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9TH INTERNATIONAL SYMPOSIUM, LJUBLJANA, 19. MAY 2023

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Program of the Symposium Socratic Lectures, May 19, 2023, 17:45 – 20:00 (Ljubljana time)

Plenary lecture

17:45 – 20:00 Marija Ipavec ; Ljubljana, Slovenia: 45 years experience with wearing an above the knee prosthesis.

Editorial

9th International Symposium Socratic Lectures was held online, 19th May 2023. It was focused on the 1.st year students of Orthotics and Prosthetics, Faculty of Health Sciences, University of Ljubljana. The plenary (and only) lecture was given by dr. Marija Ipavec, on over 45 years of experience of life with the above the knee prosthesis. On 20th May 2023 there was an accompanying cultural event marking 15 years of the Socratic lectures, held at the palace Kazina, presently the home of Academy of music, University of Ljubljana.

Dr. Ipavec was diagnosed with osteosarcoma in her first year of undergraduate study of physics and underwent amputation of the leg above the knee. She was healed of the disease and learned to live with prosthesis. She generously shared with the students her experience on this, related problems and their solutions in all Socratic lectures symposia, since 2008. When the lectures were live, she brought with her a spare prosthesis so that the students could see and touch it. It was uplifting to the participants of the lectures to meet the real winner and hero.

In the present proceedings, there are scientific contributions, repositories and a reflection on 15 years of Socratic lectures.

Veronika Kralj-Iglič and Anna Romolo

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TEXTS

Scientific contribution

The Ethmoid Labyrinth, Point of Entry to Diseases of the Nasal Cavity, Paranasal Sinuses, the Orbit and Skull Base

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Abstract:

Endoscopic endonasal surgery developed as a logical consequence of more sophisticated knowledge of the pathophysiology of the paranasal sinuses. Also endoscopic treatment of various diseases of the nasal cavity and paranasal sinuses proved to be as effective as classical methods. Ethmoidal bone is a basic anatomic area, where pathologic activities are formed or the natural extension for a minimally invasive approach from the natural nasal opening to the brain, so the knowledge of its anatomy and development is of the paramount significance. Ethmoid labyrinth starts to develop in the third week of gestation from the pharyngeal apparatus, and reach its full size only at age 12. From a surgical point of view, the most interesting are the medial turbinates, the roof of the nasal cavity, the uncinate process and the bulla ethmoidalis. Through the transethmoidal approach we can access all the cells in the ethmoid sinuses, the frontal sinus, the anterior skull base, the orbital walls and its contents. The awareness that there might be anatomical variations also carries great importance. The central location of the ethmoid bone is actually an intersection inside the nasal cavity and the basic component for understanding the anatomical relationships and the physiology of the paranasal sinuses.

Keywords: ethmoid labyrinth, embryology, anatomy, endoscopic endonasal surgery

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1. Introduction

The surgery of the nasal cavities experienced an unexpected development in the late 1970s when Messerklinger and Wigand changed the understanding of the anatomical characteristics, pathophysiology, and treatment of nasal cavity diseases. Over time, the name functional endoscopic sinus surgery (FESS) was established for the then new surgical approach to the disease, now known as chronic rhinosinusitis with nasal polyposis (CRSwNP) and chronic rhinosinusitis without nasal polyposis (CRSsNP) (Messerklinger,1978; Wigand, 1981; Wigand et al., 1978). The use of an endoscope allowed for a natural nasal opening to be used for access (Stammberger ,1986). The perspective and understanding of the surgical anatomy of the nasal cavity became completely different compared to external approaches. The key to understanding the complex anatomical relationships of the space between the middle turbinate and the posterior wall of the ethmoid sinus, the roof of the nasal cavity, and the roof of the sphenoid sinus is understanding the development and anatomy of the ethmoid sinus.

2. Embryology of the ethmoid sinus

The development of the head and neck, face, nose, and paranasal sinuses occurs simultaneously in a short period of time. By the end of the fourth embryonic week, the branchial arches, pouches, and clefts appear. In the fifth week, five protuberances form on the embryo from the mesenchyme. One of these is the frontonasal prominence, around which the nasal placodes develop. The deepening of the nasal placodes forms the nasal cavity. Between the seventh and ninth weeks of embryonic development, several bony protuberances of ectodermal origin develop on the lateral nasal wall, specifically from cells of the neural crest (Petrovič, 2002). Six bony ridges form and reduce or fuse until there are 3 to 5 of them. Each ridge has an anterior ascending and a posterior descending part. The ascending and descending parts of the first ridge will form the agger nasi cells and the uncinate process. The second and third ridges give rise to the middle and superior nasal turbinates. While the bony outgrowths form the bony structures, the spaces between them, called grooves, form the hollow spaces and openings of the sinus drainage pathways. Grooves can be divided into primary and secondary. The primary grooves give rise to the semilunar space, infundibulum, middle meatus (opening below the middle turbinate), and frontal sinus opening. The second primary groove gives rise to the superior meatus (opening below the superior turbinate). Secondary grooves form the ethmoid air cells (Petrovič, 2002; Suh, 2009). Sinuses begin to develop in the third month of embryonic development, and at birth, only the maxillary and ethmoid sinuses are present. A newborn has the same number of ethmoid air cells as an adult, but they are smaller. The ethmoid sinus reaches its final shape at 12 years of age (Elwany et al.,2013).

3. Anatomy of the ethmoid sinus

The ethmoid sinus is located between the forehead anteriorly and the sphenoid sinus posteriorly. It comprises a large part of the nasal cavity and forms the inner wall of the orbit. Its basic structure consists of four interconnected plates. The largest plate is the vertical perpendicular plate (lamina perpendicularis), which constitutes part of the bony nasal septum. On top of this plate lies the horizontally positioned cribriform plate, which forms the nasal vault and a portion of the skull base. Vertically downward from each side of the cribriform plate hang the ethmoidal labyrinth, which consists of numerous cells surrounded by thin bony walls. These cells are separated from the orbit by an extremely thin bony plate that also forms part of the orbit, known as the orbital lamina (lamina papyracea). The medial side of the labyrinth forms the upper part of the lateral nasal wall. Protruding from it are the superior and middle nasal conchae, which are curved bony structures extending downward and laterally (see **Figure 1**). The ethmoid sinus cells are divided into the anterior and posterior ethmoid sinuses by the attachment of the middle nasal concha. The superior meatus, where the posterior ethmoid cells open, is located be-

low the superior nasal concha. The middle meatus is located below the middle nasal concha and receives openings from the anterior ethmoid cells, the maxillary sinus, and the frontal sinus (Casiano, 1997; Duncavage and Becker, 2011; Kobe et al.,2007).

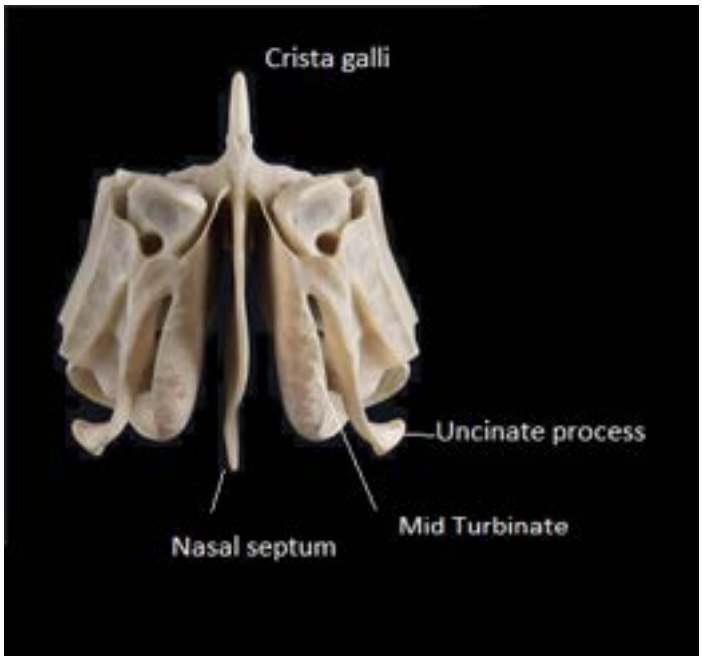

Figure 1. View of the ethmoid bone from the front. Nasal septum, middle nasal conchae, uncinate process, and cells of the ethmoid labyrinth are visible (From: https://3d4medical.com/blog/ethmoid-bone. Sneak peek from our Head & Neck update: The ethmoid bone. Complete Anatomy. 2020).

The largest of the ethmoid cells is the ethmoid bulla, which belongs to the anterior cells. Between the bulla and the middle nasal concha, there is a narrow, thin, backward and downward curved bony ridge called the uncinate process, which surrounds the semilunar hiatus. Within the hiatus, lateral and inferior to the bulla, is the entrance to the maxillary sinus. The posterior wall of the ethmoid bulla serves as a passage, through the basal lamella, into the posterior ethmoid sinus. Within it, we first find the ethmoid crest, followed by the entrance to the sphenopalatine foramen (through which the sphenopalatine artery passes). Just before the anterior wall of the bulla, there is a natural drainage pathway of the frontal sinus (frontal recess), which is surrounded by agger nasi cells on the anterior or anterolateral wall. The orbital lamina forms the bony boundary between the nasal cavity and the contents of the orbit, which are further separated by the dense connective tissue of the periorbita. Laterally to the periorbita, at the level of the anterior part of the ethmoid sinus, there is adipose tissue, followed by the medial rectus muscle of the eye. Posteriorly, there is progressively less orbital fat, and the muscle is closer to the periorbita. Two important arteries, both branches of the ophthalmic artery, traverse the ethmoid complex: the anterior and posterior ethmoidal arteries. The anterior ethmoidal artery typically courses within the roof of the ethmoid sinus. However, if there is a cell that extends above the roof of the orbit (supraorbital cell or recess), the artery may course within a bony or connective tissue mesentery. This course makes the artery more vulnerable to injury during surgical procedures. The posterior ethmoidal artery always courses within the orbital lamina. They accompany the nerves of the same name. Occasionally, a third intermediate artery may also be present (Katić and Prgomet, 2009).

4. Anatomical Variations

Knowledge of various anatomical variations of the paranasal sinuses is crucial for endoscopic surgical approaches through the nasal cavity. Onodi or sphenoethmoid cells are

posterior ethmoid air cells that extend laterally and slightly superiorly around the sphenoid sinus. They are closely related to the optic nerve, which increases the risk of injury during surgery when these cells are present. The incidence of Onodi cells ranges from 8% to 14% (Kantarci et al., 2004; Stammberger and Kennedy, 1995). Agger nasi cells are the most anteriorly located cells, situated anteriorly, laterally, and inferiorly to the frontal recess. They usually develop through the pneumatisation of the agger nasi, often via the recess. The reported incidence varies widely, ranging from 10% to 89% (Kantarci et al., 2004; Messerklinger, 1967). When these cells are large, they can alter the position of the middle nasal turbinate medially and superiorly, mechanically narrowing the frontal recess. Haller cells are ethmoid air cells that grow into the floor of the orbit, directly above and below the natural drainage pathway of the maxillary sinus. If enlarged, they can significantly narrow the ethmoid infundibulum and the opening of the maxillary sinus. The incidence of Haller cells is between 10% and 18% (Kantarci et al., 2004). Concha bullosa is a pneumatized middle nasal turbinate, occasionally involving the superior or inferior turbinate. It can cause functional narrowing of the nasal cavity due to its size. Uncinate bulla is a pneumatization of the uncinate process and is a rare anatomical variation. According to various studies, its incidence ranges from 0.4% to 5% (Kantarci et al., 2004; Rao and el-Noueam, 1998). It can significantly narrow the drainage pathways of the sinuses.

5. Modern surgical therapy of chronic inflammatory diseases, tumors of the nose, paranasal sinuses, orbit, and anterior skull base

The indications for endoscopic endonasal surgery include CRSwNP (chronic rhinosinusitis with nasal polyps), CRSsNP (chronic rhinosinusitis without nasal polyps), all intraorbital complications of acute rhinosinusitis, and other complications of acute and chronic rhinosinusitis. Furhermore, drainage of mucoceles, pyoceles, and pneumocele, resection of the tumors of the lateral wall of the nasal cavity and paranasal sinuses, and surgical access to the orbit (anterior part, including lacrimal duct surgery, extending to the cone of the orbit and optic nerve decompression) (Wilson, 2012).

Approaches to individual paranasal sinuses can vary. Previously, classical approaches involving facial incisions are mostly unsuitable for benign pathologies. However, they can be useful in cases of extensive malignancies. Endoscopic approaches through the natural openings, such as the nostrils, are now equally effective. Moreover, the boundaries of endoscopic access can extend far beyond the nose and paranasal sinuses, including the anterior skull base, orbit, retrosphenoid space, clivus, pterygopalatine fossa, and infratemporal fossa (Simmen and Jones, 2014; Wormald, 2017).

The unoperated (non-opened) labyrinth of the ethmoid bone is covered by mucosa. Imagine a coronal section through the nose (paranasal sinuses) in front of you. This provides the closest approximation to the actual endoscopic view that a surgeon sees (**Figure 2**). During a standard endoscopy, we can only see the anterior and medial surfaces of the middle turbinate, roof of the nose, uncinate process, and sometimes the ethmoid bulla. We also discuss important orientation (navigation) points that lead towards the maxillary sinus and orbit (uncinate process), towards the posterior part of the ethmoid (bulla), intracranially (roof of the nasal cavity). After removing the uncinate process, we reveal the entrance to the maxillary sinus. Moving upward, we continue into the area of the agger nasi and behind it, into the frontal recess. In this area, the ethmoid labyrinth can be composed of heterogeneous structures, which contributes to the relative complexity of the natural drainage pathway of the frontal sinus and its surgical management. By maintaining the concept of a coronal section, only the orbital wall remains laterally, while the middle turbinate remains medially. A wide surgical passage through the ethmoid into the frontal sinus represents a frontotomy. Emptying the ethmoid labyrinth behind the posterior wall of the ethmoid bulla leads us through the basal lamella into the posterior ethmoid and

ultimately into the sphenoid sinus. We return along the roof, which naturally rises forward. Usually, just before transitioning back into the frontal recess, we encounter the anterior ethmoidal artery. This emptied ethmoid labyrinth forms the basis of a complete anterior and posterior ethmoidectomy, the most commonly performed procedure in CRSwNP, where the disease affects practically all nasal cavities.

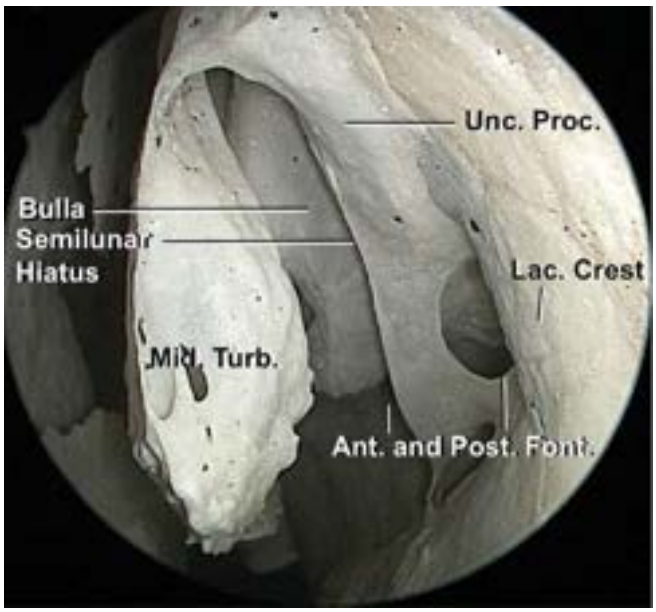

Figure 2. Endoscopic view of the parts of the ethmoid. We can see the middle nasal turbinate, uncinate process, middle nasal meatus, ethmoid bulla, and cells of the anterior ethmoid extending towards the frontal recess. Mid. Turb. – middle turbinate, Bulla – ethmoid bulla, Unc. Proc. - uncinate process, Lac. Crest – lacrimal crest, Ant. and Post. Font. – anterior and posterior fontanelle. (From: https://www.neurosurgicalatlas.com/neuroanatomy/endoscopic-view-through-the-anterior-nasal-aperture. Endoscopic View Through the Anterior Nasal Aperture.)

CRSwNP and CRSsNP are etiologically related diseases of the nasal and paranasal sinus mucosa, best defined by a yet to be precisely determined pathological inflammatory response of the mucosa to an unidentified external factor or multiple factors (Fokkens et al., 2012). The therapeutic goal in CRSsNP is to establish sinus drainage and preserve most of the natural anatomical relationships. In CRSwNP, it also involves the removal of all diseased or polypoid-transformed mucosa. In doing so, the intimate relationships of vascular and nerve structures, proximity to orbital contents, and the anatomy of the anterior cranial base must be considered (Simmen and Jones, 2014). Surgical intervention allows for the application of local medications that effectively reduce the chances of disease recurrence (Fokkens et al., 2012).

Functionally, the maxillary sinus, frontal sinus, and ethmoid sinus preserve most of their drainage capacity, provided by the active functioning of cilia in the mucosa (Duncavage and Becker, 2011). Endoscopic endonasal surgery has emerged as an equally successful and less invasive method of surgical treatment for certain benign and malignant tumors, including tumors that involve or even extend beyond the anterior cranial base intracranially (Lund et al., 2014).

In cases of malignancies in the nasal cavities, our boundaries are even broader. We aim to remove the malignant tumor in healthy tissues either in one block or in multiple divided, oriented blocks. The principles of oncologic surgery also apply to endoscopic endonasal surgery for tumors. Planning and executing the surgery in layers without tumor presence is a fundamental requirement for ensuring radicality (Lund et al., 2014).

In such cases, the lateral surface is typically the periorbita, which comparably effectively halts the local progression of the disease. This continues until reaching the optic nerve canal, which is already located in the sphenoid sinus. Moving upward in the periorbital plane, we find duplicatures for the anterior and posterior ethmoidal arteries. When viewing the roof of the ethmoid, the anterior ethmoidal artery courses lateroposteriorly to medioanteriorly, while the posterior ethmoidal artery courses lateroanteriorly to mediposteriorly. The posterior artery is in direct proximity to the optic nerve. When removing the superior orbital lamella, we start grinding the superiomedial wall of the orbit, and we can enter the most superior (frontal recess) and superoinferior (supraorbital recess) parts of the ethmoid. The roof of the ethmoid borders the dura of the cranial fossae and is considerably higher than the roof of the nasal cavity. Hence the classification according to Keros, which divides the ratio of both roofs into more or less dangerous categories from an endoscopic surgical perspective (Elwany et al.,2013; Keros, 1962).

For diseases that extend intracranially and transnasally-transethmoidally accessible diseases, the mentioned corridor can be advantageously used for successful, less invasive neurosurgical interventions (Zanation et al., 2012). The ethmoid labyrinth leads into the anterior cranial fossa. The area of the posterior wall of the frontal sinus, roof of the nose, and roof of the ethmoid is also referred to as the anterior cranial base. In a transcribriform approach, intracranially, we see the space that is laterally bounded by the left and right periorbita, with the entrance into both frontal sinuses extending intracranially with the frontal lobes of the brain and the anterior vascular network, usually after removing the cribriform plate along with both olfactory bulbs and tracts (following dural removal).

Conclusion

The ethmoid bone represents the anatomical and functional center of the nasal cavity. Various changes occur on its mucosa, leading to pathological processes of benign or malignant nature. With the advent of endoscopic nasal surgery, the ethmoid bone has become even more important, subjected to detailed surgical study, and a key aspect of surgical management for significant nasal and sinus diseases. Furthermore, corridors through the ethmoid bone have been created for accessing the anterior skull base, including intracranial regions, becoming of interest in the treatment of brain and cerebrovascular diseases.

Conflicts of Interest: The authors declare no conflict of interest.

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Scientific contribution

Spinopelvic Alignment and Sagittal Balance in Adolescent Patients with Structural Hyperkyphosis

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Abstract:

Sagittal spinopelvic configuration and global alignment have previously been investigated in healthy children, adolescents, and adults to understand biomechanics or to guide fusion surgery. However, no studies were found in the literature assessing patients with structural hyperkyphosis (HK) from the perspective of global alignment and spinopelvic parameters. The research question is: How does structural HK affect spinopelvic parameters, global tilt(GT), global alignment, and proportion (GAP) score in skeletally immature adolescents? This was a retrospective analysis of prospectively collected data. Thirty-two structural HK patients(19 male, 13 female) with a mean age of 13.8 years and an initial curve magnitude between 55-75° were included in the study. Radiographic measurements including thoracic kyphosis(TK) angle, L1-S1 lumbar lordosis(LL) and L4-S1 lower arch lordosis angles, pelvic incidence, sacral slope, global tilt were measured from lateral spine radiographs by a blinded orthopedic spine surgeon at the first visit, and GAP score was calculated using these parameters. The mean TK was 59.5°, LL was 66.7°, and the mean GT was 7.2° at baseline. There was a correlation between TK and LL, meaning that patients who have higher TK have also a higher degree of LL. There was also a correlation between PT and SS with the PI at baseline. Structural HK may affect pelvic development resulting in lower values of PI. Higher values of TK or thoracolumbar kyphosis may be sufficiently compensated by LL to maintain a neutral sagittal balance when PI has lower values. Clinicians should focus on global alignment to maintain the global sagittal balance rather than TK alone.

Keywords: Scheuermann's disease, hyperkyphosis, global alignment, sagittal balance, spinopelvic configuration

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1. Introduction

Hyperkyphosis(HK) is the structural abnormal curvature of the spine in the sagittal plane beyond the physiological limits, due to Scheuermann's disease or other causes (Tribus, 1998). Scheuermann's disease is a common cause of HK in adolescents that involves the vertebral bodies and discs of the spine. Scheuermann's disease is identified by anterior wedging greater than or equal to 5° between endplates of three or more adjacent vertebral bodies (Mansfield, 2023). Structural HK deformity develops before puberty in the thoracic or thoracolumbar spine and, it reaches the peak deformation during the adolescent growth spurt. Posterior components grow at a faster pace than their anterior counterparts during the development processes of hyperkyphotic deformity. Thus compressive forces on the anterior portion of thoracic vertebral bodies increase and resulting in an uneven distribution of forces in the thoracic region (Fotiadis et al., 2008).

Sagittal alignment and spatial pelvic parameters are directly affected in HK. Due to close anatomical relationships, the segmental alterations result in a compensated posture to minimize energy expenditure (Mac-Thiong et al., 2007; Tyrakowski et al., 2014). The interaction existing between the spine and the pelvis is a key point in the understanding of the sagittal balance of spinal deformities. In case of imbalance, the compensatory mechanisms are activated to maintain the balance. Compensation begins at the spinal level (modifications of spinal curves), and if these compensations are not sufficient, the pelvis tilts backward, increasing the PT value (Obeid et al., 2016).

Untreated hyperkyphosis in the growing spine might result in progressive deformity and back pain. The symptom severity increases with a progressive sagittal imbalance in patients with kyphosis-related back pain (de Mauroy et al., 2010). The biomechanical concept for treatment focuses to decrease the mechanical stress load on the anterior wall of the vertebral body. Thorocolumbosacral hyperkyphosis brace and hyperkyphosis-specific exercises reduce the axial load and shift the center of gravity to the posterior, and prevent the collapse of the anterior wall of the vertebral body (de Mauroy et al., 2010).

Sagittal malalignment is commonly recognized by lumbar lordosis (LL), thoracic kyphosis (TK), pelvic tilt (PT), sagittal vertical axis, and knee flexion (Le Huec et al., 2011). These parameters are used in daily practice however lumbar lordosis and thoracic kyphosis alone are insufficient to explain the effects of the spinopelvic configuration on biomechanical loading on the spine. While spinal loading can easily change in different positions depending on gravity, the angle of the kyphosis may be numerically the same (Schlösser et al., 2015). In spite of the close interaction between the spinal balance and the compensatory mechanisms, assessment of the global spinopelvic balance ignored either the spinal part or the pelvic part until the development of new global approaches. Eventually, new parameters such as the global tilt (GT) and global alignment and proportion (GAP) score were developed in recent years for the global spinopelvic balance that takes into account both the spinal part and the pelvic part (Obeid et al., 2016). The GT is a modified version of the spinopelvic angle (SPA), which analyzes malalignment, considering spinal and pelvic imbalance together. GAP score, is a new pelvic incidence-based proportional method to analyze sagittal alignment and balance, and it gives relative deviations from PI-based normative data (Gupta et al., 2021; Obeid et al., 2016; Yilgor et al., 2017a).

Sagittal spinopelvic configuration and global alignment have been investigated mostly in adults and skeletally mature young adults. For the adolescent population, studies have not been on hyperkyphosis but on adolescent idiopathic scoliosis. All of these studies have been conducted primarily to guide fusion surgery or to understand biomechanics (Yilgor et al., 2017b). Currently, no study investigated spinopelvic parameters and global alignment in skeletally immature hyperkyphosis patients with a global alignment standpoint (Aulisa et al., 2016; de Mauroy et al., 2010; Weiss et al., 2009). This study aimed to assess spinopelvic parameters, GT, GAP scores and, to determine the relationship between hyperkyphosis and these parameters in patients with structural HK.

2. Methods

This is a retrospective analysis of patients' data between December 2015-2022. Out of 44 patients diagnosed with structural HK (Scheuermann's disease or other causes), 32 of those who met the inclusion criteria were analyzed in this study. The demographic and clinical data of the patients were recorded. Physical examinations of the patient's spine were performed and the differential diagnosis of HK was made according to thoracic spine magnetic resonance imaging as described in the literature (Haddadi et al., 2018). Patients who do not meet yet Scheuermann's disease diagnosis criteria due to the early phase of the disease development, presented as structural HK. The presence of pain was recorded. The primary outcomes were a GAP score and GT.

1.1. Participants

1.1.1. Inclusion Criteria

- Subjects who were diagnosed with structural HK (Scheuermann's disease or other causes)
- Ages between 10-18 years
- Initial curve magnitude between 55-75°

1.1.2. Exclusion Criteria

- Cobb angle ≥20 in the coronal plane
- History of spinal surgery or trauma
- Patients having kyphosis due to congenital, neuromuscular, traumatic, tumor, infection causes
- Ankylosing spondylitis and other spondyloarthropathies.
- The lack of appropriate full-length lateral radiography with acceptable quality at the baseline assessment.

1.2. Radiographic measurements

Full-length (posteroanterior and lateral) standing digital radiographs of the spine were evaluated at the first visit. All radiographic measurements were performed by a blinded orthopaedic spine surgeon using Surgimap® Spine(Nemaris™ Inc, New York), a validated software (Lafage et al., 2015) . Skeletal maturity was defined according to the Risser sign using a posteroanterior spine radiograph. Risser sign is based on the iliac apophysis pattern of ossification during adolescence. It is classified on a scale from 0(immature) to 5(fusion), indicating different stages of skeletal development. The other radiographic measurements, including kyphosis angle, lumbar lordosis (L1-S1) and L4-S1 lordosis angle of the inferior arch, pelvic incidence, sacral slope and GT were measured from lateral spine radiographs and the GAP score was calculated from these parameters as described in the literature (Obeid et al., 2016; O'Brien et al., 2008; Yilgor et al., 2017b). GT is a modified version of the spinopelvic angle, which analyzes malalignment considering spinal and pelvic imbalance together (Obeid et al., 2016). GAP score is a new pelvic incidence-based proportional method developed by the European Spine Study Group (ESSG) to analyze sagittal alignment and balance. The GAP score assesses the spatial orientation of the pelvis, magnitude and distribution of lumbar lordosis, and the global spinopelvic alignment of the spine and pelvis relative to normative targets rather than as an absolute numerical value in relation to the ideal values calculated for any individual. The GAP score includes five domains, including relative pelvic version, relative lumbar lordosis,

lordosis distribution index, relative spinopelvic alignment, and age factor. The scores for each of the four domains vary from 0 to 3, and the age factor varies from 0 to 1. The total GAP score ranges from 0 to 13 points and is calculated by adding the scores of these five domains. If the total score is 0 to 2, the alignment is considered proportional, for 3 to 6, moderate disproportion, and for ≥7, severely disproportionate spine (Yilgor et al., 2017b).

1.3. Statistical analyses

Parametric data were presented as mean and standard deviation (SD). Nonparametric data were presented as median and percentage. The Spearman's correlation coefficient was used to examine correlations between parameters. A p-value of ≤0.05 was considered statistically significant. Statistical analysis was performed using IBM SPSS Statistics® (version 23; Armonk, NY: IBM Corp.)

3. Results

Among 32 patients (19 male, 13 female), 16 had Scheurmann's HK and 16 had structural HK. The mean age was 13.8 years. The baseline characteristics of the patients are shown in **Table 1.**

Table 1: Baseline Characteristics of Patients

	All	Female (n=13)	Male(n=19)	p value*
Variables	Mean±SD	Mean±SD	Mean±SD	*U*
BMI	19.7±3.7	19.4±3.7	19.9±3.8	0.985
Scheuermann/structural	16/16	5/8	11/8	
Age	13.8±1.4	12.9±1.7	14.5±0.7	**0.020**
Presence of pain(yes/no)	18/14	6/7	12/7	
Concurrent scoliosis(yes/no)	19/13	5/8	14/5	
$Cobb°_{max}$	11.4±10.6	5.6±8.2	15.3±10.5	**0.014**
$Risser_{baseline}$	2.1±1.8	1.4±1.7	2.6±1.7	0.051

BMI: body mass index, *Statistically significant at $p \leq 0.05$, *U*: Mann-Whitney *U* test

Radiographic measurements are shown in **Table 2**. The mean TK was 59.5°, LL was 66.7° and the mean GT was 7.2° at baseline. GT measurement from the x-ray results of one patient, who was taking conservative treatment for a year, presented to show the difference globally (**Figure 1**).

Table 2. Descriptive results of spinopelvic parameters in adolescents with HK

	Baseline
Variables	Mean±SD
Kyphosis angle	59.5±9.4
L1-S1 lordosis angle	66.7±11.5
L4-S1 lordosis angle	40.7±7.3
Pelvic tilt	9.06±9.1
Sacral slope	30.4±15.5
Pelvic incidence	38.4±16.1
Global tilt	7.2±12.0
GAP score	3.4±1.9

L1-S1:Lumbar1-Sacral1 vertebra levels; L5-S1 Lumbar5asfd-Sacral1 vertebra levels; GAP: global alignment and proportion

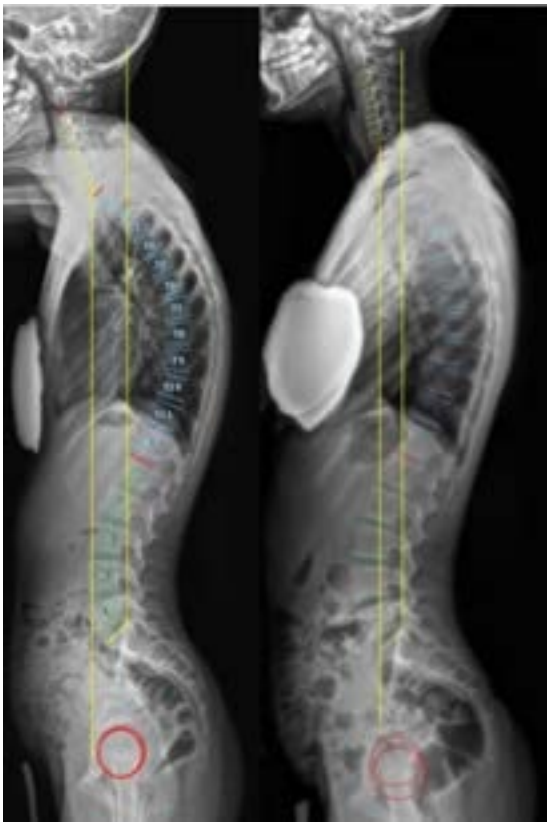

Figure 1 Sagittal spinal alignment before and after 1-year treatment in standing full-spine lateral radiography

There was a correlation between TK and LL; and also PT and SS with the PI at baseline (**Table 3**).

Table 3: Correlation Between Pelvic Incidence and Other Spinopelvic Parameters

	Baseline	
Variables	r	p value
PI vs TK	-0,253	0,162
PI vs LL	-0,053	0,774
PI vs LL_{L5-S1}	-0,255	0,159
PI vs PT	0,549**	0,002*
PI vs SS	0,490**	0,004*
PI vs GT	0,215	0,238
PI vs GAP	0,124	0,498

PI: pelvic incidence, TK: thoracic kyphosis, LL: lumbar lordosis, PT: pelvic tilt, SS: sacral slope, GT: global tilt, GAP: global alignment and proportion, *Statistically significant at $p \leq 0.05$, r: Spearman's Rho correlation coefficient

4. Discussion

Spinopelvic balance depends on the combination of pelvic and spinal shape (Roussouly and Pinheiro-Franco, 2011). Berthonnaud et al. (2005) have proposed the concept of a linear chain connecting the head to the pelvis, where the shape and orientation of each anatomical segment are closely linked and influence the adjacent segment to maintain a stable posture with minimal energy expenditure. According to this concept, a change in the shape or orientation of one anatomical segment results in a change in the shape and/or orientation of the adjacent segments of the spine and pelvis (Mac-Thiong et al., 2007)

The importance of sagittal alignment of the spine in relation to its normal function and the etiopathogenesis of spinal deformities has been increasingly recognized (Schlösser et al., 2015). The main reasons for the increasing recognition are the high rate of mechanical complications and associated morbidities and mortalities, the need for secondary surgeries, and the increased healthcare costs. It was critical to reduce mechanical complications and associated health problems. At that time, the GAP score was created using a mathematical formula that considered proportional alignment rather than using fixed numerical values for anyone. Although the restoration of normal sagittal alignment is a crucial goal of conservative and surgical treatment, it seems clinicians still focus mostly on kyphosis angle, in-brace correction, and pain (Aulisa et al., 2016; Berdishevsky, 2016; de Mauroy et al., 2010; Weiss et al., 2009). For this reason, in the present study, we wanted to provide a different viewpoint and investigated the relationship between sagittal alignment and spinopelvic parameters using relatively new measurement parameters such as GT and GAP in skeletally immature patients with structural HK.

Cil et al.(2005) found that thoracic kyphosis and lumbar lordosis increase during growth and the thoracic apex is lower in late adolescence than at younger ages in healthy children. Furthermore, they observed that the development of lumbar lordosis begins before

puberty and thoracic kyphosis develops later (Schlösser et al., 2015). Many studies have reported that during the physiological growth phase, thoracic kyphosis and pelvic incidence increase slightly, and reach adult configuration at the end of growth (Cil et al., 2005; Mac-Thiong et al., 2007; Mangione et al., 1997; Mendoza-Lattes et al., 2010). Lee et al. (2012)found that thoracic kyphosis was 31.8° and 33.4°; lumbar lordosis was 50.0° and 48.1° in 8-12 years and 13-17 years respectively. They have found a positive correlation between thoracic kyphosis and lumbar lordosis in asymptomatic young people 3-20 years of age ($r=0.485$, $p<0.001$). There was a correlation between TK and LL in our adolescent HK sample too. Weiss et al. (2009) reported that the mean kyphosis angle was 55.6° (43-80°) in patients with Scheuermann kyphosis at 12-17 years of age. Similarly, Aulisa et al.(Aulisa et al., 2016) reported the TK as 57.8° at 12 years of age. However, the LL angle was ignored in either of these studies before or after conservative treatment.

PI is a morphological pelvic parameter for the three-dimensional regulation of sagittal spinal curves. Despite some reports stating that PI may change by the end of skeletal growth, it is widely accepted that PI becomes fixed at about 10 years of age and remains constant during adolescence and adulthood unless there is a pathological process that may alter the shape of the pelvis (Tyrakowski et al., 2014). Lee et al. (2012) reported that the PI in asymptomatic adolescents was 43.6° at 8-12 years of age and 46.4° at 13-17 years of age. Tyrakowski et al. (2014) reported that the average PI in skeletally mature patients with Scheuermann's disease was 40° and significantly lower than in healthy adults and adolescents ($p < 0.0001$) . They suggested that Scheuermann's disease occurring in adolescent individuals may affect further pelvic development of the pelvis resulting in lower values of PI. In addition, higher values of TK or thoracolumbar kyphosis may be sufficiently compensated by LL to maintain a neutral sagittal balance when PI has lower values(Tyrakowski et al., 2014). Significantly lower PI values were also found in patients with post-tuberculosis or congenital thoracic and thoracolumbar kyphosis (Li et al., 2013). Similarly, PI was lower in our study than in reported healthy subjects, the mean value of PI was 38.4°, and no statistically significant correlation was found between TK, LL, and LLL5-S1 with PI, either at baseline or after treatment, and our results are consistent with the literature (Table 3).

Bracing has been used for the treatment of deformity and to relieve pain in HK. In one study, a Milwaukee brace was applied on patients with Scheuermann's disease and reported a 40% decrease in mean thoracic kyphosis, a 35% decrease in mean lumbar lordosis after an average of thirty-five months of brace wearing (Weiss et al., 2009). Weiss et al. (2009) showed that. An average in-brace correction of >15° was achieved using the kyphologic™ brace. There are a very small number of studies on the conservative treatment of structural HK and all of those studies clearly ignored the global spinopelvic alignment and balance approaches and only focused angle of kyphosis. Sagittal spinal balance was greatly affected rather than physiological kyphosis in patients with structural HK (Tyrakowski et al., 2014). Pain and other symptoms could have been caused by spinal disproportion. A patient with HK may be well adjusted so that he may be proportionally aligned even if his kyphosis angle was defined as "hyper". Each individual has their own normality and adaptations, so more than just the kyphosis angle is needed for the patient's assessment and treatment plan. Clinicians need to evaluate whole sagittal parameters and balance, taking into account the PI value, and not just the kyphosis angle or the correction by brace alone.

Although global deformity is evaluated clinically in adolescents with HK, the kyphosis angle is a determinant in deciding conservative treatment. We believe that the GAP score, which has an important place in surgical planning, could also be useful in conservative treatment. We suggest that spinopelvic parameters and integrative assessment parameters such as GT or GAP score should also be included in the guidelines for these patients.

4.1. Limitations

This study has obvious limitations including not presenting follow-up time and a small sample size. However, this result is based on baseline measurements from an ongoing study in which patients are currently taking conservative treatment. In the future, we will present the effects of treatment on global balance and spinopelvic parameters after a follow-up period of 2 years.

5. Conclusions

A sagittal profile is well studied in adults, however, there is limited knowledge on adolescents and children, especially the ones with structural HK deformity. The present study can give some more understanding of this neglected area of research. Clinicians should focus on global alignment to protect the global sagittal balance rather than kyphosis angle or in-brace correction alone.

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Institutional Review Board Statement: The study was conducted with ethical approval from the Haliç University Medical Ethics Committee (date: 4/29/2021, number:71)

Conflicts of Interest: The authors declare no conflict of interest.

Appendix A: GAP score (10).

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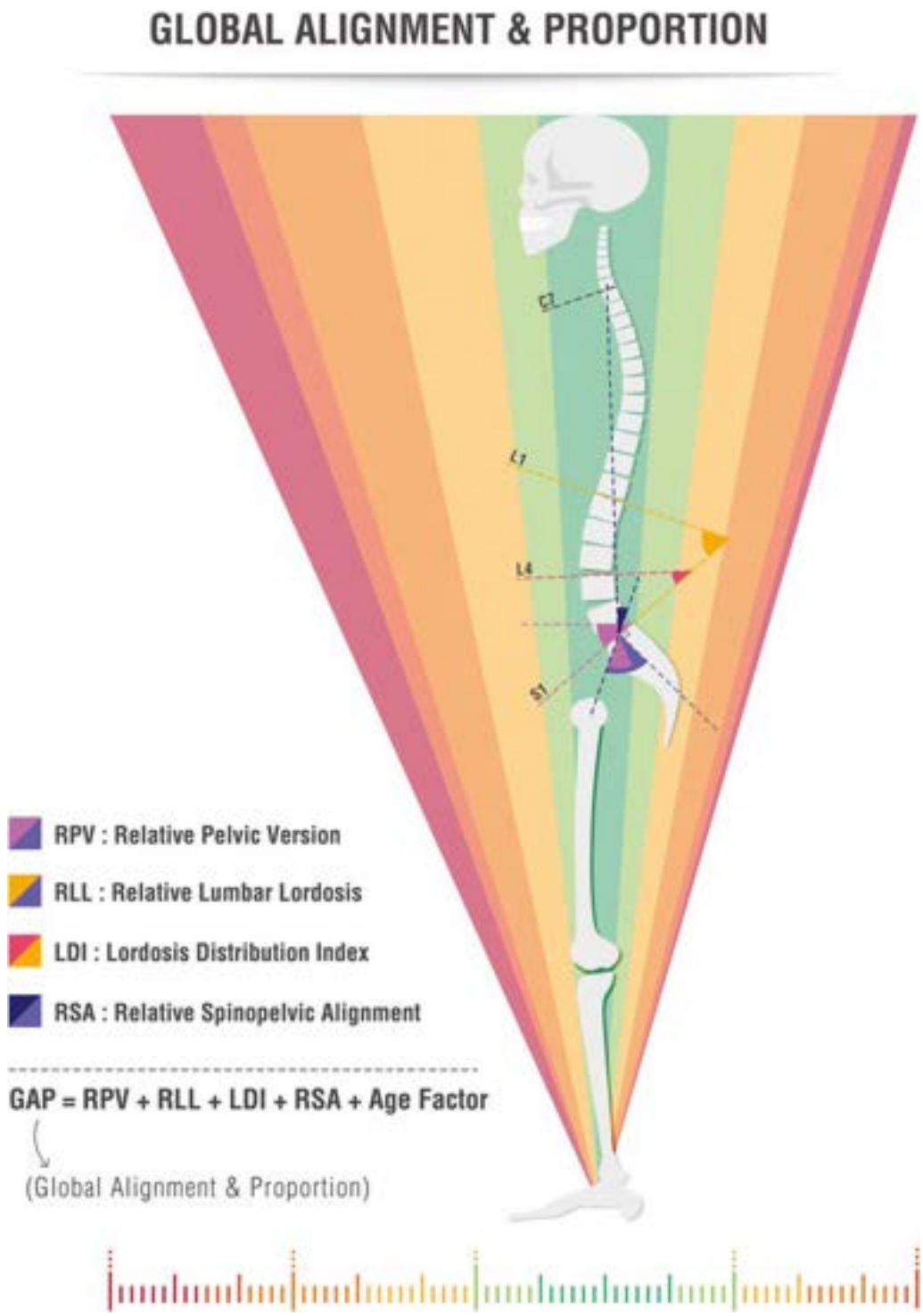
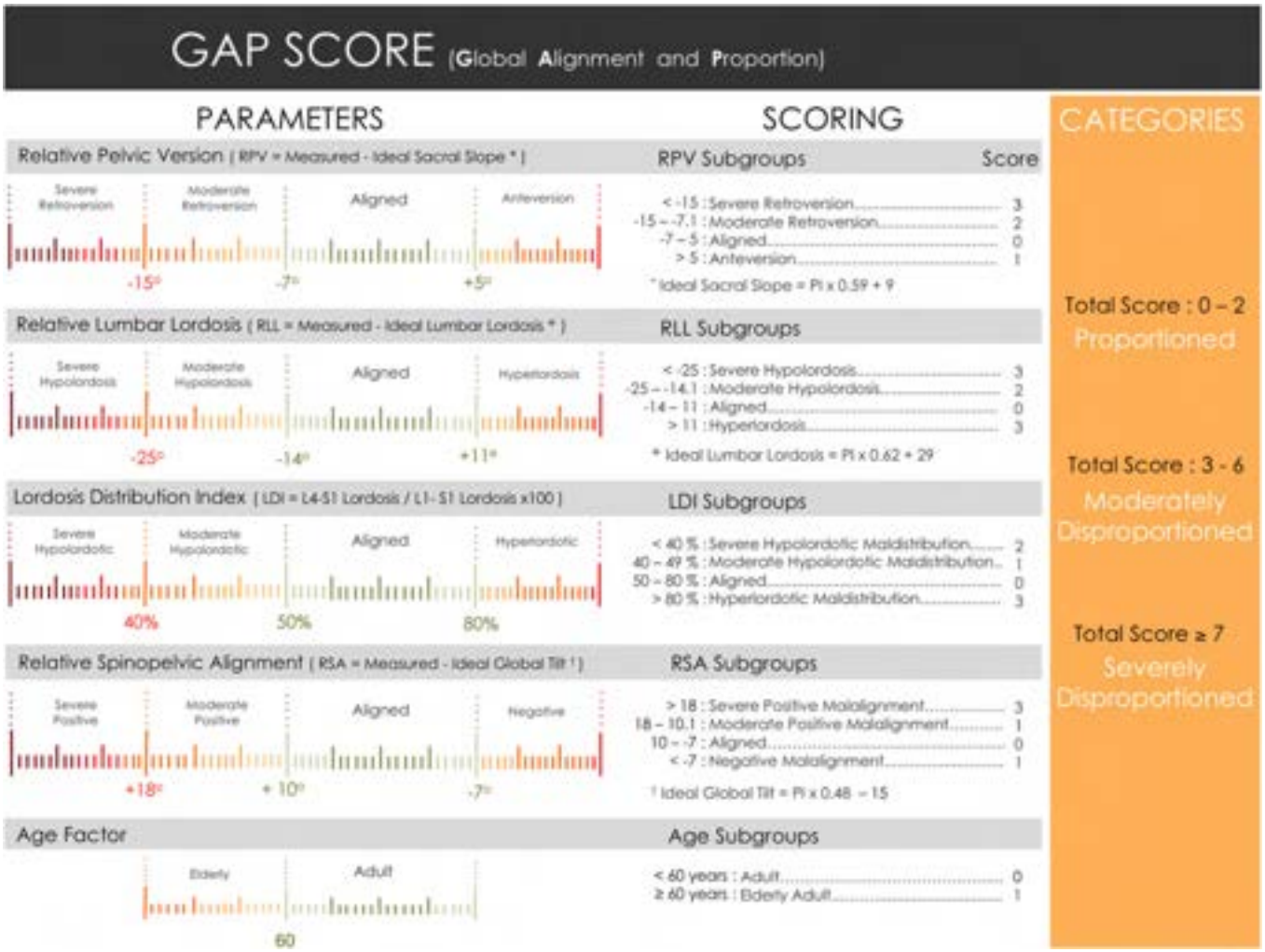
Appendix A


GLOBAL ALIGNMENT & PROPORTION
C2
L1
L4
S1
RPV : Relative Pelvic Version
RLL : Relative Lumbar Lordosis
LDI : Lordosis Distribution Index
RSA : Relative Spinopelvic Alignment
GAP = RPV + RLL + LDI + RSA + Age Factor
(Global Alignment & Proportion)



These two pictures were obtained from the original article: Yilgor C, Sogunmez N, et al., 2017b (25)

Scientific contribution

Clinical-Hematological Examination of Cattle Affected by Theileriosis

Nebieridze Shota [1], Kereselidze Maia [1*], Beruashvili Maia [1], Kvachrelishvili Vazha [1], Matskepladze Marine[1]

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Abstract:
Theileriosis is the most severe transmissible disease among the piroplasmid diseases of ruminants, which is widespread all over the world, especially in the countries with a hot climate in the south, it is also found in Georgia. The source of infection is a sick animal, and the carrier is the pasture ticks *Hyalomma Detricum* and *Hyalomma anatolicum anatolicum*. The aim of the study was to investigate the dynamics of the clinical course and solution of the disease in the case of Theileriosis in cattle, for which nine 6-month-old calves were naturally infected with ticks of the *Ixodidae species* carrying the field strain of *Theileria annulata*. Three calves were selected as a control group.During the trial, clinical observation was carried out of infected animals: morning and evening temperature control. Blood was taken from the jugular vein for hematological and serological examination. Blood smears were stained by the Romanovsky-Giemsa method. The degree of infection of erythrocytes by Theilers was determined. Serological examination of blood was performed by prolonged complement fixation reaction to detect specific antibodies.

Keywords: Theileriosis, Theileria annulate, schizont-infected cells, cattle, Infection, Hyalomma anatolicum anatolicum,

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1. Introduction

Theileriosis is the most severe transmissible disease among the piroplasmid diseases of ruminants, which is widespread all over the world, especially in the south countries with a hot climate, it is also found in Georgia.

Theileriosis is caused by the unpigmented protozoa - from the genus Theileridae. The agent of theileriosis in different species of animals is specific. It parasitizes in cattle in the form of *Theileria Annulata*, *Theileria Sergenti, Theileria Mutans*; In sheep and goats – *Theileria Ovis*, in Northern Deer – *Theileria Tarandirangiferis*. The source of infection is a sick animal, and the carrier is the pasture ticks *Hyalomma Detricum* and *Hyalomma anatolicum anatolicum*. These ticks also harbour in livestock holdings.Theileria that invades into the body of cattle goes through two stages of development: (i) Multiple dividing – schizogony, which is characterized by formation of schizont-infected cells ("pomegranatelike body") in lymph nodes, spleen, bone marrow; (ii) Simple dividing (into two, four parts) formation of erythrocyte form of *Theilers* in erythrocytes of the host. They will undergo further development in the body of *Ixodidae* ticks. The disease develops when ticks infected with Theileria attack livestock and, during blood-sucking, inoculate the Theileria into the host's blood and lymph along with saliva. (Fedorov, 1956; Nebieridze, 2009a; Nebieridze, 2009b). The damage caused by Theileriosis is determined primarily by high mortality (30-90%) with abortions, by losing weight and decreasing the quality of meat (Nebieridze, 2009a; Stepanova et al., 1987; Zablotsky, 1985).

Theileriosis was diagnosed by epizootological, clinical and laboratory tests, during which erythrocyte forms of theileriosis are seen in blood smears and macro schizonts or "pomegranatelike bodies" in the material taken from the damaged lymph nodes and spleen. The disease proceeds in an acute and subacute form, it is rarely possible to turn nto a chronic form (Fedorov, 1956; Kamanov et al., 2007).

The objective of present study was to investigate the dynamics of the clinical course and solution of the disease in the case of Theileriosis in cattle, for which nine 6-month-old calves were naturally infected with *Th. annulata* field strain carrier by ticks of the *Ixodidae species*. We included 3 calves for control.

2. Material and methods

The experiment was carried out with the informed consent of the owners of naturally infected animals, which was approved by the Bioethics Committee of the Faculty of Veterinary Medicine of the European University on April 5, 2022, registration N74817. During the trial clinical observation of the sick animals, hematological and serological examinations and temperature control was performed in the morning and in the evening. Blood was taken from the jugular vein for hematological and serological examinations. Blood smears were stained by the Romanovsky-Giemsa method. The number of erythrocytes and leukocytes was determined, as well as the hemoglobin content and the rate of infection of erythrocytes by *Theileria annulata*. Serological examination of blood was performed by prolonged complement fixation reaction to detect specific antibodies.

3. Results and discussion

The disease had subacute development while some authors (Fedorov, 1956; Kamanov et al., 2007; Lal et al., 1985) indicate the acute development of the disease. The incubation period lasted for 9-10 days. The body temperature in infected animals varied within the norm (39.3 °C - 39.4 °C). Hemoglobin content was within 100.27 - 90.75 g/l; The number of erythrocytes in 1 mm^3 of blood was 7.5 - 6.7 millions, and of leukocytes was 7.4 - 6.6 thousand. The erythrocyte forms of theilers were not yet observed in the blood.

On the 11th day after infection, the infected animals started to have a sharp increase in temperature. Constant type of fever lasted for 12 - 16 days and reached 41,5 °C, sometimes the temperature reached up to 42 °C; Hemoglobin content decreased to 80.1 g/l;

The number of erythrocytes and leukocytes is 5.6 millions, respectively-decreased to 4.5 thousand. Parasitemia in the blood was 44%.

On the 14th day after infection, a pea-sized dense nodules with a dark red center, surrounded by a relatively pale rash were observed on the skin at the site of attachment of the tick. The superficial lymph nodes near the site of invasion (groin, knee fold) were enlarged and painful to the touch.

On the 18th day after the infection (7th day after the rise in temperature), the animals were in agony, depressed, with a severe course of the disease: the hair was disheveled, multiple firm granulomas with a dark red concave center and raised edges on the surface of the skin were observed around the anus. The conjunctiva was wet and hyperemic, the mucous membrane of the nose was covered with mucus, there were pinpoint and petechiae hemorrhages. There are similar bruises on the skin of the abdomen and other bright areas of the body. Superficial lymph nodes were enlarged, hardened and painful on palpation. Sick animals stopped receiving food and water; animals stopped rumination and developed atony, constipation or diarrhea alternate; the pulse were rare - heart impulses were strengthened. Schizonts were found in the punctate smear obtained from the lymph nodes - the so-called "Pomegranatelike bodies". The same type of changes were described by other researchers (Fedorov, 1956; Nebieridze, 2009a). Sick animals with clinical signs had persistent fever (41.5 °C - 42 °C), hemodynamic disturbances, a sharp drop in hemoglobin content to 70.27 g/l, while the number of erythrocytes and leukocytes was approximately the same as on the 14th day after infection. 67% of erythrocytes were infected with theilers. N. Kamalov et al. (2007) investigated 172 cattle in southern Tajikistan, where parasitemia reached to 70-75%. Schizont-infected cells found in punctate smear obtained from the lymph nodes of infected calves on 18th day (**Figure 1**).

On the 23rd day after infection (13-15 days after the fever), a constant type of fever was still maintained, although a tendency to decrease in temperature (41.2 °C - 40.7 °C) was observed. Hemoglobin content further decreased (6.37%). The number of erythrocytes decreased to 4.8 millions. The number of leukocytes increased to 5.4 thousands, which indicated the strengthening of the defense mechanism. Parasitemia in the blood was slightly reduced to 65%.

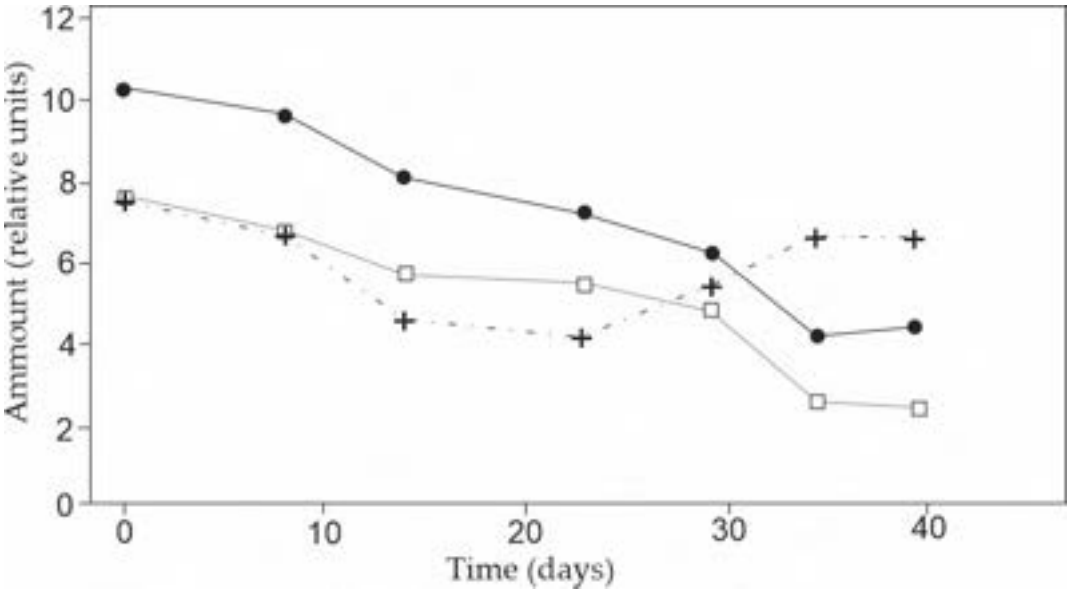

Figure 1: Hematological changes in infected calves in dependence on time. Hemoglobin concentration (circles) in mg/l, erythrocyte number density (squares) in millions and leukocyte number density in thousands per mm^3 of blood.

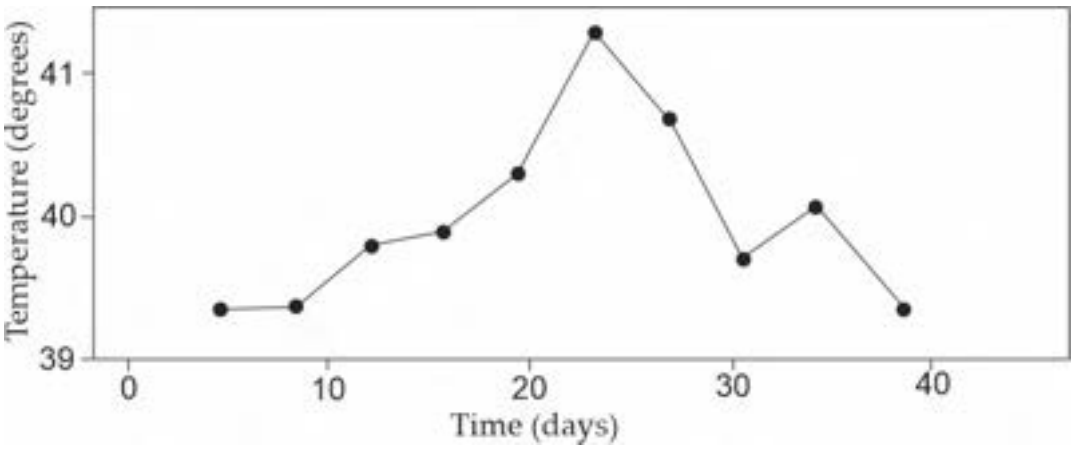

Figure 2: Temperature in infected calves in dependence on time.

In the mentioned period, progressive cachexia and weight loss of sick animals were noted. Anemia of the conjunctiva and mucous membrane of the mouth was noted together with hemorrhages. On the 23rd day after infection, the condition of four infected animals worsened, they had complete anorexia; They were in agony and culled. The carcasses and offal (internal organs) were destroyed due to pathological anatomical abrupt changes. We share the opinion of Fedorov (1956) and Lal et al. (1985) that during Theileriosis there is an activation of autoimmune reactions during which the cells of the macrophage system phagocytize not only the Theileriasis-infected, but also normal erythrocytes, which contributes to the development of anemia and, as a result, lack of oxygen in tissues and organs. In addition, an important role in the development of anemia is played by the toxins released during the activity of parasites, as well as toxic substances produced by the destruction of tissues, which enter the organs through the blood, including the bone marrow, and cause the blocking of the blood formation process. These indications agree with our results. By serological examination, specific antibodies were detected for the first time on the 23rd day after infection, the titer of which was 1:40. The antibody titer reached its peak 1:160 in the recovery stage - on the 39th day which indicates the formation of non-sterile immunity.

4. Conclusion:

On the 39th day after infection (recovery stage), normalization of temperature was observed - 39.4 °C. Hemoglobin content and the number of erythrocytes were still low against the background of an increase in the number of leukocytes. Parasitemia and general intoxication of the body slowed down, hemorrhagic diathesis weakened. The action of compensatory recovery mechanisms of the body was strengthened and the animals begun to recover clinically. Thus, as a result of our investigations, it is possible to make the following conclusions:

4.1 Incubation period of cattle theileriosis caused by *Theileria annulata* is 9 - 10 days. The disease has subacute development.

4.2 Permanent fever begins on the 10-11th day after infection and lasts for 12-28 days, which in our opinion depends on the individual condition of the animal and the virulence of the parasite.

4.3 The disease is characterized by hemorrhagic diathesis - Petechiae on visible mucous membranes and tender, visible areas of the skin.

4.4 Specific formations (granulomas) characteristic of Theileriosis in the smears made from the punctate of the lymph nodes of a diseased animal – schizont-infected cells (so-called "Pomegranatelike body") were discovered on the 3rd-4th day after the incubation period.

4.5 Theileria settle in erythrocytes, causing their lysis, which results in a sharp decrease in hemoglobin, lack of oxygen in the tissues and the development of severe anemia.

4.6 As a result of activity of theileria, toxins produced by the destruction of such tissues cause hemodynamic disorders and blocking of the blood formation process.

4.7 With the progression of the disease, on the 18th-23rd day after infection, part of the animals stopped taking water and feed, complete anorexia developed, and if the compensatory mechanisms for their protection were weak and medical intervention was not performed in time, the animals died due to a sharp heart failure. Only two from infected calves were recovered on the 39th day, in which titre of specific antibodies reached a peak of 1:160 which indicated formation of nonsterile immunity.

Conflicts of Interest: The authors declare no conflict of interest.

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Review

The Secret of the Biochemical Reaction in the Abdomen of the Beetle: Bioluminescence of the Firefly

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Abstract:
Fireflies (*Lampyridae*) belong to a family of beetles that live widely in the humid tropical and subtropical regions of the world. They are best known for using a light organ in their abdomen to produce light, which they use to communicate with each other. Species can be distinguished by the pattern of light flickering that identifies members of a species. All larvae also glow to signal to predators that they are inedible.
The light emitted by fireflies is produced by converting chemical energy into light. In this phenomenon, called bioluminescence, the substance luciferin reacts with oxygen in the presence of the enzyme luciferase. In addition, bioluminescence is difficult to study because all animals stop glowing after they are captured. Bioluminescence is a very efficient process, converting up to 90% of energy into light, also called cold light. The complex process of bioluminescence is still not fully understood, so scientists are using interdisciplinary methods (from theoretical to experimental approaches) to study the problem.

Keywords: Firefly, bioluminescence, luciferin, luciferase, mechanism, enzyme

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1. Introduction

The term *luminescence* comes from a Latin root (*lumen*: light). It was first introduced in 1888 by the German physicist and historian of science Eilhard Wiedemann as luminescence for all light phenomena that are not solely due to an increase in temperature, i.e., incandescence (Valeur et al., 2011). Before discussing the historical development of the term luminescence, it should be noted that today's definition of luminescence is: "spontaneous emission of radiation by an electronically excited species (or a vibrationally excited species) that is not in thermal equilibrium with its environment" (Valeur et al., 2011).

The different types of luminescence are classified according to the type of excitation. Photoluminescence is the emission of light that results "from direct photoexcitation of the emitting species". Fluorescence, phosphorescence, and delayed fluorescence are known forms of photoluminescence. There are other types of luminescence that differ by the type of excitation: chemiluminescence, bioluminescence, electroluminescence, cathodoluminescence, radioluminescence, sonoluminescence, thermoluminescence, triboluminescence (Valeur et al., 2011; Jeran et al., 2020). This article is about bioluminescence and explains the general mechanisms of bioluminescence in the abdomen of beetles (fireflies).

Many nocturnal fireflies use discrete pulses of bioluminescence to find mates (Lewis et al., 2008; National Geographic, 2010). In some groups (e.g., the North American common eastern firefly, *Photinus pyralis*), it is notable that both sexes use precisely timed flash signals to encode information about species and sex (**Figure 1**). Primarily, males signal this by emitting flashing signals during flight. When the female responds with a flashing signal of her own, a dialogue ensues between the two as they court each other with flashing signals. Females of this species usually light up in response to the male's signals and are often immobile, although capable of flight. This courting continues until the male comes into contact with the female. The bioluminescence of fireflies is thought to date back to an early ancestor of cantharids and served as a warning sign of inedible larvae. The original significance of bioluminescence in fireflies is therefore thought to be as a warning to larvae of potential predators, but later the system was also used for courtship (Lewis et al., 2008).

Figure 1. A biochemical reaction in the beetle's abdomen; production of bioluminescence process (Credit: Art Farmer, permission under Creative Commons license) (Tancig, 2019).

2. Development of bioluminescence in nature

The beginnings of bioluminescence research in beetles can be traced to the French physiologist Raphael Dubois, who in 1885 produced a luminescent mixture by applying cold water to the abdomen of a beetle of the genus *Elateridae* (Fraga, 2008). The light emitted by the cold water faded quickly, while he could not achieve such a result with the hot water. Dubois then noticed that the resulting solutions contained different constituents. In the cold mixture, the components were intact, but in the hot solution, the heat destroyed one of the thermolabile components that are critical to light generation. Dubois called the component consumed in the light reaction luciferin and the component destroyed by the heat in the second mixture luciferase. These definitions still designate the substrate (luciferin) and the enzyme (luciferase) responsible for light emission (Fraga, 2008).
Dubois research was continued by the American scientist Newton Harvey, who studied the relationship between luciferins and luciferases. A common aspect of all systems was their dependence on oxygen, which was first discovered by Robert Boyle in the 18th century. Using the "evacuated bell jar", he succeeded in quenching the luminescence on the rotting wood and flesh of the bacteria by depriving them of air. So, in addition to luciferin and luciferase, oxygen is also needed to trigger bioluminescence. At Princeton University, William McElroy began a lifelong study of firefly bioluminescence. Light production in fireflies occurs in the light organ, which contains specialised photocytes located between two cell types. These are located between two cell types, one of which is thin and external, while the other is internal and filled with uric acid crystals that reflect the light emitted by the photocytes. McElroy confirmed the discoveries of Dubois and Harvey and investigated the conditions that affect the production of bioluminescence, such as temperature and pH, but interpretation of the results was limited. However, they discovered that bioluminescence depends on four factors. These are: oxygen, enzyme luciferase, substrate luciferin (LH_2), and ATP-Mg^{2+} (Fraga, 2008).

3. Luciferin and luciferase

Luciferase enzymes are oxidative enzymes and belong to the class of oxidoreductase enzymes. The accepted name for the *P. pyralis* luciferase enzyme is *Photinus*-luciferin-4-monooxygenase (decarboxylating, ATP-hydrolyzing), but it is commonly referred to as firefly luciferase or simply as luciferase (Luc). The firefly luciferin substrate (LH_2) is (*S*)-2-(6′-hydroxy-2′-benzothiazolyl)-2-thiazoline-4-carboxylic acid (**Figure 2**). Luciferin occurs in the optical isomers *D*- and *L*-, but only the *D*-isomer reacts promptly via the bioluminescence pathway (Leitão et al., 2010). The general biochemical reaction catalysed by Luc is shown **Equation (1),**

$$D\text{-}LH_2 + ATP + O_2 \rightarrow AMP + PPi + CO_2 + oxyluciferin + h\nu \quad . \qquad (\mathbf{1})$$

The *D*-isomer of the luciferin substrate (*D*-LH_2) reacts with a molecule of adenosine 5′-triphosphate (ATP) in the presence of oxygen (O_2) to produce the light-emitting oxyluciferin, adenosine 5′-monophosphate (AMP), and inorganic pyrophosphate (PPi).

The overall reaction presented in **Equation (1)** proceeds in two successive steps. The first step (**Equation (2))** is an adenylation in which an enzyme-linked intermediate *D*-luciferyl adenylate (*D*-LH_2- AMP) is formed by reaction of *D*-LH_2 with ATP.

$$luciferase + D\text{-}LH_2 + ATP\text{-}Mg^{2+} \rightleftharpoons luciferase \times D\text{-}LH_2\text{-}AMP + PPi\text{-}Mg^{2+} \qquad (\mathbf{2})$$

The second step (**Equation (3**)) consists of oxidation and decarboxylation, in which the intermediate reacts with oxygen to form the product oxyluciferin, which emits light.

$$luciferase \times D\text{-}LH_2\text{-}AMP + O_2 \rightleftharpoons luciferase + AMP + CO_2 + oxyluciferin + h\nu \qquad (\mathbf{3})$$

As we could see, Luc could catalyse the reactions. In the presence of ATP, <u>*D*-</u>LH_2 is activated to *D*-LH_2-AMP, which is oxidized via a series of intermediates by O_2 to oxyluciferin,

CO_2, and AMP. In a side reaction, LH_2-AMP is oxidized by O_2 to *L*-AMP and hydrogen peroxide. *L*-AMP can be cleaved by PPi to give dehydroluciferin (L) and ATP.

Luciferase is a generic term for any enzyme that catalyzes a reaction that produces visible light. Light emission results from the formation of a product or intermediate in an electronically excited state; return to the ground state occurs by emission of a photon of light. Luciferases are so diverse that they catalyze many different reactions with very different substrates. What they all have in common is the involvement of oxygen. Luciferases are more distinct in comparison with proteases, all of which perform hydrolytic chemistry on peptide bonds. All luciferases emit light, but in very different ways. Therefore, luciferases from different organisms probably evolved independently and do not trace back to a common precursor enzyme. Bacterial luciferase, the first luciferase to be cloned and structurally characterized, is a flavin monooxygenase that uses flavin mononucleotide (FMN) to activate molecular oxygen, producing flavin C4a peroxide. Reaction of the peroxide with an aliphatic aldehyde substrate eventually yields the carboxylic acid and flavin-C4a hydroxide in the first excited singlet state. Light emission, loss of C4a hydroxide, and dissociation of FMN return the enzyme to its initial state. Firefly luciferase, on the other hand, catalyzes an oxidative reaction involving ATP, firefly luciferin, and molecular oxygen, producing the electronically excited species oxyluciferin. This excited species emits visible light, which the firefly uses for its reproductive behavior. Firefly luciferase was one of the first enzymes to be studied in detail (Baldwin, 1996).

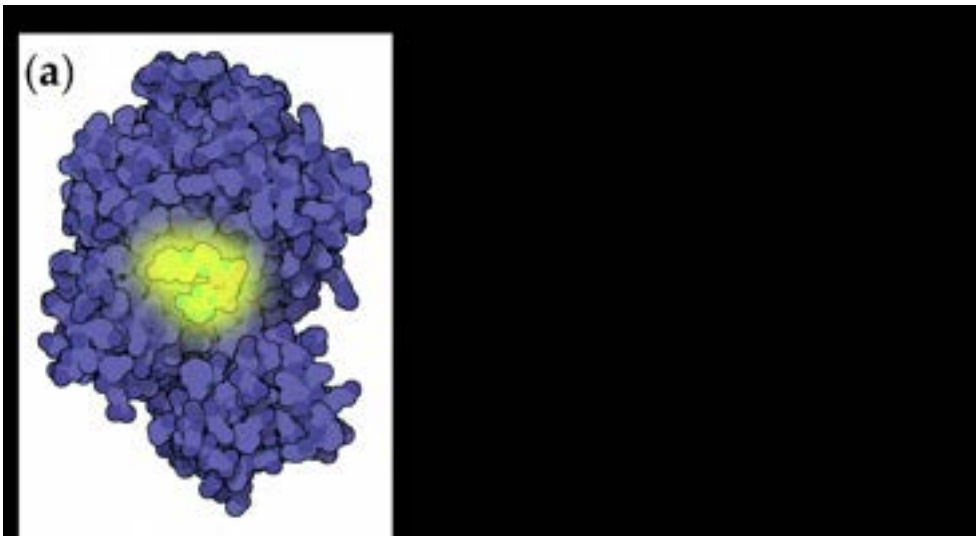

Figure 2. (**a**) Firefly luciferase with the chromophore in yellow (permission under Creative Commons license) (Goodsell, 2006). (**b**) Skeletal structure of firefly luciferin: (4*S*)-2-(6-hydroxy-1,3-benzothiazol-2-yl)-4,5-dihydrothiazole-4-carboxylic acid. The visible emission occurs when oxyluciferin transitions from the excited state to the ground state. The colour of the discharge is different even if the luciferin used is the same; this may be due to changes in pH value or differences in the structure of the luciferase involved (Hewitt et al., 2019).

The fold in which polypeptide enzymes form is unique in that it consists of two domains, a large *N*-terminal domain (containing residues 4-436) and a *C*-terminal domain (containing residues 440-544). The larger, lower domain consists of a β-barrel and two β-sheets interleaved with α-helices forming a five-layered αβαβαβ structure. The upper *C*-terminal domain, on the other hand, consists of five β-strands and three α-helices and is folded into a compact structure that is connected to the lower *N*-terminal domain by a disordered loop connecting residues 435 and 441. Between the two domains, there are also three disordered loops connecting residues 539-529 (in the *C*-terminal domain), 198-204, and 355-359 (in the *N*-terminal domain), but these are not visible in electron density. The regions with the greatest sequence conservation are most likely involved in the catalytic mechanism of the

enzyme (Baldwin, 1996). Based on previous analyzes, the active site is thought to consist of residues on the surfaces of both domains, and the two domains combine to form an active site after substrate binding. For the firefly bioluminescence reaction to proceed with high yield, water must be excluded from the active site (Baldwin, 1996).

One fascinating aspect of firefly bioluminescence is the colour of the emitted light. *Luciola mingrelica* (found in southern parts of Russia) and *Luciola cruciata* (native to the Japanese region) firefly luciferases resemble *Photinus pyralis* luciferase (from North America) with a maximum light intensity (I_{max}) of 562–570 nm, but *Luciola lateralis* (native to the Japanese region) firefly luciferase catalyzes such a reaction and emits green light with an I_{max} of 552 nm (Baldwin, 1996). Interestingly, the luciferase from the American firefly *Photinus pyralis* crystallizes in active form at low ionic strength, whereas the *Luciola firefly* luciferase is inactivated at the same conditions. They investigated both the presence and absence of proton acceptors using fluorescence emission spectra and concluded that the colored bioluminescence depends on the arrangement of the luciferase molecules. The hypothesis is that the red colour comes from the keto anion form of the product molecule and the yellow-green light comes from the enol anion form of the product molecule. This hypothesis is consistent with the emission of red light in the bioluminescence reaction at lower pH values, which led the researchers to believe that the different light colours in fireflies were due to spectral mixing of these two products. However, they later isolated luciferases from different organs of the beetle *Pyrophorus plagiophthalamus*, where they found that each emitted a different colour of bioluminescence and each spectrum showed a single peak, rather than a superposition of two or more spectra. Then they selected four luciferases from the aforementioned beetle with different colours of bioluminescence, cloned them, and sequenced their *c*DNA. The amino acid sequences of the selected luciferases were 95-99% identical, and less than two or three amino acid changes were required to produce spectral shifts of up to 50 nm at I_{max}. These isolated luciferases emit light with sharp emission spectra with spectral maxima at 546 nm (green colour), 560 nm (yellow-green colour), 578 nm (yellow colour), and 593 nm (orange colour) (Baldwin, 1996). This finding suggests that the light emission from each enzyme originates from a single molecular species in the surrounding enzymes and that the resulting colour differences are due to the different microenvironments of the enzyme complexes and oxyluciferin. A change in the colours of bioluminescence may also be caused by the tertiary structure of the luciferin molecule. In a similar study, the *c*DNA of the firefly *Luciola cruciata* was mutagenized, and five different mutants with different colours of bioluminescence were obtained. The isolated colours ranged from green to red, and the mutations consisted of single amino acid changes. The sequence changes of the mutant luciferase from the firefly *Luciola cruciata* were compared with enzymes isolated from the beetle *Pyrophorus plagiophthalamus* (Jamican click beetle (Stolz et al., 2003), and no common amino acid sequence affecting light colour was found. This suggests that bioluminescence may be affected by the overall tertiary structure of the enzyme (Baldwin, 1996).

4. Conclusion

Fireflies, which provide light in the ecosystem, have also impressed scientists. The mysterious "cold" light emission will always stimulate curious minds, and the colour issues of bioluminescence and the details of the enzyme-catalyzed reaction could be clarified through interdisciplinary approaches. Exploration of the general mechanisms will have important implications for the development of applications, especially in medicine.

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Reflection/ Review

Winner Takes it all *or* How Did Arena of International Law Fail to Decolonize the World in Versailles?

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Abstract:
We are living in a time when there is no shortage of excuses to encroach on foreign territories. This article deals specifically with the political and legal justifications for encroachments on other peoples' territories during the Peace of Versailles and the later created Mandate system. It systematically shows how the socio-political changes at the end of the 19th century and the development of international law led to the maturation of the idea of sovereignty and how it affected the empires of the Great Powers. It then shows in detail how the liberal mindset in international law is used to justify new colonial achievements and the expansion of empires even after the end of the First World War.

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Keywords: Mandate system, colonialism, level of civilisation, decolonization, mandate for Togoland, A. V. Dicey

1. Introduction

This article will examine the relationship between imperialism and winning powers of First World War (WW1) and their desire to continue to govern an empire. The hypocrisy of the Entente Powers that article will look into is their realization needed a new moral, political and philosophical explanation for their new acquisition of the territory on which they were going to exercise "political control … over the effective sovereignty of other political societies" (Doyle, 1986). This article will show that the "new" mandate system they created is just a new tool for the same practices and how did arena of international law (lawyers that were building international public law) gave them necessary academic background to do so.

2. What is imperialism?

Before delving into intentions and consequences of imperialism, we must answer a core question: what is imperialism? Defining such a broad term is in no way easy as it may encompass various internal and international attitudes of states.
Doyle accordingly concludes that "… [e]mpires are relationships of political control imposed by some political societies over the effective sovereignty of other political societies. They include more than just formally annexed territories, but they encompass less than the sum of all forms of international inequality. Imperialism is the process of establishing and maintaining an empire" (Doyle, 1986).
The author therefore outlines an empire as the goal and imperialism as its function.

3. Liberal road to mandates

3.1. Positivists influence the international law

The arena of international law at the end of the 19th century was concerned with the universality of law. The imperialist moves and the expansion of European powers suddenly made relevant the question how international law should deal with the deprivation of sovereignty of another community. To paraphrase, what excuse will the European powers use when they subjugate peoples in Africa, Oceania and elsewhere? Such an argument, at least on the surface, must appear objective in order not to undermine the legitimacy of international law. The lawyers of positivists schools of the time proudly proclaimed how their approach to international law was the most legitimate, but they had to come to grips with their own idea of sovereignty. Positivism claims that the law itself is a product of the sovereign will and that the sovereign not merely administers and executes the law. How can then a sovereign entity simply declare authority over another sovereign entity without overpowering it in war? The only possible answer was that the natives of the targeted areas were simply declared unworthy of sovereignty. If there is no sovereignty, then there is nothing to challenge the supremacy of the European powers: the average international law scholar of the time could thus be described as "… the positivist jurist who basically resolves the issue by arguing that the sovereign state can do as it wishes with regard to the non-sovereign entity which lacks the legal personality to assert any legal opposition" (Anghie, 2005). In an academic atmosphere predominated by such mentality, it was necessary to define the qualities needed to acquire sovereignty. Here, the whole arena of international law resorted to the level of civilisation as the relevant threshold. The earlier naturalist international law that prevailed in the sixteenth and seventeenth centuries claimed that all peoples, whether European or not, were subject to a universal international law derived from human reason. In contrast, positivist international law made a distinction between civilized and non-civilized states and further argued that only the sovereign states that made up the civilized "family of nations" were subject to international law (Anghie, 2005).

3.2. Level of civilisation as a criteria of international law

As an example of the application of this idea, we will look at the work of the 19th century English international law theorist John Westlake. According to Westlake, an entity's legal capacity was predetermined by the level of civilisation it had acquired. Therefore, Westlake claimed that African tribes were unable to transfer sovereignty because they could not comprehend the idea of it (Westlake, 1894). This is just an example of a theoretical approach that international jurists have created to complete the system of classifying peoples according to their level of civilisation and have attributed rights to them in international law accordingly. Westlake proposed a native people's understanding of concepts as the relevant criteria to test their level of civilisation. The peoples would thus be awarded as many rights as their understanding of the concepts of such rights would warrant. As formulated by Westlake, "… we have here a clear apprehension of the principle that an uncivilized tribe can grant by treatsuch rights as it understands and exercises, but nothing more" (Westlake, 1894). Of course, we cannot fool ourselves: that was purely an attempt to create a coherent system of classification of the "natives." In reality, the concept of 'civilization' was used as a form of exclusion of non-Western values, of non-Western identity and even of legal personality of the peoples who were targets of imperialism. The test assessing their civilisational level was precisely as accurate as the "tester" wanted it to be. It had always been carried out by forces that were more highly evolved and were checking whether their "students" understood the concepts. Systemically, therefore, it was not a system that followed the elementary concepts of fairness and did not contribute to the assessment of the level of development of nations – regardless of whether this could ever be put forward as a legitimate criterion for the acquisition of rights under international law.

3.3. Level of civilisation as a justification for imperialism

Here we will examine the view of another great liberal legal theorist, A. V. Dicey. Dicey criticizes individuals who supported imperialism as a system founded on force, privilege, and enduring class inequality. The actual supremacy of law, which was regarded as a vital element of systematic justice, was considered as being in direct conflict with these principles. The inference is that imperialism founded on the former basis was fundamentally wrong and went against liberal ideals. This criticism is part of a larger discussion about imperialism's nature and legitimacy, as well as the function of the law and justice in the exercise of political power in the late nineteen century. Dicey thought that Britain's grip on the rule of law was the most significant achievement of civilization. Ability to transmit that achievement abroad justified British imperialism (Lino, 2018).

3.4. What is the mandate system?

In response to Article 119 of the Treaty of Versailles which required Germany to renounce its colonies, the League of Nations mandate system was established. This gave the victorious powers a legal justification for taking control of the colonial territories previously administered especially by Germany.

The Mandate System was established with the Article 22 of the covenant of the League of Nations 12 in 1920. The main objective of the Mandate System was to promote the political economic, and social development of the inhabitants of the Mandate Territories and to gradually prepare them for independence.

The idea was put forward by Jan Christiaan Smuts, a South African general and the country's Prime Minister. He designed it to expand authority over the strategically important and oil-rich former Ottoman Middle East. Smuts disagreed that captured German possessions in Africa and the Pacific should be subject to such global regulation (Pedersen, 2006). He called the people in these territories barbaric, and he was completely convinced that they are not able of managing a self-governed nation state (Pedersen, 2006).

Although the League of Nations' mandate system provided some tools for managing and safeguarding the people living there, its application was in reality found to be rather limited. In managing the territories, the powers in charge of a mandate had a lot of latitude and weren't necessarily obligated to fulfil their duties to the locals. Additionally, European

nations and League of Nations officials largely held the international control over the mandated territories, with the local populace having little influence over political development and decision-making.
The League of Nations' rationale was that the mandate system constituted a significant step towards the acceptance of the necessity for political autonomy and the development of colonial territory towards self-determination. This process as it will be later explained is called decolonization.

4. What is decolonization?

What should change that we could talk of a fundamentally decolonized approach of Imperial powers to international law? Decolonization is a social and political process that aims to dismantle the structures and systems of colonialism, which have historically oppressed and exploited colonized peoples. It seeks to undo the damage caused by colonization and to restore sovereignty, dignity, and self-determination to those who have been colonized. Decolonization demands an Indigenous framework and the placement of Indigenous land, Indigenous sovereignty, and Indigenous ways of thinking into the foreground.
In case of decolonizing arena of international law, the international law should be rethought and reorganized. It should liberate the legal systems that have historically been dominated by Western powers. The aim should be to re-establish the sovereignty and self-determination of colonized peoples and to challenge the Eurocentric nature of international law.

4.1. Mandate for Togoland

Recognizing and honouring the multiplicity of legal traditions and knowledge systems is one of the main objectives of the decolonization of international law. This entails recognising and changing the continuing effects and causes of colonialism and the way colonial power structures have been maintained through the international law. We will examine if any of the goals described above are met in the in the structure that was set up in the mandate for a former German colony Togo.

4.1.1. Natives do not gain sovereignty by being "uncivilised".

As seen in Article 9:
"The Mandatory Shall have full powers of administration and legislation in the area subject to the mandate. This arca shall be administered in accordance with the laws of the Mandatory as an integral part of his territory and subject to the above provisions." (United Nations Geneva, 1922).
The main element of decolonisation, which is supposed to be the establishment of a government of the people living in the territory, and other principles of self-determination, are clearly being eroded. Sovereignty as Bodin's concept of a supreme entity without limits has therefore remained in Europe. If we combine that with Article 22 of the Covenant of the league of nations: "… that the well-being and development of such peoples form a sacred trust of civilisation and that securities for the performance of this trust should be embodied in this Covenant." (The United Nations at Geneva, 1919) which is superior to the document that establishes the mandate for Togo, we can clearly see where the root cause lies in the complete removal of any possibility of even de facto sovereign decisions. They still justify colonialism with benign liberal ideas.

4.1.2. The rights are attributed in line with the understanding of European legal concepts

The pure Westlake approach itself is no longer detectable. It could hardly be said that the mandate system, at least legally speaking, denies legal personality to anyone or denies them rights because of a misunderstanding of mental concepts. Yet governance is still based on European concepts. As the article 2 says: "… the promotion to the utmost of the material and moral well-being and the social progress of its inhabitants." (United Nations Geneva, 1922)

The attitude towards "natives" is thus still based on European concepts of well-being and, especially, social progress. The inclusion of social progress within the list of tasks can only be understood as the idea of imposing western societal rules into the local communities of Togoland, especially considering the characteristic urge of French colonialism to assimilate indigenous inhabitants of colonised territories. This is clear exclusion of non- Western values. They are clearly missing from this agreement, and I cannot show them. I can only refer the reader to the whole document and the reader can see that there is no emphasis in the whole document on the values that would be important to the people of Togo (United Nations Geneva, 1922).

4.1.3. Self-determination

Self-determination is a principle that refers to the right of a group of people to determine their own political status and to be free from external domination. The League of Nations analysed the scope of the principle of self-determination and concluded that it was a vague and general principle that could not be considered a positive rule of the Law of Nations (Lewis, 1962). However, the principle evolved into a "right" to self-determination during the decolonization period of the 1960s-1970s before that, so in the time frame relative to us, we would expect that ideas connected to it would pop-up in a document like the mandate for Togo. But there is no such a thing in the in it (United Nations Geneva, 1922).

4.1.4. Use of military under Article 3

Text of the Article 3 says:
"The Mandatory shall not establish any military or naval bases, erect fortifications, or organize any native military force in the territory except for police purposes and for the defense of the territory." (United Nations Geneva, 1922).
We observe that there is an awareness of colonial atrocities and genocides and their causal link to the presence of a large amount of coercive organs. This provision really tries to prevent such excesses. Yet is it not rather hollowed out by the second paragraph of the same article: "However, it is understood that the troops thus raised shall be utilized, in the event of a general war, to repel an attack or for the defense of the territory outside of that which is subject to the mandate." (United Nations Geneva, 1922).
When, if not during a war, will troops be needed on the ground? And it is also up to the colonial power to decide whether a state of war exists, so it can easily use the army to suppress internal unrest, because it is defending itself against attack.
However, if we look at it in a completely cynical way, we realise that it is most probably again a question of maintaining the status quo between the superpowers, who wanted to prevent the other superpowers from gaining a strategic military advantage through one of the mandates, which is also clear from the systemic interpretation, since the only ones who can bring a case against the holder of a mandate within the League of Nations at that time are the other superpowers (The United Nations at Geneva, 1919), who will most probably act in accordance with their own interests as described above.

4.1.5. Classification of peoples as being more and less developed and rights are attributed to them accordingly

The Article 2233 of the Covenant of the League of Nations categorized the regions distributed among the mandatory authorities between 1919 and 1921 into three classes: "A" mandates, "B" mandates, and "C" mandates (Wempe, 2019a) Class A mandates included areas in the Near East that had been "liberated" from the Ottoman Empire, like Iraq and Syria. The Europeans in charge of the labelling thought this class of regulations to be »civilizationally advanced enough as to reasonably pursue the construction of independent, hopefully democratic, states in the near future« (Wempe, 2019b). German colonies in East Africa, Cameroon, and Togo made up the mandates of class B. The authors categorized these territories as being too underdeveloped to expect for quick autonomy and as needing to be under European "guidance" for the foreseeable future because of their sizable African populations. Additionally, Class B mandates were anticipated to keep trade open for all

prospective League members. Class C included the remaining German colonies in the Pacific and Southwest Africa. These lands were held by Japan and the British Dominions and were basically annexed (Pedersen, 2006).

As we have shown old imperialist ideas permeated the international law that established the mandate system and did not decolonize anything. To conclude let us say that League of Nations had no interest in creating a truly decolonized system, but that the reorganisation was merely a political bargain that gave the colonies and their people false hopes of progress. The League was really a structure designed for global governance, not global reformation. It was truly League to preserve Empires (Wempe , 2019a).

5. Conclusion

As a result of colonialism, the concept of civilisation and the moral need to advance civilization are still invoked to justify interventions in and control over other countries. The mandate system, which was advertised as a different method of combating colonialism, was a continuation of the same behaviours, and the fight for decolonization is still ongoing. Additionally, we may observe the employment of similar concepts in modern discourse, such as the Responsibility to Protect (R2P) principle, which asserts that the international community has a moral duty to step in when mass atrocities occur. Despite the apparent good intentions of this concept, it is frequently invoked to justify military intervention and regime change using the same rhetoric of civilized superiority as was prevalent during the colonial era. Considering this, it is crucial to analyse critically and question the underlying presumptions of how concepts of civilization and goodness are applied in international politics. Our responsibility is to make sure that the effects of colonialism do not continue to breed injustice, exploitation, and inequality in the world. We can only hope to create a more just and equitable society by acknowledging and tackling the lingering repercussions of colonialism.

Conflicts of Interest: The authors declare no conflict of interest.

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Reflection

The Traditional Tibetan Medicine. Historical, Artistic, Clinical and Spiritual Aspects.

Vitiello Luigi*

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Abstract: The history and origins of Tibetan Medicine, has its origins in the land of Tibet in the local culture and spirit of the Tibetan people and is based on a holistic vision of the human being, who considers the body, mind and spirit as an interdependent unit. Health is the result of a balance of five elements: earth, water, fire, air and space. In the human body these elements manifest themselves through three humors: the wind, the bile, and the phlegm. When one or more of these moods are in excess or in defect, disharmonies are created that lead to diseases. Despite the fact that it is one of the oldest ethnomedicine in the world, Tibetan medicine continues to be practiced in contemporary society. Current medical and scientific research today confirms the extraordinary benefits of this ancient oriental science.

Keywords: Traditional Tibetan Medicine; Diagnostics in Tibetan Medicine; Wind; Bile; Phlegm; Tantra; Tibetan pharmacopoeia;

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Among the three most important medicines developed in Asia, Ayurveda of India, Chinese and Tibetan medicine, the latter is perhaps the least known. The origins of Tibetan medicine (MT) remain controversial. The most probable hypothesis is that they are connected to the ancient kingdom of Shang Shung that extended in the area of Mount Kailash, in western Tibet and that can be considered the cradle of Tibetan culture.

The earliest known text is a manuscript from the Bôn tradition, the religion prevalent in Tibet before the advent of Buddhism. It is the "So-rig-bum-bzhi", "The art of healing with four hundred thousand medicines": a treatise on medical subject attributed to Shenrab Miwoche who lived, according to the studies of Namkhai Norbu[1], at the beginning of the second millennium B.C. This work, reworked several times, was the reference text for Tibetan doctors until the 8th century.

The second development period of MT begins with the translation of treaties from other countries. In the 7th century, King Songtsen Gampo consolidated the unity of Tibet, which from that moment emerged as a political power. He was the first of the Tibetan rulers to convert to Buddhism and under his rule began a period of profound cultural renewal.

This ruler invited doctors from India, China and a region called Tagzig, a large area that included Persia, to compare their knowledge with that of Tibetan doctors. Unfortunately, we have not received many documents on this conference, which marks the birth of MT in its current form and which was certainly an important event, also for its modern vision of comparison and exchange of knowledge.

In the 8th century, with King Trisong Detsen (742-798), Tibetan culture flourished and Buddhism became the official religion of Tibet. During this time, an intense activity of translations was also initiated, mainly from Sanskrit, and it is thanks to these that many Buddhist texts have come down to us whose originals have been lost. In this period an official medical school was also founded, although several centers for the teaching of medicine already existed mostly connected to monasteries.

The basic text adopted for the training of doctors was still the 'Bum Bzhi', which was revised and adapted to the knowledge of the time by Yuthok Yonten Gompo 'the old' (708-833) and renamed by him as "The Four Treaties of Medicine". Yuthok also gave guidance on the ethics to be followed, specifying how each doctor should train to exercise compassion, what was the correct behavior to be held with patients and how to adjust for compensation, so that the medical profession had not only the value of a professional activity but was also a means of personal and spiritual evolution.

In the 12th century, Yuthok Yonten Gompo 'the young' (1112-1203) compared the different versions of the Four Treatises of Medicine, integrating and expanding them with the addition of new sections. Thus were born the "Four Tantras", the fundamental work of Tibetan medicine.

Another important figure in the history of the MT is Sangye Gyamtso (1653-1705), minister of the 5th Dalai Lama. In 1670, he was given the task of revising the Four Tantras and editing a new edition, a task that took him almost 25 years. During this period Gyatso also acted as regent, awaiting the installation of the 6th Dalai Lama. In 1696 he founded the Medical School called Mentsikhang on the hill called Chagpori (Iron Mountain). From his work also arose the drafting of an important commentary: the Blue Beryl, written between 1687-88.

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[1]Namkhai Norbu (Derge 1938 - Arcidosso 2018). Considered one of the most important masters of Dzogchen lived in our time. He was Associate Professor of Tibetan Language and Literature at the University Institute 'L'Orientale' in Naples from 1964 to 1993 and has published numerous texts on Tibetan history and culture and on Dzogchen Teaching.

The importance of enriching the text with illustrations that clarified its contents and made learning easier for students was felt by Sangye Gyamtso who founded the School of Medicine and Astrology on Chags-po-ri's Hill . In the colophon of his work, he states that the illustrations began to be painted in 1687 and that a year later, at the time when the text was finished, the series numbered 62 paintings.

The work was completed at the latest in 1703; in fact on this date the regent, in another work, gives the list of inscriptions that can still be found at the bottom of each of the 79 paintings that form the collection of Mentsikhang. This work deserves a special mention due to its peculiarity and the history of its discovery.

Painted in overlapping bands, similar to comics, or as anatomical tables, the 79 tangkas represent a visual documentation intended for the training of Tibetan doctors, illustrating the various aspects of traditional medicine: embryology, anatomy, physiology and pathology, diagnostic methodologies, medical matter, up to the interpretation of prognostic dreams of healing or death. (**Figure 1**)

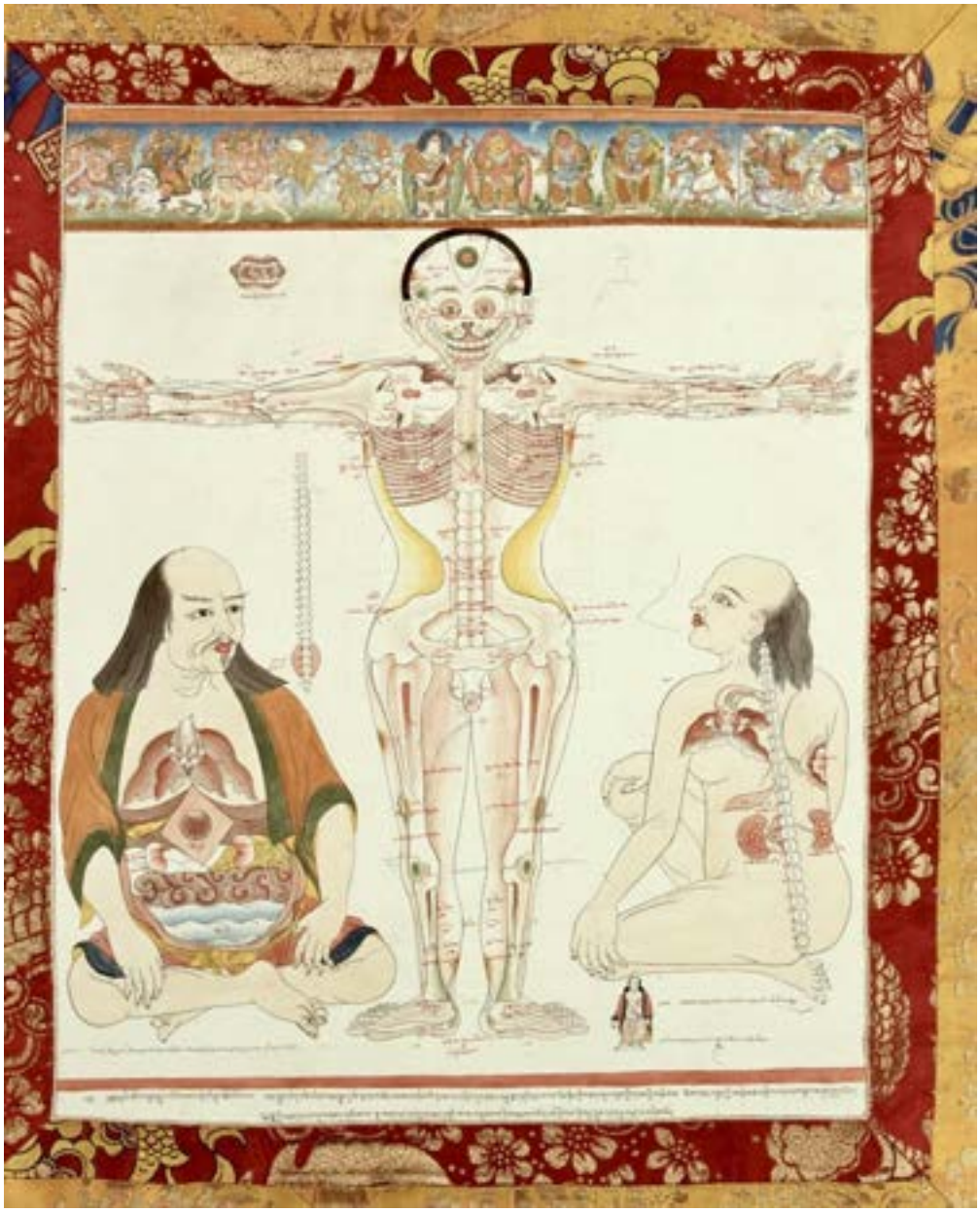

Figure 1. Anatomical *tangka* (Photo in accordance from Association for International Solidarity in Asia)

The discovery of this rich and interesting work of Tibetan medical iconography was not an easy one. The first contact that indicates its existence happened in 1904, when the Ten. Colonel Waddell, the medical officer of the Younghusband mission, visited the Chagpori medical school, which he calls the 'Temple of Medicine'.

In one of his stories we read that: "They teach a rough kind of anatomy, not by dissection, but with an imaginative body chart, ordered in tiny squares within which the positions of the internal organs are indicated" (Visit in the Temple of Tibetan Medicine in Lhasa - 1905).

Waddell must have been impressed by what he had seen during his trip because he then described it in various articles and even tried to buy some of those tangka, but the dean of the faculty did not want to deprive himself of it, so he asked to make some copies.

Certainly if we compare the anatomical table observed by Dr Waddell with those of the De humani corporis fabrica by Andrea Vesalio, precedents of over a century, the descriptive accuracy appears enormously different. But what the English doctor probably did not know is that Tibetan medicine had deepened the knowledge of the energetic aspects of the human person much more than its anatomical description, as is more evident in other *tangka.*

Around the same time, Bertold Laufer brought to the Chicago Field Museum several anatomical drawings and a painting showing Tibetan medical and surgical instruments from Beijing's famous Yonghe Gong Tibetan Buddhist temple. The only person who showed interest in this material was medical historian Karl Sudhoff, during his research on medieval anatomy in the West. He noted that the particular squatting position of one of the anatomical figures was also found in the iconography of certain Persian and European medical manuscripts of the Middle Ages and this would support the thesis of contacts between the two medicines. The surgical instruments depicted have intriguing similarities to those used in the Greek and Roman eras.

Twelve medical paintings were created by Ferdinand Lessing in Yonghe Gong for the East Asiatic Library of the University of Berkeley, California in 1947. These paintings were published in 1960 by Ilza Veith, a professor of medical history, but the connection of the tangka series to the text of Blue Beryl was only made in 1964 by Kristina Lange, who in an article dedicated to an anatomical table photographed in Ulan Bator, identical to one of the paintings of the Yonghe Gong, based on the inscriptions contained in the table, demonstrated its obvious connection with Sangye Gyamtso's commentary on the "Four Tantras" of medicine.

Lange was convinced that the author had requested the production of detailed drawings that depicted certain chapters of his book and that these paintings were utilized in medical school teaching programs (Parfionovic et al.,1994).

Among the *tangkas* in this collection, when I had the opportunity to see them at Mentsikhang in Lhasa, rather than the schematism of the anatomical ones, I was struck by the one concerning embryology (**Figure 2**). In this, in fact, we can clearly recognize the knowledge of ovulation, the segmentation of the fertilized egg and the different stages of evolution of the embryo and the fetus. This knowledge developed much later in the West: in fact, the role of the female egg in fertilization was intuited by Lazzaro Spallanzani only in the eighteenth century and it still took about one hundred years to understand the mechanism of ovulation (Lyons and Petruccelli, 1992).

Figure 2. *Tangka* illustrating the development of the embryio and fetus. (Photo Vitiello L. from MentsIkhang of Lhasa)

This iconographic collection was documented definitively only in 1979 by L. Koundanova, while a third collection, that of the Buriath Historical Museum of Ulan-Ude, was published in London in 1992 in two volumes by Yuri Parfinovic, Gyurme Dorje and Fernand Meyer (Parfinovic et al., 1994).[2]

Observing its history, we can then see how MT was born on the basis of an autochthonous system that has integrated over time the knowledge of the leading medical schools once spread between Asia and the Middle East, merging them into a synthesis that has unique and original characters and that has been continuously enriched with texts and contributions from the experience and research of doctors.

The system can be articulated at three distinct levels: somatic medicine, tantric or energetic level, and dharmic or spiritual level.

These three levels, which can also be practiced separately, are closely linked to each other and address the three essential aspects of human nature: the body, vital energy and the mind. To understand them correctly it is necessary to know the basics of medical physiology as described in the Four Tantras.

In the Tibetan cosmological view, derived from Bôn and Buddhism, all beings and the environment where they live are considered to be composed of infinitesimal particles that have the nature of the Four Great Elements: Air, Water, Fire and Earth, in perpetual reciprocal interaction according to precise laws. A fifth element, Space, is considered the base, the active matrix in which others have the possibility of manifesting and acting; in the medical field, however, only the first four are considered (Vitiello, 2013).

The term element must not mislead and must be understood in a different way from the common meaning, considering it as the potential of a function rather than as an elementary substance.

In this perspective, the Earth, the solid state, is considered the principle responsible for the structure of matter; Water, the fluid state, determines its cohesion and shape; Air, the gaseous state, allows movement and expansion; The Fire represents the plasma and dynamic state able to activate transformations.

This concept of 'primordial matter', at the basis of Buddhist cosmology and Tibetan medicine, is not foreign to our culture. The roots of Western thought arose precisely by asking the question of the nature of matter, that the first Ionic philosophers of the VI-V sec. a.c. have faced the challenge of recognizing an 'Arché', an original principle with its intrinsic vitality. Heraclitus of Ephesus, contemporary of the historical Buddha, develops some concepts close to the presuppositions of Buddhist philosophy: the uninterrupted dynamics between the elements and the close interdependence between the material and the energetic levels of reality, insights then verified and developed by modern physics.

Even the human body, in its stages of training and growth, develops its structure through the combination and balanced development of the elements: all the various parts take shape and develop to full completion; then the process begins to reverse and proceeds towards the inevitable dissolution.

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[2]A further collection known to me is that of 53 tangka currently in the possession of ASIA Onlus. The collection was commissioned in 1993 by Namkhai Norbu to the Mentsikhang of Lhasa for ASIA, an association he founded for solidarity with the Asian populations and of which he was president. These tangka were exhibited in 2000 at the Ethnographic Museum "L. Pigorini" in Rome and then in other exhibitions

On the basis of these assumptions it is considered that all phenomenal manifestations, physical sensations, emotional experiences of happiness and pain are attributable to the dynamics of the Four Elements: when these are in balance, existence is pleasant and one enjoys good health; if they come into conflict disorders and diseases arise.

If the Four Elements and their dynamics are used to describe every aspect of inert matter, passing to the biological plane, and in particular to the human condition, we speak of the Three Humors, called in Tibetan Lung, Khris-pa and Bad-kan, terms commonly translated as Wind, Bile and Phlegm. These three moods are also closely related to the Four Elements: Wind with Air; Bile with Fire; and Phlegm with the combination of Earth and Water.

The Three Humors are also in reciprocal relationship with the three main passions: Desire, Aversion and Ignorance: in particular the Wind affects desire; Bile on aversion and Phlegm on ignorance, term with which we do not mean the lack of culture but a state of ignorance, the illusory perception of reality. As already mentioned for the elements, the Three Humors should not be understood as simple substances, but above all as functions.

For each of these three humours there are five aspects with different characteristics, seats and functions: their interaction is the basis of all the pathophysiology of the Tibetan medical system.

The Lung or Wind is the mobile agent that controls breathing, blood circulation, body movements, regulates sensory perception, and expels excretions. The Wind mood is also the one most responsible for psychic processes.

The second mood is the Khris-pa, or Bile, term that commonly indicates the bile fluid present in the gallbladder: this is closely connected with metabolic processes and, through its five aspects, performs functions related to body heat, digestion and visual function.

Bad-kan or Phlegm generally refers to the moist and fluid components of the body and is particularly connected to the immune system.

The balance of all these factors generates a state of good psychophysical health; when an imbalance condition is produced by excess, defect or interference of the specific functions, they become a cause of illness. The dynamics of this system is complex, allowing precise explanations of every physiological function and pathological manifestations with a coherent deductive mechanism.

In MT it is also always considered the influence that the mind has on the body, just as this in turn affects the mind. Thanks to the knowledge of the mutual influence between the three humors and the three passions, this interdependence between psyche and soma(burden), which Western medicine often still struggles to recognize, is one of the peculiar aspects of the Tibetan medical system.

The ways of this interaction are described by tantric physiology which is based on knowledge of energy flows through a network of invisible channels.

To understand tantric or energy level medicine we don't have to look at it through the disciplines to which Western medicine refers, such as descriptive anatomy and biochemistry, but with the knowledge of physics.

Current knowledge of physical phenomena has demonstrated what was the intuition of Ionic philosophers and Tibetan doctors: at the level of infinitesimal particles, in fact, there is a continuous transition between the state of matter and the energetic one, so the boundary between the material and the immaterial dimension is blurred.

The first of the Four Tantras indicates the four causes capable of altering the physiological balance and causing diseases: these are climate, food, behaviour and 'subtle influences'.

In MT the human being is seen as an open system, constantly interconnected to the environment in which he lives.

We are in fact inseparable from the surrounding living space: our psychophysical being is a function of the food we ingest, of the air we breathe, of the climatic, social, organic and psychic influences with which we come into contact.

From this premise it is clear the importance given to the way we interact with the environment, both as individuals and as a community, how much they can affect our behavior and how everyone is responsible for their well-being.

It is not possible here to give a detailed explanation of the diagnostic techniques and therapeutic methods used by Tibetan medicine, but we would like at least to mention these two topics.

With regard to diagnosis, this is achieved through three main methods: medical history, wrist reading and urinalysis.

Each visit begins by carefully questioning the patient about his symptoms, nutrition and habits, to understand if there are eating habits and lifestyles that may have created an imbalance of the three moods. (**Figure 3**)

After this first placement, the wrists are examined. This is a peculiar technique, much more extensive and refined than that known in the West and is similar, with some differences, to that used in Ayurvedic and Chinese medicine. Through this method an experienced doctor is able to come to very precise diagnoses, but to develop this ability requires a long apprenticeship.

It begins with the study of the physiological wrists and the typical variations of sex, age and season of the year, and then passes to recognize the pathological changes due to the imbalance of the humors in the various organs.

It is interesting to note that in the case of children, where there is a disproportion between the hand of the doctor and their wrist for which it would not be possible to a precise examination, the reading takes place by observing the three main visible vascular branches looking transparently at the auricle.

The diagnostic orientation is then confirmed by urinalysis, of which the color, the smell, the foam produced after shaking and the surface film that forms with cooling are observed: all data that can give information on the state of the three moods of the patient (**Figure 4**)

Other elements taken into account to complete the diagnosis are the examination of the tongue and the pressure of the reference points for the various organs. Through these examinations the doctor is able to understand what humoral imbalance has occurred and in which organs the disease is manifested with a precision enviable even to our latest diagnostic techniques.

The resulting therapeutic interventions will tend to restore the altered balance. In the images that make up 'The Illustration of Blue Beryl', the therapeutic system is represented as a tree from which depart four main branches: nutrition, behavior, drugs and external interventions.

Figure 3: *Tangka* of wrists diagnosis (Photo in accordance from Association for International Solidarity in Asia)

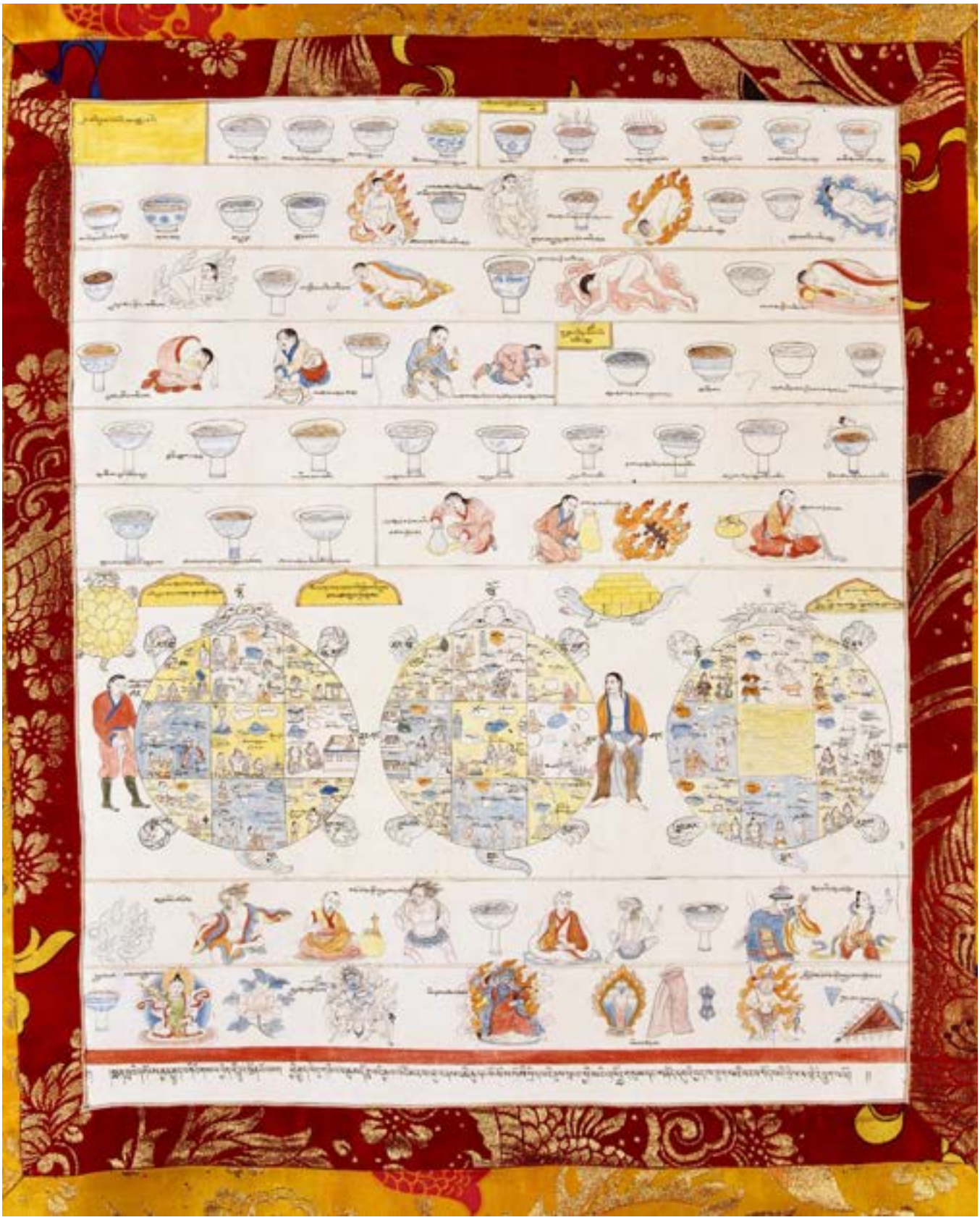

Figure 4: *Tangka* of urine diagnosis (Photo in accordance from Association for International Solidarity in Asia)

The first two points are the basis of health and directly involve the responsibility that each has towards himself, the other two are entrusted to the competence of the doctor. As we have seen before, the humoral balance depends largely on the type of nutrition and behavior during daily life, so if the doctor recognizes eating habits or life styles that are sick, will indicate to the patient the changes to be made to prevent and treat his ailments. When diet and behavior are not enough, the necessary therapies will be prescribed.

The Tibetan pharmacopoeia is particularly rich and has hundreds of remedies mostly packed in pills. The ingredients are mainly of plant origin, but many minerals and some products of animal origin are also used, which are mixed in numbers ranging from four or five to several dozen (**Figure 5; Figure 6**). These are real drugs carefully composed so that the main active ingredients on a particular organ or mood are balanced to have the maximum effect without causing imbalances on other levels.

Figure 5: *Tangka* medical materials: medicinal plants **(**Photo in accordance from Association for International Solidarity in Asia)

Figure 6: *Tangka* medical materials: mineral **(**Photo in accordance from Association for International Solidarity in Asia**)**

As for external interventions, these consist mainly in moxibustion (a therapeutic technique that consists in the stimulation of certain energy points of the body through heat), in treatments with medicated oils and ointments (Ku-Nye massage) and in bloodletting practiced with a particular technique for the drainage of toxins.(Chögyal Namkhai Norbu, 2003; Namkhai and Guarisco, 2011)

Central figure in the MT is the Buddha of Medicine (sans. Bhaiṣajyaguru) In the form with which it is represented according to the Indo-Tibetan iconographic tradition, the

body is lapis lazuli blue and appears seated in the lotus position, dressed in the monastic habit. In her left hand placed in her lap, she has a vase that contains nectar of long life, while the right hand is held in the gesture of the gift and holds a flowering branch of Arura: it is the Mirabolano, a plant with many medicinal virtues. (**Figure 7**)

Figure 7: Buddha of Medicine (Photo Vitiello L.).

In this aspect the Buddha represents the supreme curator of both the diseases that can affect the body, and the inner afflictions that derive from what are considered the 'three poisons' of existence: desire, aversion and ignorance that we have already mentioned. The meditative practice of the Medicine Buddha is considered a powerful means of developing therapeutic capacity both for himself and for others.

It is also important to remember that the historical Buddha referred to himself as a physician of spiritual suffering and his teaching as a cure. In his first public speech, held in the Deer Park near Benares, he enunciated the "Four Noble Truths", the essence of his teachings, with a method that

we could define as 'clinical'. The first truth is in fact a diagnosis that recognizes that every existence is inevitably conditioned by suffering, a word with which we translate, with some approximation, the term pāli dukkha. Buddha then describes the causes (etiology), provides for the possibility of their overcoming (prognosis) and finally indicates therapy in the Eightfold Path (Winder, 1984).

The centrality of the figure of the Buddha of Medicine indicates the high value attributed to medical practice, whose goal is to reduce the inevitable suffering that accompanies human life (**Figure 8**). For this reason in traditional teaching particular attention was given to the formation of the doctor so that at the center of his profession there was always a compassionate attention to the sick, seen in their psychophysical and spiritual unity.

Figure 8 : Mandala of the Buddha of Medicine (Photo in accordance from Association for International Solidarity in Asia)

This approach directs the vision of medicine beyond the aspect of a cold science and brings it closer to a practice that addresses the human being in all the complexity of its essence. Of the two major medical schools in Lhasa, Chagpori was destroyed during the 1959 Lhasa Uprising. This former medical college was founded in 1992 in Darjeeling, India, by Trogawa Samphel. The Mentsikhang, founded in 1916 at the behest of the 13th Dalai Lama and whose activity was greatly reduced at the time of the Cultural Revolution, Since the 1970s it has increasingly resumed its role as the center of traditional Tibetan medical science and has now been elevated to the level of University Institute, but already in 1961 the Dalai Lama had refounded it in Dharamsala H. P. where he had taken refuge in his exile.

Even if Western medicine has now reached very high targets of efficiency, these ancient medical systems, whose importance is recognized by the WHO[3], can still teach us a lot and make a valuable contribution to our well-being. Research on the effectiveness of Tibetan medicine has been underway for several years and there is a rich literature on it. In an article of the University of Minnesota it is reported that: "Scientific studies report positive findings about Tibetan medicine as a holistic system. Researchers found that Tibetan medicine had beneficial effects on quality of life, sleep, disease regression, and remission in persons with cancer and blood disorders." (https://www.takingcharge.csh.umn.edu/what-scientific-evidence-tibetan-medicine. Access: 12.09.2023)

The knowledge of MT, already spread throughout the Himalayan regions ranging from the Tibetan plateau, Ladakh, Bhutan, to the Chinese province of Sichuan, has been progressively developing in the western world. Its existence, little known in Europe until the 80s, began to spread thanks to the First International Conference of Tibetan Medicine organized in 1983 by Namkhai Norbu and held at the Cini Foundation in Venice and then in Arcidosso (Grosseto, Italy) (**Figure 9**). Unfortunately, the proceedings of this historic conference, the first held in the West, were long forgotten and were only edited in 2016. (Vv. Aa., 2016)

Figure 9: Namkhai Norbu (in the middle) with Trogawa Rinpoche (left) and Lobsang Dolma, at the first International Tibetan Medicine Conference, Venice, 1983 (Photo Archive International Dzogchen Community)

[3] Traditional and Complementary Medicine (MT&C) is an important and often undervalued component of healthcare. It is present in almost every country in the world and the demand for it is increasing." (World Health Organization. Traditional Medicine Strategy: 2014-2023) https://www.who.int/publications/i/item/9789241506096

Later, in 1985, Namkhai Norbu and Trogawa Samphel held a series of lectures in the Aula Magna of the Museo di Storia dell'Arte Sanitaria at the Ospedale di S. Spirito in Rome. These events have contributed significantly to spreading knowledge of what in Europe was the least known of the most important traditional Asian medicines (Namkhai and Trogawa, 2002).

There are currently centres of Tibetan medicine in many European cities where courses and seminars on this science are also held, so it is not difficult to meet Tibetan doctors to turn to for a visit and a cure.

I have often had the opportunity to follow some of them, recognizing their diagnostic ability and the effectiveness of their therapies. My training was not enough to be able to practice this medicine, but the knowledge of this therapeutic system has made an essential contribution to my profession, focusing more and more attention on the specific psychophysical unit of each patient rather than focusing only on the pathology to be treated.

In fact, I believe that one of the most evident limits of Western medicine, which has also reached very high goals, can be identified in having shifted more and more the interest on the disease rather than on the sick person.
The result is an often fragmentary approach, far from the complex uniqueness of each patient, and the consequences of this are increasingly perceived, awakening interest in medical systems recognized as holistic.

The doctor should never forget that each individual has a specific psychophysical identity and, as traditional Tibetan medicine indicates, his/hers role is to recognize and take care of the multiple aspects of suffering that each pathology entails, not just fight disease.

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Reflection

Homage to 15 years of Socratic Lectures

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Abstract:
We describe and comment the cultural event that was connected to 9th Socratic Lectures. The event took place May 20 in the Julij Betteto Hall, Kazina, that pertains to Academy of Music, University of Ljubljana. We reflect on the history of Socratic Lectures and underlying vision integrated in university teaching and scientific work focusing on the students. Socratic lectures initiated in 2008 with a single lecture donated by prof. Bernd Engelmann from Ludwig-Maximilian University, Munich, Germany. The lecture was integrated in the examination of students from the Faculty of Medicine, University of Ljubljana (subject Biomechanics of the hip). Socratic Lectures have been international events based on the scientific excellence and involvement of students at all levels into scientific work with the vision to promote the joy of learning and achieving amply supported by the joy of donating.

Keywords: Science-based University Teaching; University Teaching; Scientific Excellence

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1. The vision of Socratic Lectures

Going to elementary school I was almost always worried whether I would be able to fulfil my tasks. I was mostly not happy to learn the prescribed material, but wished to have good marks as not to have to endure despise and indignation of the teachers which was connected to potential problems with my parents. I learned early that albeit not much fun, having good marks is key. Also when looking at the teachers, I could not find some great joy in staying in the classroom. Some children were disrespectful to a great extent that made at certain moments the teacher's position unbearable. They had little instruments to restore order. Either they had to take the abuse or become abusers themselves. Then, there were good moments and things too, but that principal idea of learning and teaching as an instrument to make others' life miserable was nevertheless present in some extent through all my learning time making the experience somewhat sour. Sometimes when walking towards the school building, my heart accelerating in anguish, I thought how nice it would be if the teachers and the students would come to an agreement not to make each others' life miserable and that wish remained live since.

I became prone to run from school quite early. When wandering about downtown instead of going to classes, I often went to one of the libraries with things that interested me, such as stationary, coloured pencils, notebooks, music records and books. I did not wish or have any money to buy something but I took the books from the shelves, opened them randomly and took a taste of the content. Once a yellow/light blue cover with black letters attracted me particularly. In the central part there was a clear and simple title "Platon". The book was written in Serbian language which I could speak and read pretty well. Seating in the lowest shelf I created myself an intimate space to meet someone who had written the message. I have not heard of Plato before and did not even know that this word belonged to a person. I had no idea or interest to know when the book was written. Opening the book at random I learned that a man named Socrates and his pupils assembled in a place where they were able to observe the sea. They were discussing things that interested them in a pleasant atmosphere. Socrates stated and the pupils responded as to agree or disagree. Then they asked questions. It required little knowledge (in the form of data) from the pupils but a lot of curiosity and reasoning. The dialogues were respectful and polite and created a room of gentleness. It only took acknowledging and liking it to enter this room, which seemed simple and easy.

In my third year of the study of Physics, I was given a chance to become a provisional assistant at the Faculty of Medicine, Institute of Biophysics. The task was to help the students of the 1st year to solve theoretical problems and to measure and report on experiments in the subject Biophysics. The students were sometimes complaining on the subject as many found it hard and not connected to the profession that they chose. Indeed, the majority of the material taught at more or less all faculties that were not specialized for physics, was classical and partly modern physics, that seemed to be remote to medicine. Then, many students failed the exams, some of them many times. This was especially hard on some who were used to getting high marks in their previous education. It seemed that there were more than one problem underlying this situation and this did not regard the teaching only. I came to a conclusion that to be able to invite the students to use physics in medicine, the teachers themselves should first experience the integration of physics and medicine on the research level.

3. Development of HIPSTRESS method by the students and physicians

A clear goal was to integrate physics and medicine. Collaboration between the Department of Orthopaedic Surgery, University Medical Centre Ljubljana and Institute of Biophysics, Faculty of Medicine, University of Ljubljana started in 1985, when the director of the Department of Orthopaedic Surgery prof. France Srakar expressed his interest to prof. Saša Svetina, the head of the Institute of Biophysics, for development of a mathematical model that would explain why a particular operation that he was performing – the Chiary osteotomy - were successful. Namely, in Chiari osteotomy, a deficient acetabular roof is

increased by the cut iliac bone, to decrease stresses in the hip, however, the area of this augmentation is rather small. Nevertheless, to his experiences, the operation helped. Aleš Iglič had at that time started his first job at the Institute of Biophyiscs after graduating from physics at the Faculty of Physics, University of Ljubljana. Prof. Svetina suggested that Aleš Iglič, a Ph.D. student could consider this problem while prof. Srakar suggested that Vane Antolič, then a young medical doctor and a Ph.D. student should be involved. The thorough study of then existing literature was a foundation of a constructed model for resultant hip force in a one-legged stance. Some colleagues joined the team, me among them. The first recorded publication on the subject was to a local Biophysical conference (Srakar F, Iglič A, Antolič V, Kralj-Iglič V, Herman S. Mathematical modeling of the Bernoise triple pelvis osteotomy. Periodicum Biologorum 1985; 93, 2: 325-326) while five years later the model was presented in a Yugoslav journal Acta Orthopaedica Yugoslavica (Iglic A, Srakar F, Antolic V, Kralj Iglic V, Batagelj V. Mathematical analysis of Chiari osteotomy. Acta Orthop.Iugosl. 1990;20: 35-39). It took another three years to publish the first paper in a journal with Impact factor (Srakar F, Iglič A, Antolič V, Herman S. Computer simulation of the periacetabular osteotomy. Acta Orthop Scand. 1992;63: 411-412) while in 1993, two publications formed the origin of forthcoming development of collaboration between clinicians and engineers: the model for the resultant hip force (Iglič A, Srakar F, Antolič V. The influence of the pelvic shape on the biomechanical status of the hip. Clin Biomech. 1993;8: 223-224) and the model for contact hip stress (Iglič A, Kralj-Iglič V, Antolič V, Srakar F, Stanič U. Effect of the periacetabular osteotomy on the stress on the human hip joint articular surface. IEEE Transactions Rehabilitation Engineering 1993;4, 1: 207-211). The first generation mathematical model for stress was further developed to the configuration that is still being used. The first application of this model was analysis of stress during gait, for which the data were provided by prof. Richard Brand, then a Head of the Society of American Orthopaedic Surgeons and the Editor in Chief of the prestigious Journal of Biomechanics and his colleague Douglas Pedersen (Ipavec M, Brand RA, Pedersen DR, Mavčič B, Kralj-Iglič V, Iglič A. Mathematical modelling of stress in the hip during gait. J. Biomechanics. 1999;32: 1229-1235.) The first author of this publication is Marija Ipavec, a physicist who then defended her Ph. D. on the subject under the mentorship of prof. Aleš Iglič. Also included was a 3rd year student of Medicine, Blaž Mavčič, who albeit very young, was an excellent mathematician and very skilled with computer.

The method for determination of biomechanical parameters based on the two mathematical models was named HIPSTRESS. With mathematical tools prepared, considering clinical problems provided validation of the method. The first population study published was on the effect of the Chiari operation on geometrical parameters (Antolič V., Srakar F., Iglič A., Kralj-Iglič V., Zaletel-Kragelj L., Maček-Lebar A. Changes in configuration of the hip due to Chiari osteotomy. Int. Orthop. 1996;4: 183-186) followed by a comparison between resultant hip force in female and male populations with Boštjan Kersnič, an undergraduate student of Medicine, as the first author (Kersnič B., Iglič A., Kralj-Iglič V., Srakar F., Antolič V**.** Increased incidence of arthrosis in women could be related to femoral and pelvic shape. Arch. Orthop. Trauma Surg. 1997;116: 345-347). The following clinical studies provided material for the Ph.D. theses of orthopaedic surgeons and traumatologists: Dragica Smrke (bipolar hip prosthesis, 2000), Rok Vengust (Salter osteotomy, 2004), Drago Dolinar (avascular necrosis of the femoral head, 2004), Oskar Zupanc (epiphysiolysis, 2006), Robert Košak (total hip arthroplasty, 2006), Marko Kralj (Bernese osteotomy, 2006), Borut Pompe (hip dysplasia, 2007), Blaž Mavčič (hip dysplasia, 2007), Anže Kristan (rehabilitation after pelvic fracture, 2008), Gregor Rečnik (hip arthritis, 2009), Duško Spasovski (triple pelvic osteotomy), Boštjan Kocjančič (Perthes disease, 2014), Boris Rijavec (total hip arthroplasty, 2014), Andrej Moličnik (Perthes disease, 2019) and Matevž Tomaževič (displacement of hip prosthesis, 2021). The supervisors and co-supervisors of the theses were initially prof. Vane Antolič, prof. Aleš Iglič and myself, later, also those who have already became professors meanwhile. There were engineering studies corresponding to the development of the method (mathematical models) by Marija Ipavec (2002), Matej Daniel (2004) and Hana Debevec (2012). An undergraduate student of Medical Physics in Bratislava, Slovakia, Matej Daniel became another pillar to Socratic lectures. He came to Slovenia for student exchange wishing to work in a cave on Radon. He was first distributed to

a department where they considered plasma physics. As this was not exactly what he wished, he was transferred to the Medical faculty and was immediately included in theoretical and clinical analyses using the HIPSTRESS method. He graduated and defended his Ph.D. on HIPSTRESS – based problems. He was given awards and scholarships, among them the Fullbright scholarship to visit the group of Douglas Pedersen, Iowa, USA. He developed a Java computer program for calculation of biomechanical parameters with geometrical parameters that could be obtained from standard anteroposterior radiographs as input data. He is a coauthor of many papers and of a book published by the international publishing house. After becoming a professor at the Technical University in Prague, he is sending his excellent students to Ljubljana. His student Honza Pluhar was visiting Ljubljana in 2021 and his immense work on analysis of multicentric study of periacetabular osteotomy is still in progress.

I felt lucky to be involved in the development of the HIPSTRESS method. At first, I thought that the method will be useful to the medical doctors, primary for elaboration of their studies (e.g. for PhD studies and promotion). But clinical studies have shown that it could actually be useful in creation of personalized protocols for diagnostics and treatment. At the point of this realization, the undergraduate students could be invited to take part in the development of the method and see for themselves that (bio)physics can be an important tool in medicine.

4. The curriculum

In about 2005, about 10 years after the initiation of the development of the HIPSTRESS method that integrated physics and medicine, an elective subject "Biomechanics of hip" was suggested within the study of Medicine by three teachers, Veronika Kralj-Iglič from Institute of Biophysics, Vane Antolič from Chair of Orthopaedics and Matej Cimerman from the Chair of Surgery. The subject had 6 credits, which was considerable. It was open to the students from 2nd to 6th year of the study. The scope of the subject was determination of biomechanical parameters of hip with the HIPSTRESS method. The subject was put on the list of the elective subjects, however, for some years, no student had chosen it. Then, in 2008, there were 14 students who inscribed. The lectures and exercises took place in the spring semester at the Department of Orthopaedic Surgery, Zaloška 9. The first lecture was opened by prof. Antolič followed by prof. Cimerman, as to motivate the students for the study of biomechanics. Prof. Antolič on many occasions presented to the students the importance of integration of physics and medicine. As a director of Orthopedic Clinics, a mentor of Ph.D. students and a principal investigator of the HIPSTRESS-based projects financed by the Slovenian Research Agency ARRS, he was a pillar to the development of the HIPSTRESS method and of the Socratic Lectures.

All those who built and validated the HIPSTRESS method supported the subject. They donated the lectures. A new method for student examination was introduced that was customized to the HIPSTRESS method. The students were to learn the method and assess biomechanical parameters of chosen hips. Also, within the examination, there was a lecture by excellent scientists and professionals that students had to attend and enter into the discussion with. The subjects of the lectures were connected with the HIPSTRESS method. The students were free to ask the lecturers to help them compose the best answers to the exam questions.

There were two important elements that characterized the lectures: (i) they were donated, not payed; (ii) somehow, year after year, an inspiring atmosphere of interest in the subject could be felt. Remembering that room of gentleness from the book of Plato, these lectures were named Socratic lectures. Each year we have updated the contents according to the emerging new results. No student has ever failed the subject and all have deserved high marks as they were supportive to the lecturers. In the following years, the subject became quite popular reaching up to 80 students per semester. Some students elaborated the selected problems for the student Prešeren award. Some students have become coauthors of scientific papers published in distinguished journals.

4. Pioneering work on extracellular vesicles and tunelling nanotubes

The goal to integrate physics and medicine was pursued also from other directions. Although the HIPSTRESS turned out a success, my primary interest was theoretical biophysics of living systems as composed of many small particles. In considering such systems, the statistical mechanical description seemed the most appropriate. Following the emerging problems, in particular the ones connected to the vesiculation of cellular and artificial membranes, Aleš Iglič and myself started collaboration with Henry Hagerstrand from Abo Akademi University, Finland, who was studying the effect of various compounds, in particular the detergents, on vesiculation of the erythrocyte membrane (Hägerstrand, H, Isomaa, B. Vesiculation induced by amphiphiles in erythrocytes. Biochimica et Biophysica Acta, 1989, 982(2), 179–186. https://doi.org/10.1016/0005-2736(89)90053-9). Combining experimental results of the colleagues from Finland with our theoretical considerations, our first publications were on erythrocyte vesiculation (Iglič A., Hägerstrand H., Kralj-Iglič V., Bobrowska-Hägerstrand M. A possible physical mechanism of red blood cell vesiculation obtained by incubation at high pH. J. Biomech. 1997;31: 151-156) and cancer cell vesiculation (Kralj-Iglič V, Batista U, Hägerstrand H, Iglič A, Majhenc J., Sok M. On mechanisms of cell plasma membrane vesiculation. Radiol Oncol. 1998;32, 1: 119-123). In the following years the nano-sized vesicles which are free to move with liquids became recognized as a system of inter-cellular communication with important role in blood clot formation in blood vessels (Müller I, Klocke A, Alex M, Kotzsch M, Luther T, Morgenstern E, Zieseniss S, Zahler S, Preissner K and **Engelmann B**. Intravascular tissue factor initiates coagulation via circulating microvesicles and platelets. The FASEB Journal, 2003, 17: 1-20. https://doi.org/10.1096/fj.02-0574fje), autoimmune diseases (Distler JHW, Jüngel A, Huber LC, Seemayer CA, Reich CF, Gay RE, Michel BA, Fontana A, Gay S, **Pisetsky DS,** Distler O. The induction of matrix metalloproteinase and cytokine expression in synovial fibroblasts stimulated with immune cell microparticles. Proc Natl Acad Sci USA, 2005, 102(8), 2892–2897, https://doi.org/10.1073/pnas.0409781102) and cancer (Ratajczak J, Wysoczynski M, Hayek F, Janowska-Wieczorek A, Ratajczak MZ. Membrane-derived microvesicles: important and underappreciated mediators of cell-to-cell communication. Leukemia. 2006;20:1487–95).

Furthermore, existence of nano-sized network connecting membrane-enclosed compartments was suggested after observing the transport of fluorescent dye between giant phospholipid vesicles (Mathivet L, S. Cribier, and PF Devaux. Shape change and physical properties of giant phospholipid vesicles prepared in the presence of an AC electric field. Biophys J 1996,70:1112–1121). By improving the previously renowned mathematical model with inclusion of in-plane orientational ordering of the constituents, we predicted that tubular protrusions on the membrane can be stable if they are thin enough (below 100 nm) (first published in Kralj-Iglič V, Iglič A, Bobrowska-Hägerstrand M, Hägerstrand H, Peterlin P. Tethers connecting daughter vesicles and parent red blood cell may be formed due to ordering of anisotropic membrane constituents. Coll Surf A. 2001; 179: 57-64). Existence of bilayer membrane nanotubes was then experimentally proved (Kralj-Iglič V, Gomišček G, Majhenc J, Arrigler V, Svetina S. Myelin-like protrusions of giant phospholipid vesicles prepared by electroformation. Coll Surf A. 2001;181:315-318). In 2004, however, transport of matter between cells through thin tubes was published in the renowned journal Science (Rustom A, Saffrich R, Markovic I, Walther P, Gerdes HH. Nanotubular highways for intercellular organelle transport. Science. 2004; 303:1007–1010) which presented a milestone for a field of so-named tunelling nanotubes and related intercellular communication mechanisms.

Statistical mechanical approach to membranes proved key to description of membranous nanostructures (extracellular vesicles, tunneling nanotubes and other non-lamellar structures).

Socratic Lectures took advantage of early introduction of the students to new findings on intracellular communication; the pioneers Henry Hagerstrand from Abo Akademi Uni-

versity, Finland (the *ex vivo* studies) and Bernd Engelmann from Ludwig-Maximilian University, Munich, Germany (coagulation disorders) were the first to convey their views to the students at the closing event of the lectures that preceded the symposia. Following the invitation of David Pisetsky, a Ph.D. student Vid Šuštar worked about a month at the Duke University, North Dakota, USA. In due course, the plenary lecturers on the symposium were renown scientists in the field of extracellular vesicles Bernd Giebel from University of Duisburg-Essen and University Hospital Essen, Essen, Germany (extracellular vesicles as therapeutic agents), Gabriella Pocsfalvi from National Research Council of Italy, Naples, Italy (plant extracellular vesicles), Antonella Bongiovanni from National Research Council of Italy, Palermo, Italy (microalgal extracellular vesicles), Leonard Margolis from National Institute of Health, Bethesda, USA (relations of extracellular vesicles and viruses) and Sergej Tomić from Institute for the Application of Nuclear Energy, Belgrade, Serbia (extracellular vesicles as diagnostic tools).

In 2011, preceded by a conference in Paris, the International Society for Extracellular Vesicles was constituted and the first annual conference of the society took place in Gothenborg, Sweden. The Ph.D. students Eva Ogorevc and Roman Štukelj actively attended these events as well as other events of the society that took place in the early following years. Theoretical considerations were introduced into medical problems based on Ph.D. theses on isolation of extracellular vesicles from blood (Vid Šuštar, 2011, Roman Štukelj, 2014), within collaboration with the Department of Rheumatology, University Medical Centre Ljubljana on antiphospholipid syndrome (Jasna Urbanija, 2007, Mojca Frank, 2008), with Department of Gastroenterology, on gastrointestinal cancer (Rado Janša, 2012, Eva Ogorevc, 2014), with Veterinary faculty, on mastocytoma (Metka Šimundić, 2018) and with the Department of Orthopaedic Surgery, on Charcot syndrome secondary to diabetes (Karin Schara, 2021). The Ph.D. students reported on their results on the symposia, so the undergraduate students were immediately informed on the latest developments in the respective fields.

The undergraduate students generously donated blood samples for ongoing studies as to constitute the healthy control pool.

5. Experience of excellent professionals at the Socratic Lectures

To integrate biophysics and medicine, it is necessary to acknowledge both fields. In my opinion it is crucial to present within the subject of physics the substance of medicine which could only be done by excellent clinicians. Furthermore, I have asked the lecturers – clinicians to tell the students that knowing physics is good for profession. After obtaining the licence for a lecturer in 1997, I got the opportunity of conducting a course of Biophysics at the Veterinary Faculty, University of Ljubljana in 2008. Therefore, the students of Veterinary Medicine were involved in Socratic Lectures. The first invited lecturer was Pavo Zaninović, who in 1995 set up a privately-owned small animal clinic called Prva – K, Clinics for small animals. It was breathtaking when charismatic dr. Zaninović started his lecture with words: "I have treated 40000 patients." He told the students how a particular cat has touched his heart and this was the reason for his decision to treat the animals. Matevž Gorenšek was a doctor of human as well as of veterinary medicine. When we first met and discussed about lecturing to the students I asked him about the animals. He answered: "I adore animals!" He was very happy to tell this to the students. Also he highlighted that medicine is more than learning the protocols and that a good doctor is thinking about particular cases. Vida Čadonič Špelič presented to the students many examples where physics can help in the veterinary profession. Moreover, she urged the students to take an ethical standpoint for animal welfare. Metka Šimundić presented cases from her clinical work as well as the results on her research on extracellular vesicles isolated from blood of canine patients with mastocytome. By chosing the extracellular vesicles as her Ph.D. subject she showed the greatest support to the Socratic Lectures.

Duško Spasovski from Clinics Banjica, Belgrade, Serbia, has donated many excellent lectures on different subjects that have inspired the students and the colleagues. Being a

skilled lecturer and understanding the HIPSTRESS method, he immensely supported the subject and the development of curricula. In the last years he reported on the treatment of joint arthritis by mesenchymal stem cells that he was practicing himself. Also, he amply collaborated in communication with students within the exams. His contribution is a pillar to the Socratic Lectures. Kjeld Soeballe donated an experience-based lecture on periacetabular osteotomies. He presented his excellence, discipline, amazing results on a large number of patients operated and his setting of the scientific work. Also he made available the X ray images of more than 200 patients before and after the operation for analysis with the HIPSTRESS method. Boštjan Kocjančič presented a case of a patient that was implanted a hip endoprosthesis, however, his femur has previously underwent a fracture that healed in a deformed shape. There was a question whether and, if so, when, the patient should be advised to burden the leg. The surgery took place somewhat before the course began and we presented the case to the students who were requested to come to some conclusions before the symposium. The X ray images and the records of the disease were available to the students. There were no limits to the sources to gather the knowledge. At the symposium, dr. Kocjančič as well as the patient both described the whole timecourse of disease and treatment from their points of view. The patient stood on the operated leg and said that the long lasting pain is gone and that he is the luckiest person in the world. Dr. Kocjančič added that such moments are the reason why he decided to study medicine and become a physician. The students have supported this excellent moment with a long and warm applause. There were three best biomechanical analyses suggested by the students that were presented to dr. Kocjančič and the student of Radiology that suggested the best one according to dr. Kocjančič was invited to attend the total hip arthroplasty operation. One of the symposia presented the total hip arthroplasty from views of different professios: a surgeon (Drago Dolinar from the Department of Orthopaedic Surgery, University Medical Centre Ljubljana), an anesthesiologist (Anita Mrvar Brečko, Department of Anestesiology and Reanimatology, University Medical Centre Ljubljana), a nurse (Manca Pajnič from the Faculty of Health Sciences, University of Ljubljana) and a physiotherapist (Darja Rugelj from the Faculty of Health Sciences, University of Ljubljana). It was very informative to learn within the same section all the problems that a patient encounters in the way towards the restoration of the functioning. The problems of diabetes complications were presented by Karin Schara who devoted herself to treatment of the patients with Charcot syndrome. The clinical manifestations in these patients is grave and any contribution that exposes this problematics is highly warranted. Rado Janša presented treatment of pain which is an universal problem in many diseases. He amply supported Socratic lectures in many ways. To his invitation, the students could attend the seminar for physicians on overweight and cahesion where they were included in a voting system with presented cases. Also he provided sponsoring of the the catering at a symposium that took place at the Faculty of Medicine, University of Ljubljana, at a great pleasure of the students and the colleagues. During COVID-19 the lectures took place online and one of the lecturers was the leading medical doctor Bojana Beović. She was willing to donate the lecture, but was urgently called to a meeting, therefore the students have assembled again the next day (which was a Sunday). The students had a chance to discuss the emerging situation with the person in the middle of the problem solving.

The most devoted donor of the Socratic Lectures proved Marija Ipavec, a physicist, first author of the original paper presenting the mathematical model for hip stress which was the basis of her Ph.D. thesis. She donated the lectures at all events that took part, however, not on biomechanics. Having survived the osteosarcoma in her first year of undergraduate study of physics, she was generously willing to share her experiences with many generations of the students. This was particularly important for the students of Orthotics and Prosthetics as she described the problems and possible solutions with wearing the above the knee prosthesis. The questions that the students had to answer were for example: What are the possible problems encountered by the patients who wear the above the knee prosthesis? The students could ask her directly and put down the answers. Also, when the lectures were in the classroom, she brought with her a spare prosthesis so that the

students could see and touch it. It was always uplifting to the participants of the lectures to meet the real winner and hero.

6. Cultural events at Socratic Lectures

To fulfil the program within the curricula and convey to the students the message on the subject, the events sometimes took many hours. Some lecturers took more time than planned and they were not interrupted. One of the Lectures took place at the new Faculty of Medicine, Korytkova 2. It should have been completed at 8 pm, but there were a couple of lectures still to be presented and the examination test to be completed. A student came to me and asked if he could leave the symposium. He was supposed to go to a graduation dance with his girlfriend. I realized that it would be really bad if he were late. I have let him go but I was worried also for other students since we have exceeded the time. While the lectures continued, I heard disturbing noises that were coming from the hall. I went out to see and found a group of young men talking and laughing. I asked them to be quiet as our students were having the exam. They excused explaining that they did not know that someone were in the lecturing room. They told me that they are from the chorus and the director wished to rehearse with girls only, so she sent the boys out of the room. I asked them where are the director and the girls and they showed me. I entered and asked the director if they would be willing to donate a song to the participants of the symposium. They were willing and after the last lecture, I went to fetch them. They sang a gentle, happy song "When you will come to Bled". Then I announced that this song was to represent the exam and thanked the students and the participants for their attention. Seeing the effect of music on the participants, it became clear to me that music would be a great contribution to the event and since then became an obligatory part of Socratic Lectures.

Mezzosoprano Jasmina Antonić Babnik, a medical doctor, donated her performance, accompanied on the piano by Jana Jamšek, a mathematician. The text of Desanka Maksimović, a renown Serbian poet was chosen in honor of Duško and Vesna Spasovski from Belgrade, Serbia. Music on a guitar was donated by Mitja Drab, a physicst. These performances took place after the symposium in the lecturing hall. However, the main cultural activities were divided from the scientific part, as the Museum of Architecture and Design donated the rooms in the castle Fužine, for the events. There were three events in the Fužine castle (2017, 2018 and 2019). The programs were performed by professional musicians that donated their performances as well as scientists, students and friends. In the entrance level of the castle, there are two halls connected by the doors. In one of them, the acoustic is perfect and it is a pleasure to perform music in it. We brought a Yamaha clavinova 404 for the performance, Roman Štukelj taking the greatest burden to carry it up and downstairs. The chairs and tables in the hall were distributed in such way that the guests could watch and listen while eating and drinking. The programs were tentative with intervals in between the performances so that the guests could chat. There was catering, but some guests brought food and drinks too and divided them among the guests. The most distinguished were the gifts of Stanka Pezdirc from Lokvice, who brought the traditional decorated bread that was talked about long after the event. Also she brought the Slovenian cakes "potice" and cookies, all homemade. Many guests went home with her generous gifts. One of the highlights of the evening was dividing of the cake "Slice of Emona" baked by the students of the Biotechnical Education Center Ljubljana. The cake for 100 persons was put flat on a wooden desk and was until being served conserved in the room of the castle where the temperature was low enough. Namely, the cake of this size could not fit in any fridge. The cake was cut by a surgeon, Duško Spasovski. Devoted friends led by my husband Aleš were very efficient in supervising that the guests were comfortable and had enough to eat and drink. They were also very efficient in putting the hall in order after the event.

The first event that took place on April 5, 2017 at 6pm at the Fužine castle. Anita Prelovšek, a flutist and a Ph.D. of musicology performed on the flute while Jasmina Antonić Babnik and Norma de Saint Pičman, a multi-artist sung. They were accompanied by Janez Snoj, the piano player, on the piano. Norma's art was made visible on a large screen positioned at the backside of the stage. Visual art was contributed also by Nataša Ribič, a painter.

Samo Kralj, a physicist, performed A. Piazzola's Libertango at the piano. The performance of Summertime by Liviano Valesin, a mathematician, on the trombone, myself on the flute and Jana Jamšek on the piano received much interest, as Liviano was connected remote on Skype. The picture of him playing was projected live on the screen. The children donated excellent contributions. Iva Zemljič sung a song from Prekmurje and a sister and brother Nuša and Urban Levec sung and danced in French language. The highlight of the programme was the classical balet number performed by Darja Eržen, the surgeon from the Institute of Oncology accompanied by Jana Jamšek on the piano. Some pieces were performed by ensembles (Anita Prelovšek (flute), Matej Venier (violin), piano (Janez Snoj) and myself (flute)), e.g. obligatory A. Piazzola's Oblivion, D. Šostakovič's 2nd waltz and I. Albeniz' Tango. Tomaž Lampe, an engineer of Orthotics and Prosthetics, prepared a beautiful visual presentation of titanium nanostructures that he created in the laboratory and elaborated for imaging by scanning electron microscope in collaboration with Metka Benčina. The presentation was accompanied by the Adaggietto from the Mahler's 5.th symphony transcribed for two flutes and piano.
The second event was at February 10, 2018. It coincided with the carnival, so many guests came masked. A beautiful matched masks were by Stanka Pezdirc (puss in boots) and a harlequine (Boštjan Jurković), black and white fairies (Nataša Ribič and Vladimir Štefanec) and a medieval couple (Rebeka and Roman Štukelj) who after the party welcomed their first baby (Niko) the very next day. The programme included Slovene authors V. Parma, M. Sepe, J. Privšek, J. Robežnik, E. Ropas and Katalena, but also classical (A. Hačaturjan, F. Chopin, G. Bizet, B. Bartok, E. Stolzl, F. Schubert) and modern (C. Gardel, E. Marchelie, L. Guglielmi, H. Arlen, J. Lennon, G. Sviridov, C. Bolling, A. Piazzola) ones. Performances were donated by Jasmina Antonić Babnik, Janez Snoj, Elena Startseva Somun, Matej Venier, Anita Prelovšek, Metka Penko Natlačen, Dušan Ješelnik, Jana Jamšek, Livio Valesin and myself.

In December 2018, Aleš and I accepted the invitation of Gabriella Pocsfalvi to visit the National Research Council of Italy in Naples. We stayed for 3 months during which we have collaborated on the project Ves4us considering extracellular vesicles from microalgae. On that occassion the leader of the project Antonella Bongiovanni from Palermo and her colleagues came to Naples for a scientific symposium. The companion social event that was organized by Anna Romolo and ourselves took place in Palazzo Venezia, Naples on March 4, 2019. Performing were Canio Fidanza (piano) and Christopher Stanly (guitar) from Naples and Anita Prelovšek and myself (both flute) from Ljubljana. The event included catering of Francesca Schiavi that was warmly appreciated by the guests. Anna Romolo from Naples has proved an excellent organizer, having connections and experiences with proper places, catering and many things that were necessary to complete the event. Also she produced a movie that was recorded by her friend Maria Manfredi. They mounted it together to save the memory on this lovely event.

The third event in the Fužine castle was on April 25, 2019, at 8 pm. It was opened by T. Susato's Cum decore sung by chorus Evergreen (Jožica Pirc, Milan Steržaj, Metka Penko Natlačen, Barbara Knol Drobnič, Franc Drobnič, Marjan Jarnjak, Ksenija Pirc, Vojko Pirc, Jana Jamšek, Bojana Zebič). The performances that followed were donated by Anita Prelovšek (flute), Elena Startseva Somun (piano), Metka Penko Natlačen (soprano), Jana Jamšek (piano), Ilarija Griessler (flute), Aleona von Sultanova (saxophone and piano), Zoran Mosić (saxophone), Darja Božič, Manca Pajnič, Mitja Drab and Marko Jeran (chorus). Metka Penko Natlačen and Stojan Natlačen Penko showed the dances: quickstep and tango. A special guest was Canio Fidanza from Naples who played the Oblivion, 2nd waltz and Tango on the piano.

In December 2019, Aleš and I returned to Naples and the colleagues from Palermo, Naples and Ljubljana have met again. Tjaša Griessler Bulc from the Faculty of health Sciences who is also considering microalgae in her research have come from Ljubljana together with her husband and two daughters, Larina and Ilaria Amelie. The young ladies were to perform at the event, by singing and playing the flute. Also coming from Ljubljana were Anita Prelovšek, Darja Božič, a Ph.D. student and Marko Jeran. Darja Božič and Marko Jeran

were a part of the Ves4us project whereas Darja Božič is also a skilled flutist and was going to take a part in the performance.

For some pieces, piano accompaniment was necessary. The contact with Canio Fidanza who participated a year before could not be made. Anna's friend Valentina had an idea where a piano player could be found. She suggested that we go to a comedy where there will be piano accompaniment by someone she knew. Indeed, we attended a performance in a small hall in the basement of a bigger building besides the way to the sea. We were seating in the second row. The play was accompanied by popular songs played on piano, drums and guitar. The actors were giving all their energy and as the hall was small, we could feel the sweat and spit of the actors. It was very intense. After the show we went backstage to meet the piano player. Her name was Marinella Barbato. I asked her if she was willing to accompany a flutist in about 10 days, for a demanding classical piece suggested by Anita Prelovšek. Marinella said that she could not learn it on such short notice. Marinella was however willing to accompany the Shostakovic 2nd waltz and Piazzola's Oblivion. The place of performance chosen by Anna Romolo was in the convent of St Chiara, in a former church adapted for acoustics. It was a home of composer and guitar player Carlo Faiello who was happy to contribute to the program the Napoletan music. Before the event we had a rehearsal. Marinella came with a fever but was willing to play. Anita liked her accompaniment very much, however, the next day Marinella called to say that she is badly and is not fit to perform. Roberta Schmid, the organist who was to play the classical pieces saved the day by stepping in to play also the Shostakovic and the Piazzola. It was an honor and a pleasure to present in Naples Serenade by Benjamin Ipavec and some other pieces by Slovenian authors which were likely heard for the first time by the audience in Naples. Francesca Schiavi distinguished herself again by ingenious and delicious snacks that made an important contribution to the event.

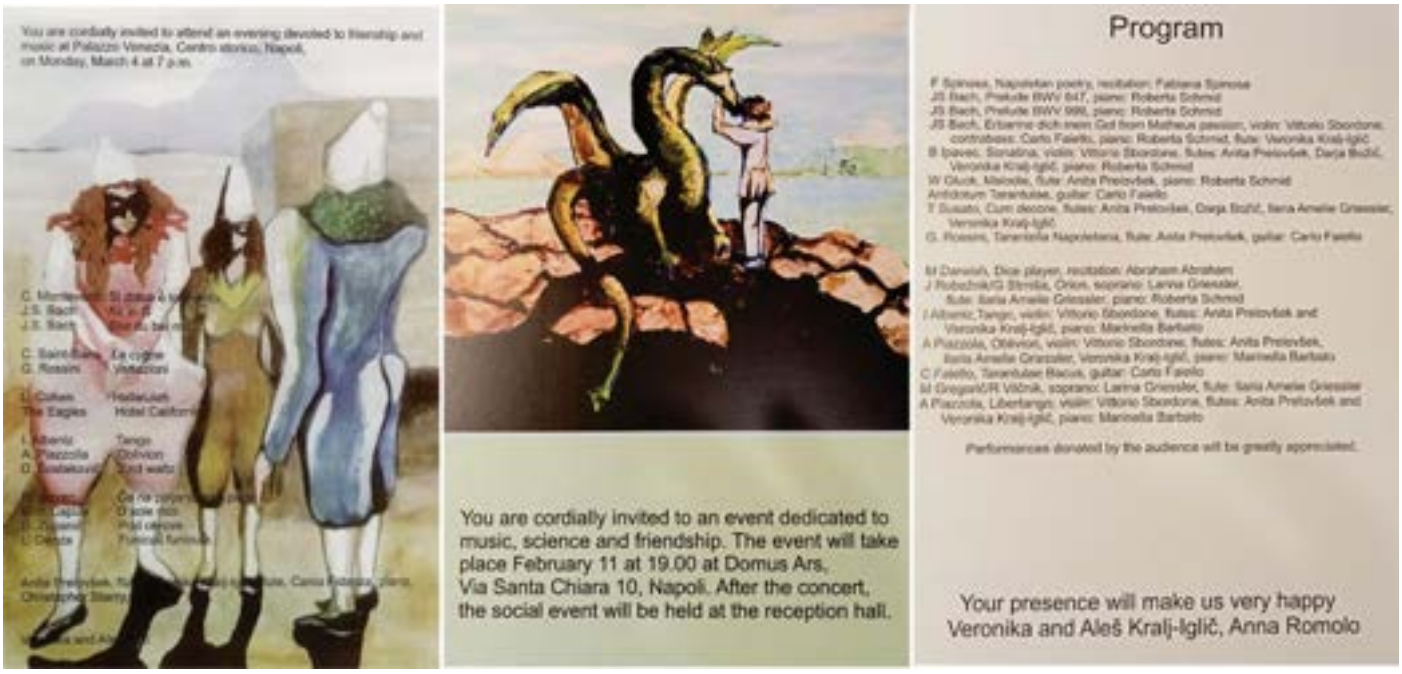

Figure 1. Invitation to the cultural events in Palazzo Venezia and Domus Ars in 2019 and 2020. The dragon and the Pulcinella symbolize the friendship between participants from Naples, Palermo and Ljubljana.

In the winter of 2020 the Socratic Lectures symposium was for the first time performed online, nevertheless, the music was included. The symposium was opened by a classical piece on the guitar by played by a student . After the sections, music was donated online by Anita Prelovšek on flute, Elena Startseva Somun on flute, Emil Somun on trombone and Vittorio Sbordone on violin. In December 11, 2021, the concert was donated live by Roberta Schimd, an organist from Naples, Italy. She performed a recital of classical pieces at the Church of St Mary's Assumption at three bridges, Ljubljana. Due to COVID-19 a

modest social event was announced as a meeting at the bar of the Fužine castle a day after the symposium, however, due to COVID-19 only a student from Prague, Honza Pluhar, and two of the organizers (Aleš and myself) appeared.

Figure 2. Invitations to social events accompanying Socratic Lectures in consecutive years 2017, 2018 and 2019.

7. Cultural and social events at 9th International symposium marking 15 years of Socratic Lectures

The 8th international symposium took place on January 2023 but the accompanying event came to be realized only associated with the next small 9th symposium in May. There was a lecture of Marija Ipavec for the students online and the focus was on the cultural and social event to honor the 15 years of Socratic Lectures. The event was financially supported by the University of Ljubljana. The concert was organized in the beautiful Julij Betteto hall of Kazina, the home of the Academy of Music, University of Ljubljana while the social event was at the club in the same building (**Figure 3**).

Vittorio Sbordone who participated at cultural events in Naples, Italy and online, sent me a message that he and his friend have learned the Beethoven sonata Spring for violin and piano and would like to perform it in Ljubljana. For this piece it is necessary to have an excellent piano. The transfer from the castle Fužine was therefore needed due to availability of a beautiful concert Stainway in the Kazina hall. The programme of the concert included F. Chopin Polonaise, Op 40, No 1 performed on the piano by Elena Startseva Somun, G. Donizetti, Sonata for flute and harp in G minor performed by Anita Prelovšek on the flute and Lara Pelikan on the harp, L. van Beethoven Sonata for violin and piano Spring performed by Vittorio Sbordone on the violin and Keith Goodman on the piano, C. Saint Saens The swan from Animal carneval performed by Anita Prelovšek on the flute and Lara Pelikan on the harp, M. Ravel Sonata for violin and piano No.2 (Allegretto and Blues, moderato) performed by Branko Brezavšček on the violin and Elena Startseva Somun on the piano, J. Ibert Entr'acte performed by Anita Prelovsek on the flute and Lara Pelikan on the harp and F. Mendelsohn The wedding march performed by Jana Jamšek on the organ.

As Vittorio and Keith needed to practice a lot, they came to my home where there is a Yamaha piano, a legacy of my mother. Anna, Aleš and I were able to listen to the beautiful Spring sonata several times during these two days as they were repeating it over and over. Keith took it very strongly keeping the rhythm while Vittorio added the romantic note. They played some other pieces as well, but kept returning to the Spring. I actually got high on it and am still listening it in my mind while writing this text.

I was contacted by a violinist Inga Ulokina who expressed her wish to be included in the concert. She told me that she and Keith Goodman have met on the internet and that it was her great wish to perform a particular piece written by Keith, accompanied by the composer himself. She said that cantilena mode that is the substance of this piece suits her very much. After some negotiation, we agreed that this piece, a Romance from a Sonata for violin and piano, will be played the last. It was to be the first performance of this piece in public. The addition to the programme was the first public performance of the K. Goodman lovely Sonata for violin and piano performed by Inga Ulokina and the composer. After the concert a social event with music took place in the Club of the Academy of Music,

featuring Baron Valvasor composed and performed by Aleona von Sultanova; the composition was first published in the Proceedings of the Socratic Lectures. Musicians and scientists performed together and created a unique atmosphere of a night that will be remembered. This time the traditional cake was skilfully cut by the otorhynolaryngology surgeon prof. Saba Battelino.

Figure 3. Invitations to cultural events of the 15.th anniversary of Socratic lectures.

Conclusions

The activities of the Socratic lectures – the curricula, excellent science and profession and intertwinning of different fields of science and art around medicine aimed at joy of learning and achieving amply supported by the joy of donating. At the beginning, these activities were performed with purity and in silence. However, after 2018, the symposia have expanded and are now organized by the Faculty of Health Sciences, University of Ljubljana, within the Lifelong Learning Centre. In 2019, the first Proceedings of the Socratic lectures was published by the University of Ljubljana Press. Future development will show how much of the spirit of the Socrates could survive in the modern world. Nevertheless, we have witnessed that the participants of Socratic lectures have created, evidenced and widely promoted it in their professional and personal development.

Proceedings of 9th Socratic Lectures **2024**

Repository

Cryogenic transmission electron images of *Dunaliela tertiolecta* isolates of small cellular particles

Bedina Zavec Apolonija[1] , Božič Darja[2,3], Iglič Aleš[3,4], Jeran Marko[2,3], Kisovec Matic[1], Kralj-Iglič Veronika[2,*], Romolo Anna[2]

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Abstract: Cryogenic transmission electron images of small cellular particles isolated from conditioned media of microalgae *Dunaliella tertiolecta* are presented. Each image is supplemented by description of the preparation of the sample and the data on the imaging technique and equipment.

The data curators of the repository are Veronika Kralj-Iglič and Anna Romolo. More data on experiments with microalgae small cellular particles can be found in (Adamo et al., 2021), (Picciotto et al., 2021) and (Božič et al., 2022).

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Keywords: Extracellular vesicles, Extracellular particles, Nanoalgosomes, Exosomes

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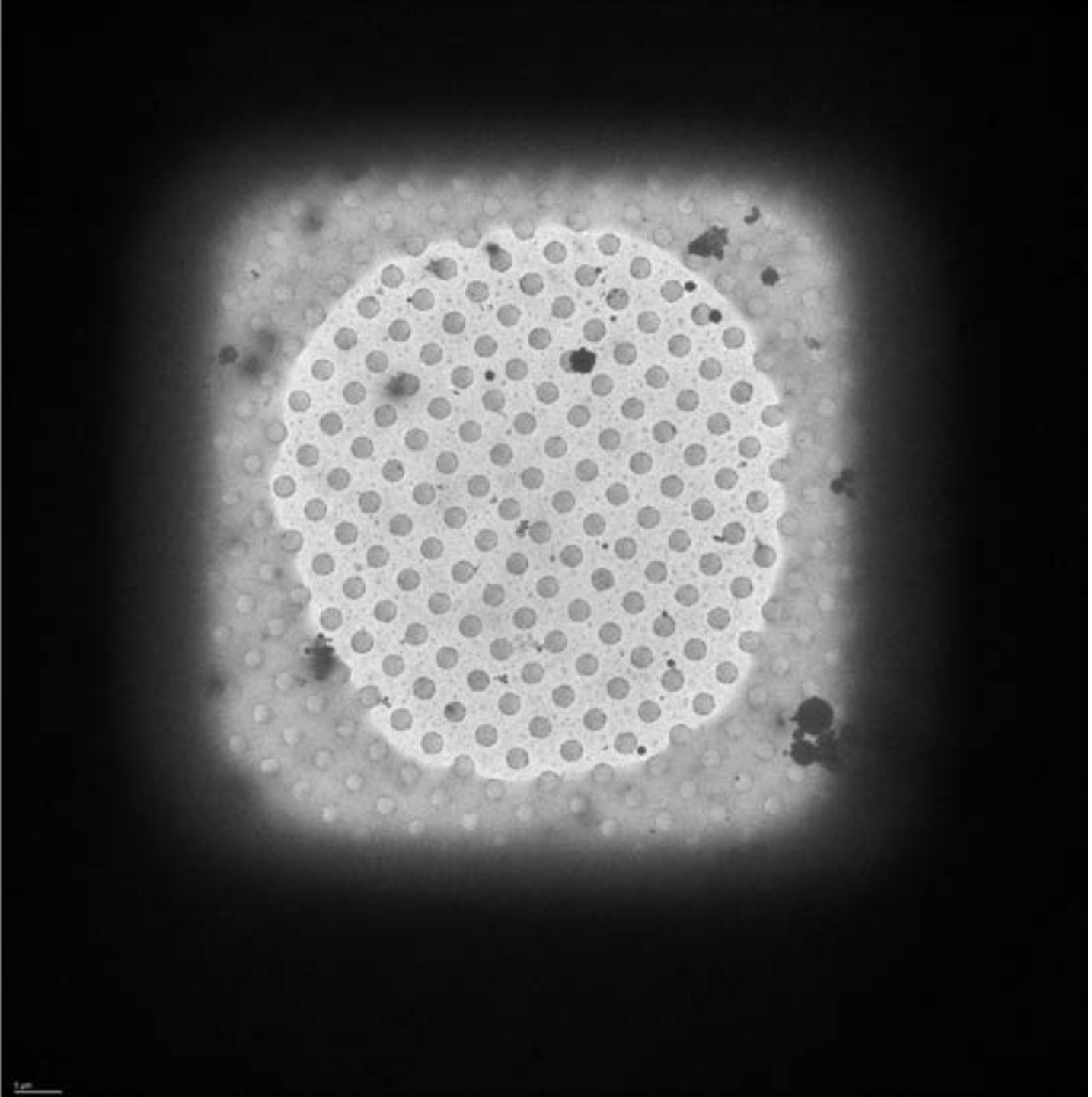

Figure *Dunnaliela tertiolecta* isolate CRYO-TEM 1.

Cultivation of the algae

Cultures of *D. tertiolecta* CCAP 19/22 from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) were grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)[17]. Cultures were grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m^2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Isolation of nanoparticles (NPs)

NPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

Cryogenic Electron Microscopy (Cryo-TEM)

Samples of NPs isolates were prepared for Cryo-TEM using Vitrobot Mark IV (Thermo Fisher Scientific, Waltham, MA, US). Quantifoil® R 2/2 (or 1.2/1.3), 200 mesh holey carbon grids (Quantifoil Micro Tools GmbH, Großlöbichau, Germany) were glow discharged for 60 s at 20 mA and positive polarity in the air atmosphere (GloQube® Plus, Quorum, Laughton, UK). Conditions were set at 4 °C, 95% relative humidity, blot time: 5 s, and blot force: 4. 2 μL of the sample with nanoparticles in suspension was applied to the grid, blotted, and vitrified in liquid ethane. Samples were visualized under cryogenic conditions using a 200 kV microscope Glacios with Falcon 3EC detector (Thermo Fisher Scientific, Waltham, MA, US).

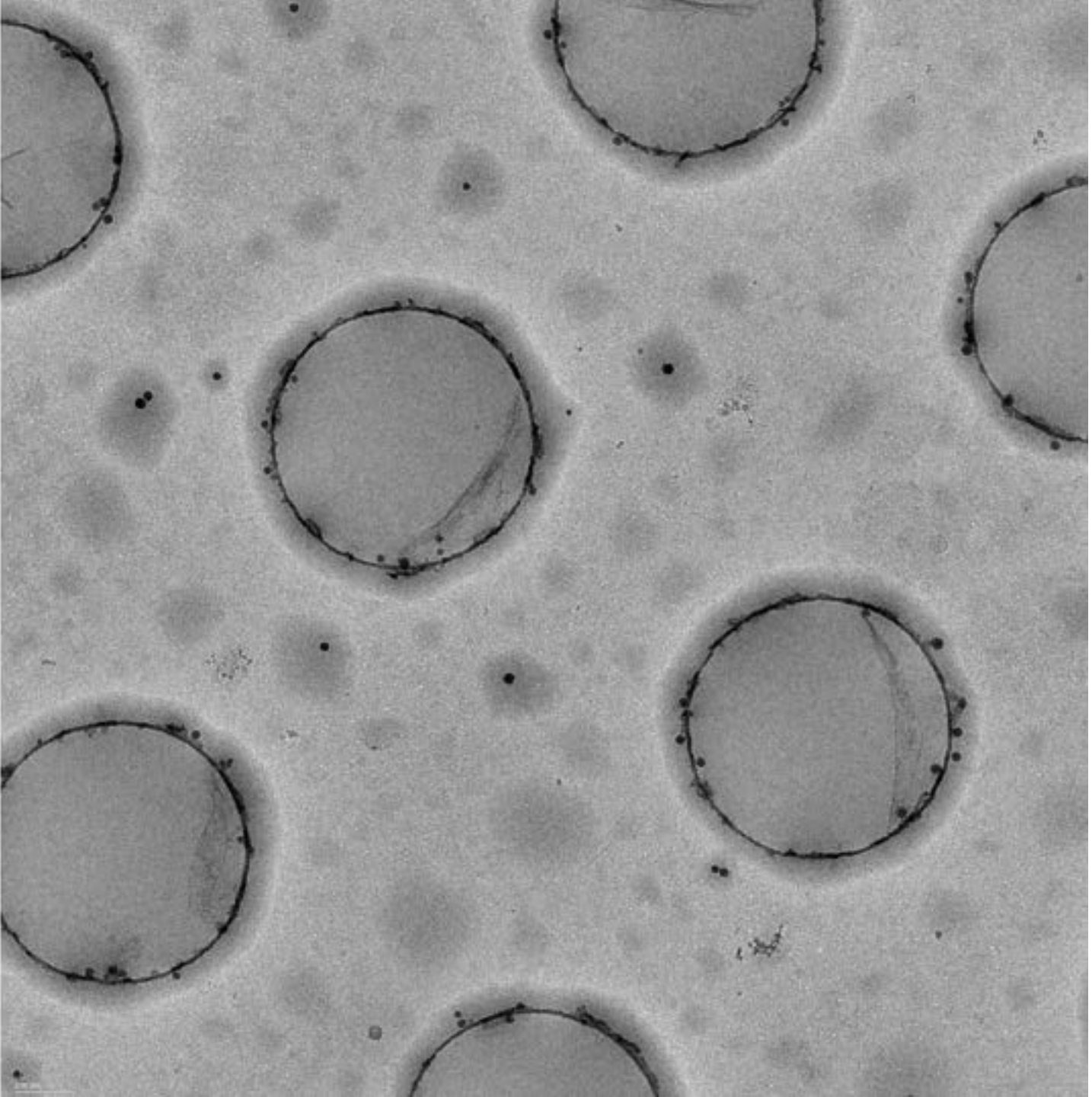

Figure *Dunnaliela tertiolecta* isolate CRYO-TEM 2.

Cultivation of the algae

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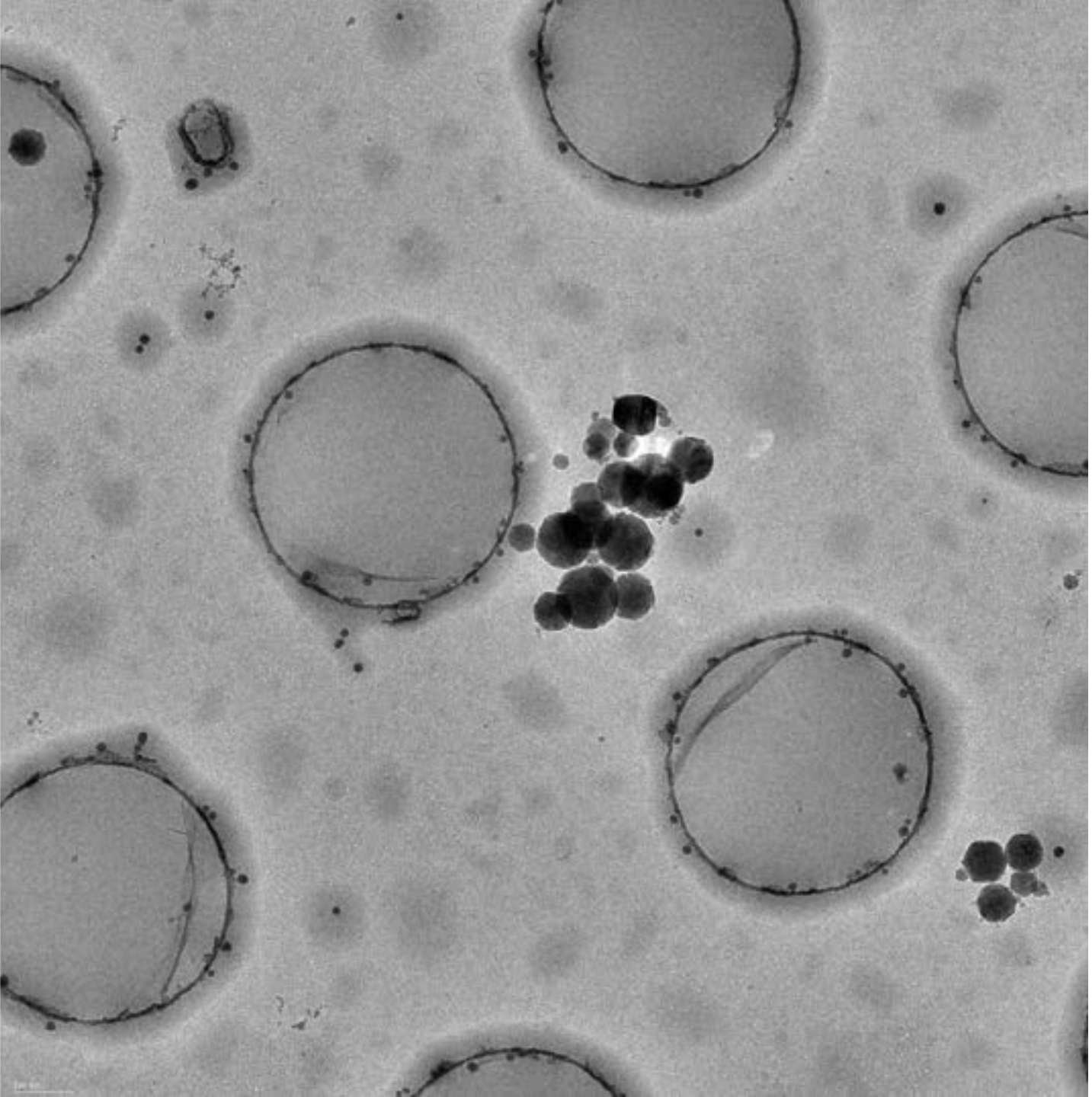

Figure *Dunnaliela tertiolecta* isolate CRYO-TEM 3.

Cultivation of the algae

Cultures of *D. tertiolecta* CCAP 19/22 from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) were grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)[17]. Cultures were grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 µmol/m^{2}s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Isolation of nanoparticles (NPs)

NPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

Cryogenic Electron Microscopy (Cryo-TEM)

Samples of NPs isolates were prepared for Cryo-TEM using Vitrobot Mark IV (Thermo Fisher Scientific, Waltham, MA, US). Quantifoil® R 2/2 (or 1.2/1.3), 200 mesh holey carbon grids (Quantifoil Micro Tools GmbH, Großlöbichau, Germany) were glow discharged for 60 s at 20 mA and positive polarity in the air atmosphere (GloQube® Plus, Quorum, Laughton, UK). Conditions were set at 4 °C, 95% relative humidity, blot time: 5 s, and blot force: 4. 2 µL of the sample with nanoparticles in suspension was applied to the grid, blotted, and vitrified in liquid ethane. Samples were visualized under cryogenic conditions using a 200 kV microscope Glacios with Falcon 3EC detector (Thermo Fisher Scientific, Waltham, MA, US).

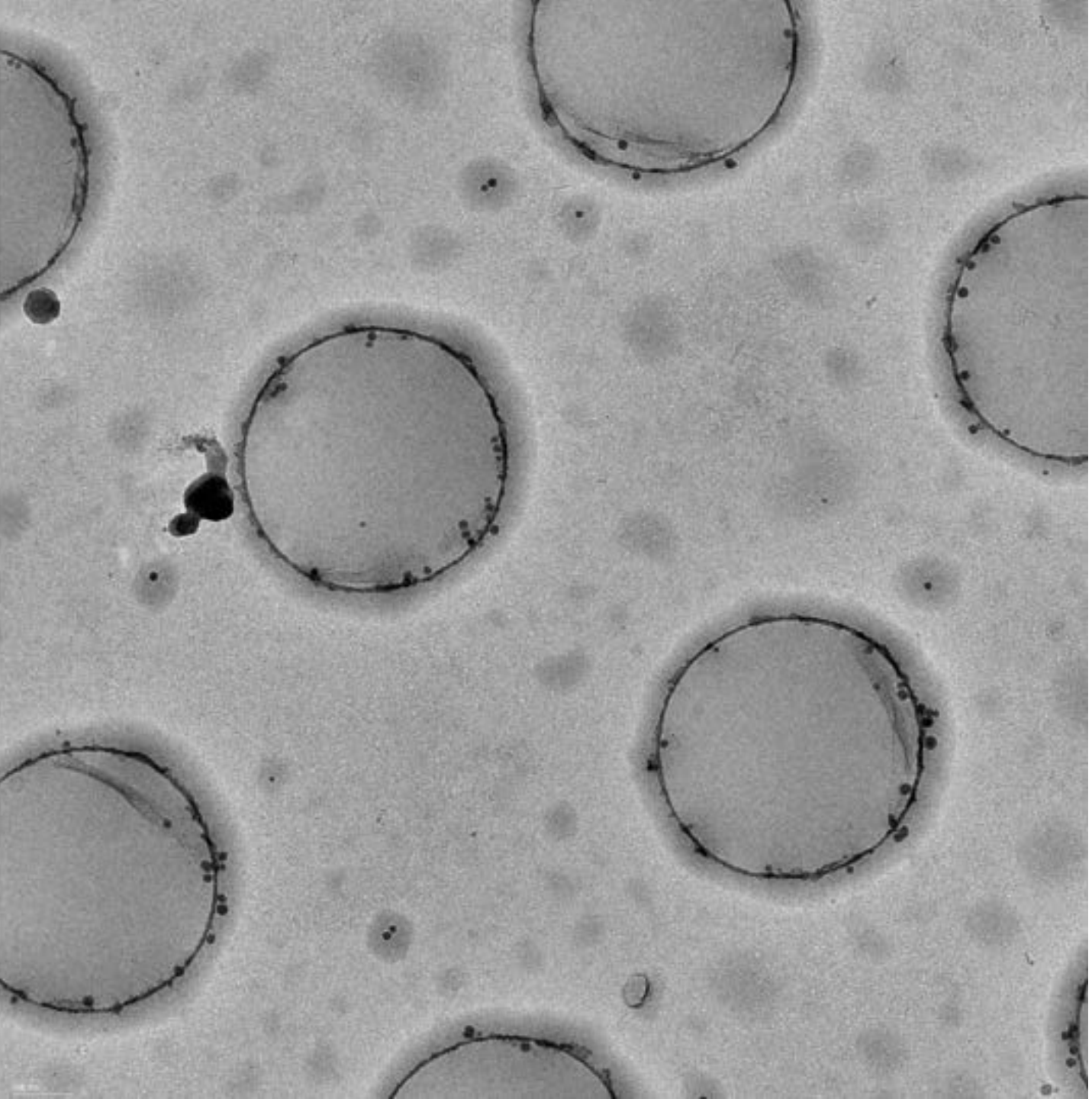

Figure *Dunnaliela tertiolecta* isolate CRYO-TEM 4.

Cultivation of the algae

Cultures of *D. tertiolecta* CCAP 19/22 from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) were grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)[17]. Cultures were grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m^2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Isolation of nanoparticles (NPs)

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Cryogenic Electron Microscopy (Cryo-TEM)

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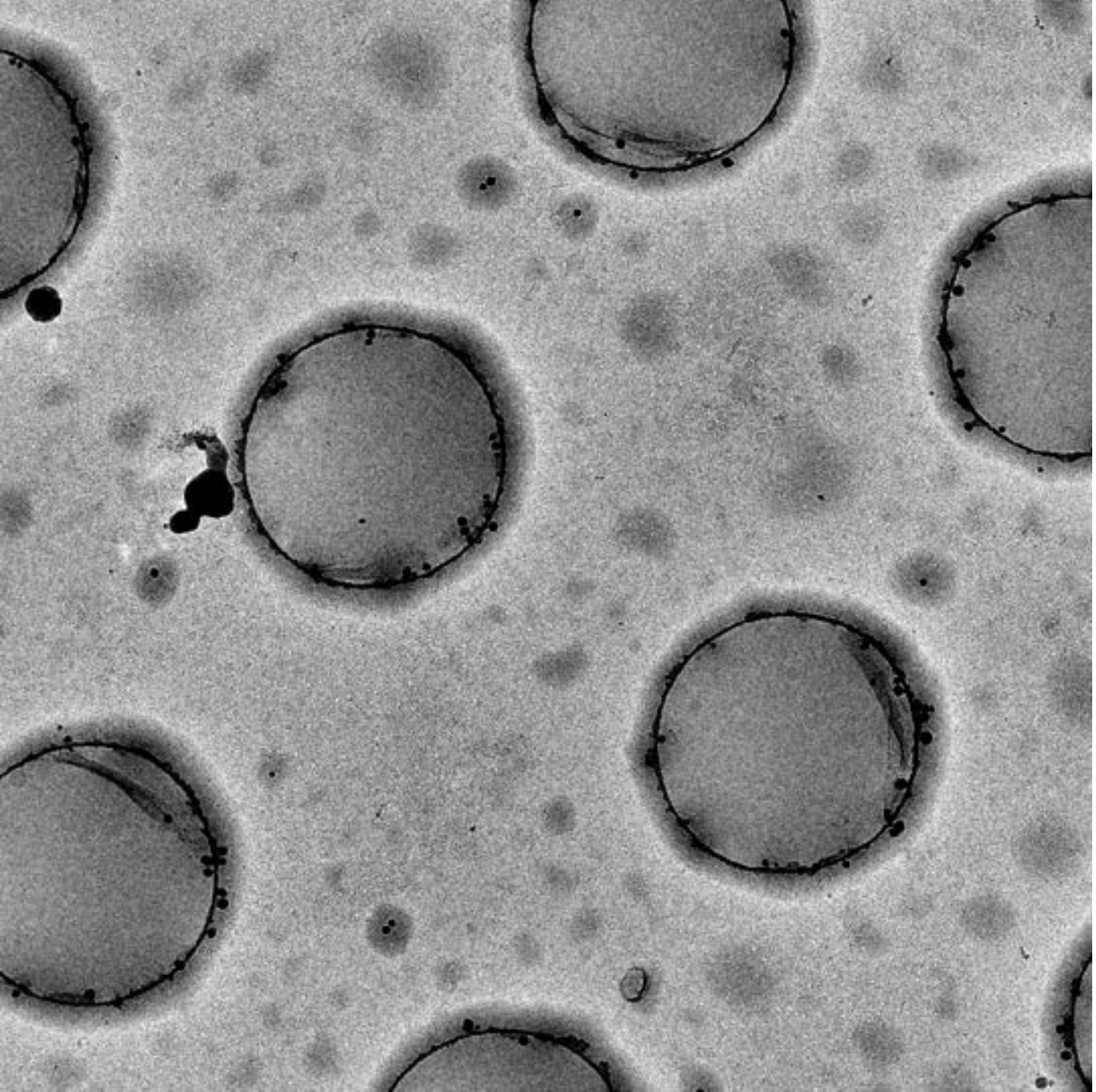

Figure *Dunnaliela tertiolecta* isolate CRYO-TEM 5.

Cultivation of the algae

Cultures of *D. tertiolecta* CCAP 19/22 from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) were grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)[17]. Cultures were grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m^2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Isolation of nanoparticles (NPs)

NPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

Cryogenic Electron Microscopy (Cryo-TEM)

Samples of NPs isolates were prepared for Cryo-TEM using Vitrobot Mark IV (Thermo Fisher Scientific, Waltham, MA, US). Quantifoil® R 2/2 (or 1.2/1.3), 200 mesh holey carbon grids (Quantifoil Micro Tools GmbH, Großlöbichau, Germany) were glow discharged for 60 s at 20 mA and positive polarity in the air atmosphere (GloQube® Plus, Quorum, Laughton, UK). Conditions were set at 4 °C, 95% relative humidity, blot time: 5 s, and blot force: 4. 2 μL of the sample with nanoparticles in suspension was applied to the grid, blotted, and vitrified in liquid ethane. Samples were visualized under cryogenic conditions using a 200 kV microscope Glacios with Falcon 3EC detector (Thermo Fisher Scientific, Waltham, MA, US).

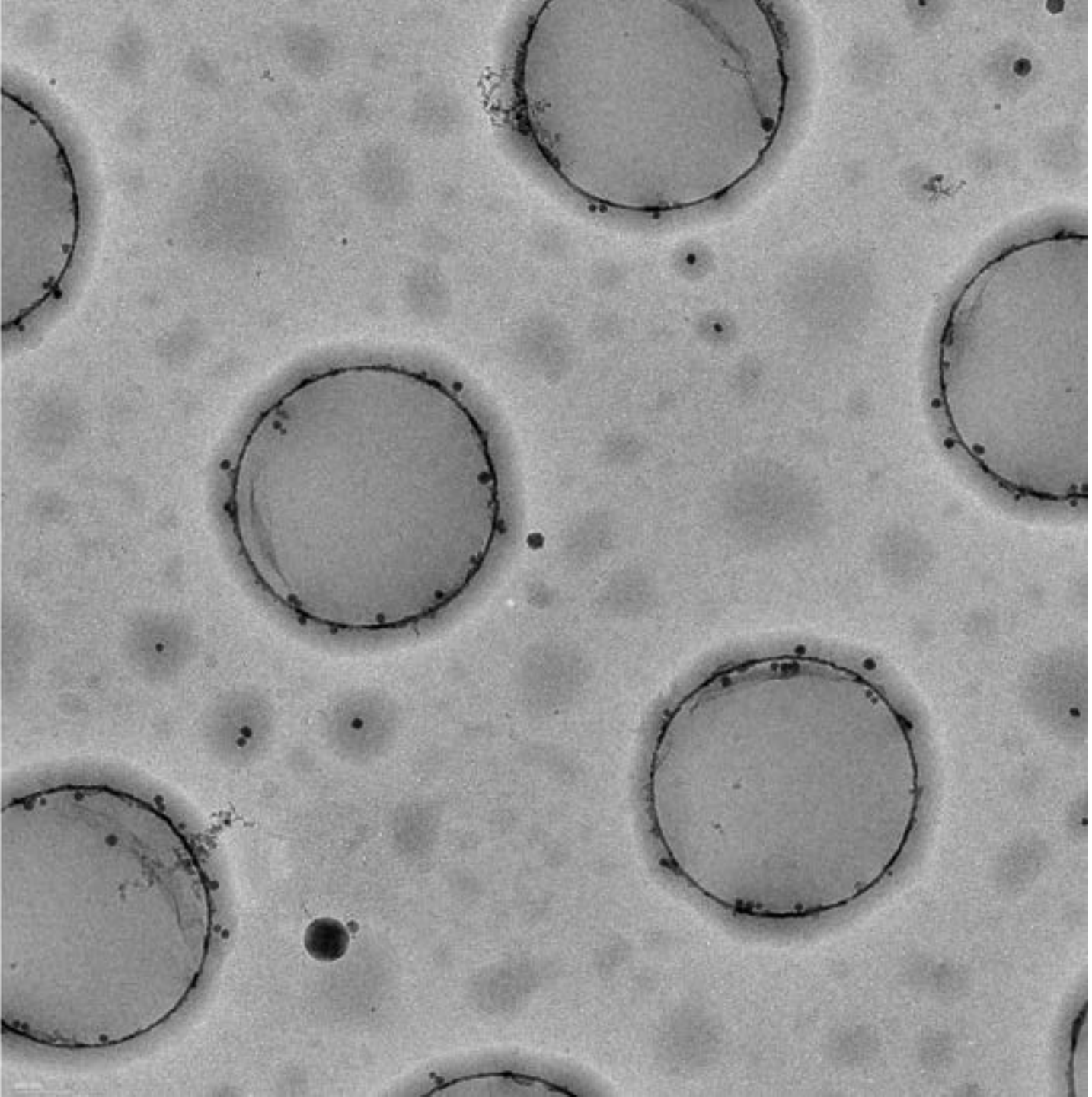

Figure *Dunnaliela tertiolecta* isolate CRYO-TEM 6.

Cultivation of the algae

Cultures of *D. tertiolecta* CCAP 19/22 from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) were grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)[17]. Cultures were grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m^{2}s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Isolation of nanoparticles (NPs)

NPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

Cryogenic Electron Microscopy (Cryo-TEM)

Samples of NPs isolates were prepared for Cryo-TEM using Vitrobot Mark IV (Thermo Fisher Scientific, Waltham, MA, US). Quantifoil® R 2/2 (or 1.2/1.3), 200 mesh holey carbon grids (Quantifoil Micro Tools GmbH, Großlöbichau, Germany) were glow discharged for 60 s at 20 mA and positive polarity in the air atmosphere (GloQube® Plus, Quorum, Laughton, UK). Conditions were set at 4 °C, 95% relative humidity, blot time: 5 s, and blot force: 4. 2 μL of the sample with nanoparticles in suspension was applied to the grid, blotted, and vitrified in liquid ethane. Samples were visualized under cryogenic conditions using a 200 kV microscope Glacios with Falcon 3EC detector (Thermo Fisher Scientific, Waltham, MA, US).

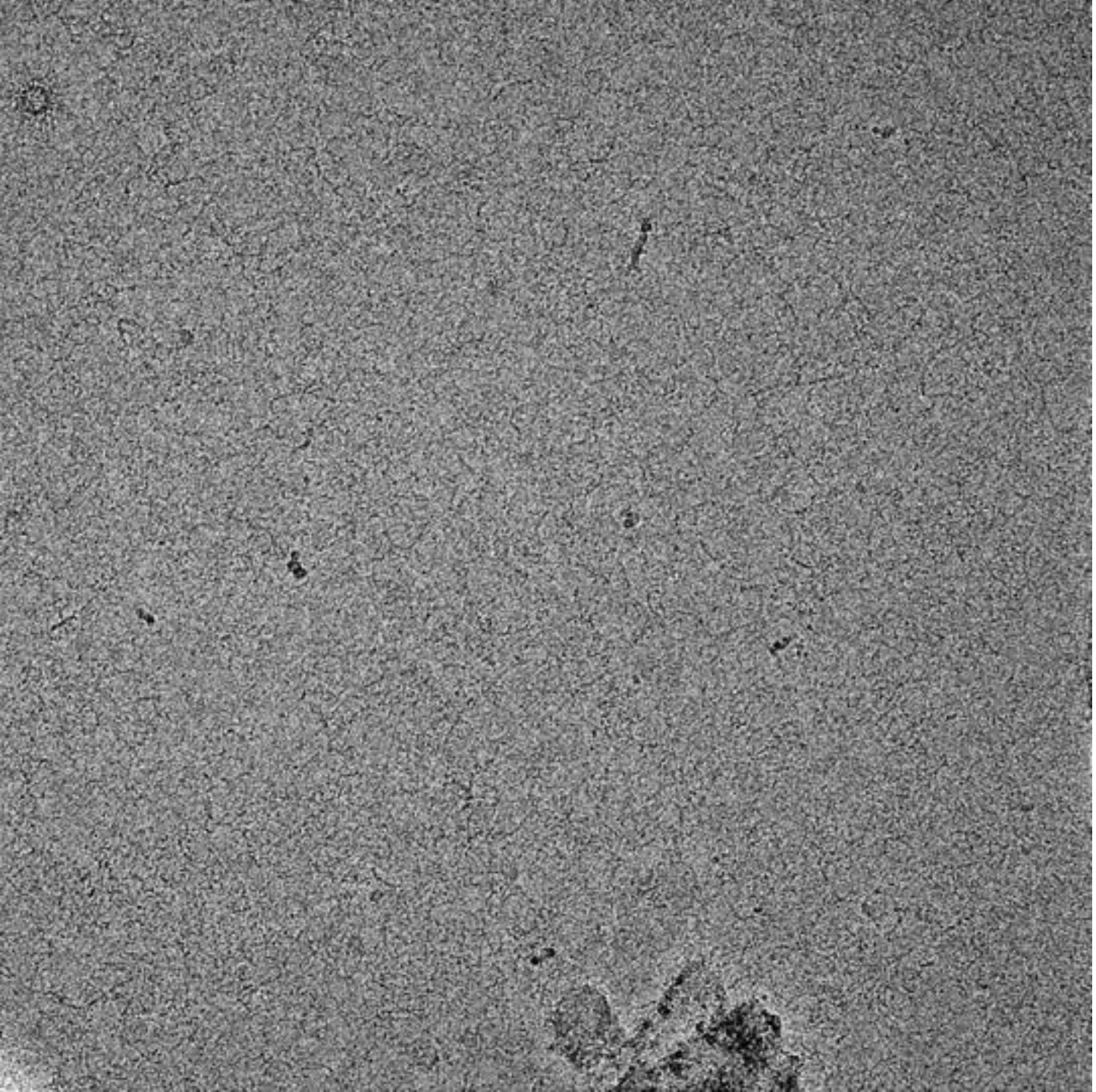

Figure *Dunnaliela tertiolecta* isolate CRYO-TEM 7.

Cultivation of the algae

Cultures of *D. tertiolecta* CCAP 19/22 from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) were grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)[17]. Cultures were grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m^2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Isolation of nanoparticles (NPs)

NPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

Cryogenic Electron Microscopy (Cryo-TEM)

Samples of NPs isolates were prepared for Cryo-TEM using Vitrobot Mark IV (Thermo Fisher Scientific, Waltham, MA, US). Quantifoil® R 2/2 (or 1.2/1.3), 200 mesh holey carbon grids (Quantifoil Micro Tools GmbH, Großlöbichau, Germany) were glow discharged for 60 s at 20 mA and positive polarity in the air atmosphere (GloQube® Plus, Quorum, Laughton, UK). Conditions were set at 4 °C, 95% relative humidity, blot time: 5 s, and blot force: 4. 2 μL of the sample with nanoparticles in suspension was applied to the grid, blotted, and vitrified in liquid ethane. Samples were visualized under cryogenic conditions using a 200 kV microscope Glacios with Falcon 3EC detector (Thermo Fisher Scientific, Waltham, MA, US).

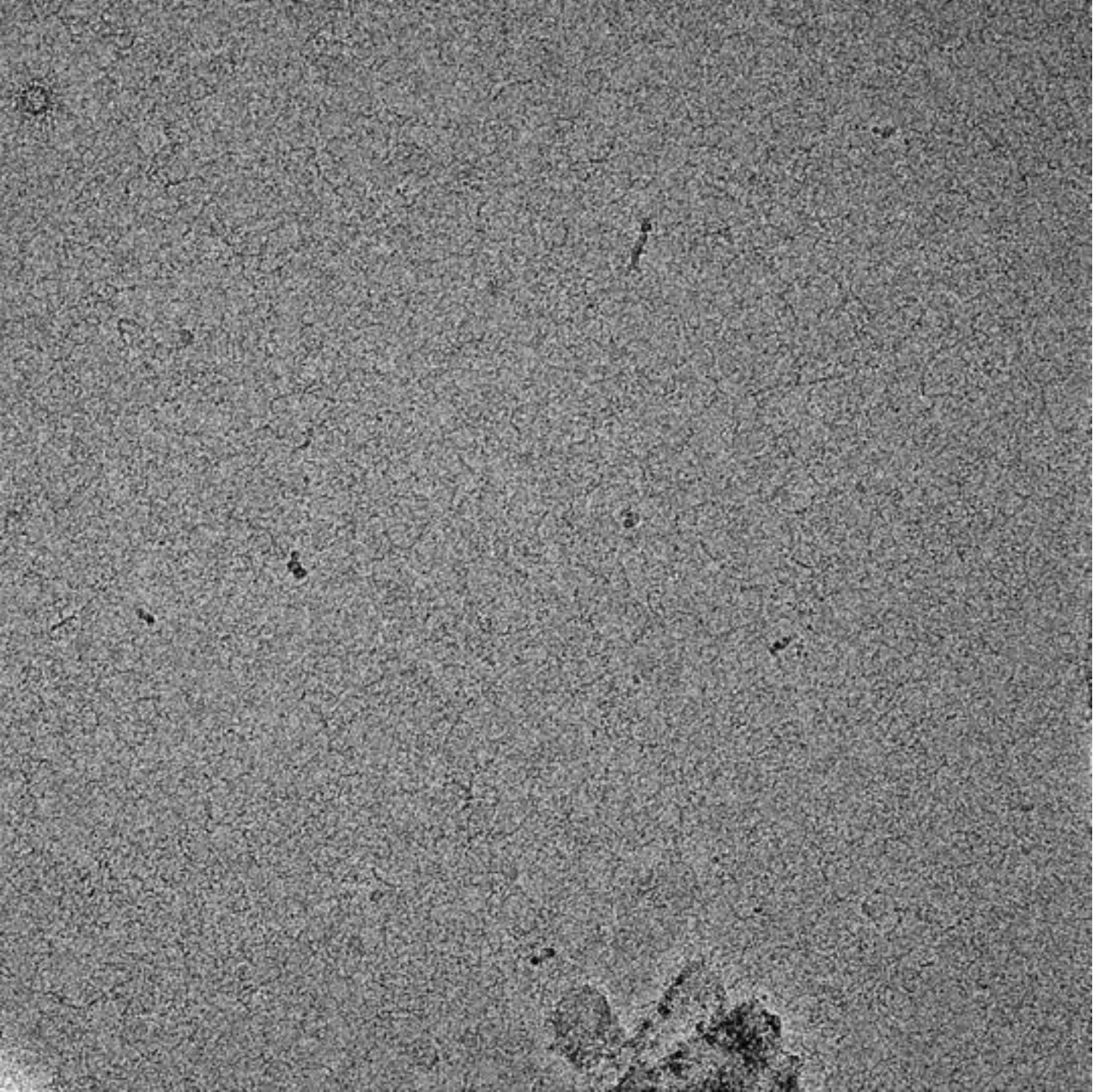

Figure *Dunnaliela tertiolecta* isolate CRYO-TEM 8.

Cultivation of the algae

Cultures of *D. tertiolecta* CCAP 19/22 from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) were grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)[17]. Cultures were grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m^{2}s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Isolation of nanoparticles (NPs)

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Cryogenic Electron Microscopy (Cryo-TEM)

Samples of NPs isolates were prepared for Cryo-TEM using Vitrobot Mark IV (Thermo Fisher Scientific, Waltham, MA, US). Quantifoil® R 2/2 (or 1.2/1.3), 200 mesh holey carbon grids (Quantifoil Micro Tools GmbH, Großlöbichau, Germany) were glow discharged for 60 s at 20 mA and positive polarity in the air atmosphere (GloQube® Plus, Quorum, Laughton, UK). Conditions were set at 4 °C, 95% relative humidity, blot time: 5 s, and blot force: 4. 2 μL of the sample with nanoparticles in suspension was applied to the grid, blotted, and vitrified in liquid ethane. Samples were visualized under cryogenic conditions using a 200 kV microscope Glacios with Falcon 3EC detector (Thermo Fisher Scientific, Waltham, MA, US).

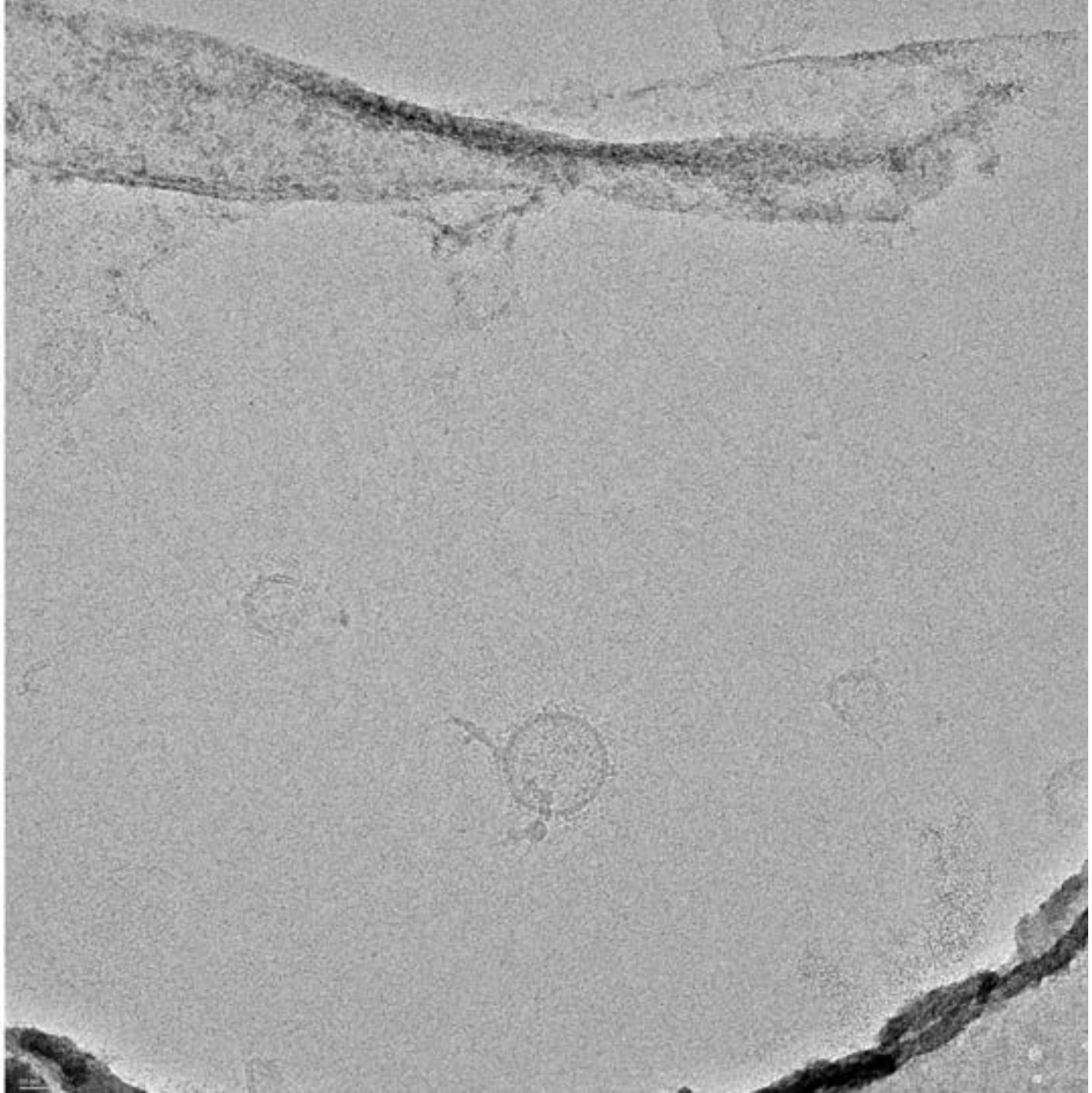

Figure *Dunnaliela tertiolecta* isolate CRYO-TEM 9.

Cultivation of the algae
Cultures of *D. tertiolecta* CCAP 19/22 from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) were grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)[17]. Cultures were grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m^{2}s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.
Isolation of nanoparticles (NPs)
NPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.
Cryogenic Electron Microscopy (Cryo-TEM)
Samples of NPs isolates were prepared for Cryo-TEM using Vitrobot Mark IV (Thermo Fisher Scientific, Waltham, MA, US). Quantifoil® R 2/2 (or 1.2/1.3), 200 mesh holey carbon grids (Quantifoil Micro Tools GmbH, Großlöbichau, Germany) were glow discharged for 60 s at 20 mA and positive polarity in the air atmosphere (GloQube® Plus, Quorum, Laughton, UK). Conditions were set at 4 °C, 95% relative humidity, blot time: 5 s, and blot force: 4. 2 μL of the sample with nanoparticles in suspension was applied to the grid, blotted, and vitrified in liquid ethane. Samples were visualized under cryogenic conditions using a 200 kV microscope Glacios with Falcon 3EC detector (Thermo Fisher Scientific, Waltham, MA, US).
From (Božič et al., 2022, https://doi.org/10.12688/openreseurope.14896.1).

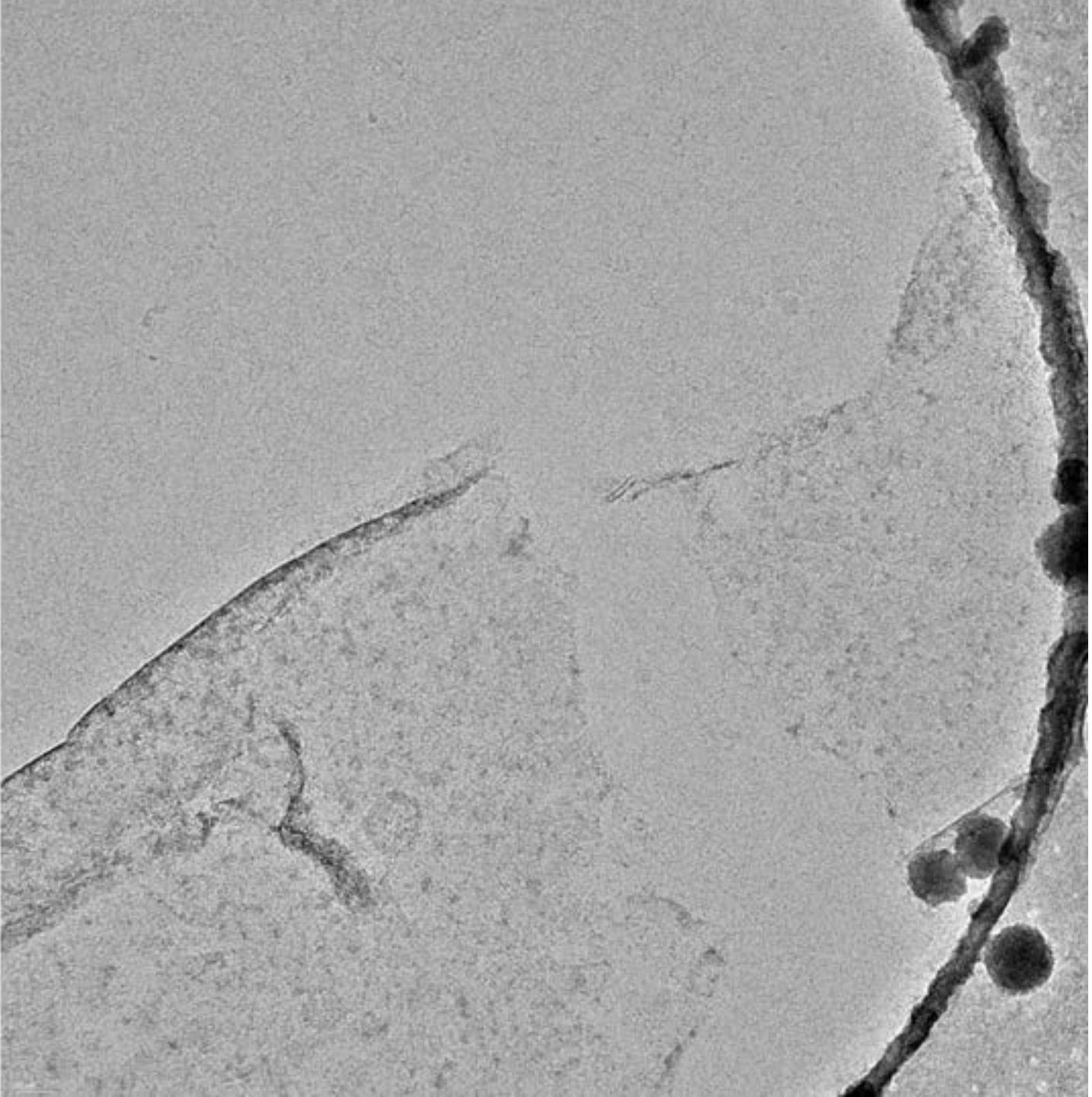

Figure *Dunnaliela tertiolecta* isolate CRYO-TEM 10.

Cultivation of the algae

Cultures of *D. tertiolecta* CCAP 19/22 from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) were grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)[17]. Cultures were grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m^2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Isolation of nanoparticles (NPs)

NPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

Cryogenic Electron Microscopy (Cryo-TEM)

Samples of NPs isolates were prepared for Cryo-TEM using Vitrobot Mark IV (Thermo Fisher Scientific, Waltham, MA, US). Quantifoil® R 2/2 (or 1.2/1.3), 200 mesh holey carbon grids (Quantifoil Micro Tools GmbH, Großlöbichau, Germany) were glow discharged for 60 s at 20 mA and positive polarity in the air atmosphere (GloQube® Plus, Quorum, Laughton, UK). Conditions were set at 4 °C, 95% relative humidity, blot time: 5 s, and blot force: 4. 2 μL of the sample with nanoparticles in suspension was applied to the grid, blotted, and vitrified in liquid ethane. Samples were visualized under cryogenic conditions using a 200 kV microscope Glacios with Falcon 3EC detector (Thermo Fisher Scientific, Waltham, MA, US).

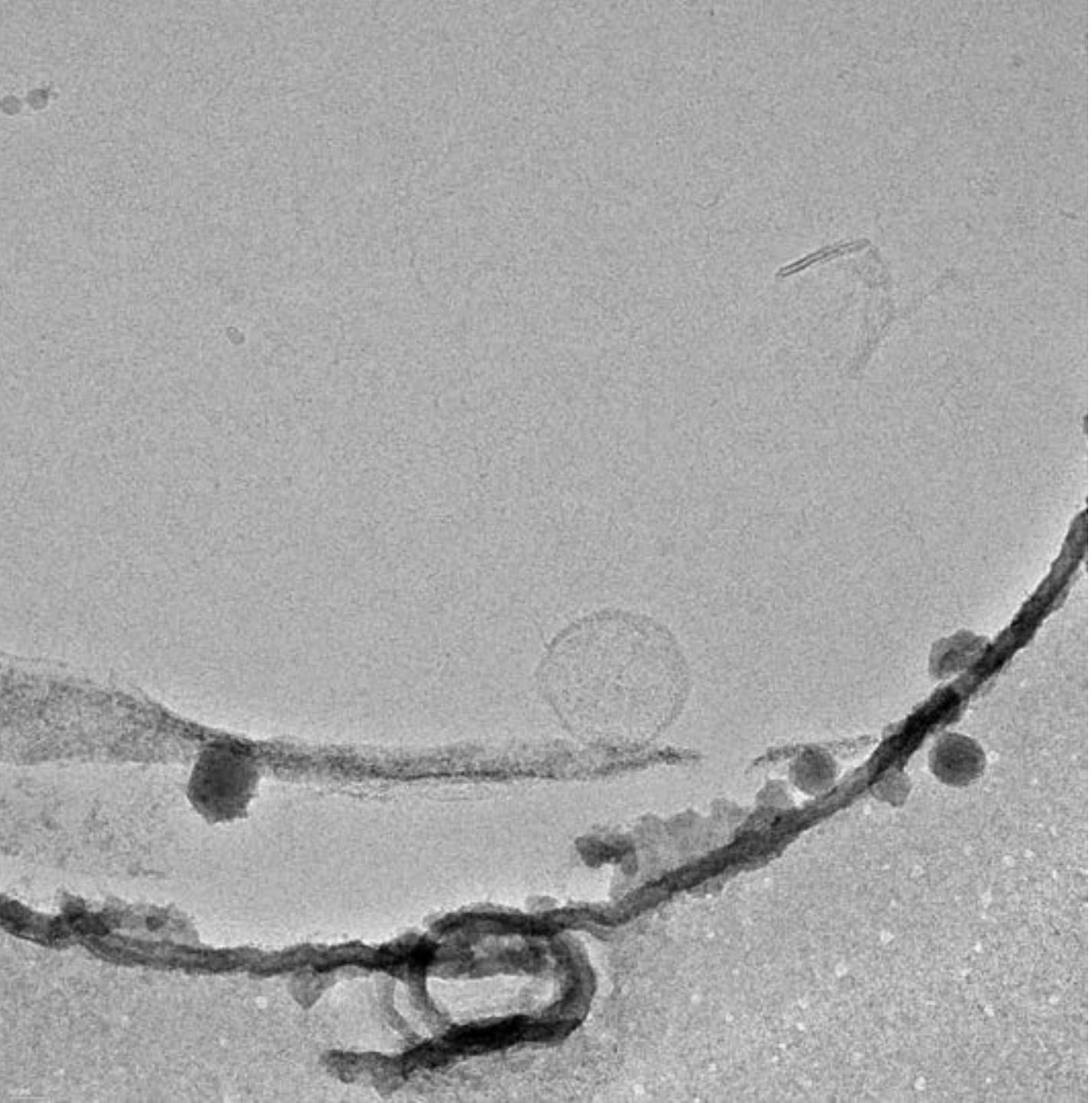

Figure *Dunnaliela tertiolecta* isolate CRYO-TEM 11.

Cultivation of the algae

Cultures of *D. tertiolecta* CCAP 19/22 from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) were grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)[17]. Cultures were grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m^{2}s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Isolation of nanoparticles (NPs)

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Cryogenic Electron Microscopy (Cryo-TEM)

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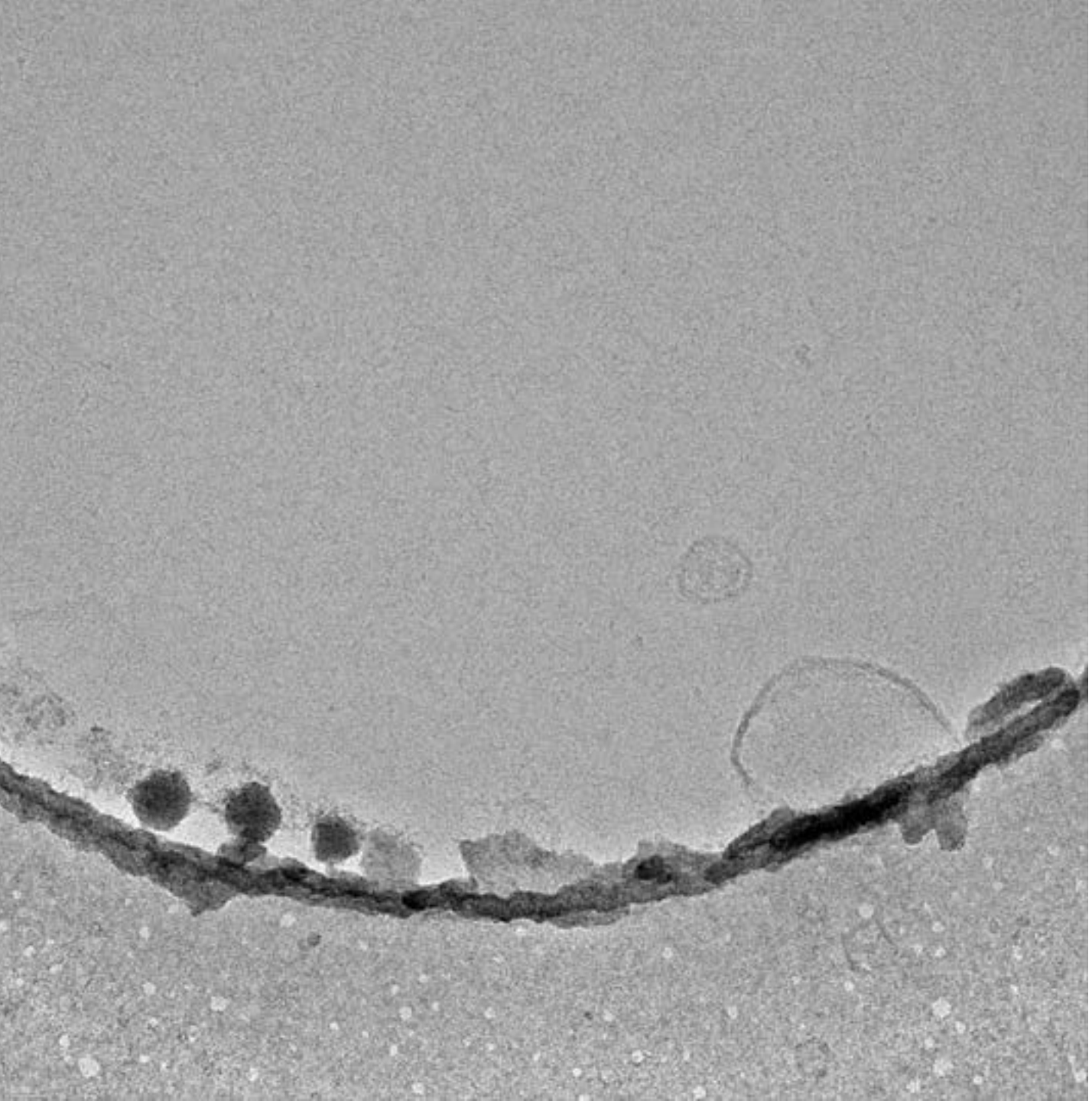

Figure *Dunnaliela tertiolecta* isolate CRYO-TEM 12.

Cultivation of the algae

Cultures of *D. tertiolecta* CCAP 19/22 from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) were grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)[17]. Cultures were grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m^{2}s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Isolation of nanoparticles (NPs)

NPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

Cryogenic Electron Microscopy (Cryo-TEM)

Samples of NPs isolates were prepared for Cryo-TEM using Vitrobot Mark IV (Thermo Fisher Scientific, Waltham, MA, US). Quantifoil® R 2/2 (or 1.2/1.3), 200 mesh holey carbon grids (Quantifoil Micro Tools GmbH, Großlöbichau, Germany) were glow discharged for 60 s at 20 mA and positive polarity in the air atmosphere (GloQube® Plus, Quorum, Laughton, UK). Conditions were set at 4 °C, 95% relative humidity, blot time: 5 s, and blot force: 4. 2 μL of the sample with nanoparticles in suspension was applied to the grid, blotted, and vitrified in liquid ethane. Samples were visualized under cryogenic conditions using a 200 kV microscope Glacios with Falcon 3EC detector (Thermo Fisher Scientific, Waltham, MA, US).

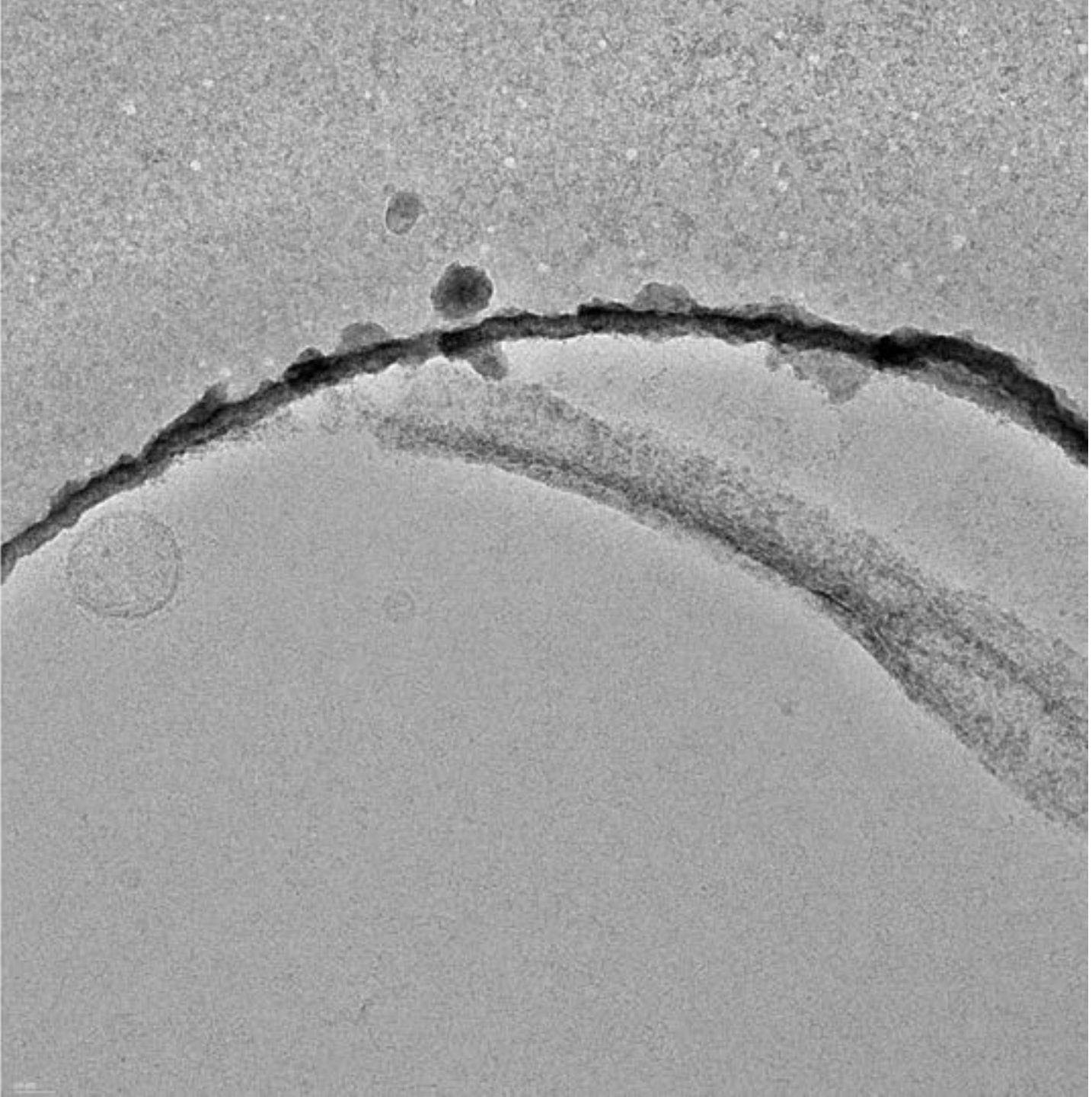

Figure *Dunnaliela tertiolecta* isolate CRYO-TEM 13.

Cultivation of the algae

Cultures of *D. tertiolecta* CCAP 19/22 from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) were grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)[17]. Cultures were grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m^{2}s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Isolation of nanoparticles (NPs)

NPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

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Samples of NPs isolates were prepared for Cryo-TEM using Vitrobot Mark IV (Thermo Fisher Scientific, Waltham, MA, US). Quantifoil® R 2/2 (or 1.2/1.3), 200 mesh holey carbon grids (Quantifoil Micro Tools GmbH, Großlöbichau, Germany) were glow discharged for 60 s at 20 mA and positive polarity in the air atmosphere (GloQube® Plus, Quorum, Laughton, UK). Conditions were set at 4 °C, 95% relative humidity, blot time: 5 s, and blot force: 4. 2 μL of the sample with nanoparticles in suspension was applied to the grid, blotted, and vitrified in liquid ethane. Samples were visualized under cryogenic conditions using a 200 kV microscope Glacios with Falcon 3EC detector (Thermo Fisher Scientific, Waltham, MA, US).

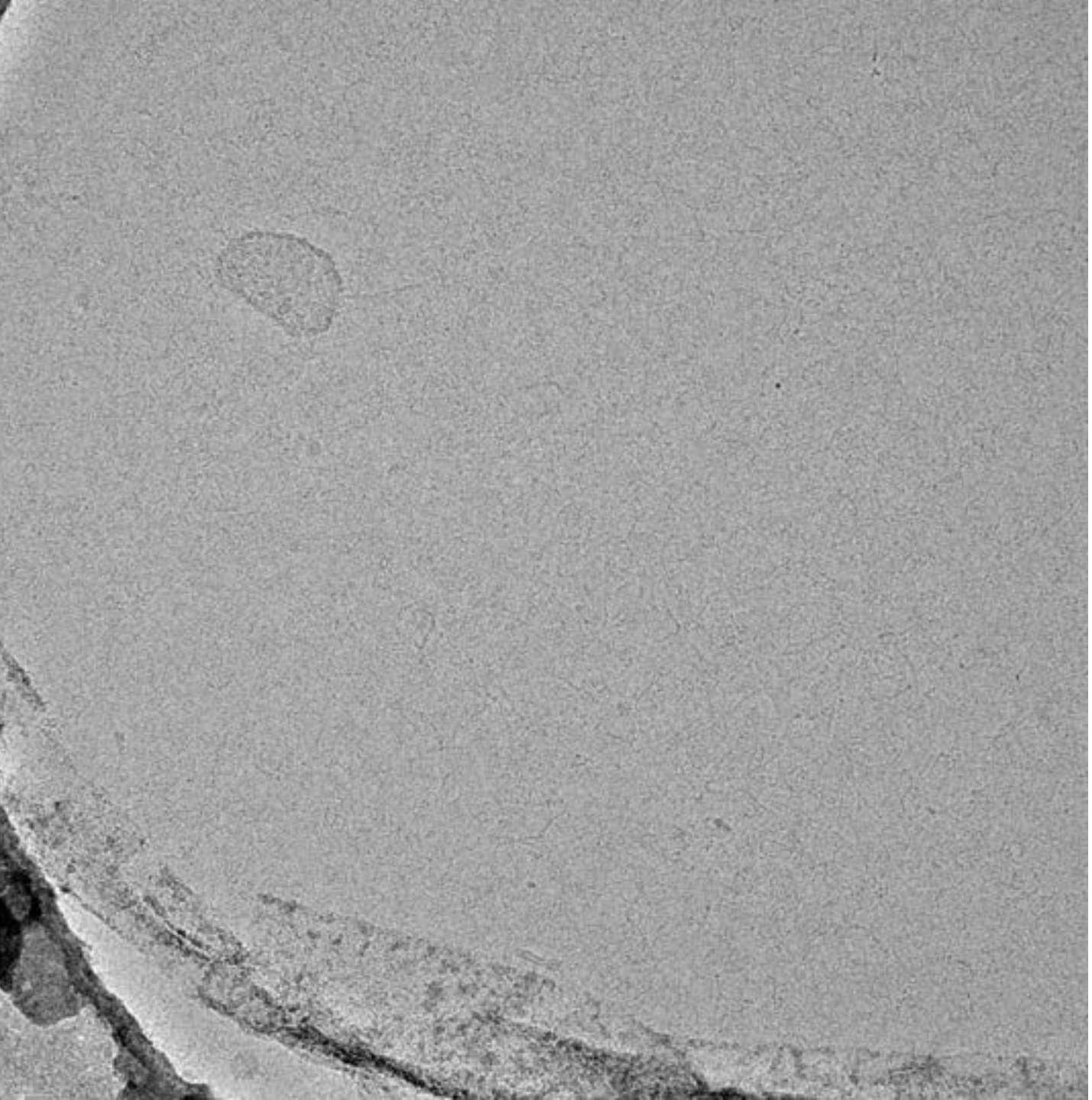

Figure *Dunnaliela tertiolecta* isolate CRYO-TEM 14.

Cultivation of the algae

Cultures of *D. tertiolecta* CCAP 19/22 from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) were grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)[17]. Cultures were grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m^2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Isolation of nanoparticles (NPs)

NPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

Cryogenic Electron Microscopy (Cryo-TEM)

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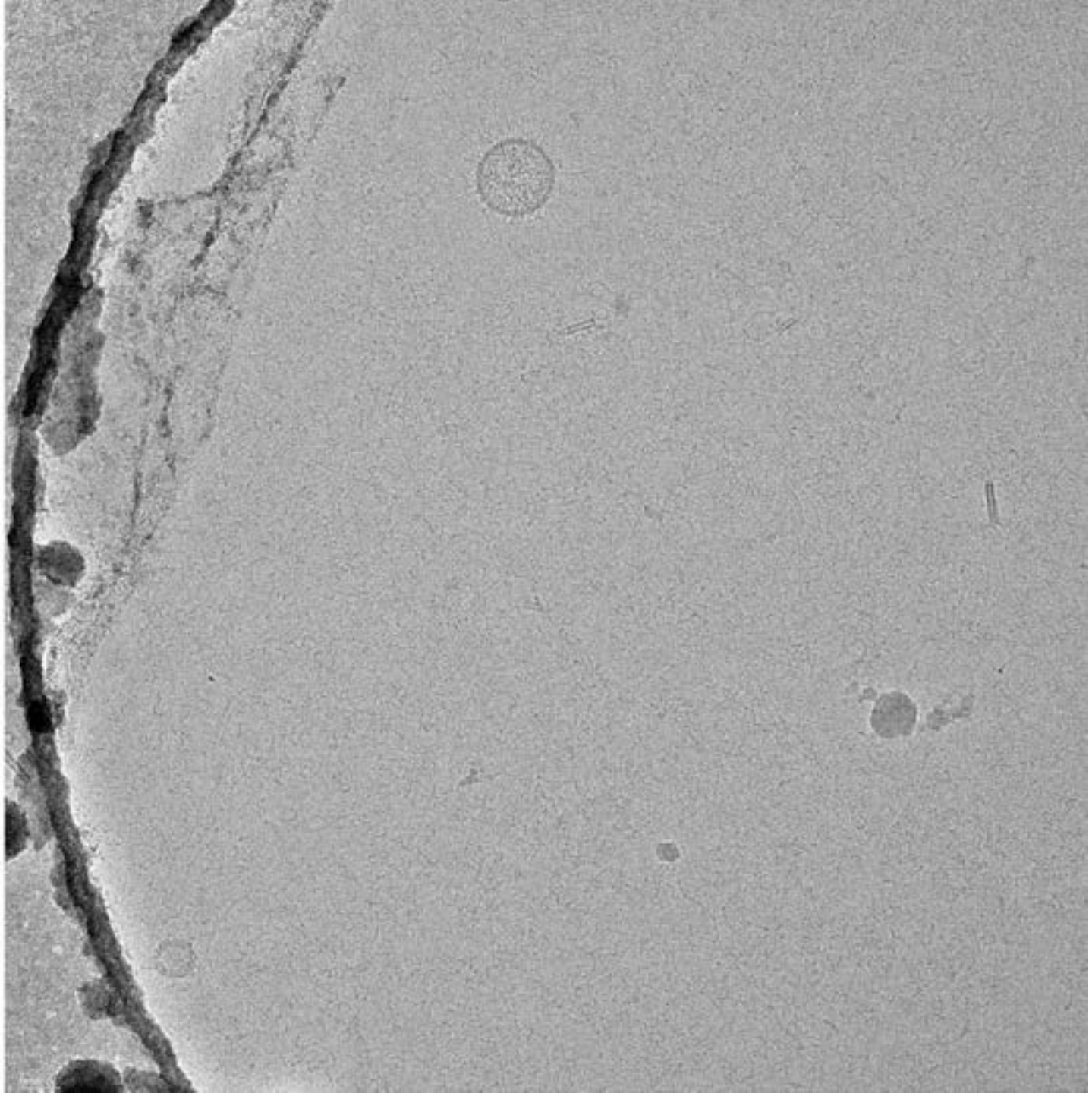

Figure *Dunnaliela tertiolecta* isolate CRYO-TEM 15.

Cultivation of the algae

Cultures of *D. tertiolecta* CCAP 19/22 from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) were grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)[17]. Cultures were grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m^2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Isolation of nanoparticles (NPs)

NPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

Cryogenic Electron Microscopy (Cryo-TEM)

Samples of NPs isolates were prepared for Cryo-TEM using Vitrobot Mark IV (Thermo Fisher Scientific, Waltham, MA, US). Quantifoil® R 2/2 (or 1.2/1.3), 200 mesh holey carbon grids (Quantifoil Micro Tools GmbH, Großlöbichau, Germany) were glow discharged for 60 s at 20 mA and positive polarity in the air atmosphere (GloQube® Plus, Quorum, Laughton, UK). Conditions were set at 4 °C, 95% relative humidity, blot time: 5 s, and blot force: 4. 2 μL of the sample with nanoparticles in suspension was applied to the grid, blotted, and vitrified in liquid ethane. Samples were visualized under cryogenic conditions using a 200 kV microscope Glacios with Falcon 3EC detector (Thermo Fisher Scientific, Waltham, MA, US).

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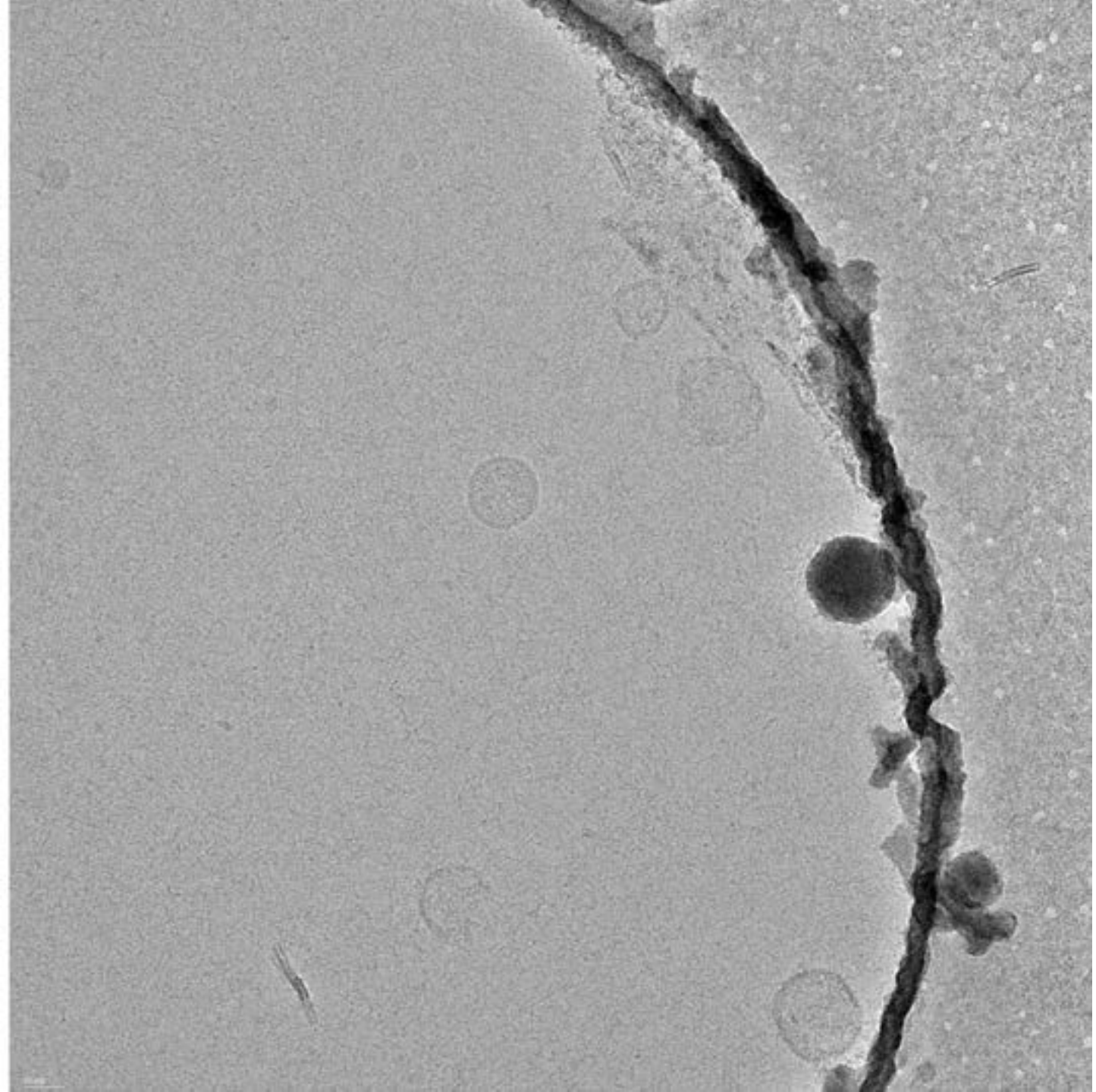

Figure *Dunnaliela tertiolecta* isolate CRYO-TEM 16.

Cultivation of the algae

Cultures of *D. tertiolecta* CCAP 19/22 from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) were grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)[17]. Cultures were grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m^2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Isolation of nanoparticles (NPs)

NPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

Cryogenic Electron Microscopy (Cryo-TEM)

Samples of NPs isolates were prepared for Cryo-TEM using Vitrobot Mark IV (Thermo Fisher Scientific, Waltham, MA, US). Quantifoil® R 2/2 (or 1.2/1.3), 200 mesh holey carbon grids (Quantifoil Micro Tools GmbH, Großlöbichau, Germany) were glow discharged for 60 s at 20 mA and positive polarity in the air atmosphere (GloQube® Plus, Quorum, Laughton, UK). Conditions were set at 4 °C, 95% relative humidity, blot time: 5 s, and blot force: 4. 2 μL of the sample with nanoparticles in suspension was applied to the grid, blotted, and vitrified in liquid ethane. Samples were visualized under cryogenic conditions using a 200 kV microscope Glacios with Falcon 3EC detector (Thermo Fisher Scientific, Waltham, MA, US).

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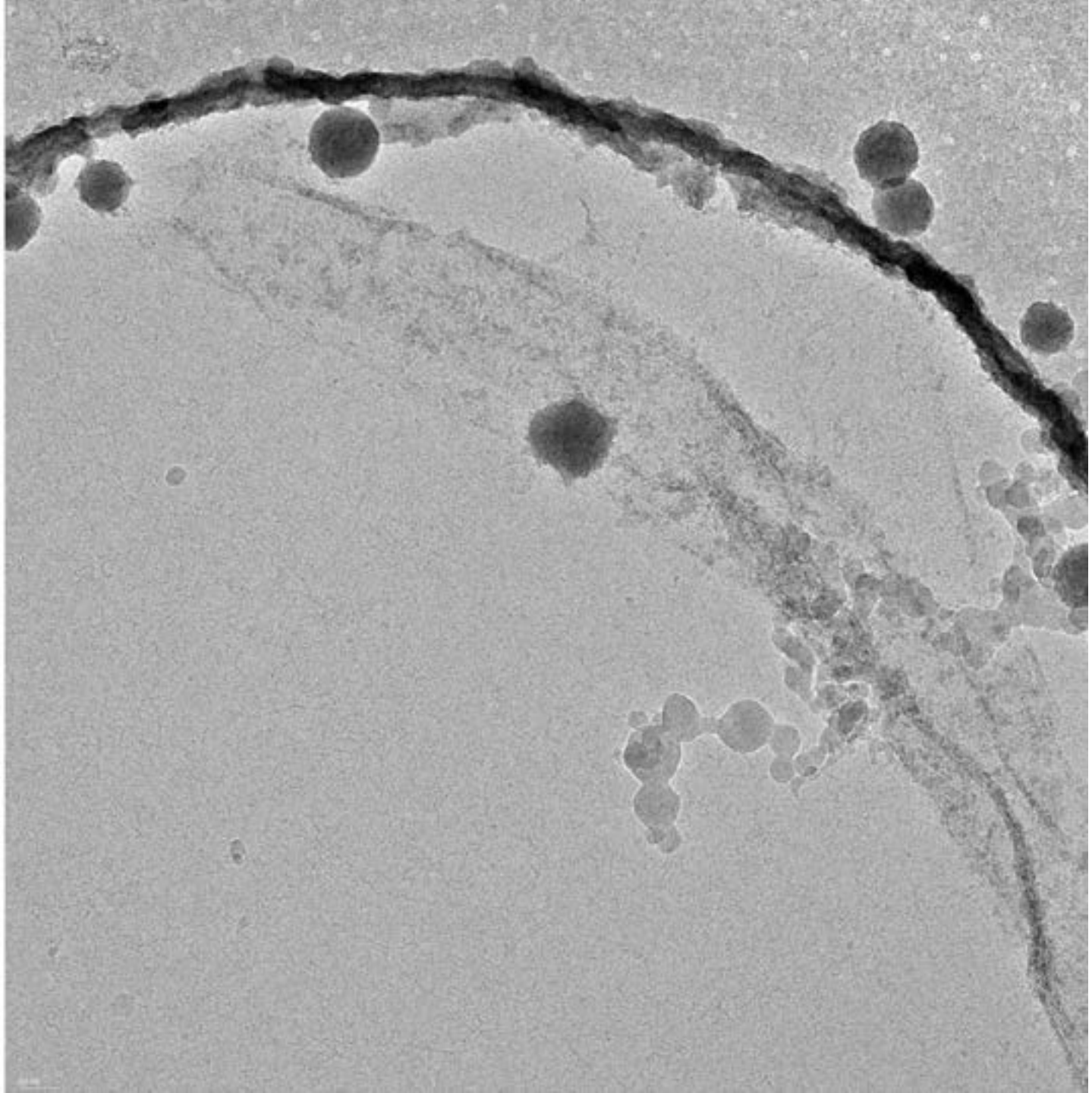

Figure *Dunnaliela tertiolecta* isolate CRYO-TEM 17.

Cultivation of the algae

Cultures of *D. tertiolecta* CCAP 19/22 from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) were grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)[17]. Cultures were grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m^2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Isolation of nanoparticles (NPs)

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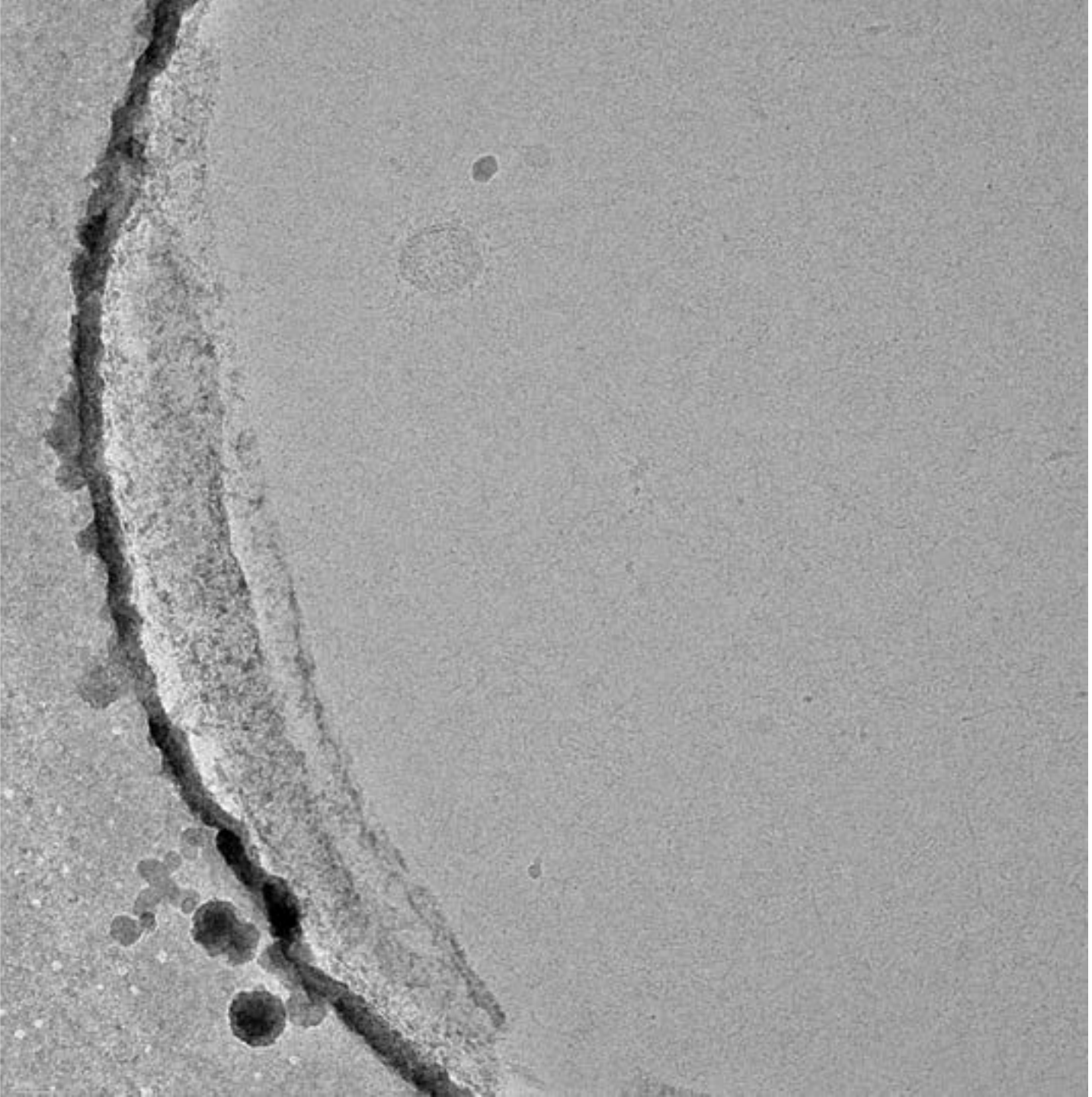

Figure *Dunnaliela tertiolecta* isolate CRYO-TEM 18.

Cultivation of the algae

Cultures of *D. tertiolecta* CCAP 19/22 from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) were grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)[17]. Cultures were grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m^2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Isolation of nanoparticles (NPs)

NPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA)) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

Cryogenic Electron Microscopy (Cryo-TEM)

Samples of NPs isolates were prepared for Cryo-TEM using Vitrobot Mark IV (Thermo Fisher Scientific, Waltham, MA, US). Quantifoil® R 2/2 (or 1.2/1.3), 200 mesh holey carbon grids (Quantifoil Micro Tools GmbH, Großlöbichau, Germany) were glow discharged for 60 s at 20 mA and positive polarity in the air atmosphere (GloQube® Plus, Quorum, Laughton, UK). Conditions were set at 4 °C, 95% relative humidity, blot time: 5 s, and blot force: 4. 2 μL of the sample with nanoparticles in suspension was applied to the grid, blotted, and vitrified in liquid ethane. Samples were visualized under cryogenic conditions using a 200 kV microscope Glacios with Falcon 3EC detector (Thermo Fisher Scientific, Waltham, MA, US).

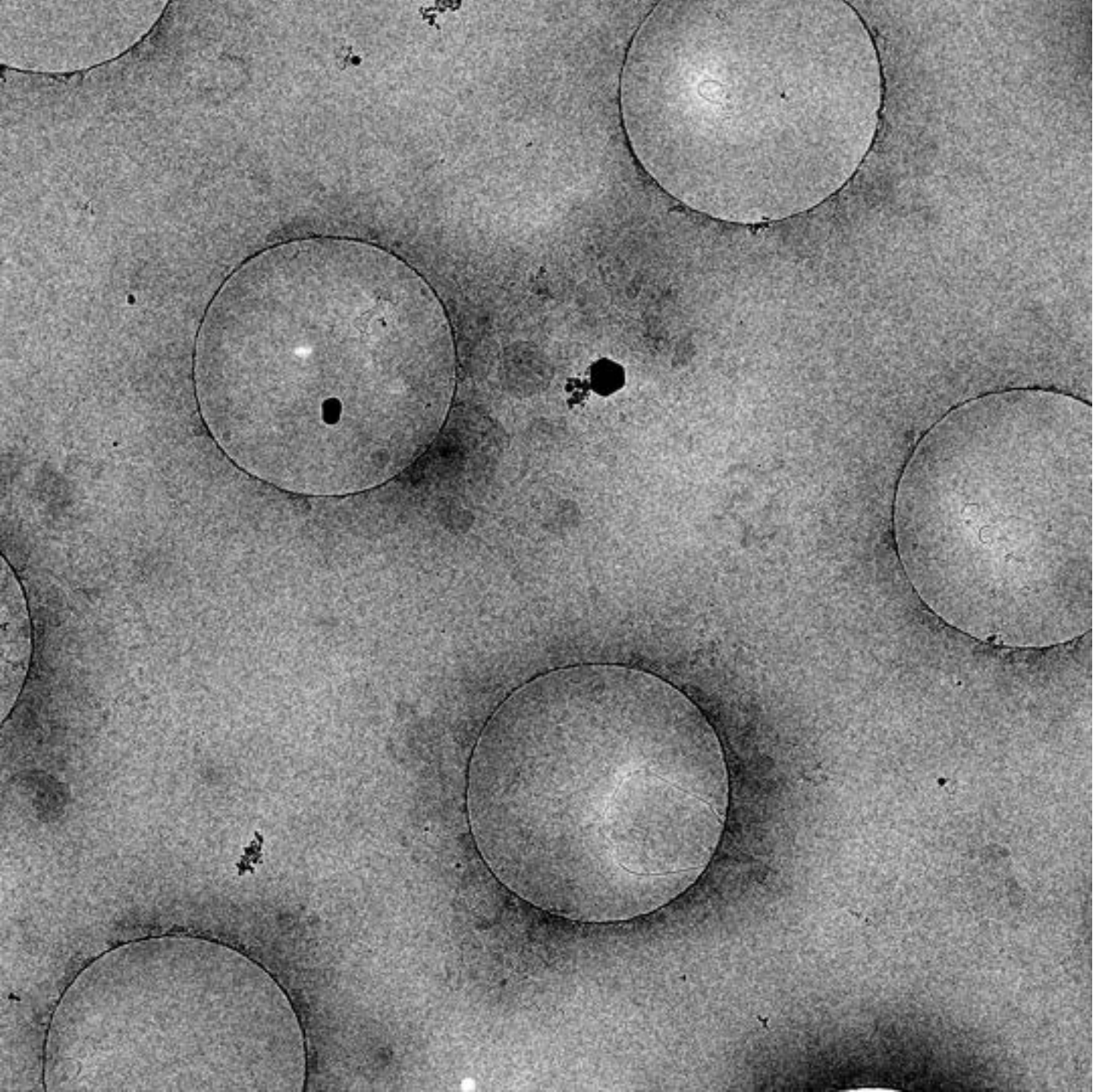

Figure *Dunaliela tertiolecta* isolate SDS CRYO-TEM 1.

Cultivation of the algae

Cultures of *D. tertiolecta* CCAP 19/22 from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) were grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)[17]. Cultures were grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m^2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Treatment of cells

Cells (taken in the exponential phase of growth) were centrifuged at 100g and 22°C for 10 minutes and suspended in solution of marine salt (22 g/L) to reach the concentration cca 10^6 cells/mL. The cells were treated with detergent sodium dodecylsulfate (SDS) at 1:50 proportion with respect to the volume of cell suspension for 1 hour.

Removal of cells

The cells from algal culture were centrifuged at 300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia) using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); then at 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice.

Isolation of nanoparticles (NPs)

The cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using 5-mL thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 50 000 g and 4°C, for 70 min in 5-mL tubes in the same type of ultracentrifuge and ultracentrifuge tubes.

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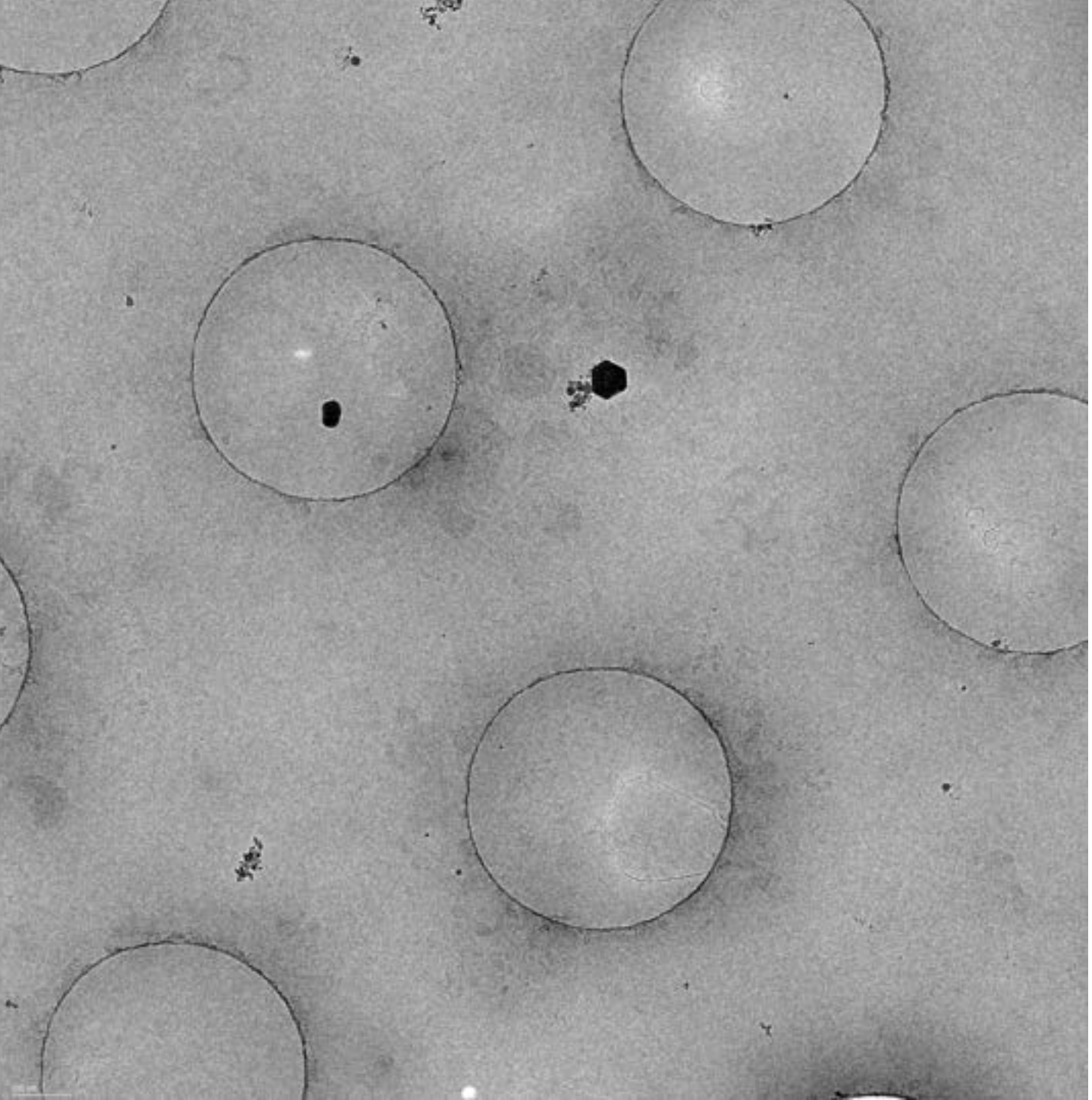

Figure *Dunaliela tertiolecta* isolate SDS CRYO-TEM 2.

Cultivation of the algae

Cultures of *D. tertiolecta* CCAP 19/22 from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) were grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)[17]. Cultures were grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m^2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

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Isolation of nanoparticles (NPs)

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Cryogenic Electron Microscopy (Cryo-TEM)

Samples of NPs isolates were prepared for Cryo-TEM using Vitrobot Mark IV (Thermo Fisher Scientific, Waltham, MA, US). Quantifoil® R 2/2 (or 1.2/1.3), 200 mesh holey carbon grids (Quantifoil Micro Tools GmbH, Großlöbichau, Germany) were glow discharged for 60 s at 20 mA and positive polarity in the air atmosphere (GloQube® Plus, Quorum, Laughton, UK). Conditions were set at 4 °C, 95% relative humidity, blot time: 5 s, and blot force: 4. 2 μL of the sample with nanoparticles in suspension was applied to the grid, blotted, and vitrified in liquid ethane. Samples were visualized under cryogenic conditions using a 200 kV microscope Glacios with Falcon 3EC detector (Thermo Fisher Scientific, Waltham, MA, US).

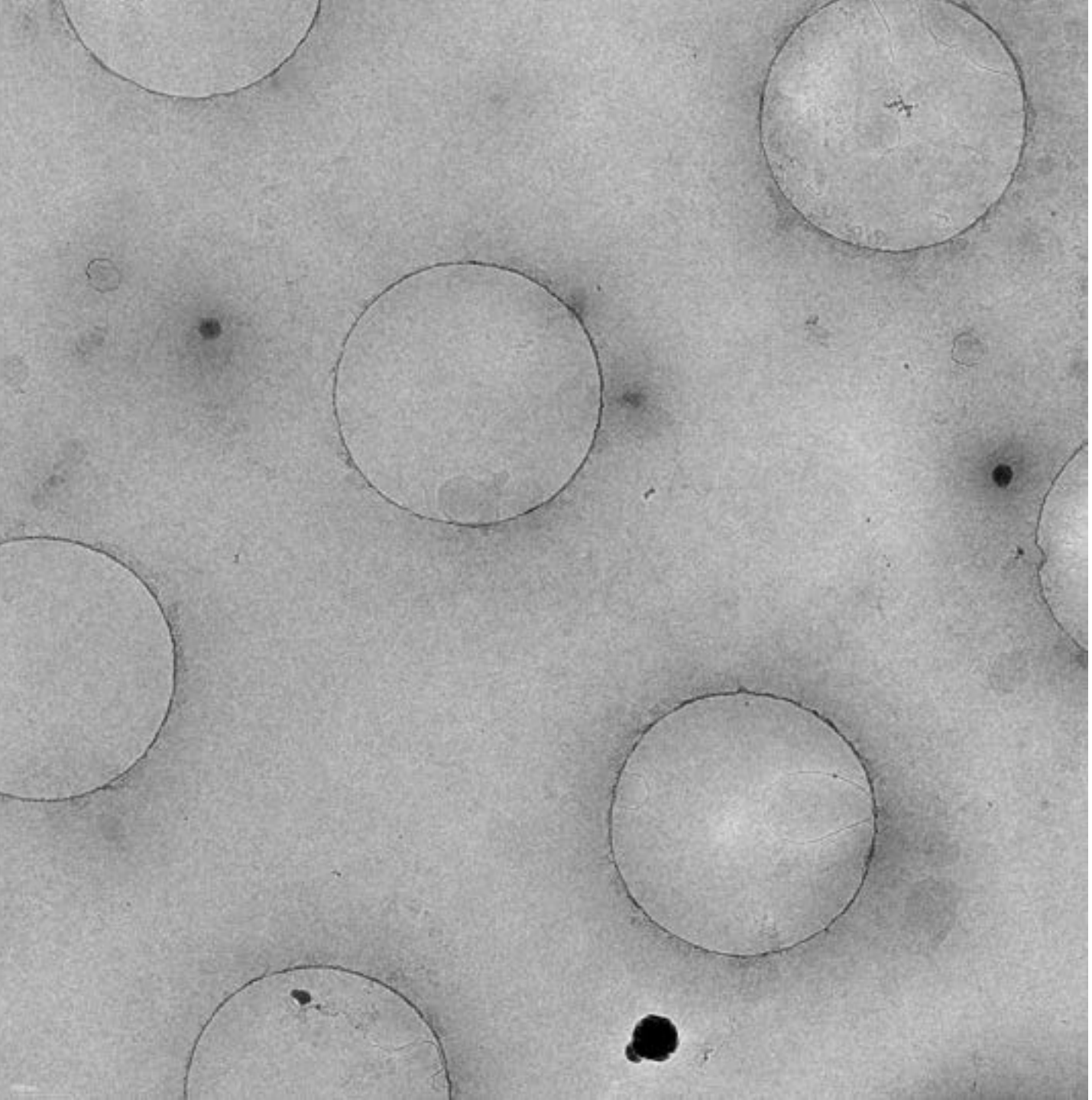

Figure *Dunaliela tertiolecta* isolate SDS CRYO-TEM 3.

Cultivation of the algae

Cultures of *D. tertiolecta* CCAP 19/22 from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) were grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)[17]. Cultures were grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m^{2}s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Treatment of cells

Cells (taken in the exponential phase of growth) were centrifuged at 100g and 22°C for 10 minutes and suspended in solution of marine salt (22 g/L) to reach the concentration cca 10^6 cells/mL. The cells were treated with detergent sodium dodecylsulfate (SDS) at 1:50 proportion with respect to the volume of cell suspension for 1 hour.

Removal of cells

The cells from algal culture were centrifuged at 300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia) using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); then at 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice.

Isolation of nanoparticles (NPs)

The cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using 5-mL thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 50 000 g and 4°C, for 70 min in 5-mL tubes in the same type of ultracentrifuge and ultracentrifuge tubes.

Cryogenic Electron Microscopy (Cryo-TEM)

Samples of NPs isolates were prepared for Cryo-TEM using Vitrobot Mark IV (Thermo Fisher Scientific, Waltham, MA, US). Quantifoil® R 2/2 (or 1.2/1.3), 200 mesh holey carbon grids (Quantifoil Micro Tools GmbH, Großlöbichau, Germany) were glow discharged for 60 s at 20 mA and positive polarity in the air atmosphere (GloQube® Plus, Quorum, Laughton, UK). Conditions were set at 4 °C, 95% relative humidity, blot time: 5 s, and blot force: 4. 2 μL of the sample with nanoparticles in suspension was applied to the grid, blotted, and vitrified in liquid ethane. Samples were visualized under cryogenic conditions using a 200 kV microscope Glacios with Falcon 3EC detector (Thermo Fisher Scientific, Waltham, MA, US).

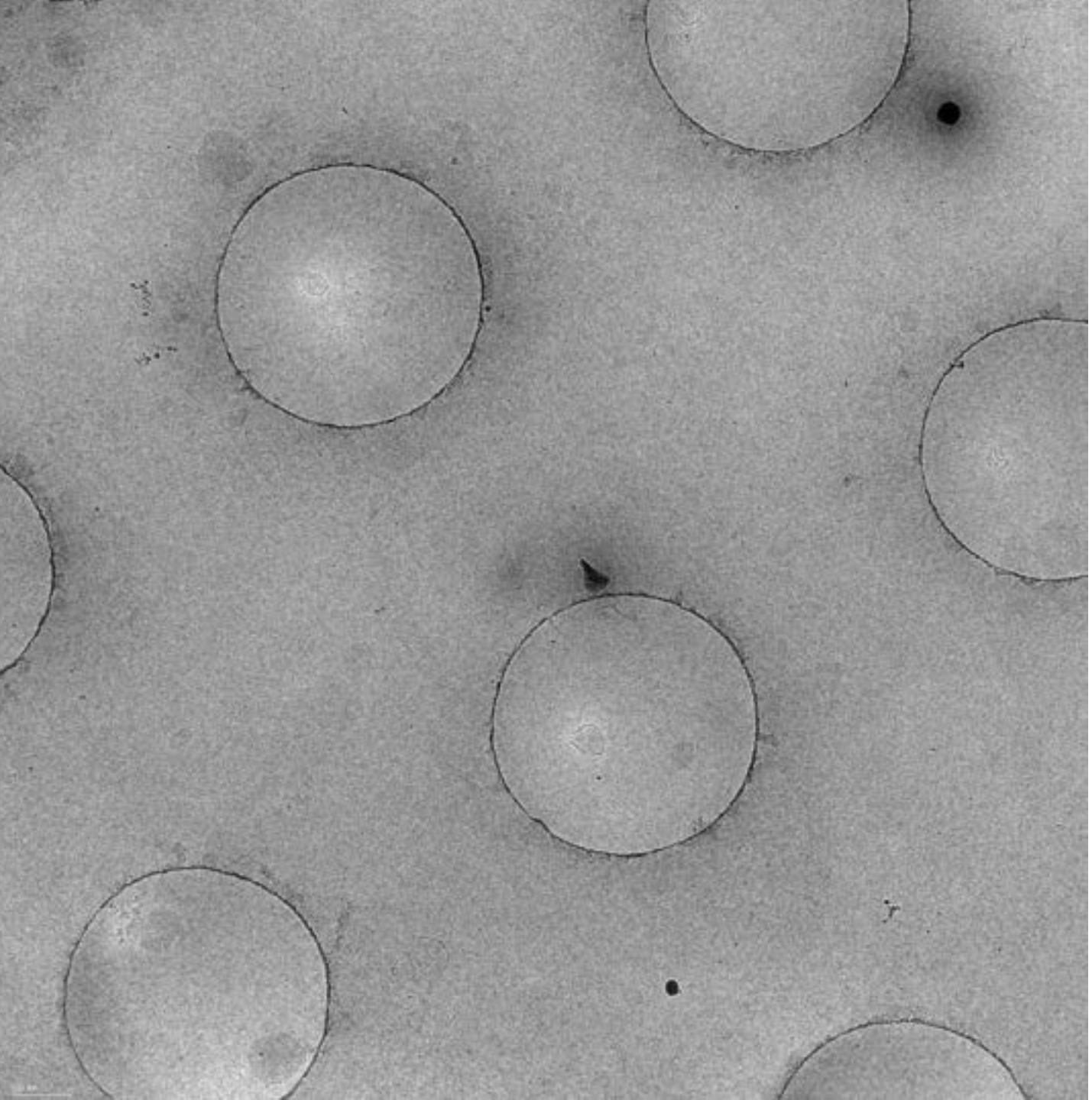

Figure *Dunaliella tertiolecta* isolate SDS CRYO-TEM 4.

Cultivation of the algae

Cultures of *D. tertiolecta* CCAP 19/22 from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) were grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)[17]. Cultures were grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m^2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Treatment of cells

Cells (taken in the exponential phase of growth) were centrifuged at 100g and 22°C for 10 minutes and suspended in solution of marine salt (22 g/L) to reach the concentration cca 10^6 cells/mL. The cells were treated with detergent sodium dodecylsulfate (SDS) at 1:50 proportion with respect to the volume of cell suspension for 1 hour.

Removal of cells

The cells from algal culture were centrifuged at 300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia) using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); then at 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice.

Isolation of nanoparticles (NPs)

The cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using 5-mL thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 50 000 g and 4°C, for 70 min in 5-mL tubes in the same type of ultracentrifuge and ultracentrifuge tubes.

Cryogenic Electron Microscopy (Cryo-TEM)

Samples of NPs isolates were prepared for Cryo-TEM using Vitrobot Mark IV (Thermo Fisher Scientific, Waltham, MA, US). Quantifoil® R 2/2 (or 1.2/1.3), 200 mesh holey carbon grids (Quantifoil Micro Tools GmbH, Großlöbichau, Germany) were glow discharged for 60 s at 20 mA and positive polarity in the air atmosphere (GloQube® Plus, Quorum, Laughton, UK). Conditions were set at 4 °C, 95% relative humidity, blot time: 5 s, and blot force: 4. 2 μL of the sample with nanoparticles in suspension was applied to the grid, blotted, and vitrified in liquid ethane. Samples were visualized under cryogenic conditions using a 200 kV microscope Glacios with Falcon 3EC detector (Thermo Fisher Scientific, Waltham, MA, US).

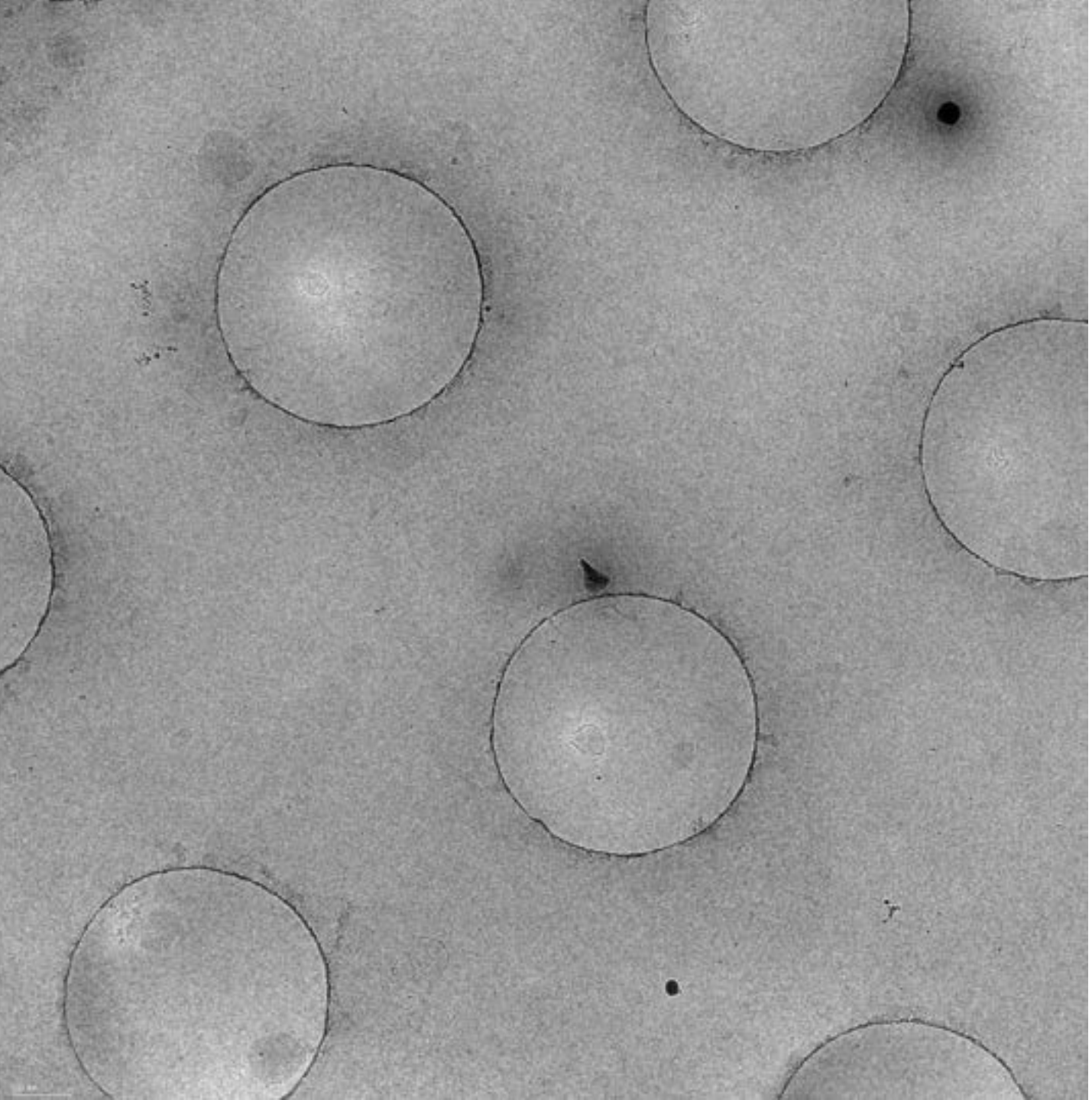

Figure *Dunaliela tertiolecta* isolate SDS CRYO-TEM 5.

Cultivation of the algae

Cultures of *D. tertiolecta* CCAP 19/22 from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) were grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)[17]. Cultures were grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m^2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Treatment of cells

Cells (taken in the exponential phase of growth) were centrifuged at 100g and 22°C for 10 minutes and suspended in solution of marine salt (22 g/L) to reach the concentration cca 10^6 cells/mL. The cells were treated with detergent sodium dodecylsulfate (SDS) at 1:50 proportion with respect to the volume of cell suspension for 1 hour.

Removal of cells

The cells from algal culture were centrifuged at 300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia) using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); then at 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice.

Isolation of nanoparticles (NPs)

The cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using 5-mL thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 50 000 g and 4°C, for 70 min in 5-mL tubes in the same type of ultracentrifuge and ultracentrifuge tubes.

Cryogenic Electron Microscopy (Cryo-TEM)

Samples of NPs isolates were prepared for Cryo-TEM using Vitrobot Mark IV (Thermo Fisher Scientific, Waltham, MA, US). Quantifoil® R 2/2 (or 1.2/1.3), 200 mesh holey carbon grids (Quantifoil Micro Tools GmbH, Großlöbichau, Germany) were glow discharged for 60 s at 20 mA and positive polarity in the air atmosphere (GloQube® Plus, Quorum, Laughton, UK). Conditions were set at 4 °C, 95% relative humidity, blot time: 5 s, and blot force: 4. 2 μL of the sample with nanoparticles in suspension was applied to the grid, blotted, and vitrified in liquid ethane. Samples were visualized under cryogenic conditions using a 200 kV microscope Glacios with Falcon 3EC detector (Thermo Fisher Scientific, Waltham, MA, US).

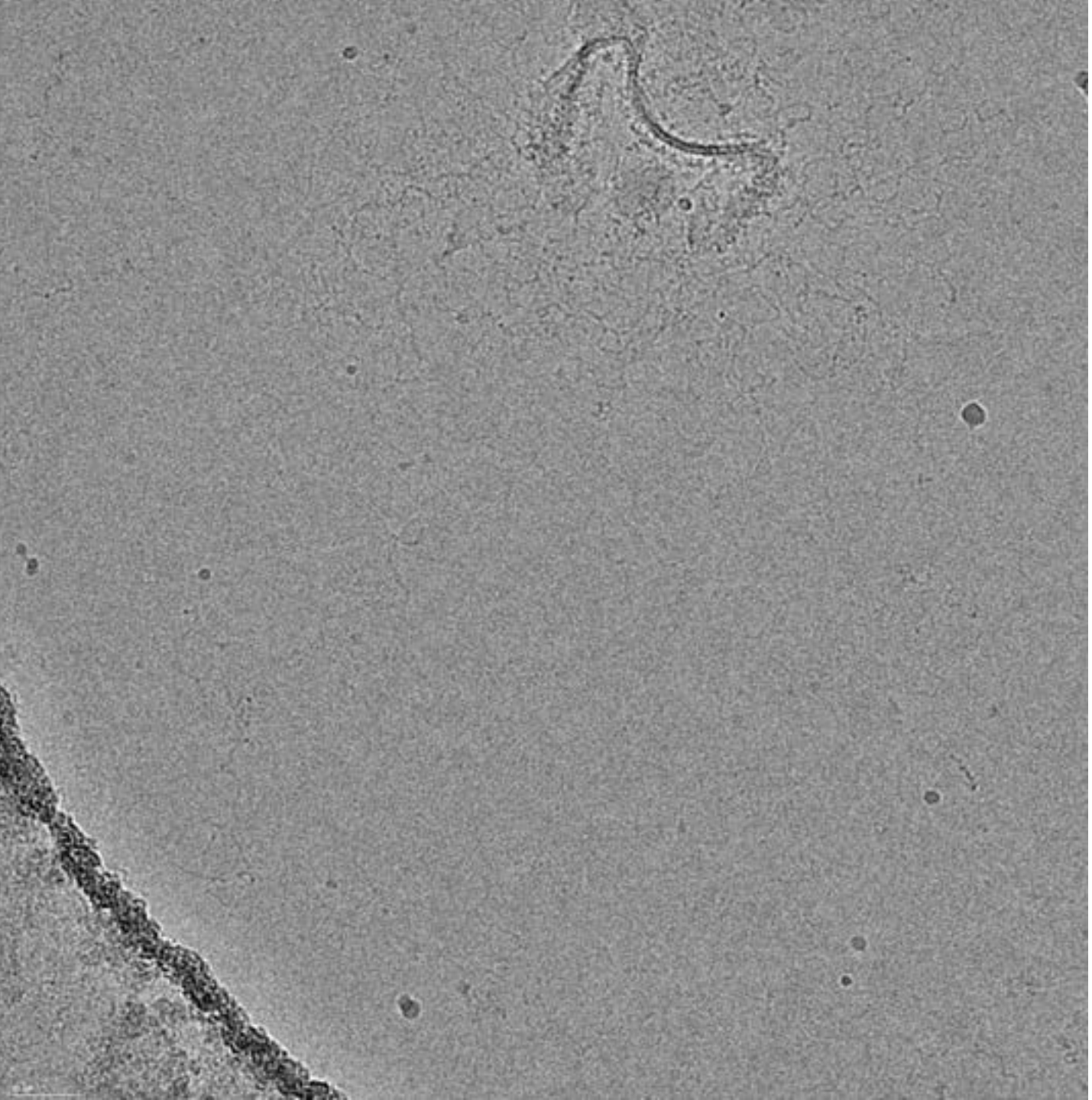

Figure *Dunaliela tertiolecta* isolate SDS CRYO-TEM 6.

Cultivation of the algae

Cultures of *D. tertiolecta* CCAP 19/22 from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) were grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)[17]. Cultures were grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m^2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Treatment of cells

Cells (taken in the exponential phase of growth) were centrifuged at 100g and 22°C for 10 minutes and suspended in solution of marine salt (22 g/L) to reach the concentration cca 10^6 cells/mL. The cells were treated with detergent sodium dodecylsulfate (SDS) at 1:50 proportion with respect to the volume of cell suspension for 1 hour.

Removal of cells

The cells from algal culture were centrifuged at 300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia) using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); then at 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice.

Isolation of nanoparticles (NPs)

The cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using 5-mL thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 50 000 g and 4°C, for 70 min in 5-mL tubes in the same type of ultracentrifuge and ultracentrifuge tubes.

Cryogenic Electron Microscopy (Cryo-TEM)

Samples of NPs isolates were prepared for Cryo-TEM using Vitrobot Mark IV (Thermo Fisher Scientific, Waltham, MA, US). Quantifoil® R 2/2 (or 1.2/1.3), 200 mesh holey carbon grids (Quantifoil Micro Tools GmbH, Großlöbichau, Germany) were glow discharged for 60 s at 20 mA and positive polarity in the air atmosphere (GloQube® Plus, Quorum, Laughton, UK). Conditions were set at 4 °C, 95% relative humidity, blot time: 5 s, and blot force: 4. 2 μL of the sample with nanoparticles in suspension was applied to the grid, blotted, and vitrified in liquid ethane. Samples were visualized under cryogenic conditions using a 200 kV microscope Glacios with Falcon 3EC detector (Thermo Fisher Scientific, Waltham, MA, US).

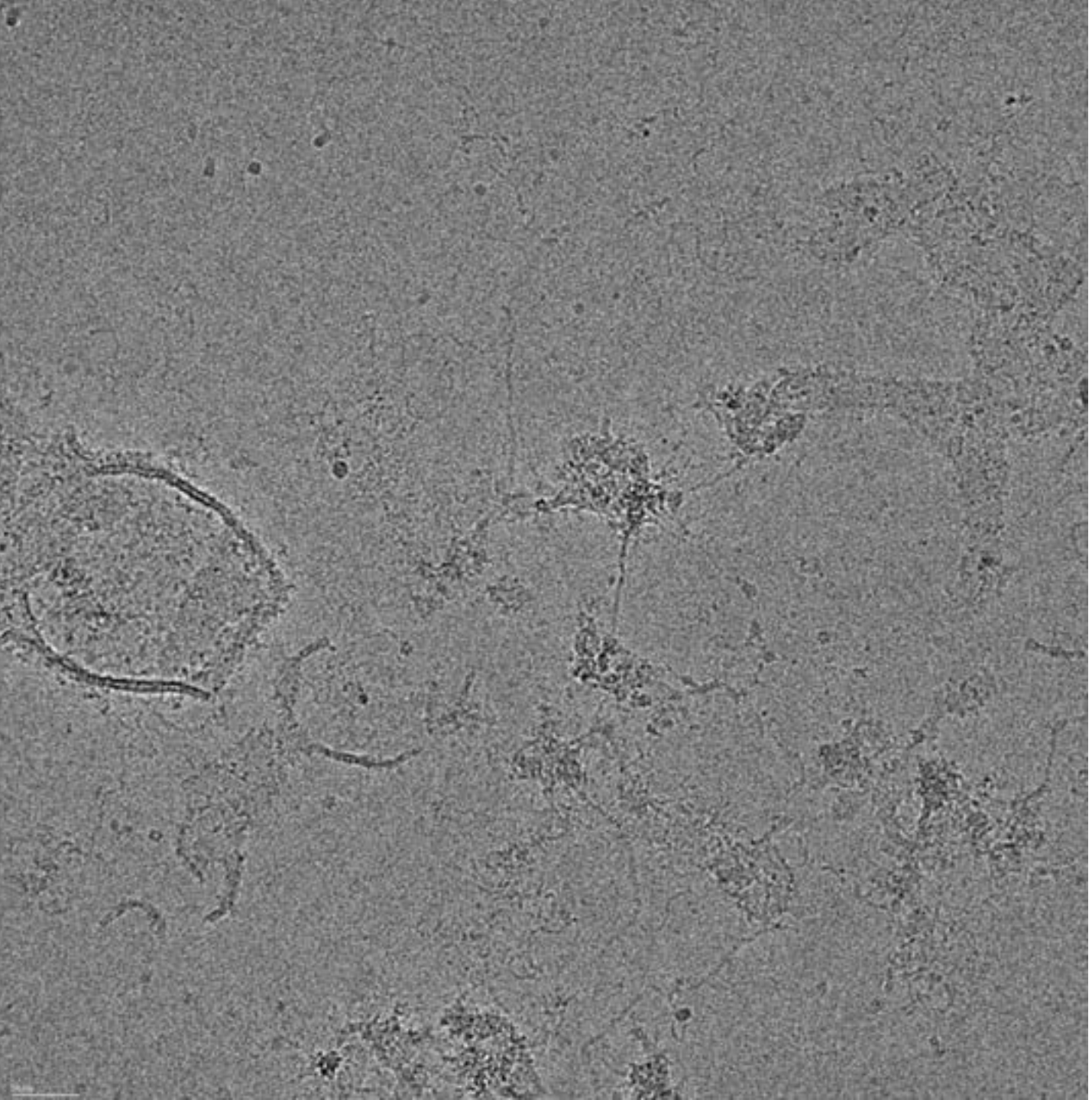

Figure *Dunaliela tertiolecta* isolate SDS CRYO-TEM 7.

Cultivation of the algae

Cultures of *D. tertiolecta* CCAP 19/22 from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) were grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)[17]. Cultures were grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 $\mu mol/m^2s$) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Treatment of cells

Cells (taken in the exponential phase of growth) were centrifuged at 100g and 22°C for 10 minutes and suspended in solution of marine salt (22 g/L) to reach the concentration cca 10^6 cells/mL. The cells were treated with detergent sodium dodecylsulfate (SDS) at 1:50 proportion with respect to the volume of cell suspension for 1 hour.

Removal of cells

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Isolation of nanoparticles (NPs)

The cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using 5-mL thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 50 000 g and 4°C, for 70 min in 5-mL tubes in the same type of ultracentrifuge and ultracentrifuge tubes.

Cryogenic Electron Microscopy (Cryo-TEM)

Samples of NPs isolates were prepared for Cryo-TEM using Vitrobot Mark IV (Thermo Fisher Scientific, Waltham, MA, US). Quantifoil® R 2/2 (or 1.2/1.3), 200 mesh holey carbon grids (Quantifoil Micro Tools GmbH, Großlöbichau, Germany) were glow discharged for 60 s at 20 mA and positive polarity in the air atmosphere (GloQube® Plus, Quorum, Laughton, UK). Conditions were set at 4 °C, 95% relative humidity, blot time: 5 s, and blot force: 4. 2 μL of the sample with nanoparticles in suspension was applied to the grid, blotted, and vitrified in liquid ethane. Samples were visualized under cryogenic conditions using a 200 kV microscope Glacios with Falcon 3EC detector (Thermo Fisher Scientific, Waltham, MA, US).

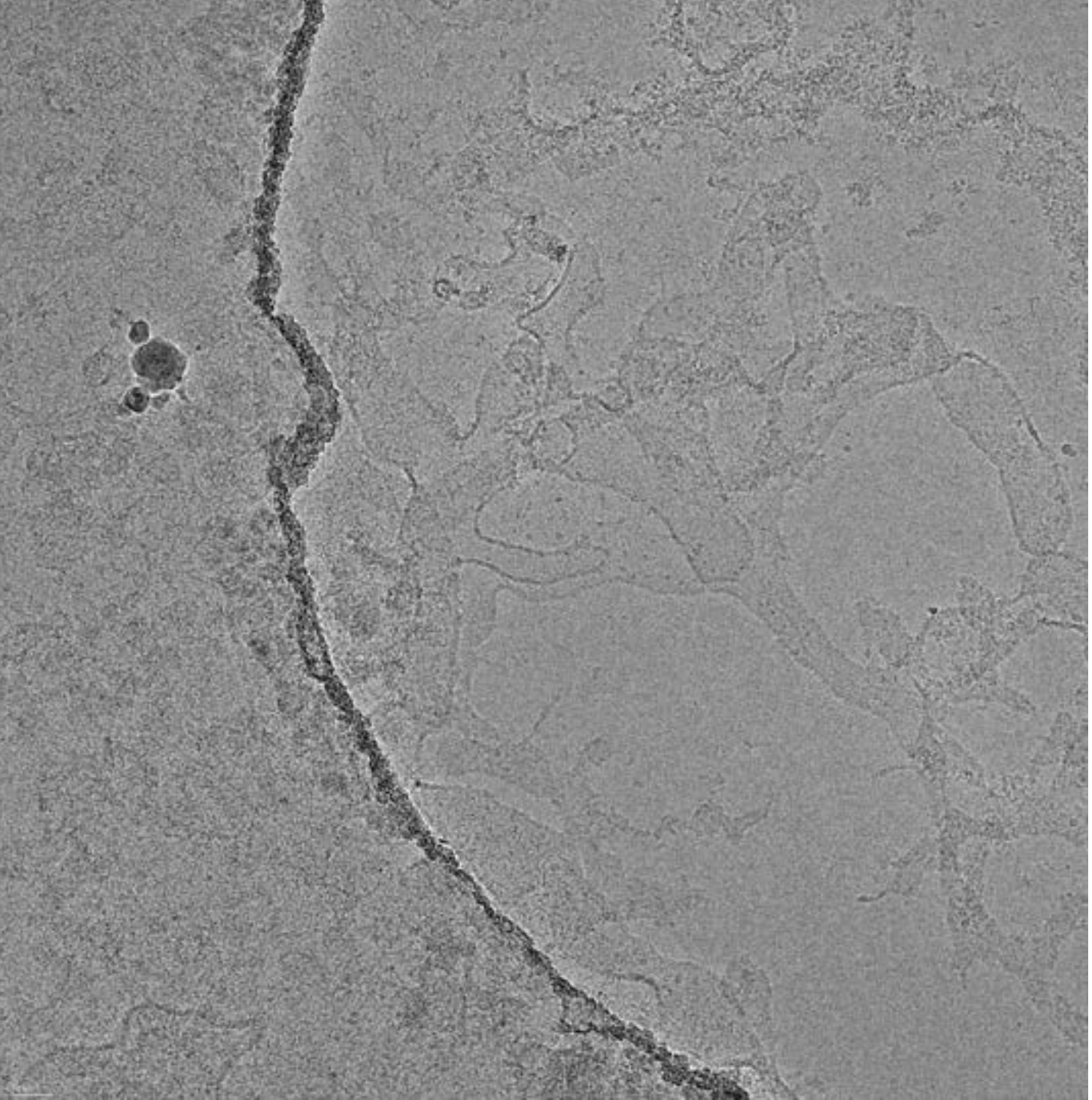

Figure *Dunaliela tertiolecta* isolate SDS CRYO-TEM 8.

Cultivation of the algae

Cultures of *D. tertiolecta* CCAP 19/22 from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) were grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)[17]. Cultures were grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m^2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Treatment of cells

Cells (taken in the exponential phase of growth) were centrifuged at 100g and 22°C for 10 minutes and suspended in solution of marine salt (22 g/L) to reach the concentration cca 10^6 cells/mL. The cells were treated with detergent sodium dodecylsulfate (SDS) at 1:50 proportion with respect to the volume of cell suspension for 1 hour.

Removal of cells

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Isolation of nanoparticles (NPs)

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Cryogenic Electron Microscopy (Cryo-TEM)

Samples of NPs isolates were prepared for Cryo-TEM using Vitrobot Mark IV (Thermo Fisher Scientific, Waltham, MA, US). Quantifoil® R 2/2 (or 1.2/1.3), 200 mesh holey carbon grids (Quantifoil Micro Tools GmbH, Großlöbichau, Germany) were glow discharged for 60 s at 20 mA and positive polarity in the air atmosphere (GloQube® Plus, Quorum, Laughton, UK). Conditions were set at 4 °C, 95% relative humidity, blot time: 5 s, and blot force: 4. 2 μL of the sample with nanoparticles in suspension was applied to the grid, blotted, and vitrified in liquid ethane. Samples were visualized under cryogenic conditions using a 200 kV microscope Glacios with Falcon 3EC detector (Thermo Fisher Scientific, Waltham, MA, US).

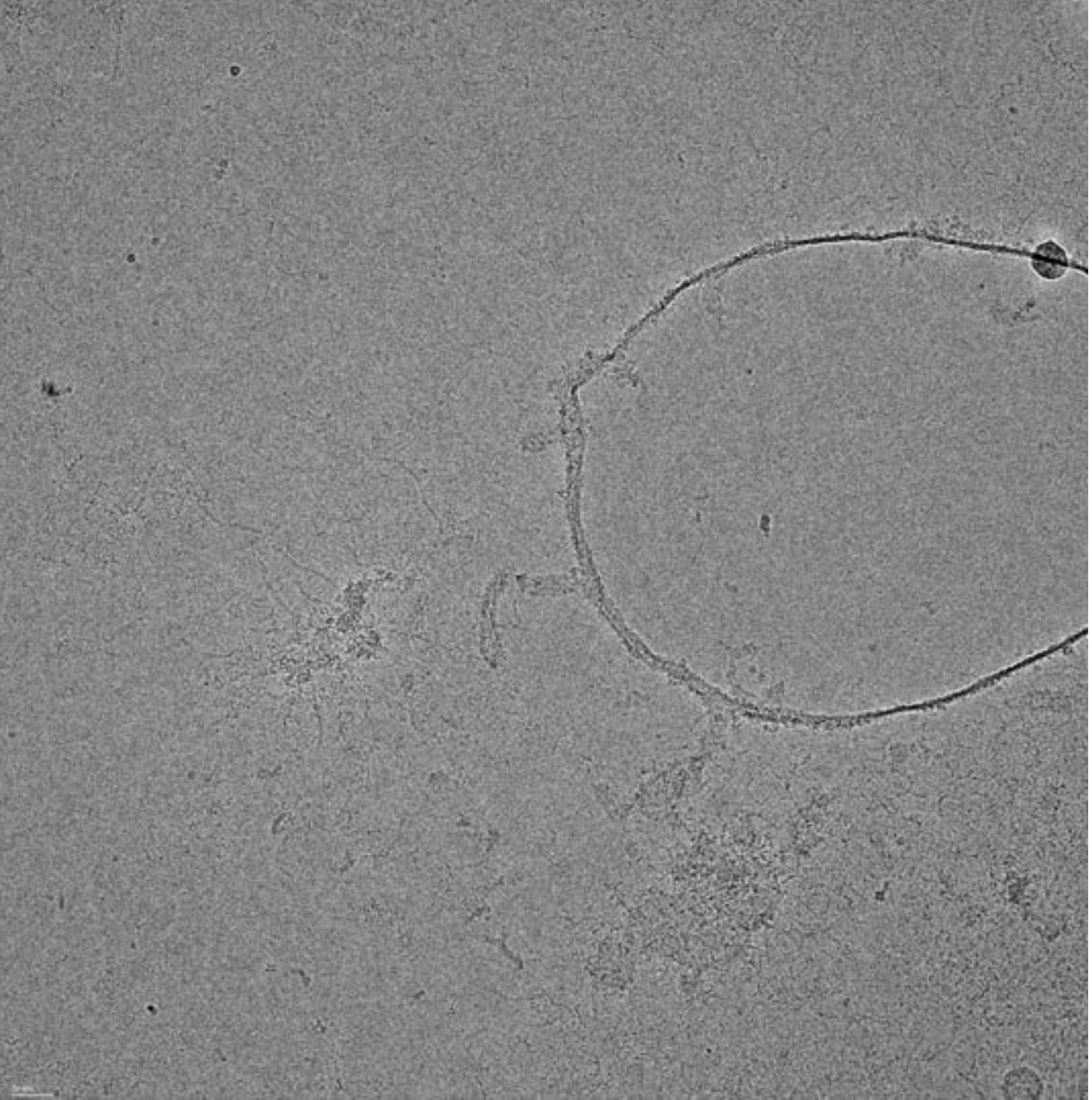

Figure *Dunaliela tertiolecta* isolate SDS CRYO-TEM 9.

Cultivation of the algae

Cultures of *D. tertiolecta* CCAP 19/22 from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) were grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)[17]. Cultures were grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m^2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Treatment of cells

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Isolation of nanoparticles (NPs)

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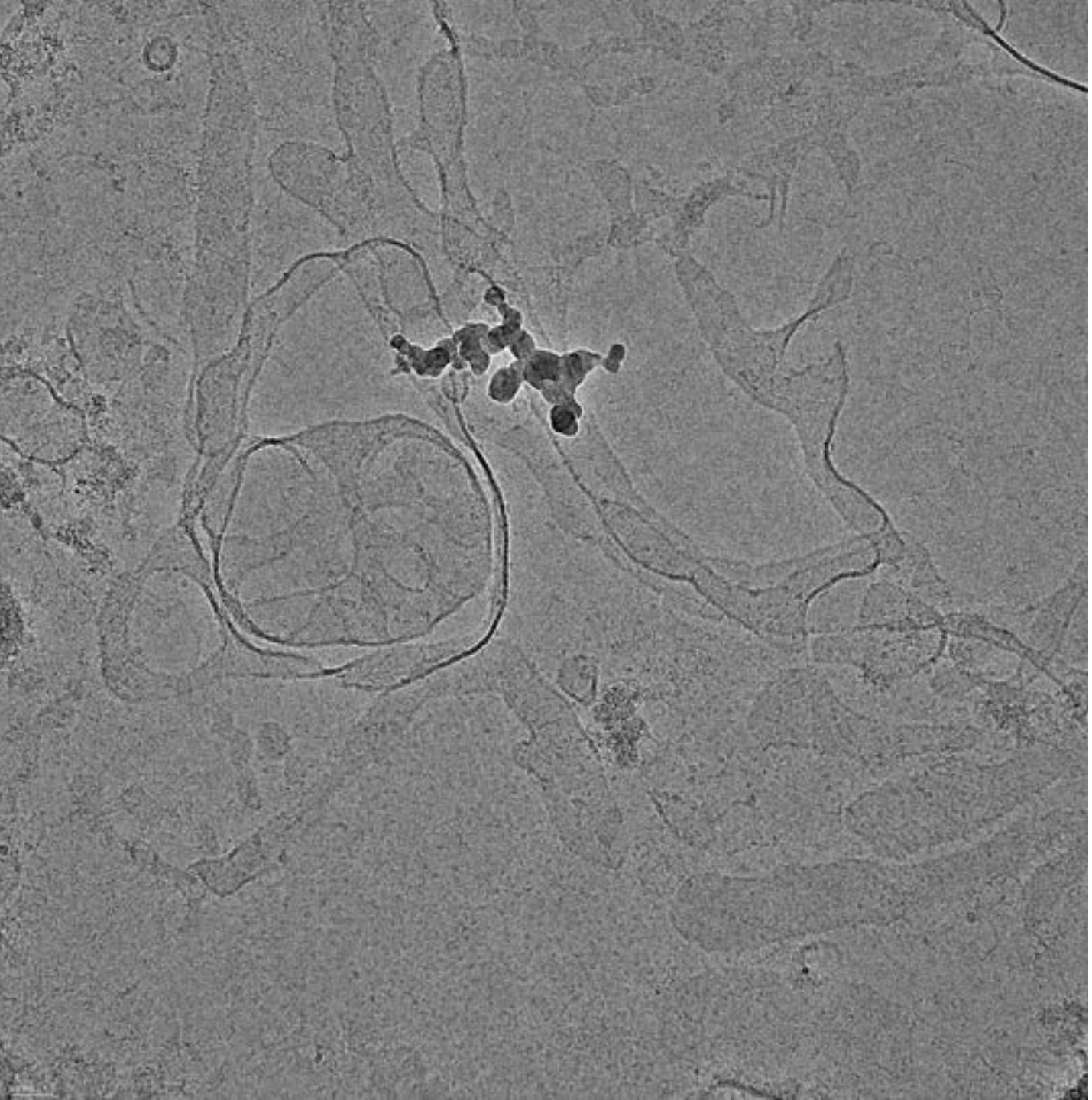

Figure *Dunaliela tertiolecta* isolate SDS CRYO-TEM 10.

Cultivation of the algae

Cultures of *D. tertiolecta* CCAP 19/22 from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) were grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)[17]. Cultures were grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m^2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Treatment of cells

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Isolation of nanoparticles (NPs)

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Cryogenic Electron Microscopy (Cryo-TEM)

Samples of NPs isolates were prepared for Cryo-TEM using Vitrobot Mark IV (Thermo Fisher Scientific, Waltham, MA, US). Quantifoil® R 2/2 (or 1.2/1.3), 200 mesh holey carbon grids (Quantifoil Micro Tools GmbH, Großlöbichau, Germany) were glow discharged for 60 s at 20 mA and positive polarity in the air atmosphere (GloQube® Plus, Quorum, Laughton, UK). Conditions were set at 4 °C, 95% relative humidity, blot time: 5 s, and blot force: 4. 2 μL of the sample with nanoparticles in suspension was applied to the grid, blotted, and vitrified in liquid ethane. Samples were visualized under cryogenic conditions using a 200 kV microscope Glacios with Falcon 3EC detector (Thermo Fisher Scientific, Waltham, MA, US).

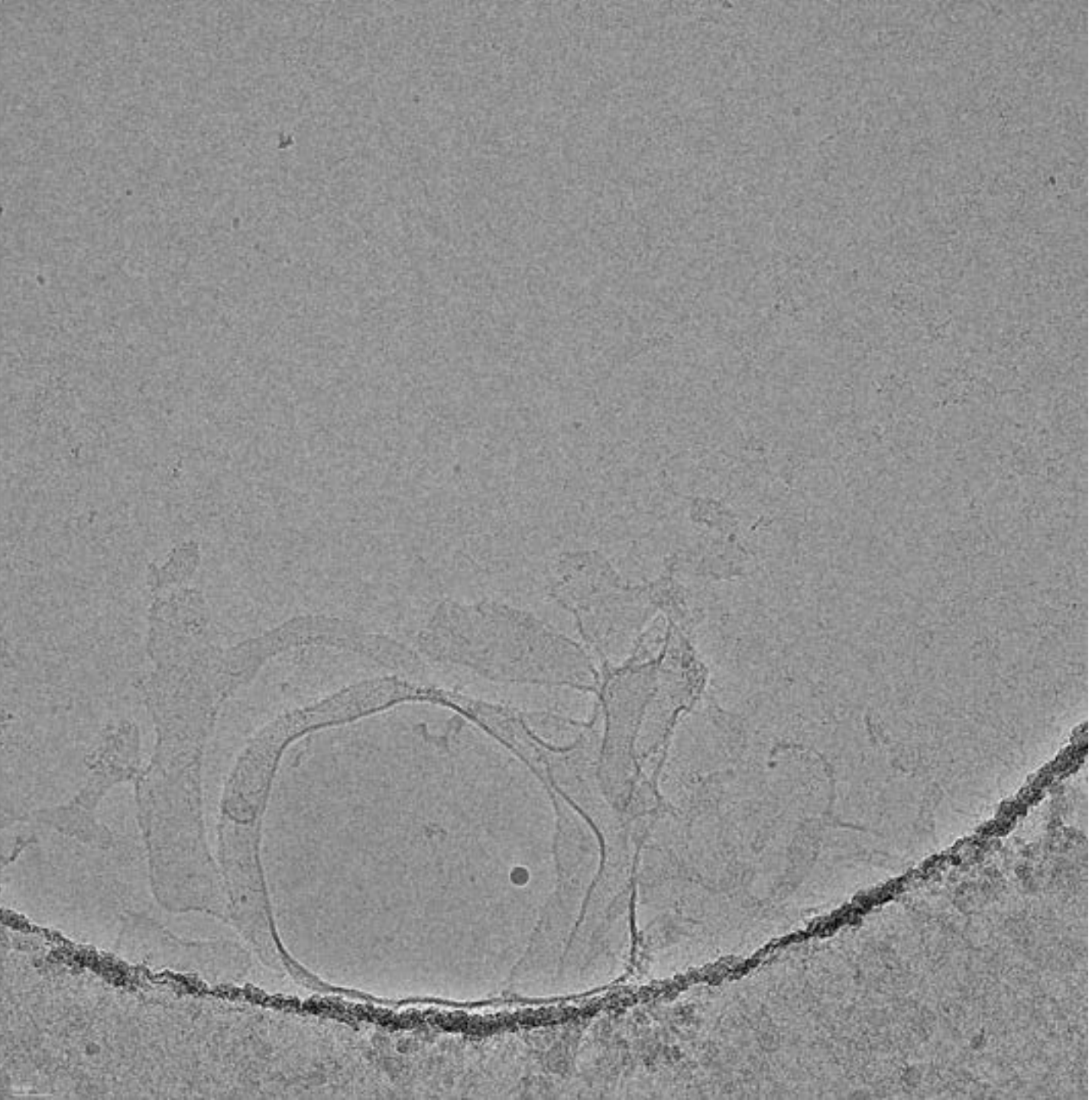

Figure *Dunaliela tertiolecta* isolate SDS CRYO-TEM 11.

Cultivation of the algae

Cultures of *D. tertiolecta* CCAP 19/22 from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) were grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)[17]. Cultures were grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m^2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Treatment of cells

Cells (taken in the exponential phase of growth) were centrifuged at 100g and 22°C for 10 minutes and suspended in solution of marine salt (22 g/L) to reach the concentration cca 10^6 cells/mL. The cells were treated with detergent sodium dodecylsulfate (SDS) at 1:50 proportion with respect to the volume of cell suspension for 1 hour.

Removal of cells

The cells from algal culture were centrifuged at 300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia) using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); then at 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice.

Isolation of nanoparticles (NPs)

The cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using 5-mL thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 50 000 g and 4°C, for 70 min in 5-mL tubes in the same type of ultracentrifuge and ultracentrifuge tubes.

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Figure *Dunaliella tertiolecta* isolate SDS CRYO-TEM 12.

Cultivation of the algae

Cultures of *D. tertiolecta* CCAP 19/22 from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) were grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)[17]. Cultures were grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m^2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

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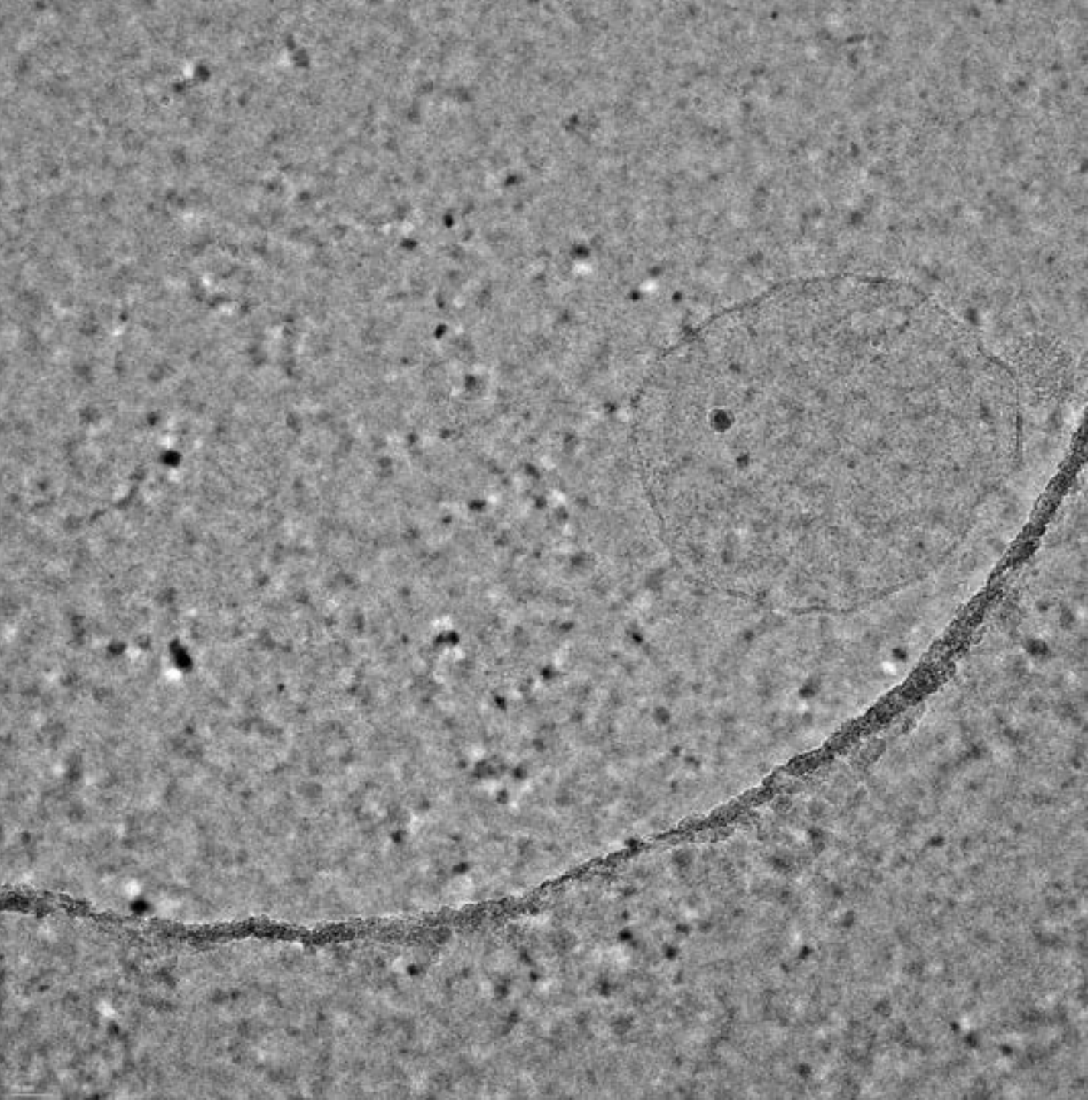

Figure *Dunaliela tertiolecta* isolate SDS CRYO-TEM 13.

Cultivation of the algae

Cultures of *D. tertiolecta* CCAP 19/22 from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) were grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)[17]. Cultures were grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m^2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Treatment of cells

Cells (taken in the exponential phase of growth) were centrifuged at 100g and 22°C for 10 minutes and suspended in solution of marine salt (22 g/L) to reach the concentration cca 10^6 cells/mL. The cells were treated with detergent sodium dodecylsulfate (SDS) at 1:50 proportion with respect to the volume of cell suspension for 1 hour.

Removal of cells

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Isolation of nanoparticles (NPs)

The cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using 5-mL thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 50 000 g and 4°C, for 70 min in 5-mL tubes in the same type of ultracentrifuge and ultracentrifuge tubes.

Cryogenic Electron Microscopy (Cryo-TEM)

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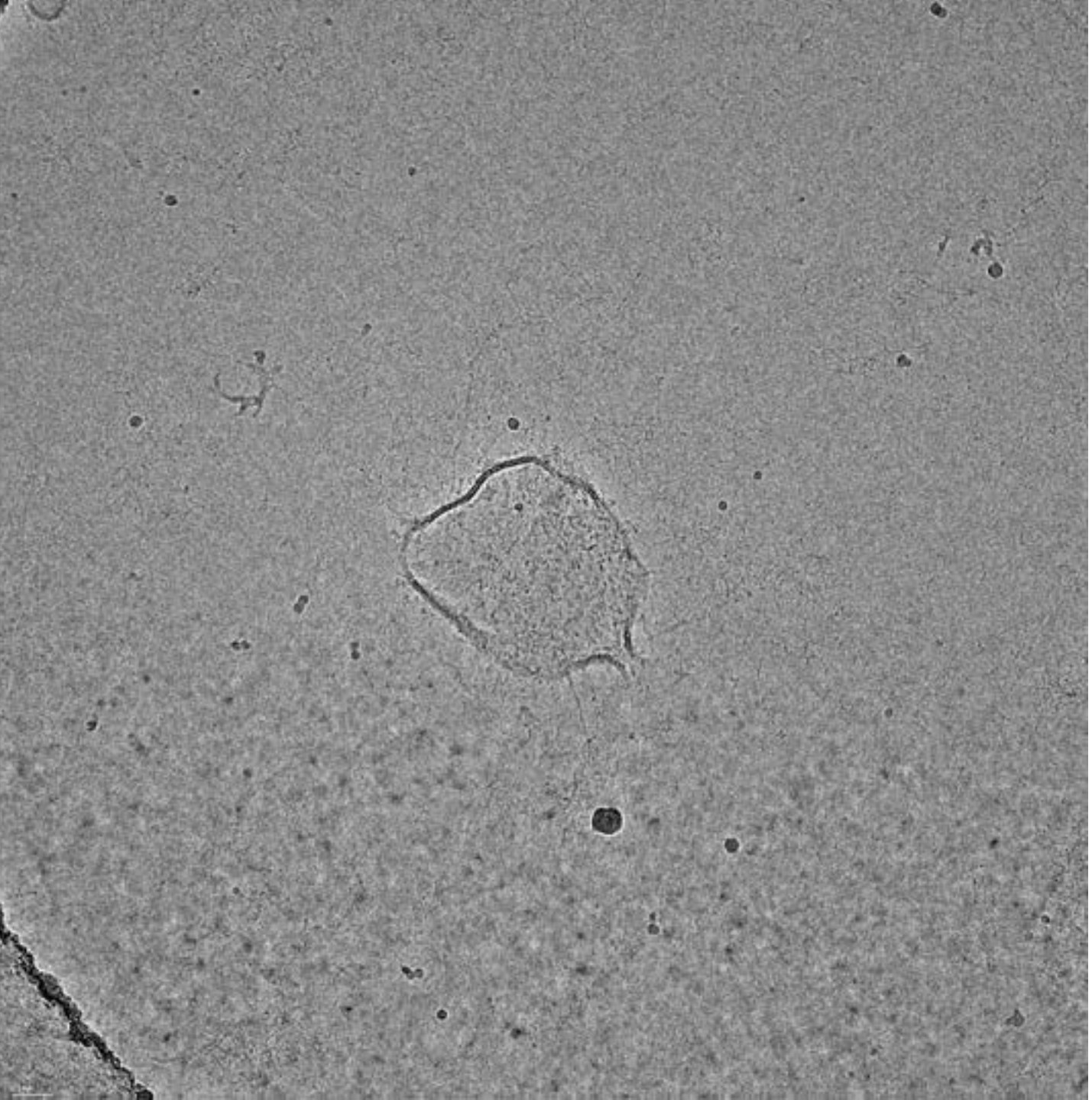

Figure *Dunaliella tertiolecta* isolate SDS CRYO-TEM 14.

Cultivation of the algae

Cultures of *D. tertiolecta* CCAP 19/22 from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) were grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)[17]. Cultures were grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m^2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Treatment of cells

Cells (taken in the exponential phase of growth) were centrifuged at 100g and 22°C for 10 minutes and suspended in solution of marine salt (22 g/L) to reach the concentration cca 10^6 cells/mL. The cells were treated with detergent sodium dodecylsulfate (SDS) at 1:50 proportion with respect to the volume of cell suspension for 1 hour.

Removal of cells

The cells from algal culture were centrifuged at 300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia) using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); then at 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice.

Isolation of nanoparticles (NPs)

The cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using 5-mL thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 50 000 g and 4°C, for 70 min in 5-mL tubes in the same type of ultracentrifuge and ultracentrifuge tubes.

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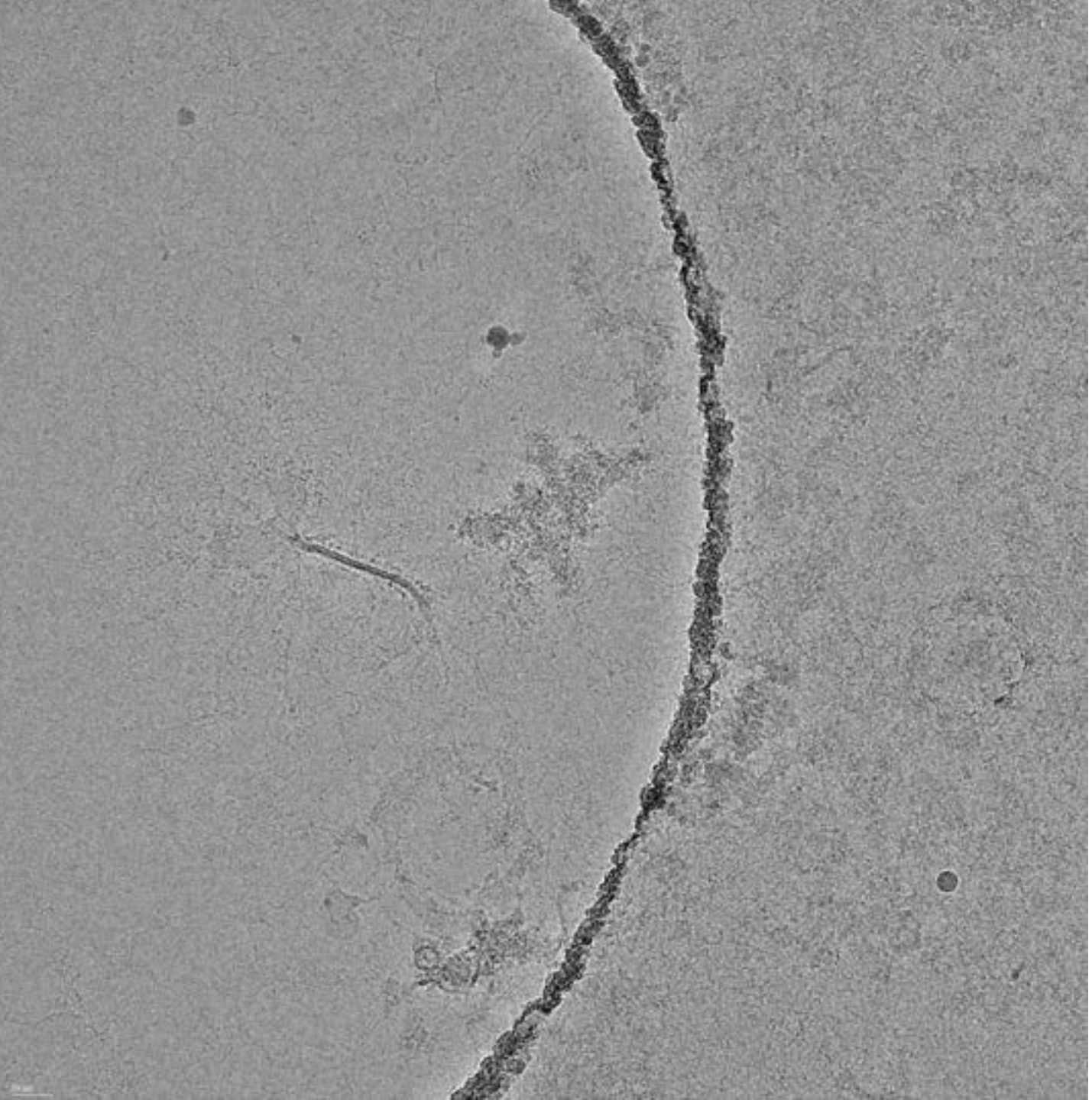

Figure *Dunaliela tertiolecta* isolate SDS CRYO-TEM 15.

Cultivation of the algae

Cultures of *D. tertiolecta* CCAP 19/22 from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) were grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)[17]. Cultures were grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m^2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Treatment of cells

Cells (taken in the exponential phase of growth) were centrifuged at 100g and 22°C for 10 minutes and suspended in solution of marine salt (22 g/L) to reach the concentration cca 10^6 cells/mL. The cells were treated with detergent sodium dodecylsulfate (SDS) at 1:50 proportion with respect to the volume of cell suspension for 1 hour.

Removal of cells

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Isolation of nanoparticles (NPs)

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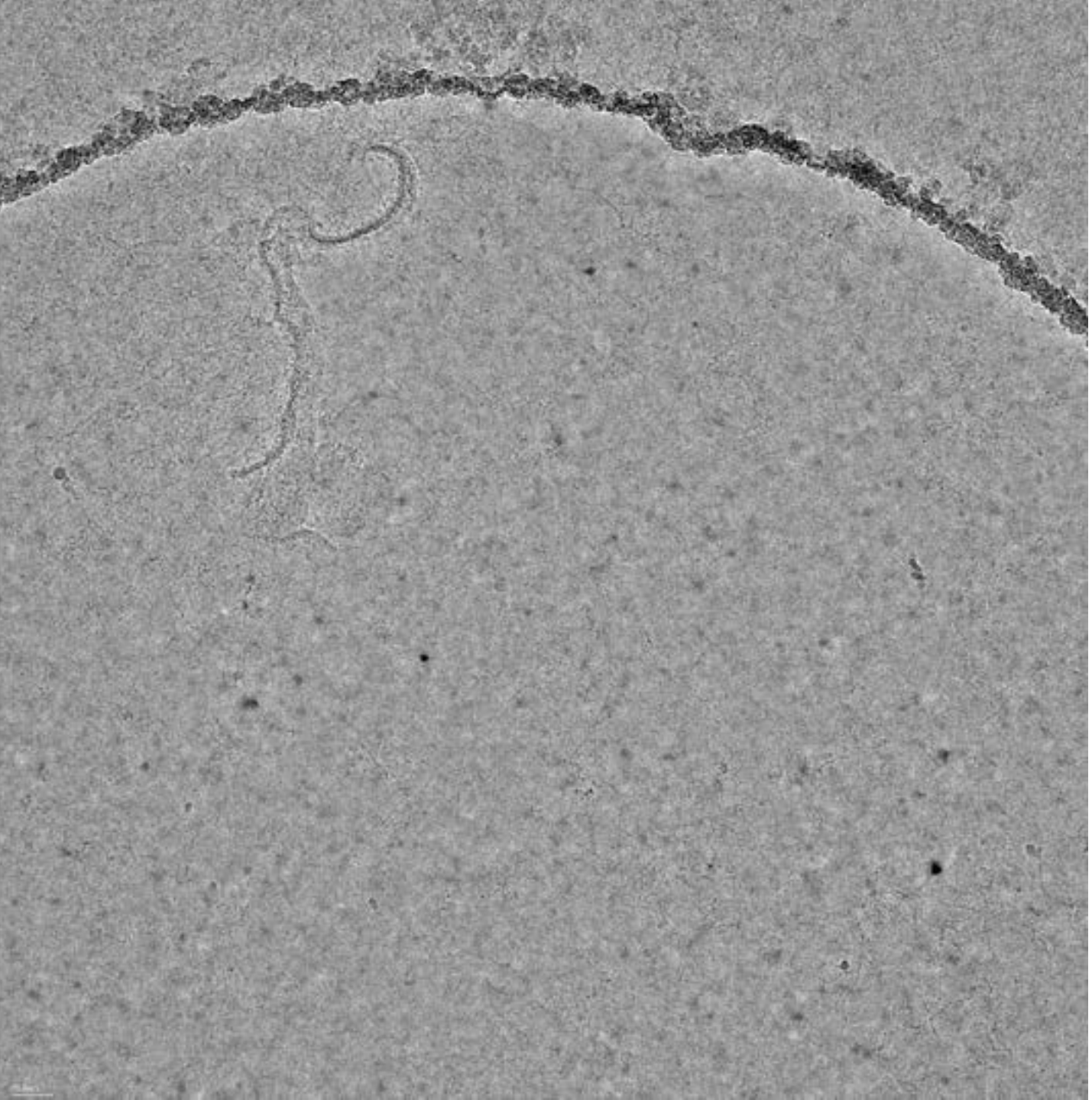

Figure *Dunaliela tertiolecta* isolate SDS CRYO-TEM 16.

Cultivation of the algae

Cultures of *D. tertiolecta* CCAP 19/22 from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) were grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)[17]. Cultures were grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m^2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

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Removal of cells

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Isolation of nanoparticles (NPs)

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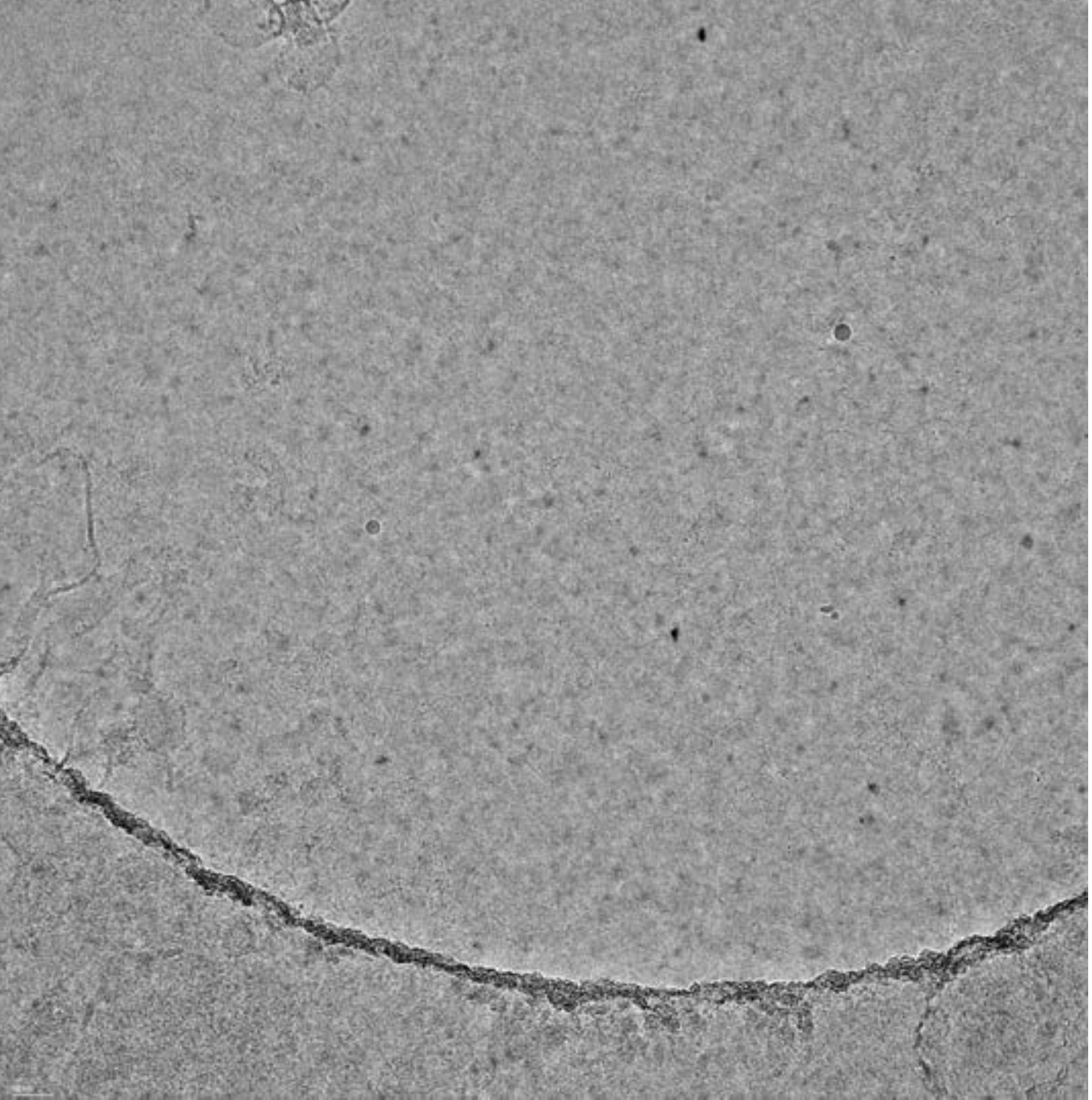

Figure *Dunaliella tertiolecta* isolate SDS CRYO-TEM 17.

Cultivation of the algae

Cultures of *D. tertiolecta* CCAP 19/22 from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) were grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)[17]. Cultures were grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m^2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

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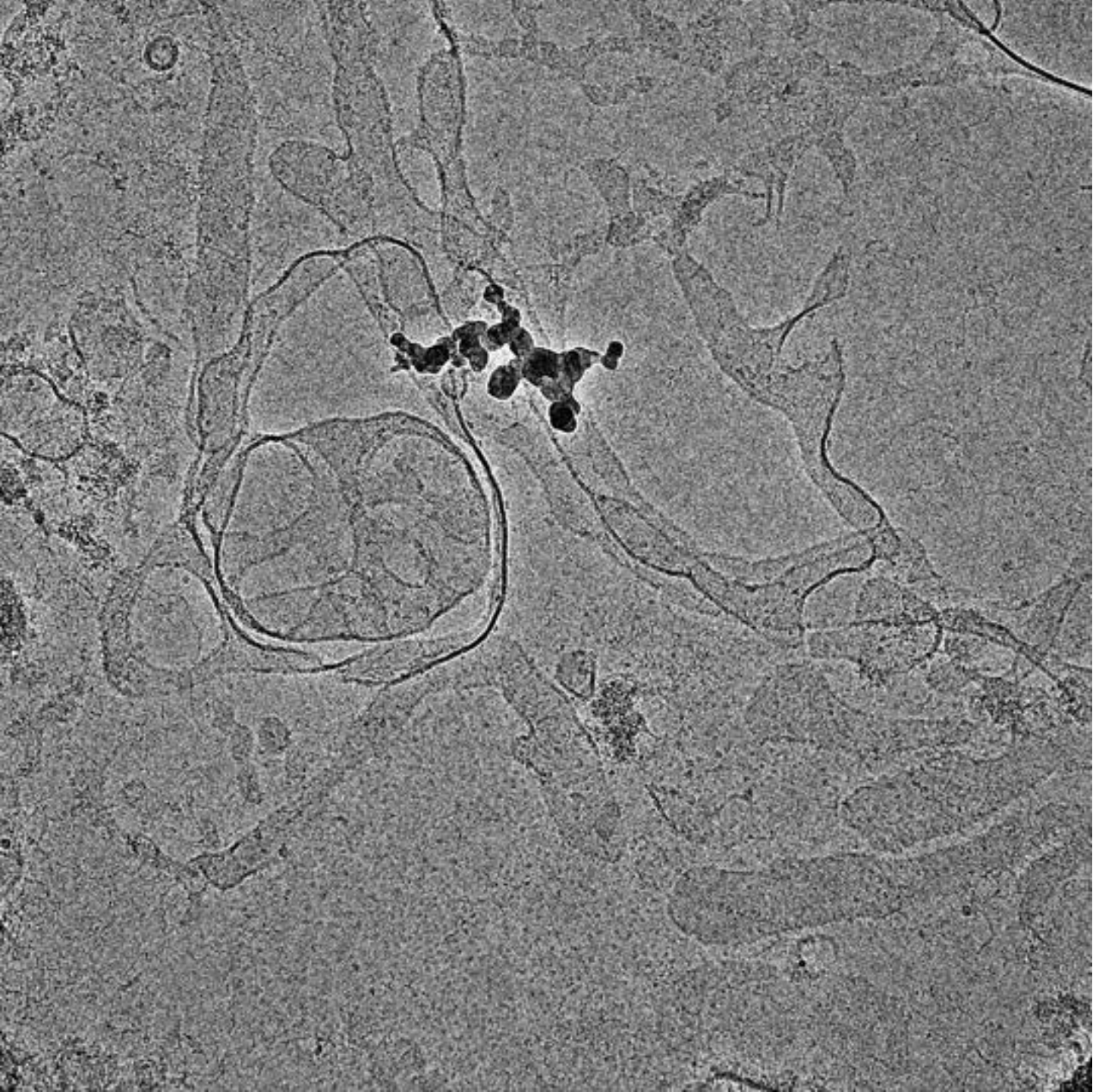

Figure *Dunaliela tertiolecta* isolate SDS CRYO-TEM 18.

Cultivation of the algae

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Proceedings of 9th Socratic Lectures **2024**

Repository

Cryogenic transmission electron images of *Phaeodactylum tricornutum* isolates of small cellular particles

Bedina Zavec Apolonija[1], Božič Darja[2,3], Iglič Aleš[3,4], Jeran Marko[2,3], Kisovec Matic[1], Kralj-Iglič Veronika[2,*], Romolo Anna[2]
[1]National Institute of Chemistry, University of Ljubljana, [2]University of Ljubljana, Faculty of Health Sciences, Laboratory of Clinical Biophysics,[3]University of Ljubljana, Faculty of Electrical Engineering, Laboratory of Physics, [4]University of Ljubljana, Faculty of Medicine, Laboratory of Clinical Biophysics, Ljubljana, Slovenia

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Citation: Bedina Zavec A, Božič D, Iglič A, Jeran M, Kisovec M, Kralj-Iglič V, Romolo A. Cryogenic transmission electron images of *Phaeodactylum tricornutum* isolates of small cellular particles. Proceedings of Socratic Lectures. **2024**, 9, 107-126. https://doi.org/10.55295/PSL.2024.D9

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Abstract: Cryogenic transmission electron images of small cellular particles isolated from conditioned media of microalgae *Phaeodactylum tricornutun* are presented. Each image is identified by the name and number that constitutes the title of the Figure, and supplemented by description of the preparation of the sample and the data on the imaging technique and equipment.

The data curators of the repository are Veronika Kralj-Iglič and Anna Romolo. More data on experiments with microalgae small cellular particles can be found in (Adamo et al., 2021), (Picciotto et al., 2021) and (Božič et al., 2022).

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Keywords: Extracellular vesicles, Extracellular particles, Nanoalgosomes, Exosomes

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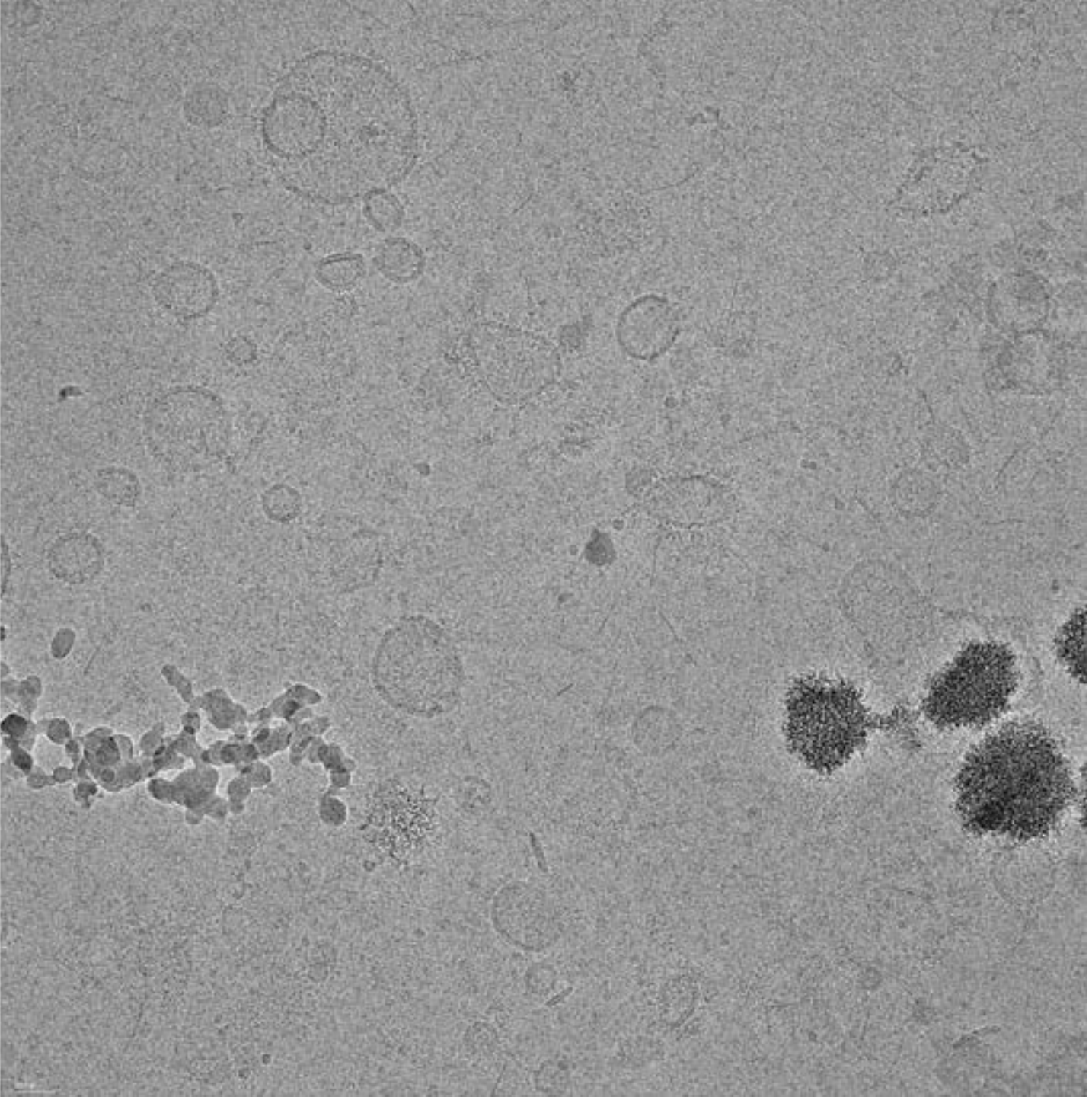

Figure *Phaeodactylum tricornutum* isolate CRYO-TEM 26.

Cultivation of the algae
Cultures of *P. tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) were grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)[17]. Cultures were grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m^2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.
Isolation of nanoparticles (NPs)
NPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.
Cryogenic Electron Microscopy (Cryo-TEM)
Samples of NPs isolates were prepared for Cryo-TEM using Vitrobot Mark IV (Thermo Fisher Scientific, Waltham, MA, US). Quantifoil® R 2/2 (or 1.2/1.3), 200 mesh holey carbon grids (Quantifoil Micro Tools GmbH, Großlöbichau, Germany) were glow discharged for 60 s at 20 mA and positive polarity in the air atmosphere (GloQube® Plus, Quorum, Laughton, UK). Conditions were set at 4 °C, 95% relative humidity, blot time: 5 s, and blot force: 4. 2 μL of the sample with nanoparticles in suspension was applied to the grid, blotted, and vitrified in liquid ethane. Samples were visualized under cryogenic conditions using a 200 kV microscope Glacios with Falcon 3EC detector (Thermo Fisher Scientific, Waltham, MA, US).
From https://zenodo.org/record/6908895. Image 16
DOI 10.5281/zenodo.6908895.

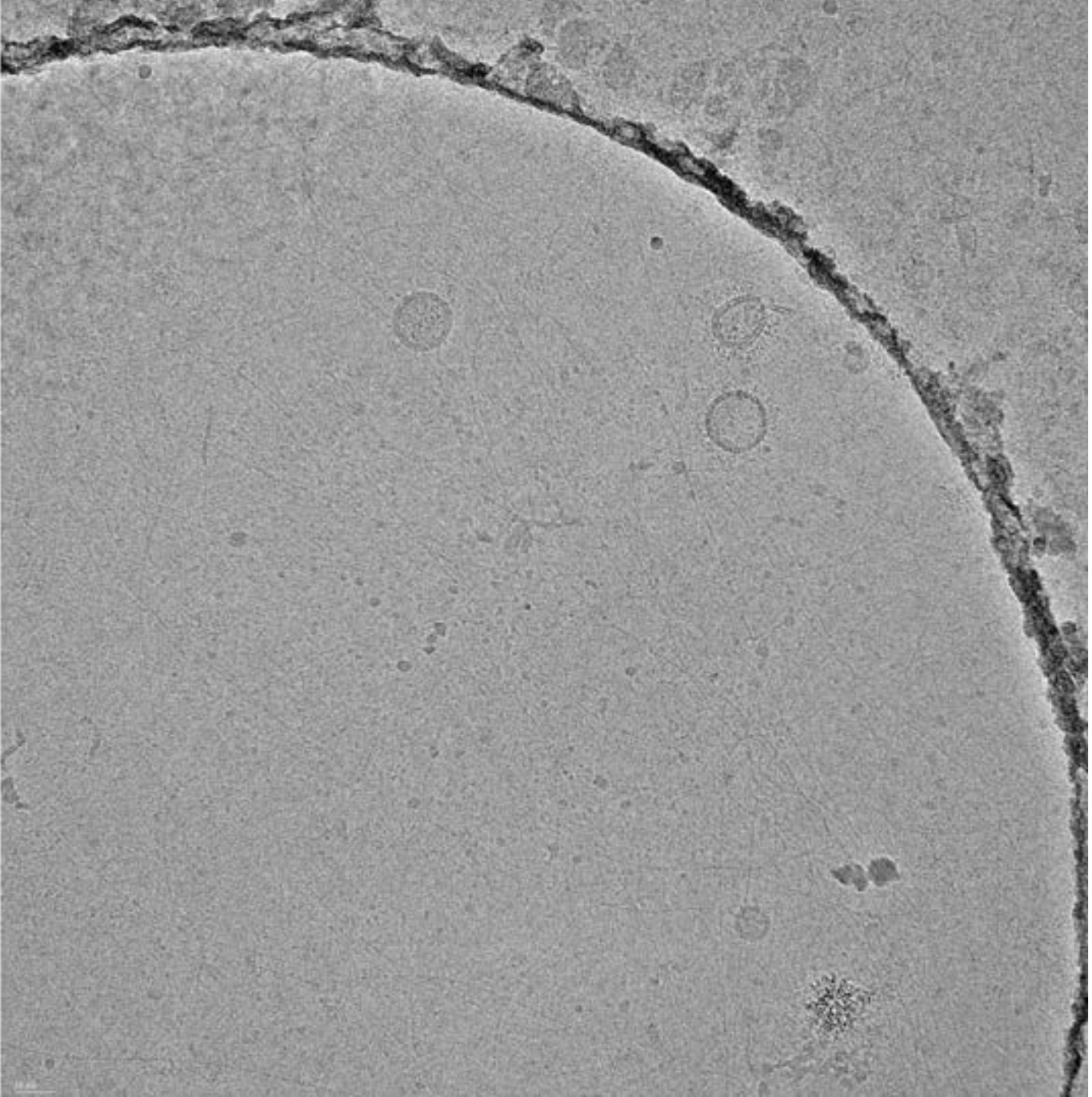

Figure *Phaeodactylum tricornutum* isolate CRYO-TEM 27.

Cultivation of the algae
Cultures of *P. tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) were grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)[17]. Cultures were grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m^2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.
Isolation of nanoparticles (NPs)
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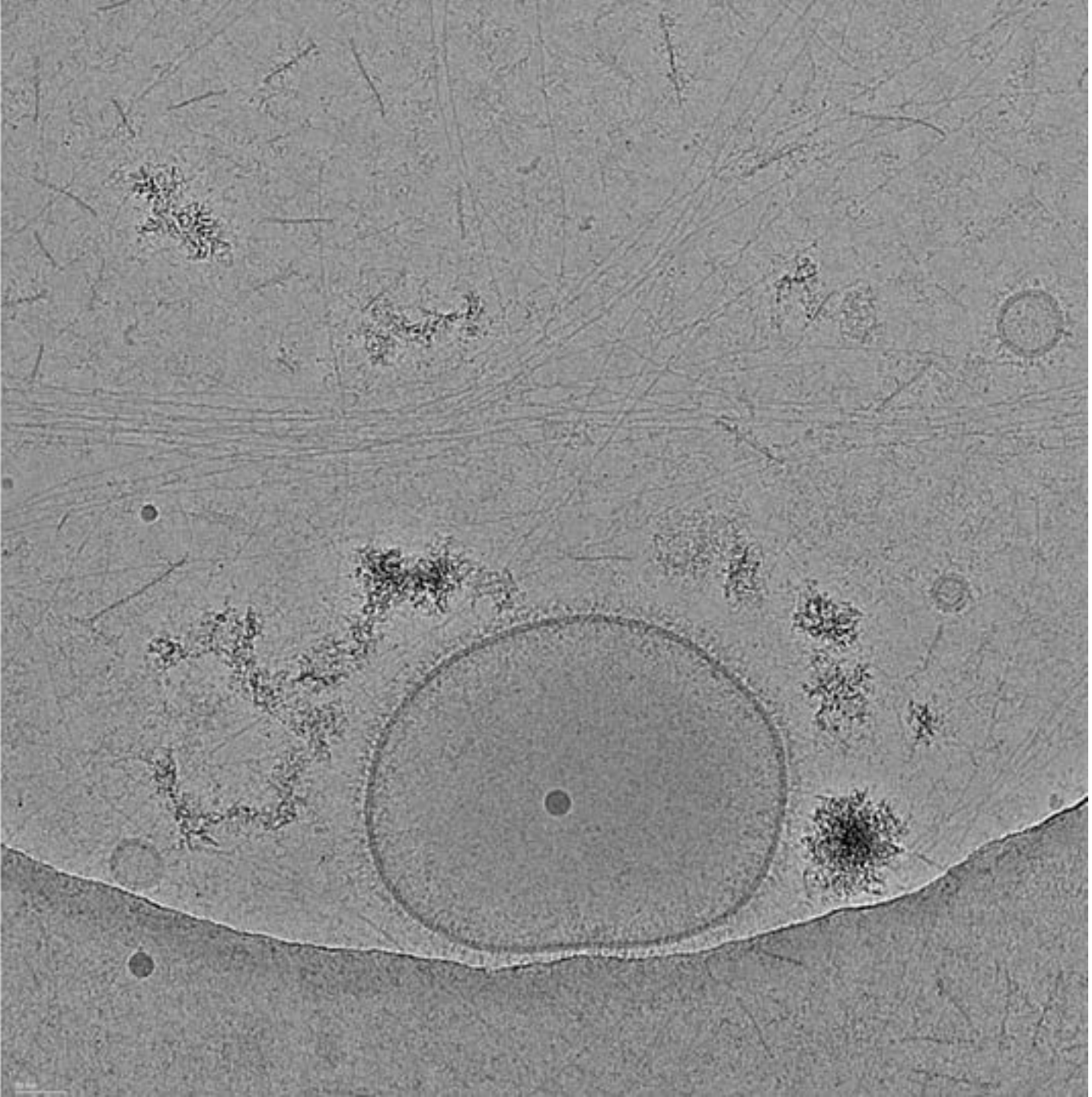

Figure *Phaeodactylum tricornutum* isolate CRYO-TEM 28.

Cultivation of the algae
Cultures of *P. tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) were grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)[17]. Cultures were grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m^2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.
Isolation of nanoparticles (NPs)
NPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.
Cryogenic Electron Microscopy (Cryo-TEM)
Samples of NPs isolates were prepared for Cryo-TEM using Vitrobot Mark IV (Thermo Fisher Scientific, Waltham, MA, US). Quantifoil® R 2/2 (or 1.2/1.3), 200 mesh holey carbon grids (Quantifoil Micro Tools GmbH, Großlöbichau, Germany) were glow discharged for 60 s at 20 mA and positive polarity in the air atmosphere (GloQube® Plus, Quorum, Laughton, UK). Conditions were set at 4 °C, 95% relative humidity, blot time: 5 s, and blot force: 4. 2 μL of the sample with nanoparticles in suspension was applied to the grid, blotted, and vitrified in liquid ethane. Samples were visualized under cryogenic conditions using a 200 kV microscope Glacios with Falcon 3EC detector (Thermo Fisher Scientific, Waltham, MA, US).
From Božič et al., 2022, https://doi.org/10.12688/openreseurope.14896.1.

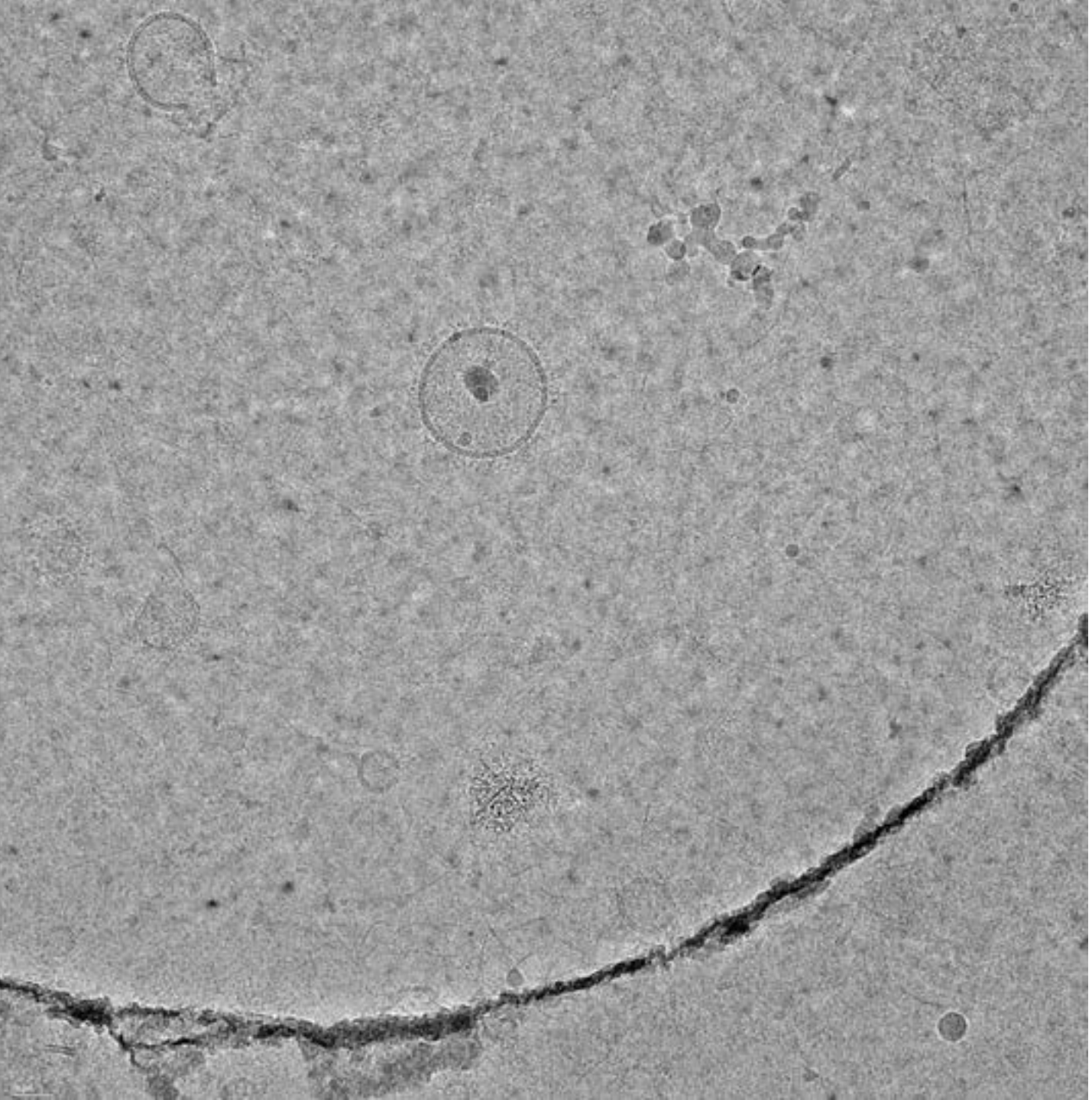

Figure *Phaeodactylum tricornutum* isolate CRYO-TEM 29.

Cultivation of the algae
Cultures of *P. tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) were grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)[17]. Cultures were grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m^{2}s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.
Isolation of nanoparticles (NPs)
NPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.
Cryogenic Electron Microscopy (Cryo-TEM)
Samples of NPs isolates were prepared for Cryo-TEM using Vitrobot Mark IV (Thermo Fisher Scientific, Waltham, MA, US). Quantifoil® R 2/2 (or 1.2/1.3), 200 mesh holey carbon grids (Quantifoil Micro Tools GmbH, Großlöbichau, Germany) were glow discharged for 60 s at 20 mA and positive polarity in the air atmosphere (GloQube® Plus, Quorum, Laughton, UK). Conditions were set at 4 °C, 95% relative humidity, blot time: 5 s, and blot force: 4. 2 μL of the sample with nanoparticles in suspension was applied to the grid, blotted, and vitrified in liquid ethane. Samples were visualized under cryogenic conditions using a 200 kV microscope Glacios with Falcon 3EC detector (Thermo Fisher Scientific, Waltham, MA, US).
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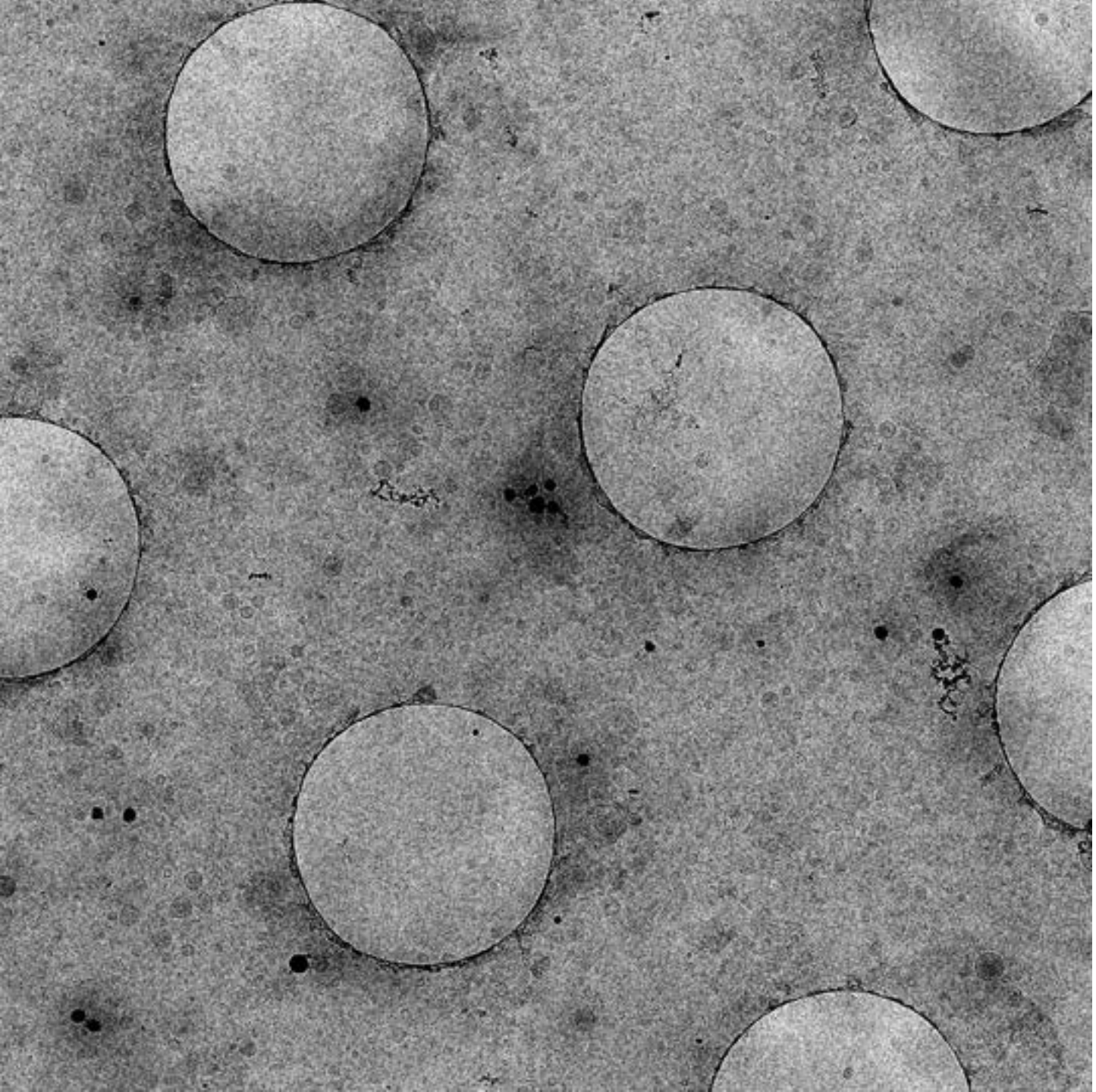

Figure *Phaeodactylum tricornutum* isolate CRYO-TEM 30.

Cultivation of the algae

Cultures of *P. tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) were grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)[17]. Cultures were grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m^2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Isolation of nanoparticles (NPs)

NPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

Cryogenic Electron Microscopy (Cryo-TEM)

Samples of NPs isolates were prepared for Cryo-TEM using Vitrobot Mark IV (Thermo Fisher Scientific, Waltham, MA, US). Quantifoil® R 2/2 (or 1.2/1.3), 200 mesh holey carbon grids (Quantifoil Micro Tools GmbH, Großlöbichau, Germany) were glow discharged for 60 s at 20 mA and positive polarity in the air atmosphere (GloQube® Plus, Quorum, Laughton, UK). Conditions were set at 4 °C, 95% relative humidity, blot time: 5 s, and blot force: 4. 2 μL of the sample with nanoparticles in suspension was applied to the grid, blotted, and vitrified in liquid ethane. Samples were visualized under cryogenic conditions using a 200 kV microscope Glacios with Falcon 3EC detector (Thermo Fisher Scientific, Waltham, MA, US).

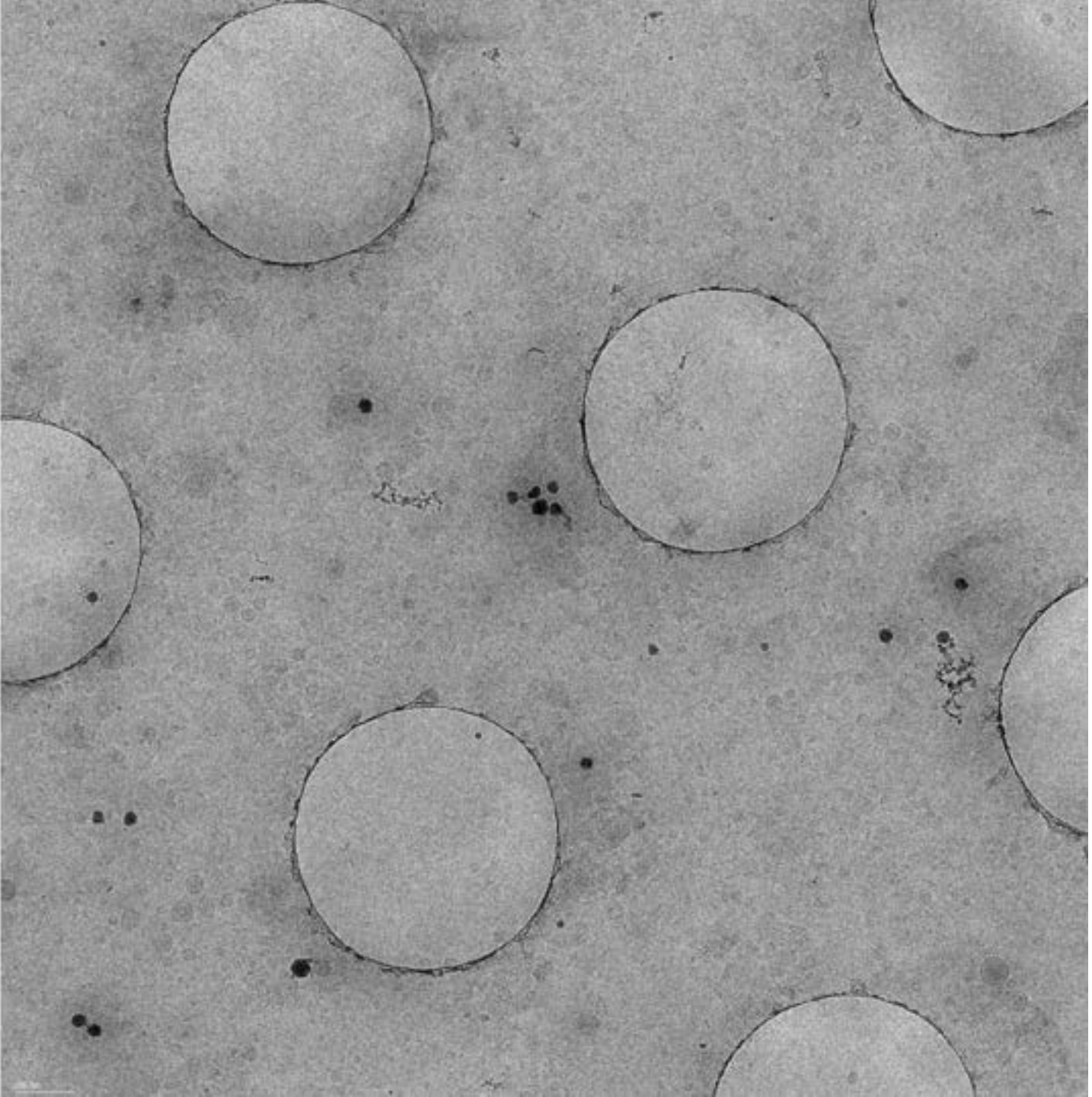

Figure *Phaeodactylum tricornutum* isolate CRYO-TEM 31.

Cultivation of the algae

Cultures of *P. tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) were grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)[17]. Cultures were grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m^2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

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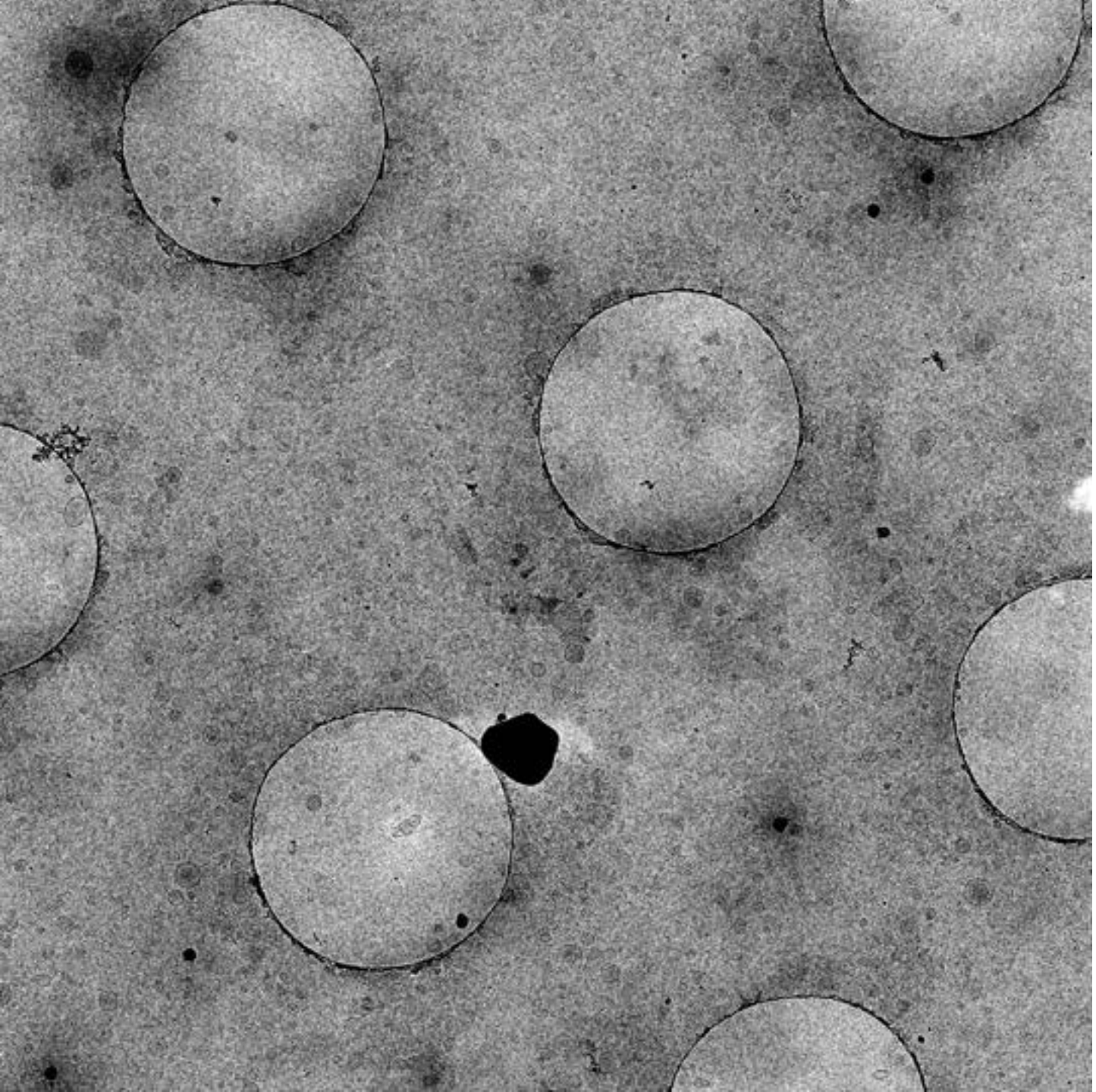

Figure *Phaeodactylum tricornutum* isolate CRYO-TEM 32.

Cultivation of the algae

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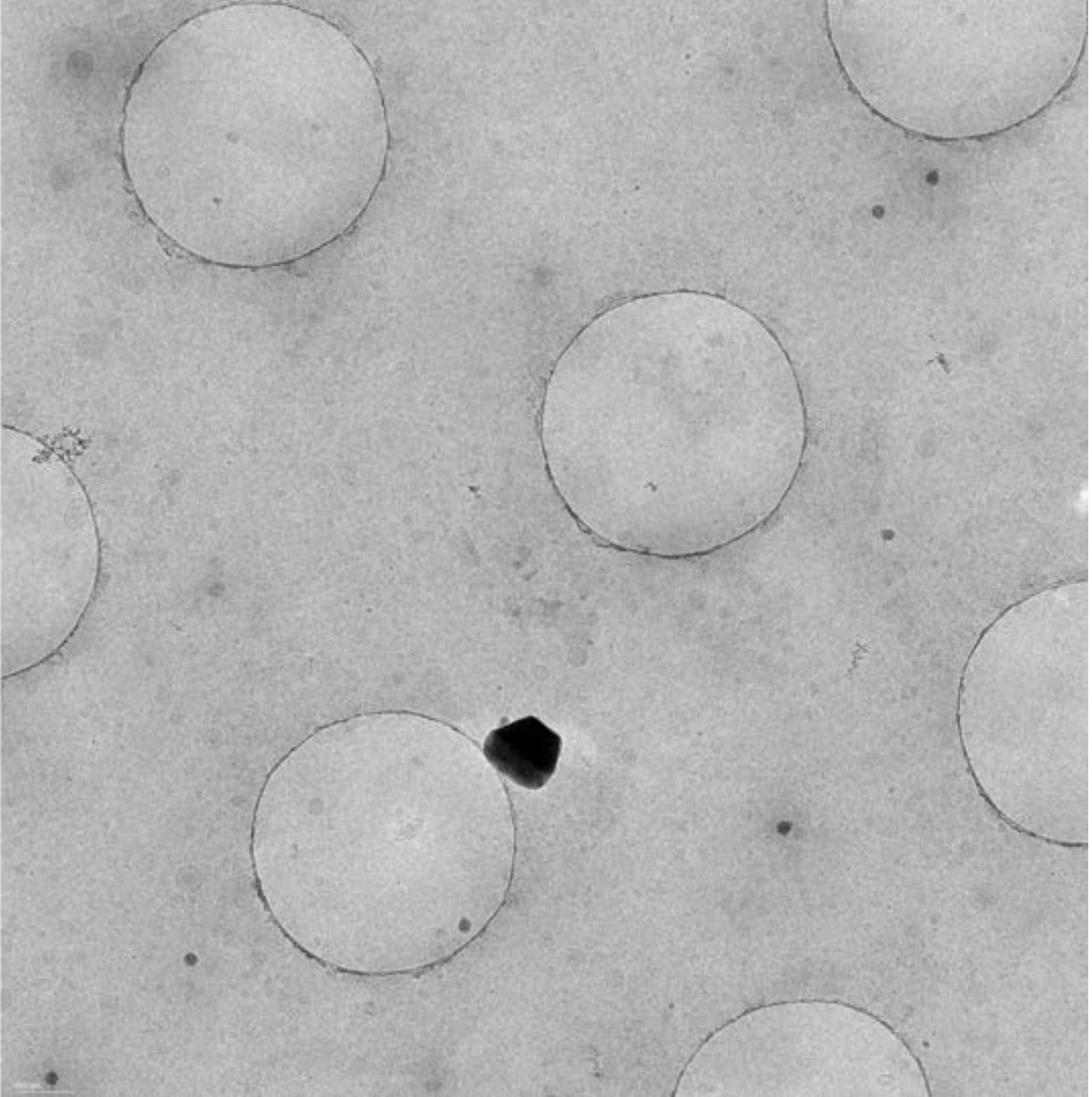

Figure *Phaeodactylum tricornutum* isolate CRYO-TEM 33.

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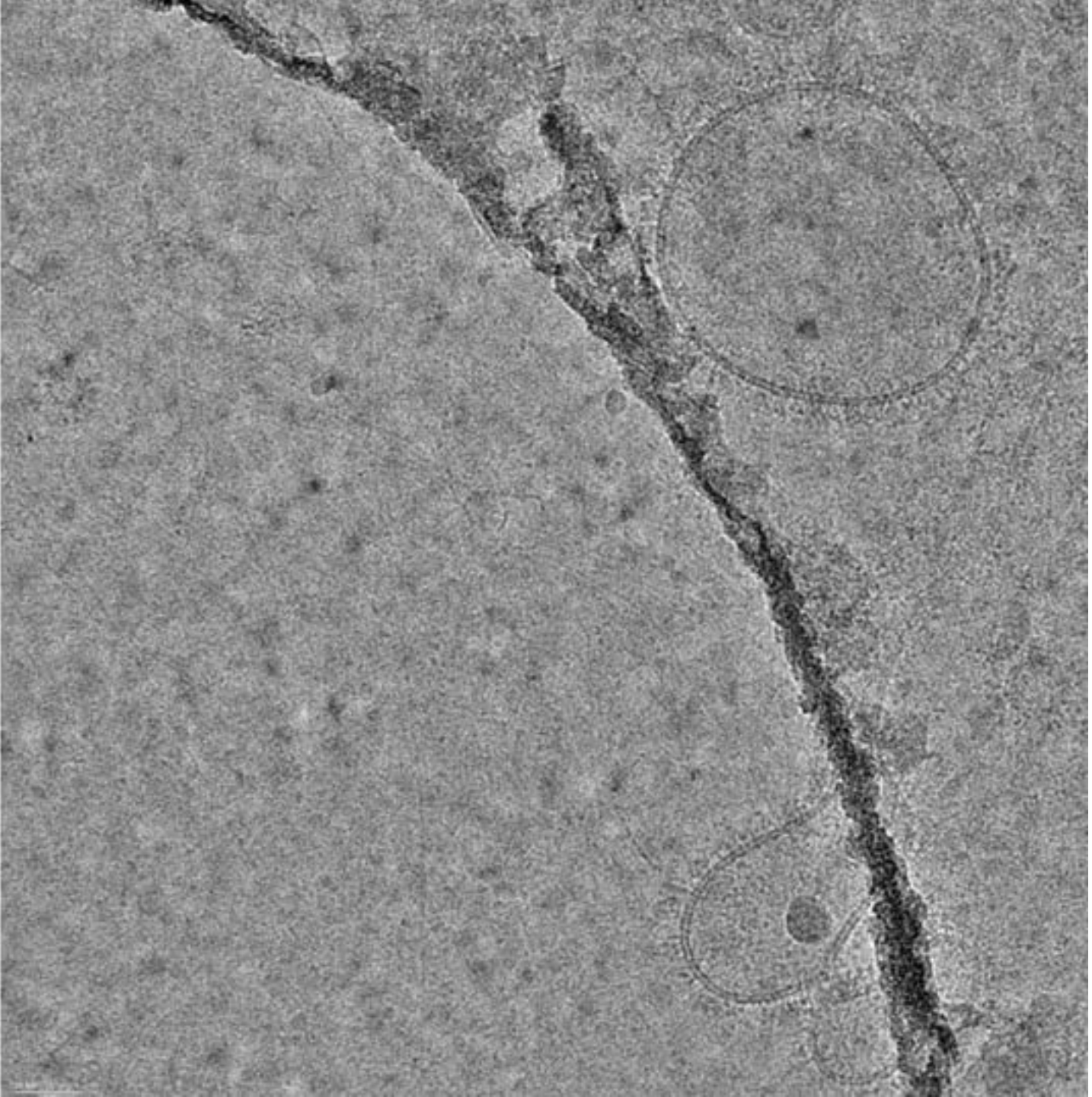

Figure *Phaeodactylum tricornutum* isolate CRYO-TEM 34.

Cultivation of the algae

Cultures of *P. tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) were grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)[17]. Cultures were grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m^2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

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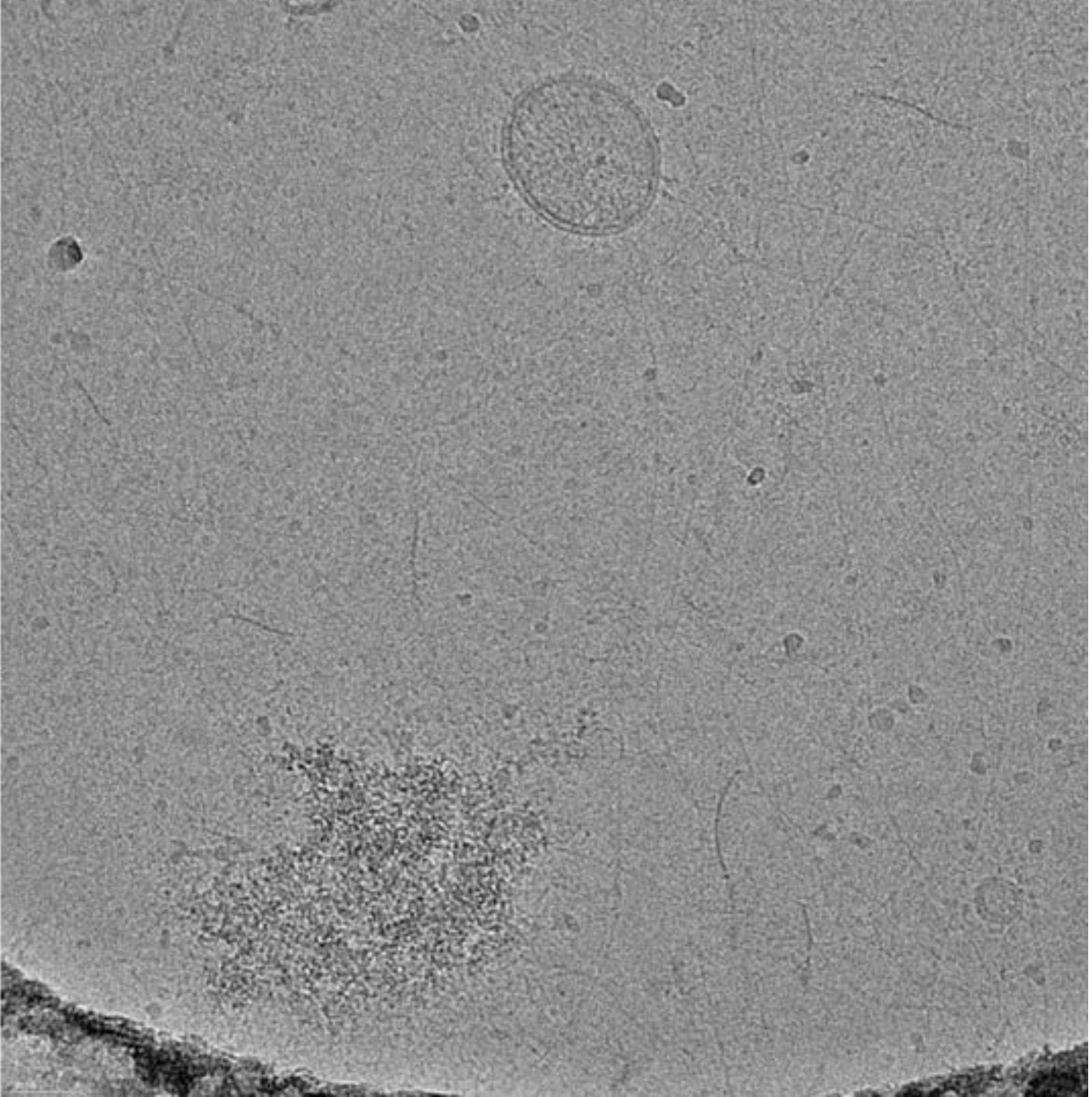

Figure *Phaeodactylum tricornutum* isolate CRYO-TEM 35.

Cultivation of the algae

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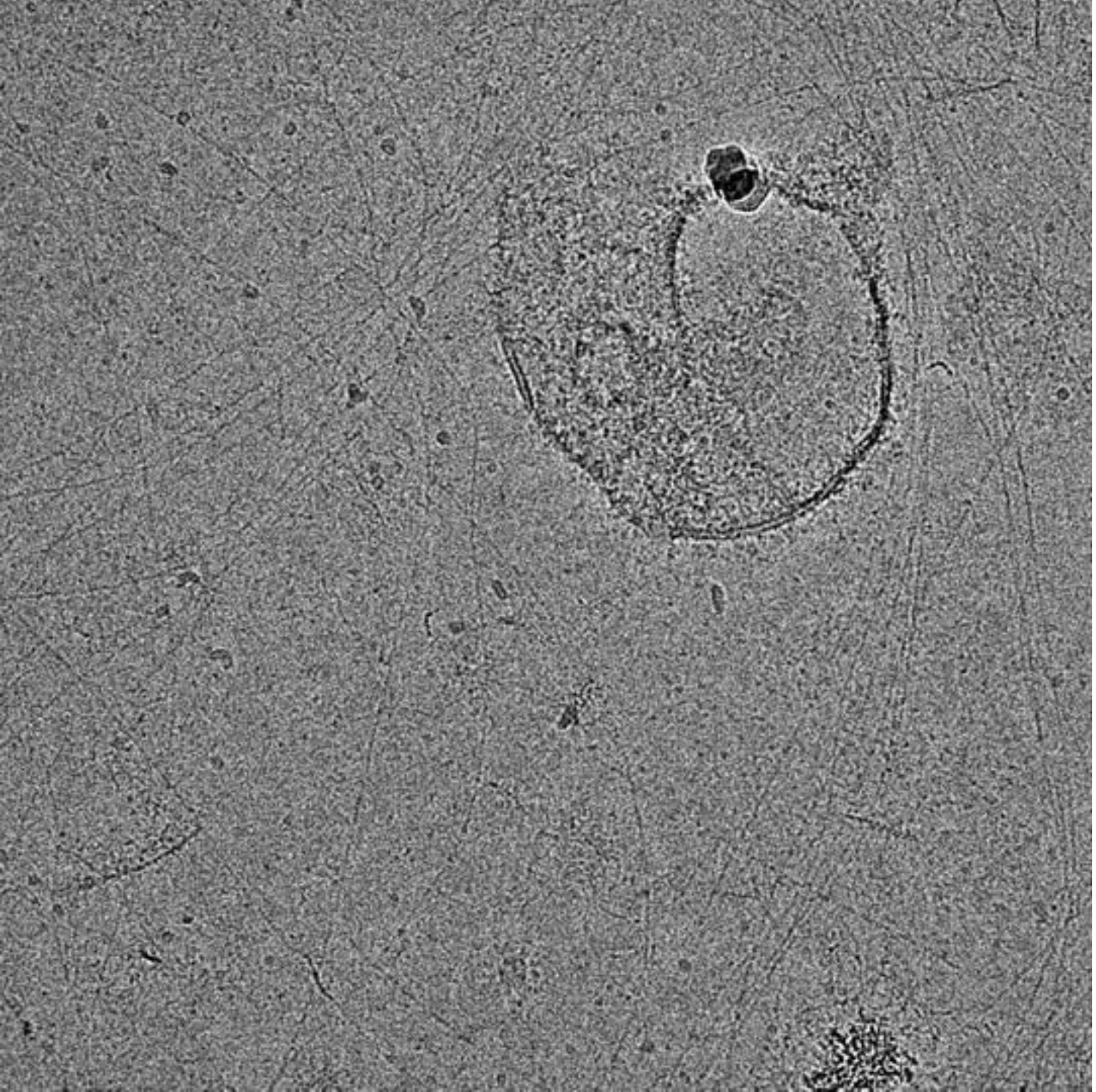

Figure *Phaeodactylum tricornutum* isolate CRYO-TEM 36.

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Cultures of *P. tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) were grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)[17]. Cultures were grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m^2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

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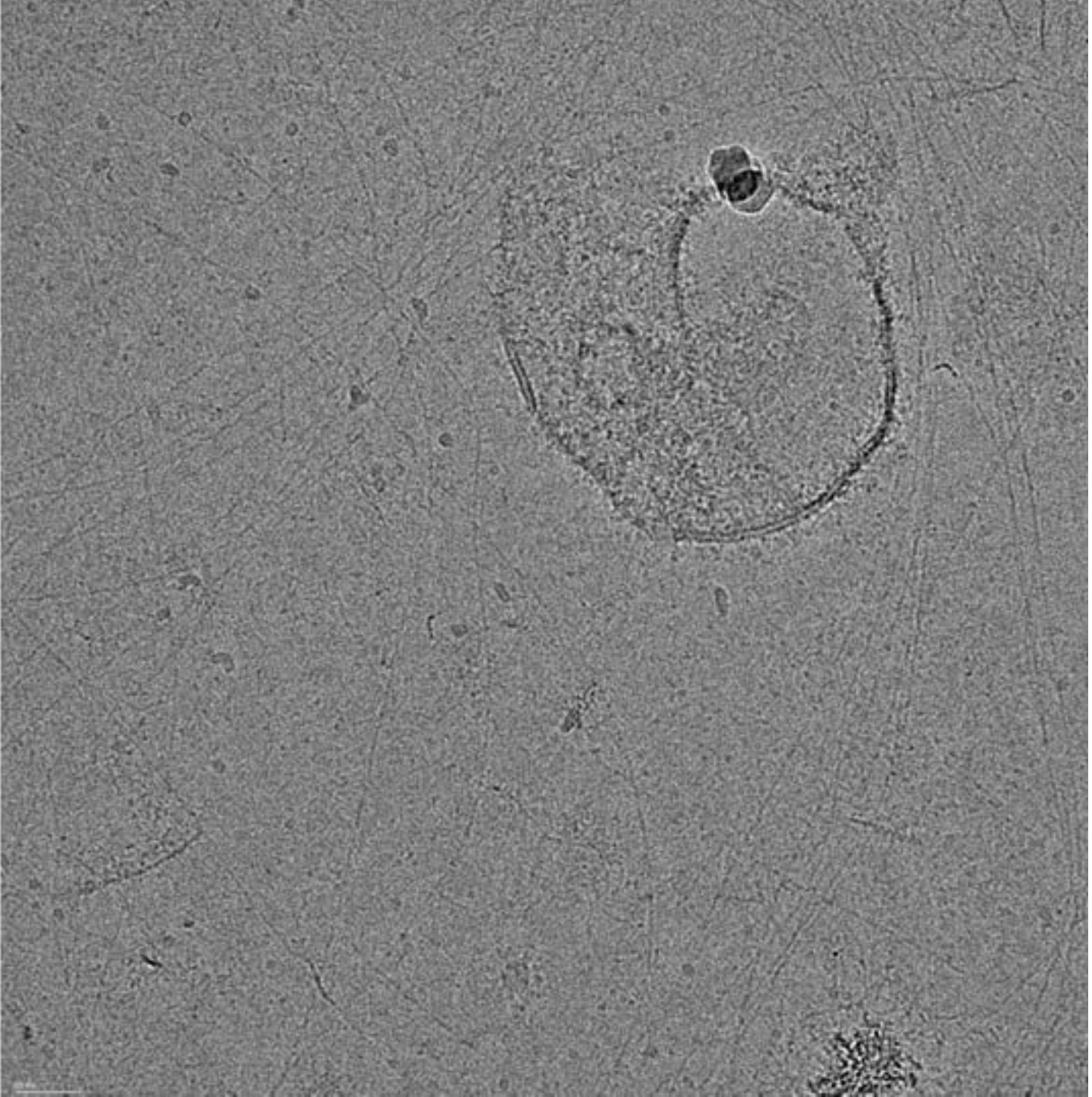

Figure *Phaeodactylum tricornutum* isolate CRYO-TEM 37.

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Cultures of *P. tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) were grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)[17]. Cultures were grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m^2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

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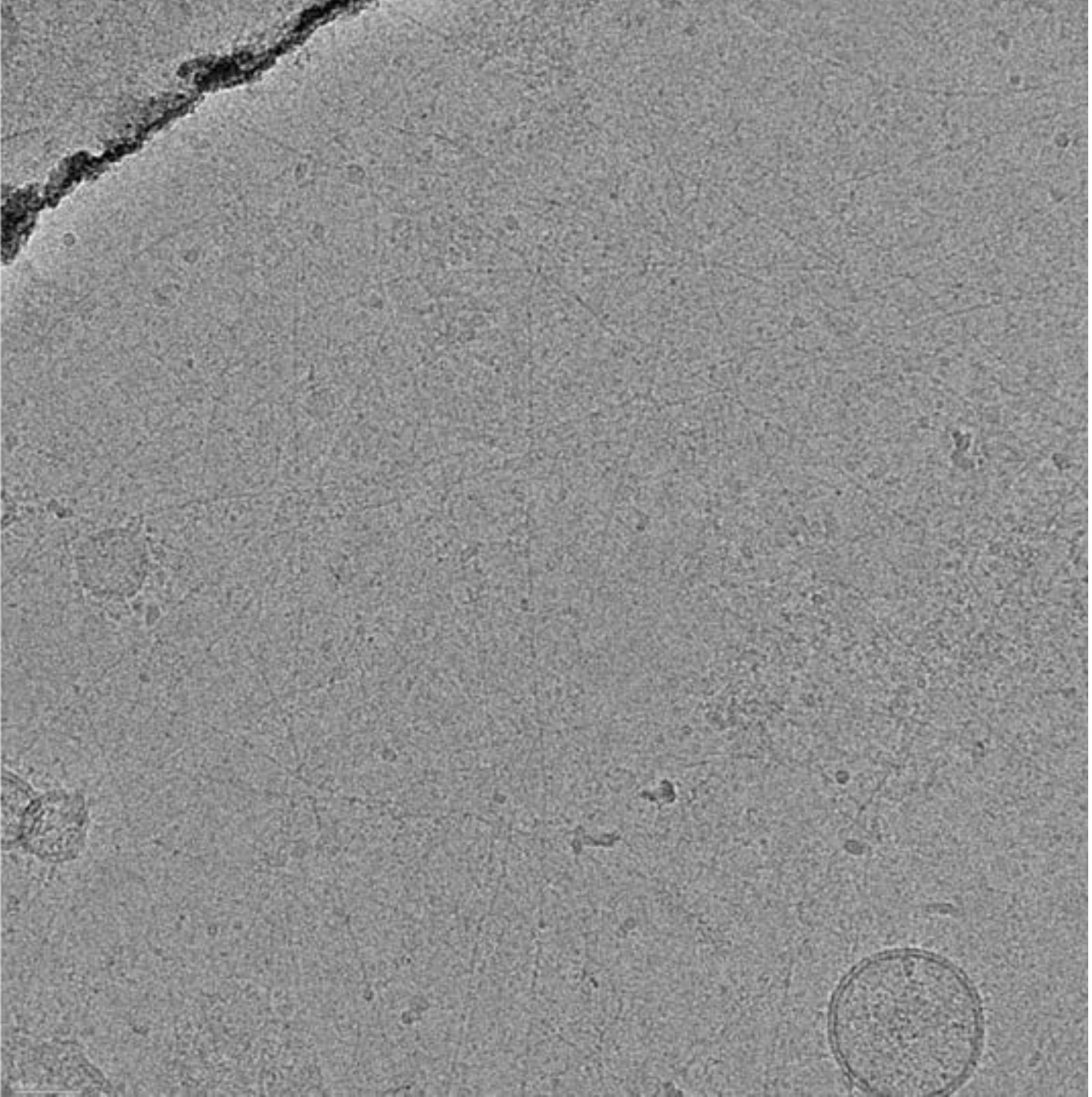

Figure *Phaeodactylum tricornutum* isolate CRYO-TEM 38.

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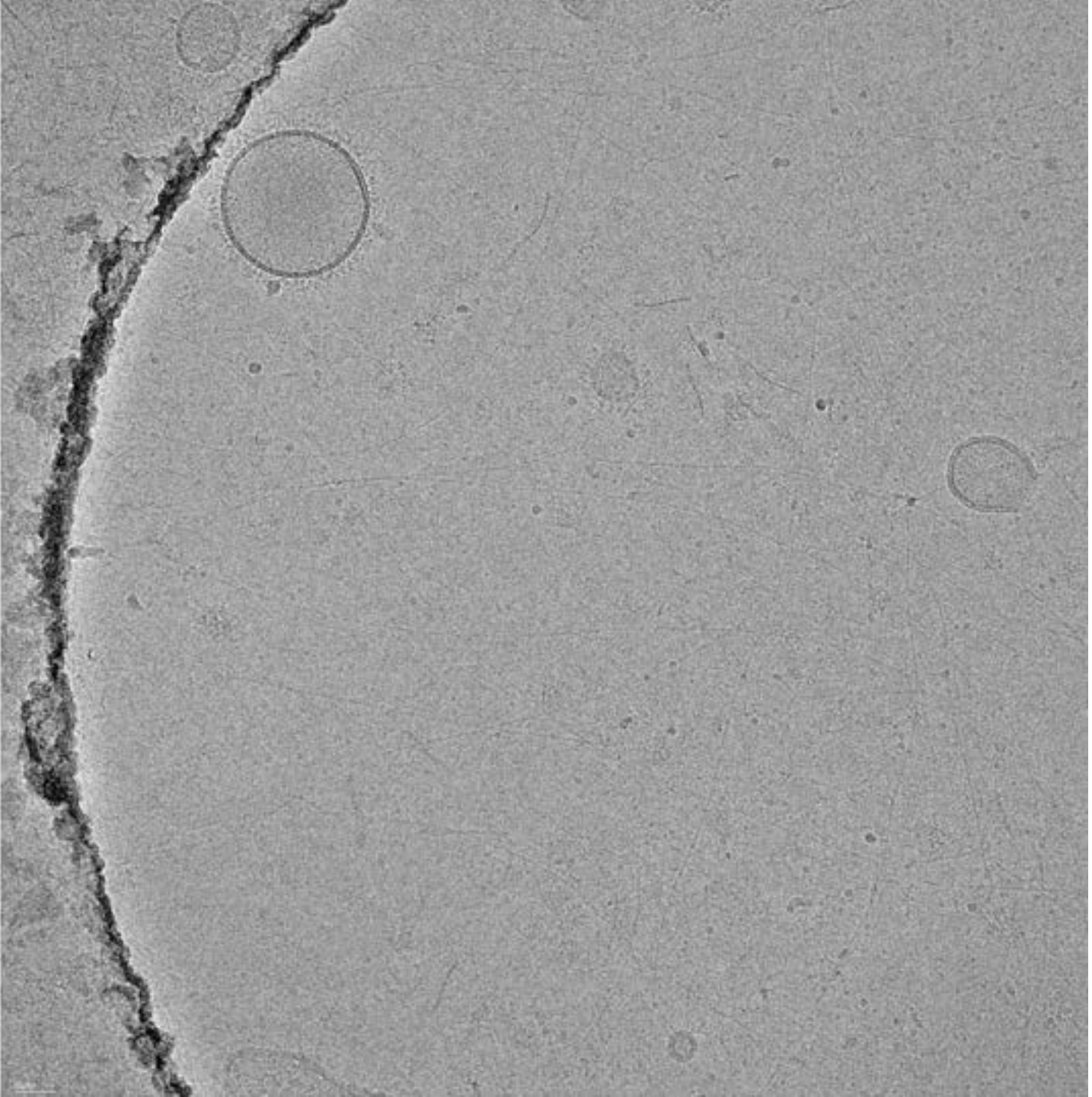

Figure *Phaeodactylum tricornutum* isolate CRYO-TEM 39.

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Cultures of *P. tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) were grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)[17]. Cultures were grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m^2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

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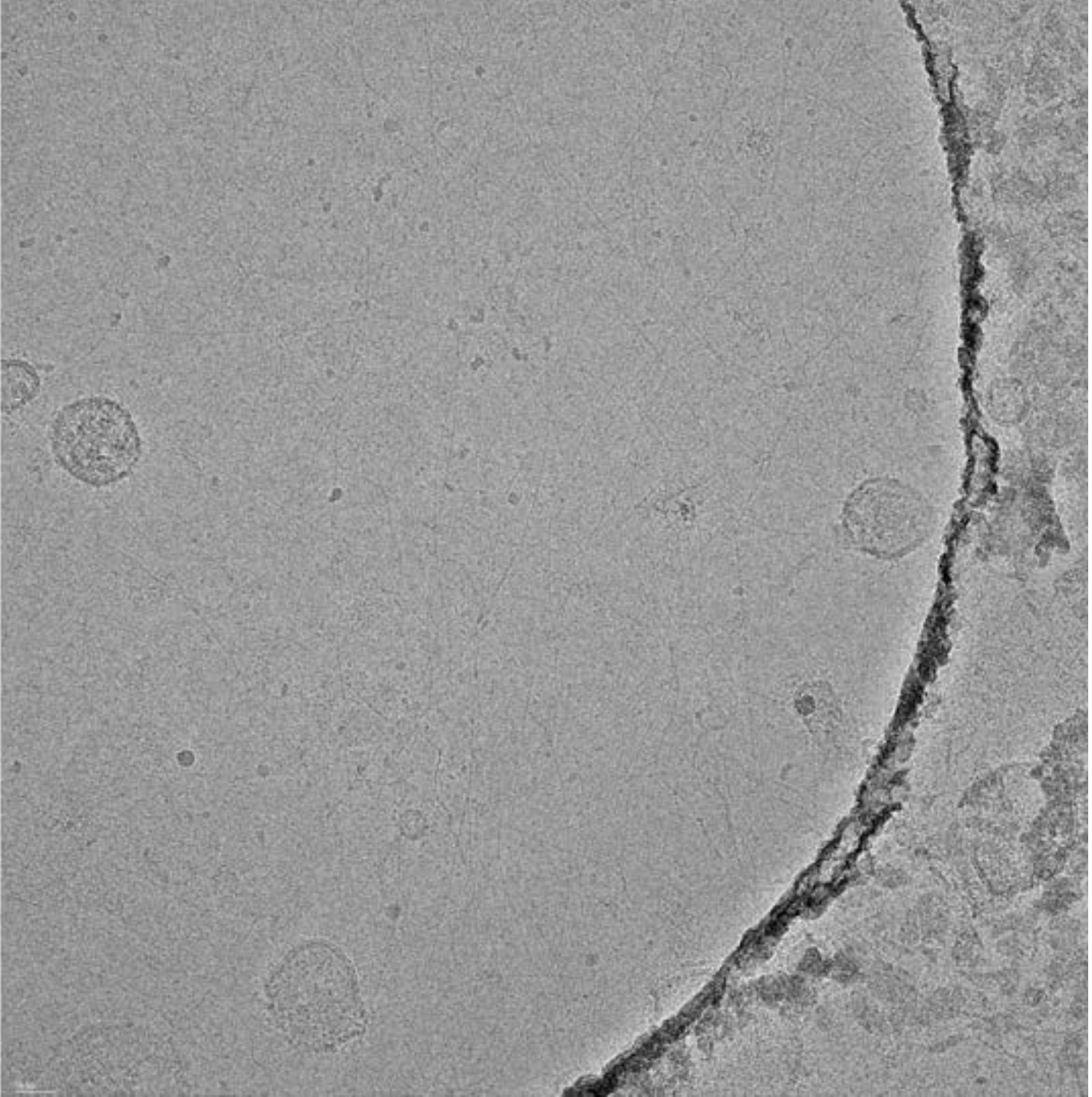

Figure *Phaeodactylum tricornutum* isolate CRYO-TEM 40.

Cultivation of the algae

Cultures of *P. tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) were grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)[17]. Cultures were grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m^2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

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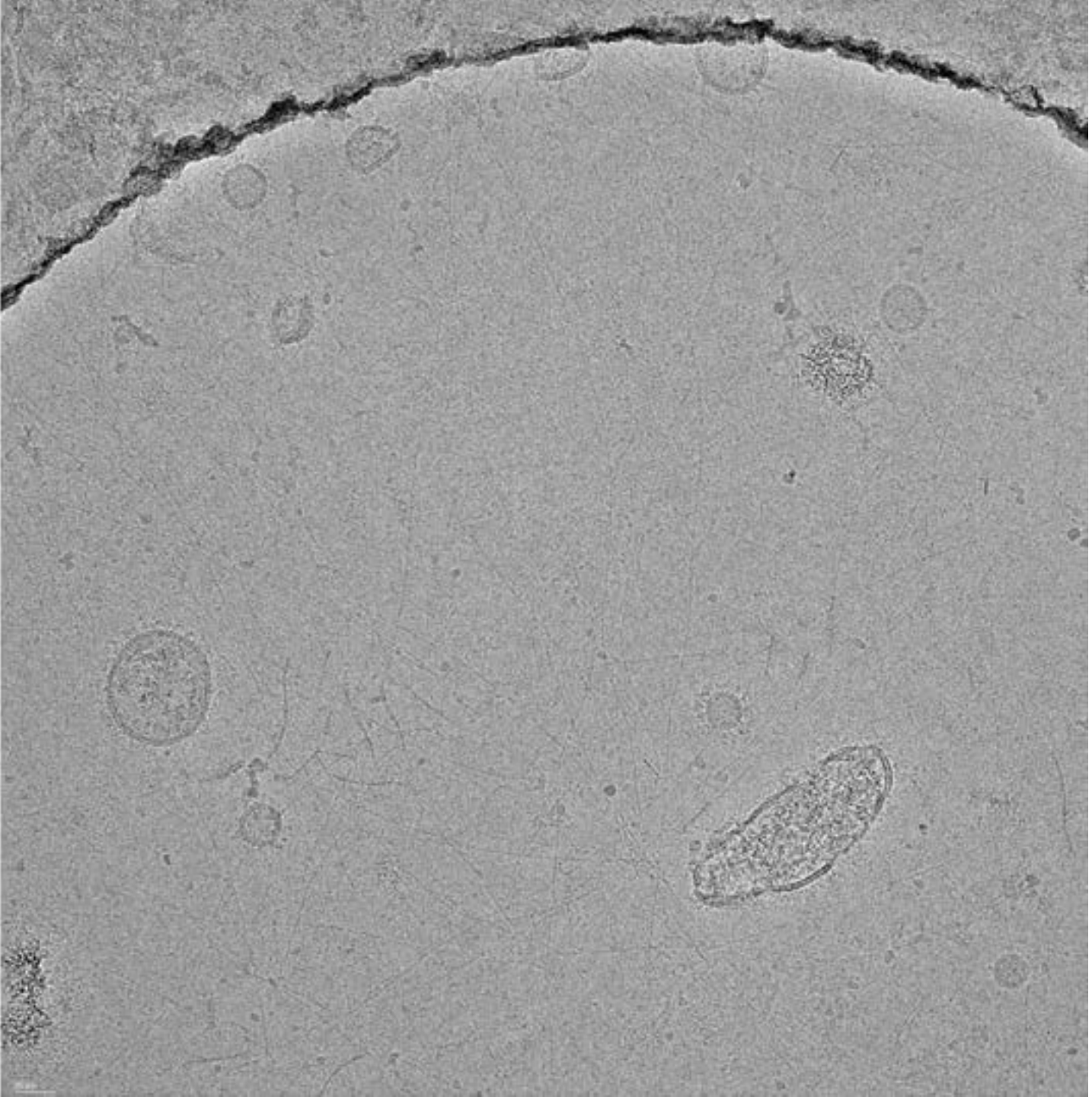

Figure *Phaeodactylum tricornutum* isolate CRYO-TEM 41.

Cultivation of the algae

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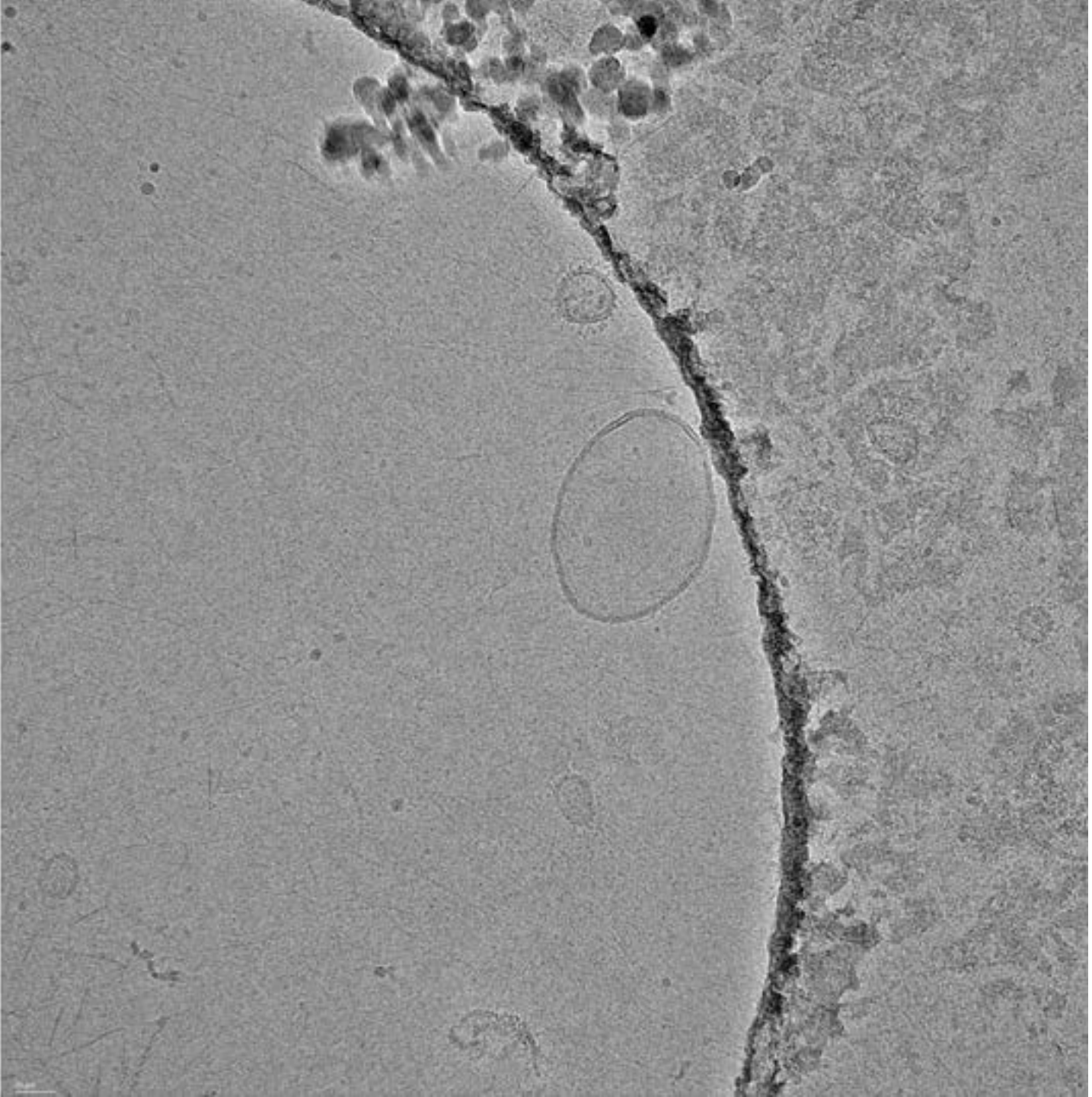

Figure *Phaeodactylum tricornutum* isolate CRYO-TEM 42.

Cultivation of the algae

Cultures of *P. tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) were grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)[17]. Cultures were grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m^2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

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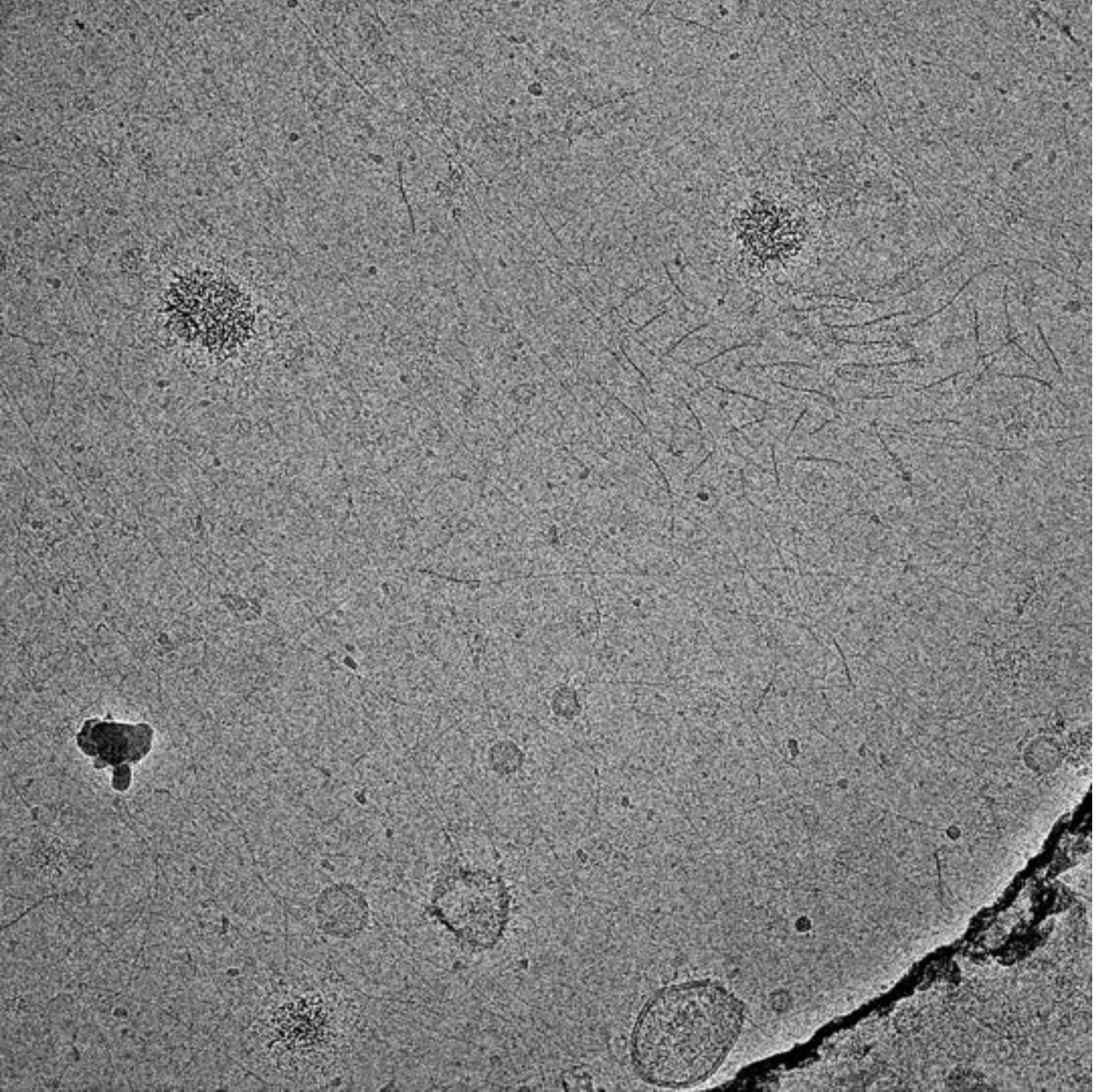

Figure *Phaeodactylum tricornutum* isolate CRYO-TEM 43.

Cultivation of the algae

Cultures of *P. tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) were grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)[17]. Cultures were grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m^2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Isolation of nanoparticles (NPs)

NPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

Cryogenic Electron Microscopy (Cryo-TEM)

Samples of NPs isolates were prepared for Cryo-TEM using Vitrobot Mark IV (Thermo Fisher Scientific, Waltham, MA, US). Quantifoil® R 2/2 (or 1.2/1.3), 200 mesh holey carbon grids (Quantifoil Micro Tools GmbH, Großlöbichau, Germany) were glow discharged for 60 s at 20 mA and positive polarity in the air atmosphere (GloQube® Plus, Quorum, Laughton, UK). Conditions were set at 4 °C, 95% relative humidity, blot time: 5 s, and blot force: 4. 2 μL of the sample with nanoparticles in suspension was applied to the grid, blotted, and vitrified in liquid ethane. Samples were visualized under cryogenic conditions using a 200 kV microscope Glacios with Falcon 3EC detector (Thermo Fisher Scientific, Waltham, MA, US).

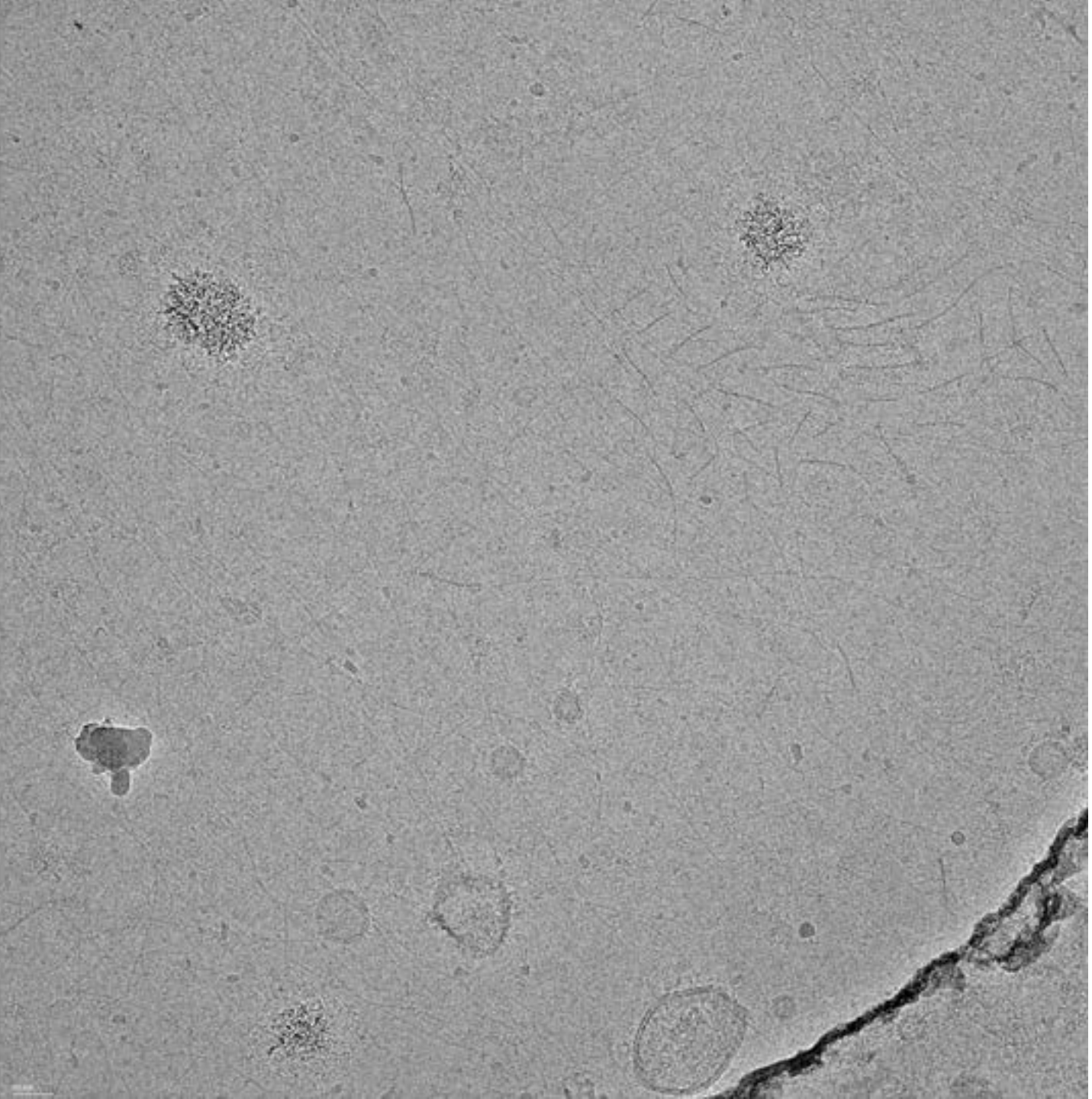

Figure *Phaeodactylum tricornutum* isolate CRYO-TEM 44.

Cultivation of the algae

Cultures of *P. tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) were grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)[17]. Cultures were grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m^2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Isolation of nanoparticles (NPs)

NPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA)) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

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Proceedings of 9th Socratic Lectures **2024**

Repository

Scanning electron microscope images of *Dunaliella tertiolecta* culture

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[1]National Institute of Chemistry, [2]University of Ljubljana, Faculty of Health Sciences, Laboratory of Clinical Biophysics, [3]University of Ljubljana, Faculty of Electrical Engineering, Laboratory of Physics, [4]Institute of Metals and Technology, [5]University of Ljubljana, Faculty of Medicine, Laboratory of Clinical Biophysics, Ljubljana, Slovenia

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Abstract: Scanning electron microscope images of small cellular particles isolated from conditioned media of microalgae *Dunaliella tertiolecta* are presented. Each image is supplemented by description of the preparation of the sample and the data on the imaging technique and equipment.

The data curators of the repository are Veronika Kralj-Iglič and Anna Romolo. More data on experiments with microalgae small cellular particles can be found in (Adamo et al., 2021), (Picciotto et al., 2021) and (Božič et al., 2022).

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Keywords: Extracellular vesicles, Extracellular particles, Nanoalgosomes, Exosomes

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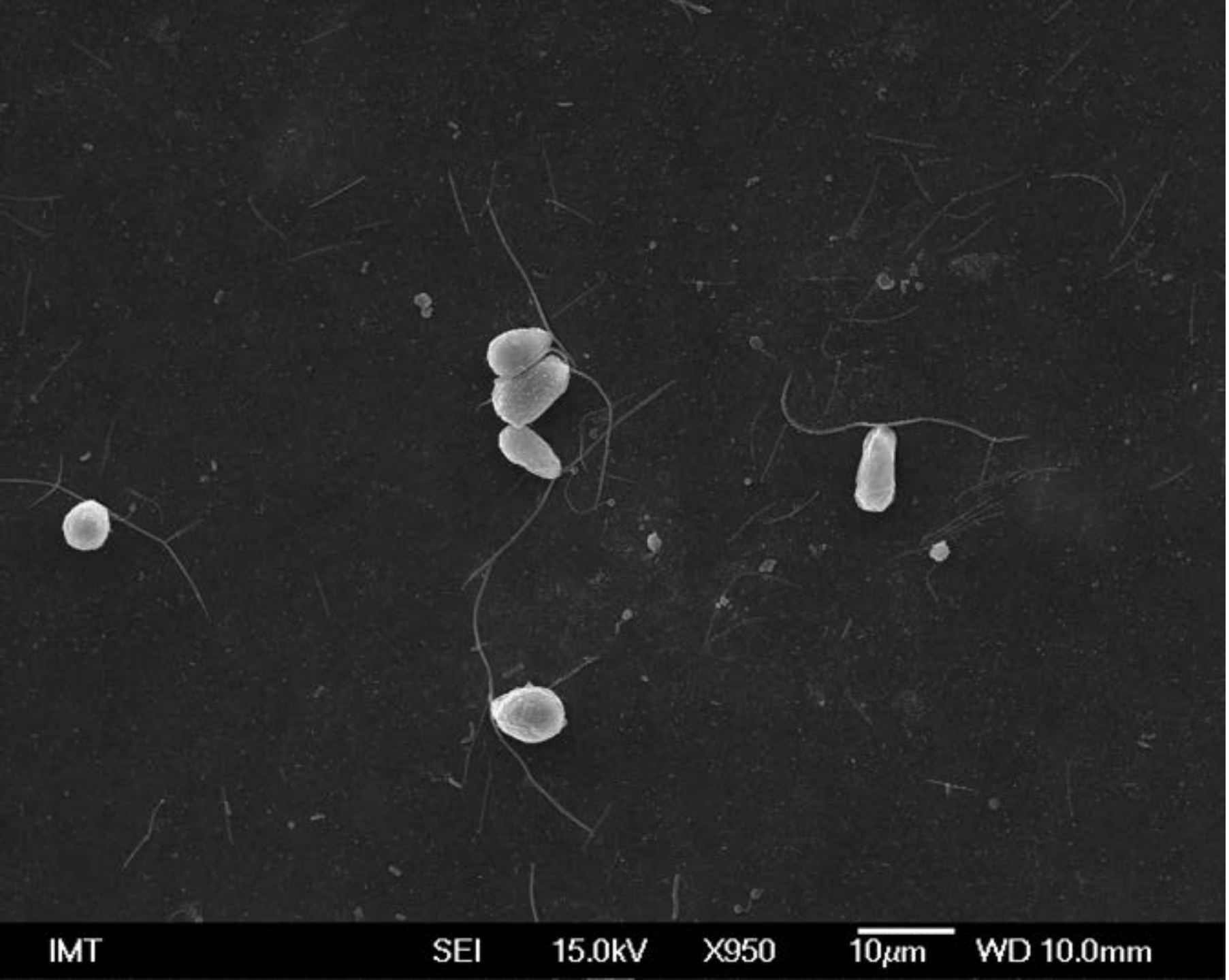

Figure *Dunaliella tertiolecta* culture SEM 1.

Cultivation of the algae
Culture of *D. tertiolecta* CCAP 19/22 from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 µmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.
Scanning Electron Microscopy (SEM)
Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

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DOI 10.5281/zenodo.6908895.

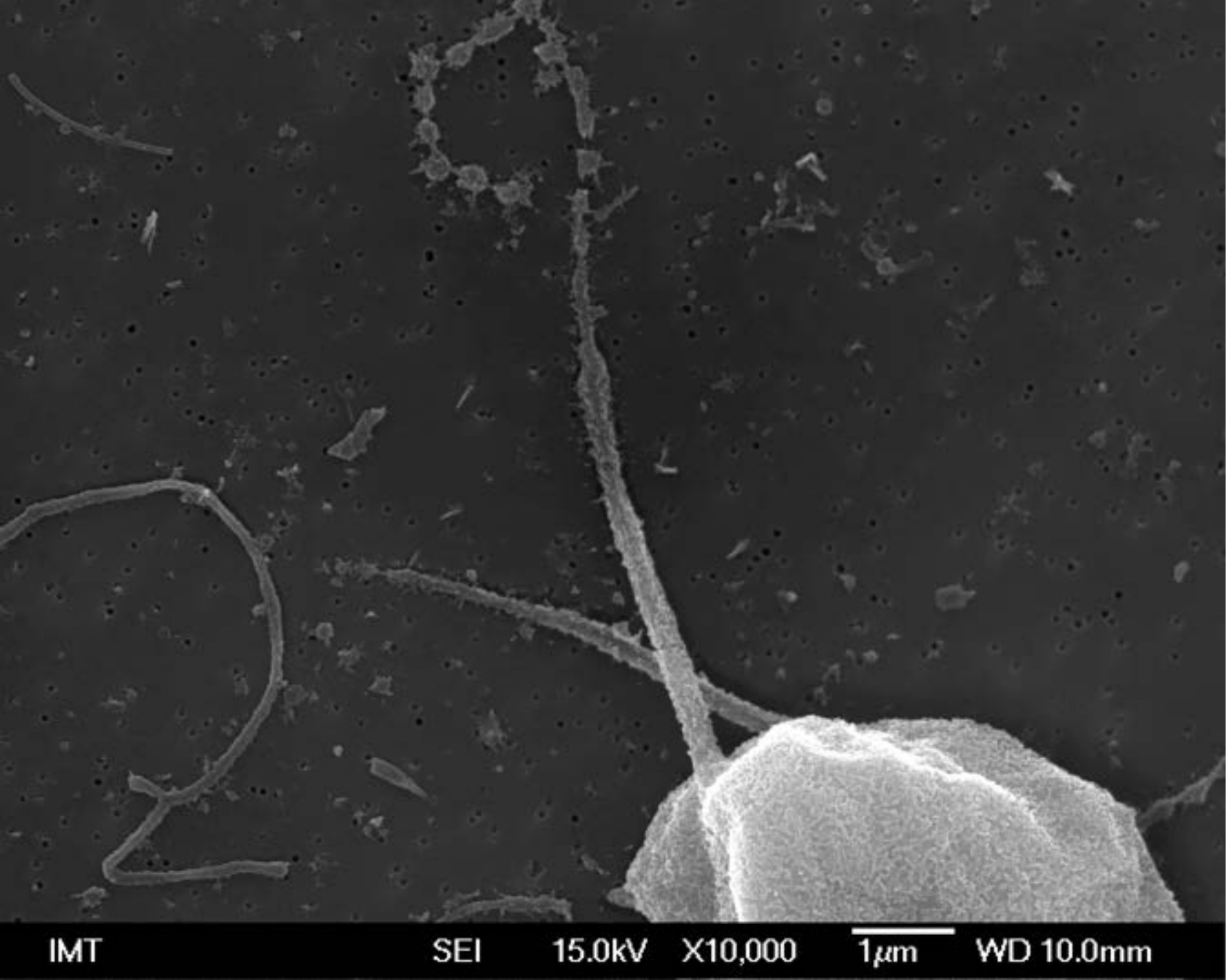

Figure *Dunaliella tertiolecta* culture SEM 2.

Culture of *D. tertiolecta* CCAP 19/22 from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

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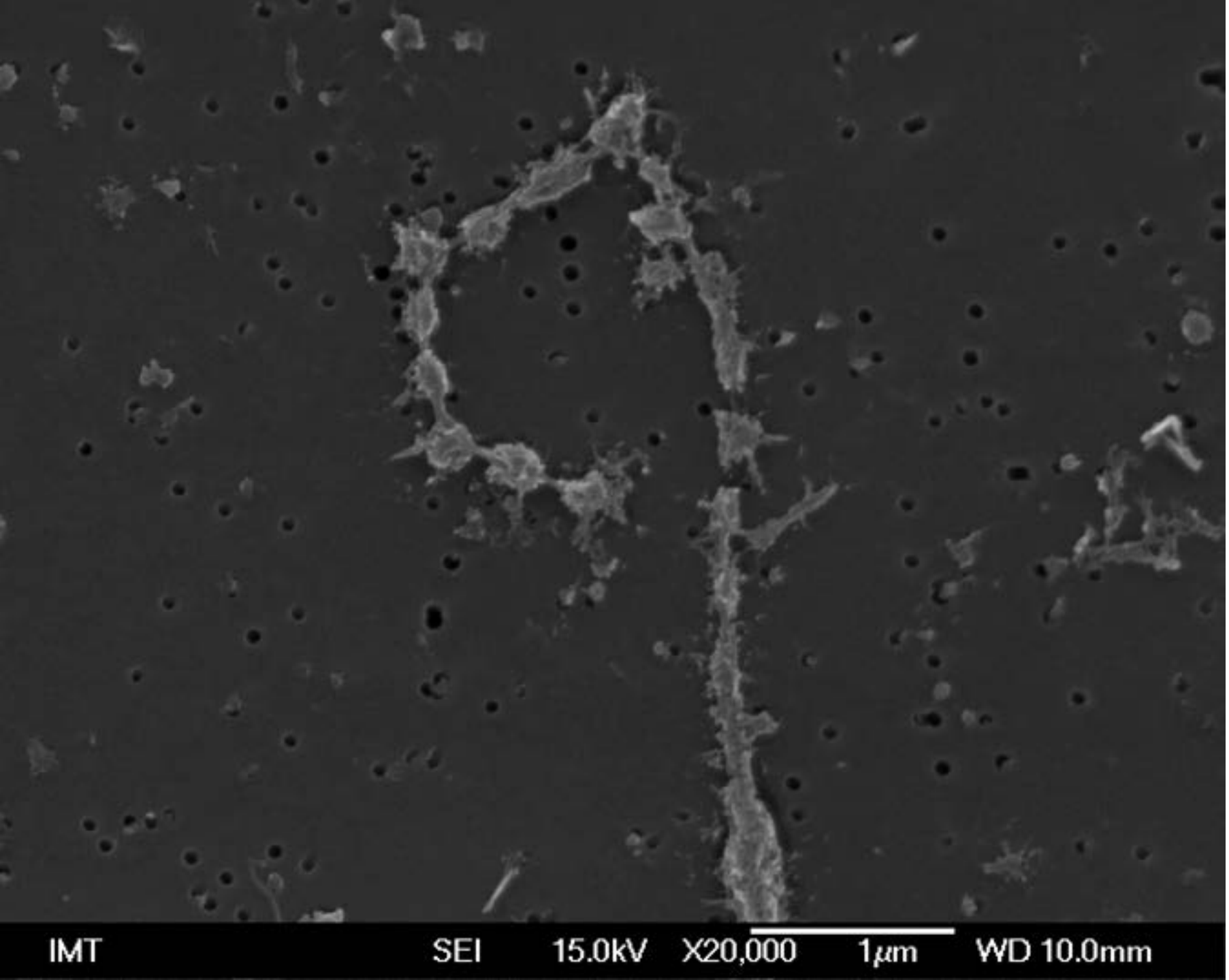

Figure *Dunaliella tertiolecta* culture SEM 3.

Cultivation of the algae
Culture of *D. tertiolecta* CCAP 19/22 from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 µmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.
Scanning Electron Microscopy (SEM)
Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

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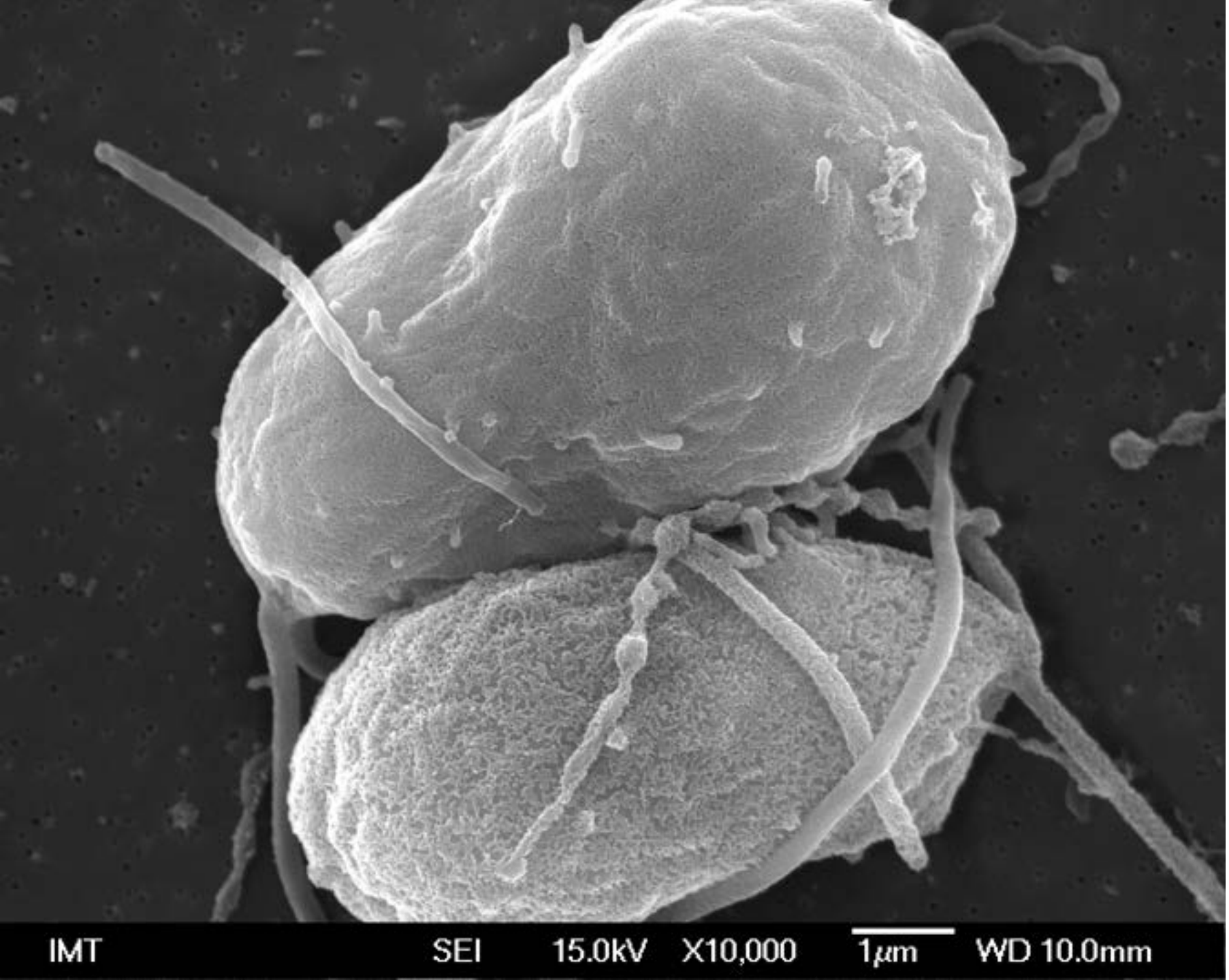

Figure *Dunaliella tertiolecta* culture SEM 4.

Cultivation of the algae
Culture of *D. tertiolecta* CCAP 19/22 from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 µmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.
Scanning Electron Microscopy (SEM)
Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

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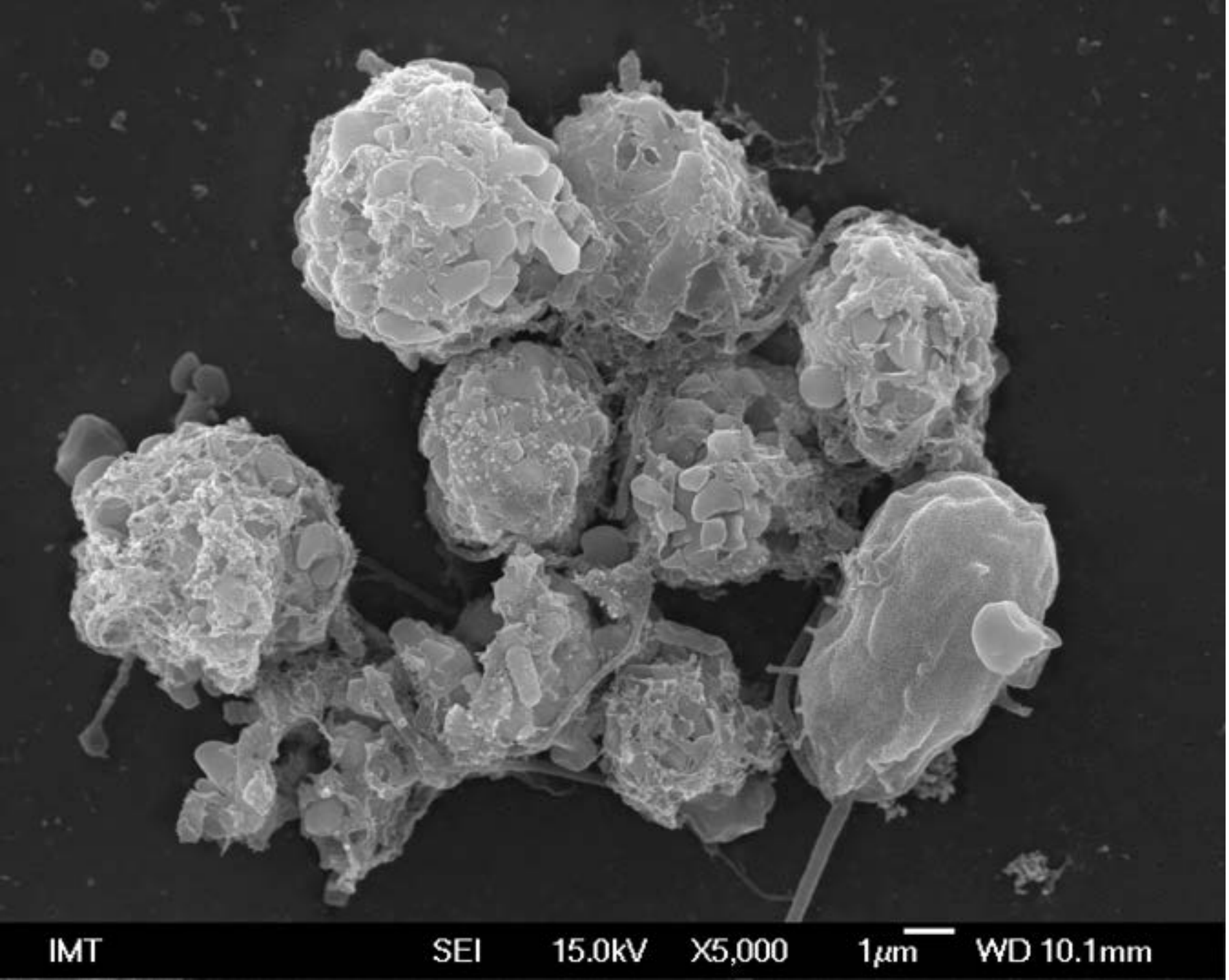

Figure *Dunaliella tertiolecta* culture SEM 5.

Cultivation of the algae
Culture of *D. tertiolecta* CCAP 19/22 from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 µmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.
Scanning Electron Microscopy (SEM)
Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

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DOI 10.5281/zenodo.6908895.

Figure *Dunaliella tertiolecta* culture SEM 6.

Cultivation of the algae

Culture of *D. tertiolecta* CCAP 19/22 from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 µmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

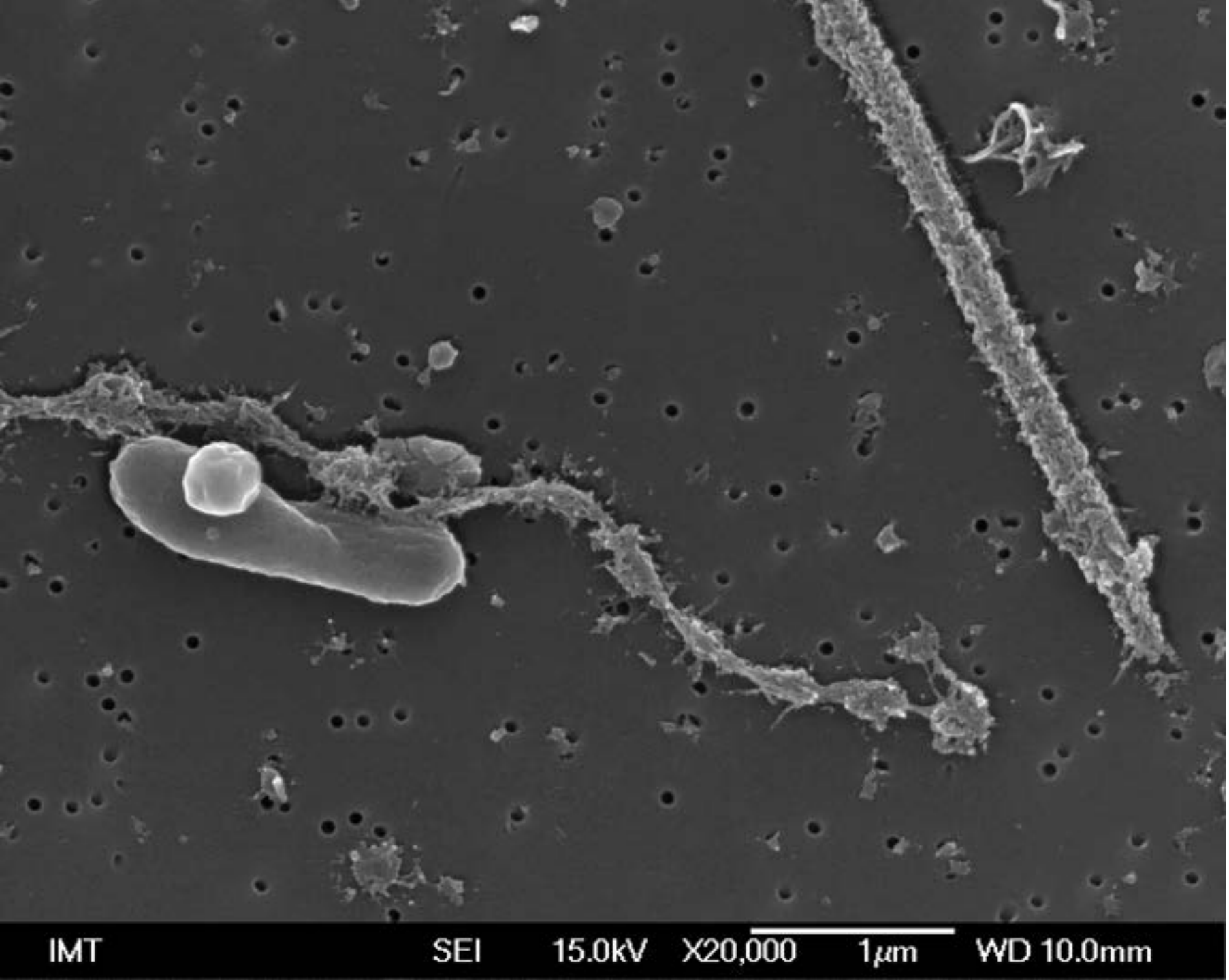

Figure *Dunaliella tertiolecta* culture SEM 7.

Cultivation of the algae

Culture of *D. tertiolecta* CCAP 19/22 from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

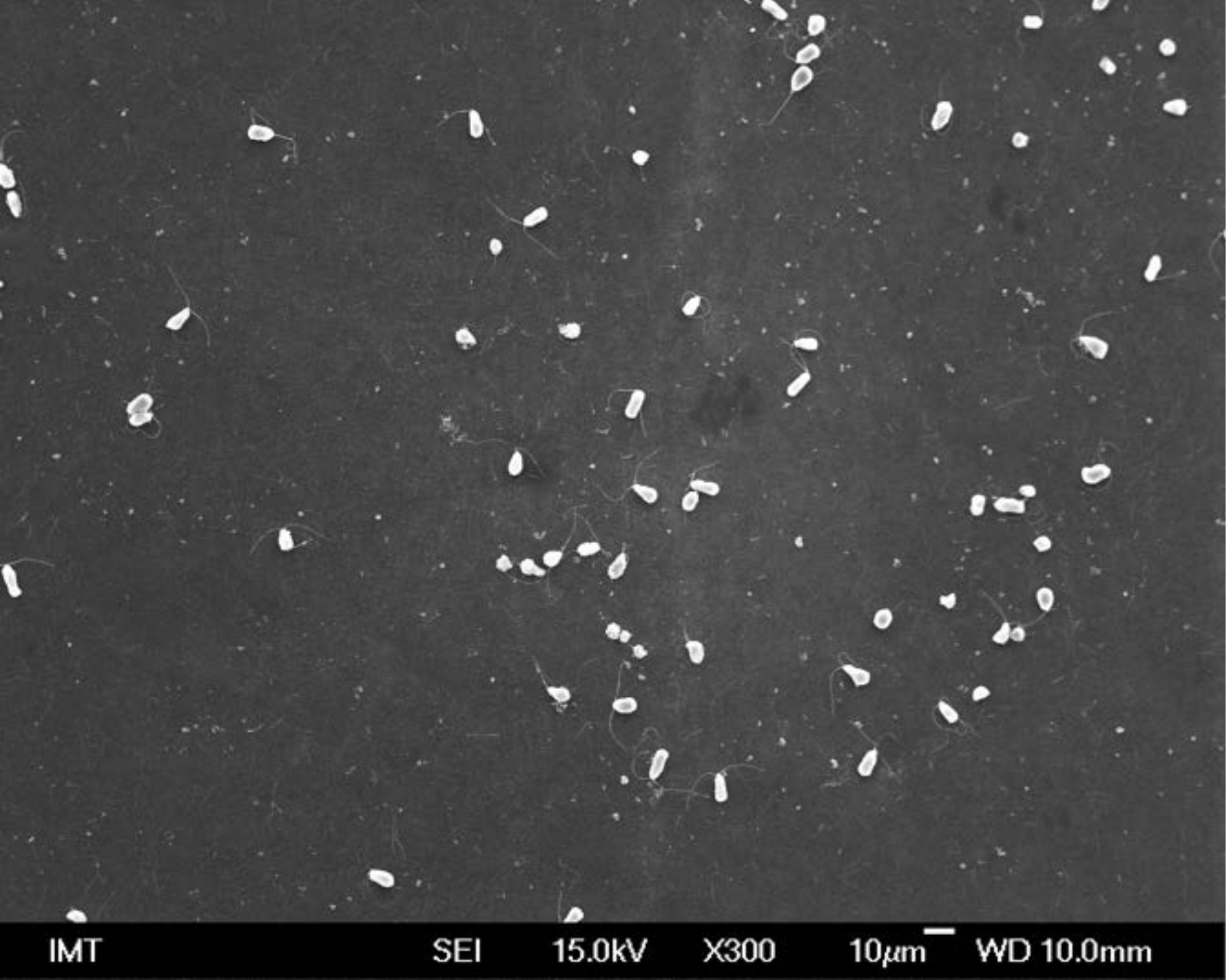

Figure *Dunaliella tertiolecta* culture SEM 8.

Cultivation of the algae

Culture of *D. tertiolecta* CCAP 19/22 from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 µmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

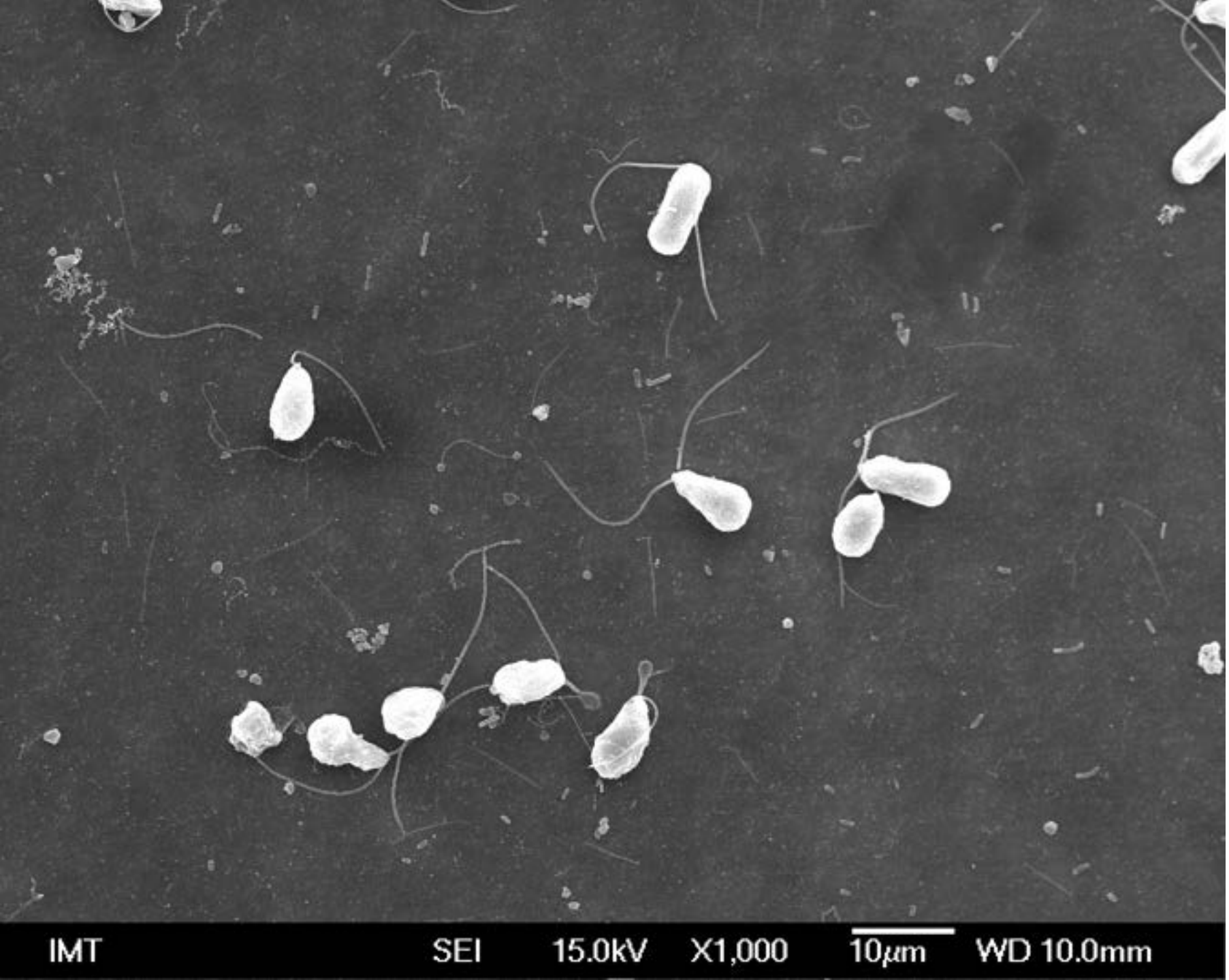

Figure *Dunaliella tertiolecta* culture SEM 9.

Cultivation of the algae

Culture of *D. tertiolecta* CCAP 19/22 from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 µmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

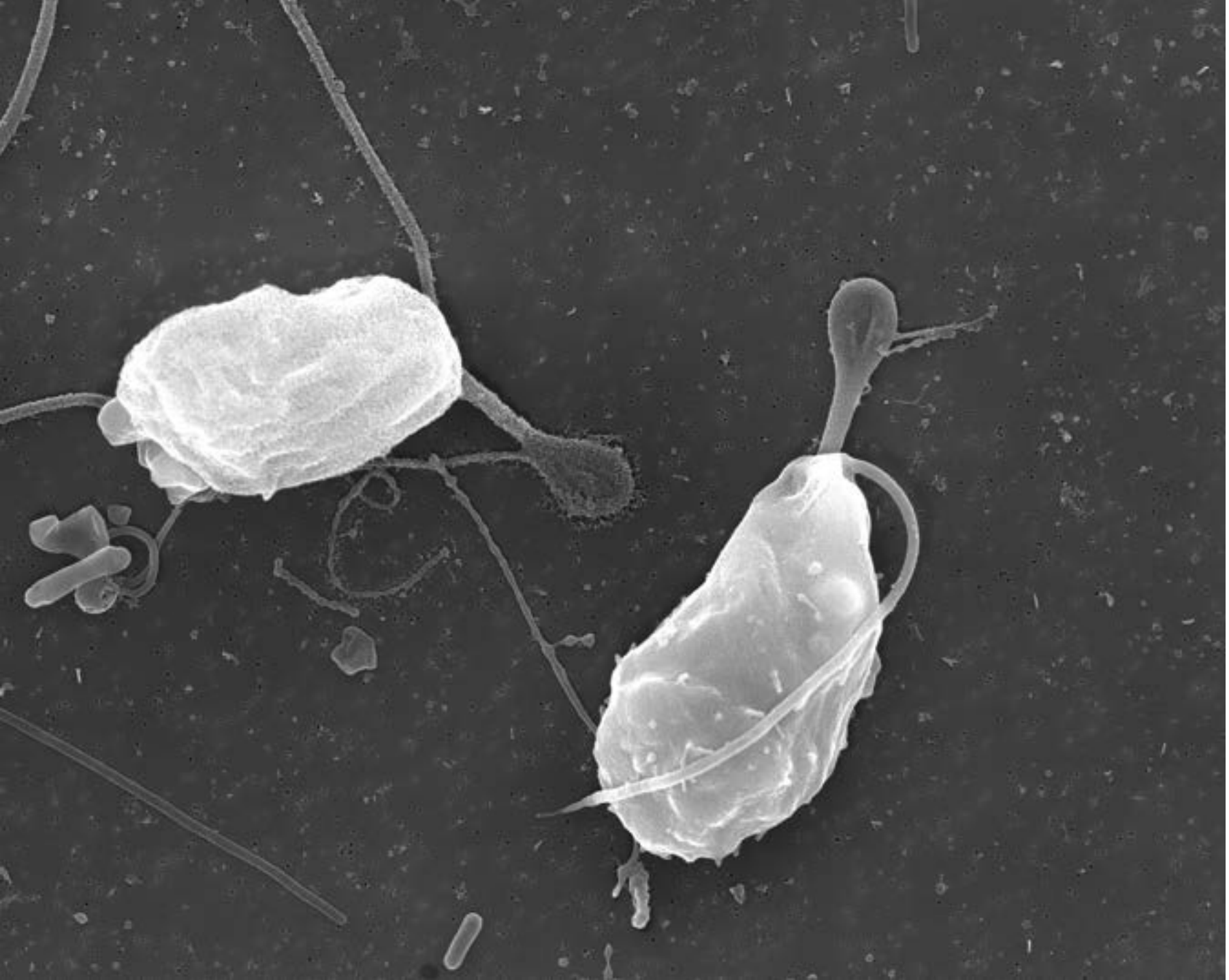

Figure *Dunaliella tertiolecta* culture SEM 10.

Cultivation of the algae

Culture of *D. tertiolecta* CCAP 19/22 from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 µmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

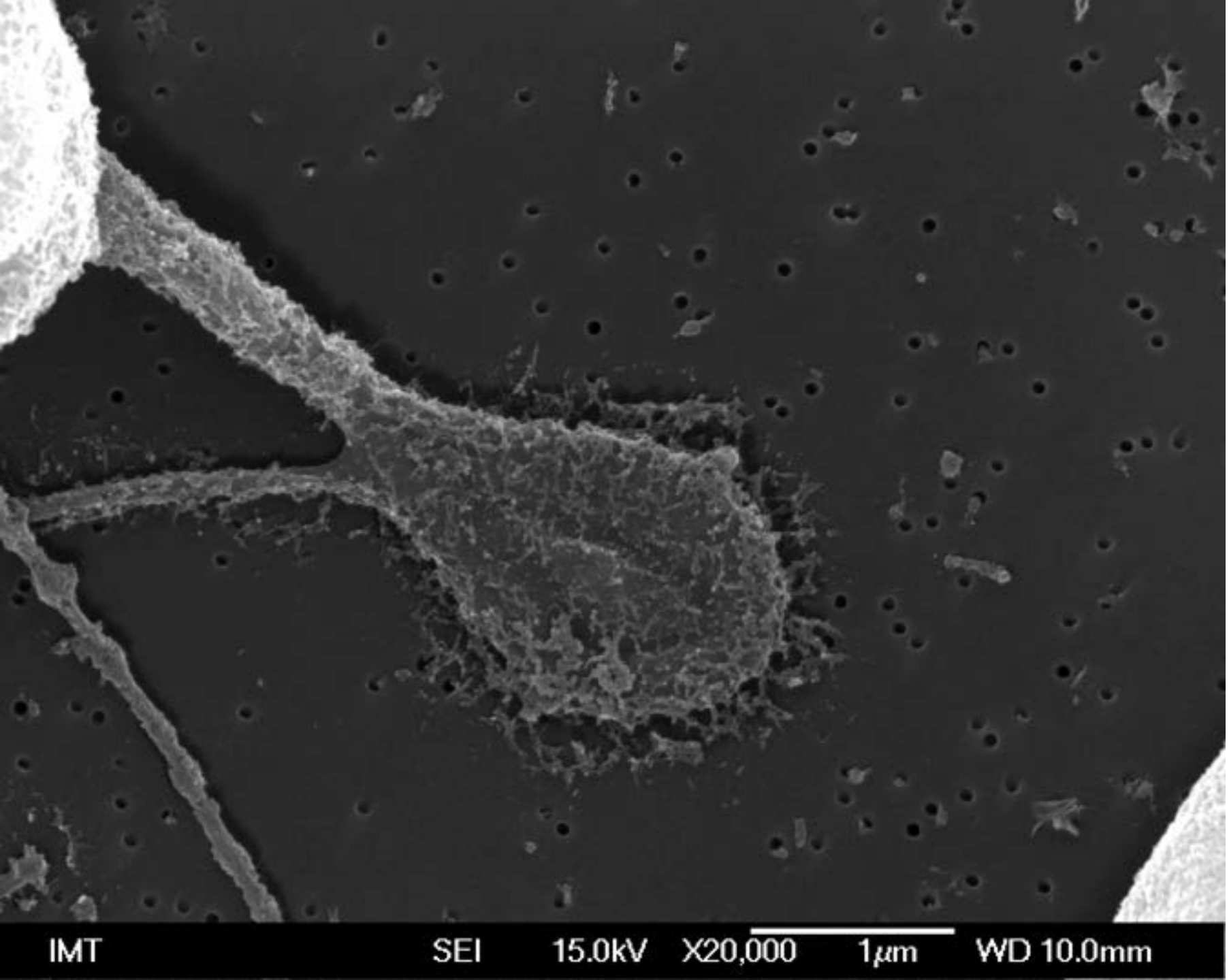

Figure *Dunaliella tertiolecta* culture SEM 11.

Cultivation of the algae

Culture of *D. tertiolecta* CCAP 19/22 from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 µmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

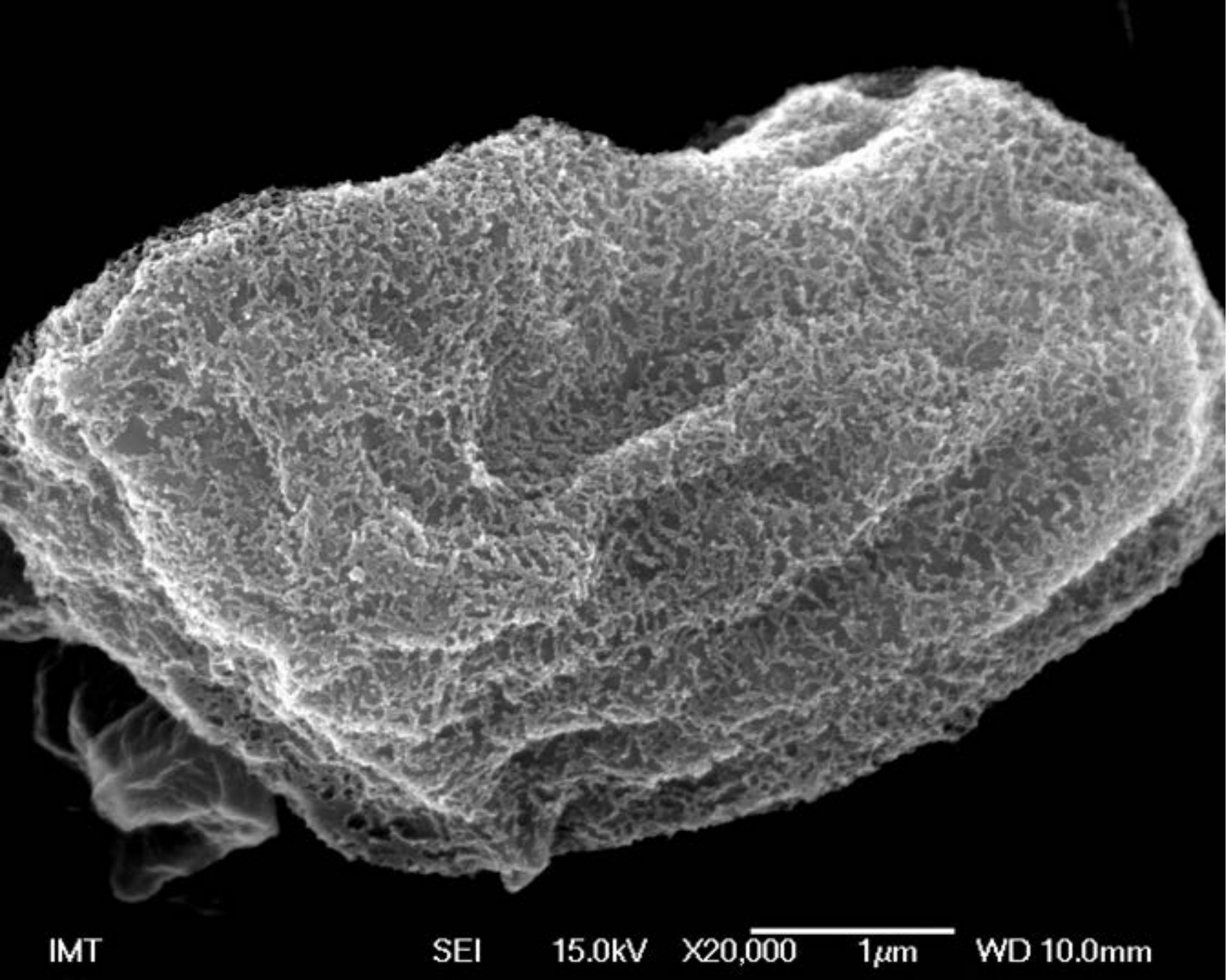

Figure *Dunaliella tertiolecta* culture SEM 12.

Cultivation of the algae

Culture of *D. tertiolecta* CCAP 19/22 from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

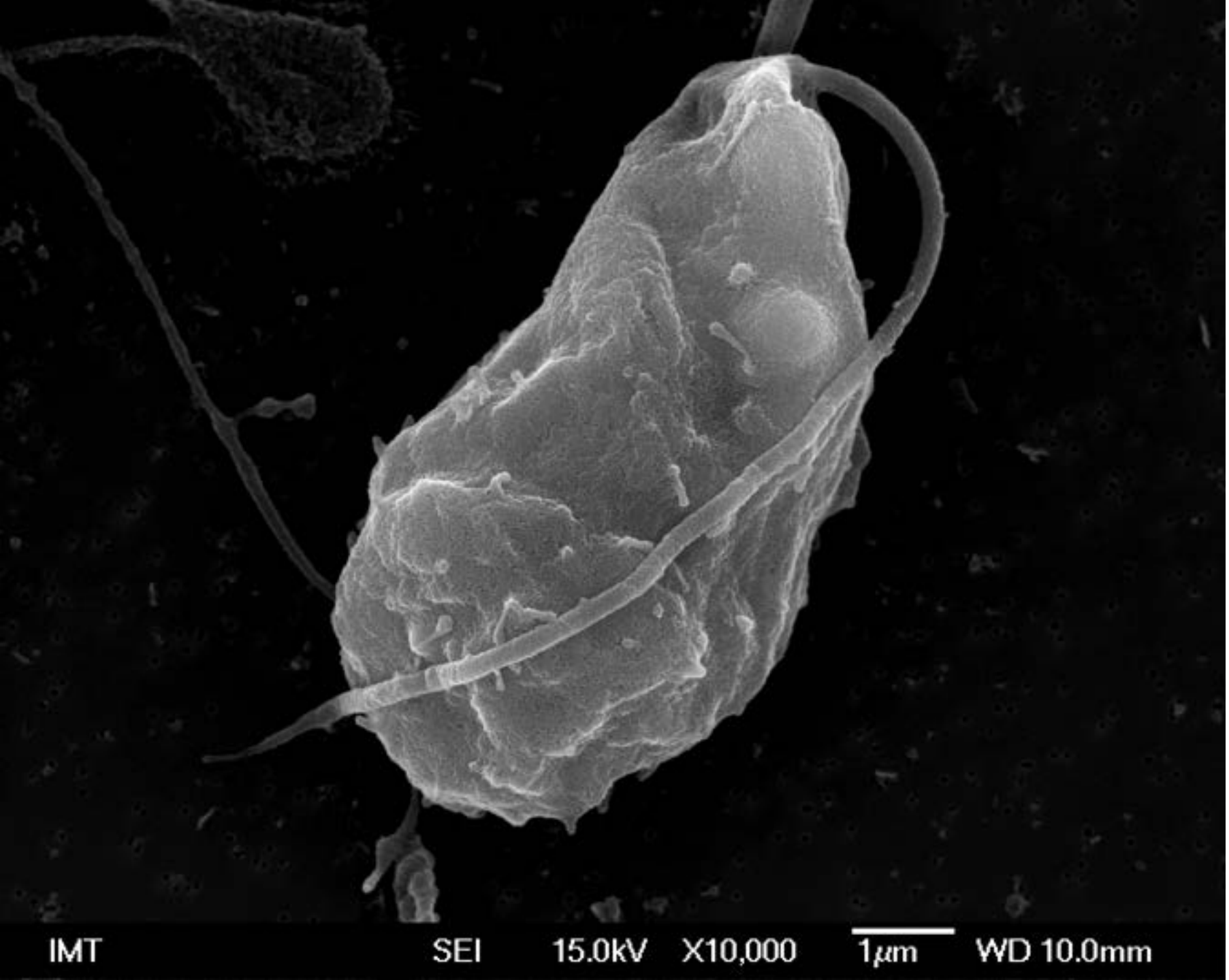

Figure *Dunaliella tertiolecta* culture SEM 13.

Cultivation of the algae
Culture of *D. tertiolecta* CCAP 19/22 from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Scanning Electron Microscopy (SEM)
Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

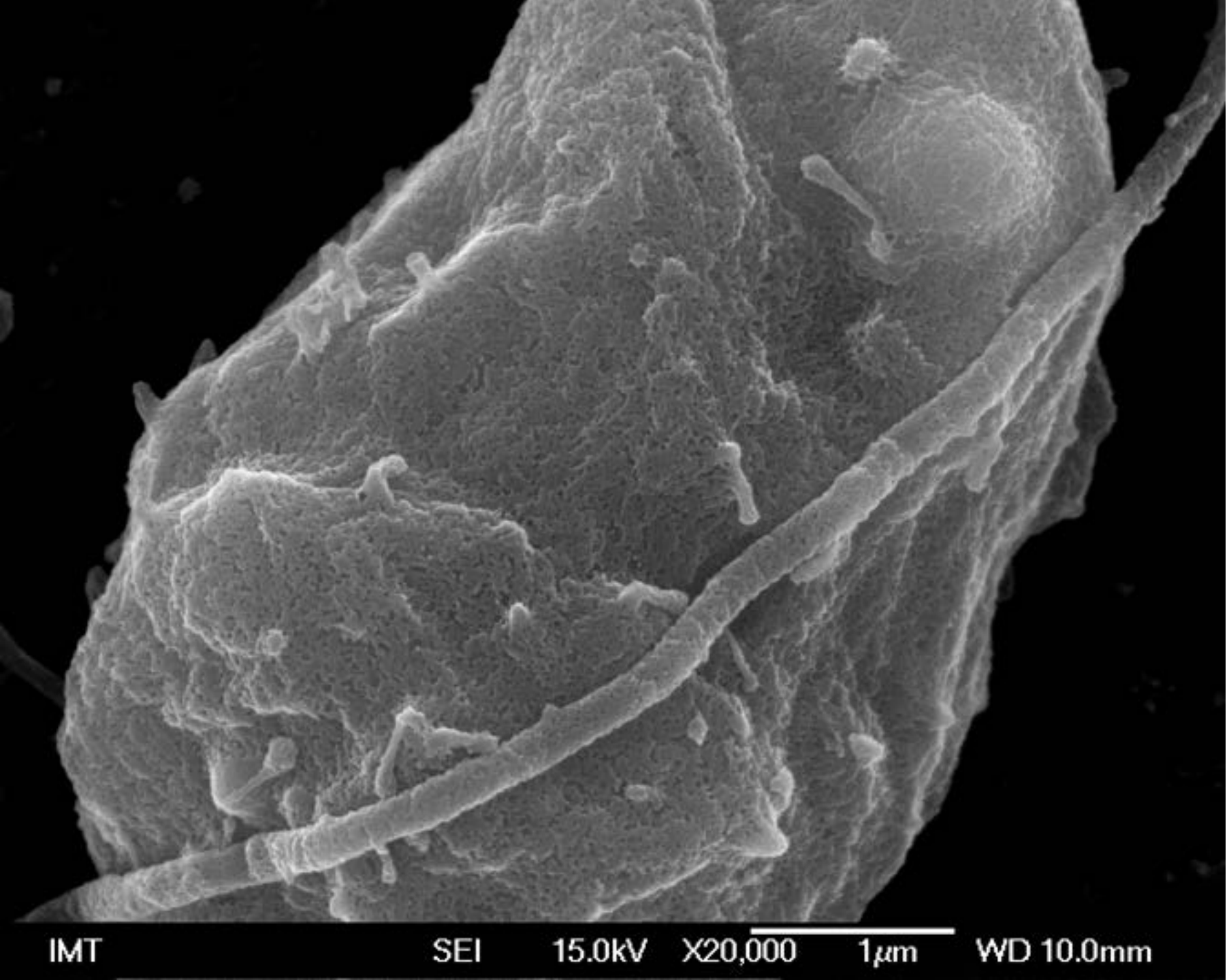

Figure *Dunaliella tertiolecta* culture SEM 14.

Cultivation of the algae

Culture of *D. tertiolecta* CCAP 19/22 from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 µmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

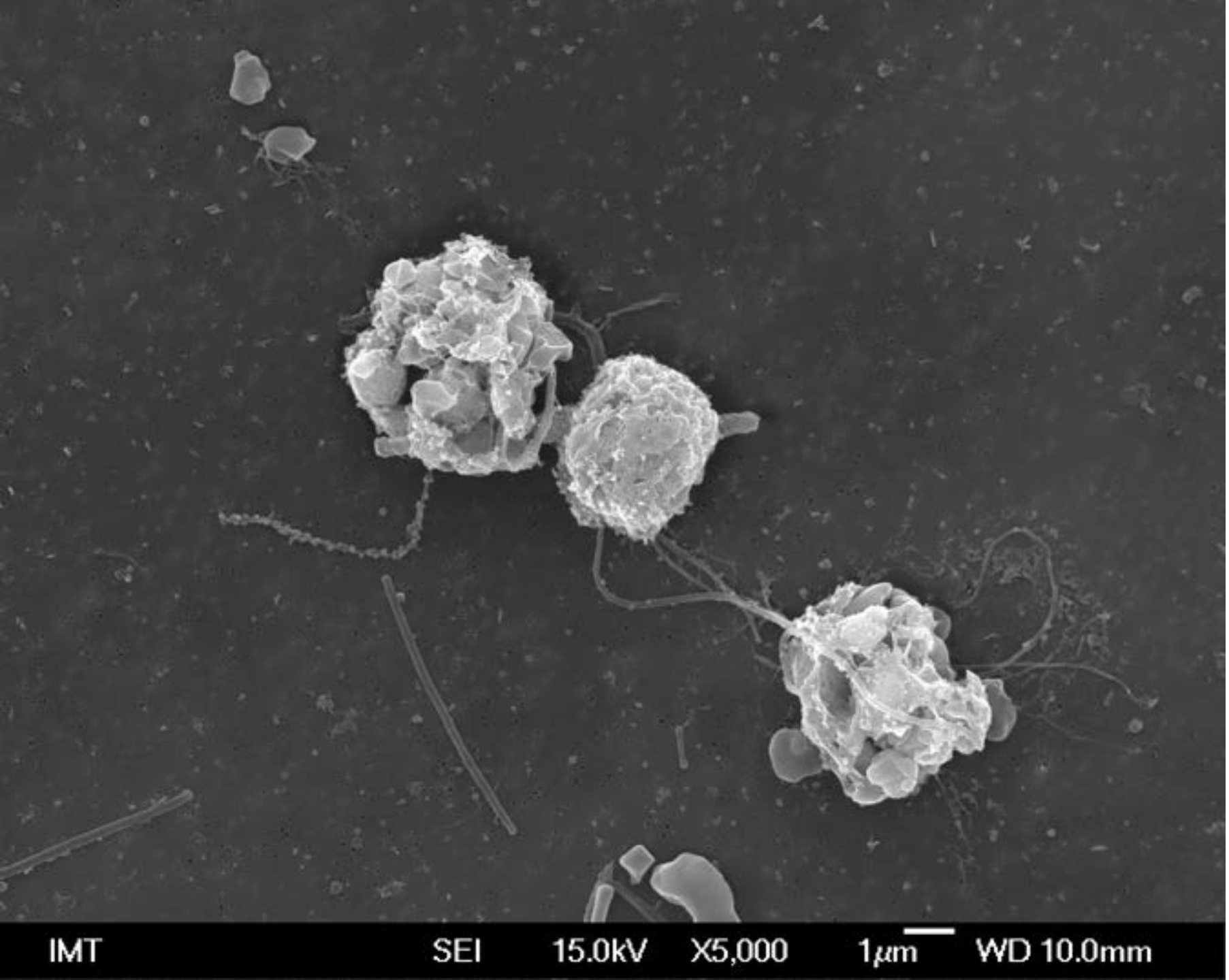

Figure *Dunaliella tertiolecta* culture SEM 15.

Cultivation of the algae

Culture of *D. tertiolecta* CCAP 19/22 from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

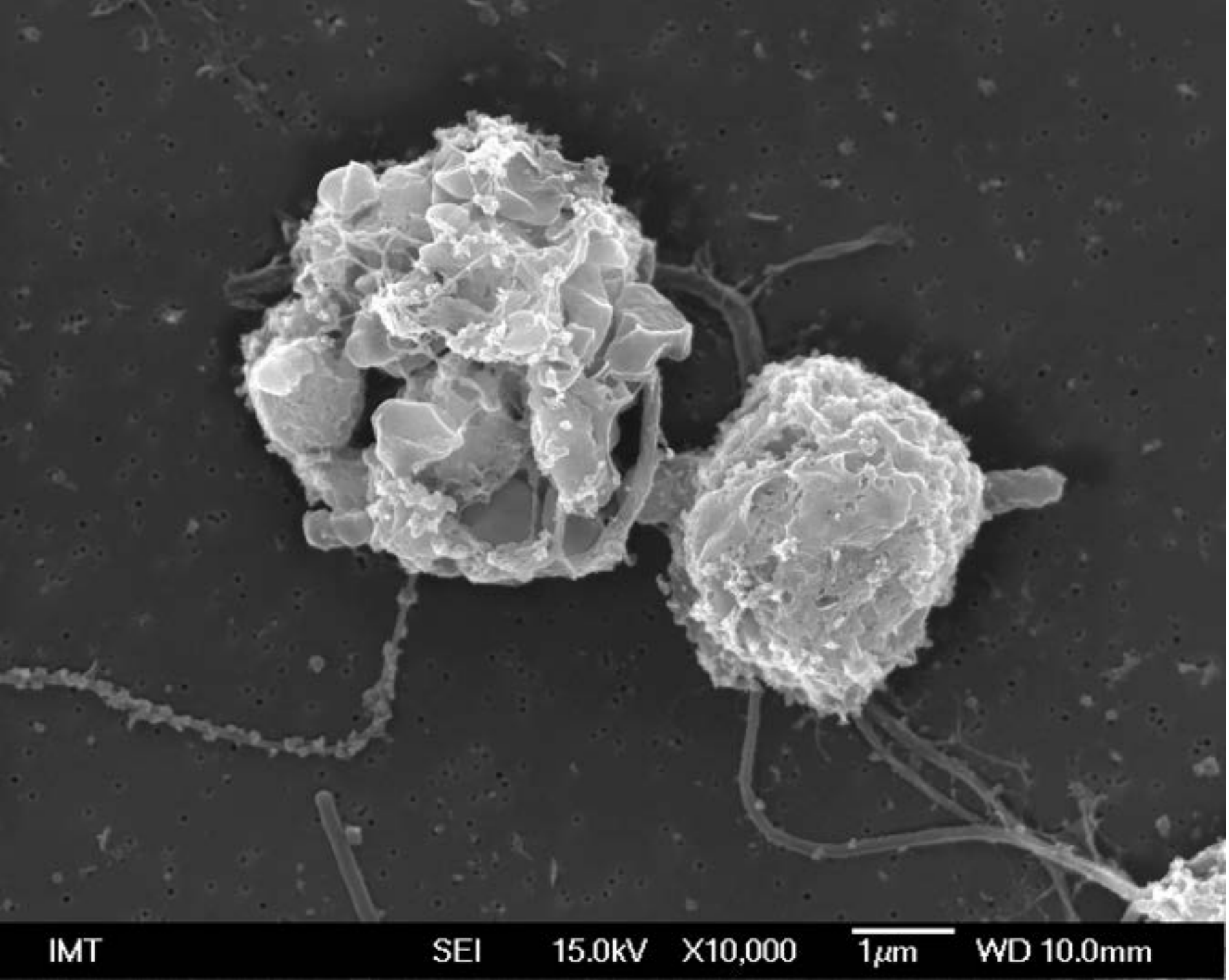

Figure *Dunaliella tertiolecta* culture SEM 16.

Cultivation of the algae

Culture of *D. tertiolecta* CCAP 19/22 from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

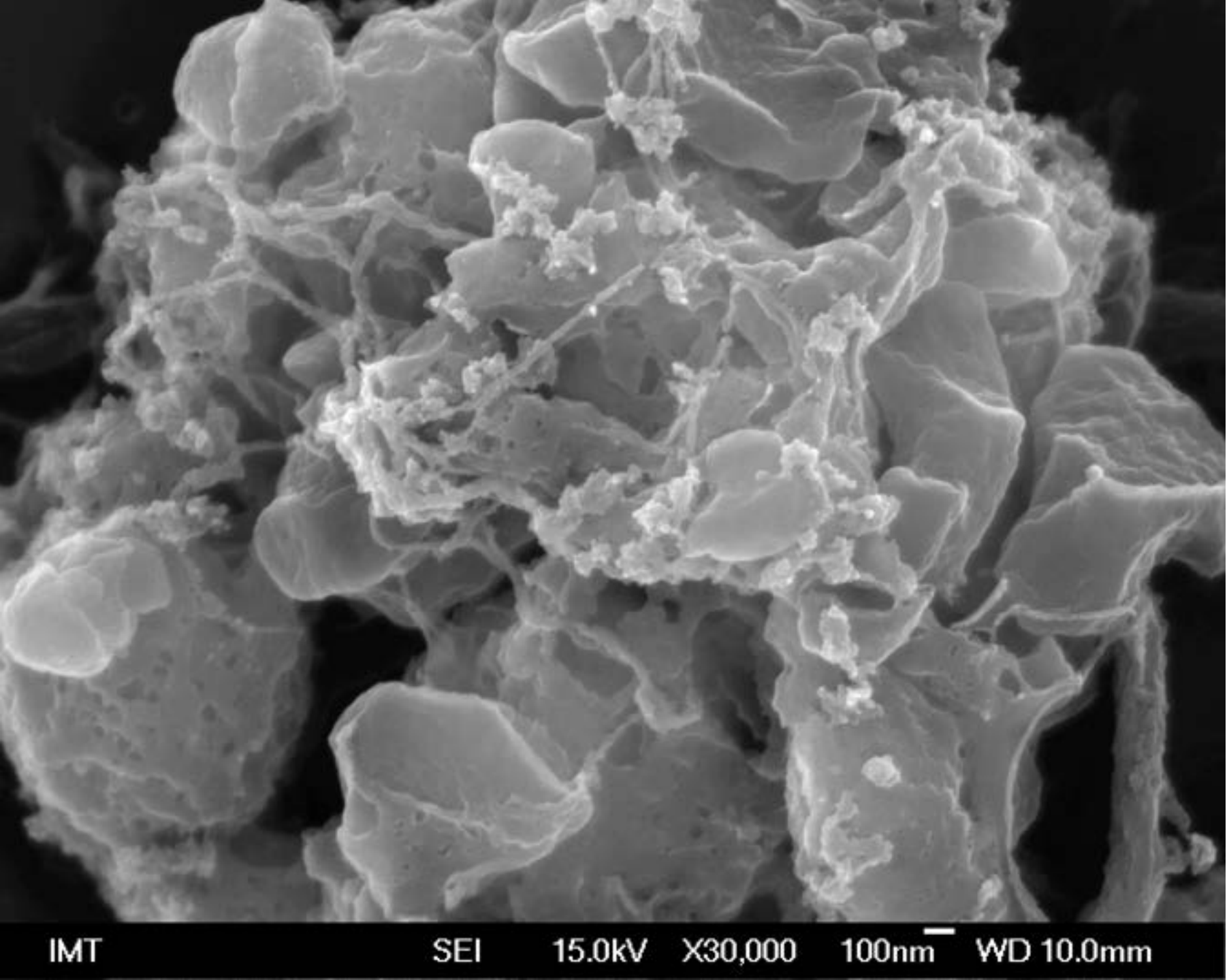

Figure *Dunaliella tertiolecta* culture SEM 17.

Cultivation of the algae

Culture of *D. tertiolecta* CCAP 19/22 from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 µmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

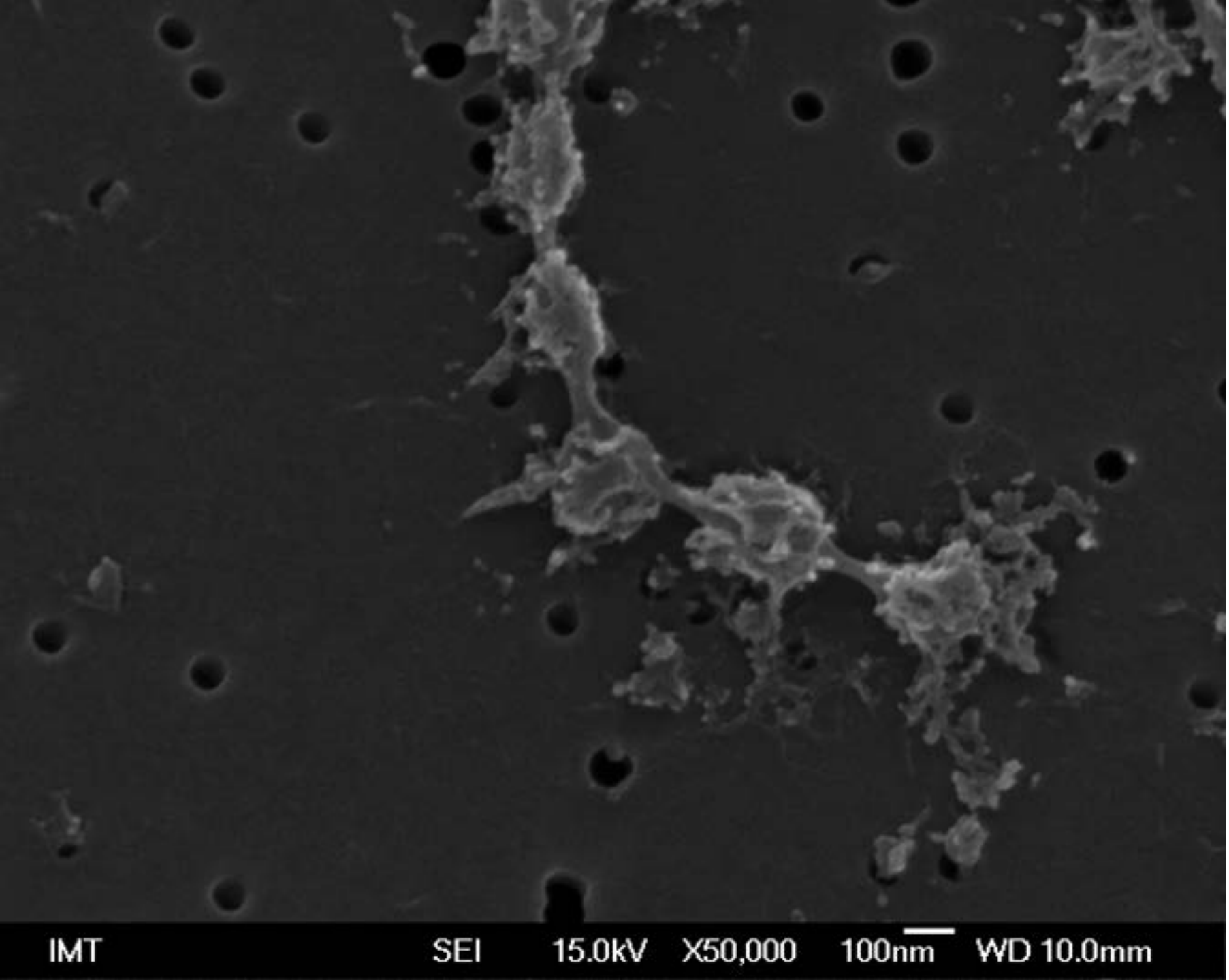

Figure *Dunaliella tertiolecta* culture SEM 18.

Cultivation of the algae

Culture of *D. tertiolecta* CCAP 19/22 from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 µmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

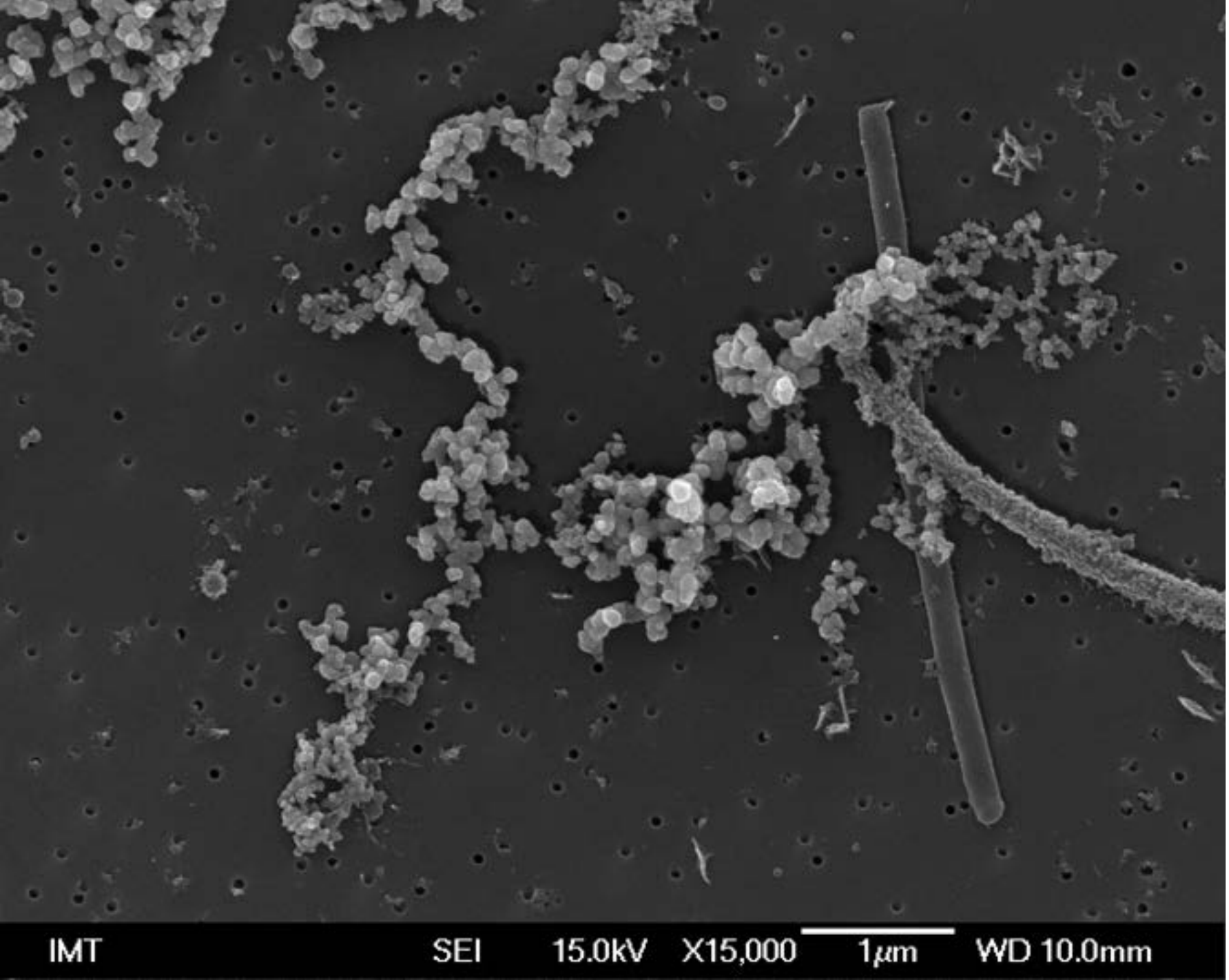

Figure *Dunaliella tertiolecta* culture SEM 19.

Cultivation of the algae

Culture of *D. tertiolecta* CCAP 19/22 from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 µmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

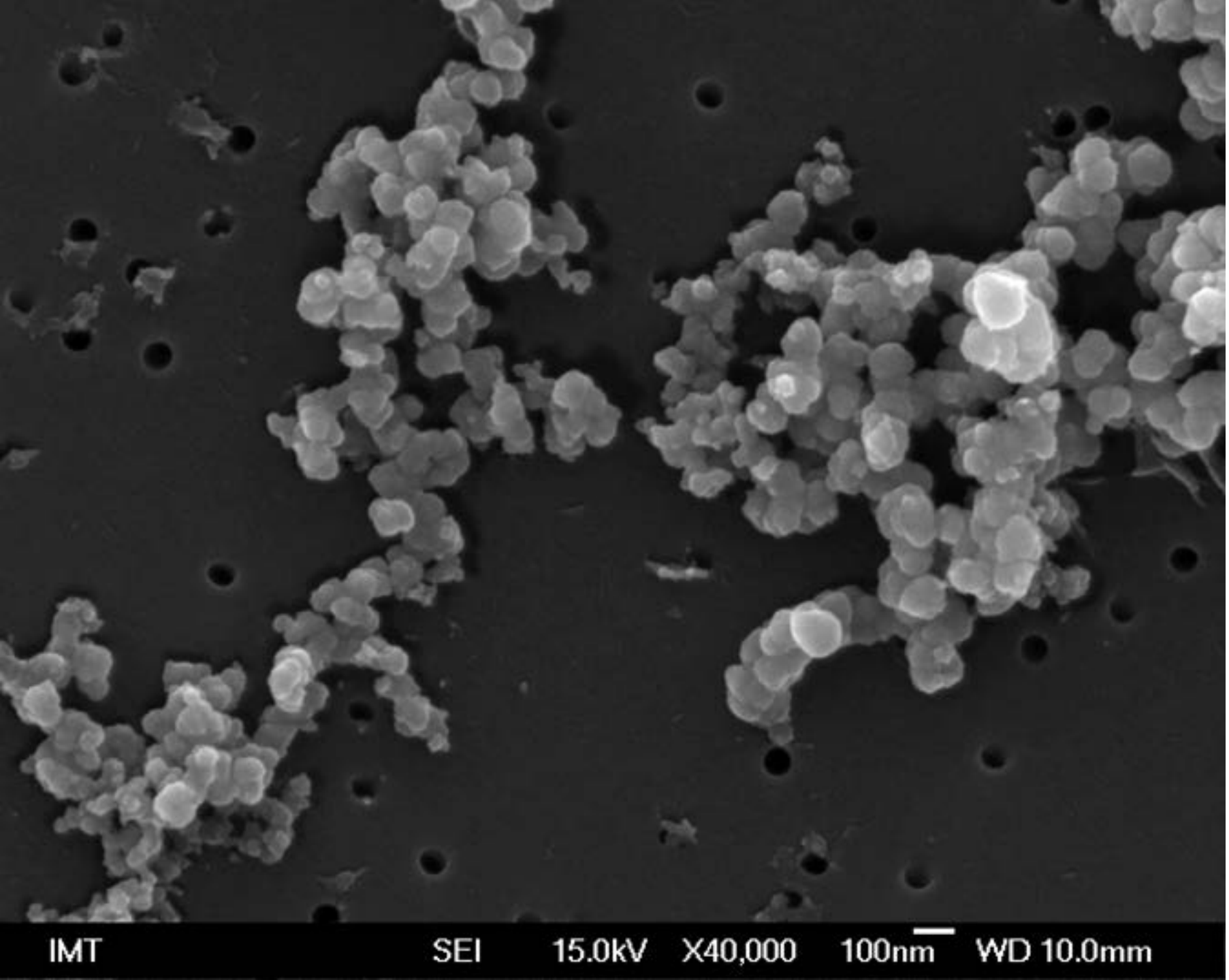

Figure *Dunaliella tertiolecta* culture SEM 20.

Cultivation of the algae

Culture of *D. tertiolecta* CCAP 19/22 from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 µmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

Figure *Dunaliella tertiolecta* culture SEM 21.

Cultivation of the algae

Culture of *D. tertiolecta* CCAP 19/22 from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 µmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

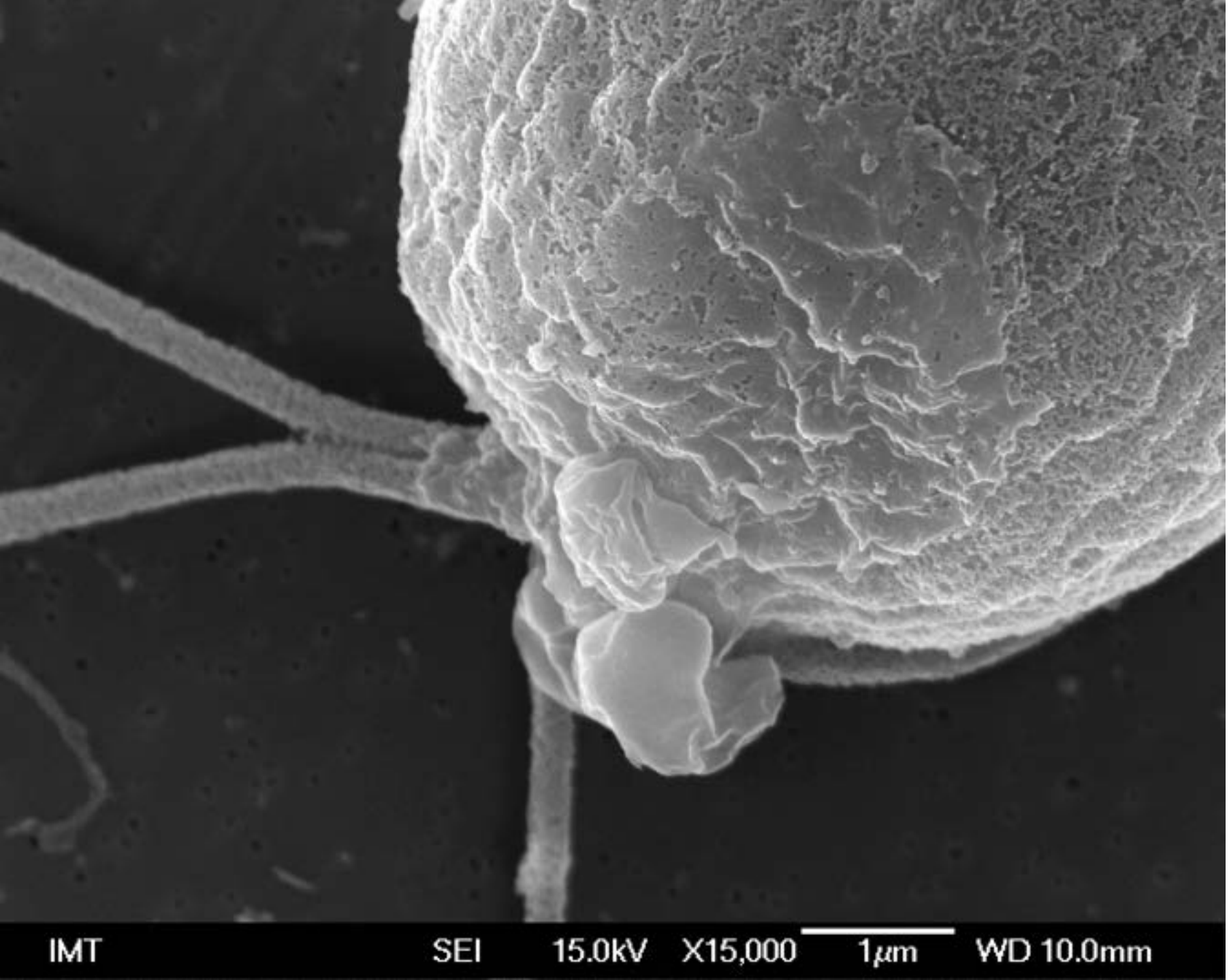

Figure *Dunaliella tertiolecta* culture SEM 22.

Cultivation of the algae

Culture of *D. tertiolecta* CCAP 19/22 from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

Figure *Dunaliela tertiolecta* culture SEM 23.

Cultivation of the algae

Culture of *D. tertiolecta* CCAP 19/22 from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 µmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

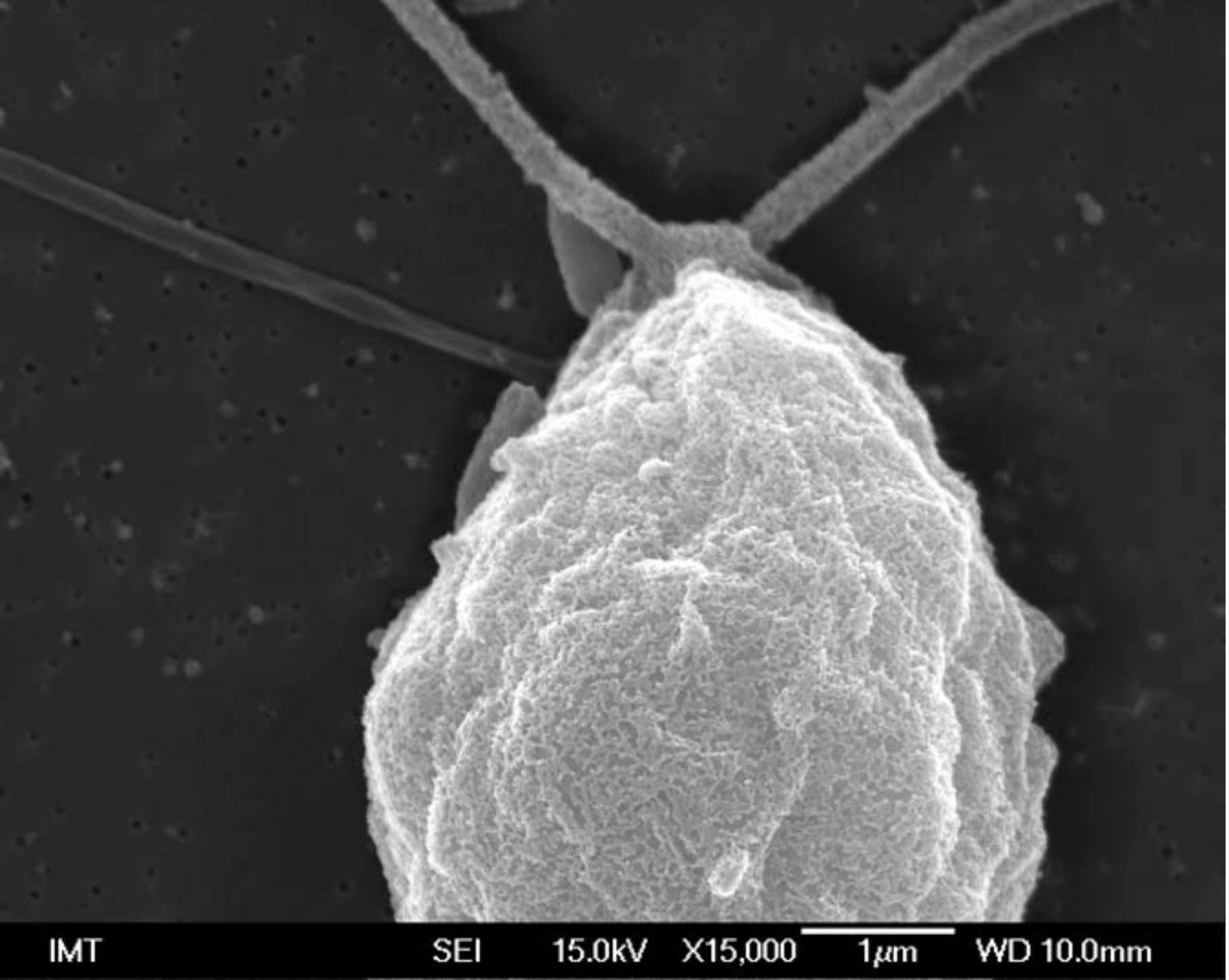

Figure *Dunaliella tertiolecta* culture SEM 24.

Cultivation of the algae

Culture of *D. tertiolecta* CCAP 19/22 from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

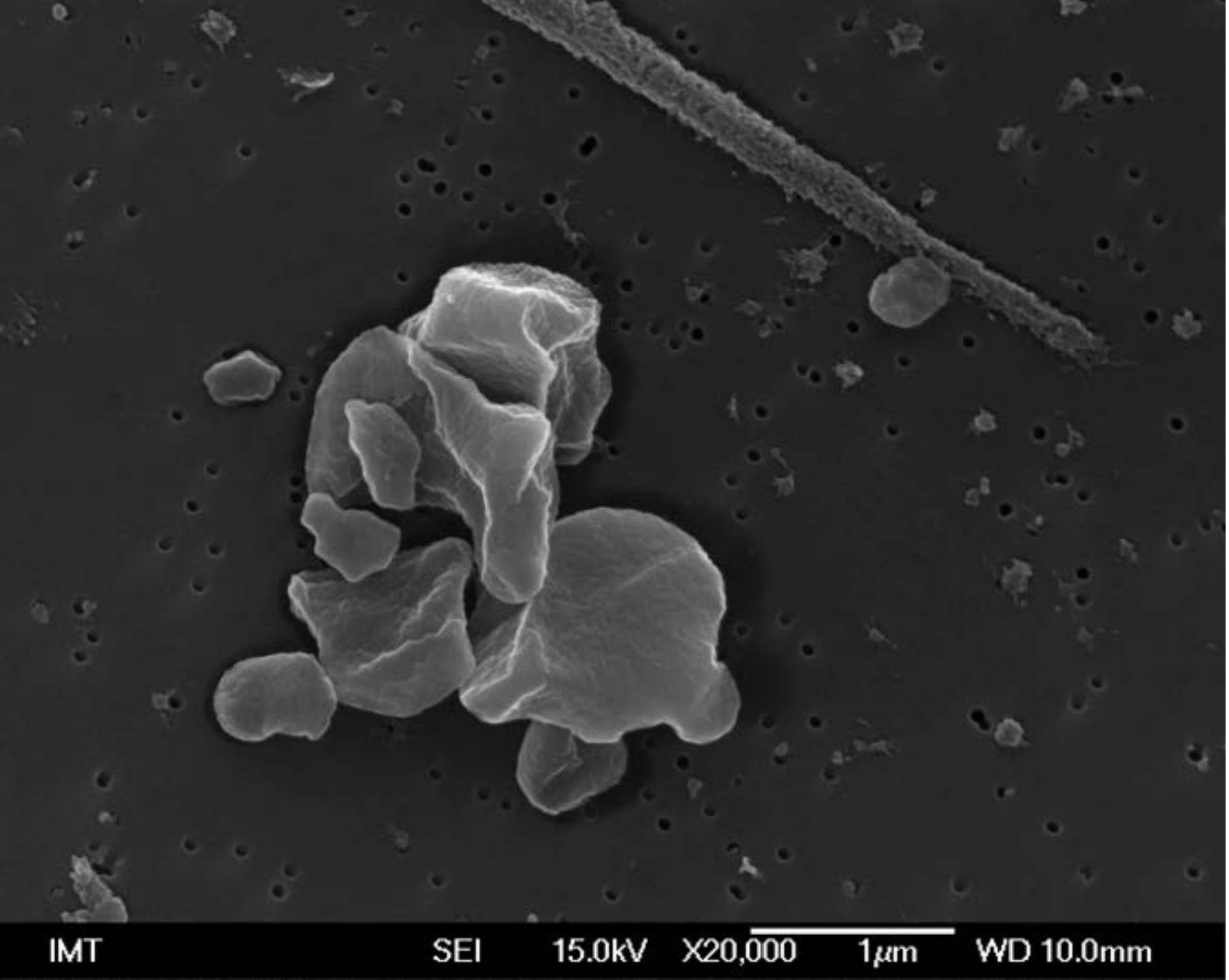

Figure *Dunaliella tertiolecta* culture SEM 25.

Cultivation of the algae

Culture of *D. tertiolecta* CCAP 19/22 from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

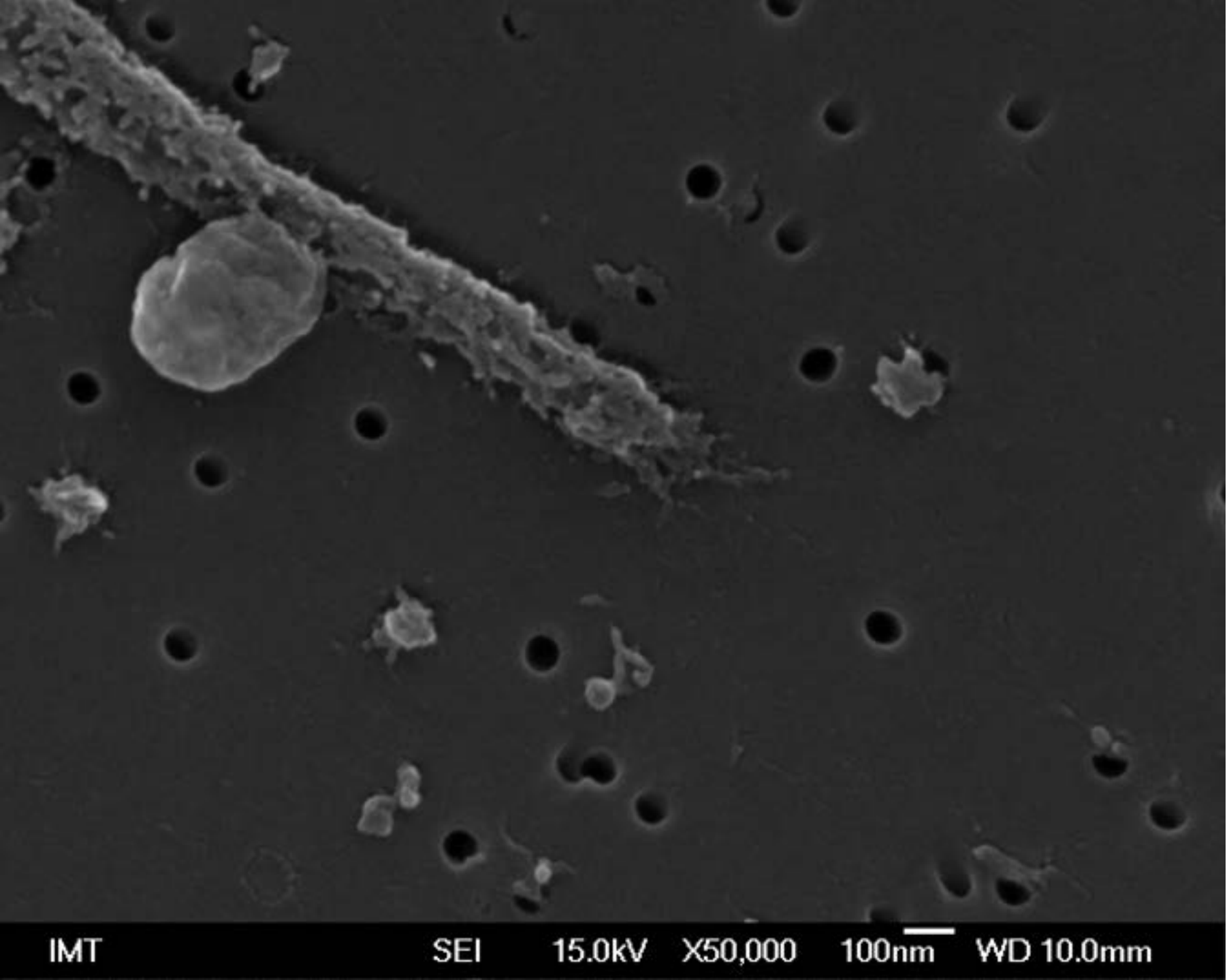

Figure *Dunaliella tertiolecta* culture SEM 26.

Cultivation of the algae

Culture of *D. tertiolecta* CCAP 19/22 from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 µmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

Figure *Dunaliella tertiolecta* culture SEM 27.

Cultivation of the algae

Culture of *D. tertiolecta* CCAP 19/22 from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

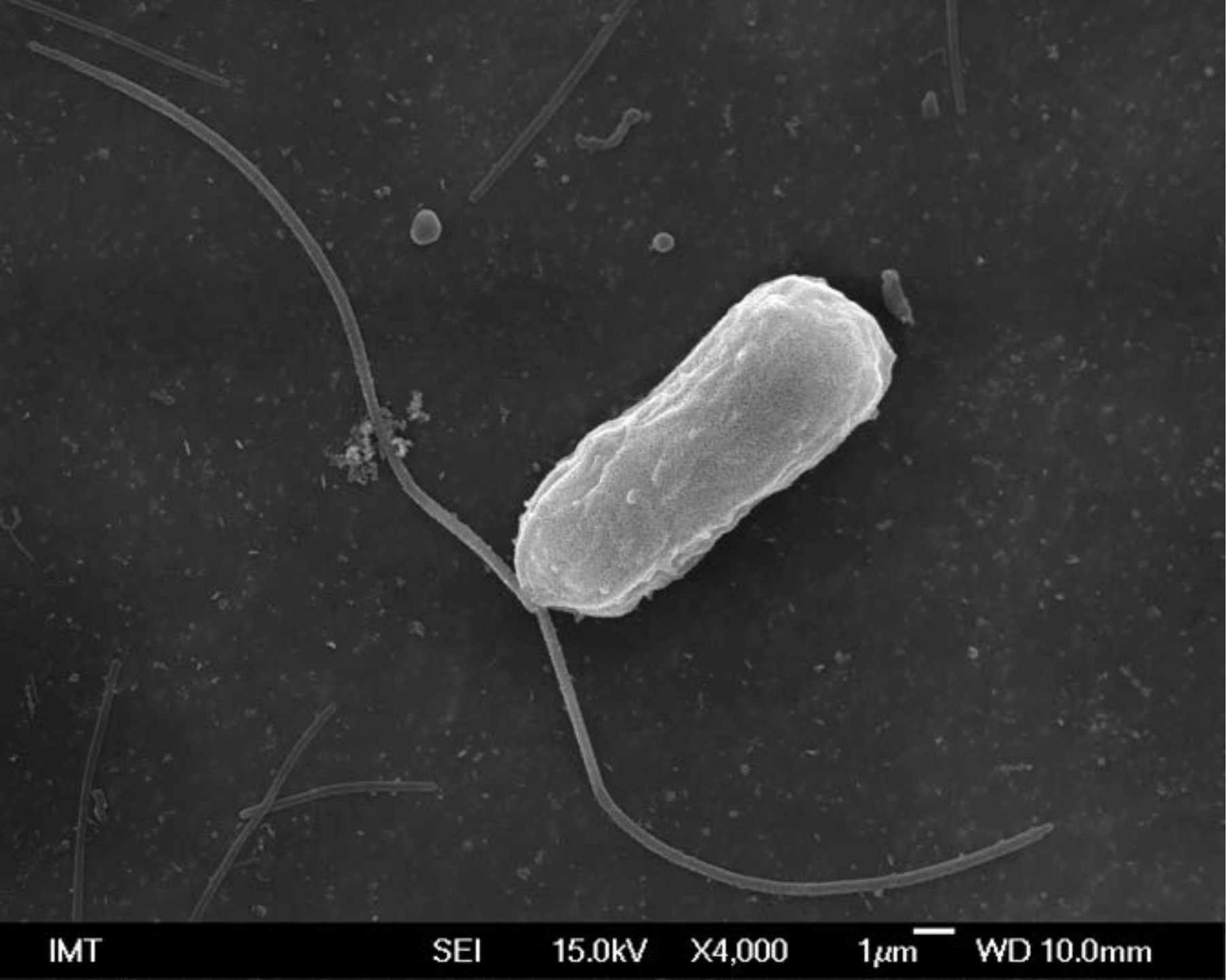

Figure *Dunaliela tertiolecta* culture SEM 28.

Cultivation of the algae

Culture of *D. tertiolecta* CCAP 19/22 from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 µmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

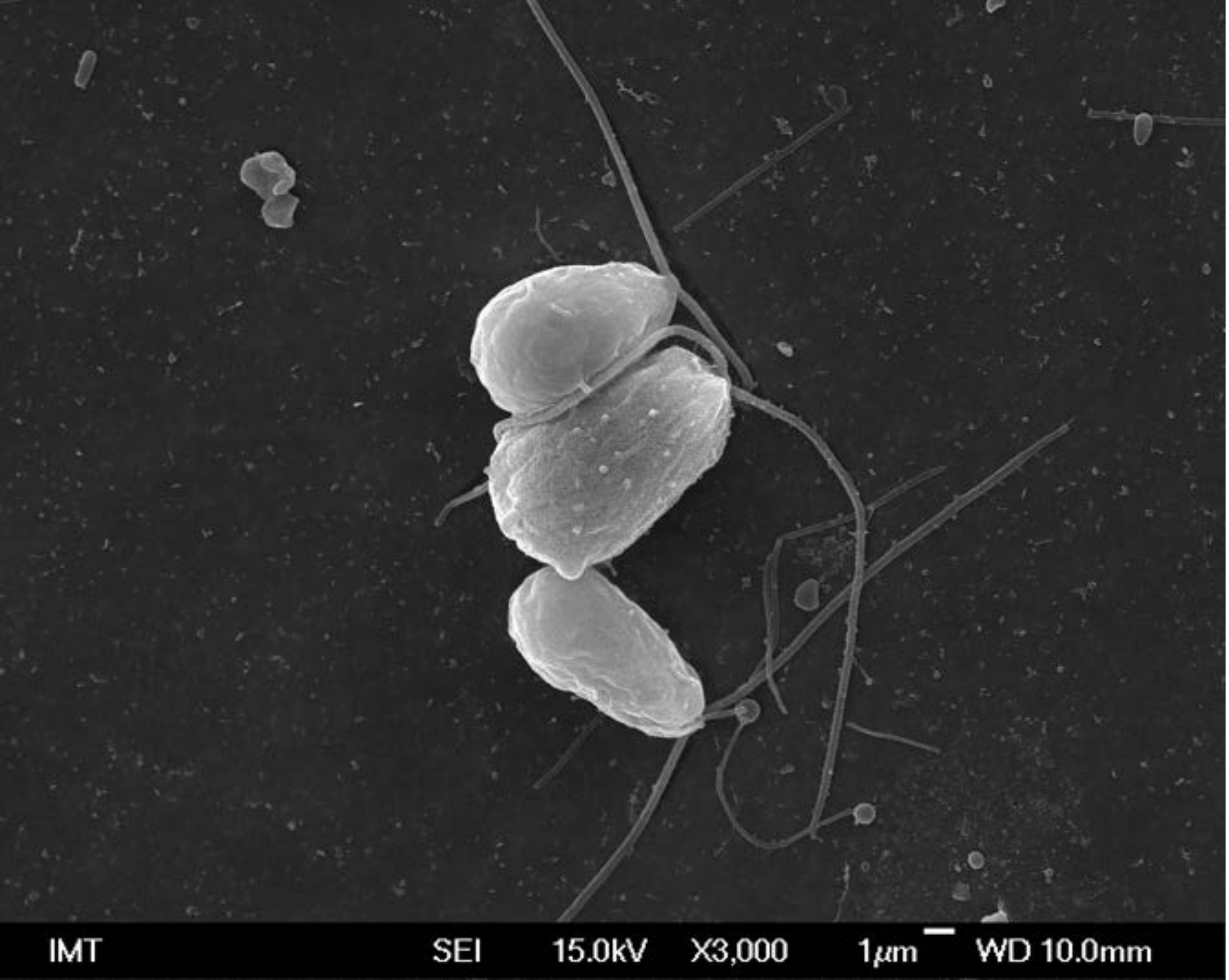

Figure *Dunaliella tertiolecta* culture SEM 29.

Cultivation of the algae

Culture of *D. tertiolecta* CCAP 19/22 from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 µmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

Figure *Dunaliella tertiolecta* culture SEM 30.

Cultivation of the algae
Culture of *D. tertiolecta* CCAP 19/22 from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 µmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.
Scanning Electron Microscopy (SEM)
Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).
From: https://zenodo.org/record/6908895. Image: 1
DOI 10.5281/zenodo.6908895.

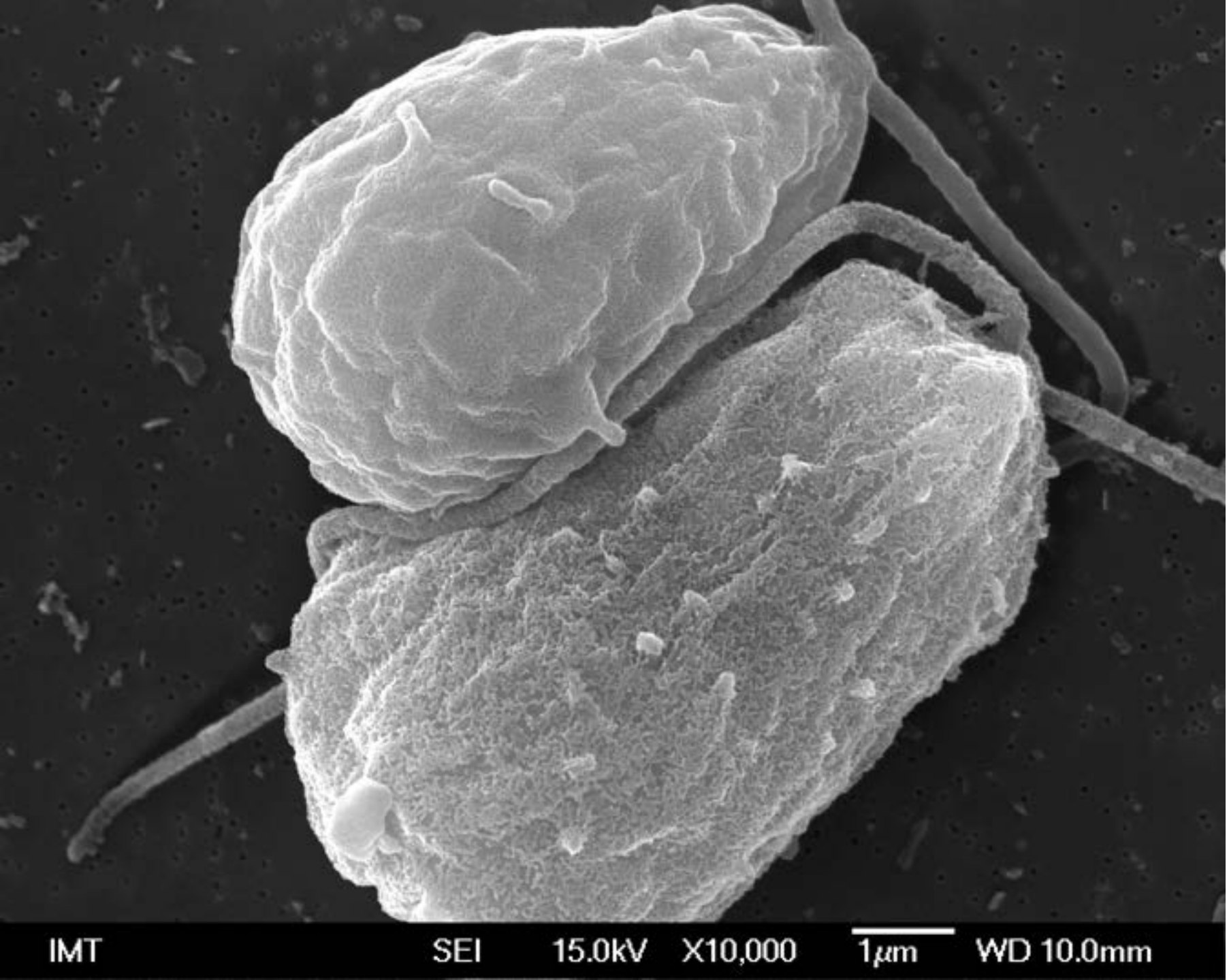

Figure *Dunaliella tertiolecta* culture SEM 31.

Cultivation of the algae
Culture of *D. tertiolecta* CCAP 19/22 from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 µmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.
Scanning Electron Microscopy (SEM)
Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).
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Proceedings of 9th Socratic Lectures **2024**

Repository

Scanning electron microscope images of *Dunaliella tertiolecta* isolates of small cellular particles

Bedina Zavec Apolonija[1], Božič Darja[2,3], Hočevar Matej[4] , Iglič Aleš [3,5], Jeran Marko[2,3], Romolo Anna[2], Kralj-Iglič Veronika[2]

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Abstract: Scanning electron microscope images of small cellular particles isolated from conditioned media of microalgae *Dunaliella tertiolecta* are presented. Each image is supplemented by description of the preparation of the sample and the data on the imaging technique and equipment.

The data curators of the repository are Veronika Kralj-Iglič and Anna Romolo. More data on experiments with microalgae small cellular particles can be found in (Adamo et al., 2021), (Picciotto et al., 2021) and (Božič et al., 2022).

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Keywords: Extracellular vesicles, Extracellular particles, Nanoalgosomes, Exosomes

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3. Božič D, Hočevar M, Jeran M et al. Ultrastructure and stability of cellular nanoparticles isolated from Phaeodactylum tricornutum and Dunaliella tertiolecta conditioned media. Open Res Europe 2022, 2:121 (https://doi.org/10.12688/openreseurope.14896.1)

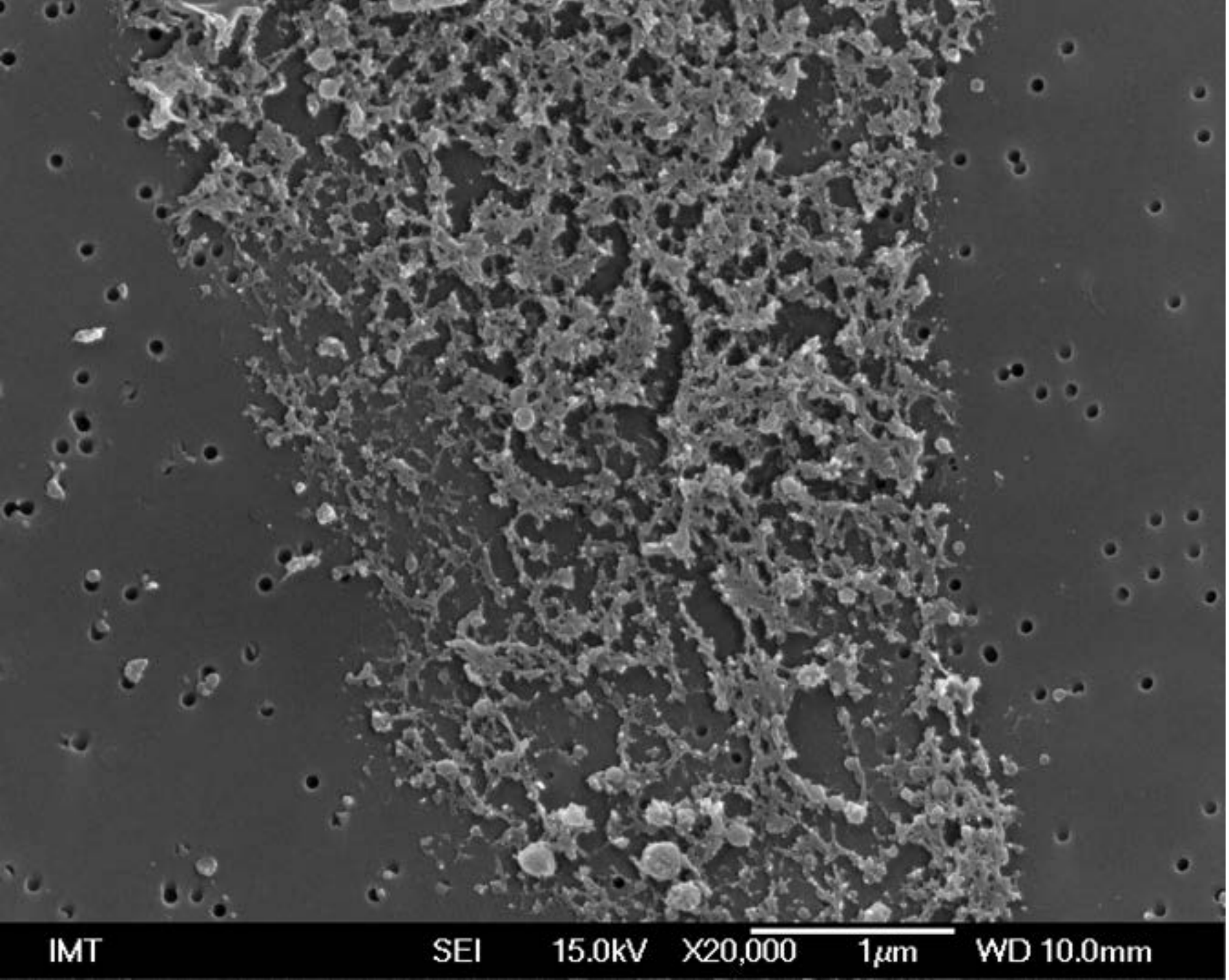

Figure *Dunaliella tertiolecta* isolate 100.000g SEM 1.

Isolation of nanoparticles (NPs)
NPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.
Scanning Electron Microscopy (SEM)
Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

From https://zenodo.org/record/6908895. Image 11

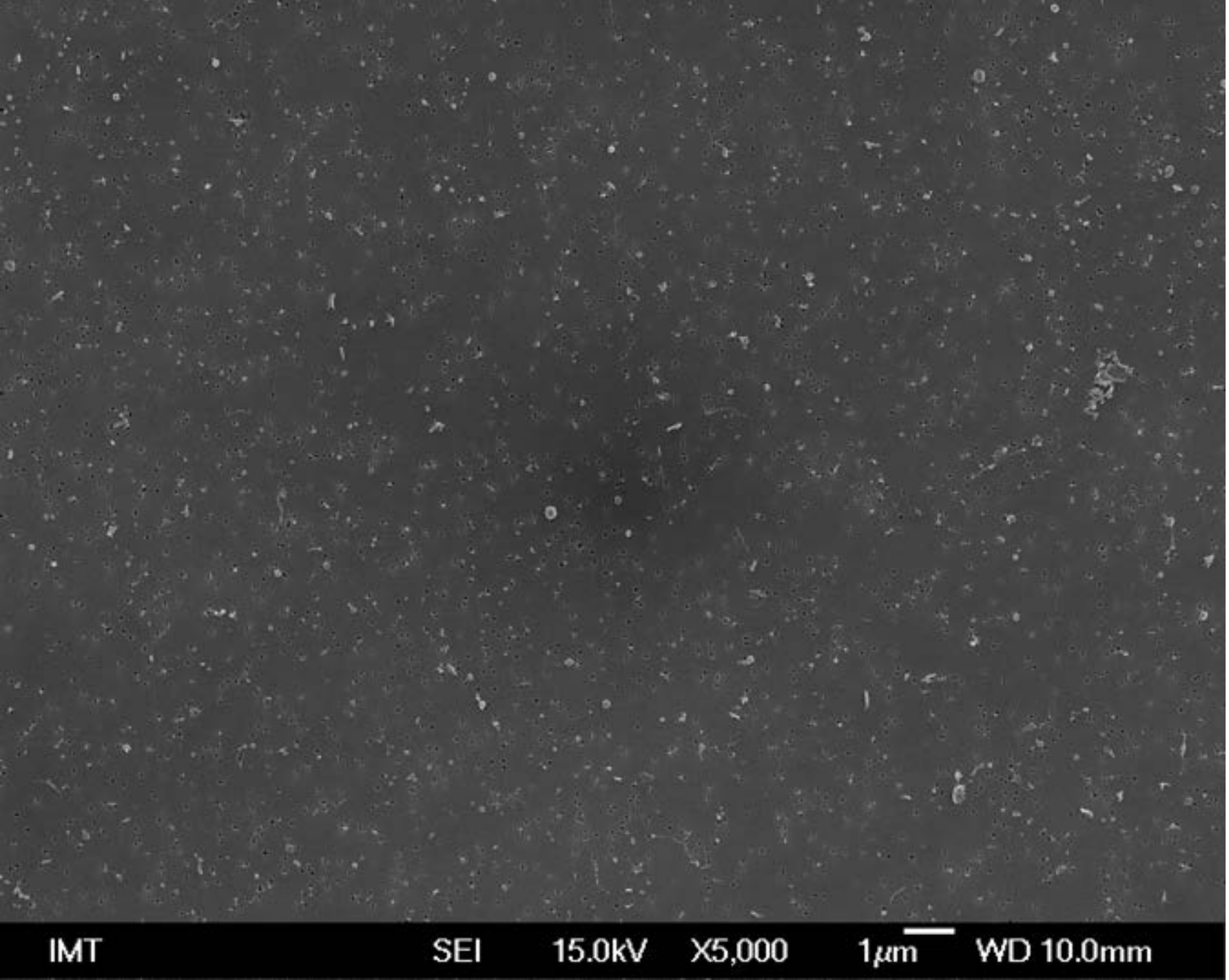

Figure *Dunaliela tertiolecta* isolate 100.000g SEM 2.

Isolation of nanoparticles (NPs)
NPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.
Scanning Electron Microscopy (SEM)
Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

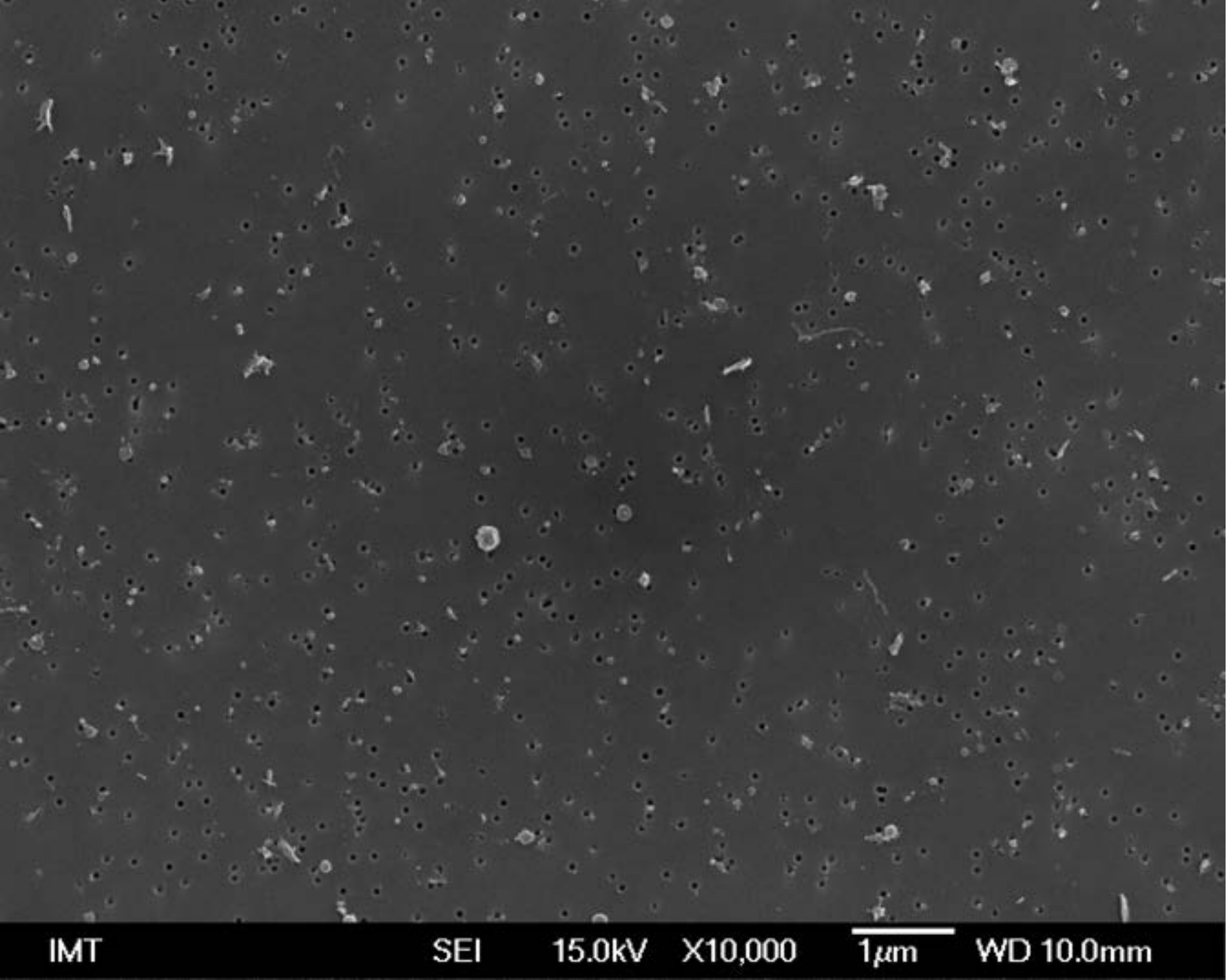

Figure *Dunaliela tertiolecta* isolate 100.000g SEM 3.

Isolation of nanoparticles (NPs)
NPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.
Scanning Electron Microscopy (SEM)
Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

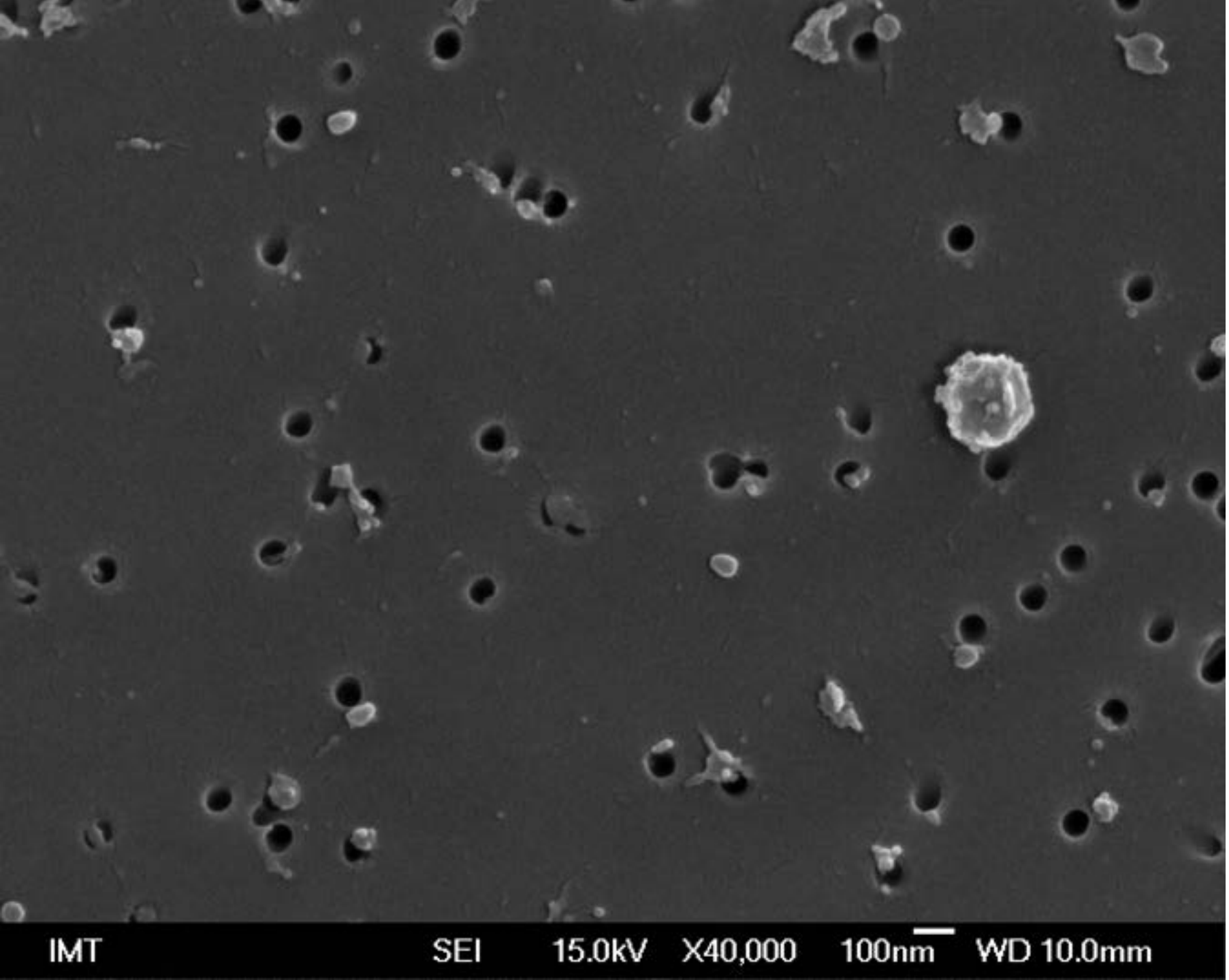

Figure *Dunaliela tertiolecta* isolate 100.000g SEM 4.

Isolation of nanoparticles (NPs)

NPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

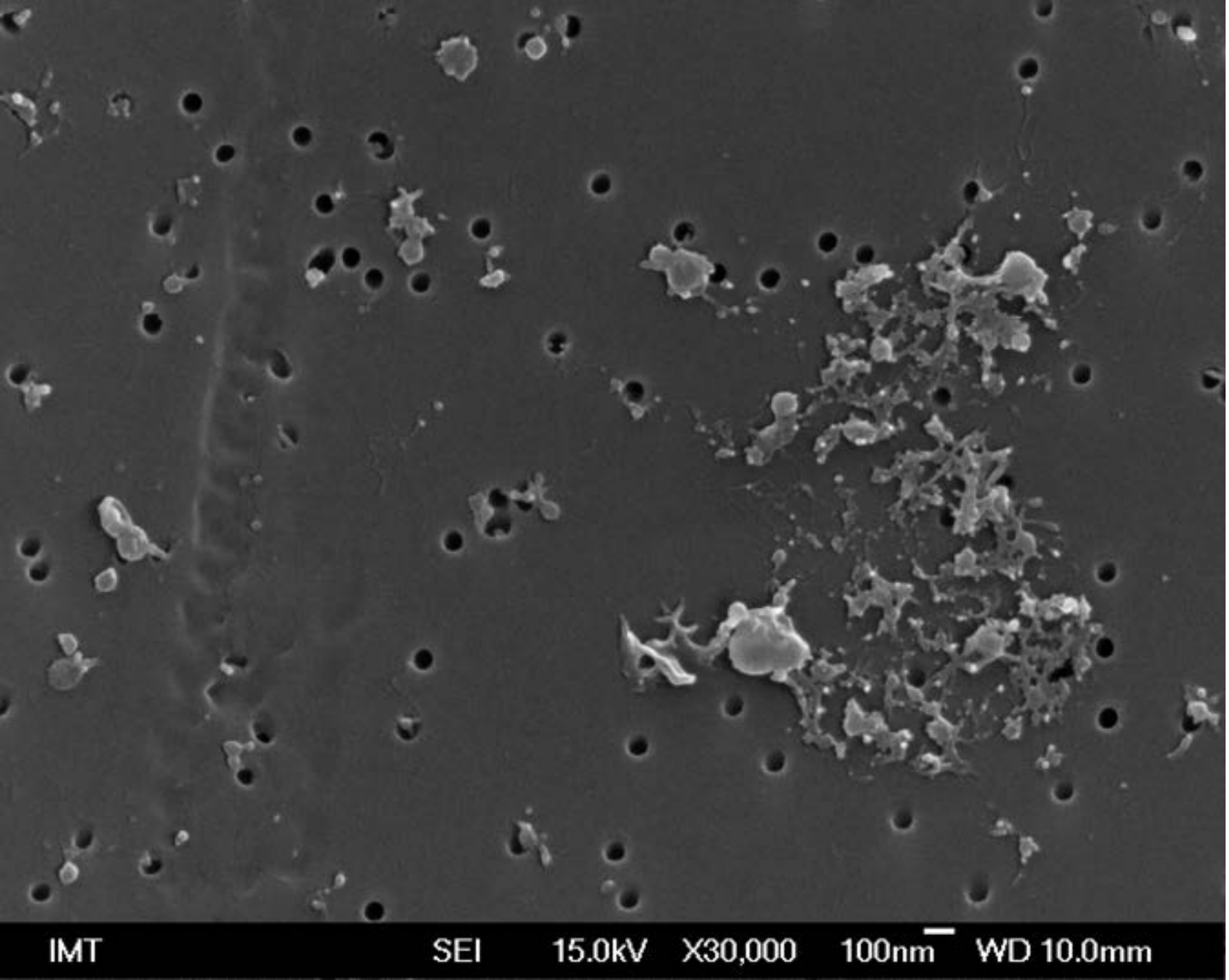

Figure *Dunaliela tertiolecta* isolate 100.000g SEM 5.

Isolation of nanoparticles (NPs)
NPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.
Scanning Electron Microscopy (SEM)
Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

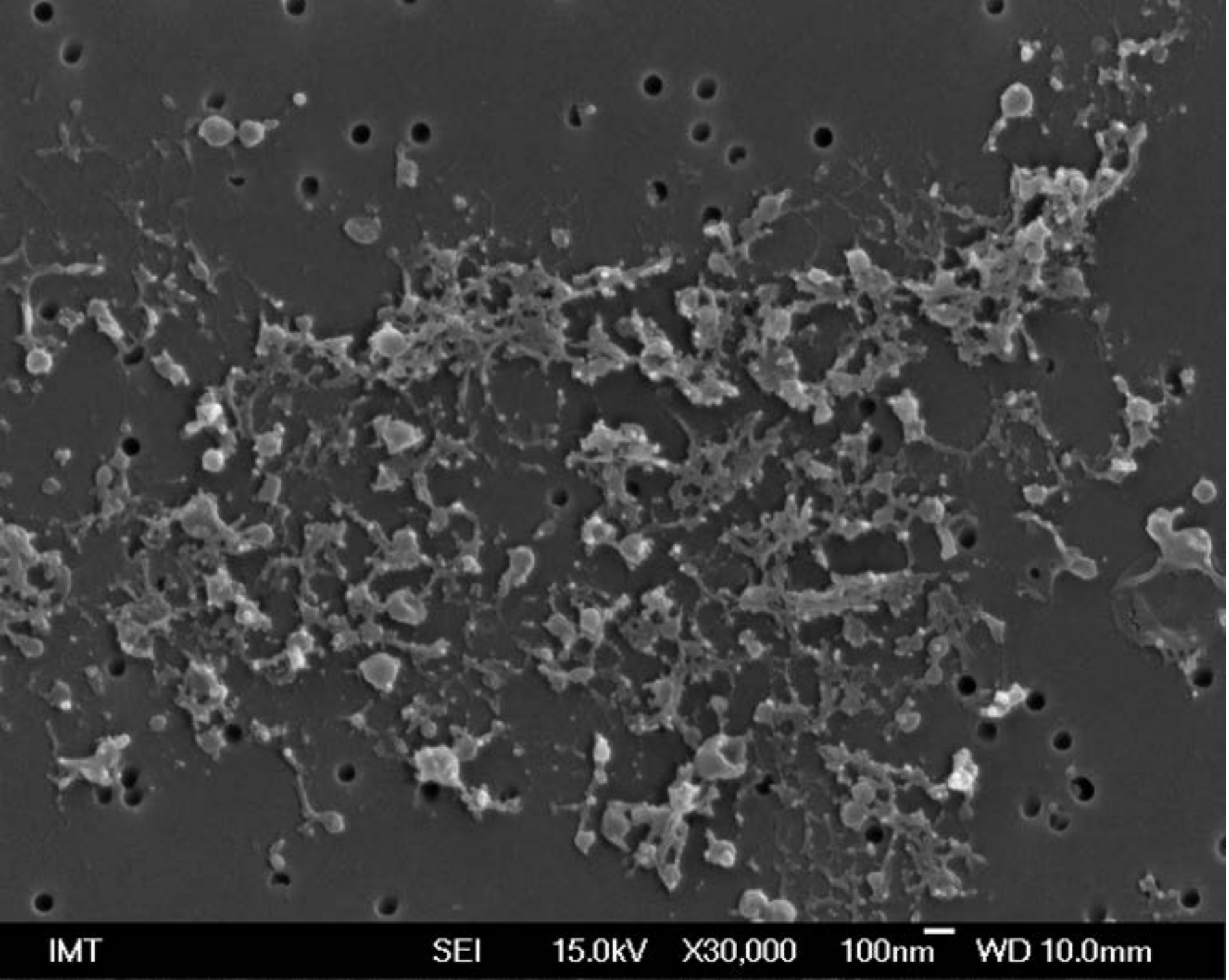

Figure *Dunaliela tertiolecta* isolate 100.000g SEM 6.

Isolation of nanoparticles (NPs)

NPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

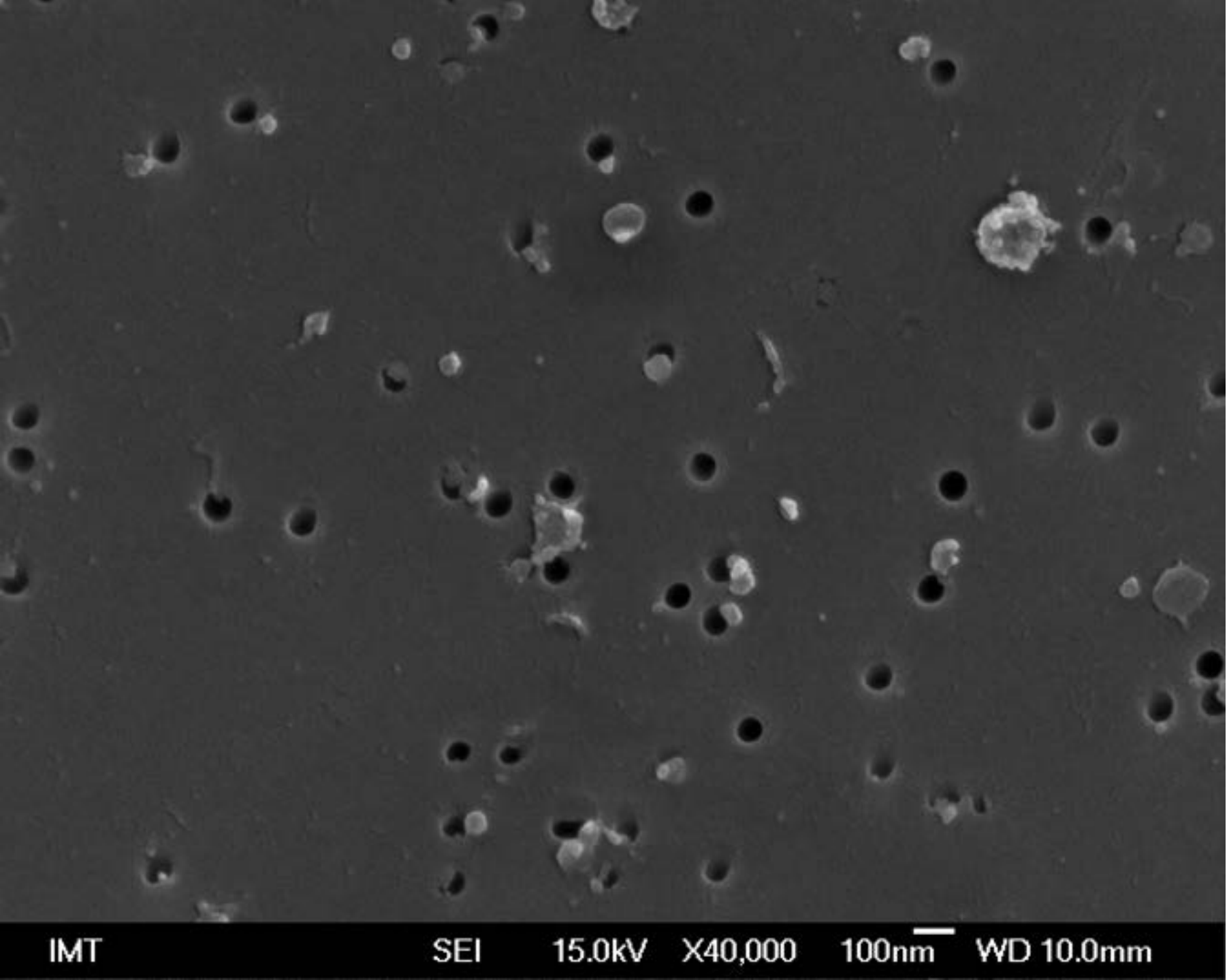

Figure *Dunaliela tertiolecta* isolate 100.000g SEM 7.

Isolation of nanoparticles (NPs)
NPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.
Scanning Electron Microscopy (SEM)
Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

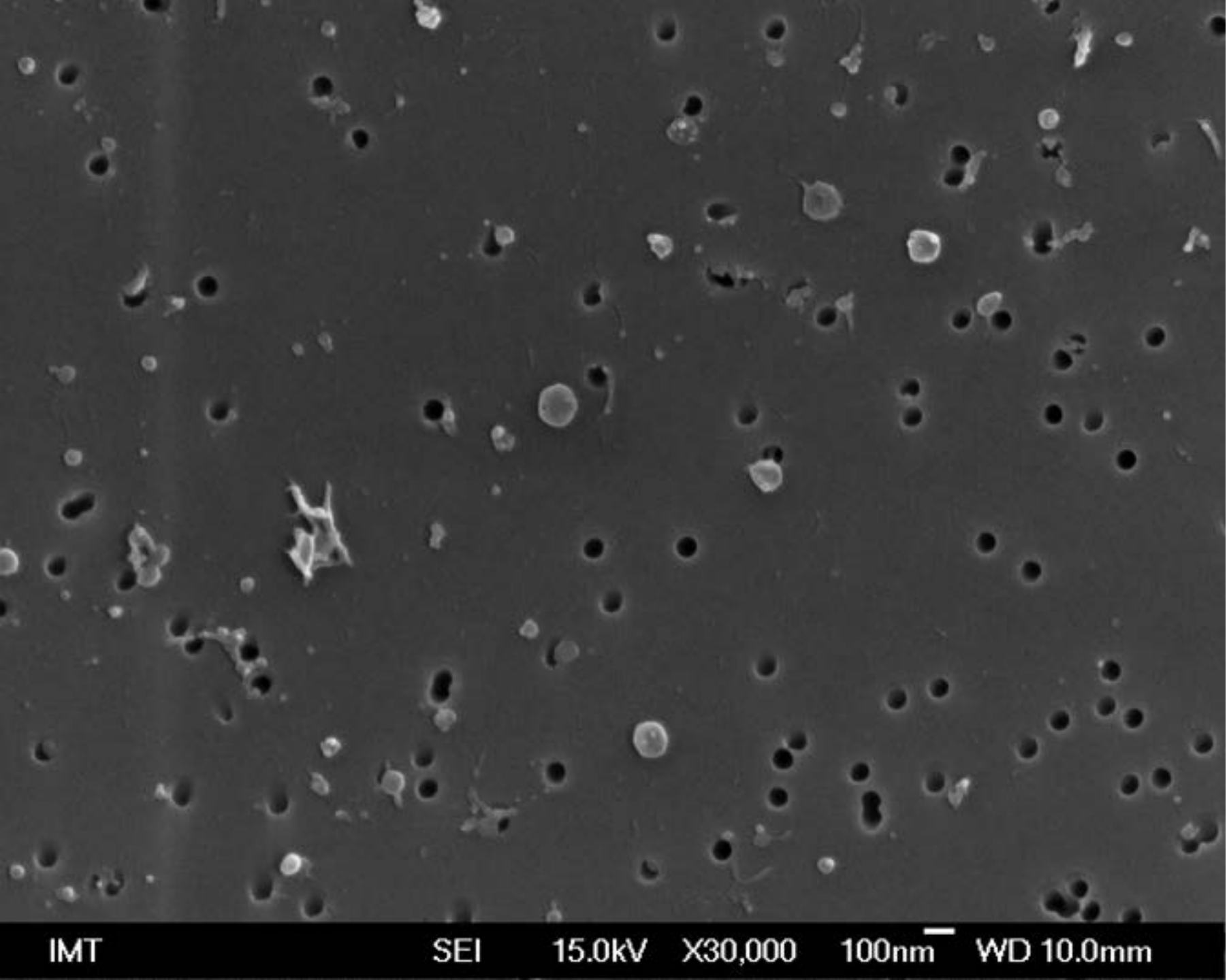

Figure *Dunaliella tertiolecta* isolate 100.000g SEM 8.

Isolation of nanoparticles (NPs)
NPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.
Scanning Electron Microscopy (SEM)
Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

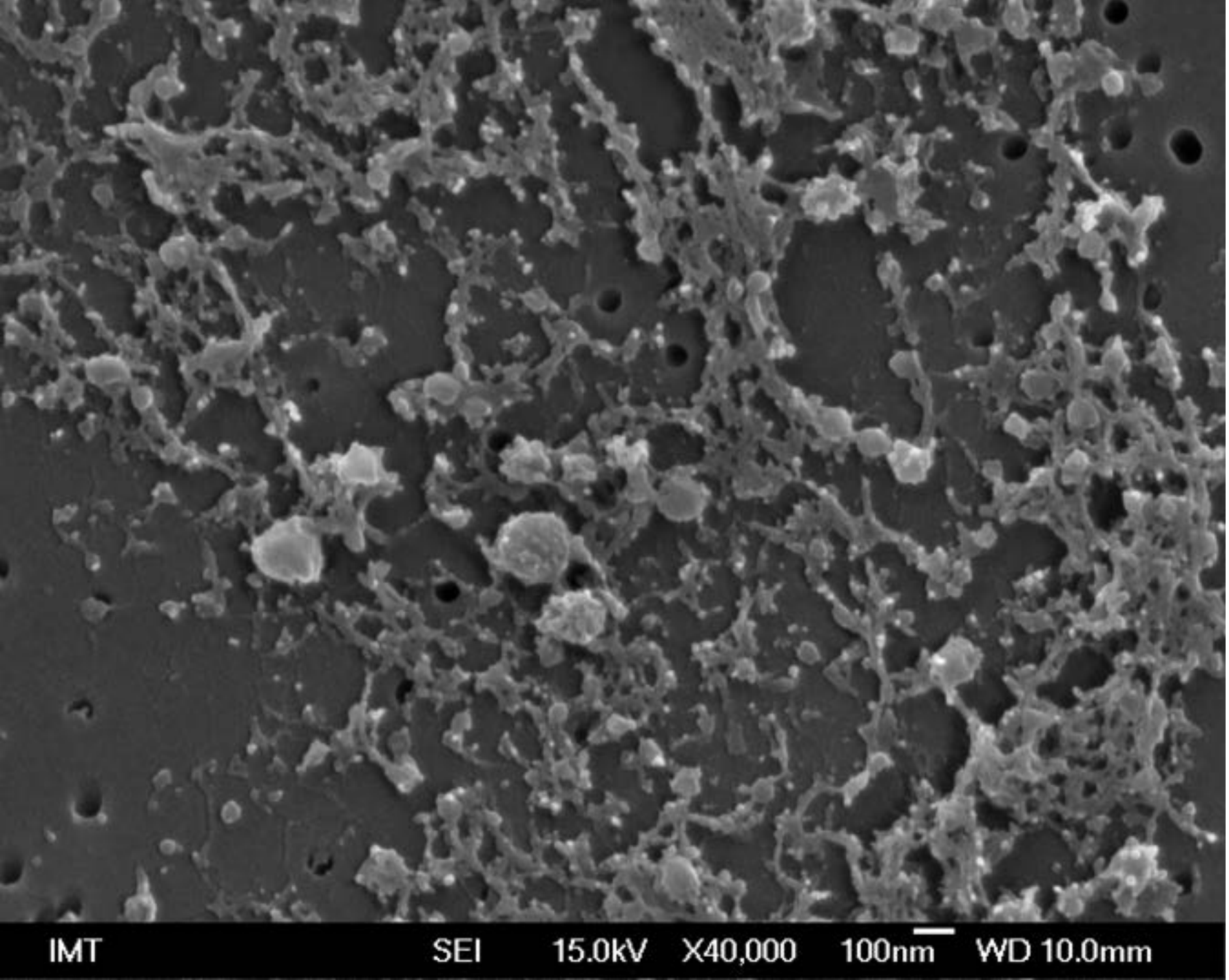

Figure *Dunaliela tertiolecta* isolate 100.000g SEM 9.

Isolation of nanoparticles (NPs)

NPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

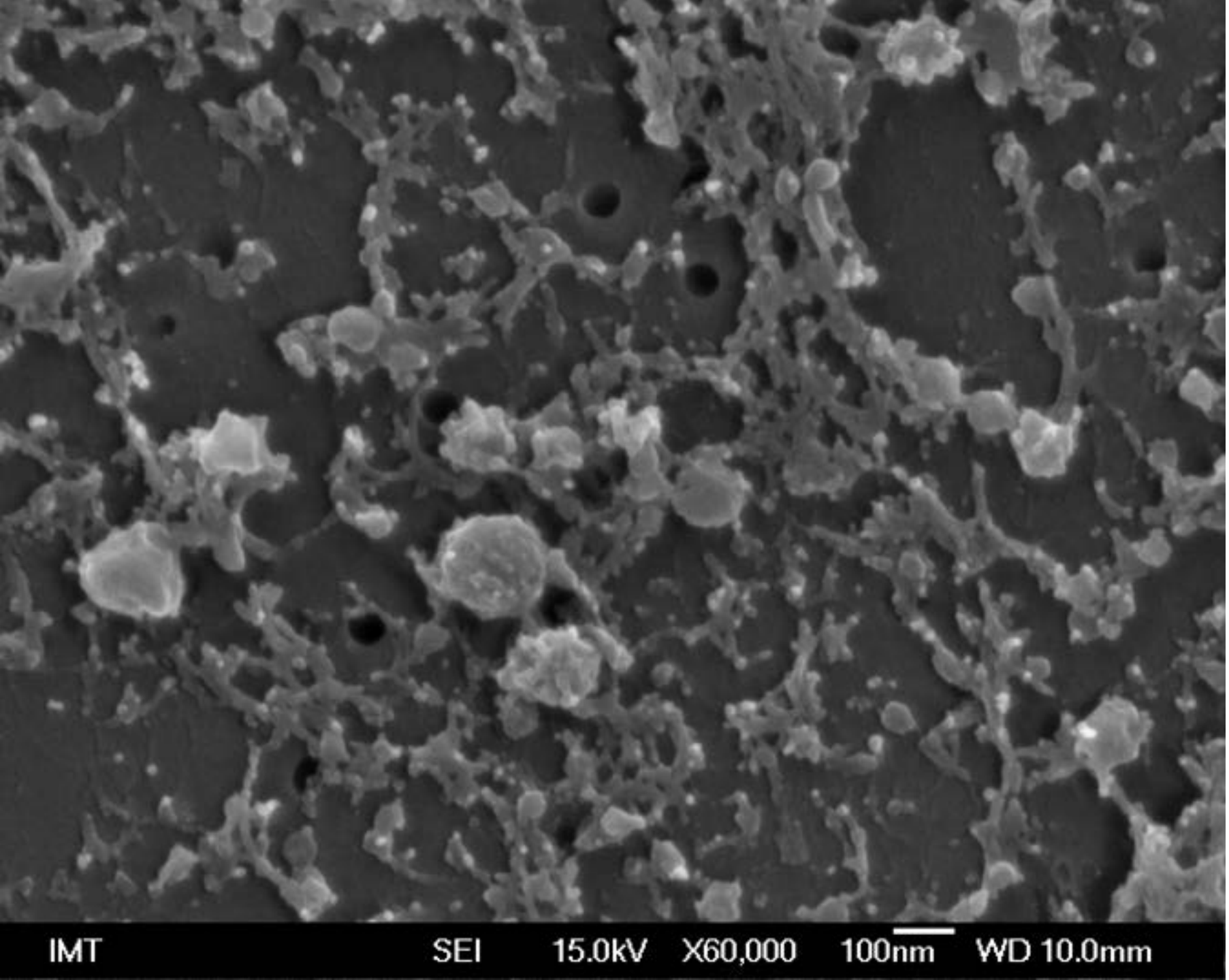

Figure *Dunaliela tertiolecta* isolate 100.000g SEM 10.

Isolation of nanoparticles (NPs)

NPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

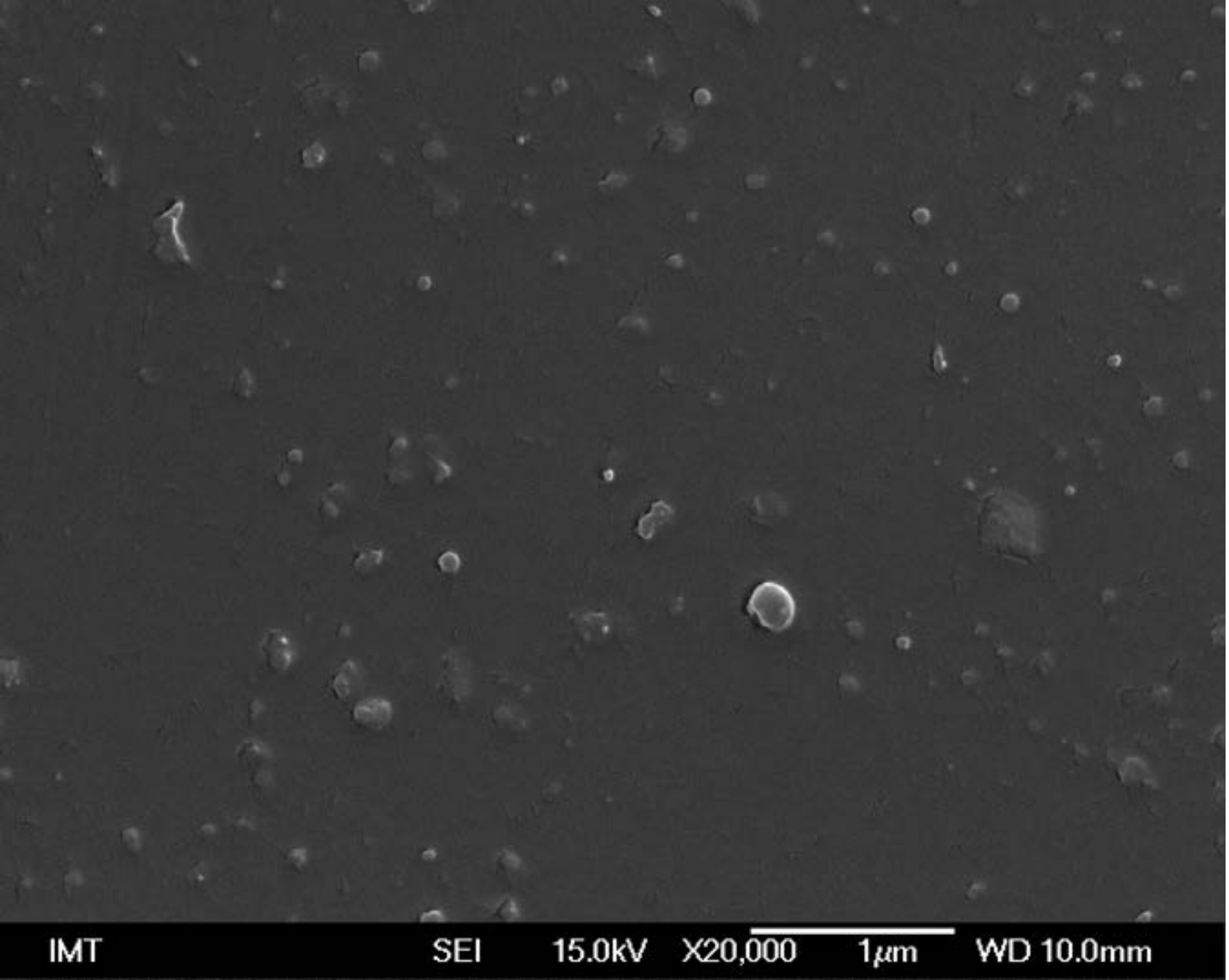

Figure *Dunaliela tertiolecta* isolate 100.000g SEM 11.

Isolation of nanoparticles (NPs)
NPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.
Scanning Electron Microscopy (SEM)
Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

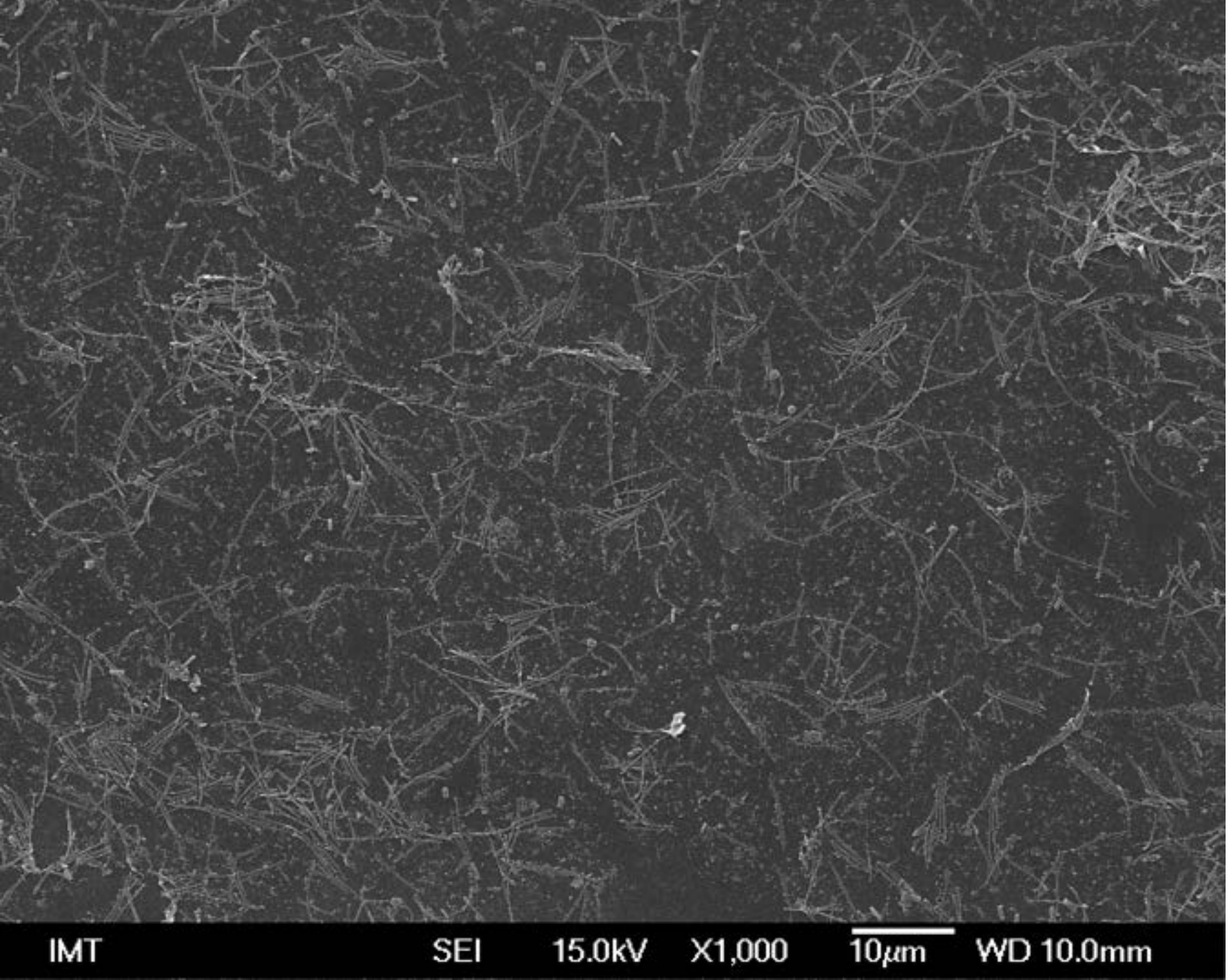

Figure *Dunaliela tertiolecta* isolate 10.000g SEM 1.

Isolation of nanoparticles (NPs)
NPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA). The isolate presents the pellet of the second centrifugation.

Scanning Electron Microscopy (SEM)
Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

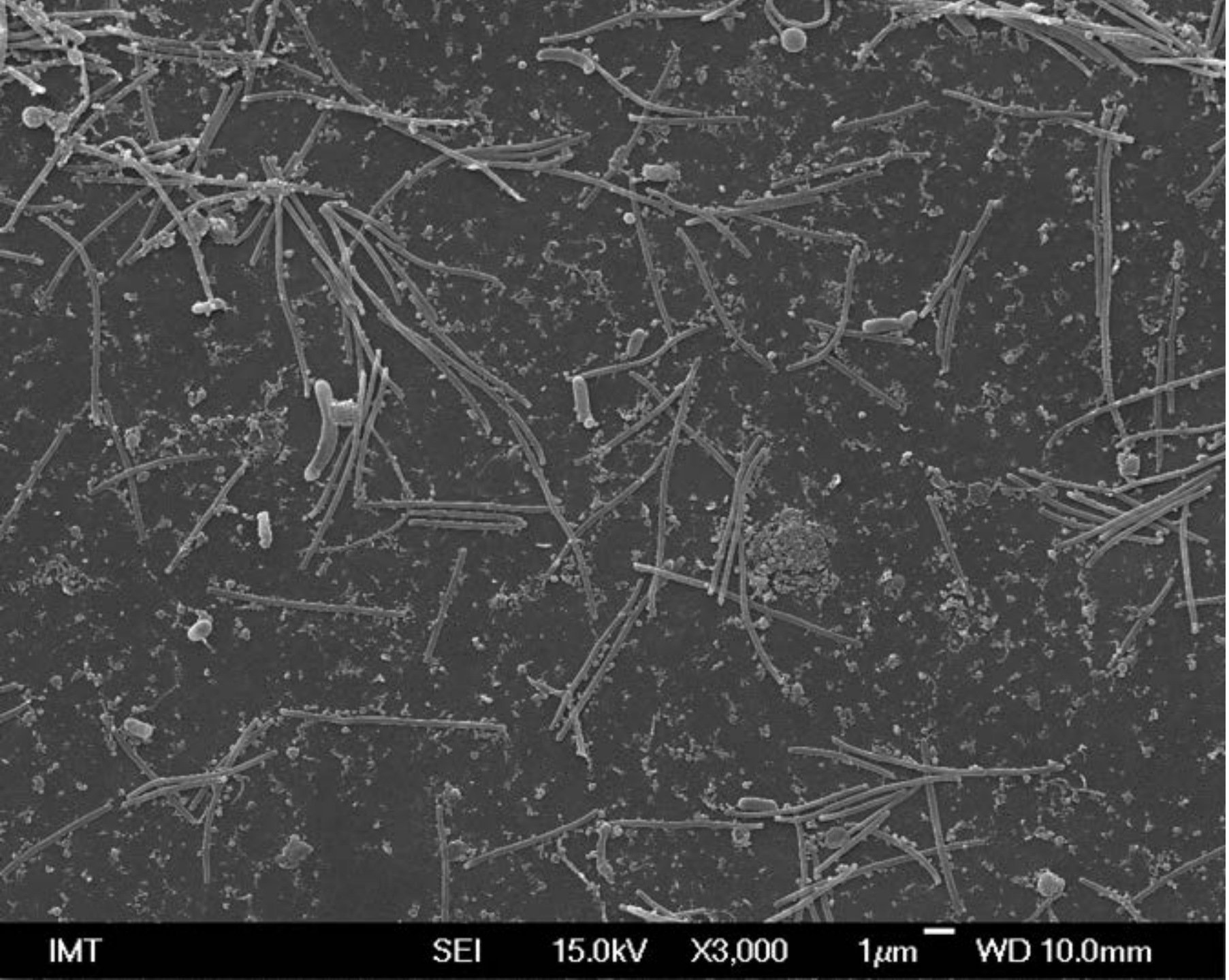

Figure *Dunaliella tertiolecta* isolate 10.000g SEM 2.

Isolation of nanoparticles (NPs)

NPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA). The isolate presents the pellet of the second centrifugation.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

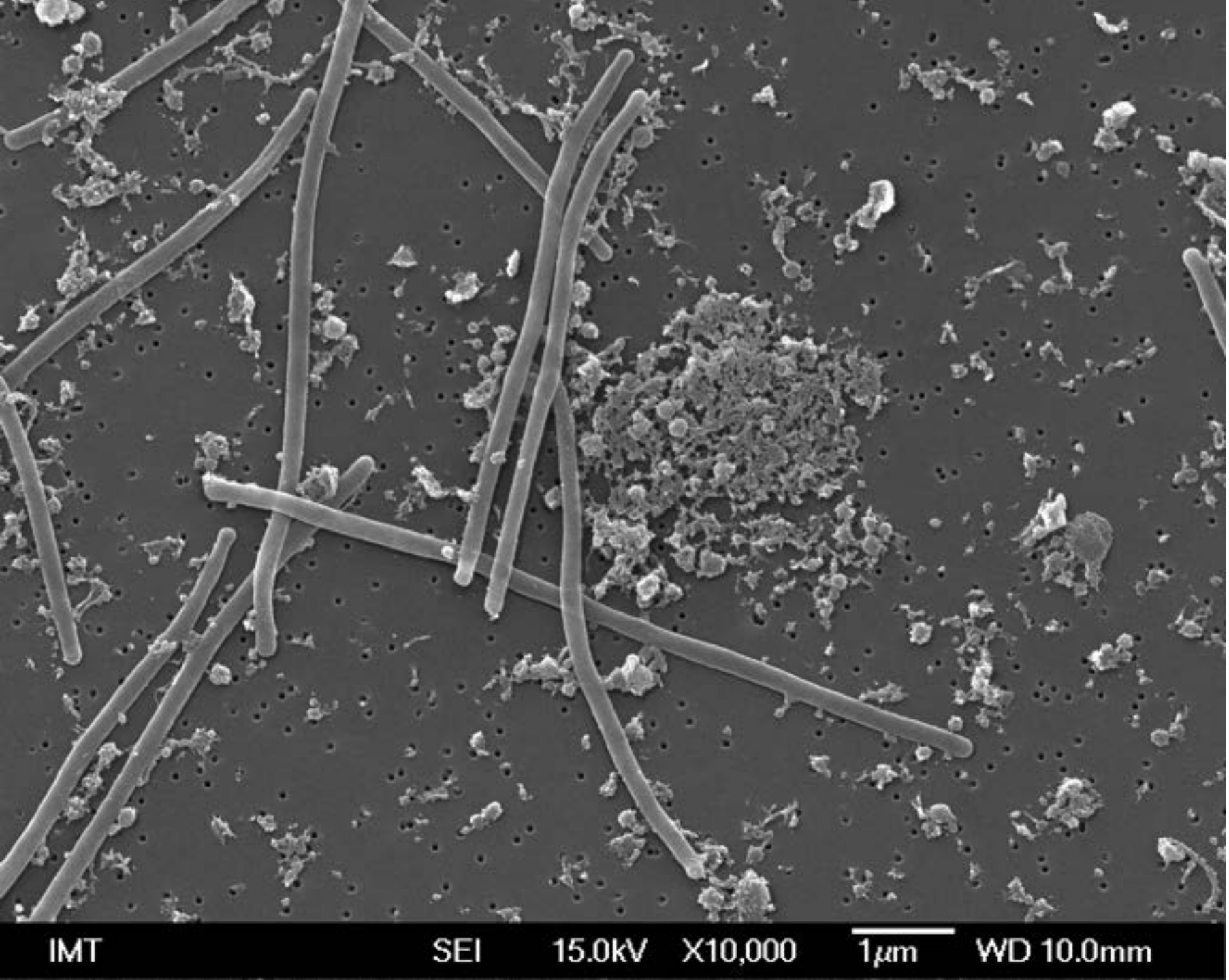

Figure *Dunaliela tertiolecta* isolate 10.000g SEM 3.

Isolation of nanoparticles (NPs)

NPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA). The isolate presents the pellet of the second centrifugation.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

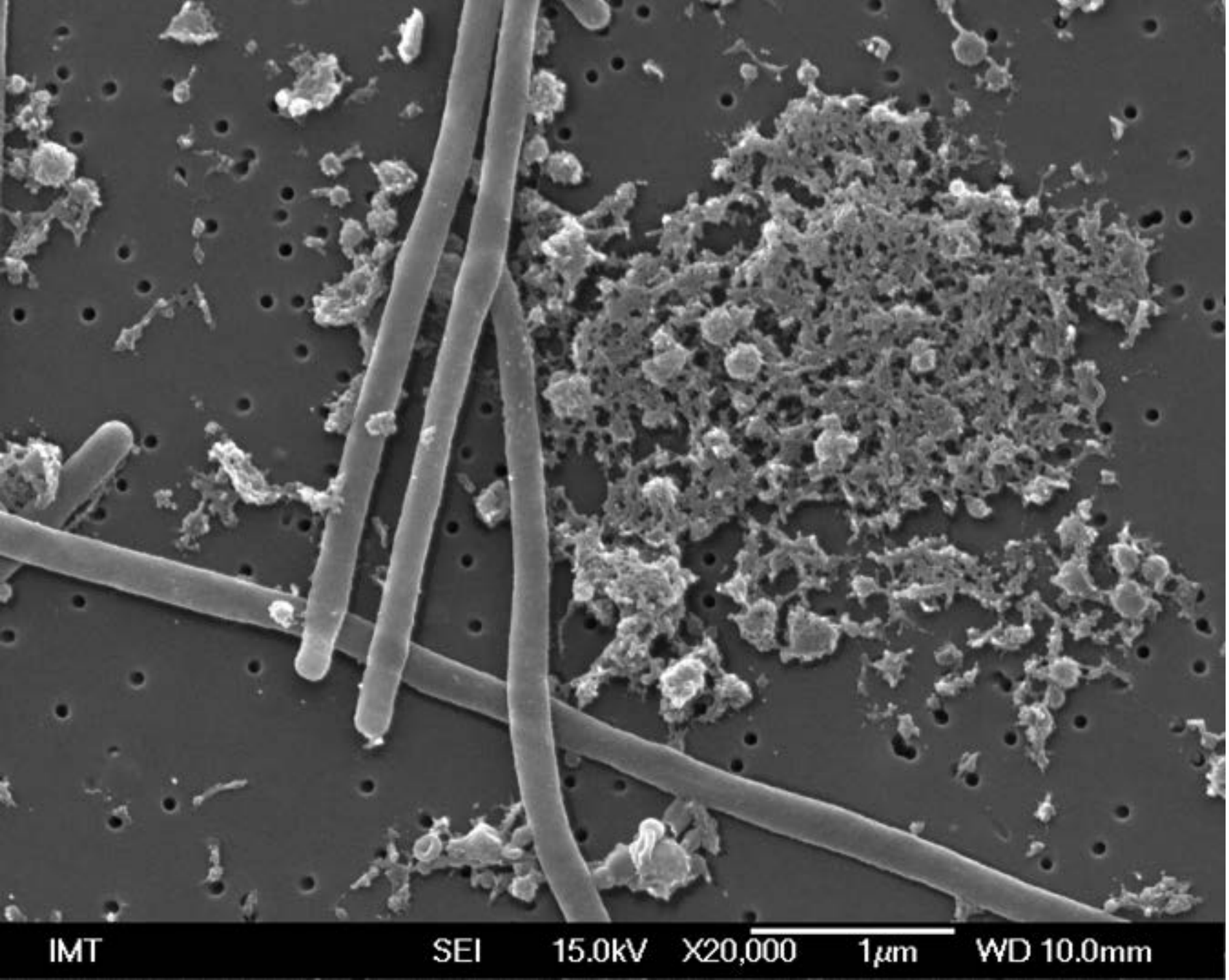

Figure *Dunaliela tertiolecta* isolate 10.000g SEM 4.

Isolation of nanoparticles (NPs)

NPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA). The isolate presents the pellet of the second centrifugation.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

Figure *Dunaliella tertiolecta* isolate 10.000g SEM 5.

Isolation of nanoparticles (NPs)

NPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA). The isolate presents the pellet of the second centrifugation.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

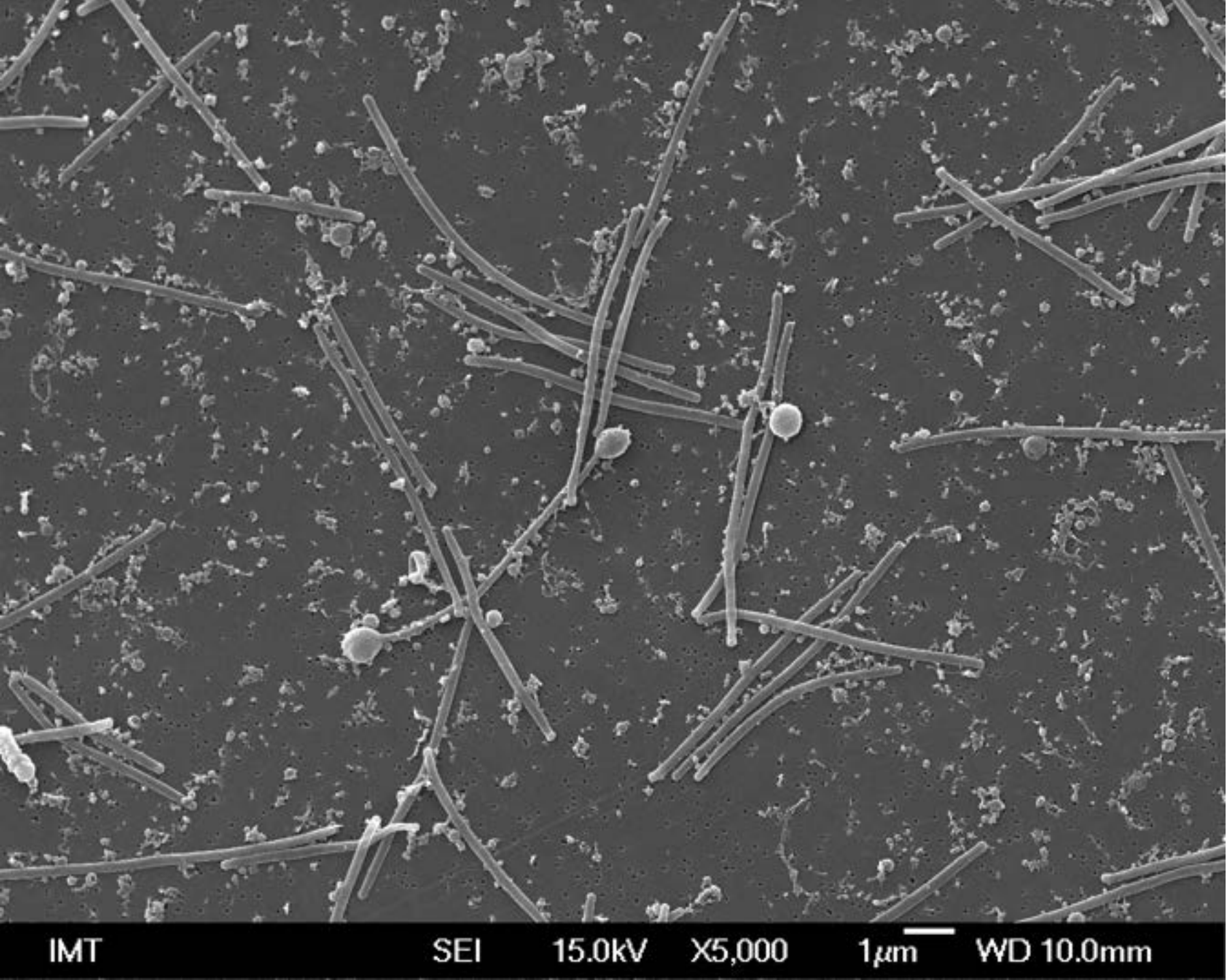

Figure *Dunaliela tertiolecta* isolate 10.000g SEM 6.

Isolation of nanoparticles (NPs)

NPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA). The isolate presents the pellet of the second centrifugation.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

Figure *Dunaliela tertiolecta* isolate 10.000g SEM 7.

Isolation of nanoparticles (NPs)

NPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA). The isolate presents the pellet of the second centrifugation.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

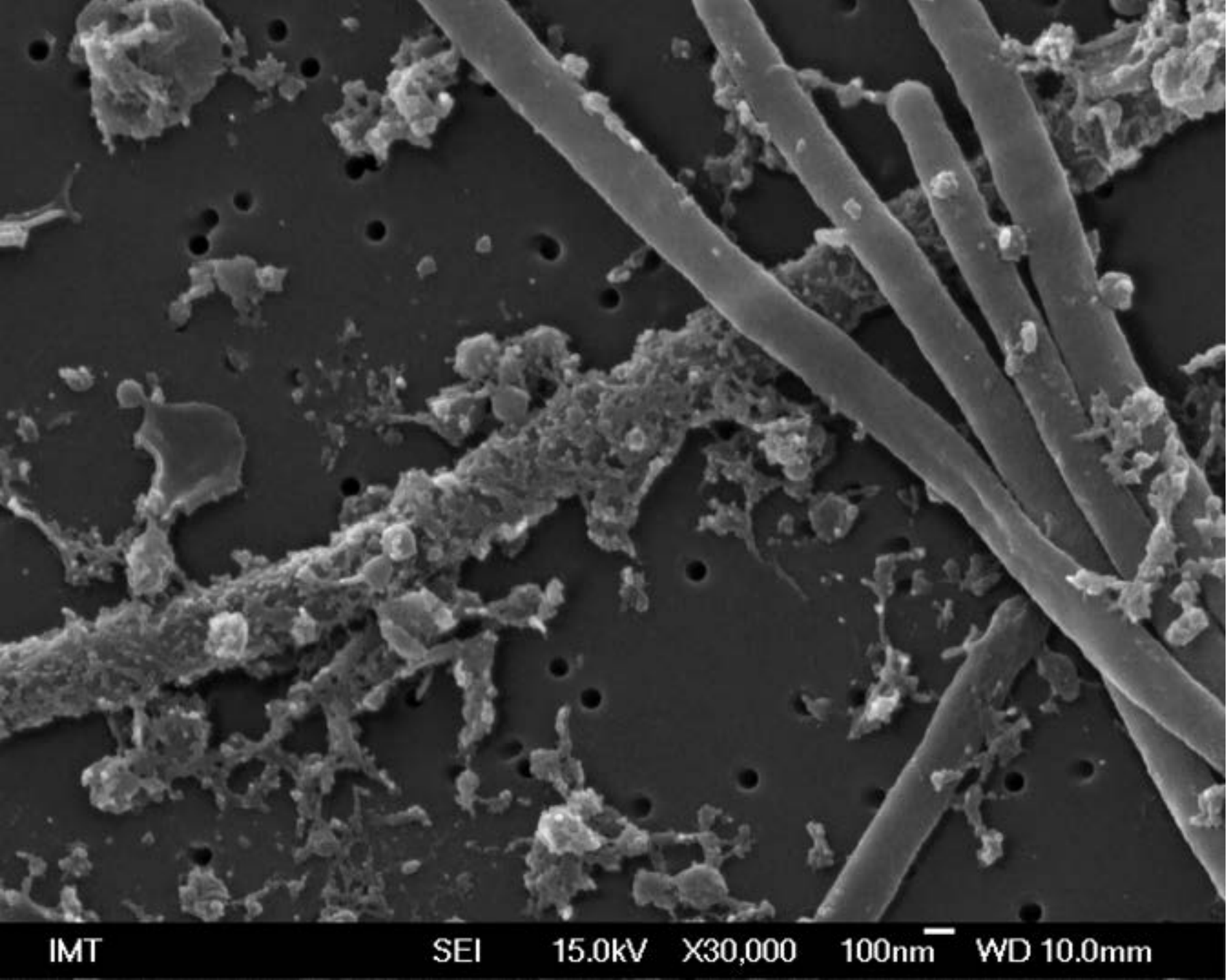

Figure *Dunaliela tertiolecta* isolate 10.000g SEM 8.

Isolation of nanoparticles (NPs)

NPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA). The isolate presents the pellet of the second centrifugation.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

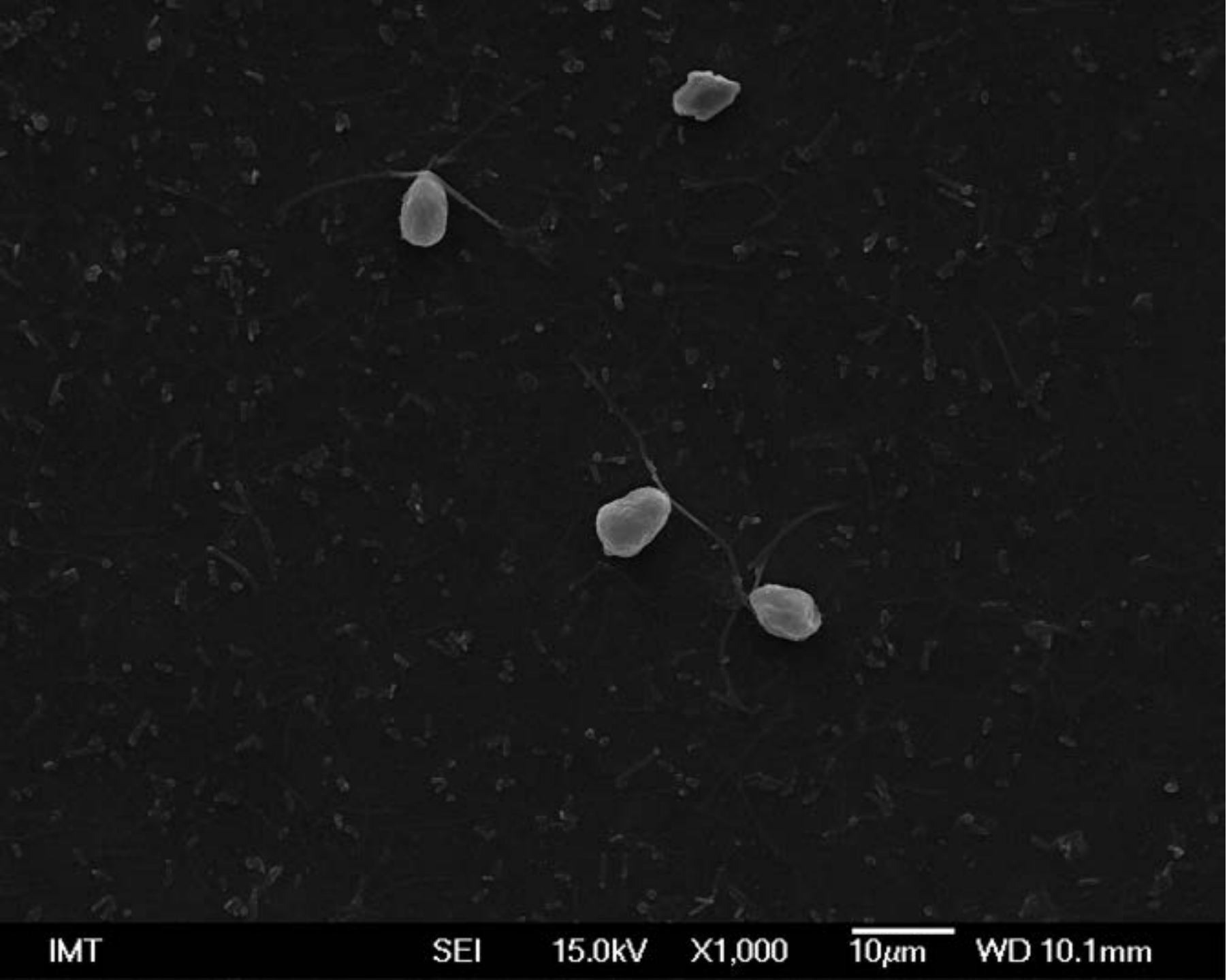

Figure *Dunaliela tertiolecta* isolate 2.000g SEM 9.

Isolation of nanoparticles (NPs)
NPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. The isolate presents the pellet of the second centrifugation.

Scanning Electron Microscopy (SEM)
Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

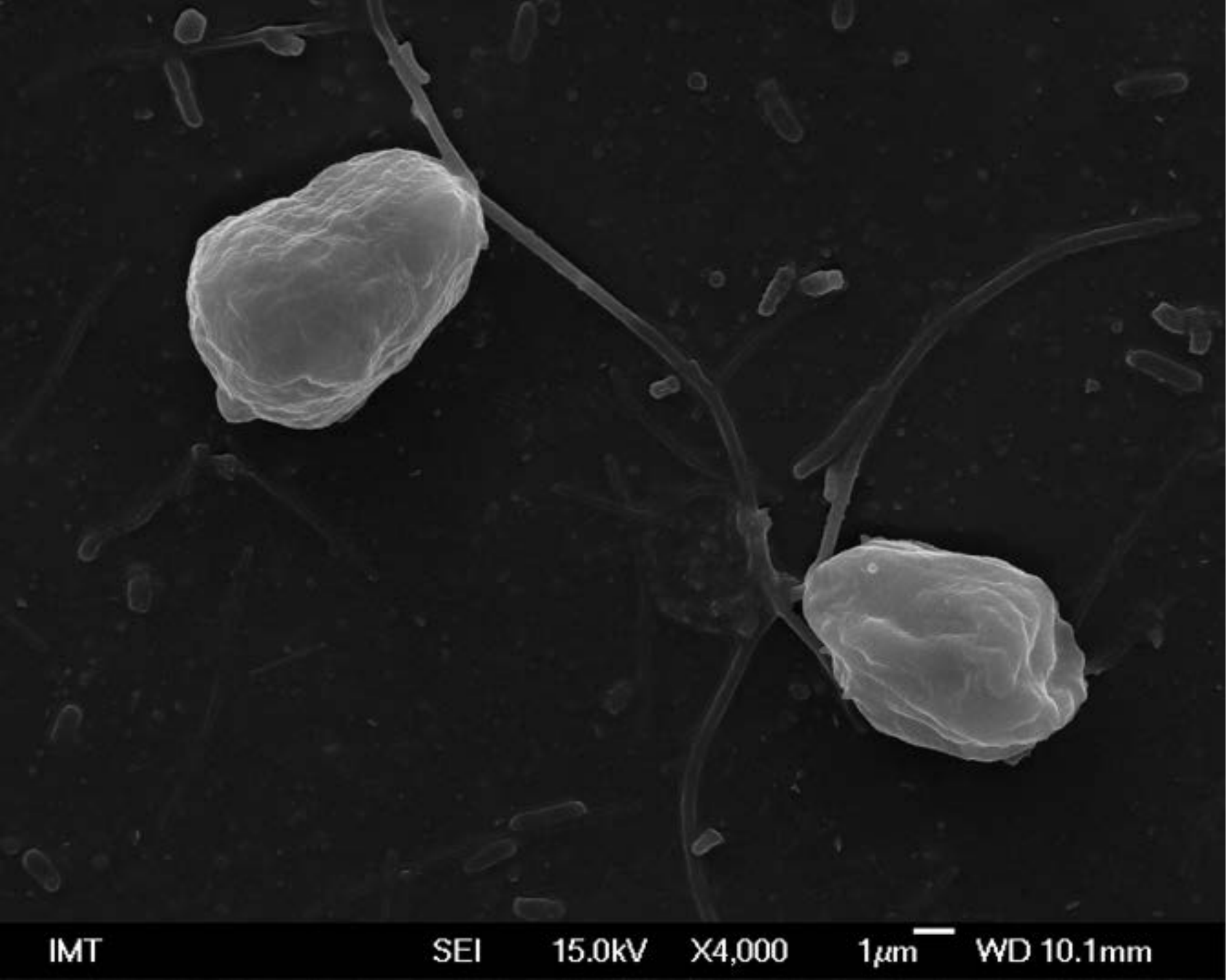

Figure *Dunaliela tertiolecta* isolate 2.000g SEM 10.

Isolation of nanoparticles (NPs)
NPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. The isolate presents the pellet of the second centrifugation.
Scanning Electron Microscopy (SEM)
Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

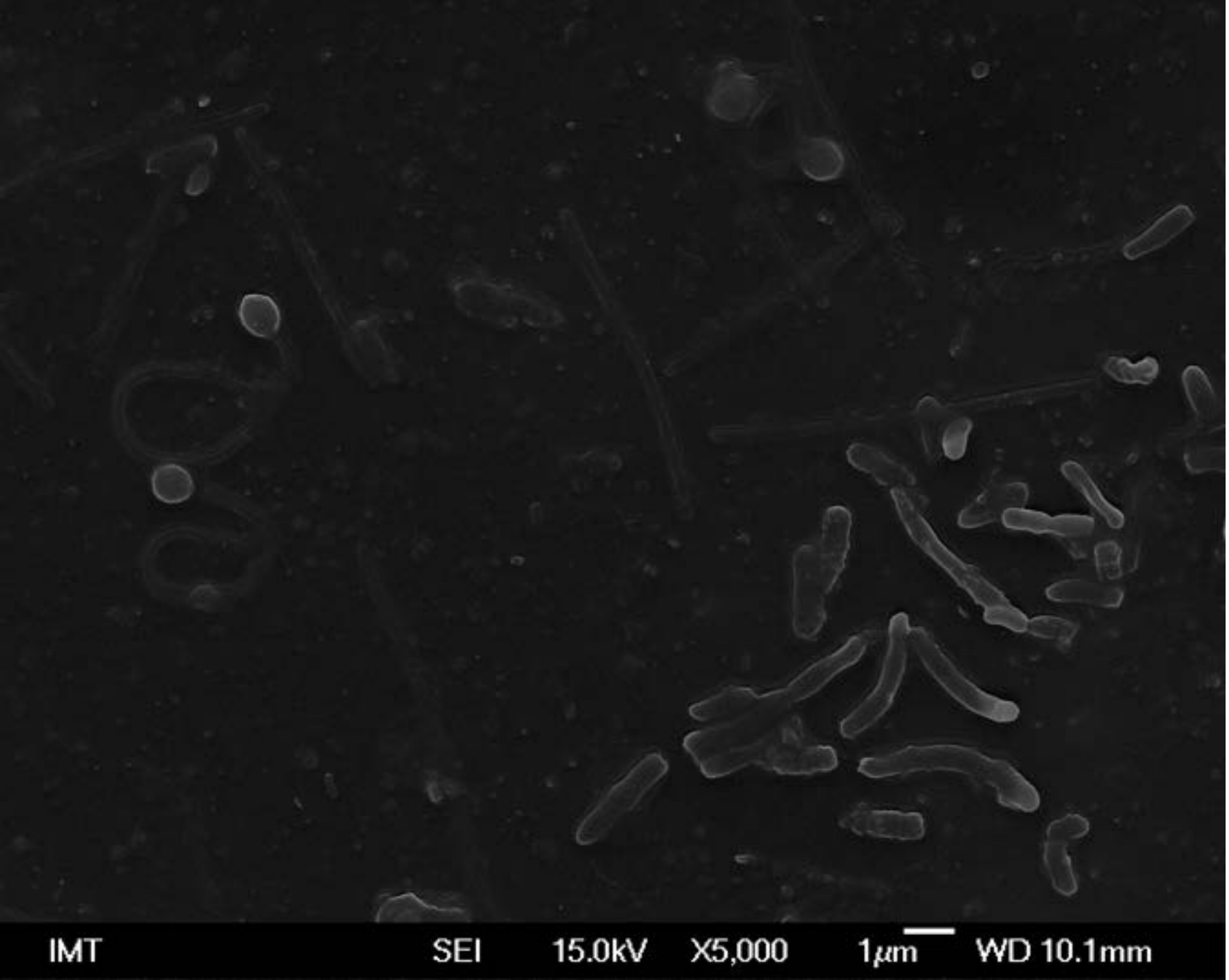

Figure *Dunaliela tertiolecta* isolate 2.000g SEM 11.

Isolation of nanoparticles (NPs)
NPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. The isolate presents the pellet of the second centrifugation.
Scanning Electron Microscopy (SEM)
Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

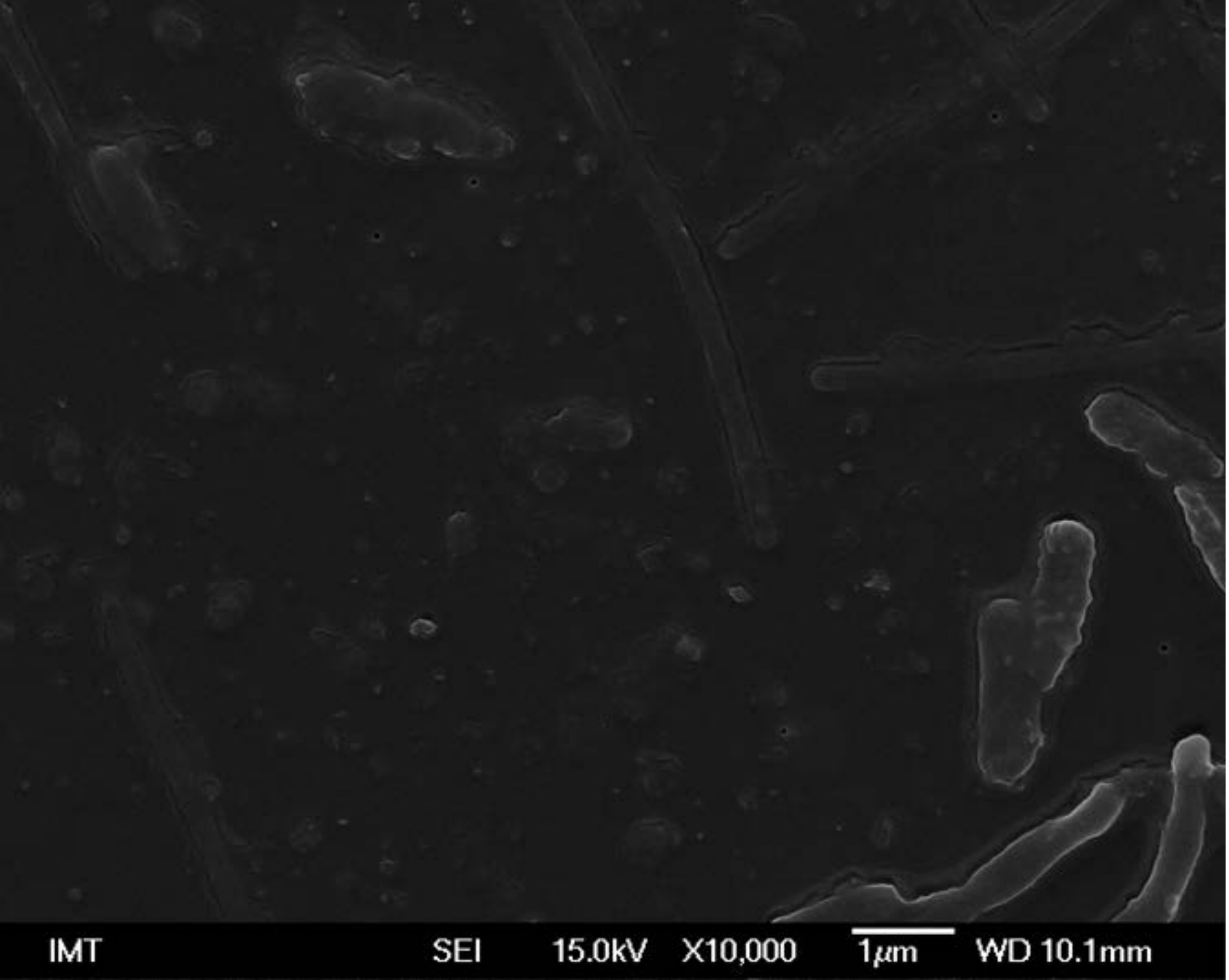

Figure *Dunaliela tertiolecta* isolate 2.000g SEM 12.

Isolation of nanoparticles (NPs)

NPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. The isolate presents the pellet of the second centrifugation.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

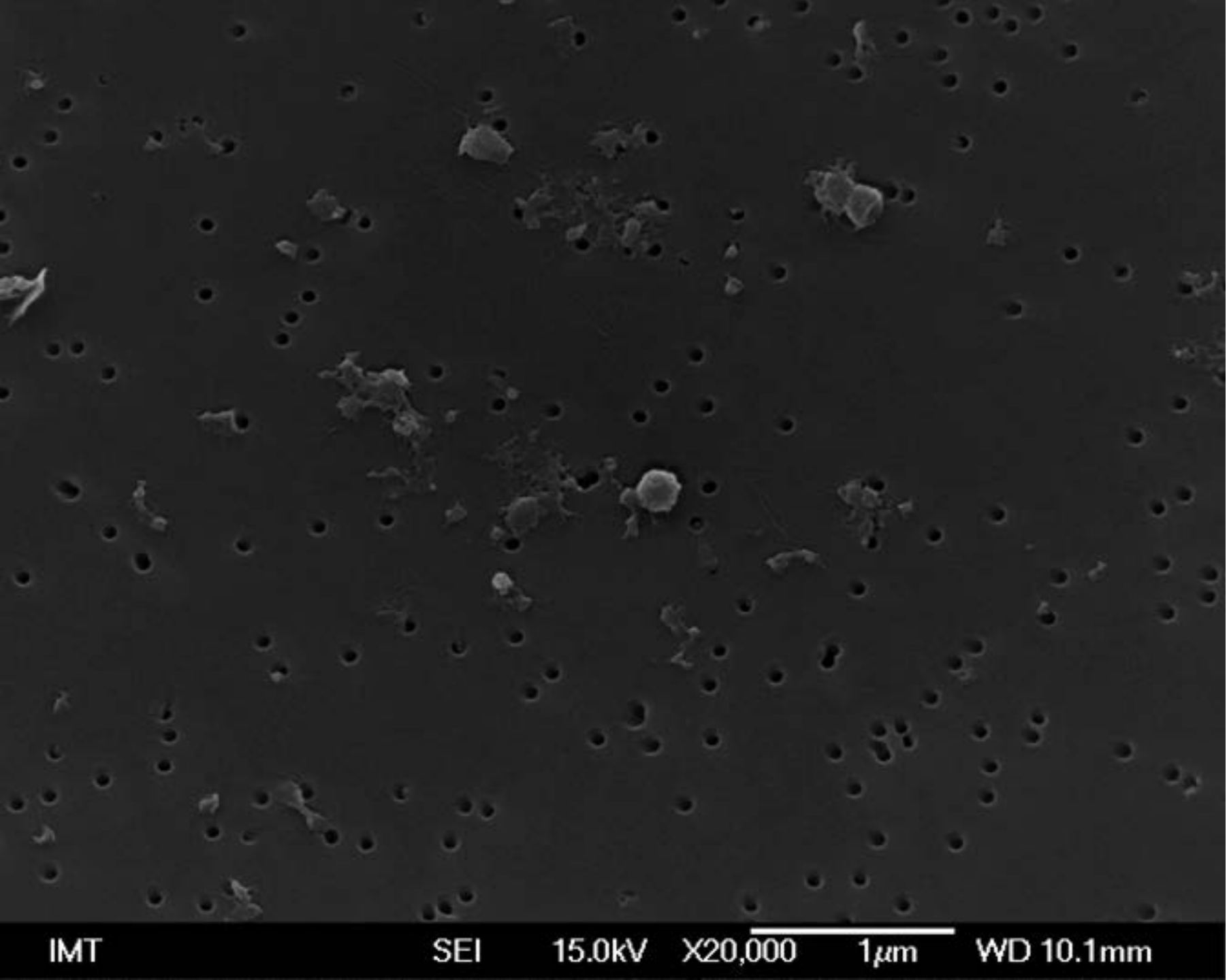

Figure *Dunaliela tertiolecta* isolate 2.000g SEM 13.

Isolation of nanoparticles (NPs)
NPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. The isolate presents the pellet of the second centrifugation.
Scanning Electron Microscopy (SEM)
Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

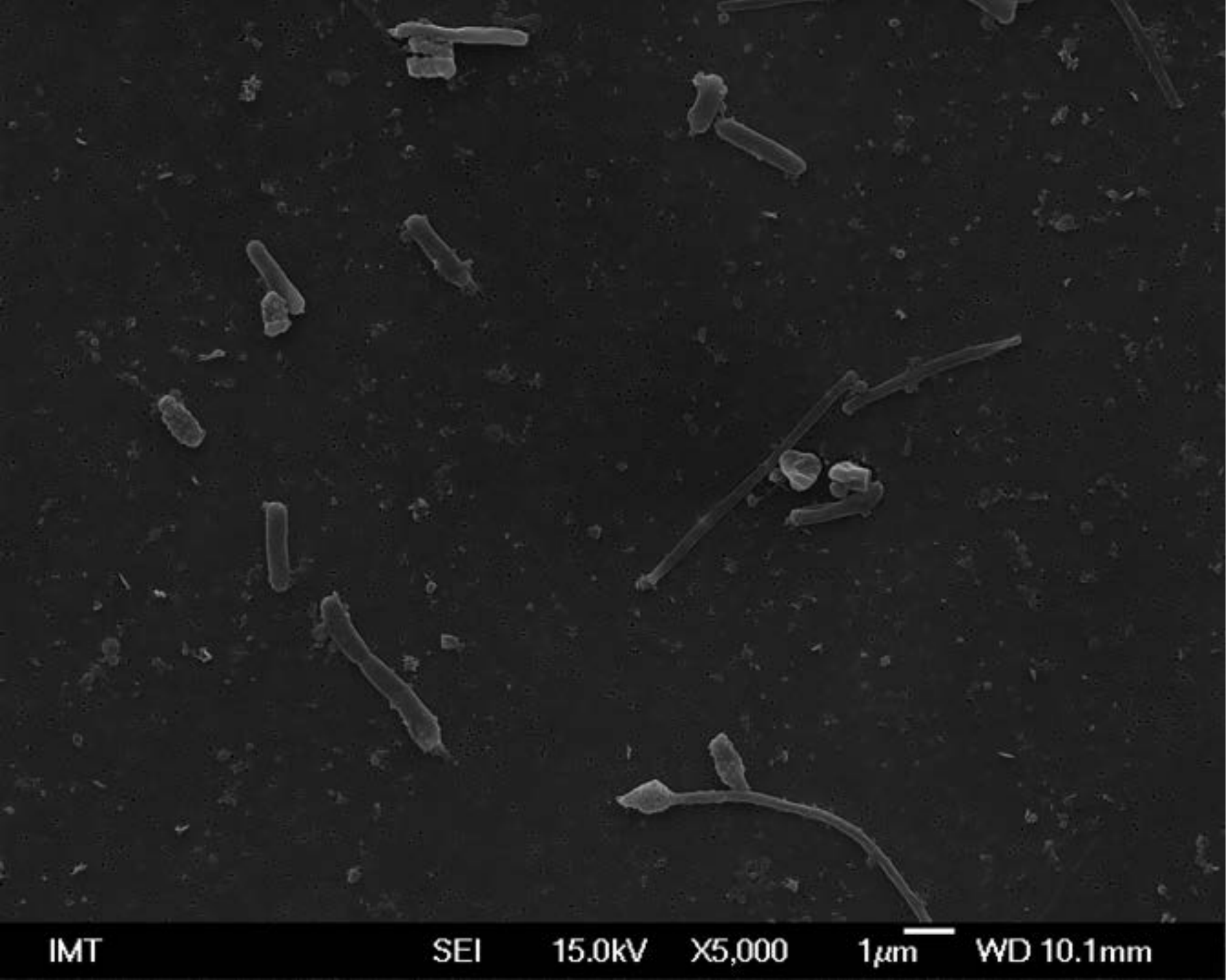

Figure *Dunaliela tertiolecta* isolate 2.000g SEM 14.

Isolation of nanoparticles (NPs)
NPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. The isolate presents the pellet of the second centrifugation.
Scanning Electron Microscopy (SEM)
Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

Figure *Dunaliella tertiolecta* isolate 2.000g SEM 15.

Isolation of nanoparticles (NPs)

NPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. The isolate presents the pellet of the second centrifugation.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

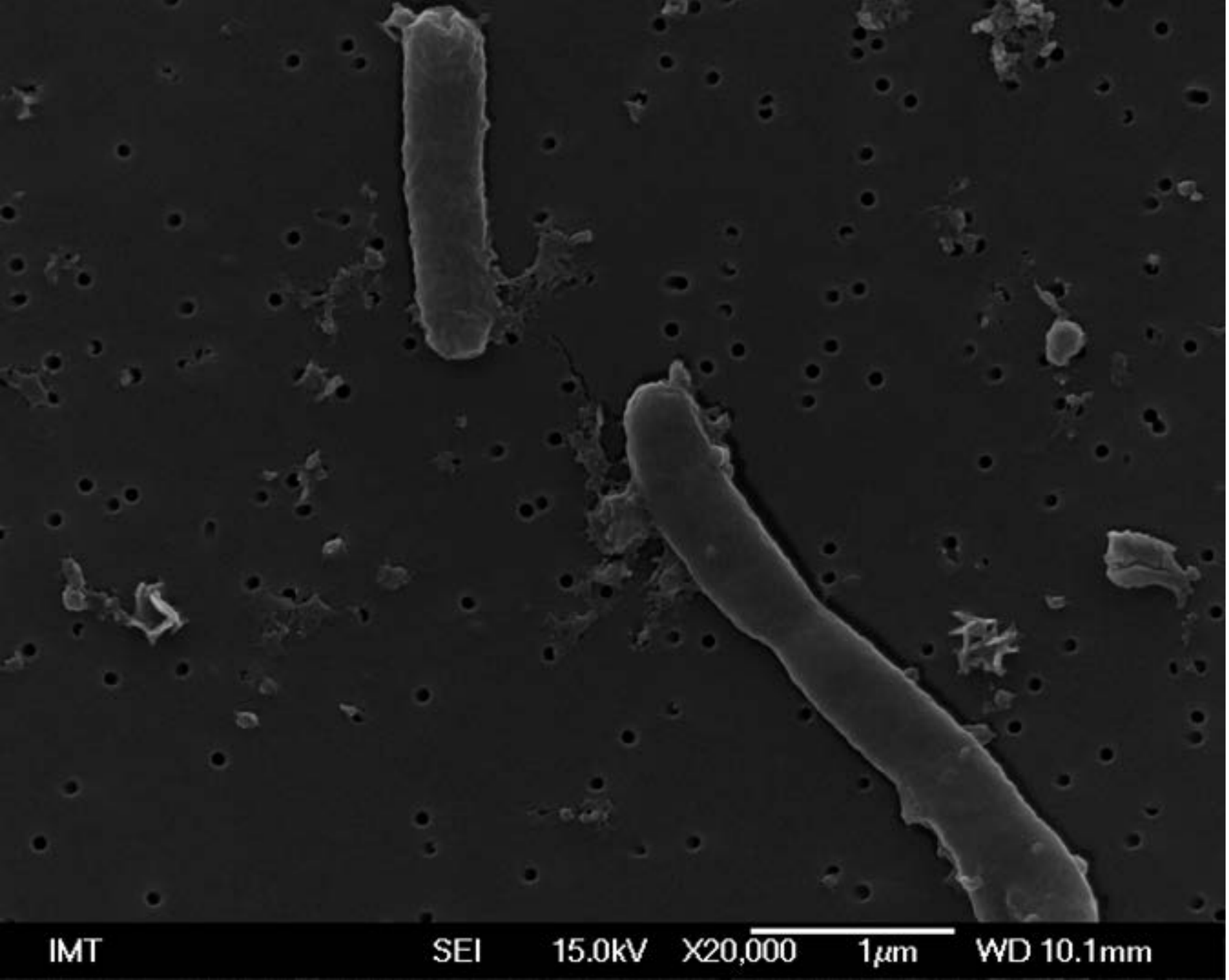

Figure *Dunaliela tertiolecta* isolate 2.000g SEM 16.

Isolation of nanoparticles (NPs)

NPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. The isolate presents the pellet of the second centrifugation.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

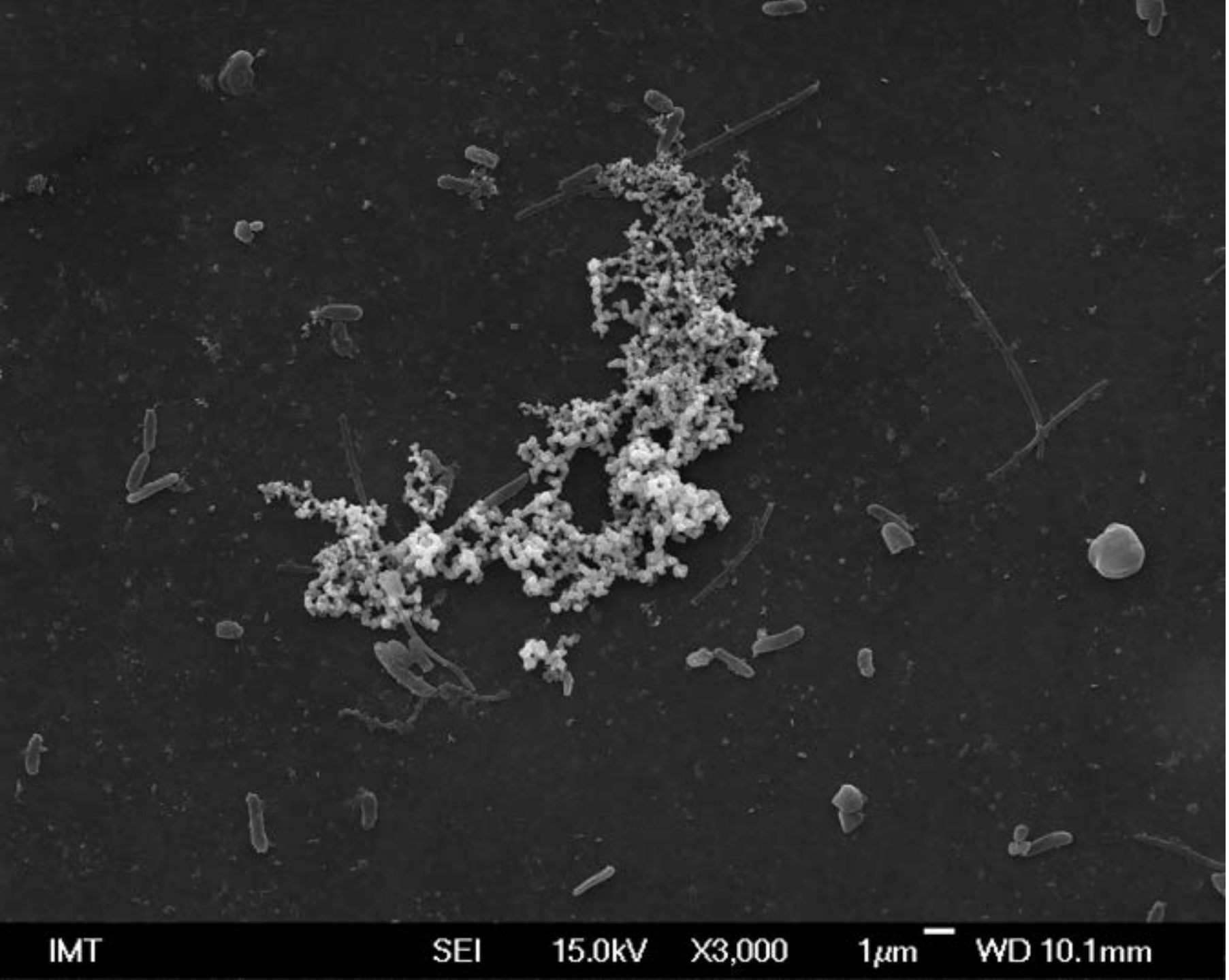

Figure *Dunaliela tertiolecta* isolate 2.000g SEM 17.

Isolation of nanoparticles (NPs)
NPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. The isolate presents the pellet of the second centrifugation.
Scanning Electron Microscopy (SEM)
Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

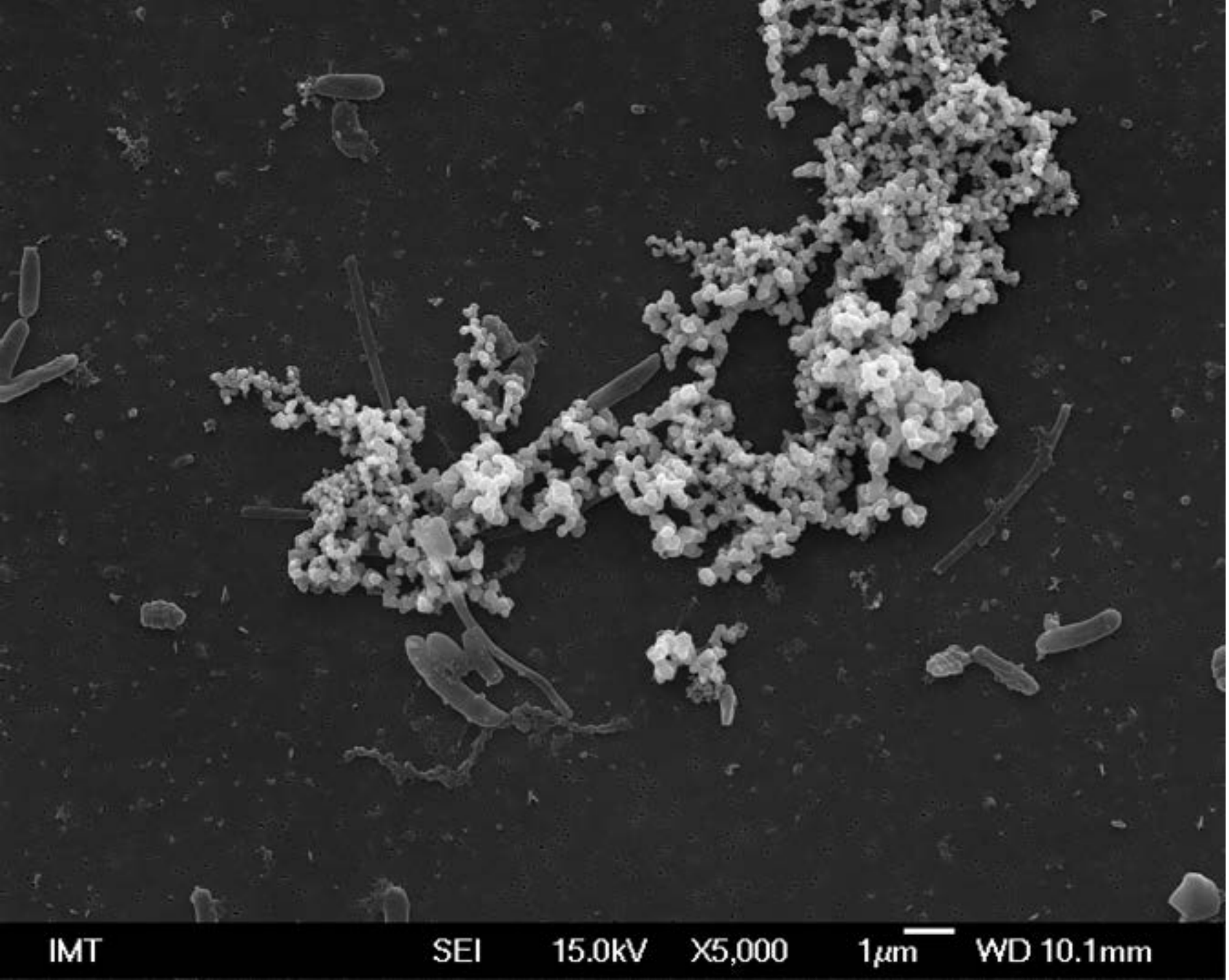

Figure *Dunaliela tertiolecta* isolate 2.000g SEM 18.

Isolation of nanoparticles (NPs)
NPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. The isolate presents the pellet of the second centrifugation.
Scanning Electron Microscopy (SEM)
Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

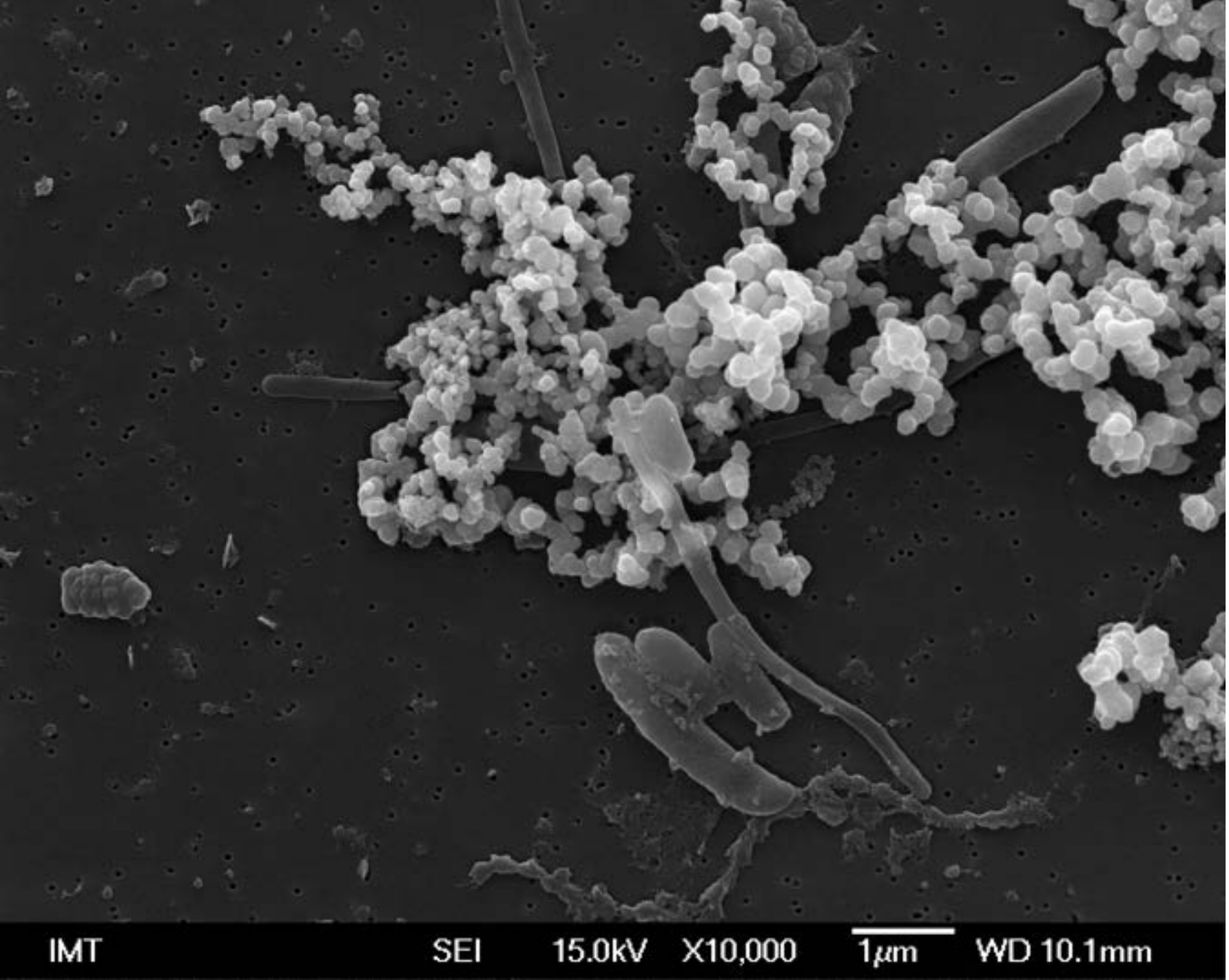

Figure *Dunaliela tertiolecta* isolate 2.000g SEM 19.

Isolation of nanoparticles (NPs)

NPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. The isolate presents the pellet of the second centrifugation.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

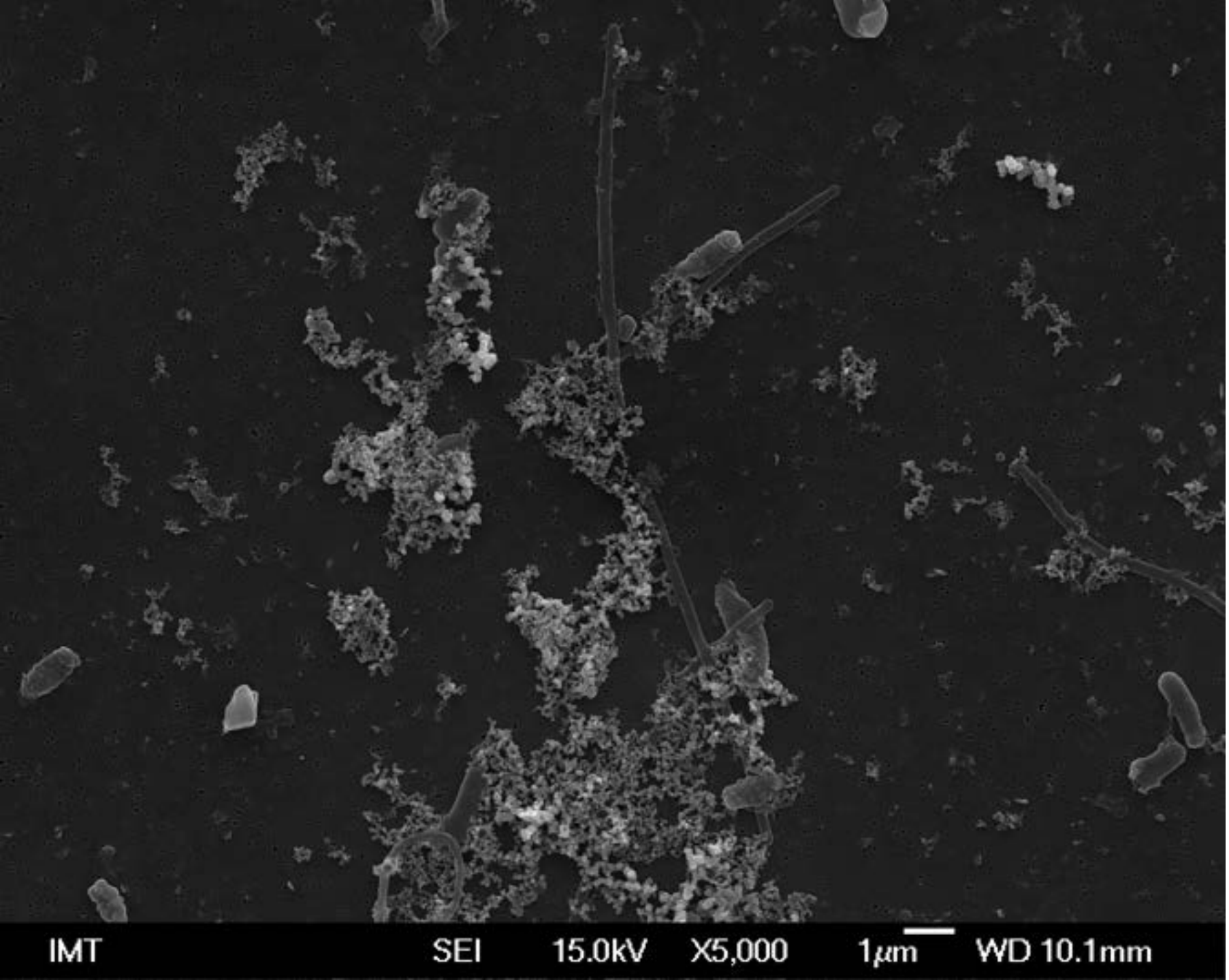

Figure *Dunaliela tertiolecta* isolate 2.000g SEM 20.

Isolation of nanoparticles (NPs)
NPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. The isolate presents the pellet of the second centrifugation.

Scanning Electron Microscopy (SEM)
Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

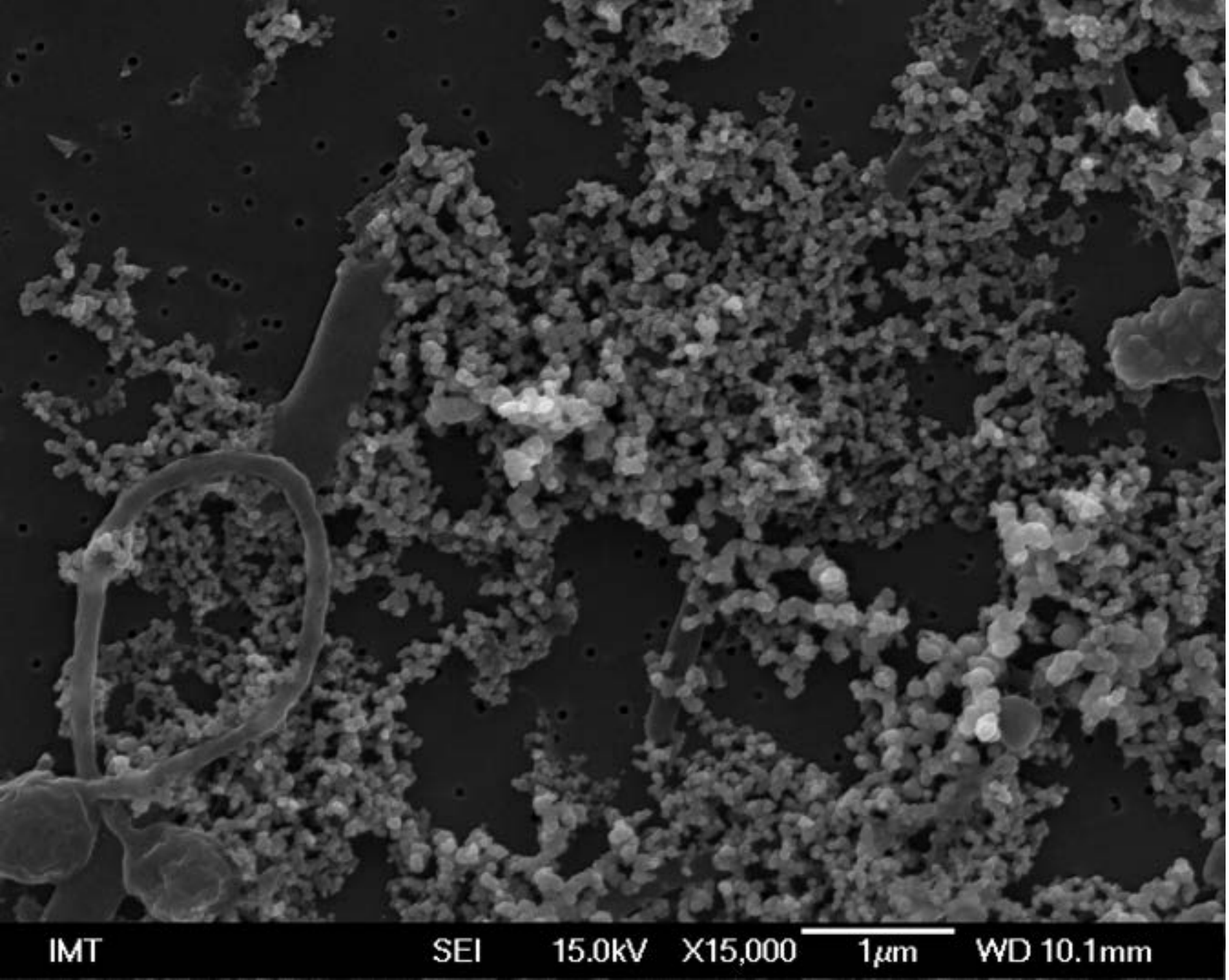

Figure *Dunaliela tertiolecta* isolate 2.000g SEM 21.

Isolation of nanoparticles (NPs)
NPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. The isolate presents the pellet of the second centrifugation.
Scanning Electron Microscopy (SEM)
Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

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Repository

Scanning electron microscope images of *Phaeodactylum tricornutum* culture

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Abstract: Scanning electron microscope images of small cellular particles isolated from conditioned media of microalgae *Phaeodactylum tricornutum* are presented. Each image is supplemented by description of the preparation of the sample and the data on the imaging technique and equipment.

The data curators of the repository are Veronika Kralj-Iglič and Anna Romolo. More data on experiments with microalgae small cellular particles can be found in (Adamo et al., 2021), (Picciotto et al., 2021) and (Božič et al., 2022).

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Keywords: Extracellular vesicles, Extracellular particles, Nanoalgosomes, Exosomes

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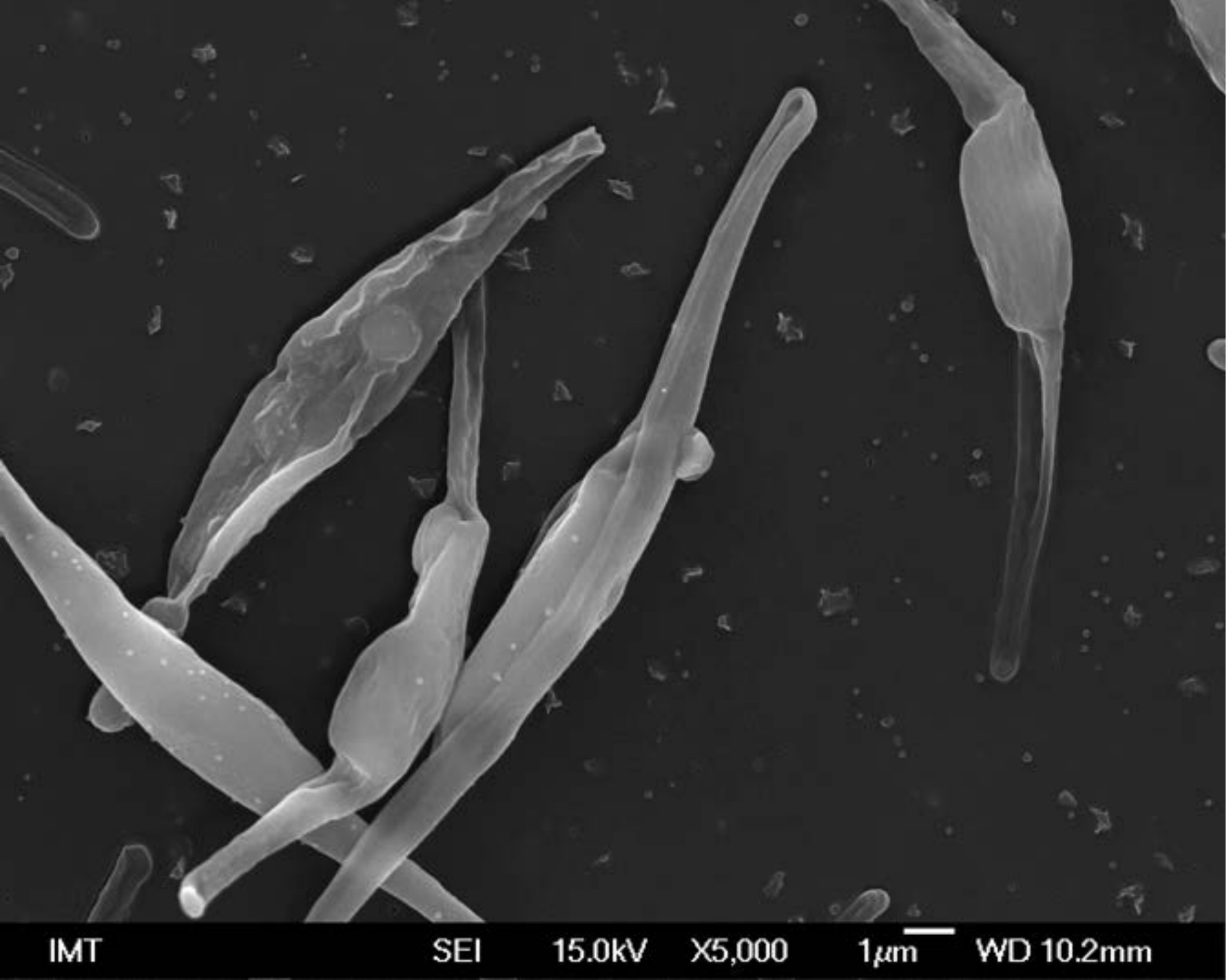

Figure *Phaeodactylum tricornutum* culture F2 SEM 1.

Cultivation of the algae
Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 µmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.
Scanning Electron Microscopy (SEM)
Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

From: https://zenodo.org/record/6908895. Image:6
DOI 10.5281/zenodo.6908895.

Figure *Phaeodactylum tricornutum* culture in F2 SEM 2.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Scanning Electron Microscopy (SEM)

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Figure *Phaeodactylum tricornutum* culture F2 SEM 3.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Scanning Electron Microscopy (SEM)

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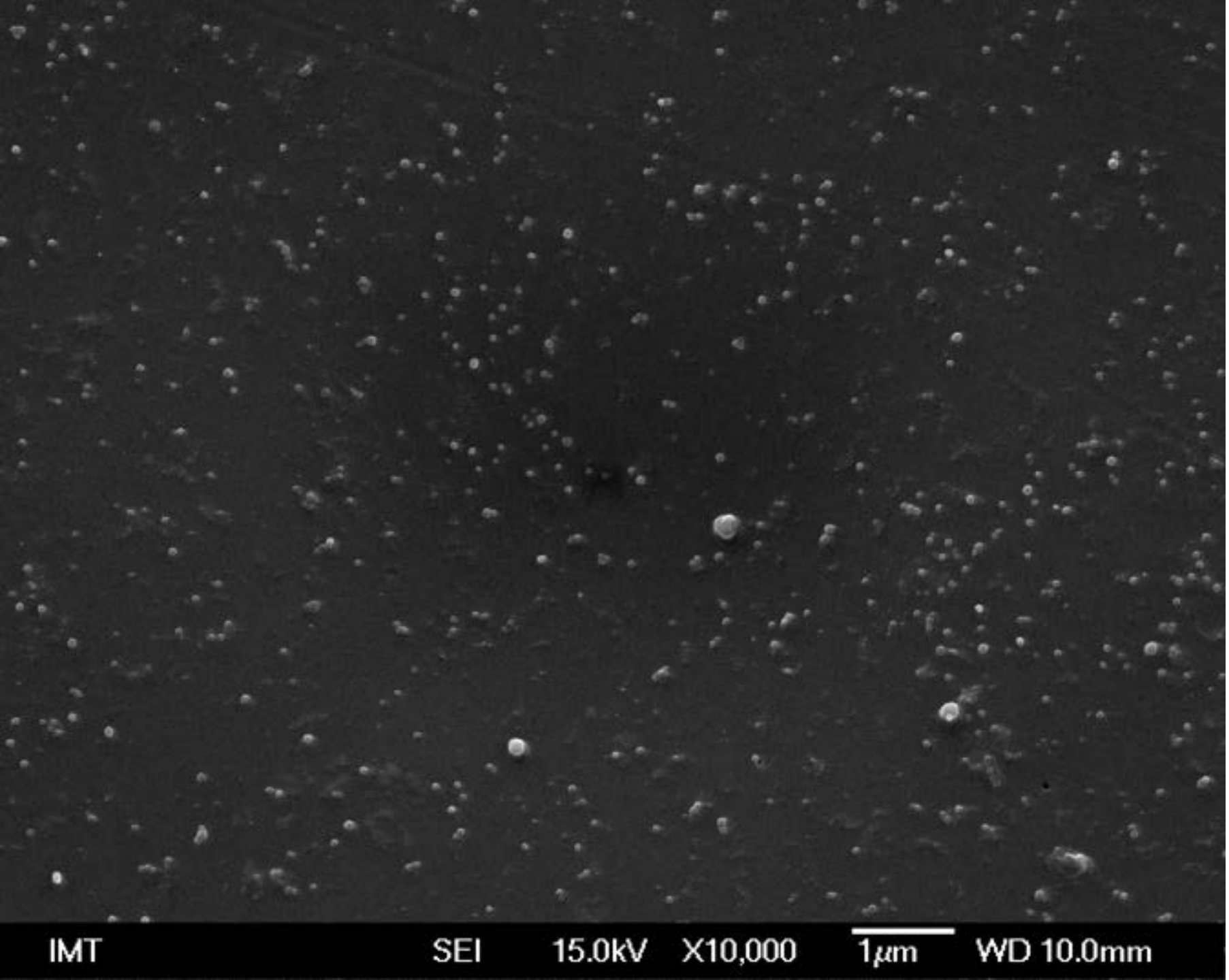

Figure *Phaeodactylum tricornutum* culture F2 SEM 4.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

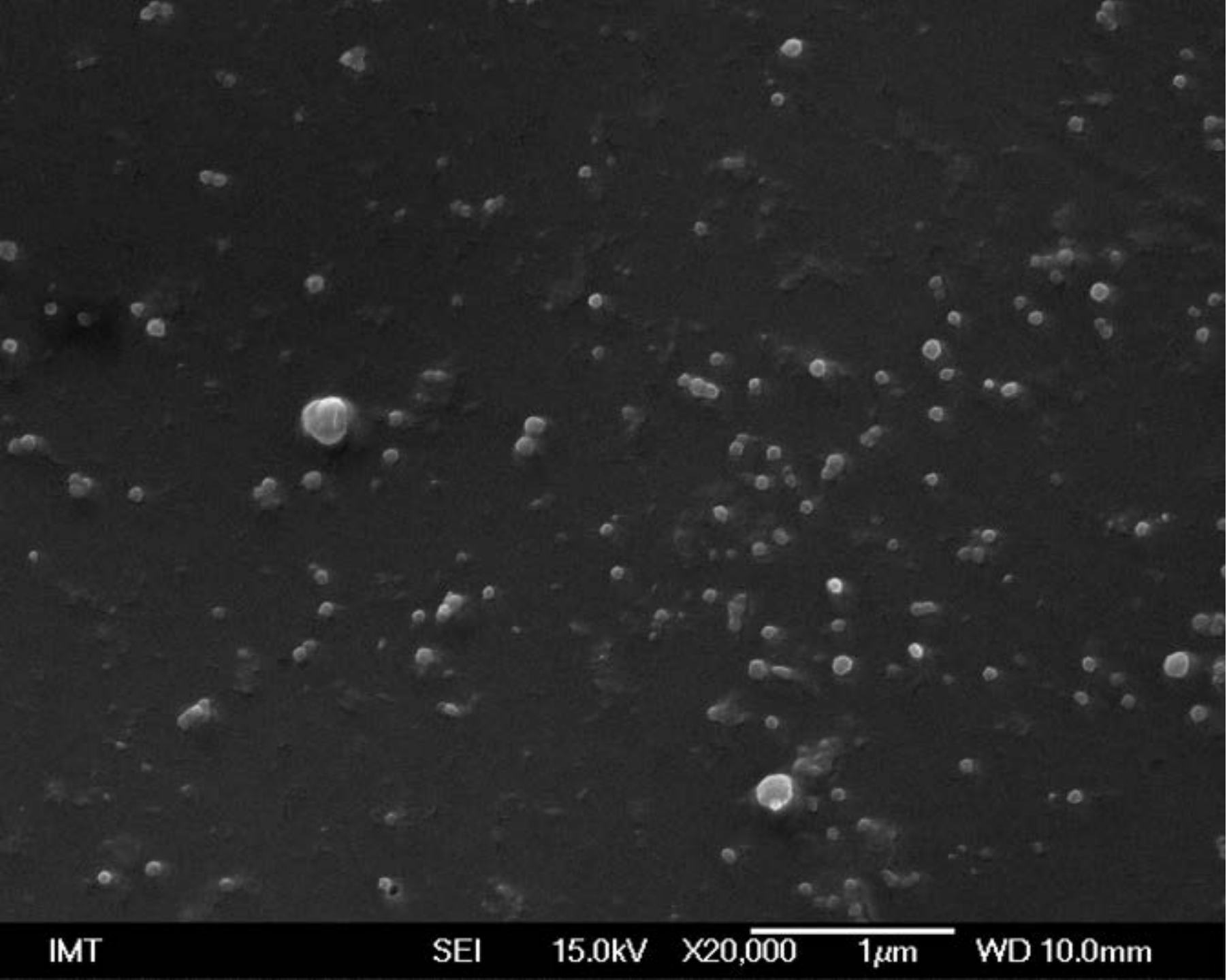

Figure *Phaeodactylum tricornutum* culture F2 SEM 5.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

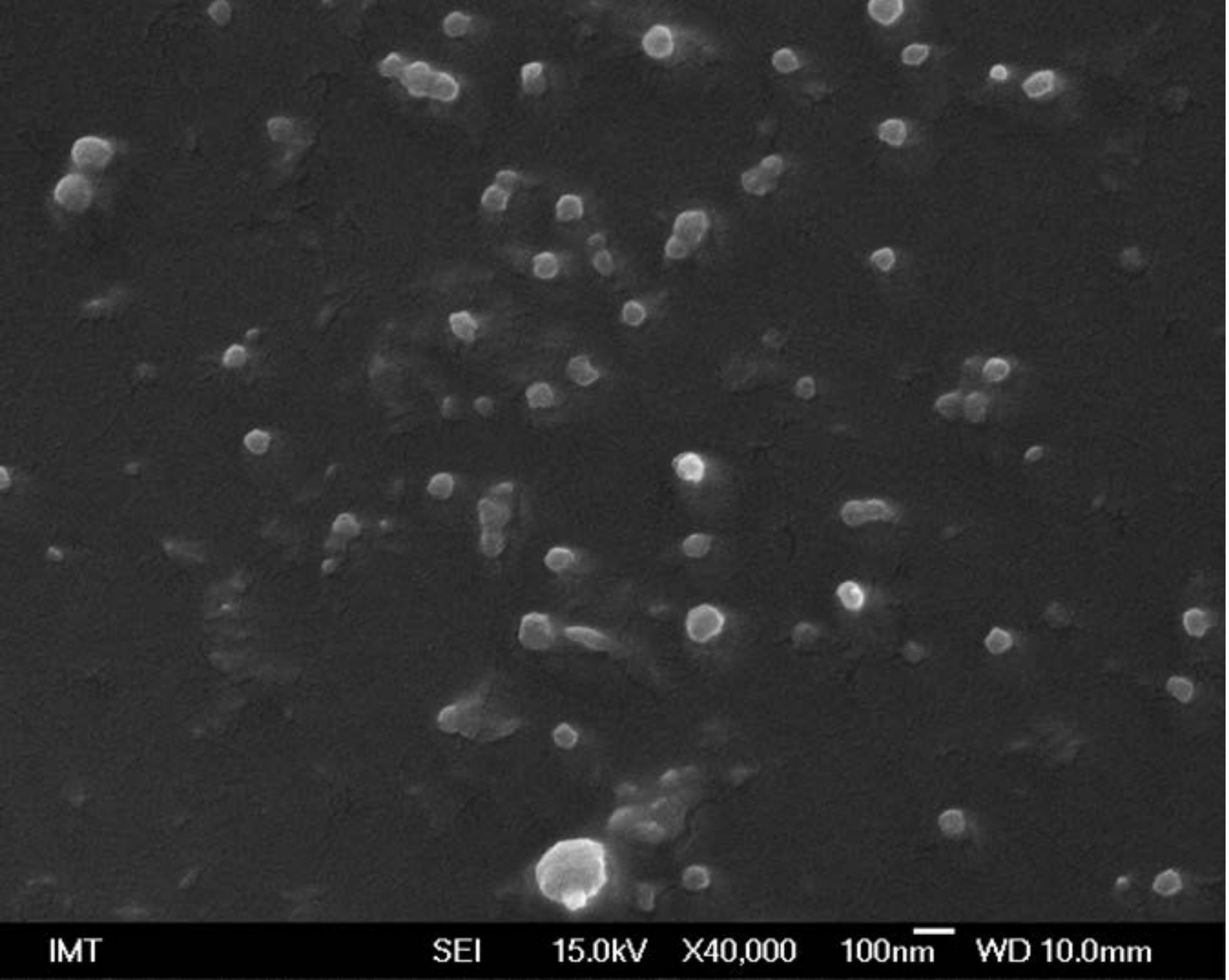

Figure *Phaeodactylum tricornutum* culture F2 SEM 6.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 µmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

Figure *Phaeodactylum tricornutum* culture F2 SEM 7.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

Figure *Phaeodactylum tricornutum* culture F2 SEM 8.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Scanning Electron Microscopy (SEM)

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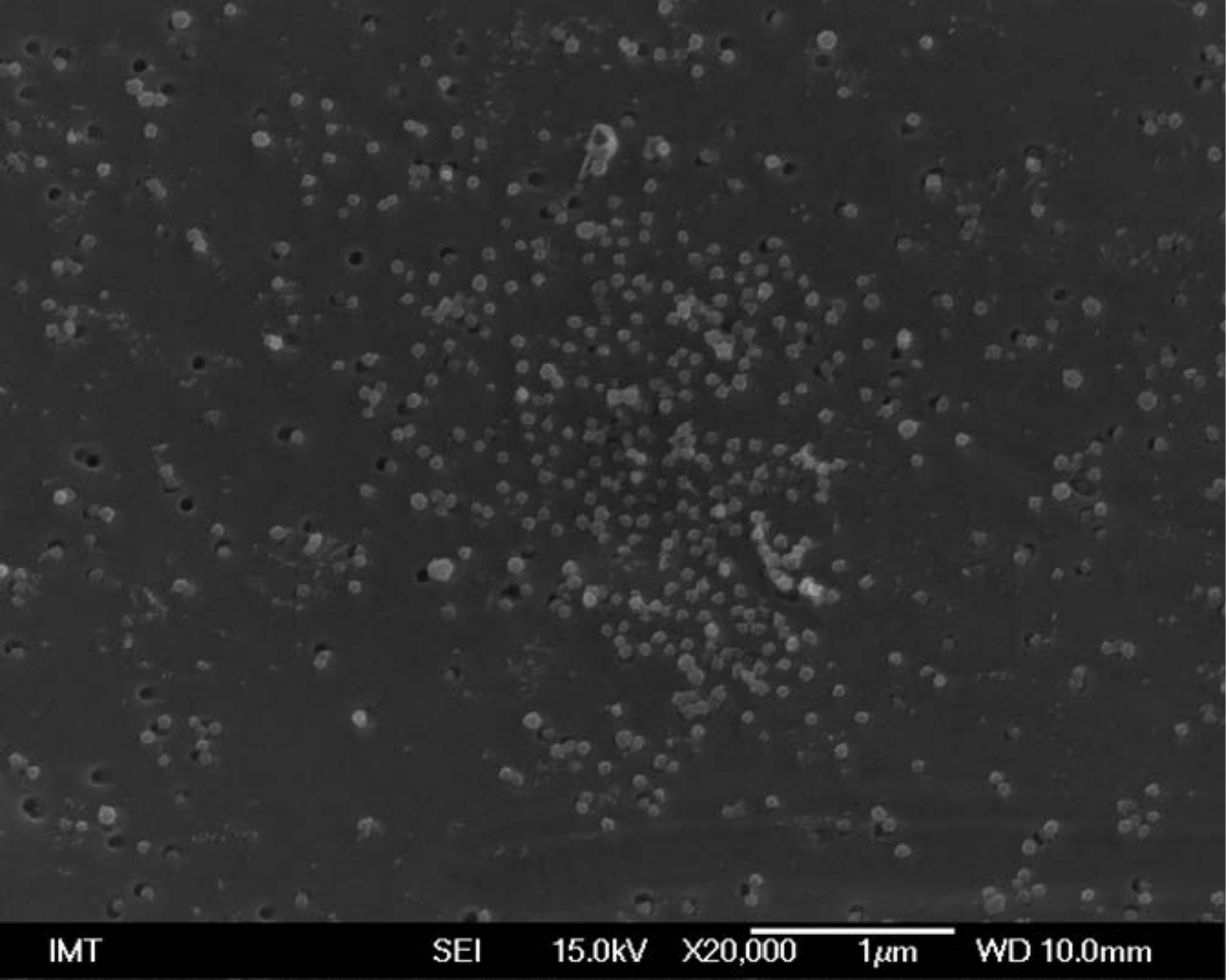

Figure *Phaeodactylum tricornutum* culture F2 SEM 9.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Scanning Electron Microscopy (SEM)

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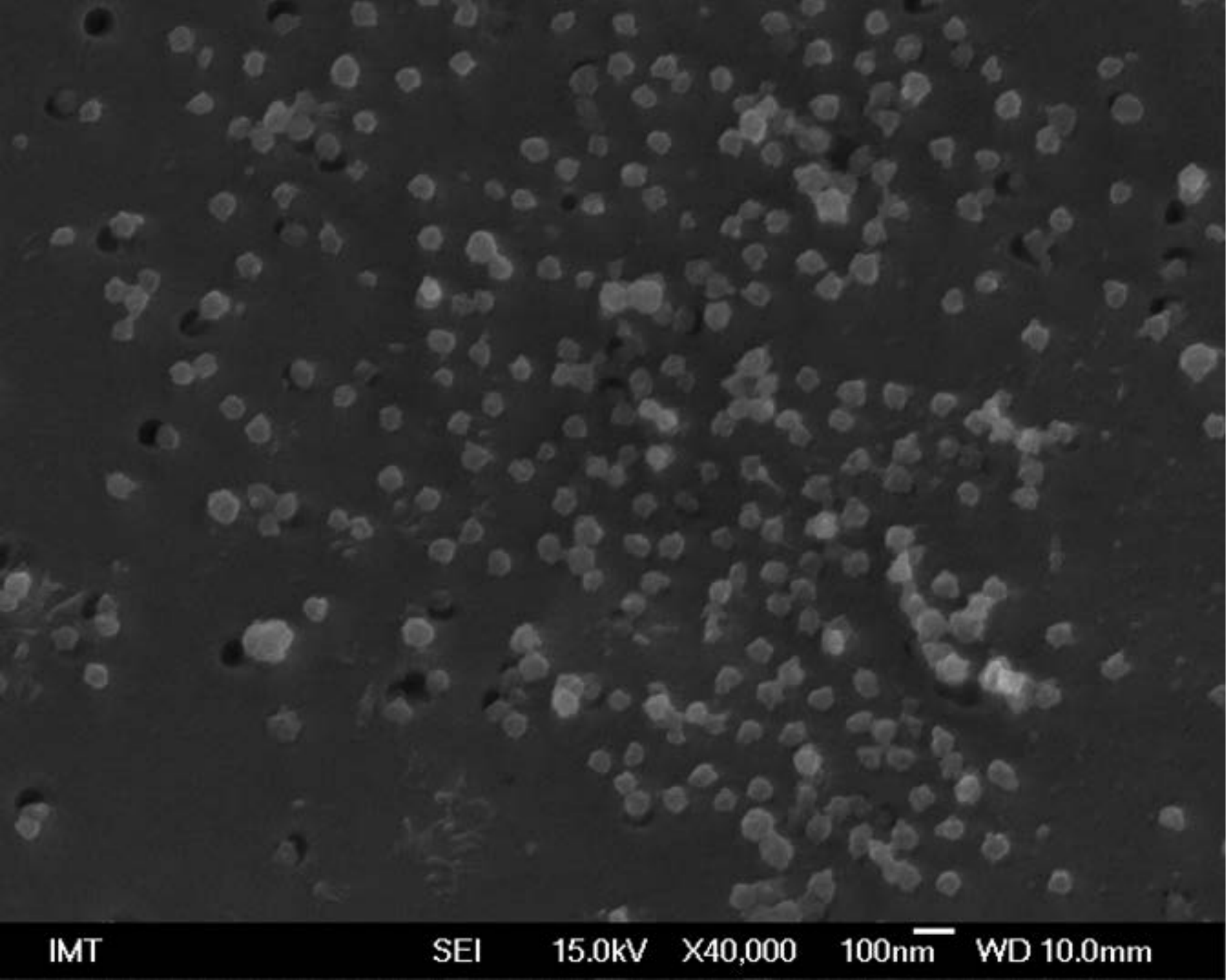

Figure *Phaeodactylum tricornutum* culture F2 SEM 10.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 µmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

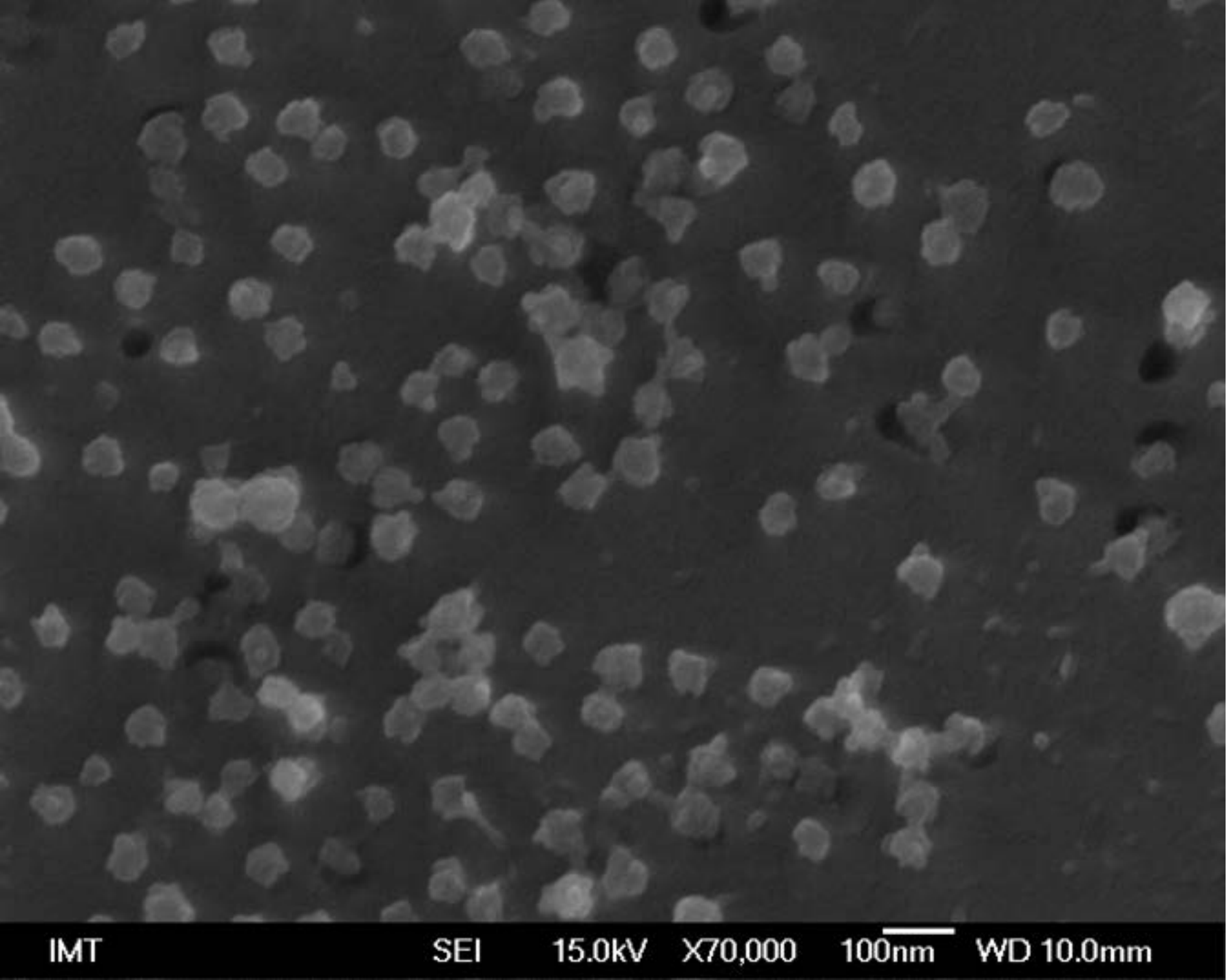

Figure *Phaeodactylum tricornutum* culture in F2 SEM 11.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Scanning Electron Microscopy (SEM)

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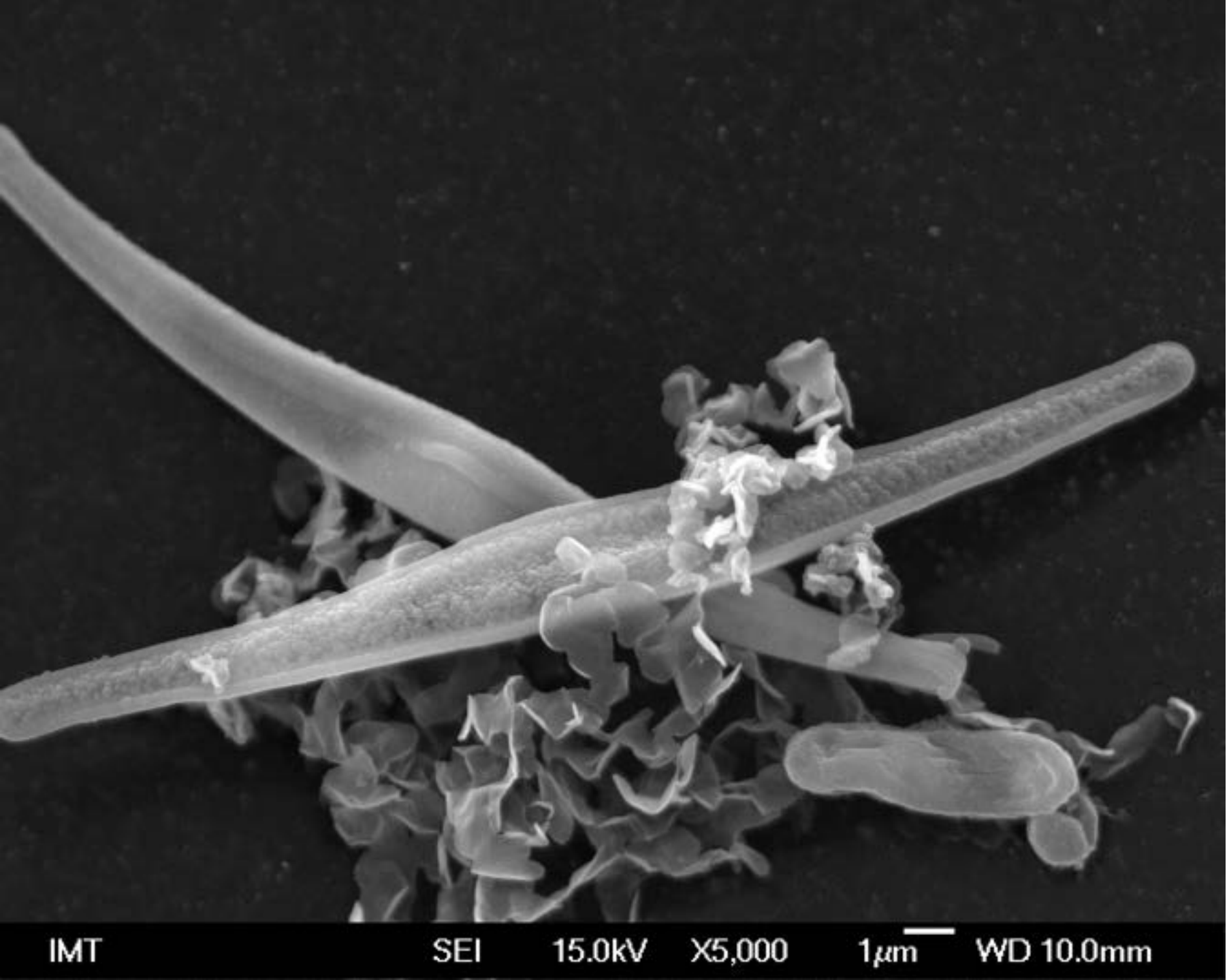

Figure *Phaeodactylum tricornutum* culture in F2 SEM 12.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Scanning Electron Microscopy (SEM)

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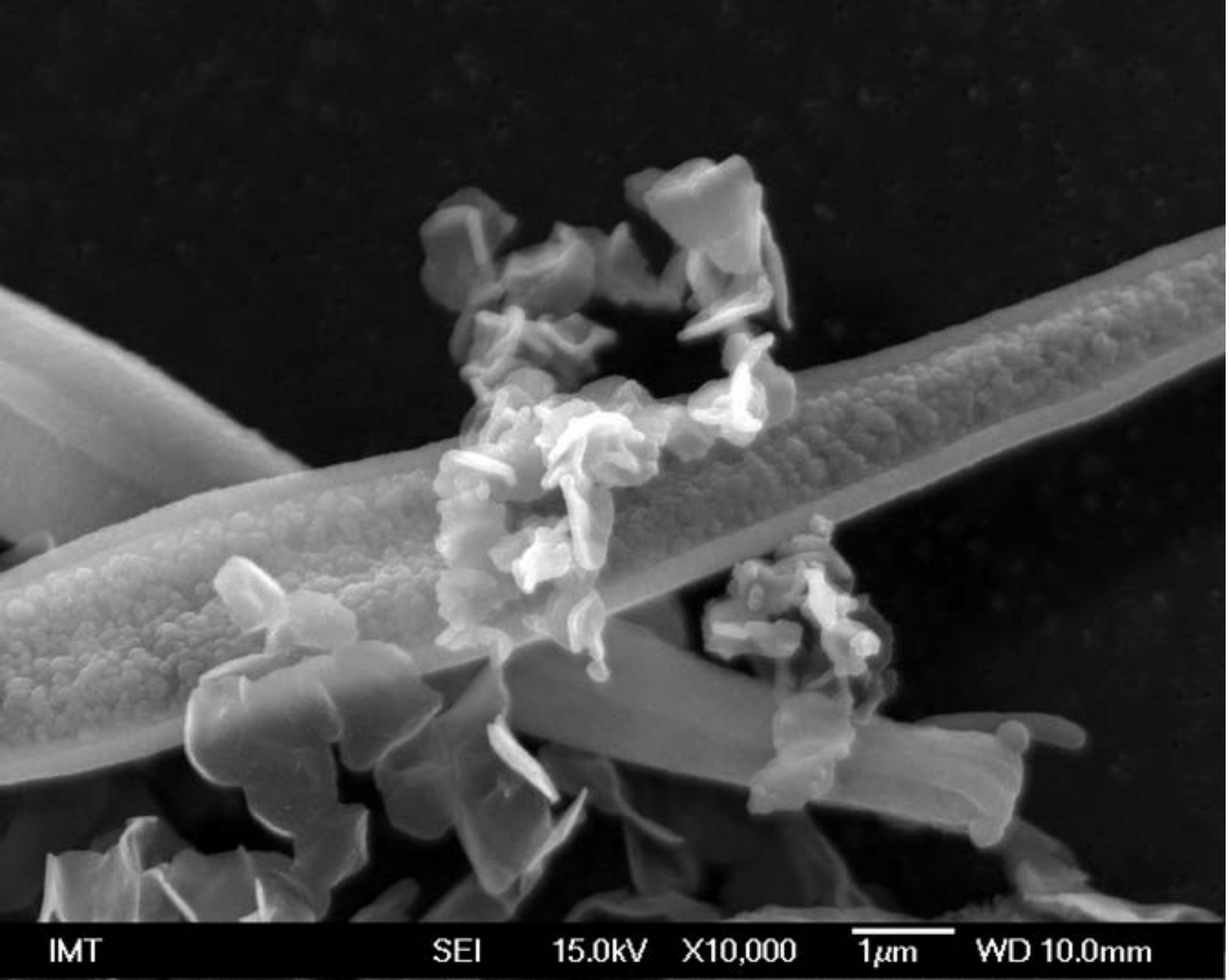

Figure *Phaeodactylum tricornutum* culture F2 SEM 13.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

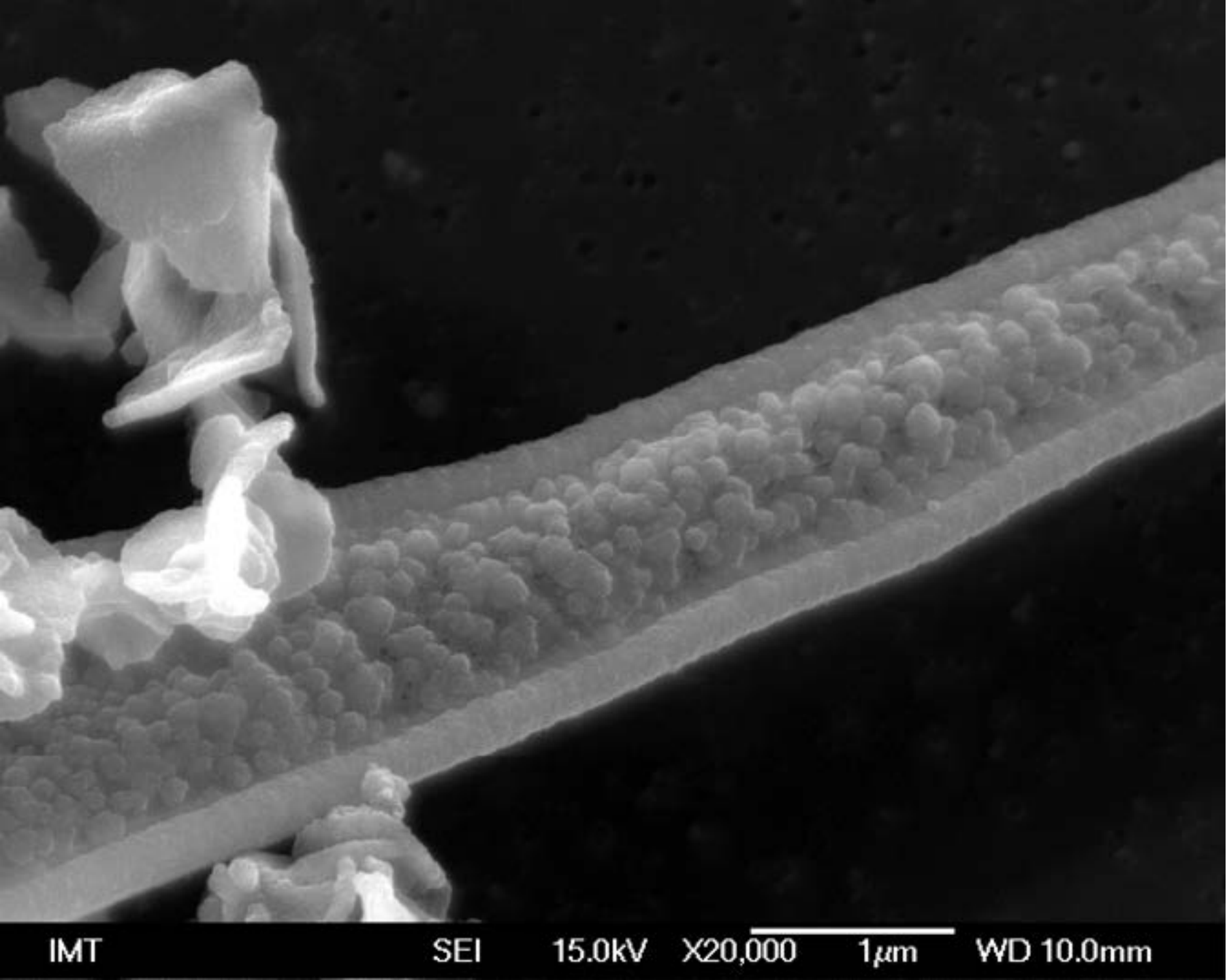

Figure *Phaeodactylum tricornutum* culture F2 SEM 14.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

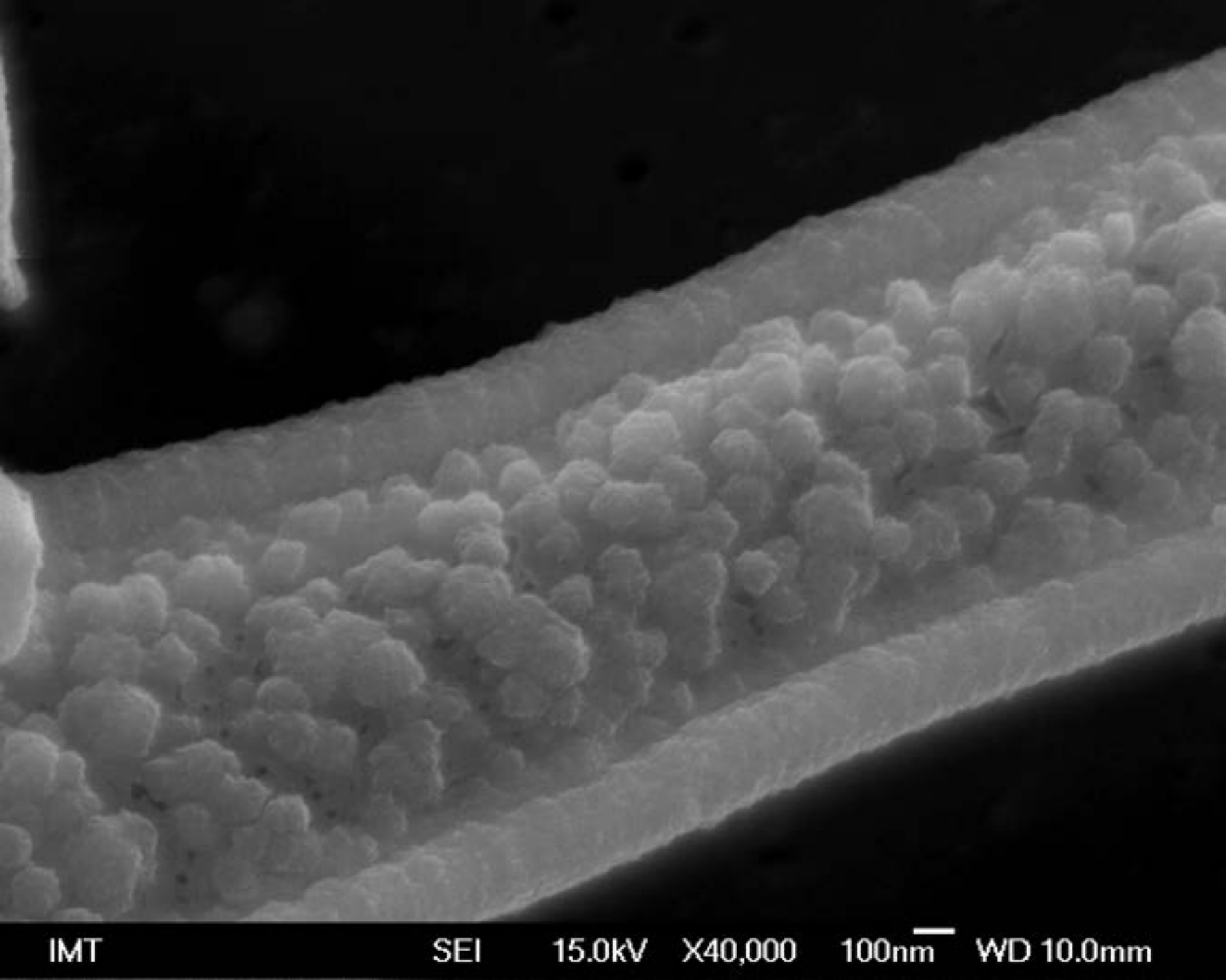

Figure *Phaeodactylum tricornutum* culture F2 SEM 15.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 µmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

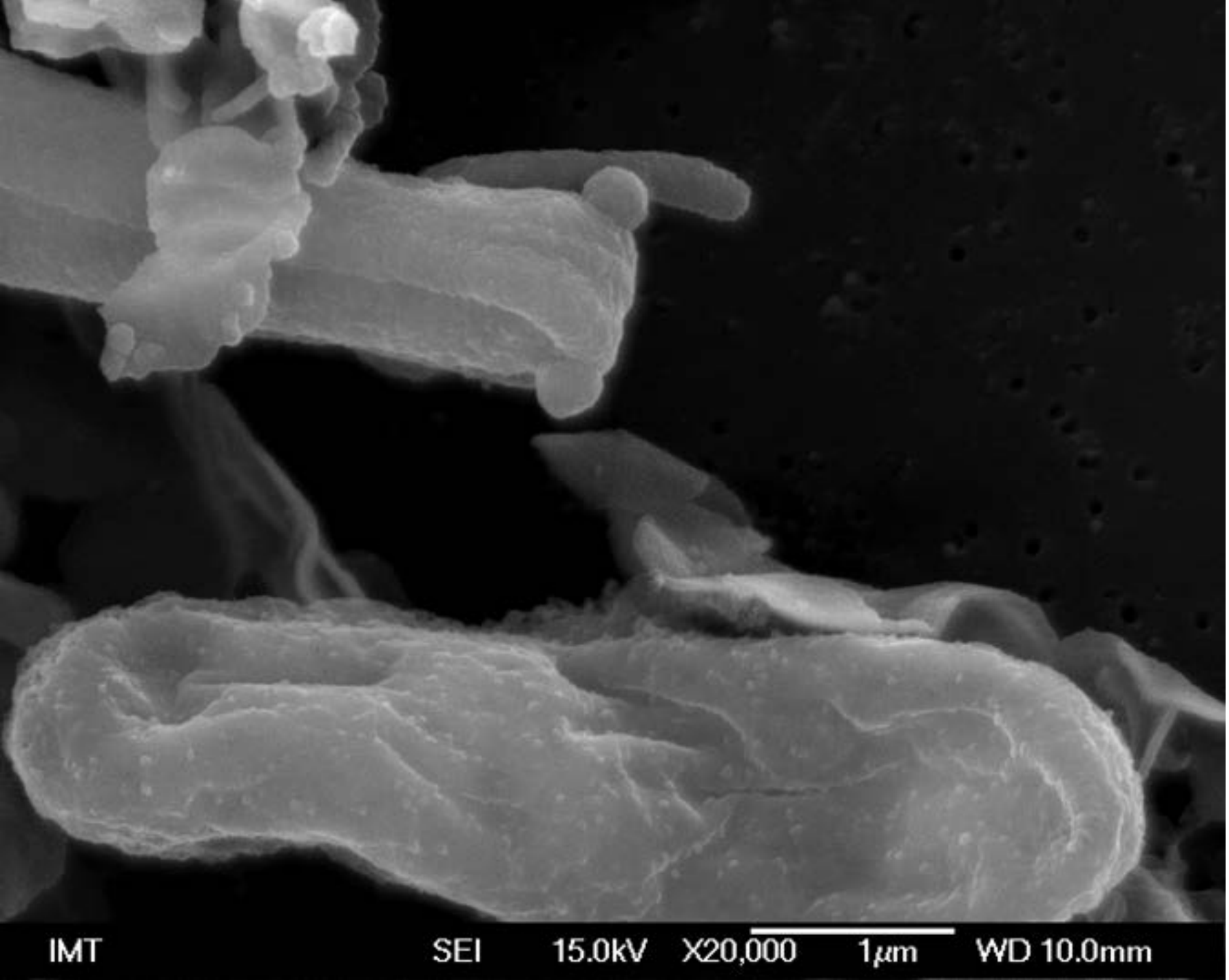

Figure *Phaeodactylum tricornutum* culture F2 SEM 16.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

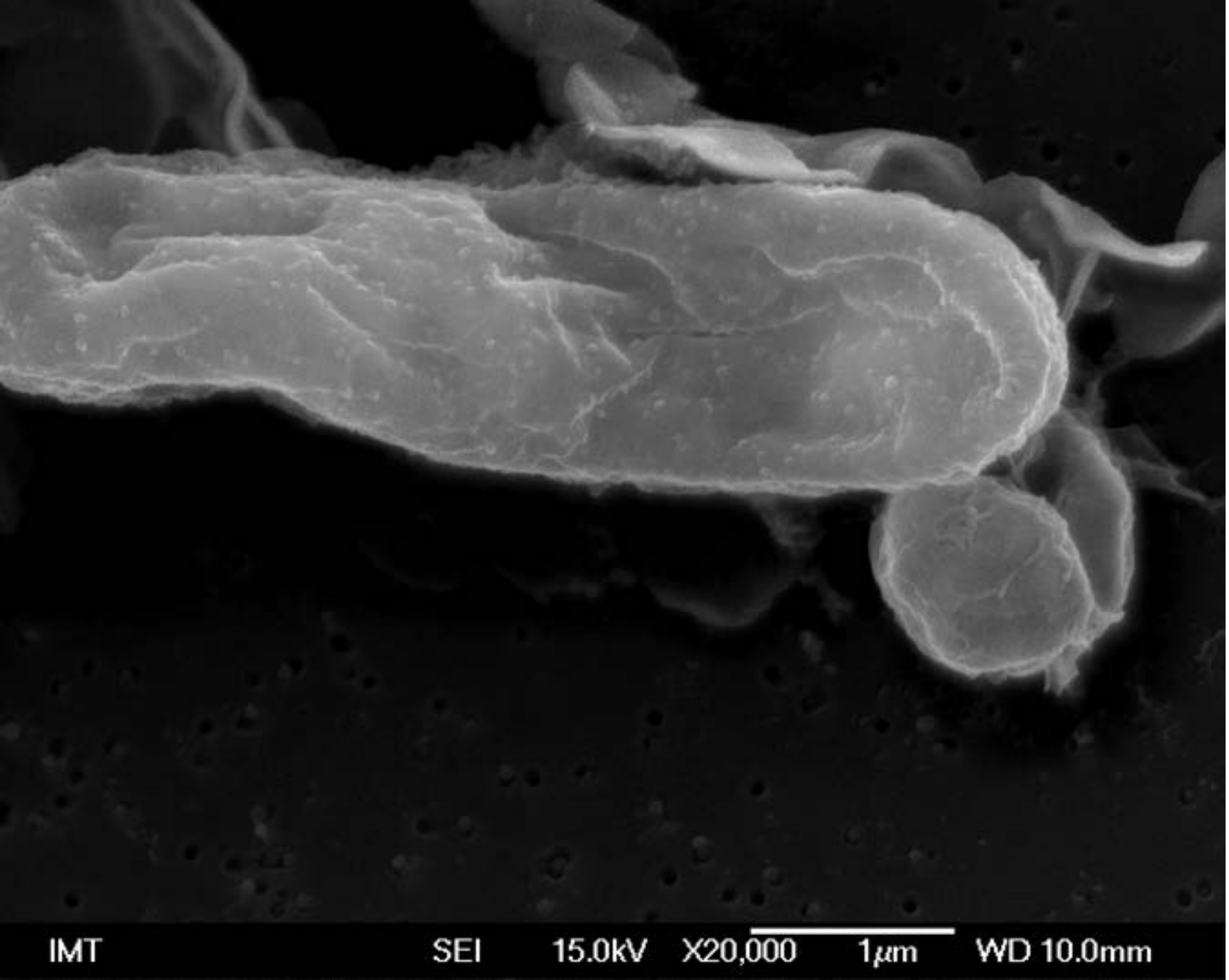

Figure *Phaeodactylum tricornutum* culture F2 SEM 17.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

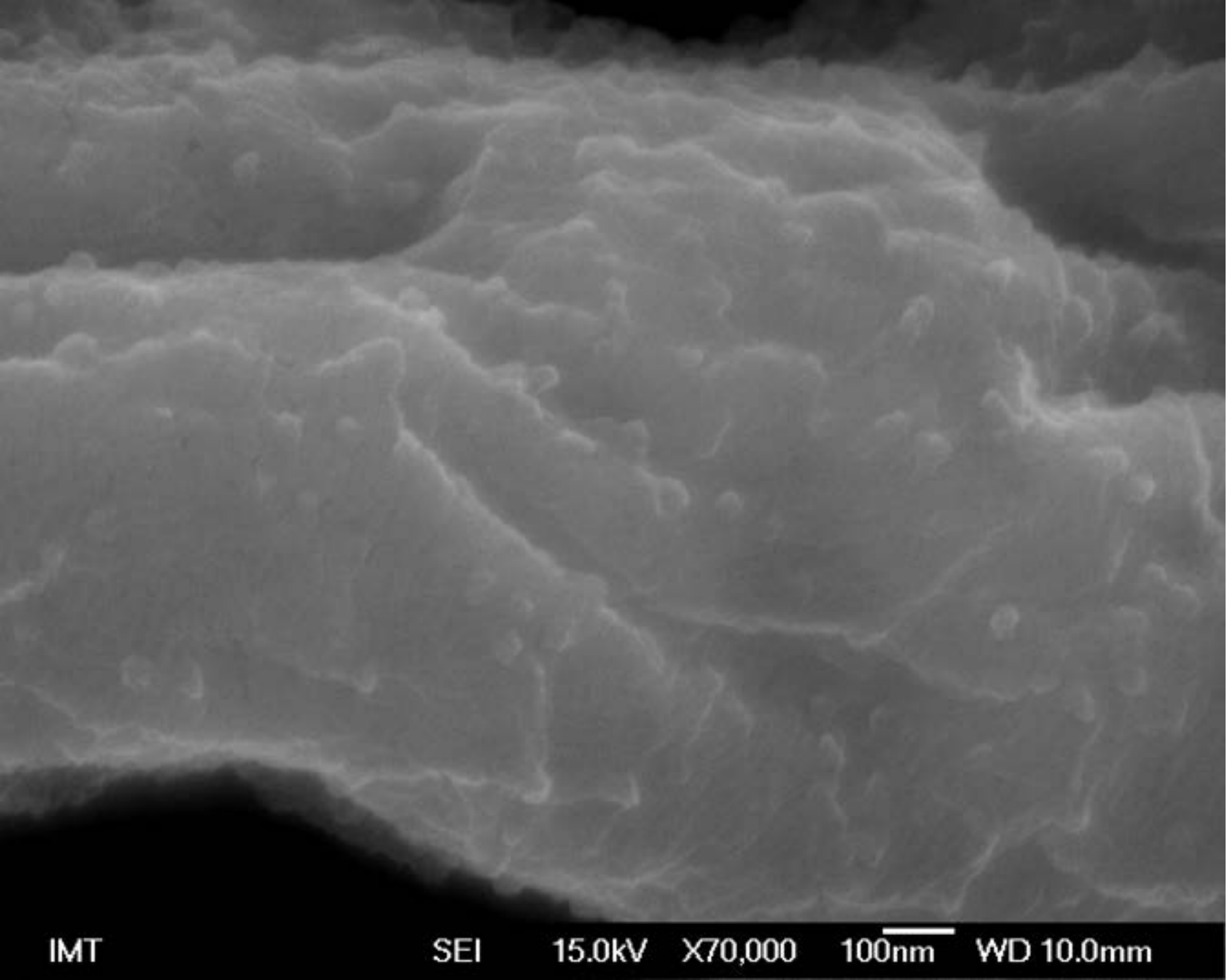

Figure *Phaeodactylum tricornutum* culture F2 SEM 18.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 µmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

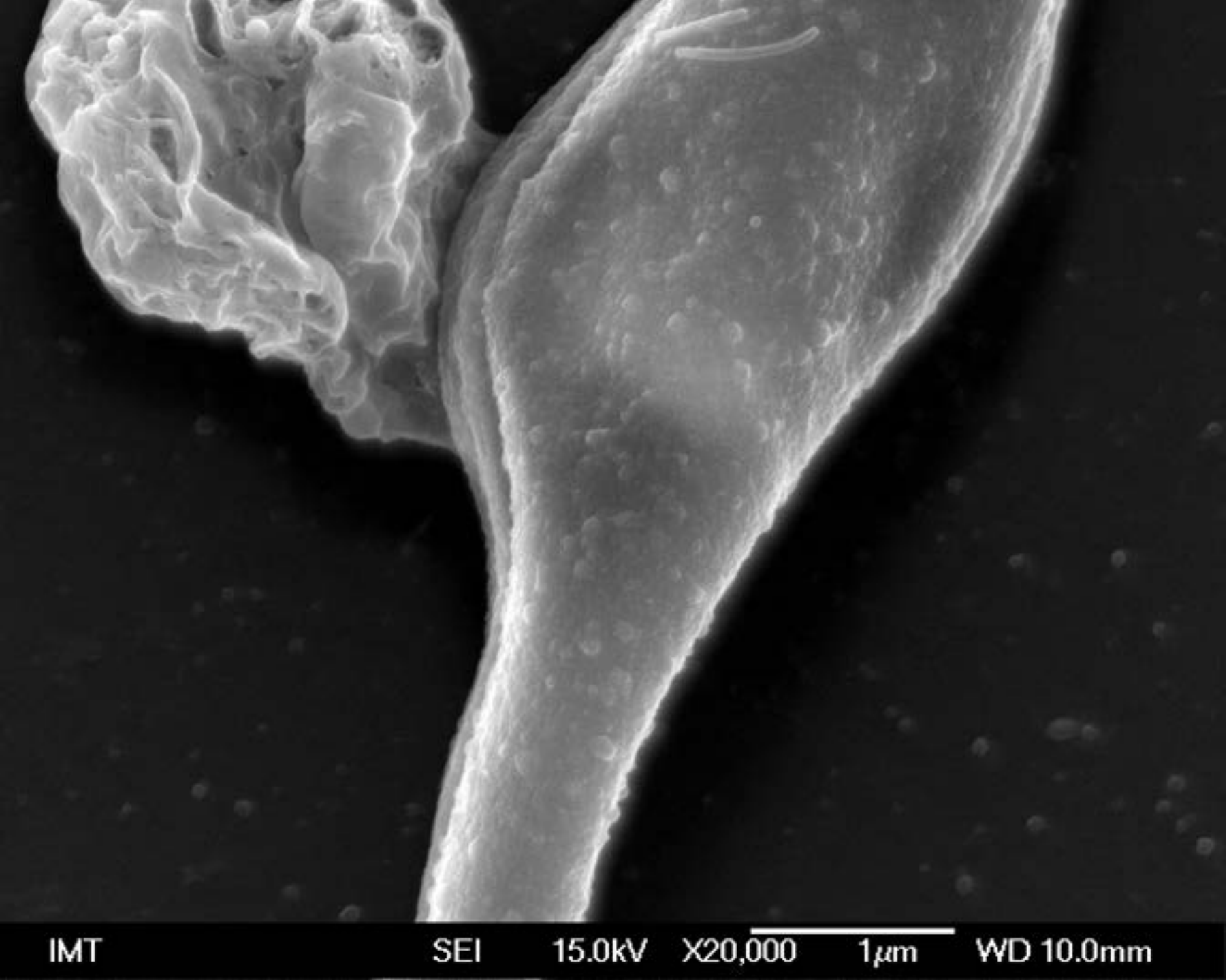

Figure *Phaeodactylum tricornutum* culture F2 SEM 19.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

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Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

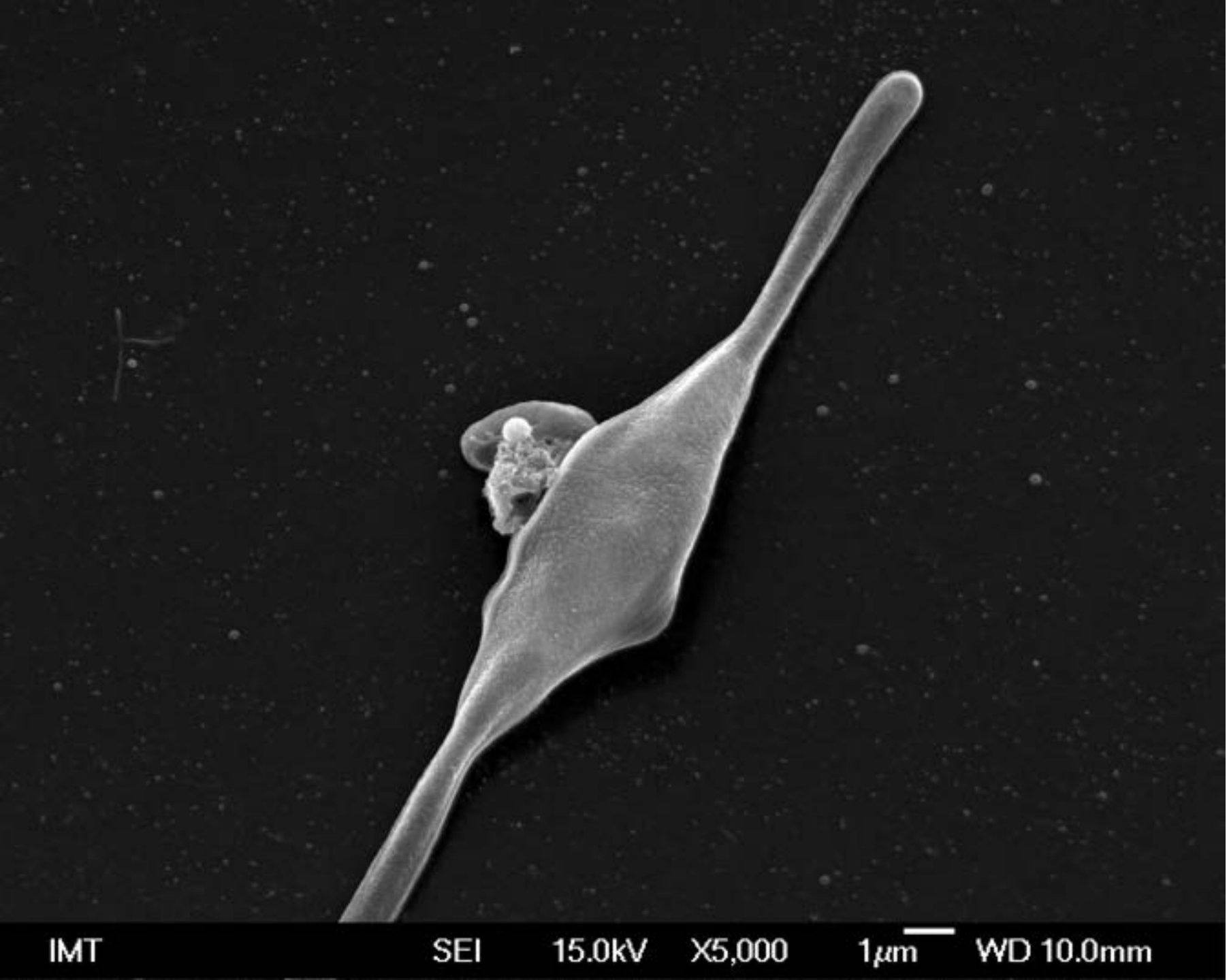

Figure *Phaeodactylum tricornutum* culture F2 SEM 20.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

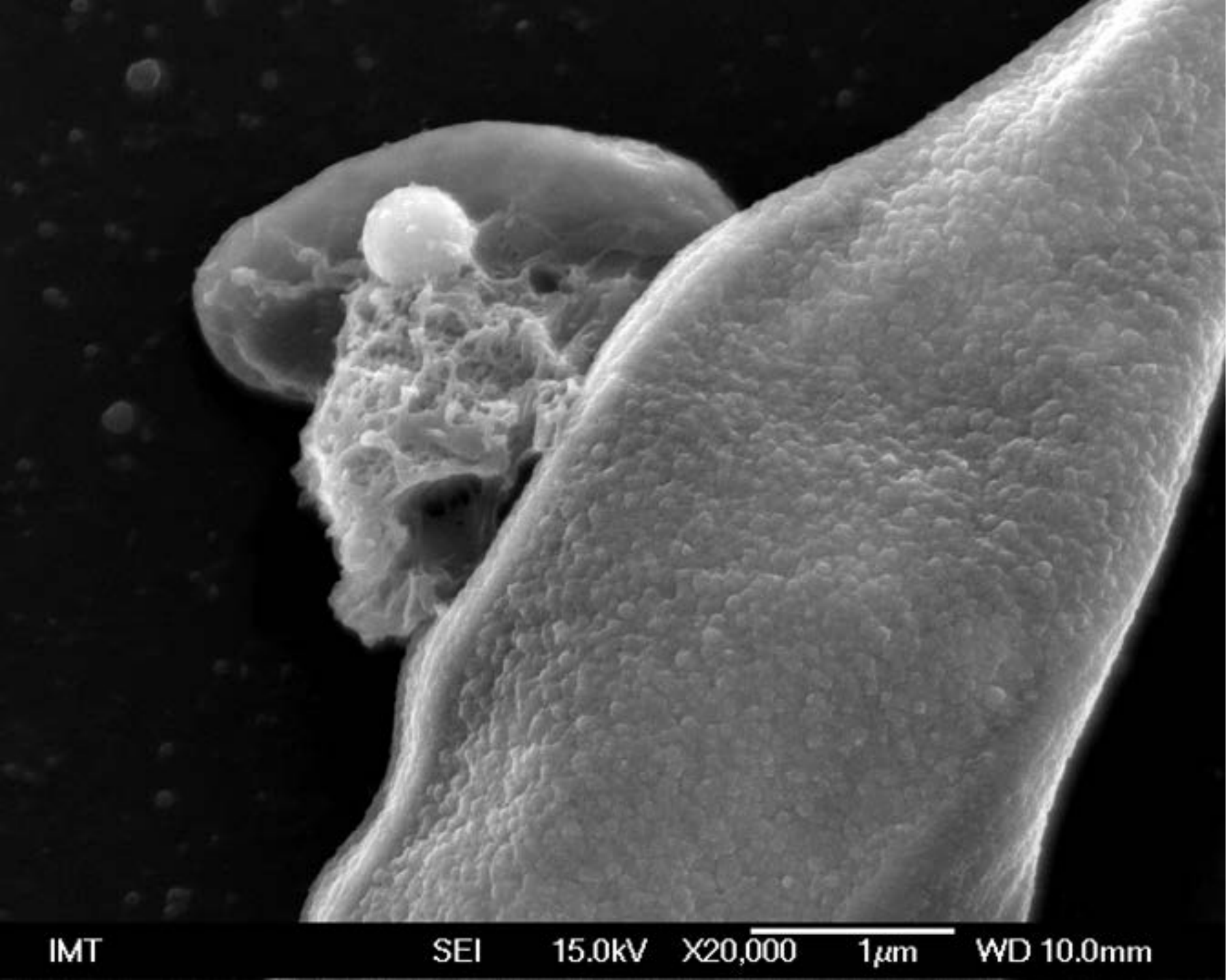

Figure *Phaeodactylum tricornutum* culturein F2 SEM 21.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Scanning Electron Microscopy (SEM)

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Figure *Phaeodactylum tricornutum* culture F2 SEM 22.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

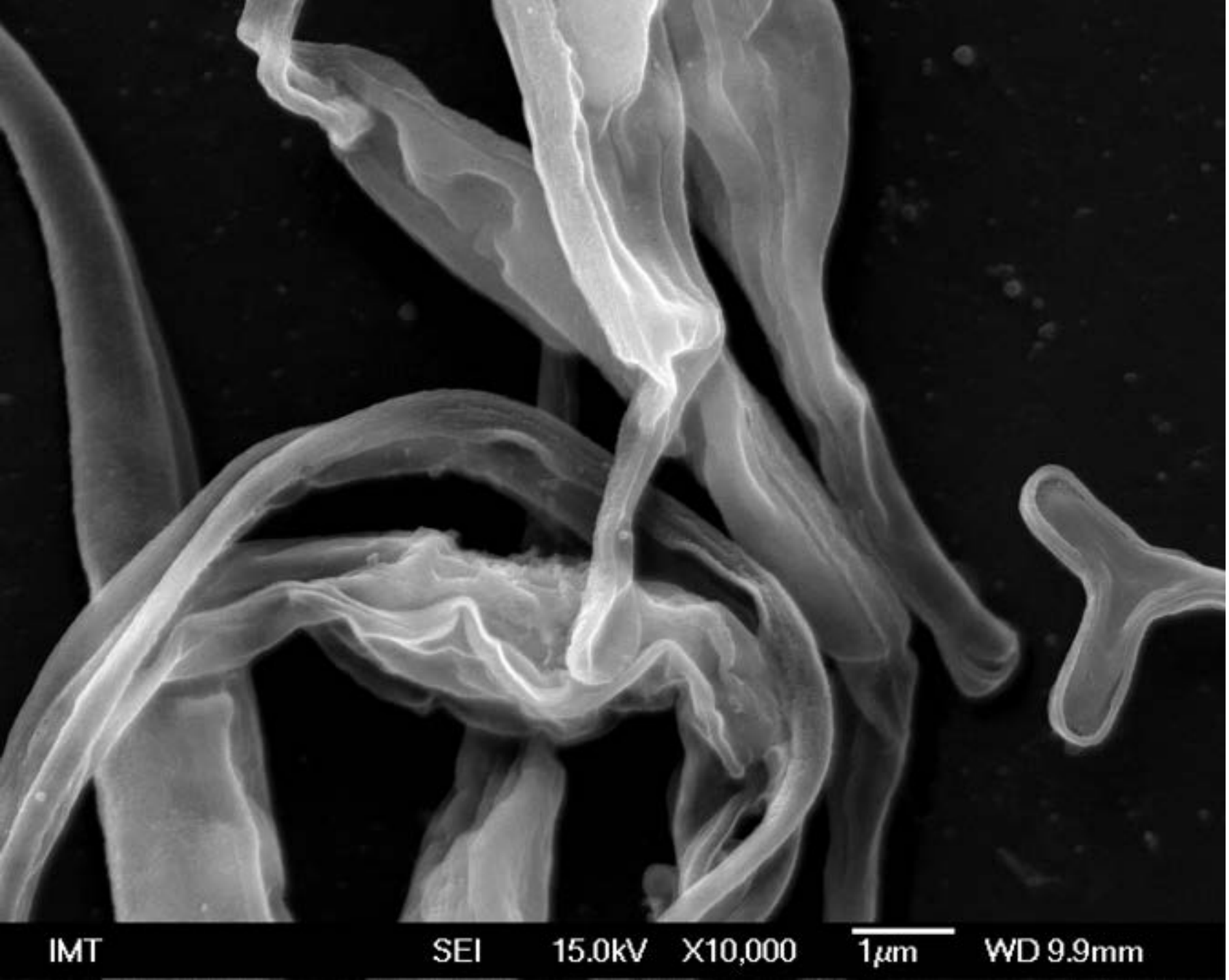

Figure *Phaeodactylum tricornutum* culture F2 SEM 23.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

Figure *Phaeodactylum tricornutum* culture F2 SEM 24.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 µmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Scanning Electron Microscopy (SEM)

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Figure *Phaeodactylum tricornutum* culture F2 SEM 25.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Scanning Electron Microscopy (SEM)

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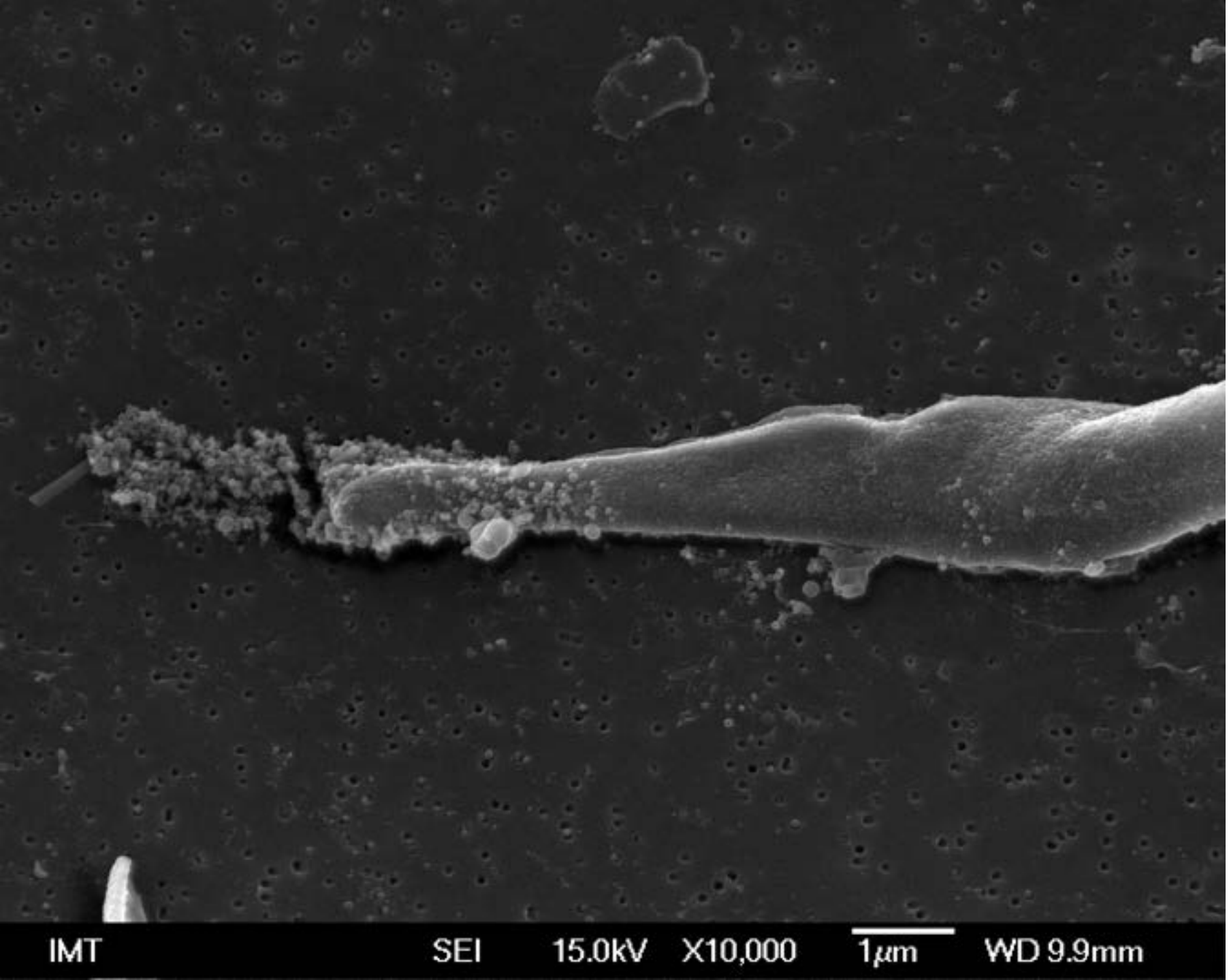

Figure *Phaeodactylum tricornutum* culture F2 SEM 26.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

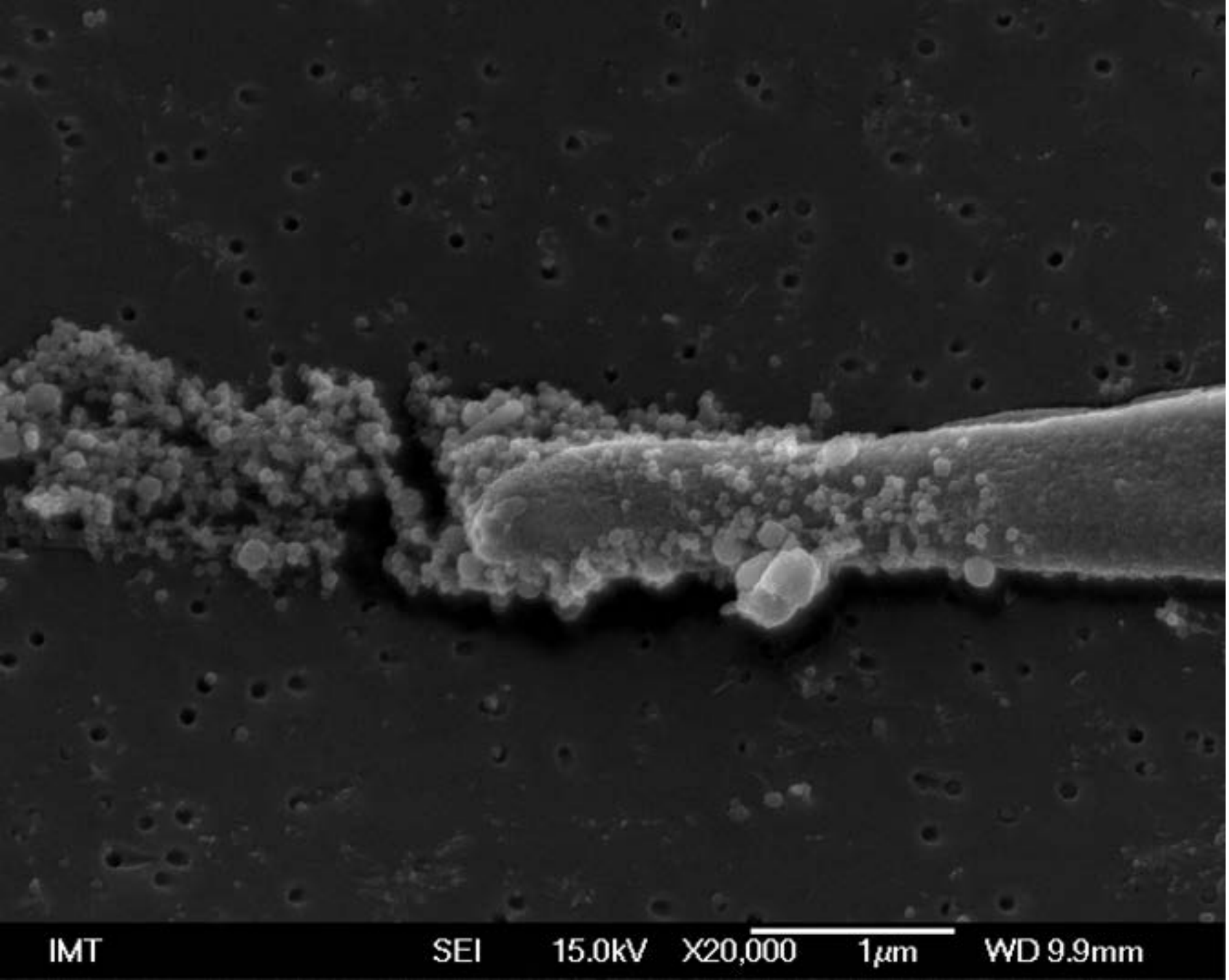

Figure *Phaeodactylum tricornutum* culture in F2 SEM 27.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

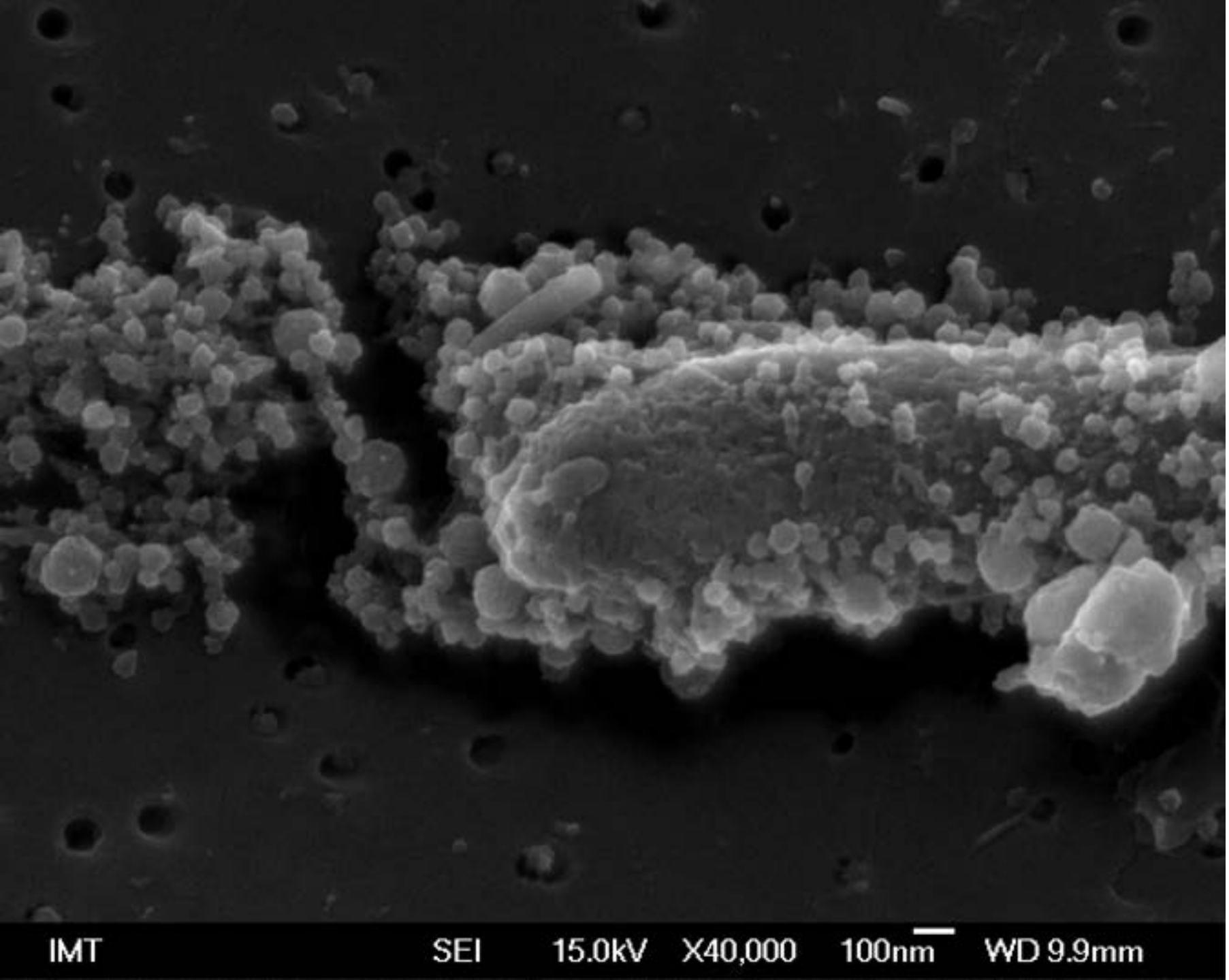

Figure *Phaeodactylum tricornutum* culture F2 SEM 28.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA). Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 µmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

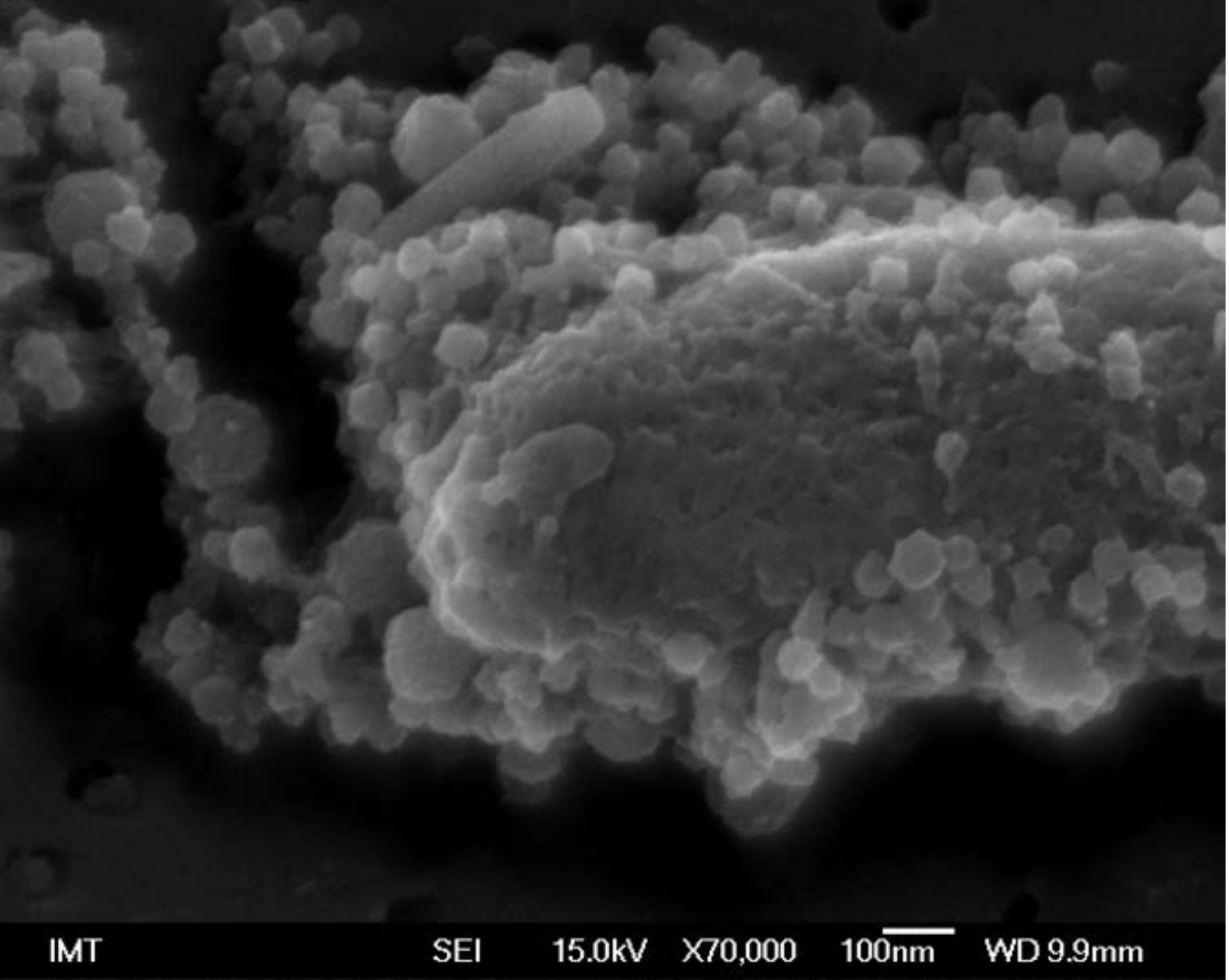

Figure *Phaeodactylum tricornutum* culture F2 SEM 29.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA). Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 µmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

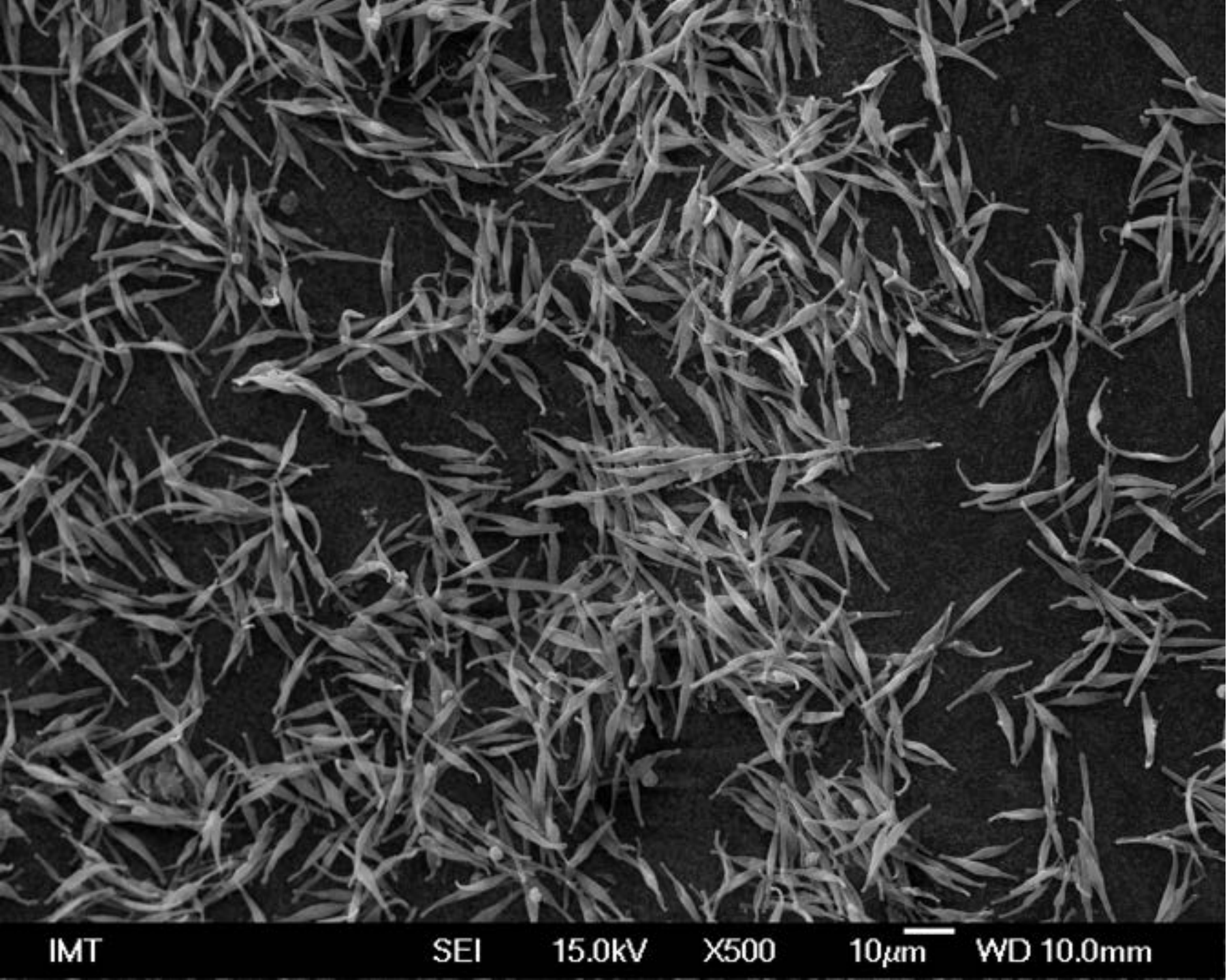

Figure *Phaeodactylum tricornutum* culture BG11 SEM 30.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with BG11 Broth (ref. 73816, Sigma Aldrich, Switzerland). Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 µmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

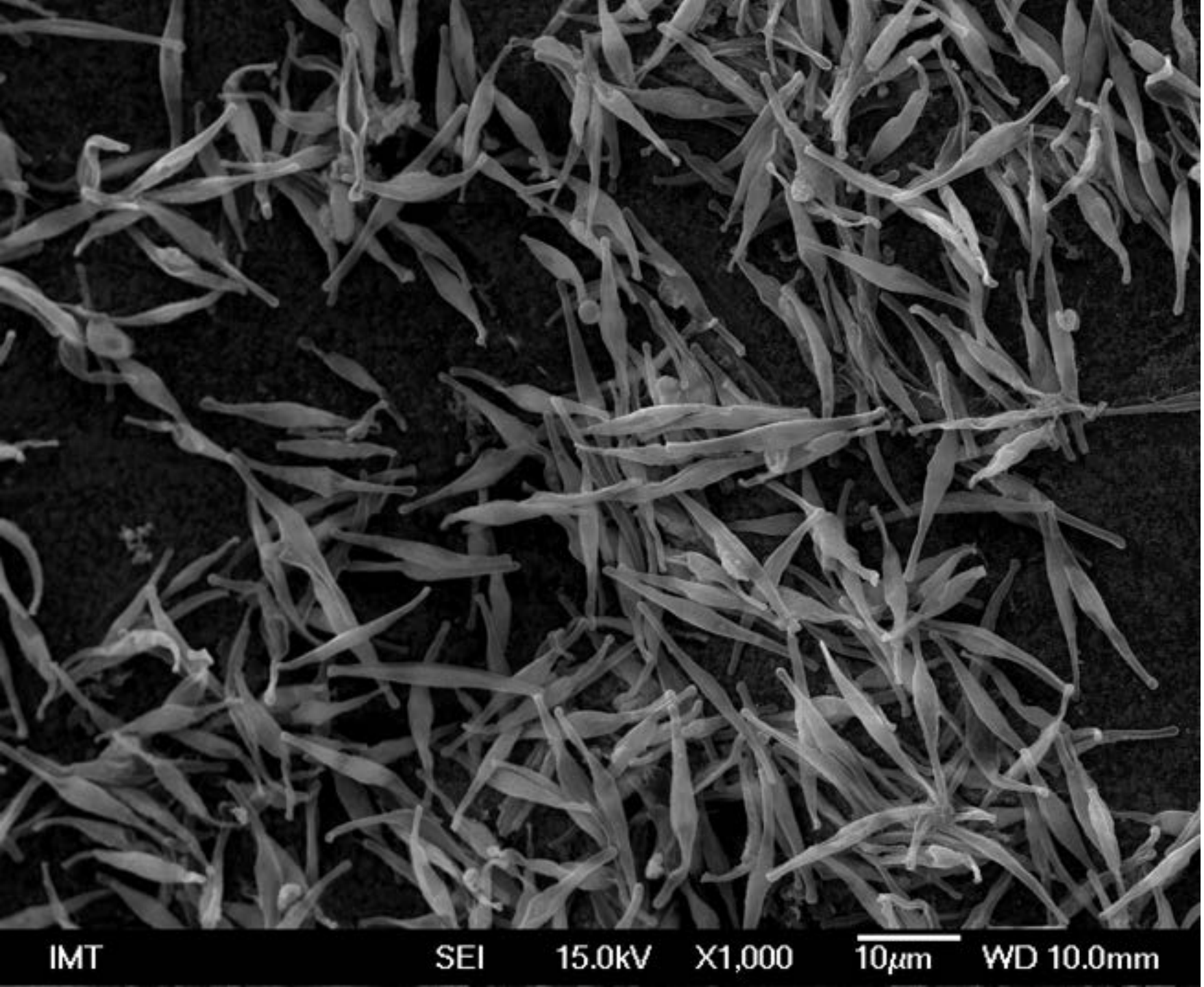

Figure *Phaeodactylum tricornutum* culture BG11 SEM 31.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with BG11 Broth (ref. 73816, Sigma Aldrich, Switzerland). Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

Figure *Phaeodactylum tricornutum* culture BG11 SEM 32.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with BG11 Broth (ref. 73816, Sigma Aldrich, Switzerland). Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

Figure *Phaeodactylum tricornutum* culture BG11 SEM 33.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with BG11 Broth (ref. 73816, Sigma Aldrich, Switzerland). Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

Figure *Phaeodactylum tricornutum* culture BG11 SEM 34.

Cultivation of the algae
Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with BG11 Broth (ref. 73816, Sigma Aldrich, Switzerland). Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.
Scanning Electron Microscopy (SEM)
Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

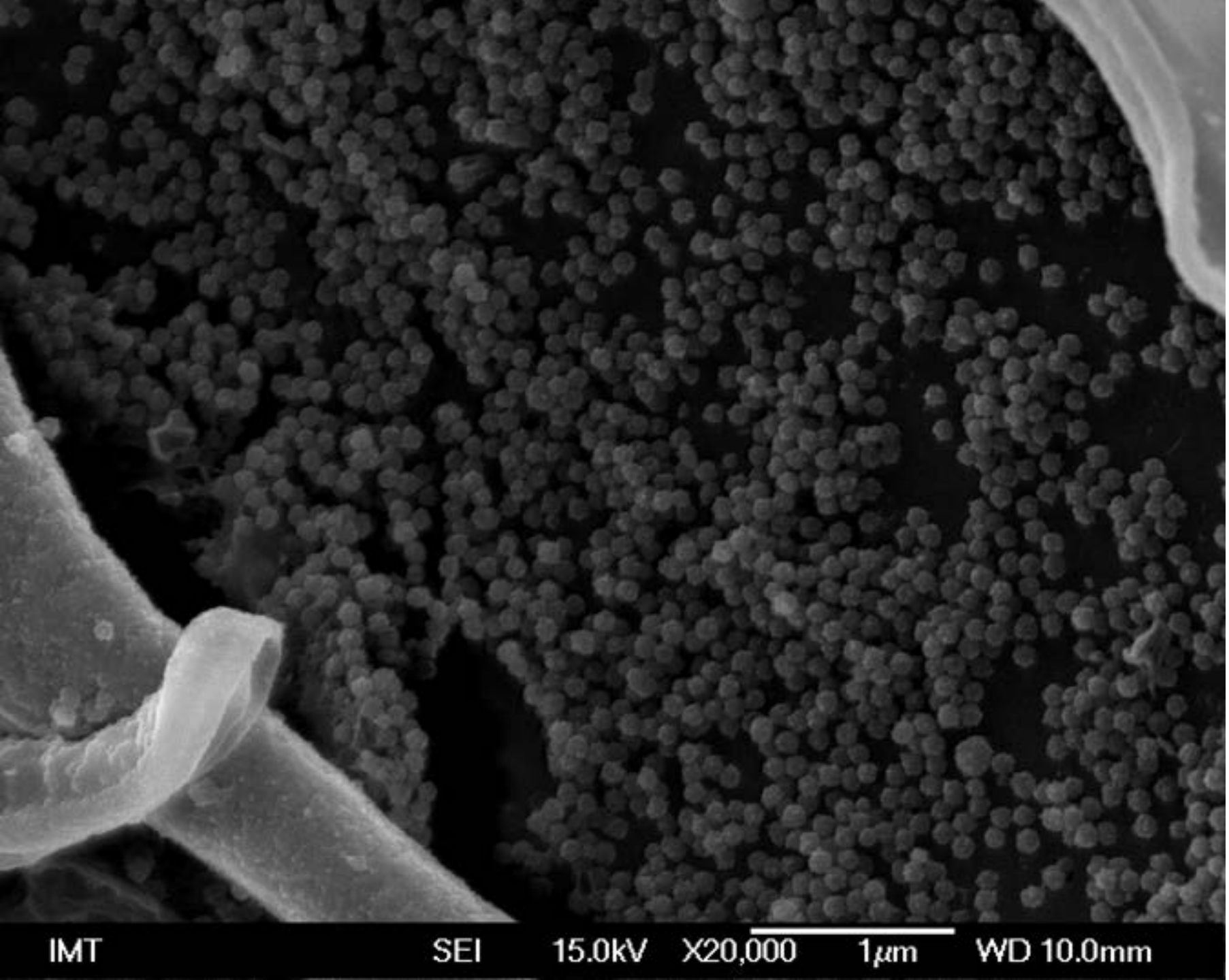

Figure *Phaeodactylum tricornutum* culture BG11 SEM 35.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with BG11 Broth (ref. 73816, Sigma Aldrich, Switzerland). Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

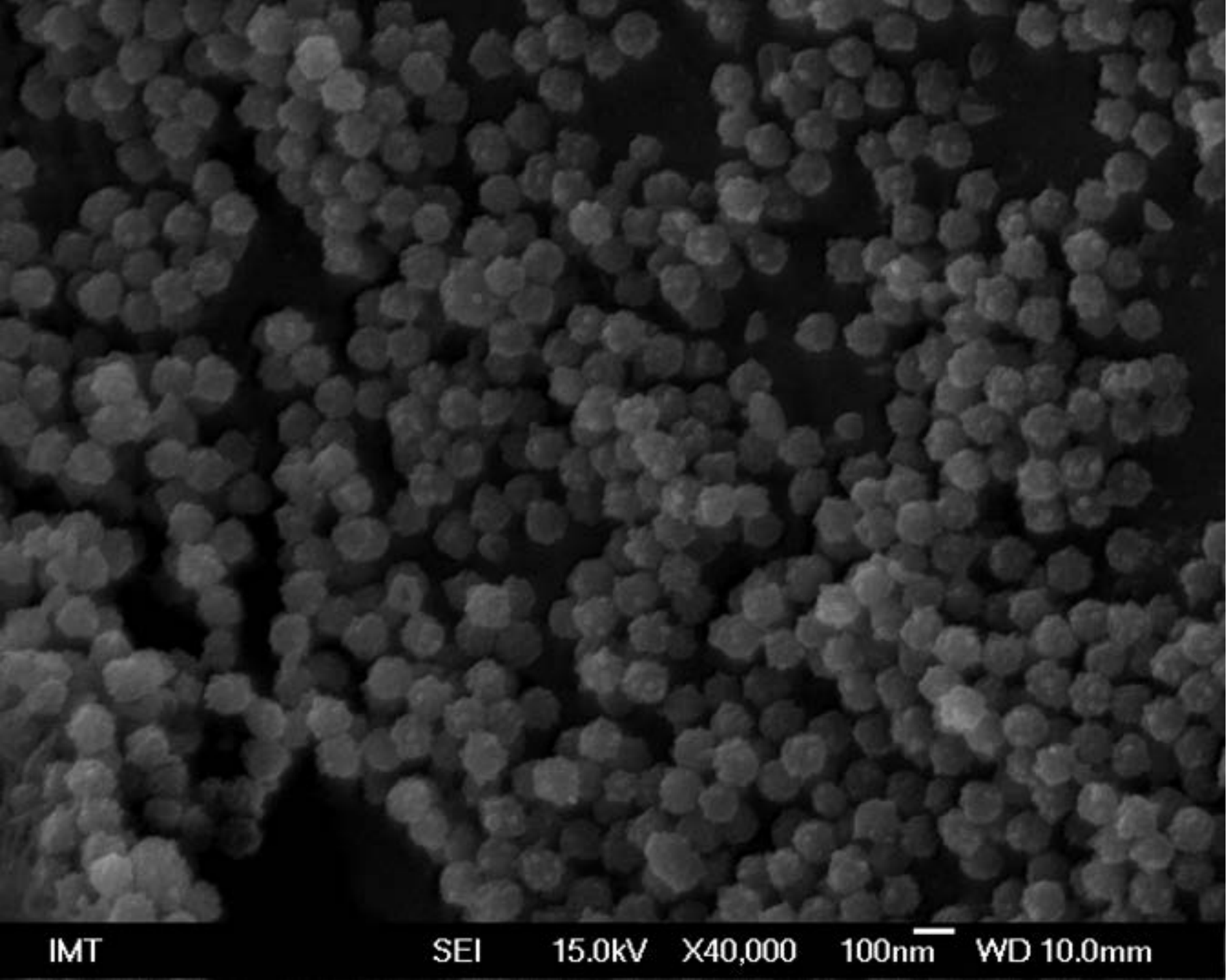

Figure *Phaeodactylum tricornutum* culture BG11 SEM 36.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with BG11 Broth (ref. 73816, Sigma Aldrich, Switzerland). Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 µmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

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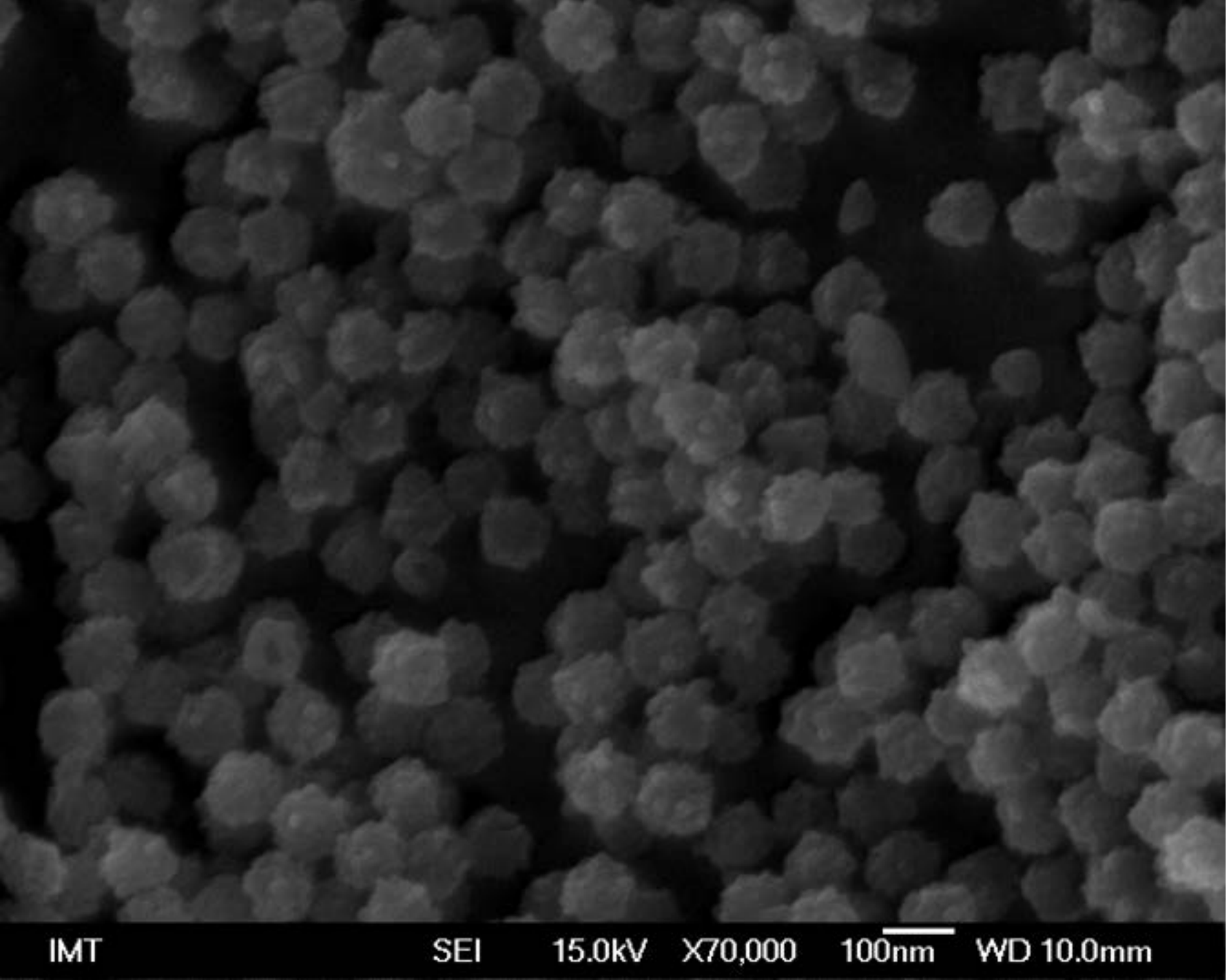

Figure *Phaeodactylum tricornutum* culture BG11 SEM 37.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with BG11 Broth (ref. 73816, Sigma Aldrich, Switzerland). Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 µmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

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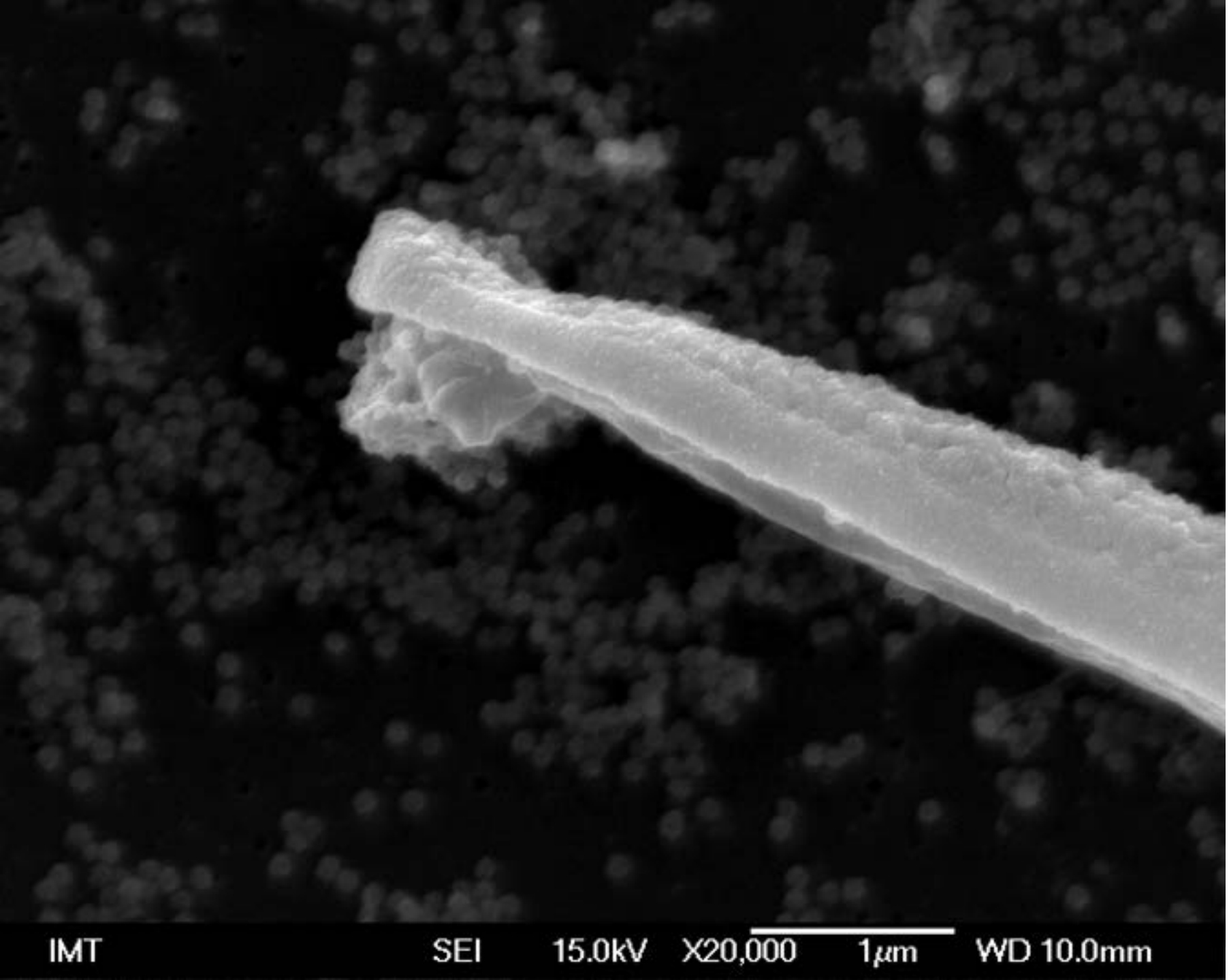

Figure *Phaeodactylum tricornutum* culture BG11 SEM 38.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with BG11 Broth (ref. 73816, Sigma Aldrich, Switzerland). Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 µmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

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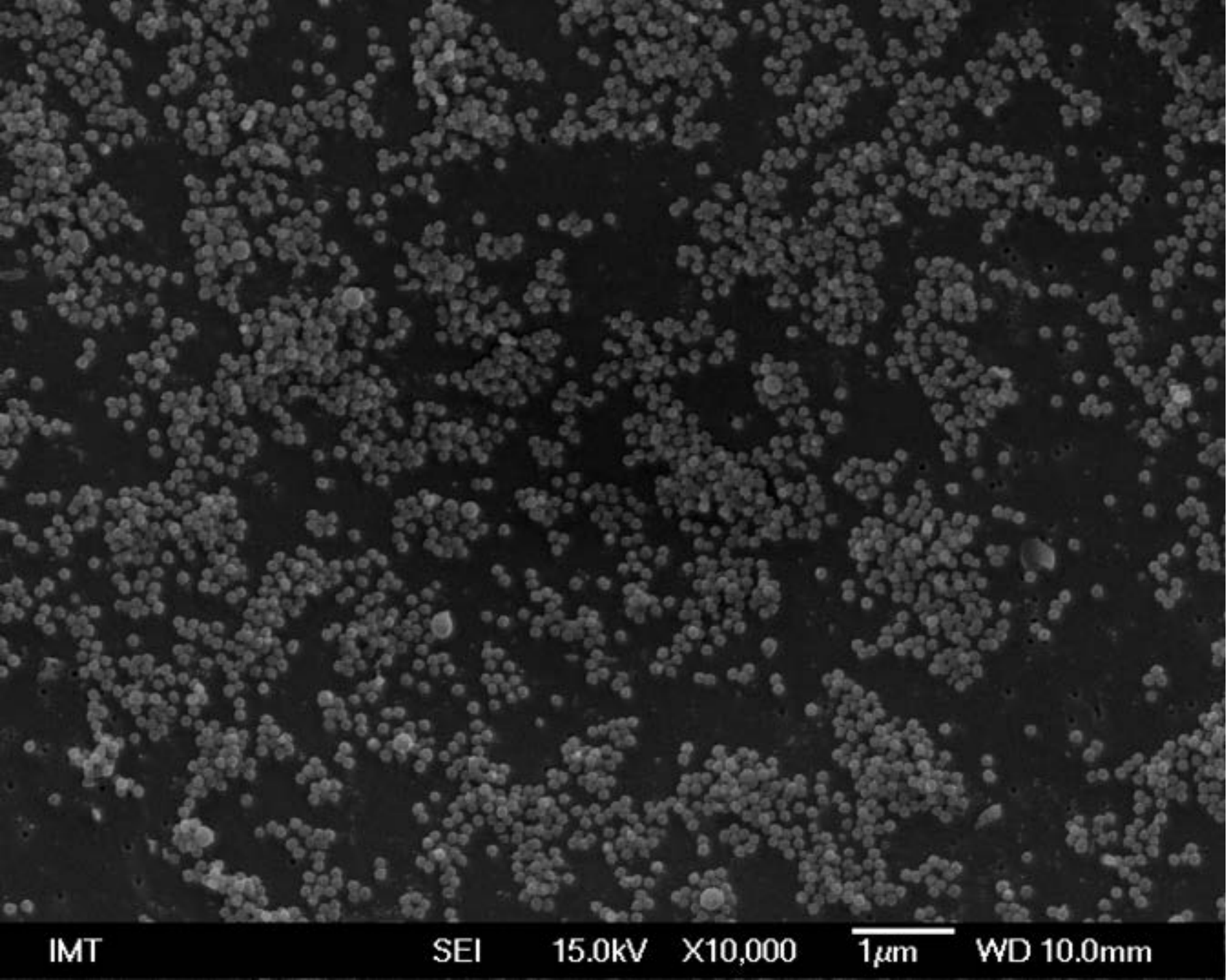

Figure *Phaeodactylum tricornutum* culture BG11 SEM 39.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with BG11 Broth (ref. 73816, Sigma Aldrich, Switzerland). Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

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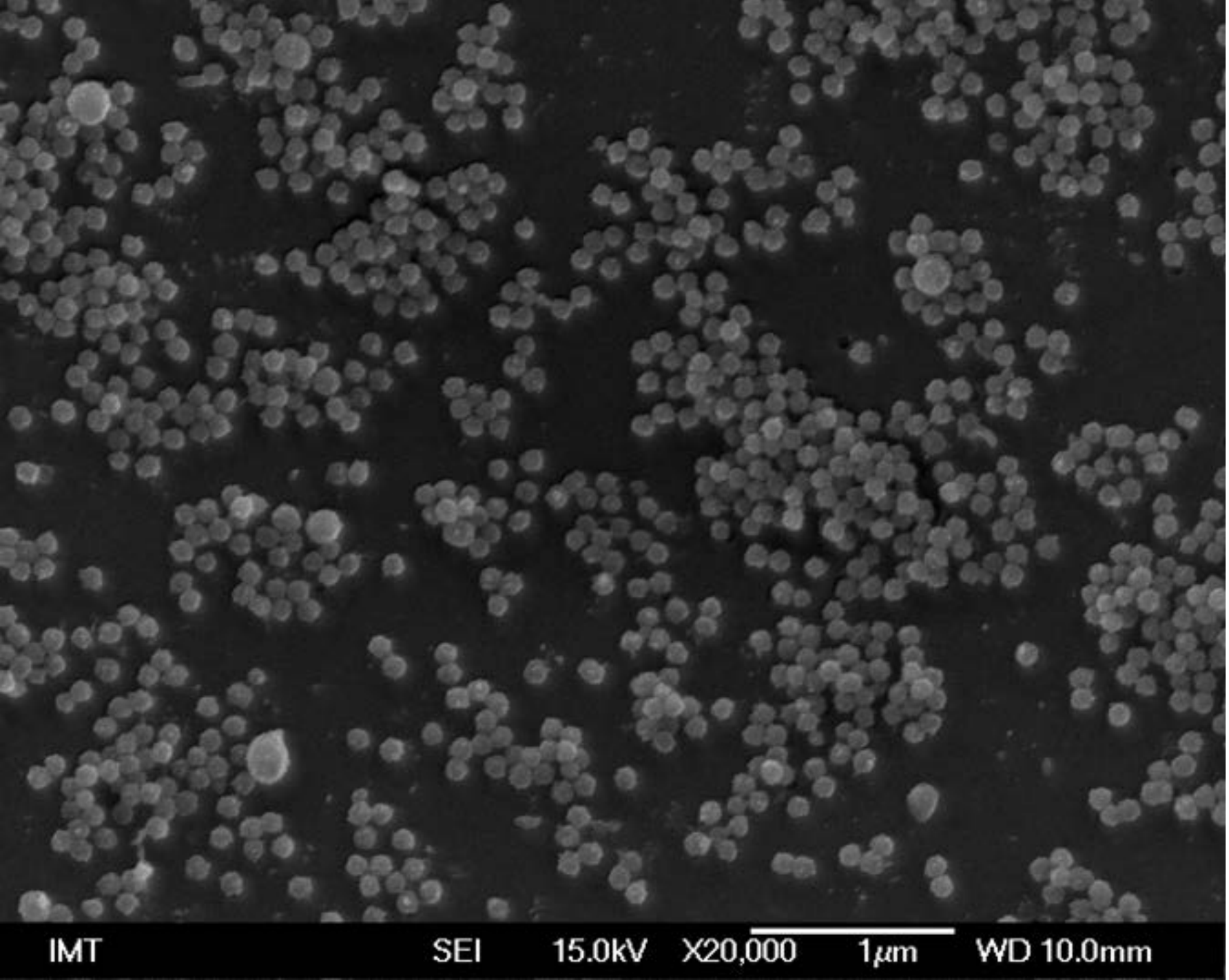

Figure *Phaeodactylum tricornutum* culture BG11 SEM 40.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with BG11 Broth (ref. 73816, Sigma Aldrich, Switzerland). Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 µmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

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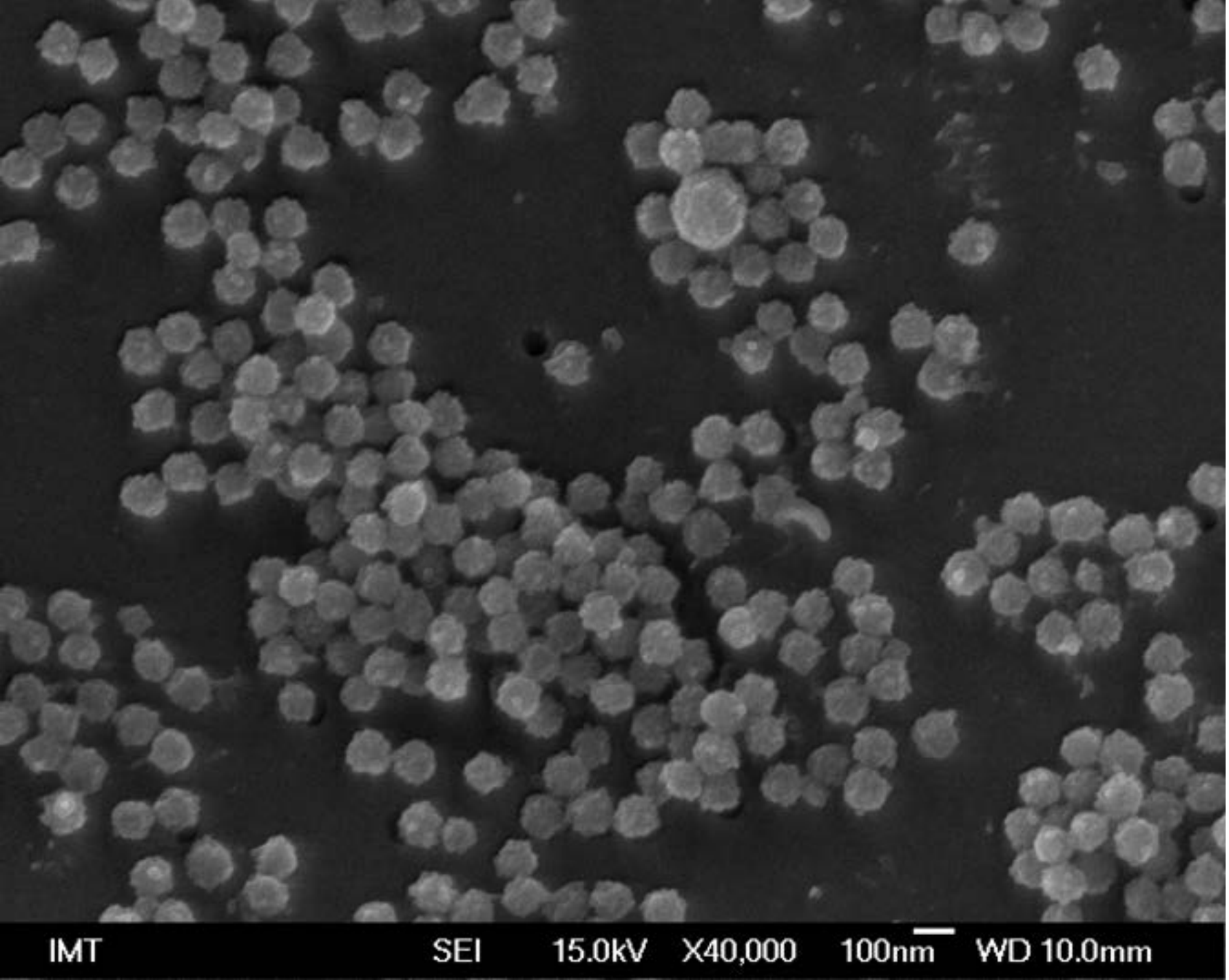

Figure *Phaeodactylum tricornutum* culture BG11 SEM 41.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with BG11 Broth (ref. 73816, Sigma Aldrich, Switzerland). Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 µmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

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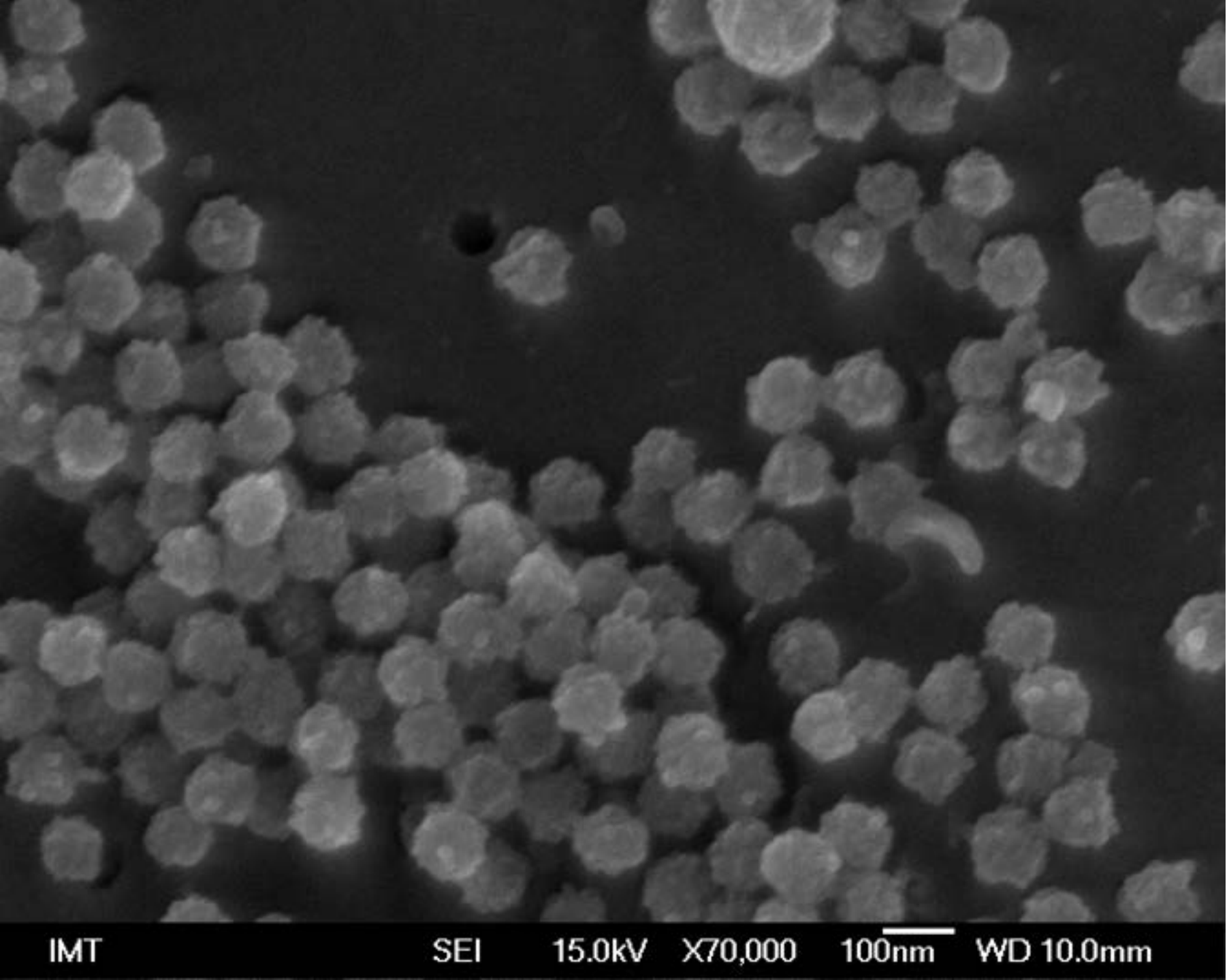

Figure *Phaeodactylum tricornutum* culture BG11 SEM 42.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with BG11 Broth (ref. 73816, Sigma Aldrich, Switzerland). Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

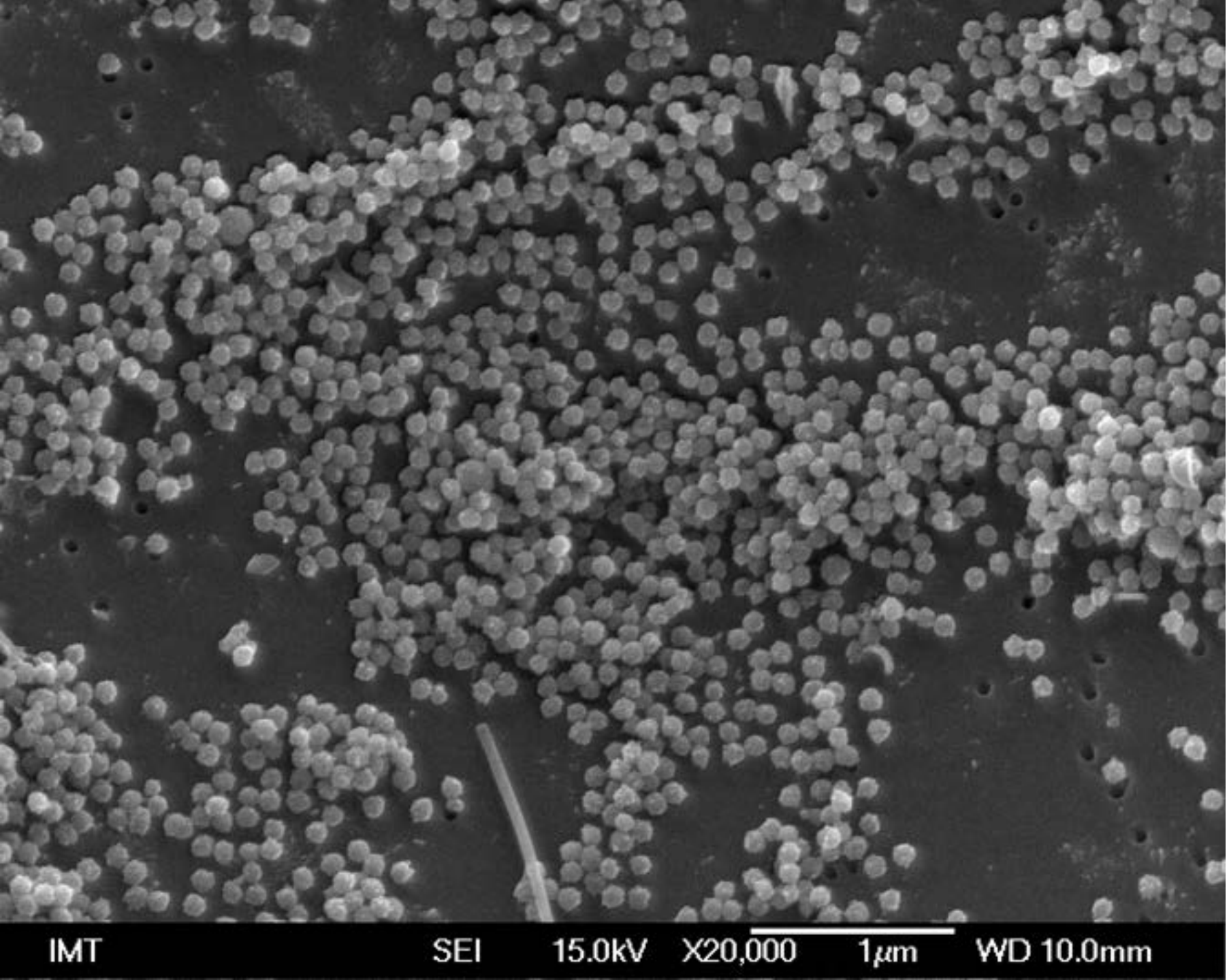

Figure *Phaeodactylum tricornutum* culture BG11 SEM 43.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with BG11 Broth (ref. 73816, Sigma Aldrich, Switzerland). Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 µmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

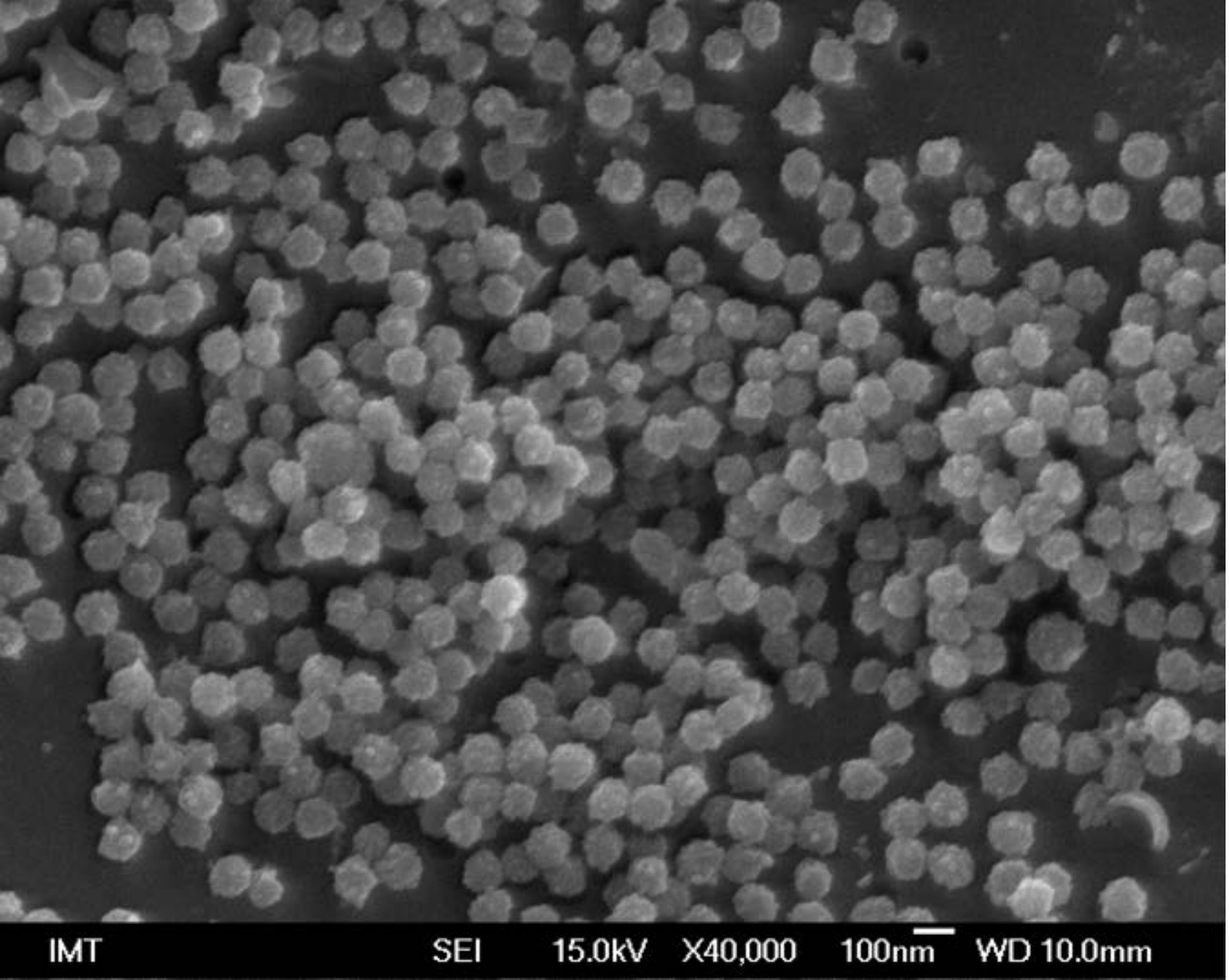

Figure *Phaeodactylum tricornutum* culture BG11 SEM 44.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with BG11 Broth (ref. 73816, Sigma Aldrich, Switzerland). Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 µmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

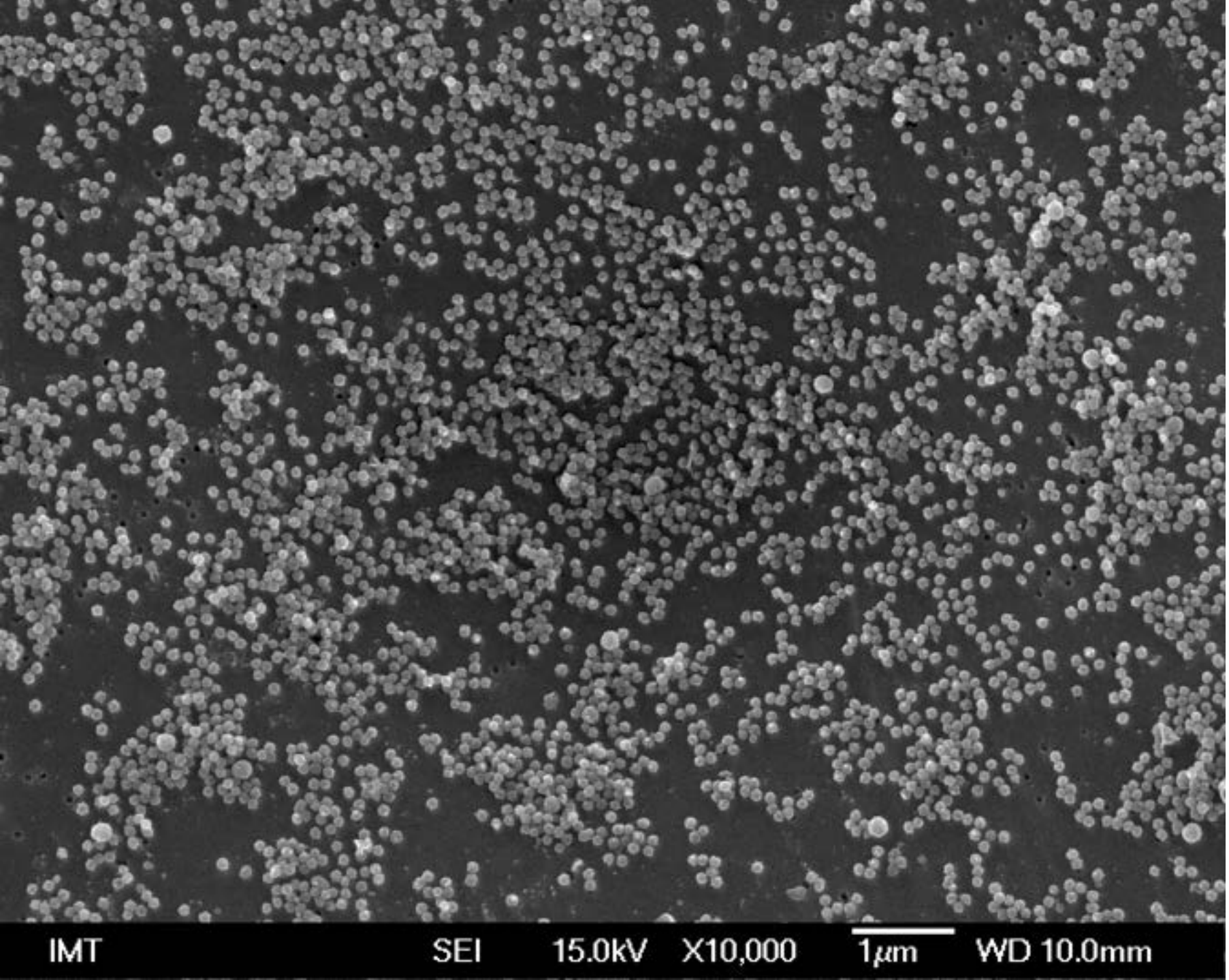

Figure *Phaeodactylum tricornutum* culture BG1 SEM 45.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with BG11 Broth (ref. 73816, Sigma Aldrich, Switzerland). Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

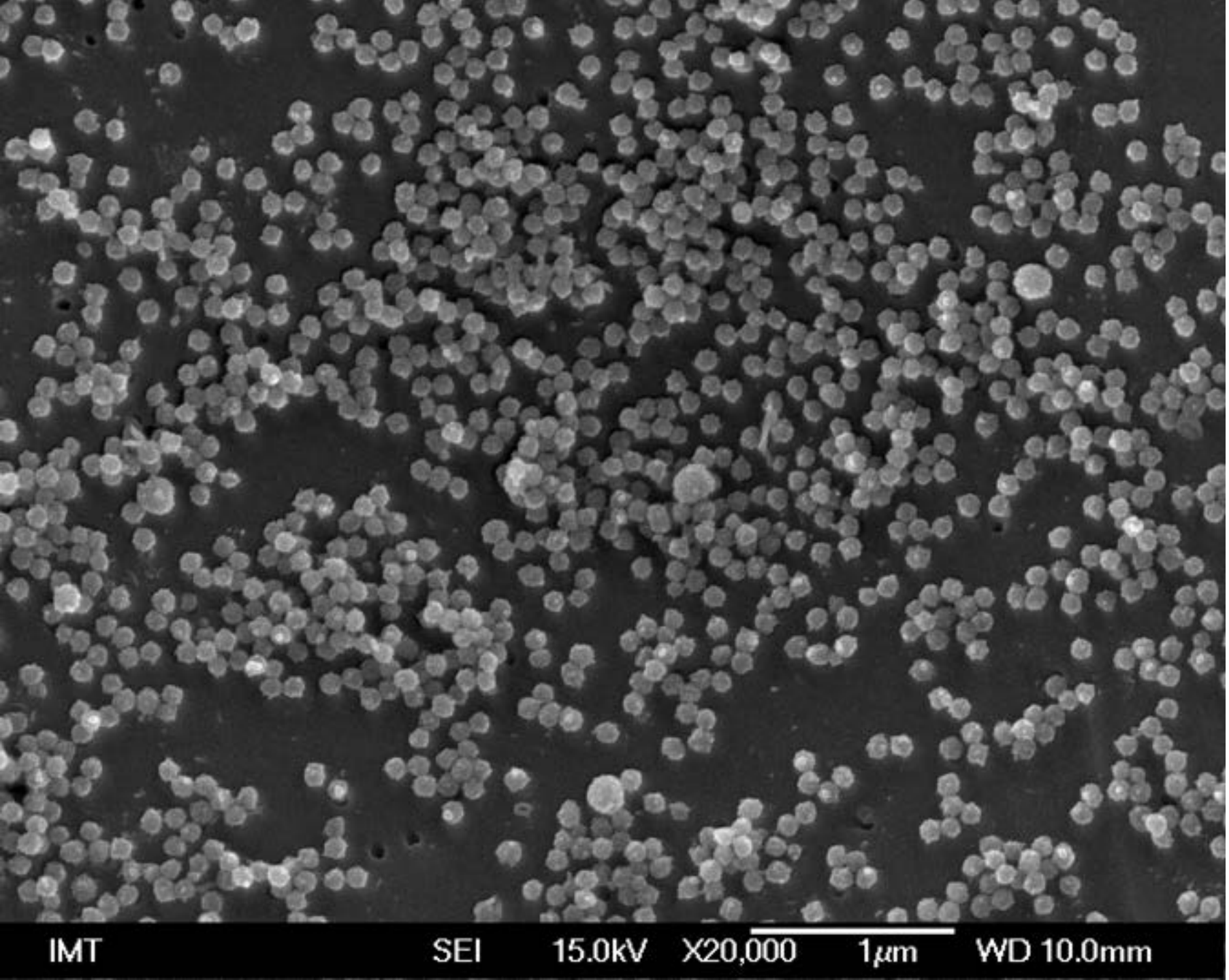

Figure *Phaeodactylum tricornutum* culture BG1 SEM 46.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with BG11 Broth (ref. 73816, Sigma Aldrich, Switzerland). Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

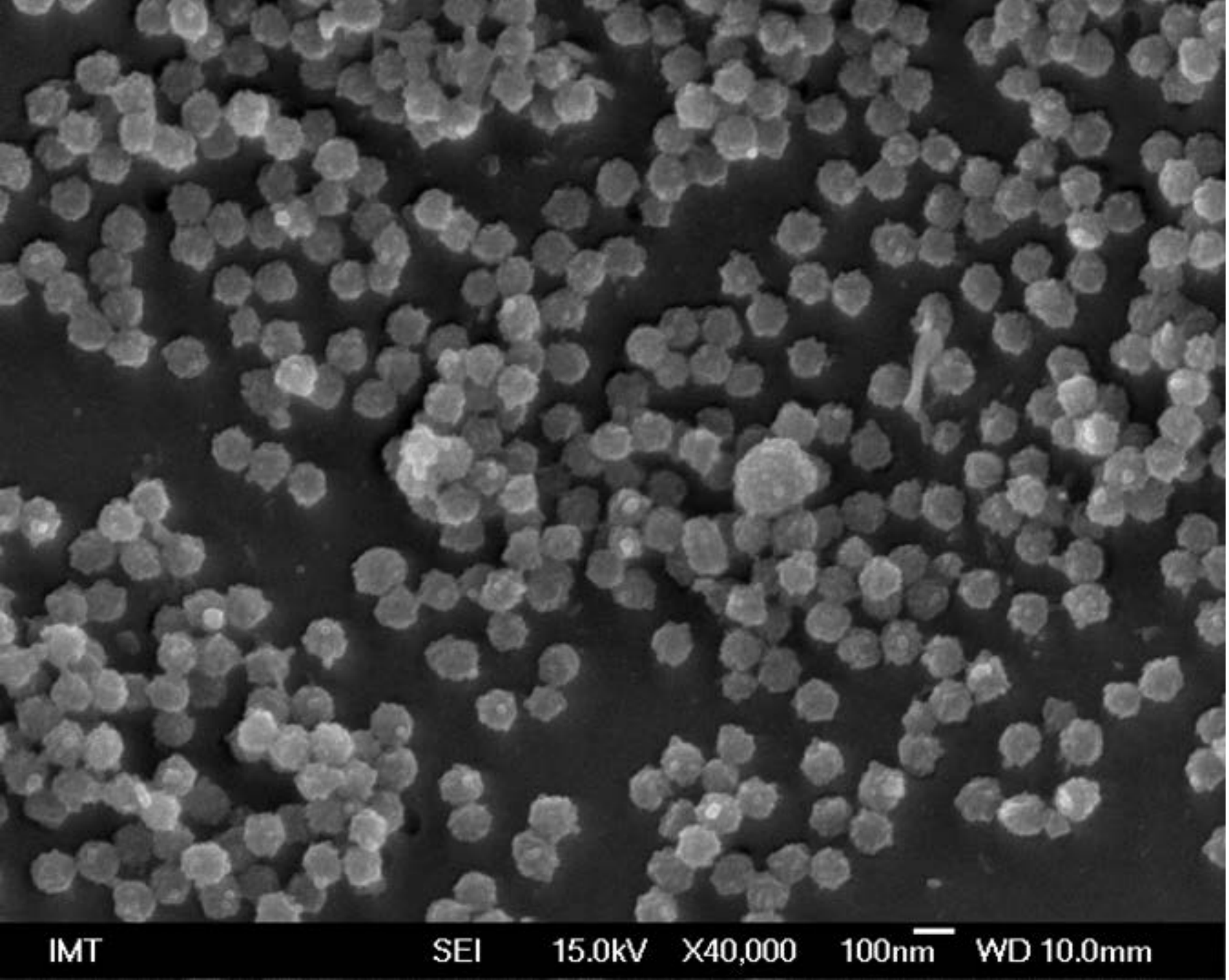

Figure *Phaeodactylum tricornutum* culture BG11 SEM 47.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with BG11 Broth (ref. 73816, Sigma Aldrich, Switzerland). Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

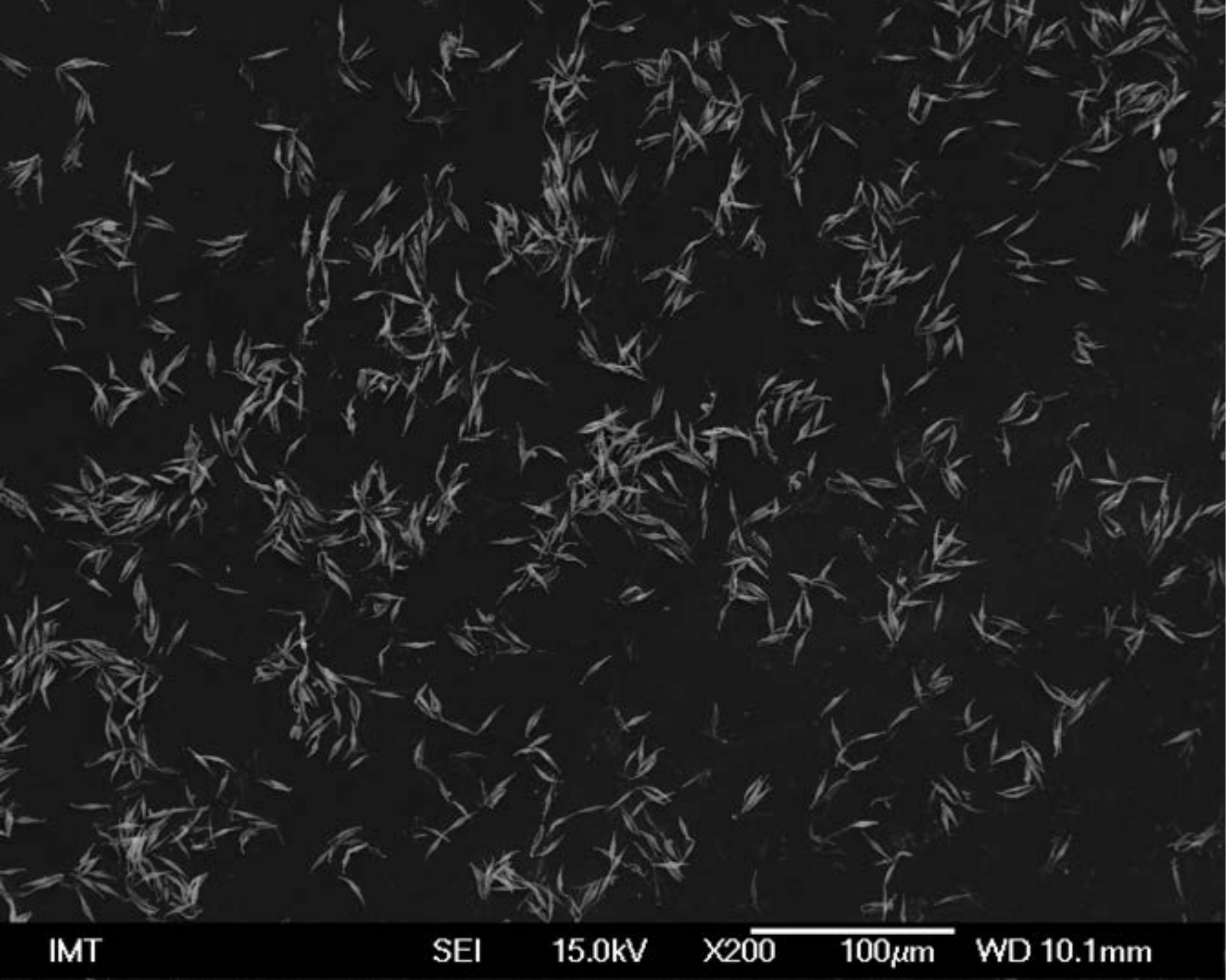

Figure *Phaeodactylum tricornutum* culture LB SEM 48.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with LB Lennox LB broth (ref. L3022, Sigma Aldrich, USA). Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

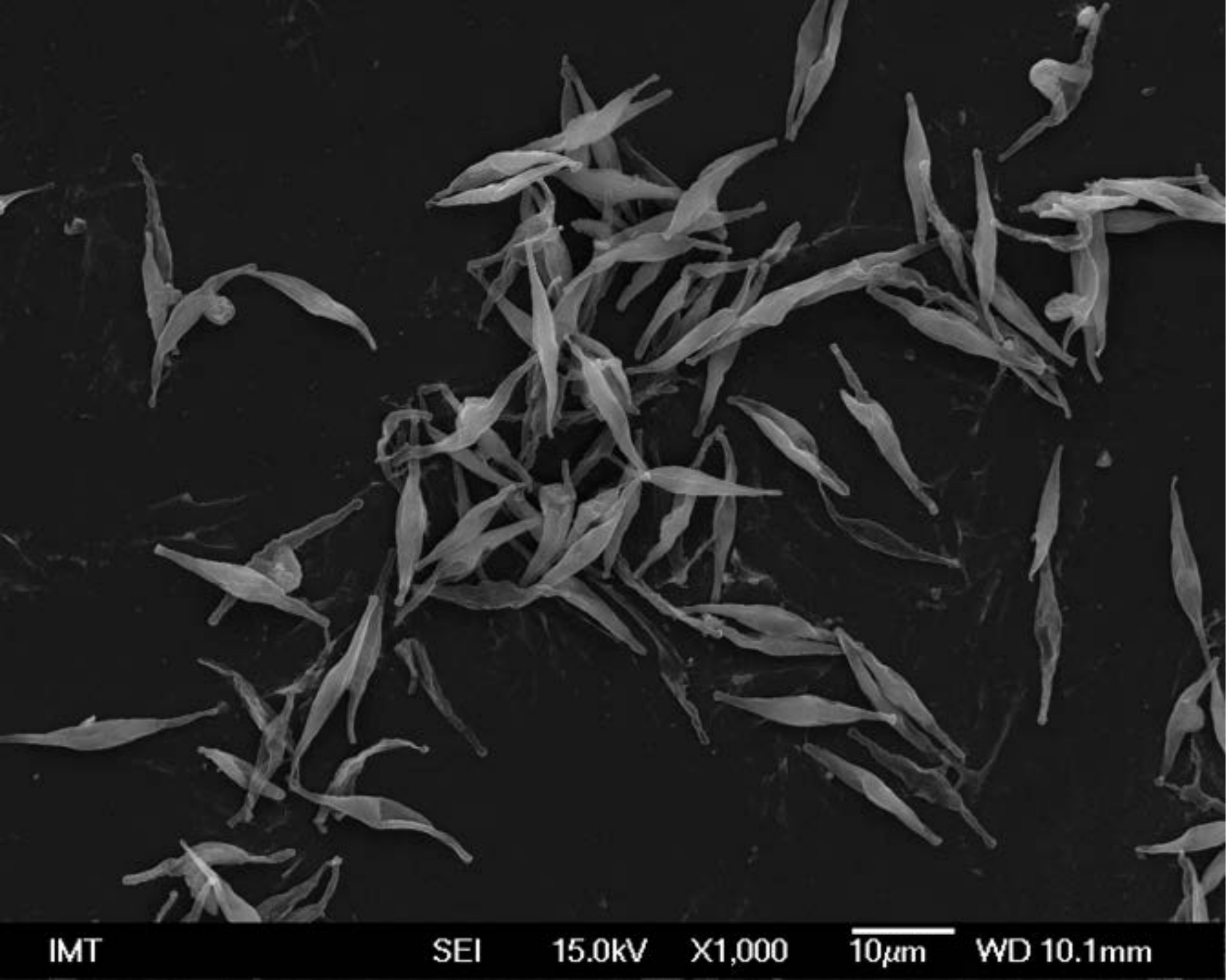

Figure *Phaeodactylum tricornutum* culture LB SEM 49.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Lennox LB broth (ref. L3022, Sigma Aldrich, USA) broth (ref. L3022, Sigma Aldrich, USA). Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

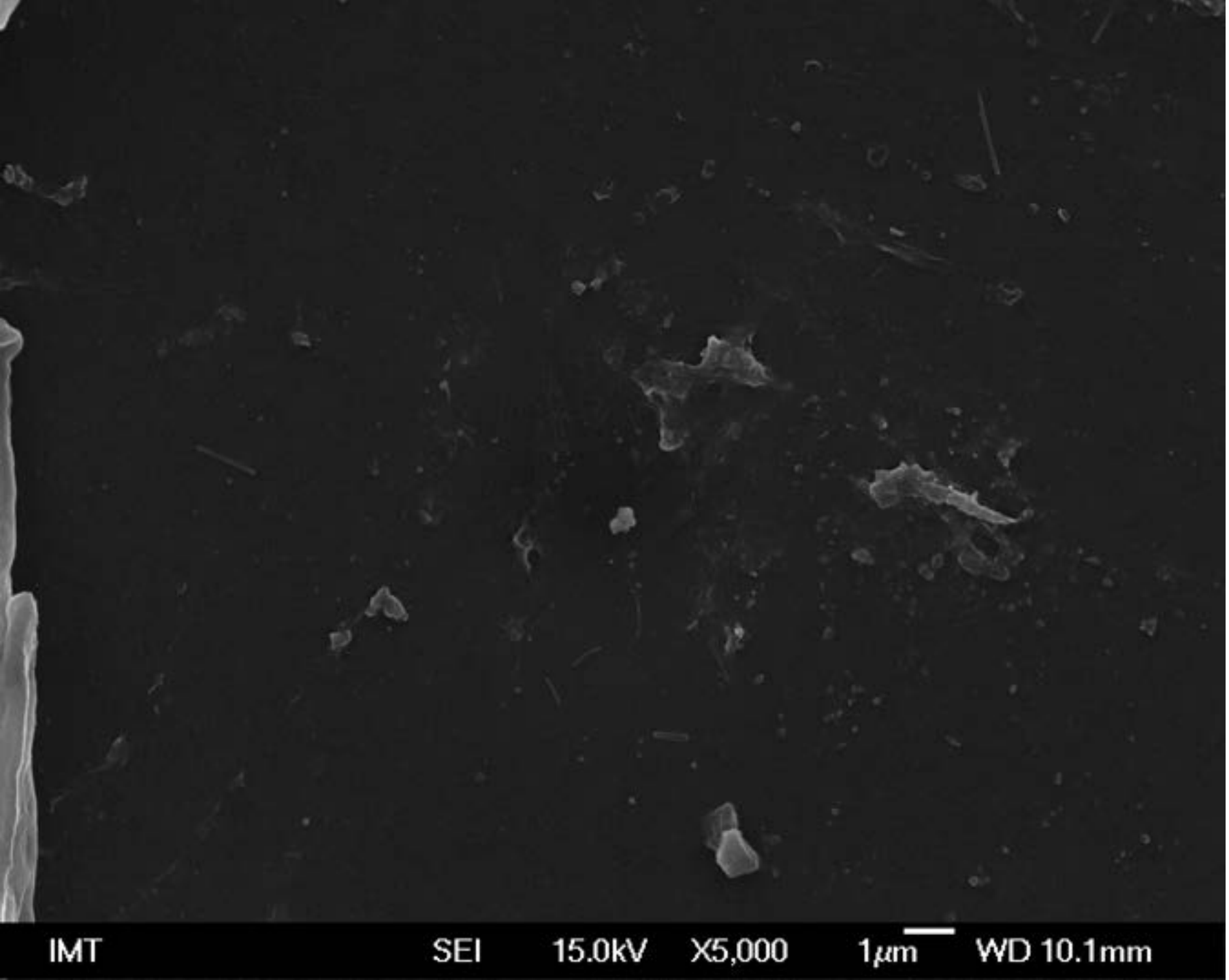

Figure *Phaeodactylum tricornutum* culture LB SEM 50.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with LB Lennox LB broth (ref. L3022, Sigma Aldrich, USA. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

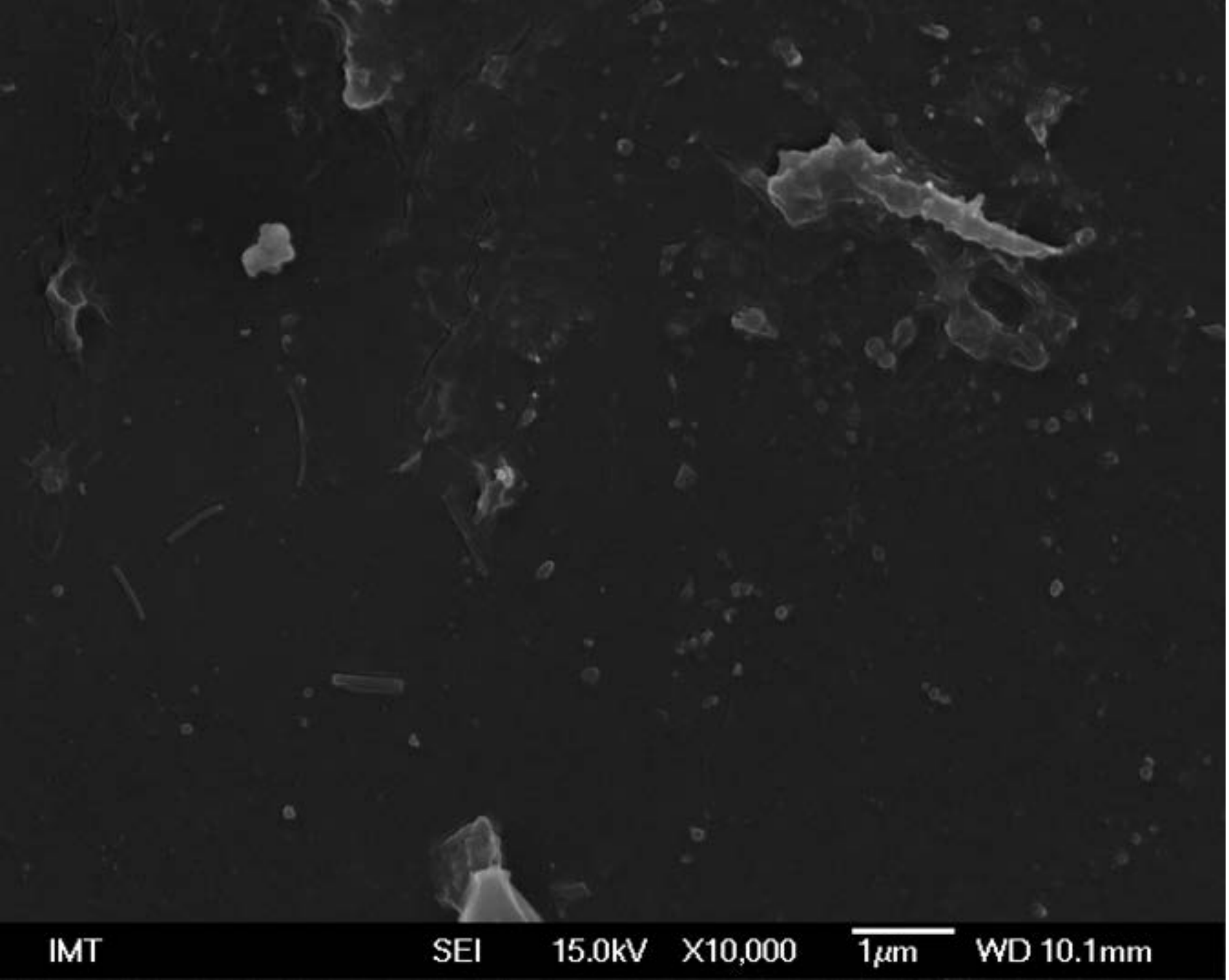

Figure *Phaeodactylum tricornutum* culture SEM LB 51.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with LB Lennox LB broth (ref. L3022, Sigma Aldrich, USA) medium. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 µmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

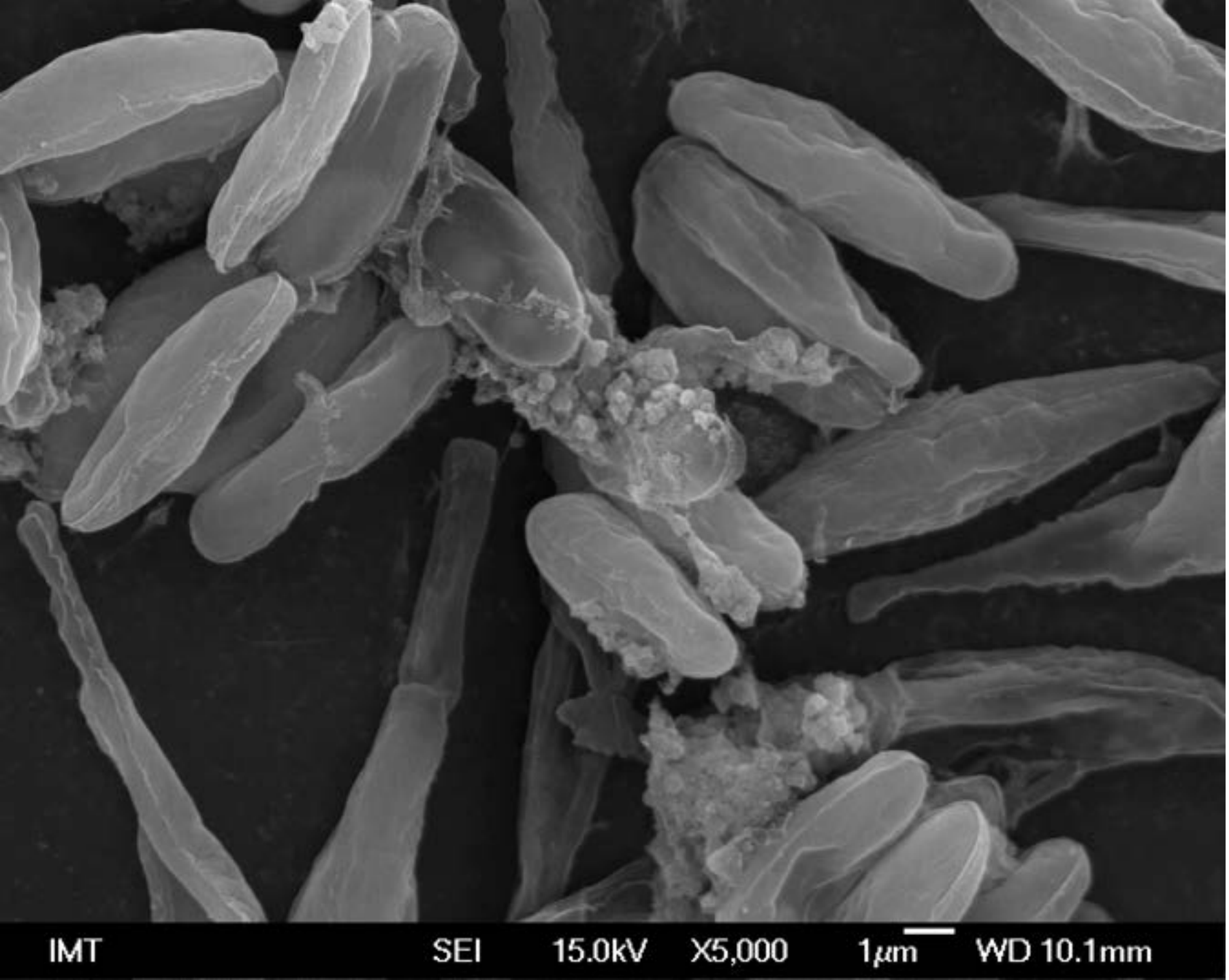

Figure *Phaeodactylum tricornutum* culture SEM LB 52.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with LB Lennox LB broth (ref. L3022, Sigma Aldrich, USA. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

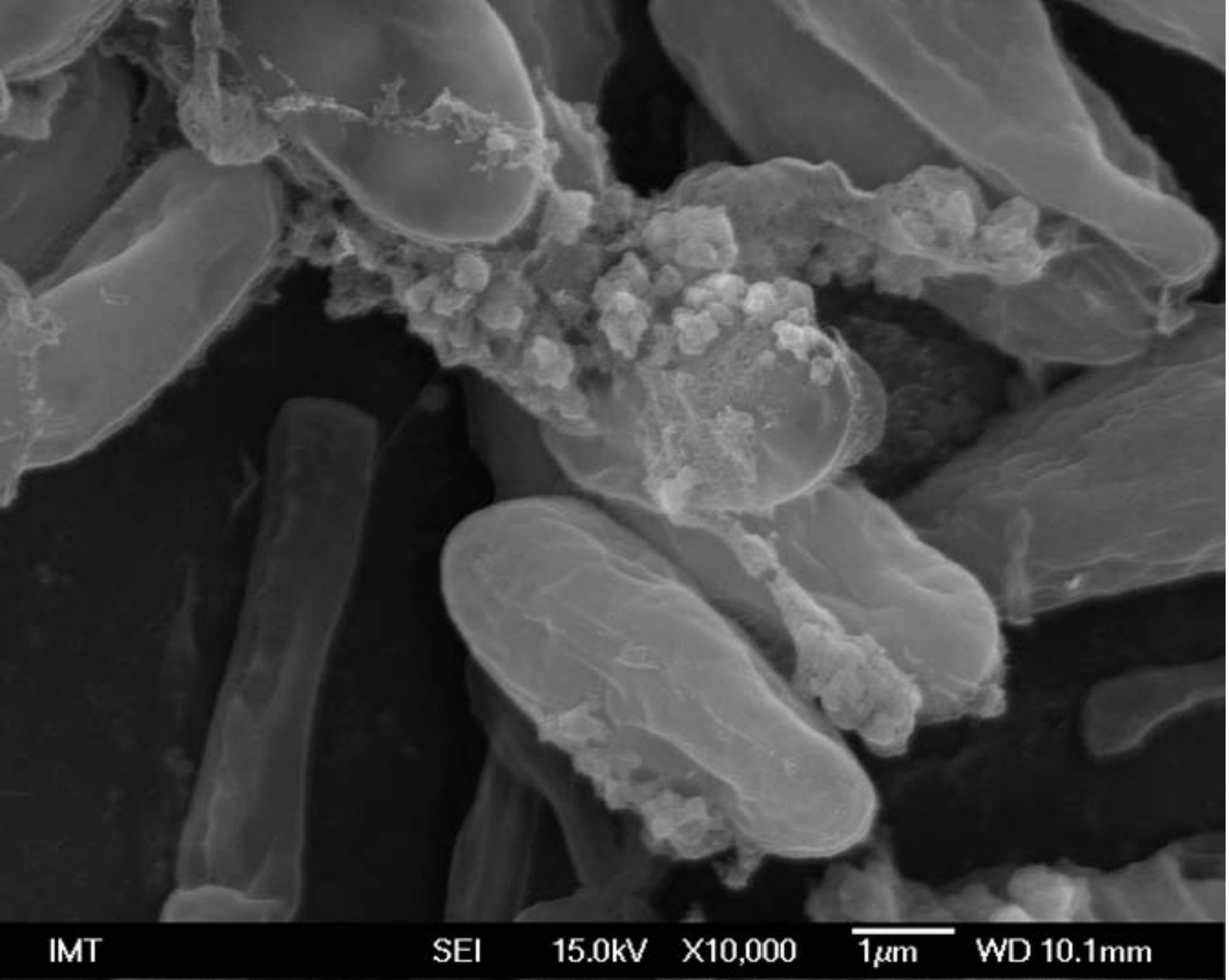

Figure *Phaeodactylum tricornutum* culture SEM LB 53.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with LB Lennox LB broth (ref. L3022, Sigma Aldrich, USA) medium. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 µmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

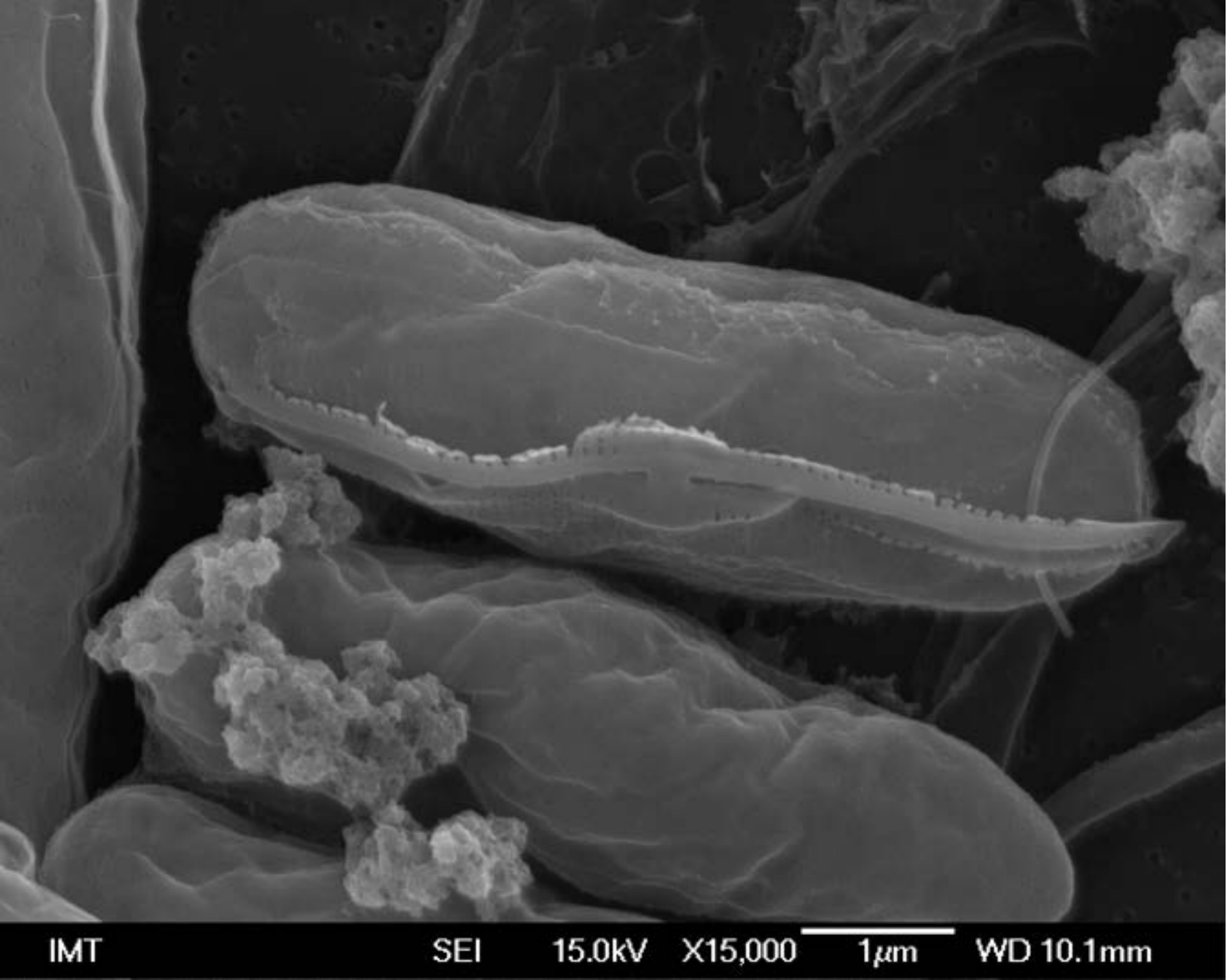

Figure *Phaeodactylum tricornutum* culture SEM LB 54.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with LB Lennox LB broth (ref. L3022, Sigma Aldrich, USA. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 µmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

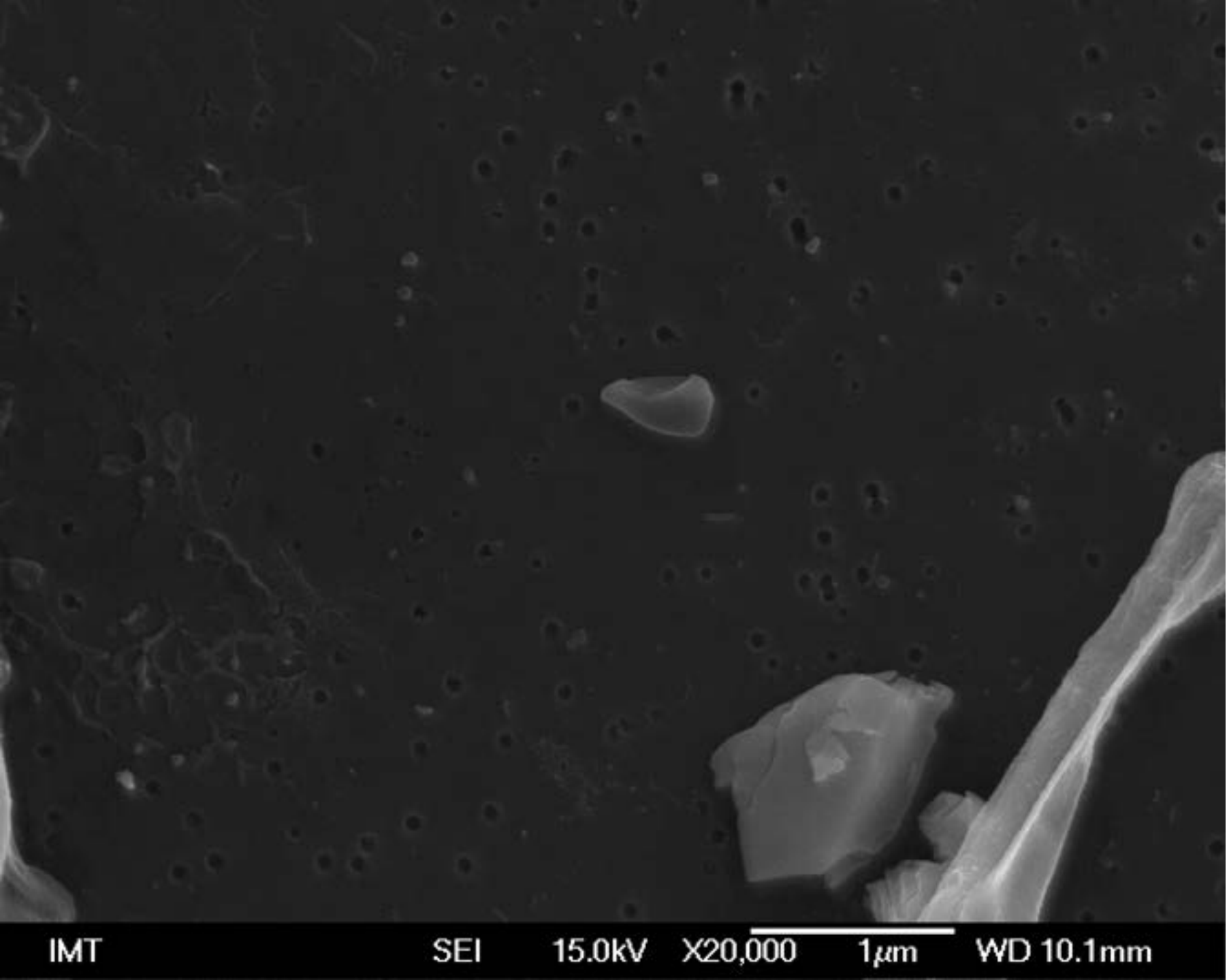

Figure *Phaeodactylum tricornutum* culture SEM LB 55.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with LB Lennox LB broth (ref. L3022, Sigma Aldrich, USA) medium. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 µmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

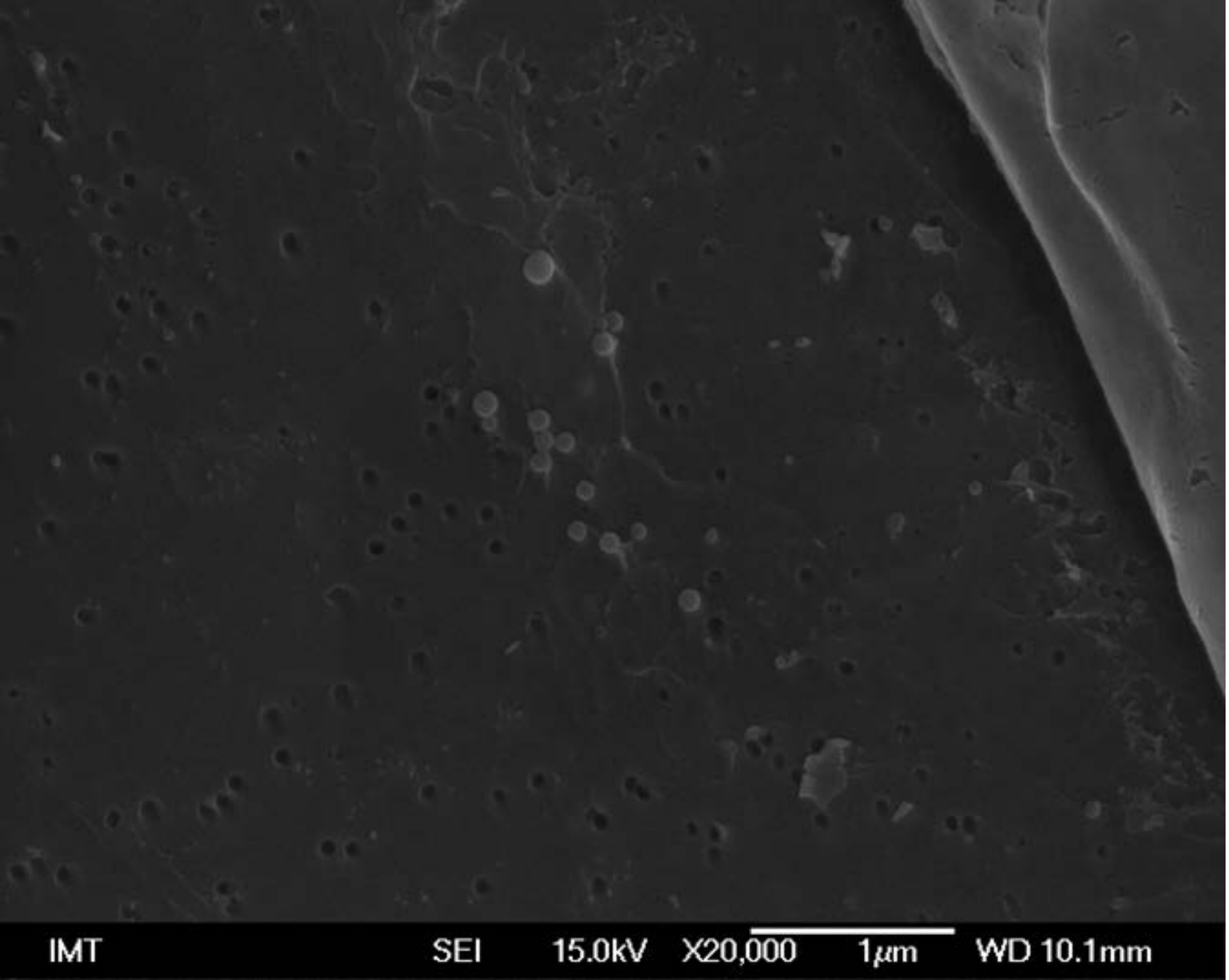

Figure *Phaeodactylum tricornutum* culture SEM LB 56.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with LB Lennox LB broth (ref. L3022, Sigma Aldrich, USA. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

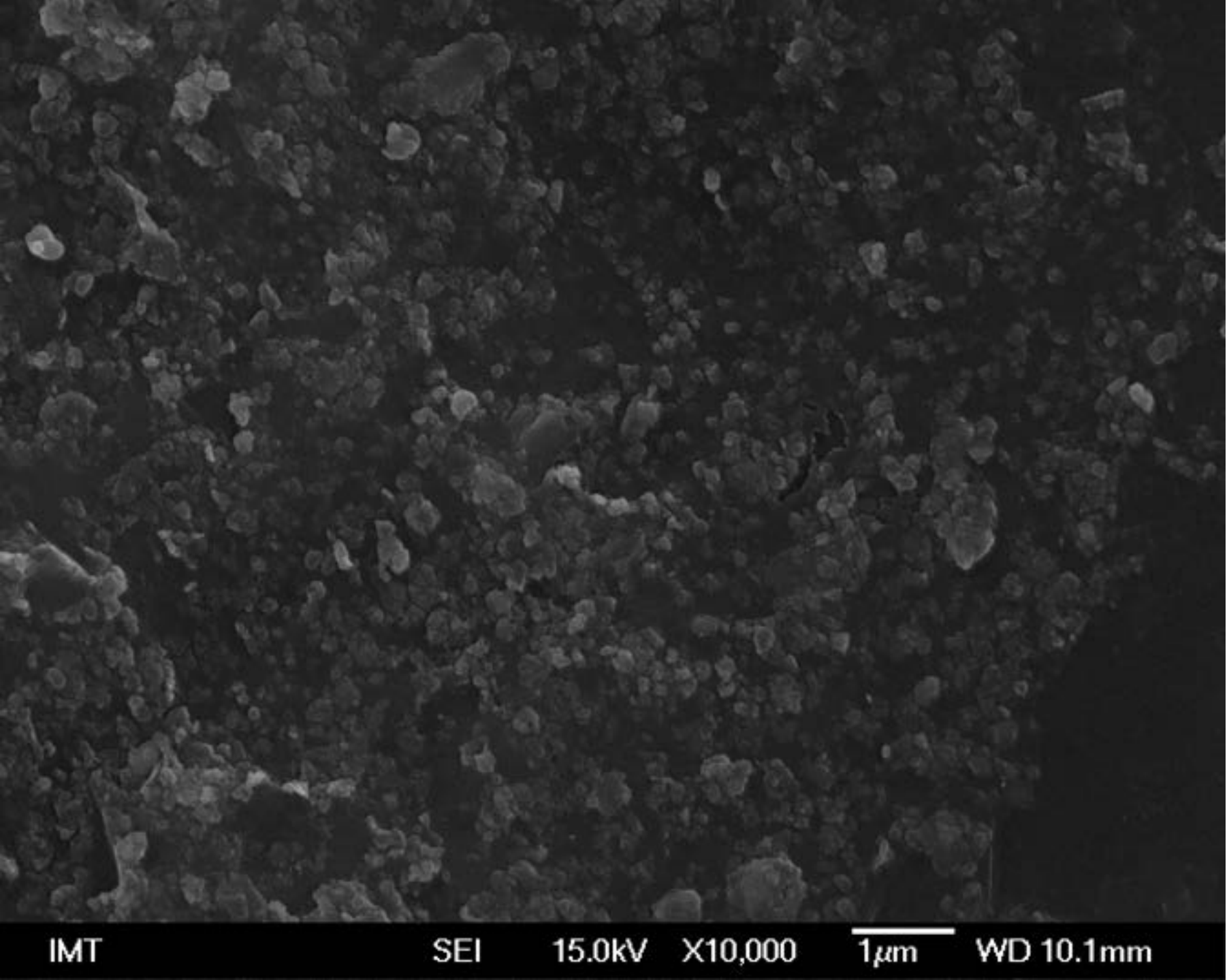

Figure *Phaeodactylum tricornutum* culture SEM LB 57.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with LB Lennox LB broth (ref. L3022, Sigma Aldrich, USA) medium. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 µmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

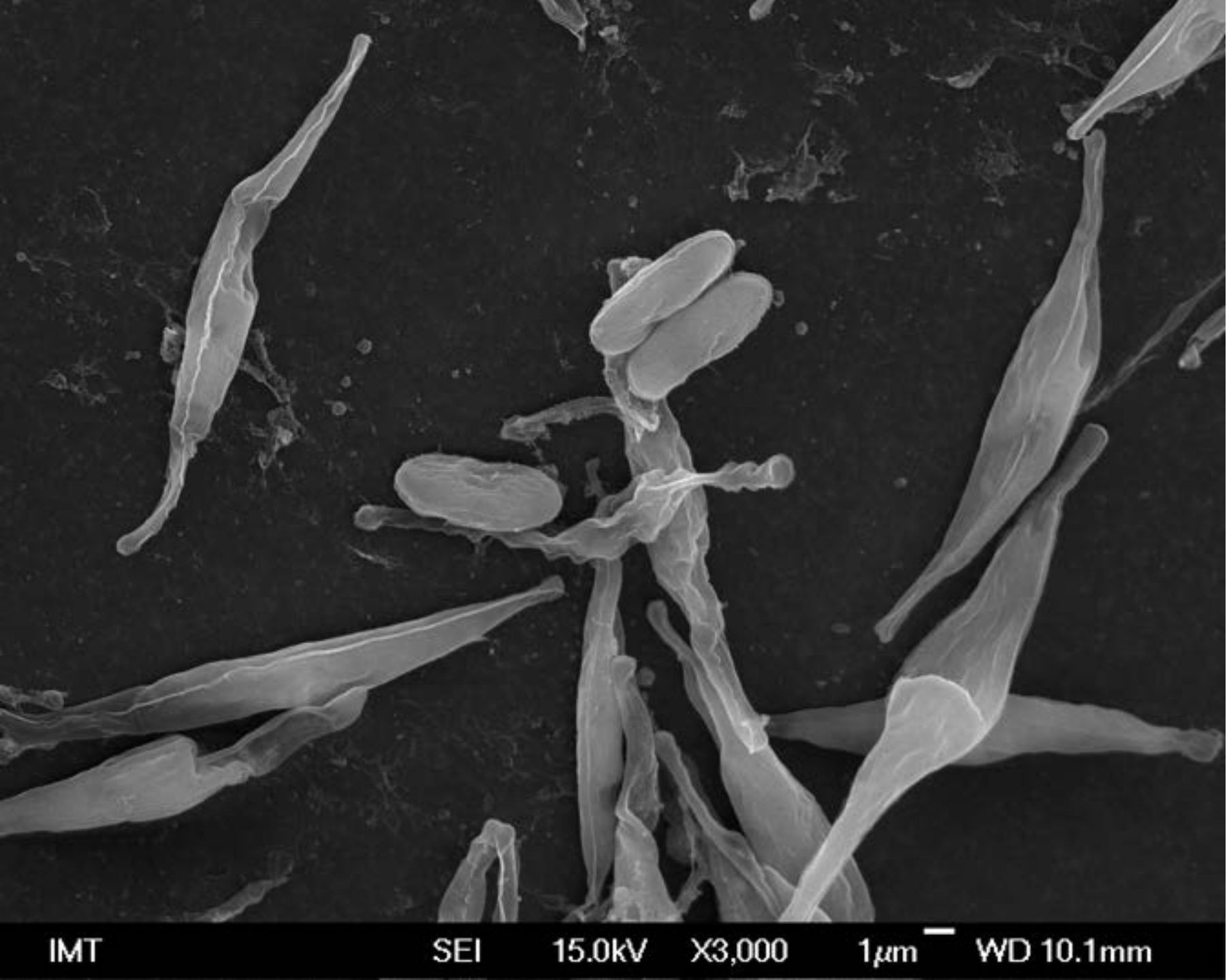

Figure *Phaeodactylum tricornutum* culture SEM LB 58.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with LB Lennox LB broth (ref. L3022, Sigma Aldrich, USA. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

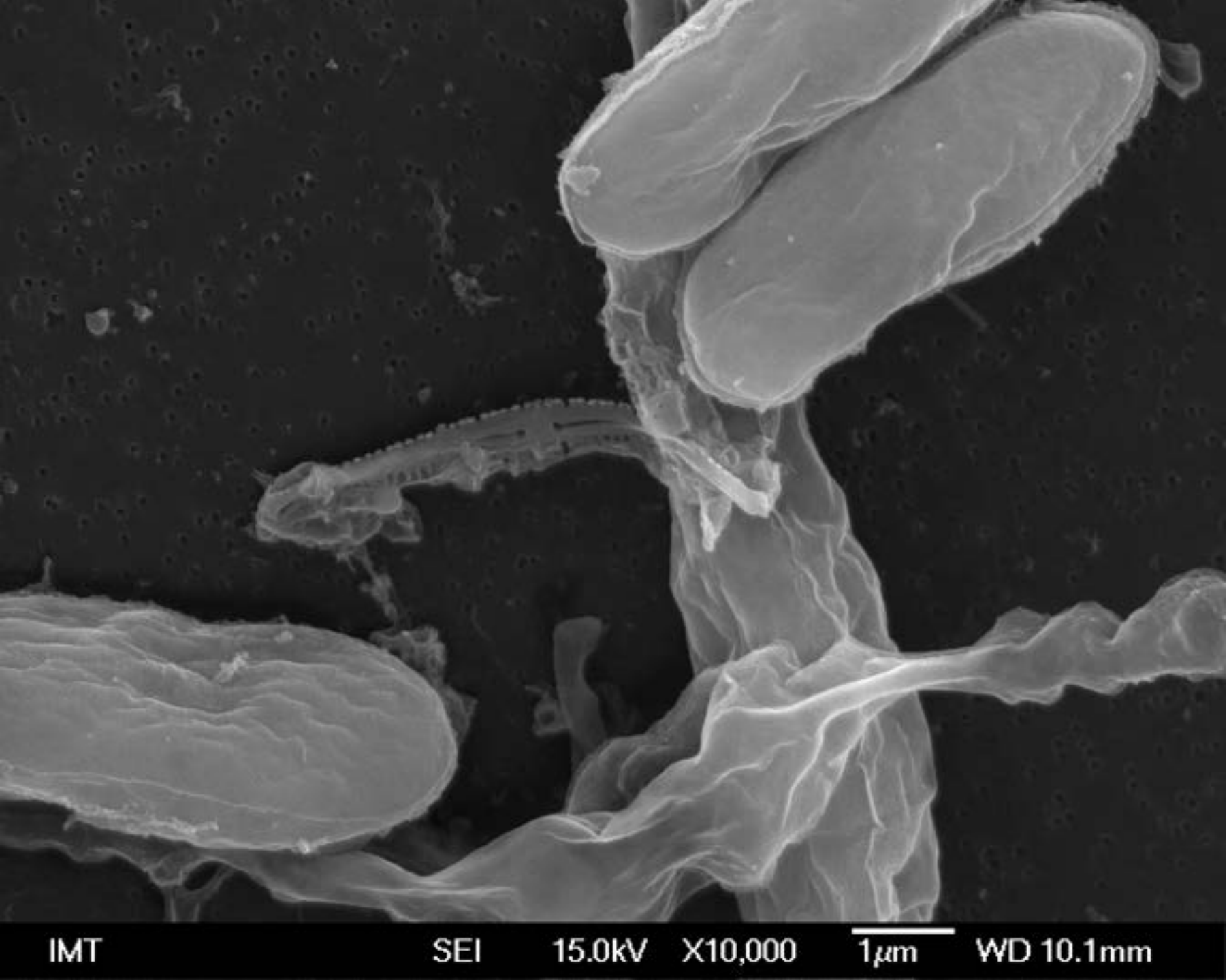

Figure *Phaeodactylum tricornutum* culture SEM LB 59.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with LB Lennox LB broth (ref. L3022, Sigma Aldrich, USA) medium. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 µmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

Figure *Phaeodactylum tricornutum* culture SEM LB 60.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with LB Lennox LB broth (ref. L3022, Sigma Aldrich, USA. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

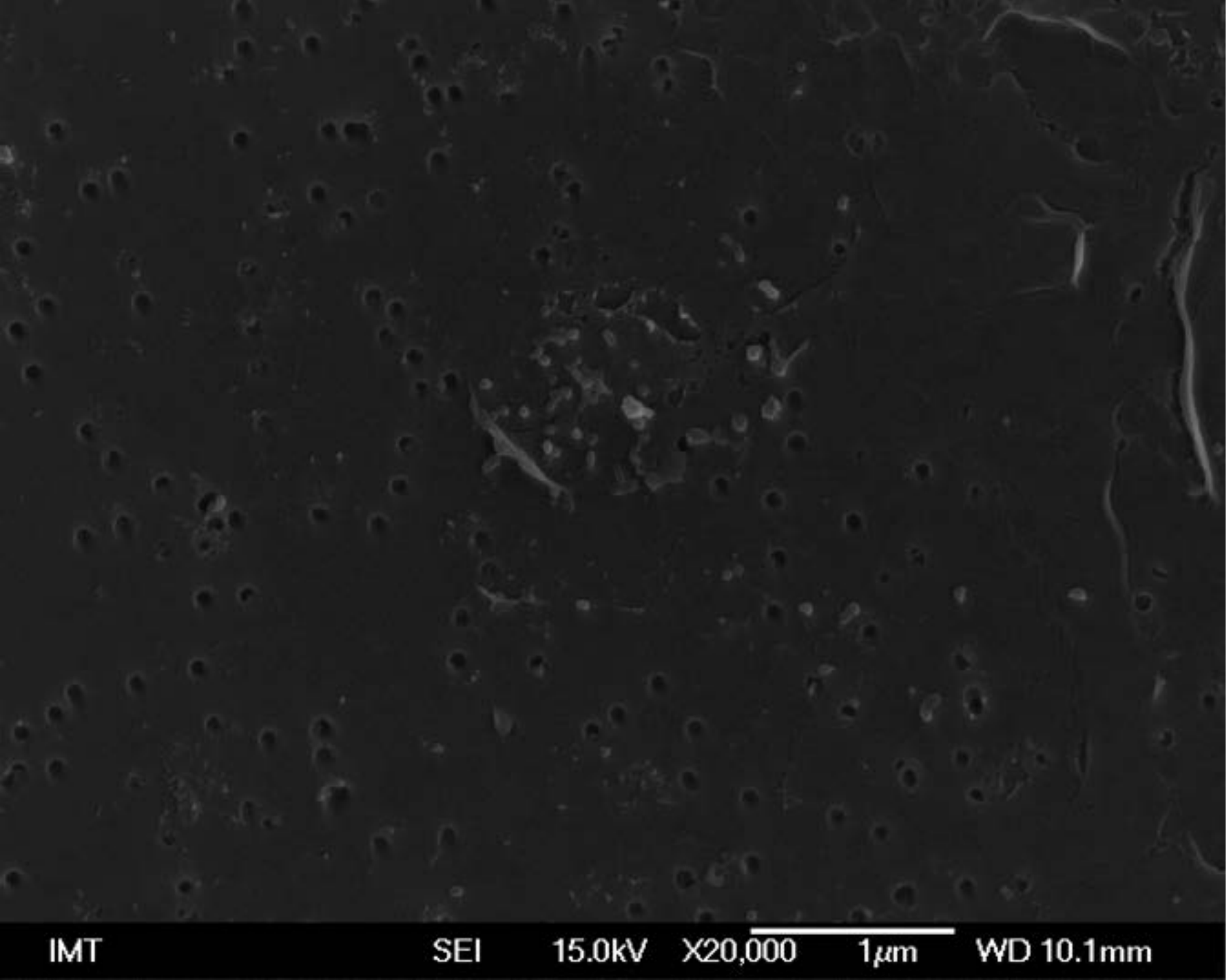

Figure *Phaeodactylum tricornutum* culture SEM LB 61.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with LB Lennox LB broth (ref. L3022, Sigma Aldrich, USA) medium. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 µmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

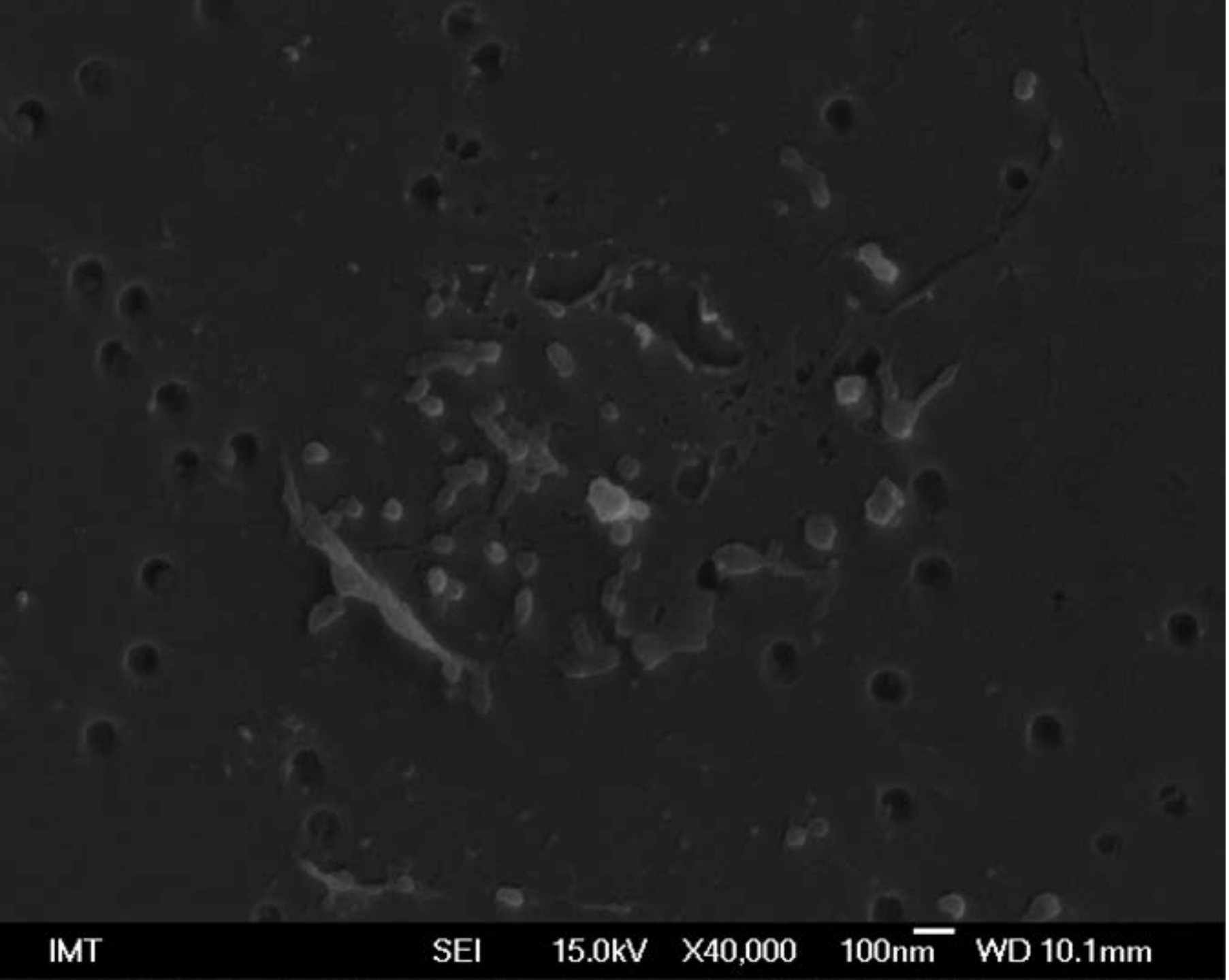

Figure *Phaeodactylum tricornutum* culture SEM LB 62.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with LB Lennox LB broth (ref. L3022, Sigma Aldrich, USA. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 µmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

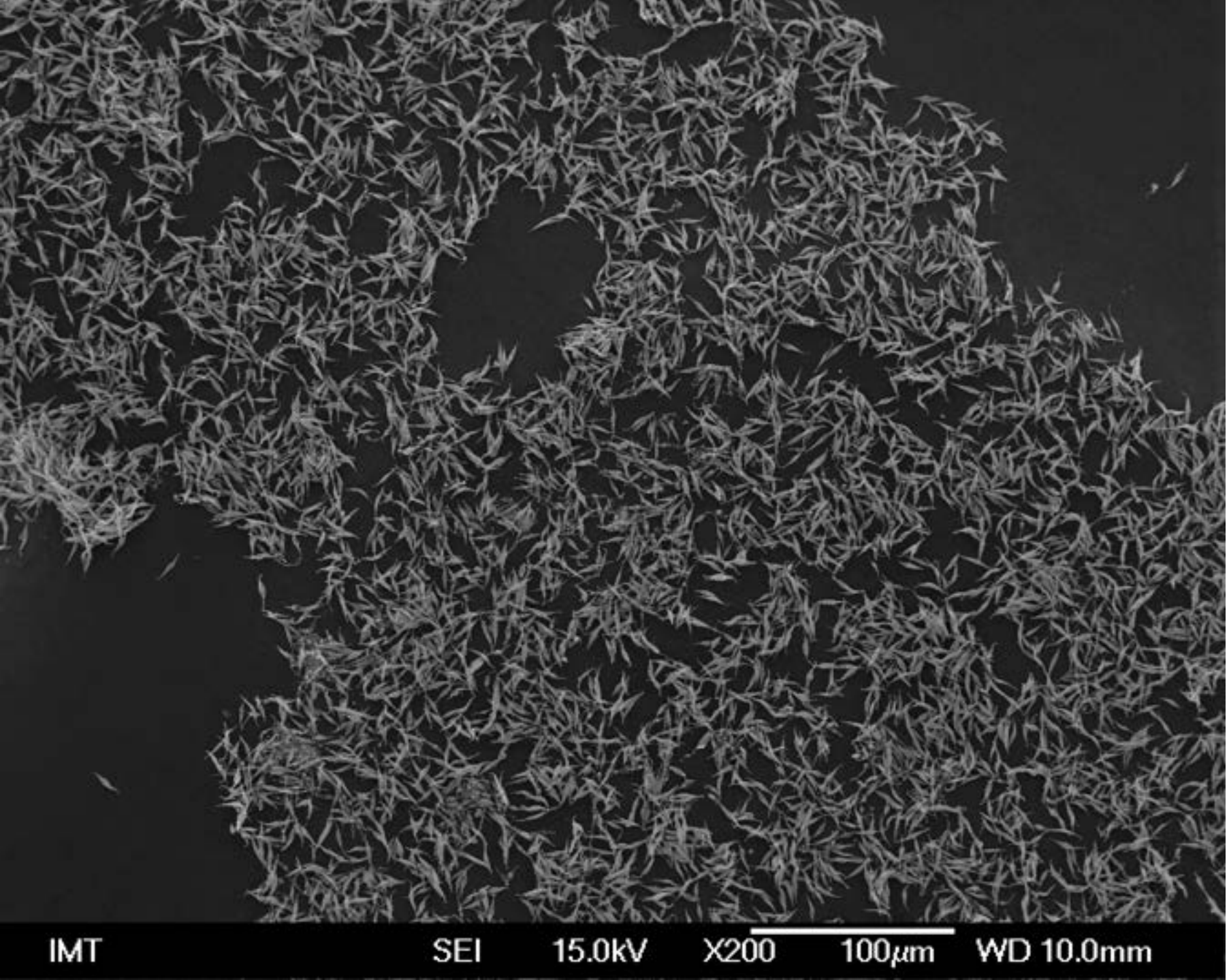

Figure *Phaeodactylum tricornutum* culture SEM 63. SEM feodaktilum netretiran #2ovoid/Darja-21-10-2021 - 10 veliko krogel dolgo na plosci

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with LB Lennox LB broth (ref. L3022, Sigma Aldrich, USA. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 µmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

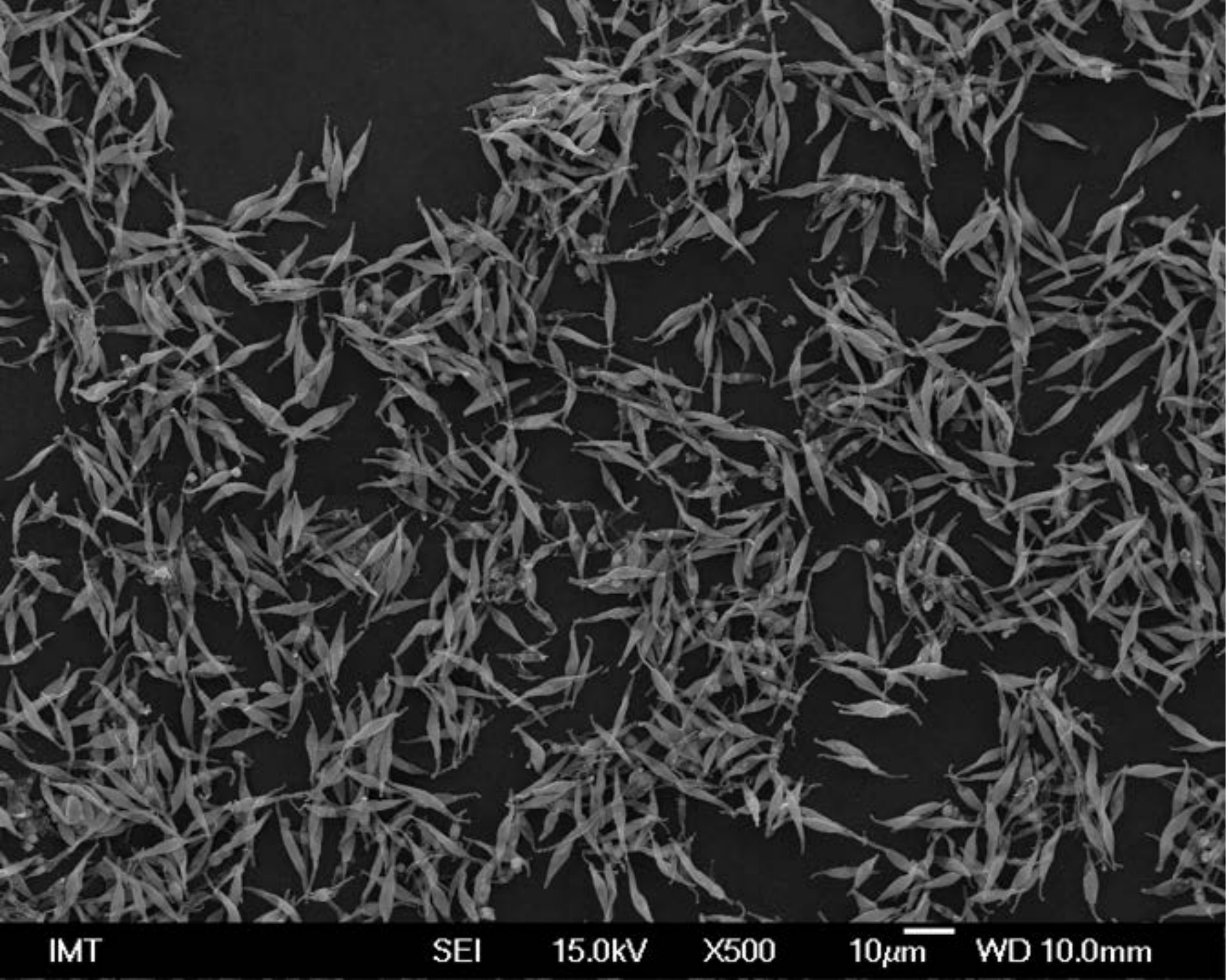

Figure *Phaeodactylum tricornutum* culture SEM 64. SEM feodaktilum netretiran #2ovoid/Darja-21-10-2021 - 10 veliko krogel dolgo na plosci

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with LB Lennox LB broth (ref. L3022, Sigma Aldrich, USA. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore^{TM}, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

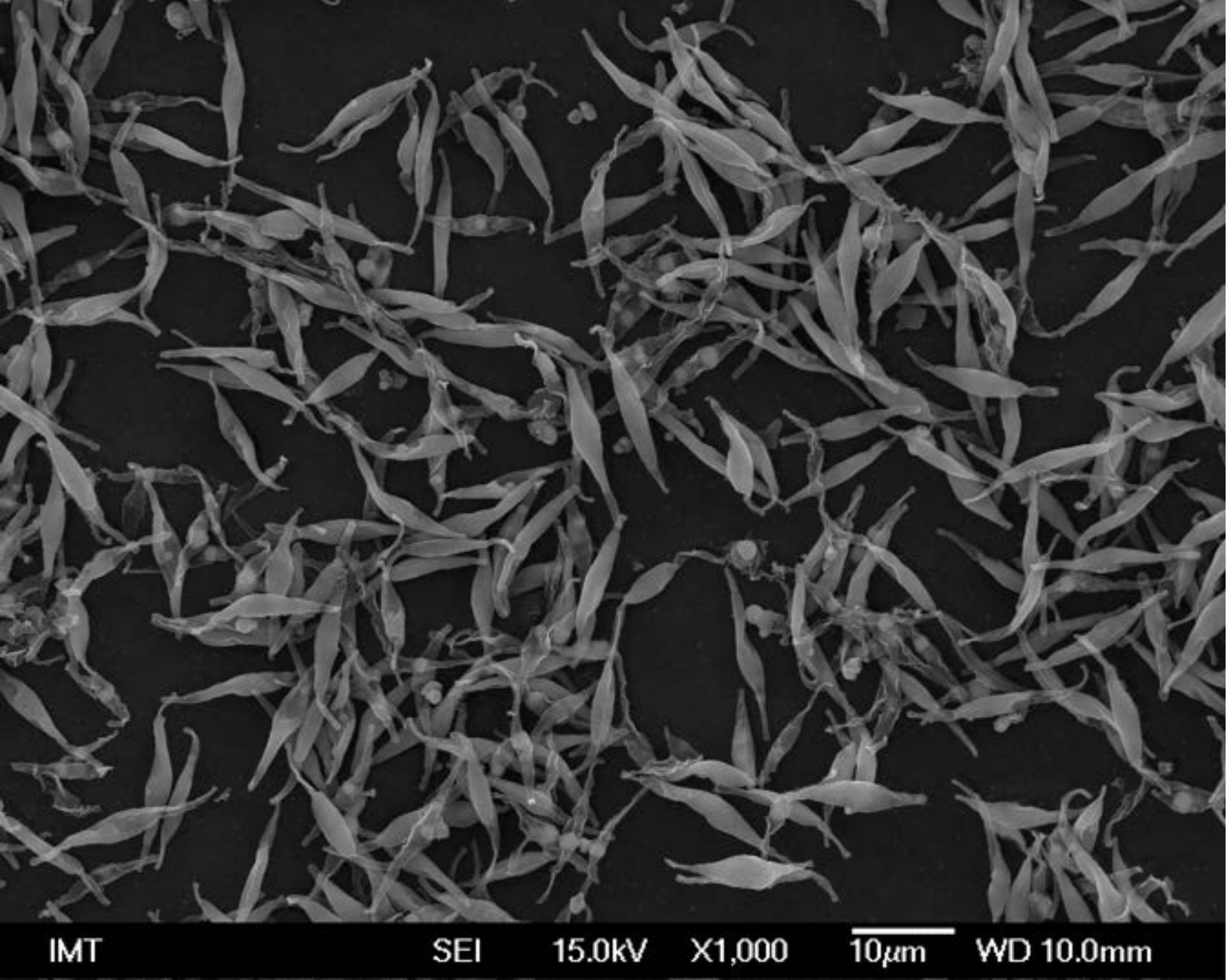

Figure *Phaeodactylum tricornutum culture* SEM 65. SEM feodaktilum netretiran #2ovoid/Darja-21-10-2021 - 10 veliko krogel dolgo na plosci

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with LB Lennox LB broth (ref. L3022, Sigma Aldrich, USA. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 µmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

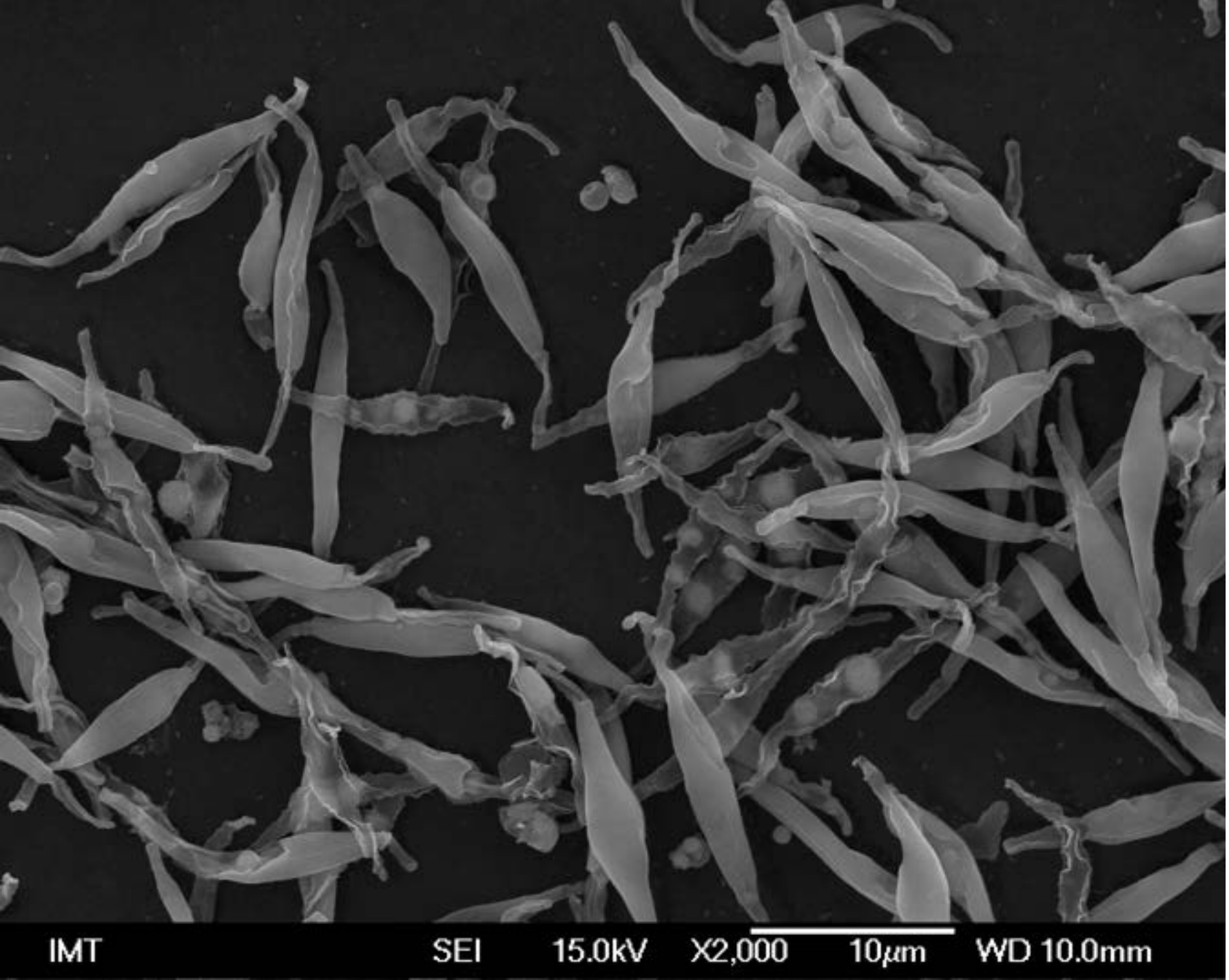

Figure *Phaeodactylum tricornutum culture* SEM 66. SEM feodaktilum netretiran #2ovoid/Darja-21-10-2021 - 10 veliko krogel dolgo na plosci

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with LB Lennox LB broth (ref. L3022, Sigma Aldrich, USA. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

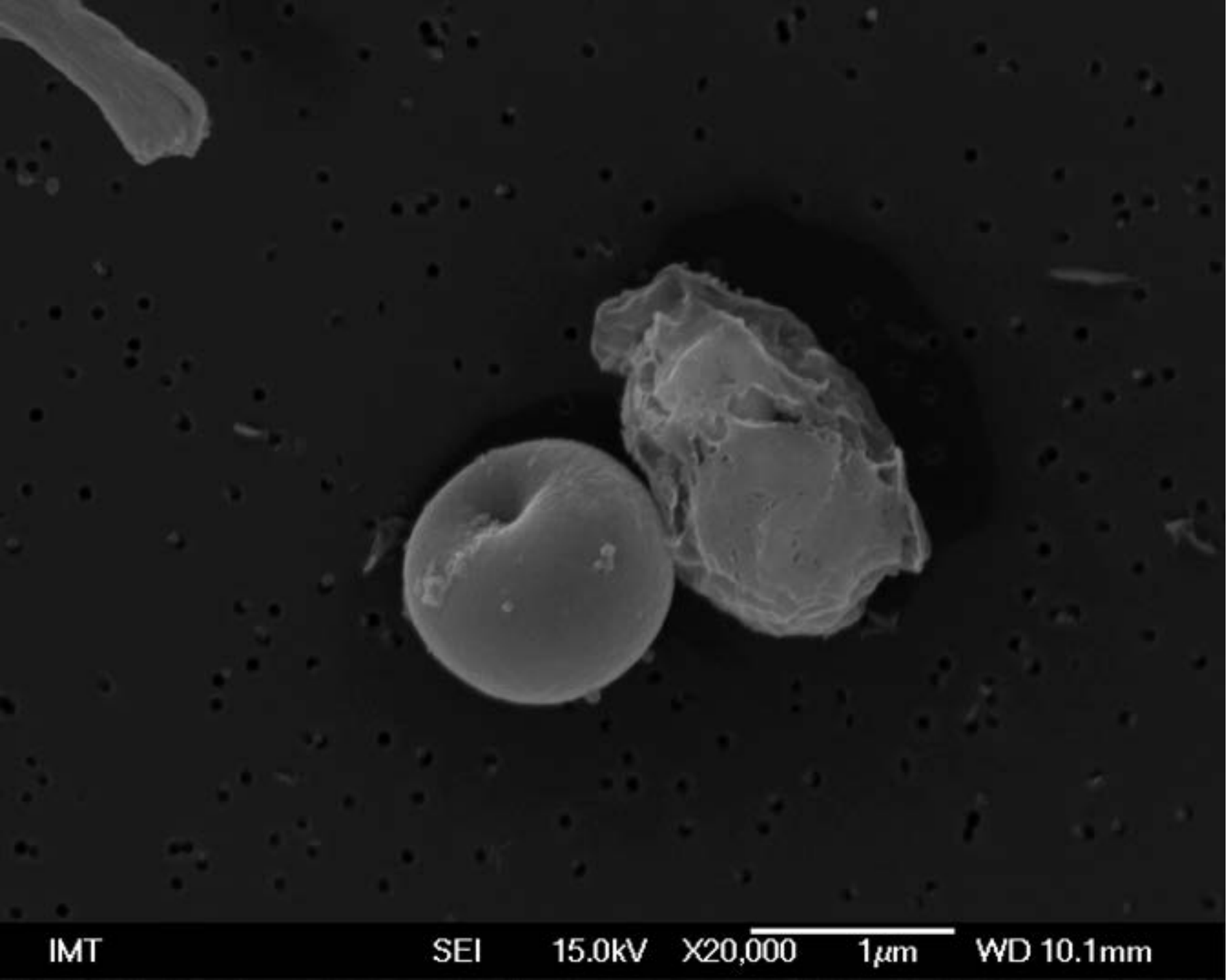

Figure *Phaeodactylum tricornutum culture* SEM 67. SEM feodaktilum netretiran #2ovoid/Darja-21-10-2021 - 10 veliko krogel dolgo na plosci

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with LB Lennox LB broth (ref. L3022, Sigma Aldrich, USA. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

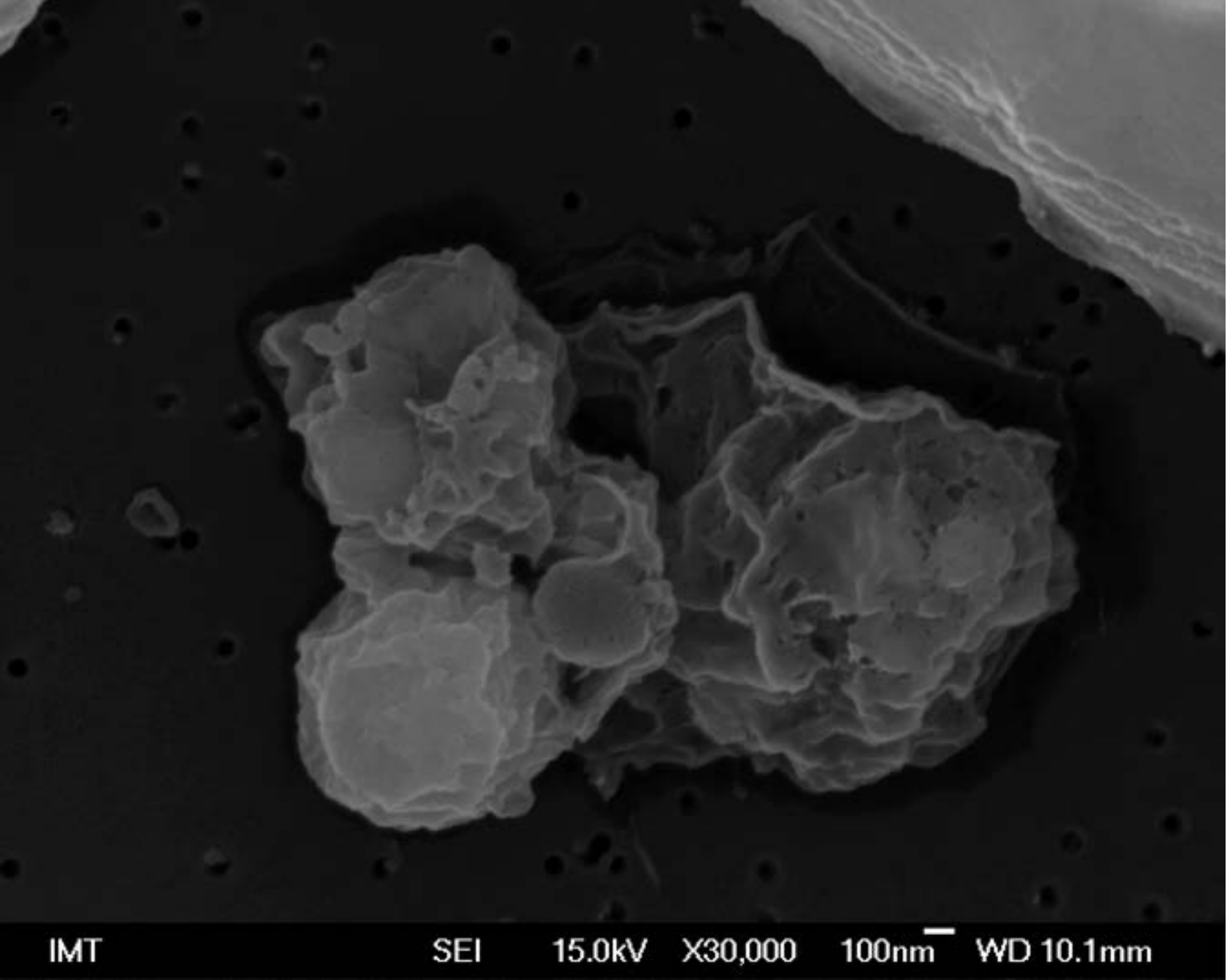

Figure *Phaeodactylum tricornutum culture* SEM 68. SEM feodaktilum netretiran #2ovoid/Darja-21-10-2021 - 10 veliko krogel dolgo na plosci

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with LB Lennox LB broth (ref. L3022, Sigma Aldrich, USA. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 µmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

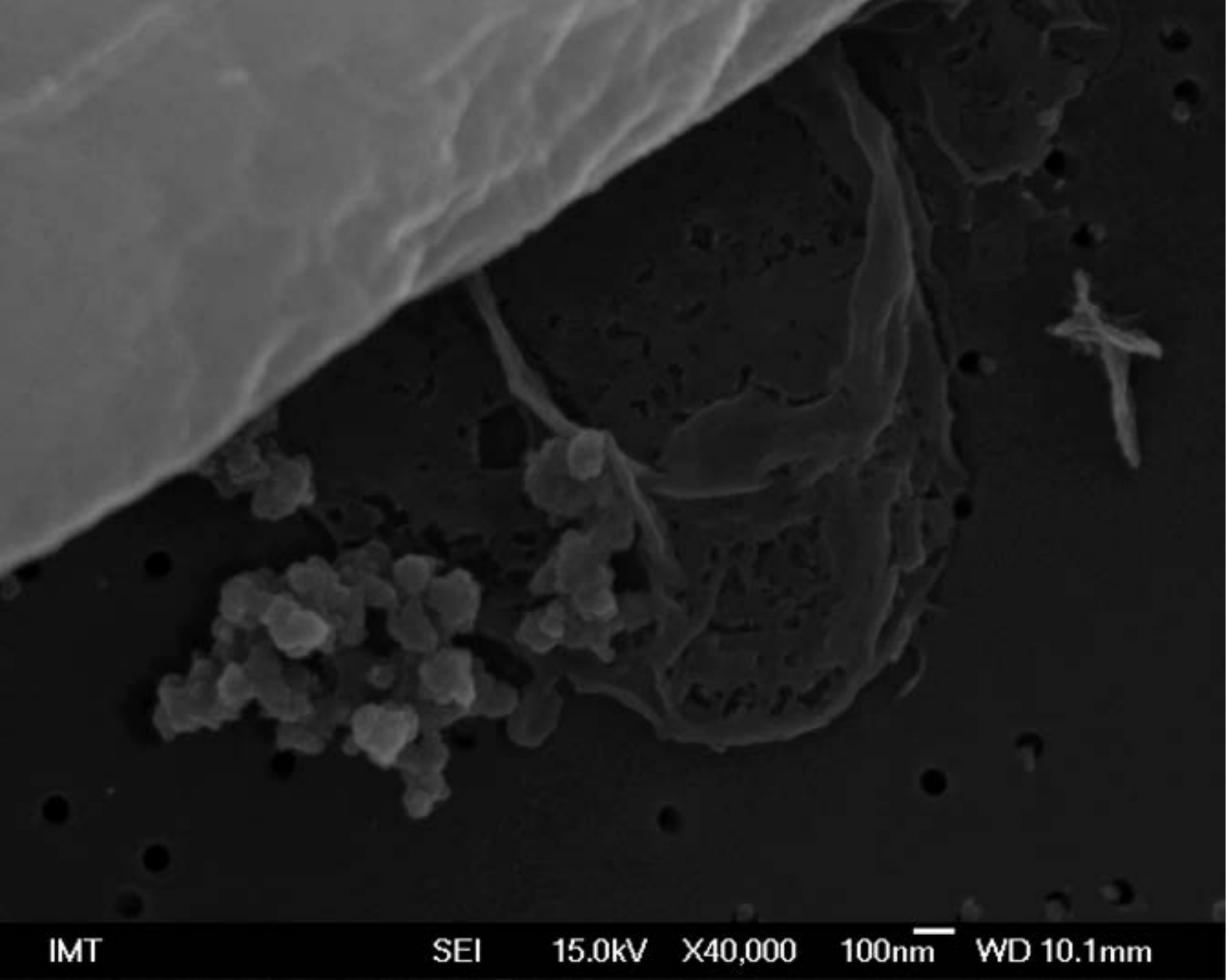

Figure *Phaeodactylum tricornutum culture* SEM 69. SEM feodaktilum netretiran #2ovoid/Darja-21-10-2021 - 10 veliko krogel dolgo na plosci

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with LB Lennox LB broth (ref. L3022, Sigma Aldrich, USA. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 µmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

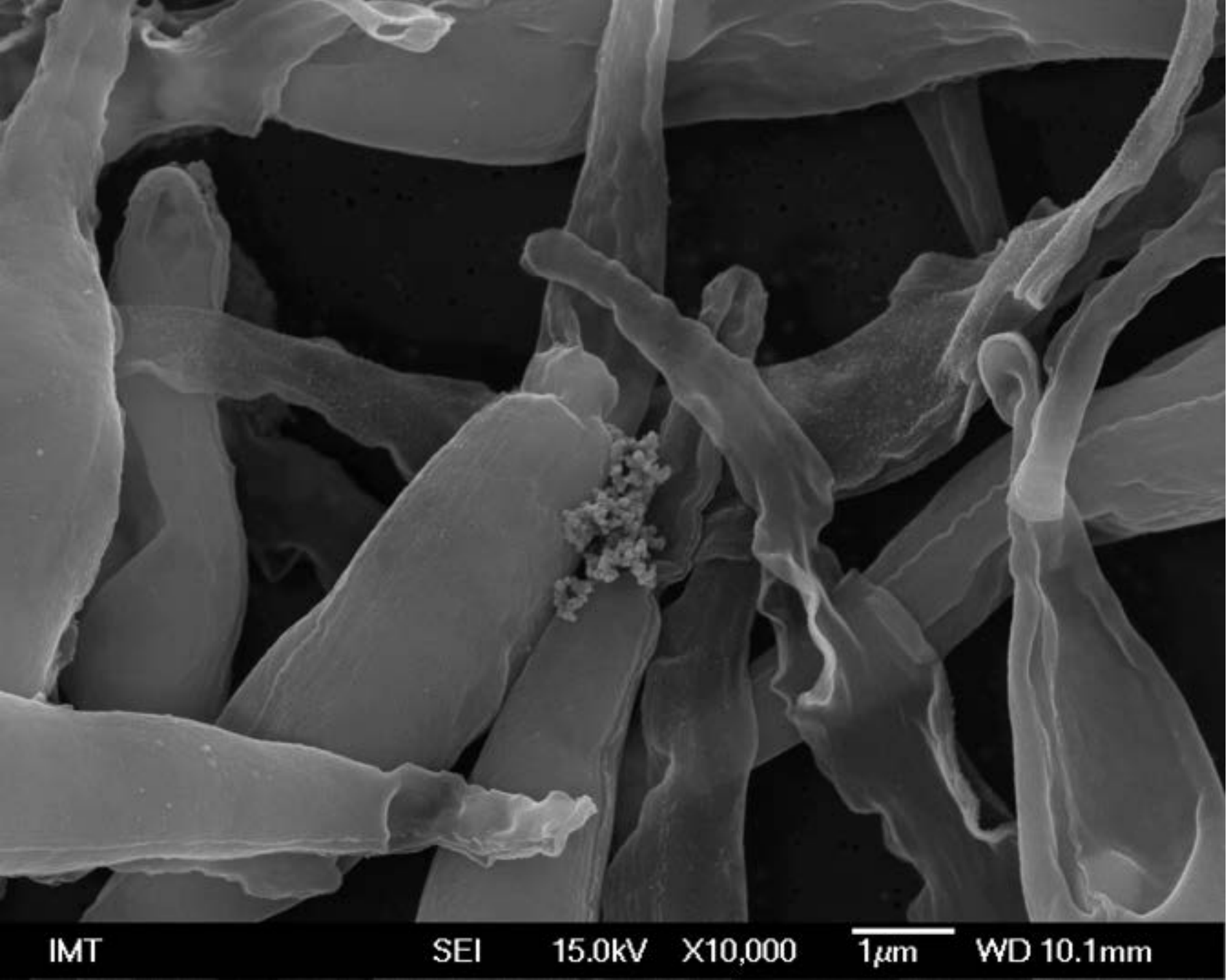

Figure *Phaeodactylum tricornutum culture* SEM 70. SEM feodaktilum netretiran #2ovoid/Darja-21-10-2021 - 10 veliko krogel dolgo na plosci

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with LB Lennox LB broth (ref. L3022, Sigma Aldrich, USA. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 µmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

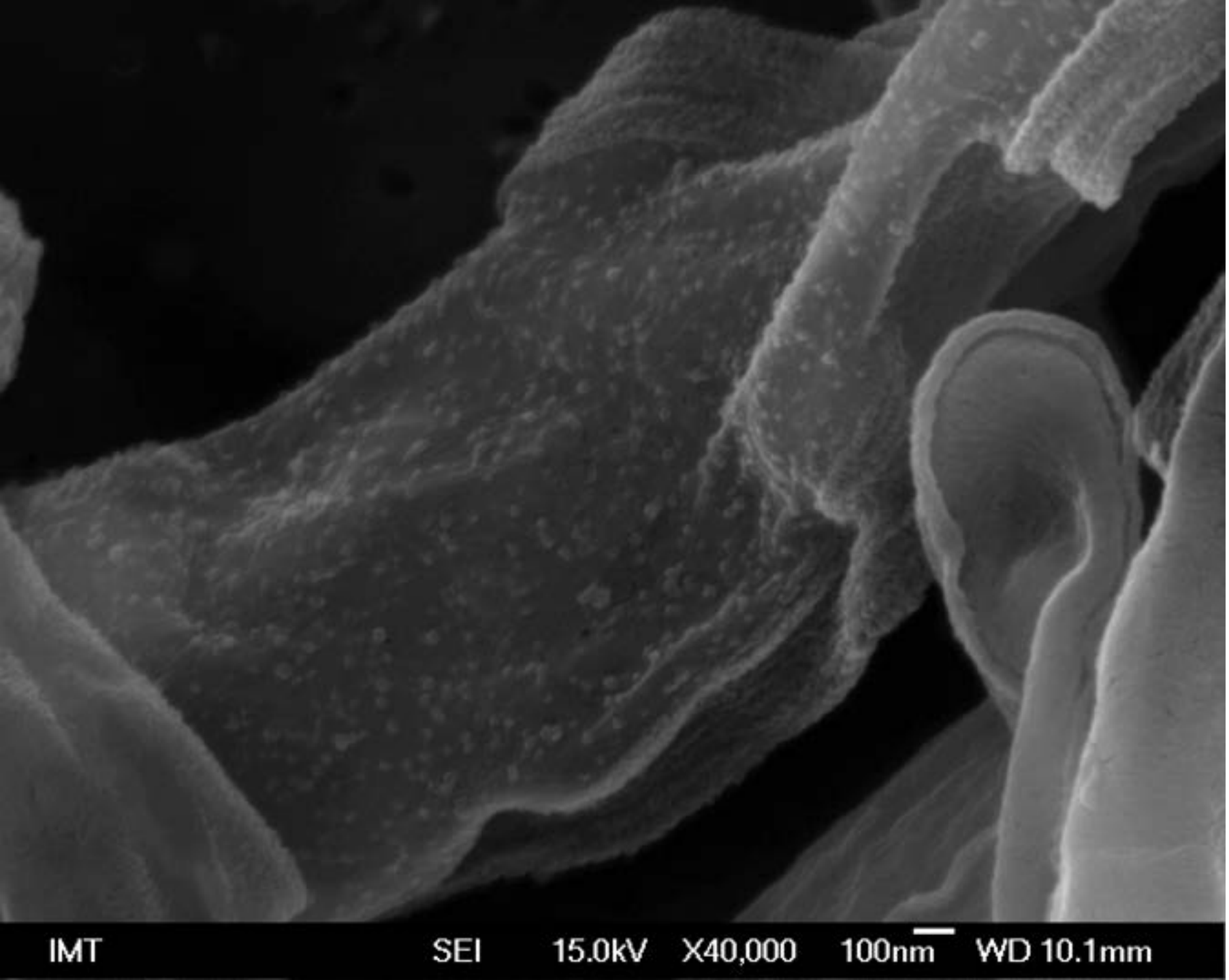

Figure *Phaeodactylum tricornutum culture* SEM 71. SEM feodaktilum netretiran #2ovoid/Darja-21-10-2021 - 10 veliko krogel dolgo na plosci

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with LB Lennox LB broth (ref. L3022, Sigma Aldrich, USA. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 µmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

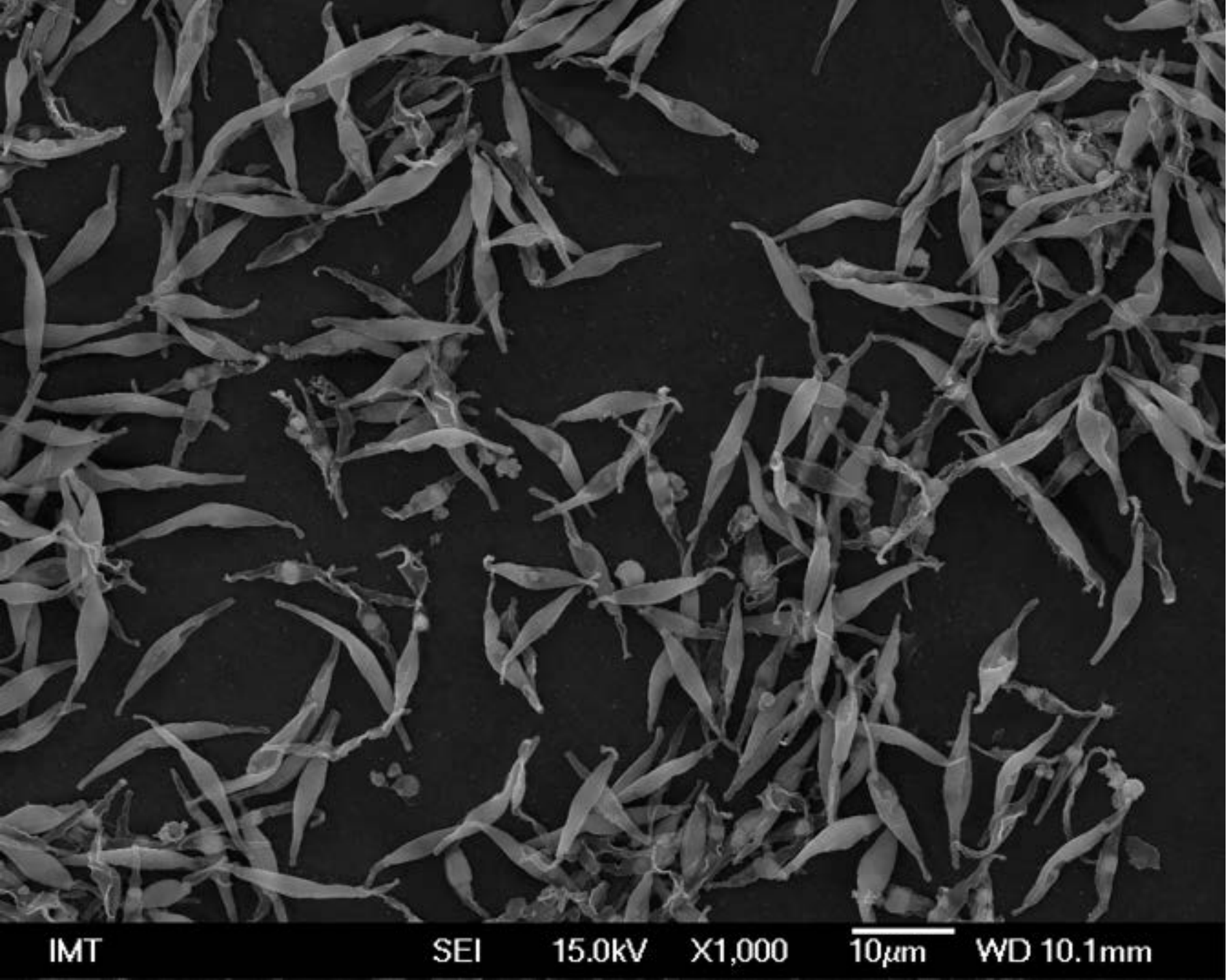

Figure *Phaeodactylum tricornutum culture* SEM 72. SEM feodaktilum netretiran #2ovoid/Darja-21-10-2021 - 10 veliko krogel dolgo na plosci

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with LB Lennox LB broth (ref. L3022, Sigma Aldrich, USA. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

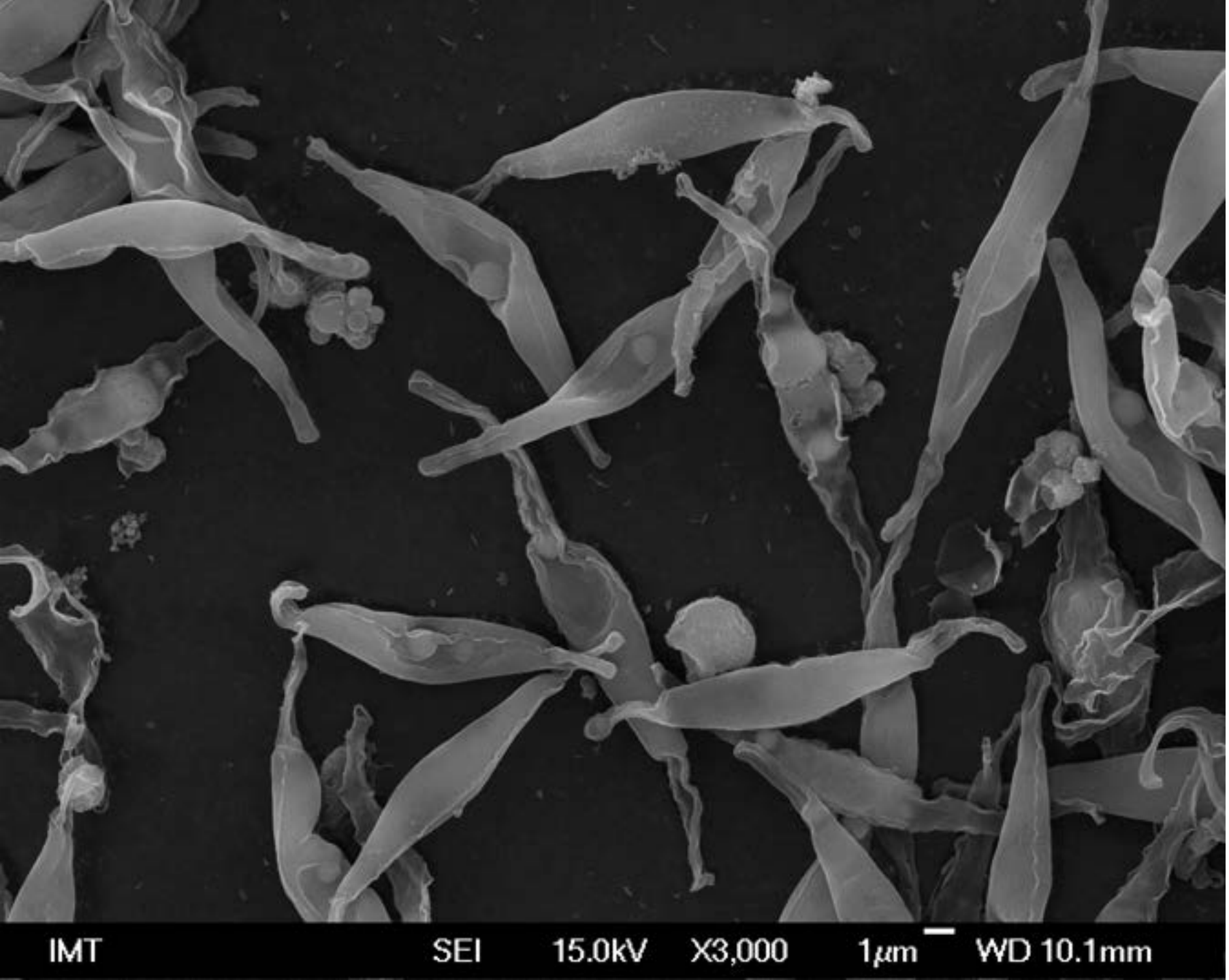

Figure *Phaeodactylum tricornutum culture* SEM 73. SEM feodaktilum netretiran #2ovoid/Darja-21-10-2021 - 10 veliko krogel dolgo na plosci

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with LB Lennox LB broth (ref. L3022, Sigma Aldrich, USA. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 µmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

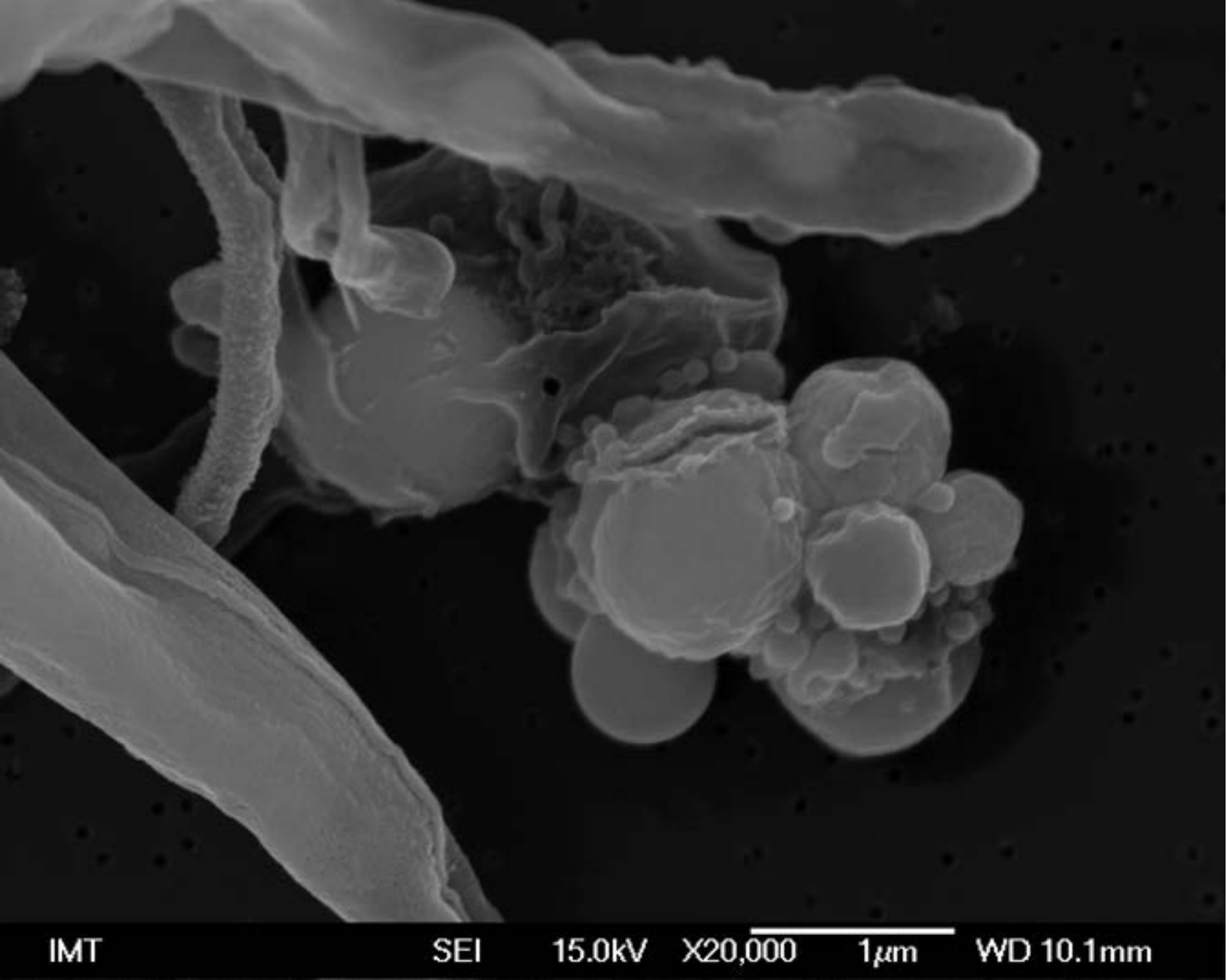

Figure *Phaeodactylum tricornutum culture* SEM 74. SEM feodaktilum netretiran #2ovoid/Darja-21-10-2021 - 10 veliko krogel dolgo na plosci

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with LB Lennox LB broth (ref. L3022, Sigma Aldrich, USA. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 µmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

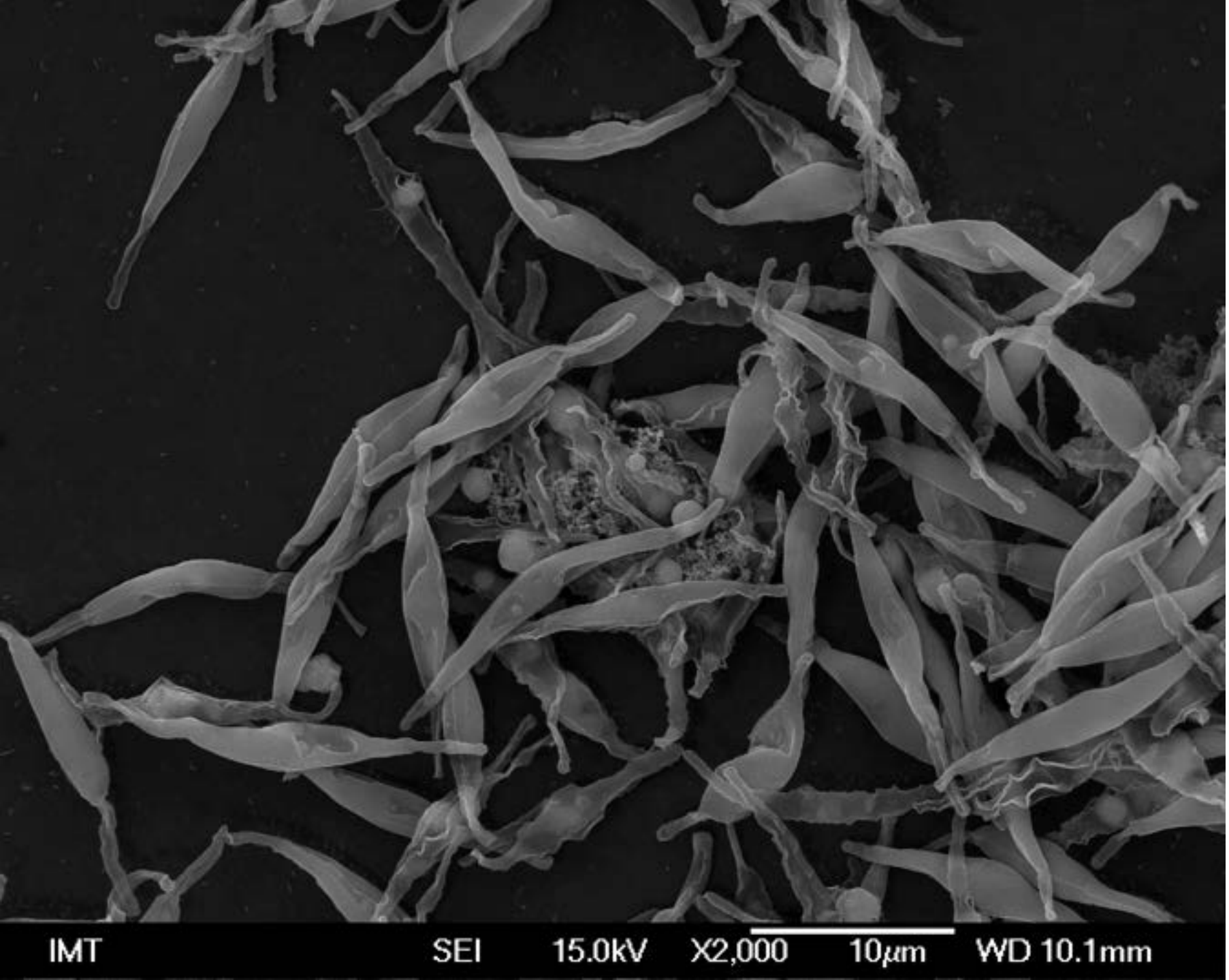

Figure *Phaeodactylum tricornutum culture* SEM 75. SEM feodaktilum netretiran #2ovoid/Darja-21-10-2021 - 10 veliko krogel dolgo na plosci

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with LB Lennox LB broth (ref. L3022, Sigma Aldrich, USA. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

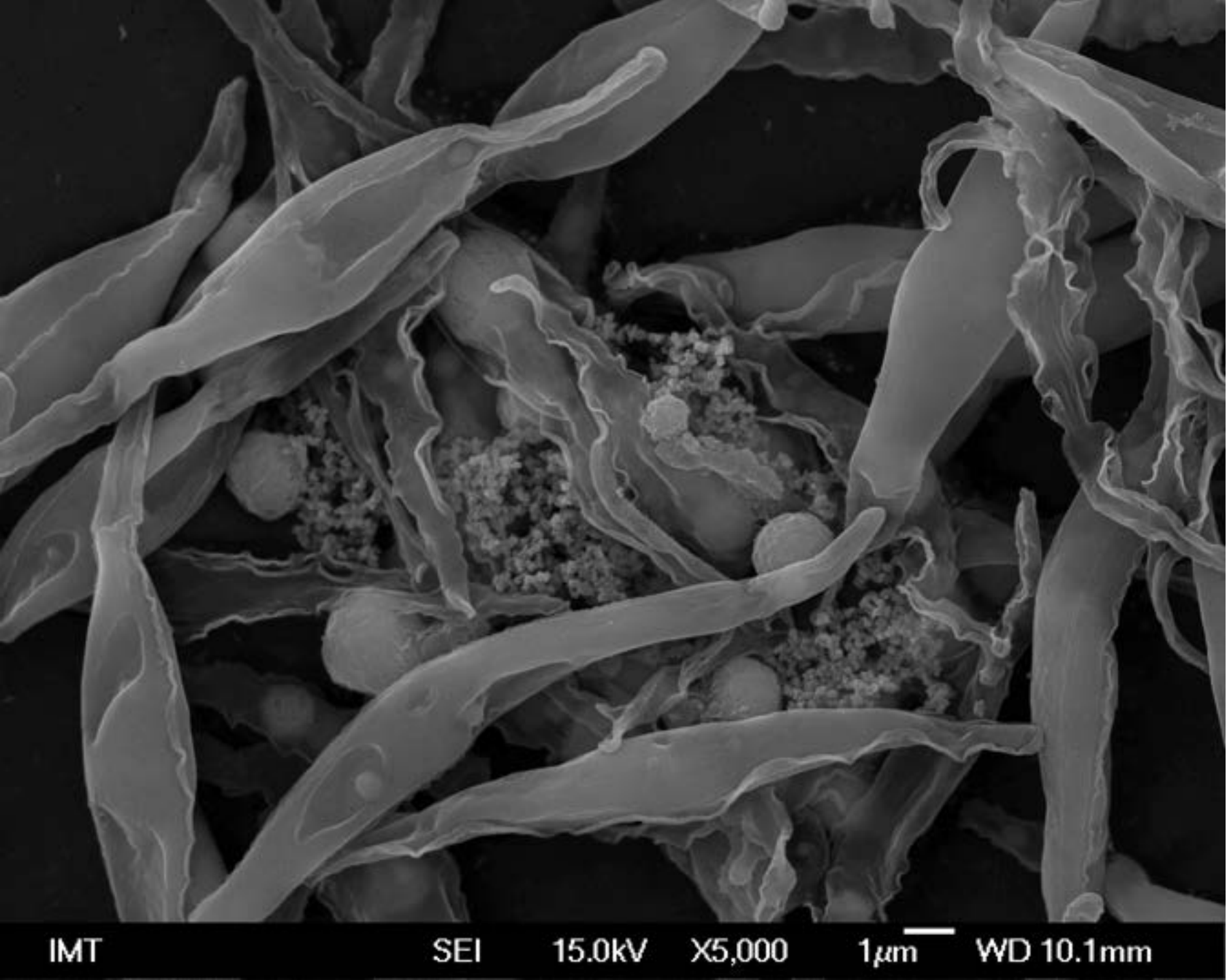

Figure *Phaeodactylum tricornutum culture* SEM 76. SEM feodaktilum netretiran #2ovoid/Darja-21-10-2021 - 10 veliko krogel dolgo na plosci

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with LB Lennox LB broth (ref. L3022, Sigma Aldrich, USA. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 µmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

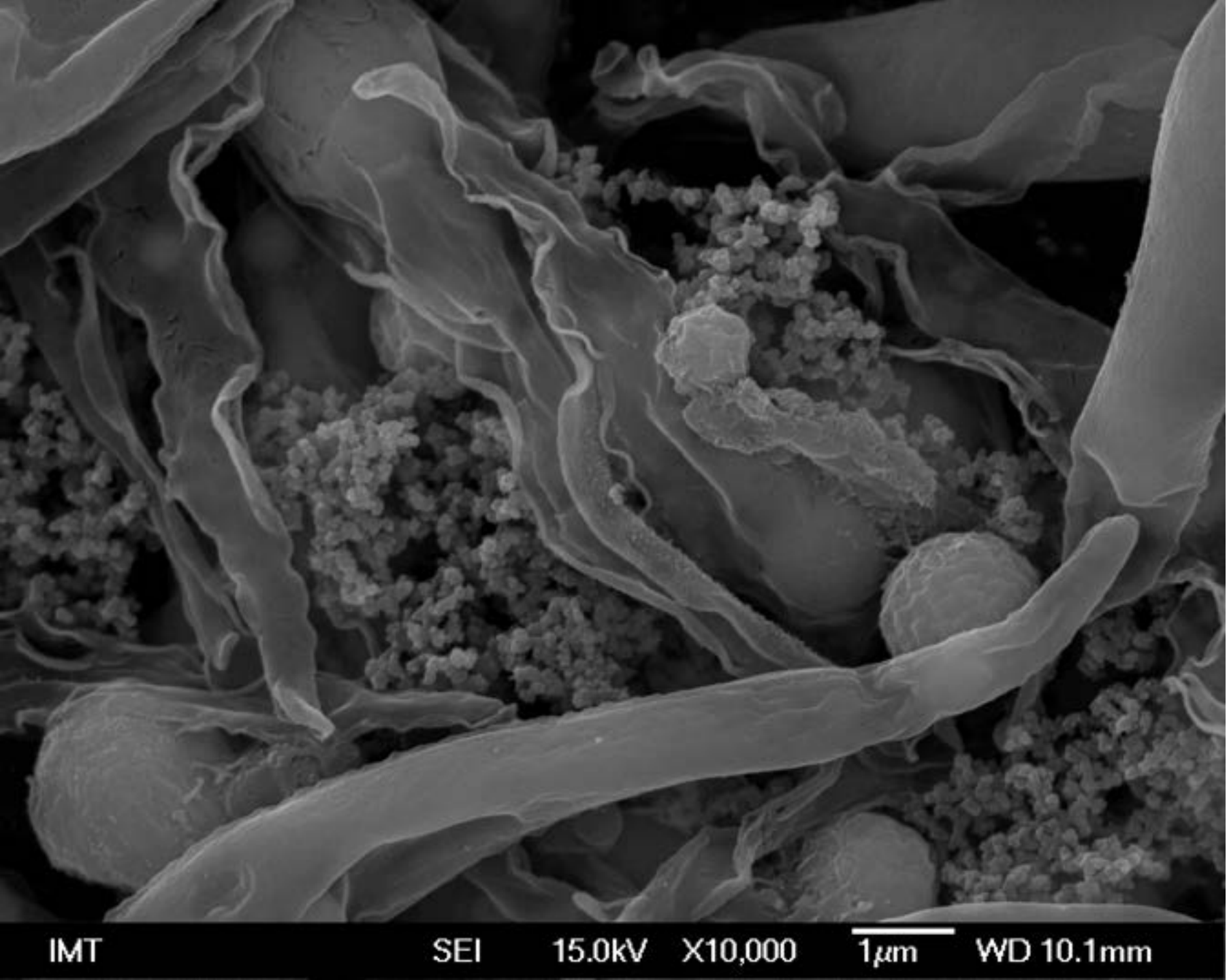

Figure *Phaeodactylum tricornutum culture* SEM 77. SEM feodaktilum netretiran #2ovoid/Darja-21-10-2021 - 10 veliko krogel dolgo na plosci

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with LB Lennox LB broth (ref. L3022, Sigma Aldrich, USA. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 µmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

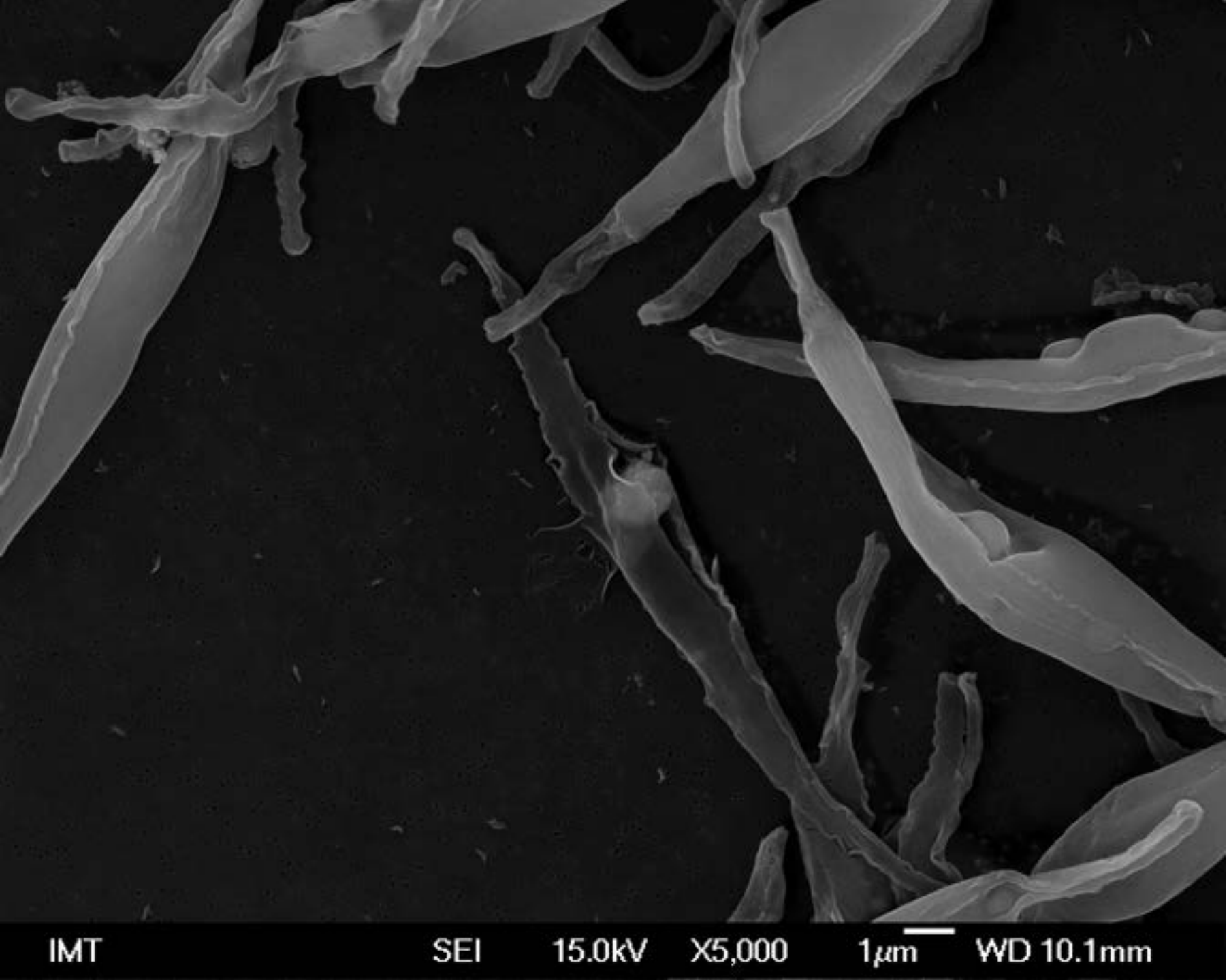

Figure *Phaeodactylum tricornutum culture* SEM 78. SEM feodaktilum netretiran #2ovoid/Darja-21-10-2021 - 10 veliko krogel dolgo na plosci

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with LB Lennox LB broth (ref. L3022, Sigma Aldrich, USA. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 µmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

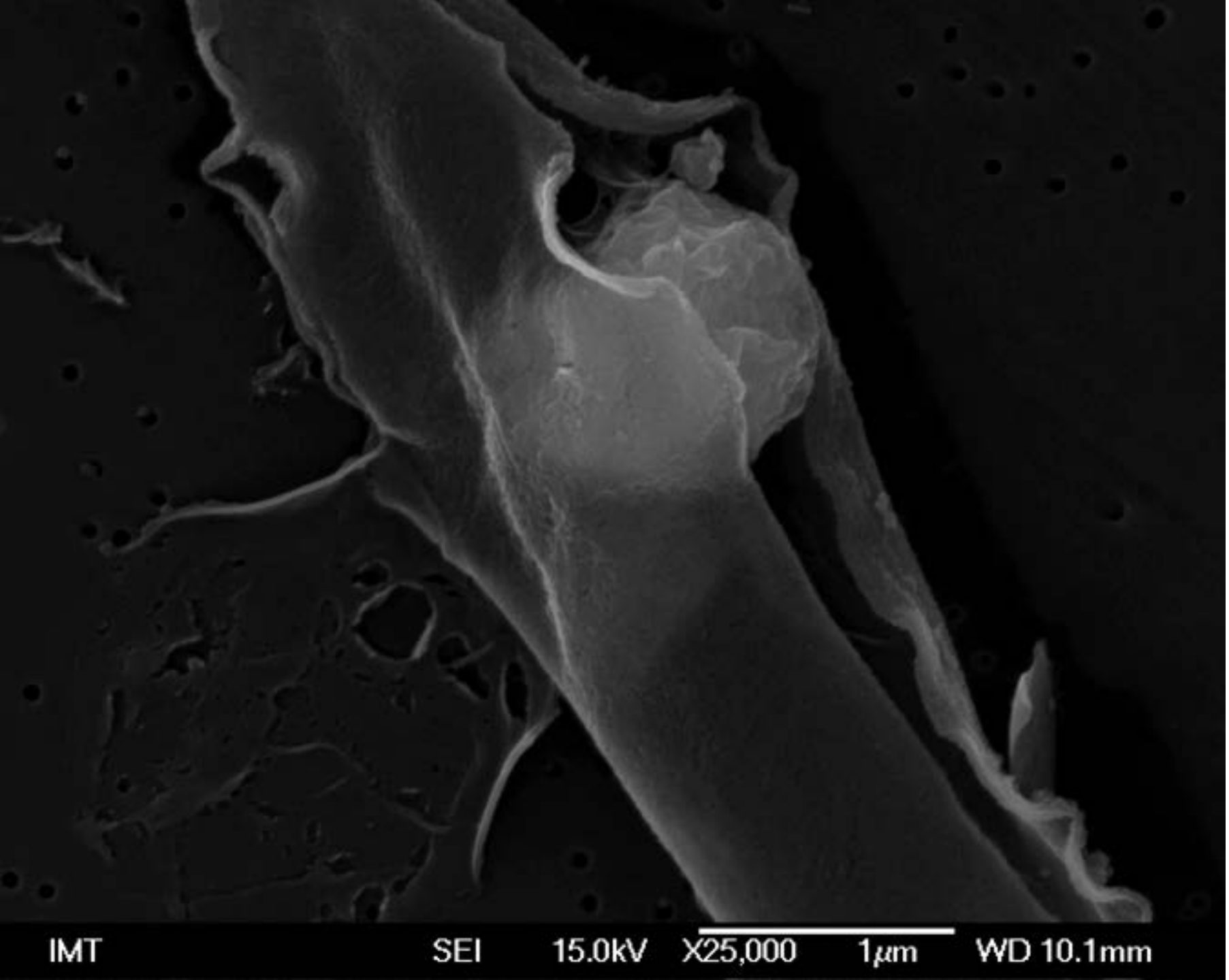

Figure *Phaeodactylum tricornutum culture* SEM 79. SEM feodaktilum netretiran #2ovoid/Darja-21-10-2021 - 10 veliko krogel dolgo na plosci

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with LB Lennox LB broth (ref. L3022, Sigma Aldrich, USA. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

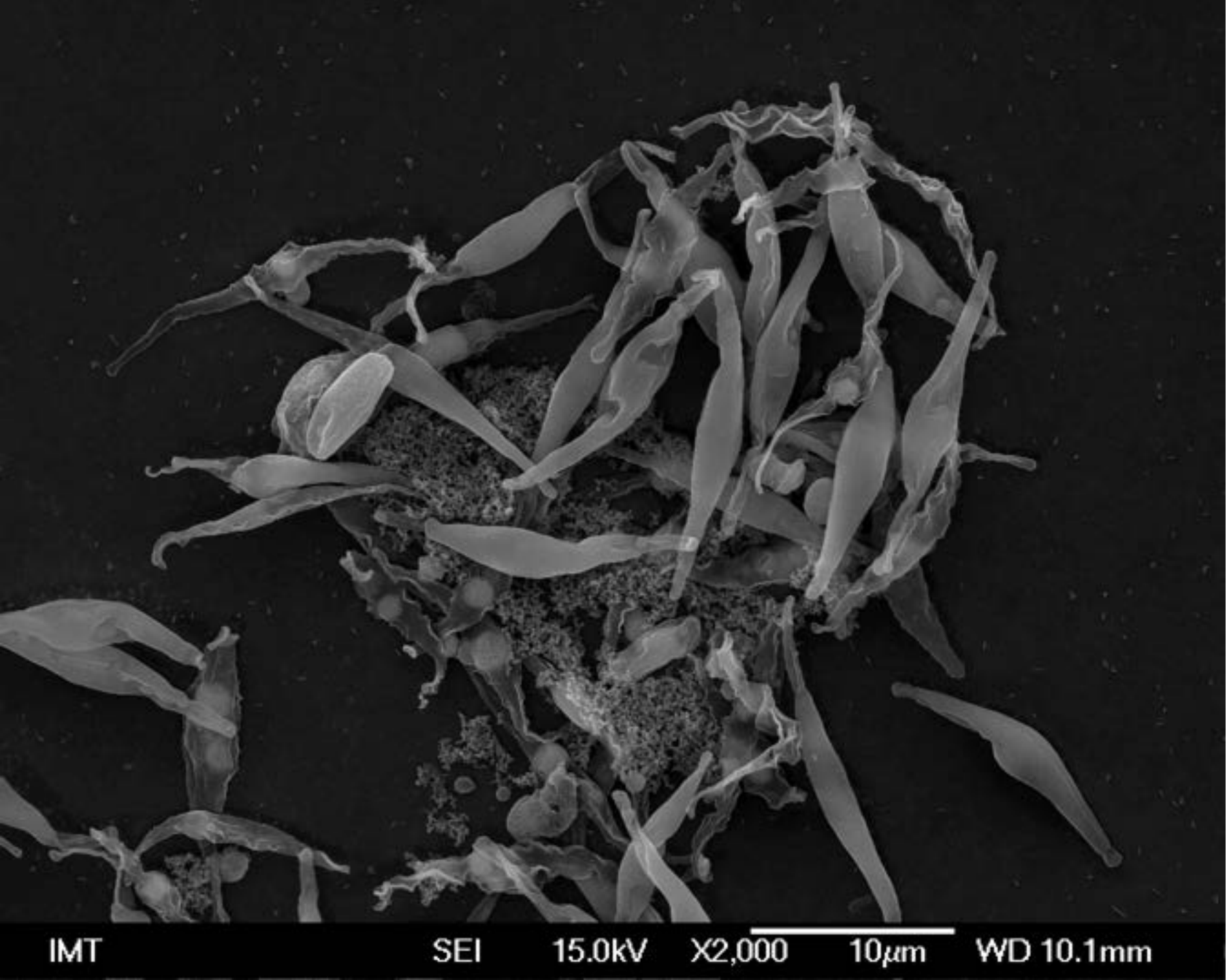

Figure *Phaeodactylum tricornutum culture* SEM 80. SEM feodaktilum netretiran #2ovoid/Darja-21-10-2021 - 10 veliko krogel dolgo na plosci

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with LB Lennox LB broth (ref. L3022, Sigma Aldrich, USA. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

Figure *Phaeodactylum tricornutum culture* SEM 81. SEM feodaktilum netretiran #2ovoid/Darja-21-10-2021 - 10 veliko krogel dolgo na plosci

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with LB Lennox LB broth (ref. L3022, Sigma Aldrich, USA. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 µmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

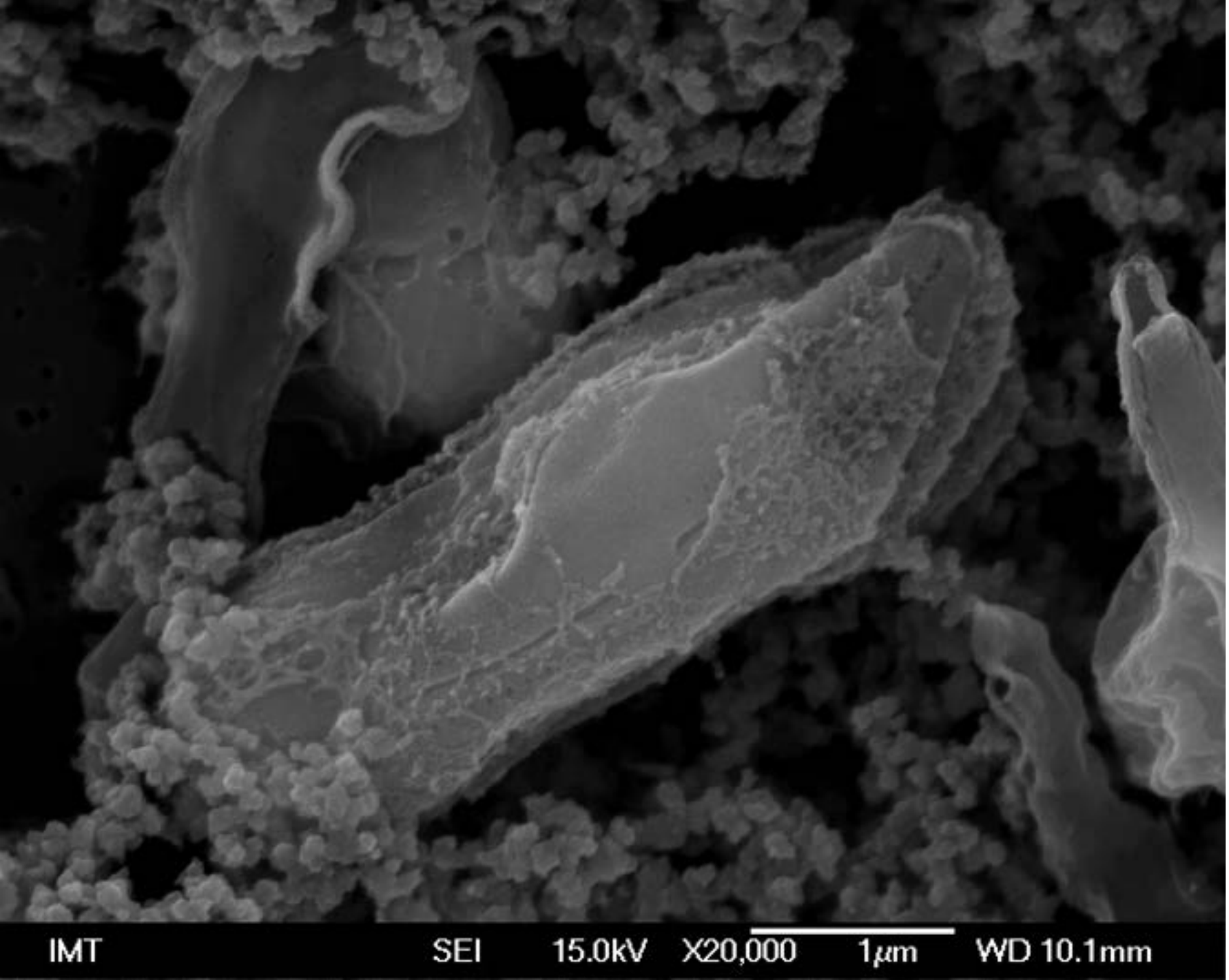

Figure *Phaeodactylum tricornutum culture* SEM 82. SEM feodaktilum netretiran #2ovoid/Darja-21-10-2021 - 10 veliko krogel dolgo na plosci

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with LB Lennox LB broth (ref. L3022, Sigma Aldrich, USA. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

Figure *Phaeodactylum tricornutum culture* SEM 83. SEM feodaktilum netretiran #2ovoid/Darja-21-10-2021 - 10 veliko krogel dolgo na plosci

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with LB Lennox LB broth (ref. L3022, Sigma Aldrich, USA. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 µmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

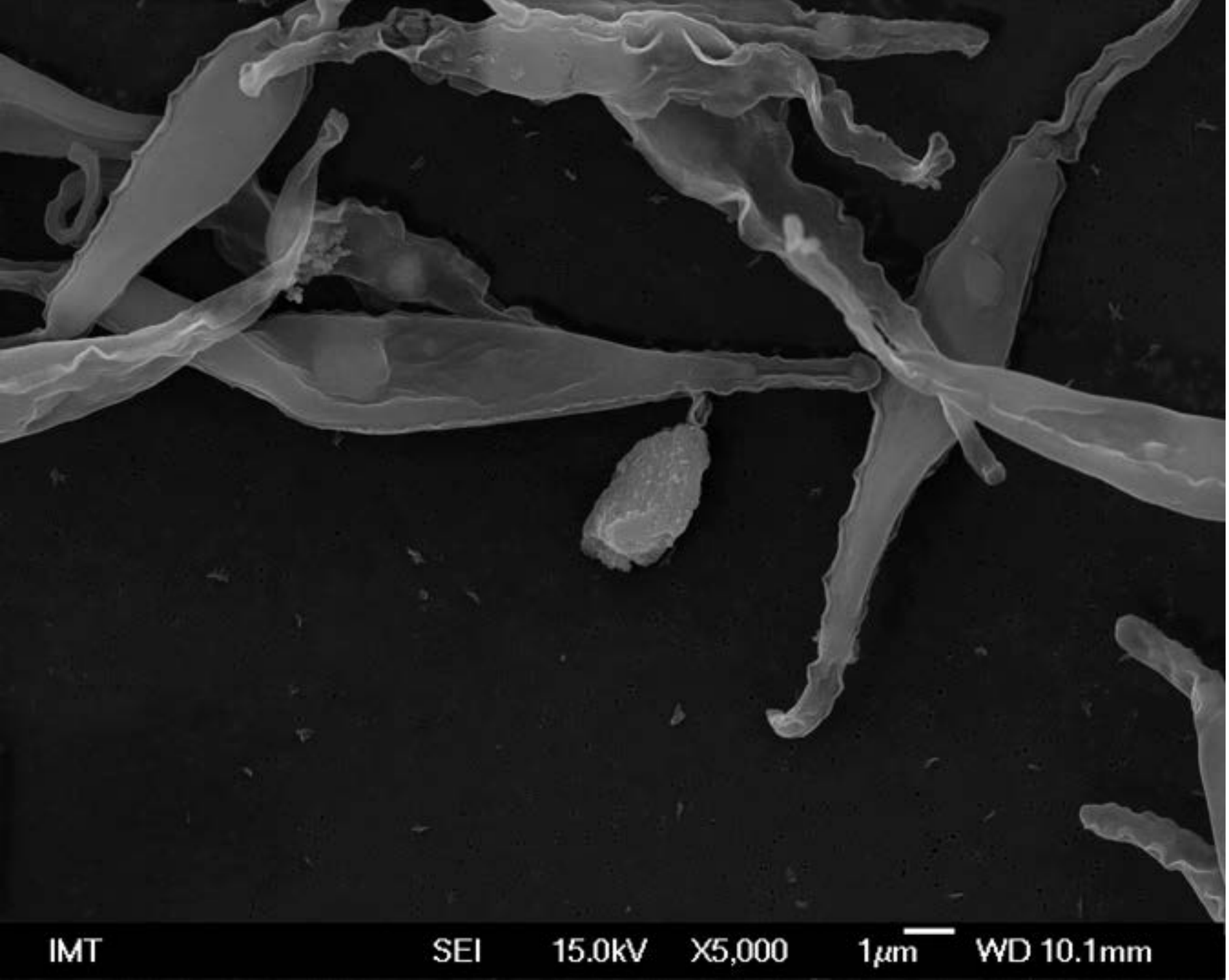

Figure *Phaeodactylum tricornutum culture* SEM 84. SEM feodaktilum netretiran #2ovoid/Darja-21-10-2021 - 10 veliko krogel dolgo na plosci

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with LB Lennox LB broth (ref. L3022, Sigma Aldrich, USA. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 µmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

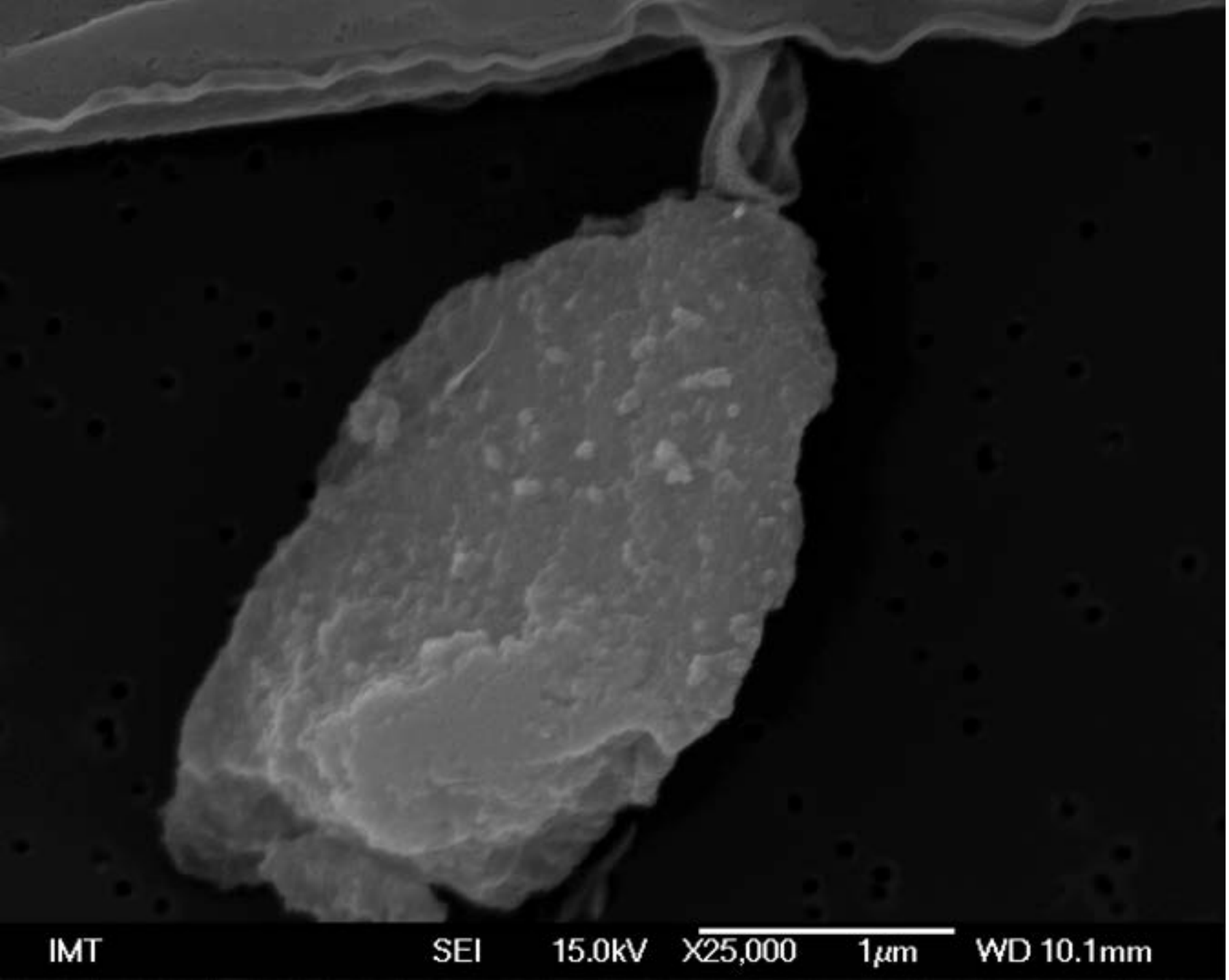

Figure *Phaeodactylum tricornutum culture* SEM 85. SEM feodaktilum netretiran #2ovoid/Darja-21-10-2021 - 10 veliko krogel dolgo na plosci

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with LB Lennox LB broth (ref. L3022, Sigma Aldrich, USA. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

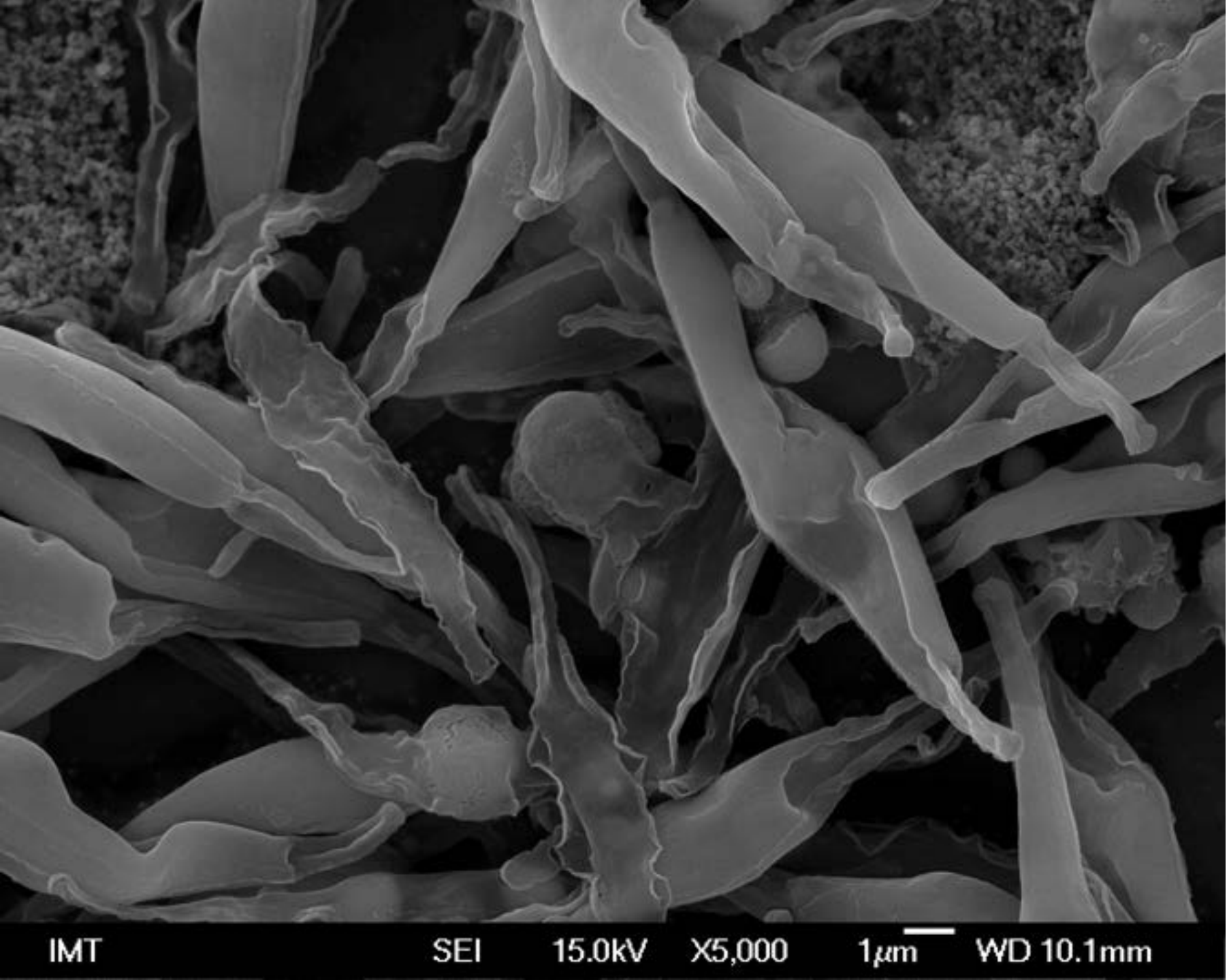

Figure *Phaeodactylum tricornutum culture* SEM 86. SEM feodaktilum netretiran #2ovoid/Darja-21-10-2021 - 10 veliko krogel dolgo na plosci

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with LB Lennox LB broth (ref. L3022, Sigma Aldrich, USA. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 µmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

Figure *Phaeodactylum tricornutum culture* SEM 87. SEM feodaktilum netretiran #2ovoid/Darja-21-10-2021

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with LB Lennox LB broth (ref. L3022, Sigma Aldrich, USA. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

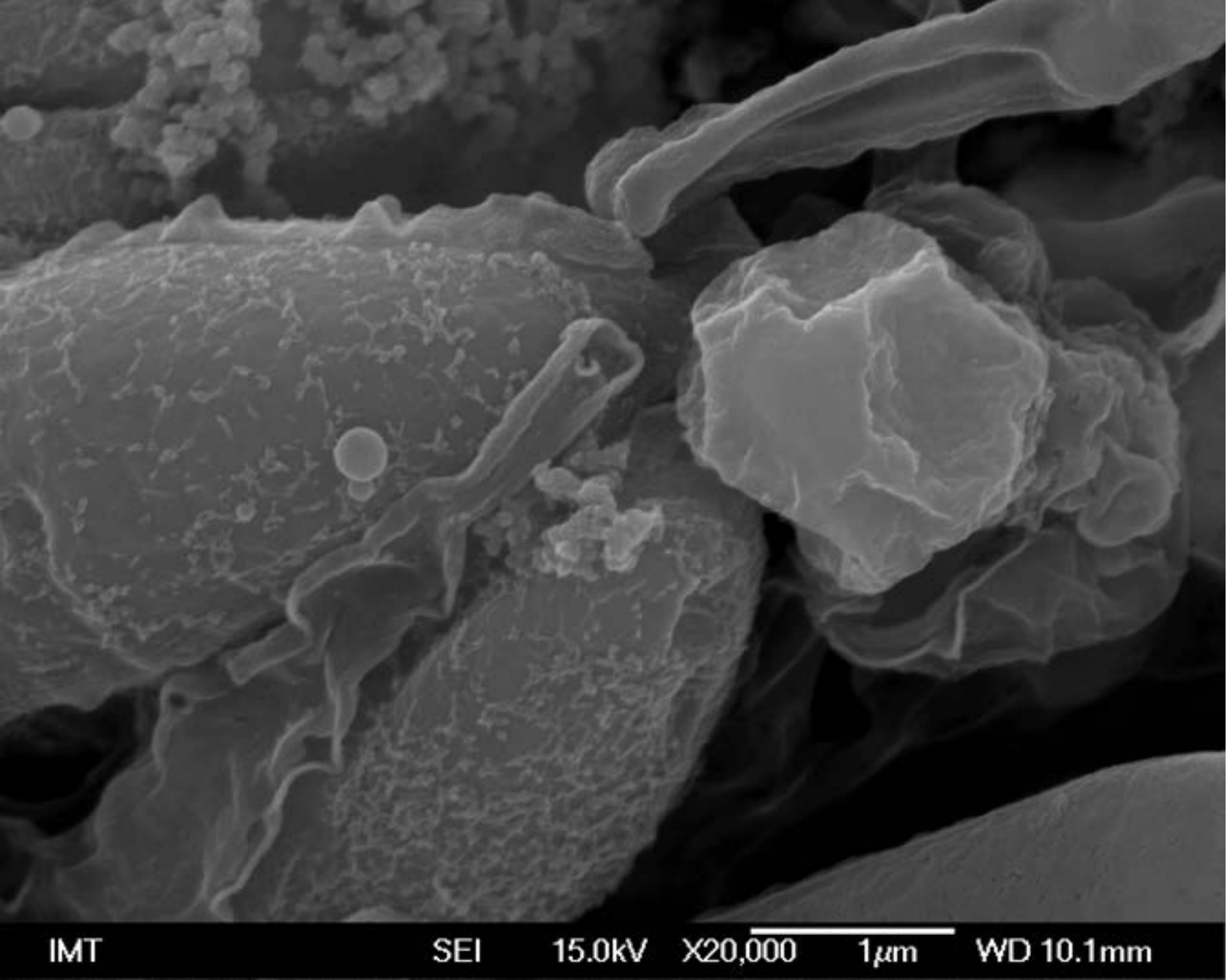

Figure *Phaeodactylum tricornutum culture* SEM 88. SEM feodaktilum netretiran #2ovoid/Darja-21-10-2021

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with LB Lennox LB broth (ref. L3022, Sigma Aldrich, USA. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

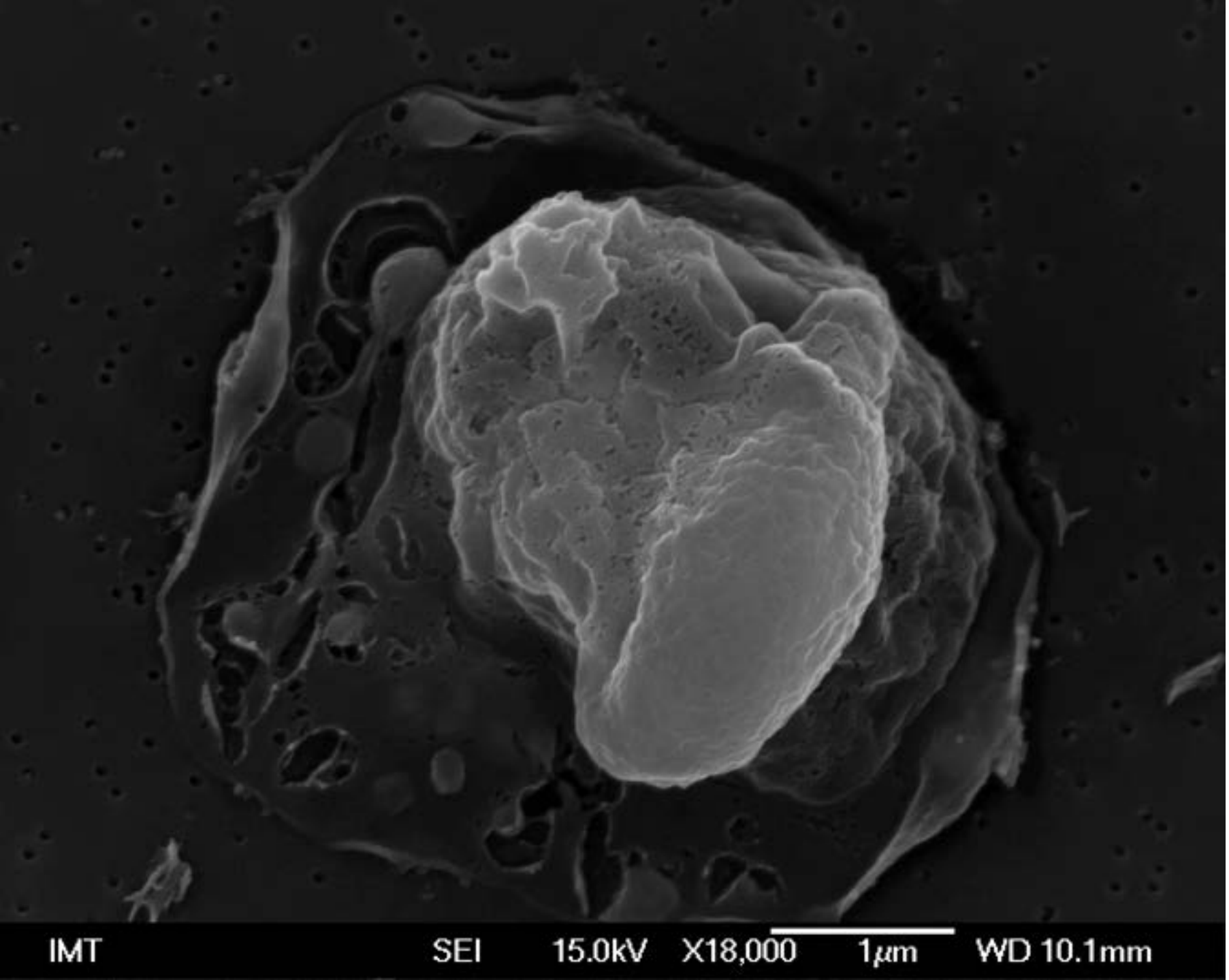

Figure *Phaeodactylum tricornutum culture* SEM 89. SEM feodaktilum netretiran #2ovoid/Darja-21-10-2021 - 10 veliko krogel dolgo na plosci

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with LB Lennox LB broth (ref. L3022, Sigma Aldrich, USA. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 µmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

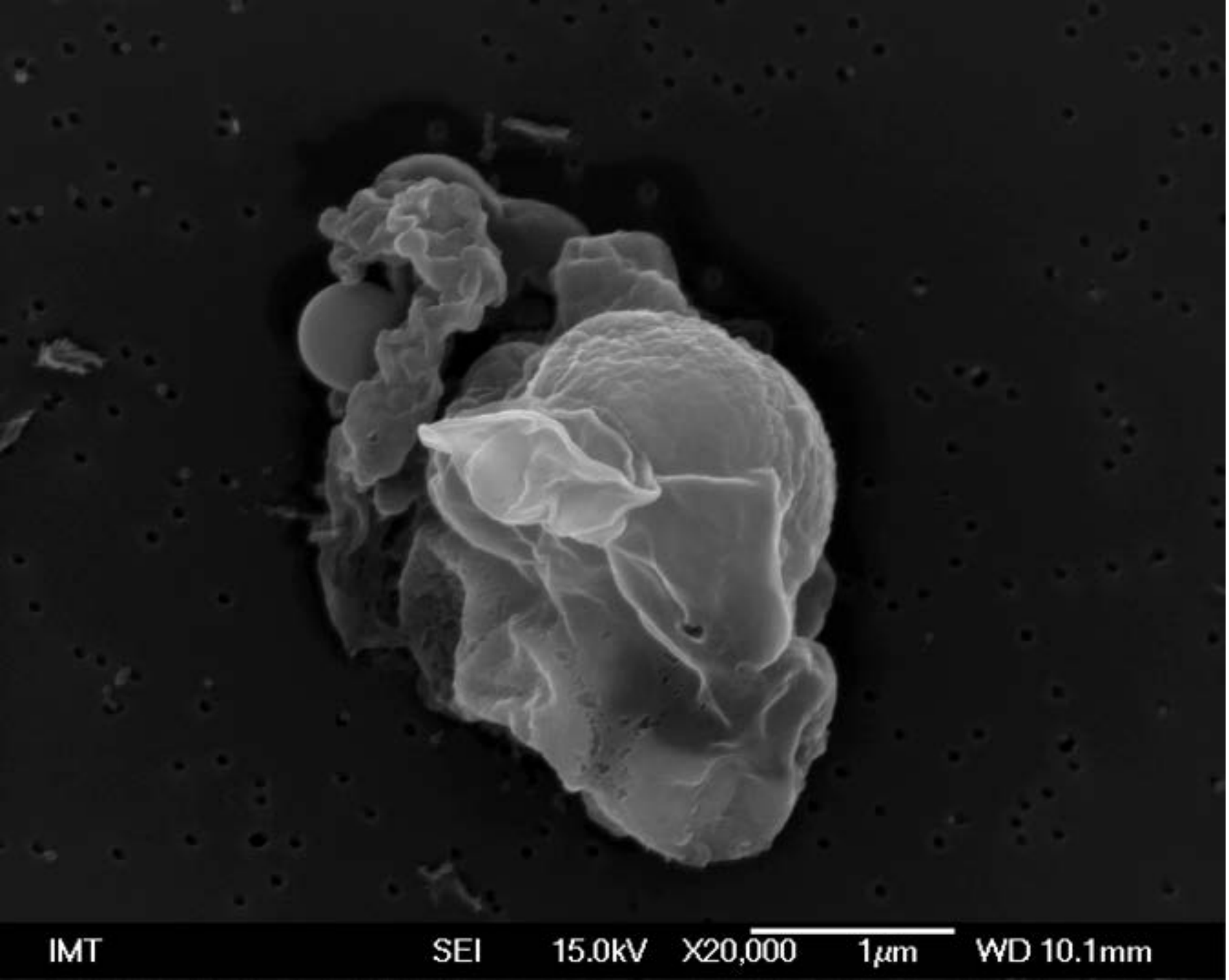

Figure *Phaeodactylum tricornutum culture* SEM 90. SEM feodaktilum netretiran #2ovoid/Darja-21-10-2021 - 10 veliko krogel dolgo na plosci

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with LB Lennox LB broth (ref. L3022, Sigma Aldrich, USA. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

Figure *Phaeodactylum tricornutum culture* SEM 91. SEM feodaktilum netretiran #2ovoid/Darja-21-10-2021 - 10 veliko krogel dolgo na plosci

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with LB Lennox LB broth (ref. L3022, Sigma Aldrich, USA. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

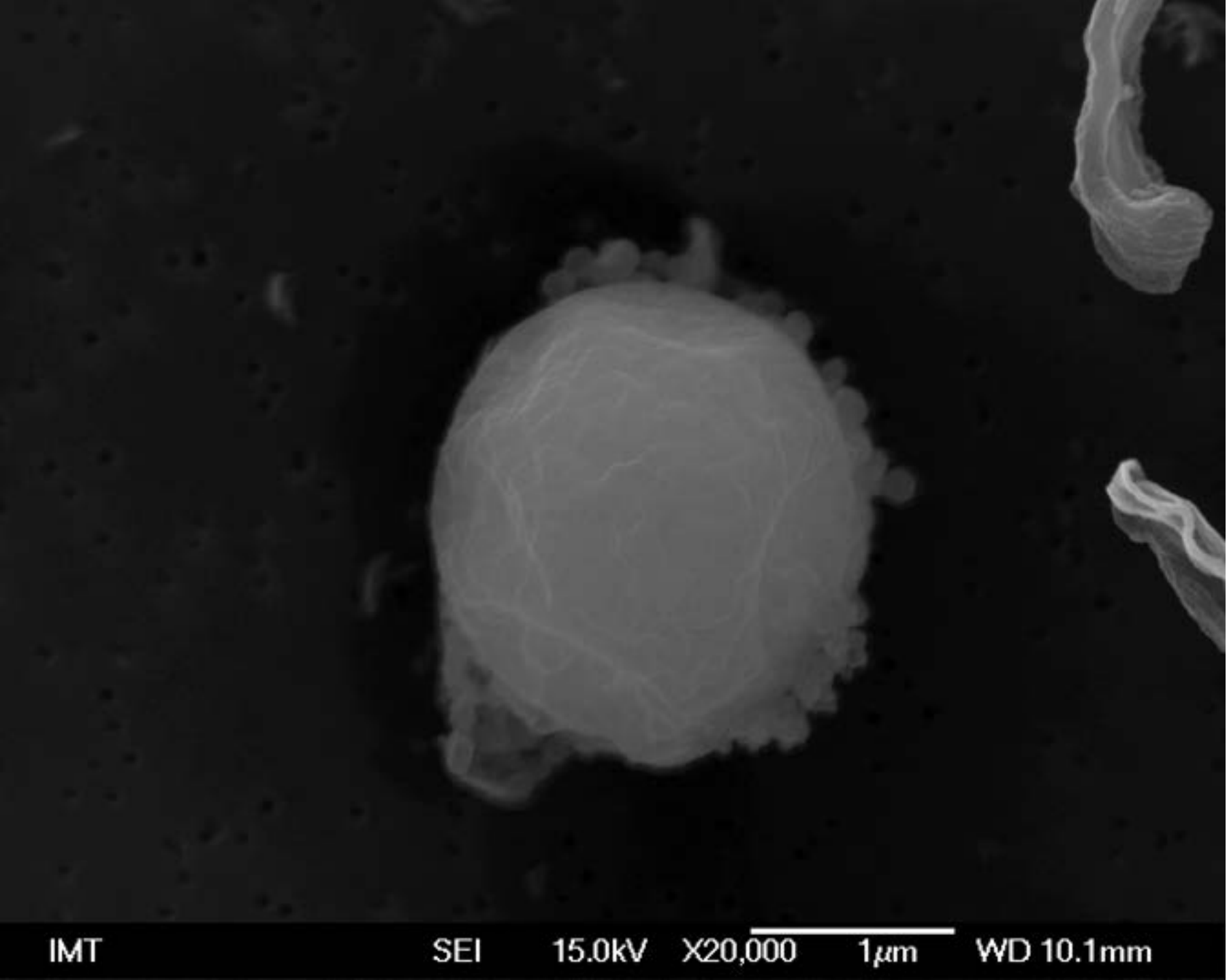

Figure *Phaeodactylum tricornutum culture* SEM 92. SEM feodaktilum netretiran #2ovoid/Darja-21-10-2021 - 10 veliko krogel dolgo na plosci

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with LB Lennox LB broth (ref. L3022, Sigma Aldrich, USA. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

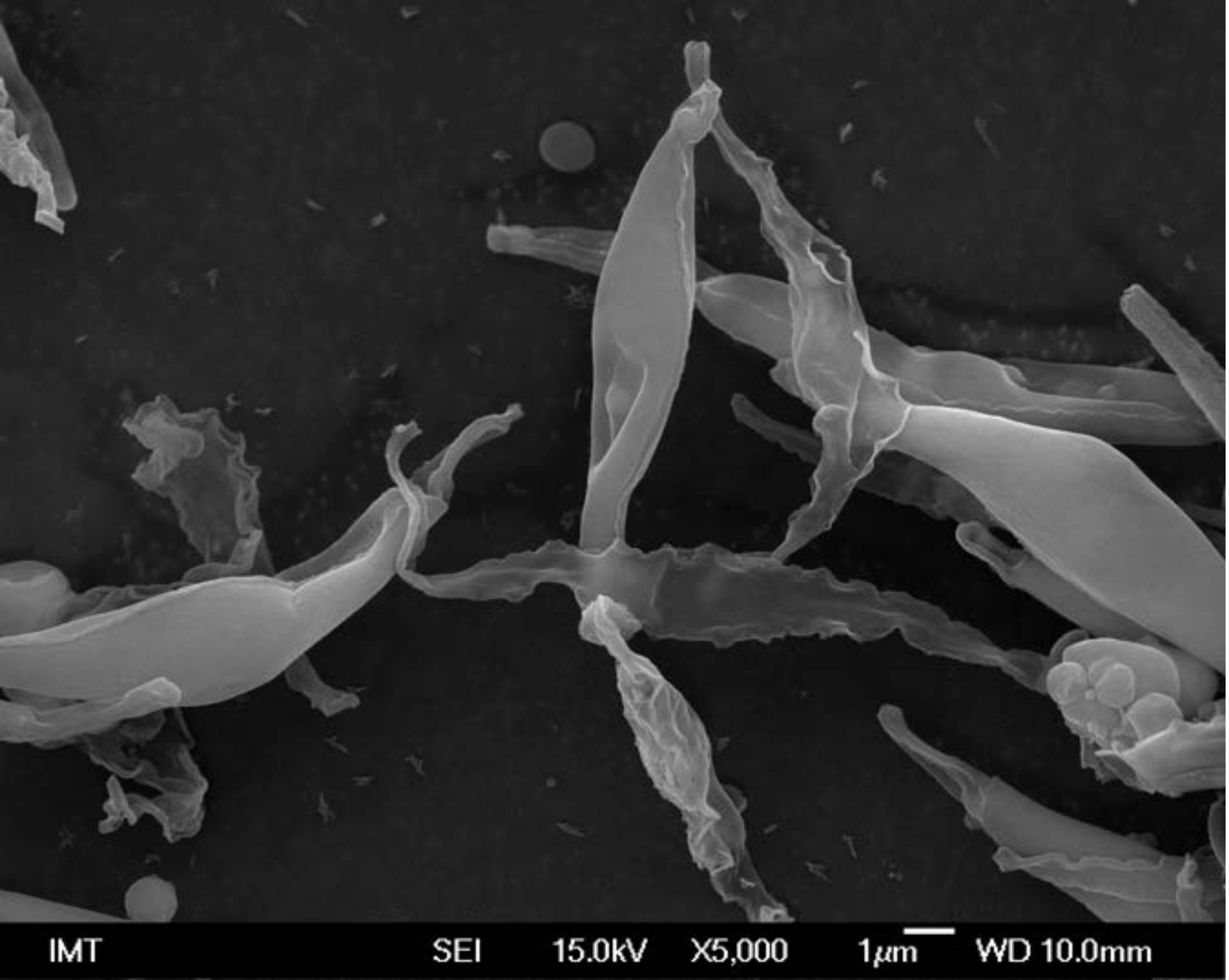

Figure *Phaeodactylum tricornutum culture* SEM 93. SEM feodaktilum netretiran #2ovoid/Darja-21-10-2021 - 10 veliko krogel dolgo na plosci

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with LB Lennox LB broth (ref. L3022, Sigma Aldrich, USA. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 µmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Scanning Electron Microscopy (SEM)

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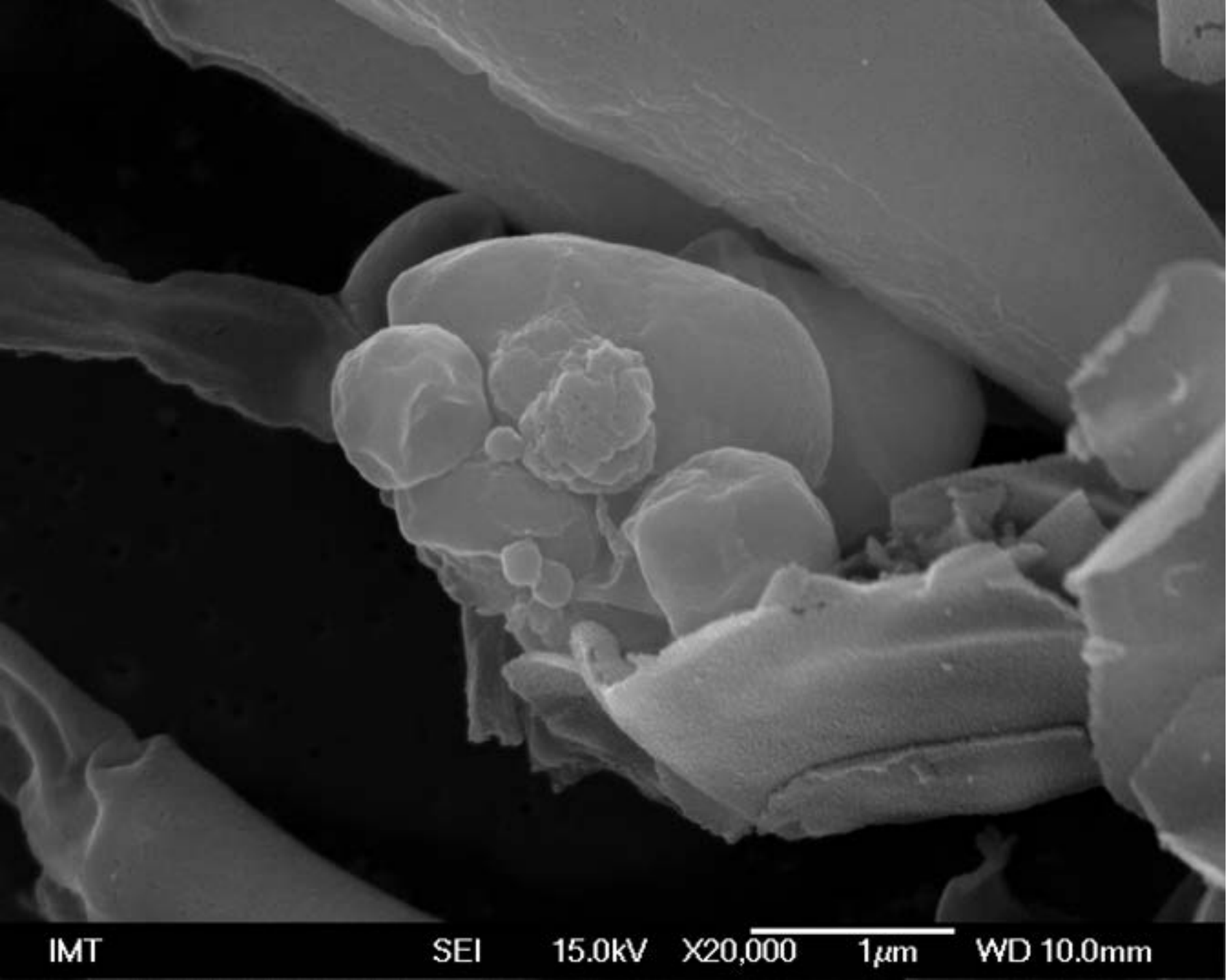

Figure *Phaeodactylum tricornutum culture* SEM 94. SEM feodaktilum netretiran #2ovoid/Darja-21-10-2021 - 10 veliko krogel dolgo na plosci

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with LB Lennox LB broth (ref. L3022, Sigma Aldrich, USA. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 µmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

Figure *Phaeodactylum tricornutum culture* SEM 95. SEM feodaktilum netretiran #2ovoid/Darja-21-10-2021 - 10 veliko krogel dolgo na plosci

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with LB Lennox LB broth (ref. L3022, Sigma Aldrich, USA. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 µmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

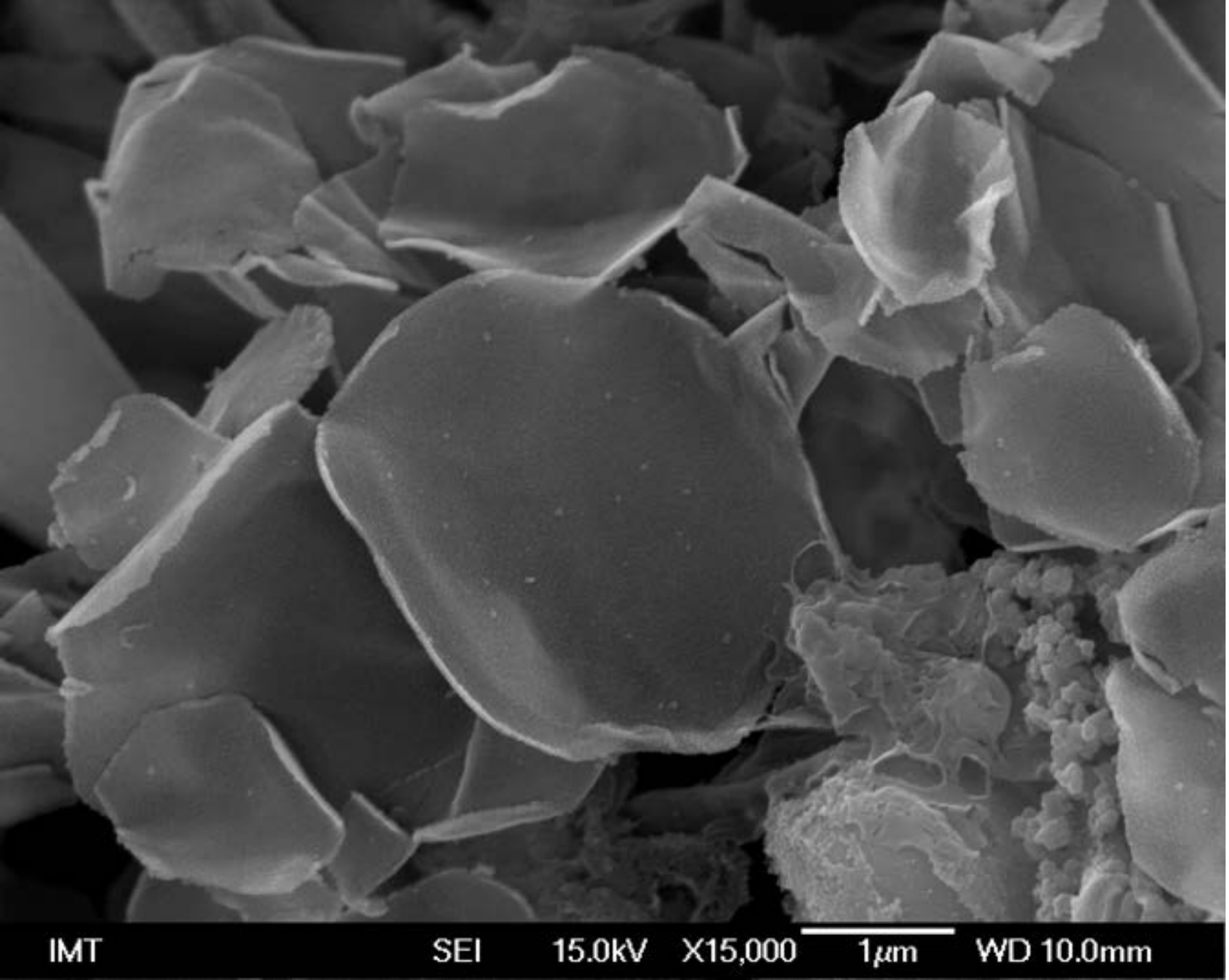

Figure *Phaeodactylum tricornutum culture* SEM 96. SEM feodaktilum netretiran #2ovoid/Darja-21-10-2021

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with LB Lennox LB broth (ref. L3022, Sigma Aldrich, USA. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

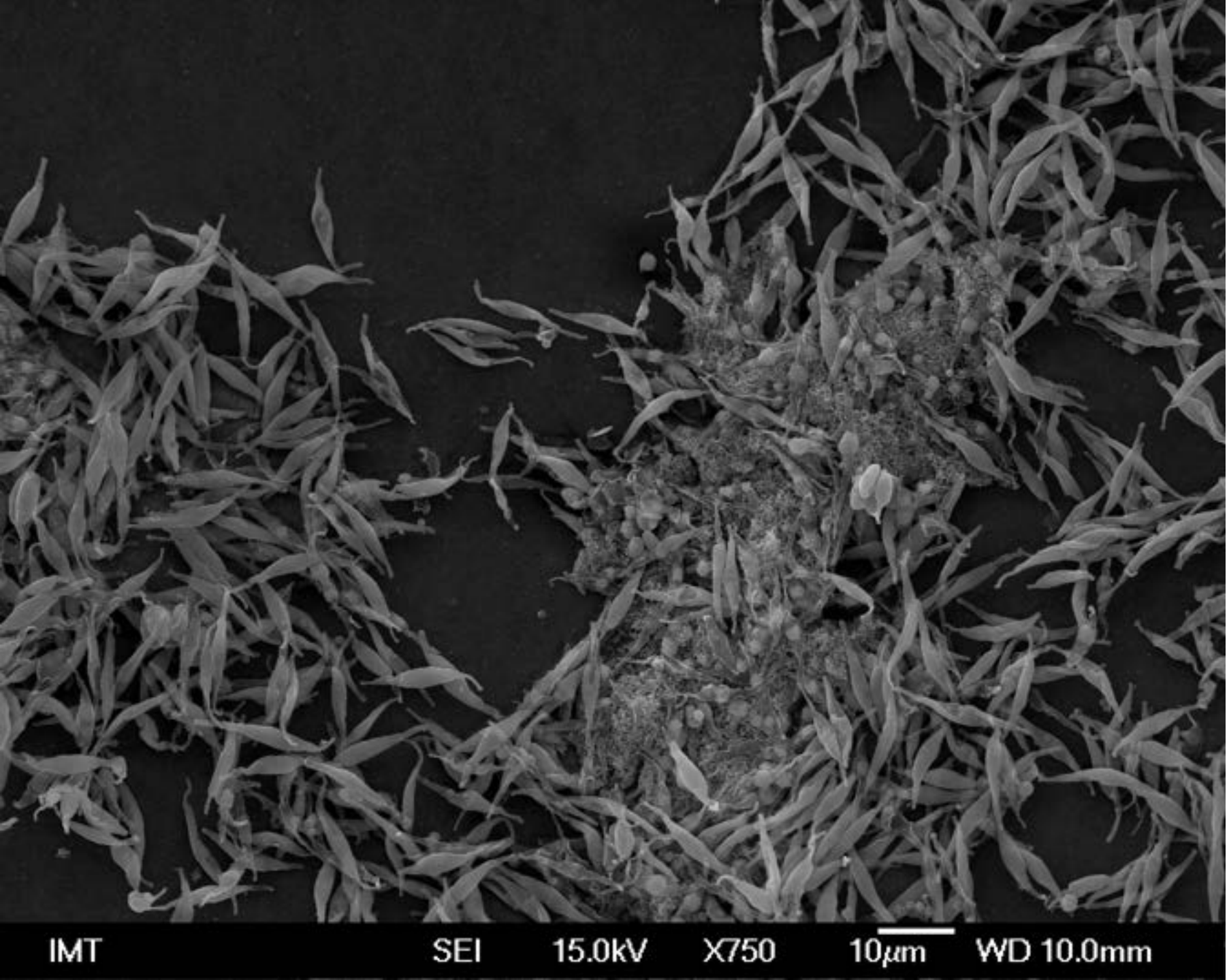

Figure *Phaeodactylum tricornutum culture* SEM 97. SEM feodaktilum netretiran #2ovoid/Darja-21-10-2021 - 10 veliko krogel dolgo na plosci

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with LB Lennox LB broth (ref. L3022, Sigma Aldrich, USA. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 µmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Scanning Electron Microscopy (SEM)

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Figure *Phaeodactylum tricornutum culture* SEM 98. SEM feodaktilum netretiran #2ovoid/Darja-21-10-2021 - 10 veliko krogel dolgo na plosci

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with LB Lennox LB broth (ref. L3022, Sigma Aldrich, USA. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

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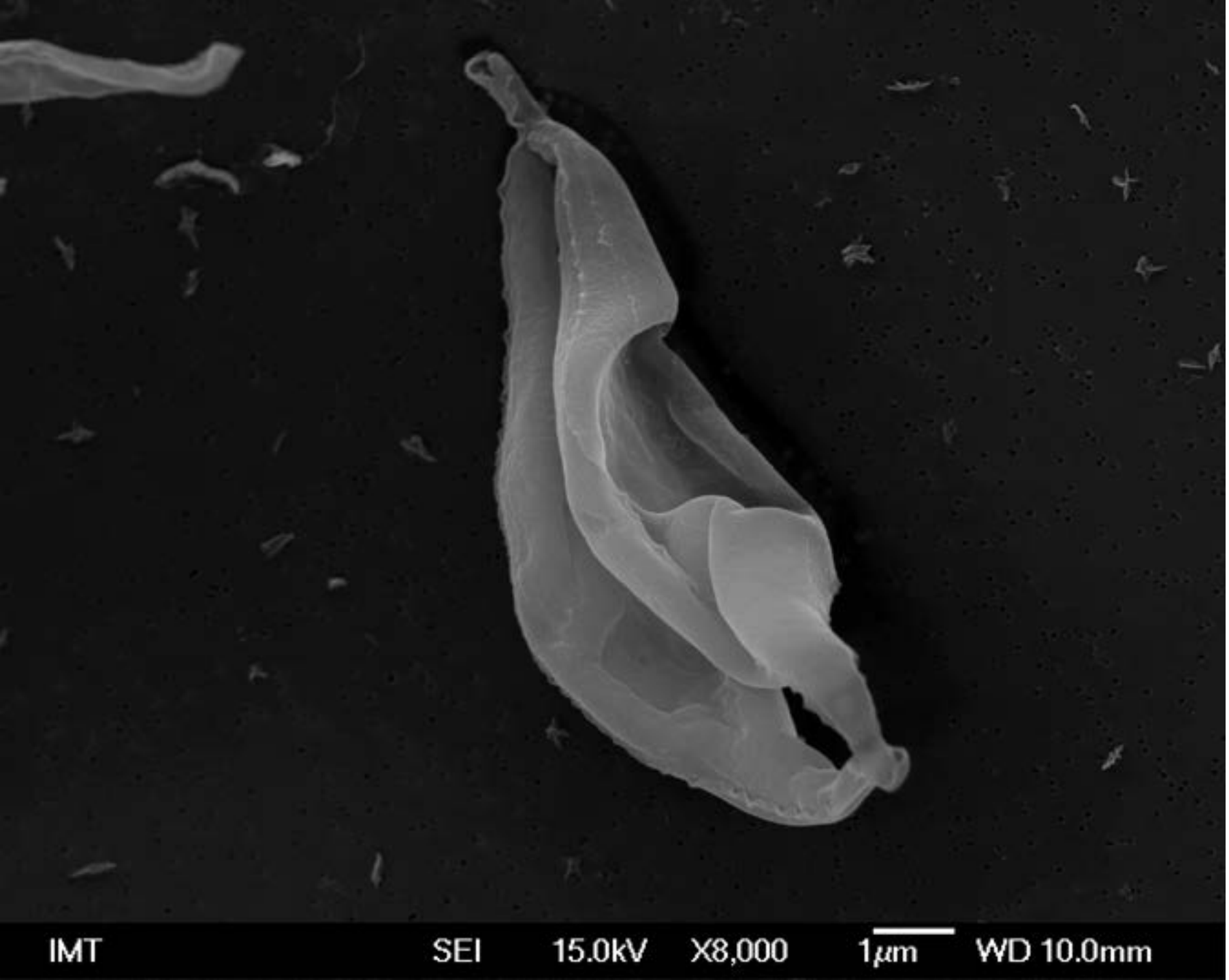

Figure *Phaeodactylum tricornutum culture* SEM 99. SEM feodaktilum netretiran #2ovoid/Darja-21-10-2021 - 10 veliko krogel dolgo na plosci

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with LB Lennox LB broth (ref. L3022, Sigma Aldrich, USA. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 µmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Scanning Electron Microscopy (SEM)

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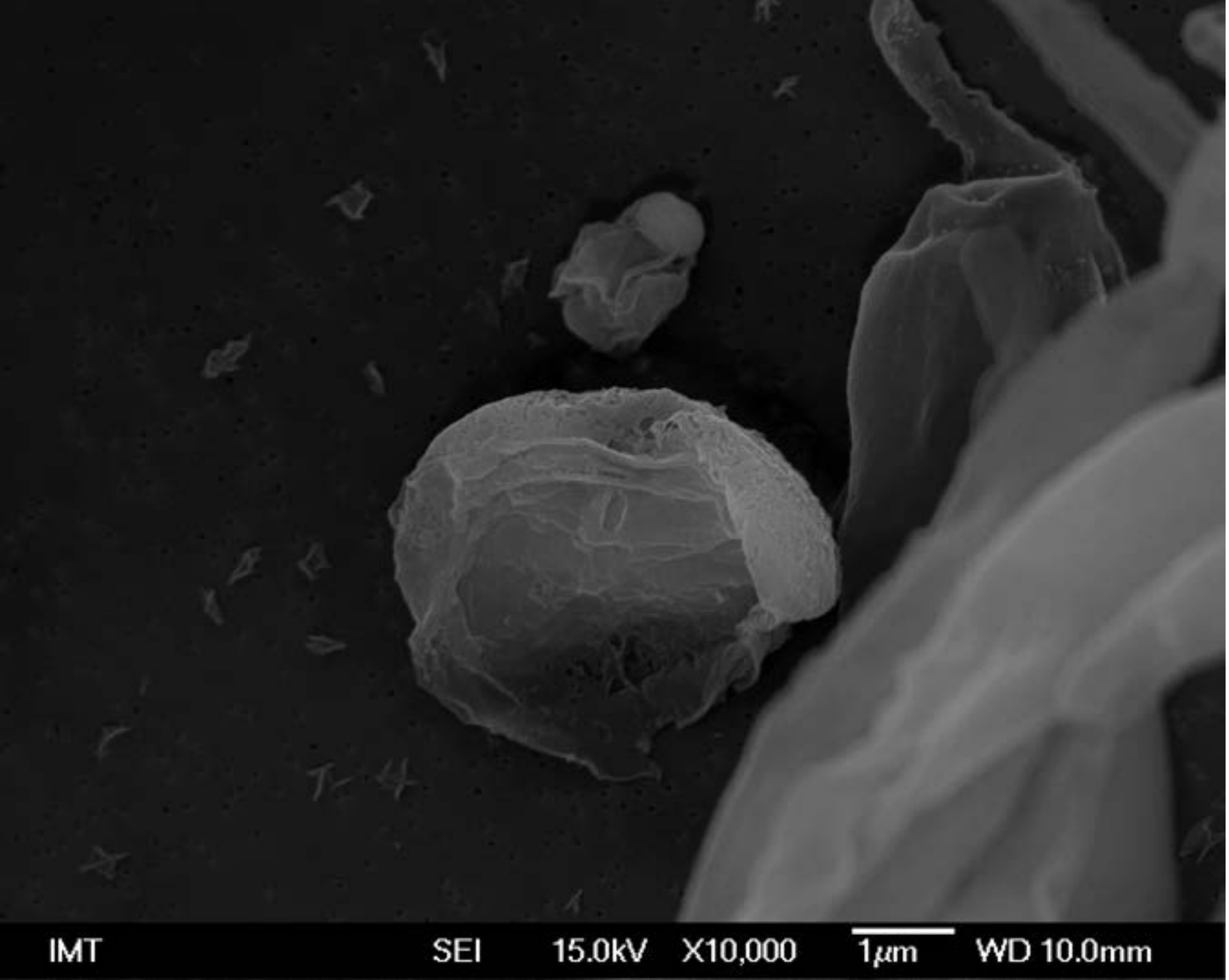

Figure *Phaeodactylum tricornutum culture* SEM 100. SEM feodaktilum netretiran #2ovoid/Darja-21-10-2021- 10 veliko krogel dolgo na plosci

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with LB Lennox LB broth (ref. L3022, Sigma Aldrich, USA. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

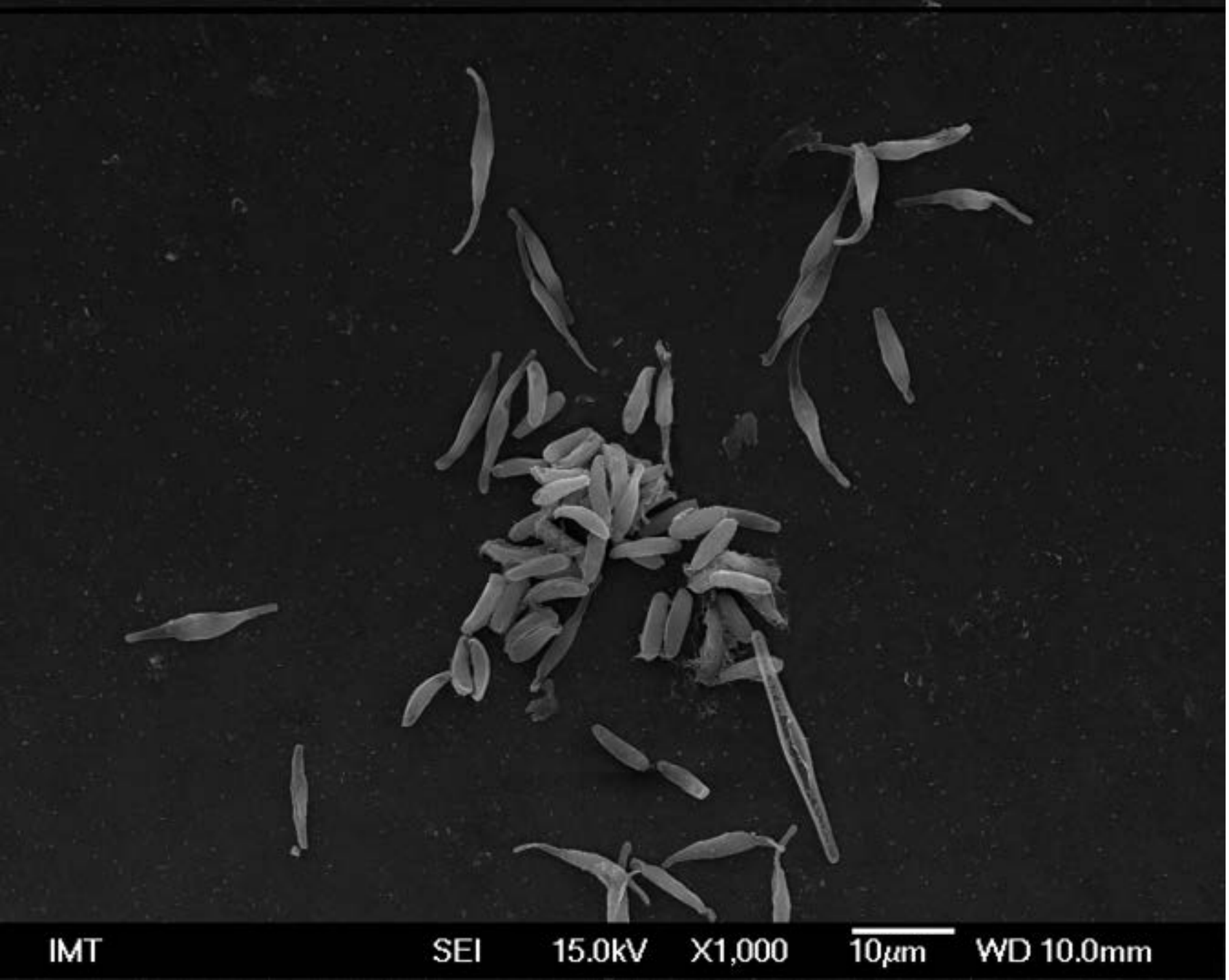

Figure *Phaeodactylum tricornutum culture* SEM 101. SEM feodaktilum netretiran #2ovoid/Darja-21-10-2021- 10 veliko krogel dolgo na plosci

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with LB Lennox LB broth (ref. L3022, Sigma Aldrich, USA. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Scanning Electron Microscopy (SEM)

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Figure *Phaeodactylum tricornutum culture* SEM 102. SEM feodaktilum netretiran #2ovoid/Darja-21-10-2021 - 11 ovalne celice s trdnega gojisca

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with LB Lennox LB broth (ref. L3022, Sigma Aldrich, USA. Culture was grown on agar at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

Figure *Phaeodactylum tricornutum culture* SEM 103. SEM feodaktilum netretiran #2ovoid/Darja-21-10-2021 - 11 ovalne celice s trdnega gojisca

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with LB Lennox LB broth (ref. L3022, Sigma Aldrich, USA. Culture was grown on agar at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

Figure *Phaeodactylum tricornutum culture* SEM 104. SEM feodaktilum netretiran #2ovoid/Darja-21-10-2021 - 11 ovalne celice s trdnega gojisca

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with LB Lennox LB broth (ref. L3022, Sigma Aldrich, USA. Culture was grown on agar at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle.

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Figure *Phaeodactylum tricornutum culture* SEM 105. SEM feodaktilum netretiran #2ovoid/Darja-21-10-2021 - 11 ovalne celice s trdnega gojisca

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with LB Lennox LB broth (ref. L3022, Sigma Aldrich, USA. Culture was grown on agar at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle.

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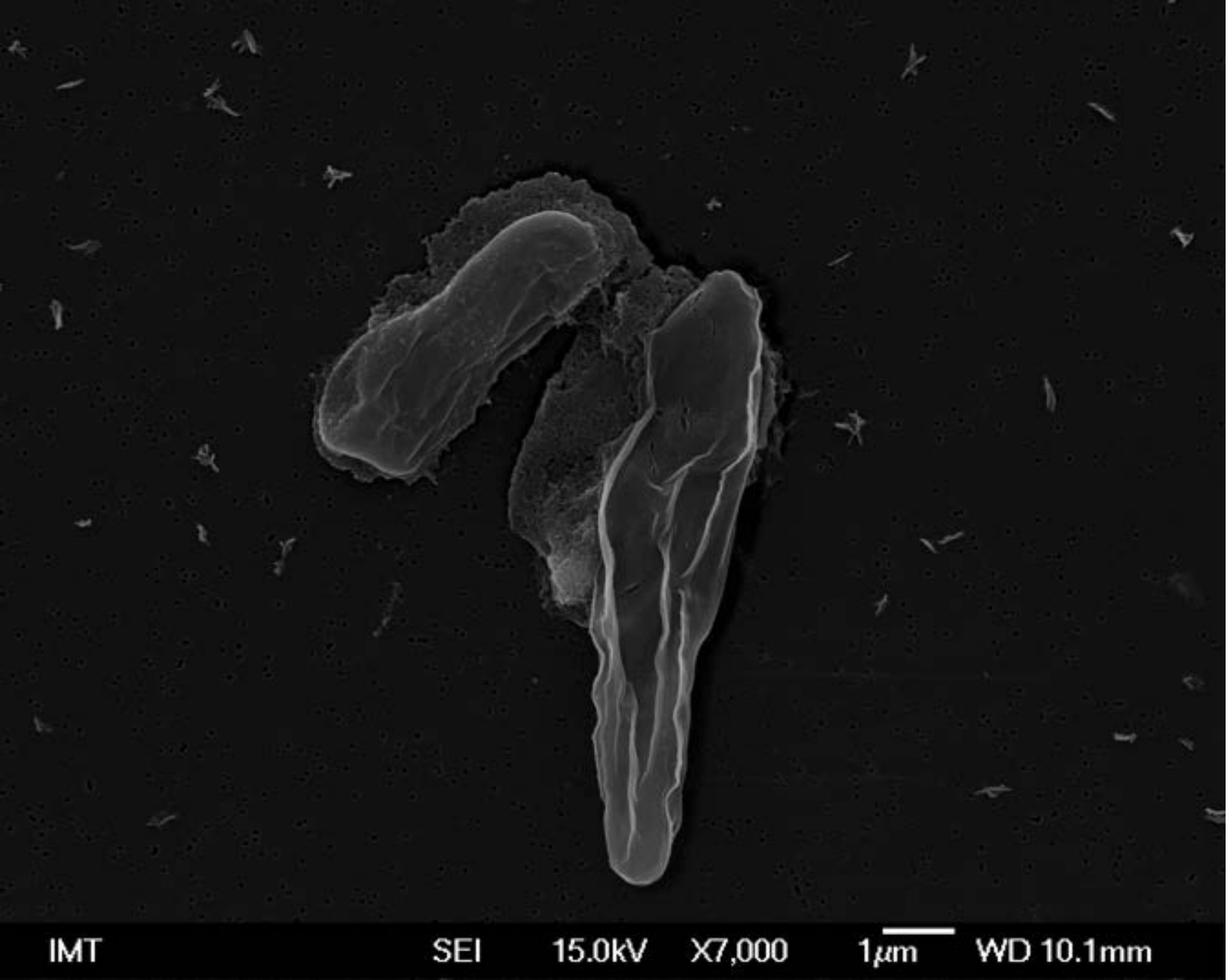

Figure *Phaeodactylum tricornutum culture* SEM 106. SEM feodaktilum netretiran #2ovoid/Darja-21-10-2021 - 11 ovalne celice s trdnega gojisca

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with LB Lennox LB broth (ref. L3022, Sigma Aldrich, USA. Culture was grown on agar at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle.

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Figure *Phaeodactylum tricornutum culture* SEM 107. SEM feodaktilum netretiran #2ovoid/Darja-21-10-2021 - 11 ovalne celice s trdnega gojisca

Cultivation of the algae

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Figure *Phaeodactylum tricornutum culture* SEM 108. SEM feodaktilum netretiran #2ovoid/Darja-21-10-2021 - 11 ovalne celice s trdnega gojisca

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with LB Lennox LB broth (ref. L3022, Sigma Aldrich, USA. Culture was grown on agar at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle.

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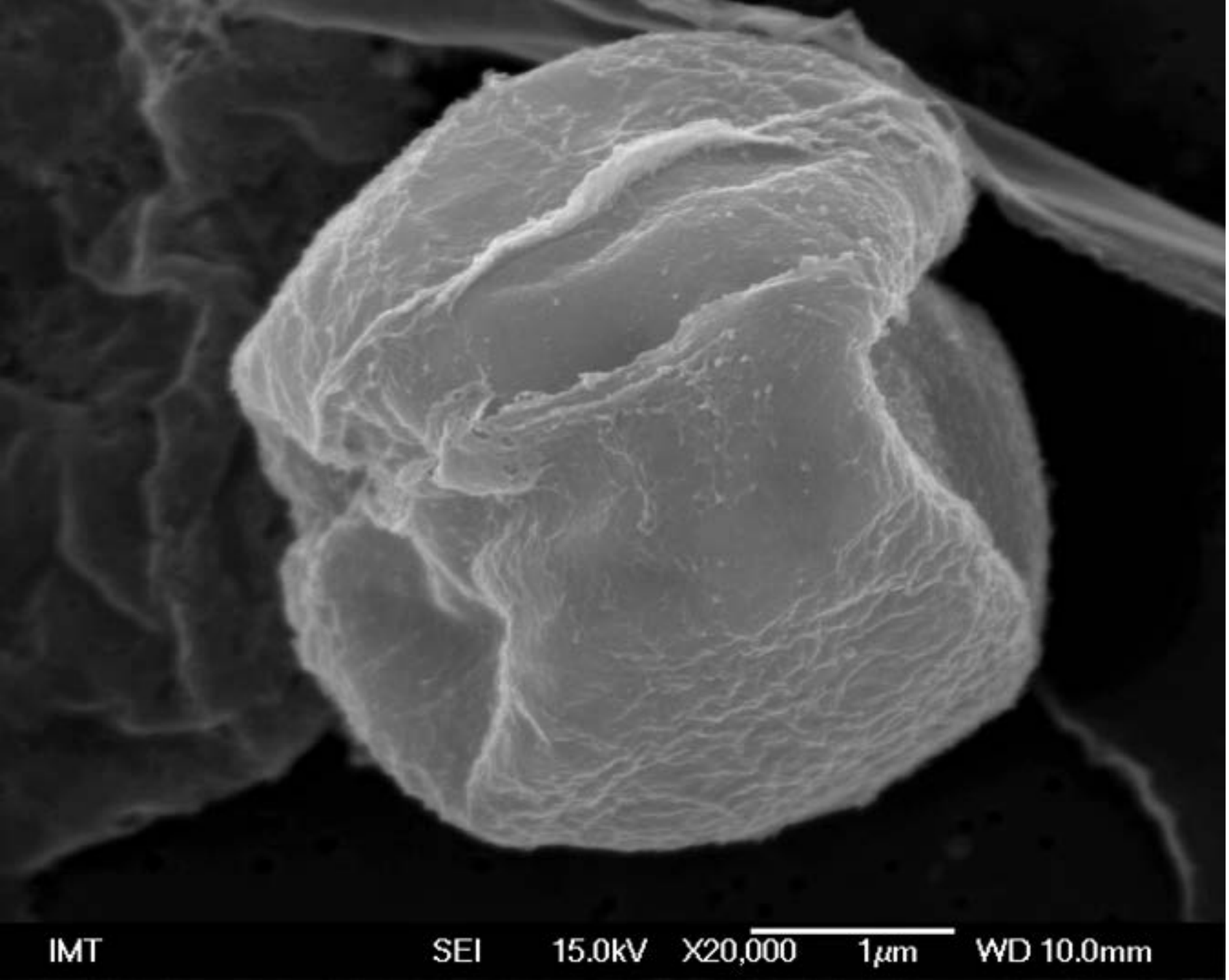

Figure *Phaeodactylum tricornutum culture* SEM 109. SEM feodaktilum netretiran #2ovoid/Darja-21-10-2021 - 11 ovalne celice s trdnega gojisca

Cultivation of the algae

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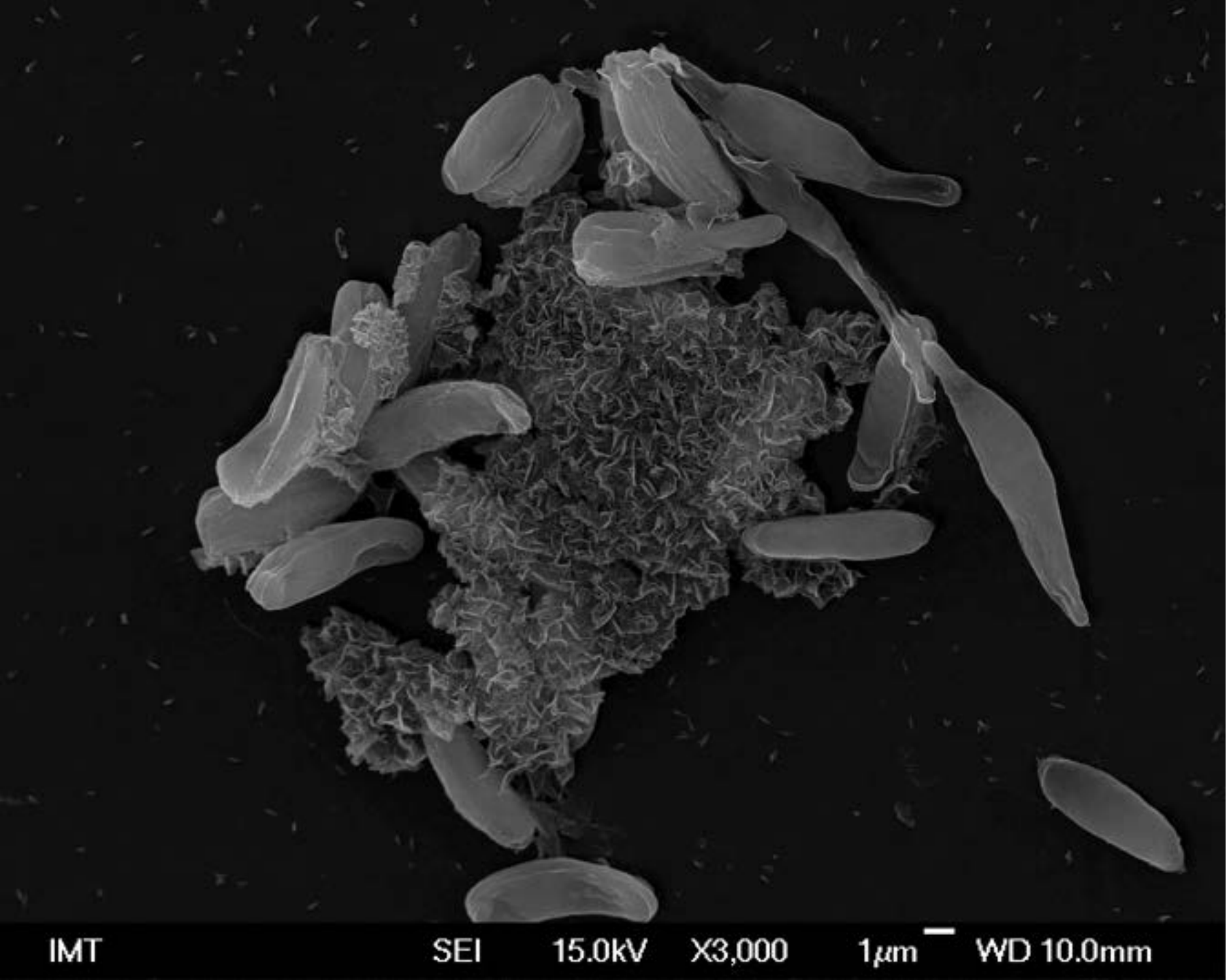

Figure *Phaeodactylum tricornutum culture* SEM 110. SEM feodaktilum netretiran #2ovoid/Darja-21-10-2021 - 11 ovalne celice s trdnega gojisca

Cultivation of the algae

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Scanning Electron Microscopy (SEM)

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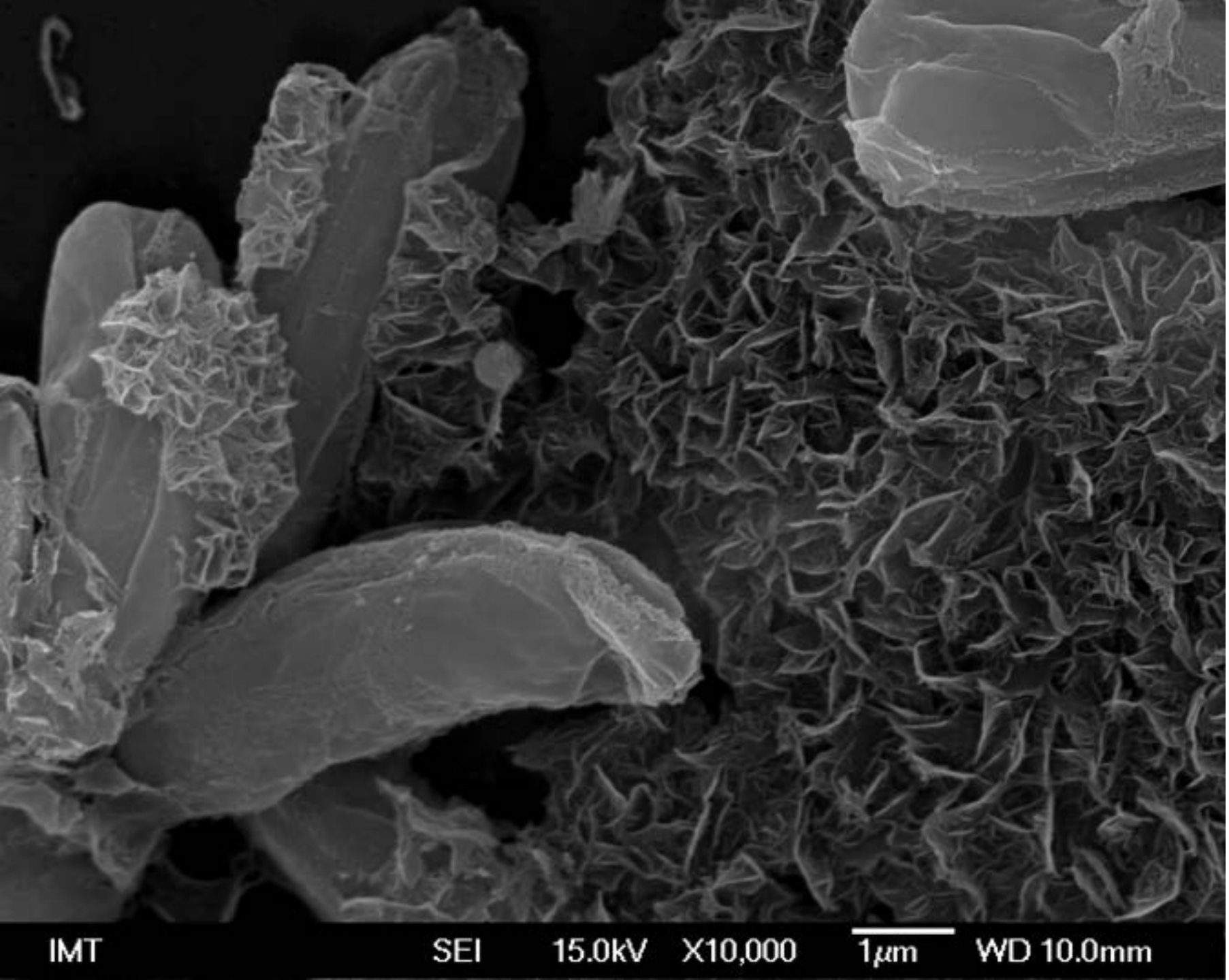

Figure *Phaeodactylum tricornutum culture* SEM 111. SEM feodaktilum netretiran #2ovoid/Darja-21-10-2021 - 11 ovalne celice s trdnega gojisca

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with LB Lennox LB broth (ref. L3022, Sigma Aldrich, USA. Culture was grown on agar at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

Figure *Phaeodactylum tricornutum culture* SEM 112. SEM feodaktilum netretiran #2ovoid/Darja-21-10-2021 - 11 ovalne celice s trdnega gojisca

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with LB Lennox LB broth (ref. L3022, Sigma Aldrich, USA. Culture was grown on agar at 20 °C and 20 % illumination (approximately 250 µmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

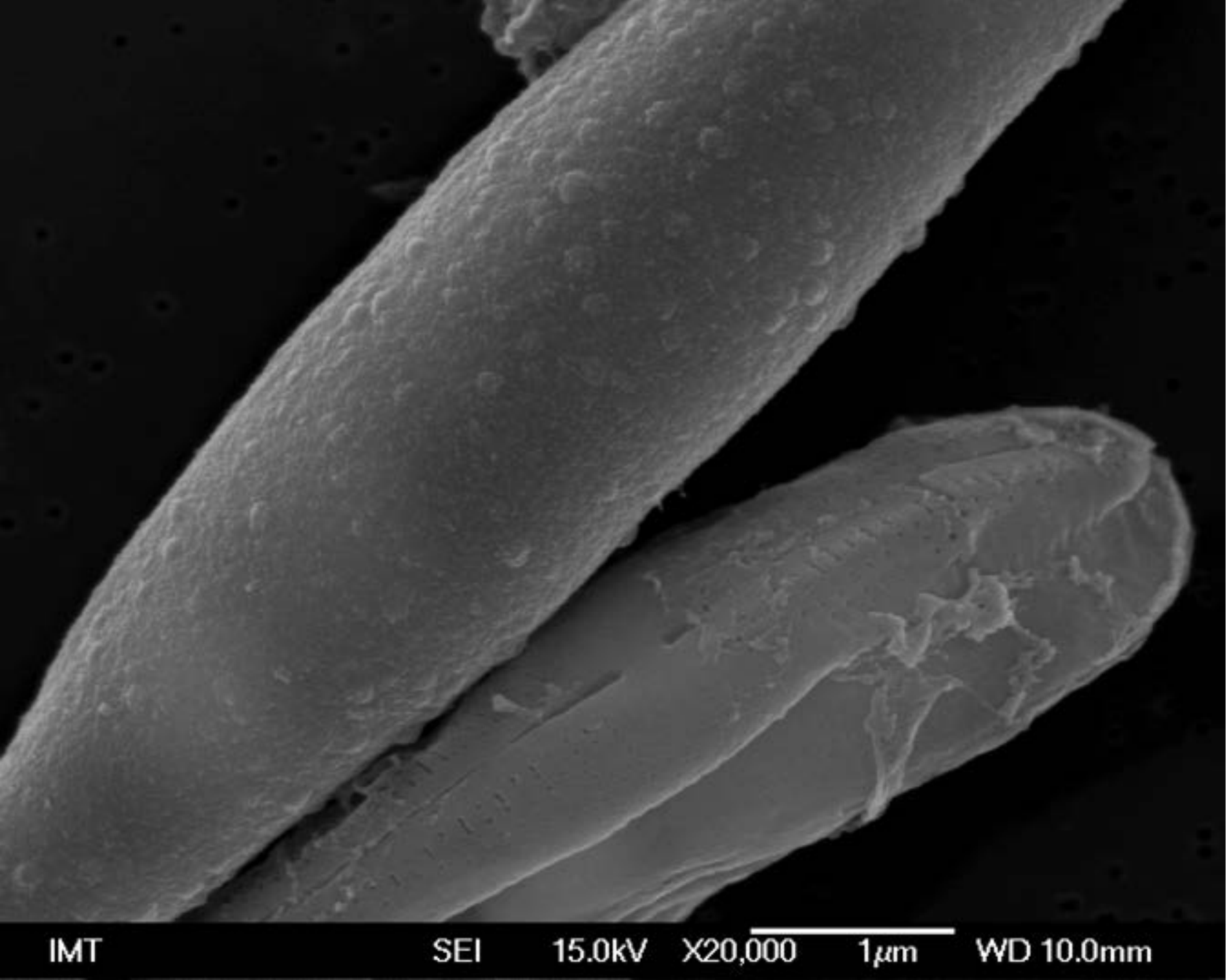

Figure *Phaeodactylum tricornutum culture* SEM 113. SEM feodaktilum netretiran #2ovoid/Darja-21-10-2021 - 11 ovalne celice s trdnega gojisca

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with LB Lennox LB broth (ref. L3022, Sigma Aldrich, USA. Culture was grown on agar at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

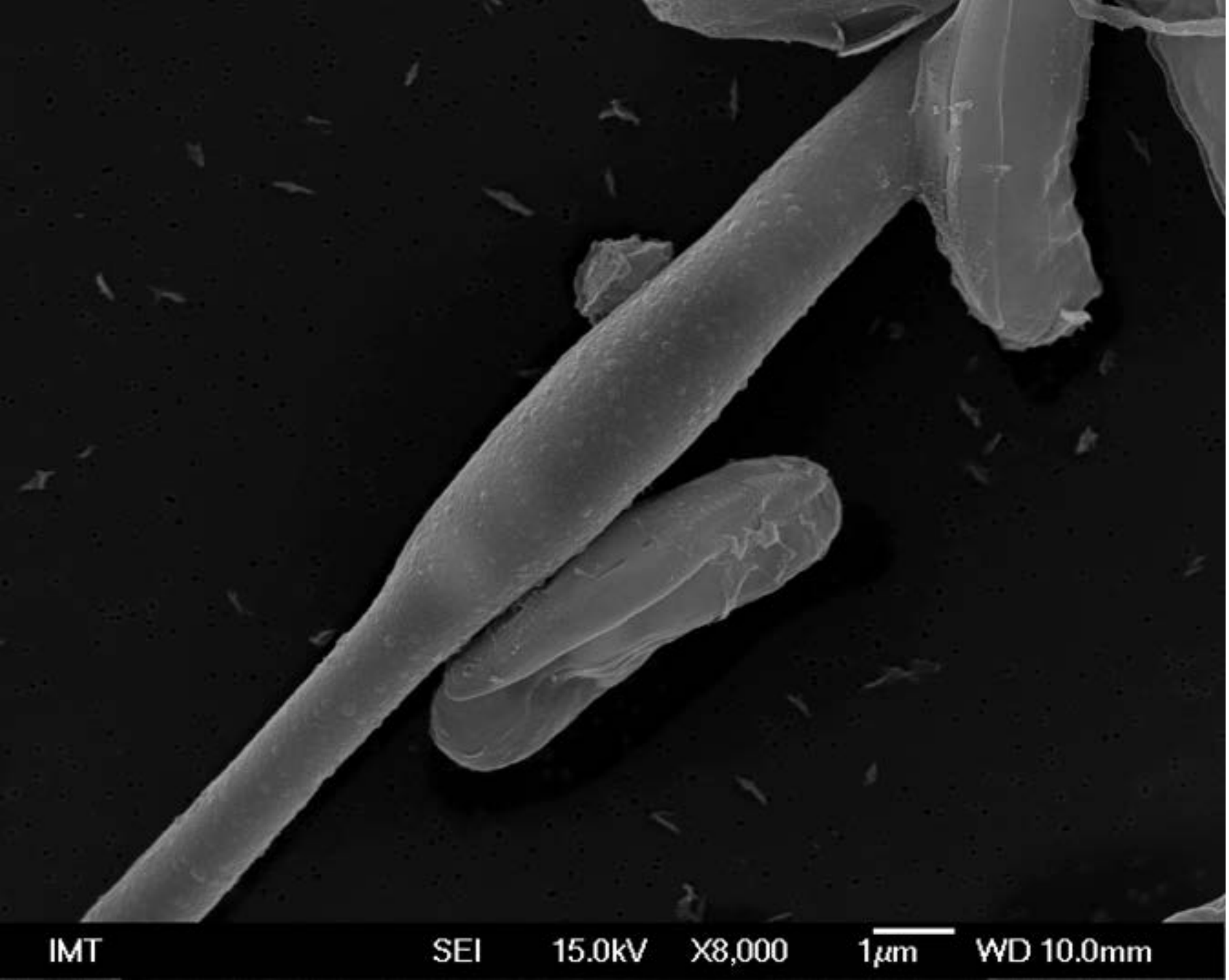

Figure *Phaeodactylum tricornutum culture* SEM 114. SEM feodaktilum netretiran #2ovoid/Darja-21-10-2021 - 11 ovalne celice s trdnega gojisca

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with LB Lennox LB broth (ref. L3022, Sigma Aldrich, USA. Culture was grown on agar at 20 °C and 20 % illumination (approximately 250 µmol/m2s) with a 14-hour light / 10-hour dark cycle.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

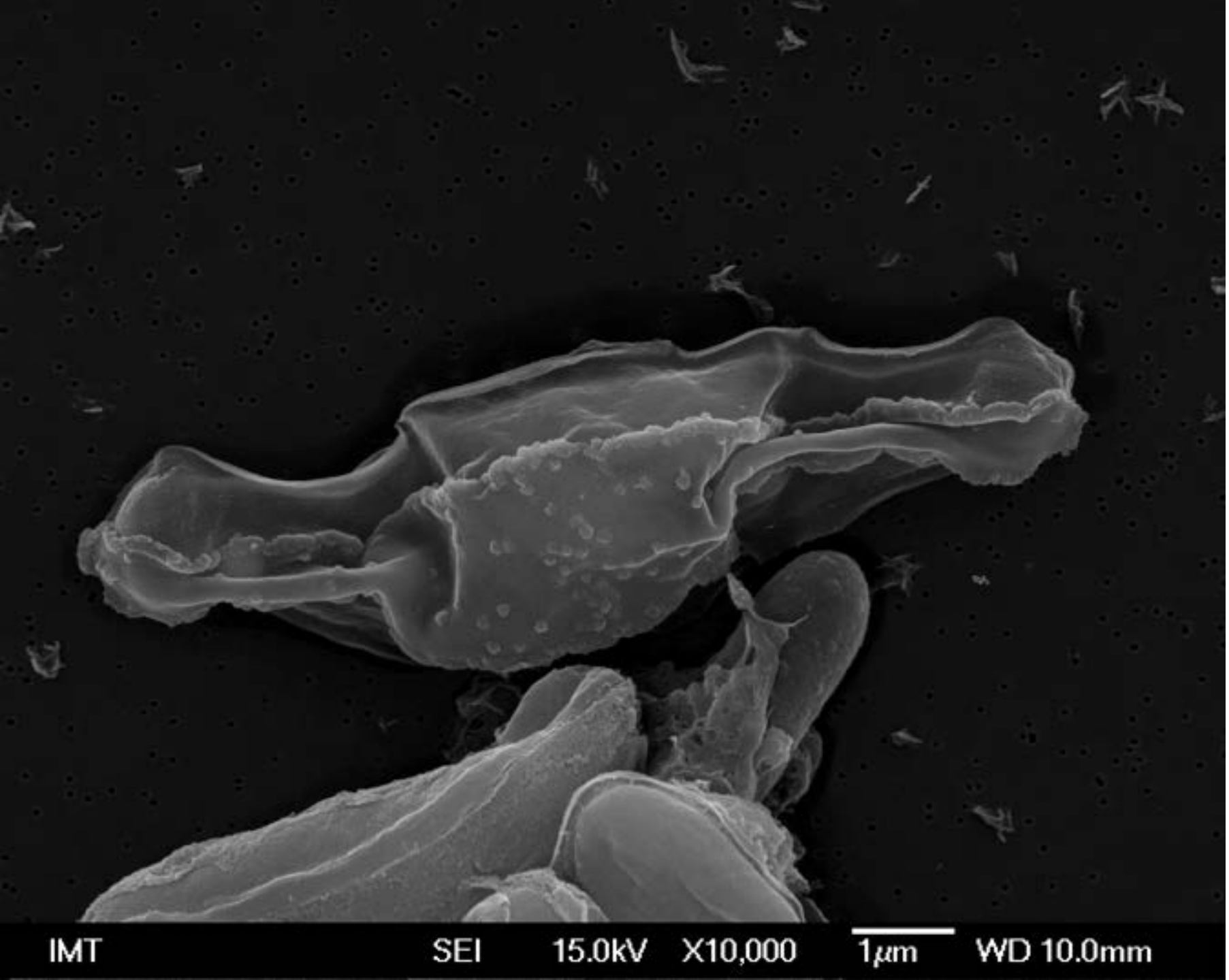

Figure *Phaeodactylum tricornutum culture* SEM 115. SEM feodaktilum netretiran #2ovoid/Darja-21-10-2021 - 11 ovalne celice s trdnega gojisca

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with LB Lennox LB broth (ref. L3022, Sigma Aldrich, USA. Culture was grown on agar at 20 °C and 20 % illumination (approximately 250 µmol/m2s) with a 14-hour light / 10-hour dark cycle.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

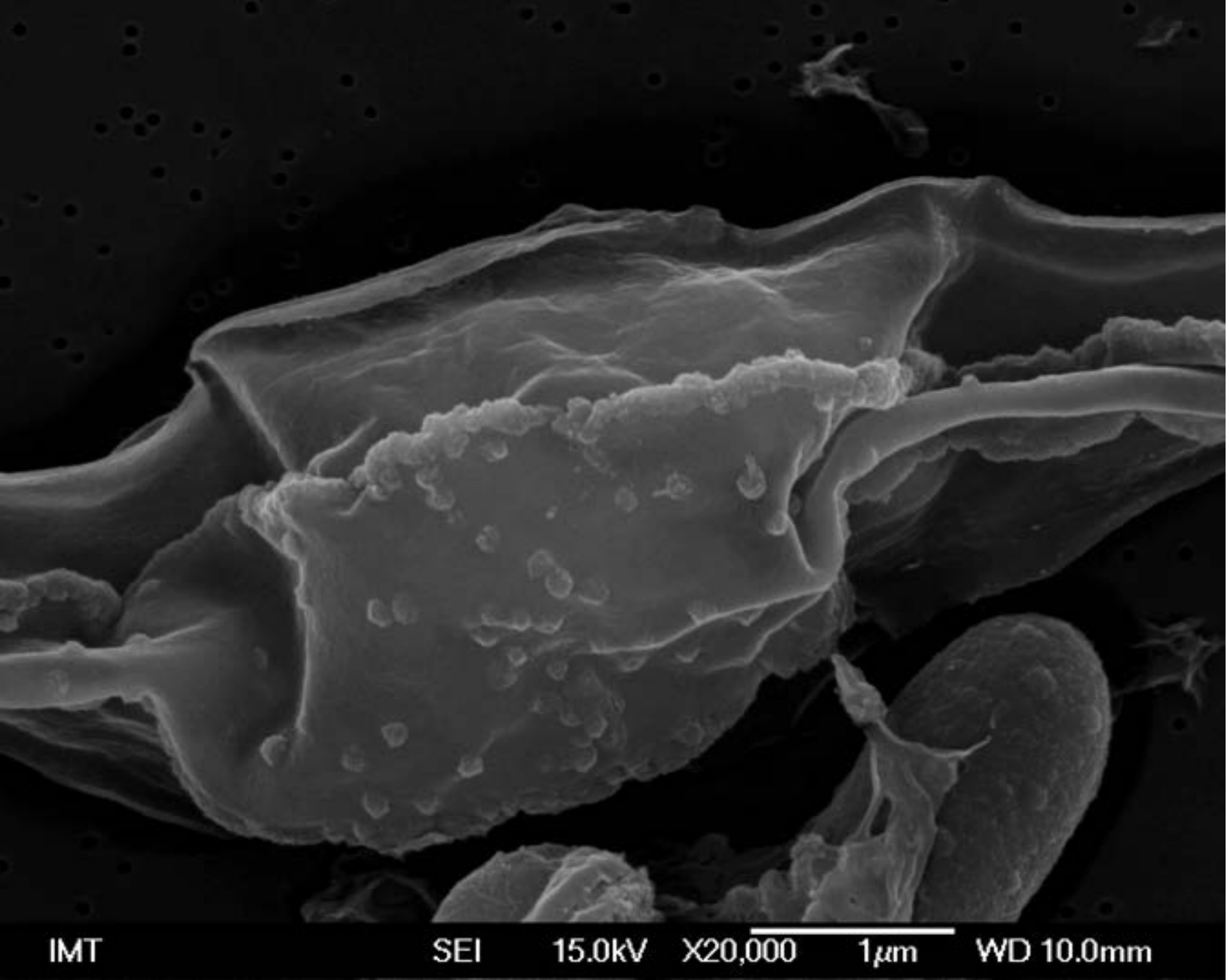

Figure *Phaeodactylum tricornutum culture* SEM 116. SEM feodaktilum netretiran #2ovoid/Darja-21-10-2021 - 11 ovalne celice s trdnega gojisca

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with LB Lennox LB broth (ref. L3022, Sigma Aldrich, USA. Culture was grown on agar at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

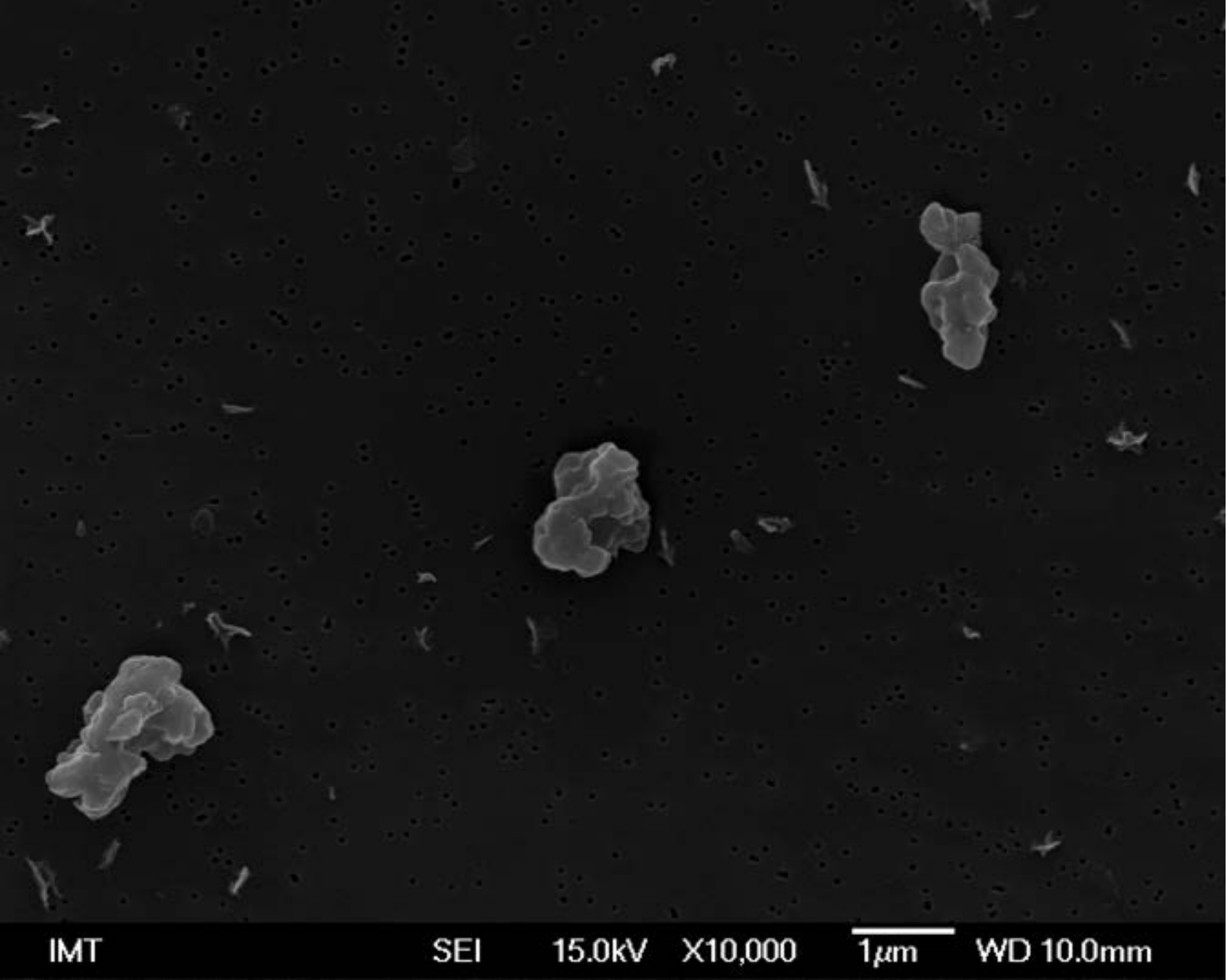

Figure *Phaeodactylum tricornutum culture* SEM 117. SEM feodaktilum netretiran #2ovoid/Darja-21-10-2021 - 11 ovalne celice s trdnega gojisca

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with LB Lennox LB broth (ref. L3022, Sigma Aldrich, USA. Culture was grown on agar at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

Figure *Phaeodactylum tricornutum culture* SEM 118. SEM feodaktilum netretiran #2ovoid/Darja-21-10-2021 - 11 ovalne celice s trdnega gojisca

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with LB Lennox LB broth (ref. L3022, Sigma Aldrich, USA. Culture was grown on agar at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle.

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Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

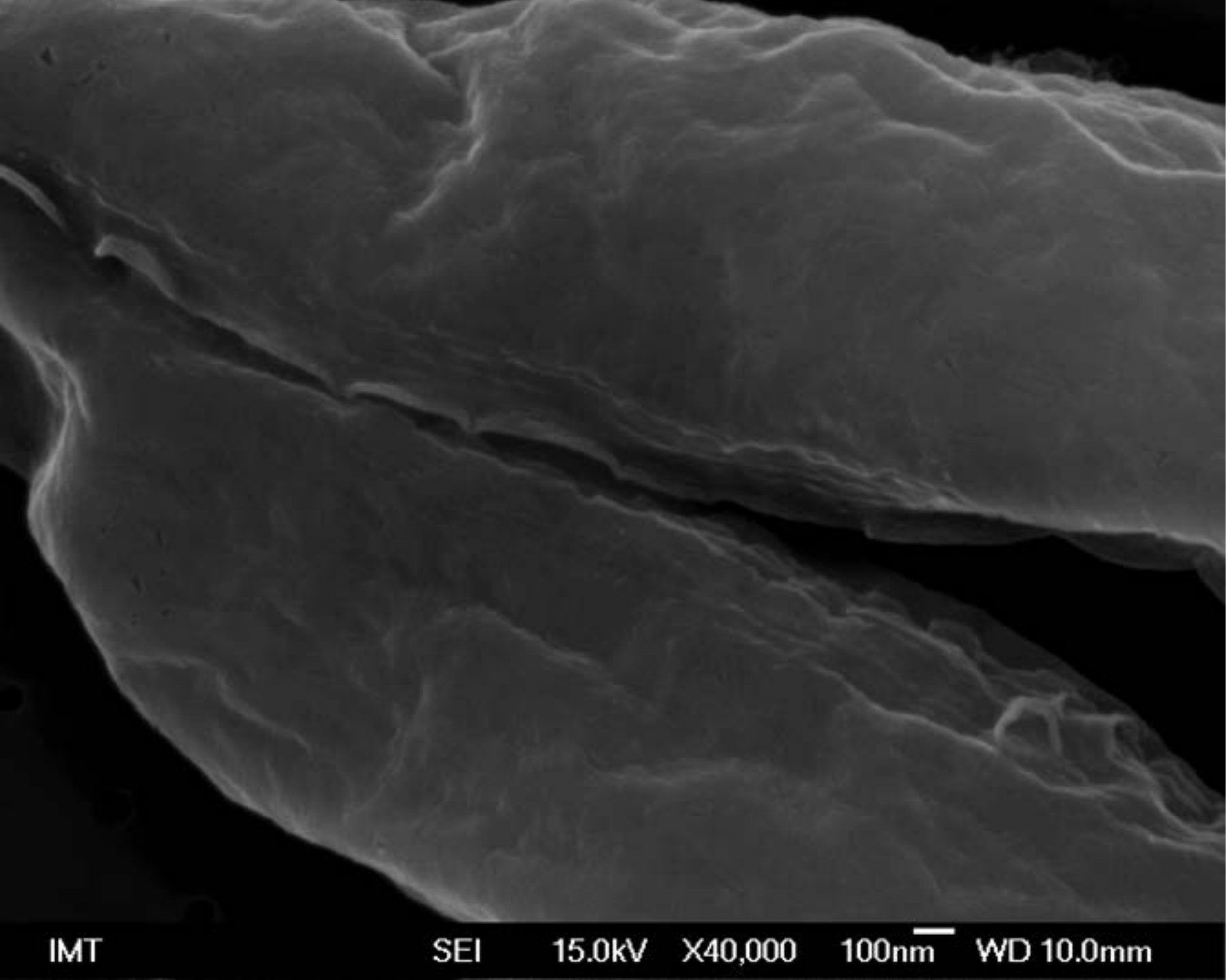

Figure *Phaeodactylum tricornutum culture* SEM 119. SEM feodaktilum netretiran #2ovoid/Darja-21-10-2021 - 11 ovalne celice s trdnega gojisca

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with LB Lennox LB broth (ref. L3022, Sigma Aldrich, USA. Culture was grown on agar at 20 °C and 20 % illumination (approximately 250 µmol/m2s) with a 14-hour light / 10-hour dark cycle.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

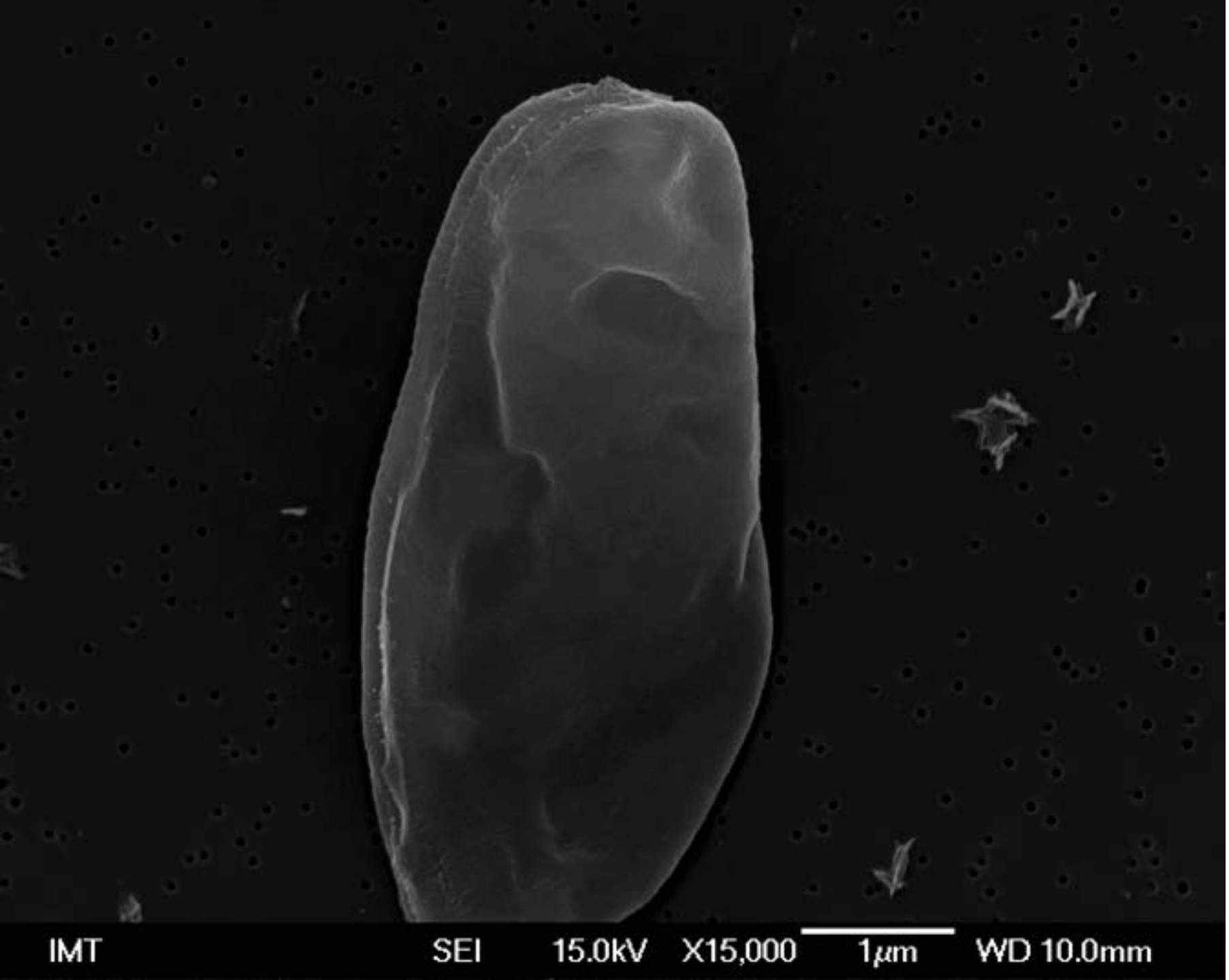

Figure *Phaeodactylum tricornutum culture* SEM 120. SEM feodaktilum netretiran #2ovoid/Darja-21-10-2021 - 11 ovalne celice s trdnega gojisca

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with LB Lennox LB broth (ref. L3022, Sigma Aldrich, USA. Culture was grown on agar at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

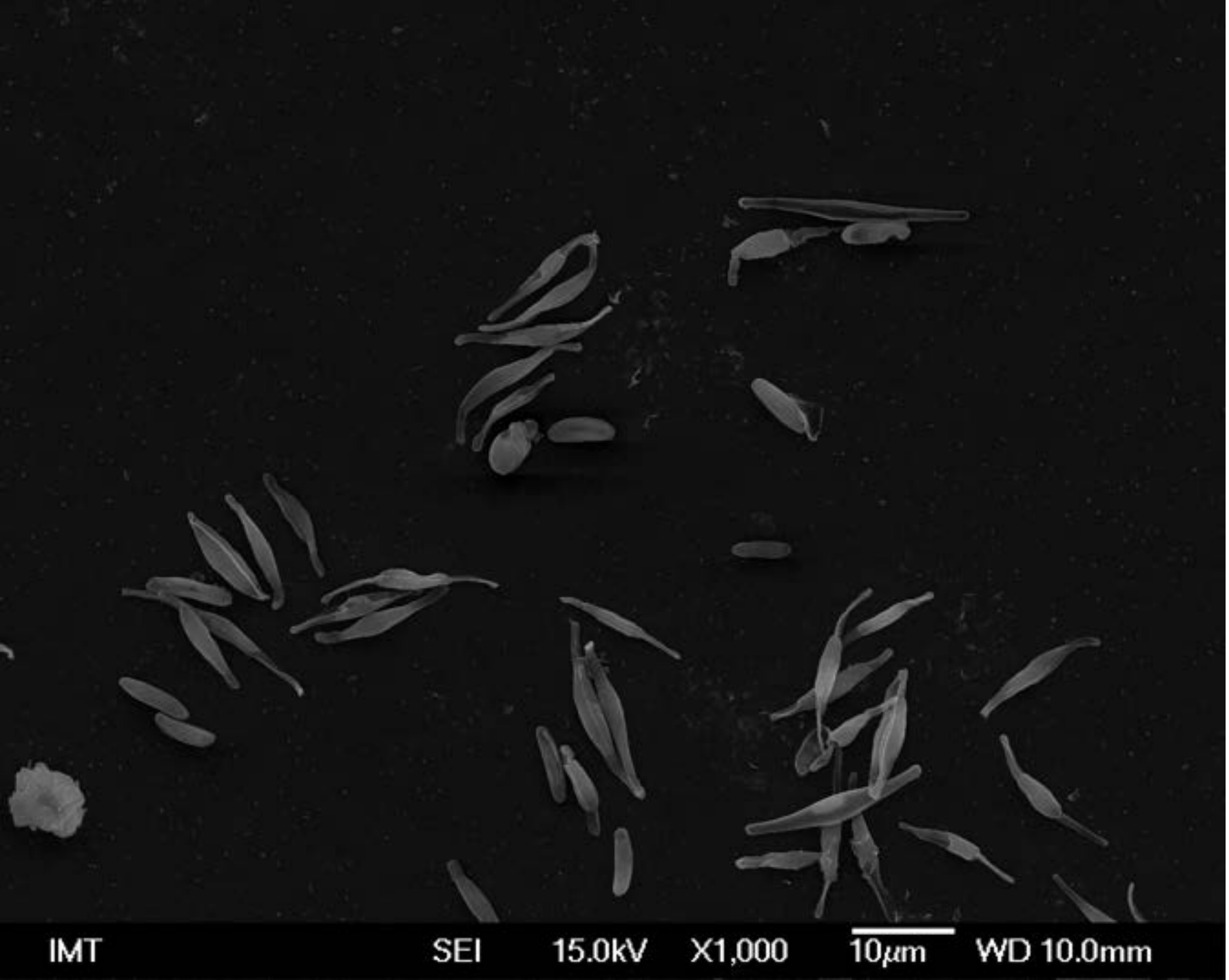

Figure *Phaeodactylum tricornutum culture* SEM 121. SEM feodaktilum netretiran #2ovoid/Darja-21-10-2021 - 11 ovalne celice s trdnega gojisca

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with LB Lennox LB broth (ref. L3022, Sigma Aldrich, USA. Culture was grown on agar at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

Figure *Phaeodactylum tricornutum culture* SEM 122. SEM feodaktilum netretiran #2ovoid/Darja-21-10-2021 - 11 ovalne celice s trdnega gojisca

Cultivation of the algae
Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with LB Lennox LB broth (ref. L3022, Sigma Aldrich, USA. Culture was grown on agar at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle.
Scanning Electron Microscopy (SEM)
Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

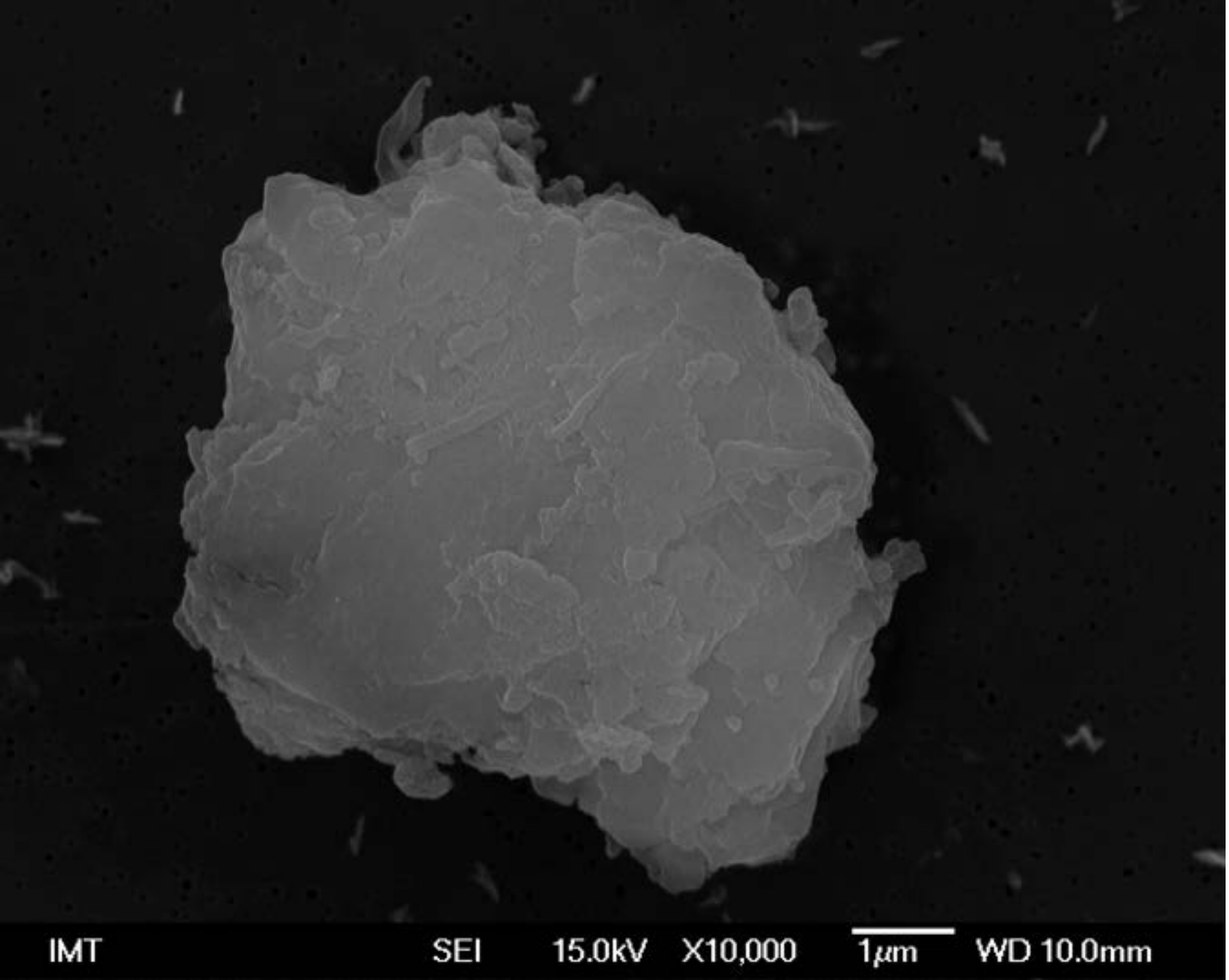

Figure *Phaeodactylum tricornutum culture* SEM 123. SEM feodaktilum netretiran #2ovoid/Darja-21-10-2021 - 11 ovalne celice s trdnega gojisca

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with LB Lennox LB broth (ref. L3022, Sigma Aldrich, USA. Culture was grown on agar at 20 °C and 20 % illumination (approximately 250 µmol/m2s) with a 14-hour light / 10-hour dark cycle.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

Figure *Phaeodactylum tricornutum culture* SEM 124. SEM feodaktilum netretiran #2ovoid/Darja-21-10-2021 - 11 ovalne celice s trdnega gojisca

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with LB Lennox LB broth (ref. L3022, Sigma Aldrich, USA. Culture was grown on agar at 20 °C and 20 % illumination (approximately 250 µmol/m2s) with a 14-hour light / 10-hour dark cycle.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

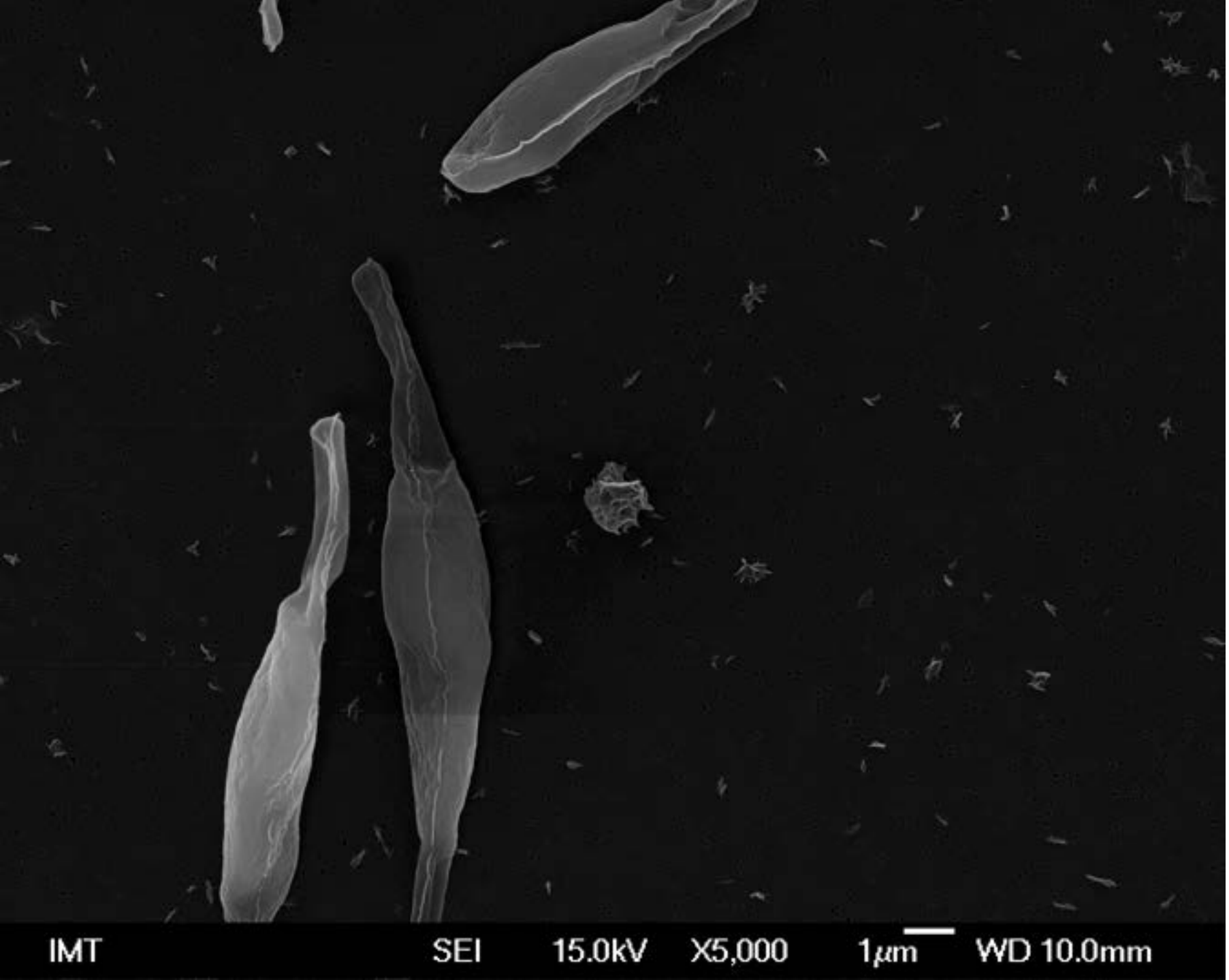

Figure *Phaeodactylum tricornutum culture* SEM 125. SEM feodaktilum netretiran #2ovoid/Darja-21-10-2021 - 11 ovalne celice s trdnega gojisca

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with LB Lennox LB broth (ref. L3022, Sigma Aldrich, USA. Culture was grown on agar at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

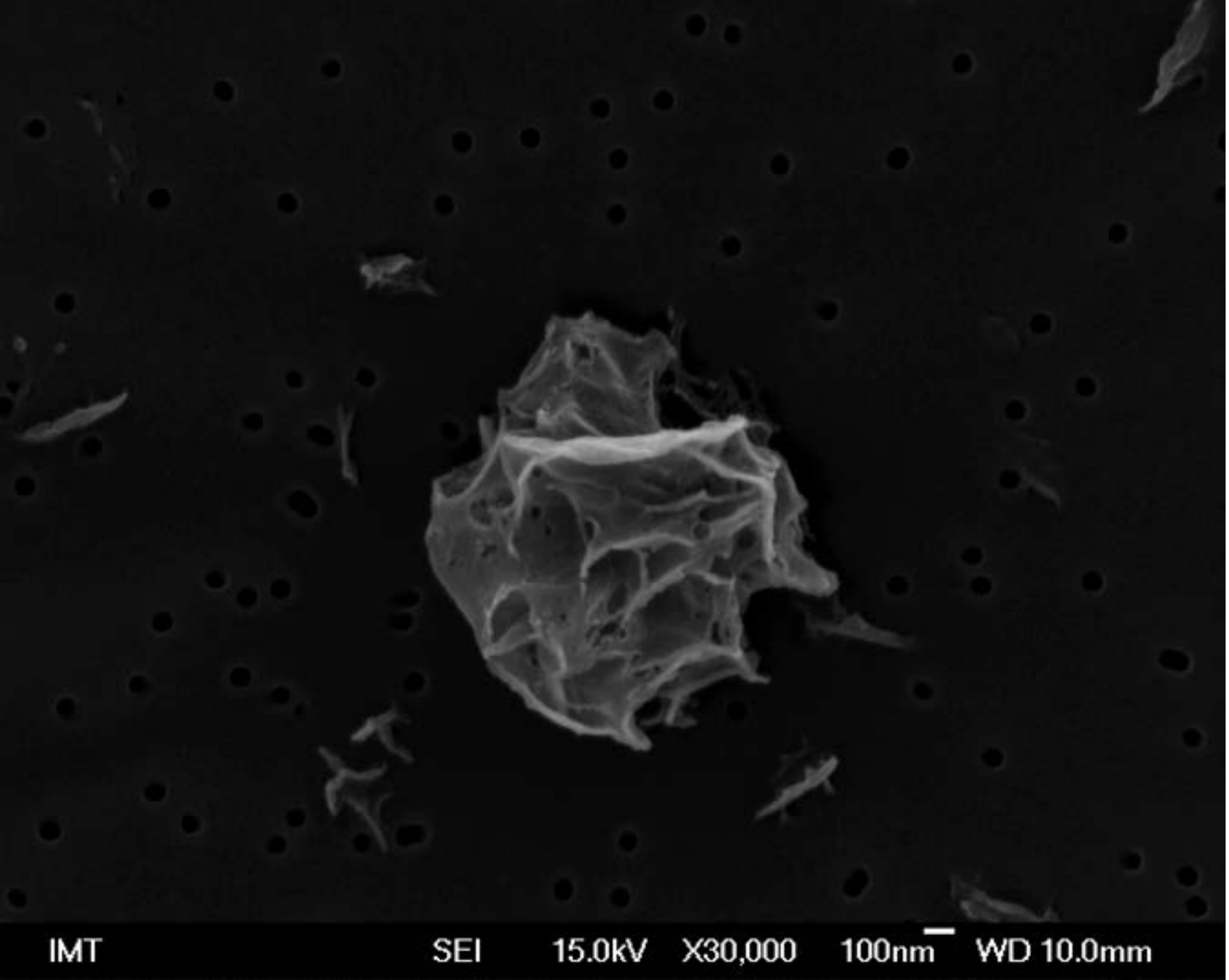

Figure *Phaeodactylum tricornutum culture* SEM 126. SEM feodaktilum netretiran #2ovoid/Darja-21-10-2021 - 11 ovalne celice s trdnega gojisca

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with LB Lennox LB broth (ref. L3022, Sigma Aldrich, USA. Culture was grown on agar at 20 °C and 20 % illumination (approximately 250 µmol/m2s) with a 14-hour light / 10-hour dark cycle.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

Figure *Phaeodactylum tricornutum culture* SEM 127. SEM feodaktilum netretiran #2ovoid/Darja-21-10-2021 - 11 ovalne celice s trdnega gojisca

Cultivation of the algae

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Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

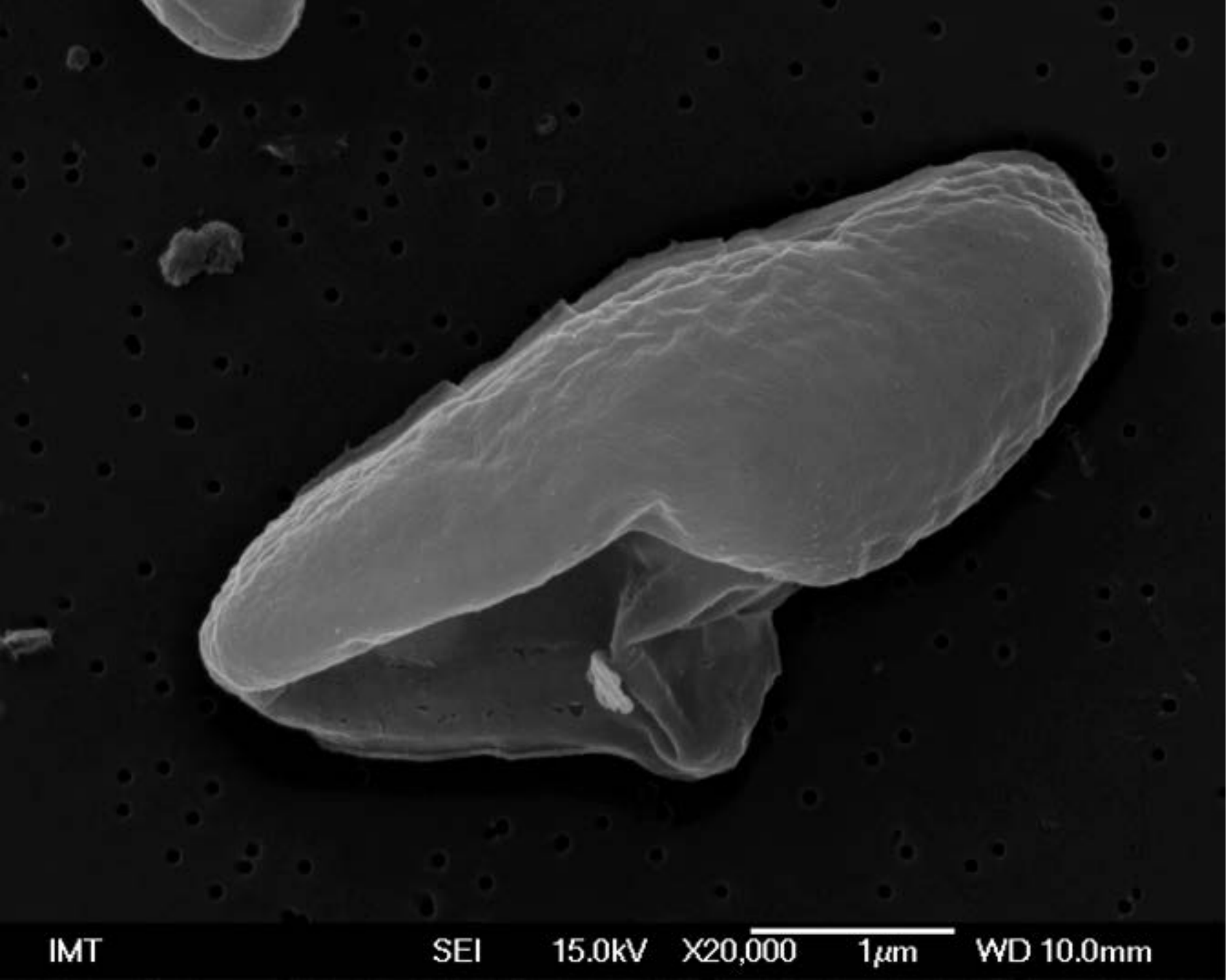

Figure *Phaeodactylum tricornutum culture* SEM 128. SEM feodaktilum netretiran #2ovoid/Darja-21-10-2021 - 11 ovalne celice s trdnega gojisca

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with LB Lennox LB broth (ref. L3022, Sigma Aldrich, USA. Culture was grown on agar at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle.

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Figure *Phaeodactylum tricornutum culture* SEM 129. SEM feodaktilum netretiran #2ovoid/Darja-21-10-2021 - 11 ovalne celice s trdnega gojisca

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with LB Lennox LB broth (ref. L3022, Sigma Aldrich, USA. Culture was grown on agar at 20 °C and 20 % illumination (approximately 250 µmol/m2s) with a 14-hour light / 10-hour dark cycle.

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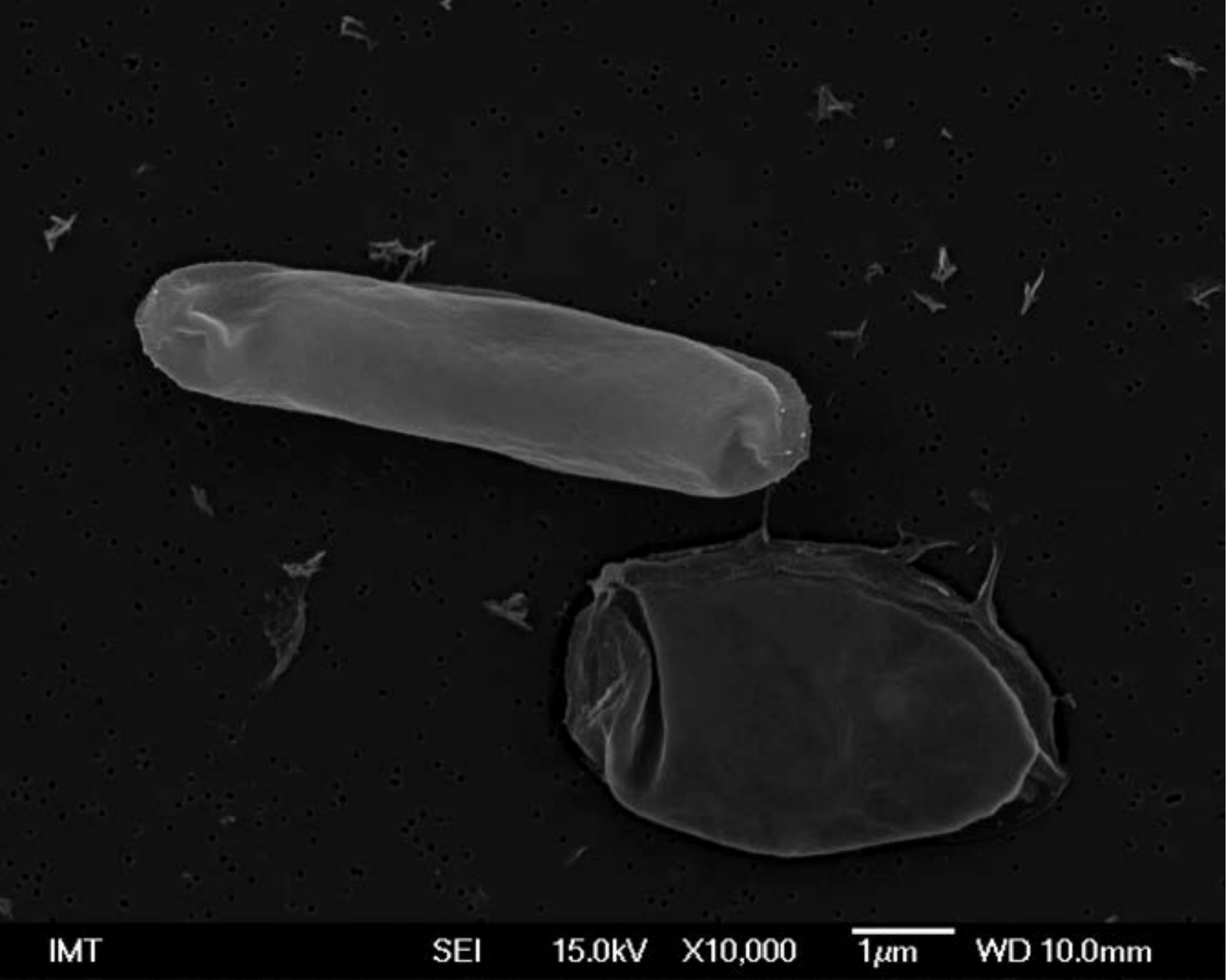

Figure *Phaeodactylum tricornutum culture* SEM 130. SEM feodaktilum netretiran #2ovoid/Darja-21-10-2021 - 11 ovalne celice s trdnega gojisca

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with LB Lennox LB broth (ref. L3022, Sigma Aldrich, USA. Culture was grown on agar at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle.

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Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

Figure *Phaeodactylum tricornutum culture* SEM 131. SEM feodaktilum netretiran #2ovoid/Darja-21-10-2021-11 ovalne celice s trdnega gojisca

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with LB Lennox LB broth (ref. L3022, Sigma Aldrich, USA. Culture was grown on agar at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle.

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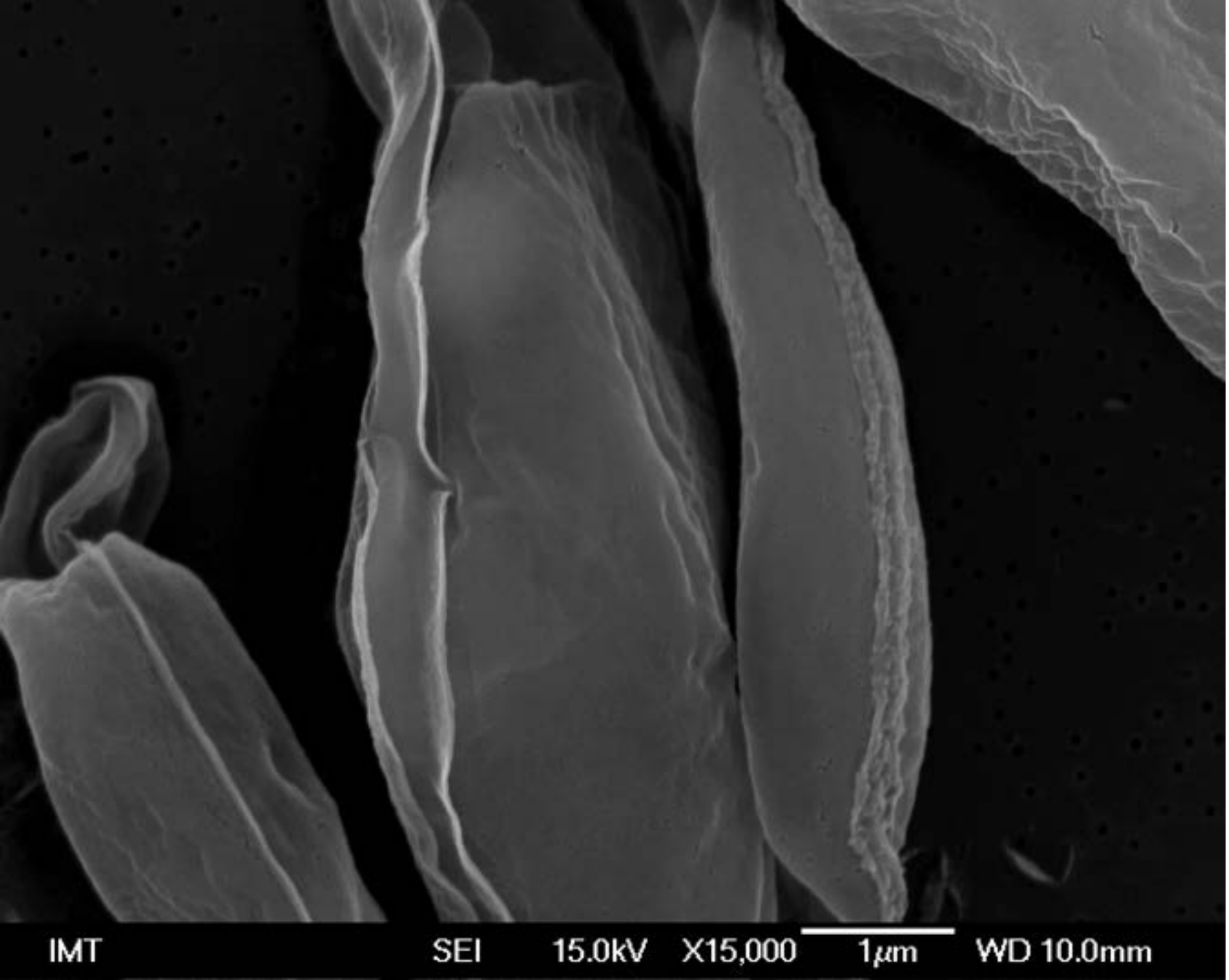

Figure *Phaeodactylum tricornutum culture* SEM 132. SEM feodaktilum netretiran #2ovoid/Darja-21-10-2021 - 11 ovalne celice s trdnega gojisca

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with LB Lennox LB broth (ref. L3022, Sigma Aldrich, USA. Culture was grown on agar at 20 °C and 20 % illumination (approximately 250 µmol/m2s) with a 14-hour light / 10-hour dark cycle.

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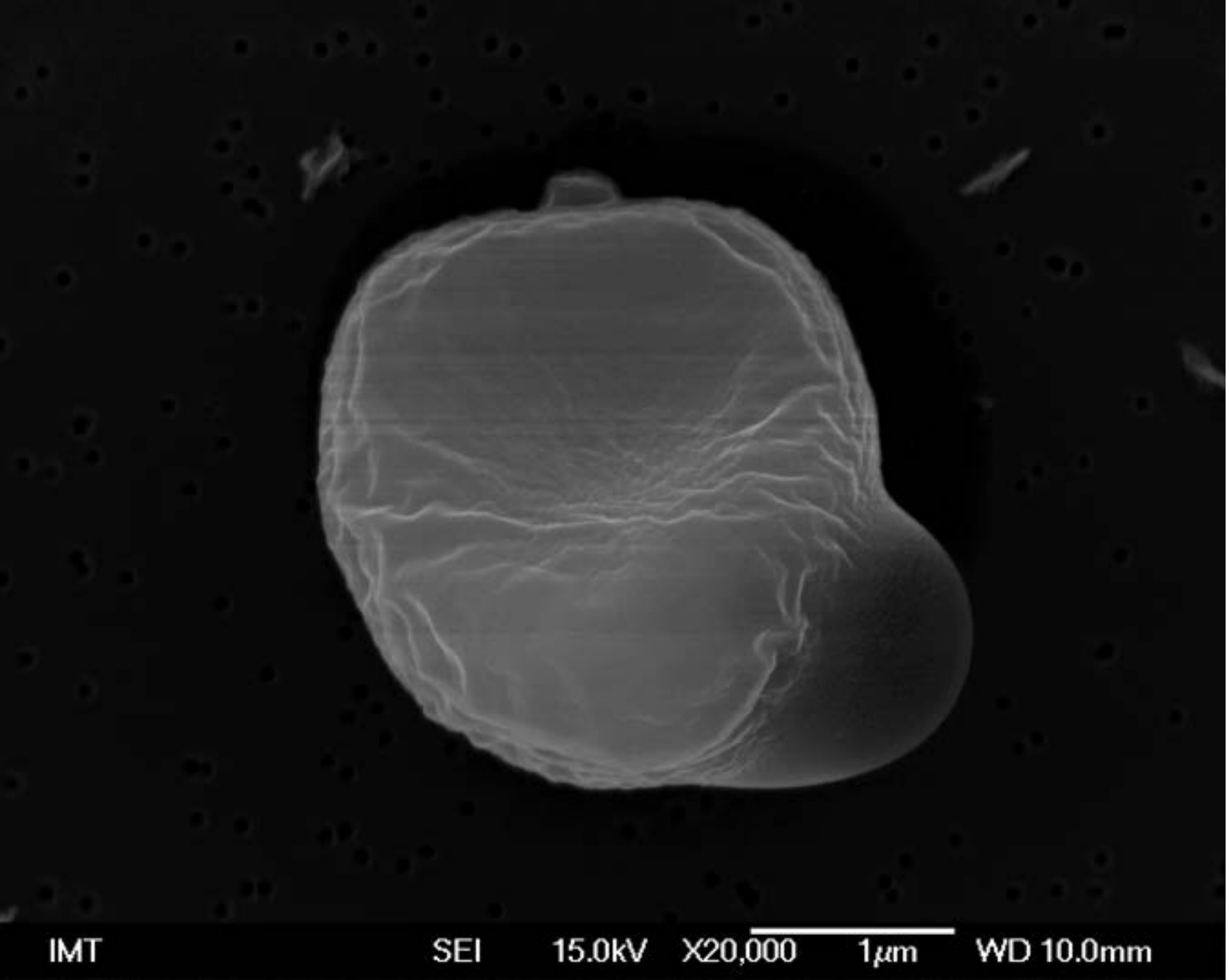

Figure *Phaeodactylum tricornutum culture* SEM 133. SEM feodaktilum netretiran #2ovoid/Darja-21-10-2021 - 11 ovalne celice s trdnega gojisca

Cultivation of the algae

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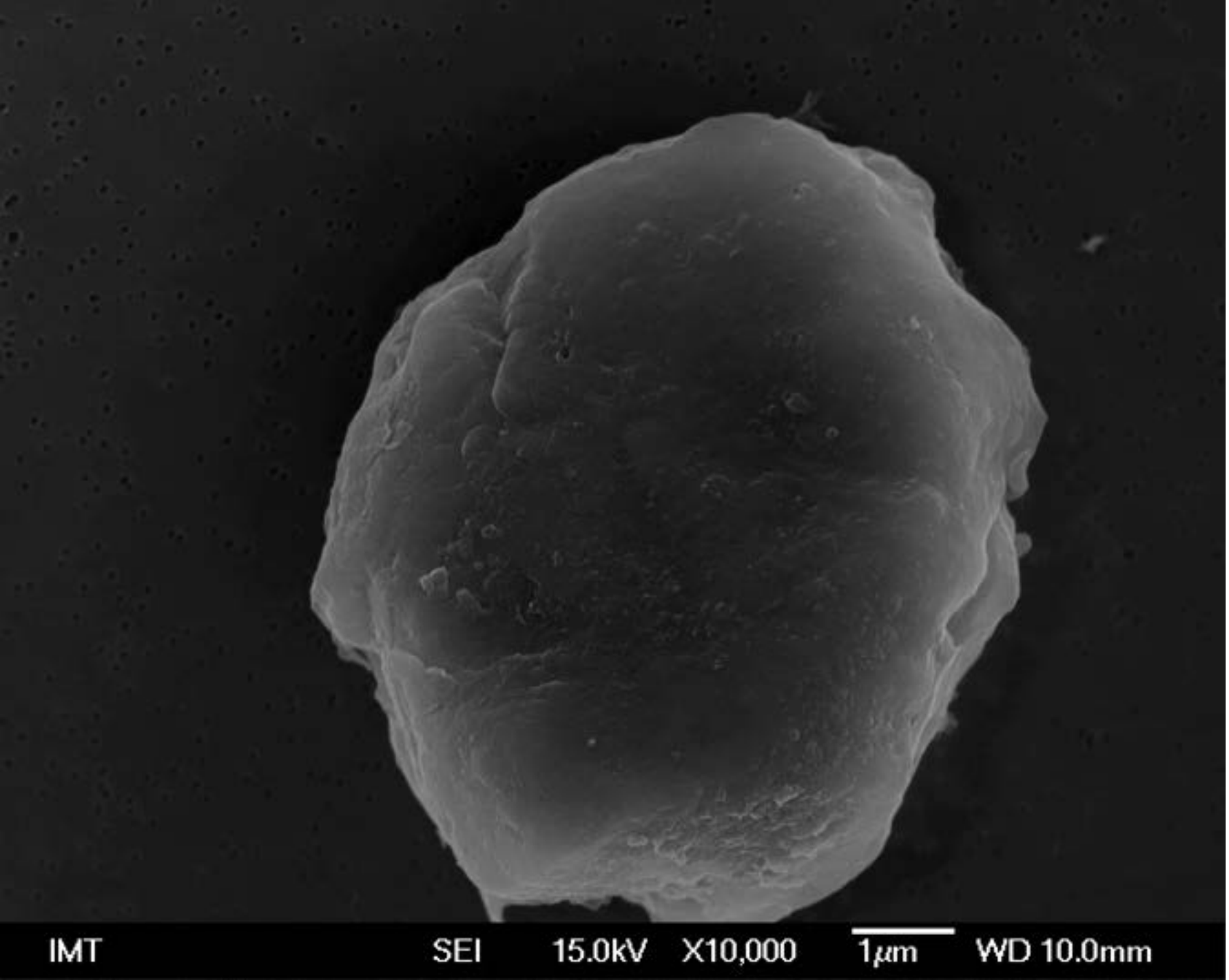

Figure *Phaeodactylum tricornutum culture* SEM 134. SEM feodaktilum netretiran #2ovoid/Darja-21-10-2021 - 11 ovalne celice s trdnega gojisca

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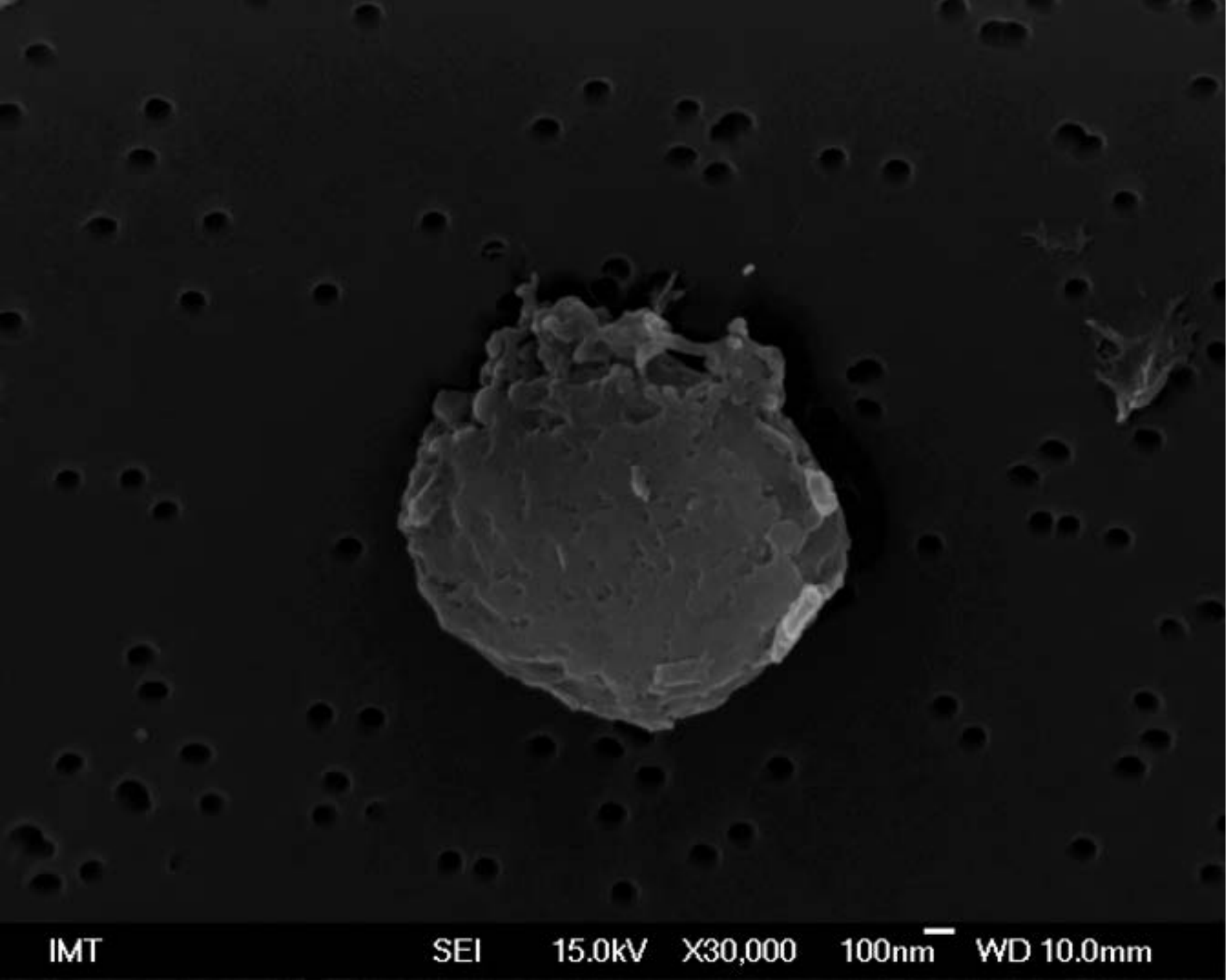

Figure *Phaeodactylum tricornutum culture* SEM 135. SEM feodaktilum netretiran #2ovoid/Darja-21-10-2021 - 11 ovalne celice s trdnega gojisca

Cultivation of the algae

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Proceedings of 9th Socratic Lectures **2024**

Repository

Scanning electron microscope images of isolates of small cellular particles *Phaeodactylum tricornutum* grown in media supplemented with Guillard's (F/2) marine water enrichment solution, BG11 broth and Lennox LB broth

Bedina Zavec Apolonija[1] , Božič Darja[2,3], Hočevar Matej[4], Iglič Aleš[3,5], Jeran Marko[2,3], Kralj-Iglič Veronika[2,*], Romolo Anna[2]

[1]National Institute of Chemistry, University of Ljubljana, [2]University of Ljubljana, Faculty of Health Sciences, Laboratory of Clinical Biophysics, [3]University of Ljubljana, Faculty of Electrical Engineering, Laboratory of Physics, [4]Institute of Metals and Technology, [5]University of Ljubljana, Faculty of Medicine, Laboratory of Clinical Biophysics, Ljubljana, Slovenia
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Citation: Bedina Zavec A, Božič D, Iglič A, Jeran M, Kisovec M, Kralj-Iglič V, Romolo A. Scanning electron microscope images of isolates of small cellular particles Phaeodactylum tricornutum grown in media supplemented with Guillard's (F/2) marine water enrichment solution, BG11 broth and Lennox LB broth Proceedings of Socratic Lectures. **2024**, 9, 328-350.
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Abstract: Scanning electron microscope images of small cellular particles isolated from conditioned media of microalgae *Phaeodactylum tricornutun* are presented. Each image is supplemented by description of the preparation of the sample and the data on the imaging technique and equipment.

The data curators of the repository are Veronika Kralj-Iglič and Anna Romolo. More data on experiments with microalgae small cellular particles can be found in (Adamo et al., 2021), (Picciotto et al., 2021) and (Božič et al., 2022).

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Keywords: Extracellular vesicles, Extracellular particles, Nanoalgosomes, Exosomes

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2. Picciotto S, Barone ME, Fierli D, et al. Isolation of extracellular vesicles from microalgae: towards the production of sustainable and natural nanocarriers of bioactive compounds. *Biomater Sci*. 2021;9(8):2917-2930. doi:10.1039/d0bm01696a
3. Božič D, Hočevar M, Jeran M et al. Ultrastructure and stability of cellular nanoparticles isolated from *Phaeodactylum tricornutum* and *Dunaliella tertiolecta* conditioned media. *Open Res Europe* 2022, 2:121 (https://doi.org/10.12688/openreseurope.14896.1)

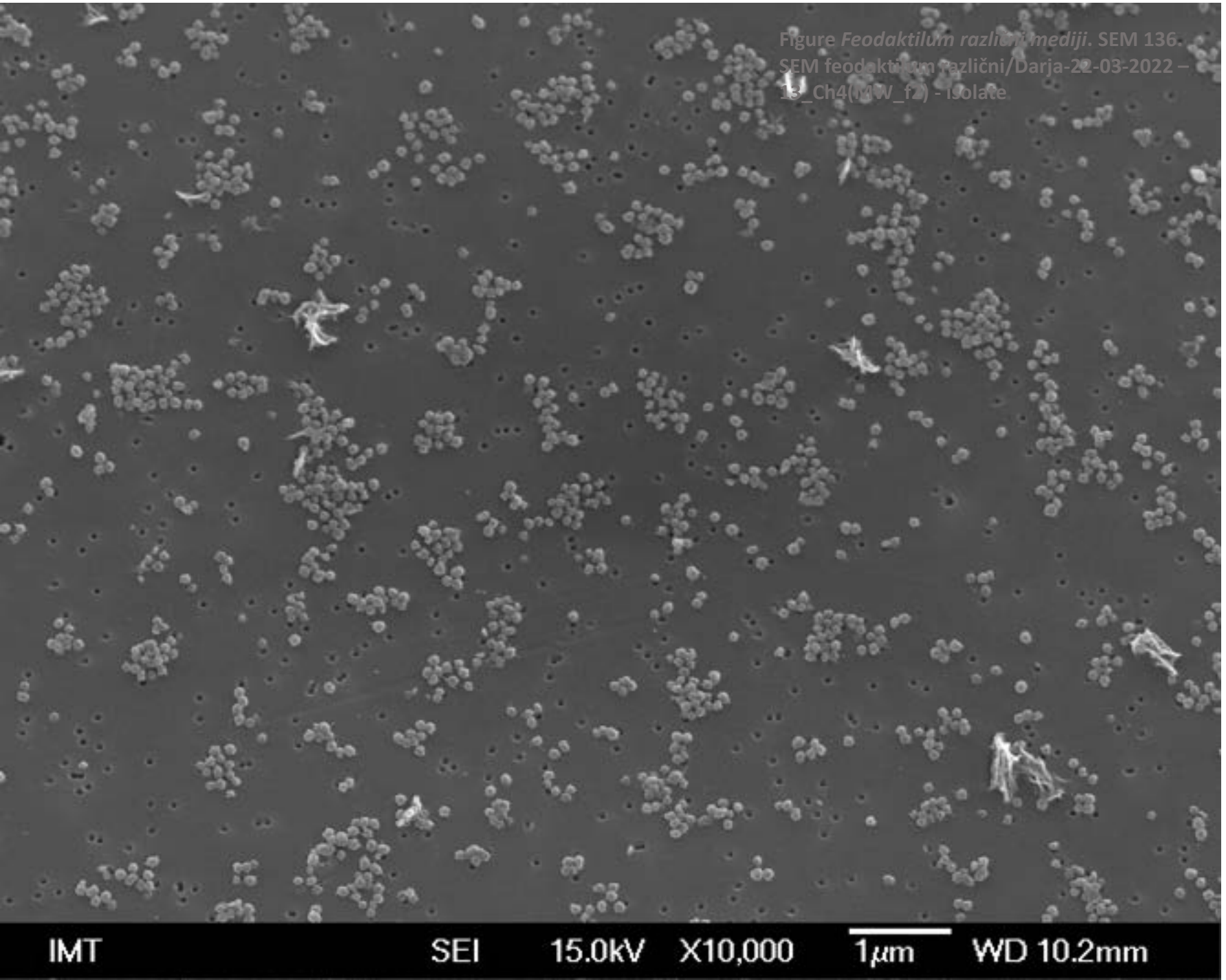

Figure *Phaeodactylum tricornutum* isolate F2 SEM 1.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with **Guillard's (F/2)** Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Isolation of small cellular particles (SCPs)

SCPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)) using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan). From: https://zenodo.org/record/6908895. Image:10

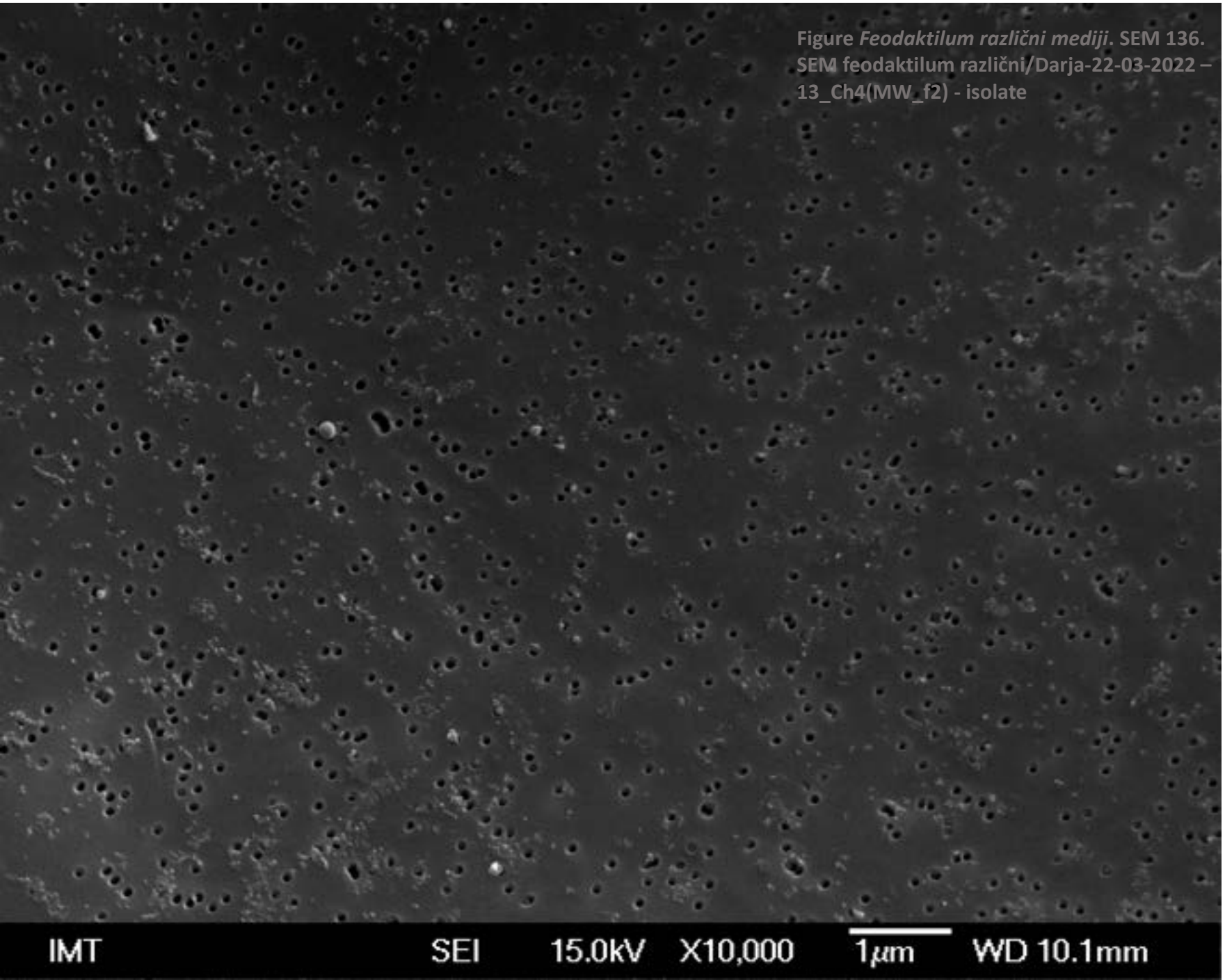

Figure *Phaeodactylum tricornutum* isolate F2 SEM 2.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Isolation of small cellular particles (SCPs)

SCPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

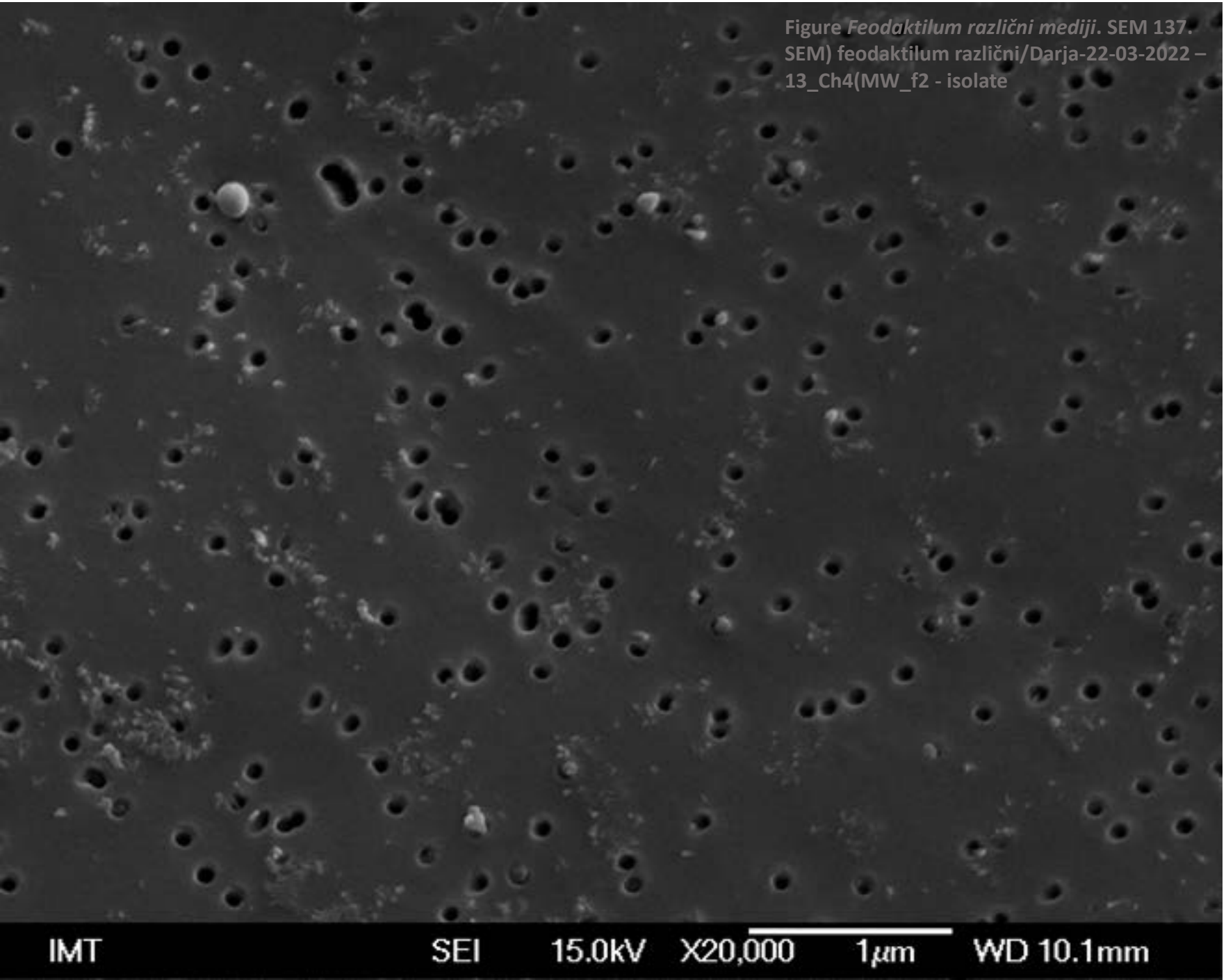

Figure *Phaeodactylum tricornutum* isolate F2 SEM 3.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Isolation of small cellular particles (SCPs)

SCPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

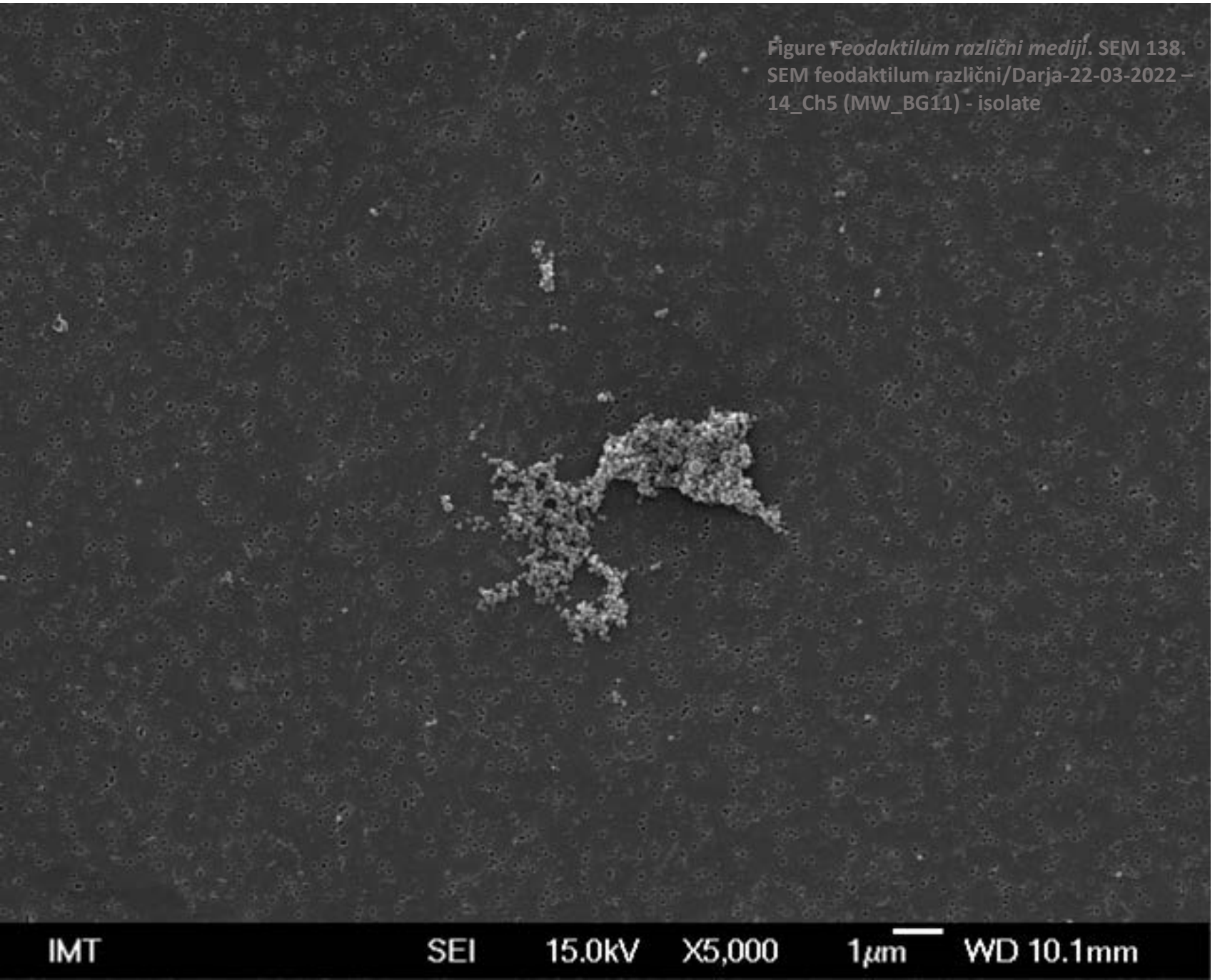

Figure *Phaeodactylum tricornutum* isolate BG11 SEM 4.

Cultivation of algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with BG11 Broth (ref. 73816, Sigma Aldrich, Switzerland). Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 µmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Isolation of Small Cellular Particles (SCPs)

SCPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

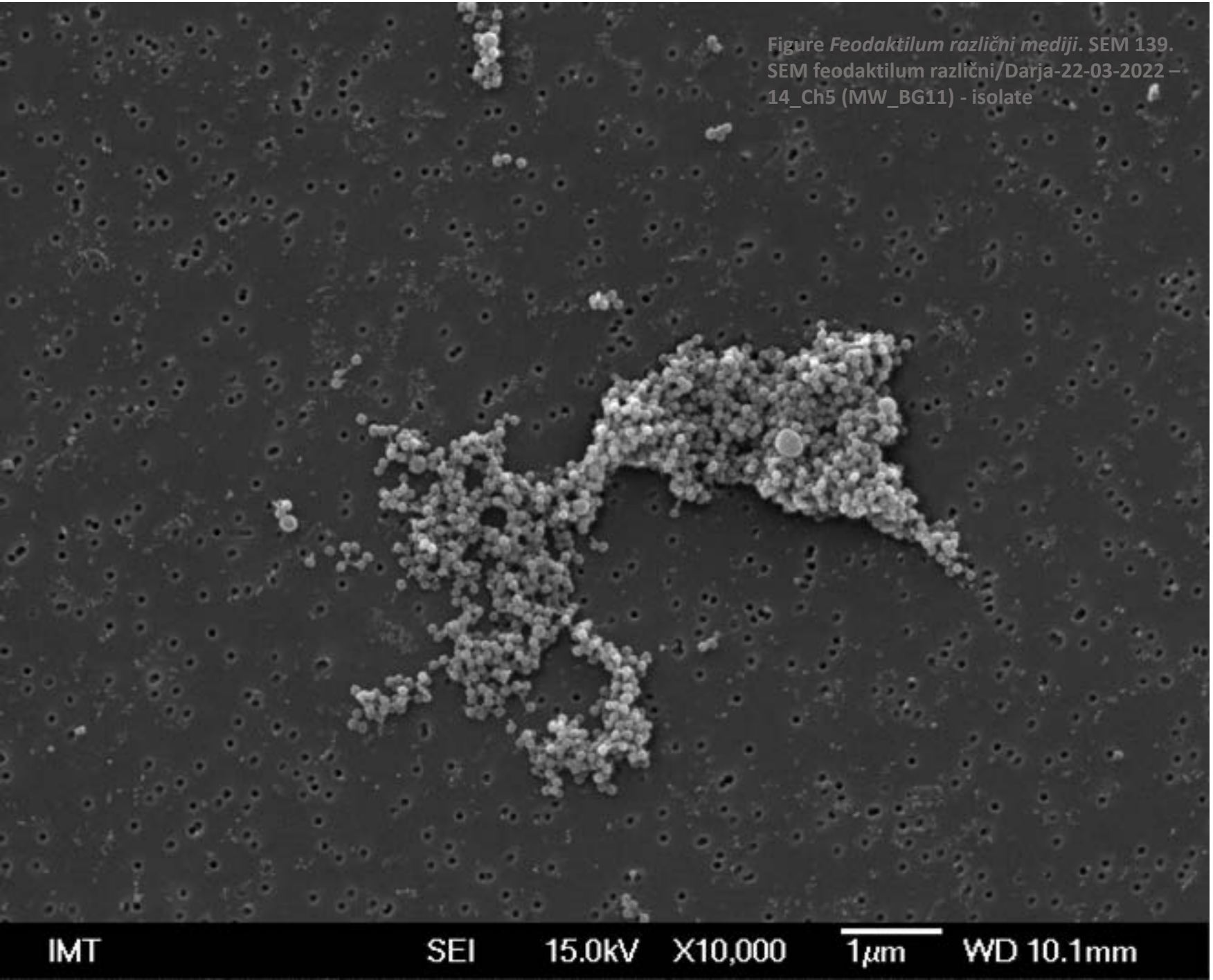

Figure *Phaeodactylum tricornutum* isolate BG11 SEM 5.

Cultivation of algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with BG11 Broth (ref. 73816, Sigma Aldrich, Switzerland). Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Isolation of Small Cellular Particles (SCPs)

SCPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

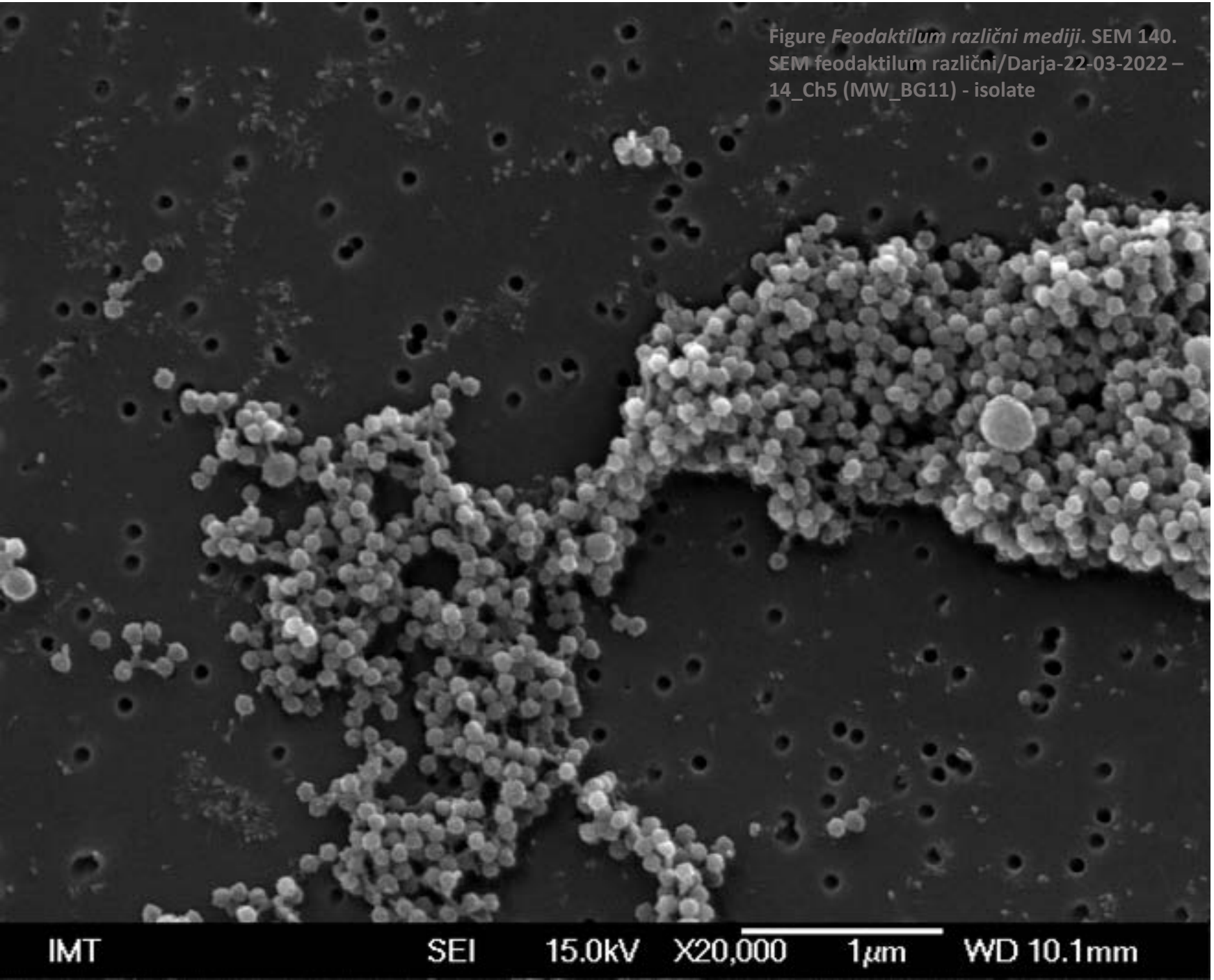

Figure *Phaeodactylum tricornutum* isolate BG11 SEM 6.

Cultivation of algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with **BG11 Broth** (ref. 73816, Sigma Aldrich, Switzerland). Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Isolation of Small Cellular Particles (SCPs)

SCPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

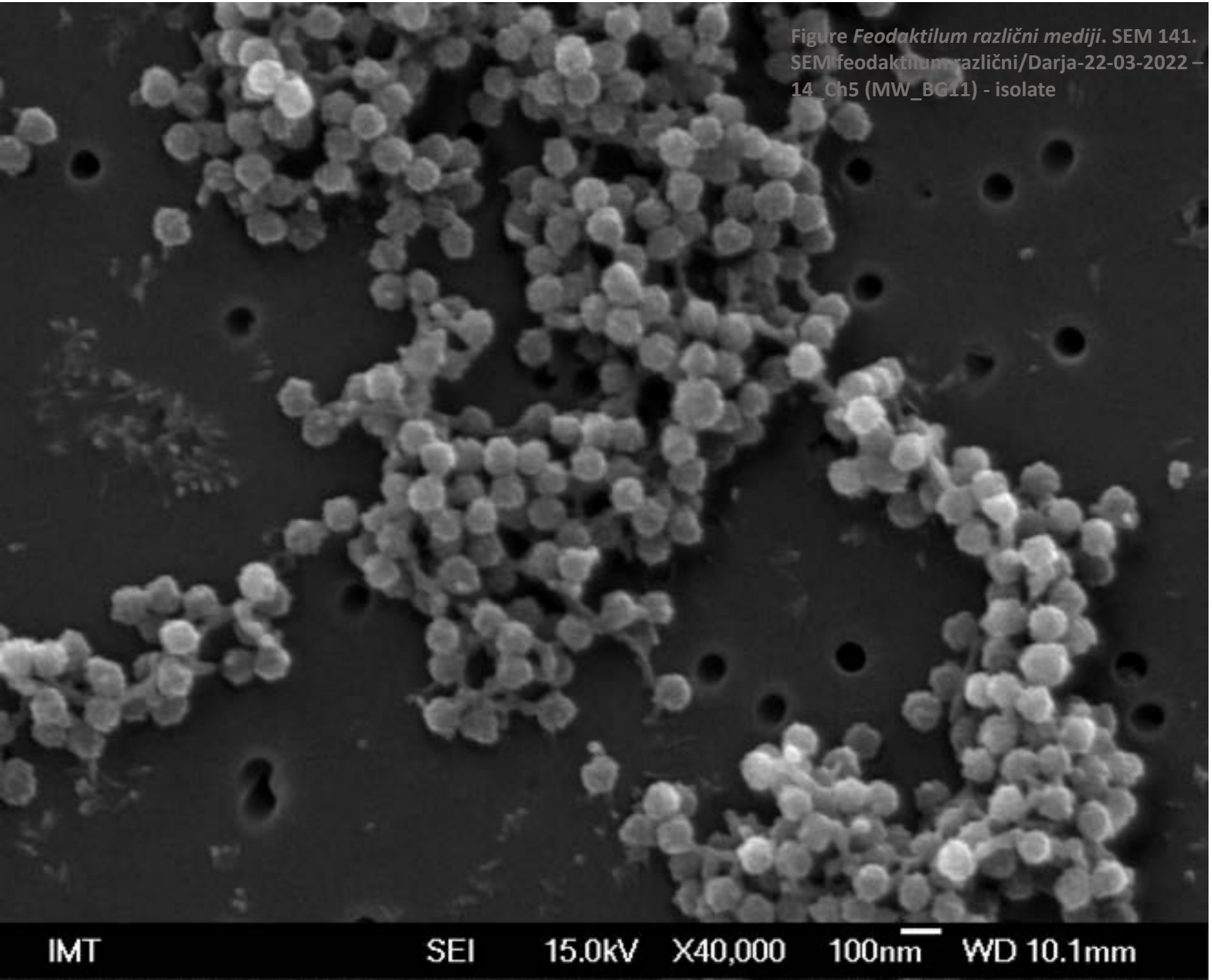

Figure *Phaeodactylum tricornutum* isolate BG11 SEM 7.

Cultivation of algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with BG11 Broth (ref. 73816, Sigma Aldrich, Switzerland). Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Isolation of Small Cellular Particles (SCPs)

SCPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

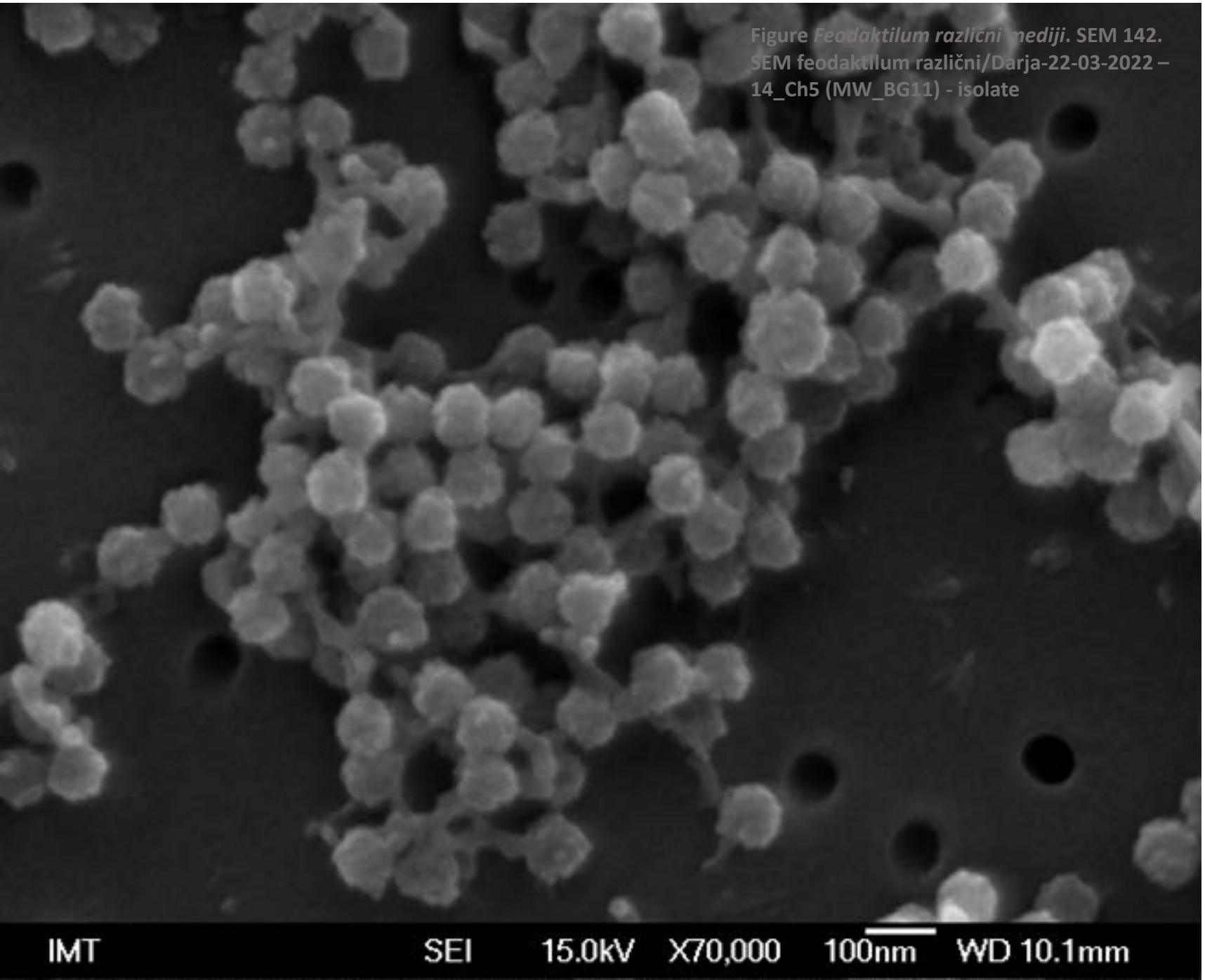

Figure *Phaeodactylum tricornutum* isolate BG11 SEM 8.

Cultivation of algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with BG11 Broth (ref. 73816, Sigma Aldrich, Switzerland). Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 µmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Isolation of Small Cellular Particles (SCPs)

SCPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

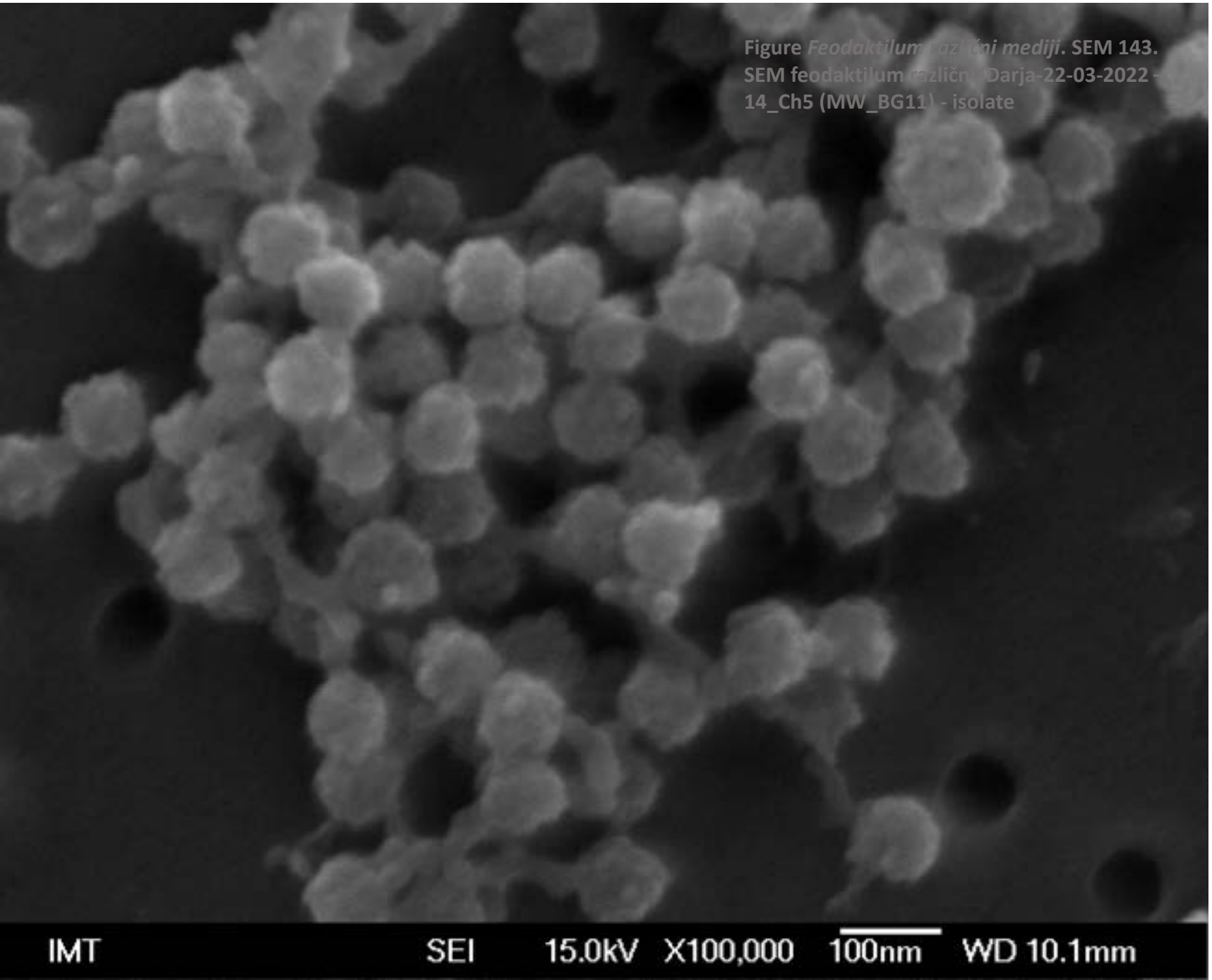

Figure *Phaeodactylum tricornutum* isolate BG11 SEM 9.

Cultivation of algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with BG11 Broth (ref. 73816, Sigma Aldrich, Switzerland). Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 µmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Isolation of Small Cellular Particles (SCPs)

SCPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

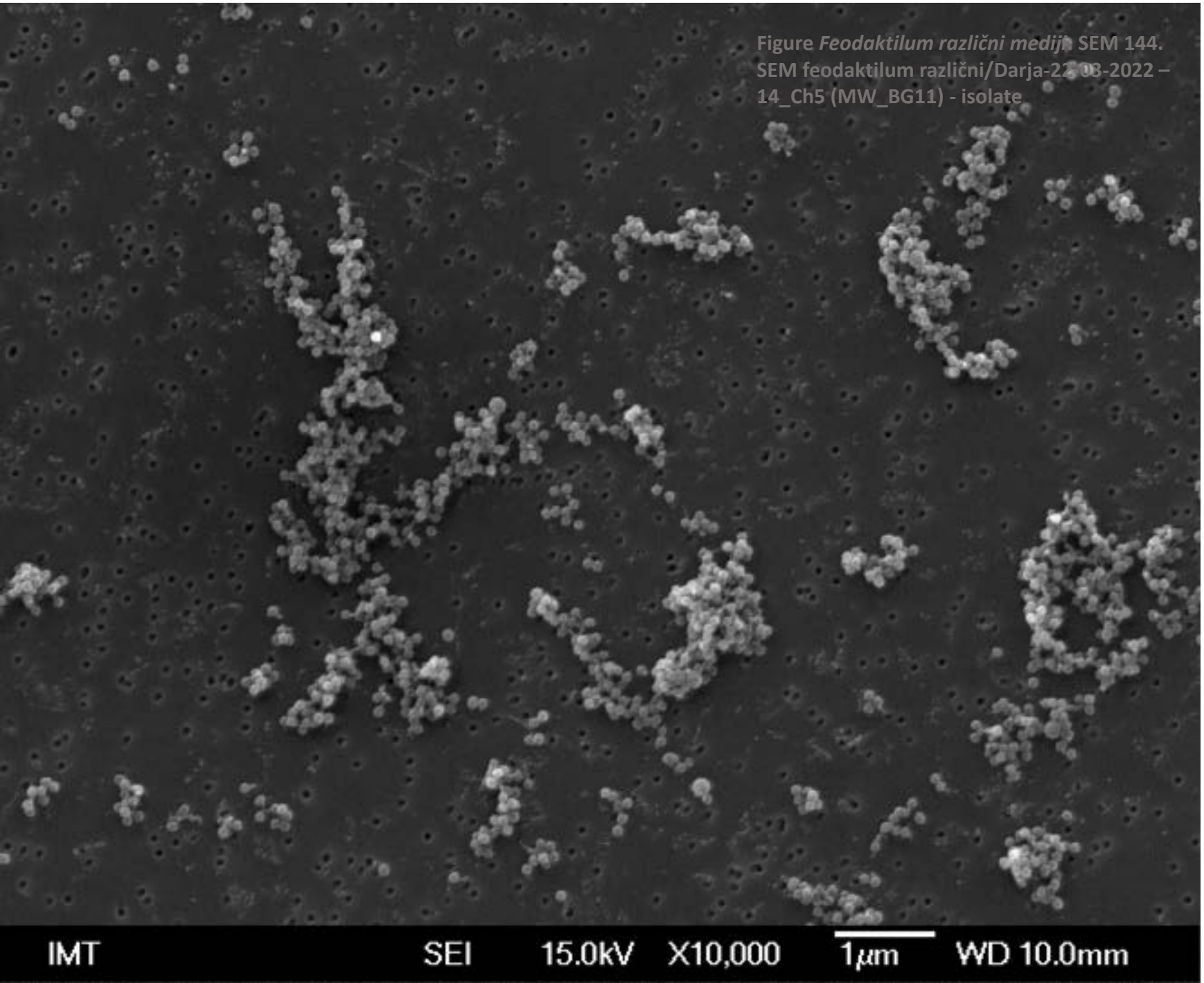

Figure *Phaeodactylum tricornutum* isolate BG11 SEM 10.

Cultivation of algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with BG11 Broth (ref. 73816, Sigma Aldrich, Switzerland). Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Isolation of Small Cellular Particles (SCPs)

SCPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

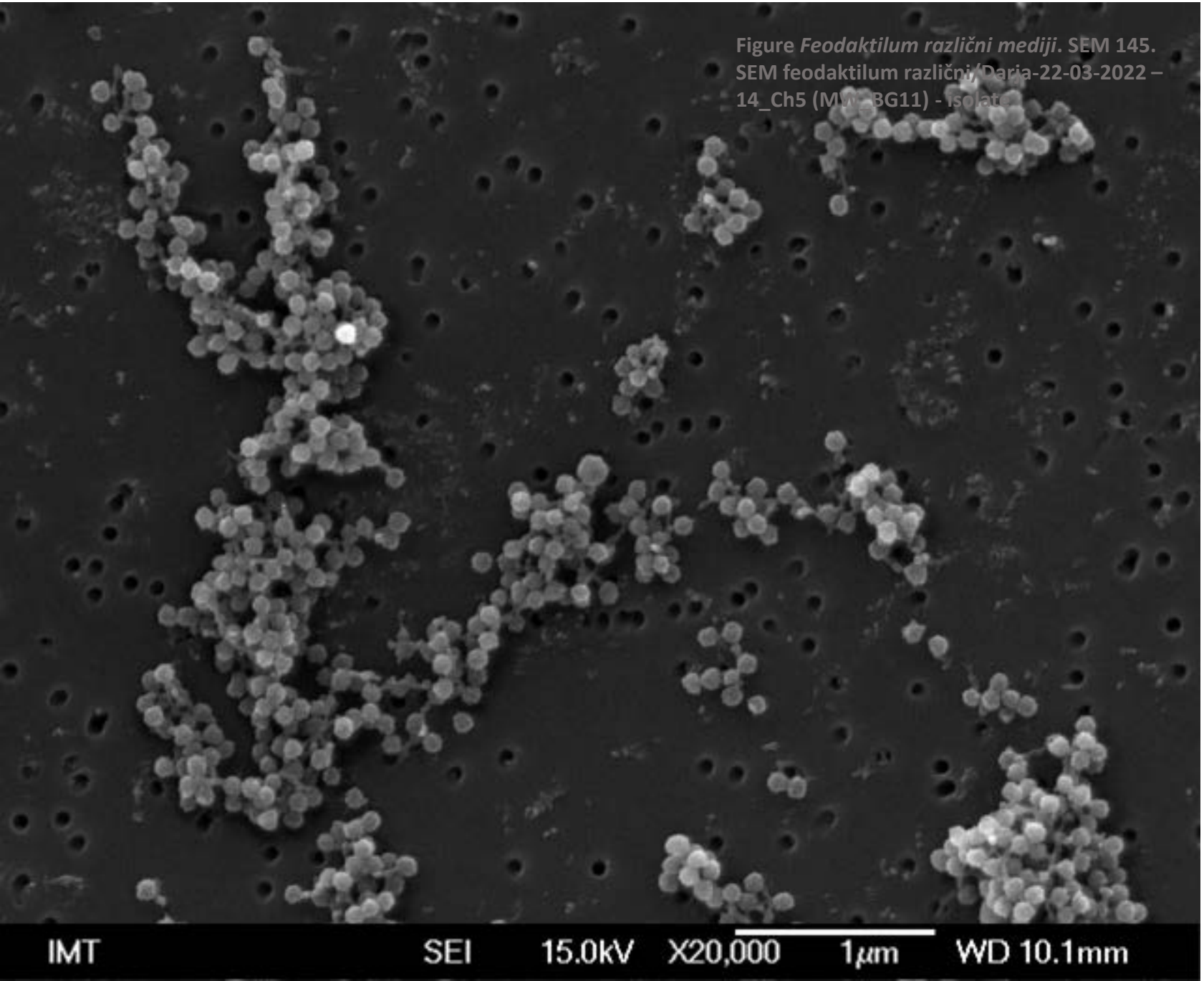

Figure *Phaeodactylum tricornutum* isolate BG11 SEM 11.

Cultivation of algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with BG11 Broth (ref. 73816, Sigma Aldrich, Switzerland). Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Isolation of Small Cellular Particles (SCPs)

SCPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

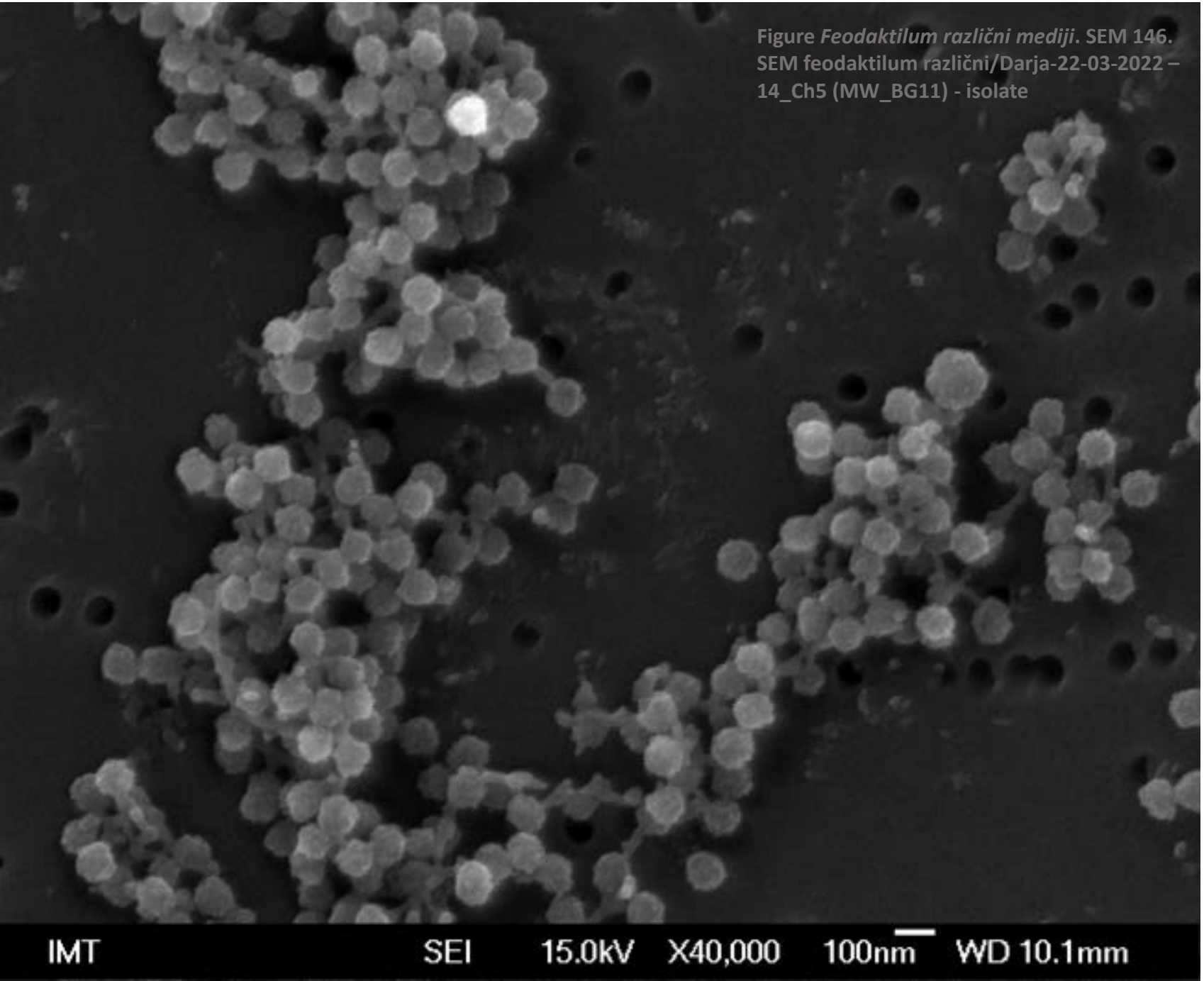

Figure *Phaeodactylum tricornutum* isolate BG11 SEM 12.

Cultivation of algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with BG11 Broth (ref. 73816, Sigma Aldrich, Switzerland). Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 µmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Isolation of Small Cellular Particles (SCPs)

SCPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

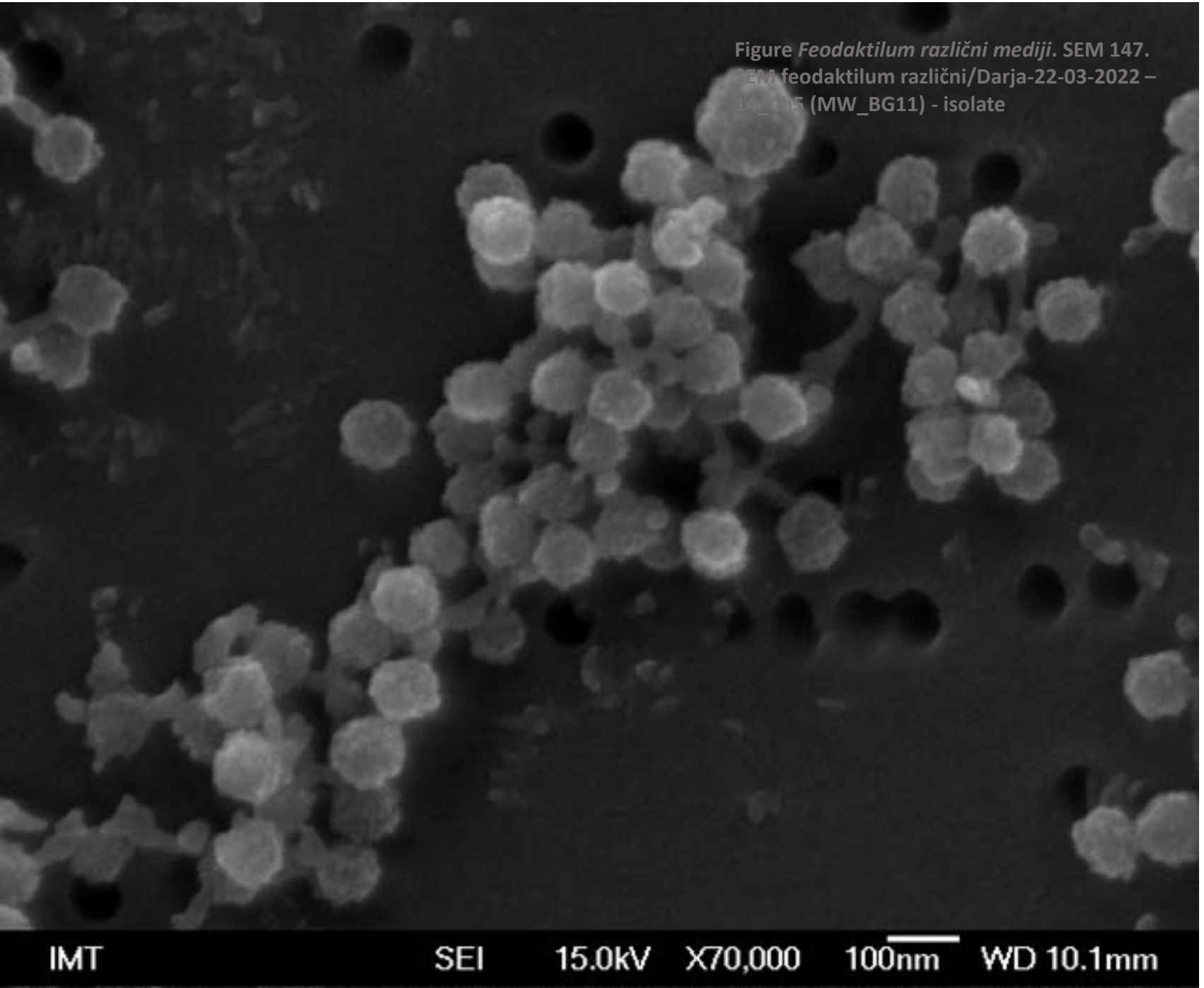

Figure *Phaeodactylum tricornutum* isolate BG11 SEM 13.

Cultivation of algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with BG11 Broth (ref. 73816, Sigma Aldrich, Switzerland). Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 µmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Isolation of Small Cellular Particles (SCPs)

SCPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isola)b Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

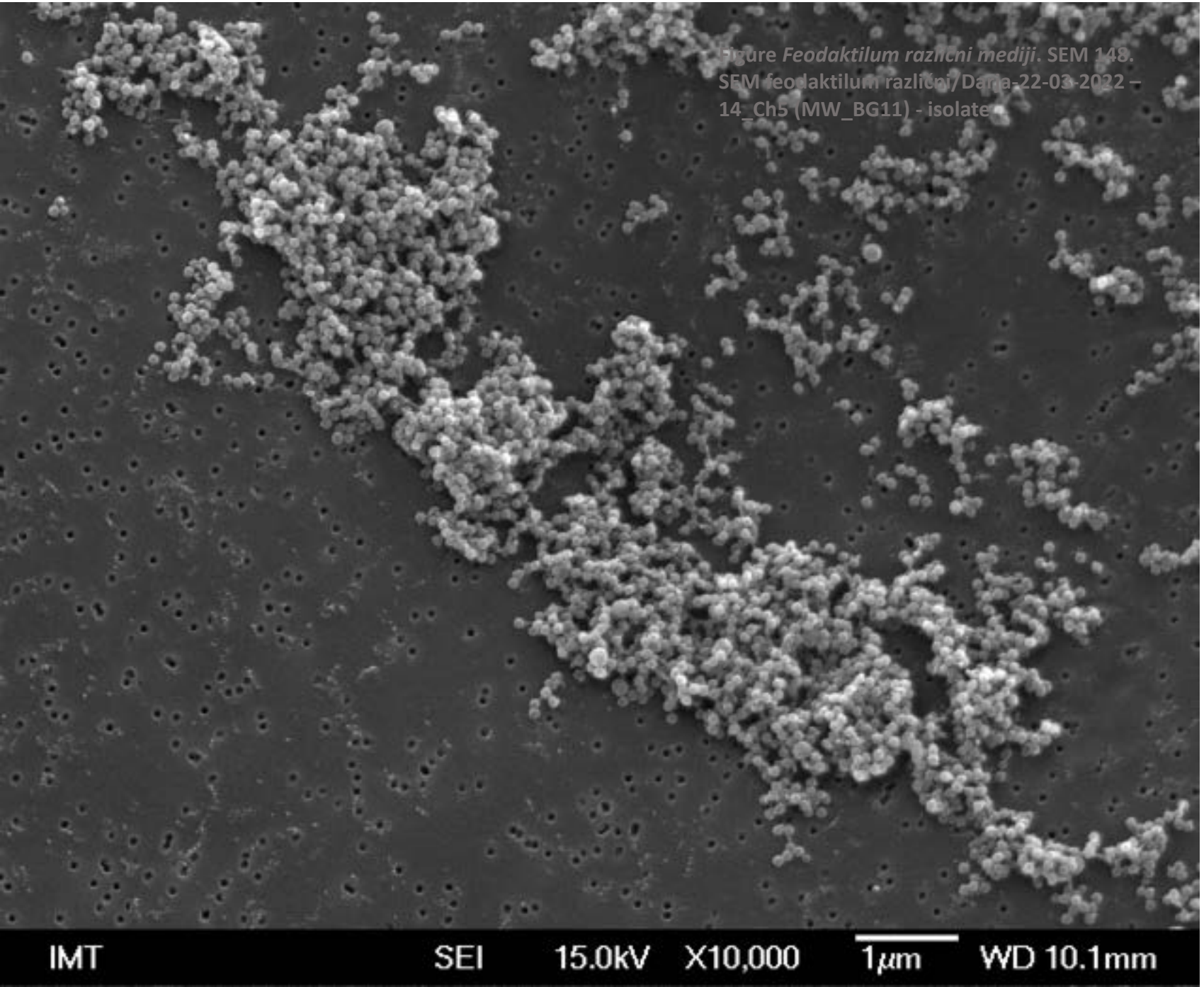

Figure *Phaeodactylum tricornutum* isolate BG11 SEM 14.

Cultivation of algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with BG11 Broth (ref. 73816, Sigma Aldrich, Switzerland). Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 µmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Isolation of Small Cellular Particles (SCPs)

SCPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

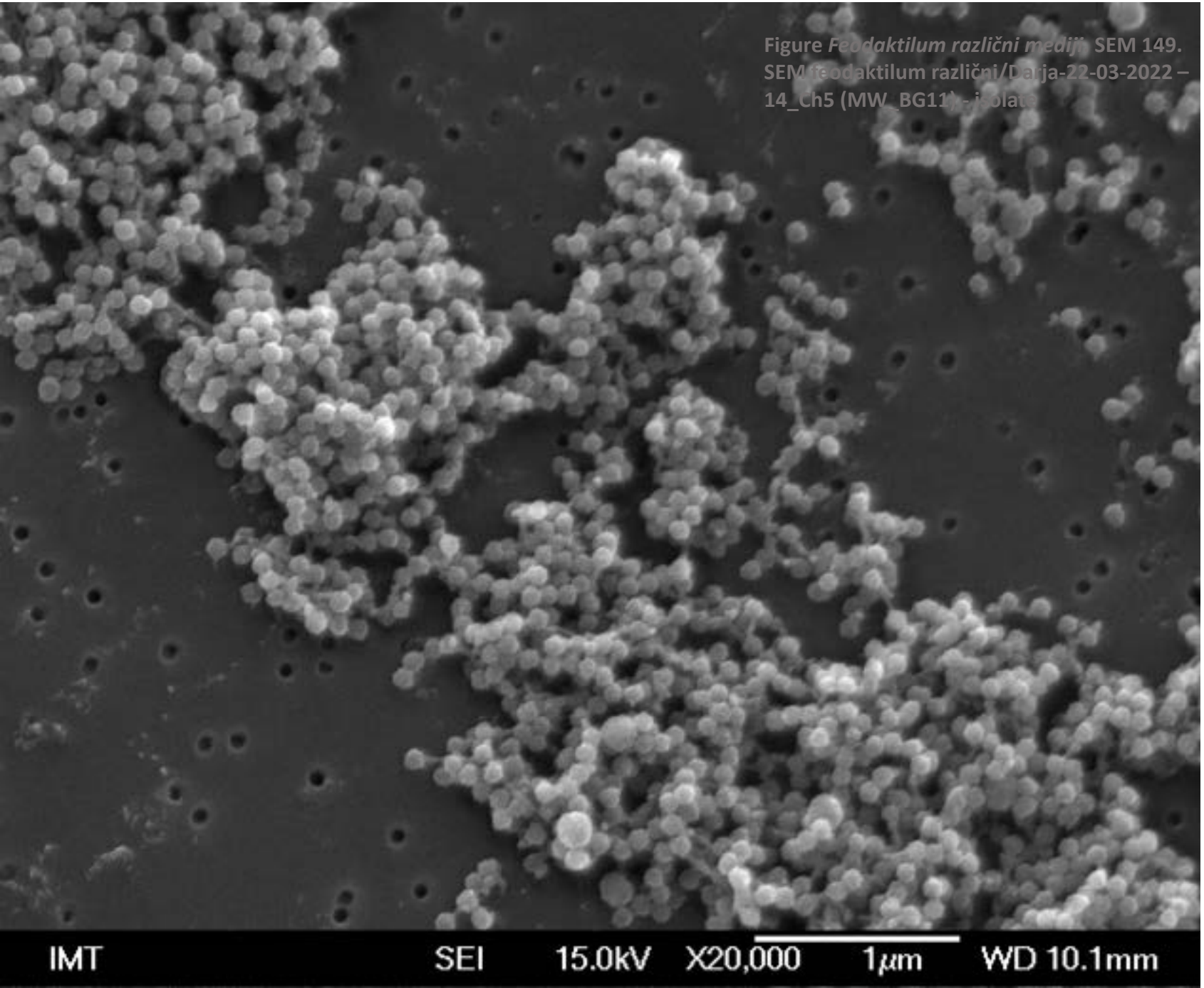

Figure *Phaeodactylum tricornutum* isolate BG11 SEM 15.

Cultivation of algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with BG11 Broth (ref. 73816, Sigma Aldrich, Switzerland). Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 µmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Isolation of Small Cellular Particles (SCPs)

SCPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

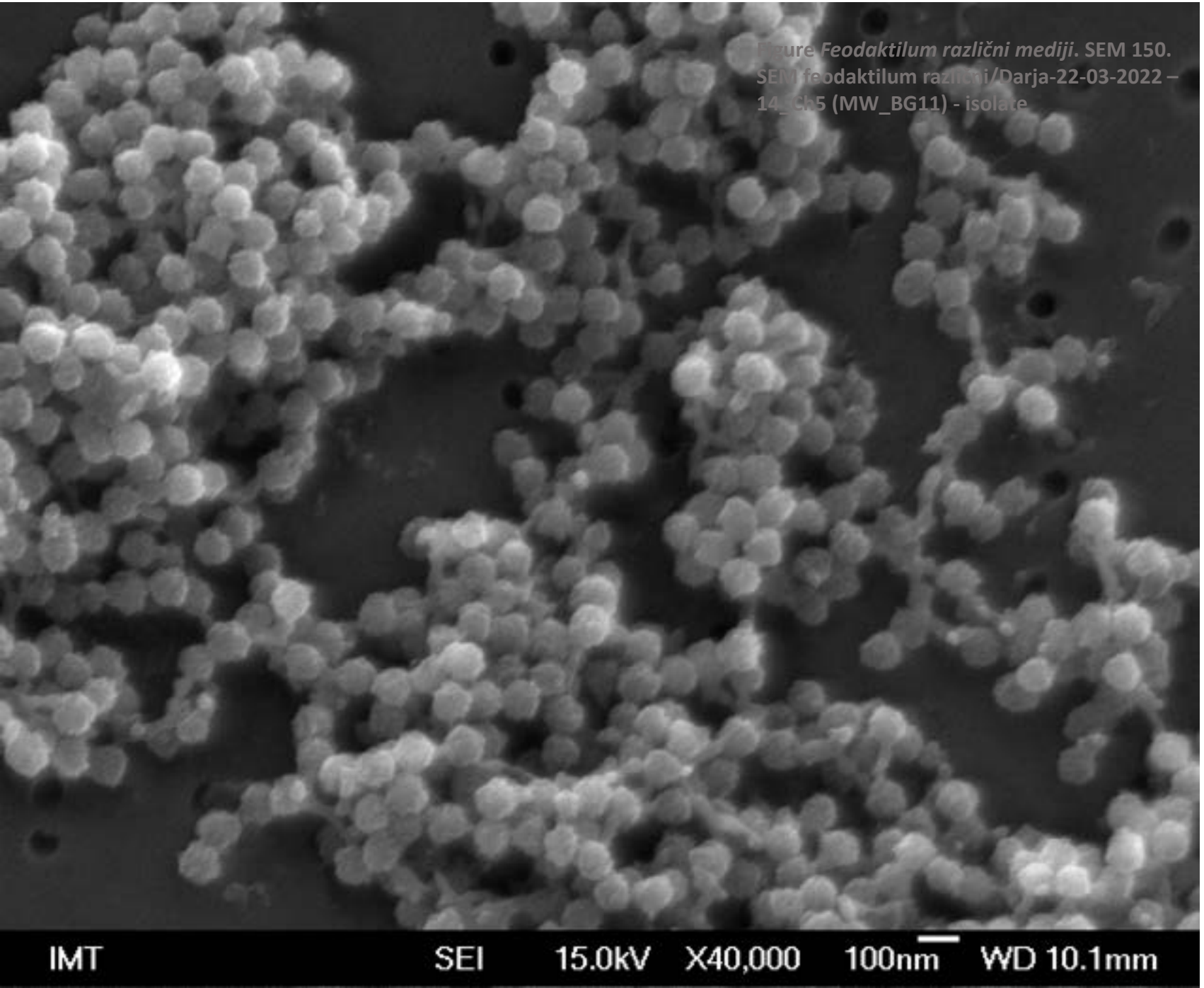

Figure *Phaeodactylum tricornutum* isolate BG11 SEM 16.

Cultivation of algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with BG11 Broth (ref. 73816, Sigma Aldrich, Switzerland). Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Isolation of Small Cellular Particles (SCPs)

SCPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

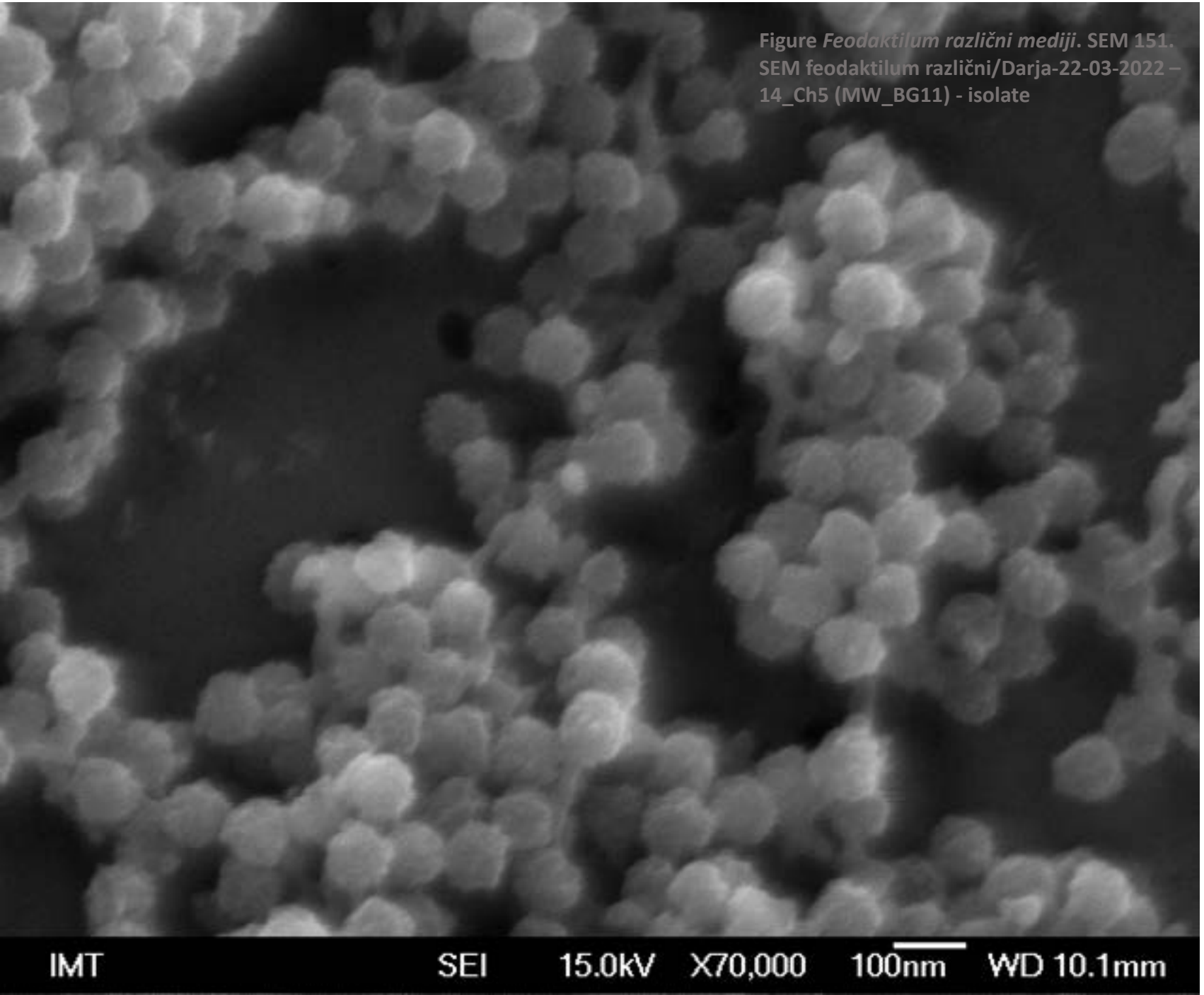

Figure *Phaeodactylum tricornutum* isolate BG11 SEM 17.

Cultivation of algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with BG11 Broth (ref. 73816, Sigma Aldrich, Switzerland). Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Isolation of Small Cellular Particles (SCPs)

SCPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

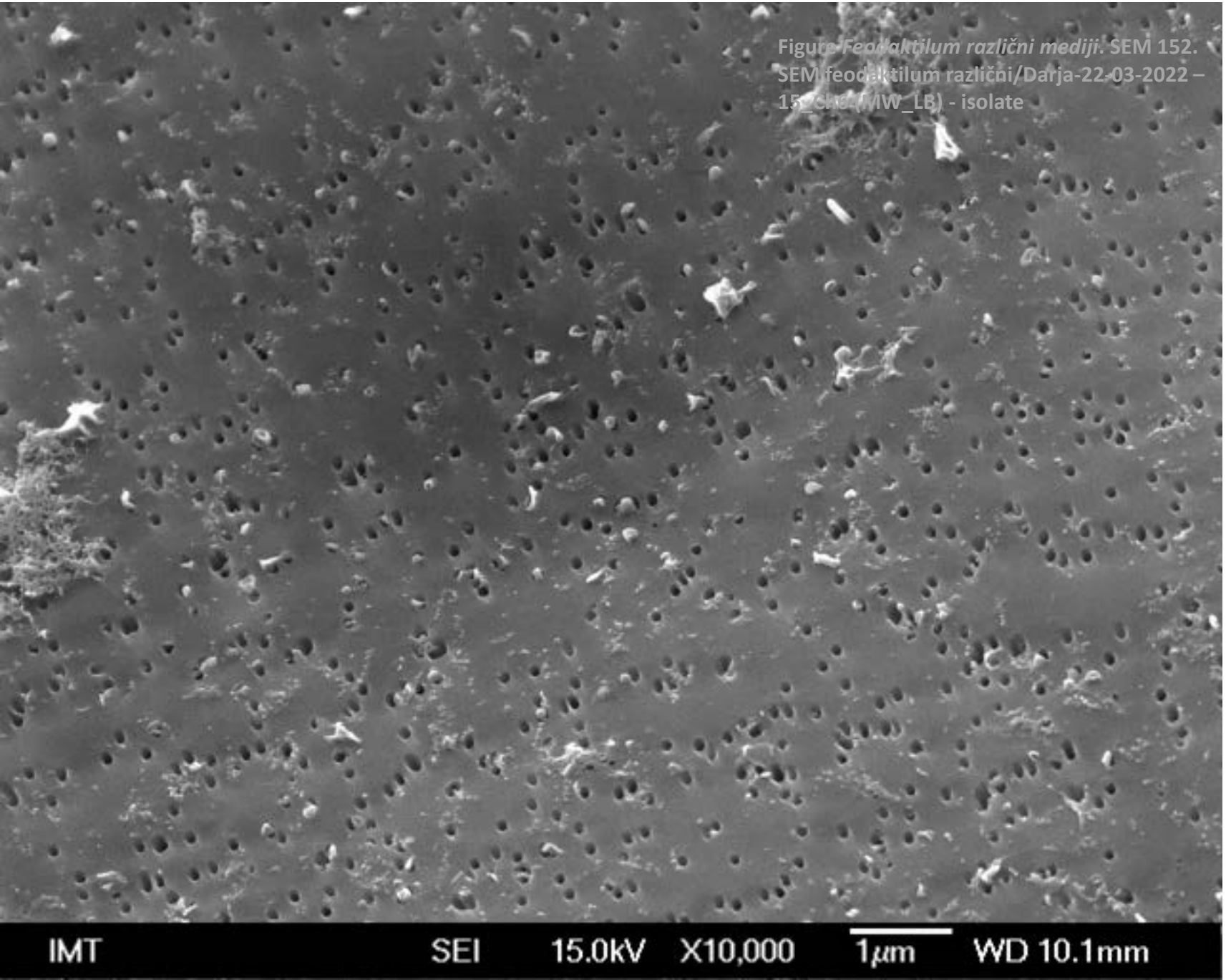

Figure *Phaeodactylum tricornutum* isolate LB SEM 18.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with LB Lennox LB broth (ref. L3022, Sigma Aldrich, USA). Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (app. 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Isolation of Small Cellular Particles (SCPs)

SCPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

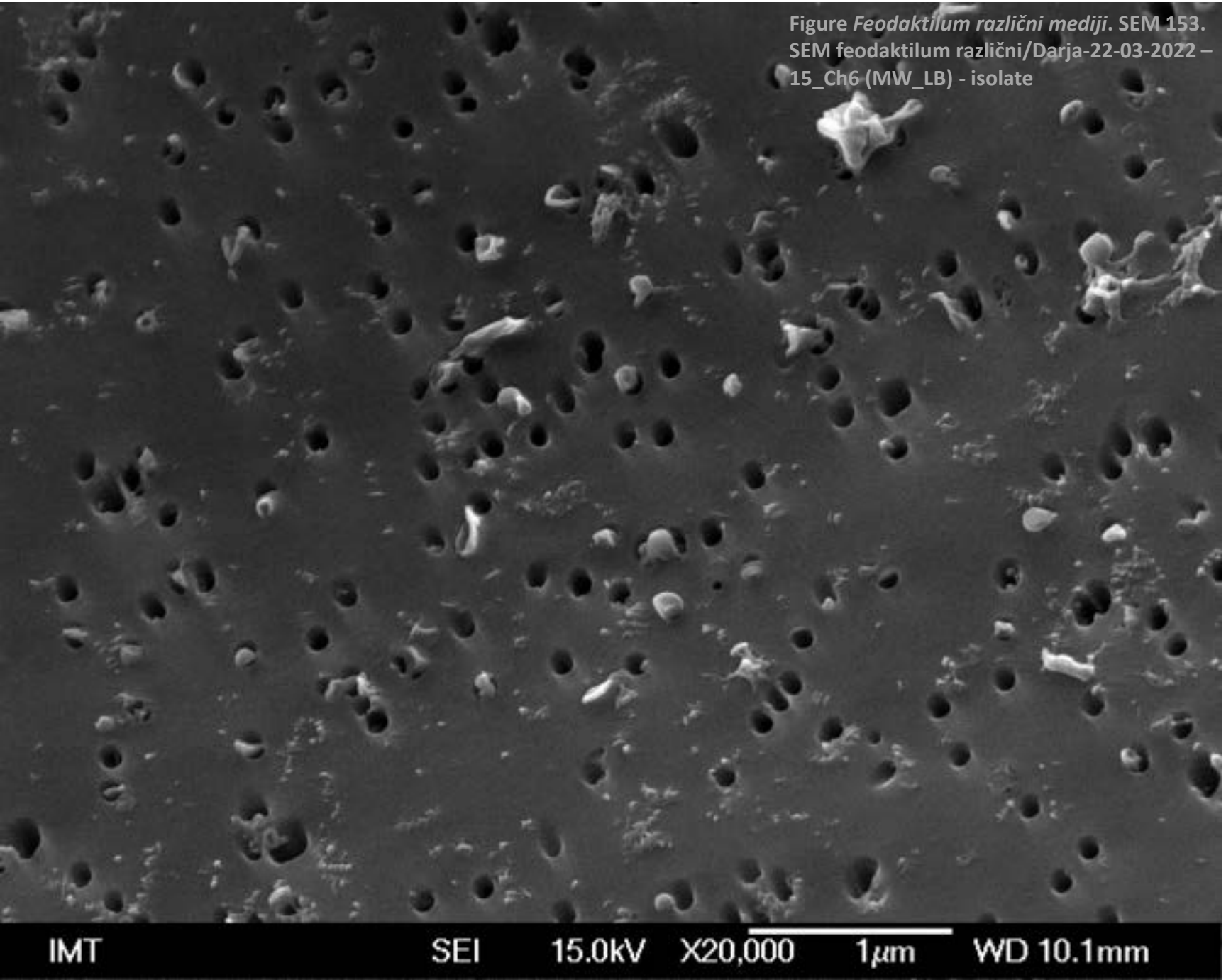

Figure *Phaeodactylum tricornutum* isolate LB SEM 19.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with LB Lennox LB broth (ref. L3022, Sigma Aldrich, USA). Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Isolation of Small Cellular Particles (SCPs)

SCPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

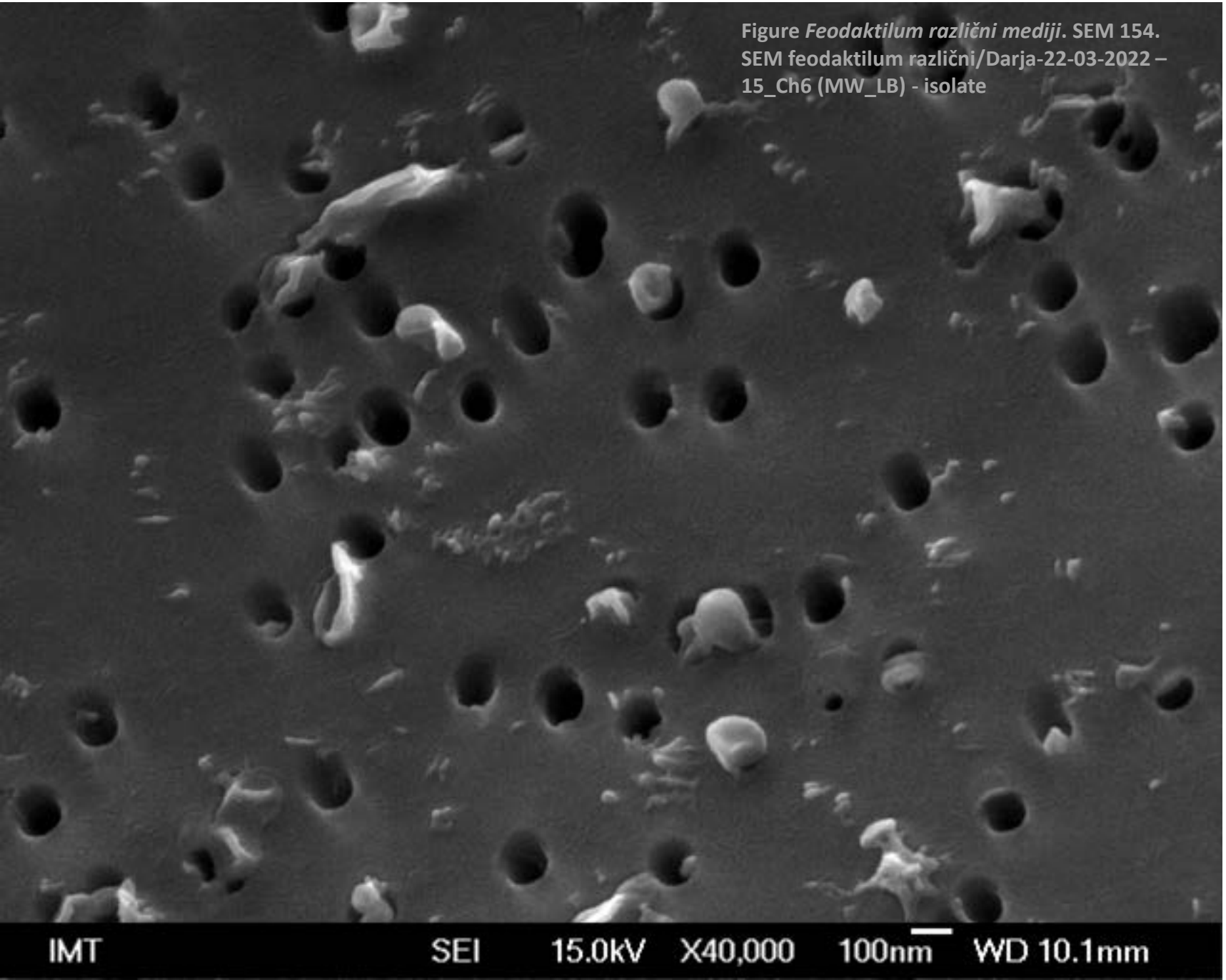

Figure *Phaeodactylum tricornutum* isolate LB SEM 20.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with LB Lennox LB broth (ref. L3022, Sigma Aldrich, USA). Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Isolation of Small Cellular Particles (SCPs)

SCPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

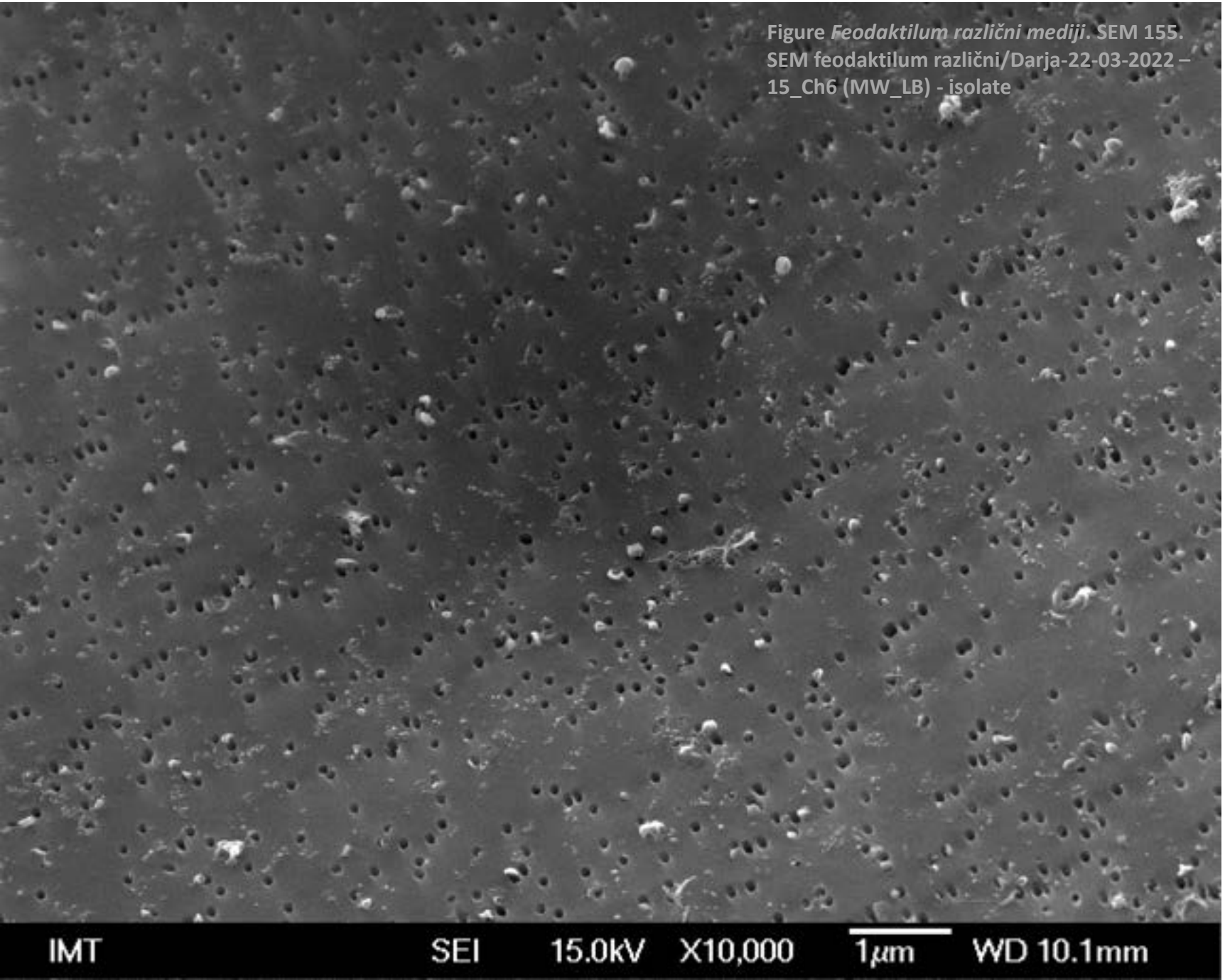

Figure *Phaeodactylum tricornutum* isolate LB SEM 21.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with LB Lennox LB broth (ref. L3022, Sigma Aldrich, USA). Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Isolation of Small Cellular Particles (SCPs)

SCPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

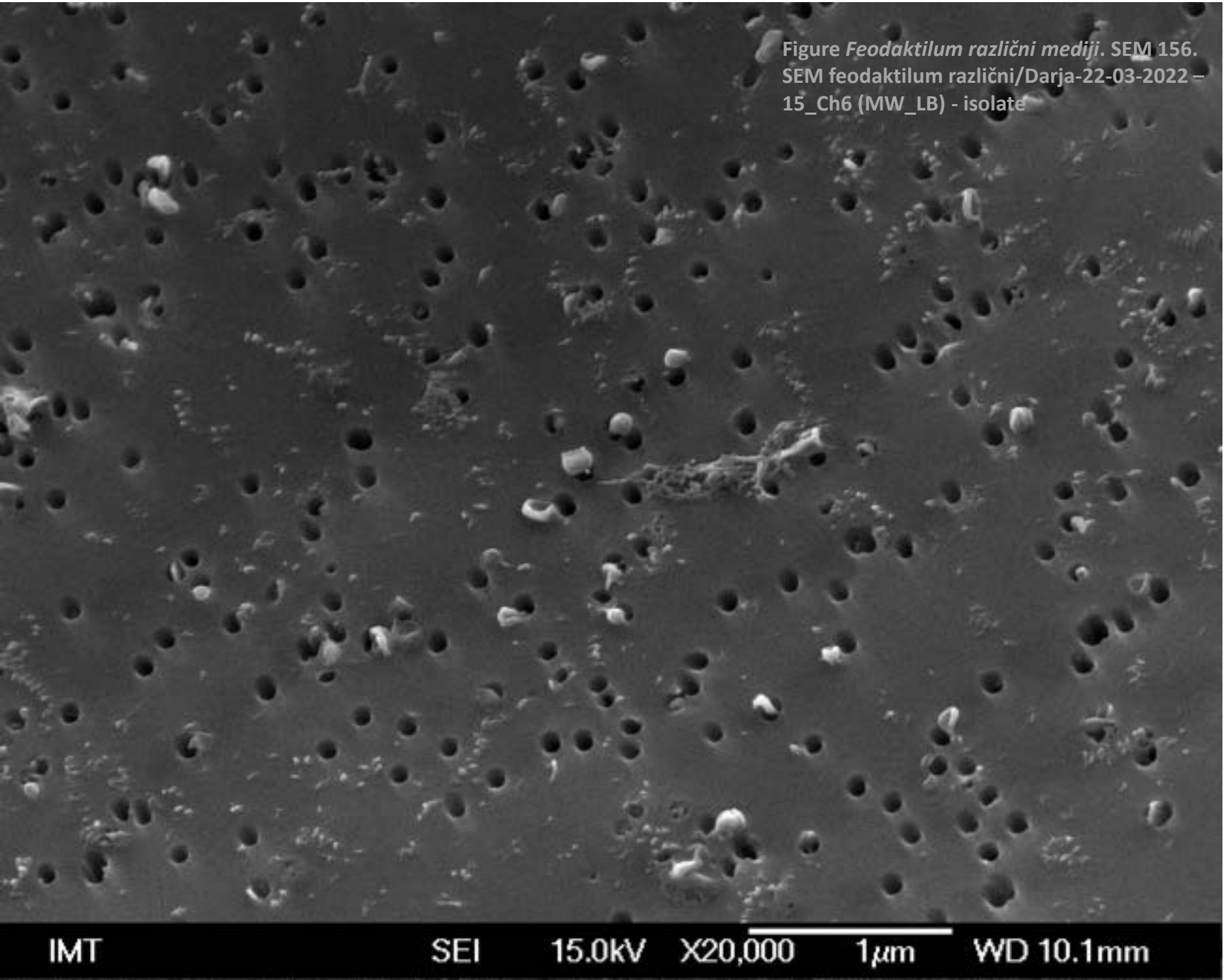

Figure *Phaeodactylum tricornutum* isolate LB SEM 22.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with **Lennox LB broth** (ref. L3022, Sigma Aldrich, USA). Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Isolation of Small Cellular Particles (SCPs)

SCPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

Proceedings of 9th Socratic Lectures **2024**

Repository

Scanning electron microscope images of small cellular particles isolated from *Phaeodactylum tricornutum* conditioned media enriched with Guillard's (F/2) and MW-BG11. Light and dark phases

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Citation: Bedina Zavec A, Božič D, Hočevar M, Iglič A, Jeran M, Kralj-Iglič V, Romolo A. Scanning electron microscope images of small cellular particles isolated from *Phaeodactylum tricornutum* conditioned media, light and dark phases. Proceedings of Socratic Lectures. **2024**, 9, 351-420. https://doi.org/10.55295/PSL.2024.D14

Abstract: Scanning electron microscope images of small cellular particles isolated from *Phaeodactylum tricornutun* conditoned media, light and dark phases are presented. Each image is supplemented by description of the preparation of the sample and the data on the imaging technique and equipment.

The data curators of the repository are Veronika Kralj-Iglič and Anna Romolo. More data on experiments with microalgae small cellular particles can be found in (Adamo et al., 2021), (Picciotto et al., 2021) and (Božič et al., 2022).

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Keywords: Extracellular vesicles, Extracellular particles, Nanoalgosomes, Exosomes

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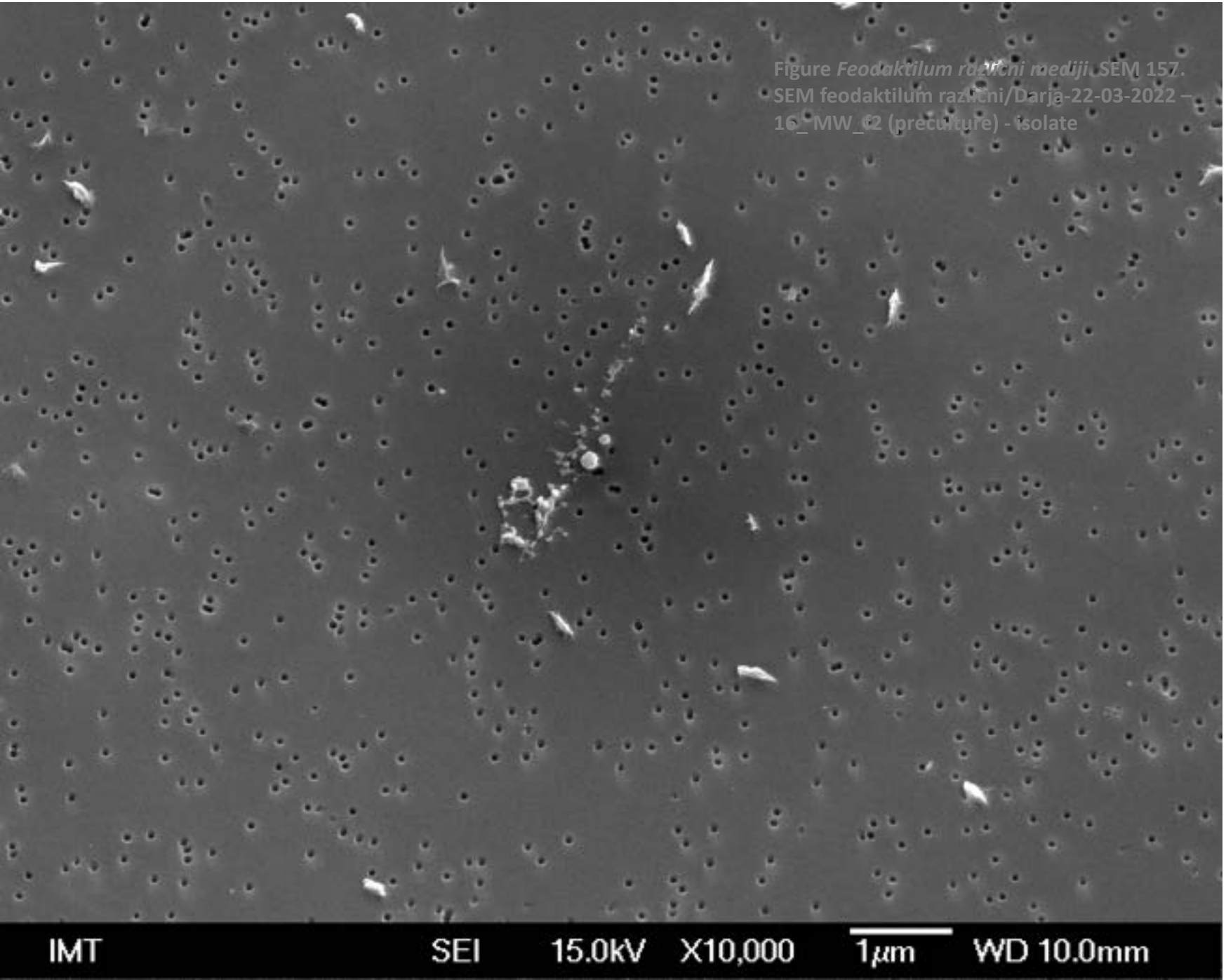

Figure *Phaeodactylum tricornutum* isolate F2 SEM 3.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with **Guillard's (F/2)** Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Isolation of small cellular particles (SCPs)

SCPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

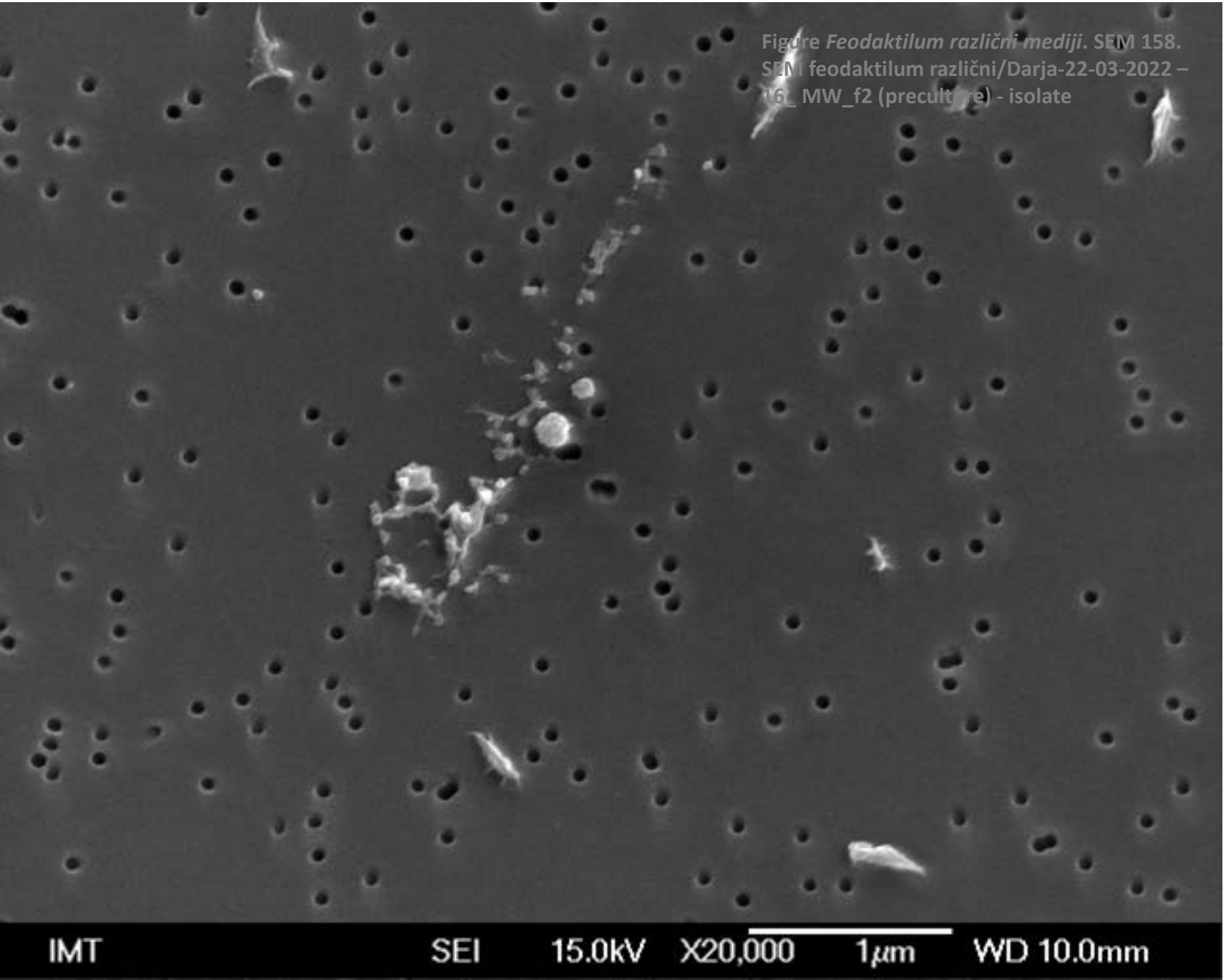

Figure *Phaeodactylum tricornutum* isolate F2 SEM 4.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with **Guillard's (F/2)** Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Isolation of small cellular particles (SCPs)

SCPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

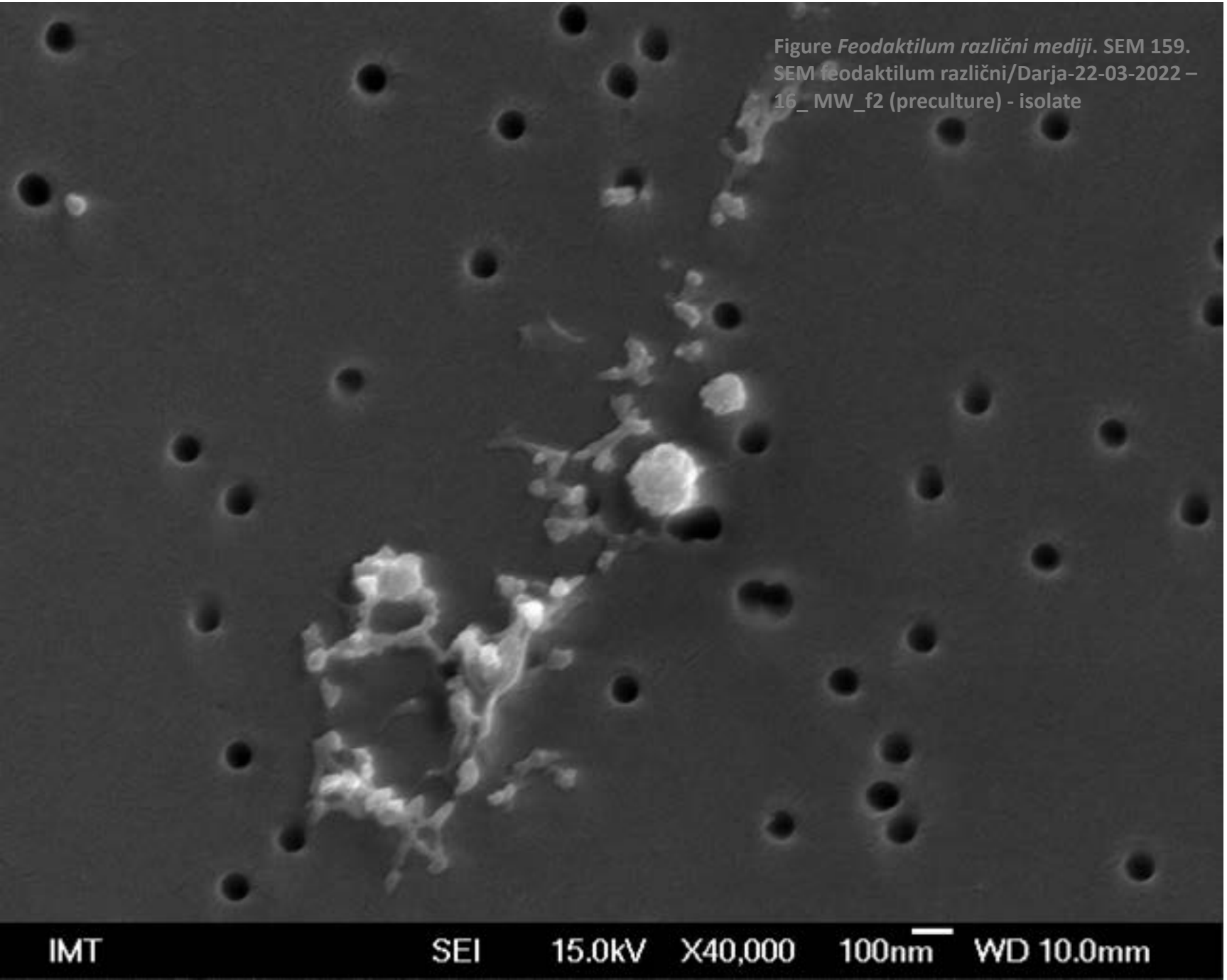

Figure *Phaeodactylum tricornutum* isolate F2 SEM 5.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with **Guillard's (F/2)** Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Isolation of small cellular particles (SCPs)

SCPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

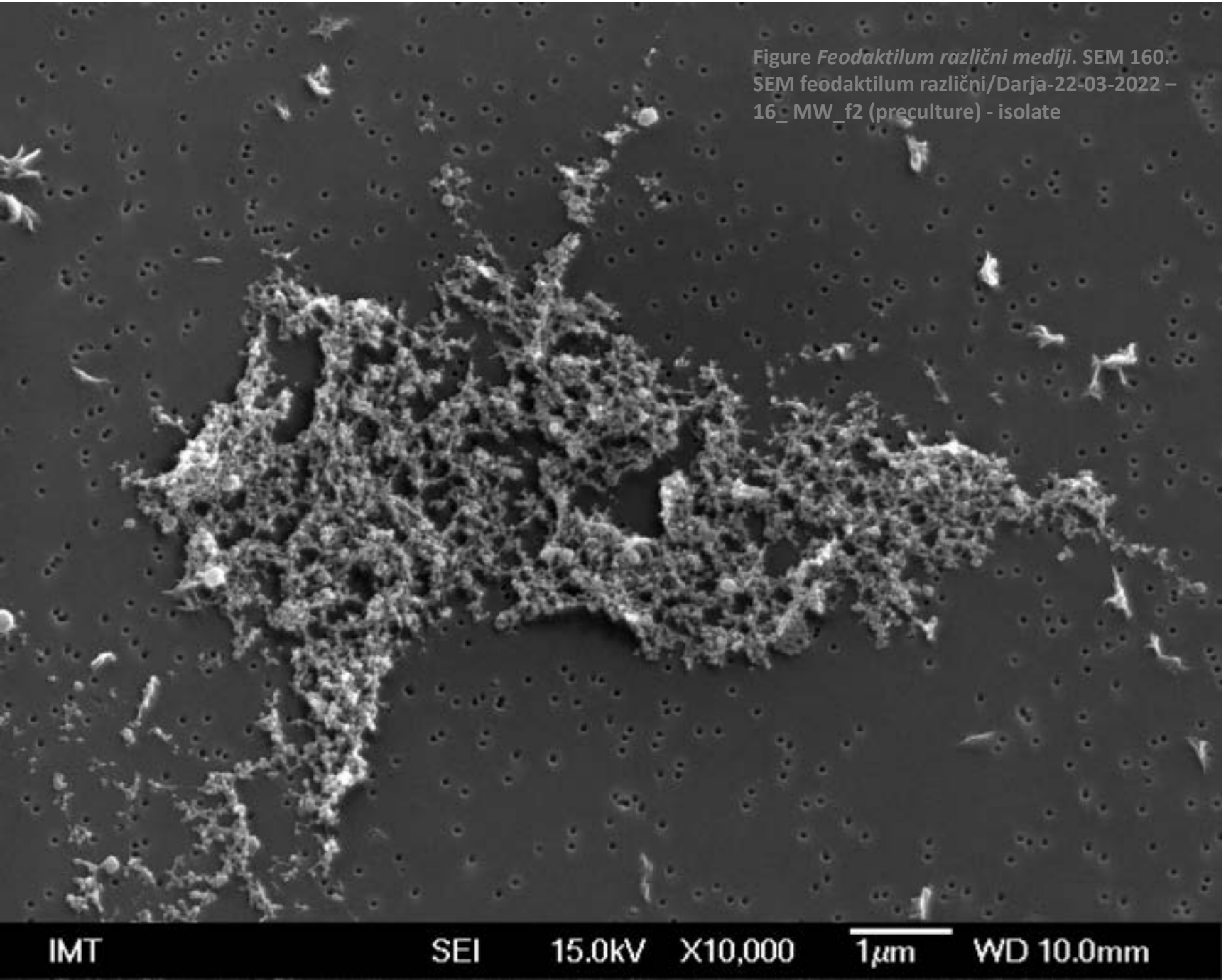

Figure *Phaeodactylum tricornutum* isolate F2 SEM 6.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with **Guillard's (F/2)** Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Isolation of small cellular particles (SCPs)

SCPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

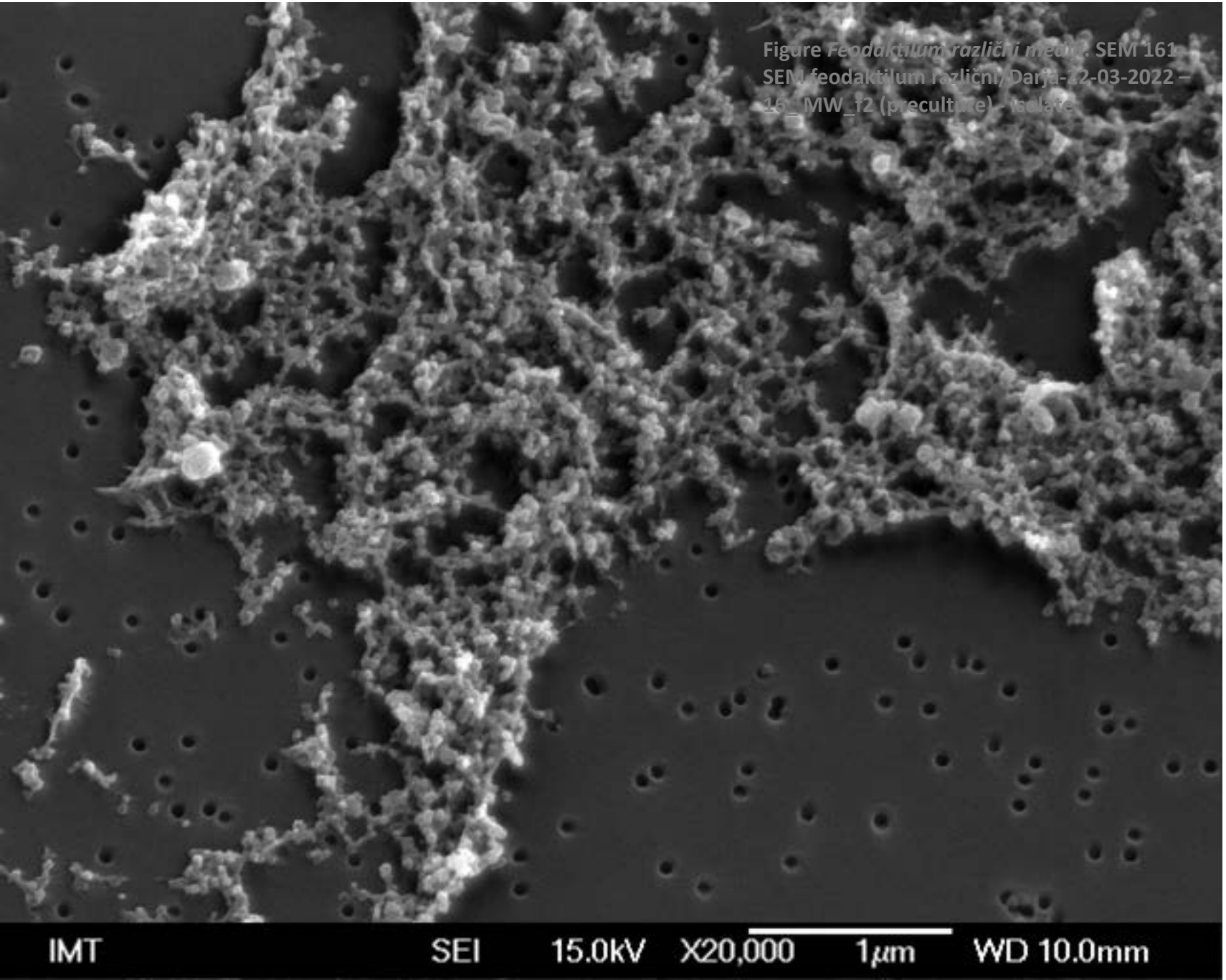

Figure *Phaeodactylum tricornutum* isolate F2 SEM 7.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with **Guillard's (F/2)** Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Isolation of small cellular particles (SCPs)

SCPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

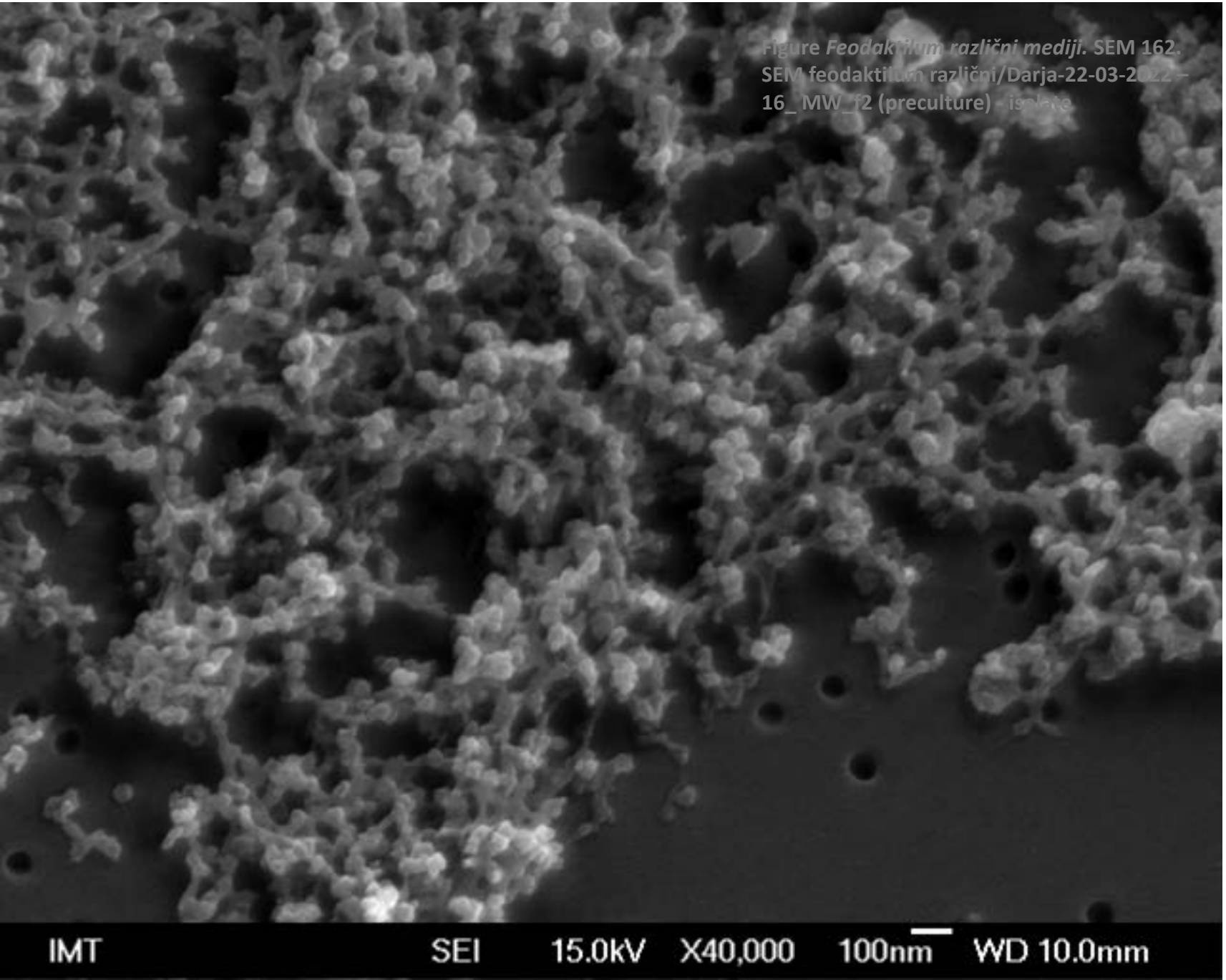

Figure *Phaeodactylum tricornutum* isolate F2 SEM 8.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with **Guillard's (F/2)** Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Isolation of small cellular particles (SCPs)

SCPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

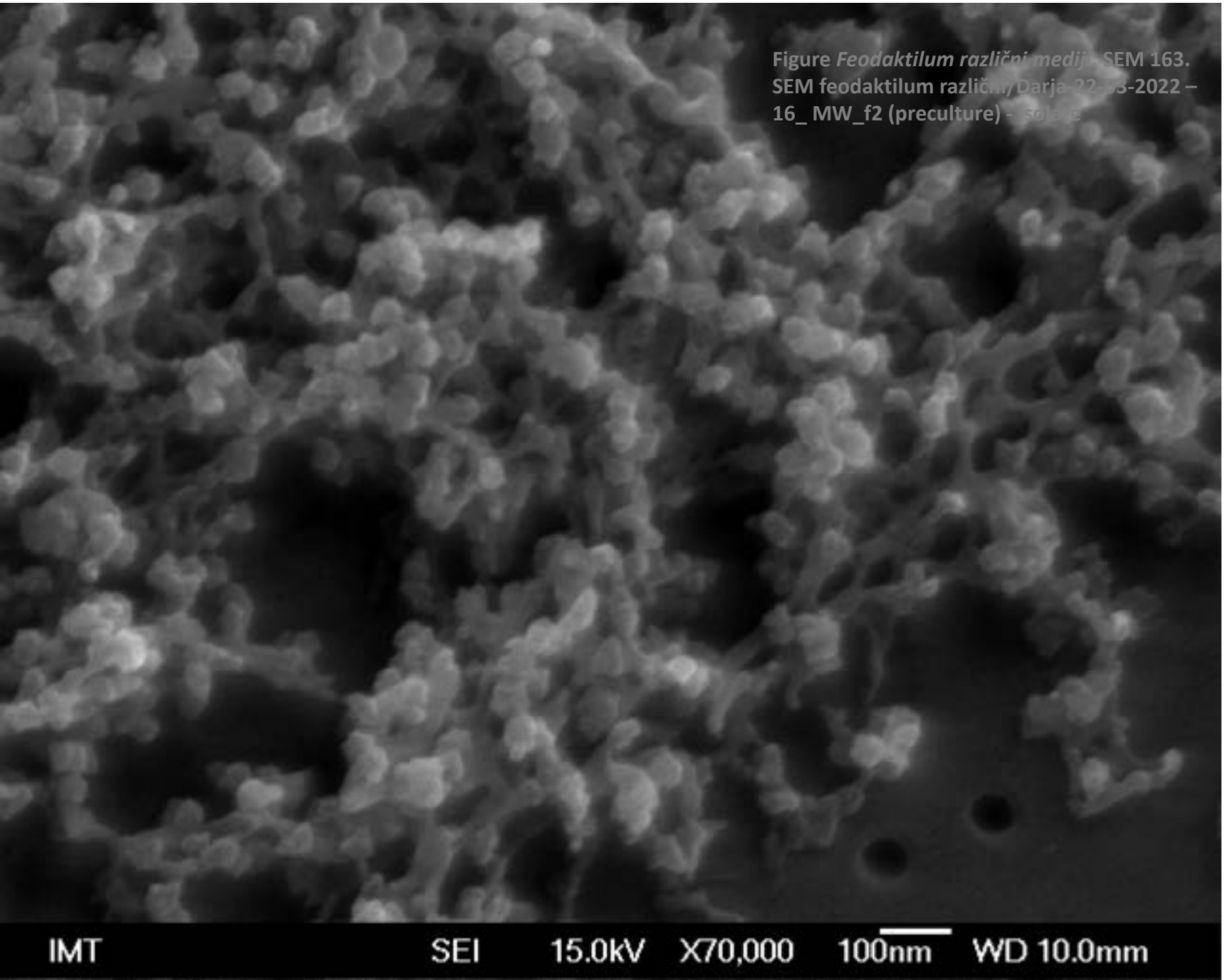

Figure *Phaeodactylum tricornutum* isolate F2 SEM 9.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with **Guillard's (F/2)** Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 µmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Isolation of small cellular particles (SCPs)

SCPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

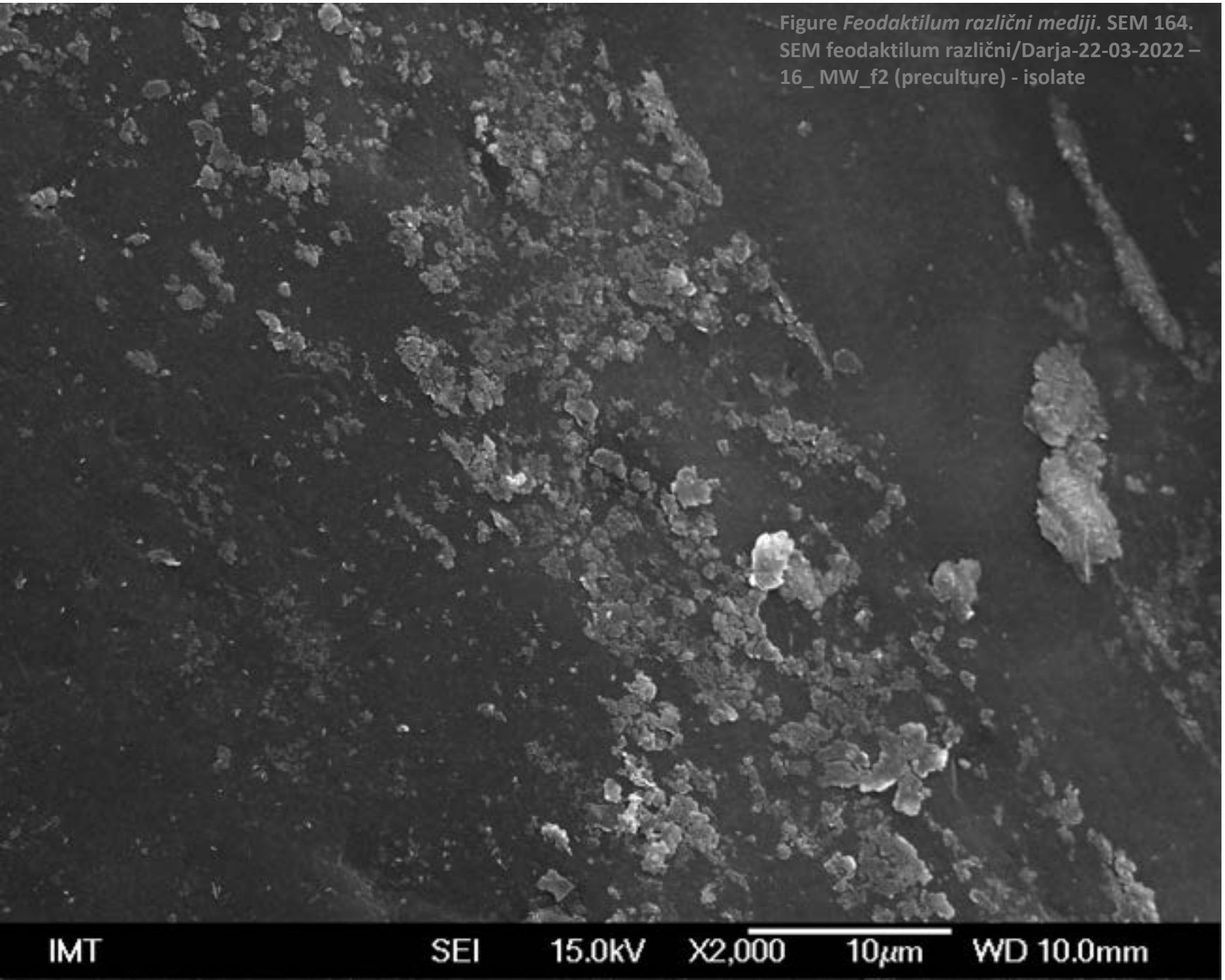

Figure *Phaeodactylum tricornutum* isolate F2 SEM 10.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with **Guillard's (F/2)** Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Isolation of small cellular particles (SCPs)

SCPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

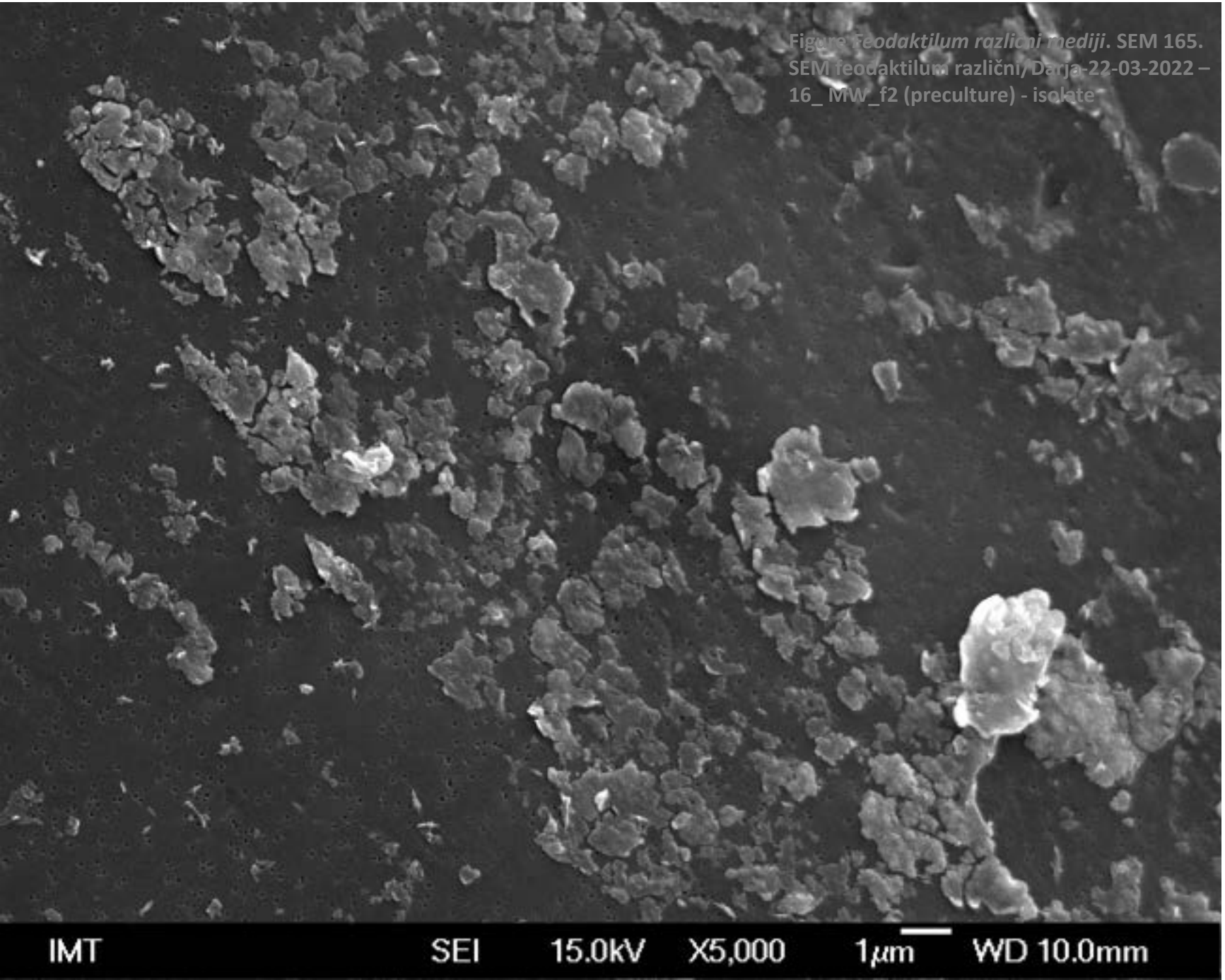

Figure *Phaeodactylum tricornutum* isolate F2 SEM 11.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with **Guillard's (F/2)** Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Isolation of small cellular particles (SCPs)

SCPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

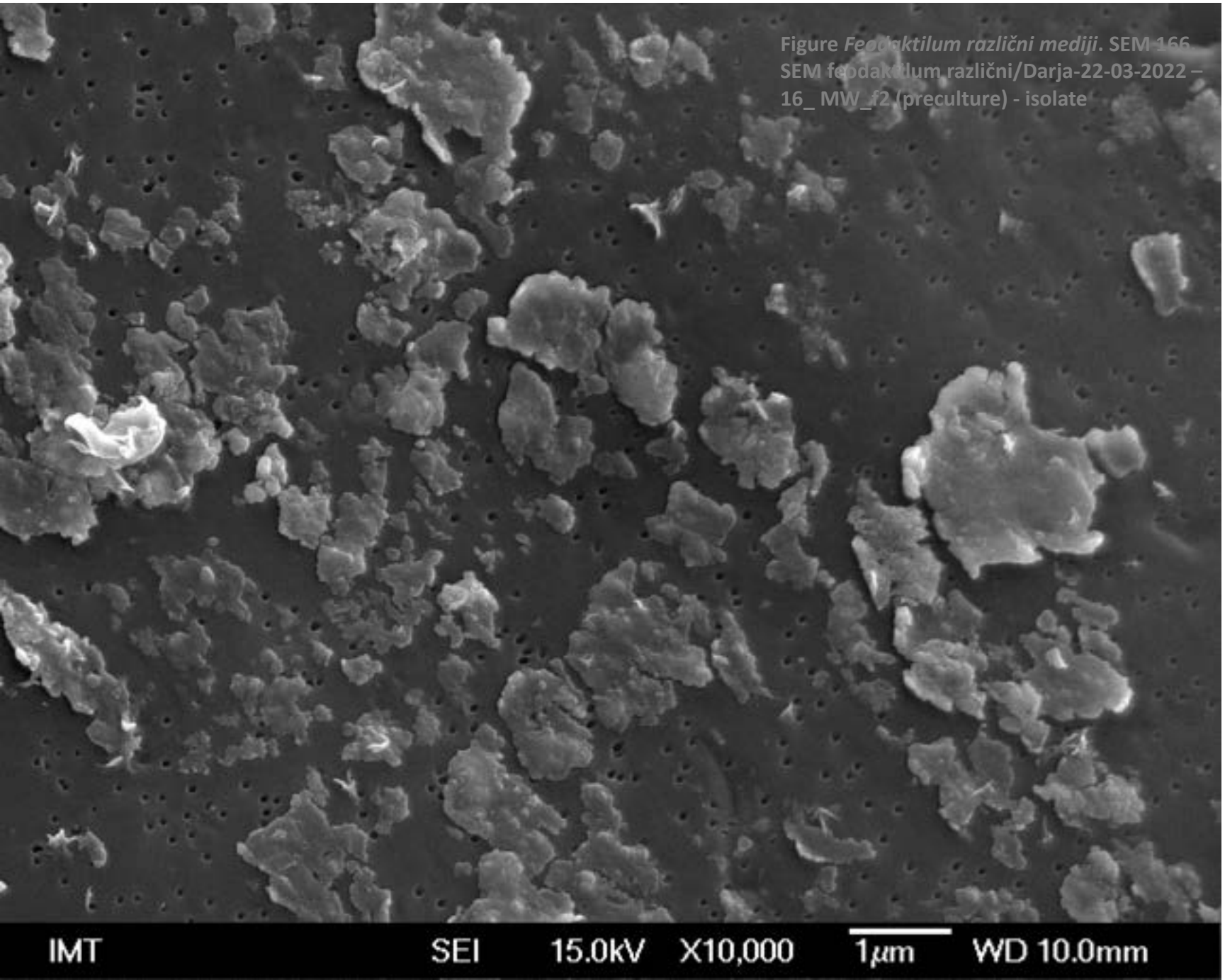

Figure *Phaeodactylum tricornutum* isolate F2 SEM 12.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with **Guillard's (F/2)** Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Isolation of small cellular particles (SCPs)

SCPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

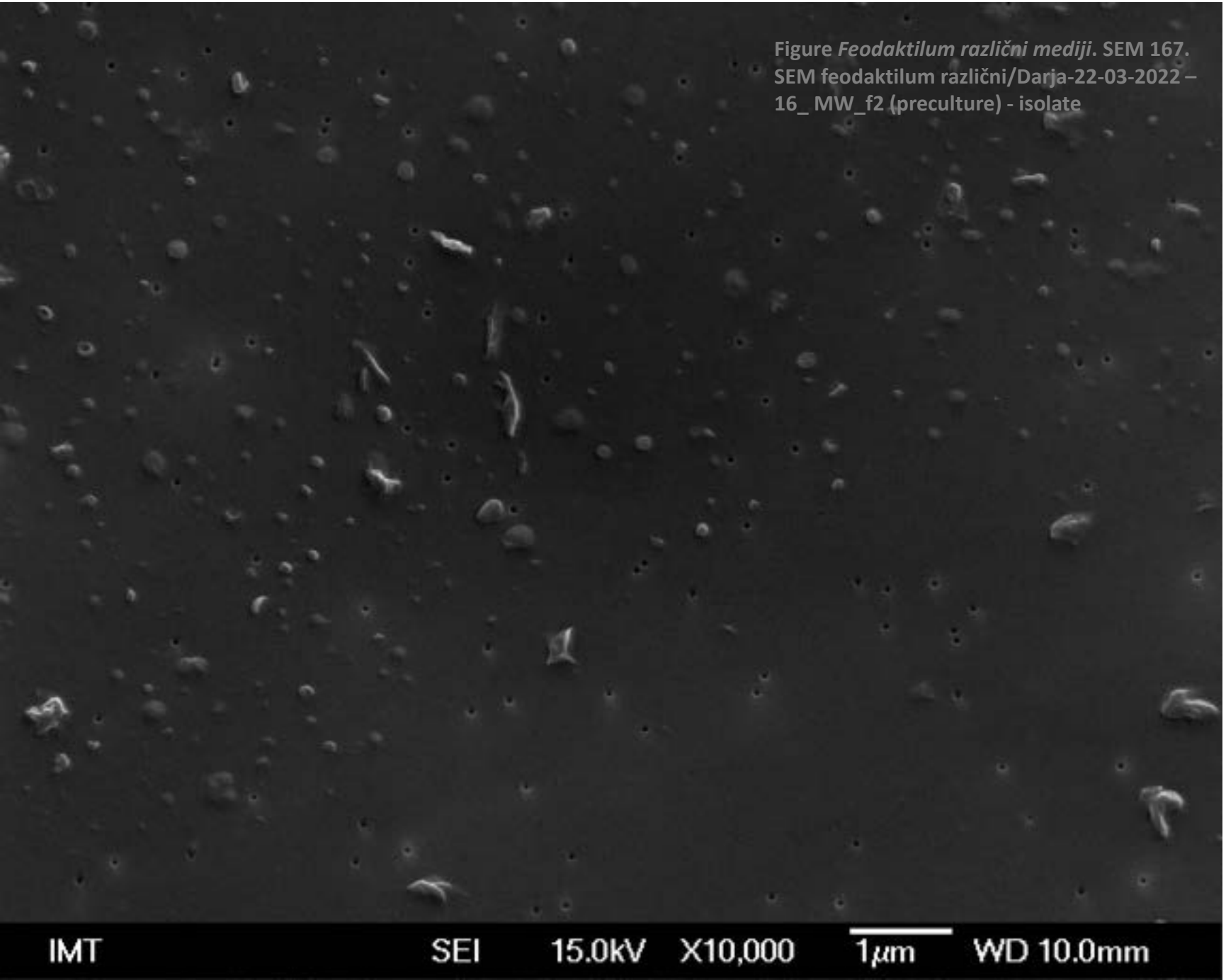

Figure *Phaeodactylum tricornutum* isolate F2 SEM 13.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with **Guillard's (F/2)** Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 µmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Isolation of small cellular particles (SCPs)

SCPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

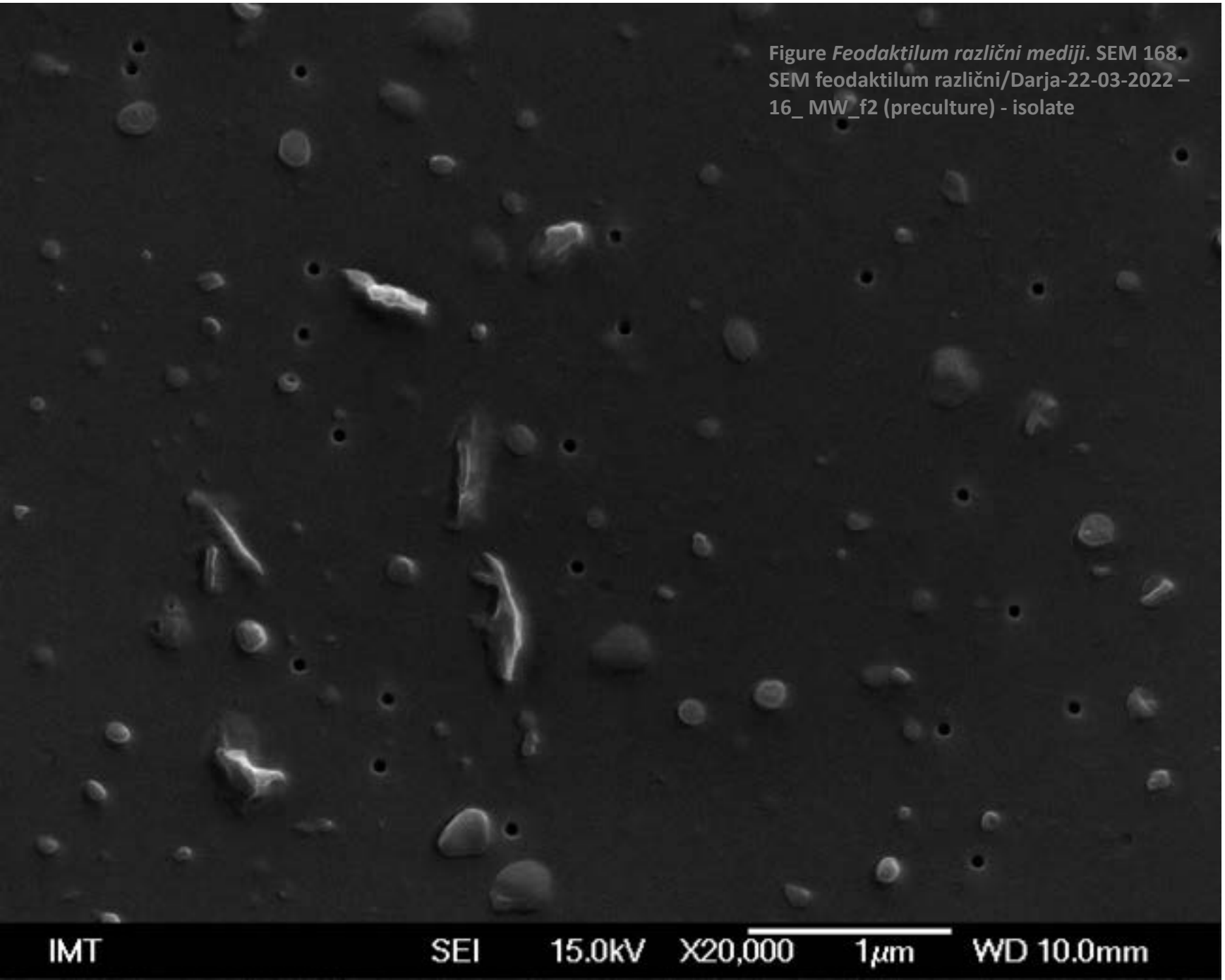

Figure *Feodaktilum različni mediji*. SEM 168. SEM feodaktilum različni/Darja-22-03-2022 – 16_ MW_f2 (preculture) - isolate

Figure *Phaeodactylum tricornutum* isolate F2 SEM 14.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with **Guillard's (F/2)** Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Isolation of small cellular particles (SCPs)

SCPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

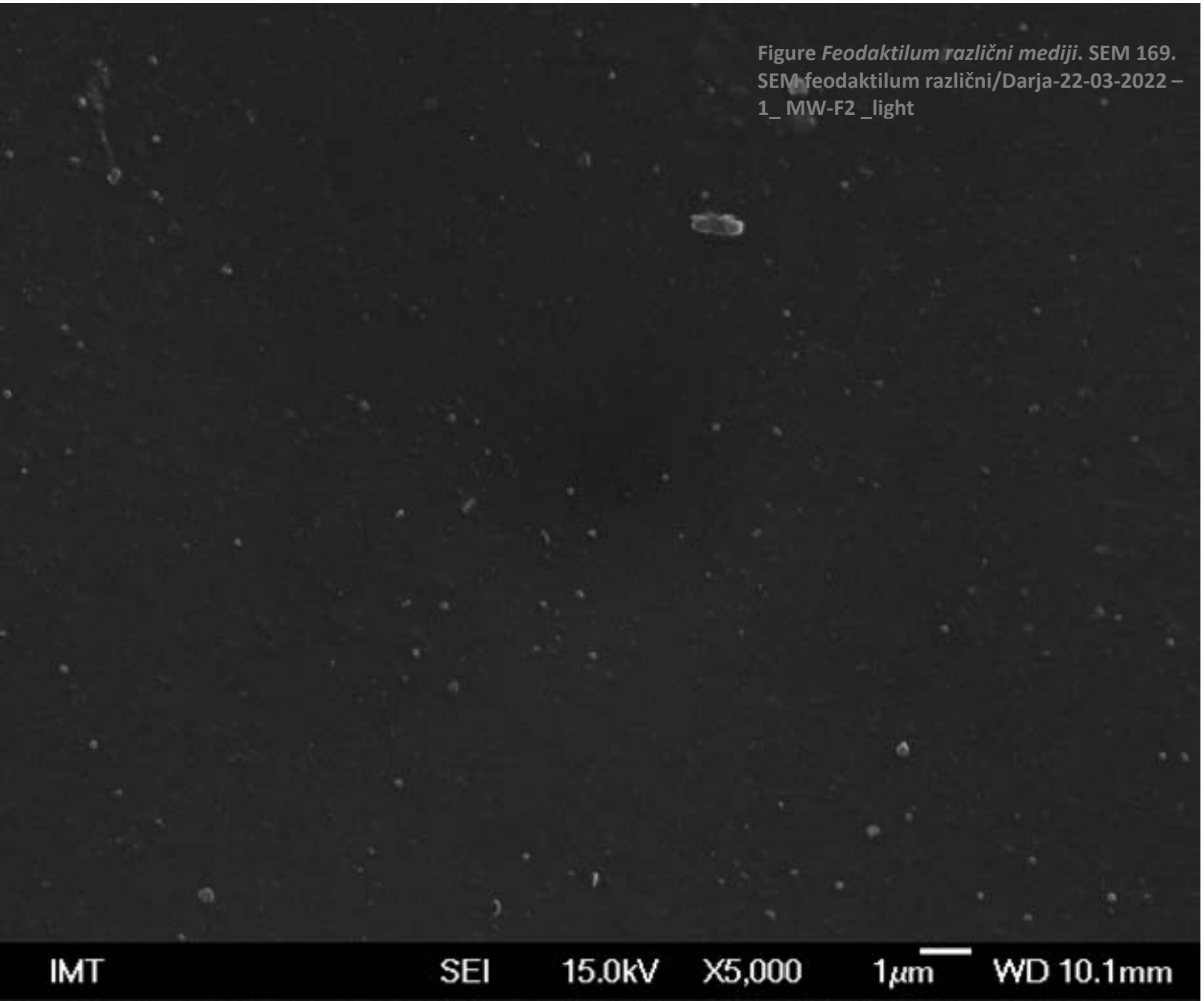

Figure *Phaeodactylum tricornutum* isolate F2 L SEM 15.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Isolation of small cellular particles (SCPs)

SCPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

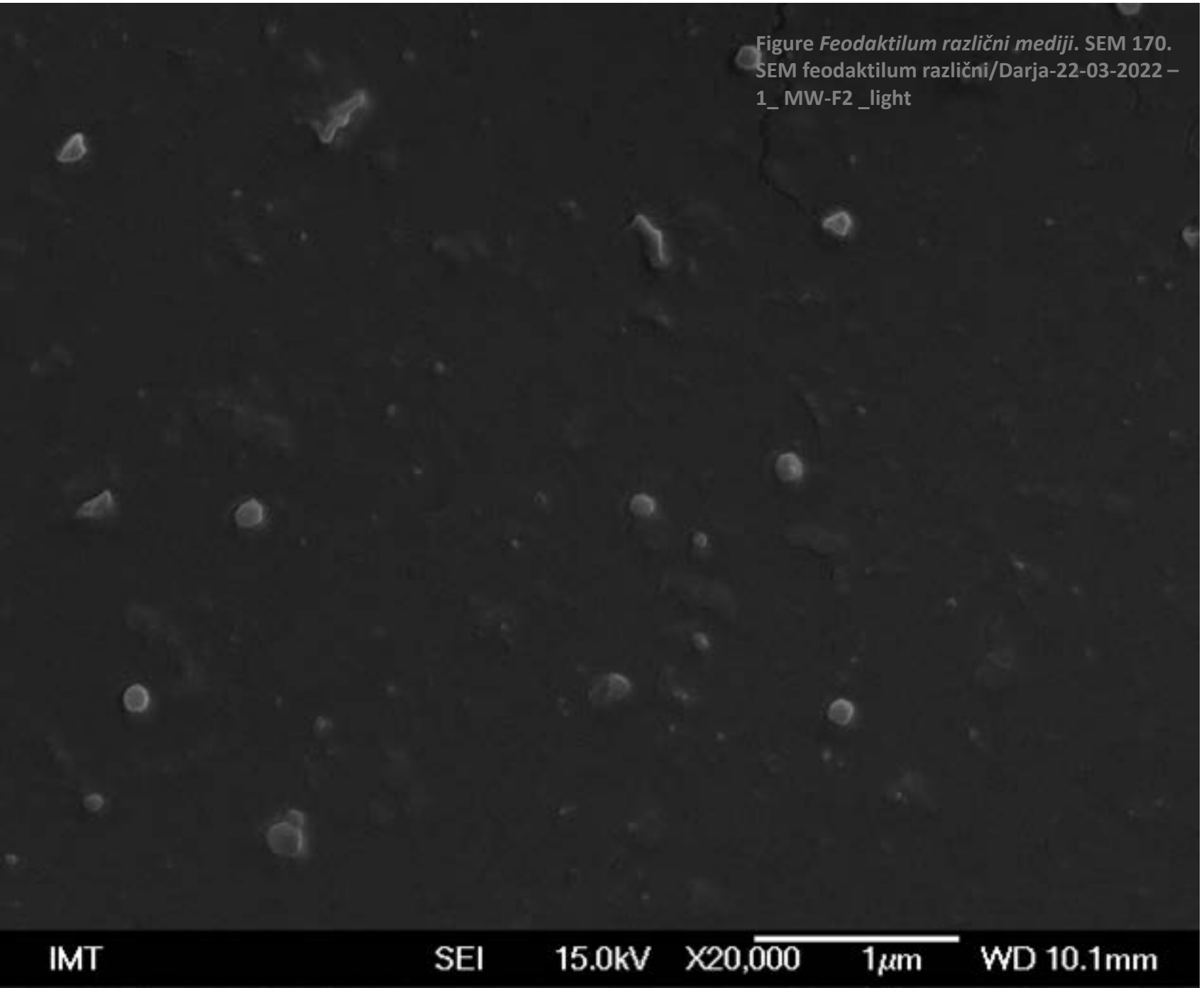

Figure *Phaeodactylum tricornutum* isolate F2 L SEM 16.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with **Guillard's (F/2)** Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Isolation of small cellular particles (SCPs)

SCPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

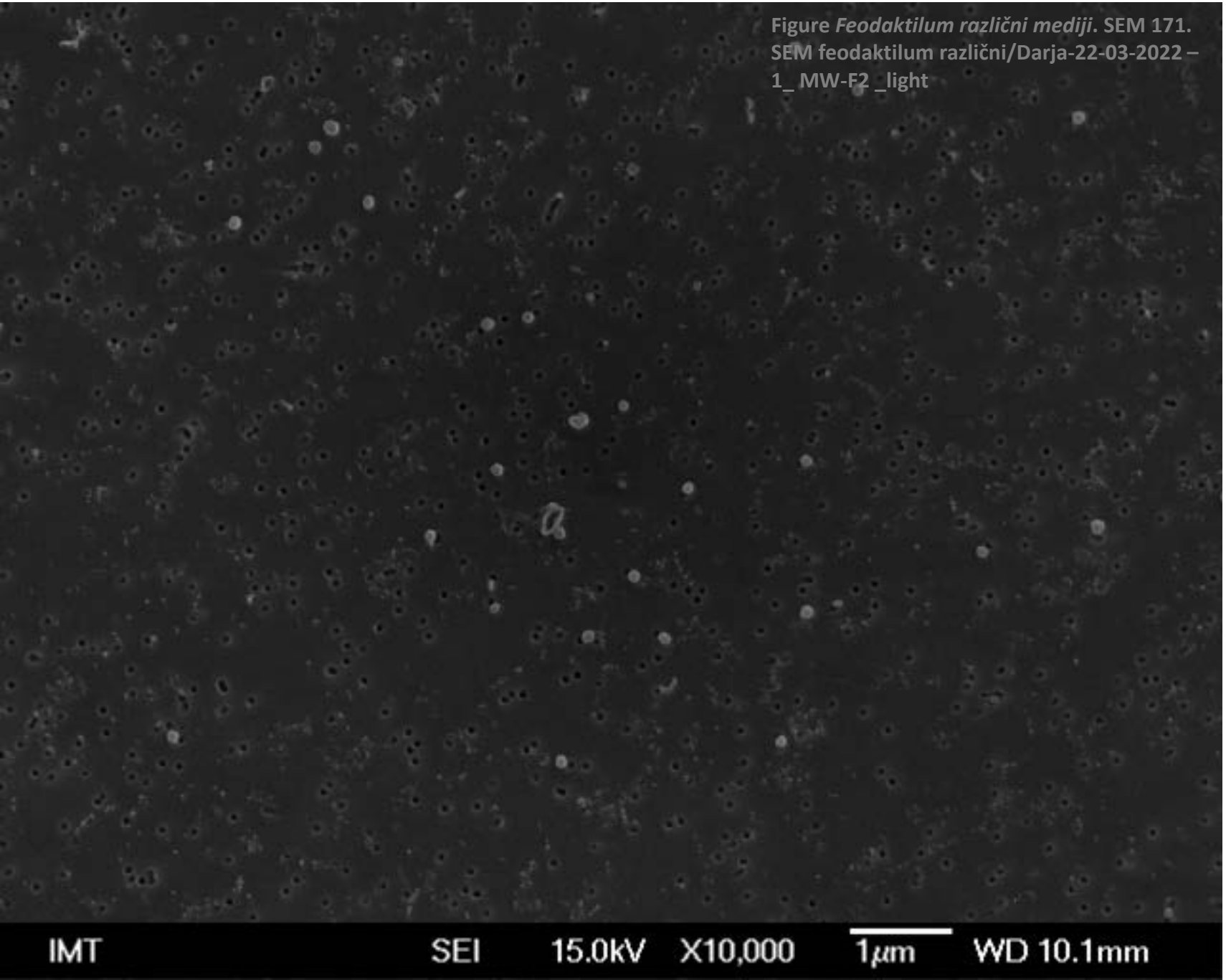

Figure *Phaeodactylum tricornutum* isolate F2 L SEM 17.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with **Guillard's (F/2)** Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Isolation of small cellular particles (SCPs)

SCPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

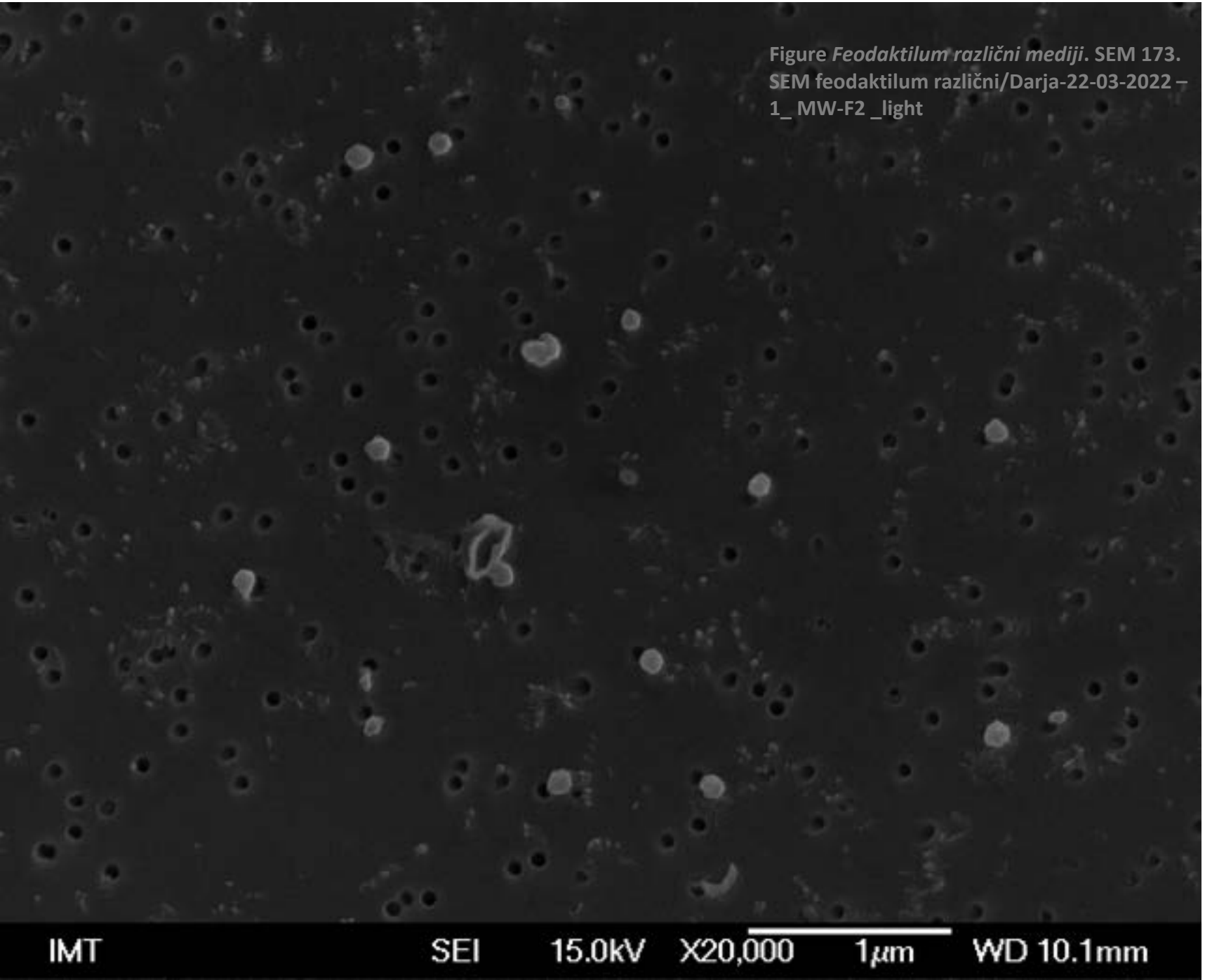

Figure *Phaeodactylum tricornutum* isolate F2 L SEM 18.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with **Guillard's (F/2)** Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 µmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Isolation of small cellular particles (SCPs)

SCPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

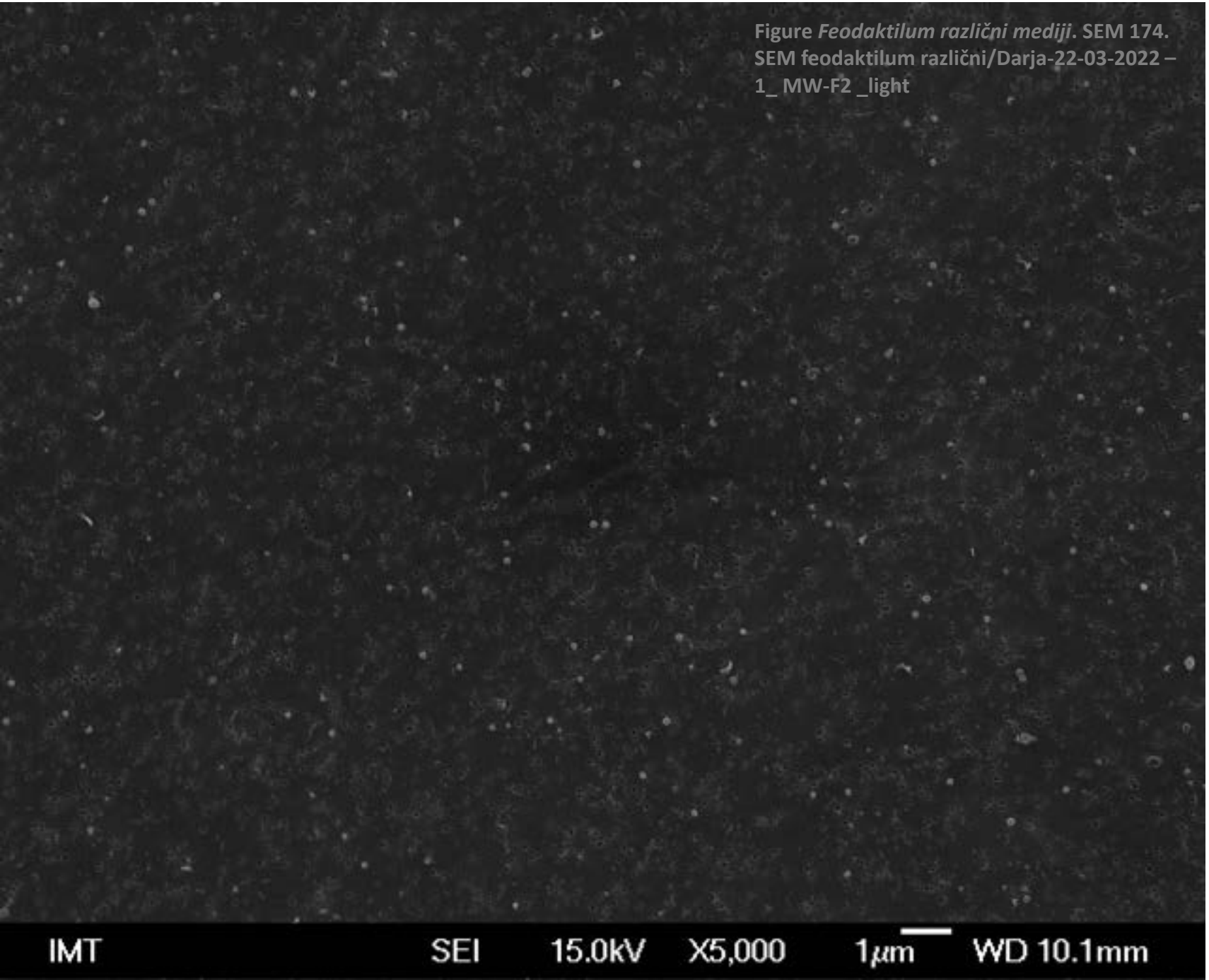

Figure *Feodaktilum različni mediji*. SEM 174. SEM feodaktilum različni/Darja-22-03-2022 – 1_ MW-F2 _light

Figure *Phaeodactylum tricornutum* isolate F2 L SEM 19.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with **Guillard's (F/2)** Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Isolation of small cellular particles (SCPs)

SCPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

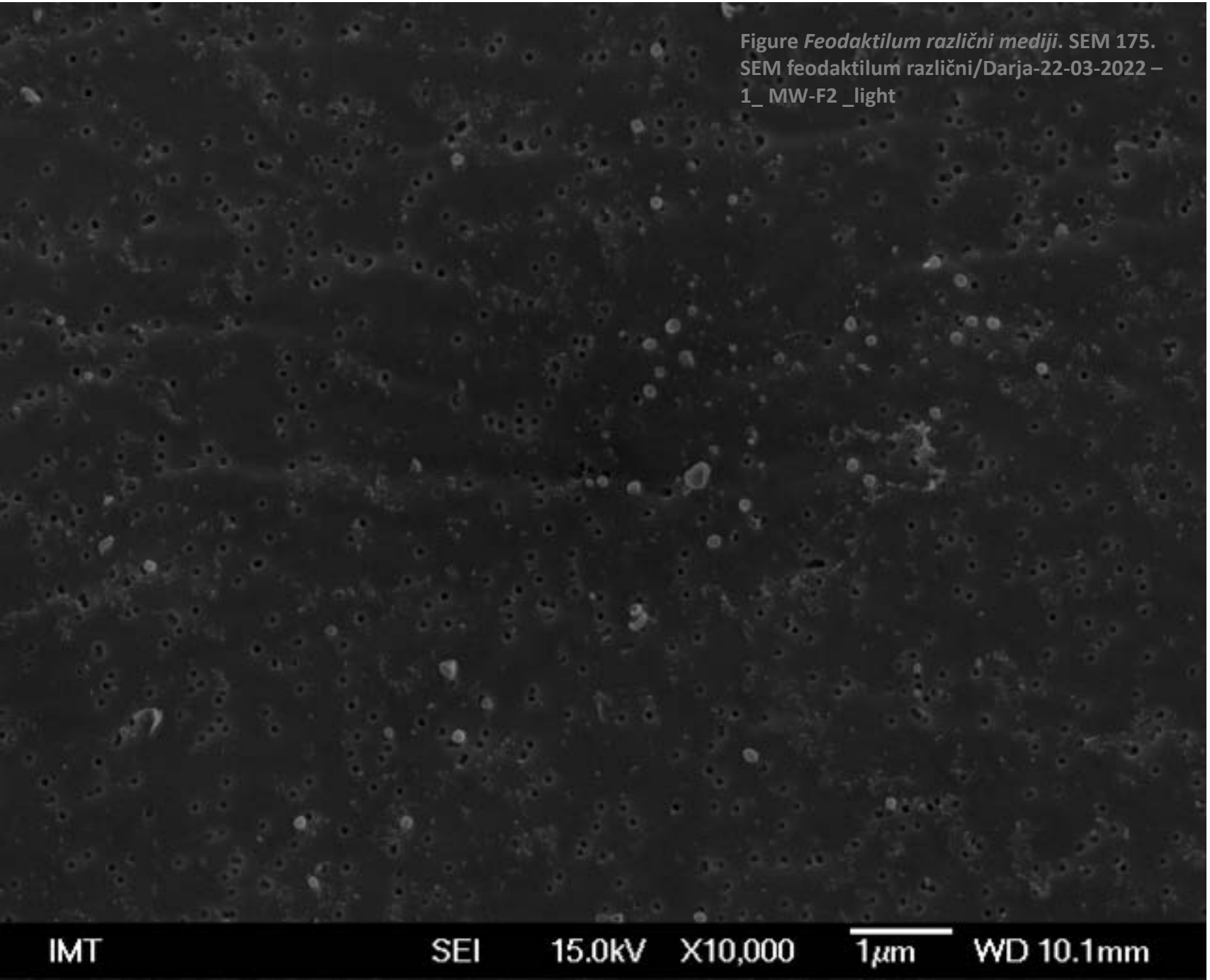

Figure *Phaeodactylum tricornutum* isolate F2 L SEM 20.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with **Guillard's (F/2)** Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Isolation of small cellular particles (SCPs)

SCPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

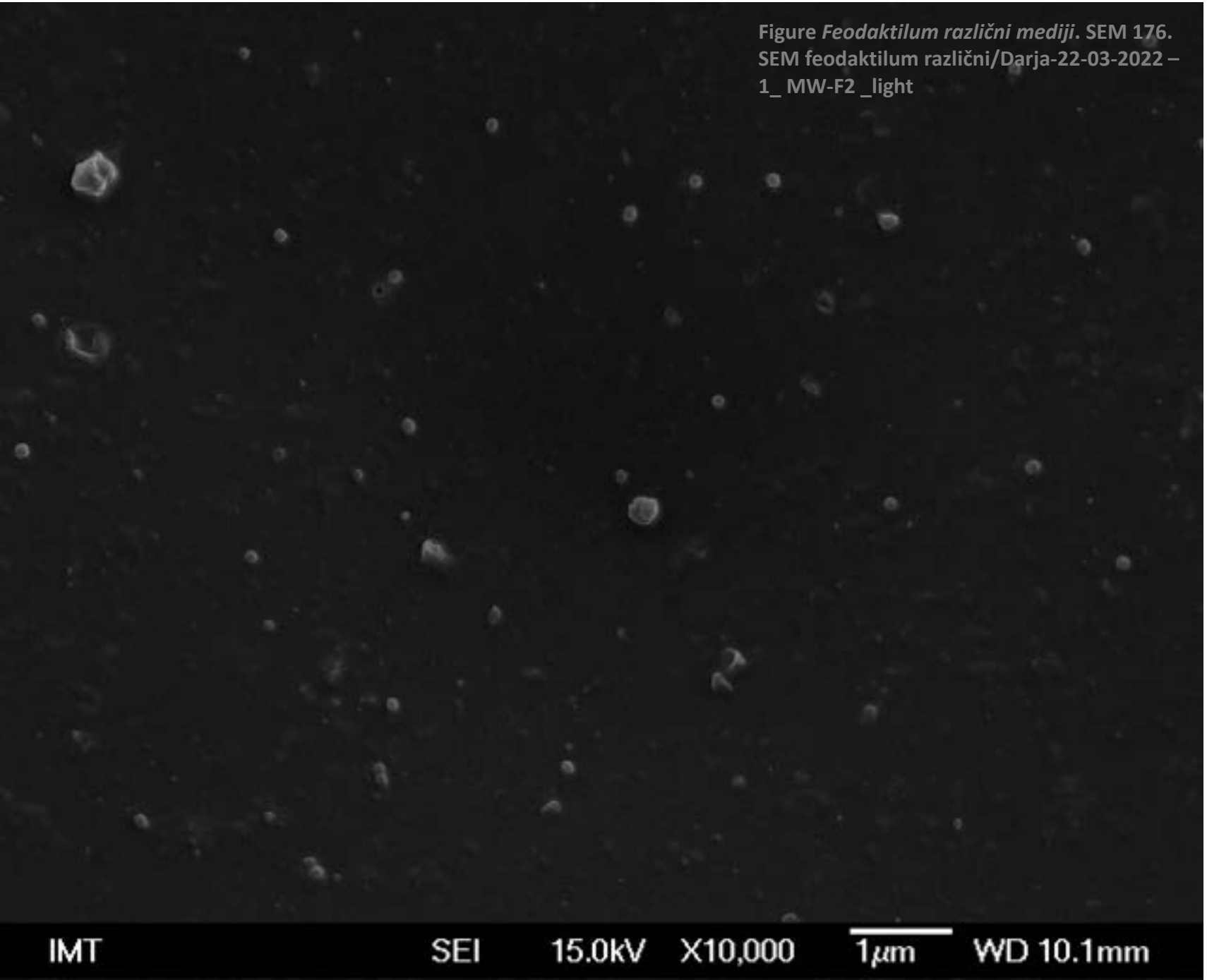

Figure *Phaeodactylum tricornutum* isolate F2 SEM L 21.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with **Guillard's (F/2)** Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Isolation of small cellular particles (SCPs)

SCPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

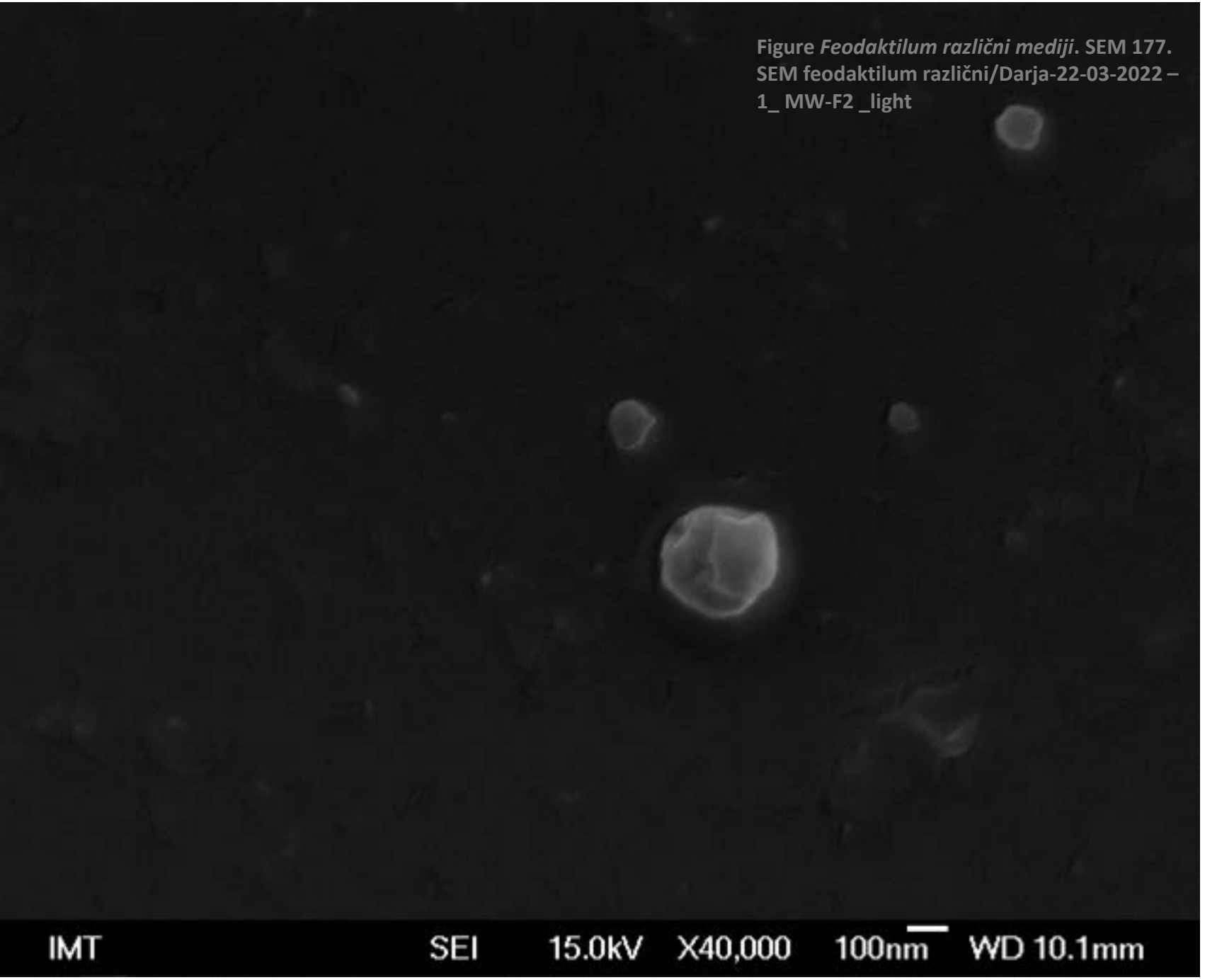

Figure *Phaeodactylum tricornutum* isolate F2 L SEM 22.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with **Guillard's (F/2)** Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 µmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Isolation of small cellular particles (SCPs)

SCPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

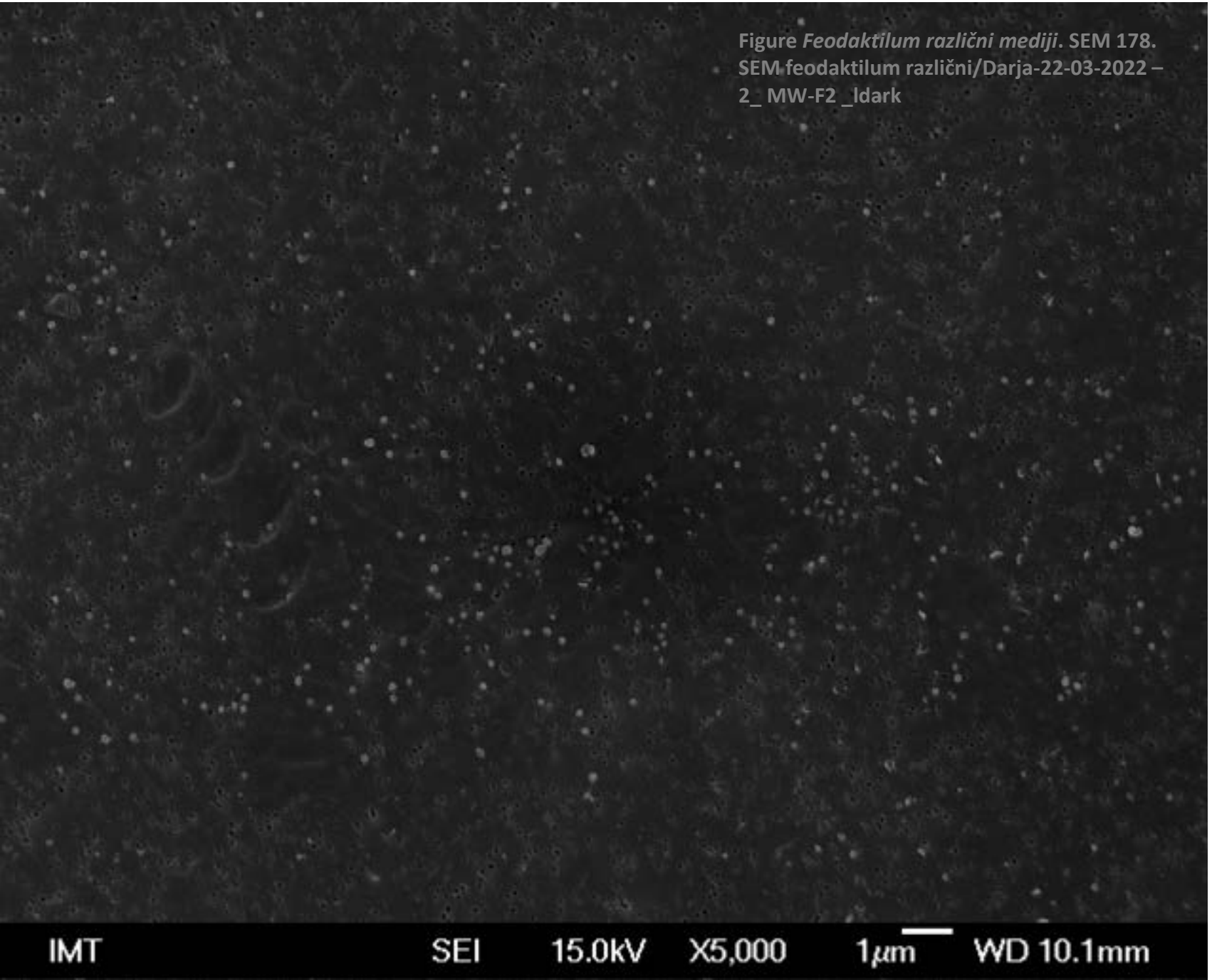

Figure *Phaeodactylum tricornutum* isolate F2 D SEM 23.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with **Guillard's (F/2)** Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 12-hour light / 12-hour dark cycle, with aeration of 0.2 L/min.

Isolation of small cellular particles (SCPs)

SCPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

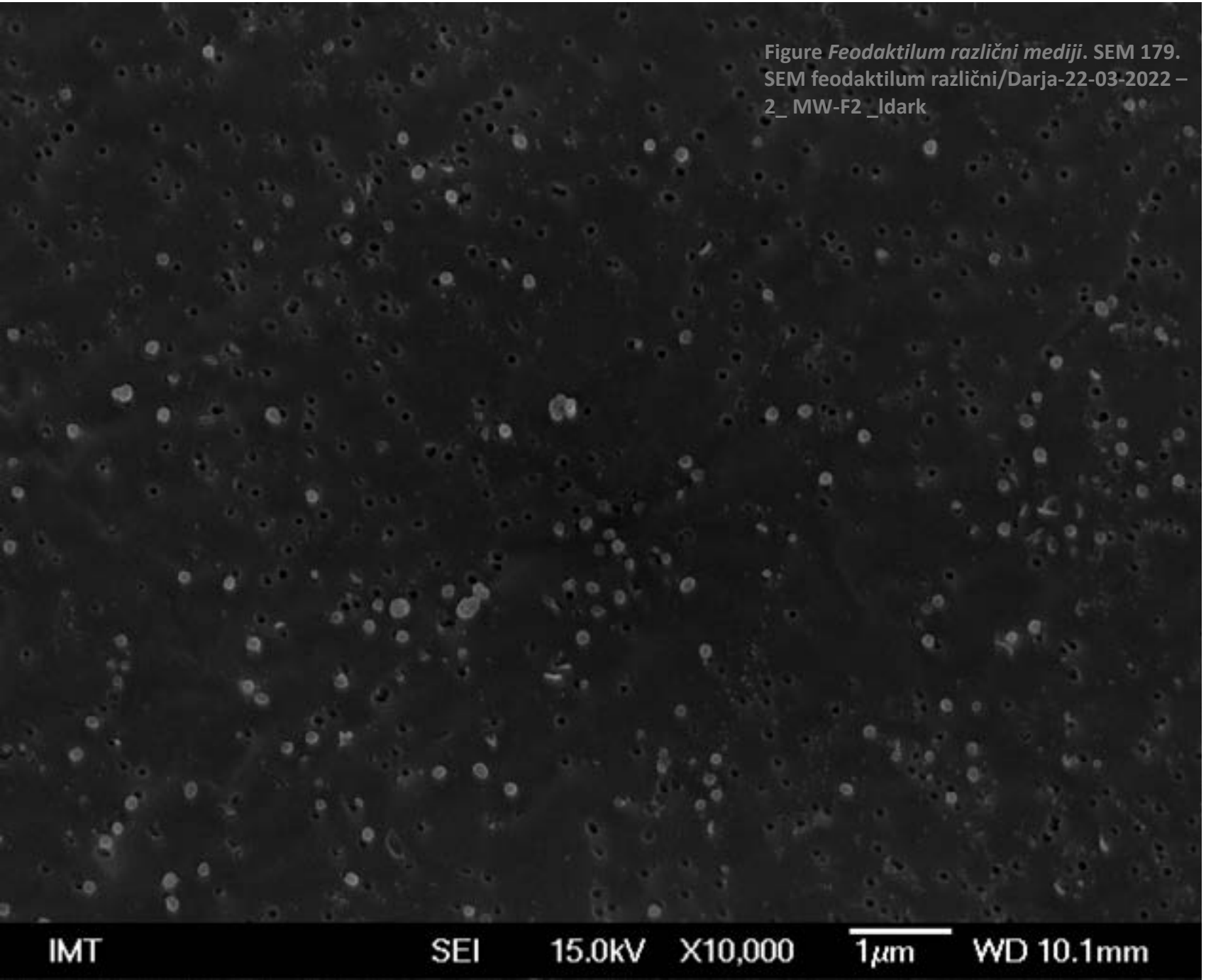

Figure *Phaeodactylum tricornutum* isolate F2 D SEM 24.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with **Guillard's (F/2)** Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 12-hour light / 12-hour dark cycle, with aeration of 0.2 L/min.

Isolation of small cellular particles (SCPs)

SCPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

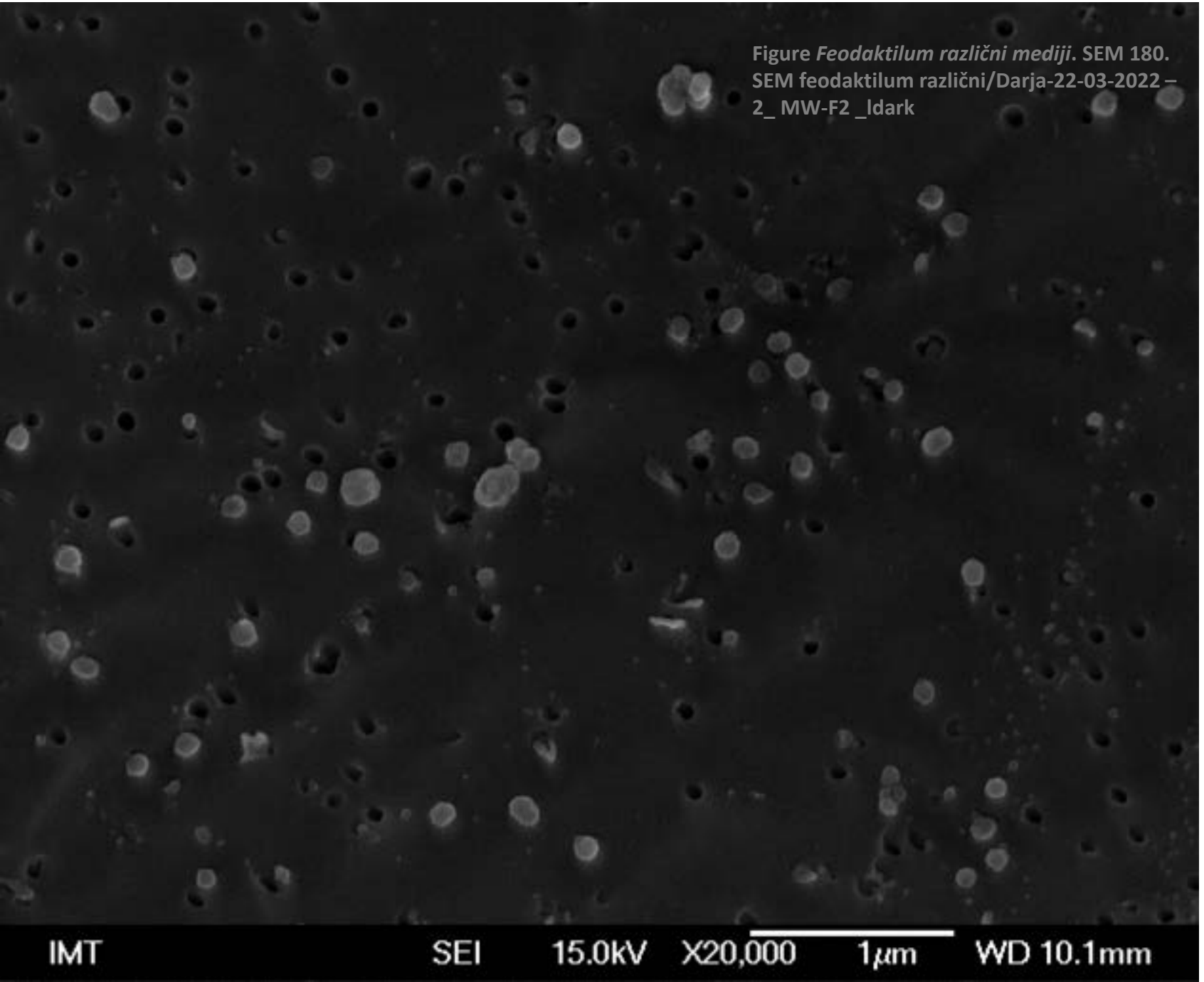

Figure *Phaeodactylum tricornutum* isolate F2 D SEM 25.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with **Guillard's (F/2)** Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 12-hour light / 12-hour dark cycle, with aeration of 0.2 L/min.

Isolation of small cellular particles (SCPs)

SCPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

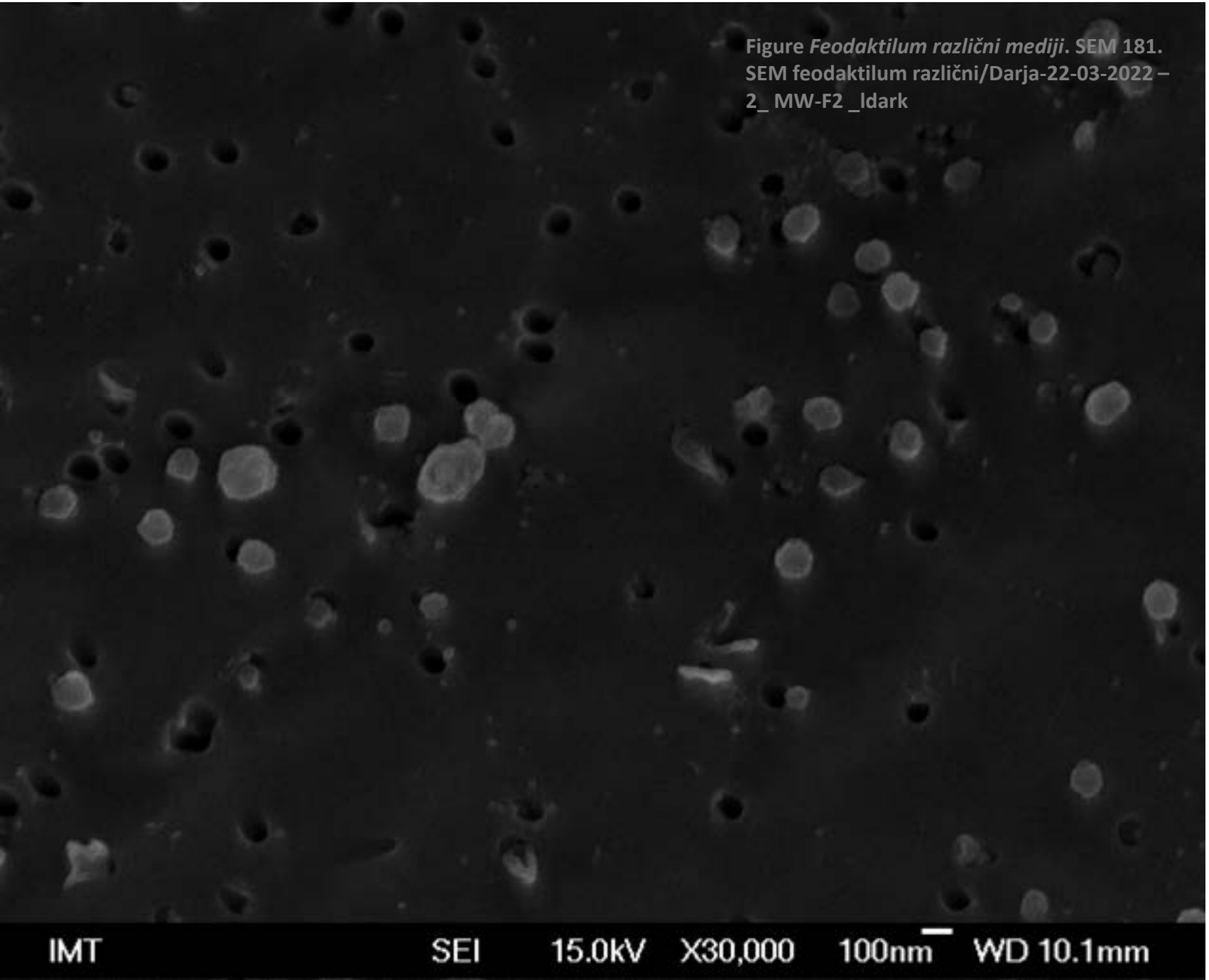

Figure *Phaeodactylum tricornutum* isolate F2 D SEM 26.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with **Guillard's (F/2)** Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 12-hour light / 12-hour dark cycle, with aeration of 0.2 L/min.

Isolation of small cellular particles (SCPs)

SCPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

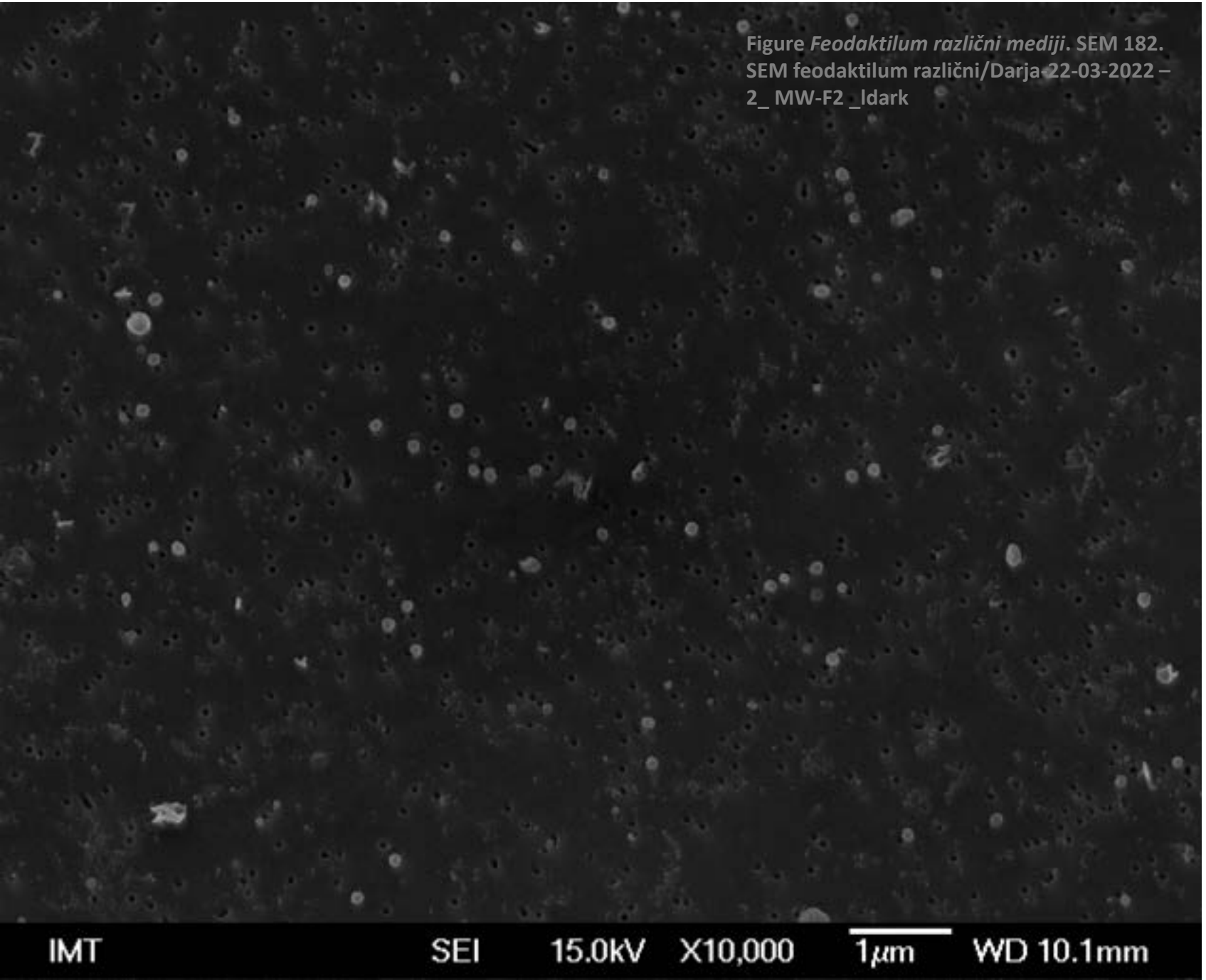

Figure *Phaeodactylum tricornutum* isolate F2 D SEM 27.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with **Guillard's (F/2)** Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 12-hour light / 12-hour dark cycle, with aeration of 0.2 L/min.

Isolation of small cellular particles (SCPs)

SCPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

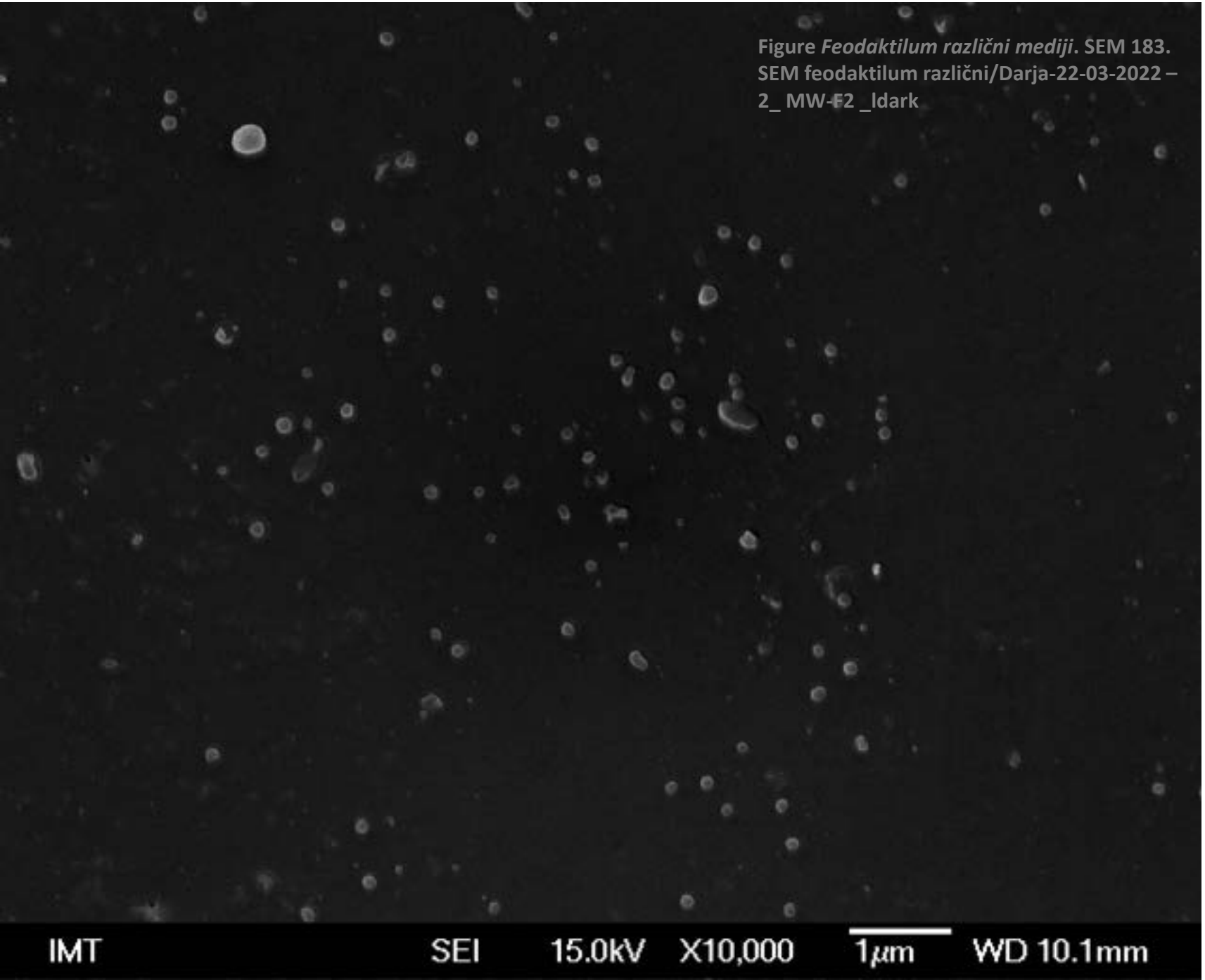

Figure *Phaeodactylum tricornutum* isolate F2 D SEM 28.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with **Guillard's (F/2)** Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 12-hour light / 12-hour dark cycle, with aeration of 0.2 L/min.

Isolation of small cellular particles (SCPs)

SCPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

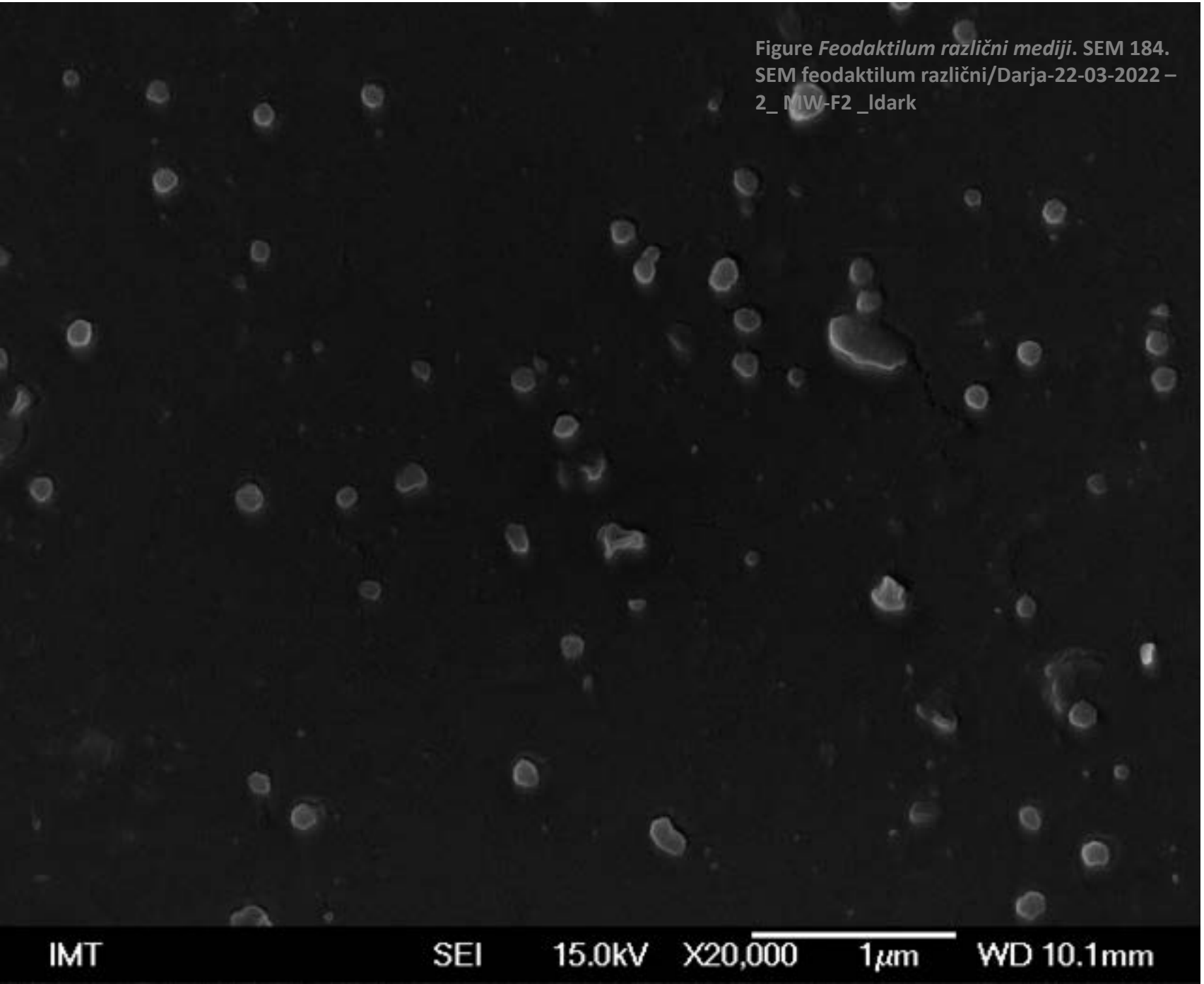

Figure *Phaeodactylum tricornutum* isolate F2 D SEM 29.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with **Guillard's (F/2)** Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 12-hour light / 12-hour dark cycle, with aeration of 0.2 L/min.

Isolation of small cellular particles (SCPs)

SCPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

Figure *Feodaktilum različni mediji*. SEM 185.
SEM feodaktilum različni/Darja-22-03-2022 – 2_ MW-F2 _ldark

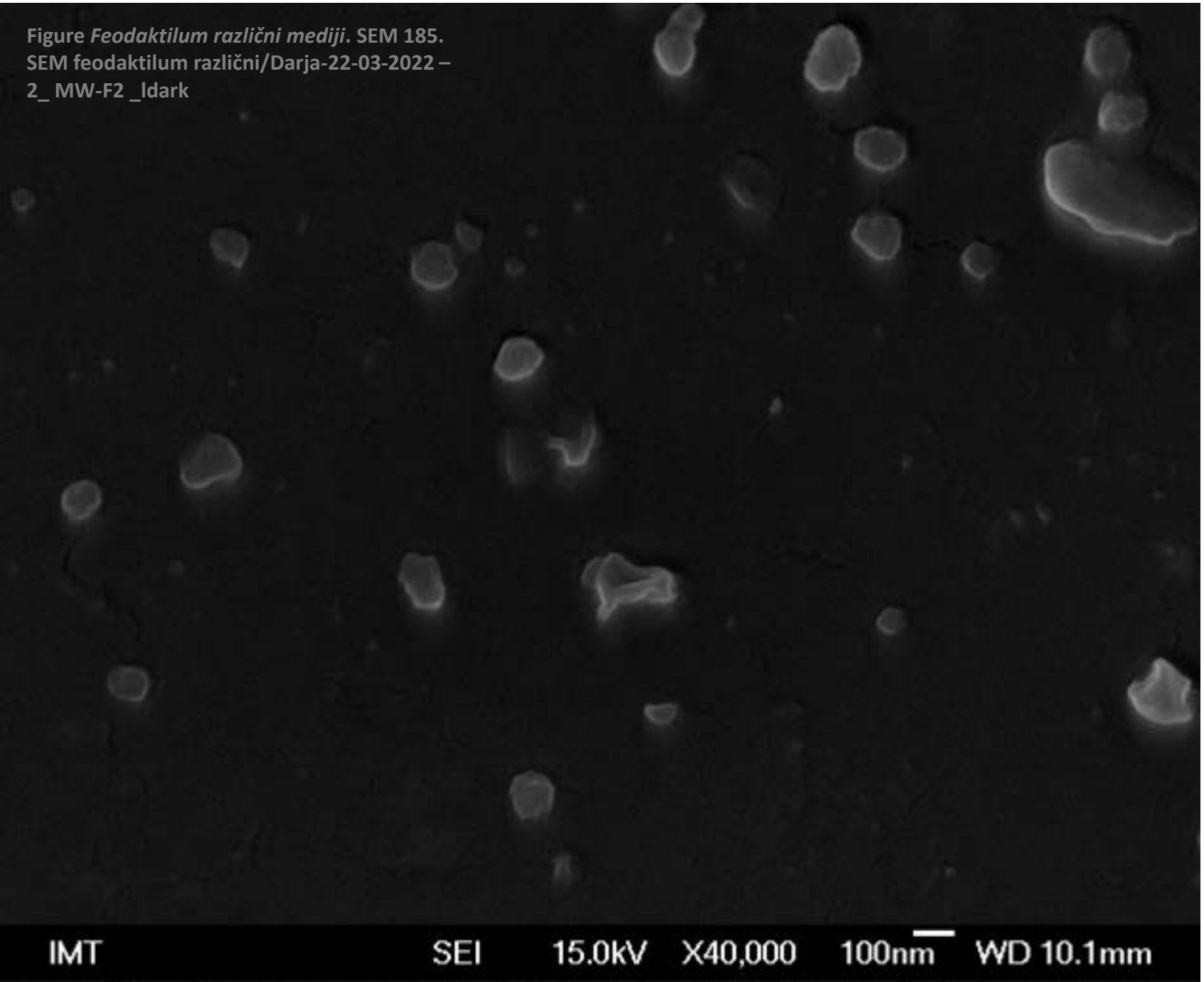

Figure *Phaeodactylum tricornutum* isolate F2 D SEM 30.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with **Guillard's (F/2)** Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 12-hour light / 12-hour dark cycle, with aeration of 0.2 L/min.

Isolation of small cellular particles (SCPs)

SCPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

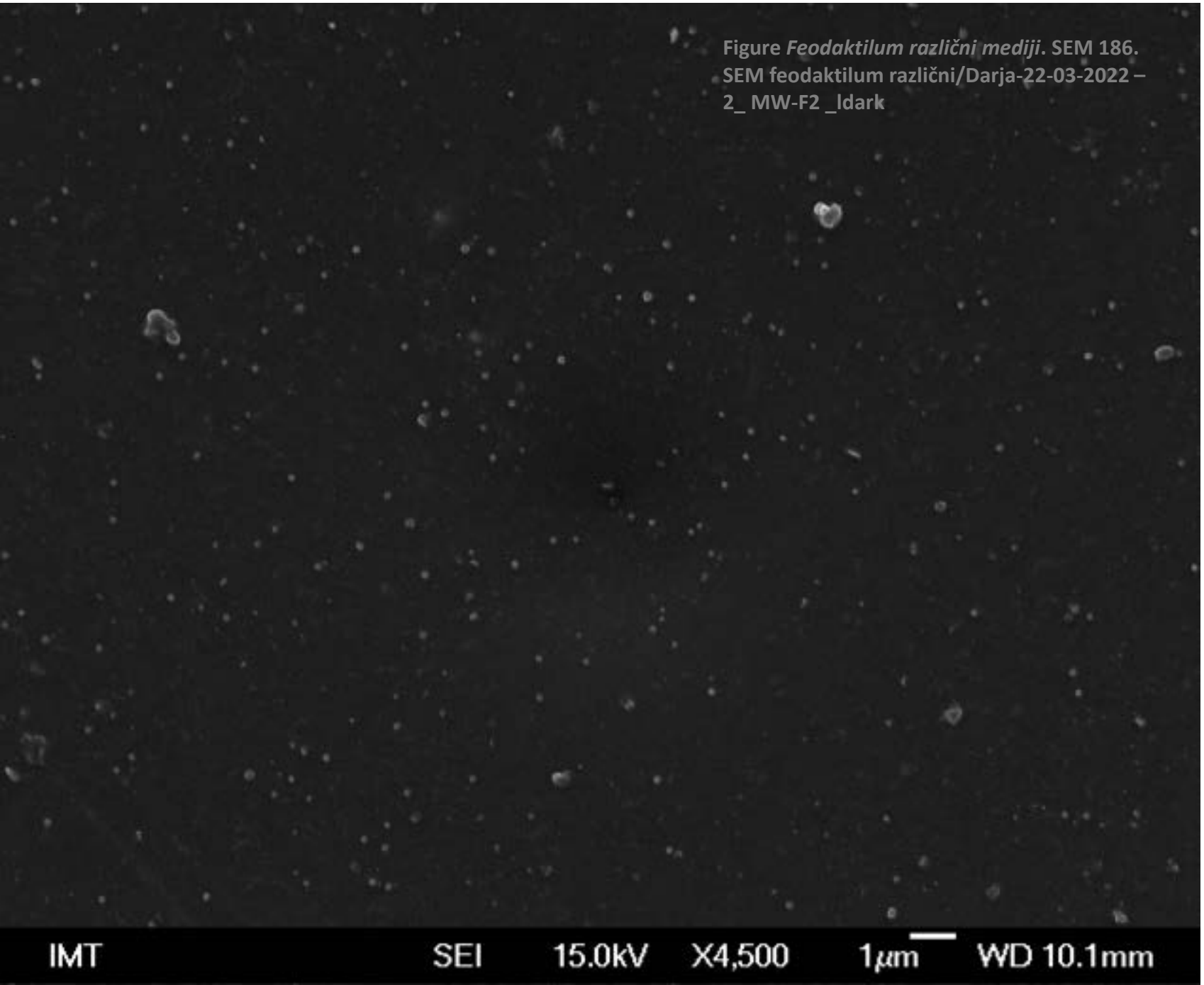

Figure *Phaeodactylum tricornutum* isolate F2 D SEM 31.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with **Guillard's (F/2)** Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 12-hour light / 12-hour dark cycle, with aeration of 0.2 L/min.

Isolation of small cellular particles (SCPs)

SCPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

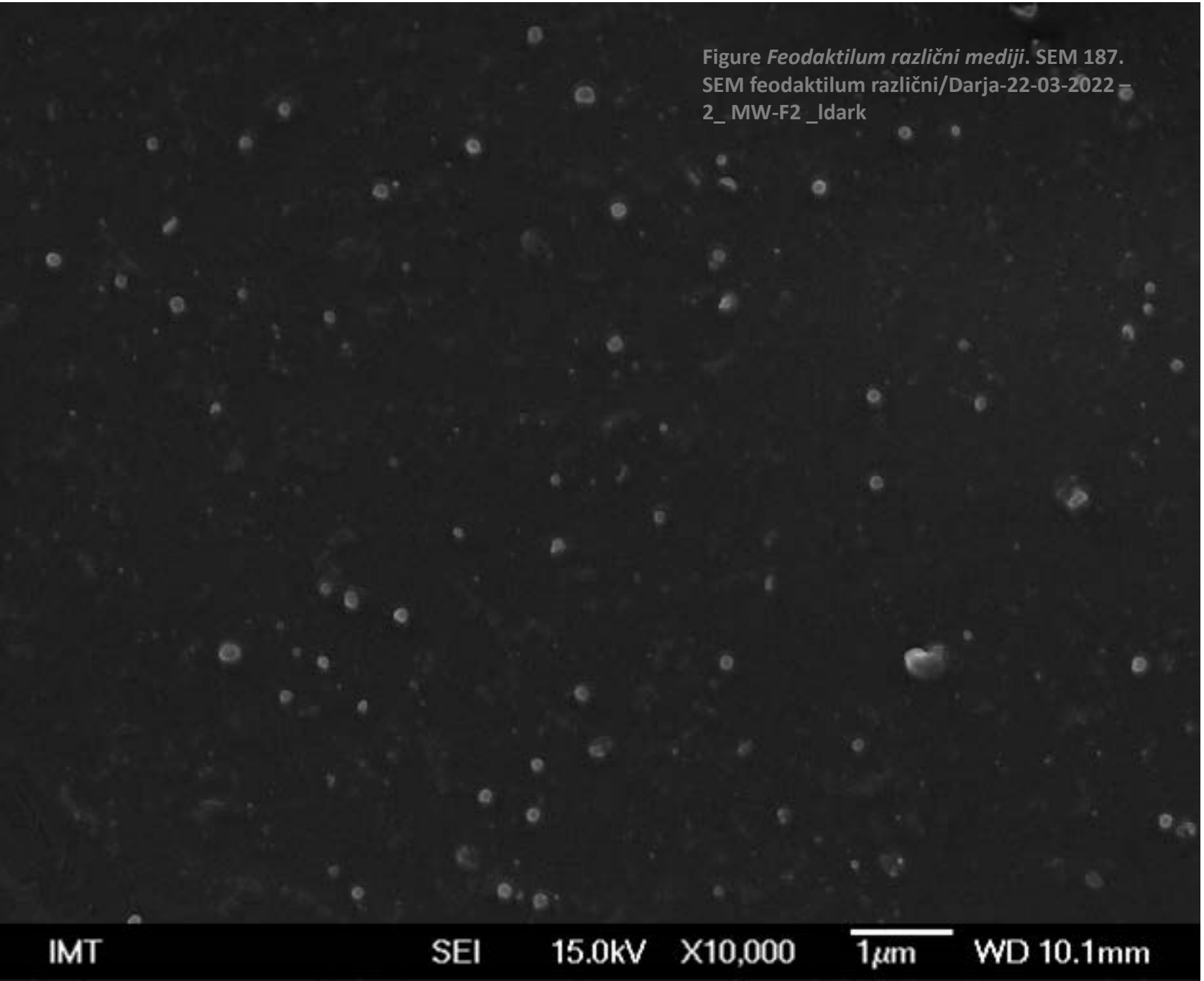

Figure *Phaeodactylum tricornutum* isolate F2 D SEM 32.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with **Guillard's (F/2)** Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 12-hour light / 12-hour dark cycle, with aeration of 0.2 L/min.

Isolation of small cellular particles (SCPs)

SCPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

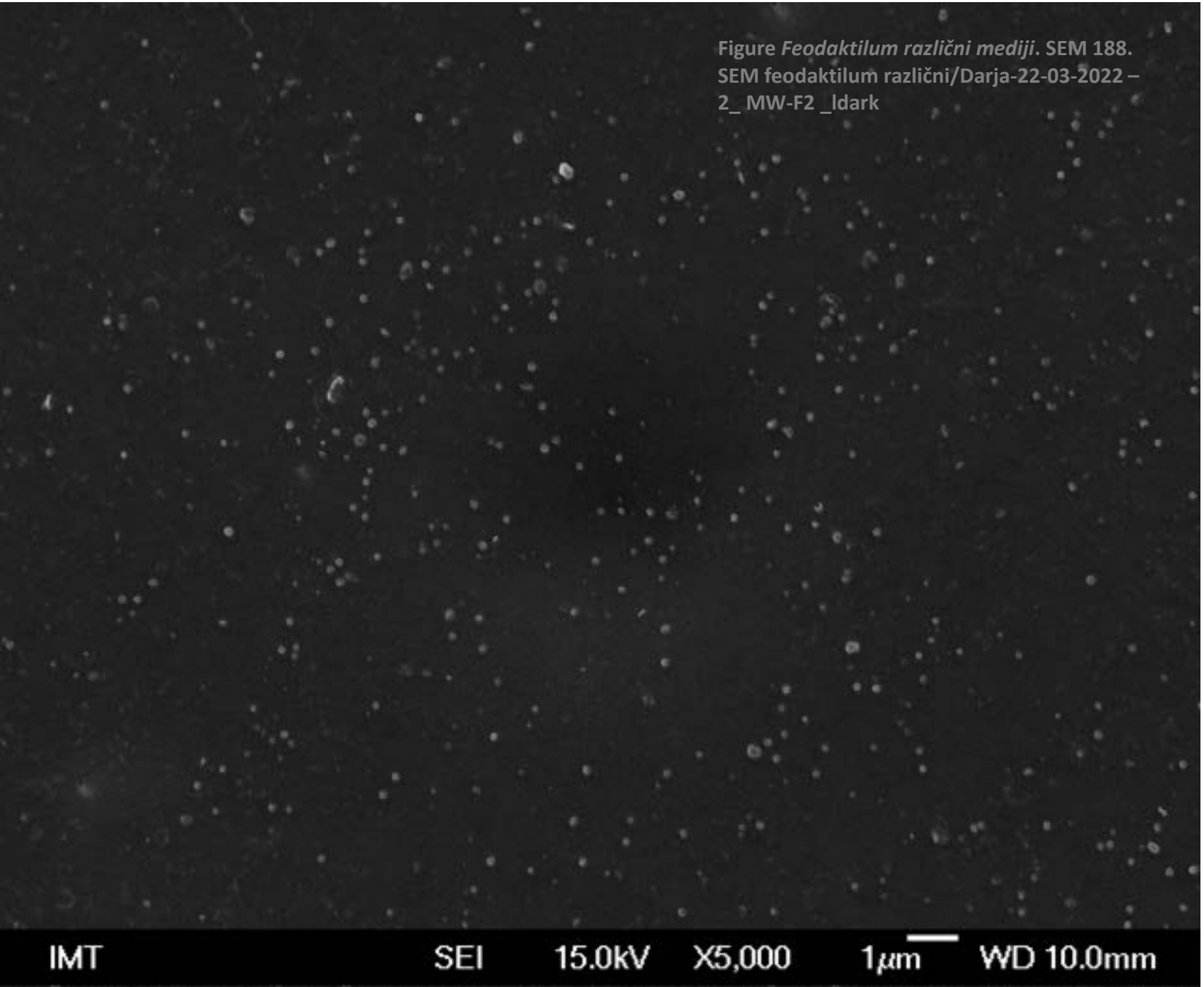

Figure *Phaeodactylum tricornutum* isolate F2 D SEM 33.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with **Guillard's (F/2)** Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 µmol/m2s) with a 12-hour light / 12-hour dark cycle, with aeration of 0.2 L/min.

Isolation of small cellular particles (SCPs)

SCPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

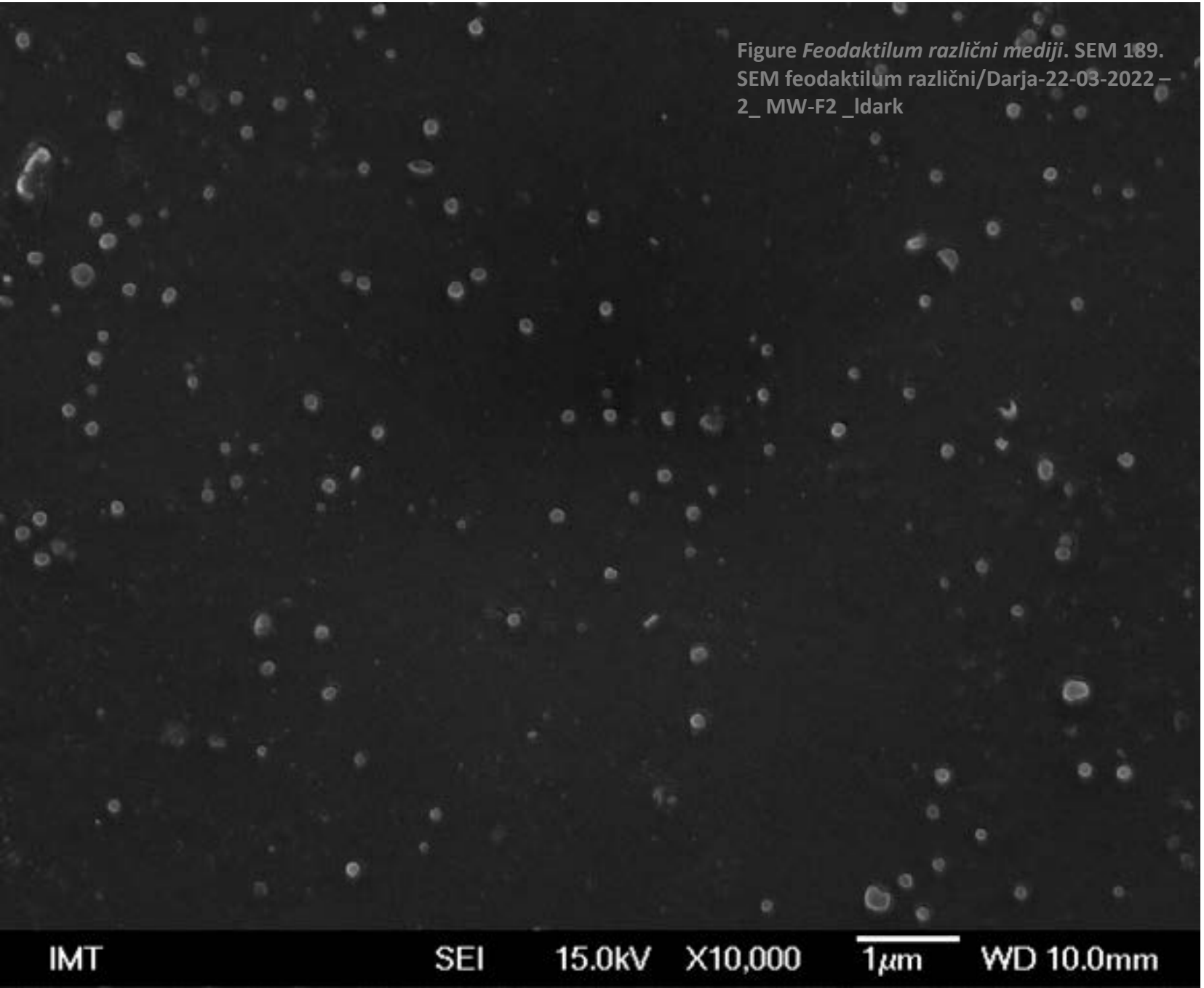

Figure *Phaeodactylum tricornutum* isolate F2 D SEM 34.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with **Guillard's (F/2)** Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 12-hour light / 12-hour dark cycle, with aeration of 0.2 L/min.

Isolation of small cellular particles (SCPs)

SCPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

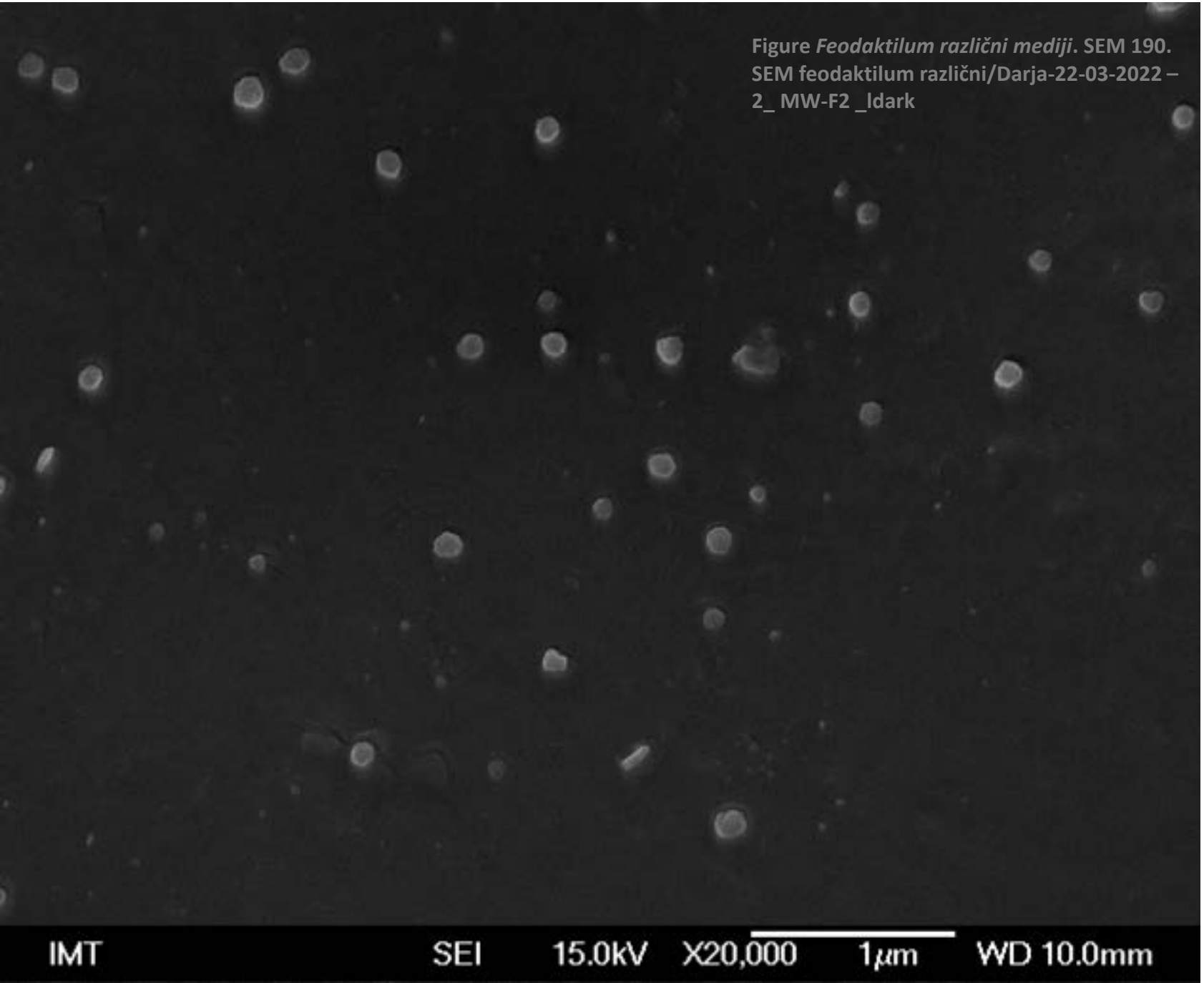

Figure *Phaeodactylum tricornutum* isolate F2 D SEM 35.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with **Guillard's (F/2)** Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 12-hour light / 12-hour dark cycle, with aeration of 0.2 L/min.

Isolation of small cellular particles (SCPs)

SCPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

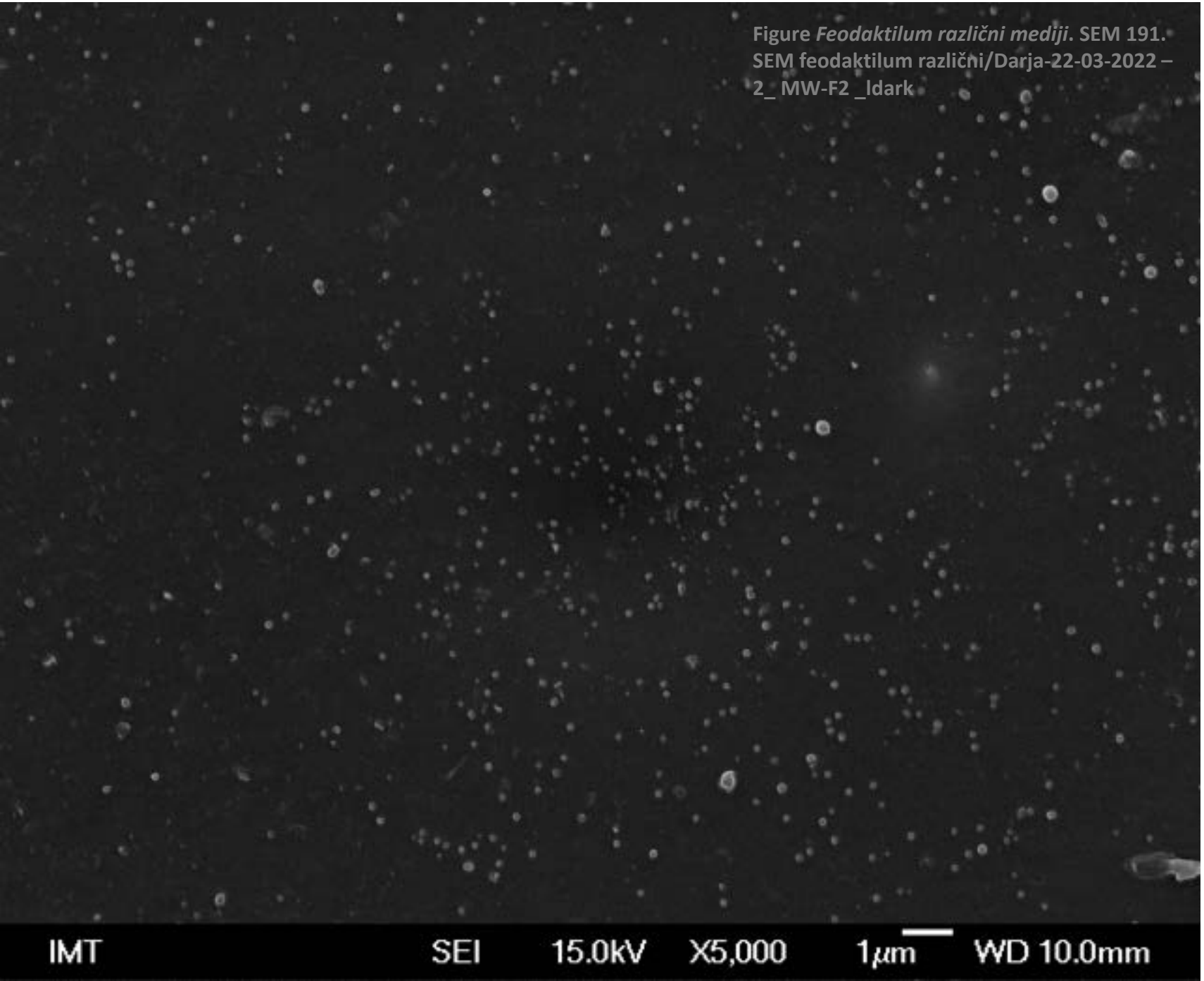

Figure *Phaeodactylum tricornutum* isolate F2 D SEM 36.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with **Guillard's (F/2)** Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 12-hour light / 12-hour dark cycle, with aeration of 0.2 L/min.

Isolation of small cellular particles (SCPs)

SCPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

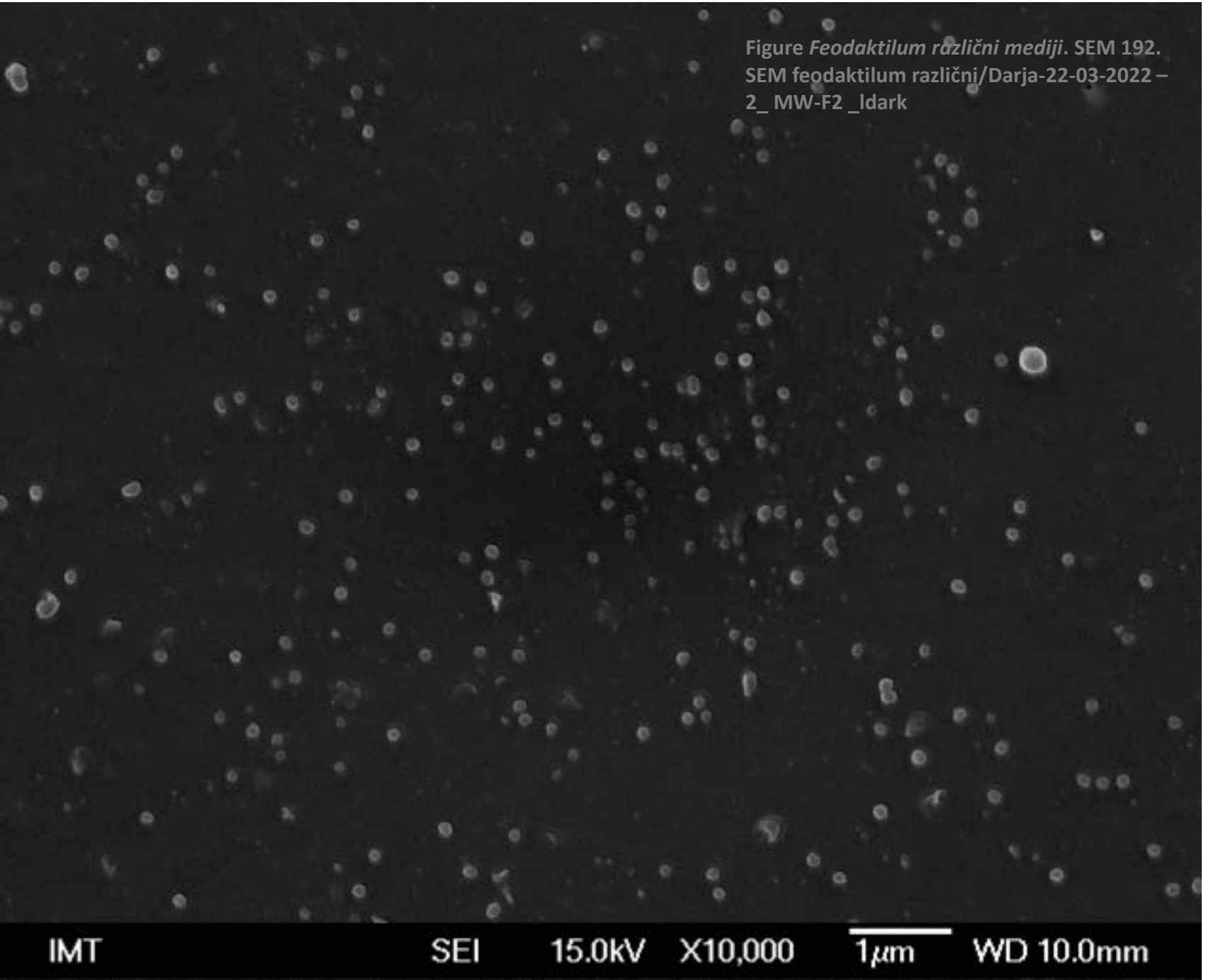

Figure *Phaeodactylum tricornutum* isolate F2 D SEM 37.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with **Guillard's (F/2)** Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 12-hour light / 12-hour dark cycle, with aeration of 0.2 L/min.

Isolation of small cellular particles (SCPs)

SCPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

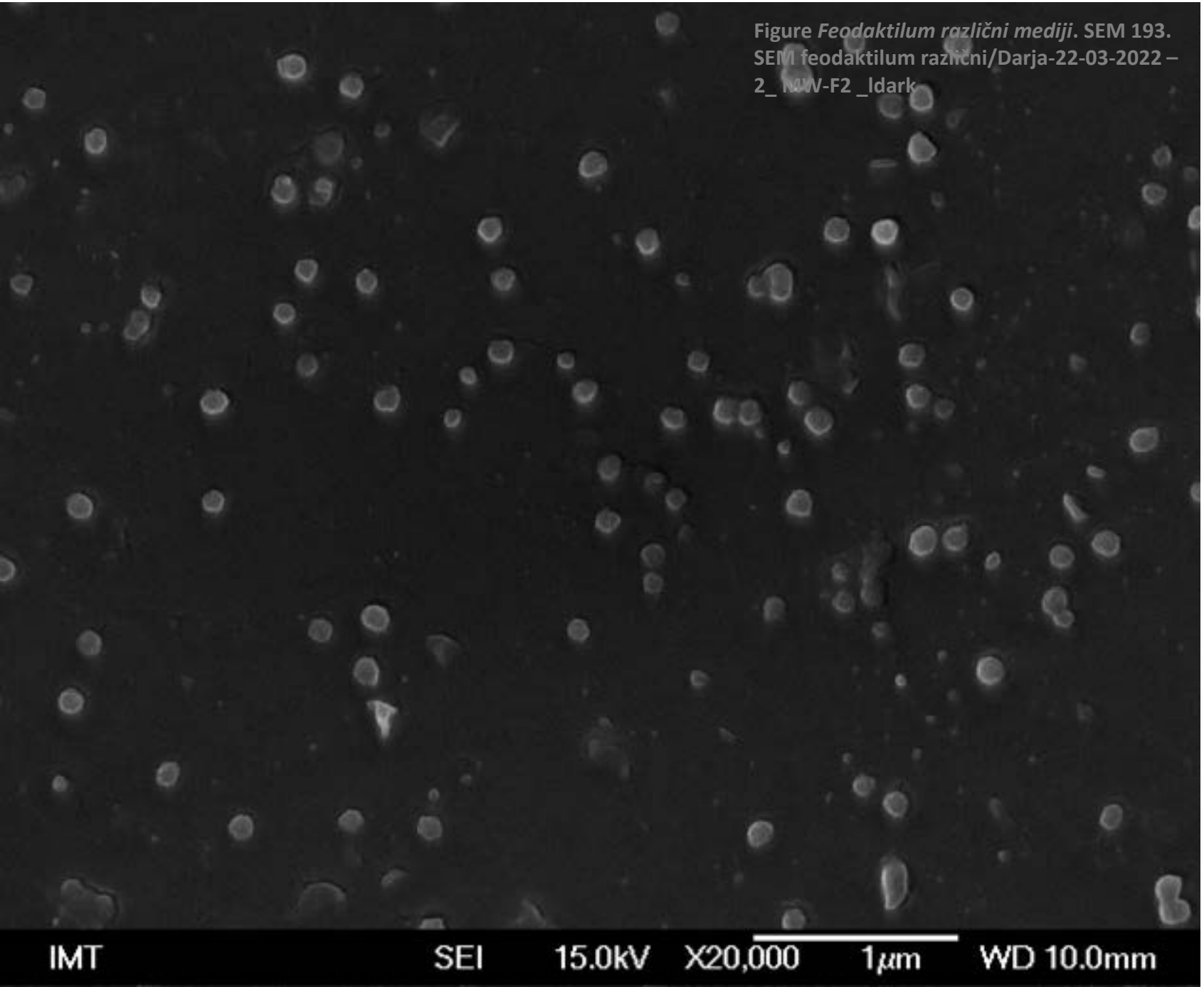

Figure *Phaeodactylum tricornutum* isolate F2 D SEM 38.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with **Guillard's (F/2)** Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 µmol/m2s) with a 12-hour light / 12-hour dark cycle, with aeration of 0.2 L/min.

Isolation of small cellular particles (SCPs)

SCPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

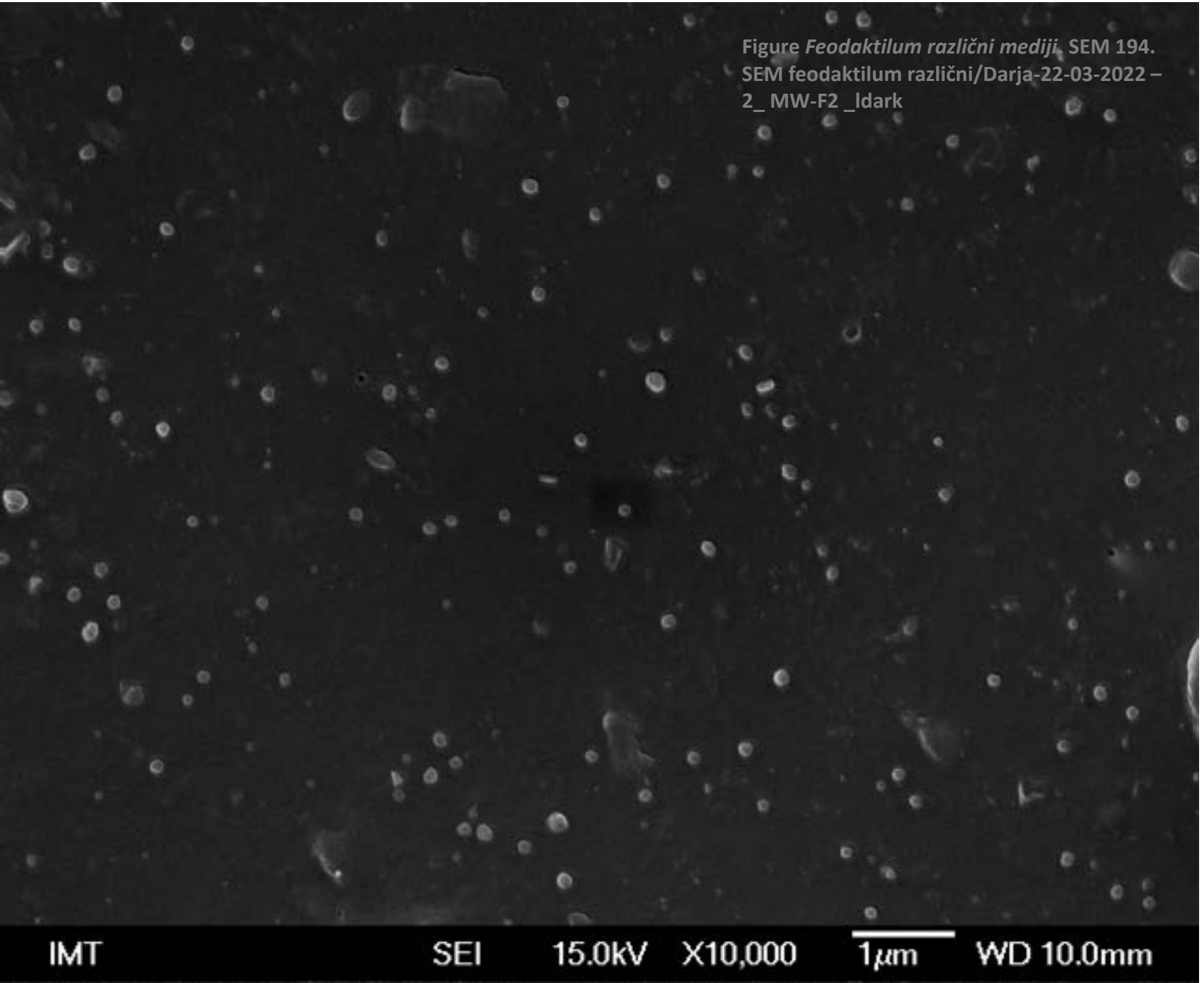

Figure *Phaeodactylum tricornutum* isolate F2 D SEM 39.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with **Guillard's (F/2)** Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 12-hour light / 12-hour dark cycle, with aeration of 0.2 L/min.

Isolation of small cellular particles (SCPs)

SCPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

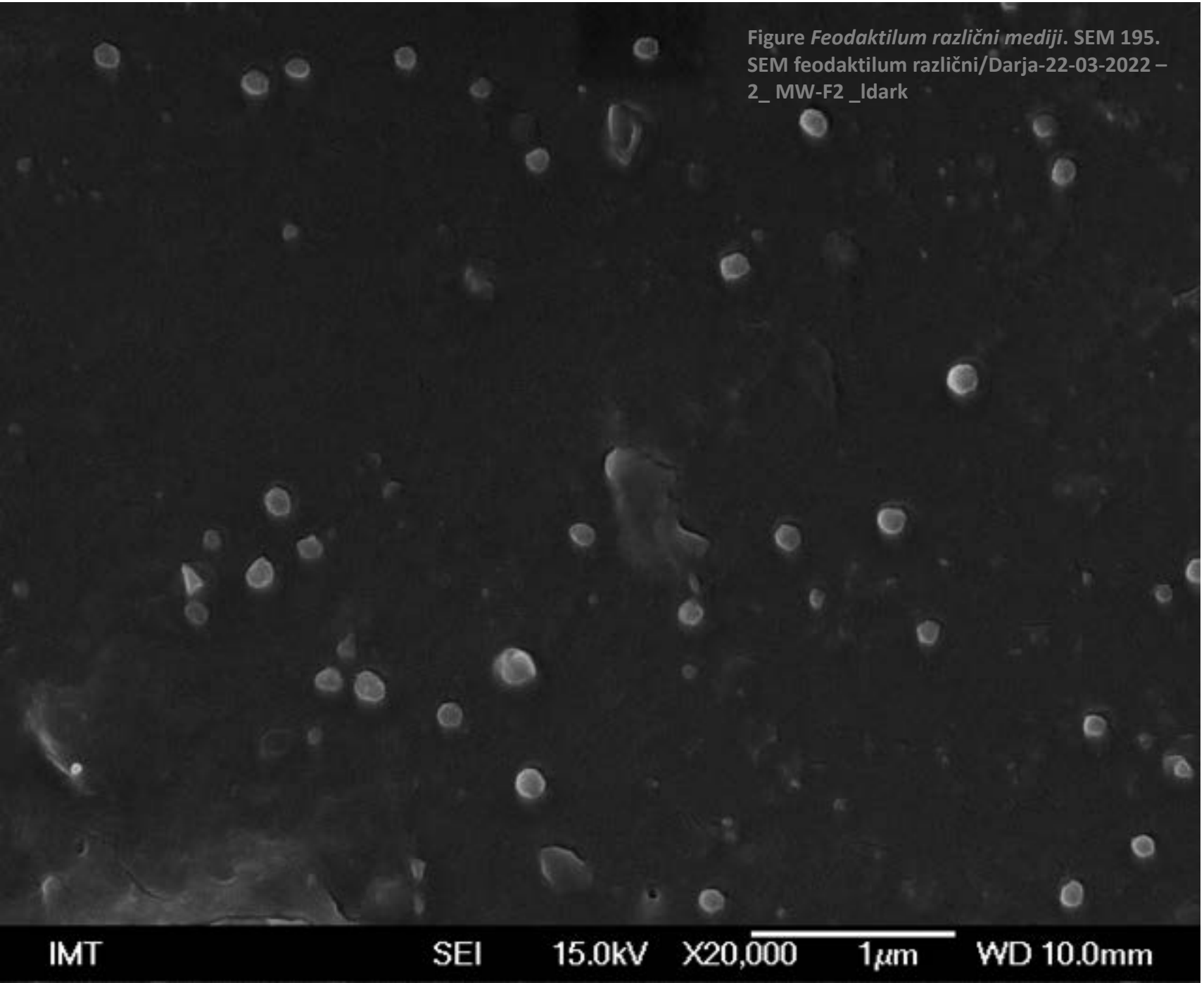

Figure *Feodaktilum različni mediji*. SEM 195. SEM feodaktilum različni/Darja-22-03-2022 – 2_ MW-F2 _ldark

Figure *Phaeodactylum tricornutum* isolate F2 D SEM 40.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with **Guillard's (F/2)** Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 12-hour light / 12-hour dark cycle, with aeration of 0.2 L/min.

Isolation of small cellular particles (SCPs)

SCPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

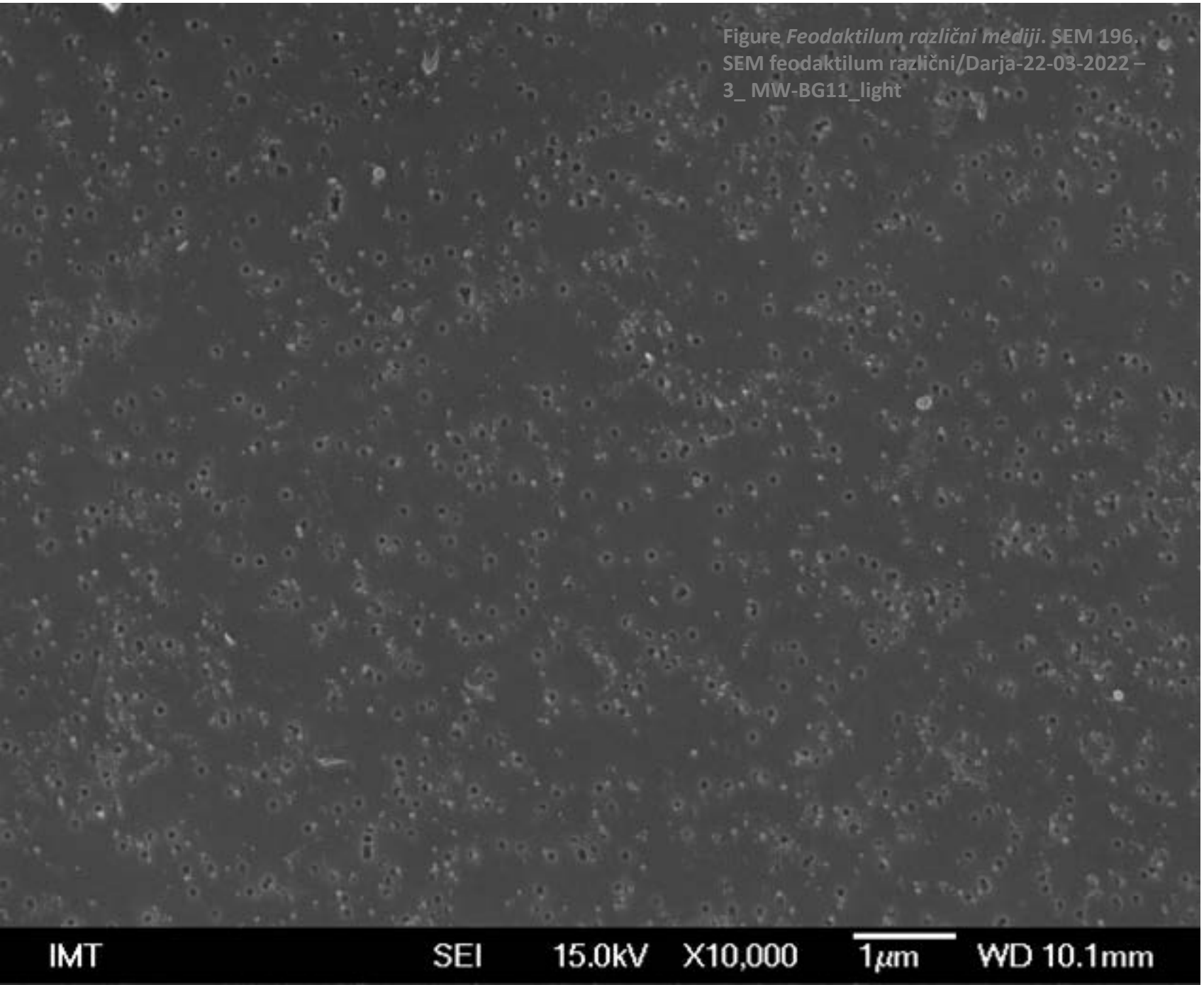

Figure *Phaeodactylum tricornutum* isolate BG11 L SEM 41.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with BG11 (ref. G0154, Sigma Aldrich, USA). Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Isolation of small cellular particles (SCPs)

SCPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, SCPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

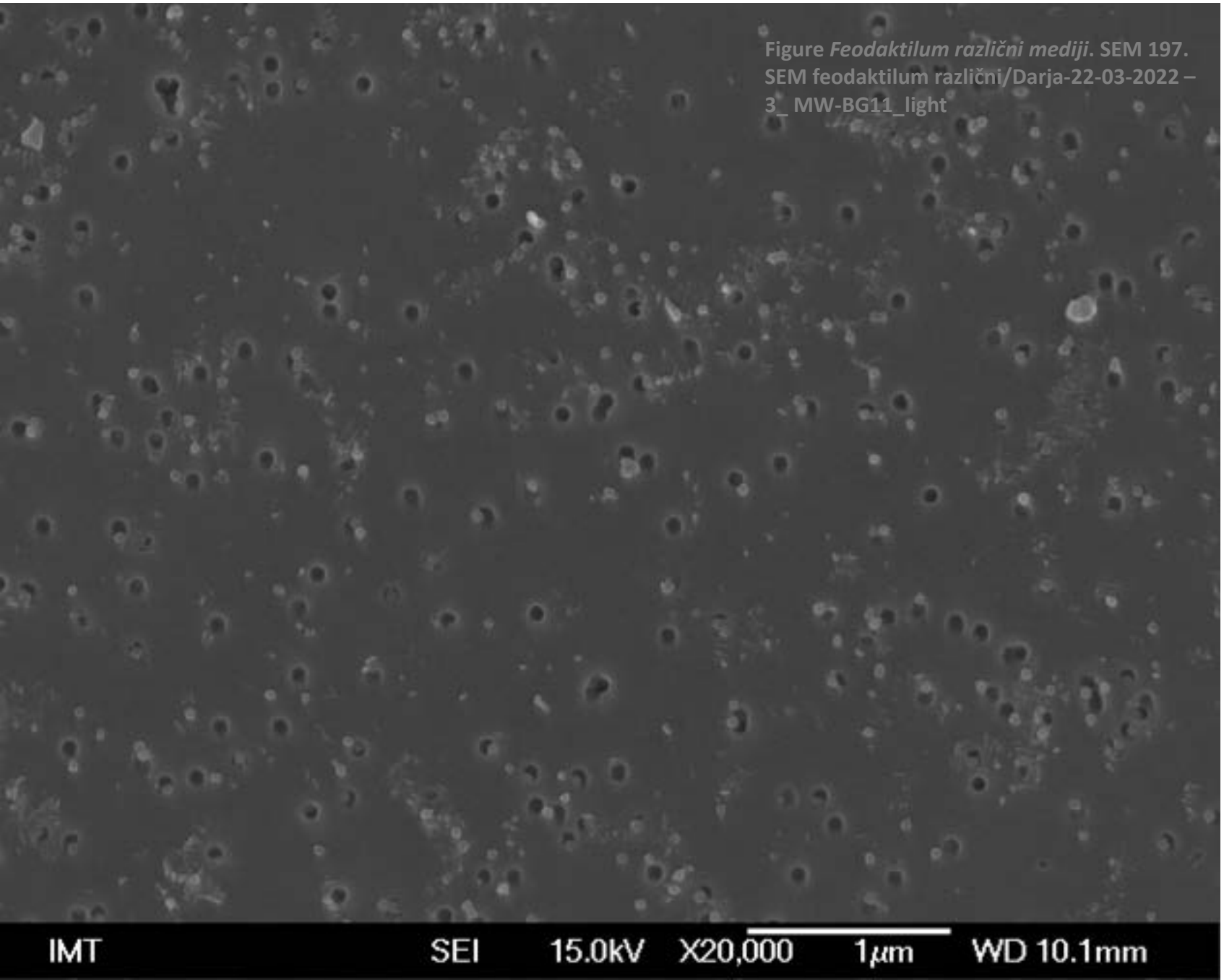

Figure *Phaeodactylum tricornutum* isolate BG11 L SEM 42.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with **Guillard's (F/2)** Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Isolation of small cellular particles (SCPs)

SCPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

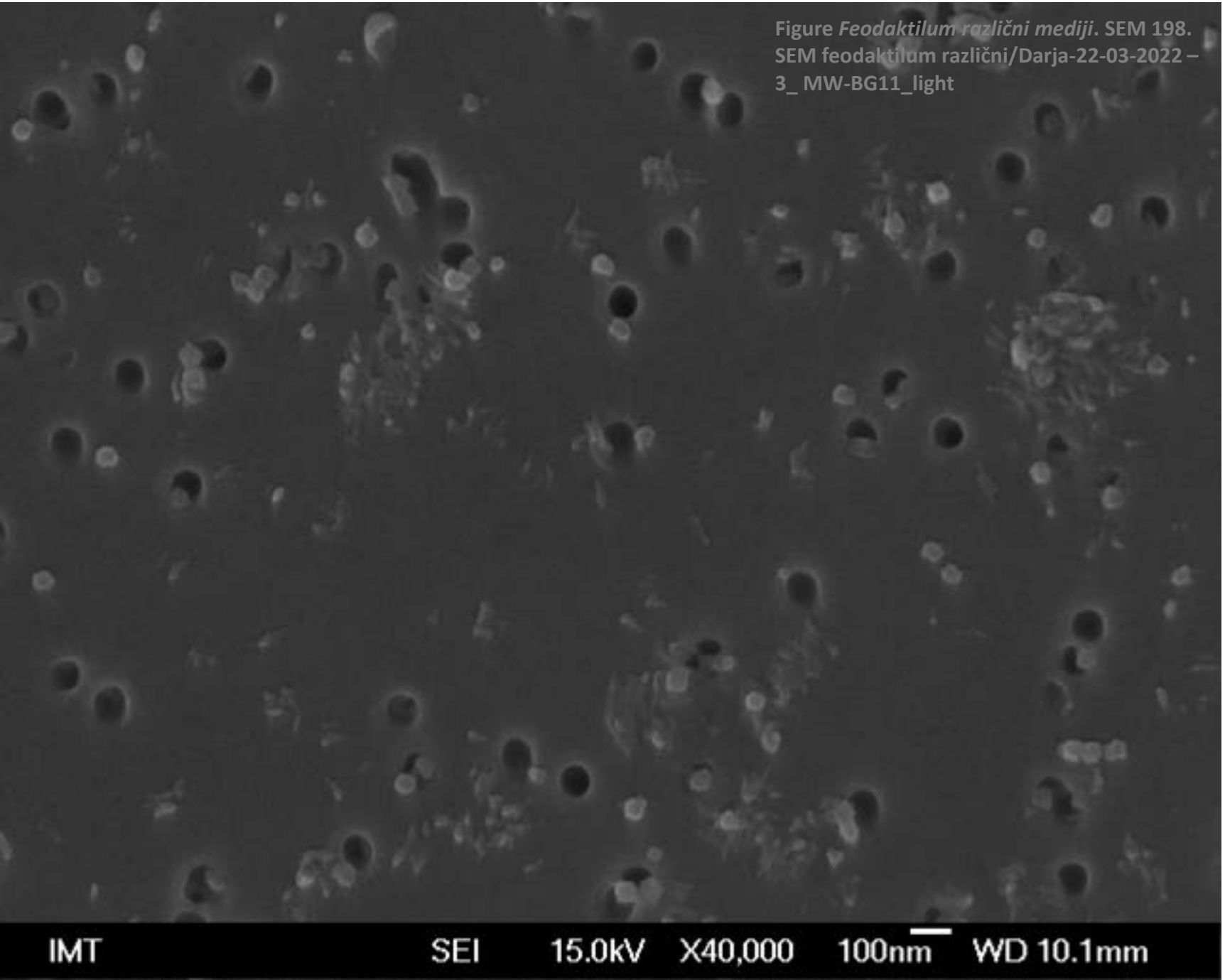

Figure *Phaeodactylum tricornutum* isolate BG11 L SEM 43.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with **Guillard's (F/2)** Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Isolation of small cellular particles (SCPs)

SCPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

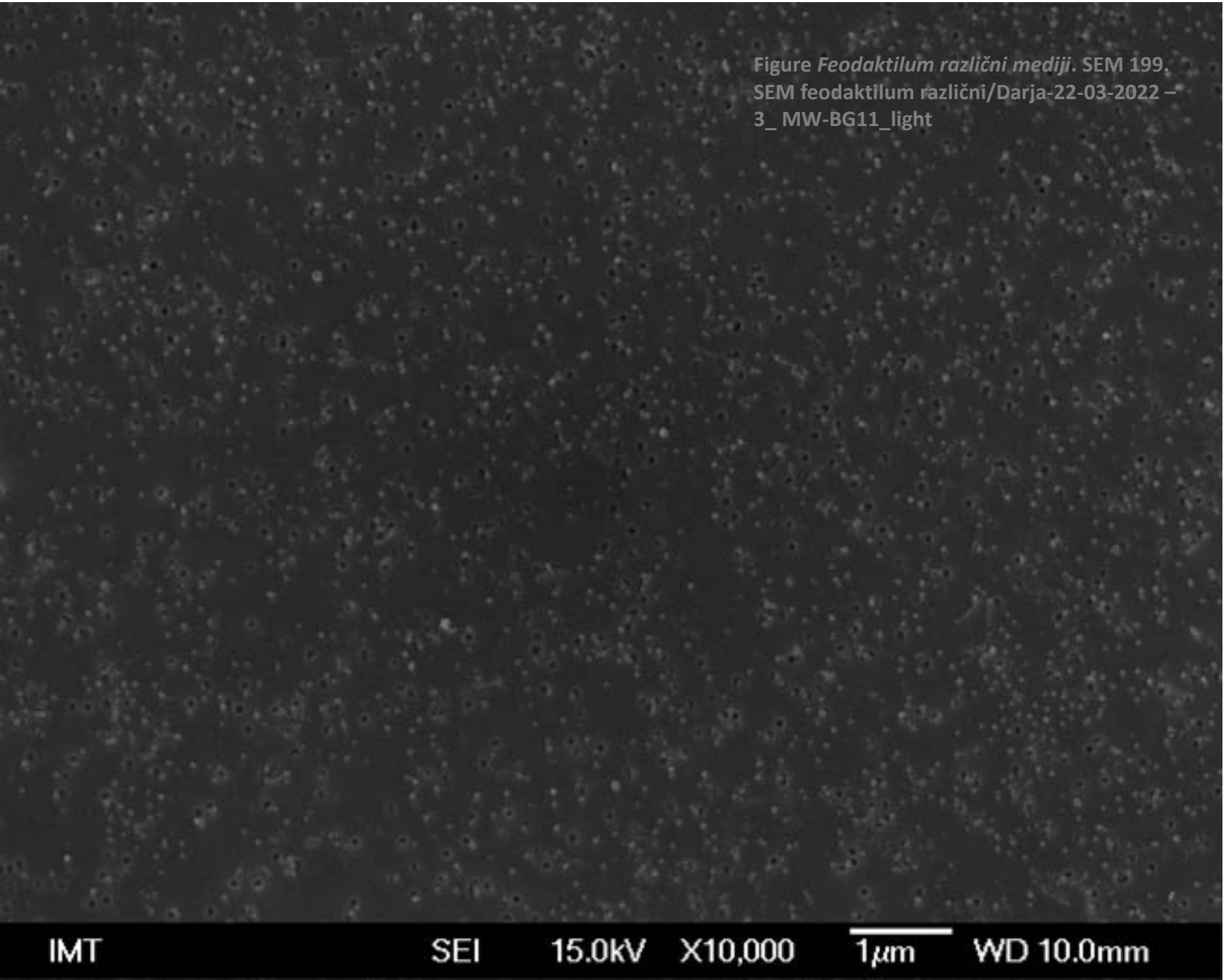

Figure *Phaeodactylum tricornutum* isolate BG11 L SEM 44.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with **Guillard's (F/2)** Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Isolation of small cellular particles (SCPs)

SCPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

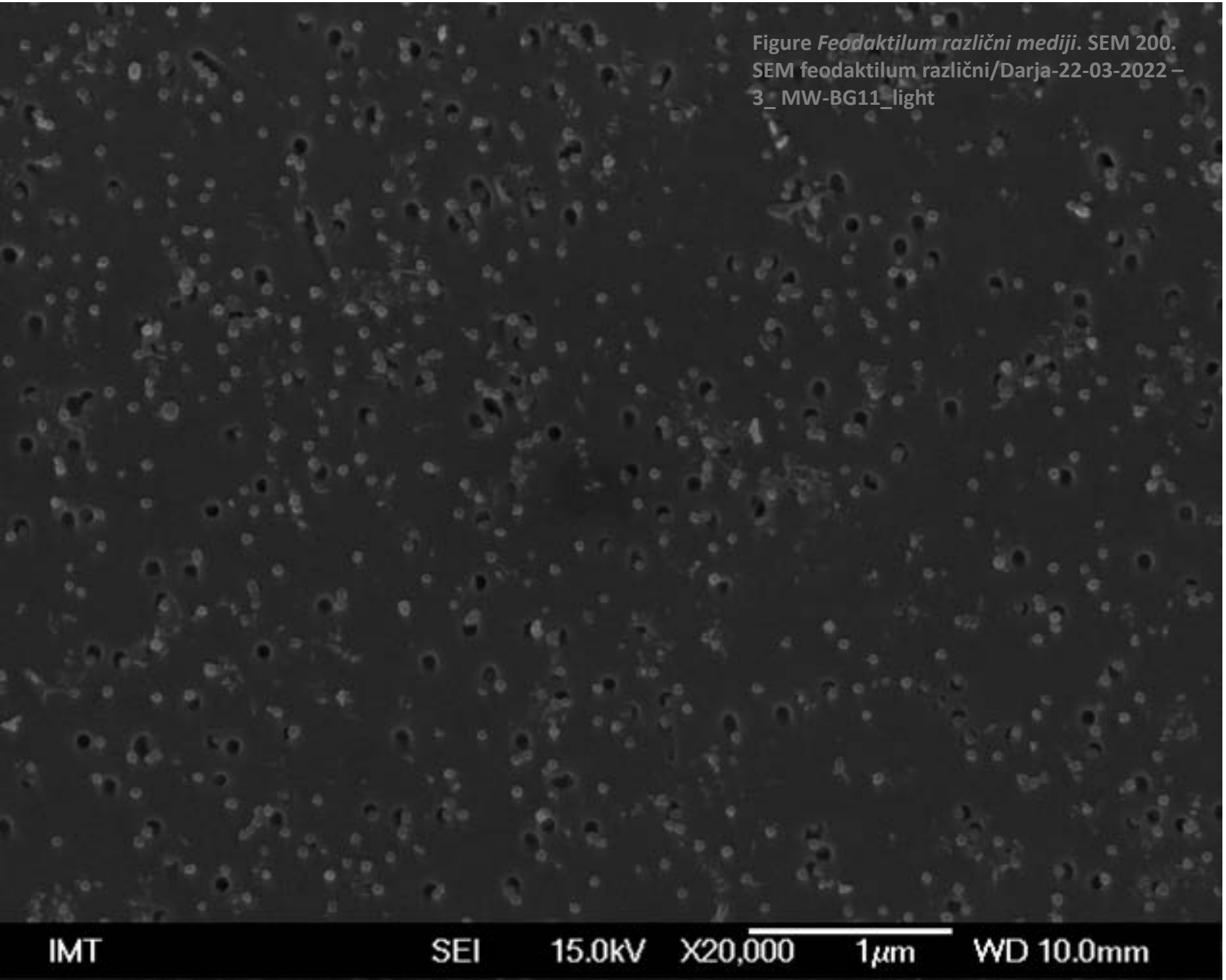

Figure *Phaeodactylum tricornutum* isolate BG11 L SEM 45.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with **Guillard's (F/2)** Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Isolation of small cellular particles (SCPs)

SCPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

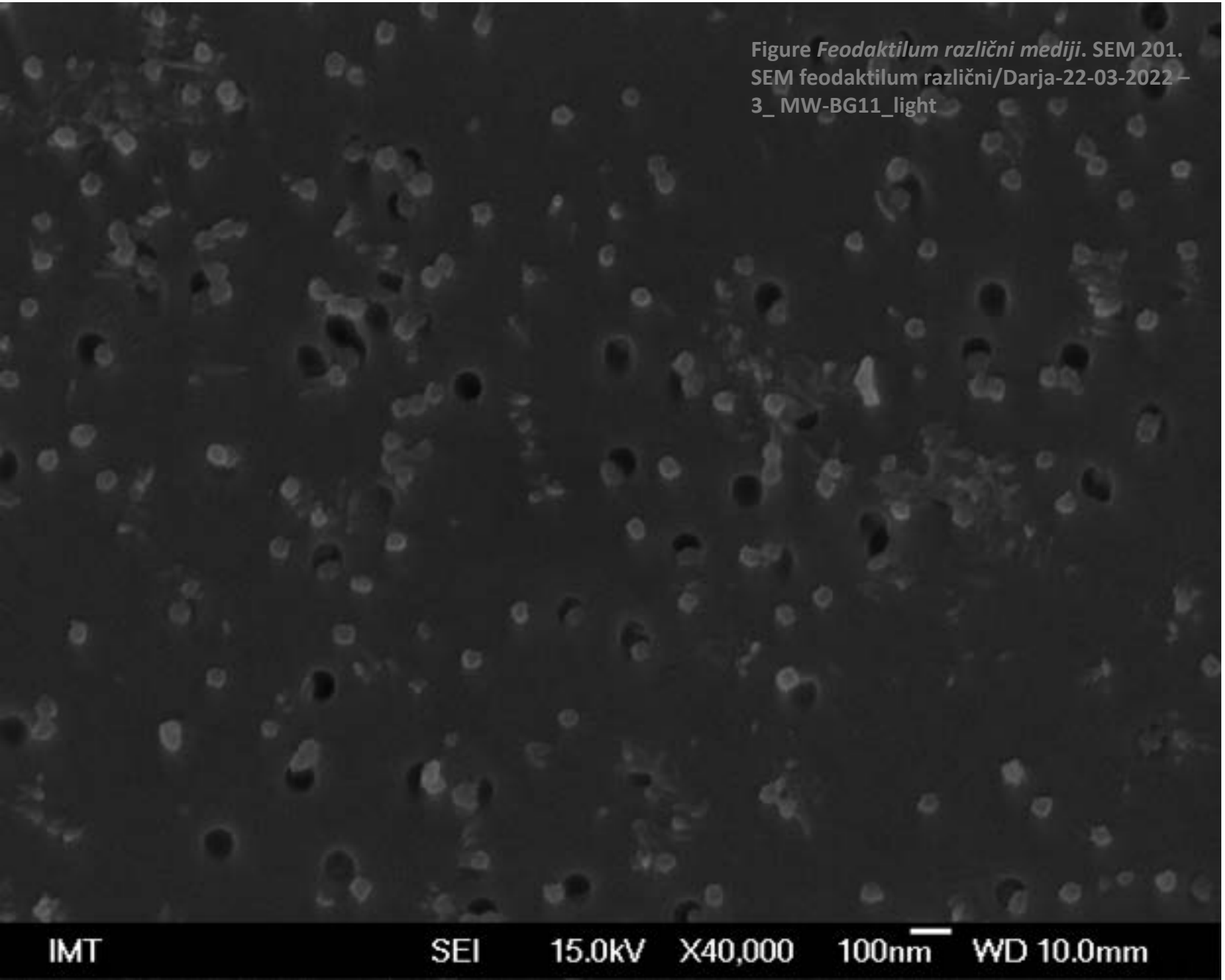

Figure *Phaeodactylum tricornutum* isolate BG11 L SEM 46.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with **Guillard's (F/2)** Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Isolation of small cellular particles (SCPs)

SCPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

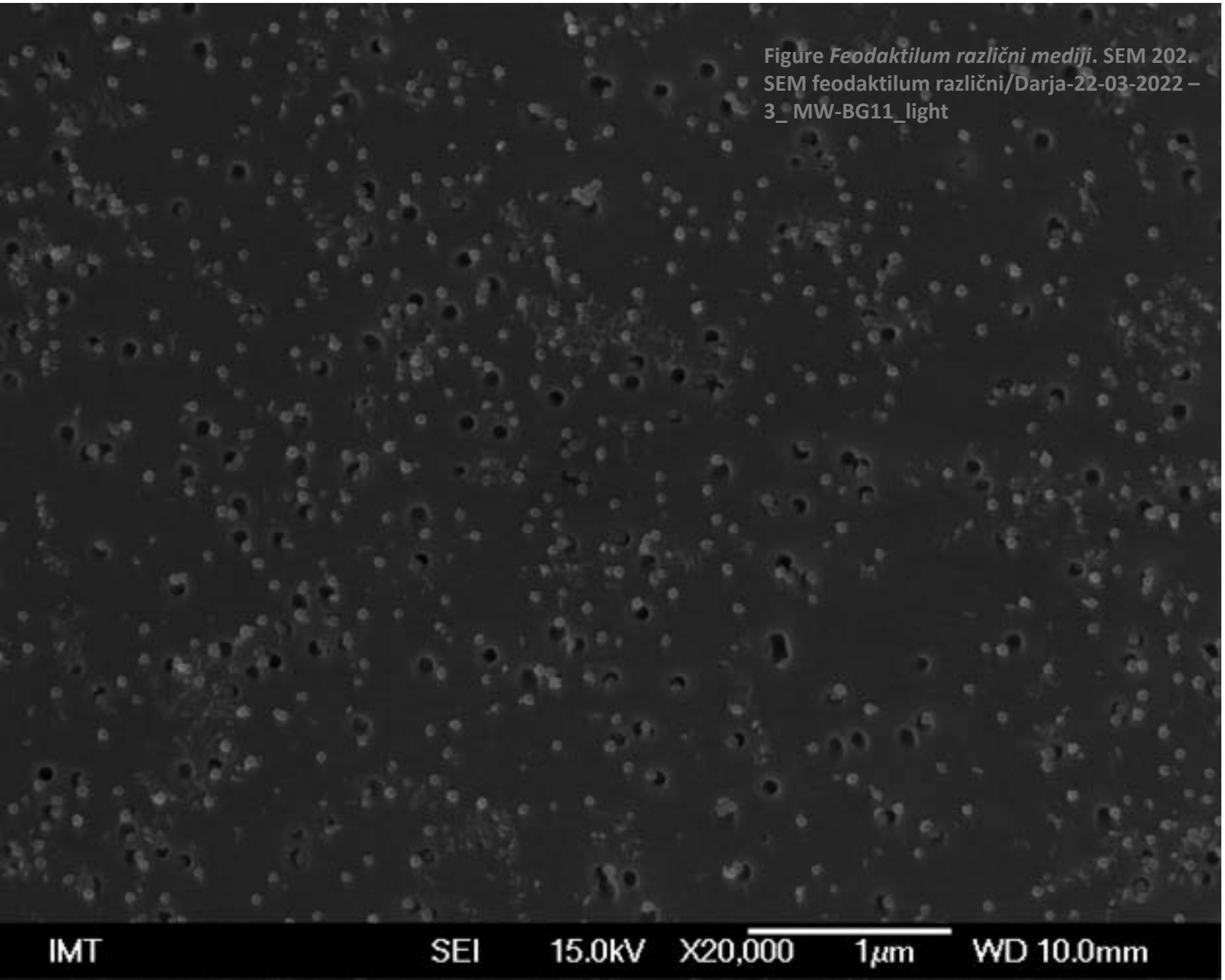

Figure *Phaeodactylum tricornutum* isolate BG11 L SEM 47.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with **Guillard's (F/2)** Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Isolation of small cellular particles (SCPs)

SCPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

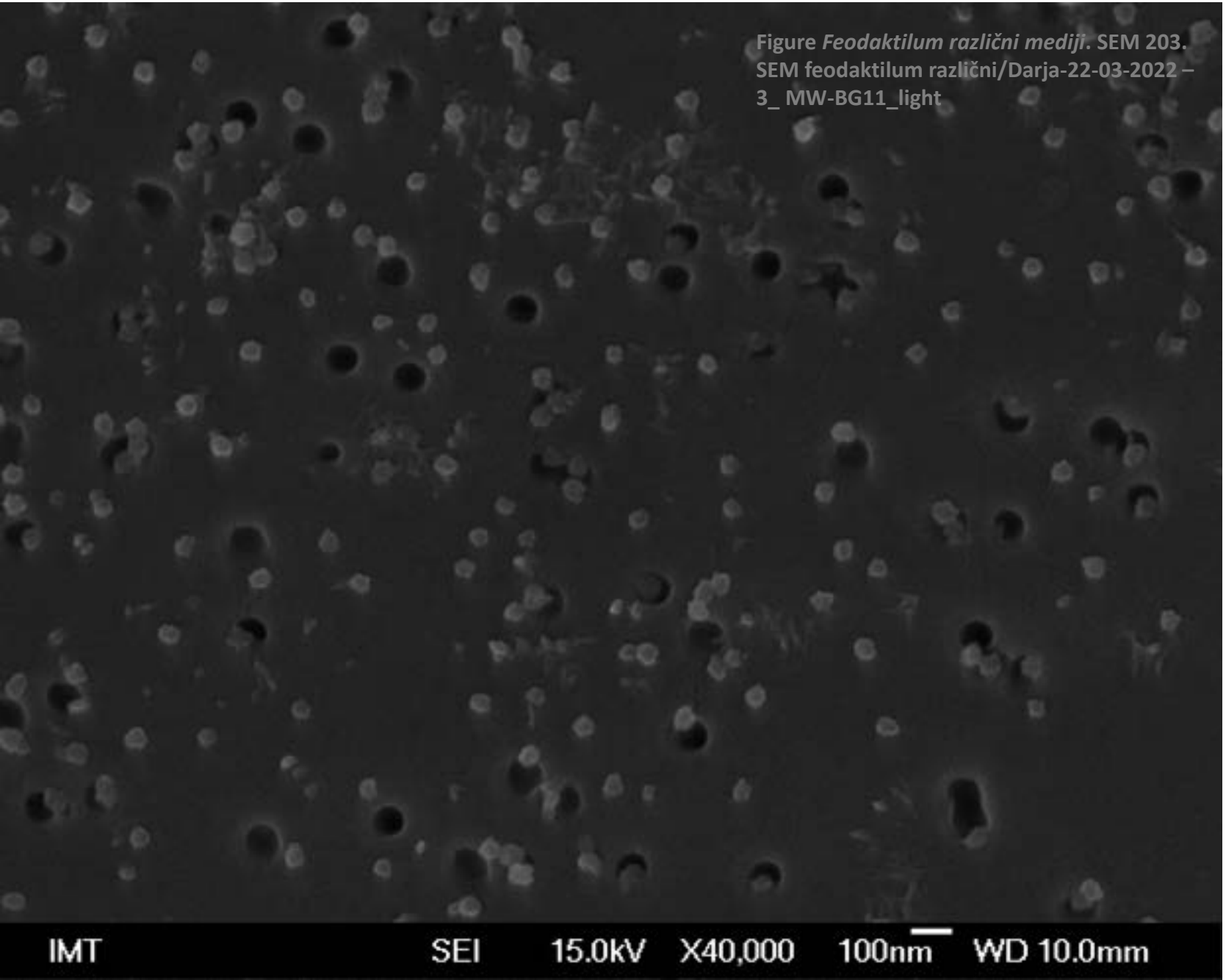

Figure *Phaeodactylum tricornutum* isolate BG11 L SEM 48.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with **Guillard's (F/2)** Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 µmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Isolation of small cellular particles (SCPs)

SCPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

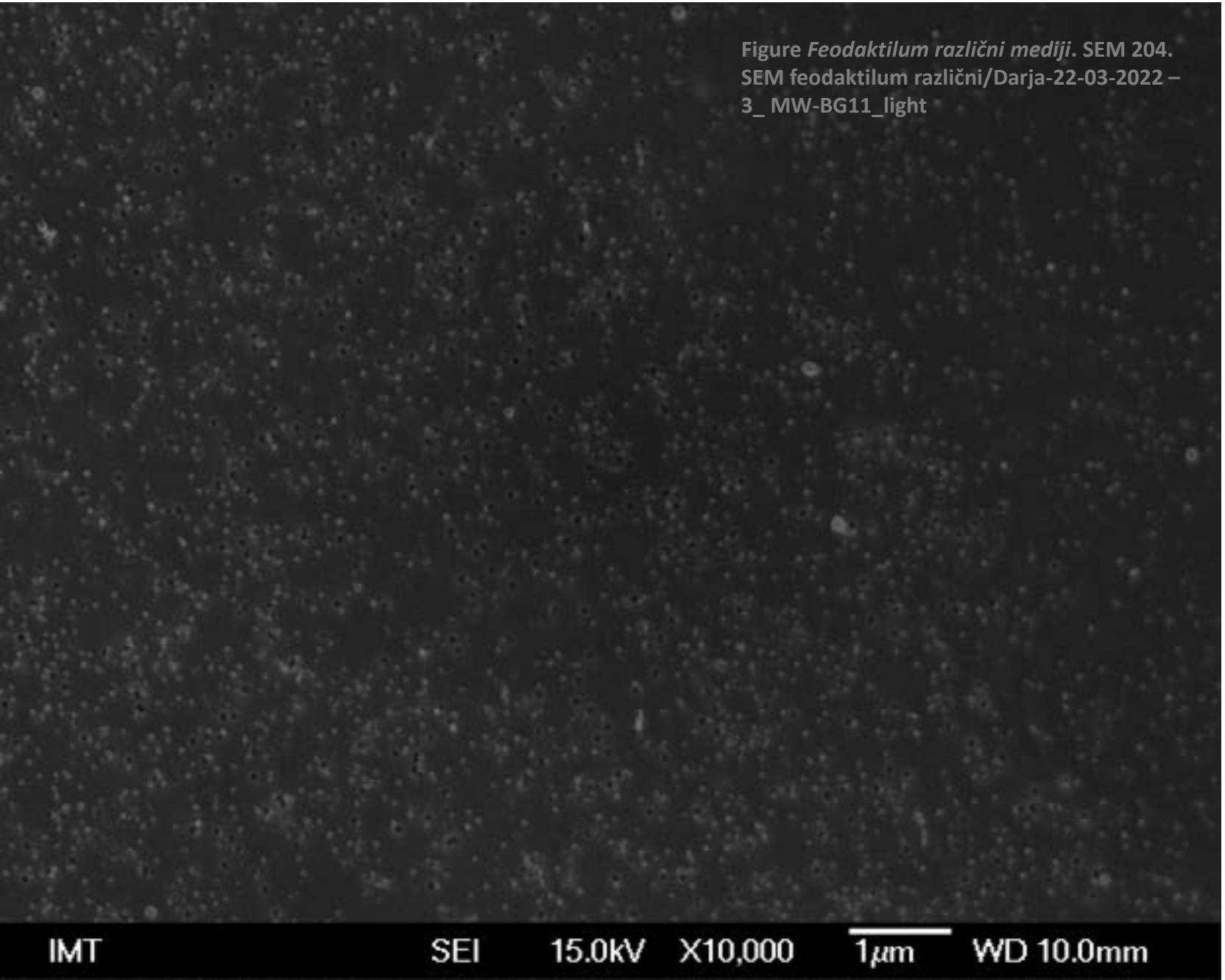

Figure *Phaeodactylum tricornutum* isolate BG11 L SEM 49.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with **Guillard's (F/2)** Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Isolation of small cellular particles (SCPs)

SCPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

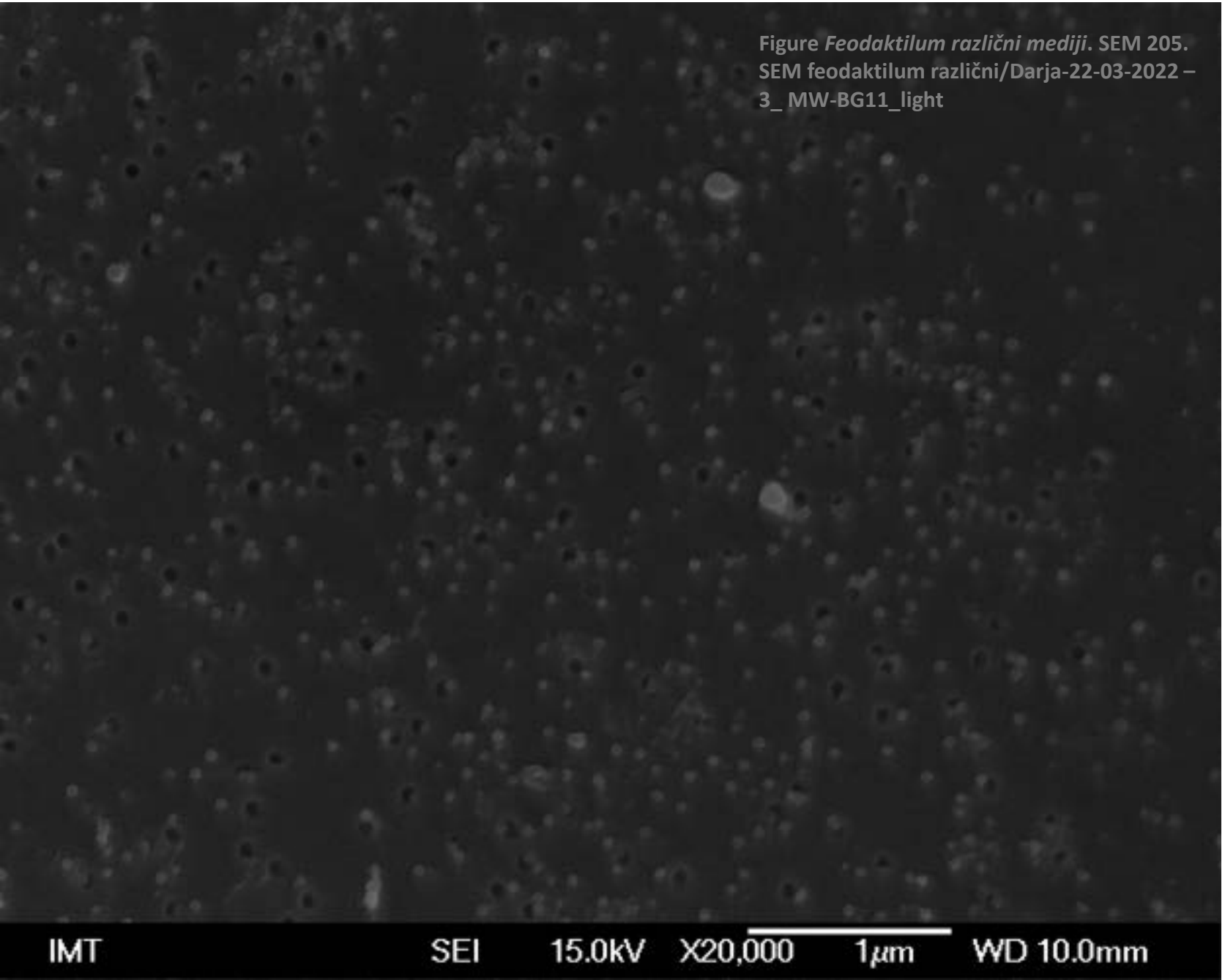

Figure *Phaeodactylum tricornutum* isolate BG11 L SEM 50.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with **Guillard's (F/2)** Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Isolation of small cellular particles (SCPs)

SCPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

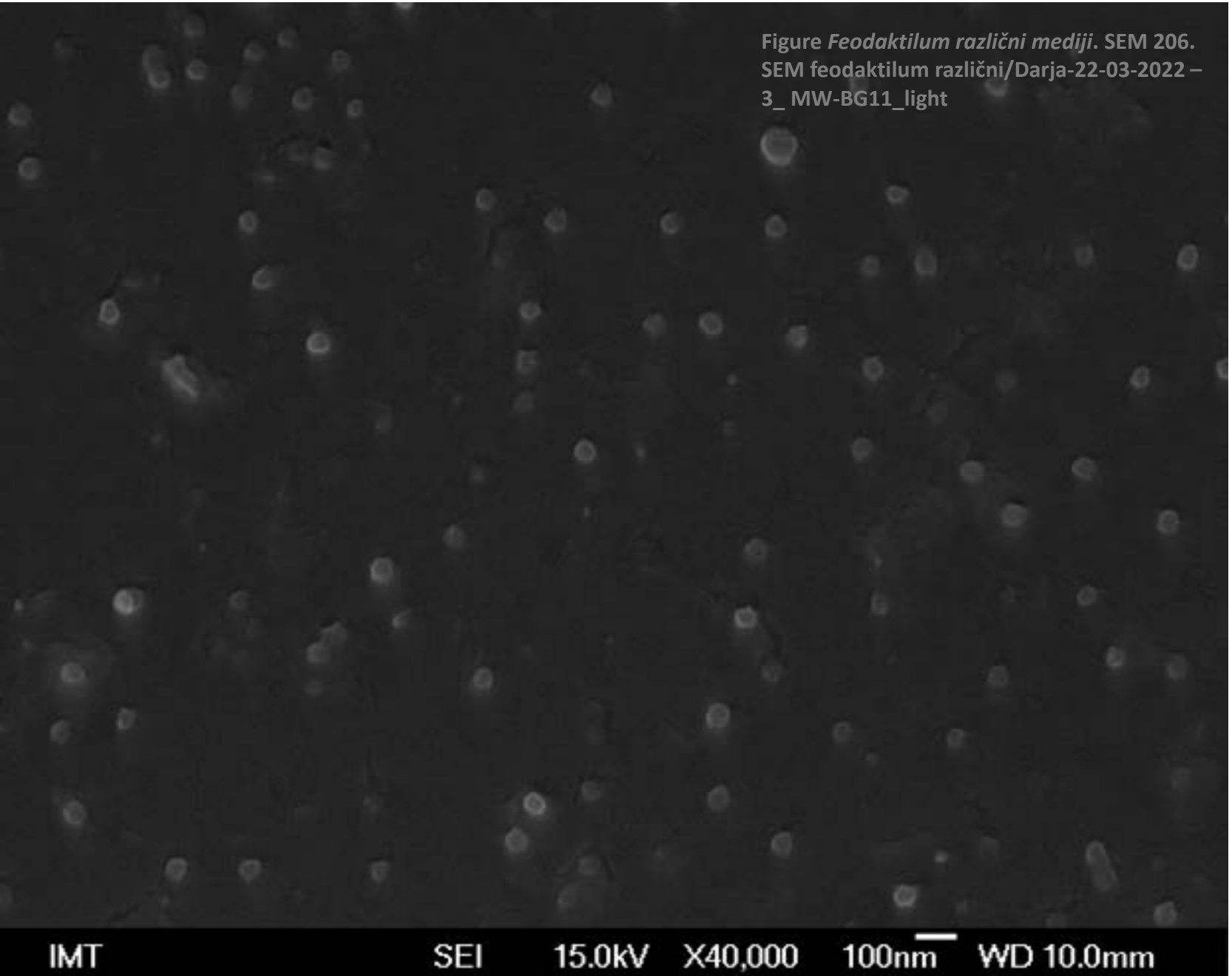

Figure *Phaeodactylum tricornutum* isolate BG11 L SEM 51.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with **Guillard's (F/2)** Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 µmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Isolation of small cellular particles (SCPs)

SCPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

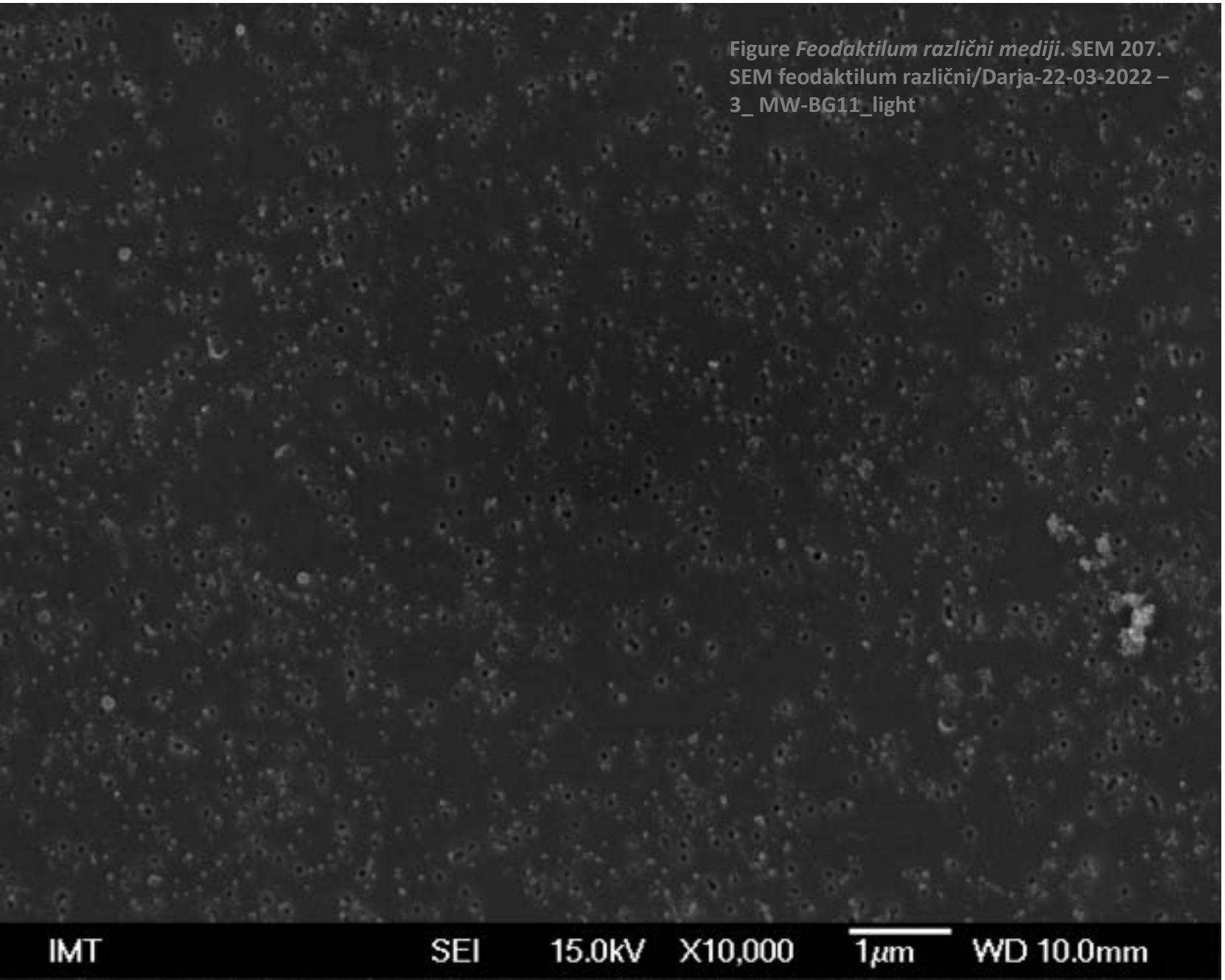

Figure *Phaeodactylum tricornutum* isolate BG11 L SEM 29.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with **Guillard's (F/2)** Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Isolation of small cellular particles (SCPs)

SCPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

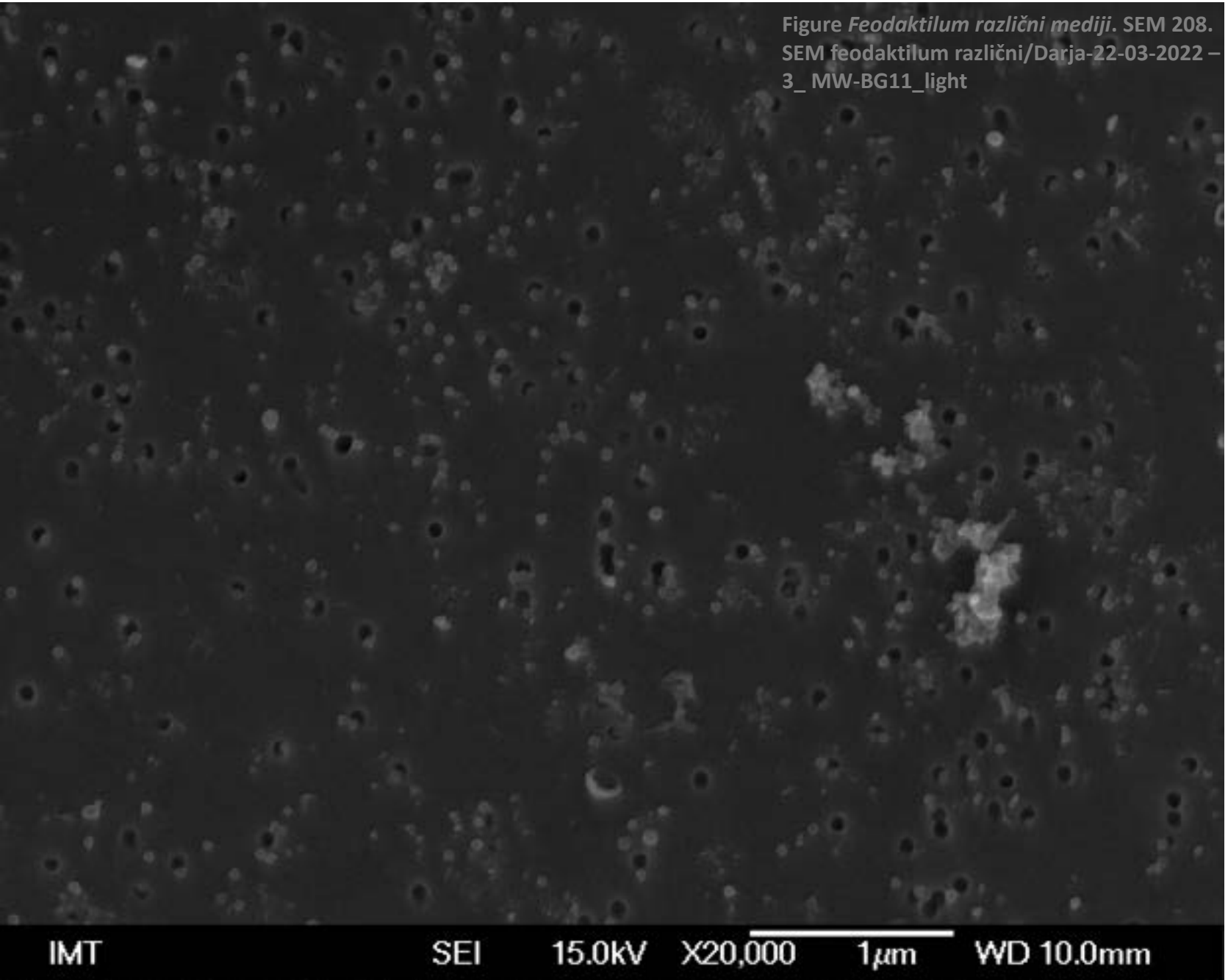

Figure *Feodaktilum različni mediji*. SEM 208. SEM feodaktilum različni/Darja-22-03-2022 – 3_ MW-BG11_light

Figure *Phaeodactylum tricornutum* isolate BG11 L SEM 29.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with **Guillard's (F/2)** Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Isolation of small cellular particles (SCPs)

SCPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

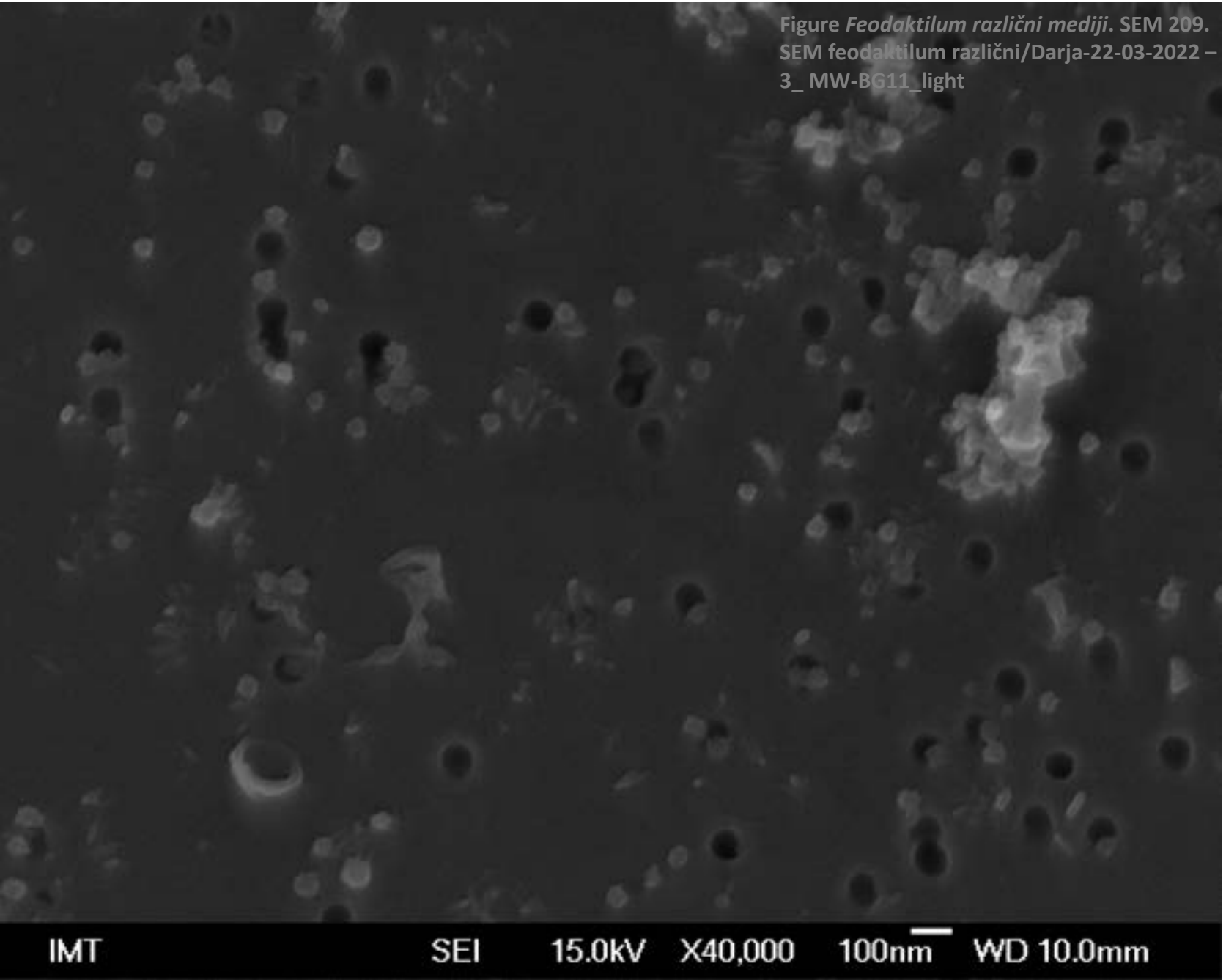

Figure *Phaeodactylum tricornutum* isolate BG11 L SEM 29.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with **Guillard's (F/2)** Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Isolation of small cellular particles (SCPs)

SCPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

Figure *Phaeodactylum tricornutum* isolate BG11 L SEM 52.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with **Guillard's (F/2)** Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Isolation of small cellular particles (SCPs)

SCPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

Figure *Phaeodactylum tricornutum* isolate BG11 L SEM 53.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with **Guillard's (F/2)** Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Isolation of small cellular particles (SCPs)

SCPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

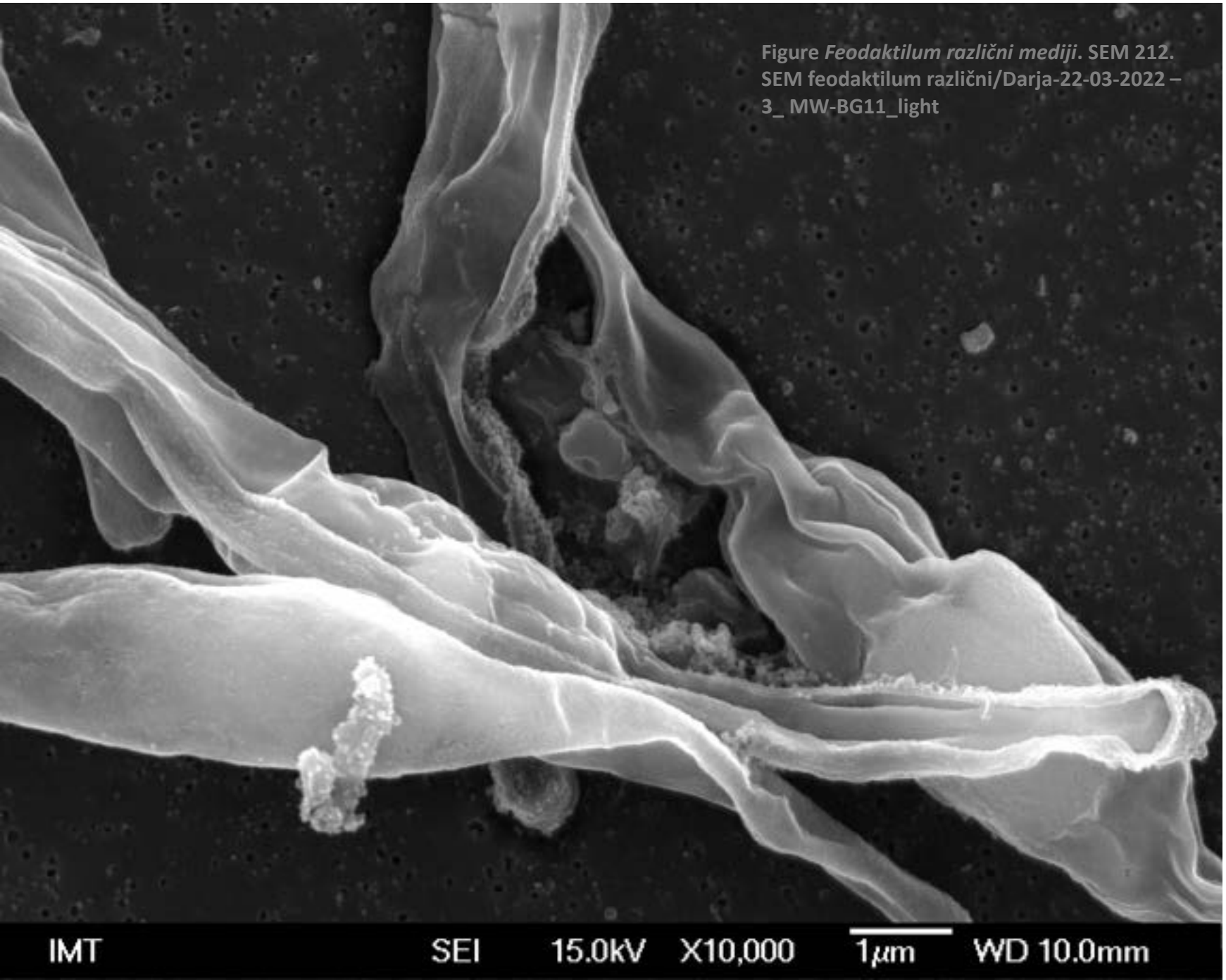

Figure *Phaeodactylum tricornutum* isolate BG11 L SEM 54.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with **Guillard's (F/2)** Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Isolation of small cellular particles (SCPs)

SCPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

Figure *Phaeodactylum tricornutum* isolate BG11 L SEM 55.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with **Guillard's (F/2)** Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Isolation of small cellular particles (SCPs)

SCPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

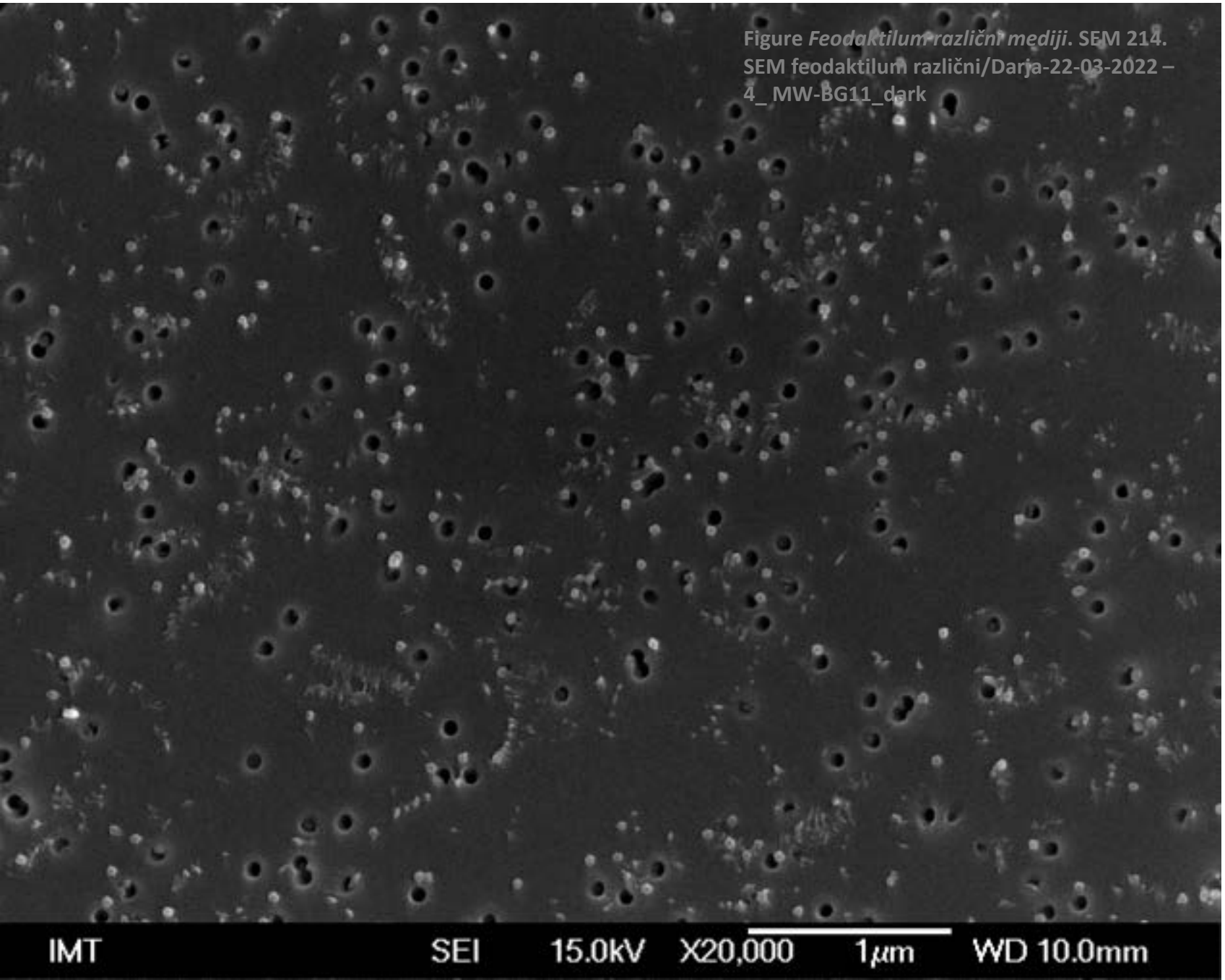

Figure *Phaeodactylum tricornutum* isolate BG11 D SEM 56.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with **Guillard's (F/2)** Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 12-hour light / 12-hour dark cycle, with aeration of 0.2 L/min.

Isolation of small cellular particles (SCPs)

SCPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

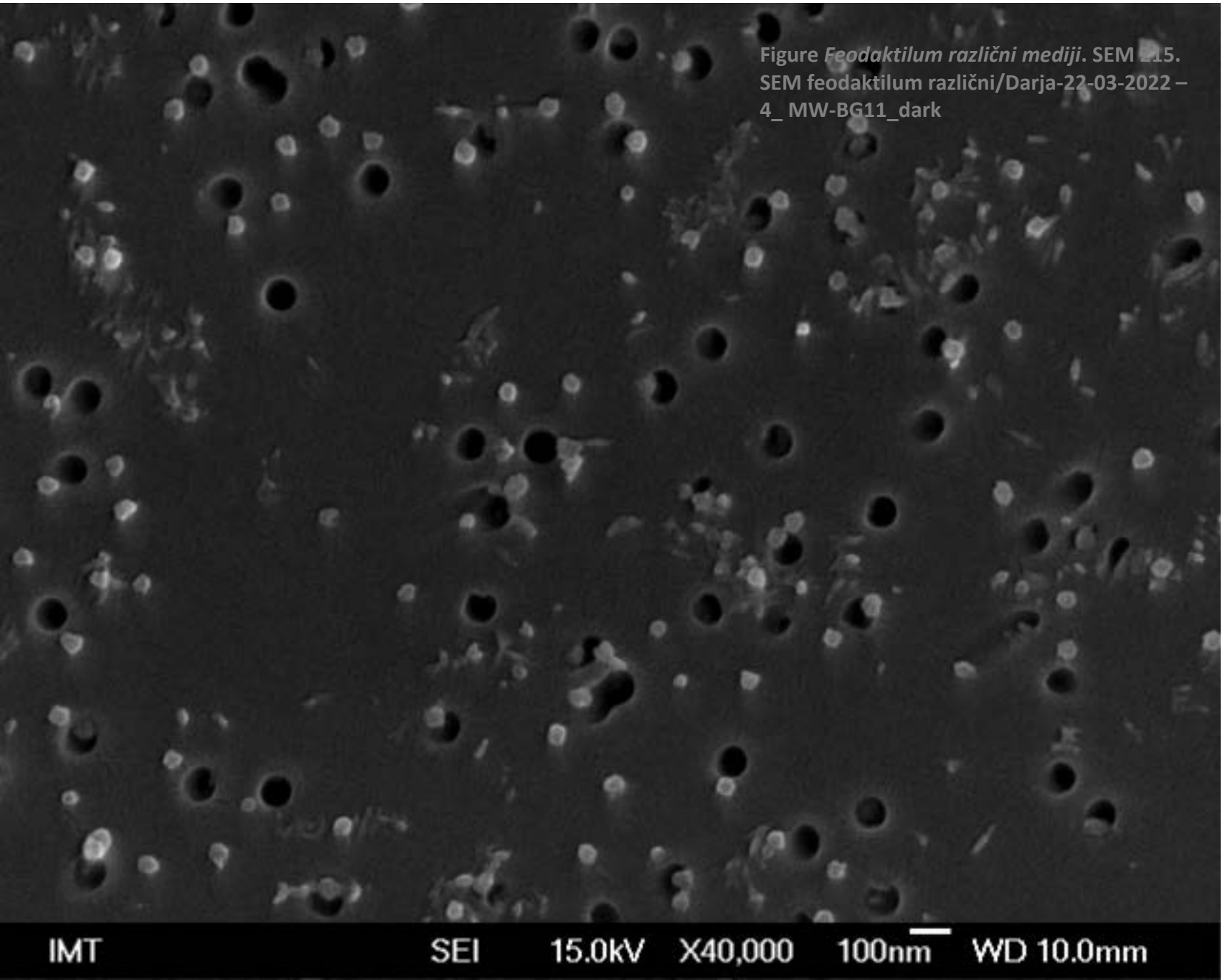

Figure *Phaeodactylum tricornutum* isolate BG11 D SEM 57.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with **Guillard's (F/2)** Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 12-hour light / 12-hour dark cycle, with aeration of 0.2 L/min.

Isolation of small cellular particles (SCPs)

SCPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

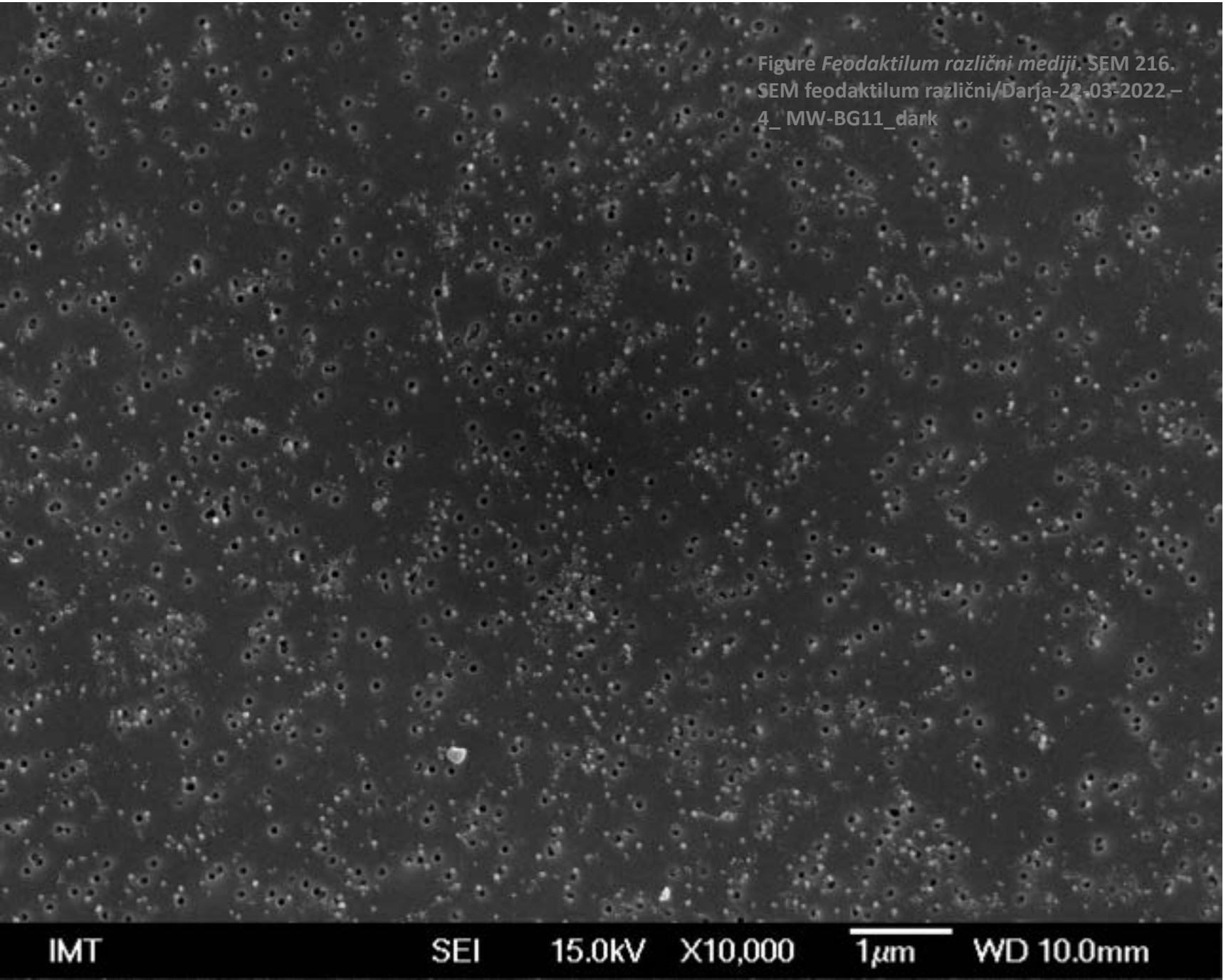

Figure *Phaeodactylum tricornutum* isolate BG11 D SEM 58.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with **Guillard's (F/2)** Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 12-hour light / 12-hour dark cycle, with aeration of 0.2 L/min.

Isolation of small cellular particles (SCPs)

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Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

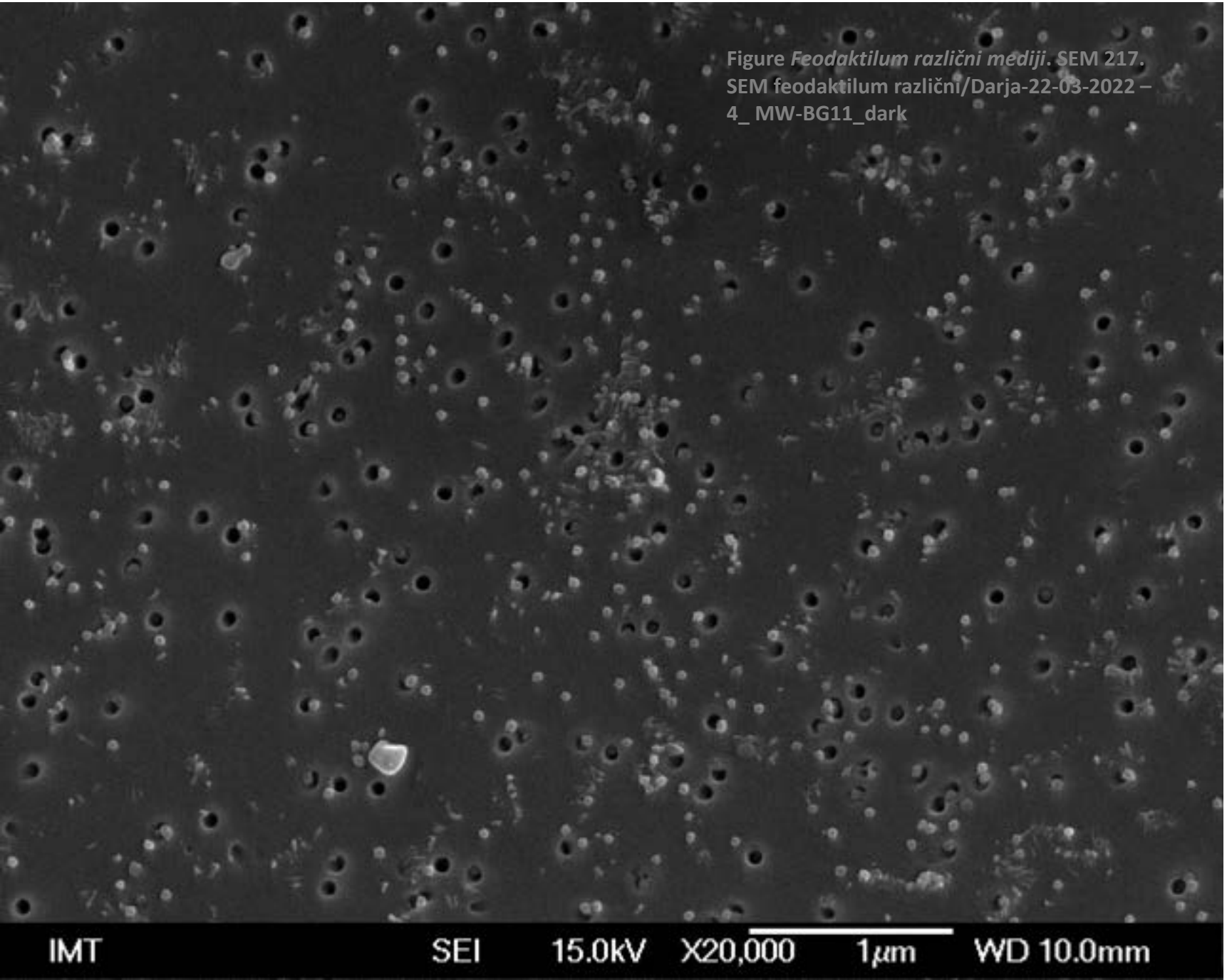

Figure *Phaeodactylum tricornutum* isolate BG11 D SEM 59.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with **Guillard's (F/2)** Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 12-hour light / 12-hour dark cycle, with aeration of 0.2 L/min.

Isolation of small cellular particles (SCPs)

SCPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

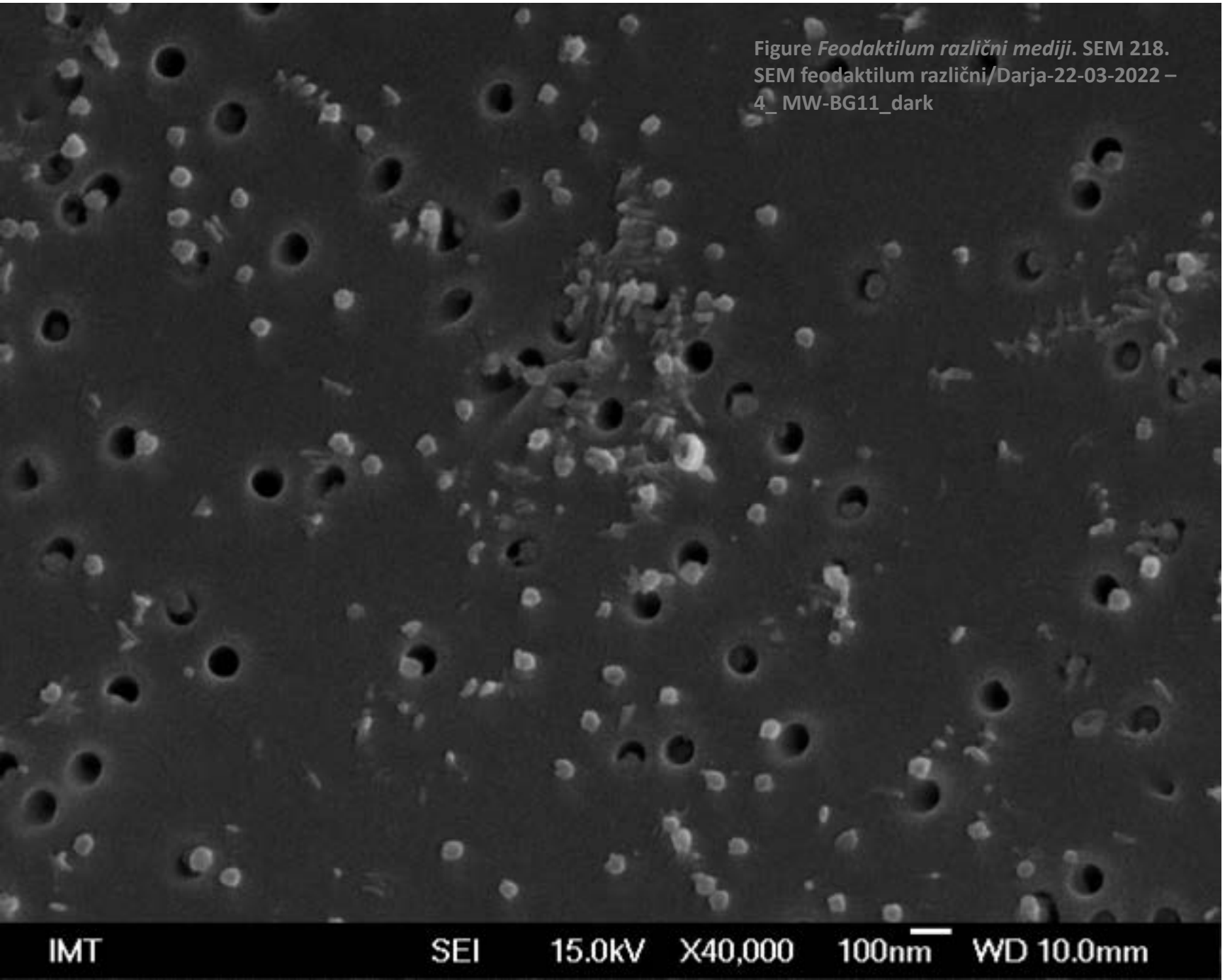

Figure *Phaeodactylum tricornutum* isolate BG11 D SEM 60.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with **Guillard's (F/2)** Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 12-hour light / 12-hour dark cycle, with aeration of 0.2 L/min.

Isolation of small cellular particles (SCPs)

SCPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

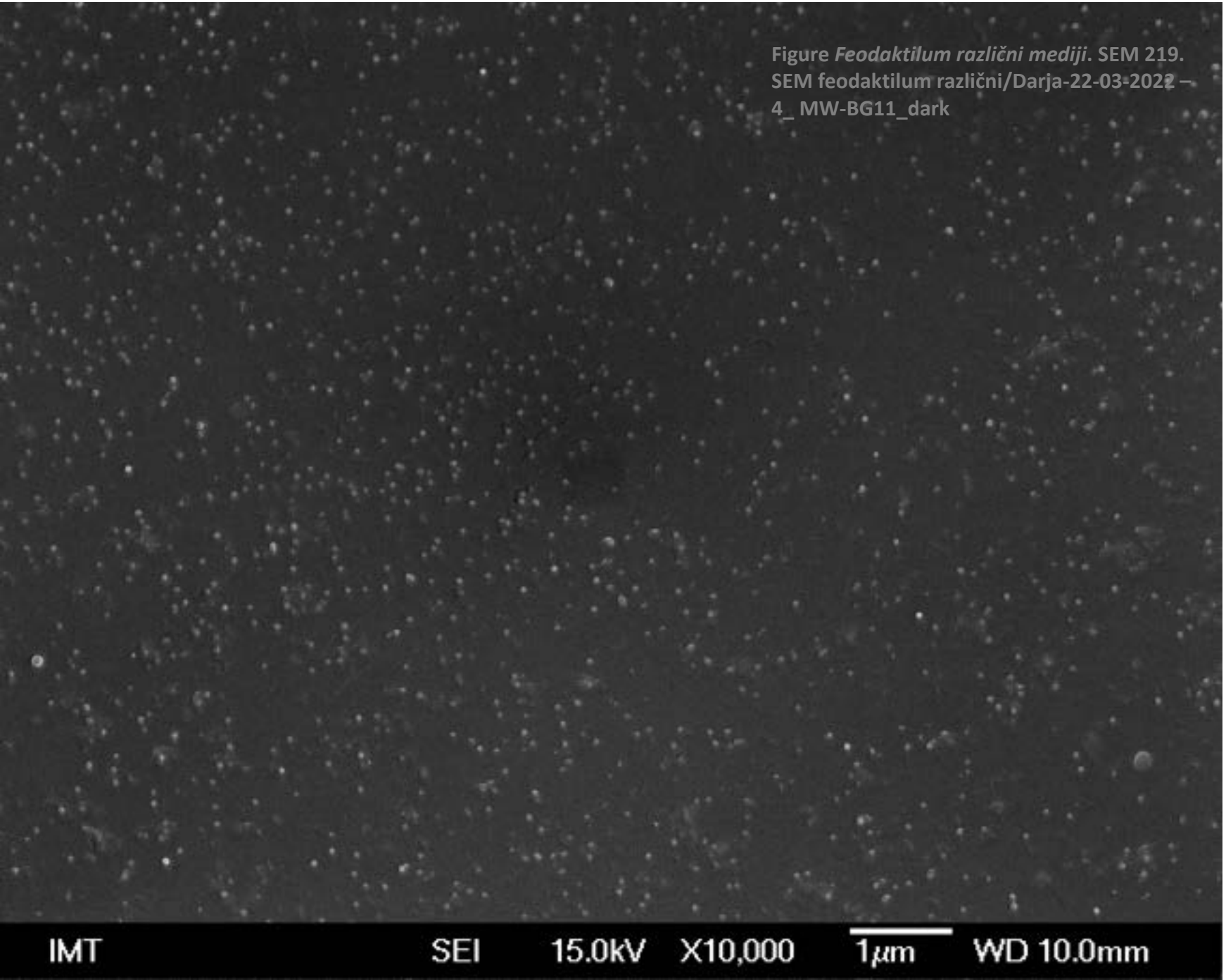

Figure *Phaeodactylum tricornutum* isolate BG11 D SEM 61.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with **Guillard's (F/2)** Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 12-hour light / 12-hour dark cycle, with aeration of 0.2 L/min.

Isolation of small cellular particles (SCPs)

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Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

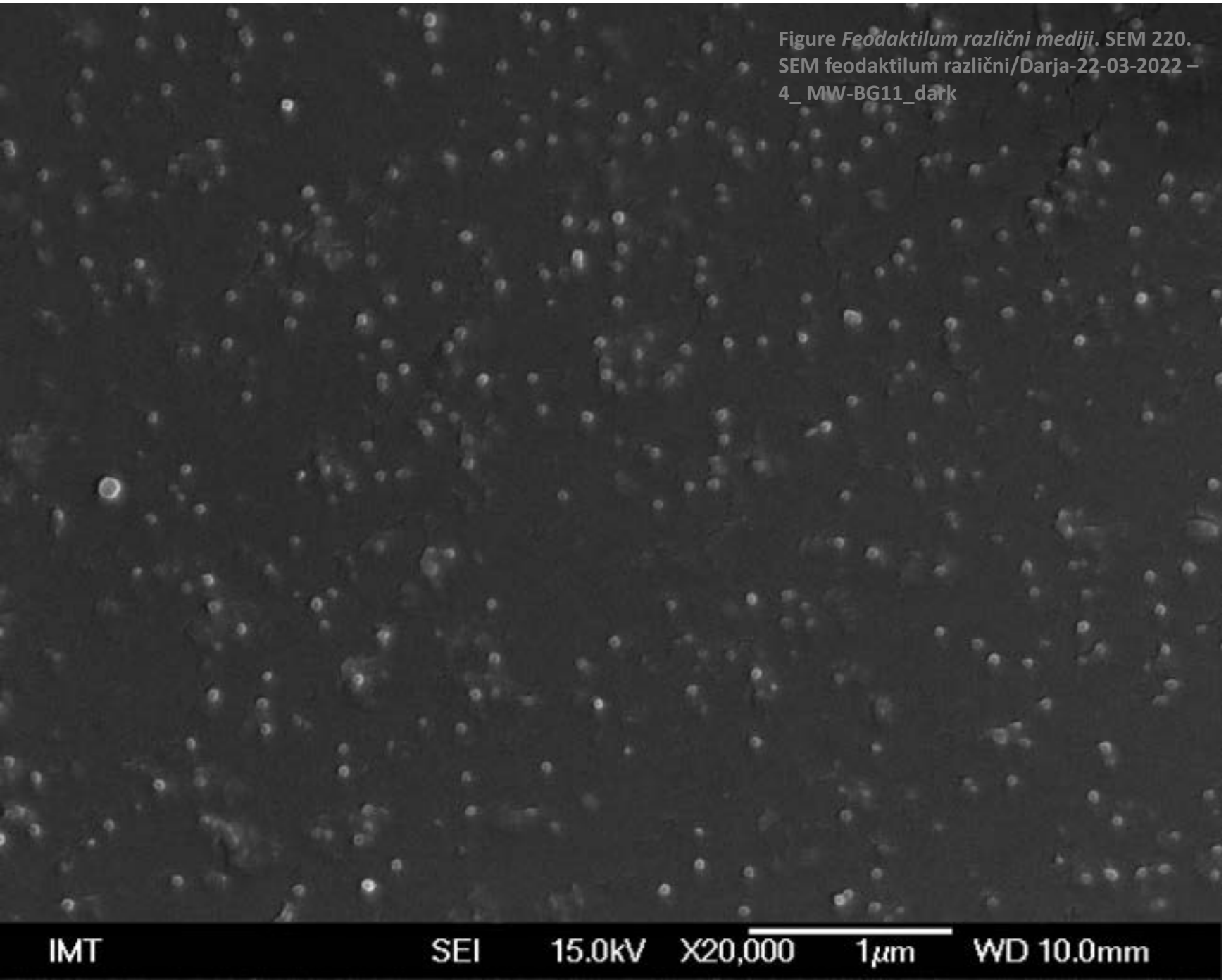

Figure *Phaeodactylum tricornutum* isolate BG11 D SEM 62.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with **Guillard's (F/2)** Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 12-hour light / 12-hour dark cycle, with aeration of 0.2 L/min.

Isolation of small cellular particles (SCPs)

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Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

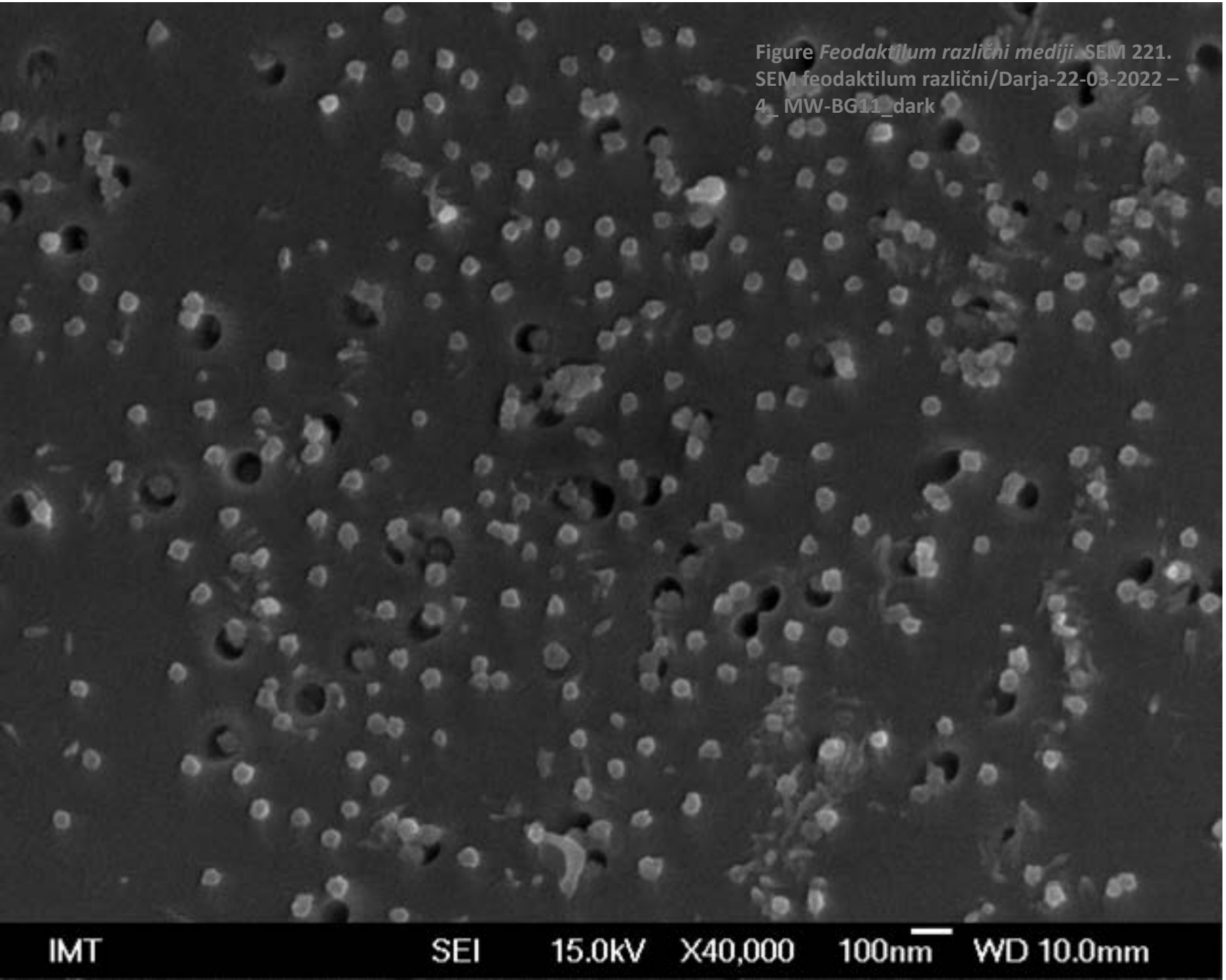

Figure *Phaeodactylum tricornutum* isolate BG11 D SEM 63.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with **Guillard's (F/2)** Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 12-hour light / 12-hour dark cycle, with aeration of 0.2 L/min.

Isolation of small cellular particles (SCPs)

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Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

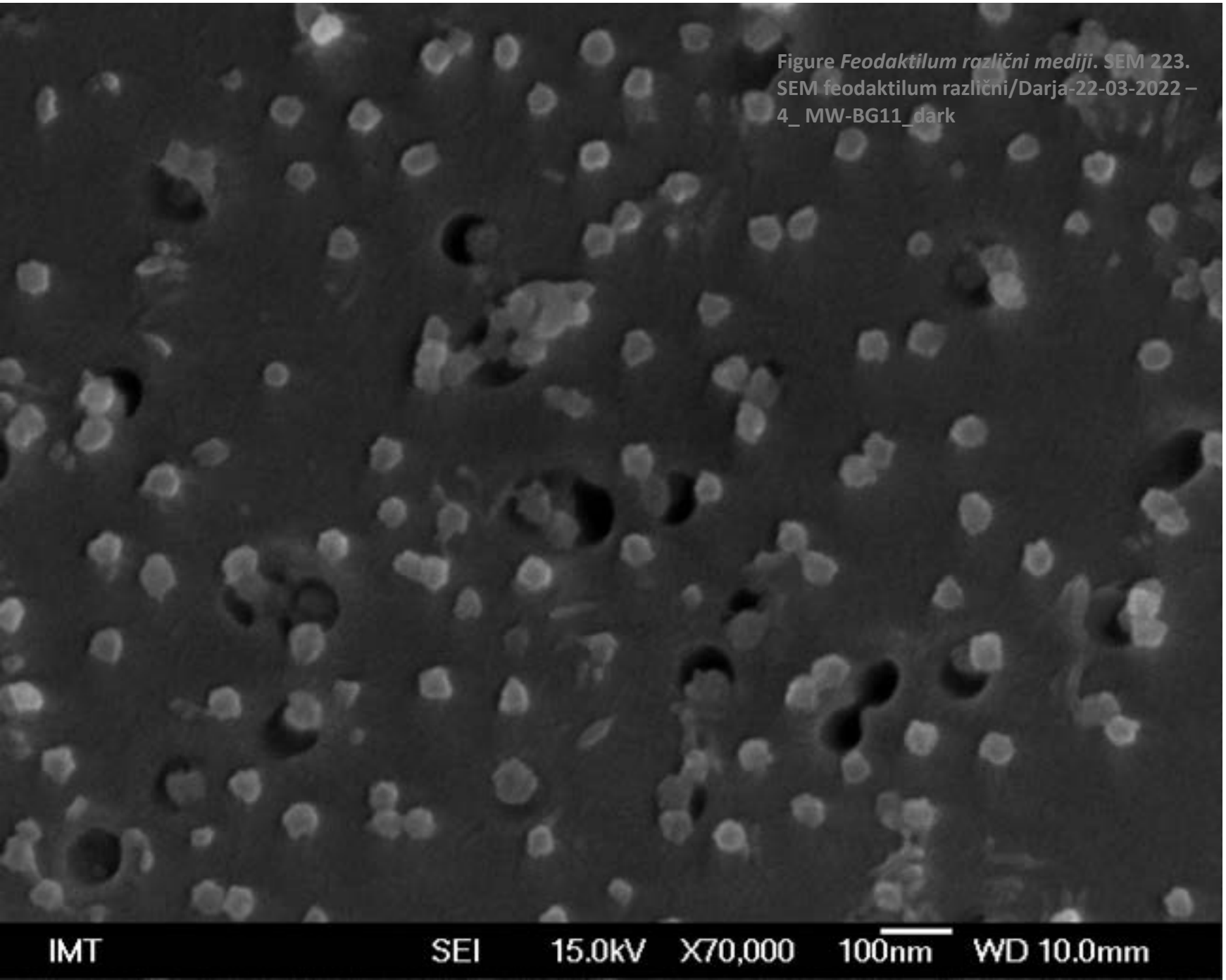

Figure *Phaeodactylum tricornutum* isolate BG11 D SEM 64.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with **Guillard's (F/2)** Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 12-hour light / 12-hour dark cycle, with aeration of 0.2 L/min.

Isolation of small cellular particles (SCPs)

SCPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

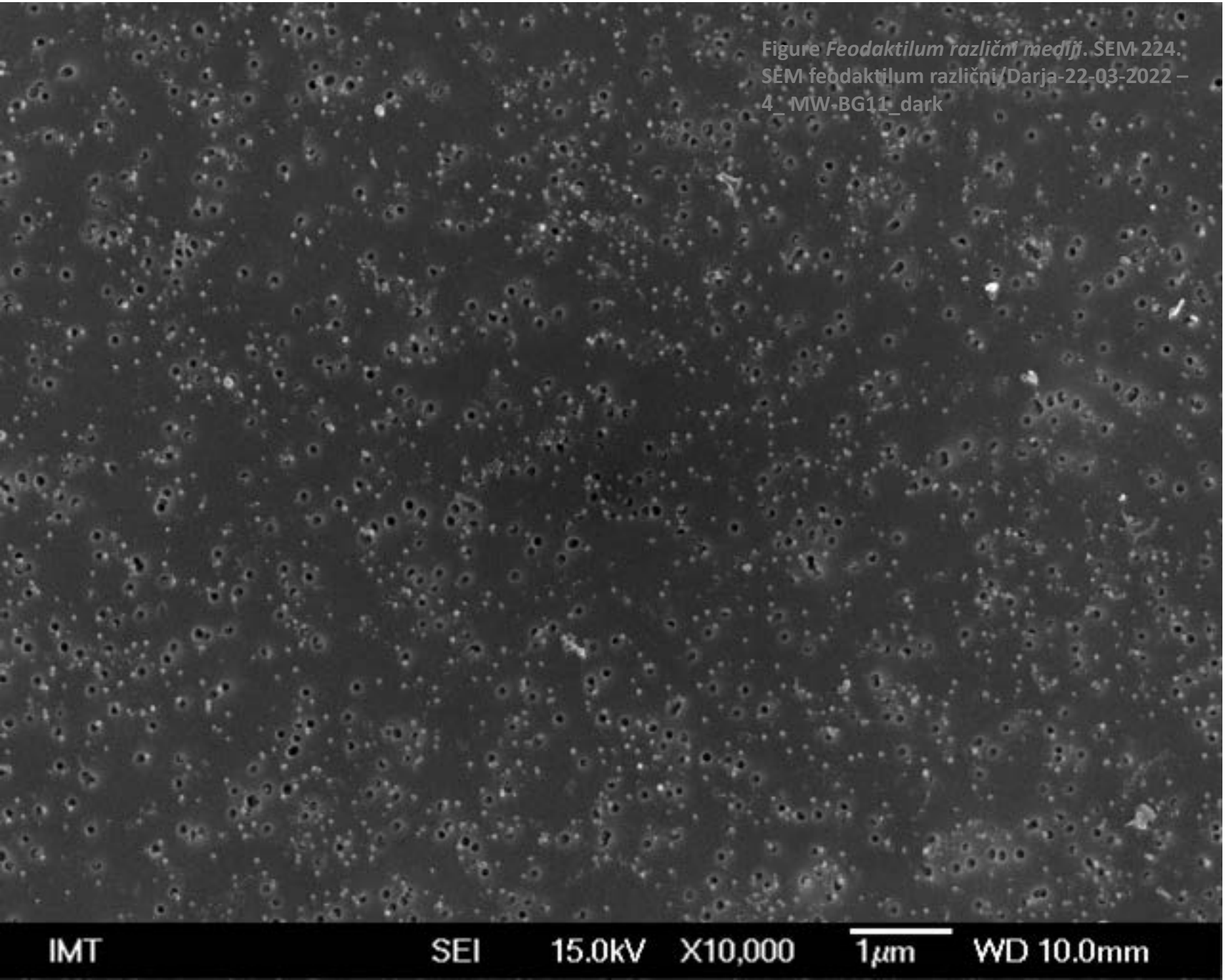

Figure *Phaeodactylum tricornutum* isolate BG11 D SEM 65.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with **Guillard's (F/2)** Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 12-hour light / 12-hour dark cycle, with aeration of 0.2 L/min.

Isolation of small cellular particles (SCPs)

SCPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

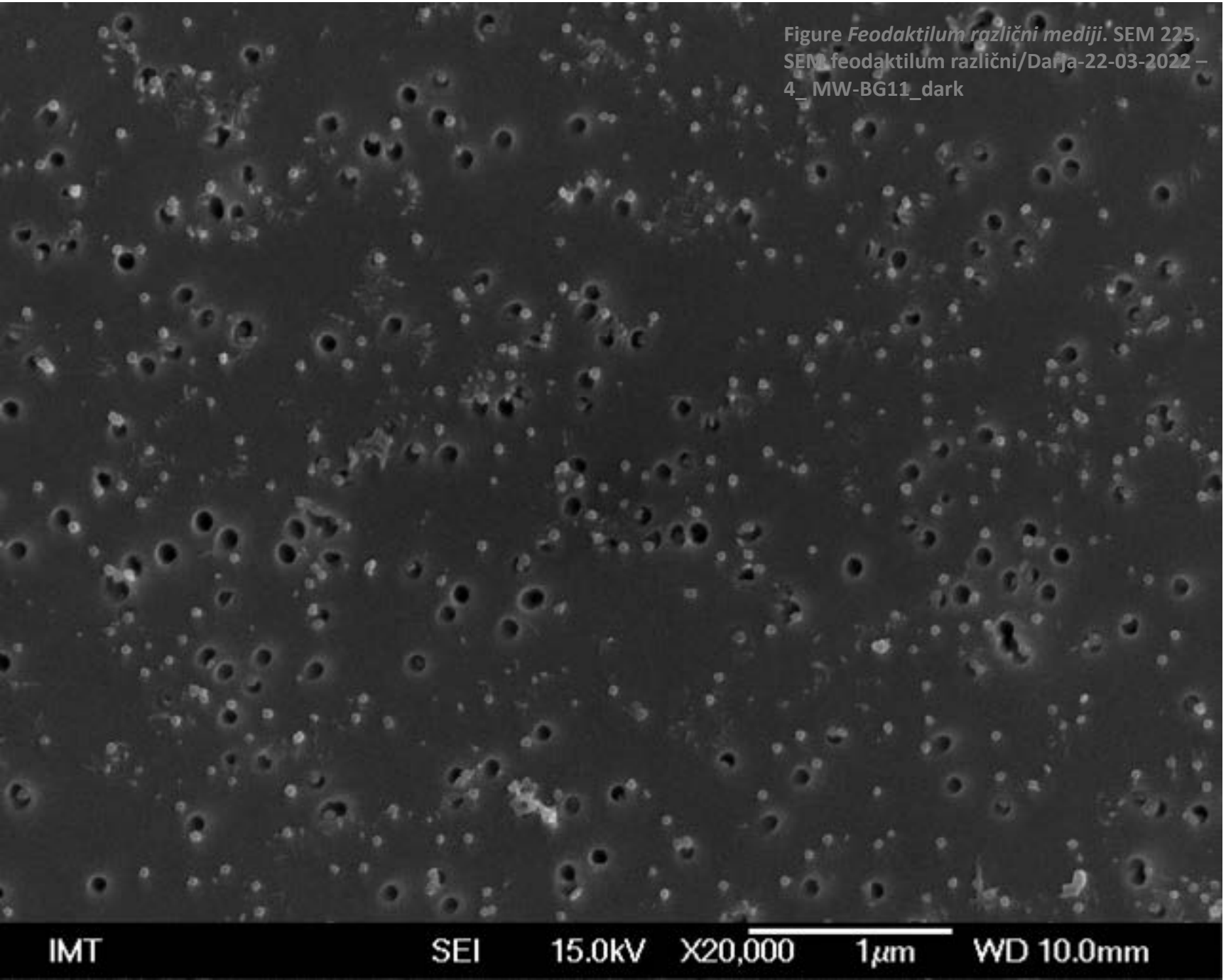

Figure *Phaeodactylum tricornutum* isolate BG11 D SEM 66.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with **Guillard's (F/2)** Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 12-hour light / 12-hour dark cycle, with aeration of 0.2 L/min.

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Figure *Phaeodactylum tricornutum* isolate BG11 D SEM 67.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with **Guillard's (F/2)** Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 12-hour light / 12-hour dark cycle, with aeration of 0.2 L/min.

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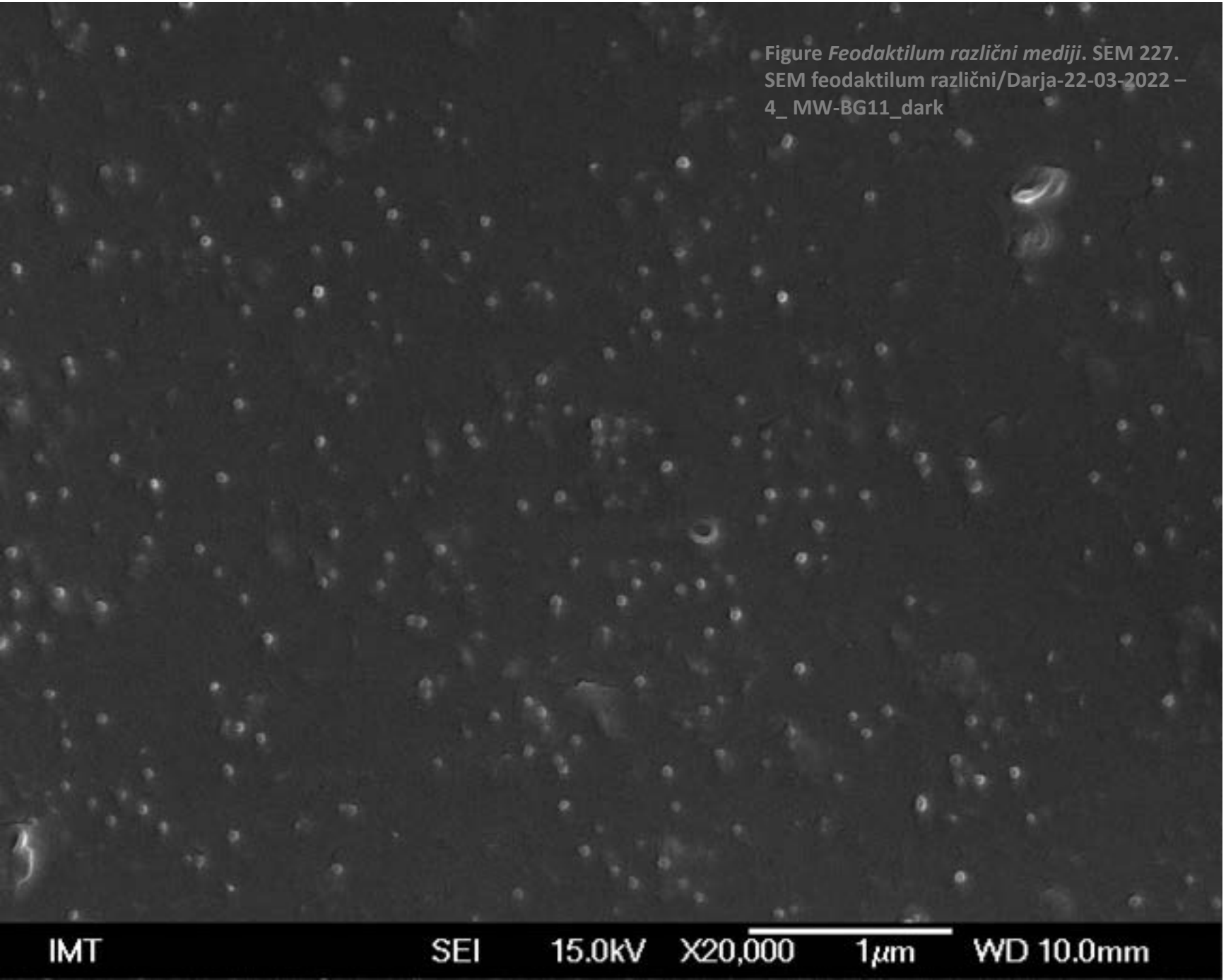

Figure *Phaeodactylum tricornutum* isolate BG11 D SEM 68.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with **Guillard's (F/2)** Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 12-hour light / 12-hour dark cycle, with aeration of 0.2 L/min.

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SCPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).