Proceedings of 9th Socratic Lectures **2024**

Repository

Scanning electron microscope images of *Dunaliella tertiolecta* culture

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, [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

* Corresponding author: Veronika Kralj-Iglič, veronika.kralj-iglic@zf.uni-lj.si

Citation: Bedina Zavec A, Božič D, Hočevar M, Iglič A, Jeran M, Kralj-Iglič V, Romolo A. Scanning electron microscope images of *Dunaliella tertiolecta* culture. Proceedings of Socratic Lectures. **2024**, 9, 127-158.
https://doi.org/10.55295/PSL.2024.D10

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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).

Acknowledgements: This project has received funding from the European Union's Horizon 2020 research and innovation program under grant agreement No 801338 and ARRS projects P1-0391, P2-0232, P3-0388, J2-4447, J2-4427, L3-2621, J3-3066, IO-0006 (A) and National Research, Development and Innovation Office (Hungary), grant number SNN 138407.

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)

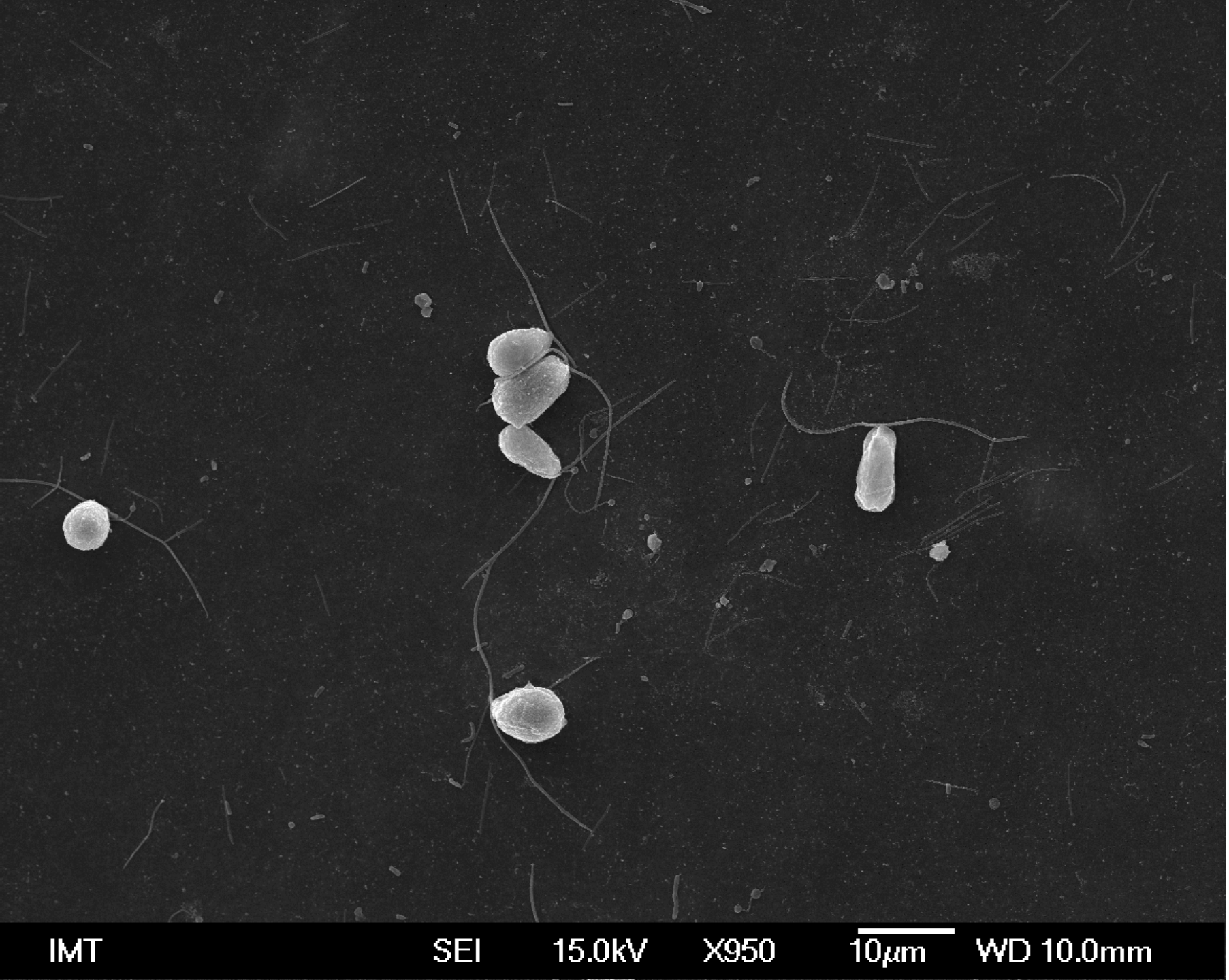

Figure *Dunaliella tertiolecta* culture SEM 1.

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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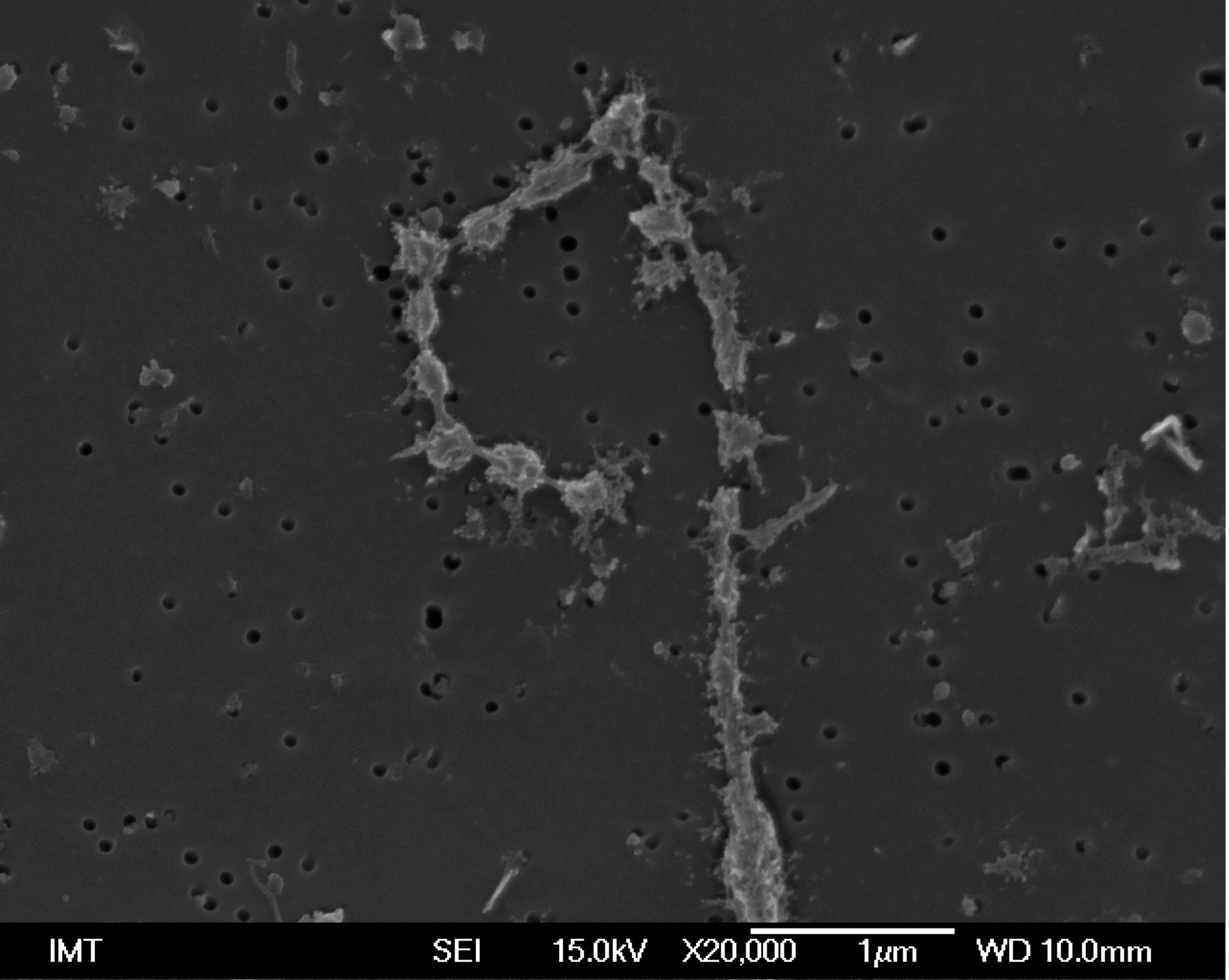

Figure *Dunaliella tertiolecta* culture SEM 3.

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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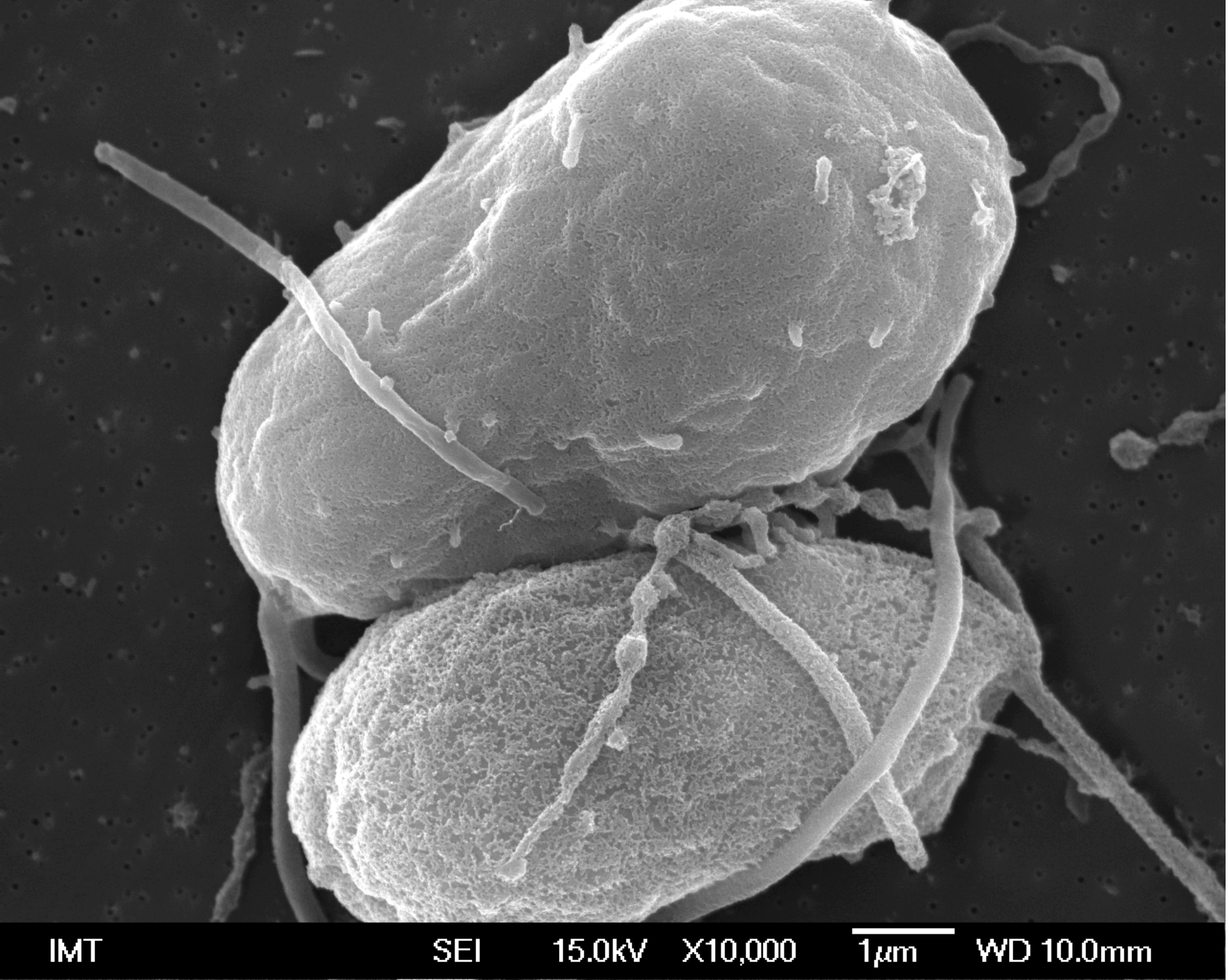

Figure *Dunaliella tertiolecta* culture SEM 4.

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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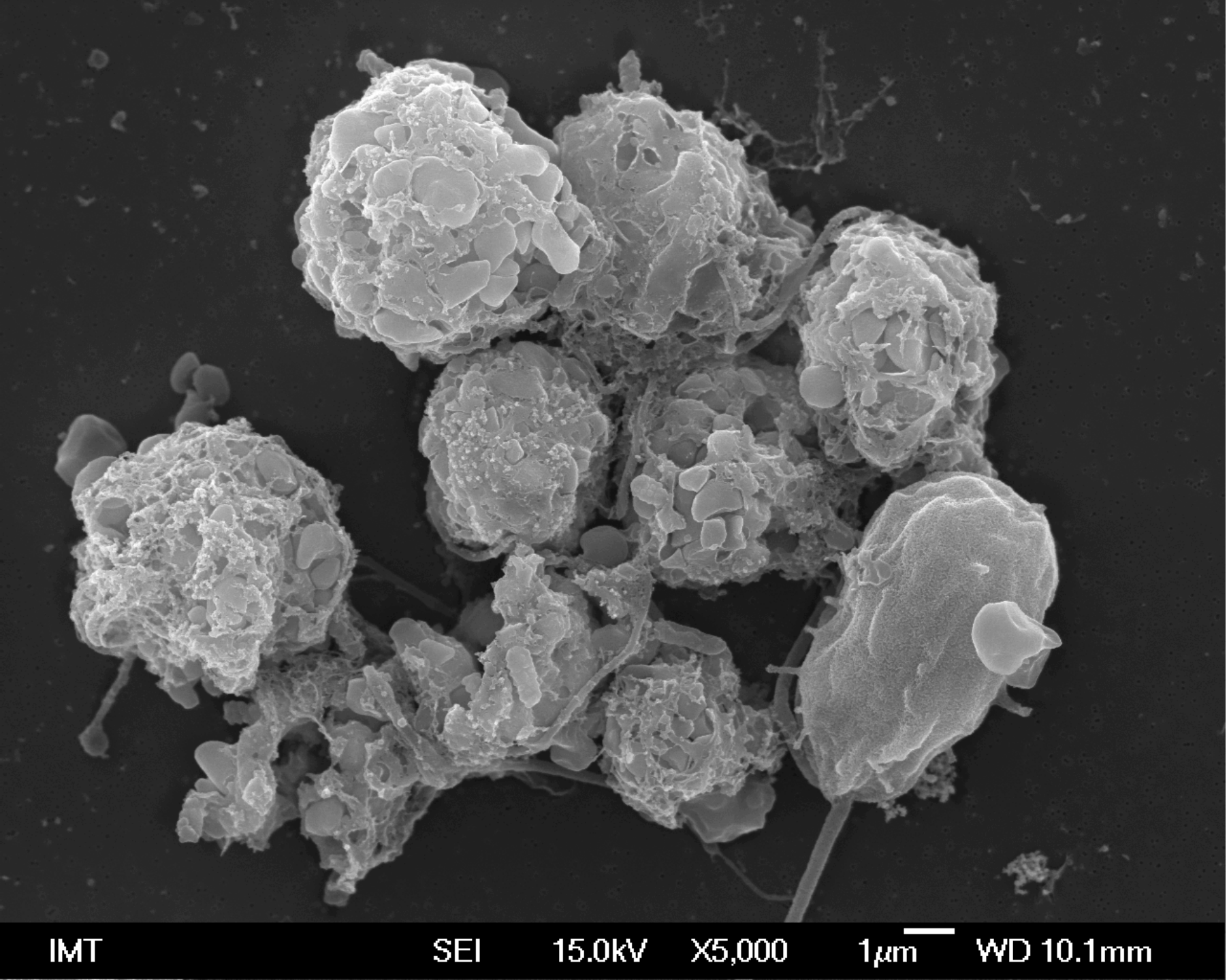

Figure *Dunaliella tertiolecta* culture SEM 5.

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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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).

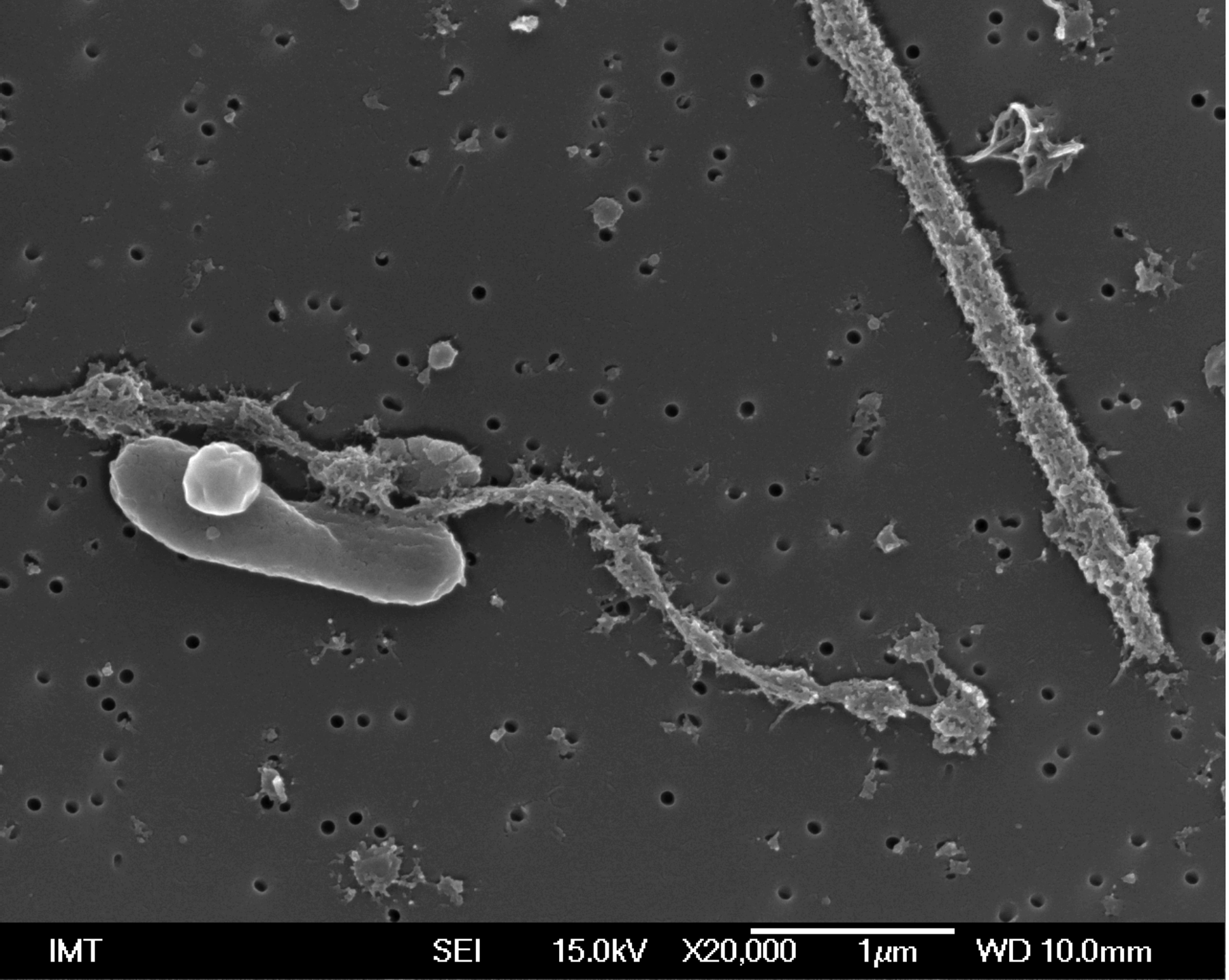

Figure *Dunaliella tertiolecta* culture SEM 7.

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).

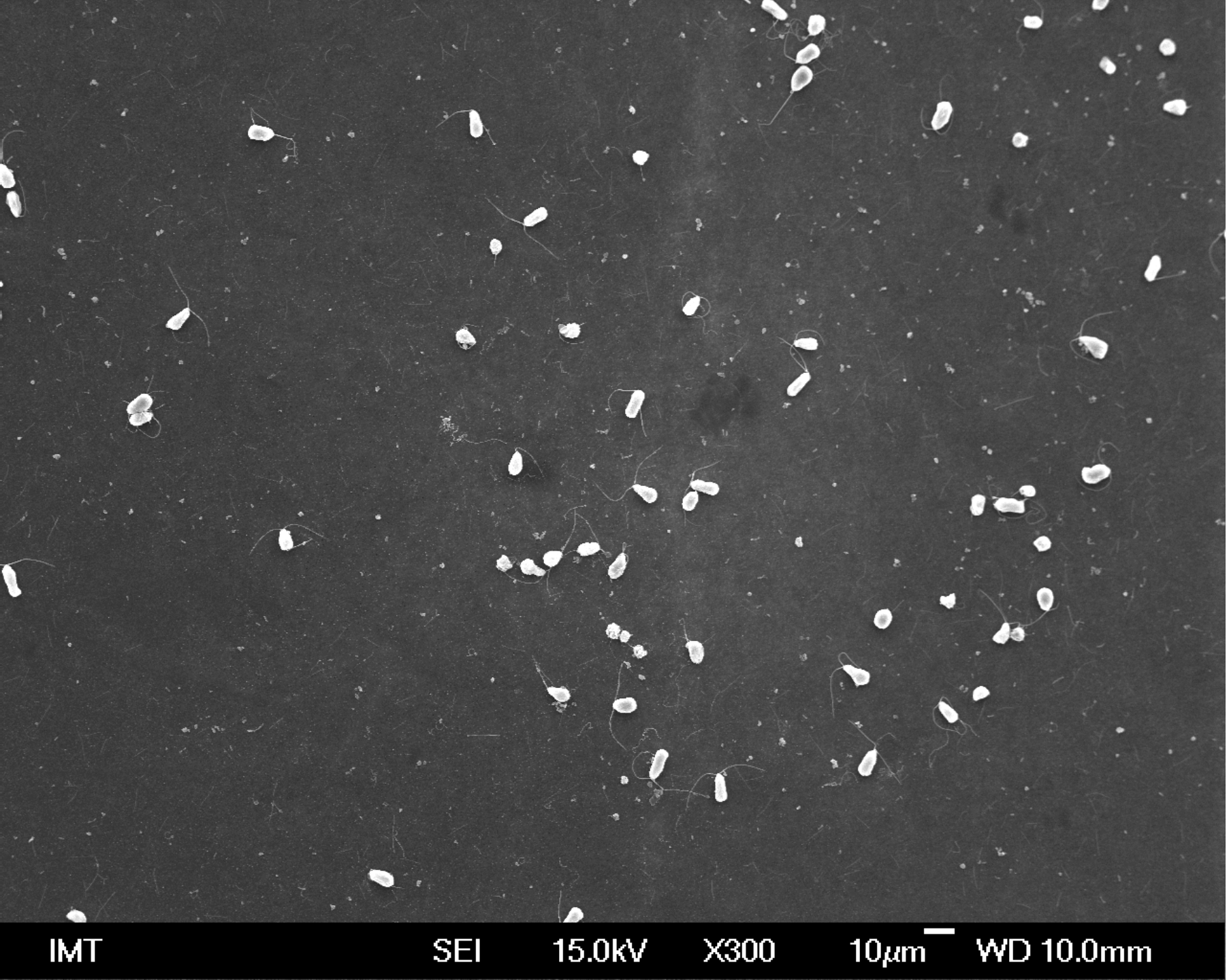

Figure *Dunaliella tertiolecta* culture SEM 8.

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).

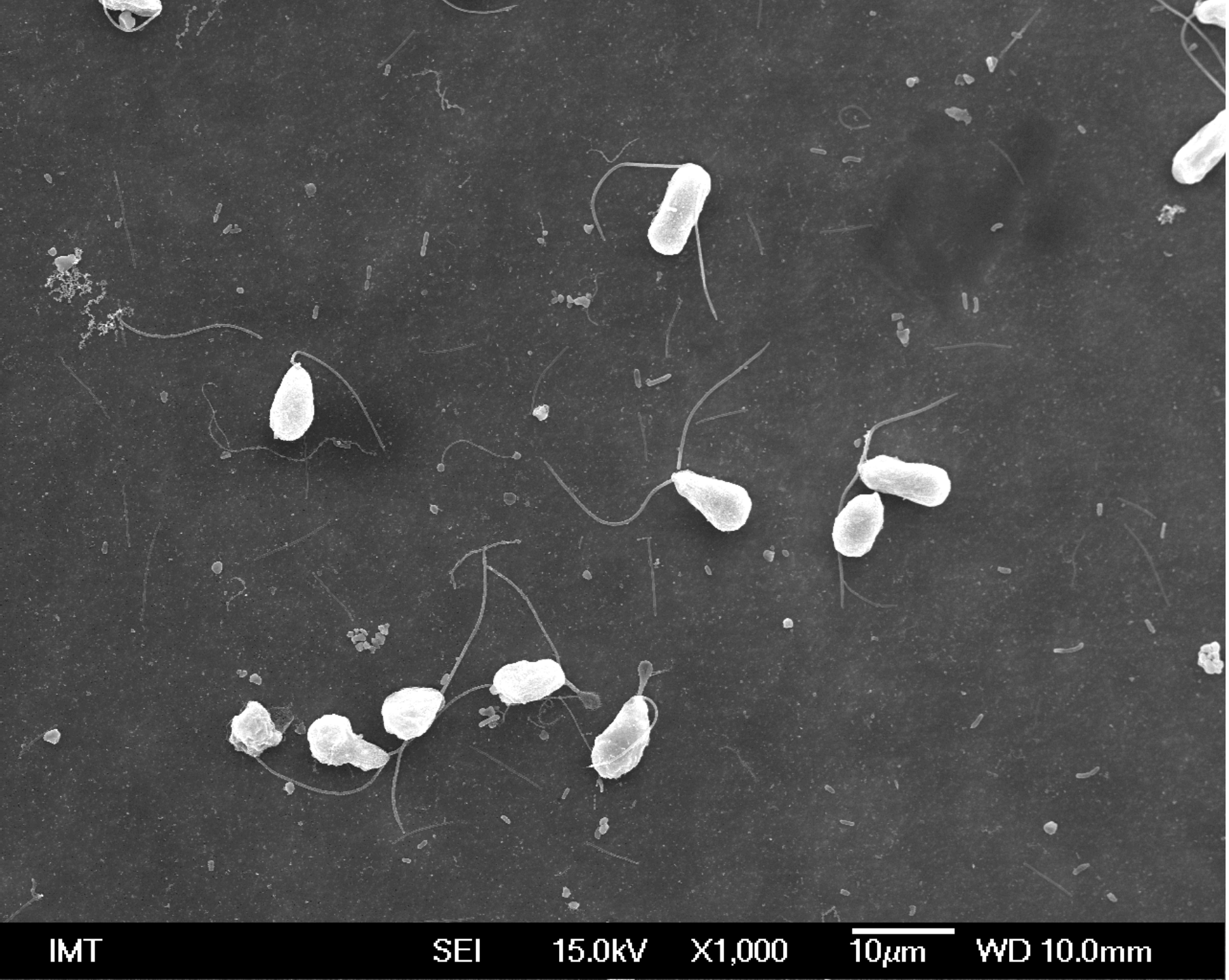

Figure *Dunaliella tertiolecta* culture SEM 9.

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).

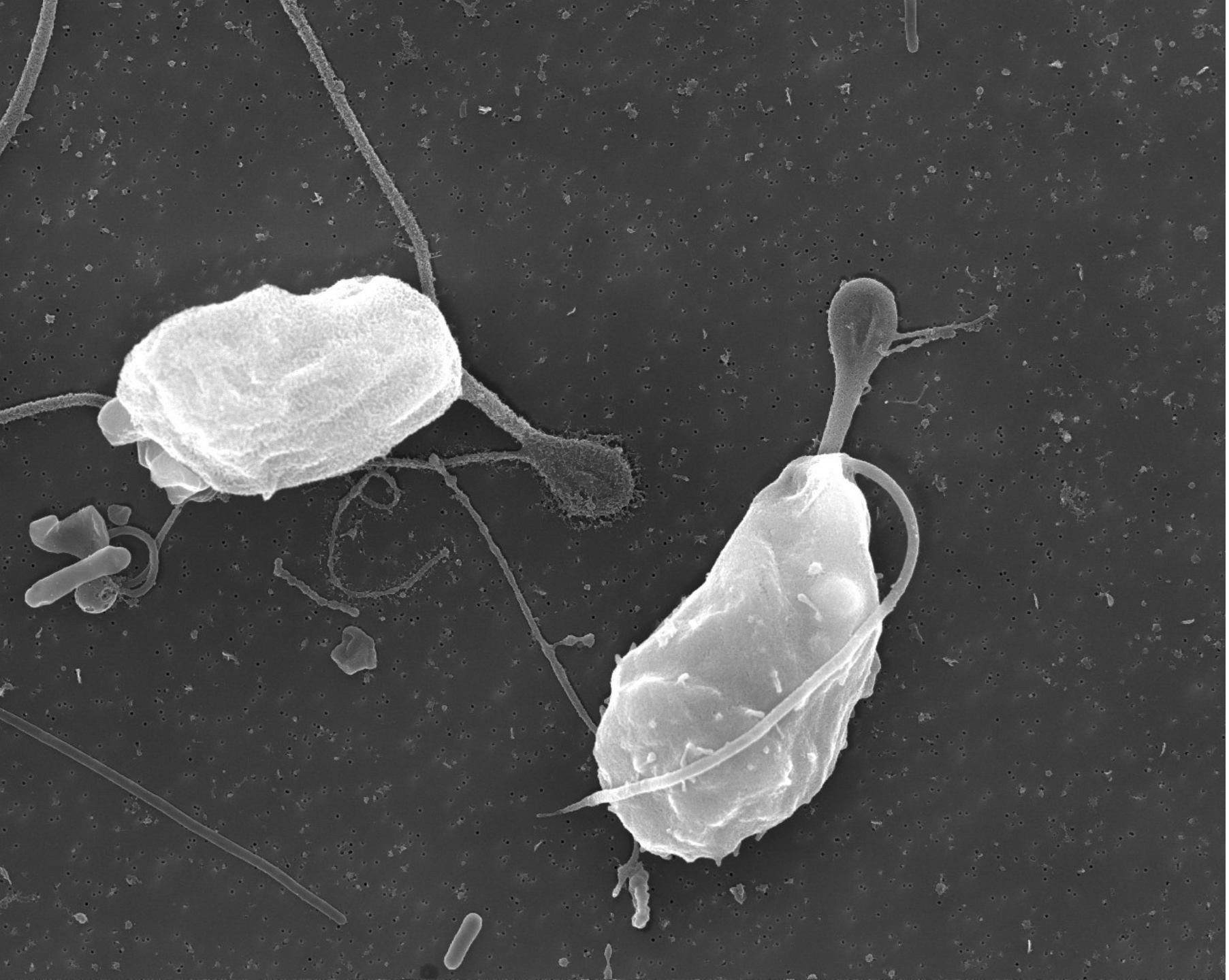

Figure *Dunaliella tertiolecta* culture SEM 10.

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).

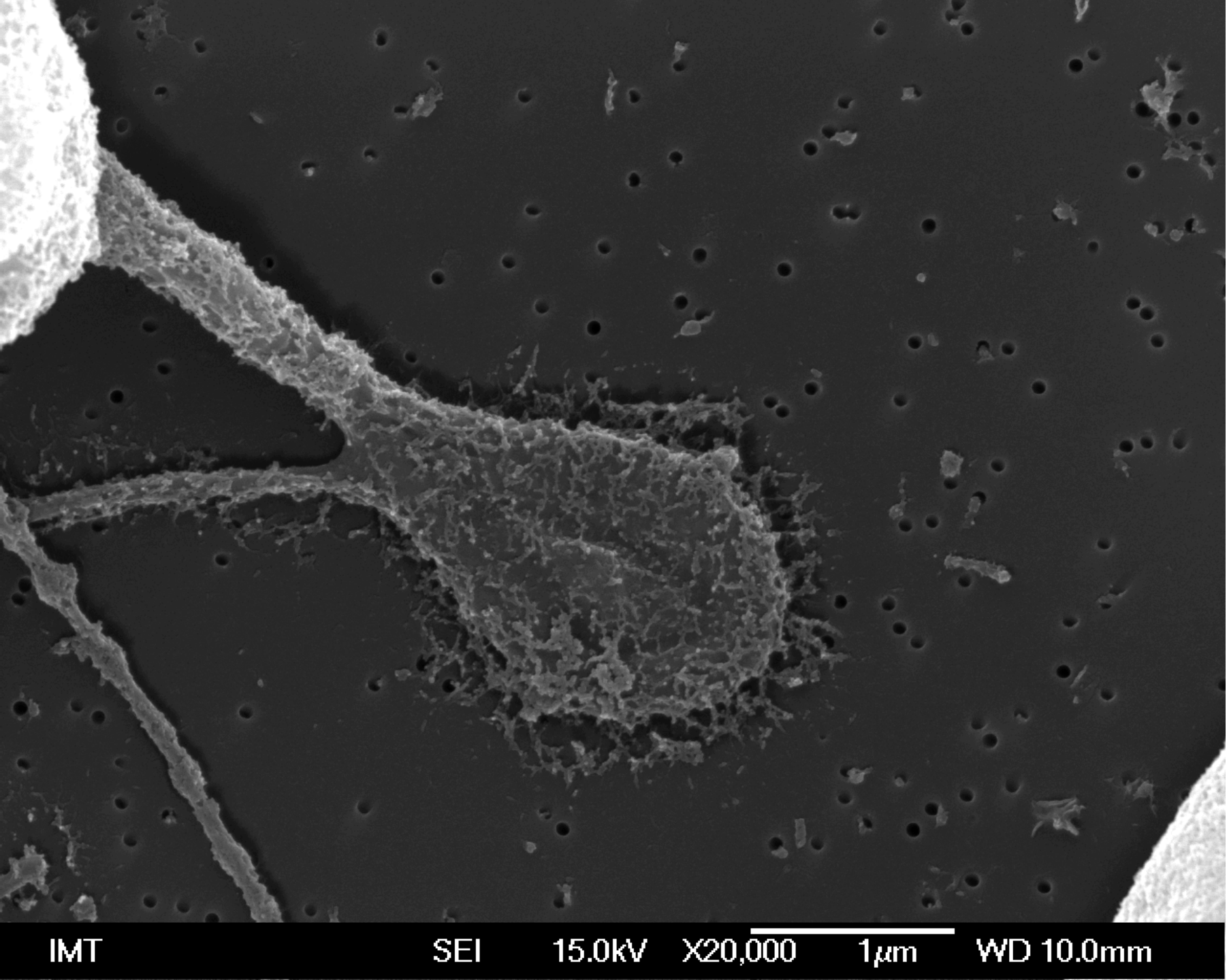

Figure *Dunaliella tertiolecta* culture SEM 11.

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).

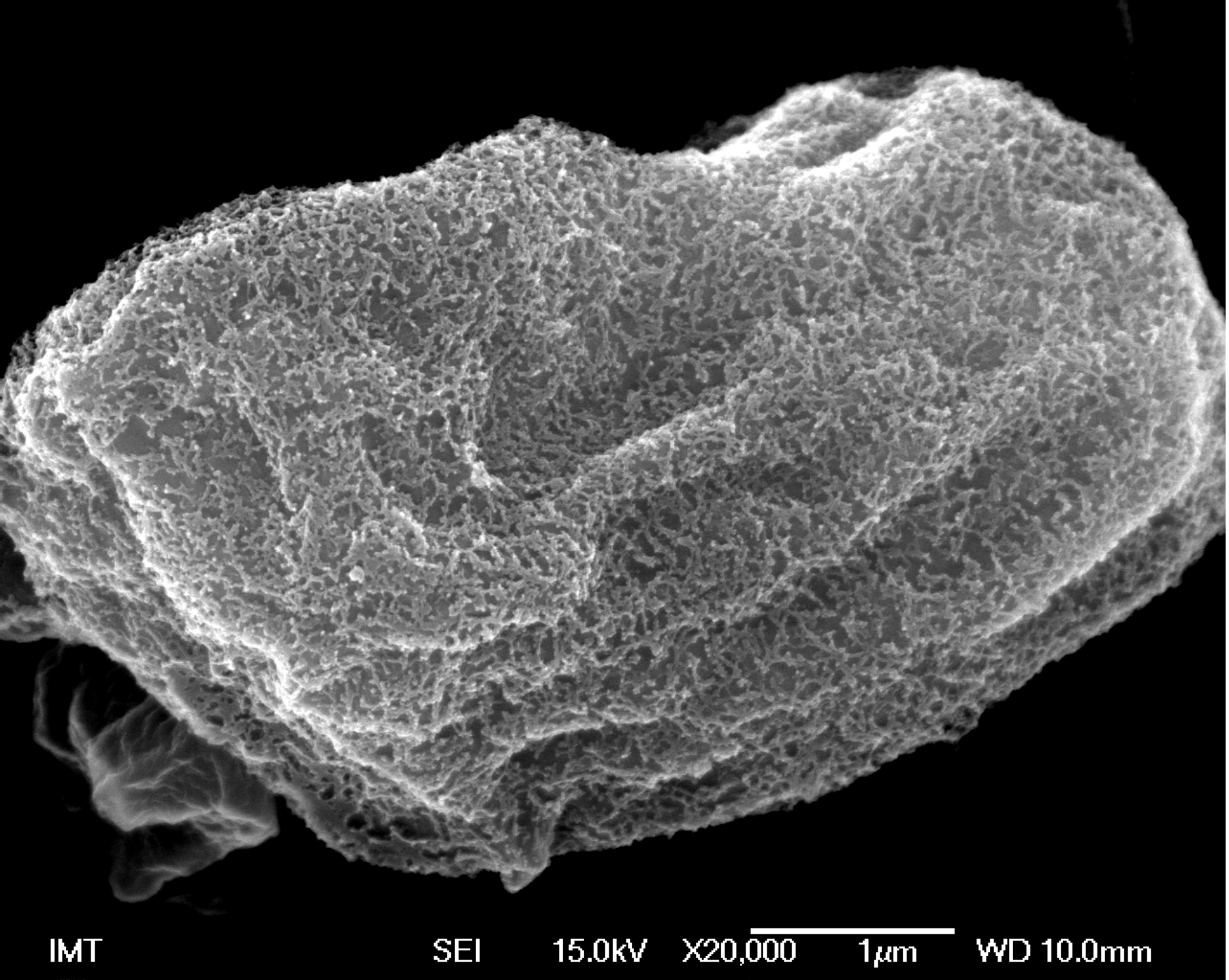

Figure *Dunaliella tertiolecta* culture SEM 12.

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).

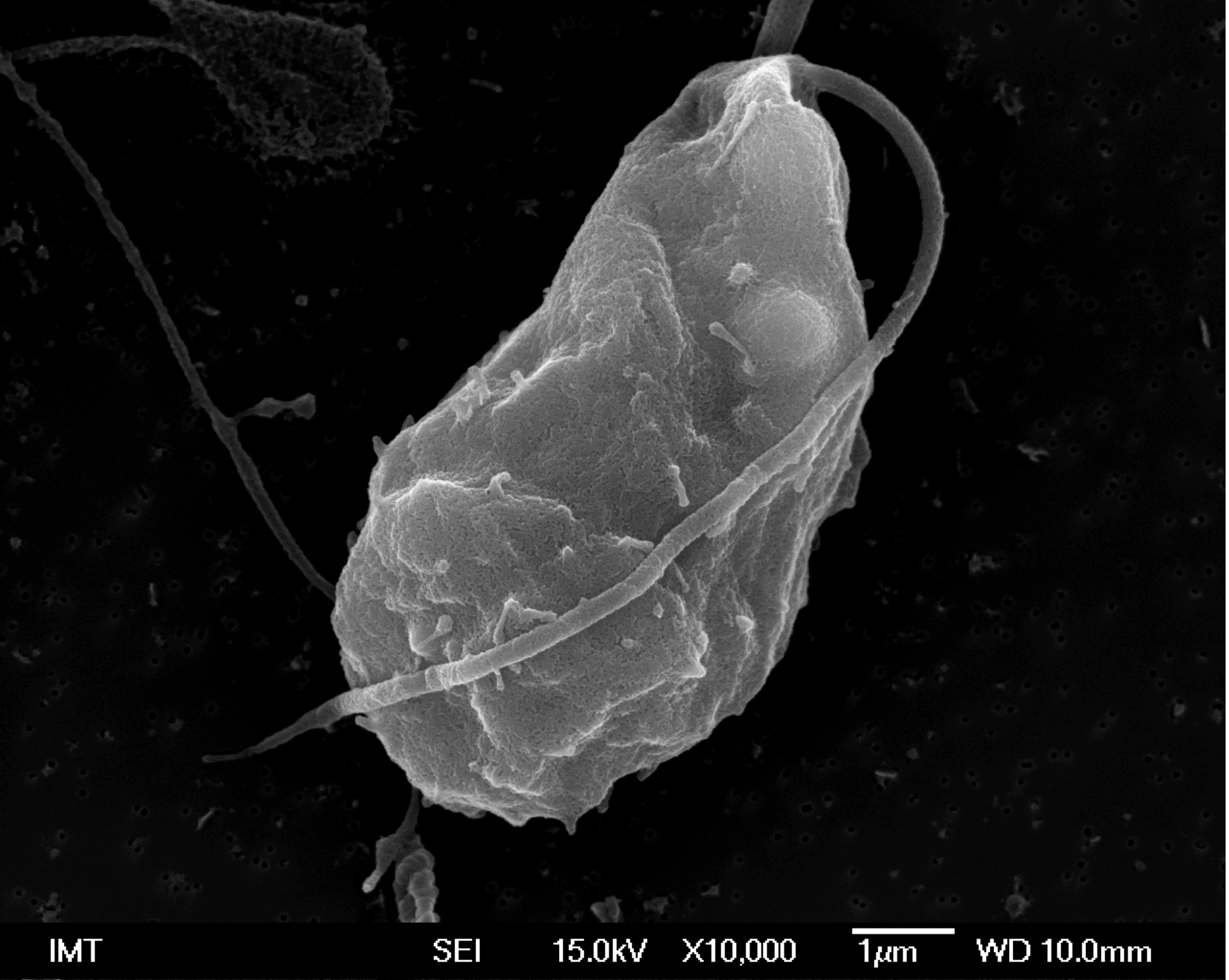

Figure *Dunaliella tertiolecta* culture SEM 13.

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).

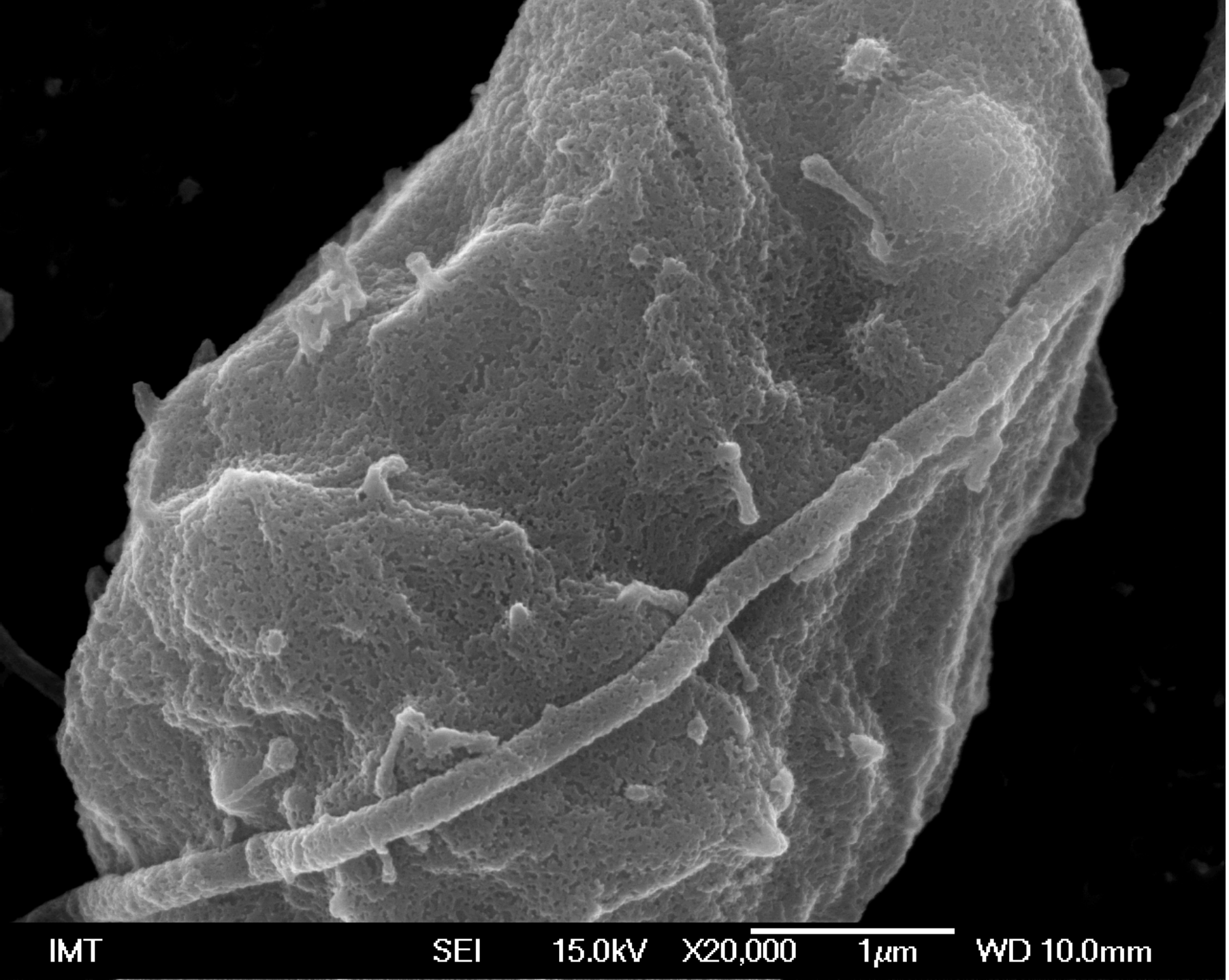

Figure *Dunaliella tertiolecta* culture SEM 14.

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).

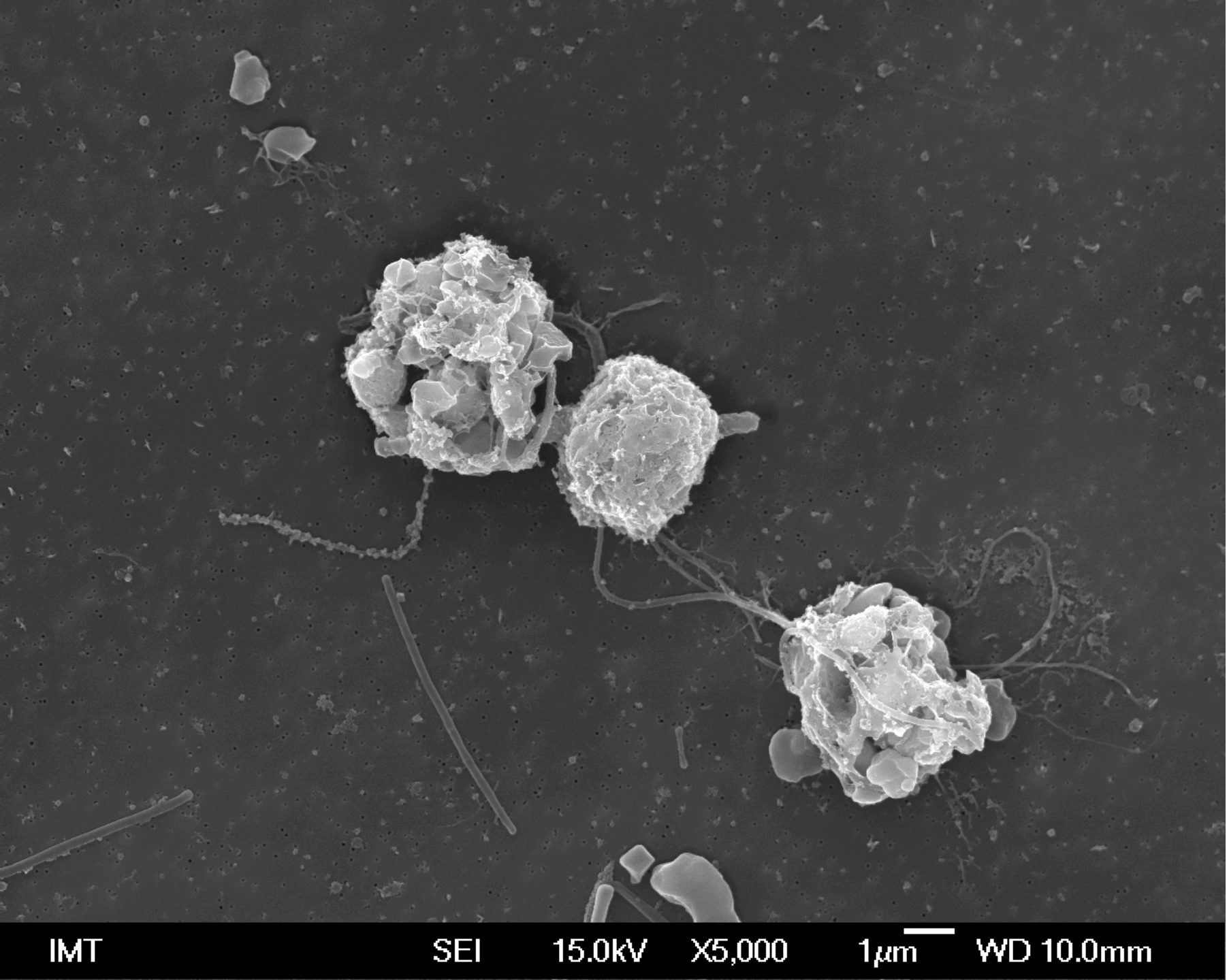

Figure *Dunaliella tertiolecta* culture SEM 15.

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).

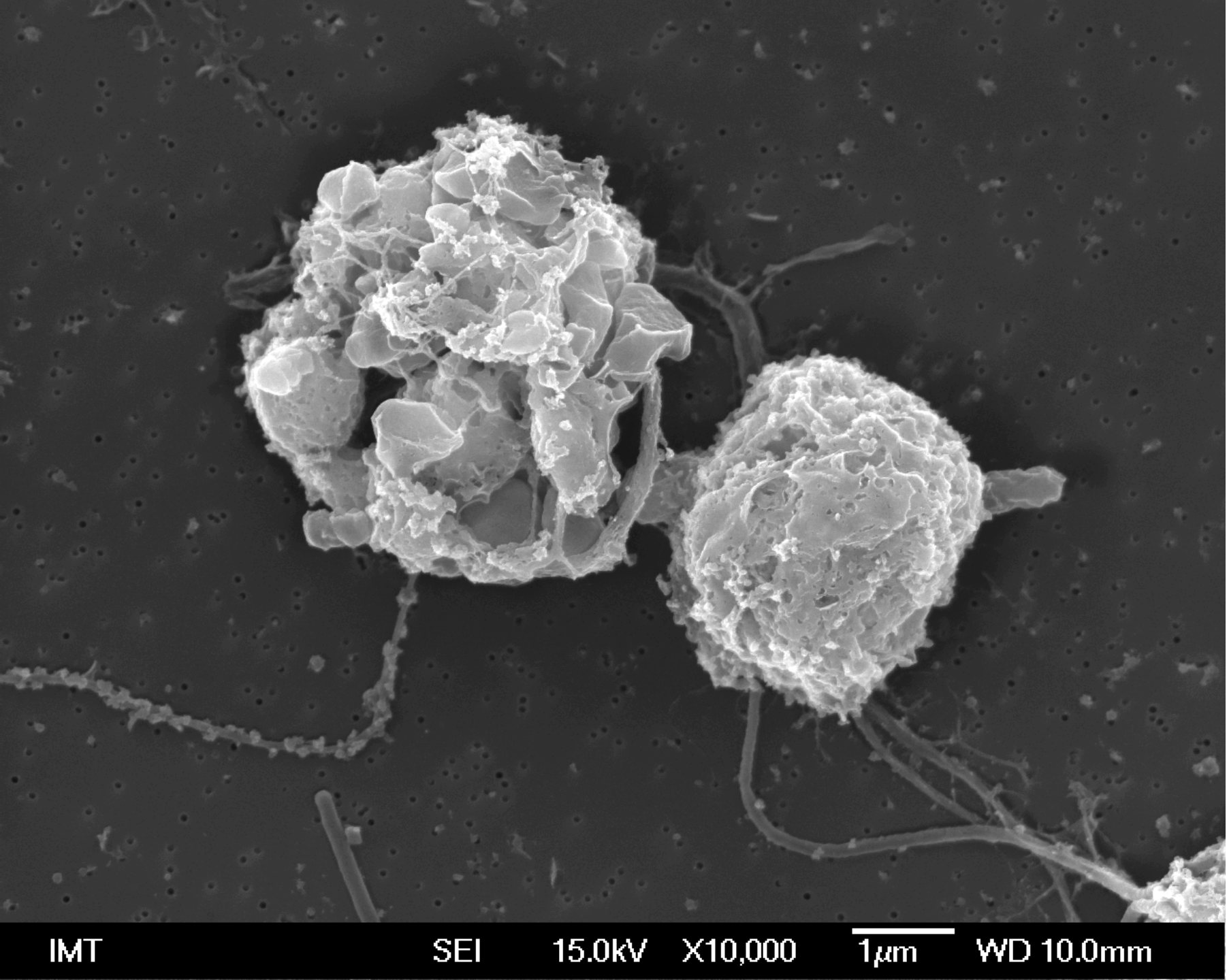

Figure *Dunaliella tertiolecta* culture SEM 16.

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).

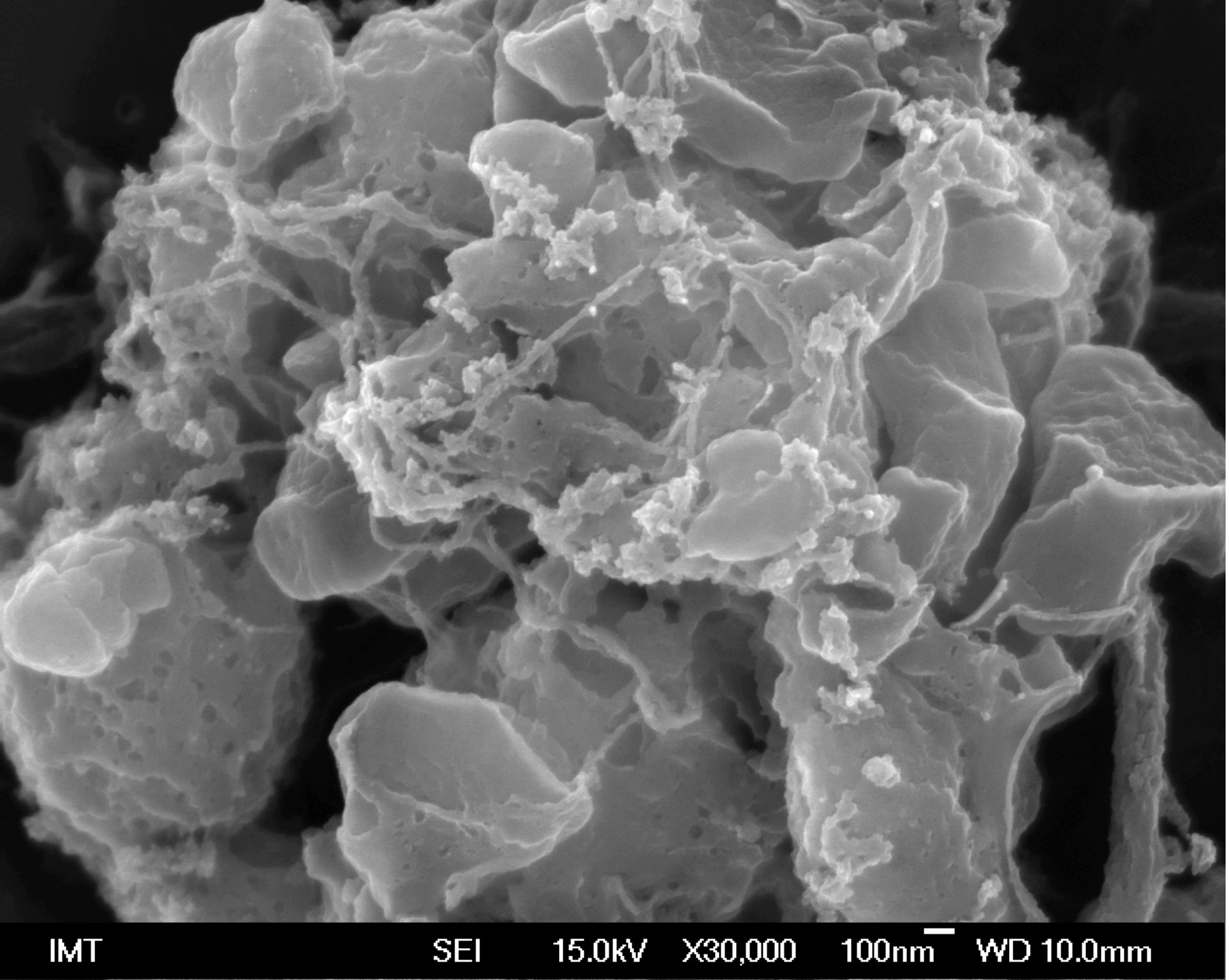

Figure *Dunaliella tertiolecta* culture SEM 17.

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).

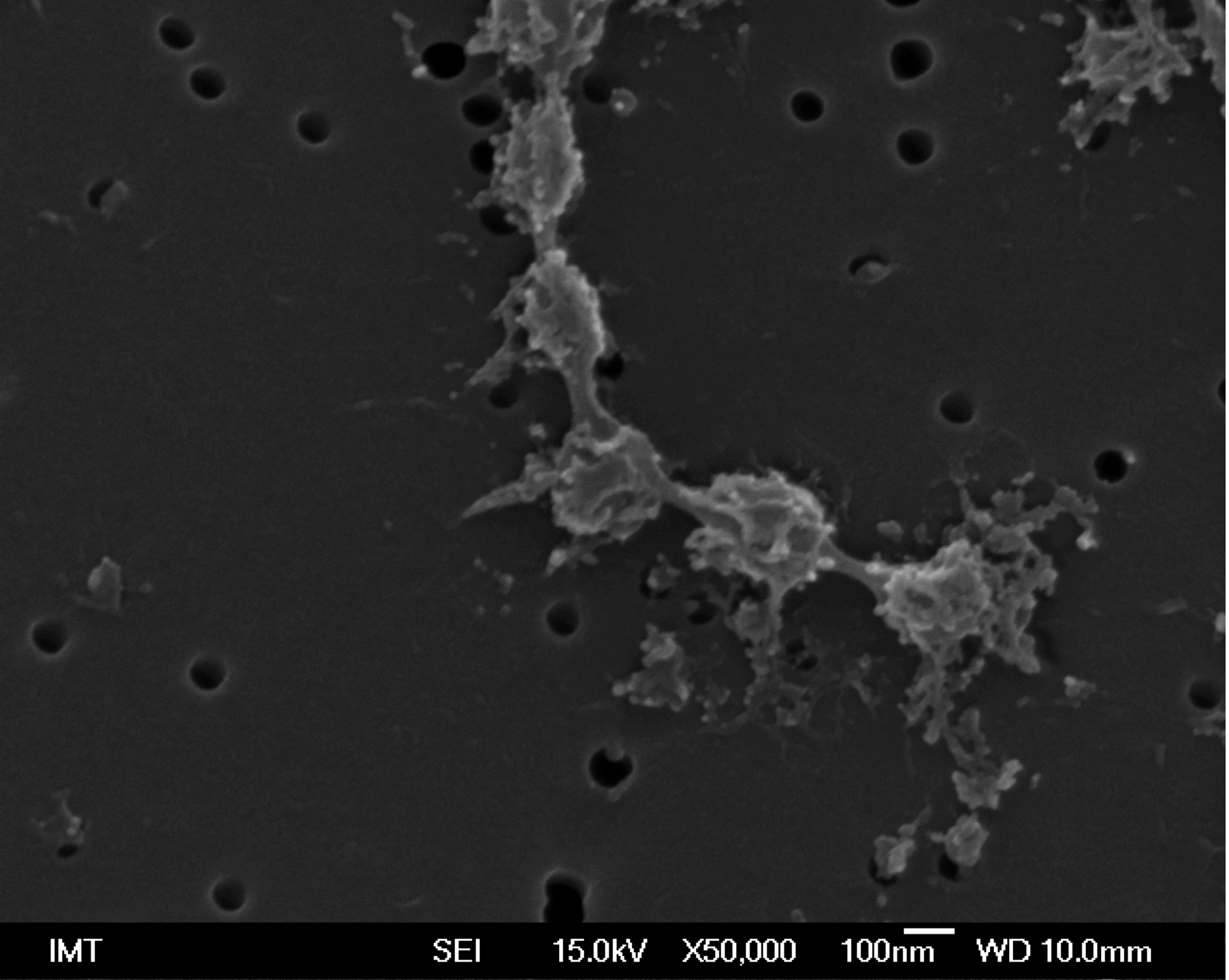

Figure *Dunaliella tertiolecta* culture SEM 18.

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).

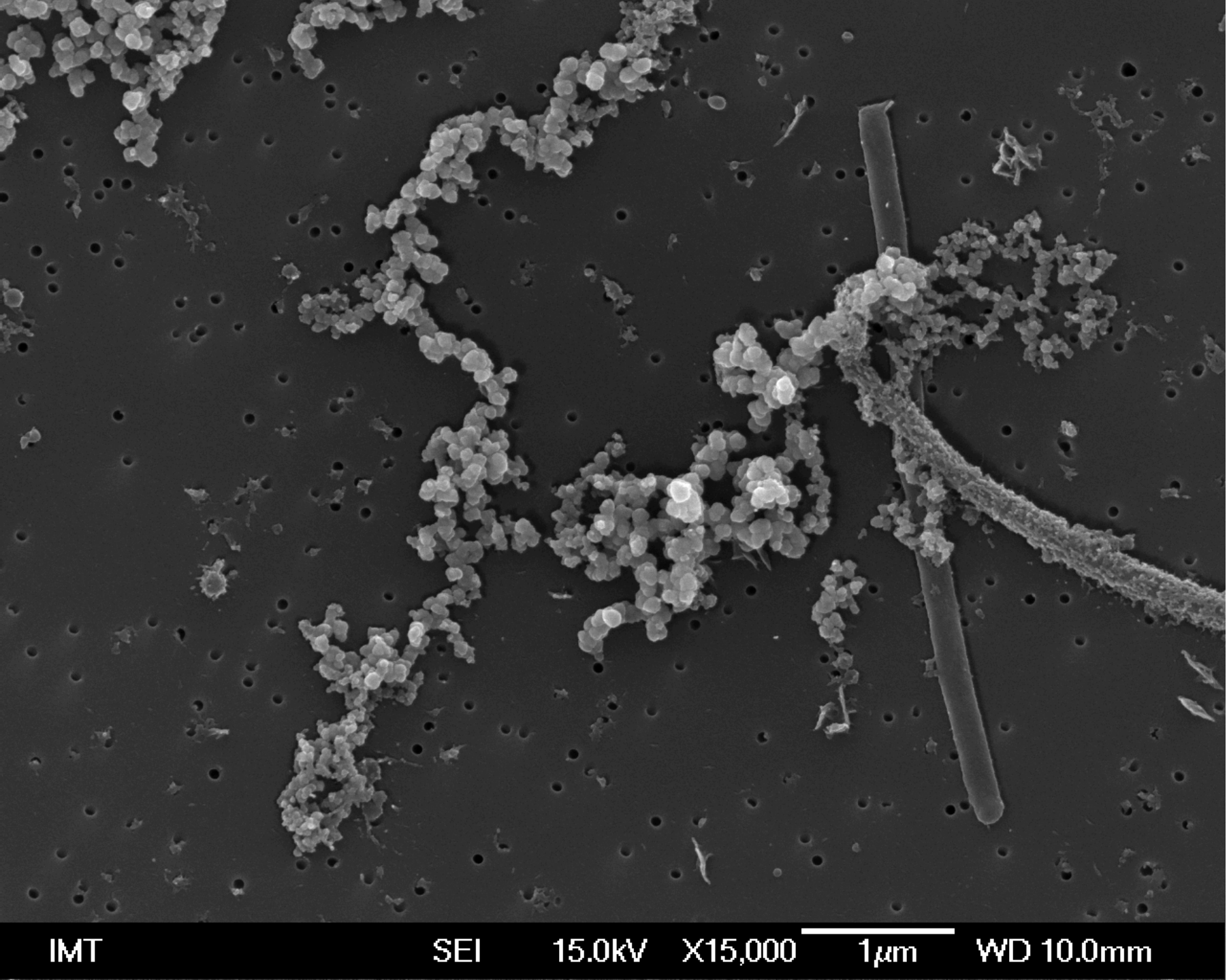

Figure *Dunaliella tertiolecta* culture SEM 19.

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).

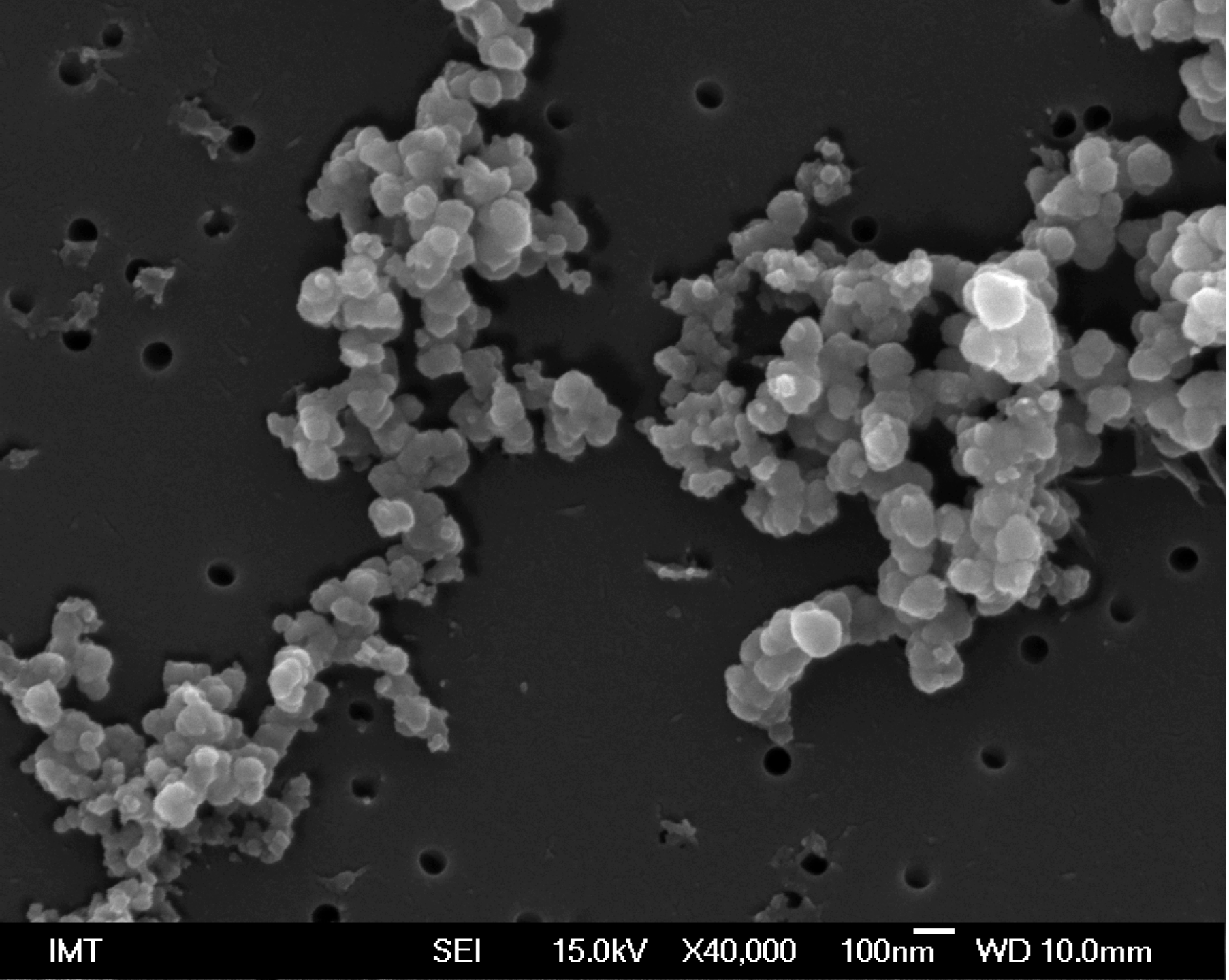

Figure *Dunaliella tertiolecta* culture SEM 20.

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).

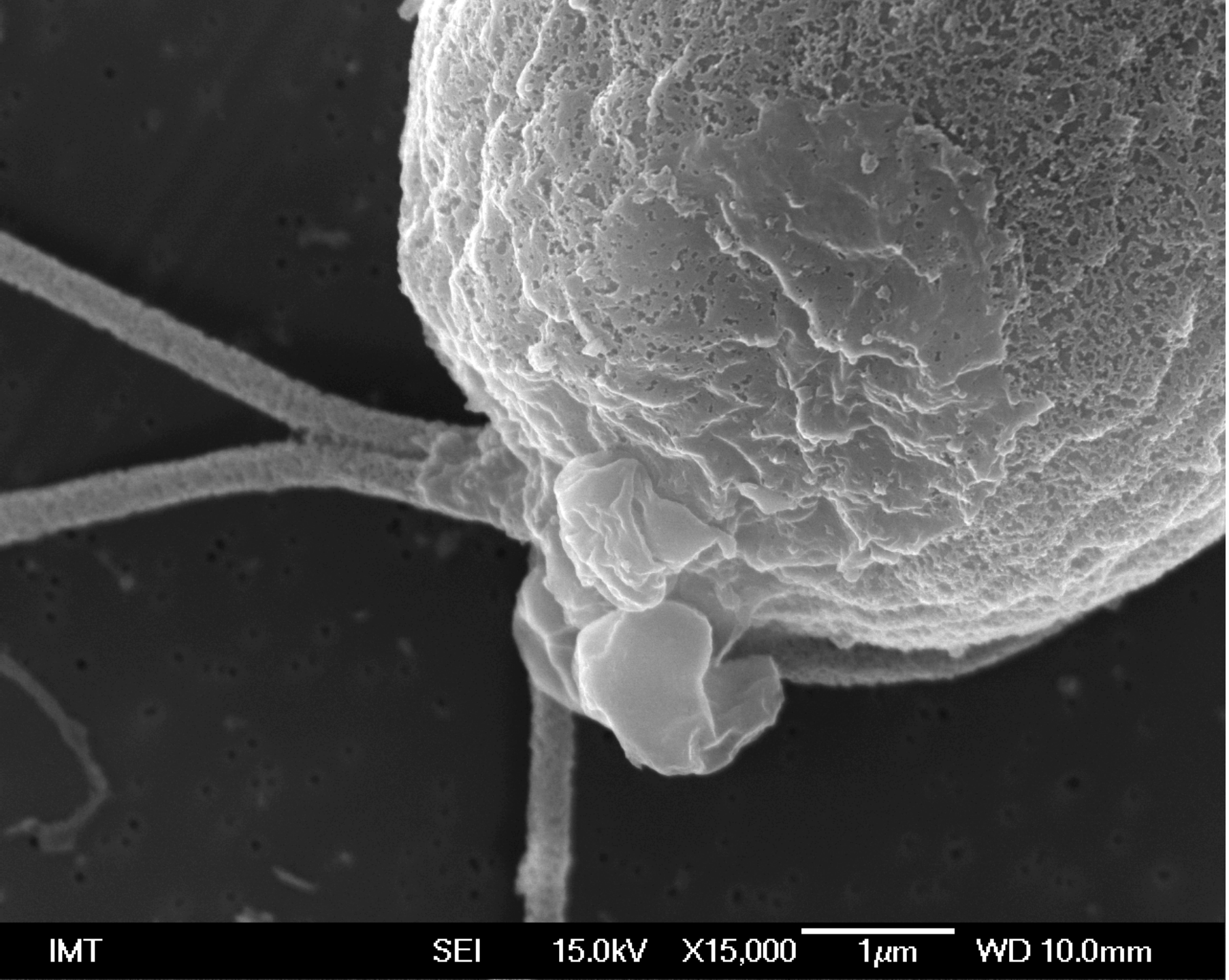

Figure *Dunaliella tertiolecta* culture SEM 22.

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).

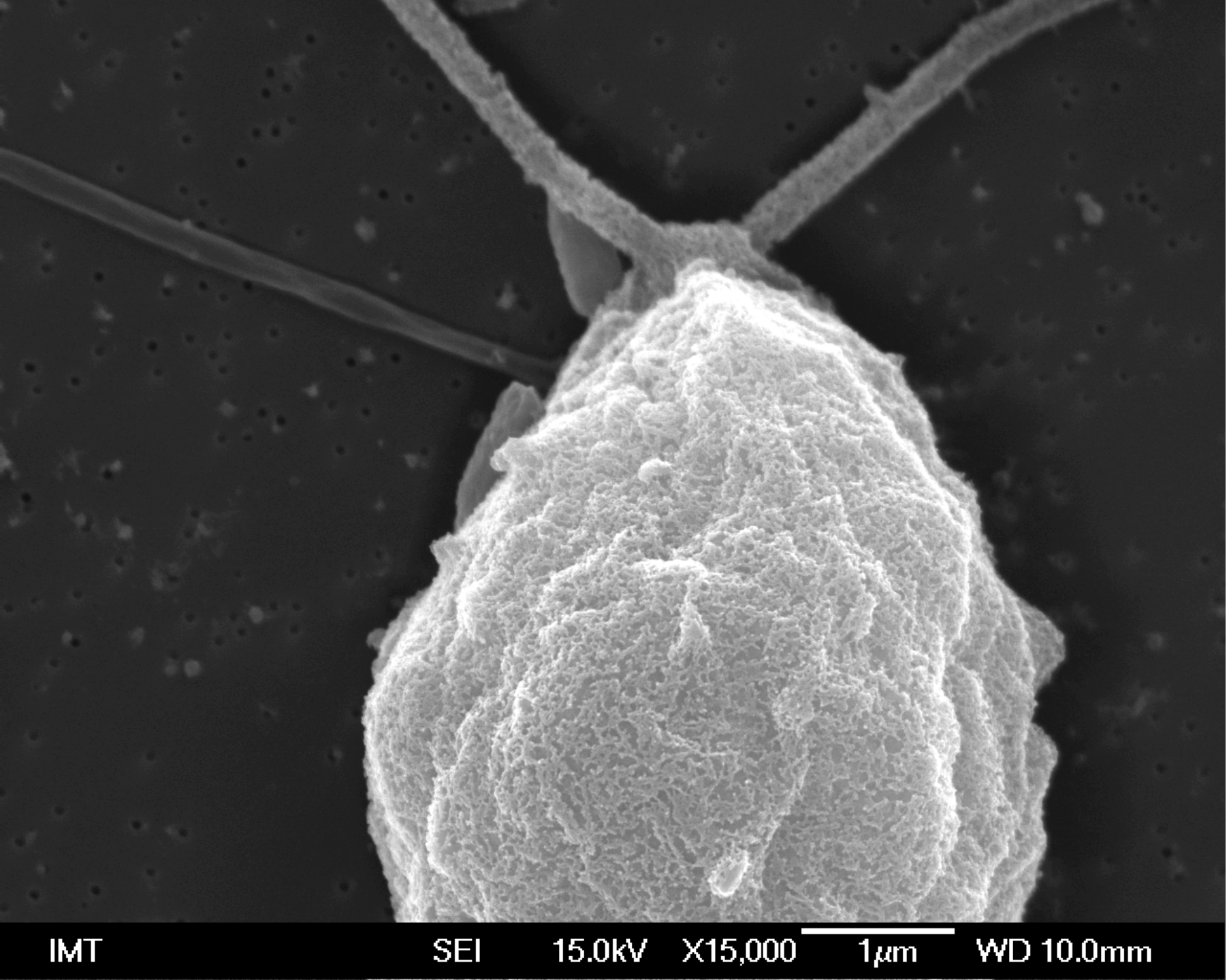

Figure *Dunaliella tertiolecta* culture SEM 24.

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).

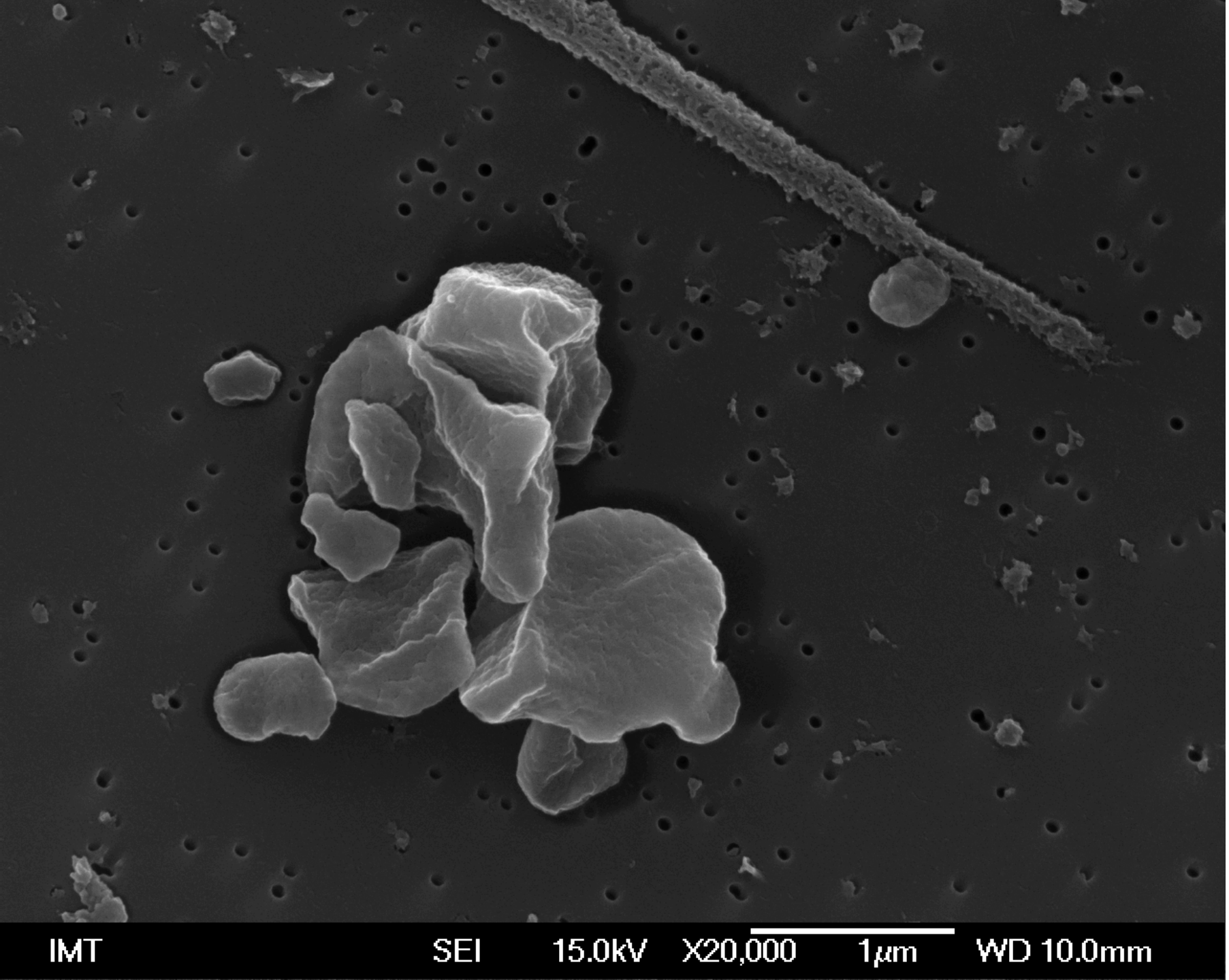

Figure *Dunaliella tertiolecta* culture SEM 25.

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).

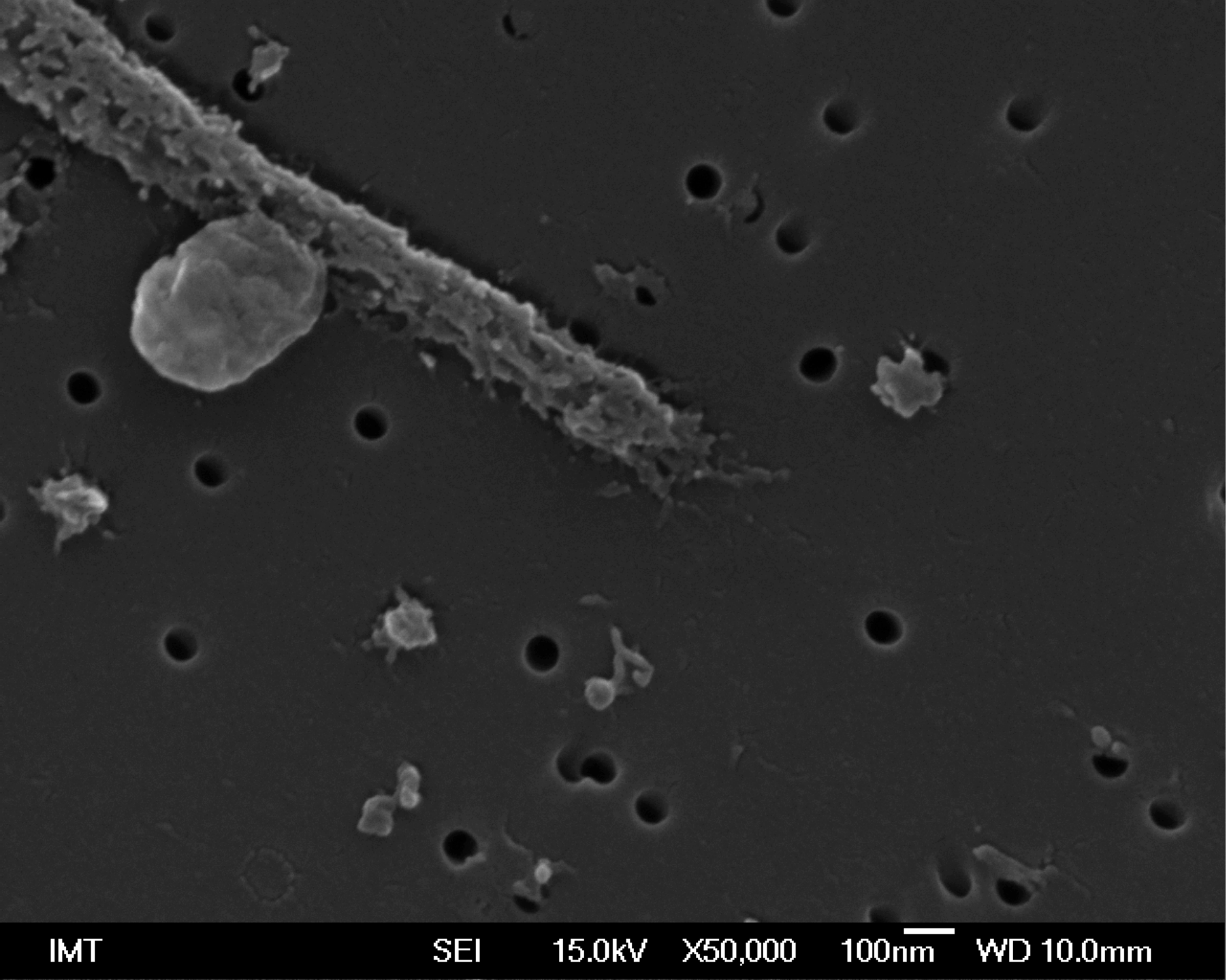

Figure *Dunaliella tertiolecta* culture SEM 26.

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).
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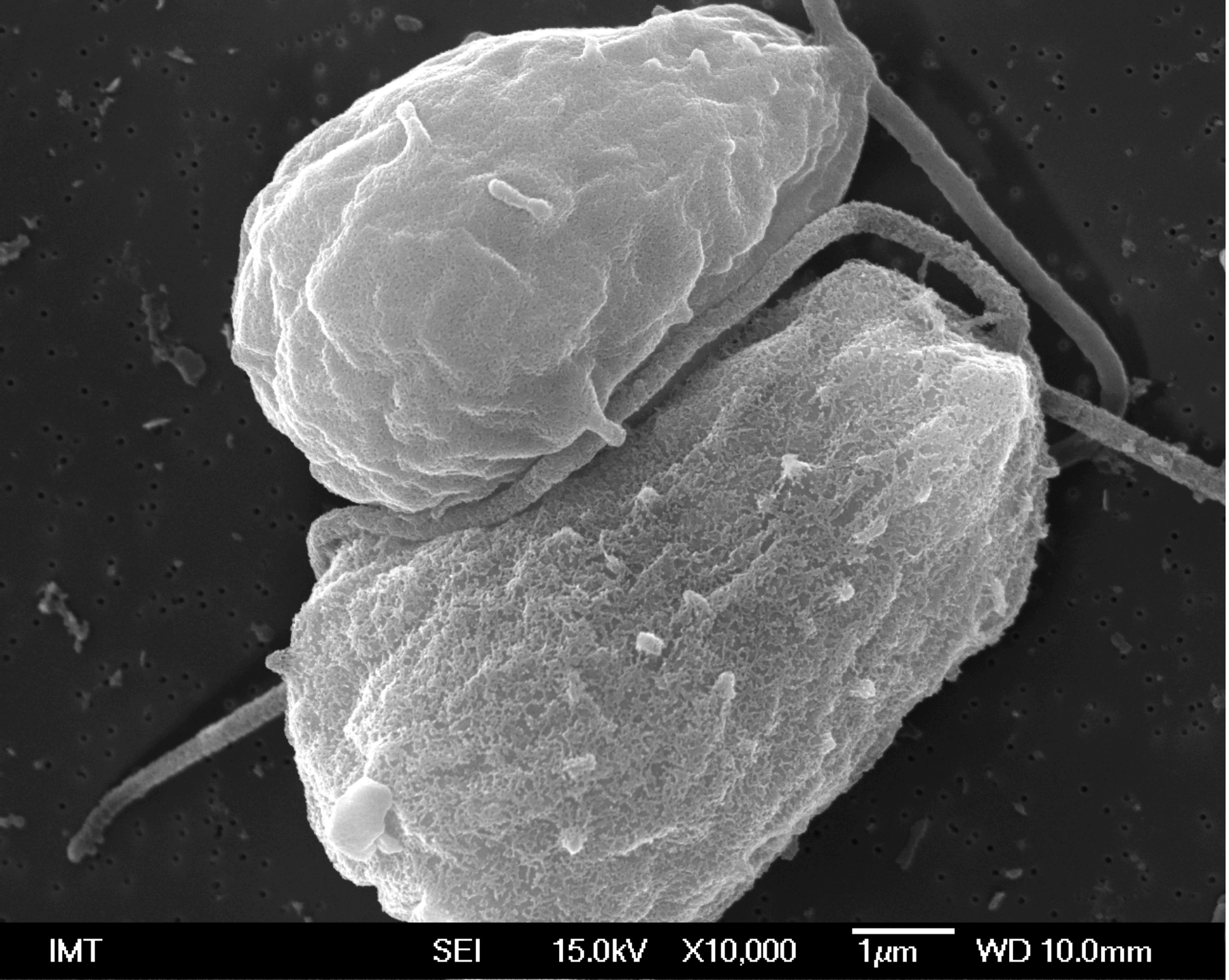

Figure *Dunaliella tertiolecta* culture SEM 31.

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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