Review

Fluorescence and Fluorescein as Pivotal Tools in Cancer Diagnosis and Therapy

Marinko Katja[1,‡], Pečan Luka Irenej[2,3,‡], Vodenik Julija[3], Petrovčič Sara[4], Lopert Lana[4], Tavčar Gašper[4], Tesovnik Tine[5,6], Silverman Julian R[7], Jeran Marko[4,*]

1. University of Ljubljana, Biotechnical Faculty, Department of Microbiology, Ljubljana, Slovenia
2. University of Ljubljana, Biotechnical Faculty, Department of Animal Science, Chair for Agrarian Economics, Policy and Law, Ljubljana, Slovenia
3. University of Trieste, Department of Life Sciences, Trieste, Italy
4. "Jožef Stefan" Institute, Department of Inorganic Chemistry and Technology, Ljubljana, Slovenia
5. University Medical Centre Ljubljana, University Children's Hospital, Clinical Institute for Special Laboratory Diagnostics, Ljubljana, Slovenia
6. University of Ljubljana, Faculty of Medicine, Department of Paediatrics, Ljubljana, Slovenia
7. Fashion Institute of Technology, Department of Science and Math, New York, USA

‡ These authors contributed equally to this work

* Correspondence: Jeran Marko; marko.jeran@ijs.si

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Abstract:
Fluorescent dyes have become important tools in various scientific and medical fields due to their unique capabilities for visualizing and analysing complex biological systems. This review focuses on the pivotal role of fluorescein and its derivatives in cancer diagnosis and therapy. Fluorescein, initially synthesized in the 19th century, has evolved into a crucial diagnostic and research tool, particularly valued for its intense fluorescence and versatility. In medical practice, fluorescein is used in high-contrast imaging techniques such as fluorescein angiography and fluorescence-guided surgery, significantly enhancing the detection and treatment of tumours, especially in oncology. This review covers the properties and applications of fluorescein, including its derivatives such as eosin and fluorescein isothiocyanate (FITC), in various medical and non-medical contexts. Special attention is given to the innovative use of these dyes in paediatrics cancer diagnostics and surgery, highlighting their potential to improve patient outcomes. The review also explores the broader applications of fluorescein dyes in chemistry, forensics, and environmental science, underscoring their ability to detect and visualize substances at very low concentrations. As research continues to advance, the scope and efficacy of fluorescent dyes are expected to expand, offering new possibilities for scientific disoveryes and medical advancements.

Keywords: Fluorescent dyes, Fluorescein, Cancer diagnostics, Paediatric oncology, High-contrast imaging, Tumor delineation

1. Introduction

Fluorescent dyes have become an indispensable tool in a variety of scientific and medical fields, providing unique capabilities for visualizing and analyzing complex biological systems. Fluorescent molecules including fluorescein absorb light at a shorter wavelength and emit light at a higher wavelength. This difference in wavelength allows observers to cast light from a source, filter this source light out, and then observe only the emitted light. As non-fluorescent molecules do not emit light of higher wavelengths at areas where the fluorescent molecules are localized, high contrast with respect to the darker areas is formed. Among such dyes, fluorescein and its derivatives stand out for their intense fluorescence and versatility (**Figure 1**). Originally synthesized in the 19th century, fluorescein has evolved into a crucial diagnostic and research tool, particularly in medical applications such as ophthalmology, oncology, and histology (Jun et al., 2020).
In medical practice, fluorescein is used for its ability to provide high-contrast images, making it invaluable in procedures such as fluorescein angiography and fluorescence-guided surgery. These applications are particularly significant in the field of oncology, where precise imaging can greatly enhance the detection and treatment of tumors. Paediatric oncology, in particular, has benefited from the use of fluorescent dyes, enabling more accurate tumor localization and minimizing damage to healthy tissues during surgical procedures (He et al., 2017).

Figure 1. Fluorescence of fluorescein dyes under UV-illumination (Photo: Marko Jeran).

Besides in medicine, fluorescein dyes play critical roles in various scientific disciplines, including chemistry, forensics, and environmental science. Their ability to detect and visualize different substances at very low picomolar concentrations makes them a powerful tool for research and diagnostics (Bell, 2009; Jeran et al., 2020; Jeran et al., 2019).
The following sections will cover the properties and applications of fluorescein, including its derivatives such as eosin and fluorescein isothiocyanate (FITC), and their usage in different medical and non-medical contexts. Special attention will be given to the innovative use of these dyes in paediatric cancer diagnostics and surgery, highlighting their potential to improve patient outcomes. As research continues to advance, the scope and efficacy of fluorescent dyes are expected to expand, offering new possibilities for scientific discovery and medical advancements (Le Guern et al., 2020).

2. Fluorescein and the use of fluorescent dyes

In fields such as medicine, forensics, and chemistry, several different fluorescent dyes are widely used, the vast majority of which are fluorescein-based (**Figure 2**). Fluorescein is a crystalline solid organic fluorophore of a yellow-orange color (National Center for Biotechnology Information, 2024). When exposed to light, it has an absorption maxima at 460 nm and emits a greenish-yellow fluorescence with an emission maxima at 515 nm

(Sigma, n. d.). This organic dye fluoresces so intensely that it can be detected even at dilutions of 1:50,000,000 (Britannica, 2018). Fluorescein is also known as D&C Yellow #7, and it was first synthesized by Adolf von Bayer in 1871, from phthalic anhydride and resorcinol. Fluorescein is poorly soluble in water and many other organic solvents, but its sodium salt easily dissolves in water (American Chemical Society, 2013). Its aqueous solutions are sensitive to prolonged exposure to light due to the degradation process (Martínek et al., 2023). Its toxicity is low (National Center for Biotechnology Information, 2024). It is also widely used as a marker in medical and biological applications, especially as a probe for localizing tumor tissues (Pothen and Parmar, 2023). Due to the strong intensity of its fluorescence, it was the first dye used in 1962 to color the Chicago River green on St Patrick's Day (American Chemical Society, 2013).

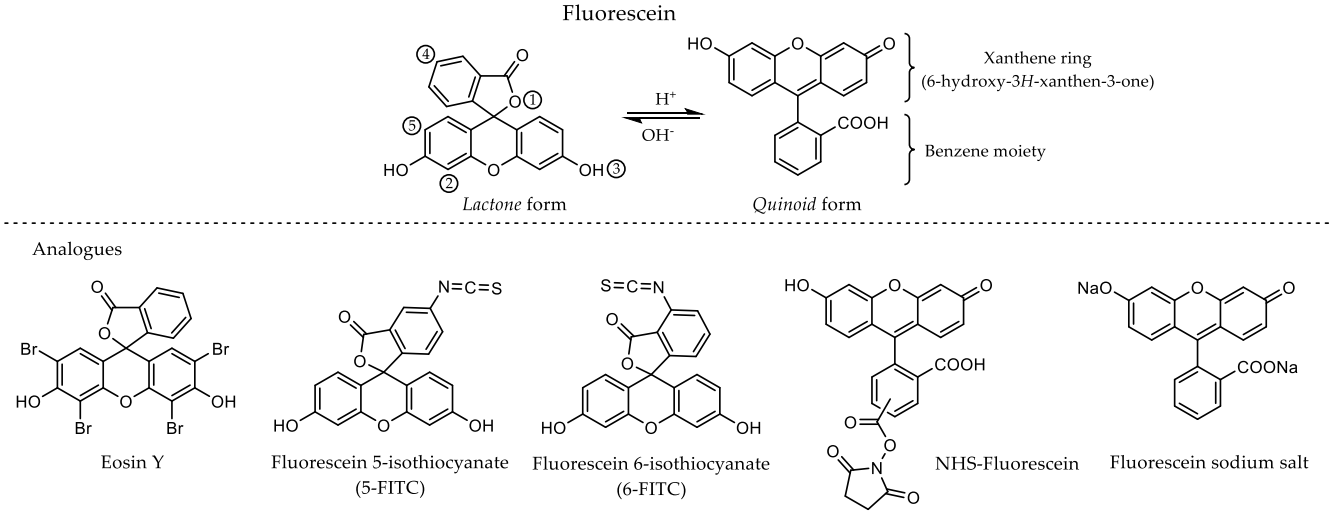

Figure 2. Fluorescein in two different forms, sites for modifications and its relevant analogues.

Fluorescein is on the World Health Organization's list of essential medicines (World Health Organization, 2019). It is a diagnostic contrast agent used mainly in various ophthalmological procedures, such as checking for possible corneal or blood vessel abnormalities. The dye produces an excellent contrast, which can be used to determine if any external corneas, epithelial keratitis, herpes simplex keratitis, or corneal foreign bodies are present. Such lesions can be detected by applying a few drops of fluorescein to the surface of the eye before the examination. Side effects of using the drug may include temporary blurred vision and discoloration of soft contact lenses (Pothen and Parmar, 2023).

Fluorescein is also used in fluorescein angiography, which is a special eye examination using fluorescein. Before the examination, a few drops of mydriatire are instilled into the eyes to dilate the pupils, and then the fluorescein is injected intravenously (through a vein in the arm). The dye then travels with the blood throughout the body and over time reaches the vessels of the retina and choroid. If the background of the eye is healthy, the blood vessels will be coloured and the dye will not come out of them, but if the vessels are damaged, the dye will either emerge or it will be unevenly retained in a certain area. Possible side effects that may occur are transient nausea, allergic reactions (rare), hematoma at the puncture site (General Hospital Celje, 2022). In a study by Huang and co-workers (2016), researchers performed aqueous angiography with fluorescein in bovine eyes. The goal of this study was to examine ventricular angiography using real-time imaging modality of the ventricular outflow tract in cow's eyes using two tracers that had different molecular characteristics. Either fluorescein or indocyanine green solution was

injected. The study showed segments of angiographic outflow patterns using fluorescein (Huang et al., 2016).

Fluorescein dyes are also used in bioimaging, where the fluorescence emitted by fluorescein provides good insight into the identification of non-diseased tissues, tumor-affected tissues or histological markers. Fluorescence can greatly assist the surgeon in removing or repairing the damaged or pathological tissues. The range of application of a fluorescein dye can vary from a bundle of nerves, to a single blood vessel, to tissue abnormalities and even at the molecular level (Pothen and Parmar, 2023). Dyes can also be a conjugated species or a biomarker, where fluorescent microscopy enables the identification of microorganisms or cellular components, such as proteins in immunohistological staining. In the enzyme-linked immunosorbent assay (ELISA), fluorescein acts as fluorescence emitting agent conjugated to secondary antibodies (Pothen and Parmar, 2023).

3. Common Fluorescein Derivatives and Their Usage

3.1. Eosin

Eosin is a derivative of brominated fluorescein (Agar Scientific, n. d.). It is often used in laboratory microscopy as a reddish dye to mark collagen, cytoplasm, muscle fibers, lymphocytes and bacteria (Clinic Sciences, n. d.). Several different types of eosin are commercially available, but the most widely used is eosin Y, which is soluble in both water and alcohol. A 0.5 or 1.0 % aqueous solution is commonly used as a cytoplasmic cell staining agent and, with the addition of thymol crystals, it is used to inhibit fungal growth. Staining with eosin can be strong after fixation with mercury, which can cause difficulties in achieving adequate differentiation (Science Direct, n. d).

For histological studies of paraffin sections, the technique of staining with eosin in combination with hematoxylin has been used for decades. In the staining process, hematoxylin is used to stain anionic components such as RNA and DNA, while eosin dye marks cationic proteins and binds to the phenolic and carboxyl groups of arginine, lysine, histidine and tryptophan residues. Eosin has been used as a selective marker for elastic fibres, muscle cells and mitotic spindle fibres but it is also a fluorescent pH indicator. This dye has also been used as a diagnostic tool to quantify liver damage resulting from hepatitis. The fluorescence pattern of eosin was assessed in a model of liver injury. They concluded that the fluorescence varies according to the health status of the tissue and the dye can be further used to help diagnose and quantify the severity of different liver diseases (Ali et al., 2017).

3.2. Fluorescein Isothiocyanate (FITC)

Fluorescein isothiocyanate (FITC) is one of the most commonly used derivatives of fluorescein. It is used to mark proteins, antibodies, peptides, hormones, amine-modified oligonucleotides, and other amine-containing molecules. FITC is also used to detect compounds that can then be observed using a fluorescence microscope (Rožman, 2012). Its use is also possible in flow cytometry (LS Bio, 2022). At the bottom of the ring, an isothiocyanate functional group (-*N*=*C*=*S*) replaces the hydrogen (-*H*) atom. This isothiocyanate group is the part of the molecule that is amine reactive (LS Bio, 2022). The isothiocyanate group on FITC reacts with primary amines to form covalent thiourea bonds that will bind the fluorescein to the biomolecule. The isothiocyanate group is reactive towards any nucleophilic site, but the dye will selectively react with *N*-terminal amines, due to these bonds being much more stable. FITC is often found as a mixture of its two isomers, namely, fluorescein 5-isothiocyanate (5-FITC) and fluorescein 6-isothiocyanate (6-FITC) (Takai et al., 2011).

In acidic environments where the pH value is less than 2, fluorescein isothiocyanate is found in cationic form. At pH value of about 3.3, it is generally in a neutral form, but when it is present in an environment where pH value is about 5.5, it can be found in the monoanion form. In basic conditions where the pH value is greater than 8, FITC can be found in the dianionic form (Casanovas et al., 2008; Rožman, 2012). In 2013, a study

evaluated the apparent permeability coefficients of model agents (fluorescently labeled FITC-dextran, rhodamine 123 and enalaprilat) in different parts of the isolated rat intestine *in vitro* using the method of bilateral Sweetana-Grass diffusion cells. For the FITC-dextran permeability, the apparent permeability coefficients in absorptive compared to the eliminator direction were not statistically significantly different. No differences between the groups or within a single part of the intestine could be detected. The latter thus indicates a comparable process of transport of the paracellular marker FITC-dextran in both directions, regardless of the part of the intestine (Šenica, 2013).

3.3. 5/6-Carboxyfluorescein succinimidyl ester, mixed isomer (NHS-fluorescein)

NHS-fluorescein is also amine-reactive and has a wide range of applications. It is most commonly used for antibody labeling, in fluorescence microscopy, immunofluorescence-based assays (*e.g.*, ELISA, Western blotting) and in flow cytometry. Unlike FITC, NHS-fluorescein is more specific for primary amines in the presence of the other nucleophiles and has a more stable binding after labelling (Thermo Fisher Scientific, n. d).

NHS-fluorescein is activated with the functional group *N*-hydroxy-succinimidyl-ester (NHS ester). Compared to FITC, the NHS ester derivative exhibits greater specificity for primary amines in the presence of other nucleophiles and results in more stable binding after labeling. Pierce amine-reactive fluorescein dyes are mixtures of isomers with reactive groups at the 5- and 6-positions of the lower ring. The properties of these isomers are indistinguishable in terms of excitation and emission spectra, and there is no need to isolate a specific isomer for protein applications (Thermo Fisher Scientific, n. d).

3.4. Fluorescein sodium salt

The sodium salt is the most common commercially used fluorescein (Mascen Labs, n. d.). The compound is also known by many other names such as resorcinolphthalein, naphthalene, uranine, acid yellow 73, D&C yellow #8 and yellow 8. The dye can be detected at dilutions up to 1:40,000,000 due to its strength (Jacobs, 1992). After injection into the bloodstream, about 80% of the dye binds to plasma proteins, mainly albumin. Then it is metabolized in the liver and kidneys, and is excreted from the body after 24 to 36 hours (Olson & Mandava, 2006). Sodium fluorescein is soluble in aqueous alkaline solutions and fluoresces under cobalt blue filtered light at 465 to 490 nm, emitting green color in the wavelength range of 520 to 530 nm. It is frequently used in opthamology in fluorescein angiography for eye examinations, in bioimaging, and as a biomarker. It is also an indispensable tool in vascular neurosurgery where it helps to track and assess blood flow (Mascen Labs, n. d.).

Sodium fluorescein was first used in 1948 to identify various brain tumors (Moore et al., 1948). Since then, its use and that of other fluorescent markers, particularly in the resection of glioblastoma multiforme, has been reported in the literature. Nevertheless, it has not been presented as an adjuvant in the resection of skull base lesions (da Silva et al., 2010).

Cerebral metastases occur in a large proportion of intracranial tumors and research tells us that the incidence of cerebral metastases is increasing. Resection has been shown to be the most effective treatment method for patients, but bright light surgery (BLS) is usually not sufficient to achieve resection. The research into the use of fluorescence guided surgery (FGS) for brain tumors has only recently started to develop. Sodium fluorescein can be used as a dye in FGS as it is effective in staining tumors such as glioblastoma, malignant melanoma and lymphoma (Xiao et al., 2018).

4. Fluorescein Sodium and its Uses in Cancer Medicine

4.1. Brain tumours

The clinical application of sodium fluorescein has already been extensively documented in diagnosing and finding brain tumors (Moore et al., 1950). Brain tumor develop from abnormal cell growth in the brain or nearby region including the nerves, pituitary glands, pineal gland, and membranes surrounding the brain. Primary brain tumors are those starting in the brain, while secondary tumors refer to its spread to other parts of the body, also known as metastatic brain tumors (Mayo Clinic, 2023). Prior to undergoing craniotomy where a section of the skull is temporarily removed to access the brain, patients

receive an injection of sodium fluorescein (Moore et al., 1950). During surgery, the brain cortex is examined under UV light, revealing yellow-green fluorescence in superficial tumors, which aids in their identification and localization of tumor boundaries (Moore et al., 1950). For deeper tumors, brain needles are used to obtain biopsy material, which is then examined for fluorescence (Moore et al., 1950). The presence or absence of fluorescence of the tissue is a reliable indicator of whether the tissue is abnormal or not (Moore et al., 1950). Even though the tumor is not directly probed, its proximity can be inferred by the fluorescence of adjacent biopsy material.

Among patients suspected of having brain tumors, those with fluorescent tumors were confirmed to have tumors upon examination, while those without fluorescence did not (Moore et al., 1950). However, limitations exist, including benign and slow-growing tumors that do not fluoresce (Moore et al., 1950). Fluorescein's utility in identifying tumor tissue is not exclusive and can also be seen in lesions that disrupt the blood-brain barrier mechanism, such as brain edema, abscess capsules, and traumatized tissue (Schebesch et al., 2016; Moore et al., 1950). Metastatic tumors consistently show bright fluorescence, even in cases with significant tumor necrosis (Moore et al., 1950).

4.2. Breast cancer brain metastasis

Fluorescein sodium applications can also be found in breast cancer brain metastasis (BCBM) surgery (Xiao et al., 2018). Breast carcinoma stands as the most common cancer type and a leading cause of cancer-related death among women (Farahani et al., 2023). Exposure to too much estrogen potentially contributes to DNA damage and genetic changes, responsible for breast cancer progression (Farahani et al., 2023). Research over the years has proven that mutations in genes BRCA1 and BRCA2, which are believed to function as tumor suppressor genes, can cause the development of breast and ovarian cancer (Casaubon et al., 2023). Metastases are responsible for 90% of cancer-related deaths and are a major cause of fatality in breast cancer (Farahani et al., 2023). The brain, axillary lymph nodes, bones, lungs, and liver are the main sites where breast cancer spreads to. Breast cancer cells (BCCs) can spread to the brain by invading their surroundings thus migrating through circulatory system and lastly multiplying inside the brain, therefore developing brain metastases (Farahani et al., 2023).

A study by Xiao et al. (2018) was conducted between May 2012 and June 2016 and it was carried out in 38 patients, who were clinically and pathologically diagnosed with breast cancer brain metastasis. Their objective was evaluating fluorescence-guided surgery as opposed to standard neurosurgical procedure. The patients were divided into two groups. Group 1, which underwent fluorescein-guided surgery, and Group 2, which underwent standard microsurgery using bright light surgery, which typically fails short in complete removal of cerebral metastases. Therefore Group 1 was intravenously injected with 5 mg/kg of fluorescein sodium following allergy testing and prior to general anesthesia, while Group 2 was not administered with fluorescein sodium. The study evaluated surgical outcomes in patients with BCBM who underwent fluorescein-guided surgery compared to standard microsurgery. Results showed that fluorescein-guided surgery yielded better results by enhancing the tumor visibility and had a more notable impact on patients' performance status as opposed to those who underwent standard microsurgery. Additionally, the overall survival after treatment of cerebral metastases (CMs) was slightly higher in patients who received fluorescein compared to those who did not, making fluorescein-guided surgery a secure and feasible approach for resecting BCBM (Xiao et al., 2018).

4.3. Gastric cancer

Stomach (gastric) cancer originates in cells which line the stomach, with the most common stomach cancer being adenocarcinoma, which originates in the mucus-producing cells located in the innermost lining of the stomach (National Cancer Institute, n. d.). In 2002 Bhunchet and co-workers (2002) did a study examining a new method for detecting gastric cancer using fluorescein electronic endoscopy, which is used for detecting early-stage gastric cancer. The study was conducted in 16 patients diagnosed with early-stage gastric cancer (using white light endoscopy and chromoendoscopy), who underwent fluorescein electronic endoscopy with fluorescein sodium before surgery. Subsequently, thorough

histopathologic evaluation was conducted on the resection specimens. Around 10 seconds following intravenous injection of fluorescein, fluorescence was noted and rapidly spread across the inner gastric surface. Early-stage gastric cancers with more abundant stroma than the surrounding normal mucosa displayed significantly stronger fluorescence as opposed to early-stage gastric cancer with less stroma, which displayed weaker fluorescence. Stronger fluorescence intensity was also present in undifferentiated early-stage gastric cancers, characterized by widened stroma due to malignant invasion. The boundaries of early-stage gastric cancers were distinct and clearly observed in all instances. During surgery, some accompanying lesions were also detected, which were previously unnoticed by routine endoscopy (Bhunchet et al., 2002).

4.4. Mouth (oral) cancer

Mouth (oral) cancer covers cancers that form in various parts of the mouth and is categorized within head and neck cancers. Treatment approaches for oral cancers are often similar to those for other cancers in this group. The primary site for mouth cancers typically originates in the flat, thin cells known as squamous cells, which line the interior of the mouth and lips (Mayo Clinic, 2024). A study was conducted in 2020 by Qaiser with co-workers (2020) involving 100 individuals displaying with 42 oral potentially malignant disorders (OPMDs), 40 oral squamous cell carcinoma (OSCC) and 18 controls. The control group consisted of people with inflammatory conditions like pericoronitis and benign fibrous polyps. It was also the first study to document the role of fluorescein in detecting oral cancer and OPMD. The patients underwent a clinical oral visual examination using white light to document the location, characteristics, and extent of the lesion. They were examined under blue light in a darkened room for autofluorescence, and any autofluorescence exhibiting lesion was excluded from the study. Later, sodium fluorescein was topically applied on the lesion and its surroundings, mouth was rinsed and checked for fluorescence from and around the lesion. The researchers indicated that fluorescein offers a swift and sensitive approach to identifying potentially malignant disorders (OPMD) and oral squamous cell carcinoma (OSCC), as it is capable of distinguishing between non-dysplasia and dysplasia/malignant lesions of the oral cavity (Qaiser et al., 2020).

4.5. Bladder cancer

Bladder cancer is still the 10th most common cancer in the world according to data from 2020, and is more prevelant in male than female populations (World Cancer Research Fund International, 2020). While abstaining from smoking, avoiding exposure to metalworking fluids, aromatic amines, and polyaromatic hydrocarbons and such decreases the risk of bladder cancer, proper screening and early medical intervention can help slow the spread and increase the chance of positive therapeutic outcome for bladder cancer treatment (World Cancer Research Fund International, 2020). Early detection of bladder cancer is of upmost importance and this can be done by using fluorescence methods. We can focus on either endogenous or exogenous fluorescence techniques for early detection of *in situ* carcinoma of the bladder. Endogenous fluorescence, also known as autofluorescence, is a phenomenon where diagnostic information is extracted from the fluorescence emitted by tissue fluorophores. The primary contributors to tissue autofluorescence include the reduced form of nicotinamide adenine dinucleotide (NADH) and collagen, particularly in *in vivo* conditions. Utilizing ultraviolet (UV) light, a comparison can be drawn between cancerous and healthy tissue (D'Hallewin et al., 2002). In individuals with bladder cancer, alterations in autofluorescence of healthy tissue occur due to heightened blood absorption, increased thickness of epithelial cells, and diminished excitation of collagen and NADH (D'Hallewin et al., 2002). Due to these changes, fluorescence intensities from carcinoma *in situ* (CIS) fall between those seen in normal tissue and papillary transitional cell carcinoma (TCC), a type of bladder cancer that grows outward from the inner lining of the bladder (D'Hallewin et al., 2002). To distinguish between normal tissue and tumorous lesions *in vivo* fluorescence intensities at two crucial wavelengths should be compared and statistically significant distinction can be made between normal tissue and tumorous lesions (D'Hallewin et al., 2002). The true challenge lies in figuring out the threshold value that separates normal epithelium from tumorous epithelium for more exact diagnosis.

While autofluorescence techniques can be used for detection of bladder cancer, it is not the most reliable, as it is influenced by factors like blood absorption and epithelial thickness. In the case of carcinoma *in situ*, such cancer can occur without visible thickened epithelium (D'Hallewin et al., 2002).

Exogenous fluorescence involves intravesical instillation of fluorophores followed by visible light excitation (D'Hallewin et al., 2002). The fluorophores used are derived from photodynamic therapy (PDT), which works on a straightforward principle. A photosensitizing drug is administered via diverse ways and begins accumulating within tumor while sparing normal tissue. Afterwards, light is employed to activate the sensitizer, leading to tumor necrosis while minimizing harm to surrounding healthy tissue. The concept of photodynamic therapy dates to 1900 when Raab and von Tappeiner described it using acridine and visible light (Raab, 1900; von Tappeiner, 1990). Policard (1924) then utilized the characteristic brick-red fluorescence of porphyrins for tumor detection. In 1942, Auler and Banzer first documented the photodynamic activity of porphyrins, with Figge and co-workers (1948) next outlining their tumor-specific localization. Schwartz and co-workers (1955) later improved this with a derivative of hematoporphyrin, enhancing its ability to localize within tumors. Kelly and Snell (1976) were the first to publish findings on using hematoporphyrins for fluorescence-guided diagnosis of bladder carcinoma *in situ* in human tissue samples. They observed bright red fluorescence in carcinoma *in situ*, dysplasia, and exophytic tumors in cystectomy specimens, while none was present in normal mucosa (D'Hallewin et al., 2002).

The concept of photodynamic diagnosis (PDD) centres on the interaction between a fluorochrome (a fluorescent dye with a strong attachment to tumorous cells) and light of a precise wavelength. Upon absorbing the light, the fluorochrome emits light at a longer wavelength, generating fluorescence which is what PDD capitalizes on to pinpoint potential abnormal tissue. The observed fluorescence can originate either from autofluorescence or from added fluorochromes. 5-Aminolevulinic acid (ALA) (**Figure 3**) has been explored since the early 1990s by researchers for its potential in fluorescent detection of urothelial cancer (Steinbach et al., 1995). ALA, a precursor in the heme biosynthesis pathway, triggers the buildup of fluorescent endogenous porphyrins, particularly protoporphyrin IX (PPIX), in epithelial tissues. PPIX serves as the key metabolite for fluorescent detection, with an excitation spectrum of 400 nm. Following intravesical administration of ALA, there is a specific accumulation of PPIX in urothelial cancer cells. This accumulation results in a striking colour contrast between red-fluorescing malignant lesions and the non-fluorescing normal mucosa, which typically appears in back-scattered blue light (Zaak et al., 2005).

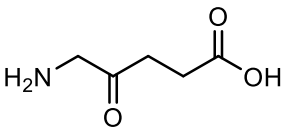

Figure 3. 5-aminolevulinic acid (ALA).

5. Fluorescence Used in Surgery

Before operating on cancer-ridden patients, preoperative imaging techniques like computed tomography (CT), magnetic resonance imaging (MRI), and positron emission tomography (PET) are used, yet the rate of surgical margin positivity has not improved in all three, as it remains between 15–60% across all types of cancers (Zheng et al., 2019). This is largely due to the subjective nature of visual inspection and palpation by surgeons, and the time-consuming process of intraoperative histopathological analysis (Zheng et al., 2019). Fluorescence-guided surgery (FGS) offers an objective and straightforward approach to define tumor margins, providing real-time imaging during surgery (Zheng et al., 2019). FGS is less expensive and easier to operate compared to traditional imaging methods and is growing in popularity (Zheng et al., 2019). FGS relies on two key components: a fluorescence probe and an imaging device. The fluorescence probe, usually

an organic molecule, needs to be able to absorb light at a specific wavelength and emit it at a longer wavelength. For FGS applications, the probe must accumulate in cancerous tissues and only a few probes are FDA - approved. Most commonly used in clinical studies are indocyanine green (ICG), methylene blue (MB), fluorescein sodium, and ALA. Of those four, ICG is the one used most often (Zheng et al., 2019). Its excitation and emission wavelengths are outside the range of most tissue autofluorescence (Zheng et al., 2019). Fluorescein sodium and MB are used less often, and ALA is unique because it is not inherently fluorescent (Zheng et al., 2019). FGS has been used to treat various cancers, such as head and neck, breast, lung, esophagus, gastric, colorectal, anal, prostate, penile cancers, hepatocellular carcinoma and melanoma (Zheng et al., 2019).

6. Fluorescent Dyes in Paediatric Cancer for Diagnosis and Surgery

The application of fluorescent dyes in paediatric cancer has emerged as a groundbreaking approach in oncology, providing critical advancements in diagnostics and treatment (Goldstein et al., 2021; Abdelhafeez et al., 2021). Fluorescent dyes, such as indocyanine green (ICG) and fluorescein, have been extensively researched for their capabilities in enhancing the visualization of cancerous tissues. These dyes exhibit unique properties that allow them to selectively accumulate in tumor cells, which can then be illuminated under specific wavelengths of light to create a vivid contrast between malignant and healthy tissues (Abdelhafeez et al., 2021). This enhanced imaging capability is particularly beneficial during surgical procedures, where it aids surgeons in accurately delineating tumor margins, thereby increasing the precision of tumor excision and reducing the risk of residual disease (Rijs et al., 2021).

Beyond surgical applications, fluorescent dyes have also shown promise in diagnostic imaging. They enhance the detection sensitivity of various imaging modalities, such as fluorescence-guided endoscopy and intraoperative imaging, facilitating earlier and more accurate diagnosis of paediatric cancers. This early detection is essential for initiating timely treatment and improving prognosis (Weiser et al., 2013).

In pediatric oncology, where the preservation of healthy tissue is paramount for the child's growth and development, the precision offered by fluorescent dye-guided surgery is invaluable. The real-time feedback provided by these dyes ensures that surgeons can achieve more complete resections while minimizing damage to surrounding healthy tissues. This is crucial in maintaining the functional integrity of vital structures and improving postoperative outcomes (Te Velde et al., 2010).

Moreover, the field has seen significant advancements with the development of targeted fluorescent probes. These probes are conjugated with antibodies, peptides, or other ligands that bind specifically to tumor-associated antigens or receptors, thereby increasing the specificity and sensitivity of tumor detection. Such targeted approaches enable the precise localization of tumors at a molecular level, which is particularly beneficial in complex cases where tumors are not easily distinguishable from normal tissues (Bertacca et al., 2023).

The integration of fluorescent dyes in paediatric oncology represents a significant leap forward in cancer management. These technologies not only enhance the accuracy and efficacy of surgical interventions but also open new avenues for non-invasive diagnostics and personalized treatment strategies. As research progresses, it is anticipated that the role of fluorescent dyes will continue to expand, offering even more sophisticated tools for tackling paediatric cancers and improving the survival rate and quality of life of young patients (Goldstein et al., 2021).

7. Other Nonmedical applications of Fluorescein

Fluorescein and its derivates can also be used for detection and optical imaging as important fluorescent probes, for which fluorescein derivates are created by introducing aldehyde groups or ester groups onto the fluorescein xanthene ring and benzene moiety. Such derivates can form complexes with analytes, resulting in colour changes and alterations in fluorescence intensity due to their high activity (Yan et al., 2017). In both aqueous solutions and living cells, fluorescein probes can be utilized to detect a range of metal ions including enviromental contaminants including copper, zinc, mercury, gold, silver, palladium, iron, magnesium, cadmium, and lead (Yan et al., 2017). Fluorescein

probes are modified across the five positions (**Figure 2**) and when merged with metal ions, a change of colour and fluorescent intensity can occur. Fluorescein probes can be modified through Schiff base and esterification reactions, providing binding sites for anions (Yan et al., 2017). When these probes react with anions, the unstable bonds within the probe-anion complex result in either fluorescence enhancement or quenching. Fluorescein probes can detect anions like hypochlorite, sulfide, nitrate radical, fluoride ions, and thiocyanate (Yan et al., 2017). There are also multifunctional probes, whose advantage is detecting both anions and cations. To detect small molecules, fluorescein probes' lipophilic properties were better enhanced by esterification and alkylation in site 3 (Yan et al., 2017). When small molecules encounter fluorescein probes, they break ester bonds present in the probes, which restores the π-conjugation structure in probes and further leads to changes in fluorescence emission (Yan et al., 2017). Fluorescein probes have the capability to detect a range of small molecules including amino acids, nitric oxide, hydrogen sulfide, reactive oxygen species, hydrazine, phosphate, and adenosine triphosphate (ATP) (Yan et al., 2017). Another use for fluorescein probes is the detection of enzymes. When enzymes hydrolyse fluorescein's phenolic groups connected to an organism's organic compounds, fluorescence recovery is observed (Yan et al., 2017).

8. Conclusion

The application of fluorescein and its derivatives has notably advanced diagnostic and therapeutic modalities, particularly within oncology. These fluorescent dyes provide essential high-contrast imaging and precise visualization capabilities, which are critical for accurate tumor detection and surgical excision. Their use in procedures such as fluorescein angiography and fluorescence-guided surgery underscores their significant role in contemporary medical practice. In paediatric oncology, the precision afforded by these dyes in delineating tumor margins is paramount, minimizing collateral damage to healthy tissues and thereby improving patient outcomes.

Furthermore, fluorescein's utility extends into environmental science and forensic analysis, where its high sensitivity facilitates the detection of trace substances. While the versatility and efficacy of fluorescein are well-documented, it is important to acknowledge the inherent limitations and potential adverse effects associated with its use. Ongoing research and advancements in fluorescent dye technology are anticipated to broaden their application spectrum, fostering new avenues for scientific discovery and clinical innovation.

In conclusion, fluorescein and its derivatives represent indispensable tools in both clinical and research domains. Their continued development and judicious application hold substantial potential for enhancing diagnostic accuracy, therapeutic efficacy, and contributions to diverse scientific fields. As the discipline advances, the innovative and careful utilization of fluorescent dyes is expected to yield significant improvements in both scientific research and medical practice.

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