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Andersson-Engels, Stefan; af Klinteberg, C; Svanberg, Katarina; Svanberg, Sune

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In vivo fluorescence imaging for tissue diagnostics

Stefan Andersson-Engels^{†‡}, Claes af Klinteberg^{†‡}, K Svanberg^{†§} and
S Svanberg^{†‡}

[†] Lund University Medical Laser Centre

[‡] Department of Physics, Lund Institute of Technology, PO Box 118, SE-221 00 Lund, Sweden

[§] Department of Oncology, Lund University Hospital, SE-221 85 Lund, Sweden

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Abstract. Non-invasive fluorescence imaging has the potential to provide *in vivo* diagnostic information for many clinical specialities. Techniques have been developed over the years for simple ocular observations following UV excitation to sophisticated spectroscopic imaging using advanced equipment. Much of the impetus for research on fluorescence imaging for tissue diagnostics has come from parallel developments in photodynamic therapy of malignant lesions with fluorescent photosensitizers. However, the fluorescence of endogenous molecules (tissue autofluorescence) also plays an important role in most applications. In this paper, the possibilities of imaging tissues using fluorescence spectroscopy as a mean of tissue characterization are discussed. The various imaging techniques for extracting diagnostic information suggested in the literature are reviewed. The development of exogenous fluorophores for this purpose is also presented. Finally, the present status of clinical evaluation and future directions are discussed.

1. Introduction and principles

The biomedical use of fluorescence-based techniques is increasing. Frequently used techniques are fluorescence microscopy, flow cytometry and cell sorting (Dressler and Bartow 1989; Herman and Lemasters 1993). These techniques are frequently based on fluorescence marking utilising externally added fluorophores which selectively bind to specific targets in tissues.

The purpose of this paper is to review the work performed for *in vivo* fluorescence imaging for bulk tissue diagnostics, a much less developed area, and to point to possible future developments in this field. We limit the scope to imaging techniques, as the field of point spectroscopy is covered by Bigio and Mourant (1997, p 803 this issue). The various imaging techniques for extracting diagnostic information suggested in the literature, from visual examination following UV excitation to advanced multispectral imaging, are included. Primary fields of application of *in vivo* fluorometry include tissue metabolic studies (Tamura *et al* 1989, Horvath *et al* 1994, Cordeiro *et al* 1995), cardiovascular diagnosis (Perk *et al* 1991, 1993, Deckelbaum 1994, Deckelbaum *et al* 1995, Papazoglou 1995, Warren *et al* 1995), ophthalmology (Docchio 1989) and oncology. Most of the published *in vivo* fluorescence imaging studies deal with oncological applications; to identify early malignant lesions, for defining tumour extent and spread to adjacent tissues and as a guide for optimizing localized treatments of solid tumours.

It is important to recognize that there are many alternative fluorescence imaging techniques available to extract the relevant diagnostic information, and, in many of the potential applications, it is not yet clear which will be optimal. In analysing this we would like to address some parameters that differ between the approaches:

(i) Firstly, the fluorescent molecule, the fluorophore, marking lesions to be visualized, should be considered. Monitoring could be based on tissue autofluorescence, that means fluorescence resulting from endogenous fluorophores, or on an externally administered marker selectively accumulated in lesions of diagnostic interest. Many drugs investigated for their photodynamic activity in the field of photodynamic therapy (PDT) have properties of interest for fluorescence tissue diagnostics. Obviously, a technique offering sufficient diagnostic information based on pure tissue autofluorescence would be preferable, as it does not require the use of any exogenous fluorophore potentially associated with risks of unwanted side-effects.

(ii) Secondly, this fluorophore must somehow alter the fluorescence characteristics of the bulk tissue examined. This difference might be found in the fluorescence excitation or emission spectrum, or in the fluorescence lifetime. The difference may be due to a change in concentration of the fluorophore between the lesion and surrounding normal tissue, but it might also be due to an alteration in the fluorescence properties of that fluorophore due to variations in the microenvironment (Gudgin *et al* 1981, Cubeddu *et al* 1989). Other parameters in fluorescence recordings, such as polarization, could possibly also be utilized (Rigler *et al* 1992).

(iii) Another issue to discuss is that the optical properties of the lesion might differ from those of the surrounding tissue. As the fluorescent light generated within the tissue is filtered by the tissue on its way to the detector, the tissue absorption and scattering properties and the detection geometry become important in addition to the primarily emitted fluorescence (Wu *et al* 1993, Ahmed *et al* 1994, Durkin *et al* 1994). This makes it difficult to compare results obtained using different illumination and detection geometries.

In order to extract diagnostic information using fluorescence, several approaches have been suggested and examined. In the simplest case, fluorescence imaging can be performed at a single excitation wavelength, λ_{ex} , and a single emission wavelength, λ_{em} . However, extracting the maximum information may require spectroscopic imaging fully utilizing the differences in the fluorescence properties. Clinical evaluations are required to find out the best compromises between information content and technical complexity for different applications. The principle of several of the suggested methods and the results generated with the techniques will be briefly described below. The fluorophores used for this purpose are also discussed.

2. Fluorophores

The phenomenon of fluorescence was first observed by Stokes (1852). That tissue fluorescence potentially could be used for diagnostic purposes was recognised much later (Stübel 1911). Much effort has been spent on investigating the origin of this tissue autofluorescence. Many fluorophores have been identified using various spectroscopic techniques, such as excitation–emission matrices, time-resolved fluorescence spectroscopy, and comparing the results obtained from fluorophores in solution and in tissues. Some of the identified fluorescent substances in various tissues are tryptophan, collagen, elastin, nicotinate adenine dinucleotide, reduced form (NADH), flavin mononucleotide (FMN) and porphyrins (Blankenhorn and Braunstein 1958, Chance *et al* 1962, Aubin 1979, Benson *et al* 1979, Visser *et al* 1984, Laifer *et al* 1988, Lohmann *et al* 1989, Baraga *et al* 1990, Hubmann *et al* 1990, Rava *et al* 1991). For a more detailed discussion and presentation of published research regarding the origin of tissue autofluorescence, we refer to the review by Andersson-Engels and Wilson (1992).

The development of exogenous fluorophores as tumour markers for fluorescence diagnostics is closely associated with that of photodynamic therapy (PDT), a tumour treatment modality utilizing production of cytotoxic radicals in photoinitiated chemical reactions. Experiments with fluorescent substances to photosensitize tissue were first performed at the very end of the nineteenth century (Raab 1899, 1900). Porphyrins were among the first naturally occurring compounds used to sensitize living organisms to visible light (Hausmann 1908, Meyer-Betz 1913).

The first quantitative study of fluorescence *in vivo* with exogenous fluorophores was performed by Winkelman and Rasmussen-Taxdal (1960) using fluorometry and spectrophotometry of porphyrins chemically extracted from tissue. In the same year, Lipson and Baldes (1960) reported on a derivative of haematoporphyrin (HpD) as a fluorescent tumour marker and photosensitizer of malignant tumours. HpD was first tested clinically in 15 patients with bronchial or oesophageal tumours and in a further 51 patients, 31 of whom had malignant lesions of cervix or vagina. All bronchial and oesophageal tumours and 29 of the cervical or vaginal lesions were examined with the unaided eye and observed to exhibit positive reddish fluorescence from the HpD following violet light excitation (Lipson *et al* 1961 1964b). In one of the two cervical or vaginal lesions in which no red fluorescence was seen the malignant lesion was found histopathologically to be covered with normal tissue.

Several other clinical fluorescence visualization studies with HpD during the period 1964–76 also showed encouraging results:

(i) Fluorescence was observed in 80% of 35 patients with bronchial or oesophageal carcinomas (Lipson *et al* 1964a).

(ii) In a study involving 226 patients, of whom 173 had malignant lesions of various types, positive HpD fluorescence was obtained in 132 (77%) (Gregorie and Green 1965, Gregorie *et al* 1968) and 22% of 53 patients with benign lesions also showed positive fluorescence.

(iii) Epithelial carcinomas of the mouth, hypopharynx, larynx or trachea all showed HpD fluorescence in a study involving 40 patients (Leonard and Beck 1971).

(iv) Eighteen of 23 patients with invasive or *in situ* cervical cancer showed positive HpD fluorescence (Gray *et al* 1967).

(v) All 12 patients with carcinoma *in situ* or dysplasia of the cervical uterus and three of four patients with squamous metaplasia showed reddish fluorescence in the lesions (Kyriazis *et al* 1973).

(vi) In a study of bladder carcinoma, lesions of 11 of 11 patients showed HpD fluorescence, and no normal tissue showed any HpD fluorescence, although a slight fluorescence was observed in the oedematous submucosa around the tumours in three patients (Kelly and Snell 1976).

It was also shown that intraocular tumours in animal models could be detected using HpD fluorescence (Cunningham and Henderson 1966, Krohn *et al* 1974). In this case interfering lens fluorescence had to be rejected to allow fluorescence to be seen in the posterior parts of the eye (Krohn *et al* 1974).

By the end of the 1970s the use of HpD as a tumour marker for fluorescence diagnostics was growing rapidly, largely due to the breakthrough in the use of HpD as a photosensitizer for photodynamic therapy (Dougherty *et al* 1972, 1975). For this latter application many questions arose regarding the composition of HpD, which is a complex mixture of various porphyrins in both monomeric and oligomeric form.

Detailed research characterizing the properties of HpD were carried out during the 1980s. Some properties of interest are the dual-peaked fluorescence emission spectrum in

the 630–700 nm region and the relatively long fluorescence lifetime of about 16 ns, making its contribution to tissue fluorescence easily extractable. However, there also exist several drawbacks with this substance. As it is not a single stable chemical compound it is difficult to fully characterize. Furthermore, it has a low fluorescence yield, a poor selectivity in malignant tissue first 24 h after the drug administration, and the patient may suffer from a skin sensitivity up to several weeks afterwards.

For these reasons alternative drugs to be used as tumour markers for fluorescence diagnostics have been considered. Most of them, like HpD, are photodynamically active substances investigated primarily for their potential as photosensitizers for PDT. Phthalocyanines are interesting, as many of them have a much stronger fluorescence than HpD, and at longer wavelengths with less overlap with the tissue autofluorescence (Spikes 1986). A better selectivity for malignant tissues has also been found (van Leengoed *et al* 1990, Peng *et al* 1991). Other PDT drugs examined for fluorescence tumour marking capabilities include chlorins (mono-aspartyl chlorin e_6 (MACE), di-aspartyl chlorin e_6 (DACE), meso-tetra hydroxyphenyl chlorin (mTHPC) and benzoporphyrin derivatives (Roberts *et al* 1988, Andersson-Engels *et al* 1993, Alian *et al* 1994). They all exhibit a strong fluorescence, but they have also a strong photosensitizing capability, which might sometimes be a drawback for pure diagnostic purposes. Also, they do not offer a much better selectivity to malignant tissue than HpD.

Several rhodamines have also been proposed (Haghighat *et al* 1992). The binding of these dyes to tissue is partly attributed to the positive charge of the rhodamines leading to attraction to transformed cells. Their drawback for this purpose is the emission wavelengths in the visible region, where the tissue autofluorescence is strong.

Recently, the introduction of δ -amino levulinic acid (ALA), a precursor to haem in the haem cycle, was a much needed boost (Malik and Lugaci 1987, Kennedy *et al* 1990, Kennedy and Pottier 1992, Svanberg *et al* 1994, Johansson *et al* 1997). Following administration of ALA, an excess amount of protoporphyrin IX (PpIX), the intermediate product just before haem in the intracellular chain reaction called the haem cycle, will build up in the tissue. Several advantages were found with this fluorescence tumour marking technique. Firstly, both ALA and PpIX are substances normally present in the body, making the toxicity issue less critical. Furthermore, the drug can conveniently be administered orally or applied topically (Kriegmair *et al* 1994, 1995, Svanberg *et al* 1994, Malik *et al* 1995, Rokahr *et al* 1995, af Klinteberg *et al* 1996). The ALA molecule is in itself a small non-photoactive substance, quickly metabolised to PpIX in tissue with a high selectivity to malignant tissue. The photophysical properties of PpIX are similar to those of HpD.

Some recent work has also been pursued to examine non-photosensitizing agents for fluorescence diagnostic of tissue. In this way one should totally eliminate the, for diagnostic purposes, drawback of light sensitization of tissues. Some compounds investigated with this idea in mind are carotenoporphyrins and chlorine derivatives (Nilsson *et al* 1994, Takemura *et al* 1994). Promising results have been obtained with these substances.

3. Fluorescence imaging techniques

At the end of the 1970s it was recognized that more sophisticated techniques than visual inspection and photometry were required (Sanderson *et al* 1972, Carpenter *et al* 1977, Profio *et al* 1977). Different types of fluorescence imaging instruments for diagnosis of malignancies are summarized below. Although most of the instruments have been developed and designed for diagnostics based on HpD fluorescence, they could, with minor changes, be used with other exogenous tumour markers or with tissue autofluorescence. Earlier

reviews of existing fluorosensors published include those by Profio (1988, 1990, 1991) and Andersson-Engels and Wilson (1992).

Improving the detection techniques, it was recognized that electronic detection allows objective fluorescence measurement and time-sharing can also be used so that both visual inspection and fluorescence recordings can be performed in real time. Further, electronic detection makes it possible to record several fluorescence signals and form any function of those, such as to subtract tissue autofluorescence and/or to form a ratio of HpD to autofluorescence to reduce geometric effects (Ankerst *et al* 1984, Profio and Balchum 1985). Alternatively, the ratio between the HpD fluorescence and the diffusely reflected excitation light (Profio *et al* 1984, Lenz 1988), can be used, but care is needed to eliminate specularly reflected light (Lenz 1988). Pulsed light sources can be used to suppress ambient background light utilizing a time-gated detector (Ankerst *et al* 1984).

The first clinical fluorescence bronchoscope using a mercury arc lamp (and later a CW krypton-ion laser) source and an image intensifier to amplify the faint fluorescence signal to a directly viewed image was presented in 1979 (Doiron *et al* 1979, Profio and Doiron 1979). One problem with this early system was that normal white light bronchoscopy could not be performed at the same time as the fluorescence examination. Rapid switching between white light and fluorescence was, therefore, developed, and a video camera was also implemented to allow easy storage of the entire examination (Profio *et al* 1983).

In general, the restriction to a single wavelength seriously limits the reliability of fluorescence diagnosis (Profio *et al* 1983). Several approaches have been used to overcome this limitation. One is to incorporate a point-measuring device with spectral resolution into an imaging instrument, so that any suspicious region on the image can be investigated spectroscopically (Hirano *et al* 1989). Another is to perform sequential or parallel imaging at several excitation or emission wavelengths to subtract autofluorescence or to form wavelength ratios (Profio and Balchum 1985, Profio *et al* 1986, Baumgartner *et al* 1987, Wagnières *et al* 1990, Palcic *et al* 1991, van den Bergh 1994). One of these systems, detecting two images in two emission bands, has been developed to a commercial product especially for endoscopic applications (Xillix Techn. Inc., BC, Canada, light-induced fluorescence endoscopy (LIFE)). This system utilizes a CW light source in the violet wavelength region, either a high-pressure mercury lamp at 405 and 436 nm or a HeCd laser at 442 nm. The diagnostic capability of this system, is based on the ratio between a red and a green autofluorescence emission band. The technique is reported to produce results of clinical interest in certain specialities (Lam *et al* 1991, 1993a, b, Harris *et al* 1995). The technique is therefore also approved by FDA for routine clinical use for endoscopic lung cancer examinations using the pure tissue autofluorescence. The reason for a change in this ratio for malignant tissues as compared with normal tissues is, however, not fully understood yet. Clinical evaluations for other specialities are also ongoing with such systems.

An alternative suggested technique combines multiple excitation and emission wavelengths. The rationale for this approach as compared with the previous ones is to be able to compensate for variations in tissue optical properties in the measurements, and thereby extract signals almost linearly dependent on an exogenous dye concentration (Sinaasappel and Sterenberg 1993, Sterenberg *et al* 1994). This technique may allow quantitative measurements of fluorophores inside turbid media, as the idea is that the tissue optical properties should not affect the signals.

A slightly more advanced system using splitting optics to provide four images of an object, filtered at different emission bands has also been developed (Montán *et al* 1985, Andersson-Engels *et al* 1990, 1991, 1994, 1995, Svanberg *et al* 1997). The four images can then be computer processed for viewing of the optimized contrast function image.

Another advantage with this system is the use of a pulsed light source and a gated detector. This concept allows the simultaneous use of fluorescence imaging and normal white light endoscopic examination. Many practitioners trained using normal white light examinations appreciate the possibility of adding the fluorescence information to the information they are used to working with rather than to replacing the white light with a fluorescence examination, or sequential examinations. As more detection wavelengths can be used, this system is more flexible in optimizing the diagnostic information extractable for various clinical applications. The system could work for tissue autofluorescence as well as for a combination for autofluorescence and fluorescence from an exogenous marker.

A further approach is to perform time-gated imaging to reduce the influence of autofluorescence. An amazingly good tumour demarcation capability has been demonstrated with very low concentrations of fluorescent tumour markers in animal studies (Cubeddu *et al* 1993, 1995). Time-gated imaging might also be interesting in the use of pure tissue autofluorescence as the diagnostic information.

Probably the most advanced and powerful system used for multispectral fluorescence imaging of tissue is presented by Malik *et al* (1996). This Fourier transform spectrometer allows recordings of the full fluorescence emission spectrum in each image pixel. With the spectral resolution provided with a system like this, it is possible to study very subtle changes in the fluorescence emission. The slight change in fluorescence emission of a fluorophore due to changes in the microenvironment can therefore be studied. Protoporphyrin bound to various subcellular compartments could thus be differentiated. This might be of specific interest for fluorescence tissue diagnostics.

Fluorescence imaging is a potential candidate for tissue diagnostics in a wide variety of clinical situations. Several techniques are suggested by which these examinations could be performed. Which one, if any, is best suited is probably going to vary with the application. All of them need to be examined separately in detail to be able to satisfactorily judge if a certain technique can provide valuable clinical information. We would therefore like to stress the need for well controlled clinical studies to evaluate the various techniques in detail. Also, since the methods vary significantly in what type of spectroscopic information they utilize, it might be difficult to judge how well the different methods would perform in a certain application, if only results from one of them have been studied. In particular, results from point-measuring systems are not directly transferable to the imaging geometry, as the tissue optical properties are much more important in the latter case due to self-filtration of the fluorescence from inside the tissue. It is thus important not to draw to general conclusions from results obtained with a certain technique.

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References

- af Klinteberg C, Nilsson A M K, Wang I, Andersson-Engels S, Svanberg S and Svanberg K 1996 Laser-induced fluorescence diagnostics of basal cell carcinomas of the skin following topical ALA application *Proc. SPIE* **2926** 32–40
- Ahmed S A, Zang Z, Yoo K M, Ali M A and Alfano R R 1994 Effect of multiple light scattering and self-absorption on the fluorescence and excitation spectra of dyes in random media *Appl. Opt.* **33** 2746–50
- Alian W, Andersson-Engels S, Svanberg K and Svanberg S 1994 Laser-induced fluorescence studies of meso-tetra(hydroxyphenyl)chlorin in malignant and normal tissues in rat *Br. J. Cancer* **70** 880–5

- Andersson-Engels S, Ankerst J, Johansson J, Svanberg K and Svanberg S 1990 1993 Laser-induced fluorescence in malignant and normal tissue of rats injected with benzoporphyrin derivative *Photochem. Photobiol.* **57** 978–83
- Andersson-Engels S, Berg R, Svanberg K and Svanberg S 1995 Multi-colour fluorescence imaging in combination with photodynamic therapy of δ -amino levulinic acid (ALA) sensitised skin malignancies *Bioimaging* **3** 134–43
- Andersson-Engels S, Johansson J, Svanberg K and Svanberg S 1991 Fluorescence imaging and point measurements of tissue: Applications to the demarcation of malignant tumors and atherosclerotic lesions from normal tissue *Photochem. Photobiol.* **53** 807–14
- Andersson-Engels S, Johansson J and Svanberg S 1990 Multicolor fluorescence imaging system for fluorescence diagnostics *Proc. SPIE* **1205** 179–89
- 1994 Medical diagnostic system based on simultaneous multi-spectral fluorescence imaging *Appl. Opt.* **33** 8022–9
- Andersson-Engels S and Wilson B C 1992 In vivo fluorescence in clinical oncology: fundamental and practical issues *J. Cell Pharmacol.* **3** 48–61
- Ankerst J, Montán S, Svanberg K and Svanberg S 1984 Laser-induced fluorescence studies of hematoporphyrin derivative (HpD) in normal and tumour tissue of rat *Appl. Spectrosc.* **38** 890–6
- Aubin J E 1979 Autofluorescence of viable cultured mammalian cells *J. Histochem. Cytochem.* **27** 36–43
- Baraga J J, Rava R P, Taroni P, Kittrell C, Fitzmaurice M and Feld M S 1990 Laser-induced fluorescence spectroscopy of normal and atherosclerotic human aorta using 306–310 nm excitation *Lasers Surg. Med.* **10** 245–61
- Baumgartner R, Fisslinger H, Jocham D, Lenz H, Ruprecht L, Stepp H and Unsöld E 1987 A fluorescence imaging device for endoscopic detection of early stage cancer—instrumental and experimental studies *Photochem. Photobiol.* **46** 759–63
- Benson R C, Meyer R A, Zaruba M E and McKhann G M 1979 Cellular autofluorescence—is it due to flavins *J. Histochem. Cytochem.* **27** 44–8
- Bigio I J and Mourant J R 1997 Ultraviolet and visible spectroscopies for tissue diagnostics *Phys. Med. Biol.* **42** 803–14
- Blankenhorn D H and Braunstein H 1958 Carotenoids in man III. The microscopic pattern of fluorescence in atheromas and its relation to their growth *J. Clin. Invest.* **37** 160–5
- Carpenter R J, Ryan R J, Neel H B and Sanderson D R 1977 Tumour fluorescence with haematoporphyrin derivative *Ann. Otol.* **86** 661–6
- Chance B, Cohen P, Jöbsis F and Schoener B 1962 Intracellular oxidation-reduction states in vivo *Science* **147** 499–508
- Cordeiro P G, Kirschner R E, Hu Q Y, Chiao J J C, Savage H, Alfano R R, Hoffman L A and Hidalgo D A 1995 Ultraviolet excitation fluorescence spectroscopy: A noninvasive method for the measurement of redox changes in ischemic myocutaneous flaps *Plast. Reconstr. Surg.* **96** 673–80
- Cubeddu R, Canti G, Taroni P and Valentini G 1993 Time-gated fluorescence imaging for the diagnosis of tumors in a murine model *Photochem. Photobiol.* **57** 480–5
- Cubeddu R, Pifferi A, Taroni P, Valentini G and Canti G 1995 Tumor detection in mice by measurement of fluorescence decay time matrices *Opt. Lett.* **20** 2553–5
- Cubeddu R, Ramponi R, Liu W-Q and Docchio F 1989 Time-gated fluorescence spectroscopy of the tumor localizing fraction of HpD in the presence of cationic surfactant *Photochem. Photobiol.* **50** 157–63
- Cunningham R D and Henderson J W 1966 Experimental evaluation of haematoporphyrin in the detection and management of intracular tumours *Am. J. Ophthalmol.* **61** 36–44
- Deckelbaum L I 1994 Cardiovascular applications of laser technology *Lasers Surg. Med.* **15** 315–41
- Deckelbaum L I, Desai S P, Kim C and Scott J J 1995 Evaluation of a fluorescence feedback system for guidance of laser angioplasty *Lasers Surg. Med.* **16** 266–34
- Docchio F 1989 Ocular fluorometry: principles, fluorophores, instrumentation and clinical applications *Lasers Surg. Med.* **9** 515–32
- Doiron D R, Profio A E, Vincent R G and Dougherty T J 1979 Fluorescence bronchoscopy for detection of lung cancer *Chest* **76** 27–32
- Dougherty T J, Grindey G B, Fiel R, Weishaupt K R and Boyle D G 1975 Photoradiation therapy II. Cure of animal tumours with haematoporphyrin and light *J. Natl. Cancer Inst.* **55** 115–19
- Dougherty T J, Kaufman J E, Goldfarb A, Weishaupt K R, Boyle D G, Mittelman A, Sanderson D R, Fontana R S, Lipsen R L and Baldes E J 1972 Photoradiation therapy for the treatment of malignant tumours haematoporphyrin as a diagnostic tool—a preliminary report of new techniques *Cancer Res.* **30** 1368–72
- Dressler L G and Bartow S A 1989 DNA flow cytometry in solid tumors: practical aspects and clinical applications *Sem. Diagn. Pathol.* **6** 55–82

- Durkin A J, Jaikumar S, Ramanujam N and Richards-Kortum R 1994 Relation between fluorescence spectra of dilute and turbid samples *Appl. Opt.* **33** 414–23
- Gray M J, Lipson R, Maecck J V S, Parker L and Romeyn D 1967 Use of haematoporphyrin derivative in detection and management of cervical cancer—a preliminary report *Am. J. Obstet. Gynecol.* **99** 766–71
- Gregorie H B and Green J F 1965 Haematoporphyrin derivative fluorescence in malignant neoplasms *J. Sci. Med. Assoc.* **61** 157–64
- Gregorie H B, Horger E O, Ward J L, Green J F, Richards T, Roberts H C and Stevenson T B 1968 Haematoporphyrin-derivative in malignant neoplasms *Ann. Surg.* **167** 820–8
- Gudgin E, Lopez-Delgado R and Ware W R 1981 The tryptophan fluorescence lifetime puzzle. A study of decay times in aqueous solution as a function of pH and buffer composition *Can. J. Chem.* **59** 1037–44
- Haghighat S, Castro D J, Lufkin R B, Fetterman H R, Soudant J, Ward P H and Saxton R E 1992 Laser dyes for experimental phototherapy of human cancer: comparison of three rhodamines *Laryngoscope* **102** 81–7
- Harries M L, Lam S, MacAuley C, Qu J and Palcic B 1995 Diagnostic imaging of the larynx: autofluorescence of laryngeal tumours using the helium-cadmium laser *J. Laryngol. Otol.* **109** 108–10
- Hausmann W 1908 Über die sensibilisierende Wirkung tierischer Farbstoffe und ihre physiologische Bedeutung *Biochem. Z.* **14** 275–8
- Herman B and Lemasters J J 1993 *Optical Microscopy—Emerging Methods and Applications* (Orlando: Academic)
- Hirano T et al 1989 Photodynamic cancer diagnostics and treatment system consisting of pulse lasers and an endoscopic spectro-image analyzer *Lasers Life Sci.* **3** 1–18
- Horvath K A, Schomacker K T, Lee C C and Cohn L H 1994 Intraoperative myocardial ischemia detection with laser-induced fluorescence *J. Thorac. Cardiovasc. Surg.* **107** 220–5
- Hubmann M R, Leiner M J P and Schaur R J 1990 Ultraviolet fluorescence of human sera: I. Sources of characteristic differences in the ultraviolet fluorescence spectra of sera from normal and cancer-bearing humans *Clin. Chem.* **36** 1880–3
- Johansson J, Berg R, Svanberg K and Svanberg S 1997 Laser-induced fluorescence studies of normal and malignant tumour tissue of rat following intravenous injection of δ -amino levulinic acid *Lasers Surg. Med.* at press
- Kelly J F and Snell M E 1976 Haematoporphyrin derivative: a possible aid in the diagnosis and therapy of carcinoma in the bladder *J. Urol.* **115** 150–1
- Kennedy J C and Pottier R H 1992 Endogenous protoporphyrin IX, a clinically useful photosensitizer for photodynamic therapy *J. Photochem. Photobiol. B: Biol.* **14** 275–92
- Kennedy J C, Pottier R H and Pross D C 1990 Photodynamic therapy with endogenous protoporphyrin IX: Basic principles and present clinical experience *J. Photochem. Photobiol. B: Biol.* **6** 143–8
- Kriegmair M, Baumgartner R, Knuechel R, Steinbach P, Ehsan A, Lumper W, Hofstädter F and Hofstetter A 1994 Fluorescence photodetection of neoplastic urothelial lesions following intravesical instillation of 5-aminolevulinic acid *Urology* **44** 836–41
- Kriegmair M, Steep H, Steinbach P, Lumper W, Ehsan A, Stepp H G, Rick K, Knuechel R, Baumgartner R and Hofstetter A 1995 Fluorescence cystoscopy following intravesical instillation of 5-aminolevulinic acid: A new procedure with high sensitivity for detection of hardly visible urothelial neoplasias *Urol. Int.* **55** 190–6
- Krohn D L, Jacobs R and Morris D A 1974 Diagnosis of model choroidal malignant melanoma by haematoporphyrin derivative fluorescence in rabbits *Invest. Ophthalmol.* **13** 244–55
- Kyriazis G A, Balin H and Lipson R L 1973 Haematoporphyrin-derivative-fluorescence test colposcopy and colpophotography in the diagnosis of atypical metaplasia, dysplasia, and carcinoma *in situ* of the cervix uteri *Am. J. Obstet. Gynecol.* **117** 375–80
- Laifer L, O'Brien K, Stetz M, Gindi G, Garrand T and Deckelbaum L 1988 Biochemical basis for the difference between normal and atherosclerotic arterial fluorescence *Circulation* **80** 1893–901
- Lam S, Hung J and Palcic B 1991 Mechanism of detection of early lung cancer by ratio fluorometry *Lasers Life Sci.* **4** 67–73
- Lam S, MacAuley C, Hung J, LeRiche J, Profio A E and Palcic B 1993a Detection of dysplasia and carcinoma *in situ* with a lung imaging fluorescence endoscopic device *J. Thorac. Cardiovasc. Surg.* **105** 1035–40
- Lam S, MacAuley C and Palcic B 1993b Detection and localization of early lung cancer by imaging techniques *Chest* **103** 12S–4S
- Lenz P 1988 Endoscopic fluorescence detector *Rev. Sci. Instrum.* **59** 930–3
- Leonard J R and Back W L 1971 Haematoporphyrin fluorescence: an aid in diagnosis of malignant neoplasms *Laryngoscope* **81** 365–77
- Lipson R L and Baldes E J 1960 The photodynamic properties of a particular haematoporphyrin derivative *Arch. Dermatol.* **82** 508–16
- Lipson R L, Baldes E J and Olsen A M 1961 Haematoporphyrin derivative: a new aid for endoscopic detection of malignant disease *J. Thorac. Cardiovasc. Surg.* **42** 623–9

- Lipson R L, Baldes E J and Olsen A M 1964a Further evaluation of the use of haematoporphyrin derivative as a new aid for the endoscopic detection of malignant disease *Dis. Chest* **46** 676–9
- Lipson R L, Pratt J H, Baldes E J and Dockerty M B 1964b Haematoporphyrin derivative for detection of cervical cancer *Obstet. Gynecol.* **24** 78–84
- Lohmann W, Mussmann J, Lohmann C and Künzel W 1989 Native fluorescence of the cervix uteri as a marker for dysplasia and invasive carcinoma *Eur. J. Obstet. Gynecol. Reprod. Biol.* **31** 249–53
- Malik Z, Dishi M and Garini Y 1996 Fourier transform multipixel spectroscopy and spectral imaging of protoporphyrin in single melanoma cells *Photochem. Photobiol.* **63** 608–14
- Malik Z, Kostenich G, Roitman L, Ehrenberg B and Orenstein A 1995 Topical application of 5-aminolevulinic acid, DMSO and EDTA: protoporphyrin IX accumulation in the skin and tumours of mice *J. Photochem. Photobiol. B: Biol.* **28** 213–18
- Malik Z and Lugaci H 1987 Destruction of erythroleukaemic cells by photoinactivation of endogenous porphyrins *Br. J. Cancer* **56** 589–95
- Meyer-Betz F 1913 Untersuchungen über die biologische (photodynamische) Wirkung des Hämatoporphyrins und andere Derivate des Blut- und Gallenfarbstoffs *Deutsch. Arch. Klin. Med.* **112** 476–50
- Montán S, Svanberg K and Svanberg S 1985 Multi-color imaging and contrast enhancement in cancer tumor localization using laser-induced fluorescence in hematoporphyrin derivative (HpD)-bearing tissue *Opt. Lett.* **10** 56–8
- Nilsson H, Johansson J, Svanberg K, Svanberg S, Jori G, Reddi E, Segalla A, Gust D, Moore A L and Moore T A 1994 Laser-induced fluorescence in malignant and normal tissue in mice injected with two different carotenoporphyrins *Br. J. Cancer* **70** 873–9
- Palcić B, Lam S, Hung J and MacAuley C 1991 Detection and localization of early lung cancer with imaging techniques *Chest* **99** 742–3
- Papzogou T G 1995 Malignant and atherosclerotic plaque diagnosis—is laser induced fluorescence spectroscopy the ultimate solution? *J. Photochem. Photobiol. B: Biol.* **28** 3–11
- Peng Q, Moan J, Kongshaug M, Anholt H and Rimington C 1991 Sensitizers for photodynamic therapy of cancer: a comparison of the tissue distribution of Photofrin II and aluminum phthalocyanine tetrasulfonate in nude mice bearing a human malignant tumor *Int. J. Cancer* **48** 258–64
- Perk M, Flynn G J, Gulamhusein S, Wen Y, Smith C, Bathgate B, Tulip J, Parfrey N A and Lucas A 1993 Laser-induced fluorescence identification of sinoatrial and atrioventricular nodal conduction tissue *Pace* **16** 1701–12
- Perk M, Flynn G J, Smith C, Bathgate B, Tulip J, Yue W and Lucas A 1991 Laser-induced fluorescence emission: I. The spectroscopic identification of fibrotic endocardium and myocardium *Lasers Surg. Med.* **11** 523–34
- Profio A E 1988 Review of fluorescence diagnosis using porphyrins *Proc. SPIE* **907** 150–6
- 1990 Fluorescence diagnosis and dosimetry using porphyrins *Photodynamic Therapy of Neoplastic Disease* ed D Kessel (Boca Raton, FL: CRC Press) pp 77–89
- 1991 Endoscopic fluorescence detection of early lung cancer *Proc. SPIE* **1426** 44–6
- Profio A E and Balchum O J 1985 Fluorescence diagnosis of cancer fluorescence diagnosis of cancer *Methods in Porphyrin Photosensitization* ed D Kessel (New York: Plenum) pp 43–50
- Profio A E, Balchum O J and Carstens F 1986 Digital background subtraction for fluorescence imaging *Med. Phys.* **13** 717–21
- Profio A E and Doiron D R 1979 Laser fluorescence bronchoscope for localization of occult lung tumors *Med. Phys.* **6** 523–5
- Profio A E, Doiron D R, Balchum O J and Huth G C 1983 Fluorescence bronchoscopy for localization of carcinoma *in situ Med. Phys.* **10** 35–9
- Profio A E, Doiron D R and Huth G C 1977 Fluorescence bronchoscope for lung tumour localization *IEEE Trans. Nucl. Sci.* **24** 521–4
- Profio A E, Doiron D R and Sarnaik J 1984 Fluorometer for endoscopic diagnosis of tumors *Med. Phys.* **11** 516–20
- Raab O 1899 Untersuchungen über die Wirkung fluoreszierender Stoffe *Z. Biol.* **39** 16
- 1990 Über die Wirkung fluoreszierenden Stoffe auf Infusoria *Z. Biol.* **39** 524
- Rava R P, Richards-Kortum R, Fitzmaurice M, Cothren R M, Petras R E, Sivak M V, Levin H and Feld M S 1991 Early detection of dysplasia in colon and bladder tissue using laser induced fluorescence *Proc. SPIE* **1426** 68–78
- Rigler R, Widengren J and Mets Ü 1992 Interactions and kinetics of single molecules as observed by fluorescence correlation spectroscopy *Fluorescence Spectroscopy: New Methods and Applications* ed O S Wolfbeis (Heidelberg: Springer) pp 13–24
- Roberts W G, Shiau F Y, Nelson J S, Smith K M and Berns M W 1988 *In vitro* characterization of mono-aspartyl chlorin e6 (MACE) and diasparyl chlorin e6 (DACE) for photodynamic therapy *J. Natl. Cancer Inst.* **80** 330–6

- Rokahr I, Andersson-Engels S, Svanberg S, D'Hallewin M A, Baert L, Wang I and Svanberg K 1995 Optical detection of human urinary bladder carcinoma utilising tissue autofluorescence and protoporphyrin IX-induced fluorescence following low-dose ALA instillation *Proc. SPIE* **2627** 2–12
- Sanderson D R, Fontana R S, Lipson R L and Baldes E J 1972 Haematoporphyrin as a diagnostic tool—a preliminary report of new techniques *Cancer* **30** 1368–72
- Sinaasappel M and Sterenberg H J C M 1993 Quantification of the hematoporphyrin derivative by fluorescence measurement using dual-wavelength excitation and dual-wavelength detection *Appl. Opt.* **32** 541–8
- Spikes J D 1986 Phthalocyanines as photosensitizers in biological systems and for the photodynamic therapy of tumors *Photochem. Photobiol.* **43** 691–9
- Sterenberg H J C M, Motamedi M, Wagner R F, Thomsen S and Jacques S L 1994 *In vivo* fluorescence spectroscopy and imaging of human skin tumors *Lasers Med. Sci.* **9** 191–201
- Stokes G G 1852 Über die Änderung der Brechbarkeit des Lichtes *Phil. Trans. R. Soc.* **107** 11
- Stübel H 1911 Die Fluoreszenz tierischer Gewebe in ultravioletten Licht *Pflügers Arch. Physiol.* **142** 1
- Svanberg K, Andersson T, Killander D, Wang I, Stenram U, Andersson-Engels S, Berg R, Johansson J and Svanberg S 1994 Photodynamic therapy of non-melanoma malignant tumours of the skin utilizing topical δ -amino levulinic acid sensitization and laser irradiation *Br. J. Dermatol.* **130** 743–51
- Svanberg K et al 1997 Clinical multi-colour fluorescence imaging of malignant tumours—initial experience *Acta Rad.* at press
- Takemura T, Nakajima S and Sakata I 1994 Tumor-localizing fluorescent diagnostic agents without phototoxicity *Photochem. Photobiol.* **59** 366–70
- Tamura M, Hazeki O, Nioka S and Chance B 1989 *In vivo* study of tissue oxygen metabolism using optical and nuclear magnetic resonance spectroscopies *Ann. Rev. Physiol.* **51** 813–34
- van der Bergh H 1994 Photodynamic and photodetection of early cancer in the upper aerodigestive tract, the tracheobronchial tree, the oesophagus and the urinary bladder *Hardrontherapy in Oncology* ed U Amaldi and B Larsson (Amsterdam: Elsevier) pp 577–621
- van Leengoed E, Versteeg J, van der Veen N, van den Berg-Blok A, Marijnissen H and Star W 1990 Tissue-localizing properties of some photosensitizers studied by *in vivo* fluorescence imaging *J. Photochem. Photobiol. B: Biol.* **6** 111–19
- Visser A J W G, Sanetema J S and van Hoek A 1984 Spectroscopic and dynamic characterization of FMN in reversed micelles entrapped water pools *Photochem. Photobiol.* **39** 11–16
- Wagnières G, Depeursinge C, Monnier P, Savary M, Cornaz P, Chatelain A and van den Bergh H 1990 Photodetection of early cancer by laser induced fluorescence of a tumour-selective dye: apparatus design and realization *Proc. SPIE* **1203** 43–52
- Warren S, Pope K, Yazdi Y, Welch A J, Thomsen S, Johnston A L, Davis M J and Richards-Kortum R 1995 Combined ultrasound and fluorescence spectroscopy for physico-chemical imaging of atherosclerosis *IEEE Trans. Biomed. Eng.* **42** 121–32
- Winkelman J and Rasmussen-Taxdal D S 1960 Quantitative determination of porphyrin uptake by tumour tissue following parenteral administration *Bull. Johns Hopkins Hosp.* **107** 228–33
- Wu J, Feld M S and Rava R P 1993 Analytical model for extracting intrinsic fluorescence in turbid media *Appl. Opt.* **32** 3585–95