Laser-based spectroscopic methods in tissue characterization

Svanberg, Katarina; af Klinteberg, C; Nilsson, A; Wang, I; Andersson-Engels, Stefan; Svanberg, Sune

Published in:
ADVANCES IN OPTICAL BIOPSY AND OPTICAL MAMMOGRAPHY

DOI:
10.1111/j.1749-6632.1998.tb08193.x

1998

Laser-Based Spectroscopic Methods in Tissue Characterization

KATARINA SVANBERG,[a,c] CLAES AF KLINTEBERG,[b]
ANNIKA NILSSON,[b] INGRID WANG,[a]
STEFAN ANDERSSON-ENGELS,[b]
AND SUNE SVANBERG[b]

[a]*Department of Oncology*
Lund University Hospital
S-221 85 Lund, Sweden
and
[b]*Department of Physics*
Lund Institute of Technology
P.O. Box 118
S-221 00 Lund, Sweden

INTRODUCTION

Although there is a variety of conventional diagnostic modalities for the localization of human malignancies, such as X-ray and magnetic resonance imaging of the body, the identification of early tumor growth can hardly be accomplished with the conventional diagnostic modalities available. For hollow organs endoscopically based investigations are performed, and areas showing alterations in topography, shape and color can be identified. However, even for the trained eye it might be difficult to assess the tissue condition only by looking at changes in the reflected light. In several clinical situations where an obvious site for biopsy sampling cannot be identified, samples are collected randomly according to a 'map' defined for each organ.

Complementary methods for identification of early malignancies and also premalignant conditions would be of great value. In particular the development of techniques that can be used for guiding the biopsy sampling procedure would be of importance. One technique that has shown a potential for tissue characterization is laser-induced fluorescence (LIF). LIF can be used for noninvasive spectroscopic identification of biological tissue and is of special interest for early tumor detection. The basis for this 'optical biopsy' method is the interaction of the laser light with tissue chromophores, such as tryptophan, collagen, elastin, the reduced form/oxidized form of nicotinamide adenine dinucleotide ($NADH/NAD^+$), β-carotene and flavins. The UV-excited fluorescence that arises from the endogenous chromophores, the autofluorescence, has a broad distribution, peaking at about 490 nm with a lower intensity in malignant and premalignant tissue as compared to normal tissue. The tumor detection potential is enhanced by the distribution of exogenously administered tumor marking agents, such as different hematoporphyrin derivatives. Recently, the heme precursor δ-amino levulinic acid (ALA) administered topically,

[a] Corresponding author. Tel: 46 46 222 40 48; fax: 46 46 222 31 77; email: katarina.svanberg@fysik.lth.se

orally or intravenously was introduced for the induction of protoporphyrin IX (PpIX) tissue sensitization. Time-integrated laser-induced fluorescence measurements utilizing a point-monitoring fluorosensor and a multicolor fluorescence imaging system were performed *in vivo* and sometimes *ex vivo* in patients with various malignant tumors. The autofluorescence as well as the PpIX-related fluorescence signals were monitored, and dimensionless tumor demarcation functions were calculated for different human malignant and premalignant tumors, such as malignant tumors in the urinary bladder, in the head and neck region and in the skin. Also different pathological conditions in the large intestine were spectrally characterized.

FLUORESCENCE EQUIPMENT

A fiber-based clinical fluorosensor was used for the point-monitoring measurements, in which the full spectral information was collected. The equipment is described in detail in Ref. 1. Conventional biopsy sampling was performed and a correlation between spectral shape and histopathology was performed. A multicolor imaging system was utilized for the investigations of larger tissue areas, and the system as well as initial clinical experience with the system is described in Ref. 2. Examples from point-monitoring measurements as well as tissue fluorescence imaging will be given in some clinical specialities.

NONMELANOMA MALIGNANT SKIN TUMORS

Topical application of ALA on nonmelanoma malignant skin tumors, such as basal cell and squamous cell carcinomas and cutaneous T-cell lymphoma, was utilized in laser-induced photodynamic therapy (PDT). By monitoring the PpIX-related fluorescence the tumor borders might be demarcated more precisely than by visual judgment only. It is well known that there are several recurrences in the border zone of the tumor. One example recorded from a basal cell carcinoma is shown in FIGURE 1. The tumor area and the surrounding skin was topically applied with 20% ALA 6 hours *prior* to the point-monitoring measurements. As seen in the figure, the autofluorescence peaking at about 500 nm is decreasing in the tumor area. The PpIX-related signal with a dual-peaked emission at about 635 and 700 nm is clearly seen within the tumor area and the border zone. The same tumor scan was recorded after the laser irradiation with a total light dose delivery of 60J/cm^2. As illustrated in the figure, the PpIX fluorescence signal is decreased after the treatment, which is due to a photo-induced bleaching. Monitoring bleaching might be useful for dosimetry in the treatment procedure.

URINARY BLADDER TUMORS

Urinary bladder cancer, mainly transitional cell carcinoma, is the most frequent malignant tumor in the urinary tract and accounts for about 2% of cancer deaths in the United States. Typical presentation of a urinary bladder malignancy includes gross hematuria, which is the most common clinical finding and the first sign in approximately 75% of patients. Microscopic hematuria is present in almost all cases. For the diagnosis, urine cytology is performed followed by cystoscopy. The most

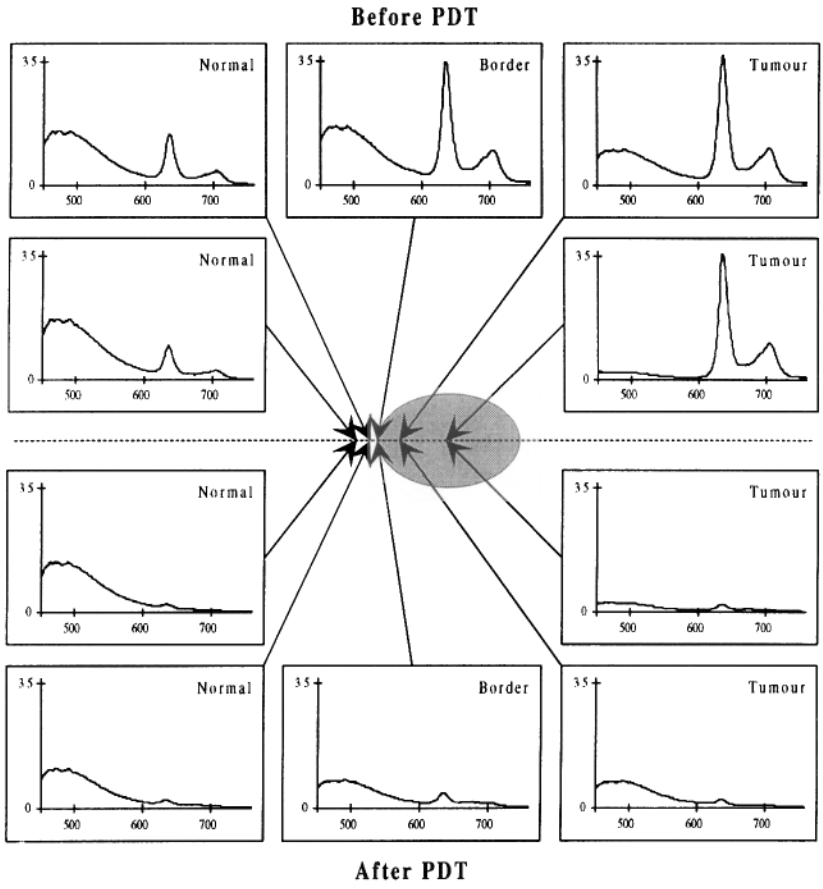

FIGURE 1. A point-monitoring fluorescence scan through a basal cell carcinoma and surrounding normal skin in a patient. The whole area had been topically treated with 20% ALA 6 hours before the investigation. The PpIX-related fluorescence, peaking at 635 and 700 nm, shows high signal within the tumor area and the border zone. A photo-induced PpIX bleaching is recorded after the laser treatment. The autofluorescence peaking at about 500 nm shows higher intensity in the normal skin as compared to the tumor.

important factor in predicting the ultimate prognosis for the patient is the depth of tumor penetration into the bladder wall. Often urinary bladder malignancies appear multifocally. One main appearance is the papillary tumor, which is easy to identify in the bladder wall. However, besides these easily recognized tumors, multifocal growth may also include flat noninvasive carcinomas *in situ*. These tumors may appear only as a reddening in the mucosa and can easily be mistaken for an inflammatory area. However, these tumors are aggressive and have a potential to grow through the bladder wall and develop into a life-threatening disease if untreated. Therefore, these lesions are of special interest from a diagnostic point of view, as they are hard to discover.

In a series of clinical studies, intravenous injection of low-dose hematoporphyrin derivative (HpD)[3] and more recently topical application of ALA as tumor-marking agents were used for the induction of porphyrin-related fluorescence.[4,5] Also the autofluorescence was utilized for tumor demarcation.[5] An example from Ref. 5 is given in FIGURE 2, in which an *in vivo* bladder scan is shown recorded from a patient 4 hours after the instillation of a 1% ALA solution. The evaluated ratios are also given for the corresponding points. Two spectra in the figure (14 and 15) were recorded from a suspicious area. As seen in the figure they both show a low red/blue fluorescence ratio, and the histopathology did not reveal any malignant or premalignant tissue. Detailed results from the study are presented in Ref. 5.

HEAD AND NECK CANCER

There are a lot of diagnostic problems in the detection of carcinomas in the head and neck region for which a tool for guiding the biopsy sampling would be of great value. In early glottic cancer it might be required to perform repeated diagnostic biopsy procedures which increases the risk of affecting the voice quality. Also in cases of early oral malignancy, which often is multifocal, it would be of importance to be able to direct the tissue sampling for histopathological examination. Low doses of ALA (5–15 mg/kg b.w.) were administered in patients with oral or laryngeal lesions. The ALA-induced PpIX fluorescence was detected as well as the autofluorescence. Kinetic studies were also performed, in which the build-up of the PpIX in different tissue types was investigated at different time intervals.[6] An example from an *in vivo* measurement from a patient who had been given 5 mg/kg b.w. of oral ALA 4 hours prior to the investigation is shown in FIGURE 3. The spectra shown in the figure were recorded from normal buccal mucosa and an area of the vocal cord with severe dysplasia. The premalignant area shows a very low autofluorescence as compared to the normal mucosa. On the other hand, the dysplasia exhibits the dual-peaked PpIX-related fluorescence. Detailed results from the study are under evaluation and will be presented elsewhere.[6]

DISCUSSION

All medical diagnostic procedures aim at tissue characterization, and in most cases biopsy sampling for a histopathological investigation is used to confirm the diagnoses. For early tumor identification and detection of premalignant tissue, randomly performed biopsy sampling is sometimes used as there may only be a subtle distinction in the tissue appearance, such as a slight reddening in the surface Optical characterization of tumor tissue has a great advantage of being noninvasive and providing real-time information. By developing LIF techniques as a guiding tool for biopsy sampling, precision in the procedure might be enhanced. With the full spectral information in the point monitoring, full spectral characterization is obtained, and the relevant wavelengths can be selected for imaging monitoring of larger tissue areas.

SUMMARY

Laser-based spectroscopic techniques were developed for tumor tissue characterization utilizing different tumor-localizing substances. In particular, sensitization

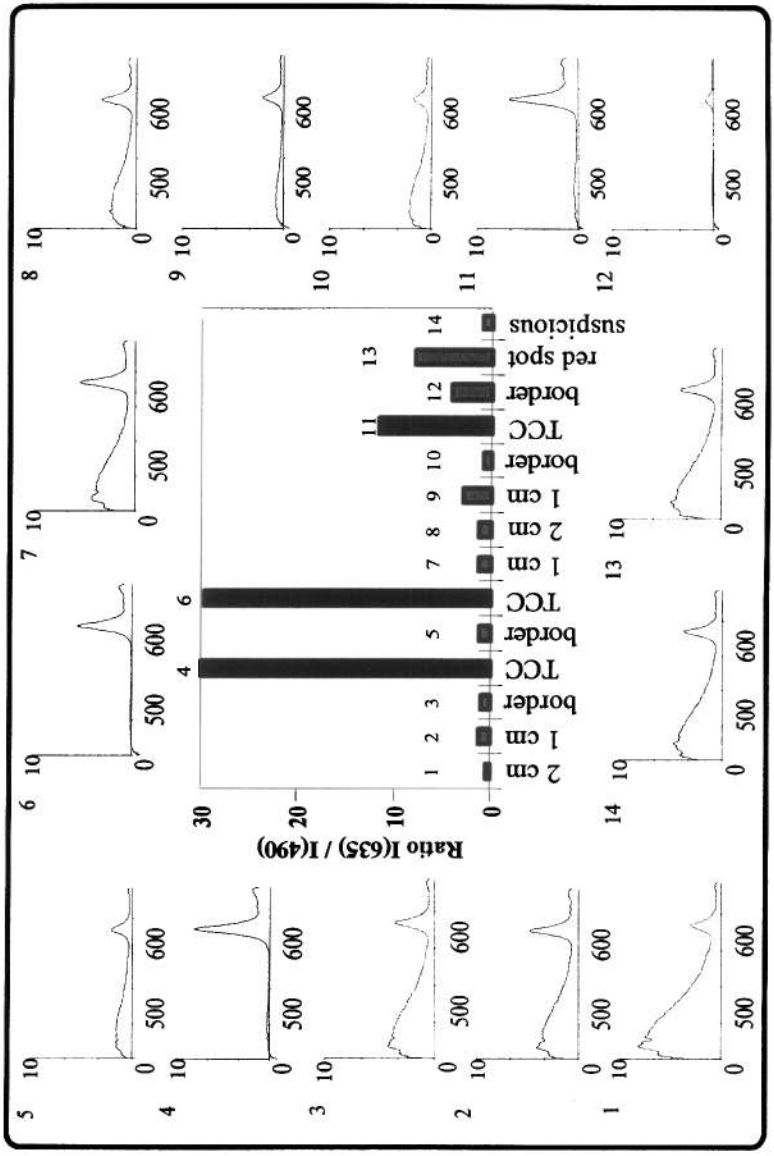

FIGURE 2. Fluorescence spectra and the corresponding histogram of the intensity ratio I (635 nm)/I (490 nm) recorded in a patient with transitional cell carcinoma lesions and normal mucosa. A solution of 1% ALA was instilled 4 hours *prior* to the investigation. The excitation wavelength was 405 nm. (From Rokahr *et al.*[5] Reprinted by permission from *SPIE Proceedings*.)

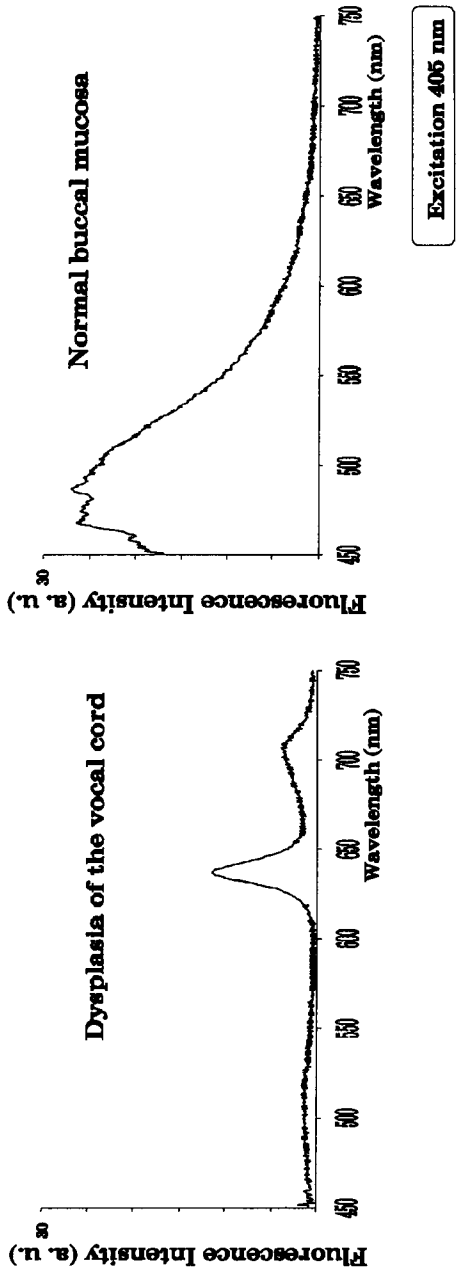

FIGURE 3. *In vivo* fluorescence recordings in a patient with severe dysplasia of the vocal cord (*left*). A fluorescence spectrum from normal buccal mucosa is also shown (*right*). The spectrum from the dysplastic mucosa is clearly dominated by the PpIX-related dual-peaked fluorescence, which is not at all seen in the spectrum from the normal buccal mucosa. On the other hand, the autofluorescence from the dysplasia shows very low intensity. The patient had been given ALA at a dose of 5 mg/kg b.w. orally 4 hours *prior* to the investigation. The excitation wavelength was 405 nm. (From Wang *et al.*[6])

with the heme precursor δ-amino levulinic acid (ALA) administered topically, orally or intravenously was used for the induction of protoporphyrin IX (PpIX). The autofluorescence as well as the PpIX-related fluorescence signals were monitored, and tumor demarcation functions were calculated for different human malignant tumors, such as tumors in the urinary bladder and the prostatic gland, in the head and neck region, in the breast and in the gastrointestinal tract. In the gastrointestinal tract, colon tumors were examined as well as tumors and dysplastic lesions in the esophagus, where patients with Barrett's esophagus were examined. Time-integrated laser-induced fluorescence measurements utilizing a point monitoring fluorosensor and a multicolor fluorescence imaging system were performed *in vivo* in patients in different clinical specialities.

REFERENCES

1. ANDERSSON-ENGELS, S., Å. ELNER, J. JOHANSSON, S.-E. KARLSSON, L. G. SALFORD, L. G. STRÖMBLAD, K. SVANBERG & S. SVANBERG. 1991. Clinical recordings of laser-induced fluorescence spectra for evaluation of tumour demarcation feasibility in selected clinical specialities. Lasers Med. Sci. **6:** 415.
2. SVANBERG, K., I. WANG, S. COLLEN, I. IDVALL, CH. INGVAR, R. RYDELL, D. JOCHAM, H. DIDDENS, S. BROWN, G. GREGORY, S. MONTÁN, S. ANDERSSON-ENGELS & S. SVANBERG. 1997. Clinical multi-colour fluorescence imaging of malignant tumours—initial experience. Acta Radiol. **38.** In press.
3. BAERT L., R. BERG, M. A. D'HALLEWIN, J. JOHANSSON, K. SVANBERG & S. SVANBERG. 1993. Localization of superficial bladder tumours using low-dose HpD and laser-induced fluorescence. Urology **41:** 322.
4. KRIEGMAYR, M., R. BAUMGARTNER, R. KNÜCHEL, H. STEPP, F. HOFSTÄDTER & A. HOFSTETTER. 1997. Detection of early bladder cancer by 5-aminolevulinic acid induced porphyrin fluorescence. J. Urol. **157:** 357–361.
5. ROKAHR I., S. ANDERSSON-ENGELS, S. SVANBERG, M. A. D'HALLEWIN, L. BAERT, I. WANG & K. SVANBERG. 1995. Optical detection of human urinary bladder carcinoma utilising tissue autofluorescence and protoporphyrin IX-induced fluorescence following low-dose ALA instillation. SPIE Proceeding BiOS'95. **2627:** 2.
6. WANG I., L. PAIS CLEMENTE, R. M. G. PRATAS, E. CARDOSO, M. PAIS CLEMENTE, S. MONTÁN, S. SVANBERG & K. SVANBERG. Fluorescence diagnostics and kinetic studies in malignant, premalignant and benign lesions in the head and neck area utilising δ-aminolevulinic acid sensitisation. To appear.