JOURNAL OF OPTOELECTRONICS AND ADVANCED MATERIALS Vol. 8, No. 1, February 2006, p. 396 - 399

Auto-fluorescence spectroscopy of colorectal carcinoma: *ex vivo* study

L. HORAK[a*], J. ZAVADIL[b], V. DUCHAC[a], S. JAVORSKY[b], F. KOSTKA[b], A. SVEC[c], D. LEZAL[d]
[a]*Surgical Clinic of the III[rd] Medical Faculty, Charles University, Prague, Srobarova 55, 100 00 Praha 10, Czech Republic*
[b]*Institute of Radio Engineering and Electronics, Academy of Sciences of the Czech Republic, Chaberská 57, 182 51 Praha 8, Czech Republic*
[c]*Institute of Pathology, of the III[rd] Medical Faculty of the Charles University Prague, Šrobárova 55, 100 00 Praha 10, Czech Republic*
[d]*Institute of Inorganic Chemistry, Academy of Sciences of the Czech Republic, 250 68 Rez u Prahy, Czech Republic*

Ex-vivo investigation of colorectal tissue by autofluorescence spectroscopy is reported. Wet microscopic mounts of healthy colon mucosa were examined and compared with those prepared from colon mucosa affected by adenocarcinoma. The diagnosis of adenocarcinoma was verified by using clinical and histology means. Fluorescence spectra of tissue samples, excited by blue emitting semiconductor diode have been studied by using portable spectrometer with fixed grating and fibre optic system compatible with endoscope. The study demonstrates differences in both the spectral shape and in the signal intensity in photoluminescence spectra emitted from tissue affected by adenocarcinoma as compared to that of healthy colon mucosa. The overall decrease and the shift of autofluorescence intensity to longer wavelengths have been observed for malignant tissue samples.

(Received after revision January 24, 2006; accepted January 26, 2006)

Keywords: Autofluorescence spectroscopy, Carcinoma

1. Introduction

A goal of painless, rapid diagnostics using photonics is desirable and has now reached the stage where it can be a clinical reality. The benefits of using optics directly for biopsies are clearly preferable to the conventional approach. The use of fibre optics further allows such techniques to be accomplished within interior regions of the body. Optics in these measurements require light sources, coupling optics and analysis with efficient detectors. The endoscopic techniques Nd:YAG laser and argon plasmatic coagular, were studied and compared in [1].

Colorectal carcinoma is supposed to develop in premalignant lesions following a sequence of events, based on genetic and environmental factors, which result in defects of mucosa that can evolve from flat dysplasia to adenomatous polyp and finally to carcinoma [2,3]. Thus the alterations due to the neoplastic transformation will result in changes of some optical characteristics of transformed cells. Hence, the scattering, absorption or emission of light can yield information about the presence of transformed cells. The ability to detect subtle lesions still confined to the mucosa would then permit their cure by either endoscopic ablation or minimally invasive surgery.

It is well recognized that conventional endoscopy, using white light, does not detect early stages of dysplasia and this subtle lesions may be missed. Thus, there is a strong motivation to develop and test new optical systems compatible with endoscope that would complement white light endoscopy. The fluorescence spectroscopy provides a unique and sensitive tool to reveal changes in the physical and chemical properties that occur in healthy and abnormal cells in tissues. There are well known intrinsic fluorophors bound to proteins within cells that fluoresce in a wide visible and near infrared spectral regions, after being excited by suitable external light source. The return of excited molecules of the tissue to their equilibrium state is accompanied by emission of corresponding optical radiation. The energy or wavelength of emitted photons roughly corresponds to the energy difference between excited and equilibrium states of the tissue molecular system. The emitted spectral shape is sensitive to the microenvironment such as pH level, redox potential, binding sites, polarity, ion concentration etc. Natural fluorophores within cells emit luminescence - so called autofluorescence (AF) - and display a unique set of spectral features which characterise the state of cells making up the tissues. Depending on the environment, flavins and porphyrins are known to fluoresce in 510-560 nm and 630-690 nm regions, respectively. The experiments proved the higher concentration of porphyrins in malignant tissues in comparison with that in the normal ones. The excited porphyrins produce the fluorescence in red part of the spectrum [4,5]. Tissues also contain molecules called chromophores that absorb light without re-emission of fluorescence. The main chlorophore in gastrointestinal tissues in the visible wavelength (400 – 700 nm) range is hemoglobin.

AF spectroscopy, sometimes called optical biopsy, consists in recording an autofluorescence spectrum from a small area of tissue of interest excited with a narrow beam of exciting light. Thus the optical biopsy could be viewed as point measurement technique as compared to imaging. The fluorescence point spectroscopy [6-8] involves the use of a contact optical fibre probe that delivers excitation light to the tissue surface and collects the resulting fluorescence light. Typically, a central delivery fiber illuminates the tissue, while the surrounding circular array of fibres collects the emitted fluorescence. Optical filters block the detection of scattered excitation light. The fiber bundle probe, typically 1-2 mm in diameter, is delivered via the biopsy channel of the endoscope and placed in contact with the tissue surface. Fluorescence light is separated into component colours by a spectrometer and displayed as a fluorescence intensity versus wavelength curve.

A quantitative analysis detects intensity changes of fluorescence. A number of reports [9] relay on the observation that under the same excitation and collection geometry the fluorescence intensity of lesions is almost always lower than that of the surrounding normal tissue. Consequently most of the imaging techniques concentrate on the proper calibration technique to correct the effects of AF signal caused by the varying illumination/collection geometry across the imaged tissue surface. We believe that quantitative analysis, in general, is not as reliable as the analysis and understanding of spectral changes seen in the AF due to precancerous and cancerous changes in the tissue. To be able to detect small changes in the spectral shape we need not only sensitive photoluminescence (PL) spectrometer but also optimal transfer of both exciting and fluorescence radiation to the tissue and the spectrometer, respectively.

Most of measuring systems previously described in the literature for spectroscopic diagnosis of colorectal tissue, both *ex-vivo* and *in-vivo*, utilised lasers [10-15] or filtered lamps [16] as the light source.

In this paper we report *ex-vivo* investigation of colorectal tissue by measuring the AF emission in the range 550-800 nm excited by spontaneous semiconductor blue Light Emitting Diode (LED) emitting at 450 nm.

2. Experimental

The experimental set-up used to collect autofluorescence spectra of colorectal tissue is schematically shown in Fig. 1. Fluorescence was induced by light source equipped with blue emitting semiconductor diode and analysed by portable Avantes spectrometer *AvaSpec-2048* with fixed grating, fibre connector and CCD linear array detector with 2,048 elements. A specially designed filter holder has been placed in front of the spectrometer to block the reflected and scattered exciting light from entering the spectrometer. High transmission interference long-pass (LP) filters were used to take advantage of their very narrow transition edge. Various filters were tested and the most suitable have been found the Spectrogon 530 and MIT 500 nm. The filter holder also enables proper optical coupling to fibre connectors of both the fibre system and the spectrometer. Fibre-optic system with three terminals has been prepared and used for excitation and collection of AF signal. Polymer optical fibres with the diameter in the range 500 – 1000 μm were used. Optical connectors were placed at two ends connected to the light source and the spectrometer, while the third one was in a form of a special probe tip. Various types of fibre systems, schematically shown in Fig. 2, were tested. Reported AF spectra were measured by using the two-fibre system. The spectrometer is controlled and data collected by a notebook via the USB port.

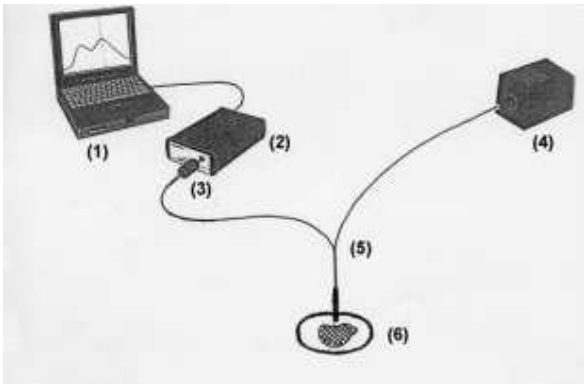

Fig. 1. Optical system used for the excitation and collection of AF spectra. (1) notebook for the control of the spectrometer and collection of measured data; (2) spectrometer AvaSpec-2048; (3) specially designed housing for LP filter and coupling optics between the fibre and spectrometer input connectors; (4) excitation light source; (5) fibre optic system with three terminals; (6) the measured tissue sample.

Photoluminescence spectrum of the light source used for tissue excitation and spectrum of the same source scattered on inert material and filtered by LP filter 530 nm are shown in Fig. 3 by curves (a) and (b), respectively. A detailed inspection of the diode spectrum (curve (a)) at 700 nm revealed that a very weak luminescence band at this wavelength is inherent to the light source used. The bands marked by arrows (1) and (2) are due to "filtering" effect and thus similar bands observed in tissue AF spectra should be excluded from consideration. It follows from Fig. 3 that only spectra in the range 560—800 nm bear relevant information in case of spontaneous semiconductor diode used. This disadvantage, caused by a broad emission band of LED source as compared to a laser diode, is partially compensated by its low price.

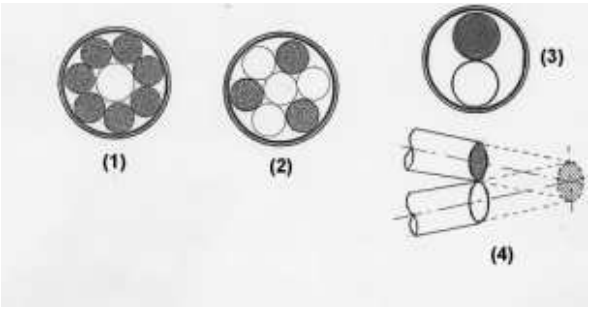

Fig. 2. Cross sections of probe tips of various fibre optic systems used for AF experiments. Fibres with shadowed cross sections are used for collection of emitted radiation. (1) bundle consisting of 8 fibres with the central one used for excitation; (2) a 7 fibre system with four fibres used for excitation and three for collection; (3) a two fibre system; (4) angle geometry of the 2 fibre system that enhances the collection efficiency.

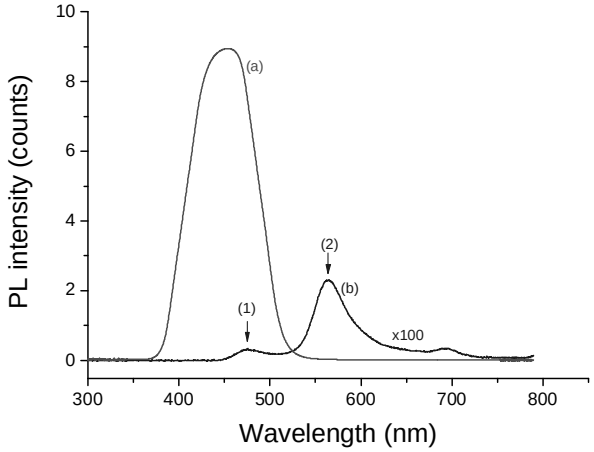

Fig. 3. Fotoluminescence spectrum of the blue Light Emitting Diode (LED) used for tissue excitation is shown by curve (a). The spectrum of the same LED scattered on optically neutral material and filtered by LP filter 530 nm is shown by curve (b). The arrows (1) and (2) are due to filtering and correspond to the arrows in Fig. 4.

Autofluorescence spectra emitted by normal colon mucosa and other tissue components have been compared with those emitted by tissue affected by colorectal carcinoma. The probes were taken during radical surgeries performed in order to treat the colorectal carcinoma. The wet probes were taken from tumour centre and from oral edge of the cut piece that should have consisted of healthy mucosa. Native samples were cooled and examined immediately after the surgical operation. First of all, both the histology of healthy mucosa and histology of adenocarcinoma were examined by using quick biopsy. The final histological examination followed.

We did not intend to investigate statistically meaningful set of tissue samples but rather to demonstrate that relatively cheap light source, equipped with LED, could be used for detection of AF spectral changes, i.e. the changes in the intrinsic fluorescence of the tissue layers with disease due to alterations in their biochemical composition.

3. Results and discussion

Only thick samples (of the order of 1-2 mm) turned out to be useful in order to supply a measurable luminescence signal. Thin samples routinely used for optical histology analysis could not be measured even on highly sensitive laboratory PL spectrometer [15].

Several samples of normal and malignant tissues have been measured. Two sets of typical samples with spectra measured *ex-vivo* are shown in Fig. 4. As stated above, the two bands described by arrows (1) and (2) are due to convolution of spectral dependences of the used LP filter with measured AF signal. The curves (a), (b), (c) and (d), (e), (f) in Fig. 4, correspond to the emission from normal and malignant tissues, respectively. It follows from all measurements that AF intensity from malignant tissues is decreased as compared to the normal one and relative contributions of major AF bands are also changed – thus changing the spectral shape. Major contributions to the tissue AF appear in three bands around 560, 620 and 690 nm. Spectra taken from the healthy parts are dominated by fluorescence band around 560 nm, while spectra collected from malignant portions are dominated by bands at 620 and 690 nm. Also intensity ratios of observed fluorescence bands differ for normal and malignant portions. This observation corresponds to the fact that excited porphyrins produce the fluorescence in red part of the spectra and consequently the red bands (620 and 690 nm) are enhanced in spectra collected from malignant parts. The inherent difficulty with LED as an excitation source comes from the fact that the long-wavelength tail of LED overlaps with the investigated AF signal.

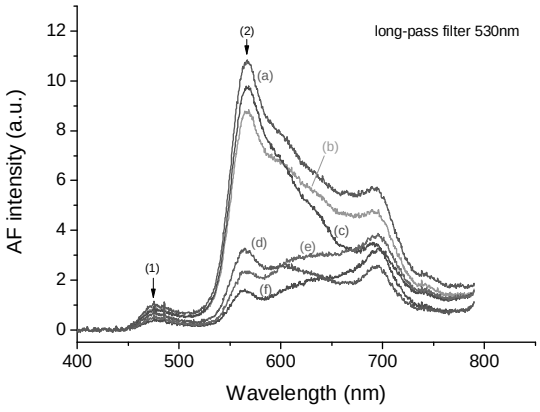

Fig. 4. AF spectra of native colon tissue excited by blue LED. Curves (a), (b) and (c) correspond to normal tissue, while curves (d), (e) and (f) correspond to the malignant one. Both the decrease of overall intensity and the relative increase in bands at 620 and 690 nm is observed in malignant samples. Band (1) marked by arrow corresponds to maximum LED intensity passed through the LP filter and band (2) is an AF band distorted due to convolution with LP filter.

We can conclude that in most observed samples the alterations in biochemical composition due to disease proliferation is manifested by overall decrease in AF intensity accompanied by relative decrease of intensity in the band around 560 nm, and by the relative increase in intensity in the bands around 620 and 690 nm.

4. Conclusions

It has been shown that the portable set-up that employs LED as an excitation source enables to measure AF spectral changes induced by microscopic abnormalities in tissues of the colon and rectum. The overall decrease and the shift of AF intensity to longer wavelengths have been observed for most of malignant tissue samples. This red shift, together with the changed intensity ratio of observed AF bands could serve as an indication of malignant changes in the tissue.

More experiments and careful comparison of luminescence spectra with optical histological examinations of samples with different compositions are needed to understand changes in the tissue that are manifested by considerable decrease of AF intensity (when going from healthy to tumour tissue) without significant change in the spectral dependence, observed in same samples. On the other hand, the observed spectral differences are attributed to the transformation of local environment surrounding the fluorophors assigned to flavins and porphyrins in the normal and cancerous tissues. However, conditions when cancerous and healthy tissues yield different AF spectra and when AF signals differ only by intensity, remains to be clarified.

Fibre optic system has been used for both the excitation and collection of AF signal, so that endoscopic *in-vivo* tests could also be attempted. The endoscopic *in-vivo* system would improve the surgical treatment of rectal carcinoma due to potential precise determination of resection line. Further work is needed to be able to detect the malignant colon tissue and differentiate it from the healthy colon tissue by using AF spectroscopy coupled to endoscope.

Acknowledgement

The cooperation with P. Gladkov, I. Kasik and M. Fruhauf is greatly appreciated. The work was supported by the Grant Agency IGA MH CR, grant No. ND/7581-3.

References

[1] L. Horák, R. Švek, R. Grill, V. Duchàč, V. Štukavec, J. Optoelectron. Adv. Mater. **7**(6), 3219 (2005).
[2] T. Mutto, H. J. R. Bussey, B. C. Morson, Cancer **36**, 2251 (1975).
[3] B. C. Morson, H. J. R. Bussey, Br. J. Surg. **72**, 23 (1985).
[4] G. Bottiroli, A. C. Croce, D. Locatelli et al., Lasers in Surgery and Medicine **16**, 48 (1995).
[5] J. Nakhosteen, Prog Respir Res basel, Karger, , **30**, 236 (2000).
[6] B. W. Chwirot, S. Chwirot, Optica Applicata **32**, 624 (2002), and references there in.
[7] M. A. Mycek, B. W. Pogue, Handbook of Biomedical Fluorescence, (Marcel Dekker, Inc. New York), 2003, and references there in.
[8] M. A. Mycek, P. Urayama, W. Zhonget al, Diagnostic Optical Spectroscopy in Biomedicine II, Proceedings of SPIE-OSA Biomedical Optics, SPIE **5141**, 70 (2003).
[9] J. Y.Qu, J. Hua, Appl. Phys. Lett. **78**, 4040 (2001).
[10] K. T. Schomacker, J. K. Frisoli, C. C. Compton, T. J. Flotte, J. M. Richter, N. S. Nishioka, T. F. Deutsch, Lasers Surg. Med. **12**, 63 (1992).
[11] K. T. Schomacker, J. K. Frisoli, C. C. Compton, T. J. Flotte, J. M. Richter, T. F. Deutsch, N. S. Nishioka, Gastroenterology **102**, 1155 (1992).
[12] M. A. Mycek, K. T. Schomacker, N. S. Nishioka, Gastrointest. Endosc. **48**, 390 (1998).
[13] G. S. Fiarman, M. H. Nathanson, A. B. West, L. I. Decelbaum, L. Kelly, C. R. Kapadia, Dig. Dis. Sci. **40**, 1261 (1995).
[14] R. M. Cothren, M. V. Sivak, J. van dam, R. E. Petras, M. Fitzmaurice, J. M. Crawford, J. Wu, J. F. Brennan, R. P. Rava, R. Manoharan, M. S. Feld, Gastrintest. Endosc. **44**, 168 (1996) .
[15] J. Zavadil, D. Lezal, L. Horak, V. Duchac, M. Prochazka, J. Optoelectron. Adv. Mater. **7**, 1023 (2005).
[16] B. Mayinger, M. Jordán, P. Horner, C. Berlách, S. Muehldorfer, B. R. Bittorf, K. E. Matzel, W. Hohenberger, E. G. Hahn, K. Guenther, J. Photochemistry and Photobiology B: Biology **70**, 13 (2003).

*Corresponding author: lad.horak@volny.cz