Optica Applicata, Vol. XXXII, No. 4, 2002

Fluorescence detection of human tumours without photosensitisers

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Autofluorescence detection of human cancer has made rapid progress with several groups reporting very promising data for the detection of premalignant and malignant lesions in several locations. The coming years will most probably see a transfer of the autofluorescence methods to clinical practice, especially if the research and development of new instruments will be supported by fundamental studies on the origin of the autofluorescence. Controlled multicentre trials and outcome analysis studies are still necessary to prove the benefits but the results obtained up to now have been very optimistic

1. Introduction

Cancer can be considered a price paid by multicellular organisms for their extreme complexity. Cancer is not a civilisation disease, although an increase in the incidence has been brought by a progress of our civilisation. Bone tumours were found in fossils, for instance on a skeleton of a dinosaur, in a 1,500,000 year old human jaw or on a skeleton of a Neolithic man [1]. Tumours were mentioned in several historical sources. Different tumours and methods for treatment were described in a papyrus document as old as 1,500 B.C. [1]. On the other hand, the increase in the incidence of cancer is no doubt related to the increase in the average age of a population. In most cases cancer develops slowly and most commonly arise in older people because of time needed for multiple required mutations to accumulate in a single cell. For instance, in man it may take over 20 years for tumours to develop after exposure to industrial carcinogen [2]. The average age at the time of a diagnosis is for all the tumours approximately 67 years [3]. Therefore, it is thought that in the future the average risk of developing cancer will rise due to the increase of a life expectancy of human population.

Last century was a period of an unprecedented effort in cancer research. However, with all the information and knowledge gathered up to now the main

questions still remain unanswered and no or little success has been achieved in reducing mortality caused by most cancers. Nowadays cancer is seen as a group of diseases characterised by uncontrolled cellular proliferation. With a progress in molecular cell biology it has become clear that a network of mechanisms driving and controlling cellular proliferation, functioning and death is extremely complex and sophisticated. All those mechanisms are crucial for a normal development and functioning of any multicellular organism and it is them that are perturbed in cancer cells.

Thus, despite all the progress in cancer research, the lines of defence against cancer have not changed and are the same as many years ago. The best form of reducing cancer related mortality remains a prevention and the next best is early detection. Unfortunately, because of the complexity of processes of carcinogenesis even those two approaches have a limited efficacy. It is now clear that carcinogenesis is a multistep process, can be initiated by many different factors and is to a large extent of a stochastic nature. Therefore, on a mass scale the prevention means in practice initiatives like smoking control, recommendations on dietary habits or on avoiding excessive exposure to solar ultraviolet light.

Also, a screening and early detection of cancer are still very limited. Biological and chemical methods used for the screening have a limited specificity due to a fact that typically the tumour cells do not express genes specific to the disease. The altered cells are usually characterised by shifted levels of the expression of some factors and receptors involved in a control of cellular growth. Such changes are extremely difficult to detect. On the other hand, physical methods based on absorption, scattering or emission of all kinds of radiation have a sensitivity too low to allow for a sufficiently early detection of tumour cells. Clinically detectable tumour of a diameter of 5 mm already consists of about $10^8 - 10^9$ cells. Tumours of a diameter $2-3$ mm can already be able to stimulate angiogenesis. At the stage of the clinical detection some of the cells may already be able to invade the surrounding tissue and to metastasise to other organs of a body. Thus, all around the world there has been a strong, continuous multidisciplinary effort to develop new approaches and methods allowing for efficient detecting of human cancers at possibly early stages of their development.

In this paper, we present a relatively new group of techniques of detecting premalignant and malignant lesions using endogenous fluorescence – autofluorescence of cells and tissues. The first two papers presenting a feasibility of such an approach were published in 1987 [4], [5]. In 1990's there has been an increasing interest in developing autofluorescence techniques for detecting neoplastic tissues in all organs of human body accessible to such examinations. We should like to stress here that it is not our intention to review all the work done in the field during last fifteen years but rather to present the different approaches and to illustrate them with selected examples reflecting the development and current status of the field.

2. Rationale

Carcinogenesis is a multistep process driven by the accumulation of mutations resulting in errors in key regulatory mechanisms. Due to those errors the altered cells, initially premalignant, then malignant acquire new characteristics that give them advantage over the neighbours and allow for uncontrolled proliferation. Therefore, it is obvious that both a metabolism and a structure of such cells are different than of the cells of a tissue of their origin. All the molecules constituting a cell can interact with light and their optical properties are determined by both their nature and by properties of their microenvironment. One can thus reasonably expect that the alterations due to the neoplastic transformation will result in changes of some optical characteristis of the transformed cell. Hence, scattering, absorption or emission of light can yield information about a presence of transformed cells. In fact, this is just such phenomena that enable clinical diagnosis based on direct or endoscopic visual examinations.

Fluorescence techniques are well known for their sensitivity. Two main factors contribute most to the senstivity: firstly, fluorescence can be detected at conditions of a very low background and secondly, the detection can be optimised by a proper selection of the excitation and detection wavelengths. Using ultraviolet or visible light it is possible to excite the fluorescence of many biomolecules. At the same time, it can be expected that the neoplastic transformation results in either a production of a new fluorophore or in a change of relative concentration of fluorophores existing in normal cells. The main idea of the fluorescence methods of tumor detection consists in a determination of such characteristics of the autofluorescence of the transformed cells that might allow for a differentiation between such cells and the host cells from their neighbourhood. The reason why it is so difficult to implement that simple idea in practice is the extreme complexity and the variability of living systems at every level of their organisation. Moreover, the natural variability is additionally entangled with changes due to age, history of diseases and treatments of each individual. For such reasons the real progress in the field of fluorescence detection of cancerous tissues is relatively slow in spite of very promising results reported at the early stages of the research.

3. Methods

3.1. Optical biopsy

Optical biopsy was a method of choice at early stages of the research on the fluorescence detection of tumours. The method, deeply rooted in a traditional molecular spectroscopy, consists in recording a spectrum of the autofluorescence of a small area of a tissue of interest excited with a narrow beam of exciting light (Figs. 1 and 2**a**–**c**). It was hoped that it should be possible to select such conditions of the excitation that the normal and the neoplastic cells would emit the autofluorescence of different spectra. First results seemed to confirm the expec-

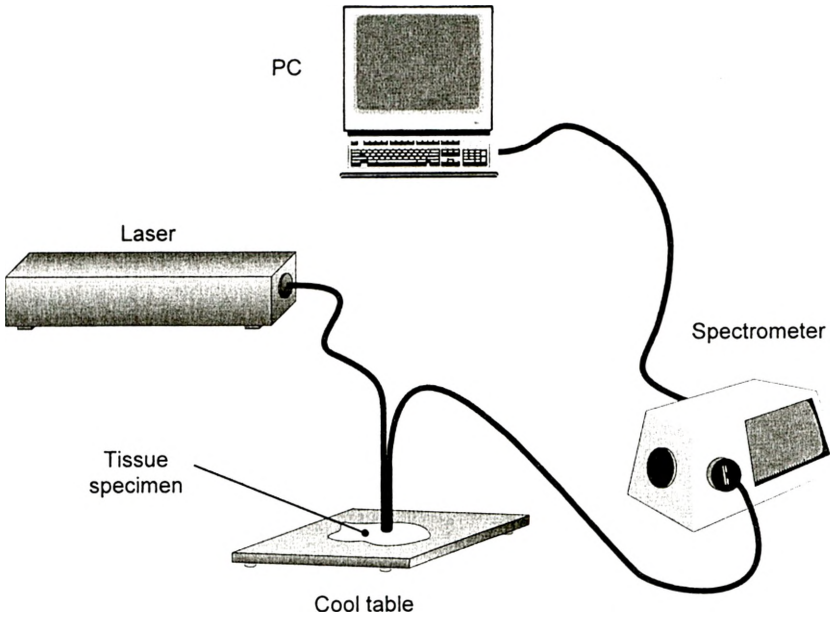

Fig. 1. Schematic representation of a typical set-up used for *ex vivo* optical biopsy experiments.

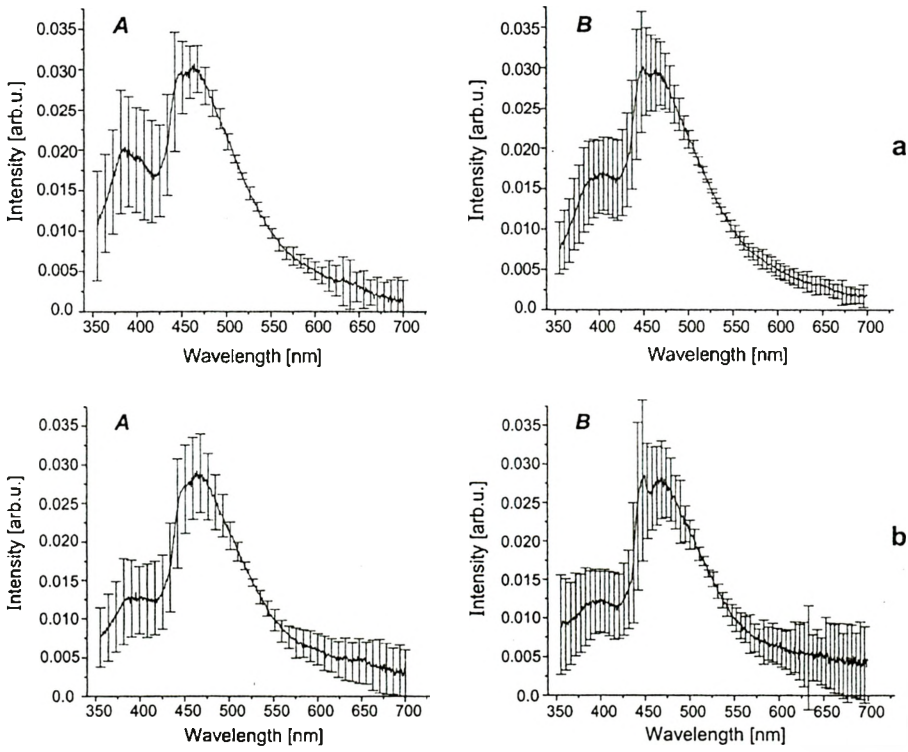

Fig. 2a,b

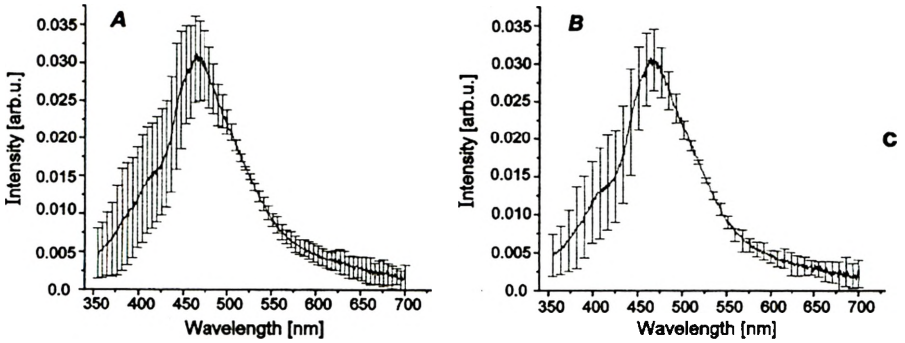

Fig. 2. Examples of the averaged spectra of the autofluorescence excited with 325 nm line of He-Cd laser obtained for normal mucosa and cancerous lesions in human colon in two separate studies A and B (n — number of cases): **a** — normal mucosa (A: $n = 48$, B: $n = 36$), **b** — adenocarcinoma (A: $n = 55$, B: $n = 46$), **c** — adenoma (A: $n = 12$, B: $n = 8$).

tations. ALFANO *et al.* [4] reported clearly distinct patterns of maxima and minima in the autofluorescence spectra of normal and cancerous tissues of human breast and lung examined *ex vivo*. Similarly promising results were obtained by YAKSHE *et al.* [6]. However, with a growing number of data on the autofluorescence spectra obtained from different patients it became clear that the differences were of a more subtle character. The spectra showed also a dependence on experimental factors such as intensity of the exciting radiation (effective depth of the excitation) and geometry of the excitation and detection of the autofluorescence (for instance, single or separated excitation/detection fibres *etc.*). Several approaches were used to correct for such factors and to ensure a sensitive and right differentiation between normal and diseased tissues. KAPADIA *et al.* [7] elaborated a diagnostic algorithm resulting in the so-called LIF score (quantitative laser-induced fluorescence score) calculated as a weighted sum of contributions of six selected emission bands to the total emission intensity. The six spectral bands were selected on a basis of a statistical analysis of the spectra obtained in a study of the training set of specimens of colonic tissues of well-known histological characteristics. A similar approach was adopted by SCHOMACKER *et al.* [8], [9]. Those authors, however, fitted the autofluorescence spectra with the *in vitro* emission spectra of three potential fluorophores (crystalline collagen, crystalline nicotinamide adenine dinucleotide (NADH), oxidised flavin adenine dinucleotide (FAD)) and with the absorption spectrum of hemoglobin and calculated the LIF score from contributions of the four components. At the same year Alfano's group [10] demonstrated that it is possible to differentiate between the normal and tumour tissues using a much simpler algorithm based on ratios of the intensity of the autofluorescence in adequately selected spectral bands. All the diagnostic algorithms discussed above were used in different forms by different research groups but it seems that the fluorescence ratioing is best suited for practical applications and ensures a highest reproducibility of the results.

It should also be noted that in the case of the optical biopsy techniques, similarly to classical biopsies, it is the examiner who decides on a selection of suspicious areas subject to the fluorescence examination. Hence, the optical biopsy may assist in assessing the character of the lesions of interest facilitating the correct decision on a treatment.

3.2. Autofluorescence imaging

There is a fundamental difference between the autofluorescence imaging (Figs. 3 and 4 **a**,**b**) and the optical biopsy. The examined areas have typically diameters on the order of centimetres rather than millimetres as in the case of the optical biopsy approach. The main goal of the imaging approach is rather to label the

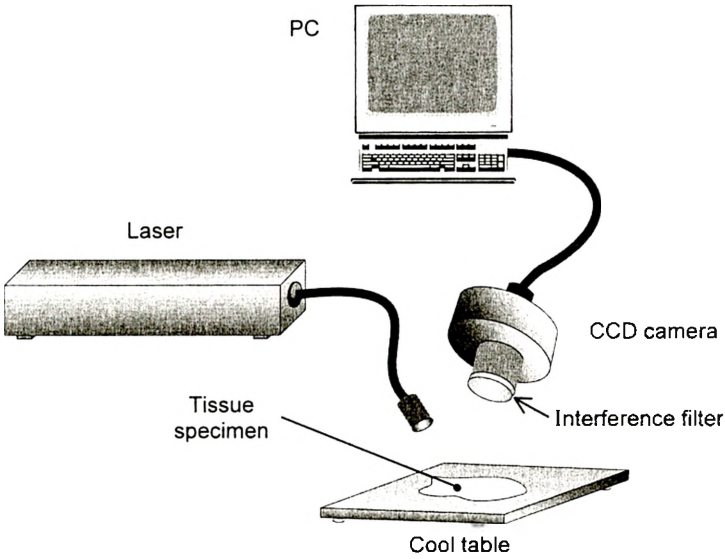

Fig. 3. Schematic representation of a typical set-up used for *ex vivo* autofluorescence imaging experiments.

places of a supposedly neoplastic nature within the examined area and to guide to them the examiner. That does not exclude of course a high positive predictive value of the autofluorescence imaging algorithms but it is a high sensitivity towards neoplastic lesions that is of primary importance. The autofluorescence imaging approach is therefore better suited for screening applications and gives a better chance of enhancing a sensitivity of the detection of the premalignant and malignant lesions. The optical biopsy methods can help in optimising the diagnostic algorithms based on the autofluorescence imaging by providing the information useful for a determination of the best excitation and imaging conditions (spectral bands, intensity of the exciting radiation). It is also possible to image several times the autofluorescence of the same region using different excitation and imaging bands. If only one excitation wavelength is used but images

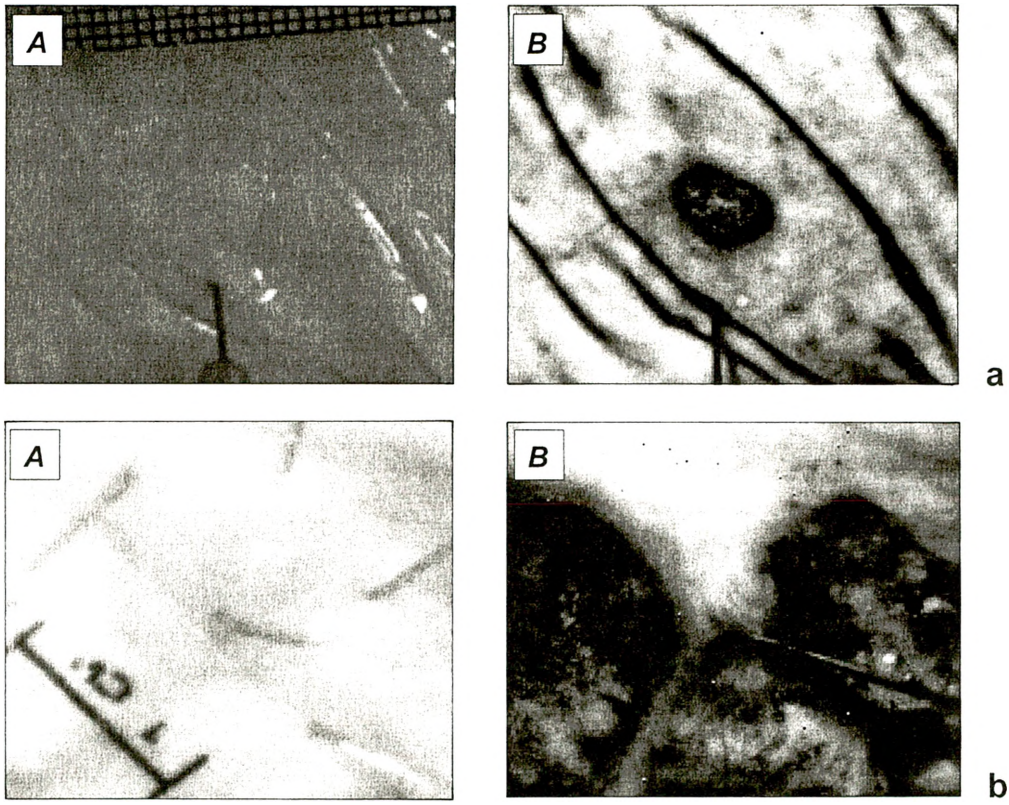

Fig. 4. Examples of the images of the autofluorescence of human colonic tissues excited with 325 nm line of He-Cd laser: **a** – tubular adenoma with dysplasia of II° (A – white light illumination, B – autofluorescence imaged in 440 nm band), **b** – tubular adenoma (A – white light illumination, B – autofluorescence imaged in 475 nm band).

are recorded photometrically in different spectral bands then it is possible to calculate the intensity ratios similarly as in the optical biopsy approach. The application of fluorescence imaging to lung cancer detection was described as early as in 1989 by HIRANO *et al.* [11]. Those authors, however, studied the fluorescence of an exogenously administered photosensitiser. Two years later PALCIC *et al.* [12] used a diagnostic approach based on a ratio of two spectrally resolved images of the autofluorescence excited with the 442 nm line of He-Cd laser and were able to detect the premalignant and malignant lesions in a bronchial tree with both a sensitivity and a specificity better than in the case of a classical white-light bronchoscopy or of a photosensitised fluorescence bronchoscopy. Other algorithms used for a detection of neoplastic regions in the autofluorescence images involve the analysis of a spatial distribution of the intensity of a total- [13] and of a spectrally resolved autofluorescence [14]–[17] or the analysis of the intensity ratios for images obtained with and without using a photosensitiser [18]. Images of the autofluorescence are

typically recorded with CCD cameras but other systems are also used like, for instance, the flying-spot scanner described in paper [19].

3.3. Time-resolved autofluorescence spectroscopy

There are only a few reports (see, for instance, [20]) on studies attempting to use a temporal decay analysis to differentiate between the normal and neoplastic tissues. Differences in a decay kinetics may result from differences in a composition of fluorophores present in the two classes of the tissues or from a change of a molecular microenvironment influencing, for instance, kinetics of quenching processes. One can also expect different kinetics of the fluorescence decay if the neoplastic tissues are labelled with photosensitiser molecules of a decay constant different from that of the natural autofluorescence. WAGNIERES *et al.* [21] described recently a very interesting experimental system for the time-resolved studies of the autofluorescence.

4. Autofluorescence detection of tumour tissues at specific locations in the body

Practically all organs of a human body accessible to the fluorescence examinations have been studied up to now from a point of view of detecting the premalignant and malignant lesions.

4.1. Gastrointestinal tract

Tumours of the gastrointestinal tract are a major health problem worldwide. Mortality attributed to those cancers is second only to a lung cancer. Early studies on a fluorescence detection of malignant lesions in the human stomach, colon and rectum were carried out using the optical biopsy approach.

4.1.1. Stomach

First studies on a differentiating between the neoplastic and normal stomach tissues were carried out by YUANLONG *et al.* [5] who suggested that the malignant transformation in the human stomach results in the occurrence of new emission maxima associated with increased concentration of porphyrin compounds in the neoplastic cells. Other studies did not confirm the hypothesis and now it is believed that the porphyrin emission is most probably due to the presence of some bacteria. In 1997 CHWIROT *et al.* [15] suggested that tumour tissues can be detected in the human stomach by imaging in the three spectral bands (395, 440 and 590 nm) the autofluorescence excited with 325 nm line of He-Cd laser. The sensitivity of such an approach applied *ex vivo* to macroscopic specimens removed during surgical operations was 96%. The method has still to be tested *in vivo* in gastroscopic fluorescence examinations of patients suspected of stomach cancer. KOBAYASHI *et al.* [16] achieved similar sensitivity *in vivo* using imaging and diagnostic algorithm based on the analysis of color patterns of the autofluorescence.

4.1.2 Colon and rectum

Several groups have tried to develop the fluorescence methods for detecting the neoplastic lesions in the human colon and rectum since early 1990's. All the early attempts applied different variants of the optical biopsy approach with continuous and pulsed, ultraviolet and violet light used for the excitation of the autofluorescence [7], [8], [22]–[25]. First preliminary results obtained by the digital imaging of a total autofluorescence of adenomatous polyps in tissue samples obtained from three patients were published in [13]. CHWIROT *et al.* [17], [25] carried out systematic *ex vivo* investigations of a diagnostic potential of the autofluorescence imaging for a detection of the neoplastic lesions. The authors recorded the autofluorescence images in 6 spectral bands and were able to correctly classify 23 of 26 polyps selecting the suspected areas as emitting with the intensity lower than normal mucosa. Similar sensitivity was achieved *in vivo* by BRAND *et al.* [26] who recorded the images of the autofluorescence with the lung imaging fluorescence endoscope (LIFE) system, originally developed for the autofluorescence detection of cancerous tissues in a bronchial tree [12]. WANG *et al.* [27] reported that using the digital imaging of the total visible autofluorescence and a simple diagnostic algorithm also based on the fluorescence threshold, they were able to identify colonic dysplasia in vivo with a sensitivity of 83%.

4.2. Lung and upper aerodigestive tract

Lung cancer is the most common cause of cancer related deaths and cancers of the upper aerodigestive tract are also a serious health problem. For many years there has been no significant progress in therapeutic treatment of those cancers and thus there has been increasing interest in the early detection. Promising results were obtained in 1980's by groups working on the early detection of the bronchial neoplastic lesions by the digital imaging of the malignant lesions labelled with exogeneously applied photosensitiser (see, for instance, [28]). However, the photosensitising drugs render the skin of patients sensitive to light. Studies on a determination of a minimum dose required for the succesful imaging and detection of the cancerous lesions yielded a surprising result. It was shown that the neoplastic areas could be detected with high efficacy without administering the photosensitising drugs using a ratio fluorometer and a diagnostic algorithm involving a determination of the ratio of the intensities of a "green" and "red" fluorescence [29]. A new method to image early lung cancer was then developed and the LIFE system [12] became the first commercially available instrument for the autofluorescence detection of human cancer. The autofluorescence was excited with 442 nm line of a He-Cd laser. The images were recorded in two spectral bands analysed in real time. The diagnostic algorithm incorporated ratioing both to enhance differences in the autofluorescence emission in the selected bands and to correct for effects of the excitation and detection geometry and for reflective properties of the tissues.

Clinical tests of the LIFE system confirmed that the sensitivity of the autofluorescence tumour detection was higher than of the classical white-light bronchoscopy [30]–[34].

BETZ *et al.* [35] reported that mucosal neoplasias of the oral cavity could be detected with a high sensitivity by imaging of a green autofluorescence excited with a violet light. QU *et al.* [36] described a more sophisticated system for detecting nasopharyngeal malignancies. The authors used both the imaging and the analysis of the spectra obtaining a sensitivity of 98% and a specificity of 95%. Other authors using different excitation and detection schemes [37]–[40] also reported similarly high sensitivities of the autofluorescence detection of cancers in the head and neck.

4.3. Bladder

Cancers of the bladder are the fifth cause of cancer related deaths of men in Poland [41]. In the United States it is the fourth most common cancer in men and the eighth in women [42]. Both the precancerous lesions and the early cancers are often invisible or very difficult to detect visually. To overcome those problems several groups have been working on elaborating new methods enhancing the sensitivity of the detection of dysplasias, carcinomas *in situ* and small papillary tumours of the bladder *in vivo*. In 1994 D'HALLEVIN *et al.* [43] demonstrated a potential of the optical biopsy approach for detecting the neoplastic lesions in the human bladder without using exogenous fluorophores. Two years later, ANIDJAR *et al.* [44] suggested a detection scheme involving the excitation with ultraviolet 308 line of an excimer XeCl laser and the ratioing of the autofluorescence emitted in 385 nm and 440 nm bands, while KOENIG *et al.* [42] described a similar approach but used 337 nm line of a nitrogen laser for the excitation and the ratio of the autofluorescence emitted in 385 nm and 455 nm bands for the detection. Using that diagnostic algorithm in examinations of 75 patients in whom the bladder cancer was suspected, the authors demonstrated that the method detects the malignant lesions with a sensitivity of 95% and substantially decreases the number of biopsies obtained from a nonmalignant tissue [45].

4.4. Cervix

In many countries cytology screening programmes resulted in spectacular decrease in both incidence and mortality rates from cervical cancer. In the United States the mortality declined by 70% over a period of 40 years. Similar trend was seen in Scandinavian countries [46]. Two methods commonly used in the screening for the cervical neoplasia are Papanicolau (Pap) smear and colposcopy. False – negative results from both methods can be as high as 20–40% [47], [48]. The errors are associated with sampling and reading errors. Thus, the improvement in the screening and in the detection is clearly needed. Already in 1992 GLASSMAN *et al.* [6] investigated *in vitro* the autofluorescence spectra of tissues of the gynecological tract and suggested that the neoplastic lesions could be differentiated on the basis of the spectroscopic characteristics. Most of the work in that field has been done

by Richards-Kortum and her coworkers [49]–[53] who demonstrated both *in vitro* and *in vivo* that the autofluorescence could be used to diagnose the cervical neoplasia with high sensitivity and specificity. Other attempts to diagnose such lesions using the optical biopsy approach were described in [54], [55].

4.5. Skin

Among the skin cancers melanoma is a cause for most serious concern because it is potentially fatal and the early detection is crucial for a survival of a patient. The incidence of melanoma has been growing worldwide with a doubling time of 10–15 years. Clinical diagnosis of melanoma is often difficult. Initial assessment relies on the visual examination of the suspected lesions. Statistical data indicate that up to 50% of melanomas can be missed in routine clinical examinations, while the experts achieve 80–90% of correct results [56]. Since the percentage of correct diagnoses depends to a large extent on the experience of the examiners only 35–50% of melanomas identified in mass screening programmes is confirmed in later histopathological examinations [57]. In 1988 LOHMAN and PAUL [58] carried out a feasibility study on a possibility of *in situ* detecting melanomas by spectroscopic analysis of the autofluorescence. The authors suggested that melanomas generate a characteristic spatial distribution of the autofluorescence intensity characterised by a strong increase of the intensity in a transition zone between the melanoma and the normal skin. Later the same authors tested their hypothesis in a study of larger number of cases and reported that a presence of the characteristic maximum of the emission was sufficient to differentiate between melanomas and pigmented naevi with high sensitivity and specificity [59]. Other authors, [14], [60], were not able to confirm the existence of the characteristic spatial distribution of the autofluorescence observed by LOHMANN *al.* [58], [59]. CHWIROT *et al.* [14] reported in 1998 that human cutaneous melanoma could be detected *in situ* with a sensitivity of 85% using the digital imaging of a spectrally resolved autofluorescence excited by a low intensity UVA radiation. Multicentre validation study confirmed the sensitivity of the method [61]. The UV excited autofluorescence of the skin was recently applied by BRANCALEON *et al.* [62] for *in situ* detection of nonmelanoma skin cancers. The authors found that both basal cell carcinomas and squamous cell carcinomas were characterised by a higher level of the autofluorescence associated with tryptophan residues and by a lower emission due to dermal collagen crosslinks.

5. Summary and concluding remarks

The autofluorescence detection of the premalignant and malignant lesions in human tissues has been attracting increasing interest for more than a decade. Several groups reported very promising results and at least three techniques were subject to multiple clinical tests (lung cancer – [12], [30], [31], cancers of human gastrointestinal tract [63], and melanoma – [61]). One can reasonably expect that in the near future similar tests will be carried out for the autofluorescence methods developed for the detection of the neoplastic lesions in bladder and cervix.

The autofluorescence examinations are practically non-invasive and the side -effects are negligible or at least comparable to other endoscopic techniques. The methods does not require taking tissue biopsies and the information on a physiological state and on a histological status of the tissues of interest is obtained from the light emitted by optically excited endogenous fluorophores. Since the excited molecules are natural components of the cells and the tissues the autofluorescence methods avoid photosensitizing the patients. Such side-effects turned out to be a main obstacle in developing fluorescence diagnostic techniques based on using photosensitisers accumulating preferentially in the neoplastic cells.

The rapid development of the field of the autofluorescence diagnostics has been to a large extent facilitated by a technological progress. Digital imaging equipment has become readily available and relatively cheap in the recent decade, while modern computing systems allow fast processing of large bodies of data and the efficient real time analysis of multiple images and spectra. Up to now the methods of the autofluorescence detection and diagnostics of human cancer have been developed mostly empirically. Such an approach seems to have exhausted the resources for further development. At the same time, however, the relation between the autofluorescence and the cell and tissue metabolism, biochemistry and structure is poorly understood. Therefore, it seems that a necessary condition for a qualitative progress in the field is to achieve a better understanding of biological background of the different properties of the autofluorescence of the normal and neoplastic cells and tissues, especially of a nature and of a localisation of endogenous fluorophores. Other important questions concern a degree of a natural variability of the autofluorescence both in individuals and in populations, as well as variations related to a progress of the disease and a role of biological processes accompanying the cancer, like immunologic response, inflammation, necrosis *etc.*

Systematic research on all those problems has been relatively scarce. Proper model systems and methodologies have not been fully developed yet. The nature of potential endogenous flurophores was discussed in most of the publications and there seems to be a general agreement that the potential candidates are NAD(P)H, NADH, flavins, collagen and elastin with other candidates like specific cellular lipid elements and eosinophiles also considered by some authors (see, for instance, [8], [63]–[71]). There were also attempts to model the excitation and emission processes for the autofluorescence (for instance, [8], [73]–[75]), but the main problems of the origin and of the role of tissue architecture remain unresolved.

Autofluorescence detection has proven able to differentiate with a high sensitivity between the normal and the neoplastic tissue in many organs. The coming years will most probably see a transfer of the autofluorescence methods to clinical practice, especially if the research and development of new instruments will be supported by fundamental studies on the origin of the autofluorescence, especially on a nature and a localisation of endogenous fluorophores and on factors influencing the spectra and the intensity of their fluorescence. The future techniques will widely use the imaging approach. The suitably processed images can guide the examiner to suspicious areas,

improving not only the diagnosis of cancer but also the efficiency of the procedures. Practically all the algorithms elaborated for the optical biopsy approach can now be transferred to procedures involving multispectral imaging. It should not be expected that the autofluorescence methods will replace current histopathological and clinical examinations. They may however and probably will become an important auxiliary tool assisting and facilitating the correct diagnosis. The primary field of wide application of the autofluorescence methods of cancer detection may become a screening of large populations and a selection of patients who should seek advise of a specialist. Controlled multicentre trials and outcome analysis studies are still necessary to prove the benefits but the results obtained up to now have been very optimistic.

Acknowledgment – This work was supported by a grant 514-B from Nicholas Copernicus University, Toruń, Poland.

References

[1] LADIK J., FOERNER W., *The Beginnings of Cancer in the Cell.* Springer-Verlag, Berlin, Heidelberg, New York 1994.
[2] FRANKS L.M., TEICH N.M, *Introduction to the Cellular and Molecular Biology of Cancer,* Oxford University Press, Oxford, New York, Tokyo 1995.
[3] COLEMAN W.B., TSONGALIS G.J.. *The Molecular Basis of Human Cancer,* Humana Press, Totowa, New Jersey 2002.
[4] ALFANO R.R., TANG G.C., PRADHAN A., LAM W., CHOY D.S.J.,OPHER E., IEEE J. Quantum Electron. **23** (1987), 1806.
[5] YUANLONG Y., YANMING Y., FUMING L., YUFEN L., PAOZHONG M., Lasers Surg. Med. **7** (1987), 528.
[6] YAKSHE P.N., BONNER R.F., COHEN P., LEON M.B., FLEISCHER D.E., Gastrointest. Endosc. **35** (1989), 184.
[7] KAPADIA C.R., CUTRUZZOLA F.W., O'BRIEN K.M., STETZ M.L., ENRIQUEZ R., DECKELBAUM L.I., Gastroenterology **99** (1990), 157.
[8] SCHOMACKER K.T., FRISOLI J.K., COMPTON C.C., FLOTTE T.J., RICHTER J.M., NISHIOKA N.S., DEUTSCH T.F., Lasers Surg. Med. **12** (1992), 63.
[9] SCHOMACKER K.T., FRISOLI J.K., COMPTON C.C., FLOTTE T.J., RICHTER J.M., DEUTSCH T.F., NISHIOKA N.S., Gastroenterology **102** (1992), 1155.
[10] GLASSMAN W.S., LIU C.H., TANG G.C., LUBICZ S., ALFANO R.R., Lasers Life Sci. **5** (1992), 49.
[11] HIRANO T., ISHIZUKA M., ISHIDA K., SUZUKI K., MIYAKI S., Lasers Life Sci. **3** (1989), 1.
[12] PALCIC B., LAM S., HUNG J., MACAULAY C., Chest **99** (1991), 742.
[13] WANG T.D., VAN DAM J., CRAWFORD E.A., PREISINGER E.A., WANG Y., FELD M.S., Gastroeneterology **111** (1996), 1182.
[14] CHWIROT B.W., CWIROT S., REDZIŃSKI J., MICHNIEWICZ Z., Eur. J. Cancer **34** (1998), 1730.
[15] CHWIROT B.W., CHWIROT S., JĘDRZEJCZYK W., JACKOWSKI M., RACZYŃSKA A.M., WINCZAKIEWICZ J., DOBBER J., Lasers Surg. Med. **21** (1997), 149.
[16] KOBAYASHI M., TAJIRI H., SEIKE E., SHITAYA M., TOUNOU S., MINE M., OBA K., Cancer Lett. **165** (2001), 155.
[17] CHWIROT B.W., KOWALSKA M., SYPNIEWSKA N., MICHNIEWICZ Z., GRĄDZIEL M., J. Photochem. Photobiol. B: Biol. **50** (1999), 174.
[18] ANDERSSON-ENGELS S., CANTI G., CUBEDDU R., EKER C., KLINTEBERG A., PIFFERI A., SVANBERG K., SVANBERG S., TARONI P., VALENTINI G., WANG I., Lasers Surg. Med. **26** (2000), 76.
[19] RAMANUJAN N., CHEN J.X., GOSSAGE K., RICHRDS-KORTUM R., CHANCE B.. IEEE Trans. Biomed. Eng. **48** (2001), 1034.

[20] MYCEK M.A., SCHOMACKER K.T., NISHIOKA N.S., Gastrointest. Endoscopy **48** (1998), 390.
[21] WAGNIERES G., MIZERET J., STUDZINSKI A., VAN DEN BERGH H.J., Fluorescence **7** (1997), 5.
[22] RICHARDS-KORTUM R., RAVA R.P., PETRAS R.E., FITZMAURICE M., SIVAK M., FELD M.S., Photochem. Photobiol. **53** (1991), 777.
[23] MARCHESINI R., BRAMBILLA M., PIGNOLI E., BOTTIROLI G., CROCE A.C., DAL FANTE M., SPINELLI P., DI PALMA S., J. Photochem. Photobiol. B: Biol. **14** (1992), 219.
[24] COTHREN R.M., SIVAK M.V., VAN DAM J., PETRAS R.E., FITZMAURICE M., CRAWFORD J.M., WU J., BRENNAN J.F., RAVA R.P., MANOHARAN R., FELD M.S., Gastrointest. Endosc. **44** (1996), 168.
[25] CHWIROT B.W., MICHNIEWICZ Z., KOWALSKA M., NUSSBEUTEL J., Photochem. Photobiol. **69** (1999), 336.
[26] BRAND S., STEPP H., OCHSENKUEHN T., BAUMGARTNER R., BARETTON G., HOLL J., VON RITTER C., BAUMGARTNER G., SACKMANN M., Int. J. Colorect. Dis. **14** (1999), 63.
[27] WANG T.D., CRAWFORD J.M., WANG Y., ITZKAN I., VAN DAM J., Gastrointest. Endosc. **49** (1999), 447.
[28] PROFIO E.A., Med. Phys. **10** (1982), 35.
[29] LAM S., HUNG J., MACAULAY C., JAGGI B., PALCIC B., IEEE **12** (1990), 1142.
[30] SMID Z.M., BUBNIC F.I., LENARCIC J., OBLAK P., Acta Otolaryngol. (Stockh.) S **527** (1997), 125.
[31] LAM S., MACAULAY C., HUNG J., LERICHE J., PROFIO A.E., PALCIC B.J., Thorac. Cardiovasc. Surg. **105** (1993), 1035.
[32] VENMANS B.J.W., VAN BOXEM T.J.M., SMIT E.F., POSTUMS P.E., SUTEDJA T.G., Ann. Otol.. Rhinol. Laryngol. **110** (2001), 635.
[33] ZARGI M., FAJDIGA A., SMID L., Eur. Arch. Oto.-Rhino.-Laryngol. **257** (2000), 17.
[34] KUSUNOKI Y., IMAMURA F., UDA H., MANO M., HORAI T., Chest **118** (2000), 1776
[35] BETZ G.S., MEHLMANN M., RICK K., STEPP H., GREVERS G., BAUMGARTNER R., LEUNING A., Lasers Surg. Med. **25** (1999), 323.
[36] QU J.Y., WING P., HUANG Z., KWONG D., SHAM J., LEE S.L., HO W.K., WIE W.I., Lasers Surg. Med. **26** (2000), 432.
[37] KULAPADITHAROM B., BOONKITTCHAROEN V., Ann. Otol. Rhinol. Laryngol. **107** (1998), 241.
[38] INAGUMA M., HASHIMOTO K., Cancer **86** (1999), 2201.
[39] ONIZAWA K., YOSHIDA H., SAGINOYA H., Int. J. Maxillofacial Surg. **29** (2000), 42.
[40] MAJUMDER S.K., MOHANTY S.K., GHOSH N., GUPTA P.K., JAIN D.K., KHAN F., Current Sci. (Bangalore) **79** (2000), 1089.
[41] DIDKOWSKA J., WOJCIECHOWSKA U., TARKOWSKI W., ZATOŃSKI W., *Cancer in Poland*, Polish National Cancer Registry, Warszawa, 2002.
[42] KOENIG F., MCGOVERN F.J., ALTHAUSEN A.F., DEUTSCH T.F., SCHOMACKER K.T., J. Urology **156** (1996), 1597.
[43] D'HALLEVIN M.A., BAERT L., VANHERZEELE H., J. Am. Paraplegia Soc. **17** (1994), 161.
[44] ANIDJAR M., ETTORI D., CUSSENOT O., MERIA P., DESGRADCHAMPS F., CORTESSE A., TEILLAC P., LE DUC A., AVRILLIER S., J. Urology **156** (1996), 1590.
[45] KOENIG F., MCGOVERN F.J., ENQUIST H., LARNE R., DEUTSCH T.F., SCHOMACKER K.T., J. Urology **159** (1998), 1871.
[46] MERA S.L., Brit. J. Biomed. Sci. **52** (1995), 120.
[47] KOSS L.G., Cancer **71** (1993), 1406.
[48] MITCHELL M.F., Consul. Obstet. Gynecol. **6** (1994), 70.
[49] RAMANUJAM N., MITCHEL M.F., MAHADEVAN A., THOMSEN S., SILVA E., RICHARDS-KORTUM R., Clin. Gynecol. Oncology **52** (1994), 31.
[50] MAHADEVAN A., MITCHEL M.F., SILVA E., THOMSEN S., RICHARDS-KORTUM R., Lasers Surg. Med. **13** (1993), 647.
[51] RAMANUJAM N., MITCHEL M.F., MAHADEVAN A., THOMSEN S., SILVA E., RICHARDS-KORTUM R., [In] Proc. Natl. Acad. Sci. U.S.A. **91** (1994), 10193.
[52] RAMANUJAM N., MITCHEL M.F., MAHADEVAN-JANSEN A., THOMSEN S., STAELKER G., MALPICA A., WRIGHT T., ATKINSON N., RICHARDS-KORTUM R., Photochem. Photobiol. **64** (1996), 720.

[53] AGRAWAL A., UTZINGER U., BROOKNER C., PITRIS C., MITCHELL M.F., RICHARDS-KORTUM R., Lasers Surg. Med. **25** (1999), 237.
[54] CHEN C.T., HUANG C.C., CHEN R.J., LIN H.H., WANG C.Y., LEE Y.S., CHOW S.N., J. Formos. Med. Assoc. **96** (1997), 247.
[55] KOUMANTAKIS E., VASILEIOU A., MAKRIGIANNAKIS A., UNSOELD E., PAPAZOGLOU T.G., J. Photochem. Photobiol. B: Biol. **40** (1997), 183.
[56] GRIN C.M., KOPF A.W., WELKOVICH B., BART R.S., LEVENSTEIN M.J., Arch. Dermatol. **126** (1990), 763.
[57] LEFFEL D.J., CHEN Y.T., BERWICK M., BOLOGNA J.L., J. Am. Dermatol. **28** (1993), 1003.
[58] LOHMANN W., PAUL E., Naturwissenschaften **75** (1988), 201.
[59] LOHMANN W., NILLES M., BOEDEKER R.H., Naturwissenschaften **78** (1991), 456.
[60] STERENBORG H.J.C.M., THOMSEN S., JACQUES S.L., MOTAMEDI M., Melanoma Res. **5** (1995), 211.
[61] CHWIROT B.W., CHWIROT S., SYPNIEWSKA N., REDZIŃSKI J., MICHNIEWICZ Z., KURZAWSKI G., RUKA W., J. Invest. Dermatol. **117** (2001), 1449.
[62] BRANCALEON L., DURKIN A.J., TU J.H., MENAKER G., FALLON J.D., KOLLIAS N., Photochem. Photobiol. **73** (2002), 178.
[63] ROEMER T.J., FITZMAURICE M., COTHREN R.M., RICHARDS-KORTUM R., PETRAS R., SIVAK M.V., KRAMER J.R., Am. J. Gastroenterology **90** (1995), 81.
[64] BOHORFOUSH A.G., Endoscopy **28** (1996), 372.
[65] RICHARDS-KORTUM R., SEVICK-MURACA E., Ann. Rev. Phys. Chem. **47** (1996), 555.
[66] MARCHESINI R., BRAMBILLA M., PIGNOLI E., J. Photochem. Photobiol. B: Biol. **14** (1992), 219.
[67] FIARMAN G.S., NATHANSON M.H., WEST A.B., DECKELBAUM L.I., KELLY L., KAPADIA C.R., Digest. Dis. Sci. **40** (1995), 1261.
[68] BANERJEE B., MIEDEMA B., CHANDRASEKHAR H.R., J. Med. Sci. **316** (1998), 220.
[69] BANERJEE B., MIEDEMA B.E., CHANDRASEKHAR H.R., J. Invest. Med. **47** (1999), 326.
[70] PRADHAN A., PAL P., DUROCHER G., VILLENEUVE L., BALASSY A., BABAI F., GABOURY L., BLANCHARD L., J. Photochem. Photobiol. B: Biol. **31** (1995), 101.
[71] BROOKNER C.K., FOLLEN M., BOIKO J., GALVAN J., THOMSEN S., MALPICA A., SUZUKI S., LOTAN R., RICHARDS-KORTUM R., Photochem. Photobiol. **71** (2000), 730.
[72] IZUISHI K., TAJIRI H., BOKU N., OHTSU A., OHNISHI T., RYU M., KONOSHITA T., YOSHIDA S., Endoscopy **31** (1999), 511.
[73] CHEONG W.-F., PRAHL S.A., WELCH A.J., IEEE J. Quantum Electron. **26**, (1990), 2166.
[74] ZONIOS G.I., COTHREN R.M., ARENDT J.T., WU J., VAN DAM J., CRAWFORD J.M., MANOHARAN R., FELD M.S., IEEE Trans. Biomed. Eng. **43** (1996), 113.
[75] ZENG H., MACAULAY C., MCLEAN D.I., PLACIC B., J. Photochem. Photobiol. B: Biol. **38** (1997), 234.

Received July 31, 2002
in revised form September 25, 2002