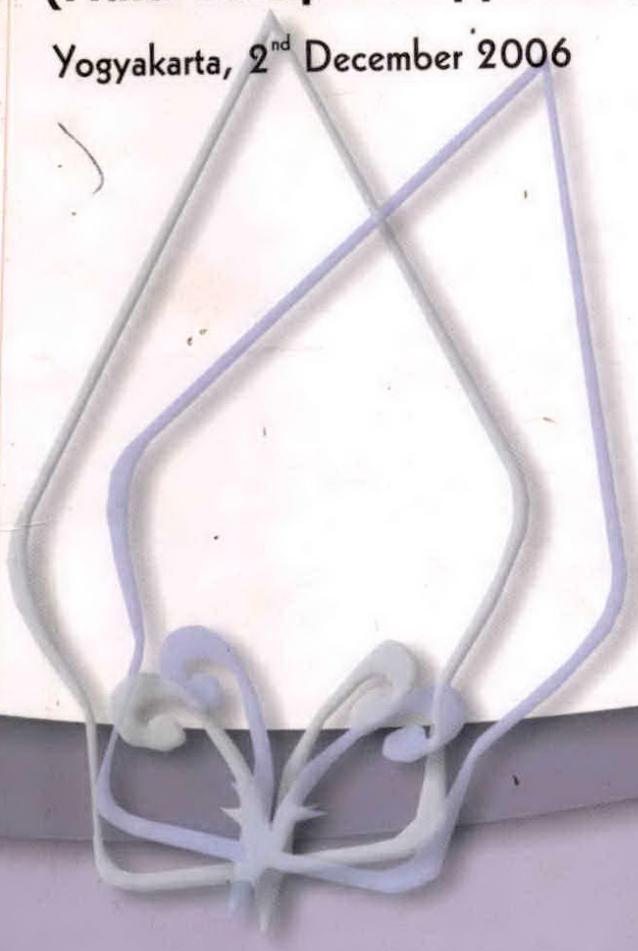
ISBN 979-3700-10-6

Proceeding

International Joint Seminar

Muslim Countries and Development :
Achievements, Constraints and Alternative Solutions
(Multi-Discipline Approach)

Yogyakarta, 2nd December 2006



Organized by:

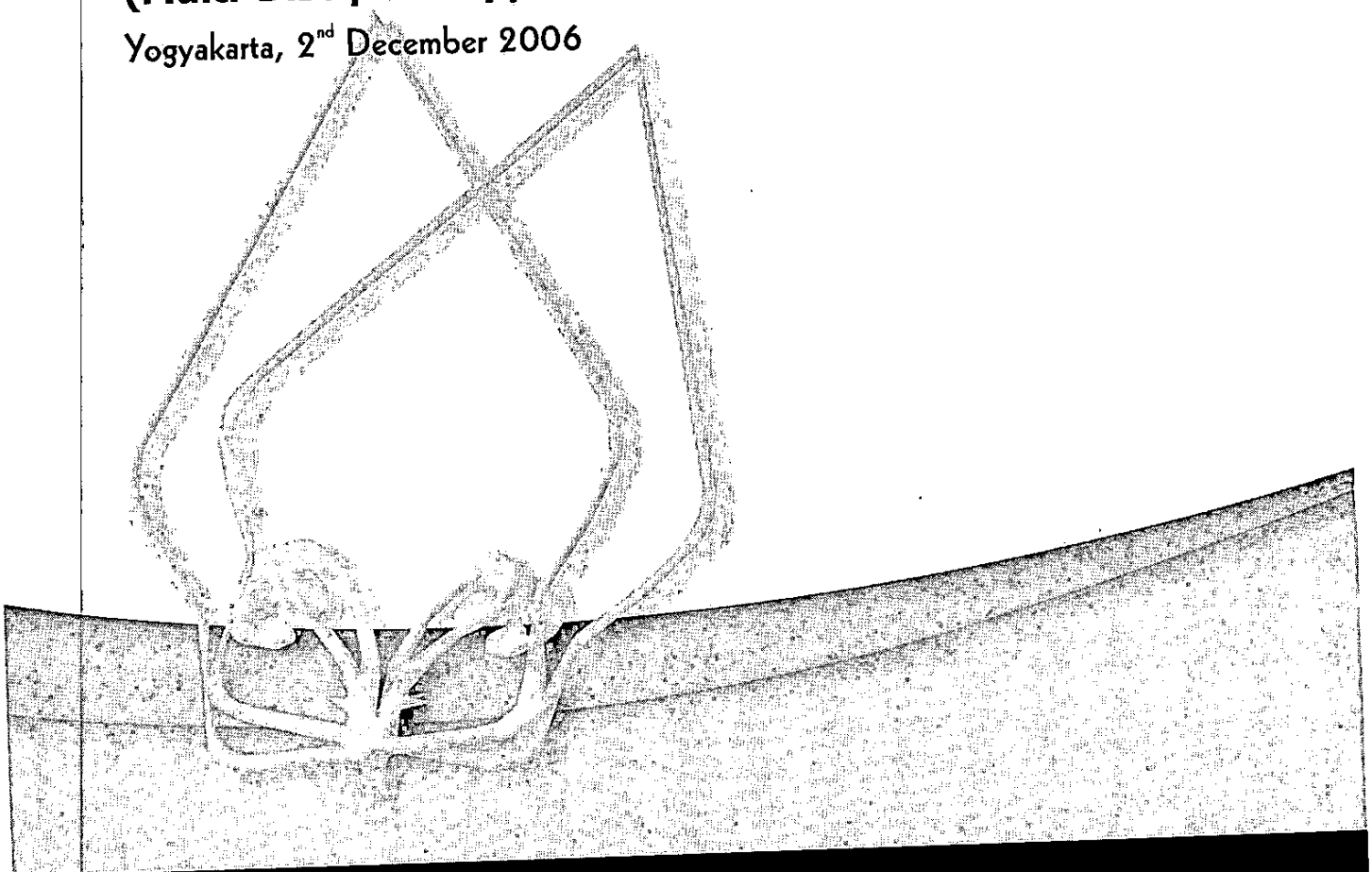
ISBN 979-3700-10-6

Proceeding

International Joint Seminar

Muslim Countries and Development :
Achievements, Constraints and Alternative Solutions (Multi-Discipline Approach)

Yogyakarta, 2nd December 2006



Organized by:

UMY
Universitas Muhammadiyah Yogyakarta

IIUM
International Islamic University Malaysia

Education and Cultural Attache Embassy of The Republic Indonesia in Malaysia

MESSAGE FROM THE RECTOR OF UNIVERSITAS MUHAMMADIYAH YOGYAKARTA (UMY)

Assalamu'alaikum warahmatullahi wabarakatuh

All praise be to Allah SWT, Lord of the world. Peace and blessings on Muhammad SAW, His Servants and Messenger.

First of all, as the rector of Universitas Muhammadivah Yogyakarta (UMY), I would like to welcome to the honourable guests, Rector, Dean of Postgraduate Studies (CPS), Dean of ISTAC, Dean of IRKHS, Deputy Deans and Head Departments from various Kulliyah, lecturers, postgraduate students of International Islamic University Malaysia (IIUM), and all participants in this joint seminar.

Academic cooperation between UMY and IIUM started several years ago. The cooperation between us is based on a solid foundation; both us are Islamic universities having same missions to develop Islamic society, to prepare future generations of Islamic intellectuals, and to cultivate Islamic civilization. In fact, improving academic quality and strengthening our position as the producers of knowledge and wisdom will offer a meaningful contribution to the development of Islamic civilization. This responsibility is particularly significant especially with the emergence of the information and knowledge society where value adding is mainly generated by the production and the dissemination of knowledge.

Today's joint seminar signifies our attempts to shoulder this responsibility. I am confident to say that this joint program will be a giant step for both of us to open other pathways of cooperation. I am also convinced that through strengthening our collaboration we can learn from each other and continue learning, as far as I am concerned, is a valuable ingredient to develop our universities.

I sincerely wish you good luck and success in joining this program

Wassalamu'alaikum Wr, Wb.

Dr. Khoiruddin Bashori
Rector, UMY

MESSAGE FROM THE RECTOR OF INTERNATIONAL ISLAMIC UNIVERSITY MALAYSIA (IIUM)

Assalamu'alaikum warahmatullahi wabarakatuh

In the name of Allah, the most Gracious and the most Merciful. Peace and blessings be upon our Prophet Muhammad (S.A.W).

First and foremost, I felt honoured, on behalf of the university to be warmly welcomed and to be given the opportunity to work hand in hand, organizing a respectable conference. Indeed, this is a great achievement towards a warmers bilateral tie between the International Islamic University Malaysia (IIUM) and Universitas Muhammadiyah Yogyakarta (UMY) after the MoU Phase.

I would also like to express my heartfelt thanks to Centre for Postgraduate Studies (CPS), Postgraduate Students Society (PGSS), contributors, paper presenters, participants and our Indonesian counterpart for making this program a prestigious event of the year.
This educational and cultural visit is not only an avenue to foster good relationship between organizations and individuals and to learn as much from one another but a step forward in promoting quality graduates who practices their ability outdoor and master his or her studies through first hand experience. The Islamic platform inculcated throughout the educational system namely the Islamization of knowledge, both theoretical and practical, will add value to our graduates. This comprehensive excellent we strived for must always be encouraged through conferences, seminars and intellectual-based activities in line with our lullaby: The journey of a thousand miles begin by a single step, the vision of centuries ahead must start from now.

My utmost support is with you always. Looking forward to a fruitful meeting.

Ma'assalamah
Wassalamu'alaikum Wr, Wb.

Prof. Dato' Dr. Syed Arabi Idid
Rector, IIUM

MESSAGE FROM EDUCATION AND CULTURAL ATTACHE
EMBASSY OF THE REPUBLIC OF INDONESIA
KUALA LUMPUR

Assalamu'alaikum warahmatullahi wabarakatuh

All praise be to Allah SWT. This is the moment where implementation of MoU between Universitas Muhammadiyah Yogyakarta (UMY) and International Islamic University Malaysia (IIUM) comes in the form of action by organizing this Joint Seminar. The efforts of both sides to implement the MoU are highly appreciated, especially, in the context of which both universities effort to enhance the quality of education.

Substantially, I believe that this Joint Seminar will bring many benefits. In term of the development of knowledge, it is a means for developing academic quality, for exchanging of information on academic development, as well as for constructing intellectual atmosphere at both universities. In term of international relations, both universities have taken part in increasing close relationship between Malaysia and Indonesia. RUM and UNIY as well are using 'soft power' to increase bilateral relations among citizens which brings a lot of benefits for both nations.

Therefore, I hope that both RUM and UMY can make use of this program as a 'kick-off' for other programs in the future, especially in using UMY's vast networks with other Muhammadivah Universities in various cities in Indonesia as well as IIUM's network. The support of IIUM for UMY also means a progress for IIUM and UMY. I hope such joint program will continue in future for betterment of both Indonesia and Malaysia. Embassy of the Republic of Indonesia in Kuala Lumpur will always support these efforts.
To our honorable guests, Rector, Dean of Postgraduate Studies (CPS), Dean of ISTAC, Dean of IRKHS, Deputy Deans and Head Departments from various Kulliyah, lecturers and students of IIUM, I warmly welcome you to Yogyakarta. I hope you enjoy your stay in the cultural city of Yogyakarta.
Finally, as the Attache of Education and Cultural, Embassy of the Republic of Indonesia, Kuala Lumpur, I sincerely wish you good luck *and a* successful program with unforgettable memories.

Wabillahit Taufiq Wal Hidayah
Wassalamu'alaikum warahmatullahi wabarakatuh.

M.Imran Hanafi

Education and Cultural Attache Embassy of the Republic of Indonesia

MESSAGE FROM DEAN CENTRE FOR POSTGRADUATE STUDIES

Assalamu'alaikum warahmatullahi wabarakatuh

Praise be to Allah. May the peace and blessings of Allah be on the last prophet and messenger, our master Muhammad and on his household and companions. It is a great privilege for me to foreword this message to this wonderful event that is jointly organized by the Universitas Muhammadiyah Yogyakarta (UMY) and International Islamic University (IIUM).

First and foremost I would like to record my special gratitude to management of Universitas Muhammadiyah Yogyakarta for their co-operation.

In order to obtain comprehensive excellence, the Centre for Postgraduate studies has always facilitates postgraduate students of the university to achieve the highest quality in their academic work. This seminar is one of the many programs that Centre for postgraduate studies has to ensure quality graduates.

I would therefore like to thank all the participants and programme coordinators who have worked hard to realize this event.

May Allah SWT shower His blessing upon us.

Wassalamu'alaikum Wr, Wb.

Prof. Dato' Dr.Wan Rafaei Abdul Rahman
Dean, Centre For Postgraduate Studies

MESSAGE FROM THE ACTI
PRESIDENT OF POSTGRADUATE STUDE

Assalamu'alaikum warahmatullahi wabarakatuh

On behalf of Postgraduate Students' Society (PGSS), my gratitude and appreciation to our beloved Dean of Studies, the Embassy of Indonesia in Kuala Muhammadiyah Yogyakarta and the organizing com IIUM and the Universitas Muhammadiyah Yogyakarta huge success. Postgraduate Students' Society (PGSS) u supervision of the Center for Postgraduate Studies (CP this event.

As I strongly believe that the initial stages of unity a and building the new generation, who will represent t more, such programs, not only achieve the missio universities but to achieve the global mission and Therefore, I believe today, we have to have understa and then only we can appreciate our diverse cult acknowledge the different strengths posses in us an weaknesses through knowledge in this age of informa sure this joint seminar will initiate unity among the fut along with integrating them.

Thank you,

Mohd Nabi Habibi

Acting President Postgraduate Students' Society (PGSS

MESSAGE FROM PROGRAM DIRECTOR

Assalamu'alaikum warahmatullahi wabarakatuh.

Praise be to Allah. May the peace and blessings of Allah be on the last Prophet and Messenger, our master Muhammad and on his household and companions.

Honestly speaking, we are pleased to be trusted by Postgraduate Students' Society (PGSS) and Centre for Postgraduate Studies (CPS) to organize the programme named Educational and Cultural Visit to Yogyakarta, Indonesia. For this, We express our gratitude to the management of both PGSS and CPS. This programme is of immense value. It has the potentials to promote intellectual endeavor, develop leadership capabilities and enrich cross-cultural understandings. We sincerely believe and hope that program of this kind will be organized in a regular fashion in future.

It is a great privilege for us to play twofold role in organizing this event: *as a host* and as *guest*. In fact, this is a fascinating experience to manage this event. Since our inception here, we have found meaningful interaction of students in an interweaving of cultures into complicated, yet beautiful, embroidery of social fabric. We are proud to say that this dearly loved university has produced graduates of high quality, who are distinct from those of the local universities.

Finally, we wish to express our special thanks to Bapak M.Imran Hanafi, Education and Cultural Attache of Indonesian Embassy, Bapak Herdaus, S.H., Assistant of Immigration Attache of Indonesian Embassy, Bapak Tharian Taharuddin for their immensely valuable assistance and co-operation in making this program a success. I sincerely appreciate all local committees at Yogyakarta, the colleagues and program coordinators and committee members who worked diligently to materialize this event. We wish to pass on good wishes to the PGSS for their valuable efforts it expended for this event.

May Allah s.w.t shower His blessing upon us.

Wassalam,

Nasrullah
Programme Director

Todi Kurniawan
Co-Programme Director

Contents

SCIENCES, TECHNOLOGY AND EDUCATION HUMAN RESOURCES DEVELOPMENT ISSUES

ECONOMICS AND DEVELOPMENT ISSUES

POLITICS AND LEGAL ENFORCEMENT ISSUES

SOCIAL, RELIGIOUS AND CULTURAL ISSUES

Fluorescence Detection of Human Premalignant and Malignant Lesions

Torla Hassan*
International Institute of Islamic Thought and Civilization (ISTAC)
International Islamic University Malaysia

Abstract

Diagnostic potential of autofluorescence spectroscopy and imaging for early detection of precancerous and malignant lesions has been actively investigated for almost twenty years. Fluorescence diagnosis based on excitation of the fluorescence of endogenous fluorophores can detect human neoplasia with high sensitivity and specificity. The autofluorescence methods may provide an important auxiliary tool assisting and facilitating the correct diagnosis but their diagnostic merit still has to be proven in properly performed randomized studies.

Keywords: Diagnostic potential; autofluorescence; spectroscopy; precancerous and malignant lesions; fluorophores; neoplasia.

Introduction

Cancer is one of the leading causes of mortality worldwide. According to WHO mortality data in the year 2000, 10 million people developed malignant tumours and more than 6 million died of cancer [1]. For instance, in the United States, 23% of deaths were related to cancer. Similar rates for United Kingdom and Japan were respectively 24% and 30% [2]. The average age at the time of a diagnosis is for all the tumours approximately 67 years [3]. It is thought that in a future the average risk of developing cancer will increase due, to a large extent, to the increase of a life expectancy of human population. The current trends indicate that by the year 2020 the number of new cases will increase by 50% to 15 million [1].

Last century was a period of unprecedented effort in cancer research. However, with all the information and knowledge gathered up to now no or little success has been achieved in treatment of the most common cancers. Thus, despite all the progress in cancer research, the lines of a defence against cancer have not changed and are the same as many years ago. The best form of reducing cancer related mortality remains prevention and the next best is early detection. The prevention could reduce the cancer incidence by 2 million by the year 2020 and 6.5 million by 2040 [1].

Unfortunately, because of a 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.

*Corresponding Author: torla242@yahoo.com

Medical knowledge permits at present the cure of one-third of all cancers on a condition of early detection and administration of a proper therapy [1]. On the other hand, despite all the progress in diagnostic radiology and advances in elaborating new diagnostic modalities there is still a need for more sensitive techniques for early sensitive and specific detection of premalignant and malignant lesions.

In this paper we present a review of studies on developing a new group techniques of early detection of human cancers based on endogenous fluorescence – autofluorescence of human cells and tissues. The field has been developing rapidly since mid-1990's with many research groups involved in investigating diagnostic potential of the autofluorescence for early detection and visualisation of neoplastic lesions in all anatomic sites accessible for optical examinations.

Principles of the fluorescence detection of neoplastic lesions

Cancer is a cellular disorder and a visible tumour is the result of a series of molecular changes which often require many years to develop. Carcinogenesis is a multistep process of accumulation of errors in key regulatory mechanism controlling cellular proliferation and differentiation. Premalignant and later malignant cell acquires in that process new properties which give it advantage over the surrounding normal cells and allow for its uncontrolled proliferation, invasion of neighbouring tissues and metastasis to distant anatomic sites. Thus, it is obvious that both metabolic processes and a structure of the transformed cell are different from those of cells of its parental tissue. All the molecules of a cell can interact with light and their optical properties may be significantly influenced by the local molecular environment. One can, therefore, reasonably expect that carcinogenic transformation results also in changes in optical properties of the cell. If so, the processes like light scattering, reflection, absorption and re-emission may provide information on occurrence of a pathologic process. In fact, the changes in scattering, reflection and absorption of light have been for ages enabling clinical diagnoses based either on direct or endoscopic visual examinations.

Fluorescence techniques are well-known for their high sensitivity and often used for studying molecules which are present in very small amounts. The fluorescence can be detected with very small background signals and the procedures can be optimized by a proper selection of both excitation and detection wavelengths. Many (if not all) biomolecules emit fluorescence while excited with ultraviolet or visible light. It can also be expected that malignant transformation may result either in producing new fluorophores (absent in normal cells) or in changes in relative concentrations of fluorescent molecules present also in original cells. The rationale behind the fluorescence techniques of early cancer detection is that fluorescence spectra may be used to distinguish displastic or malignant tissue from its normal counterparts and/or other noncancerous pathologic lesions. The research is thus oriented on defining the fluorescence characteristics unique for neoplastic cells of any given tumour which differ them from normal cells of the original tissue and/or from other tissues of the host.

Practical implementation of such a fundamentally straightforward approach has met several obstacles. The excitation and emission spectra of endogenous cellular and tissue fluorophores are wide, have little structure and often overlap. Moreover, living organisms are enormously complex and dynamic at any level of the organization. The individual variability is additionally modified by factors related to ageing, history of medical problems and treatments. Therefore, despite great efforts undertaken by many research groups and very promising results of several feasibility studies the progress in the field has been relatively slow.

Endogenous fluorophores

Fluorescence techniques, similarly to other optical approaches used for medical examinations belong to methods of tissue spectroscopy or just of biomedical optics. A common goal of all such methods is finding information relevant to pathologic processes in optical signals resulting from interactions of light with biomolecules contained in the cells and tissues.

The autofluorescence of animal cells and tissues is mostly due to different kinds of proteins, enzymes and pigments. Among the best known endogenous fluorophores are NADP (H) and flavins [4-13]. The two types of the fluorophores emit in visible range of spectrum while excited with ultraviolet light, their emission bands partially overlap and positions of emission maxima depend on molecular microenvironment (i.e. physiological status of the cells). At a subcellular level NADP (H) emissions are assigned mainly to mitochondria [4-12].

Other commonly occurring fluorophores include collagen and elastin [4, 5, 14,15], porphiryns [5] and lipopigments [16-18]. Data on many potentially useful endogenous fluorophores can be found both in original papers and in references [19, 20]. A comprehensive list of excitation-emission matrices of many biomolecules relevant for tissue spectroscopy can be found in reference [21] and in the interactive database developed by the authors (http://www.aspjournal.com/). At a cellular and tissue level white blood cells [22], especially eosinophils [22-24] are often listed as intensely fluorescent both *in vitro* and *in situ*.

Practically any biological material contains substances demonstrating the fluorescence while illuminated with ultraviolet light. At enormous chemical complexity of living organisms and changing molecular microenvironment of the emitting molecules it is often very difficult to unequivocally link the observed autofluorescence with particular biomolecules. The excitation-emission spectra obtained *in vitro* often are different from those observed *in vivo*. Moreover, the apparent spectra observed *in vivo* depend not only on physical properties of the emitters and their near environment but also on processes such as scattering and absorption of both the exciting and emitted light. The latter depend on wavelengths, cellular and tissue architecture, composition etc. The excitation-emission spectra are very difficult to model and different models proposed in the literature may be lead to various interpretations [5, 25-33].

Methods

Optical biopsy

Optical biopsy also referred to as point spectrometry was widely used in early years of research on the fluorescence detection of cancer. At such an approach, clearly related to a traditional molecular spectroscopy and also to clinical biopsy tissue sampling, the autofluorescence is excited in a restricted area and analyzed for its spectrum. It was hoped that at appropriate excitation conditions it would be possible to find characteristic spectral features of the autofluorescence allowing for a differentiation between neoplastic and normal tissues. Alfano *et al.* [34] reported clear differences in maxima and minima of the autofluorescence spectra of normal and malignant tissues of human lung and breast examined *ex vivo*. Similarly promising data were obtained also by Yuanlong *et al.* [35] and Yakshe *et al.* [36]. However, studies involving larger number of tissue samples and patients demonstrated that spectral differences between normal and neoplastic tissues were more variable and subtle than those observed in the first feasibilty studies [37-40].

Moreover, apparent spectral patterns were influenced by several factors such as intensity of the exciting light (effective excitation depth) and geometry of both the excitation and detection (for instance single fiber and separate fibers used for a delivery and collection of the excitation light and the fluorescence). Several approaches were then suggested to account for systematic effects and to correct the data. Kapadia *et al.* [37] developed a quantitative LIF score (laser – induced fluorescence score) based on weighted sum of relative contribution of six emission bands to the total normalized autofluorescence spectrum. The six wavelengths were selected empirically as yielding optimum classification of normal and adenomatous tissue specimens of the so-called training set of tissue samples. Schomacker *et al.* [38] used a similar approach. However, *instead* of using empirically selected wavelengths the authors decomposed the autofluorescence spectra using the *in vitro* fluorescence spectra of collagen, reduced nicotinamide dinuctleotide and oxidized flavin adenine dinucleotide, corrected the fitted spectra for hemoglobin reabsorption and calculated relative contributions of the three endogenous fluorophores to the normalized spectra. At the same year Alfano's group [41] demonstrated that it is possible to differentiate between normal and tumour tissues using a much simpler algorithm based on ratios of the intensity of the autofluorescence in adequately selected spectral bands. Another approach based on using spectra of the autofluorescence excited with different excitation wavelengths was proposed by Richards-Kortum's group [42]. All the diagnostic algorithms discussed above were used in different forms by other 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 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.

Autofluorescence imaging

There is a fundamental difference between approaches using the autofluorescence imaging and the optical biopsy. The imaging provides wide-area surveillance and 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 localize places of supposedly neoplastic nature within the examined area and to guide to them the examiner (often in a real time). Therefore, the imaging techniques can indeed help in achieving better sensitivity of detection of neoplastic lesions. Earlier or parallel optical biopsy studies play a great role in establishing best, from a point of view of tumour detection, conditions for exciting and recording the autofluorescence images. Similarly as in the case of the optical biopsy, the imaging can be carried out at different excitation and detection bands and several images can be used for calculating the emission intensity ratios just as in algorithms developed for the optical biopsy examinations.

First studies on using fluorescence imaging were carried out in 1989 for detecting lung cancer by Hirano *et al.* [43] who, however, studied the fluorescence of an exogenously administered photosensitiser. Two years later Palcic *et al.* [44] reported that exciting the autofluorescence with 442 nm line of He-Cd laser and using diagnostic algorithm based on a ratio of two spectrally resolved images of the autofluorescence they 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 classical white – light bronchoscopy or of a photosensitised fluorescence bronchoscopy. Other algorithms used for the

detection of neoplastic regions in the autofluorescence images involve analysis of a spatial distribution of the intensity of a total – and of a spectrally resolved autofluorescence [45-49] or the analysis of the intensity ratios for images obtained with and without using a photosensitiser [50]. Images of the autofluorescence are typically recorded with CCD cameras. More advance imaging systems allow for simultaneous multispectral imaging [51]. There has been a continuous effort to develop more practical systems (see for instance [52]) and in recent years it is also possible to buy complete systems developed for the autofluorescence imaging (like for instance autofluorescence videoscopic system described in ref. [53]).

Time-resolved autofluorescence spectroscopy

There are only few reports on applications of time-resolved spectroscopic methods for distinguishing different tissue types. Decay times of the tissue fluorescence may change with changes in a composition of endogenous fluorophores, due to treatment with exogenous labelling molecules and also due to changes in a molecular composition of the cells resulting from carcinogenic transformations. First feasibility studies on using autofluorescence decays as a means of *in vivo* distinguishing colonic adenomas from non-adenomatous polyps were reported in 1998 by Mycek *et al.* [54]. More recent tests involve a similar approach but using time-resolved imaging with timing resolution reaching even picosecond timing resolution [10, 55, 56].

Using autofluorescence in differentiation between normal and neoplastic tissues by organ site

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 by optical biopsy and/or autofluorescence imaging.

1. Gastrointestinal tract

Tumours of gastrointestinal tract are a major health problem worldwide. Mortality attributed to those cancers is second only to a lung cancer. Among the top causes of death worldwide the cancers of stomach, liver, colon and rectum respectively ranked 14th, 22nd and 24rth in 1999 [2]. Early studies on the fluorescence detection of malignant lesions in human stomach, colon and rectum were carried out using the optical biopsy approach while the more recent work concentrates mostly on applications of the autofluorescence imaging.

Stomach

First fluorescence data of Yuanlong *et al.* [35] indicated that malignant transformation of stomach tissues is associated with occurrence of new maxima in the autofluorescence spectra. The authors suggested that that new emission of cancerous cells was related to porphyrin compounds but that conclusion was not confirmed by other researchers. Chwirot *et al.* [46] reported in 1997 that using multispectral imaging (395, 440 and 590 nm) of the autofluorescence excited with 325 nm line of He-Cd laser malignant stomach tissues could be detected *ex vivo* with a sensitivity of 96%. The method was not tested *in situ*, however, Kobayashi *et al.* [48] reported similar sensitivity for *in vivo* imaging of the stomach tissue autofluorescence with commercial LIFE-GI system. Other groups also

tested the LIFE-GI system with respect to its potential for detecting the stomach cancer and found that the sensitivity of such examinations changed with both a stage and histologic type of the cancer [57]. The sensitivity was 57.5% for invasion to the mucosa, 74.3% for invasion to the submucosa and 88.1% for invasion to the muscularis propria. Detection rates for different histologic types were 82% and 61% for differentiated and undifferentiated cancer, respectively.

Colon and rectum

Fluorescence techniques for detection of colorectal dysplasia and early-stage carcinoma have been developed since early 1990's mainly using different schemes of the optical biopsy method and both continuous and pulsed ultraviolet and violet light [37-39, 58,59]. First imaging studies carried out *ex vivo* for a total ultraviolet - excited autofluorescence of adematous polyps from 3 patients were reported only in 1997 [45]. Chwirot *et al.* [49, 60] performed systematic *ex vivo* studies of diagnostic potential of the autofluorescence imaging using a more sophisticated procedure of collecting spectrally-resolved images in six spectral bands. They found that a simple algorithm using a cut-off value of the intensity of the fluorescence normalized to the emission intensity of normal mucosa was sufficient for a correct classification of 88% (23 of 26) adenomatous polyps. The high sensitivity of the approaches using the ultraviolet-excited autofluorescence was confirmed by *in* vivo imaging- and optical biopsy studies [62,63]. Brandt *et al.* [64] used *in* vivo the LIFE system originally developed for the autofluorescence imaging of bronchial neoplastic lesions [44] and showed that imaging of blue-light (430-470 nm) excited autofluorescence may improve the detection of colonic dysplasia. Multi-centre feasibility studies on the detection of gastrointestinal neoplastic lesions with a modified LIFEII system confirmed that autofluorescence endoscopy has the ability to detect early-stage lesions not observable with white-light endoscopy [65].

2. Lung and the upper aero-digestive tract

Lung cancer is the major cause of death from cancer worldwide [1] and cancers of upper aero-digestive tract also are a serious health problem. Despite advances in treatment of many cancers the survival rates for lung and upper aero-digestive tract cancers are still low. Most of such cancers is detected at an advanced stage and there is a clear need for more sensitive and specific diagnostic techniques. Promising results were obtained in 1980' by groups working on early detection of the bronchial neoplastic lesions by the digital imaging of malignant cells labelled with exogenously applied photosensitiser [see for instance 76]. However, the photosensitising drugs render the skin of patients sensitive to light. Studies on a determination of minimum dose required for the successful imaging and detection of the cancerous lesions led to a conclusion that the bronchial neoplastic areas could be detected with high efficacy without administering the photosensitising drugs, using a diagnostic algorithm involving determination of a ratio of intensities of the "green" (560±10 nm) and "red" (560±10 nm) autofluorescence [77]. That discovery led to a first commercial imaging device (LIFE) designed for the autofluorescence imaging and detection of human neoplasia [44]. The autofluorescence was excited with 442 nm line of He-Cd laser, the images recorded in two spectral bands and automatically converted to a diagnostic image guiding the examiner to suspicious areas. Clinical tests of the LIFE system confirmed that the fluorescence imaging facilitates early detection of cancerous lesions and improves both the sensitivity and specificity of bronchoscopic examinations [78-83]. For instance, in the study of Kusunoki et al. [80] the sensitivity of

white- light bronchoscopy for severe dysplasia was 61.2% and with LIFE added it was 89.8%. Venmans et al. [83] in a similar study found for detection of intraepithelial neoplastic lesions sensitivities of 21% and 57%, respectively.

The present status of autofluorescence detection and classification of malignancies of the oral mucosa was recently reviewed by de Veld *et al.* [84]. The authors concluded that autofluorescence imaging and spectroscopy may be helpful in detection of mucosal cancerous lesions, especially of those difficult to find by visual inspection.

Alfano *et al.* [85] carried out *ex vivo* studies of a diagnostic potential of fluorescence imaging of head and neck tumours and also concluded that the technique could be used in clinical setting with a resulting increased likelihood of early detection. Three groups studied the reliability of the autofluorescence imaging with the LIFE system for laryngeal cancer and found it a useful complementary method for a standard laryngoscopy [86-89]. Qu *et al.* [90] designed a new system for fluorescence detection of nasopharyngeal carcinoma. The authors carried out *in vivo* spectroscopic studies of the autofluorescence and elaborated two- and three-wavelength algorithms allowing for a correct classification of 98% of cancerous lesions with a specificity of 95%. In 1995 Panjehpour *et al.* [91] reported spectroscopic measurements of the autofluorescence of oesophagus excited with 410 nm line of a dye laser. The diagnostic tests carried out *in vivo* had a sensitivity and specificity of 100% and 98%, respectively. Using the same approach for a detection of high-grade dysplasia in Barrett's oesophagus the authors obtained 100% of correct diagnoses [92]. Fluorescence detection of Barrett's oesophagus was discussed also in references [65, 93, 94]. The whole field of the fluorescence detecting neoplasia of the aero-digestive tract was reviewed in 1997 in references [95, 96].

3. Urinary bladder

Bladder cancer accounts for ca 66% of all cancers of urinary tract [1]. Both precancerous lesions and early cancers are often invisible or very difficult to detect visually. There has been a continuous effort to overcome those problems and several groups have been working of 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.* [97] reported on differences in the intensities of the autofluorescence of normal and cancerous areas of human bladder and concluded that for 308 nm excitation a clear diagnosis could be achieved without absolute intensity measurements. Two years later Anidjar *et al.* carried out spectroscopic *in vitro* measurements of the autofluorescence of human urothelial cells [98] followed by *in vivo* studies of a new diagnostic algorithm based on using 308 nm excitation (XeCl excimer laser) and ratioing the intensities of the autofluorescence emitted at 360 nm and 455 nm [99-101]. Koenig *et al.* [102] suggested a similar algorithm (autofluorescence intensities at 385 nm and 455 nm with excitation by 337 nm line of the nitrogen laser) and demonstrated *in vivo* that such an approach substantially decreased the number of unnecessary biopsies from nonmalignant areas during bladder cytoscopy [103]. In that study the sensitivity, specificity and positive predicting value for differentiating malignant from nonmalignant lesions were 97%, 98% and 93%, respectively. In conclusion the ultraviolet - excited autofluorescence undoubtedly is efficient technique in the detection of cancerous lesion of the bladder [104, 105] and may also be helpful in detecting malignant renal tissues [106].

4. Cervix

Cervical cancer is the second most common cancer of women [1] . In several countries cytology screening programmes resulted in marked decreases 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 [1, 107]. However, the two most common screening tests, the Papanicolau smear and colposcopy have recognized limitations due mostly to high rates of false negative results [108, 109]. The errors are associated with sampling and reading errors. Already in 1992 Glassmann *et al.* [41] carried out *in vitro* spectroscopic studies of tissues of the gynecological tract and described differences in the spectra of normal and cancerous tissues. Majority of publications on the fluorescence detection of cervical neoplasia has come from the group of Richards-Kortum [42, 110-116]. The group obtained very promising results. Tests in colposcopy clinic demonstrated sensitivity and specificity of 86% and 74% while tests in the screening setting showed accordingly 75% and 80%. Other reports on studies of diagnostic potential of the autofluorescence for the detection of cervical premalignant and malignant lesions were also reported by Chen *et al.* [117] and Koumantakis *et al.* [118].

5. Skin

Malignant melanoma

The incidence of melanoma has been growing world – wide with a doubling time of 10 – 15 years [1]. Survival rates are closely correlated with a stage of melanoma at the time of detection and surgical removal. Clinical diagnosis of melanoma is often difficult. Literature data indicate that up to 50% of melanomas can be missed in routine clinical examinations while the experts achieve 80-90% of correct results [119]. Clinical diagnosis is based on morphological features of the lesions and thus the percentage of correct diagnoses depends to a large extent on experience of the examiners. Only 35-50% of melanomas identified in mass screening programmes is confirmed in later histopathological examinations [120]. Therefore, there is a clear need for elaborating new sensitive and objective diagnostic tools suitable for screening for melanoma.

In 1988 Lohman and Paul [121] reported first study on a possibility of *in situ* detecting melanomas by spectroscopic analysis of the autofluorescence. The authors found that melanomas generated characteristic spatial distributions of the autofluorescence intensity with a strong increase of the intensity in a transition zone between the melanoma and the normal skin. In the next study the authors tested that hypothesis investigating a larger number of cases and reported that a presence of such a localized maximum of the emission was sufficient to differentiate between melanomas and pigmented naevi with high sensitivity and specificity [122]. Independent study of Sterenborg *et al.* [123] did not confirm the results of Lohman's group and similar results were obtained by other groups [47, 124]. Chwirot *et al.* [47] 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. Ebert *et al.* [124] who used similar conditions of the excitation and spectrally resolved imaging obtained the sensitivity of 74% but their diagnostic algorithm differed from that of Chwirot *et al.* [47]. Multicentre validation study of the technique described in [47] confirmed its high sensitivity [125]. Recently Paraskevas *et al.* [126] published a case report describing utility of visual observation of the ultraviolet - excited autofluorescence in clinical diagnostic of pigmented lesions.

Nonmelanoma skin cancer

Skin cancer other than melanoma (basal cell carcinoma and squamous cell carcinoma) is the most prevalent human malignancy world-wide. In 1985, the newly diagnosed squamous and basal cell carcinomas accounted for 30% of all newly diagnosed cancer [1]. Andersson-Engels *et al.* [127] demonstrated *in vivo* that a clear demarcation of basal cell carcinomas of the skin was possible with imaging of the autofluorescence excited with 405 nm light of a dye laser. Brancaleon *et al.* [128] carried out *ex vivo* and *in vivo* studies of the autofluorescence emitted in ultraviolet band of the spectrum and found that nonmelanoma skin cancers (both basal cell and squamous cell carcinomas) emit with higher intensity than the normal skin. The authors suggested that the higher autofluorescence intensity of the cancerous lesions was due to a lower presence of collagen cross-links in the affected areas of the skin. Hewett et al [129] found that multispectral fluorescence imaging might be useful for *in vivo* monitoring of photodynamic therapy of superficial skin cancers.

6. Other organs

Proven ability of the autofluorescence to distinguish malignant lesions from normal tissues in many anatomic sites has been a stimulus for exploring other possibilities of similar applications.

In the case of breast cancer (first among cancers affecting women throughout the world) the research is still at *in vitro* stage. Alfano *et al.* [34] demonstrated differences in spectra of the autofluorescence of normal and cancerous tissues using 300 nm excitation. Gupta *et al.* [130] used 337 nm excitation and demonstrated that *ex vivo* the intensity of total autofluorescence of malignant breat tissues was about three times higher than of normal tissues. Similar, although significantly smaller differences were observed by Hage *et al.* [131] who excited the autofluorescence with visible blue light (458 nm). The authors developed a multiparameter diagnostic algorithm that distinguished *ex vivo* the cancerous lesions from the normal tissues with both asensitivity and a specificity of 100%.

Mueskens *et al.* [132] reported that the autofluorescence may be useful for differentiating between choroidal melanoma and choroidal nevus.

Izuishi *et al.* [133] carried out first evaluation using the autofluorescence to detect bile duct cancer. The authors used GI-LIFE system and found that cancerous lesions displayed colours different from normal epithelium, dark-red and light-blue, respectively.

Very interesting report on clinical tests using the autofluorescence for brain tumour demarcation was published by Lin i wsp. [134] who found that a two-step diagnostic algorithm based on the autofluorescence and a diffuse reflectance differentiated infiltrating tumour margins from normal brain tissues with a sensitivity of 100% and a specificity of 76%.

Concluding remarks

Novel approaches using the autofluorescence provide a promising practically non-invasive, real-time diagnostic tool to detect precancerous and malignant lesions. 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. In principle, the non-invasive spectroscopic examinations of the whole cells and tissues are carried out at molecular

level and similar data cannot be obtained (or in some cases would be difficult to obtain) with other modalities.

Clinical applications of optical techniques including the autofluorescence spectroscopy and imaging have been facilitated in recent years by rapid technological progress and growing availability of lasers, spectrum analysers, fluorescence imagers and efficient computing systems. Most of the published results have shown a great potential of the autofluorescence spectroscopy and imaging for detecting cancerous and precancerous lesions of many organs. Several algorithms have also been tested in clinical settings. Increased use of fluorescence techniques in clinical medical diagnostics, and perhaps also in histopathological diagnostics, can be anticipated in forthcoming years.

At the same time, it seems that a necessary condition for a significant progress in this field is reaching 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 the endogenous fluorophores. The relations between the autofluorescence and the cell and tissue metabolism, biochemistry and structure are poorly understood. Other important questions concern a degree of a natural variability of the autofluorescence both in individuals and in populations as well as changes of its properties related to a progress of the disease and a role of biological processes accompanying the cancer like immunologic response, inflammation, necrosis etc. At present all the diagnostic algorithms are empirical.

The future applications 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 biopsy procedures. Practically all the algorithms elaborated for the optical biopsy approach can now be transferred to procedures involving multispectral imaging. The objective autofluorescence methods will provide an important auxiliary tool assisting and facilitating the correct diagnosis although their diagnostic merit still has to be proven in properly performed randomized studies.

References

[1] Stewart, B., W. and Kleihues P. (Eds.) 2003, World Cancer Report, IARC Press, Lyon..

[2] Morgan, W. D., Forssmann, U., F. and Nakada M.T. 2004, Cancer and Inflammation, Birkhaeuser Verlag, Basel, Boston, Berlin.

[3] Coleman, W., B. and Tsongalis, G., J. 2002, The Molecular Basis of Human Cancer, Humana Press, Totowa, New Jersey.

[4] Pradhan, A., Pal, P., Durocher, G., Villeneuve, L., Balassy, A., Babai, F., Gaboury, L. and Blanchard, L. J. 1995, Photochem. Photobiol. B: Biol., 31, 101.

[5] Richards-Kortum, R. and Sevick-Muraca, E., S. 1996, Ann. Rev. Phys. Chem., 47, 555.

[6] Dellinger, M., Geze, M., Santus, R., Kohen, E., Kohen, C., Hirschberg, J., G. and Monti, M. 1998, Biotechnol. Appl. Biochem. 28, 25.

[7] Andersson, H., Baechi, T., Hoechl, M. and Richter, C. 1998, J. Microsc., 191, 1.

[8] Esposti, M., D., Hatzinisiriou, I., Mc Lennan, H. and Ralph S. 1999, 274, 29381.

[9] Pitts, J., D., Sloboda, R., D., Dragnev, K., H., Dmitrovsky, E. and Mycek, M.-A. 2001, J. Biomed. Optics, 6, 31.

[10] Sud, D., Zhong, W., Beer, D., W. and Mycek M.-A. 2006, Optics Express, 14, 4412.

[11] Salmon, J.-M., Kohen, E., Viallet, P., Hirschberg, J., G., Wouters, A., W., Kohen C. and Thorell, B. 1982, Photochem. Photobiol., 36, 585.

[12] Galeotti, T., van Rossum, G.D.V., Mayer, D., H. and Chance, B. 1970, Eur. J. Biochem., 17, 485.

[13] Schuchmann, S., Kovacs, R., Kann, O., Heinemann, U. and Buchheim, K.2001, Brain Res. Protocols, 7, 267.

[14] Izuishi, K., Tajiri, H., Fujii, T., Boku, N., Ohtsu, A., Ohnishi, T., Ryu, M., Kinoshita, T. and Yoshida, S. 1999, Endoscopy, 31, 511.

[15] Banerjee, B., Miedema, B., E. and Chandrasekhar, H., R. 1999, J. Invest. Med., 47, 326.

[16] Tanhuanpaa, K., Virtanen, J. and Somerharju, P. 2000, Biochim. Biophys. Acta, 1497, 308

[17] Elleder, M. and Borovansky J. 2001, Histochem. J., 33, 273.

[18] Da Costa, R., S., Andersson., H., Cirocco, M., Marcon, N., E. and Wilson, B., C. 2005, J. Clin. Pathol., 58, 766.

[19] Rost, F.W.D. 1995, Fluorescence microscopy, Cambridge University Press, Cambridge.

[20] Wolfbeis, O.S. 1985 Molecular luminescence spectroscopy. Methods and Applications: Part 1, S.G. Schulman (Ed.), John Wiley&Sons, New York.

[21] DaCosta, R., Andersson, H. and Wilson, B., C. 2003, Photochem.Photobiol., 78, 384.

[22] Monici, M., Pratesi, R., Bernabei, P., A., Caporale, R., Ferrini, P., R., Croce, A., C., Balzarini, P. and Bottiroli G.1995, J. Photochem. Photobiol. B: Biol., 30, 29.

[23] Samoszuk, M., K. and Espinoza F., P. 1987, Blood, 70, 597.

[24] Barnes, D., Aggarwal, S., Thomsen, S., Fitzmaurice, M. and Richards-Kortum R. 1993, Photochem. Photobiol., 58, 297.

[25] Wilson, B., C., Jeeves, W., P. and Lowe, D., M. 1985, Photochem.Photobiol., 42, 53.

[26] Qu, J., MacAulay, S. and Palcic, B. 1995, Optical Enginnering, 34, 3334.

[27] Zeng, H., MacAulay, C., McLean, D., I. and Palcic, B. 1995, Photochem. Photobiol., 61, 639.

[28] Zeng, H., MacAulay, C., McLean, D., I. and Palcic, B. 1997, J. Photochem. Photobiol.B: Biol., 38, 234.

[29] Kumar, G. and Schmidt, J., M. 1997, Appl. Optics, 36, 2286.

[30] Contini, D., Zaccanti, G., Martelli, F. and Sassaroli, A. 1997, Physica Scripta, T72, 76.

[31] Gandjbakhche, A., H., Bonner, R., F., Nossal, R. and Weiss, G. H. 1997, Appl. Optics, 36, 4613

[32] Pogue, B., W. and Burke, G. 1998, Appl. Optics, 37, 7429.

[33] Viswanath, K., Pogue, B. and Mycek, M.-A. 202, Phys. Med. Sci., 47, 3387.

[34] Alfano, R., R., Tang, G., C., Pradhan, A., Lam, W., Choy D., S., J. and Opher E. 1987, IEEE J. Quant. Electr., QE-23, 1806.

[35] Yuanlong, Y., Yanming, Y., Fuming, L., Yufen, L. and Paozhong, M. 1987, Lasers Surg. Med., 7, 528.

[36] Yakshe, P., N., Bonner, R., F., Cohen, P., Leon, M., B. and Fleischer, D., E. 1989, Gastrointest. Endosc., 35, 184.

[37] Kapadia, C., R., Cutruzzola, F., W., O'Brien, K., M., Stetz, M., L., Enriquez, R.and Deckelbaum, L.,I. 1990, Gastroenterology, 99, 157.

[38] Schomacker, K., T., Frisoli, J., K., Compton, C., C., Flotte, T., J., Richter, J., M., Nishioka, N., S. and Deutsch, T., F. 1992, Lasers Surg. Med. 12, 63.

[39] Richards-Kortum, R., Rava, R., P., Petras, R., E., Fitzmaurice, M., Sivak, M. and Feld, M., S. 1991, Photochem Photobiol., 53, 777.

[40] Chwirot, B., W., Kowalska, M., Płóciennik, N., Piwiński, M., Michniewicz, Z. and Chwirot, S. 2003, Ind. J. Exp. Biol.41, 500.

[41] Glassman, W.,S., Liu, C., H., Tang, G.,C., Lubicz, S. and Alfano, R.,R. 1992, Lasers Life Sci. 5, 49.

[42] Ramanujam, N., Mitchell, M., F., Mahadevan-Jansen, A., Thomsen, S., L., Staerkel, G., Malpica, A., Wright, T., Atkinson, N. and Richards-Kortum, R. 1996, Photochem. Photobiol., 64, 720.

[43] Hirano, T., Ishizuka, M., Ishida, K., Suzuk,i K. and Miyaki S. 1989, Lasers Life Sci. 3, 1.

[44] Palcic, B., Lam, S., Hung, J. and MacAulay, C. 1991, Chest, 99, 742

[45] Wang, T., D., Van Dam, J., Crawford, E., A., Preisinger, E., A., Wang, Y.and Feld, M.,S. 1996, Gastroeneterology, 111, 1182.

[46] Chwirot, B., W., Chwirot, S., Jędrzejczyk, W., Jackowski, M., Raczyńska, A., M., Winczakiewicz, J.and Dobber, J. 1997, Lasers Surg. Med., 21, 149.

[47] Chwirot, B., W., Chwirot, S., Redziński, J.and Michniewicz, Z. 1998, Eur. J. Cancer, 34, 1730.

[48] Kobayashi, M., Tajiri, H., Seike, E., Shitaya, M., Tounou, S., Mine, M.and Oba, K. 2001, Cancer Lett., 165, 155.

[49] Chwirot, B.,W., Kowalska, M., Sypniewska, N., Michniewicz, Z.and Grądziel, M., 1999, J. Photochem.Photobiol. B: Biol. 50, 174.

[50] Andersson – Engels, S., Canti, G., Cubeddu, R., Eker, C., Klinteberg, A., Pifferi, A., Svanberg, K., Svanberg, S., Taroni, P., Valentini, G.and Wang I. 2000, Lasers Surg. Med., 26, 76.

[51] Hewett, J., Nadeau, V., Ferguson, J., Moseley, H., Ibbotson, S., Allen, J., W., Sibbett, W. and Padget, M. 2001, Photochem. Photobiol., 73, 278.

[52] Ramanujan, N., Chen, J., X., Gossage, K., Richards – Kortum, R.and Chance, B. 2001, IEEE Trans. Biomed. Eng. 48, 1034.

[53] Nakaniva, N., Namihisa, A., Ogihara, T., Ohkawa, A., Abe, S., Nagahara, A., Kobayashi, O., Sasaki, J. and Sato, N. 2005, Digest. Endoscopy, 17, 235.

[54] Mycek, M.-A., Schomacker, K., T. and Nishioka, N., S. 1998, Gastrointest. Endoscopy, 48, 390.

[55] Urayama, P., Zhong, W., Beamish, J., A., Minn, F., K., Sloboda, R., D., Dragnev, K., H., Dmitrovsky, E. and Mycek, M.-A. 2003, Appl. Phys. B, 76, 483.

[56] Munro, I., McGinty, J., Galletly, N., Requejo-Isidro, J., Lanigan, P., M., P., Elson, D., S., Dunsby, C., Neill, M., A., A., Lever, M., J., Stamp, G., W., H. and French, P., M., W. 2005, J. Biomed.Optics, 10, 051403-1

[57] Abe, S., Izuishi, K., Tajiri, H., Kinoshita, T. and Matsuoka, T. 2000, Endoscopy, 32, 281.

[58] Marchesini, R., Brambilla, M., Pignoli, E., Bottiroli ,G., Croce, A., C., Dal Fante, M., Spinelli, P.and Di Palma S., J. 1992, Photochem. Photobiol. B: Biol. 14, 219.

[59] 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.and Feld, M.,S. 1996, Gastrointest.Endosc., 44, 168.

[60] Chwirot, B., W., Michniewicz, Z., Kowalska, M.and Nussbeutel, J. 1999, Photochem. Photobiol. 69, 336.

[61] Wang, t., D., Crawford, J., M., Feld, M., S., Wang, Y., Itzkan, I. and Van Dam, J. 1999, 49, 447.

[62] Eker, C., Montan, S., Jaramillo, E., Koizumi, K., Rubio, C., Andersson- Engels, S., Svanberg, K., Svanberg, S. and Slezak, P. 1999, Gut, 44, 511.

[63] Chich-Yu, W., Jen-Kou, L., Be-Fong, C. and Kenny, C., H. 1999, J. Formosan Med. Ass. 98, 837.

[64] Brand, S., Stepp, H., Ochsenkuehn, T., Baumgartner, R., Baretton, G., Holl, J., von Ritter, C., Baumgartner, G.and Sackmann, M. 1999, Int. J. Colorect Dis. 14, 63.

[65] Haringsma, J., Tytgat, G., N., J., Yano, H., Iishi, H., Tatsuta, M., Ogihara, T., Watanabe, H., Sato, N., Marcon, N., Wilson, B., C. and Cline, R., W. 2001, Gastrointest. Endosc. 53, 642.

[66] Bottiroli, G., Dal Fante, M., Marchesini, R., Croce, A.C., Cuzzoni, C., Di Palma, S., Pignoli, E. and Spinelli P. 1992, Photodynamic Therapy and Biomedical Lasers, P. Spinelli, M. Dal Fante and R. Marchesini (Eds.), Elsevier Science Publishers, New York, 898.

[67] Marchesini, R., Pignoli, E., Tomatis, S., Fumagalli, S., Sichirollo, A., E., Di Palma, S., Dal Fante, M., Spinelli P., Croce, A. and Bottiroli G. 1994, Lasers Surg. Med. 15, 351.

[68] Firman, G., S., Nathanson, M. H., West, B., Deckelbaum, L., I., Kelly, L. and Kapadia, C., R. 1995, Digest.Sci., 40, 1261.

[69] Bottiroli, G., Croce, A., C., Locatelli, D., Marchesini, R., Pignoli, E., tomatis, S., Cuzzoni, C., Di Palma, S., Dalfante, M. and Spinelli, P. 1995, Lasers Surg.Med., 16, 48.

[70] Roemer, Tj., J., Fitzmaurice, M., Cothren, R., M., Richards-Kortum, R., Petras, R., Sivak, M., V. and Kramer, J., R. 1995, Am. J. Gastroeneterology, 90, 81.

[71] Zonios, G., I., Cothren, R., M., Arendt, J., T., Wu, J., Van Dam, J., Crawford, J., M., Manoharan, R. and Feld, M.S. 1996, IIIE Trans. Biomed. Eng., 43, 113.

[72] Banerjee, B., Miedema, B. and Chandrasekhar, H., R. 1998, Am. J. Med., 16, 220.

[73] Bohorfoush, A., G. 1996, Endoscopy, 28, 372.

[74] Stepp, H., Sroka, R.and Baumgartner, R. 1998, Endoscopy, 30, 379.

[75] Prosst, R., L. and Gahlen, J. 2002, Int. J. Colorectal Dis. 17, 1.

[76] Profio, A. E., Balchum, O., J. and Huth, G., C. 1982, Med. Phys. 10, 35.

[77] Lam S., Hung J., MacAulay C., Jaggi B., Palcic B. IEEE **12**, 1990, 1142.

[78] Lam, S., MacAulay, C., Hung, J., LeRiche, J., Profio, A., E. and Palcic, B. 1993, J. Thorac. Cardiovasc. Surg.105, 1035.

[79] Lam, S., Kennedy, T., Unger, M., Miller, Y., E., Gelmont, D., Rusch, V., Gipe, B., Howard, D., LeRiche, J., C., Coldman, A. and Gazdar, A., F. 1998, Chest, 113, 696.

[80] Kusunoki, Y., Imamura, F., Uda, H., Mano, M. and Horai T. 2000, Chest, 118, 1776.

[81] Sheski, F., D. and Mathur, P., N. 2000, Cancer Control, 7, 35.

[82] Sutedja, T., G., Codrington, H., Risse, E., K., Breuer, R., H., van Mourik, J., C., Golding, R., P. and Postmus P., E.2001, Chest, 120, 4.

[83] Venmans, B., J., W., van Boxem, T., J., M., Smit, E., F., Postmus, P., E. and Sutedja, T., G. 2001, Ann. Otol. Rhinol. Laryngol.,110, 635.

[84] de Veld, D., C., G., Witjes, M., J., H., van der Waal, J., E., Sterenborg, H., J., C., M. and Roodenburg, J., L., N. 2005, Oral Oncol. 41, 117.

[85] Katz, A., Savage, H., E., Scantz, S., P., McCormick, S., A. and alfano, R., R. 2002, Technol. Cancer Res. Treatm. 1, 9.

[86] Harries, M., L., Lam., s., Macaulay, C., Qu, J. and Palcic, B. 1995, J. Laryngol. Otol. 109, 108.

[87] Kulapaditharom, B. and Boonkittichaeroen, V. 1998, Ann. Otol. Rhinol. Laryngol.,107, 241.

[88] Zargi, M., smid, L., Fajdiga, I., Bubnic, B., Lenarcic, J. and Oblak, P. 1997, Acta Otolaryngol. (Stockh), S527, 125.

[89] Zargi,. J., Fajdiga, I. and Smid, L. 2000, Eur. J. Otorhinolaryngol., 257, 17.

[90] Qu, J., Y., Wing, P., Huang, Z., Kwong, D., Sham, J., Lee, s., L., Ho, W., K. and Wei, W., I. 2000, Lasers Surg. Med., 26, 242.

[91] Panjehpour, M., Overholt, B., F., Schmidhammer, J., L., Farris, C., Buckley, P., F. and Vo-Dinh, T. 1995, Gastrointest. Endoscopy, 41, 577.

[92] Panjehpour, M., Overholt, B., F., Vo-Dinh, T., Haggit, R.C., Edwards, D., H. and Buckley III, F., P. 1996, Gastroenteroly, 111, 93.

[93] Messmann, H. 1999, Acta Endoscopica, 29, 583.

[94] Niepsuj, K., Niepsuj, G., Cebula, W., Zieleznik, W., Adamek, M., Sielanczy-Adamczyk, J., Kurek, J. and sieron, A. 2003, Gastrointest. Endosc. 58, 715.

[95] Scantz, S., P., Savage, H., E., Sacks, P. and Alfano, R., R. 1997, Environ. Health Persp. 105(Suppl 4), 941.

[96] Gillenwater, A., Jacob, R. and Richards-Kortum, R. 1998, Head Neck, 20, 556.

[97] D'Hallewin, M., A., Baert, L. and Vanherzeele, H. 1994, J. Am. Parapleg. Soc., 17, 161.

[98] Anidjar, M., Cussenot, O., Blais, J., Bourdon, O., Avrillier, S., Ettori, D., Villete, J.-M., Fiet, J., Teillac, P. and Le Luc, A. 1996, T. Urol., 155, 1771.

[99] Avrillier, S., Ettori, D. and Anidjar, M. 1996, Abstracts of 28th EGAS Conference, L. Windholz (Ed.), Graz.[100] Anidjar, M., Ettori, D., Cussenot, O., Meria, P., Desgrandchamps, F., Cortesse, A., Teillac, P., Le Duc, A. and Avrillier. S. 1996, J. Urol., 156, 1590.

[101] Avrillier, S., Tinet, E., Ettori, D. and Anidjar, M. 1997, Physica Scripta, T72, 87.

[102] Koenig, F., McGovern, F., J., Althausen, A., F., Deutsch, T., S. and Schomacker, K., T. 1996, J. Urol., 156, 1597.

[103] Koenig, F., McGovern, F., J., Enquist, H., Larne, R., Deutsch, T., F. and Schomacker, K., T. 1998, J. Urol., 159, 1871.

[104] Zaak, D., Stepp, H., Baumgartner, R., Schneede, P., Waidlich, R., Frimbe, D., artmann, A., Kunchel, R., Hofstetter, A. and Hohla, A. 2002, Urology, 60, 1029.

[105] Zheng, W., Lau, W., Cheng, C., Soo, K., C. and Olivo, M. 2003, Int. J. Cancer, 104, 477.

[106] Parekh, D., J., Lin, W.-C. and Herrell, D. J. Urol., 174, 1754.

[107] Mera, S., L. 1995, Brit. J. Biomed.Sci., 52, 120.

[108] Koss, L., G. 1993, Cancer, 71, 1406.

[109] Mitchell, M., F. 1994, Consult. Obstet Gynecol., 6, 70.

[110] Mahadevan, A., Mitchell, M., F., Silvoa, E., S., Thomsen, S. and Richards-Kortum, R. 1993, Lasers Surg. Med., 13, 647.

[111] Ramanujam, N., Mitchell, M., F., Mahadevan, A., Warren, S., Thomsen, S., Silva, E. and Richards-Kortum, R. 1994, Proc. Natl. Acad. Sci. USA, 91, 10193.

[112] Ramanujam, N., Mitchell, M., F., Mahadevan, Thomsen, S., Malpica, A., Wright, T., Atkinson, N. and Richards-Kortum, R. 1996, Lasers Surg. Med., 19, 46.

[113] Ramanujam, N., Mitchell, M., F., Mahadevan, Thomsen, S., Malpica, A., Wright, T., Atkinson, N. and Richards-Kortum, R. 1996, Lasers Surg. Med., 19, 63.

[114] Agrawal, A., Utzinger, U., Brookner, C., Pitris, C., Mitchell, M., F. and Richards-Kortum, F. 1999, Lasers Surg. Med. 25, 237.

[115] Brookner, C., K., Follen, M., Boiko, J., Galvan, J., Thomsen, S., Malpica, A., Suzuki, S., Lotan, R. and Richards-Kortum, R. 2000, Photochem.Photobiol. 71, 730.

[116] Chang, S., K., Dawood, M., Y., Staerkel, G., Utzinger, U., Atkinson, E., N., Richards-Kortum, R., R. and Follen, M. 2002, J. Biomed. Optics, 7, 595.

[117] Chen, C., T., Huang, C., C., Chen, R., J., Lin, Y., H., Chiang, H., H., Wang, C., Y., Lee, Y., S. and Chow, S., N. 1997, Formos. Med. Assoc., 96, 247.

[118] Koumantakis, E., Vasileiou, A., Makrigiannakis, A., Unsoeld, E.and Papazoglou, T., G. 1997, J. Photochem. Photobiol. B: Biol., 40, 183.

[119] Grin, C., M., Kopf, A., W., Welkovich, B., Bart, R.,S. and Levenstein M., J. 1990, Arch. Dermatol., 126, 763.

[120] Leffel, D., J., Chen, Y., T., Berwick, M. and Bologna, J., L. 1993, J. Am. Dermatol., 28, 1003.

[121] Lohmann, W. and Paul, E. 1988, Naturwissenschaften, 75, 201

[122] Lohmann, W., Nilles, M. and Boedeker R., H. Naturwissenschaften, 78, 456. [123] Sterenborg, H., J., C., M., Thomsen, S., Jacques, S.,L. and Motamedi, M. Melanoma Res. 5, 1995, 211

[124] Ebert, B., Kohl, M., Sukowski, U., Rinneberg, H., Winter, H., Bellmann, K.-P. and Audring, H. 1988, Lasers Med. Sci. 13, 204.

[125] Chwirot, B., W., Chwirot, S., Sypniewska, N., Redziński, J., Michniewicz, Z., Kurzawski, G. and Ruka, W., J. 2001, Invest. Dermatol. 117, 1449.

[126] Paraskevas, L.-R., Halpern, a., C. and Marghoob, A., A. 2005, Brit. J. Dermatol., 135, 1031.

[127] Andersson-Engels, S., Canti, G., Cubeddu, R., Eker, C., Klintenberg, C., Pifferi, A., Svanberg, K., Svanberg, S., Taroni, P., Valentini, G. and Wang, I. 2000, Lasers Surg. Med. 26, 76.

[128] Brancaleon, L., Durkin, A., J., Tu, J., H., Menaker, G., Fallon, J., D. and Kollias, N. 2001, Photochem.Photobiol., 73, 178.

[129] Hewett, J., Nacleau, V., Ferguson, J., Moseley, H., Ibbotson, S., Allen, J., W., Sibbet, W. and Padgett, M. 2001, Photochem. Photobiol., 73, 278.

[130] Gupta, P., K., Majumder, S., K. and Uppal, A. 1997, Lasers Surg. Med. 21, 417.

[131] Hage, R., Galhanone, P., R., Zangaro, R., A., Rodrogues, K., C., Pacheco, M., T., T., Martin, A., A., Netto, M., M., Soares, F., A. and da Cunha, I., W. 2003, Lasers. Med. Sci., 18, 171.

[132] Mueskens, R., P., H., van Best, J., A., Bleeker, J., C. and Keunen J., E., E. 2001, Br. J. Ophtalmol., 85, 662.

[133] Izuishi, K., Tajiri, H., Furuse, J., Maru, Y., Inoue, K., Konishi, M. and Kinoshita, T. 1999, Hepato-Gastroenterology, 46, 804.

[134] Lin, W.-C., Toms, S., A., Johnson, M., Jansen, E., D. amd Mahadevan-Jansen, A. 2001, Photochem. Photobiol., 73, 3.