

OPTICAL BIOPSY – TOOL FOR INITIAL CANCER DIAGNOSIS AND MONITORING OF THERAPY

E. Borisova^{1*}, E. Pavlova², P. Troyanova², B. Nikolova³, I. Tsoneva³

¹Institute of Electronics, Bulgarian Academy of Sciences, Sofia, Bulgaria; ²University Hospital “Queen- Jiovan-na- ISUL”, Sofia, Bulgaria; ³ Institute of Biophysics and Biomedical Engineering, BAS, Sofia, Bulgaria

Abstract

Our investigations are a part of a clinical trial for introduction of optical biopsy spectral diagnostic system for skin cancer detection. We apply autofluorescence and diffuse reflectance spectroscopy to several different classes of malignant non-melanoma cutaneous lesions. Autofluorescence spectroscopy is very attractive tool for early diagnosis of cancer due to its high sensitivity, possibilities for real time measurements and noninvasive tumor detection. However, when the lesion is highly pigmented diffuse reflectance spectroscopy is applied, including malignant melanoma diagnosis.

Skin optical biopsy diagnostic clinical trial is currently under implementation and we expect to receive objective tool for detection and evaluation of skin lesion type, which could become a basis for reliable system for skin cancer detection. Based on the results obtained during initial diagnosis we start additional task – to monitor the lesion treatment process, where the non-invasive character of the optical biopsy procedure is acclaimed by the patients. Autofluorescence detection is applied for monitoring of electrochemotherapy of tumours. The therapeutic procedure itself - electrochemotherapy (ECT) combines chemotherapy and electroporation to increase locally anticancer drug delivery into the cancer cells.

The origins of diagnostically significant spectral peculiarities are evaluated on more than 400 patients up to now and possible differentiation features useful for skin cancer detection and evaluation of their stage and sub-type are discussed in our report.

Introduction

Biomedical optics is one of the fastest growing areas of research. The non-ionizing nature of light applied for investigation and detection of abnormalities in human tissues make this area very attractive for development of new diagnostic techniques and modalities [1]. The optical spectra provide biochemical and morphological information about the tissue under

* Presenting author: borisova@ie.bas.bg

investigation based on its absorption, reflectance, fluorescence and elastic scattering properties [1, 2].

Optical biopsy technique is a term used in the last few years in biophotonics, as a parallel with the standard histological sampling of the tissues under investigation. It could be any optical technique, which give diagnostic information about the nature and type of given pathology, but mostly is used for fluorescence and reflectance spectroscopy of biological tissues. Promises of this technique for rapid, non-invasive, highly-sensitive detection of tissue changes allow to detect morphological and biochemical changes occurred in early stages of pathology development [1-3].

Tissue autofluorescence, where signal from naturally occurred fluorophores is detected is absolutely non-invasive and extremely sensitive technique, which theoretically could detect single tumor cell, appeared in this tissue. Problems related to its applicability are due to not enough sensitivity of the detectors applied and appearance of superposition of fluorescent signals from variety of compounds in the tissue under investigation. Autofluorescence spectra are observed mainly in blue-green spectral region for the most of the human tissues. The overlapping of the absorption and emission spectra of the most of diagnostically important compounds leads to slight differences in the observed fluorescence spectra for different pathological conditions and variations from patient to patient, due to significant dependence from the anatomic area investigated, general health status, ages, sex, even from the light sources and detectors applied [1-5]. For highly pigmented pathologies autofluorescence spectroscopy has low specificity and suboptimal diagnostic value [6]. This diagnostic modality is extremely sensitive on early changes of the tissues and if proper differentiation algorithms are applied, significant diagnostic information could be extracted from the spectra detected [1-6].

In general the fluorescence detection gives a possibility for real-time, noninvasive diagnosis of tumors and other tissue pathologies with high sensitivity on early stage of the lesion growth. Autofluorescence spectroscopy is used for investigation of different skin lesions, as well as for differentiation between malignant and benign cutaneous neoplasia. Fluorescent technique is also widely applied for other cutaneous lesions' investigations, including erythema [5], psoriasis, and vitiligo [1, 4, 7]. This method gives information about biochemical composition of the tissue under study.

Diffuse reflectance spectroscopy from other side is responsible mainly about morphological information, which could be received from the tissues. Scattering intensity and spectral distribution of the signals detected could give information about scatterers' size and

distribution (cells, nuclei, etc.). As the detected diffuse reflectance signal is superposition from diffuse scattering and absorption from tissues' pigments, the resultant spectrum also reveal information about main absorbers in the biological tissues, like hemoglobin and melanin in the skin and its pathologies [2, 6]. Diffuse reflectance in the field of dermatology has been applied to evaluate skin color and erythema doses, as well as for skin cancer diagnosis [7]. Reflectance spectra could be used for quantitative and qualitative evaluation of major tissue pigments, such as melanin, hemoglobin, oxy-hemoglobin, bilirubin [1, 8].

There are some attempts to introduce such diagnostic systems into standard clinical practice, such as fiber-based fluorimeter - SkinScan system (JobinYvon, France), where fluorescence of endogenous aminoacids is used for cutaneous lesions' investigations [9], or more recently developed DYADERM system (Biocam GmbH, Germany), which is applied for photodynamic diagnosis with exogenous photosensitizers [10]. However, up to our days there is no such universal clinical apparatus, based on autofluorescence and/or reflectance detection of skin surface, which could be used as a general tool for early cancer detection and differentiation. The reasons for such instrument absence in the field of clinical equipment based on the optical biopsy of skin cancer are very complex.

Problems for development of such reliable universal diagnostic fluorescence system for skin cancer detection are related to the great variety of benign and malignant forms of cutaneous pathologies, for example basal cell carcinoma lesions have more than 15 sub-types, squamous cell carcinoma lesions, have about 10 different subtypes, and all of them have variety of benign and dysplastic forms. Positive is the fact that due to these changes, depending on the lesion growth, we could use light-induced autofluorescence spectroscopy (LIAFS) for evaluation of the lesion stage, negative is that we will need to compare this exact situation with great variety of other possibilities, such as lesion kind, stage of growth, and even patient skin general conditions, such as influence of medicines, ages, cutaneous phototype, typical work conditions, etc.

However, that variety of spectral information, which we could obtain from different skin pathologies and its diversity, does not mean that we could not use optical biopsy, as a tool for early cancer detection. Moreover, exactly due to high sensitivity of fluorescence, LIAFS could be applied, as a very precise tool for initial diagnosis, for planning, and monitoring of therapeutic procedures. In the current report we will present several practical applications of LIAFS system as an initial diagnostic tool, as well as a tool for therapeutic monitoring and a decision tool for treatment planning.

Materials and Methods

This investigation is a part of a clinical trial for introduction of spectral diagnostic system for skin cancer detection in the daily practice of the dermatological department of University Hospital “Queen Jiovanna” [8], as well as for monitoring of electrochemotherapy procedures in the frames of National Oncological Center. Autofluorescence and diffuse reflectance spectroscopy are applied to several different classes of malignant non-melanoma cutaneous lesions. Initially, they were classified visually and dermatoscopically. Second step was detection of lesion’ and surrounding normal skin autofluorescence and reflectance spectra, using different excitation wavelengths, namely 365, 385, and 405 nm for the first technique – narrow-band light-emitting diodes (LEDs) and broad-band halogen lamp for irradiation in the region of 400-900 nm for the second technique. In the end for every lesion histological examination is used as a “gold standard” for all our investigations. The spectra and dermatoscopic evaluations were obtained from more than 400 patients up to now. Spectral properties of variety of benign cutaneous lesions are also evaluated for development of more precise discrimination algorithms for diagnosis of cancer lesions. The origins of diagnostically significant spectral features are evaluated and differentiation schemes are developed.

Optical fiber probe is used to deliver the light from LEDs and lamp and to collect the fluorescence signals from the skin surface. It consists of 7 fibers in circular geometry. Central fiber is used for autofluorescence and reflectance signal detection and it is connected to microspectrometric system and surrounding six fibers are used for delivery of excitation/illumination light from the LEDs and lamp to the skin under investigation.

Both kinds of spectra – autofluorescence signals and diffuse reflectance signal are recorded and stored using a fiber-optic microspectrometer (USB4000, Ocean Optics, Dunedin, FL, USA). A personal computer is used to control the system and to store and display the data using the specialized microspectrometer software OOI Base (“Ocean Optics”, Inc., Dunedin, FL, USA). Normal tissue spectra detected are used as a basis for comparison with the pathologies observed.

Results and discussion

Typically the patients observed have one cutaneous tumour which needed to be diagnosed. Major trends in the autofluorescence signals obtained are related to changes in the fluorescence intensity, as well as appearance of secondary fluorescent maxima, depending

from the tumour subtype, and observation of increased absorption from the major skin pigments – melanin and hemoglobin, related to the tissue conditions. Basal cell carcinoma (BCC) lesions have decreased fluorescence intensity than surrounding normal skin, squamous cell carcinoma (SCC) in opposite, has revealed fluorescence intensity usually comparable and higher than that of normal skin tissues. Compounds, which fluoresce are collagen type I – at 400-405 nm; its cross-links – at 460 – 490 nm; elastin – with maxima at 400-420, 460 nm; elastin cross-links – about 500 nm; NADH – at 440-470 nm; keratin – at 430 – 460 nm, and around 500-520 nm, and flavins.

Reflectance spectra have not many specific spectral shape changes and were used in comparison of melanin-pigmented pathologies, such as dysplastic nevi and malignant melanoma lesions, where the reflectance was applied as additive diagnostic tool to the autofluorescence measurements, which improve the diagnostic accuracy of the diagnostic procedure. Reflectance spectra also reveal some specific features, useful for differentiation algorithms development for benign and malignant cutaneous lesions. Significant differences are observed in comparison of papilloma, keratocanthoma, BCC and SCC, as well as in comparison with other reflectance data, including amelanotic malignant melanoma lesions and atypical nevi, which could be misdiagnosed very easily in the process of initial clinical examination.

In the cases of advanced BCC lesions, a red fluorescence, related to endogenous porphyrins accumulation is also observed. This feature is useful and has been applied in our clinical practice for development of treatment planning for patients, having multiple BCC lesions, which clinical condition does not allow simultaneously treatment of all pathologies. We planned our treatment according received fluorescence data for the lesions, which correlate with the stage of growth and severity of tumour itself.

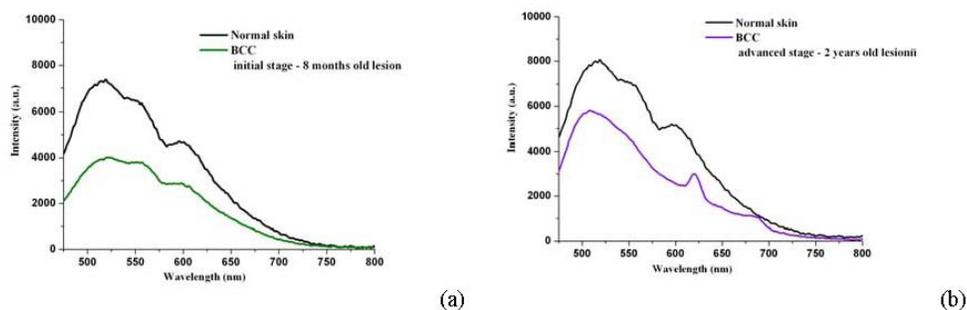


Figure 1. Fluorescence spectra of normal skin and two BCC lesions of one patient, second lesion has appeared about eight months before the light-induced fluorescence measurements carried out (a) and the first about two years before the observation (b)

Convenient fact is that when we compare spectra of multiple lesions from same kind in one patient, we do not need to develop compensation procedures, related to inter-patient differences. Based on fluorescence spectra results initially more advanced lesion was treated, and after patient recover – initial tumour was also treated. The benefits for treatment planning, using fluorescence data from the lesions are very obvious when the patient has multiple lesions on different stage of growth. For example, such “porphyrin-like” signals in advanced stages of the BCC lesions allow developing mixed treatment plan for one 69 years old patient. Based on the fluorescence spectra obtained, lesions #2 and #5 (see figure 2), which were on the most advanced stage, where surgically removed and chemotherapy was applied as well. Lesion #4 – intermediate stage was treated using chemo- and radiotherapy, and lesions #1 and #3 – initial stage – where treated using local chemotherapy 3 months later.

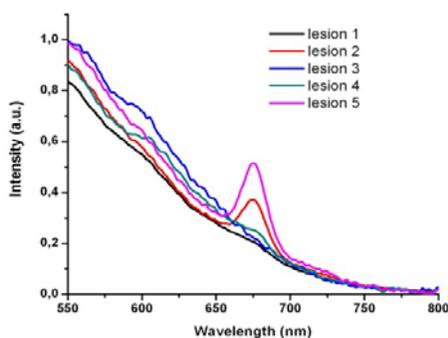


Figure 2. Red part of the fluorescence signal of BCC lesions on one patient, having multiple BCC tumours on different stage of growth. Excitation at 405 nm is applied.

Autofluorescence detection is applied for monitoring of electrochemotherapy of tumours as well. The therapeutic procedure itself - electrochemotherapy (ECT) combines chemotherapy and electroporation to increase locally cytostatic drug delivery in the cancer cells. The electroporator is battery supplied, associated with isolated ECG signals amplifier, QRS detection and synchronization circuits. The injection of local anesthetic (1% lidocain) and cytostatic drug (Bleomycin®) in very small concentration direct to the tumor lesion, which is followed by application of electrical pulses. Drug delivery conditions (electric field intensity) and dose of cytostatic drug are personal for every single case. To monitor the effects of application of the electrochemotherapy fluorescence spectra are taken from the lesion and surrounding healthy skin, prior to, immediately after treatment and at the control check-ups.

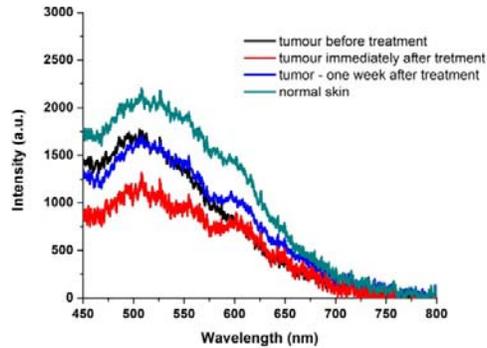


Figure 3. Fluorescence spectra of normal skin, BCC lesion before and immediately after ECT procedure, as well as one-week follow up of the lesion.

Patients are followed up at the first week after treatment, the first month and third month. On the fig.3 are presented results from such therapeutic monitoring, using 365 nm excitation of BCC lesion and normal skin. BCC tumour has lower intensity than normal tissue. It is clearly observed immediate reaction after therapeutic procedure application – appearance of specific minima at 543 and 575 nm, related to increased hemoglobin absorption. One week later the fluorescence intensity of the lesion area is higher and approach to the “normal skin” spectral shape, which is indication for successful treatment of the tumour.

Conclusion

It was received a good correlation between histological analysis of the skin and repeatability of the features of the fluorescence and reflectance signals from patient to patient with one-type lesion obtained. Although the number of reported cases does not permit us to create general diagnostic algorithms for all variety of cutaneous tumours, on the base of the observed spectral changes, results of the present study suggest that the used approach, related to creation of algorithms between specific wavelengths of obtained lesions and normal skin spectra can provide useful information on the given lesions, that could be transformed into diagnostic algorithms for clinical usage. Clinical trial is currently under implementation and with broadening of the database with fluorescence and reflectance spectra of major skin benign and malignant pathologies we expect to receive objective tool for detection and evaluation of skin lesion type, as well as noninvasive treatment monitoring modality, based on tissues’ spectral properties.

Acknowledgements

This work is supported by the National Science Fund of Bulgarian Ministry of Education, Youth and Science under grant MU-03-46/2011 “Development and introduction of optical biopsy for early diagnosis of malignant tumours” and EU COST Action TD 1104 “European network for development of electroporation-based technologies and treatments (EP4Bio2Med)”.

References

1. Wang L., Wu H. Biomedical optics: principles and imaging, Wiley-Interscience, 2007.
2. Tuchin V., Handbook of Biomedical Diagnostics, Bellingham: SPIE Press, 2002.
3. Svanberg S. Environmental and medical applications of photonic interactions, *Physica Scripta*2004; T110:39-51.
4. Sinichkin Yu, Utz S, Mavliutov A, Pilipenko H. In vivo fluorescence spectroscopy of the human skin: experiments and models. *J Biomed Opt*1998; 3:201-211.
5. Borisova E, Troyanova P, Avramov L. Fluorescence spectroscopy for early detection and differentiation of cutaneous pigmented lesions, *OAM-RC* 2007; 1:388-394.
6. Kollias N, Zonios G, Stamatas G. Fluorescencespectroscopyofskin, *Vibrational Spectroscopy* 2002; 28:17 - 23.
7. Marchesini R, Bono A, Baroli C, Lualdi M, Tomatis S, Cascinelli N. Optical imaging and automated melanoma detection: questions and answers, *Melanoma Res* 2002; 12:279-286.
8. Pavlova P, Borisova E, Avramov L, Petkova E, Troyanova P. Investigation of Relations Between Skin Cancer Lesions‘ Images and Their Reflectance and Fluorescent Spectra, *Melanoma in the Clinic - Diagnosis, Management and Complications of Malignancy*, Prof. Mandi Murph (Ed.), InTech, 2011; ch. 6:87-104.
9. Valeur B. From well-known to underrated applications of fluorescence, in *Fluorescence of supermolecules, polymers and nanosystems*, Springer, 2008; 4(A):21-43.
10. de Leeuw J, van der Beek N, Neugebauer WD, Bjerring P, Neumann HA. Fluorescence detection and diagnosis of non-melanoma skin cancer at an early stage, *Las Surg Med*, 2009; 41(2):96-103.