

DEVELOPMENT AND INTRODUCTION OF OPTICAL BIOPSY SYSTEM FOR EARLY DIAGNOSTIC OF MALIGNANT TUMORS

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E Borisova

Institute of Electronics, Bulgarian Academy of Sciences,
72, Tsarigradsko Chaussee, 1784 Sofia, Bulgaria.

Project Coordinator:

Assoc. Prof. Dr. Ekaterina Georgieva Borisova.

Project team members:

Irina Angelova Bliznakova, PhD student, IE-BAS;

Aleksandra Zhivkova Zhelyazkova, PhD student, IE-BAS;

Momchil Dimitrov Keremedchiev, PhD student, Queen Giovanna – ISUL University Hospital;

Liliya Plamenova Angelova, IE- BAS.

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1. Main objectives

- Development and optimization of optoelectronic instrumentation and methodology for optical biopsy and their approbation and introduction into the clinical practice for early diagnosis of malignant tumors of the skin and mucosa.
- Optimization of fluorescent and reflective diffuse spectroscopic techniques for obtaining maximum diagnostic accuracy in identifying the main types of malignant neoplasias in dermatology and gastroenterology.

2. Specific project objectives

- To achieve a maximum diagnostic accuracy in identifying the main types of malignant neoplasias in dermatology and gastro-enterology. To apply the developed 1-D and 2-D systems for spectroscopic analysis to local measurement of the spectral characteristics and the topography of skin and mucosa tumors, and to intraoperative monitoring of tumor

excisions (surgical removal) allowing precise determination of the tumor borders and metastasized cells in tissue and adjacent lymph nodes.

- To introduce into the clinical practice of Queen Giovanna – ISUL University Hospital the systems and methods developed for diagnosis and monitoring. To determine basic spectral parameters of biological tissues in norm and pathology, and expand the database of human tissues optical properties. To obtain original data on fluorescence temporal parameters of the main types of cancers of the skin and the gastrointestinal tract.
- To assist the career progress of the young scientists in the project team by publishing the scientific research results in peer-reviewed journals and presenting them at prestigious international conferences. To include the work on systems development and the results of the clinical studies as integral and significant parts of the dissertations of the PhD students - members of the team: A. Zhelyazkova

(systems and methods for spectral analysis), and M. Keremedchiev (clinical research and approbation of systems for fluorescence diagnostic and intraoperative monitoring of GIT tumors).

3. Methods

Fluorescence analysis methods are used – both autofluorescence and introduction of exogenous markers, as well as time- and frequency-domain spectroscopy. Diffuse reflectance spectroscopy methods are used to expand the diagnostic capabilities and applied to pigmented neoplasms. The measurements are performed with fiber optic probes – one-dimensional point spectroscopy (1-D) and topographic (2-D) images of the distribution of fluorescence signals from tissues in norm and pathology. Databases of fluorescent and reflective properties are created and computer-processing methods are introduced, namely, development of diagnostic algorithms to differentiate the main types of malignancies by type, stage of development and from benign pathologies. The spectral data are compared and verified with histological results included in a "gold standard".

4. Results expected

Systems will be developed for optical biopsy of skin and mucosa malignant neoplasias, for primary diagnosis and for intraoperative monitoring. Methods for diagnosis of the main types of skin neoplasias and their differentiation from benign and dysplastic lesions will be introduced. A system and methods for fluorescent intraoperative monitoring of tumor resection of the colon and rectum will be established. The methodology developed in previous research for fluorescence diagnosis of tumors of the upper and lower gastrointestinal tract will be expanded and improved. The devices and methodologies will be clinically approved.

The research results will be published in specialized international journals and reported at conferences as necessary for their dissemination; this will contribute to the overall career development of the project team members. The methods and equipment developed will be used in the dissertations of two young scientists.

5. Preliminary results

5.1. Measurements "in vivo"

The term "optical biopsy" can be found in the description of different optical spectral techniques, such as fluorescence, absorption, scattering, reflectance, Raman scattering, NIR luminescence of biological tissues, but its most popular usage is when light-induced autofluorescence (LIAF) and diffuse reflectance spectroscopic techniques for tissue detection and determination are described. We applied these two techniques in a clinical trial investigation in view of introducing a skin cancer optical biopsy system into the clinical practice of Queen Giovanna – ISUL University Hospital.

In the LIAFS measurements, multiple wavelength excitation was used of the endogenous fluorescence of benign and malignant cutaneous lesions. Initially, the lesions were classified visually by an experienced dermatologist and dermatoscopically using ABCD scoring criteria. The second step was detection of autofluorescence originating from the lesion and the surrounding normal skin using different excitation wavelengths, namely 365, 385, and 405 nm, emitted by several narrow-band light-emitting diodes. An optical fiber probe was used to deliver the light from the LEDs and to collect the fluorescence signals from the skin surface. It consists of 7 fibers in a circular geometry. The central fiber is used for autofluorescence signal detection; it is connected to a microspectrometric system, while the surrounding six fibers are used to deliver the excitation light from the LEDs to the skin under investigation. The same fiber-optic probe was used for reflectance

measurements. In the DRS mode of examination, the illumination source was a broad-band halogen lamp. We thus acquired cutaneous diffuse reflectance spectra within the 380-900 nm spectral region.

Both the LIAFS and DRS spectra were recorded and stored using a fiber-optic microspectrometer (USB4000, Ocean Optics Inc., Dunedin, FL, USA). A personal computer was used to control the system and store and display the data using the specialized microspectrometer software OOI Base (Ocean Optics Inc., Dunedin, FL, USA). Fluorescent data acquired *in vivo* from cutaneous lesions are presented in figure 1.

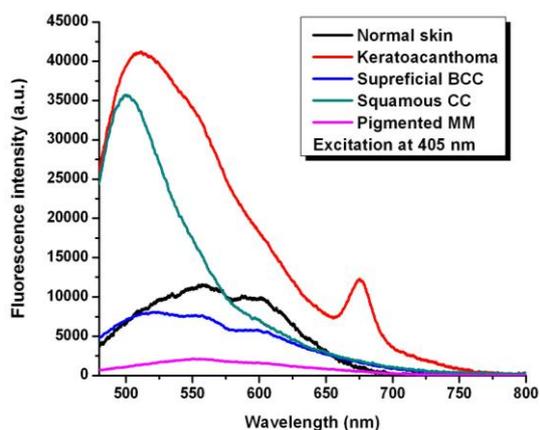


Figure 1. Autofluorescence spectra of normal skin, compared with these of different dysplastic and malignant cutaneous lesions using excitation at 405 nm.

After these initial medical and spectroscopic measurements, cytological and/or histological samples were taken from each lesion. The histological examination results were used as a “gold standard” to which all data acquired were compared. All subjects were Caucasian volunteers – patients of Queen Giovanna – ISUL University Hospital, with skin phototypes I, II and III according to Fitzpatrick classification.

Our work proceeded with assessing the origins of diagnostically significant spectral features; differentiation schemes were developed and examined. A clinical trial for initial diagnosis of skin cancer is currently being performed at Queen Giovanna – ISUL University Hospital; by

expanding the database of fluorescence spectra of major skin benign and malignant pathologies, we expect to develop an objective tool for cancer detection and treatment monitoring. In order to clarify the autofluorescence origins of the signal detected, excitation – emission matrices were constructed using *in vivo* measurements on normal human skin, figure 2. One can thus address the major fluorophores and compare these data to all EEM data for different cutaneous pathologies.

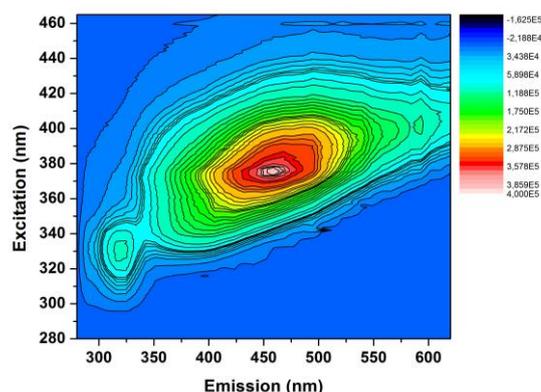


Figure 2. Excitation-emission matrix of normal skin, phototype II.

We are currently processing the spectral data obtained for subsequent medical statistical analysis. The results indicate that the optical biopsy method yields extremely high sensitivity (> 95 %) and specificity (~97 %) in differentiating carcinoma from other pathologies, and SE = 92 % and SP = 100 %, in differentiating malignant melanoma. The results also revealed a very good correlation with the histological skin analysis, as well as a repeatability of the features of the fluorescence and reflectance signals from patient to patient, with the same type of lesion being obtained by the LIAFS and DRS measurements.

5.2. Measurements “*ex vivo*”

Autofluorescence spectroscopy of human tissues is a very attractive tool for early diagnosis of cancer due to its high sensitivity, easy-to-use measurement methodology, lack of need for contrast

agents application on the tissue under investigation, possibilities for real-time measurements and non-invasive tumor detection. However, no reliable and universal systems for fluorescence detection of skin cancer have so far been offered on the medical market. The problems in developing such a system are related to the great variety of benign and malignant forms of skin pathologies; e.g., basal cell carcinoma lesions have more than 15 sub-types, squamous cell carcinoma lesions have about 10 different subtypes, and all of them have a variety of benign and dysplastic forms; moreover, they are different, including by their fluorescence properties, at the different stages of the lesion growth. Another very important disadvantage is the fact that different endogenous fluorophores appear in the integral autofluorescence signal originating from the skin under excitation at different wavelengths, which makes this type of spectra difficult to analyse and compare with the fluorescence signals detected from various pathological skin lesions.

The use of autofluorescence spectroscopy for early detection of cutaneous pathologies has been reported in a large number of publications. To stimulate *in vivo* autofluorescence emission from skin tissues, the research groups have applied a variety of excitation sources (and wavelengths), such as lasers, LEDs, narrow-filter broad-band lamps. However, a comprehensive review of the autofluorescence spectra emitted *in vivo* by normal human skin under excitation at different wavelengths is yet to appear in the literature.

In the framework of this project, in the beginning of 2012, we started investigations on determining the endogenous fluorescent properties of cutaneous and mucosal (lower gastrointestinal tract) tissues – normal and diseased.

The studies involved two systems – for point spectroscopic measurements and for 2-D imaging of the tissues and lesions

investigated. Excitation-emission matrices were constructed for a variety of tumors and normal samples.

The excitation applied in the point-measurements was in the range from 250 to 550 nm, with a 5-nm step between the excitation bands (2 nm); the emission was detected in the 260 – 800 nm region using a FluoroLog3 spectrofluorimetric system in a steady-state mode with a F3000 fiber-optic stand and a probe attached to the spectrometric system (HORIBA Jobin Yvon, France). In the EEMs formed, one can address all detected *in vivo* major skin fluorophores existing in the normal cutaneous tissues based on their coordinates (excitation max; emission max).

As an example, figure 3 presents an EEM of basal cell carcinoma *ex vivo*, where the signal originating from structural compounds is much higher than that from the co-enzyme component, due to enzymes reduction in excised tissues.

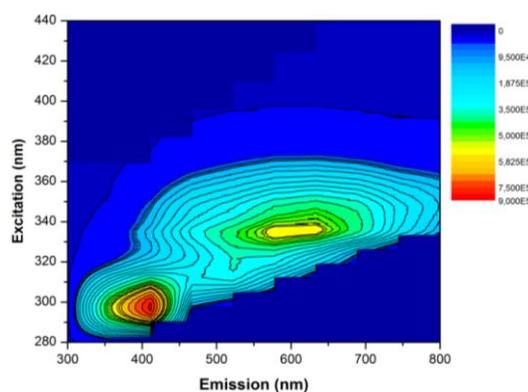


Figure 3. EEM of basal-cell carcinoma *ex vivo*.

A complete picture is thus obtained *in vivo* of the autofluorescence properties of normal Caucasian skin; it can be used as a diagnostic basis for comparison with autofluorescence signals from different cutaneous pathologies. Detection of EEM data from cutaneous tumors and mucosal neoplasia is in progress, as our first results showed very good repeatability for the same type of samples with specific changes that correlate with our previous investigations and our knowledge on the biochemical and morphological changes occurring in pathological tissues.

Publications

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3. Borisova E, Pavlova E, Troyanova P and Avramov L 2012 Optical biopsy of cutaneous tumours *Proc. 2nd Int. Conf. Laser Surgery and Medicine CLSM'2012* (23-30 April 2012 Yokohama Japan) **CLSM6-2** pp 2-3
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Conferences

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3. Borisova E, Pavlova E, Troyanova P, Keremedchiev M, Vladimirov B and
7. Borisova E, Autofluorescence spectral features of normal skin – basis for comparison with cutaneous pathologies, *Int. School on Optics, Lasers and Biomedical Photonics (Saratov Fall Meeting - SFM'2012)*, 23-30 Sept. 2012, Saratov, Russia.
8. Borisova E, Pavlova E, Troyanova P, Nikolova B and Tsoneva I, Optical biopsy – tool for initial cancer diagnosis and monitoring of therapy, *European Medical Phys. Conf. 18-20 October 2012, Sofia, Bulgaria.*