STSM REPORT

STSM Application number: COST-STSM-ECOST-STSM-BM1205-250916-080234

STSM Grantee: Dr. Ekaterina Borisova

STSM title: Optical spectroscopy of cutaneous benign, dysplastic and malignant cutaneous lesions in UV-VIS-NIR spectral ranges

Home institution: Institute of Electronics, Bulgarian Academy of Sciences, Sofia, Bulgaria

Host Institution: Saratov National Research State University, Saratov, Russian Federation

STSM period: 25.09.2016-16.10.2016

STSM purpose: Joint investigations using fluorescence, diffuse reflectance and transmission spectroscopy of skin tumour samples ex vivo and histological slices in visible and near-infrared spectral ranges.

Description of the work carried out during the STSM:

Many optical techniques are applied recently in clinical practice for obtaining qualitatively and quantitatively new data. Due to their high sensitivity in detection of small changes, spectroscopy techniques are widely used for detection of early changes in biological tissues. Such techniques based on the recent progress in optics have been developed for laboratorial and clinical applications. The human skin is a complex, multilayered and inhomogeneous organ with spatially varying optical properties. Analysis of cutaneous optical spectra could be a very complicated task; therefore researchers apply complex mathematical tools for data evaluation, or try to find some specific approaches, that would simplify the spectral analysis. Another approach to increase the diagnostic accuracy of skin tumour detection is to apply several spectroscopic modalities and to search for specific optical fingerprints in the spectra obtained in a broad spectral region.

During the current STSM optical spectroscopic measurements with a few different modalities were performed by the grantee Ekaterina Borisova, in collaboration with the scientific research group at the Saratov National Research State University, Optics and Biophotonics Department and under the supervision of Prof. Valery Tuchin.

Measurements were made on two types of samples – surgically excised formalin fixed cutaneous tumour tissues and hematoxylin-stained, prepared for histology analysis glass slices with different skin tumour types, which were obtained from University Hospital “Tsaritsa Ioanna-ISUL”, Sofia, under collaboration with the Institute of Electronics. All ethical issues and approvals for work with these tissue samples were received from the ethical committee of the hospital. The measurements allowed enriching the spectral information achieved by the spectral techniques available in Institute of Electronics-BAS, Sofia, with these ones, available in Saratov National Research State University, Russian Federation.

Spectrometers used for the measurements of absorption and transmission properties of different skin pathologies were Shimadzu spectrophotometer system, working at the spectral region from 250 to 700 nm and a block fiberoptic system, with a broad-band light source and

detector - microspectrometer USB4000 (OceanOptics) in the region from 345 nm to 1040 nm. NIR transmission measurements in the region of 900-2150 nm were performed using NIRQuest spectrometer (OceanOptics Inc.) fiber-optic modular system.

Spectral investigations of transmission and absorption modes of the samples were carried out in the visible and near-infrared spectral region. The results obtained would be analyzed and compared with the data received in Ultraviolet and visible spectral range, which could be obtained on the biophotonics laboratory facilities in Sofia, Bulgaria. As a result – a new knowledge about several very widespread cutaneous tumour optical properties are received, which would be used in the development of discrimination algorithms needed for development of diagnostic algorithms for detection of skin neoplasia – by type and stage of growth.

Possibility to obtain new knowledge about the optical and spectral properties for skin benign, dysplastic and malignant tissues improved the quality of the performed measurements and could contribute to a better understanding of the observed spectral peculiarities, which could be used for development of differentiation algorithms for skin tumour analysis.

The several tasks were performed by Dr. Ekaterina Borisova to implement the goals of the STSM, as follow:

1) Preliminary preparation of tissue samples, obtained from surgical excision under the collaboration of BG laboratory of Biophotonics, Institute of Electronics, with the University hospital “Tzaritsa Ioanna-ISUL” for the spectrometric and topographic measurements;
2) Introduction and training for work on the spectroscopy equipment available in Optics and Biophotonics department in Saratov National Research State University, Russian Federation;
3) Obtaining of a set of spectra in absorption, reflectance and transmission modes for each ex vivo tissue sample in VIS-NIR spectral regions;
4) Analysis and comparison between the spectral data obtained for the different pathologies investigated;
5) Evaluation of the diagnostically – important spectral features of malignant melanoma (MM) lesions vs. dysplastic nevi and non-melanoma cutaneous malignancies, such as basal cell carcinoma, and squamous cell carcinoma;
6) Discussion of results obtained and conclusions made with the host scientists and draft preparation of a research paper for a following publication in a scientific journal. This last task is currently under development

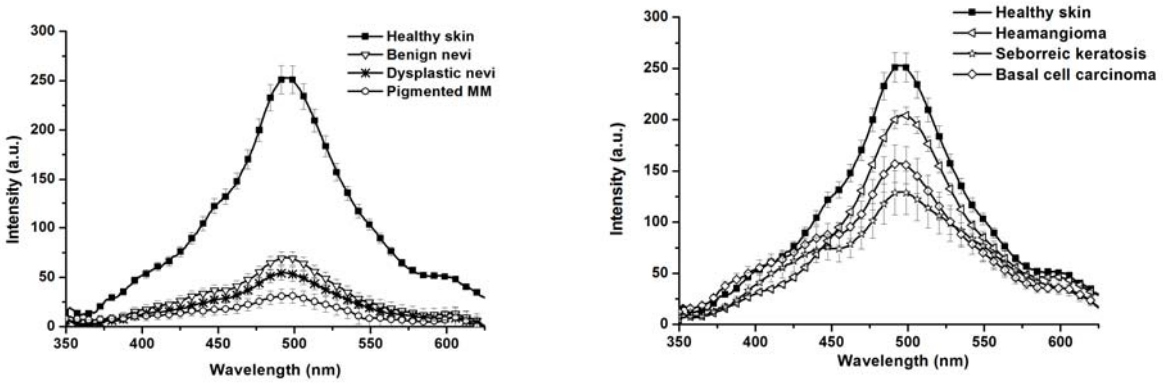
Description of the main results obtained:

The completed measurements provided spectral data with specific characteristics, whose diagnostic value could be evaluated through comparison and analysis of the optical properties of the tissues investigated.

The investigated samples are cutaneous tumours ex vivo, obtained after surgical removal and kept in a formalin solution and histological sections from biopsy tissue samples, which were routinely processed for histological analysis, during which they were stained with hematoxylin and eosin.

In fluorescence mode (excitation at 337 nm) are compared spectra of benign, dysplastic nevi and pigmented malignant melanoma lesions, as well as non-melanoma skin tumour – basal cell

carcinoma with benign non-melanin pigmented pathologies – heamangioma and seboreic veruca. On figure 1 are presented fluorescence spectra, averaged for the given set of tissue samples, grouped by the type of pathology, evaluated by histology examination in advance, which is used as a “gold standard” in the comparative spectroscopic studies.



(a) (b)

Fig.1 Autofluorescence spectra of melanin-pigmented lesions (a) and non-melanin pigmented lesions (b), including comparison with the fluorescence signal from healthy skin tissue. Spectra are averaged by the set of samples of different pathologies investigated.

Fluorescence spectra reveal statistically significant differences between the different benign, dysplastic and malignant lesions by the level of emission intensity, as well as by spectral shape, which are fingerprints applicable for differentiation algorithms for detection and evaluation of cutaneous neoplasia investigated.

In reflectance mode the most significant differences are related to the influence of skin pigments – melanin and hemoglobin from the blood. They are responsible for the shape of the reflectance spectra observed in the region of 350-1000 nm. On figure 2 are presented averaged, compared by pathology type lesions vs. normal skin reflectance. Malignant melanoma (MM) lesions (4 samples) had the lowest reflectance intensity in the whole spectral range in VIS-NIR region. The dysplastic nevi reflectance is similar by intensity levels and could not be easily separated by values from MM, but the spectral shape differences observed, related to a negative slope in the region of 600-900 nm is typical for the non-melanoma pigmented lesions, as well as for normal skin.

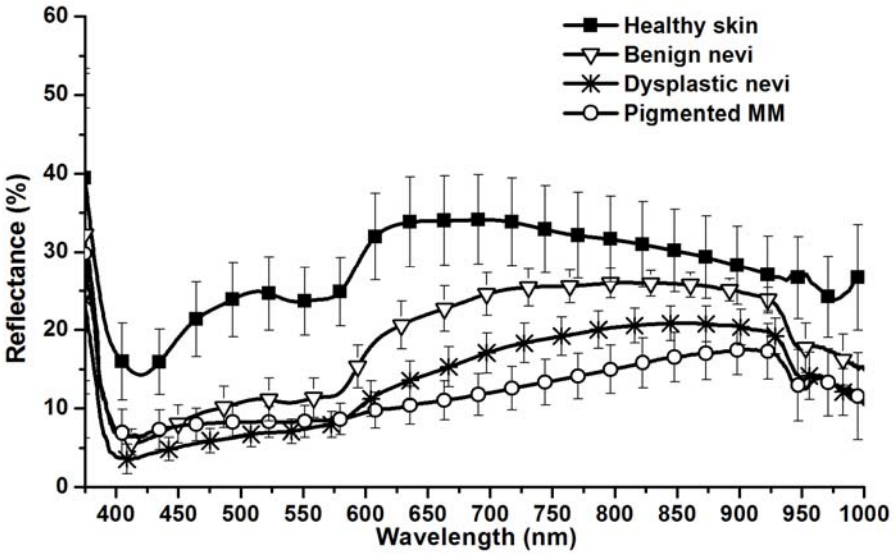


Fig.2 Diffuse reflectance spectra of melanin-pigmented lesions vs. healthy skin tissue reflectance. Spectra are averaged by the set of samples of different pathologies investigated and the standard deviation is presented as error bars.

Reflectance spectroscopy could give information not only for morphological information from the tissues. In many cases it is used to receive indirectly the pigments' content in the biological tissues investigated. Absorption of different skin pigments hemoglobin and melanin, can yield valuable information about pathological conditions, Absorption of these pigments cause significant distortions in the spectral shape of the diffuse reflected signals coming from the tissues and that changes have high diagnostic value. Reflectance in skin cancer investigations is applied mainly for the melanin-pigmented cutaneous pathologies, including malignant melanoma (MM), as well as a part of the combination of other spectral techniques simultaneously with fluorescence, which allow increasing of the diagnostic accuracy in general for all pathologies investigated. The reflectance of non-melanoma cutaneous lesions did not reveal statistically significant differences, useful for a development of appropriate discrimination algorithm between them. However, the MM reflectance spectrum is statistically significantly ($p<0,05$) different from these ones of basal cell carcinoma and squamous cell carcinoma lesions.

Transmission spectroscopy mode gave complementary optical properties information about the tissue samples investigated to that one of reflectance and absorption spectroscopy. It could be used in the cases of ex vivo and histological samples investigations – for better evaluation of the absorption and scattering coefficients for given spectral range. However, it is not very convenient for in vivo tissue examination in the case of cutaneous tumour detection and diagnostics. On figure 3 are presented compared transmission spectra of different histological slices, representing different types of benign and malignant skin pathologies.

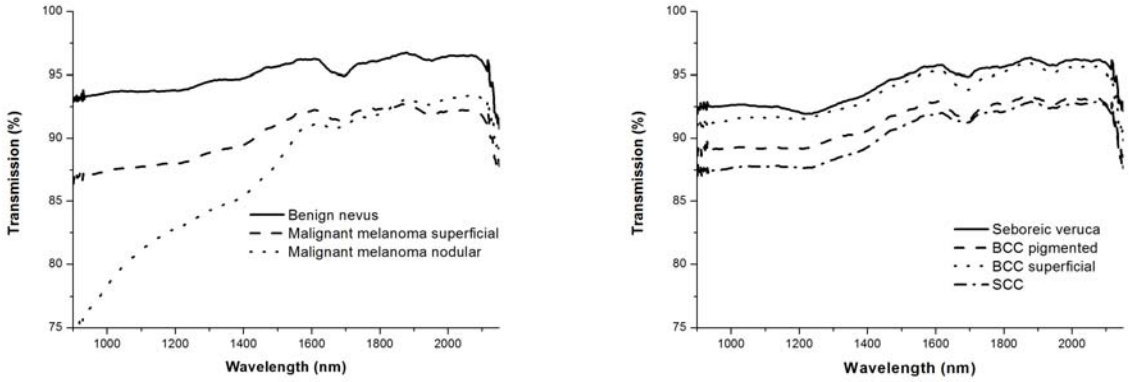


Fig.3 NIR Transmission spectra in the 900-2150 nm spectral region of melanin-pigmented lesions (a) and non-melanoma skin neoplasia (b). Spectra are averaged by the set of samples of different pathologies investigated.

The most important for every diagnostic procedure developed is its possibility to differentiate malignant from non-malignant lesions. Where it is absolutely accurate, than 100 % of the lesion types would be predicted. But every diagnostic test is imperfect in its own way – one procedure will miss many cases and make a few false diagnoses, another will miss a few cases, but the number of false diagnoses will be much higher. Using autofluorescence detection of skin benign and malignant lesions we obtain very good diagnostic performance for distinguishing of non-melanoma lesions from other simulating benign and malignant pathologies. Using diffuse reflectance and transmission spectroscopy we obtain significant tool for pigmented pathologies differentiation, but it is a tool with moderate sensitivity for non-melanoma lesions detection. When several spectral detection techniques are applied in common and multispectral algorithms for diagnosis and differentiations are applied we could rapidly increase the diagnostic accuracy of the received combined "optical biopsy" method. Results obtained in the current study would be used for development of such combined diagnostic algorithms for skin neoplasia differentiation.

Mutual benefits for the Home and Host Institution:

The collaboration between the "Biophotonics" laboratory of the Institute of Electronics, Bulgarian Academy of Science and Optics and Biophotonics Department of Saratov National Research State University give the opportunity of the grantee Ekaterina Borisova to take a part in performing spectroscopic measurements with different techniques (transmission, absorbtion, reflectance) in near-infrared range. The measurements enriched the spectral information that could be achieved by the spectral techniques available in Institute of Electronics-BAS, Sofia, with these ones, available in Saratov National Research State University, Russian Federation. The obtained spectral data were shared between the groups and added to their knowledge about the specific optical properties of the cutaneous tumours and the optical spectroscopic techniques, which could be applied for cancer diagnostics. – UV-VIS-data of the skin cancer optical properties are coming from IE-BAS, Bulgaria and VIS-NIR data - from SNRSU, Russian Federation, respectively.

Future collaboration with the Host institution:

Future collaboration between the Home and Host institutions has been discussed and will be realized, if an opportunity arises.

In the current moment – a bilateral proposal is under preparation for a current call of Bulgarian Science Fund of Ministry of Education and Science, and Russian Foundation for Fundamental Research, with a deadline for proposals' submissions – 15.11.2016. Both groups involved in this STSM now try to prepare such bilateral project with a draft title "Development of new methods of improvement of diagnostic quality of gastric cancer."- for application of optical techniques for detection and evaluation of gastrointestinal tumours.

Foreseen journal publications or conference presentations expected to result from the STSM:

After a precise analysis of the spectral data obtained, under investigations carried out publications in research specialized journals are planned.

STSM OUTCOME FORM

STSM application number	Home institution & country	Host institution & country	BM1205 WG	Objective of the collaboration	Results of the collaboration
COST-STSM-ECOST-STSM-BM1205-250916-080234	Saratov National Research State University, Saratov, RUSSIAN FEDERATION	Institute of Electronics, Bulgarian Academy of Sciences, Sofia, BULGARA	WG 4	Spectroscopic evaluation using fluorescence, diffuse reflectance and transmission spectroscopy of skin tumour samples ex vivo and histological slices in visible and near-infrared spectral ranges.	Connection between specific spectral features in UV-VIS-NIR spectral ranges and pathological condition for different types of cutaneous tumours was observed.

CONFIRMATION

STSM Applicant: Dr. Ekaterina Borisova
Home Institution: Institute of Electronics, Bulgarian Academy of Sciences
Host Institution: Saratov National Research State University, Russian Federation

Herewith I would like to confirm the successful realization of the STSM applied by Dr. Ekaterina Borisova, under the topic of "Optical spectroscopy of cutaneous benign, dysplastic and malignant lesions in UV-VIS-NIR spectral ranges".

Dr. Borisova has worked at the Optics and Biophotonics Department of Saratov National Research State University, Russian Federation, in the period of 25.09.2016 to 16.10.2016.

Her work was related to investigations using optical spectroscopy techniques (fluorescence, diffuse reflectance, and transmission) of skin tumour samples ex vivo and histological slices in visible and near-infrared spectral ranges.

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