STSM REPORT

STSM Application number: COST-STSM-BM1205-25207
STSM Grantee: Aleksandra Zhivkova Zhelyazkova
STSM title: Investigating different skin and gastrointestinal tract (GIT) pathologies (in vivo and ex vivo) by optical imaging
Home Institution: Institute of Electronics, Bulgarian Academy of Sciences, Sofia, 1784, Bulgaria
Host Institution: Institute of Atomic Physics and Spectroscopy/ Physics Department, University of Latvia, Riga, Latvia
STSM period: 2015-03-29 to 2015-04-10
STSM purpose:

The aim of the current project is to investigate different skin and gastrointestinal tract (GIT) pathologies (in vivo and ex vivo) by optical imaging, for this purpose, it was used a digital microscope Dino-Lite modified with 4 blue (450 nm), 4 green (545 nm), 4 red (660 nm) and 4 infrared (940 nm) diodes and adapted for the monitoring of skin and GIT pathologies. Blue light (450 nm) penetrates less than 1 mm in depth and provides information about superficial layers of the skin. Green light (545 nm) provides information about blood distribution and the red light (660 nm) penetrates into the skin tissue several millimeters in depth, which provides information about melanin. Infrared light (940 nm) gives us the information about the deeper skin layers. A prototype device "SkImager" with polarized LED light at several spectral regions will be used for illumination of ex vivo samples (different malignancies) obtain after surgical removal from different patients and round skin spot of diameter 34 mm or 11 mm, imaged by a CMOS sensor via cross-oriented polarizing filter. That investigations allow to obtain RGB image at white LED illumination for revealing subcutaneous structures; four spectral images at narrowband LED illumination (450, 540, 660, and 940 nm) for mapping of the main skin chromophores and diagnostic indices and autofluorescence images under UV (365 nm) LED irradiation for mapping of the skin fluorophores. The comparison with the data obtained from fluorescence excitation-emission matrices (EEM) measurements. The fluorescence excitation-emission matrix data were acquired using a commercial spectrofluorometer (FluoroLog3, Horiba Jobin Yvon). The system is composed of five parts: light source, double grating excitation monochromator, sample compartment, double-grating emission monochromator and photomultiplier tube (PMT) detector.

Description of the work carried out during the STSM:

The tissue samples obtained after surgical excision was preliminary preparated for the spectrometric and topographic measurements. Ethical approval for our investigations is received from the Ethical Committee of University Hospital "Tsaritsa Yoanna-ISUL" – Sofia, where from the samples are obtained as well. The samples was fixed in formalin solution and transported from Sofia to Riga. Based introduction how to use the equipment for 2-D fluorescence topography for tissue imaging in the host organization and trained in special software developed in MatLab to control the Dino-lite camera and SkImager. It was obtained a set of images for each *ex vivo* tissue samples using different excitation wavelengths on 2-D fluorescence system. *Ex vivo* point-by-point measurements were taken from the excised tumour lesions and outwards from surrounding skin. Autofluorescence using different excitation wavelengths for differentiation of tummor and healthy tissue is detected, forming excitation–emission matrix of data. Analysis and comparison between 1D spectroscopic and 2D tomographic data. Analysis and comparison with the data obtained from fluorescence EEM measurements.

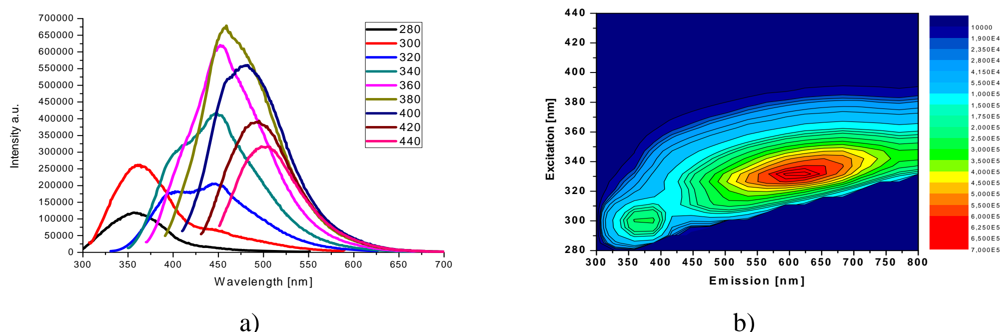
Description of the main results obtained:

The second most commonly diagnosed type of cancer is this of skin worldwide, gastrointestinal tract (GIT) tumours also are in the “top ten” positions. Most of them could have better prognoses for the patients, if earlier and precise diagnosis is applied. According to the Bulgarian National Cancer Registry 2010, there are 6 098 registered new GIT cancer cases and 4 919 registred new skin cancer cases in Bulgaria[1].

It is important to develop and combine diagnostic techniques for accurate early stage diagnosis to improve the chances for curative action for the skin and GIT tumours. Optical methods are very promising for noninvasive diagnosis of skin and mucosa tumours, leveraging the advantages of deep imaging depth, high resolution, fast imaging speed, and reach endogenous fluorophores.

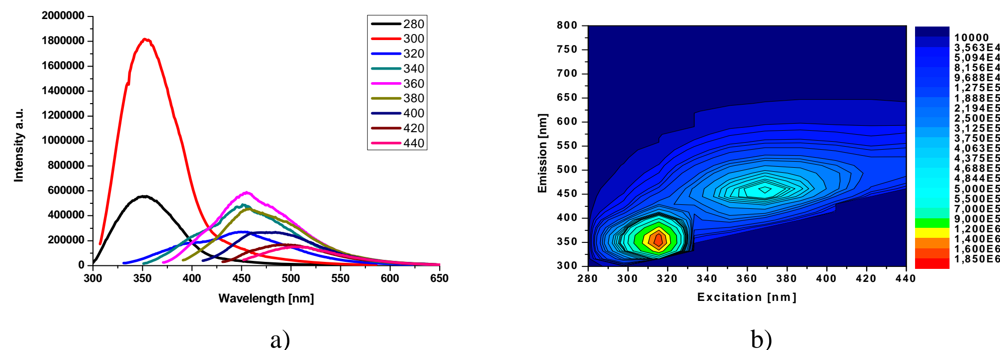
The non-invasive skin imaging techniques has become a principal tool in the diagnosis of different skin lesions. The optical magnification of the region-of-interest makes subsurface structures more visible than conventional macroscopic images. However, it has also been demonstrated and well known that the dermoscopy may have low diagnostic accuracy in the hands of inexperienced dermatologists. Therefore, computerized image understanding tools in combination with autofluorescence techniques are needed to minimize the diagnostic errors generally caused by the complexity of the subsurface structures and the subjectivity of visual interpretations[2].

We do not expect to have strong signals from co-enzymes such as NADH, NADPH, FAD and flavins, due to their fast degradation in excised tissues. We expect to measure fluorescence signals from structural compounds in the skin and its lesions. Steady-state autofluorescence and EEM data for different excitation wavelengths of normal skin are presented in Fig.1 a) and b). On the next two figures are presented our results, when lesion was investigated – Basal Cell Carcinoma (BCC) Fig. 2 a) and b). In the spectra detected we observed signals from amino acids – tyrosine and tryptophan, structural proteins such as collagen and elastin, their cross-links, as well as keratin.



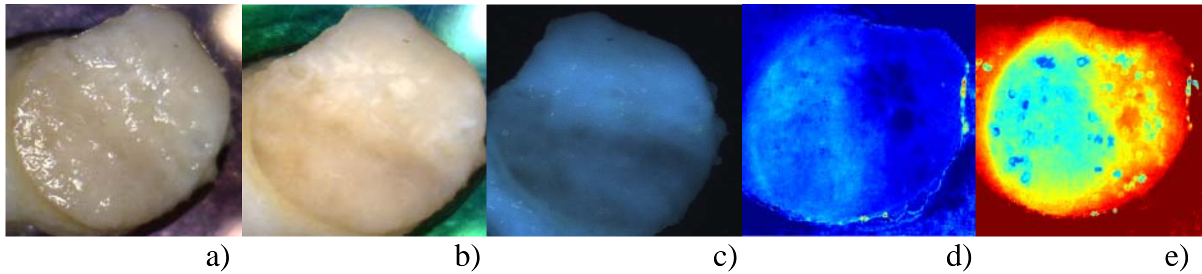
a) b)

Figure 1. a) Steady-state autofluorescence data on different excitation wavelengths–280-440 nm step 20 nm, b) excitation-emission matrix of healthy skin



a) b)

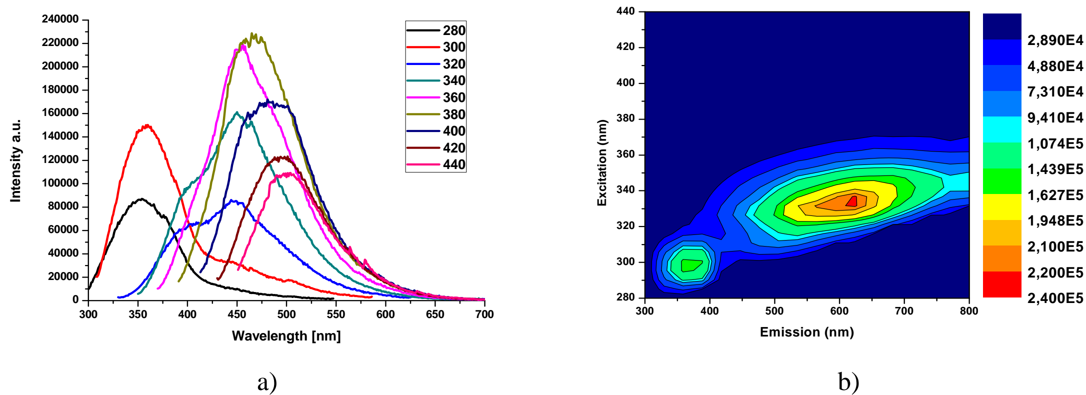
Figure 2. a) Steady-state autofluorescence data on different excitation wavelengths, b) excitation-emission matrix of Basal Cell Carcinoma (BCC)



a) b) c) d) e)

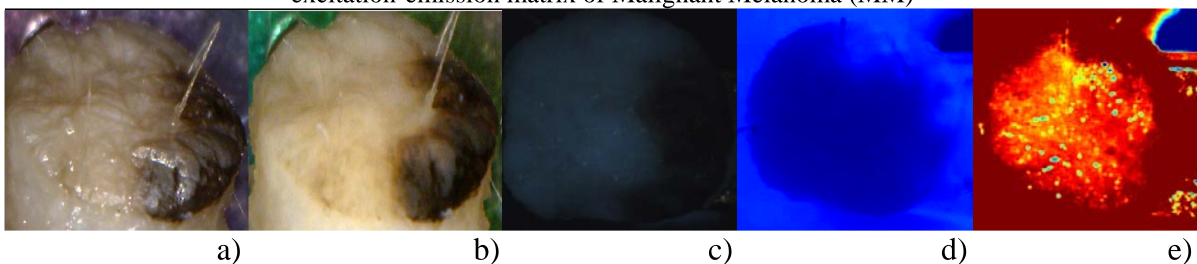
Figure 3. a) Picture of BCC obtain with Dino-Lite Digital Microscope after illumination with white light, b) picture with Dino-lite after illumination with polarized white light and c) picture obtained with SkImager after illumination with UV light 365nm, d) calculations for dermal melanin, e) calculations for index of dermal melanin

The fluorescence spectrum in the range 300-350 nm with excitation in the UV range (260-280 nm) is dominated by the aromatic amino acids tyrosine, phenylalanine and tryptophan. In addition, NADH metabolic co-factor excitation maximum at 340 nm and emission range 450-470 nm and FAD, flavins excitation in the range 420-460 nm and fluorescence spectrum in the range 500-520 nm are detected. Collagen is the protein found in the skin when excitation maximum is around 320-390 nm and an emission maximum observed is around 390-440 nm. Elastin is a protein with excitation maxima around 280 and 360 nm and emission maxima centered around 350 and 410 nm, protein cross-links excitation maximum is around 380-420 nm and the emission maxima are found at the regions of 390-440 nm and 460-500 nm. The main differences observed between the fluorescence spectra of healthy skin and cancerous tissue are in the intensity of the fluorescence from the amino acids – tyrosine and tryptophan, the enzymes and coenzymes NADH and FAD, and from the structural proteins elastin and collagen.



a) b)

Figure 4. a) Steady-state autofluorescence data on different excitation wavelengths–280-440 nm step 20 nm, b) excitation-emission matrix of Malignant Melanoma (MM)



a) b) c) d) e)

Figure 5. a) Picture of BCC obtain with Dino-Lite Digital Microscope after illumination with white light, b) picture with Dino-lite after illumination with polarized white light and c) picture obtained with SkImager after illumination with UV light 365nm, d) calculations for dermal melanin, e) calculations for index of dermal melanin

The decrease in the fluorescence intensity of the malignant tissues (see figure 4 a) and b)) is due to cancer-induced destruction of collagen and elastin cross-links surrounding the tumor cells, as the tissues progress towards malignancy. Tissue scattering is also changed due

to the structural changes of the connective tissue associated with tumor progression. Numerous studies of tumour tissue demonstrated that the collagen matrix is reduced at the locations where tumor cells reside. Collagen structures in the tumor are associated with collagenase, which suggesting a role of the collagenase in facilitating extracellular matrix breakdown and further tumor invasion. The collagen in the dermis surrounding the tumor is reduced. Therefore, the scattering should decrease and become lower than in the normal dermis. Collagen and elastin cross-links are major fluorophores of the connective tissue. Therefore, decreased collagen fluorescence in the tumor tissue is associated with a reduced number of collagen cross-links in the tumor stroma, which is suggested to be the result of the degradation of collagen fibers by the collagenases secreted by the tumor and stromal cells[3].

The chromophore maps of BCC and MM with Dino-Lite Digital Microscope after illumination with white light reflectance image and picture with polarized light (see fig. 3a), b) and fig. 5a), b)) for observing the sub dermal layer of skin was obtained. Set of pictures with SkImager after illumination with UV light 365nm (fig.3c) and 5c)), blue, green, red and infrared were obtained. The chromophore maps reflect the spatial chromophore concentration distribution in skin and GIT samples. The combinations of monochrome images were used to calculate three chromophore maps: hemoglobin, bilirubin, and melanin. The hemoglobin map was calculated by comparison of skin optical density in the green (540 nm, where hemoglobin absorption is high) and red (about 650 nm, where hemoglobin absorption is low) spectral bands. The bilirubin map was calculated by comparison of skin optical density in the blue (450 nm) and green (540 nm) spectral bands, melanin map was calculated by comparison of skin optical density in the red (660 nm) and near infrared (940 nm) spectral bands, and the dermal melanin map was calculated from the near infrared spectral image. The parametric maps of the illuminated skin spot are available: epidermal melanin distribution, dermal melanin distribution (see fig. 3d) and 5c)), index of dermal melanin (see fig. 3e) and 5e)), total haemoglobin distribution, bilirubin distribution, erythema index map and fluorescence intensity distribution, several images can be overlapped to obtain more detailed diagnostic information[4].

We can clearly distinguish healthy from tumor tissue in those set of the images. We find it easier to delineate the boundaries of the tumor after the calculations than with the naked eye. The visual contrast between the regions of BCC, MM and the surrounding normal skin on the false-colour maps is a result of the difference in the fluorescence calculated for the pixels from the two areas in the image. In the future, AF spectra and chromophore maps could have a role for both the localization of small or poorly visible tumors, and for delineating lesions to aid precise surgical excision.

We will increase the images taken under illumination with UV light for better comparisons between the autofluorescence spectra and chromophore maps.

REFERENCES

[1] “Bulgarian National Cancer Registry”, Volume XXI, (2012)

[2] Maryam Sadeghi, “Towards Prevention and Early Diagnosis of Skin Cancer: Computeraided Analysis of Dermoscopy Images” PhD thesis

[3] Yuan, Y. and Relue, P., „Enzymatic degradation of human skin dermis revealed by fluorescence and reflectance spectroscopy”, Opt. Express. 16(13):9857-68 (2008).

[4] Janis Spigulis, Uldis Rubins, Edgars Kviesis-Kipge, and Oskars Rubenis, “SkImager: a concept device for in-vivo skin assessment by multimodal imaging”, Proceedings of the Estonian Academy of Sciences, 2014, 63, 3, 301–308 doi: 10.3176/proc.2014.3.02 Available online at www.eap.ee/proceedings

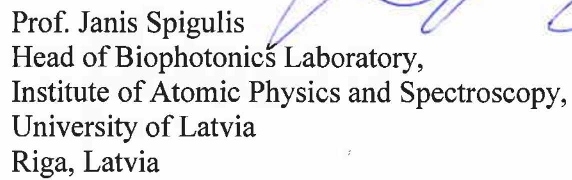
Mutual benefits for the Home and Host institutions:
We had a great opportunity for scientific exchanges, to gain new knowledge and to share experience. In the frames of STSM visit in the laboratory in Riga, Latvia, I had the opportunity to use their equipment for fluorescence topography of biological tissues detection, which allows me to complete the cycle of experiments on cancer spectral detection. The scientific communication between two laboratories also was improved and we exchange knowledge and technical information about possibility to make twinning of the activities in the field of 2D fluorescence topography of bio-tissues. It is expected that obtained results will become more adapted for medical purposes. Closer cooperation between both research groups is expected.

Future collaboration with the Host institution (if applicable):
It has new possibilities to exchange a young scientists and postdoc researchers.

Foreseen journal publications or conference presentations expected to result from the STSM (if applicable):
The results of these investigations will be published as research article and would be presented on the specialized scientific events as research reports, the measurements made would be added as a part of my Ph.D. thesis.

Confirmation

I would like to confirm the completion of the STSM applied by Aleksandra Zhelyazkova. She worked in this Project from 29th March to 10th April 2015 and fulfilled the objectives of the STSM work plan in the Institute of Atomic Physics and Spectroscopy, University of Latvia.



Prof. Janis Spigulis
Head of Biophotonics Laboratory,
Institute of Atomic Physics and Spectroscopy,
University of Latvia
Riga, Latvia

STSM outcome form

STSM application number	Home institution & country	Host institution & country	BM1205 WG	Objective of the collaboration	Results of the collaboration
COST-STSM-BM1205-25207	Institute of Electronics, Bulgarian Academy of Sciences	Institute of Atomic Physics and Spectroscopy/ Physics Department, University of Latvia	WG4	Investigating different skin and gastrointestinal tract (GIT) pathologies (in vivo and ex vivo) by optical imaging	The chromophore maps and AF spectra of skin and GIT samples were obtained. The parametric maps of the illuminated skin spot and steady-state autofluorescence data on different excitation wavelengths and excitation-emission matrix are available.