Scientific Report for Dr. Eric Tkaczyk

Short Term Scientific Mission (STSM) to Confocal Laser Skin Imaging Clinic at the University of Modena and Reggio Emilia (UNIMORE)
November 9 to 17, 2016

STSM application number: COST-STSM-BM1205-091116-081383
STSM grantee: Eric Tkaczyk, M.D., Ph.D.
STSM period: Nov 9 – Nov 17, 2016
Home institution & country: University of Tartu, Tartu, Estonia
Host institution & country: University of Modena and Reggio Emilia, Modena, Italy
Objective of the collaboration: Confocal Laser Skin Imaging Pattern Analysis for Detection of Skin Cancers

Description of the work carried out during the STSM and main results:
Optical instrumentation for imaging is an important component of biological and medical science progress. The range of applications of biomedical optics devices is very broad, from medical diagnostics, monitoring and treatment to clinical research (1). Medical imaging has already dramatically transformed the practice of medicine in a variety of clinical settings. Optical methods and devices are indeed advantageous, being non-invasive, portable, and relatively inexpensive, with the potential to provide rapid feedback to the practitioner.
In the field of dermatology, non-invasive imaging technologies are particularly useful for skin disease diagnostic and monitoring, including skin cancer detection. This is especially the case when cutaneous conditions present ambiguous features, leading to delays in treatment. Many optical imaging methodologies are currently developed to improve the diagnosis of a variety of skin conditions without the need for biopsy, which is a high burden for patients. The current standard of care concerning skin neoplasms is indeed to perform a biopsy to establish a diagnosis (2-4).

A much studied potential application is melanoma, for which new and better treatments are now available but are also very expensive (can exceed $400,000 USD/year/treatment of a single patient). In addition, melanoma incidence has been increasing for 30 years and while accounting for only 5-10% of skin cancer, it is responsible for around 75% of deaths by this disease worldwide (5). Early detection and accurate diagnosis of melanoma is therefore critical for a good prognosis and appropriate treatment. Current standard diagnosis is based on biopsy and histopathologic examination, a method that is invasive and which accuracy is highly dependent upon physician experience. In addition, significant discordance among pathologists have been often documented for the classification of melanocytic neoplasms (6, 7), resulting in meaningful change in clinical management in about 18% of cases (7). This observation highlighted the urgent need to find more consistent skin tumour staging parameters to improve the accuracy of diagnosis decisions by dermatologists.

During this STSM, I initially became acquainted with the daily implementation of confocal microscopy and the clinic organization. Together, we reviewed the major results of eleven different optical technologies for skin cancer diagnosis, which will be published and copyrighted at the next American Academy of Dermatology Annual Meeting in Orlando, Florida (Table that will be the audience handout attached to this document).

One particularly important technique to compare to confocal is multiphoton microscopy (MPM). MPM is a femtosecond laser scanning microscopy technique that relies on nonlinear light-matter interactions such as two-photon excited autofluorescence (AF) and second

harmonic generation (SHG) to achieve 3-dimensional (3D) images with submicron resolution (8). These contrast mechanisms produce images of endogenous biomolecules in the tissue, without using specific fluorescent labels. Indeed autofluorescence resulting from the distribution of endogenous fluorophores in tissue yields predictive structural and biochemical information without fixation or staining procedures. The main sources of fluorescence in skin are reduced nicotinamide adenine dinucleotide (NADH), flavine adenine dinucleotide (FAD), keratin, melanin, collagen, and elastin fibres, whereas SHG is used to visualize collagen fibres in the dermis. Multiphoton microscopy technology is therefore capable of non-invasive in vivo imaging of human skin with sensitivity to the epidermis and superficial dermis, to provide label-free morphological and molecular information. A few groups are exploring the ability of multiphoton microscopy to provide qualitative and quantitative information for diagnosis of skin lesions. As an example, it was used by Balu and colleagues a pilot clinical study to establish specific criteria to distinguish melanocytic nevi at different stages (8): common nevi with no dysplastic changes, dysplastic nevi, and melanoma. A quantitative algorithm was derived from in vivo MPM measurements to quantify the histopathologic feature of melanocytic nevi. Most of the histologic criteria for diagnosis of dysplastic nevi and melanoma, such as cellular atypia, lentiginous hyperplasia, elongated dermal papilla, ascending melanocytes, and Pagetoid spread, were identified by MPM. The authors managed to derive a numerical multiphoton melanoma index (MMI), combining 3 criteria: the melanocyte dendrites density, and the measurement of collagen and autofluorescence intrinsic changes (8). Current MPM are unfortunately not suitable for widespread clinical use due to several limitations. Practical limitations include firstly an extraordinary high cost (>400 000 EUR, quotation obtained 2015 from Dermainspect) of MPM systems, partially due to the expense of the required femtosecond laser. Secondly, scan times are several orders of magnitude larger than for reflectance confocal microscopy, which provides similar resolution but no compositional information due to lack of discrimination of autofluorescence. Finally, technical challenges include limited field of view (about 250×250 μm^2) and penetration depth (about 200- 300 μm). Scanning large areas of the lesions is important to avoid false-negative diagnoses because lesions are often non-uniform, presenting focal dysplasia and/or malignant neoplasm. The field of view can be increased by implementing a mosaic feature (acquisition of adjacent field of views) or by redesigning the optical components but is unlikely in the foreseeable future to be able in a matter of minutres to capture entire lesions on the skin that are several mm^2, which by contrast is the case for confocal imaging. Therefore, MPM will need to undergo substantial advances before it can be used clinically for cancer diagnosis. Therefore, we continue to focus in our Italy-Estonia COST collaboration on confocal microscopy.

While in Modena, I attended several educational lectures and obtained one-on-one tutoring in confocal image acquisition and basic image interpretation. Finally, together we analyzed over 40 confocal image data sets of volunteer patients and also of patients with melanoma skin cancer. This confocal data helped refine my skills. Finally, we planned out joint collaboration and in particular a potential project imaging skin of oncology patients with visiting professor Marco Ardigo from Rome who coincided with my visit to Modena. Example confocal images that were analyzed are shown in the figures.

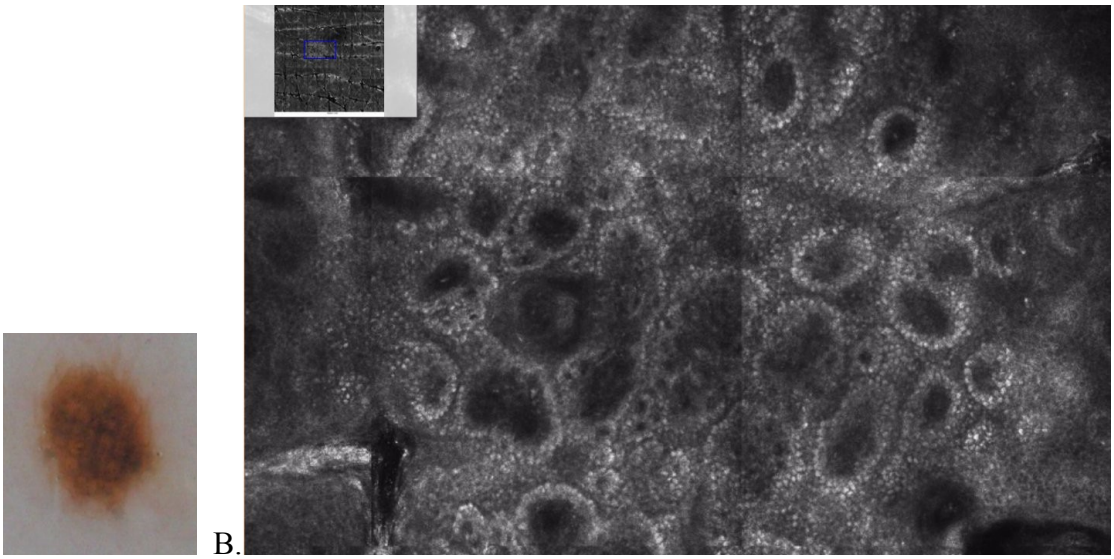
B.

Figure 1. A. Dermoscopy of confocal case 1143 revealing an irregular pigment network suggesting a possible melanocytic malignancy requiring surgery. B. Confocal image of the dermal-epidermal junction revealing a predominantly ringed pattern and edged papillae. These benign features make the diagnosis of melanoma unlikely and saved this patient a surgical procedure.

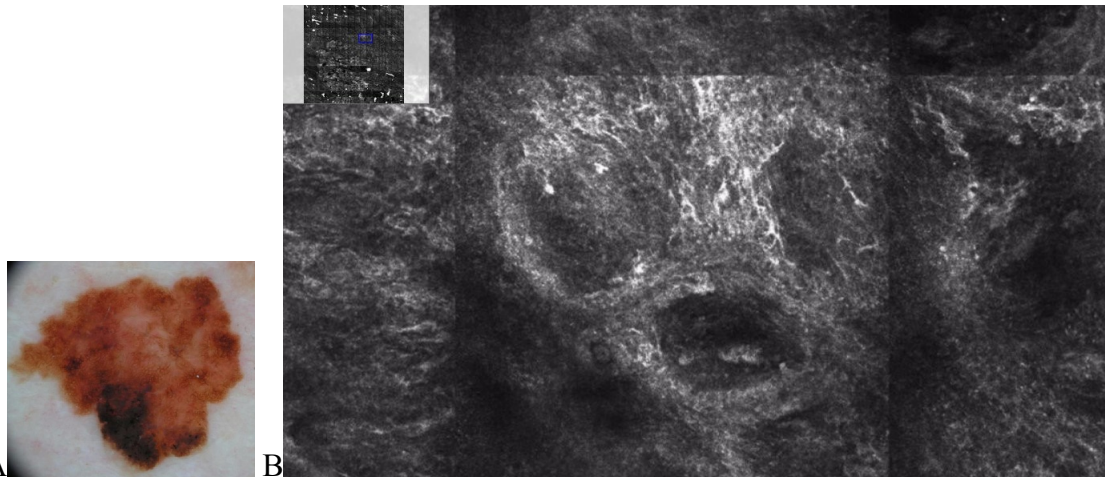
A B

Figure 2. A. Dermoscopy of confocal case 1158 revealing regression and eccentric pigment suggesting a possible melanocytic malignancy requiring surgery. B. Confocal image of dermal-epidermal junction reveals irregular junctional thickenings, non-edged papillae, and pleomorphic atypical cells with numerosity >10 per square mm infiltrating the papillae – malignant features which were consistent with the diagnosis of melanoma confirmed by excisional biopsy.

Mutual benefits for the Home and Host institutions: This visit resulted in a transfer of knowledge, examination of many features of confocal microscopy for cancer detection, and several ideas and connections for future collaboration.

Future collaboration with the Host institution (if applicable): We are continuing to collaborate by discussing and analyzing additional confocal skin images at a distance. We are also working on a new project with broader patient base to longitudinally monitor evolving skin lesions.

Foreseen journal publications or conference presentations expected to result from the STSM (if applicable): Dr. Pellacani and I have been accepted to speak together about our experiences with noninvasive detection of skin cancer at the March 2017 American Academy of Dermatology Annual Meeting in Orlando, USA. Additionally, I am submitting some of the text of this scientific report as an article for *Acta Dermato-Venereologica*.

Conclusion

Medical imaging has dramatically transformed how clinicians evaluate, diagnose, monitor, and treat disease. The highly visual nature of cutaneous diseases makes digital imaging extremely valuable in everyday practice of dermatologists. The appended table summarizes the clinical uses, advantages and limitations of all optical imaging technologies that we are aware of in dermatology, including the four techniques reviewed here. In practice, no single imaging method will resolve all skin conditions, but many clinical problems will require multimodal in vivo imaging. With advancements and improved standardization of non-invasive imaging in dermatology, clinical practitioners may be able to better capture and monitor skin conditions over time and achieve better diagnostic accuracy, resulting in fewer biopsies, decreased morbidity and ultimately less cost. Moreover, since great innovations for skin imaging have often been the result of the interplay between engineers and clinicians, they have to continue to work together and to define common roadmaps to provide the best benefit of innovative technologies to the actual clinical needs. Their joint forces continue to serve the ultimate goal to improve patients' care.

References:

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STSM Outcome Form

Confocal Laser Skin Imaging Clinic at the University of Modena and Reggio Emilia (UNIMORE), November 9 to 17, 2016

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COST-STSM-BM1205-091116-081383	University of Tartu, Tartu, Estonia	University of Modena and Reggio Emilia, Modena, Italy	Confocal Laser Skin Imaging Pattern Analysis for Detection of Skin Cancers	Dr. Tkaczyk became acquainted with the daily implementation of confocal microscopy and learned the principles of the technique. Features of melanoma and non-melanoma skin cancer relative to benign tumors were studied with the technique. Together, confocal image sets from 40 different skin lesions were analyzed.

I acknowledge that the described short-term scientific mission (STSM) was successfully carried out in the conditions here specified. Prospects of potential further collaborations on topics related to confocal skin imaging are expected in the coming months.

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