Diagnostic Aspects of Lichen Sclerosus and Skin Cancer Studied with Laser Scanning Microscopy

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Despoina Kantere conducted her PhD studies at the Department of Dermatology and Venereology, Sahlgrenska University Hospital, Gothenburg, Sweden during the period 2010–2020. Ann-Marie Wennberg Larkö was her main supervisor, and the co-supervisors were Professor Marica B. Ericson and Associate Professor Petra Tunbäck. The opponent was Professor Merete Haedersdal, University of Copenhagen, Copenhagen, Denmark. Complete dissertation can be found at https://gupea.ub.gu.se/handle/2077/64517.

Histopathologic examination of tissue biopsies is the current gold standard for the diagnosis of dermatological problems. Similarly, in oncology, sentinel lymph node (SLN) biopsy is the state-of-the-art diagnostic method for metastasis screening. Although these methods are safe, they are associated with some limitations, particularly because they are invasive, labour-intensive, and time-consuming. Moreover, histopathological analysis does not always lead to conclusive results. Therefore, there is a need for the development of fast, accurate, and non-invasive diagnostic procedures, complementary to these standard techniques. It seems that laser scanning optical microscopy modalities have the potential to meet this need. Regarding this, the present study was conducted to explore the efficiency of two of these methods, namely reflectance confocal microscopy (RCM) and multiphoton microscopy (MPM), in dermatological and oncological applications. Particular focus was given to lichen sclerosus (LS), basal cell carcinoma (BCC), and malignant melanoma (MM) metastases, all of which are important disorders requiring improved diagnostic methods.

This thesis builds upon the work reported in 5 papers. The first two papers involved the investigation of LS. In the first paper, we reported the clinical signs of LS, namely hypopigmentation, petechiae, and preputial constriction, based on which the diagnosis of LS was established. In the second paper, it was found that RCM could visualize the thick fibre structures corresponding to sclerosis in the dermis, thereby facilitating the differentiation of LS from normal penile skin. In the third paper, it was observed that the application of methyl-aminolaevulinic acid (MAL) on BCCs could not increase the contrast when imaged with *ex vivo* MPM. Furthermore, it was found that MAL-induced fluorescence can-

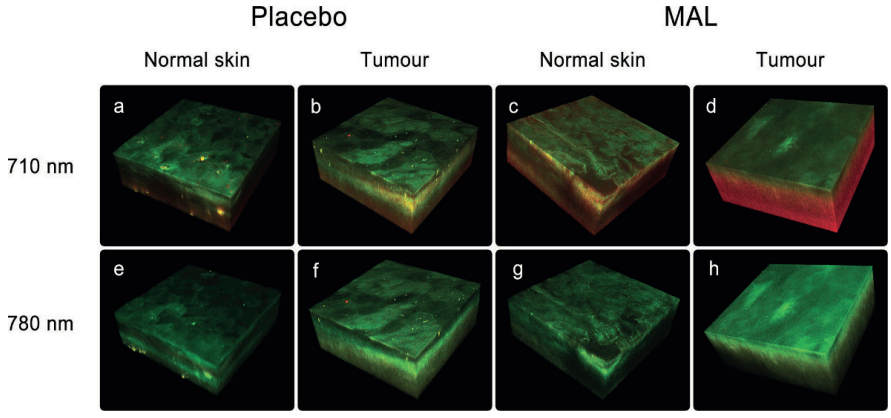

Fig. 1. Three-dimensional reconstruction of multiphoton microscopy z-stacks obtained from two different superficial basal cell carcinomas (b, f, and d, h) and the corresponding surrounding normal skin (a, e and c, g). In the upper row, anti-stokes 710 nm excitation was used, and in the bottom row, 780 nm. The lesions had either been exposed to placebo (a, e, and b, f) or to methyl-aminolaevulinic acid (MAL) (c, g and d, h). Field of view for each image: 213 x 213 x 90 mm. Images used with permission from J Biophotonics 2013; 6: 409–415, paper II).

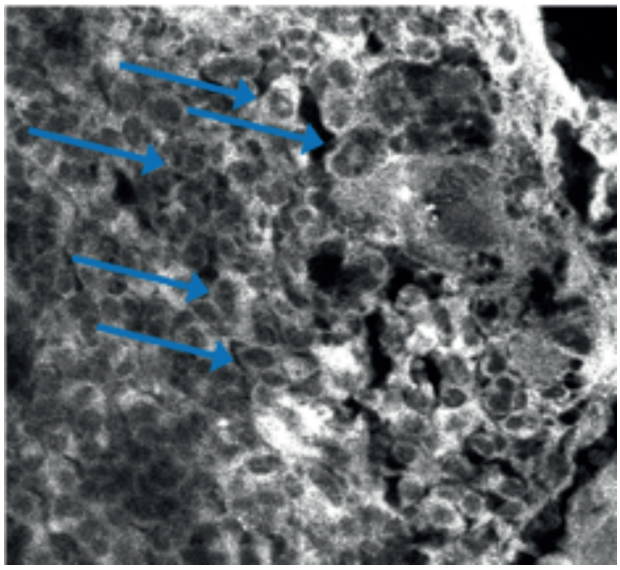

Fig. 2. This figure illustrates multiphoton microscopy data obtained from a lymph node with malignant melanoma metastases. Arrows shows atypical cells corresponding to malignant cells. Photo: Despoina Kantere.

not be excited by the expected two-photon excitation route when studied with MPM; rather, a one-photon process (i.e., anti-Stokes fluorescence) takes place (Fig. 1). This finding is important not only for diagnostic aspects but also for future work in which photodynamic effects might be required. The 4th and 5th papers involved the investigation of MM metastases in lymph node tissues. It was found that particularly *ex vivo* MPM can enable the visualization of the characteristic morphologic features in this type of tissue (Fig. 2). Furthermore, by extending the spectroscopic information to include also fluorescence life-time, the latter has the potential to increase the diagnostic ability.

Taken together, the obtained results were indicative of the potential of laser scanning microscopy techniques as diagnostic tools for the detection of LS, BCCs, and MM metastases to lymph nodes. Future studies are encouraged to fully explore the potential of these techniques to be used for dermatological and oncological investigations in a non-invasive/intravital manner.

List of original publications

I. Kantere D, Löwhagen G-B, Alvengren G, Månesköld A, Gillstedt M,Tunbäck P. The clinical spectrum of lichen sclerosus in male patients—a retrospective study. Acta Derm Venereol 2014; 94: 542–546.

II. Kantere D, Neittaanmäki N, Maltese K, Wennberg Larkö A-M, Ericson MB, Tunbäck P. Exploring laser scanning microscopy as a non-invasive diagnostic tool for genital lichen sclerosus. In manuscript

III. Kantere D, Guldbrand S, Paoli J, Goksör M, Hanstorp D, Wennberg Larkö AM et al. Anti-Stokes fluorescence from endogenously formed protoporphyrin IX – Implications for clinical multiphoton diagnostics. J Biophotonics. 2013; 6: 409–415.

IV. Kantere D, Siarov J, De Lara S, Parhizkar S, Olofsson Bagge R, Wennberg Larkö A-M, Ericson MB. Label-free laser scanning microscopy targeting sentinel lymph node diagnostics – A feasibility study ex vivo. Translational Biophotonics 2020; https://doi.org/10.1002/tbio.202000002.

V. James J, Kantere D, Enger J, Siarov J, Wennberg Larkö A-M, Ericson MB. Report on fluorescence lifetime imaging using multiphoton laser scanning microscopy targeting sentinel lymph node diagnostics. J Biomed Opt. 2020; 25: 1–8. doi: 10.1117/1.JBO.25.7.071204.