

Rapid vertical tissue imaging with clinical multiphoton tomography

Hans Georg Breunig^{a,b}, Benjamin Sauer^a, Ana Batista^{a,b}, Karsten König^{a,b}

^aJenLab GmbH, Straße zum Müggelhort 36, 12559 Berlin, Germany

^bSaarland University, Biophotonics and Laser Technology, Campus A5.1, 66123 Saarbrücken, Germany

E-mail: breunig@jenlab.de

KEY WORDS: multiphoton imaging, second harmonic generation, skin imaging, laser scanning.

Two-photon fluorescence imaging has advanced the traditional methods of biomedical microscopy with its non-invasive, label-free three-dimensional (3D) imaging capability and subcellular resolution. It is also a useful clinical diagnostic method, in particular for the investigation of skin pathologies based on autofluorescence. Furthermore, by multimodal imaging setups, which include non-linear Raman techniques, even important non-fluorescent and non-second harmonic generating molecules like lipids can be visualized. Until now, most of the applications of multiphoton tomography were horizontal tissue imaging based on the well-known laser scanning setups with two scanning mirrors for x and y directional scanning. Vertical information can then only be retrieved indirectly from 3D representations of the tissue. We present a novel modification of the clinical multiphoton tomograph MPT*flex* that allows the direct acquisition of vertical images of the skin non-invasively, label free, and within seconds. The rapid image acquisition is achieved by combining the laser scanning with timed adjustments of the z position of the focussing optics during image acquisition. By simultaneously recording fluorescence intensity and lifetime (FLIM), a single vertical image, generated in this way, provides information on the epidermal cell morphology and metabolism as well as the collagen-elastin fibre network of the upper dermis. With the vertical imaging capability, more direct comparisons of multiphoton images with standard light micrographs of histologic specimen are possible. The vertical imaging capability, hence, accommodates requirements of dermatologist who are familiar with vertical histological sections. The whole imaging system is mobile (on wheels), flexible (articulated mirror arm with movable scan head) and compact. Imaging examples of *in vivo* and *ex vivo* samples are presented.

We gratefully acknowledge financial support under the project FLIM-Vertico (E!10689 FLIM-VERTICO) from the Eurostars program.

Reference

König, K. (Ed.), Multiphoton Microscopy and Fluorescence Lifetime Imaging. Applications in Biology and Medicine. Berlin, Boston: De Gruyter (2018).