POINT SCANNING AND STRUCTURED-ILLUMINATION MULTI-PHOTON AND CONFOCAL MICROSCOPY OF HUMAN HAIR FOLLICLES AND SKIN

Zoltan Cseresnyes, Raluca Niesner(1), Jennifer Kloepper(2), Ralf Paus(2), Anje Sporbert

Confocal and 2-Photon Microscopy Core Facility (MCF)
Max-Delbrück-Center for Molecular Medicine
Robert-Rössle-Str. 10, 13125 Berlin, Germany
email: zoltan.cseresnyes@mdc-berlin.de

(1) Deutsches Rheuma-Forschungszentrum, Berlin
(2) Universitätsklinikum Schleswig-Holstein, Experimentelle Dermatologie, Lübeck

KEY WORDS: skin, hair follicle, confocal microscopy, two-photon microscopy, striped-illumination

Traditional morphological methods in dermatology require embedding and slicing the samples, followed by slice-by-slice imaging and digital image reconstruction. Confocal and multi-photon microscopy are much more time efficient and structurally less damaging to dermatological samples, which include intact hair follicles (HFs) and skin patches. Today’s confocal microscopes are characterized by very efficient light delivery and collection, thus allowing high spatial resolution imaging of relatively thick samples. In a current report [1] we successfully imaged half- and entire intact human hair follicles using single-photon point scanning confocal microscopy. We improved the optical conditions of the HFs by infusing the samples with 2,2’-Thiodiethanol (TDE), which decreased refractive index mismatches in tissue and thus the spherical aberration. In the HFs we were able to visualise the hair shaft, the inner and outer root sheath, the connective tissue sheath, as well as the medulla, the cortex and the cuticle of the hair shaft.

We now show that two-photon laser scanning microscopy (TPLSM) can be even more successful in studying skin and HFs, due to higher penetration depth as well as reduced photobleaching and photodamage at the out-of-focus regions, which allows non-invasive imaging through an entire HF. Furthermore, the use of TPLSM enables the observation of fibrillar structures within the tissue, especially collagen and elastin, due to their second and third-harmonic generation signal (SHG, THG). We also demonstrate better morphology visualization of the HF with a novel method, i.e. striped-illumination multi-beam two-photon laser-scanning microscopy (SI-MB-TPLSM), which improves the spatial resolution of standard TPLSM up to 3 fold at large imaging depths within tissue.

The relatively short total scan times and the high quality of the acquired image stacks make confocal and multi-photon microscopy versatile tools in dermatological studies, including intact hair follicles and skin patches, under normal and pathological conditions.