

Providing optical biopsies

At the 2010 Prism Awards for photonics innovation, myself and my JenLab team received the Prism Award 2010 in the Life Sciences and Biophotonics category for our medical femtosecond laser tomograph – MPTflex. The clinical multiphoton tomograph provides a fast microscopic view into the skin without taking any tissue. Tissue labelling is not required. The MPTflex overcomes the poor resolution of current skin imaging methods, such as dermoscopy, ultrasound, and optical coherence tomography. Using two-photon technology, the tomograph provides *in vivo*, marker-free, 3D optical biopsies with subcellular submicron resolution. Femtosecond laser systems have so far been used in refractive eye surgery (Femtosecond LASIK), but not in medical diagnostics. Jenlab's tomographs are the first medical femtosecond laser diagnostic tools.

Multiphoton effects were predicted by the PhD student and later Nobel prize winner Maria Göppert more than 80 years ago. It took 30 years to prove her theory by using the novel light source laser in 1961 to demonstrate two-photon fluorescence in crystals. A further three decades were required to apply this technology to cell biology and to develop the first two-photon microscope. Now, CE-marked multiphoton tomographs have finally performed the jump from the lab to the clinical bedside.

The multiphoton tomograph MPTflex is a compact tunable near-infrared 80MHz picojoule femtosecond laser system with an articulated arm and a flexible scan head that includes galvoscaners, piezodriven high numerical aperture optics, active beam stabilisation, and two single photon counting detectors. Simultaneously, the photomultipliers measure the two-photon excited autofluorescence of endogenous biomolecules such as NAD(P)H, keratin, melanin, and elastin, as well as the second-harmonic generation (SHG) from collagen. Optical en face sections are typically taken within two to eight seconds with mean powers between two (skin surface) and 49mW (dermis). The non-linear excitation of intratissue biofluorophors occurs only in a sub-femtolitre volume. By moving the laser spot within the tissue of interest in all three



Karsten König, head of the department of biophotonics and laser technology at the Saarland University and CEO of Jenlab, explains the Prism Award-winning medical femtosecond laser tomography

dimensions and subsequent image processing, pinhole-free (non-confocal) high-resolution imaging can be performed.

The stack of multiphoton fluorescence/SHG sections provides 3D information on the morphology, chemical fingerprints, and cellular metabolism. The different skin tissue layers can be easily differentiated and cells such as keratinocytes, macrophages, and dendritic cancer cells can be imaged. Furthermore, even single fluorescent intracellular mitochondria, the dark-non-fluorescent nuclei, and single elastin fibres can be detected with a lateral resolution of better than 500nm. The fast, precise look into the skin without any surgery – and without any application of fluorescent markers – has become reality.

A major application of multiphoton

tomographs is the early diagnosis of skin cancer. Researchers in hospitals in London, Modena, Brisbane, Jena, and Berlin are using the tool to find malignant melanoma non-invasively on a cellular level. Dermatologists at hospitals in Münster and Mannheim are using the tomographs to optimise the treatment of patients suffering from dermatitis and actinic keratosis. Professor Roberts from the Princess Alexandra Hospital in Queensland, Australia, employs Jenlab's multiphoton tomograph to measure the accumulation of zinc oxide in the skin of volunteers. ZnO nanoparticles are major components in a variety of sunscreens and should not penetrate into deep tissue, which could risk circulation through blood and subsequent accumulation in the liver.

Interestingly, important costumers of the multiphoton tomographs are cosmetic companies in France, Germany, Japan, the US, and the UK. The high-resolution imaging tools provide exact information on the extracellular matrix components elastin and collagen. The elastin network can be imaged based on two-photon autofluorescence, whereas collagen structures can be detected by back-scattered SHG radiation at half the laser wavelength. The ratio of elastin emission and collagen SHG determines the skin age parameter SAAID. Multiphoton tomographs provide SAAID values. They are used to measure the effect of cosmetic anti-aging products.

Long-term multiphoton imaging studies of up to three months are performed on volunteers who are taking active ingredients versus placebos. It was shown that some of these creams can indeed stimulate the biosynthesis of collagen, whereas other substances completely fail to show either an effect on cellular metabolism as measured by NAD(P)H fluorescence or a modification of the collagen pattern and amount as detected by SHG. Multiphoton tomographs are also in use to measure the effect of UV exposure in tanning studios, of smoking, and of using hormones on the skin. Researchers at the Beckman Laser Institute at the University of California Irvine (UCI) use the detection of elastin and collagen over long periods to understand the process of wound healing.

So far, about 2,000 volunteers and patients have been investigated with Jenlab's skin imaging devices. Current developments focus on the integration of a Coherent Anti-Stokes Raman spectroscopy (CARS) module to detect non-fluorescent lipids and water with the clinical multiphoton tomograph. ●

For further information

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