SPECTRALLY-RESOLVED MULTIPHOTON IMAGING OF IN VIVO AND EXCISED MOUSE SKIN TISSUES

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The deep-tissue penetration and submicron spatial resolution of multi-photon microscopy and the high-detection efficiency and nanometer spectral resolution capability of a sensitive, broadband spectrograph were combined to study the intrinsic emission of mouse skin post mortem biopsy and section, and in vivo tissue samples.

Different layers of skin could be clearly distinguished based on both their spectral signature and morphology. Auto fluorescence could be detected from both cellular and extra cellular structures. The fluorescence arises from native chromophores such as NADPH, elastin, collagen and flavins. In addition SHG from collagen and a narrowband spectral emission band related to collagen were observed. The narrowband emission shifts with excitation wavelength and is therefore related to Raman scattering.

Visualization of the spectral images in RGB color allowed us to identify tissue structures such as epidermal cells, lipid-rich keratinocytes and intercellular structures, hair follicles, collagen, elastin, and dermal fibroblasts. The results also showed morphological and spectral differences between the mouse skin post mortem biopsy and in vivo samples which explained by biochemical differences, specifically of NAD(P)H. Overall, spectral imaging provided a wealth of information not easily obtainable with present conventional multi-photon imaging methods.


In-vivo spectral imaging of mouse tissue. Imaging depth 30 µm, excitation wavelength 764 nm. In the emission spectra the SHG of collagen is visible, including Raman scattering. Furthermore, the emission spectra of elastin fibers and a hair shaft follicle in the image are shown.