RAPID ACQUISITION AND ANALYSIS OF MULTIPHOTON SPECTRAL IMAGES OF IN-VIVO HUMAN SKIN

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KEY WORDS: Multiphoton microscopy, spectral imaging, in vivo tissue imaging, image analysis, spectral unmixing, phasor analysis.

Multiphoton microscopies provide useful tools in e.g. tissue imaging, especially when combined with spectroscopic detection. Here, we present an optimized system for fast, high-resolution spectral imaging of in vivo human skin. The spectrograph is composed of a dispersive prism in combination with an electron multiplying CCD camera. Spectra of autofluorescence and second harmonic generation (SHG) are acquired at a rate of 8 kHz. Image quality is significantly enhanced by the simultaneous recording of background spectra. In vivo spectral images of 224x224 pixels were acquired, background corrected and previewed in real RGB color in 6.5 seconds.

In the second part of the talk, advanced segmentation and unmixing methods to analyze spectral images are compared: RGB visualization, spectral phasor analysis and blind source separation by nonnegative matrix factorization (NMF). The methods are optimized and tested for their performance in analyzing spectral images of skin.

Combined, these methods provided valuable and fast tools for the acquisition and analysis of 3D spectral images of in vivo human skin. Various structures in the skin could be easily distinguished, including Stratum Corneum, epidermal cells and dermis. Interestingly, for epidermal cells two distinct populations could be separated, the difference being the melanin content.

Figure: Multiphoton spectral images of autofluorescence of in vivo human skin (at 50-56 µm depth). Spectral unmixing in three channels (dermis, epidermis and melanized epidermis) is performed by spectral phasor analysis. Each image was recorded in 6.5 seconds; also the unmixing took a few seconds.