DUAL-CHANNEL MICROSCOPE FOR OCULAR TISSUE ANALYSIS: LINEAR CONFOCAL AND SECOND HARMONIC GENERATION IMAGING

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During the last two decades, there has been an increasing interest in the development of imaging techniques to provide improved visualization of ocular tissues (both in-vivo and ex-vivo). Confocal scanning laser microscopes (CLSMs) are often used in corneal pathology diagnosis and to explore surgical outcomes with increased axial and lateral resolutions. In particular, CSLMs may differentiate the corneal layers, such as epithelium, keratocytes and endothelium. However, the stroma that represents the 90% of the corneal thickness and it is composed of collagen fibers, cannot be imaged with linear CSLMs. On the other hand, Second Harmonic Generation (SHG) is a multiphoton imaging technique able to visualize collagen-based tissues without labelling techniques. Here we proposed a combined dual imaging system (linear confocal and SHG imaging) able to obtain images of the whole cornea.

A compact research prototype of a dual-channel microscope was designed and implemented. It consists of two illumination paths, a femtosecond laser (800 nm, 76 MHz) and a continuous diode laser (532 nm), coupled to a non-resonant galvomirror system to scan the sample. A long-working distance objective (NA=0.5) was attached to a piezo-motor for optical sectioning. Different ex-vivo ocular tissues, especially corneas, were used as samples. The system is fully-controlled via custom C++ software.

The effectiveness and performance of the dual imaging system was evaluated. The specimen was first imaged in the confocal linear mode, then, a flip mirror allowed to switch to the multiphoton imaging mode while the z-piezo motor was activated to correct the defocus chromatic difference. As an example, Figure 1 shows the comparison of a confocal and a SHG image corresponding to an ex-vivo human sclera fixed in paraffin. The acquisition time for a complete image was ~0.30 s (100x100 px^2 with a resolution of 1µm/px). The combination of both signals (i.e. linear and non-linear) allows obtaining integral imaging of the analyzed tissue. This combined approach might be a promising tool in clinical research and diagnosis of ocular diseases.

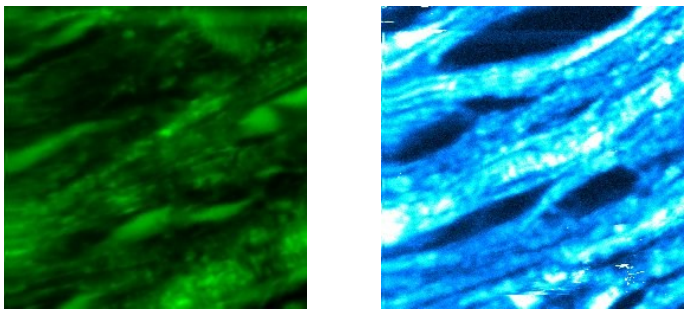

Figure 1: Linear confocal (left) and SHG (right) images of an ex-vivo human sclera fixed in paraffin. Image size: 100x100 μm^2.

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