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Internship project

Mapping of Human corneal 3D structure using Second Harmonic microscopy

Development of **nonlinear optical microscopy** has strongly improved three-dimensional (3D) imaging of biological tissues. Notably, Second Harmonic Generation (SHG) signals enable the visualization of fibrillar collagen with unequalled contrast and without any labeling, which is not possible using conventional techniques [1]. Collagen is the main component of connective tissues in mammals: it is synthetized as triple helices that spontaneously self-assemble to form fibrils (10-300 nm diameter) that further organize and form various structures with sizes 1 µm to 1 mm, which are specific for every type of tissue. The size and 3D distribution of collagen fibrils determine the biophysical and biomechanical response of tissues: opacity and compliance of skin, transparency and rigidity of cornea, etc... *In situ* characterization of collagen 3D distribution is therefore a major biomedical challenge, specifically to understand the very specific architecture of organs such as cornea, to get insight into pathological dysfunction and to develop new diagnosis tools.

In this context, we have implemented SHG microscopy experiments and characterized in a quantitative way the lamellar structure of *ex vivo* Human corneas and *in vivo* Rat corneas [2, 3]. However, we have only visualized small regions of interest because of the limited field of view of optical microscopy (less than 1 mm²), while full-size imaging of cornea is necessary to identify structural changes from center to periphery, and in the anterior versus posterior regions. This internship therefore **aims to carry out a detailed mapping of the full 3D structure of cornea**, in close collaboration with the *Banque Française des Yeux* and *Hôpital des 15-20* (Paris). The work will comprise 3 steps: (i) the implementation of a motorized stage to acquire automatically tiles mapping the full cornea (≈ 1 cm² x 0.5 mm) at µm resolution; (ii) multiphoton imaging of the full volume of Human corneas, using epi- and trans-detection of SHG signals and acquisition of polarization-resolved SHG images, which provide complementary information about the direction and organization of fibrils at sub-µm scale; (iii) the development of automated image processing tools to calculate relevant parameters for quantitative assessment of the cornea structure.

This internship may be continued by a PhD about similar measurements of structural parameters as a function of the intra-ocular pressure, in healthy corneas and dystrophic corneas (keratoconic corneas) with disrupted mechanical properties.

Recent related publications (see also http://www.lob.polytechnique.fr/):

[1] Bancelin et al, *Determination of collagen fibril size via absolute measurements of SHG signals*, Nat. Commun. 5, art 4920 (2014)
[2] Latour et al, *In vivo imaging of the cornea by polarization-resolved SHG microscopy,* Biomed. Opt. Express **3** (2012)
[3] Grieve et al, *Stromal striae: a new insight into corneal physiology and mechanics,* Sci. Rep. *in press* (2017).

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