# *IN SITU* VISUALIZATION OF COLLAGEN ARCHITECTURE IN BIOLOGICAL TISSUES USING POLARIZATION-RESOLVED SHG MICROSCOPY

**Marie-Claire SCHANNE-KLEIN**

*Laboratory for Optics and Biosciences*
*Ecole Polytechnique – CNRS - INSERM U696*
*F-91128 Palaiseau, France*

marie-claire.schanne-klein@polytechnique.edu

Multiphoton microscopy has been shown to provide three-dimensional (3D) cell-scale contrasted images of biological tissues. In particular, Second Harmonic Generation (SHG) microscopy has emerged as a unique tool to probe the 3D distribution of collagen fibrils within unstained tissues (see fig. 1) because this second order coherent signal is highly specific for dense non-centrosymmetrical macromolecular structures. Such a 3D imaging technique is of great interest for biological and biomedical studies since collagen is the main component of the extra-cellular matrix in mammals. This structural protein is characterized by triple helical domains, which self-assemble into fibrils that further form fibers, lamellae or other 3D networks essential for the architecture of organs. This hierarchical organization of collagen is responsible for the biophysical and mechanical properties of tissues, for instance the mechanical strength of the tendon, the transparency of the cornea, etc…It is also involved in a variety of pathologies, including genetic diseases such as Elhers-Danlos syndrome, fibrosis, cancer, or any abnormal tissue remodeling, etc…

In the recent years, we focused on the development of polarization-resolved SHG imaging to access the sub-micrometer scale distribution of collagen fibrils [1]. We used a multiscale non-linear optical formalism to show that this technique can probe both the main direction of collagen fibrils and their orientational disorder in the focal volume [2]. We validated this theoretical approach by imaging rat-tail tendons subjected to controlled mechanical stretching, which increasingly straightened and aligned the collagen fibrils. Finally, we used polarization-resolved SHG microscopy to map the 3D distribution of nanometer-sized collagen fibrils in corneal stroma [3]. We successfully retrieved structural information from *ex vivo* human corneas and *in vivo* rat corneas, while raw backward-detected SHG images were spatially homogenous.

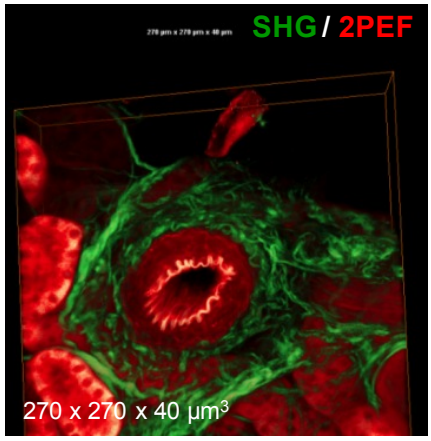


*Fig. 1: multiphoton imaging of unstained arcuate artery from fibrotic mice*

Our work also aimed to develop *quantitative* SHG imaging of the collagen fibrillar network. As a first step, we measured the nonlinear response of the collagen triple helix using Hyper Rayleigh scattering experiments [4]. Then, we assessed the sensitivity of SHG microscopy by measuring the minimum size of fibrils that can be detected using SHG microscopy. For that purpose, we correlated SHG images to TEM images of the same fibrils, for the first time to the best of our knowledge. It showed that SHG microscopy allows imaging of fibrils with a diameter down to 30 nm in our setup, much below the optical resolution.

[1] I. Gusachenko et al, Opt. Express **18**, 19339-19352 (2010).
[2] I. Gusachenko et al, Biophys. J. **102**, 2220-2229 (2012).
[3] G. Latour et al, Biomed. Opt. Express **3**, 1 (2012).
[4] A. Deniset-Besseau et al, J. Phys. Chem. B, 113(40), 13437-13445 (2009).