

# SIMULATING SECOND HARMONIC IMAGING OF COMPLEX BIOLOGICAL SAMPLES

M. Strupler and M.-C. Schanne-Klein

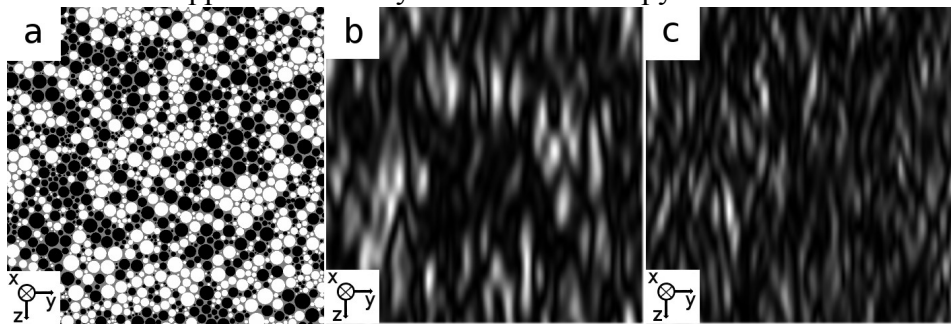
Laboratoire d'Optique et Biosciences, Ecole Polytechnique – CNRS – INSERM U696,  
91128 Palaiseau, France.

[mathias.strupler@polytechnique.edu](mailto:mathias.strupler@polytechnique.edu)

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Recently, we demonstrated that second harmonic generation (SHG) microscopy is a valuable tool to assess collagen fibrosis severity [1]. However, we used a phenomenological score because of the complex relationship between collagen content and SHG signal. In order to quantify collagen concentration and organization more precisely, additional theoretical work on SHG from biological tissues is required.

SHG theoretical background was mainly developed for laser frequency doubling in crystals [2]. However, SHG imaging of biological samples usually deals with inhomogeneous distribution of harmonophores and with tightly focused beams. In that regard, conventional theoretical work is not applicable directly to SHG microscopy.



**Figure 1** : Simulation of SHG images from a tendon **(a)** Position, size and orientation of the collagen fibrils used for simulation (direction :  $x+$  = white /  $x-$  = black) **(b)** Normalized forward-SHG image **(c)** Normalized backward-SHG image. Incident laser propagating along the  $z$  axis, excitation wavelength : 860 nm, objective : 20x 0.9NA, collection angle :  $45^\circ$  in both directions, image size :  $10 \times 10 \mu\text{m}^2$ .

To take these two features into account, SHG from biological samples has been simulated by summing up all the contributions of infinitesimal units of volume in the focal region [3, 4, 5]. While this approach is accurate and explains most experimental data, it is time consuming for complex arrangements of molecules.

We thus developed a novel model to simulate SHG radiation from biological samples. We used three-dimensional (3D) Fourier transform of the spatial organization of collagen within the sample, in analogy with methods used in X-ray crystallography. This technique is faster than conventional simulations and gives a better insight into the relationship between 3D distribution of harmonophores and SHG radiation pattern. We then applied this technique to tendon like structures (as seen in Figure 1) and compared our results with experimental data.

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