

# DISCRIMINATION BETWEEN COLLAGEN AND MUSCLE USING POLARIZATION SECOND HARMONIC IMAGING WITH PIXEL RESOLUTION

Sotiris Psilodimitrakopoulos<sup>a</sup>, Guadalupe Soria<sup>b</sup>, Ivan Amat-Roldan<sup>a</sup>, David Artigas<sup>c</sup>  
Anna M. Planas<sup>b</sup>, and Pablo Loza-Alvarez<sup>a</sup>

<sup>a</sup>ICFO-Institut de Ciències Fotòniques,

Mediterranean Technology Park, 08860 Castelldefels (Barcelona), Spain

<sup>b</sup> IIBB-Institut d'Investigacions Biomèdiques de Barcelona, CSIC- Consejo Superior de Investigaciones Científicas, IDIBAPS-Institut d'Investigacions Biomèdiques August Pi-Sunyer, Rossello 161, 08036, Barcelona, Spain

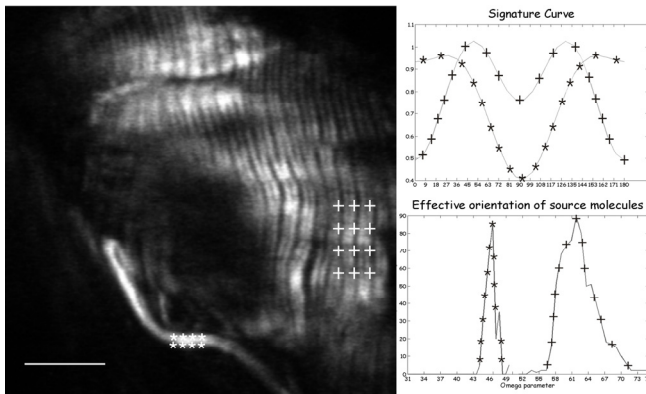
<sup>c</sup> UPC- Universitat Politècnica de Catalunya,

Department of signal theory and communications, 08034, Barcelona, Spain

E-mail: sotiris.psilodimitrakopoulos@icfo.es

**KEY WORDS:** Polarization second harmonic generation, discrimination, collagen, muscle.

Polarization sensitive second harmonic generation (SHG) imaging can provide information unreachable by intensity only SHG imaging. It works as a contrast mechanism and has the potential to characterise different SHG active structures<sup>1</sup>. The information is obtained by quantifying the contrast provided by the SHG intensity variation on the incoming polarization. This response is unique for each SHG active molecular architecture (called harmonophore). The experimental data is fitted into a biophysical model that assumes hexagonal symmetry in the  $\chi^{(2)}$  tensor of the sample. This allows calculating the ratio of the non-vanishing tensor elements. By expanding the model to the microscopic level, the effective orientation of the harmonophores can be estimated. In this study we developed and used this technique to retrieve submicron structural information and to discriminate point by



point in the same image collagen from muscle. We used tissue from the left temporalis muscle of rats, in which both skeletal striated muscle and fibrillar collagen type I can be found.

Figure 1: Left: Temporalis muscle of rat, imaged with SHG. Right: Signature and effective orientation curves which are referring to the ROIs shown with (\*) and (+), respectively. Scale bar is 10 $\mu$ m.

The images were fitted pixel by pixel using an algorithm based on the above mentioned model where a coefficient of determination of  $r^2 > 85\%$  was chosen as a filtering mechanism. For the selected ROIs we then retrieved the distribution of effective orientations,  $\theta_e$ , of the harmonophores. These are shown next the SHG image along with their signature curves. For the muscle the distribution is centered at  $\theta_0 = 60.2^\circ$  and has a bandwidth of  $\Delta\theta_e = 8^\circ$  (FWHM), and for collagen these values are  $\theta_0 = 47^\circ$  and  $\Delta\theta_e = 3^\circ$ . In this work we show that it is possible to quantitative discriminate between collagen and muscle, without the use of any markers or any sample treatment. This represents the first demonstration of discrimination between harmonophores in the same image, without the use of analyzer. Moreover, the use of a mammalian tissue demonstrates its great potential for biomedical applications.

[1] F. Tiaho, G. Recher, D. Rouede, "Estimation of helical angles of myosin and collagen by second harmonic generation imaging microscopy", *Opt. Express*, **15**, 12286-12295, (2007).