

# MUELLER POLARIMETRY REVEALS INTERNAL COLLAGEN ORGANIZATION FROM SECOND HARMONIC MICROSCOPY IMAGES

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Second Harmonic Generation (SHG) microscopy is used to image collagen-based samples (micro-scale). The analysis of collagen external spatial arrangement has been carried out by different methods including the Fourier transformation, the structure tensor or polarization-sensitive (PS) approaches [1-3]. However, the characterization of the internal organization (nano-scale) is not straightforward and it is still a topic of interest. The ratio of hyperpolarizabilities ( $\rho = \beta_{xxx}/\beta_{xyy}$ ) is closely related to the internal collagen distribution (nano-scale) [3,4]. PS-SHG microscopy has been reported to be a useful tool to extract information on both the parameter  $\rho$  and the molecular orientational distribution [4,5]. On the other hand, the internal triple helices of the collagen molecules originate intrinsic chirality [6], which can be studied by measuring the circular dichroism (CD). Since the Mueller matrix (MM) contains the full polarimetric response of a sample, in this work we propose to characterize the collagen organization at internal and external scales by combining MM polarimetry and SHG microscopy.

A polarization state generator was incorporated into the illumination path of a custom-built multiphoton microscope [7]. Four polarimetric SHG images corresponding to independent incident polarization states were acquired. These images were used to compute the spatially resolved MM elements. Some polarimetric maps such as the dichroic ratio (DR), the CD, or the diattenuation (D) were computed from the MM elements.

Results show that the internal collagen structure (i.e. the ratio  $\rho$ ) can be computed from the DR and is related to the external organization (measured by means of the structure tensor) through a parabolic function. There is also a significant linear correlation between the parameter  $\rho$  and the CD. Moreover, the sign of  $\rho$  can be directly determined from the sign of CD. In addition, collagen-based tissues behave as diattenuators (i.e. non-null D values) with the axis parallel to the preferred orientation of the fibers. This means that the parameter D presents a linear dependence with the external organization, what means that a sample with a high structural dispersion of the collagen fibers has low polarization sensitivity. Since mechanical effects, trauma and disease can modify collagen arrangement at both scales, this approach based on MM polarimetry allows an integral measurement and collagen characterization that might help in early diagnosis or tracking of pathologies.

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