CIRCULARLY-POLARIZED SECOND-HARMONIC MICROSCOPY OF FIBROUS PROTEINS

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Collagens are the most abundant proteins in mammals, especially found in flesh and fibrous tissues. In total, 28 types of collagen are known of which type I is the most common. Structurally, a single type I collagen consists of three intertwined chiral α-helices coiled together, which self-assemble to form microfibrils and even larger fibril aggregates. Due to this supramolecular ordering, fibrillar collagens such as type I or II, are also second-harmonic generation (SHG) active [1]. SHG is a second-order nonlinear process, which has been shown to be sensitive to the chirality of type I collagen, originating from the micrometer-scale structural organization [2]. More recently, polarized SHG microscopy was performed to provide morphological contrast between types I and II of collagen [1].

Here, we propose that SHG microscopy with circular polarization could also provide morphological contrast for imaging fibrillar proteins, such as different collagen types. The technique is simple needing only two input polarizations, namely the left-handed (LHCP) and right-handed (RHCP) circular polarization, and relies on measuring second-harmonic generation circular-difference (SHG-CD) responses of the sample [3]. Since the SHG and SHG-CD responses depend sensitively on the structure of the sample, the SHG-CD response can provide morphological contrast similar to polarized SHG microscopy.

To evaluate the feasibility of the new imaging modality, we modelled SHG microscopy (NA=0.8 and wavelength of 1060 nm) of fibril aggregates [Fig. 1a] consisting of two rod-like helical microfibrils, with pitch-angles (ϕ) for the helices chosen as ϕ=45° and ϕ=70°. The SHG responses from each sample pixel were calculated using Green’s function approach[4] for RHCP [Fig. 1b] and LHCP [Fig. 1c] inputs and in Fig. 1d) is shown the correspondingly calculated SHG-CD response image.

To conclude, we have proposed that SHG microscopy with circularly-polarized light could be utilized to provide morphological contrast for imaging of fibrous proteins, such as collagen. The technique is extremely simple and could be implemented in any existing SHG microscope and therefore be a useful imaging tool.