POLARIZATION RESOLVED SECOND HARMONIC GENERATION IMAGING AND MODELLING PROBES PROTEIN MOLECULAR STRUCTURE

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Keywords: Second Harmonic Generation, Non-linear optics, Multiscale modeling, Protein structure

Polarization resolved second-harmonic-generation (pSHG) microscopy is increasingly used for mapping organized arrays of non-centrosymmetric proteins such as collagen, myosin and tubulin, and holds potential for probing their molecular structure and supramolecular organization in intact tissues. However, the contrast mechanism of pSHG is complex, and the development of applications in the life sciences is hampered by the lack of models accurately relating the observed pSHG signals to the underlying molecular and macromolecular organization. In this work, we establish a general multi-scale numerical framework relating the micrometer-scale SHG measurements to the atomic-scale and molecular structure of the proteins under study and their supramolecular arrangement. We first develop a new method to automatically analyze pSHG signals independently of the protein type and fiber orientation. We then characterize experimentally pSHG signals in live zebrafish larvae, and show that they can be used to distinguish collagen, myosin and tubulin structures in intact tissues. We then introduce a numerical model that considers the peptide bond (PB) as the elementary SHG source in proteins, and takes into account the three dimensional distribution (3D) of PB to predict the second order hyperpolarizability tensor β of proteins, as well as the SHG efficiency and pSHG response of an arbitrary macromolecular assembly. We show that this model accurately reproduces pSHG measurements obtained from collagen, myosin, microtubule and actin structures, revealing the precise dependence of SHG signals on the 3D distribution of PB within protein assemblies. We then use our model to analyze pSHG from a 3D distribution of microtubules assemblies as a function of out-of-plane angles, angular disorder and polarity. Finally, we demonstrate that our model predicts SHG from different molecular conformations of tubulin that are highly relevant from a biomedical point of view as associated with to microtubules (de)polymerization. By bridging scales from the molecular bonds to the optical wavelength, our model provides an accurate interpretation of SHG signals in terms of protein structure and supramolecular organization.

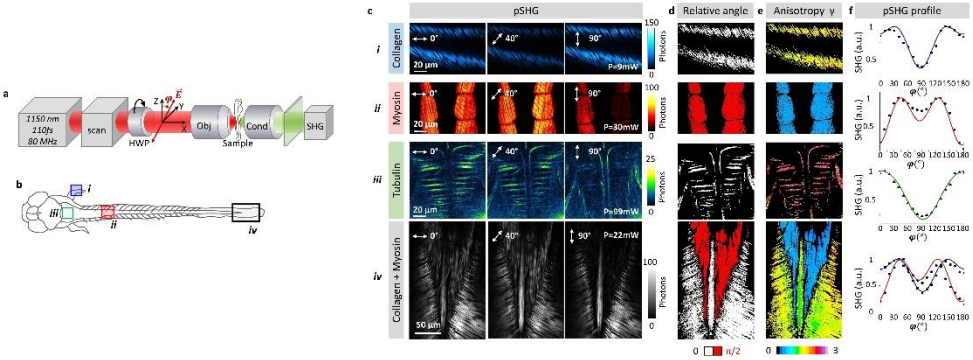

Fig. 1: Polarization-resolved SHG (pSHG) measurements on different fibrillar structures in a live zebrafish larva at 5 dpf. (a) pSHG microscopy setup. A rotating half waveplate (HWP) is installed before the objective and is used to control the direction of the incident linear polarization in the yz imaging plane of the laboratory system. (b) Scheme of a zebrafish larva at 5 dpf. Collagen and myosin signals are observed in the pectoral fins (i) and in the trunks (ii), while tubulin signals are observed in the brain and spinal cord (iii). (c)

pSHG images recorded at different polarization angles as indicated by the arrows. (d) Maps of the relative angle of the maximum (resp. highest minimum) SHG intensity with respect to the fiber axis for one-peaked (resp. two-peaked) pSHG profiles extracted automatically with the structure tensor analysis and FFT-based analysis. (e) Maps of the anisotropy factor γ for the three proteins calculated with the FFT-based analysis taking in account the relative angle of the maximum (resp. highest minimum) SHG intensity. pSHG distinguishes myosin and collagen within the same image based on the anisotropy factor. (f) Dependence of the SHG intensity in a region of interest as a function of the incident polarization angle $\varphi - \varphi 0$, where $\varphi 0$ is the angle of the protein fiber axis.

Asadipour, B., et al., *Modelling and predicting second harmonic generation from protein molecular structure.* Phys. Rev. X, 2024. **14**: p. 011038.