Stokes Vector Based Second Harmonic Microscopy and it's applications

Nirmal Mazumder,[1,*] Fu-Jen Kao[2]

[1]Department of Biophysics, School of Life Sciences, Manipal Academy of Higher Education, Manipal, Karnataka, India-576104

[2]Institute of Biophotonics, National Yang-Ming University, Sec. 2, Linong St., Taipei 112, Taiwan

*Corresponding Email: nirmaluva@gmail.com

Keywords: optical scanning microscopy, second harmonic, Stokes vector, polarization.

Second Harmonic Generation (SHG) microscopy is an effective analytical tool for detailed investigation of microscopic structure of non-centrosymmetric molecules. We developed a four-channel photon counting based Stokes polarimeter integrated to SHG microscope for spatial characterization of polarization effects in SH signal. We have implemented a calibration technique allowing quantitative measurement of various polarization parameters, such as the degree of polarization (DOP), the degree of linear polarization (DOLP), the degree of circular polarization (DOCP), and anisotropy from the 2D second harmonic Stokes images in a pixel-by-pixel manner. Use of Stokes polarimetry is critical in determination of the full polarization state of light, and enables discrimination of material properties not possible with conventional crossed-polarized detection schemes. In this abstract, we will be discussing about various applications of the technique from our published results.

The changes of the starch morphology during heating and the degree of crystallinity of dry and hydrated granules are investigated using SHG based Stokes polarimetry. The SH signal from hydrated and dry starch on heating differed significantly in DOLP and DOCP values, indicating that hydrated starch has a greater degree of ultrastructural amylopectin disorder. The detail of denaturation and the phase transition of hydrated starch demonstrate the significant influence of thermal processing. Thus, the combination of SHG microscopy and Stokes polarimetry makes a powerful tool to characterize the polarization properties and to investigate the structural order of collagen type-I, skeletal muscle fibers and starch granules.

References:
1. X. Chen, O. Nadiarynkh, S. Plotnikov, and P. J. Campagnola, Second harmonic generation microscopy for quantitative analysis of collagen fibrillar structure, Nat. Protoc. 7(4) 654–669 (2012).
2. N. Mazumder, G. Deka, W.W. Wu, A. Gogoi, G.Y. Zhuo, F.J. Kao, Polarization Resolved Second Harmonic Microscopy, Methods 128, 105-118 (2017).
3. N. Mazumder, J. Qiu, M. R. Foreman,C. M. Romero, C.W. Hu, H.R. Tsai, P. Török, and F.J. Kao, Polarization resolved second harmonic generation microscopy with a four-channel Stokes-polarimeter, Opt. Express. 20(13) 14090–14099 (2012).
4. N. Mazumder, Y.X. Lu, J. Qiu, and F. J. Kao, Investigating starch gelatinization through Stokes vector-resolved second harmonic generation microscopy, Sci. Rep. 7 45816 (2017).