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Revealing molecular structure and orientation with Stokes vector resolved second harmonic generation microscopy

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ABSTRACT

We report on measurements and characterization of polarization properties of Second Harmonic (SH) signals using a four-channel photon counting based Stokes polarimeter. In this way, the critical polarization parameters can be obtained concurrently without the need of repeated image acquisition. The critical polarization parameters, including the degree of polarization (DOP), the degree of linear polarization (DOLP), and the degree of circular polarization (DOCP), are extracted from the reconstructed Stokes vector based SH images in a pixel-by-pixel manner. The measurements are further extended by varying the polarization states of the incident light and recording the resulting Stokes parameters of the SH signal. In turn this allows the molecular structure and orientation of the samples to be determined. 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. The combination of SHG microscopy and Stokes polarimeter hence makes a powerful tool to investigate the structural order of targeted specimens.

1. Introduction

Second harmonic generation (SHG), a second order nonlinear coherent optical process, has been widely used for imaging non-centrosymmetric molecular structures, partly due to the lack of photobleaching [1–8]. SHG provides a unique contrast mechanism on a wide range of materials, as well as the capacity to image with higher spatial resolution and at sub-millimeter depths [1–5]. SHG imaging is commonly used in conjunction with two-photon-excited (TPE) fluorescence for optical diagnostics on complex cellular assemblies, such as skin or organ tissues [6,7]. Recently, polarization resolved SHG microscopy was used to investigate the relative molecular orientation and disorder in the structure of human tissues, such as the dermis [9], cornea [10,11], and myosin of the skeletal muscle [12–14]. SHG anisotropy was studied through the measurement of the SH intensity whilst rotating the polarization of the incident linearly polarized excitation beam [2,4,15,16]. The nonlinearity of SH enables a higher extinction ratio in polarization microscopy, as can prove beneficial in, for example, overcoming the depolarization due to high NA optics [17]. In general, multiphoton microscopy is expected to enhance polarization effects induced on the sample, especially for nonlinear optical contrasts based upon higher order susceptibilities.

Polarization analysis of a SH signal can be carried out using either Jones calculus or Stokes algebra [18]. In most applications cross-polarized two-channel detection is employed and hence use of Jones calculus is used. Strictly, the Jones method is however, only applicable for perfectly polarized light beams [18,19] and is thus unsuitable in many scenarios. For example, use of cross-polarized two-channel detection does not allow the relative ratio of the polarized and un-polarized components of the field to be determined. Additionally, the full polarization state of the polarized component cannot be found due to an ambiguity over the phase difference between the two measured polarization basis vectors. In contrast, the full polarization state of a general optical signal, including partially polarized or unpolarized fields, can be characterized using Stokes algebra [20,21]. Accordingly, a four-channel Stokes polarimeter can be constructed so as to measure all four Stokes parameters. In this article, Stokes vector based SHG microscopy is therefore reviewed [22–24]. Knowledge of the optical polarization properties of biological tissues, which can be inferred from a set of measured Stokes parameters, has a number of biomedical applications since it provides insight into molecular

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structure and tissue organization. The polarization parameters can for instance, be correlated with the corresponding structural symmetry of tissue samples. A full Mueller matrix formalism, a 4×4 matrix, has long been shown as a powerful method in the context of linear optics [24]. However, in the case of nonlinear optics, the Mueller matrix formalism and associated physical interpretation [24], including the decomposition and the corresponding interpretation, are very involved and may be counter-intuitive [25]. Therefore, developing a full formalism of nonlinear optics based Mueller matrix for correlating the polarization states of the input beam with the nonlinear output optical signals, despite of the envisioned beauty of such a theory, is likely beyond practicality. Although we believe the presented treatment to be generally applicable, we choose to demonstrate it through characterization of the polarization properties of SH signal from potassium m dihydrogen phosphate (KDP) and type-I collagen.

2. Materials and methods

2.1. Instrumentation

A schematic diagram of an experiment arrangement, suitable for measuring the polarization properties of SH signal of SHG microscopy, is described in detail in [22,23] and is shown in Fig. 1. The illumination and excitation optics, used to generate a SH signal from a sample of interest, is very much the same as other multiphoton microscope, and is formed by integrating an ultrafast laser with a scanning microscope. The detection optics, comprising a Stokes polarimeter incorporated into the output of a scanning microscope (operating in a transmitting modality), and the associated calibration is, however, the main focus of this work.

A femtosecond Ti: Sapphire (Coherent Mira Optima 900-F) laser oscillator was used to generate linearly polarized ~150 fs pulses with central wavelength of 800 nm and average power ~550 mW at a repetition rate of ~76 MHz. Our polarization setup includes a polarization state generator (PSG), sample and polarization state analyzer (PSA). The various linear and circular polarization states are generated using the PSG, which is a combination of a linear polarizer (LPUV 100-MP, Thorlabs), a half wave-plate ($\lambda/2$) (AHWP05M-600, achromatic HWP, Thorlabs), and a quarter wave-pate ($\lambda/4$) (AQWP05M-600, achromatic QWP, Thorlabs). A dichroic mirror reflects the laser pulses into the microscope objective lens (UPlanFLN 40X/N.A. 1.3, Olympus Co., Japan). In our experiment, the diameter of the laser beam before the objective lens is 5 mm, which is less than the back aperture of the objective lens, which is approximately 10 mm. We chose not to fill the aperture such that (1) transmission loses and (2) depolarization associated with high NA focusing was reduced. The laser beam was focused onto a sample mounted upside-down on an XYZ stage and scanned with a laser scanning unit (Olympus, FV300). Collected SH signals were analyzed by our polarization state analyzer (PSA), commonly known as a four-channel Stokes-polarimeter.

2.2. Working principle of four channel Stokes-polarimeter

Of great importance in this work are the Stokes parameters on the light scattered from a sample, regardless of whether their origins are attributed to linear or nonlinear process. As mentioned earlier, there is no well accepted theory or formalism on nonlinear optics based Mueller matrix. We would like to point out that Mueller matrix formalism becomes a practical approach only when Lu-Chipman decomposition [24] is also implemented to make a functional connection between the input and output polarization states. For nonlinear optics, the corresponding decomposition method does not exist and would regardless be overwhelmingly complicated to be intuitive and practical. Fig. 2 shows the schematic in explaining the difference between Stokes polarimetry, as compared to Mueller polarimetry. Given the discussion above, a full Mueller matrix formalism remains a powerful method only within the context of linear optics [24,26].

A Stokes polarimeter consists of a light source (in our case the SH signal), polarization state analyzer (PSA) and intensity based detection (I). Specifically, a Stokes polarimeter measures the polarization state of the incoming optical signal, as parameterized by the associated Stokes parameters, or collectively the Stokes vector $S_{out} = [S_0, S_1, S_2, S_3]^t$. Each Stokes parameter can be accorded a physical meaning by noting [27,28].

$$S = \begin{bmatrix} S_0 \\ S_1 \\ S_2 \\ S_3 \end{bmatrix} = \begin{bmatrix} I_{0^\circ} + I_{90^\circ} \\ I_{0^\circ} - I_{90^\circ} \\ I_{45^\circ} - I_{-45^\circ} \\ I_{RCP} - I_{LCP} \end{bmatrix} \quad (1)$$

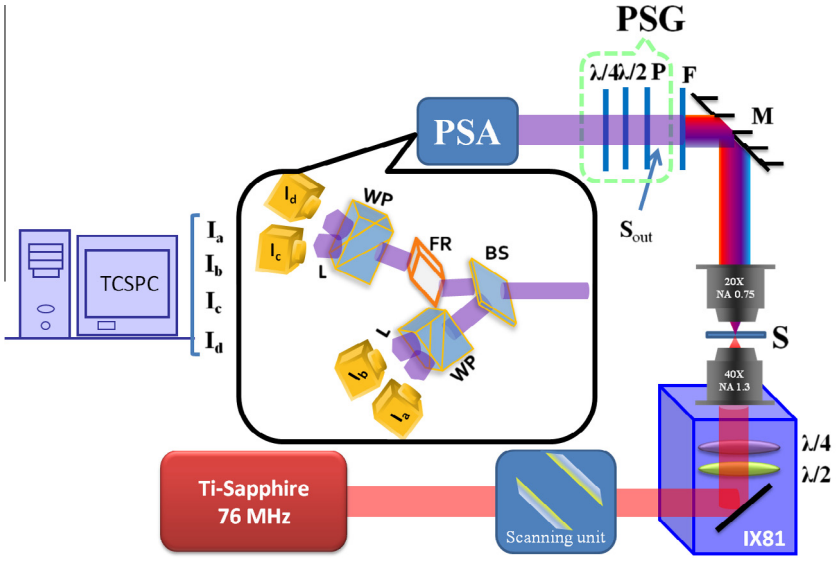

Fig. 1. The schematic diagram of polarization resolved second harmonic generation four-channel Stokes-polarimeter setup. The setup is module based. PSG is inserted for the calibration of PSA and is removed afterward. $\lambda/2$: half wave-plate, $\lambda/4$: quarter wave-plate, S: sample, M: mirror, F: filter, BS: Beam splitter, FR: Fresnel rhomb, WP: Wollaston prism, L: focusing lens, I_a, I_b, I_c, I_d: photo-multiplier tubes (PMTs), TCSPC: Time correlated single photon counting.