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Synchronous-digitization for Video Rate Polarization Modulated Beam Scanning Second Harmonic Generation Microscopy

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Abstract

Fast beam-scanning non-linear optical microscopy, coupled with fast (8 MHz) polarization modulation and analytical modeling have enabled simultaneous nonlinear optical Stokes ellipsometry (NOSE) and linear Stokes ellipsometry imaging at video rate (15 Hz). NOSE enables recovery of the complex-valued Jones tensor that describes the polarization-dependent observables, in contrast to polarimetry, in which the polarization stated of the exciting beam is recorded. Each data acquisition consists of 30 images (10 for each detector, with three detectors operating in parallel), each of which corresponds to polarization-dependent results. Processing of this image set by linear fitting contracts down each set of 10 images to a set of 5 parameters for each detector in second harmonic generation (SHG) and three parameters for the transmittance of the fundamental laser beam. Using these parameters, it is possible to recover the Jones tensor elements of the sample at video rate. Video rate imaging is enabled by performing synchronous digitization (SD), in which a PCIe digital oscilloscope card is synchronized to the laser (the laser is the master clock.) Fast polarization modulation was achieved by modulating an electro-optic modulator synchronously with the laser and digitizer, with a simple sine-wave at 1/10th the period of the laser, producing a repeating pattern of 10 polarization states. This approach was validated using Z-cut quartz, and NOSE microscopy was performed for micro-crystals of naproxen.

Keywords

Microscopy; Nonlinear optics; polarization; second harmonic generation

1. INTRODUCTION

Polarization dependent second harmonic generation (SHG) microscopy has previously been used in analysis of ordered biological motifs^{1,2}, cell membranes³, and the analysis of collagen^{4–6}. Polarization based SHG imaging has also seen success in characterization of inorganic materials^{7–9}, in surface analysis^{10,11} and crystal domain imaging¹².

Current approaches for polarization based imaging suffer from significant limitations. Typically, polarization modulation is achieved by mechanical rotation of a waveplate/polarizer, which can suffer from many potential pitfalls. Due to the relatively long time

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required for manual rotation, significant $1/f$ noise may be introduced into the measurement. Additionally, sample movement (especially relevant in the case of *in vivo* imaging), or thermal effects induced by laser absorption, can result in systematic bias and increased uncertainty in the extracted parameters.

In this present study, quantitative analysis in SHG microscopy is demonstrated based on nonlinear optical Stokes ellipsometry (NOSE)¹³. By combining high speed (8MHz) polarization modulation using an electro-optic modulator (EOM) and expanding on our previously reported technique of synchronous digitization (SD) for lock-in amplification¹⁴, the time for NOSE microscopy was reduced to as low as 150ns per pixel, enabling video rate polarization dependent imaging.

2. METHODS

All experiments were performed on a custom built microscope described in detail previously^{9,13–15}. In short, a 80 MHz ~100 fs MaiTai Ti:Sapphire laser (SpectraPhysics) operating at 800nm was used, with average powers between 20–220mW at the sample. The beam was passed through a custom electro optic modulator (EOM) rotated 45° from its fast-axis. A Soleil-Babinet compensator was placed after the EOM and before the microscope to correct for slight polarization changes induced by the beam path. The beam was directed through an in-house built scan head consisting of a resonant mirror operating at ~7.8 KHz (EOPC) and a galvanometer mirror (CambridgeTech). Immediately following the scan head, the beam was directed through telecentric lens in a 4f configuration, with a 4x beam expansion. The laser was focused onto the sample using a 10× objective (Nikon). SHG and fundamental wavelengths were separated using a dichroic mirror and collected in the transmitted direction. The laser fundamental and SHG were re-collimated using a paired 10× objective in the transmitted direction. The SHG signal was separated into its horizontal and vertical components with a Glan-Taylor polarizer, which were detected on two photomultiplier tubes (PMTs) (Hamama-tsu H12310-40) with bandpass filters (HQ 400/20m-2p; Chroma Technology) to further reject the fundamental. The transmitted fundamental light was detected with a photodiode (Thorlabs DET-10A) after passing through a Glan-Taylor polarizer set to pass horizontal, allowing for simultaneous polarized laser transmittance imaging and SHG imaging.

Due to the high quality factor (Q) of the resonant mirror ($Q > 250$), an in-house electronic package was developed to maintain phase stability. The custom driving electronics were controlled by an 8-bit microcontroller (Silicon Laboratories, C8051f120), running at 80 MHz derived from an external 10 MHz phase-lock loop (PLL) synchronous with the 80 MHz master clock from the Ti:Sapphire laser, allowing for precise knowledge of the number of laser pulses per resonant mirror trajectory. The feedback signal from the resonant mirror was analyzed using custom software by the box, and corrections to the driving phase were made to maintain a stable phase relationship. Phase corrections were performed to a precision of two clock cycles (25 ns). A copy of the monitor output of the resonant mirror was sent to the data acquisition cards to trigger line position in acquisition. Additionally, another copy of the resonant mirror output was sent to a custom built ramp generator, controlling the stepping of the galvanometer mirror.

Data were acquired with two PCIe digitizer cards (AlazarTech ATS-9350), running synchronously with the laser (synchronous-digitization, SD)¹⁴. The 80MHz clock signal from the laser was divided by 8 to produce a 10MHz signal, which was used to clock the digitizer cards directly. The PCIe digitizer cards were set to take the 10MHz PLL signal and upscale to 80 MHz, resulting in 80 million samples per second per channel, synchronized with the laser. The signal transients from each individual detector were flash digitized synchronously with every laser pulse. A 2–13 ns electronic delay stage was placed between the 10MHz PLL and the PCIe digitizer card to allow for adjustment of phase of the digitization event relative to the firing of the laser, to account for time delays in the optical path between the laser and detectors. The data were binned following a sine-wave trajectory and an image reconstructed using custom in-house built Matlab software. Due to the nature of the sine-wave trajectory of the resonant mirror, sample density near the edges (turning points) are higher than in the middle, and consequently the resulting data were normalized by the total number of laser event per pixel per polarization state.

High speed, synchronous polarization modulation was performed using a custom-built extended length EOM (Conoptics). A function generator was used to generate an 8 MHz sine-wave, which was amplified using a high voltage amplifier (AR Worldwide KAW1040) and a custom built resonant tank circuit. The function generator was synchronized to the laser using a 10MHz phase-locked loop (PLL), resulting in the same 10 polarizations being cycled through synchronously with the laser and digitization cards. To insure every pixel in the image sampled every polarization, the period of the resonant mirror was chosen to ensure that the number of laser pulses per image was an integer multiple of the number of polarizations used.

Polarization dependent imaging was performed using z-cut quartz as a reference standard, due to its well-established non-linear optical properties and lack of birefringence when probed along its z-axis. Images were acquired with 20 mW power at 800 nm and 32 frames were averaged together. NOSE imaging was also performed on naproxen crystals, with an average laser power of 95 mW. The crystals were recrystallized in isopropanol prior to imaging. Index matching of naproxen crystals was performed using Type A immersion oil. Video-rate imaging of the naproxen crystals moving across the field of view was performed by physical translation of the sample using an automated stage (Prior Instruments). Imaging was also performed on stationary crystals by averaging 64 frames together for increased S/N.

3. RESULTS AND DISCUSSION

Analysis of the polarization-dependent nonlinear optical response was performed. Full details on the recovery of the Jones tensor elements from polarization dependent SHG are out of the scope of this manuscript, but have been described previously in great detail^{10,13}. The intensity of the detected SHG can be described by equation 1,

$$I_{\text{det}}^{2\omega} \propto A \cos^4\left(\frac{\Delta}{2}\right) + B \cos^3\left(\frac{\Delta}{2}\right) \sin\left(\frac{\Delta}{2}\right) + C \cos^2\left(\frac{\Delta}{2}\right) \sin^2\left(\frac{\Delta}{2}\right) + D \cos\left(\frac{\Delta}{2}\right) \sin^3\left(\frac{\Delta}{2}\right) + E \sin^4\left(\frac{\Delta}{2}\right) \quad (1)$$

where Δ is the time dependent phase of the EOM given by equation 2.

$$\Delta(t) = A \sin(2\pi f_0 t + \delta) + B \quad (2)$$

Where A is the amplitude of modulations, f_0 is the modulation frequency (cycles/s), δ is the phase shift between the driving frequency and actual frequency of the EOM, and B is a residual phase difference inherent to the EOM.

Measurements of the polarization of the fundamental beam allowed for determination of the time dependent phase angle of the EOM, which determines the polarization state of the incident light. Polarized SHG and laser transmittance images are obtained simultaneously. Additionally, conformation of the phase angle of the EOM was established via analysis of the SHG response of Z-cut quartz. Detailed characterization and validation of this approach is given in a previous manuscript¹³. In short, the 10 polarization dependent SHG data sets are turned into a set of five Fourier coefficients, which can further be converted into three unique Jones tensor elements (six total, taking advantage of the interchangeability of the latter two subscripts in SHG). To succinctly display all five coefficients on the image, the five different coefficients were assigned to the intensity of a five channel color map. A visual representation of this process is depicted in Figure 1 for naproxen crystals. The results in Figure 1 were performed with signal averaging, in which 64 frames were averaged together. Additionally, Figure 2 demonstrates the same naproxen crystals across all detection channels, including the polarized laser transmittance imaging, and recovery of the linear birefringent coefficients as detailed previously¹³. Though signal averaging was performed, this analysis approach is capable of being performed at video rate. Several example frames of a video-clip of the color-coded polarization maps of the naproxen crystals are shown in Figure 3. In the case of Figure 3, the sample was not index matched to provide more contrast in the birefringence imaging results. The video was acquired at 15 Hz.

It is apparent from the color maps images that the polarization-dependence of SHG varies significantly for different naproxen crystals, depending on their relative orientation. The overall similarity of colormap hues for different crystals at similar orientations is consistent with this explanation. Such good agreement is encouraging, given that the azimuthal rotation angle is just one of the three possible rotation angles. Variability in the tilt and twist angles are expected to introduce polarization dependent responses. The lack of diversity in polarization dependence is attributed to templating by the glass interface. Low free energy barriers are introduced at the crystal/glass interface resulting in heterogeneous nucleation. Additionally, the interfacial energy at the crystal/glass interface will depend on the crystal plane position, such that thermodynamics will favor a specific twist and tilt angle relative to the interface. Under these conditions, only azimuthal rotation is fully unconstrained.

4. CONCLUSIONS

By incorporating fast polarization modulation with synchronous digitization, we demonstrated a technique to provide real-time ellipsometric imaging. Validation of this technique was performed using the well characterized system z-cut quartz. This technique was used to generate the Fourier coefficient images for naproxen crystals showing evidence of crystal orientation, i.e. similar color for similar apparent orientations. Additionally, it was demonstrated that coupling the fast polarization modulation synchronously with the

detection, the S/N of the measurement was sufficient to perform video rate (15 Hz) imaging with as little as 150 ns per pixel integration time and still recover Fourier coefficient images. The key enabling feature of this technique was the synchronizing of all devices, including data acquisition, to the laser serving as the master clock, which allowed for precise knowledge of every location and polarization of the individual laser pulses as it is scanned across the sample.

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Figure 1. (Adapted from Ref. ¹³) Summary of image construction. An overall intensity image a) and the same image separated out into its 10 unique polarization-dependent images b) are shown. The five Fourier coefficient images are shown in c), with each coefficient representing a unique color (red, green, blue, cyan and magenta for coefficients a, b, c, d and e, respectively). The five coefficient images were then merged into a single 5-color image, shown in d).

Figure 2. (Adapted from Ref. ¹³). Integrated intensity images of index matched naproxen crystals for a) horizontally polarized SHG, b) vertically polarized SHG and c) polarized laser transmittance, with corresponding coefficient colormaps in d), e) and f).

Figure 3.
(Adapted from Ref. ¹³). Video of color encoded polarization maps for naproxen crystals being translated in the X/Y plane. Polarization data were acquired in real time simultaneously on all three channels (horizontal-SHG, vertical-SHG and laser transmittance). The sample was translated using an automated prior-stage during imaging.