Fast Mueller linear polarization modality at the usual rate of a laser scanning microscope

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ABSTRACT

Mueller microscopes enable imaging of the optical anisotropic properties of biological or non-biological samples, in phase and amplitude, at sub-micrometer scale. However, the development of Mueller microscopes faces instrumental challenges: whilst adjusting the microscope, the operator needs a polarimetric image as guidance and the production of polarimetric parameters must be sufficiently quick to ensure fast imaging. To mitigate this issue, in this paper, a full Mueller scanning microscope based on spectral encoding of polarization is presented. The spectrum collected every 10 μs for each position of the optical beam on the specimen, incorporates all the information needed to produce the full Mueller matrix, which allows simultaneous images of all the polarimetric parameters at the unequalled rate of 1.5 Hz (for an image of 256×256 pixels). The design of the optical blocks allows for the real-time display of linear birefringent images which serve as guidance for the operator. In addition, the instrument has the capability to easily switch its functionality from a Mueller to a Second Harmonic Generation (SHG) microscope, providing a pixel-to-pixel matching of the images produced by the two modalities. The device performance is illustrated by imaging various unstained biological specimens.

Keywords: Mueller polarization, microscopy, Second Harmonic Generation microscopy

1. INTRODUCTION

Among numerous optical microscopy imaging modalities, polarized light microscopy[1] is dedicated to the observation of biological structures that exhibit intrinsic optical anisotropic properties in phase (linear/circular birefringence) and amplitude (linear/circular diattenuation) at sub-micrometer scale. The usual white light polarization microscope, in which the sample is placed between crossed polarizers, is routinely used for qualitative imaging but sometimes provides ambiguous image contrasts when several effects occur simultaneously. To obtain quantitative information and automate the measurement process, other schemes have been proposed[2,3] assuming usually that the specimens exhibit a pure polarimetric effect. As the need has arisen to take mixed polarimetric effects into account, confocal scanning Mueller microscopes[4] and full-field transmission Mueller polarimetric microscopes[5] have been successfully devised and then used for imaging biological tissues. Nevertheless, such microscopes still have limited performances due to: (i) the complexity of the polarization state generation and analysis and (ii) the time delay, typically from a few dozens of seconds to few minutes, for recording enough images to retrieve the 16-elements of the Mueller matrix with a suitable signal-to-noise ratio. Another limitation, usually not mentioned in most studies, is the difficulty of displaying these polarimetric parameters in real time, which is a huge issue when the operator adjusts the microscope to find a region of interest. Indeed, in addition to the acquisition time, a long post-processing stage is necessary to compute the Mueller matrices, to correct numerically the polarimetric signature of the microscope itself and to interpret Mueller matrices physically. Due to these limitations, Mueller microscopy is not widespread yet despite its interesting use in imaging histological sections. Recently, we have demonstrated as a proof of concept, the first full Mueller polarimetric scanning microscope based on an optical frequency swept-laser source implemented within a commercial confocal-like microscope[6]. The device did not use any moving parts for the polarized state generator (PSG) and analyzer (PSA) blocks, but only static polarizers and retarders to spectrally encode light polarization. From the channeled spectrum measured by a single-pixel detector, the full Mueller matrix was determined at each point of the specimen. The tuning speed of the swept laser, (10 μs in our case) determined the data acquisition time of our device. The paper was a proof-of-concept, involving averaging of data, and more importantly, only static specimens were imaged.

This communication presents a full Mueller scanning microscope based on new designs of passive PSA and PSG blocks allowing to display in real-time linear birefringence and its orientation or more generally the change of

polarization induced by the specimen as guidance for the operator whilst adjusting the microscope. In addition, the instrument is provided with another functionality allowing the operator to switch easily from a Mueller to a Second Harmonic Generation (SHG) imaging modality, while keeping a perfect point-to-point matching of the images. The performance of our new Mueller microscope is illustrated through imaging various unstained biological specimens and liquid crystals at frame rates of 1.5 Hz and 0.4 Hz from respectively single-scan 256×256 and 512×512 images. Finally, specimens have been scanned using both Mueller and SHG modalities in order to illustrate how a well-designed Mueller modality is able to reveal unstained birefringent arrangements – such as fibrillar collagen within thin tissue sections – and competes with a much more expensive nonlinear SHG modality.

2. EXPERIMENTAL SET-UP

The measurement of the Mueller matrix of a specimen, allowing access to its complete polarimetric information, requires generating several input polarization states and analyzing these states modified by the specimen. Instead of carrying out the measurements by active PSG and PSA blocks using rotating optical elements or polarization modulators, PSG and PSA blocks are made of thick chromatically dispersed retarders associating each polarization state to a wavelength and permitting to perform both generation and analyzing in a passive way. The microscope is based on a standard upright confocal microscope with these PSG and PSA blocks respectively at the input and the output of the microscope in such a way that polarization states are generated and analyzed for each wavelength delivered in time by the swept laser. They incorporate: a linear polarizer (Pol) oriented at 0° or 90°, YVO_4 thick retarders oriented at 45° and 0°, achromatic quarter wave-plates at 45°, all the optical elements in the PSG and PSA being fixed. The YVO_4 thickness is e = 0.4 mm for PSG and $5e$ = 2 mm for PSA. The swept-source, (SSOCT-1060, Axsun Tech. Inc.) sweeps the optical frequency over a spectral range of 100 nm (before digitization) around 1050 nm at a rate of 100 kHz. The electrical signal delivered by an avalanched photodiode (APD module C12703SPL, Hamamatsu) equipping a standard upright confocal microscope (Olympus BX51W1-FV300) is digitized by a data acquisition board (DAQ, ATS9350 digitizer, AlazarTech) in synchronism with the galvo-scanners the microscope is equipped with. The digitized signal includes a series of channeled spectra whose modulation is related to the polarimetric signature of each pixel of the image with a pixel-dwell time equal to 10 μs.

3. RESULTS AND DISCUSSION

The channeled spectrum $I_{x,y}(t)$ measured in transmission (Fig. 1(a)) is the combination of 12 modulations from f_0 to 12 f_0 whose amplitudes and phases depend on the elements m_{ij} of the Mueller matrix related to the polarimetric signature at each point of the specimen, the microscope itself and the PSG and PSA blocks. The acquisition of the spectrum takes 10 μs per image pixel, according to the tuning speed of the swept-source used in these experiments, which allows for producing the Mueller components at the usual frame rate of optical scanning microscopes, once raw data are processed. Three modalities have been implemented in the microscope:

(i) Display of specific images for guidance for the microscope operator through a software developed using LabVIEW 2017: the amplitude and the phase of the modulations of $I_{x,y}(t)$ are measured using harmonic analysis instead of Fourier transform. This strategy is more efficient to tackle real-time issues, as the axial range of interest is shorter than the one obtained via Fast Fourier Transform. To measure the complex values of the peaks P_k associated to the 12 modulations (k=0 to 12), $I_{x,y}(t)$ is only multiplied with 12 numerical oscillators $Exp(i2\pi k f_0 t)$, and the difference between the peaks ΔP_k with and without the specimen is calculated. Figures 1(c), 1(d) and 1(e) are images of a potato starch granule specimen obtained by displaying different peaks ΔP_k. Figure 1(c) corresponds to the peak ΔP_0 in grey levels that is mainly sensitive to the transmittance of the sample. Figure 1(d) is an image for which the phase of the peak ΔP_9 is color-coded in Hue-Saturation-Value (HSV) space, while the brightness of colors is regulated by the amplitude of ΔP_9, the saturation being set to 1. The peak ΔP_9 has been chosen because it theoretically depends on the linear retardance $R_{L(x,y)}$ and its azimuth $\alpha_{R(x,y)}$ through the expression $\Delta P_9 = 2\sin R_{L(x,y)}\, Exp\left(-i\,2\alpha_{R(x,y)}\right)$. Figure 1(e) corresponds to $\Sigma_{k=1,12}$ [$|\Delta P_k|$] in green levels that images the starch granule on a dark background and is sensitive to an overall change of polarization. This parameter is less sensitive to the orientation of the birefringence and exhibits a low contrasted Maltese cross.

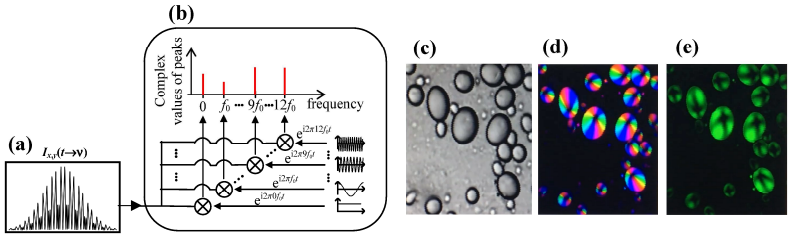

Fig. 1. Signal processing for real-time display. (a) Channeled spectrum measured versus time by the detector for the Mueller microscopy modality, originating from a single point on the specimen. (b) Extraction of the signal modulations by local oscillators. (c) Grey level image of the DC component. (d) HSV image of the 9th peak. Hue determined by the phase of the peak and brightness by its amplitude. (e) Green level image of all the modulations $\Sigma_{k=1,12}\,[|\Delta P_k|]$ except the DC component.

(ii) Record of all the polarimetric response (linear/circular retardance, linear/circular diattenuation, depolarization) of the specimens according to the different functionalities of the scan head: images at different frame rates and sizes, or in point mode at 100 kHz.

(iii) Use of the same microscope for both Mueller matrix measurement and SHG detection: this allows a pixel-to-pixel comparison of the images while the usual practice consists in imaging specimen with two different devices. As a demonstration, images of the same area of an unstained human liver specimen embedded in paraffin are presented with both Mueller and SHG modalities (Fig. 2). However, paraffin has not been correctly removed from the preparation and pollutes retardance image of the specimen. By using a binary mask based on the degree of alignment of birefringence axes, it is possible to select only collagen fibers.

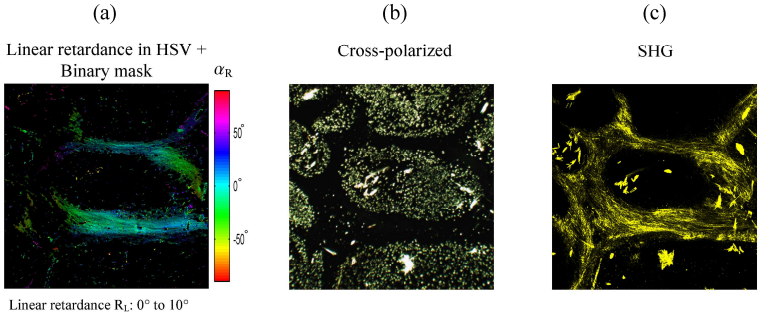

Fig. 2. Comparison between Mueller, cross-polarized and SHG images of an unstained human liver specimen (~50µm-thick fixed slice). (a) Linear retardance image in HSV (Hue = azimuth of retardance α_R, Saturation = 1, Value = linear retardance R_L) with a binary mask based on degree of alignment of birefringence axes. (b) Same area imaged with a wide field polarized microscope (crossed polarizers). (c) Same area imaged in second harmonic generation (SHG). Dots and hot regions in (b) correspond to paraffin.

All 3 modalities implemented in the microscope will be presented in details at the conference together with convincing demonstration with images and videos.

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