Polarization and Nonlinear Microscopy

Image Improvement of Ocular Tissue

2-photon imaging of collagen-based biological tissues might benefit from the choice of particular incident polarization states. Here we further explore the effects of polarization on the visualization of ocular structures. Theoretical and experimental results suggest that linear polarization optimizes the imaging of samples containing mostly parallel elongated structures. However this type of polarization might not be the most appropriate when tissues present random distributed collagen patterns.

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Introduction

Second harmonic generation (SHG) is a particular technique of 2-photon (nonlinear) microscopy able to extract information from collagen-based tissues without fixation or labeling procedures [1]. SHG signal originates from the simultaneous absorption of two infrared photons and the emission of a visible photon with one-half of the incident wavelength.

Unlike regular confocal microscopy, SHG signal is only generated in a reduced volume of the focal plane. This provides auto-confocality and optical sectioning capabilities, reducing photobleaching and phototoxicity side effects in the surrounding areas. Despite this intrinsic confocality, 2-photon microscopy performance is limited by aberrations and scattering (mainly within the sample), which lead to a significant decrease in image quality when deeper planes are imaged [2]. The use of adaptive optics techniques yielded substantial improvements in the images obtained with 2-photon microscopy [3].

Since some ocular tissues exhibit a significant dependence on polarization, ophthalmic microscopy often uses this property to explore structures that cannot be visualized with regular techniques [4,5]. In 2-photon microscopy, polarization-sensitive approaches have been used for the analysis of tissues providing SHG signal [6,7].

It has been recently reported by these authors a model to characterize the spatial distribution of SHG signal originated from collagen for focused elliptical polarized light [8]. The model provided an analytical expression for the SHG intensity signal as a function of the incident polarization state and the fundamental wavelength as well as the numerical aperture of the microscope objective. This model was based on a single collagen fiber and restricted to particular polarization states. The generalization of the model to any possible polarization state and its experimental implementation might provide interesting results when visualizing collagen-based tissues using 2-photon microscopy.

Theory

Let us consider a light beam from a fs-laser system with wavelength λ_ω. This passes a generator of polarization states (PSG) composed of a fixed linear polarizer and a rotating $\lambda/4$ plate. When the plate rotates, different elliptically polarized states (azimuth, χ; ellipticity, Ψ) are produced, covering well defined locations on the Poincaré Sphere [4]. This light enters the microscope and is focused through a

Keywords

2-photon Microscopy, Second Harmonic Generation, Polarization

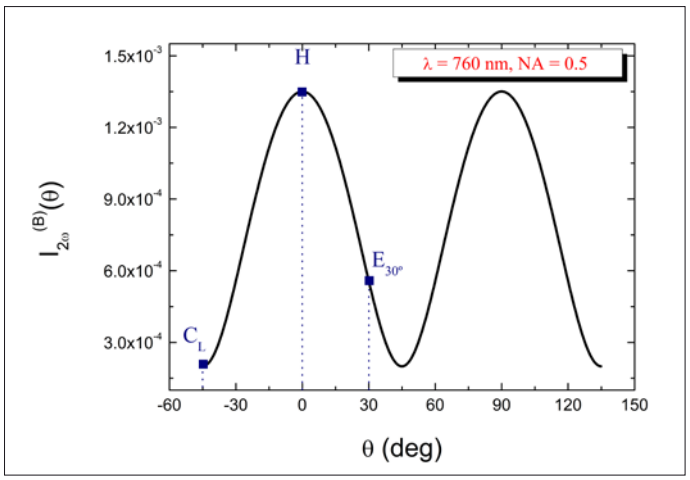

Fig. 1: Total B-SHG as a function of the orientation of the fast axis of the λ/4 of the PSG. Data correspond to λ_ω = 760 nm and NA = 0.5. Results have been normalized to those obtained for λ_ω = 1000 nm and NA = 1.0. Three independent polarization states are also labelled in the plot for later comparisons: horizontal, H; left-handed circular, C_L and elliptical, $E_{30°}$.

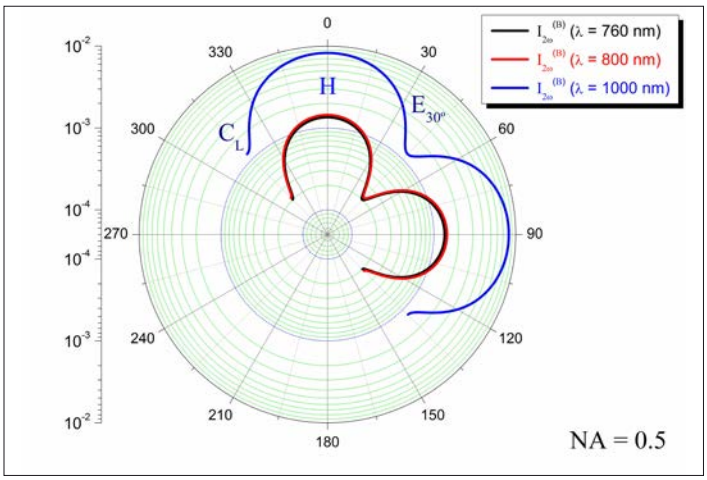

Fig. 2: B-SHG intensity as a function of the incident polarization state for different values of λ_ω and NA = 0.5. For a better comparison, the curves have been normalized in the same way as figure 1.

microscope of numerical aperture NA on a collagen fiber (lying along the X direction). The emerging SHG intensity in the backward direction (B-SHG) will be given by:

$$I_{2\omega}^{(B)} \propto K\cdot\left(\sin^2\mu\cdot\sin^2\varphi+\cos^2\mu\right)\cdot A(\lambda,NA,\mu)\cdot B(\chi,\Psi,\beta)$$

where (μ,φ) are the spatial coordinates in the backward direction, A is a term depending on λ_ω, NA and μ, and B includes dependencies on the polarization parameters of the incident light and the hyperpolarizabilities of the medium (β).

Figure 1 shows the total SHG intensity as a function of the rotating angle of the λ/4 fast axis. Unlike previous literature, our configuration produces a unique linearly polarized state. The rest are, in general, elliptically polarized (including also both circular ones). It can be observed that the maximum B-SHG intensity value occurs when horizontal polarized light (H) reaches the sample (i.e. direction parallel to the collagen fiber). On the opposite, circularly polarized states provided minimum signal values.

It is also interesting to investigate how λ_ω affects the B-SHG signal for different incident polarization states. In particular, figure 2 presents the polar diagram where the B-SHG signal has been plotted as a function of the angle of the λ/4 fast axis for three λ_ω values. The change between 800 and 760 nm is ~6%, however this increases noticeably when λ_ω = 1000 nm.

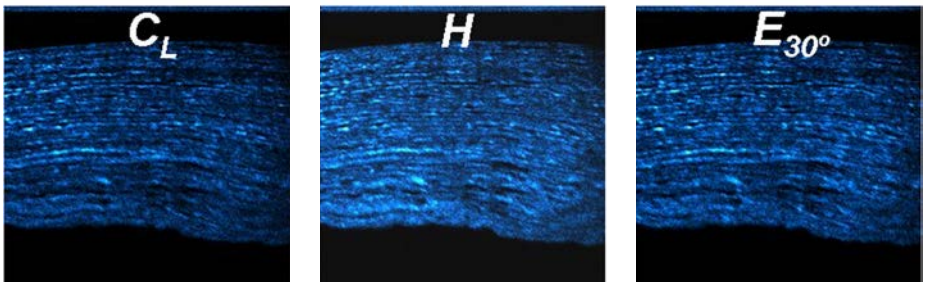

Fig. 3: Polarization dependence of the B-SHG signal. Images correspond to a sample of fixed non-stained rabbit cornea and subtend 360 x 360 μm.

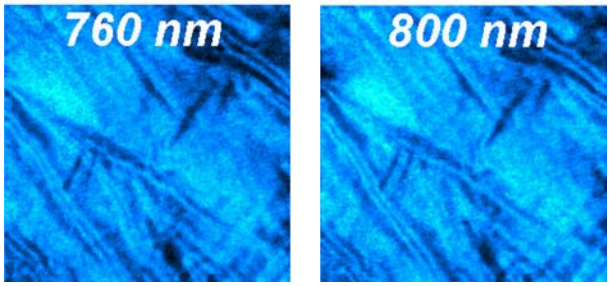

Fig. 4: Effect modifying the incident wavelength on B-SHG images (*ex vivo* human cornea) when keeping the polarization state fixed. Images subtend 210 x 210 μm.

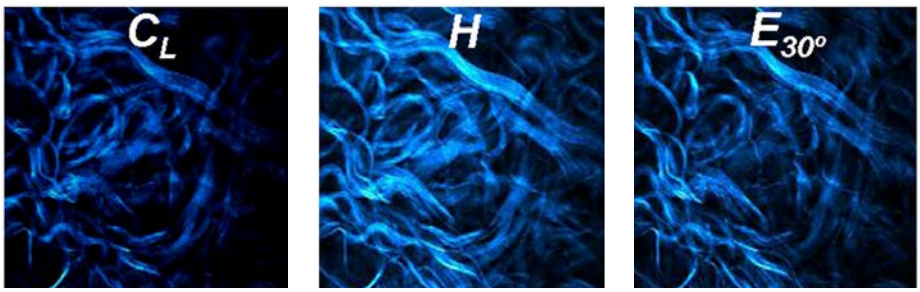

Fig. 5: B-SHG images of an *ex vivo* bovine sclera for three independent incident polarization states. Images subtend 250 x 250 μm.

Experimental Implementation

A custom 2-photon microscope [9] has been modified to include a PSG as describe above. B-SHG images of different ocular tissues (mainly composed of collagen) were recorded for different polarization states produced by the PSG. Unless indicated, the laser beam was set to λ_ω = 760 nm. All SHG images were acquired using a microscope objective of NA = 0.5.

Figure 3 presents a set of B-SHG images corresponding to a fixed non-stained rabbit cornea (histological section) for three independent polarization states (C_L, H and $E_{30°}$). Overall these re-

sults agree with the model above explained. Since the sample presents a well-defined orientation of collagen structures (horizontal in this case), the highest B-SHG signal corresponds to H polarized light. A minimum values is reached for C_L polarized light.

Figure 4 corroborates the small effects of changing λ_ω from 760 nm to 800 nm (~60 %). SHG signals are according to data showed in figure 2. Both images were recorded using H polarized light.

Despite the simplicity of our theoretical model (a single fiber), these results seem to agree well. However, a question arises now: could this model also predict the results obtained from structures with more complex collagen patterns (and not just a set of collagen fibers aligned in the same direction)? In order to answer this question, figure 5 presents an example on the effects of incident polarization for a non-organized tissue: *ex vivo* bovine sclera (neither fixed, nor stained). It can be observed that C_L also provides the image with the lowest signal. However, for this particular case the images corresponding to the polarization states H and $E_{30°}$ provided similar levels of SHG signal.s

Conclusions

Polarization can improve the visualization of ocular tissues containing collagen when imaged with 2-photon microscopy. A theoretical model has been combined with preliminary experimental results to investigate which polarization states provide optimum B-SHG signal.

Results showed that SHG signal from tissues composed of parallel-arranged collagen structures can be maximized when using linearly polarized light. On the contrary, circularly polarized light provides worse images. However, if the tissue lacks of a well-defined arrangement, this behavior might change and the optimum polarization state may not necessarily be linear. Moreover, polarization-sensitive 2-photon microscopy is also able to provide additional spatial resolved features.

Although results here reported are limited to single planes, the analysis of the effects of particular polarization states on B-SHG images from deeper locations within the samples could be of interest. These findings might also be useful in clinical environments, enhancing ocular tissue imaging, what is essential for early diagnosis of pathologies in the eye.

Acknowledgements

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Super-resolution Microscopy Technique without Fluorescent Dyes

Researchers from Purdue University (USA) have found a way to see synthetic nanostructures and molecules using a new type of super-resolution optical microscopy that does not require fluorescent dyes, representing a practical tool for biomedical and nanotechnology research.
The imaging system, called saturated transient absorption microscopy, or STAM, uses a trio of laser beams, including a doughnut-shaped laser beam that selectively illuminates some molecules but not others. Electrons in the atoms of illuminated molecules are kicked temporarily into a higher energy level and are said to be excited, while the others remain in their "ground state." Images are generated using a laser called a probe to compare the contrast between the excited and ground-state molecules. The researchers demonstrated the technique, taking images of graphite "nanoplatelets" about 100 nanometers wide.

Original publication:

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More information:
www.purdue.edu/newsroom/releases/2013/Q2/super-resolution-microscope-possible-for-nanostructures.html