

ENHANCEMENT OF SECOND HARMONIC MICROSCOPY IMAGES USING RADIALLY POLARIZED BEAMS

Rosa M. Martínez-Ojeda, Carlos Hernández-García,* Pablo Artal and Juan M. Bueno
Laboratorio de Óptica, Universidad de Murcia, Spain
Laboratorio de Óptica, Universidad de Murcia, 30100 Murcia, Spain
*Dept. Física Aplicada, Universidad de Salamanca, 37008 Salamanca, Spain
E-mail: bueno@um.es

KEYWORDS: Second harmonic generation, radial polarization

Polarization-resolved multiphoton microscopy has been reported to be very useful in both biological imaging and structural molecular analysis [1]. In particular, the visualization of collagen-based tissues imaged with second harmonic generation (SHG) microscopy strongly depends on the incident polarization (mainly linear) [2]. The lateral resolution of confocal laser scanning microscopy has been reported to improve when using radially polarized illumination [3]. Theoretical studies have also shown higher spatial resolutions in SHG and THG signals [4, 5]. However to our knowledge, the effect of radial polarization on experimental SHG images has not been reported yet. In that sense the aim of this study is to analyze the performance of radial polarized light in SHG microscopy. Images will be compared to those acquired with circular polarized light. Unlike linear polarization, circular light minimizes the influence of the orientation of the sample's collagen fibers.

Stacks of SHG images were acquired in collagen-based tissues using both radial and circular polarized light with a customized SHG microscope. An S-waveplate in the illumination pathway allows linear polarization to be converted into radially polarized light [3]. Parameters such as acutance, image sharpness and speckle noise, were used as image quality metrics. Results show an improvement in the different metrics of SHG images recorded with radial polarization when compared to those acquired with circular light. The actual enhancement depended on each particular sample and ranged between 20 and 35%. This effect was also found at different depth locations within the tissue.

These findings indicate that radial polarization tightens the focused spot providing an improvement of the SHG effectiveness, an increase in the image resolution and an extended depth of focus, what enhances the capability of multiphoton microscopes.

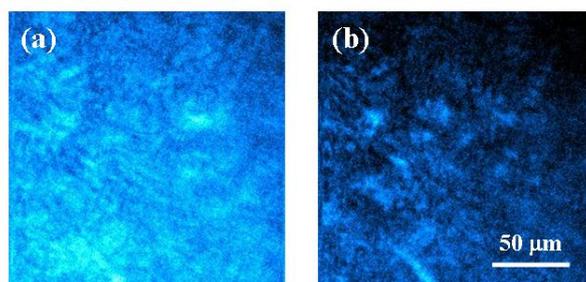


Fig. 1: SHG images of a rabbit cornea acquired with radial (a) and circular (b) polarized light..

ACKNOWLEDGEMENTS: Supported by grants FIS2016-76163-R and FIS2015-71933-REDT (SEIDI, Spain) and 19897/GERM/15 (Fundación Séneca, Murcia, Spain).

- [1] Brasselet, *Adv. Opt. Phot.* **3**, 205 (2011).
- [2] Stanciu et al., *Sci. Rep.* **7**, 15476 (2017).
- [3] Kozawa et al., *Opt. Express* **19**, 15947 (2011)
- [4] Yang & Zhan. *J. Opt. A: Pure Appl. Opt.* **10**, 125103 (2008).
- [5] Kozawa & Sato, *Opt. Express* **23**, 2076 (2015).