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Widefield sensitive birefringence microscopy by spectral encoding of the polarization

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Abstract

Birefringence microscopy is a label-free imaging technique that is well-suited for the observation of thin biological samples presenting highly ordered structures such as collagen fibers or actin filaments in the cell cytoskeleton. However, the detection of weakly linear birefringent structures requires more specific and sensitive techniques than usually employed techniques that perform exhaustive polarimetric measurements.
We have recently developed a highly sensitive measurement technique of linear birefringence and its orientation based on the spectral encoding of the polarization implemented to a laser scanning microscope using a swept source [1]. The spectral encoding of the polarization consists in encoding the polarimetric response of the specimen in the transmitted light spectrum with passive polarization elements. This technique allowed the acquisition in “real-time” of the birefringence map of the specimen thanks to the wavelength sweep speed of the source and the short dwell time of the laser spot on the specimen (10 µs). However, this technique does not allow to perform widefield imaging of the birefringence information of the sample.
To this end, we present two techniques to extract the birefringence information from the transmitted spectrum and their implementations in a widefield microscope. Both techniques still rely on the spectral encoding of the polarization. The first technique uses a white light source and a hyperspectral camera to perform a spectral detection [2]. A Fourier analysis at each pixel allows access to the birefringence parameters. However, the detection of an intensity map at each spectral channel of the camera and the analysis by Fourier transform are time consuming and not compatible with real-time imaging.
To perform sensitive and quantitative measurement of the linear birefringence information and widefield imaging of the birefringence map at video rate, we developed a second technique that does not require any spectral detection or Fourier domain analysis. This technique is based on the spectral structuration of the illumination with passive polarization elements based on the principle of spectral encoding [3]. The injection in the microscope of at least three illuminations with different spectra allows access to the birefringence information after successive detection by a monochromatic camera. The high commutation speed of the three illuminations (up to 20 µs) makes it possible to image the linear birefringence of the specimen and its orientation in real-time at video rate.

References

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