QUANTITATIVE BIREFRINGENCE IMAGING USING QUADRI-WAVE LATERAL SHEARING INTERFEROMETRY (QWLSI)

Sherazade Aknoun1-2, Pierre Bon1, Julien Savatier1, Benoit Wattellier2 & Serge Monneret1

1 Institut Fresnel, CNRS, Aix-Marseille Université, Ecole Centrale Marseille, Campus de Saint-Jérôme, 13397 Marseille, France

sherazade.akhnoun@fresnel.fr

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Phase contrast imaging can be considered as a powerful method for the label-free imaging of semi-transparent biological samples. Recent techniques give access to a quantitative measurement of optical thickness.

Changes in the refractive index inside the samples are the main contrast sources in living cells. However, some biological structures inside cells are optically anisotropic and thus scatter light differently depending on the illumination light polarization. In polarized light microscopy, the contrast created by anisotropic elements has been widely used to reveal ordered structures of biological samples without staining or labeling but most techniques are purely qualitative or experimentally hard to implement when one wants to obtain quantitative measurements or do not allow the study of living specimens. Measurement of phase shifts introduced with different incident polarization angles, on an anisotropic sample, would give access to quantitative values of both its linear birefringence and orientation of its optical axes.

We propose here to use QWLSI to measure a set of polarization-dependent phase shifts, in order to reveal collagen fibers local structure and some components like actin stress fibers in living cells samples. The high-resolution wave front sensor is mounted on a non-modified transmission microscope to measure characteristic optical path difference (OPD) of the sample [1]. The very simple setup is composed by a single rotating polarizer placed in the illumination light path before the sample that leads to record one quantitative phase image for each excitation polarization angle. The set of those images, recorded in few seconds so as to deal with living samples, is numerically computed to obtain what we call "Quantitative Birefringence Images" which represents specific local linear birefringence and orientation of the optical axis of the sample. Results on collagen fibers and COS-7 cells will be presented.