QUANTITATIVE PHASE MICROSCOPY USING A HIGH-RESOLUTION WAVEFRONT SENSOR

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KEY WORDS: Phase-shift measurements, Wavefront-sensing, Living cells, White-light microscopy.

1. CONSIDERED TECHNIQUE AND SETUP

Marker-free cell visualization requires a specific setup as Zernike phase contrast or Nomarski-DIC equipments. Those techniques implicitly use the fact that light passing through a sample accumulates phase shift. Accessing to the actual value of this phase shift is very difficult, explaining that such techniques are commonly used as contrast enhancer only. We describe here the use of quadri-wave lateral shearing interferometry (QWLSI) [1] for wavefront sensing, in order to measure quantitatively the local phase shift within a sample.

We use a SID4-HR wavefront sensor (Phasics) and we get a 300x400 sampling points on the sample, with both phase and intensity information. The method is easy to implement on a conventional microscope: it requires only a bright-field illumination and a wavefront sensor in the microscope image plane.

2. EXPERIMENTS ON MICROSCOPIC SAMPLES

By comparing the measured and the expected phase-shift of microscopic beads, we have checked the accuracy and reliability of the technique.

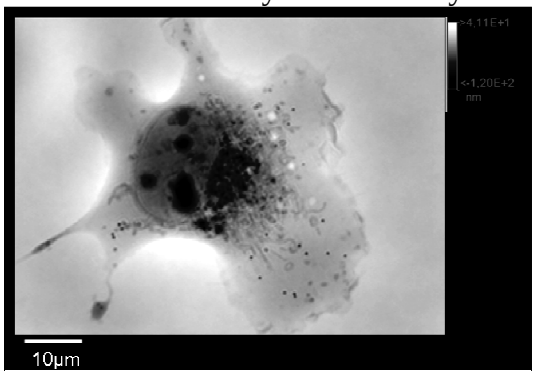

Figure 1 : Phase-shift map of a COS-7 cell. X150 , NA=1.3

The study of transparent adherent COS-7 cells is also considered – figure (1). The visualization of intracellular elements is clearly possible with a high contrast. By using image processing on these phase-shift maps, we are able to simulate DIC images or detect very small phase-shifting elements such as lipid bilayer. Cellular dynamic studie is also demonstrated as the acquisition time is only depending on the frame rate of the camera.

This paper will present the measurement principle. This will be illustrated on various cell types and interesting image processing on cell phase map.

[1] J. Primot; N. Guérineau, "Extended Hartmann Test Based on the Pseudoguiding Property of a Hartmann Mask Completed by a Phase Chessboard", *Appl. Opt.*, **39**, 5715-5720 (2000).