SIMULTANEOUS FLUORESCENCE AND QUANTITATIVE PHASE IMAGING OF LIVING CELLS WITH A HIGH RESOLUTION WAVEFRONT SENSOR ON A CONVENTIONAL MICROSCOPE

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ABSTRACT:

Phase visualization of cells has been used for decades for cell, tissue or organism imaging, with setups such as Zernike phase contrast or Nomarski-DIC equipments. Those techniques use the fact that light passing through a sample accumulates phase shift. Nevertheless, they don’t enable to give information on the quantitative phase shift of each pixel of the image and are commonly used only to increase contrast. Here we describe the relevance of quadri-wave lateral shearing interferometer [1], using a Modified Hartmann Mask, for wavefront sensing in order to measure quantitatively the local phase shift within a sample, with a high sensitivity (up to 1 nm of difference in optical pathway).

We use a high resolution wavefront sensor as a simple camera and we get a 300x400 sampling points on the sample, with both phase and intensity information. The method is direct and can be implemented on a conventional wide-field microscope: its native bright-field Köhler illumination system is used as the light source and the wavefront sensor has only to be mounted on a video port. We don’t need a reference arm. We have the possibility to combine phase and fluorescence imaging simultaneously with appropriate dichroic mirrors and fluorescence cubes (one colour + phase image at a time). Since it is an achromatic interferometric technique, we can use wavelengths between 750 and 850 nm for phase imaging and keep shorter ones for fluorescence.

Different cell lines are tested, either stable or transiently transfected, with different expression levels of fusion proteins. The goal is to look at the correlation between phase and fluorescence images of specific organelles, such as mitochondria or different kinds of vesicles. The quantitative local phase shift can be used to obtain the refractive index of objects of known dimensions, thus enabling us to identify some organelles only looking at the phase image, thanks to appropriate numerical filtering.