Quantitative phase contrast, retardance imaging and phase tomography by wavefront sensing

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1. INTRODUCTION

Quantitative phase imaging techniques are now conventionally used in microscopy for measuring specific properties of semi-transparent samples without any labelling. With the differential wavefront measurement we developed, we are able to produce quantitative contrast of living cells, but also to extend the technique to retardance imaging. At last, by modifying the illumination scheme of the microscope, we also are able to optically section the sample in order to reach 3D phase tomography of tissue slices.

2. RESULTS

2.1 Quantitative phase contrast microscopy by lateral shearing interferometry

Lateral shearing interferometry is a well-known technique to measure the phase gradients of light beams. The incident wavefront is replicated into identical but tilted wavefronts. After propagation, their mutual interference pattern is recorded with a CCD camera. The phase gradients are recovered from the fringe deformation, by means of an inverse Fourier transform around the interferogram fringe frequency. In case of quadriwave lateral shearing interferometry (QWLSI), two gradients along two perpendicular directions are measured and then integrated to determine the field intensity and phase, i.e. the wavefront of the incident beam. Unlike classical interferometers where a coherent reference arm is mandatory, QWLSI is self-referenced and achromatic. Hence, measurement is particularly insensitive to environmental vibrations.

The QWLSI wavefront sensor we used (SID4Bio, Phasics S.A., Saint Aubin, France) has been specifically optimized for biology applications. Figure 1 shows the experimental setup we realized to produce quantitative phase images.

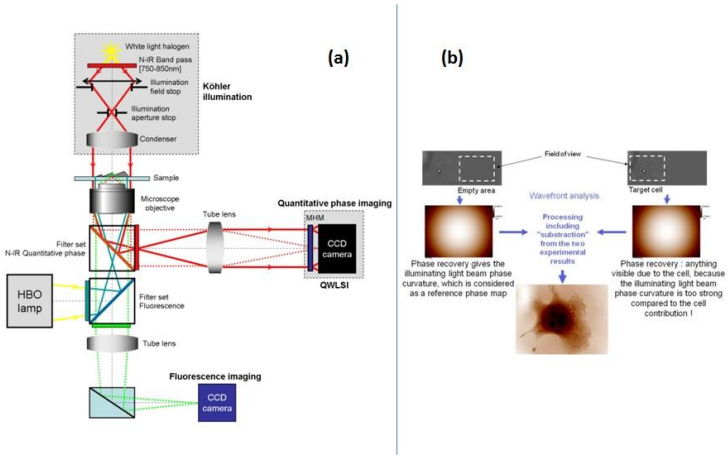

Figure 1. (a) Experimental setup. (b) General principle of the technique. A first reference wavefront is recorded without any cell in the field of view. Then a second one is recorded, with the target cell imaged on the CCD plane of the system. Both recorded wavefronts seem identical, but specific post-processing based on subtraction between them gives a highly contrasted phase image of the cell. Such a process also allows eliminating most of the phase distortion due to the optical system.

The QWLS interferometer is plugged onto the microscope back exit port and measures the exit wavefront on the image plane in the N-IR spectral band. Comparison of the given wavefront with a reference one, obtained in an empty area as explained in fig. 1(b), allows defining a quantitative phase map of the actual sample. This can be used to determine dry mass of living cells.

2.2 Quantitative retardance imaging

It is possible to improve the technique in order to perform quantitative linear birefringence measurements on biological samples. The system combines a set of quantitative phase images with different excitation polarizations to create birefringence images. These give information about the local retardance and structure of dynamic biological anisotropic components such as actin fibers. By introducing a liquid crystal retarder to rotate the incident polarization, we are able to take a retardance image in less than 1 second.

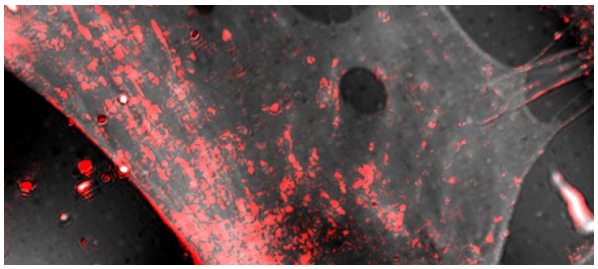

Figure 2. Multichannel image of a living COS7 cell reconstructed from the images derived by our technique. The gray channel corresponds to the quantitative phase image. The red channel corresponds to birefringence retardance image that reveals anisotropic components (stress fibers) inside the cell.

2.3 Phase imaging and tomography of tissue slices

By using spatially incoherent illumination, it is also possible to create lateral resolution increase and optical sectioning in order to image thick samples with intracellular resolution. The 3D volume is imaged by axially scanning the sample. The main advantages of this approach are its easy implementation, compared to the other state-of-the-art diffraction tomographic setups, and its speed which makes even 3D living sample imaging (such as cells) possible. Figure 3 gives some examples of phase images of tissue slices (skin).

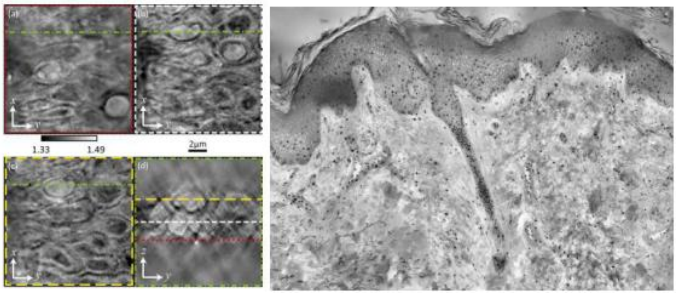

Figure 3. Left: Refractive index maps of a fixed mouse epithelial skin-cell tissue, embedded in Mowiol. The different dashed lines represent the sectional drawings, each one associated to its own image (same dashed line around the image). (a) z = 1 µm plane, (b) z = 7 µm plane, (c) z = 14 µm plane, (d) x = 30 µm plane. Right : phase image of a fixed mouse epithelial skin-cell tissue, embedded in Mowiol (millimeter size, obtained by merging a complete set of microscopic images).

3. CONCLUSION

In this presentation, we make a review of three biological applications of wavefront sensing as a phase imaging tool. In all of these experiments, QWLSI was very simply added to existing setups, thanks to its “camera-like” design.

REFERENCES

[1] Bon, Maucort, Wattellier, and Monneret, "Quadriwave lateral shearing interferometry for quantitative phase microscopy of living cells," Optics Express **17**, 13080-13094 (2009).

[2] Aknoun, Bon, Savatier, Wattellier, Monneret, "Quantitative Retardance Imaging of biological samples using Quadri-Wave Lateral Shearing Interferometry", soumis (2015).

[3] Bon, Aknoun, Monneret, Wattellier, "Enhanced 3D spatial resolution in quantitative phase microscopy using spatially incoherent illumination", Optics Express **22** (7), 8654-8671 (2014).