Quantitative Phase Microscopy

Non-interferometric Method Using a Refractive Pyramid

Classical phase contrast microscopy techniques allow only non-quantitative partial visualization of transparent samples. In recent years, a variety of techniques have appeared for complete phase determination with different levels of complexity. **A new simple non-interferometric method able to provide real time quantitative optical path difference information is presented.** It is based on the use of a refractive pyramid to measure the transillumination wavefront gradient using incoherent light.

Introduction

In microscopy, unstained biological media are often transparent; in such cases, the full determination of cellular structures using transillumination light is only possible through measuring the imprinted changes in the wavefront generated by spatial variations in the refractive index. Interferometric techniques [1-3] have been applied for the quantitative determination of these samples although their disadvantages include their reliance on the use of temporal coherent light, sophisticated instrumentation, mechanical stability and complex data processing. An alternative is direct sensing, which avoids the need of combination with a reference beam. Self-interference techniques based on the use of gratings have recently been proposed [4, 5] to obtain the phase gradient for subsequent integration, the main advantages being the simpler setups involved, the removal of the dependence on temporal coherent and real time operation. However, their reliance on interferograms still present drawbacks.

The Pyramid Wavefront Sensor

To directly determine quantitatively the phase using a non-interferometric simple optical setup, we have propose [6] the use of the pyramid wavefront sensor (PWS) [7]. Related with the Foucault-knife method, the PWS was introduced to detect atmospheric wave aberration in astronomy and it is based on generating the Fourier transformation of the optical perturbation on a given input-plane and its division on the Fourier plane. As figure 1 shows, the splitting is carried out by a four-sided refractive pyramid driving the divided beams in diverging directions.
from the optical axis.

After the light has passed through the pyramid, an additional optical system re-images the input plane onto a new conjugate plane, where a CCD registers four laterally displaced images of the input plane. If a plane wave with propagation direction along the optical axis arrives to the lens L1, an Airy diffraction amplitude distribution will be generated on the pyramid (for simplicity a circular aperture limits the input plane) and four approximate uniform discs each of which with a quarter of the input intensity will be detected on the CCD thanks to the lens L2. If the propagation direction of the plane wave slightly changes, the amplitude will be unequally distributed and an intensity imbalance between the four sub-images will be registered as in a quad-cell position sensor.

More explicitly, two images, \( S_x \) and \( S_y \) proportional to the wavefront gradient in orthogonal directions can be obtained by combining the images, \( I_i \). In the case of a general optical perturbation, each four corresponding pixels in \( I_i \) will function as a quad-cell and the sensor generate an array of signals proportional to the local gradient at each input plane coordinates. This gradient determination can be applied to the transillumination wavefront of a microscopic sample optically transported to the input-plane.

**Instantaneous Dynamic Range Extension**

A difficulty with the PWS is the saturation for high gradient values. This occurs, for example, when the tilt in the above plane wave is so high that the diffracted field occupies only one pyramid facet. A method to avoid it is to mechanically introduce a displacement of the pyramid in such a way that the apex follows a circular trajectory around the optical axis (whose radius will be responsible for the sensor gain and range) during the acquisition time. Other option that generates the same signal and valid for transparent objects is to induce an oscillation in the illumination beam. This solution can be refined to avoid the need for moving parts by the use of a source able to generate all the required tilted plane waves simultaneously and incoherently and can be easily implemented using an extended incoherent disc-shaped emitter.

Such a source can be modeled as a composition of elemental annular sources of different radius, each composed of points emitting independently. If placed in the front focal plane of a lens, each point will generate, on the exit pupil of the lens, a
plane wave with the propagation direction determined by its lateral position coordinates. In this way, the PWS response to an elemental annular illumination source will be equivalent to the response of an oscillating pyramid following a circular path around the optical axis. Assuming a perfectly uniform emission, an analytic expression for the expected sensor response can be obtained by integration of the elemental annular responses with respect to the radius (fig. 2).

**Experimental Results**

Figure 3 shows the set-up built for proof-of-concept demonstration. It is divided into three parts. The first is the above described illumination method composed of a collimated white led, L, (6500 K) passing through a diffuser, D, close to a circular aperture, A. The diffuser is placed on the front focal plane of a lens, L1. The plane corresponding to the exit pupil of this lens is transported to the sample plane, S, by using a relay (lenses L2 and L3). The second part is a microscope consisting of an objective, MO, (Nikon 40x NA 0.60) and a doublet as tube lens, L4. This lens forms a scaled image of the microscopic sample on a plane that corresponds to the camera port of a conventional microscope. The third part of the system is the PWS whose input-plane is the previous image plane where a diaphragm, DI, is placed to control the field extension. Adjacent, a lens, L5, generates the Fourier transform on its back focal plane where a refractive pyramid is placed. Finally, the lens L6 re-images the sample on a CCD.

To test the system, we built artificial samples simulating cellular tissue using polystyrene ($n_s = 1.60$) microspheres of 43.3 µm Ø which were transferred to a calibrated host liquid ($n_h = 1.56$) inside a sealed glass chamber. As panel (a) on figure 4 shows, without the pyramid, the optical system behaves as a conventional microscope (DI fully open). Figure 4 (b) shows the image containing the sub-images $I_i$ for a sample consisting of three microspheres in the field when the pyramid is in place and closing DI to avoid overlapping. Panels (c) and (d) show the images corresponding to $S_x$ and $S_y$ obtained using the image on (b). Note that information of the light absorption by the sample is not lost given that the algebraic sum of the four sub-images is the conventional microscope image that can be simultaneously displayed.

Figure 5 represents the comparison between the optical path difference (OPD) of a single sphere obtained using an algorithm for gradient data integration and the expected OPD (solid line). Figure 5 (b) shows the OPD map obtained for the images on the figure 4 (c).

**Final Remarks**
A new non-interferometric phase microscope that uses a pyramid sensor with dynamic range extension generated by a disc-shaped incoherent source has been presented. The system instantaneously provides high-resolution sampling of the phase gradient suitable for the effective numerical integration of structurally complex OPD maps. It has no moving parts, is resilient to noise and mechanical stability is not required. In conjunction with a fast integration algorithm, the microscope can provide OPD information in real time, which may be of interest for the study of structural changes in dynamic biological processes. Work is in progress to avoid the prototype's field limitation which is not intrinsic of the method and in an alternative beam splitting mode in order to use the CCD sensor area more effectible.

References

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