QUANTITATIVE OPTICAL PHASE MICROSCOPY

A.Barty, K.A.Nugent, D.Paganin and A. Roberts

School of Physics,
University of Melbourne,
Parkville 3052, Victoria, Australia

We present a new method for the extraction of quantitative phase data from microscopic phase samples using partially coherent illumination and an ordinary transmission microscope. The technique produces quantitative images of the phase profile of the sample without phase unwrapping. The technique is able to recover phase even in the presence of amplitude modulation making it significantly more powerful than existing methods of phase microscopy. We demonstrate the technique by providing quantitatively correct phase images of well characterised test samples, and show that the results obtained for more complex samples correlate with structure observed using Nomarski DIC techniques.

Many objects of interest to biologists and materials scientists are phase objects. The phase structure can be made visible by spatial filtering, as is done in phase contrast microscopy, or by interferometric techniques such as Nomarski differential interference contrast (DIC) microscopy. Both of these techniques are commonly used in commercial microscopes to render the phase structure visible, but, in general, this intensity distribution does not quantitatively map the phase distribution. Furthermore, these techniques entangle the phase and intensity images and so have limitations where both phase and intensity information is required.

The technique we present here overcomes a number of these limitations, and at the same time dispenses with the need for specialised optics - the phase information is obtained by analysing the way in which propagation of light is affected by the phase sample. The generality of this approach in principle enables us to tackle phase microscopy with electrons and x-rays as well as visible light. In this paper we describe our phase imaging technique and then describe a quantitative experimental demonstration of the technique.

Consider a perfect, aberration-free optical microscope of high numerical aperture and a coherently illuminated object with both phase and amplitude structure. The field leaving the object may be described by the complex function:

$$O(\vec{r}) = A(\vec{r})\exp[i\phi(\vec{r})] \qquad (1)$$

where, in the case of a two-dimensional object, $A(\vec{r})$ represents the object's absorption profile and $\phi(\vec{r})$ its phase profile. Within the paraxial approximation the image plane contains a magnified form of the field described by equation (1) distorted by the imaging properties of the microscope. In the case of incoherent illumination, for example, the image is convolved with a point spread function. In this letter, for convenience, we will ignore the distorting effects of the imaging system. Within this approximation the intensity distribution in the image plane is given by:

$$I_{\text{Image}}(\vec{r}) = \left| A\left(\frac{\vec{r}}{M}\right)\right|^2 I_{\text{Illum}}(\vec{r}) \qquad (2)$$

where $I_{\text{Illum}}(\vec{r})$ is the intensity distribution in the absence of the object and *M* is the image magnification. The introduction of a small amount of defocus is mathematically equivalent to a differential propagation of the field and may be described by the so-called transport of intensity equation[1].

$$k\frac{\partial I_{\text{Image}}(\vec{r})}{\partial z} = -\nabla\bullet\left(I_{\text{Image}}(\vec{r})\nabla\phi\left(\frac{\vec{r}}{M}\right)\right) \qquad (3)$$

If the intensity distribution given by equation 2 is uniform so that $I_{image}(\vec{r})$ is a constant, this equation can be readily solved for the phase using Fourier transform methods published elsewhere[2]. To deal with the case of non-uniform illumination we note that, if there are no phase singularities in the optical field, we can write[3]

$$I_{\text{Image}}(\vec{r})\nabla\phi\left(\frac{\vec{r}}{M}\right) = \nabla\psi(\vec{r}) \qquad (4)$$

for some function $\psi(\vec{r})$. In this case equations 3 & 4 may be solved uniquely for $\psi(\vec{r})$, and subsequently for the phase $\phi(\vec{r})$, using the known intensity distribution, equation 2 above. Significantly the phase obtained does not have to be unwrapped, as is required for interferometry.

Thus to obtain a quantitative phase image one collects an in-focus image and a very slightly positively and negatively defocused images and use this data to estimate the differential with respect to the defocus of the image. The resulting data is described by eq(3) and may be solved to yield the phase distribution $\phi(\vec{r})$ using a Fourier transform implementation of the methods described in reference (3).

The above analysis is based on coherent illumination with a non-uniform intensity distribution. In practice the illumination will be partially coherent. Consider Köhler illumination using a planar source. In this configuration, the object is illuminated by plane waves from

a range of directions. If we again assume a perfect imaging system the sample field is transported to the image plane unchanged, and the mutual intensity function of the partially coherent image is described by:

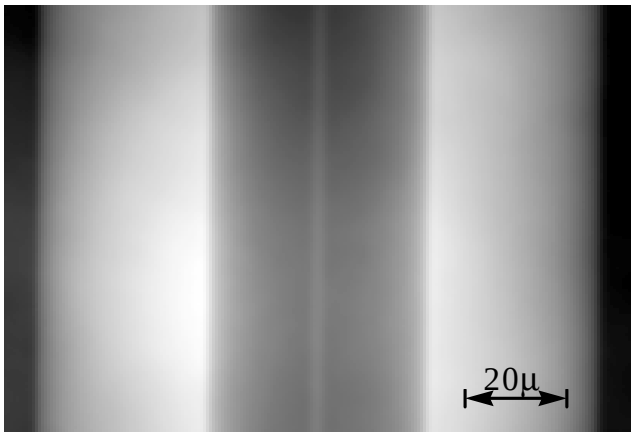
$$J(\vec{r}_1, \vec{r}_2) = A\left(\frac{\vec{r}_1}{M}\right)A\left(\frac{\vec{r}_2}{M}\right)\exp\left[i\left\{\phi\left(\frac{\vec{r}_1}{M}\right) - \phi\left(\frac{\vec{r}_2}{M}\right)\right\}\right]g(\vec{r}_1 - \vec{r}_2) \quad (5)$$

where $A(\vec{r})$ and $\phi(\vec{r})$ are the amplitude and phase transmissions of the object respectively and, where $\vec{r}_1$ and $\vec{r}_2$ are vectors in the plane of observation and $g(\vec{r}_1 - \vec{r}_2)$ is a correlation factor directly related to the original source distribution. It is possible to directly calculate the intensity derivative with respect to defocus and, to within the approximations adopted, the result is identical to the coherent case, eq(3), provided that the irradiance distribution in the planar incandescent source shows inversion symmetry ($I_{source}(\vec{q}) = I_{source}(-\vec{q})$ where $\vec{q}$ is the position vector in the illuminating source distribution) and the illumination is at least partially coherent ($g(\vec{x}) \neq \delta(\vec{x})$). A full discussion of these issues, removing the simplifications in this discussion and taking into account the properties of a real microscope, will be presented in a future publication.

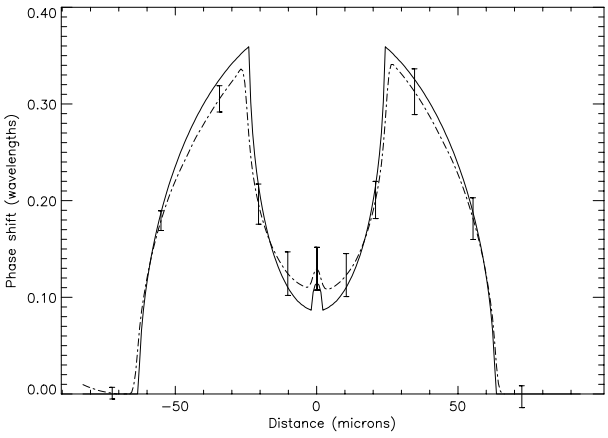
In addition to being able to use partially coherent illumination, we are able to perform phase retrieval using broadband polychromatic radiation. In equations (3) and (4) the phase always appears in the combination $\lambda\nabla\phi(\vec{r},\lambda)$. In the case that $\lambda\nabla\phi(\vec{r},\lambda) \approx$ constant over the wavelength range of interest (i.e. dispersion is negligible) the data may be interpreted entirely in terms of this product.

To demonstrate our technique quantitatively we imaged a well characterised optical fibre, 3M F-SN-3224 single-mode optical fibre (3M optical fibres, West Haven, Connecticut, USA) immersed in Cineole (a form of Eucalyptus oil, Ajax chemicals, Sydney, Australia) index-matching fluid illuminated by narrow-bandwidth light obtained by passing white light from a thermal source through an interference filter with a pass-band centred on 625nm and a spectral width of 10nm. Images were taken using an Olympus UMPlan 20x, 0.4NA objective on an Olympus BX-60 microscope using a Kodak KAF1400 CCD chip in a Photometrics CH250A camera. The sample was illuminated in transmission mode using Köhler illumination with an incandescent bulb as the source. Displaced intensity measurements were obtained by moving the sample in the z-direction using a stepper motor on the microscope focus control knob. Images were taken at various positive and negative displacements, and processed in pairs using our phase recovery technique.

Figure 1(a) shows the phase image recovered from a pair of images at ±(8.3±0.5)μm either side of best-focus. All layers of the fibre including the core are clearly resolved. The optical fibre used has been characterised in detail by us using atomic force microscopy[4] and by commercial fibre profiling techniques (York Technologies P102 analyser, York Technology, Chandlers Ford, UK). We then were able to use our detailed characterisation to predict the image of the optical fibre; a comparison of the predicted and experimental profiles across the fibre is shown in figure 1(b) and the recovered phase profile can be seen to quantitatively match the predicted profile. Note that the uncertainties indicated in the plot arise from variations in the observed profile from one part of the fibre to the next and represent one standard deviation of the data along the length of the fibre. We attribute these differences primarily to inhomogeneities in the microscope cover slip.



(a)

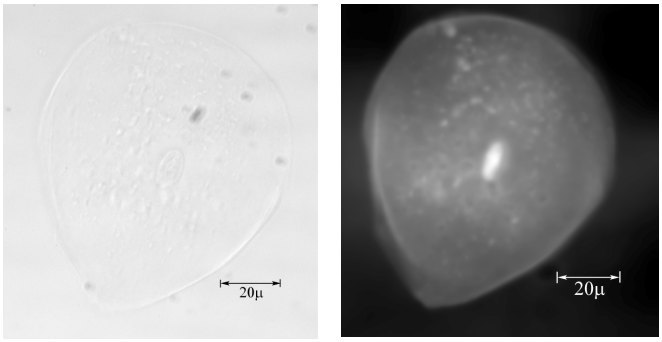


(b)

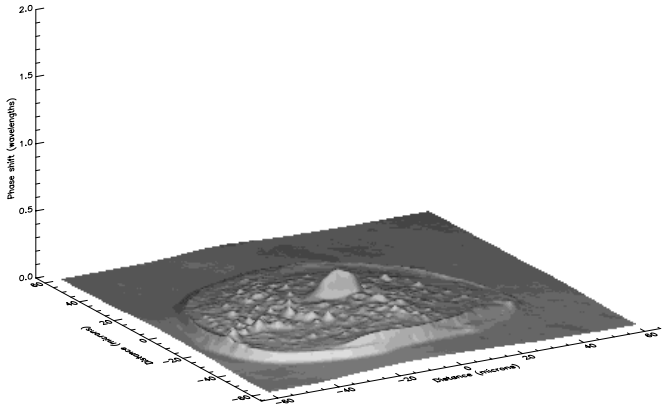
Fig 1: Panel (a) shows the recovered phase of the test optical fibre. Panel (b) shows a section through the above image (dashed line) along with the theoretical phase shift one would expect based on the known refractive index profile of this fibre (solid line). Significantly the small refractive index changes involved (less than 0.3% between adjacent regions) demonstrate the sensitivity of our technique to small phase shifts.

To demonstrate the capability of our phase-amplitude technique with more complex objects illuminated by a broadband source we imaged unstained cheek cells using a Carl Zeiss x40, 0.8NA objective on a Zeiss inverted microscope with a Kodak KAF1400 CCD chip in a Photometrics SenSys camera. The absorption of this object varied from 5% to 37% due to structure within the cell, and up to 58% in places due to dust on the microscope slide. Figure 2 shows a comparison of the Nomarski DIC picture (a) with the recovered phase map of the same cell (b), obtained from images at ±(2.0±0.2)μm either side of best focus. Our recovered phase is entirely consistent with the phase structure seen using DIC, but the clarity is

significantly improved. Note also how the optically thick nucleus and mitochondria in the cytoplasm appear distinctly in our image, as seen in both the grey-scale and surface plots of our data.



(a) **(b)**



(c)

Fig 2: Comparison of the recovered phase-amplitude image of an unstained cheek cell recovered from images taken at $\pm(2\pm0.5)\mu$ either side of best focus with the sample illuminated by polychromatic illumination. Panel (a) shows a Nomarski DIC image of the cell; panel (b) shows the recovered phase image. The surface plot in panel (c) demonstrates that the artefact level outside of the cell is low, and that both the nucleus and mitochondria within the cell membrane are clearly resolved.

The technique we describe here provides a simple and general method for making visible the magnitudes of the phase shifts in microscopic objects using existing, readily available microscope hardware in the form of an ordinary transmission microscope equipped with a CCD camera, and should apply equally well to both transmission electron microscopy and x-ray microscopy. We have shown that the recovered phase profile is in quantitative agreement with the profile produced by independent calculations, and that the spatial resolution of our technique is high enough to easily image the core of an optical fibre using a 20x objective. The technique works equally well when the objects show substantial absorption so that true phase and amplitude images are possible. We have also shown that this method produces results which are consistent with existing, accepted phase imaging techniques when applied to biological samples. These properties mean that our technique should find ready application in both biological and materials science microscopy.

This research was supported by a grant from the Australian Research Council. We acknowledge valuable discussions with Dr Peter Harris of the Department of Physiology at the University of Melbourne.

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