Invited Paper

Quantitative Phase Imaging in Microscopy

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1 Introduction

There are many different alternative methods that can be used for phase contrast imaging of objects at a microscopic scale [1]. Some of these are more amenable than others to obtaining quantitative information. The most important methods are:

Digital holographic microscopy (DHM)
Defocus
Transport of intensity equation (TIE)
Differential Interference Contrast (DIC)
Differential phase contrast (DPC)
Interference microscopy
Optical coherence tomography (OCT)

Phase imaging can be used in a reflection or transmission geometry. In biology it has the advantage of being marker-free. It can measure physical shape, and refractive index variations, ideally in three dimensions (3D). In transmission, phase is related to optical path difference. The phase difference after travelling through a thickness t in a medium with refractive index n as compared with the ambient refractive index n_0 is $\phi = (n - n_0)k_0 t$, where k_0 is the free-space wave number $2\pi / \lambda_0$. Thus optical path can be used to determine the thickness if we know the refractive index, or the refractive index if we know the thickness. Refractive index is important in biology because it can be used to determine concentration or dry mass [2]. In reflection, phase is also related to optical path, and can be used to reconstruct surface profiles or the optical thickness of layers. Note that refractive index also influences the amplitude of the reflected beam, by the Fresnel equations.

In tomography, in the first Born approximation the object scatters with a scattering potential given by [3]

$$F(\mathbf{r}) = -k_0^2 \left[n^2(\mathbf{r}) - n_0^2 \right] \tag{1}$$

Note that here we have replaced the background refractive index by n_0. It is well-known that the Born approximation can be invalid for many practical samples, e.g. in biology. The Rytov approximation can be an improvement [4]. For imaging in reflection, another approach based on the Kirchhoff approximation gives a scattering potential [5, 6]

$$F(r) \propto \nabla^2 \left\{ \ln \left[\frac{n(r)}{n_0} \right] \right\} \tag{2}$$

It is seen that in this model the signal comes from the changes in refractive index, rather than the refractive index itself.

2 Coherent methods

Imaging methods can be simply classified into coherent and partially-coherent methods. The main coherent method is digital holographic microscopy (DHM). A major advantage of DHM performed in an off-axis arrangement, is that the complete phase and amplitude information can be recovered from a single hologram. According to the Abbe theory of microscope image formation, an object can be resolved into grating components, which are filtered by the aperture stop of the imaging lens. It is well-known that imaging in a coherent system can be analyzed in terms of a coherent transfer function (CTF), which is a scaled version of the pupil function. For an in-focus circular pupil, all spatial frequencies less than the cut-off $(n_0 \sin\alpha)/\lambda$, where we take n0 to be the immersion medium, are imaged perfectly. These principles can be extended to the case of 3D imaging, and the CTF now becomes a 3D CTF. As was shown by Wolf [3], the 3D CTF for coherent imaging is given by the cap of a sphere of radius $n_0 k$, the Ewald sphere of x-ray diffraction theory, with the angle subtended by the cap being equal to the semi-angular aperture α of the imaging lens. The sphere passes through the origin of Fourier space. Then the only grating vectors present in the object that can be imaged are those that lie on the surface of the sphere. Thus we recognize that holography, and other forms of coherent imaging, result in some 3D information including surface height information, sometimes called $2\frac{1}{2}$D, but not complete 3D volume information [7]. The 2D hologram is, in fact, not large enough to contain the full 3D information. Different paprts of the hologram contain information on different views, but full resolution (given by the size of the complete hologram) is not present for the different views.

3 Partially coherent methods

Incoherent imaging is described by an optical transfer function (OTF), given by the scaled autocorrelation of the pupil function. As is well known the spatial frequency cut-off of an incoherent system is twice that of a coherent system. For a weak object, spatial frequencies twice as large can be imaged in an incoherent as compared with a coherent imaging system. However, it is not completely fair to say that imaging is superior in an incoherent system, as the OTF decreases in value for no-zero spatial frequencies, and higher spatial frequencies are present in the intensity object. For 3D imaging, the 3D OTF is given by a toroidal pass-band, which now includes a volume of imaged spatial frequencies, but exhibits a missing cone of information around the longitudinal spatial frequency axis. Incoherent imaging systems, however, cannot image phase information.

Partially-coherent imaging systems are, unfortunately, much more difficult to analyze. In general, we have to introduce a partially coherent transfer function, or transmission cross-coefficient, that depends on spatial frequency pairs. For the simpler case of a weak object, such that we neglect interference of diffracted light with diffracted light, the 3D transfer function was presented by Streibl [8]. The form of the weak object transfer function (WOTF) depends on the aperture of the condenser lens. In the limit of a small condenser aperture, it tends to the normal CTF for a coherent system, while if the condenser aperture is equal to that of the objective, it has the same form as the OTF. In general the WOTF is not symmetric about the origin of axial spatial frequencies, the even part imaging amplitude information and the odd part imaging phase information.

3.1 Defocus

This suggests that phase information can indeed be present in a partially coherent image, but that defocus is necessary to obtain this information. In fact defocus was used many years ago as a way of imaging phase information. A way to make this quantitative is to take two images at opposite defocus settings [9]. This phenomenon is based on the fact that the image wave field must satisfy the wave equation.

3.2 Transport of intensity equation

The above method is based on the assumption that the object is weakly scattering, but even if it is not the phase can be recovered using the transport of intensity equation [10, 11]. However, the method recovers the phase of the wave-field rather than the phase of the object, and extracting the phase for the partially-coherent case is not so simple, especially for a 3D object [12].

3.3 Differential interference contrast (DIC)

One of the most used methods for phase imaging is differential interference contrast (DIC). This uses a shearing interferometer to compare two closely-spaced images of the sample. The images are added with a relative phase difference called the bias. Theory for imaging based on diffraction theory which takes account of vignetting effects has been developed [13-16]. By using different values of bias, a phase-shifting algorithm can be used to extract the phase gradient, and by measuring phase gradient in two directions the phase can be recovered [15].

3.4 Differential phase contrast (DPC)

Differential phase contrast (DPC) was first introduced for scanning microscopy, and uses a pair of semicircular detectors. The two images are subtracted to produce a DPC image and summed to give an amplitude contrast image [17]. A similar method can be used in the conventional microscope, by using condenser pupil masks and processing the images digitally [18]. We call this technique asymmetric illumination DPC (AIDPC). Fig. 1 shows an example, where the object exhibits strong amplitude and phase information that can be separated by the technique. The middle row of the figure shows the partially coherent transfer function, that is useful to investigate the imaging properties of the system.

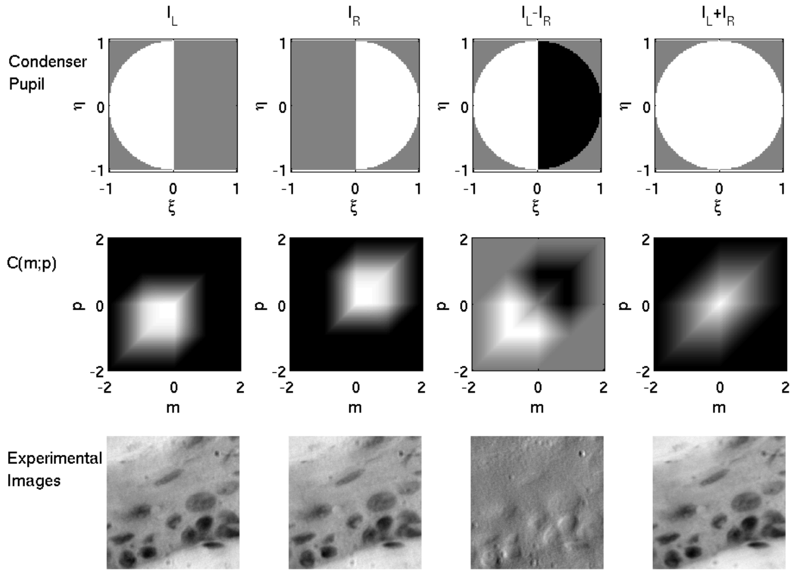

Fig. 2. Condenser pupil structures (top row), partially coherent transfer function in direction of differentiation (middle row), and experimental images (bottom row) obtained with AIDPC. The sample is skin H&E stained section courtesy Graham Wright, TLL and Declan Lunny, IMB.

3.5 Interference microscopy

Holographic microscopy when performed as image plane holography can be regarded as a form of interference microscopy. A special feature of DHM is that the illumination is spatially coherent, or close to spatially coherent. When interference microscopy is performed with a spatially incoherent source and an appreciable condenser aperture, the imaging properties become very different [13]. The 3D imaging properties depend on the aperture of the condenser in a similar way to partially coherent imaging considered earlier [19]. In reflection there is an optical sectioning effect, similar to that in confocal imaging, that results from the correlation of the object and reference waves [20]. The 3D CTF is thus also similar to that in confocal imaging [7]. Using a source of low temporal coherence can also be used to improve image formation in the depth direction [21, 22]. This is, of course, also the basis of optical coherence tomography, which is usually performed in a scanning modality.

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