

- Determine the lateral and axial resolution of a quantitative phase imaging (QPI) system
- Can utilise the resolution target to objectively compare QPI techniques

1. Introduction

The Phasefocus livecyte cell imaging system offers the advantage of quantitative phase images with fields of view that are not limited by camera chip size.

In order to assess the performance of a quantitative phase imaging system, a transparent resolution target is required. A transparent target can be used to test the resolution, noise, sensitivity, and accuracy of the phase values of the images. Moreover, it can be used to inspect the transfer of spatial frequencies in the imaging system. All of the above are needed for accurate segmentation of live cells, and most importantly for the accurate determination of properties such as cell volume, mass and thickness.

2. The resolution target

The features on the target are a series of horizontal and vertical rectangles etched into glass. The lateral size of these features varies from $2\mu\text{M}$ to $600\mu\text{M}$, giving a large spread of spatial frequencies over which to measure the phase shifts. Each rectangle gives a different optical path length to its surrounding, leading to a phase shift which is proportional to the depth of the etch.

3. Measurement of the resolution target

Figure 1(a) and (b) show images of the modulus and phase of the transparent target respectively. The modulus is the square root of the intensity as if it were measured on a brightfield optical microscope. The etched features are transparent in the brightfield (a), but have very high contrast on the phase image (b). We measure the same phase shift for all the lateral feature sizes, which demonstrates the uniform transfer of accurate phase values for all spatial frequencies. As a result, this makes image segmentation very accurate, and moreover, allows the quantitative measure of the thickness values for all feature sizes. This is unlike phase contrast and many other quantitative phase imaging techniques where the images have artefacts, such as halos or shade-off, or loss of contrast with decreasing feature size.

4. Analyses

We can use images of the targets for critical analyses of the imaging system. The sharpness of the edges of the etched features can be used to determine the resolution, which we find to be diffraction limited by the objective lenses used. The contrast of the phase values can be used to assess the coherent transfer function (transfer of spatial frequencies). The noise in the background can be used to determine the sensitivity. Finally, the known values of the etch depths can be used to assess the accuracy and repeatability of the quantitative phase values in the images. The known values are independently certified to traceable measurement standards by the UK National Physical Laboratory. The accuracy of the phase values directly affects the accuracy of dry mass, thickness and volume of cells in real world experiments. Table 1 shows specifications of a Phasefocus system determined from measurements of the target. Further details can be found in the Optics Express paper on this work [1]

Fig.1 a)

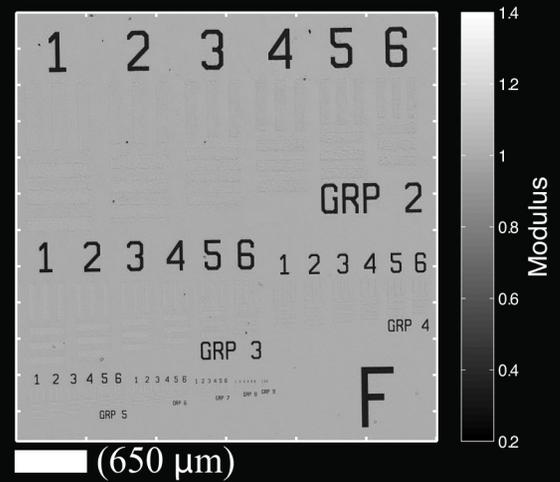


Fig.1 b)

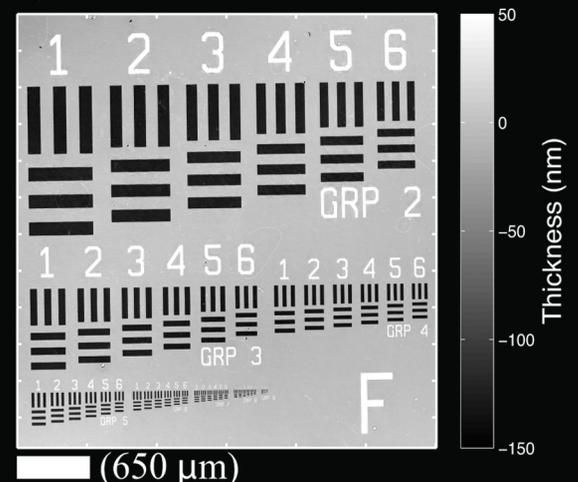


Fig1. Example images of the transparent target. (a) is the modulus and (b) the phase.

Magnification	Lateral Resolution of images (nm)	Sensitivity to thickness (phase sample) (nm)	Sensitivity to thickness (rad)	Transfer of spatial frequencies
10x (NA = 0.25)	1.3	11	$2\pi / 126$	Unity
20x (NA = 0.40)	0.81	11	$2\pi / 126$	Unity
40x (NA = 0.65)	0.50	11	$2\pi / 126$	Unity

Wavelength (nm) : 650
 $n_{\text{phase sample}}$: 1.466405
 n_{air} : 1

5. References

[1] T. M. Godden, A. Muñiz-Piniella, James Claverley, Andrew Yacoot, and M. J. Humphry, A phase calibration target for quantitative phase imaging with ptychography, Optics Express (submitted) 2016.



For more information on the benefits of the Livecyte system, to access application notes and for additional product information, please visit: www.phasefocus.com/livecyte

A sample of time-lapse videos can be found at: www.youtube.com/phasefocuslimited

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