A Phase-Shifting DIC Technique for Measuring 3D Phase Objects:  
Experimental Verification

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ABSTRACT

Experimental verification of our previously proposed linear phase imaging technique for differential interference contrast microscopy (DIC) microscopy is presented. This technique first applies phase-shifting methods to DIC to acquire linear phase gradient images in two orthogonal directions. A special Fourier integration algorithm is then applied to the combined phase gradient images to create a single linear phase image in which intensity is proportional to phase. This overcomes the limitations of traditional DIC, which cannot accurately measure the phase (i.e. refractive index or thickness) of embedded 3D phase objects. The linear phase imaging technique is implemented using a standard DIC microscope altered to allow controlled phase shifting, a low noise CCD camera, and post-processing in Matlab. The results presented confirm the linear proportionality of intensity to phase in these images.

Keywords: DIC, phase, phase gradient, phase imaging, spiral phase, interference microscopy

1. INTRODUCTION

DIC microscopy is well known for its ability to image transparent phase objects that otherwise produce very little contrast in bright-field microscopy. Its particular advantages over other phase imaging techniques include: applicability at high numerical apertures, ability to image phase objects embedded within a transparent material, and lack of aberrations.

Until recently, one disadvantage of DIC was its non-linear intensity versus phase gradient response. In other words, intensity of a DIC image was not linearly proportional to the differential phase of the object. In 1997 Cogswell et al. showed that geometric phase-shifting techniques could be applied to DIC to remove this non-linearity.1

Theoretically, integration of images created using this technique leads to final images in which intensity is linearly proportional to the true phase of the object. Therefore, phase-shifting DIC (PS-DIC) seems an ideal starting point for measurement of 3D phase objects. However, previous attempts to integrate PS-DIC images using numerical integration produced inadequate phase image results. These images showed directional artifacts due to the unknown constant of integration.2

A newly proposed method of integration, developed by our research collaborators, has shown promising results in simulation.3 Based on a Fourier filtering technique; this method has been dubbed spiral phase integration due to the spiral nature of the filtering function’s phase angle. The symmetry of the spiral phase filtering function prevents the directional artifacts seen in numerical integration. Intensity of phase images created using this technique in simulation is linear with the phase of the object, if one allows for some error off-axis and at image and object edges. The sources of error are discussed in detail by Arnison et al.3 In the following section we present experimental results of this technique. Important points to consider in experimental application are discussed in section three.

2. EXPERIMENTAL RESULTS

In order to image real objects of a known phase delay, sets of gold bars on silicon were fabricated using MUMPs® technology which is a precise multilayer polysilicon surface machining process. These were imaged with a 20x, 0.5
NA objective with a focal depth of approximately 1.5μm. The resultant image after application of the spiral phase integration algorithm is shown in figure 1. For comparison, the gold bars were also measured with a Zygo interferometric profilometer. In figure 2, normalized comparison of the phase image with the interferometric profilometer data shows good agreement in height and width of the gold bars. As predicted by simulation, however, the data shows a poor agreement at the image and object edges. A mirror reflecting image processing technique is sometimes included in the spiral phase integration algorithm in an effort to overcome these edge discontinuity effects. However, comparison of data integrated with and without this step, shown in figure 2, shows its improvement of edge effects is minimal. Additionally, there is a disagreement in background phase, which is not present in simulation results.

Figure 1: Linear phase image of gold bars. Height of bars in z = 0.5μm, width of each bar in y = 10μm, imaged with a 20x, 0.5 NA objective with a focal depth of approximately 1.5μm. The spiral phase integration algorithm (including mirror reflection step) was applied to a complex combination of cropped and padded PS-DIC images representing two orthogonal directions of DIC shear.

Figure 2: Comparison of normalized Zygo interferometer profile with normalized vertical profile through gold bar phase image (along direction of arrows in figure 1). Note that there is good agreement with respect to height and position of bars but poor
agreement at the image and object edges as expected, with additional disagreement in background phase partially due to the sensitivity of PS-DIC to the silicon substrate surface roughness.

3. CRITICAL EXPERIMENTAL CONSIDERATIONS

A poor signal to noise ratio in the original DIC images will degrade the final phase image. The effects of lighting, exposure and gain settings, which mainly influence the amplitude of the light passing through the object, are calculated out of the phase image. However, effects of all three also influence the signal to noise ratio. Due to the nature of the spiral phase function modulus, low frequency noise is boosted slightly more than high frequency noise. Additional system noise can be avoided by requiring that images not be aliased by digital camera sampling and by windowing the spiral phase function so that frequencies outside the system cut-off are zeroed.

The effects of misfocus can also degrade the final phase image and must be considered carefully when imaging large objects. In a second measurement gold bumps were dropped onto a gold plated silicon wafer and partially flattened to 20μm height. These bumps were imaged with a 20x, 0.5 NA objective with a focal depth of approximately 1.5μm, shown in figure 3, and measured with a Zygo interferometric profilometer. The plane of focus was the bottom edge of the gold bump with the top detail blurred. Figure 4 is a normalized comparison of the phase image with the interferometric profilometer data showing good agreement in width of the gold bump to a height within, and slightly higher than, the focal depth. Low frequencies from well outside the focal plane, but still present in the original PS-DIC images, only partially reconstruct the phase outside the focal depth. Therefore, it is critical to be aware of the axial size of the object being studied with respect to the focal depth of the system.

![Figure 3: Linear phase image of gold bump. Height of bump in z = 20μm, width of gold bump in y = 130μm, imaged with a 20x, 0.5 NA objective with a focal depth of approximately 1.5μm. Focus was at the bottom edges of the bump with the top detail blurred. The spiral phase integration algorithm (including mirror reflection step) was applied to a complex combination of cropped and padded PS-DIC images representing two orthogonal directions of DIC shear.](image-url)
Figure 4: Comparison of normalized Zygo interferometer profile with normalized vertical profile through gold bump phase image (along direction of arrows in figure 3). Note there is good agreement in width to a height within, and slightly higher than, the focal depth. Low frequencies from outside the focal plane only partially reconstruct the phase outside the focal depth.

It is important to recall that DIC images the differential phase of an object with respect to its background. As a result, the linear phase gradient of the PS-DIC image, and therefore also the linear phase of the integrated image, is a measure with respect to the object background. Therefore, if the background of an object is also varying with phase, it is critical to interpret the intensity of the image accordingly. Figure 5 shows the linear phase image of a single mode fiber imaged in transmission using a 40x oil objective with a NA = 0.75 and index matching oil. Effects due to the edges of the image are more prominent due the object’s very smoothly varying phase.

Figure 5: Linear phase image of Corning SMF-28 fiber, $n_{\text{core}} = 1.46008$, $n_{\text{cladding}} = 1.45482$, core diameter = 8µm, cladding diameter = 125µm, imaged with a 40x oil objective with a NA = 0.75 and index matching oil, $n_{\text{oil}} = 1.518$. Note that effects due to the edges of the image are more prominent due the object’s very smoothly varying phase. The spiral phase integration algorithm (including mirror reflection step) was applied to a complex combination of cropped and padded PS-DIC images representing two orthogonal directions of DIC shear.
The cylindrical fiber core is embedded in a cylindrical cladding. In this case, linear phase is measured primarily with respect to the oil background. However, the phase of the smaller core is measured with respect to the cladding as well. If the background phase variation due to the cylindrical shape is divided out, the expected refractive index change of a step index fiber is revealed inverted. The inversion is due to the fact that the index matching oil used has a higher index than either the cladding or the core. The resulting index difference between the oil and the core is less than the index difference between the oil and the cladding, creating an inverted step index.

Figure 7: Diagonal profile through linear phase image of SMF-28 fiber with background phase variation due to the cylindrical shape divided out. The inversion is due to the fact that the index matching oil used has a higher index than either the cladding or the core: $n_{\text{oil}} = 1.518 > n_{\text{core}} = 1.46008 > n_{\text{cladding}} = 1.45482$. 

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4. CONCLUSIONS

With careful attention to signal to noise ratio, aliasing and system frequency cut-offs, phase images created using this technique experimentally are similar in quality to those created by Arnison et al. through simulation.\(^3\) Intensity of phase images is linear with the phase of the object allowing for some error off-axis and at object edges. Interpretation of these images must be done carefully, taking into consideration the effects of misfocus and background phase variations.

In the future, error may be reduced through a revision of the filter function to reflect a more rigorous model of the DIC point spread function. Additionally, calibration of the proportionality between the linear and true phase will provide images in which intensity equals phase.

With only minor alterations to the traditional DIC microscope and no difficult alignment, this technique can be used in transmission to quantitatively image phase objects embedded in transparent material or in reflection to quantitatively image surface profile. Image processing is non-iterative and takes only seconds, producing no phase wrapping unless extreme phase gradients over the size of the DIC shear dimension are present.

REFERENCES

