Real-time quantitative differential interference contrast (DIC) microscopy implemented via novel liquid crystal prisms

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ABSTRACT

A phase shifting differential interference contrast (DIC) microscope, which provides quantitative phase information and is capable of imaging at video rates, has been constructed. Using a combination of phase shifting and bi-directional shear, the microscope captures a series of eight images which are then integrated in Fourier space. In the resultant image the intensity profile linearly maps to the phase differential across the object. The necessary operations are performed by various liquid crystal devices (LCDs) which can operate at high speeds. A set of four liquid crystal prisms shear the beam in both the x and y directions. A liquid crystal bias cell delays the phase between the e- and o-beams providing phase-shifted images. The liquid crystal devices are then synchronized with a CCD camera in order to provide real-time image acquisition. Previous implementation of this microscope utilized Nomarski prisms, a rotation stage and a manually operated Sénarmont compensator to perform the necessary operations and was only capable of fixed sample imaging. In the present work, a series of images were taken using both the new LCD prism based microscope and the previously implemented Sénarmont compensator based system. A comparison between these images shows that the new system achieves equal and in some cases superior results to that of the old system with the added benefit of real-time imaging.

Keywords: Differential interference contrast (DIC) microscopy, quantitative phase imaging, liquid crystal devices, live cell imaging

1. INTRODUCTION

Quantitative phase microscopy provides essential information for biological imaging of cellular dynamics. This is especially true for transparent specimens which cannot be easily imaged using bright field or fluorescence microscopy. Common methods of imaging phase, namely Nomarski differential interference contrast (DIC) microscopy, only provide qualitative information about the phase differential for a given sample. The main problem being that the intensity variations in a Nomarski DIC image do not linearly vary with the true phase differential of the object. A number of different methods for quantitative phase imaging have been proposed and demonstrated\(^1\)\(^-\)\(^3\). The following discussion will be specifically focused on improving quantitative phase imaging achieved through phase shifting DIC\(^4\) (PS-DIC) microscopy. PS-DIC features a number of convenient properties over alternative methods such as no need for phase unwrapping, low computational expense and easy integration into commercial biological microscopes.

1.1 Background on phase shifting DIC microscopy

Phase shifting differential interference contrast microscopy utilizes principles from optical metrology to reconstruct a quantitative phase profile of a given object. The differential phase information along a given shear direction is collected by taking four traditional Nomarski DIC images with a phase bias of 0°, 90°, 180° and 270° on each of the respective images. The linearized phase difference and amplitude across the image can then be found using Eqs. 1-2,

$$\Delta \phi = \tan^{-1} \left( \frac{I_{90} - I_{270}}{I_{0} - I_{180}} \right)$$

(1)
where $\Delta \theta$ is the linearized phase gradient, $I$ is the intensity of each of the four phase-shifted images, and $a_1, a_2$ represent the image amplitude.$^{4,5}$

The sample is then rotated and the process repeated to collect information along the orthogonal shear direction. The two orthogonal images are then integrated to provide a quantitative measure of the true optical path profile of an object. In PS-DIC images, areas of brighter intensity linearly map to optically thicker portions in the sample and conversely dark areas represent optically thin portions of the sample. The phase shifts used in this technique are generated by a Sénarmont compensator$^6$ consisting of a quarter wave plate and a rotating analyzer. Figure 1 shows a schematic of a PS-DIC microscope.

Figure 1. Schematic of a phase shifting DIC microscope that utilizes a Sénarmont compensator to perform the phase shifts.

The main drawback of the Sénarmont compensator method for phase shifting is that it requires the manual rotation of the analyzer to produce the four bias images in one shear direction followed by manual rotation of the microscope stage to generate four more phase-shifted images with orthogonal shear. This prevents any real-time imaging of live samples or cellular dynamics. The rotation of the sample also leads to image registration problems and a reduced field of view as a result of the post processing. To overcome these challenges we have constructed a new system that automatically preforms the phase shifts and shears the beam in orthogonal directions.

2. LIQUID CRYSTAL DEVICE IMPLEMENTATION OF PS-DIC

The development of novel liquid crystal devices has allowed for the construction of a new phase shifting DIC microscope capable of real-time imaging. In this new system the Nomarski prisms are replaced with pairs of liquid crystal prisms (LCPs). The quarter wave plate and rotating analyzer used to preform phase shifts are replaced with a singular LCD phase bias cell (PBC). Each of these parts works in unison to automate the image acquisition process and to create a PS-DIC microscope capable of real-time imaging.

2.1 Liquid crystal prisms

The liquid crystal prisms offer a number of advantages over the traditional Nomarski prisms. The most dramatic improvement is that the LCPs can vary the shear applied to the beams. In a standard Nomarski prism the shear is fixed, so a separate prism is needed to match the different magnification and numerical aperture for each objective. The
strength of the shear in the liquid crystal prism is varied by the amount of voltage applied. This allows for one set of prisms to be used with a wide range of objectives reducing the number of components and cost to do DIC microscopy. The other major advantage of the LCPs is that one set of prisms are used to shear the beams in x while another set are used to shear the beam in y. In conjunction the two sets of prism pairs can also shear the beam at any desired angle. This eliminates the need to manually rotate the sample to obtain the phase differential in the orthogonal directions, significantly increasing the acquisition speed and eliminating minor misalignments. Avoiding rotation of the sample also improves the quality of the post-processing algorithm by removing the need for registration between the orthogonal shear images. Figure 2 shows two close up views of a liquid crystal prism.

![Figure 2](image1.png)

**Figure 2. Close up of a liquid crystal prism.** Separate pairs of prisms are used to shear and recombine the beam in both x and y respectively. A variable amount of voltage can be used to adjust the magnitude of the shear.

### 2.2 Phase bias cell

The addition of the phase bias cell completes the modification of the old PS-DIC microscope by replacing the quarter wave plate and rotating analyzer in the Sénarmont compensator. This allows for the automatic collection of the four phase-shifted images in each direction of shear as opposed to the manual rotation of the analyzer required in the previous implementation. The PBC works by altering the relative index difference between the orthogonally polarized beams. The phase delay is linearly related to the voltage applied to the cell. Figure 3 below shows a close up image of the phase bias cell with the optics axis oriented at a 45° angle.

![Figure 3](image2.png)

**Figure 3. Close up of a liquid crystal phase bias cell.** The voltage applied to the bias cell linearly applies a phase delay to the extraordinary beam with respect to the ordinary beam.
2.3 Experimental Setup

The experimental setup used to test and implement the new liquid crystal devices is shown in Fig. 4 below. The system is essentially the same as the phase shifting DIC setup previously shown. The only differences being that fixed crystal optics in the original setup have been replaced with the new liquid crystal devices. Since the shear of the LCPs is variable, it also allows for more freedom in their placement. Standard Nomarski prisms have a fixed shear and must be placed at the appropriate planes in the microscope to properly recombine the two polarization states, whereas the liquid crystal prisms can be adjusted to fit wherever is most convenient for the user. Real-time imaging is achieved by syncing the voltages applied to the various liquid crystal devices with the CCD camera acquisition software.

Figure 4. Schematic of the experimental setup for the phase shifting DIC microscope based on liquid crystal devices.

3. RESULTS

The new PS-DIC system was tested using a human cheek cell as the sample. The cell was imaged with a 20x/0.5 NA Zeiss objective. Figure 5 shows the four phase-shifted images acquired for each direction of shear using the liquid crystal setup. The phase-shifted images clearly demonstrate the ability of the liquid crystal prisms to create DIC images and demonstrate the ability of the phase bias cell to accurately shift the phase bias between the two beams. The linearized differential phase profiles for each shear direction were then extracted from the eight original images using Eq. 1 and integrated using a spiral phase integration (SPI) algorithm\(^7\). The process was then repeated using the Sénarmont compensator PS-DIC microscope. Figure 6 shows a comparison of the quantitative phase images generated from each microscope. The systems used different cameras and different tube lenses so the magnifications are not equal. The automatic phase shifting microscope shows comparable results compared to the Sénarmont compensator. The nucleus and the feature in the lower left of the cell exhibit a longer optical path length and are thus represented as brighter than the rest of the cell. These results with biological samples validate that the new phase shifting DIC microscope has equivalent performance to prior art with the benefit of a vastly increased image acquisition rate.
Figure 5. Four phase-shifted images of a human cheek cell (20x/0.5 NA) acquired from the liquid crystal PS-DIC microscope for the (a) x shear and (b) y shear. Starting from the top left and moving clockwise the 0°, 90°, 180° and 270° phase biased images are shown.
Figure 6. Comparison of human cheek cells imaged with a 20x/0.5 NA Zeiss objective. The result from the liquid crystal prism microscope, (a), shows that it is comparable to that of the Sénarmont compensator microscope, (b). Due to different cameras and tube lenses in the respective setups there is a difference in magnification between the two images.

4. CONCLUSIONS

We have demonstrated a new phase shifting DIC microscope that can acquire quantitative phase images in real time. This system improves upon prior art by replacing manually operated components with new liquid crystal devices. The traditional Nomarski prisms have been replaced by novel LC prisms that allow for a variably adjusted shear. In addition, the Sénarmont compensator has been replaced by a liquid crystal phase bias cell that can shift the phase by simply applying a voltage. These new devices coupled with a CCD camera and customized software allow for real-time acquisition of quantitative phase images. The automated phase shifting microscope has been tested with biological samples and has shown comparable performance to previous implementations. The liquid crystal system has no moving parts and is much more robust to focus drift and image registration problems.

Future work includes imaging live biological samples in real time. The liquid crystal devices are also being modified so that they can be easily integrated into existing commercial microscope systems. This will allow biological researchers that already have microscopy setups to easily expand the performance of their systems.

REFERENCES


ACKNOWLEDGEMENTS

We would like to thank and acknowledge the National Institutes of Health, Grant #5 R43 RR028192-02, for their support.