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Orientation-Independent Differential Interference Contrast Microscopy

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The image in a regular DIC microscope reflects the orientation of the prism shear direction and the optical path gradients in a phase specimen. If the shear direction lies parallel to the specimen boundary no contrast is generated. Also a bias retardance is generally introduced, which creates a gray background and reduces image contrast. Here we describe the theoretical foundation for a new DIC technique, which records phase gradients independently of their orientation and with the digitally generated gradient magnitude image as well as the optical path distribution image free from the gray background. Separate images can show the magnitude distribution of the optical path gradients and of the azimuths, or the two images can be combined into one picture e.g., with the brightness representing magnitudes and color showing azimuths respectively.

For experimental verification of the proposed technique we investigated various specimens such as glass rods embedded in Permount, Siemens star nano-fabricated in 90-nm thick silicon oxide layer, Bovine pulmonary artery endothelial cell, etc, using regular DIC optics on a microscope equipped with a precision rotating stage. Several images were recorded with the specimen oriented in different directions, but with the prism bias unchanged, followed by digital alignment and processing of the images.

At first we explored a model specimen, which is optically similar to transparent filaments in living organisms. We took short segments of glass rods, which are used as spacers in liquid crystal cells, and embedded them in Fisher Permount* Mounting Medium (http://www.fishersci.com). The refractive indices of the glass rods and Permount at wavelength 546 nm were measured with a Jamin-Lebedeff microscope (Zeiss, Germany). They measured 1.554 and 1.524 respectively. A drop of the suspension was placed between microscope slide and a 0.17 mm thick coverslip, and the preparation was placed on the precision rotating microscope stage.

One of the images taken by regular DIC optics is shown in Fig.1 on the left picture. Also the figure contains line scans of intensities in two sections, one of them (AA’) is scanned along the shear direction and another (BB’) is perpendicular to the shear direction. Both scans are normalized to the maximal intensity in the image and multiplied by 100.

Grayscale images of gradient magnitude are given in Fig.1 on the second picture. Here brightness is linearly proportional to the magnitude. The gradient magnitude values measured along lines A-A’ and B-B’ are shown below the gradient image. In contrast to the left figure, the two linear scans of magnitude now exhibit virtually no difference. The gradient magnitude image clearly shows the rod boundaries independently of orientation.

Both gradient magnitude and azimuth images can be combined into one, color shaded picture e.g., with the brightness representing magnitude and color showing azimuth respectively (Fig.1, third picture). As can be seen from the shaded color, a gradient vector on rod boundaries is always oriented toward the rods. That means the rod’s refractive index is higher than the refractive index of the mounting medium.

Using the simple numerical contour integration, we computed the two-dimensional phase distributions of rays transmitted through the glass rods (Figure 1, right). The reconstructed phase image contains some streaks, which can be caused by misalignment of the raw DIC images. The normalized phase values along lines A-A’ and B-B’ are shown below the picture.

The results demonstrate that the proposed DIC technique can successfully image and measure phase gradients of transparent specimens, independent of the directions of the gradient. The orientation-independent DIC data obtained can also be used to compute the quantitative distribution of specimen phase or to generate enhanced, regular DIC images with any desired shear direction.

We are currently developing a new device using special DIC prisms, which allows the bias and shear directions to be switched rapidly without the need to mechanically rotate the specimen or the prism (US Patent Application 2005-0152030). With the new system an orientation independent DIC image should be obtained in a fraction of a second.

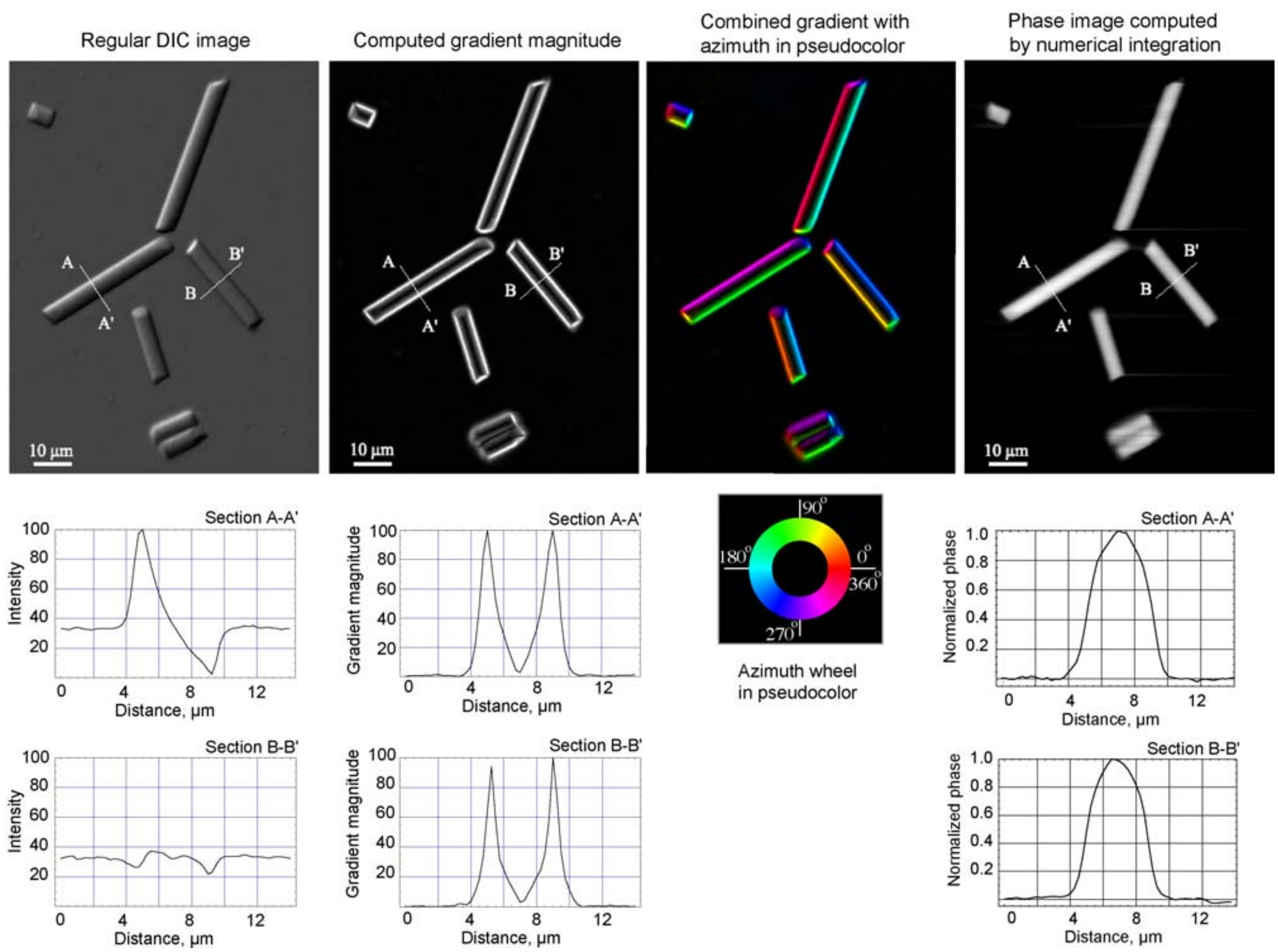

Fig.1. Regular DIC image of glass rods immersed in Permount (left picture), computed gradient magnitude image of the rods (second picture), combined gradient image with azimuth in pseudocolor (third picture), and computed phase (right). DIC shear direction is parallel to AA’ section. Linear scans of normalized intensity, gradient magnitude, and computed phase in sections A-A’ and B-B’ are shown below the corresponding images. The color wheel below the third image depicts azimuth.