

ITERATIVE DECONVOLUTION OF DIFFERENTIAL INTERFERENCE CONTRAST MICROSCOPY IMAGES

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Nomarski Differential-Interference-Contrast (DIC) microscopy is an important imaging modality widely used both in motility analysis and, more generally, for structural studies of live biological specimens. DIC image contrast is generated by detecting local differences in the optical path length (OPL) throughout the specimen. The OPL is the product of the refractive index and the geometrical distance (thickness) traversed by a light beam between two points along the optical path. DIC microscopy utilized two closely spaced partially coherent, but orthogonal, wavefronts separated by a distance less than the lateral resolution of the objective lens to detect orientation-specific changes in OPL. Figure 1 shows a DIC image of yeast cells. We present here an iterative optimization solution to estimate a specimen's OPL distribution from its DIC image.

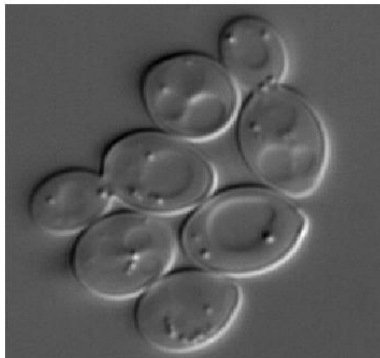


Fig 1: DIC Image of yeast cells

DIC imaging is essentially a directional differentiation of the specimen's OPL, and the DIC image spectrum results in a *null* region, perpendicular to the shear direction, that is contaminated by noise. To recover the OPL data, the frequency information residing in the null region is estimated, and the DIC image formation process is reversed. This is achieved by iteratively optimizing a cost function that minimizes the difference between the observed DIC image and the reconstructed data after it has passed through the DIC imaging model, suppressing streaking artifacts that appear parallel to the shear orientation, and penalizing negative values in the reconstructed data. The initial estimate is determined by Wiener filtering the DIC image.

The regularization filter (RF) used in the reconstruction process smooths over the streaks in the OPL image caused by the null in the image spectrum. However, excessive noise in the null region can cause problems. In such cases, the null region is suppressed and frequencies outside the null that are less corrupted are utilized. Frequency selective filtering based on noise can be achieved using a Wiener filter (WF) approach. The two filters, RF and WF, act as compliments of each other, filtering different frequencies by different amounts. By controlling the contribution of each filter to the estimation process, the OPL data can be reconstructed for most DIC images.

Figure 2 shows the estimated OPL data for the DIC image in Figure 1. The pixel intensities represent relative OPL values with respect to the background medium. The reconstructed data can then be used with general image analysis algorithms, such as segmentation, morphometric measurement or object tracking. Reconstruction of 3D DIC volumes enables visualization using standard methods, such as a maximum intensity projection.

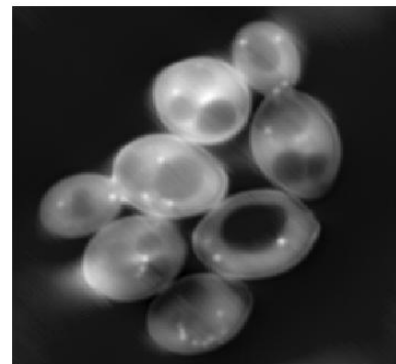


Fig 2: OPL Reconstruction