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Quantitative Phase Imaging and Differential Interference Contrast for Biological TEM

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Workers in transmission electron microscopy have developed a number of approaches to phase recovery for the purpose of quantitative comparison with models of materials structure. These approaches typically rely on the acquisition of through focal series[1]. Considerable success has been achieved in this work but it has not been applied to the study of biological samples.

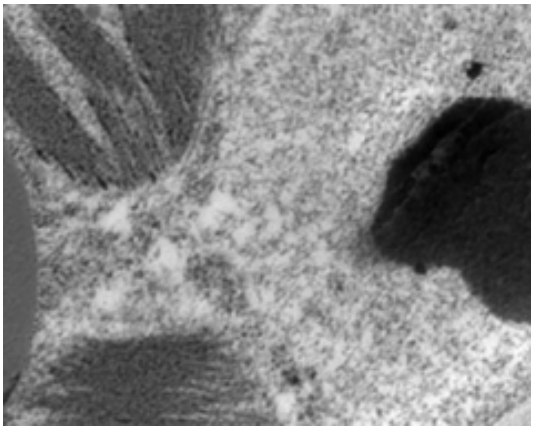
In this example we demonstrate that conventional transmission electron microscope (TEM) images of biological samples may be acquired and processed to present images entirely analogous to the differential interference contrast (DIC) images familiar to the optical microscopist.

Starting with conventional, digital, bright field TEM images of the sample, our non-recursive algorithm based on the Transport of Intensity equation[2] provides quantitative phase information independent of the sample's bright field intensity image. This independent phase and intensity information is then used to simulate a range of conventional experimental phase visualization modalities. Additionally, all of these can be implemented independent of the intensity.

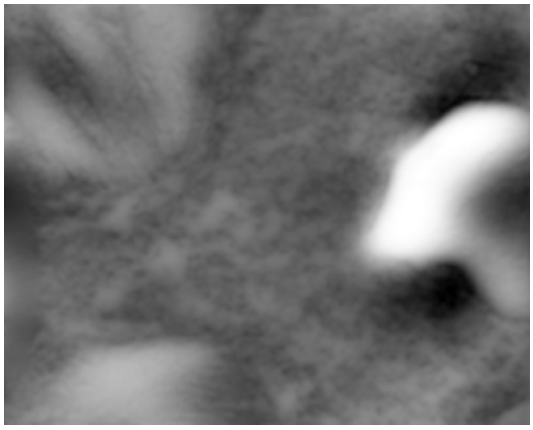
In order to apply this technique to a biological specimen, a sample of *Radula* sp.[3] (liverwort spore) was used. These spores were prepared using glutaraldehyde fixation, dehydration in a graded acetone series followed by infiltration and embedding in LR white. 75 nm thick sections were stained with Uranyl Acetate. Data was collected using a SIS Megaview II digital camera on a Philips CM 100 TEM. The in-focus image obtained at a magnification of 19,000 times is shown in figure A. The recovered phase image, shown in figure B is calculated from the composite data from the in- focus and defocussed images. A defocus of +/- 12.3μm was used.

Using this recovered phase contrast data it is possible to simulate an electron DIC image by simply differentiating the phase data. The result is shown in figure C. The intensity data was included in figure D. The images both display the characteristic DIC surface relief appearance.

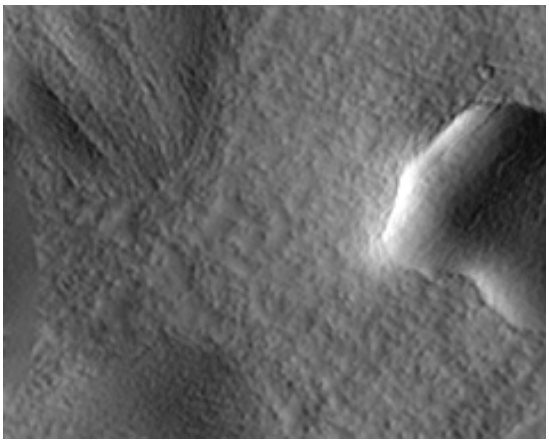
The biological information present in these images is not going to be discussed here. But we point out that the features of the DIC image shown in figure C are a result of biological structure; none are an artifact of the staining process.



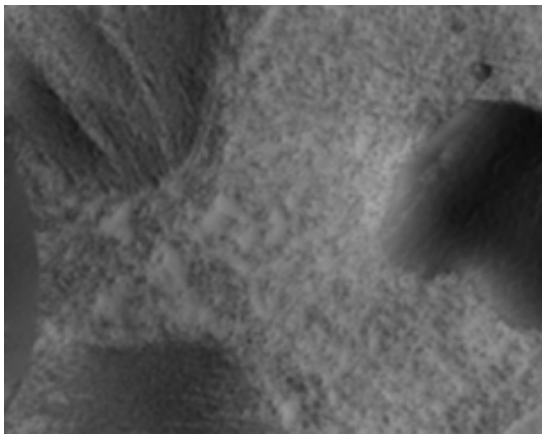
A: Bright field Image



B: QPe Phase Image



C: QPe DIC (Without Intensity)



D: QPe DIC (with 75% Intensity)

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