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Powerful Tools for 3D Microscopy Image Analysis, Processing and Visualization Based on 2D and 3D Fourier Transforms

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In recent years we have developed an experimental confocal microscope that can acquire images in confocal transmission modes, such as transmission brightfield and transmission Nomarski differential interference contrast (DIC) (Cogswell et al. 1994, Cogswell and O'Byrne 1992, Dixon and Cogswell 1995). We have configured the microscope with point-like laser illumination, with two photomultiplier detectors (PMTs) in the transmitted image (detection) path, and have used a beam splitter to direct half of the light to each PMT. When a pinhole spatial filter is placed in front of one PMT detector, this portion of the microscope behaves as a confocal transmission system. If the second PMT is left as a large-area detector (i.e. no pinhole is inserted) then it produces images similar to a conventional transmission microscope. Using both detectors provides us the opportunity to obtain both a conventional and a confocal transmission image simultaneously, for each plane of focus within the specimen, which is essential for precise comparison and evaluation of the two modes.

Initial experimental results with this microscope system show, particularly when using DIC optics, that the confocal configuration helps reject flare from out-of-focus scattering objects as compared to the conventional large-area-detector microscope configuration. In addition, the confocal transmission DIC mode appears to give an improvement in the ability of the system to delineate the edges and surfaces of objects in the axial direction as compared to a conventional DIC microscope. However, in the two-dimensional (2D) optical sections of a confocal focus series, these improvements appear as well-illuminated features that go out of focus more rapidly than in the conventional transmission DIC microscope. Thus the images are rather different from a typical confocal reflection mode in which out-of-focus features contribute little to the image and therefore appear dark.

Because of the non-typical appearance of the confocal transmission DIC images produced in our experiments, our initial attempts to apply standard confocal image processing and 3D visualization techniques to analyze the conventional versus confocal DIC images, or to extract and display image features, were unsuccessful. In order to help alleviate these problems, we have begun to investigate alternative methods for image analysis using Fourier transform techniques. In this paper, we describe a digital 3D fast Fourier transform (FFT) method for measuring and displaying the spatial frequencies which comprise our 3D image volumes. This 3D spatial frequency information can then be used to evaluate the axial and transverse resolution of our confocal versus conventional transmission images. The 3D FFT was developed by our group as a subroutine to expand a 2D FFT algorithm in a commercial software package (Vital Images VoxelMath) so that it would produce a 3D FFT of the full image volume. Besides evaluating resolution, the 3D FFT procedure is useful for determining if small shifts in registration have occurred between successive 2D sections in a 3D image stack.

In addition to the 3D FFT analysis, we also show that digital Fourier transform methods, such as the Hilbert transform (Oppenheim and Schafer 1989), can be successfully employed to overcome the difficulty of visualizing differentially-shaded phase objects, in 3D, that are typical of images acquired using transmission DIC optics. The Hilbert transform has the property of inverting one side of the differential shading gradient while at the same time maintaining the high spatial frequencies of the original DIC image (Cogswell et al. 1995). This is in contrast to a standard (directional) integration algorithm which tends

to act as a low pass filter, removing the high spatial frequency information (i.e. fine detail) in the image at the same time as it removes the shading gradient of features typical of DIC. The Hilbert transform algorithm allows us to extract object features from our 2D transmission Nomarski DIC image slices, in such a way that they can be further processed with a high boost axial filter, and then clearly visualized as a full 3D volume.

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