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Simultaneous Blur and Image Restoration in 3-D Optical Microscopy

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The point spread function (PSF) in three-dimensional (3-D) optical microscopy including widefield and confocal (fluorescence, brightfield reflective and transmission) is spatially variant especially in the axial direction. Further, the PSF depends upon various parameters such as the refractive indices of the materials along the light path, wavelength of the light and the depth of the volume of interest from the coverslip (Hell et al. 1993). Even though empirically derived PSF is an estimate of the true PSF, one must be aware of the caveat that it may not be an accurate estimate when optical conditions change. Rather than depending on an unreliable, empirically determined PSF, a type of image restoration called blind deconvolution has been developed and introduced in the literature (Holmes 1992, Krishnamurthy et al. 1992). In this paper, we present a novel, iterative algorithm for simultaneous blur and image restoration (SBIR) in 3-D optical microscopy. The method described here requires fewer iterations than those published in literature without sacrificing numerical accuracy of the results. Moreover, all the constraints necessary for the restoration are derived from the data itself.

The process begins by sectioning data into overlapping volumes so that overlap save method of convolution can be performed (Oppenheim and Schafer 1975). Since the PSF changes rather slowly in the axial direction but never abruptly, it can be modeled as spatially shift invariant within a given subvolume, but shift variant across subvolumes. Each subvolume can be processed independently as follows: The desired result is initialized to the observed data while the PSF is initialized to the Dirac delta function. Next, the input image subvolume is examined to determine the constraints on the PSF. The spatial extent of the PSF is usually overestimated in x-, y-, and z-directions. The spatial extent of the image subvolume is limited to the non-zero voxel intensities within the volume after background subtraction. The spatial frequency bounds are determined by examining sections of the 3-D power spectrum (periodogram) of the data in three mutually orthogonal directions. This estimation is possible since it is the PSF and not the true specimen which determines the bandlimits. The bandlimit estimation is done as follows: First we notice the lowpass nature of the power spectral sections. The magnitude function pertaining to extreme high frequency portions of the plotted spectral sections can be assumed to represent underlying noise processes and the bandlimit at each of the axis is determined by locating the point where the image frequency content drops off to the noise content as one traverses from low frequency end to the high frequency end. Generally, we overestimate this cut-off frequency since significant artifacts can result if the bandlimits are underestimated.

The iterative SBIR algorithm uses a generalized Landweber-type method (Strand 1974) for both the PSF and the image update in the Fourier domain. Each iteration begins by computing a PSF estimate in the Fourier domain given a specimen estimate. Bandlimit constraints estimated from the observed data are applied to the PSF in the frequency domain. The PSF estimates are further constrained to have unity gain with numbers being non-imaginary and positive in the spatial domain. Next the specimen update is obtained in the frequency domain by using the PSF estimate from the previous step. Constraints such as spatial bounds and positivity are applied in spatial domain. Acceptable results can usually be obtained within ten such iterations.

The SBIR algorithm was validated using simulated 3-D images. Test images were obtained by using a known computer generated specimen which was corrupted by convolving with a known PSF and by adding Gaussian noise. Blur and noise variances were varied to simulate different conditions generally encountered in practice. Blurry and noisy images were then restored with the SBIR algorithm and the quality of restoration was studied using a normalized mean squared error criterion. Next fluorescent latex beads of known diameter were used for the validation of the SBIR algorithm under more realistic conditions. The test specimen consisted of spherical fluorescent beads of 4μm diameter (hollow) and 1μm diameter (solid). These beads were next to each other in the axial direction. From the raw data, one could not resolve these two beads nor determine that the 4μm bead was hollow. In the processed data, both these limitations were resolved as both lateral and axial resolution of the image were improved. Finally, the algorithm was applied to unknown specimens. The preliminary results have shown that successful restorations could be obtained using the SBIR algorithm even when the specimen is located deeper in the tissue.

In conclusion, the SBIR algorithm improved lateral and axial resolution for different kinds of specimens. Since all the constraints were based only on the observed image data, the proposed algorithm was applicable for improving the quality of images at arbitrary depths. The algorithm provided acceptable results in fewer iterations than the methods described in literature. Furthermore, this procedure has potential to become a turnkey system to deblur images in 3-D optical microscopy.

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