

FAST AUTOMATED BLIND DECONVOLUTION OF 2D OPTICAL MICROSCOPE IMAGERY

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KEY WORDS: Optical microscopy, image deconvolution, MLEM method, wavelet denoising

Image deconvolution has been widely used in optical microscopy to improve image resolution and enhance contrast, mainly for 3D optically sectioned images. 2D images can be deconvolved in a similar way, and in many circumstances, such processing is either necessary or sufficient. Confocal microscope image slices can be processed as 2D images, especially if the pinhole is opened slightly to increase signal intensity while sacrificing some resolution [1]. Widefield microscope images of thin specimens with a thickness less than the depth of field of the objective lens, and TIRF (Total Internal Reflection Fluorescence) microscope images, in which only structures on the slide surface are illuminated, are also very suitable for 2D deconvolution.

The point spread function (PSF) is assumed unknown, so an iterative blind deconvolution approach is used, based on the maximum likelihood expectation maximization (MLEM) method [2]. A vector extrapolation algorithm is employed for acceleration [3], and together with an efficient algorithm implementation, has reduced processing times for a 256x256 pixel image to 2 seconds (for 20 iterations) with a Pentium IV 2GHz CPU. In time-series processing, once the PSF has been estimated from a single frame it can be reused with subsequent frames, further reducing processing times. In addition, a real-time linear filtering method has been developed that allows 512 by 512 pixel images to be processed at up to 30 frames per second. The resulting increase in image contrast enhances low-light level imaging by revealing fine features while the user is exploring the specimen, and potentially enabling the illumination intensity to be reduced.

Hybrid methods have been developed that combine the robustness of MLEM, with the speed of Gold's algorithm, resulting in a 3-5 times faster convergence than the standard MLEM. Wavelet based denoising methods have been adapted to handle the Poisson noise typical in low-light microscope images and integrated in the deconvolution framework. These denoising methods significantly improve the overall deconvolution quality, and stabilize the result from deteriorating when over-iterated. A dynamic stopping criterion is essential in automating the deconvolution. To achieve this, a set of characteristics including the signal-to-noise ratio, the total variation, and relative image change are computed, and a voting procedure is applied to determine whether to terminate the computation. Combined with the stabilized performance introduced by denoising, automatic termination near the optimal result can be achieved.

Results from a variety of microscope modalities, including multi-channel and time-lapse imagery will be presented.

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