

## METHOD AND APPARATUS FOR THREE-DIMENSIONAL DECONVOLUTION OF OPTICAL MICROSCOPE IMAGES

### BACKGROUND OF THE INVENTION

The present invention relates generally to the field of microscopy. More specifically, the present invention relates to a method and apparatus for three-dimensional deconvolution of optical microscope images.

It is known in light microscopy to use fluorescent dyes to study the spatial distribution of specific cellular elements. The use of fluorescent dyes allows examination of cellular elements that would not otherwise be discernible with conventional light microscopy. With the use of some very specialized dyes, specific cellular elements can be tagged for imaging, e.g., Hoechst 33258 is a dye for labeling DNA, see, Agard, D. A., Hiraoka, Y., Shaw, P., Sedat, J. W., *Methods in Cell Biology—Volume 30, Chapter 13 “Fluorescence Microscopy in Three Dimensions,”* San Diego, Calif., Academic Press, pp. 353–377, 1989. A class of reversible cationic redistribution dyes, tetramethylrhodamine ethyl (TMRE) and methyl esters (TMRM) are also used. These dyes are used for the study of mitochondria since the dyes produce intensities proportional to membrane potentials, see, Loew, L. M., Tuft, R. A., Carrington, W., Fay, F. S., “Imaging in Five Dimensions: Time-Dependent Membrane Potentials in Individual Mitochondria,” *Biophysical Journal*, Volume 65, pp. 2396–2407, December, 1993.

Three-dimensional imaging of a fluorescently dyed substrate is performed by collecting a series of x-y images along the an optical z-axis. The data is recorded by charge-coupled-device (CCD) arrays. The CCD array is a two-dimensional arrangement of CCD elements that collects an image or an image slice in the x-y plane. The optical focus of the system is moved to an adjacent plane where the next image slice in the x-y plane is acquired. This is continued until the desired number of planes (image slices) have been acquired for the three-dimensional data set.

Two types of microscope systems are typically used for image acquisition: a three-dimensional wide-field microscope and a scanning confocal microscope. Both of these microscope systems are considered to be diffraction-limited. Diffraction theory describes the effects of light passing through a finite aperture to an image plane. The resulting image displays the effects of the wave nature of light. The end result is that light emanates from a point as a spherical wave front and scatters through a finite aperture. Various treatments of diffraction theory have been presented, e.g., see Goodman, J. W., *Introduction to Fourier Optics*, New York, McGraw-Hill, pp. 101–136, 1988. The most commonly accepted treatments of diffraction theory are Fresnel approximations for near field (i.e., Fresnel diffraction) and Fraunhofer approximations for far field (i.e., Fraunhofer diffraction). In either case, the resulting image due to a diffraction-limited system can be described by the product of the Fourier transforms of a source image and aperture distribution. In other words, the resulting image is a result of a convolution of the source object’s light with an aperture of the imaging system. Further, the system transfer-function is obtained directly by taking the Fourier transform of the aperture. It will be appreciated that the blurring effects due to convolution exist in two-dimensions only, i.e., the x-y planes. The point-spread-function (PSF) is the expression used to describe the convolutional blurring in two-dimensions. The PSF physically results from imaging a point source. The Fourier transform of the PSF is the system

transfer-function, which is obtained by convolving the system transfer-function with a Dirac-delta function. A point source is the physical equivalent of a Dirac-delta function, and, in the frequency domain, the Dirac-delta function is a unity operator across the spectrum. Therefore, the Fourier transform of the PSF should be the Fourier transform of the aperture. However, the PSF contains noise and blurring due to other effects such as aberrations.

Achieving high resolution imaging requires the use of a high numerical aperture lens (on the order of N.A.=1.2 to 1.4 or greater). This is one of the basic problems with three-dimensional wide-field microscopy. A high numerical aperture lens, although needed to obtain high spatial resolution with adequate sensitivity, causes the undesirable effect of corrupting the data with out-of-focus light. The very large cone angle associated with a high N.A. lens results in a very limited depth-of-focus (e.g., possibly 0.4  $\mu\text{m}$  or less), see, Agard, D. A., Hiraoka, Y., Shaw, P., Sedat, J. W., *Methods in Cell Biology—Volume 30, Chapter 13 “Fluorescence Microscopy in Three Dimensions,”* San Diego, Calif., Academic Press, pp. 353–377, 1989. Consequently, such images are plagued with out-of-focus light from planes above and below the plane being imaged, resulting in reduced resolution and sensitivity of the three-dimensional wide-field data sets.

Of the aforementioned six types of aberrations (i.e., the five Seidel aberrations and the chromatic aberration), spherical aberrations are the most significant. Spherical aberrations have little effect in two-dimensional imaging but are very prevalent in three-dimensional imaging. This is due to the fact that objective lenses are designed to image at a specific plane, usually the immediate underside of the coverslip. Therefore, the other planes that are imaged show significant blurriness from spherical aberrations.

Another major contribution to blurriness is the point-spread-function (PSF). This is the actual subject of deconvolution, it is the only contributor to blurriness that is a result of a convolution. As discussed hereinbefore, PSF occurs as a separate process in each of the two-dimensional planes. The combination of these three different effects plus noise contributes to the overall blurriness in a three-dimensional data set. The filter, although called a deconvolution filter, is actually designed to filter out the effects of out-of-focus light, aberrations, PSF and noise all at the same time. The term deconvolution is somewhat of a misnomer since only the PSF blurriness requires deconvolving.

The imaging process is greatly improved by using a scanning confocal microscope, wherein a laser is illuminated through a pinhole focused on a point in an object. The point is, in turn, focused through a pinhole to a detector. The term confocal describes the fact that the illumination and detection are commonly focused to the same point in an object. The object is scanned in three-dimensions to generate the three-dimensional data set. The same lens may be used to achieve confocal conditions.

Methods for improving the resolution of the scanning confocal microscope over what can be achieved with conventional microscopy are known. By way of example, assuming a circular aperture, the intensity distribution of the main lobe is reduced in width by a factor of 1.389 compared to conventional microscopy, see, Agard, D. A., Hiraoka, Y., Shaw, P., Sedat, J. W., *Methods in Cell Biology—Volume 30, Chapter 13 “Fluorescence Microscopy in Three Dimensions,”* San Diego, Calif., Academic Press, pp. 353–377, 1989. In three-dimensions, this translates to  $(1.389)^3$  or an improvement of 2.64 in the voxel intensity