

repeatable data was required. Devising a repeatable test method allows a quantitative comparison to other methods. The basis of universal or general testing of the algorithms was to use a phantom point spread generated by an easily reproducible function. The filter performance was then measured by tracking the overall mean-square-error for every iteration. The final phase of testing involved collecting real image data of a biological cell with companion PSF data. The PSF data was used to generate the filter which was then applied to (convolved with) the image data.

The image data was converted from a TIFF format (in which such image data are typically stored) to a format that could be easily read by a C program for subsequent processing, as is known. The data was then separated into individual x-y data sets for each of the z-planes that comprised the image. This facilitates displaying the data in two-dimensions.

Several different synthesized desired data sets were used for iterating the filters. The desired data basically consisted of a 3x3x3 cube with a single voxel of maximum intensity located in the center with the remaining eight voxels of the cube at half intensity. All other voxels were set to zero intensity. Among the variations to this general scheme for establishing the desired data set were to generate it mathematically using an exponential function, e.g., the desired PSF was set by the following expression: $\text{desired}[x,y,z]=65535 \exp[-r^2]$, where r^2 is the radial distance squared away from the center. Increasing the exponent of r results in higher resolution of the restored image data.

Once convergence of the filter was achieved, various parameters were experimented with to view the effects on stability, processing speed and performance. The parameters that were varied include: initializing and stepping of μ ; the number of filter taps in x , y and z ; the number of iterations; and, varying the desired function. FIGS. 6A-AJ show a representative PSF and desired-PSF with some of the filtering results. More specifically, a series of central z-plane slices of the PSF response to a micro-sphere are shown. In actuality the point-spread occurs in x - y only with out-of-focus blur and other aberrations occurring in z . The PSF (les36psf.xxx) is 68x58x36 in x , y , and z respectively with a synthesized desired data set (desr36.xxx). Several different filters were experimented with. The results of two filters are shown here. The first one shown (ls36ftr2.xxx), is 21x21x9 taps in the filter kernel with 1200 iterations. The second filter results shown (ls36ftr4.xxx), is 21x21x21 taps with 1200 iterations. The ".xxx" indicates a corresponding plane identifier. It will be appreciated from these views that increasing the number of taps in z results in better performance in eliminating un-wanted light in the outer planes.

Testing was done by creating a blurred point-spread-function using the following equation:

$$f(x,y,z)=\exp(-(x^2+y^2+z^2)/\sigma^2) \text{ for } x,y,z \in [-0.5 \text{ to } +0.5] \quad (61)$$

where x,y,z is discretized into a 64x64x64 volume and σ is the attenuation coefficient. Two test cases were run a first with $\sigma=0.1$ and a second with $\sigma=0.5$. After numerous attempts to make the deconvolution stable for the $\sigma=0.5$ case, it was clear that the attenuation was too gradual to maintain stability in the recursive iterations. Consequently, the second test case was changed to $\sigma=0.12$. FIGS. 7A-AR show the even x - y planes for the PSF @ $\sigma=0.1$ (exp_psf1.xxx) with the corresponding filtered plane (expsf_f1.xxx) and for the PSF @ $\sigma=0.12$ (exp_psf2.xxx) with the corresponding filtered plane (expsf_f2.xxx), where ".xxx" indicates a corresponding plane identifier. The MSE was calculated and stored to a file after each iteration as the filter

was converging. The desired function for these test cases was a single voxel of maximum intensity corresponding to the center of the PSF. In this example, each iteration took approximately 4 minutes, 6 seconds. An iteration is defined as a complete pass through the 64x64x64 element data set. Technically, each filter tap is iterated for every point in the data set, or approximately 250,000 times for each complete pass. The test parameters could be modified for more rapid attenuation, which would be more representative of actual PSFs and compatible with the stability requirements for the iterative process. The C programs for deconvolving the data sets and the printouts of the convergence tracking are set forth hereinafter. The convergence tracking is show in the following order: iteration number, MAX value of filtered data, MIN value, and mean-square-error (MSE).

The data set is a three-dimensional series of cell mitochondria with a companion PSF. The PSF was generated from a 90 nm (0.09 μm) sub-resolvable fluorescent microbead. The voxel size is approximately 0.25 to 0.33 μm (0.25 μm nominal) in x , y and z . The numerical aperture of the lens is N.A.=1.4 @ $\eta=1.52$ with a 67° lens cone ½ angle. The data set size is 68x58x36 voxels. The data was filtered with an 11x11x11 kernel iterated to a desired PSF that was generated from an exponential function of r^3 . FIGS. 8A-L, 9A-R and 10A-B show acquired image data of actual cell mitochondria with companion PSF data and the filtering results. FIGS. 8A-L show a series of central z-plane slices from a three-dimensional data set of cell mitochondria. The acquired cell data are designated, cell_byt.xxx and the filtered results are designated, cell_ftr.xxx, with ".xxx" indicating a corresponding plane identifier. The filter is 11x11x11 (1331) coefficients applied to (convolved with) the image data set. FIGS. 9A-R show a series of central z-plane slices of the PSF corresponding to cell mitochondria of FIGS. 8A-L. The PSF data are shown in psf_byt.xxx, the synthesized desired data are shown in desr_psf.xxx, and the filtered results are shown in psf_ftr.xxx, where ".xxx" indicating a corresponding plane identifier. The filter is 11x11x11 (1331) coefficients iterated 1200 times through the PSF data set. The results illustrate the high performance of this method with excellent convergence to the desired data set with extremely low noise. FIG. 10A shows a filtered x - y plane of cell mitochondria designated cell_ftr.024, with all resulting negative values are set to zero (equivalent to positive ½ wave rectification). FIG. 10B shows a filtered x - y plane of cell mitochondria designated cell_str.024, which is identical to that of FIG. 10A except that it is displayed by setting the most negative value to zero intensity, or "tstretched" over the full range of intensities. FIG. 11A is a volume rendering of the original image of the cell mitochondria data shown in FIGS. 8A-L and FIG. 11B is a volume rendering of the restored image of the cell mitochondria data shown in FIGS. 8A-L. FIG. 12A is a volume rendering of the point spread function shown in FIGS. 9A-R and FIG. 12B is a volume rendering of the restored image of the point spread function shown in FIGS. 9A-R. These volume renderings show the high level of un-wanted light prior to filtering.

The results show excellent convergence in deconvolving the PSFs with no discernible noise. This could not be expected with a frequency-domain solution, an example of this is shown in FIGS. 13A and B. FIGS. 13A and B show a comparison of frequency-domain versus time-domain deconvolution of ultrasound data, FIG. 13A is a plot of a typical frequency domain deconvolution with A designating raw data and B designating deconvolved data and FIG. 13B is a plot of a time-domain deconvolution deconvolution with