Three Dimensional Deconvolution of Microscope Data

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Advances in computer processing power have enabled new three-dimensional image enhancement and visualization techniques. Data storage capacities of a gigabyte or more are affordable and commonplace. The Power Macintosh computers are RISC based processors which perform graphical and computational tasks many times faster than the older 68000 based systems. These new systems are ideal platforms for three-dimensional deconvolution of microscope data sets.

Deconvolution Algorithms

There are basically three different deconvolution methods. All of them significantly reduce out-of-focus haze in microscope data sets.

1. Single image deconvolution
2. Deconvolution using 3 images (nearest neighbor deconvolution)
3. Deconvolution using the whole volume

Single image deconvolution is used when the acquired image or images are not a volume scan. For example, researchers studying fast events must keep the image at the same focal plane and quickly capture an image. Each image is a snapshot of the specimen at a unique time sample. These images can be deconvolved by a single image deconvolution method to remove some of the out-of-focus haze.

Nearest neighbor deconvolution is useful when the specimen can be imaged along the optical axis and a series of images captured and stored to disk. The resulting data set is a volume representation of the object. The image can be deconvolved using the nearest neighbor method. In this approach, three consecutive images are used to deconvolve the middle image. The image on the top and bottom of the triplet can be thought of as windows in which out-of-focus haze from all the images above and below the processed image must pass to reach the middle image. This technique produces excellent results when:

1. The images are sampled at the proper frequency along the z axis. For large lens NAs, such as 1.4, sampling should be at 0.25 microns while for a low NA of 0.7, sampling size can be 1.0 microns.
2. The scanned volume is thin (50 microns, larger distance if sample is very transmissive).

Constrained iterative deconvolution is the third method. This method uses a whole volume during deconvolution. The mathematics is as follows:

1. Convolve a focused volume with the point spread function (PSF) to create the original acquired volume.
2. Compare the difference between the original volume and the blurred focused volume.
3. Use the error difference to correct the focused volume.
4. Go back to step one and repeat the procedure.

How do you get the first focused volume? Most procedures use the original volume as a first guess. During this iterative procedure one prevents any pixel from being negative - this is the well known positivity constraint. Differences between various constrained iterative algorithms are due to differences in step 3 where the focused volume is corrected and the rate of convergence is determined. All constrained iterative methods decrease the error computed in step 2.

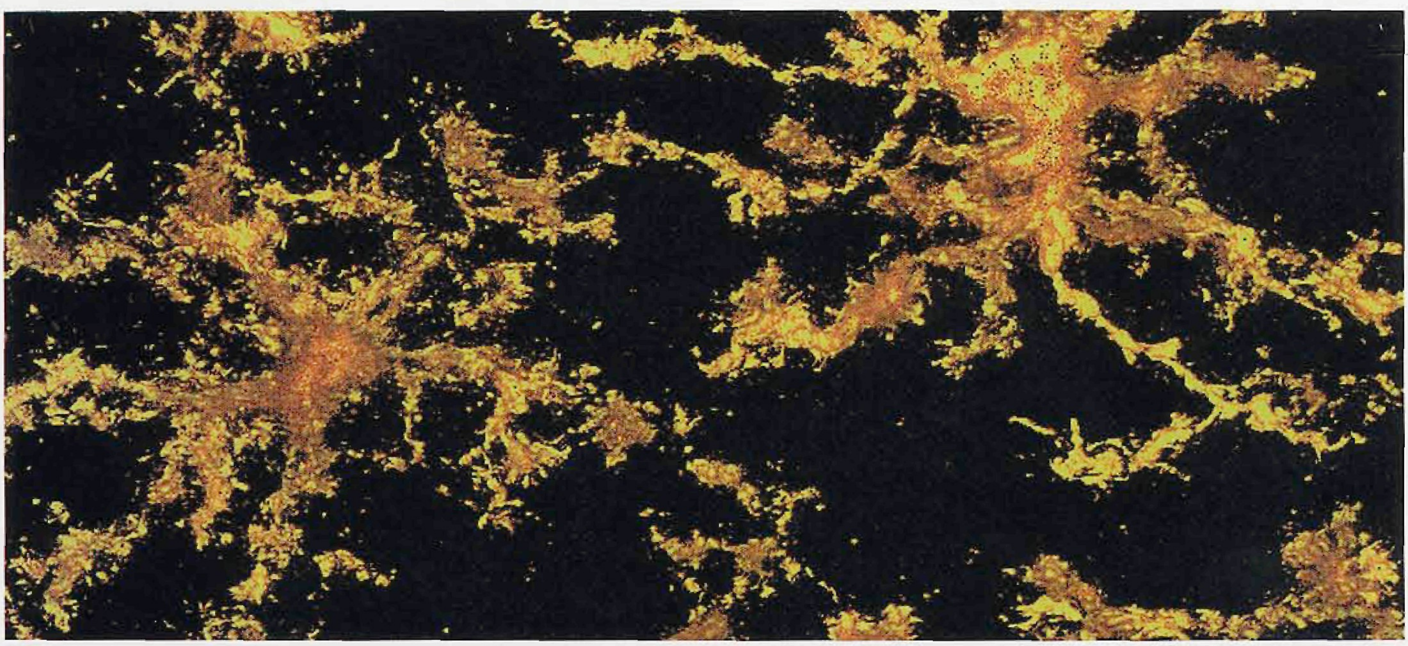
The PSF is a very important factor when using a constrained iterative method. Both theoretical and experimental deconvolution PSF's can be used. However experimental PSF determination is very difficult. Typically very small fluorescent beads about 0.2 microns in diameter are used to get a direct PSF representation. Accurate imaging of such small beads is difficult and the beads must be implanted in a medium with the same optical characteristics as the specimen for the PSF to be optimal. For experimental PSF's to be used reliably with an iterative method requiring large amounts of processing time, great care must be exercised when measuring the PSF.

Memory Requirements

For all machines, memory is an extremely important factor in determining how large a volume can be processed. To determine the required memory, three volumes must be present at one time: the original blurred volume, the 3D PSF and the deconvolved volume. For floating point representation, this means that you multiply the size of the input volume by 24 to calculate total memory requirements.

This leads to the following table:

Volume Size	Required Memory
5123	3.2 gigabytes
2563	402 megabytes
1283	50 megabytes
643	6.3 megabytes
323	785 kilobytes



Rodent microglia stained with the OX-42 antibody. Created using a 12 bit electronic camera (Photometrics), and a 63x oil immersion lens with a 1.42 numerical aperture. Out-of-focus haze removed with VayTek's MicroTome™. Background corrected and pixel values uniformly raised to reduce noise using Image-Pro™ (Media Cybernetics). Individual images assembled with VayTek's VoxBlast™ into the three dimensional composite. Image courtesy of Dr. Richard Kraig, University of Chicago, Department of Neurology.

This means that computers that deconvolve volumes must either have lots of RAM or very efficient file swapping for an effective virtual memory capability.

System Requirements

We expect RAM cost will decrease and virtual memory efficiency will increase for both Windows and Macintosh based systems. In addition to an appropriate computer platform with adequate RAM, a good three dimensional deconvolution/microscope system has the following components:

1. High quality image acquisition equipment. This may include a CCD camera and a high quality microscope.
2. Excellent microscope control for precise z stage movement and optical sectioning; control of excitation shutters to minimize photo bleaching of fluorescent specimens.

The adage "Garbage In, Garbage Out" applies to all data acquisition, but is particularly apt for 3D deconvolution of microscope data. Sophisticated mathematical algorithms cannot add what is not in the data. For collection of accurate 3D data, precise techniques must be applied at every step of the image acquisition. The new, more powerful computers with generous amount of RAM and hard disk space can provide the precision and control needed to acquire and deconvolve the quality of images needed to push scientific research forward.

Current Software

VayTek, Inc. of Fairfield, Iowa, has implemented single image, three image and constrained iterative deconvolution programs for Power Macintosh and Windows platforms. The single and three image deconvolution is based on the work of Agard & Sedat, USCD. The constrained iterative technique uses a Janson-van Cittert correction method originally developed by van Cittert (van Cittert, 1930) and modified by Jansson (Jansson, Hunt et al., 1970). All these deconvolution programs use a theoretical 3D PSF. An experimental PSF module has also been developed.

For more information about these programs and for additional educational materials regarding image acquisition and deconvolution, see VayTek's web site at http://www.vaytek.com ■

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