What should I know about the 3D Restoration module?
Improvision Technical Note No. 142
Last updated: 28 June, 2000

Topic

This technical note discusses some aspects of iterative deconvolution and how to use the Openlab 3D Restoration module for optimum results.

Discussion

What is the 3D Restoration module?

Improvision Openlab currently offers three deconvolution modules:

- Nearest Neighbour
- Volume Deconvolution
- 3D Restoration

Although each of the deconvolution techniques significantly reduces out-of-focus haze, the three techniques have very different features.

Openlab Nearest Neighbour Module

The Improvision Openlab Nearest Neighbour module uses a fast Nearest Neighbour algorithm to remove the blurring effects of the microscope optics from captured images.

Openlab Volume Deconvolution Module

The Improvision Openlab Volume Deconvolution module is an extension of the fast Nearest Neighbour algorithm which allows you to vary the number of neighbours involved in the calculation. This dramatically improves the XY resolution obtained from the Nearest Neighbour algorithm and visually approaches the XY resolution which can be obtained from a Laser Scanning Confocal Microscope.

Volume Deconvolution is a fast algorithm which does not require you to completely section your sample from top to bottom. This makes it the preferred choice when fast, single slice imaging is required over time with significant improvement in XY resolution.

Openlab 3D Restoration Module

The Improvision Openlab 3D Restoration module processes the Z stack of images as a complete data set, converting pixels to voxels. It uses a sophisticated Maximum Entropy technique to remove the blurring effects of the microscope optics from captured 3D images. The module has a number of notable advantages:

▪ The Openlab 3D Restoration module acts upon those features for which there is significant evidence in the blurred data and that are spatially distributed through the data in a way that is consistent with the microscope psf. The deconvolved image will therefore only contain features for which there is consistent evidence in the original data.

▪ The blurring due to the optics of a microscope is most severe along the optical axis. The Improvision Openlab 3D Restoration module "reassigns photons where they belong". The result of this is an increase in resolution in the z-direction.

▪ The Openlab 3D Restoration module rejects noise robustly and automatically. Noise results from the physics of all cameras, and is inevitably present in the acquired images. Noise is a limiting factor in the resolution available in all deconvolved images. The clear separation of noise from signal, to increase confidence in the resulting deconvolved 3D images, is a significant design feature of this Openlab module.

How does it work ?

The aim of this module is to produce a deconvolved image. The process begins by producing an "estimate" of the final deconvolved image. When this estimate is blurred with the microscope psf, the estimate should look like the original "raw" data but without the out-of-focus blur.

Each iteration moves the estimate in a sophisticated way gradually towards the final deconvolved image.

At the point at which there is negligable difference between the original raw data and the blurred estimate, the final deconvolved image has been achieved.

For any specific amount of error between the blurred estimate and the original images, there are an infinite number of possible deconvolved estimate images. The effect of the Maximum Entropy part of the algorithm is to choose the single smoothest image as the estimate. This is probably the deconvolved image for which there is most evidence in the original data and is one of the reasons that the module has such powerful noise rejection characteristics.

What's important during the acquisition of images?

Openlab 3D Restoration requires you to completely section the object of interest from top to bottom. We strongly recommend that you over sample the number of sections and for optimum results, capture a number of images above and below the sample. Sections should be as closely spaced as possible.
The 3D Restoration technique is susceptible to changes in the uniformity and stability of the illumination light to the sample. This can be due to poor alignment of the UV lamp

used to illuminate the sample or due to position of the arc changing between sections. It is therefore important that the microscope is set up for constant and homogeneous illumination.

It is also important that the acquired images do not contain saturated pixels as it is impossible to determine the intensity values in the subsequent deconvolution process.

How do I use the Openlab 3D Restoration module?

The performance of the 3D Restoration module is highly dependent on the amount of memory allocated to Openlab and on the number of voxels in the data set. (A voxel is a volume element.)

The amount of memory required for the module to work is calculated as

$$\text{memory (MB)} = \frac{(\text{image width} + 2) \times \text{image height} \times (\text{No. of layers} + 14) \times 16}{1 \times 10^{6}}$$

To use 3D Restoration with less than the calculated amount of memory, you will need to either add Virtual Memory (but this is the slower option) or reduce the number of voxels in the data set - this can be achieved either by cropping the document around the sample or by reducing the number of layers.

Note

1. Reducing the number of layers may compromise the performance of the module as you should ideally include as many images as possible.

2. The image depth is not a performance criteria.

If you do not have enough memory for the data set and still attempt to use the module, Openlab will display a dialogue box warning that the process is not possible.
Having acquired a series of images that you wish to deconvolve select **all** of the layers in the Layers Manager. If you do not select all of the data set the resultant deconvolution will be of sub-optimum quality.

In Openlab, select "3D Restoration" from the Image menu.
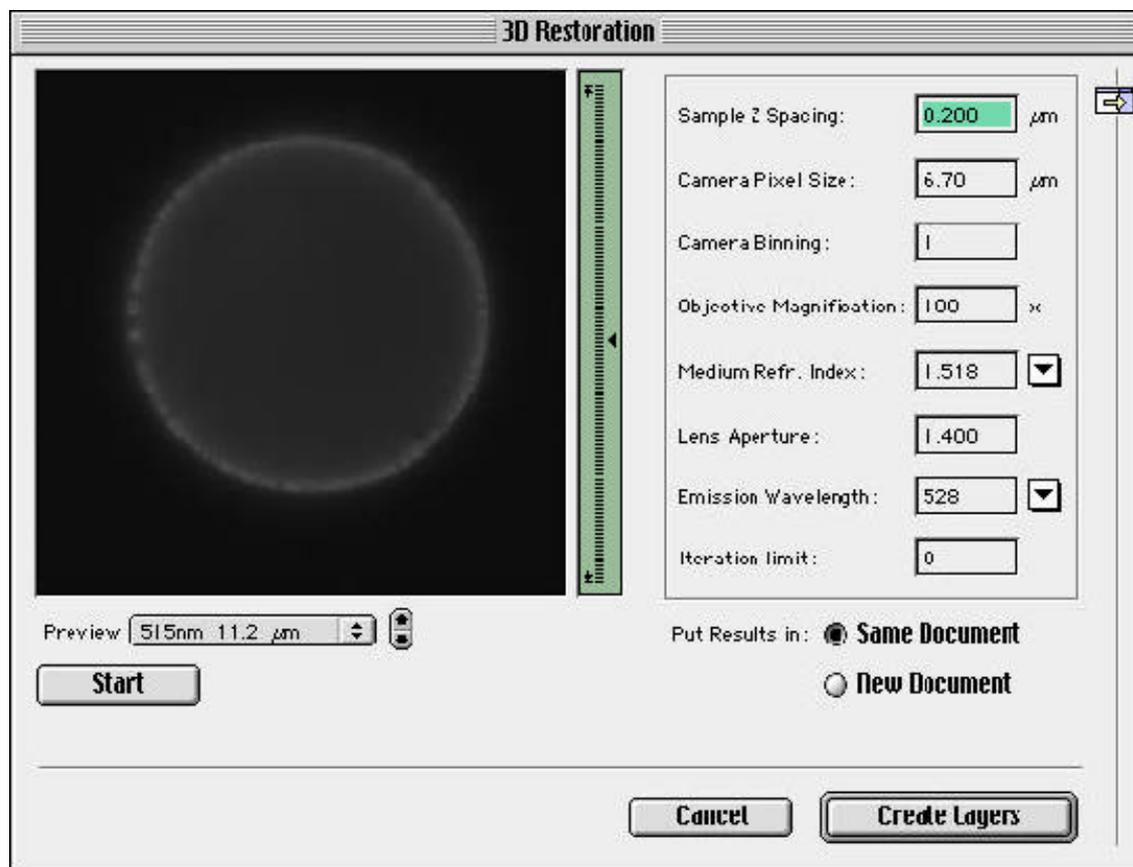
Enter the exact psf values in the appropriate boxes and enter an "Iteration limit". It is suggested that a number between 20 – 30 is used. Select "Start".

You may enter a small number for the "Iteration limit" to begin with and when these are complete, enter a final "Iteration limit". The module will then add on the appropriate extra number of iterations to reach that limit.

You may stop the process at any time by clicking once on the "Stop" button or by using z. once. The process will then stop after completion of the current iteration; using either of these options twice will cancel the stop request.

When the process has finished select the destination document for the deconvolved images from either "Same Document" or "New Document" and click on the "Create

Layers" button. All the deconvolved images have the (dci) suffix.
Why does the image after the first iteration look blurred?

The first estimate for the deconvolution is a blank image which when blurred with the psf produces a new image that appears to contain no data. However, subsequent iterations refine the estimate to produce the final deconvolved image.

Why is the image in the preview window pixelated?

The image preview window is 256 x 256 pixels. An image stack which has a smaller resolution than this will be scaled to fit the preview window. Pixelation in the preview may therefore be evident as a function of the zoom.