

## **Practical work no. 8: Deconvolution**

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### **Background**

Epifluorescence microscope: In epifluorescence microscopes, light from a lamp is reflected to the sample through an objective by a dichroic mirror. The emitted wavelengths from the sample are collected with the objective and now the dichroic mirror allows a different range of wavelengths to pass through (compared to reflected ones).

Deconvolution: Deconvolution is an image restoration method that improves contrast and increases resolution of an image set using mathematical and computational techniques. For deconvolution, reversed point spread function (PSF) of the microscope is used to identify the origin of out-of-focus blurred signal in the image, and reassign it to an in-focus location. PSF is a mathematical function that describes the distortion of light when it goes through the instrument. Every point source contributes a small area of fuzziness in the final image.

Convolution is a mathematical operation that uses the PSF of the instrument to model how the object will look like in the image. Its reversed operation (deconvolution) applies  $\text{PSF}^{-1}$  to model how the imaged object actually looks like. If PSF can be determined, it is then a matter of computing its inverse function, and applying that to the acquired image. The result is an undistorted image. Deconvolution sets certain requirements for image acquisition. The factors that must be taken care of are saturation, signal-to-noise ratio, background correction, as well as xy and z sampling frequencies.

There are several deconvolution algorithms. The more simple ones are called deblurring algorithms. They remove out-of-focus haze by filtering, thus discarding information. Image restoration algorithms utilize the out-of-focus signal, and the so-called constrained iterative algorithms can improve resolution of the image.

Also, the following criteria must be met:

- noise levels must be tolerable;
- optical alignment of the microscope must be set properly;
- optical section spacing is at optimum or oversampled. Factors that influence this are the numerical aperture of the objective, refractive index of the immersion medium, and the emission wavelength.

### **Instrument and software used:**

- Imaging Workstation I (room A503b)
- AutoQuant software

### **Short description of the work**

In this work, we will use the z-stack you previously imaged with a wide-field epifluorescence microscope (work 1). Z-stack means that you have taken images of the same sample by focusing at different levels of the object. To obtain better resolution by reassigning the blurred (out-of-focus) information, the image set is run through AutoQuant X deconvolution software.

How the results should be presented: you should show the effect of deconvolution as a side-by-side comparison where the original and deconvolved image sets are shown as maximum projections. Include also a single z-slice comparison from each image set.

## Work instructions:

1. Open your previously saved zvi-file from FILE > OPEN, or use the specific icon on the menu bar (Fig. 1).

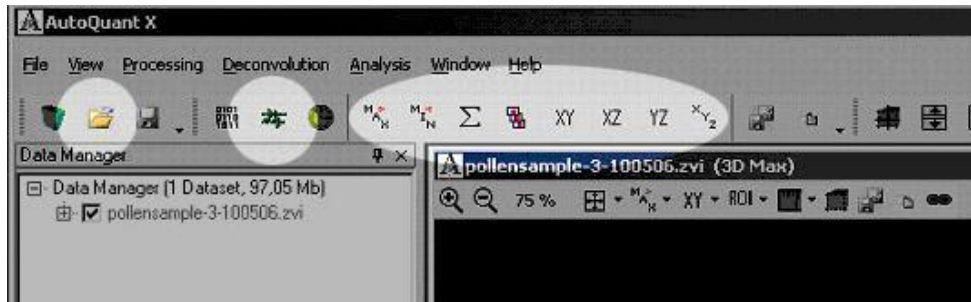


Figure 1. Selections for opening a file, display status, and alternative XYZ view modes.

2. Browse through your image set by selecting SLICE VIEWER from the main menu bar (Fig. 1). You can also choose different modes to view your data set: Max, Min, or Sum projections, Slice Viewer, and XYZviews.

3. From DECONVOLUTION menu, choose 3D DECONVOLUTION.

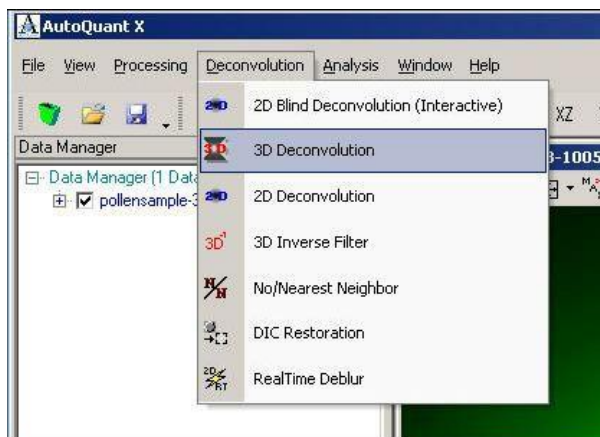


Figure 2a

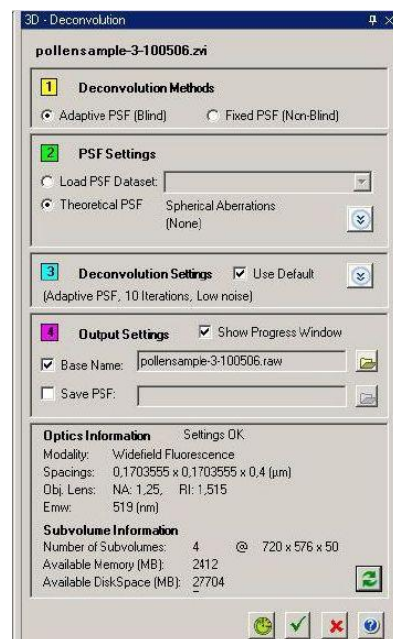


Figure 2b

a. 3D Deconvolution Menu bar appears on the right side; Adaptive PSF (blind) and Theoretical PSF should be selected (Fig. 2b). Blind deconvolution estimates PSF at the same time as it estimates the object, and can respond to PSF changes between different specimens unlike previously measured PSF. (Blind deconvolution is one form of constrained iterative algorithms.)

b. In Deconvolution Settings (Fig. 2b), the number of iterations can be adjusted downwards, if time is a critical factor (default is 10 = duration ~10 min. depending on data set). The number of iterations tells how many times the computational operations are done to each image.

c. Optical and microscope settings are imported from the zvi-file directly. You can see them in Figs. 2b and 3.

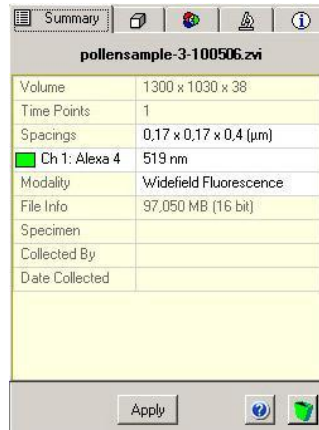


Figure 3. Optics Information

d. Press OK button to begin deconvolution.

e. The deconvolution process and the remaining time can be followed choosing VIEW – DISPLAY STATUS (Fig. 1).

4. After deconvolution is done, choose MAX PROJECTION from main menu bar (or alternatively from image window bar) (Fig. 4). The deconvoluted image has been saved automatically to the data source folder as a rawfile.

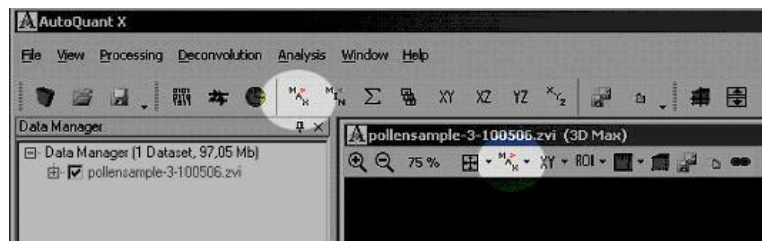


Figure 4. Selecting Maximum Projection

5. Save the deconvoluted image stack as a maximum projection presentation in tiff file format using SAVE CURRENT VIEW button (Fig. 5). Save to Images/Mcbserver1 > Workshop06 and also to your USBstick.

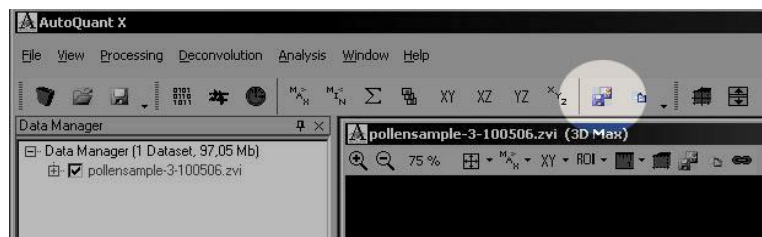


Figure 5. Save Current View button

6. Do the same maximum projection to your original image set and save it as in step 5.

**Links:**

Deconvolution theory: <http://en.wikipedia.org/wiki/Deconvolution>

AutoQuant X AutoDeblur software: <http://www.mediacy.com/aqi/index.asp>