

## **Practical work no. 6: 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). Excitation and emission filters further limit wavelengths used for excitation/detection.

Deconvolution: Deconvolution is an image restoration method that improves contrast and increases resolution of an image set using mathematical and computational techniques. For deconvolution, the reversed point spread function (PSF) of the microscope is used for identifying 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  $PSF^{-1}$  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 (lobby of room B501a)
- AutoQuant X Autodeblur software

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

In this work, we will use the z-stack you previously acquired 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.

**Be prepared to answer the questions marked in bold during the summary session.**

**Work instructions:**

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

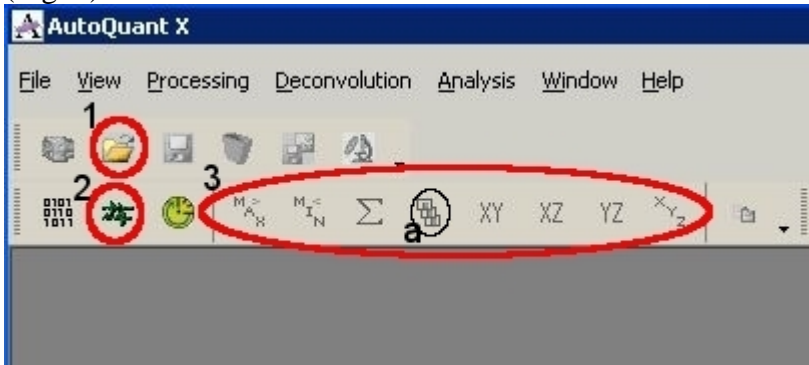


Figure 1. Selections for opening a file (1), display status (2), and alternative XYZ view modes (3).

2. Browse through your image set by selecting SLICE VIEWER from the main menu bar (Fig. 1, icon 3a). You can also choose different modes to view your data set: Max, Min, or Sum projections, and XYZ views.

3. From DECONVOLUTION menu, choose 3D DECONVOLUTION.

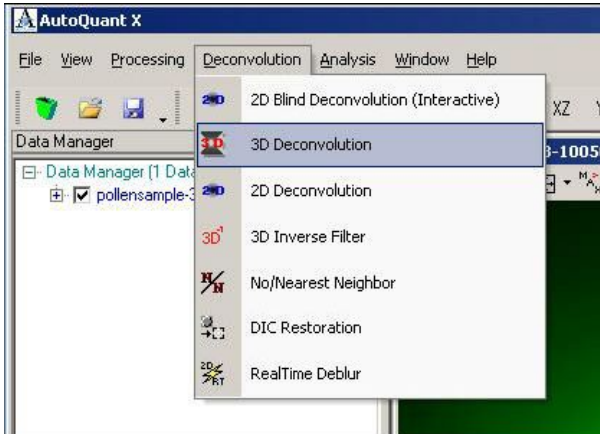


Figure 2a

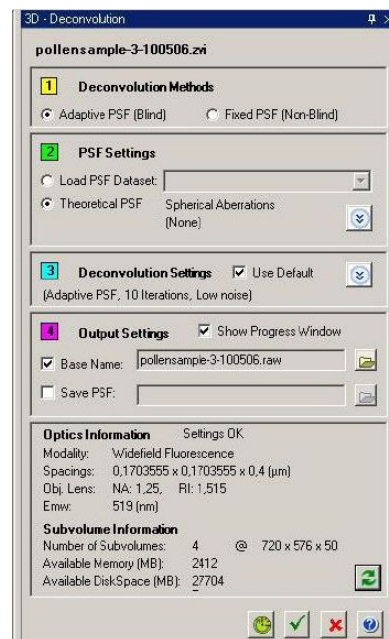


Figure 2b

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 PSF 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.

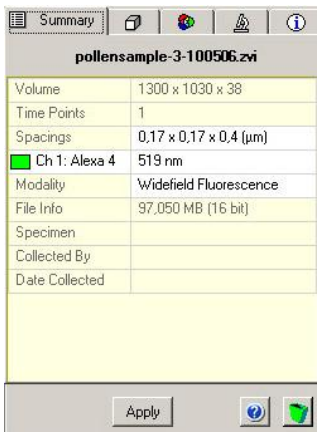


Figure 3. Optics Information

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, you can compare the before and after images side by side by viewing through the stacks. **How do these two stacks compare? What has been the effect on the fluorescent signal? How about the background?**

5. Now choose MAX PROJECTION from main menu bar (or alternatively from image window bar) (Fig. 4) for both stacks. **How do these images compare?**

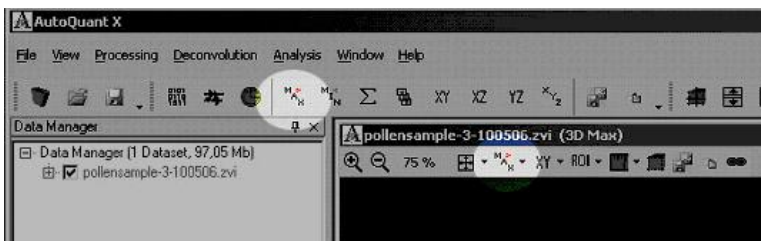


Figure 4. Selecting Maximum Projection

### Further questions for summary

Different so called filters are often used in image processing to enhance an image according to what we want to analyze or show with an image. **How does deconvolution compare with filters used for example for background subtraction or noise reduction? For what type of analyses would you use noise reduction and background subtraction filters? And when would you use deconvolution?**

### Links:

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

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