King's College London : Wohl Cellular Imaging Centre

Deconvolution using NIS-Elements

A Step by Step guide for acquisition and processing

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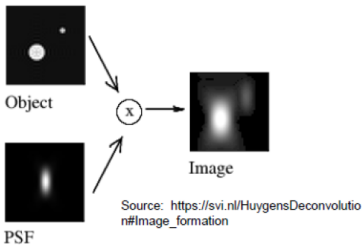
Scope

This user Guide is to describe the necessary steps for how to acquire data for and how to process data using 3D Deconvolution in NIS-Elements

What is Deconvolution and Why Do it?

- Images acquired with any optical system are *convoluted* by various factors:
  - Low S/N
  - Differences in refractive index
  - Scattering
  - Background noise
  - Imaging depth
  - Optical aberrations



- The convolution of a single point light source is described by a Point Spread Function (PSF)
- **Deconvolution** is a algorithm-based image processing technique that aims to reverse the effect of these optical distortions
- To recover the true image, a theoretical or experimental estimation of the PSF is used within the deconvolution algorithm

Figure 1 – Points of what is Deconvolution From Nikon Enhancing Confocal Resolution Document

When deconvolving data correctly you can expect an improvement in the presentation of details and contrast in your image data. This can mean that you can observe more easily the features that were present in the data and makes for a clearer interpretation of that information.

Step By Step Instructions

Step 1 – How to image your sample, so you can use deconvolution on the images afterwards.

The general rule for acquisition of 3D data for 3D deconvolution is to set the z-step distance to be smaller than the recommended minimum.

This is called Oversampling.

Correct sampling ensures that all possible points of the depth you are imaging would be adequately visible in the dataset and all known

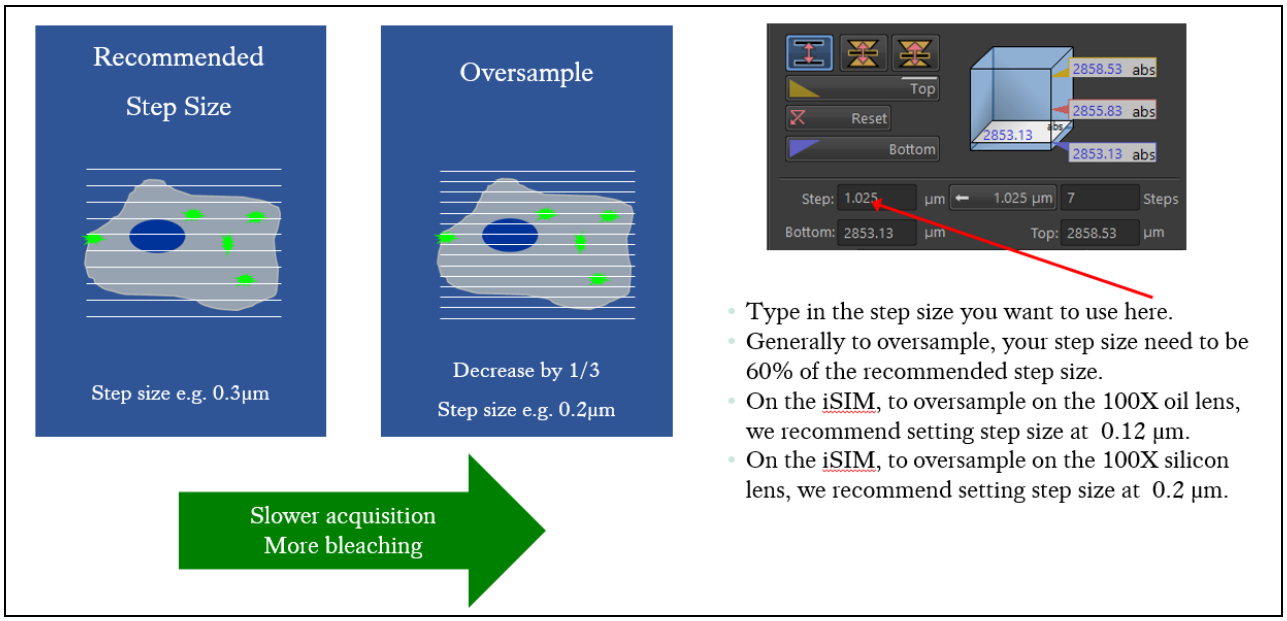


Figure 2- Comparison of Z- step size when oversampling

If using a confocal… use a small pinhole size and oversample xy as well

(for more in-depth imaging instructions, please see specific microscope user guides here - https://www.kclwcic.co.uk/instructions)

The smaller the pinhole size, the more out of focus light is eliminated, which results in a higher resolution image, but this also means, the smaller the pinhole size, the more your signal intensity will drop.

Standard pinhole size is 1AU (note: due to hexagonal pinhole shape, 1.2AU on A1 is the same area as 1 square AU on other confocal systems)

Pinhole Size (AU)	Resolution
1	1
0.8	1.10 x
0.6	1.19 x
0.4	1.28 x
0.2	1.34 x
0.0	1.40 x

Smaller pinhole improves Z-sectioning and XYZ resolution, but decreases signal intensity

Close pinhole only if sample allows it; required SNR > 1:4
Note: Beyond 0.6AU, signal intensity drops dramatically!

Try **0.8 or 0.6 AU** for best trade-off:
(and adjust z-step accordingly!)

Measurement	1 AU	0.8AU	0.6 AU	0.2 AU
Lateral PSF (resolution)	100% (200nm)	+10% (180nm)	+17% (165nm)	+25% (150nm)
Axial PSF (resolution)	100% (450nm)	+10% (410nm)	+17% (385nm)	+17% (385nm)
Signal intensity (SNR)	83% (8:1)	-15% (5:1)	-30% (3:1)	-90% (1.5:1)

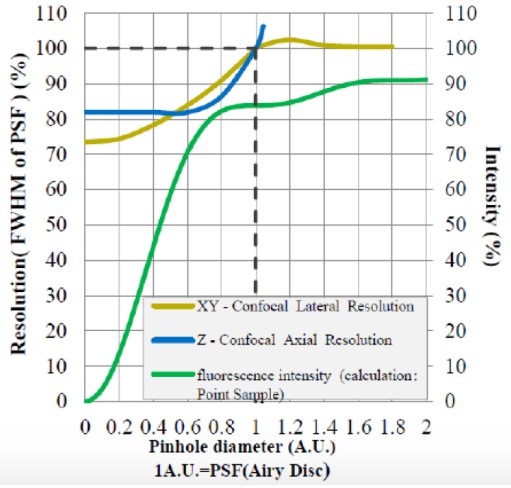
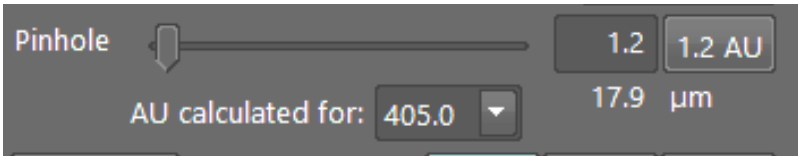


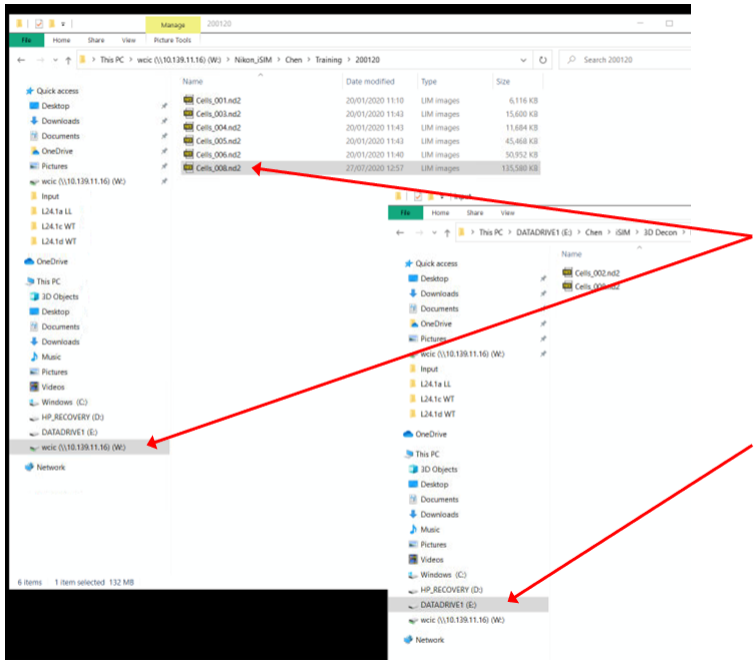
Figure 2 – Pinhole size, resolution and signal intensity



1. Select a channel you want to use to set your pinhole size.
2. Type in the pinhole size you want to use. 0.6 to 1 AU is recommended.
    a. You may find imaging this way quite a challenge!
3. Set the optical zoom to past the Nyquist XY (2.2x smaller than xy resolution) by a factor of 1/3; so about 3x the xy resolution. For excitation through a 1.40NA oil lens (100x or 60x) this would be something like 0.06-0.08µm/pixel

STEP 2: Open your image file on Workstation 1 NIS-Elements

Transfer your files onto the local computer before starting

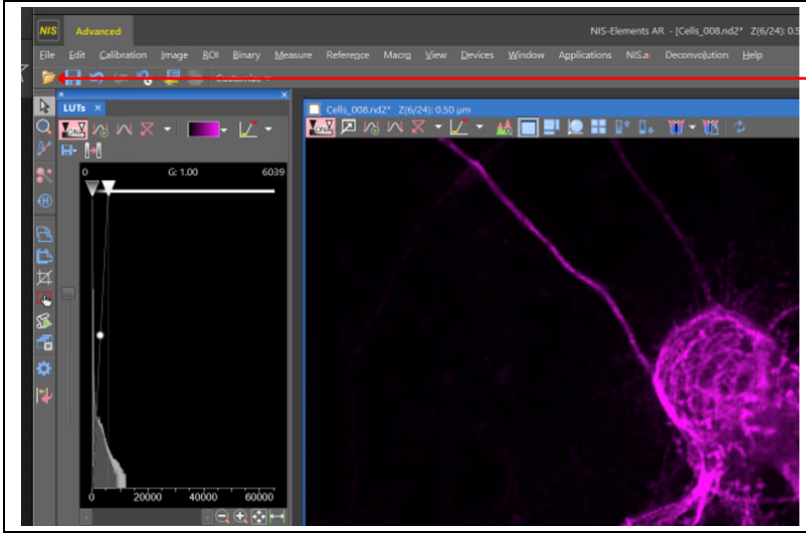


1
Login to Workstation 1

2
Select the files from their saved location, i.e. the shared network drive.

3
Copy and paste them onto the local computer, on Workstation 1, please save them in DATADRIVE1 > Your own folder

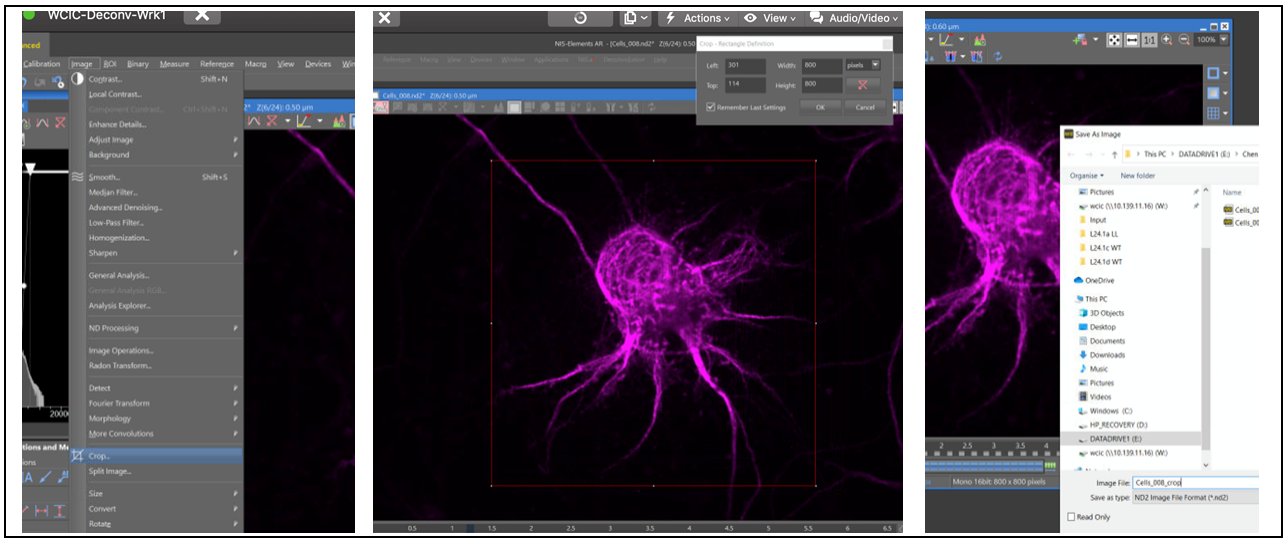
Then open your image in NIS-Elements



Open your image.

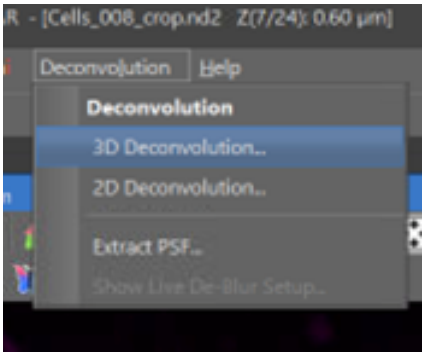
Optional STEP 3: Crop images to reduce file size

Deconvolution can take a long time and if it is only one part of the image that you actually want to use then crop the file before deconvolving it all.



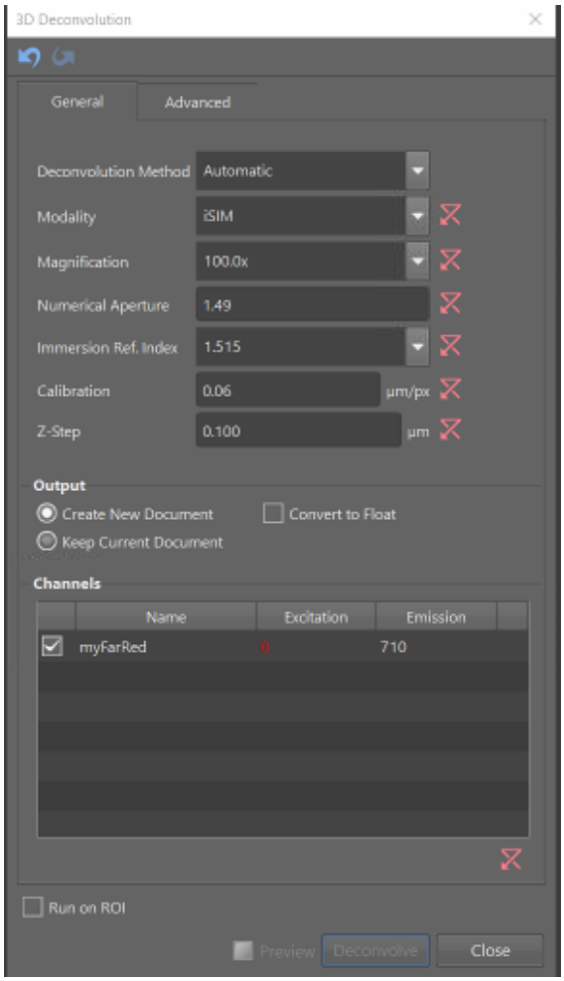
1) Choose the menu item: Image→Crop
2) Draw the rectangular region you want to crop to and right click to confirm or select OK
3) Save the cropped region as a new file

Step 4: Deconvolution



Select the menu option for 3D Deconvolution

If your data is 2D then 2D deconvolution can enhance details but has a limited amount of improvement.



If you acquired your data on a Nikon microscope and saved it as an ND2 format then most of the details in the menu will already be filled out. If you have imported some Tiff files there may be some missing details . You will need to know these to use this.

You will also need to know the emission filter middle wavelength and the excitation wavelengths used.

You will need to select the deconvolution algorithm (or method)

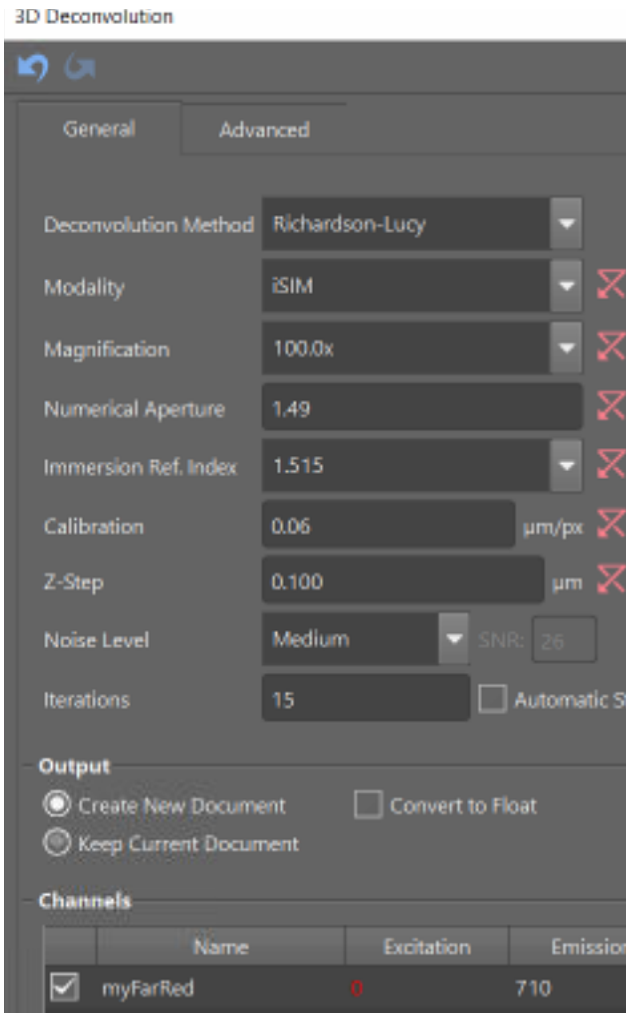
Each method has pros and cons (speed vs quality):

	Richardson-Lucy	Landweber	3D Blind
Quality	Good	Reasonable	Excellent
Speed	Good	Fastest	Reasonable
Noise Reduction	Excellent	Reasonable	Good (but may amplify noise in some cases)
PSFs	New (assumes oversampled data)	New (assumes oversampled data)	Old
Spherical Aberration correction	New	New	Old
Output	Floating Point	Floating Point	Floating Point
Comments	Stable method, usually produces good results on biological samples		Not optimized for GPU processing

- **Richardson-Lucy** usually offers the best trade-off

For ideal results it is recommended that you try to acquire data that has at least 600 integer units above the background for deconvolution – this isn't always feasible but will significantly improve the results that you obtain. Ideally 1000s above background so if your camera/detector has an option for 16bit acquisition this is recommended to activate and try to fill your histogram about ¼ to ⅓ the way up and not all the way. When you run deconvolution it will increase the intensity values and this can go past the saturation point of the 16bit range. Typically there are very few applications where the whole 16 bit range data needs to be fully utilised

If you can attain this then the Blind method will give you the best response and you will have some great looking data. Otherwise the Richardson Lucy method is better for noisier data or lower signal data (less than 600)



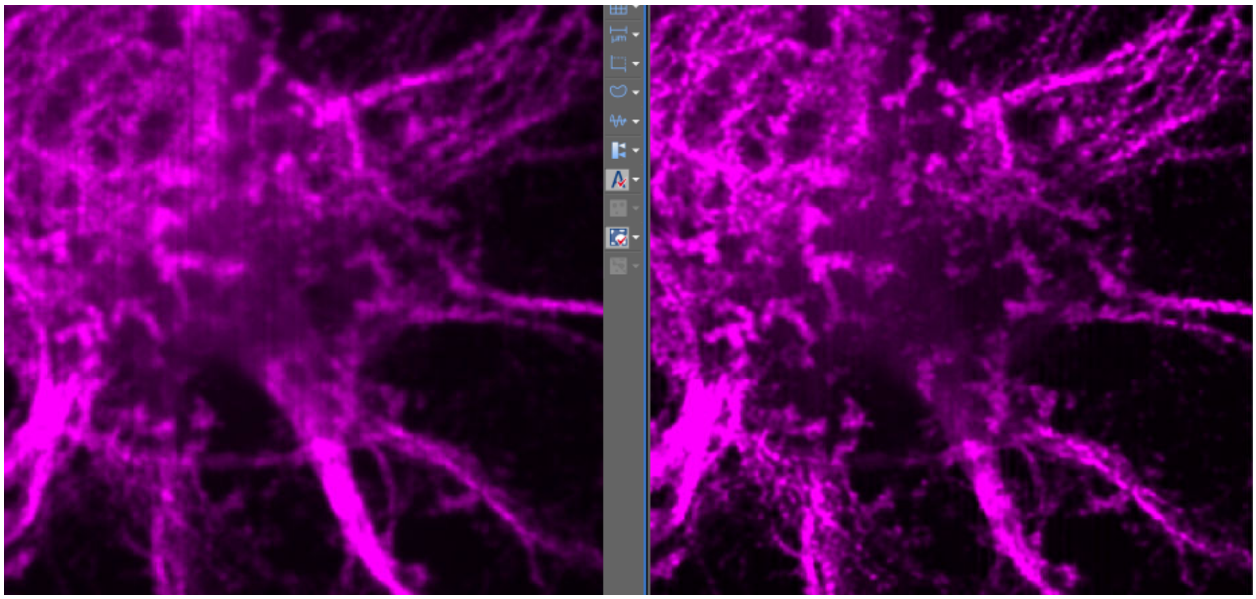
You will be required to input the estimated noise level if choosing all but the automatic deconvolution method. Aim for the lowest noise level you can. If you choose lower than the algorithm can handle it will fail to complete/stop.

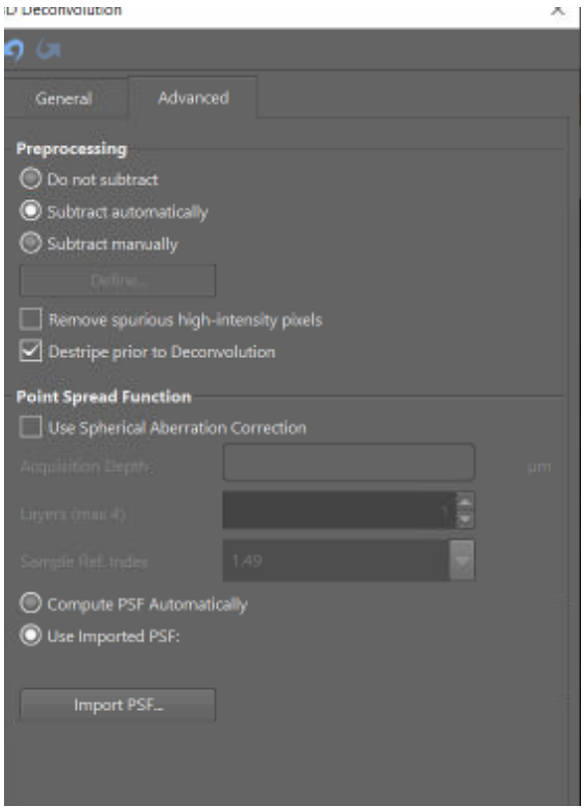
You will also be required to input the iterations that the processing will perform. Deconvolution is not a linear process and results will appear sharply when sufficient iterations are applied. However you can do too many!

Ideally try for about 15-25 iterations.

Using fewer may not provide any visible results. Too many will result in artifacts

Too many iterations (below right) will create noise artifacts which are not real!

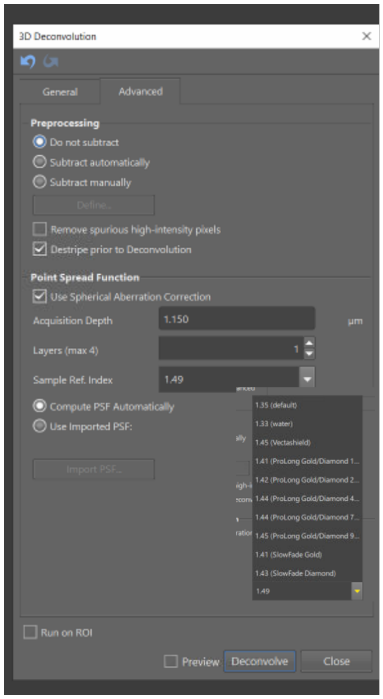




The second tab of the deconvolution window should also be checked before you use the processing – the last user may have left things differently to how you want to run them.

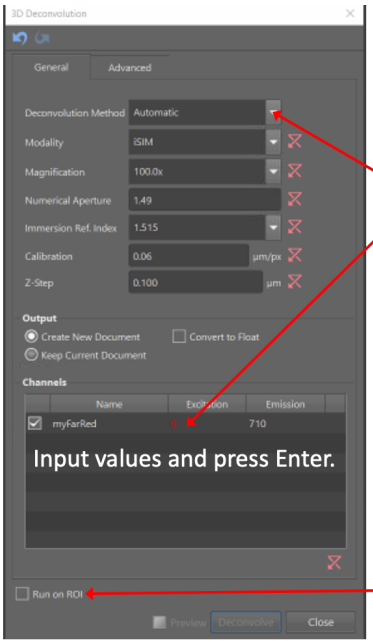
The top option of subtracting background signal can be useful in instances where a lot of background is present (epifluorescent mic for example) but is typically left to “Do Not Subtract” in most cases.

The Point Spread Function option will allow you to perform some correction for spherical aberration that may be present if you don’t match the refractive index of your sample perfectly to the immersion of the lens (1.52 for oil immersion lenses).

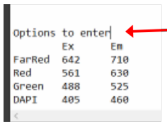


You can either leave this unchecked and not perform the correction or set the refractive index value to that of your mounting medium if you know it.

For images taken on the iSIM

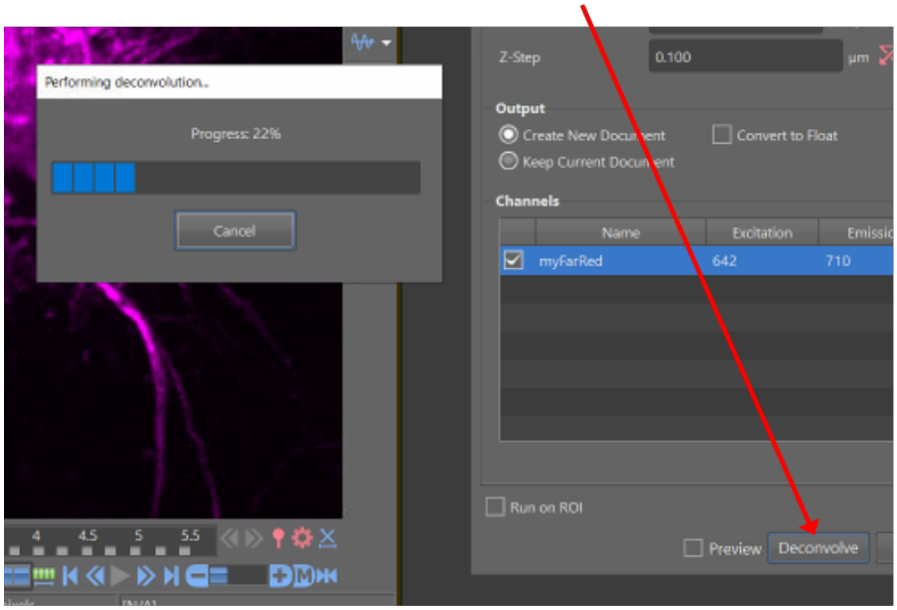


3D deconvolution set up, you need to select the deconvolution method (**STEP 4**) and input the excitation and emission wavelength if you used the iSIM microscope. Everything else is automatically selected for you based on the information collected during your imaging such as lens details and Z-step size.



iSIM wavelengths can be found in:
WCIC shared drive > Nikon_iSIM > filers iSIM_WCIC

You can either cropping your image or selecting regions and interest (ROI). I prefer to crop image, but if you do use ROI, please check this box.

And finally…

Click on Deconvolve