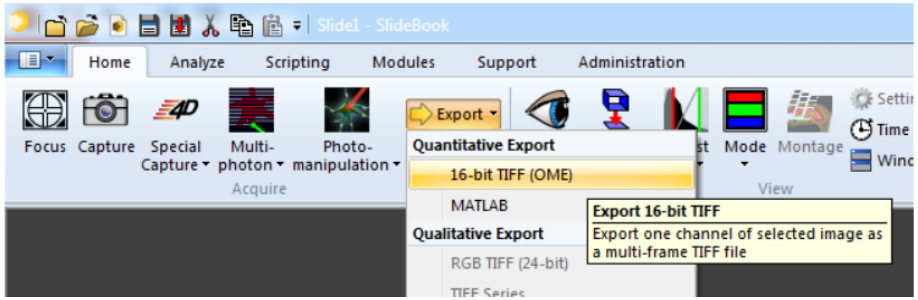
Steps for Deconvolution processing of diSPIM data with Huygens (SPIM, Deconvolution & Fusion) software.

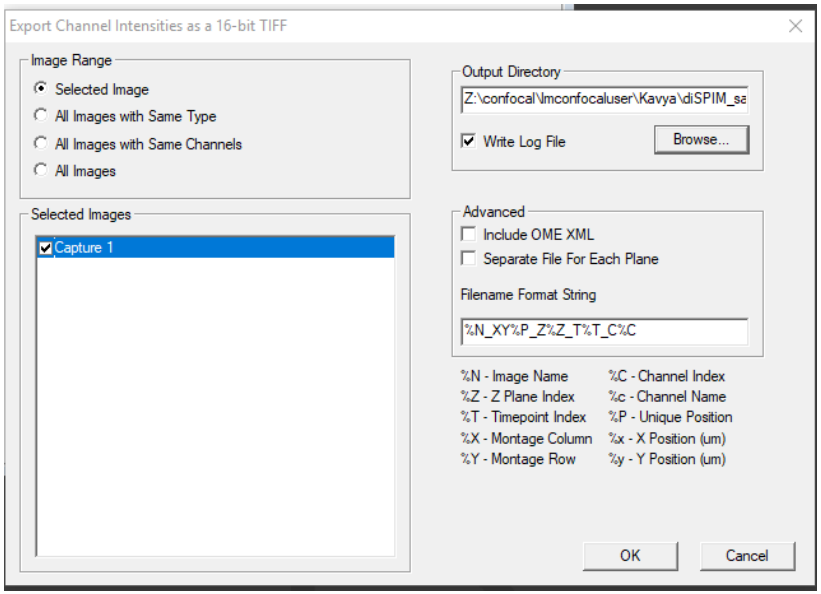
The following steps are applicable for islice data (slice scan – single channel acquisition)

1) **Export of raw Data from Slidebook:**

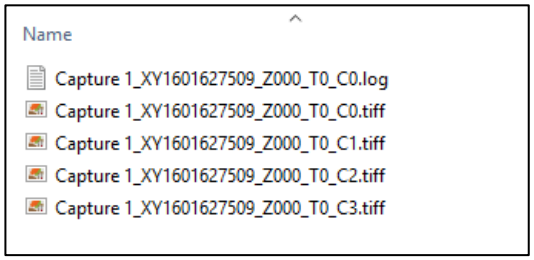
i. Go to Home> Export>16-bit TIFF.



ii. Select the default options and choose the folder (kraken\confocal\lmconfocaluser\...) you would want to export to.



iii. The exported files consist of .tiff files for each channel, and a .txt file with exported data log.

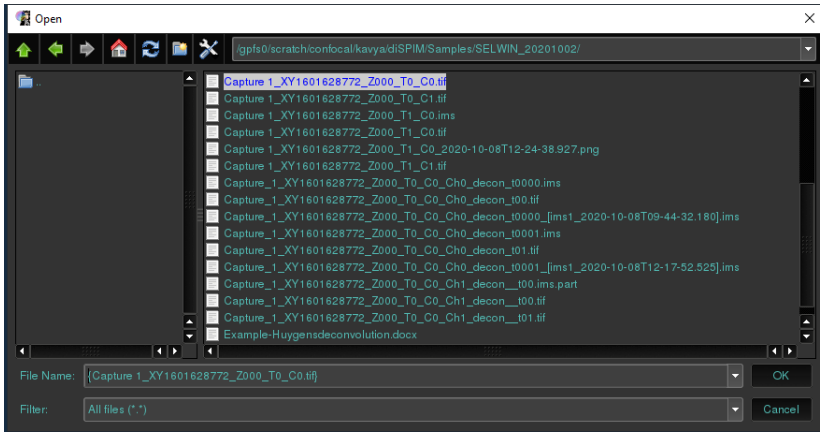


2. Processing Deconvolution with Huygens Software:

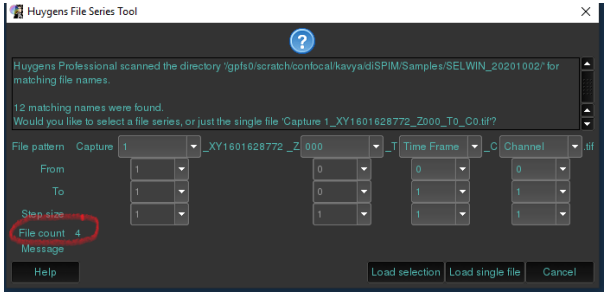
a) Importing files

i. File -> Open

Select the first channel Tiff file exported (**C0.tif**).

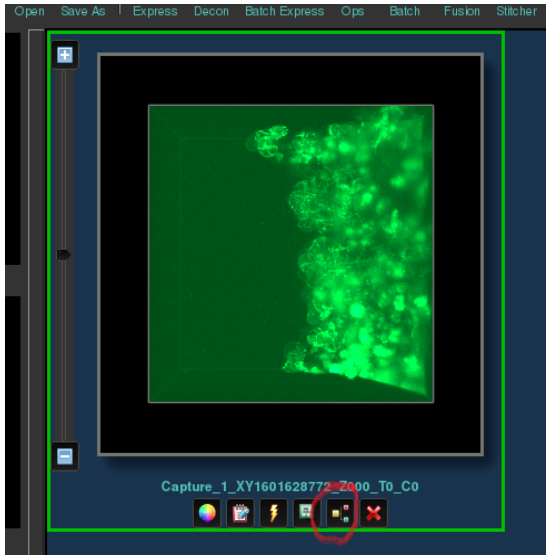


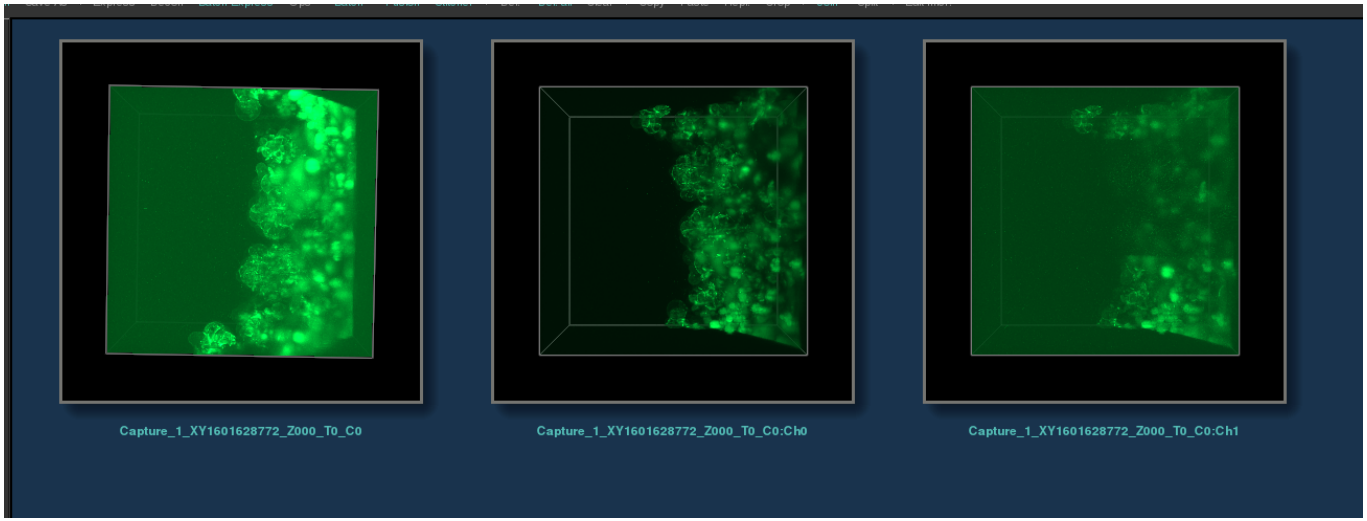
The first channel file C0.tif is a **stack image** that is the image sequence of all the images under the capture file exported.



Click ‘Load selection’ to load all the files in the stack

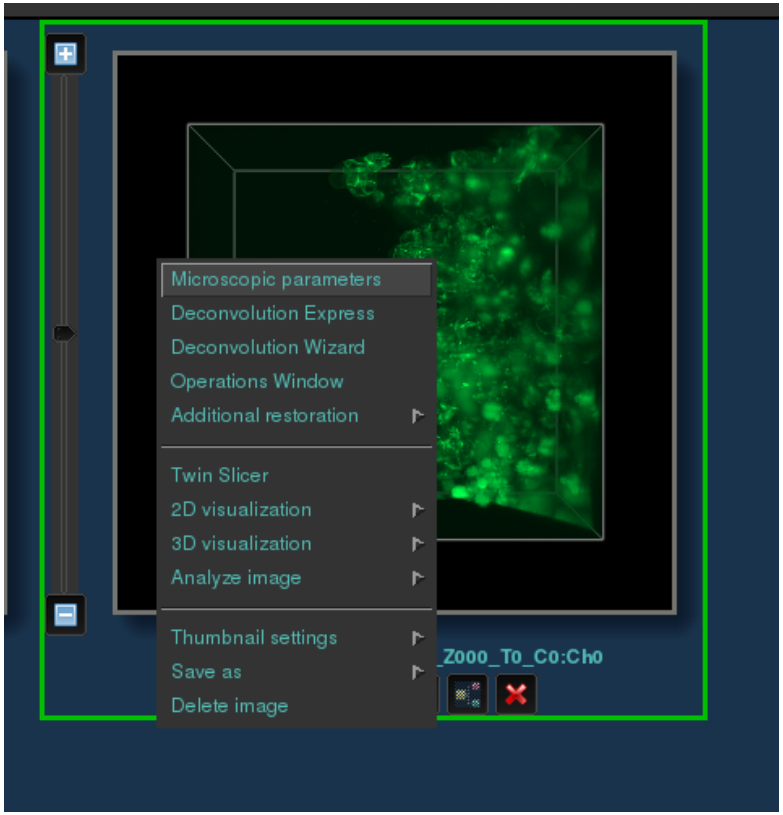
ii. After the image stack is opened, click the ‘Spilt channels’ icon for splitting of the images (channels) for processing





b) Set microscope parameters for the image according to the acquisition protocol.

i. Right click on the image and select ‘Microscopic parameters’



ii. Enter the parameters based on the acquisition setup. Information for the particular channel can be found in the .txt file (X, Y, Z sampling intervals and wavelength used).

For diSPIM, X and Y sampling interval = 162.5nm

Z sampling interval as set in experiment (found in .txt file). When acquistion ensure z sampling interval <480nm to match Nyquist criterion of sampling

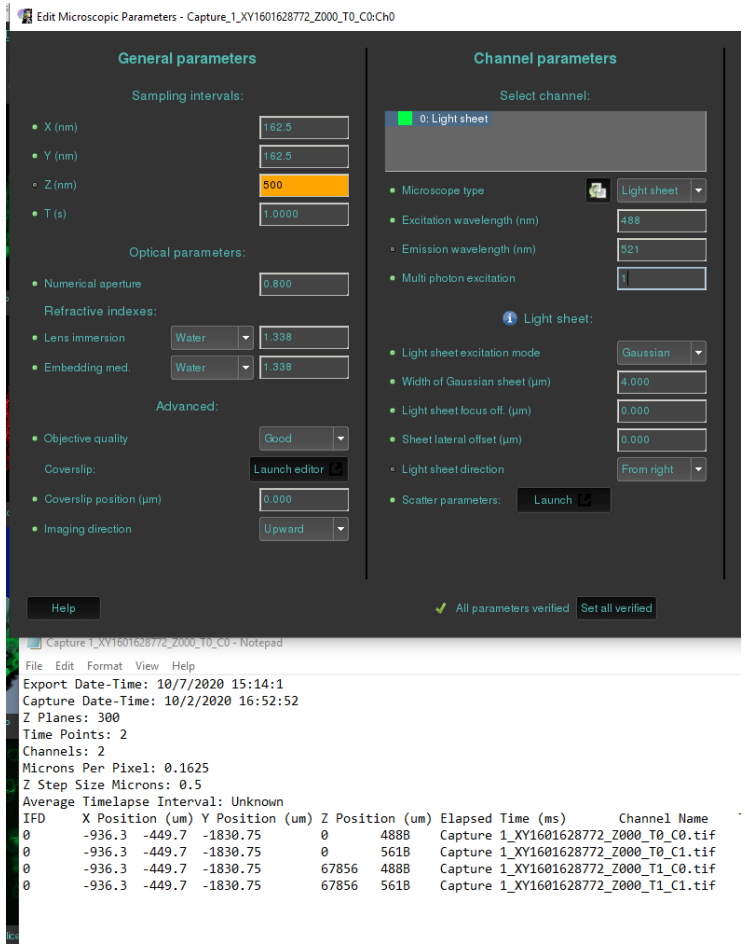
For excitation wavelength: 405nm emission wavelength to be set: 440nm

For excitation wavelength: 488nm emission wavelength to be set: 521nm

For excitation wavelength: 561nm emission wavelength to be set: 601nm

For excitation wavelength: 640 nm emission wavelength to be set: 700nm

Scattering parameters can be set to 'No scattering' for thin samples without much scattering effect of light.

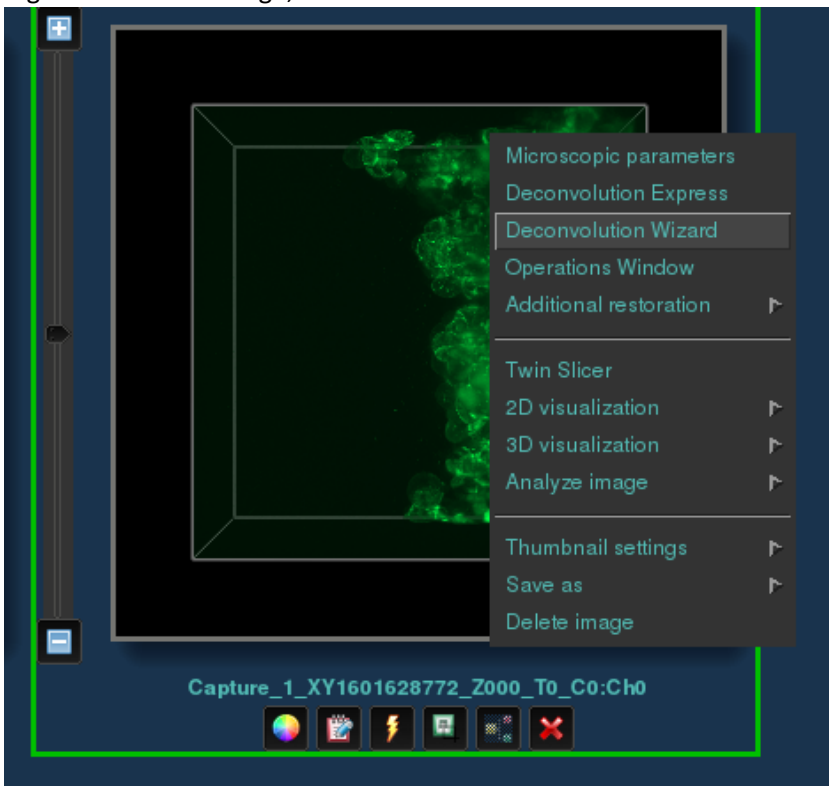


CHANNEL A: LIGHT SHEET DIRECTION – LEFT

CHANNEL B: LIGHT SHEET DIRECTION - RIGHT

c) Using deconvolution wizard

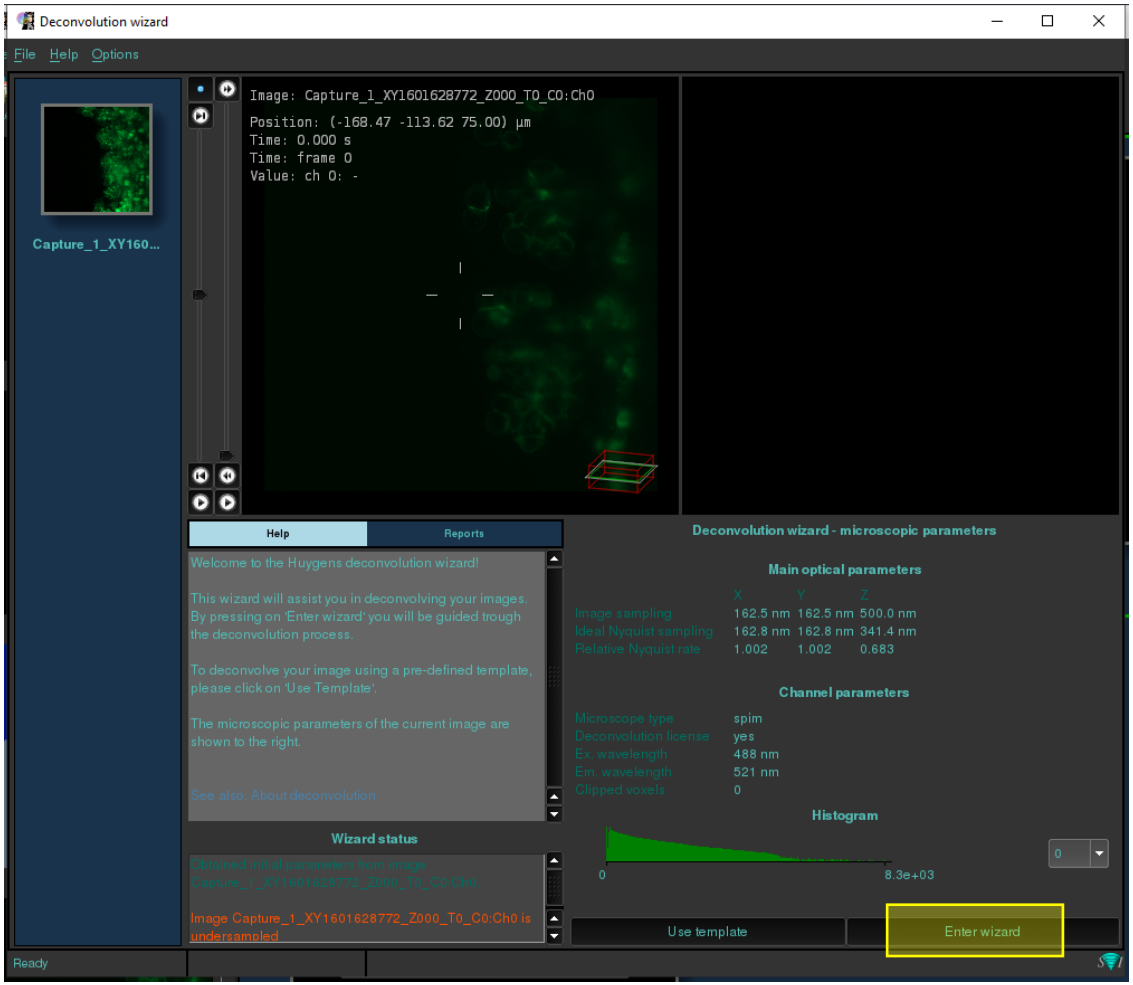
i. Right click on the image, select ‘Deconvolution wizard’



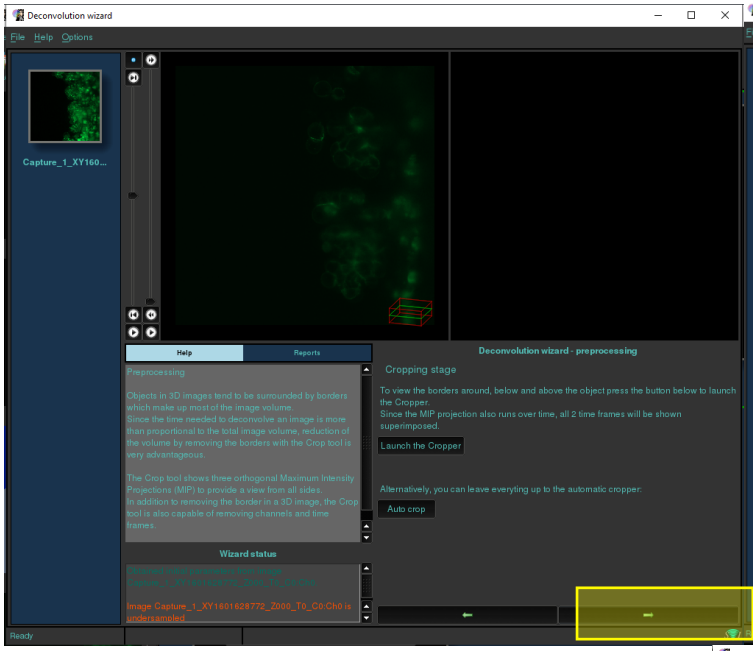
*Note: For stage scans additional preprocessing are required before deconvolution

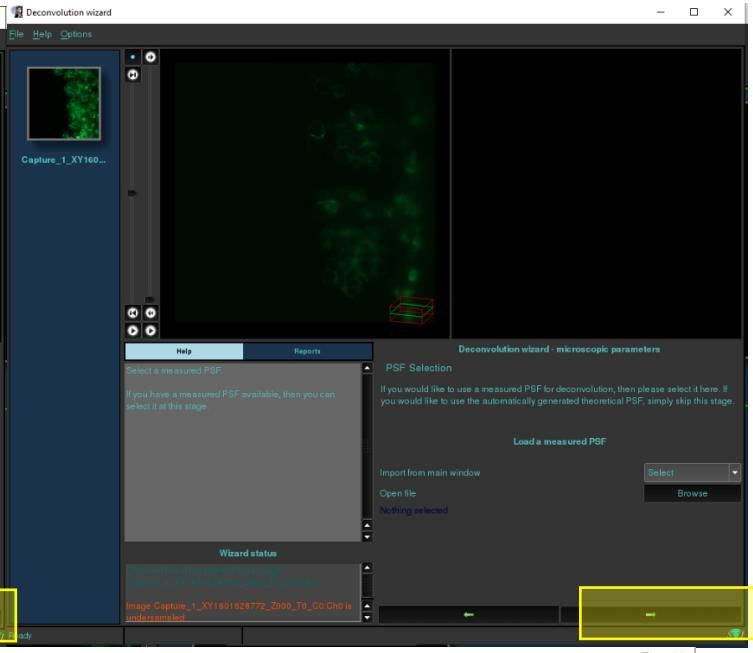
ii. Go through the deconvolution wizard steps. Theoretical PSF is used by default for the deconvolution. Check the microscopic parameters set for the image (X, Y, Z sampling, wavelength information etc.)

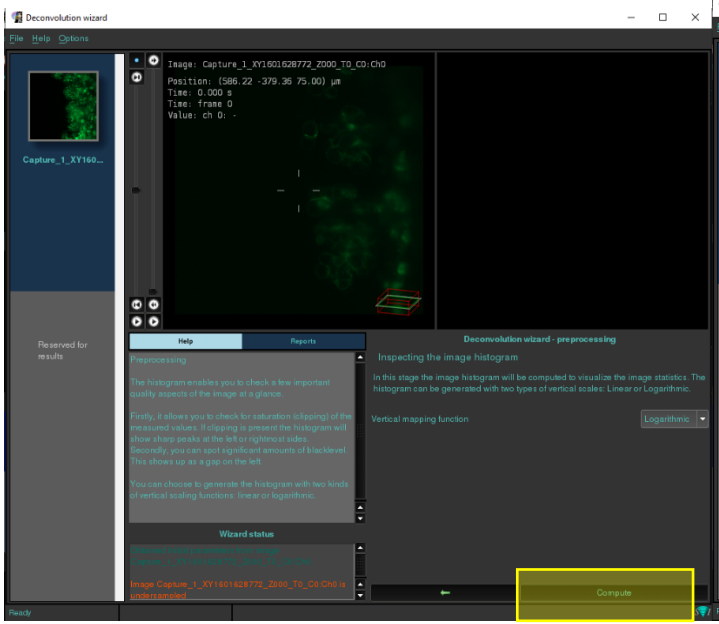
Enter wizard

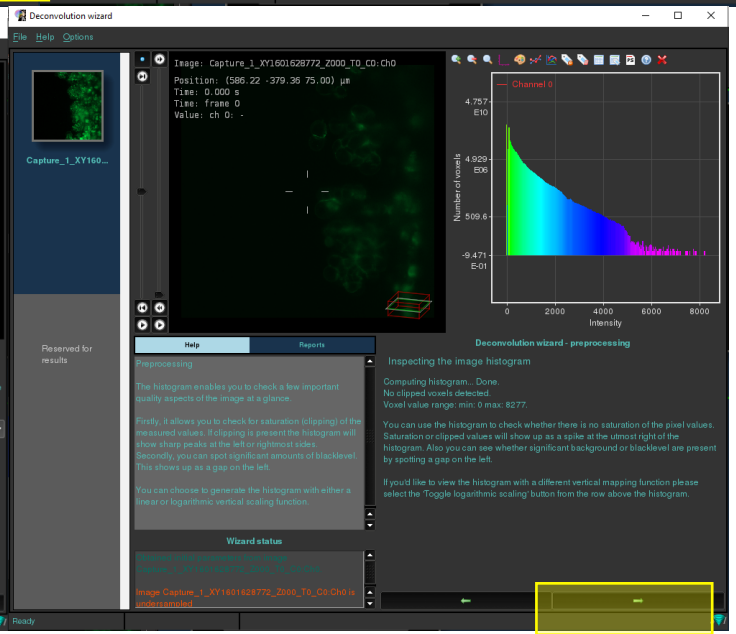


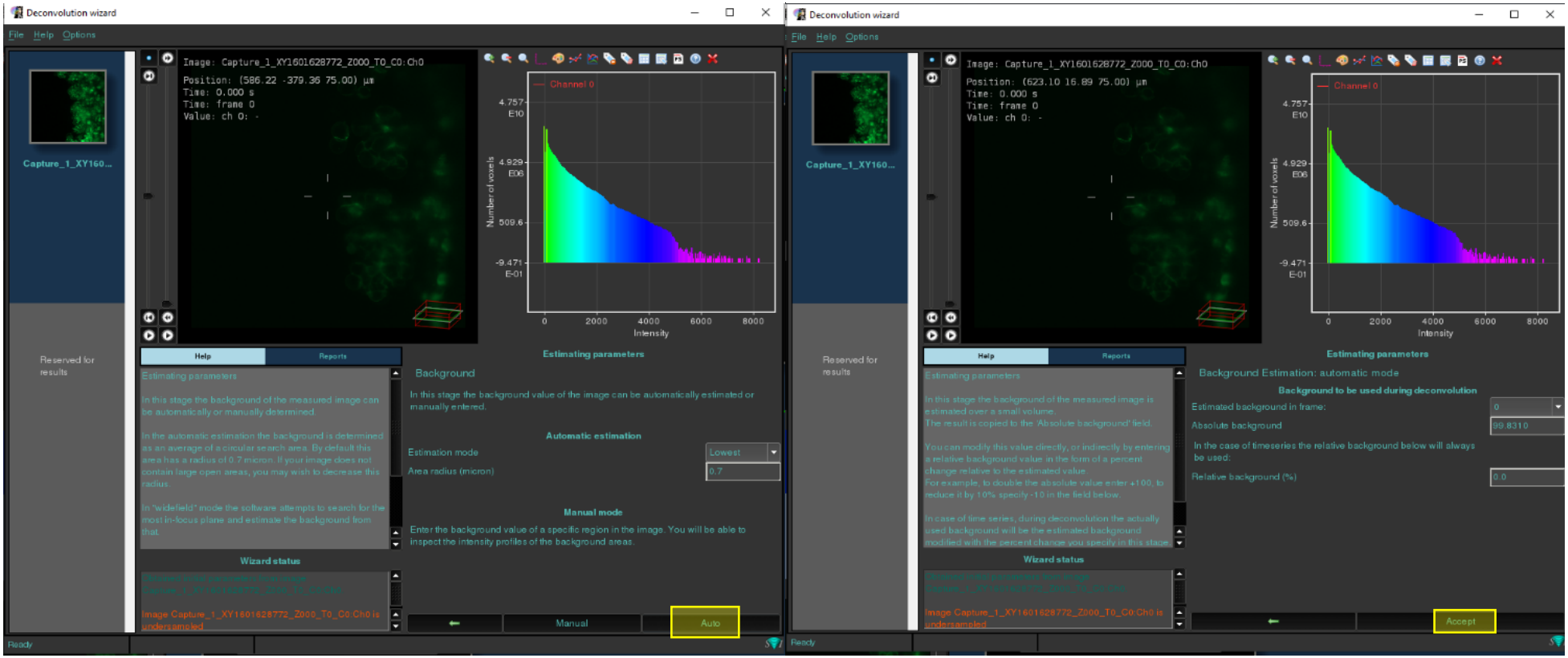
Follow the default steps highlighted in yellow.

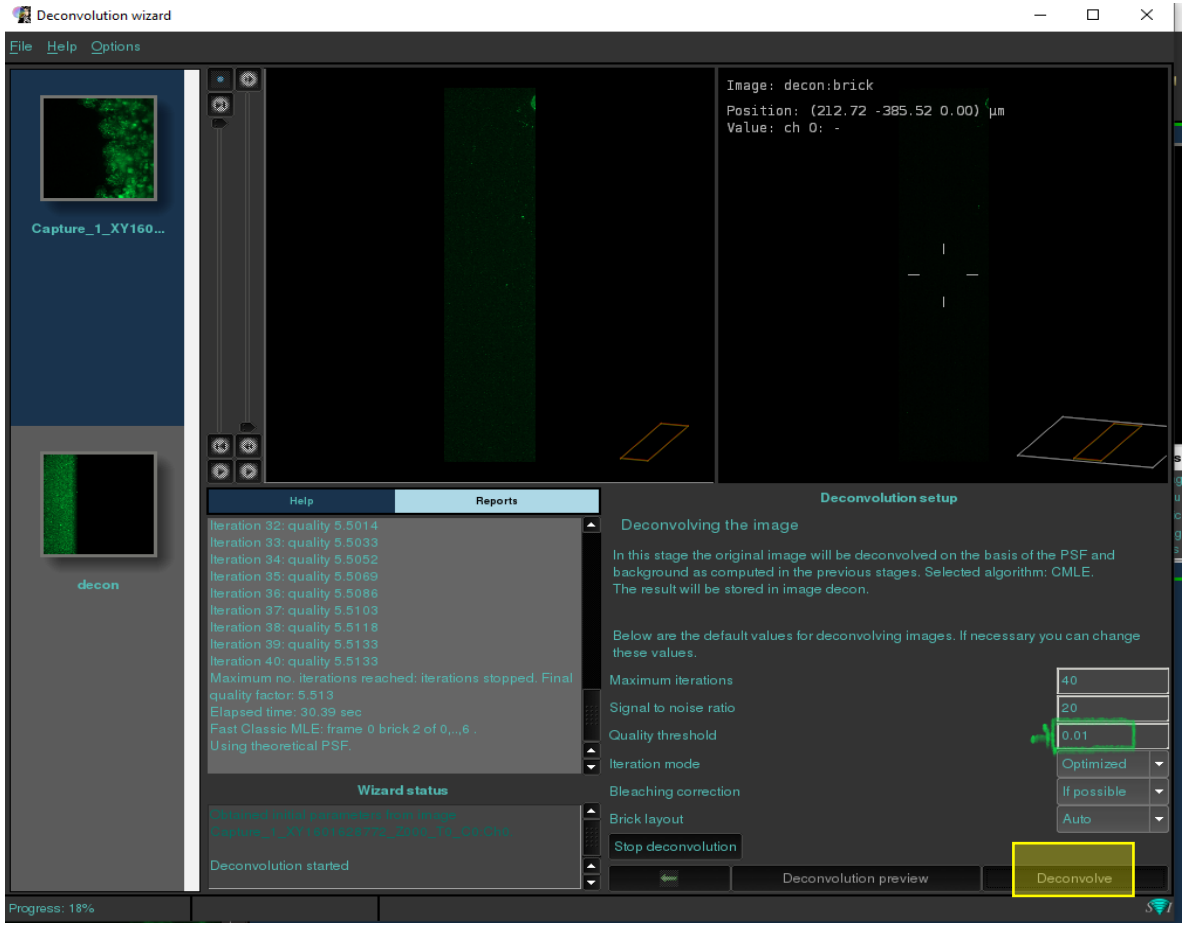

Deconvolution wizard
File Help Options
Capture_1_XY160...
Help
Reports
Deconvolution wizard - preprocessing
Cropping stage
To view the borders around, below and above the object press the button below to launch the Cropper.
Since the MIP projection also runs over time, all 2 time frames will be shown superimposed.
Launch the Cropper
Alternatively, you can leave everyting up to the automatic cropper:
Auto crop
Wizard status
Image Capture_1_XY1601628772_Z000_T0_C0:Ch0 is undersampled
Ready


Deconvolution wizard
File Help Options
Capture_1_XY160...
Help
Reports
Deconvolution wizard - microscopic parameters
PSF Selection
If you would like to use a measured PSF for deconvolution, then please select it here. If you would like to use the automatically generated theoretical PSF, simply skip this stage.
Load a measured PSF
Import from main window
Select
Open file
Browse
Nothing selected
Wizard status
Image Capture_1_XY1601628772_Z000_T0_C0:Ch0 is undersampled
Ready


Deconvolution wizard
File Help Options
Image: Capture_1_XY1601628772_Z000_T0_C0:Ch0
Position: (586.22 -379.36 75.00) µm
Time: 0.000 s
Time: frame 0
Value: ch 0: -
Capture_1_XY160...
Reserved for results
Help
Reports
Deconvolution wizard - preprocessing
Inspecting the image histogram
In this stage the image histogram will be computed to visualize the image statistics. The histogram can be generated with two types of vertical scales: Linear or Logarithmic.
Vertical mapping function
Logarithmic
Wizard status
Image Capture_1_XY1601628772_Z000_T0_C0:Ch0 is undersampled
Compute
Ready


Deconvolution wizard
File Help Options
Image: Capture_1_XY1601628772_Z000_T0_C0:Ch0
Position: (586.22 -379.36 75.00) µm
Time: 0.000 s
Time: frame 0
Value: ch 0: -
Capture_1_XY160...
Reserved for results
Channel 0
4.757 E10
4.929 E06
509.6
-9.471 E-01
Number of voxels
0
2000
4000
6000
8000
Intensity
Help
Reports
Deconvolution wizard - preprocessing
Inspecting the image histogram
Computing histogram... Done.
No clipped voxels detected.
Voxel value range: min: 0 max: 8277.
You can use the histogram to check whether there is no saturation of the pixel values. Saturation or clipped values will show up as a spike at the utmost right of the histogram. Also you can see whether significant background or blacklevel are present by spotting a gap on the left.
If you'd like to view the histogram with a different vertical mapping function please select the 'Toggle logarithmic scaling' button from the row above the histogram.
Wizard status
Image Capture_1_XY1601628772_Z000_T0_C0:Ch0 is undersampled
Ready

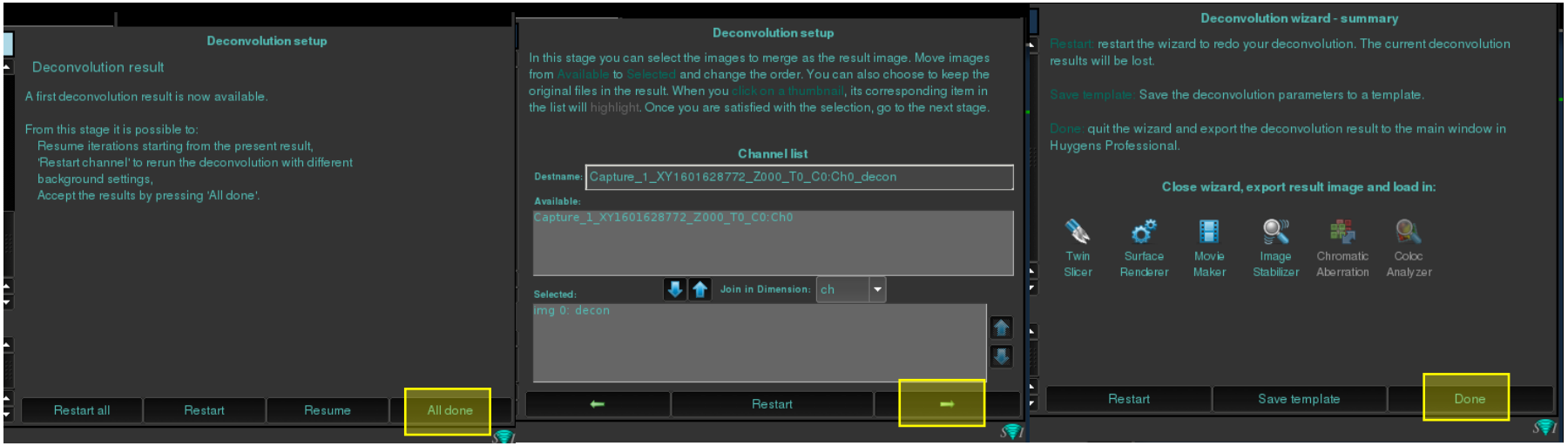

Deconvolution wizard
File Help Options
Capture_1_XY160...
Reserved for results
Image: Capture_1_XY1601628772_Z000_T0_C0:Ch0
Position: (586.22 -379.36 75.00) µm
Time: 0.000 s
Time: frame 0
Value: ch 0: -
Channel 0
Number of voxels
Intensity
Help
Reports
Estimating parameters
In this stage the background of the measured image can be automatically or manually determined.
In the automatic estimation the background is determined as an average of a circular search area. By default this area has a radius of 0.7 micron. If your image does not contain large open areas, you may wish to decrease this radius.
In "widefield" mode the software attempts to search for the most in-focus plane and estimate the background from that.
Wizard status
Image Capture_1_XY1601628772_Z000_T0_C0:Ch0 is undersampled
Background
In this stage the background value of the image can be automatically estimated or manually entered.
Automatic estimation
Estimation mode
Lowest
Area radius (micron)
0.7
Manual mode
Enter the background value of a specific region in the image. You will be able to inspect the intensity profiles of the background areas.
Manual
Auto
Ready
Deconvolution wizard
File Help Options
Capture_1_XY160...
Reserved for results
Image: Capture_1_XY1601628772_Z000_T0_C0:Ch0
Position: (623.10 16.89 75.00) µm
Time: 0.000 s
Time: frame 0
Value: ch 0: -
Channel 0
Number of voxels
Intensity
Help
Reports
Estimating parameters
In this stage the background of the measured image is estimated over a small volume.
The result is copied to the 'Absolute background' field.
You can modify this value directly, or indirectly by entering a relative background value in the form of a percent change relative to the estimated value.
For example, to double the absolute value enter +100, to reduce it by 10% specify -10 in the field below.
In case of time series, during deconvolution the actually used background will be the estimated background modified with the percent change you specify in this stage.
Wizard status
Image Capture_1_XY1601628772_Z000_T0_C0:Ch0 is undersampled
Background Estimation: automatic mode
Background to be used during deconvolution
Estimated background in frame:
0
Absolute background
99.8310
In the case of timeseries the relative background below will always be used:
Relative background (%)
0.0
Accept
Ready



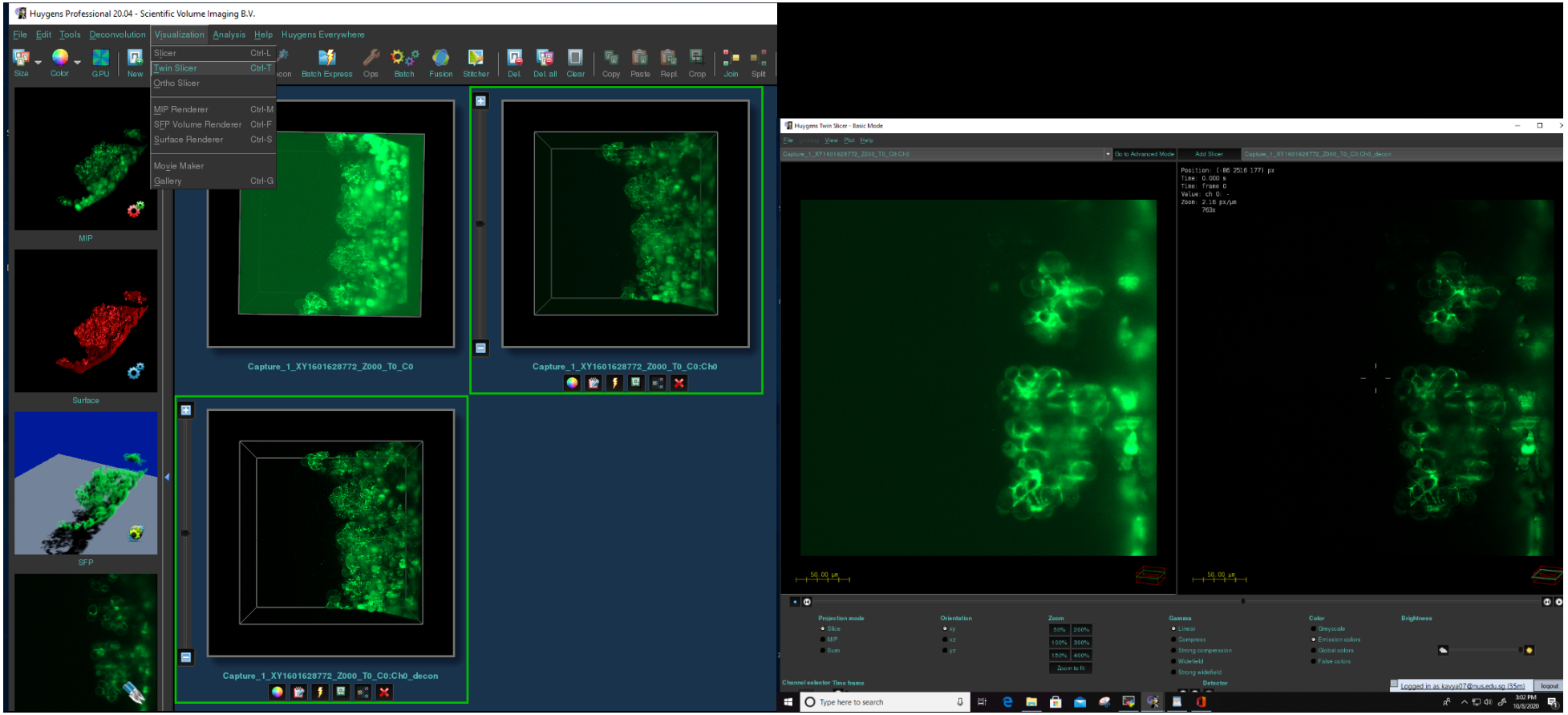
Decrease the quality threshold to 0.01 for better quality of deconvolution.

Other default values are suitable for the sample data.

For further improvisation, increase maximum iteration number and/or change iteration mode to ‘Classic’



Name the deconvolved file.



To compare the two images before and after deconvolution, select the two images.

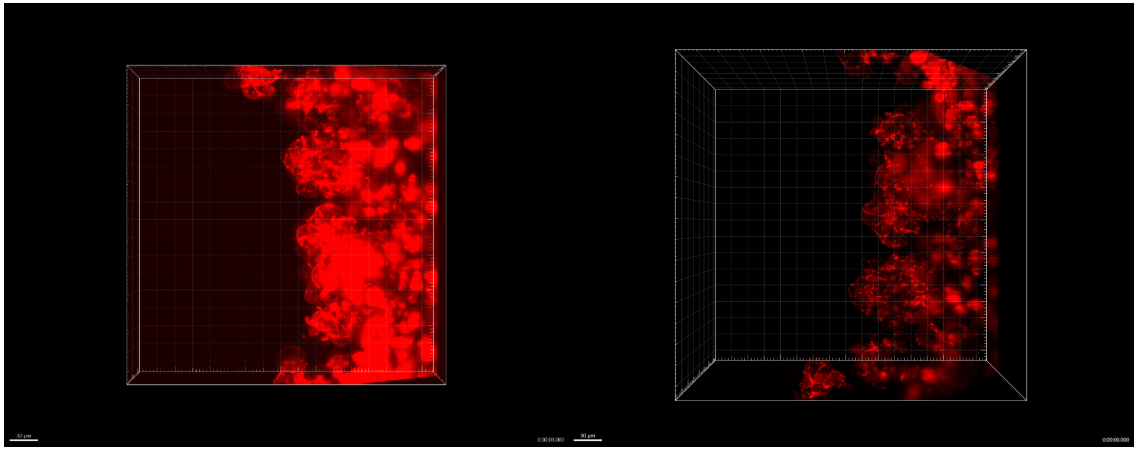
Visualization -> Twin slicer

Comparison after deconvolution can be visualized with win slicer.

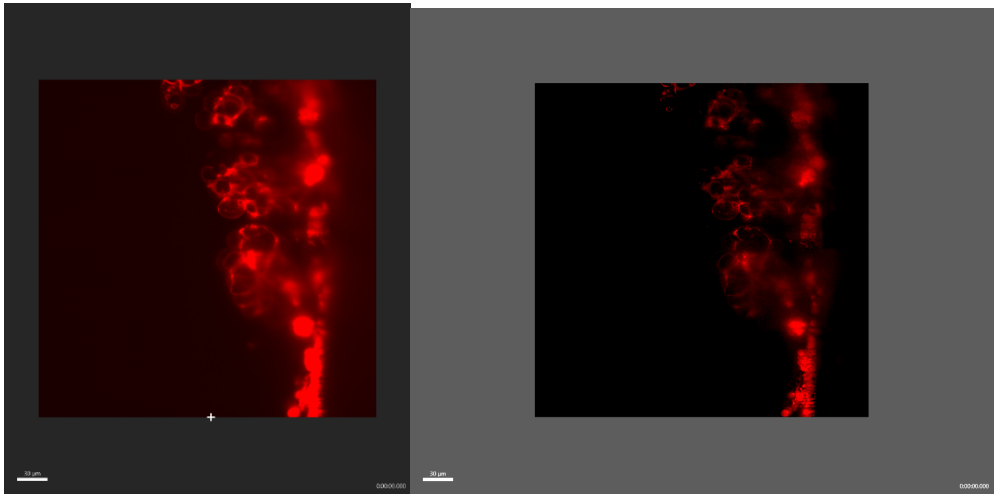
If deconvolution result is satisfactory, save the file.

Example image Deconvolved

3-D volume view



Slice view



Note: The above steps are suitable for islice scans image prcessing with dispim.
Additional steps
For dislice (slice scans with two orthogonal views) fusion after deconvolution will need to be performed
For istage/ distage scans deskewing of data will need to be performed additionally as pre-processing.