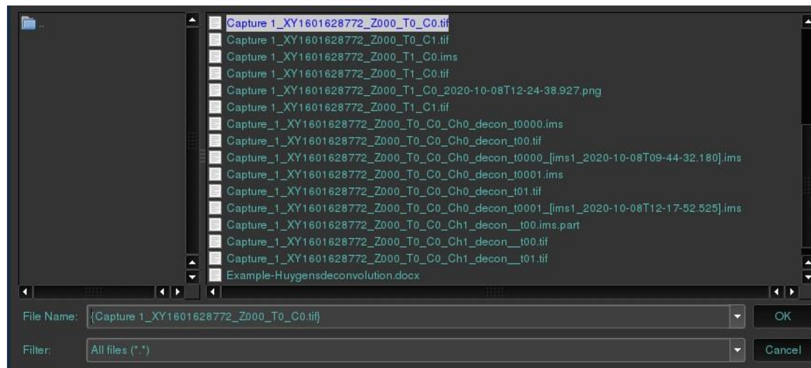
Huygens Deconvolution wizard

*Important: Please copy the metadata of the acquired samples with information on microscope type, wavelengths of excitation and emission, numerical aperture, immersion medium, sampling intervals, back projected pinhole and specific microscope settings before using Huygens.

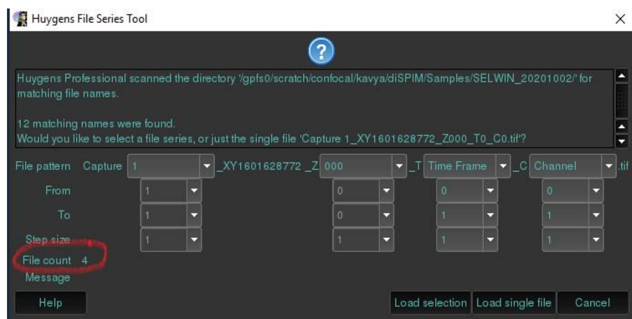
a) Importing files

File → Open

Select the image to be deconvolved.



If the image is a **stack image,** a pop-up window to select the channels, Z-frames and time-frames will appear.

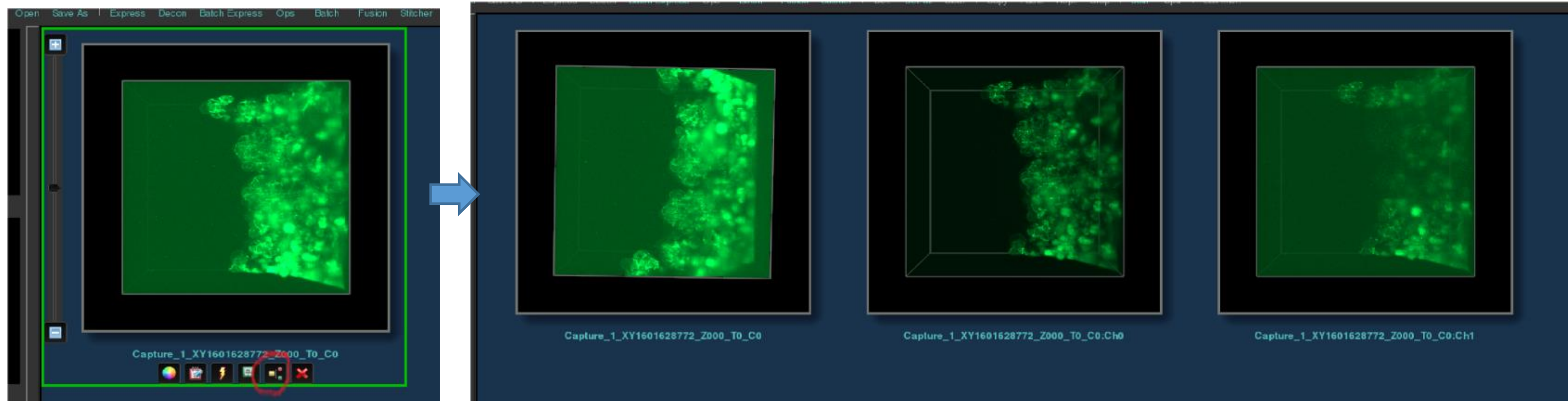


Specify your selection in 'From' to 'To' in each File pattern.

Click 'Load selection' to load the selected images in the stack

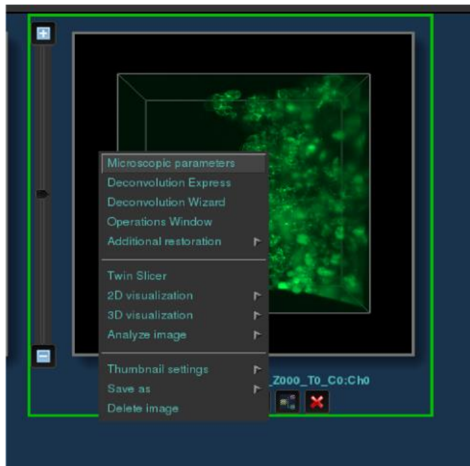
Huygens Deconvolution Wizard
NUS CBIS

The image stack is opened in the Huygens interface.
If you have an image with multiple channels, 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 Scattering parameters can be set to 'No scattering' for thin samples which have not much scattering effect of light.

c) Using Deconvolution Wizard

i. Right click on the image, select 'Deconvolution wizard'

ii. Go through the deconvolution wizard steps.
Follow the steps (default highlighted in yellow here).

Huygens Deconvolution Wizard
NUS CBIS

Enter wizard

Deconvolution wizard

File Help Options

Capture_1_XY160...

Image: Capture_1_XY1601628772_Z000_T0_C0:Ch0
Position: (-168.47 -113.62 75.00) µm
Time: 0.000 s
Time: frame 0
Value: ch 0: -

Help | Reports

Welcome to the Huygens deconvolution wizard!

This wizard will assist you in deconvolving your images. By pressing on 'Enter wizard' you will be guided trough the deconvolution process.

To deconvolve your image using a pre-defined template, please click on 'Use Template'.

The microscopic parameters of the current image are shown to the right.

See also: About deconvolution

Wizard status

Image Capture_1_XY1601628772_Z000_T0_C0:Ch0 is undersampled

Deconvolution wizard - microscopic parameters

Main optical parameters

	X	Y	Z
Image sampling	162.5 nm	162.5 nm	500.0 nm
Ideal Nyquist sampling	162.8 nm	162.8 nm	341.4 nm
Relative Nyquist rate	1.002	1.002	0.683

Channel parameters

Microscope type	spim
Deconvolution license	yes
Ex. wavelength	488 nm
Em. wavelength	521 nm
Clipped voxels	0

Histogram

0 8.3e+03

Use template | Enter wizard

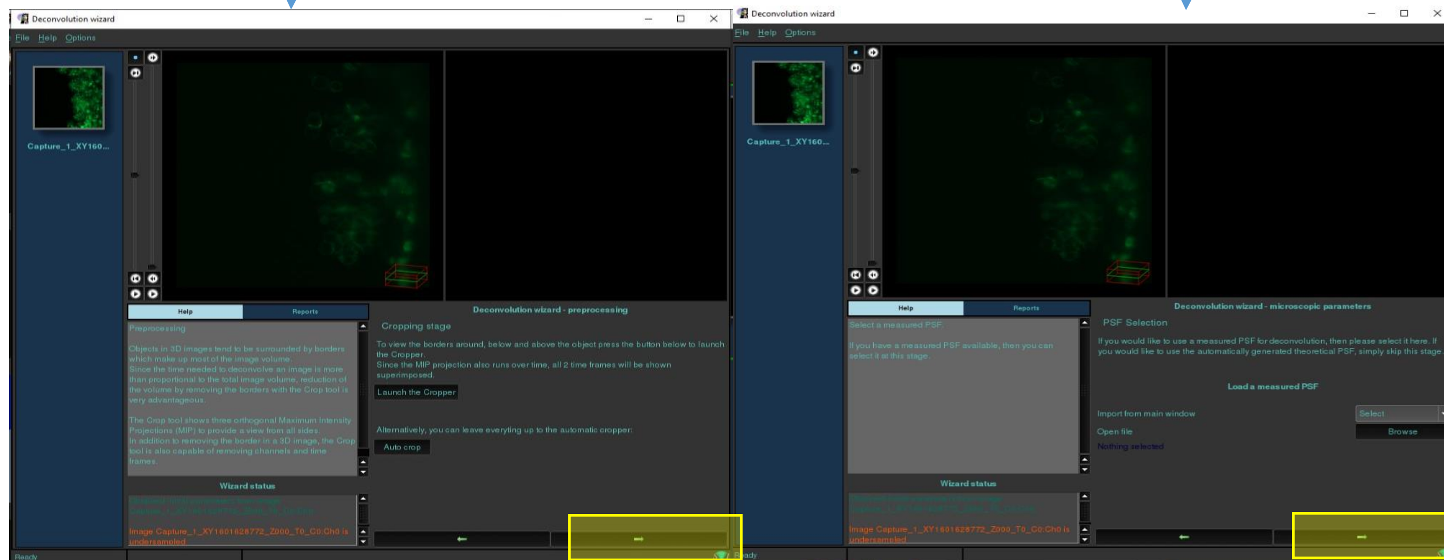
Ready

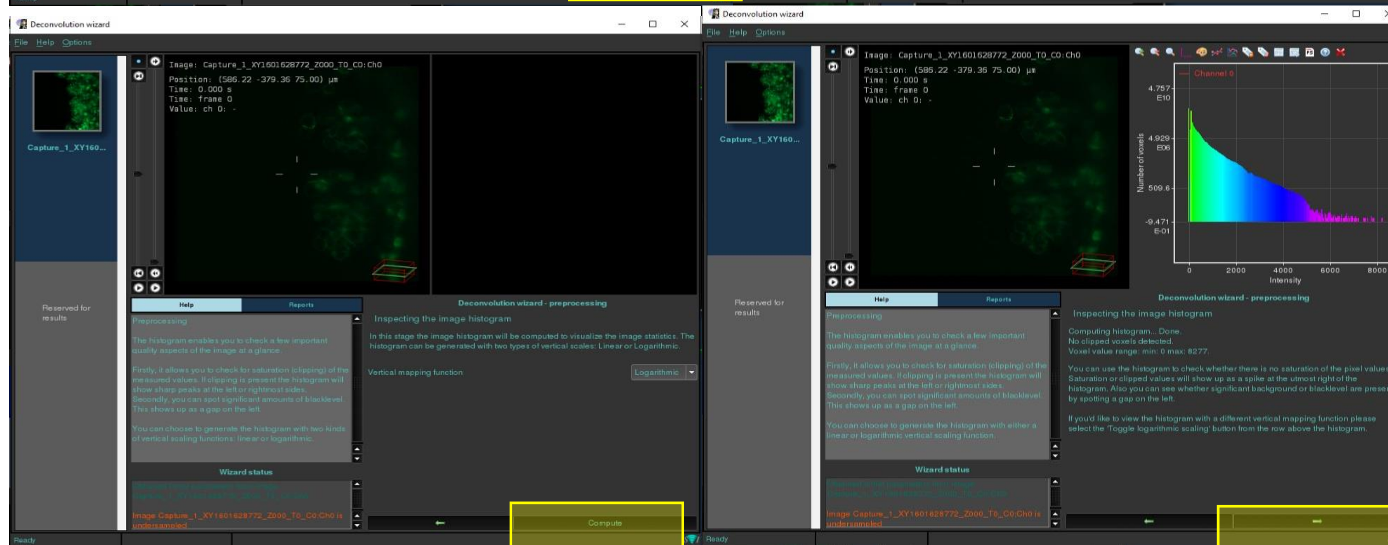
Check the microscopic parameters set for the image

Huygens Deconvolution Wizard
NUS CBIS

Crop image if necessary (to reduce size and processing time)

Load a measured PSF (distilled) if you want to use a measure PSF. If not skip, Theoretical PSF will be generated default



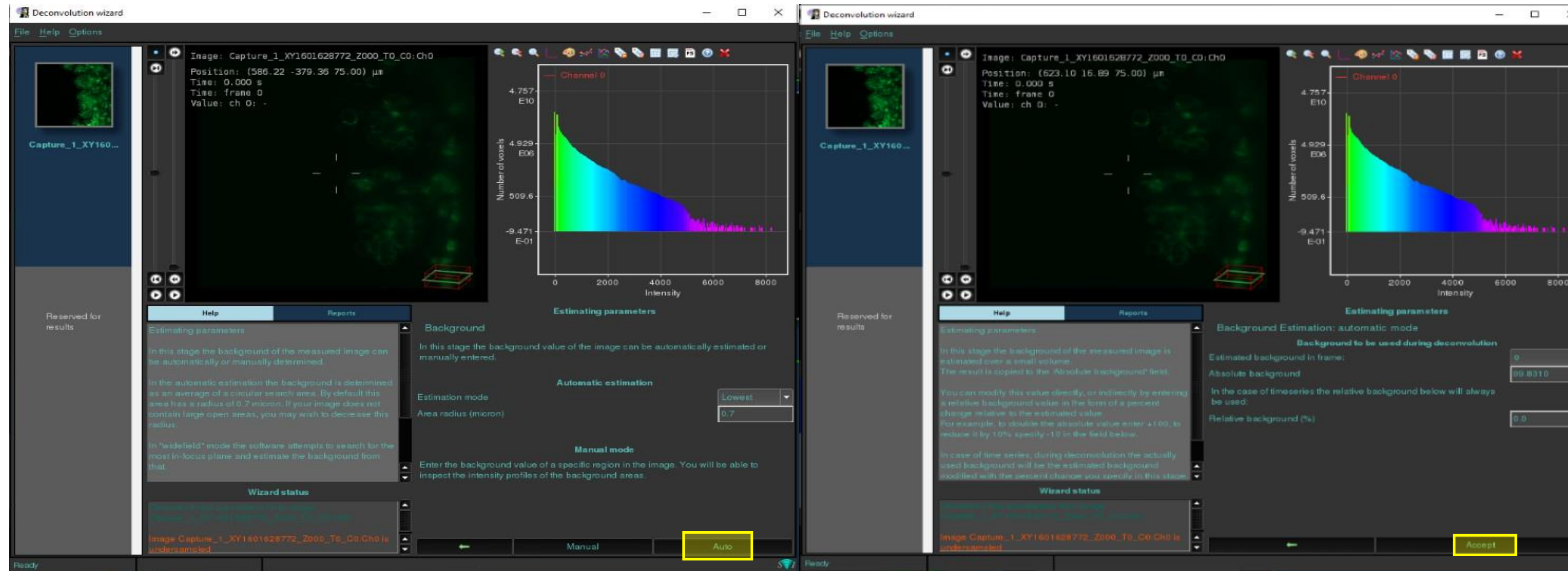


Histogram is computed for background subtraction.

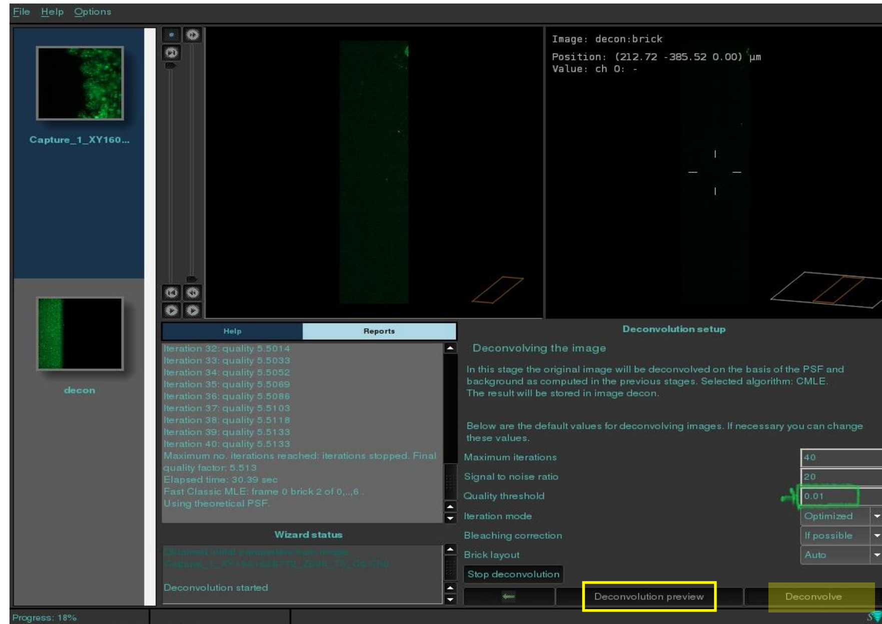
Huygens Deconvolution Wizard
NUS CBIS

Background can be estimated **Auto.** (Automated method performs well.
In exceptional case, where the background is not subtracted efficiently, use Manual.)

Background estimation results are generated.
Modify if necessary



Huygens Deconvolution Wizard
NUS CBIS



- Start with default no. of iterations (40).
- Enter the correct signal to noise ratio of your image. (Note: If SNR estimated higher, more artifacts is visible, if SNR estimated lower, deconvolution is not effective. See next pg. for SNR calculation)
- Use default quality threshold and iteration mode.
- Click **Deconvolution Preview.**

Based on observations try the following for each trial of deconvolution preview:

If artifact/noise is seen, reduce no. of iterations, reduce SNR and increase quality threshold.

If deconvolution not satisfactory, increase no. of iterations, increase SNR and reduce quality threshold. Change to Classic iteration mode.

Once satisfactory, click **Deconvolve**

Huygens Deconvolution Wizard
NUS CBIS

SNR calculation with FIJI (for reference)

1. Open the TIFF image with FIJI.
2. Ensure that selected images are not saturated as the SNR does not account for saturation effects. Saturated images should be discarded and re-acquired in different conditions.
3. Select a Region of the tissue containing positive staining for the marker of choice
4. Draw a line on that region covering the areas with positive staining (see Figure 1 as an example).
5. Press Ctrl K to obtain the intensity profile for the pixels located underneath the yellow line.
6. Save the obtained values as Signal Intensity Profile pressing the Save button underneath the graph, this will generate a csv file, that can be opened with Microsoft Excel or another preferred software.
7. Select an ROI containing the tissue in absence of positive staining from the marker under evaluation and draw a line (see Figure 1 as an example).
8. Press Ctrl K and obtain the corresponding intensity profile.
9. Save the obtained values pressing the Save button underneath the graph as Noise Intensity Profile, this will generate another csv file.
10. Using the software of choice, open the two csv files.
11. Extract the local maximum values from the Signal Intensity profile file and calculate their average. • Average all intensity values reported in the Noise Intensity Profile file.
12. Divide the average signal by the average noise to obtain the SNR.
13. This procedure can be repeated in multiple regions for more accurate results.

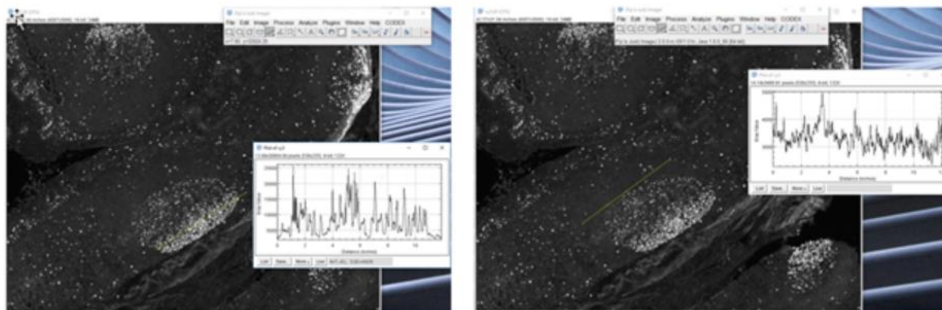


Figure 1.

Huygens Deconvolution Wizard
NUS CBIS

Deconvolution setup

Deconvolution result

A first deconvolution result is now available.

From this stage it is possible to:
Resume iterations starting from the present result,
'Restart channel' to rerun the deconvolution with different background settings,
Accept the results by pressing 'All done'.

Restart all | Restart | Resume | All done

Deconvolution setup

In this stage you can select the images to merge as the result image. Move images from Available to Selected and change the order. You can also choose to keep the original files in the result. When you click on a thumbnail, its corresponding item in the list will highlight. Once you are satisfied with the selection, go to the next stage.

Channel list

Destname: Capture_1_XY1601628772_Z000_T0_C0:Ch0_decon

Available: Capture_1_XY1601628772_Z000_T0_C0:Ch0

Selected: img 0: decon

Join in Dimension: ch

Restart

Deconvolution wizard - summary

Restart: restart the wizard to redo your deconvolution. The current deconvolution results will be lost.

Save template: Save the deconvolution parameters to a template.

Done: quit the wizard and export the deconvolution result to the main window in Huygens Professional.

Close wizard, export result image and load in:

Twin Slicer | Surface Renderer | Movie Maker | Image Stabilizer | Chromatic Aberration | Coloc Analyzer

Restart | Save template | Done

Name the deconvolved file.

d) Viewing and saving results

To compare the two images before and after deconvolution, select the two images (Ctrl) in main Huygens window.

GO to Menu: Visualization → Twin slicer

To save the deconvolved image result, select the image. Menu: File → Save as