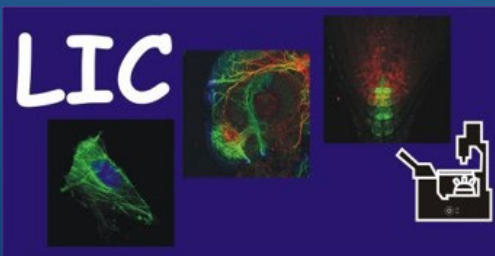


Tutorial - Basic

Huygens Professional

Life Imaging Center

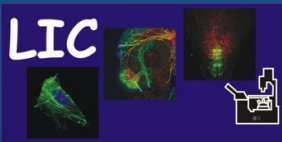


Open - Huygens Professional

Access: on all LIC Workstation in the LIC

Licenses available:

- Deconvolution for
 - Confocal
 - Multiphoton
 - Spinning disk
 - STED
 - Light-sheet
 - Widefield
 - Airyscan (ZEISS)
- Colocalization Analysis
- Light-sheet Fusion
- Object Stabilizer
- Time-series



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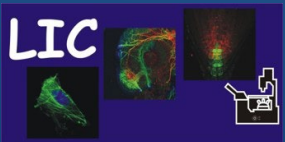
Deconvolution Process in Short !

You have no Microscopic Parameter and Deconvolution Templates

- Step A: Open image files and edit and verify **Microscopic Parameter**:
 - Write the specific properties of your images into a template, save and / or accept
- Step B: Open **Deconvolution Wizard**, it will guide through the deconvolution process
 - Define the preferred deconvolution settings in a template, save this template and run it
- Step C: **Save** your deconvolved image

You have Microscopic Parameter and Deconvolution Templates

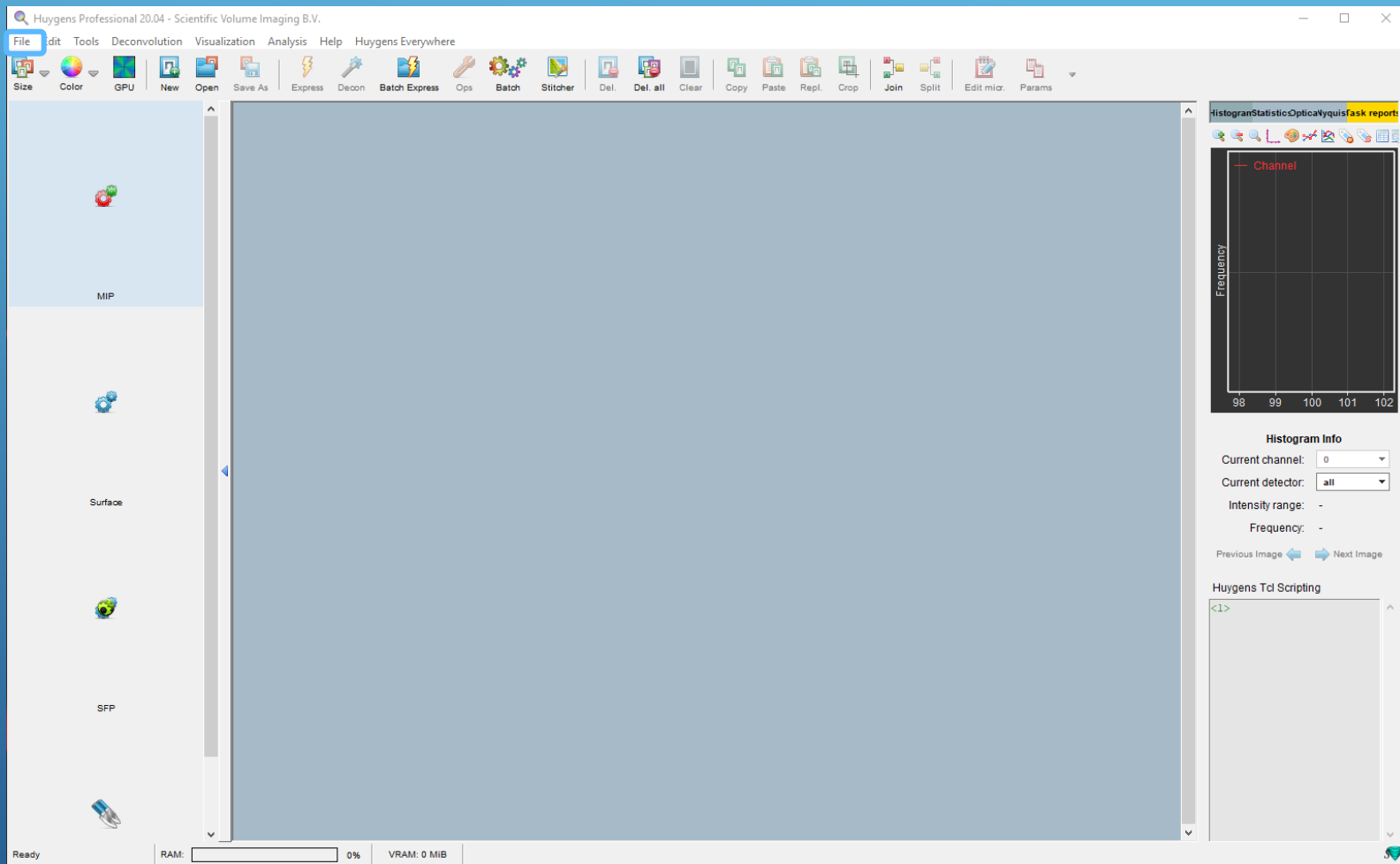
- Step A: Open image file, load and apply template of **Microscopic Parameter**
- Step B: Load and apply template of **Deconvolution Wizard**
- Step C: **Save** your deconvolved image



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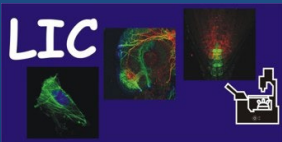


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Open Image File (File menu)

or

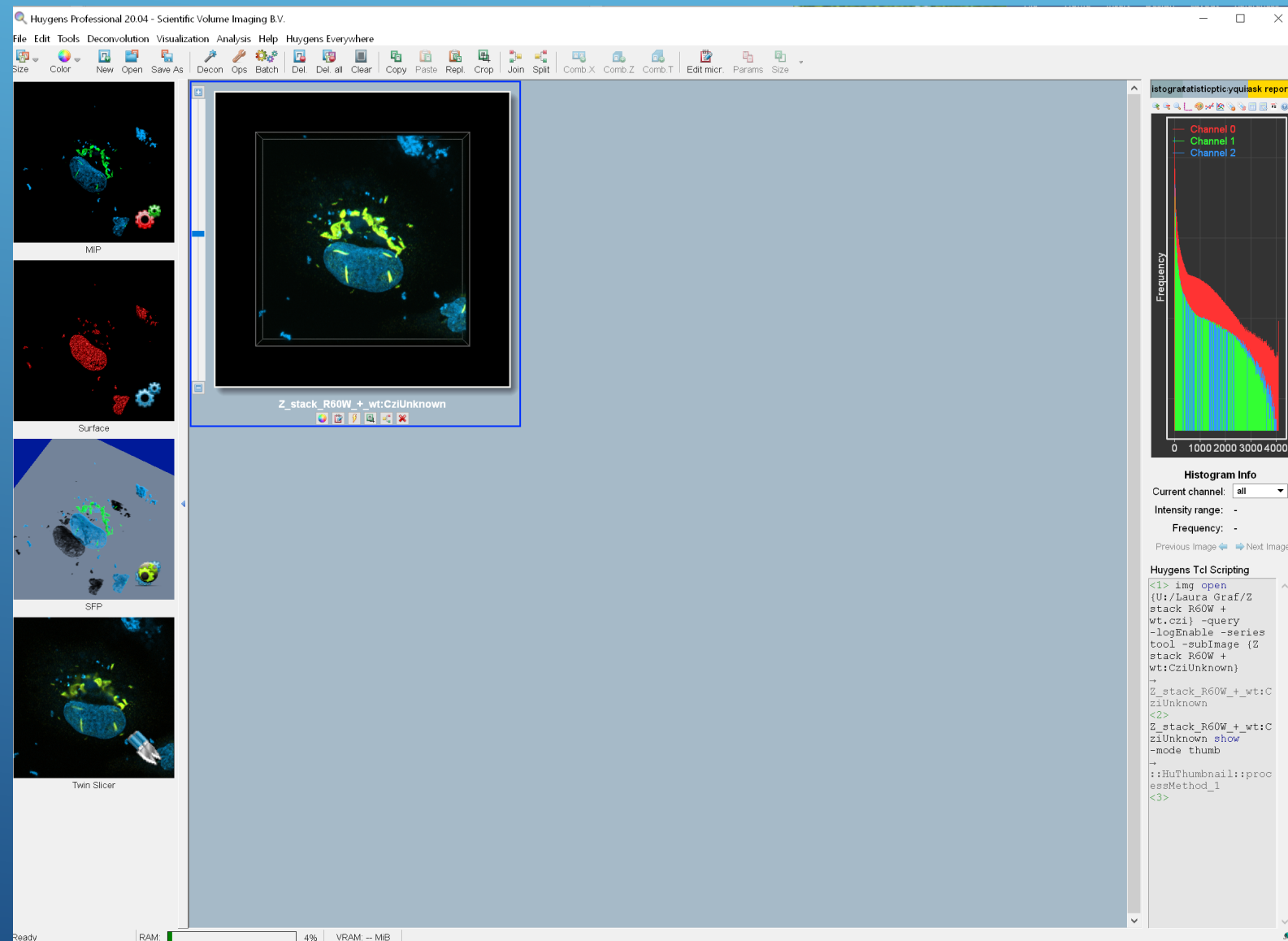
Drag and Drop



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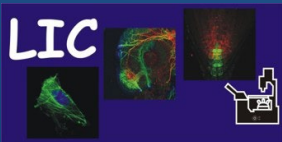
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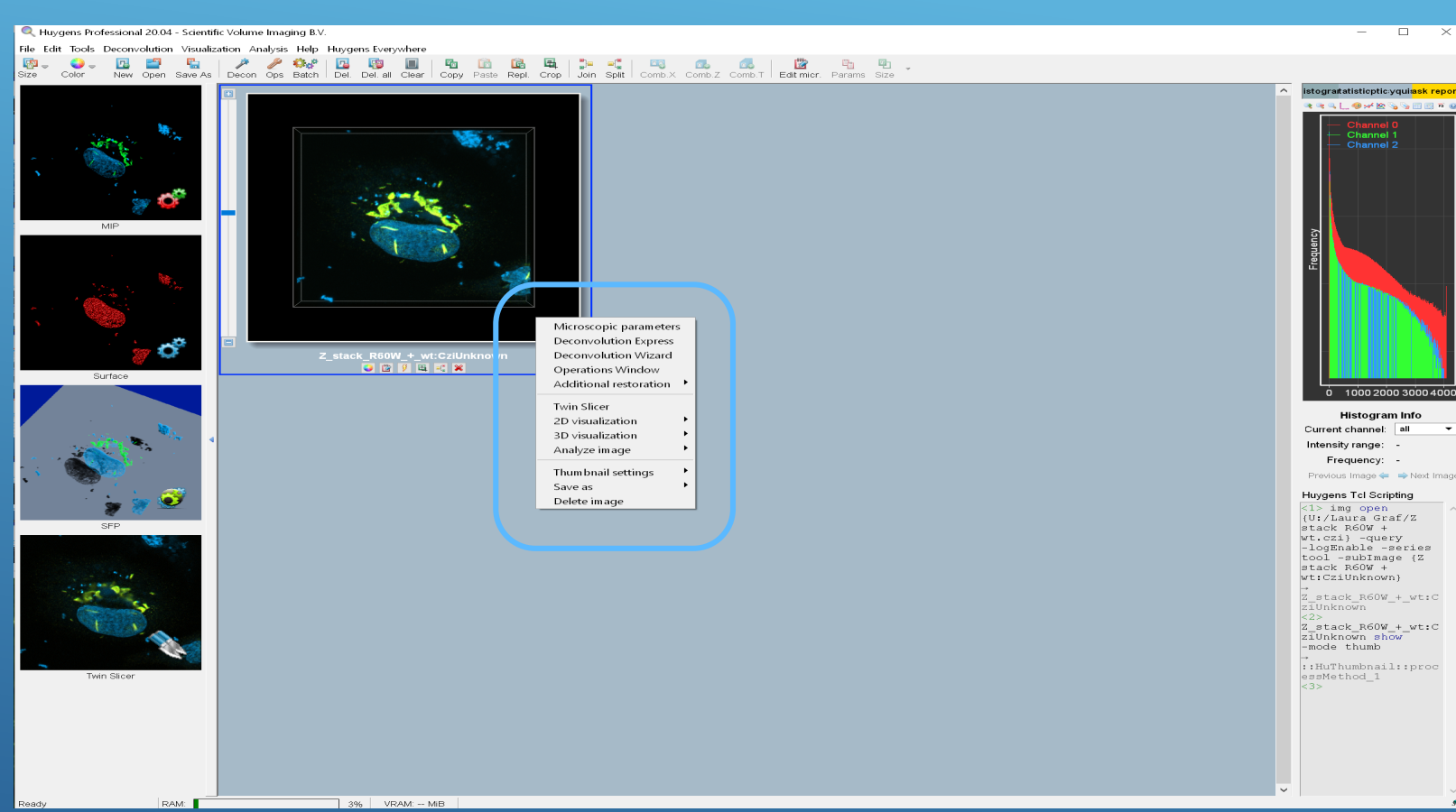
Select Image



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Right Mouse Click on Image

- A menu appears

Start **Microscopic parameters**

Edit Microscopic Parameters - Z_stack_L542S_+_wt:CziUnknown

General parameters

Sampling intervals:

X (nm) 70.681

Y (nm) 70.681

Z (nm) 364.796

T (s) 1.000000

Optical parameters

Numerical aperture 1.400

Refractive indexes:

Lens immersion Oil 1.515

Embedding med. Oil 1.515

Advanced:

Objective quality Normal

Coverslip: Launch editor

Coverslip position (µm) 5.472

Imaging direction Downward

Channel parameters

Select channel:

0: Confocal

1: Confocal

2: Confocal

Microscope type Confocal

Backprojected pinhole (nm) 254

Excitation wavelength (nm) 405

Emission wavelength (nm) 450

Multi photon excitation 1

Excitation fill factor 2.00

Image properties

Z_stack_L542S_+_wt:CziUnknown

Dimensions: 1260×1260×20×1

Channels: 3 (stacked)

Data type: 16 bit unsigned integer

Size: 181.7 MiB

Templates: Load Save

Reports:

The X sampling is too large.
The Y sampling is too large.
The Z sampling is FAR too large.
Updated coverslip position to data top at 7.296 micron
Please consult the Nyquist Calculator for optimal sampling intervals.

Revert Cancel Accept

Name	Fluo-cells_z-stack_for_Deconvolution
Description	
Acquisition Date	3/13/2019 16:30:35
Notes	
User	schulung
Scaling X	0.085 µm
Scaling Y	0.085 µm
Scaling Z	0.350 µm
Dimensions	x: 1000, y: 1000, z: 13, channels: 3, 12-bit
Image size	x: 85.02 µm, y: 85.02 µm, z: 4.20 µm
Scan Mode	stack
Zoom	2.5
Objective	Plan-Apochromat 40x/1.4 Oil DIC M27
Position (xyz)	Position 1: x: -22806.2 µm, y: -2423.4 µm, z: -30.5 µm
Pixel dwell	0.44 µs
Average	line 2
Master gain	488 Ch1 : 257 561 Ch2 : 700 405 Ch3 : 800
Digital gain	488 Ch1 : 1.00 561 Ch2 : 1.00 405 Ch3 : 1.00
Digital offset	488 Ch1 : 0.00 561 Ch2 : 0.00 405 Ch3 : 0.00
Pinhole	488 Ch1 : 53 µm 561 Ch2 : 53 µm 405 Ch3 : 601 µm
Filters	488 Ch1 : 508 - 553 561 Ch2 : 597 - 695 405 Ch3 : 415 - 480
Beam splitters	MBS : MBS 488/561/633 MBS 2006 : MBS 690+ DMS1 : Mirror
Lasers	488 488 nm : 0.8000 % 561 561 nm : 0.8000 % 405 760 nm : 1.0 %

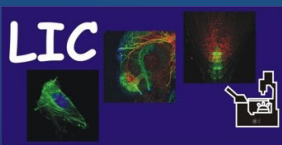
Open Microscopic Parameter

Huygens will read image meta data but some values have to be specified

Therefore **control all imaging values** used during acquisition and **fill in the missing** values, too

- Check **Sampling Values** (pixel size xyz)
- Specify **Embedding medium -Refractive Index** of your sample
- Specify **objective quality** (use normal or good)
- **Launch Coverslip Editor**

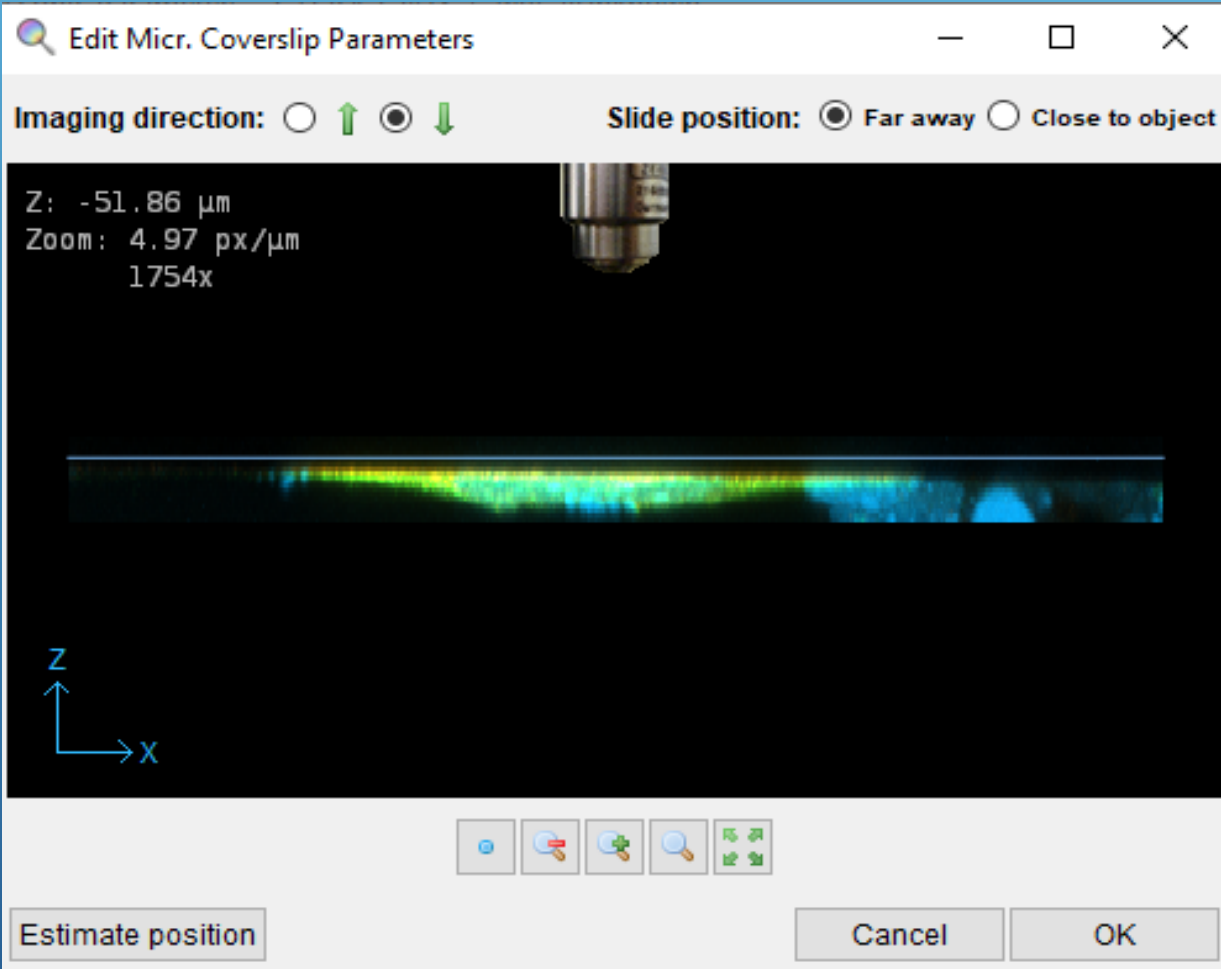
If you are not sure how you have acquired your data, open your data file in the **Acquisition software** to look up the parameter



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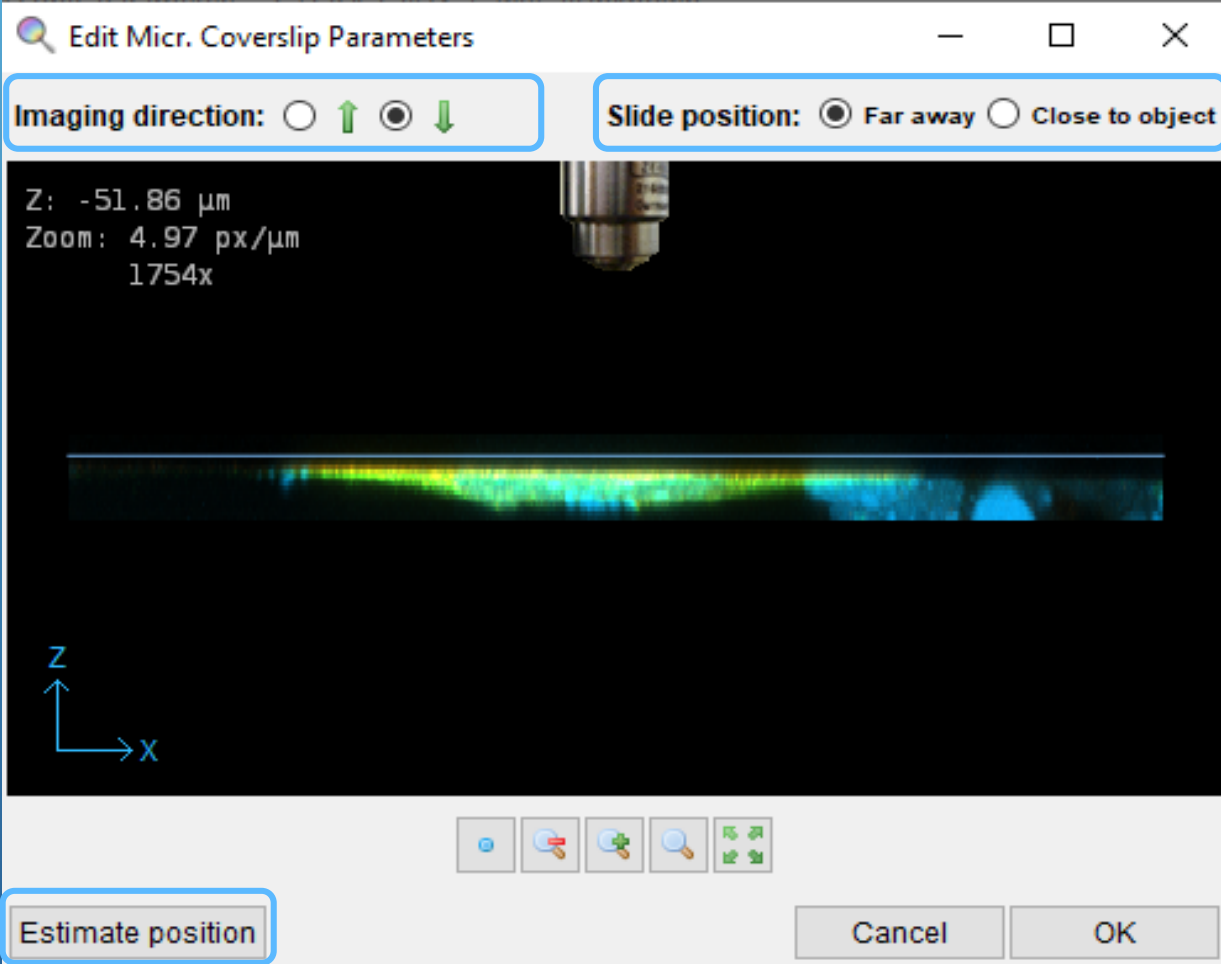


Coverslip Editor

Coverslip position parameter can be used to optimize spherical aberration correction by defining the distance between the coverslip surface and the image plane.

As deeper layers in the specimen are imaged, moving away from the coverslip, this distortion will progressively worsen.

Therefore it is important to set the first plane in the microscopic parameter editor.



Coverslip Editor

Choose **image direction**

- in an **inverted microscope** the objective lens points **upwards**

Choose **slide (not coverslip) position to object**

- When the specimen is **mounted on the coverslip**, the distance from the object to the slide is **far away**

Estimate position or move the blue line manually

- Fortunately, it is often easy to spot the flat side of the object where it adheres to the glass on which it was mounted , so the orientation can be verified

Go ON - **OK**

Edit Microscopic Parameters - Z_stack_L542S+_wt:CziUnknown

General parameters

Sampling intervals:

X (nm) 70.681

Y (nm) 70.681

Z (nm) 364.796

T (s) 1.000000

Optical parameters:

Numerical aperture 1.400

Refractive indexes:

Lens immersion Oil 1.515

Embedding med. Oil 1.515

Advanced:

Objective quality Normal

Coverslip: Launch editor

Coverslip position (µm) 5.472

Imaging direction Downward

Channel parameters

Select channel:

0: Confocal

1: Confocal

2: Confocal

Microscope type Confocal

Backprojected pinhole (nm) 254

Excitation wavelength (nm) 405

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Multi photon excitation 1

Excitation fill factor 2.00

Image properties

Z_stack_L542S+_wt:CziUnknown

Dimensions: 1260×1260×20×1

Channels: 3 (stacked)

Data type: 16 bit unsigned integer

Size: 181.7 MiB

Templates: Load Save

Reports:

The X sampling is too large.
The Y sampling is too large.
The Z sampling is FAR too large.
Updated coverslip position to data top at 7.296 micron
Please consult the Nyquist Calculator for optimal sampling intervals.

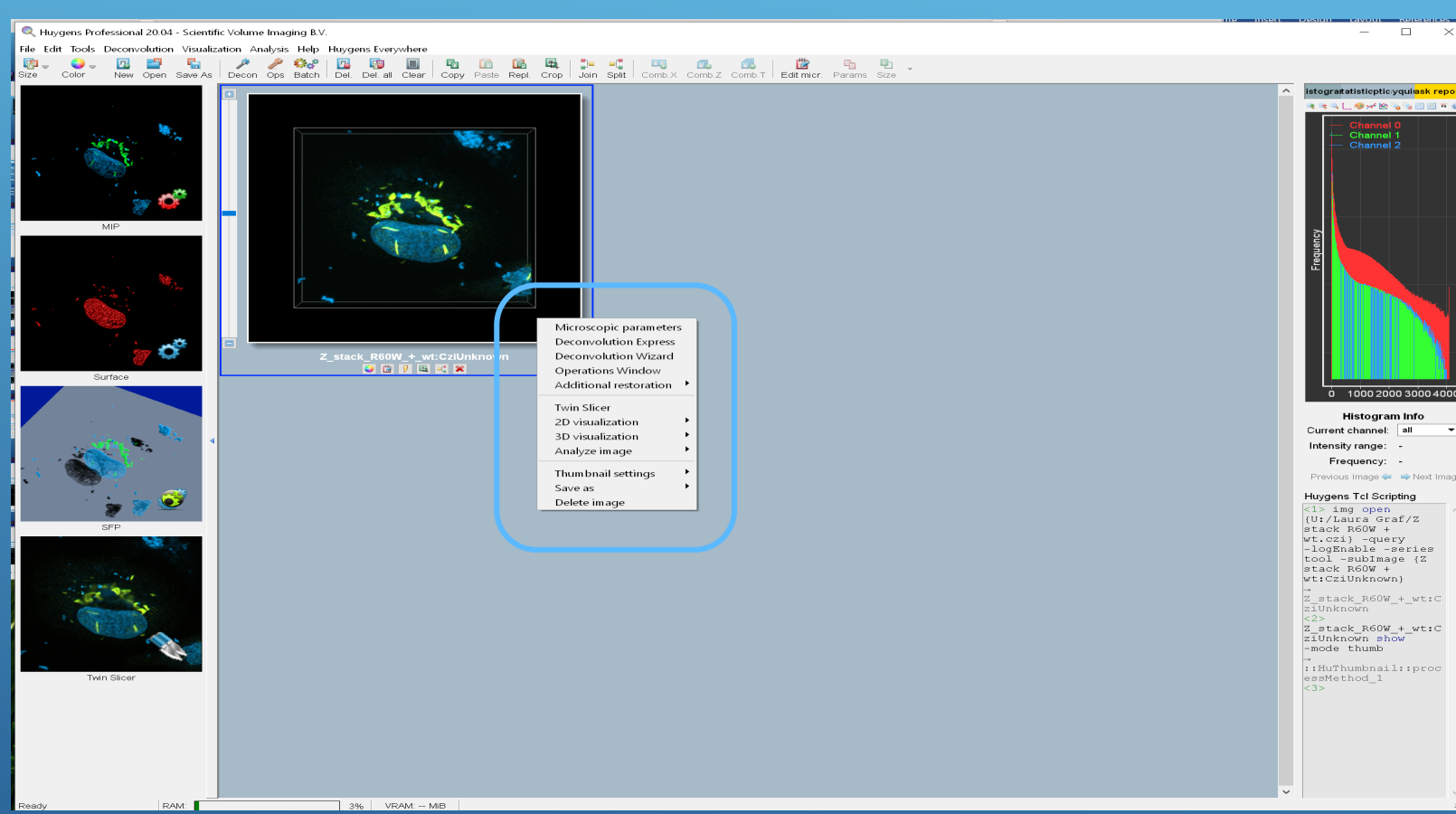
Help

Not all parameters verified Set all verified

Revert Cancel Accept

Microscopic Parameter

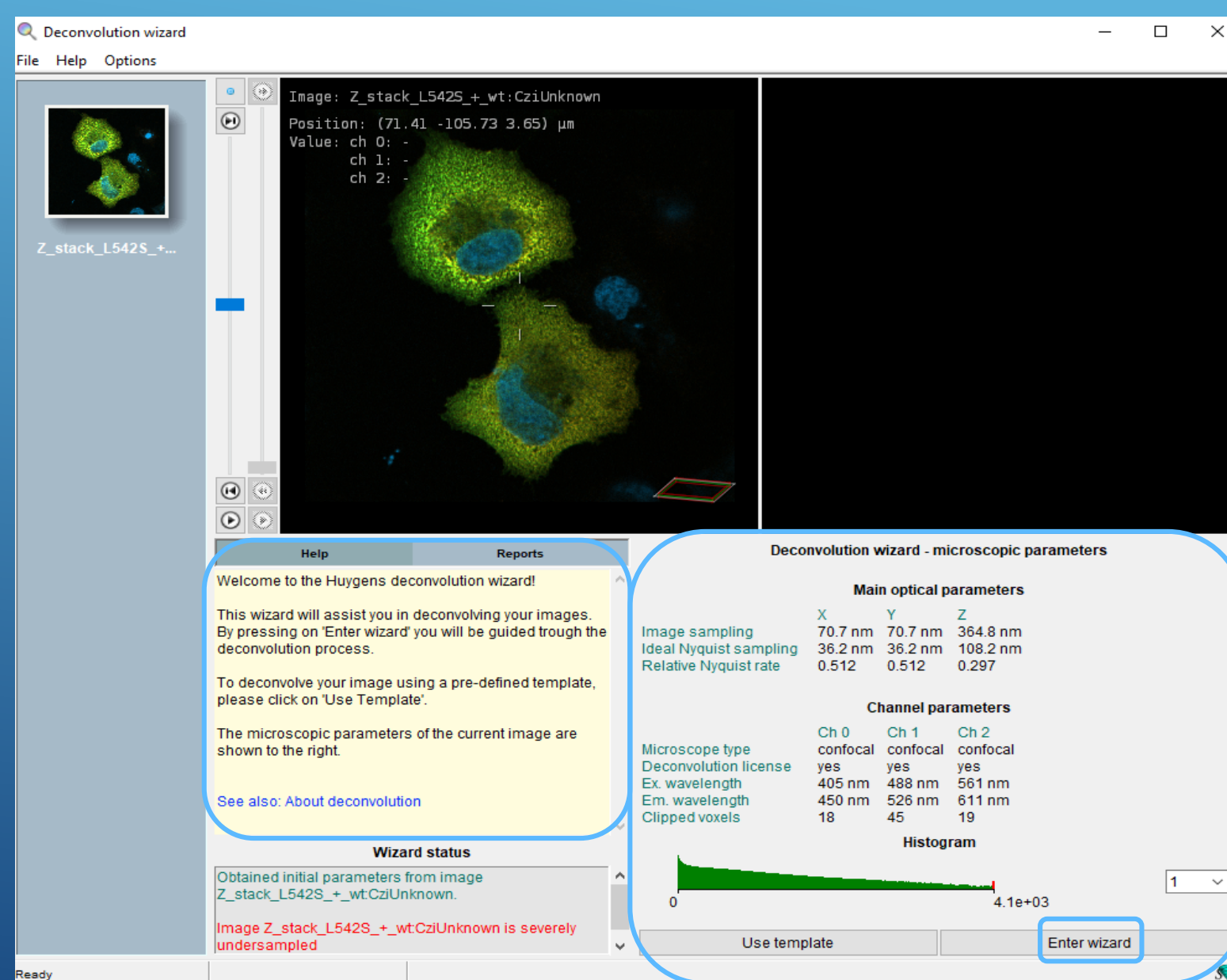
- Control **Excitation and Emission** (peak value)
- Set **all verified**
- You could **save the template**
- **Go On - Accept**



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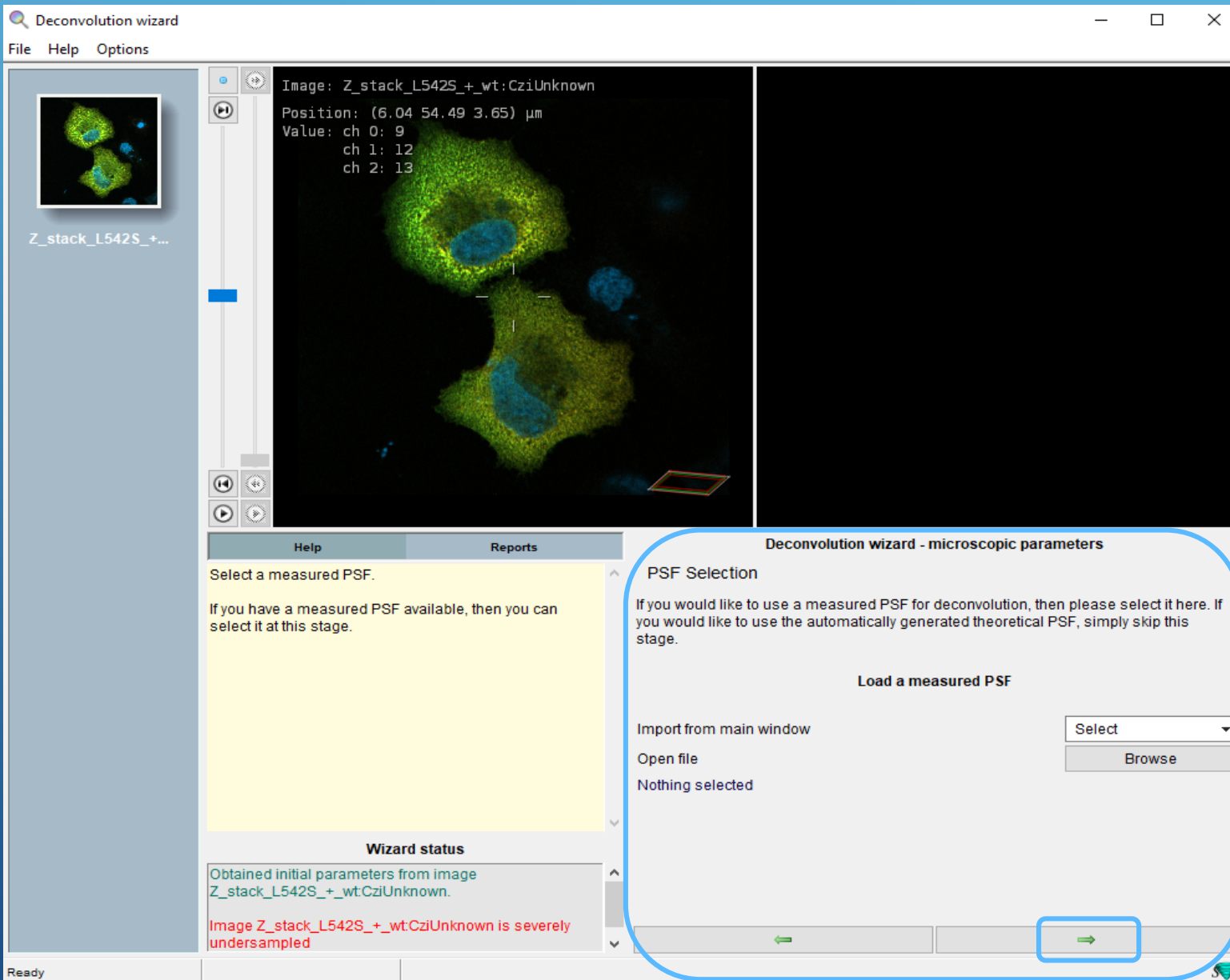
Again **Right Mouse Click** on Image

Start **Deconvolution Wizard**



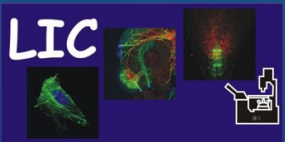
Open Deconvolution Wizard

- On the left side - **Help explanations** concerning the wizard step
- Enter Wizard**



Deconvolution Wizard

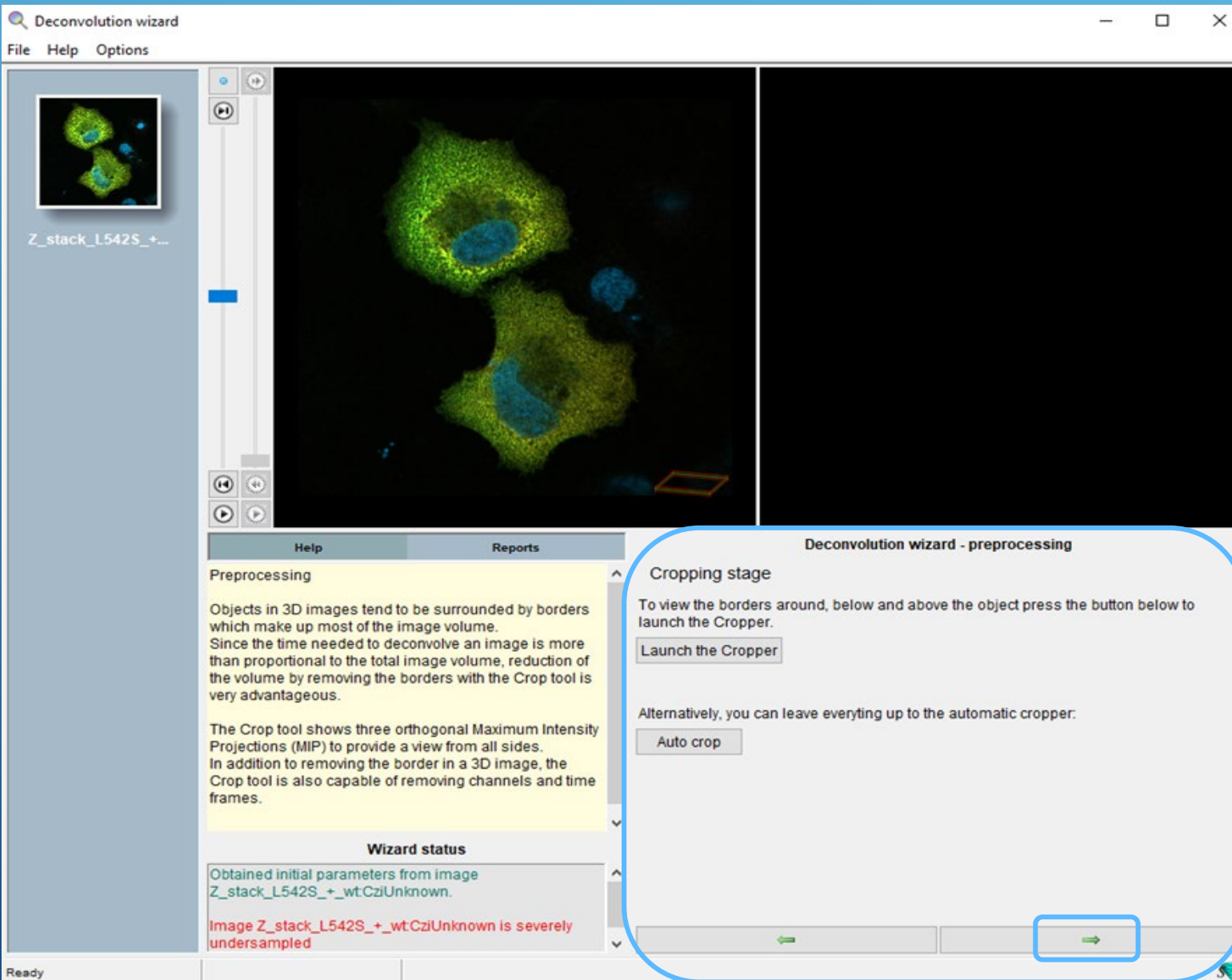
- If you have measured a **PSF** load this here, otherwise software use a **theoretical PSF** (default setup)
- **Go On**



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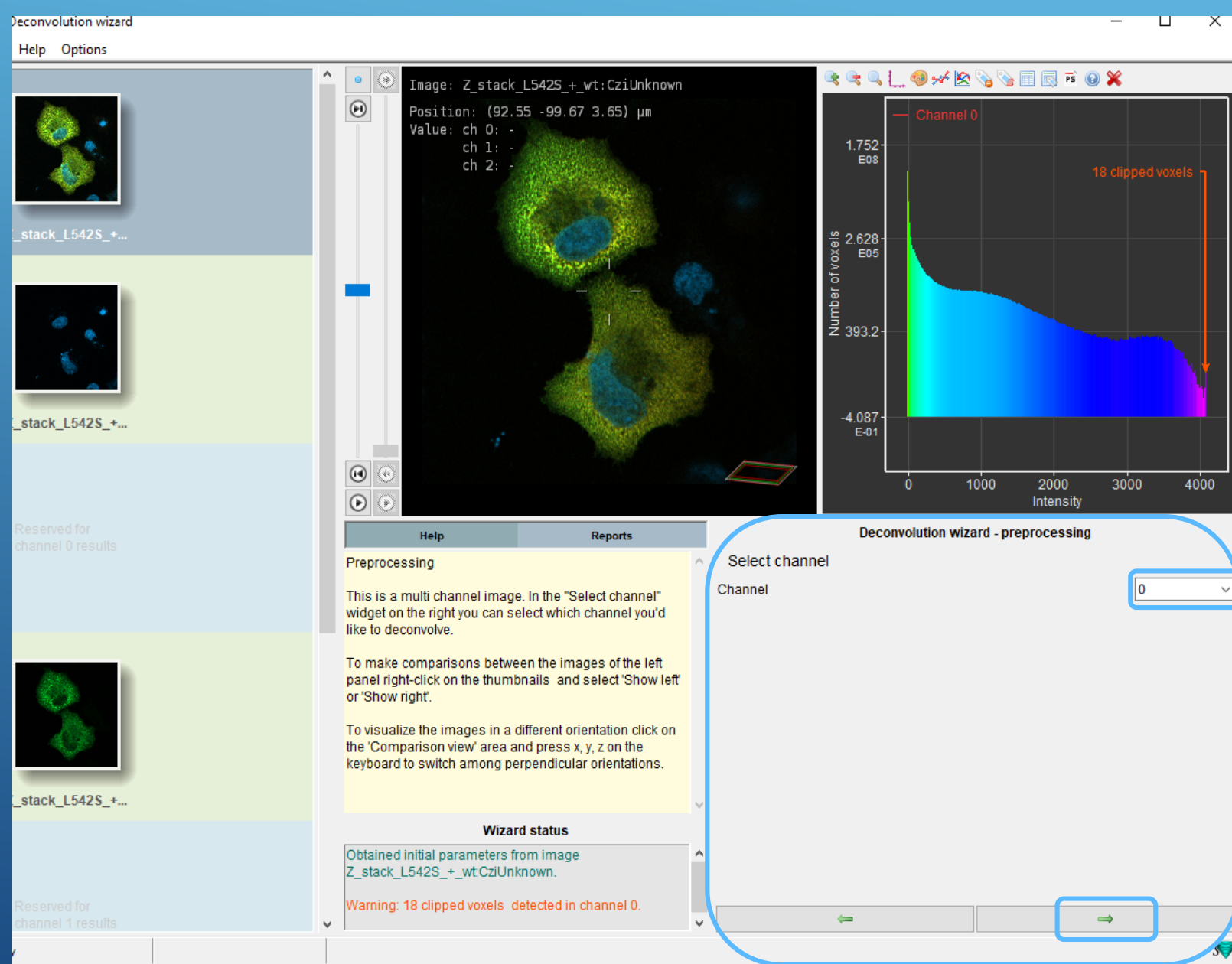
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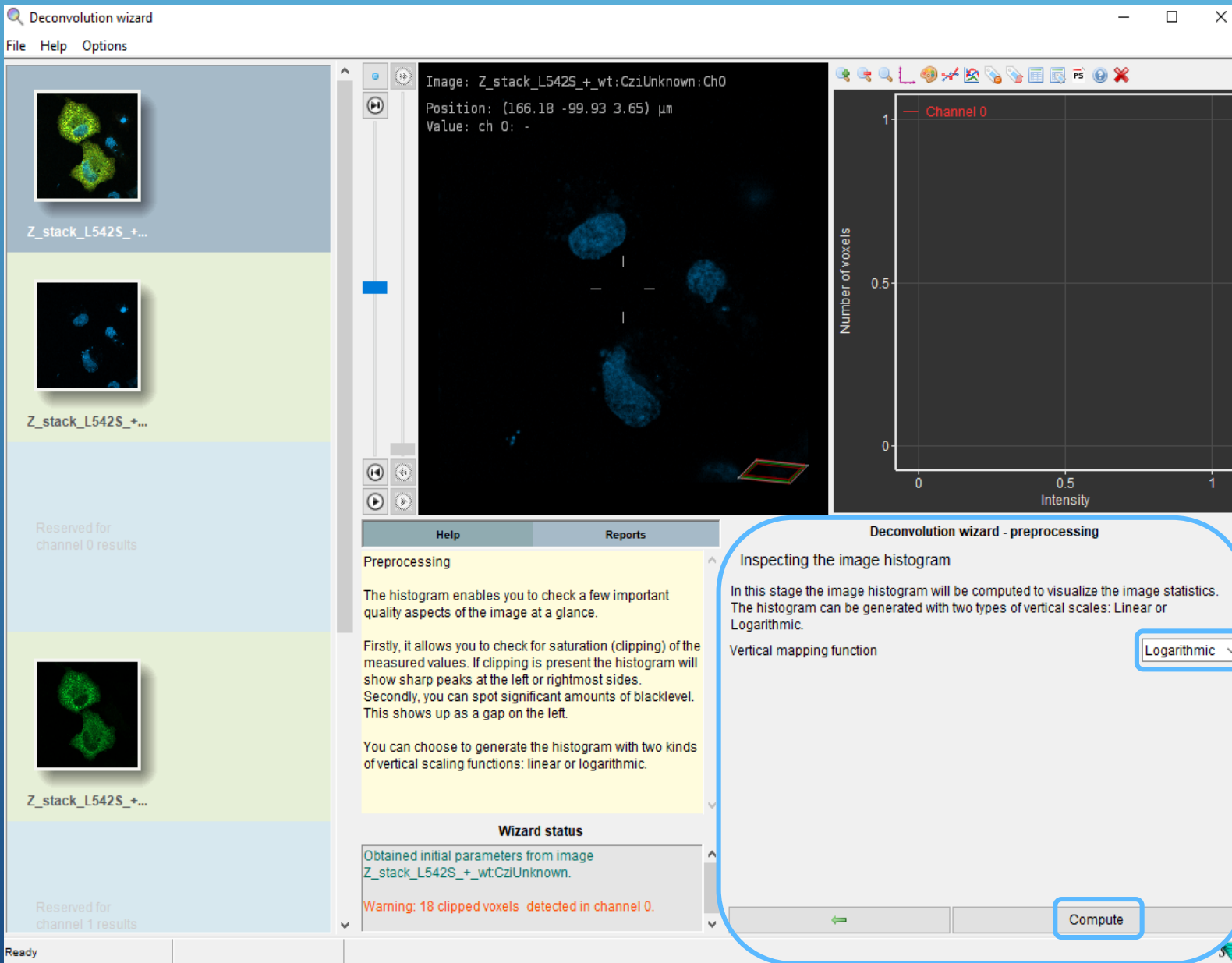
Deconvolution Wizard

- **Crop** sample if necessary
- **Go On**



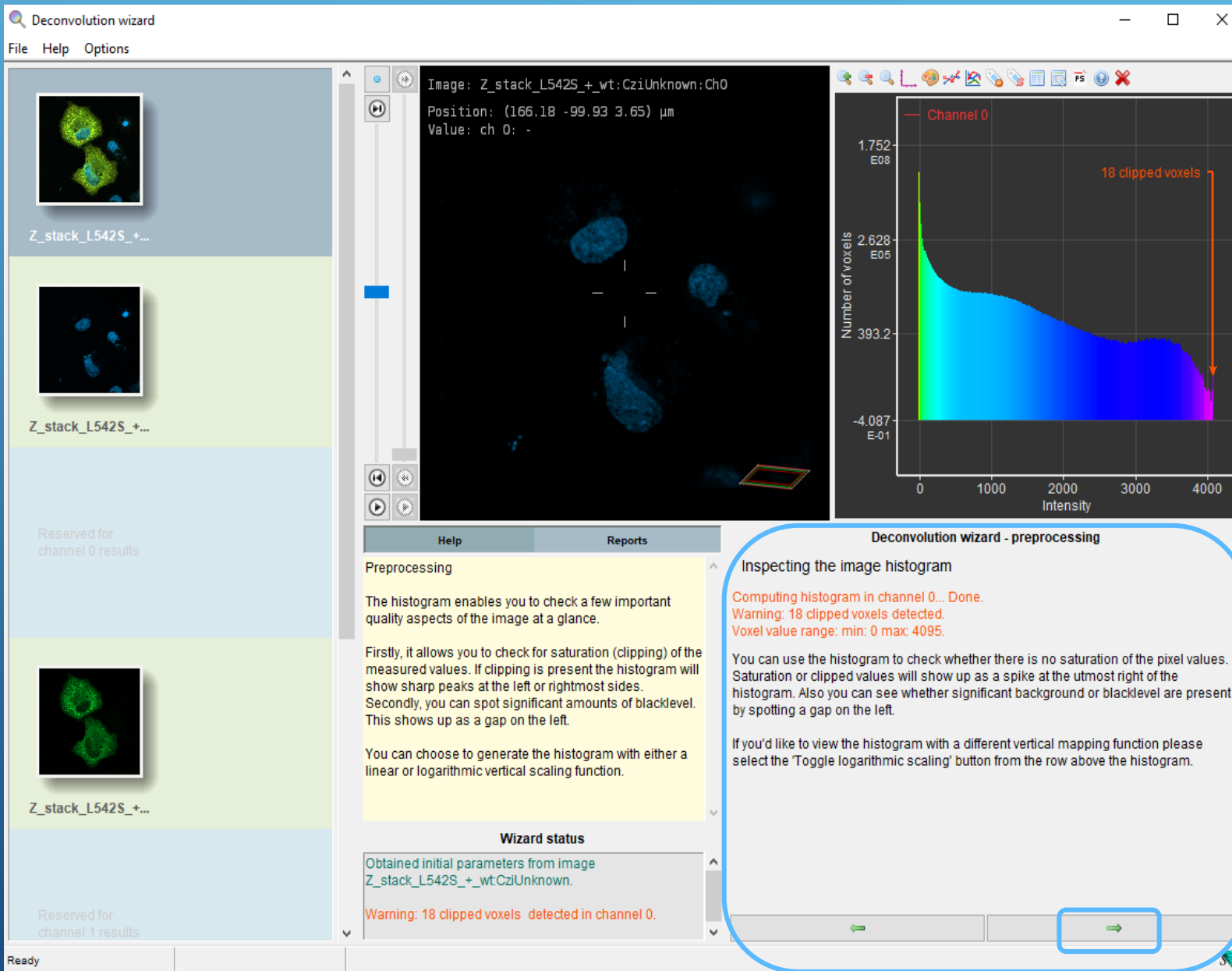
Deconvolution Wizard

- The **channel number counting** in Huygens is different
 - Huygens will start with Channel 0 - this corresponds to Channel 1
- Go On



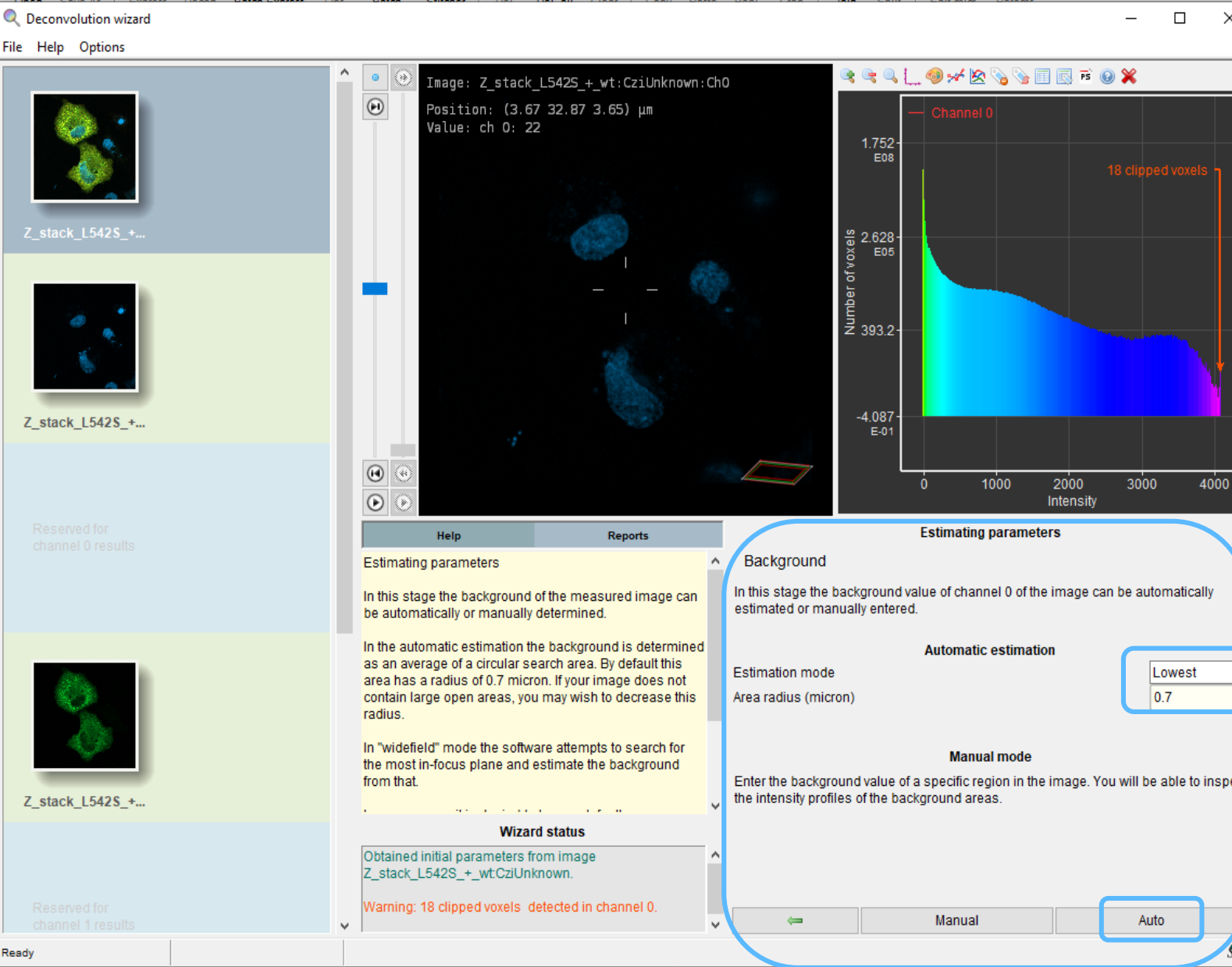
Deconvolution Wizard

- **Image histogram** is computed and displayed during the deconvolution process **to let you spot problems** that might have occurred during the image recording as clipping and Quantization Noise.
 - It has no meaning for the deconvolution process that follows.
- **Compute** with **logarithmic function**
- **Go On**



Deconvolution Wizard

- Go ON



Deconvolution Wizard

- The **mean background** can be estimated **automatically** in Huygens Professional with the Estimate background tool in the Analysis menu of the Operations window.
- Normally use „lowest“ or as estimation mode „In/near object “
- Use a search area of **0.7 micron radius**.
- If your image does not contain large open areas, decrease the radius.
- Go On - Auto

Deconvolution Wizard

- Go On - Accept

File Help Options

Image: Z_stack_L542S+_wt:CziUnknown:Ch0
Position: (3.67 32.87 3.65) μm
Value: ch 0: 22

Channel 0

Number of voxels

Intensity

18 clipped voxels

Estimating parameters

Background Estimation: automatic mode

Background to be used during deconvolution

Absolute background 0.1999

Relative background (%) 0.0

☐ Log absolute background instead of relative in template.

Select deconvolution algorithm

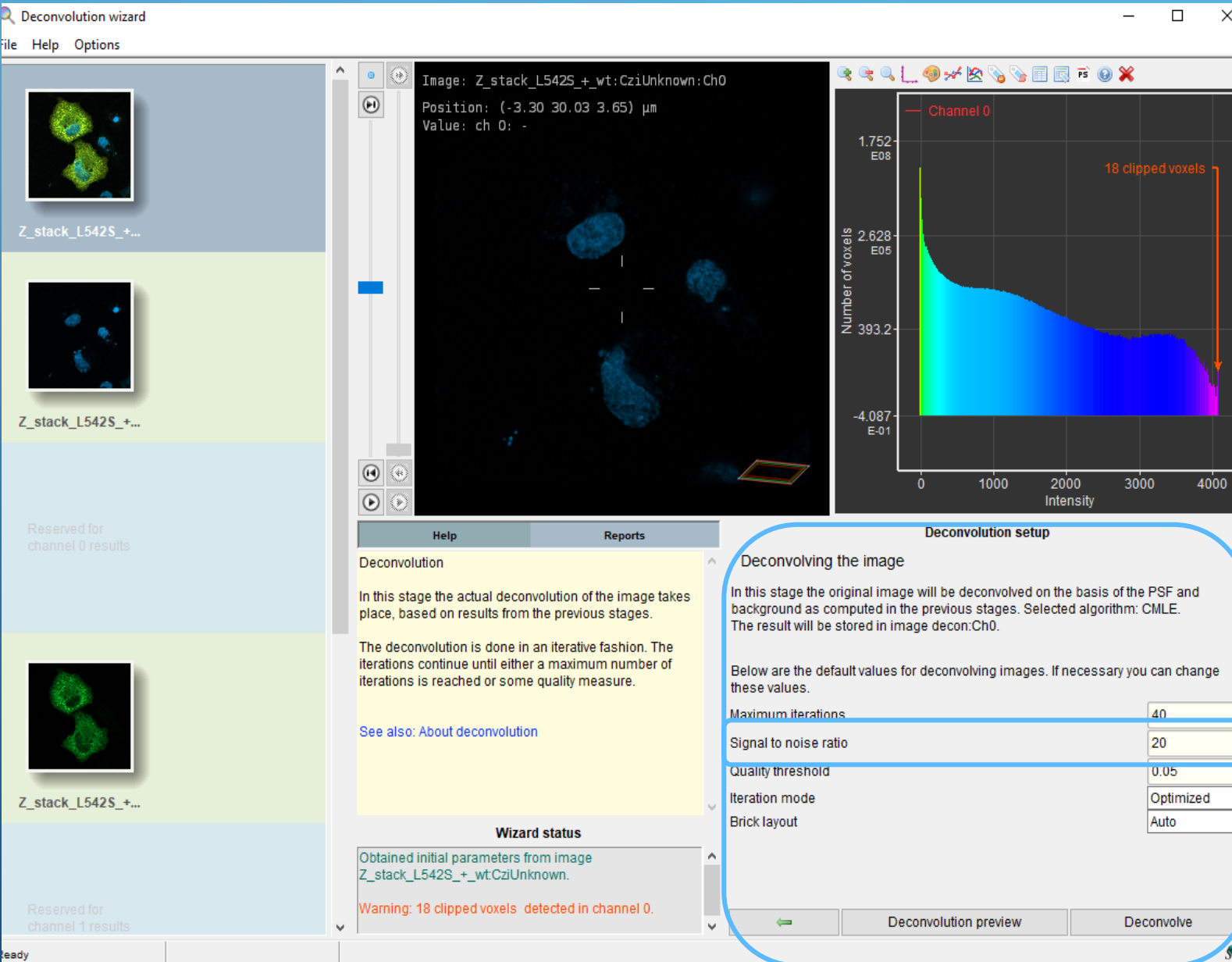
Deconvolution algorithm CMLE

Wizard status

Obtained initial parameters from image
Z_stack_L542S+_wt:CziUnknown.

Warning: 18 clipped voxels detected in channel 0.

Accept

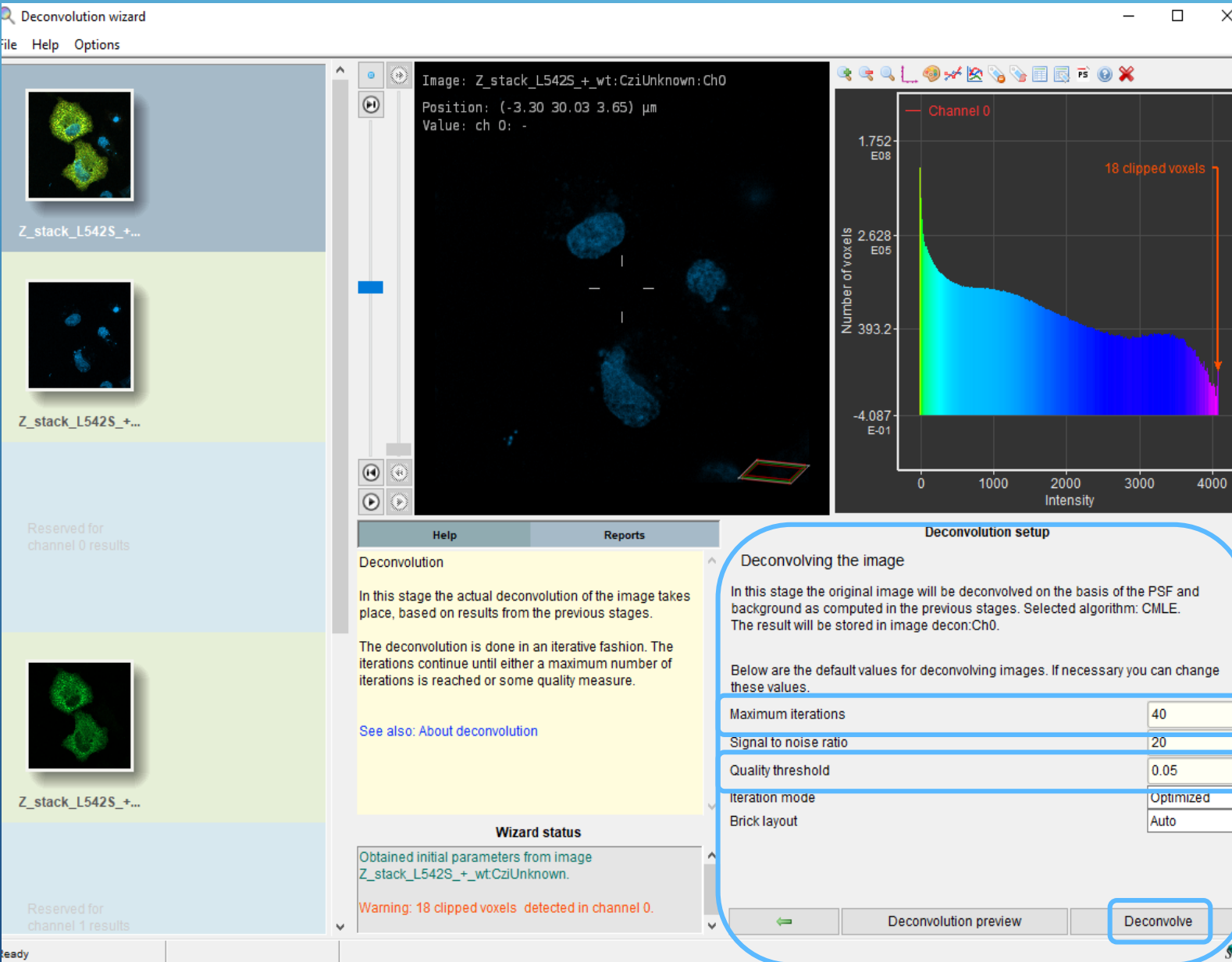


Deconvolution Wizard

Estimate the Signal to Noise Ratio

Using a too large SNR value might be risky when restoring noisy originals, because you could be just enhancing the noise.

- For a noise-free **widefield image** use SNR values **higher than/ = 40**
- A noisy **confocal** image can have values **lower than/= 20**
- For noisy **STED** use values **below/= 7**

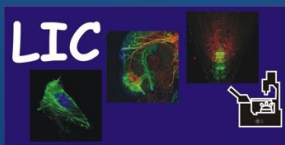


Deconvolution Wizard

Define the **stopping criteria** of the algorithm

- The **Maximum Number of Iterations** is a Restoration Parameter of the Huygens Software that puts a limit to the iterative deconvolution.
- Another limit is established by the Quality Change **Threshold parameter**.
- For an **initial run** you can use **40 iterations** and **quality change 0.05**

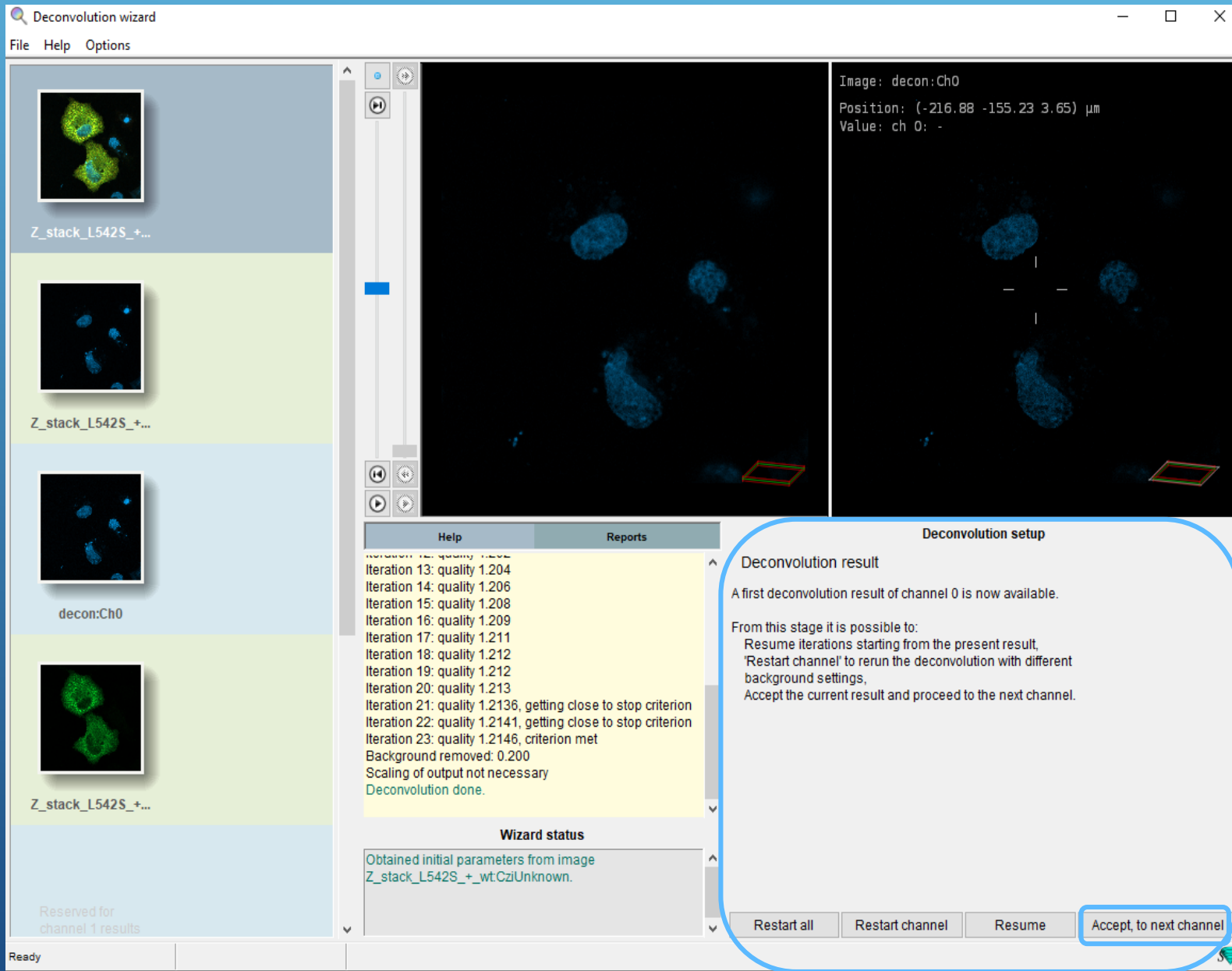
Go ON - Deconvolve



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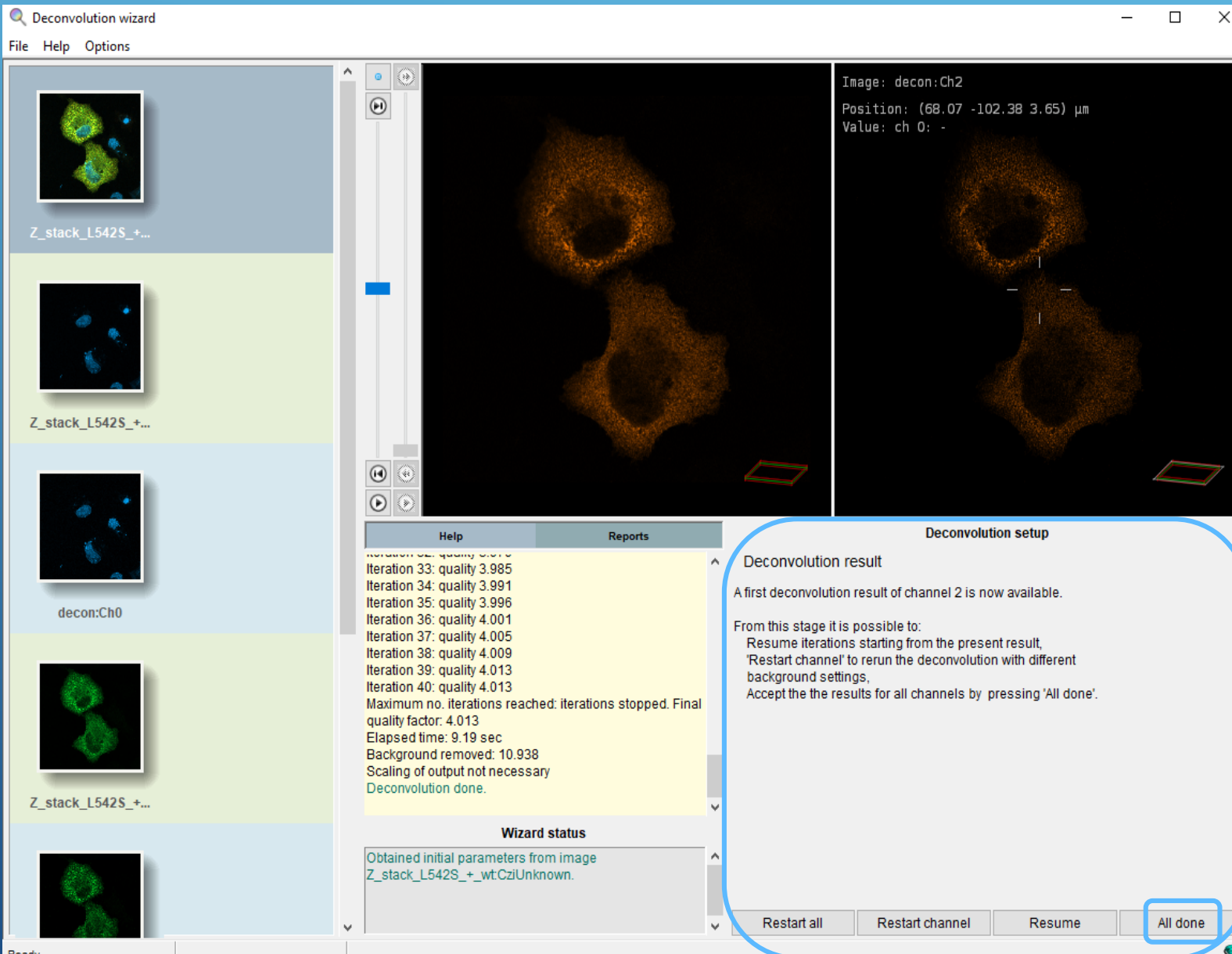
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Deconvolution Wizard

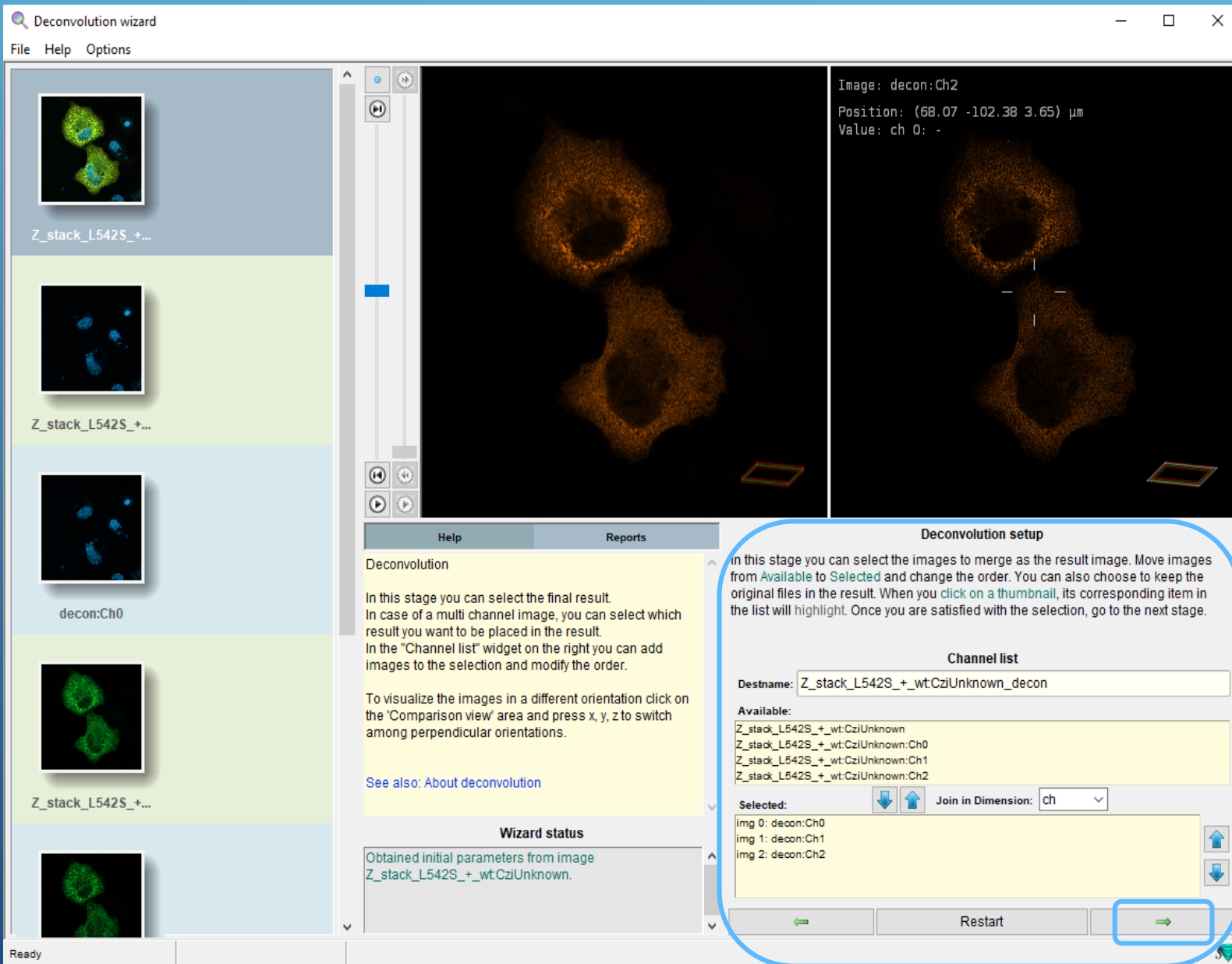
- **Accept** , to next channel

Then **repeat** with **all channels**



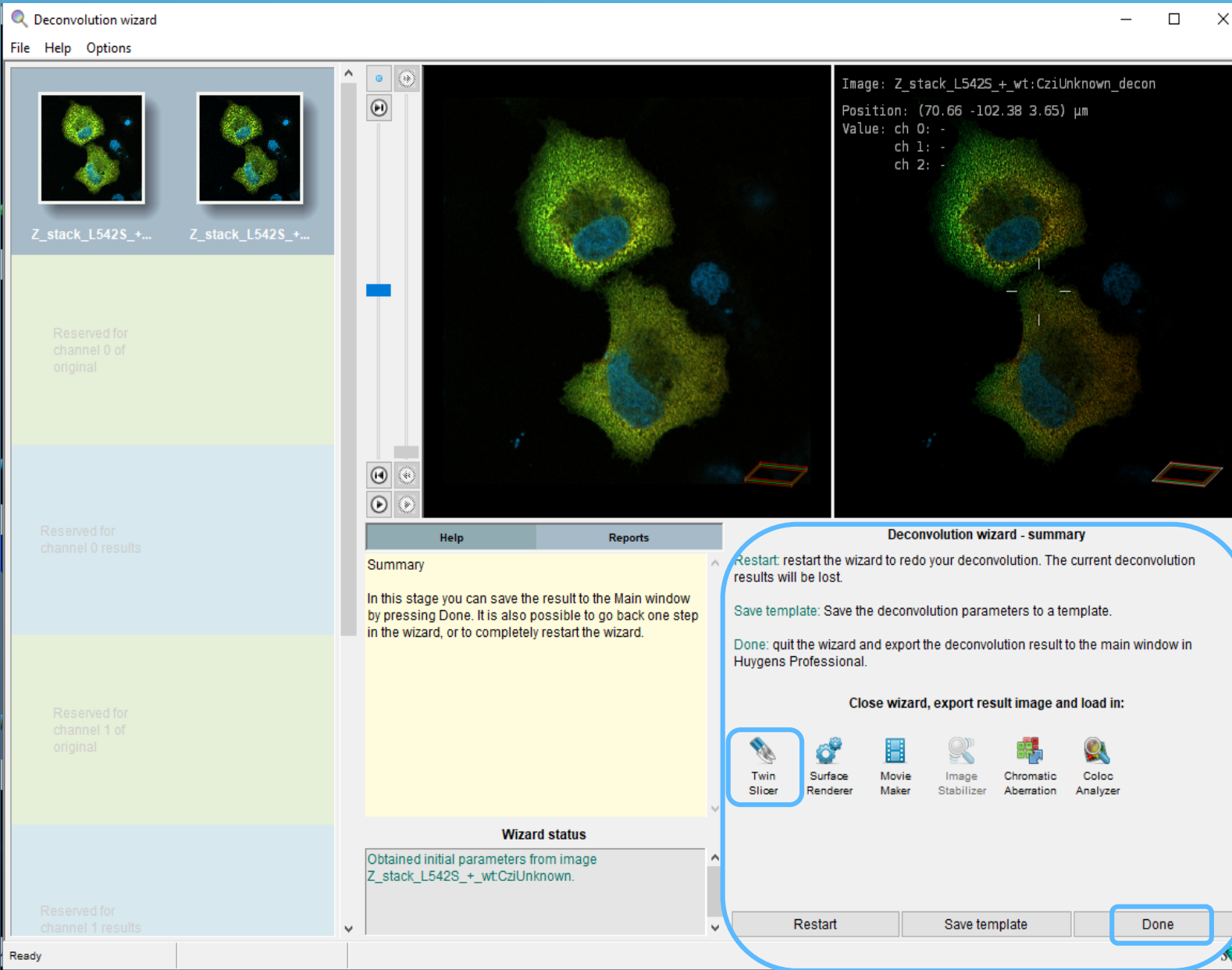
Deconvolution Wizard

- After **all Channels** are **processed**
 - Go On - All done



Deconvolution Wizard

- Go On



Deconvolution Wizard

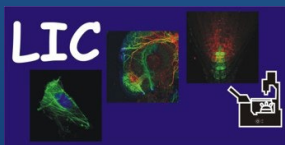
- **Go On – Done**

The processed data will now be shown in the Huygens Professional Main Window

- **or**

Use e.g. the **Twin Slicer** to look at your data

The Twin Slicer allows to synchronize views of two images, measure distances, plot line profiles and more



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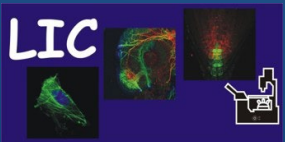
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Save your processed data for further analysis

- **ICS or ICS2** is the recommended format - provides good dynamic range and necessary meta data infrastructure to save all the image parameters - **creates more than one file for each restored image**, all have to be saved and/or copied!
- **IMS (Imaris classic)** – also ok, creates only one file for each restored image, but always **only 8bit images**

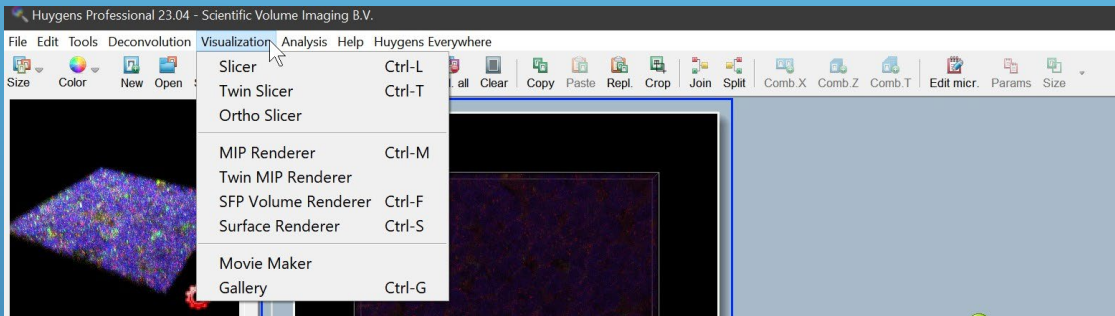
Further details: <https://svi.nl/FileFormats>



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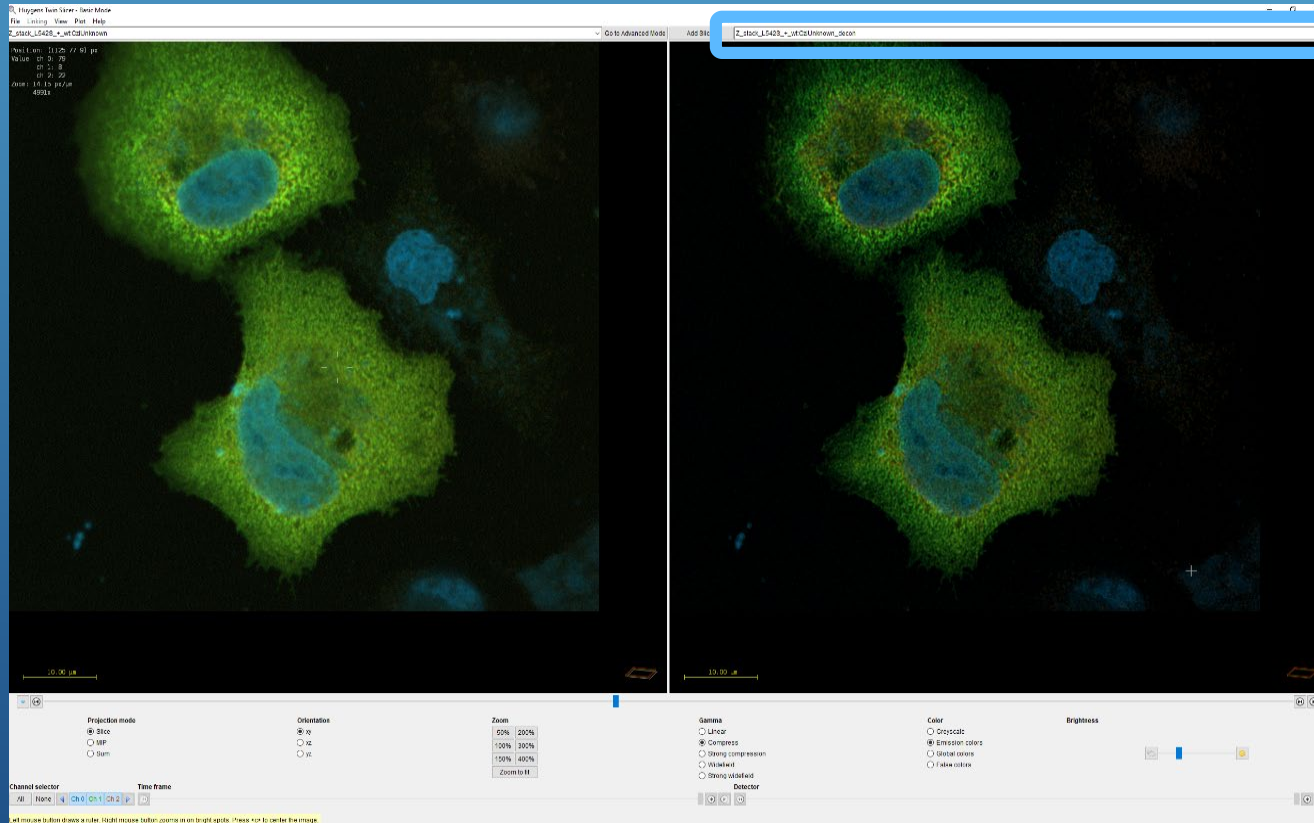
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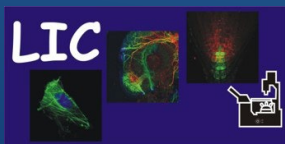


In the main menu in “**Visualization**” use e.g. the **Twin Slicer** to look at your data

- To view **another image** in an open slicer, click the image name in the **drop-down menu**, all open images are listed



The Twin Slicer allows to synchronize views of two images, measure distances, plot line profiles and more.

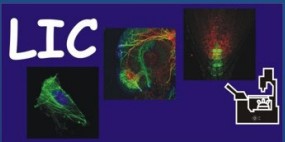


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Thanks for viewing



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