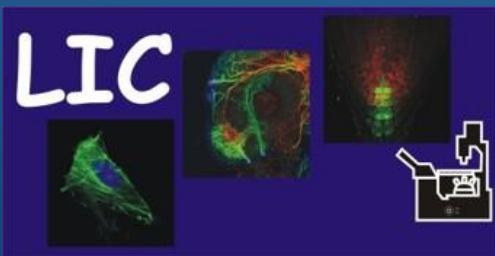


# Tutorial - Basic

## Huygens Professional

Life Imaging Center 2020



## Open - Huygens Professional



**Specification:** Huygens Core server (HRM) for deconvolution (256 GB RAM, 2 CPUs, 28 cores, 24 GB GPU VRAM, 6 GB SSD RAID 5, 28 TB storage RAID 5)

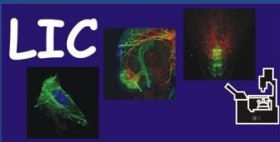
**Access:** on all LIC Workstation in the LIC

### 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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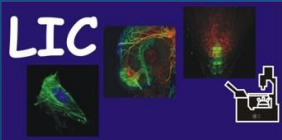
## Deconvolution Process in Short !

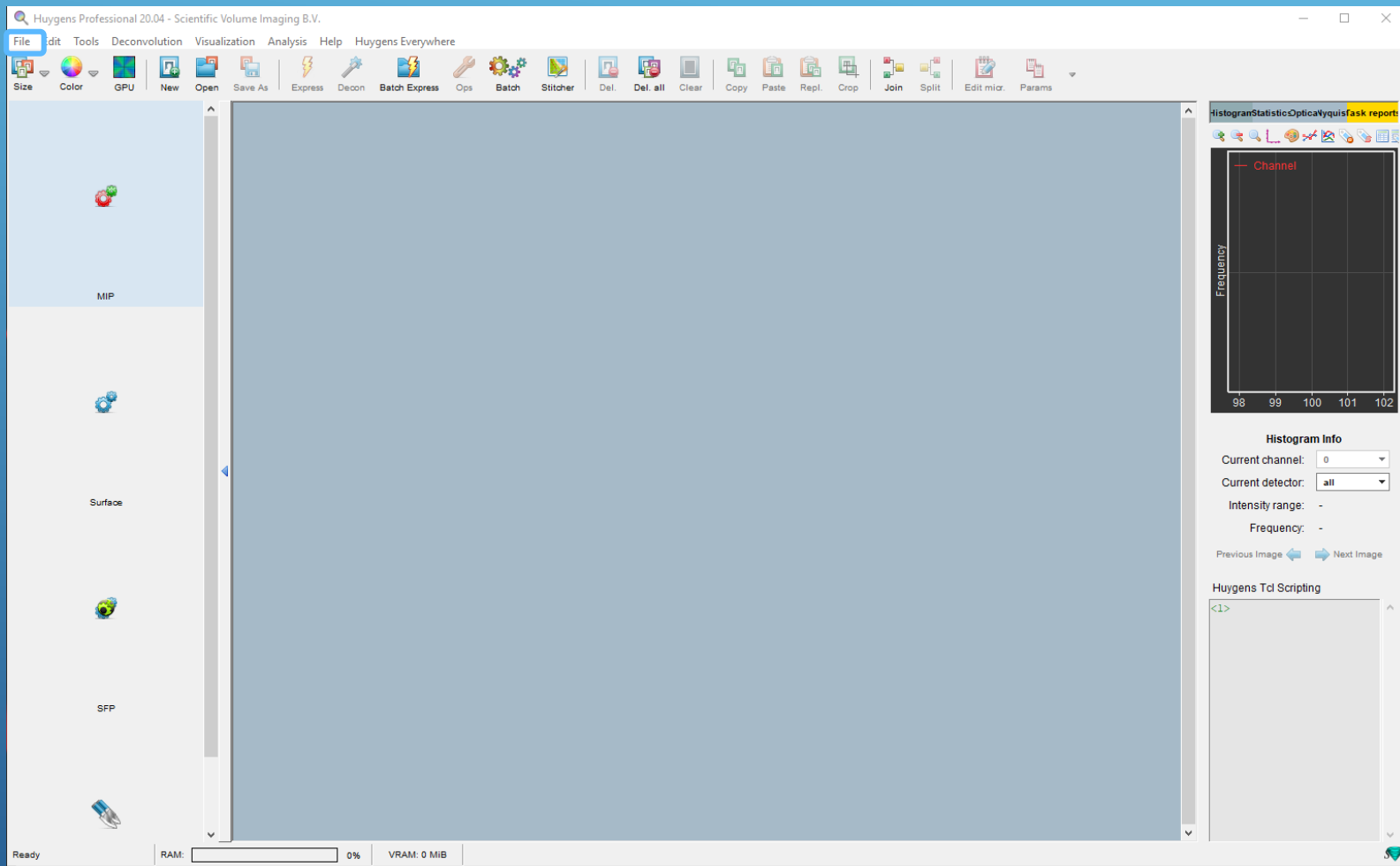
### You have no Microscopic Parameter and Deconvolution Templates

- 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



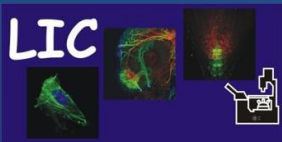


# Huygens Professional

Open Image File (File menu)

or

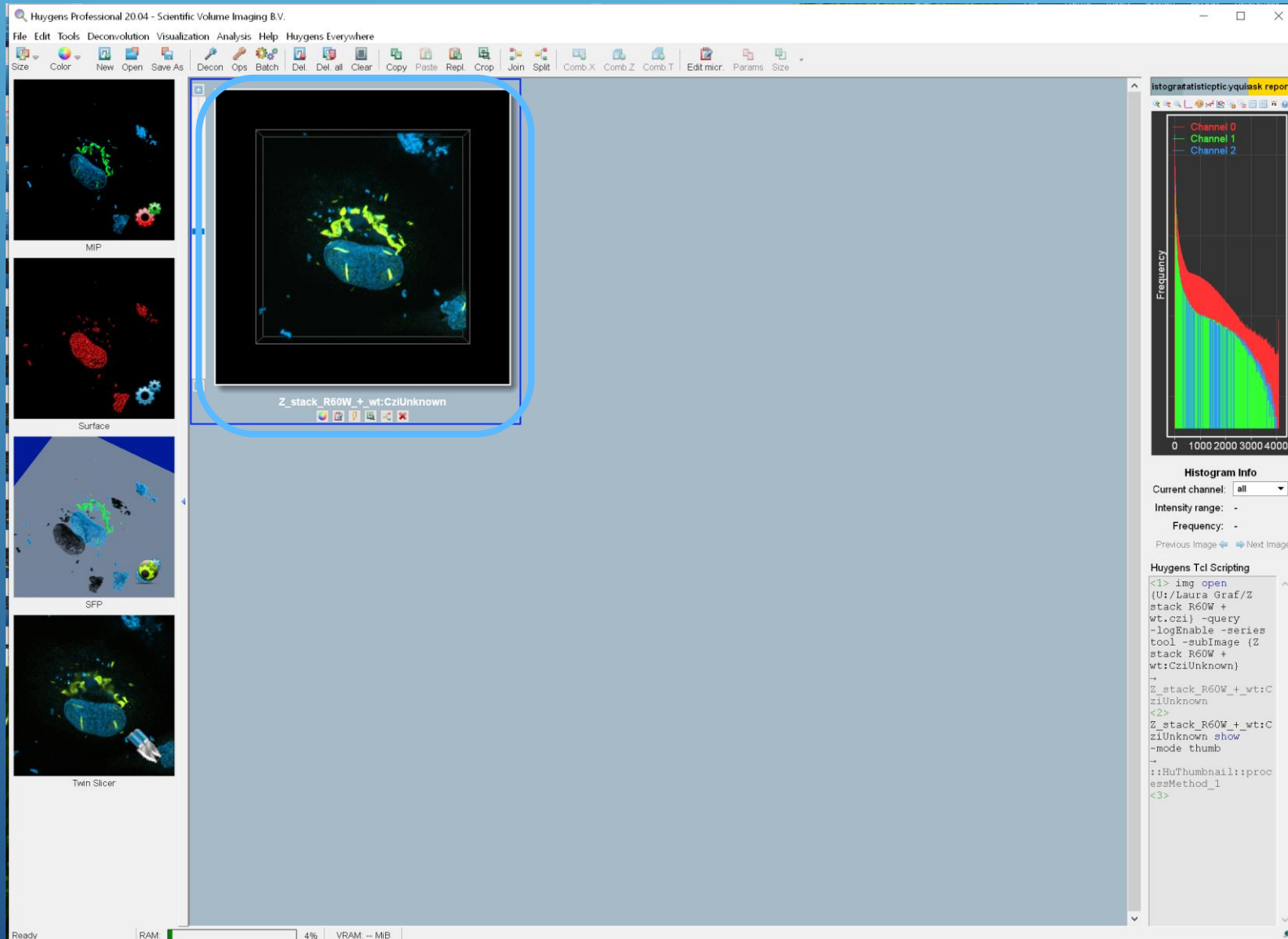
Drag and Drop



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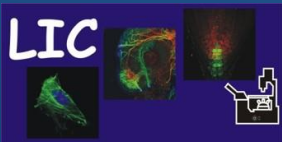
Huygens Professional





# Huygens Professional

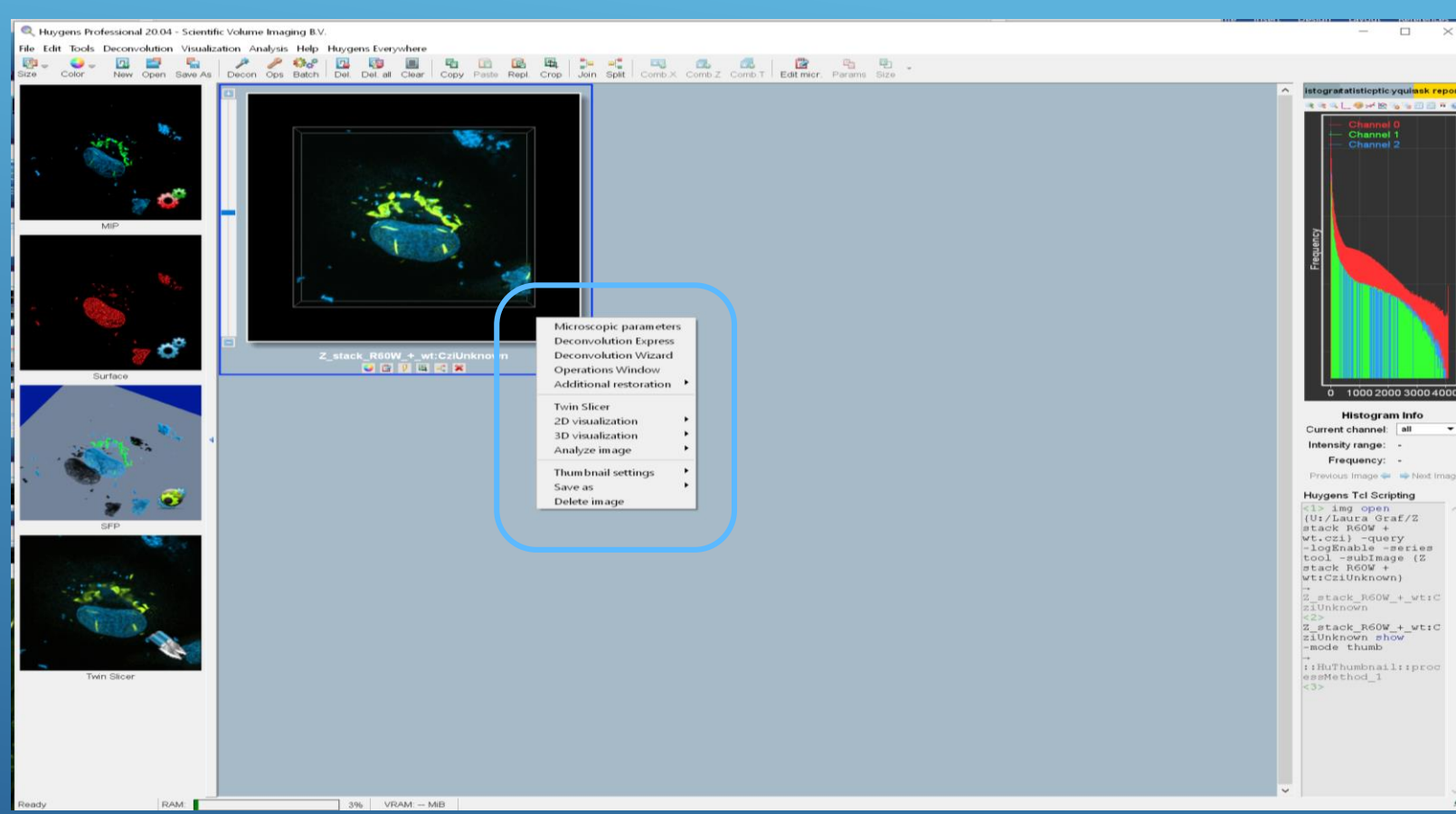
Select Image



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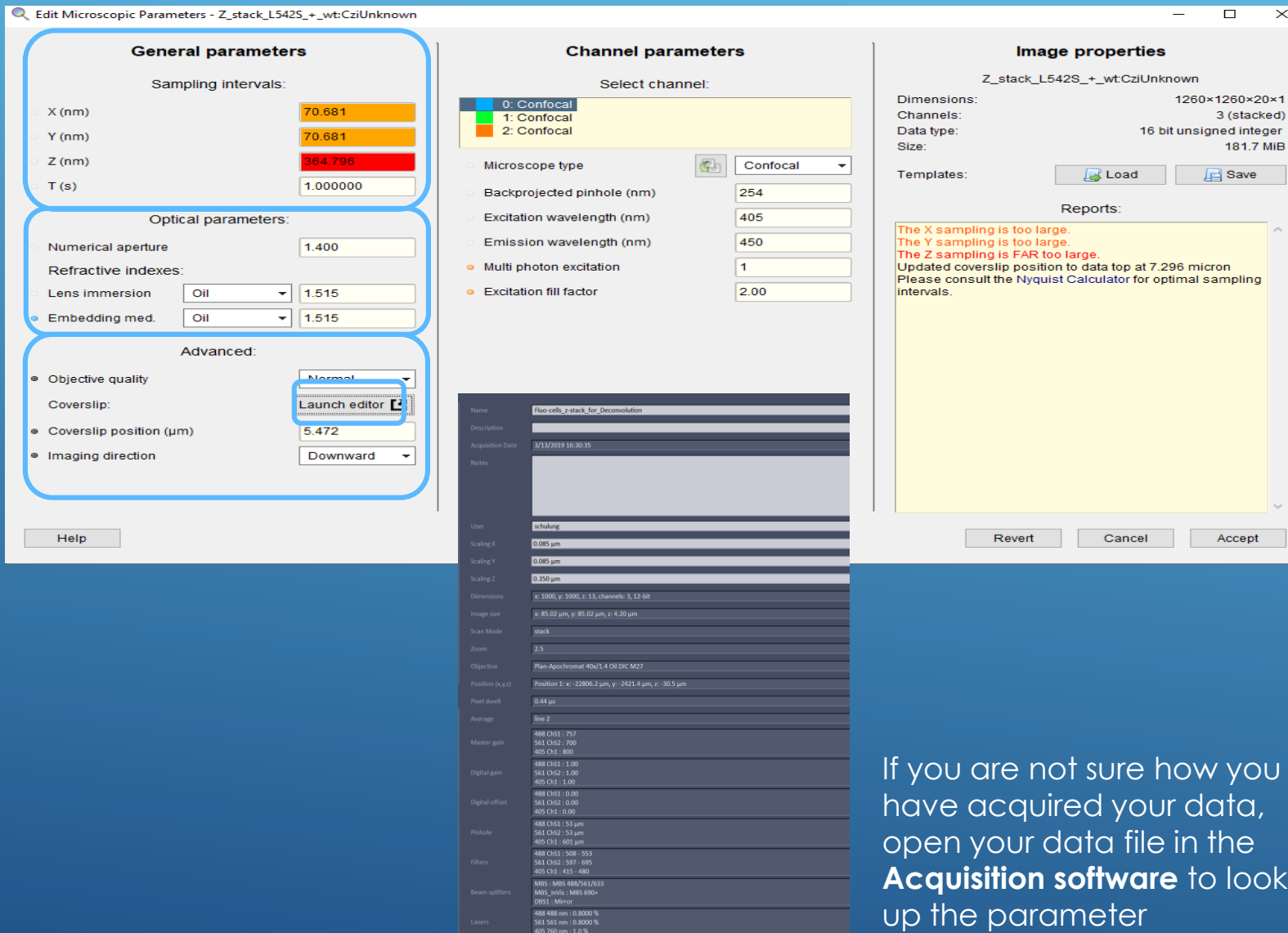


# Huygens Professional

Right Mouse Click on Image

- A menu appears

Start **Microscopic parameters**



## Open Microscopic Parameter

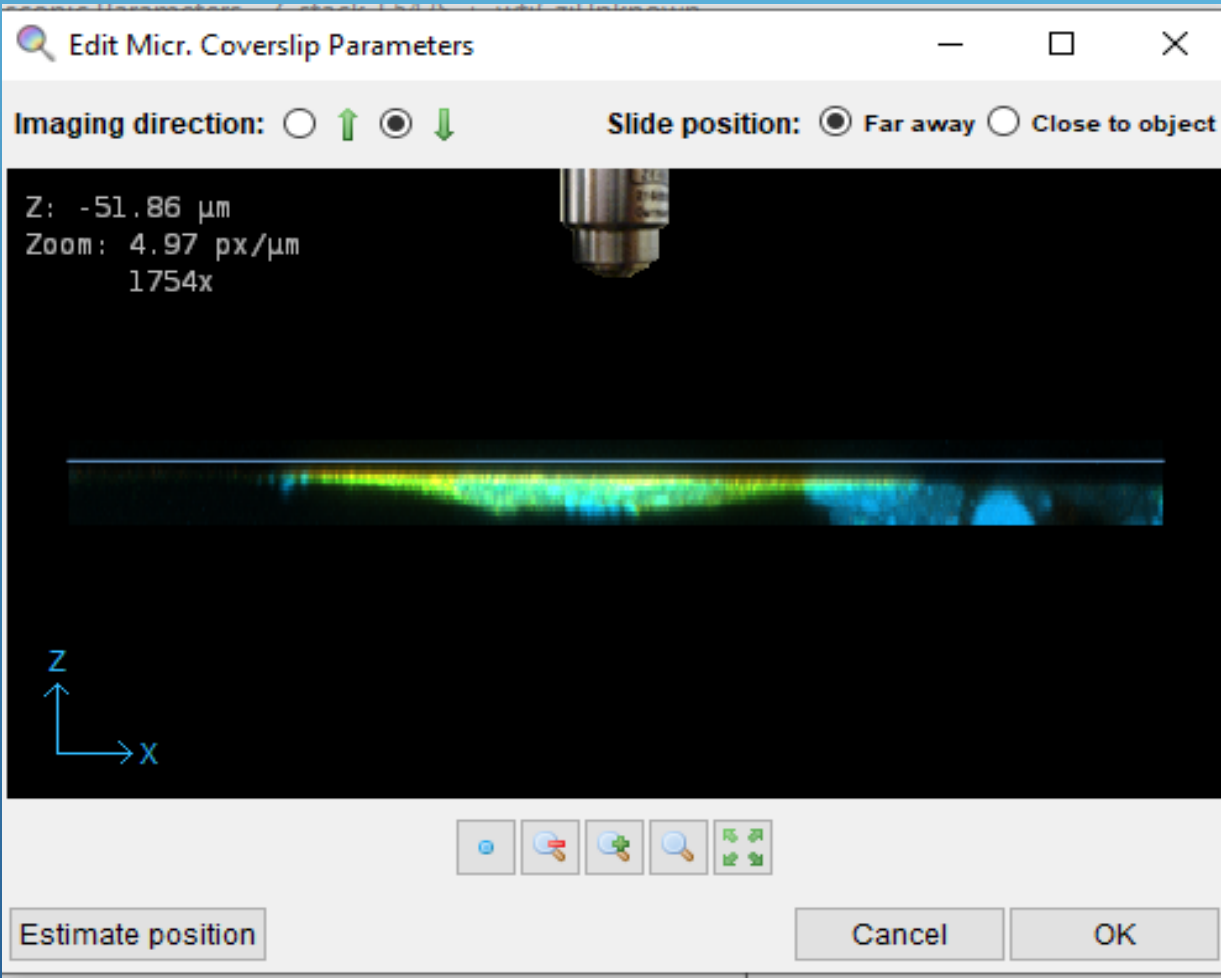
Huygens will read image meta data but some values have to be specified

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





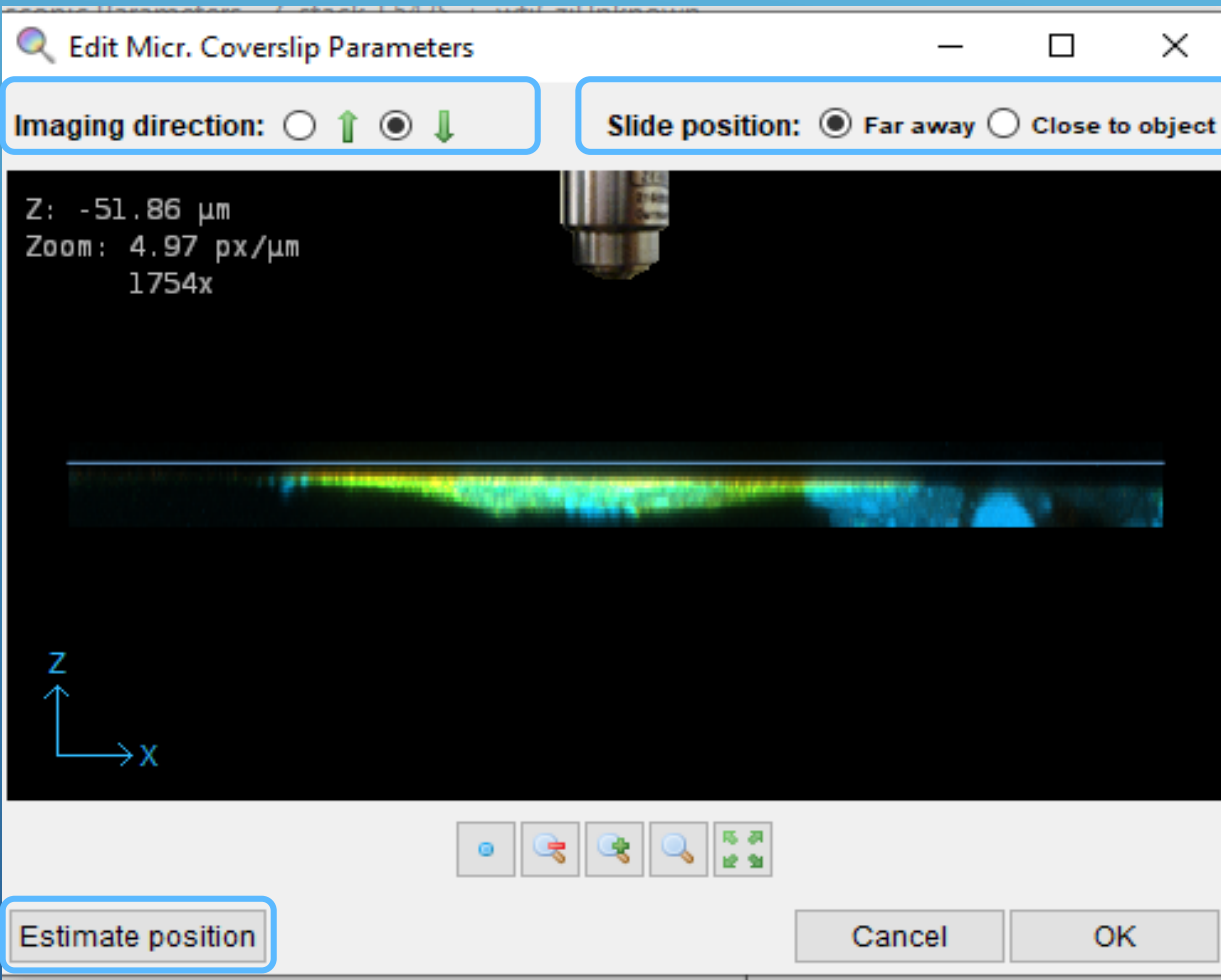
## Coverslip Editor

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): 864.796
- 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.

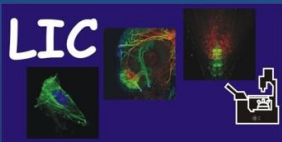
Help

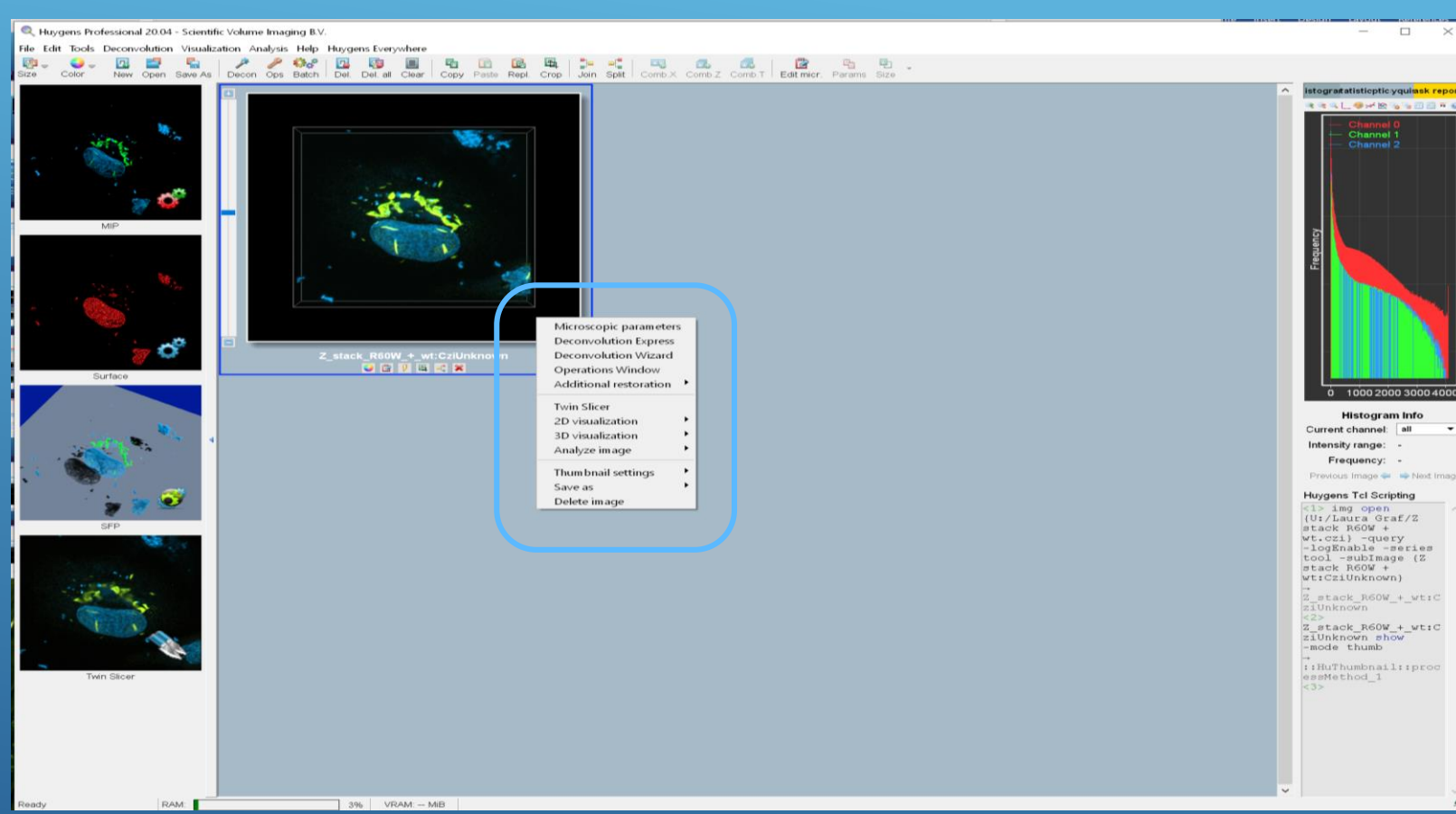
Not all parameters verified Set all verified

Revert Cancel Accept

## Open Microscopic Parameter

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

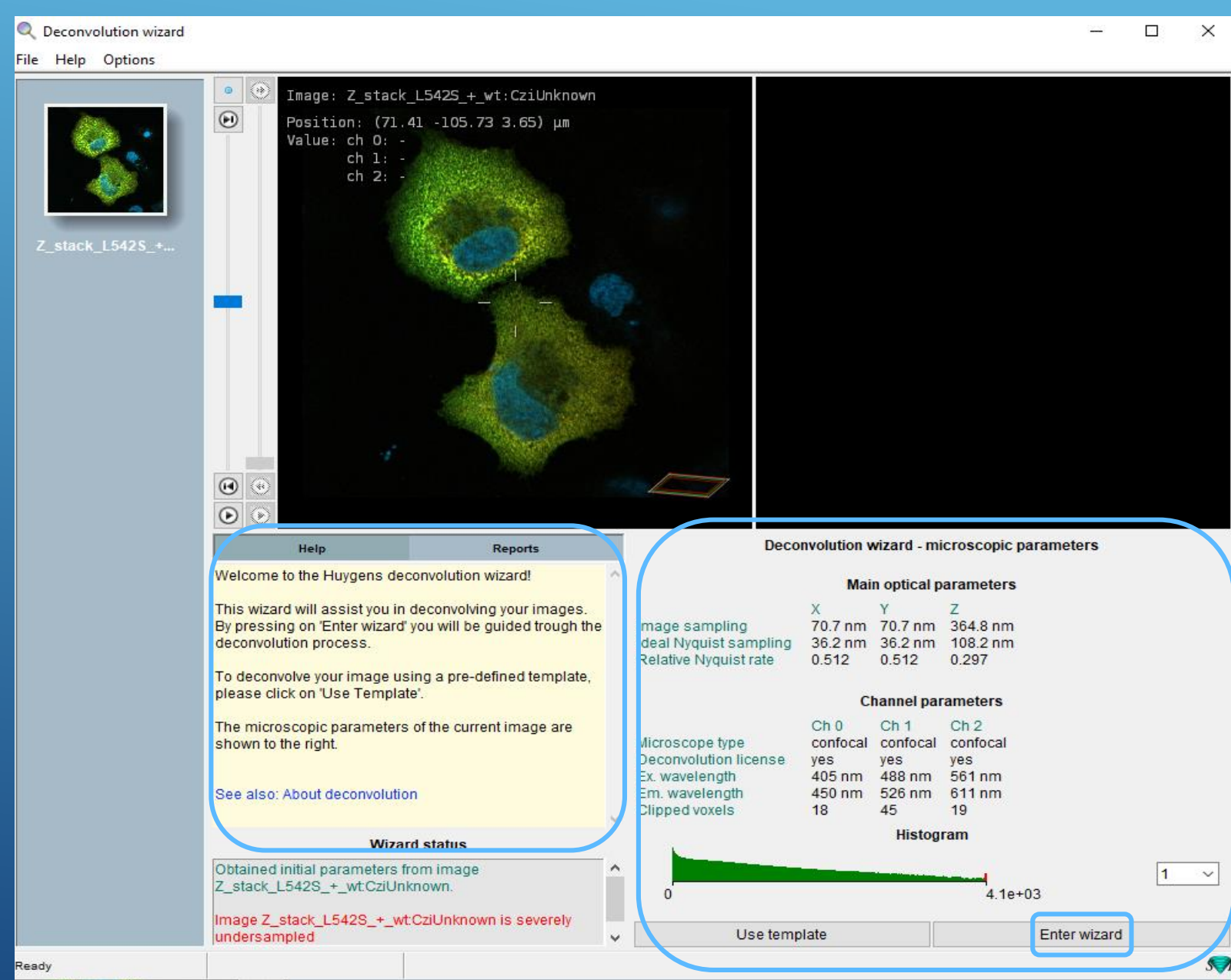




## Huygens Professional

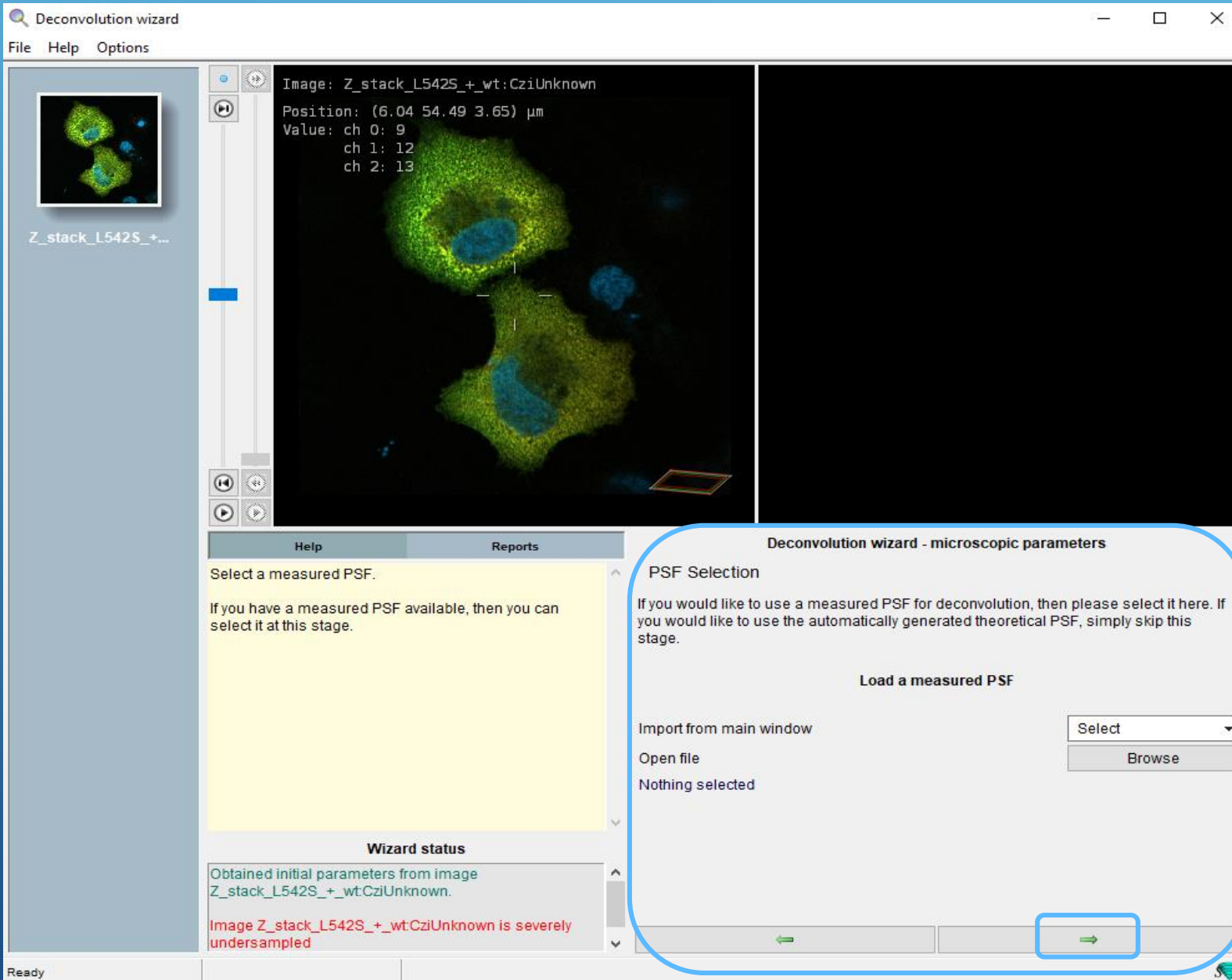
Again **Right Mouse Click** on Image

Start **Deconvolution Wizzard**



## Open Deconvolution Wizzard

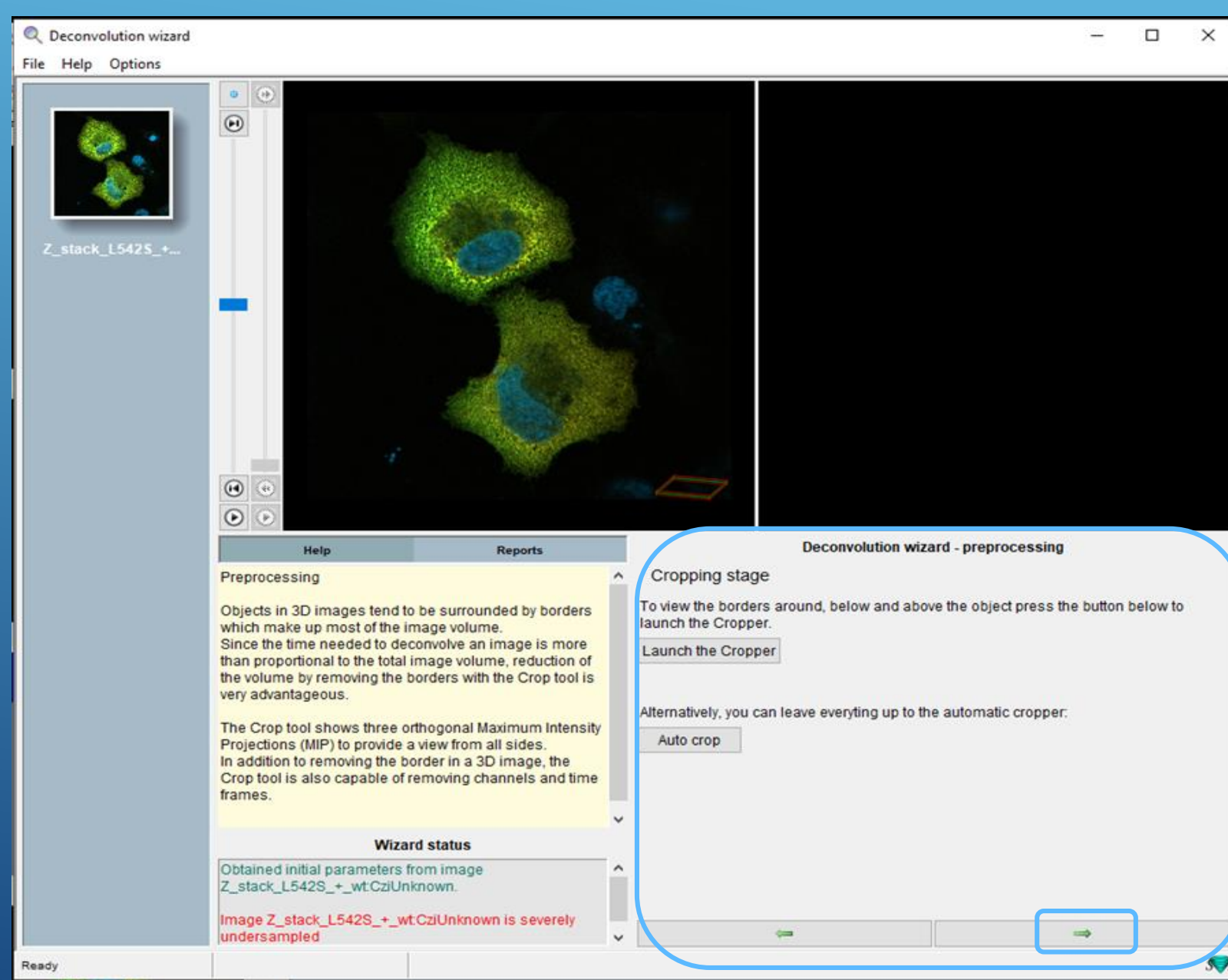
- On the left side - **Help explanations** concerning the wizard step
- Enter Wizzard**



## Open Deconvolution Wizard

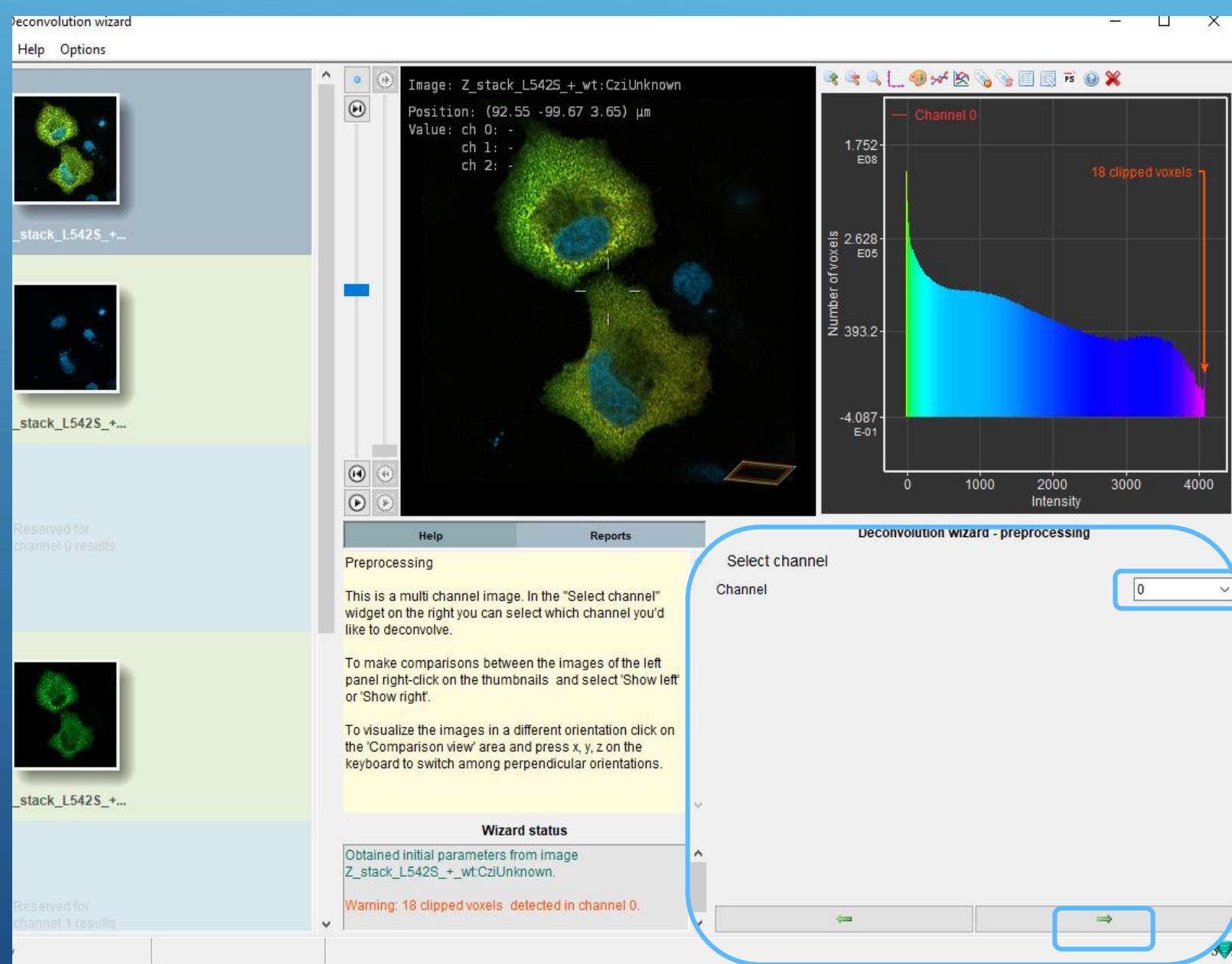
- If you have measured a **PSF** load this here, otherwise software use a **theoretical PSF** (default setup)
- **Go On**





## Open Deconvolution Wizzard

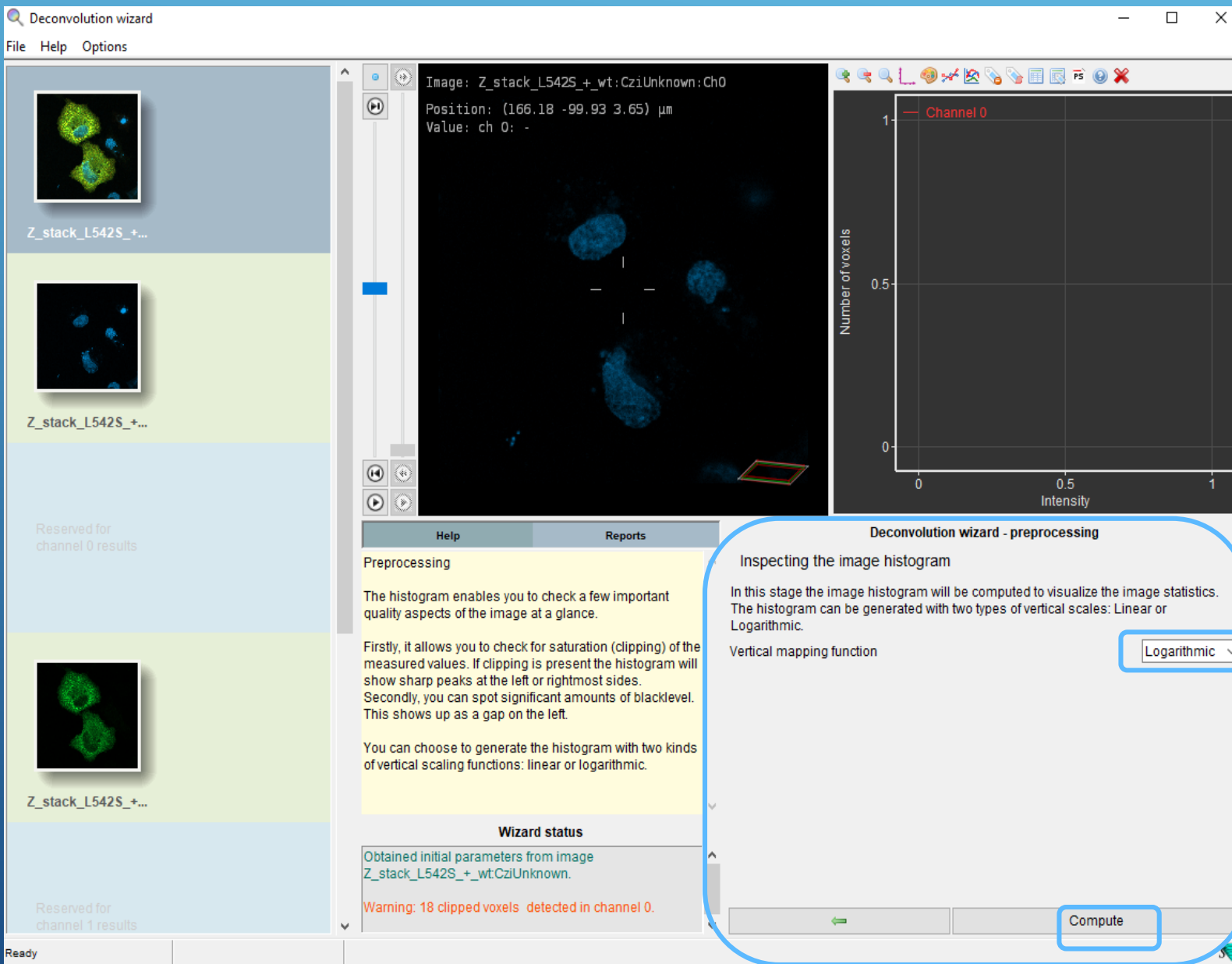
- **Crop** sample , if necessary
- **Go On**



## Open Deconvolution Wizard

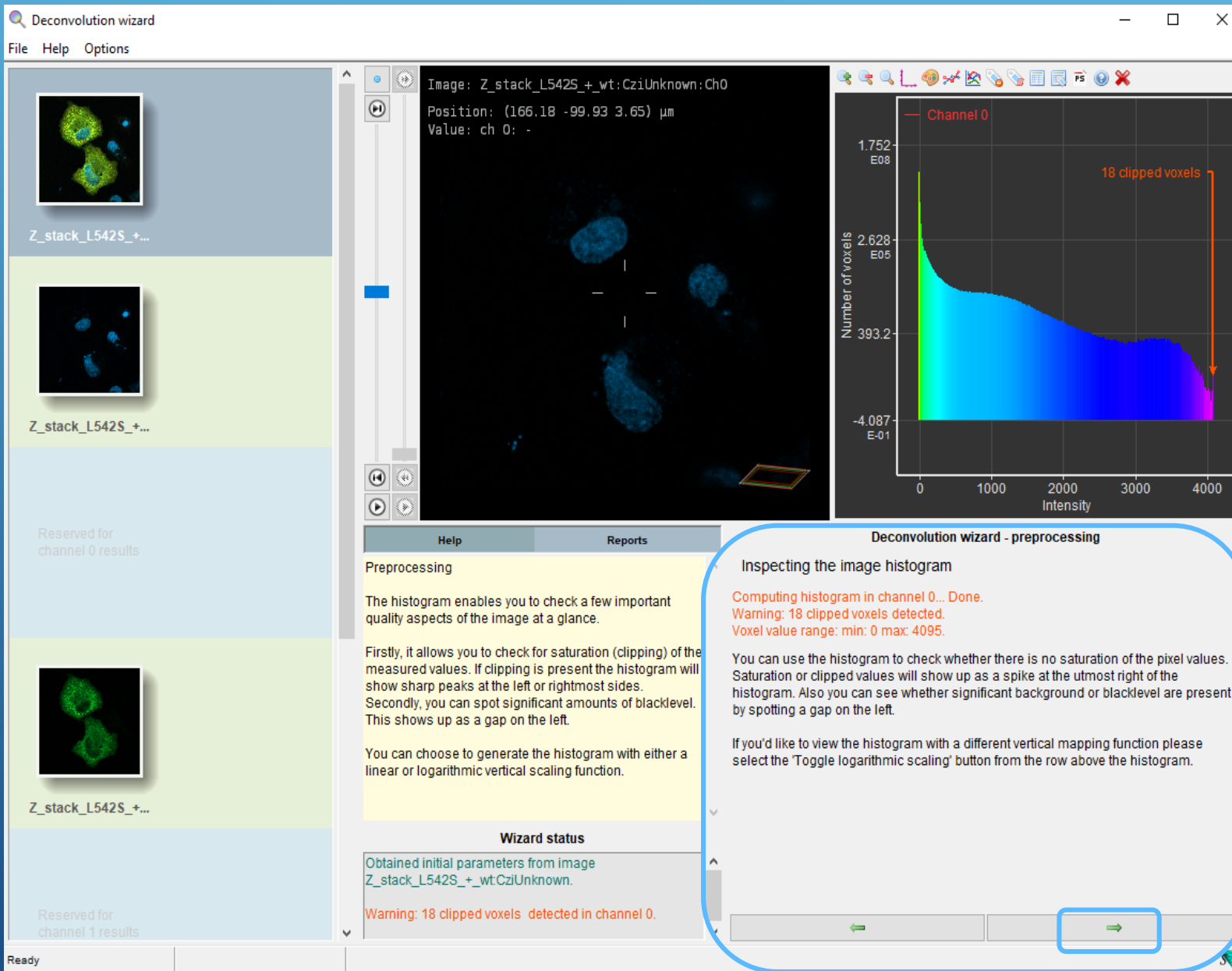
- The **channel number counting** in Huygens is different
  - Huygens will start with Channel 0 - this corresponds to Channel 1
- Go On





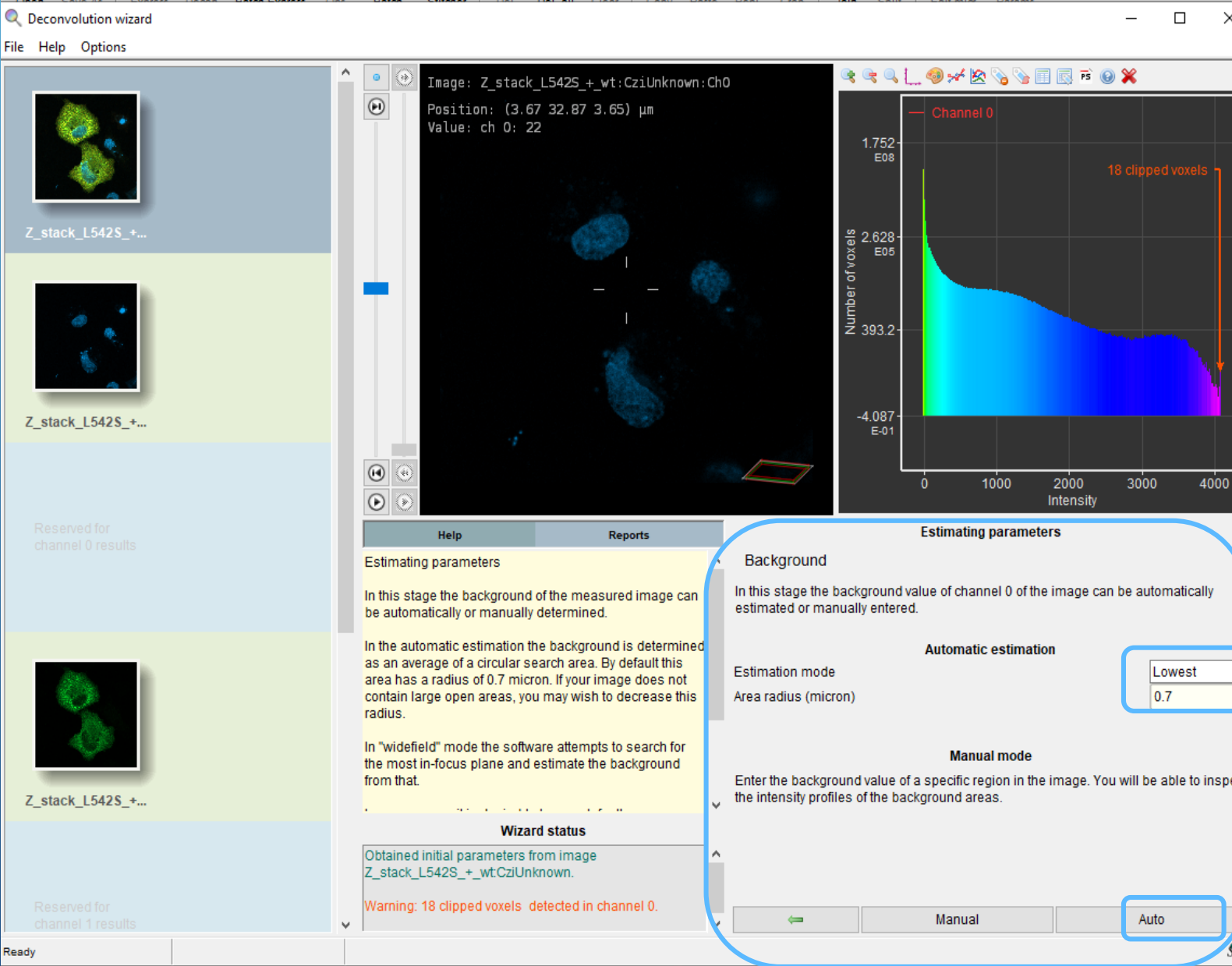
## Open Deconvolution Wizzard

- **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**



## Open Deconvolution Wizard

- Go ON



## Open Deconvolution Wizzard

- The **mean background** can be estimated **automatically** in Huygens Professional with the Estimate background tool in the Analysis menu of the Operations window.
- Use a search area of **0.7 micron radius**.
- If your image does not contain large open areas, decrease the radius.
- Go On - Auto

## Open Deconvolution Wizzard

- Go On - Accept

File Help Options

Image: Z\_stack\_L542S+\_wt:CziUnknown:Ch0  
Position: (3.67 32.87 3.65)  $\mu\text{m}$   
Value: ch 0: 22

Channel 0

Number of voxels

Intensity

18 clipped voxels

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.

Since this is 3D confocal data you have the choice between either the fast Good's roughness GML method or the robust CMLE method.

Wizard status

Obtained initial parameters from image Z\_stack\_L542S+\_wt:CziUnknown.

Warning: 18 clipped voxels detected in channel 0.

Background Estimation: automatic mode

Background to be used during deconvolution

Absolute background 0.1999

Relative background (%) 0.0

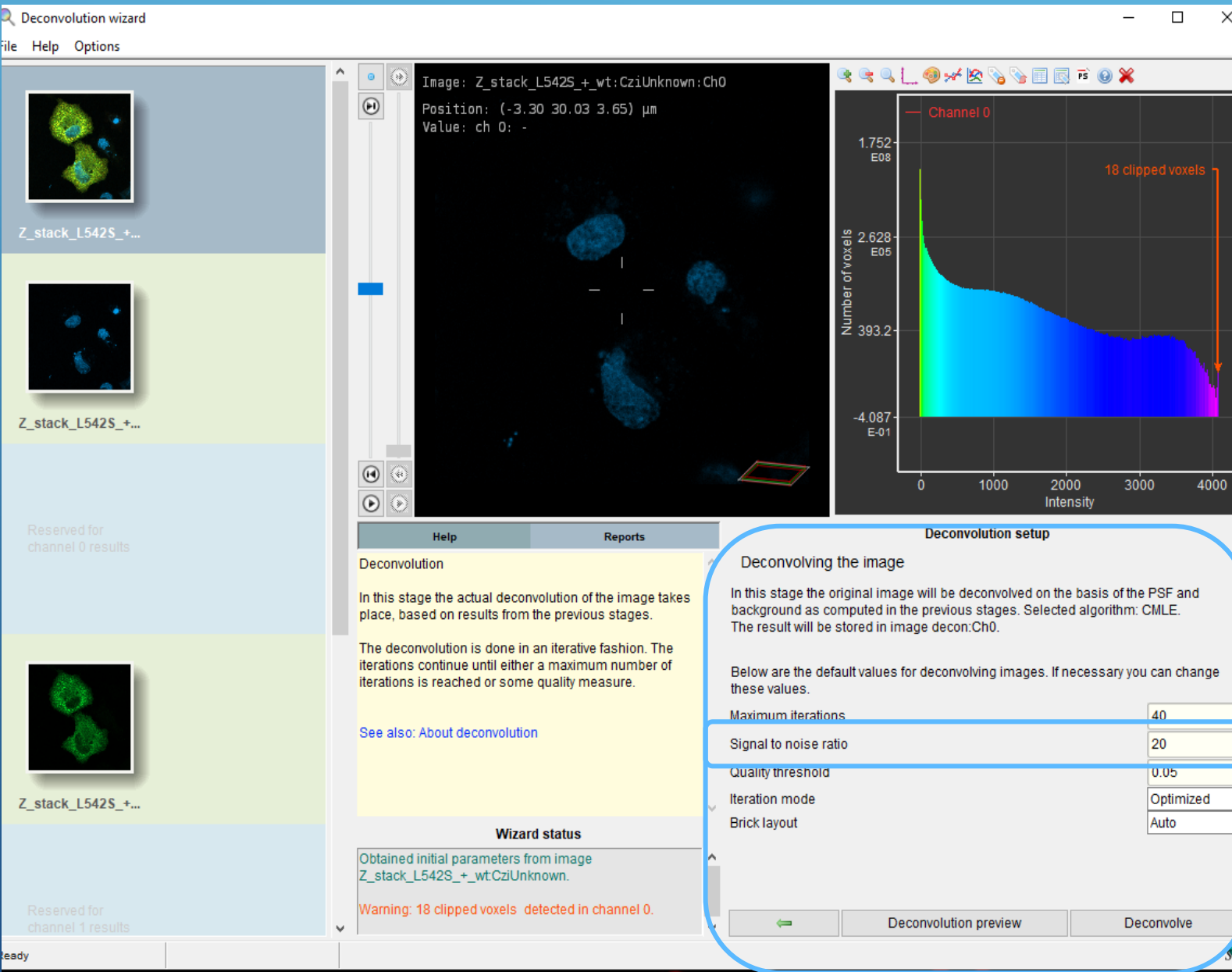
☐ Log absolute background instead of relative in template.

Select deconvolution algorithm

Deconvolution algorithm CMLE

Accept





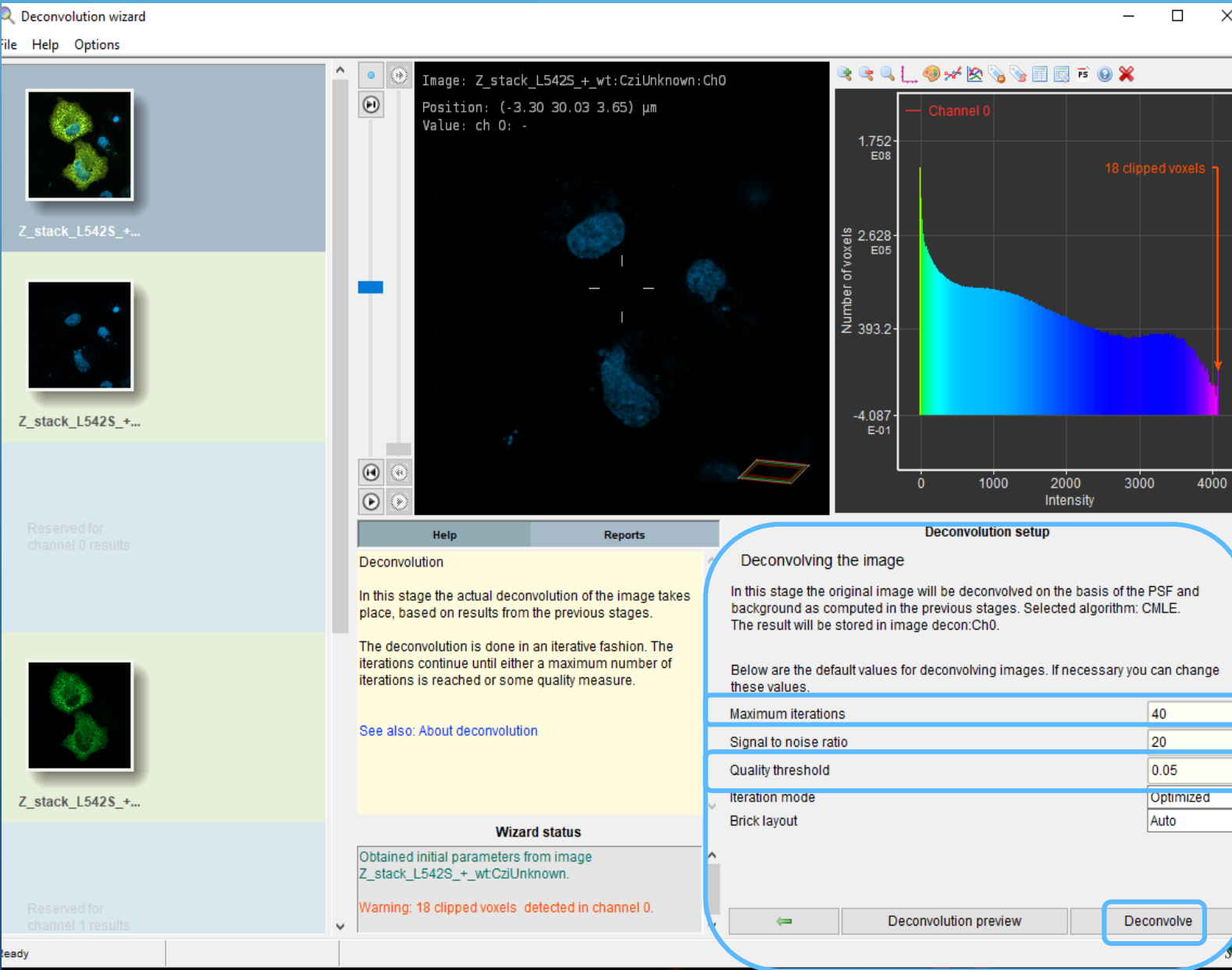
## Open Deconvolution Wizard

Estimate the **Signal to Noise Ratio** with the integrated tool - follow the instructions of the estimator

- The **Signal-to-Noise ratio (SNR or S/N)** controls the **sharpness** of the restoration result- as higher this value, as sharper the restored image will be.

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/ = 50**.
- A noisy **confocal** image can have values **lower than/ = 20**
- For a noisy **STED** values **below/ = 12**

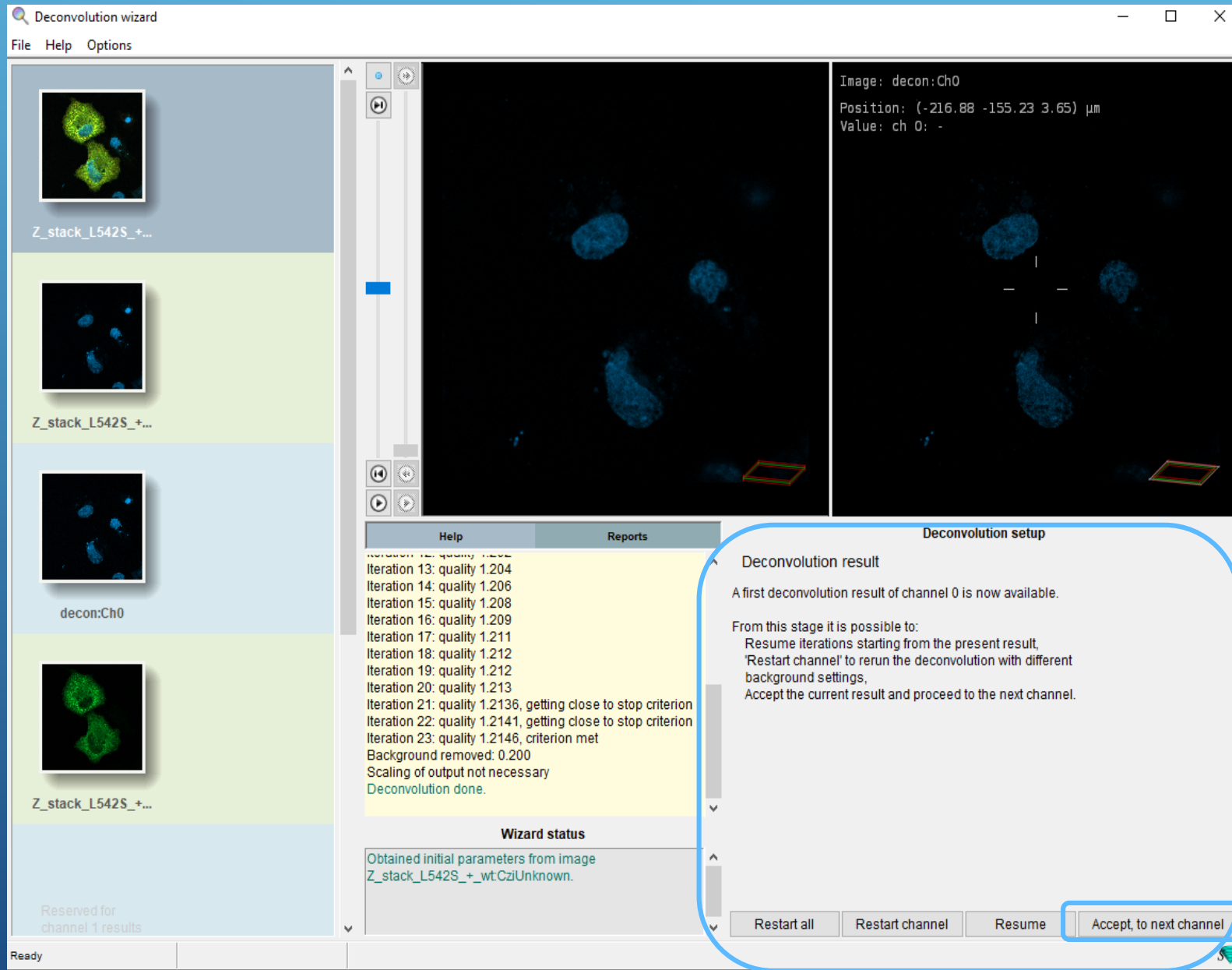


## Open Deconvolution Wizard

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 iteration** and **quality change 0.05**.

Go ON - Deconvolve

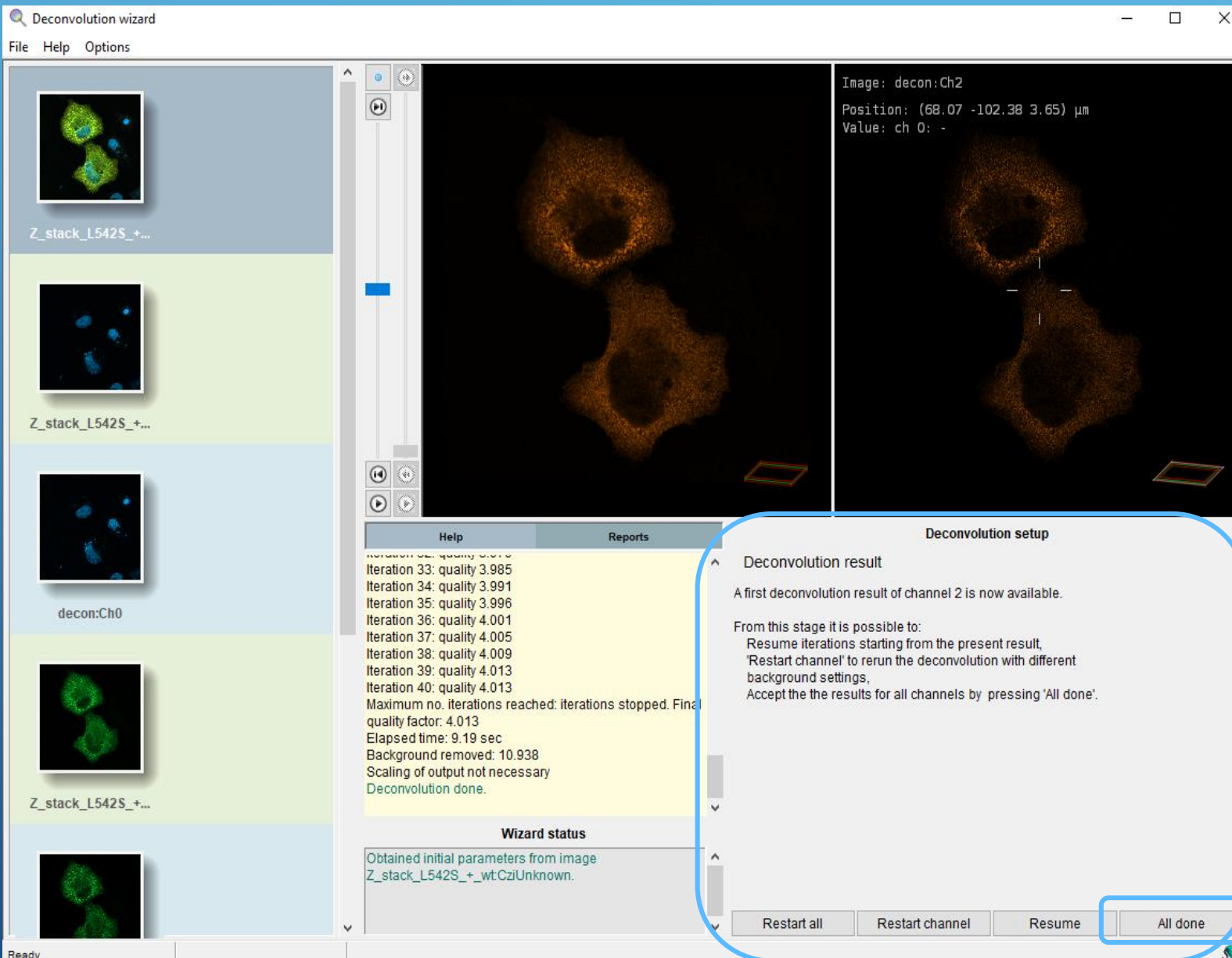


## Open Deconvolution Wizzard

- **Accept** , to next channel

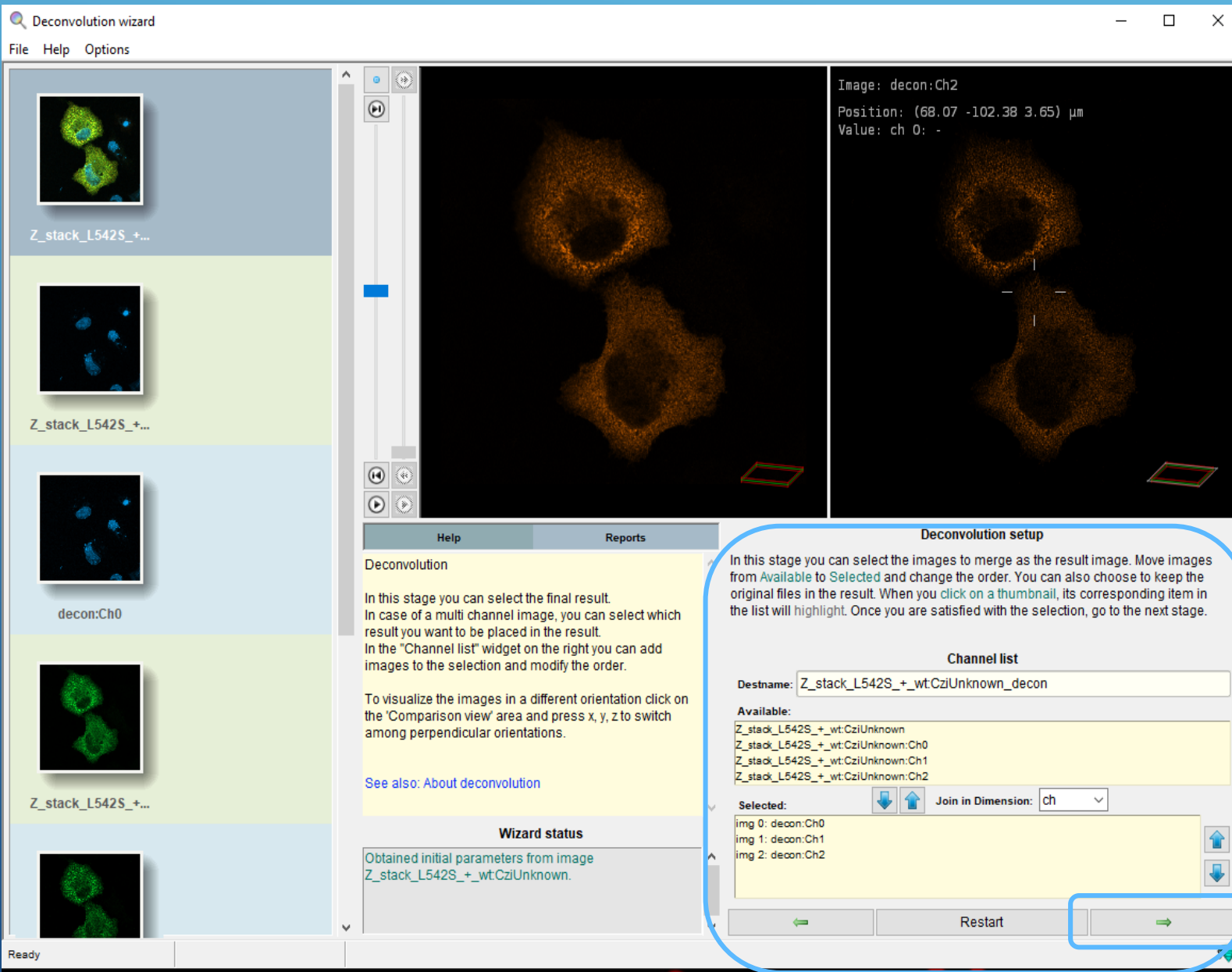
Then **Go On** as before.





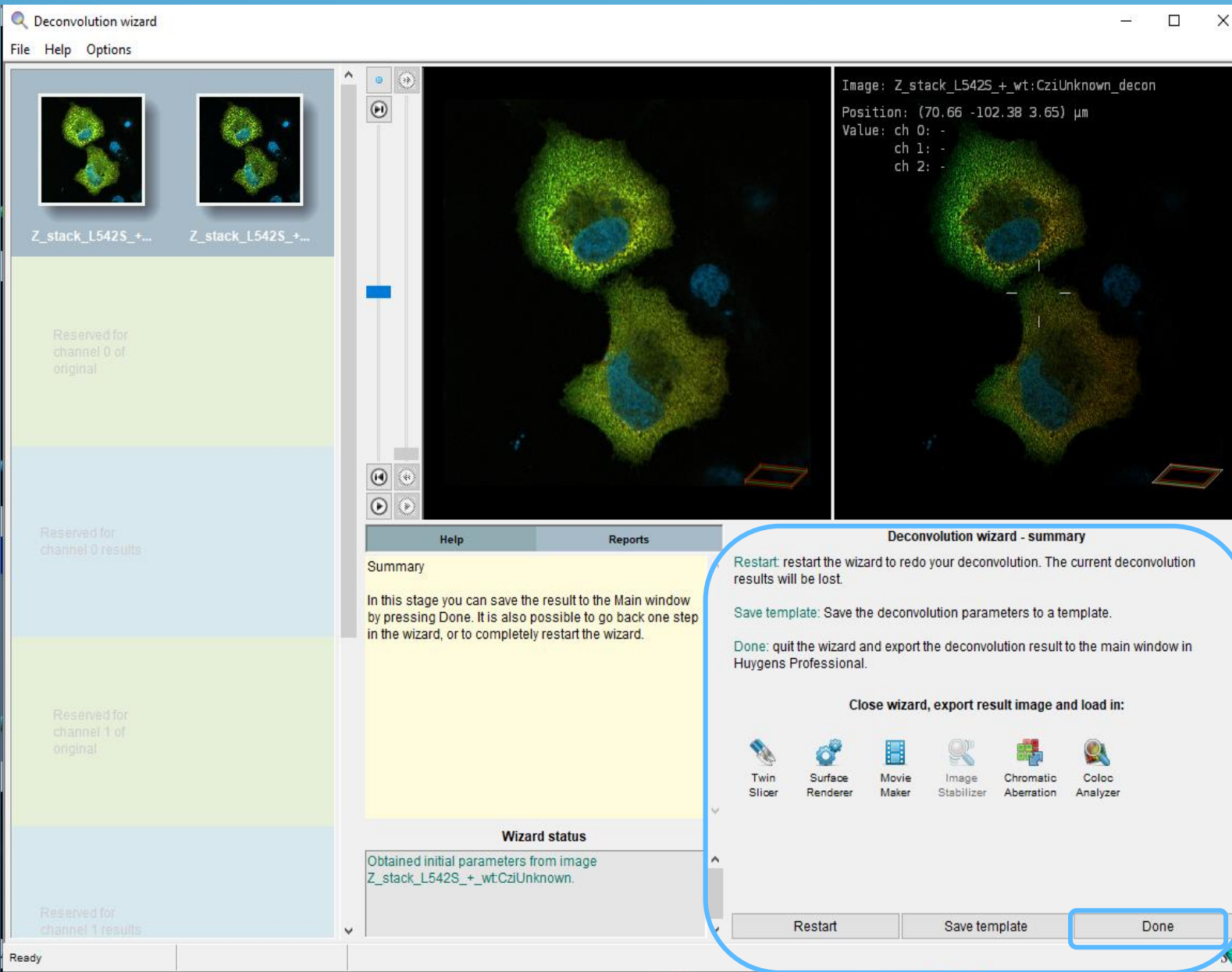
## Open Deconvolution Wizzard

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



## Open Deconvolution Wizzard

- Go On



## Open Deconvolution Wizzard

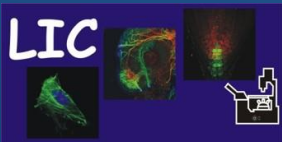
- Go On – Done

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

## Save you 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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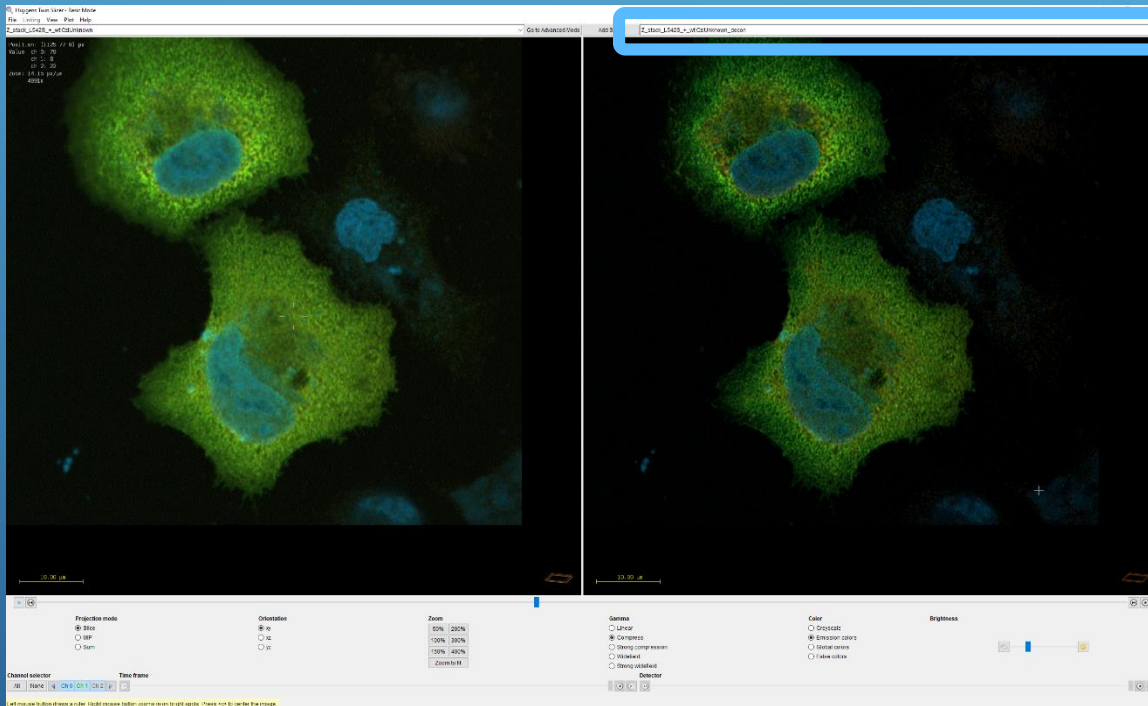




- **select** image - **choose** 'Twin Slicer' from the 'Visualization' menu in the **main menu**

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

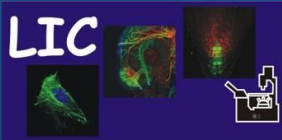
The Twin Slicer allows to synchronize views of two images, measure distances, plot line profiles and more.



Thanks for viewing

For further information

[i.E Online HRM - User Manual](#)



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