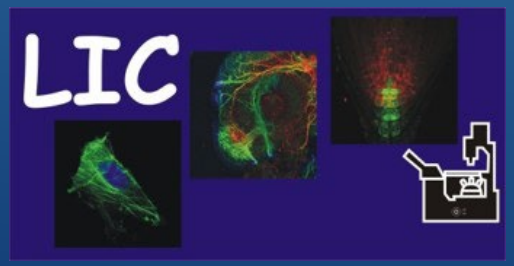
Tutorial - Basic

# Huygens Professional

Life Imaging Center

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# 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

# 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

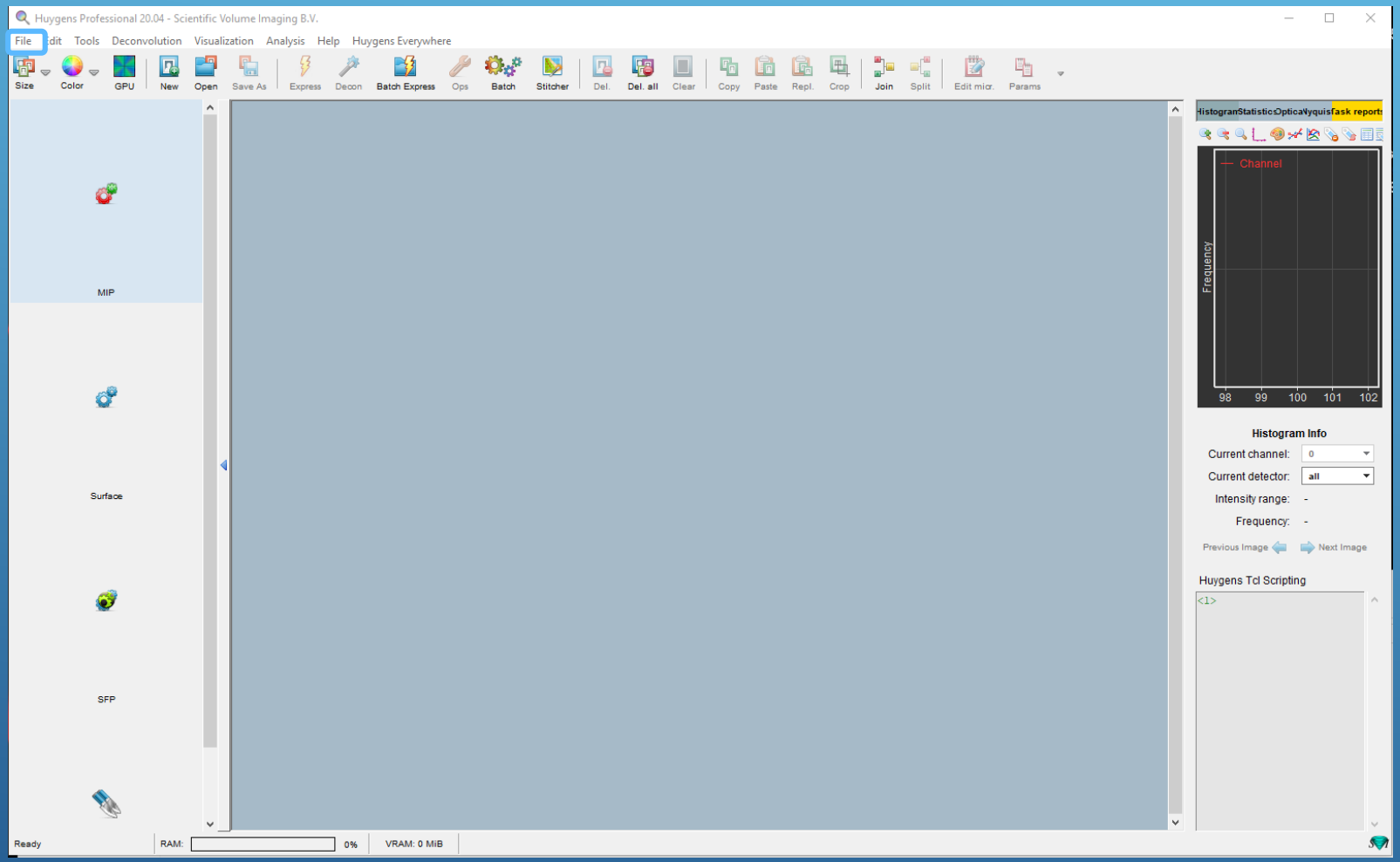
- Step A: Open image file, load and apply template of **Microscopic Parameter**
- Step B: Load and apply template of **Deconvolution Wizard**
- Step C: **Save** your deconvolved image

# Huygens Professional

**Open** Image **File** (File menu)

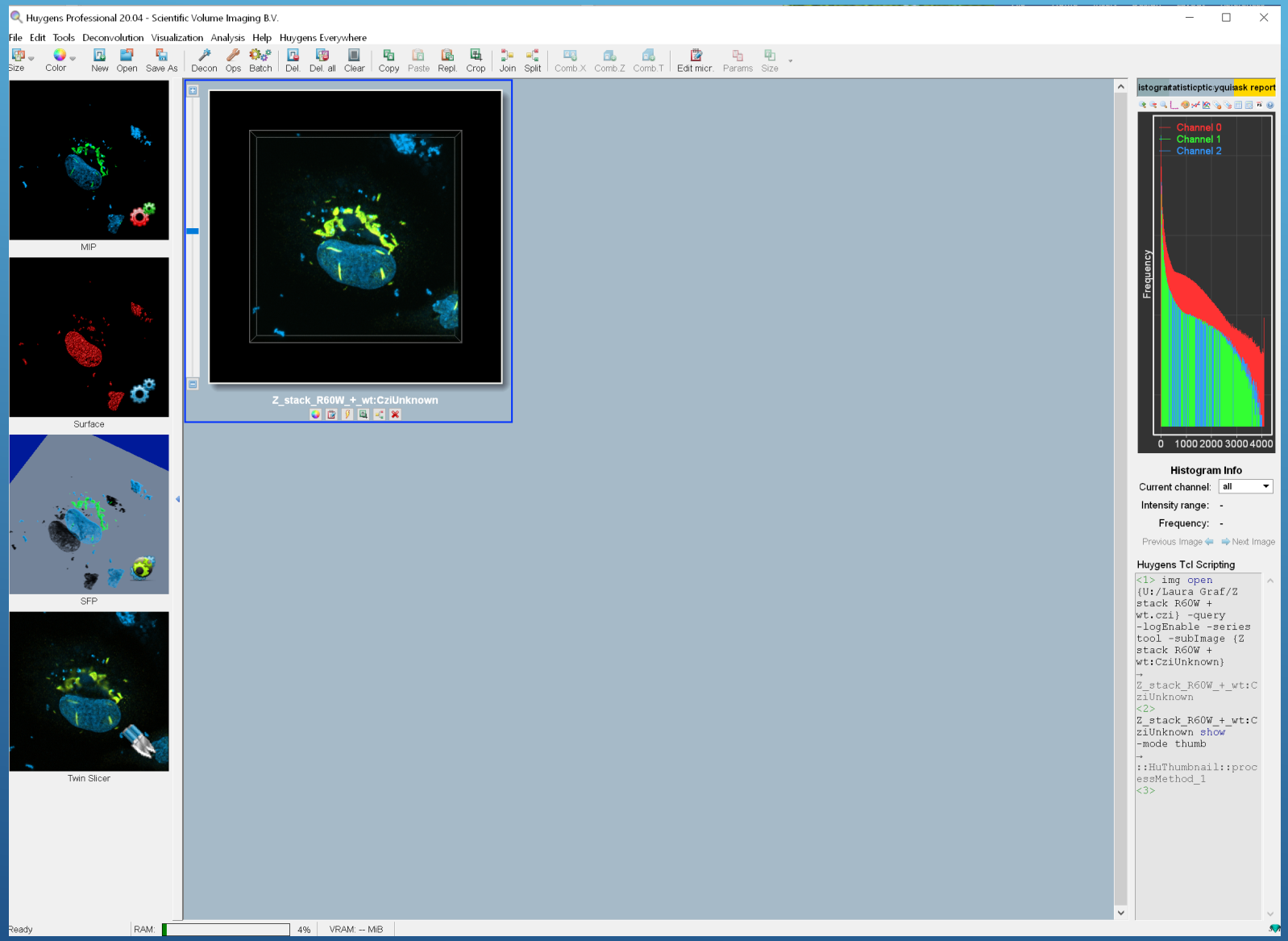
or

**Drag** and **Drop**



# Huygens Professional

**Select** Image

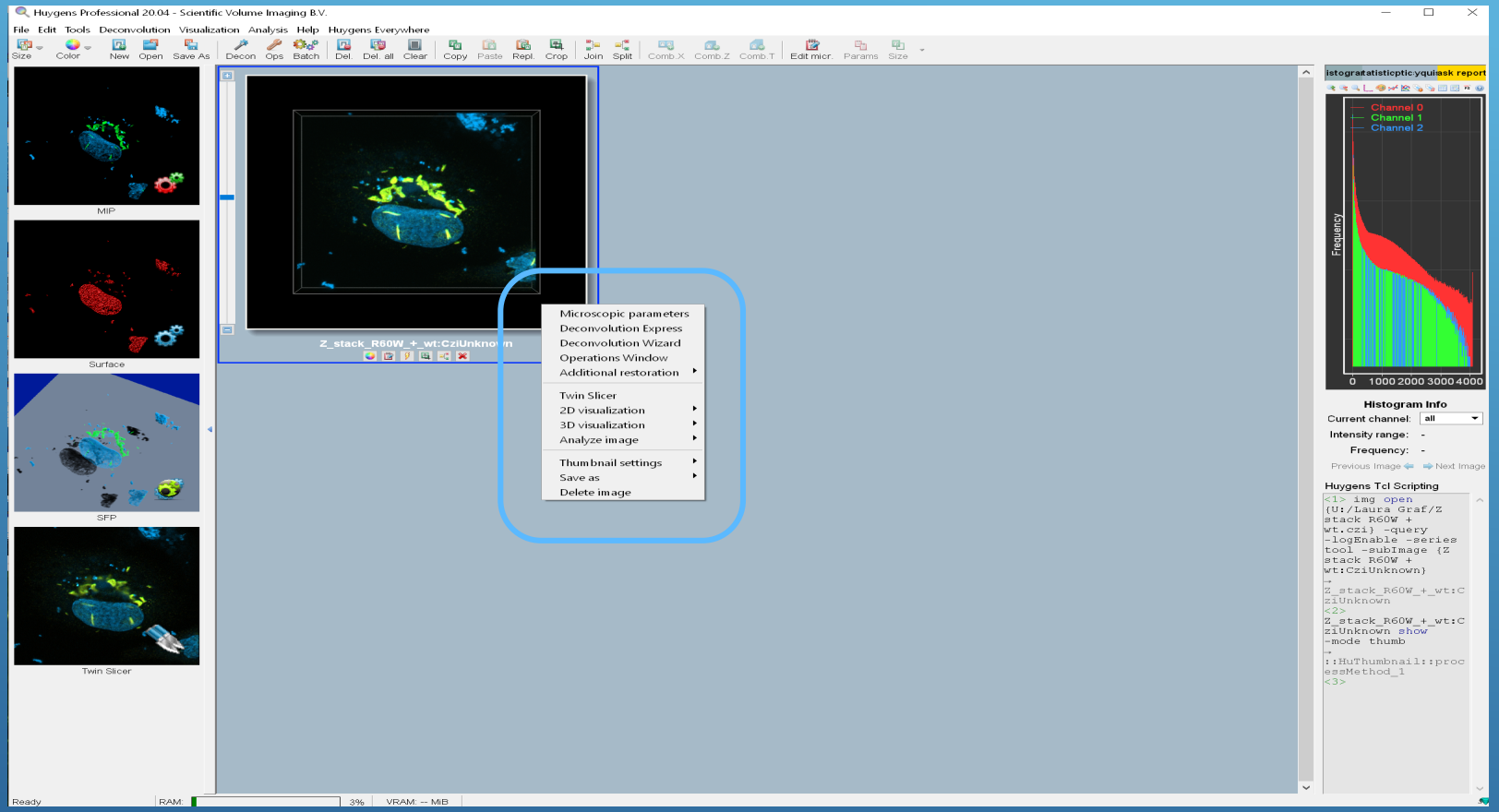


# Huygens Professional

**Right Mouse Click** on Image

- A **menu** appears

Start **Microscopic parameters**

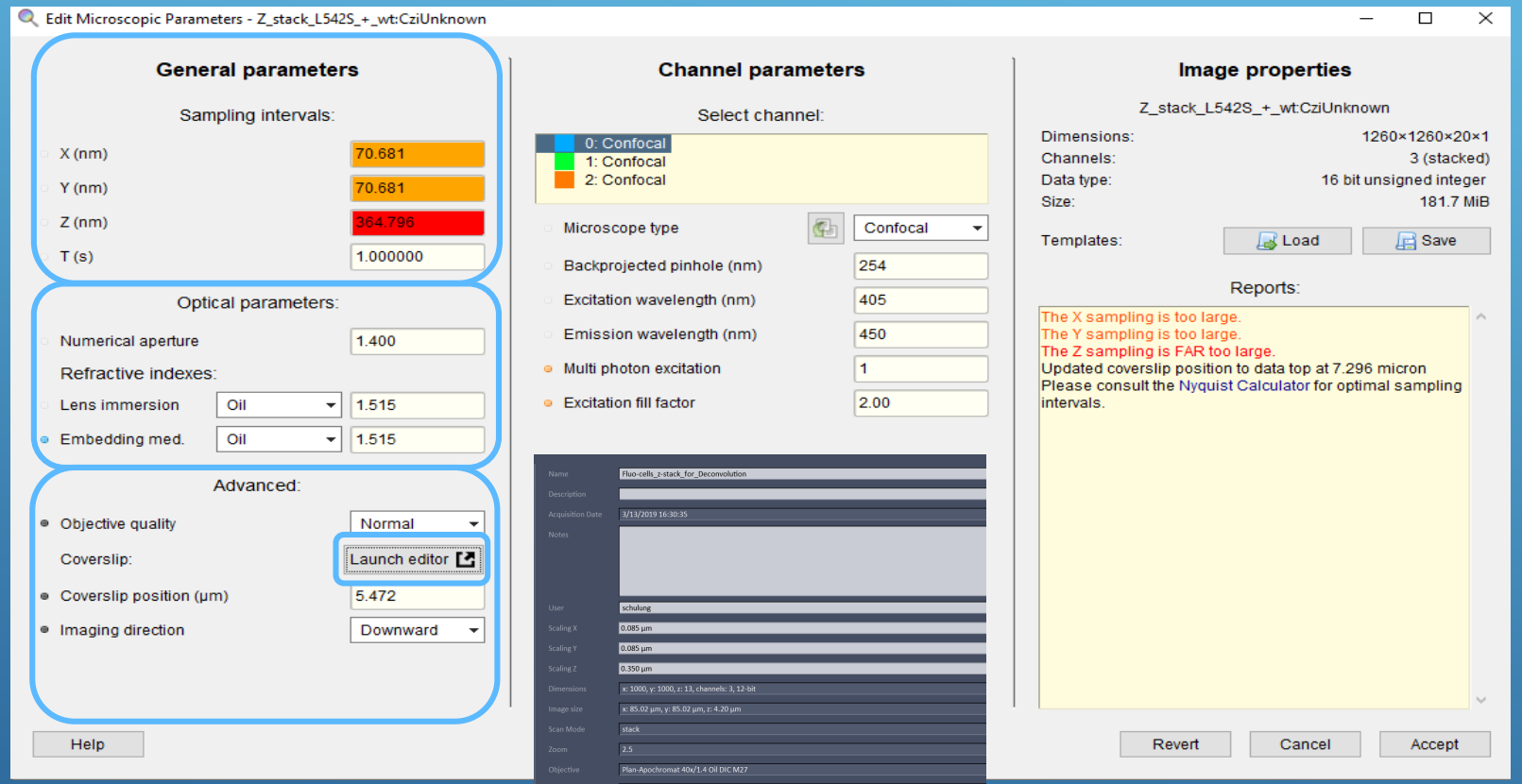


# Open Microscopic Parameter

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

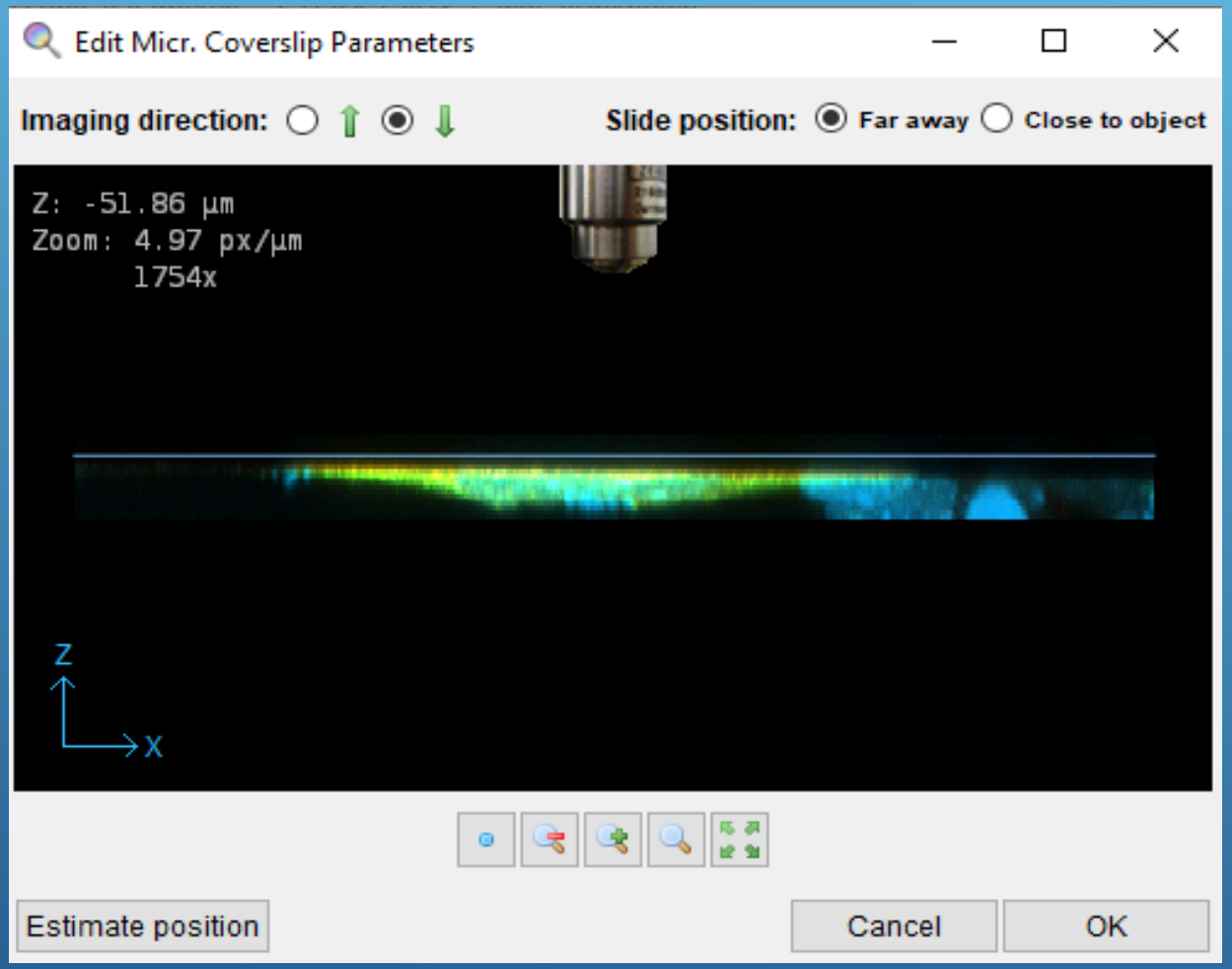
Therefore **control all imaging values** used during acquisition and **fill in the missing** values, too

- 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

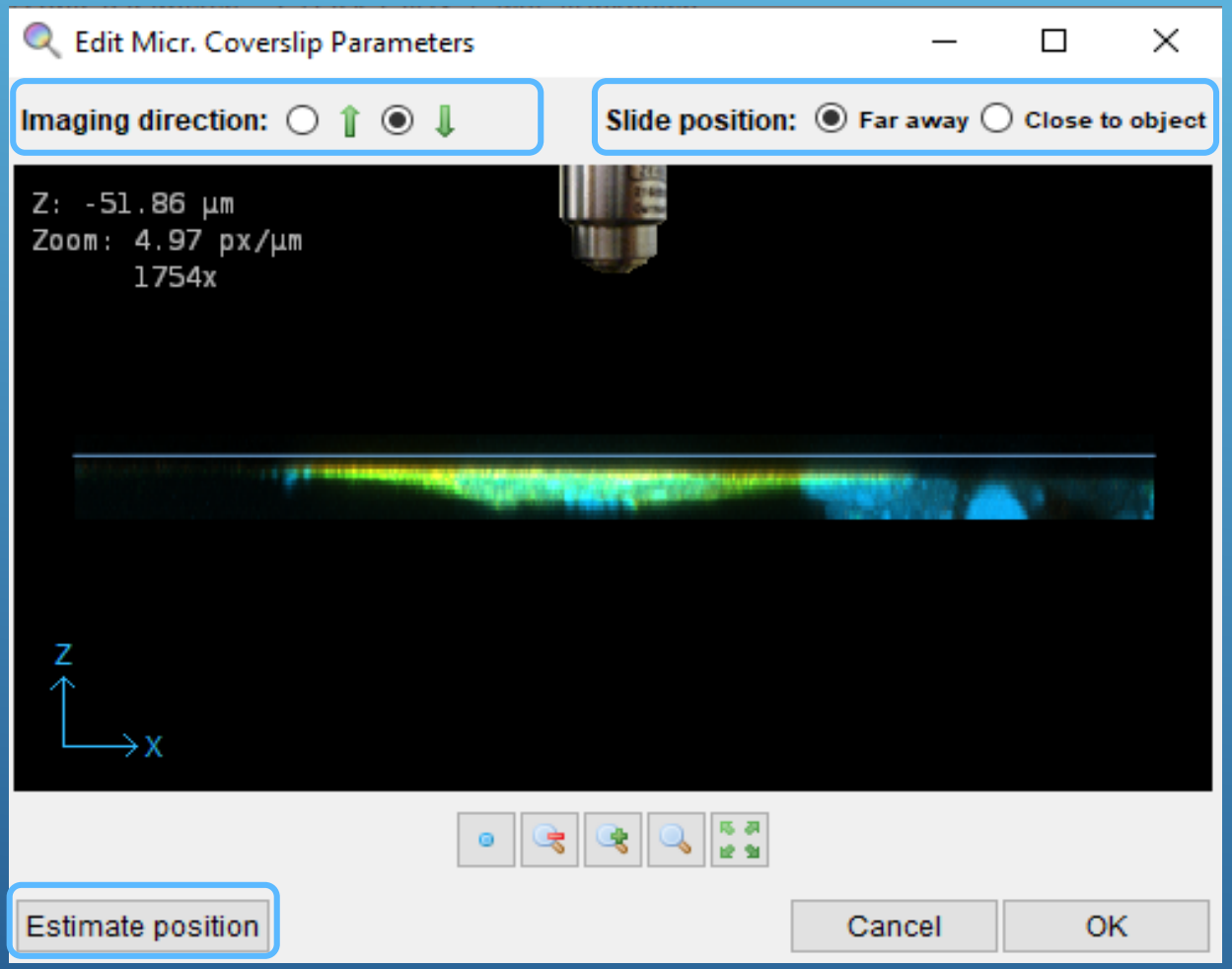



# Coverslip Editor

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**

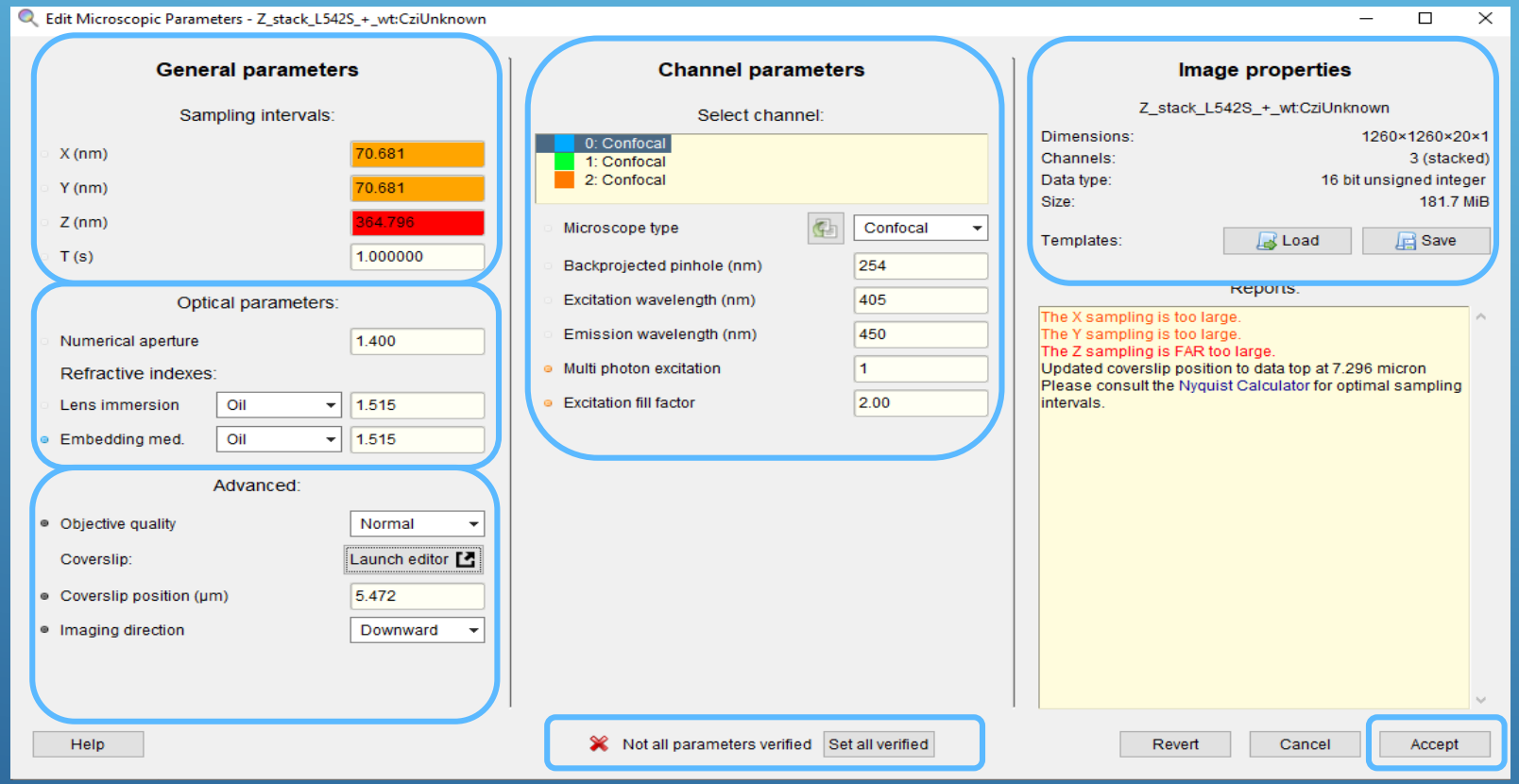
Choose **slide (not coverslip) position to object**

- 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**



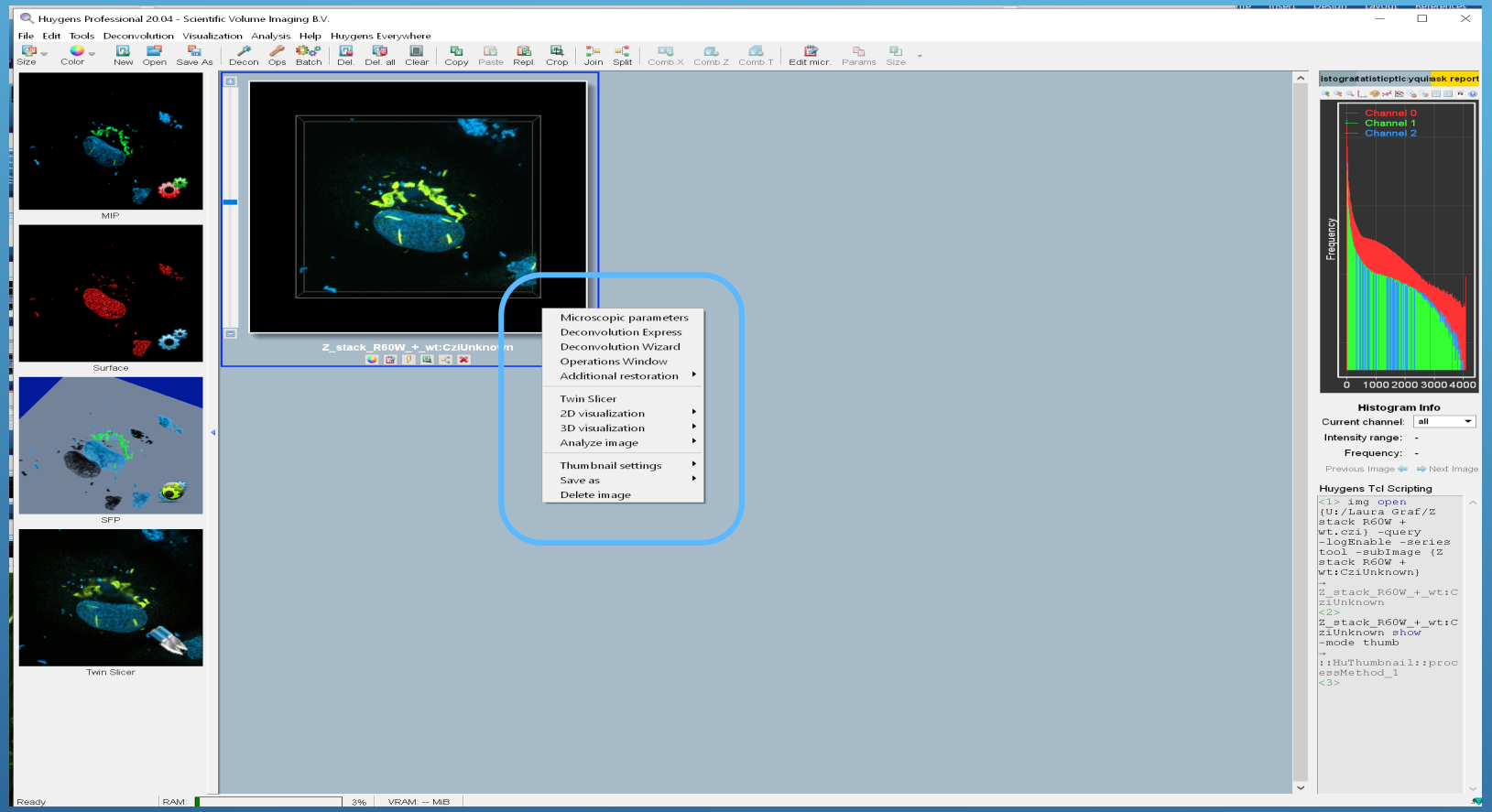

# Microscopic Parameter

- 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 Wizard**



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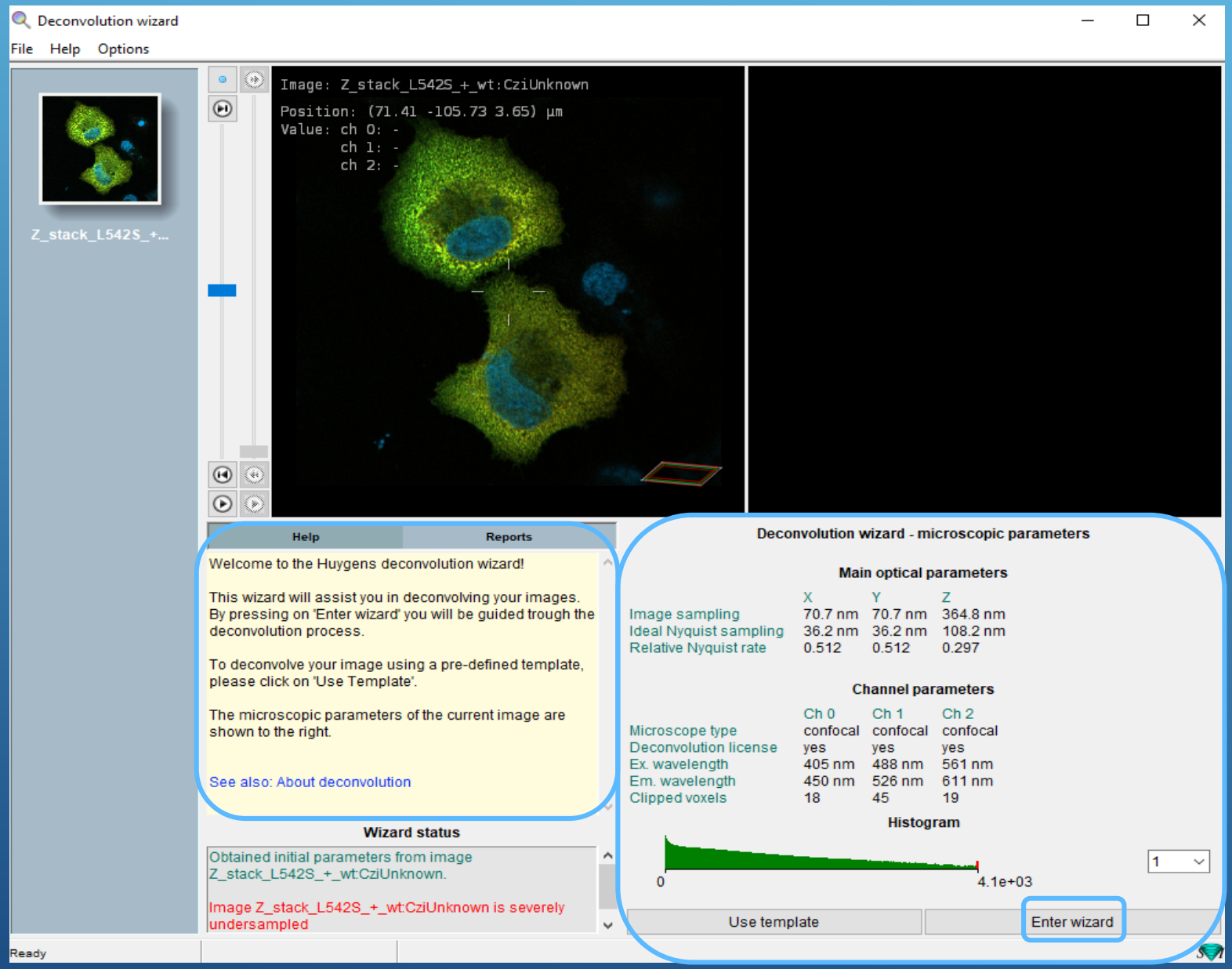
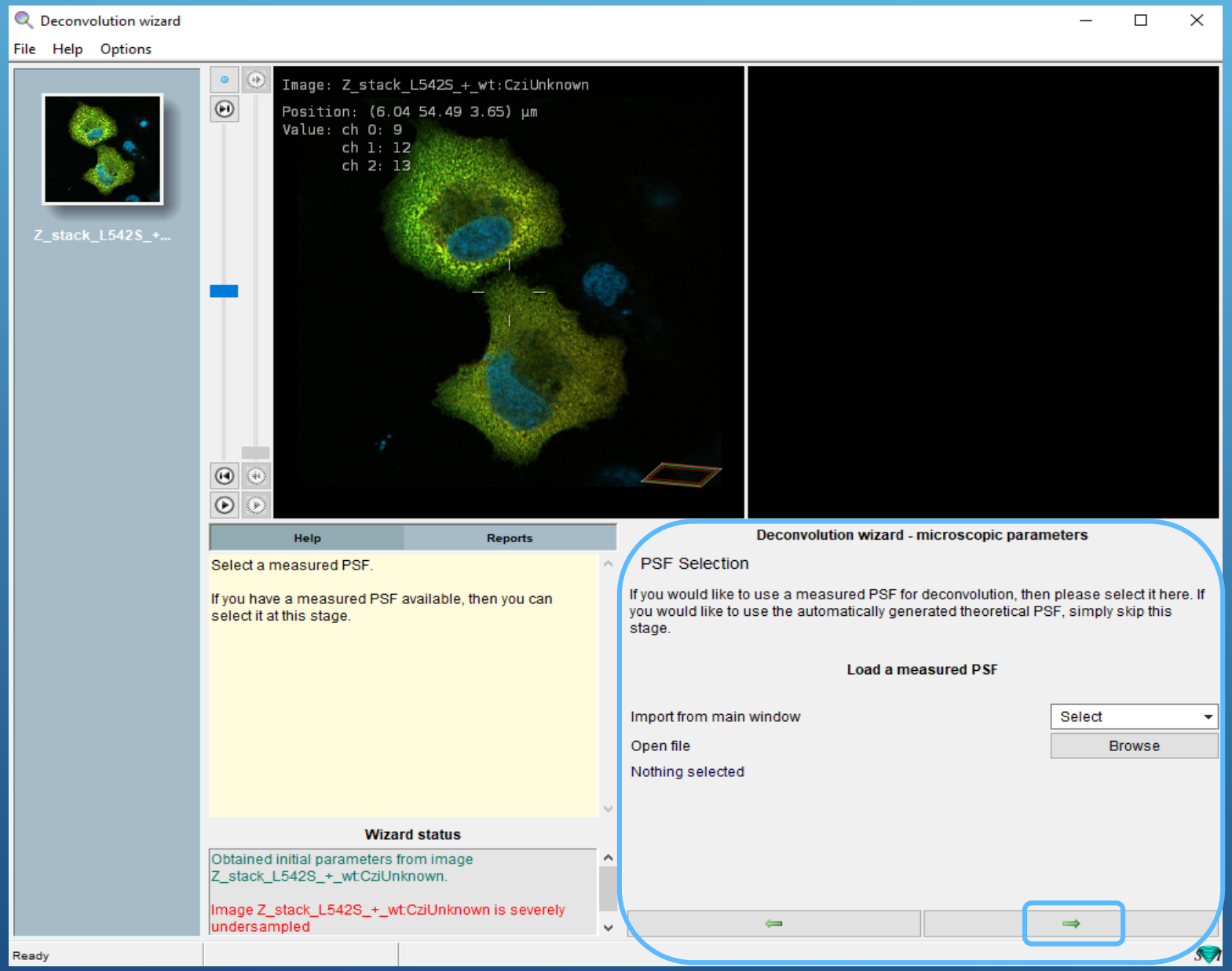
# Open Deconvolution Wizard

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



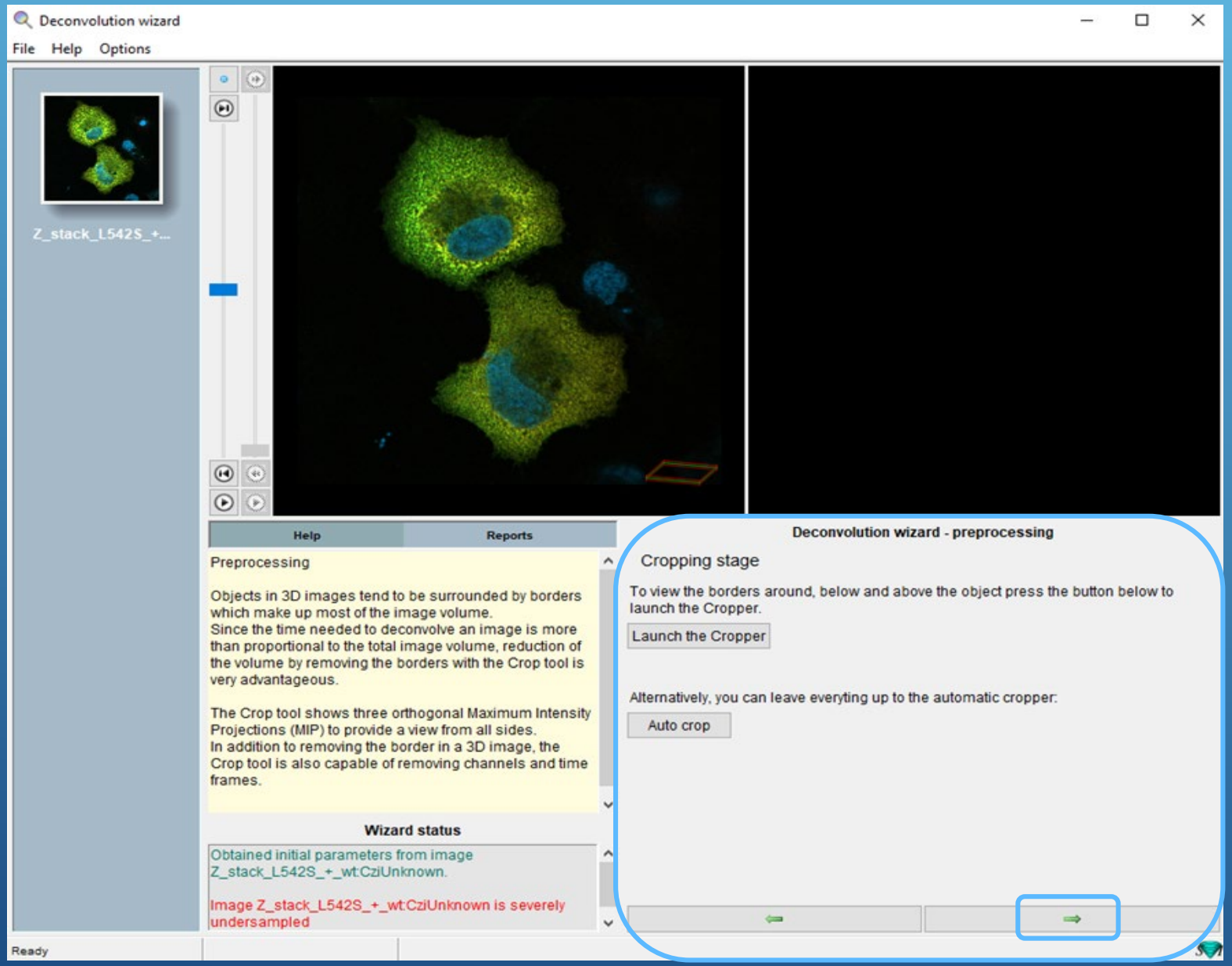
# Deconvolution Wizard

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



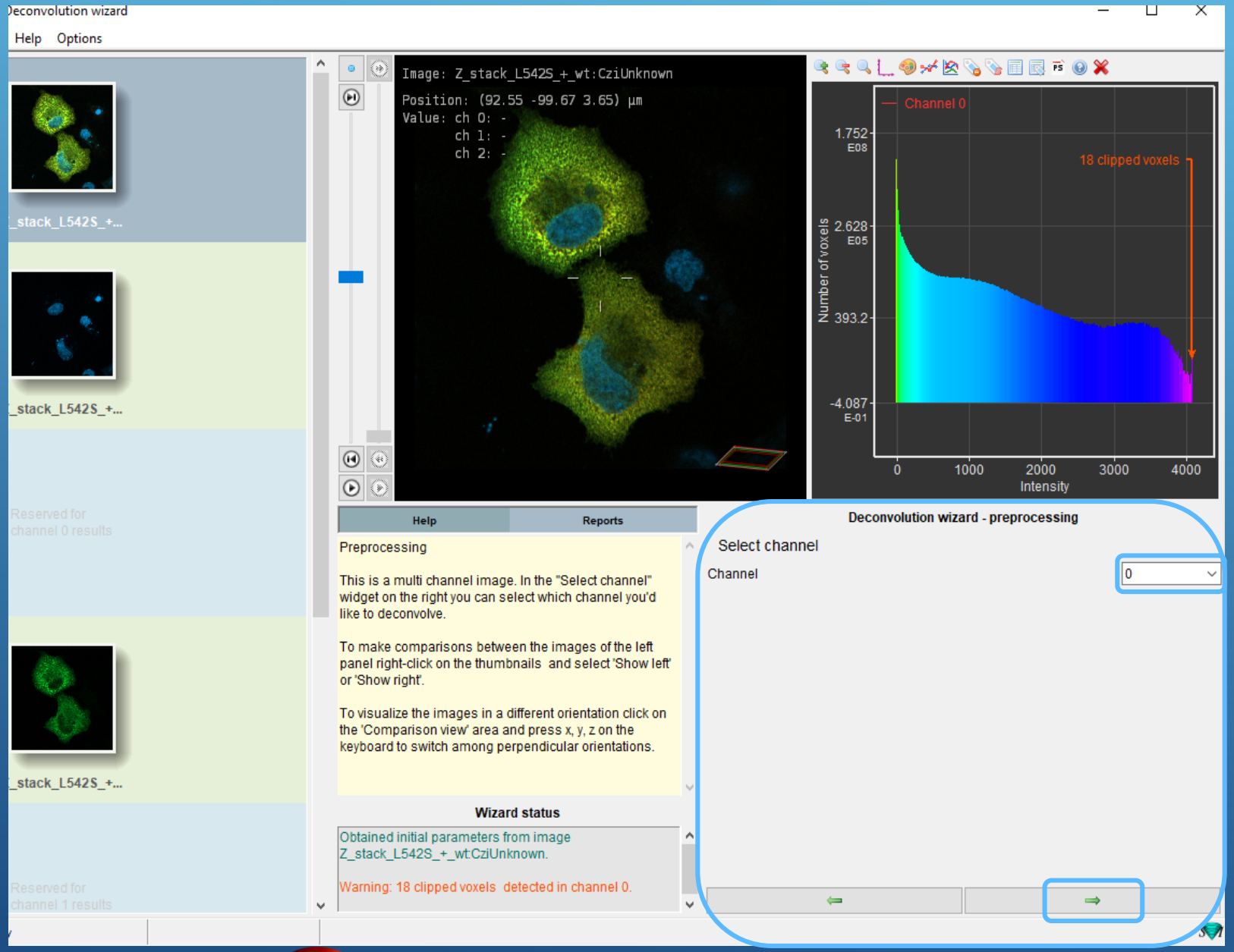
# Deconvolution Wizard

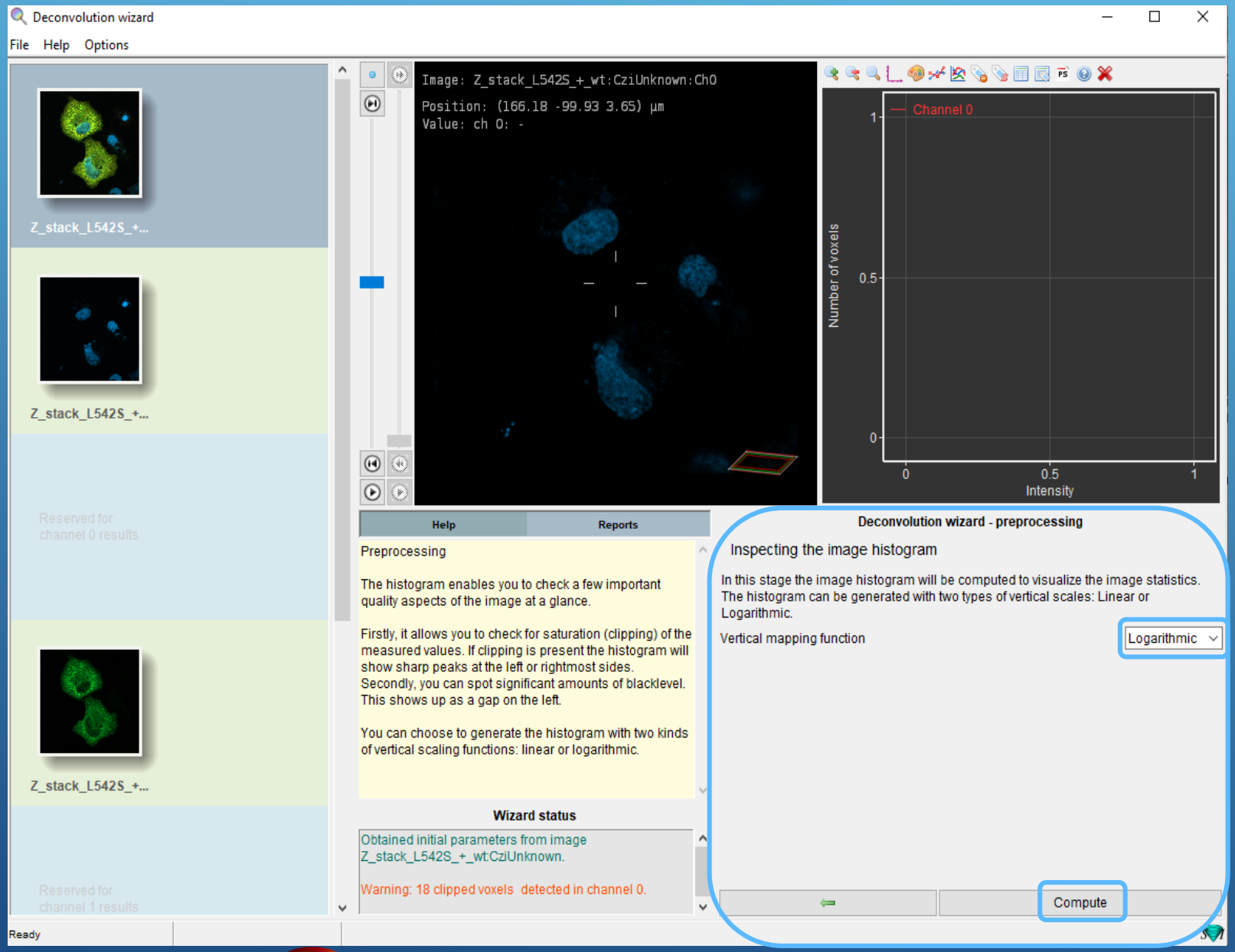
- **Crop** sample if necessary
- **Go On**



# Deconvolution Wizard

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



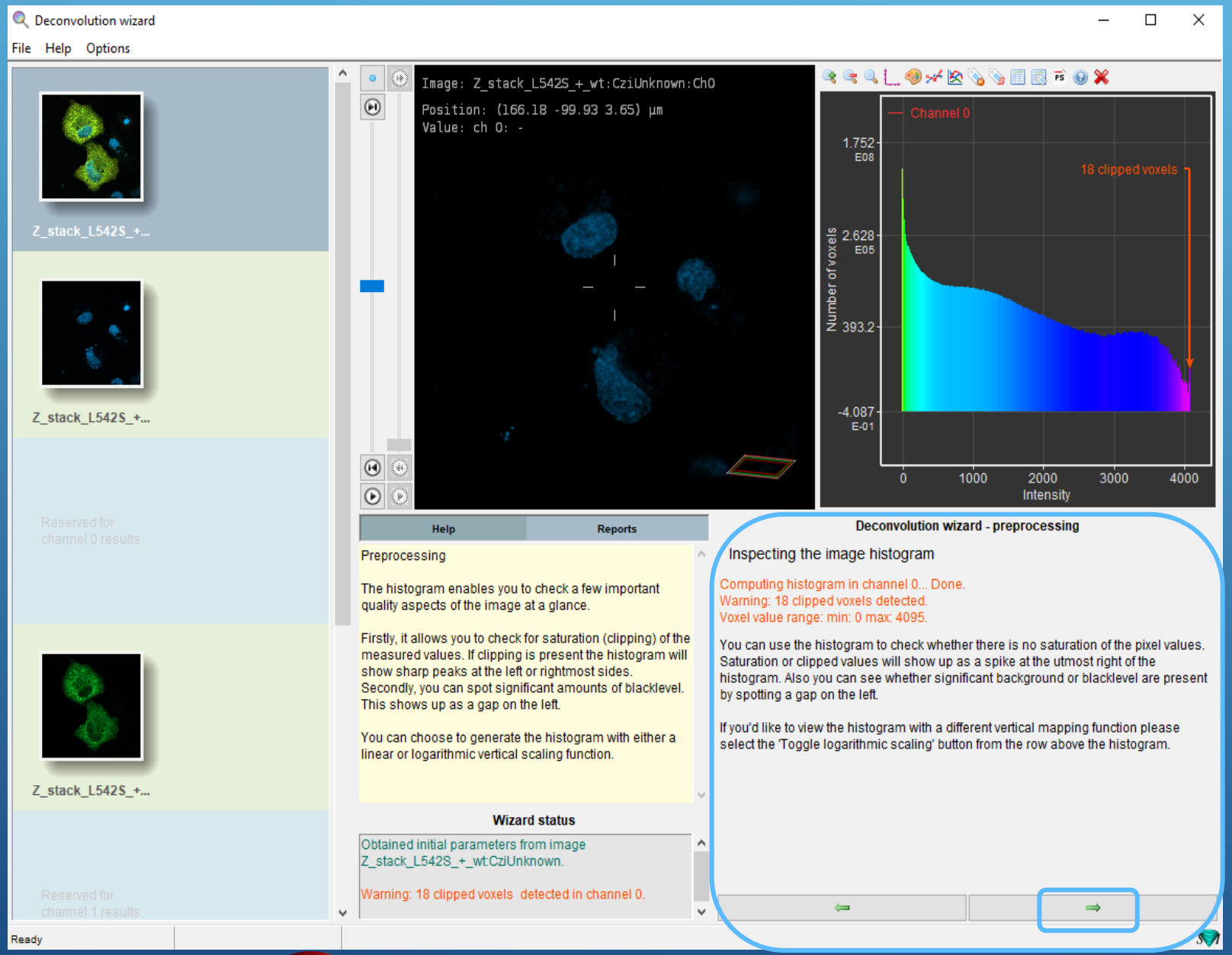



# Deconvolution Wizard

- **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**

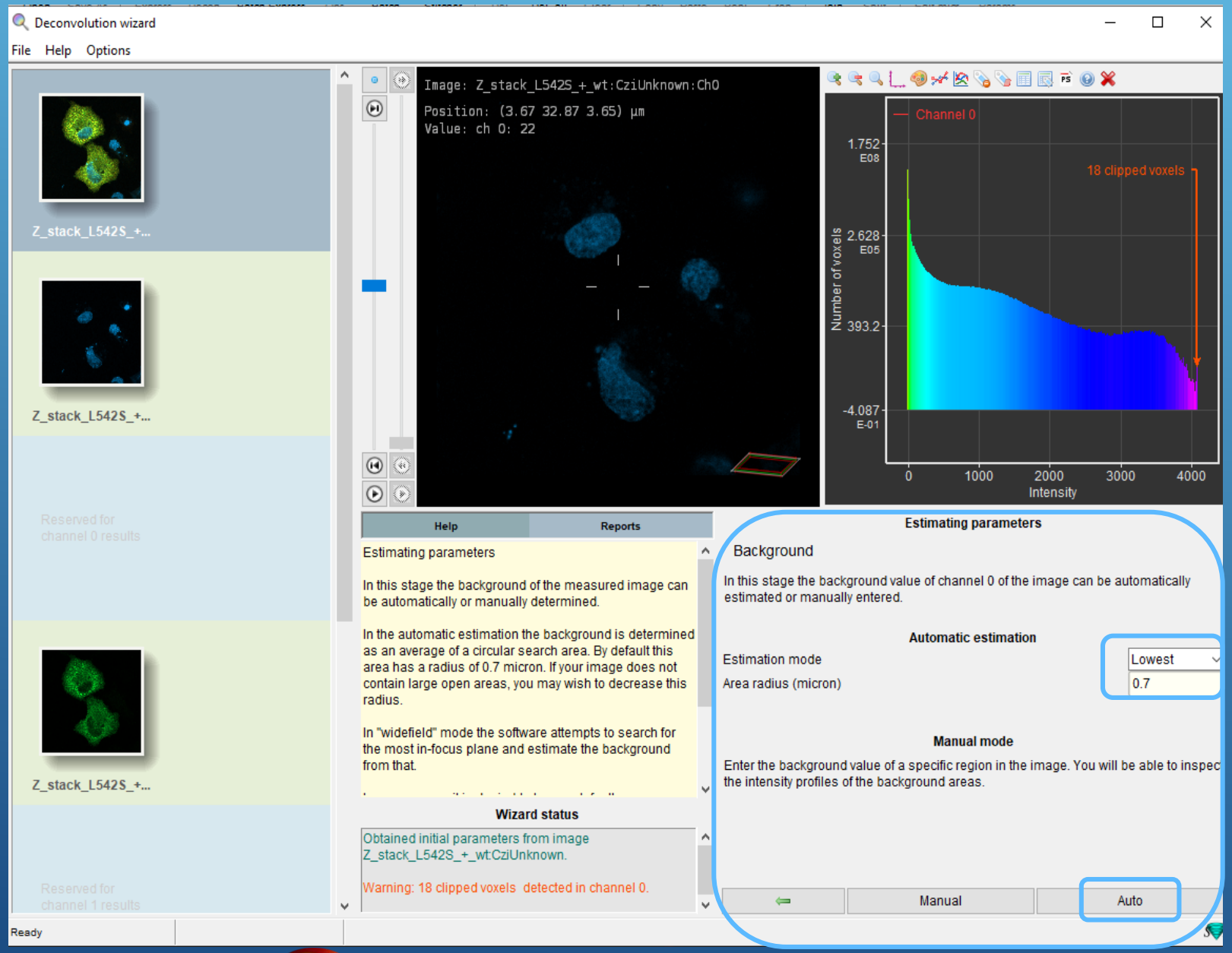
# Deconvolution Wizard

- Go ON



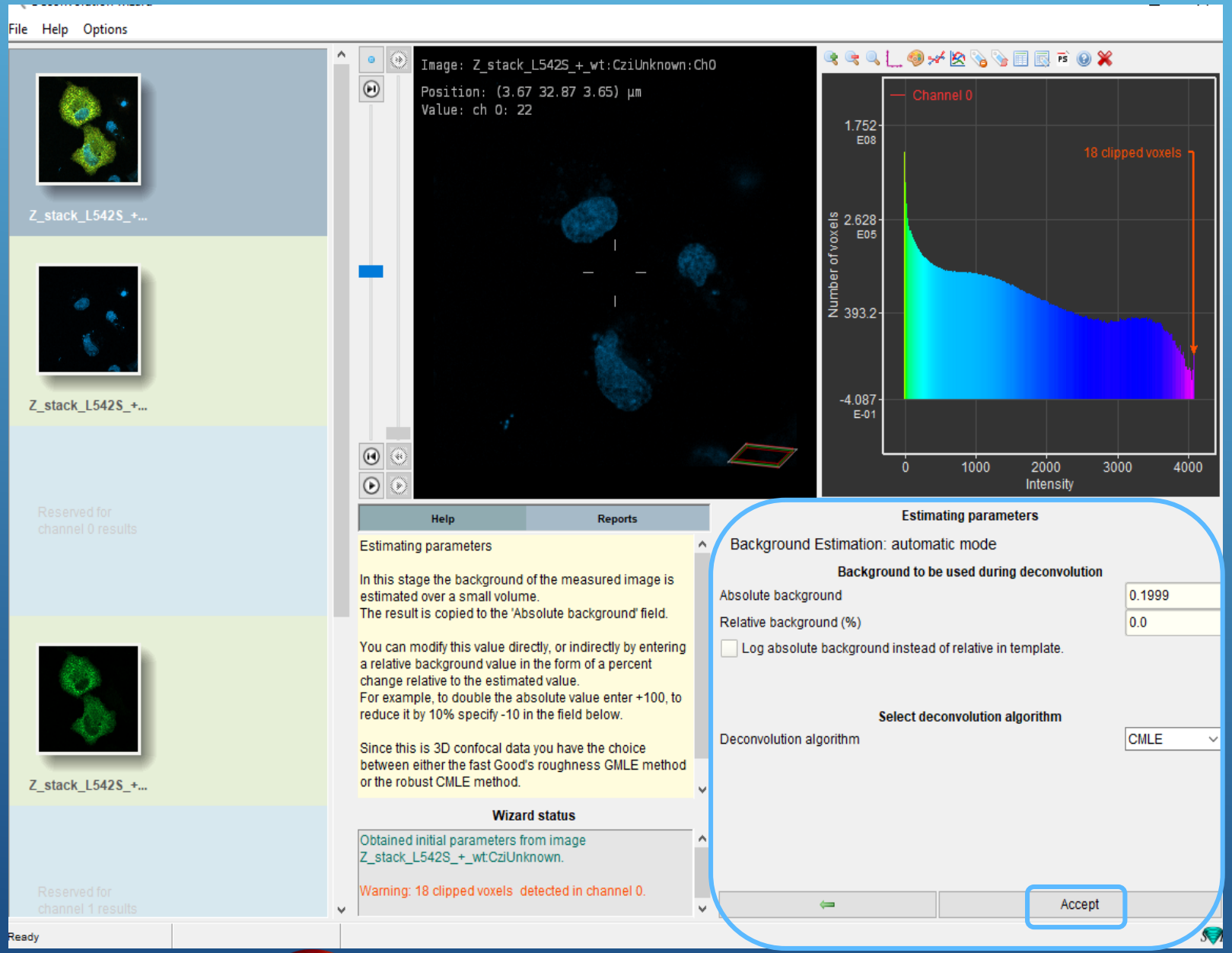
# Deconvolution Wizard

- 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

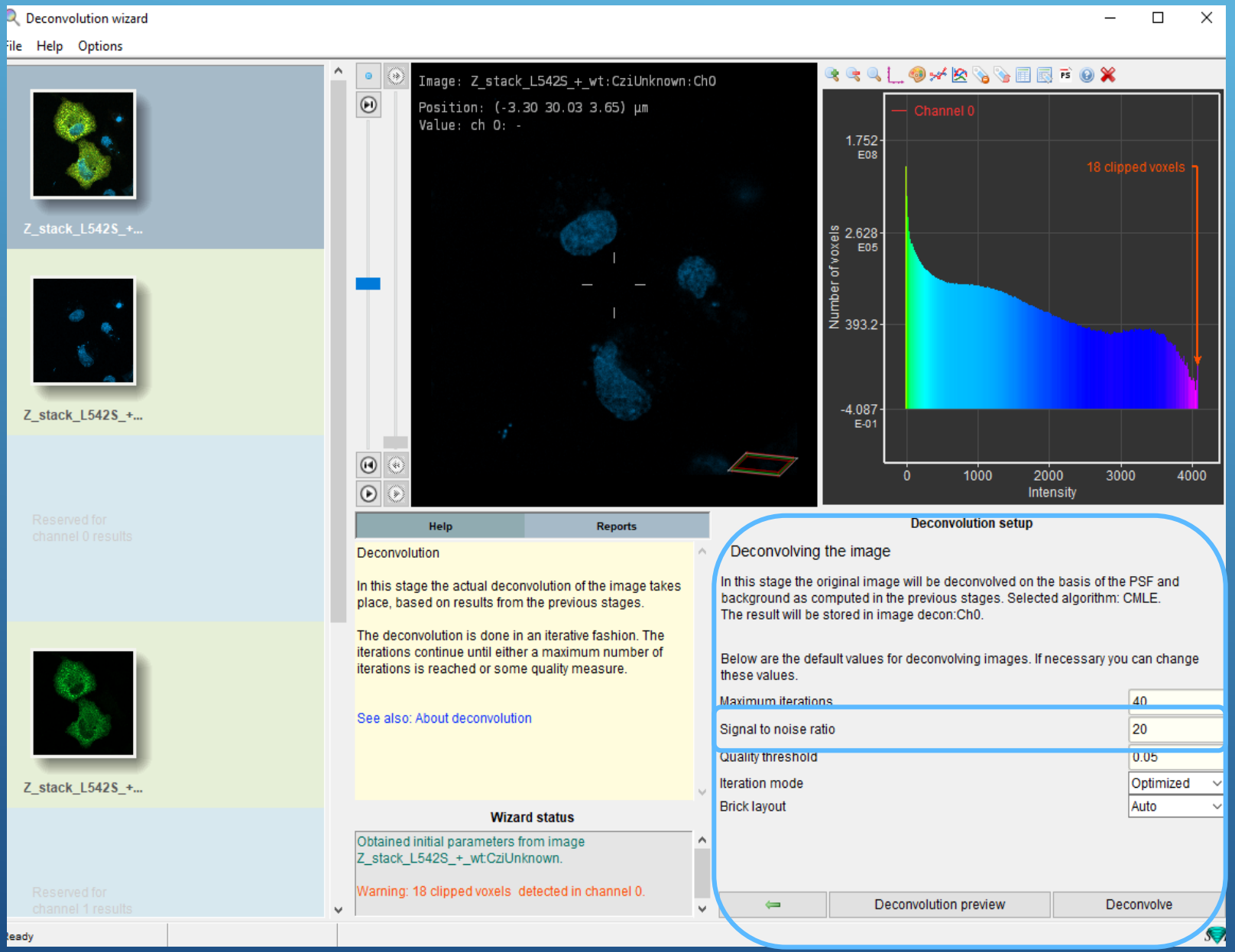


# Deconvolution Wizard

## 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**

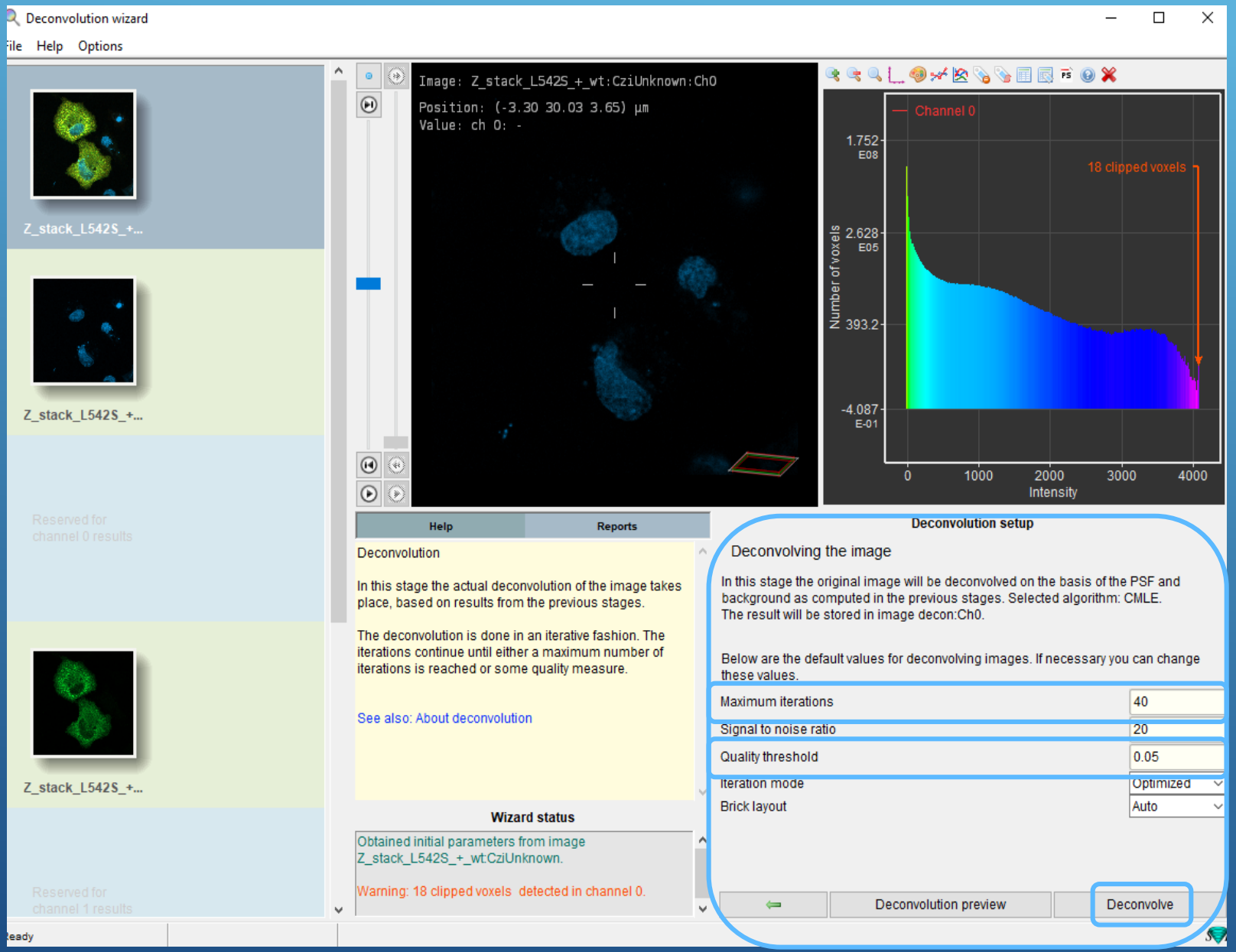


# Deconvolution Wizard

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**

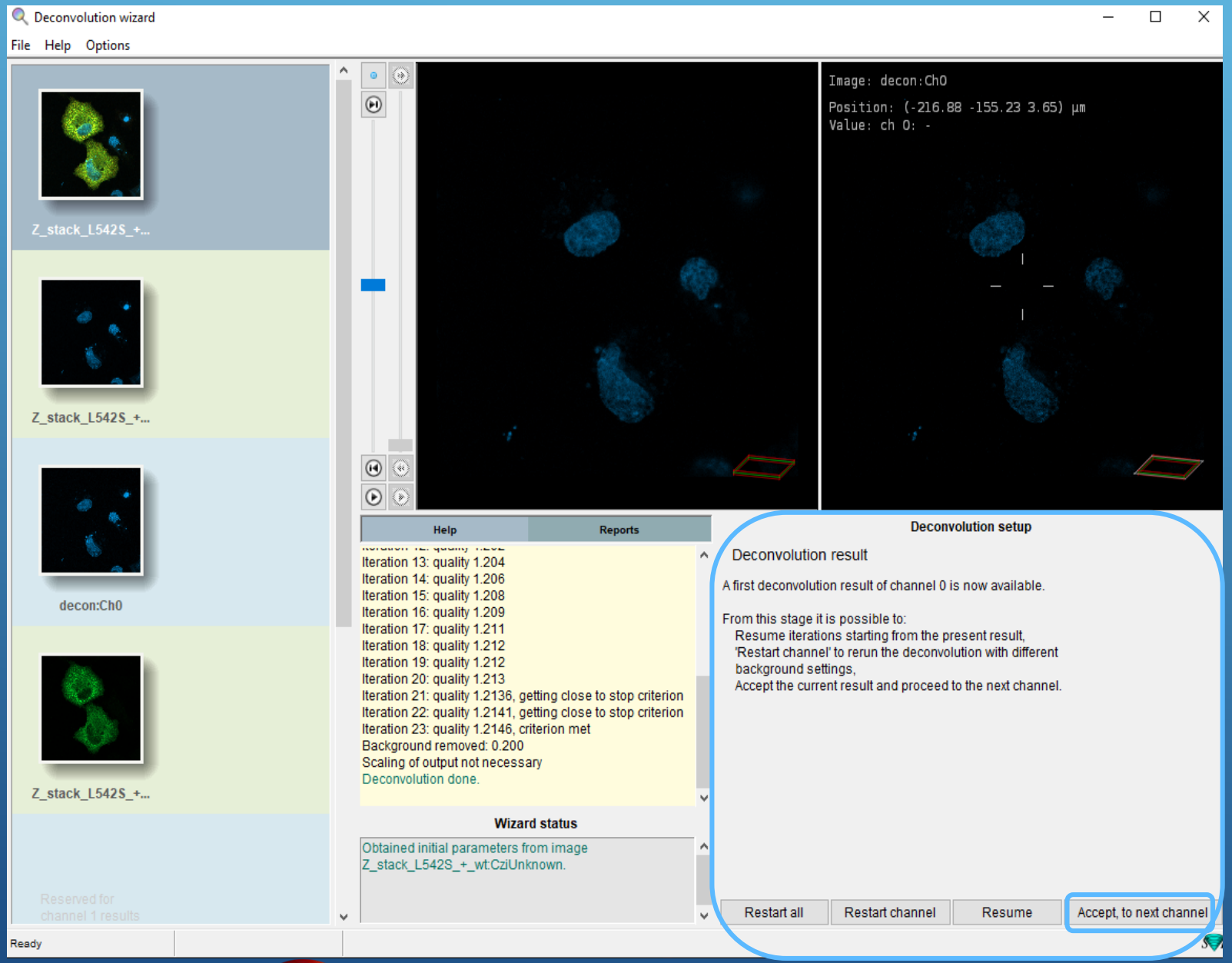
**Go ON - Deconvolve**



# Deconvolution Wizard

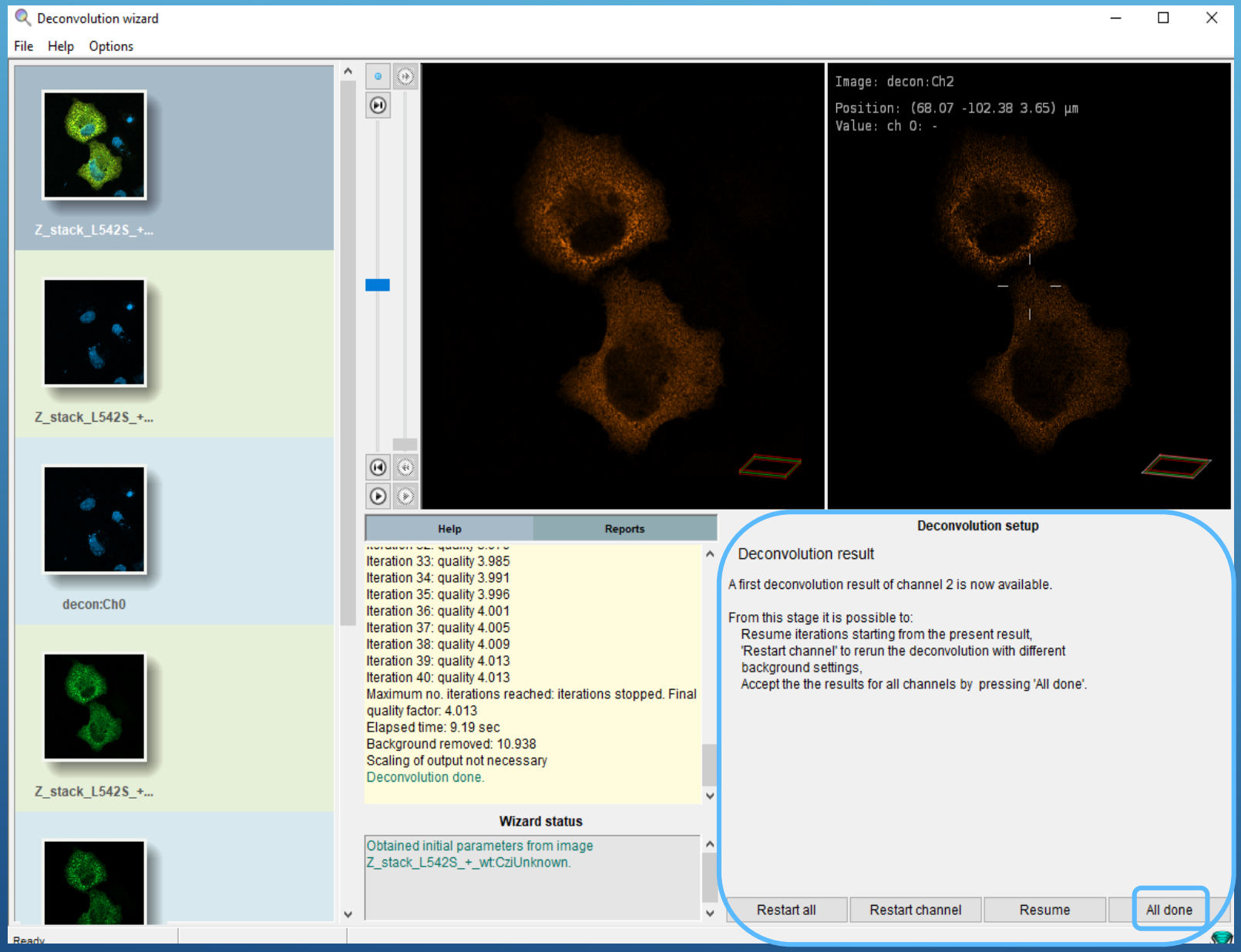
- **Accept** , to next channel

Then **repeat with all channels**



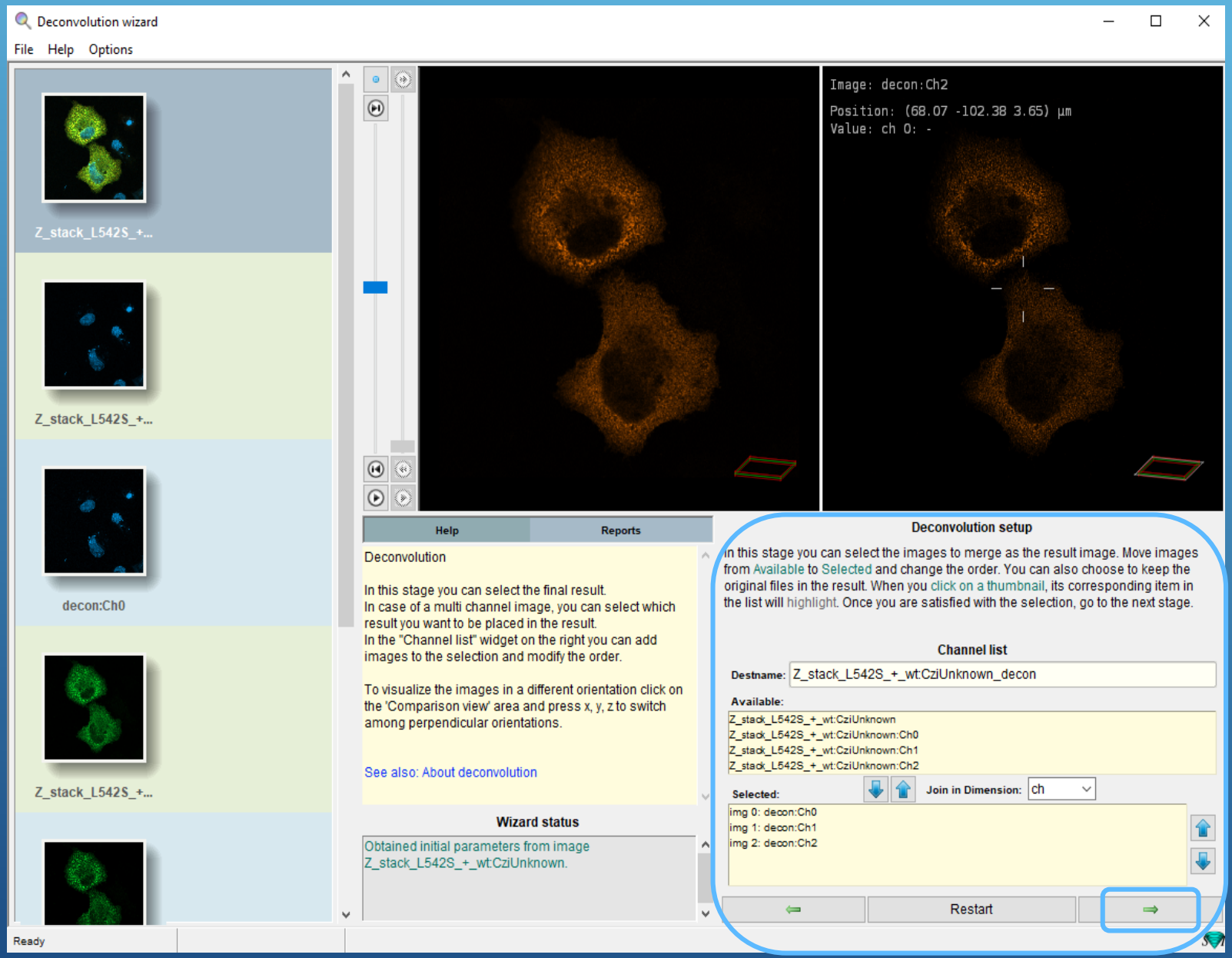
# Deconvolution Wizard

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



# Deconvolution Wizard

- Go On



# Deconvolution Wizard

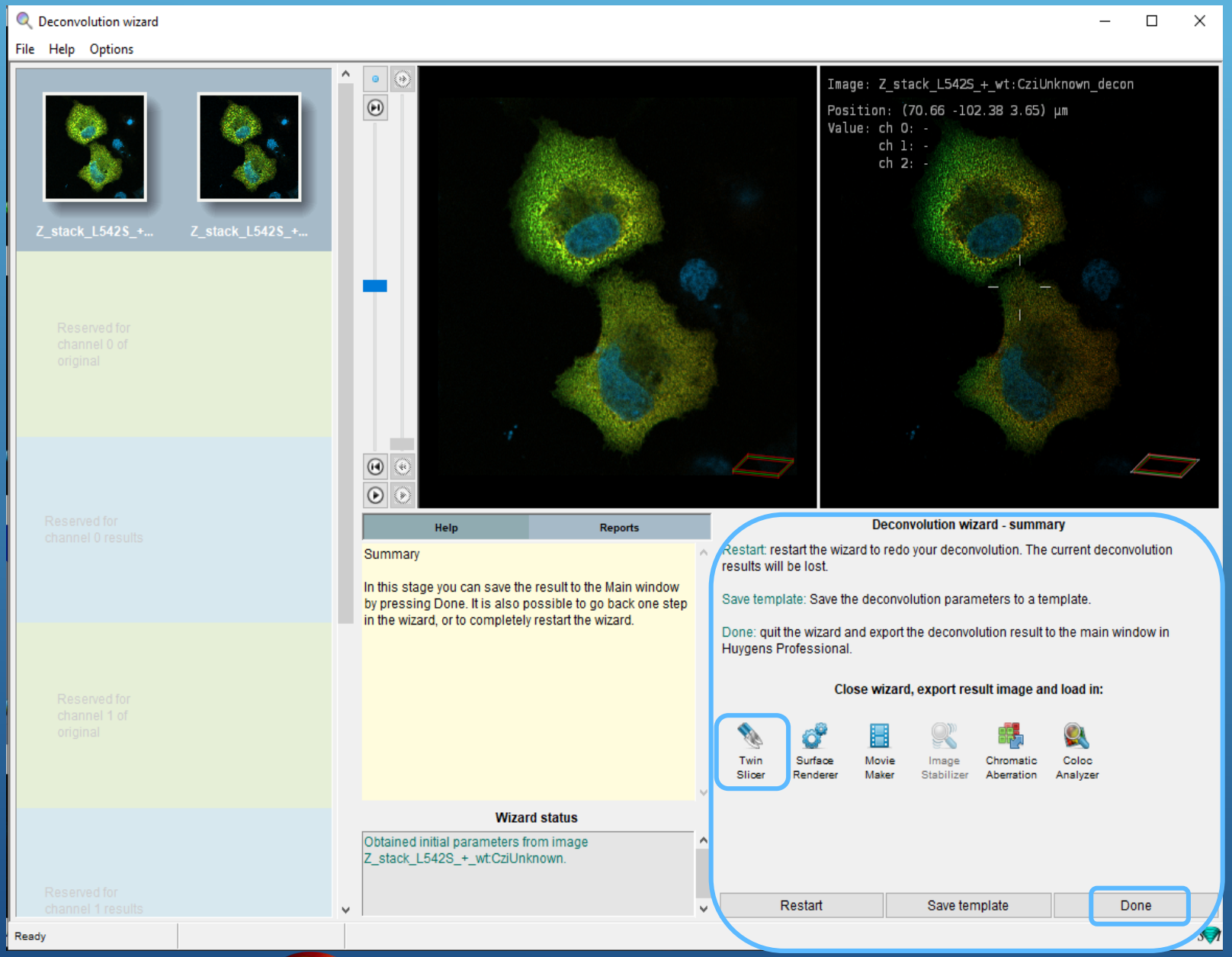
- **Go On – Done**

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

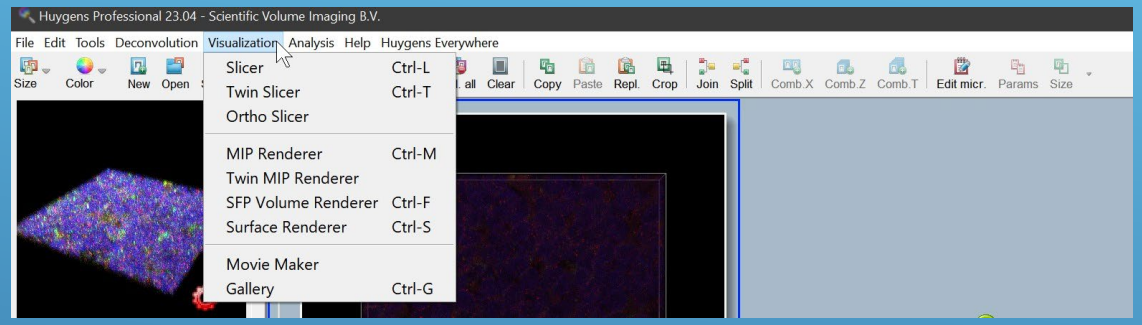


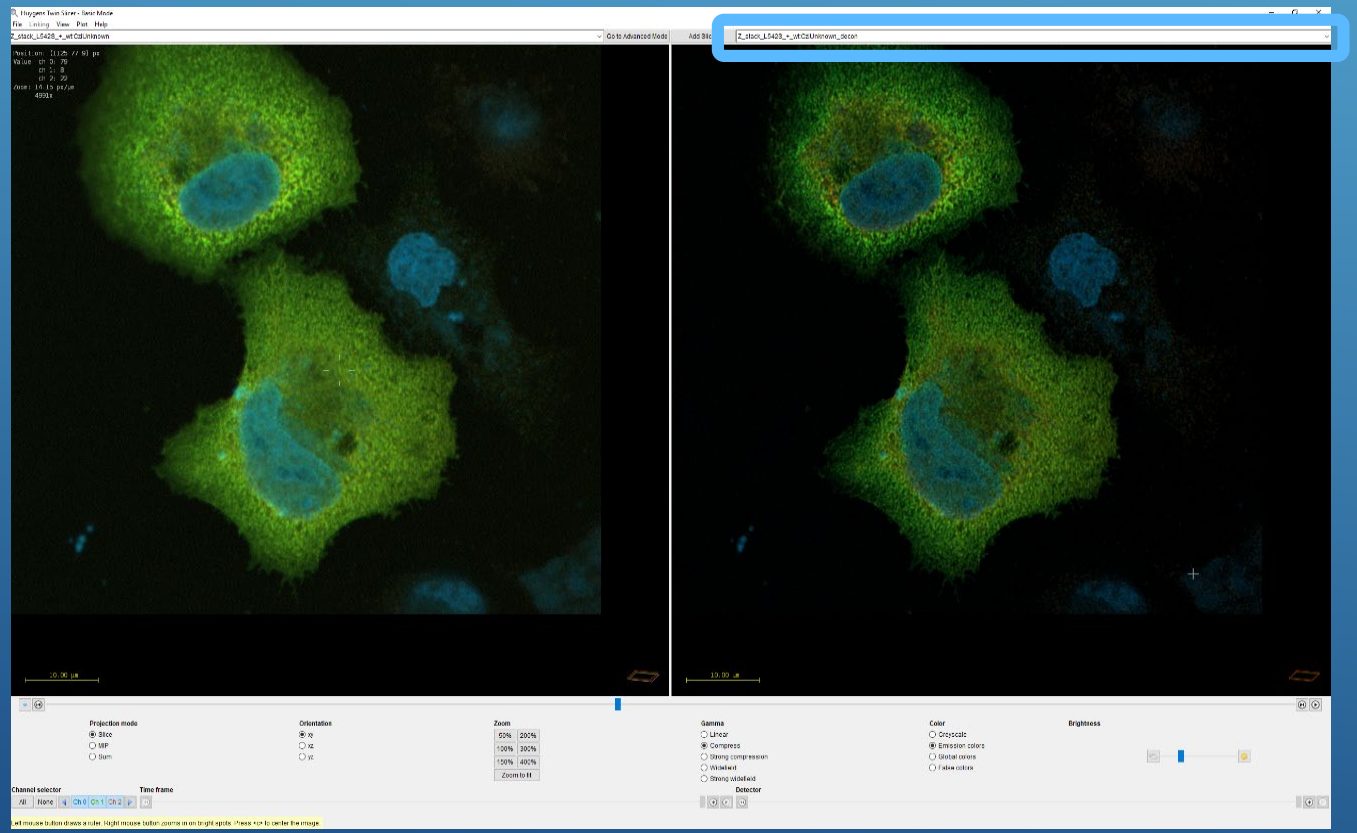
## Save your processed data for further analysis

- **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



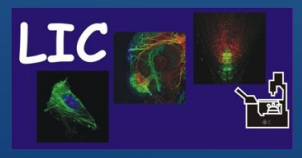



In the main menu in "**Visualization**" use e.g. the **Twin Slicer** to look at your data

- 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.

# Thanks for viewing





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