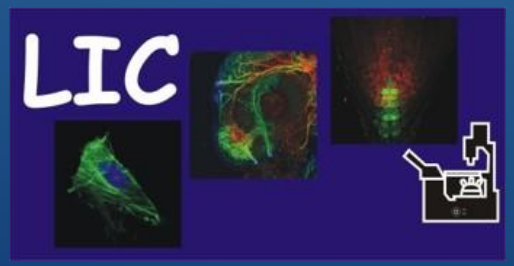
Tutorial - Basic

Huygens Remote Manager

Life Imaging Center 2020



Open - Huygens Remote Manager

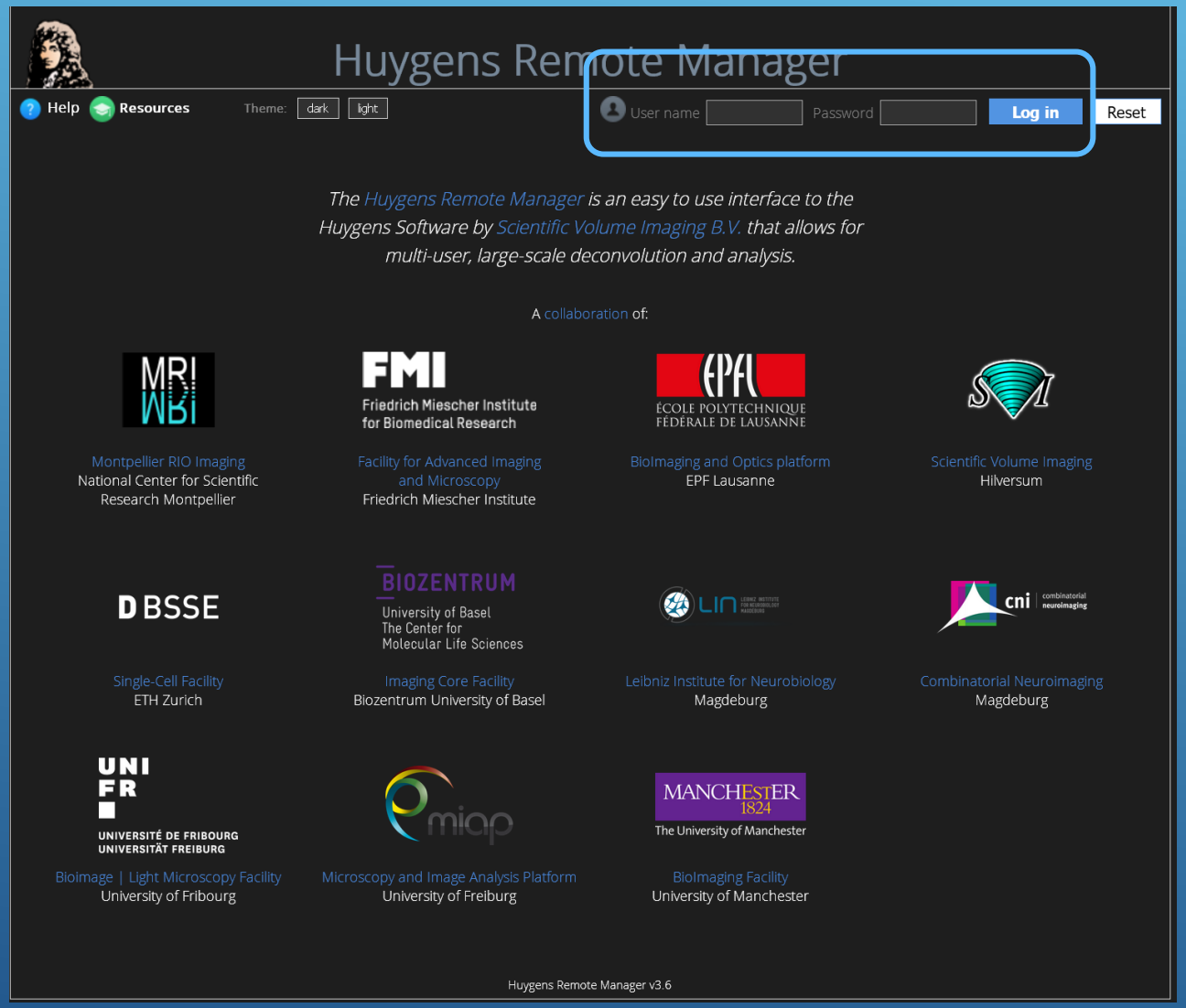
Specification: Huygens Core server (HRM) for deconvolution (256 GB RAM, 2 CPUs, 28 cores, 24 GB GPU VRAM, 6 GB SSD RAID 5, 28 TB storage RAID 5)

Access: http://rod.lic.zbsa.privat/hrm

- only accessible within the network of Freiburg University

Licenses available:

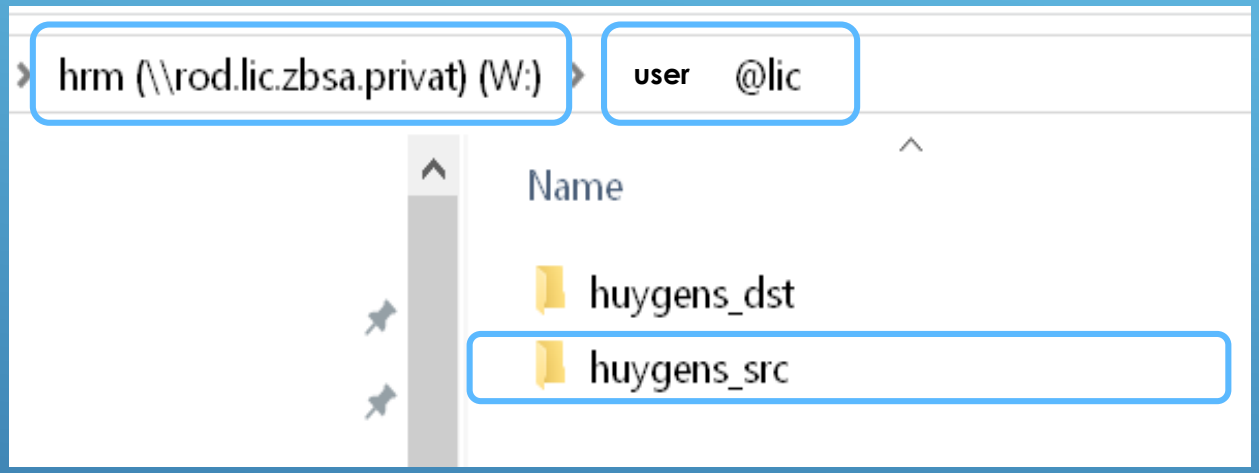
- Deconvolution for
  - Confocal
  - Multiphoton
  - Spinning disk
  - STED
  - Light-sheet
  - Widefield
  - Airyscan (ZEISS)
- Colocalization Analysis
- Light-sheet Fusion
- Object Stabilizer
- Time-series



Login - Huygens Remote Manager

- **Login**: **username@lic** and **password**

- **IMPORTANT**:
  - Please use the same username and password as for your LIC login.
  - Additional the username has to be supplemented with @ and the logon-domain - all in small letters (i.e. username@lic)



Upload Imaging Data to Huygens Remote Server

Down- and **Upload** image data to a special **network drive W**

After your first login on the HRM you will find a folder with your username on **drive W**: containing

- (**huygens_dst**) – folder for restored data
- (**huygens_src**) - folder for raw data

Copy your raw image data into the **source folder (scr)**

After completion of your HRM job always clean up the disc space

- Remove raw and processed data
- We have only limited storage disk space!

Deconvolution Process in Short !

You have no Parameter and Restoration Templates

- Step A: Launch a Job, then select image files and create and define Image Parameter Settings:
  - Write the specific properties of your images into a template, save this template and select it
- Step B: Restoration settings:
  - Write the preferred deconvolution settings in a template, save this template and select it
- Step C. Start deconvolution – Start JOB

You have Parameter and Restoration Templates

- Step A: Start a Job, then select images files:
  - Select the images and the corresponding templates and go through the process.
- Step B: Start deconvolution - Start JOB

Welcome Page - Huygens Remote Manager

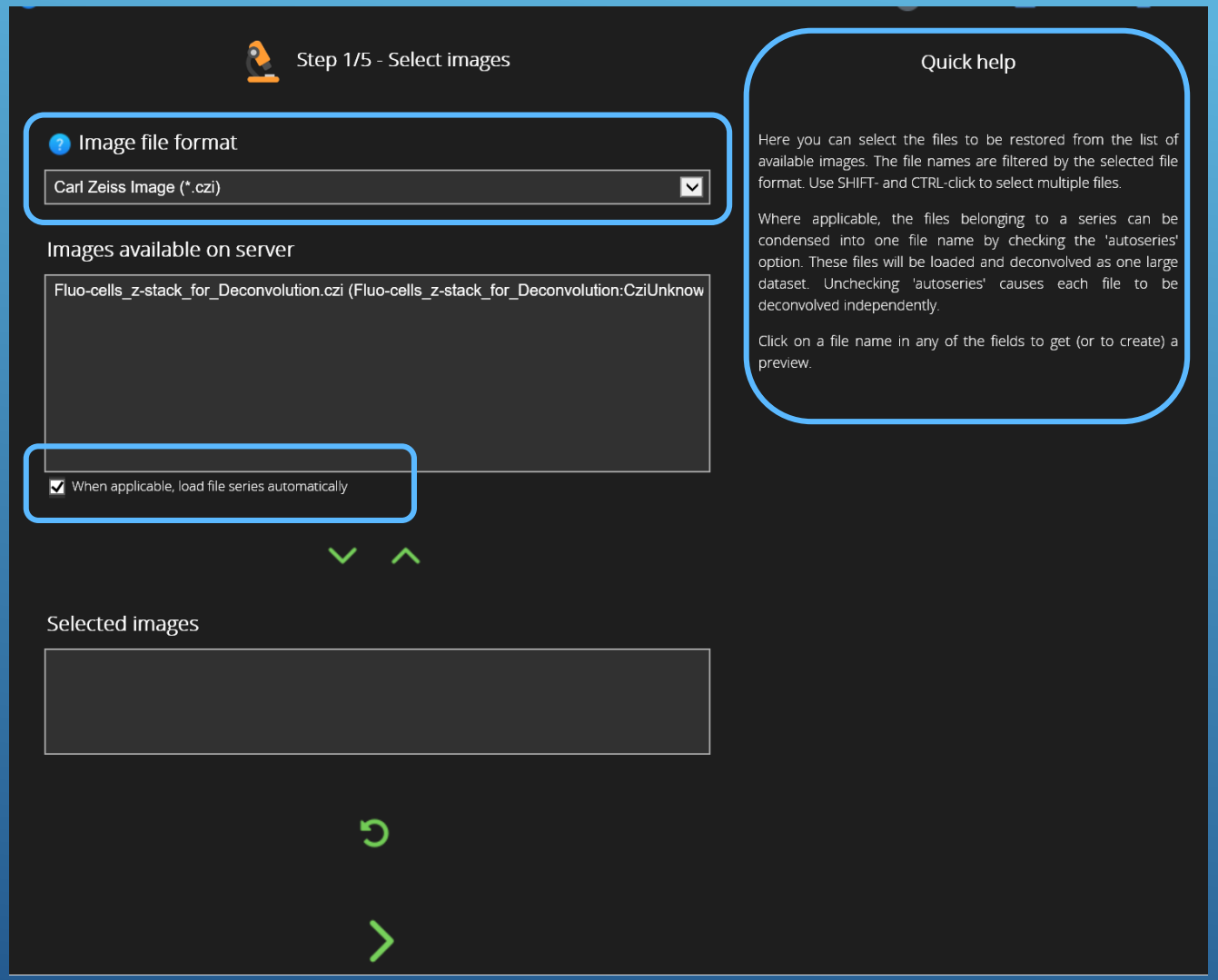
After Login you enter the **Home Panel – Welcome Page**

- To manage here the deconvolution jobs as well as other HRM administrative tasks

i.e. following shortcuts are available:

Launch a job
Here you create the image parameter and restoration templates

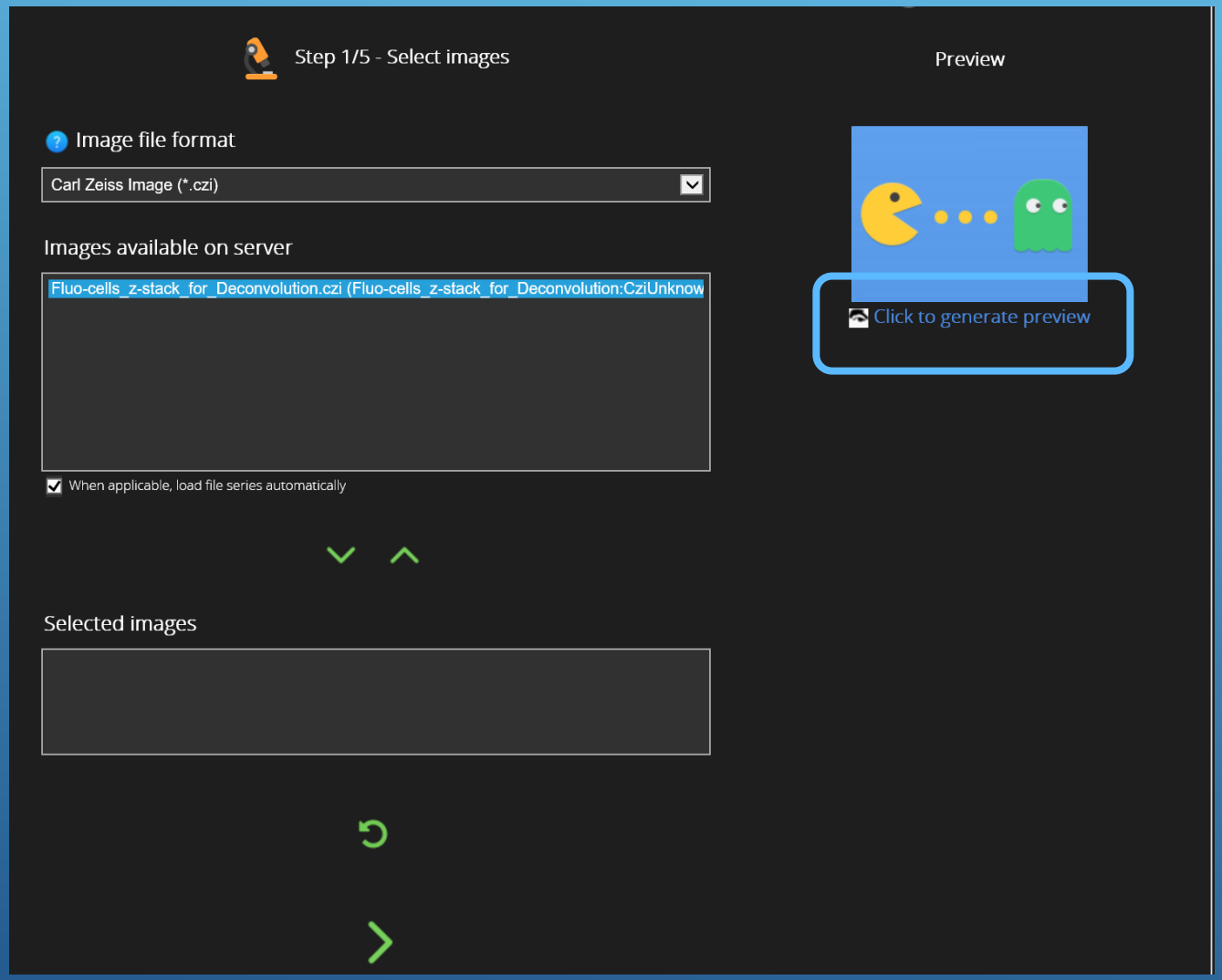
Raw images
Here you could see you data on Drive W, we do not use the integrated upload function



Step 1/5 - Select Images

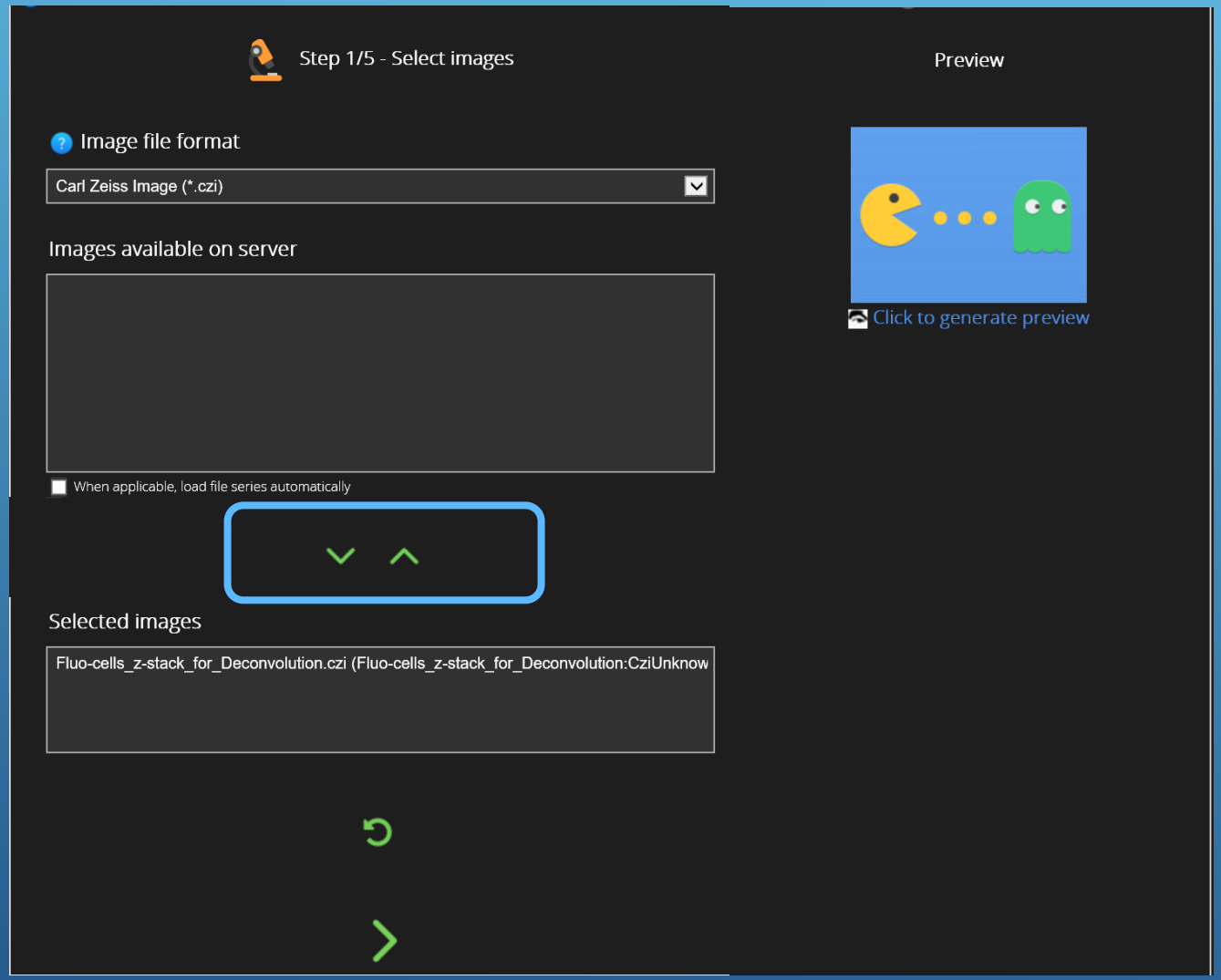
- **Select** the **file format** of the images you want to process
- **Activate automatically loading**

You always get on the right side of the Huygens software a short **help menu**, placing the mouse pointer over one program icon - the specific help is visible on the right side



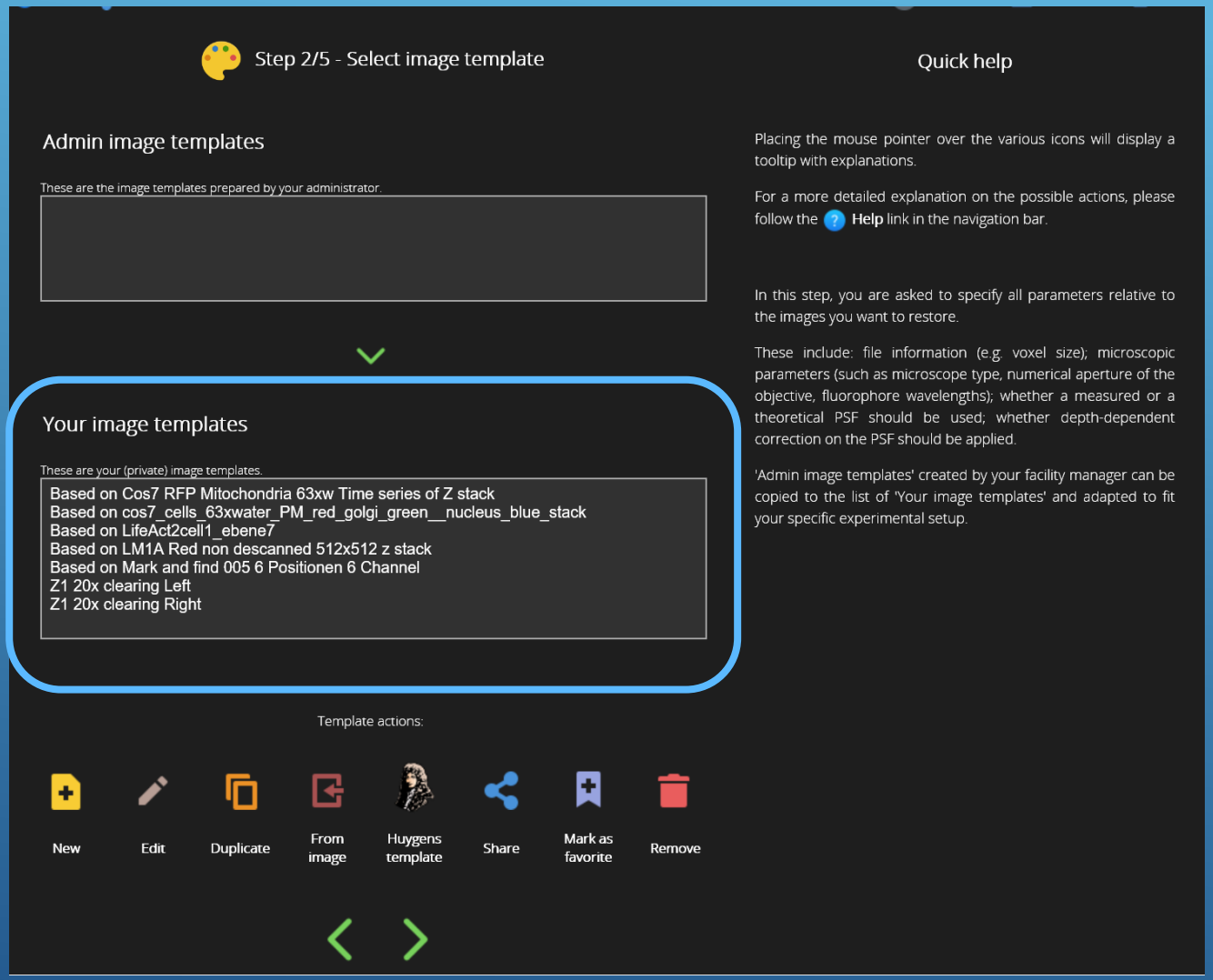
Step 1/5 - Select Images

- You can generate a **Preview of your data** by using the link ‘click to generate preview’



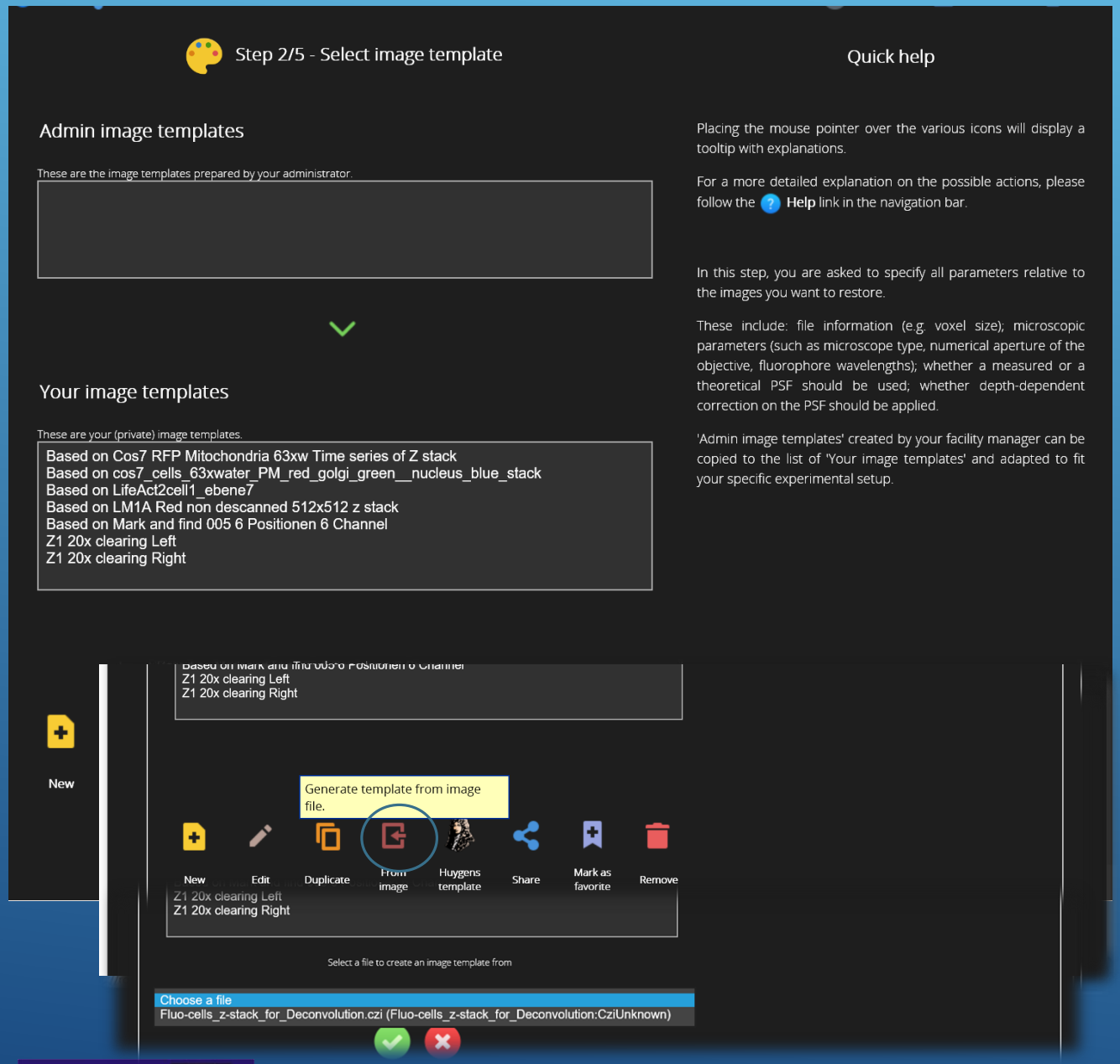
Step 1/5 - Select Images

- **Move data** to the **'selected images' folder**
  - **Use the Down Arrow and Up Arrow** icons to add/remove images to/from the selection
- You can move several files, important image acquisition conditions and parameters have to be the same for all
- **Go on**



Step 2/5 - Image Parameters - Create Image Template

- Specify the **imaging parameters** used during image acquisition
  - These include :
    - File information (geometry, voxel size)
    - Microscope parameters such as Microscope type, numerical aperture of the objective, fluorophore wavelength,….

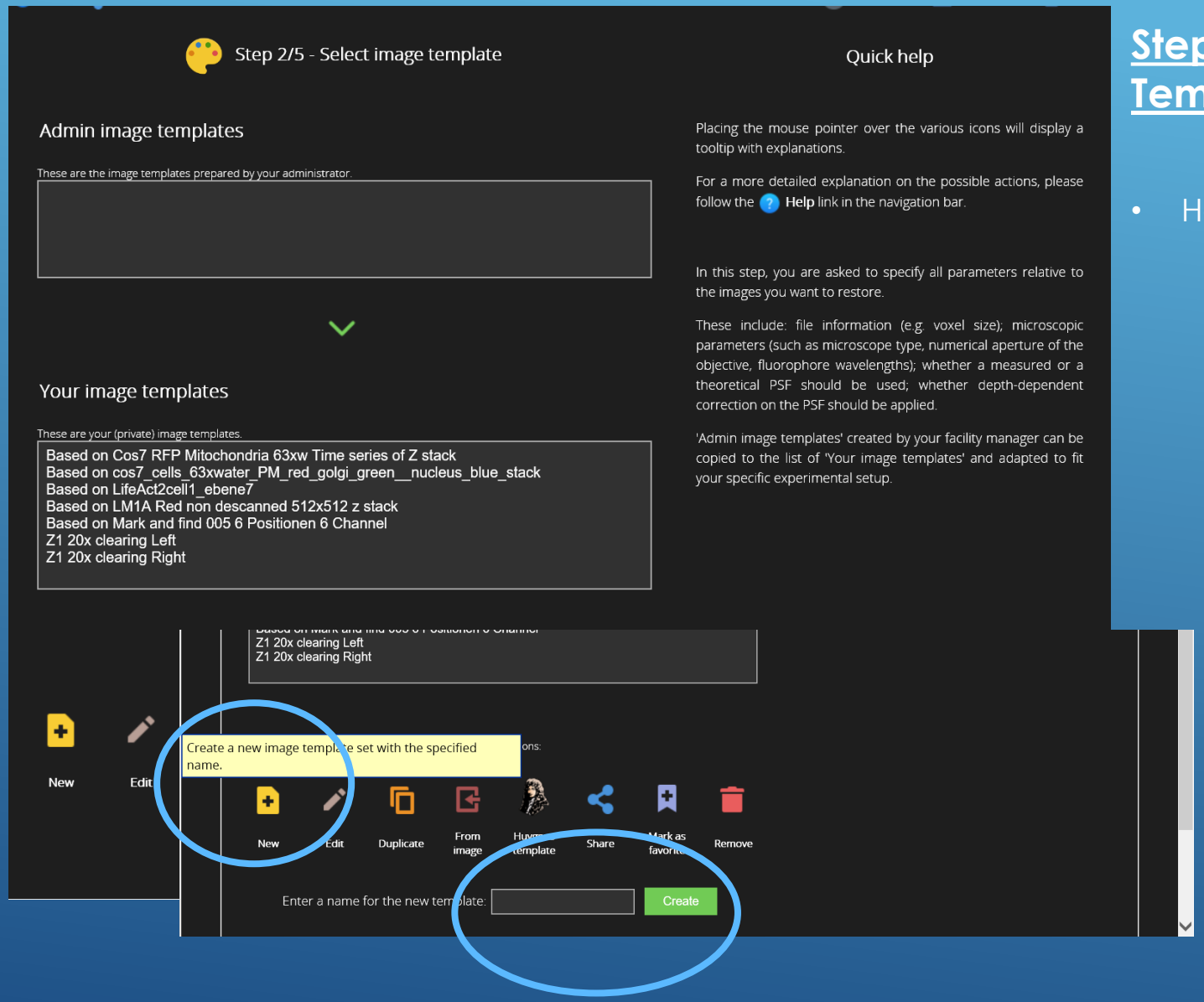


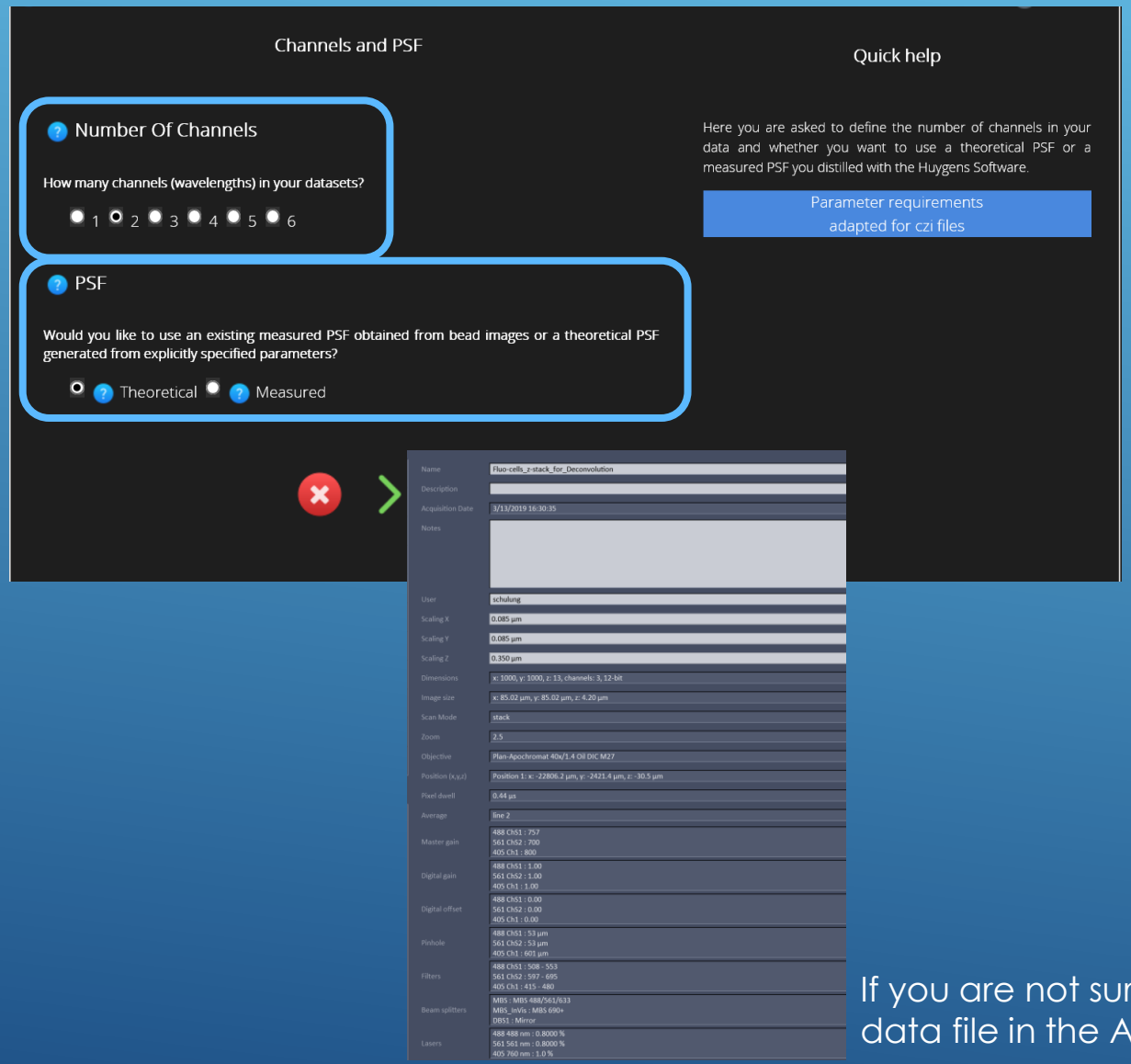
Step 2/5 - Image Parameters - Create Image Template

- To create a new Image Parameter Template you have different options:
  - Create a new one
  - Edit and change an old one
  - Duplicate and edit the new duplicate
  - Generate template from image file, will read meta data and use this as presets in new template

Step 2/5 - Image Parameters - Create Image Template

- Here we **create** a **NEW One from scratch !!!**
  - Click the button '**New**'.
  - Type a **name** for the new template
  - Click '**Create**'. The template editor opens





Step 2/5 - Image parameters - Create Image Template

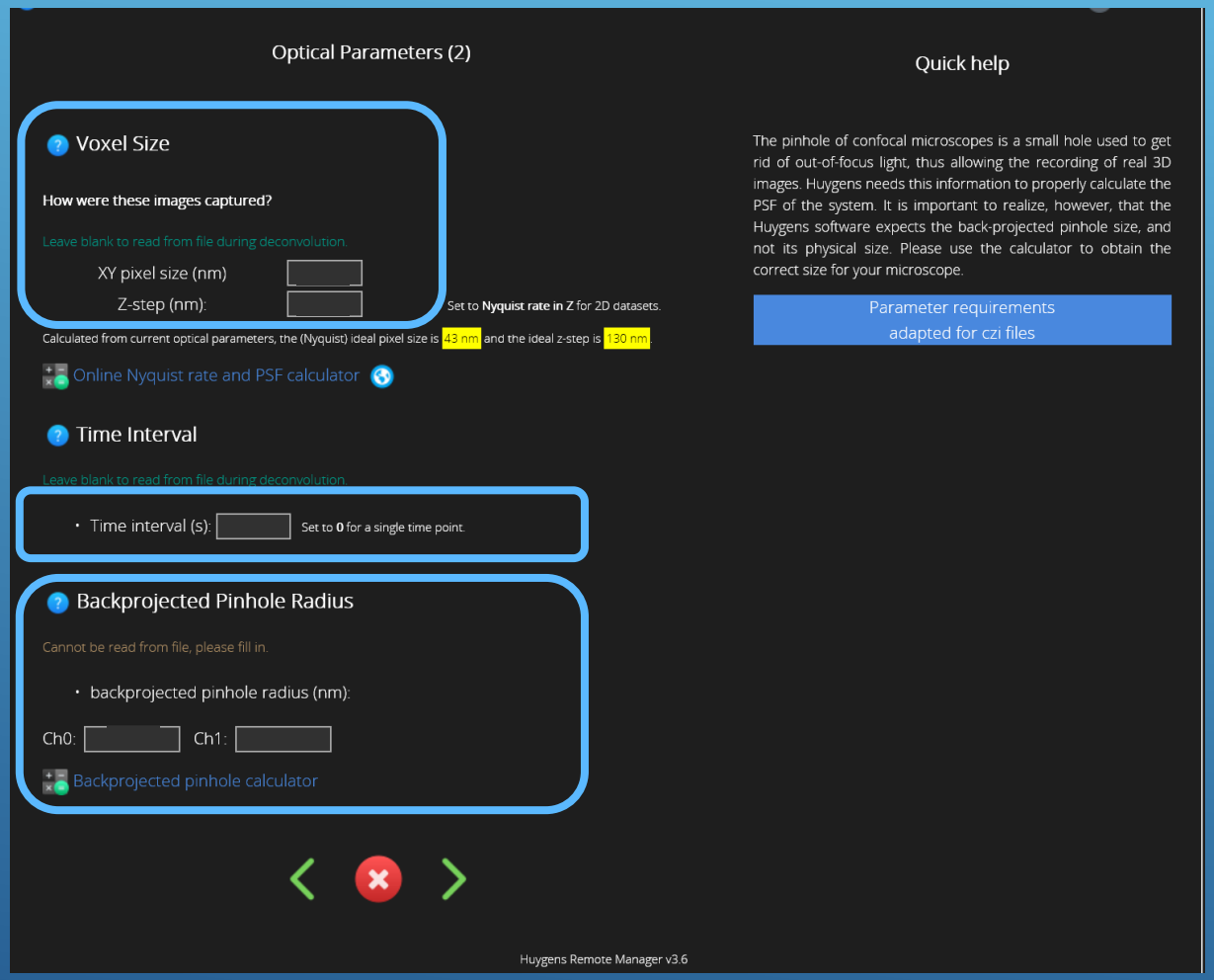
Specify the imaging conditions used during acquisition

- Select the correct **number of channels**
- **Select PSF type** - because you have not measured a PSF with Beads in your sample use – **theoretical** PSF

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

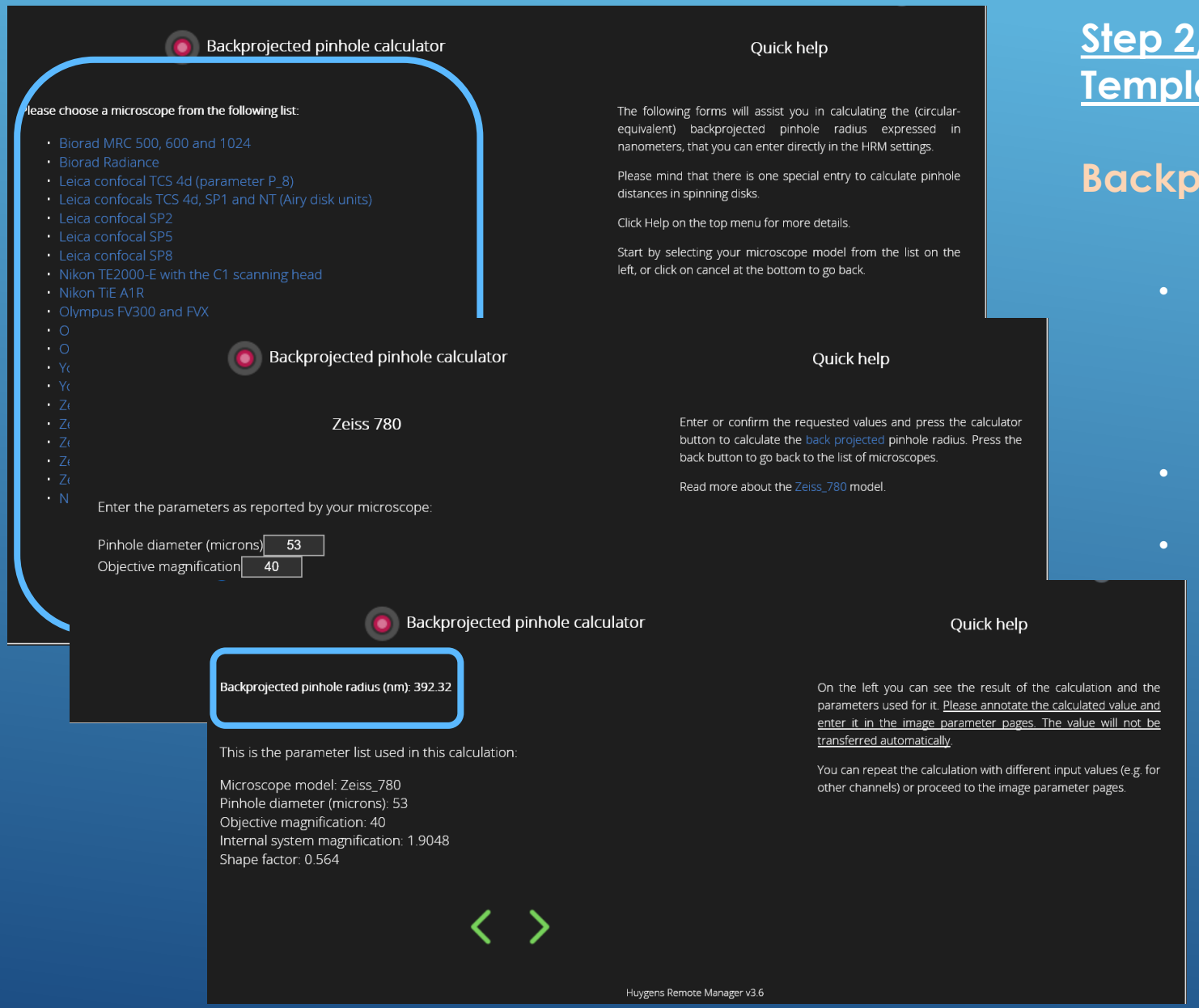
Step 2/5 - Image Parameters - Create Image Template

- Select the **correct microscope type**
- Specify the **numerical aperture** of the objective
- Specify the **wavelength** of each channel and **emission maximum peak**
- Specify the **Objective type**
- Select **Refractive Index of Embedding Medium**
  - Help about the refractive index of the sample embedding medium can be found in the literature of the mounting or can be measured at LIC



Step 2/5 - Image Parameters - Create Image Template

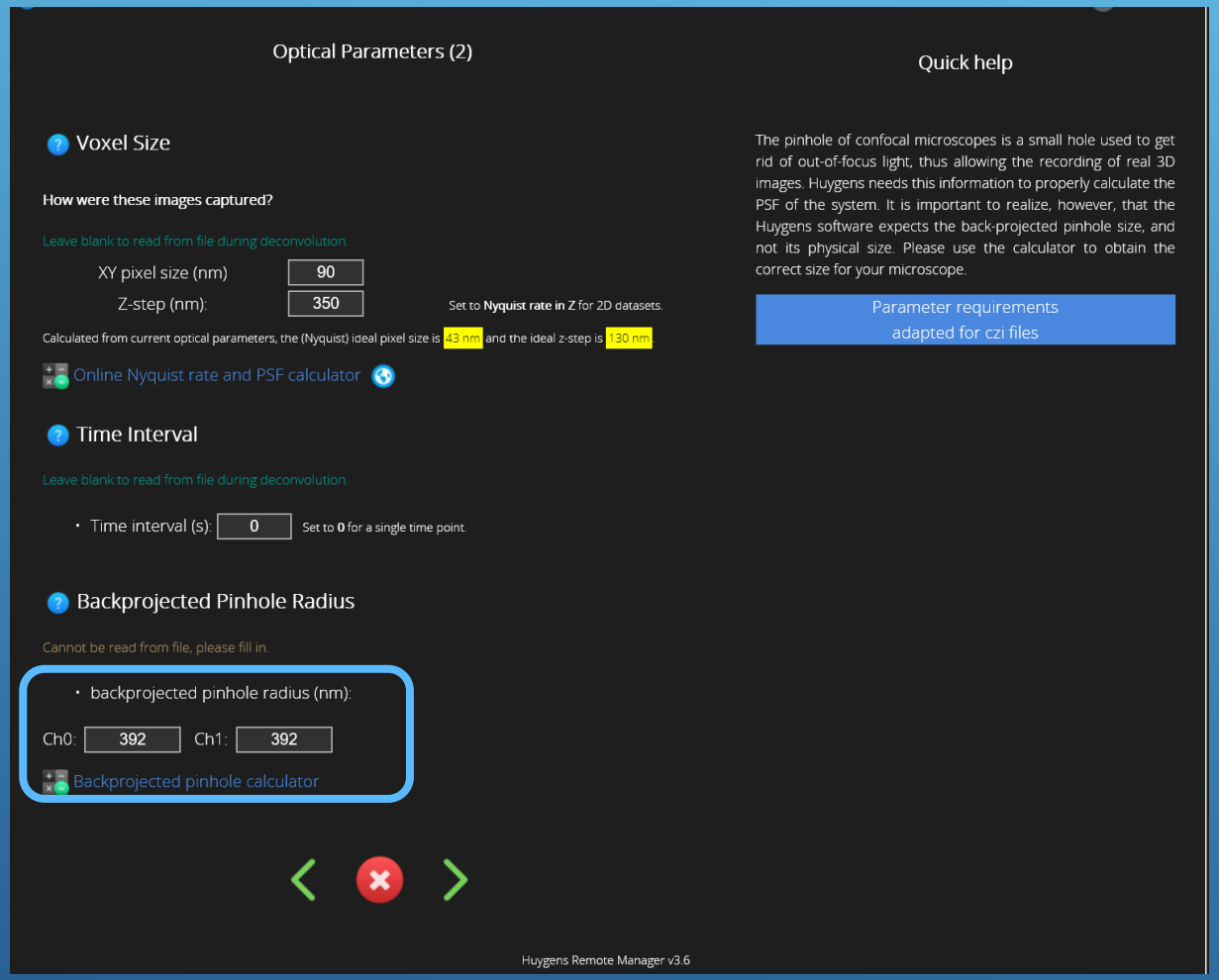
- Remember you can find all asked values when you open your data file in the Acquisition software
- If your raw data are **no time series use 0** for the interval.
- **Open the Backprojected pinhole Calculator** to get the correct values



Step 2/5 - Image Parameters - Create Image Template

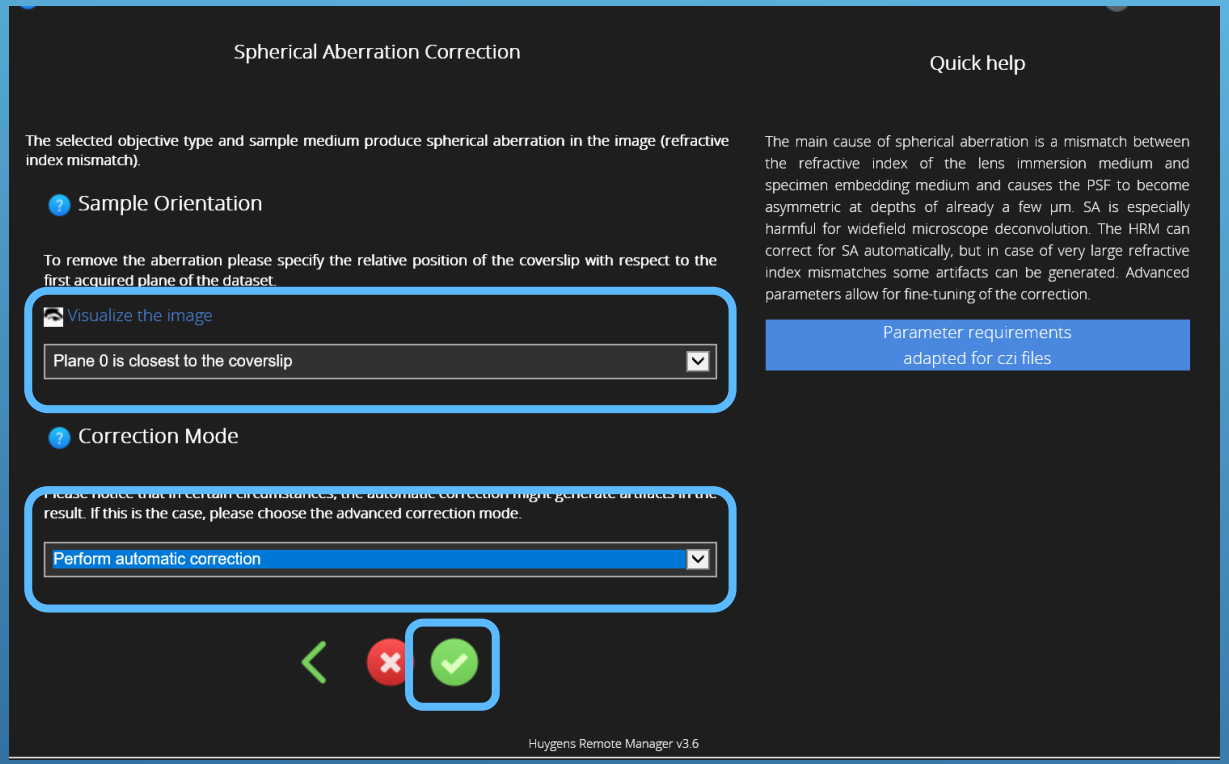
Backprojected Pinhole Calculator

- **Choose** the **microscope type** from the list
  - To check the used microscope type, go to www.miap.eu
- **Follow the instructions** of the Quick help
- **Write down and go on to transfer the value** to the main sheet



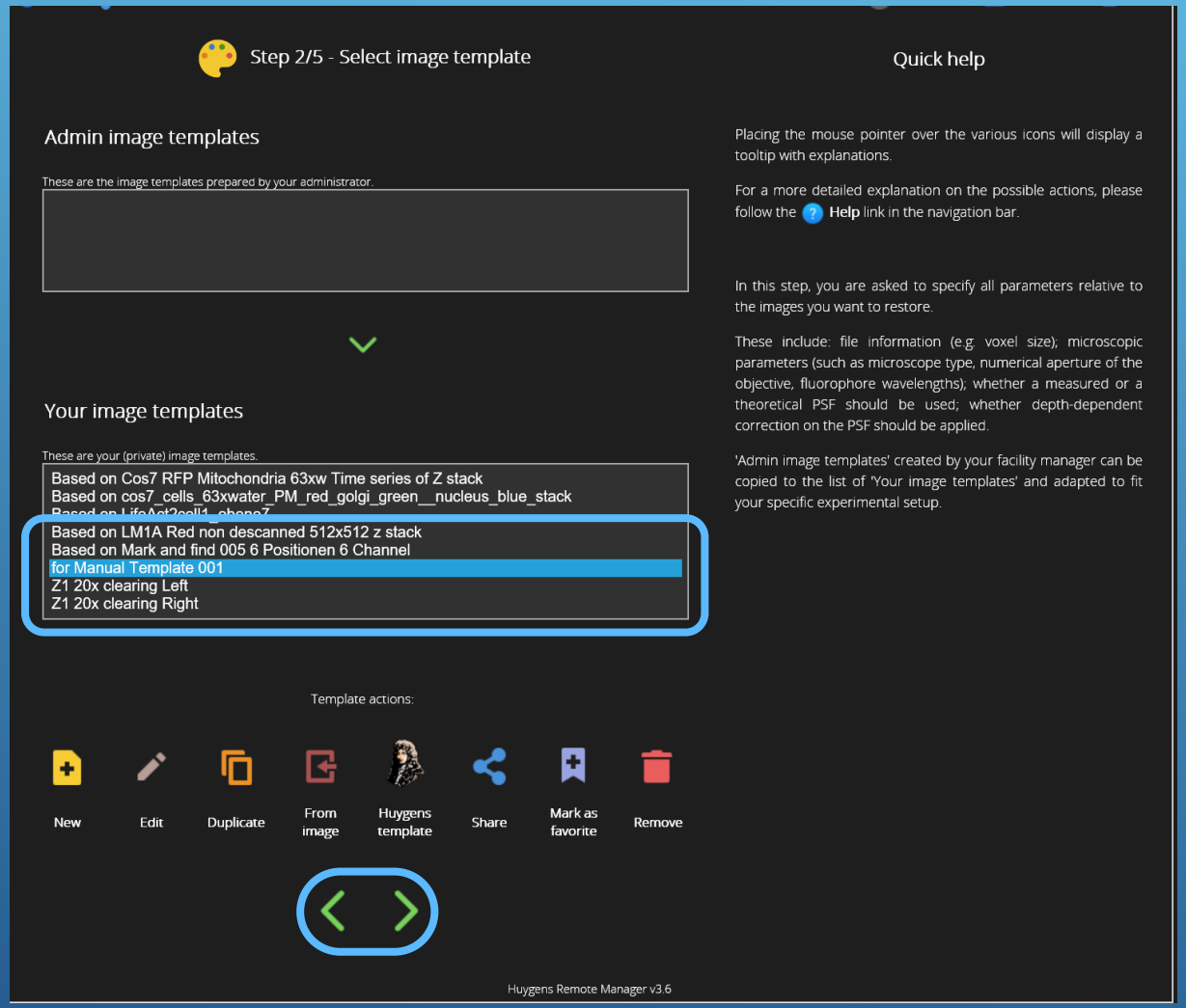
Step 2/5 - Image Parameters - Create Image Template

- Insert the **calculated radius** values of the backprojected pinhole
- Go on



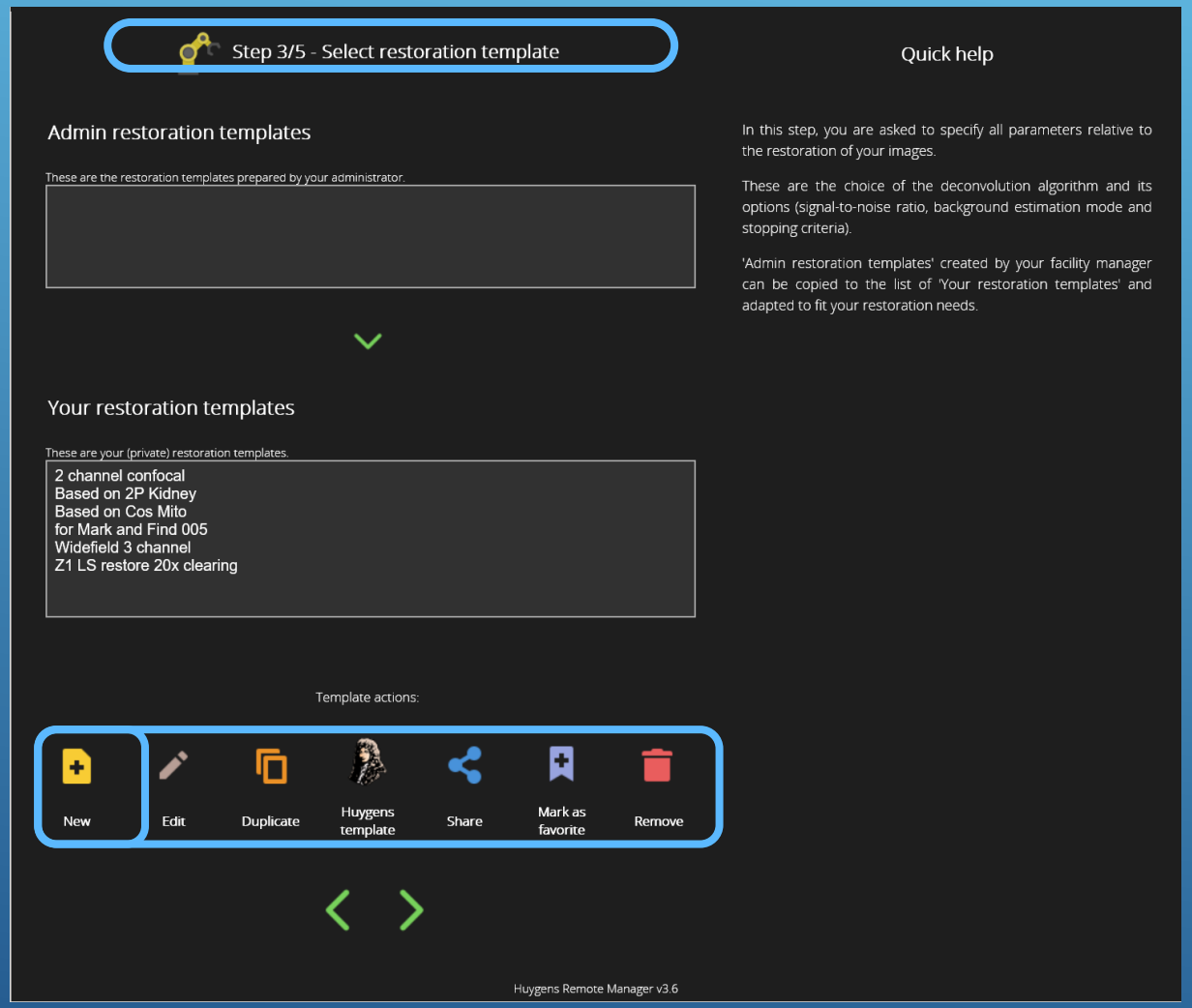
Step 2/5 - Image Parameters - Create Image Template

- To remove spherical aberration created by objective type and sample medium **define the orientation of sample plane 0 to the coverslip**
- Perform **automatic correction**
- **Finish** Template creation.



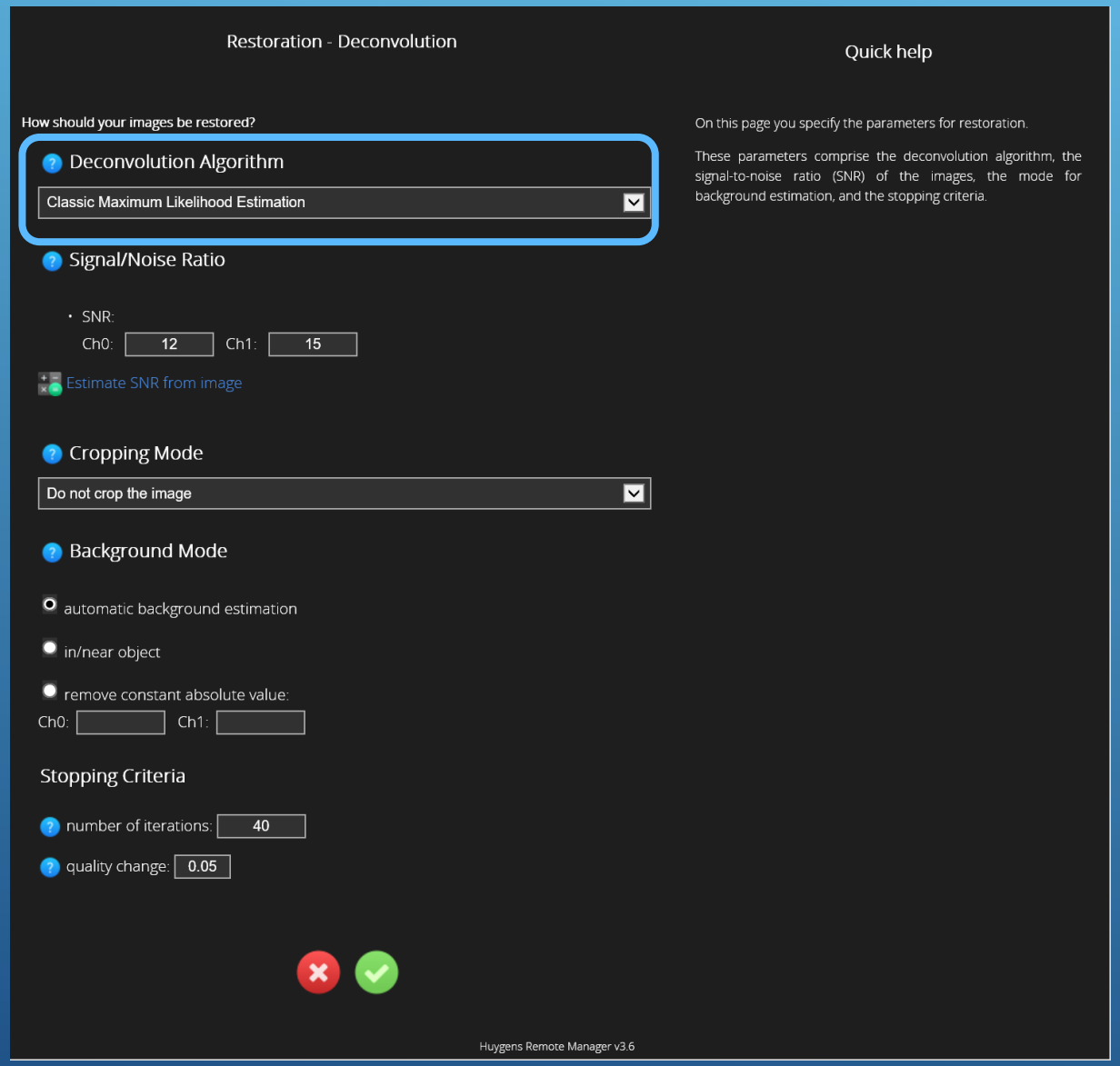
Step 2/5 - Image Parameters - Create Image Template

- **Select** your new Image Parameter Template
- Go on…



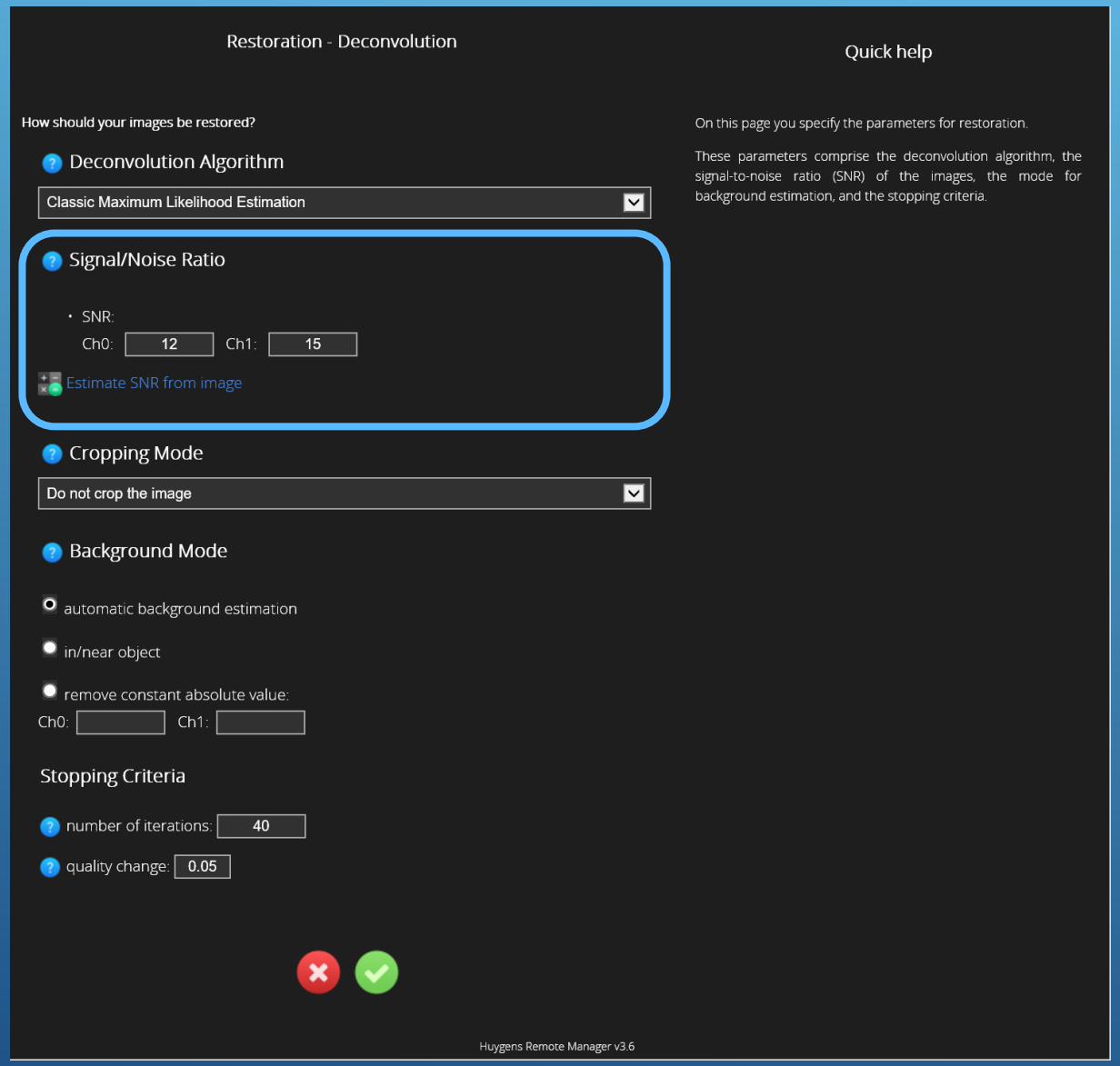
Step 3/5 - Restoration Parameters - Create Restoration Template

- Now create a **Restoration template**
  - specify parameters relative to the restoration of you image (choice of deconvolution algorithm, signal-to-noise ratio, background estimation ….)
- As before for the Image Parameter Template **you have different options**
- Now we **Create New as before with a new name**



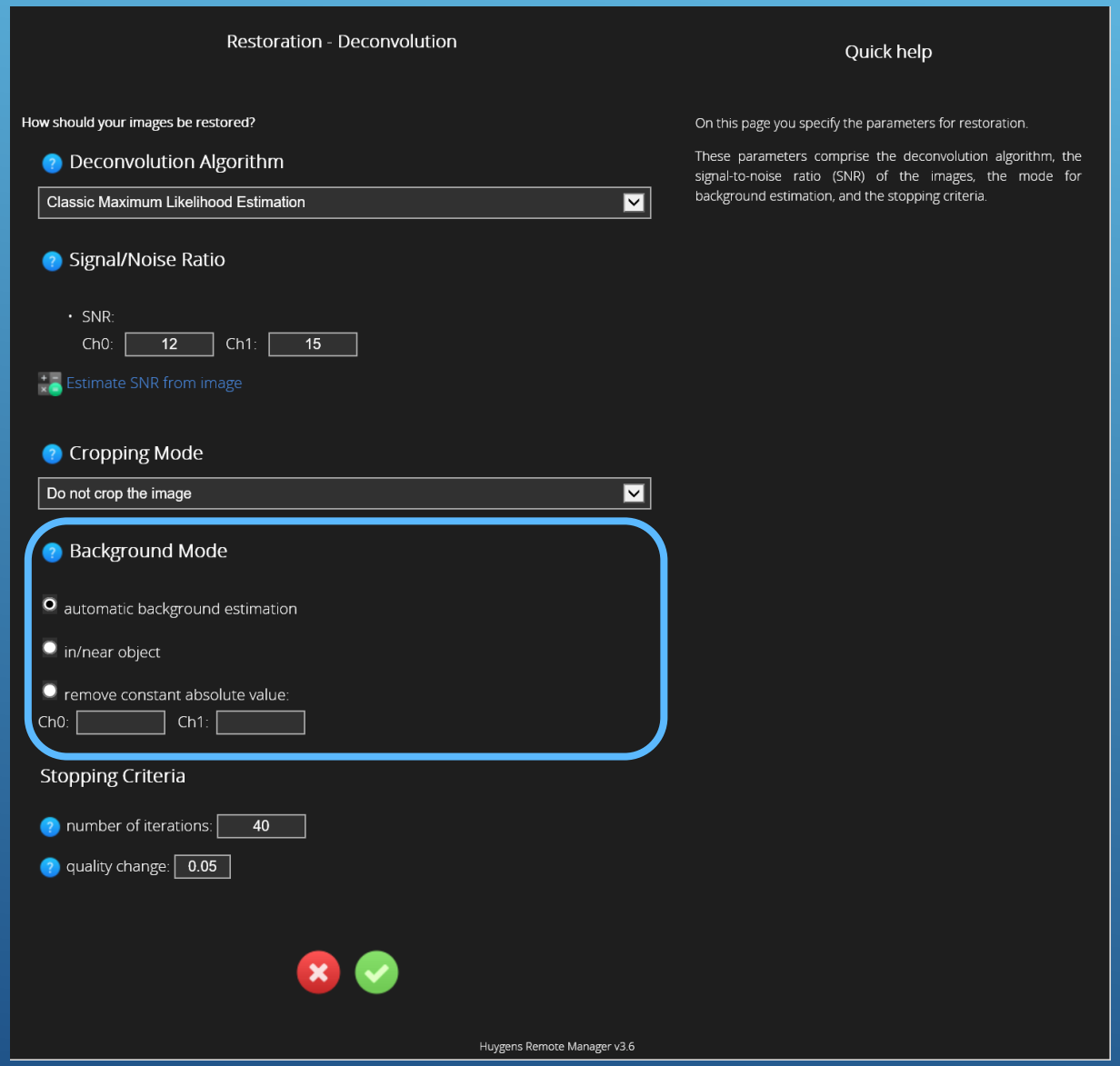
Step 3/5 - Restoration Parameters - Create Restoration Template

- Define the **Deconvolution Algorithm**
  - The **CMLE** is the most default general deconvolution algorithm available in Huygens, that provides good results for a variety of imaging conditions (**confocal, widefield**).
  - The **GMLE** is a deconvolution algorithm optimized to handle high-noise images, as **STED** or confocal images.
  - The **QMLE** is a deconvolution algorithm that gives good and fast deconvolution results on **low-noise widefield images.**



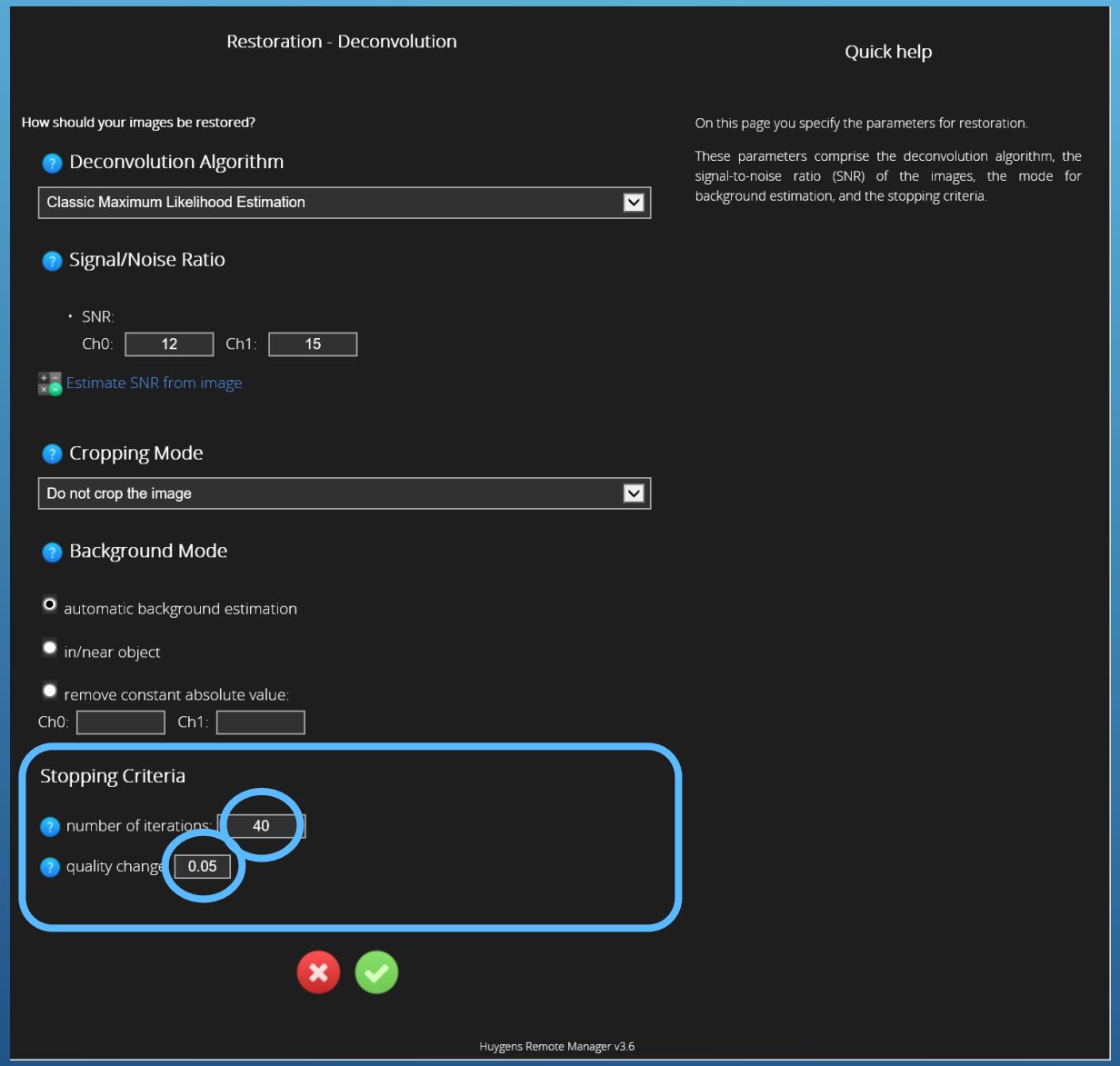
Step 3/5 - Restoration Parameters - Create Restoration Template

- 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.
  - A **noise-free widefield image** usually has SNR values higher than **50.**
  - A **noisy confocal image** can have values lower than **20**
  - A noisy **STED** values below **12**



Step 3/5 - Restoration Parameters - Create Restoration Template

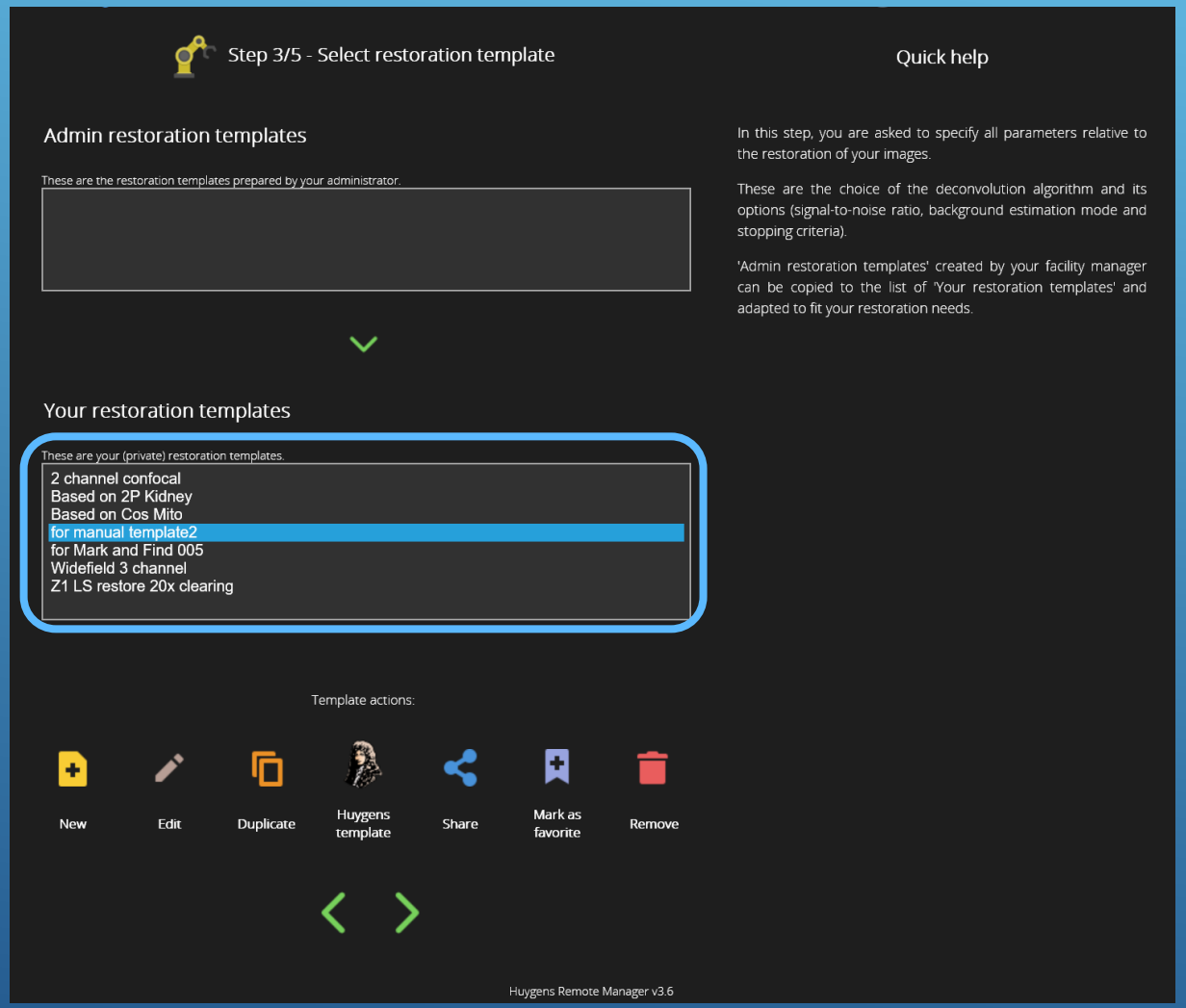
- Set the **background** mode to **automatic**



Step 3/5 - Restoration Parameters - Create Restoration Template

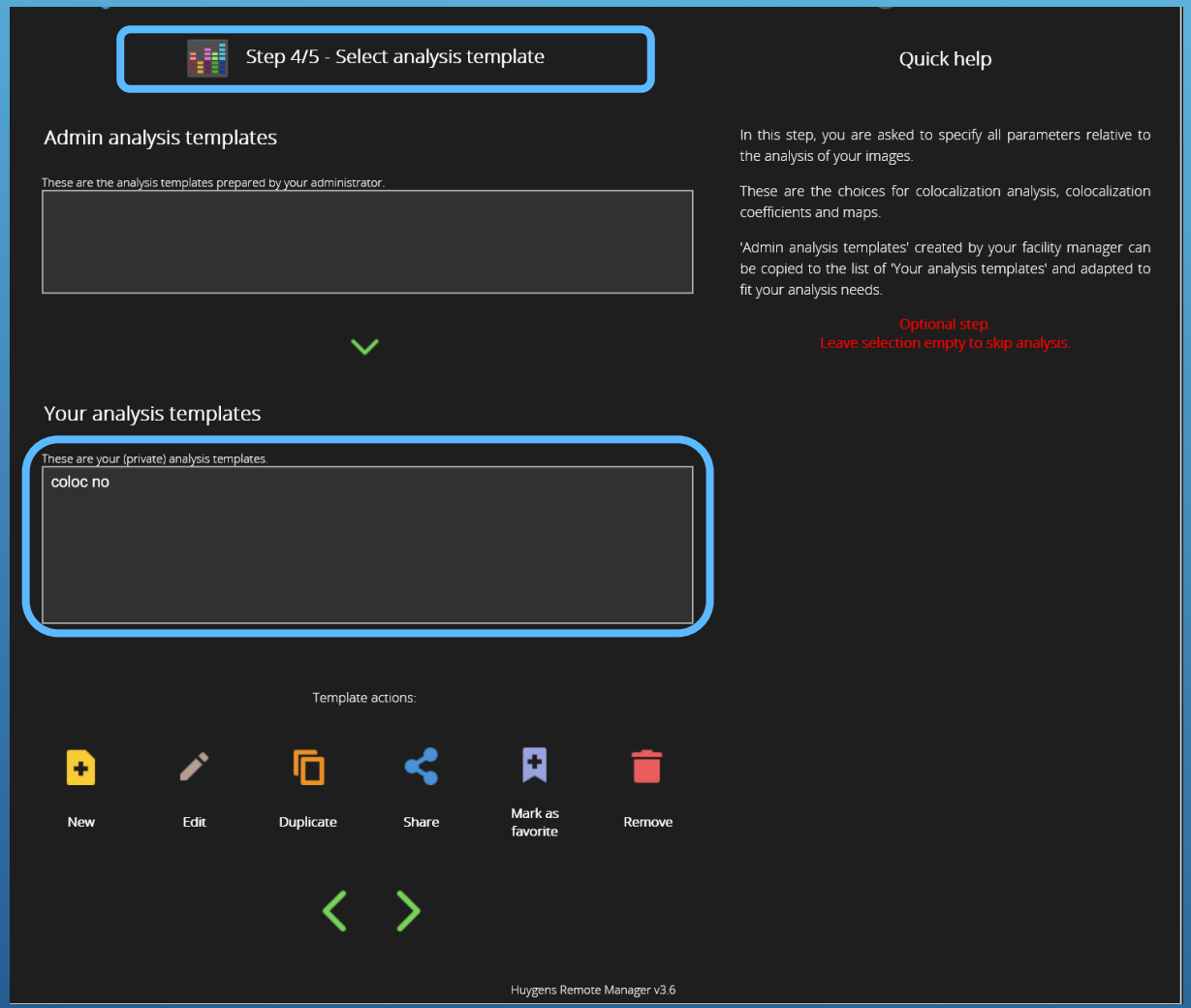
- Define the **stopping criteria** of the algorithm
  - The **Maximum Number of Iterations** is a Restoration Parameter of the Huygens Software that puts a limit to the iterative deconvolution.
  - Another limit is established by the Quality Change **Threshold parameter.**
  - **For an initial run you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

To achieve best result you can increase or decrease values



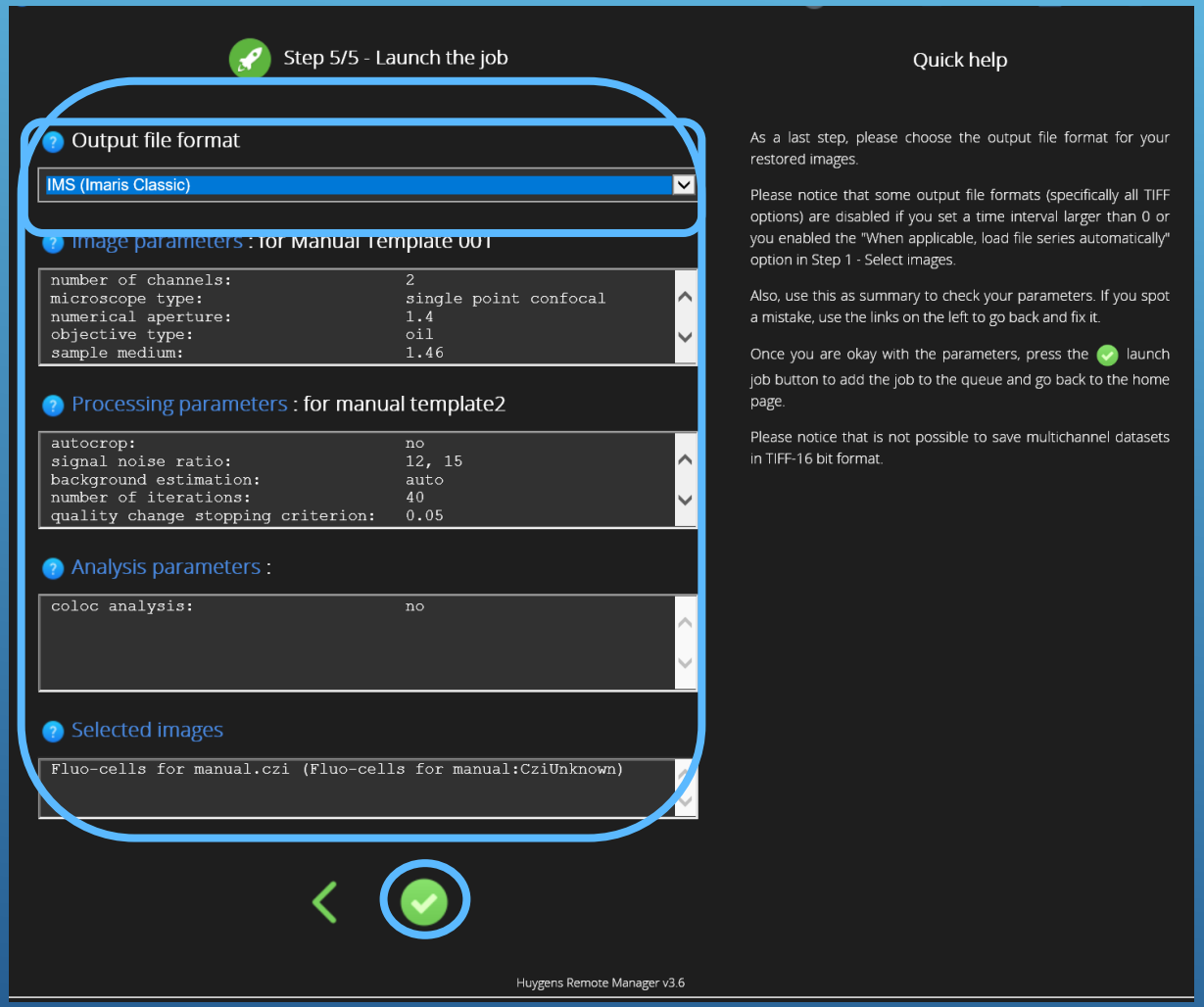
Step 3/5 - Restoration Parameters - Create Restoration Template

- **Select** the **new Restoration Template**
- Go on



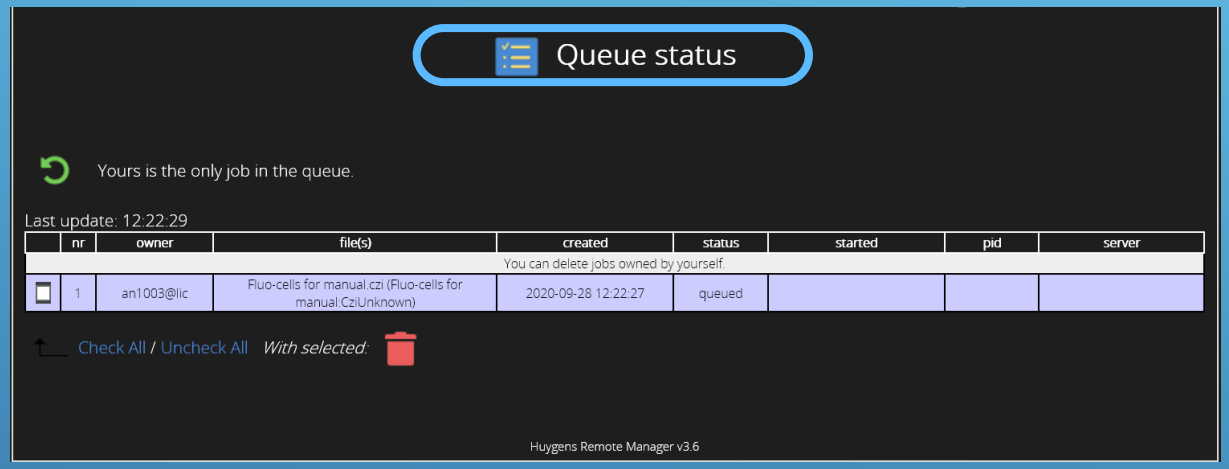
Step 4/5 - Analysis Template

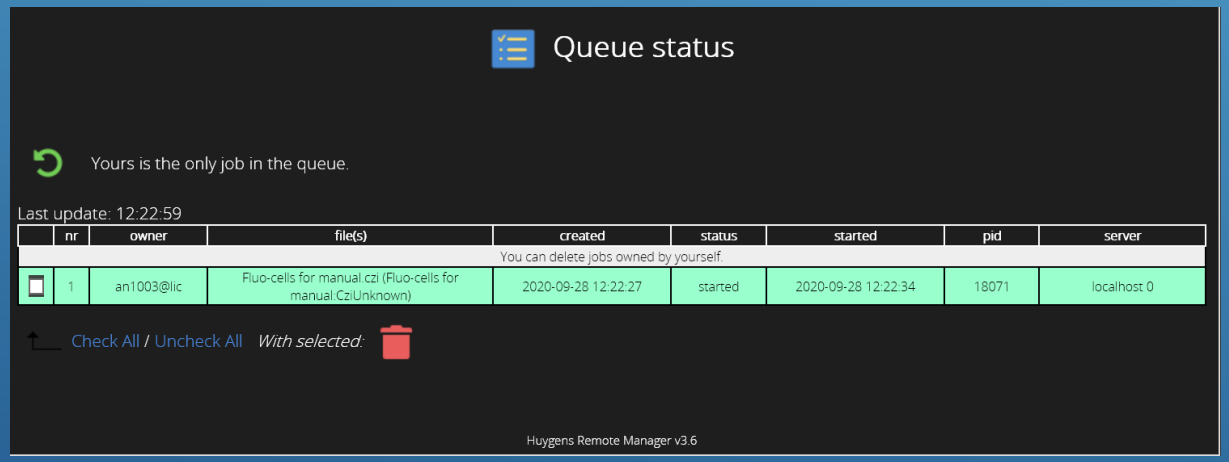
- This step is only active when 2 or more channels have been specified in the previous steps - allows the user to execute Colocalization analysis on the deconvolved images
    - **Select/create a template or skip**
    - **Created** with i.e. Click ' **New** ', **type** a name and click '**Create**'.
        - Select whether colocalization should be enabled. For a colocalization run select the channels to be analyzed as well as the colocalization coefficients of interest. Set the threshold to "Automatic estimation" and the Colocalization map to "Pearson". In order to further fine-tune the Colocalization parameters please refer to Advanced deconvolution in HRM.
- Go to next step



Step 5/5 – Launch a Job

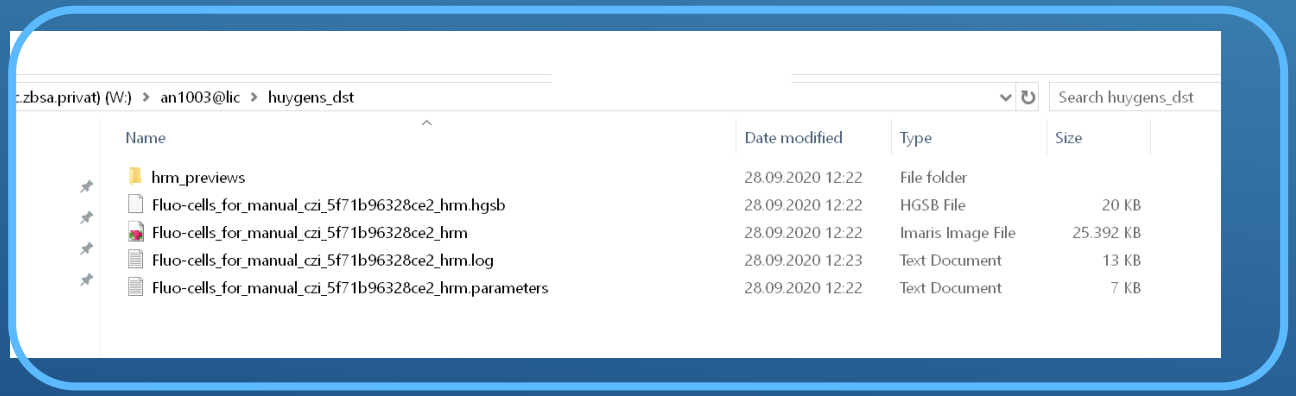
- As last step **Define Output file format** for the restored images
  - **ICS** recommended format - provides good dynamic range and necessary meta data infrastructure to save all the image parameters - creates more than one file for each restored image, all have to be saved and/or copied!
  - **IMS** (Imaris classic) – also ok, creates only one file for each restored image, but always only 8bit images
  - Further details: https://svi.nl/FileFormats
- **Control parameter** summaries as well as the image selection
- Then **start job**





Queue Status

- After submitting the job HRM returns to the Home panel.
  - Click **Queue Button** in **Home panel - Welcome Page**
- Deconvolution will run automatically and sequentially if there are jobs of other user too.
  - When a job is finished a notification email is sent to you with a link to the result.



Restored Images

- **Access to the restored images** via HRM home panel - click on – '**Result** Button'

or

- Via **File explorer** on **you computer** - File manager: data on drive W/username@lic/: **huygens_dst** (in this case an Imaris file)

Thanks for viewing

For further information

Detailed - Online HRM - User Manual