

Huygens quick start guide

Once you have loaded data into Huygens (File → open → select file):

- Right click the image series you wish to deconvolve and select “Microscope parameters”
- Here you will be able to check that Huygens has correctly read the metadata parameters associated with your data acquisitions. The things to pay particular attention to, and correct if necessary, are:
 - The XYZ pixel sizes in nm
 - The numerical aperture of the lens used
 - The lens immersion
 - The embedding medium
- Once the microscope parameters have been checked, click “Set all as verified” at the bottom of the window. Then close the window by clicking “accept”.
- Right click the image series you wish to deconvolve again and select “Deconvolution Express”
- A new window will open showing your data
 - Down the RHS you have sliders to play through the z-stack and/or timecourse
 - Toggle active channels
 - Change display colours
 - Change brightness and contrast
- In the section under the window you can select the deconvolution algorithm
 - Standard – this will be appropriate for most users
 - Aggressive – this is appropriate for high SNR images
 - Conservative – this is more appropriate for low SNR image
- Click “ROI Deconvolution” to create a small test region for deconvolution.
 - Use the slider to change the deconvolution algorithm parameters and see a real time output of the results in the ROI area selected
 - The ROI area can be moved by clicking and dragging to an area of interest in your sample
- Once happy with the slider position, click “Express Deconvolution” to deconvolve.
- Once deconvolution is finished you can explore and compare to the raw data using the “Twin Slicer” option, or click “Done” and the processed data will be visible on the Huygens start page appended with the words “_decon”
- You can then right click the processed data series and select “Save As” to save the data
 - HDF5 format is Huygens preferred format to read so we recommend saving as this
 - Also save data as TIFF format 16bit.
 - Select option “One per channel”
 - Select option “Clip”