Huygens PSF Distillation

Using Huygens PSF Distillation Wizard – steps:

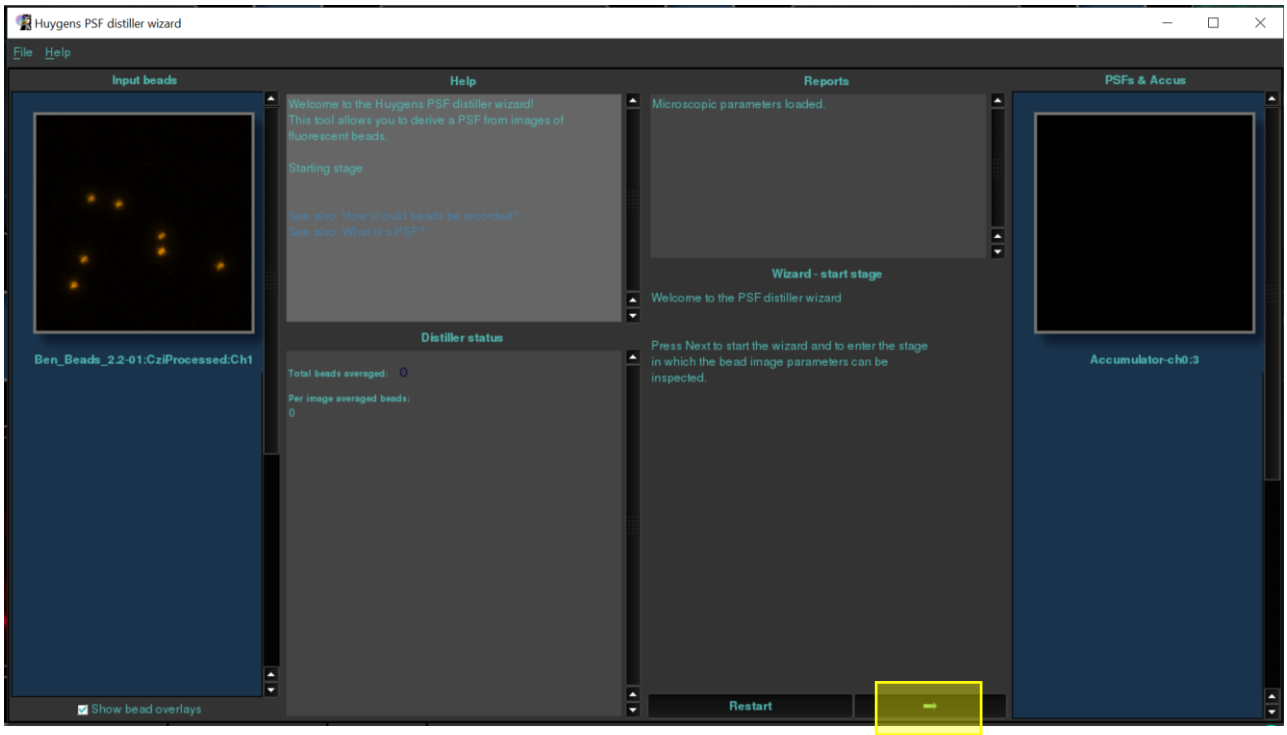
1. Loading an image.

   Open the beads image via **FILE→OPEN.**

2. Load microscopic parameters:
   Right click on the image.

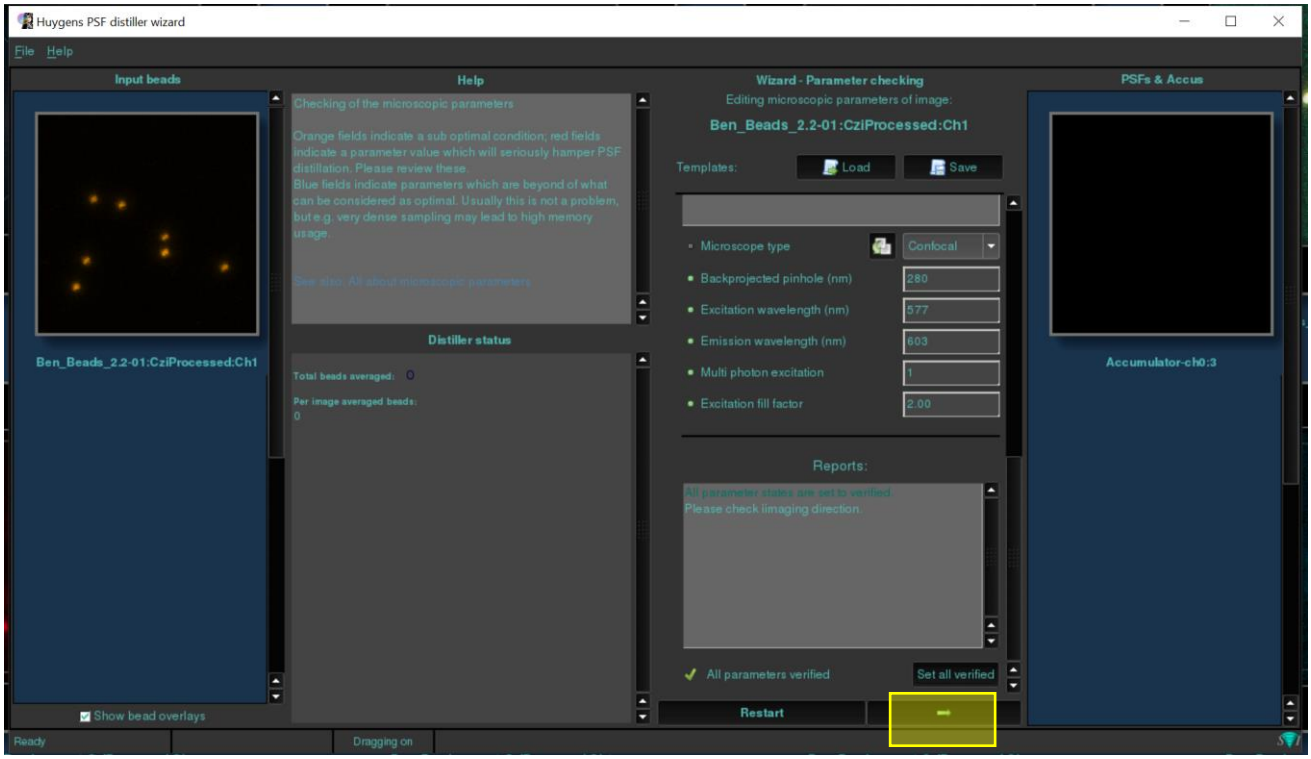
Based on the acquisition settings, load the microscopic parameters and check. It is in particular important to check the sampling densities. Do not use undersampled bead images. If any of the entry fields for the sampling density turns orange or red, the data is unusable for distilling PSF'

3. Start the PSF Distiller via the menu **DECONVOLUTION -> PSF DISTILLER**
   Follow the steps below (default options highlighted in yellow).



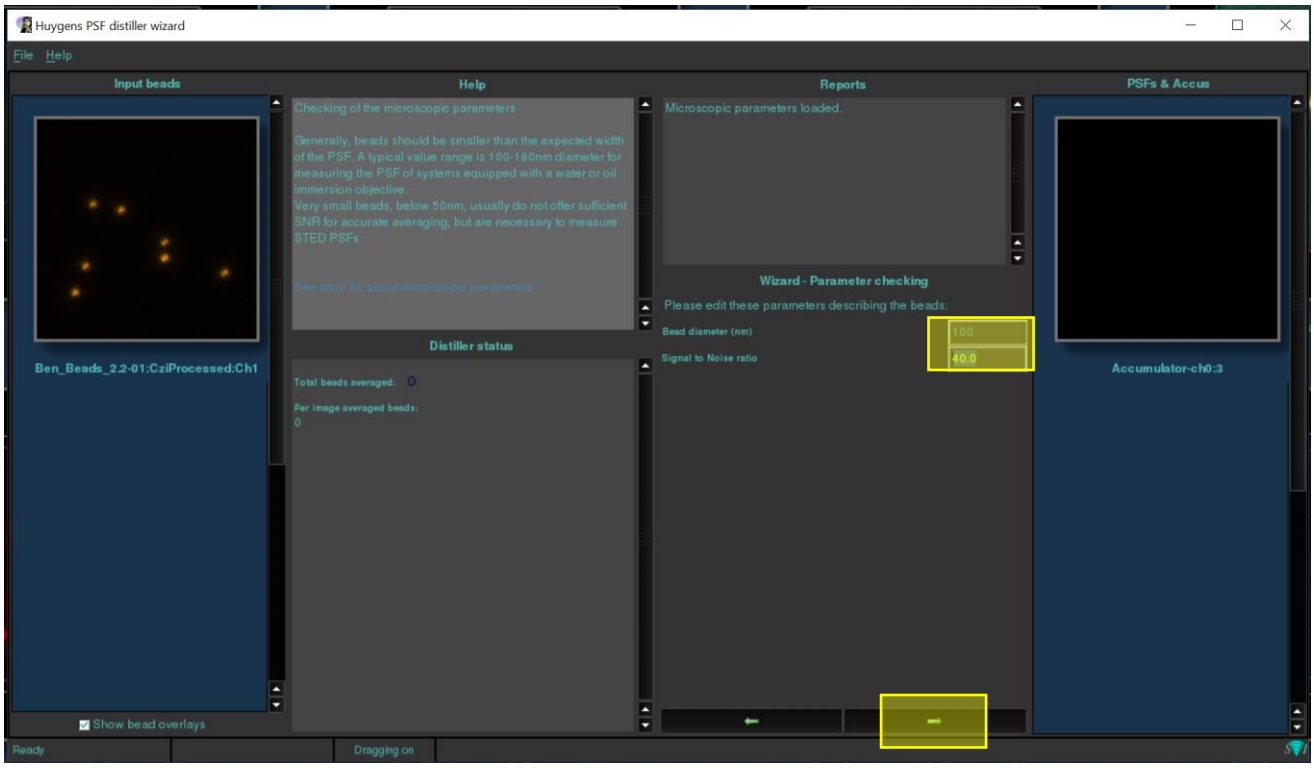
Huygens PSF Distillation
NUS CBIS

Check the microscopic parameters and verify. Click next



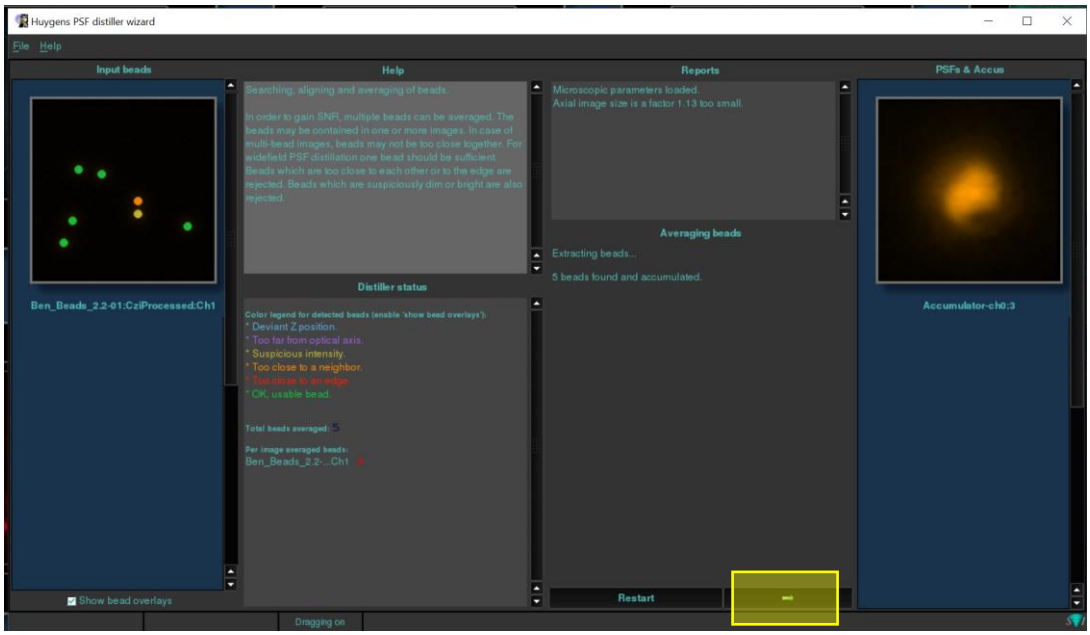
Enter size of beads and SNR value of beads images

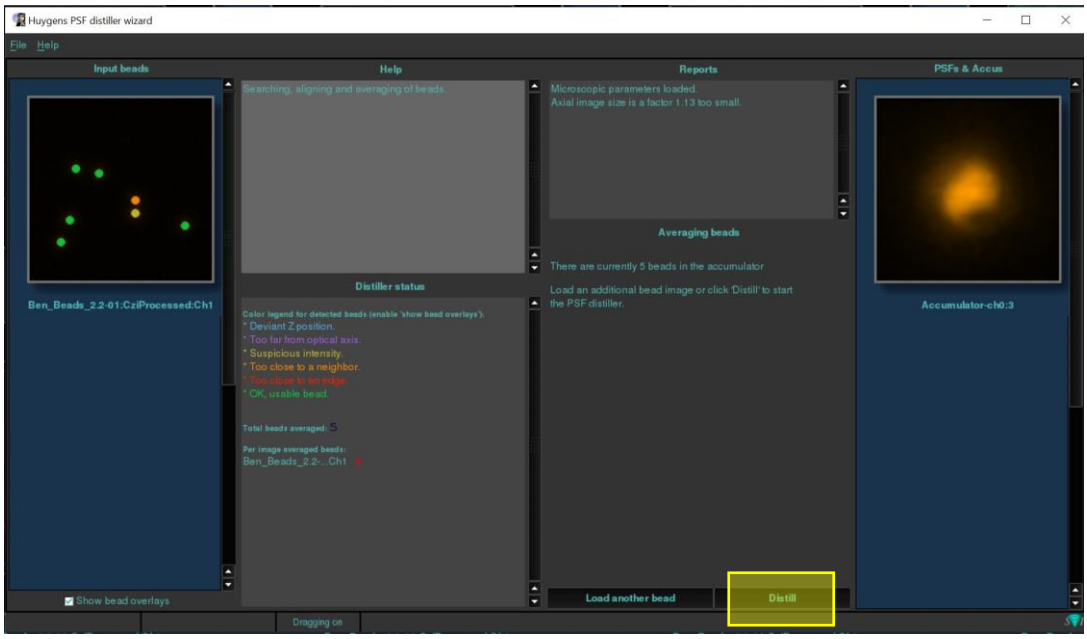
Click Next



Huygens PSF Distillation
NUS CBIS

Averaging Stage:





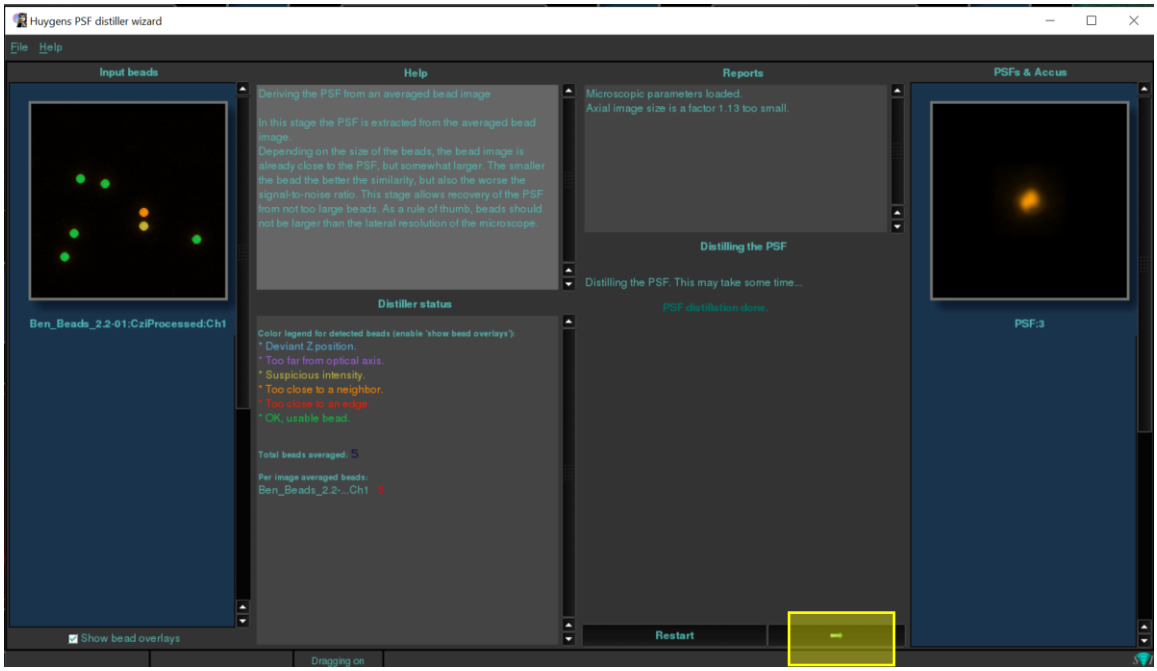
The beads which have been identified for accumulation and usable are observed in the panel.

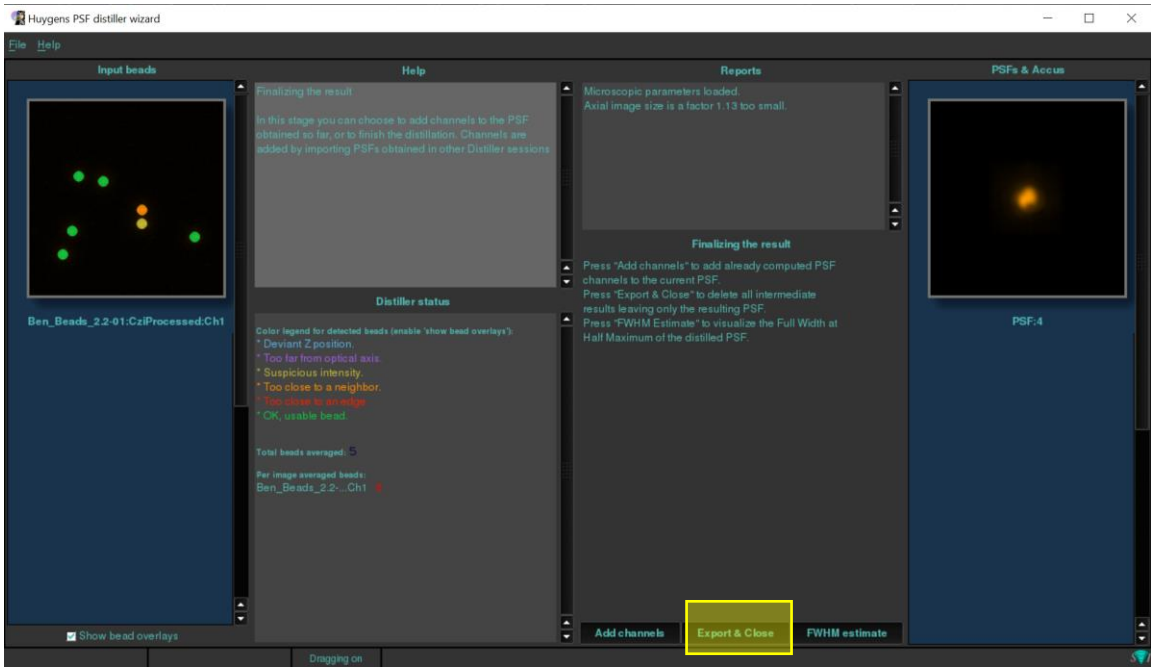
Only beads that meet the following selection criteria can be used:

•A bead should not be too close to another bead. If a bead is too close to another bead.
•A bead should not be too close to an image edge. After all, another bead might be located just over the image edge.
•The intensity of a bead should not deviate too much from the median intensity of all beads. If it is brighter then it may be a cluster of two or more beads. If it is dimmer then it is not likely to be a bead.

If you are face issues in the above criteria, and face an error in this section:
Go back to the main menu. Select the beads images in main window, in menu go to TOOLS→ CROP.
Crop the images and ensure you select 4-5 beads that do not overlap with each other and are not at the corner of the image. Try the PSF distiller wizard again, with the cropped beads image.

Distillation Stage: in this stage the PSF is measured from the averaged beads, for all available channels.





Add channels: in case it is desired to combine results from earlier distillations with the current result to obtain a multi channel PSF, an earlier result can be added here.

FWHM estimate: Full width half maximum calculation of the distilled PSF is visualised, for reference.

Click **Export & Close**

Measured PSF is generated. Save the result to use it for deconvolution.

Huygens PSF Distillation
NUS CBIS

Note:
Beads ranging from 120 to 250 nm can be used. Typically beads with a diameter of 160 nm perform very well for many types of microscopy.
Beads should be recorded with the same microscopic parameters that you will use later to image your specimens (ensure Nyquist sampling).