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'

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



Check the microscopic p verify. Click next	parameters and		
🕵 Huygens PSF distiller wizard			- 🗆 X
<u>File H</u> elp			
	Help	Wizard - Parameter checking	PSFs & Accus
Ben_Beads_2.2-01:CziProcessed:Ch1	 Checking of the microscopic parameters Orange fields indicate a sub optimal condition, red fields indicate a parameter value which will seriously hamper PS distillation. Please review these. Blue fields indicate parameters which are beyond of what can be considered as confined. Usually this is not a point of the parameters which are beyond of what can be considered as confined. Usually this is not a point of the parameters which are beyond of what can be considered as configured as configur	Editing microscopic parameters of image: Ben_Beads_2.2.01: Cz/Processed:Ch1 Templates: Image: Microscope type Microscope type Backprojected pinhole (nm) Backprojected pinhole (nm) Excitation wavelength (nm) Emission wavelength (nm) Excitation fill factor Load Reports: All parameters allers are pet to ventiond Please check liminging direction.	Accumulator-ch0:3
🖬 Show bead overlays	● ▼		

Enter size of beads and SNR value of beads images Click Next

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Input beac		Help	Reports	PSFs & Accus
Ben_Beads_2.2-01:Czil	Processed:Ch1	rep Precking of the microscopic parameters Senerally, beads should be smaller than the expected width the PSF A typical value range is 100-160nm diameter for neasuring the PSF of systems equipped with a water or oil mears on objective. Yery small beads, below 50nm, usually do not offer sufficient SNP for accurate averaging, but are necessary to measure STED PSFs. See alter: All about microscopic parameters Distiller statue	Wicroscopic parameters loaded. Wizard - Parameter checking Please edit these parameters describing the beads: Bead diameter (nm) Signal to Noise ratio	Accumulator ch0:3
Show bead ov	rerlays	d'ull beach averaged beads:)		
Ready		Dragging on		57

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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 \rightarrow 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.

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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.

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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).