

# How to acquire an experimental PSF

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## *Introduction*

In all cases the Huygens software will use a psf in order to deconvolve the experimental sample. This can either be a PSF that has been calculated from the image parameters taking into consideration the objective, the fluorescence properties of the dye, the refractive indices etc (theoretical psf) or it can be measured experimentally. The advantage of an experimentally determined psf is that it contains additional information about small optical aberrations of the objectives etc, parameters that are not considered in a theoretical psf. Practically this implies that the total improvement in optical resolution will be higher when using an experimentally determined psf, although a theoretical psf will as well result in a substantial gain in resolution. The disadvantage of a measured PSF is that it needs to be measured (☺), requiring additional experimental effort. Therefore the measurement of the psf is especially recommended in situations, where the final resolution is very critical.

## *Bead image acquisition(SP5 confocal)*

An experimental point spread function, which can be used for deconvolution is usually acquired using fluorescent beads. Suitable beads should be bright, rather small (<200 nm diameter) and are available from the biooptics manager. Before or after acquisition of the sample one needs to acquire the respective beads, for each color separately under the same imaging conditions, **same meaning**

- Same laser line
- Same PMT
- Same wavelength emission detection range
- Same zoom (@Nyquist sampling rate)
- Same objective (!!!)
- Same mounting medium (!!!)
- Same coverslip

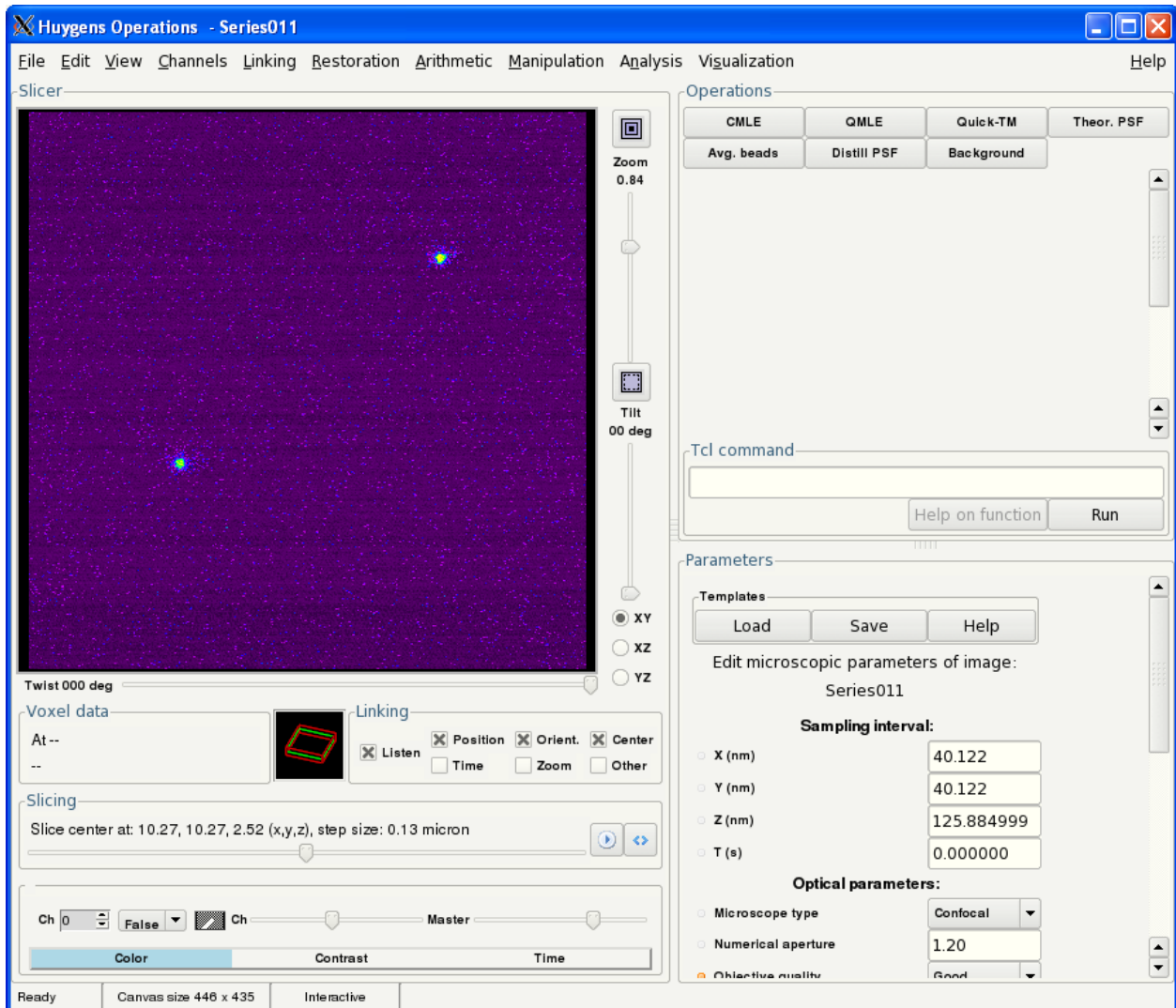
It is, however, **acceptable to change the following parameters**

- Laser power (beads are often more intense)
- PMT gain and offset
- Averaging (more averages can be used)
- More than one bead may be present per image, but spacing between the beads should be rather large

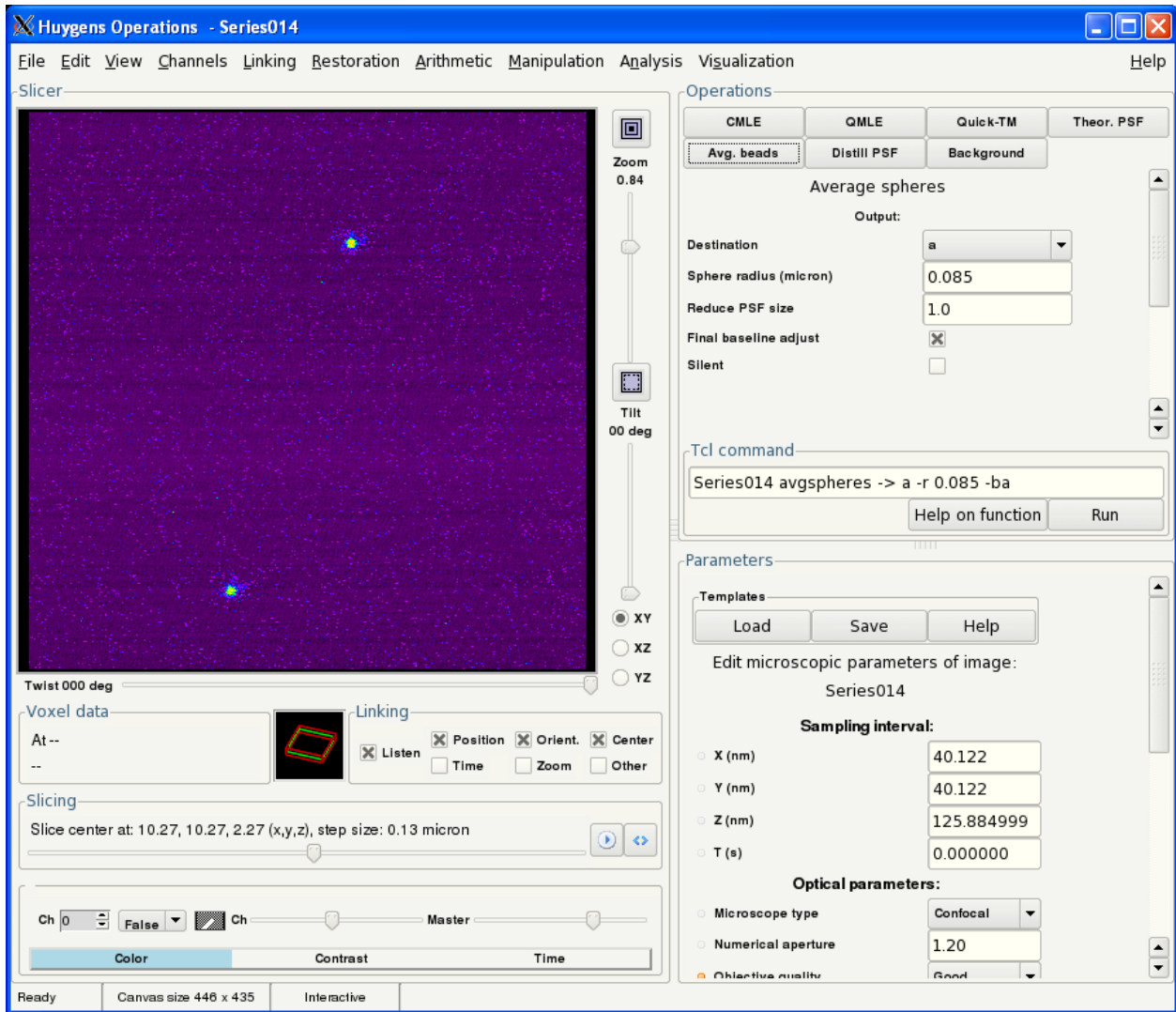
Bead images should be acquired as z-stacks at optimal Nyquist sampling rate in all three (xyz) directions. All other guidelines concerning proper imaging also need to be followed (saturation, gain, offset, bleaching....).

## Data handling in Huygens

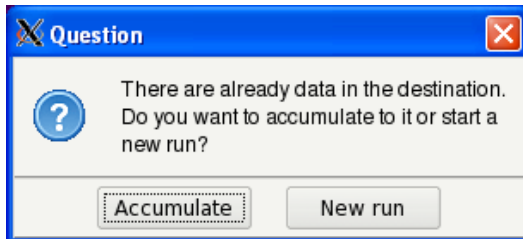
- Load bead images & enter image parameters as usual
- Choose operations window
- “Avg.beads”



- Enter the bead radius (the manufacturer supplies *the bead diameter* on the box!)
- Run



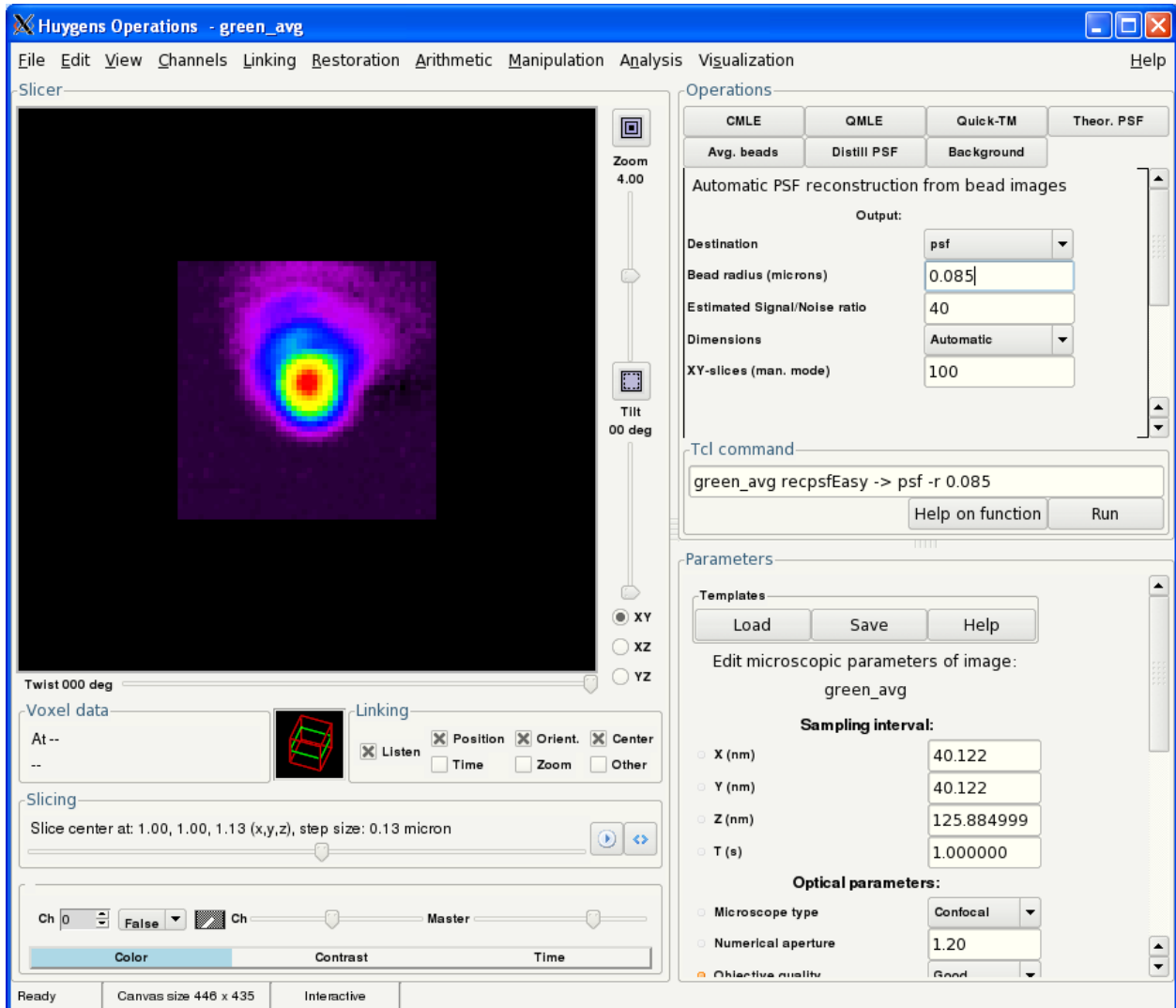
- Repeat this for each bead image and accumulate the data in one image



Accumulate several bead stacks into one final averaged bead image (eg in a). The image in a does NOT yet represent the measured PSF, by definition the PSF is the fluorescence obtained from an infinitesimal small object if imaged through the scope. Until now we have just made an average bead image. Huygens now needs to do an inverse deconvolution in order to get the psf, this requires again knowledge about the bead geometry (size)...

- Now click the average bead image, select “Operations window” again

- Take “Distill PSF”
- Enter the bead radius (not diameter!)
- Enter the estimated signal-noise-ratio
- Run



- Save the psf and the average bead image
- Close
- Repeat all steps for each channel
- Load the psfs for each channel and make one multi-channel psf out of it
- “Copy” (ALT-C) one psf
- Click the next psf and “Join”(ALT-J)
- Proceed like that for all channels
- Save the final PSF

- Use this psf instead of the theoretical one for deconvolving your sample: load the psf into one of the windows and during entering the deconvolution parameters choose that window as “psf” ; for theoretical psf this window is usually left empty...!

### *Further information*

Theoretical and experimental psf: <http://www.svi.nl/TheoVsExpPsf>

Recording a psf with beads: <http://www.svi.nl/RecordingBeads>