Huygens Professional

Installation:

Download and install Huygens Professional from the SVI website: https://svi.nl/Download

To install the license, launch Huygens Professional version and go to 'License overview' at the pull-down of 'Help'. Click on the 'Add new license' button, paste the license in and press 'Add license'. Close the program and reopen it. Now the license is activated. If you are using a GPU, please check if the GPU option is running. Click on the 3rd Icon from the left and follow the instruction if necessary.

License:
Please contact the Imaging Network to get the actual license.

Deconvolution:

First check your microscopic parameters:
Use right click on your image and go to 'Microscopic parameters'. If necessary correct your parameters. If something is red or orange, this setting is not optimal for deconvolution. Keep this in mind next time you are imaging.

Find the optimal sampling (pixel size):
You can find out the optimal sampling for deconvolution by using the Nyquist calculator:
https://svi.nl/NyquistCalculator
(also available as app for smartphones, and as link on the Imaging Network webpage.)
For a confocal with 1.4 NA lens and 488 excitation the optimal sampling is xy: 43nm, z: 130nm.
This is about 2x the resolution which the software of the microscope indicates as optimal.

How much undersampling is still ok?

For a point-scanning confocal microscope (1.3 lens, 488 excitation) good sampling is around 50nm x 150nm. Laterally 70nm is fine, you shouldn't go beyond 100nm, axially it is best to stay at 150-200nm. If you sample around the 70nm x 150nm you will see that the restoration is capable to reduce noise considerably. As a result you might need less signal than you thought before. If practical considerations (bleaching, data size) don't allow these sampling densities, you'll just have to do the best you can. In our experience, unless you undersample dramatically, the restoration will always improve your image.

Deconvolution Wizard:

Use the Deconvolution Wizard by clicking the magic wand in the menu icon list or right-click on your image. Read the explanations and click next. The 'Auto' modes working fine for most images.
At the 'Deconvolution Setup' window press 'Deconvolution preview' to check your current settings. If the result is to noisy, lower the signal to noise ratio value. Increase the value to get a higher resolution. As a very basic approach, the following values can be used as a first estimation of the SNR of different image types:

Bad quality confocal image: SNR = 10

Normal confocal image: SNR = 20

Good quality confocal image: SNR = 30-60

Good quality widefield image: SNR > 40

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If the image noise is clearly amplified after the restoration then the SNR value provided to the deconvolution algorithm was too high. On the contrary, if the restored image looks too smooth then the SNR value was too low. In both cases, please proceed to restore the raw data again with an adapted SNR value.

How to deconvolve TIRF images?

TIRF (Total Internal Reflection Fluorescence) deals mostly with 2D images. Huygens can handle 2D images by internally treating them as part of a 3D stack from which most planes happen to be missing.
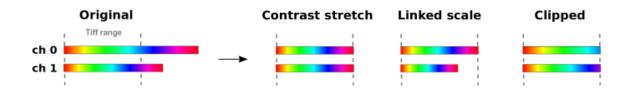
Strictly speaking Huygens does not generate TIRF Theoretical PSFs, but customers report good results with high NA confocal PSFs. Proceed by setting the Microscope type of your image to confocal and NA ~ 1.4.

What should I keep in mind when using hybrid detectors (HyD)?

Use the hybrid detector (HyD) in counting mode only, as they don´t have a physical gain! Instead of using average, you should use accumulation to improve the dynamic range of your image.

Save image:

You can save your image in different formats. The ICS (2) format is an open source format from SVI and can be read by Fiji. If you want to save as 16bit Tiff, you may have to decide how to scale your image to the Dynamic Range.

Contrast stretch (positive data range for each channel is adapted to the Tiff range, the different per-channel factors are not reported)

Linked scale (as stretch, but now the relation between channels is retained: the same factor is applied to all channels that stretches the one with maximum intensity to the Tiff range. The factor is reported by Huygens.)

Clip (positive data outside the Tiff range is clipped to that range)