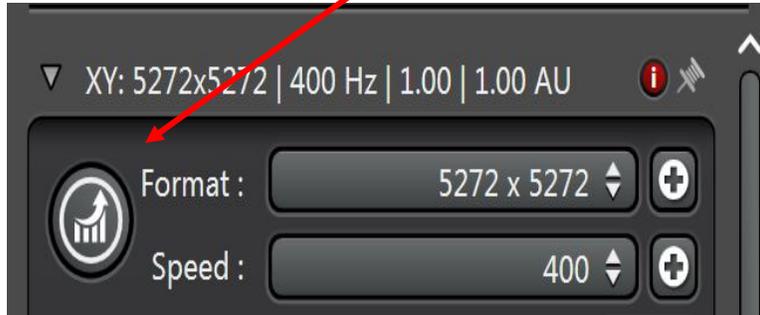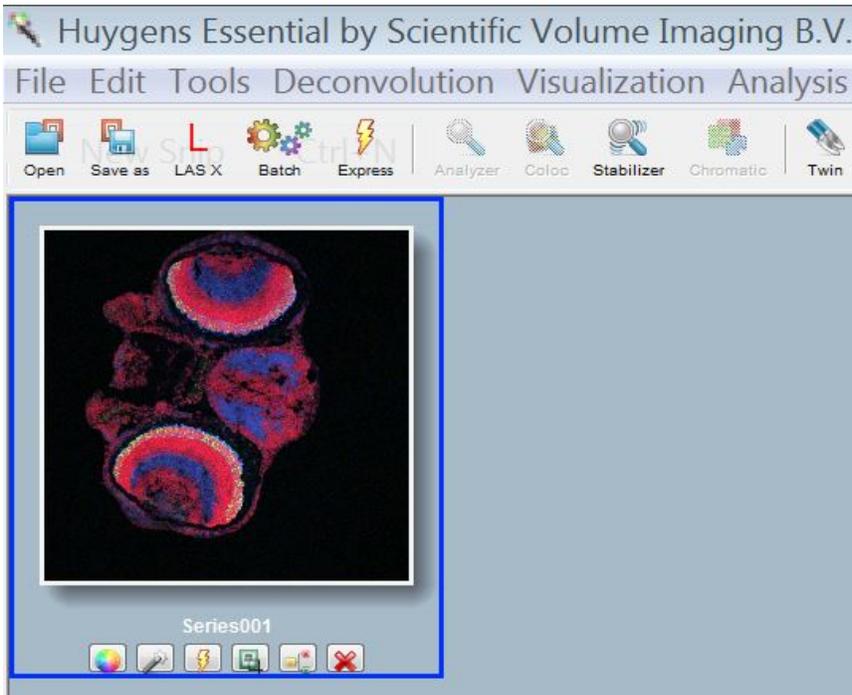# Huygens Deconvolution

The Huygens software would like to have (**3x**) oversampling as opposed to 2.3 for Nyquist. For Leica confocal images this can be accomplished by using the XY Adjust button in LASAF to oversample perfectly for zoom, etc. You would then also have to make sure that Nyquist is met for the number of slices in your z-stack as well.


You can export directly from the Leica software on the SP8 by right clicking on a series and then choosing Export to Huygens Essential. **NOTE:** There is no progress bar in the Huygens software to indicate that the file is being imported.

When the Huygens software opens click on the imported series to highlight it.

Then choose Huygens > Deconvolution Wizard > Parameter Editor. The Parameter Editor will open as below:
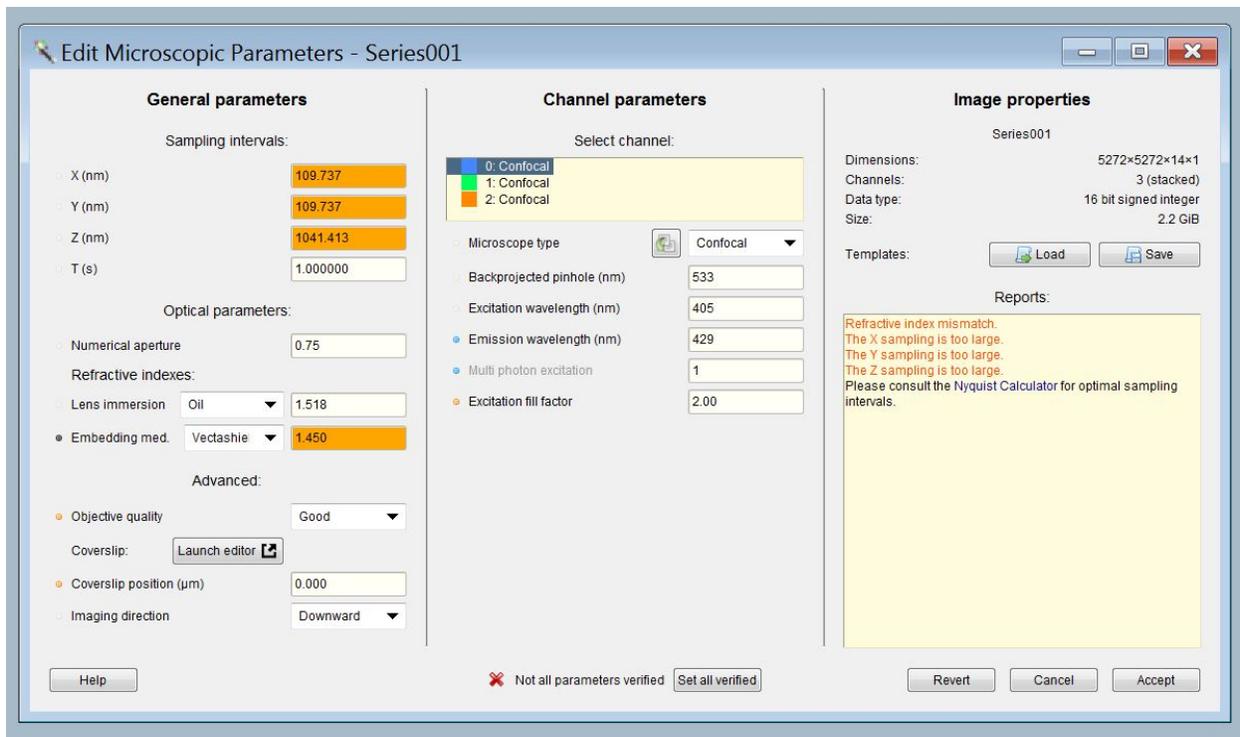
Anything that is orange means that the value is not quite optimal but this is not a real concern.

If it's red, or extremely under sampled, you can increase the S/N ratio but not really the resolution with deconvolution.

Coverslip Position – Don't worry about this in most cases.
Objective Quality – Leave as good.

The other parameters should be derived from the Leica file except for the embedding media which you can change if necessary.

Choose **Set all verified** then **Accept** at the bottom of the window once you are satisfied with all of the settings.

Enter Wizard. Click green arrow at the bottom right to move to the next step of the deconvolution process when you are satisfied with the current settings.

Cropper – You may use this to remove unwanted parts of your scan, i.e., portions that don't contain useful information to speed up the deconvolution process.

PSF – You can use the automatically generated PSF.

For multi-channel images choose the channel to deconvolve on the far right, leave the setting on logarithmic and then click Compute at the bottom of the screen.

Compute – Looks for saturation in your image. If there are saturated pixels they will appear as a spike on the far right side of the generated histogram. The software will tell you if there are saturated pixels, how many there are, and that they are being clipped. > Next
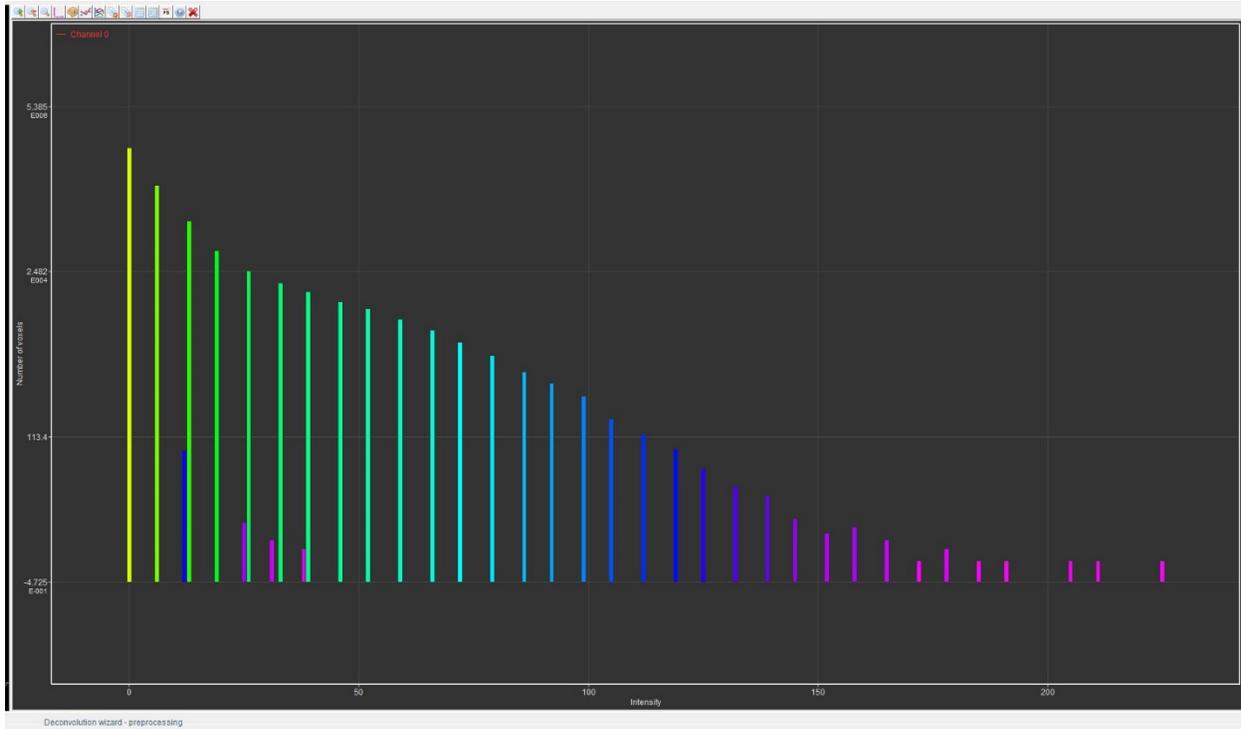
NOTE: With HyD on the SP8 confocal you will get a histogram that looks like individual bars or bins of photons, (as seen in the example), unless you average a lot.

Use as little gain as possible with the HyD. With a gain of 50 you may need 2x averaging, with a gain of 100 you may need 4x averaging.

With a PMT you should get a much smoother and continuous histogram.

Background – The Huygens software would like to use an area with no sample for the background. Draw a line in an area of your image where there is little or no fluorescence and follow the onscreen instructions to obtain and enter the background. Then click accept. Alternatively you can choose "Auto" and the software will enter the background for you.

The next step is to set the final parameters for doing the deconvolution. Adjust these settings as indicated below and then click deconvolve.

Max iteration – leave at 40
Quality threshold – 0.05

S/N – 15 to 20 is average for confocal. You can divide the average signal by the average background or draw a line through the color on the image you're deconvolving and visually figure out the average.

Iteration mode – leave alone
Brick layout – leave alone

You can use the mouse wheel to zoom both the original and deconvolved images side by side.

Once the system has deconvolved the first channel of a multichannel image you have four options. 1. Resume the iterations from the current result. 2. Restart the deconvolution for the channel and use different settings. 3. Accept the result and continue to the next channel. 4. Choose "All Done" to stop and not deconvolve the remaining channels.

Choose **Accept** to go to the next channel and repeat the same steps as above beginning with the "**Compute**" step.

When you are finished choose "**All Done**" and then click on the image that you wish to export. Then click the **LasX** icon at the top of the Huygens toolbar.
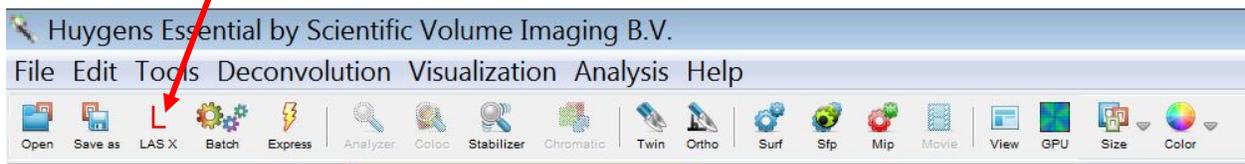
Once again there will be no progress bar to indicate that the software is processing your request. You have to go back to the Leica software and wait for the deconvolved image to appear.