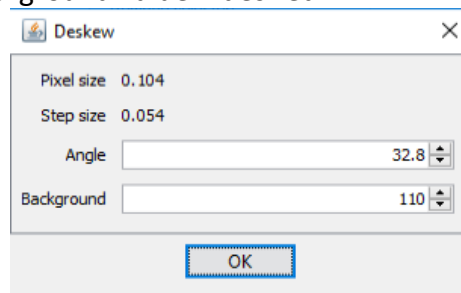
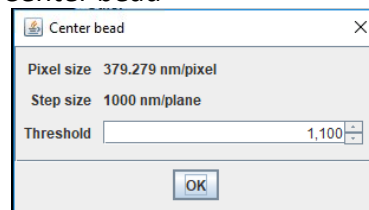


LLSM Deconvolution with Measured PSFs

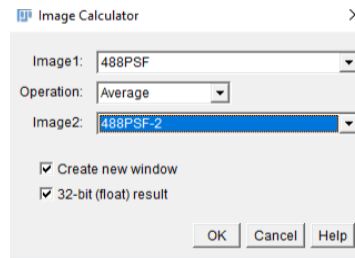
1. First, ensure that you capture the Dataset and fluorescent bead PSF under Nyquist conditions. <https://svi.nl/NyquistCalculator>
2. Open FIJI/ImageJ
3. Open the Slidebook file containing all of the PSF files
4. Select the Sample Scan PSF files you require, ie: 488 and 560nm etc
 - a. Do not use the Slidebook deskewed PSFs as they contain edge artefacts
 - b. Ideally you should have more than one PSF for each wavelength so that you can average them to reduce noise and artefacts.
5. Goto Image/Properties, take note of the Pixel Height, Width and Voxel depth, compare with your original data and correct if necessary.
6. Ensure the Image Type is 32bit
7. Measure the background value using your cursor
8. Plugins/Microvolution/Deskew
 - a. Ensure the angle is 32.8 degrees
 - b. Enter the background value if desired



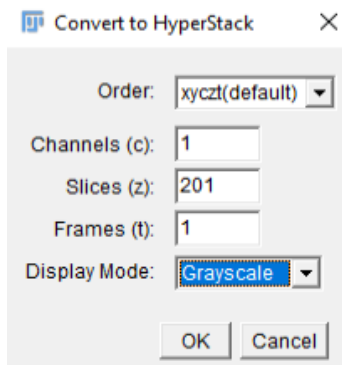
9. A new window will appear with the deskewed PSF, close the original non-deskewed PSF file.
10. Goto Image/Properties to confirm the dimensions of the new deskewed PSF file are correct.
11. Plugins/Microvolution/PSF/Center bead



12. Enter the background value if desired and press "OK"
13. A new window will appear with the centered PSF, close the original non-centered PSF file.
14. Repeat steps 5-12 for all of the remaining PSF files.
15. Average each wavelengths PSFs by going to Process/Image Calculator
 - a. Select the first wavelengths PSFs
 - b. Select Average
 - c. Create a new window
 - d. 32bit Float result

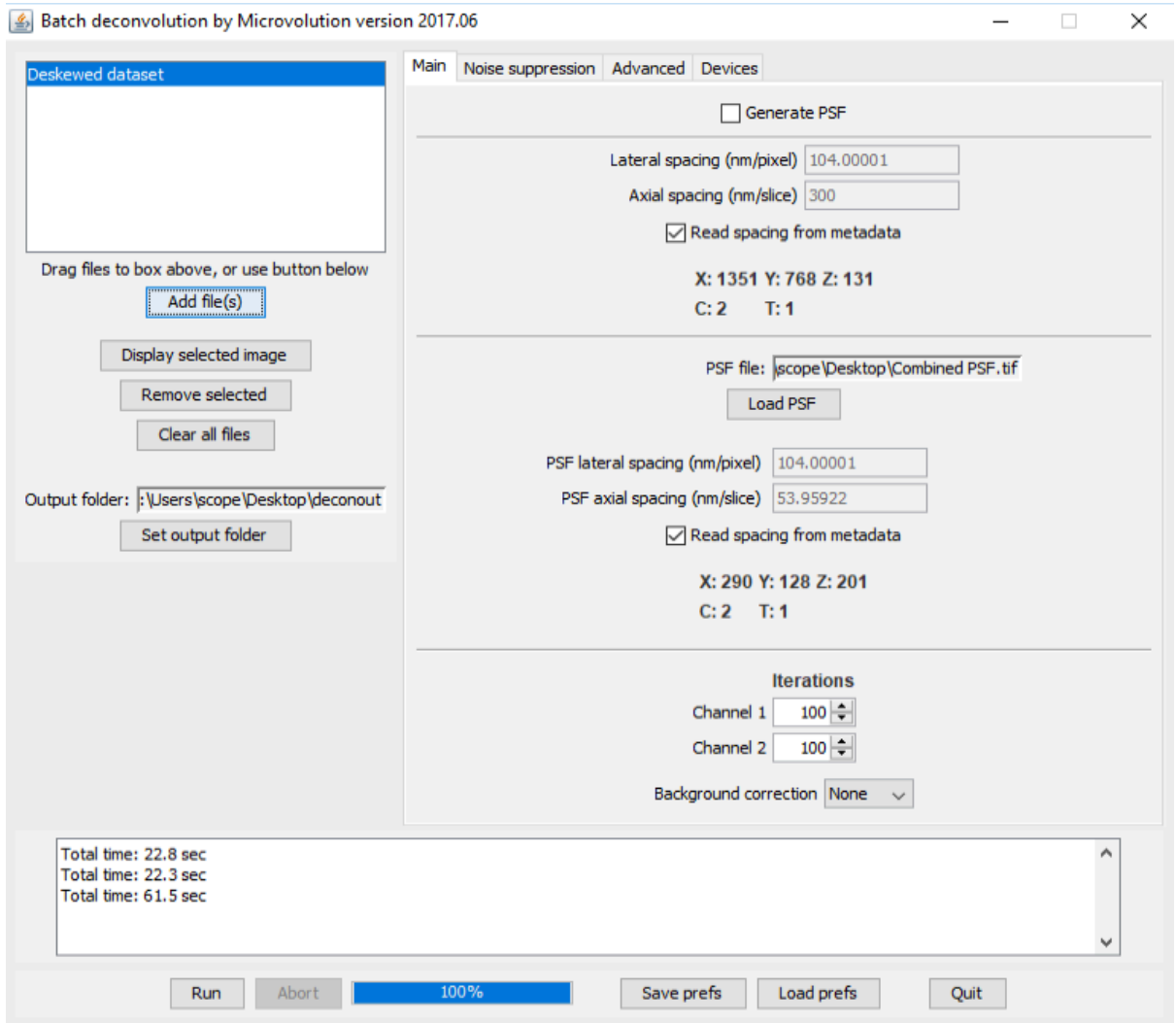


16. Repeat step 14 for the other wavelengths, so that you end up with a deskewed, centred and averaged PSF for each wavelength. Close all other files.
17. Plugins/Microvolution/PSF/Combine to combine the averaged PSFs from each channel into a single file. Slidebook saves the output dataset with the lowest (bluest) wavelength first, so make sure you do the same with the PSF file.
18. Make sure the output PSF file is Image/Type/32bit and also ensure it is a grayscale hyperstack -> Image/Hyperstacks/Stack to Hyperstack



19. Save the output multi-channel PSF file and close it.

20. Plugins/Microvolution/Batch Deconvolution

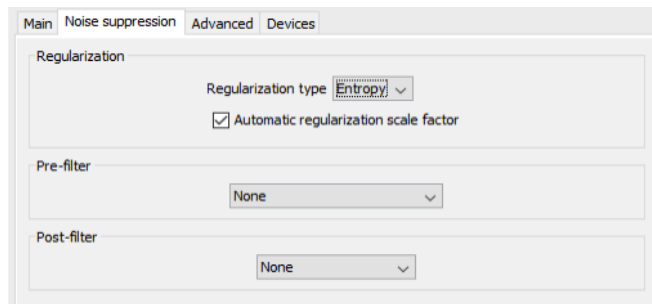


21. Add your dataset files (they must only contain a single dataset per Slidebook file)
22. Set your output folder
23. Untick "Generate PSF"
24. Load your PSF file
25. Ensure that the lateral and axial spacings for both the dataset and PSF are correct.
 - Lateral spacing is the Width and Height or XY pixel size, Axial spacing is the Voxel Depth or Z-stack spacing
26. If the Z and T dimensions are flipped for either the dataset or the PSF, go to the "Advanced Tab" and tick the "swap Z and T" tickboxes as required. You will then have to clear and reload your dataset and/or PSF file.
27. Adjust the number of iterations as desired, set any channels you don't want to deconvolve to zero iterations
 - a. 10 is a good starting point
 - b. Up to around 50 for a good confocal image
 - c. 50-500 for a good widefield image

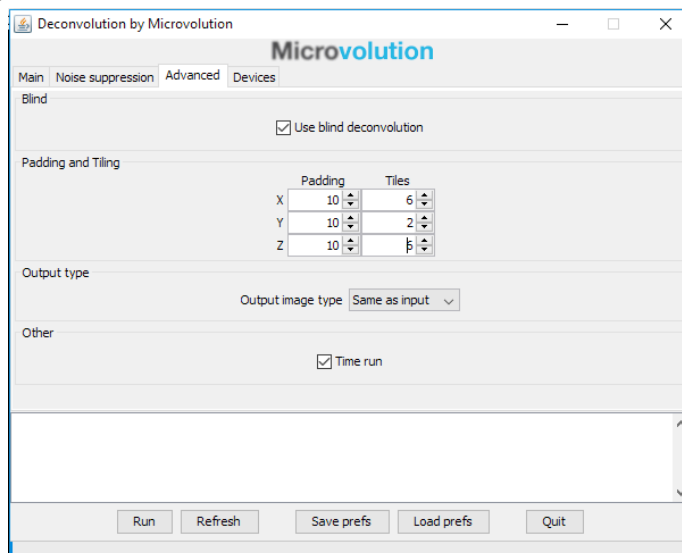
28. Measure the background of the dataset with your cursor (click display selected file to load dataset), and adjust the “background correct” setting as necessary

29. “Noise suppression” Tab

- a. Regularisation helps to reduce artifacts, and Entropy is the most advanced algorithm.
- b. Try the Pre and Post filtering algorithms if you have a lot of noise in your dataset



30. “Advanced” Tab



- c. You may choose to use “Blind Deconvolution” to improve the original PSF file and possibly enhance your deconvolved image further at the expense of processing time.
- d. Adjust the padding and tiles as appropriate for the dataset size, Padding helps to reduce artefacts by ignoring pixels at the edges of your dataset which don’t have enough information to be deconvolved properly
- e. Tiling cuts up the dataset into smaller blocks so that it fits into the VRAM on the computers graphics card (GPU). If you don’t use enough tiling the deconvolution will fail or slow down as the dataset may be bigger than the available GPU VRAM. The biggest dataset you can fit into the 8GB VRAM is around 2048x2048x50Z.
- f. A typical Zebrafish stitched image of 3 x 2048x2048x180Z z-stacks will probably require the following settings: 5,5,5 for padding and 3,3,1 for tiling. Generally,

try not to use Z Tiling as you're more likely to see artifacts such as banding in the Z plane.

- g. Output image type can be set to "32bit (default)" or "Same as input" which is usually 16bit and helps to reduce the output file size.

31. "Devices" Tab

- a. Allows the user to select how many GPUs to use for the deconvolution, the more the merrier.

32. You can "Save prefs" if you wish to use these settings again on another dataset.

33. Press Run, and wait for the deconvolution to finish.

34. The deconvolved image is saved automatically, you'll need to open it in FIJI or Imaris to view it.