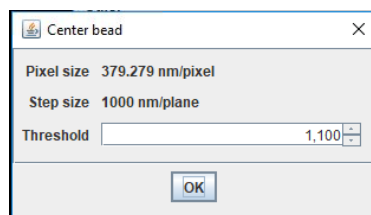
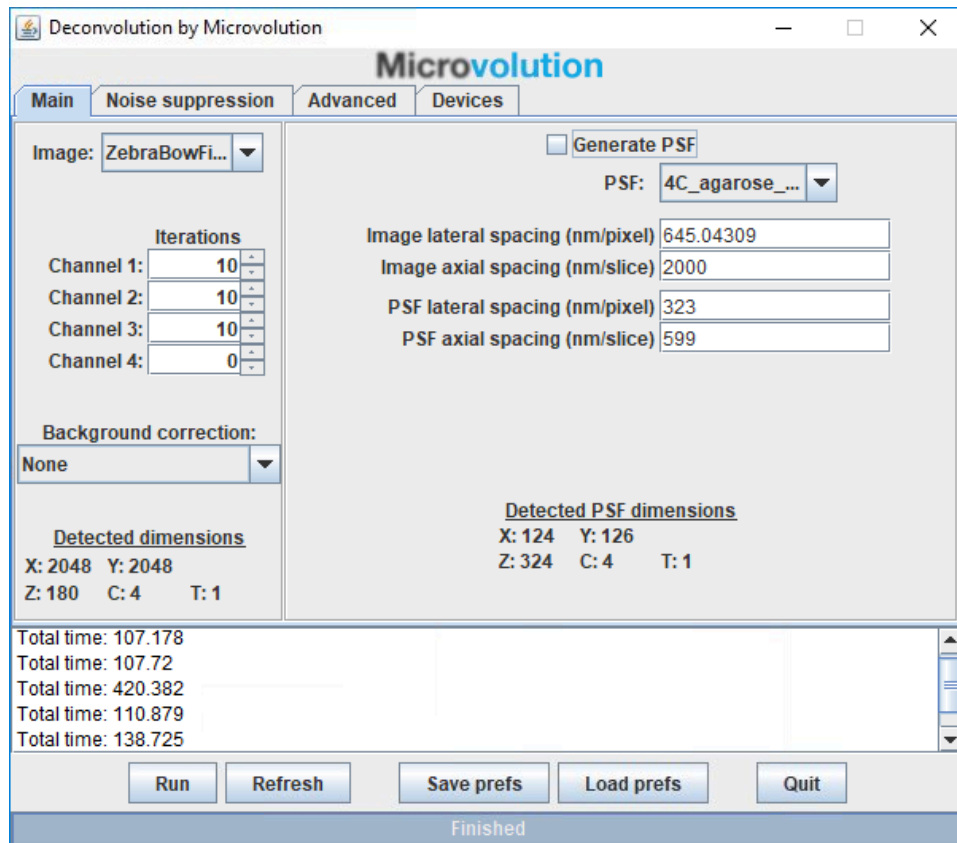


Deconvolution with Measured PSFs

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 multi-channel PSF file
4. Image/Properties, take note of the Pixel Height, Width and Voxel depth, compare with your original data and correct if necessary.
5. Image/Color/Split Channels
A new window for each PSF channel will appear, with the last channel at the front
6. Measure the background value using your cursor
7. Plugins/Microvolution/PSF/Center bead

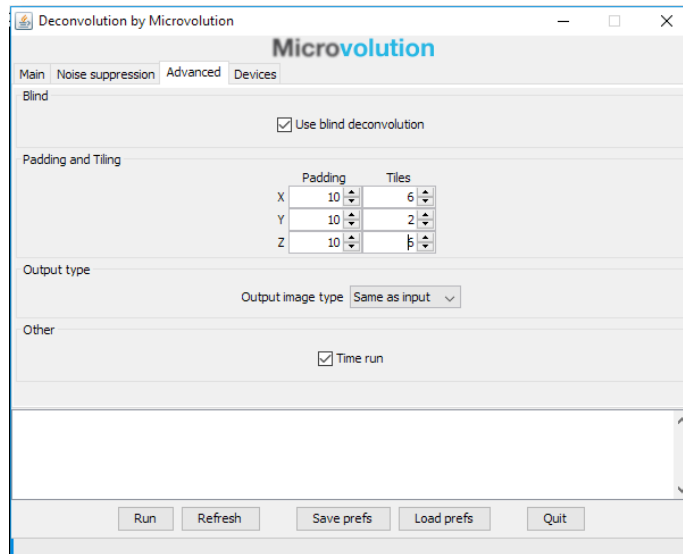


8. Enter the background value and press "OK"
9. A new window will appear with the centered PSF, close the original non-centered PSF file.
10. Repeat steps 5-8 for all of the remaining PSF files.
11. Image/Color/Merge Channels and ensure "Create Composite" is ticked, Click "OK" to merge the images together
12. Save the aligned PSF file and leave it open in FIJI.
13. Open the dataset to Deconvolve
14. Plugins/Microvolution/Deconvolution



15. Ensure that the correct image dataset for deconvolution is selected in the Image drop-down selection box in the top left corner.
16. Adjust the number of iterations as desired, set the brightfield channel 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
17. Measure the background of the dataset with your cursor, and adjust the “background correct” setting as necessary
18. Untick “Generate PSF” and select the PSF file
19. 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
20. “Noise suppression” Tab
 - a. Regularisation helps to reduce artifacts, and Entropy is the most advanced algorithm.

21. “Advanced” Tab



- 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.
- 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
- 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.
- 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.
- 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.

22. “Devices” Tab

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

23. You can “Save prefs” if you wish to use these settings again on another dataset.

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

25. Save the deconvolved image

26. Open Imaris/Surpass/FIJI/Get Image from FIJI.

This will transfer the dataset across to Imaris which is quicker and reduces format issues that may occur if you open a saved file. “Export” to Save the dataset to Imaris .ims format.