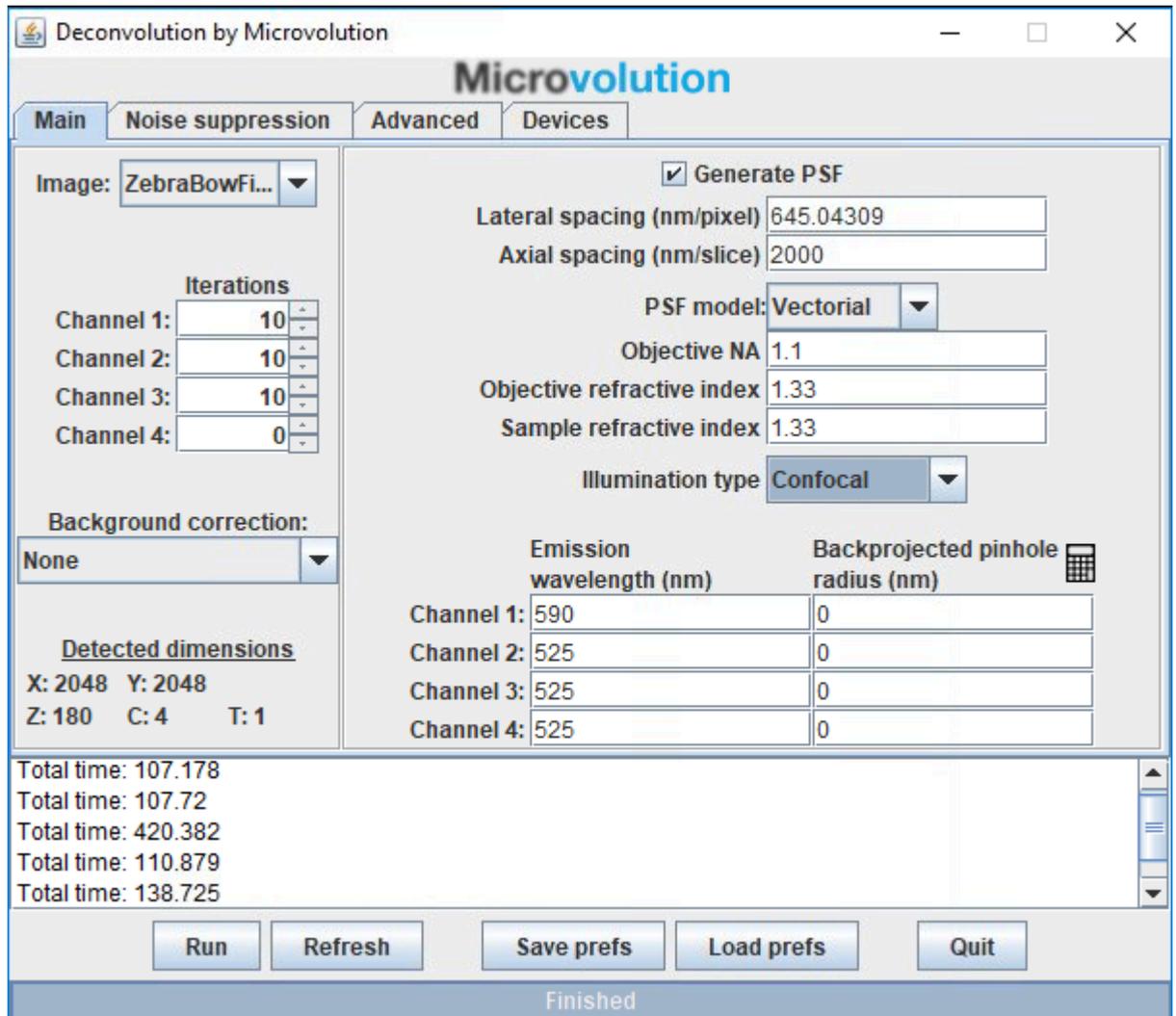


Deconvolution with Generated PSFs

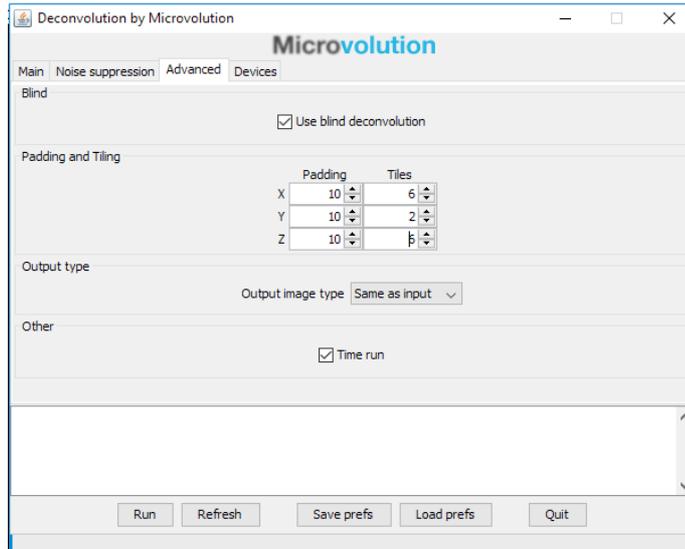
1. Open Fiji/ImageJ
2. Open the dataset to Deconvolve
3. Open Plugins/Microvolution/Microvolution



4. Ensure that the correct image dataset for deconvolution is selected in the Image drop-down selection box in the top left corner.
5. 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
6. Measure the background of the dataset with your cursor, and adjust the "background correct" setting as necessary

7. Tick "Generate PSF"
8. Check that the Lateral and Axial spacings are correct for the dataset
Lateral spacing is the Width and Height or XY pixel size, Axial spacing is the Voxel Depth or Z-stack spacing
9. Select a PSF model, Vectorial is generally best
10. Select the Illumination type, Widefield/Confocal etc
11. Enter the Objective NA and other details
12. Enter the Emission wavelength for each channel
13. If you're using a confocal or Spinning Disc, you will need to enter the Backprojected pinhole radius.
 - a. You can obtain this from the Zeiss Zen software, or using FIJI under Image/Show Info, Control+F and type "PinholeDiameter". The number is in meters and needs to be converted to microns, so multiply by 1×10^6 .
 - b. Alternatively, you can use the Pinhole Calculator in Microvolution. Zeiss use a square pinhole.
14. All of this information is generally available in the metadata of your dataset, which you can read in the acquisition software such as Zeiss Zen or NIS-Elements, alternatively you may be able to find it in FIJI under Image/Show Info
15. "Noise suppression" Tab
 - a. Regularisation helps to reduce artifacts, and Entropy is the most advanced algorithm.

16. “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.

17. “Devices” Tab

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

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

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

20. Save the deconvolved image

21. 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.