

Image Restoration Computer Instructions

1. Shake the mouse to wake up the computer and make sure that the acquisition computer is turned on.
2. Select "Bio User"
3. Move your image files from the acquisition computer to this computer.
 - A. Click "Start" → "Run" → inverted/dchp.wfu.edu
 - B. Copy and paste the files you want to move into "My Documents".
4. Open "Volocity" and select to "Open an existing library".
5. Once your library is open, select an image sequence to work with.
 - A. Double-click on the desired sequence.
(A new window will pop open showing the image.)
 - B. The "Image" tab will allow views of the X, Y, and Z planes of the image volume.
 - C. Clicking in the image and moving the mouse will allow the different planes to be viewed.
6. Make or Import the appropriate Point Spread Function.
 - A. Go back to the library window.
 - B. To Import the PSF:
 - File >> Import >> My documents >> PSF Folder >> select appropriate PSF
 - C. To make the PSF:
 - Tools >> Calculate PSF
 - Use the table beside the acquisition computer to input the emission wavelength, the z step, and the objective NA. The refractive index of air is 1 and the scope is "widefield".
7. Make allowable image adjustments before performing restoration if needed.
 - A. Crop: do if the data volume is too large to be deconvolved as is.
 - make a duplicate of the image volume before cropping
 - select the rectangular ROI and box in the desired area
 - Action >> Crop to selection
 - B. Correct illumination: do if there are bad lamp intensity fluctuations (see in the Z plane)
 - Tools >> correct illumination
 - C. Noise removal: do if the data set is particularly noisy
 - Tools >> Remove Noise >> fine filter
8. Run the image restoration (the deconvolution)
 - A. Tools >> Iterative Restoration (this will open a new window)
 - select the appropriate PSF (it will only show the ones in your library)
 - set the confidence between 98% and 99.9%
 - set the iterations at 40 (can select pause and increase the number if needed)
 - Click "Start"
9. Examine the results of the restoration compared to the original image.
 - A. Select your image sequence from the library.
 - B. Click the "image" tab to view the image planes.

- C. Click show/hide channels (on the right panel) to see the original or the restored version of each image plane.
- To see each separate Z-plane: Image >> XYZ planes
 - To see the max projection: Image >> Extended Focus
 - To add scale bars if desired: Image >> Show scale (these bars can be adjusted)
10. Extract and save individual 2-D image files.
- There are two different ways to do this and I will describe each.
- A. Split-volumes method.
- In the library window, select the volume of interest
 - Tools >> Split volume
 - Window will pop open; select “single folder” and click “split”
 - A new folder will appear in the library
 - Click this folder to see all the individual files within it (double click to see the files as a gallery)
 - Select the desired 2-D file
 - File >> Export >> select desired folder and file type (TIFF) and click “Save”
- B. Capture Snapshot method.
- In the “Image” window, select the desired X, Y, and Z positions
 - Click “Image” >> Capture Snapshot
 - A new folder will show up in the library containing four images:
XY, XZ, YZ, and the composite
 - Select the desired plane
 - File >> Export >> select desired folder and file type (TIFF) and click “Save”
- Make notes about your image manipulations in the “Notes” tab of each active image sequence.