

- Scan post-processing and segmentation -

Stack alterations and enhancements

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Stack alterations in Fiji

For users not familiar with Fiji, basic instructions for editing and cropping stacks can be found here –

<https://micro.uark.edu/micro-instructions-for-working-with-image-stacks-in-fiji-fiji-is-just-imagej/>

Before beginning segmentation in Avizo, it is highly preferable to adjust your region of interest so that it is in a standard orientation. To do this you can use the Plugins > Stack > Crop 3D and the Image > Transform > Rotate... functions. In the Rotate function, changing the number of gridlines so that you have gridlines going through your region of interest, can help you to orient the stack in a standardised way. Once you are happy with the orientation, save this new cropped, rotated version so that it can be used for segmentation. After the stack is in the orientation you would like for segmentation, you can apply filters that will enhance boundaries between tissue types and perform alterations on the histogram.

For your segmentation KEEP THE ORIGINAL STACK and add new, altered stacks to your project so that you can switch between them for different purposes. Stacks altered in different ways can be useful for thresholding in different ways. For example, applying a gamma transformation can increase the greyscale value differences between the brightest and darkest tissues, which can make thresholding easier for structure that differ more subtly in their values. Applying an unsharp mask can enhance boundaries between structures, and may make following particular structures within the stack (e.g. nerves) easier.

Cropping irregular objects using Fiji

Useful for cropping curved objects out from one another – e.g. cropping the tail or head of a snake out from the coils of the body.

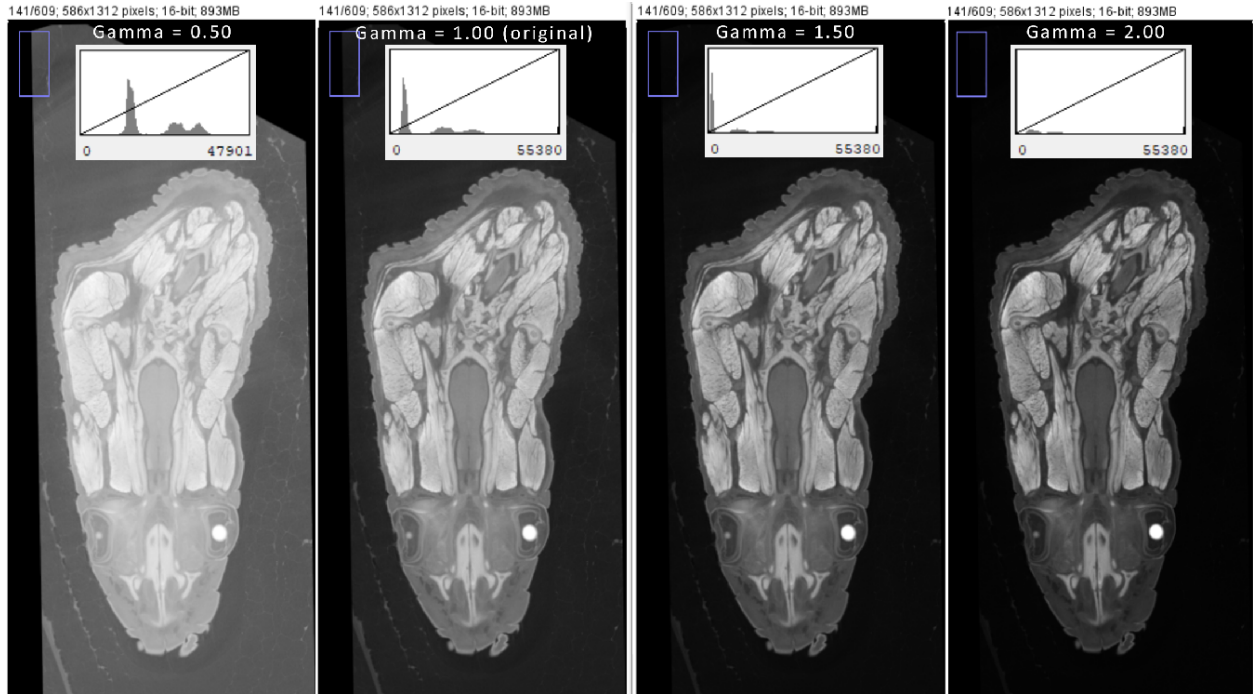
1. Select polygon tool and draw around ROI by clicking around it. You can add as many points as you want to the polygon – these will be used to adjust its size and shape.
2. Scroll through your stack and continue to adjust polygon so that your ROI is always inside it. Usually its best to start with a small polygon and continue to make it bigger (never smaller).
3. Once you have your ROI completely inside the polygon go to Edit > Clear Outside. This will turn any space outside the polygon to black.
4. Go to Image > Crop. This will crop a square around your ROI, making the images in the stack as small as possible.
5. Go to File > Save As > Image Sequence... to save your new stack

Stack enhancements

Gamma (Process > Math > Gamma)

From <https://imagej.net/Image_Intensity_Processing#Non-linear_contrast_stretching>

Gamma performs a non-linear histogram adjustment. Faint objects become more intense while bright objects do not (gamma <1). Also, medium-intensity objects become fainter while bright objects do not (gamma > 1). The intensity of each pixel is "raised to the power" of the gamma value and then scaled to 8-bits or the min and max of 16-bit images.

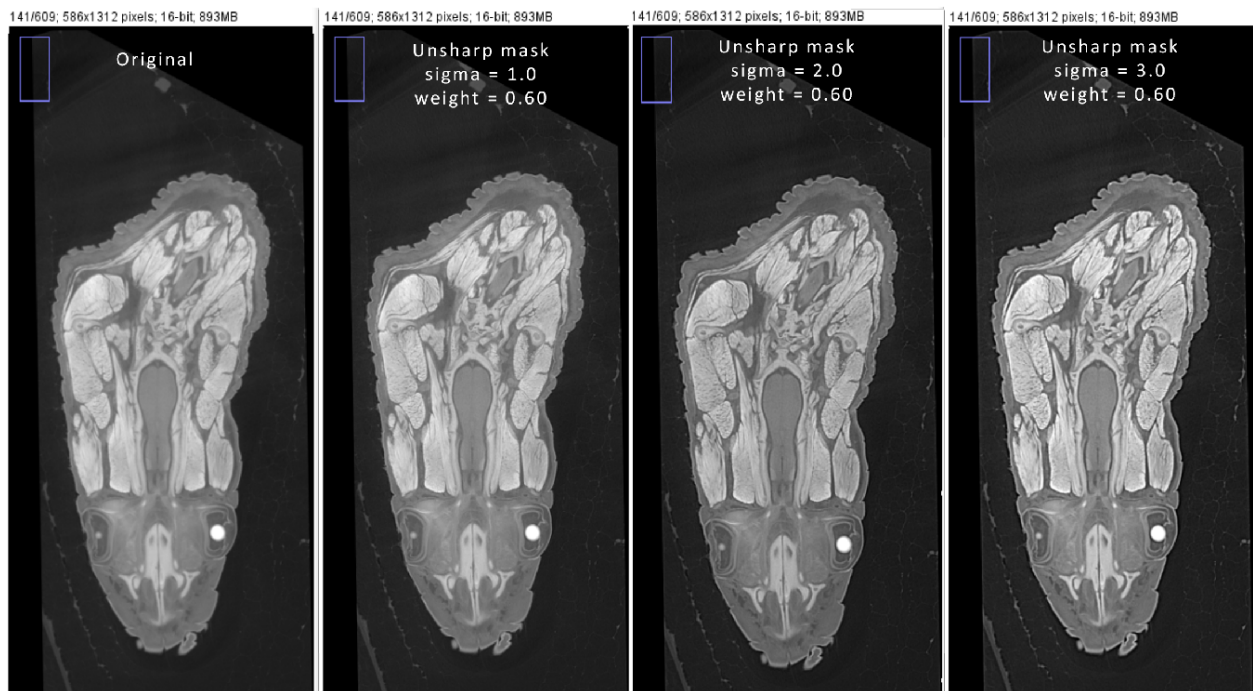


Unsharp mask (Process > Filters > Unsharp Mask)

From <<http://homepages.inf.ed.ac.uk/rbf/HIPR2/unsharp.htm>>

The unsharp filter is a simple sharpening operator which derives its name from the fact that it enhances edges (and other high frequency components in an image) via a procedure which subtracts an unsharp, or smoothed, version of an image from the original image.

Changing the **sigma value** (changes depth of the boundaries, can make magic wanding in Avizo easier):



Changing the **mask weight** (strength of filtering):

