

## Quantitative Trait Loci Mapping Part II: Measuring the Total Brain Volume Using Image J

*Correcting for Differential Shrinkage of Olfactory Bulb.* A great deal of the didactic value of this exercise grapples with the concept of variance in data—what variance is, how variance can affect possible conclusions, and how it can be eliminated. One source of variance is differential shrinkage of olfactory bulbs across individuals, which happened during histological processing—details of histological procedures can be obtained from the Mouse Brain Library:

([http://www.mbl.org/procedures/tissue\\_proc.html](http://www.mbl.org/procedures/tissue_proc.html)).

This problem can be controlled by mathematically “reversing” the shrinkage and arriving at an estimate of the original volume.

This differential shrinkage of the olfactory bulbs gets introduced in the embedding and sectioning processes. The brains were shrunk during the embedding process before sectioning. If the shrinkage is variable across individuals, which it is, we need to correct for shrinkage. We can do this because the weights of the brains before they were processed are published by the MBL and are provided in the “Collected RIS mouse data” spreadsheet available on our course website. Different parts of the brain do not seem to shrink differentially in this sort of processing (Bush, & Allman, 2004), so proportions within a brain should stay roughly the same when shrunk during histology.

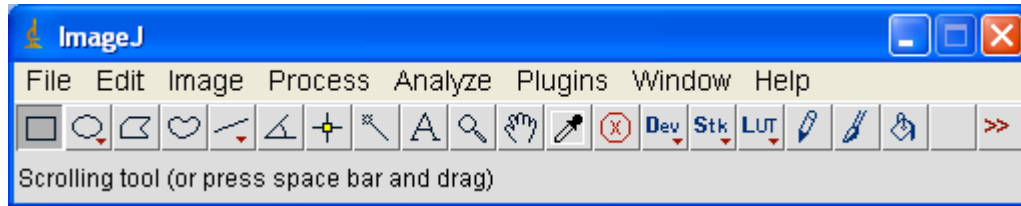
This differential shrinkage can be corrected by dealt with by using information about the density of brains, the fresh weight of the brain for that individual, the volume of the brain, and olfactory bulbs in their shrunken state. If we make the assumption that brains have a density slightly greater than water, say  $1.05\text{mg/mm}^3$  (Beatty & Laughlin, 2006), we can determine the volume of the unshrunk brain in  $\text{mm}^3$  using a simple ratio-and-proportion problem (see procedure below).

To solve for unshrunk OB volume, one will need to measure the shrunk OB volume, which is described above, figure out the unshrunk total brain volume using the weight of the freshly dissected brain and the density, and know the shrunk volume of the total brain, which one may do by the procedure described below .

*Take Home Points Part II (Testable Material)*

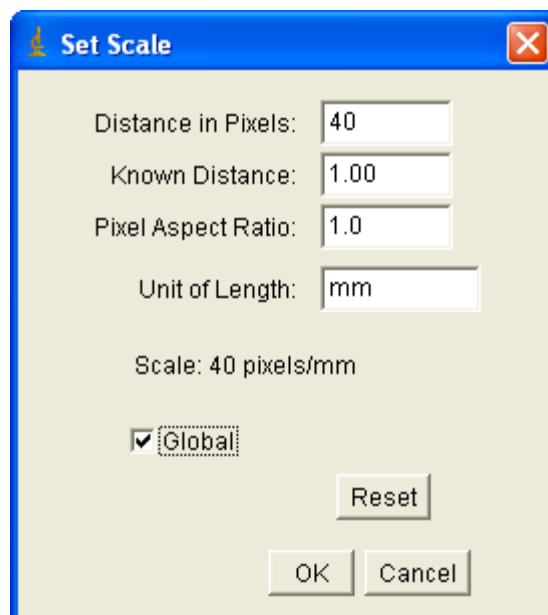
- How do we calculate the shrunken brain area for each mouse?
- When we determine the volume of our brain, why do we multiply the area by 0.3 mm?
- How and why do we have to calculate the unshrunk brain volume for our analysis?

1. Open **Image J** by double-clicking the desktop icon. An **Image J** menu bar will appear on the screen.



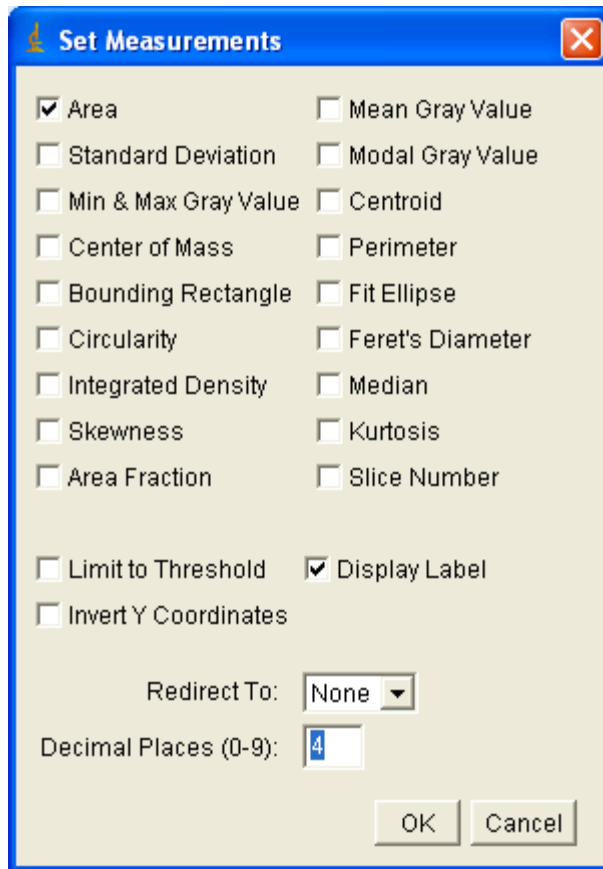
2. Open your first image file by selecting **File**, then **Open...** from the menu bar.

3. Select **Analyze**, then **Set Scale...** from the menu bar. A **Set Scale** window will appear. Change any values and checks in the window so that it looks identical to the one that appears below. Make sure the 'Distance in Pixels' is 40, the 'Unit of Length' is millimeters, and the 'Global' box is checked.



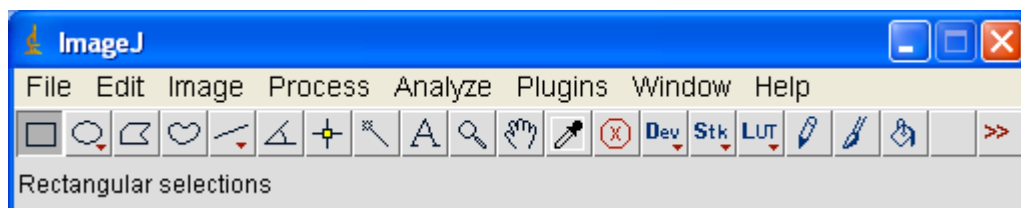
Select **OK** to save the changes and close the window. (Most monitors have a pixel aspect ratio of 1.0—pixels are the same width as height—but one should check this.)

5. Select **Analyze**, then **Set Measurements...** from the menu bar. A **Set Measurements** window will appear. Change and values and checks in the window so that it looks identical to the one that appears below. Make sure the 'Area' box is checked and the number of 'Decimal Places (0-9)' is 4.



Select **OK** on the **Set Measurements** window to save the changes and close the window.

6. Select the 'Rectangular selections' tool from the menu bar.



Use the tool to create a box around all of the brain slices. **Try not to include too much extra space (edge markings, labels, etc).**

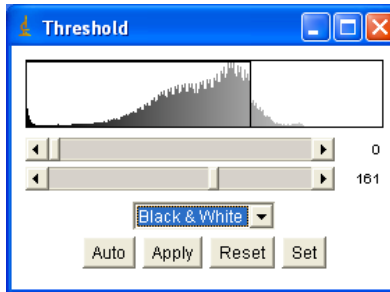


6. Copy the selected region by selecting **Edit**, then **Copy** from the menu bar.

7. Open the copied region in a new window by selecting **File**, then **New**, then **Internal Clipboard** from the menu bar. A **Clipboard** window will appear with the copied region already pasted into it.

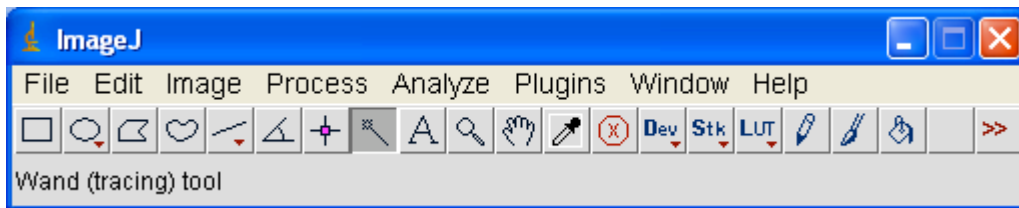


8. Make sure the entire **Clipboard** window is selected (and not any other window), then select **Image**, then **Adjust**, then **Threshold...** from the menu bar. A **Threshold** window will appear. Set the threshold to 'Black & White,' and adjust the lower bar to darken the brain regions if necessary—brain tissue should go black.

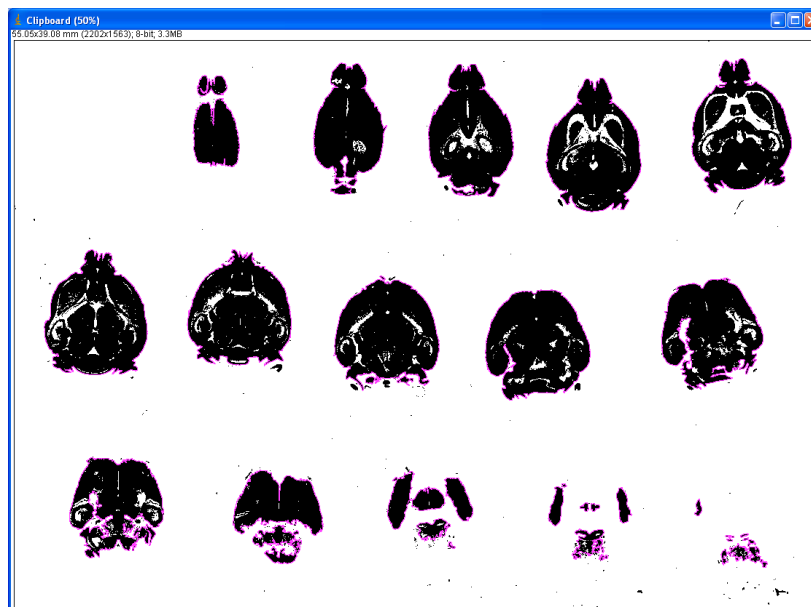


Select **Apply** to save the changes.

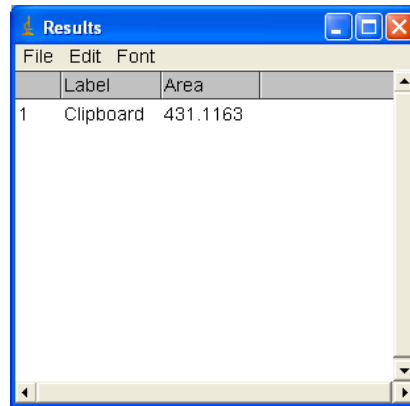
9. Select the '**Wand (tracing)**' tool from the menu bar.



While holding down the 'Shift' key (to allow for multiple selections), use the tool to select ALL of the brain regions in the image file by clicking on the brain sections (including all small particles that also belong to the brain). These regions will be outlined if selected correctly.



10. Measure the outlined brain regions by selecting **Analyze**, then **Measure** from the menu bar. A **Results** window will appear which will provide the area, in  $\text{mm}^2$ , of the various outlined particles in the **Clipboard** window.



Record the total area given in the **Results** window, and label it as ‘**Shrunk Brain Area.**’ on your data sheet.

Close the windows in the **Image J** program and **DO NOT** save any files.

11. Determine the total volume of the brain by multiplying the total area (from the previous step) by 0.3mm. This will yield a value in  $\text{mm}^3$ .

**Record the total volume of the brain, and label it as ‘Shrunk Brain Volume’ on your data sheet.**

Explanation: The mouse brain was sliced at a thickness of  $30\mu\text{m}$ , and every tenth slice was mounted, stained, and captured in the image file. Thus the image file only represents a portion of the whole mouse brain. To approximate the volume of the whole brain, the area of the brain slices in the image file must be multiplied by both the thickness of each slice and the sampling interval.

$$\text{VOLUME } \text{mm}^3 = \text{AREA } \text{mm}^2 * \text{THICKNESS } \text{mm/slice} * \text{SAMPLINGINTERVAL slice}$$

$$\text{VOLUME } \text{mm}^3 = \text{AREA } \text{mm}^2 * [ 30 \mu\text{m/slice} * \text{mm}/10^3\mu\text{m}] * 10 \text{ slice}$$

$$\text{VOLUME } \text{mm}^3 = \text{AREA } \text{mm}^2 * 0.3 \text{ mm}$$

12. Determine the unshrunk brain volume by dividing the brain weight by  $1.05\text{mg}/\text{mm}^3$ . You can find the brain weight by looking at the brain data handout and searching for your assigned number. This will yield a value in  $\text{mm}^3$  (that should be larger than the volume obtained in the previous step).

**Record the total volume of the unshrunk brain, and label it as ‘Unshrunk Brain Volume’ on your data sheet**

Explanation: The mouse brain was weighed and recorded immediately following its removal. Because the brain is subject to shrinking, this value can help determine the original brain volume (unshrunk) when it is divided by its density.

$$\text{VOLUME mm}^3 = \text{BRAINWEIGHT mg} / \text{DENSITY (mg/ mm}^3)$$

$$\text{VOLUME mm}^3 = \text{BRAINWEIGHT mg} / 1.05 \text{ mg/mm}^3$$

13. Determine the unshrunk olfactory bulb volume by solving the following equation for ‘Unshrunk Olfactory Bulb Volume.’

$$\frac{\text{SHRUNK OB VOLUME}}{\text{SHRUNK BRAIN VOLUME}} = \frac{\text{UNSHRUNK OB VOLUME}}{\text{UNSHRUNK BRAIN VOLUME}}$$

Record the total volume of the unshrunk olfactory bulb, and label it ‘Unshrunk OB Volume’ on your data sheet.

Provide your instructor with the results you obtained, and then close all the windows in the **Image J** program and DO NOT save any files.

**Please hand in your data sheets so that I can compile the data and run the statistics. I will hand this back to you at the next class so that you can have it for your records. You will have to turn it in again at the end of the semester.**