

# BIRD SONG SYSTEM

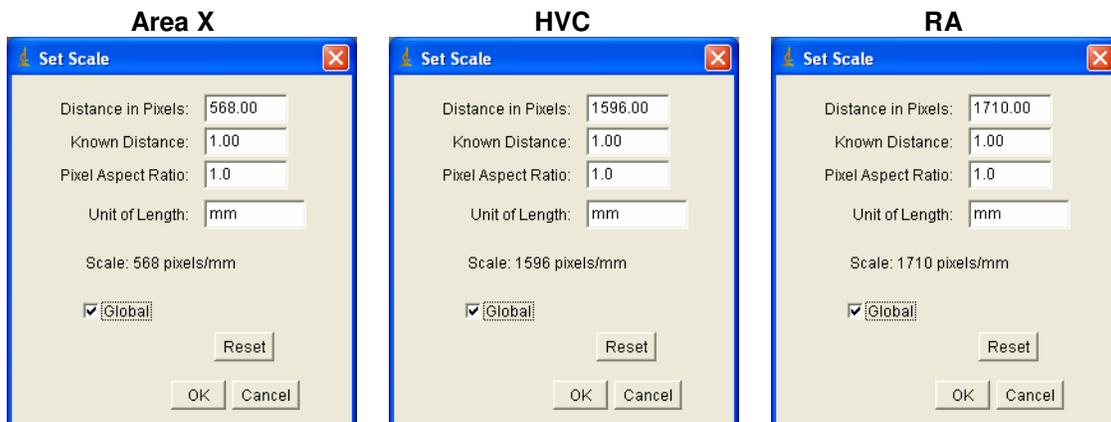
## Using Image J to Measure Areas

- (1) Open the **Image J** software by double-clicking the **Image J** icon found on the desktop.
- (2) Open the image file to be measured within **Image J** (File > Open...).
  - For **Area X**, you can find image files by browsing:  
BSS - Image Database > Area X > *find folder for assigned bird* > *find first image file*
  - For **HVC**, you can find image files by browsing:  
BSS - Image Database > HVC > *find folder for assigned bird* > *find first image file*
  - For **RA**, you can find image files by browsing:  
BSS - Image Database > RA > *find folder for assigned bird* > *find first image file*

Be sure the image opens onto the screen.

- (3) When the file is open, use the **Image J** menu bar to select:  
Analyze > Set Scale...

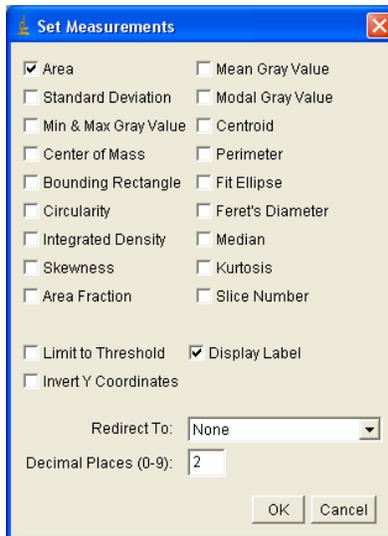
In the **Set Scale** window that appears, enter the following information, based on what set of images you are working on.



Be sure the **Global** option is checked, and click **OK**.

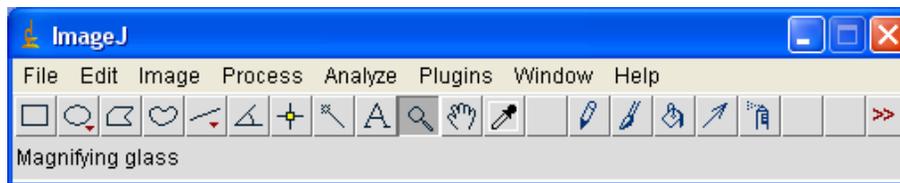
- (4) Use the **Image J** menu bar to select:  
Analyze > Set Measurements...

In the **Set Measurements** window that appears, check only the **Area** box and the **Display Label** box, and uncheck any other boxes (as shown below).



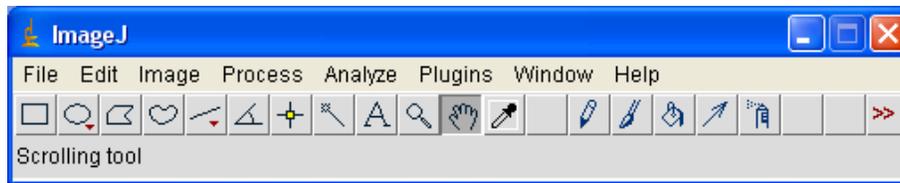
Be sure to set the number of decimal places to **2**, and click **OK**.

(5) Select the **Magnifying glass** tool from the **Image J** menu bar.



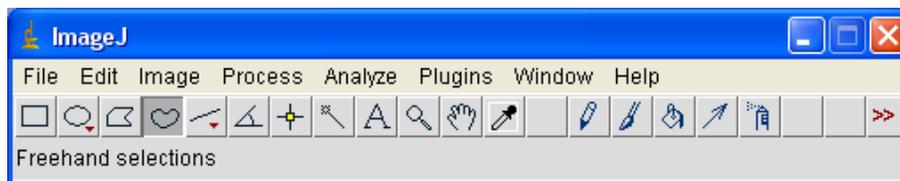
If your image is not zoomed in to around 25%, click the center of the image with the left mouse button until this is so.

If the region of interest is not centered on the screen, select the **Scrolling** (hand-shaped) tool from the **Image J** menu bar.



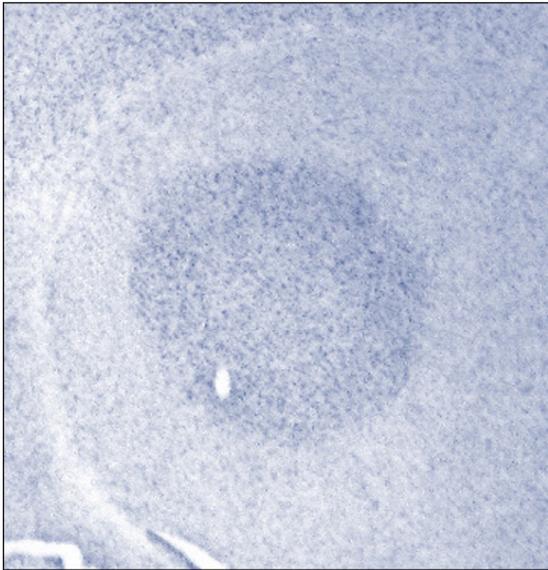
By holding down the left mouse button, you can drag the image about the window.

(6) Select the **Freehand selections** (bean-shaped) tool from the Image J menu bar.

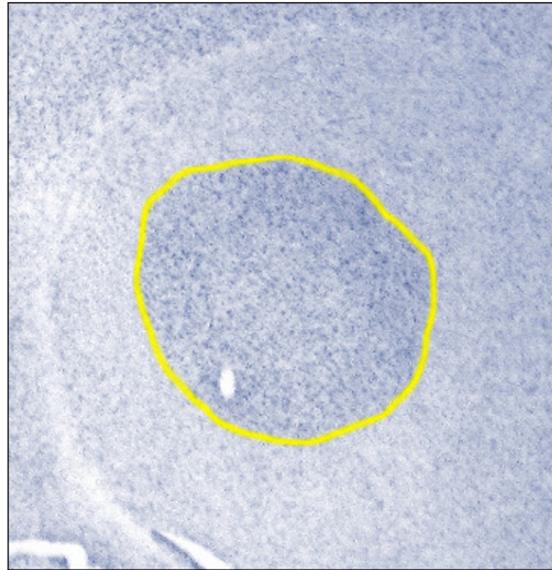


Use the left mouse button to carefully trace around the perimeter of the region of interest. Be as precise as possible! This will leave a trace around the region.

**Before Trace**

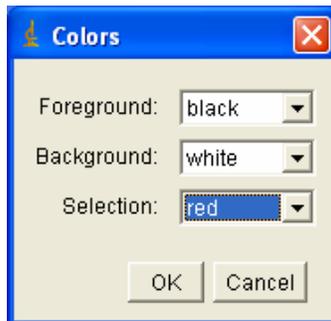


**After Trace**



If you would like to change the color of your trace so that is easier to see, select:  
Edit > Options > Colors...

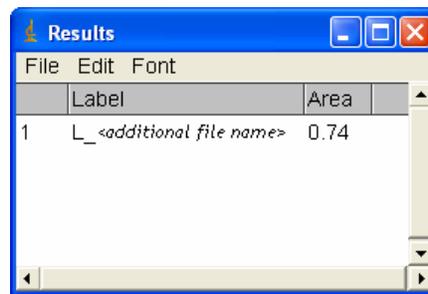
In the **Colors** window that appears, change only the selection color.



Click **OK** to apply the new color.

(7) Use the **Image J** menu bar to select:  
Analyze > Measure

A **Results** window will appear containing the area of the region you just traced.



Copy these data in a new **Excel** spreadsheet (in case the **Image J** program crashes and you lose this information), and save the spreadsheet on the Computer Desktop with your name in the title.

(8) If you have more images to measure, repeat the **Steps 2-7** until you have measured all the necessary images for your assigned specimen.

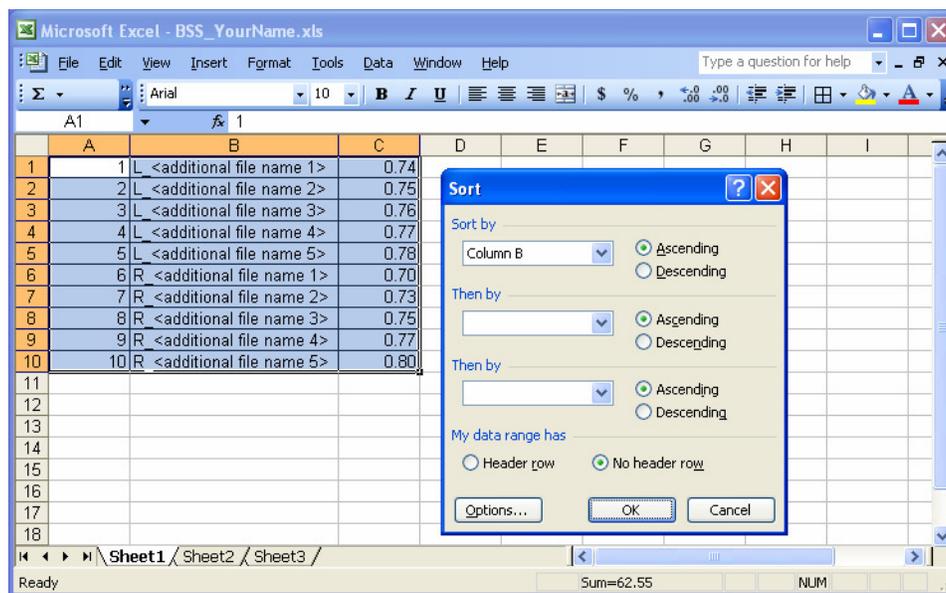
If **Image J** crashes or runs out of memory while you are working (which is probable when working with numerous images), begin these instructions over **FROM THE BEGINNING** with the next image file that needs to be measured (possibly one you were already using when Image J crashed).

If you have finished measuring all the necessary images, make sure that all of the data have been copied exactly once into an **Excel** spreadsheet, save with your name in the title. If so, you may close **Image J**. Be sure you **DO NOT SAVE** anything through Image J.

(9) Select all of the data in the **Excel** spreadsheet (Ctrl+A).

Use the **Excel** menu bar to select:  
Data > Sort...

In the **Sort** window that appears, sort in ascending order by the column that contains the file names (which begin with either 'L' or 'R'), and click **OK**.



(10) Sum up the areas in the **Excel** spreadsheet for each hemisphere; for each bird you should have one area for the left hemisphere and one area for the right hemisphere.

Left Hemisphere - Total Area					Right Hemisphere - Total Area								
IF	A	B	C	D	E	F	IF	A	B	C	D	E	F
1	L	<additional file name 1>	0.74				1	L	<additional file name 1>	0.74			
2	L	<additional file name 2>	0.75				2	L	<additional file name 2>	0.75			
3	L	<additional file name 3>	0.76				3	L	<additional file name 3>	0.76			
4	L	<additional file name 4>	0.77				4	L	<additional file name 4>	0.77			
5	L	<additional file name 5>	0.78	=SUM(C1:C5)			5	L	<additional file name 5>	0.78			
6	R	<additional file name 1>	0.70	SUM(number1, [number2], ...)			6	R	<additional file name 1>	0.70			
7	R	<additional file name 2>	0.73				7	R	<additional file name 2>	0.73			
8	R	<additional file name 3>	0.75				8	R	<additional file name 3>	0.75			
9	R	<additional file name 4>	0.77				9	R	<additional file name 4>	0.77			
10	R	<additional file name 5>	0.80				10	R	<additional file name 5>	0.80	=SUM(C6:C10)		
11							11				SUM(number1, [number2], ...)		
12							12						
13							13						
14							14						
15							15						
16							16						
17							17						
18							18						

Remember that each area is represented in mm<sup>2</sup>.

(11) Multiply each hemispheric area by the 0.12 mm.

Explanation: The bird brain was sliced at a thickness of 40 μm, and every third slice was mounted, stained, and captured in the image file. Thus, the image file only represents a portion of the whole bird brain. To approximate the respective volumes of Area X, HVC, and RA, the area of the brain slices in the image files must be multiplied by both the thickness of each slice and the sampling interval:

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

$$\text{VOLUME mm}^3 = \text{AREA mm}^2 * [40 \mu\text{m/slice} * 1 \text{ mm}/10^3\mu\text{m}] * 3 \text{ slice}$$

$$\text{VOLUME mm}^3 = \text{AREA mm}^2 * 0.12 \text{ mm}$$

(12) Calculate the average (mean) volume of the left and right hemisphere.

(13) Save a copy of your data for your own reference, and notify your instructor of the values you measured and calculated.