Procedural Guide

STEP A: Setup, Calibration, Placing the ROI

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1) Open the Image in ImageJ

Software can be downloaded for free at: https://imagej.nih.gov/ij/download.html

Open ImageJ. Click *File>Open* and select the X-ray image file.

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2) Calibrate the Image

Aluminum step wedges are used as an image scale and have been included in each example X-ray image; each step of the step wedge measures 10.0mm. Always include a scale in your images.
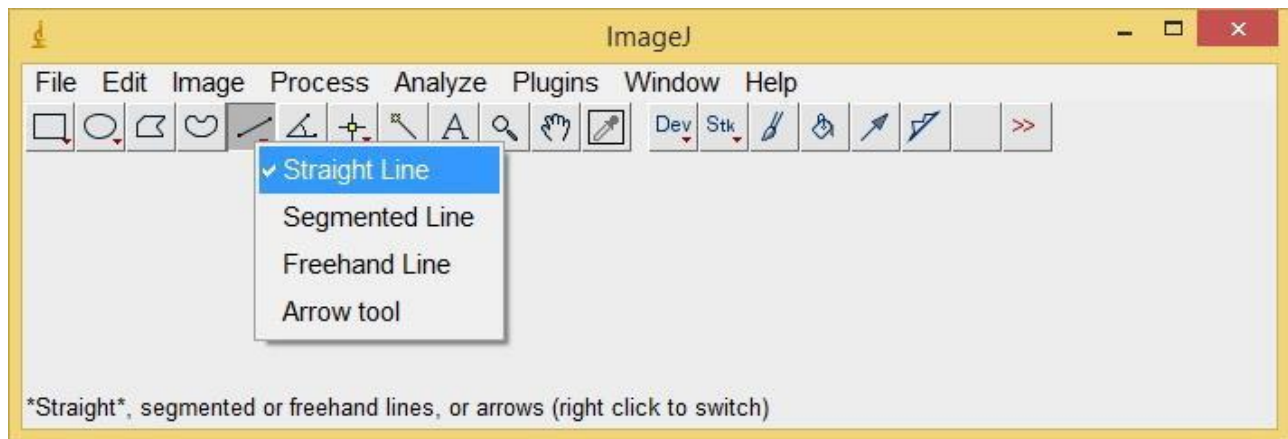
Zoom-in on a step wedge in the radiograph.

To **zoom in** click the '+' keyboard key

To **zoom out**, click the '-' key

To **adjust the position of the image**, hold the spacebar and drag the image to the desired location.

Select the 'Straight Line' Tool (see image below).

Using the 'Straight Line' Tool, draw a line along one step of the step wedge (indicated below with a blue arrow).

In this example, one step of the aluminum step wedge is equivalent to 10 millimeters (indicated below with a blue arrow); in these radiographic images, 10mm is typically equivalent to 70 pixels.

a) Click *Analyze>Set Scale*. The Set Scale dialogue box appears with the 'Distance in pixels' already filled out.

b) Enter **10.0** in the '**Known distance**' box and **1.0** in the '**Pixel aspect ratio**' box.

c) Enter **mm** in the '**Unit of length**'.

d) Click 'OK'.

Note: The line is used to calibrate the image; once calibration is complete the line may disappear.
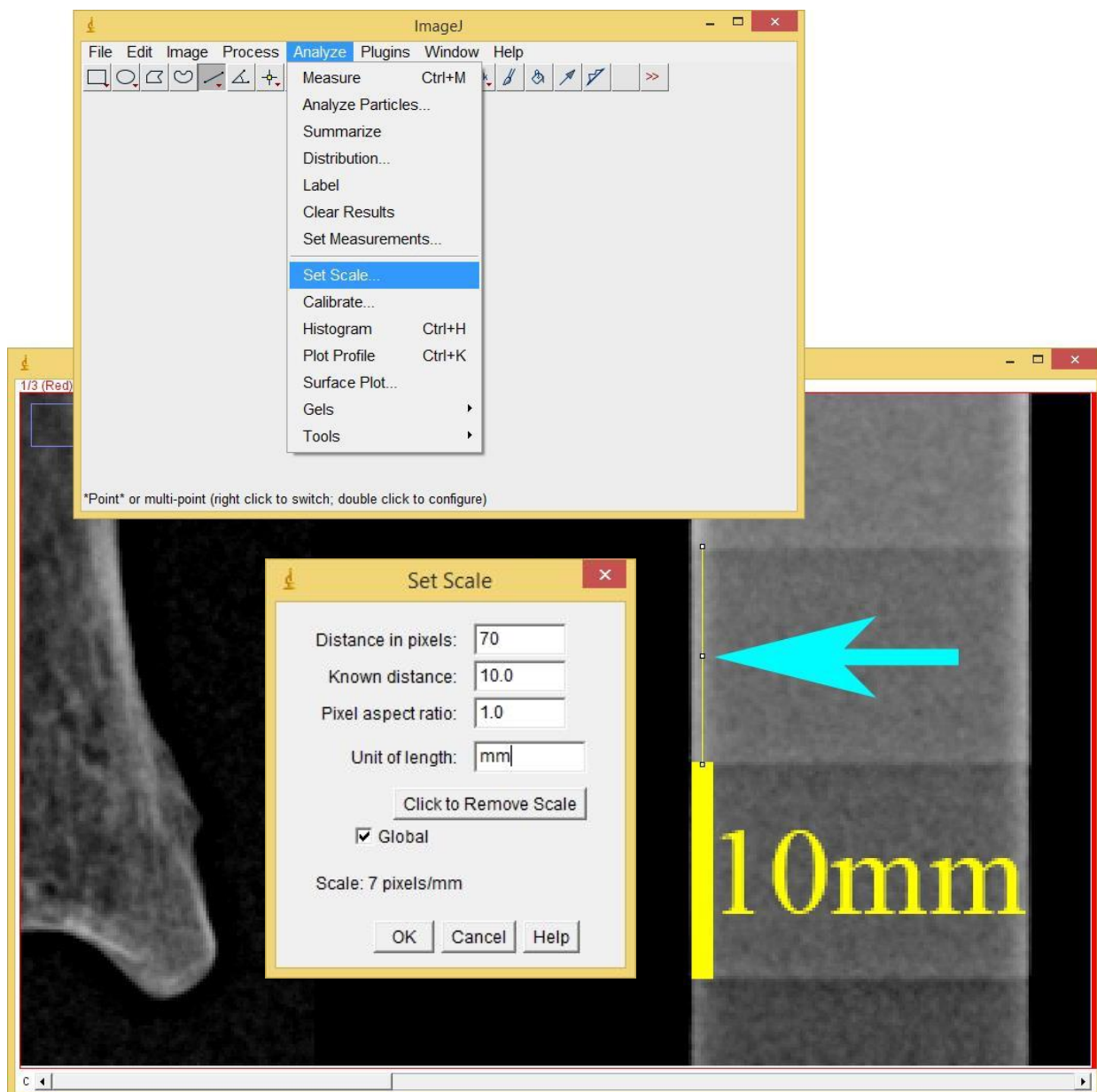
3) Create & Place a Region of Interest (ROI)

In the toolbar, select the 'Rectangle Tool' (indicated with a blue arrow in the image below).

Create a 19mm^2 ROI guide. The ROI size is based on a clinical standard, in which a 19mm^2 standard-length ROI is used for all second metacarpals:

a) Click *Edit>Selection>Specify*.
b) In the 'Specify' dialogue box, check the 'Scaled units (mm)' box.
c) Enter **19.0** in **_both_** the 'Width' and 'Height' boxes.
d) Click 'OK'.

With the 'Rectangle Tool' selected, click and drag the square ROI to place it over the **narrowest part** of the second metacarpal bone shaft.
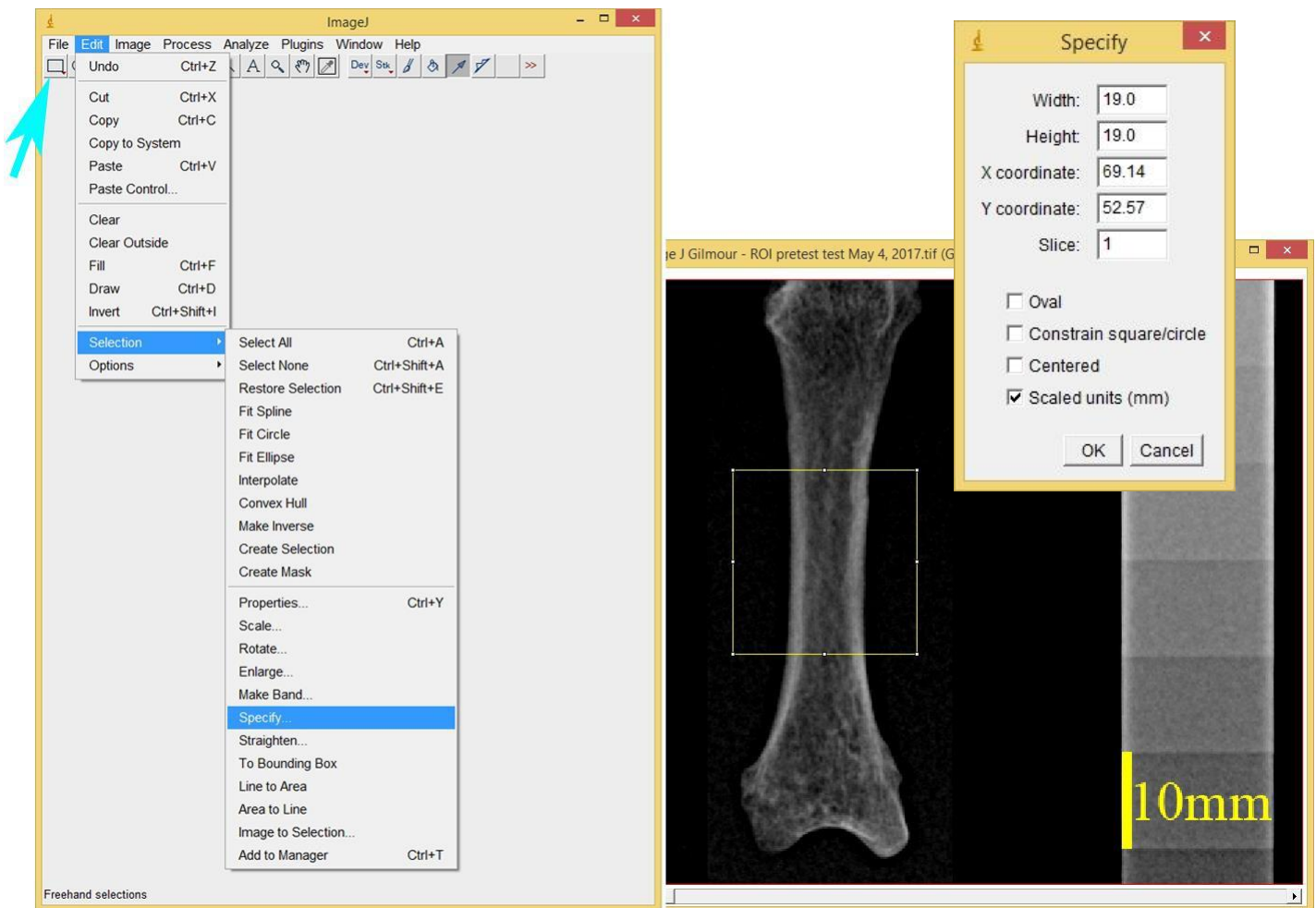
4) Add the ROI rectangle to the ROI Manager

Open the ROI Manager dialogue box by clicking *Analyze>Tools>ROI Manager*. You will use the ROI manager to save and keep track of all your selected regions (including the square region of interest, as well as the selected cortical bone) for later measurement.

In the ROI Manager, click the 'Add [t]' button to add the ROI rectangle to the ROI manager.

Shortcut: Click 't' (on the keyboard) to add the ROI rectangle to the 'ROI Manager'. When using the shortcut, it is not necessary to first open the ROI Manager dialogue box, it will open automatically when you press 't'.
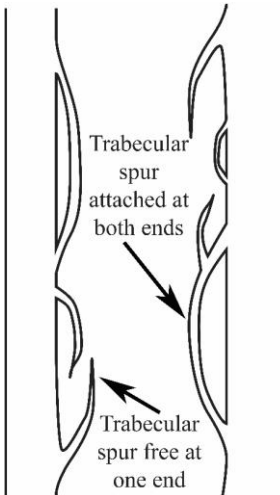
It is very important to add your selected regions to the ROI manager to save time later
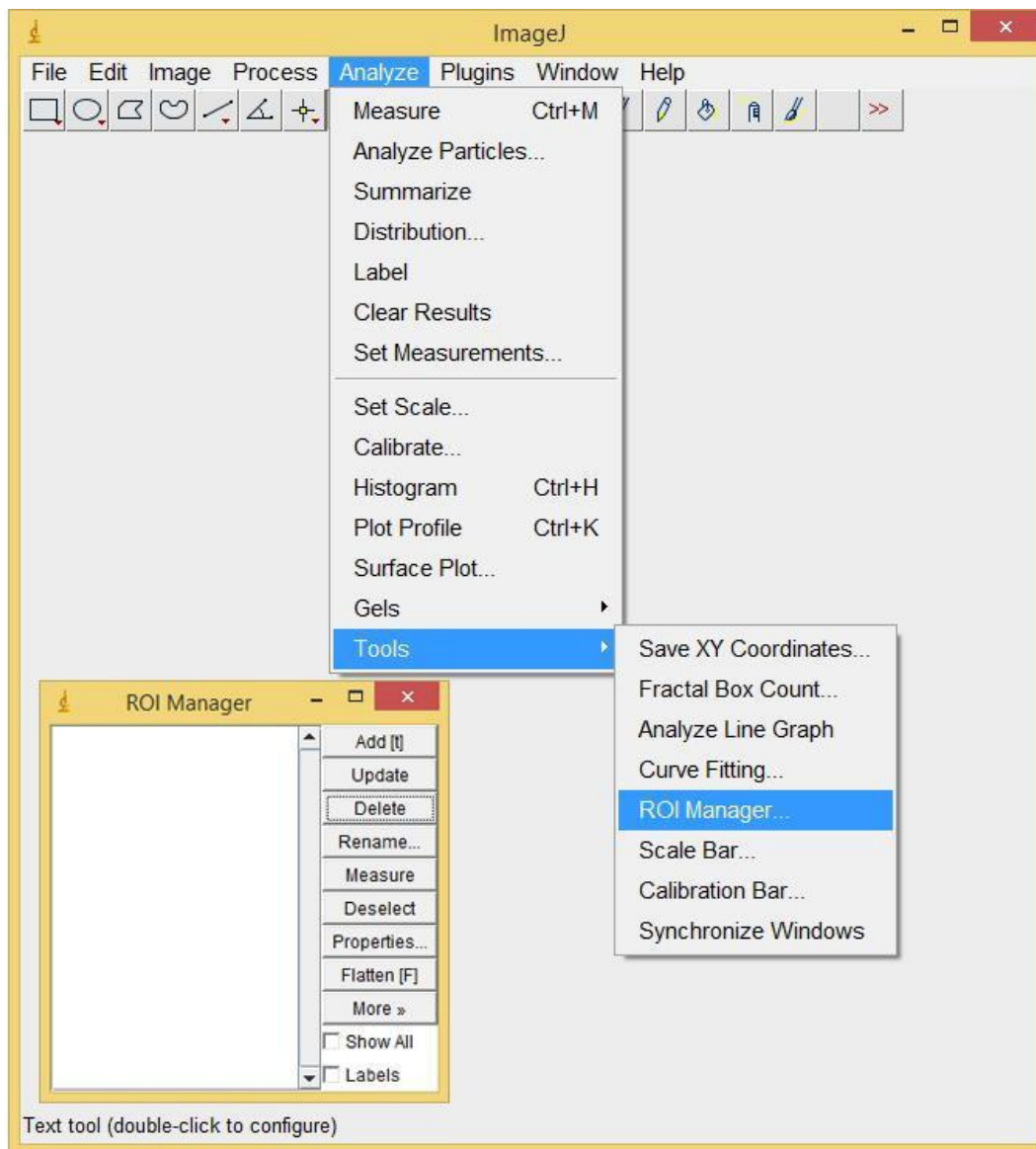
# STEP B: Measure the Cortical Bone Amount

Before Selecting Cortical Bone, an Important Note:
### WHAT CLASSIFIES AS CORTICAL BONE?

Trabecular bone on the endosteal margin must be carefully considered. When a trabecular spur was joined to the cortex on both ends, the spur was included as cortical bone. When a trabecular spur was free on one or both ends, it was not counted as cortical bone and not selected.

The image to the left depicts trabecular spurs that are attached at both ends, as well as free at one end.
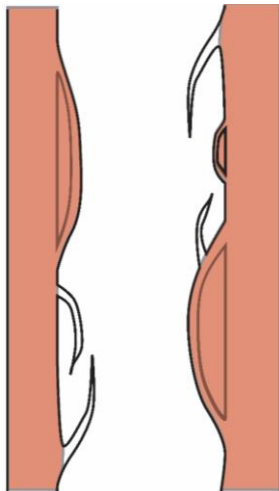
The image on the left depicts how these trabecular spurs are recorded when selecting the cortical bone walls. The red areas indicate the areas that should be selected and counted as cortical bone.
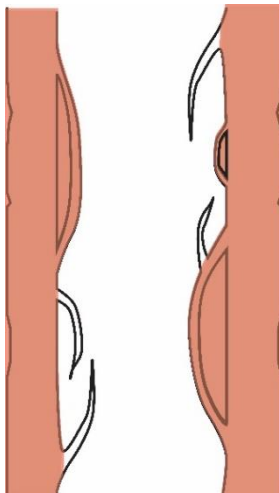
The diagram on the left shows cortical bone with some damage to the external cortical bone surface (e.g., divots caused by root etching). Trinkaus and Ruff (1999)[1] suggest that external bone contours can be interpolated radiographically by extending surfaces between more well-preserved areas of subperiosteal cortical bone. This premise allows bones with small regions of damage to be included, and is applied in the ROI method when slight damage to the external surface is present.

The red areas in the image indicate the regions that should be selected and counted as cortical bone. The adjacent, undamaged cortical bone surface contours have been extended to allow for small amounts of damage to the external bone surface.

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[1] Trinkaus E, and Ruff CB. 1999. Diaphyseal Cross-Sectional Geometry of near Eastern Middle Palaeolithic Humans: The Tibia. *Journal of Archaeological Science* 26:1289-1300.

5) Selecting the Cortical Bone

a) In the toolbar, right click on the oval or elliptical tool and select the 'Selection Brush Tool' (indicated below with the blue arrow).

Double click the 'Selection Brush Tool' to set your brush width to approximately 2 pixels and click 'OK'. You may adjust your brush width as necessary to fill in more or less bone.
Tip: I set my brush to 6 pixels when I am first filling in a cortical bone wall; I then decrease the brush size to 1 or 2 pixels to carefully add or remove areas as needed.
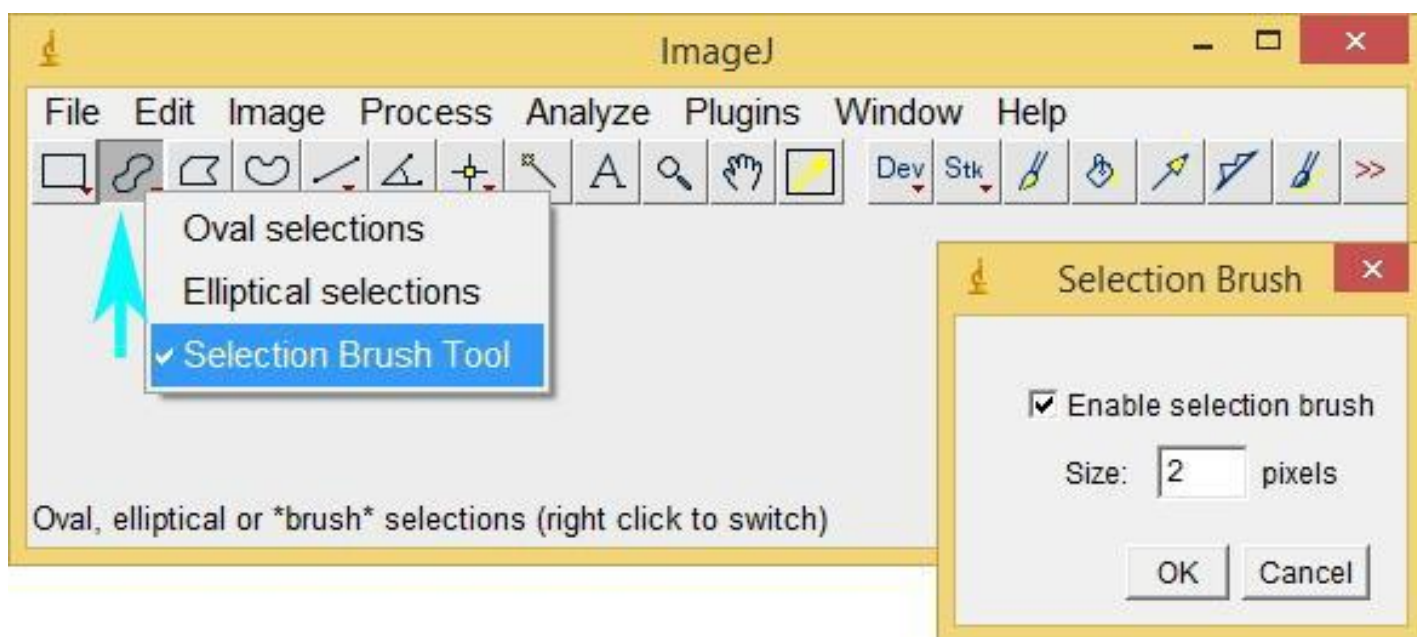
b) Zoom in on the cortical bone within the ROI rectangle (Click +)
While working to select the cortical bone, you may zoom in and out, and reposition the image as much as needed.

Reminder:

To **zoom in** click the '+' keyboard key

To **zoom out**, click the '-' key

To **adjust the position of the image**, hold the spacebar and drag the image to the desired location.

5 c)   You will have to **trace each cortical wall separately**, starting with either the left or the right side. With the 'Selection Brush Tool' selected, click, hold, and trace to fill in <u>all</u> of the cortical bone within the square ROI (colour in between the lines – i.e., between the periosteal and endosteal bone margins so that all the cortical bone is selected). See '**Trouble Shooting**' below if you have difficulties working the selection brush tool.

It is not necessary to add all the cortical bone in a single pass. In order to add and remove cortical bone from the selection:

c.1)   To **remove** from the selection, click **outside** the selection and drag inside the selection.

c.2)   To **add** to the selection, click **inside** the selection and drag outside the selection.

NOTE Please take your time and be attentive to the area you are selecting; only highlight what classifies as cortical bone.
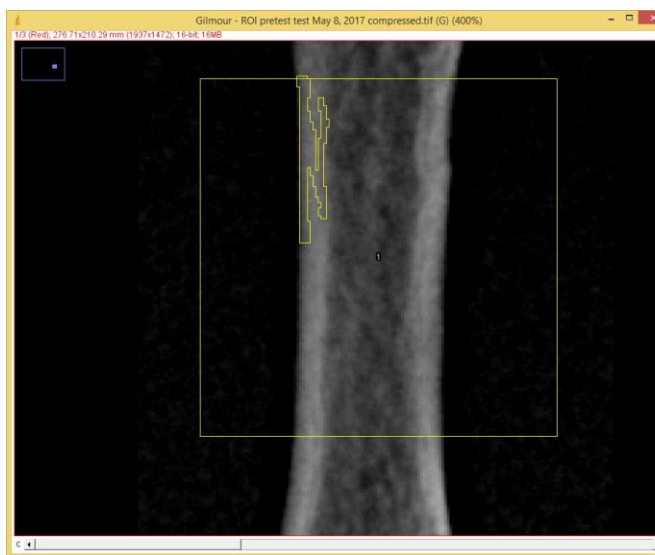
Above: The left cortical bone wall is *in the process* of being selected. The selected area is indicated by a yellow line. The selected edges are not yet defined and all the cortical bone in the left cortical wall has not yet been selected.
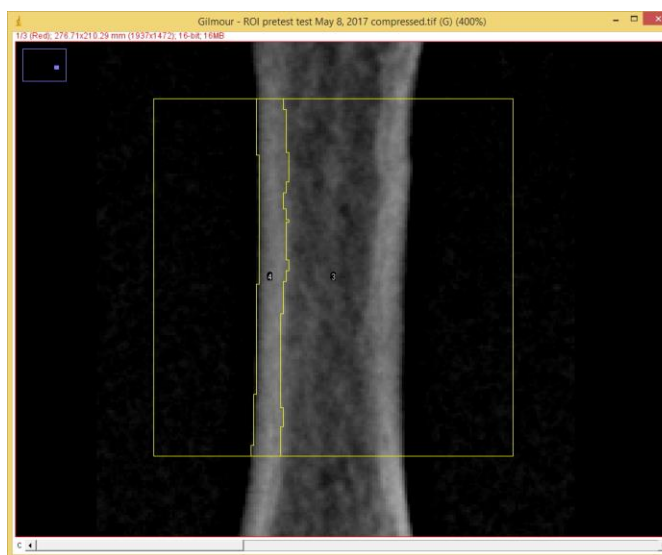
Above: The left cortical bone wall is completely selected. The selected area is indicated by a yellow line.

Trouble Shooting: If you are having difficulties getting the 'Selection Brush Tool' to work, try clicking and unclicking 'Show All' in the ROI manager (click it again if the ROI rectangle you positioned disappeared). This should allow you to create new selections using the 'Selection Brush Tool'. Repeat this troubleshooting step as needed.

d)   Add the first selected cortical bone wall to the ROI Manager dialogue box by pressing 't' on the keyboard.

e) Next, to select the *opposite* cortical bone wall, click 'Show All' in the ROI Manager (described in the 'Trouble shooting' above).

f) Choose the 'Selection Brush Tool' and select the opposite cortical bone wall (follow step c).

g) Add the second cortical bone selection to the ROI manager (Click 't').

It is very important to add your selections to the ROI Manager dialogue box for measurements later
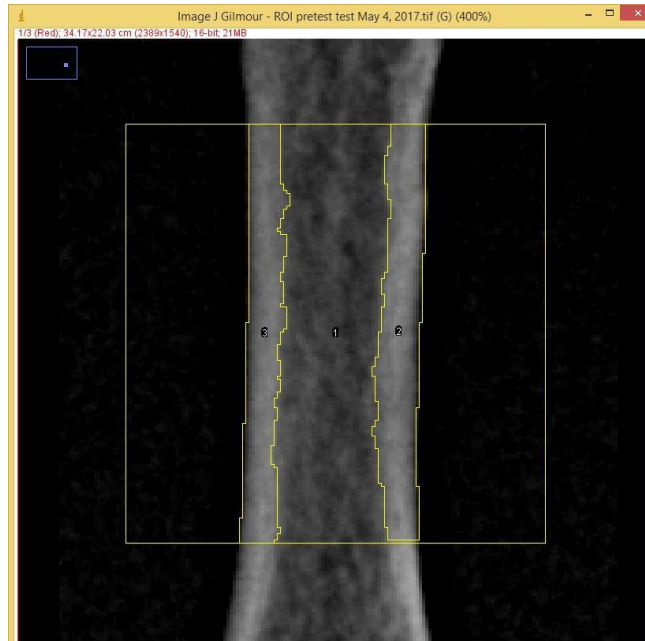
Above: Both cortical walls are selected within the 19mm^2 ROI. The selected areas are indicated by yellow lines.

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Note about the ROI Manager:

At this point, you should have three selections added to the ROI Manager dialogue box:

1) The 19mm^2 ROI box
2) The left cortical bone wall
3) The right cortical bone wall

These are all listed in the ROI manager dialogue box according to their different image coordinates.

When you select one of the numbers in the ROI Manager list (indicated with a red arrow in the image), it will highlight the selection in the image in blue (see image to the right). If you are unsure which items in the list are cortical bone wall selections, click the list items individually to see which selected region turns blue.
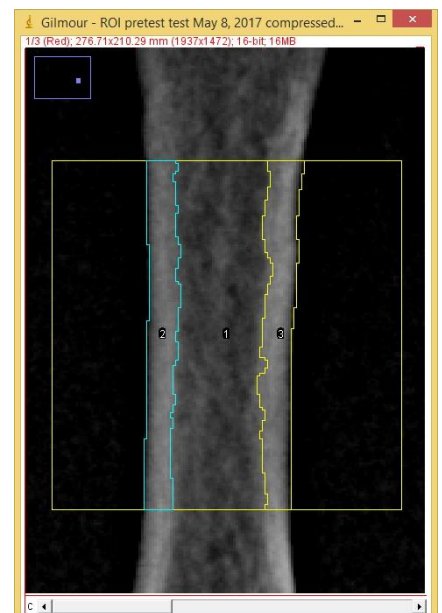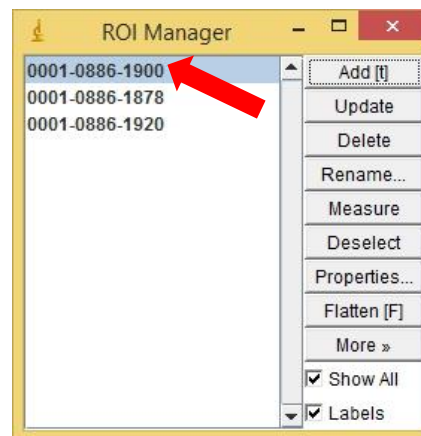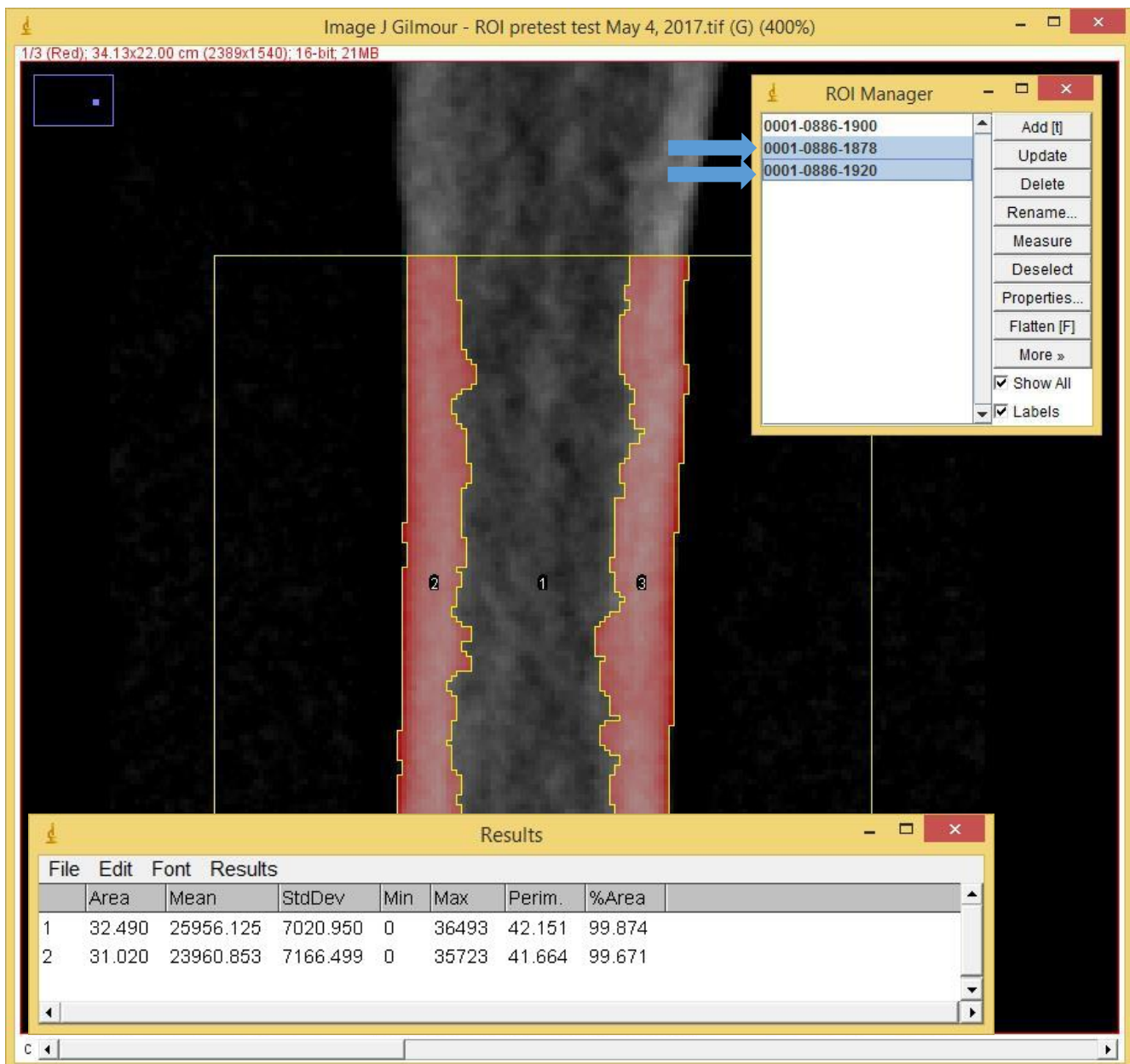
6) Measure the Amount of Cortical Bone Selected

Once the cortical bone is completely selected and has been added to the ROI Manager, crtl+click to choose both of the saved cortical wall selections in the ROI Manager (indicated with blue arrows in the image below).

Click 'Measure' in the ROI Manager dialogue box.

A new dialogue box ('Results') will appear, reporting the area of each selection in mm^2. To report the total amount of cortical bone within the 19mm^2 ROI square, add both entries under the 'Area' column together. For the purposes of this test, please report the area with as many significant figures as possible.

For example: in the Results dialogue box shown below, the measured areas are 32.490 and 31.020. Added together, these areas total 63.510. In this example, 63.510 would be reported.

8) Preserve your Measurements for Later Reconsideration

The final step is to fill the selected cortical bone area so that your measurements can be replicated if necessary.

Select one cortical bone wall region that you saved in the ROI manager. Click *Edit>Fill*.

Repeat for the second cortical bone wall region.

9) Save the image