**MRI Analysis**

**Standard Operating Procedures**

**Loading an Image**

1. Open the ITKSnap program.
2. Select the File drop down menu at top left, then select Open Main Image. Browse to select the desired MRI image. When prompted click Next twice and Finish.

**Changing the Image Contrast (Optional and can be done at any step)**

1. Select the Tools drop menu at the top left, then select Image Contrast and choose Auto-Adjust Contrast (all layers).
2. If the Auto-Adjust doesn’t provide adequate image contrast, then select the Tools drop down menu, select Image Contrast and then Contrast Adjustment and an Image Layer Inspector window will pop-up. Slide the top node representing image intensity to the left from 2500 closer to 1000 by Left-Clicking and holding down the cursor. See FIGURE below.

A screenshot of a computer screen

Description automatically generated

**Rotating an Image for View Standardization**

1. Select the File menu again and choose Add Another Image and load the same file selected as the main image. When prompted select Next twice and on the following screen make sure “As a separate image” is selected before clicking Next a third time and then select Finish.
2. Select the Tools drop down menu and then click Registration. A new menu will appear on the right side of the screen. *Tips:* *To change the slice being viewed, scroll with the mouse wheel.* *You can change the display configuration so that you view all 4 panels or an expanded view of 1 panel by selecting the either the A (axial), S (sagittal) or C (coronal) at the top Right corners of the panels. Once one panel is expanded the option changes to a grid icon and by selecting that icon it will go back to viewing all four panels.*
3. Select Manual and an interactive tool will appear with a sundial overlaid on the image. When you hover the cursor on the sundial, it turns yellow and you can rotate the image by holding down the left-click mouse button. Rotate the image so that the tibiae are oriented at the top and are level [See FIGURE at the top of page 2].
4. Select the Save button at the bottom of the Registration menu on the right side of the screen and save the image as Image#\_Rotated.txt in the same folder as the main image is located.

A screen shot of a computer

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**Identifying Proximal and Distal Tibia-Fibula Junctions**

1. Using the mouse wheel, scroll down towards the knee to the slice where the fibula makes contact with the tibia. Make note of the slice number for the proximal Tib-Fib junction in the Excel File for the appropriate study (titled SCI-X MRI Triceps Surae Worksheet located in the MRI folder within the Study folder). See FIGURE below; proximal Tib-Fib junction on slice 24.

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1. Scroll up towards the ankle to the slice displaying where the fibula contacts the tibia and enter the slice number in the Excel file for the distal Tib-Fib junction. See FIGURE below; the distal Tib-Fib junction is on slice 7.

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**Drawing Cross Sectional Area (CSA) of the triceps surae**

**NOTE:** CSAs will be drawn for 8 consecutive slices relative to the proximal Tib-Fib junction. Draw CSA’s on slices that are 8-15 slices away from the proximal Tib-Fib junction.

1. Select the Polygon tool under the Main Toolbar on the upper left side of the screen, then select Smooth curve under the Polygon Inspector.
2. The Segmentation Labels menu is located on the lower left screen. Under Active label, the current label will likely be Label 1. Select the Segmentation drop down menu at the top left of the screen and choose Label Editor. Click on Label 1 and once it is highlighted, move the cursor to Description and change the name to Left TS (triceps surae) and choose the color as red if not already. Under Active label, make sure Left TS is selected.
3. In the Segmentation Labels menu, the Paint over label should be All labels.
4. There are a few approaches to drawing the desired area. You can start drawing the CSA by holding down the left mouse button and continuously move the mouse in a free hand style. Alternatively, you can use several separate clicks covering a small distance in order to construct consecutive small lines until the area is complete. If you are not satisfied with the line drawn, you can choose clear or undo last point (as many points as you like can be deleted). Once the general shape is drawn select accept at the bottom right of the screen, if accept is not available then the shape did not connect to starting point, so choose complete and then accept. You can draw several more objects of any size to more accurately fill in areas of CSA as needed. If you draw too large of an area, you can select the Active Label to be the Clear label. Draw an object and select accept and the Left TS area will be erased. *See* FIGURE on next page. *Tips: You can zoom in by holding down the right-click mouse button and move the mouse up or down until the desired view is obtained. The hamstrings run on the medial side and often the border between the TS and hamstrings in not clear. By scrolling through the slides moving up and down, you can often predict where the border is between the muscles when it is visible on the slice that you are working with.*
5. Once satisfied with the area, select the Segmentation drop down menu and choose Volumes and Statistics. Enter the value for the Left TS in the Excel File indicating the slice distance from the proximal Tib-Fib junction.
6. Draw the next CSA on the desired slice. When you select Volumes and Statistics, the volume displayed will now be a volume for both slices, so you need to subtract the 2nd volume from the first. Continue to draw and calculate the CSA for each slice until all 6 slices are done.
7. Repeat Steps 2-6 for the right side (change Label 2 to Right TS and choose the color as green).
8. Select the Volumes and Statistics option and Export the file as Image#\_Volumes.txt and save it to the same folder as the main image is located. See FIGURES on page 4.
9. Select the Segmentation drop down menu and choose Save Segmentation Image and name the file Image#\_Segmentation.txt in the same folder as the main image is located. This allows someone to revisit the images with the CSAs drawn at any future time.
10. To check the shape of the CSAs, select the grid icon on the upper left corner if you were viewing only the axial panel so that all 4 panels are being displayed. Select update on the lower left panel designated for volume (this panel is blank until you choose update). You can then zoom and rotate the image by holding down the Right-Click and Left-Click on the mouse, respectively.

See FIGURE on page 4. If unable to zoom to the desired area, place the crossbar in panel A in the middle of the right or left triceps surae and then zoom in the 3D panel.

A screenshot of a computer

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**General Image Information and Tips for Using ITKSnap**

* MRI images have 40 axial slices that are 1 mm thick each.
* **Left-Clicking** on the mouse anywhere on a panel will move the **Crosshair** to that location.
* To **Zoom In/Out** on a panel, hold down the **Right-Click** and move the mouse up or down.
* To **Rotate** the 3D image around each axis, hold down the **Left-Click** and either move the mouse side-to-side or up-down. To Rotate around a single-axis hold down Control + Left-Click on the mouse and move mouse as desired.
* To **Pan** the 3D image press **Shift + Left-Click** at the same time and move the mouse to the desired view.

A screenshot of a cell phone

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