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| **Getting Started** | Open DICOM image file, to do this select **FILE** then **OPEN**. To increase the size of the DICOM image go to **IMAGE** then **In[+]** or **Out[-]**. The **ImageJ** window will increase or decrease accordingly. |
| **Waist circumference** | **Step 1.** To assess waist circumference using the abdominal perimeter of the cross-sectional image.   1. Start at **IMAGE** then **ADJUST** then **THRESHOLD**. 2. Threshold box opens, click **SET** and change **LOWER** threshold to ‘250’ and **UPPER** threshold to ‘1000.’ 3. Click OK to Exit this window. 4. ~~S~~elect ‘*Wand (Tracing) Tool*’ icon on the IMAGEJ control box. 5. Click on the outer surface of threshold-highlighted area. The tracing tool has now delineated the abdominal perimeter. 6. To set the correct measurement unit, go to **ANALYZE** then **SET MEASUREMENTS** and select **AREA** and **PERIMETER**. 7. To quantify the abdominal perimeters, click on **ANALYZE** then **MEASURE**. A new **RESULTS** window emerges on computer screen with measures for **AREA** and **PERIMETER**. The unit for **AREA** (mm2) and **PERIMETER** unit is in centimeters (mm). |
| **Outer muscle perimeter for skeletal muscle mass** | **Step 2.** The outer perimeters of the abdominal muscles is needed for assessment of skeletal muscle mass.   1. Go to Threshold box, **ADJUST** then **THRESHOLD**. 2. Threshold box opens, click **SET** and change **LOWER** threshold to ‘-250’ and **UPPER** threshold to ‘-250.’ This returns image to original grey color and allows the anatomical features at the L3 to be easily determined. 3. Exit this window by clicking **OK**. 4. Click on *‘Freehands selection’* tool and click on image to remove the yellow line surrounding abdominal perimeter. 5. Using the stylus, trace the outer muscle perimeter. Then, return to **THRESHOLD** box and change **LOWER** value to ‘-29’ and **UPPER** value to ‘+150.’ 6. To set the correct measurement units, go to **ANALYZE,** then **SET MEASUREMENTS** and select **AREA**, **PERIMETER** and **LIMIT TO THRESHOLD**. 7. To quantify the outer muscle perimeter, click on **ANALYZE** then **MEASURE**. 8. In the **RESULTS** window, a second set of measurements will appear for **AREA** and **PERIMETER**. The unit for **AREA** (mm2) and **PERIMETER** unit is in centimeters (mm). |
| **Inner muscle perimeter for skeletal muscle mass** | **Step 3.** The inner perimeters of the abdominal muscles is needed for assessment of skeletal muscle mass.   1. Go to Threshold box, **ADJUST** then **THRESHOLD**. 2. Threshold window opens, click **SET** and change **LOWER** threshold to ‘-250’ and **UPPER** threshold to ‘-250.’ 3. Exit this window by clicking **OK**. 4. Using the stylus, trace the inner muscle perimeter. Then, return to **THRESHOLD** window and change **LOWER** value to ‘-29’ and **UPPER** value to ‘+150.’ 5. To quantify the inner muscle perimeter, click on **ANALYZE** then **MEASURE**. 6. In the **RESULTS** window, a third set of measurements appear. |
| **Unit conversion process to cm and cm2** | **Step 4**. Transfer of data to excel spreadsheet for unit conversions.   1. Copy and paste these measurements to an EXCEL spreadsheet. 2. To copy and paste the data, in **RESULTS** window, highlight each measurement with the mouse then right click on mouse, select **COPY**. 3. Open Excel spreadsheet and paste the measurements. 4. Plug in the data into the following calculations to derive waist and skeletal muscle areas. 5. **For waist circumference (WC) do the following:** Measurement 1 (Perimeter, mm) divided by 10. 6. **For Skeletal Muscle Mass (SM) do the following:**  Measurement 2 (outer area) – Measurement 3 (inner area) divided by 100. |
| **Closing IMAGEJ** | To close **ImageJ**, click on ⌧ in corner of **ImageJ** window. All windows associated with **ImageJ** will close. |

**Figure 4. Summary of steps for body composition analysis using National Institutes of Health ImageJ.**