

## Calculating Area of Cells and Length of Projections in Zeiss Images Using Image J

1. Open Image J.
2. File --> Open --> Select .ism image you wish to analyze.

*If necessary:*

a) *Adjust brightness/contrast:*

*Image --> Adjust --> Brightness/Contrast*

b) *Zoom image:*

*10th icon from left. Magnifying glass icon. Click then click on image to zoom. Also can use +/- to increase or decrease zoom.*

c) *Move around image to get different view field:*

*11th icon from left. Hand icon. Click then click on image and see hand with index pointer. Left click then move around image.*

3. Use 3rd icon on the left to draw around the cell. Single click to close the circle when you come back to the beginning and the line is in the box that appears at the origin point. Slowly move mouse away from the area to make sure circle is "set".

4. To measure the circle:

*Analyze --> Measure . A results box opens with calculations. If you do not see the feature you want: In the results box - Results --> Results --> Set Measurements and check the calculation you want.*

**To move on to another measurement:**

- 5. Edit --> Draw --> Yes . You will have to select yes only if your image is a Z stack. Single stack or maximum projection images will just need Edit --> Draw. Line will not change to solid and different color line until you go to draw the next feature. This will save your drawing features.**

**You can also add text to label the cell:**

**9th icon from left, the "A" text box. Click then click on image to create text box and type in text. Remember to do the**

**Edit --> Draw step to save the text to the image.**

- 6. To measure the projections:**

**5th Icon from left. Right click to select straight or segmented line depending on how straight your projections are. Draw a line along your projection. Double click left click to save line.**

**Repeat steps 4 to 6**

**Repeat until finished with the cell.**

- 7. To create a line break in the Results to separate cell data:**

**7th icon from left, circle with lines out of it. Click then click on image. Best to click inside cell you just finished.**

**Do Step 4 and Step 5. Data creates a break to indicate different cell will follow. By selecting the area that is the cell this will give you coordinate in results that could be useful in tracking where your data came from in the image.**

**When finished with whole image.**

**To Save Image with Overlays:**

**Plugins --> Utilities --> Capture Image**

**then**

**File --> Save As --> Name image file and select .tif or .jpg**

**To Save Results Box:**

**In Results Box: File --> Save As --> Name file which will be saved as excel file.**