

Quantification of the distance
between lysosomes and nucleus

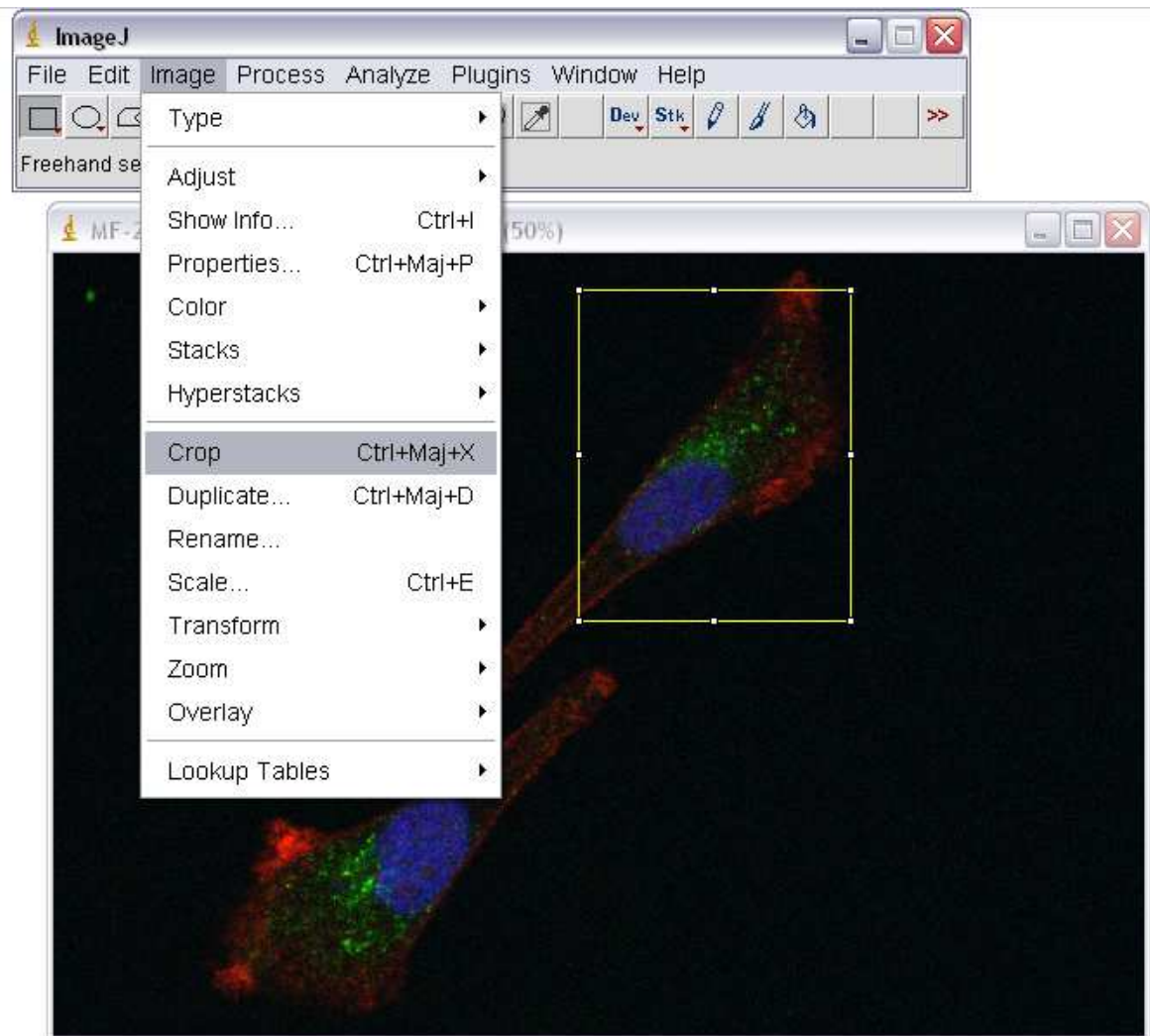
1. Selection of cells to quantify

Open image



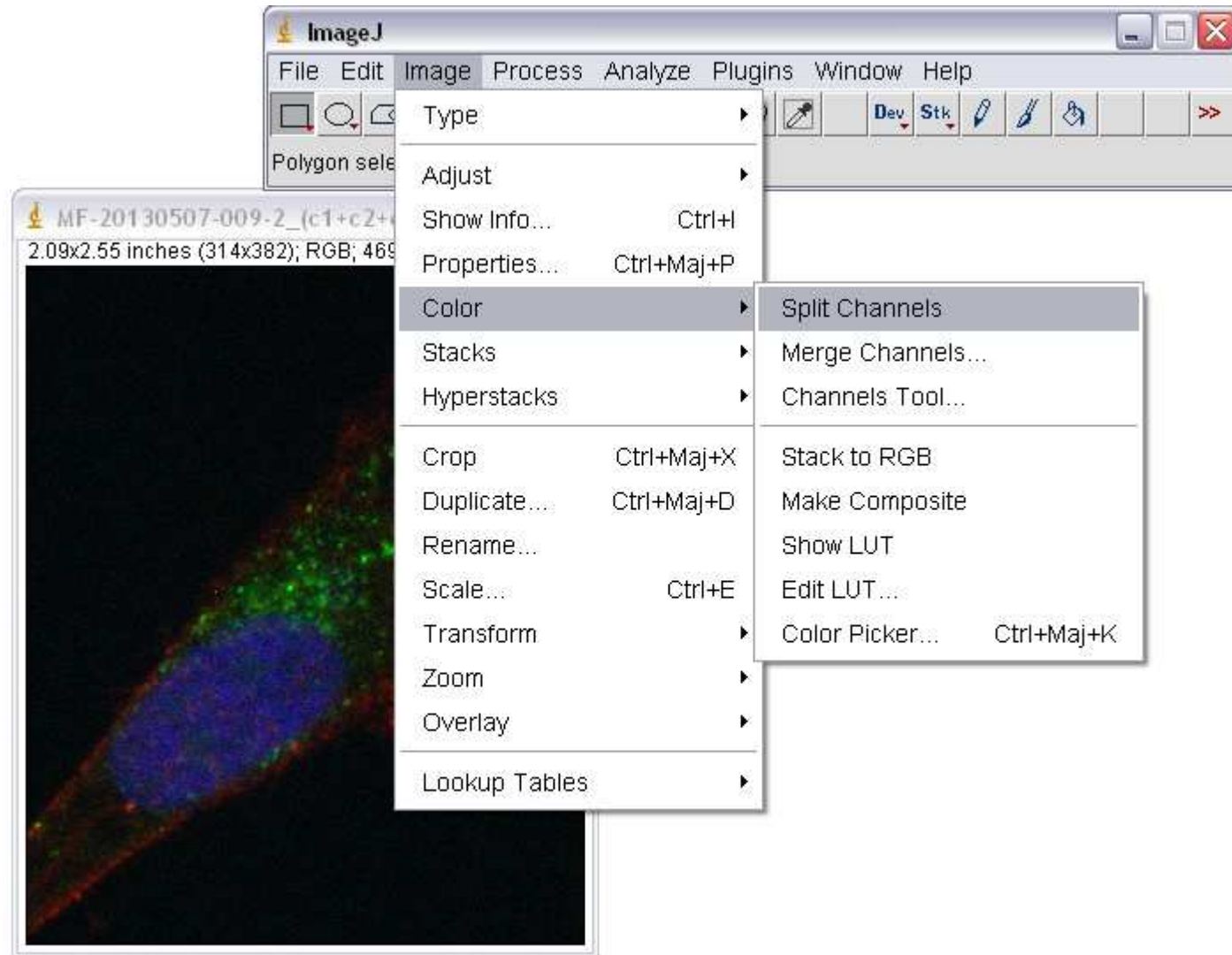
lysosomes labelled by anti- LAMP1 (green)
Nuclei labelled with DAPI (blue)

Select 1 cell and isolate it (crop)

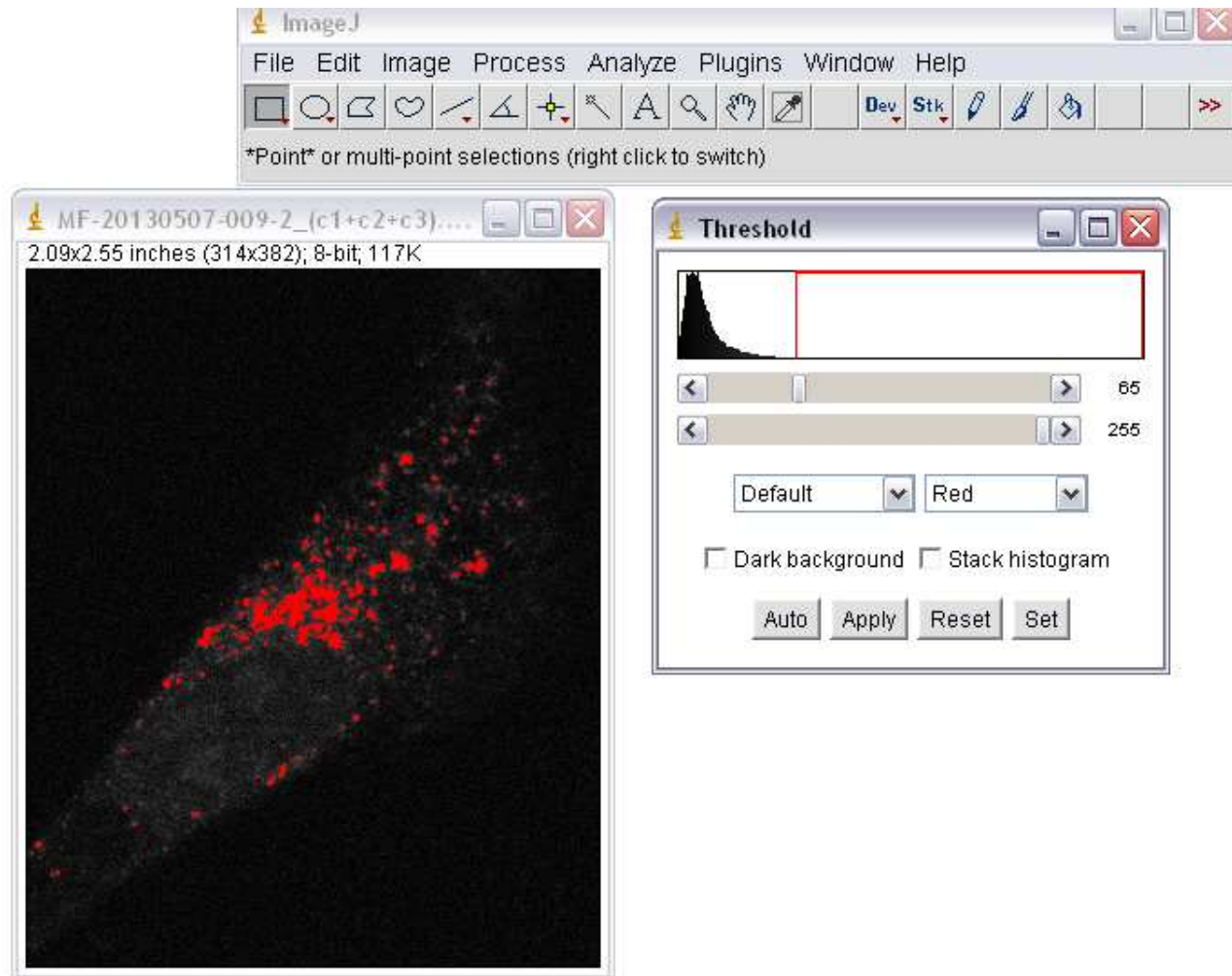


2. Preparation of image

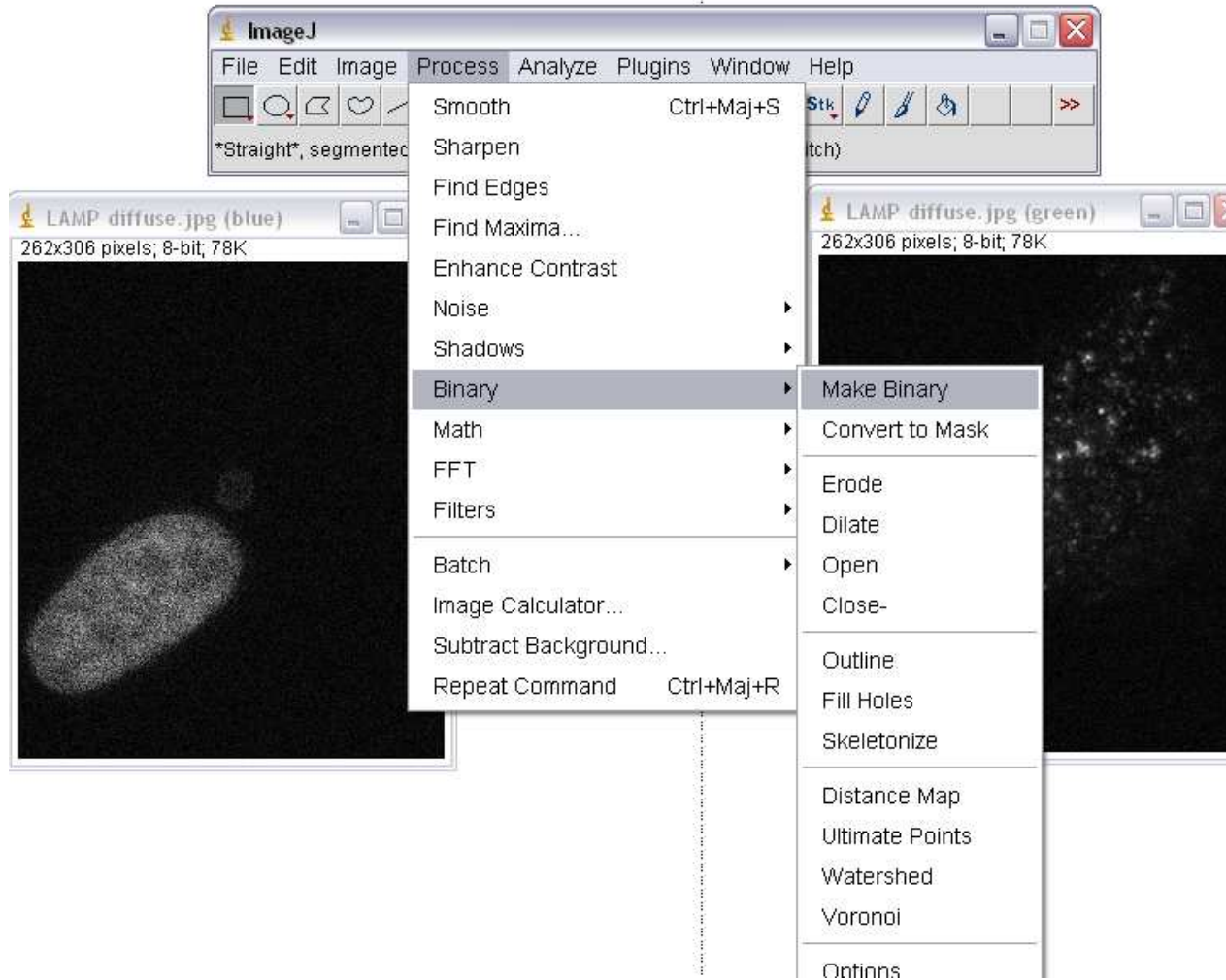
Separate color channels



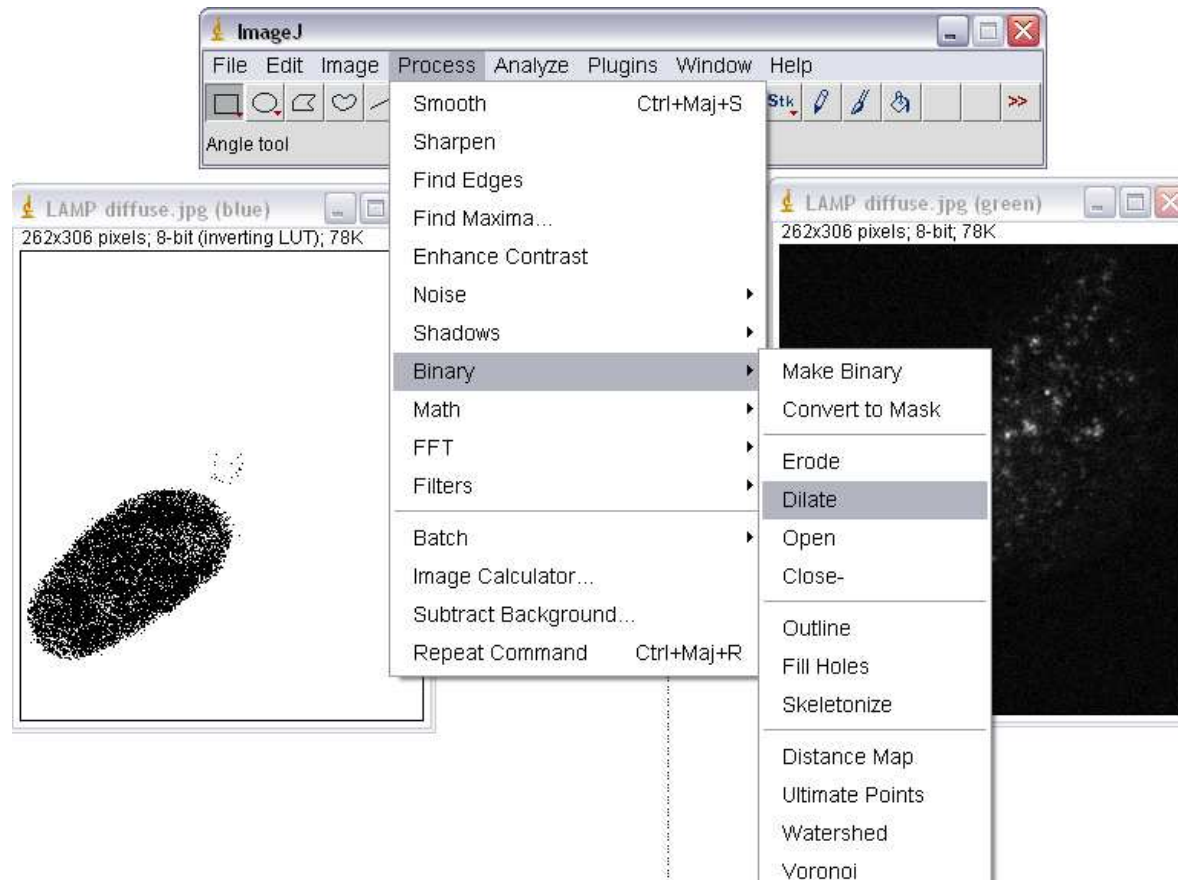
Transform the green image (lysosomes) into binary image:
set the threshold and click on *Apply*



Transform the blue image (nucleus) into binary image

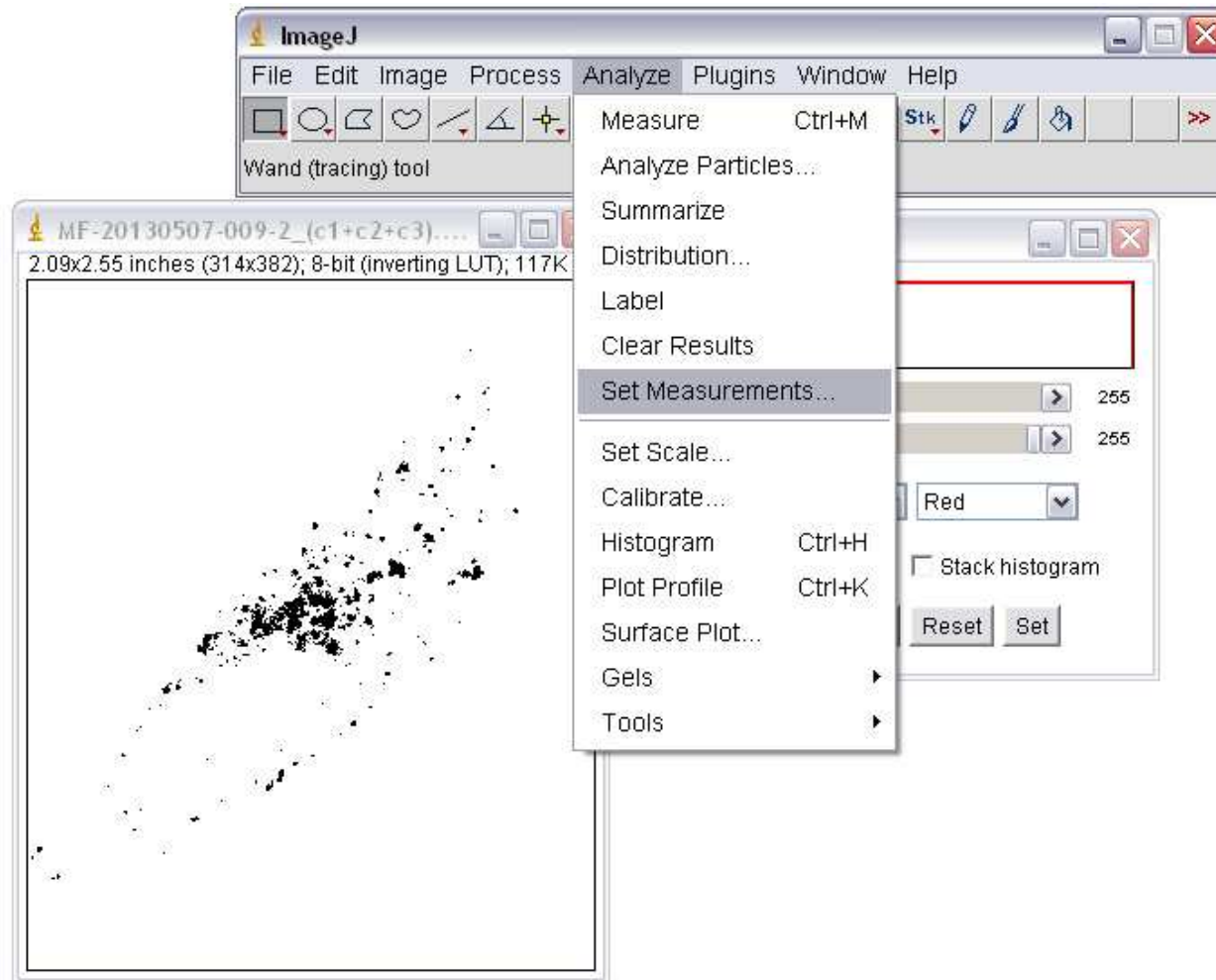


Dilate in order to have 1 large particle representing the nucleus

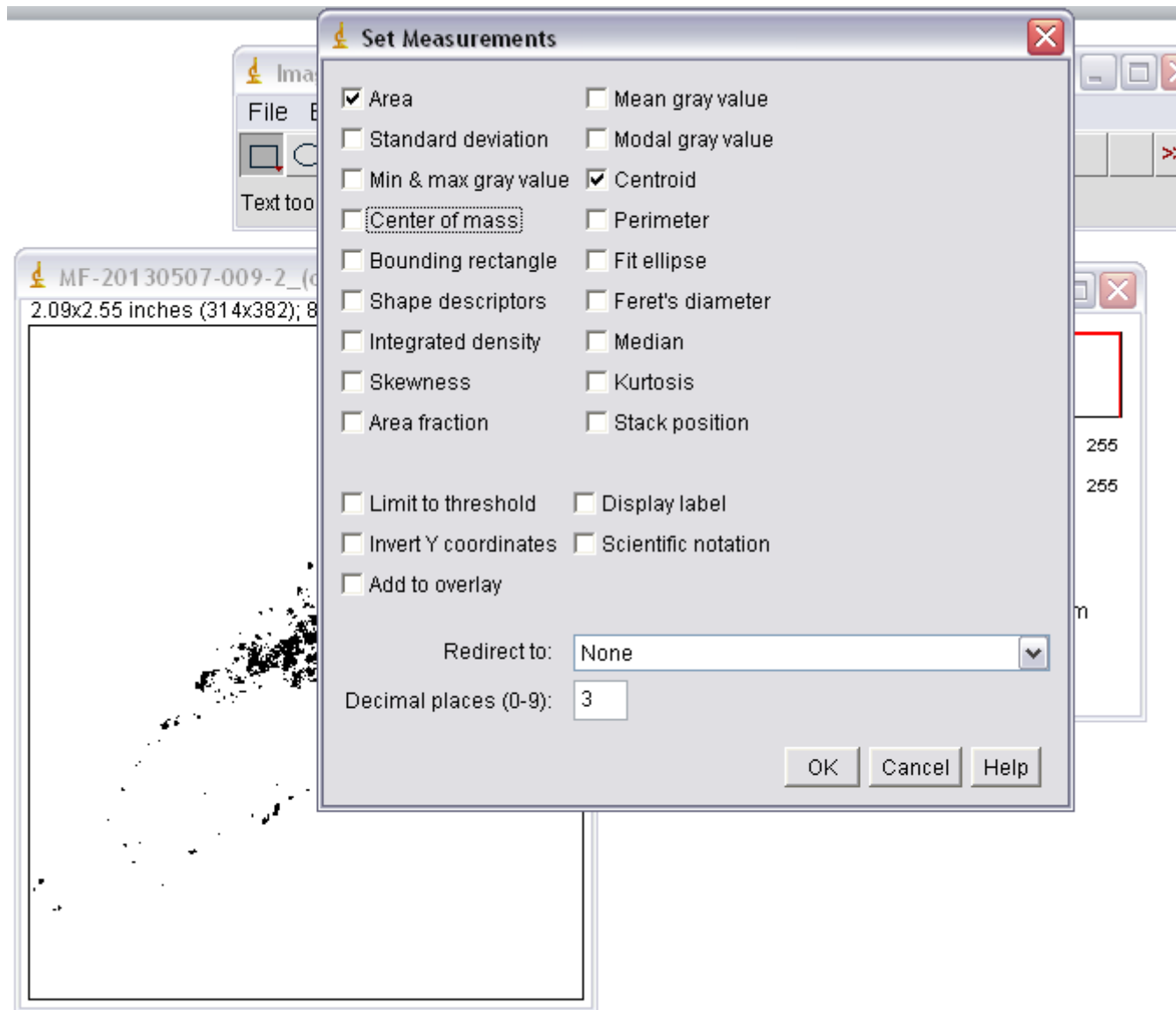


3. Determine X / Y coordinates of nucleus and lysosomes

Define measurements

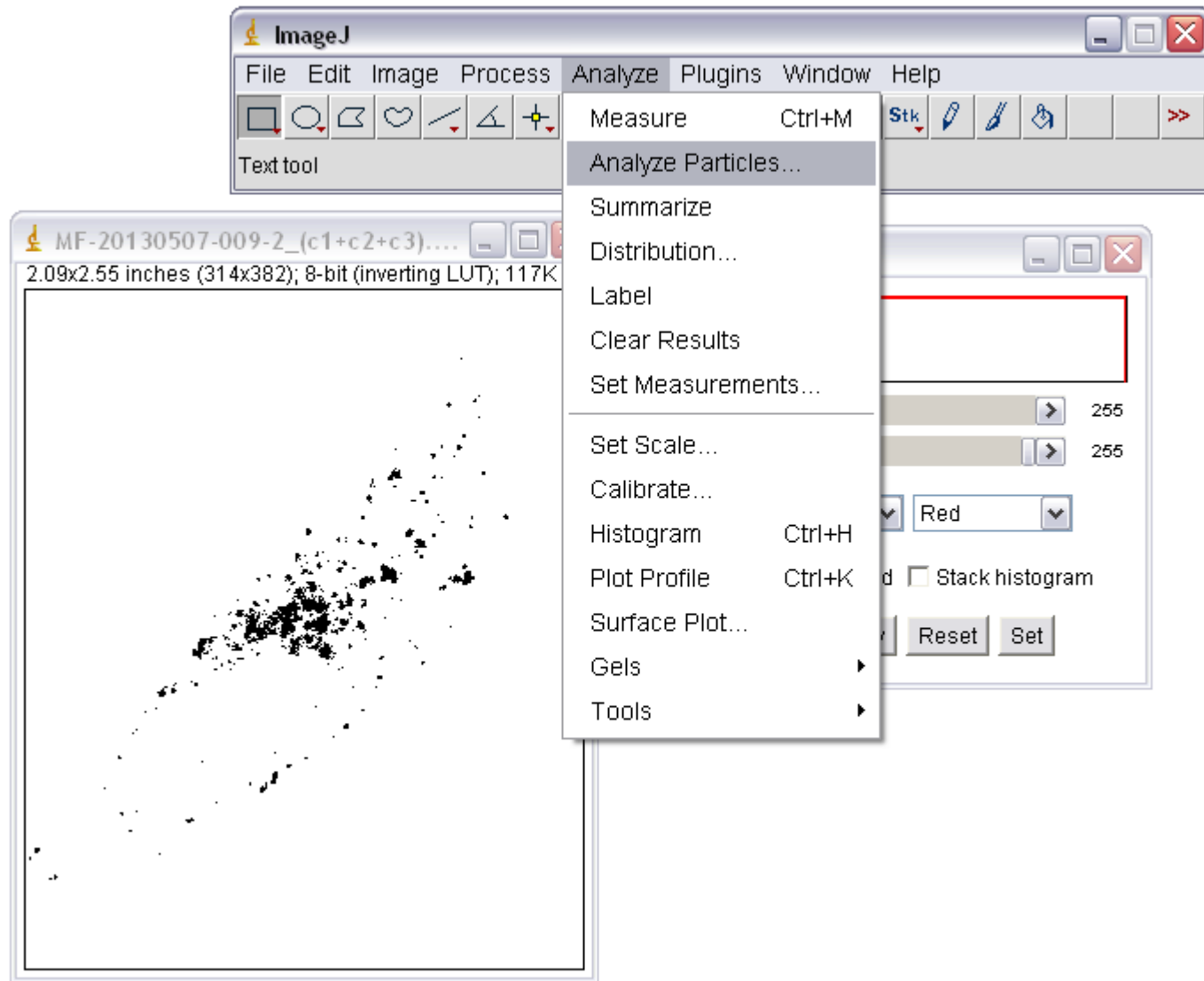


Select Centroid

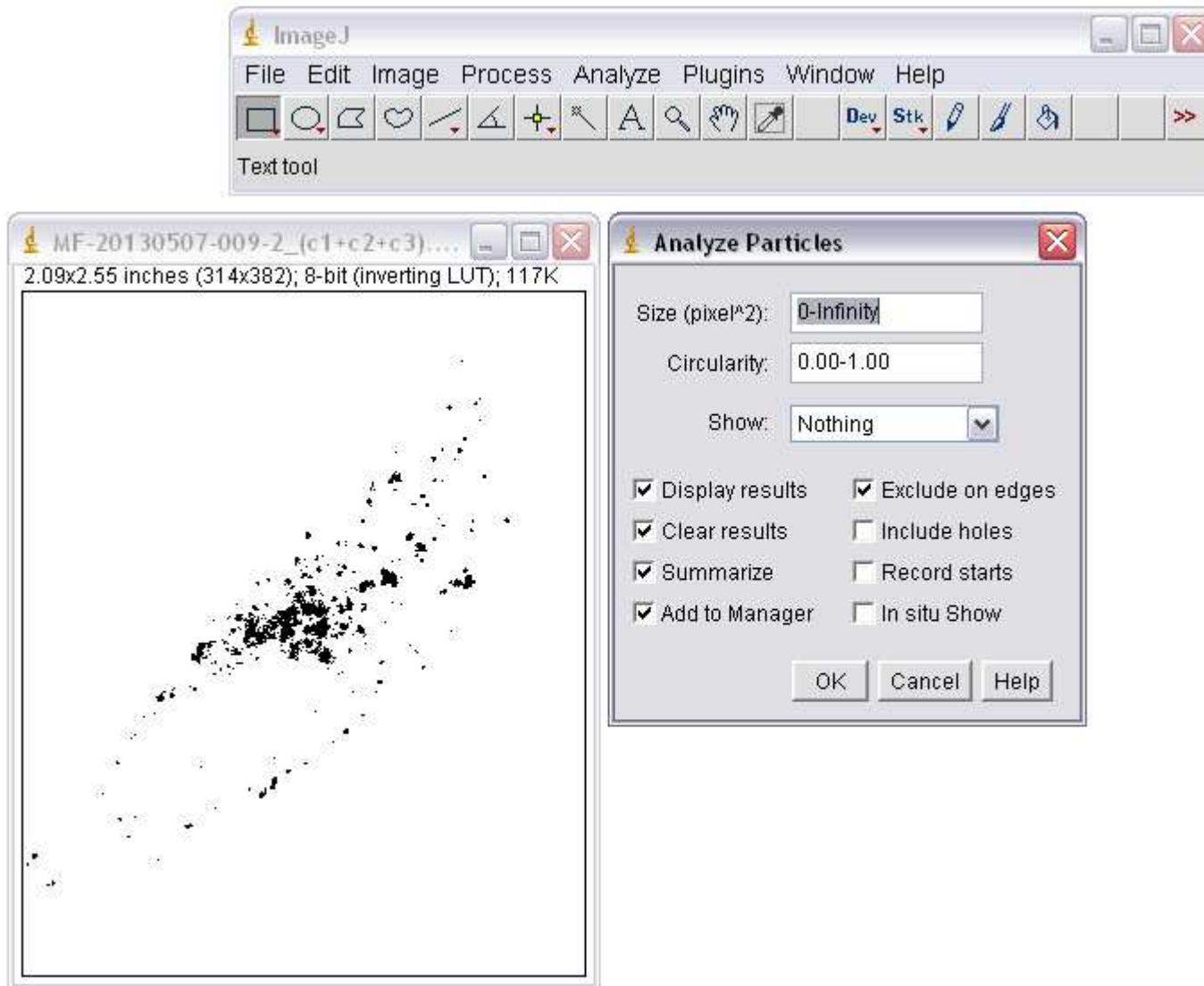


Centroid - The center point of the selection. This is the average of the x and y coordinates of all of the pixels in the selection.
Center of Mass - This is the brightness-weighted average of the x and y coordinates all pixels in the selection.

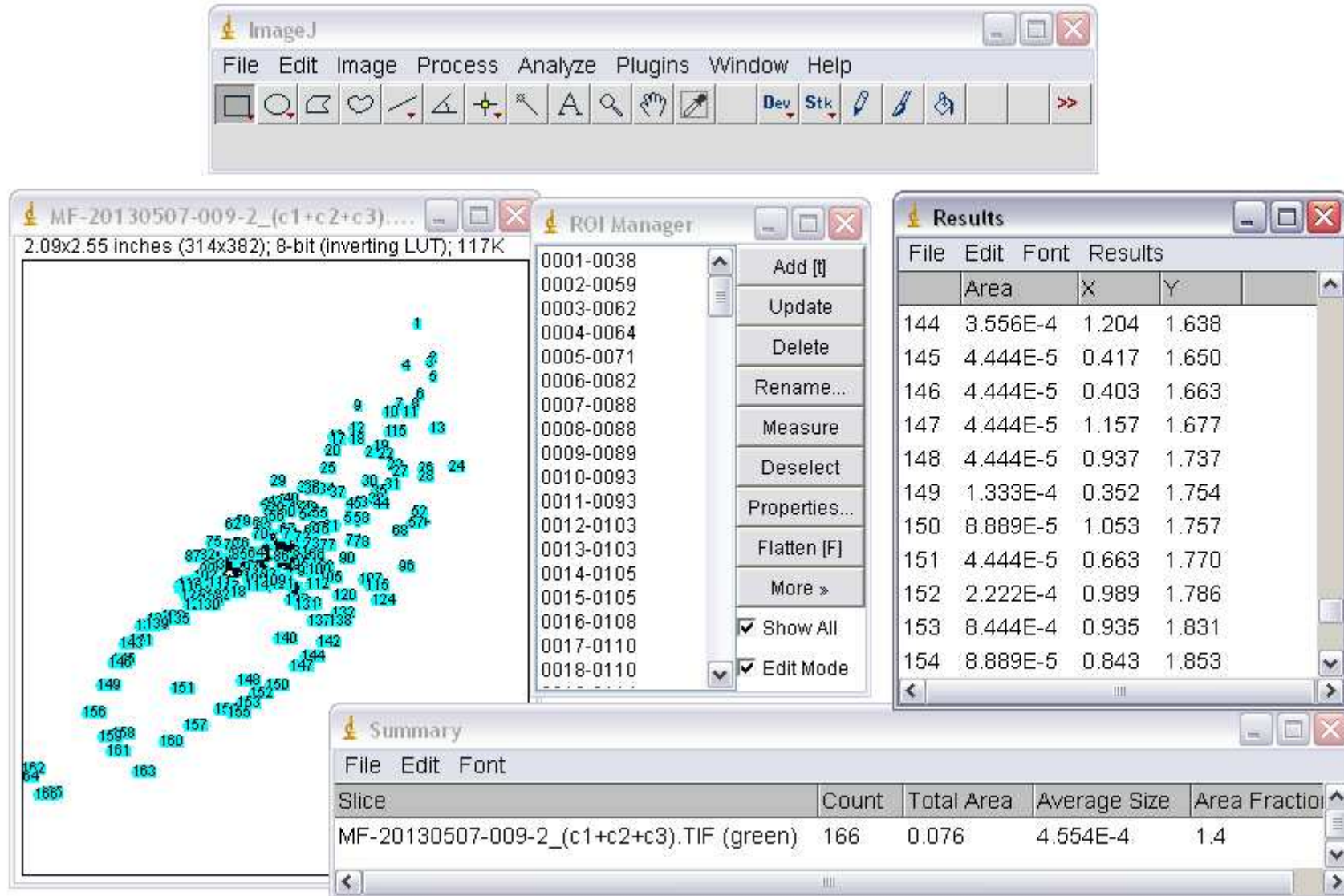
For the green image, Analyze particles



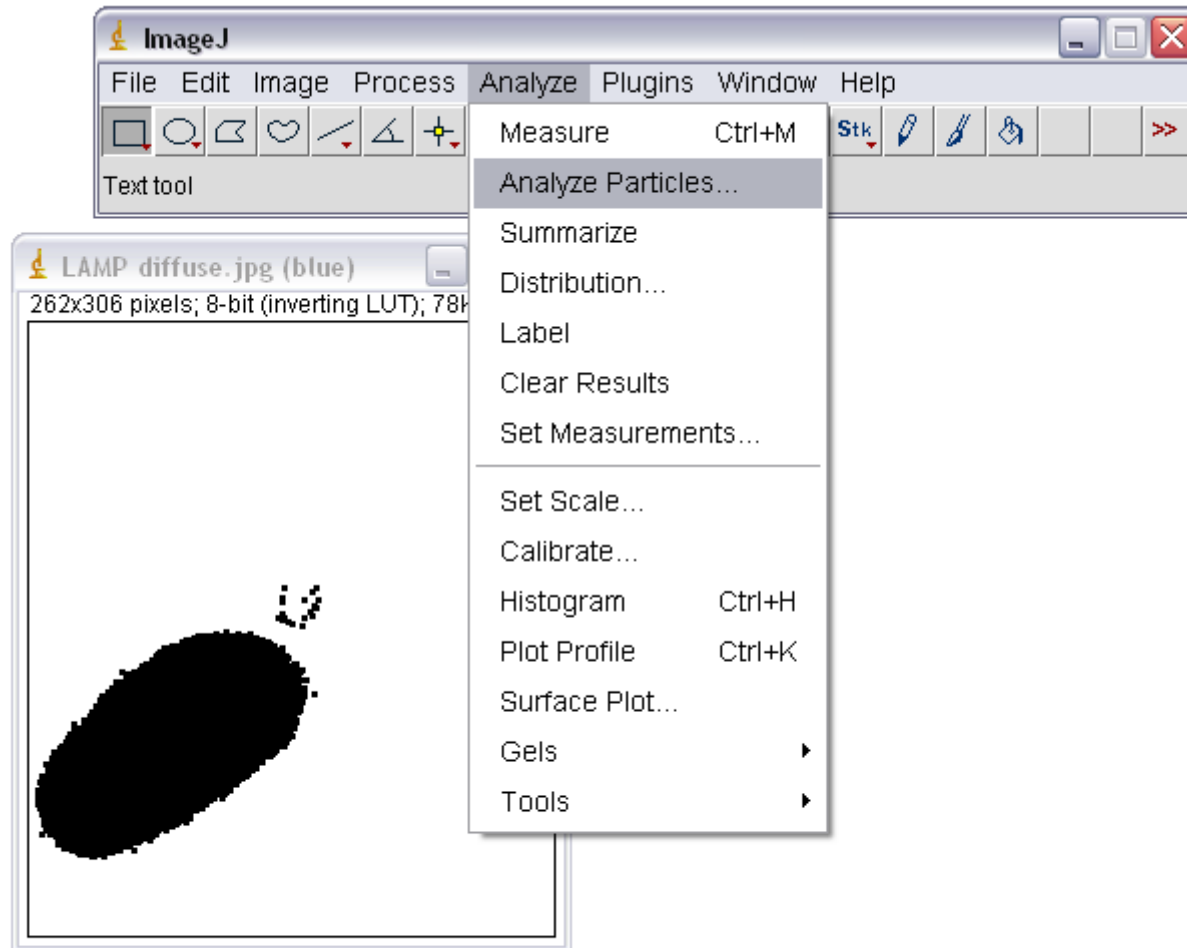
If required, limit the particle size



Copy data presented in the *Result* window (including X and Y coordinates) and paste them in an excel sheet (or save results)



For the blue image, Analyze particles



Copy data corresponding to the largest particle (nucleus) in the *Result* window (including X and Y coordinates) and paste them in excel (or save results)

The screenshot shows the ImageJ interface with the following components:

- ImageJ Window:** The main application window with a menu bar (File, Edit, Image, Process, Analyze, Plugins, Window, Help) and a toolbar.
- LAMP diffuse.jpg (blue) Window:** Displays a grayscale image of a nucleus with a cyan ROI. The image size is 262x306 pixels, 8-bit (inverting LUT), 78K.
- ROI Manager Window:** Lists 9 particles with their IDs (0001-0132 to 0009-0185). Particle 8 is highlighted. Buttons include Add [t], Update, Delete, Rename..., Measure, Deselect, Properties..., Flatten [F], and More ». Checkboxes for Show All and Edit Mode are checked.
- Results Window:** A table with columns Area, X, and Y. Row 8 is highlighted.
- Summary Window:** A table showing overall statistics for the 9 particles.

File	Edit	Font	Results
	Area	X	Y
1	9	127.500	132.500
2	17	142.500	133.500
3	15	126.500	138.500
4	9	137.500	138.500
5	48	141.583	143.542
6	42	127.762	148.214
7	9	136.500	151.500
8	9780	70.313	209.314
9	9	142.500	185.500

Slice	Count	Total Area	Average Size	Area Fraction
LAMP diffuse.jpg (blue)	9	9938.000	1104.222	12.4

4. Calculate the distance lysosomes-nucleus (in excel)

For 2 points:

Point A: coordinates (x_A, y_A)

Point B: coordinates (x_B, y_B)

Distance between A and B =

$$AB = \sqrt{(x_B - x_A)^2 + (y_B - y_A)^2}$$