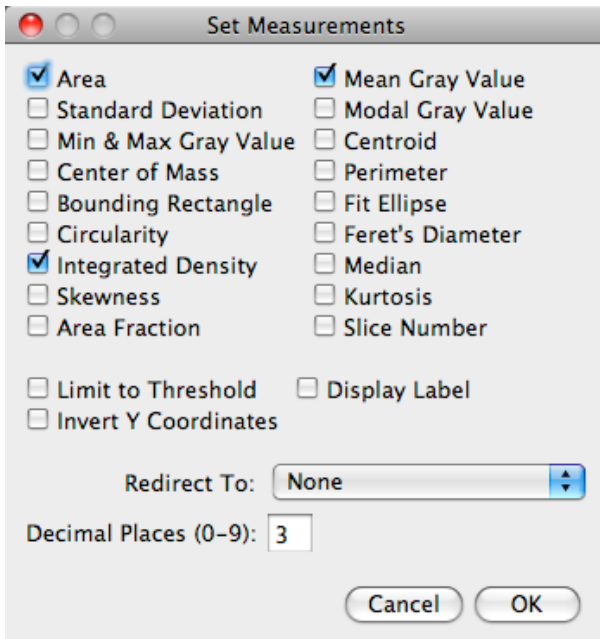


Background Subtraction to Obtain Total Fluorescence per Cell, using ImageJ and Excel

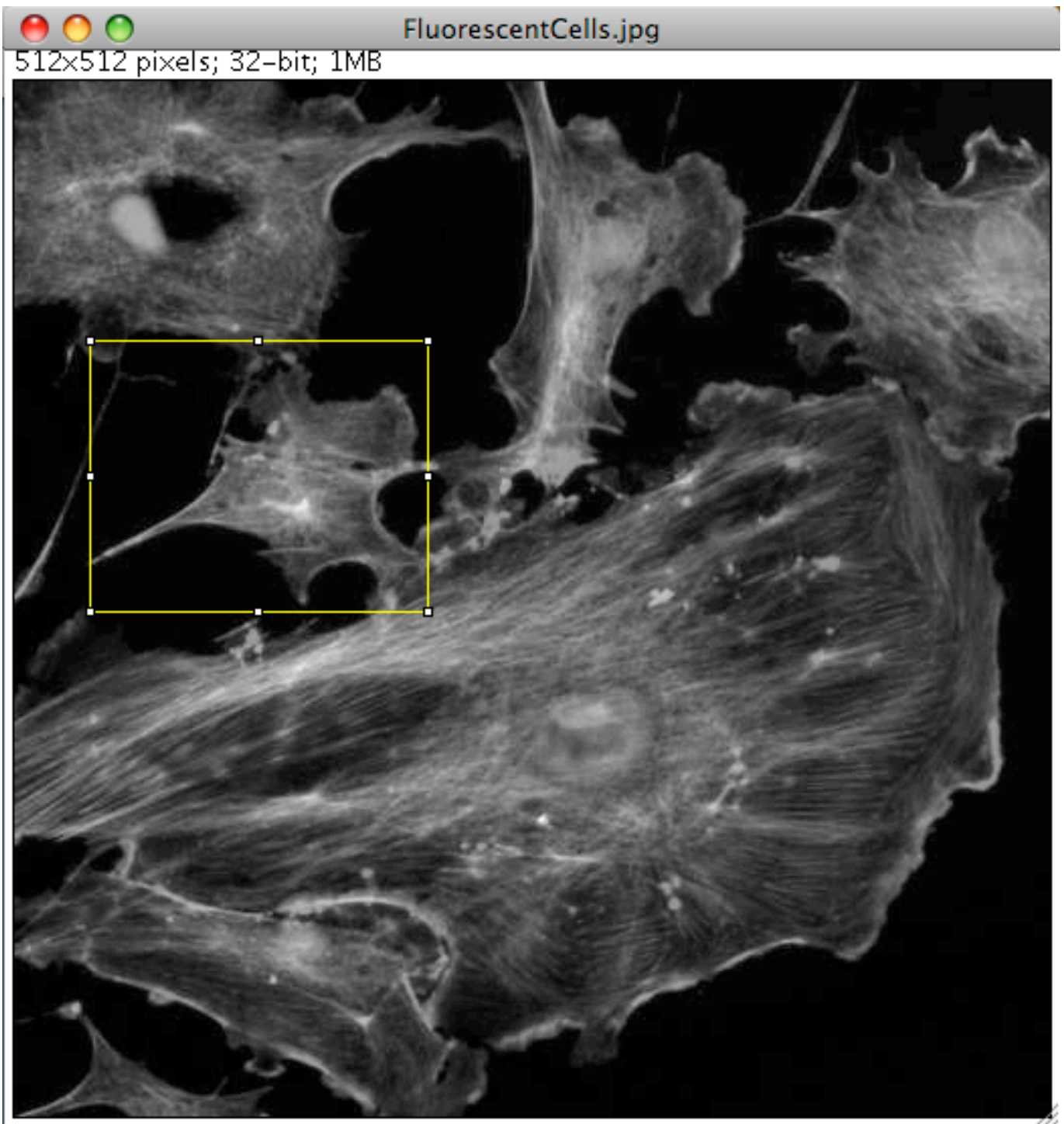
1. Collect Data using ImageJ.

Open images in ImageJ as usual.

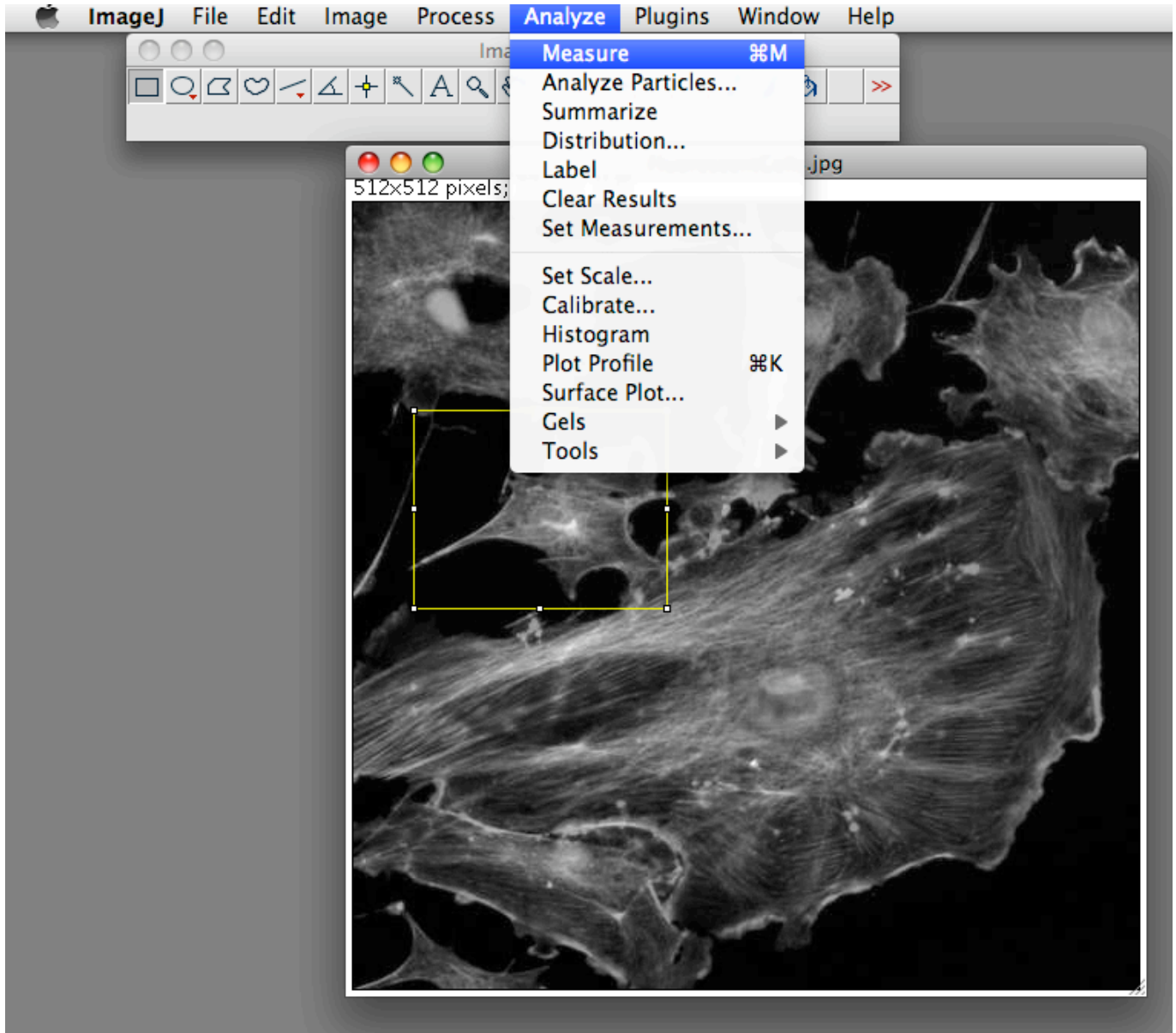
Under the menu **Analyze : Set Measurement...** Select Area, Mean Gray Value and Integrated Density



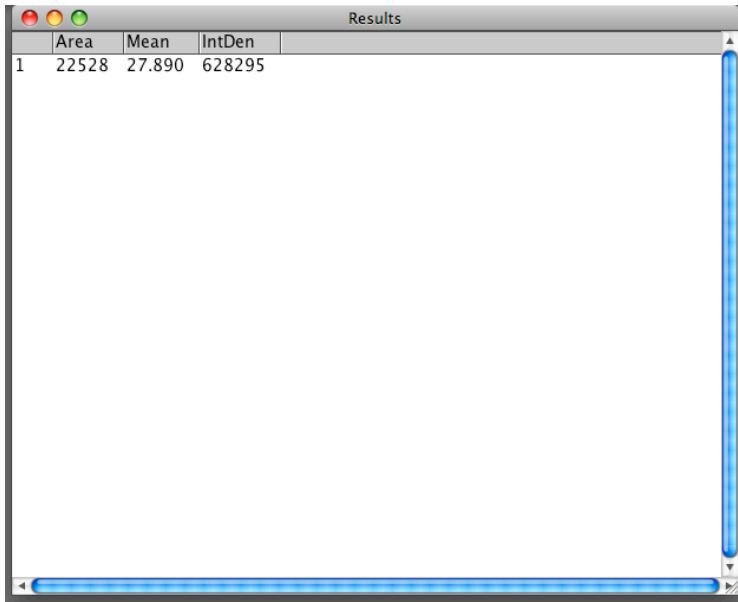
Draw a box around the one cell for which you want to know the fluorescence value. Note that ImageJ will let you adjust the size and position of the box after you create it, by clicking and dragging.



Do Analyze : Measure (Keyboard Shortcut Command-M), like this...



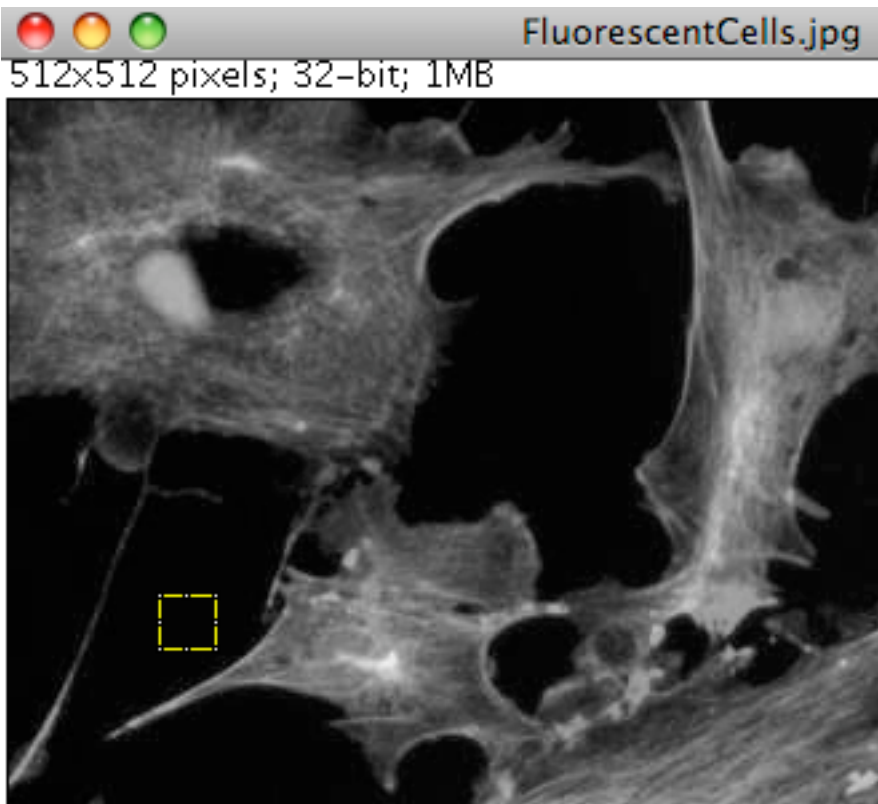
That creates the Results window...



The screenshot shows a window titled "Results" with a table containing the following data:

	Area	Mean	IntDen
1	22528	27.890	628295

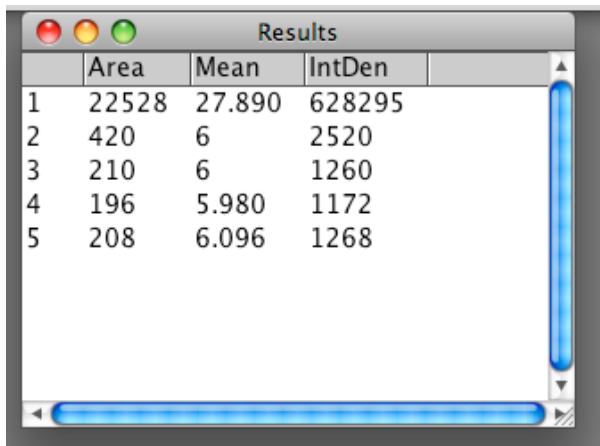
Draw a small box near your cell, which you think is a good representation of the background fluorescence. Such as this...



Do **Analyze : Measure (Command-M)**.

Repeat this for a few (from one to seven) small boxes around and near, but not on, the cell.

After doing four small boxes, the Results window looks like this.

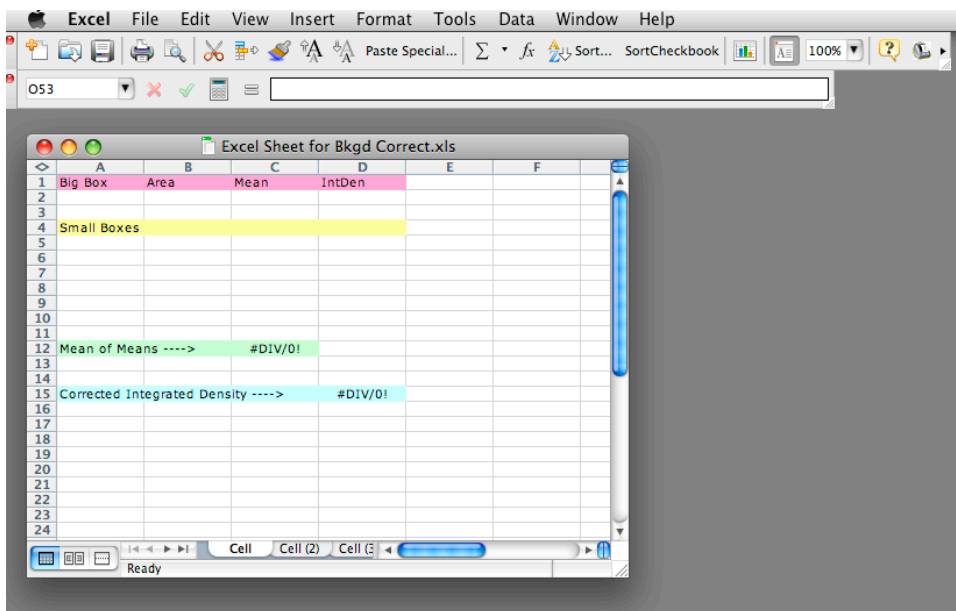


	Area	Mean	IntDen
1	22528	27.890	628295
2	420	6	2520
3	210	6	1260
4	196	5.980	1172
5	208	6.096	1268

Calculation of Corrected Integrated Density (i.e. the value you want - total fluorescence per cell).

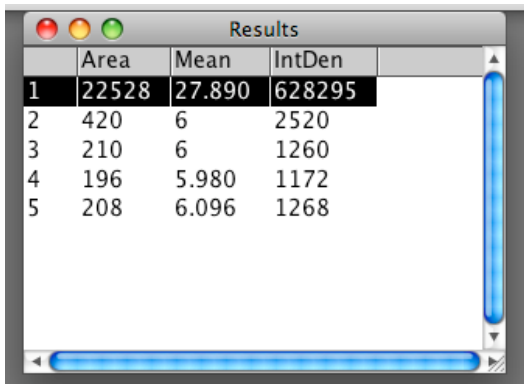
We are going to use Excel to average the values for the background and subtract that value from the value for the cell. To average the values, we want the “Mean” value for the small boxes, and we are going to average them (calculate the mean of the Means).

Open the file “Excel Sheet for Bkgd Correct.xls”



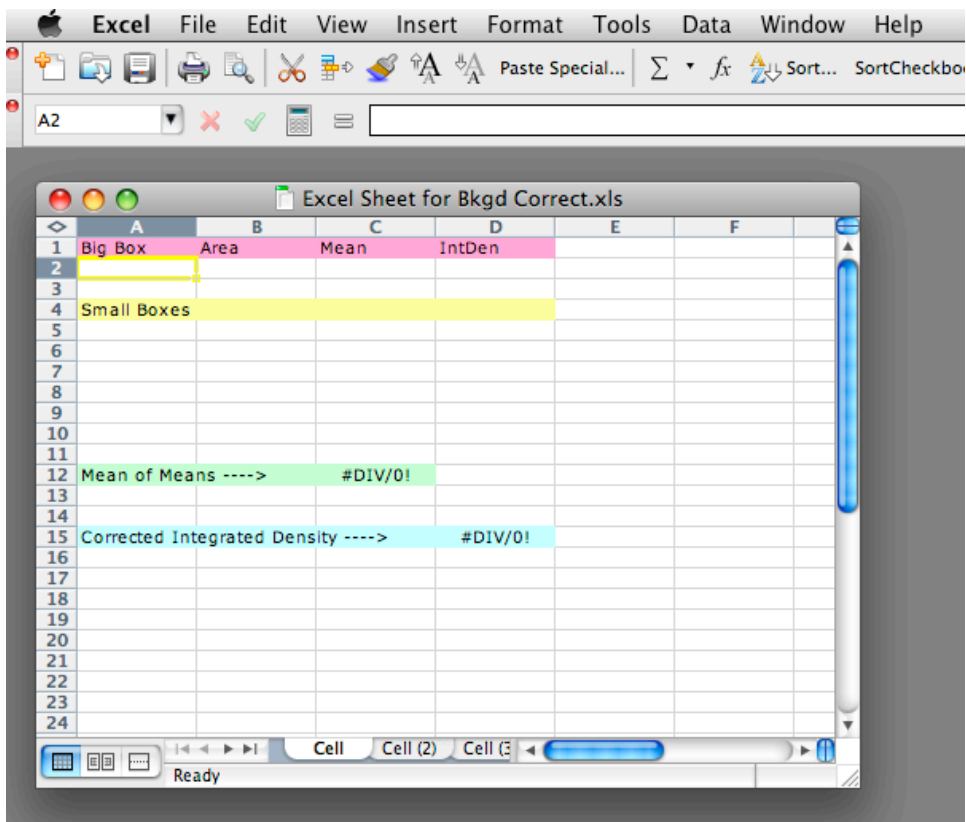
	A	B	C	D	E	F
1	Big Box	Area	Mean	IntDen		
2						
3						
4	Small Boxes					
5						
6						
7						
8						
9						
10						
11						
12	Mean of Means ---->		#DIV/0!			
13						
14						
15	Corrected Integrated Density ---->		#DIV/0!			
16						
17						
18						
19						
20						
21						
22						
23						
24						

In ImageJ, copy the first row of the Results window.



	Area	Mean	IntDen
1	22528	27.890	628295
2	420	6	2520
3	210	6	1260
4	196	5.980	1172
5	208	6.096	1268

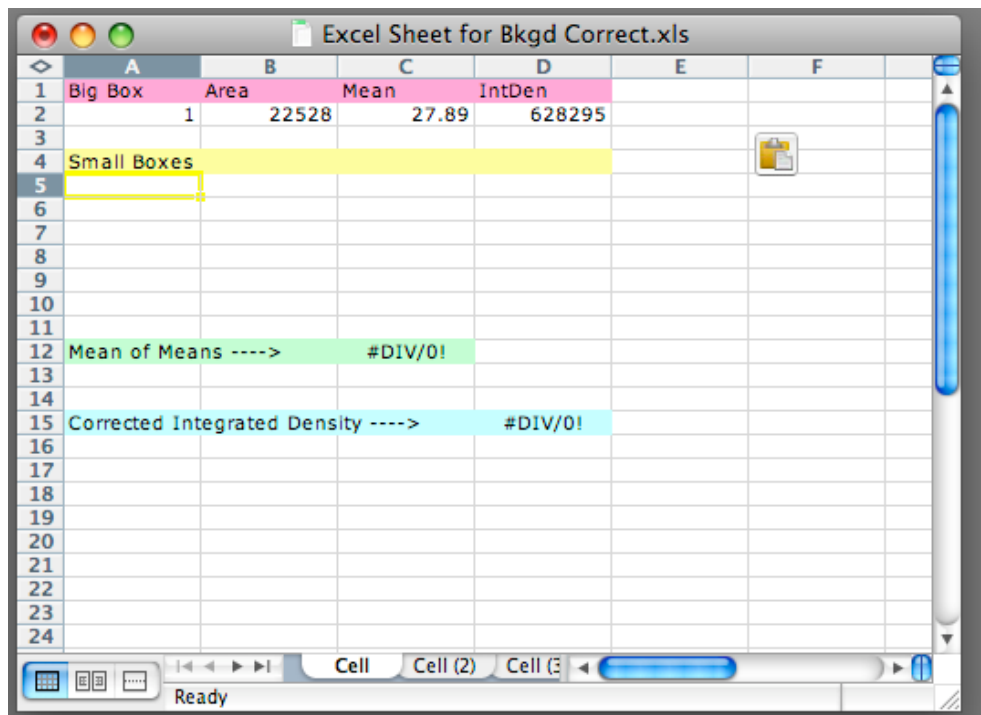
In Excel, click in cell A2



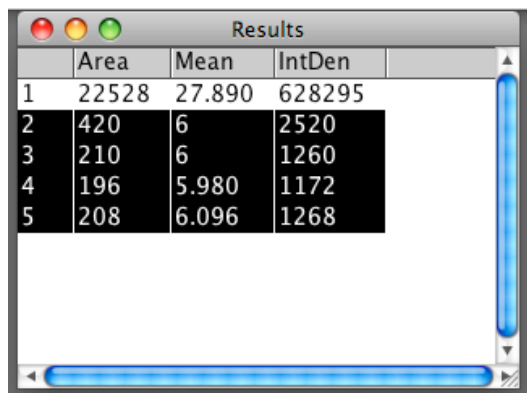
Excel Sheet for Bkgd Correct.xls

	A	B	C	D	E	F
1	Big Box	Area	Mean	IntDen		
2						
3						
4	Small Boxes					
5						
6						
7						
8						
9						
10						
11						
12	Mean of Means ---->		#DIV/0!			
13						
14						
15	Corrected Integrated Density ---->		#DIV/0!			
16						
17						
18						
19						
20						
21						
22						
23						
24						

Then Paste...



In ImageJ, copy the data for the small boxes, i.e. rows 2 and higher. You can have any number of rows from one to seven, but not zero and not eight. This example uses four.



In Excel, click in cell A5, then Paste.

	A	B	C	D	E	F
1	Big Box	Area	Mean	IntDen		
2	1	22528	27.89	628295		
3						
4	Small Boxes					
5	2	420	6	2520		
6	3	210	6	1260		
7	4	196	5.98	1172		
8	5	208	6.096	1268		
9						
10						
11						
12	Mean of Means ---->		6.019			
13						
14						
15	Corrected Integrated Density ---->		492699			
16						
17						
18						
19						
20						
21						
22						
23						
24						

The value you want is “Corrected Integrated Density” in cell D15.

The file contains several copies of the blank worksheet, fyi.

JAC, 5/26/08