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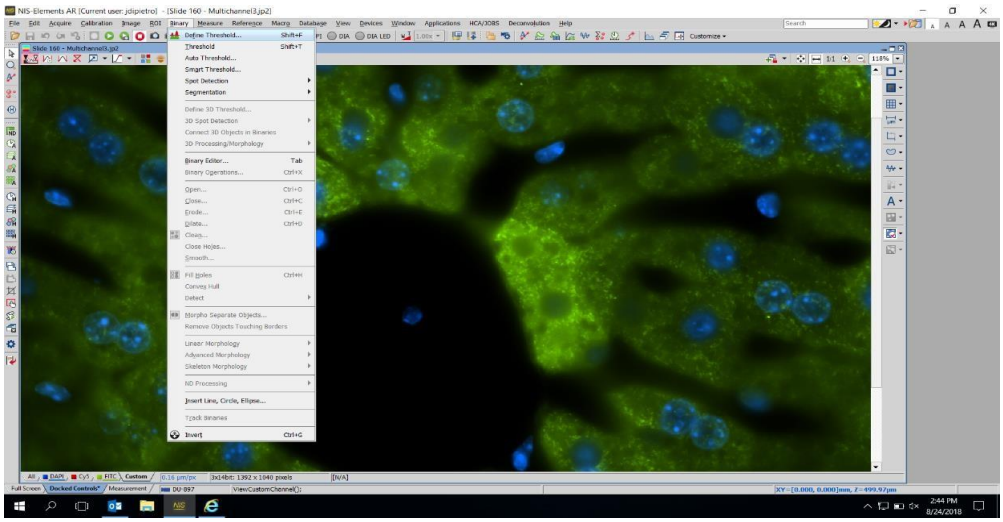
Make Binary & Intensity Measurements on Colocalized Channels

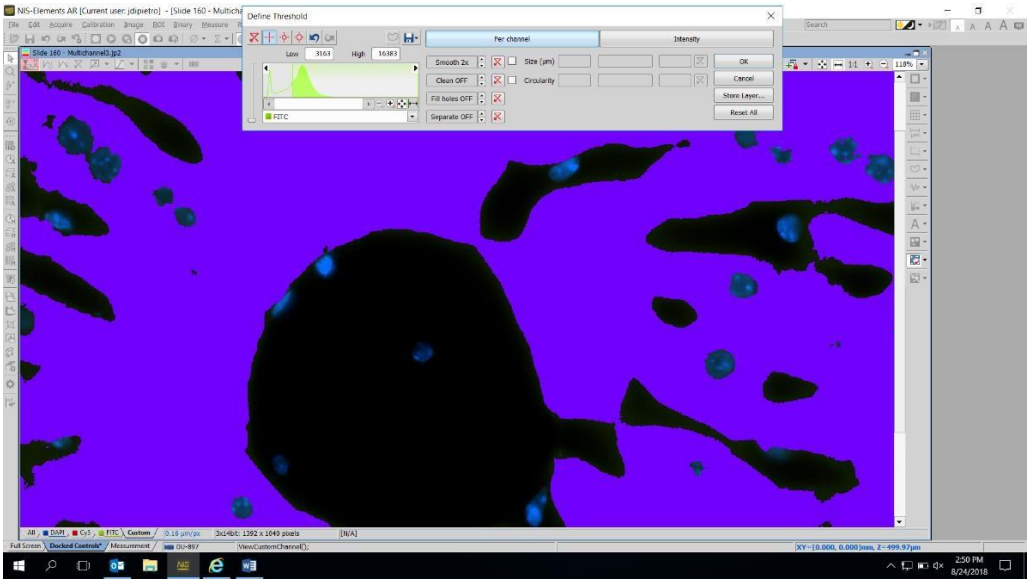
Nis-Elements Software

For the below example we will extract intensity values of the blue structures, but only those that are colocalized with the green channel:

1) Go to Binary->Define Threshhold



2) Select the “per channel ” option and select the first channel you wish to use by using the selector on the bottom left of the window. Use the slide bar and other tools to create a binary that covers the first channel (in this case, green). When satisfied, click OK.



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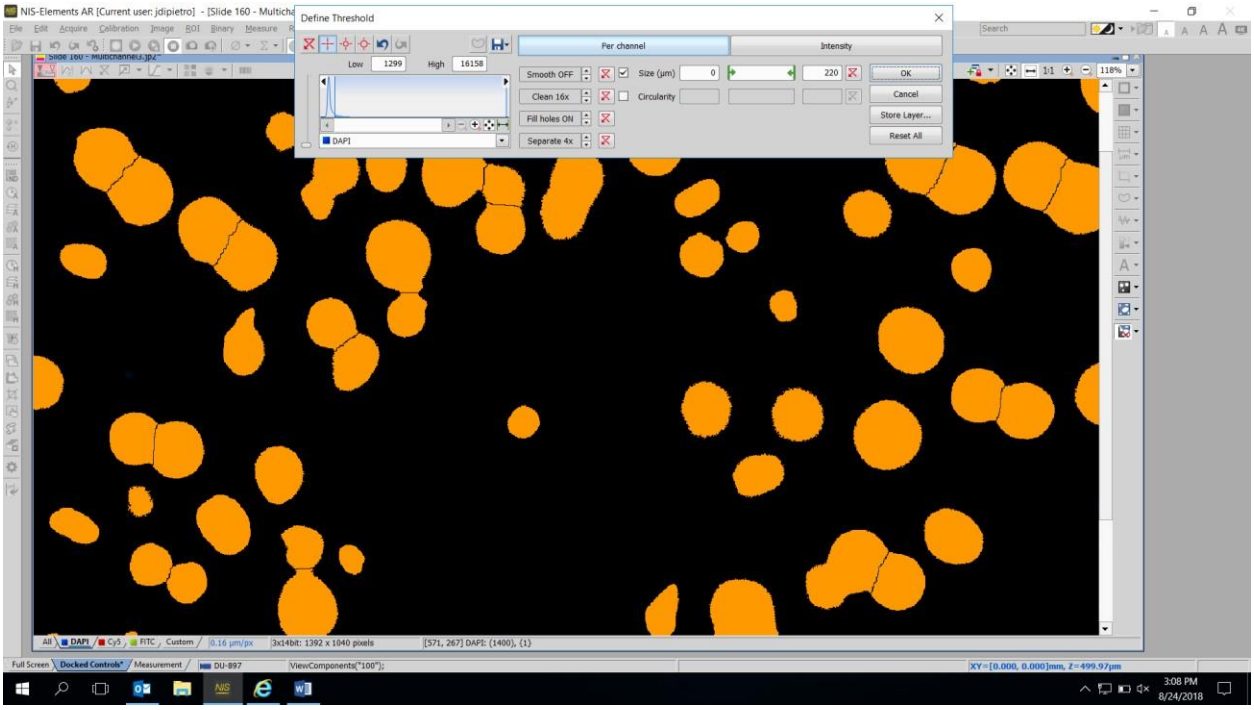
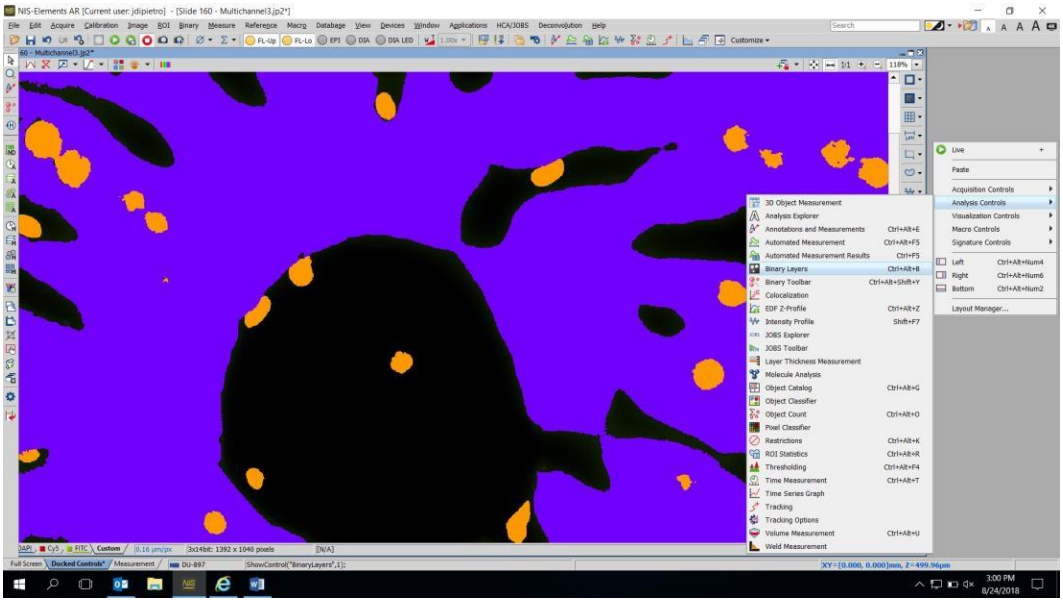
3) Repeat #2 with the second channel (the one you wish to make the measurement. You should now have 2 binaries, one for each channel



4) In an empty area in elements, right click and go to Analysis Controls->Binary Layers



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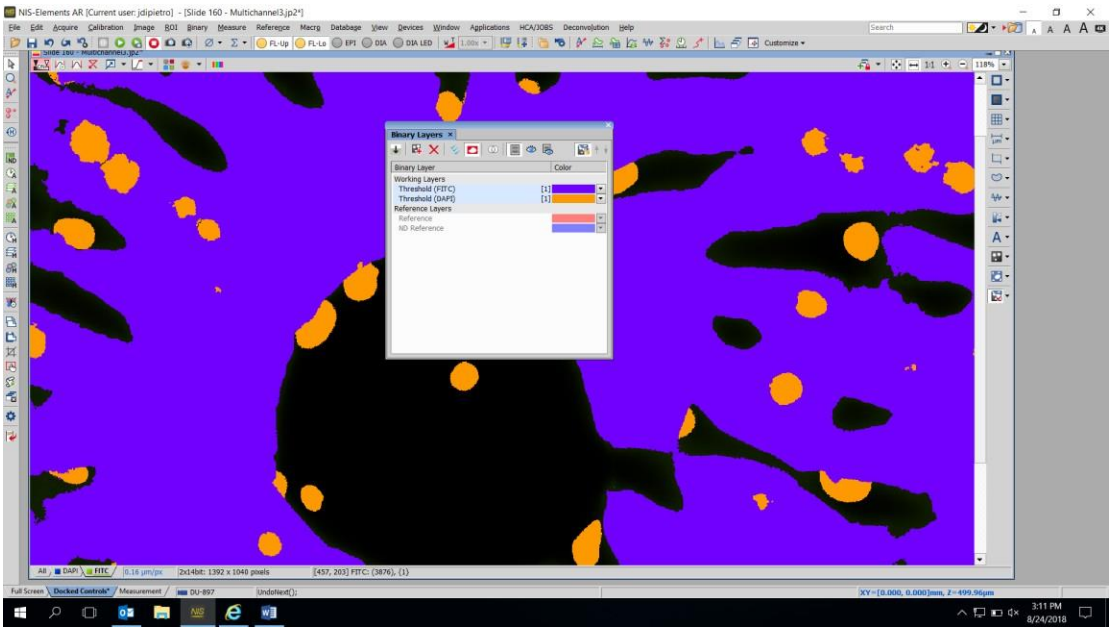
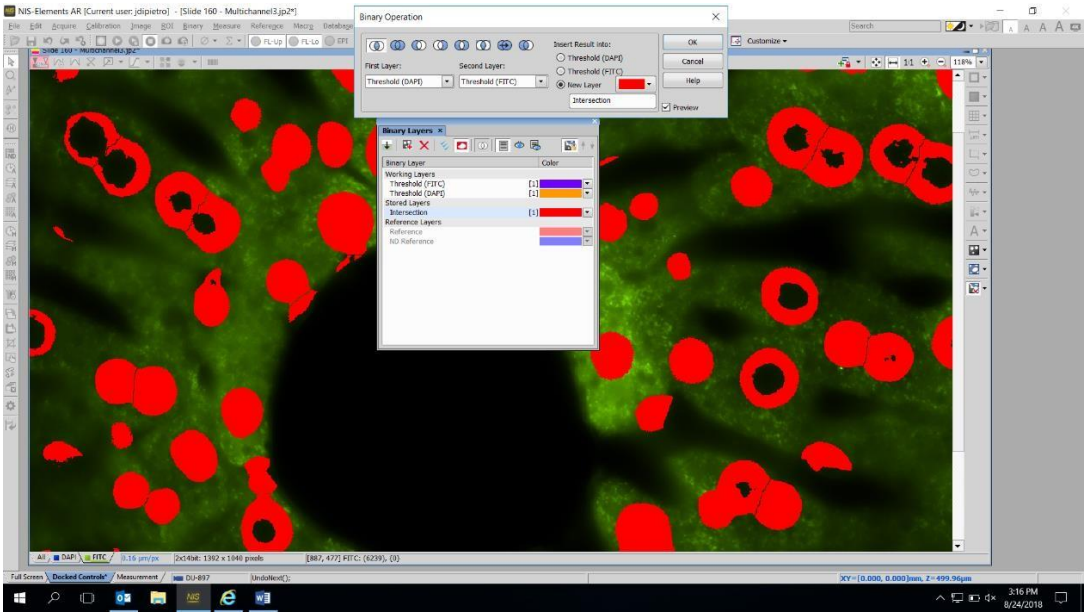
5) Select both binaries by holding SHIFT and click each so they are both highlighted. Then click the "Binary Options Dialog button in the top center of the Binary Layers window (looks like 2 overlapping circles)



6) Make the binary associated with the channel you with to measure (in this case, blue) the first layer and the other only the second layer. Then select the AND operation and click OK.



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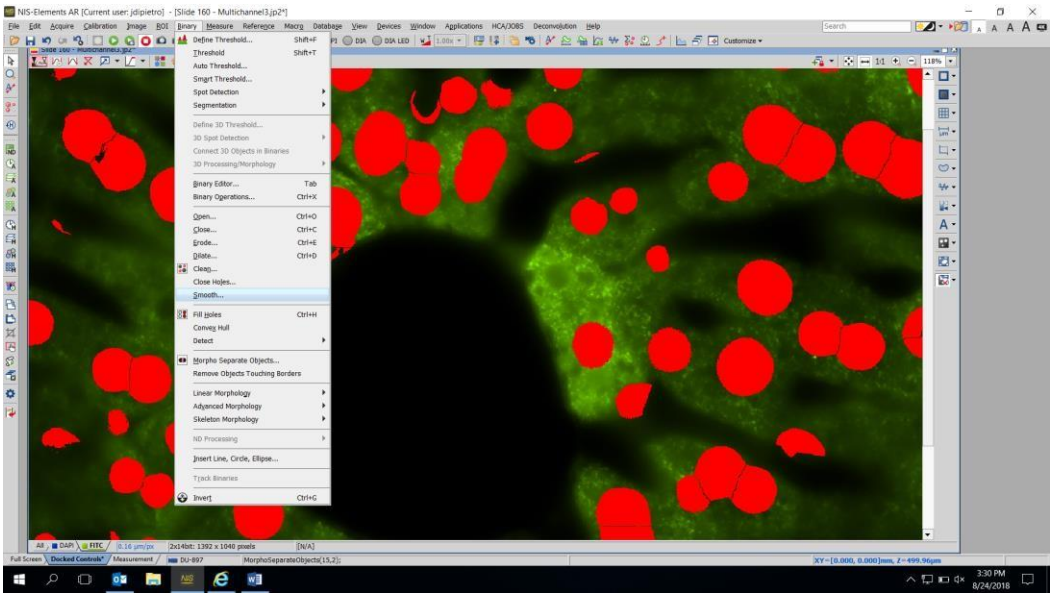
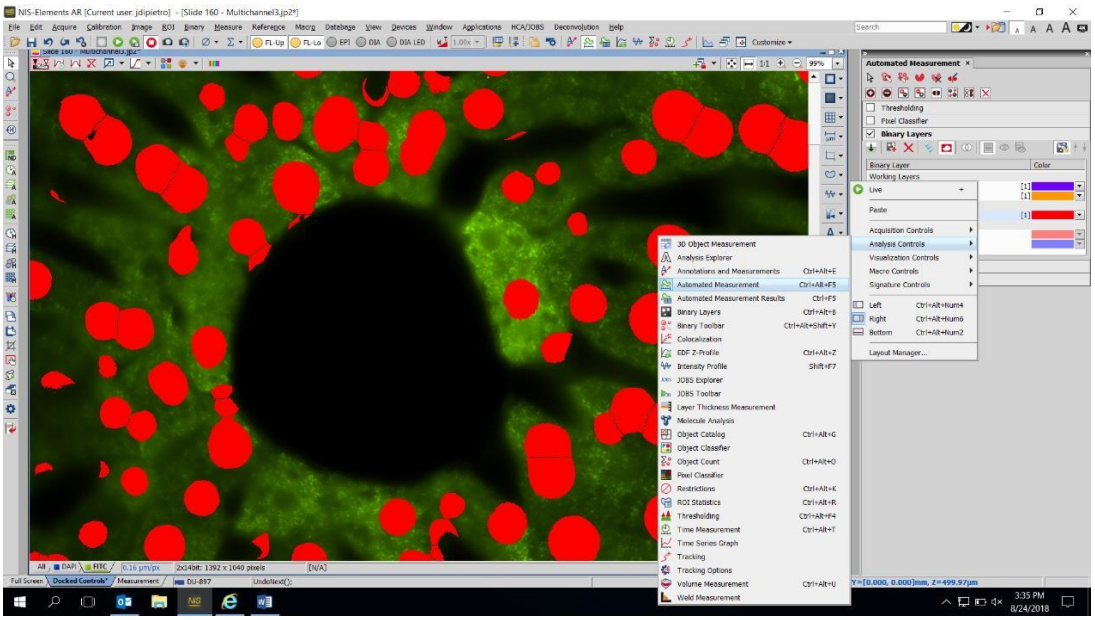
7) You now have a binary that represents the overlap…you can use tools in the binary drop down menu to clean up this resulting binary…(NOTE!!!: making/editing binaries takes some practice. Do not be afraid to play around for a while to figure out the best combination of parameters to make a good binary) the better your first 2 binaries are, the better your overlap binary will be and the less work you will have to do to touch it up).



8) Right click on an empty area and select Analysis-> Automated Measurement



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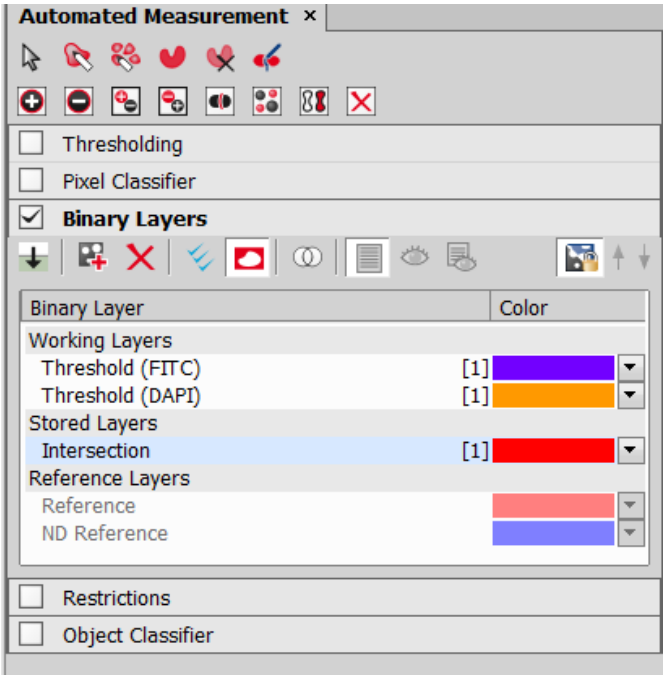
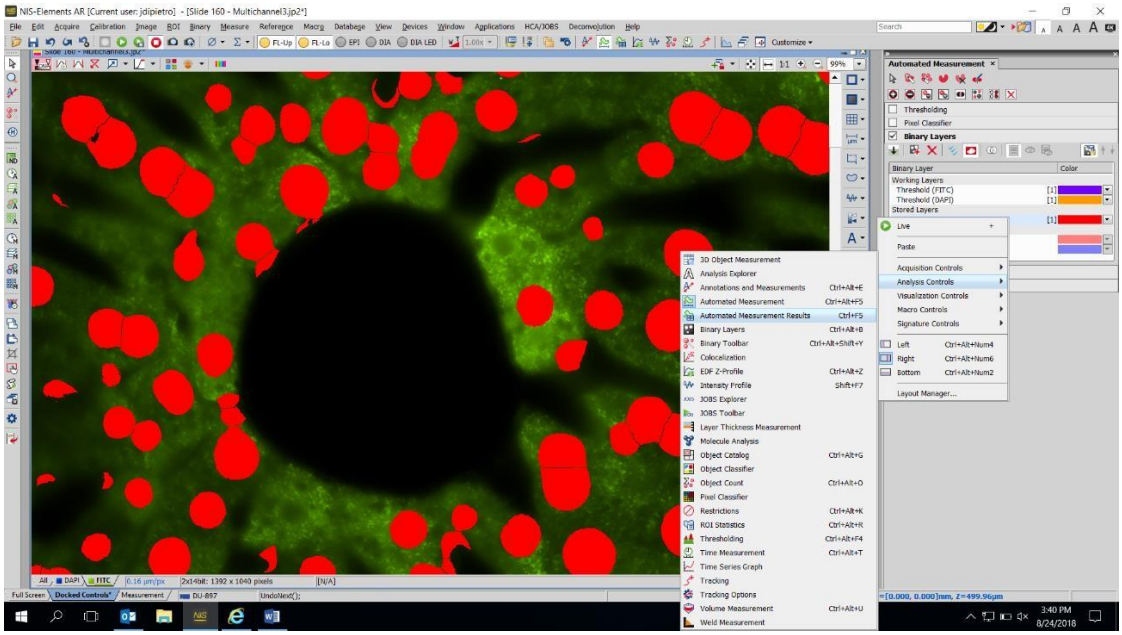
9) Check the box for binary layers ONLY. Then highlight the Intersection layer by clicking on it.



10) Right click on an empty area and select Analysis-> Automated Measurement Results



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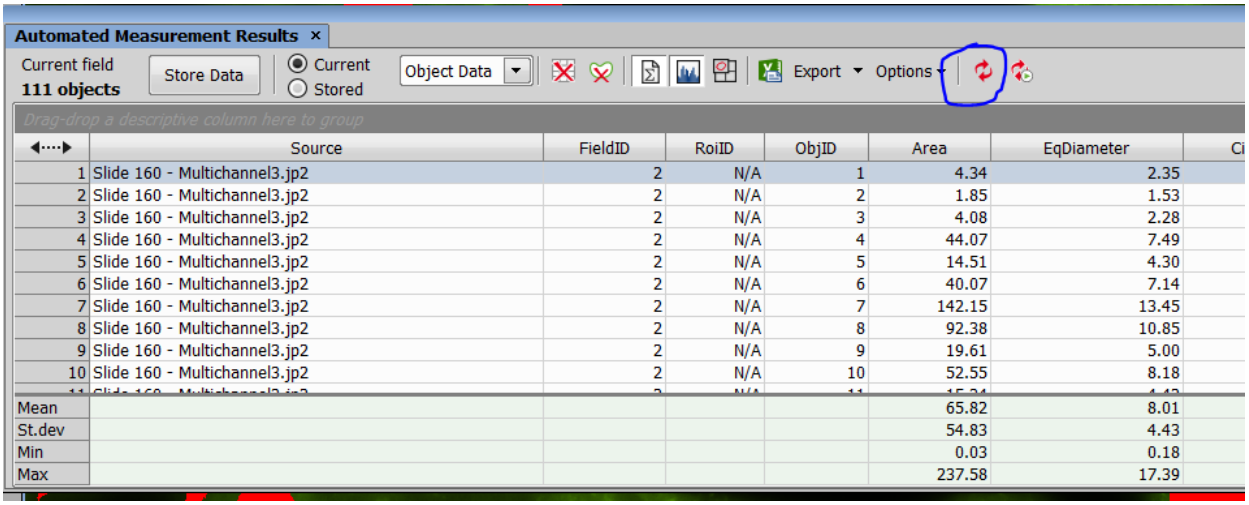
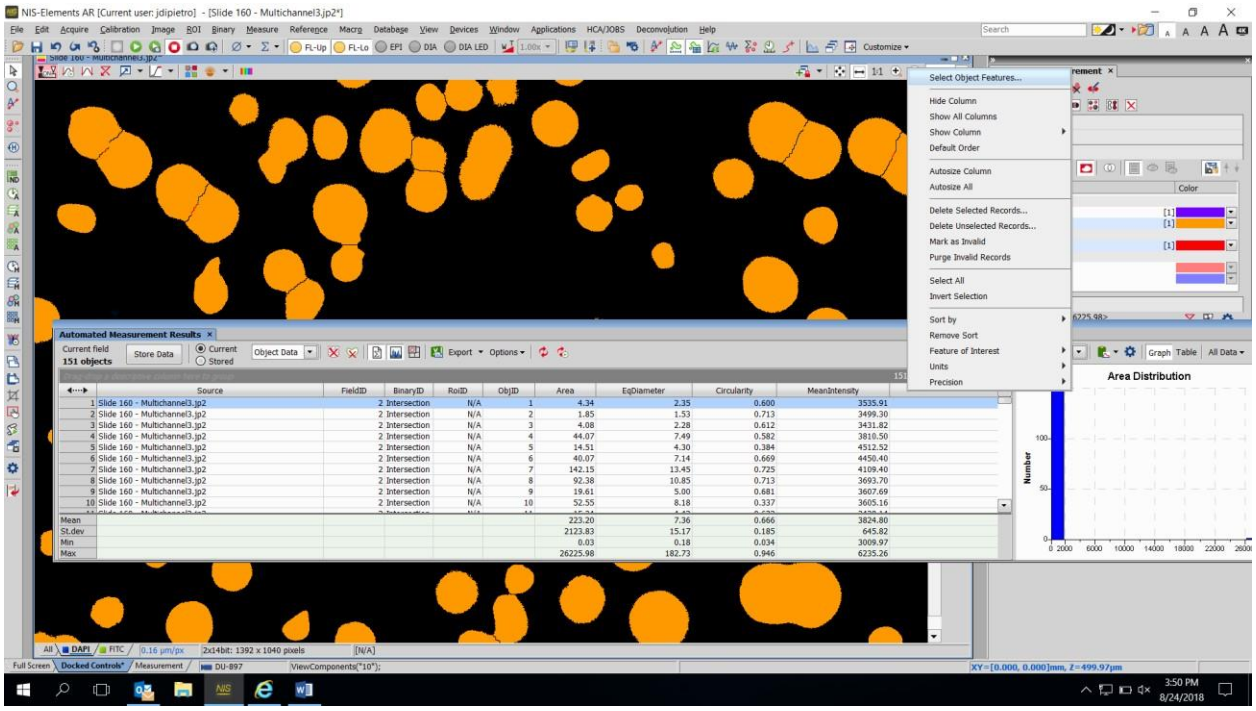
11) Make sure the tab for the channel you are trying to measure is selected (if All tab is selected, you will get measurements for both channels combined). In the Automated Measurements Results Window, click the Update Measurement button (circled in blue below). This will make measurements for the selected channel.



	Source	FieldID	RoiID	ObjID	Area	EqDiameter
1	Slide 160 - Multichannel3.jp2	2	N/A	1	4.34	2.35
2	Slide 160 - Multichannel3.jp2	2	N/A	2	1.85	1.53
3	Slide 160 - Multichannel3.jp2	2	N/A	3	4.08	2.28
4	Slide 160 - Multichannel3.jp2	2	N/A	4	44.07	7.49
5	Slide 160 - Multichannel3.jp2	2	N/A	5	14.51	4.30
6	Slide 160 - Multichannel3.jp2	2	N/A	6	40.07	7.14
7	Slide 160 - Multichannel3.jp2	2	N/A	7	142.15	13.45
8	Slide 160 - Multichannel3.jp2	2	N/A	8	92.38	10.85
9	Slide 160 - Multichannel3.jp2	2	N/A	9	19.61	5.00
10	Slide 160 - Multichannel3.jp2	2	N/A	10	52.55	8.18
Mean					65.82	8.01
St.dev					54.83	4.43
Min					0.03	0.18
Max					237.58	17.39

12) Right click on an empty column and choose “Select Object Features”.



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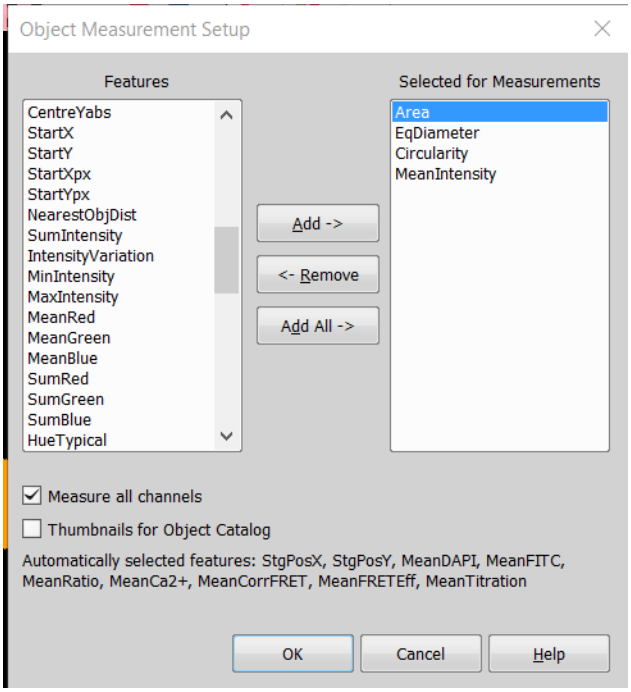
13) Use the window to add the features (such as mean intensity or maxIntensity) that you would like to measure. Then click OK.



YOU CAN NOW EXPORT THE TABLE INTO EXCEL CONTAINING THE DATA FOR EACH OBJECT!!!