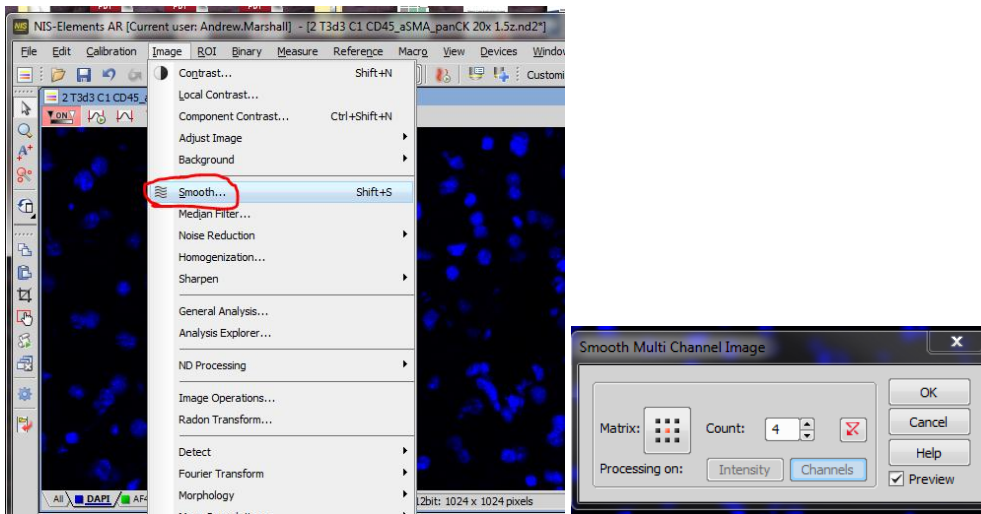
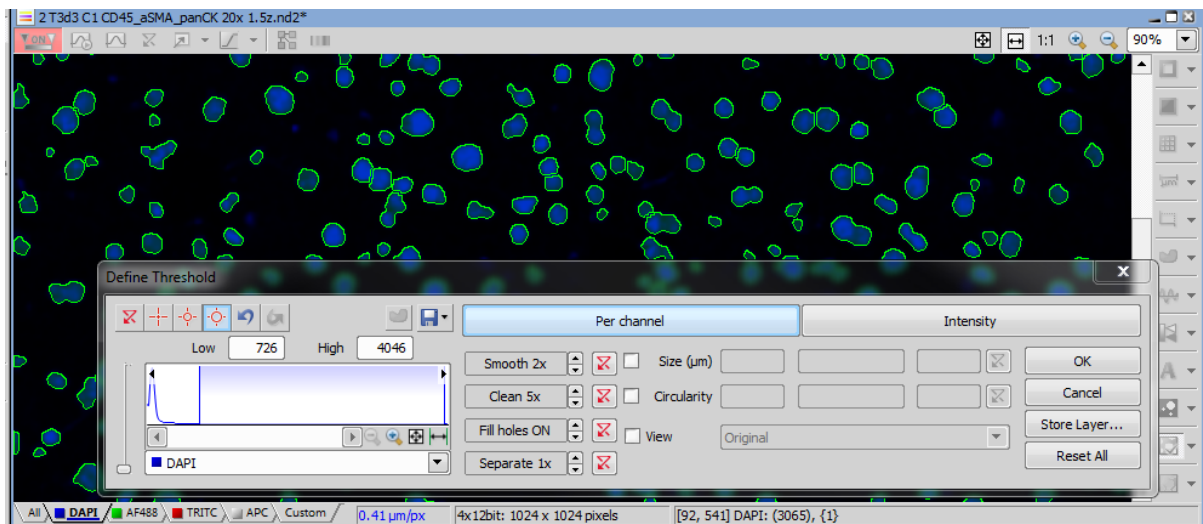


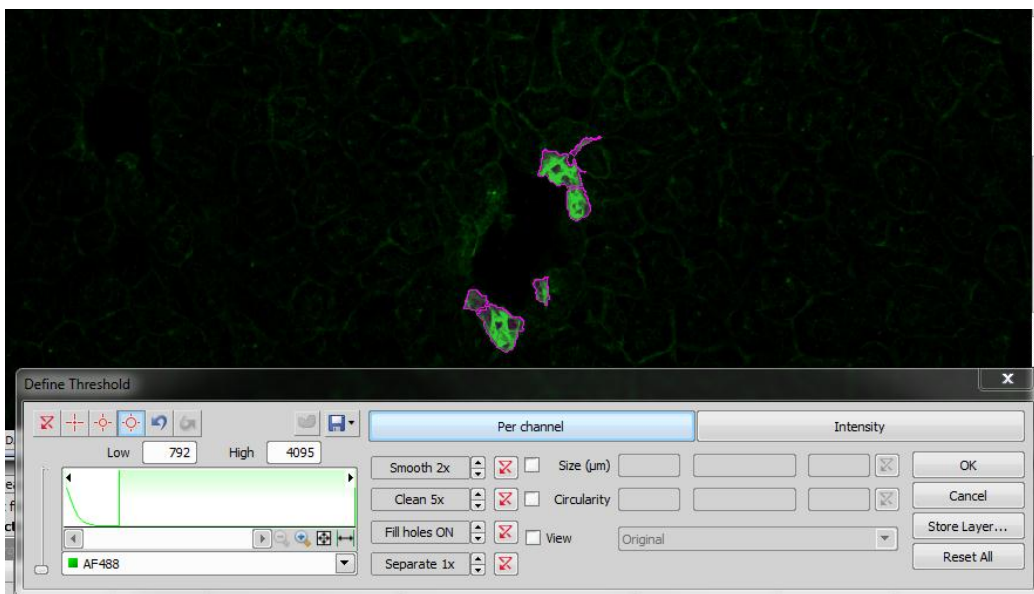
1. Firstly the image of DAPI needs some pre-processing so I used a smoothing filter




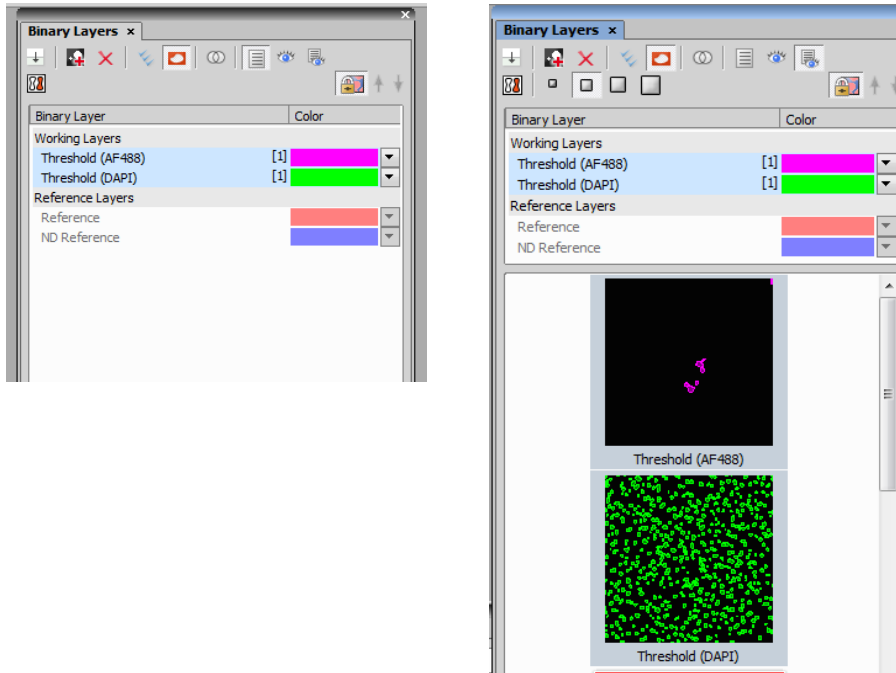
2. Then define the threshold for DAPI




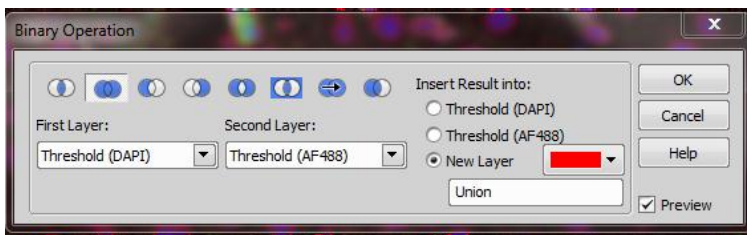
3. Then define the threshold for AF488



4. Open the binary layer GUI, you can display the layers by selecting this button 

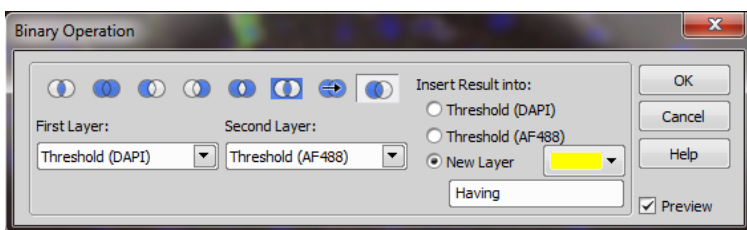


5. Select the binary operations button  to bring up the following

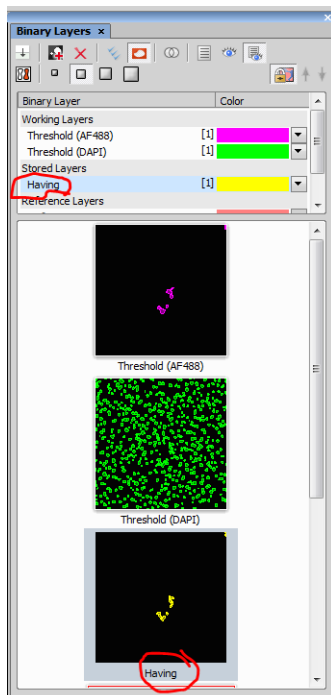


6. Select the button for HAVING 

7. Then you want to have first layer as DAPI and second layer as AF88. That way the equation is show all DAPI having AF488 in it. And make sure it is a new layer



8. You should be able to see it in the binary layer GUI



9. Highlight the HAVING binary layer and in Automated measurements update the result for the Object data to get the number of DAPI objecting having AF488. Here you can see 11 objects as the result.

The screenshot shows the main software interface. The top part is a microscopy image with blue (DAPI) and green (AF488) channels. The 'Binary Layers' window is open on the right, showing the 'Having' layer highlighted. Below the image is the 'Automated Measurement Results' window. The 'Object Data' dropdown menu is circled in red, and the '11 objects' count is also circled in red. The table below shows the results of the automated measurements.

Source	FieldID	ObjID	Area	EqDiameter	Circularity	MeanIntensity
1 2 T3d3 C1 CD...	1	1	10.78	3.70	0.835	927.59
2 2 T3d3 C1 CD...	1	2	26.86	5.85	0.826	1171.61
3 2 T3d3 C1 CD...	1	3	26.86	5.85	0.906	556.94
4 2 T3d3 C1 CD...	1	4	85.19	10.41	0.795	832.95
5 2 T3d3 C1 CD...	1	5	68.60	9.35	0.700	1278.55
6 2 T3d3 C1 CD...	1	6	48.24	7.84	0.954	1369.56
7 2 T3d3 C1 CD...	1	7	35.41	6.71	0.961	514.21
8 2 T3d3 C1 CD...	1	8	65.69	9.15	0.845	807.24
9 2 T3d3 C1 CD...	1	9	37.46	6.91	0.852	1153.64
10 2 T3d3 C1 CD...	1	10	100.41	11.31	0.613	1198.60
11 2 T3d3 C1 CD...	1	11	104.18	11.52	0.722	1311.96
Mean			55.42	8.05	0.819	1011.17
St.dev			30.10	2.39	0.102	286.87
Min			10.78	3.70	0.613	514.21
Max			104.18	11.52	0.961	1369.56