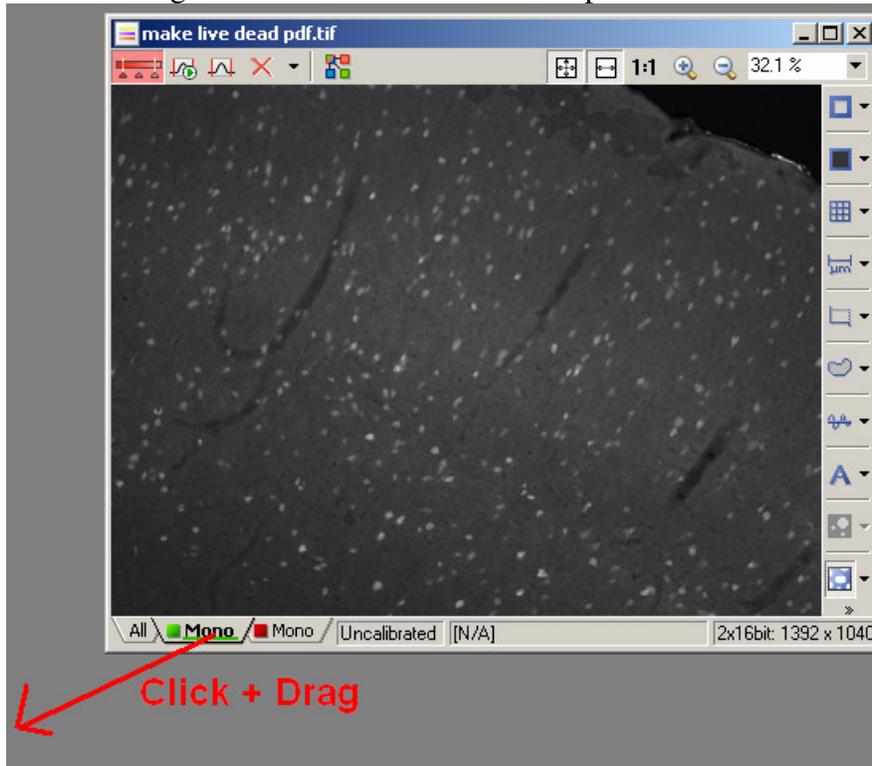Colocalization of Green / Red fluorescence

1. Acquire two image channels (Green and Red).

2. If channels are combined into one image, click+drag each channel into a
    new image. You should now have two separate monochrome images.


3. Select the Green channel. Click the Binary menu and click "Define
    Threshold".

4. Set the threshold for the green channel so that only cell signal is colored. Close the threshold window when finished.
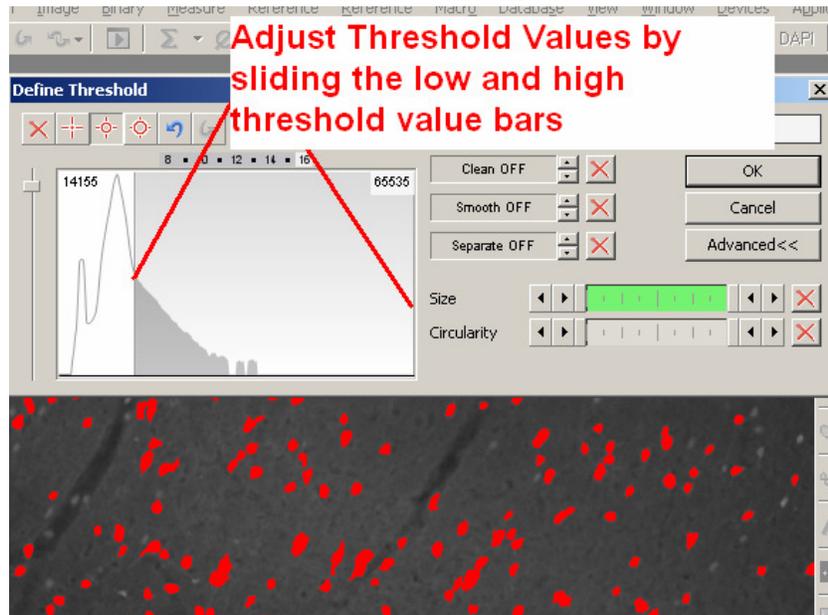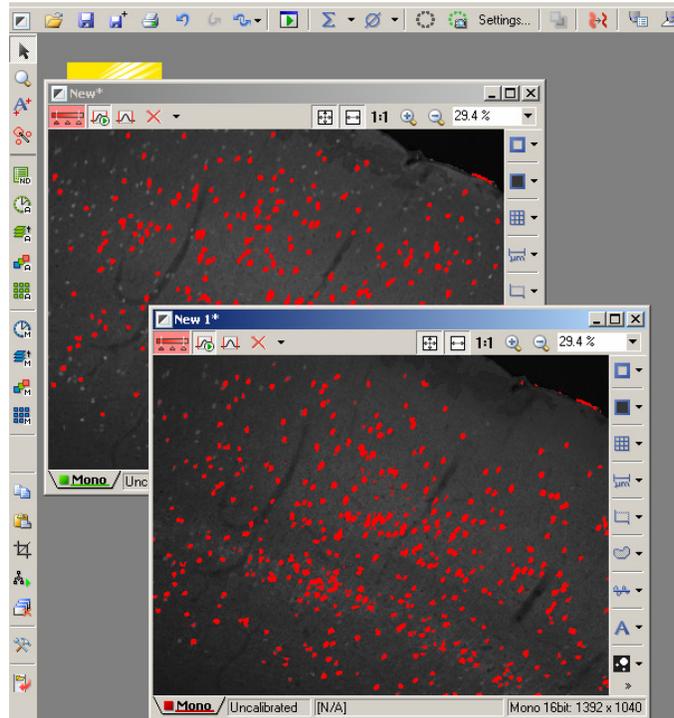

5. Under the "Reference" Menu select "Binary -> RefBinary"
    i. This operation takes the current binary layer and saves it in memory as a "reference". We can later access this reference for image to image arithmetic.
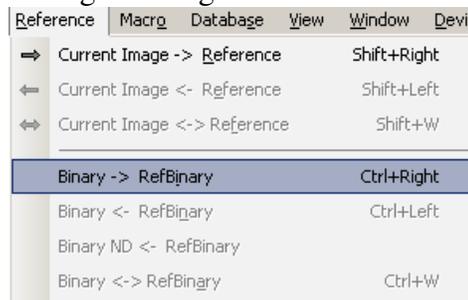

6. Select the Red channel image.
7. Again select the Binary menu, then "Define Threshold". Repeating steps 3 and 4 above using the red channel instead of green.

8. You should now have 2 images with thresholds on them as in the screen shot below:


9. Under the reference menu, select "Binary Operations". In the new window that appears select the "(A) AND (B)" operation icon as seen in the screenshot below.
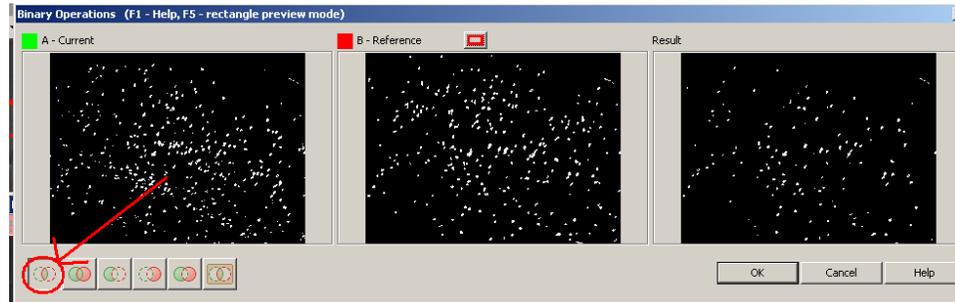
10. Press OK to create the result binary. You now have a binary overlay that only exists where green channel and red channel signal overlap.
11. To confirm that the result binary is highlighting the correct cells we can make a visual layer. To do this:

12. Select the arrow next to the "Binary Layers" button on the right side of the image. Click "Copy Binary".
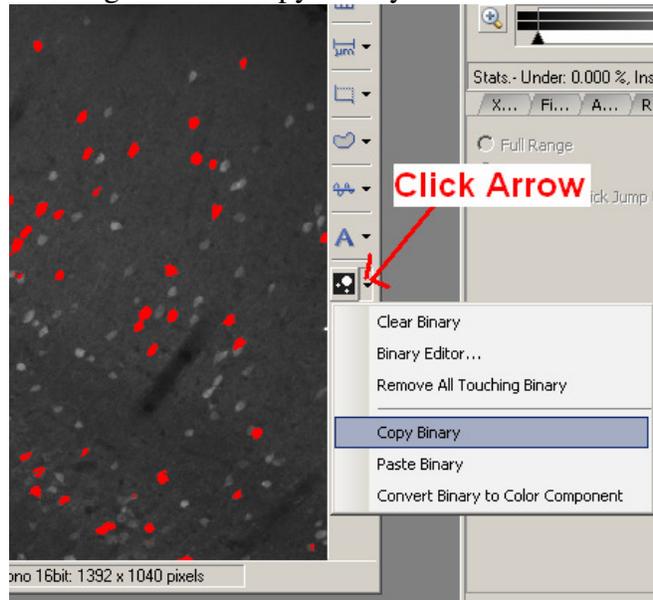

13. Re-Merge the green channel into the red channel. Click + Drag the green channel tab into the red image. You will now have the combined image we started with.
14. Under the Edit menu, select "Paste as New Image". This will paste the binary layer into a new image.
15. We want to overlay the binary on top of the green and red channels, but we don't want the intensity of the binary image to overwhelm the green and red colors. We need to reduce the brightness of the binary layer. Under the Image Menu, select "Convert" then "Binary to Color".

16. Next find the bit depth of your original image. It will either be 14 bit or 16 bit. The bit depth can be found in the lower right side of the original image.

17. Once you know the bit depth of the original image, select the Image menu, then "Convert", then "Change Color Depth". Select the bit depth of your original image and click OK.
18. To reduce the intensity of the binary image we need to subtract some of the brightness. To do this select the Image Menu, then "Adjust Image", then "Intensity Transformation".
19. In the new window that appears select the operation "Subtract" and enter a value that reduces the brightness of the binary image. 14 bit images will need to subtract ~ 8,000. 16 bit images will need ~30,000. Click OK to complete the operation.
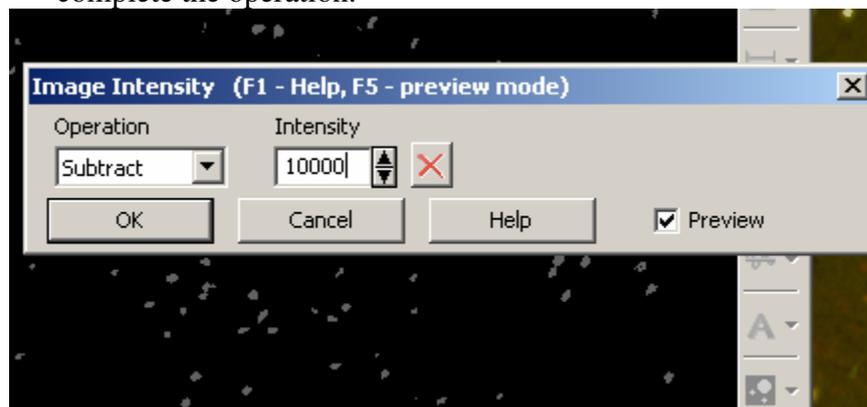
20. The last step is to click the "mono" tab at the bottom right side of the binary layer, and click+drag that tab into the original image. You will now have a 3-channel image that you can view while controlling the brightness of the "colocalized" layer. Adjust the colocalized layer brightness using the LUT tool.

Result Example