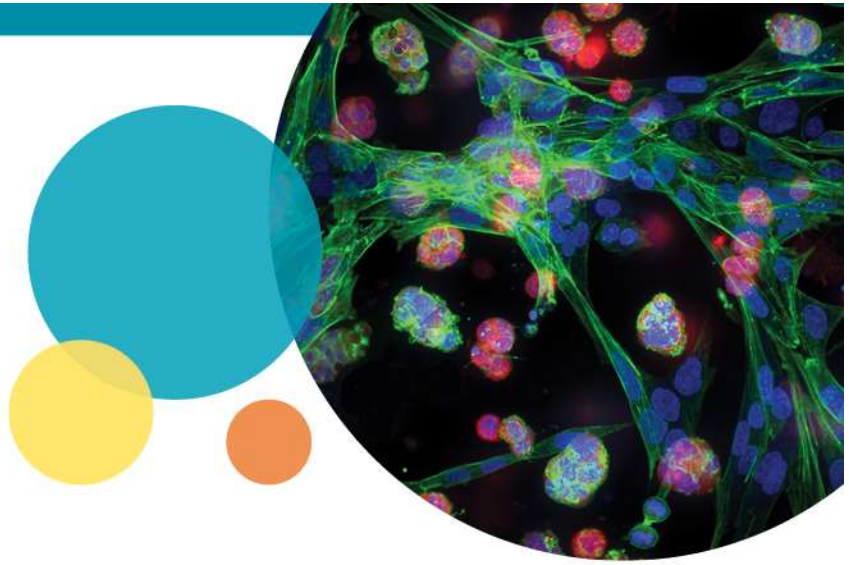

MetaXpress Custom Module Editor

Background Subtraction for Complex Images

Rev A 2018-08-21

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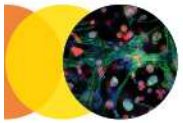
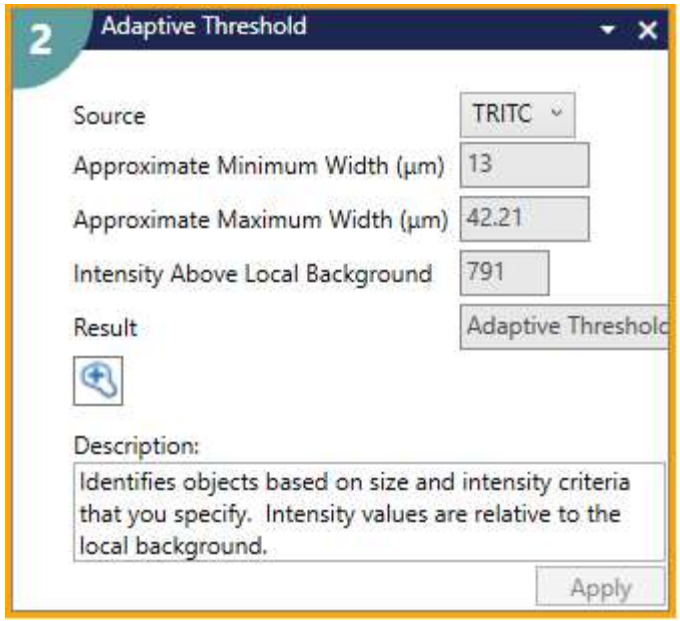
Chapter Purpose

- This guide explains a method for doing background subtraction within the Custom Module Editor. This method is suitable for more complex images/objects, such as cytoplasmic or mitochondrial stains where cells are touching one another.
- The resulting object intensities will have the background subtracted. This may help in comparing results from plate to plate, where the fluorescent staining may vary.



Step 1: Find Objects > Adaptive Threshold

- Use the Click to Find tool to define settings.
- This should find all objects, even irregularly-shaped ones.



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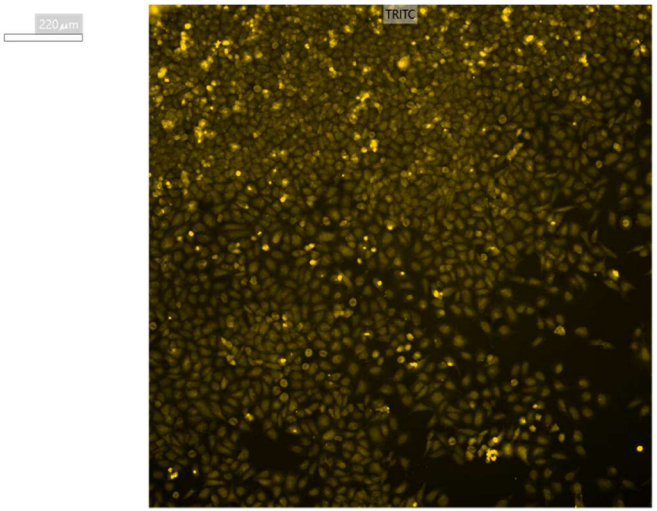
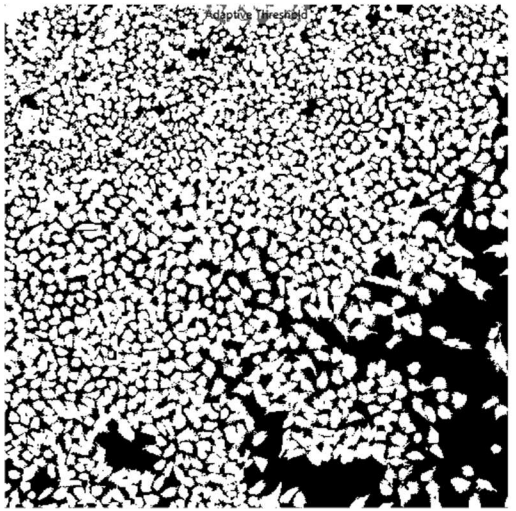
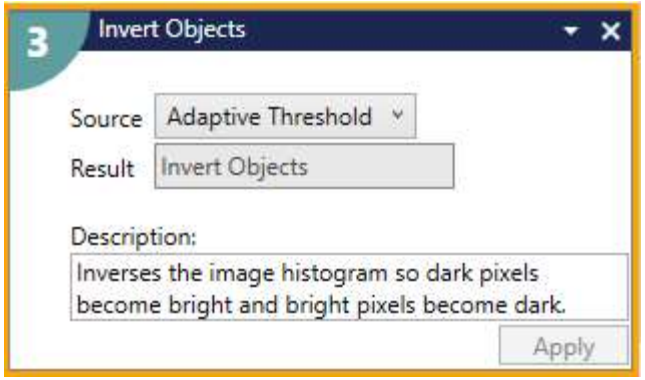




Step 2: Modify Objects > Invert Objects

- Invert the objects mask, so that all of the background area is selected.



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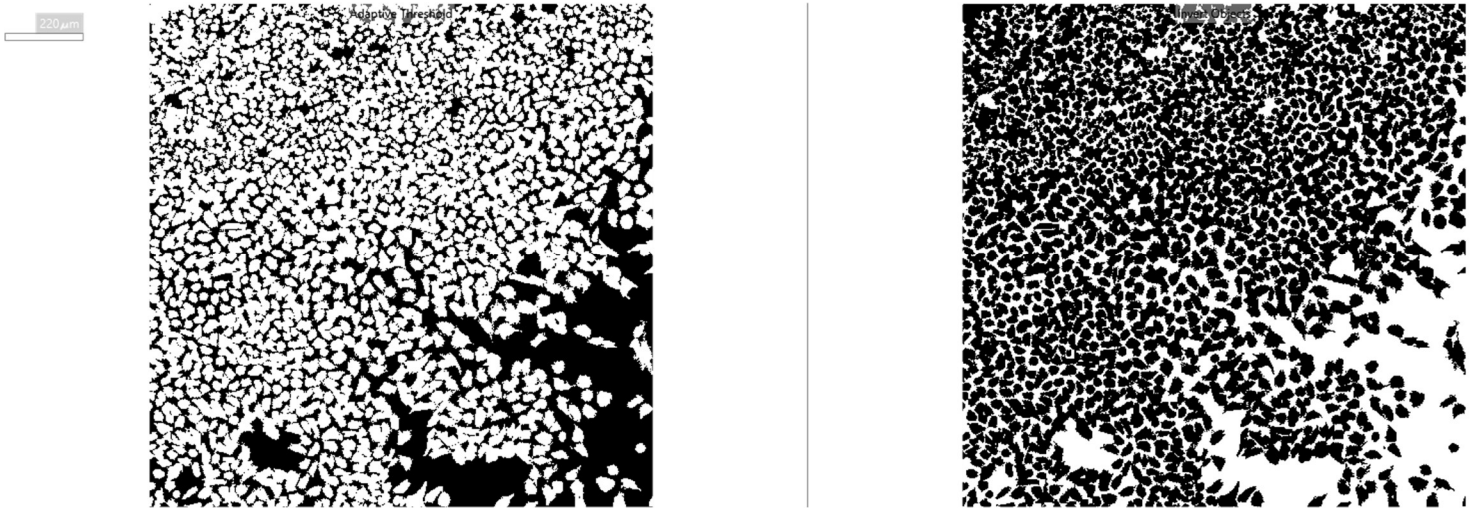
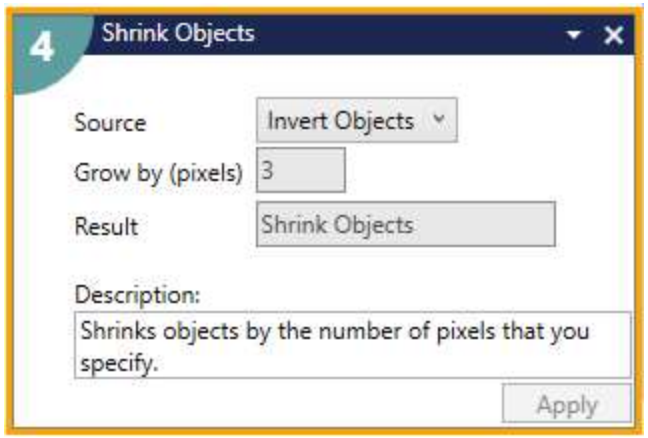


Step 3: Modify Objects > Shrink Objects

- Shrink the background area by a few pixels, to avoid edge effects.



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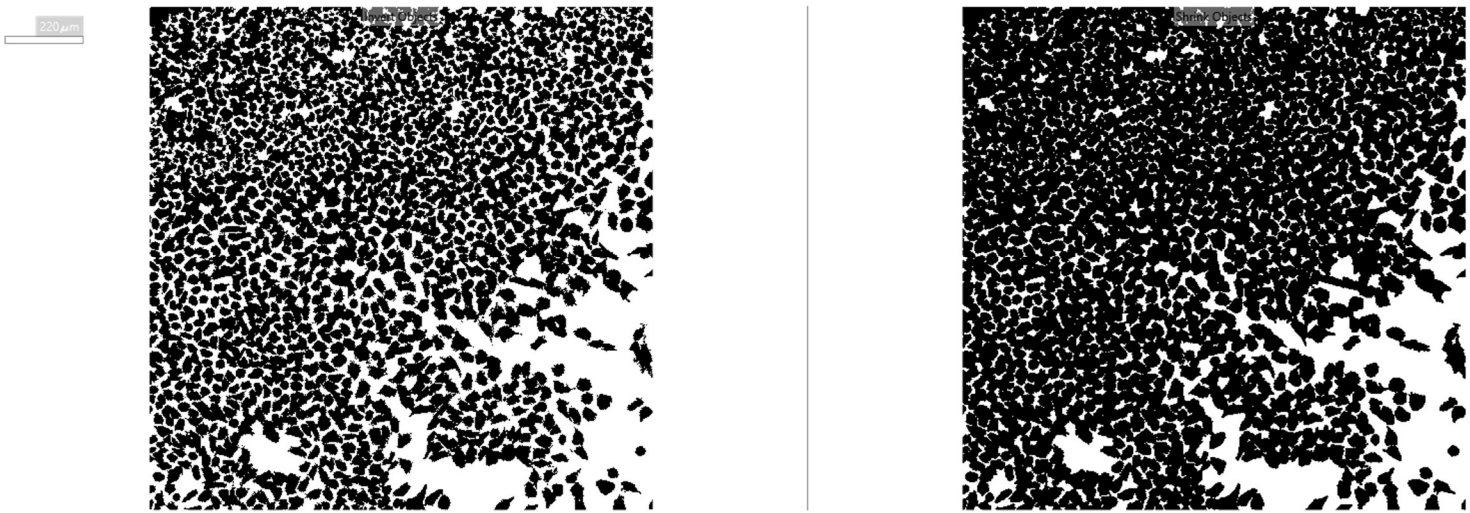
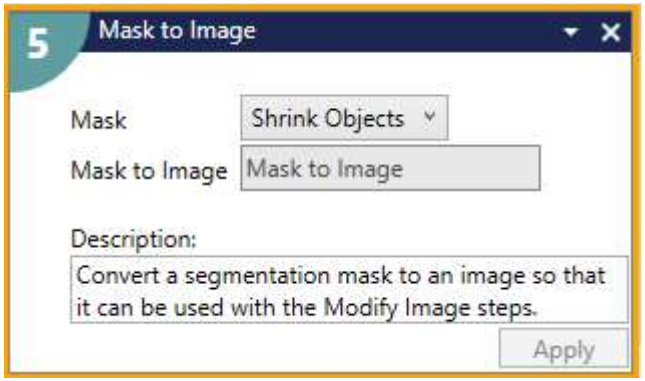


Step 4: Modify Image > Mask to Image

- Convert the Shrink Objects from a binary mask to a 16-bit image so that it can be used for further image calculations



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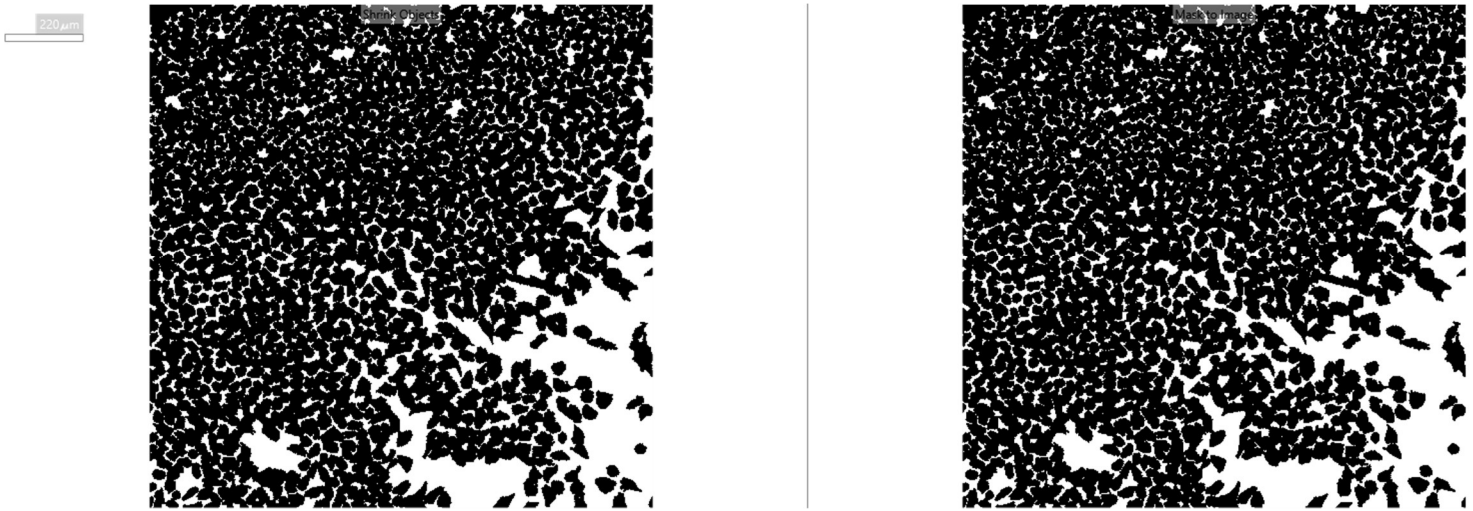
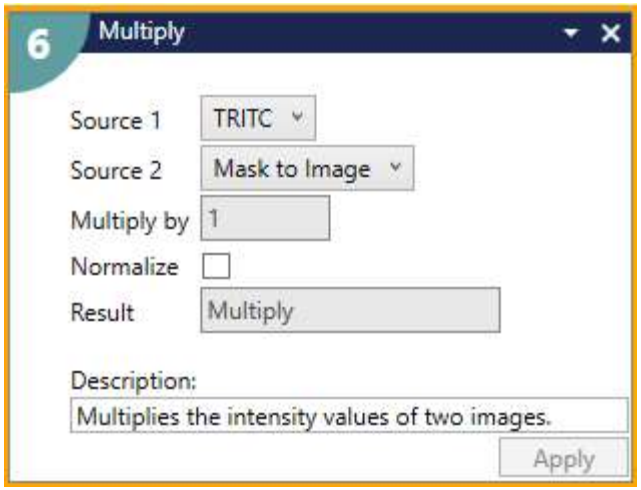


Step 5: Modify Image > Multiply

- Multiply the original image by the Mask to Image. The background pixels are kept, and the object pixels are changed to zero.



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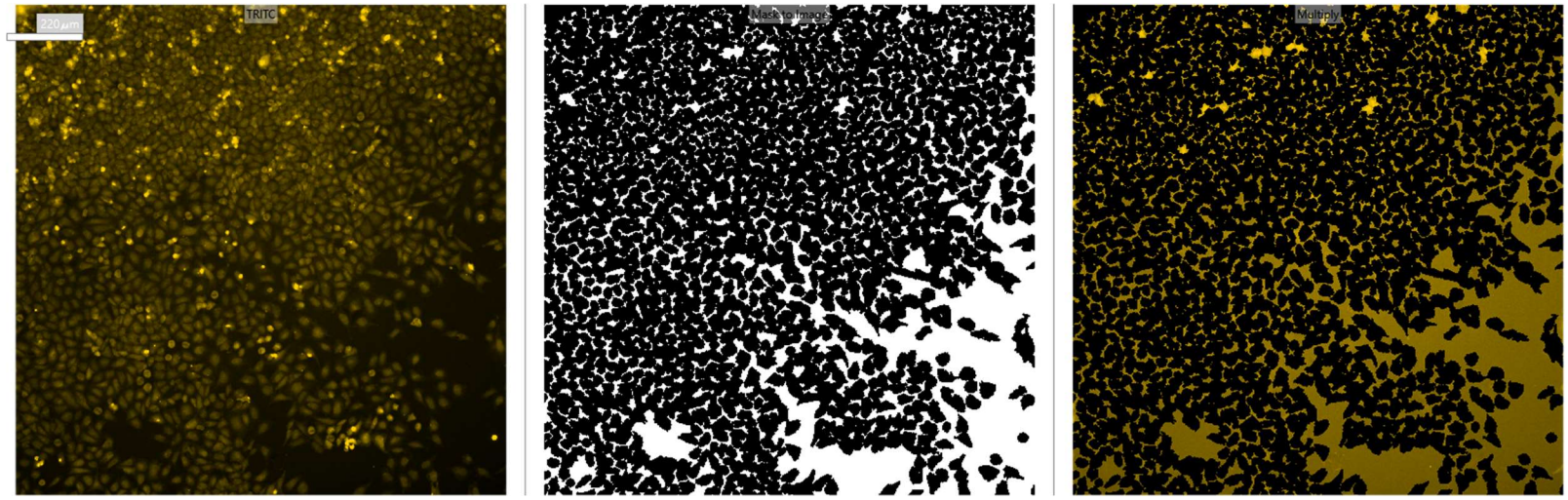
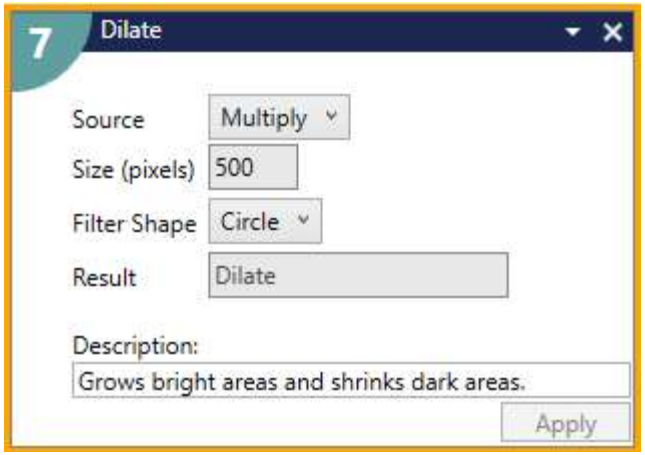


Step 6: Modify Image > Dilate

- Dilate the Multiply result by a large number of pixels, so that the background area is expanded to cover the object area.



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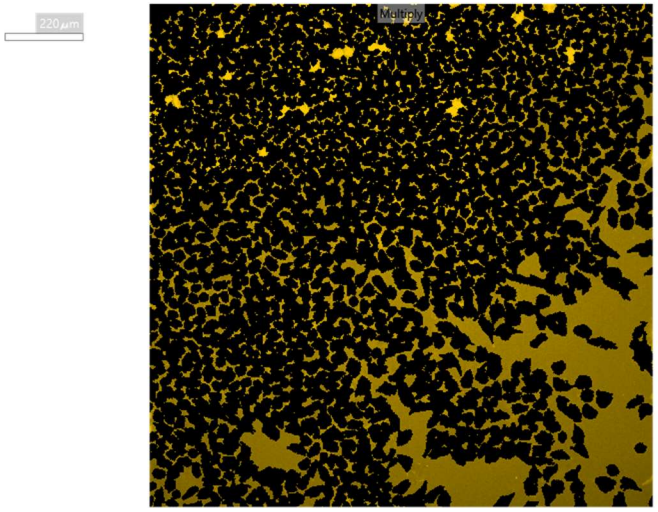
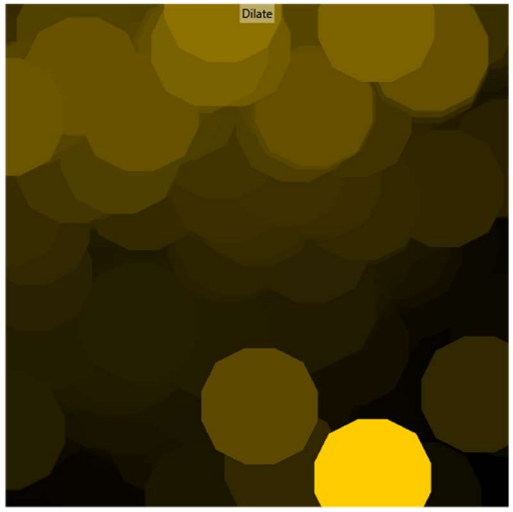
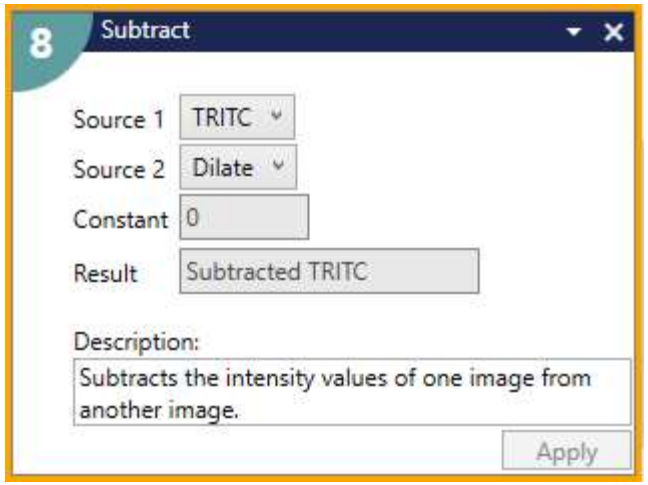




Step 7: Modify Image > Subtract

- Subtract the Dilate result from the original image.
- The resulting image will have a background at or very close too zero.



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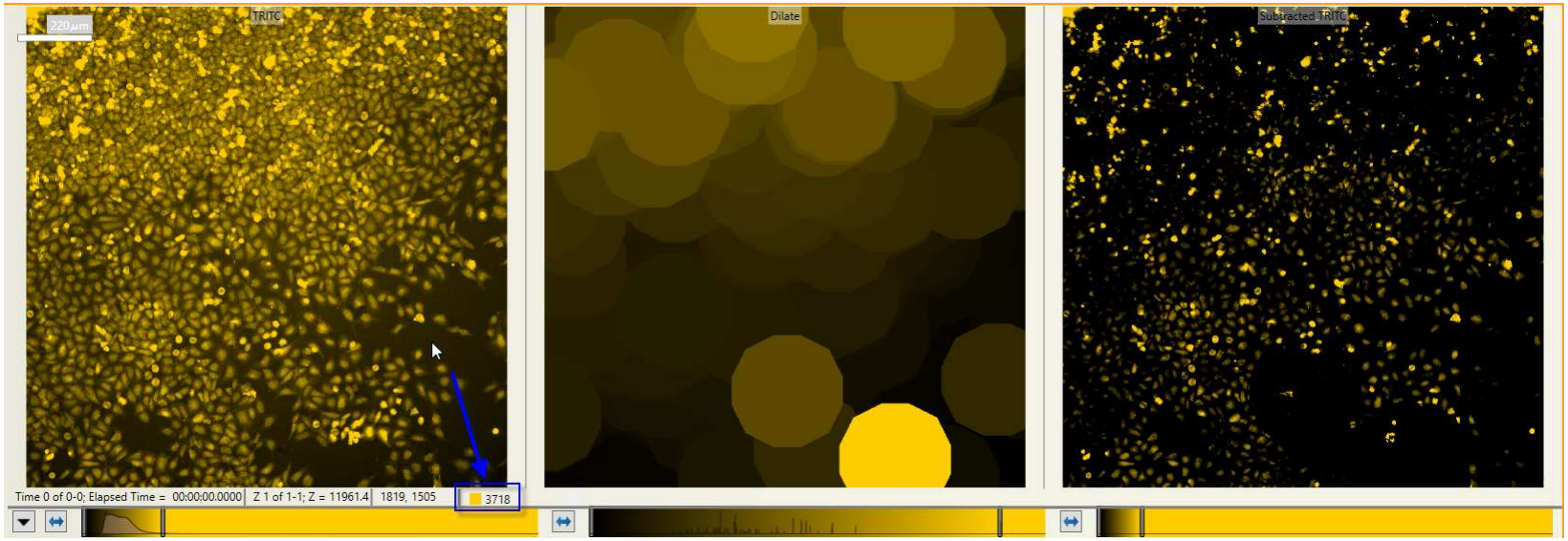

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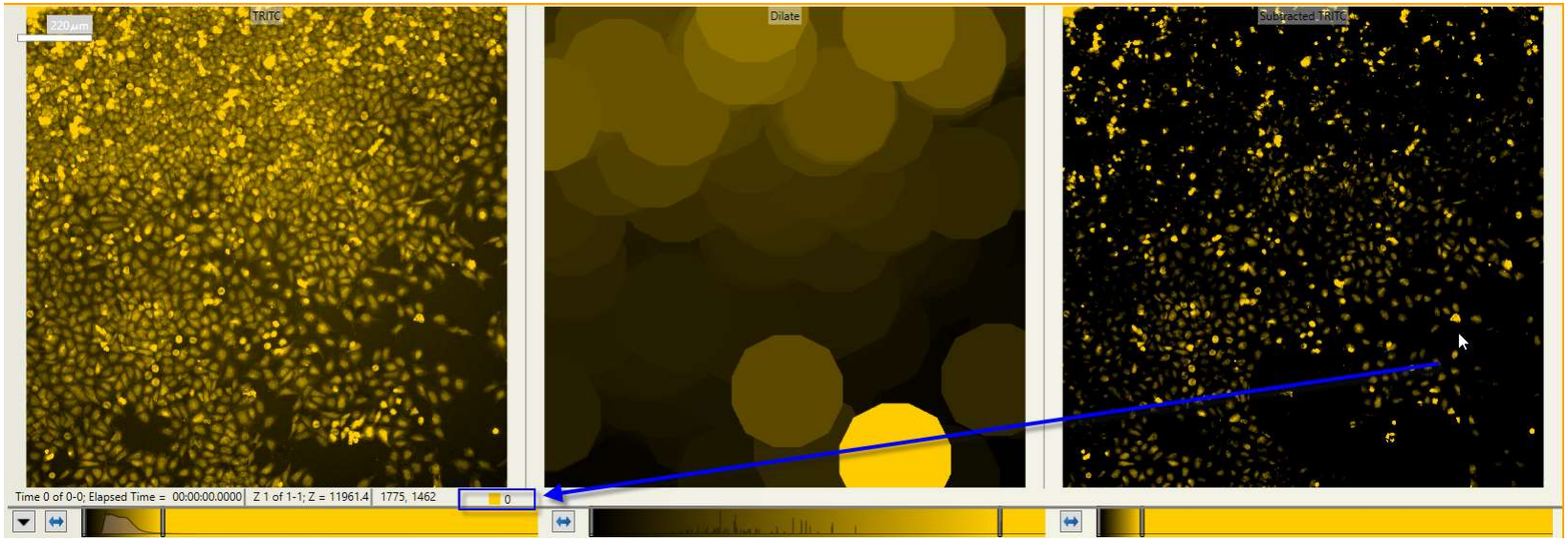
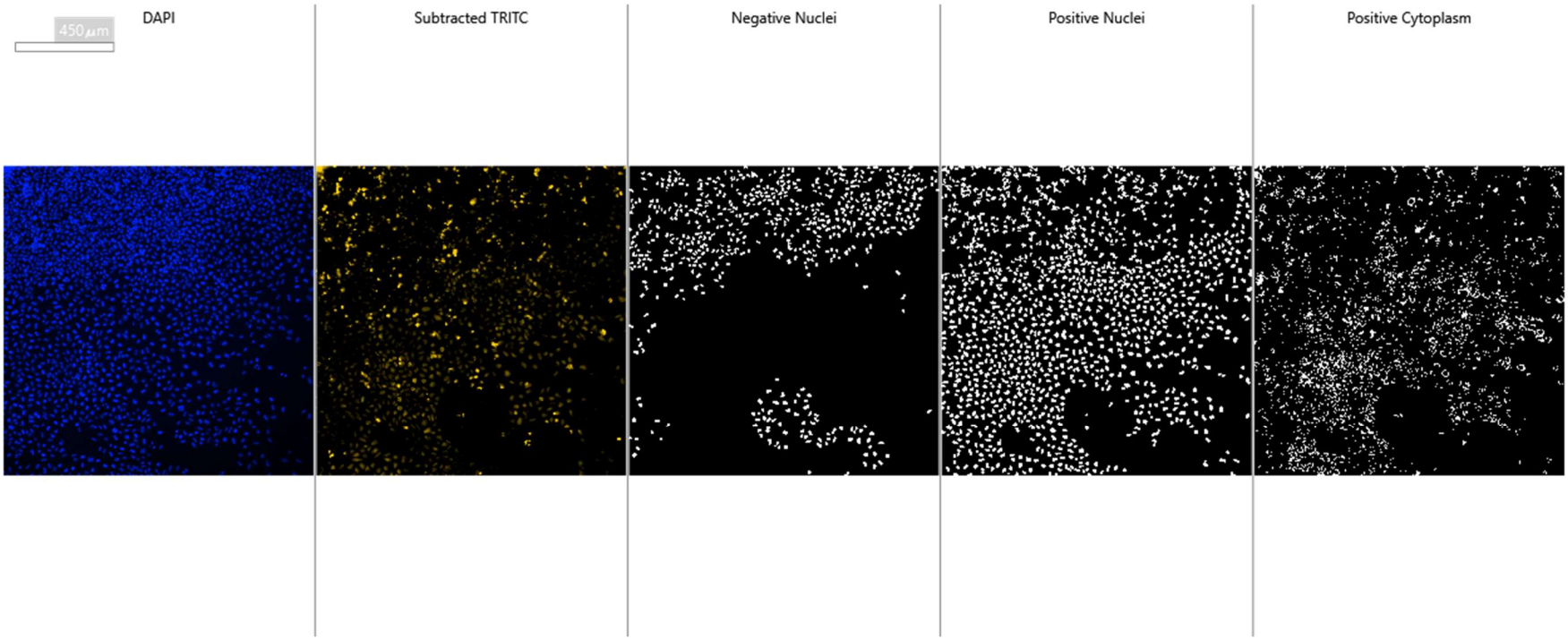


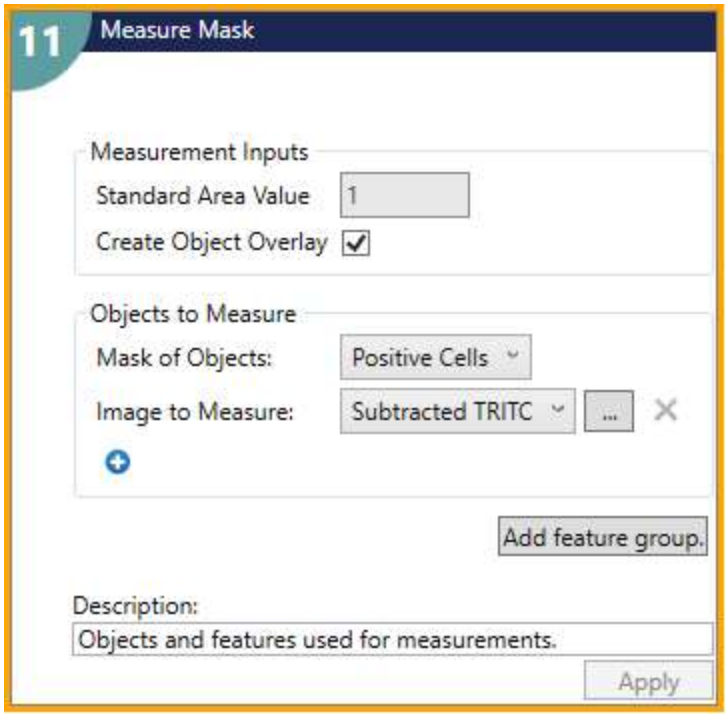
Step 8: Find Objects

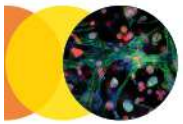
- Add segmentation routine of interest.



Step 9: Measure

- Set up measurements, making sure to select the subtract result image for intensity measurements





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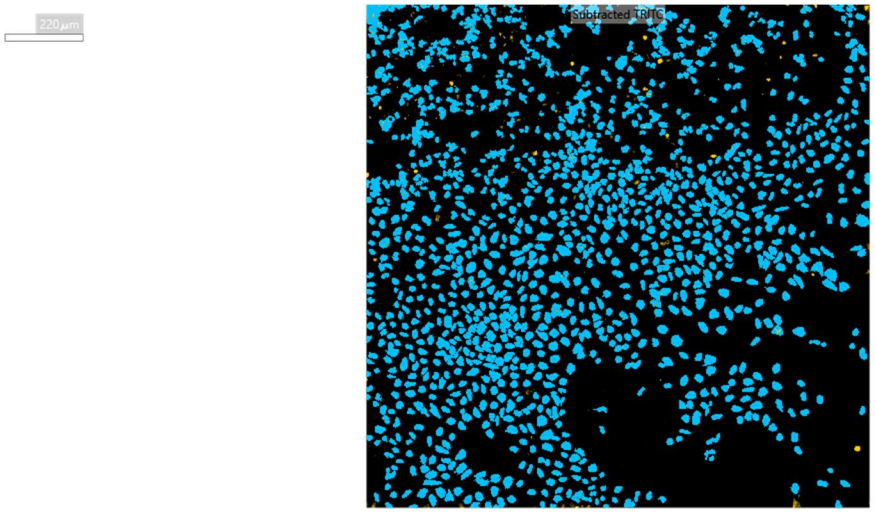

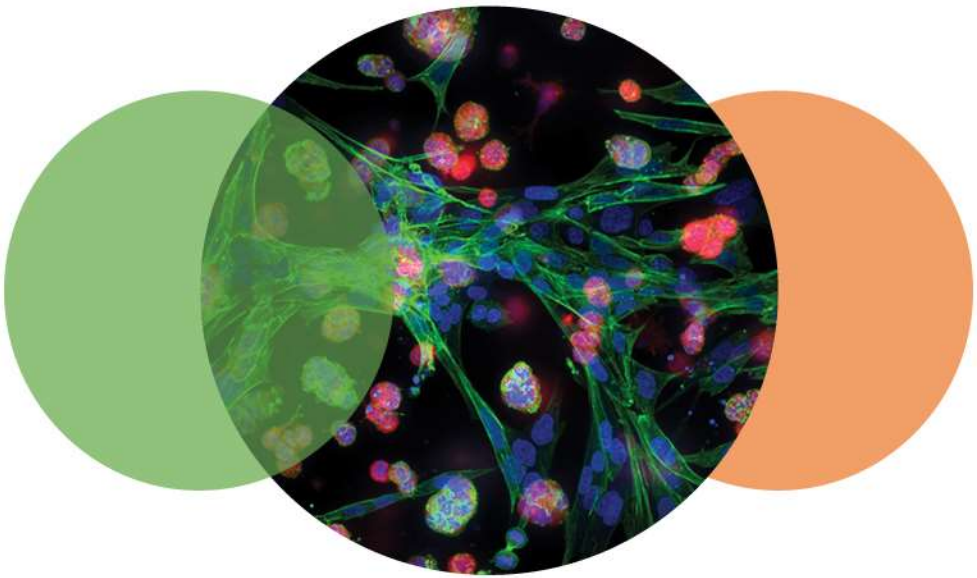

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