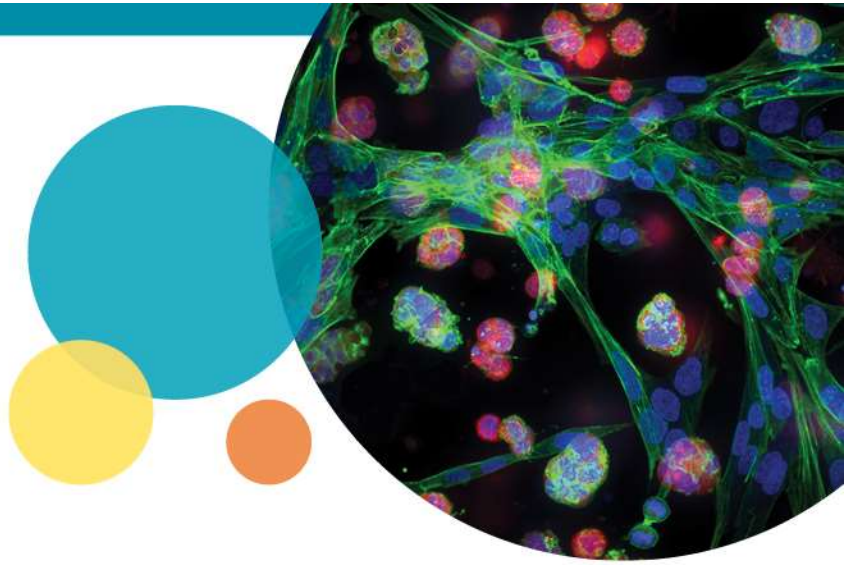


MetaXpress Custom Module Editor

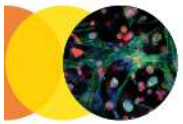
Measure Background Intensity in Well Area

Rev A 2018-08-22

MOLECULAR DEVICES
ADVANCING DISCOVERY

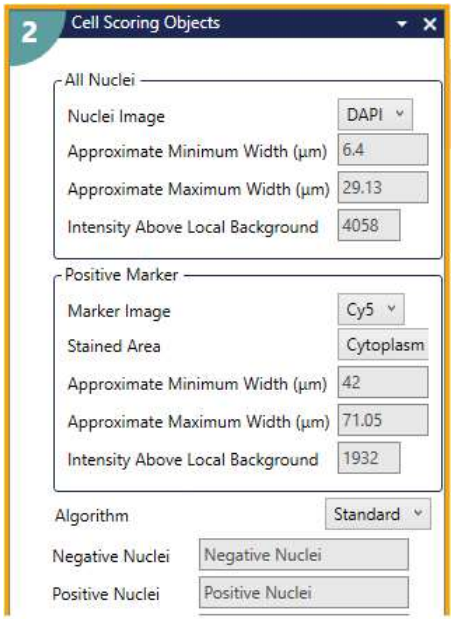
Chapter Purpose

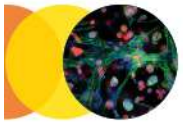
- This guide explains a method to measure background intensity within the Custom Module Editor. This method will find the intensity of the background across the well area, which might be less than the whole image.



Step 1: Find Objects

- Use a suitable Find Objects or Application Module step to identify all objects. Cell Scoring, Simple Threshold, or Adaptive Threshold often works well.





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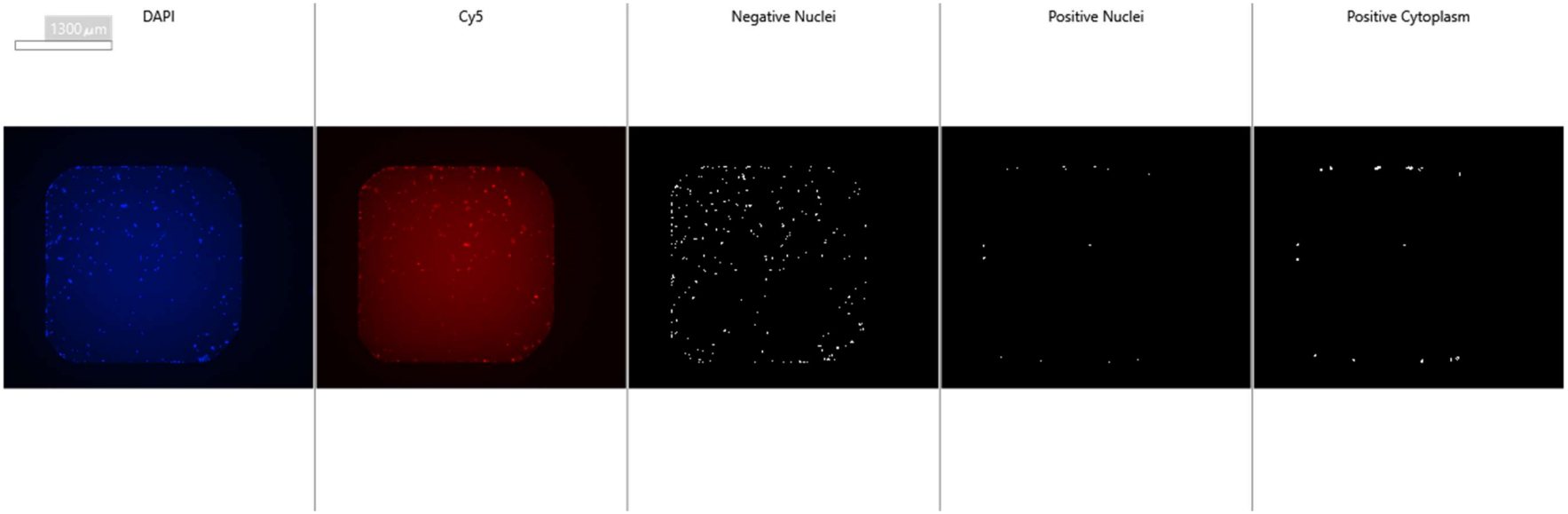
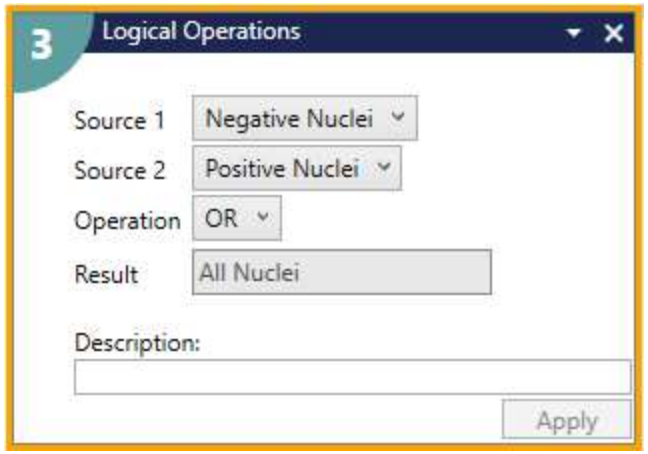


Step 2 (optional): Modify Objects > Logical Operations

- If you have multiple masks, use Logical OR steps to combine them into a single mask representing all objects.



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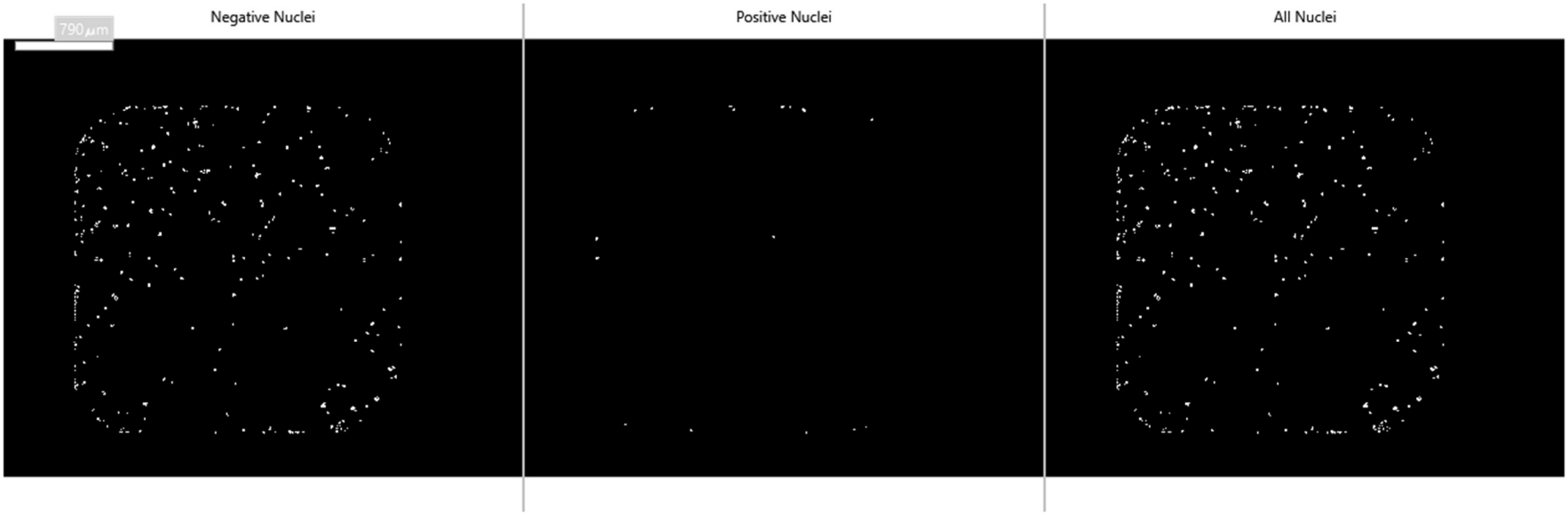

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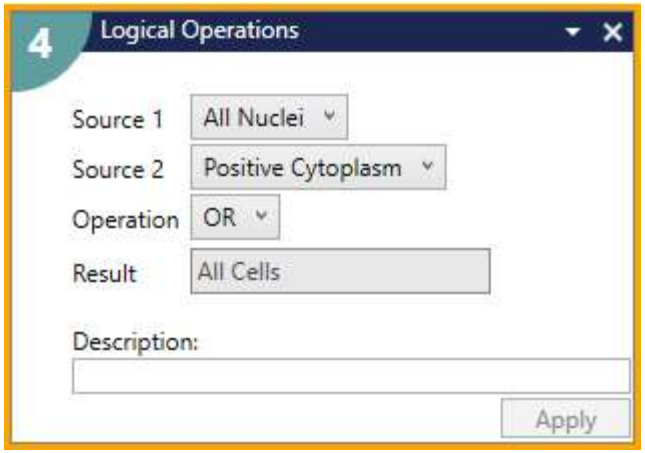

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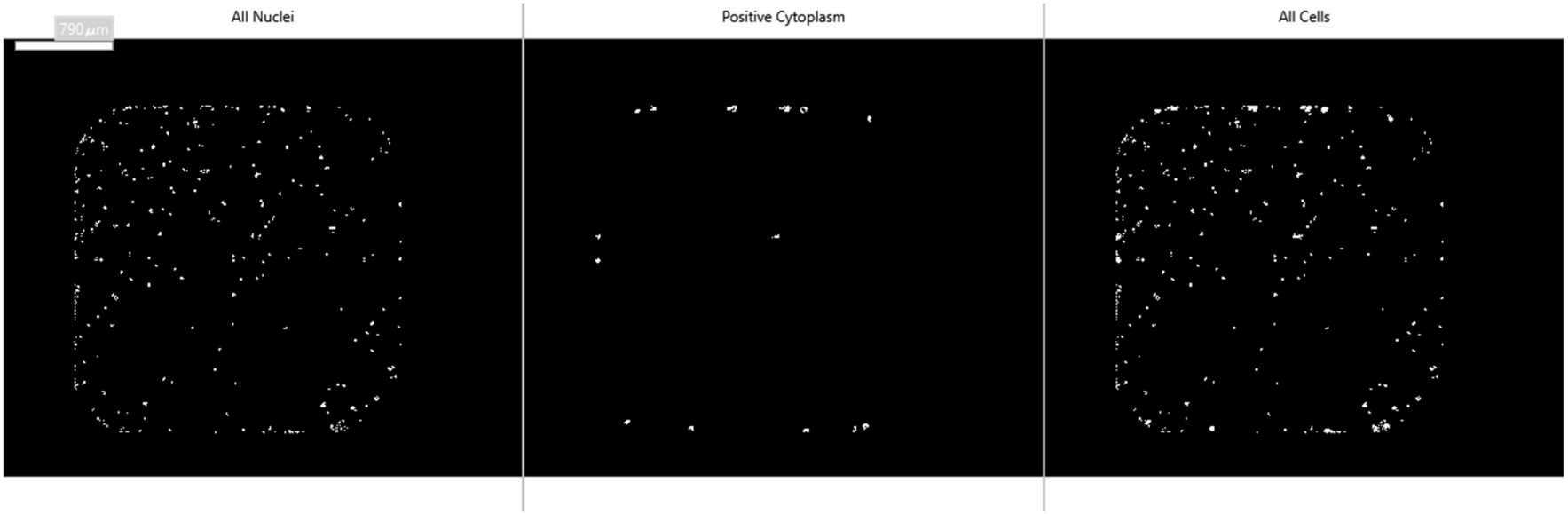
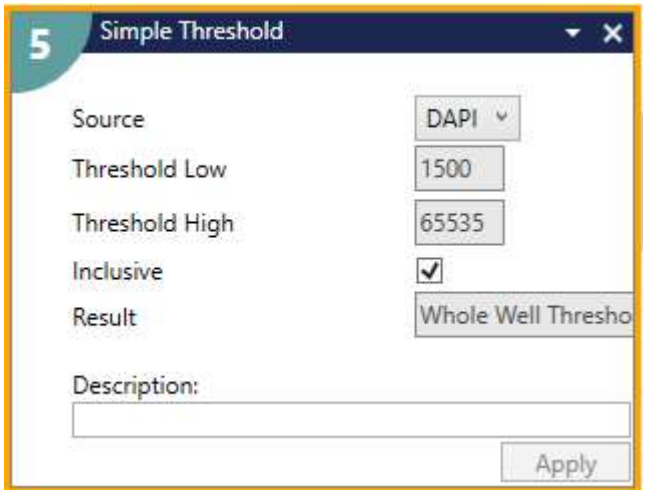


Step 3: Find Objects > Simple Threshold

- Use Simple Threshold on a suitable channel to identify the well area.



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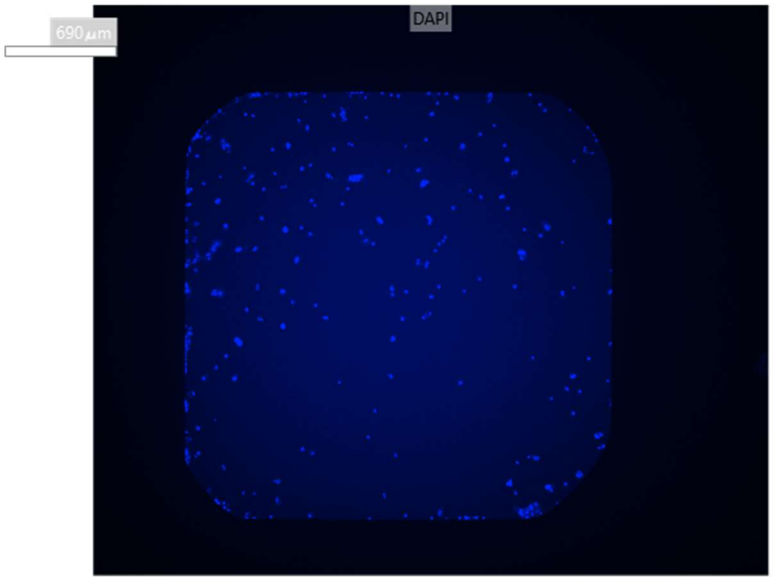

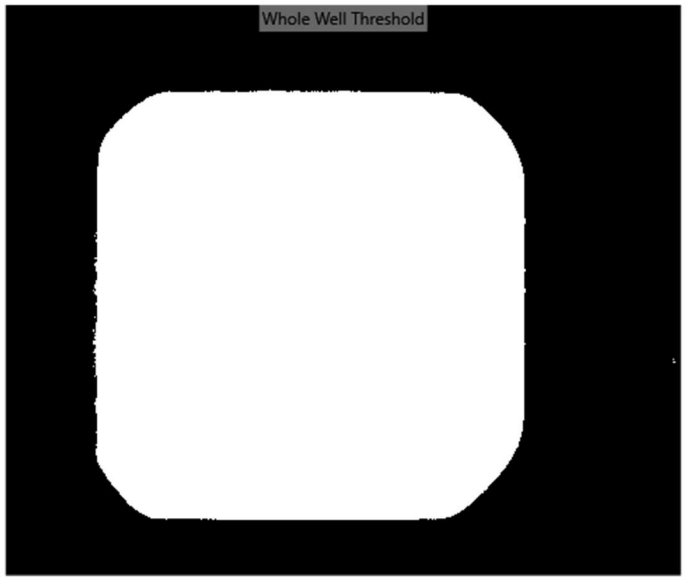

Step 3: Modify Objects > Fill Holes

- Fill Holes in the well mask. This may be needed because of objects/cells that are outside the well threshold range.

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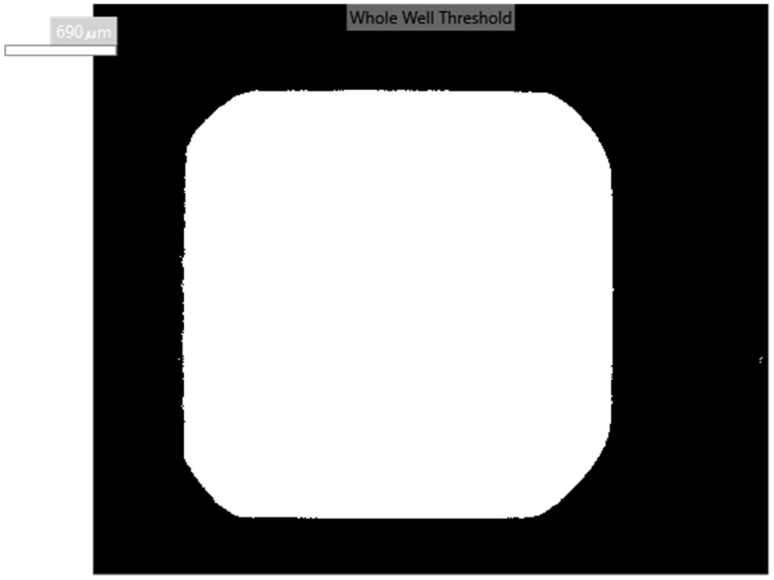
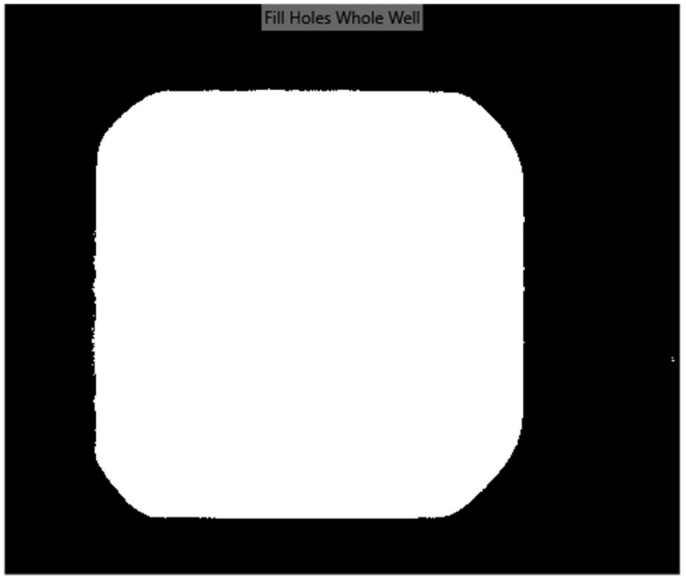
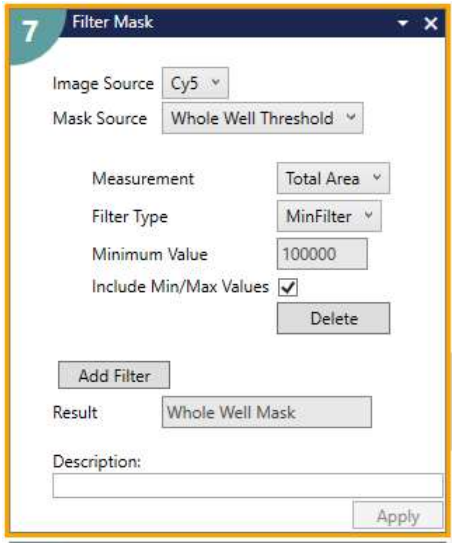




Step 4: Modify Objects > Filter Mask

- Filter the filled well mask by area to remove small artifacts located outside of the well.



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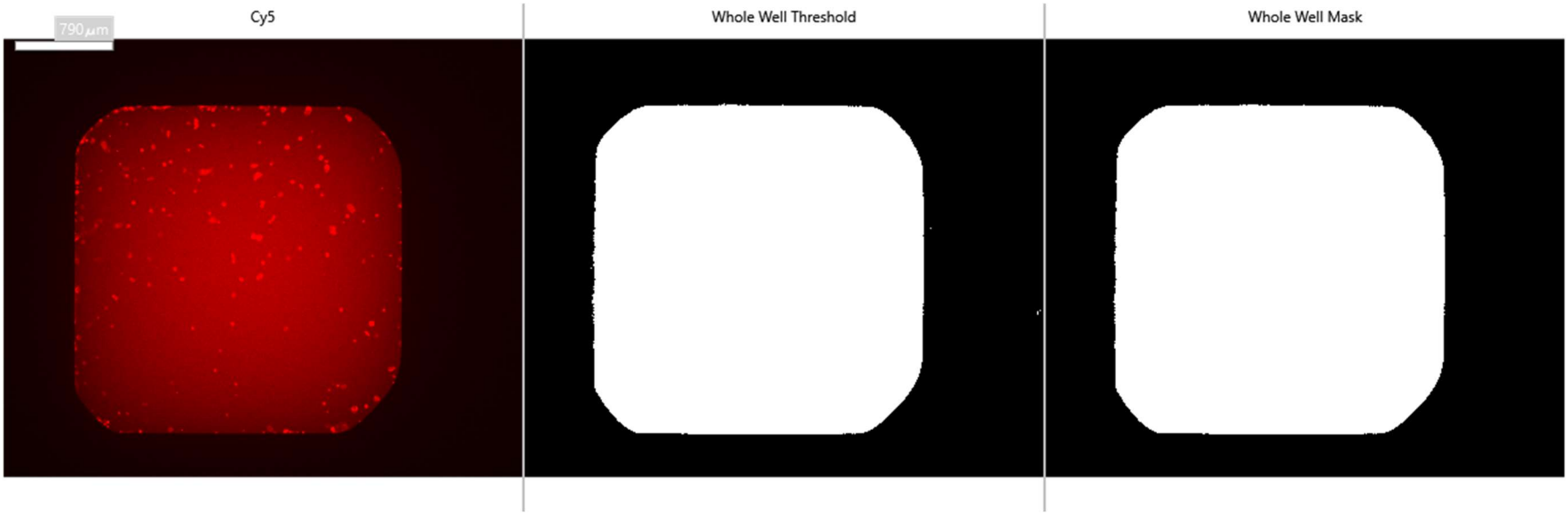
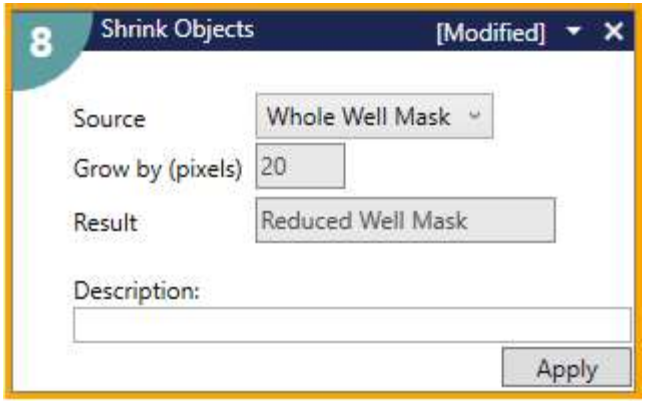


Step 5: Modify Objects > Shrink Objects

- Shrink the well area to avoid edge effects at the well borders.



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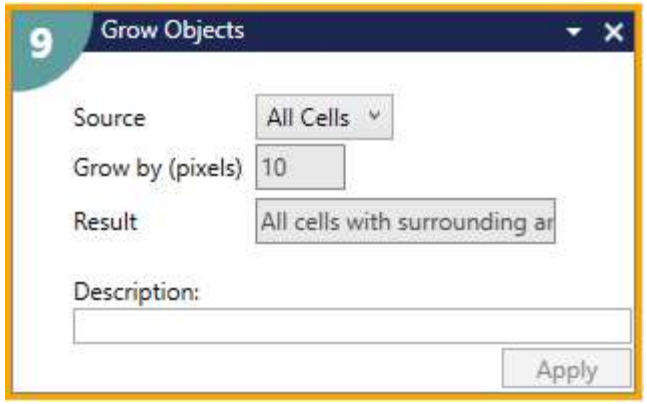
Step 6: Modify Objects > Grow Objects

- Grow the all objects mask to avoid edge effects at the cell borders



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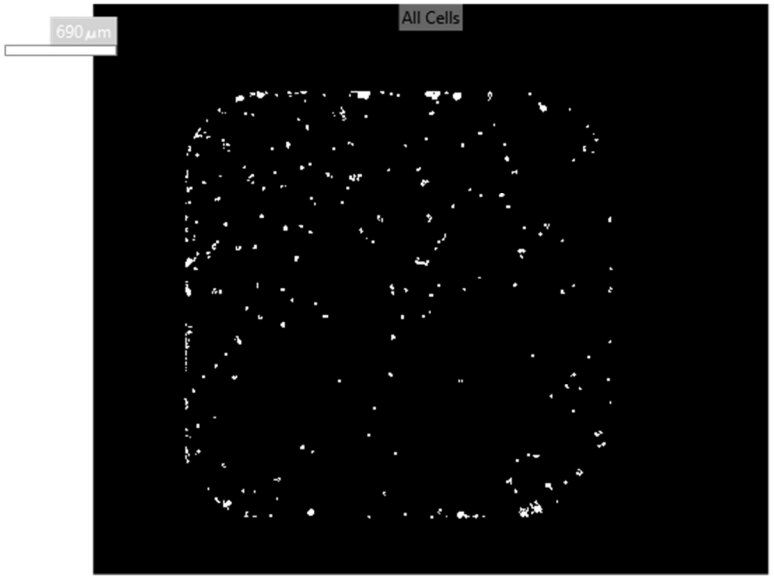
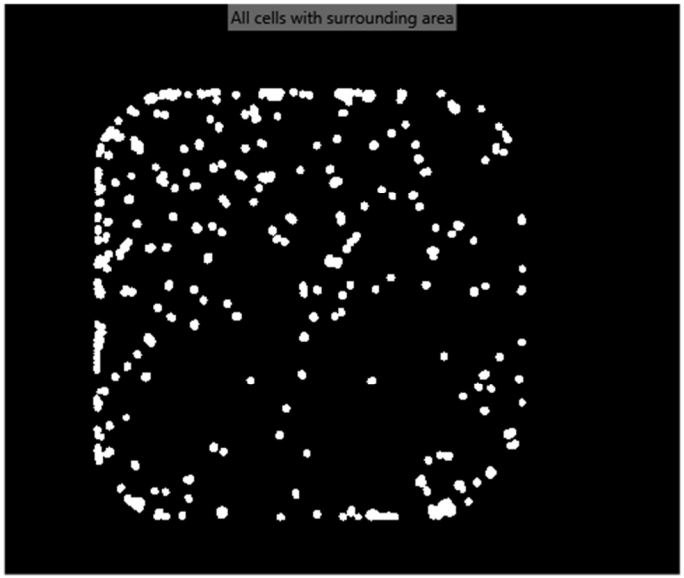
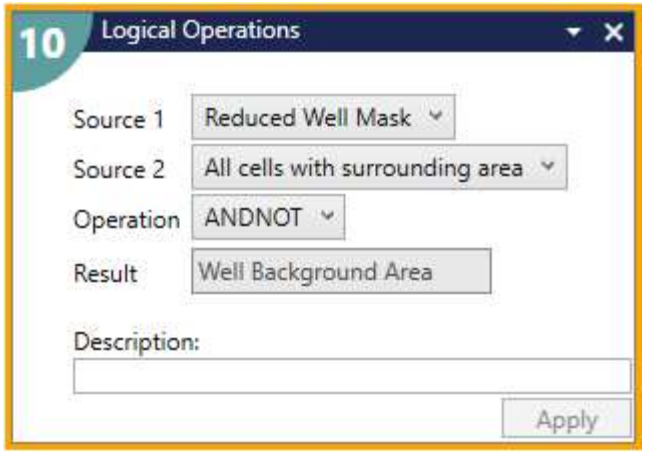




Step 7: Modify Objects > Logical Operations

- Use the Logical ANDNOT Operation to remove the grown cell area from the shrunk well area. This will leave the background well area.



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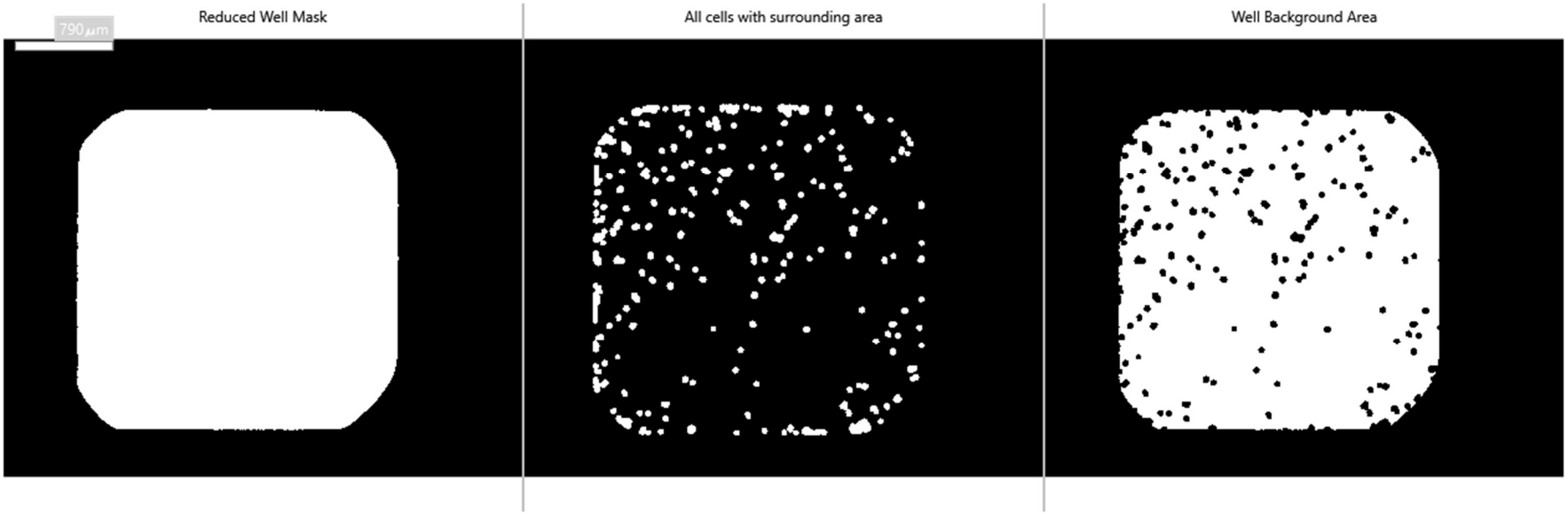
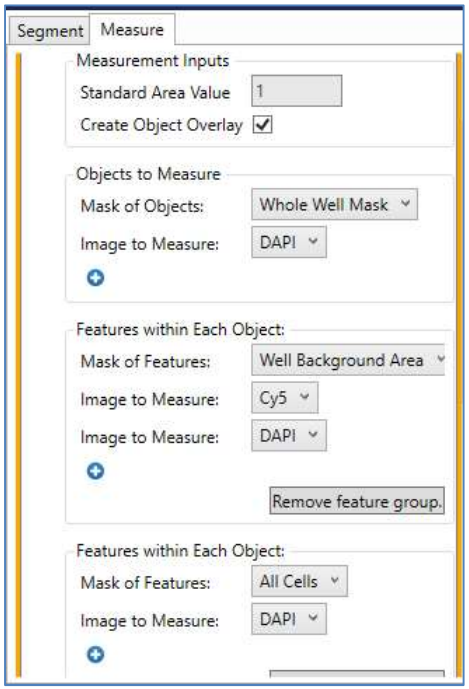
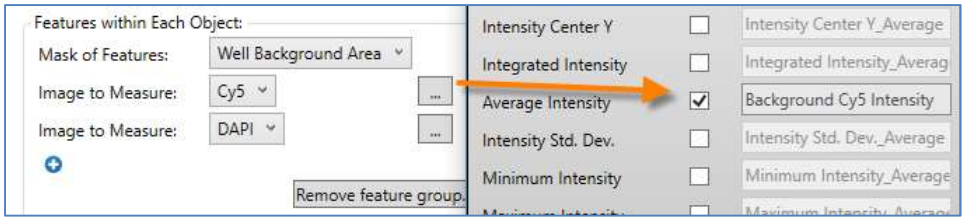
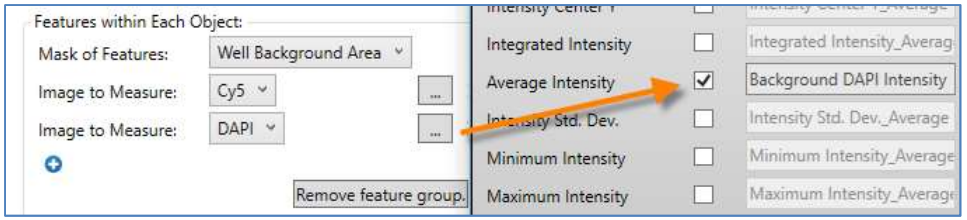


Step 8: Measure

- For Objects to Measure, select the Whole Well Mask. No measurements are needed unless you need intensity across the whole well.
- Include the well background area as one of the feature groups and define the Average Intensity_Average measurement as your background intensity output(s).







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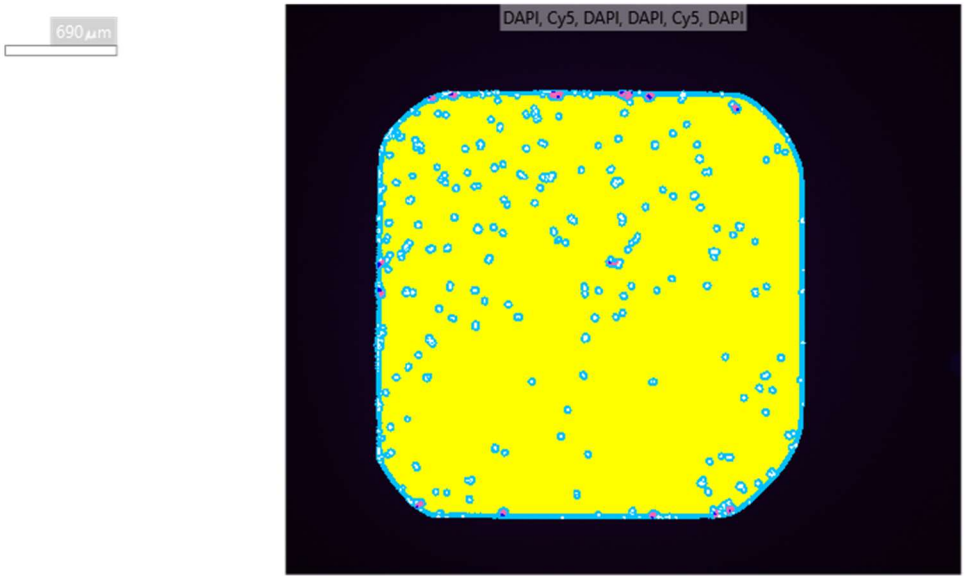
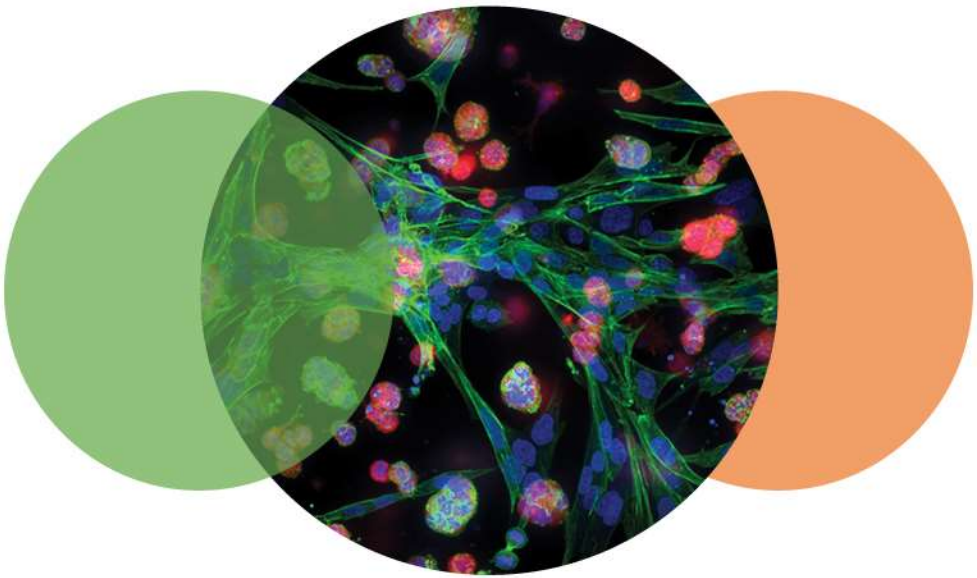


Mask Legend

Layer	Color	Mask Name
1		Whole Well Mask
2		Well Background Area
3		All Cells
4		Positive Cytoplasm
5		Negative Nuclei



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