

MetaXpress Custom Module Editor

Measure Background Intensity in Well Area

Rev A 2018-08-22



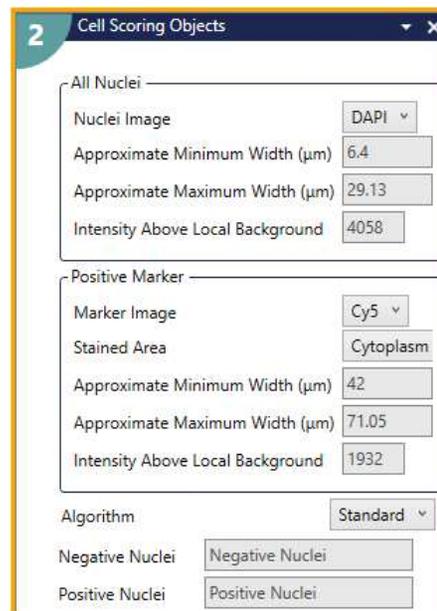
Chapter Purpose

- 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.



The screenshot shows a software window titled "2 Cell Scoring Objects" with a close button. It contains two main sections: "All Nuclei" and "Positive Marker".

All Nuclei

- Nuclei Image: DAPI
- Approximate Minimum Width (μm): 6.4
- Approximate Maximum Width (μm): 29.13
- Intensity Above Local Background: 4058

Positive Marker

- Marker Image: Cy5
- Stained Area: Cytoplasm
- Approximate Minimum Width (μm): 42
- Approximate Maximum Width (μm): 71.05
- Intensity Above Local Background: 1932

Algorithm: Standard

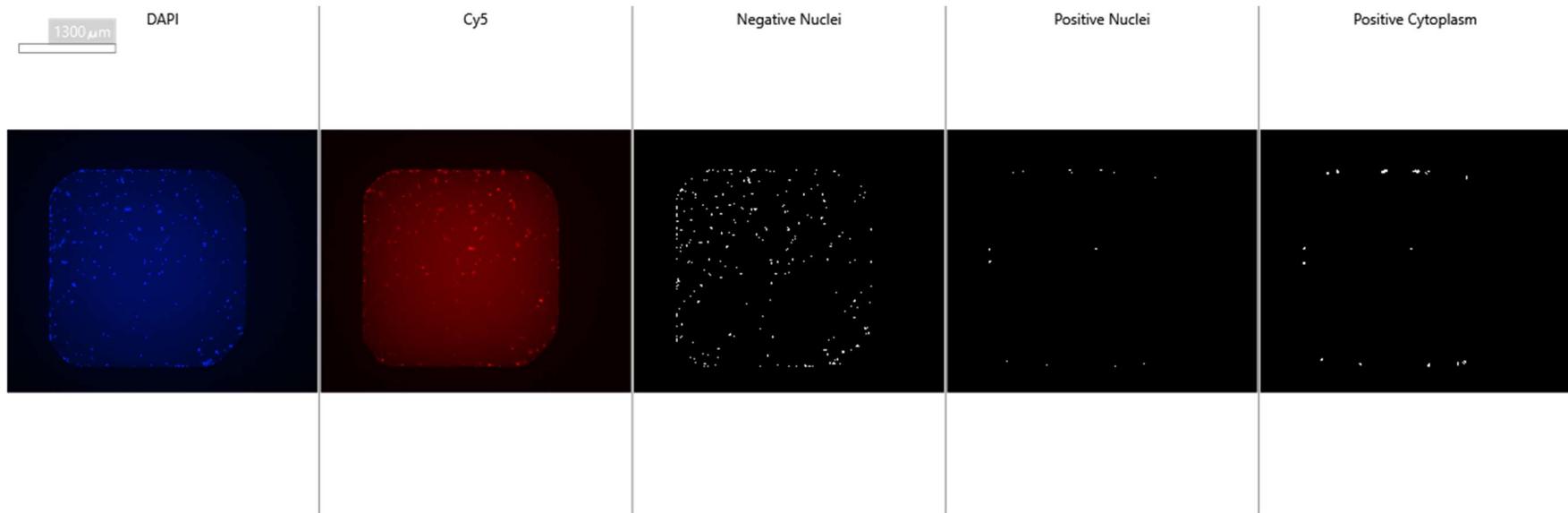
Negative Nuclei: Negative Nuclei

Positive Nuclei: Positive Nuclei



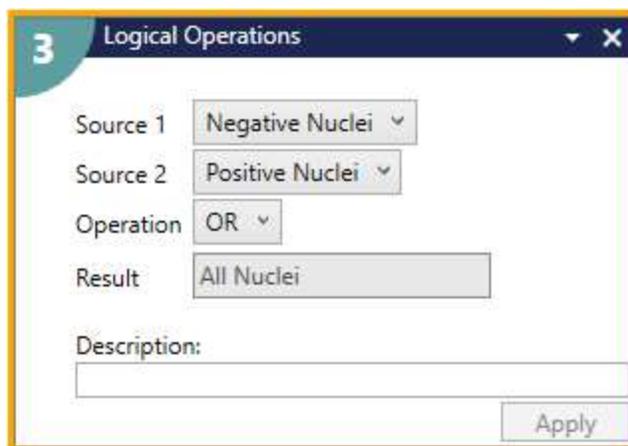
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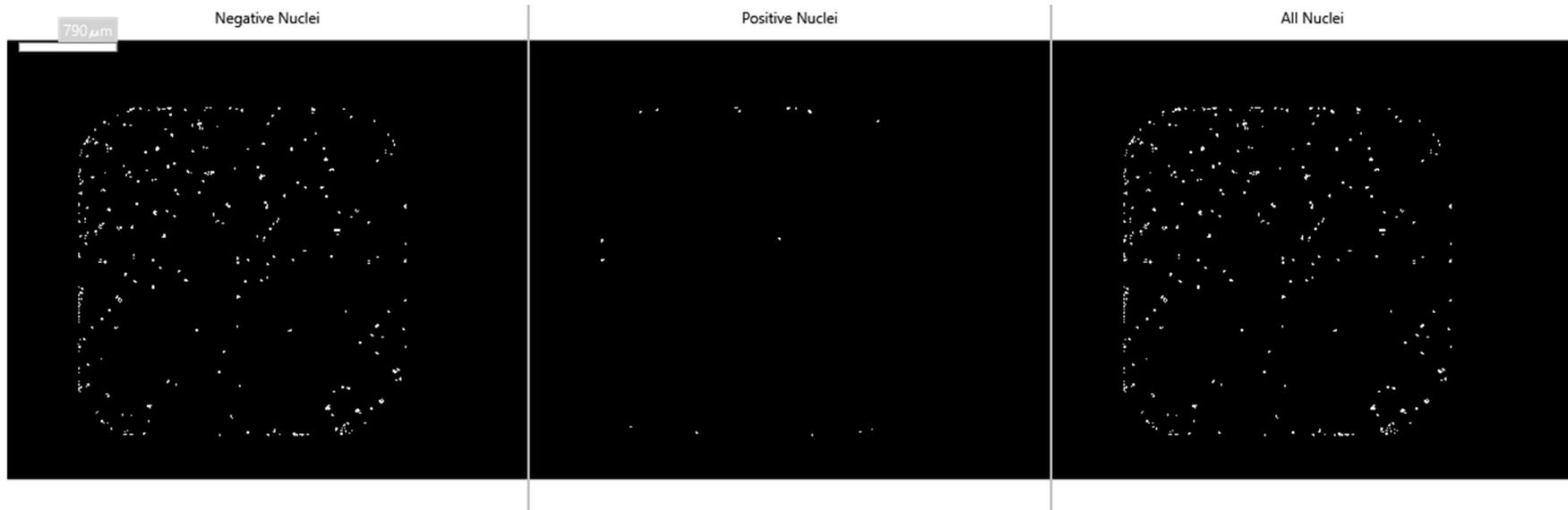
Step 2 (optional): Modify Objects > Logical Operations

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



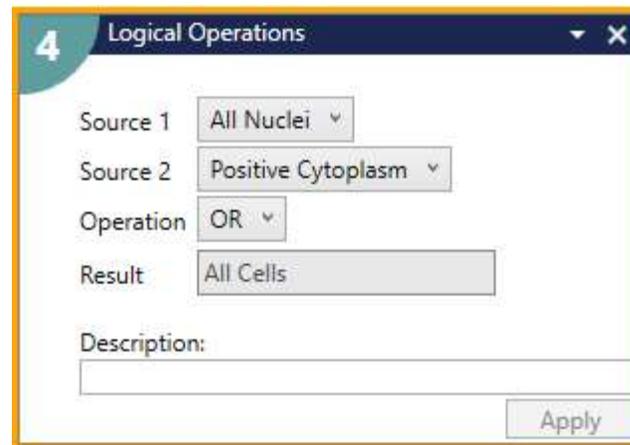
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4 Logical Operations

Source 1 All Nuclei

Source 2 Positive Cytoplasm

Operation OR

Result All Cells

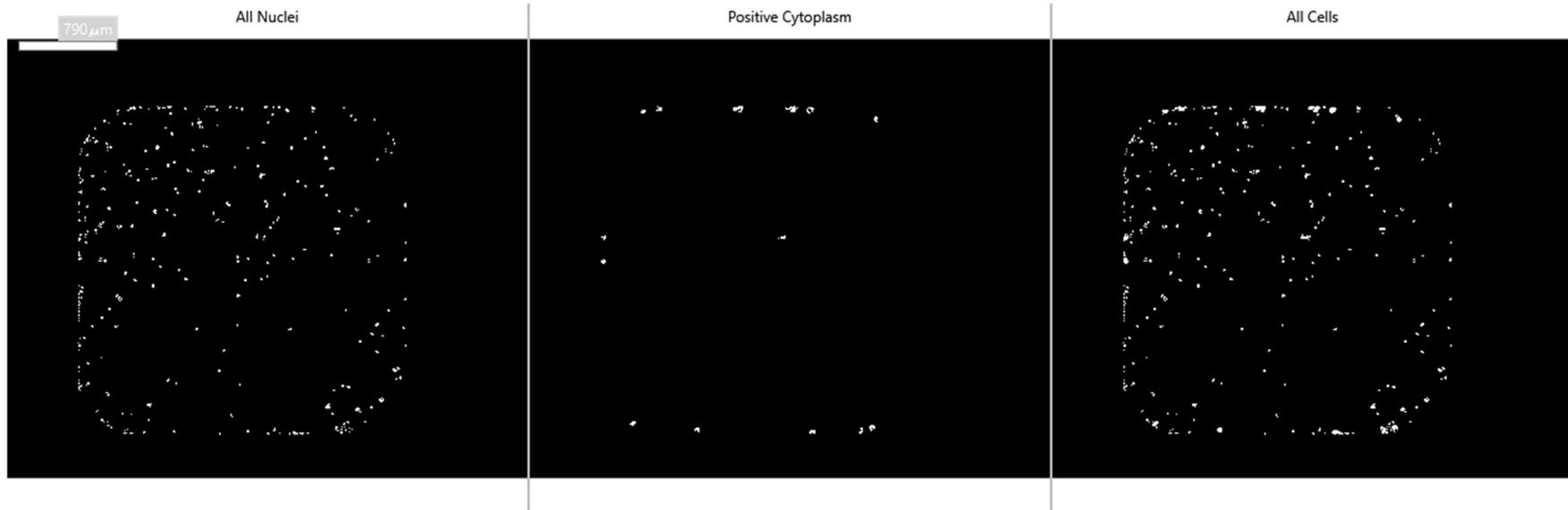
Description:

Apply



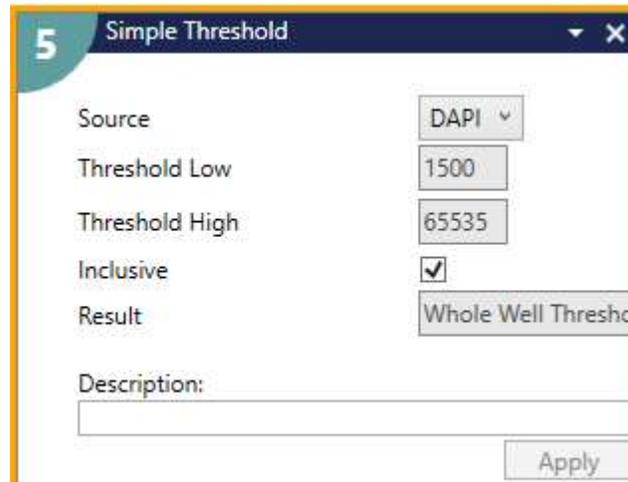
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Step 3: Find Objects > Simple Threshold

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



5 Simple Threshold

Source: DAPI

Threshold Low: 1500

Threshold High: 65535

Inclusive:

Result: Whole Well Thresho

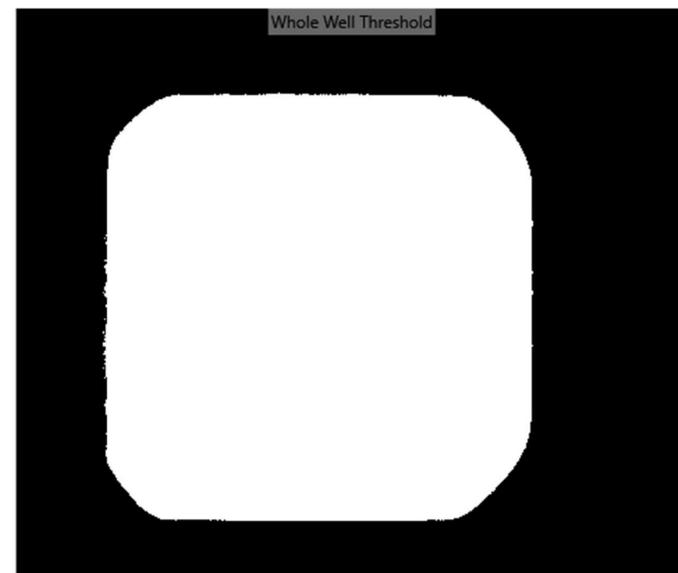
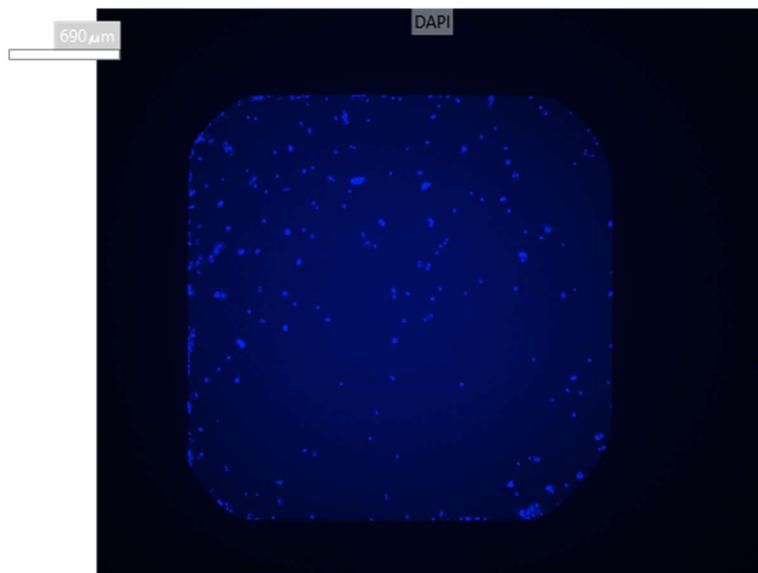
Description:

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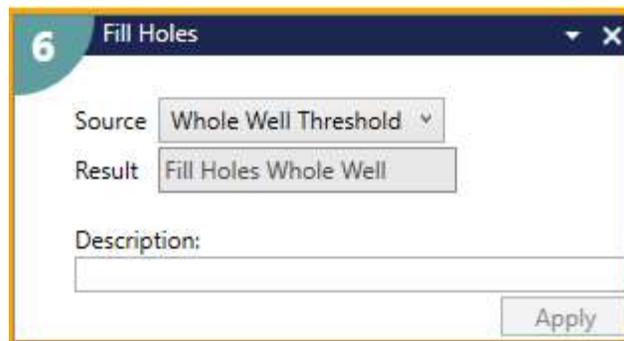
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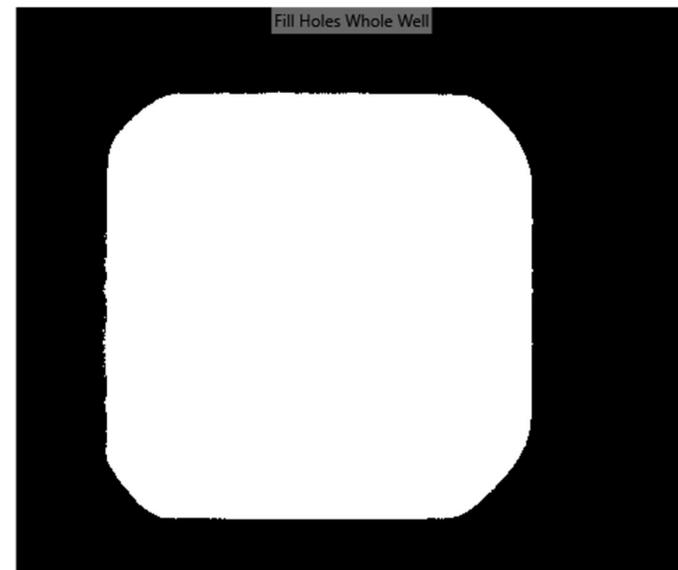
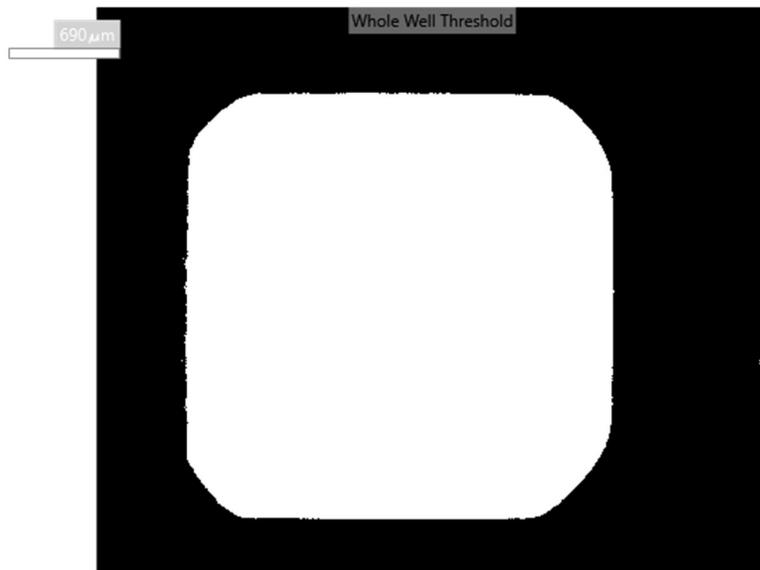
Step 3: Modify Objects > Fill Holes

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



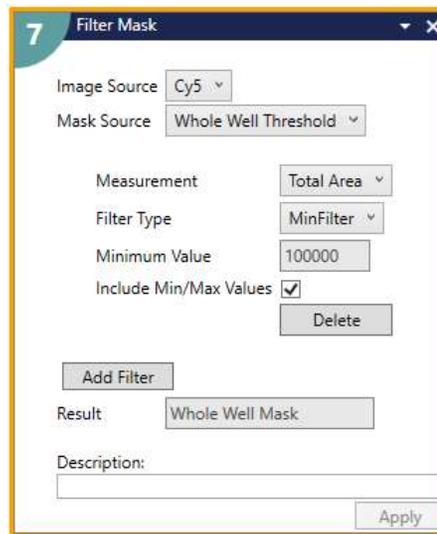
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Step 4: Modify Objects > Filter Mask

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



7 Filter Mask

Image Source: Cy5

Mask Source: Whole Well Threshold

Measurement: Total Area

Filter Type: MinFilter

Minimum Value: 100000

Include Min/Max Values:

Delete

Add Filter

Result: Whole Well Mask

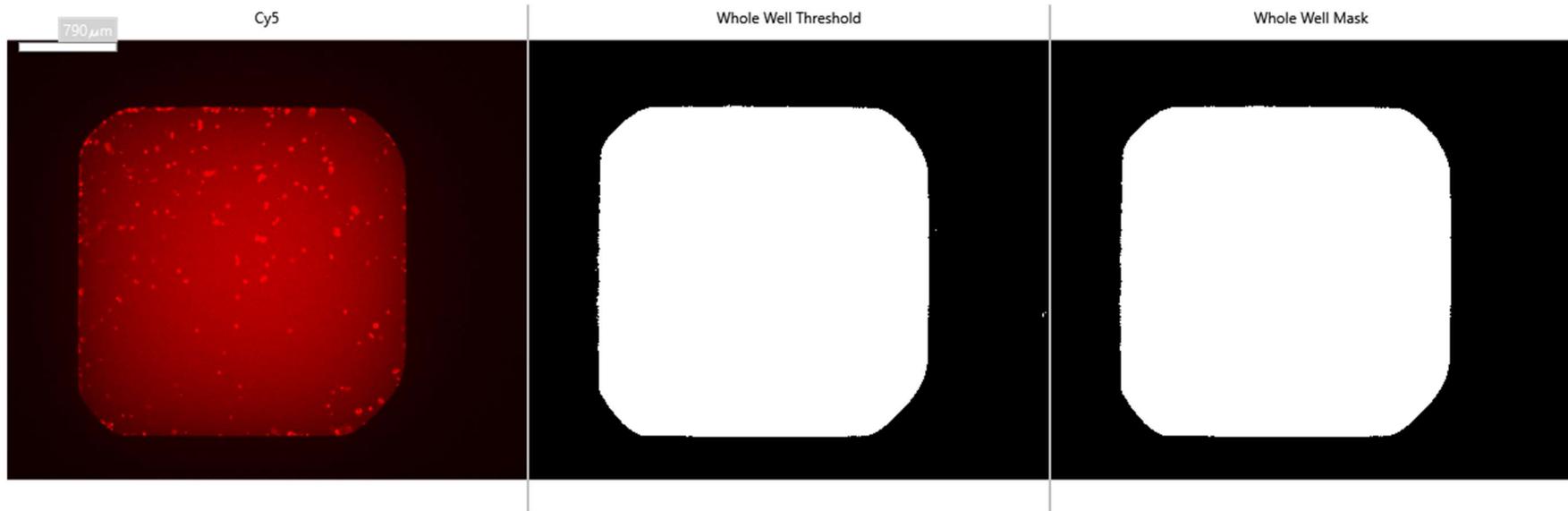
Description:

Apply



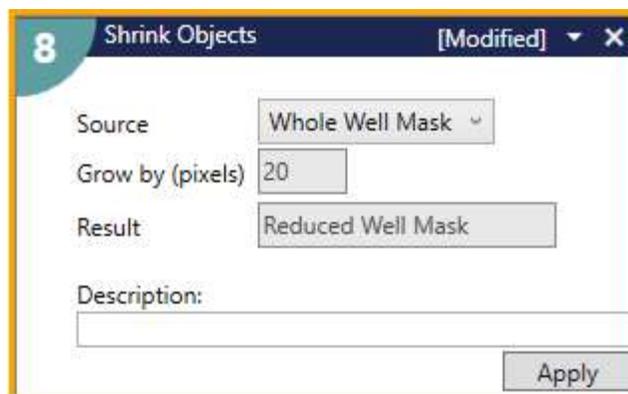
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- Filter the filled well mask by area to remove small artifacts located outside of the well.



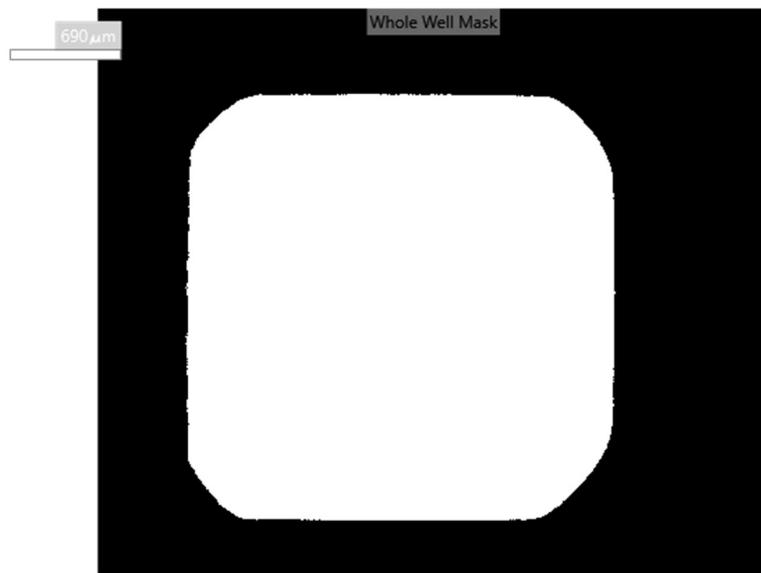
Step 5: Modify Objects > Shrink Objects

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



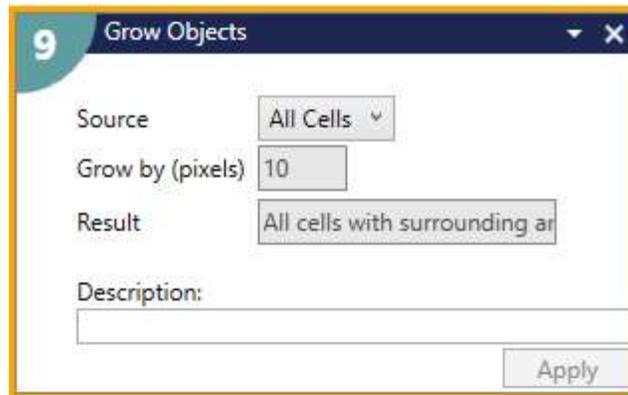
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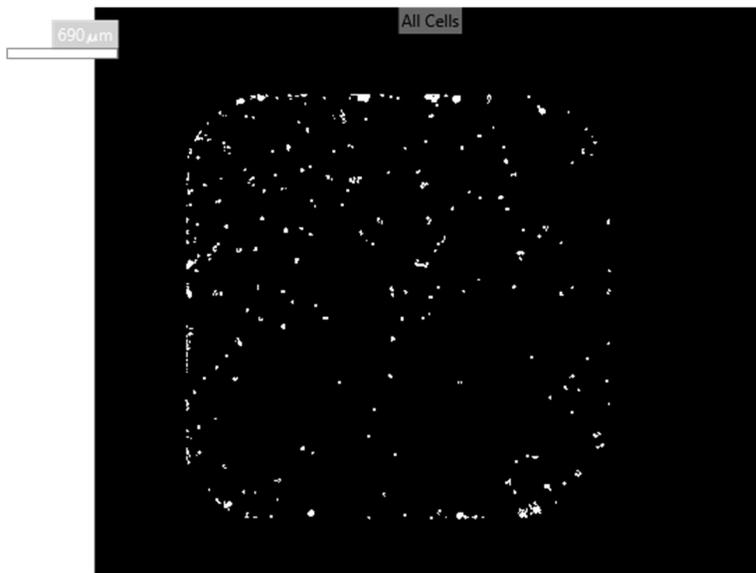
Step 6: Modify Objects > Grow Objects

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



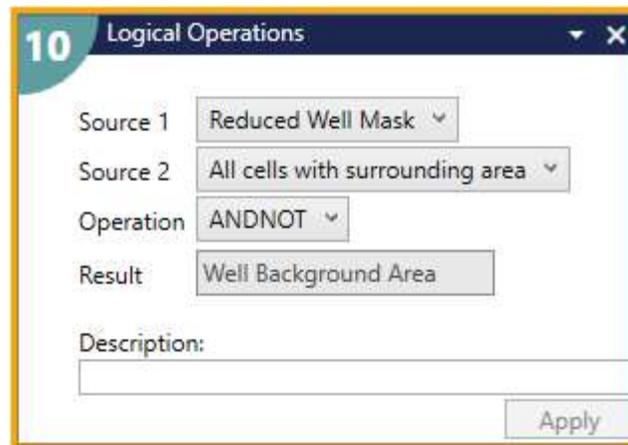
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- Grow the all objects mask to avoid edge effects at the cell borders



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.



10 Logical Operations

Source 1: Reduced Well Mask

Source 2: All cells with surrounding area

Operation: ANDNOT

Result: Well Background Area

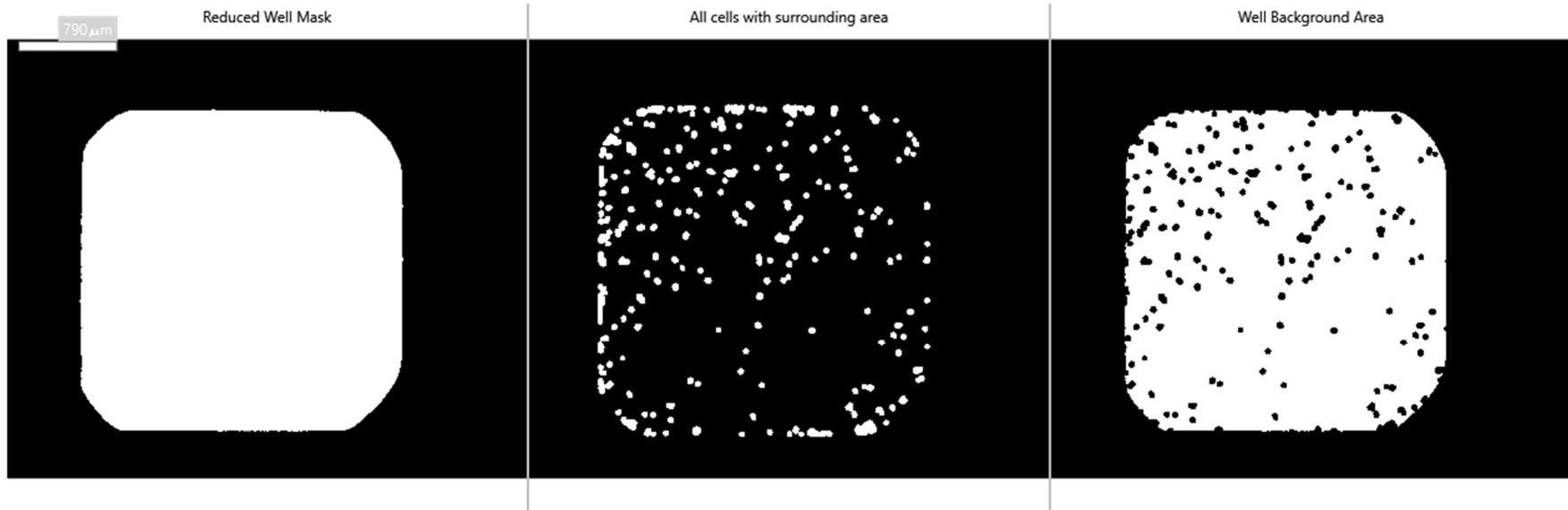
Description:

Apply



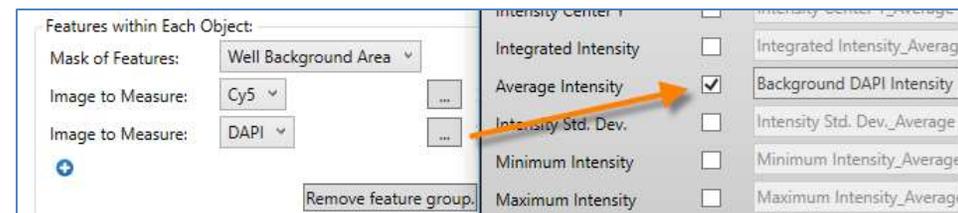
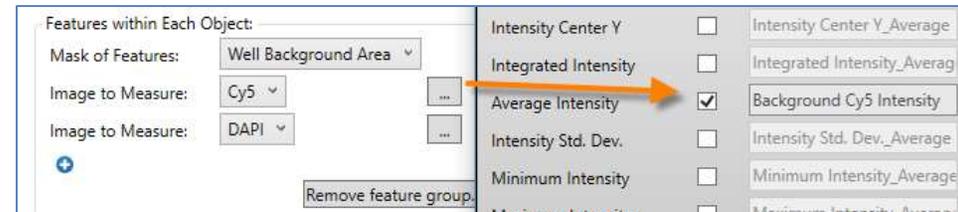
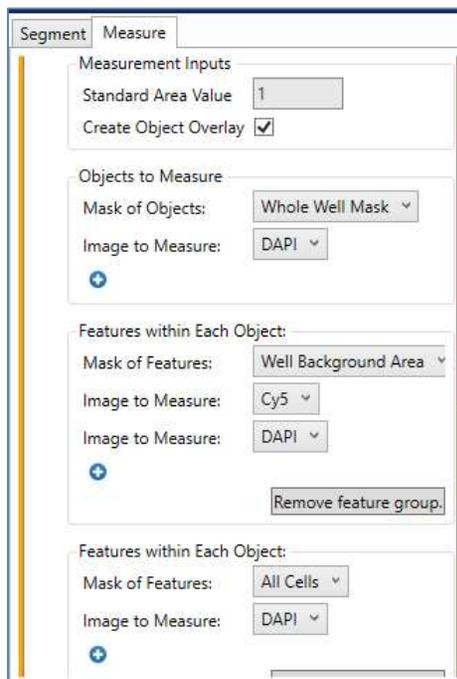
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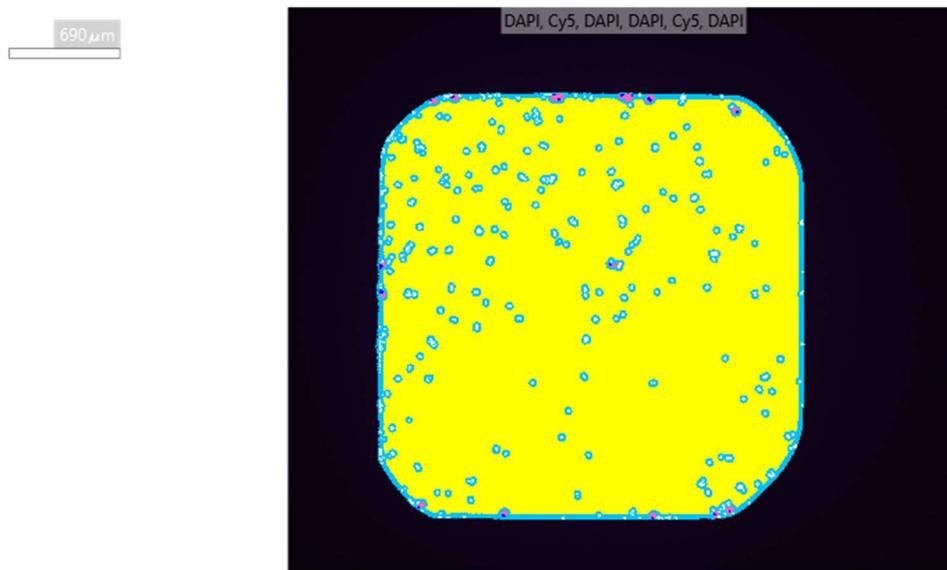
Step 8: Measure

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



