

# MetaXpress Custom Module Editor

## Background Subtraction for Simple Images

Rev A 2018-08-21



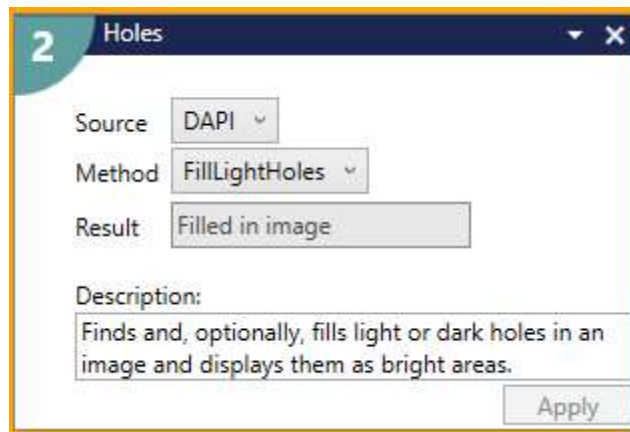
# Chapter Purpose

- This guide explains a method for doing background subtraction within the Custom Module Editor. This method is suitable for simpler images/objects, such as nuclei or beads.
- 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.



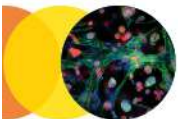
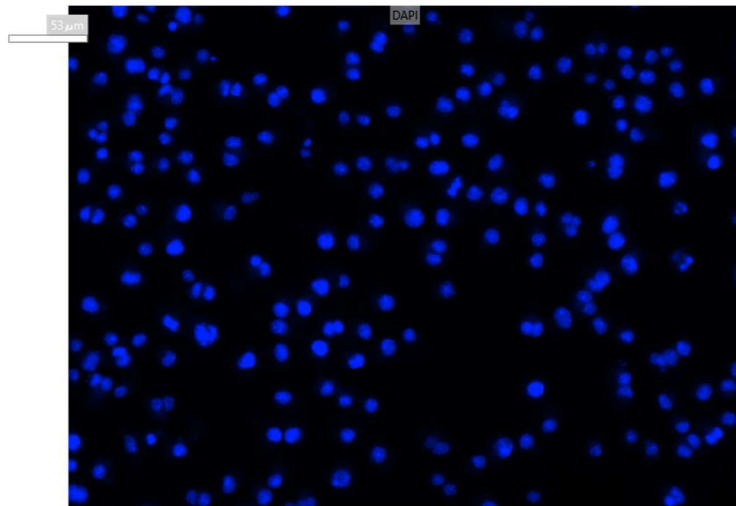
# Step 1: Modify Image > Holes

- Use the FillLightHoles option.
- This will give an image which is mostly just the background, except around the border.



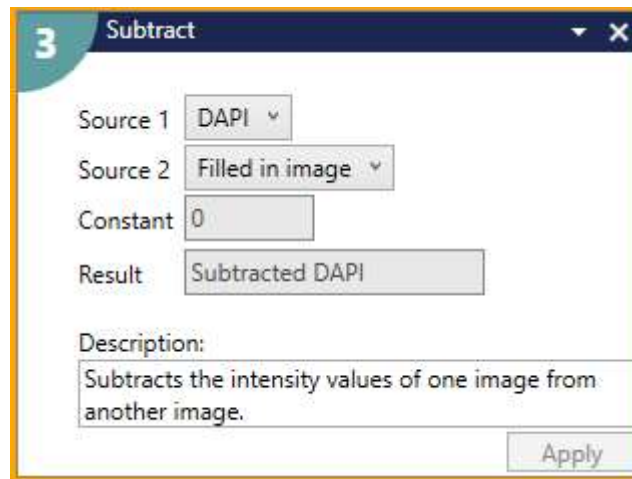
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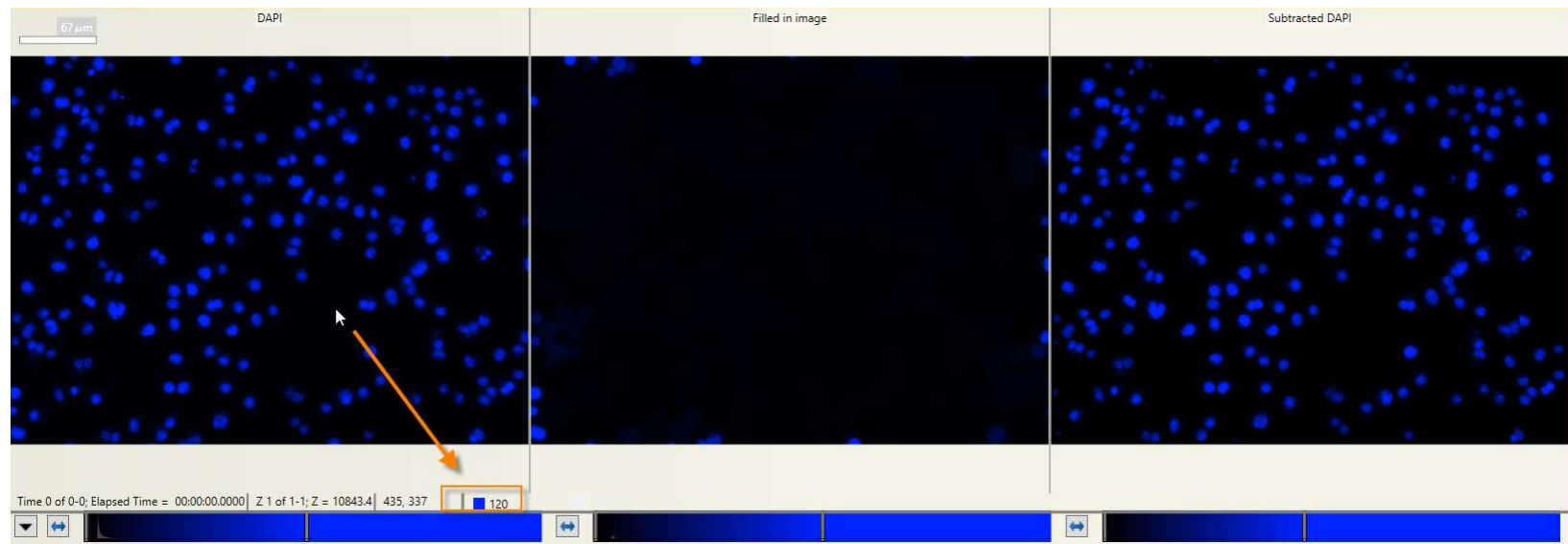
## Step 2: Modify Image > Subtract

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



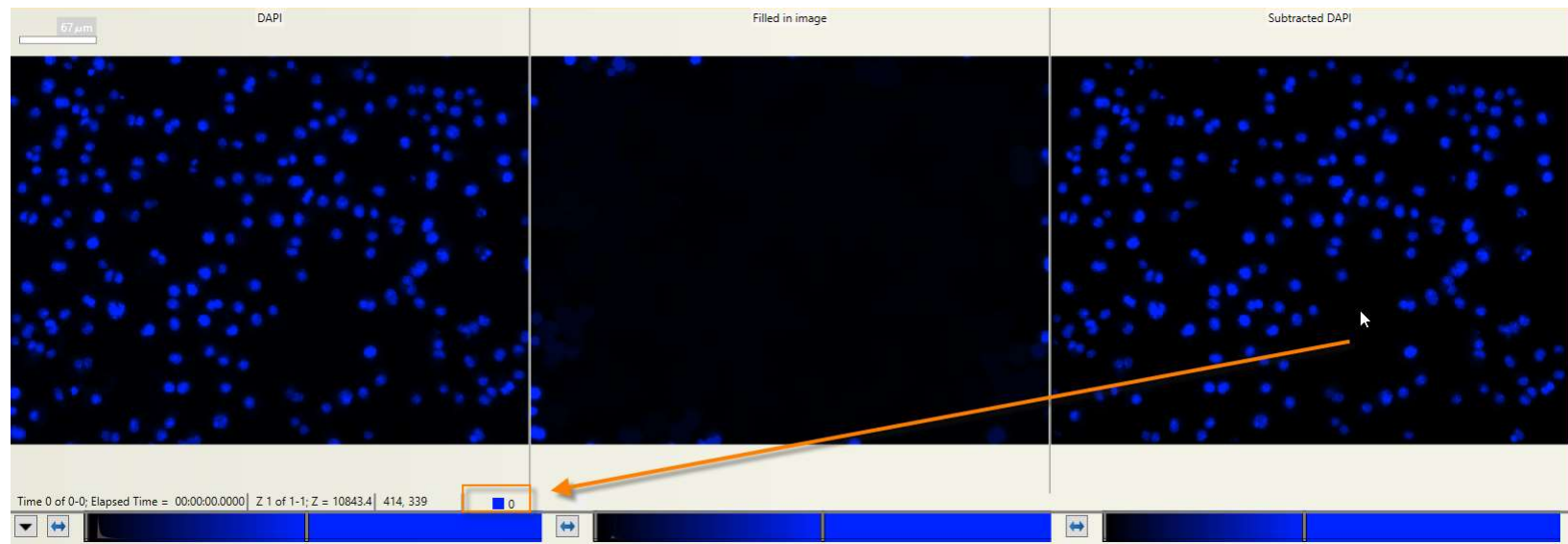
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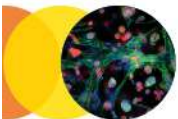
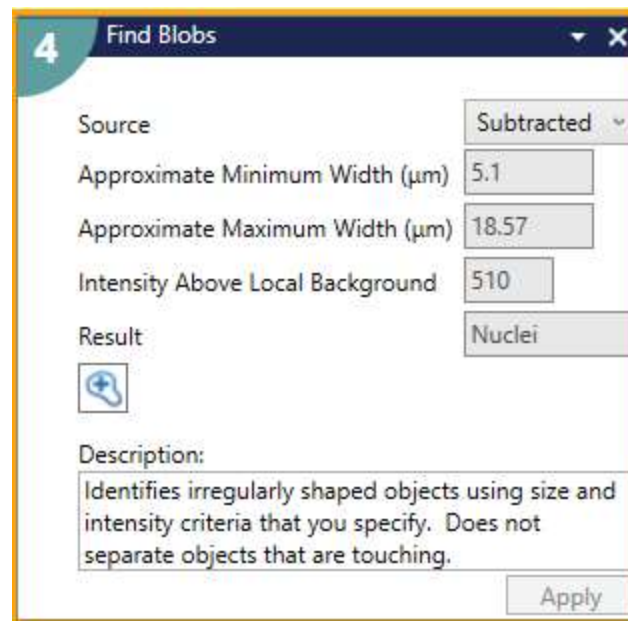
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## Step 3: Find Objects

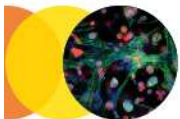
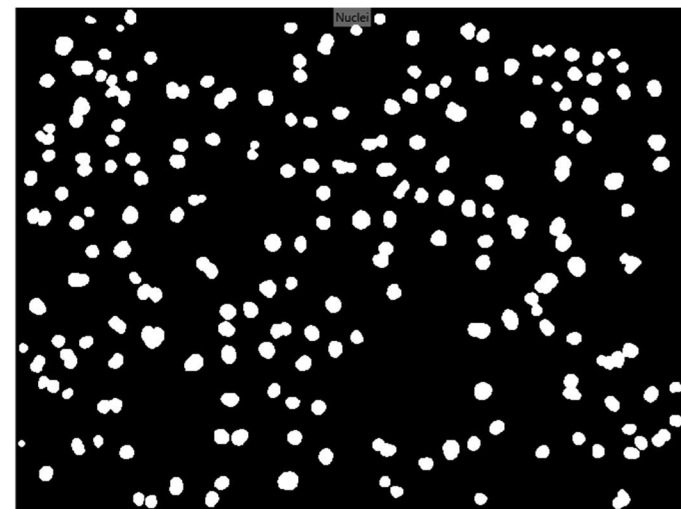
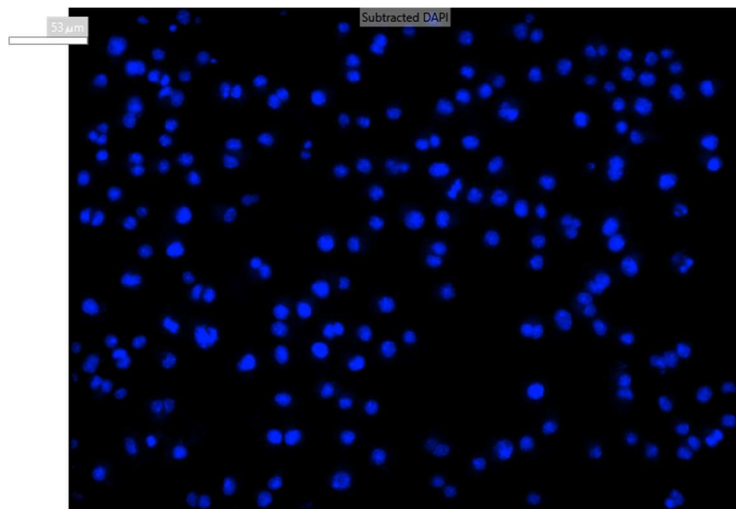
- Use a suitable segmentation step, such as Find Blobs.





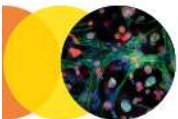
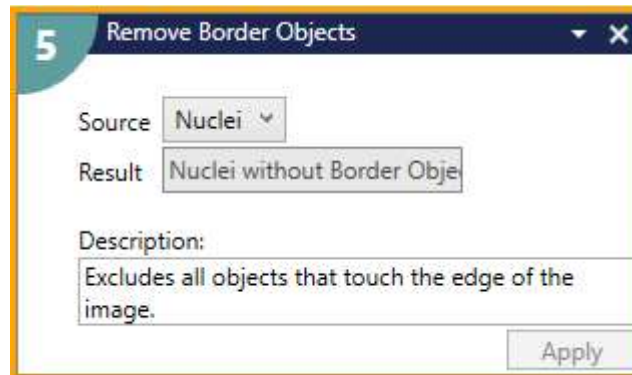
## Step 3: Find Objects

- Use a suitable segmentation step on the subtract result, such as Find Blobs.



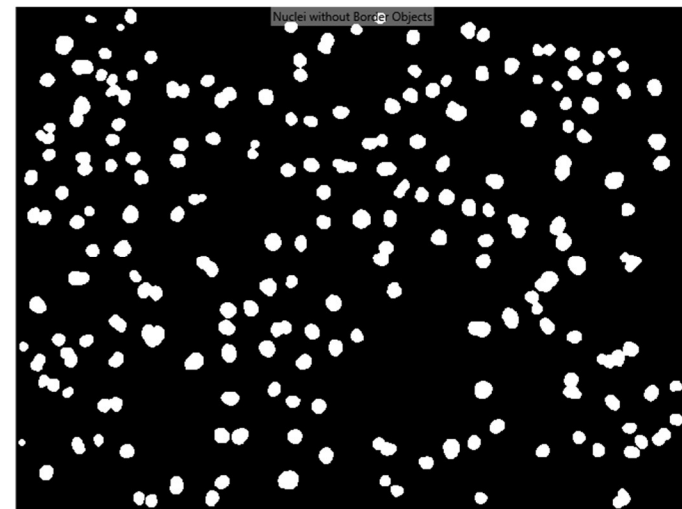
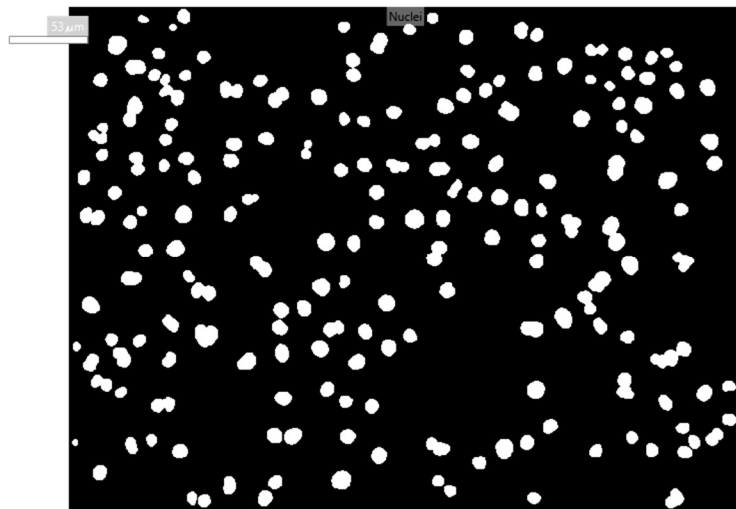
## Step 4: Modify Objects > Remove Border Objects

- Since the objects on the border might not have subtracted correctly, these are removed from the final result so that they will not be measured.



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# Step 5: Measure

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

6 Measure Mask

Measurement Inputs

Standard Area Value: 1

Create Object Overlay: ☒

Objects to Measure

Mask of Objects: Nuclei without Border Objects

Image to Measure: Subtracted DAPI

+ Add feature group.

Description: Objects and features used for measurements.

Apply



# Step 5: Measure

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

53 µm

