

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









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

Mask	Shrink Objects 💉	
Mask to Image	Mask to Image	
Description		





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









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.

Source 1	TRITC Y	
Source 2	Mask to Image Y	
Multiply by	1	
Normalize		
Result	Multiply	





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









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.

Source 1	TRITC Y	
Source 2	Dilate 👻	
Constant	0	
Result	Subtracted TRITC	
Descriptic Subtracts	n: the intensity values of	one image from





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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- 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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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

Measurement Inputs Standard Area Value	1
Create Object Overlay	
Objects to Measure	
Mask of Objects:	Positive Cells 👻
Image to Measure:	Subtracted TRITC ~
0	
	Add feature on







Step 9: Measure

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











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