

MetaXpress Custom Module Editor

Measure Background Intensity in Whole Images

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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 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.

All Nuclei —		-71	
Nuclei Image		Су5 ~	
Approximate Mi	nimum Width (µm)	9.1	= 28 pixels
Approximate Ma	aximum Width (μm)	28.24	= 87 pixels
Intensity Above	Local Background	4117	Ð
Positive Marker			
Marker Image		GFP ~	
Stained Area		Both ~	
Approximate Minimum Width (µm) Approximate Maximum Width (µm) Intensity Above Local Background		9.7	= 30 pixels = 170 pixel
		55.16	
		6599	Ð
Algorithm		Fast 👻	
Nega <mark>t</mark> ive Nuclei	Negative Nuclei		
Positive Nuclei	Positive Nuclei		





Step 1: Find Objects

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







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Positive Nuclei



Positive Cytoplasm



Positive Cells





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Step 3: Modify Objects > Grow Objects

 Grow the All objects mask by a few pixels to avoid measuring background at the edges of the objects.

Grow Objects		+ >
Source Grow by (pixels)	All Cells *	
Result	All Cells with Surrounding A	
Description:		
Expands objects objects to touch	by the number of pixels that you	u specify. Allow
		Apply





Step 3: Modify Objects > Grow Objects

 Grow the All objects mask by a few pixels to avoid measuring background at the edges of the objects.







Step 4: Modify Objects > Invert Objects

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







Step 4: Modify Objects > Shrink Objects

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Step 5: Modify Objects > Invert Objects

 Invert the grown objects mask to select the background area







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 Invert the grown objects mask to select the background area







Step 6: Find Objects > Simple Threshold

• Set the threshold to 0-65535 on any of the input images to define a mask which represents the entire image area.







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Step 7: Measure

Segment Measure

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

atures within Each C Jask of Features: nage to Measure: nage to Measure:	Dbject: Background Area V GFP V Cy5 V	Intensity Center Y Integrated Intensity Average Intensity		Intensity Center Y_Average Integrated Intensity_Average
nage to Measure: nage to Measure:	Background Area × GFP × Cy5 ×	Integrated Intensity Average Intensity		Integrated Intensity_Avera
nage to Measure: nage to Measure:	GFP ~ *	Average Intensity		
nage to Measure:	Cy5 ~ 🗙	2 C C C C C C C C C C C C C C C C C C C	hand	GFP Background Intensity
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0		Minimum Intensity		Minimum Intensity_Avera
	Remove feature gr	ou Maximum Intensity		Maximum Intensity_Aver
atures within Each C	Dbject:	Intensity Center Y		Intensity Center Y_Aver
atures within Each O	Dbject:	Integrated Intensity		Integrated Intensity Av
nage to Measure:	GFP X	Average Intensity	•	Background Cy5 Intens
nage to Measure:	Cv5 ~	Intensity Std. Dev.		Intensity Std. DevAve
9		Minimum Intensity		Minimum Intensity_Ave
	Remove feature gr	oup. Maximum Intensity		Maximum Intensity_Av
	4) - 2290		1. mark	
	atures within Each (lask of Features; nage to Measure: nage to Measure:	Remove feature gr atures within Each Object: lask of Features: Background Area nage to Measure: GFP mage to Measure: Cy5 Remove feature gr	Minimum Intensity Remove feature grot Maximum Intensity Intensity Center Y Intensity Std. Dev. Minimum Intensity Remove feature group. Maximum Intensity	Remove feature grou Minimum Intensity Autres within Each Object: Intensity Center Y lask of Features: Background Area hage to Measure: GFP Cy5 Remove feature group Intensity Center Y Integrated Intensity Average Intensity Intensity Std. Dev. Intensity Std. Dev. Minimum Intensity Minimum Intensity



Step 7: Measure

- For Objects to Measure, select the Whole Image Mask. No measurements are needed unless you need intensity across the whole image.
- Include the 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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