

## **MetaXpress® 6 Software Guide**

Custom Module Editor Example: Objects within Objects

UNLEASH YOUR BRILLIANCE

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#### **Custom Module Editor Exercise Purpose**

The purpose of this exercise is to step the user through creating a custom module designed to measure cell based on morphology: round versus elongated cells

You will need the EX4 Cell Morphology data set to complete this exercise.





## **Getting Started**

- 1. Import the EX4 Cell Morphology image data set into MetaXpress
  - In the main menu, select Plate Data Utilities > Import Images
  - Click on **Select Directory** and navigate to the location of the image set
  - Select the EX4 Cell Morphology HTD file and enter a name for the Experiment Set
  - Click Import
- 2. Open the **Review Plate Data** dialog and select the EX4 Cell Morphology plate
- 3. Left-click and drag over the wells with images to open the thumbnail montage
- 4. Click on the thumbnail for well F01, Site 1







### **Getting Started**

- 5. On the **Run Analysis** tab, click on the **Create Custom Module** button
- The goal of this exercise is to create a custom module that classifies the cells based on round vs. elongated morphology
  - CY5: Cell marker
  - DAPI: Nuclear marker
- 7. We will be measuring the following parameters:
  - Nuclear Count, Average Area, Average Intensity
  - Round Cell Count, Average Area, Average Intensity
  - Elongated Cell Count, Average Area, Average Intensity





#### 8. Identify all objects

We need to create a segmentation of each cell. Although this can be done through multiple tools, using the nuclear marker (DAPI) can aid in better cytoplasmic segmentation.

- You will notice that both the DAPI and CY5 images have a high background. We can reduce this using the **Top Hat** tool under the **Modify Image** section in the ribbon.
- Use the **Top Hat** tool for both DAPI and CY5



2 Top Hat	<b>-</b> ×
Source	Cy5 (Alexa 647) 🔻
Size (pixels)	30
Filter Shape	Circle 🔻
Grayscale Reconstruction	
Result	Top Hat
Description:	
Finds small bright spots and size.	based on a filter shape
	Apply





- 8. Identify all objects continued
  - Next, use the Cell Scoring Application Module the Top Hat images of DAPI and CY5 to identify cells based on the nuclear (DAPI) and positive (CY5) marker.



Cell Scoring Objects	[Modified] 👻 🗙
All Nuclei —	
Nuclei Image	Clean DAPI 🔻
Approximate Minimum Width (µm)	5.2
Approximate Maximum Width (µm)	21.85
Intensity Above Local Background	1800
Positive Marker	
Marker Image	Clean Cy5 💌
Stained Area	Both 💌
Approximate Minimum Width (µm)	5
Approximate Maximum Width (µm)	36.23
Intensity Above Local Background	3000





- 8. Identify all objects continued
  - Use the **Logical Operations** tools found under the **Modify Objects** tools section in the ribbon to create a whole cell mask
  - Use the **OR Operation** to combine the positive nuclei and positive cytoplasm masks



Algorithm		Fast 🔻	
Negative Nu	uclei	Negative Nuclei	]
Positive Nuc	lei	Positive Nuclei	]
Positive Cyte	oplasm	Positive Cytoplasm	]
Description:			
			Apply
Logical O	peratior	ns [Mo	dified] 🔹 🕽
Logical O	peratior Positive	ns [Mo Nuclei v	dified] 🔹 🕽
Logical O Source 1 ( Source 2 (	peration Positive Positive	ns [Mo Nuclei ▼ Cytoplasm ▼	dified] 🔻
Logical O Source 1 ( Source 2 ( Operation (	Positive Positive OR 👻	ns [Mo Nuclei ▼ Cytoplasm ▼	dified] 🔻
Logical O Source 1 ( Source 2 ( Operation ( Result	Positive Positive OR -	ns [Mo Nuclei ▼ Cytoplasm ▼	dified] 🔻





9. Generate subpopulation (round vs. elongated) segmentation masks based on shape factor (roundness)

Add a **Filter Mask** step located under the **Modify Objects** tools to filter objects based on shape factor

• Set filter type to **MinFilter** and use a value of 0.7. This will create a mask of round objects.

Next we need to create a segmentation mask of the rest of the objects (elongated). Add a **Remove Marked Objects** step under the **Modify Objects** section

 Use the Logical Operations mask from step 8 for Objects and the Filter Mask from the previous step to create the Elongated cells segmentation mask



Image Sour	rce Top Hat	•		
Mask Sourc	Logical Op	perations 🔻		
Measu	urement	Shape Factor 💌		
Filter Type		MinFilter 🔻		
Minim	num Value	0.7		
Includ	le Min/Max Val	ues 🔲		
		Delete		
Add Filt	er	Delete		
Add Filt Result	Filter Mask	Delete		
Add Filt Result Description	er Filter Mask			
Add Filt Result Description Remove o values of t	er Filter Mask bjects from the be objects.	mask based on measureme		



Marker Source	Filter Mask 🔻
1	
lesult	Remove Marked Objects
Description:	
Compares the c	objects in two images. If any part of a





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#### The Measure Tab: Hierarchy of Measurement

- 10. Steps 8 and 9 have resulted in several masks
- 11. The next step is to make measurements. Click on the Measure tab and select the mask and images as shown below from the drop-down menus

Objects: mask of all objects that will eventually be measured (i.e. whole cell mask)

Features: masks that contain objects or subpopulations found in the mask of all objects (i.e. nuclei, round cells, elongated cells)

8	Measure Mask	[Modified]
-		
	Measurement Inputs	·
	Standard Area Value	1
	Create Object Overlay	r 📝
	Objects to Measure	
	Mask of Objects:	Whole Cell Mask 🔻
1	Image to Measure:	Cy5 (Alexa 647) 🔹 📖 🗙
1	0	
	Features within Each C	Dbject:
	Mask of Features:	Positive Nuclei 🔻
	Image to Measure:	DAPI • ×
	0	
		Remove Feature Group
Í	Features within Each C	bject:
	Mask of Features:	Round Cells 🔻
	Image to Measure:	Cy5 (Alexa 647) 🔹 📖 🗙
	0	
		Remove Feature Group
	Features within Each C	bject:
	Mask of Features:	Elongated Cells 🔻
	Image to Measure:	Cy5 (Alexa 647) 🔹 🛄 🗙
	0	
		Remove Feature Group
		Add Feature Group
	Descriptions	
	Description:	
		Apply





#### The Measure Tab: Configure measurements

- Click on the ellipses (...) button to display the Measurement Selection Configuration dialog
- There are over 50+ Available measurements whose names are customizable
- The measurements are arranged in **Average** and **Sum** columns
- Measurements under the Average column give statistics for the average of the objects being measured
- Measurements under the Sum column give statistics for the sum of the objects being measured
- For example:
  - For **Objects to Measure**, Average and Sum statistics will be the same
  - For **Features within Each Object**, Average statistics will give the average of the objects found and sum will give you the total

Measurement Name	Average	Column Label	Sum	Column Label	
Total Area		Total Area_Average		Total Area_Sum	
Hole Area	<b>V</b>	Hole Area_Average		Hole Area_Sum	
Area		Area_Average		Area_Sum	
Relative Hole Area		Relative Hole Area_Average		Relative Hole Area_Sum	
Standard Area Count	<b>V</b>	Standard Area Count_Avera		Standard Area Count_Sum	
Width		Width_Average		Width_Sum	
Height		Height_Average		Height_Sum	
Centroid X		Centroid X_Average		Centroid X_Sum	
Centroid Y		Centroid Y_Average		Centroid Y_Sum	
Intensity Center X		Intensity Center X_Average		Intensity Center X_Sum	
Intensity Center Y	V	Intensity Center Y_Average		Intensity Center Y_Sum	
Integrated Intensity	V	Integrated Intensity_Averag		Integrated Intensity_Sum	
Average Intensity	✓	Average Intensity_Average		Average Intensity_Sum	
Intensity Std. Dev.		Intensity Std. DevAverage		Intensity Std. DevSum	
Minimum Intensity	✓	Minimum Intensity_Average		Minimum Intensity_Sum	
Maximum Intensity		Maximum Intensity_Average		Maximum Intensity_Sum	
Perimeter	✓	Perimeter_Average		Perimeter_Sum	
Shape Factor		Shape Factor_Average		Shape Factor_Sum	
Fiber Length		Fiber Length_Average		Fiber Length_Sum	
Fiber Breadth		Fiber Breadth_Average		Fiber Breadth_Sum	
Length		Length_Average		Length_Sum	
Orientation		Orientation_Average		Orientation_Sum	
Breadth		Breadth_Average		Breadth_Sum	
Ell. Form Factor		Ell. Form Factor_Average		Ell. Form Factor_Sum	
Divel Central V		Divel Centroid V Average		Dival Controld V Sum	



#### **Configuring Measurements**

- 12. Click on the ellipses (...) button next to **Objects to Measure** (Nuclei)
  - Deselect the Average column
  - Select the following under the Sum column
    - Total Area
    - Average Intensity
- Name each measurement as desired
- 13. Click on the ellipses (...) button next to **Features Within Each Object** (Nuclei, Round Cells, Elongated Cells)
  - Select the following under the Average column
    - Total Area
    - Average Intensity
  - Select the following under the Sum column
    - Feature Count
  - Name each measurement as desired
  - Repeat this for each feature
- 14. You can now run, save, and test on other wells the custom module to make sure settings are optimized.

Measurement Name	Average	Column Label	Sum	Column Label
Total Area	<b>v</b>	Total Area_Average		Total Area_Sum
Hole Area	<b>v</b>	Hole Area_Average		Hole Area_Sum
Area	<b>V</b>	Area_Average		Area_Sum
Relative Hole Area	<b>V</b>	Relative Hole Area_Average		Relative Hole Area_Sum
Standard Area Count		Standard Area Count_Avera		Standard Area Count_Sum
Width	<b>V</b>	Width_Average		Width_Sum
Height	<b>V</b>	Height_Average		Height_Sum
Centroid X		Centroid X_Average		Centroid X_Sum
Centroid Y		Centroid Y_Average		Centroid Y_Sum
Intensity Center X		Intensity Center X_Average		Intensity Center X_Sum
Intensity Center Y		Intensity Center Y_Average		Intensity Center Y_Sum
Integrated Intensity		Integrated Intensity_Averag		Integrated Intensity_Sum
Average Intensity		Average Intensity_Average		Average Intensity_Sum
Intensity Std. Dev.		Intensity Std. DevAverage		Intensity Std. DevSum
Minimum Intensity	<b>V</b>	Minimum Intensity_Average		Minimum Intensity_Sum
Maximum Intensity	<b>V</b>	Maximum Intensity_Average		Maximum Intensity_Sum
Perimeter		Perimeter_Average		Perimeter_Sum
Shape Factor	<b>V</b>	Shape Factor_Average		Shape Factor_Sum
Fiber Length	<b>V</b>	Fiber Length_Average		Fiber Length_Sum
Fiber Breadth	<b>V</b>	Fiber Breadth_Average		Fiber Breadth_Sum
Length		Length_Average		Length_Sum
Orientation		Orientation_Average		Orientation_Sum
Breadth		Breadth_Average		Breadth_Sum
Ell. Form Factor		Ell. Form Factor_Average		Ell. Form Factor_Sum
Divel Central V		Dival Controid V Average		Dival Controid V Sum





#### Final Segmentation Mask Example

Cy5 (Alexa 647)

Cy5 (Alexa 647)









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#### Support Resources

- F1 / HELP within MetaXpress® Software
- Support and Knowledge Base: <u>http://mdc.custhelp.com/</u>
- User Forum: <a href="http://metamorph.moleculardevices.com/forum/">http://metamorph.moleculardevices.com/forum/</a>
- Request Support: <u>http://mdc.custhelp.com/app/ask</u>
- Technical Support can also be reached by telephone:
  - 1 (800) 635-5577
  - Select options for Tech Support → Cellular Imaging Products → ImageXpress Instruments





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