

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 Puncta in Nuclei (objects within objects).

You will need the EX1 NuclearFoci data set to complete this exercise.





CME Exercise: Puncta in Nuclei

- 1. Import the EX1 Nuclear Foci 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 folder and click **OK**
 - Select the **EX1 Nuclear Foci** HTD file and enter a name for the **Experiment Set**
 - Click on the Import button
- 2. Open the **Review Plate Data** dialog and select the EX1NuclearFoci plate
- 3. Left-click and drag over the wells with images to open the thumbnail montage
- 4. Click on the thumbnail for well C08







CME Exercise: Puncta in Nuclei

- 5. On the **Run Analysis** tab, click on the **Create Custom Module** button
- 6. The goal of this exercise is to create a custom module that measures puncta in the nucleus only. In this image set:
 - CY5: Nuclei
 - FITC: Antibody aggregates (puncta)
- 7. We will be measuring the following parameters:
 - Nuclear area
 - Nuclear average intensity
 - Puncta count
 - Puncta average area
 - Puncta average intensity
 - Puncta total intensity









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Suggested Workflow

8. Identify primary objects (Cy5 channel)

We are only interested in spots found within the nuclei, so our first steps should be to identify nuclei. This can be done using

- Auto-Find Blobs
- Find Blobs
- Count Nuclei Application Module

Select the **Find Blobs icon** under the **Find Objects** menu in the ribbon. Click-to-Find tool to select nuclei. You may need to adjust the parameters manually to find the optimal values.

NOTE You can pre-process the image using the **LoG** tool under the **Modify Image** section to help optimize segmentation. This step must be added before the **Find Blobs** step.



2 Find Blobs	* ×
Source	Cy5 v
Approximate Minimum Width (μm)	7
Approximate Maximum Width (μm)	20
Intensity Above Local Background	3000
Result Contemporation:	Find Blobs
Identifies irregularly shaped objects	s using size and intensity
criteria that you specify. Does not s	separate objects that are
touching.	Apply





Suggested Workflow

9. Identify Puncta (FITC channel)

The next step is to identify all the puncta (antibody aggregates). This can be done using:

- Find Round Objects
- Granularity Application Module

If using the Granularity Application Module, it may not be necessary to do step # 9 finding the nuclei.

At this point, you should have two segmentation masks: Nuclei and Puncta

(OPTIONAL): You can use the **Filter Mask** tool to remove large artifacts in the segmentation

NOTE You can pre-process the image using the **Top Hat** tool under the Modify Image section to help optimize segmentation.



Source	FITC -
Approximate Minimum Width (µm)	0.5
Approximate Maximum Width (µm)	2
Intensity Above Local Background	500
Result	Find Round Objects
Rescription:	
Identifies small, symmetrically round	d objects using size and





The Measure Tab: Hierarchy of Measurement

- 10. Steps 8 and 9 have resulted in two segmentation masks that identify all of our objects of interest
- 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

Measurement Inputs Standard Area Value 1 Create Object Overlay Objects to Measure Mask of Objects: Nuclei Image to Measure: Cy5 	×	Objects: outer area or larger objects containing everything we want to measure (i.e. Nuclei)
Peatures within Each Object: Mask of Features: Puncta ▼ Image to Measure: FITC ▼	×	Features: inner or smaller thing inside the larger objects (i.e. Puncta)

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	V	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	V	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 X 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
 - Features Count
- Name each measurement as desired
- 13. Click on the ellipses (...) button next to Features Within Each Object (Puncta)
 - Select the following under the Average column
 - Total Area
 - Average Intensity
- Select the following under the Sum column
 - Integrated Intensity
 - Feature Count
 - Name each measurement as desired
- 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		Total Area_Average		Nuclear Area
Hole Area		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		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		Intensity Center Y_Average		Intensity Center Y_Sum
Integrated Intensity		Integrated Intensity_Averag		Integrated Intensity_Sum
Average Intensity		Average Intensity_Average		Nuclear Average Intensity
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
Pixel Centroid X		Pixel Centroid X_Average		Pixel Centroid X_Sum
Pixel Centroid Y		Pixel Centroid Y_Average		Pixel Centroid Y_Sum
Line Length		Line Length_Average		Line Length_Sum
Features Count				Nuclei Count





Final Segmentation Mask Example







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Support Resources

- F1 / HELP within MetaXpress® Software
- Support and Knowledge Base: <u>http://mdc.custhelp.com/</u>
- User Forum: <u>http://metamorph.moleculardevices.com/forum/</u>
- 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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