

## **MetaXpress® 6 Software Guide**

Using Application Module Objects Tools in CME

UNLEASH YOUR BRILLIANCE

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#### **Chapter Purpose**

The purpose of this chapter is to describe the **Application Module Objects** tools available in the **Custom Module Editor** (CME) plugin.

**Application Module Objects** tools analyze grayscale (8 or 16-bit) images and generate a segmentation (1-bit binary) mask using the same principles as **Application Modules** in MetaXpress. The segmentation mask that is created can then be used to make measurements or modified further using tools available in CME.

For detailed descriptions of each **Application Module**, refer to the corresponding chapters. Note that certain Image and Cell Measurements available in the Application Modules through **Review Plate Data** may not be available in **CME**.





#### **Application Module Objects Tools Overview**



#### 16-Bit Image



#### **Binary Mask**



- Use **Application Modules** to find objects of interest with predefined algorithms
- Objects are identified by size and/or pixel intensity
- Source image must be grayscale (8-bit or 16-Bit) image
- Result image is a segmentation (1-Bit binary) image that can be used to make measurements or can be modified further in CME





#### **Ribbon: Application Module Objects Tools**



Clicking on an **Applications Module Objects** tool icon will add a step card on the panel to the left





## **Application Module Objects Basic Descriptions**

- Identifies and differentiates tubules and nodes
- Identifies cells as positive or negative for a secondary marker
- Classifies cells as live or dead based on stains that mark all or live cells, and dead cells
  - Detects, analyzes, and quantifies mitotic cells with monopolar and bipolar spindles
- Determines if a specific fluorescent probe can be detected both outside and inside one or more compartments, with fine control over the size of the compartments and overlapping areas
- Identifies the cell cycle phase of each nucelus in an image
- Detects cells using a nuclear stain

Angiogenesis Objects

Cell Scoring Objects

Live Dead Objects

Monopole Objects

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Translocation Enhanced Objects

Cell Cycle Objects

Count Nuclei Objects

Micronuclei Obiects

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Neurite Outgrowth Objects

Translocation Objects

Cell Health Objects

Granularity Objects

Mitotic Index Objects

Transfluor Objects

- Identifies interphase cells containing micronuclei, bi-nucleated and multi-nucleated cells, and optional stains for additional wavelengths to facilitate cell identification.
- Identifies neurite extensions from the cell body of neurons
- Determines if a specific fluorescent probe can be detected both outside and inside one or more compartments
- Classifies cells as live, early apoptotic, late apoptotic, or necrotic based on markers for apoptosis and necrosis (dead cells)/
- Identifies puncta (granules) and nuclei in cells
- Identifies cells as Mitotic or in Interphase
- Identifies two categories of puncta (Pits/Vesicles) based on size and nuclei in cells



## Workflow of an Application Module Card

Application Module Objects tools have a similar workflow as Application Modules in Review Plate Data.



## **Click-to-Find Tool**





- Click-to-Find is enabled when s is highlighted orange
- Use crosshairs to click on objects of interest in the source image.
- To delete objects, deselect the **Click-to-Find** tool, highlight the object and press **Delete** on the keyboard
- Size and intensity parameters in the step card will be updated (see below).
- Select 5-7 objects then click **Apply**.
- Examine results. If necessary, adjust numbers manually or use **Click-to-Find** tool to select more objects.



## Application Modules: Review Plate Data vs. CME

In **Review Plate Data**, all objects identified in the application module can be associated with a single cell. In the example below, the orange segmentation represents a single cell with its associated granules.



In **CME**, objects identified with an application module are NOT associated with a single cell. Instead, a separate segmentation mask is created for each object type. In this example, two masks are created: one for nuclei and one for granules







## **Application Module Objects: Count Nuclei**



- Count Nuclei can be used to find nuclei (or other similarly shaped round objects)
- Uses one source (wavelength) image
- Count Nuclei Objects can be used instead of the Find Blobs or Auto Find Blobs in the Find Objects section.





#### Application Module Objects: Cell Scoring







**Cell Scoring** can be used to identify cells as positive or negative for a secondary marker

Uses two source images: one for nuclei and the other for the positive marker

Select the **Stained Area** appropriate for the positive marker: Nuclear, Cytoplasmic, or Both

- Creates three masks:
  - Positive Nuclei: nuclei that are associated with cells positive for marker
  - Positive Cytoplasm: cytoplasmic area of cells positive for marker

• **Negative Nuclei:** nuclei that are NOT associate with cells positive for marker \*NOTE\* Combine the **Positive** and **Negative Nuclei** images using logical operations to create a mask of all nuclei. Refer to chapter on **Modify Objects** for details.



### **Application Module Objects: Granularity**

Source images









- **Granularity** can be used to find puncta (granules) and nuclei in cells \*Granules may be any subcellular compartment that is round or blobbed (Examples include vesicles, lysosomes, autophagosomes, fragmented ER or mitochondria, etc.)
- Uses two source images: one for Granules the other for Nuclei Creates two masks for each category
- Granularity Objects can be used instead of the Find Round Objects and Auto Find Blobs / Find Blobs steps under the Find Objects section



#### **Application Module Objects: Transfluor**



Transf	luor Objects	[Modified] 🔻 🗙
Pits And	Vesicles Image	FITC 🔻
- 🗸 Use	Pits	
Appro	kimate Minimum Width (μm)	1
Appro	kimate Maximum Width (μm)	4
Intensi	ty Above Local Background	1000
- 🔽 Use	Vesicles	
Appro	kimate Minimum Width (μm)	4
Approximate Maximum Width (µm)		10
Intensity Above Local Background		2000
- Nuclea	r Image —	
Nuclear Image		DAPI -
Approximate Minimum Width (µm)		5
Appro	kimate Maximum Width (μm)	30
Intensi	ty Above Local Background	100
Algorith	m (	Fast 🔻
Nuclei	Nuclei	
Pits	Pits	
Vesicles	Vesicles	
Descripti	on:	
Detects characte	G-Protein Coupled Receptor eristics.	(GPCR) cycling
		Apply

- **Transfluor** can be used to find two categories of puncta (pits/vesicles) that are different sizes and the nucleus \*Pits and Vesicles are any subcellular compartments that are round or blobbed. The difference between them is their size.
- If only one population of subcellular compartments is desired, either use the **Granularity** application module or deselect the **Use Vesicles** box
- Uses two source images: one for Pits/Vesicles and the other for Nuclei



#### **Application Module Objects: Cell Cycle**

DNA Content — DNA Content Ch Approximate Mir				
DNA Content Ch Approximate Mir				
Approximate Mir	annel	Cv5 -		
	nimum Width (um)	5		
Approximate Ma	ximum Width (um)	30		
Intensity Above I	ocal Background	100	æ	
-Background Su	btraction			
Background Su	btraction	AutoConstant 💌	Gray Levels	
Classification P	· Interested Interest		1941	
Classification b	y integrated intensi	(x 1000)		
G0/G1 (2N)		200		
5 Phase		200		
Mitotic Classificat	tion			
Method		MitoticSpecificStaining		
Mitotic Classifica	Mitotic Classification Channel			
Intensity Above L	ocal Background	100		
	Classification			
Apontotic Classif	ication Channel	(v5 x		
Stained Area	cation channel	Both •		
Stained Area		5		
Approximate Mar	vinum Width (um)	30		
Intensity Above I	ocal Background	100	(F)	
Intensity Above L	ocal background	100		
Algorithm	F	ast 💌		
Cell Cycle Objects	Cell Cycle Objects			
G0G1	G0G1			
S Phase	S Phase			
G2	G2			
Early M	Early M	_ ' <b>`</b>		
Late M	Late M			
Apoptotic	Apoptotic			
Description				
Identifies the cell of	cycle phase of each	cell in an image.		



- **Cell Cycle** can be used to classify cells in the stages of the cell cycle using the **DNA Content Channel**
- Classifies cells as mitotic with a specific stain or using **DNA Content Channel** Image
- (Optional) Classify cells as **Apoptotic** using a separate apoptotic stain image
- Uses integrated intensity measurements to classify cells into the stages of interphase

\*NOTE\* Molecular Devices recommends determining values of parameters through the application module in **Review Plate Data** and then entering them in CME





- **Neurite Outgrowth** can be used to measure neurite extensions from the cell body
- Uses two source images: one for neurons and the other for Nuclei (optional). Images can be transmitted light or fluorescence. Nuclear Stain image helps identify cells more easily
- Identify nuclei, cell bodies, and outgrowths by size and intensity measurements
  - Use Minimum Area parameter to exclude small cell bodies that were identified
  - Use Minimum Cell Outgrowth parameter to exclude short outgrowths from the final measurements





#### Application Module Objects: Micronuclei

Micronuclei Objects		[Modified] 👻 🗙	
Nuclear Channel	6	N5 •	
- Nuclei	L	.95 *	
Approximate Minimu	m Width (μm)	5	
Approximate Maximu	30		
Intensity Above Local	100		
Maximum Distance o	f Nuclei in Polynucleated Cells (um)	7	
Mitotic Cell Minimum	Intensity	500	
Mitotic Cell Minimum	50		
Exclude Border Nucle			
c Micronuclei			
Approximate Minimu	m Width (um)	1	
Approximate Maximu	um Width (um)	10	
Intensity Above Local	Background	100	
Minimum Distance Er	rom Main Nucleus (um)		
Maximum Distance F	rom Main Nucleus (µm)	10	
	ion main nacieus (pin)	10	
Apoptotic-specific	Staining		
Apoptotic Channel		Cy5 -	
Minimum Intensity		100	
Minimum Coverage (	%)	50	
Necrotic-specific S	Staining		
Necrotic Channel	Necrotic Channel		
Minimum Intensity		100	
Minimum Coverage (	(96)	50	
Probe A			
Probe A Channel		Cy5 •	
Minimum Intensity		100	
Minimum Coverage (	(96)	50	
Probe B			
Probe B Channel		Cy5 •	
Minimum Intensity		100	
Minimum Coverage (	(%)	50	
Mononucleated Mono	onucleated		
Binucleated Binuc	leated		
Multinucleated Multin	nucleated		
Mitotic Mitot	ic		
Micronuclei Micro	nuclei		
Apoptotic Apop	totic		
Necrotic Necro	otic		
Probe A Probe	A		
Probe B Probe	B		
Probe AB Probe	AB		
Description: Identifies interphase of nucleated cells, and op cell identification.	ells containing micronuclei, bi-nucle ptional stains for additional wavelen	ated and multi- gths to facilitate	
		Apply	



- **Micronuclei** can be used to measure the number of nuclei per cell, the formation of micronuclei, and mitotic state
  - Micronuclei are identified based on the user-specified distance
  - Determine number of nuclei per cell by setting the max distance nuclei can be separated by and still be considered one cell
- Optionally can identify up to 4 additional stains: apoptosis, necrosis, Probe A, and Probe B



#### **Application Module Objects: Live Dead**

Apply

Description:

Identifies both live and dead cells in an image.



- cells
  - Identify either the nuclei, cytoplasm, or both for each channel Creates three masks that can be modified further or used to make measurements with
    - Live Dead Objects: mask of all identified live and dead cells
    - Live: cells categorized as live
    - Dead: cells categorized as dead





#### **Application Module Objects: Monopole**

2 Monopole Objects [Modified] • ×	Source images	Monopole Objects	Interphase
DNA Structures DNA Channel Approximate Minimum Width (µm) 5 Approximate Maximum Width (µm) 30 Intensity Above Local Background 100 Microtubules Microtubule Channel Classification by Correlation with DNA Image			
Bipole Correlation Level 0.3 Monopole Correlation Level 0.6	Bipole	Monopole	All Masks
Algorithm Fast  Monopole Objects Interphase Interphase Bipole Bipole Monopole	<b>\$</b>		
Description: Detects, analyzes, and quantifies mitotic cells with monopolar and bipolar spindles. Apply	/	•	

- **Monopole** can be used to classify cells as interphase or mitotic. If mitotic, cells are secondarily classified as having bipolar spindles or monopole spindles.
- Uses two source images: one for DNA stain and the other for Microtubules
- Classify as interphase, bipolar, or monopolar by adjusting the correlation levels (can have values -1 to 1).





#### 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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#### ADVANCING PROTEIN AND CELL BIOLOGY