MetaXpress® 6 Software Guide

Using Application Module Objects Tools in CME

Date Revised 07/10/15 Version B

MOLECULAR DEVICES
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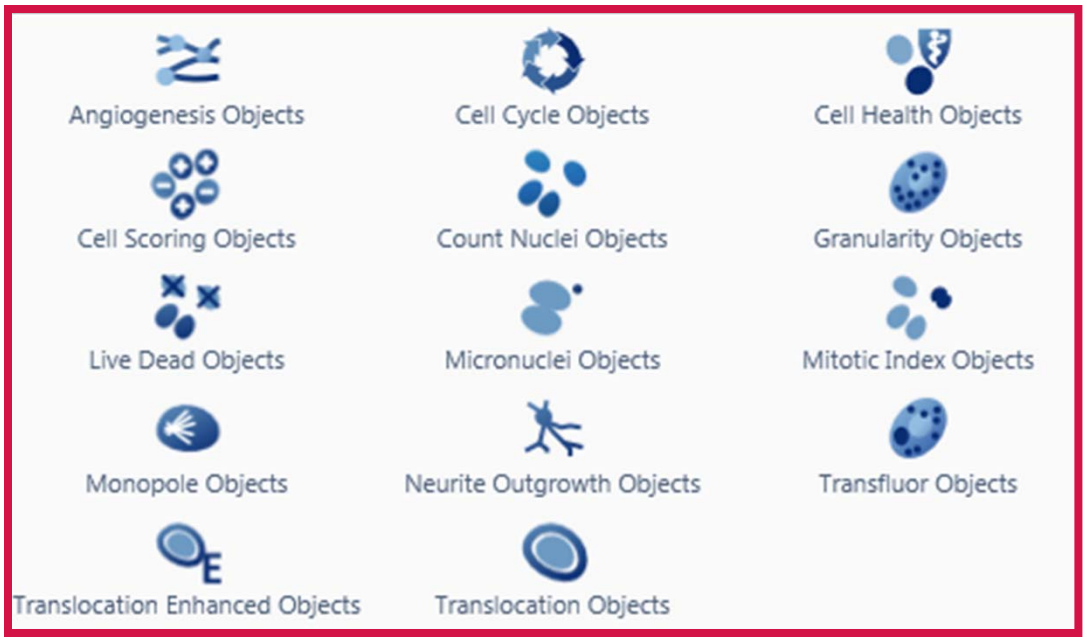
Chapter Purpose

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

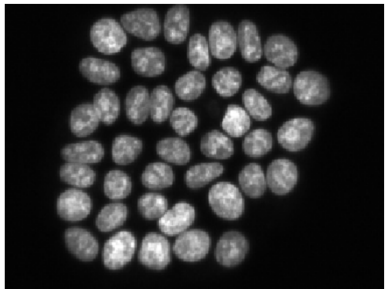
Application Module Objects tools analyze grayscale (8 or 16-bit) images and generate a segmentation (1-bit binary) mask using the same principles as **Application Modules** in MetaXpress. The segmentation mask that is created can then be used to make measurements or modified further using tools available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

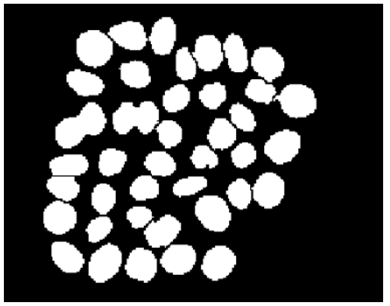
Application Module Objects Tools Overview



16-Bit Image

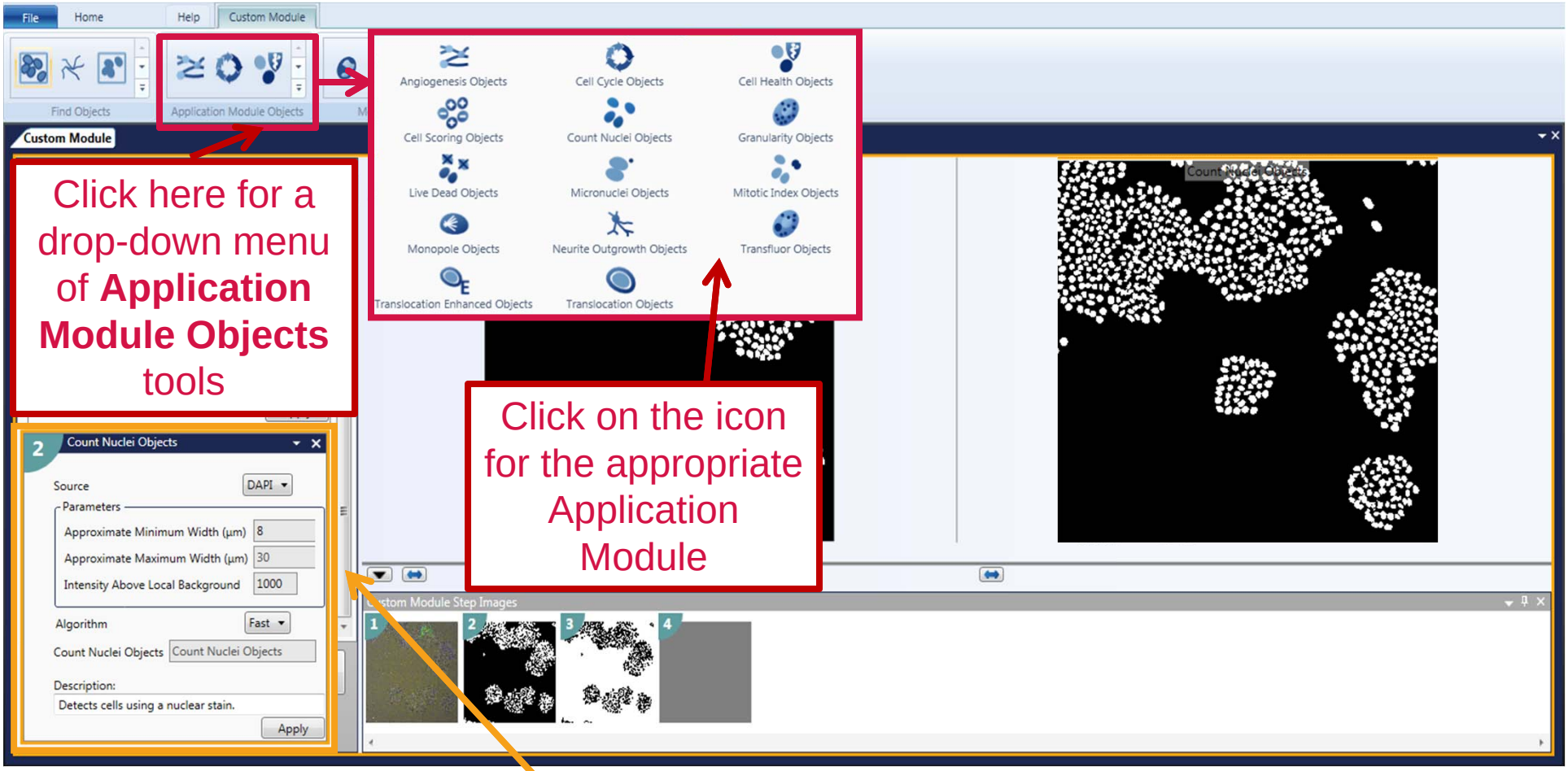


Binary Mask



- Use **Application Modules** to find objects of interest with pre-defined 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

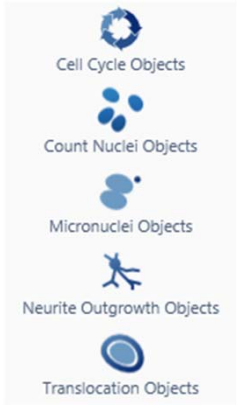
Ribbon: Application Module Objects Tools



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

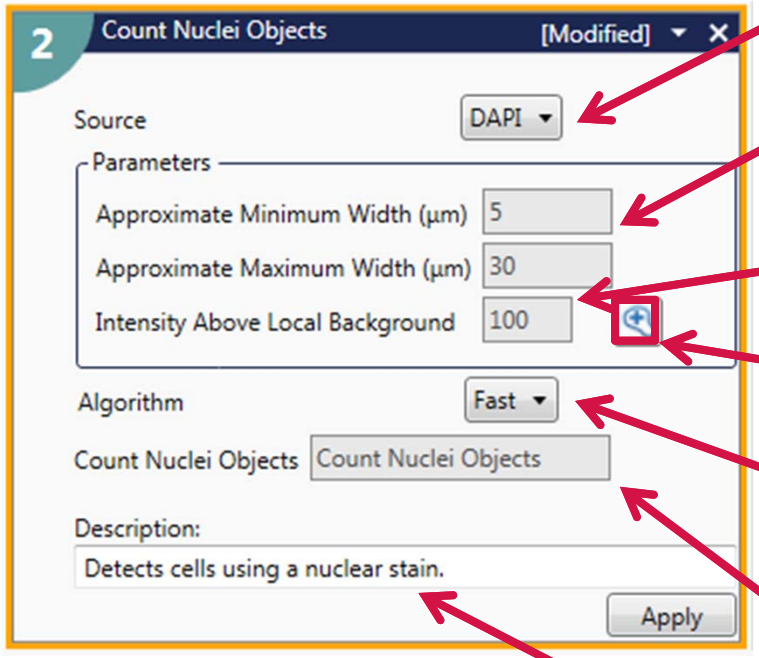


- Identifies the cell cycle phase of each nucelus in an image
- Detects cells using a nuclear stain
- Identifies interphase cells containing micronuclei, bi-nucleated and multi-nucleated cells, and optional stains for additional wavelengths to facilitate cell identification.
- Identifies neurite extensions 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.**



Select image to use for finding objects

Define the size of objects

Set intensity threshold for identifying objects

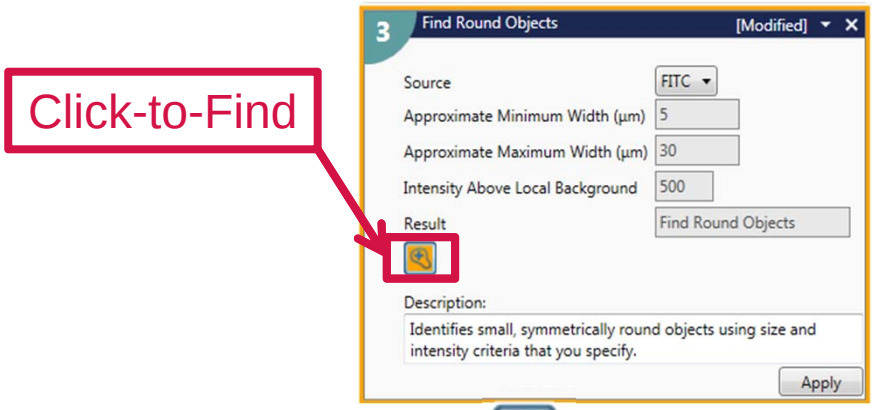
Click-to-Find tool: see next section

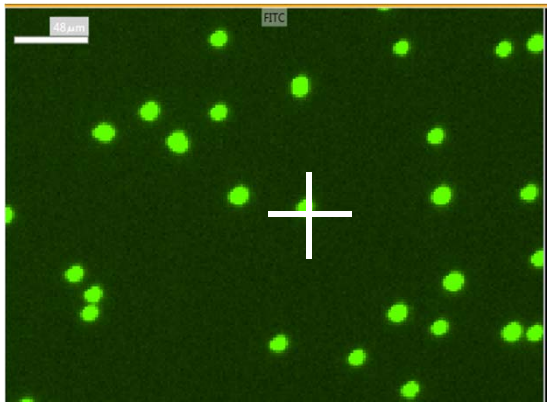
Select **Fast** or **Standard Algorithm**

Specify the name of the resulting image so that you may reference it later

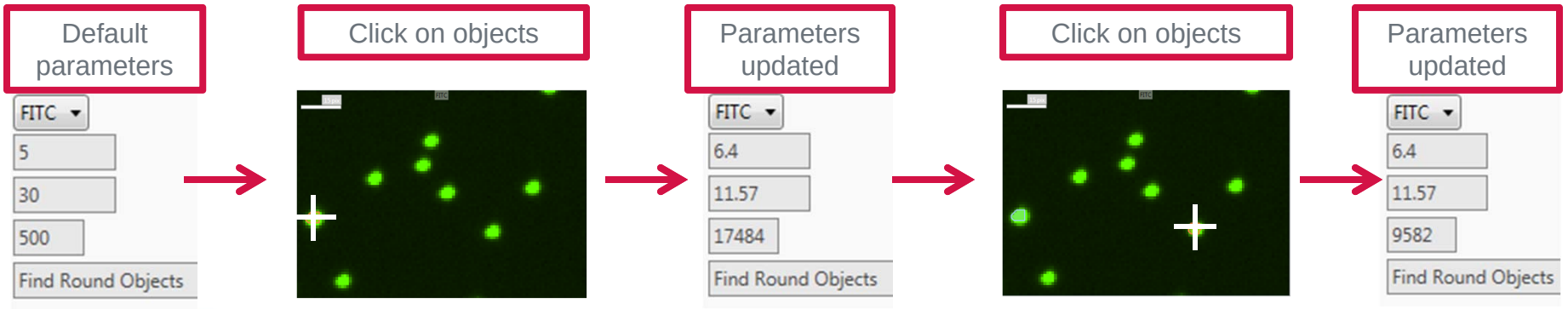
If desired, enter a note. Default text is a description of the step.

Click-to-Find Tool



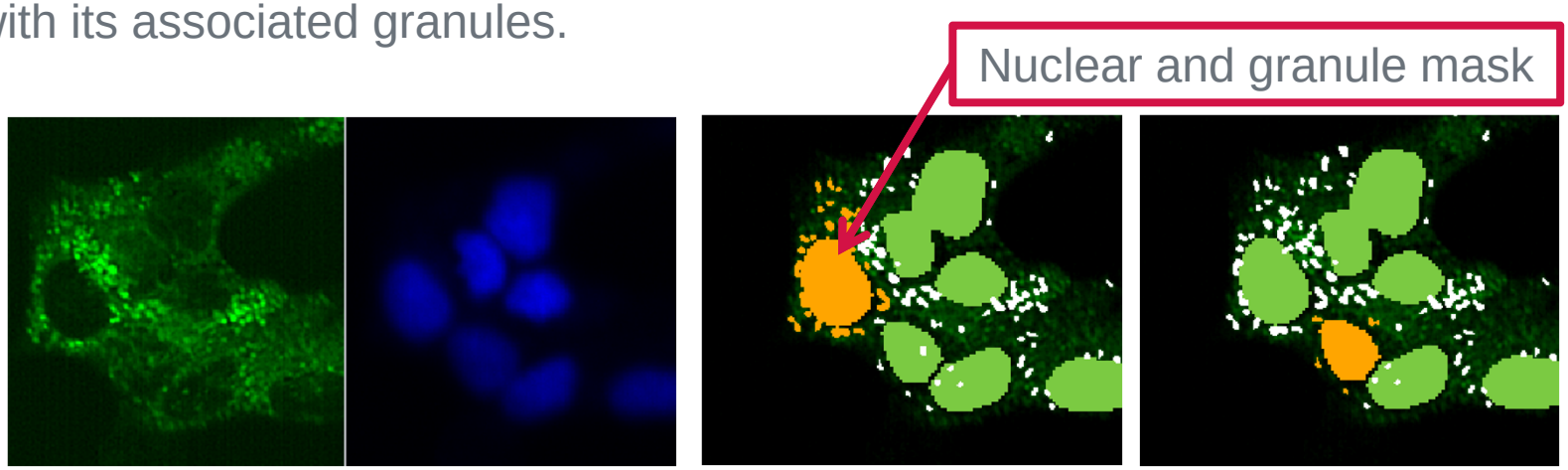


- **Click-to-Find** is enabled when  is highlighted orange
- Use crosshairs to click on objects of interest in the source image.
- To delete objects, deselect the **Click-to-Find** tool, highlight the object and press **Delete** on the keyboard
- Size and intensity parameters in the step card will be 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.

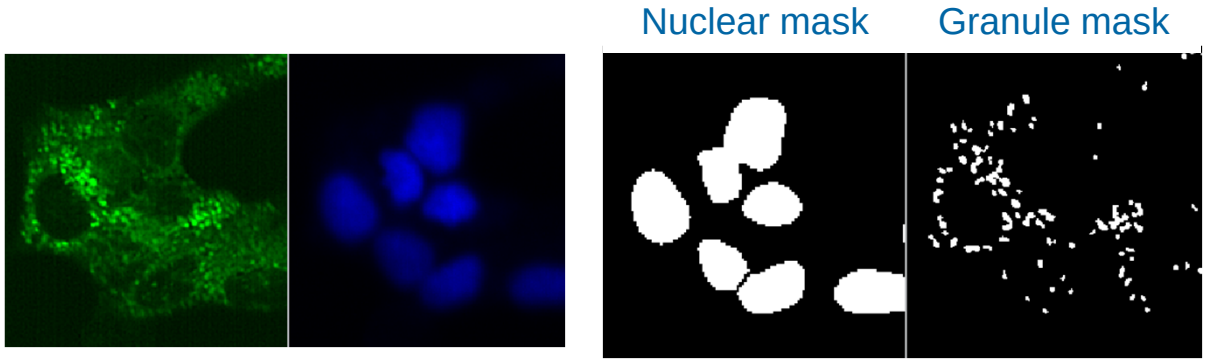


Application Modules: Review Plate Data vs. CME

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.

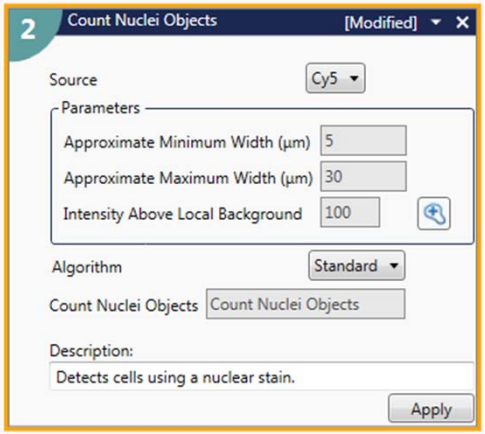


In **CME**, objects identified with an application module are NOT associated with a single cell. Instead, a separate segmentation mask is created for each object type. In this example, two masks are created: one for nuclei and one for granules

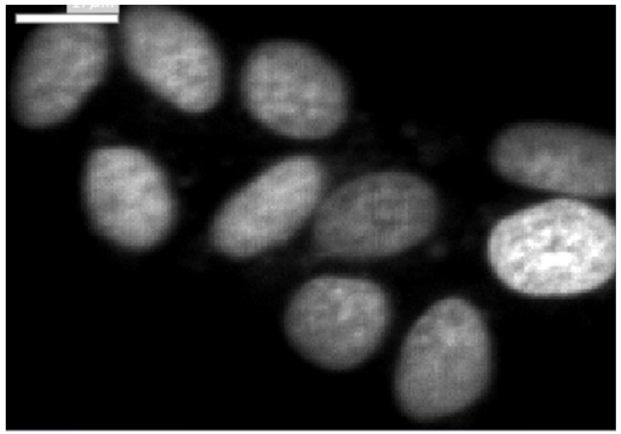


Application Module Objects: Count Nuclei

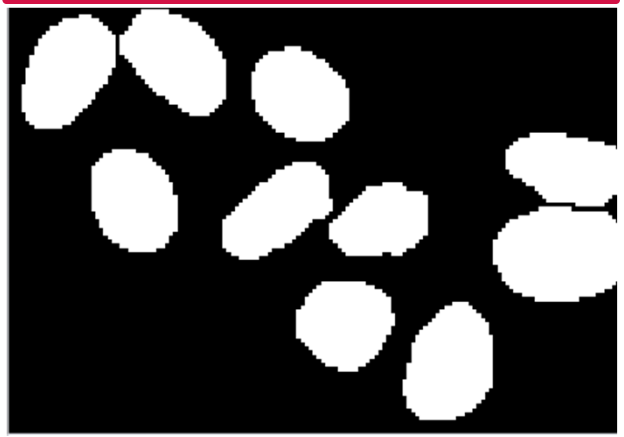
Count Nuclei Objects Tool



Source Image



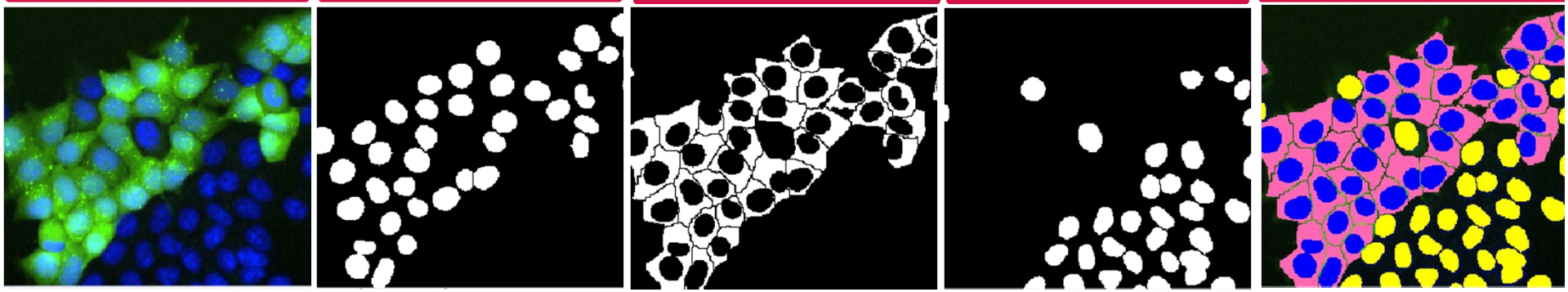
Resulting Segmentation Mask

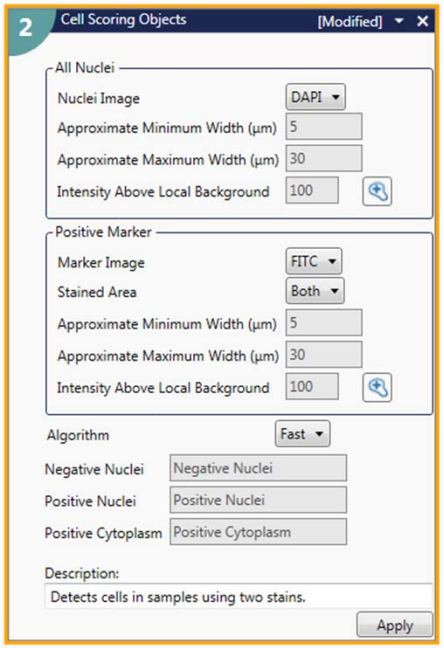


- **Count Nuclei** can be used to find nuclei (or other similarly shaped round objects)
- Uses one source (wavelength) image
- **Count Nuclei Objects** can be used instead of the **Find Blobs** or **Auto Find Blobs** in the **Find Objects** section.

Application Module Objects: Cell Scoring

Source images	Positive nuclei	Positive Cytoplasm	Negative Nuclei	All Masks



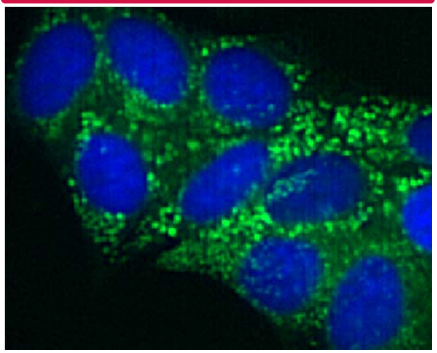


- **Cell Scoring** can be used to identify cells as positive or negative for a secondary marker
- Uses two source images: one for nuclei and the other for the positive marker
- Select the **Stained Area** appropriate for the positive marker: Nuclear, Cytoplasmic, or Both
- Creates three masks:
  - **Positive 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

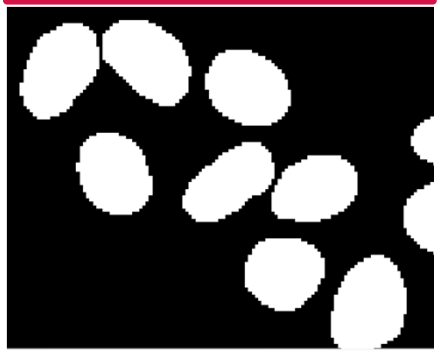
NOTE Combine the **Positive** and **Negative Nuclei** images using logical operations to create a mask of all nuclei. Refer to chapter on **Modify Objects** for details.

Application Module Objects: Granularity

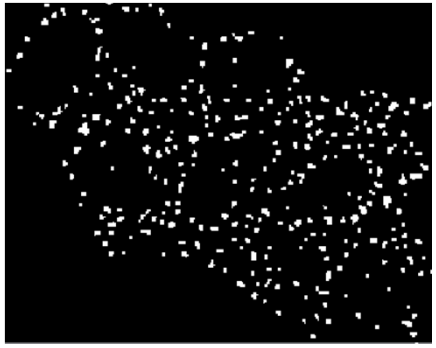
Source images



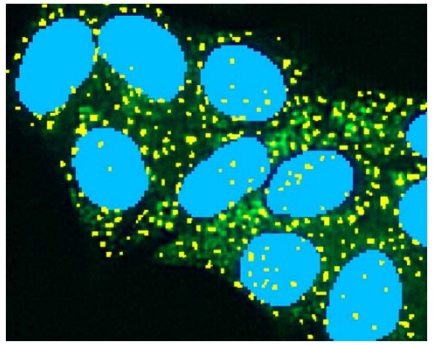
Nuclei

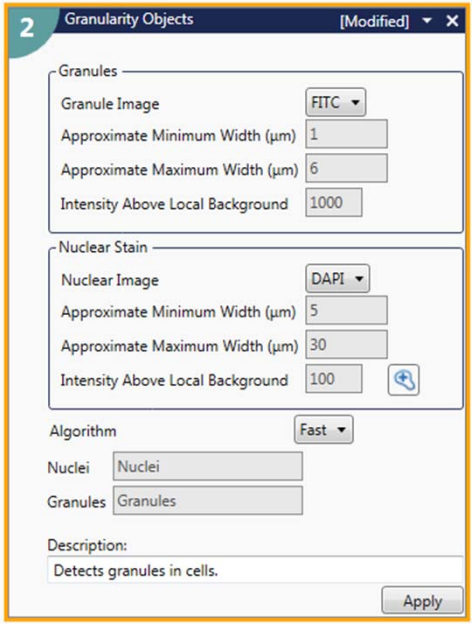


Granules



All Masks

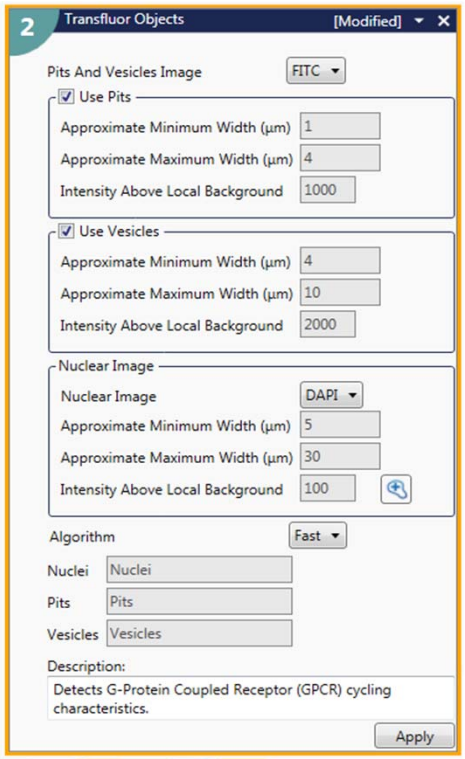




- **Granularity** can be used to find puncta (granules) and nuclei in cells
  *Granules may be any subcellular compartment that is round or blobbed (Examples include vesicles, lysosomes, autophagosomes, fragmented ER or mitochondria, etc.)
- Uses two source images: one for Granules the other for Nuclei
- 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

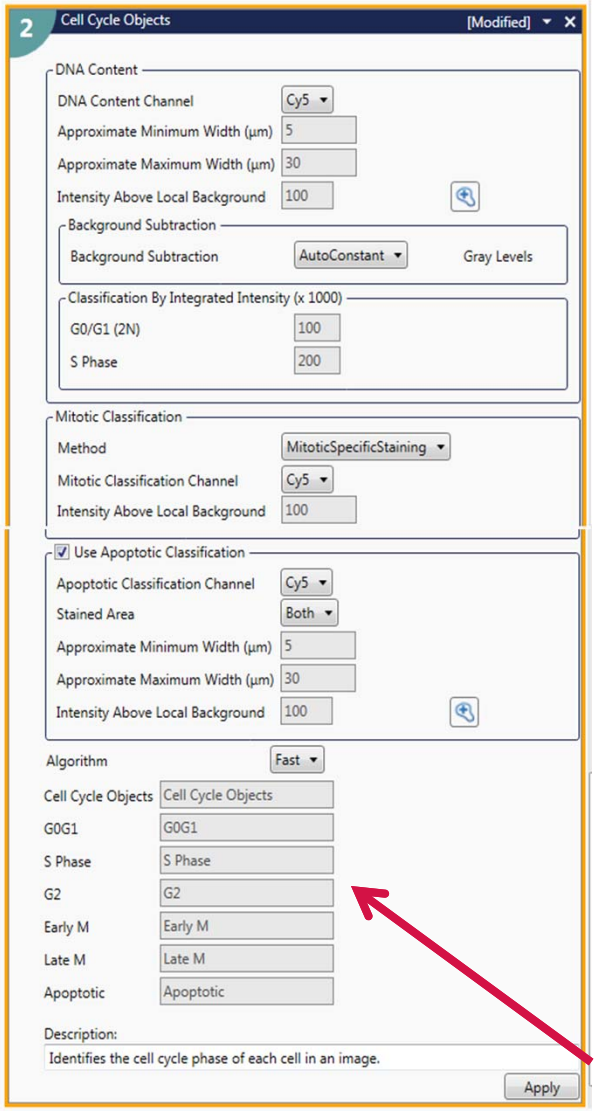
Application Module Objects: Transfluor

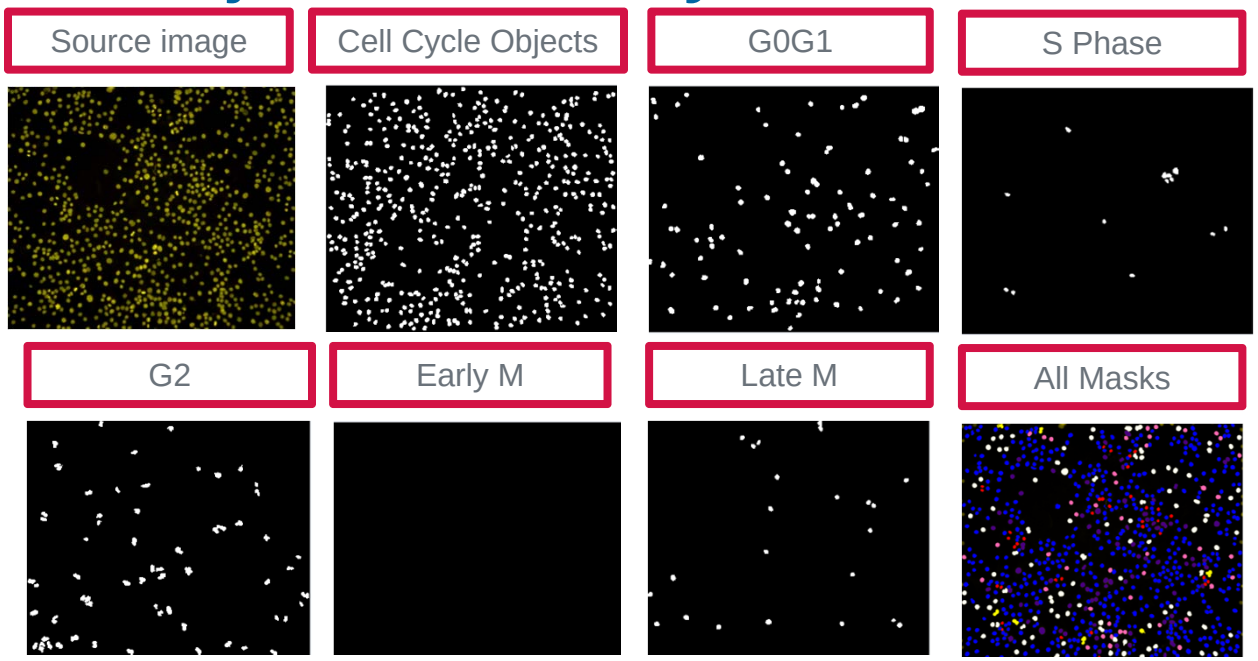
Source images	Nuclei	Pits	Vesicles	All Masks



- **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

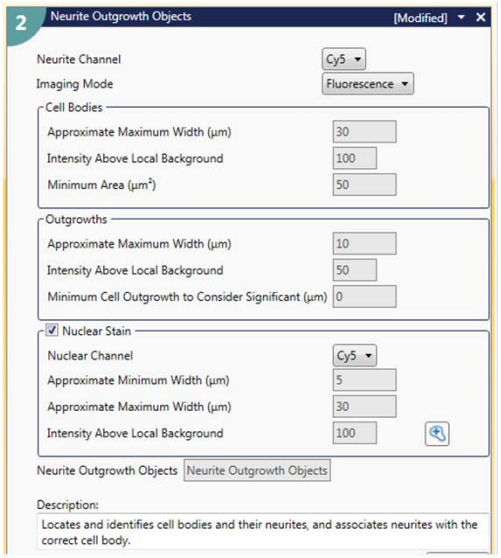




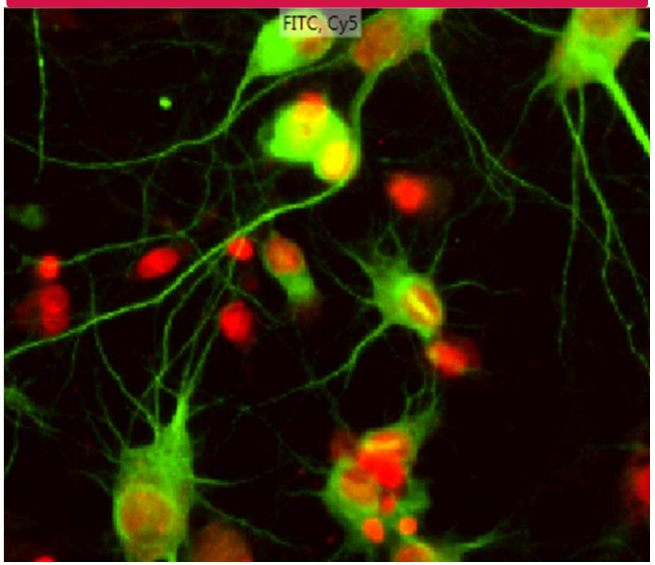
- **Cell Cycle** can be used to classify cells in the stages of the cell cycle using the **DNA Content Channel**
- Classifies cells as mitotic with a specific stain or using **DNA Content Channel** Image
- (Optional) Classify cells as **Apoptotic** using a separate apoptotic stain image
- Uses integrated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

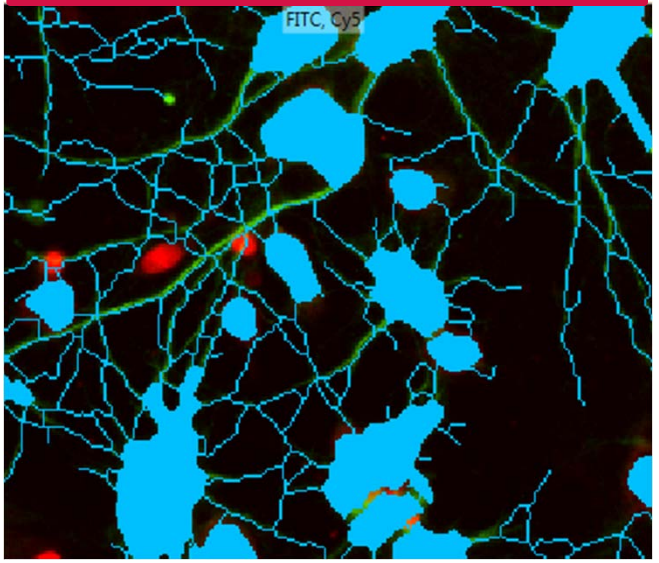
Application Module Objects: Neurite Outgrowth



Source Image

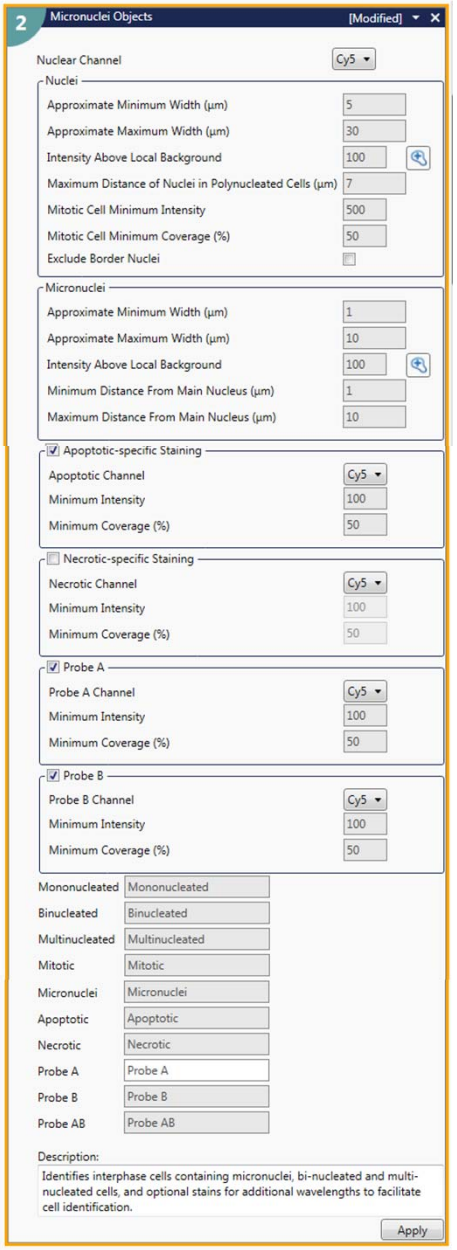


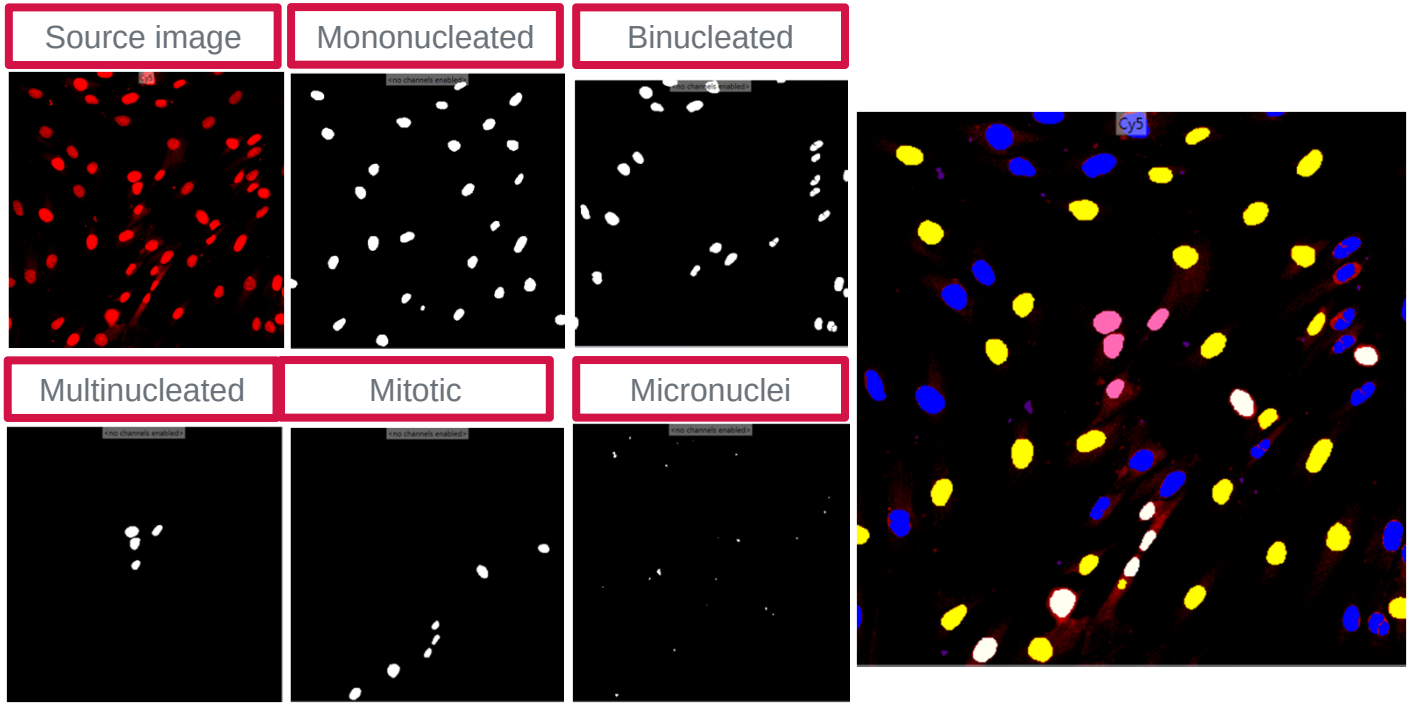
Neurite Outgrowth Mask



- **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

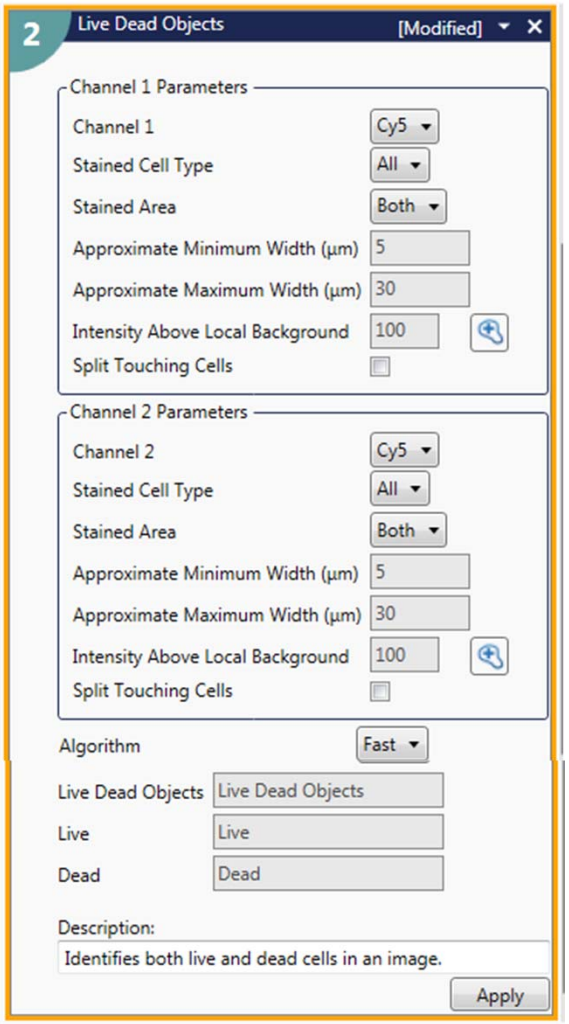
Application Module Objects: Micronuclei

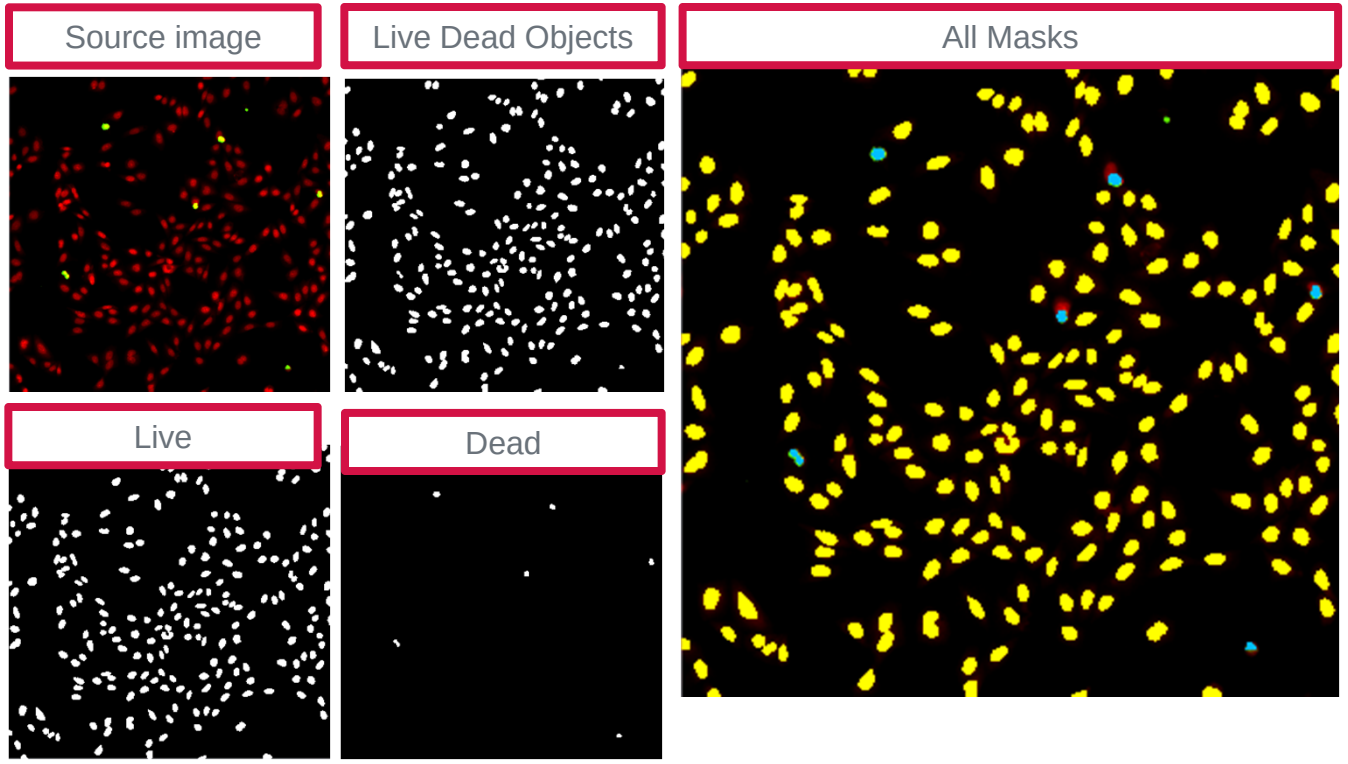




- **Micronuclei** can be used to measure the number of nuclei per cell, the formation of micronuclei, and mitotic state
  - Micronuclei are identified based on the user-specified distance
  - Determine number of nuclei per cell by setting the max distance nuclei can be separated by and still be considered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

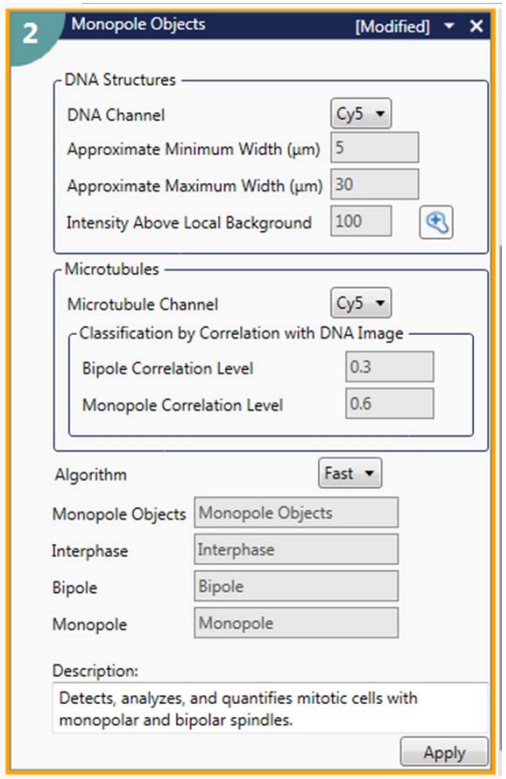
Application Module Objects: Live Dead

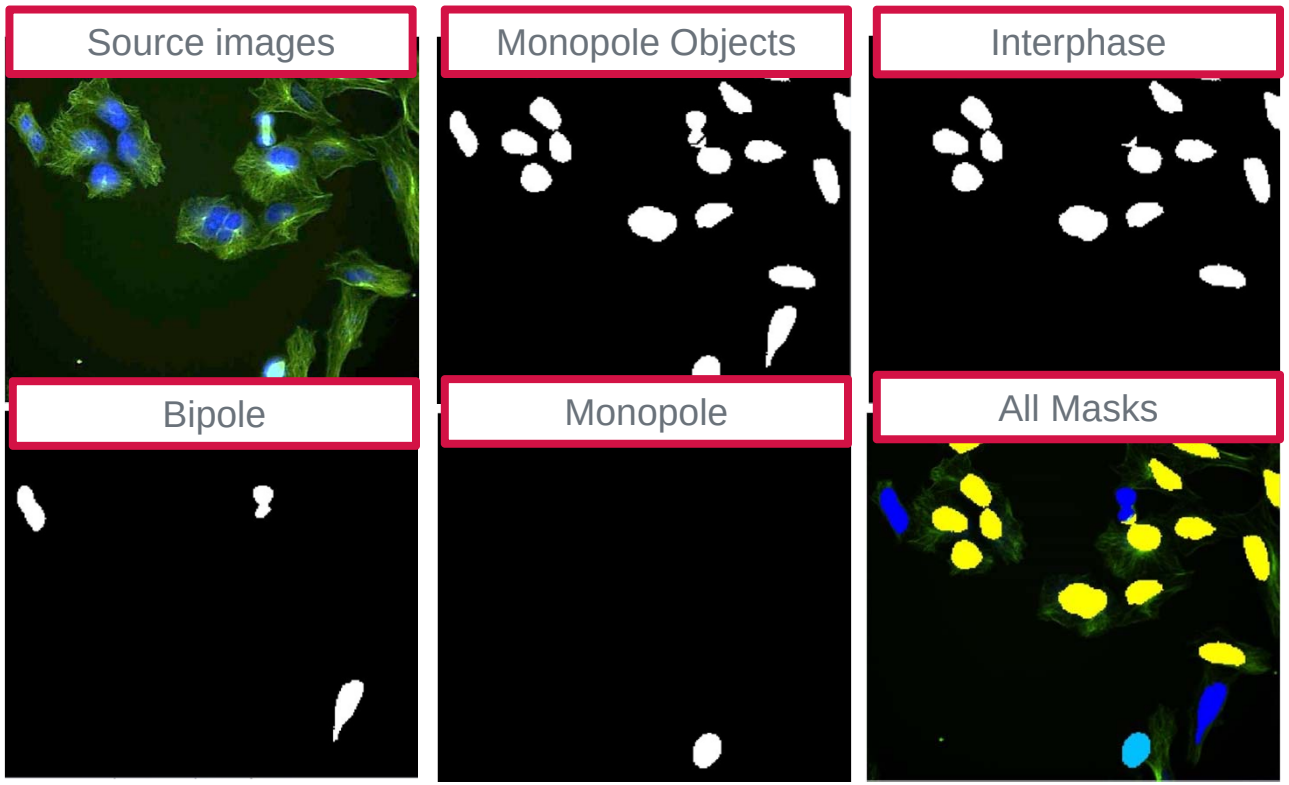




- **Live Dead** can be used to categorize cells as live or dead
- Uses two source (wavelength) images to mark all, live, or dead cells
- Identify either the nuclei, cytoplasm, or both for each channel
- Creates three masks that can be modified further or used to make measurements with
  - **Live Dead Objects:** mask of all identified live and dead cells
  - **Live:** cells categorized as live
  - **Dead:** cells categorized as dead

Application Module Objects: Monopole





- **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: http://mdc.custhelp.com/
- User Forum: http://metamorph.moleculardevices.com/forum/
- Request Support: http://mdc.custhelp.com/app/ask
- Technical Support can also be reached by telephone:
  - 1 (800) 635-5577
  - Select options for Tech Support → Cellular Imaging Products → ImageXpress Instruments