MetaXpress® 6 Software Guide

Custom Module Editor Example: Objects within Objects

Date Revised 07/10/15 Version B

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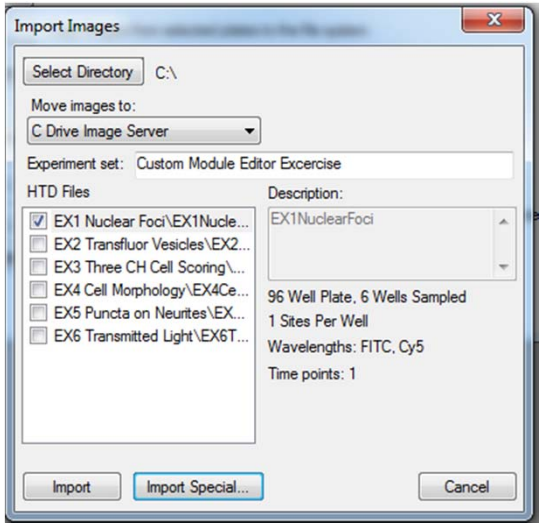
Custom Module Editor Exercise Purpose

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

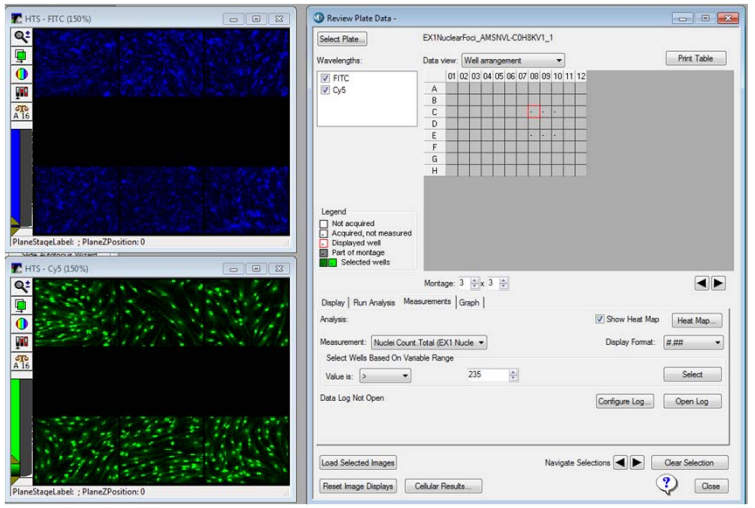
1. Import the EX1 Nuclear Foci image data set into MetaXpress
   - In the main menu, select **Plate Data Utilities > Import Images**
   - Click on **Select Directory** and navigate to the location of the image set folder and click **OK**
   - Select the **EX1 Nuclear Foci** HTD file and enter a name 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**

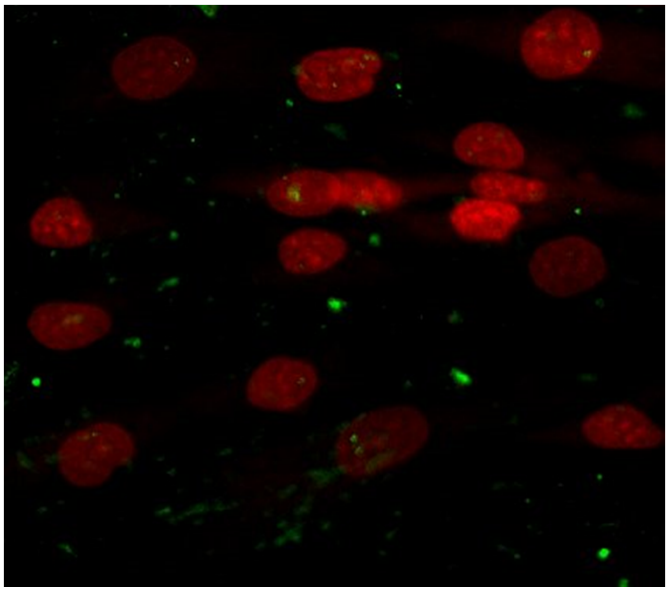


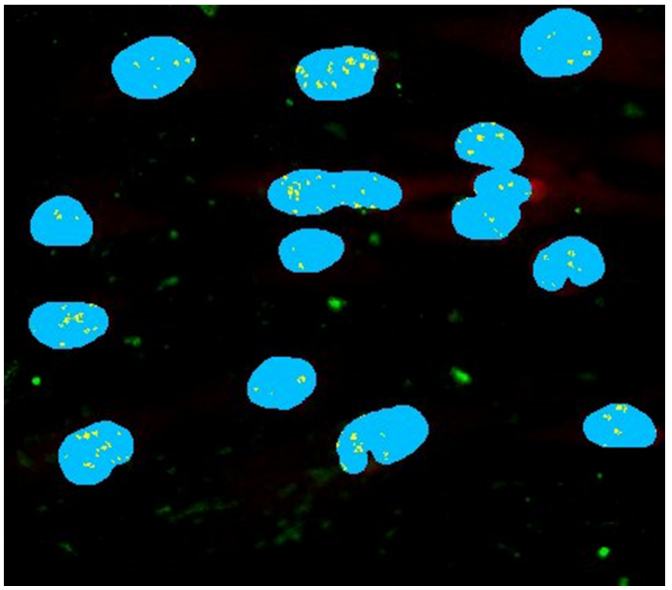
CME Exercise: Puncta in Nuclei

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





Suggested Workflow

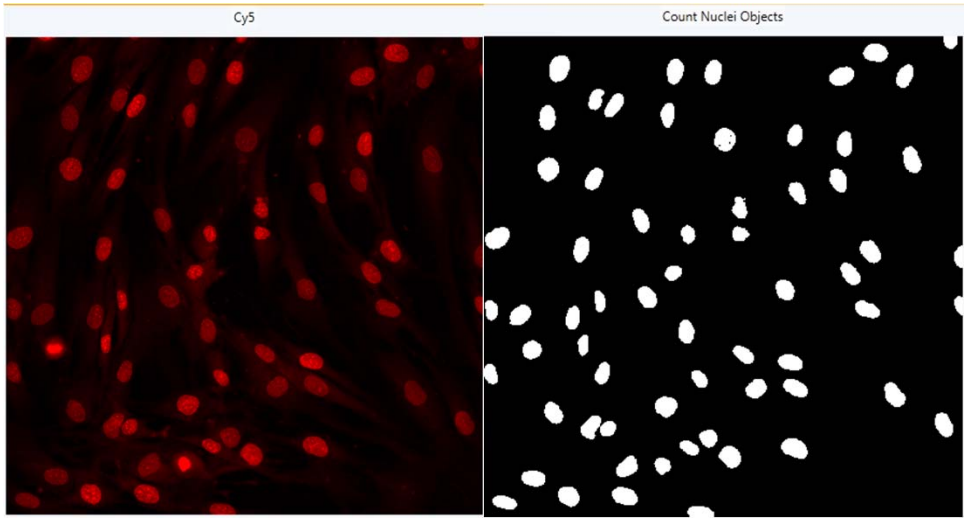
8. Identify primary objects (Cy5 channel)

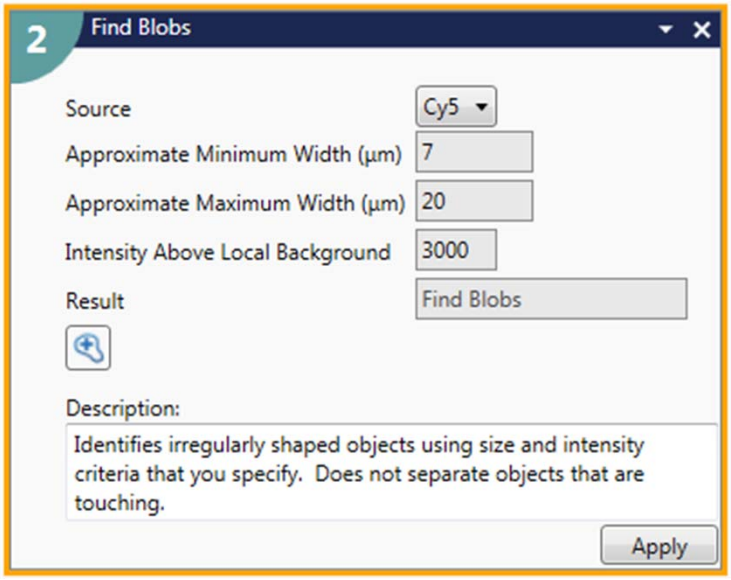
We are only interested in spots found within the nuclei, so our first steps should be to identify nuclei. This can be done using

- **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.





Suggested Workflow

9. Identify Puncta (FITC channel)

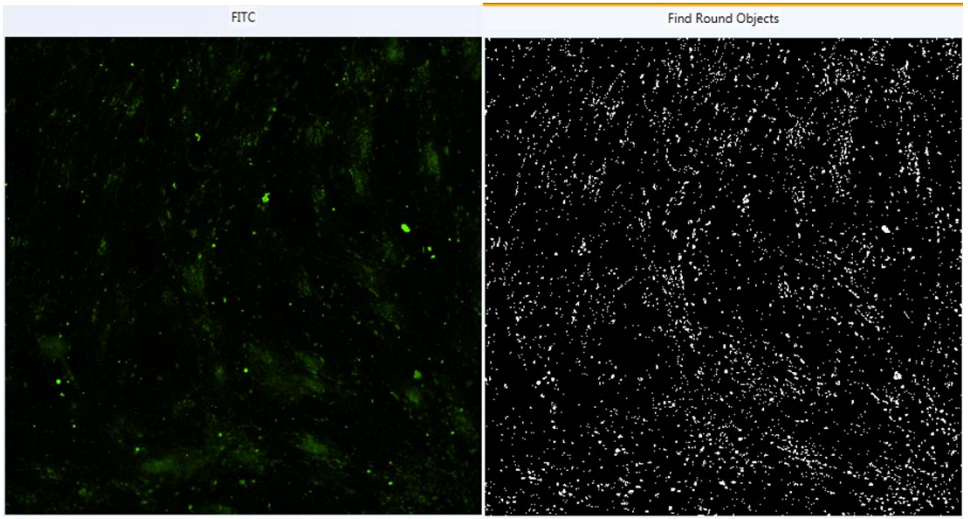
The next step is to identify all the puncta (antibody aggregates). This can be done using:

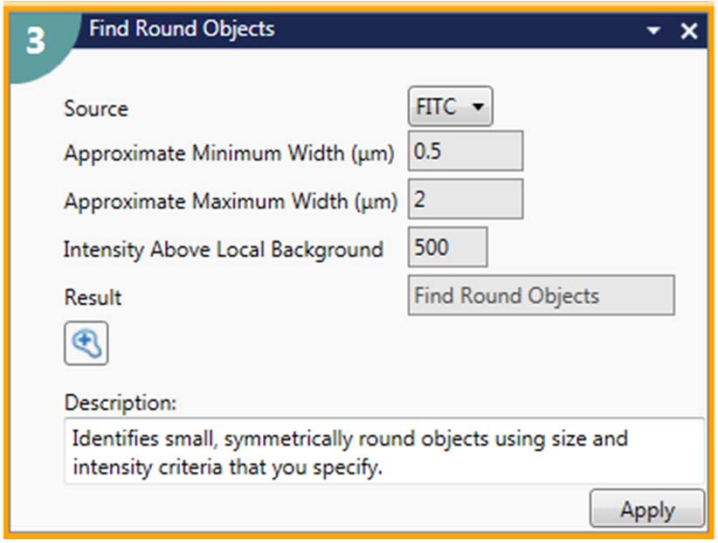
- **Find Round Objects**
- **Granularity Application Module**

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



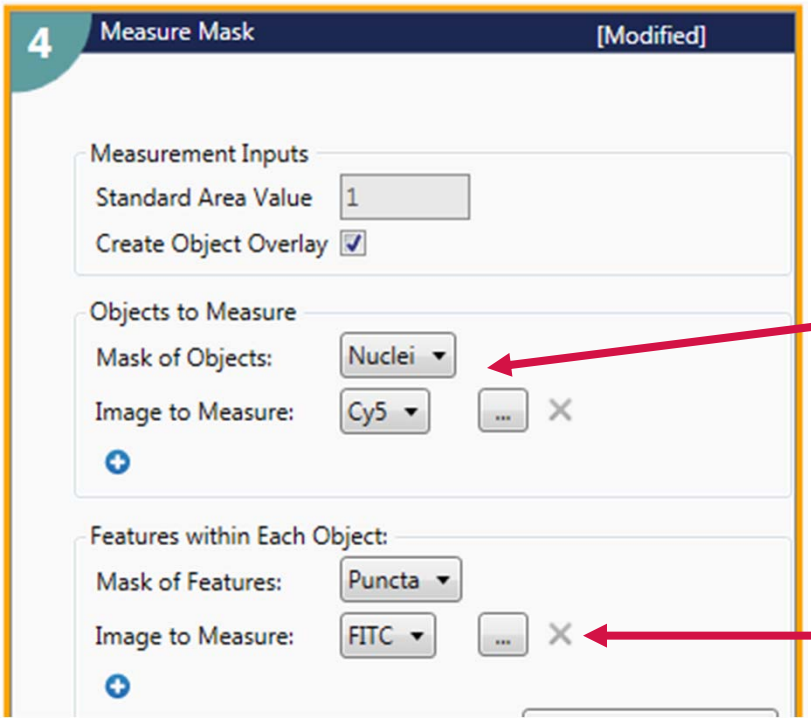


NOTE You can pre-process the image using the **Top Hat** tool under the Modify Image section to help optimize segmentation.

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

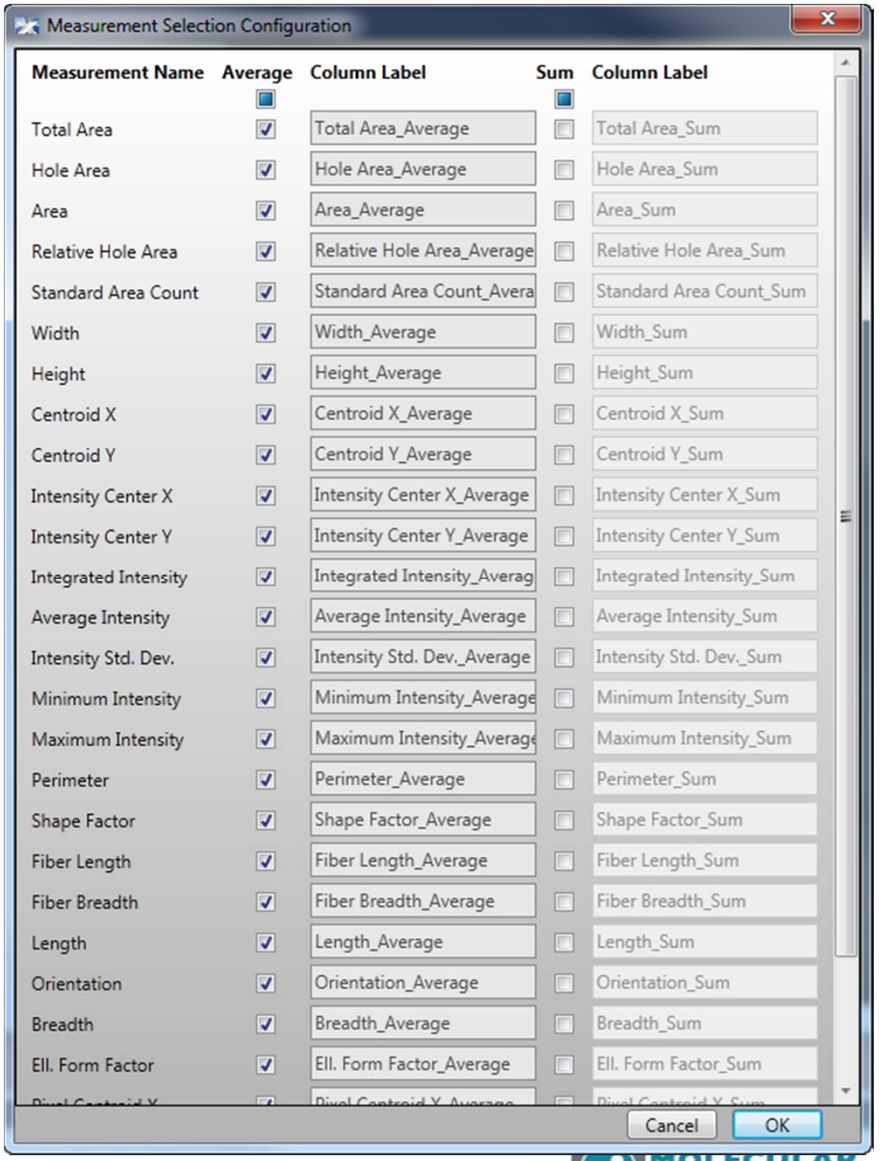


Objects: outer area or larger objects containing everything we want to measure (i.e. Nuclei)

Features: inner or smaller things 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

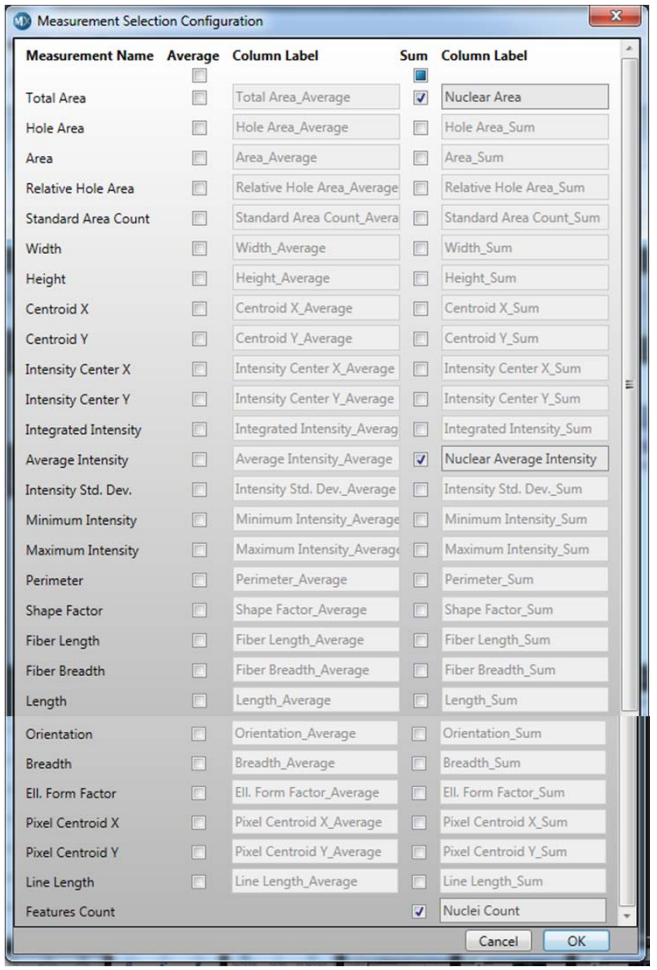


Configuring Measurements

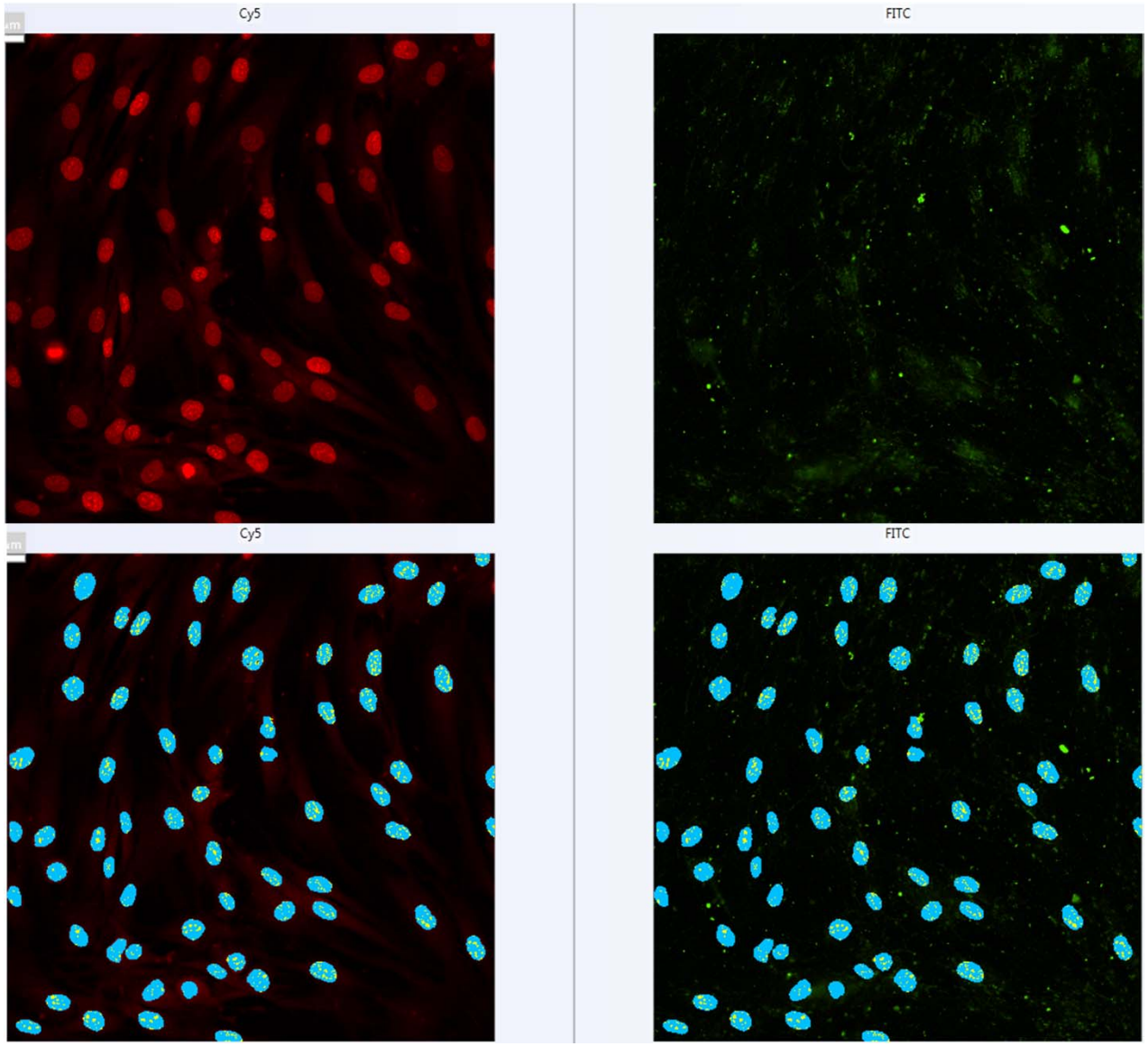
12. Click on the ellipses **(…)** button next to **Objects to Measure** (Nuclei)
    - Deselect the **Average** column
    - Select the following under the **Sum** column
        - **Total Area**
        - **Average Intensity**
        - **Features Count**
- Name each measurement as desired

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.



Final Segmentation Mask Example



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