MetaXpress® 6 Software Guide

Quick Start Guide for Custom Module Editor

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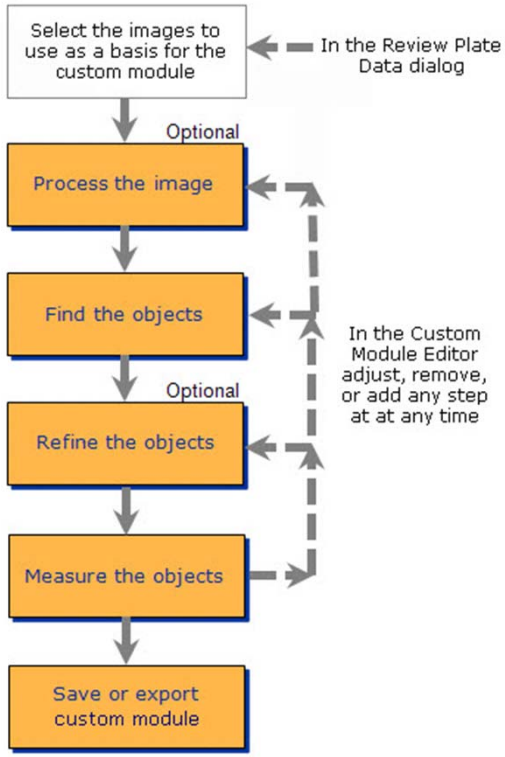
Why Use the Custom Module Editor?

Not all images can be analyzed using just an Application Module. In some cases, you may want to process the images or filter out unwanted objects before measuring. You may want to run more than one application module to generate the desired measurements. For these situations, the **MetaXpress Custom Module Editor** can be used to build a custom analysis that is ran through the **Review Plate Data** dialog just like an application module. Custom modules can also be run through **MetaXpress PowerCore™** Software.

The workflow for using the **Custom Module Editor (CME)** is shown on the right. The key steps are:

- Select your images and open the Editor
- Add steps to the analysis to find objects in the images
- Measure the objects
- Save the module and run analysis on the plate



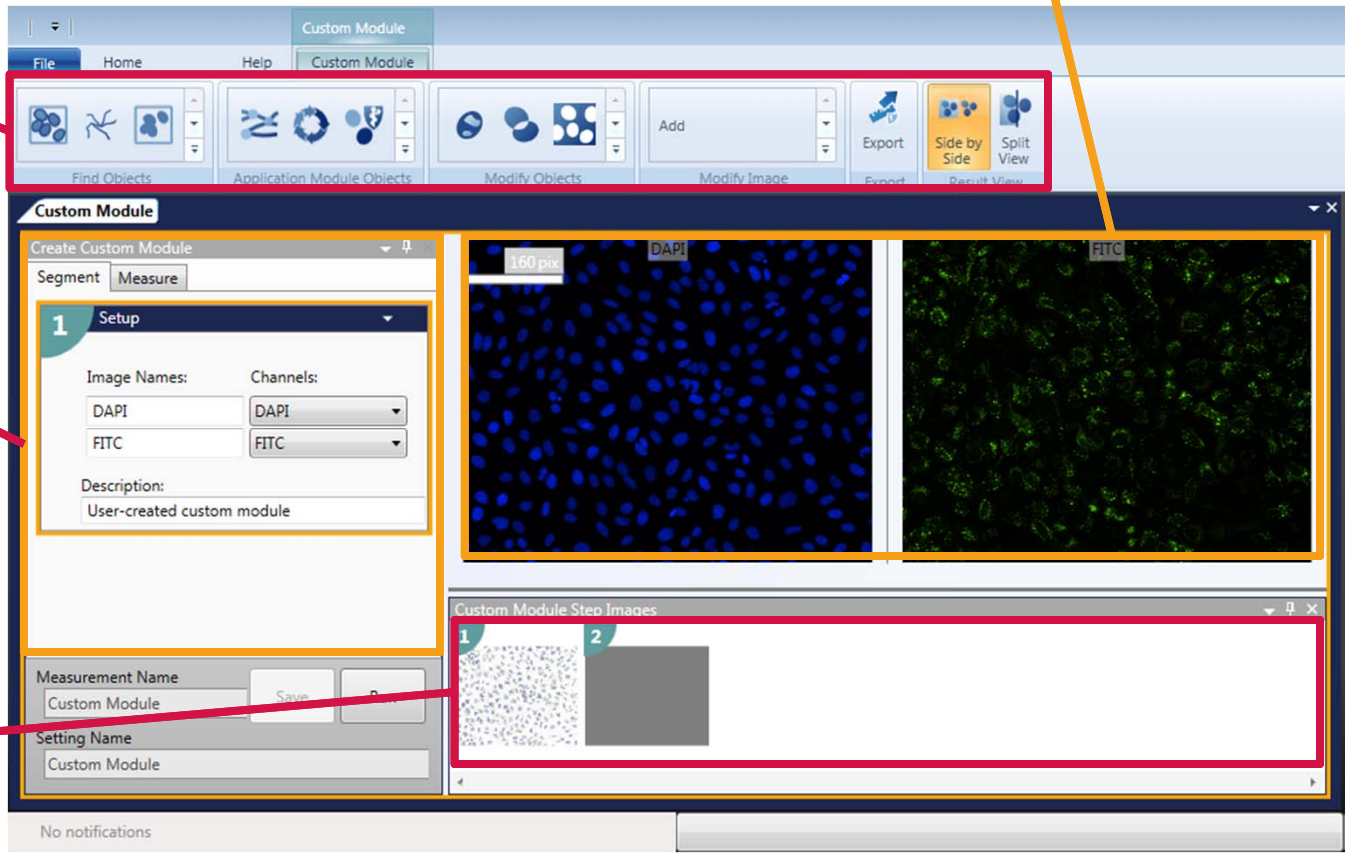
The Custom Module Editor Interface

Image grid: images and corresponding segmentation images appear here

Ribbon: contains tools for image segmentation and processing

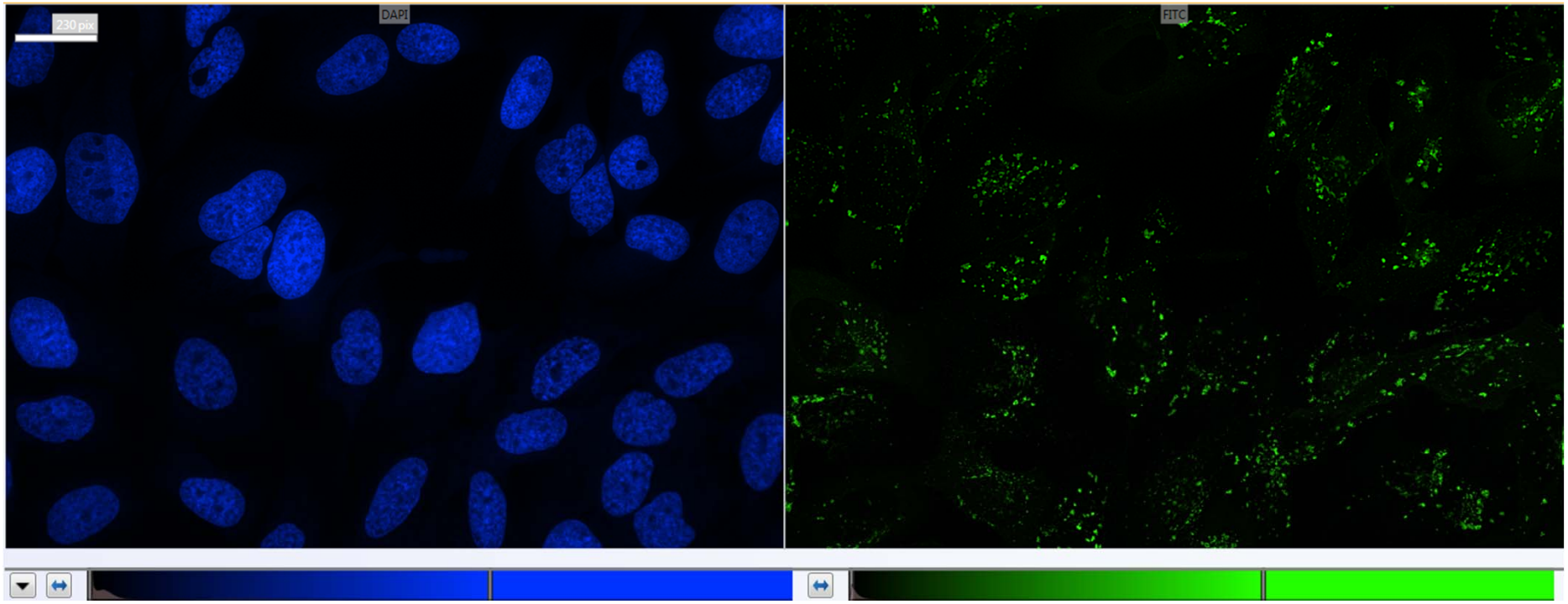
Cards: a new card appears for each step added from the Ribbon

Filmstrip view: a thumbnail representing each step appears here



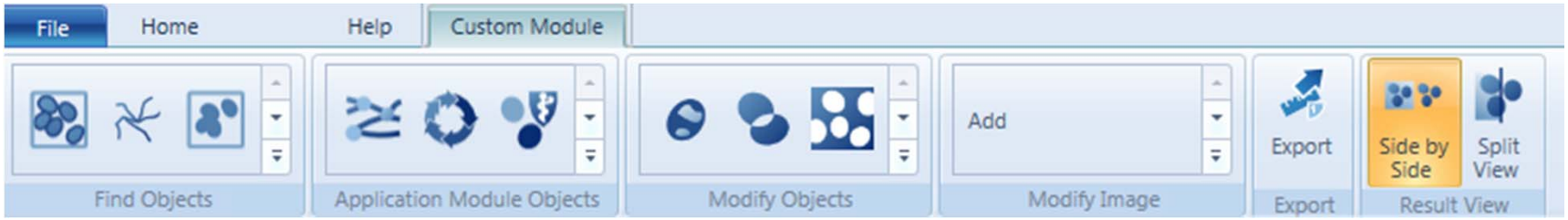
CME Interface: Image Grid

The **Image Grid** shows the starting and result images for each step in the analysis. The intensity histogram is shown directly below each image and can be used to adjust scaling in the display.



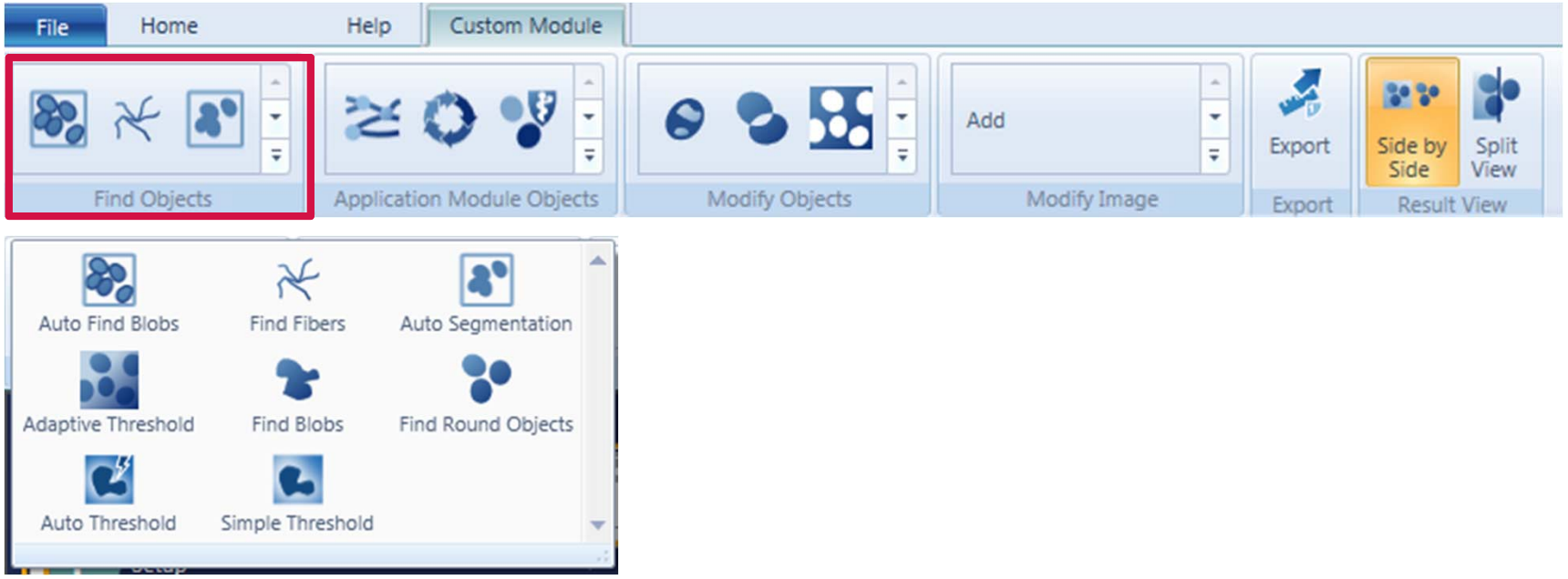
CME Interface: Ribbon

The **Ribbon** contains all the tools available for image segmentation and processing. The tools are separated into categories for **Find Objects**, **Application Module Objects**, **Modify Objects**, and **Modify Image**.



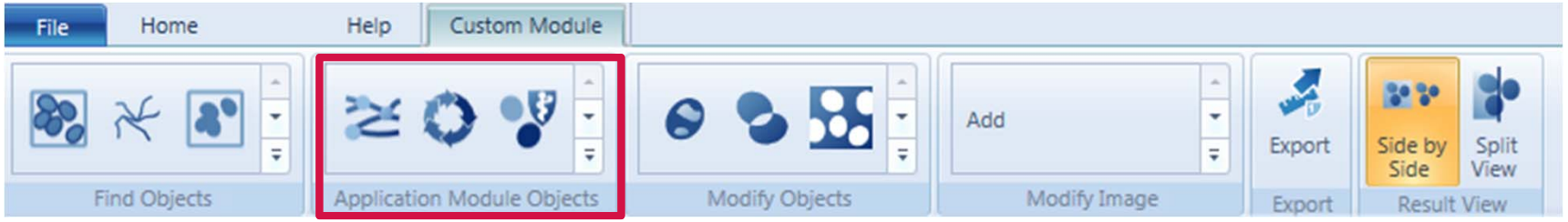
CME Interface: Find Objects Tools

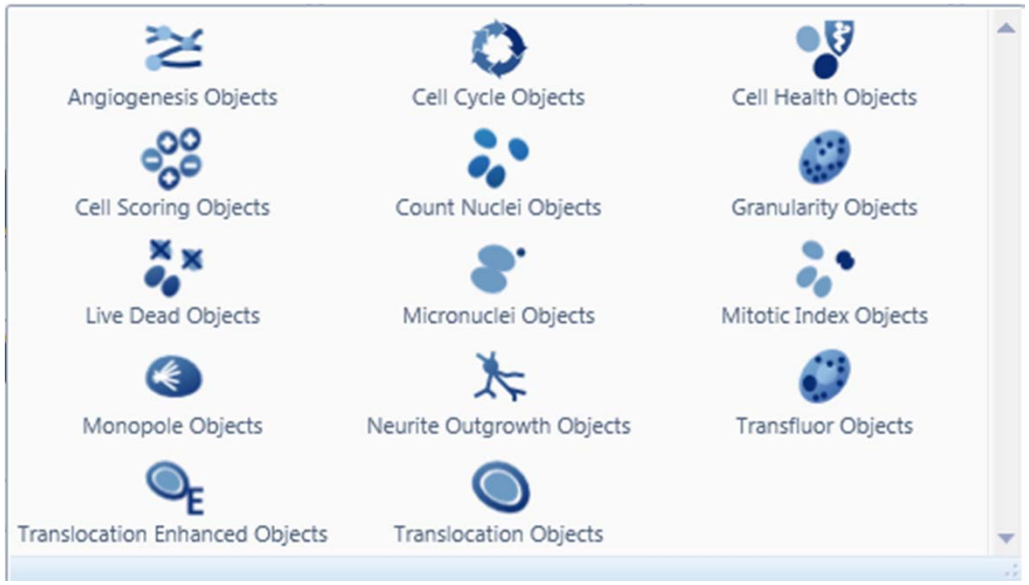
The **Find Objects** tools are used for finding objects in grayscale images and creating masks for them. For example, use **Find Blobs** to create a mask for nuclei, **Find Round Objects** to find small puncta, and **Find Fibers** to find neurites.



CME Interface: Application Modules Objects

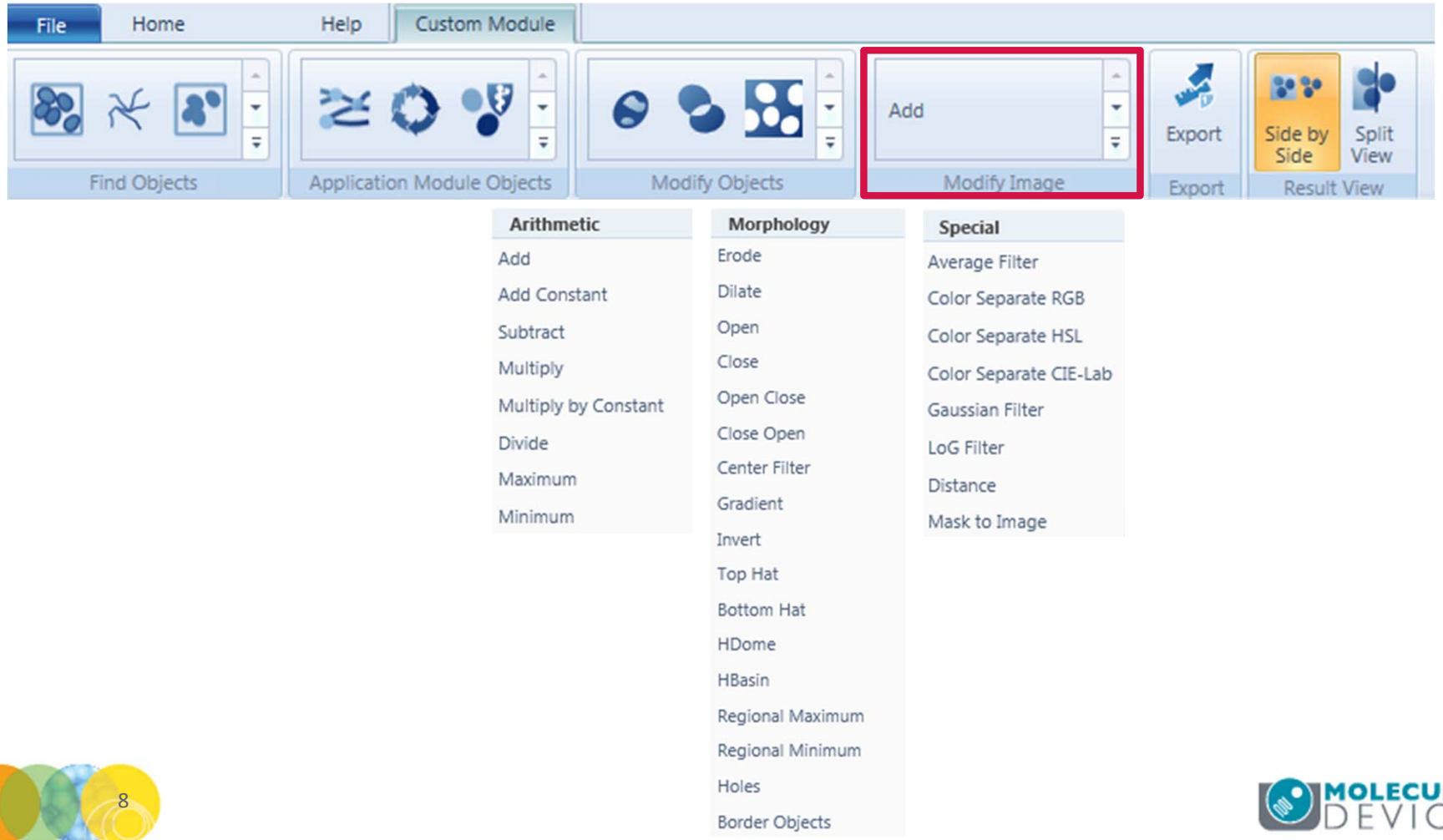
The **Application Module Objects** tools run the built-in application modules as a step to generate object masks from grayscale images. You will only see the application modules that you have purchased here.





CME Interface: Modify Image Tools

The **Modify Image** tools are used to modify or process grayscale images before creating a mask. The tools are grouped into **Arithmetic**, **Morphology**, and **Special** categories.

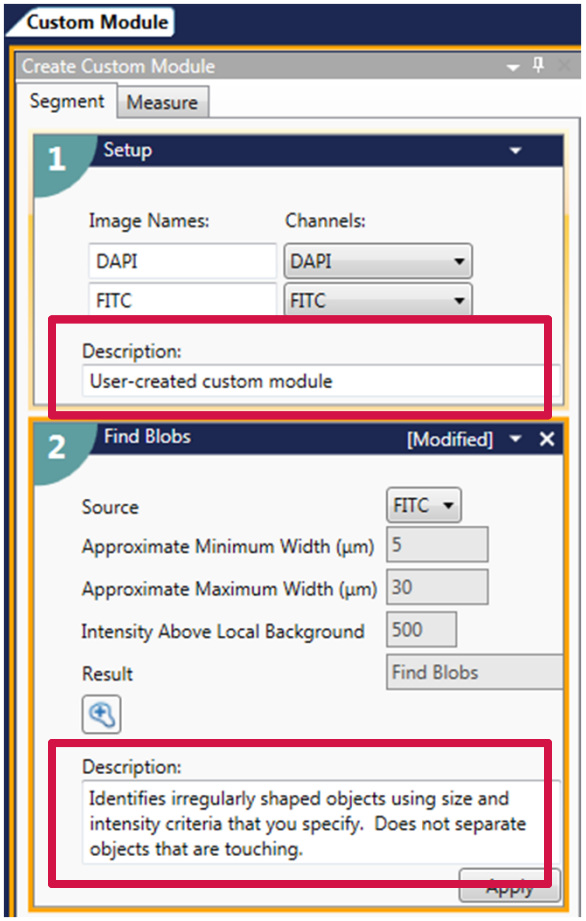


CME Interface: Step Cards

The left side of the CME window shows the steps in the analysis. Each step is called a **Card** and appears in hierarchal order.

Cards contain the specific parameters necessary to find a particular type of object in the images.

Additionally, a description can be added to each step card. This is a great way to remember the logic of steps undertaken as well as to have collaborators follow a given workflow. The default description identifies the function of that card.

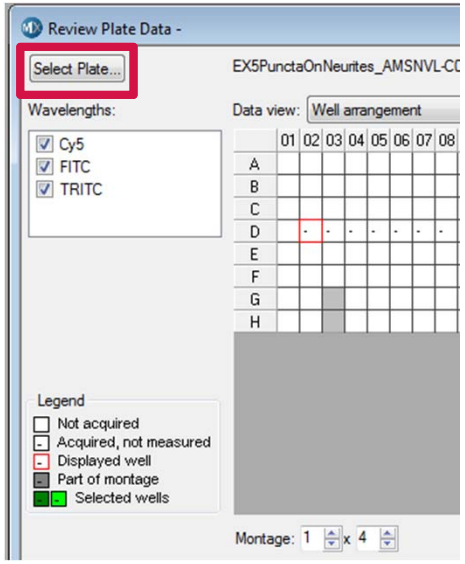


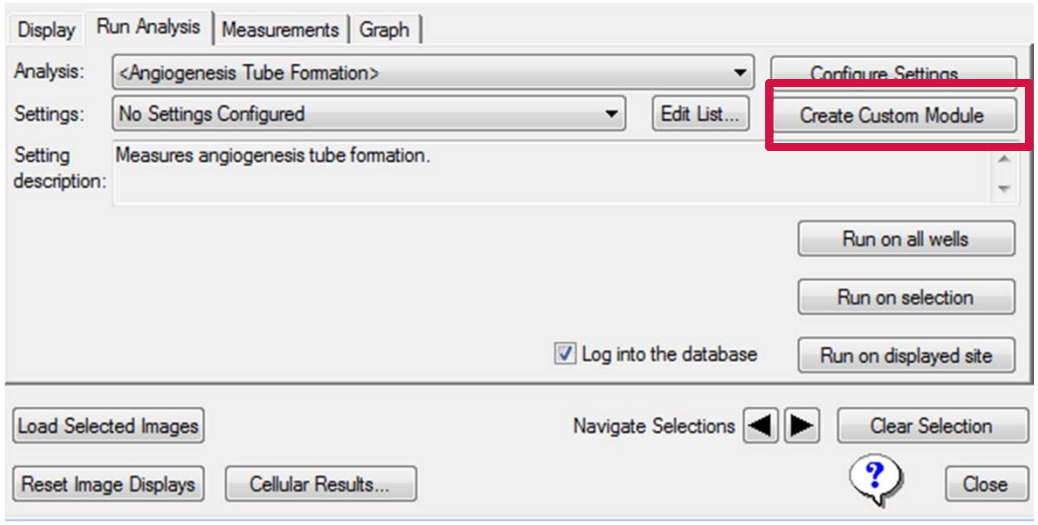
CME Interface: Filmstrip

The **Filmstrip** shows thumbnail views of the result images for each step. Double-clicking on a thumbnail displays that card in the Editor and displays the result image in the image grid.

Creating a Custom Module

1. Open the Review Plate Data dialog
2. Click on **Select Plate** and browse for the plate of interest
3. From the thumbnail montage images, select a site/well to determine analysis steps (i.e. control well)
4. Select the **Run** tab
5. Click on **Create Custom Module**

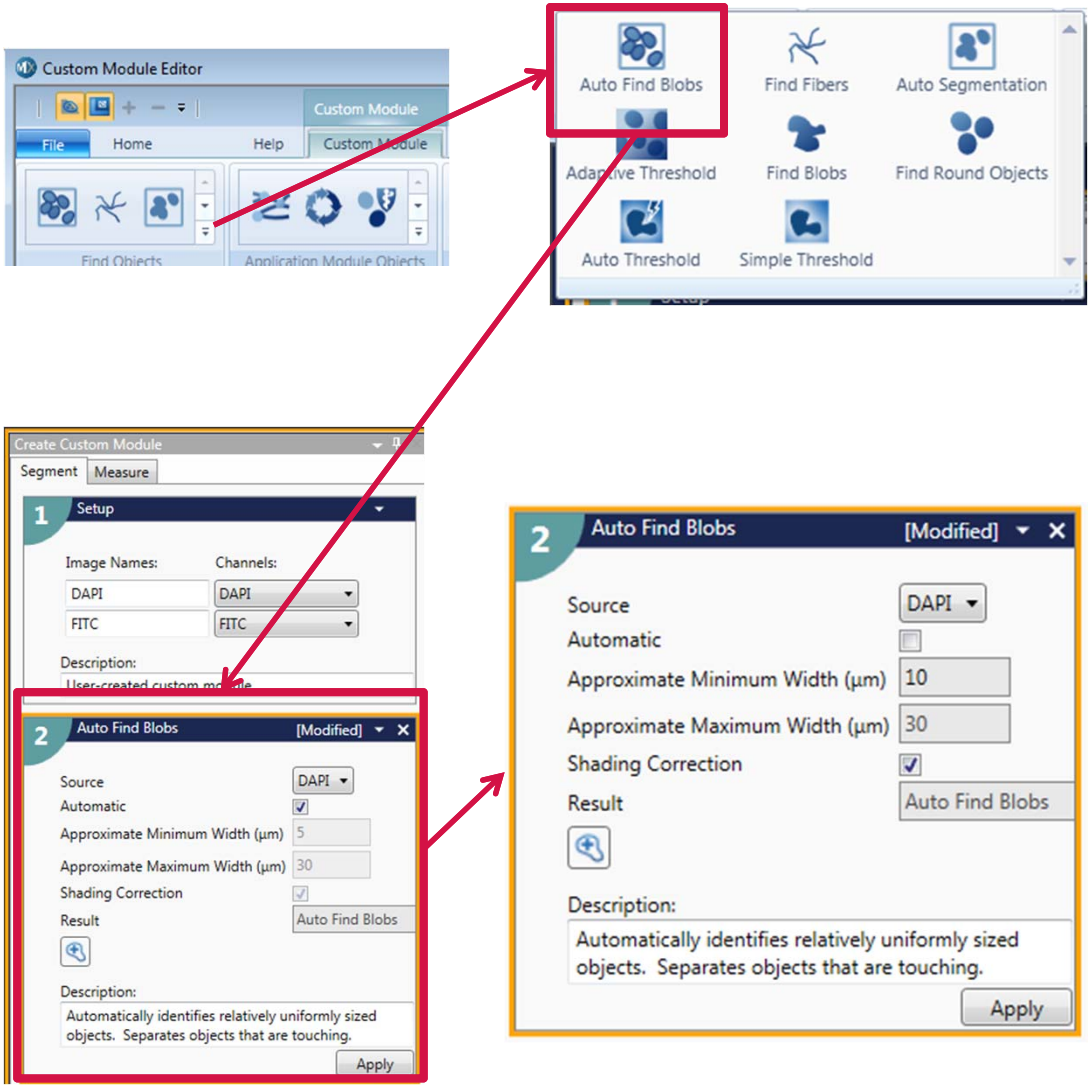




Creating a Custom Module

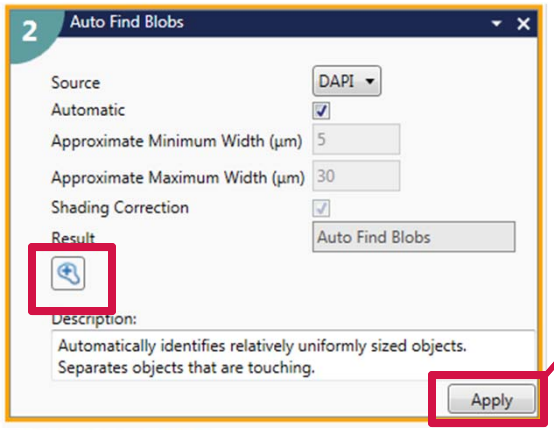
6. Add steps to complete the desired analysis
   - On the **Custom Module** tab, select a tool from the ribbon. This will add a corresponding card to the analysis steps on the left. The currently selected step will be outlined in orange.
     - In the example to the right, the **Auto Find Blobs** tool is added to find nuclei. DAPI is selected from the **Source** drop-down menu. Appropriate min/max width values are entered to find the nuclei.

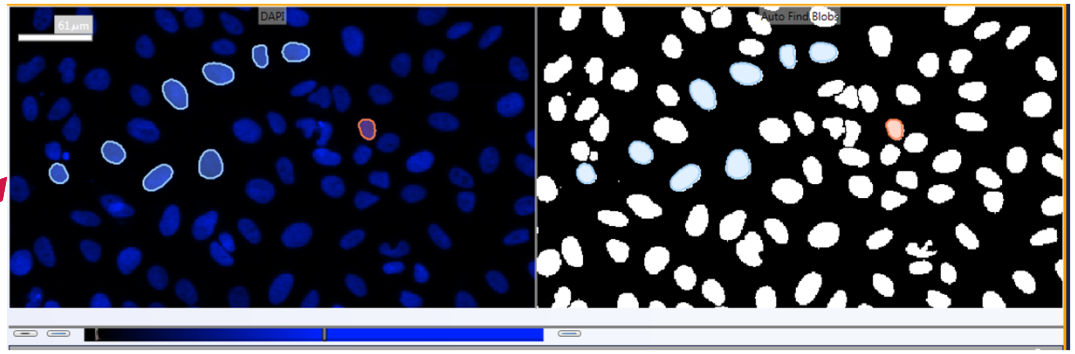
NOTE Not all cards have the same available parameters



Creating a Custom Module

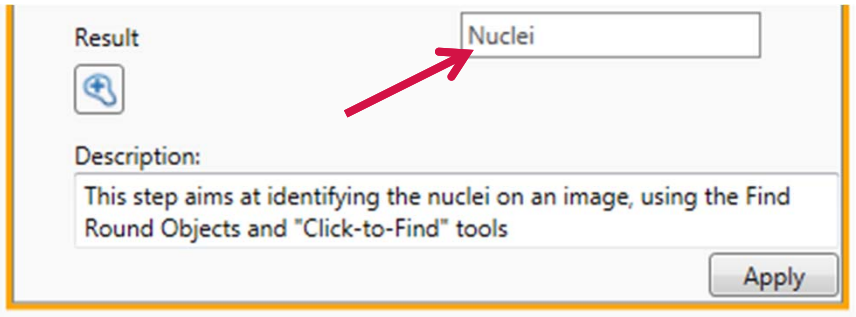
7. Many of the step cards require entering values for parameters. The **Auto Find Blobs** tool requires **Approximate Minimum** and **Maximum widths.** There are 3 ways to determine these values:
   i. Check the **Automatic** box and click **Apply**. The software will attempt to automatically determine the correct values
   ii. Manually measure the smallest and largest objects in the image and enter the values
   iii. Use the **Click-To-Find Tool** to interactively determine the values
      - Enable the tool (highlighted in orange) and click on 4-6 representative objects in the image grid
      - The software automatically finds the boundary of the object and populates the **Minimum** and **Maximum Width** values
      - Click **Apply** to use the values across the entire image. The image grid will show a resulting binary mask all objects found



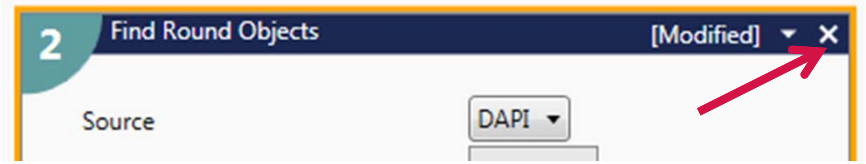


Creating a Custom Module

8. In the result box, change the default name of the result image to more closely represent the objects identified. For example, the result image in the previous example is called “Nuclei” below.



9. To remove a step card from the analysis, click on the “x” at the top right hand corner of the card



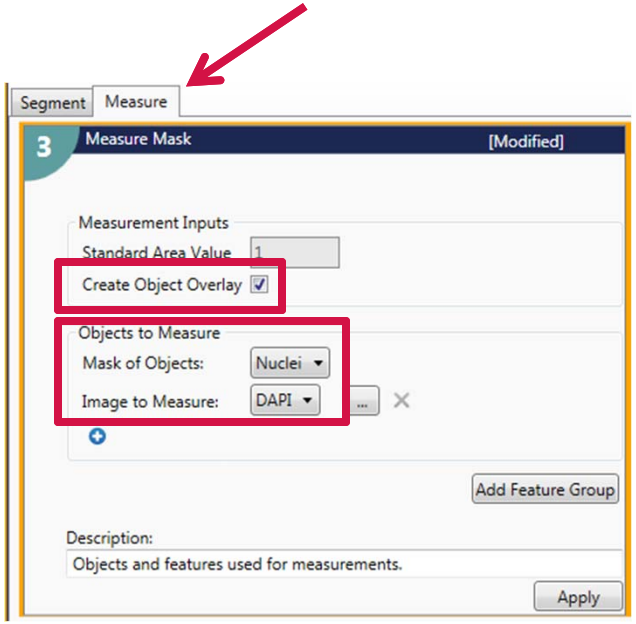
Creating a Custom Module

10. Once you have found all the objects of interest (created masks for each type of object), you can specify which measurements to make on those objects. The Custom Module Editor allows measurements of objects within objects and the **Measure** tab is organized to take advantage of this. Specify the larger objects first, then the smaller objects within the larger ones.

- Select the **Measure** tab located above the step cards
- Choose to select/deselect the check box next to **Create Object Overlay.**

NOTE Saving cell segmentation can take up significant space in the database.

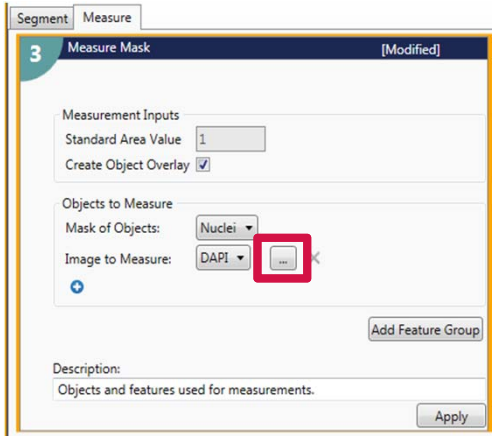
- Under the **Objects to Measure** section:
  - Select the mask covering the largest area from the **Mask of Objects** drop-down menu. For example, a mask covering the whole cell to measure smaller objects within the cell.
  - Select the appropriate gray scale image to apply the mask and measure from the **Image to Measure** drop-down menu.

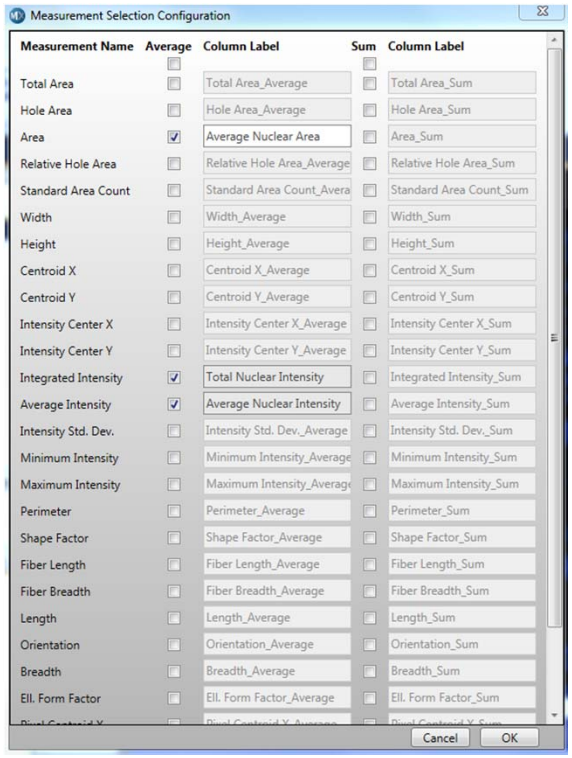


Creating a Custom Module

11. Click on the **Ellipsis** button (…) to display a list of all possible measurement outputs for this step. The measurements are grouped into average values and sum values. There are more than 50 outputs available for each object found in the image.

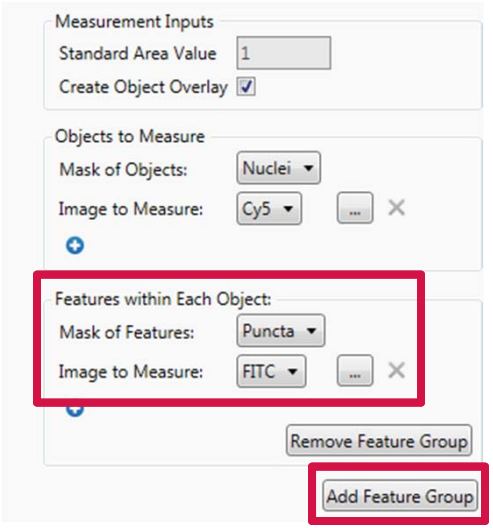
    - Check the box next to the desired measurements for each object
    - Edit the names of the measurements to more accurately reflect the assay
    - Click the **OK** button





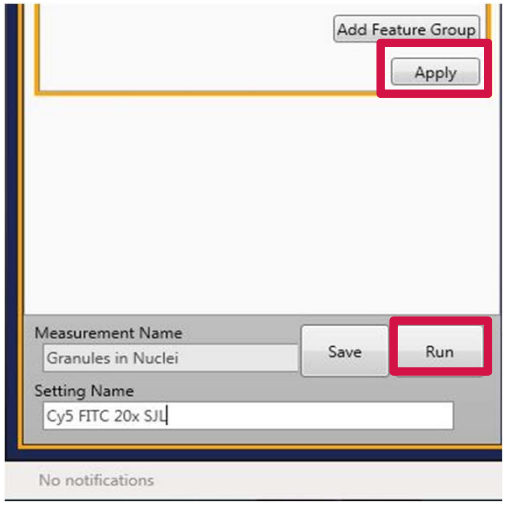
Creating a Custom Module

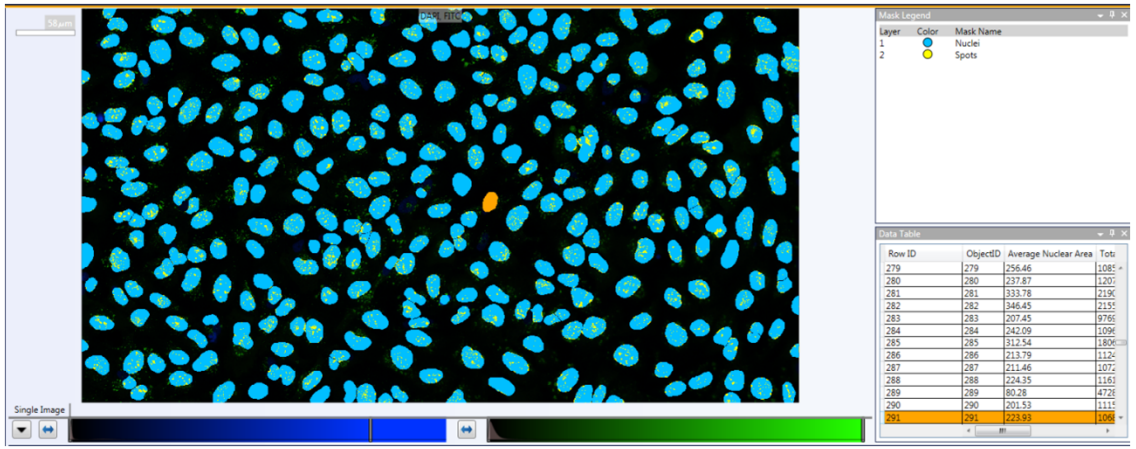
12. If measuring more than one type of object (i.e., puncta in the nucleus, nuclei in multi-nucleated cells, etc.), then click on the **Add Feature Group** button to access measurements for the next type of object.
    - Under the **Features within Each Object** section
        - Select the appropriate mask of smaller objects (features) to measure from the **Mask of Features** drop-down menu
        - Select the grayscale from the **Image to Measure** drop-down menu. This most often will be one of the original images and can be the same image as specified in the **Objects to Measure** section above
    - Click the **Ellipsis** (…) button once again to select the desired measurements and change the names as appropriate for the assay.
    - At the bottom of the sum column, select the **Features Count** measurement to count all the objects in the mask. If the features you are measuring in this step lie within the **Mask of Objects** specified in step 11, then this option will count the number of smaller features (i.e. puncta) within the larger objects (i.e. nuclei).
    - Continue to add **Feature Groups** as desired



Creating a Custom Module

13. Test the custom module with control images to determine if analysis steps and parameters are optimized for the assay
    - To test run the custom module, click the **Apply** button on the **Measure** tab or the **Run** button at the bottom of the dialog.
    - A color segmentation overlay is applied to the images selected for measurement, and a table to the right of the Image Grid shows the specified measurements.
    - Each row in the table represents an object (from step 11-12 above) in the images
    - Clicking on a row in the table will highlight that object in orange in the segmentation overlay and vice versa
    - A legend specifying the colors attributed to the different object and features classes is displayed in the top right corner

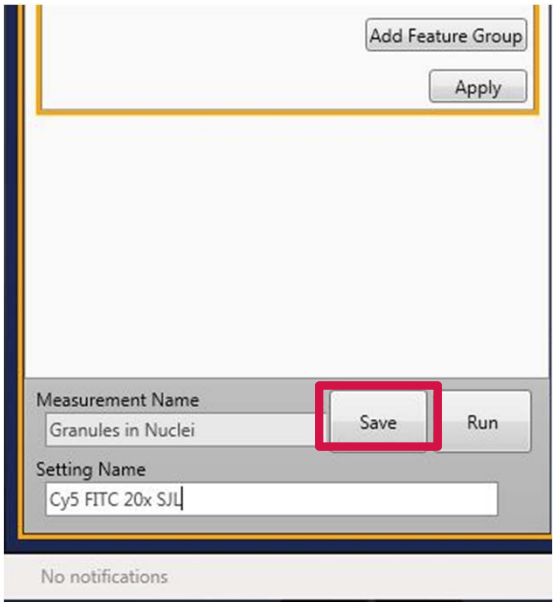


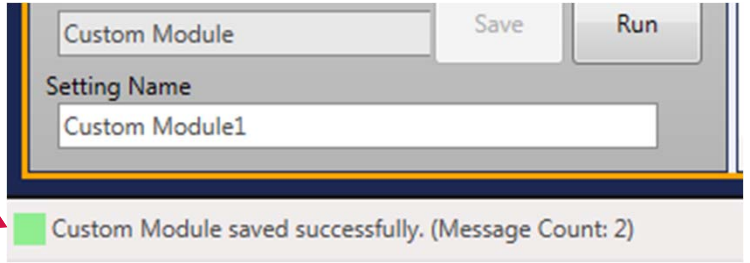


Creating a Custom Module

14. Save the custom module to the database in order to run the analysis on other images

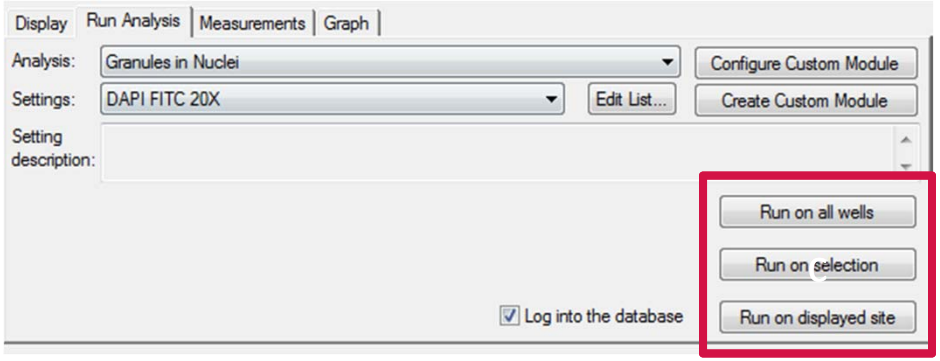
    - Enter a **Measurement Name** and a **Setting Name**. Multiple **Setting Names** can be given for one **Measurement Name**.
    - Click the **Save** button to automatically add the custom module to the database
    - Verify that the custom module has been saved by noting the message at the bottom of the **Custom Module Interface**





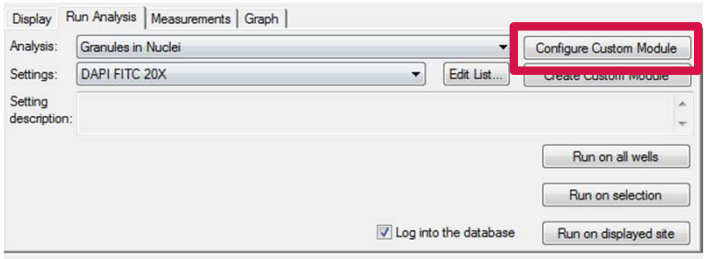
Creating a Custom Module

15. Run the custom module created through the **Review Plate Data** dialog
    - On the **Run Analysis** tab, select the custom module and settings from the corresponding drop-down menus
    - Select the appropriate button to run the analysis on:
        - **Run on all wells**: runs analysis on all sites/wells in the plate
        - **Run on selection**: runs analysis on sites/wells selected in the plate grid (right-click in the plate grid to highlight wells of interest in green)
        - **Run on displayed site**: run analysis on the site displayed as high resolution images (right-click on the thumbnail montage to select)
    - When complete, analysis results will be shown in the plate grid of the **Review Plate Data** dialog

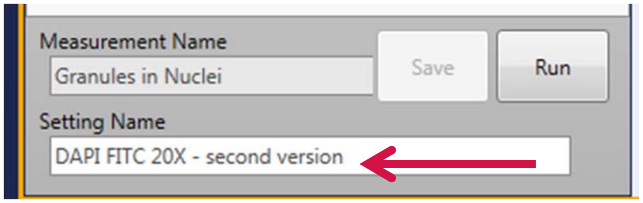


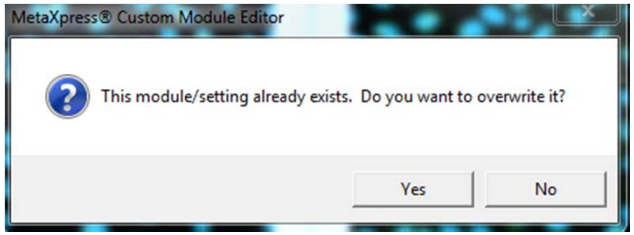
Creating a Custom Module

15. To edit an existing custom module, select the **Run Analysis** tab:
    - Select the custom module and settings from the corresponding drop-down menus
    - Click on the **Configure Custom Module** button



16. To overwrite an existing custom module, keep the **Measurement Name** and **Setting Name** the same as the existing custom module and click **Save**. A window asking confirmation for overwriting the module will appear, click **Yes**.





17. To keep the original custom module in the database, save the changes under a new **Measurement Name** and/or **Setting Name** and click **Save**

NOTE: Changing only the **Setting Name** will result in two versions of the custom module having the same **Measurement Name** in the database.

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