The MetaXpress® 5.0
Custom Module Editor

Quick Start Guide
The MetaXpress® 5.0 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 is used to build a custom analysis that can be run 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:
A. Select your images and open the Editor.
B. Add steps to the analysis to find objects in the images.
C. Measure the objects.
D. Save the module and run analysis on the plate.

Custom Module Editor Interface
• The **Image Grid** shows the starting and result images for each step in the analysis. The intensity histogram is shown directly below each image.

![Image Grid Example](image)

• 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**.

![Ribbon Example](ribbon)

• 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.

![Find Objects Example](find_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.

![Application Module Objects Example](application_modules)
- The **Modify Objects** tools are used for resizing, selecting, or reshaping objects in a mask.

- 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.

- The left side of the CME window shows the steps in the analysis. Each step is called a **card**. Cards contain the specific parameters necessary to find a particular type of object in your images.

- The **Filmstrip** shows thumbnail views of the result images for each step. Double-clicking on a thumbnail displays that card in the custom module and displays the result image in the image grid.
Creating a New Custom Module in MetaXpress® 5.0 Software

Open the **Custom Module Editor**

1. Open MetaXpress Software version 5.0 and log into the MDCStore™ Database as described in the ImageXpress® Micro or ImageXpress Ultra System Quick Start Guide.

2. Open the **Review Plate Data** dialog and select a plate.

3. Select images to analyze.

4. Go to the **Run Analysis** tab

5. To create a new custom module, click on the **Create Custom Module** button.
Adding and Removing Steps to the Analysis

6. Select a tool from the ribbon to add that card to the analysis steps on the left. The currently selected step will be outlined in orange.

7. For example, to find nuclei, you can use the **Auto Find Blobs** card. Select the appropriate **Source** image and specify the **Approximate Minimum Width** and **Approximate Maximum Width**. **Note:** Not all cards have the same available parameters.

![Auto Find Blobs Card](image)

8. There are 3 ways to generate the minimum width and maximum width values.

   a. Check the **Automatic** box and press **Apply** and the software will attempt to automatically detect the correct values.
   b. Manually measure the smallest and largest objects in the image and enter the values into the card.
   c. Use the **Click-To-Find Tool** to interactively determine the values.

9. Using the **Click-To-Find Tool**.

   a. Click on the **Click-To-Find Tool**. It will be highlighted in orange.
b. Select objects of interest in the image.

c. The software automatically finds the boundary of the object and populates the minimum and maximum width values (and Intensity Above Local Background for some cards) based on your selections. 
d. Click **Apply** to use the values across the entire image. The image grid will show a resulting binary mask all objects found.

10. In the result box, you can change the default name of the result image to more closely represent the objects identified. For example, call the result image for this step “Nuclei.”

11. To remove a step card from the analysis, click on the “X” at the top right corner of the card.
Measuring Objects

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

12. Click on the Measure tab above the analysis cards.

13. To save segmentation overlays to the database, check the Create Object Overlay box. **Note:** This will take up significant space in the database.

14. For Mask of Objects, select the mask covering the largest area. For example, a mask covering the whole cell to measure smaller objects within the cell.

15. Select the grayscale Image to Measure. This should be one of your original images.

16. Click on the Ellipsis button (…) to view 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.
17. Check the box next to the measurements you would like to make on each object. You can edit the name of the measurements to more accurately reflect your specific experiment.

18. When you have finished selecting measurements, click the **OK** button.

19. If you are measuring more than one type of object (i.e., foci 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.

20. For **Mask of Features**, select the appropriate mask of smaller objects (features) to measure.

21. Select the grayscale **Image to Measure**. This should be one of your original images and can be the same image as specified in the **Objects to Measure** section above.

22. Again, click on the **Ellipsis** button (…) to access the measurement selection dialog.

23. Select the desired measurements and change the names as appropriate for your experiment. At the bottom of the sum column, you can also select the **Features Count** measurement. If the features you are measuring in this step lie within the Mask of Objects specified in step 13, then this option will count the number of smaller features (i.e., foci) within the larger objects (i.e., nuclei).

24. Click **OK** to close the dialog.
Testing a Custom Module

25. To test run the custom module, click the **Apply** button on the **Measure** tab or the **Run** button.

26. 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.

27. Each row in the table represents an object (from step 13 above) in the images. Clicking on a row in the table will highlight that object in orange in the segmentation overlay.
Saving a Custom Module

28. To save the custom module, enter a **Measurement Name** and a **Setting Name**. You can have multiple Setting Names for a given Measurement Name.

29. Click the **Save** button to automatically add the custom module to the database. **NOTE:** Clicking save will also sever the connection with the Review Plate Data dialog so that any further edits cannot be saved. We recommend saving only after you have completed your custom module. In order to re-establish the connection with Review Plate Data, follow the directions in the next section, **Editing an Existing Custom Module in the Custom Module Editor**.

30. To verify the custom module is saved, return to the Review Plate Data dialog (or minimize the CME window) to see a verification dialog.

31. Once you have finished and saved your custom module, close the Custom Module Editor window and return to the Review Plate Data dialog.

Running a Custom Module on a Plate

32. In the **Review Plate Data** dialog, select your custom module in the **Analysis** drop-down list.

33. Select the appropriate **Settings** name in the drop-down list.

34. To run the custom module on all wells, click on the **Run Analysis for All Positions** button.
35. To run the custom module only on selected wells, click on the Run Analysis for Selections button.

36. To run the custom module only on the currently selected site, click on the Run Analysis for Site button.

37. When complete, analysis results will be shown in the Plate grid of the Review Plate Data dialog.

Editing an Existing Custom Module in the Custom Module Editor

Opening an Existing Custom Module

1. Open MetaXpress Software version 5.0 and log into the MDCStore Database as described in the ImageXpress Micro or ImageXpress Ultra System Quick Start Guide.

2. Open the Review Plate Data dialog and select a plate.

3. Select images to analyze.

4. Go to the Run Analysis tab

5. Select the custom module name in the Analysis drop down list. Select the setting name in the Settings drop down list. Click on the Configure Custom Module button.

6. The MetaXpress Custom Module Editor window will open.
Saving an Existing Custom Module

7. To overwrite an existing custom module:
   
a. Keep the **Measurement Name** and **Setting Name** the same as the existing custom module and click **Save**.

   b. Minimize the CME window and you will see a warning that you are replacing an existing custom module. Click **OK** to accept.

   ![Warning dialog]

   c. Next you will see the save confirmation dialog.

   ![Confirmation dialog]

8. To keep the original custom module in the database, save the changes under a new **Measurement Name** and/or **Setting Name**. **NOTE**: Changing only the **Setting Name** will result in two versions of the custom module having the same **Measurement Name** in the database.

   ![Custom module settings]

9. Minimize the CME window to see the confirmation in step 7c above.
Help and Assistance

10. Within CME, press F1 to bring up the help system. The help system is searchable by keyword and provides how to’s, information about each of the CME tools, and two example custom modules.

11. Find product support including software updates, product manuals, FAQs, and technical notes in the Molecular Devices® Knowledge Base: http://www.moleculardevices.com/Support/Knowledge-Base.html

12. Visit our User Forum (http://metamorph.moleculardevices.com/forum/) to ask questions and to share information and custom modules with our support personnel and other MetaXpress users.

13. Call Molecular Devices Support at 1-800-635-5577.