



MetaXpress[®] 6 Software Guide

Custom Module Editor Overview

Date Revised 07/09/15 Version B



© 2012-2015. Trademarks property of Molecular Devices, LLC or their respective owners.
For research use only. Not for use in diagnostic procedures.

Chapter Purpose

The purpose of this chapter is to provide an overview of the **Custom Module Editor** (CME) analysis plugin.

CME is an analysis plugin that enables users to go beyond the standard **Application Modules** available in MetaXpress (i.e. Cell Cycle, Count Nuclei, etc.) to build a custom analysis routine from a suite of image processing tools.

Examples of custom modules include removing image artifacts, counting cells in transmitted light, multiplexing application modules, measuring objects within objects, and many more.



Working With CME

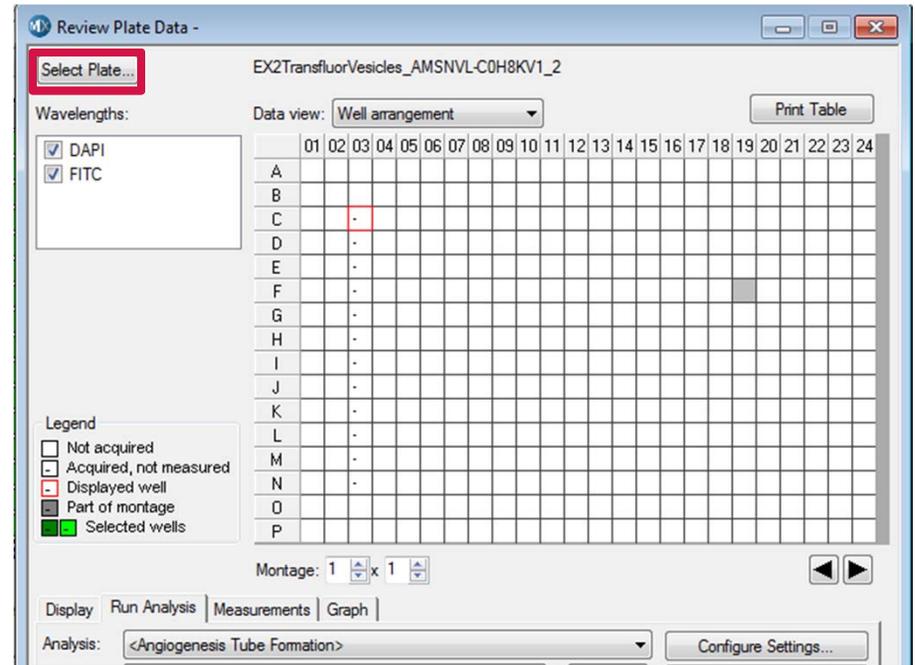
1. Open **Review Plate Data**

- In the main toolbar click on



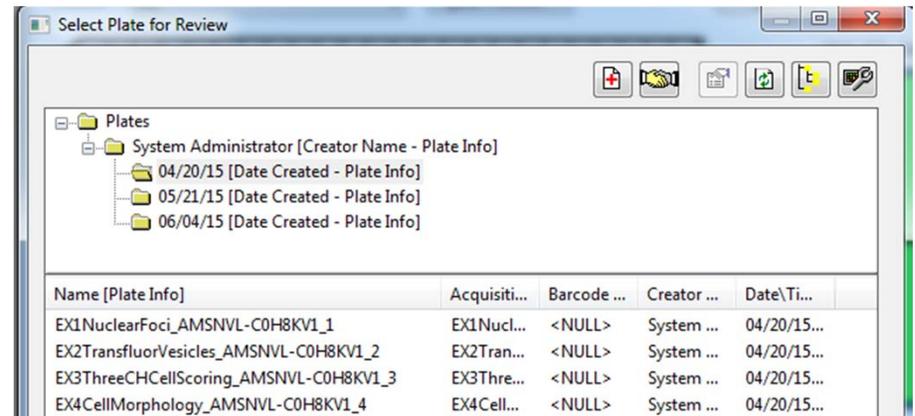
OR

- Under the **Screening** menu, select **Review Plate Data**



2. Click on the **Select Plate** button

3. Browse through the folders to open the plate of interest



Working With CME

4. Select Wells to View in Montage

5. Click on the desired site/well

6. Review High Resolution Images

- Molecular Devices suggests when setting up a custom module for the first time to start with an image from a control well
- If modifying an existing custom module, select any appropriate image



Working With CME

7. Select the **Run Analysis** tab
8. If creating a new module, click on **Create Custom Module**
9. If modifying an existing custom module:
 - Select the desired module from the **Analysis** and **Settings** drop-down menus
 - Application modules have <> brackets
 - Custom modules and journals do not
 - Click on **Configure Custom Module**

Display | **Run Analysis** | Measurements | Graph

Analysis: Organoids

Settings: Organoids Colon

Setting description:

Buttons: Configure Custom Module, Edit List..., Create Custom Module



CME Interface: Custom Module Tab

The screenshot displays the CME software interface with the 'Custom Module' tab selected. The ribbon contains several tool groups: 'Find Objects', 'Application Module Objects', 'Modify Objects', 'Modify Image', 'Export', 'Side by Side', and 'Split View'. A 'Custom Module' panel is open on the left, showing a 'Find Round Objects' module with parameters like 'Source: FITC', 'Approximate Minimum Width (µm): 1', 'Approximate Maximum Width (µm): 3', and 'Intensity Above Local Background: 2983'. The main workspace shows an 'Image Grid' with two panels: 'DAPI' (blue) and 'FITC' (green). Below the grid is a 'Custom Module Step Images' filmstrip showing three steps: 1. Original image, 2. Thresholded image, and 3. Final processed image. Red callout boxes highlight the ribbon, the cards in the ribbon, the image grid, and the filmstrip view.

Custom Module

File Home Help Custom Module

Find Objects Application Module Objects Modify Objects Modify Image Export Side by Side Split View Result View

Custom Module

Create Custom Module

Segment Measure

User-created custom module

2 Find Round Objects

Source FITC

Approximate Minimum Width (µm) 1

Approximate Maximum Width (µm) 3

Intensity Above Local Background 2983

Result Puncta

Description: Identifies small, symmetrically round objects using size and intensity criteria that you specify.

Apply

Measurement Name Custom Module Save Run

Setting Name Custom Module

Image Grid

Filmstrip View of All Image Processing Steps

78 µm DAPI

FITC

1 2 3



CME Interface: Image Processing Tools

Find Objects

- Auto Find Blobs
- Find Fibers
- Auto Segmentation
- Adaptive Threshold
- Find Blobs
- Find Round Objects
- Auto Threshold
- Simple Threshold

Application Module Objects

- Angiogenesis Objects
- Cell Cycle Objects
- Cell Health Objects
- Cell Scoring Objects
- Count Nuclei Objects
- Granularity Objects
- Live Dead Objects
- Micronuclei Objects
- Mitotic Index Objects
- Monopole Objects
- Neurite Outgrowth Objects
- Transfluor Objects
- Translocation Enhanced Objects
- Translocation Objects

Modify Objects

- Fill Holes
- Logical Operations
- Invert Objects
- Remove Border Objects
- Watershed
- Grow Objects
- Keep Marked Objects
- Remove Marked Objects
- Shrink Objects
- Grow Objects Without Touching
- Filter Mask

Modify Image

Arithmetic

- Add
- Add Constant
- Subtract
- Multiply
- Multiply by Constant
- Divide
- Maximum
- Minimum

Morphology

- Erode
- Dilate
- Open
- Close
- Open Close
- Close Open
- Center Filter
- Gradient
- Invert
- Top Hat
- Bottom Hat
- HDome
- HBasin
- Regional Maximum
- Regional Minimum
- Holes
- Border Objects

Special

- Average Filter
- Color Separate RGB
- Color Separate HSL
- Color Separate CIE-Lab
- Gaussian Filter
- LoG Filter
- Distance
- Mask to Image

Refer to corresponding chapters for more details on each of the image processing tools seen here



CME Interface: Side by Side View

Click on the **Side by Side** button to change display so that before / after images are displayed next to each other



CME Interface: Split View

Click on the **Split View** button and adjust the vertical slider back and forth to view before / after images



CME Interface: Home Tab

The screenshot displays the CME software interface. At the top, a ribbon menu includes 'File', 'Home', and 'Help'. The 'Home' tab is active, showing various tool icons such as 'Zoom To Fit Space', 'Single View', 'View Mode', 'Export to PowerPoint', 'Show Image Info', 'Channel Display', 'Reset All Scaling', 'Object Overlay', 'Threshold Overlay', 'Zoom Preview', 'Regions', 'Calibration Bar', and 'Saturation Markers'. Below the ribbon, the 'Custom Module' window is open, featuring a 'Create Custom Module' panel with 'Segment' and 'Measure' tabs. The 'Setup' section includes fields for 'Image Names' (DAPI, FITC) and 'Channels' (DAPI, FITC). The 'Find Blobs' section contains parameters for 'Source', 'Approximate Minimum Width (µm)', 'Approximate Maximum Width (µm)', and 'Intensity Above Local Background'. A 'Measurement Name' and 'Setting Name' field are also present. The main workspace shows two image channels: 'DAPI' (blue) and 'FITC' (green). A red box highlights the 'Tools for Customizing the Display' area, which includes the 'Channel Display' and 'Object Overlay' icons in the ribbon and the 'Custom Module Step Images' panel at the bottom.

Tools for Customizing the Display



CME Home Tab: Setting and Adjusting Zoom

The screenshot displays the CME Home Tab software interface. The top menu bar includes File, Home, Help, and Custom Module. The ribbon contains various tools: Zoom To Fit Space (147.8, 100%), View Mode, Export to PowerPoint, Show Image Info, Channel Display, Reset All Scaling, Channel Display (DAPI, FITC, Unassigned Hidden, Unassigned Hidden), Object Overlay, Threshold Overlay, Zoom Preview, Regions, Calibration Bar, and Saturation Markers. The Zoom Preview button is highlighted with an orange box and labeled "Enable Zoom Preview".

The main workspace shows a "Custom Module" window with two tabs: "Segment" and "Measure". The "Segment" tab is active, showing a "Setup" section with "Image Names" (DAPI, FITC) and "Channels" (DAPI, FITC). Below this is a "Find Blobs" section with parameters: Source (DAPI), Approximate Minimum Width (9.9), Approximate Maximum Width (30.58), and Intensity Above Local Background (9905). At the bottom of the "Segment" tab are "Measurement Name" (Custom Module) and "Setting Name" (Custom Module) fields, along with "Save" and "Run" buttons.

The main image area displays two channels: DAPI (blue) and FITC (green). A red box highlights the zoom controls in the top left, and a red box highlights the "Zoom Preview" button. A red box highlights the "Adjust zoom here OR by using mouse scroll wheel over the images" text. A red box highlights the "Click and drag to move around the image" text. A red box highlights the "Zoom Preview" button. A red box highlights the "Adjust zoom here OR by using mouse scroll wheel over the images" text. A red box highlights the "Click and drag to move around the image" text.

At the bottom of the software interface, there is a "Custom Module Step Images" window showing four steps of the process: 1. Original image, 2. Thresholded image, 3. Segmented image, and 4. Final image.



CME Home Tab: Color Overlay

Click View Mode to display images side by side

Click Single View to overlay images

Zoom To Fit Space 100% Zoom 59.4

File Home Help Custom Module

Single View View Mode Export to PowerPoint Show Image Info Channel Display Reset All Scaling DAPI FITC Unassigned Hidden Unassigned Hidden

Object Overlay Threshold Overlay Zoom Preview Regions Calibration Bar Saturation Markers

Custom Module

Create Custom Module

Segment Measure

1 Setup

Image Names: DAPI FITC

Description: User-created custom

2 Find Blobs

Source

Approximate Minimum Width (μm) 9.9

Time vs Z

Time vs Channel

Channel vs Z

Multi Time

Multi Z

Multi Channel

Single Image

Custom Module Step Images

1 2 3 4



CME Home Tab: Adjusting Image Display

The screenshot shows the CME Home Tab software interface. The top menu bar includes File, Home, Help, and Custom Module. The ribbon contains various tools like Zoom, Dataset View, Image Info, Channel Display, and Overlay. The Channel Display window is open, showing a list of channels (DAPI, FITC, Unassigned 2) and their corresponding images. The FITC channel is selected, and its intensity scale is being adjusted using a histogram slider. The Custom Module window is also open, showing the Channel Display settings, including Hide Scale Bar, Scale Mode (Fixed, Normalize, Camera), and Channels (Show DAPI, Show FITC, Show Unassigned 1, Show Unassigned 2). The Custom Module Step Images window is visible at the bottom.

Click Channel Display to modify image display

Change color display

Click down arrow for display options

Adjust the intensity scale using the histogram slider

Dock or Auto-Hide windows by clicking the down arrow



CME Home Tab: Show Image Info

The screenshot displays the CME software interface. A red box highlights the 'Show Image Info' button in the top toolbar. A red callout box with an arrow pointing to the button contains the text: "Click Show Image Info to show information about how image was acquired".

The 'Image Info' table is shown on the right side of the interface. A red arrow points to the 'ZStep' row in the table.

Name	DAPI
TStep	0
ZStep	0
ChannelStep	0
StagePositionStep	0
StagePositionX	0
StagePositionY	0
AbsoluteZPosition	0
AbsoluteZ2Position	
StageLabel	I03 : Site 1
Wavelength	447
IlluminationSettingName	
ExposureTimeMilliseconds	
Date	3/30/2005
CreationTime	13:26:09.472
Image Directory	C:\Users\Hamidah.Sultan\Docum
Image Name	t0s0z0c0.tif
Annotation	;Group(s)=Public;Camera(s)=T;Cli
CameraBinningX	1
CameraBinningY	1
CameraChipOffsetX	0
CameraChipOffsetY	0
Width	640



CME Home Tab: Show Line Scan

The screenshot displays the CME software interface. At the top, the 'Regions' button is highlighted with a red box and a red arrow pointing to it. A red text box above it reads: "Use **Regions** to determine size and intensity values across objects".

In the center, two microscopy images are shown: a DAPI channel (blue) and a FITC channel (green). A yellow line is drawn across the DAPI image, with a yellow text box above it stating: "Double click on line to adjust length. Click and drag to move".

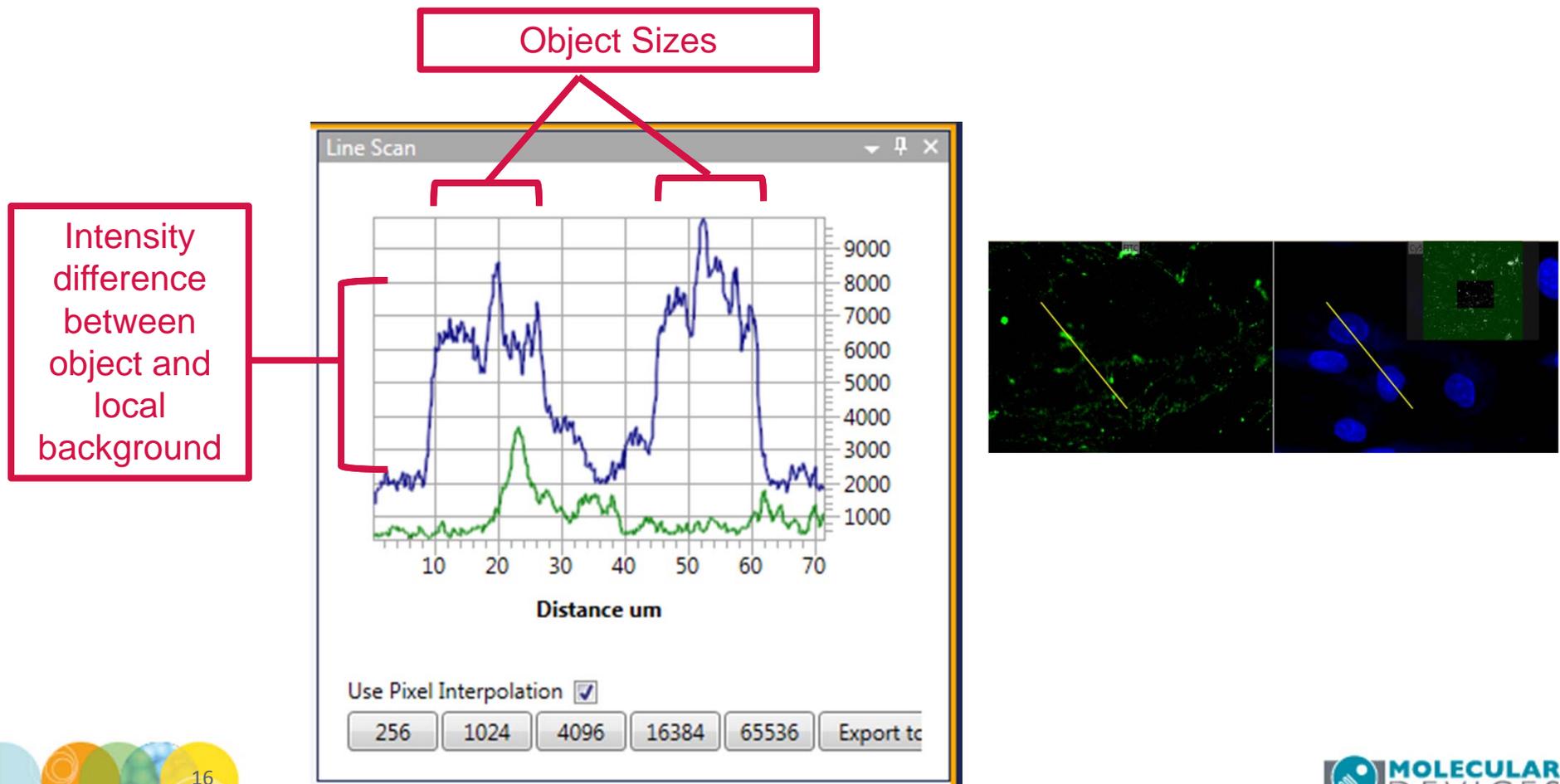
To the right, a 'Line Scan' graph is displayed. The x-axis is labeled 'Distance um' and ranges from 0 to 140. The y-axis ranges from 0 to 20000. The graph shows two intensity profiles: a blue line for the DAPI channel and a green line for the FITC channel. A red arrow points from the yellow line in the image to the corresponding blue line in the graph.

At the bottom left, a red text box contains the instruction: "Right click on image, select **Show Line Scan**".



Show Line Scan

- The **Line Scan** graph can be used to determine distances across objects as well as intensity levels
- This useful when setting up step cards that require **Minimum** and **Maximum widths** as well as **Intensity above local background**



CME Home Tab: Show Calibration Bar

The screenshot displays the CME software interface. The top toolbar contains various icons, with the 'Calibration Bar' icon highlighted in a yellow box. The main workspace shows two channels: 'DAPI' (blue) and 'FITC' (green). A measurement bar is visible on the DAPI channel, indicating a length of 69 μm. A text box with an orange border and arrow points to the Calibration Bar icon, stating: "Click Calibration Bar to display calibration bar (shown in μm)".



CME Home Tab: Export to PowerPoint

The screenshot displays the CME software interface. The top toolbar includes the 'Export to PowerPoint' button, which is highlighted with an orange box. A callout box with an orange border and text points to this button, stating: "Click here to export current display in image grid to PowerPoint". The main window shows a grid of two images: a blue-stained DAPI channel on the left and a green-stained FITC channel on the right. The 'Custom Module' panel on the left is open, showing settings for 'Segment' and 'Measure' tabs. The 'Measure' tab is active, with 'Objects to Measure' set to 'Nuclei' and 'Image to Measure' set to 'DAPI'. The 'Features within Each Object' section has 'Mask of Features' set to 'Puncta' and 'Image to Measure' set to 'FITC'. The 'Measurement Name' and 'Setting Name' fields are both set to 'Custom Module'. The bottom of the interface shows a color calibration bar with blue and green segments.



CME Home Tab: Export to PowerPoint

The screenshot displays the Microsoft PowerPoint interface. The title bar reads "Presentation1 - Microsoft PowerPoint". The ribbon includes tabs for File, Home, Insert, Design, Transitions, Animations, Slide Show, Review, and View. The Home tab is active, showing options for Clipboard, Slides, Font, Paragraph, Drawing, Editing, and WebEx. The Slides pane on the left shows a single slide titled "Custom Module: Custom Module: 7/10/2015 1:44:57 AM" with a thumbnail image. The main slide area contains the following text and images:

Custom Module: Custom Module:
7/10/2015 1:44:57 AM

Below the text are two side-by-side microscopy images. The left image is labeled "DAPI" and shows blue-stained nuclei. The right image is labeled "FITC" and shows green-stained cells. A scale bar in the top left of the DAPI image indicates "10 μm".

At the bottom of the slide area, there is a text box that says "Click to add notes". The status bar at the bottom of the window shows "Slide 1 of 1", "Office Theme", and a zoom level of 87%.



CME Home Tab: Object Overlay

After the custom module has been ran, a color-coded segmentation image overlay will be produced. Toggle **Object Overlay** to turn segmentation on and off.



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





ADVANCING PROTEIN AND CELL BIOLOGY