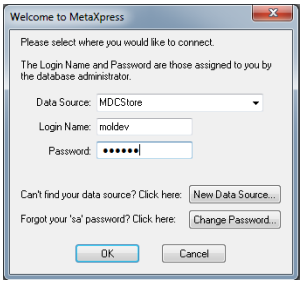
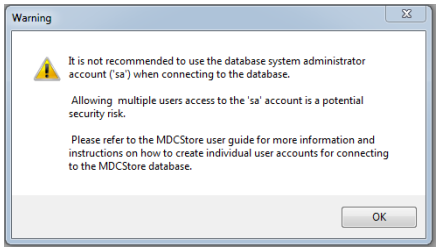
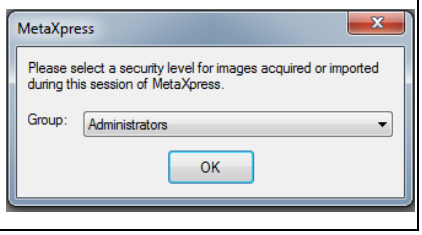
ImageXpress® Micro Confocal & MetaXpress® 6.5

The purpose of this guide is to briefly describe:

I. Turn on System and Acquire Plate with Saved Settings

1.	Turn on the system: • IXM power supply controller box • Computer and Monitor
2.	Go to the MetaXpress folder and double-click on the appropriate hardware profile shortcut 
3.	Login to MDCStore database with username and password Username: ***moldev*** Password: ***moldev*** *NOTE* Your database, username, and password may be different. Refer to your administrator for this information 
4.	If you log in as system administrator (sa), the next window is a warning regarding security risks; click **OK** 
5.	Group (security level) and click **OK** 

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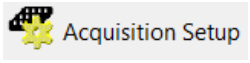
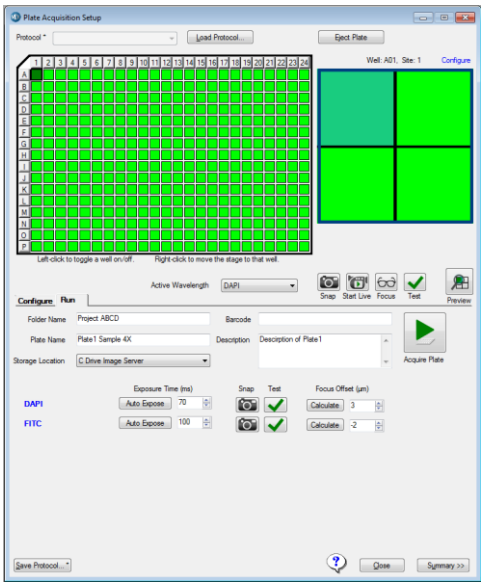
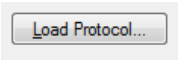
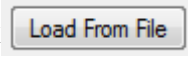
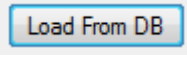
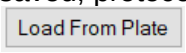
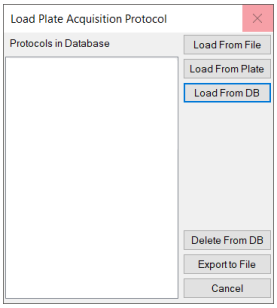
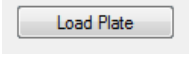
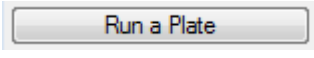
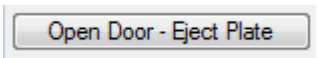
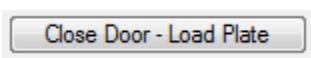
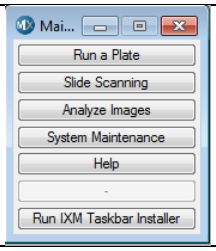
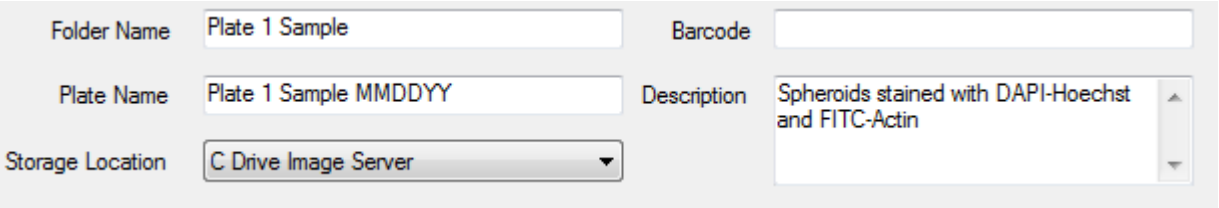
6.	In the main toolbar, click  or in the main menu select **Screening > Acquisition Setup** 
7.	To load a previous saved protocol, click on  in **Plate Acquisition Setup**
8.	• Click  to search windows for the appropriate .hts file. • If the settings file is saved to the database, highlight the protocol and click  • If no settings have been saved, protocols can be loaded from an existing plate by clicking  
9.	• Click  to open the door and place the plate in the in the system • Click  to close the door
10.	Alternatively, you can use the **Main Taskbar** to open and close the door. • Click  • Click  or  

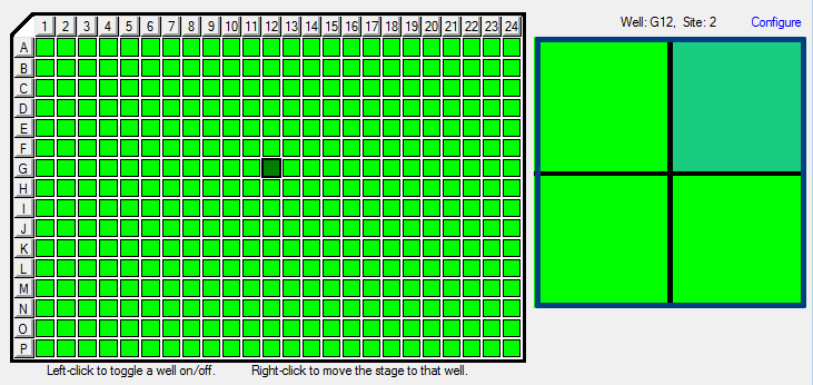
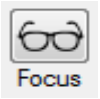
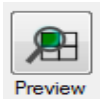
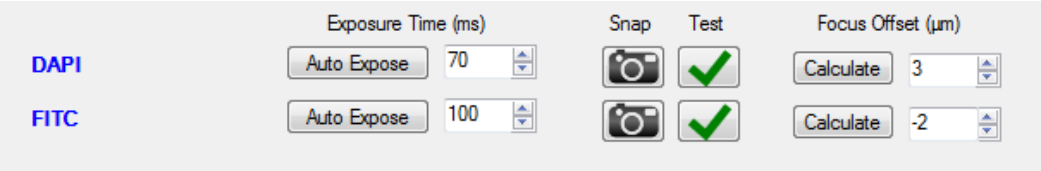
11. On the **Run** tab, update the folder name, plate name and description as desired

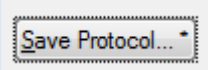
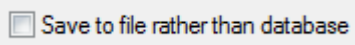
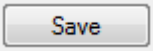


12. Click 

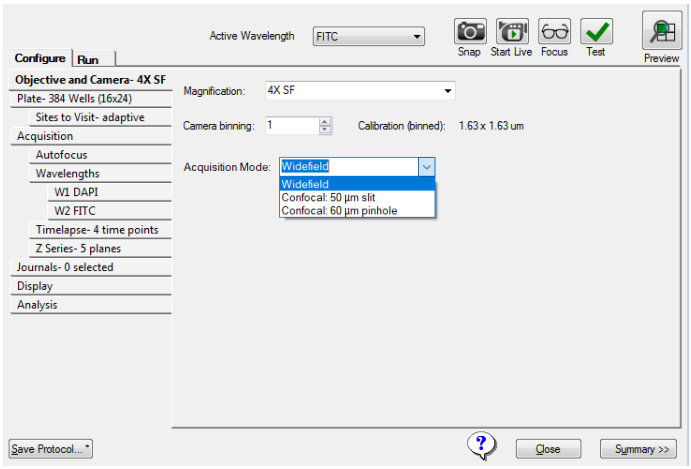
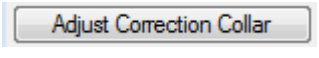
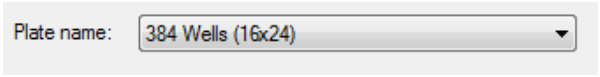
to begin acquiring the plate

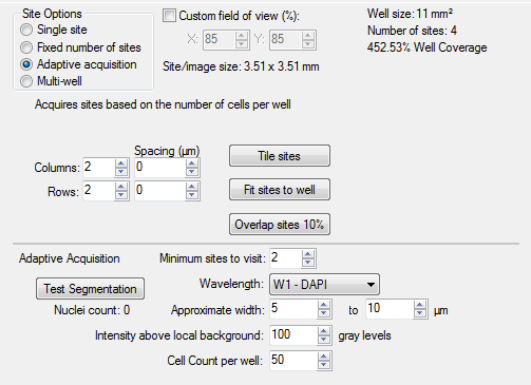
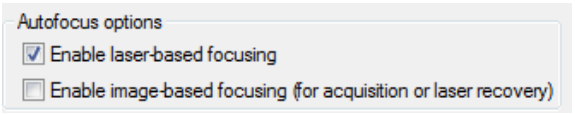
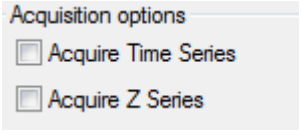
II. Test Acquisition Settings

1.	Open **Plate Acquisition Setup**
2.	In the plate and site section of **Plate Acquisition Setup**, right-click on the desired well and/or site to move the plate to that position (indicated by a dark green color) 
3.	Test the acquisition settings by clicking: •  to perform a large-range autofocus and snap image routine •  to perform a focus and snap image routine (if Z series has been activated, all planes will be acquired) •  to perform an autofocus and snap image routine all for all wavelengths (if Z series has been activated, all planes will be acquired)
4.	Adjust the acquisition settings, if necessary, within the **Run** tab: • Adjust the focus offset by clicking Calculate or adjust the number manually • Adjust the exposure time by clicking Auto Expose or change the number manually  *NOTE* Click on the wavelength name to open the corresponding wavelength tab for advanced options

5.	When you have optimized settings, click  • Molecular Devices recommends enabling  • Click  to search for a location on the hard drive.
6.	Click  to begin acquiring the plate

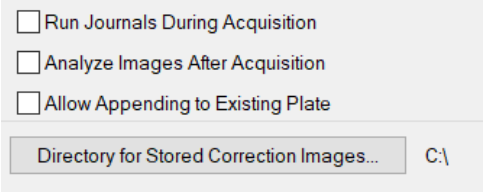
III. Define New Acquisition Settings

1.	Open **Plate Acquisition Setup**
2.	Select the **Configure** tab
3.	Select the **Objective and Camera** tab i. Select the appropriate magnification from the drop-down menu ii. Set binning (2 for cell counting and cell scoring; 1 for fine sub-cellular detail) iii. Select Acquisition Mode: Widefield or Confocal 3 confocal options depending on system configuration: 50 µm slit, 60 µm pinhole, 42 µm pinhole 
4.	Adjust the objective correction collar, if necessary, (setting on objective should match physical plate bottom thickness in mm). On the **Run a Plate Taskbar**, click on  to step through the process.
5.	Select the **Plate** tab and select the appropriate plate type from the drop-down list 

6.	Select the **Sites to Visit** tab and select the appropriate number of sites • **Single Site**: image one site per well in the center • **Fixed number of sites**: image the number of selected sites for every well. Adjust number and spacing of sites. Left-click on sites to select (green) and deselect (grey). Right-click on any site to move the plate to that site position (dark green) • **Adaptive acquisition**: collect the minimum number of sites to image at least the cell count indicated by the user. The Adaptive Acquisition section will appear allowing the user to choose wavelength, size and threshold settings, and desired minimum count for cells • **Multi-well**: collect multiple wells within one image which is then cropped to define single wells automatically • **Custom field of view (%):** reduce the size of each image by the percentage entered. This is useful when the field of view covers more than the site/well area desired 
7.	Select the **Acquisition** tab to select Autofocus and Acquisition options
8.	Autofocus options: • Always select **Enable laser-based focusing** • **Enable image-based focusing** for thick samples or those with different focal planes from site-to-site or well-to-well 
9.	Acquisition options: • Enable **Acquire Time series** for timelapse experiments • Enable **Acquire Z series** for Z step acquisition 

10.

Other options:



- If running a journal during acquisition, enable this option to activate the **Journals** tab
- If an analysis has already been setup, enable **Analyze Images After Acquisition**

  NOTE this requires an offline computer to be in Auto-run mode or running PowerCore software
- To enable appending time points, enable **Allow Appending to Existing Plate**
- If using the **Legacy Correction** shading correction option for any wavelengths, click **Directory for Stored Correction Images** and select the appropriate directory where shading correction images are saved

11.

Select the **Autofocus** tab:

i. Set **Well to well autofocus** to Focus on well bottom This is the default acquisition setup, however when imaging thin-bottom plates with low magnification objectives (4x and below) or microscope slides, select Focus on plate bottom, then offset by bottom thickness

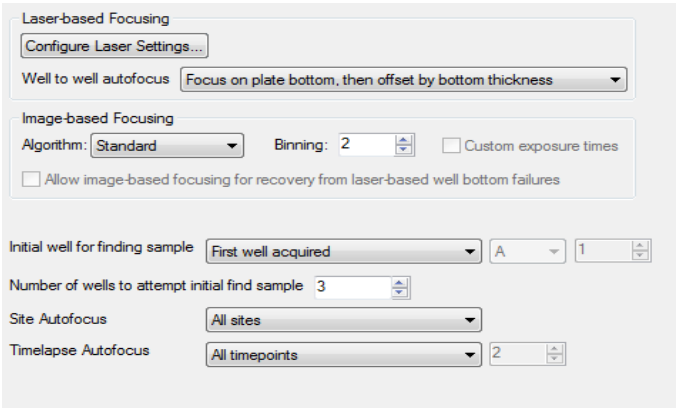
ii. For **Image-based Focusing** refer to corresponding MetaXpress 6 Software Guide modules for suggested settings

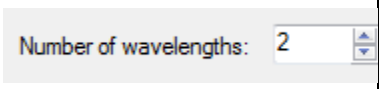
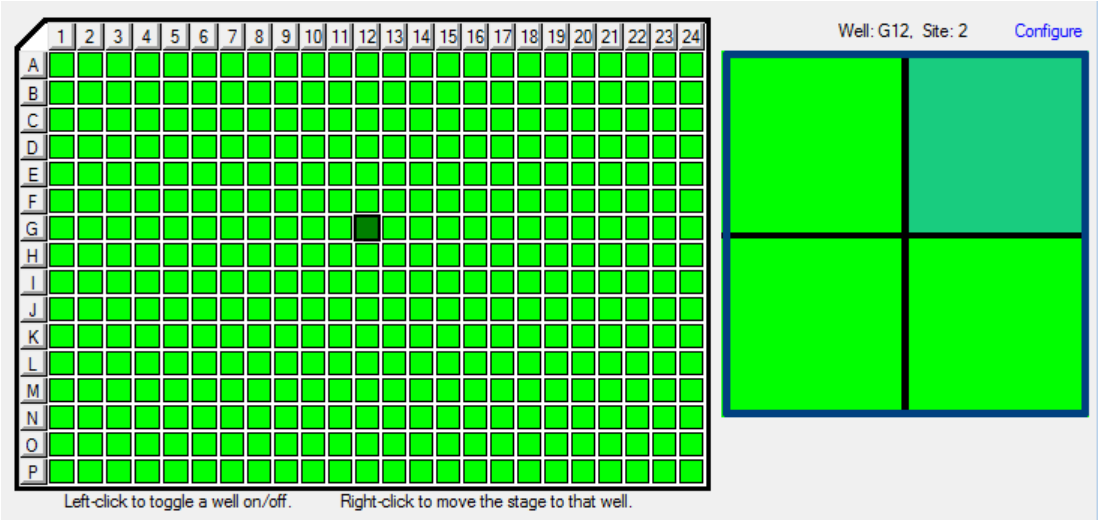
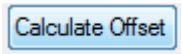
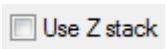
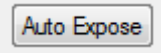
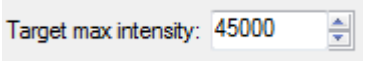
iii. Set **Initial well for finding sample** to First well acquired

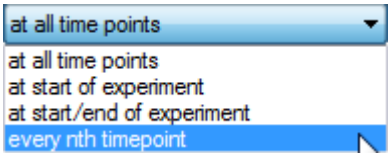
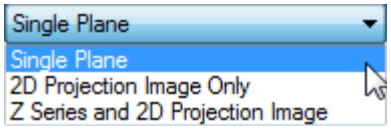
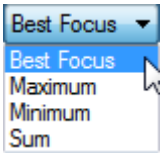
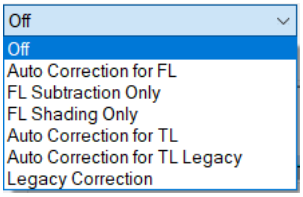
iv. Set **Number of wells to attempt initial find sample** to 3

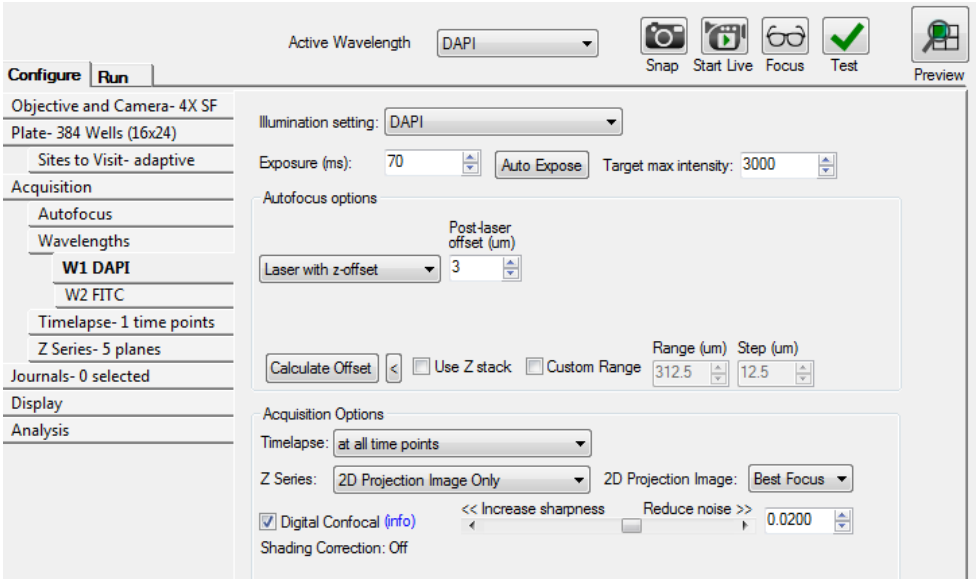
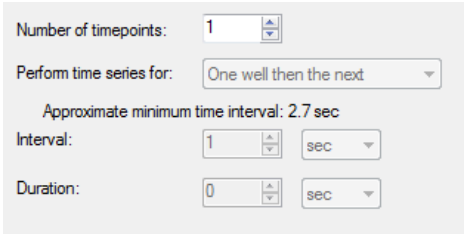
v. If more than one site is acquired, set **Site Autofocus** to All sites

vi. If timelapse is enabled, set **Timelapse Autofocus** to All timepoints for long term timelapse, and First timepoint only for fast kinetic experiments

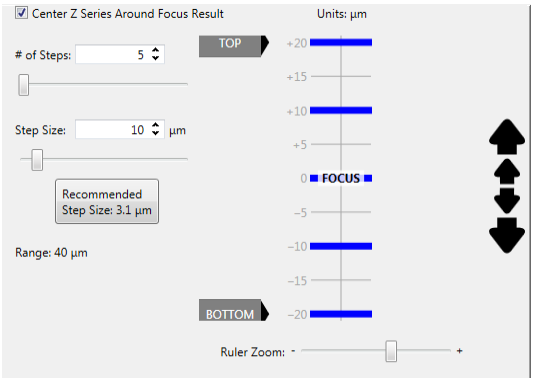


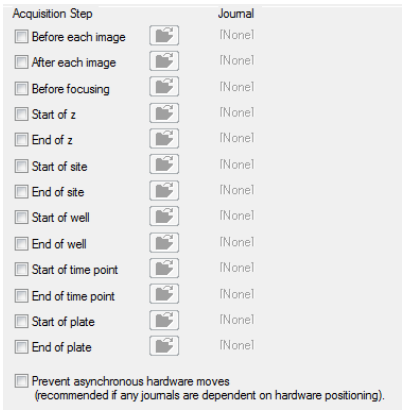
12.	Select the **Wavelengths** tab and select the number of wavelengths (colors) including transmitted light that you would like to acquire 
13.	In the plate and site section of **Plate Acquisition Setup**, right-click on the desired well (typically a control well) and/or site to move the plate to that position (indicated by a dark green color) 
14.	Select the **W1** (wavelength) tab i. Select the desired filter set from the drop-down menu under **Illumination setting** ii. Click  iii. Examine the image a. If the image appears to be dim or saturated, first adjust the image scaling, then adjust exposure time if necessary b. If a blank or snowy image appears, this can indicate that a plate is not in the system or laser autofocus settings are incorrect iv. Click the  to perform an automatic focus determination a. For more control, enable  and follow the prompts b. If necessary, enable  v. Click  again to test the new post-laser offset. Image should now be in focus. vi. Examine the image for brightness a. If necessary, click  with  set to **33000 – 45000** b. You can also increase or decrease exposure manually

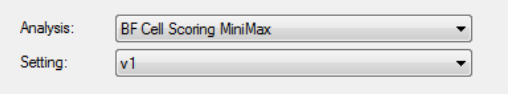
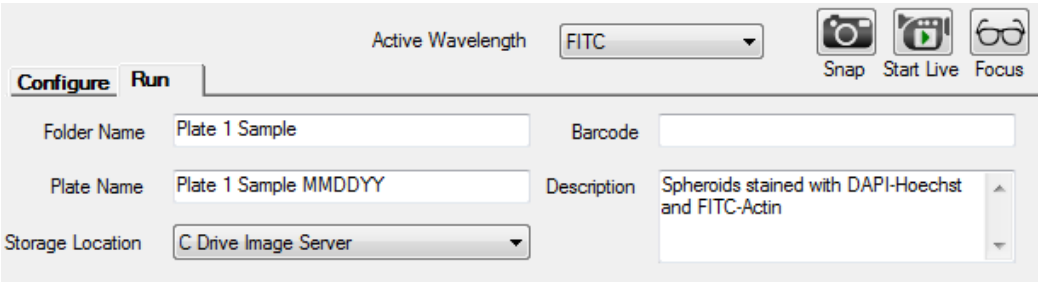
15.	If acquiring a Timelapse, select how often to acquire this image from the drop-down menu 
16.	If acquiring a Z Stack, select the appropriate setting for image collection  *NOTE* Z Series and 2D Projection Image is not available when acquiring a Timelapse If saving the 2D Projection Image, select the appropriate projection method (press F1 for more information)  *NOTE* Best Focus is not recommended for comparison of intensity measurements
17.	If the option is available, you can enable **Digital confocal** and select the appropriate K value using the slider bar (press F1 for more information)
18.	Apply a shading correction option for your wavelength, if needed.  For Fluorescent wavelengths: **FL Shading Only** generally works well for most assays. Other options include **Auto Correction for FL**, **FL Subtraction Only**, **Legacy Correction** (requires the use of preset reference images), or **Off** (no shading correction) For Brightfield or Phase Contrast wavelengths: **Auto Correction for TL** generally works well for most assays. Other options include **Auto Correction for TL Legacy**, **Legacy Correction** (requires the use of preset reference images), or **Off** (no shading correction)

19.	Repeat for each subsequent wavelength 
20.	If acquiring with Timelapse, select the **Timelapse** tab i. Enter the number of **Time points** desired ii. Set **Interval** as the time between each time point iii. Set **Duration** as the total time of the experiment iv. Set **Perform time series** for: • **One well then the next**: entire timelapse is run for one well before acquiring next well • **One column then the next**: entire timelapse is run for one column before the next • **One row then the next**: entire timelapse is run for one row before acquiring next row • **All selected wells**: all wells are imaged before continuing with next time point 

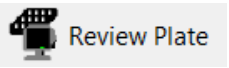
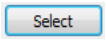
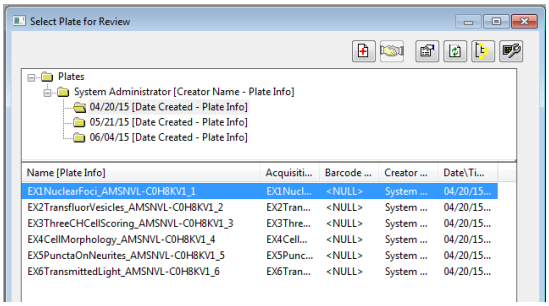
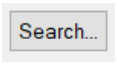
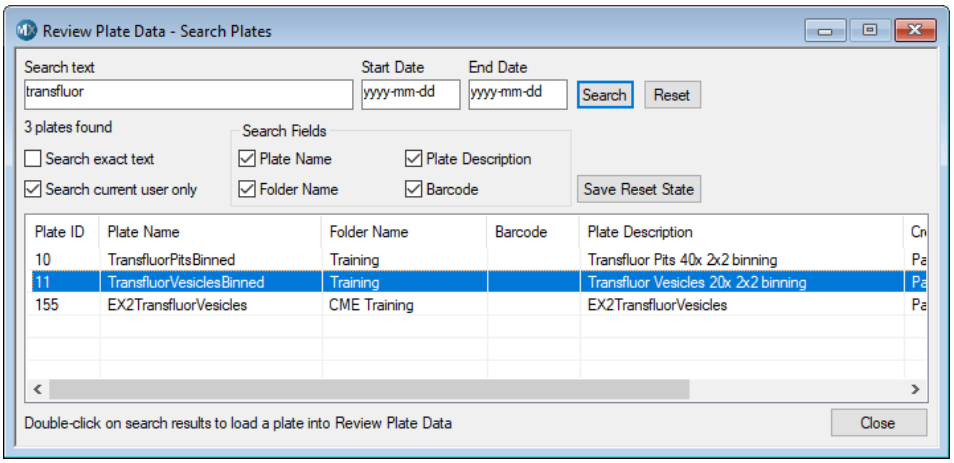
21.	If acquiring a Z Series, select the **Z Series** tab i. Deselect Center Z Series Around Focus Result ii. Adjust **Step size** for spacing between each Z plane iii. Click Focus to determine the Z start position iv. Click to start **Live Mode** v. Use the large and small arrows to move to the top of the focus range for the sample and click Set T vi. Use the large and small arrows to move to the bottom of the focus range for the sample and click Set B vii. Click F2:Stop to stop **Live Mode** viii. Click Test to perform focus and snap image routine to acquire all Z steps for the active wavelength. The last image in the stack will be the selected 2D projection image. ix. Click Preview to perform focus and snap image routine to acquire all Z steps for all wavelengths. The last image in the stack will be the selected 2D projection image.
22.	Select the **Journal** tab (enabled on acquisition tab) and activate journals where appropriate. It might also be necessary to enable Prevent asynchronous hardware moves (recommended if any journals are dependent on hardware positioning). for certain journals (refer to documentation accompanying journals for details)

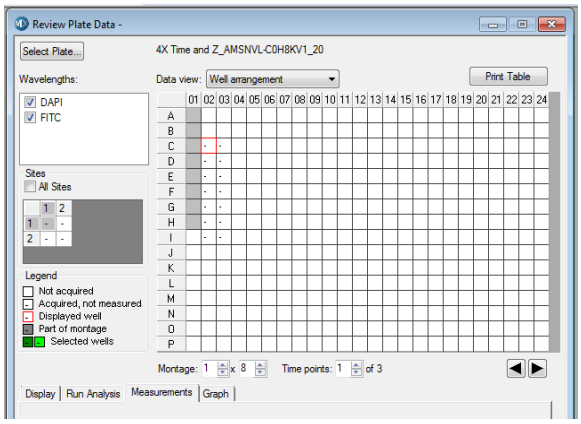
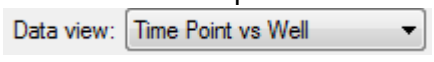
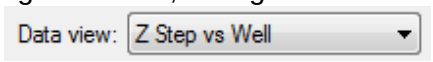
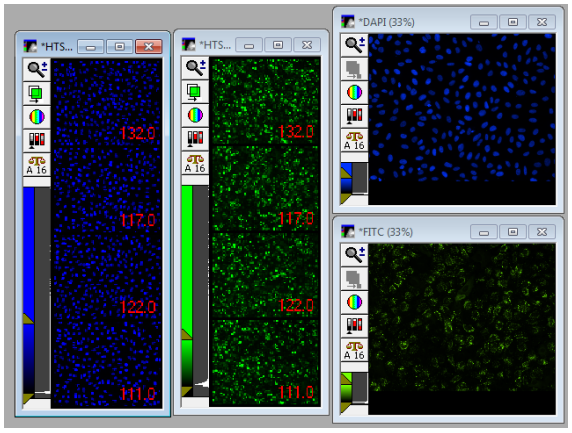
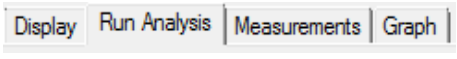


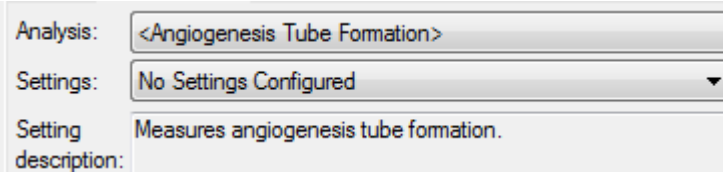
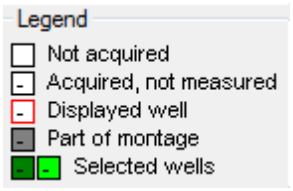
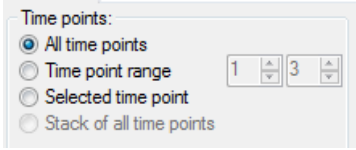
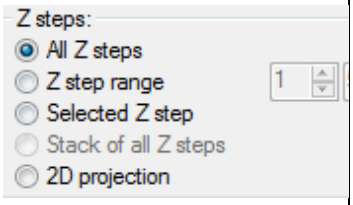


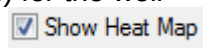
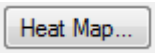
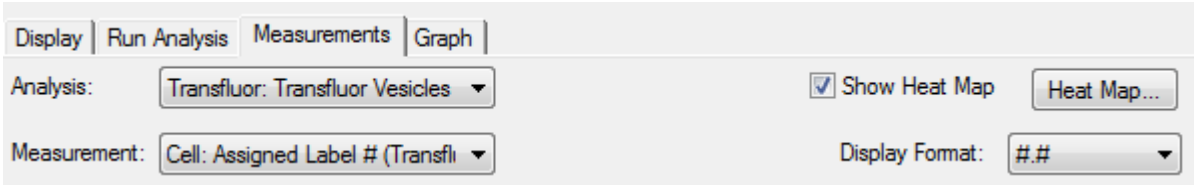
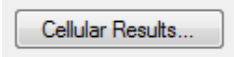
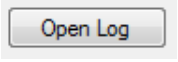
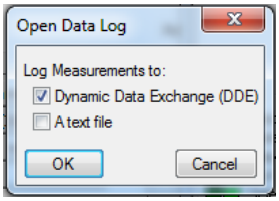
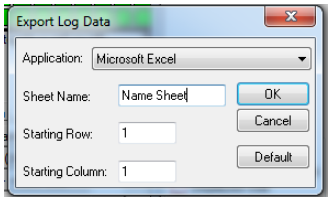
23.	Select the **Display** tab to: • Auto Arrange Images will use default settings to arrange displayed images • Display Acquisition Layout manually adjust image display prior to acquisition • Enable ☑ Display images during autofocus • Enable ☑ Display images during acquisition • (Optional) Enable ☑ Display a color overlay of wavelength images during acquisition
24.	Select the **Analysis** tab (enabled on the acquisition tab) to specify the appropriate optimized **Analysis** routine and **Settings** from the drop down-menus *NOTE* This requires an offline computer set in Auto-run mode or running PowerCore software 
25.	Under the **Run** tab, enter: • **Folder Name**: Project name, your name, PI, etc. All your plates will go under this name. • **Plate Name**: Name of this experiment • **Storage location**: Select appropriate server for image storage. *NOTE* There may only be one choice. • **Barcode**: Enter a barcode if desired • **Description**: Any text regarding the experiment 
26.	When you have optimized settings, click Save Protocol... • Molecular Devices recommends enabling ☐ Save to file rather than database • Click Save for a location on the hard drive.
27.	Click on  to begin acquiring the plate

IV. Review Images and Run an Analysis

1.	In the main toolbar, click  or in the main menu select **Screening > Review Plate**
2.	On the **Review Plate Data** dialog, click 
3.	Navigate through the folders to find the plate of interest. Highlight the plate and click  
4.	If you cannot find your plate, on the **Review Plate Data** dialog, click  • Enter the Search text and optional date range • Enable or disable search options • Click **Search**. • Highlight the plate and double-click it to open. *NOTE* Search results can remain open, in case multiple plates need to be checked. 

5.	In the Plate View section, you will see a '-' in each well that was imaged. Left-click and drag across the wells you want to view. A thumbnail montage of these wells will open for each wavelength. 
6.	If there are multiple sites per well, select an appropriate site to view, or enable **All Sites**. The image montages will automatically adjust. 
7.	To view all Timelapse or Z Series images at once, change the **Data view** to  or  , respectively.
8.	Left-click on a single thumbnail to view full resolution images (all wavelengths) 
9.	To run or set up an analysis, select the **Run Analysis** tab 

10.	If analysis settings have already been optimized, select the analysis routine (application module, custom module, or journal) and settings from the drop-down menus 
11.	Under the **Run Analysis** tab, select the appropriate button to run the analysis: • Run on all wells analysis we be run on all acquired images • Run on selection analysis will be run on selected wells (selected wells are indicated in green; to select wells, right click well(s) in the plate section or image montage) • Run on displayed site analysis will be run only on the currently displayed site 
12.	For a Timelapse data set, select the appropriate option for analysis under the **Time points** section • **All time points**: run analysis on all time points in the data set • **Time point range**: run analysis on a consecutive range of time points • **Selected time point**: run analysis on only one time point that is select in the **Time point** section below the plate layout Time points: 1 of 3 • **Stack of all time points**: use if, in the **Analysis** field, you select a timelapse journal which analyzes the planes in a stack 
13.	For a Z Series data set where all Z planes were saved, select the appropriate option in the **Z steps** section • **All Z Steps**: run analysis on all Z planes • **Z Step range**: run analysis on consecutive range of Z planes • **Selected Z step**: run analysis on only one Z plane that is selected in the **Z step** section below the plate layout Z steps: 3 of 5 • **Stack of all Z steps**: run an analysis with a journal that requires a stack of images • **2D projection**: only run analysis on the saved 2D projection image 

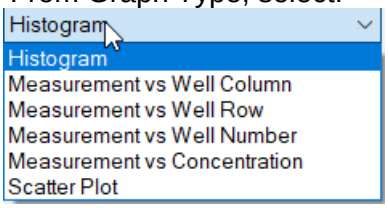
14.	If the selected analysis has already been run on the plate, a warning will appear asking to overwrite the data. If you are not sure, save the analysis settings with a new name before analyzing your plate.
15.	To view analysis results, select the **Measurements** tab i. Select the **Analysis** (module and settings name) from the drop-down menu ii. Select a measurement from the drop-down menu. The values will be shown in the plate layout. • Measurements starting with a "Cell" are cell-by-cell data and will give the average of all cells in the displayed site(s) for the well iii. Activate the heat map by enabling  iv. Configure the heat map by clicking on  *NOTE* In the plate view, summary measurements, such as counts, are displayed as an average of all sites in the well, rather than a sum. To obtain sum values, the data can be exported via Plate Data Utilities. 
16.	To view the cell-by-cell data, click  at the bottom of the **Review Plate Data** dialog. Data will be automatically updated based on the well and site selected in the montage view
17.	To export data to Excel: i. On the **Measurements** tab, click on  ii. Select only **Dynamic Data Exchange** iii. Select **Microsoft Excel** and name worksheet as desired. This opens an empty worksheet. iv. Click  . Currently viewed data will be logged into the Excel sheet.  

18.

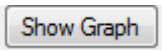
To create simple graphs in MetaXpress:

i. Go to **Graph** tab

ii. From Graph Type, select:



iii. Select measurements to plot from the drop-down menu

iv. Click 

v. Right-click on the graph for more options

NOTE For Measurement vs Concentration, the plate must first be annotated.