MOLECULAR DEVICES

Quick Start Guide

ImageXpress® Micro Confocal & MetaXpress® 6

The purpose of this guide is to briefly describe:

I. Turn on system and acquire plate with saved settings
II. Test acquisition settings
III. Define new acquisition settings
IV. View images and run an analysis

I. Turn on System and Acquire Plate with Saved Settings

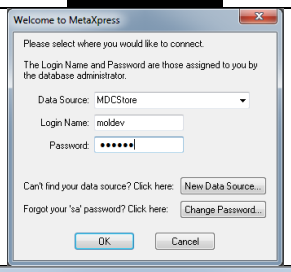
1. Turn on the system:
   - Light source (if not already on)
   - IXM Power Supply/Options Controller Box (Also controls Transmitted Light, Environmental Control or Fluidics modules)
   - Computer and Monitor

2. Go to the MetaXpress folder and double-click on the appropriate hardware profile shortcut

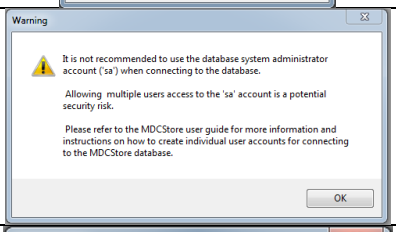
3. Login to MDCStore database with username and password

Username	***moldev***
Password	***moldev***

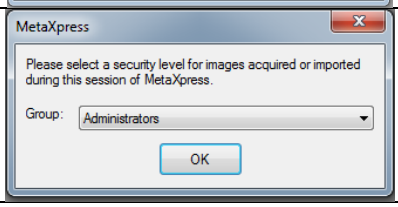
NOTE Your database, username, and password maybe different. Refer to your administrator for this information



4. If you log in as system administrator (sa), the next window is a warning regarding security risks; click **OK**

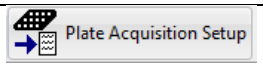


5. Select Group (security level) and click **OK**

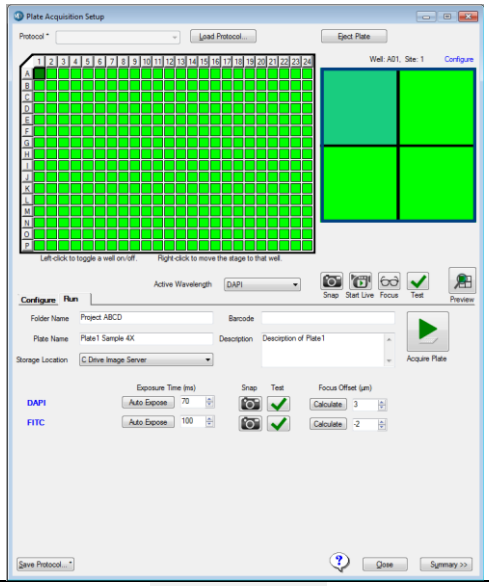


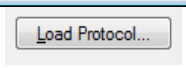
1311 Orleans Drive
Sunnyvale, CA 94089
toll-free 1.800.635.5577
phone 408.747.1700
fax 408.747.3601

moleculardevices.com

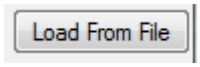
6. In the main toolbar, click 

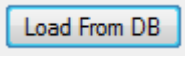
or in the main menu select **Screening > Plate Acquisition Setup**

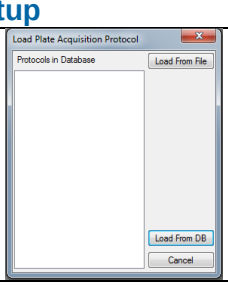


7. To load a previous saved protocol, click on 

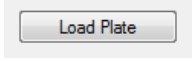
in **Plate Acquisition Setup**

8.
- Click 

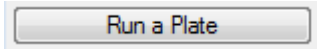
to search windows for the appropriate .hts file.
- If the settings file is saved to the database, highlight the protocol and click 

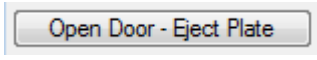


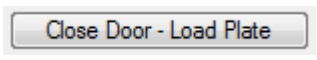
9.
- Click 

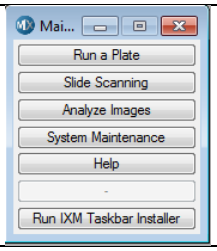
to open the door and place the plate in the in the system
- Click 

to close the door

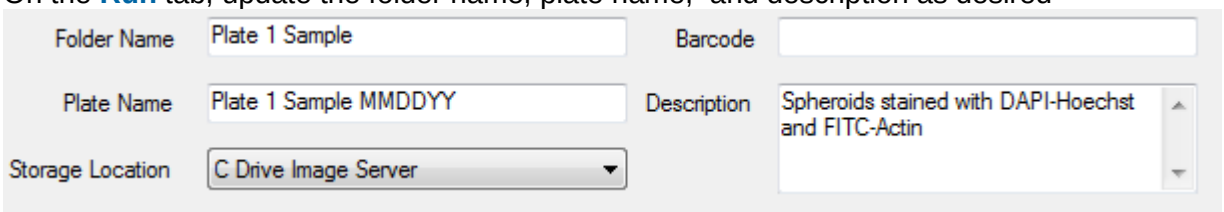
10. Alternatively, you can use the **Main Taskbar** to open and close the door.
- Click 

- Click 

or 



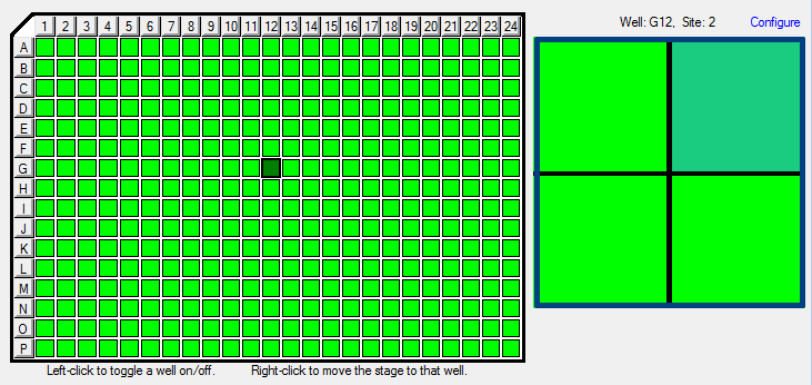
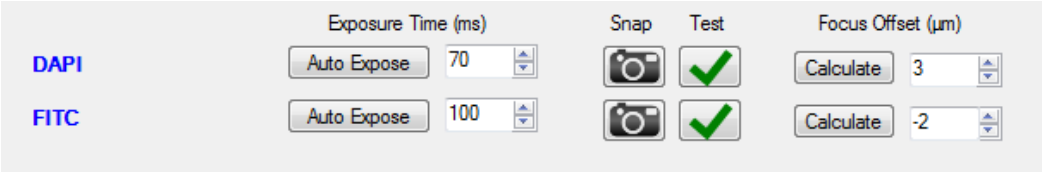
11. On the **Run** tab, update the folder name, plate name, and description as desired



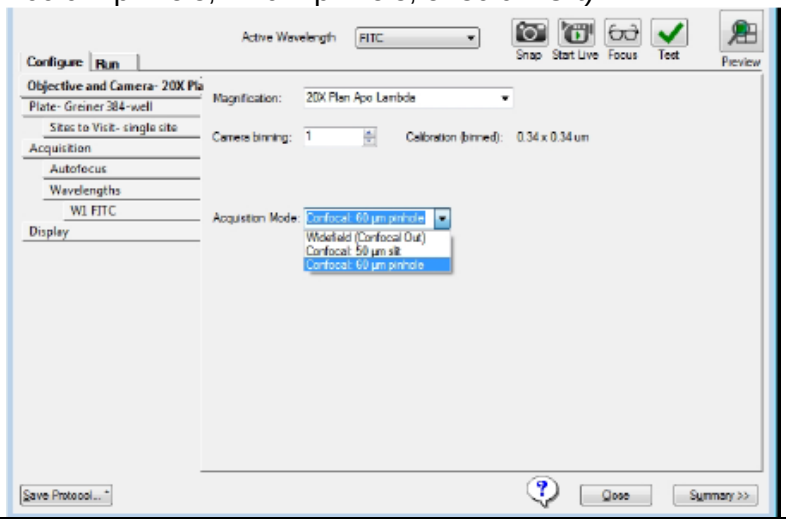
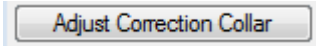
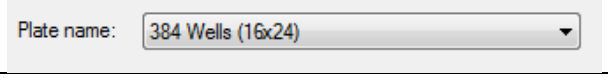
12. Click 

to begin acquiring the plate

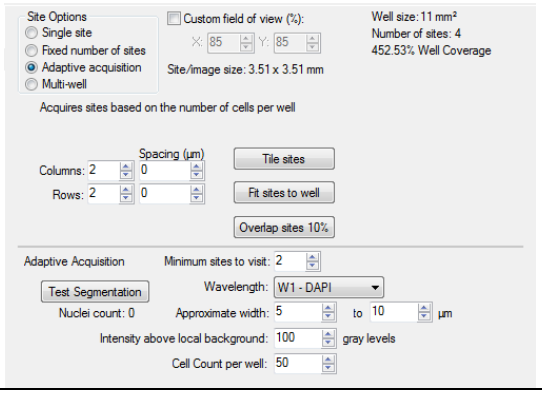
II. Test Acquisition Settings

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 • Focus to perform a large range autofocus and snap image routine • Test to perform a focus and snap image routine (if Z series has been activated, all planes will be acquired) • Preview 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: • Adjust the focus offset by clicking Calculate or adjusting the number manually • Adjust the exposure time by clicking Auto Expose or changing 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 Save Protocol... • Molecular Devices recommends enabling Save to file rather than database • Click Save to search for a location on the hard drive.
6.	Click Acquire Plate to begin acquiring the plate

III. Define New Acquisition Settings

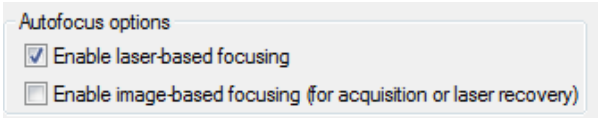
1.	Open **Plate Acquisition Setup**
2.	Select the **Configure** tab
3.	Select the **Objective and Camera** tab i. Select the appropriate magnification from the drop-down menu ii. Set binning (2 for cell counting and cell scoring; 1 for fine sub-cellular detail) iii. Select Acquisition Mode: Widefield or Confocal (3 possibilities depending on system configuration: 60 um pinhole, 42 um pinhole, or 50 um slit) 
4.	Adjust the objective correction collar if necessary (setting on objective should match physical plate bottom thickness in mm X refractive index of material – 1.59 for Plastic, 1.52 for Glass). 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

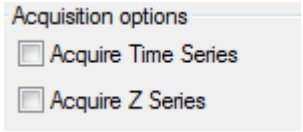


7. Select the **Acquisition** tab to select Autofocus and Acquisition options

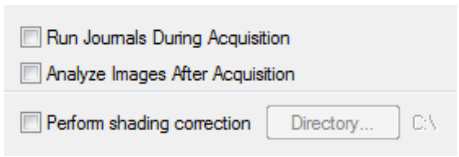
8. Autofocus options:
   - Always select **Enable laser-based focusing**
   - **Enable image-based focusing** for thick samples or those with different focal planes from site-to-site or well-to-well



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 correct for uneven background, enable **Perform shading correction** and select the appropriate directory where shading correction images are saved



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

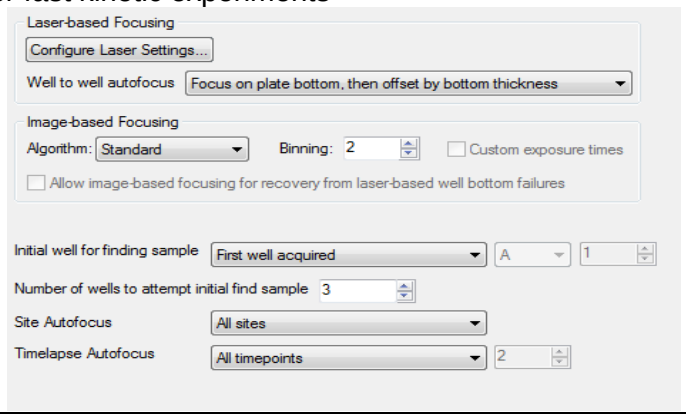
ii. For **Image-based Focusing** refer to corresponding MetaXpress 6 Software Guide modules for suggested settings

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

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

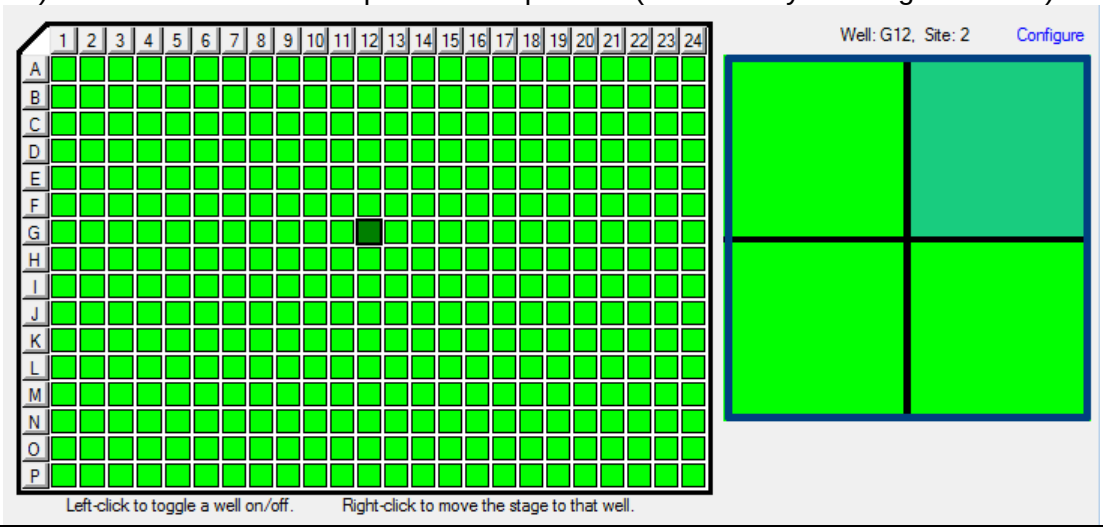
v. If more than one site is acquired, set **Site Autofocus** to All sites

11. vi. If timelpase 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)



Select the **W1** (wavelength) tab

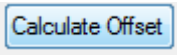
i. Select the desired filter set from the drop-down menu under **Illumination setting**

ii. Click 

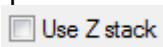
iii. Examine the image

a. If the image appears to be dim or saturated, first adjust the image scaling, then adjust exposure time if necessary. *(Be sure to look at the histogram on the left of your image window to judge exposure – should peak at 50-75% of the total dynamic range.)*

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

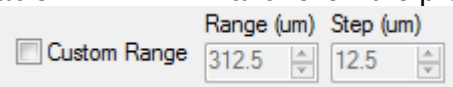
iv. Click the 

button to perform an automatic focus determination

a. For more control, enable 

and follow the prompts

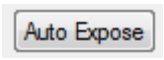
14.

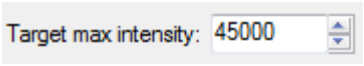
b. If necessary, enable 

v. Click 

again to test the new post-laser offset. Image should now be in focus.

vi. Examine the image for brightness

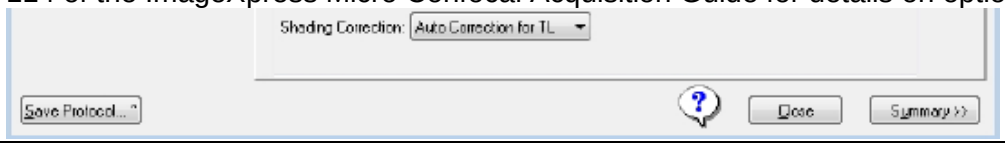
a. If necessary, click 

with 

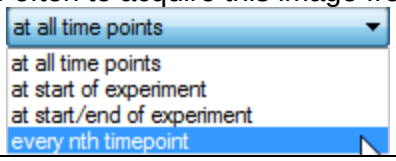
set to **33000 – 45000**

b. You can also increase or decrease exposure manually

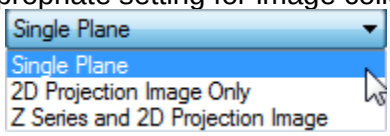
vii. Select the appropriate shading correction option for your wavelength (see Table 5-5, page 114 of the ImageXpress Micro Confocal Acquisition Guide for details on options):



15. If acquiring a Timelapse, select how often to acquire this image from the drop down menu

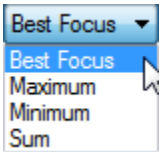


If acquiring a Z Stack, select the appropriate setting for image collection



NOTE Z Series and 2D Projection Image is not available when acquiring a Timelapse

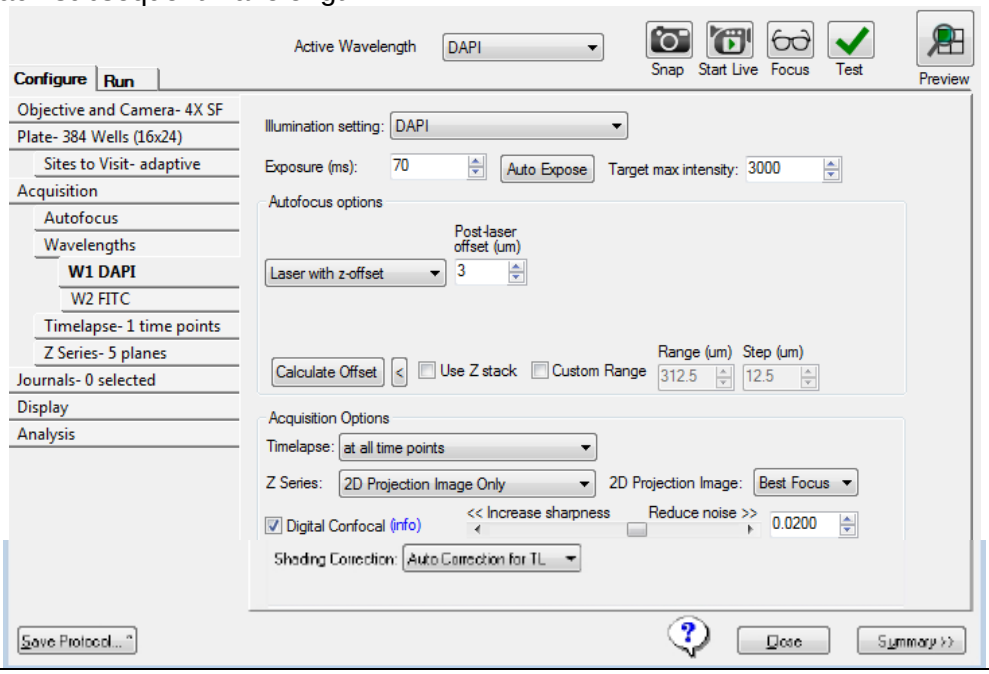
16. If saving the 2D Projection Image, select the appropriate projection method (press F1 for more information)



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)

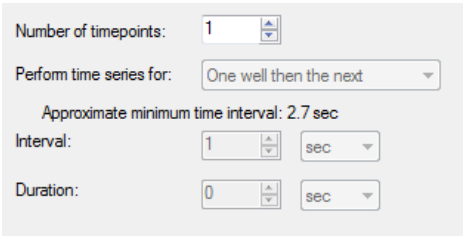
18. Repeat for each subsequent wavelength



19. 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 acquiring next column
- **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



20.

If acquiring a Z Series, select the **Z Series** tab

i. Deselect 

ii. Adjust **Step size** for spacing between each Z plane

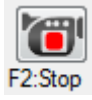
iii. Click 

to determine the Z start position

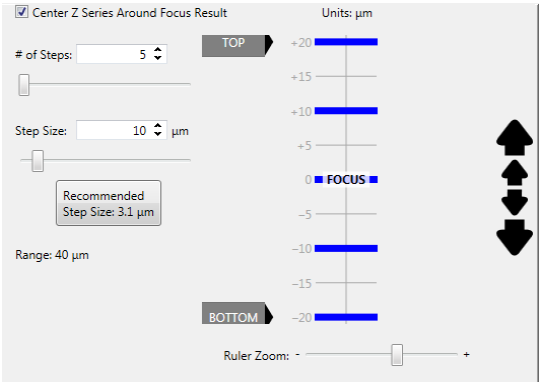
iv. Click  to start **Live Mode**

v. Use the large and small arrows to move to the top of the focus range for the sample and click 

vi. Use the large and small arrows to move to the bottom of the focus range for the sample and click 

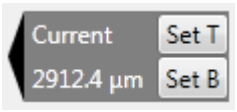
vii. Click 

to stop **Live Mode**



- Click 

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.

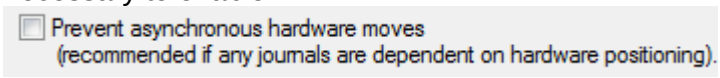


- Click 

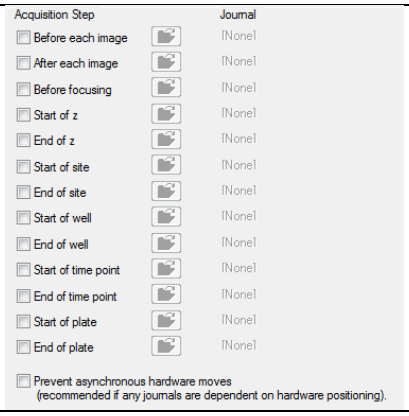
to perform focus and snap image routine to acquire all Z steps for all wavelengths. The last image in the stack will be the selected 2D projection image.

21.

Select the **Journal** tab (enabled on acquisition tab) and activate journals where appropriate. It might also be necessary to enable



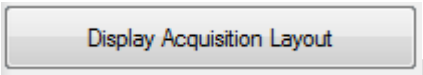
for certain journals (refer to documentation accompanying journals for details)

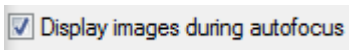


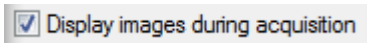
22.

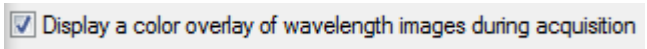
Select the **Display** tab to:

- 

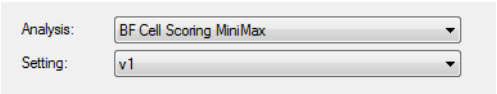
will use default settings to arrange displayed images
- 

manually adjust image display prior to acquisition
- Enable 

- Enable 

- (Optional) Enable 

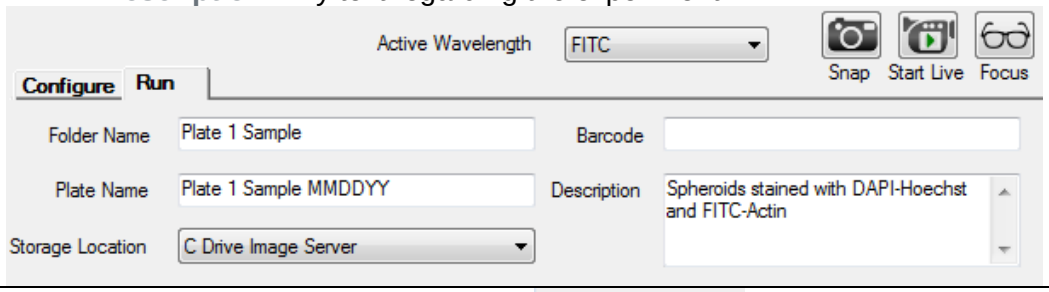
23. Select the **Analysis** tab (enabled on the acquisition tab) to specify the appropriate optimized **Analysis** routine and **Settings** from the drop down-menus

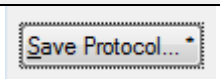


NOTE This requires an offline computer set in Auto-run mode or running PowerCore software

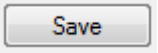
24. Under the **Run** tab, enter:

- **Folder Name**: Project name, your name, PI, etc. All your plates will go under this name.
- **Plate Name**: Name of this particular 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



25. When you have optimized settings, click 

- Molecular Devices recommends enabling 

- Click 

to search for a location on the hard drive.

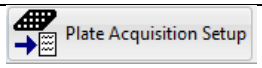
26. Click on 

to begin acquiring the plate

Key to ImageXpress Micro Confocal Status Lights:

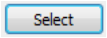
Color	Instrument Status
Orange	The instrument is powered on without software control.
Blue	The instrument is powered on with software control and is ready to use.
Green	The instrument is acquiring data.
Red	The instrument is in an error state or cannot communicate with the software.

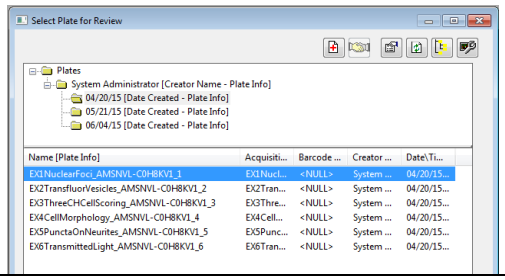
IV. Review Images and Run an Analysis

1. In the main toolbar, click 

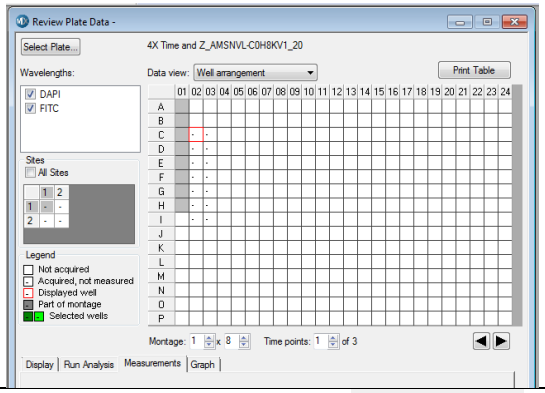
or in the main menu select **Screening > Plate Acquisition Setup**

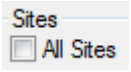
2. On the **Review Plate Data** dialog, click 

3.
- Navigate through the folders to find the plate of interest.
- Highlight the plate and click 

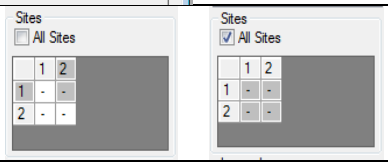


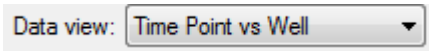
4. In the Plate View section, you will see a "-" in each well that was imaged. Left-click and drag across the wells you want to view. A thumbnail montage of these wells will open for each wavelength

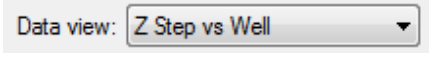


5. If there are multiple sites per well, select an appropriate site to view, or enable 

. The image montages will automatically adjust.

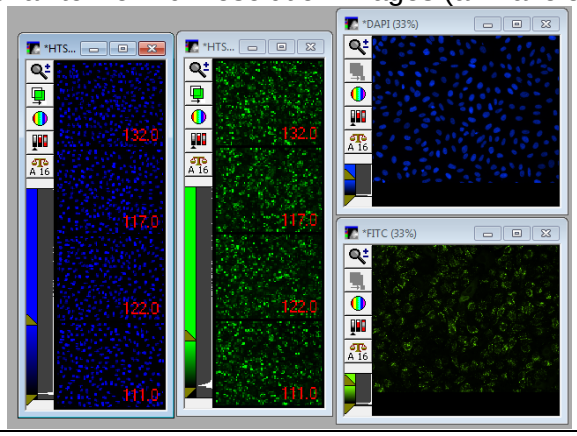


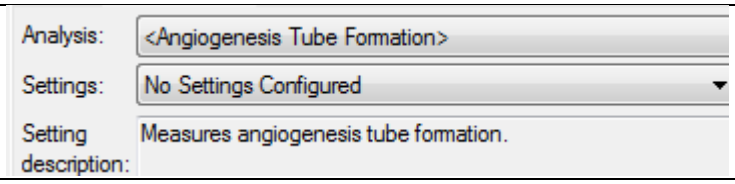
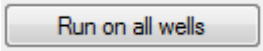
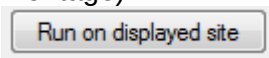
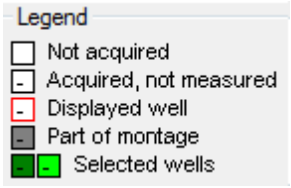
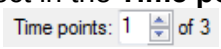
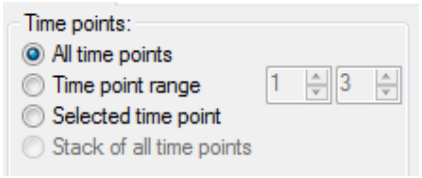
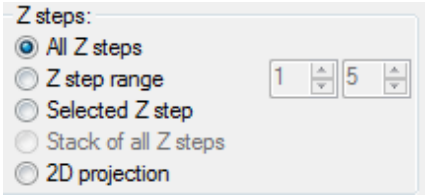
6. To view all Timelapse or Z Series images at once, change the **Data view** to 

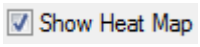
or 

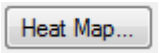
, respectively.

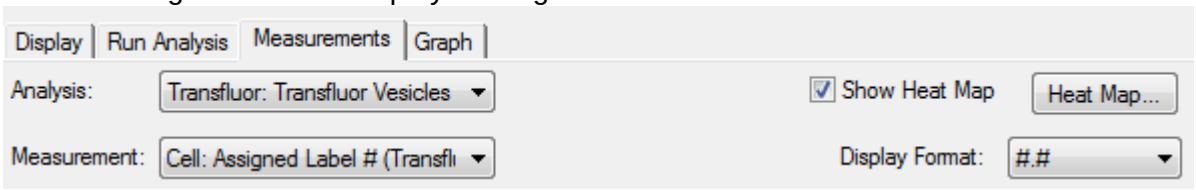
7. Left-click on a single thumbnail to view full resolution images (all wavelengths)

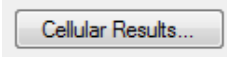


8.	To run or set up an analysis, select the the **Run Analysis** tab	
9.	If analysis settings have already been optimized, select the analysis routine (application module, custom module, or journal) and settings from the drop-down menus	
10.	Under the **Run Analysis** tab, select the appropriate button to run the analysis: •  analysis we be run on all acquired images •  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) •  analysis will be run only on the currently displayed site	
11.	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  • **Stack of all time points**: use if, in the **Analysis** field, you select a legacy timelapse journal which analyzes the planes in a stack	
12.	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  • **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	
13.	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.	

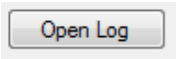
14. To view analysis results, select the **Measurements** tab
    i. Select the **Analysis** (module and settings name) from the drop down menu
    ii. Select a measurement from the drop-down menu. The values will be shown in the plate layout.
        a. Measurements starting with a "Cell" are cell-by-cell data and will give the average of all cells in the well
    iii. Activate the heat map by enabling 

    iv. Configure the heat map by clicking on 



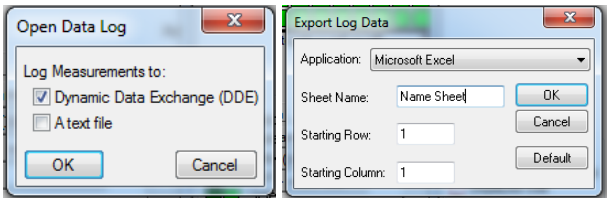
15. To view the cell-by-cell data, click 

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

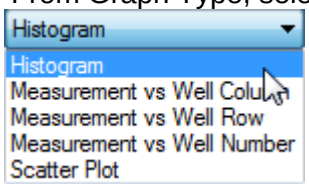
16. To export data to Excel:
    i. On the **Measurements** tab, click on 

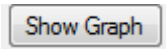
    ii. Select only **Dynamic Data Exchange**
    iii. Select **Microsoft Excel** and name worksheet as desired. This opens an empty worksheet.
    iv. Click 

. Currently viewed data will be logged into the Excel sheet.



17. 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