

ImageXpress[®] Micro XLS & MetaXpress[®] 6.5



The purpose of this guide is to briefly describe:

- I. Turn on system and acquire plate with saved settings (p. 1)
- II. Test acquisition settings (p. 4)
- III. Define new acquisition settings (p. 6)
- IV. View images and run an analysis (p. 14)

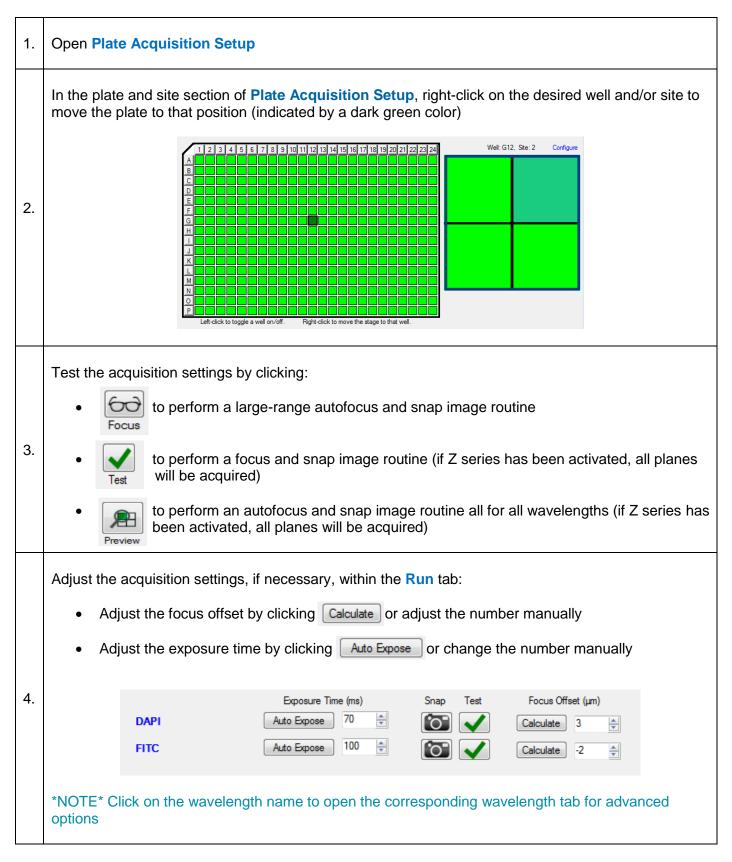
I. Turn on System and Acquire Plate with Saved Settings

1.	 Turn on the system: Light source (if not already on) IXM power supply controller box IXM options controller box (for Transmitted light, Environment Computer and Monitor 	al control or Fluidics modules)
2.	Go to the MetaXpress folder and double-click on the appropriate hardware profile shortcut	MetaXpress
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	Welcome to MetaXpress X Please select where you would like to connect. The Login Name and Password are those assigned to you by the database administrator. Data Source: MDCStore Login Name: moldev Password: •••••• Can't find your data source? Click here: New Data Source Forgot your 'sa' password? Click here: Cancel OK Cancel
4.	If you log in as system administrator (sa), the next window is a warning regarding security risks; click OK	Warning 83 It is not recommended to use the database system administrator account ('sa') when connecting to the database. Allowing multiple users access to the 'sa' account is a potential security risk. Please refer to the MDCStore user guide for more information and instructions on how to create individual user accounts for connecting to the MDCStore database. OK
5.	Group (security level) and click OK	MetaXpress X Please select a security level for images acquired or imported during this session of MetaXpress. Group: Administrators OK

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

	On the Run tab, update the folder name, plate name and description as desired					
	Folder Name	Plate 1 Sample	Barcode			
11.	Plate Name	Plate 1 Sample MMDDYY	Description	Spheroids stained with DAPI-Hoechst and FITC-Actin	*	
	Storage Location	C Drive Image Server 🔹			-	
12.	Click Lo k	begin acquiring the plate				

II. Test Acquisition Settings



	When you have optimized settings, click
5.	 Molecular Devices recommends enabling Save to file rather than database
	Click Save to search for a location on the hard drive.
6.	Click Legin 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) Image: Contract of the sub-cellular detail Image: Contract of the sub-cellular detail </td
4.	Adjust the objective correction collar, if necessary, (setting on objective should match physical plate bottom thickness). On the Run a Plate Taskbar , click on Adjust Correction Collar to step through the process.
5.	Select the Plate tab and select the appropriate plate type from the drop-down list

	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 		
6.	• 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		
	Site Options Outsom field of view (%): Well size: 11 mn² Single site X B5 III X B5 III Number of sites: 4 Adaptive acquisition Ster/mage size: 3.51 x 3.51 mm Hell Size: 11 mn² Multi-well Acquires sites based on the number of cells per well He sites Columns: Spacing (µn) Tile sites Rows: 2 III IIII Overlap sites 10% IIII Adaptive Acquisition Minimum sites to visit: 2 Image size: Verlap sites 10% Verlap sites 10% Verlap sites 10% Columns: Image size: Columns: Image size: Columns: Image size: Image size: Image size: Number of sites Image size: Columns: Image size: Image size: Image size: Overlap sites 10% Image size: Verlap sites: Image size: Image size: Image size:		
7.	Select the Acquisition tab to select Autofocus and Acquisition options		
8.	 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 Acquisition options Acquire Time Series 		
	Enable Acquire Z series for Z step acquisition		

	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 C:\
10.		*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
	Select	t 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 timelapse is enabled, set Timelapse Autofocus to All timepoints for long term timelapse, and First timepoint only for fast kinetic experiments
		Laser-based Focusing Configure Laser Settings Well to well autofocus Focus on plate bottom, then offset by bottom thickness
		Image-based Focusing Algorithm: Standard Binning: 2 Image-based focusing for recovery from laser-based well bottom failures
		Initial well for finding sample First well acquired A I I Number of wells to attempt initial find sample I I Site Autofocus All sites I Timelapse Autofocus All timepoints I I I I I I I I I I I I I I I I I I I I I I I I I I I I I I I I I I I I I I I I I I I I I I I I I I I I I I I I I I I I I I I I I I I I I I I I I I I I I I I
12.		t the Wavelengths tab and select the number of wavelengths s) including transmitted light that you would like to acquire

	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)
13.	1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 22 23 24 Well: G12, Ste: 2 Configure A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A </td
	 Select the W1 (wavelength) tab i. Select the desired filter set from the drop-down menu under Illumination setting ii. Click Focus 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
14.	 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 Calculate Offset to perform an automatic focus determination a. For more control, enable Use Z stack and follow the prompts
	 b. If necessary, enable Custom Range 312.5 12.5 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 Auto Expose with Target max intensity: 45000 Set to 33000 - 45000
	 You can also increase or decrease exposure manually

	If acquiring a Timelapse, select how often to acquire this image from the drop-down menu		
15.	at all time points at all time points at start of experiment at start/end of experiment every nth timepoint		
	If acquiring a Z Stack, select the appropriate setting for image collection		
	Single Plane Single Plane 2D Projection Image Only Z Series and 2D Projection Image		
10	*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) Best Focus Best Focus Maximum Minimum Sum		
	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)		
	Apply a shading correction option for your wavelength, if needed.		
	For Fluorescent wavelengths:		
	FL Shading Only generally works well for most assays.		
18.	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)		

	Repea	at for each subsequent wavelength
		Active Wavelength DAPI Active Wavelength DAPI Configure Run
19.		Objective and Camera-4X SF Plate-384 Wells (16x24) Sites to Visit- adaptive Acquisition Autofocus Wavelengths Wavelengths W2 HTC Timelapse-1 time points Z Series - 5 planes Journals-0 selected Display Analysis Timelapse: at all time points Z Series: 2 D Projection Image Only Z Series: 2D Projection Image Only Digtal Confocal (info) Y Digtal Confocal (info) Y Bidation Confocal (info)
	-	uiring with Timelapse, select the Timelapse tab
	i. ii.	Enter the number of Time points desired 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: Number of timepoints: 1 (*) Perform time series for: One well then the next •
20.		• 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

	 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 Coord to determine the Z start position iv. Click Coord 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 	✓ Center Z Series Around Focus Result # of Steps: 5 \$ Step Size: 10 \$ µm Range: 40 µm Focus Bortrom Commended Less Less Less Range: 40 µm Less <pless< p=""> <pless< p=""></pless<></pless<></pless<></pless<></pless<></pless<></pless<></pless<></pless<></pless<></pless<></pless<></pless<></pless<></pless<></pless<></pless<></pless<></pless<></pless<></pless<></pless<></pless<></pless<></pless<></pless<>			
21.	 vi. Use the large and small arrows to move to the bottor 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. 	2912.4 μm Set B			
	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.				
	Select the Journal tab (enabled on acquisition tab) and activate journals where appropriate.	Acquisition Step Journal Before each image INonel After each image INonel Before focusing INonel Start of z INonel End of z INonel			
	It might also be necessary to enable	Start of site [None]			
22.	Prevent asynchronous hardware moves	Start of well			
	(recommended if any journals are dependent on hardware positioning).	End of well INone1 Start of time point INone1			
	for certain journals (refer to documentation accompanying journals for details)	End of plate INone1 INone1 Inone1 Inone1 Inone1 Inone1 Inone1 Inone1			
		Prevent asynchronous hardware moves (recommended if any journals are dependent on hardware positioning).			

	Select the Display tab to:
23.	 Auto Arrange Images Multiple Arrange Images Multiple Arrange Images Multiple Arrange Images Multiple Arrange Images
	 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
	 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
25.	Active Wavelength FITC Active Wavelength FITC FITC Snap Start Live Focus
	Folder Name Plate 1 Sample Barcode Plate Name Plate 1 Sample MMDDYY Description Spheroids stained with DAPI-Hoechst
	Storage Location C Drive Image Server
	When you have optimized settings, click
26.	 Molecular Devices recommends enabling Save to file rather than database Click Save for a location on the hard drive.
26.	Click on Login acquiring the plate
San Jo	N First Street toll-free 1.800.635.5577 moleculardevices.com ose, CA 95134 office 408.747.1700 Page 13 of 18 I States fax 408.747.3601 Page 13 of 18

IV. Review Images and Run an Analysis

1.	In the main toolbar, click Review Plate or in the main menu select Screening > Review Plate				
2.	On the Review Plate Data dialog, click Select Plate				
3.	Navigate through the folders to find the plate of interest. Highlight the plate and click select Image: Select Plate for Review Image: Select Plate for Review </th				
4.	If you cannot find your plate, on the Review Plate Data dialog, click search. 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				

	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.	O Review Plate Data Image: 1 A Print Table Wavelengths: Data vers: (Well arrangement		
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 Data view: Time Point vs Well or Data view: Z Step vs Well , 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 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	ation. Legend Not acquired Acquired, not measured Displayed well Part of montage Selected wells			
12.	 displayed site 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 for 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 d 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 	Z steps: All Z steps Z step range Selected Z step Stack of all Z steps 2D projection 			

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 Show Heat Map iv. Configure the heat map by clicking on Heat Map *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. Display Run Analysis Measurements Graph Analysis: Transfluor: Transfluor Vesicles Display Format: ## Display Format: ## 		
16.	To view the cell-by-cell data, click Cellular Results 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 Open Log ii. Select only Dynamic Data Exchange iii. Select Microsoft Excel and name worksheet as desired. This opens an empty worksheet. iv. Click Log Data . Currently viewed data will be logged into the Excel sheet. 		

	To create simple graphs in MetaXpress:				
	i.	Go to Graph tab			
18.	ii.	Measurement vs Well Row Measurement vs Well Number Measurement vs Concentration Scatter Plot	ay Run Analysis Measurements Graph ysis: Transfluor: Transfluor Vesicles ▼ raph view: Plate Multiple graphs of displayed wells Single Well		
	iii. iv.	Select measurements to plot from the drop- down menu Click Show Graph	oh type: Histogram 🔻		
	v.	Right-click on the graph for more options			
		TE* For Measurement vs Concentration, the must first be annotated.			