



# ImageXpress<sup>®</sup> Micro XLS & MetaXpress<sup>®</sup> 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.	<ul> <li>Turn on the system:</li> <li>Light source (if not already on)</li> <li>IXM power supply controller box</li> <li>IXM options controller box (for Transmitted light, Environmental</li> <li>Computer and Monitor</li> </ul>	control or Fluidics modules)			
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	Welcome to MetaXpress     Email       Please select where you would like to connect.     The Login Name and Passwood en thore assigned to you by the disbase administed.       Data Source:     MDCStore       Login Name:     Inddata connect       Cent lind your data source?     Click here:       Darget your 'sa' password?     Cancel			
4.	If you log in as system administrator (sa), the next window is a warning regarding security risks; click <b>OK</b>	Warning         It is not recommended to use the database system administrator account ('sa') when connecting to the database.           Allowing multiple uses access to the 'sr' account is a potential sectivity mix.           Piece refer to the MDCStore user guide (or more information and instructions on how to create individual user accounts for connecting to the MDCStore database.           OK			
5.	Select Group (security level) and click <b>OK</b>	MetaXpress Please select a security level for images acquired or imported during this session of MetaXpress. Group: Administrators OK			

	In the main toolbar, click Plate Acquisition Setup or in the main menu select Screening > Plate Acquisition Setup
6.	
7	Load Protocol
/.	To load a previous saved protocol, click on in Plate Acquisition Setup
8.	<ul> <li>Click Load From File to search windows for the appropriate .hts file.</li> <li>If the settings file is saved to the database, highlight the protocol and click Load From DB</li> </ul>
9.	<ul> <li>Click Feet Plate to open the door and place the plate in the in the system</li> <li>Click Load Plate to close the door</li> </ul>
10.	Alternatively, you can use the Main Taskbar to open and close the door.
	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 <ul> <li></li></ul>
12.	Click Acque Piece to begin acquiring the plate

# **II. Test Acquisition Settings**

1.	Open Plate Acquisition Setup			
	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)			
2.	1       2       3       4       5       6       7       8       9       10       11       12       13       14       15       16       17       18       19       20       22       22       22       24       Well: G12. Ste: 2       Corrigure         A       B<			
	Test the acquisition settings by clicking			
3.	<ul> <li>to perform a large range autofocus and snap image routine</li> <li>Test to perform a focus and snap image routine (if Z series has been activated, all planes will be acquired)</li> <li>Preview to perform an autofocus and snap image routine all for all wavelengths (if Z series has been activated, all planes will be acquired)</li> </ul>			
	Adjust the acquisition settings, if necessary:			
	Adjust the focus offset by clicking     Calculate     or adjusting the number manually			
	Auto Emose			
	Adjust the exposure time by clicking or changing the number manually			
4.	Exposure Time (ms)     Snap     Test     Focus Offset (µm)       DAPI     Auto Expose     70     Image: Calculate     3     Image: Calculate     -2       FITC     Auto Expose     100     Image: Calculate     -2     Image: Calculate     -2			
	*NOTE* Click on the wavelength name to open the corresponding wavelength tab for advanced			
	options			
5.	<ul> <li>When you have optimized settings, click</li> <li>Molecular Devices recommends enabling</li> <li>Click Save to search for a location on the hard drive.</li> </ul>			
6. Click Acquire Plate to begin acquiring the plate				

### III. Define New Acquisition Settings

1.	Open Plate Acquisition Setup		
2.	Select the Configure tab		
	Select the <b>Objective and Camera</b> 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. If necessary set gain (gain of 2 for binning of 1, gain of 1 for binning of 2 or higher) Active Wavelength FITC State Focal Test Focal Test		
3.	Objective and Camera-4X SF     Magnification:     4X SF       Plate-384 Wells (16x24)     Camera bining:     1       Sites to Tisk-adaptive     Camera bining:     1       Acquisition     Calibration (pinned):     1.63 x 1.63 um       Mavelengths     Uk     Uk       Wit bAPI     Uk     Uk       Journals-0 selected     Display       Analysis     Image: Selected		
	Save Protocol."		
4.	Adjust the objective correction collar if necessary (setting on objective should match physical plate bottom thickness). On the <b>Run a Plate Taskbar</b> , click on Adjust Correction Collar to step through the process		
5.	Select the <b>Plate</b> tab and select the appropriate plate name: 384 Wells (16x24)		
6.	<ul> <li>Select the Sites to Visit tab and select the appropriate number of sites</li> <li>Single Site: image one site per well in the center</li> <li>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)</li> <li>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</li> <li>Multi-well: collect multiple wells within one image which is then cropped to define single wells automatically</li> <li>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</li> <li>Were acquisition: Collect 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</li> <li>Select the Acquisition tab to select Autofocus and Acquisition options</li> </ul>		

 
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	Autofocus options:					
	<ul> <li>Always select Enable laser-based</li> </ul>	Autofocus options				
0	focusing					
8.	• Enable image-based focusing for thick					
	samples or those with different focal	Enable image-based focusing (for acquisition or laser recovery)				
	planes from site-to-site or well-to-well					
	Acquisition options:					
	Epoble Acquire Time cories for	Acquisition options				
0	Ellable Acquire Time series for     timelence experimente	Acquire Time Series				
9.	Innelapse experiments	Acarica Z Sarias				
	• Enable Acquire Z series for Z step	Acquire 2 Series				
	acquisition					
	Other options:					
	<ul> <li>If running a journal during acquisition,</li> </ul>					
	enable this option to activate the Journal	5				
	tab					
	<ul> <li>If an analysis has already been setup,</li> </ul>					
	enable Analyze Images After	Run Journals During Acquisition				
10	Acquisition	Analyze Images After Acquisition				
10.	*NOTE* this requires an offline computer					
	to be in Auto-run mode or running	Perform shading correction Directory C:\				
	PowerCore software					
	<ul> <li>To correct for uneven background, enable</li> </ul>					
	Perform shading correction and select	Perform shading correction and select				
	the appropriate directory where shading	the appropriate directory where shading				
	correction images are saved					
	Select the Autofocus tab:					
	Sciect the Autorocus tab.					
	i. Set Well to well autofocus to	This is the default acquisition setup.				
	however when imaging thin-bottom plates	with low magnification objectives (4x and below) or				
		m then effect by bettern thickness				
	microscope slides, select	In, their birset by bottom thickness				
	ii. For Image-based Focusing refer to corre	esponding MetaXpress 6 Software Guide modules				
	for suggested settings					
	Firs	t well acquired				
	iii. Set Initial well for finding sample to					
	iv Set Number of wells to attempt initial fi	nd complete				
	IV. Set number of wells to attempt initial fi	nd sample to				
	v. If more than one site is acquired, set Site	Autofocus to Al sites				
11.	vi. If timelpase is enabled, set Timelapse Au	itofocus to All timepoints for long term timelapse, and				
	First timepoint only					
	for fast kinetic experiments					
	Laser-based hocusing					
	Well to well autofocus. Focus on plate both	om then offset by bottom thickness				
	Well to well autofocus Focus on plate bottom, then offset by bottom thickness  Image-based Focusing Algorithm: Standard  Binning: 2  Custom exposure times Allow image based foo wing for recovery from laser-based well bottom failures					
	Allow image-based rocusing for recovery from laser-based well bottom failures					
	Initial well for finding sample					
	Number of wells to attempt initial find sample 3					
	Site Autorocus Al sites					
	Timelapse Autofocus Al timenoints					

	Select the Wavelengths tab and select the number of		
12.	wavelengths (colors) including transmitted light that you would like Number of wavelengths: 2		
	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)			
	A A A A A A A A A A A A A A A A A A A		
13			
15.			
	Left-click to toggle a well on/of. Right-click to move the stage to that well.		
	Select the W1 (wavelength) tab		
	i. Select the desired filter set from the drop-down menu under Illumination setting		
	60		
	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 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		
	Calculate Offset		
14.	iv. Click the button to perform an automatic focus determination		
	a. For more control, enable Use Z stack and follow the prompts		
	Range (um) Step (um)		
	b. If necessary, enable		
	60		
	v. Click Focus again to test the new post-laser offset. Image should now be in focus		
	vi. Examine the image for brightness		
	Auto Expose Target max intensity: 45000		
	a. If necessary, click with set to 33000 - 45000 set to 33000 - 45000		
	D. You can also increase or decrease exposure manually If acquiring a Timelapse, select how often to acquire this image from the drop down monu-		
	at all time points		
15.	at all time points		
_	at start 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				
	XOTE* 7 Series and 2D Projection Image is not available when acquiring a Timelapse				
	NOTE 2 denes and 20 Trojection image is not available when acquiring a	lineapse			
16.	16. If saving the 2D Projection Image, select the appropriate projection method (press F1 for more information)				
	Best Focus 💌				
	Best Focus				
	Minimum				
	Sum				
	*NOTE* Best Focus is not recommended for comparison of intensity measure	ements			
17.	If the option is available, you can enable <b>Digital contocal</b> and the slider bar (press E1 for more information)	select the appropriate K value using			
	Repeat for each subsequent wavelength				
	Repeat for each subsequent wavelength				
	Active Wavelength DAPI -				
	Configure Run	Snap Start Live Focus Test Preview			
	Objective and Camera- 4X SF Plate- 384 Wells (16x24) Illumination setting: DAPI				
	Sites to Visit- adaptive Exposure (ms): 70 Auto Expose Target max intensity: 3000				
	Acquisition Autofocus options				
	Autorocus Post-laser Wavelengths offset (um)	Autofocus Post-laser Wavelengths Gfset (m)			
18.	W1 DAPI Laser with z-offset V 3	W1 DAPI Laser with z-offset V 3 🜩			
	W2 FITC Timelanses 1 time points				
	Z Series- 5 planes	Range (um) Step (um)			
	Journals- 0 selected Calculate Offset	ournals- 0 selected Calculate Offset Cal			
	Display Acquisition Options	Acquisition Options			
	Timelapse: at all time points				
	Z Series: 2D Projection Image Only V 2D Pro	ection Image: Best Focus			
	Digital Confocal (info)     Standing Composition: Off	↓ 0.0200 ÷			
	Shading Conection. On				
	If acquiring with Timelapse, select the <b>Timelapse</b> tab				
	i. Enter the number of <b>Time points</b> desired				
	II. Set Interval as the time between each time point				
	iv Set <b>Perform time series</b> for:				
	One well then the next: entire timelanse	Number of timepoints: 1			
	is run for one well before acquiring next	Perform time series for:			
10	well	Anonyvimate minimum time interval: 2.7 sec			
19.	One column then the next: entire	Interval: 1 sec v			
	timelapse is run for one column before	Province and a lab			
	acquiring next column				
	One row then the next: entire timelapse				
	is run for one row before acquiring next				
	All selected wells: all wells are imaged     before continuing with post time point				
	before continuing with next time point				



	Select the <b>Analysis</b> tab (enabled on the acquisition tab) to specify the appropriate optimized <b>Analysis</b> routine and <b>Settings</b> from the drop down-menus		n						
23.				Analysis:	BF Cell Scoring	MiniMax	-		
	*NOTE* This re	quires an offline computer :	set in Auto-run moo	de or	Setting:	<u>v</u> 1		•	
	Under the Ru	un tab. enter:							
	Folde	er Name: Project name	e, your name, P	l, etc. Al	l your	plates will	go unde	er this name.	
	Plate	Name: Name of this p	particular experi	ment	,		0		
	<ul> <li>Stora</li> </ul>	ige location: Select a	ppropriate serve	er for ima	age sto	orage.			
	*NOTE	* There may only be one c	hoice.						
	Barco	ode: Enter a barcode i	IT DESIFED	mont					
24	• Desc	ription. Any text regai	raing the expen	ment					
24.		Adive Wa	avelength FITC	•			60		
	Configure Run				Sr	ap Start Live	Focus		
	Folder Name	Plate 1 Sample	Barcode						
	Plate Name	Plate 1 Sample MMDDYY	Description	Spheroids st and FITC-Ad	ained with	DAPI-Hoechst			
	Storage Location	C Drive Image Server	•				*		
			Save Proto	col*					
05	When you have optimized settings, click								
25.	<ul> <li>Molecular Devices recommends enabling Save to file rather than database</li> </ul>								
	Click Save to search for a location on the hard drive.								
26.									
	Click on Acquire Plate to begin acquiring the plate								

## IV. Review Images and Run an Analysis

1.	In the main toolbar, click Plate Acquisition Setup or in the main menu select Screening > Plate Acquisition Setup		
2.	On the Review Plate Data dialog, click Select Plate		
	Navigate through the folders to find the plate of interest.		
	Highlight the plate and click Select		
3.	Partes     Partes     Organizationary (Creator Name - Plate Info)		
	Name (Plate Info)         Acquisitis.         Barcode         Creator         Date/Til           EXINAL         Coll Not         CRAINIL         CRAINIL		
	Diz (Institutor Vender), and Vender), and De Vantania Conductor System – OV/2015 DizTwerc/Hc/Bellogy, AMSWN-CONBRO, 3 EXTens. – OVLD System – OV/2015 DXCeMbrophology, AMSWN-CONBRO, 4 EX7Cell OVLD System – OV/2015 EVEnerst-ON-Anstitutes AMSWN-CONBRO, 5 EX7Sure. – OVL2015		
	D/8TransmittedLight_AMSNL-C0H8XV1_6 D/8Tran (NULL> System 04/20/15		
	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		
	Review Plate Data		
	Select Filter A Time and Z_MISVYC_CHENYY_CO		
	V         DAPI         00 ccc0 04 00 00 07 00 00 00 11 12 13 14 15 16 17 16 15 20 21 22 22 44         22 22 44           V         FITC         8         6 </td		
4.	D         -           M Stes         F		
	Learnd K		
	CALCARENCIA TRADUCTO     CONSIDERATION     Prot of motope     Selected weble p		
	Mortage: 1 🔯 k 8 🖄 Time points: 1 🗟 of 3 🖉 Display   Run Analysis Measurements   Graph		
	If there are multiple sites per well, select an		
5.	Sites		
•	appropriate site to view, or enable view adjust		
	To view all Timelapse or 7 Series images at once, change the <b>Data view</b> to		
6.	Data view: Time Point vs Well   Or Data view: Z Step vs Well  respectively.		
	Left-click on a single thumbnail to view full resolution images (all wavelengths)		
7.	1720		

 
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8.	To run or set up an analysis, select the <b>Run Analysis</b> tab	Display Run Analysis Measurements Graph		
	If analysis settings have already been optimized, select the analysis routine	Analysis: <a>Angiogenesis</a> Tube Formation>		
9.	(application module, custom module,	Settings: No Settings Configured		
	or journal) and settings from the drop-	Setting Measures angiogenesis tube formation.		
	down menus	description:		
10.	<ul> <li>Inder the Run Analysis tab, select the a to run the analysis:</li> <li>Run on all wells analysis we b acquired images</li> <li>Run on selection analysis will wells (selected wells are indicated wells, right click well(s) in the plat montage)</li> <li>Run on displayed site analysis will currently displayed site</li> </ul>	be run on selected d in green; to select se section or image be run only on the		
11.	<ul> <li>For a Timelapse data set, select the appropriate option for analysis under the Time points section</li> <li>All time points: run analysis on all time points in the data set</li> <li>Time point range: run analysis on a consecutive range of time points</li> <li>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</li> <li>Stack of all time points: use if, in the Analysis field, you select a legacy timelapse journal which conducted the plane at a stack.</li> </ul>			
12.	<ul> <li>For a Z Series data set where all Z planes were saved, select the appropriate option in the Z steps section</li> <li>All Z Steps: run analysis on all Z planes</li> <li>Z Step range: run analysis on consecutive range of Z planes</li> <li>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 dof 5</li> <li>Stack of all Z steps: run an analysis with a journal that requires a stack of images</li> <li>2D projection: only run analysis on the saved 2D projection image</li> </ul>			
13.	<ul> <li>If the selected analysis has already been run on the plate, a warning will appear asking to</li> <li>13. overwrite the data. If you are not sure, save the analysis settings with a new name before analyzing your plate.</li> </ul>			

	To view and wie would be also take Management	and a tab				
	I o view analysis results, select the <b>Measurements</b> tab					
	i. Select the Analysis (module and setting	I. Select the Analysis (module and settings name) from the drop down menu				
	II. Select a measurement from the drop-do	whitehu. The values will be shown in the plate				
	a Mossuromonts starting with a "C	oll" are cell-by-cell data and will give the average				
	of all cells in the well	en ale cen-by-cen data and win give the average				
14.	iii. Activate the heat map by enabling					
	iv. Configure the heat map by clicking on Heat Map					
	Display Run Analysis Measurements Graph					
	Analysis: Transfluor: Transfluor Vesicles 🔻	Show Heat Map Heat Map				
	Measurement: Cell: Assigned Label #(Transfli 💌	Display Format: #.#				
15	To view the cell-by-cell data click	at the bottom of the Review Plate Data dialog				
15.	Data will be automatically updated based on the	e well and site selected in the montage view				
	To export data to Excel:					
	i. On the <b>Measurements</b> tab, click on					
	Open Log					
		Open Data Log				
1.0	II. Select only Dynamic Data Exchange	Log Measurements to:				
16.	III. Select Microsoft Excel and name	A text file     Cancel				
	worksheet as desired. This opens an	OK Cancel Default				
	empty worksheet.	Starting Column: 1				
	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					
	ii. From Graph Type, select:					
	Histogram 🔻	Display Run Analysis Measurements Graph				
	Histogram	Analysis: Transfluor: Transfluor Vesicles				
17.	Measurement vs Well Coluly Measurement vs Well Row	Graph view:				
	Measurement vs Well Number	Plate O Multiple graphs of displayed wells O Single Well				
	Scatter Plot	Graph type: Histogram 💌				
	III. Select measurements to plot from the					
	arop-aown menu					
	iv. Click Show Graph					
	v. Right-click on the graph for more options	3				