



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 Transm Control or Fluidics modules) Computer and Monitor 	itted Light, Environmental
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 maybe different. Refer to your administrator for this information	Welcome to MetaXpress Please elect where you would like to comment. The Login Yama and Passmod are those assigned to you by the delabore odminister. Data Source, MDCStore Login Yama and Passmod are those assigned to you by Password. Password. Can't find your data source? Click here: Con't find your 'sa' pastword? Click here: DK Cancel
4.	If you log in as system administrator (sa), the next window is a warning regarding security risks; click OK	Warning XX 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.	Select Group (security level) and click OK	MetaXpress

	In the main toolbar, click Plate Acquisition Setup or in the main menu select Screening > Plate Acquisition Setup
6.	
7.	To load a previous saved protocol, click on 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
9.	 Click Eject 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 Open Door - Eject Plate or Close Door - Load Plate
11.	On the Run tab, update the folder name, plate name, and description as desired Folder Name Plate 1 Sample Barcode Plate Name Plate 1 Sample MMDDYY Description Spheroids stained with DAPI-Hoechst and EUC Actin
	Storage Location C Drive Image Server
12.	Click Acquire Plate 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 9 20 21 22 23 24 Well: G12. Ste: 2 Configure A A B C </th		
	Test the acquisition settings by clicking		
3.	 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) 		
	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 menually		
4.	Exposure Time (ms) Snap Test Focus Offset (µm)		
	DAPI Auto Expose 70 🚔 🏹 Calculate 3 🖨		
	FITC Auto Expose 100 🚖 🏹 Calculate -2 🜩		
	NOTE Click on the wavelength name to open the corresponding wavelength tab for advanced		
	options		
	Save Protocol		
	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.			
	Ulick requerise to begin acquiring the plate		

III. Define New Acquisition Settings

1.	Open Plate Acquisition Setup		
2.	Select the Configure tab		
	 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) 		
3.	Active Wavelength Singo Start Live Focus Test Objective and Camera-200X Pit Magnification: 20X Plan Apo Lambda Plate-Griener 384-well Singo Start Live Focus Test Plate-Griener 384-well Camera branka Singo Start Live Focus Test Plate-Griener 384-well Singo Start Live Focus Test Plate-Griener 384-well Acquisition Camera branka Camera branka Camera branka With FTC Acquisition Model Official (Opin parending) Opin parending Display With FTC Acquisition Model Opin parending Opin parending Save Precosel* Save Precosel* Save Precosel* Save Save Save Save Save Save Save Save		
4. Adjust the objective correction collar if necessary (setting on objective should match physic bottom thickness in mm X refractive index of material – 1.59 for Plastic, 1.52 for Glass). C			
	Plate Taskbar, click on the ended as the end		
5.	Select the Plate tab and select the appropriate Plate name: 384 Wells (16x24)		

	Select the Sites to Visit tab and select the appropriate number of sites		
6.	 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 Water image the number of sites to image the number of sites to image by the percentage entered. This is useful when the 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 Water image the number of stere image is a strain strain image is the number of stere is the 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 Water image image is a strain strain image is the strain image is t		
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 		
	Acquisition options:		
9.	 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	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		
	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	
	٧.	If more than one site is acquired, set Site Autofocus to	
11.	vi.	If timelpase 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	
		Algorithm: Standard Binning: 2 Custom exposure times Allow image-based focusing for recovery from laser-based well bottom failures	
		Initial well for finding sample First well acquired	
		Number of wells to attempt initial find sample 3	
		Timelapse Autofocus All timepoints	
12.	Select	t the Wavelengths tab and select the number of engths (colors) including transmitted light that you would like Number of wavelengths: 2	
	to acq	uire	
	contro	bl well) and/or site to move the plate to that position (indicated by a dark green color)	
		1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 Well: G12, Site: 2 Configure	
13.			
	Left-click to toggle a well on/off. Right-click to move the stage to that well.		

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	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. (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.	
		Calculate Officer	
iv. Click the Calculate Offset button to perform an automatic focus determination			
		a. For more control, enable Use Z stack and follow the prompts	
14.		Range (um) Step (um)	
		b If necessary enable □Custom Range 312.5 🔄 12.5 🔄	
	V. vi	Click Focus again to test the new post-laser offset. Image should now be in focus.	
	vi.		
		a. If necessary, click Auto Expose with with arget max intensity: 45000 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):	
	Shading Correction: Auto Correction for TL 🔷		
	If acqu	uiring a Timelapse, select how often to acquire this image from the drop down menu	
45		at all time points	
15.		at start of experiment	
		at start/end of experiment	
	If aca	uiring a Z Stack, select the appropriate setting for image collection	
		Single Plane	
		Single Plane	
		2D Projection Image Only b≩ Z Series and 2D Projection Image	
	*NOTE	* Z Series and 2D Projection Image is not available when acquiring a Timelapse	
16.	lf savi	ng the 2D Projection Image, select the appropriate projection method (press F1 for more	
	inform	nation)	
		Best Focus 🔻	
		Best Focus	
	Maximum K Minimum		
		* Rest Focus is not recommanded for comparison of intensity measurements	
	If the option is available, you can enable Digital confocal and select the appropriate K value using		
17.	the sli	der bar (press F1 for more information)	

	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: UAPI	
	Sites to Visit- adaptive	Exposure (ms): 70 🚔 Auto Expose Target max intensity: 3000 🚔	
	Acquisition	Autofocus ontions	
	Autofocus	Pret Jacar	
	Wavelengths	offset (um)	
	W1 DAPI	Laser with z-offset 🔻 3	
40	W2 FITC		
18.	Timelapse- 1 time points		
	Z Series- 5 planes	Range (um) Step (um)	
	Journals- 0 selected		
	Display	Acquisition Options	
	Analysis	Timelapse: at all time points	
		7 Series: 2D Projection Image Only	
		✓ Digital Confocal (info) ✓ ✓ 0.0200	
		Shading Correction: Auto Correction for TL 🔫	
	Save Protocol"		
	If acquiring with Timelapse, sele	ect the Timelapse tab	
	i. Enter the number of Tim	ne points desired	
	ii Set Interval as the time I	between each time point	
	iii Set Duration as the total	between each time point	
	III. Set Duration as the total	a time of the experiment	
	iv. Set Perform time series	s for:	
	 One well then th 	ne next: entire timelapse	
	is run for one wel	Il before acquiring next Perform time series for: One well then the next	
10	well	Approximate minimum time interval: 2.7 sec	
19.	One column the	an the next: entire	
	timelence is run f	for one column before	
	umeiapse is fuil i		
	acquiring next co	Diumn	
	 One row then the 	ne next: entire timelapse	
	is run for one row	v before acquiring next	
	row		
		lles ell welle ere impred	
	All Selected wells, all wells are illidged		
	before continuing	j with next time point	



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	Analysis: BF Cell Scoring MiniMax ▼ Setting: v1 ▼			
	 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: Approximate the experiment 				
24.	Active Wavelength FITC Configure Run Barcode Folder Name Plate 1 Sample Barcode Plate Name Plate 1 Sample MMDDYY Description Storage Location C Drive Image Server Image Server	Is stained with DAPI-Hoechst			
25.	 When you have optimized settings, click Molecular Devices recommends enabling Click Save to file rather than database Click 				
26. Kev	Click on Acquire Plate to begin acquiring the plate ev to ImageXpress Micro Confocal Status Lights:				

Color	Instrument Status	
Orange	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 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
	Dates
3.	GV/20/15 [Date Created - Plate Info]
	Name (Plate Info) Acquisiti Barcode Creator Date/Ti
	EXtractical end and an
	EXX-cellMorphology_AMSMVL-CoH8VX1_4 EXX-cell < VNUL.> System 04/2015 EXSPunctaOnNeuriter_AMSNVL-COH8VX1_5 EXSPunc < VNUL.> System 04/2015 EXSTansmittedUpt_AMSNVL-COH8VX1_6 EXStans < vNUL.> System 04/2015
	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 Plate 4X Time and Z_AMS/VU_CDH8/V/1_20 Wavelengths: Data view: Well arrangement Print Table
	IV DAPI 01 02 03 04 05 06 07 08 09 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 IV FITC A
4.	C •
	Legend
	Displayer well 0 Sectod wells 0
	Montage: 1 🔯 k 8 🖄 Time points: 1 🖄 of 3 🗾 Display Run Analysis Measurements Graph
	If there are multiple sites per well, select an
5.	
	appropriate site to view, or enable Al Sites .
	The image montages will automatically adjust.
6.	Detensions The Detension Well
Data view: Lime Point vs Well or Or Data view: Z Step vs Well , respectively.	
7	
1.	■ ■ ■ ■ ■

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8.	To run or set up an analysis, select the the Run Analysis tab	Display Run Analysis Measurements Graph	
	If analysis settings have already been	Analysis: <pre></pre> <pre></pre> Analysis: <pre></pre> <pre></pre> <pre>Analysis: </pre> <pre></pre>	
0	optimized, select the analysis routine	Settings: No Settings Configured	
9.	(application module, custom module, or journal) and settings from the dron-		
	down menus	description:	
10.	Under the Run Analysis tab, select the a to run the analysis: Run on all wells analysis we b acquired images Run on selection analysis will wells (selected wells are indicated wells, right click well(s) in the plat montage) Run on displayed site analysis will currently displayed site	be run on selected d in green; to select e section or image be run only on the	
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 Time points: 1 of 3 Stack of all time points: use if, in the Analysis field, you select a legacy timelapse journal which 		
12.	 For a Z Series data set where all Z plane select the appropriate option in the Z stee All Z Steps: run analysis on all Z Z Step range: run analysis on co Z planes Selected Z step: run analysis on that is selected in the Z step sect layout Z steps: 3 0 of 5 Stack of all Z steps: run an analysis projection: only run analysis projection image If the selected analysis has already been 	s were saved, ps section planes nsecutive range of only one Z plane ion below the plate ysis with a journal on the saved 2D run on the plate, a warning will appear asking to	
13.	 overwrite the data. If you are not sure, save the analysis settings with a new name before analyzing your plate. 		

The view encloses a short the Management of the				
	I o 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.			
14.	of all cells in the well			
	iii. Activate the heat map by enabling Show Heat Map			
	iv. Configure the heat map by clicking on Heat Map			
	Display Run Analysis Measurements Graph			
	Analysis: Transfluor: Trans	fluor Vesicles 🔻	Show Heat Map Heat Map	
	Measurement: Cell: Assigned La	abel # (Transfli 🔻	Display Format: #.#	
15	5. To view the cell-by-cell data, click Cellular Results at the bottom of the Review Plate Data dia			
	Data will be automatically	will be automatically updated based on the well and site selected in the montage view		
	To export data to Excel:			
16.	i. On the Measurements tab, click on			
	ii Calact ank Dura	mia Data Evakanga		
	iii. Select Microsoft	Find Data Exchange	Log Measurements to:	
	worksheet as desired. This opens an		A text file	
	empty worksheet		OK Cancel Statuting now.	
	iv. Click Log Data	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	e, select:		
17.	Histogram	-	Display Run Analysis Measurements Graph	
	Histogram		Analysis: Transfluor: Transfluor Vesicles	
	Measurement vs Well Colul _v A Measurement vs Well Row Measurement vs Well Number		Graph view:	
			In the Company of the second secon	
	Scatter Plot		Graph type: Histogram	
	iii. Select measurements to plot from the drop-down menu		indogram	
iv. Click Show Graph		J		
1	v. Right-click on the graph for more options			