

MetaXpress® 6 Software Guide

Setting up a Basic Acquisition (without Timelapse or Z Series)



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Chapter Purpose

The purpose of this chapter is to guide the user through setting up a basic acquisition with two wavelengths. This includes selecting objectives, plates, wavelengths, and focal position.

Acquiring Timelapse or Z Series images <u>will not</u> be covered in this chapter. Refer to corresponding chapters for details on these processes.





- 1. Open Plate Acquisition Setup
 - In the main toolbar click on



OR

- Under the Screening menu, select Plate Acquisition Setup
- 2. Select the **Configure** tab





- 3. Select the **Objective and Camera** tab
- 4. Select the appropriate Magnification from the drop-down menu
 - You may need to adjust the correction collar of the objective; refer to the Main Taskbar to do this.
 - Select **Camera Binning** (refer to next section for guidance)
 - Pixel size is automatically calculated based on magnification and binning
 - Set Camera Binning to 1 to acquire unbinned images maximum resolution
- 5. If the **Gain** option is available, it is suggested to start with gain set to **Low**

Objective and Camera- 4X S Flu		44.0 5		-	
Plate- Corning 1536-well Black-	Magnification:	4X S Fluor	•		
Sites to Visit- multi-well	Comoro binoina:	1	Calibration (binned):	1.61 × 1.61 um	
Acquisition	Camera birrining.		Calibration (binned).	1.01X 1.01 UIII	
Autofocus	Gain	Low -			
Wavelengths	Gain.	Low			
W1 DAPI					
W2 FITC					
Display					
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What is Binning?

Combining groups of pixels into a single pixel during image acquisition



Example of 2x2 Binning

Each pixel records an intensity







4 Pixels are summed to make one larger pixel





Why Bin?

Brighter pixels

• The resultant pixel is brighter than any of the 4 component pixels

Save Space

• 2x2 binning reduces file size 4-fold

Increase Speed

- Faster image transfer from MetaXpress to database
- Faster image analysis

When to Bin

- You do not need to see intricate sub-cellular detail
- Cell counting
- Scoring cells positive or negative for fluorescent markers
- Measuring overall cell intensity





- 6. Select the **Plate** tab
- 7. Select the appropriate Plate Type from the drop-down menu







- 8. In the **Plate Section**, select the wells you would like to acquire
 - Left click and drag your mouse to select wells (wells do not need to be contiguous)
 - Click on "All" (top left corner), row letters, column numbers, or individual wells
 - Gray wells are deactivated, green wells are activated and will be imaged.
 - Right click on a well to move the stage to that position (well turns dark green)







9. Select the Sites to Visit tab

- Select Single Site to acquire one site in the middle of the well
- To acquire a single site elsewhere in the well, refer to the next section on setting up multiple sites

Objective and Camera- 10X Plar Plate- Greiner 384-well thin bot:	Site Options Single site Fixed number of sites	Custom field of view (%): X: 50 ♀ Y: 50 ♀	Well size: 11 mm² Number of sites: 1 17.82% Well Coverage		
Sites to Visit- single site	Adaptive acquisition	Site/image size: 1.39 x 1.39 mm			
Acquisition	Multi-well				
Autofocus	Acquires a single site ce	entered in each well			
Wavelengths					
W1 DAPI					
W2 FITC					
Display					
Wavelengths W1 DAPI W2 FITC Display	nuquies a single site of				







9. On the Sites to Visit tab

- Select **Fixed number of sites** to acquire multiple sites
- Build site grid by specifying number of Columns and Rows
- Spacing defines the x-y spacing between sites

Objective and Camera- 10X Plan	Site Options	Custom field of view (%):	Well size: 11 mm ²
Plate- Greiner 384-well thin bot:	 Single site 	※ 50 ♦ ¥: 50 ♦	Number of sites: 2
Sites to Visit- multi-site	 Adaptive acquisition 	Site (image cize: 1.20 x 1.20 mm	35.65% Well Coverage
Acquisition	 Multi-well 	Site/inage size: 1.55 X 1.55 min	
Autofocus	Acquires a fixed number	of sites in each well	
Wavelengths			
W1 DAPI	Con		
W2 FITC	Columns: 2 🖨 0	Tile sites	
Display	Rows: 2 🔶 0	Fit sites to well	
		Overlap sites 10%	

- **Tile sites** places sites edge to edge
- Fit sites to well spreads sites to well edge
- Overlap sites 10% overlaps edges of sites for stitching







NOTE Left clicking on site selects (green) or deselects (gray) for imaging. Right click moves stage to that position (dark green)

- 9. On the Sites to Visit tab
 - Refer to corresponding chapters for details on Adaptive acquisition and Multi-well options

Objective and Camera- 10X Plar Plate- Greiner 384-well thin bot: Sites to Visit- single site Acquisition	Site Options Single site Fixed number of sites Adaptive acquisition Multi-well	Custom field of view (%): X: 50 Y: 50 X Site/image size: 1.39 x 1.39 mm	Well size: 11 mm ² Number of sites: 1 17.82% Well Coverage
Autofocus	Acquires a single site ce	ntered in each well	
Wavelengths			
W1 DAPI			
W2 FITC			
Display			





10. Select the Acquisition tab

- Always Enable laser-based focusing
- For certain samples it may be necessary to Enable mage based focusing
- Disable Acquire Time Series
- Disable Acquire Z Series
- Optionally, enable Perform shading correction

NOTE Some of the choices shown below may not appear in your version of MetaXpress

Objective and Camera- 10X Plar	Autofocus options	
Plate- Greiner 384-well thin bot:	Enable laser-based focusing	
Sites to Visit- multi-site	Enable image-based focusing (for acquisition or laser recovery)	
Acquisition	Acquisition options	
Autofocus	Acquire Time Series	
Wavelengths	Acquire 7 Series	
W1 DAPI		
W2 FITC		
Display	Use Fluidics	
	Run Journals During Acquisition	
	Analyze Images After Acquisition	
	Perform shading correction Directory C:\Shading Images	



11. Select the Autofocus tab

- Select the appropriate option from the **Well to well autofocus** drop-down menu:
 - Focus on well bottom: most scenarios using 10X and higher objective
 - Focus on plate bottom then offset by bottom thickness: for low magnification objectives (2X, 4X), thin plates, or microscope slide/coverslip.
 - Focus on plate and well bottom: for warped plates (plate bottom variation is more than half the optical thickness)

Objective and Camera- 10X Plan	Laser-based Focusing
Plate- Greiner 384-well thin bot:	Configure Laser Settings
Sites to Visit- multi-site	
Acquisition	Vveli to well autorocus
Autofocus	Image-based Focusing Focus on plate bottom, then offset by bottom thickness
Wavelengths	Algorithm: Standard Focus on plate and well bottom
W1 DAPI	
W2 FITC	Allow image-based focusing for recovery from laser-based well bottom failures
Display	
	Initial well for finding sample First well acquired A I I I I I I I I I I I I I I I I I I
	Site Autofocus All sites
13	DEVIC

11. On the Autofocus tab

- Set Initial well for finding sample to First well acquired
 - This serves as a check to verify a plate is loaded
 - Only disable for very specific applications (i.e., oil immersion objectives)
- Set Number of wells to attempt initial find sample to 3

Objective and Camera- 4X SF	Laser-based Focusing			
Plate- 384 Wells (16x24)	Configure Laser Settings			
Sites to Visit- multi-site				
Acquisition	Well to well autofocus Focus on plate bottom, then offset by bottom thickness			
Autofocus	Image-based Focusing			
Wavelengths	Algorithm: Standard Binning: 2 A Custom exposure times			
W1 DAPI				
W2 FITC	Allow image-based focusing for recovery from laser-based well bottom failures			
Display				
	Initial well for finding sample First well acquired A I			
	Site Autofocus All sites			
14				



11. On the Autofocus tab

- Select the appropriate option for **Site Autofocus** from the drop down menu
 - Select **First site only** or **Center of well** only for faster acquisition at lower magnification or with high quality, flat plates.
 - Select All sites for greater focusing accuracy (recommended).

Objective and Camera- 4X SF	Laser-based Focusing			
Plate- 384 Wells (16x24)	Configure Laser Settings			
Sites to Visit- multi-site				
Acquisition	Well to well autorocus Focus on plate bottom, then offset by bottom thickness			
Autofocus	Image-based Focusing			
Wavelengths	Algorithm: Standard Binning: 2 A Custom exposure times			
W1 DAPI				
W2 FITC	Allow image-based focusing for recovery from laser-based well bottom failures			
Display				
	Initial well for finding sample First well acquired A I A I I I I I I I I I I I I I I I I			
	Site Autofocus All sites First site only Center of well only All sites			
	View Focusing Details			





12. Select the Wavelengths tab

- Select the number of wavelengths or channels that you will acquire on this plate
 - A separate W tab will appear below for each channel
 - You can select up to 8 wavelengths

Objective and Camera- 10X Pla	Number of wavelengths:	
Plate- Greiner 384-well thin bot		
Sites to Visit- multi-site		
Acquisition		
Autofocus		
Wavelengths		
W1 DAPI		
W2 FITC		
Display		





13. Select the W1 tab

- Select the desired **Illumination Setting** from the drop-down menu
- Right-click to select a site/well that should contain the highest signal for the wavelength chosen in the Plate map

Objective and Camera- 10X Plan Plate- Greiner 384-well thin bot:	Illumination setting: DAPI
Sites to Visit- multi-site	Exposure (ms): 50 - Auto Expose Target max intensity: 33000
Acquisition	Autofocus options
Autofocus	Poet Jacer
Wavelengths	offset (um)
W1 DAPI	Laser with z-offset 12.36
W2 FITC	
Display	
	Calculate Offset ✓ ✓ ✓ ✓ ✓ ✓ Kange (um) Step (um) 138.89 ↓ 5.56 ↓





13. On the W1 tab

- Click on the **Calculate offset** button to perform an automatic routine for finding the best focal position (post-laser offset value)
 - Enable **Use Z Stack** for an interactive option to select the focus position. The software will acquire a Z stack of images and allow you to select the most in-focus image.
 - Enable **Custom Range** to specify a custom range and step size for the focus search

Objective and Camera- 10X Plan		0.40				
Plate- Greiner 384-well thin bot:	Illumination setting:	DAPI		•		
Sites to Visit- multi-site	Exposure (ms):	50	Auto Expose	Target max intensity:	33000	
Acquisition	Autofocus ontions					
Autofocus		Post	0.00			
Wavelengths		offset	(um)			
W1 DAPI	Laser with z-offset	▼ 12.36				
W2 FITC						
Display						
	Calculate Offset	< 🔽 Use Z sta	ack 🔲 Custom	Range (um) 138.89	Step (um) 5.56 ⊋	
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What is a Post- Laser Offset?

Post-laser offset is the Z distance between the bottom of the well and the sample

- Laser autofocus routine finds the well bottom, NOT the biological sample of interest
- You may need to empirically determine the offset (or distance) between the well bottom and the sample
- Very wavelength dependent (chromatic aberration)
- Offset can be positive or negative
- Molecular Devices recommends to checking multiple wells for consistency





13. On the W1 tab

- Enter an **Exposure** (ms) time and click on the **Focus** button
 - Evaluate the image for pixel intensities (bit range)
 - Optionally, click on the **Auto Expose** button to determine exposure automatically (i.e. avoid saturation or very dim signal)
 - Set **Target max intensity** between 33000-45000 for a 16 bit camera (3000-3500 for 12 bit camera). The Auto Expose routine will attempt to attain this value for the brightest pixel in the image.
 - Molecular Devices recommends check exposure times for both positive and negative control wells

Objective and Camera- 10X Plan	Illumination setting: DAPI
Sites to Visit- multi-site	Exposure (ms): 50 - Auto Expose Target max intensity: 33000
Acquisition	Autofocus options
Autofocus	Part lass
Wavelengths	offset (um)
W1 DAPI	Laser with z-offset 🔹 12.36 🚔
W2 FITC	
Display	
	Calculate Offset Image Stack Image Custom Range Range (um) Step (um) 138.89 5.56
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- 14. Select the **W2** tab (and subsequent W tabs)
 - Select desired **Illumination Setting** from the drop-down menu
 - Right-click to select a site/well that should contain the highest signal for the wavelength chosen in the plate map
 - Calculate Focus offset
 - Determine Exposure time

Objective and Camera- 10X Plar Plate- Greiner 384-well thin bot:	Illumination setting: FITC
Sites to Visit- multi-site	Exposure (ms): 400 - Auto Expose Target max intensity: 33000
Acquisition	Autofocus options
Autofocus	Addredd options
Wavelengths	Offset (um)
W1 DAPI	Z-offset from W1 2.76
W2 FITC	
Display	
	Calculate Offset ≤ ✓ Use Z stack Custom Range Range (um) Step (um) 138.89 ⇒ 5.56 ⇒





15. Select the **Display** tab to configure:

- Auto Arrange Images: Software automatically determines the arrangement and size of images shown in MetaXpress
- Click on Display Acquisition Layout: Manually configure how the images will look during acquisition (position, size, scaling, monochrome or color).
- **Display images during autofocus** should be checked to help with finding post-laser offset
- **Display images during acquisition** displays images according to the settings determined using **Auto Arrange Images** or **Display Acquisition Layout**
- **Display a color overlay of wavelength images during acquisition**: Will create a color composite of the first 3 wavelengths selected.

Plate- Greiner 384-well thin bot:		
Sites to Visit- multi-site	Auto Arrange images	
Acquisition	Display Acquisition Layout	
Autofocus		
Wavelengths	Display images during autofocus	
W1 DAPI	Diaday impage during peruitation	
W2 FITC		
Display	Display a color overlay of wavelength images during acquisition	



- 16. Click on the **Save Protocol** button at the bottom of the **Plate Acquisition Setup** dialog
 - A star on the Save Protocol button indicates there are unsaved changes to the protocol
 - Molecular Devices recommends to save your settings to a file, rather the database
 - Click on the **Save** button, name the protocol, and navigate through windows to save the file (bto)





- 17. Select the Run tab and enter:
 - Folder Name: folder your plates go in in the database (i.e. project or PI)
 - **Plate Name**: the name of the plate to be imaged (i.e. specific experiment)
 - Barcode (optional): manually enter the plate barcode
 - **Storage Location**: select where you want images to be stored (there may only be one choice)
 - **Description**: enter any identifying information you would like to store with the plate

Configure Run	Active Wavelength	FITC	•	Snap Start Live	60 Focus	Test	Preview
Folder Name	Transfluor	Barcode					
Plate Name	Transfluor 10x	Description	Transfluor plate		*		
Storage Location	Local File Server				~	Acquire Plate	
	Exposure Time (ms)	Snap	Test	Focus Offset (µm)			
DAPI	Auto Expose 50 🖨	[`O"		Calculate 12.36	•		
FITC	Auto Expose 400 🖨	[`0"		Calculate 2.76	•		
24						MOL	ecular VICES

18. Click on the **Acquire Plate** button to begin acquisition of the plate

Configure Run	Active Wavelength	FITC	•	Snap Start Live	Focus	Test	Preview
Folder Name	Transfluor	Barcode					
Plate Name	Transfluor 10x	Description	Transfluor plate		*		
Storage Location	Local File Server				-	Acquire Plate	
	Exposure Time (ms)	Snap	Test	Focus Offset (µm)			
DAPI	Auto Expose 50 🚔	` O`		Calculate 12.36	-		
FITC	Auto Expose 400 🖨	[`O]		Calculate 2.76	▲ ▼		





Support Resources

- F1 / HELP within MetaXpress® Software
- Support and Knowledge Base: <u>http://mdc.custhelp.com/</u>
- User Forum: <u>http://metamorph.moleculardevices.com/forum/</u>
- Request Support: <u>http://mdc.custhelp.com/app/ask</u>
- Technical Support can also be reached by telephone:
 - 1 (800) 635-5577
 - Select options for Tech Support → Cellular Imaging Products → ImageXpress Instruments





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