

MetaXpress® 6 Software Guide

Acquiring a Plate with Timelapse Enabled (without Z Series)



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Chapter Purpose

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

Timelapse acquisition should be enabled when the user wants to observe and measure changes over time in a live cell experiment.



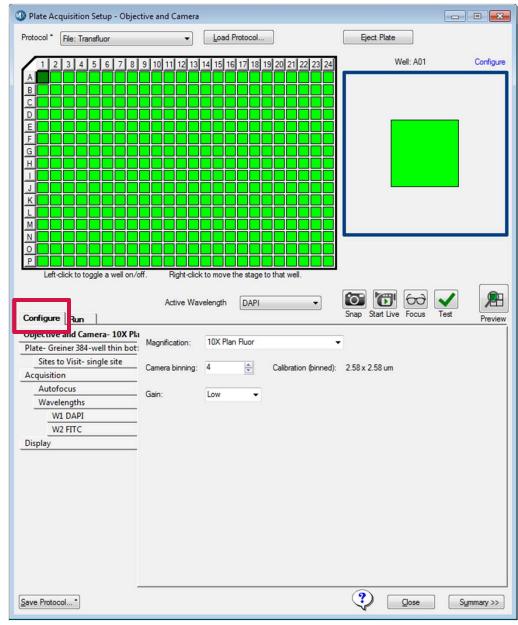


- 1. Open Plate Acquisition Setup
 - In the main toolbar click on



OR

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



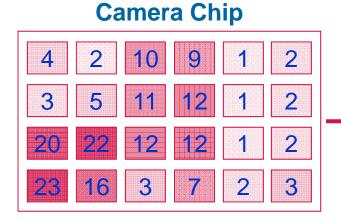


- 3. Select the **Objective and Camera** tab
- 4. Select the appropriate Magnification from the drop-down menu
 - You may need to adjust the correction collar of the objective; refer to the Main Taskbar to do this.
 - Select **Camera Binning** (refer to next section for guidance)
 - Pixel size is automatically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

					1
Objective and Camera- 4X S Flu	Manafaatiaa	4X S Fluor		1	
Plate- Corning 1536-well Black-	Magnification:	4A 3 FILLOF	•		
Sites to Visit- multi-well	Camera binning:	1	Calibration (binned):	1.61 × 1.61 um	
Acquisition	Camera birning.	1	Calibration (binned):	1.61 x 1.61 um	
Autofocus	Gain:	Low -			
Wavelengths	Gain.	Low			
W1 DAPI					
W2 FITC					
Display					
	For rea	coreb use only. Not for u	a in diagnostia procedures		DEVICES
	For res	earch use only. Not for u	se in diagnostic procedures.		

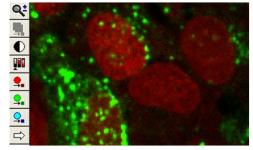
What is Binning?

Combining groups of pixels into a single pixel during image acquisition

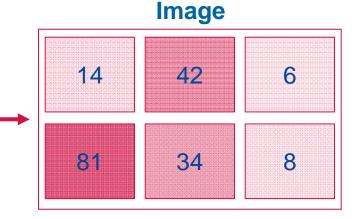


Example of 2x2 Binning

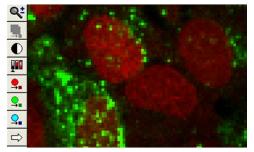
Each pixel records an intensity







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

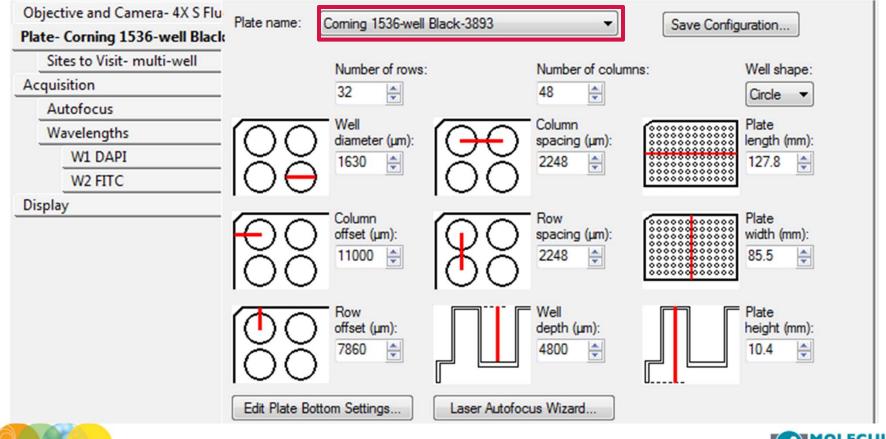
When to Bin

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





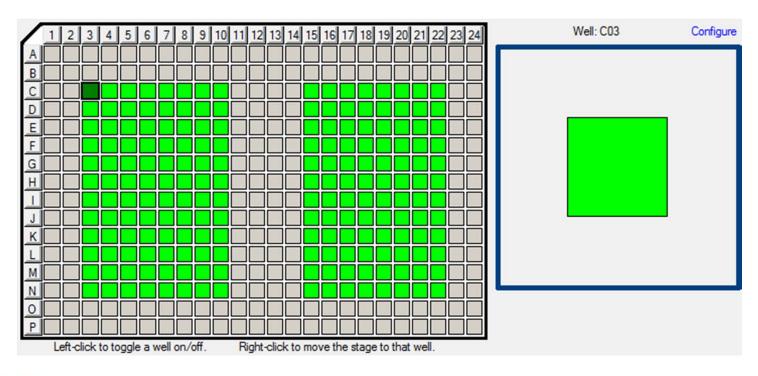
- 6. Select the Plate tab
- 7. Select the appropriate Plate Type from the drop-down menu







- 8. In the plate section, select the wells you would like to acquire
 - Left click and drag mouse to select wells
 - 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)



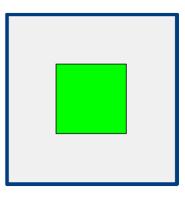




9. Select the Sites to Visit tab

- 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	Custom field of view (%): X: 50 ♀ Y: 50 ♀	Well size: 11 mm ² Number of sites: 1
Sites to Visit- single site	 Fixed number of sites Adaptive acquisition 	Site/image size: 1.39 x 1.39 mm	17.82% Well Coverage
Acquisition	Multi-well	Site/indge size. 1.55 X 1.55 min	
Autofocus	Acquires a single site ce	entered in each well	
Wavelengths			
W1 DAPI			
W2 FITC			
Display			





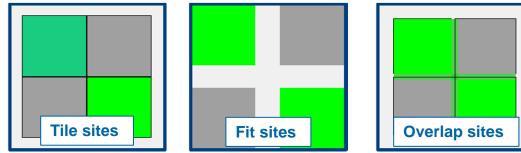


9. On the Sites to Visit tab

- 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 Plar	Site Options	Custom field of view (%):	Well size: 11 mm ²
Plate- Greiner 384-well thin bot:	Single site	×: 50 🜩 Y: 50 🌩	Number of sites: 2
Sites to Visit- multi-site	 Fixed number of sites Adaptive acquisition 	Site/image size: 1.39 x 1.39 mm	35.65% Well Coverage
Acquisition	Multi-well	Site/inage size. 1.55 X 1.55 min	
Autofocus	Acquires a fixed number	r of sites in each well	
Wavelengths	_		
W1 DAPI	Con	voine (um)	
W2 FITC	Columns: 2 🚔 0	icing (µm) 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 more details on the 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 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
- Enable Acquire Time Series
- Optionally, enable **Perform shading correction**
- *NOTE* Some of the choices shown may not appear in your version of MetaXpress

Objective and Camera- 10X Plan Plate- Greiner 384-well thin bot:	Autofocus options Image: Second sec	
Sites to Visit- multi-site	Enable image-based focusing (for acquisition or laser recovery)	
Acquisition	Acquisition options	
Autofocus	Acquire Time Series	
Wavelengths	Acquire Z Series	
W1 DAPI		
W2 FITC		
Timelapse- 1 time points	Use Fluidics	
Display	Run Journals During Acquisition	
	Analyze Images After Acquisition	
	Perform shading correction Directory C:\Shading Images	



11. Select the Timelapse tab

- From the drop-down menu, select an option to **Perform time series for**:
 - **One well then the next** for fast kinetic time lapse in a single well (or site). All time points will be collected in one well before moving to the next well.
 - All selected wells for long-term time lapse experiments. All wells are acquired during each time point.
 - One row then the next or One column then the next for specialized cases (most common for fluidics experiments).

Objective and Camera- 10X Plar	Number of times with a	2
Plate- Greiner 384-well thin bot:	Number of timepoints:	2
Sites to Visit- multi-site	Perform time series for:	One well then the next
Acquisition	Approximate minimum	One well then the next
Autofocus	Interval:	One row then the next One column then the next
Wavelengths		All selected wells
W1 DAPI	Duration:	10 🚔 sec 🔻
W2 FITC		
Timelapse- 2 time points		
Display		





11. On the **Timelapse** tab

- Enter the Number of timepoints
- Set the **Interva**I: time between each image taken (ms, sec, min, or hr)
- Set the **Duration**: total time of experiment (ms, sec, min, or hr). This is equivalent to Interval x Number of timepoints

Objective and Camera- 10X Plan Plate- Greiner 384-well thin bot:	Number of timenointe:	2
Sites to Visit- multi-site	Perform time series for:	One well then the next 🔹
Acquisition	Approximate minimum	m time interval: 2.6 sec
Autofocus	Interval:	10 🚖 sec 🔻
Wavelengths		
W1 DAPI	Duration:	10 🚖 sec 🔻
W2 FITC		
Timelapse- 2 time points		
Display		





12. Select the Autofocus tab

- Select the appropriate option from the **Well to well autofocus** drop-down menu:
 - Focus on well bottom: most scenarios using a 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 Plar	Laser-based Focusing
Plate- Greiner 384-well thin bot:	Configure Laser Settings
Sites to Visit- multi-site	
Acquisition	Well to well autofocus Focus on well bottom
Autofocus	Image-based Focusing Focus on well bottom, then offset by bottom thickness
Wavelengths	Focus on plate and well bottom
W1 DAPI	Algorithm: Standard V Binning: 2 V Custom exposure times
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
15	

12. 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		2
Acquisition	Well to well autofocus Focus on well bottom	J
Autofocus	Image-based Focusing	
Wavelengths	Algorithm: Standard Binning: 2 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	×
	Number of wells to attempt initial find sample 3	
	Site Autofocus All sites 👻	





12. 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).

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 Custom exposure times
Allow 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 1
Site Autofocus All sites
Timelapse Autofocus First site only Center of well only All sites





12. On the Autofocus tab

- Select the appropriate option for **Timelapse Autofocus** from the drop down menu:
 - **First timepoint only** for fast kinetic time lapse in a single well (i.e., the stage does not move between time points. Use this setting when selecting One well then the next on the Timelapse tab
 - All timepoints for long-term time lapse where the stage moves from well to well between time points. Use this setting when selecting All selected wells on the Timelapse tab
 - Every Nth timepoint for slower or longer kinetic experiments in a single well to periodically verify focal position. This setting is recommended when selecting One well then the next on the Timelapse tab

Display	Initial well for finding samp	First well acquired	• A	v 1 ×	
	Number of wells to attemp	ot initial find sample 1			
	Site Autofocus	All sites	•		
	Timelapse Autofocus	First timepoint only	- 2	A V	
		First timepoint only			
		All timepoints Every Nth timepoint			



- 13. Select the Wavelengths tab
 - Enter 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 enter up to **8** wavelengths

Objective and Camera- 10X Plar		
Plate- Greiner 384-well thin bot:	Number of wavelengths:	2
Sites to Visit- multi-site		
Acquisition		
Autofocus		
Wavelengths		
W1 DAPI		
W2 FITC		
Display		





14. Select the W1 tab

- Select the desired Illumination Setting from the drop-down menu
- In the plate Map, right-click to select a site/well that should contain the highest signal for the wavelength chosen

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	Post-laser
Wavelengths	offset (um)
W1 DAPI	Laser with z-offset
W2 FITC	
Display	
	Calculate Offset ✓ Use Z stack Custom Range Range (um) Step (um) 138.89 ↓ 5.56 ↓





14. 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 Plar	
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	Post-laser
Wavelengths	offset (um)
W1 DAPI	Laser with z-offset
W2 FITC	
Display	
	Range (um) Step (um) Calculate Offset ✓ Use Z stack □ Custom Range 138.89 ↓ 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 checking multiple wells for consistency





14. On the W1 tab

- Enter an Exposure (ms) time and click on the Focus button
 - Evaluate the image for pixel intensity (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 checking exposure times for both positive and negative control wells

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	Post-laser
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 Image Step Stack Image Stack Image Stack Image Stack Image Stack
23	DEVICE





14. On the W1 tab

- Select the appropriate option from the **Acquisition Options** drop-down menu to specify how often to collect the selected wavelength
 - At all time points acquires this wavelength at each time point
 - At start of experiment acquires this wavelength only at time point 1
 - At start/end of experiment acquires this wavelength at only the first and last time points
 - Every nth time point acquires this wavelength every nth time point (2nd, 5th, 6th, etc.) throughout the time lapse experiment

W1 DAPI W2 FITC	Laser with z-offset I2.36	
Timelapse- 2 time points		
Display	Calculate Offset Image Use Z stack Custom Range Range (um) Step (um) Acquisition Options Image Step Stack Image Step Step Step Step Step Step Step Ste	
24	at all time points at start of experiment at start/end of experiment every nth timepoint	LAR

- 15. Select the W2 tab (and subsequent W tabs)
 - Select the desired Illumination Setting from the drop-down menu
 - In the plate Map, right-click to select a site/well that should contain the highest signal for the wavelength chosen
 - Calculate Focus offset
 - Determine **Exposure** time
 - Select the appropriate acquisition options from the **Timelapse** drop-down menu

Objective and Camera- 10X Plar	Illumination setting: FITC
Plate- Greiner 384-well thin bot:	indrimitation setting.
Sites to Visit- multi-site	Exposure (ms): 100 🖨 Auto Expose Target max intensity: 33000 🖨
Acquisition	Autofocus options
Autofocus	
Wavelengths	Offset (um)
W1 DAPI	Z-offset from W1 2.76
W2 FITC	
Timelapse- 2 time points	
Display	Calculate Offset Image Stack Image Custom Range Range (um) Step (um) Acquisition Options Acquisition Options
	Timelapse: at all time points





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

- Auto Arrange Images: Software automatically determines the arrangement and size of images shown during acquisition.
- 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.

Objective and Camera- 10X Plan		
Plate- Greiner 384-well thin bot:		
Sites to Visit- multi-site	Auto Arrange Images	
Acquisition	Display Acquisition Layout	
Autofocus	Display Acquisition Layout	
Wavelengths	Display images during autofocus	
W1 DAPI		
W2 FITC	Display images during acquisition	
Display	Display a color overlay of wavelength images during acquisition	



- 17. 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 saving settings to a file rather then the database
 - Click on the **Save** button, name the protocol, and navigate through windows to save the file (.hts)

Configure Run	Active Wavelength FITC	• Sn	ap Start Live	Focus	Test Prev		
Objective and Camera- 10X Plan							
Plate- Greiner 384-well thin bot:	Auto Arrange Images						
Sites to Visit- multi-site	Auto Arrange images						
Acquisition	Display Acquisition Layout			(1	_
Autofocus					Save Acquisition	on Protocol	
Wavelengths	Display images during autofocus				Course to file	with an it has a data	
W1 DAPI	Display images during acquisition				Save to file i		base
W2 FITC	Display a color overlay of wavelength in		141		Protocol Name:		
					Stored Protocol	•	
Save Protocol*		(?	Close	Save	Ca	ncel



- 18. 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 Rur	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 🖨	` 0"		Calculate 12.36			
FITC	Auto Expose 400 🖨	[`0"		Calculate 2.76			
28						MOL	ECULA

19. Click on the **Acquire Plate** button to begin acquisition of 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				Ŧ	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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