

MetaXpress® 6 Training

Setting up Timelapse and Z Series Acquisition



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Chapter Purpose

The purpose of this chapter is to guide the user through setting up an acquisition with both Timelapse and Z Series. This includes selecting objectives, plates, wavelengths, and focal position. Note that you cannot save both time point and z step images in the same experiment. If you use Timelapse, then you *must* convert the Z Series to a 2D projection for saving.

Optimizing Z Series options are NOT included in this chapter. Refer to the corresponding chapters for guidance on setting up Z Series acquisition.





- 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	AX C Eluca		1	
Plate- Corning 1536-well Black-	Magnification:	4A 3 FILLOF	•		
Sites to Visit- multi-well	Comora hispina:	1	Calibration (binned):	1.61 × 1.61 um	
Acquisition	Camera binning.		Calibration (binned).	1.01 X 1.01 UIII	
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 us	se in diadnostic brocedures.		

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 saving
- 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 whole 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; 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	Custom field of view (%):	Well size: 11 mm² 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	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

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

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 mm ² Number of sites: 2 35.65% Well Coverage
Sites to Visit- multi-site	Adaptive acquisition	Site/image size: 1.39 x 1.39 mm	
Acquisition	Multi-well		
Autofocus	Acquires a fixed number	r 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





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

Objective and Camera- 10X Plar Plate- Greiner 384-well thin bot:	Site Options Single site	Custom field of view (%):	Well size: 11 mm ² Number of sites: 1
Sites to Visit- single site	Adaptive acquisition	Site /image size: 1.29 x 1.29 mm	17.82% Well Coverage
Acquisition	Multi-well	Site/inage size. 1.55X 1.55 min	
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 image based focusing
- Enable Acquire Time Series
- Enable Acquire Z Series
- Optionally, enable **Perform shading correction**

Objective and Camera- 10X PF Plate- 384 Wells (16x24)	Autofocus options Image: Second state Image:
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- 6 time points	
Z Series- 10 planes	Run Journals During Acquisition
Display	Analyze Images After Acquisition
	Perform shading correction Directory C:\



11. Select the Autofocus tab

- Select the appropriate option for **Well to well autofocus** from the 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, u-bottom 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 PF	Laser-based Focusing	
Plate- 384 Wells (16x24)	Configure Laser Settings	
Sites to Visit- multi-site		
Acquisition	Well to well autorocus Focus on well bottom	
Autofocus	Image-based Focusing Focus on plate bottom, then offset by bottom thickness	
Wavelengths	Algorithm: Standard	
W1 DAPI		
W2 FITC	Allow image-based focusing for recovery from laser-based well bottom failures	
Timelapse- 6 time points		
Z Series- 10 planes		
Display	Initial well for finding sample First well acquired	
	Number of wells to attempt initial find sample 3	
	Site Autofocus All sites 💌	
	Timelapse Autofocus First timepoint only	
	View Focusing Details	s



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- 10X PF	Laser-based Focusing
Plate- 384 Wells (16x24)	Configure Laser Settings
Sites to Visit- multi-site	
Acquisition	Vell to well autorocus Focus on well bottom
Autofocus	Image-based Focusing
Wavelengths	Algorithm: Standard Binning: 2
W1 DAPI	
W2 FITC	Allow image-based focusing for recovery from laser-based well bottom failures
Timelapse- 6 time points	
Z Series- 10 planes	
Display	Initial well for finding sample First well acquired
	Number of weils to attempt initial find sample 3
	Site Autofocus All sites
	Timelapse Autofocus First timepoint only





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- 10X PF	Laser-based Focusing
Plate- 384 Wells (16x24)	Configure Laser Settings
Sites to Visit- multi-site	
Acquisition	Well to well autorocus Focus on well bottom
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
Timelapse- 6 time points	
Z Series- 10 planes	
Display	Initial well for finding sample First well acquired
	Number of wells to attempt initial find sample 3
	Site Autofocus All sites
	Timelapse Autofocus First site only Center of well only All sites
	View Focusing Details





11. On the Autofocus tab

- Select the appropriate option for **Timelapse Autofocus** from the drop down menu:
 - **First timepoint only** for fast kinetic timelapse 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 timelapse 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

Z Series- 10 planes Display	Initial well for finding samp	First well acquired	▼ A	v) 1 (*)
	Number of wells to attemp	t initial find sample 3		
	Site Autofocus	All sites	•	
	Timelapse Autofocus	First timepoint only	- 2	
		First timepoint only All timepoints		
		Every Nth timepoint		
			1	View Focusing Details





12. Select the Wavelengths tab

- Enter the number of wavelengths or channels you wish to acquire
 - A separate W tab will appear below for each channel
 - You can enter up to 8 wavelengths

Objective and Camera- 10X PF				
Plate- 384 Wells (16x24)	Number of wavelengths:	2		
Sites to Visit- multi-site				
Acquisition				
Autofocus				
Wavelengths				
W1 DAPI				
W2 FITC				
Timelapse- 6 time points				
Z Series- 10 planes				
Display				





13. Select the W1 tab

- 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

Objective and Camera- 10X PF	
Plate- 384 Wells (16x24)	Illumination setting: DAPI
Sites to Visit- multi-site	Exposure (ms): 50 - Auto Expose Target max intensity: 3000
Acquisition	Autofaque aptiene
Autofocus	Autorocus options
Wavelengths	Post-laser offset (um)
W1 DAPI	Laser with z-offset 🔹 3
W2 FITC	
Timelapse- 6 time points	
Z Series- 10 planes	Range (um) Step (um)
Display	Calculate Offset < Use Z stack Custom Range 138.89 5.56
	Acquisition Options Timelapse: at all time points Z Series: 2D Projection Image Only Digital Confocal (info) </td
	Shading Correction: Off





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
- For Z Series acquisition, Molecular Devices recommends setting the post-laser offset to find the approximate middle of the sample

W1 DAPI W2 FITC	Laser with z-offset
Timelapse- 6 time points	
Z Series- 10 planes Display	Calculate Offset Image Step (um) Calculate Offset Image Step (um) Image Step (um) Image Step (um)
	Timelapse: at all time points
	Z Series: 2D Projection Image Only
	□ Digital Confocal (info) << Increase sharpness





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





13. On the W1 tab

- Enter an Exposure time and click 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 (2000-3000 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 PF	
Plate- 384 Wells (16x24)	Illumination setting: DAPI
Sites to Visit- multi-site	Exposure (ms): 50 - Auto Expose Target max intensity: 3000
Acquisition	Autofocus options
Autofocus	Part lager
Wavelengths	offset (um)
W1 DAPI	Laser with z-offset 🔹 3 🚖
W2 FITC	
Timelapse- 6 time points	
Z Series- 10 planes	Range (um) Step (um)
Display	Calculate Offset





13. 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 timelapse experiment

W1 DAPI W2 FITC	Laser with z-offset
Timelapse- 6 time points Z Series- 10 planes	Range (um) Step (um)
Display	Calculate Offset Use Z stack Custom Range 138.89 5.56 Acquisition Options Timelapse at all time points at start of experiment at start of experiment at start/end of experiment at start/end of experiment Bigital Projection Image: Best Focus Best Focus Calculate Offset Output Acquisition Options Timelapse at all time points at start of experiment at start/end of experiment every nth timepoint Best Focus Custom Range Digital Correction: Off





13. On the W1 tab

- Under Acquisition Options, select the appropriate option for saving Z Series
 - When acquiring Timelapse you will not have the option to save the individual steps in the Z Series
 - **Single Plane**: only the image taken at the post-laser offset will be saved
 - **2D Projection Image Only**: only the 2D Projection image will be saved *NOTE* The above options will be available on each W tab. It is not necessary to acquire and save images the same way for each wavelength

W1 DAPI W2 FITC	Laser with z-offset
Timelapse- 6 time points	
Z Series- 10 planes	Range (um) Step (um)
Display	Calculate Offset < Use Z stack Custom Range 138.89 = 5.56
	Acquisition Options Timelapse: at all time points Z Series: 2D Projection Image Only Single Plane Digital C 2D Projection Image Only Shading Correction. On





13. On the W1 tab

- Under Acquisition Options, select the appropriate option for 2D Projection Image
 - **Best Focus**: estimates the regions of best focus in an image stack to within onetenth pixel accuracy along Z. Two resolution grid sizes are used to enhance the criterion of focus through the stack (recommended for counting, not for comparing pixel intensities)
 - **Maximum**: For each corresponding pixel position in the images, the Maximum operation finds the pixel that has the highest intensity value out of all the values in all the planes, and outputs this value to the new image (not recommended for samples with high background)
 - **Minimum**: For each corresponding pixel position in the images, the Minimum operation finds the pixel that has the lowest intensity value out of all values in all the planes, and outputs this value to the new image (often used with Transmitted light)
 - Sum: For each corresponding pixel position, the Sum operation adds the intensities of the pixels in the stack planes, and outputs this value to the new image. This operation is useful for combining images

NOTE The above options will be available on each W tab. It is not necessary to apply the same projection algorithm to each wavelength

	focal (info)	<< Increase sharpness	s Reduce noise >	Best Focus Maximum
Shading Corre	ction: Off			Minimum Sum





2D Projection Images

- These are examples of 2D Projections generated from the same stack of images
- The optimal choice for 2D projection will depend on your sample type and the analysis goal







- 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
 - Determine Acquisition Options
 - **Timelapse**: select the appropriate acquisition type from the drop-down menu
 - **Z Series**: select which z plane to save or the type of 2D projection

Objective and Camera- 10X PF	
Plate- 384 Wells (16x24)	Illumination setting: FIC
Sites to Visit- multi-site	Exposure (ms): 100 - Auto Expose Target max intensity: 3000
Acquisition	
Autofocus	- Autorocus options
Wavelengths	Offset (um)
W1 DAPI	Z-offset from W1 -2
W2 FITC	
Timelapse- 6 time points	
Z Series- 10 planes	Range (um) Step (um)
Display	Calculate Offset
	Acquisition Options
	Timelapse: at all time points
	Timelapse: at all time points Z Series: 2D Projection Image Only 2D Projection Image Only 2D Projection Image:
	Timelapse: at all time points Z Series: 2D Projection Image Only 2D Projection Image: Best Focus Digital Confocal (info) Reduce noise >>





15. Select the Z Series tab

- Enter **# of Steps**: # of Z steps acquired
- Enter Step Size: spacing (µm) between each Z step
- Center Z Series Around Focus result:
 - If checked, # of Steps and Step Size will be centered around the post-laser offset configured on the W tab
 - If unchecked, you will need to manually set the **Top** and **Bottom** Z positions. These Z positions are the customized Z heights from the focus result that will be used for every site/well (refer to the ruler to determine µm spacing from the focus result)

Configure Run		Snap Start Live Focus Test Previe
Objective and Camera- 4X S Flu	Center Z Series Around Focus Result	Units: µm
Plate- Corning 96-Well U-Botto		+200
Sites to Visit- single site	# of Steps: 10 🗘	
Acquisition		+150 Current Set T
Autofocus	IOP	9589.6 μm Set B
Wavelengths		+100
W1 DAPI	Step Size: 30 🗘 µm	
W2 FITC	D	+30
W3 CY5		0 - FOCUS -
Z Series- 10 planes	Recommended	
Display	Step Size: 3.1 µm	-50
	Range: 270 µm	-100
	воттом	-150
		-200
	Ruler	700mi +







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16. 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

Diste- Greiner 284-well thin bot		
Sites to Visit- multi-site	Auto Arrange Images	
Acquisition	Display Association Laws 4	
Autofocus	Display Acquisition Layout	
Wavelengths	Display images during autofocus	
W1 DAPI		
W2 FITC		
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 your settings to a file rather than the database
 - Click on the **Save** button, name the protocol, and navigate through windows to save the file (.hts)

Configure Run	Active Wavelength FITC	Snap S	Start Live Focu	s Test	Preview	
Objective and Camera- 10X Plar					1	
Plate- Greiner 384-well thin bot:						
Sites to Visit- multi-site	Auto Arrange Images					
Acquisition	Display Appricition I must		,			
Autofocus	Display Acquisition Layout		Í	Save Acou	isition Protocol	
Wavelengths	Display images during autofocus			Save Acqu	insicion Protocor	
W1 DAPI				Save to	file rather than data	aba
W2 FITC	 Display images during acquisition 			Protocol N	ame:	-
Display	Display a color overlay of wavelength images	during acquisition			anto.	-
				Stored Prot	iocols:	
Save Protocol*		$\langle \rangle$	Close	Same		
				Save		1110



- 18. Select the Run tab and enter:
 - Folder Name: folder to organize your plates in (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	60 Focus	Test P	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 🚔	[`0"		Calculate 12.36	•		
FITC	Auto Expose 400 🚔	` 0`		Calculate 2.76	•		
31							olecui E V I C

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				-	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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