



MetaXpress® 6 Software Guide

Setting up a Z Series Acquisition
(Option to Save All Z Series Images with Projection)

Date Revised 06/24/15 Version A



Chapter Purpose

The purpose of this chapter is to guide the user through setting up an acquisition with Z Series. This includes selecting objectives, plates, wavelengths, focal position, and optimizing Z steps.

Acquiring timelapse images with Z Series will NOT be covered in this chapter. Refer to corresponding chapter for this process.



Setting Up a Z Series Acquisition

1. Open Plate Acquisition Setup

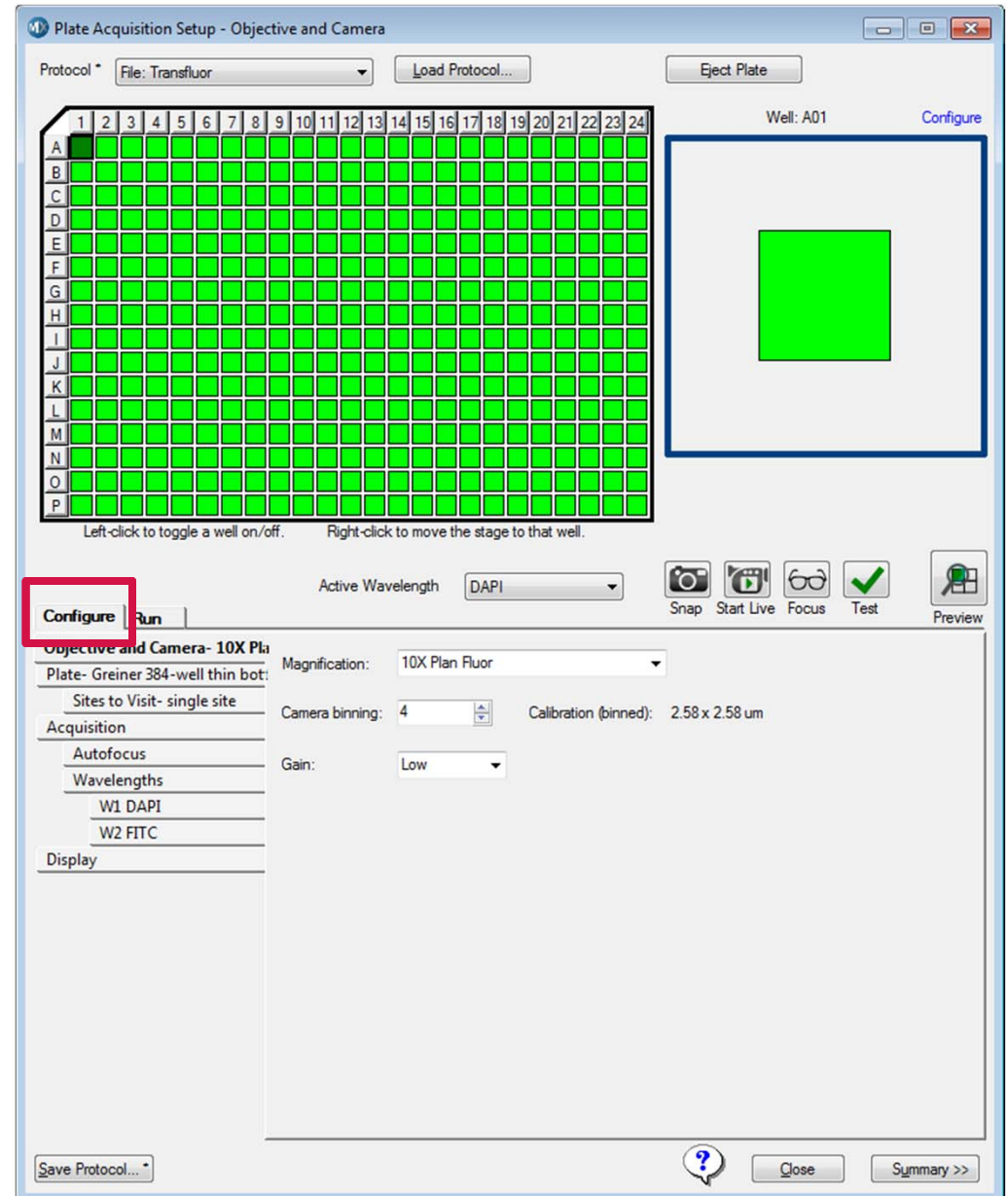
- In the main toolbar click on



OR

- Under the **Screening** menu, select **Plate Acquisition Setup**

2. Select the **Configure** tab



Setting Up a Z Series Acquisition

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 section on binning 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, it is suggested to start with gain set to **Low**

Objective and Camera- 4X S Flu

Plate- Corning 1536-well Black-
Sites to Visit- multi-well
Acquisition
Autofocus
Wavelengths
W1 DAPI
W2 FITC
Display

Magnification: 4X S Fluor

Camera binning: 1 Calibration (binned): 1.61 x 1.61 um

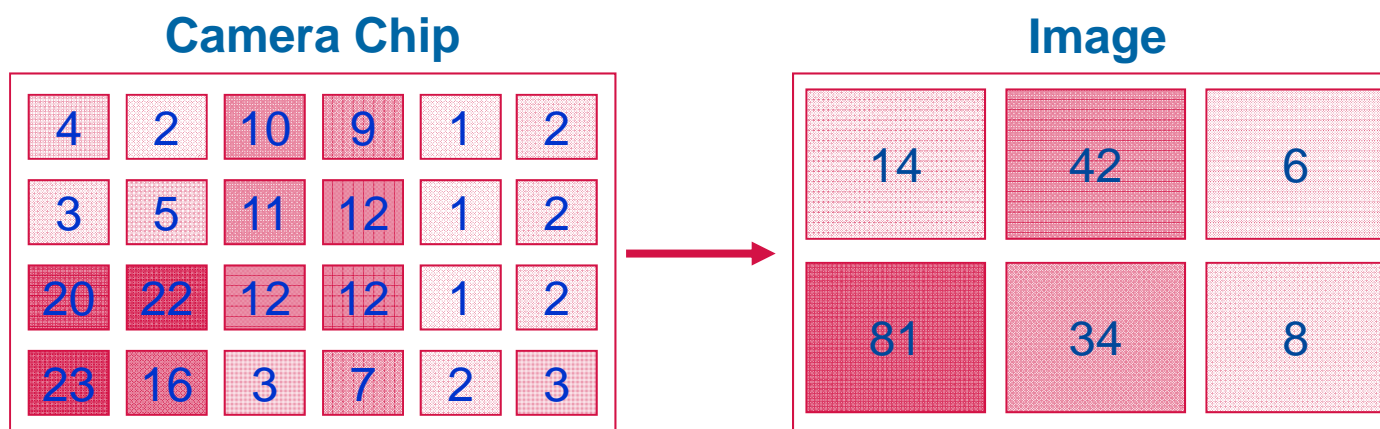
Gain: Low



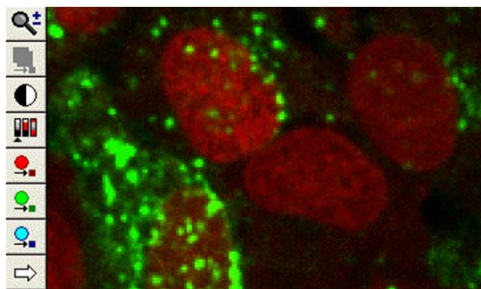
What is Binning?

Combining groups of pixels into a single pixel during image acquisition

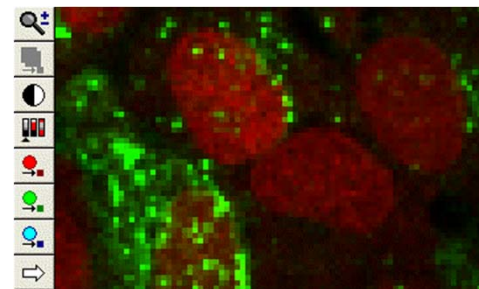
Example of 2x2 Binning



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



Setting Up a Z Series Acquisition

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

Objective and Camera- 4X S Flu

Plate- Corning 1536-well Black

Sites to Visit- multi-well

Acquisition

Autofocus

Wavelengths

W1 DAPI

W2 FITC

Display

Plate name: **Coming 1536-well Black-3893** Save Configuration...

Number of rows: 32

Number of columns: 48

Well shape: Circle

Well diameter (μm): 1630

Column spacing (μm): 2248

Plate length (mm): 127.8

Column offset (μm): 11000

Row spacing (μm): 2248

Plate width (mm): 85.5

Row offset (μm): 7860

Well depth (μm): 4800

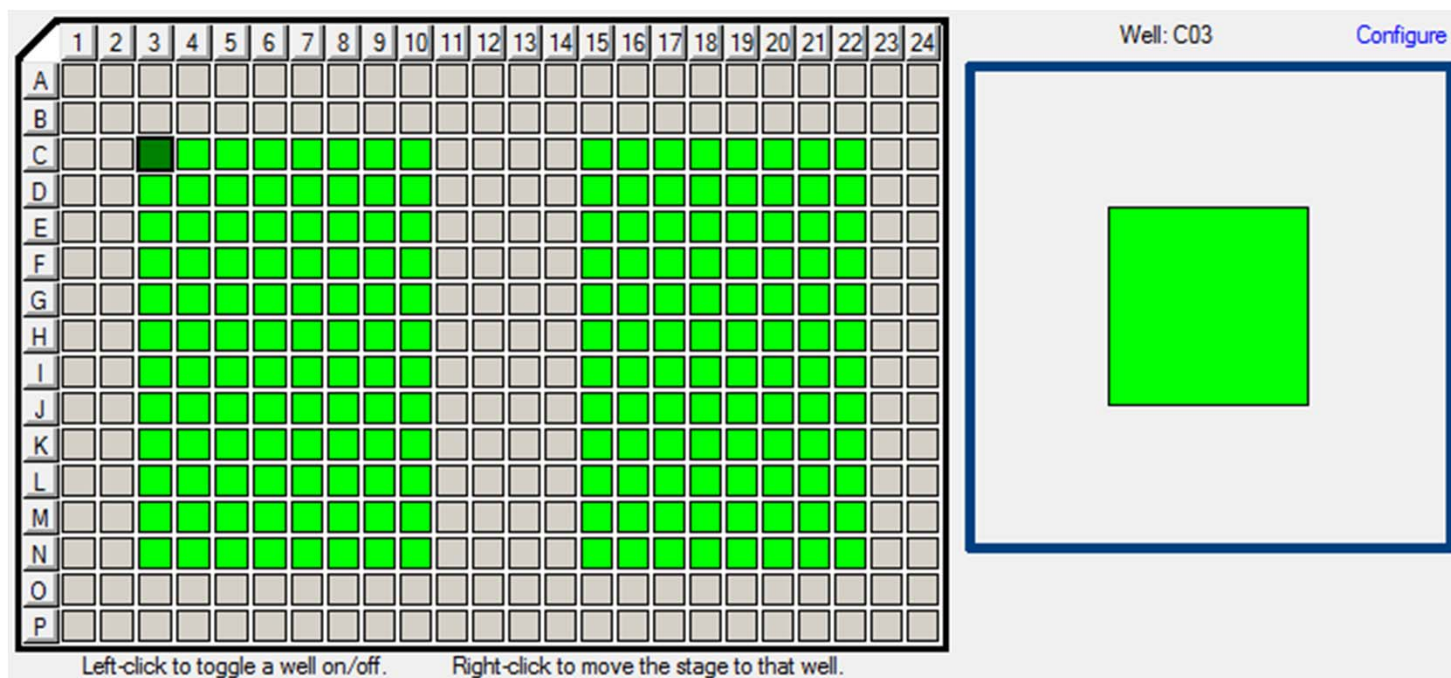
Plate height (mm): 10.4

Edit Plate Bottom Settings... Laser Autofocus Wizard...



Setting Up a Z Series Acquisition

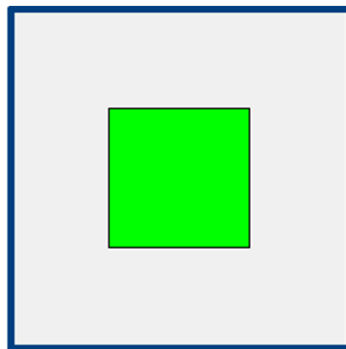
8. In the **Plate Section**, select the wells you would like to acquire
- Left click and drag mouse to select wells (selections do not need to be contiguous)
 - Click on “All” (top left corner), row letters, column numbers, or individual wells
 - Gray wells are deactivated, green wells are activated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



Setting Up a Z Series Acquisition

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 section on setting up multiple sites

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



Setting Up a Z Series Acquisition

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 Plan
Plate- Greiner 384-well thin bot:
Sites to Visit- multi-site
Acquisition
Autofocus
Wavelengths
W1 DAPI
W2 FITC
Display

Site Options
☐ Single site
☒ Fixed number of sites
☐ Adaptive acquisition
☐ Multi-well

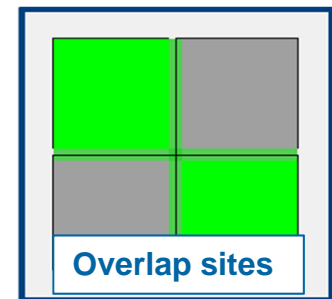
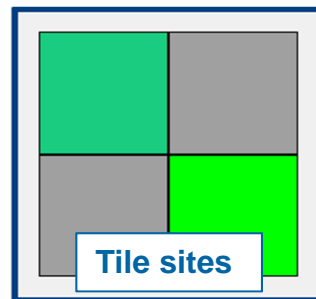
☐ Custom field of view (%):
X: 50 Y: 50
Site/image size: 1.39 x 1.39 mm

Well size: 11 mm²
Number of sites: 2
35.65% Well Coverage

Acquires a fixed number of sites in each well

Columns: 2 Spacing (µm): 0
Rows: 2
Tile sites
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



Setting Up a Z Series Acquisition

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

Objective and Camera- 10X Plan
Plate- Greiner 384-well thin bot:

Sites to Visit- single site

Acquisition

Autofocus

Wavelengths

W1 DAPI

W2 FITC

Display

Site Options

- ☒ Single site
- ☐ Fixed number of sites
- ☐ Adaptive acquisition
- ☐ Multi-well

Acquires a single site centered in each well

☐ Custom field of view (%):

X: 50 Y: 50

Site/image size: 1.39 x 1.39 mm

Well size: 11 mm²
Number of sites: 1
17.82% Well Coverage



Setting Up a Z Series Acquisition

10. Select the **Acquisition** tab

- Always **Enable laser-based focusing**
- For certain samples it may be necessary to **Enable image based focusing** (recommended if samples are in different focal planes or using u-bottom plates)
- Disable **Acquire Time Series**
- Enable **Acquire Z Series**
- Optionally, enable **Perform shading correction**

Objective and Camera- 10X PF
Plate- 384 Wells (16x24)
Sites to Visit- multi-site

Acquisition

Autofocus
Wavelengths
W1 DAPI
W2 FITC
Z Series- 3 planes
Display

Autofocus options

- ☒ Enable laser-based focusing
- ☐ Enable image-based focusing (for acquisition or laser recovery)

Acquisition options

- ☐ Acquire Time Series
- ☒ Acquire Z Series

☐ Run Journals During Acquisition
☐ Analyze Images After Acquisition
☐ Perform shading correction

Directory... C:\



Setting Up a Z Series Acquisition

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, or microscope slide/coverslip.
 - **Focus on plate and well bottom:** for warped plates (plate bottom variation is more than half the optical thickness)

The screenshot shows the software interface for setting up a Z Series Acquisition. On the left, a sidebar contains several tabs: 'Objective and Camera- 10X PF', 'Plate- 384 Wells (16x24)', 'Sites to Visit- multi-site', 'Acquisition', 'Autofocus' (which is selected), 'Wavelengths', 'W1 DAPI', 'W2 FITC', 'Z Series- 3 planes', and 'Display'. The main panel is titled 'Laser-based Focusing' and contains a 'Configure Laser Settings...' button. Below this, the 'Well to well autofocus' dropdown menu is open, showing three options: 'Focus on well bottom' (highlighted in blue), 'Focus on well bottom' (highlighted in blue), and 'Focus on plate bottom, then offset by bottom thickness'. Below the dropdown, there is an 'Image-based Focusing' section with an 'Algorithm' dropdown set to 'Standard', a 'Binning' dropdown set to '2', and a checkbox for 'Custom exposure times'. There is also a checkbox for 'Allow image-based focusing for recovery from laser-based well bottom failures'. At the bottom, there are three fields: 'Initial well for finding sample' set to 'First well acquired', 'Number of wells to attempt initial find sample' set to '3', and 'Site Autofocus' set to 'All sites'. A 'View Focusing Details...' button is located at the bottom right of the main panel.



Setting Up a Z Series Acquisition

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**

The screenshot displays the 'Autofocus' tab in the software interface. On the left, a sidebar lists various settings: 'Objective and Camera- 10X PF', 'Plate- 384 Wells (16x24)', 'Sites to Visit- multi-site', 'Acquisition', 'Autofocus' (selected), 'Wavelengths', 'W1 DAPI', 'W2 FITC', 'Z Series- 3 planes', and 'Display'. The main panel is divided into sections for 'Laser-based Focusing' and 'Image-based Focusing'. The 'Laser-based Focusing' section includes a 'Configure Laser Settings...' button and a 'Well to well autofocus' dropdown set to 'Focus on well bottom'. The 'Image-based Focusing' section shows 'Algorithm' set to 'Standard', 'Binning' set to '2', and checkboxes for 'Custom exposure times' and 'Allow image-based focusing for recovery from laser-based well bottom failures'. A red rectangular box highlights the 'Initial well for finding sample' dropdown (set to 'First well acquired'), the 'Number of wells to attempt initial find sample' spinner (set to '3'), and the 'Site Autofocus' dropdown (set to 'All sites').



Setting Up a Z Series Acquisition

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)

The screenshot displays the 'Autofocus' tab in the software interface. On the left, a sidebar contains navigation options: 'Objective and Camera- 10X PF', 'Plate- 384 Wells (16x24)', 'Sites to Visit- multi-site', 'Acquisition', 'Autofocus' (selected), 'Wavelengths', 'W1 DAPI', 'W2 FITC', 'Z Series- 3 planes', and 'Display'. The main panel is divided into sections for 'Laser-based Focusing' and 'Image-based Focusing'. Under 'Laser-based Focusing', there is a 'Configure Laser Settings...' button and a 'Well to well autofocus' dropdown set to 'Focus on well bottom'. The 'Image-based Focusing' section includes an 'Algorithm' dropdown set to 'Standard', a 'Binning' value of 2, a checkbox for 'Custom exposure times' (unchecked), and a checkbox for 'Allow image-based focusing for recovery from laser-based well bottom failures' (unchecked). Below these, 'Initial well for finding sample' is set to 'First well acquired' with column 'A' and row '1'. 'Number of wells to attempt initial find sample' is set to 3. The 'Site Autofocus' dropdown is open, showing options: 'All sites' (highlighted in blue), 'First site only', 'Center of well only', and 'All sites' (repeated at the bottom). A 'View Focusing Details...' button is located at the bottom right of the main panel.



Setting Up a Z Series Acquisition

12. Select the **Wavelengths** tab

- Enter the number of wavelengths or channels that 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	Number of wavelengths: <input type="text" value="2"/>
Plate- 384 Wells (16x24)	
Sites to Visit- multi-site	
Acquisition	
Autofocus	
Wavelengths	
W1 DAPI	
W2 FITC	
Z Series- 3 planes	
Display	



Setting Up a Z Series Acquisition

13. Select the **W1** tab

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

The screenshot displays the software interface for setting up a Z Series acquisition. On the left, a sidebar contains a tree view with the following items: 'Objective and Camera- 10X PF', 'Plate- 384 Wells (16x24)', 'Sites to Visit- multi-site', 'Acquisition', 'Autofocus', 'Wavelengths', 'W1 DAPI' (highlighted in bold), 'W2 FITC', 'Z Series- 3 planes', and 'Display'. The main panel on the right is titled 'Illumination setting: DAPI' and is enclosed in a red rectangular box. Below this, the 'Exposure (ms)' is set to 50, with an 'Auto Expose' button and a 'Target max intensity' of 3000. The 'Autofocus options' section includes a 'Laser with z-offset' dropdown menu set to '3' and a 'Post-laser offset (um)' input field. At the bottom, there are checkboxes for 'Calculate Offset', 'Use Z stack', and 'Custom Range', along with 'Range (um)' and 'Step (um)' input fields showing values of 138.89 and 5.56 respectively.



Setting Up a Z Series Acquisition

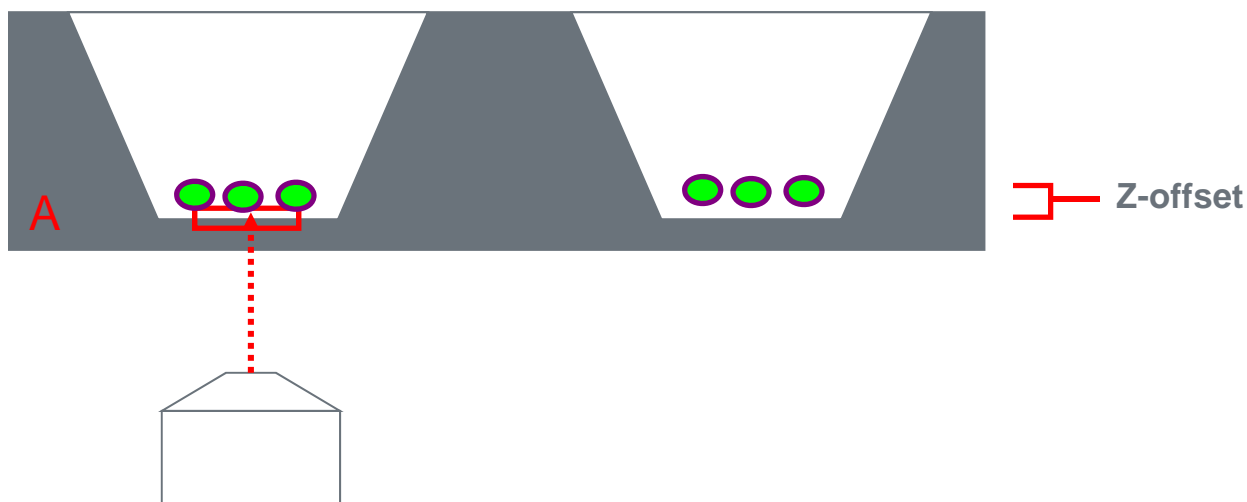
13. On the **W1** tab

- 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 to set the **Post-laser offset** to find the approximate middle of the sample

The screenshot shows the software interface for setting up a Z Series acquisition. On the left is a sidebar with a tree view containing the following items: 'Objective and Camera- 10X PF', 'Plate- 384 Wells (16x24)', 'Sites to Visit- multi-site', 'Acquisition', 'Autofocus', 'Wavelengths', 'W1 DAPI' (selected), 'W2 FITC', 'Z Series- 3 planes', and 'Display'. The main panel is titled 'Illumination setting: DAPI'. Below this are 'Exposure (ms): 50' and 'Auto Expose' button, and 'Target max intensity: 3000'. The 'Autofocus options' section contains a 'Laser with z-offset' dropdown set to '3' and a 'Post-laser offset (um)' input field. At the bottom, a red-bordered box highlights the 'Calculate Offset' button, a left arrow button, and two checkboxes: 'Use Z stack' and 'Custom Range'. To the right of these are 'Range (um)' and 'Step (um)' input fields with values 138.89 and 5.56 respectively.



What is a Post- Laser Offset?




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

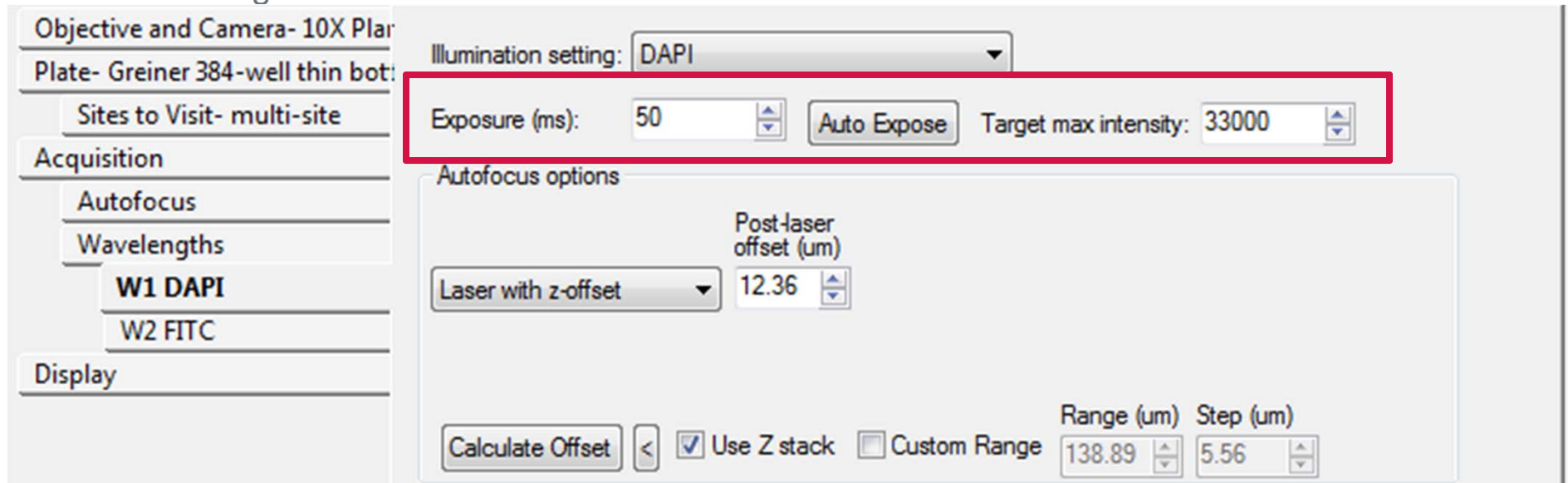
- Laser autofocus routine finds the well bottom, NOT the biological sample of interest
- You may need to empirically determine the offset (or distance) between the well bottom and the sample
- Very wavelength dependent (chromatic aberration)
- Offset can be positive or negative
- Molecular Devices recommends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



Setting Up a Z Series Acquisition

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 Plan

Plate- Greiner 384-well thin bot

Sites to Visit- multi-site

Acquisition

Autofocus

Wavelengths

W1 DAPI

W2 FITC

Display

Illumination setting: DAPI

Exposure (ms): 50 Target max intensity: 33000

Autofocus options

Laser with z-offset ☒ Use Z stack ☐ Custom Range Range (um) 138.89 Step (um) 5.56



Setting Up a Z Series Acquisition

14. On the **W1** tab

- Under **Acquisition Options**, select the appropriate option for saving 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
 - Z Series and 2D Projection Image**: Every Z Series plane as well as 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

Objective and Camera- 10X PF
Plate- 384 Wells (16x24)
Sites to Visit- multi-site
Acquisition
Autofocus
Wavelengths
W1 DAPI
W2 FITC
Z Series- 3 planes
Display

Illumination setting: DAPI
Exposure (ms): 50 Auto Expose Target max intensity: 3000
Autofocus options
Post-laser offset (um)
Laser with z-offset 3
Calculate Offset < Use Z stack Custom Range Range (um) Step (um) 138.89 5.56
Acquisition Options
Z Series: 2D Projection Image Only
Single Plane
2D Projection Image Only
Z Series and 2D Projection Image
Digital C Shading
2D Projection Image: Best Focus
Reduce noise >> 0.200

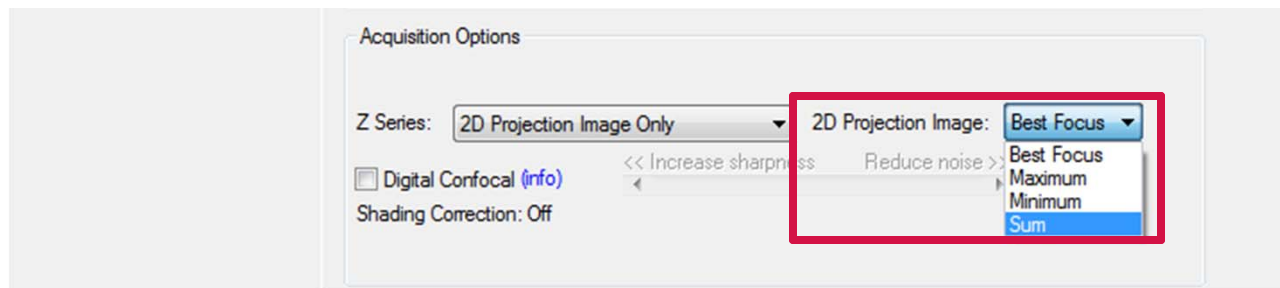


Setting Up a Z Series Acquisition

15. On the **W1** tab

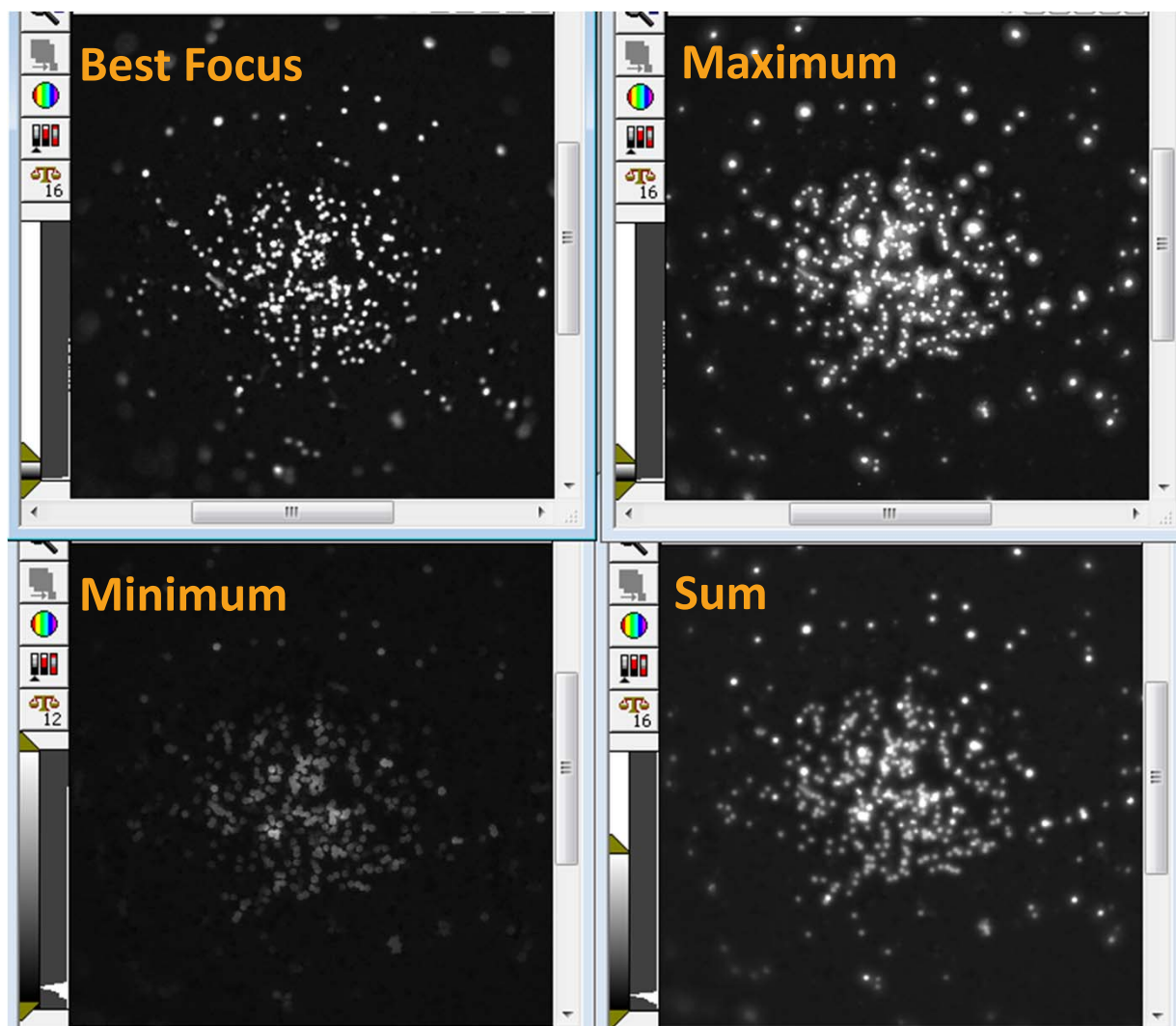
- Under **Acquisition Options**, select the appropriate option for **2D Projection Image**
 - Best Focus:** estimates the regions of best focus in an image stack to within one-tenth pixel accuracy along Z. Two resolution grid sizes are used to enhance the criterion of focus through the stack (*use only for counting or scoring, 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 image to each wavelength*



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



Setting Up a Z Series Acquisition

16. 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** for Z Series: images to save and 2D projection algorithm

The screenshot shows the software interface for setting up a Z Series Acquisition. On the left is a sidebar with a tree view containing the following items: Objective and Camera- 10X PF, Plate- 384 Wells (16x24), Sites to Visit- multi-site, Acquisition, Autofocus, Wavelengths, W1 DAPI, **W2 FITC** (highlighted), Z Series- 3 planes, and Display. The main panel is divided into several sections. The 'Illumination setting' is set to 'FITC'. 'Exposure (ms)' is 100, with an 'Auto Expose' button and 'Target max intensity' of 33000. The 'Autofocus options' section shows 'Z-offset from W1' selected with an 'Offset (um)' of -2. Below this are buttons for 'Calculate Offset', '<', 'Use Z stack', and 'Custom Range', followed by 'Range (um)' of 138.89 and 'Step (um)' of 5.56. The 'Acquisition Options' section shows 'Z Series' set to 'Z Series and 2D Projection Image' and '2D Projection Image' set to 'Best Focus'. There is a checkbox for 'Digital Confocal (info)', a slider for 'Increase sharpness' and 'Reduce noise' with a value of 0.200, and 'Shading Correction' is set to 'Off'.



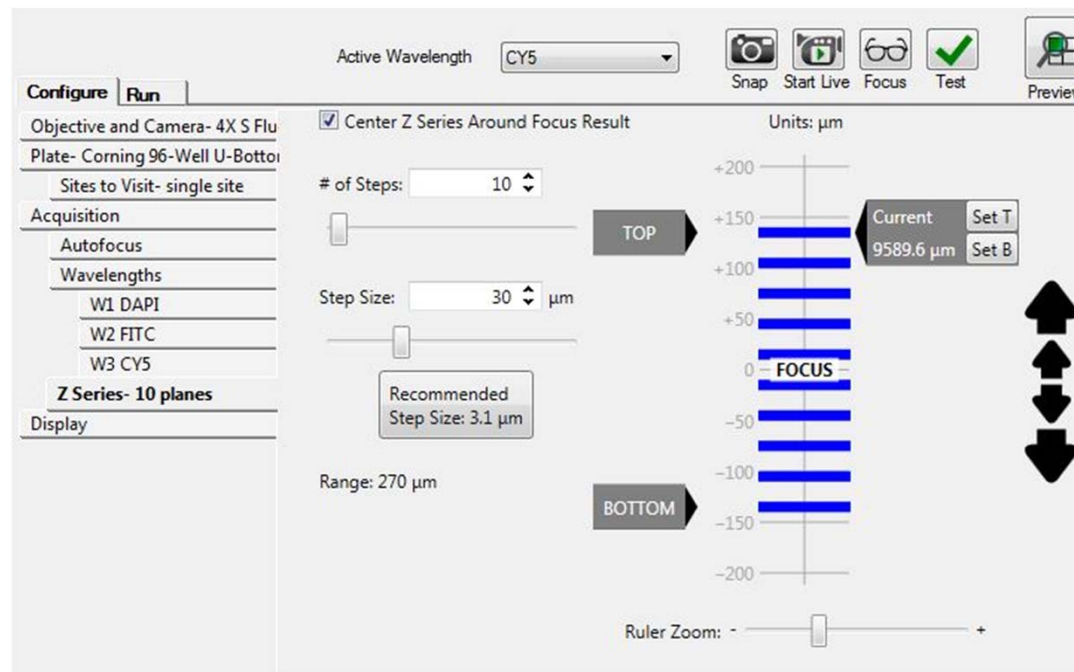
24

For research use only. Not for use in diagnostic procedures.

Setting Up a Z Series Acquisition

17. 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 center around the post-laser offset configured on each **W** tab.
 - If unchecked, you will need to set the **Top** and **Bottom** Z positions. These Z positions are the distances (see ruler) from the post-laser focus offset



The Z Series Tab

The screenshot shows the 'Z Series' configuration window. On the left is a sidebar with tabs: 'Configure', 'Run', 'Acquisition', 'Wavelengths', 'Z Series- 10 planes', and 'Display'. The 'Configure' tab is active. The main area displays settings for the Z series. At the top, 'Active Wavelength' is set to 'CY5'. Below it, the checkbox 'Center Z Series Around Focus Result' is checked. The '# of Steps' is set to 10. The 'Step Size' is set to 30 μm , with a 'Recommended Step Size: 3.1 μm ' box below it. The 'Range' is 270 μm . On the right, a vertical ruler shows the Z range from -200 to +200 μm . The 'Current' position is 9589.6 μm . The ruler has 'TOP' at +200 and 'BOTTOM' at -200. The 'FOCUS' point is at 0. To the right of the ruler are four arrows: two pointing up and two pointing down. At the bottom right, there are 'Set T' and 'Set B' buttons. A 'Ruler Zoom' slider is at the bottom center.

Use buttons to set Top and Bottom when **Center Z Series Around Focus Result** is unchecked. These are the Z heights that will be used at every site/well from the focus result

Displays current Z position after clicking on the **Focus**, **Test**, or **Preview** buttons

Use slider bars to adjust or manually enter values

Click these icons to move Z stage based on Step Size

Click here to set step size to recommended value based on objective properties

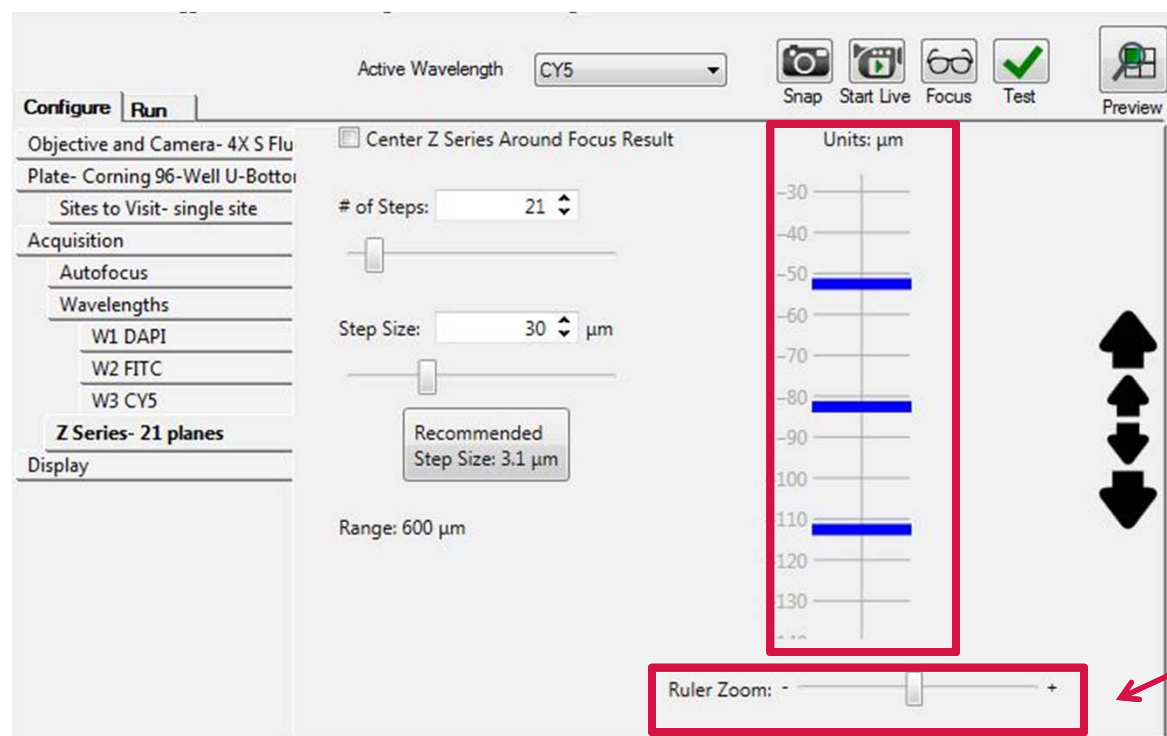
Indicates range of Z series with current settings

Adjust slider to zoom in and out on ruler

Click these icons to move half the focal distance of the objective



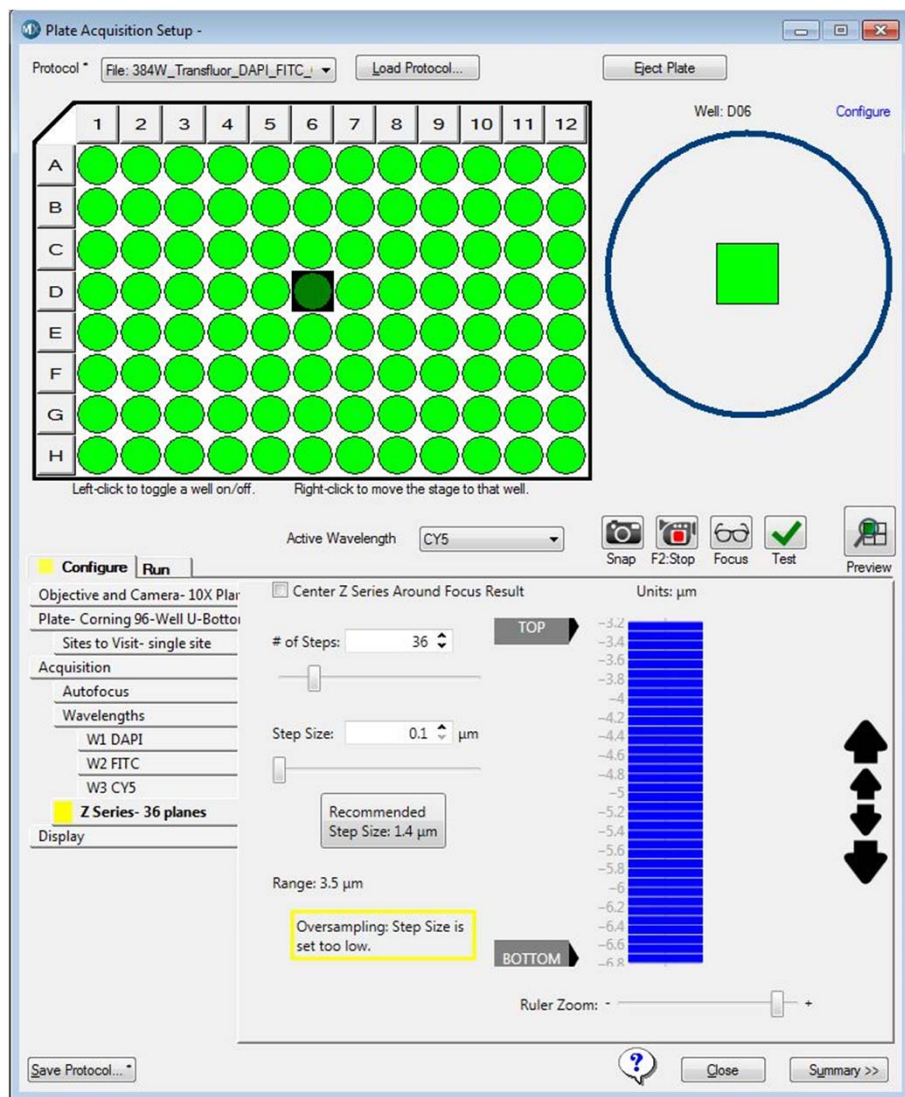
Z Series Tab – Missing Current Position Indicator



If after clicking the **Focus**, **Test**, or **Preview** buttons, the current position indicator does not appear, adjust the **Ruler Zoom** to the left



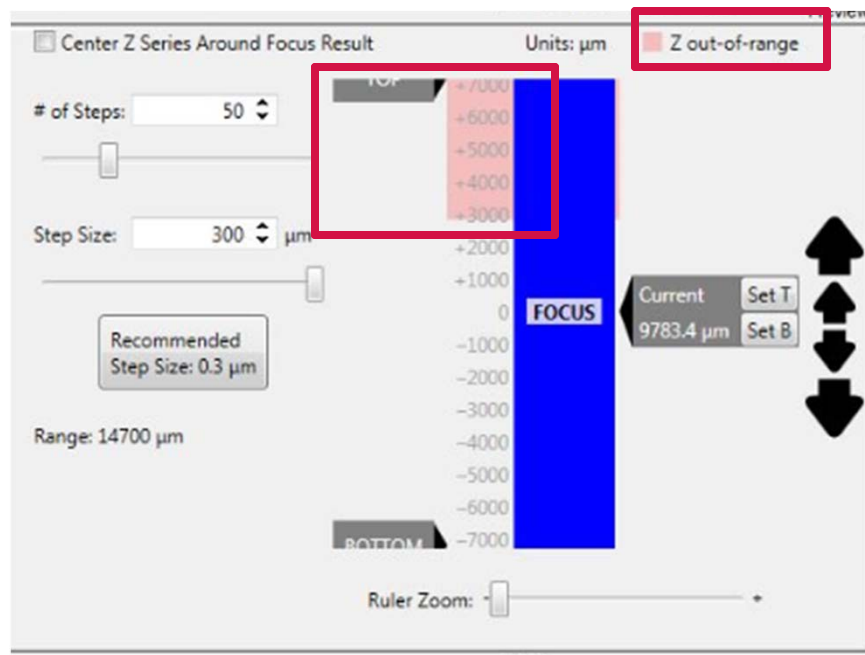
Z Series Tab – Oversampling Message



A yellow box will appear on the **Configure** and **Z Series** tabs when step size chosen is too small (oversampling) based on objective properties. Increase step size to get rid of the message.



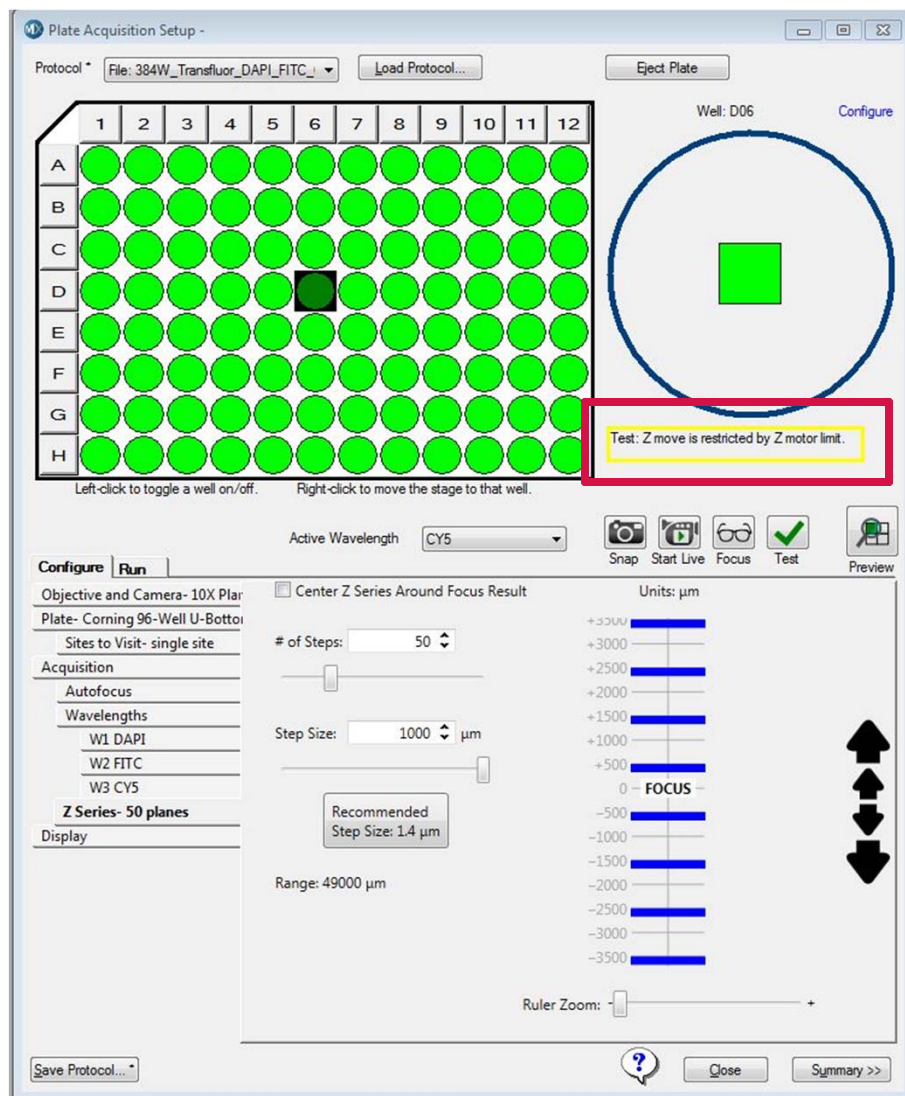
Z Series Tab – Z Out of Range



If Step Size and/or # of Steps results in the Z-series being outside of the physical range of the objective, a pink overlay will appear. Reduce the **# of steps**, adjust **Step Size** or set z-series to a position to below the pink area to get rid of the error



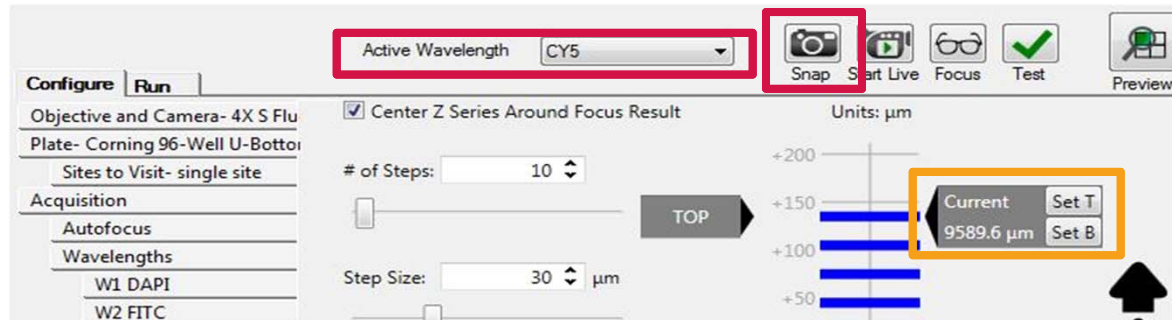
Z Series Tab – Z Motor Limit



An error message will appear if you click the **Test** button while a Z out of range condition exists. Adjust **# of steps**, **Step size** or Z top position to remove the error message.

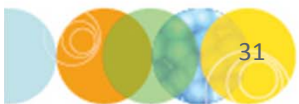


Optimizing Z Series Acquisition . . . Snap Method

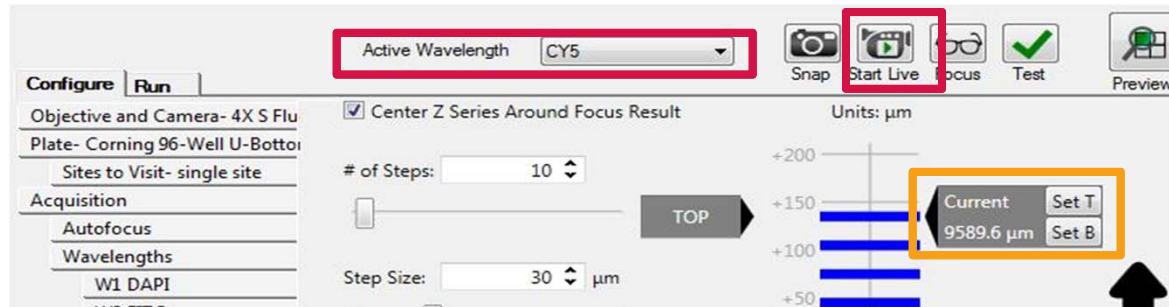


17. On the **Z Series** tab

- Click on the **Recommended Step Size** button (or adjust manually)
- Click on the **Focus** button to display the current position indicator
- To adjust the Z height:
 - Drag the current position indicator using your mouse and click **Snap** to take an image at the new Z position
 - Click the small and large arrow buttons, then click **Snap** to take an image
- Adjust **# of Steps** to cover the range desired, or
- If **Center Z Series around Focus result** is deselected, set the Top and Bottom Z positions
- Click on the **Test** button to verify settings
- Test the settings for each wavelength by changing the **active wavelength** from the drop down menu and clicking **Test**. You may also click on the **Preview button** (only 2D projection image is shown for all wavelengths).



Optimizing Z Series Acquisition . . . Live Method



17. On the **Z Series** tab

- Click on the **Recommended Step Size** button (or adjust manually)
- Click on the **Focus** button to display the current position indicator
- Click the **Start Live** button

NOTE In this method, the sample is constantly exposed to light which can lead to phototoxicity or photobleaching

- To move the Z Stage:
 - Drag the current position indicator using your mouse
 - Click the small and large arrow buttons
- Adjust **# of Steps** to cover the range desired, or
- If **Center Z Series around Focus result is deselected**, set the Top and Bottom Z positions
- Click on the **Test** button to verify settings
- Test the settings for each wavelength by changing the **active wavelength** from the drop down menu and clicking **Test**. You may also click on the **Preview** button (only 2D projection image is shown for all wavelengths).



Setting Up a Z Series Acquisition

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

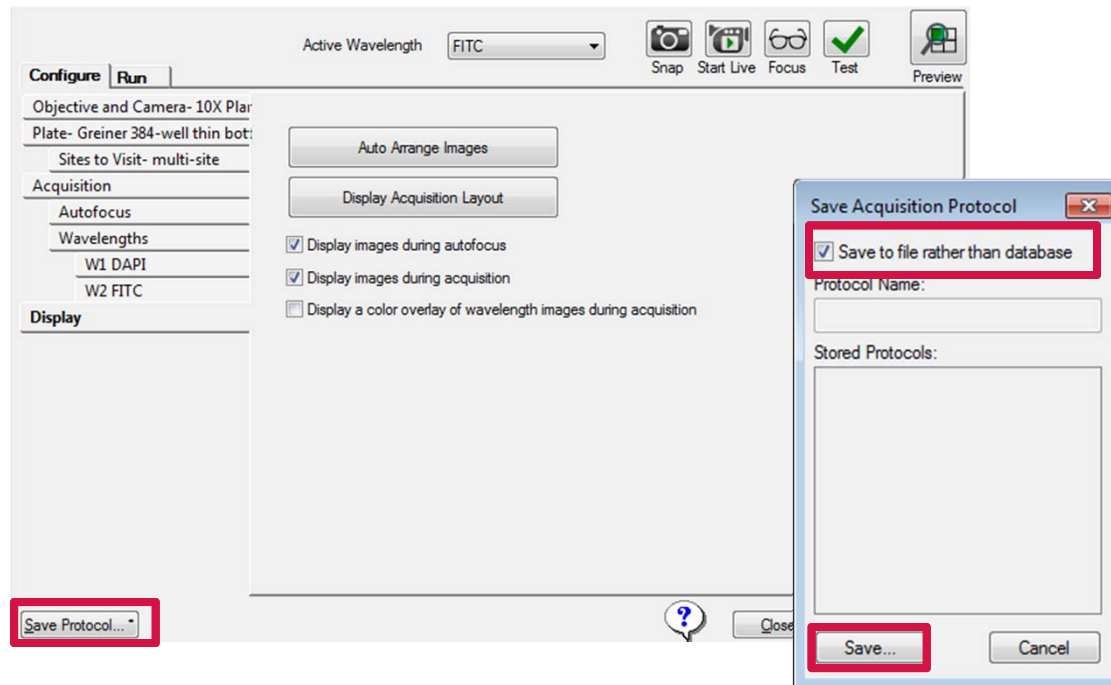
- **Auto Arrange Images:** Software automatically determines the arrangement and size of images shown in MetaXpress
- **Click on Display Acquisition Layout:** Manually configure how the images will look during acquisition (position, size, scaling, monochrome or color)
- **Display images during autofocus** 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

The screenshot shows the MetaXpress software interface. On the left is a sidebar with a tree view containing the following items: 'Objective and Camera- 10X Plan', 'Plate- Greiner 384-well thin bot', 'Sites to Visit- multi-site', 'Acquisition', 'Autofocus', 'Wavelengths', 'W1 DAPI', 'W2 FITC', and 'Display' (which is highlighted). The main panel on the right is titled 'Display' and contains two buttons at the top: 'Auto Arrange Images' and 'Display Acquisition Layout'. Below these buttons are three checkboxes: 'Display images during autofocus' (checked), 'Display images during acquisition' (checked), and 'Display a color overlay of wavelength images during acquisition' (unchecked).



Setting Up a Z Series Acquisition

19. 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 settings to a file rather than the database
 - Click on **Save** button, name the protocol, and navigate to the directory where you want to save the file (.hts)



Setting Up a Z Series Acquisition

20. 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

Active Wavelength: FITC

Buttons: Snap, Start Live, Focus, Test, Preview

Configure | **Run**

Folder Name: Transfluor | Barcode: | Description: Transfluor plate

Plate Name: Transfluor 10x

Storage Location: Local File Server

Acquire Plate

	Exposure Time (ms)	Snap	Test	Focus Offset (µm)
DAPI	Auto Expose 50			Calculate 12.36
FITC	Auto Expose 400			Calculate 2.76



Setting Up a Z Series Acquisition

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

Active Wavelength: FITC

Configure **Run**

Snap Start Live Focus Test Preview

Folder Name: Transfluor Barcode: [Empty]

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			Calculate 12.36
FITC	Auto Expose 400			Calculate 2.76

Support Resources

- F1 / HELP within MetaXpress® Software
- Support and Knowledge Base: <http://mdc.custhelp.com/>
- User Forum: <http://metamorph.moleculardevices.com/forum/>
- Request Support: <http://mdc.custhelp.com/app/ask>
- 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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