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.

Setting Up Timelapse & Z Series Acquisition

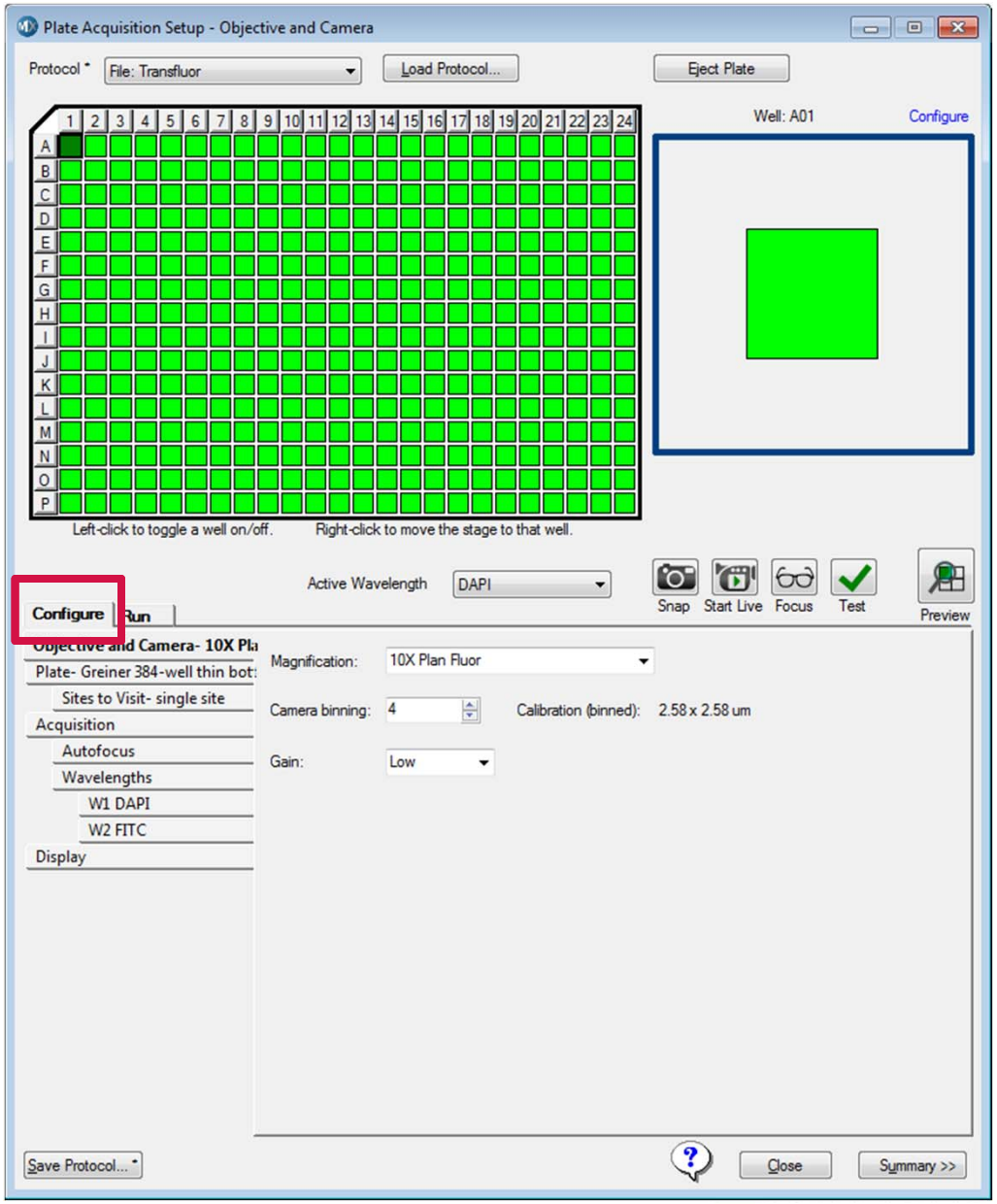
1. Open **Plate Acquisition Setup**

   - In the main toolbar click on

   OR

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

2. Select the **Configure** tab

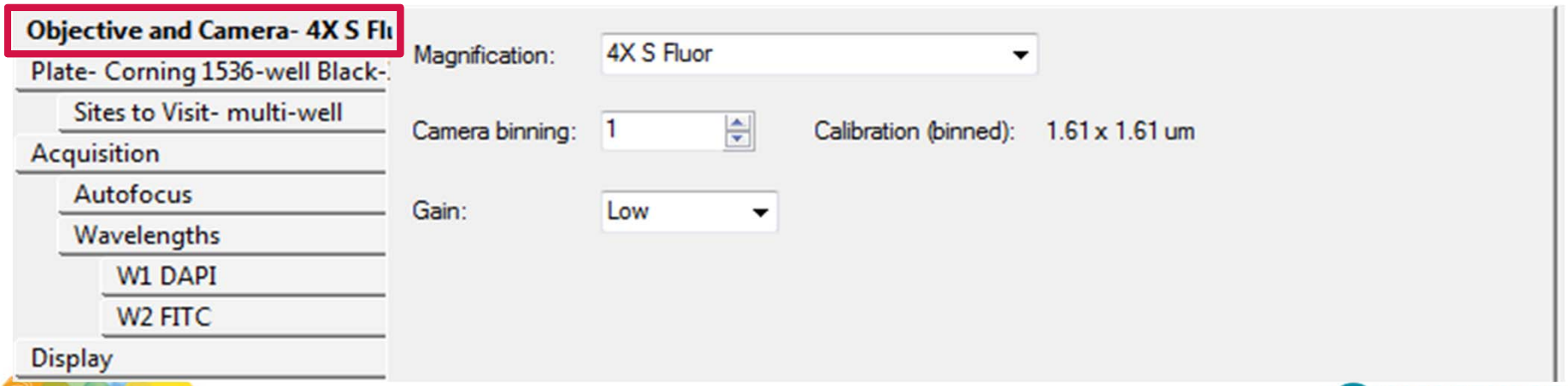


Setting Up Timelapse & Z Series Acquisition

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 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

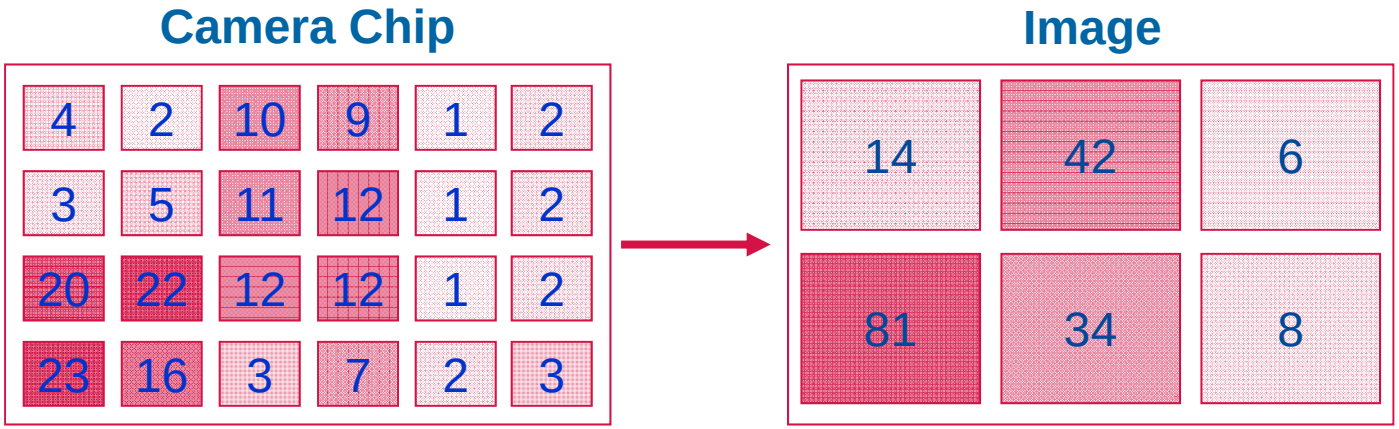
5. If the **Gain** option appears, start with gain set to **Low**



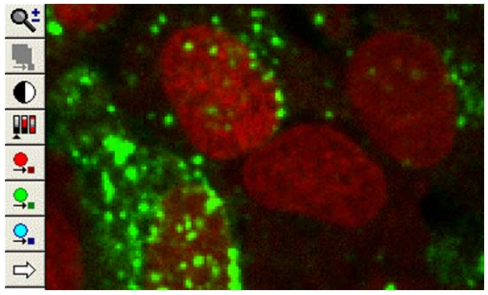
What is Binning?

Combining groups of pixels into a single pixel during image acquisition

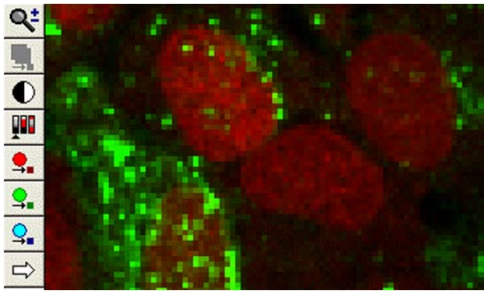
Example of 2x2 Binning



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

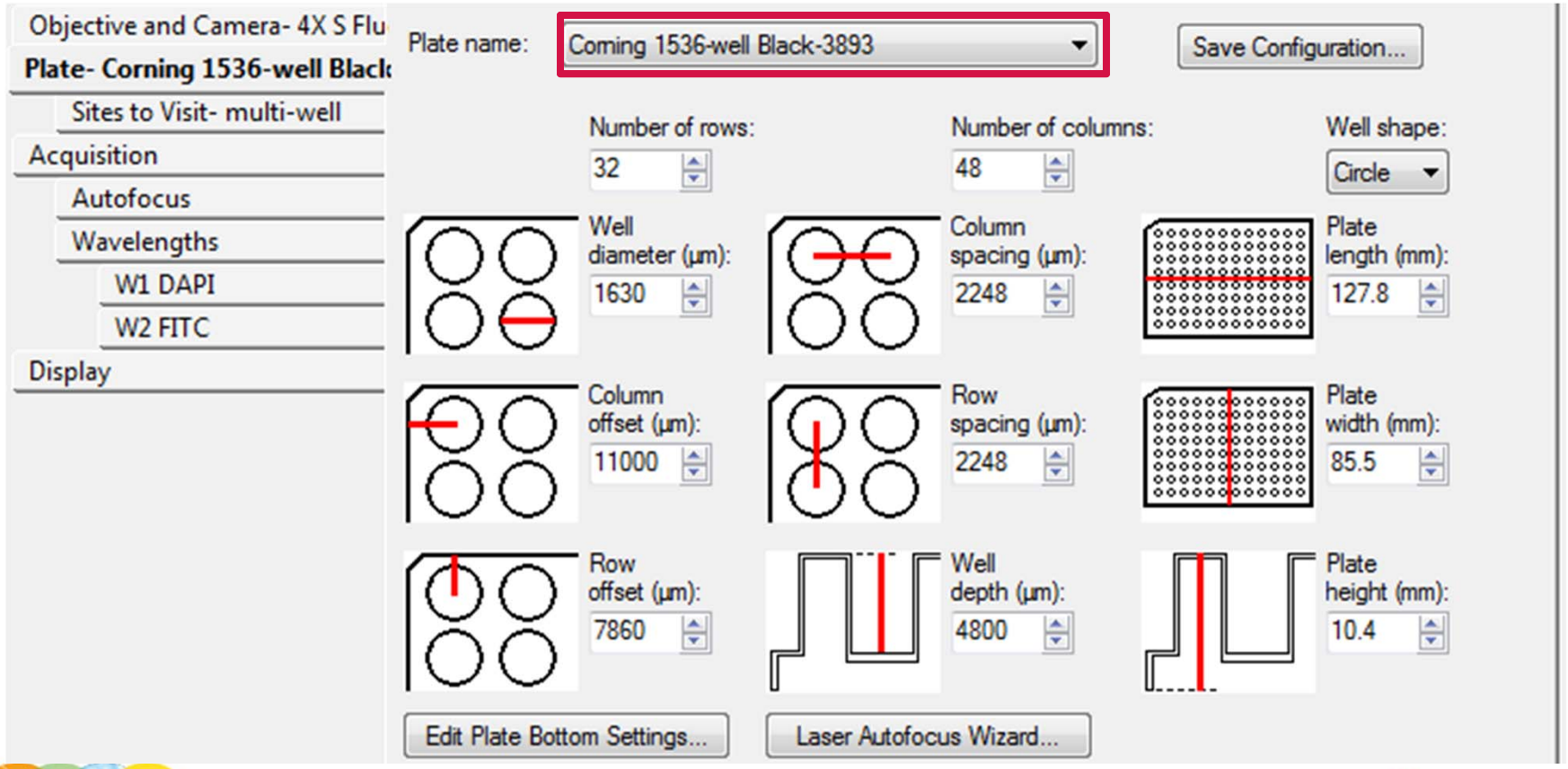
When to Bin

- 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 Timelapse & Z Series Acquisition

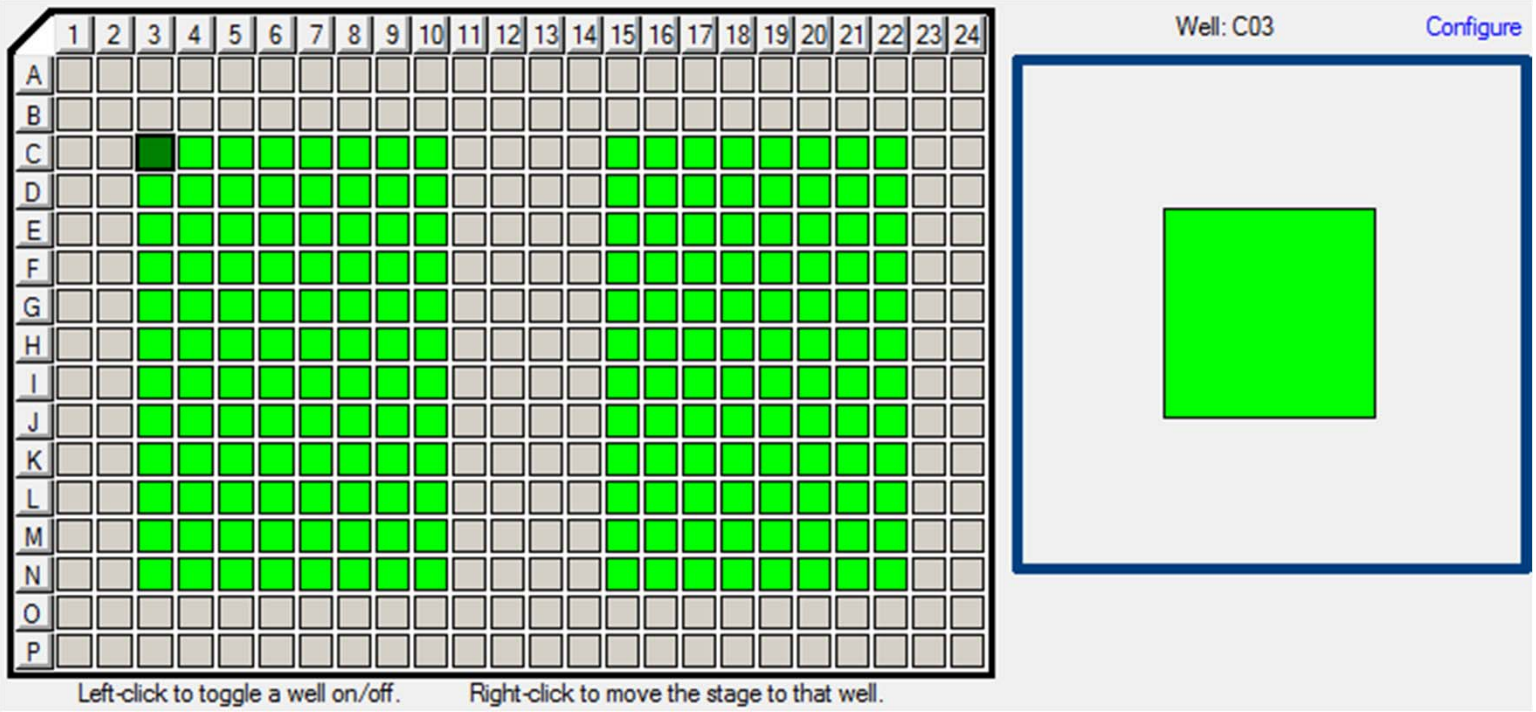
6. Select the **Plate** tab

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



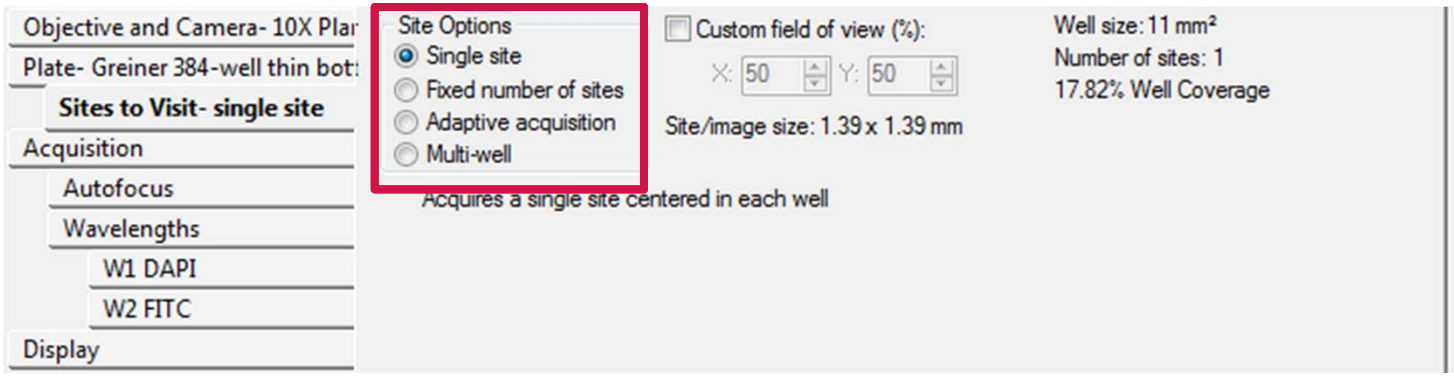
Setting Up Timelapse & Z Series Acquisition

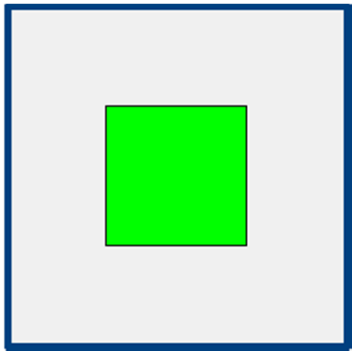
8. In the **Plate Section**, select the wells you would like to acquire
   - Left click and drag mouse to select wells; wells do not need to be contiguous
   - Click on “All” (top left corner), row letters, column numbers, or individual wells
   - Gray wells are deactivated, green wells are activated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



Setting Up Timelapse & 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 next section on setting up multiple sites

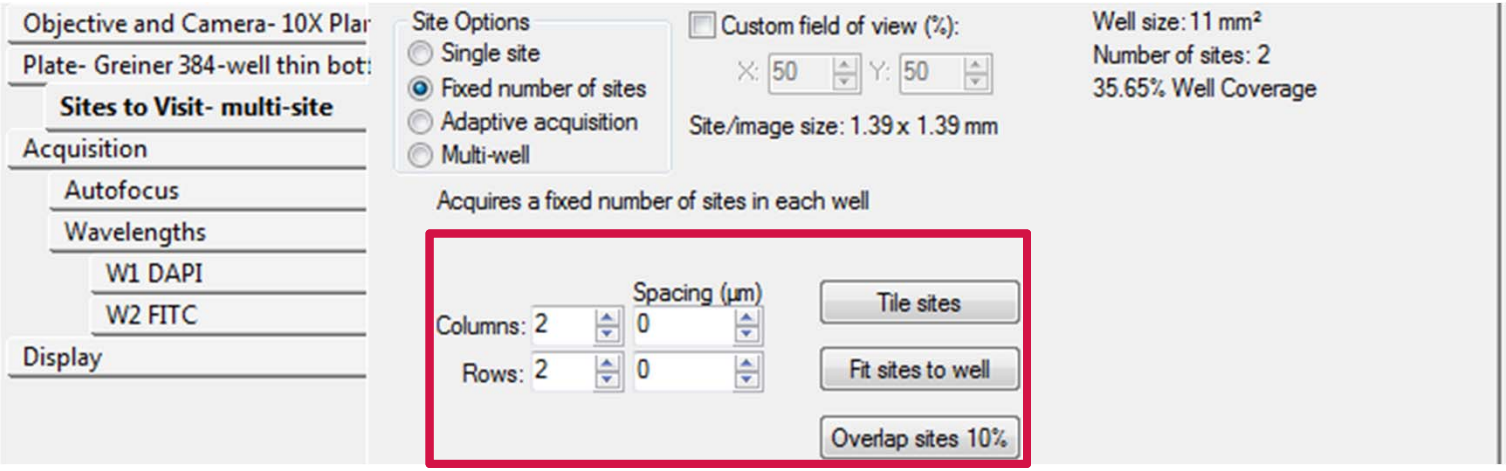




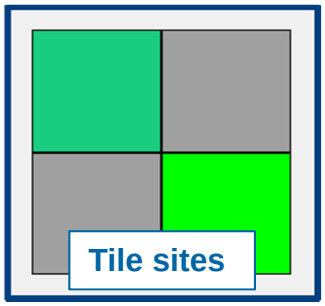
Setting Up Timelapse & Z Series Acquisition

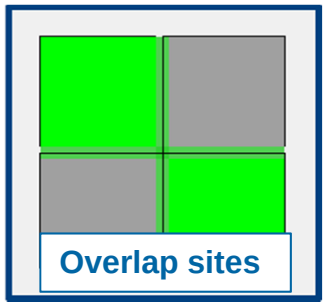
NOTE Left clicking on site selects (green) or deselects (gray) for imaging. Right click moves stage to that position (dark green)

9. On the **Sites to Visit** tab
   - 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



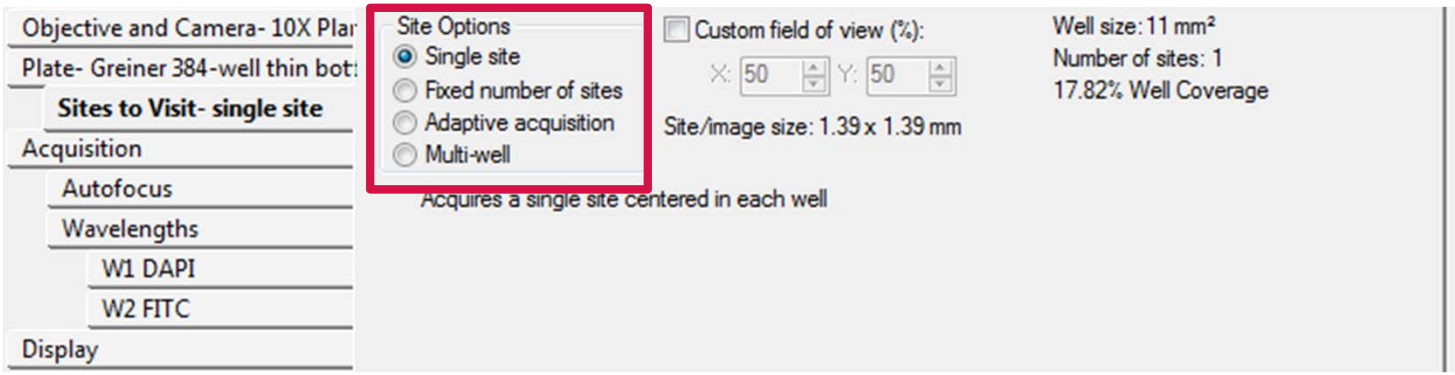
- **Tile sites** places sites edge to edge
- **Fit sites to well** spreads sites to well edge
- **Overlap sites 10%** overlaps edges of sites for stitching





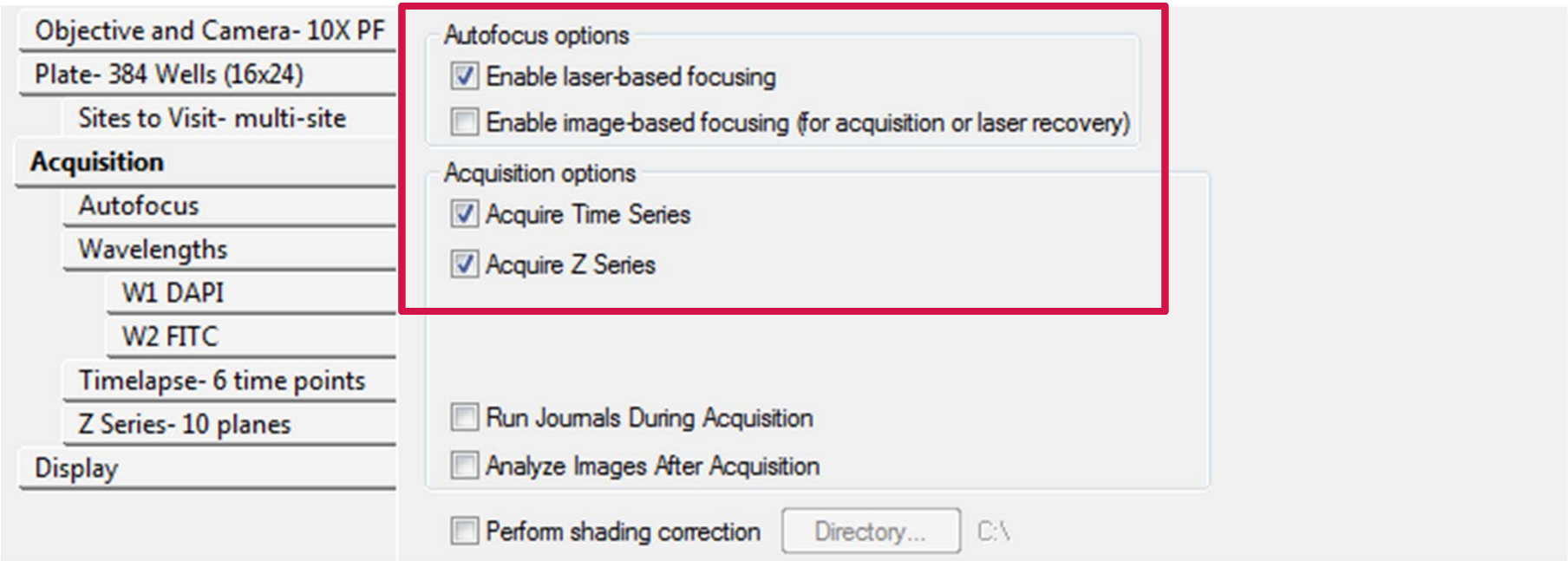
Setting Up Timelapse & Z Series Acquisition . . .

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



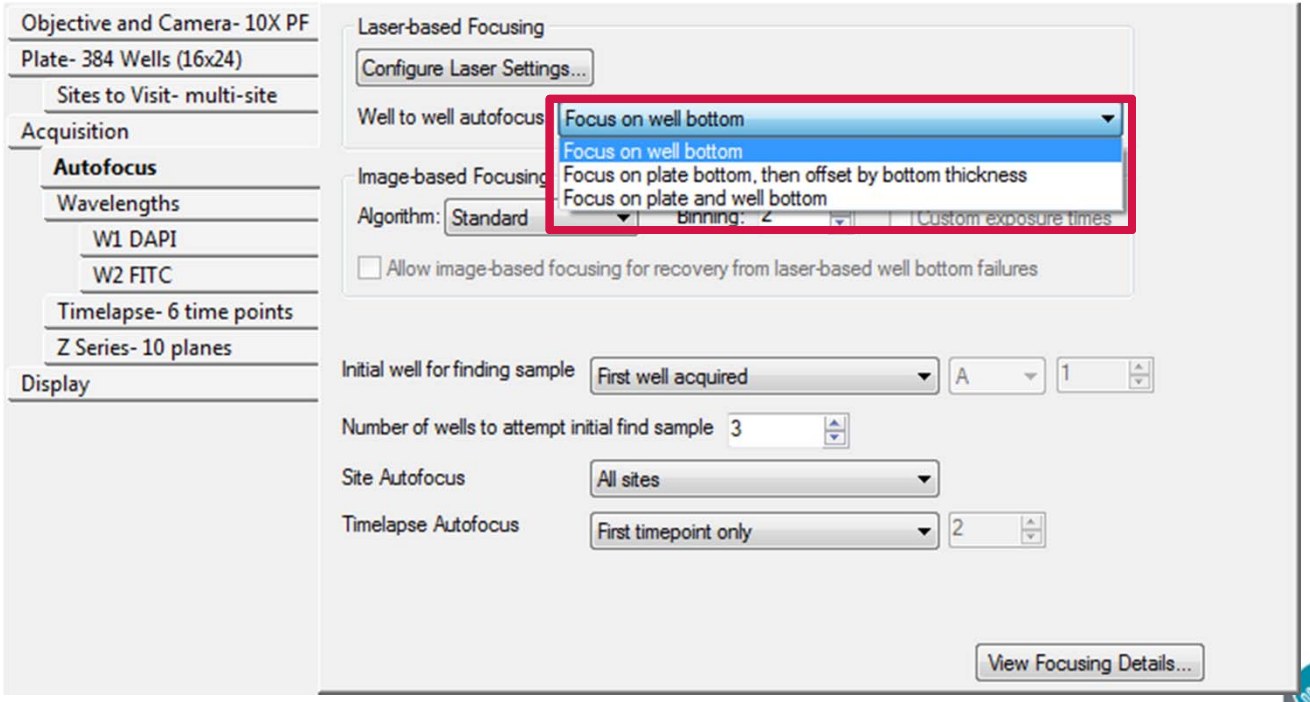
Setting Up Timelapse & Z Series Acquisition

10. Select the **Acquisition** tab
    - Always **Enable laser-based focusing**
    - For certain samples it may be necessary to **Enable image based focusing**
    - Enable **Acquire Time Series**
    - Enable **Acquire Z Series**
    - Optionally, enable **Perform shading correction**



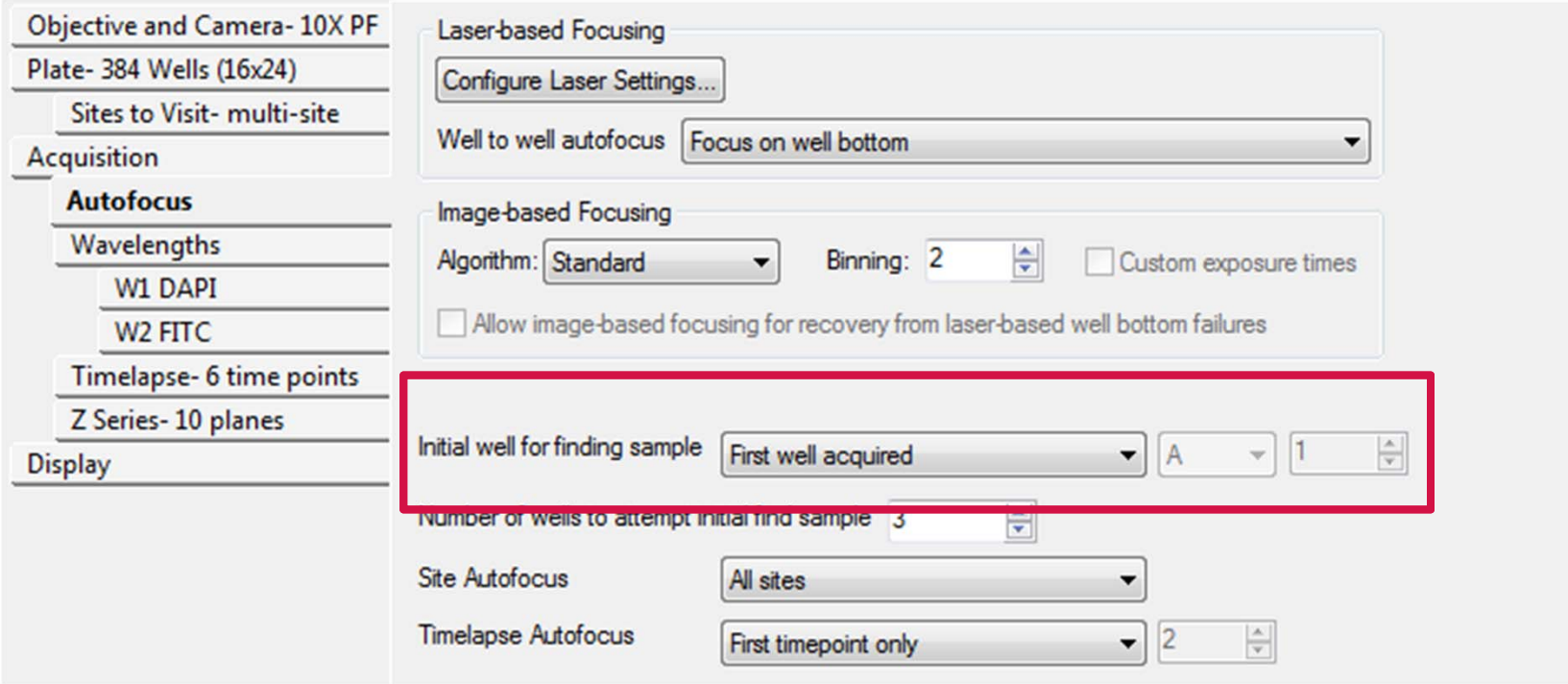
Setting Up Timelapse & Z Series Acquisition

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, 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)



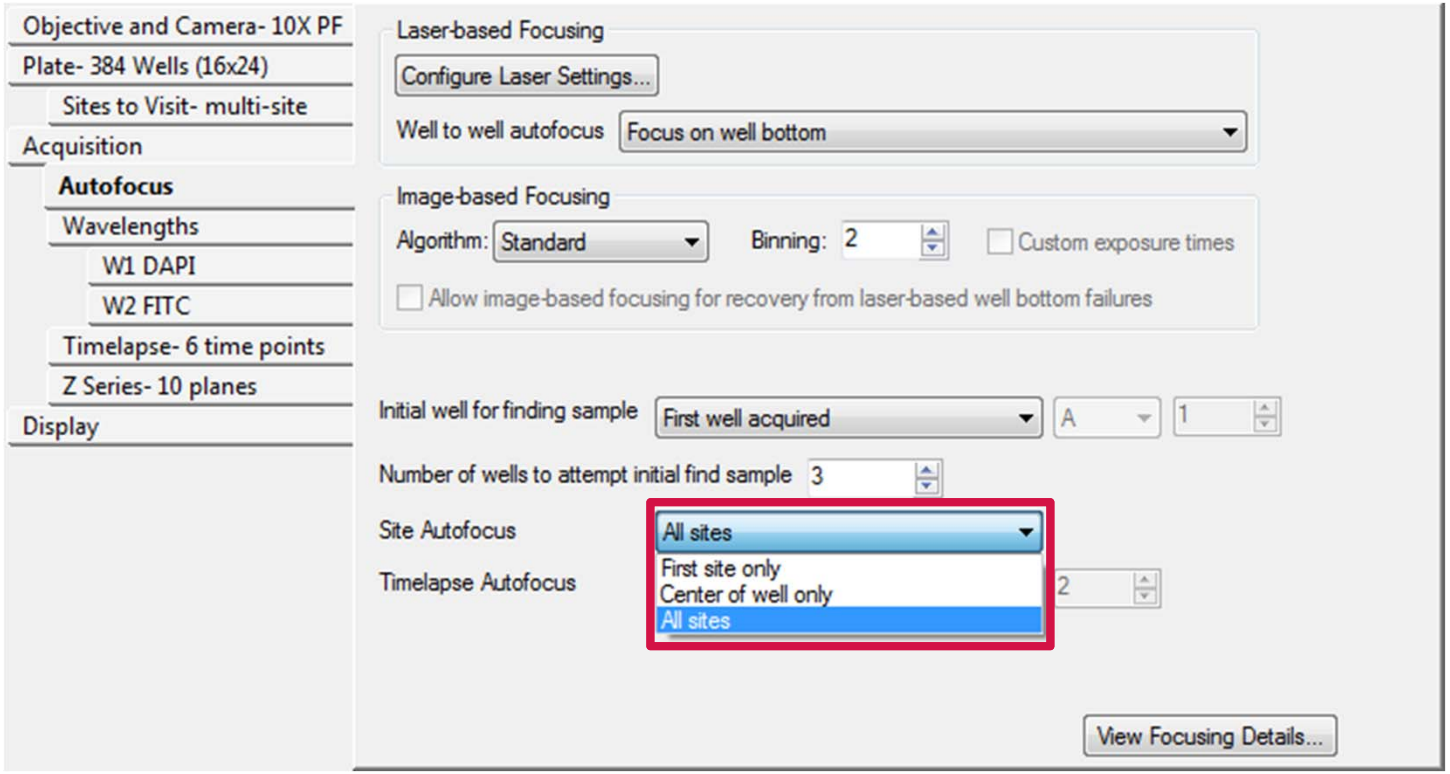
Setting Up Timelapse & Z Series Acquisition

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**



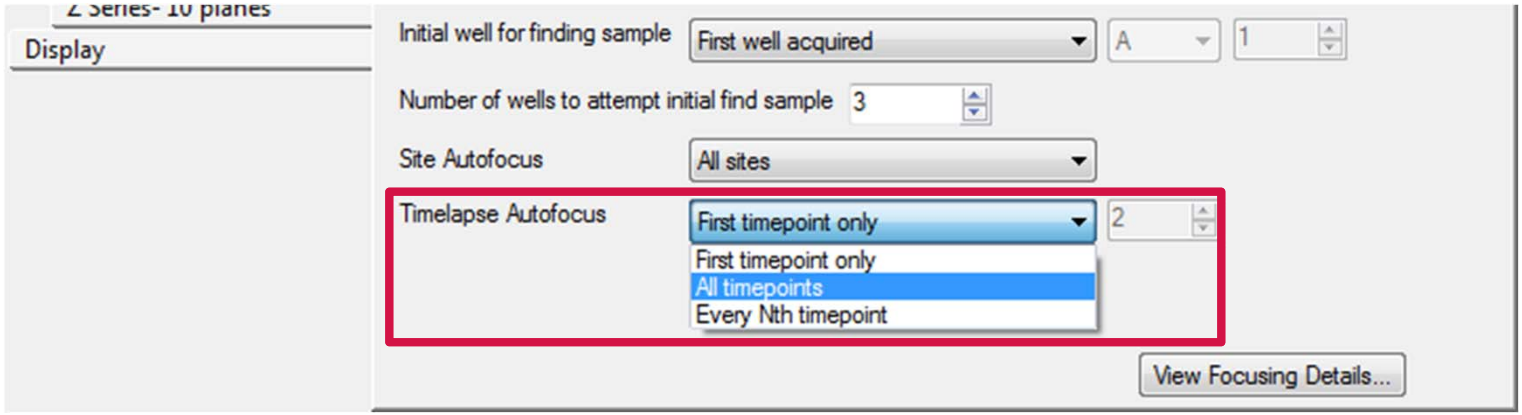
Setting Up Timelapse & Z Series Acquisition

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).



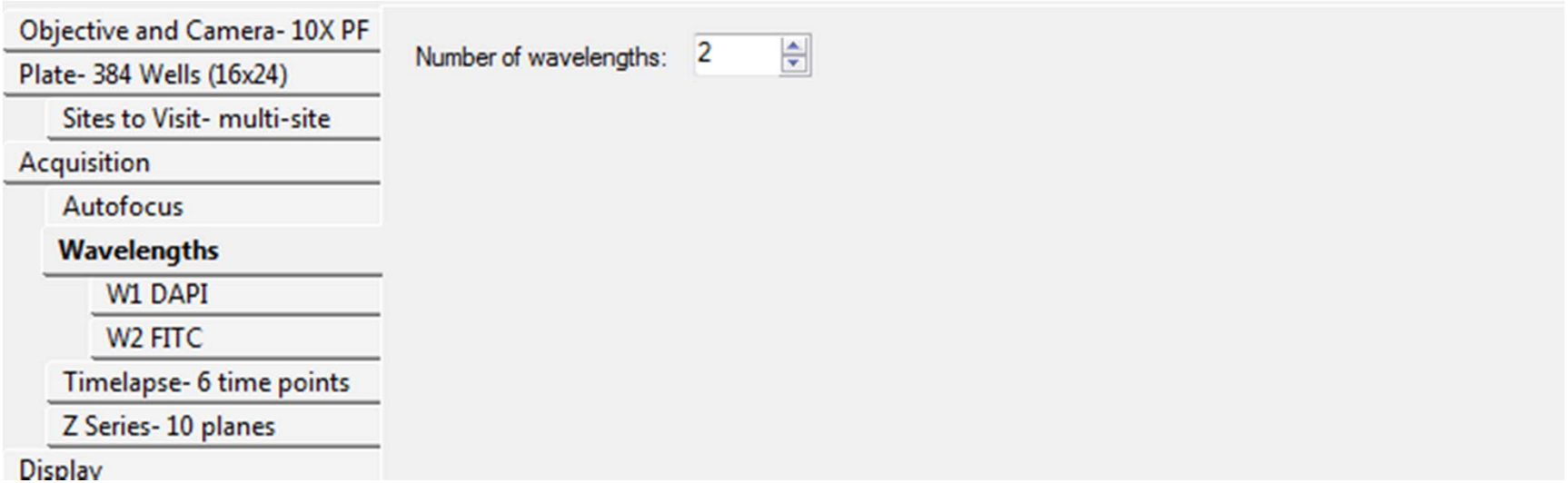
Setting Up Timelapse & Z Series Acquisition

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



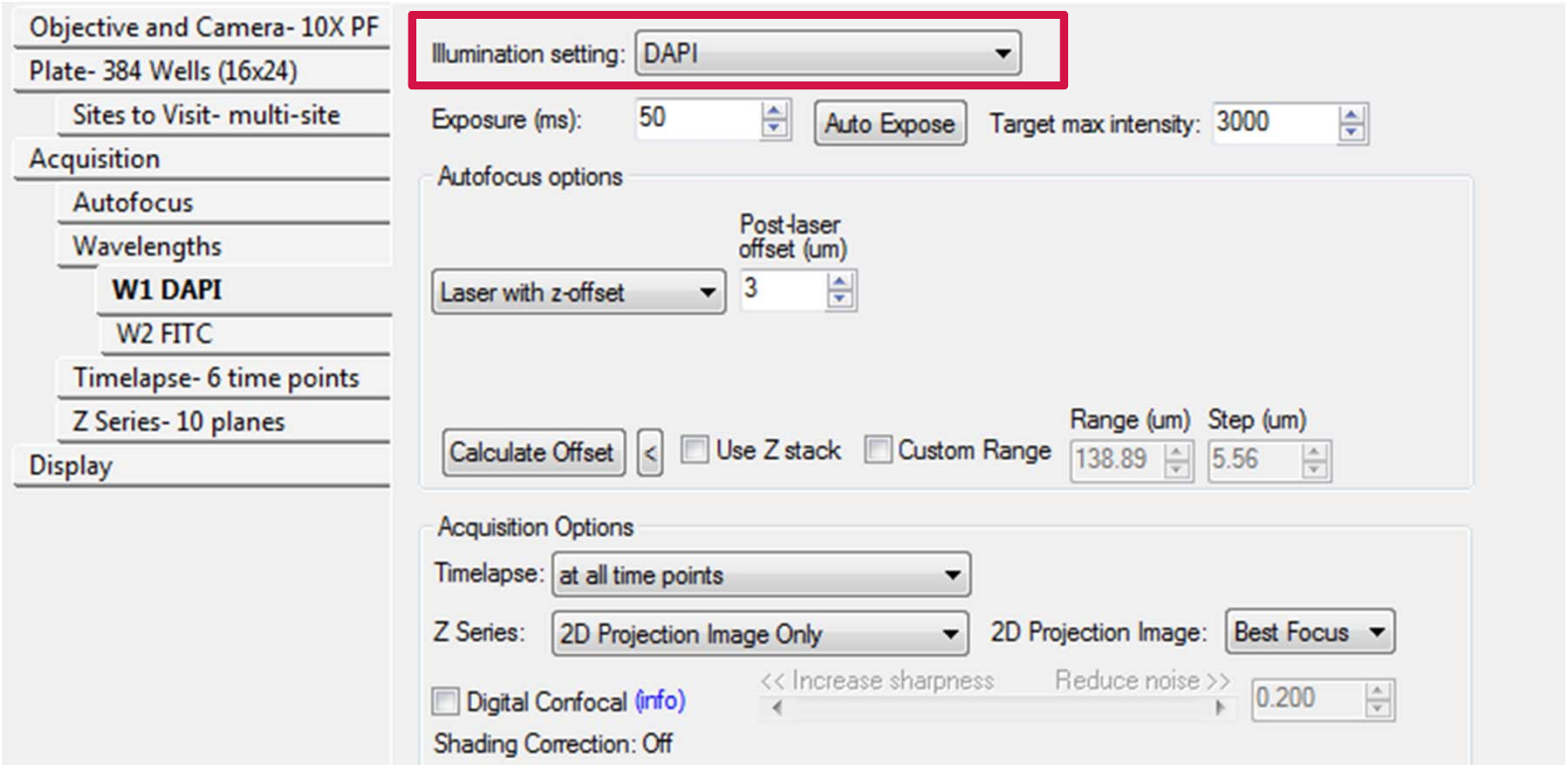
Setting Up Timelapse & Z Series Acquisition

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



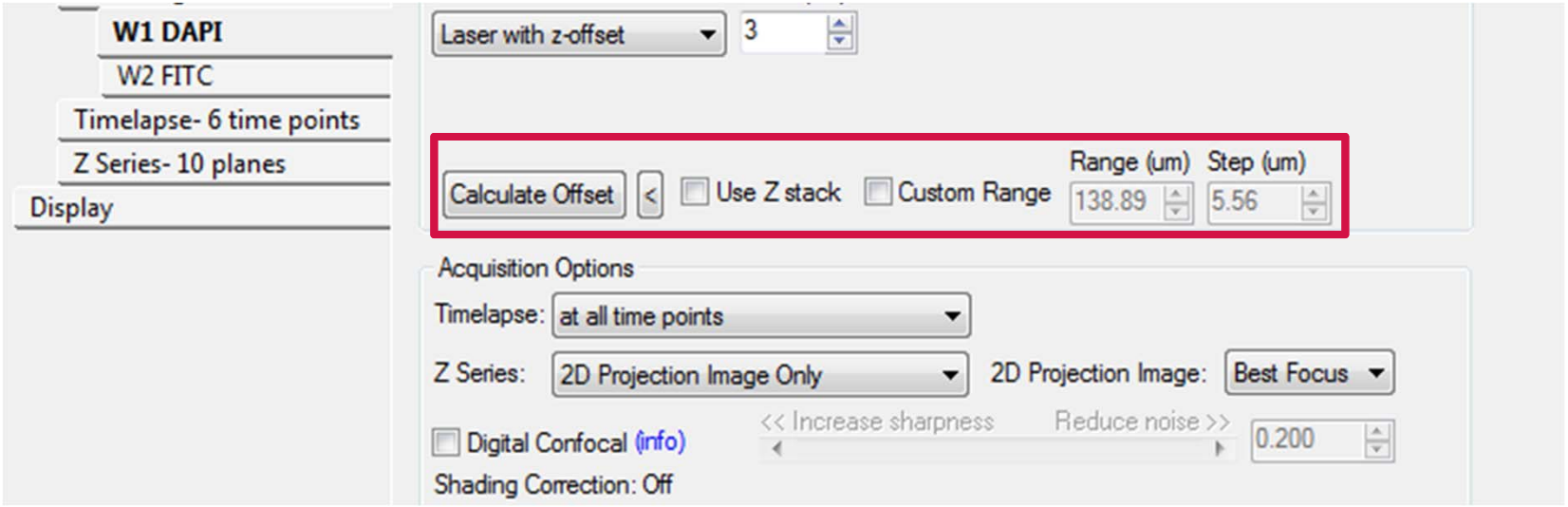
Setting Up Timelapse & Z Series Acquisition

13. Select the **W1** tab
    - Select desired **Illumination Setting** from the drop-down menu
    - Right-click to select a site/well that should contain the highest signal for the wavelength chosen in the plate map

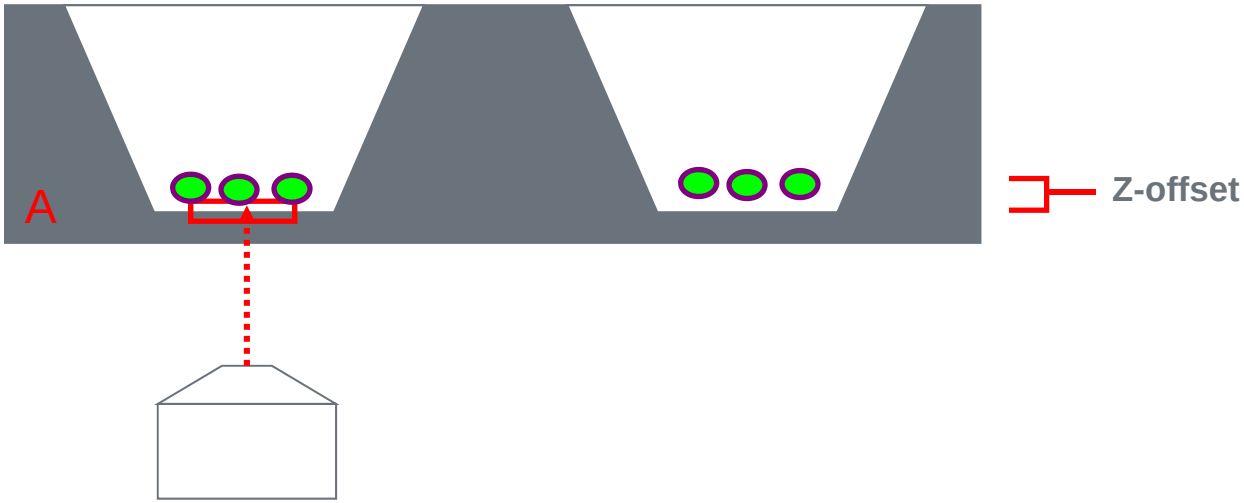


Setting Up Timelapse & Z Series Acquisition

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 setting the post-laser offset to find the approximate middle of the sample



What is a Post- Laser Offset?



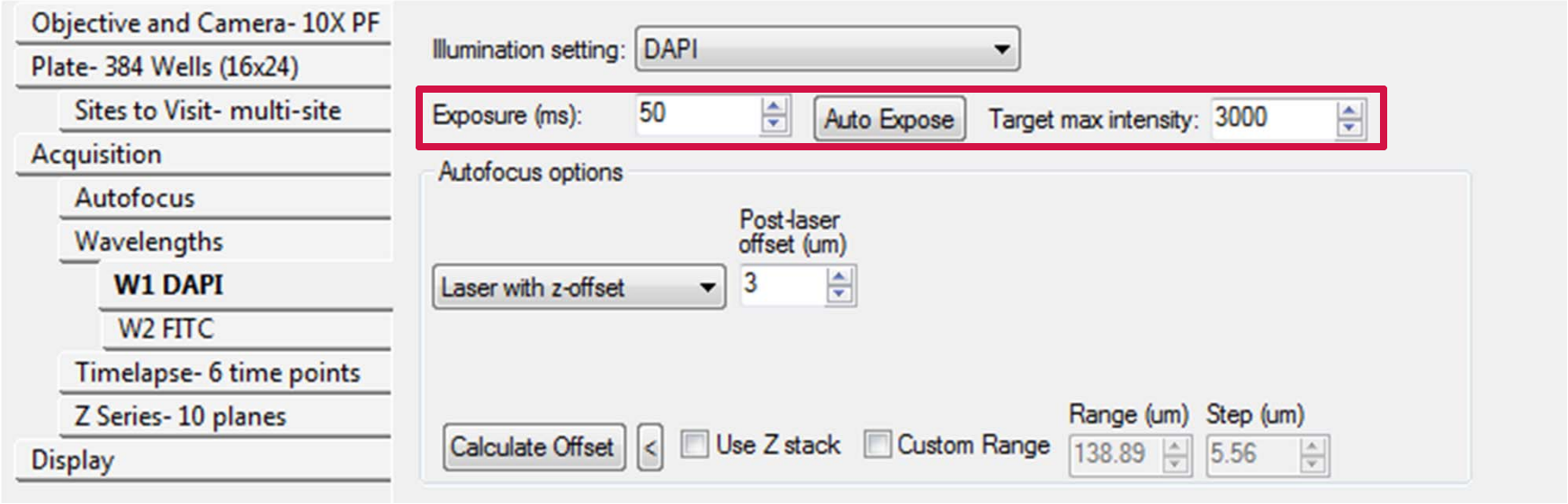
Post-laser offset is the Z distance between the bottom of the well and the sample

- 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 Timelapse & Z Series Acquisition

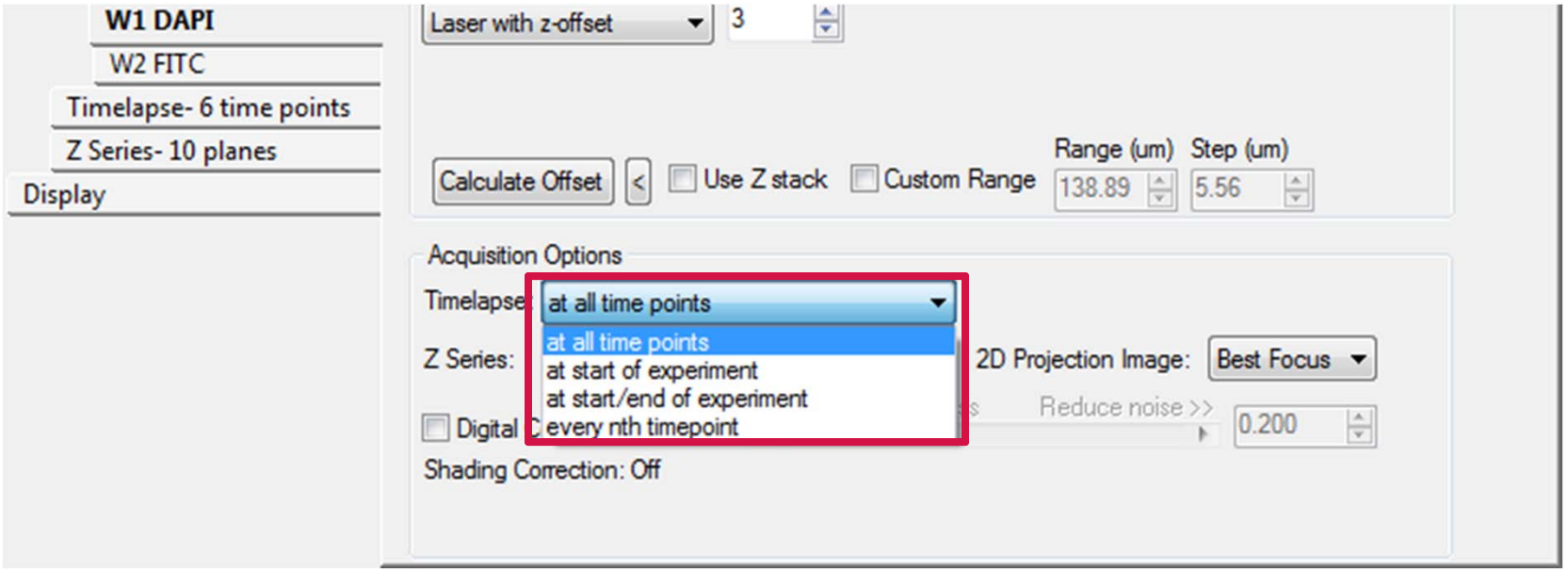
13. On the **W1** tab
    - Enter an **Exposure** time and click the **Focus** 

 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



Setting Up Timelapse & Z Series Acquisition

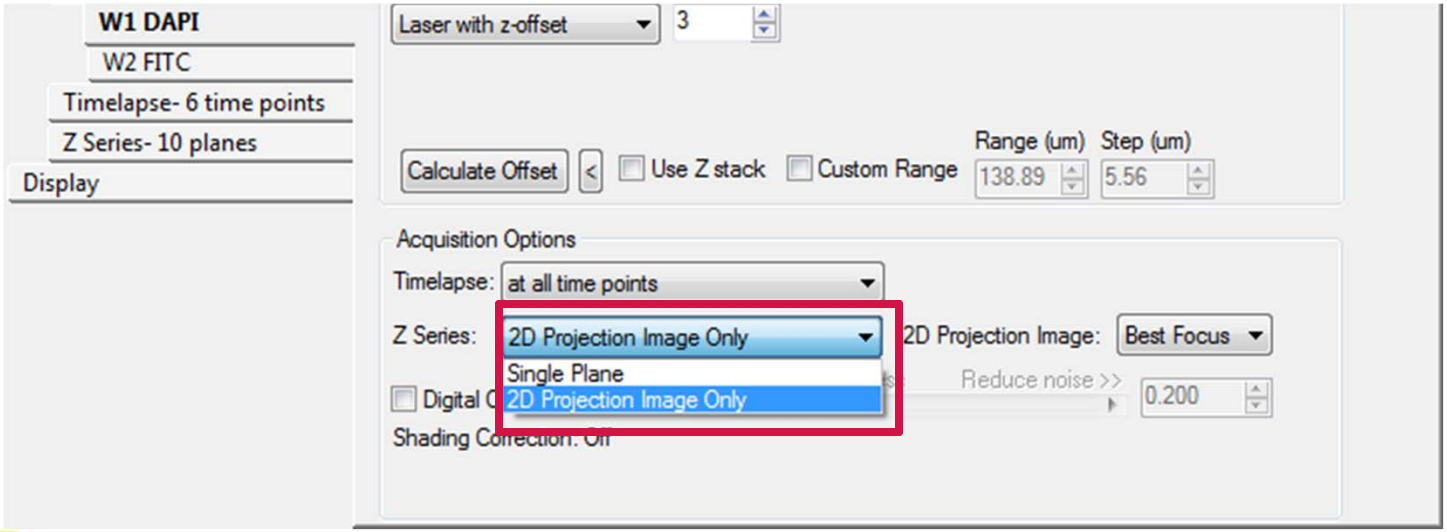
13. On the **W1** tab
    - Select the appropriate option from the **Acquisition Options** drop-down menu to specify how often to collect the selected wavelength
        - **At all time points** acquires this wavelength at each time point
        - **At start of experiment** acquires this wavelength 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



Setting Up Timelapse & Z Series Acquisition

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

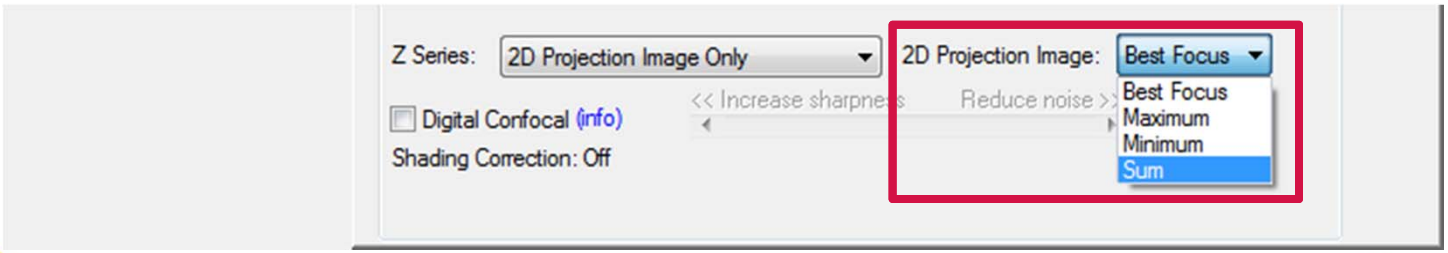
    NOTE The above options will be available on each W tab. It is not necessary to acquire and save images the same way for each wavelength



Setting Up Timelapse & Z Series Acquisition

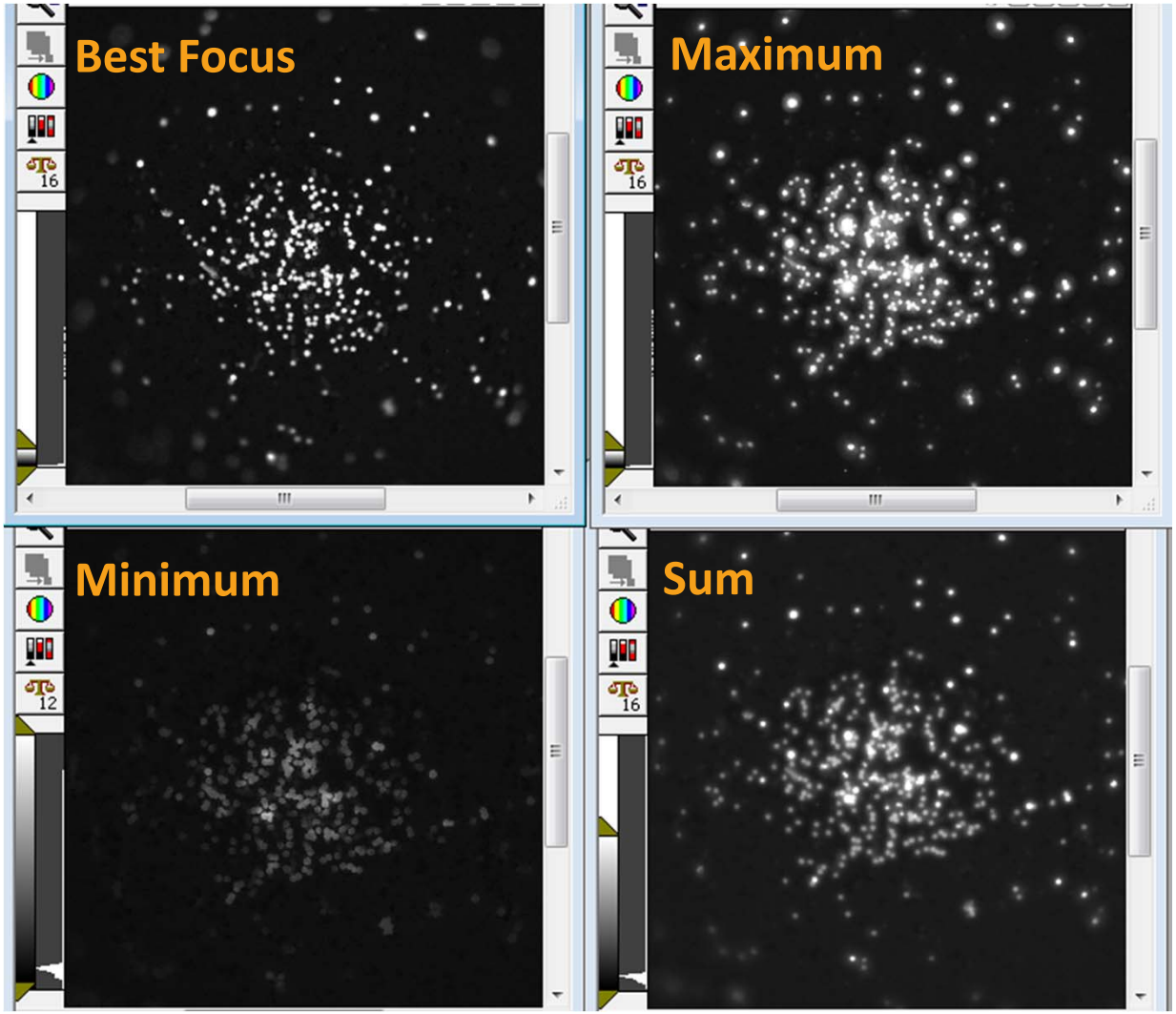
13. On the **W1** tab
    - Under **Acquisition Options**, select the appropriate option for **2D Projection Image**
      - **Best Focus**: estimates the regions of best focus in an image stack to within one-tenth pixel accuracy along Z. Two resolution grid sizes are used to enhance the criterion of 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



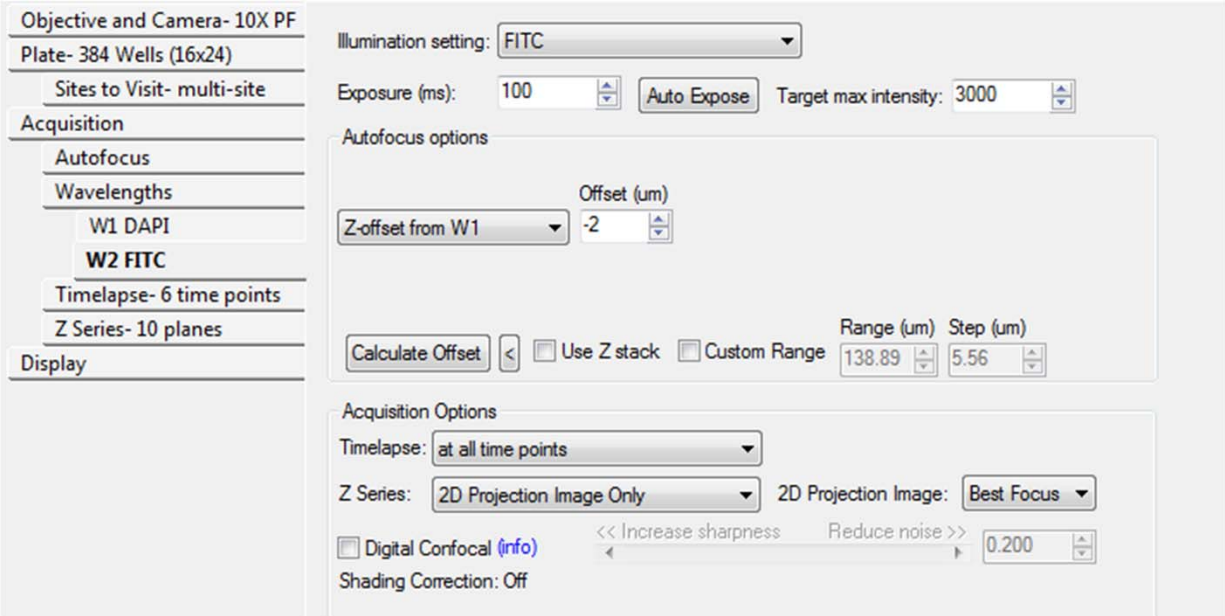
2D Projection Images

- 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 Timelapse & Z Series Acquisition

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



Setting Up Timelapse & Z Series Acquisition

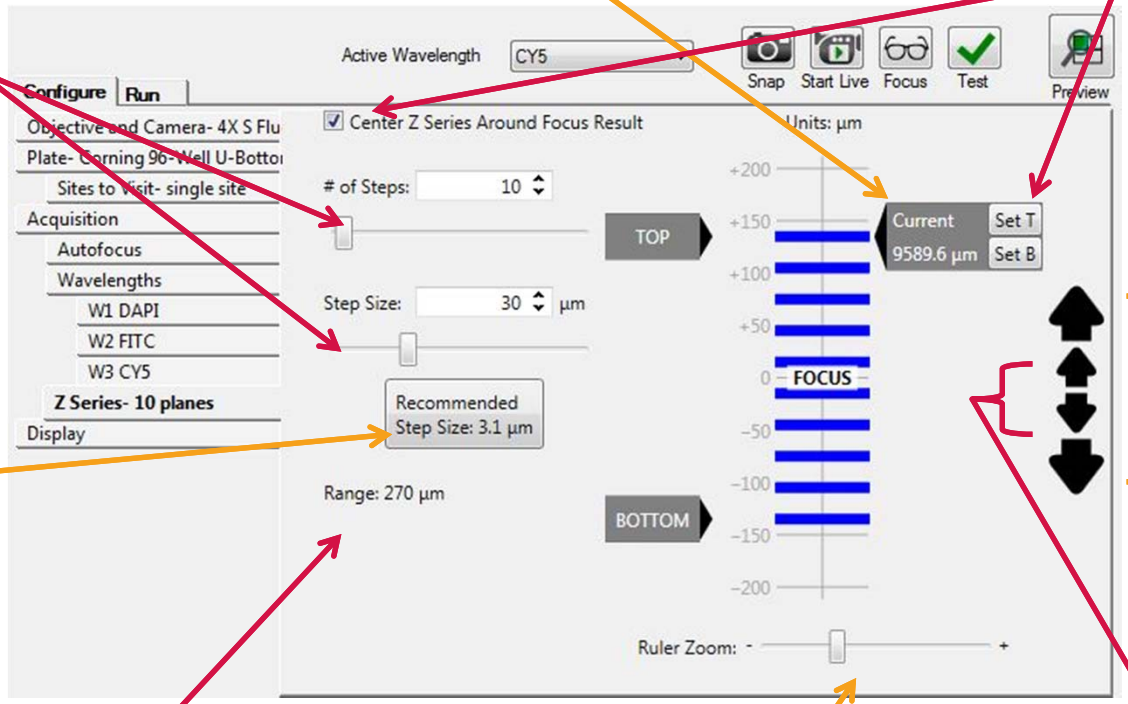
15. Select the **Z Series** tab
    - Enter **# of Steps**: # of Z steps acquired
    - Enter **Step Size**: spacing (µm) between each Z step
    - **Center Z Series Around Focus result**:
        - If checked, # of Steps and Step Size will be centered around the post-laser offset configured on the **W** 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)

The Z Series Tab

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

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

Use slider bars to adjust or manually enter values



Click on these icons to move Z stage based on Step Size

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

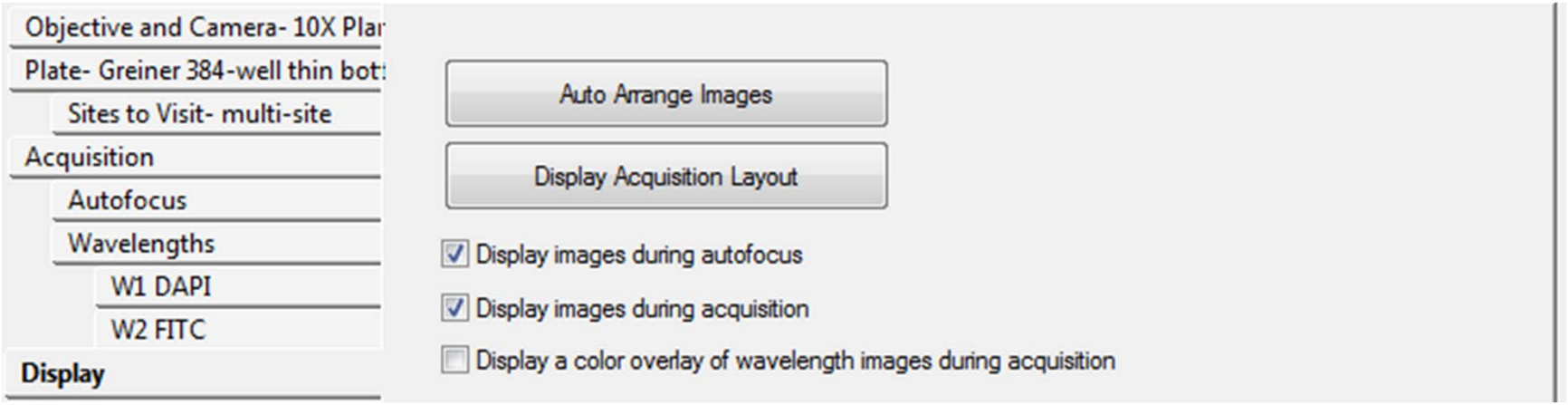
Indicates range of Z series with current settings

Adjust slider to zoom in and out on ruler

Click on these icons to move Z stage half the focal distance of the objective

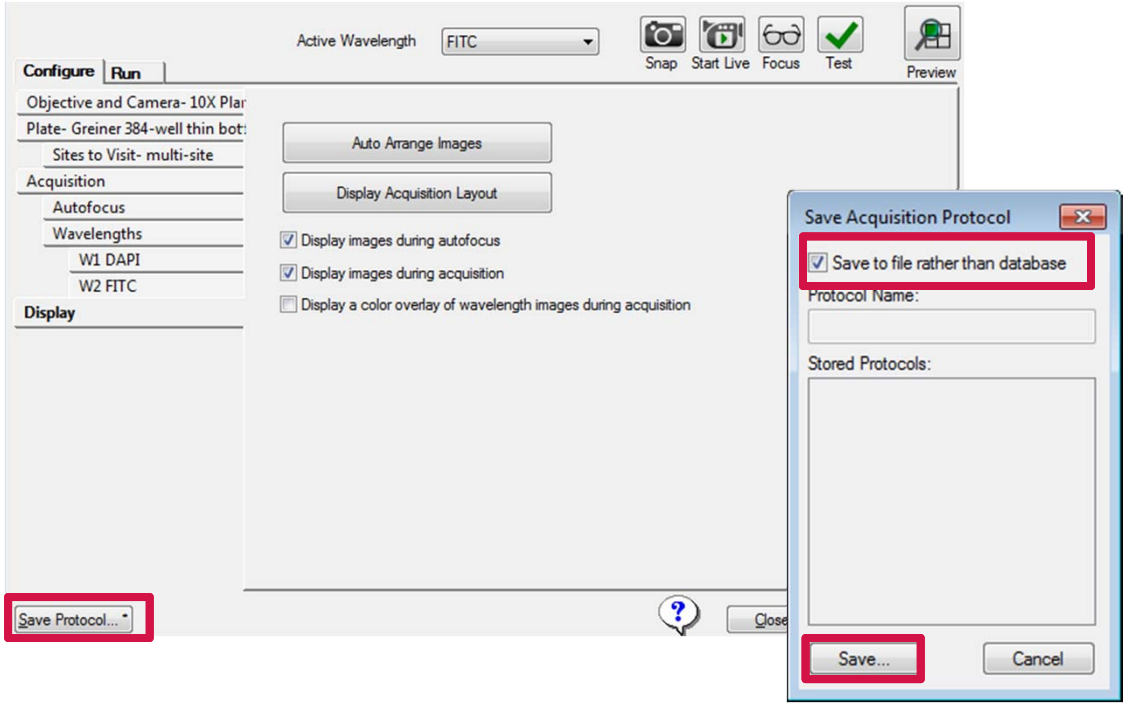
Setting Up Timelapse & Z Series Acquisition

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



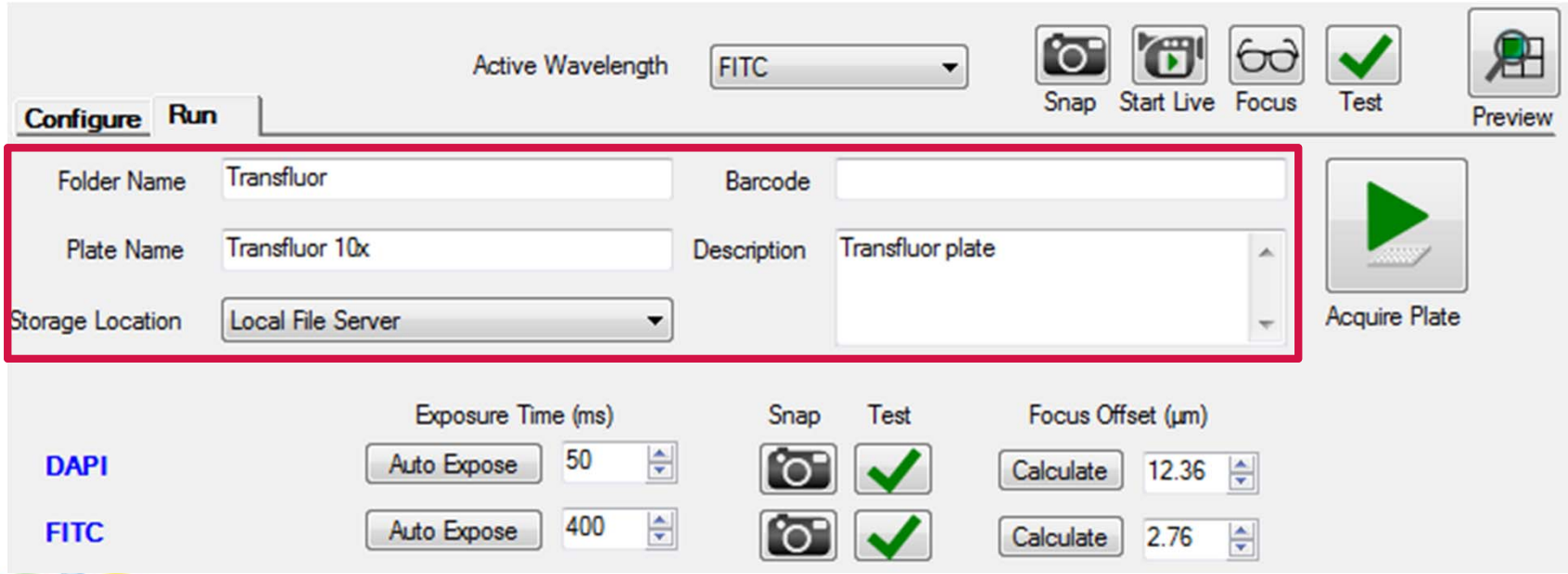
Setting Up Timelapse & Z Series Acquisition

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)



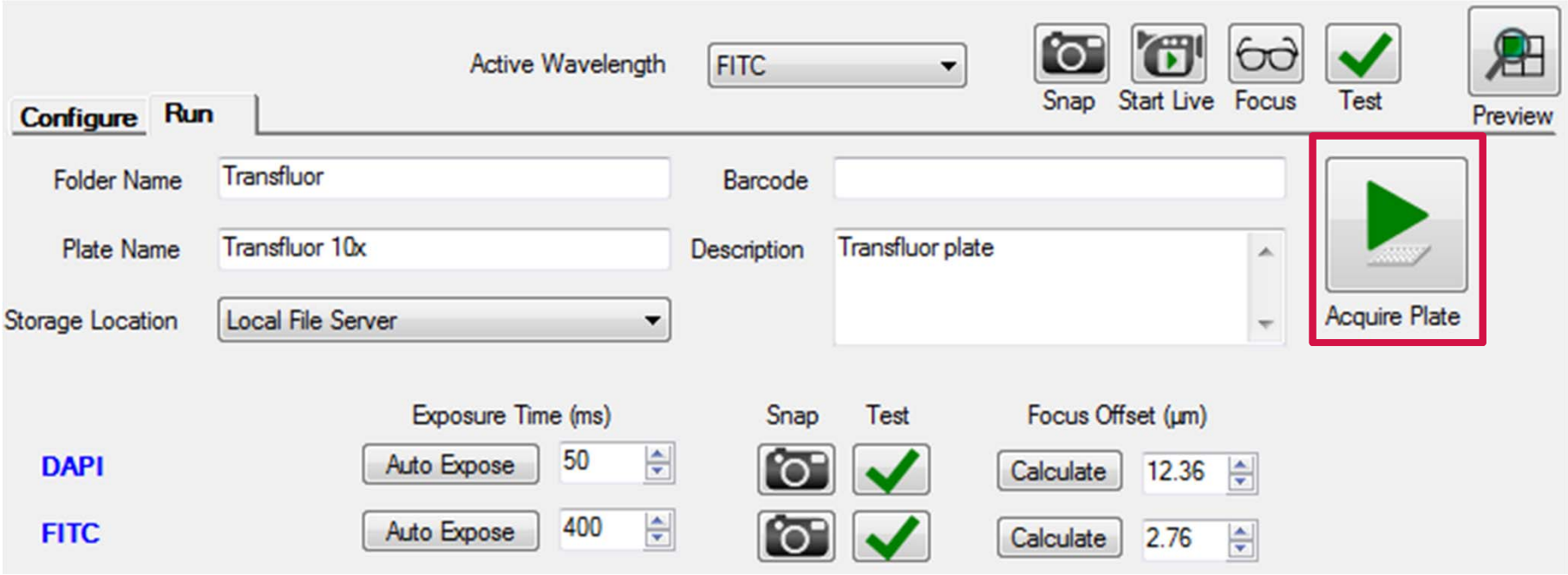
Setting Up Timelapse & Z Series Acquisition

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



Setting Up Timelapse & Z Series Acquisition

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



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