MetaXpress® 6 Software Guide

Acquiring a Plate with Timelapse Enabled (without Z Series)

Date Revised 6/08/15 Version B

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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.

Setting Up a Timelapse Acquisition

1. Open **Plate Acquisition Setup**

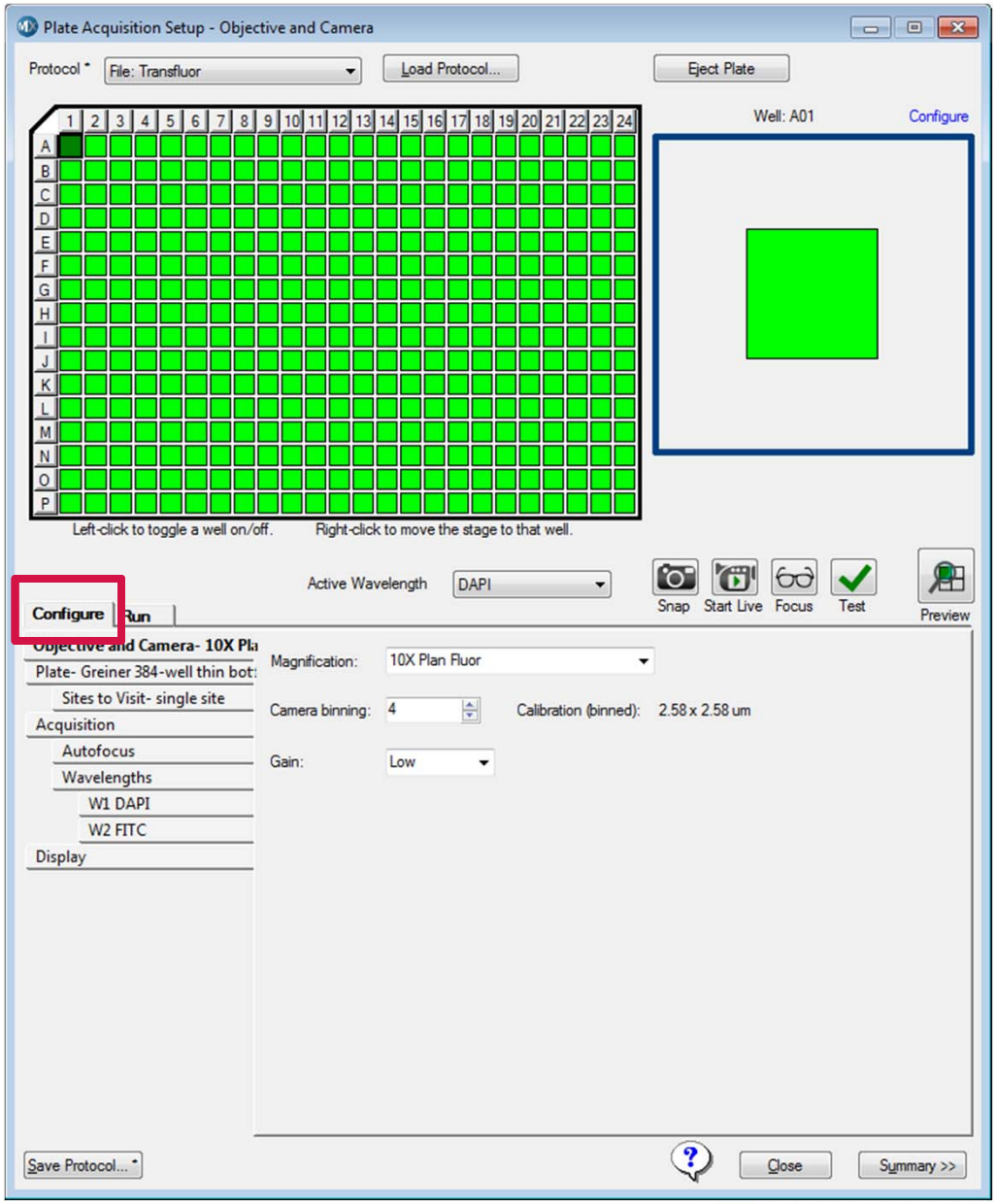
   - In the main toolbar click on

     OR

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

2. Select the **Configure** tab

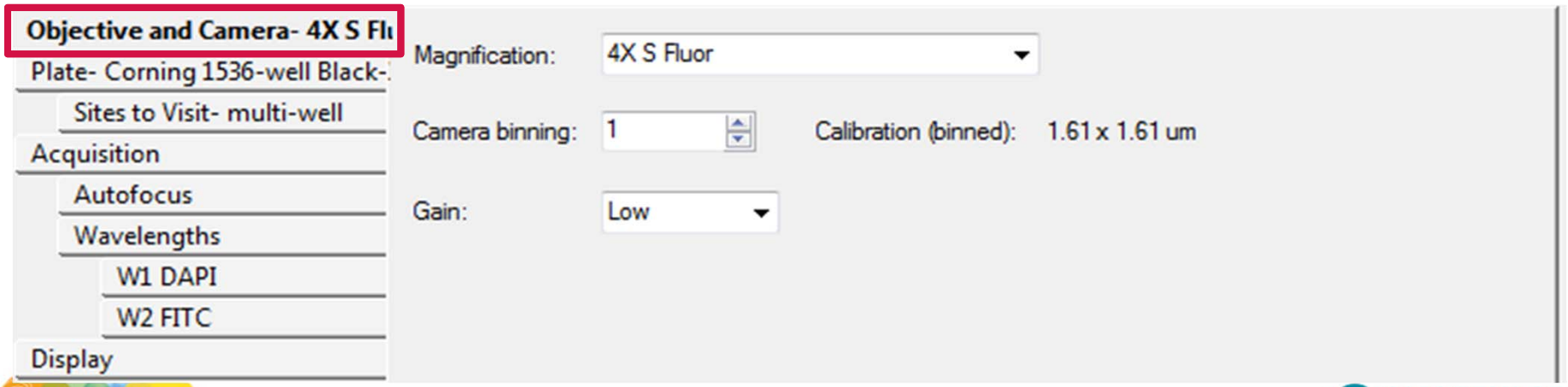


Setting Up a Timelapse Acquisition

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

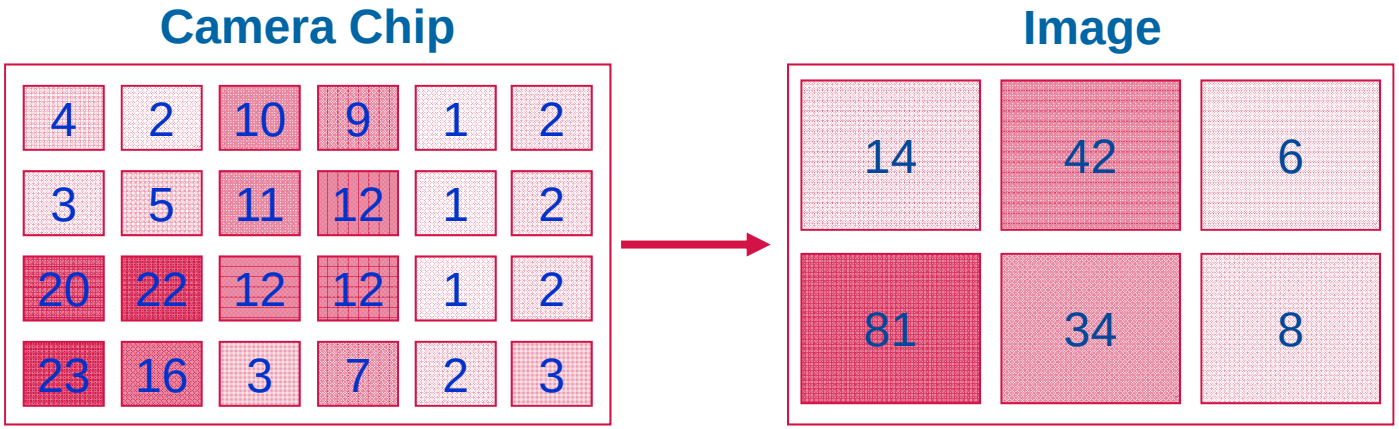
5. If the **Gain** option appears, start with gain set to **Low**



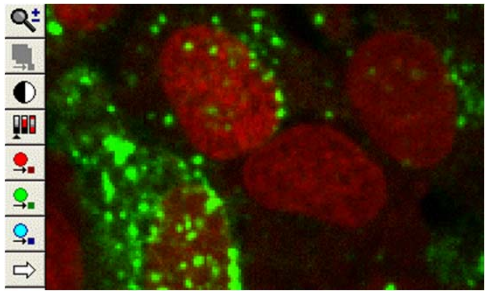
What is Binning?

Combining groups of pixels into a single pixel during image acquisition

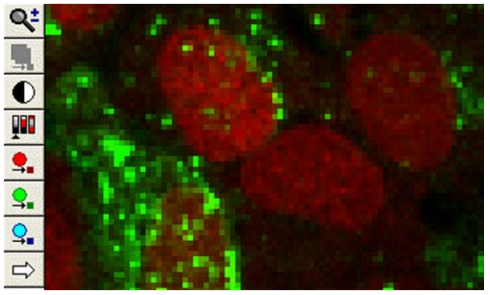
Example of 2x2 Binning



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 transfer from MetaXpress to database
- Faster image analysis

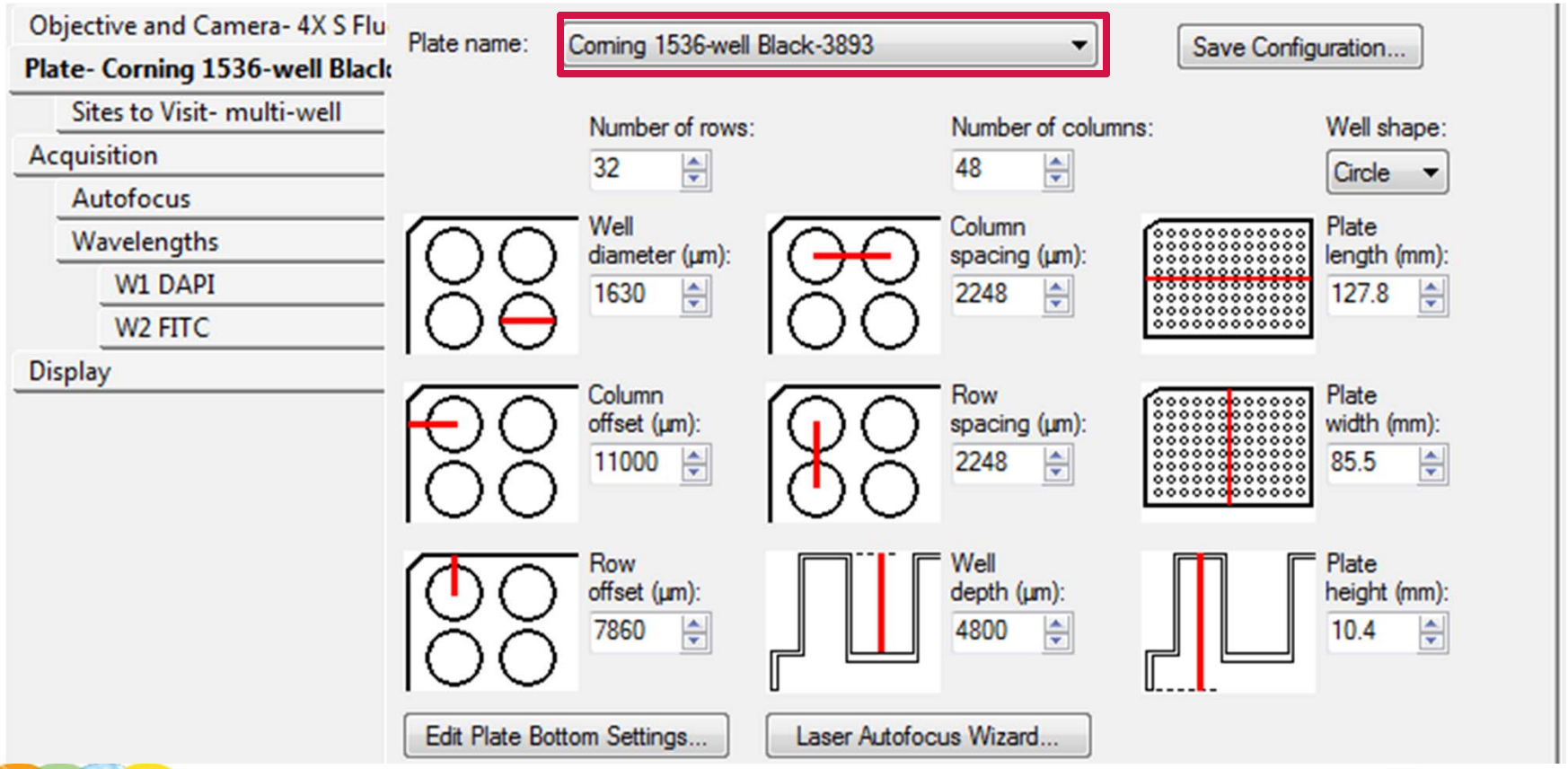
When to Bin

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

Setting Up a Timelapse Acquisition

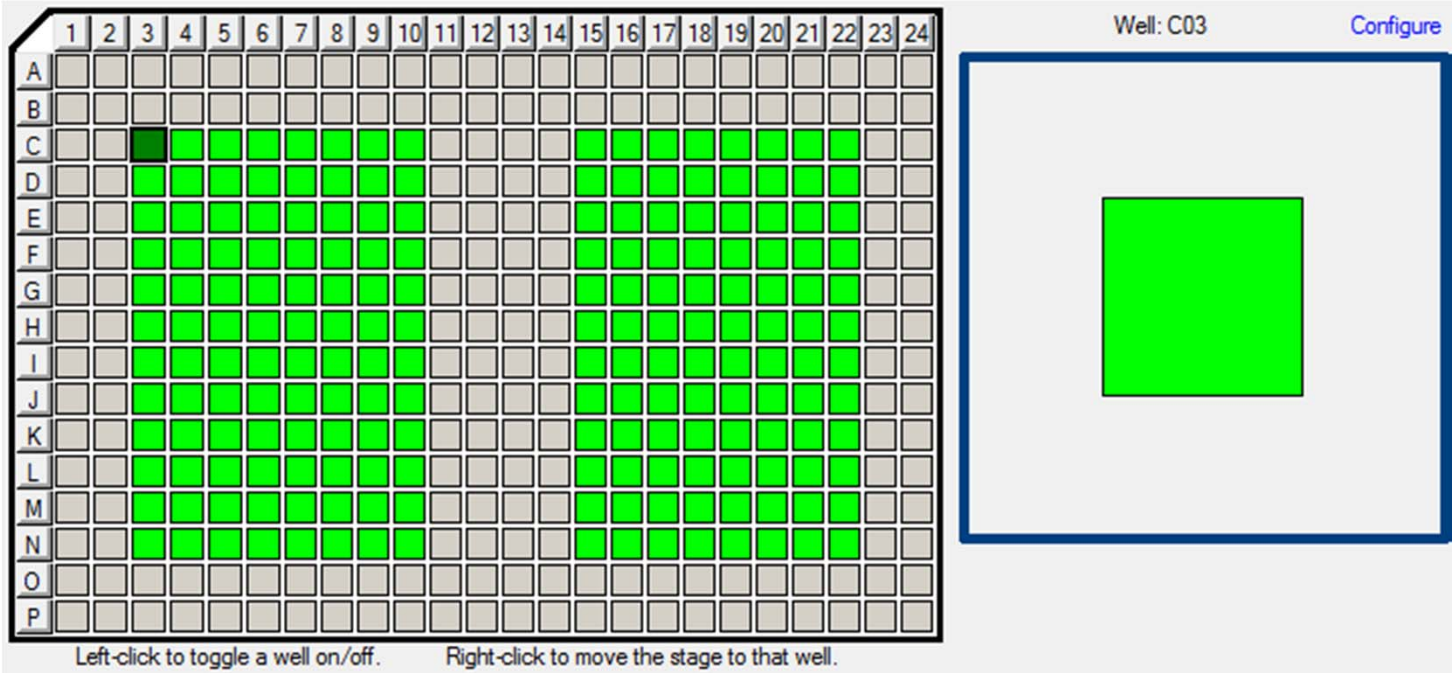
6. Select the **Plate** tab

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



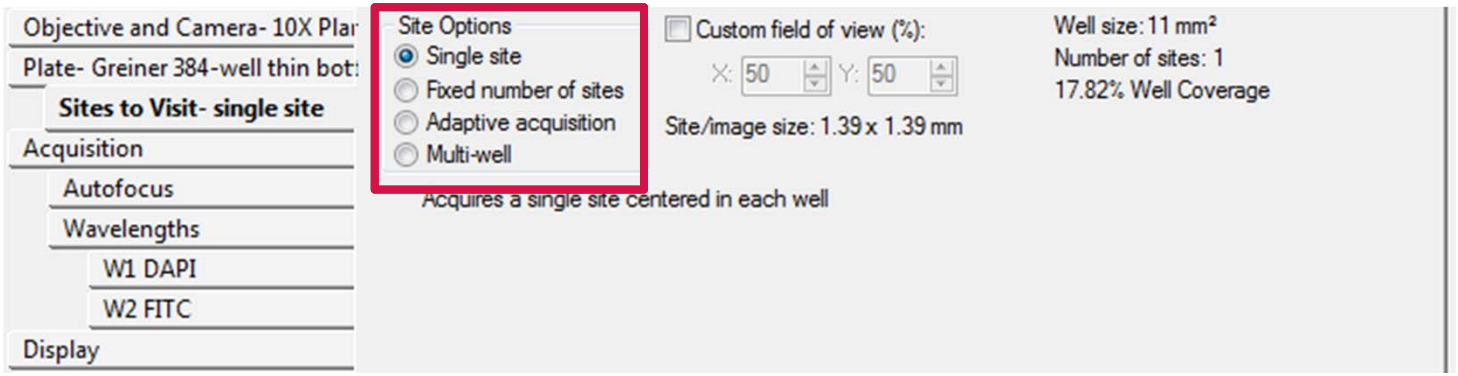
Setting Up a Timelapse Acquisition

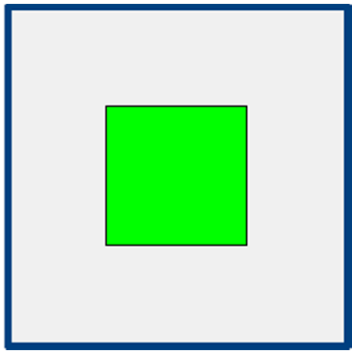
8. In the plate section, select the wells you would like to acquire
   - Left click and drag mouse to select wells
   - Click on “All” (top left corner), row letters, column numbers, or individual wells
   - Gray wells are deactivated, green wells are activated and will be imaged
   - Right click on a well to move the stage to that position (well turns dark green)



Setting Up a Timelapse 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

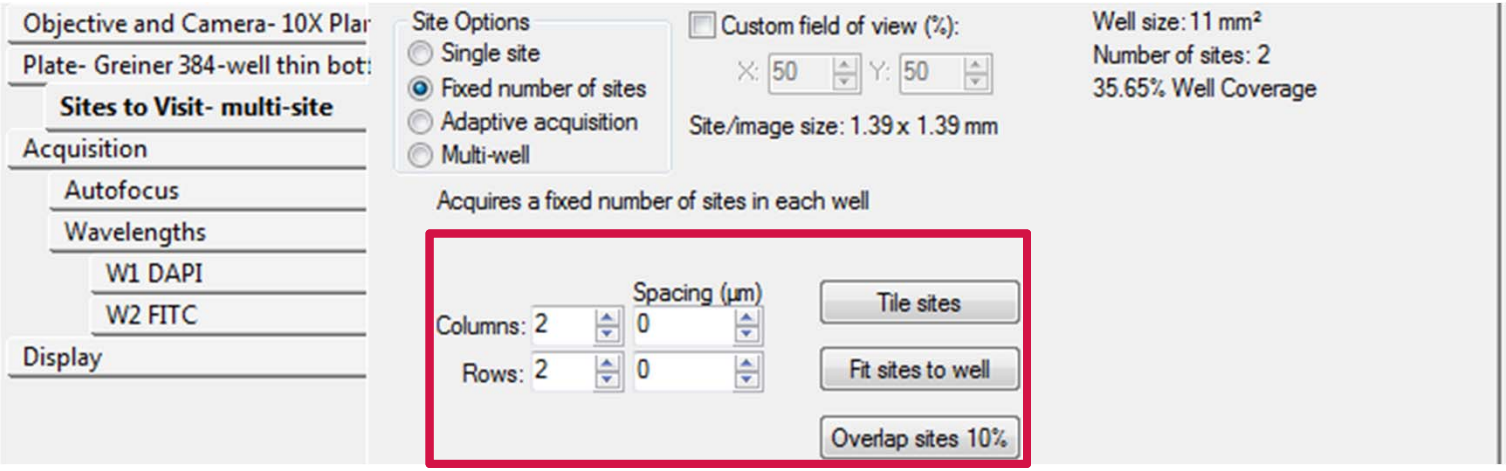




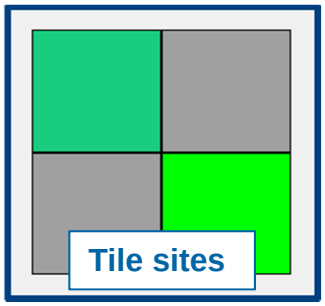
Setting Up a Timelapse Acquisition

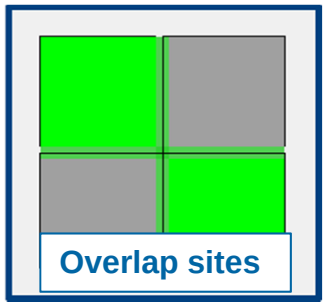
NOTE Left clicking on site selects (green) or deselects (gray) for imaging. Right click moves stage to that position (dark green)

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



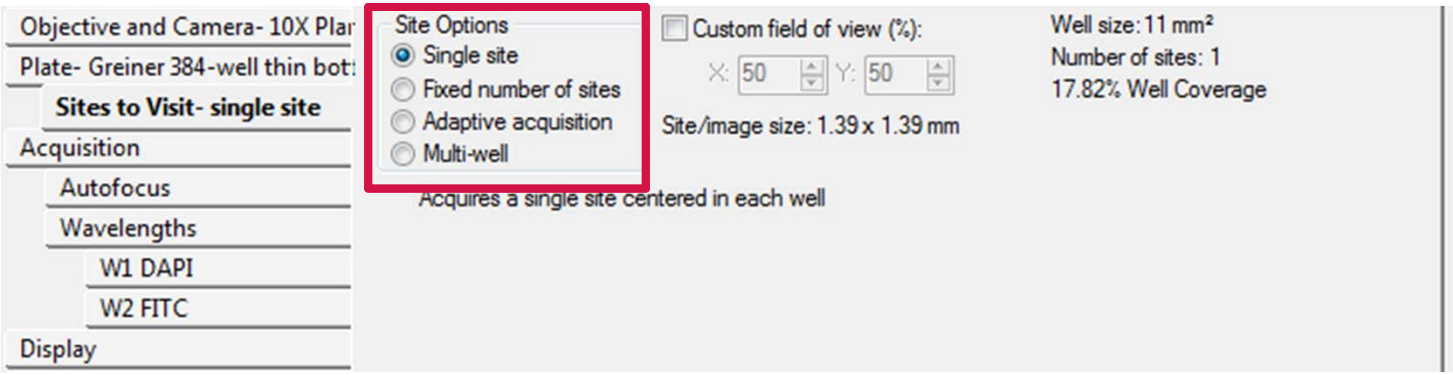
- **Tile sites** places sites edge to edge
- **Fit sites to well** spreads sites to well edge
- **Overlap sites 10%** overlaps edges of sites for stitching





Setting Up a Timelapse Acquisition

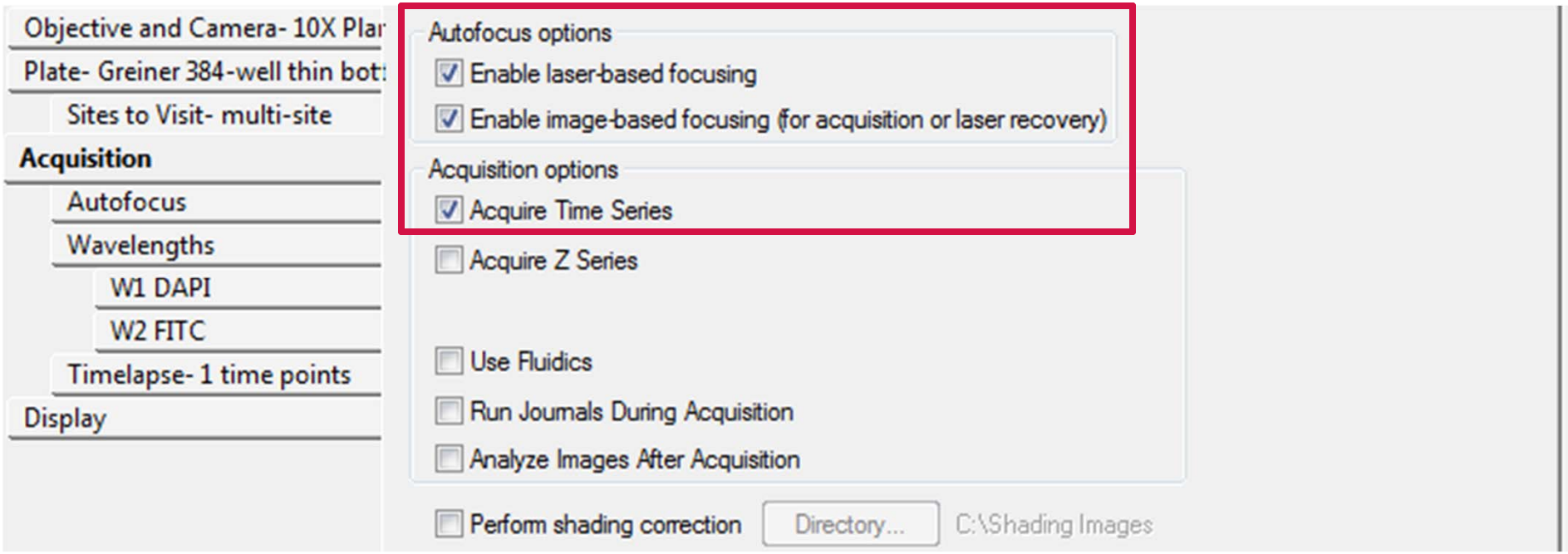
9. On the **Sites to Visit** tab
   - Refer to corresponding chapters for more details on the **Adaptive acquisition** and **Multi-well** options



Setting Up a Timelapse Acquisition

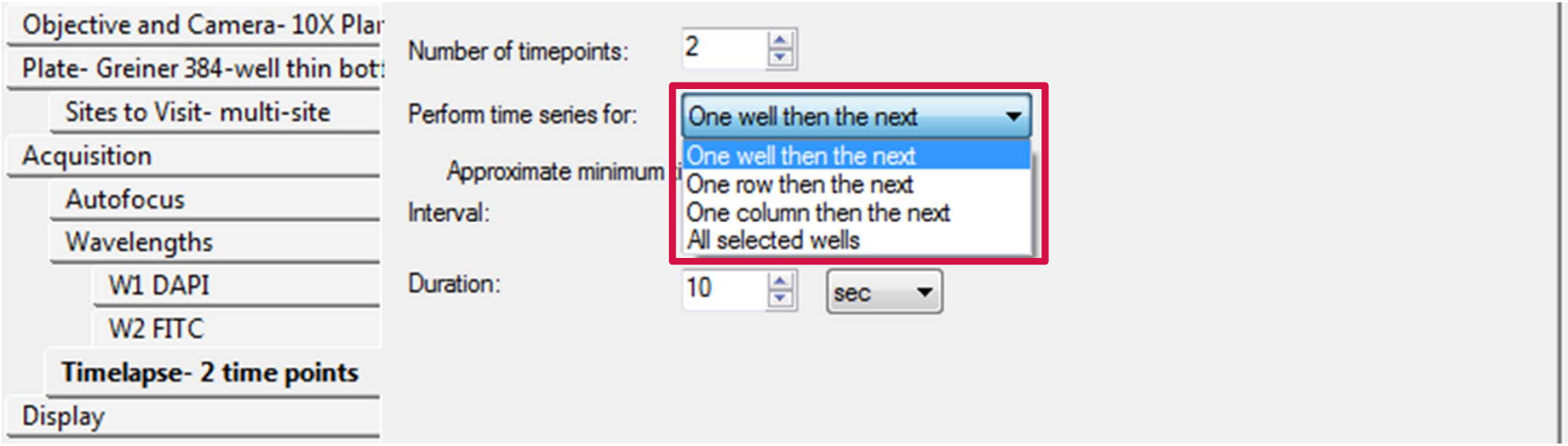
10. Select the **Acquisition** tab
    - Always **Enable laser-based focusing**
    - For certain samples it may be necessary to **Enable mage based focusing**
    - Enable **Acquire Time Series**
    - Optionally, enable **Perform shading correction**

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



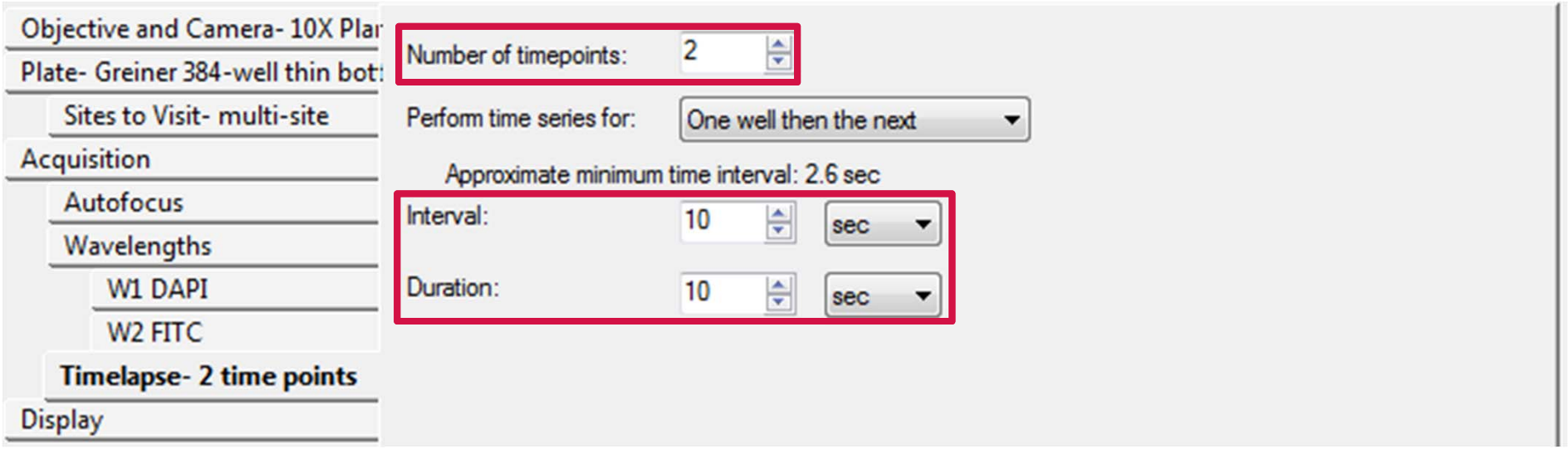
Setting Up a Timelapse Acquisition

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).



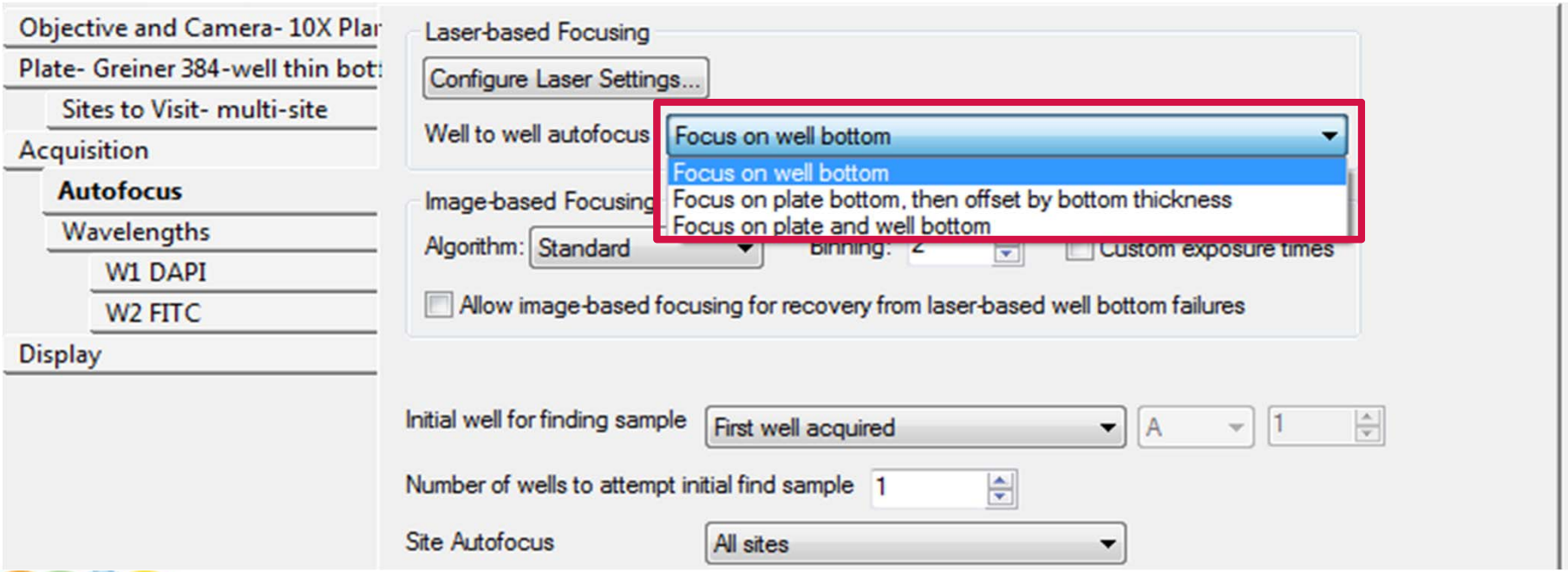
Setting Up a Timelapse Acquisition

11. On the **Timelapse** tab
    - Enter the **Number of timepoints**
    - Set the **Interval**: 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



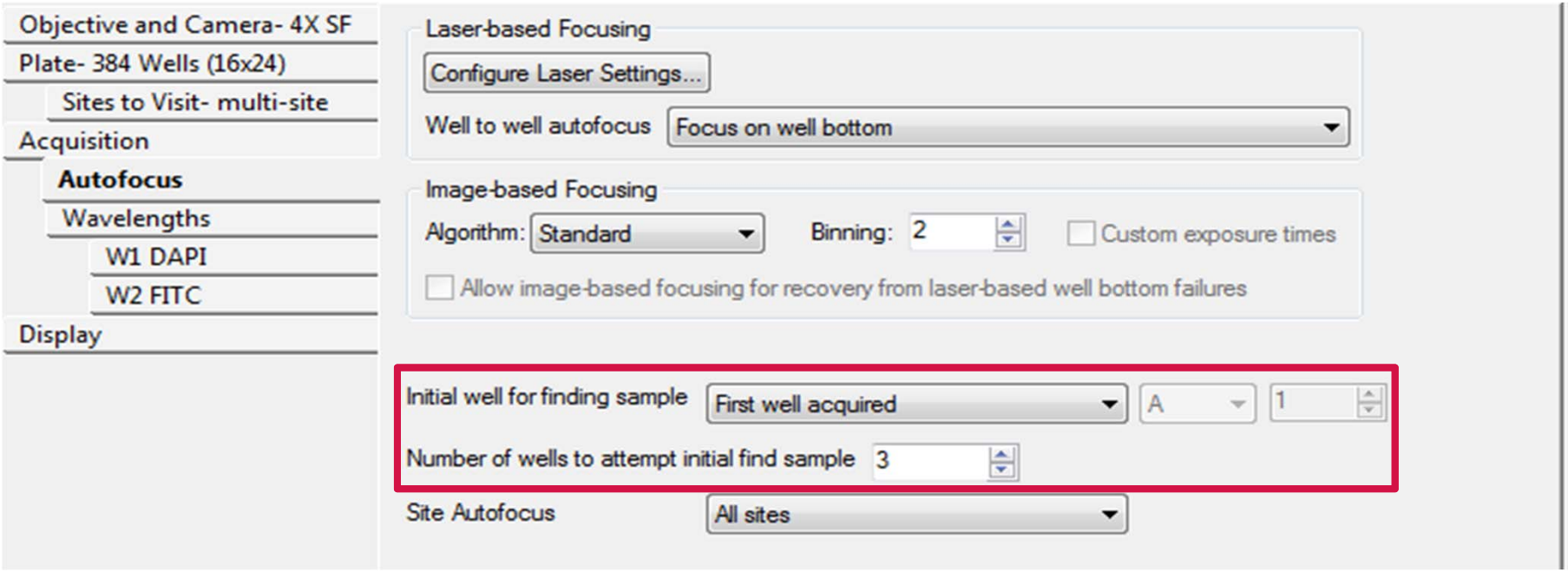
Setting Up a Timelapse Acquisition

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)



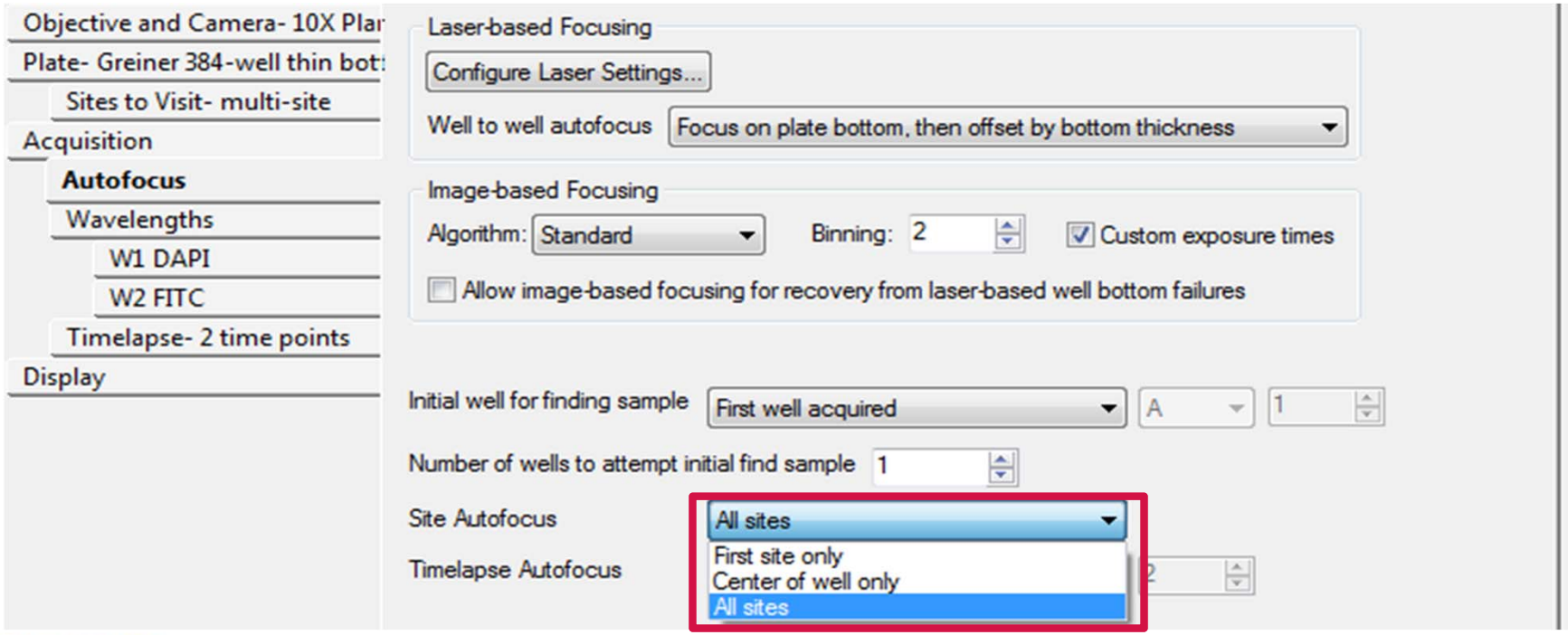
Setting Up a Timelapse Acquisition

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**



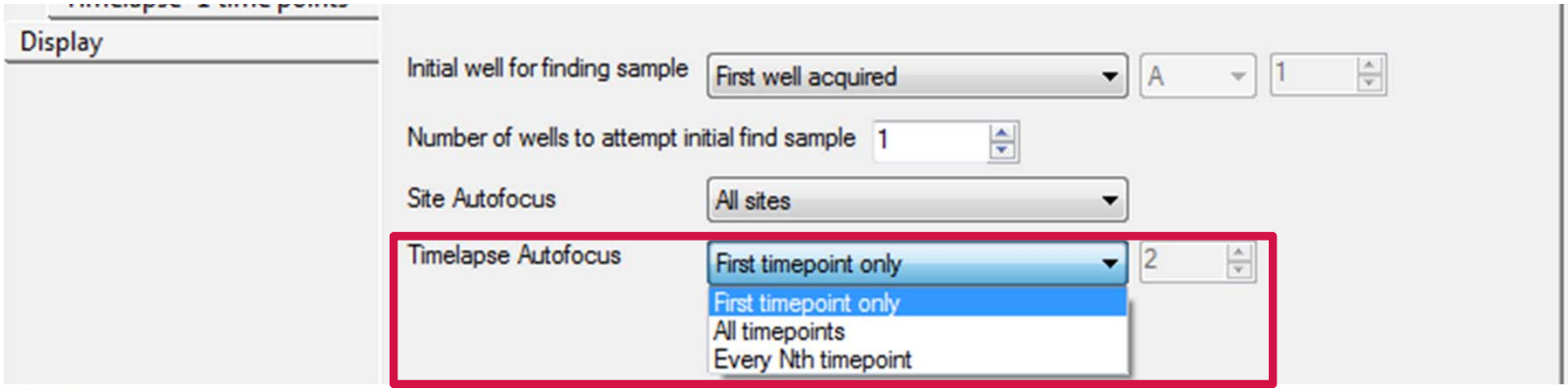
Setting Up a Timelapse Acquisition

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).



Setting Up a Timelapse Acquisition

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

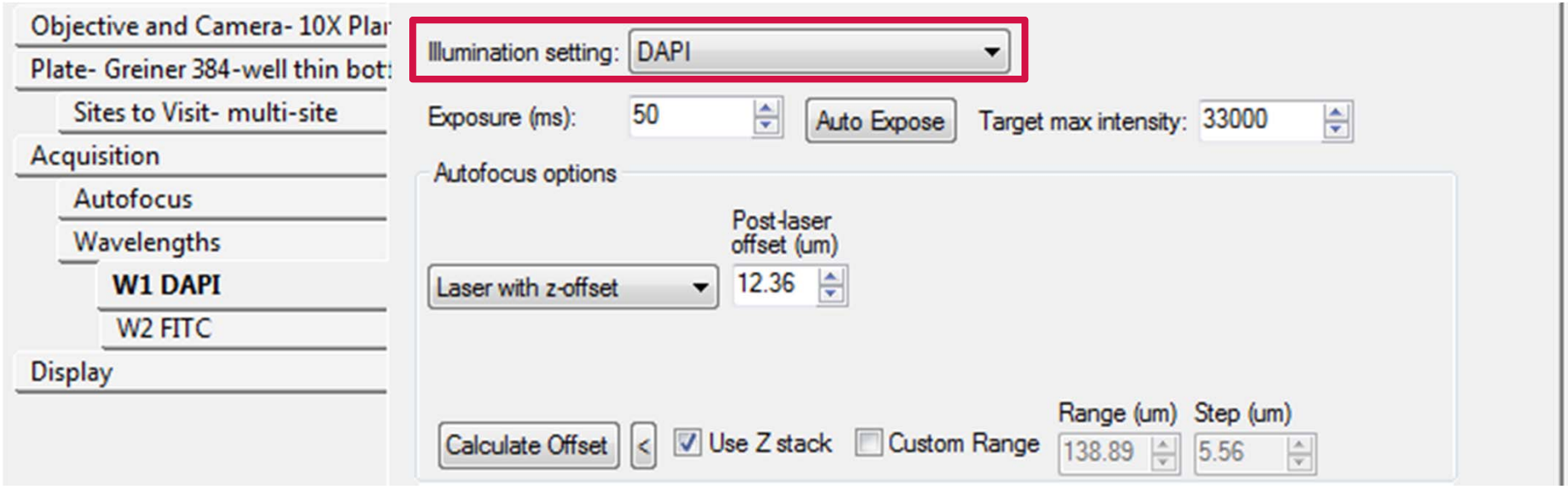


Setting Up a Timelapse Acquisition

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

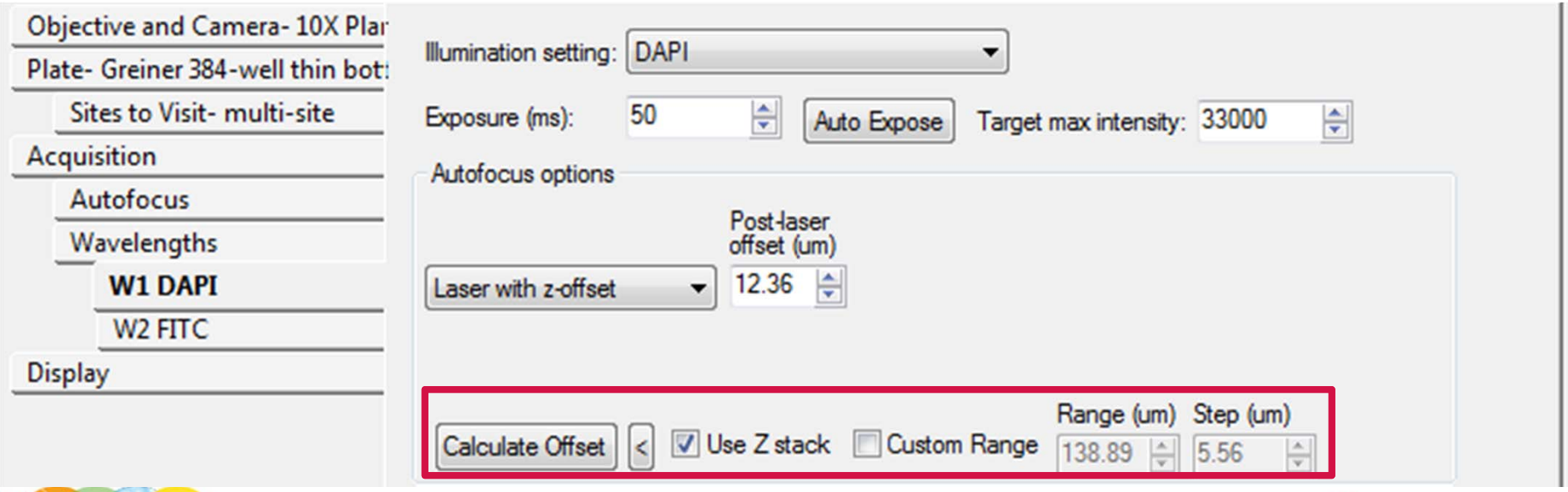
Setting Up a Timelapse Acquisition

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

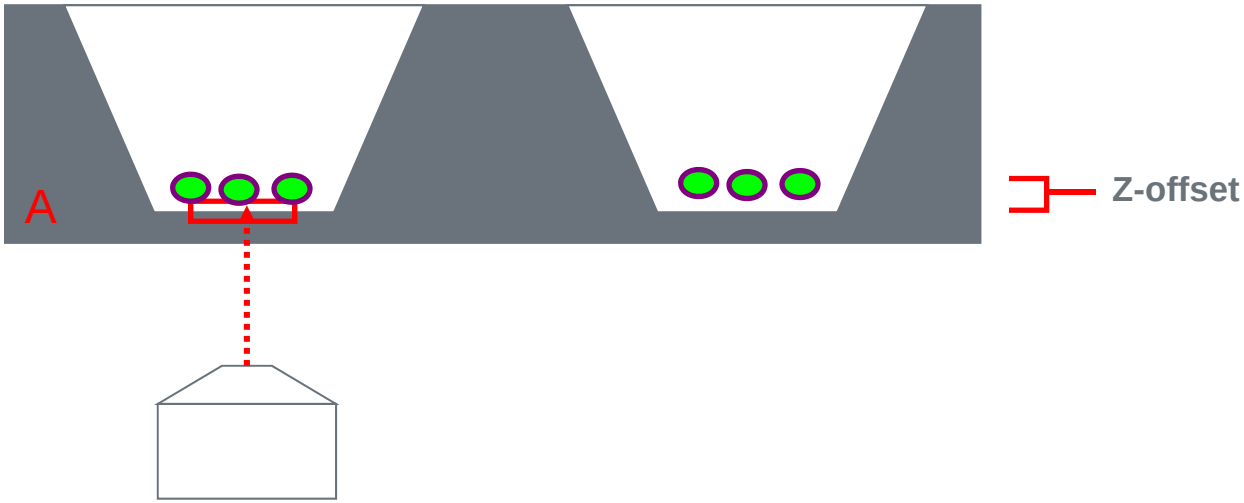


Setting Up a Timelapse Acquisition

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



What is a Post- Laser Offset?



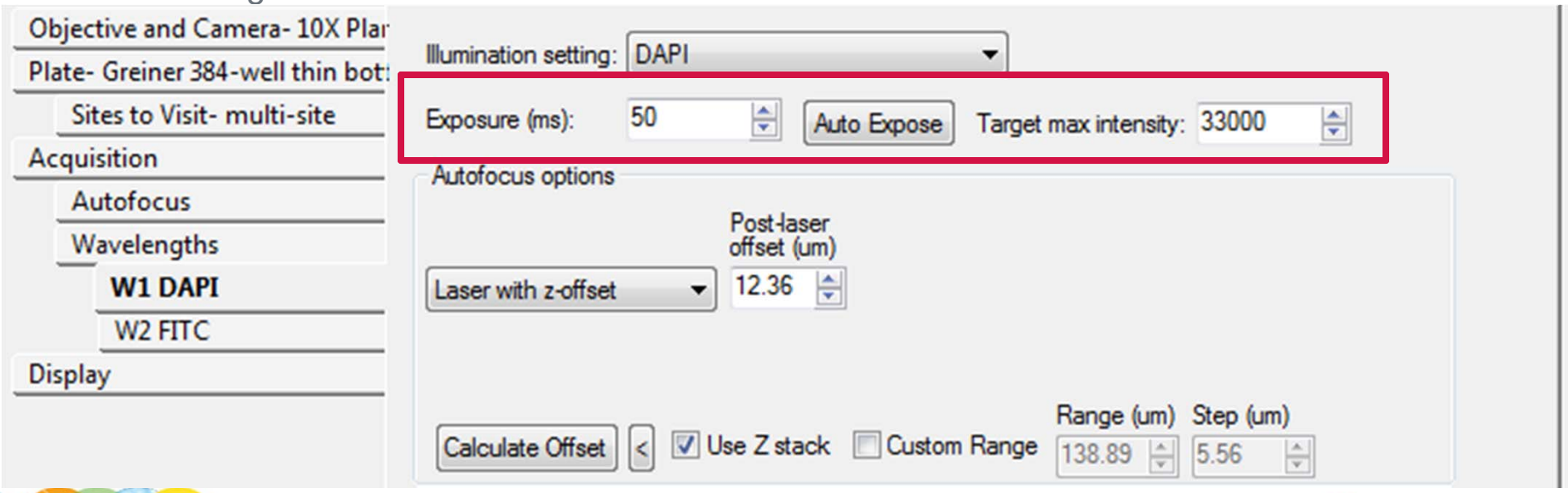
Post-laser offset is the Z distance between the bottom of the well and the sample

- 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 Timelapse Acquisition

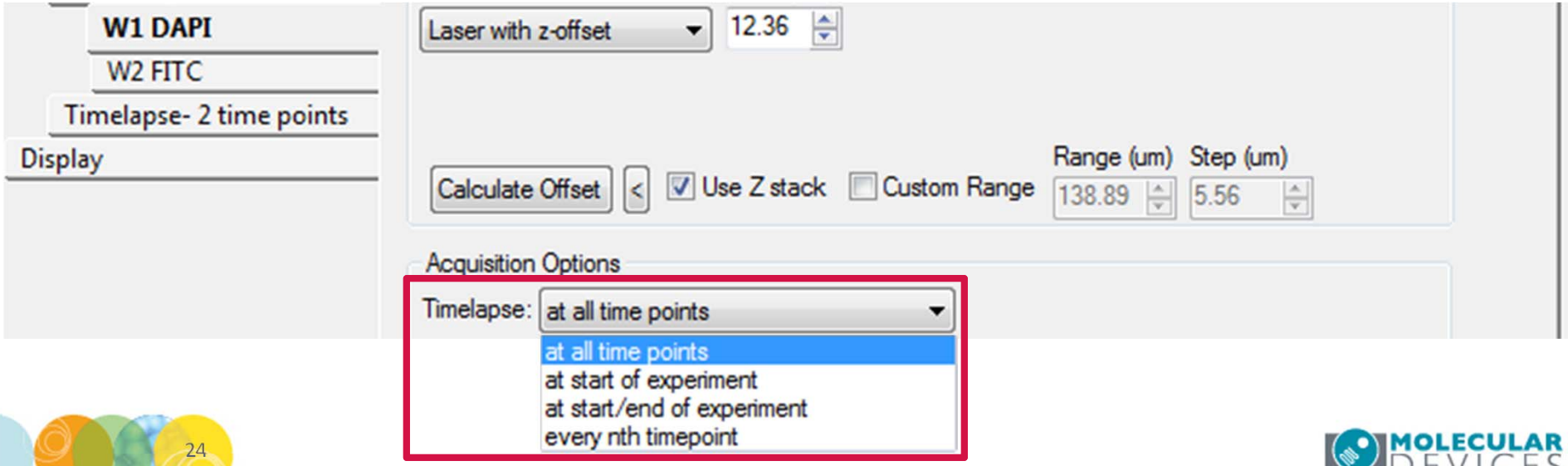
14. On the **W1** tab
    - Enter an **Exposure** (ms) time and click on the **Focus** button 

      - 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



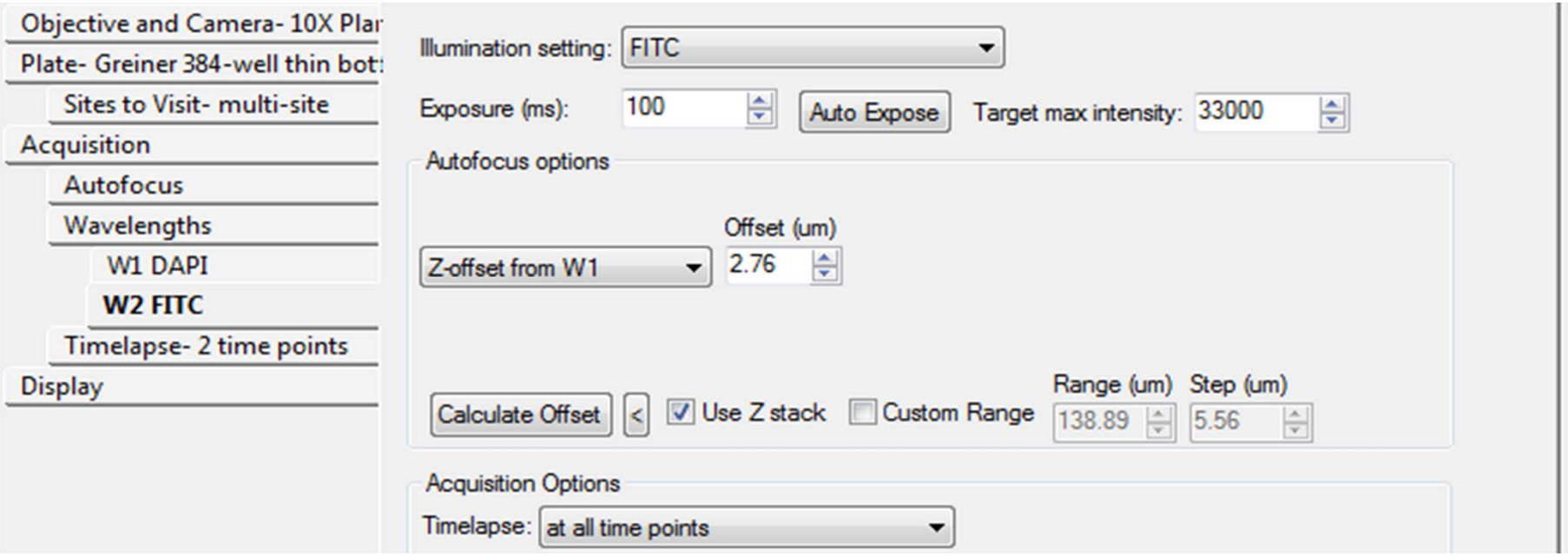
Setting Up a Timelapse Acquisition

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



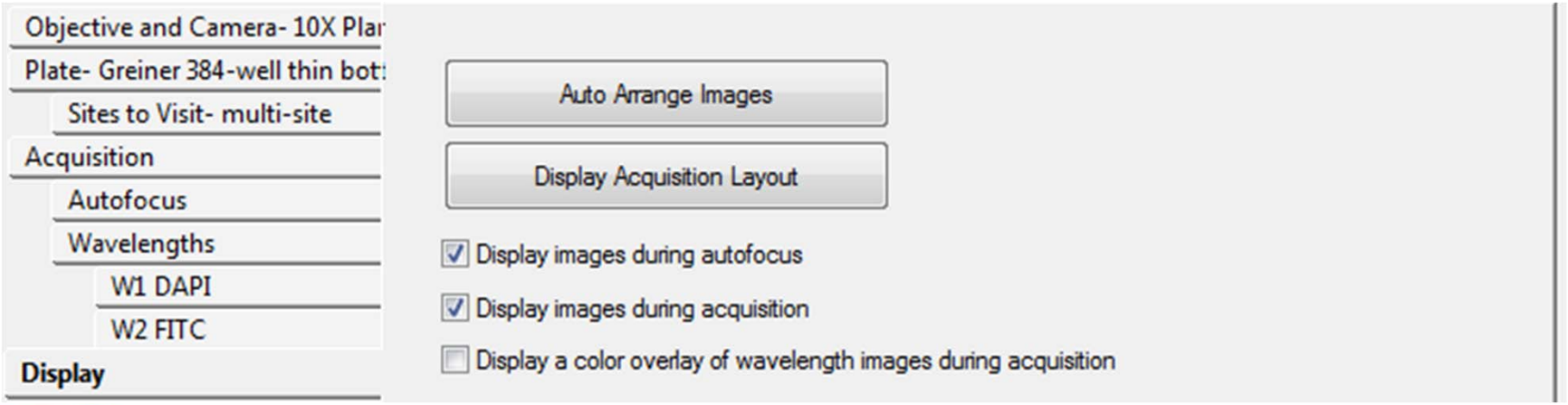
Setting Up a Timelapse Acquisition

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



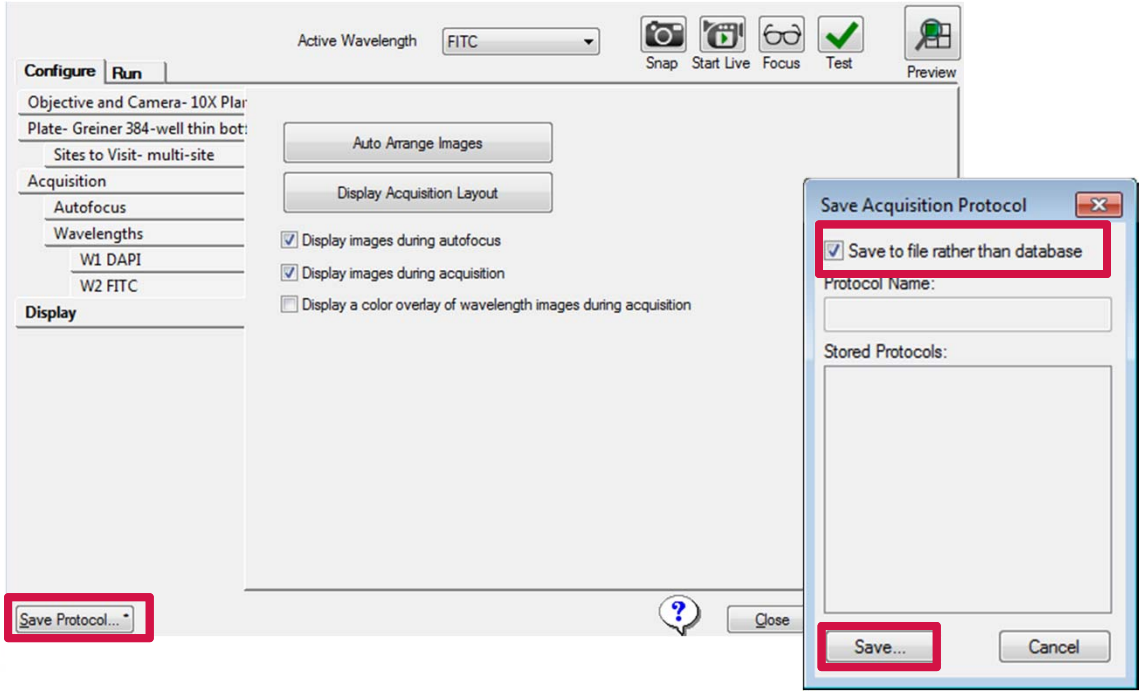
Setting Up a Timelapse Acquisition

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.



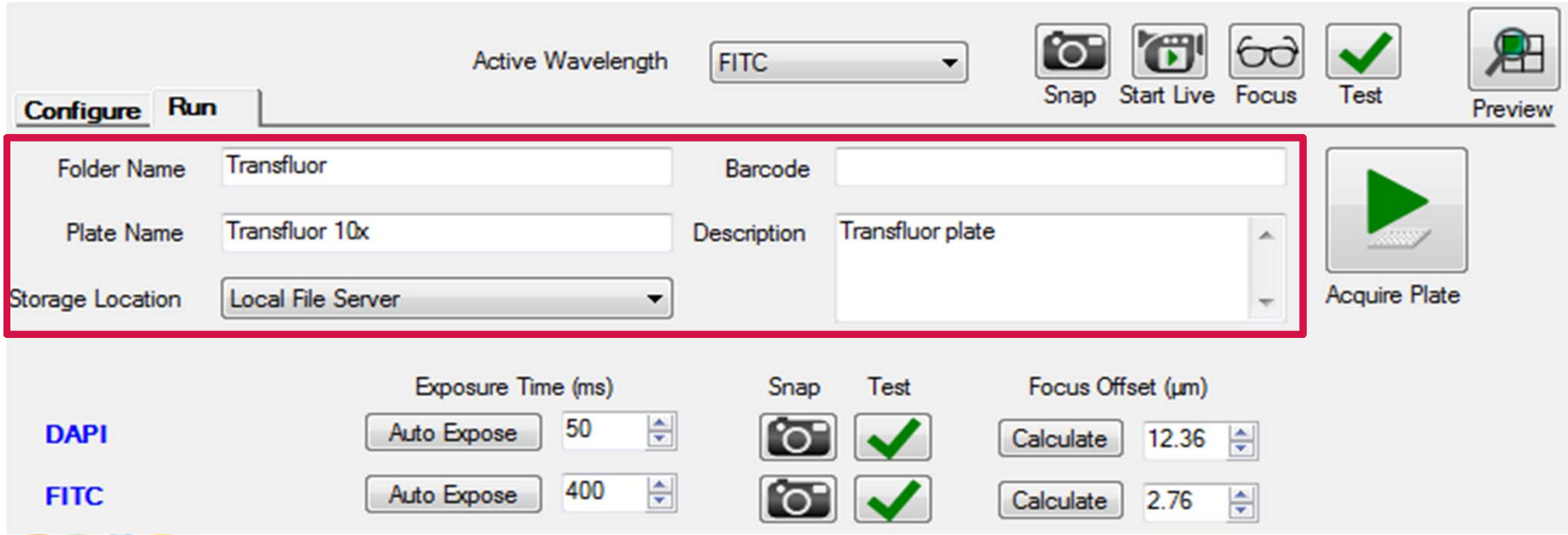
Setting Up a Timelapse Acquisition

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)



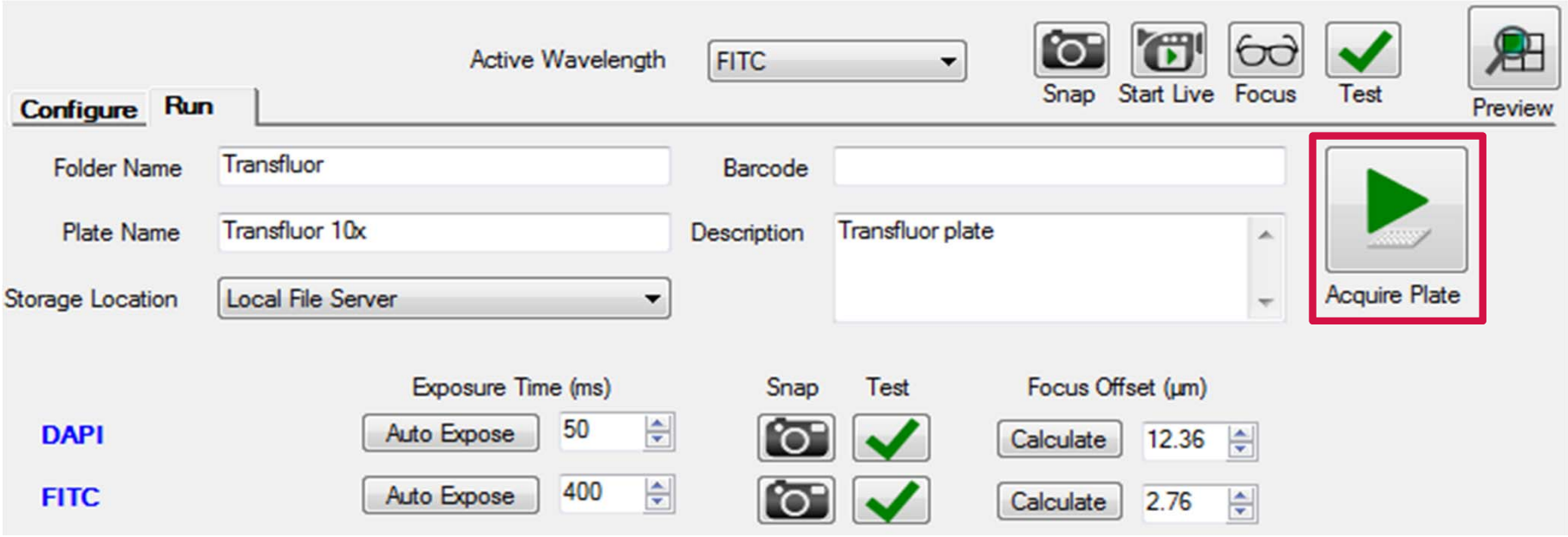
Setting Up a Timelapse Acquisition

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



Setting Up a Timelapse 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

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