MetaXpress® 6 Software Guide

Acquiring a Plate with Timelapse and Fluidics Enabled

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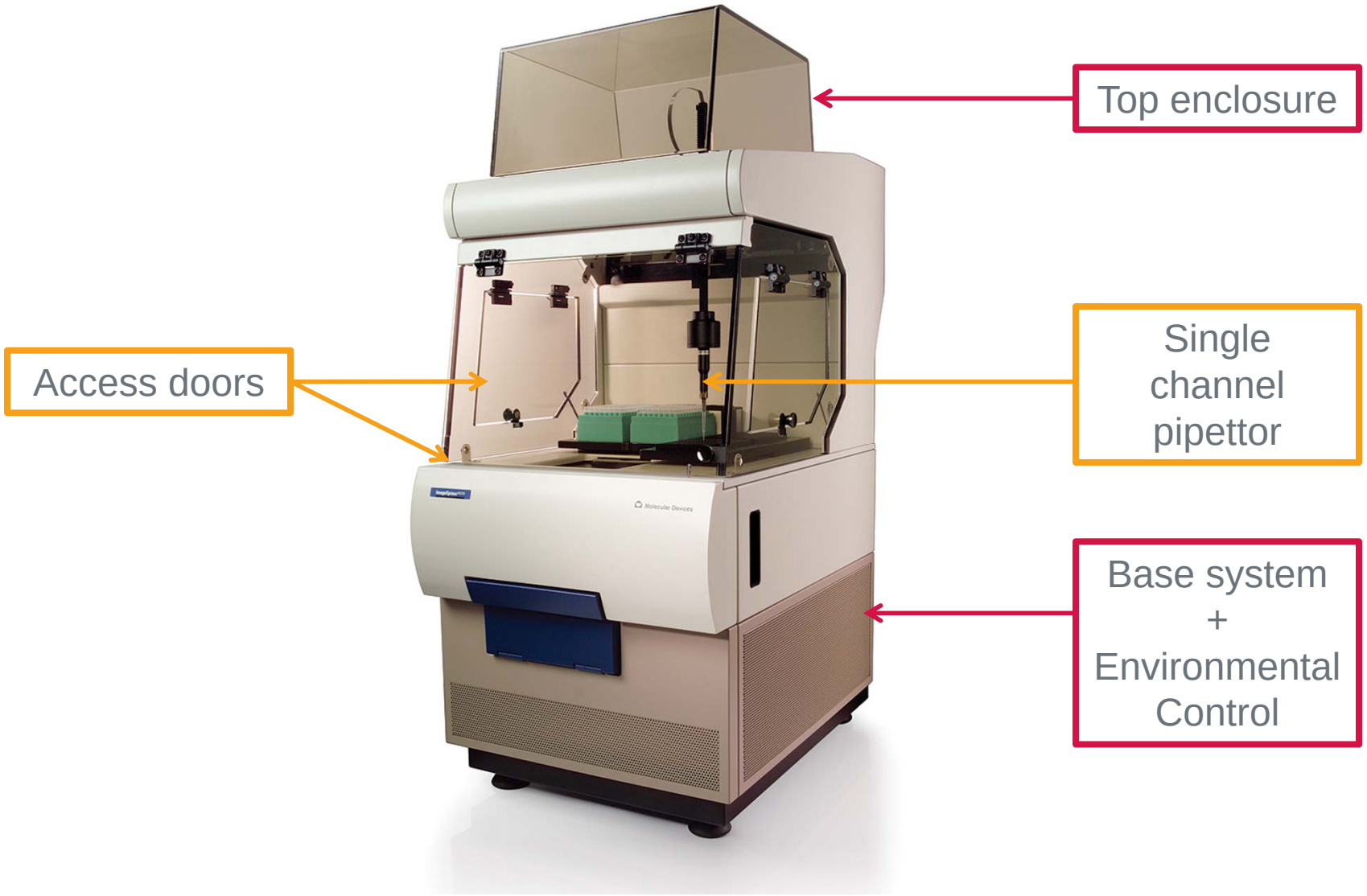
Chapter Purpose

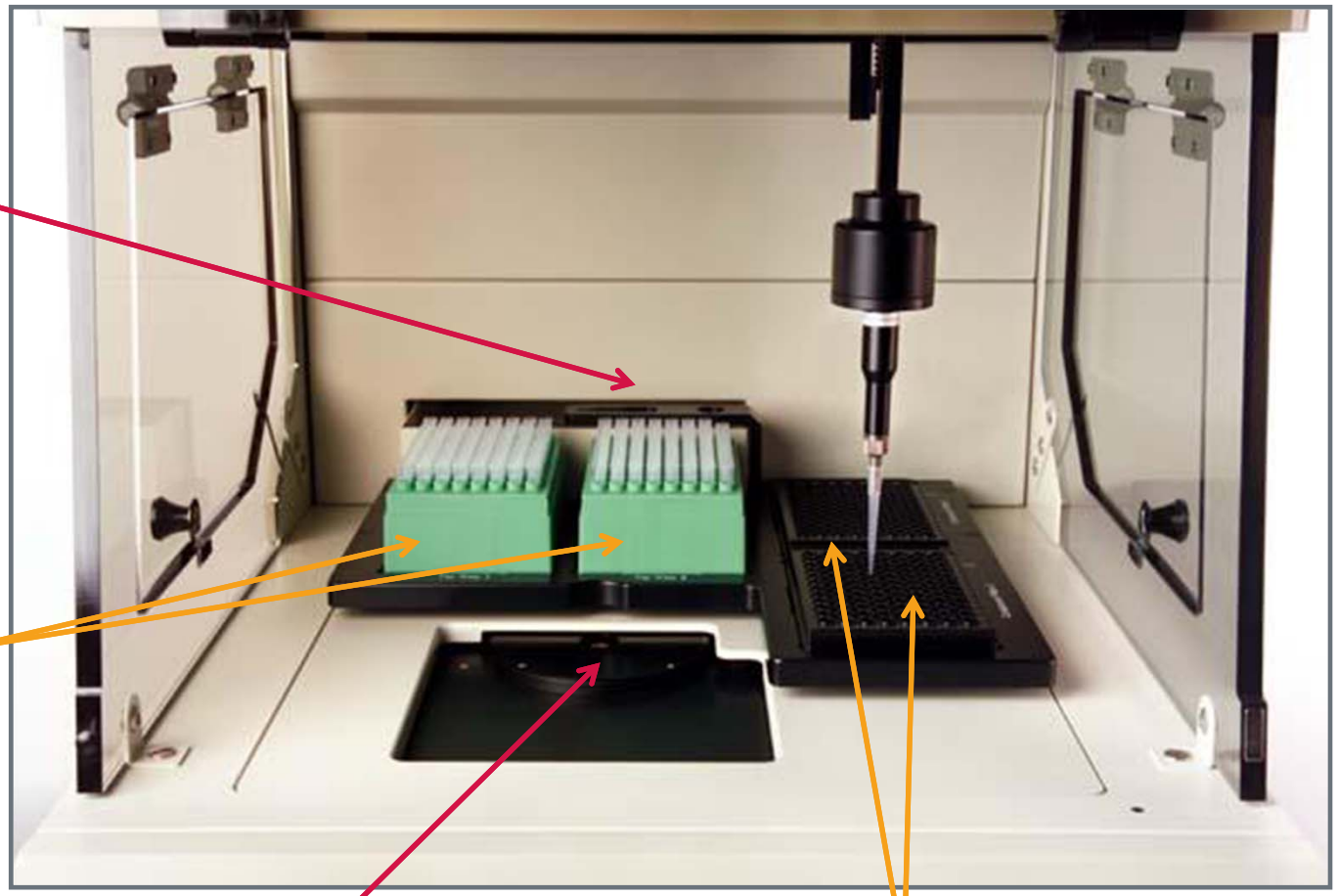
The purpose of this chapter to guide the user through setting up a timelapse acquisition with fluidics events. This includes selecting objectives, plates, wavelengths, focal position, and configuring fluidics events.

The fluidics option has a single channel pipettor that can be used to add compounds, exchange media, and wash cells as part of an imaging experiment.

Fluidics Option: Hardware Components



Fluidics Option: 96-Well Plate Setup



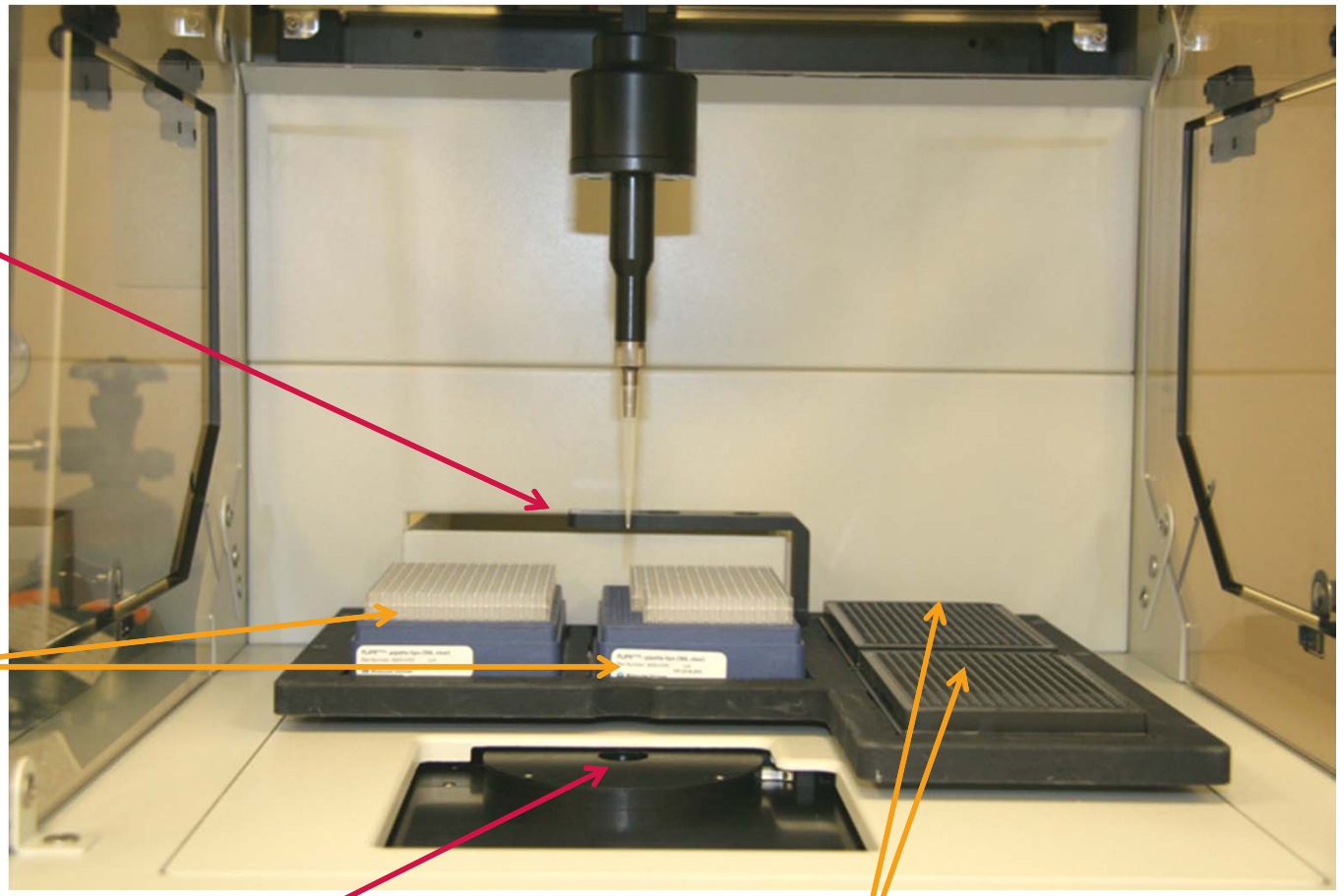
Tip remover and waste bin

2 Tip Rack Positions

Shutter for sample access

2 heated Compound Plate Positions

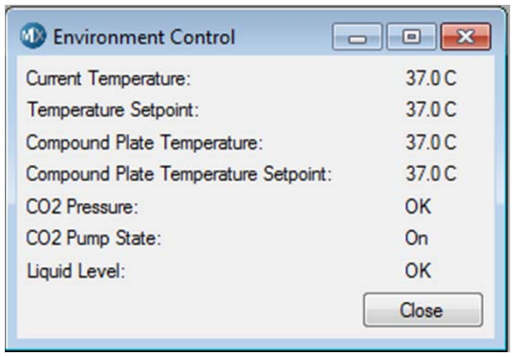
Fluidics Option: 384-Well Plate Setup



Preparing for a Fluidics Experiment

Preparation

1. Turn on the ImageXpress Micro system at least two hours before experiment to allow the system temperature to stabilize
2. Set sample and compound plate temperature on the options controller box
3. Make sure system has the appropriate objective(s) and filter sets
4. Place the EC sealing ring in the system
5. (optional) Put your plate in the system



After system has equilibrated

1. Check status of environmental control (temperature/CO_2/ Humidity). In the main menu, select **Control > ImageXpress > Environment Control**
2. Load tips and compound plate(s)
3. Load sample plate without lid if not already in the system
4. If using CO_2 / Humidity:
   - Place EC sealing ring sample plate inside system
   - Check gas tank and regulator
   - Check liquid level in water reservoir

NOTE: Set Compound and Sample Plate Temperatures on the options controller box. Out-of-focus images may result if plates are not equilibrated. If plates need to be equilibrated, place plates in system with sealing ring for at least 20 minutes prior to experiment.

Setting Up a Timelapse Acquisition with Fluidics

1. Open **Plate Acquisition Setup**

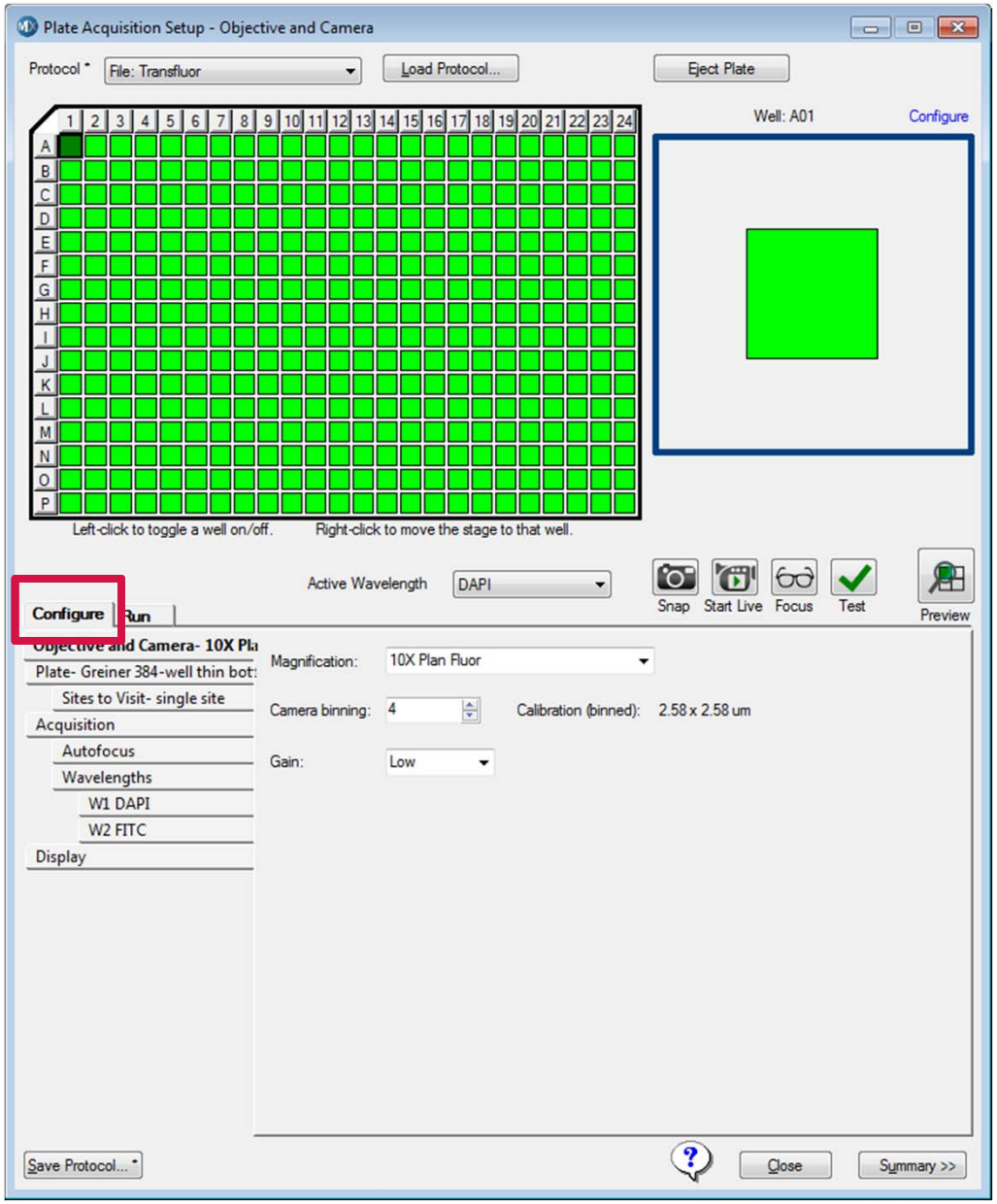
   - In the main toolbar click on

     OR

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

2. Click on the **Configure** tab

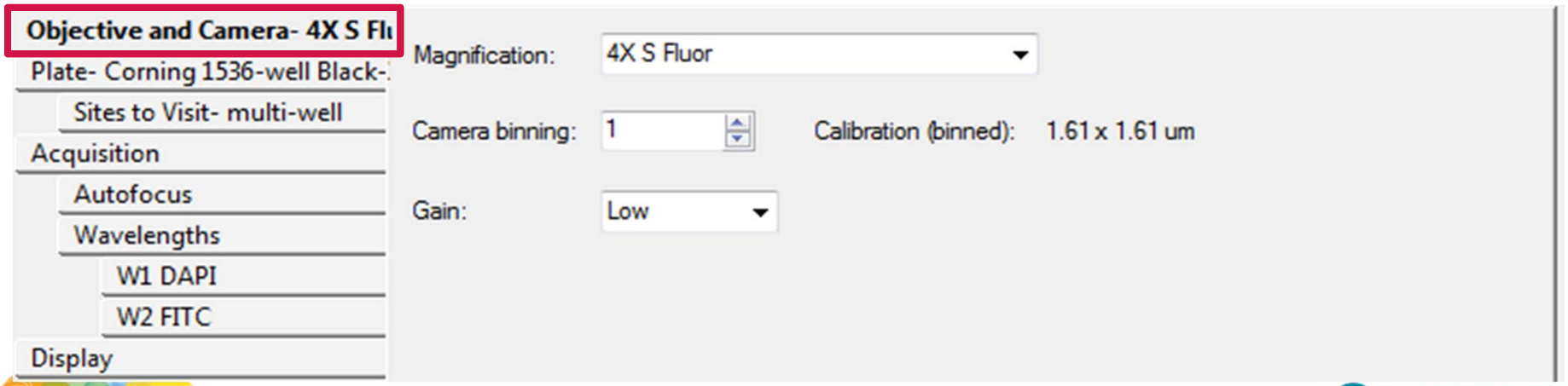


Setting Up a Timelapse Acquisition with Fluidics

3. Select the **Objective and Camera** tab

4. Select the desired **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** (please see next slides for guidance)
   - Pixel size is automatically calculated based on magnification and binning
   - Set Camera Binning to **1** to acquire unbinned images – maximum resolution

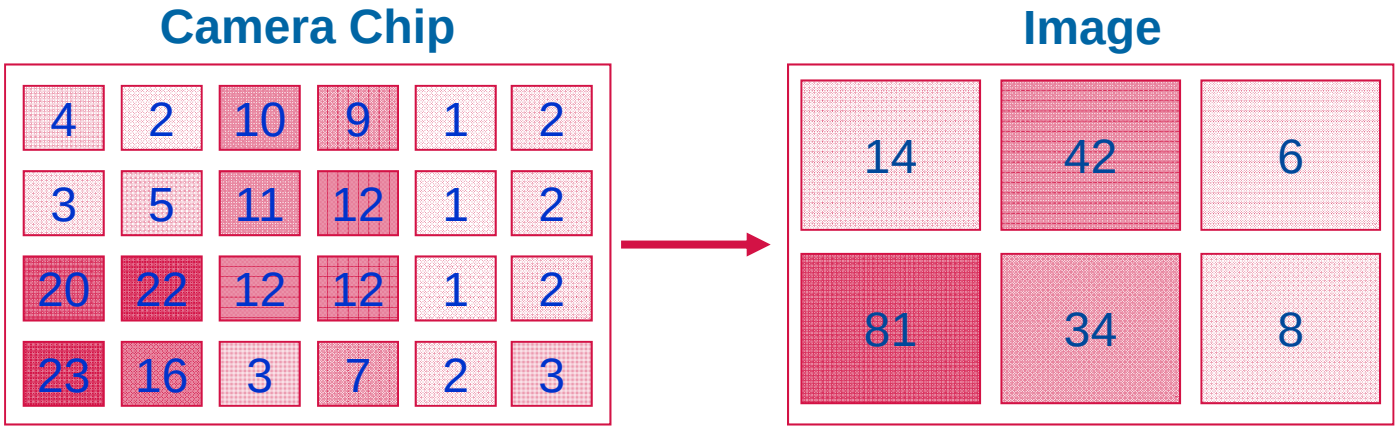
5. If the **Gain** option appears, start with gain set to **Low**



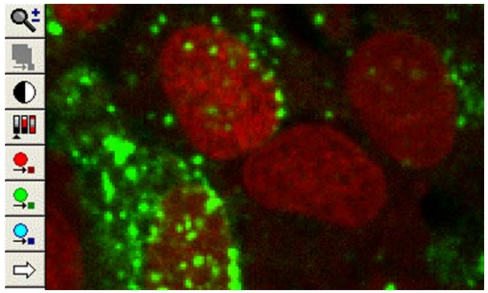
What is Binning?

Combining groups of pixels into a single pixel during image acquisition

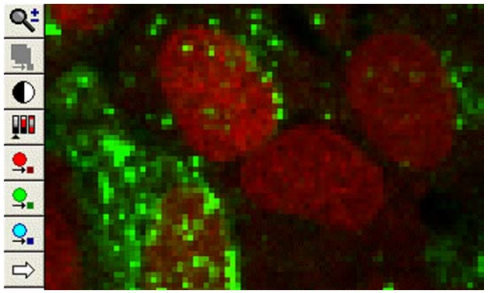
Example of 2x2 Binning



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

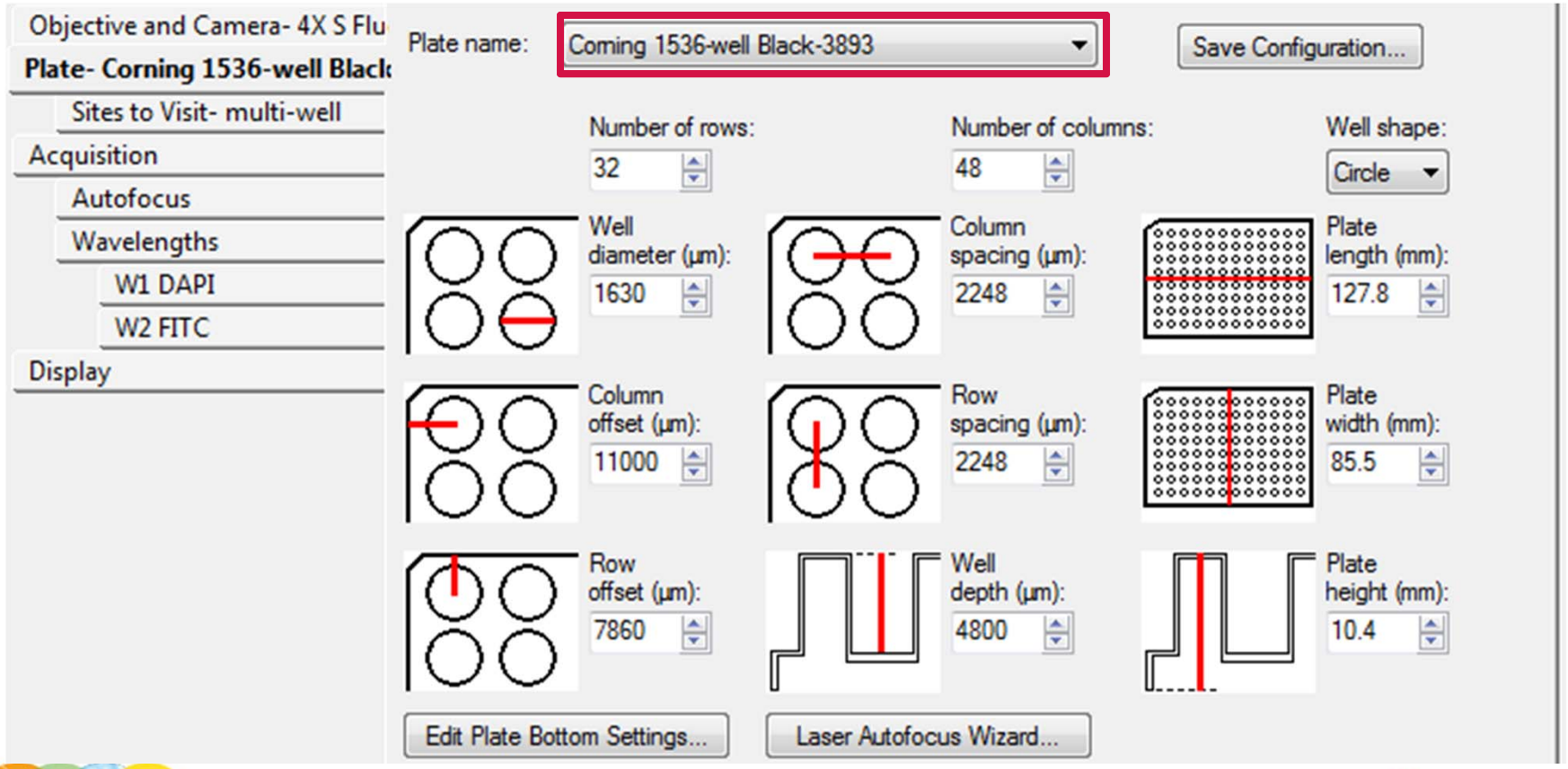
When to Bin

- 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 with Fluidics

6. Select the **Plate** tab

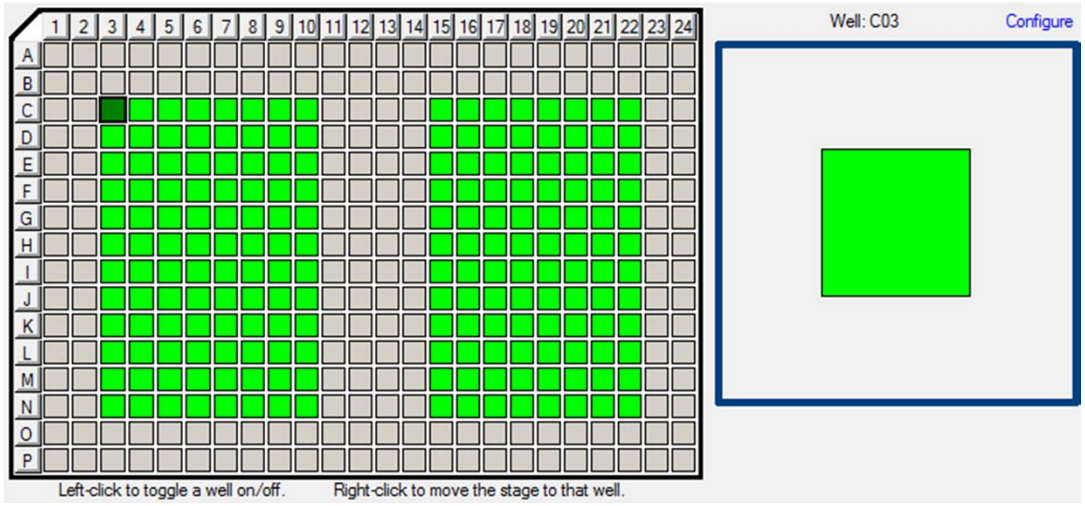
7. Select the appropriate **Plate Type** from the drop-down menu



Setting Up a Timelapse Acquisition with Fluidics

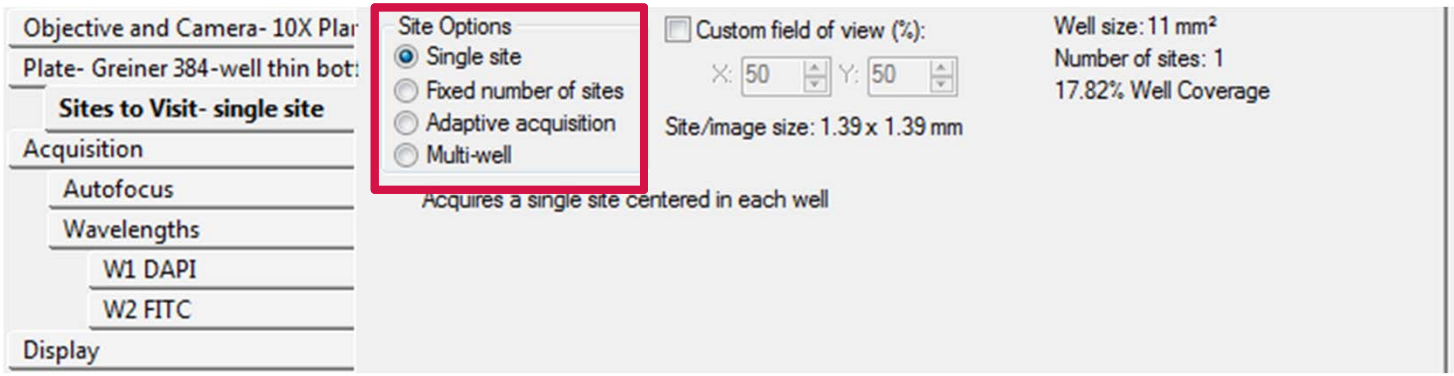
8. In the Plate Section, select the wells you would like to acquire
   - Left click and drag mouse to select wells
   - Click on “All” (top left corner), row letters, column numbers, or individual wells
   - Gray wells are deactivated, green wells are activated and will be imaged
   - Right click on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

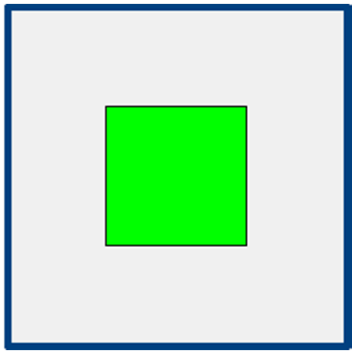
NOTE For experiments involving fluidics, select all wells to be acquired. Well selection for individual fluidics events will be done separately.



Setting Up a Timelapse Acquisition with Fluidics

9. Click on the **Sites to Visit** tab
   - Select **Single Site** to acquire one site in the middle of the well (recommended for fast kinetic experiments)
   - To acquire a single site elsewhere in the well, refer to the next section on setting up multiple sites

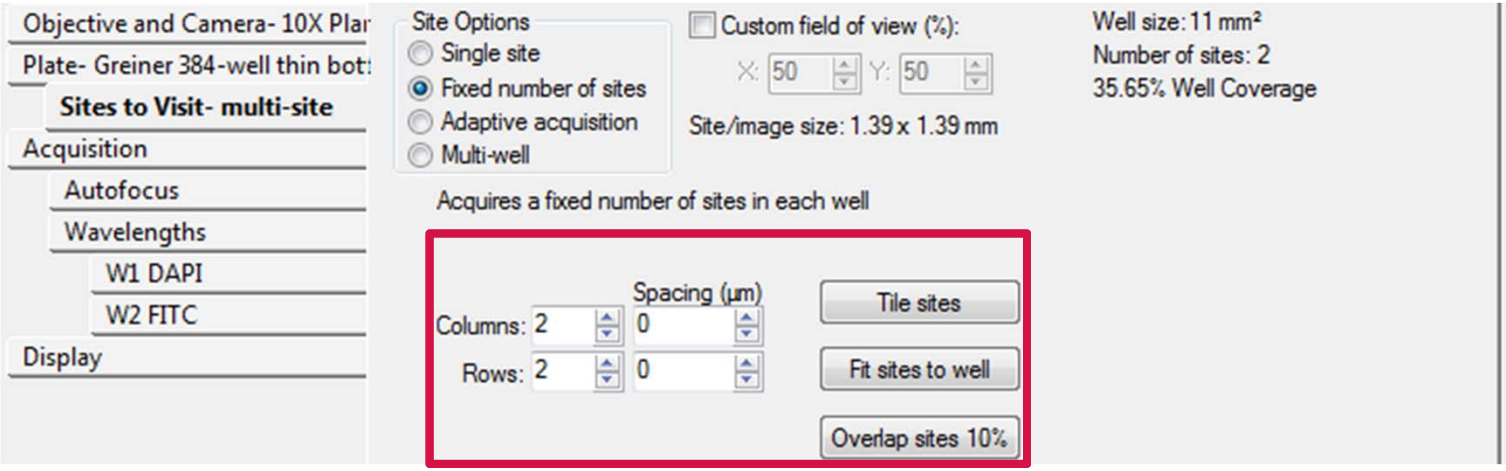




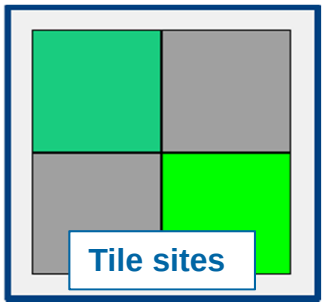
Setting Up a Timelapse Acquisition with Fluidics

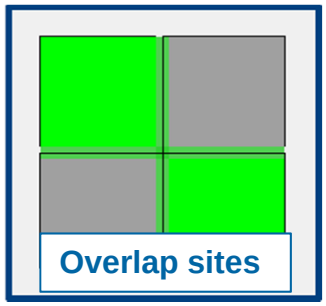
NOTE Left clicking on site selects (green) or deselects (gray) for imaging. Right click moves stage to that position (dark green)

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



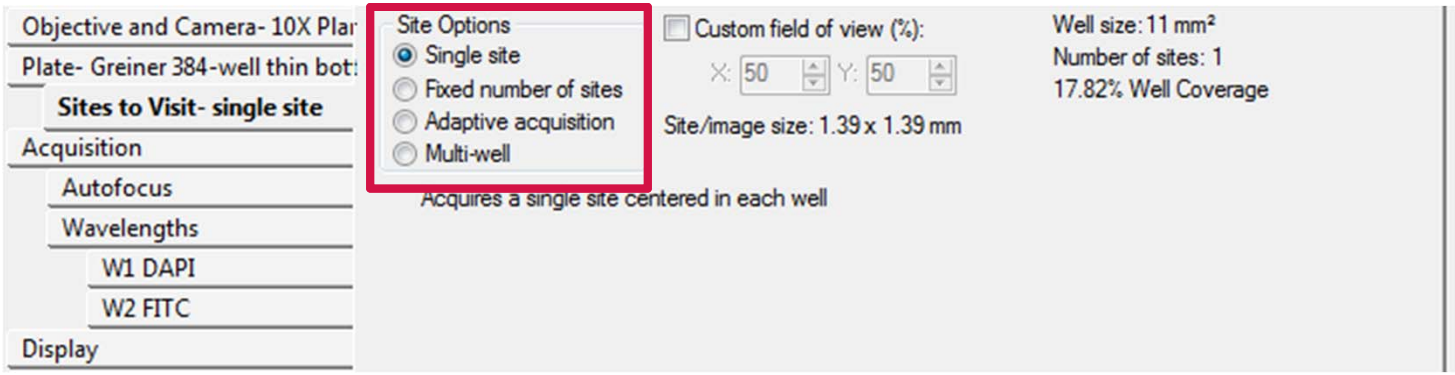
- **Tile sites** places sites edge to edge
- **Fit sites to well** spreads sites to well edge
- **Overlap sites 10%** overlaps edges of sites for stitching





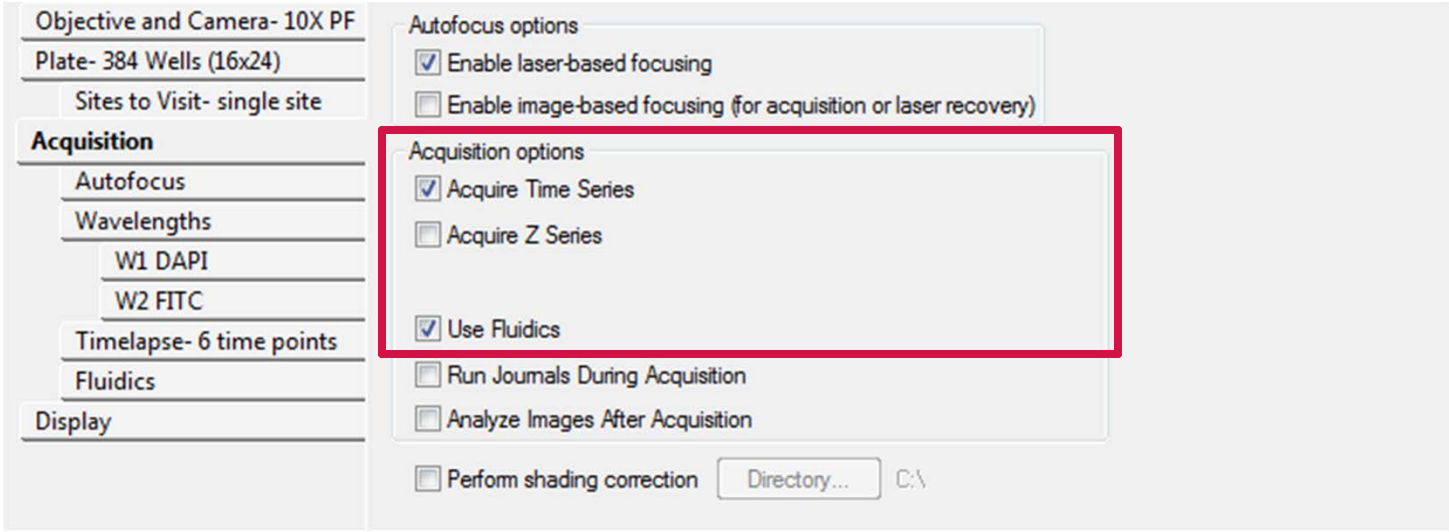
Setting Up a Timelapse Acquisition with Fluidics

9. On the **Sites to Visit** tab
   - The Adaptive acquisition and Multi-well options are not compatible with fluidics events



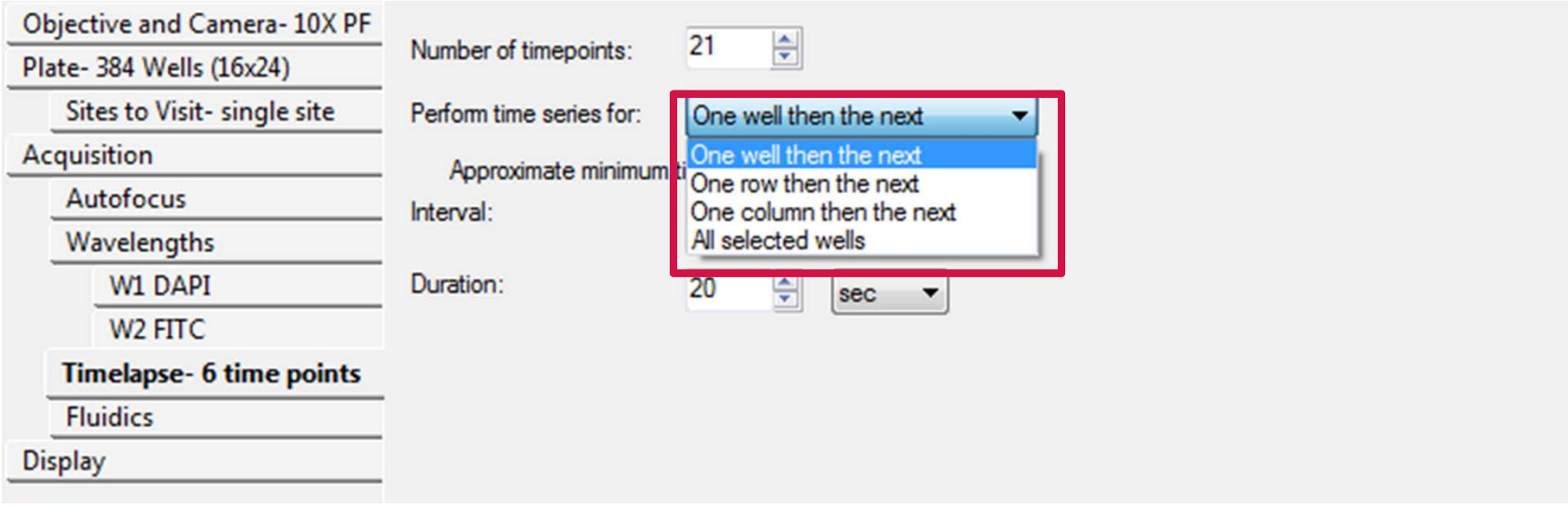
Setting Up a Timelapse Acquisition with Fluidics

10. Click on the **Acquisition** tab
    - Always **Enable laser-based focusing**
    - Enable **Acquire Time Series**
    - Enable **Use Fluidics**
    - Optionally, enable **Perform shading correction**



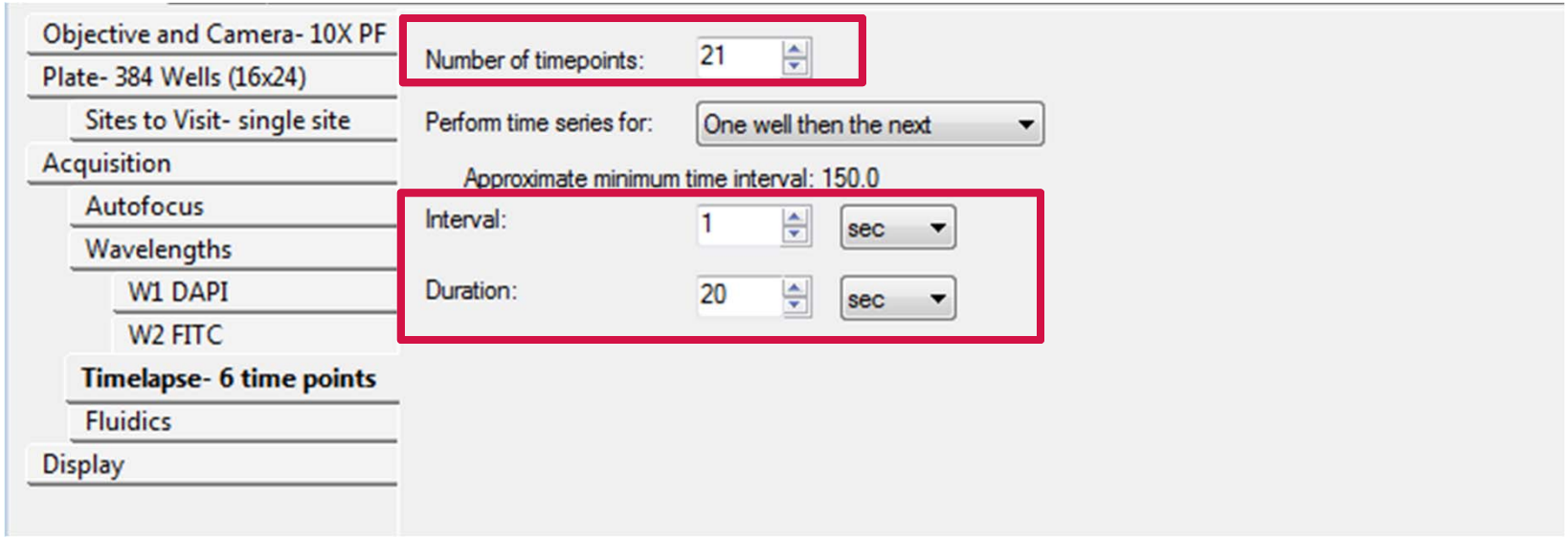
Setting Up a Timelapse Acquisition with Fluidics

11. Click on 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 (uncommon for fluidics experiments).



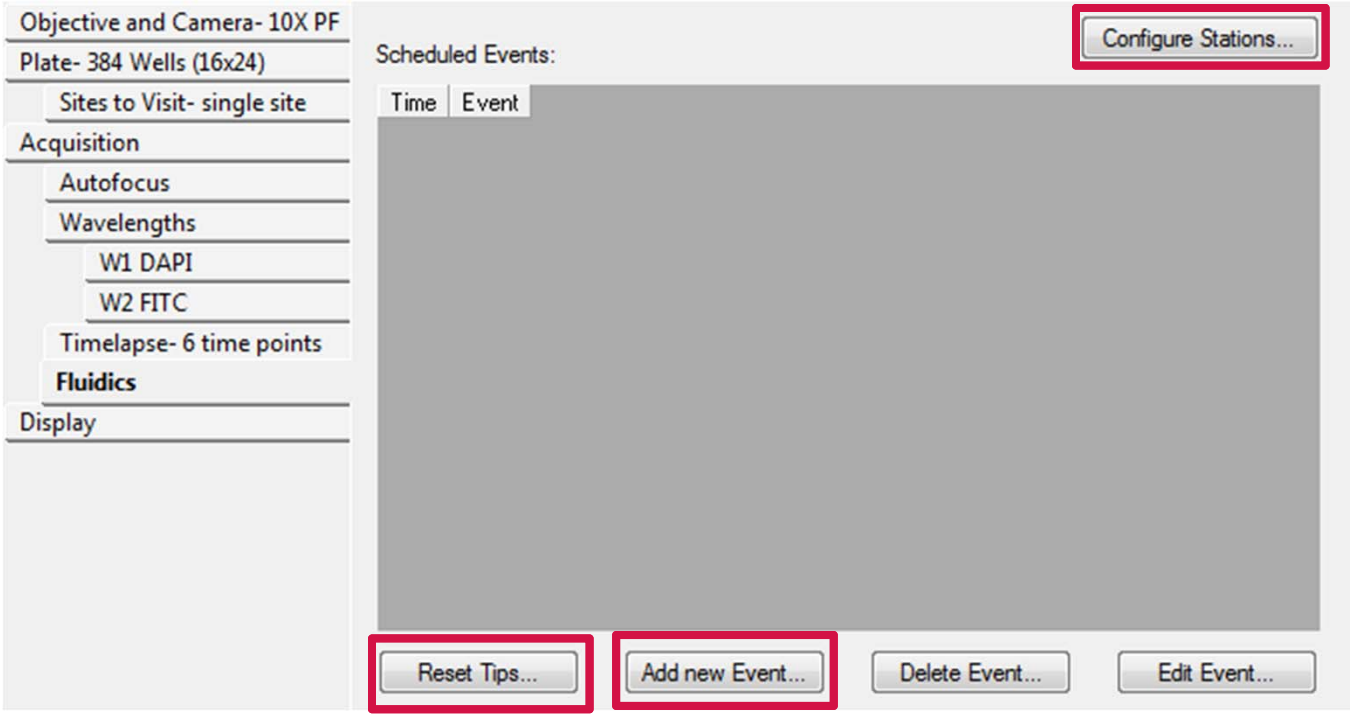
Setting Up a Timelapse Acquisition with Fluidics

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



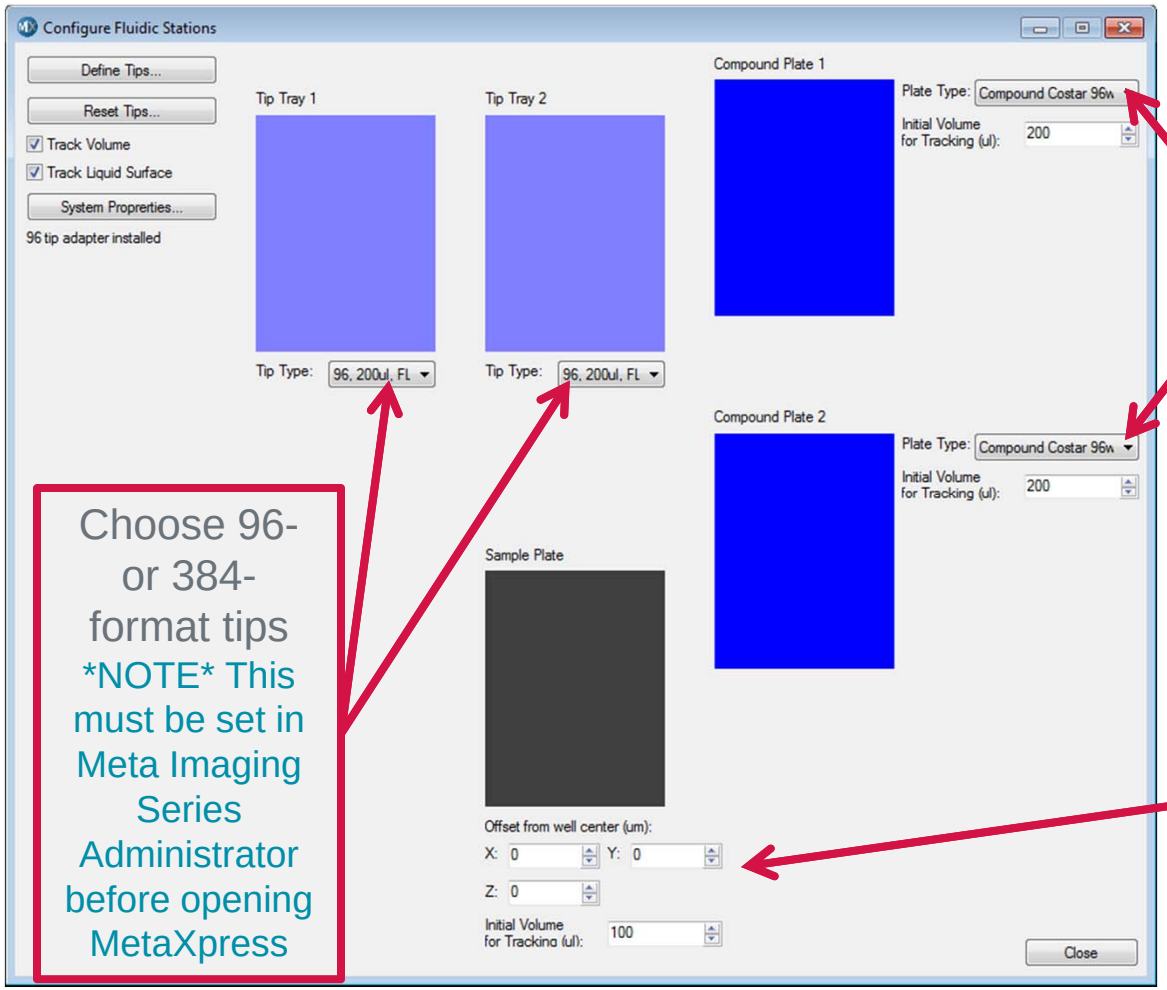
Setting Up a Timelapse Acquisition with Fluidics

12. Click on the **Fluidics** tab to:
    - **Configure Stations**: configure the deck layout, select tips and compound plate type, set global pipetting parameters
    - **Add New Event**: schedule fluidics events
    - **Reset tips**: reset tip and liquid levels



Configuring Stations

On the **Fluidics** tab, click on the **Configure Stations** button



Choose 96- or 384-format tips

NOTE This must be set in Meta Imaging Series Administrator before opening MetaXpress

Select compound plate type. Plate types are configured on the **Plate** tab.

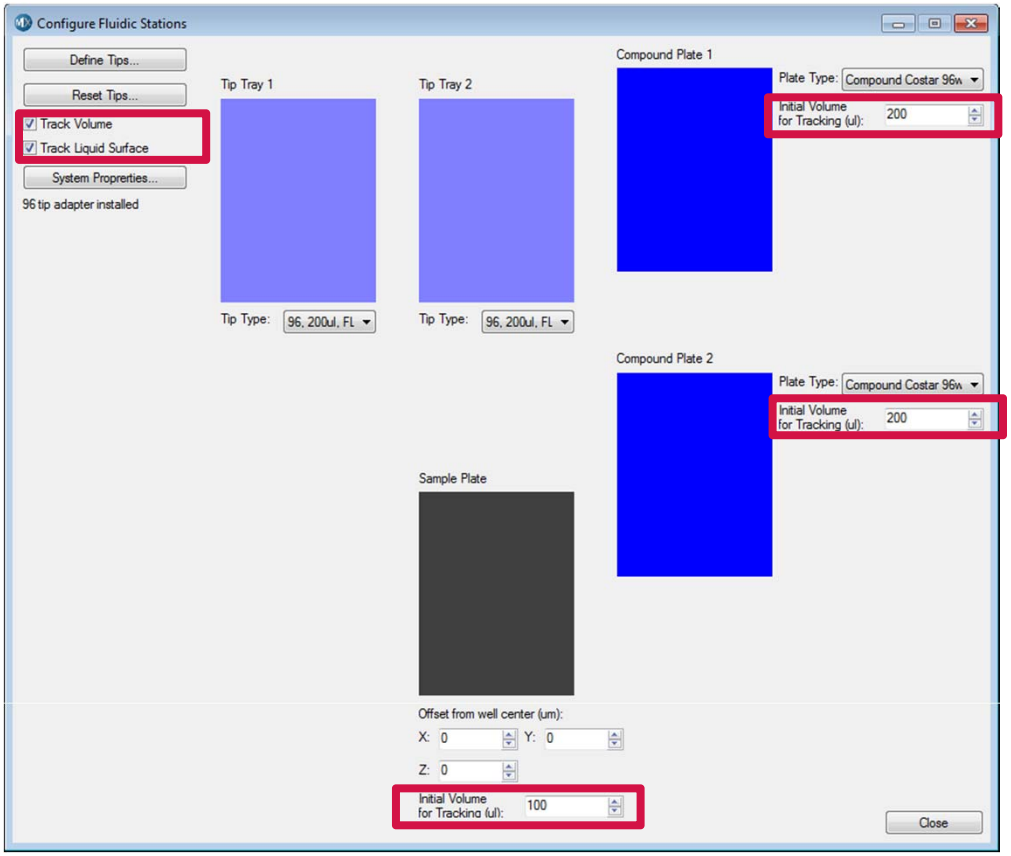
NOTE For washing or media exchange, wells in a compound plate should be filled with media only.

Offset pipetting position in sample plate to minimize cell disturbance. Positive Z offset results in the tip inserting into the well higher than default; negative Z offset results in tip inserting into the well lower than default

Tracking Liquid Levels

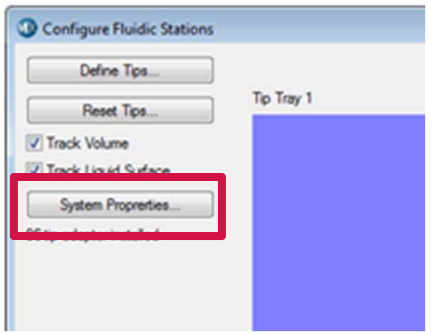
In the **Configure Fluidic Stations** dialog Molecular Devices recommends enabling **Track Volume** and **Track Liquid Surface**

- Enabling **Track Volume** will track available volume and allow the software to warn you if there is not enough volume for scheduled fluidic events
- Enter the appropriate values for **Initial Volume for Tracking** in compound plates and sample plate
- Enabling **Track Liquid Surface** allows the system to draw or dispense liquid at the surface and move the tip up or down as the liquid level changes. This requires enabling **Track Volume**, an accurate plate template, and correct **Initial Volume** settings



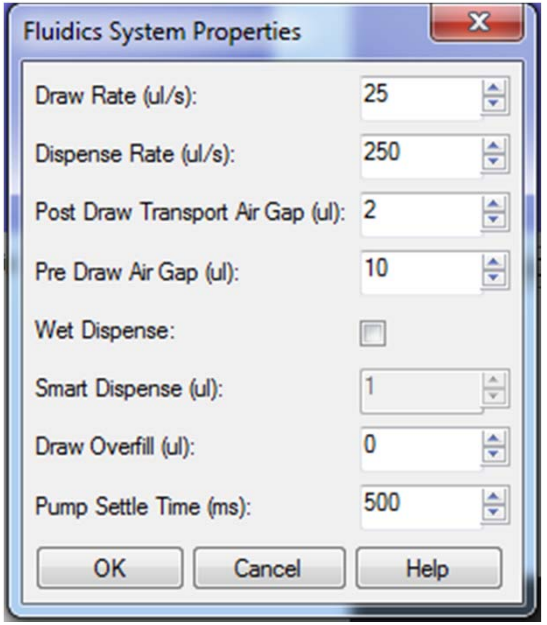
NOTE If **Track Liquid Surface** is not checked, the pipet tip will be inserted near the bottom of the well for draw and dispense events

Configuring System Properties



1. In the **Configure Fluidic Stations** dialog, click on **System Properties** button

2. Click on the **Help** button for explanations on each property

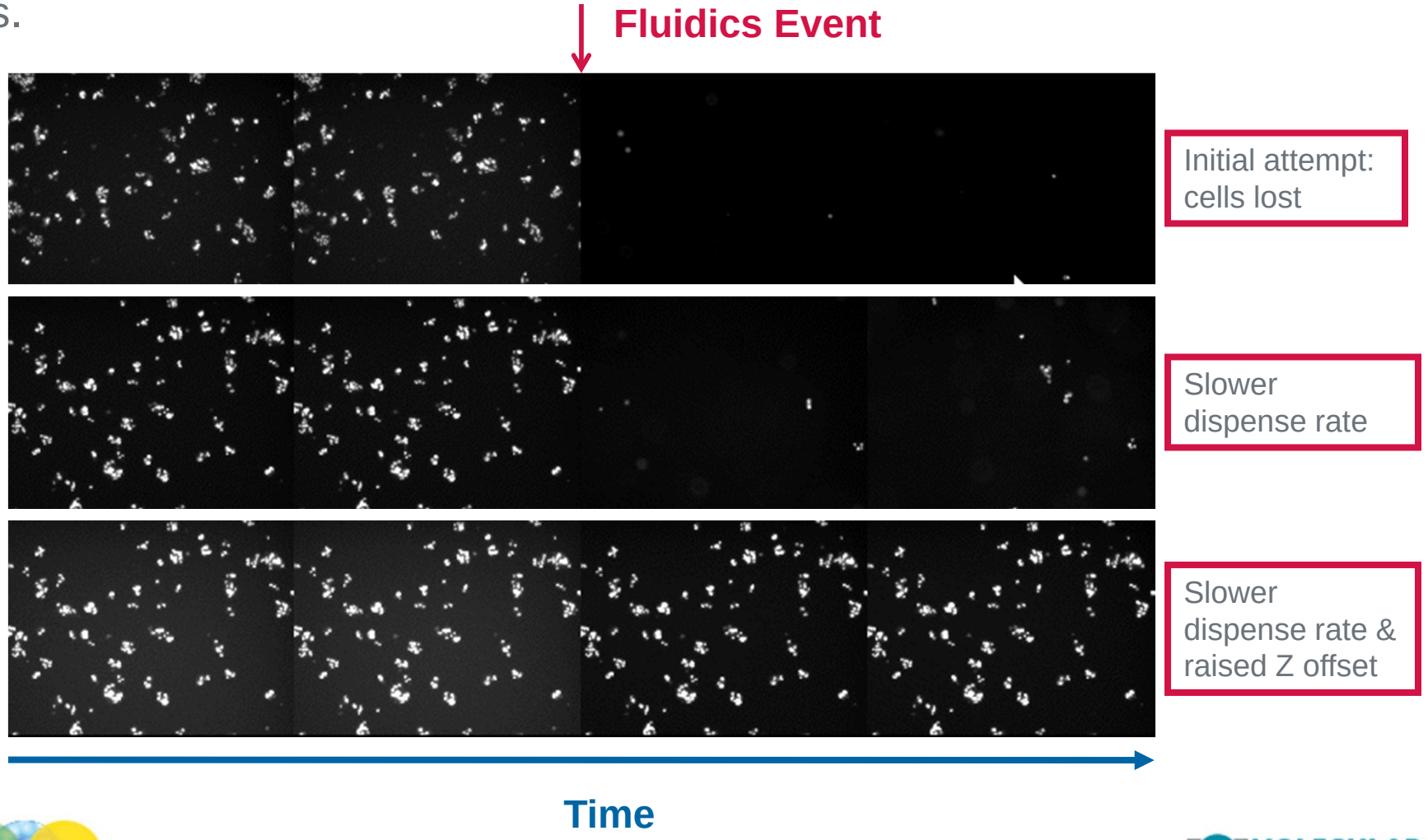


3. Items to note:
   - **Draw Rate** and **Dispense Rate** can affect:
     - Length of experiment, volume accuracy, disturbance to cells
   - **Post Draw Transport Air Gap** minimizes fluid dripping
   - **Pre Draw Air Gap** and **Smart Dispense** help push fluid out
   - When **Wet Dispense** is enabled
     - Tip is just below liquid surface in sample plate when **Track Liquid Surface** is on
     - Tip is just above well bottom in sample plate when **Track Liquid Surface** is off
   - When **Wet Dispense** is disabled
     - Tip is just above the liquid in sample plate
   - **Draw Overfill** draws extra compound for better volume accuracy
   - Molecular Devices suggests using the **Pump Settle Time** default value

NOTE the settings in **Fluidics System Properties** are saved with the protocol and used for all fluidics events

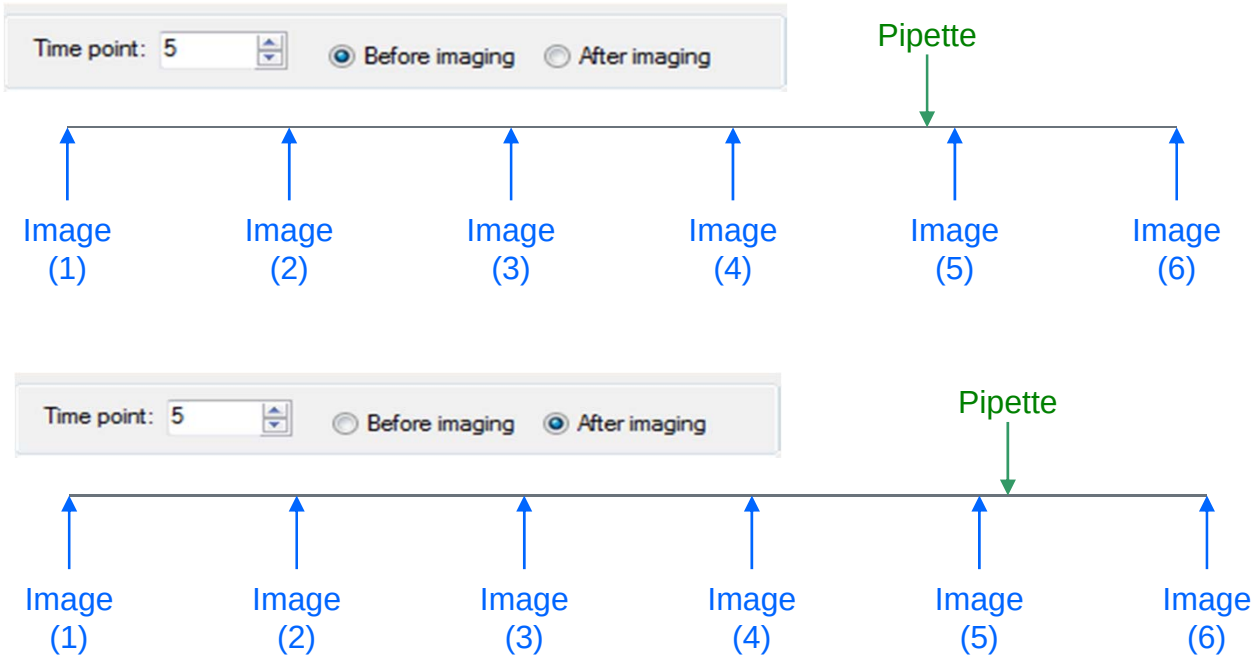
Optimizing System Properties

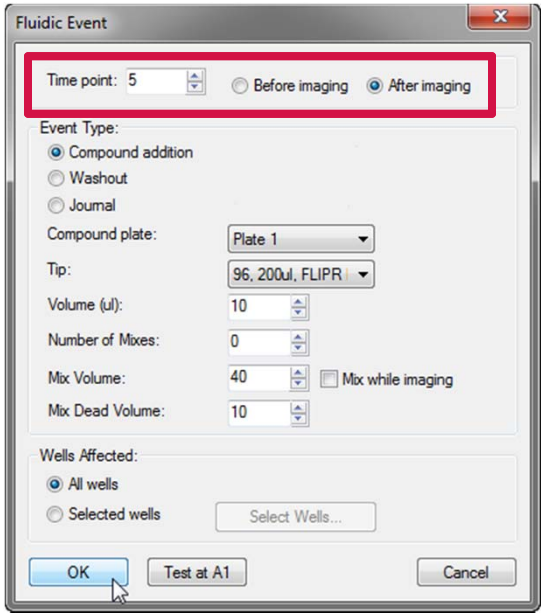
Molecular Devices highly recommends testing and optimizing fluidics settings for any experiment. As the example below shows, multiple properties can affect results.



Event Scheduling

12. On the **Fluidics** tab, click on the **Add New Event** button to begin setting up a fluidics event
    - Events can be scheduled to occur before or after any time point (see diagram below)
    - If time points are close together, a fluidics event may delay acquisition of subsequent time points

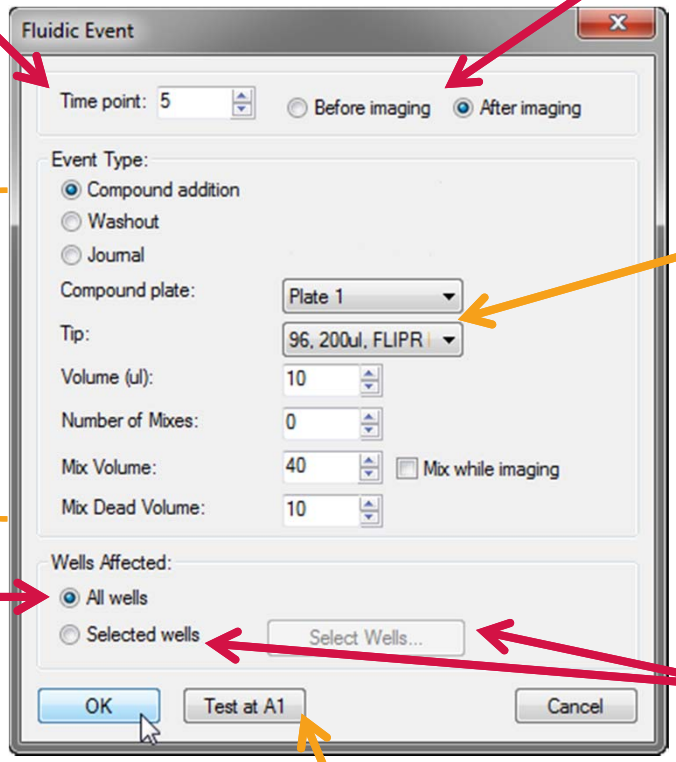




Fluidic Event Dialog

Select time point for the scheduled fluidics event to occur

Select if fluidics event occurs **Before imaging** or **After imaging** of selected time point



Select **Event Type** and enter details for liquid transfer (see next sections for more details)

Select **Compound Plate** and **Tip type** from drop-down menus. Compound plates are configured on the **Plate** tab of **Plate Acquisition Setup** dialog

Select **All wells** to have fluidics event occur in all wells selected in the Plate Map of the **Plate Acquisition Setup** dialog

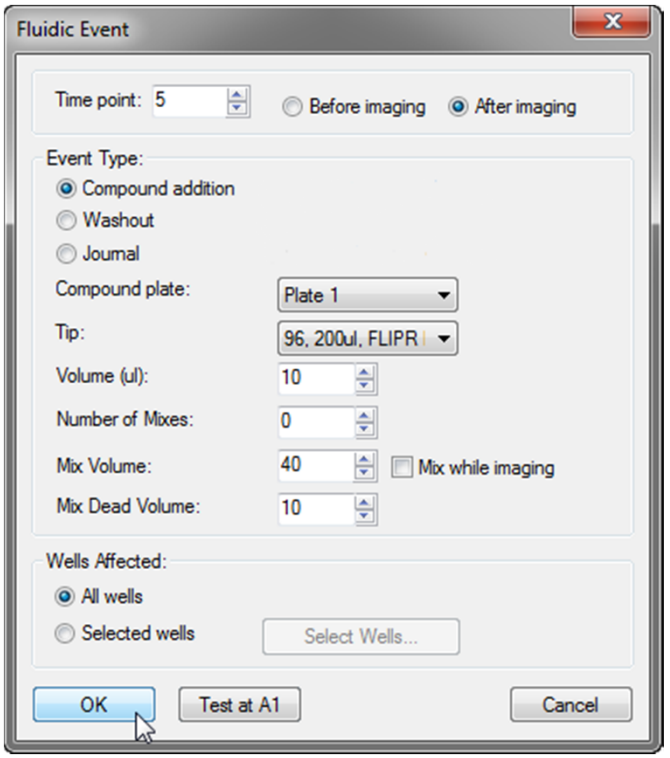
To apply the fluidics event to only particular wells, select **Selected wells** and then click on the **Selected wells** button to highlight the desired well(s) in the plate map.

Test A1 button will apply these fluidics event settings to well A1, but does not schedule the event. Use this option to perform an initial test of your settings.

Event Scheduling – Compound Addition Event

Compound addition adds reagent to the sample plate at the designated **Time point**:

i. Imaging is paused
ii. Pipettor loads a tip
iii. Aspirates reagent from compound plate
iv. Adds reagent to the corresponding well in sample plate (and optionally mixes)
v. Disposes tip
vi. Imaging continues



To configure this dialog:

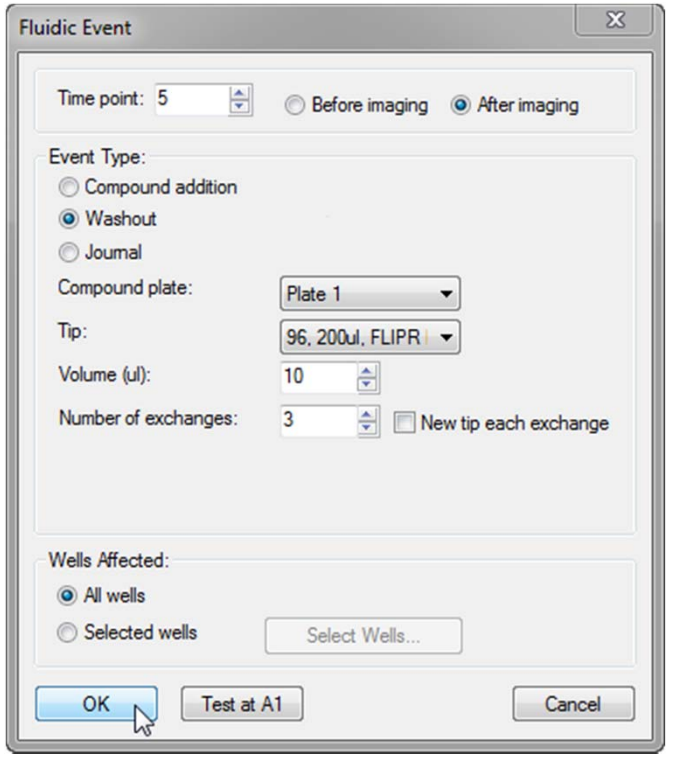
- Enter the **Volume** to be drawn from the compound plate and dispensed into the sample plate
- Optionally select **Number of Mixes** and **Mix Volume**
- Enter a value for **Mix Dead Volume** – volume that remains in tip during mix to prevent bubbles and is dispensed out at the end of mix cycle
- Optionally enable **Mix while imaging**
- Select wells to apply these parameters to

NOTE REagent drawn from the compound plate is dispensed to the corresponding well in the sample plate. For example, draw from compound plate A01 and dispense to sample plate A01.

Event Scheduling – Wash Out Event

A wash out event performs a wash (or media refresh) in the sample plate. At the designated **Time point**:

i. Imaging is paused
ii. Pipettor loads a tip and aspirates liquid from sample plate
iii. Disposes liquid (and optionally tip)
iv. Aspirates liquid (new tip optional) from corresponding well of compound plate
v. Dispenses liquid into sample plate
vi. Repeats ii – v as specified in **Number of exchanges**
vii. Disposes of tip
viii. Imaging continues



To configure this dialog:

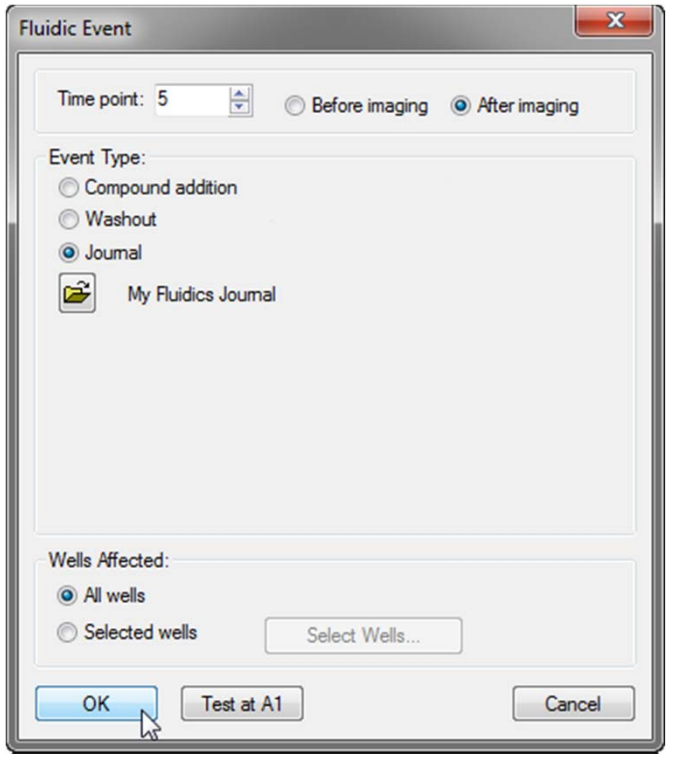
- Enter the **Volume** to aspirate and dispense
- Specify the **Number of exchanges**
- Optionally Enable **New tip for each exchange**
- Select wells to apply these parameters to

NOTE Reagent drawn from the compound plate is dispensed to the corresponding well in the sample plate. For example, draw from compound plate A01 and dispense to sample plate A01.

Event Scheduling – Journal Event

A journal event runs a journal (macro) at the specified time point. A journal:

i. Is a script written to customize fluidics steps in a protocol
ii. Can be used to serial dispense compound from one well into multiple sample wells
iii. Can be used to save media from sample plate for later analysis
iv. Can be used for staggering pipetting events
v. …. And more



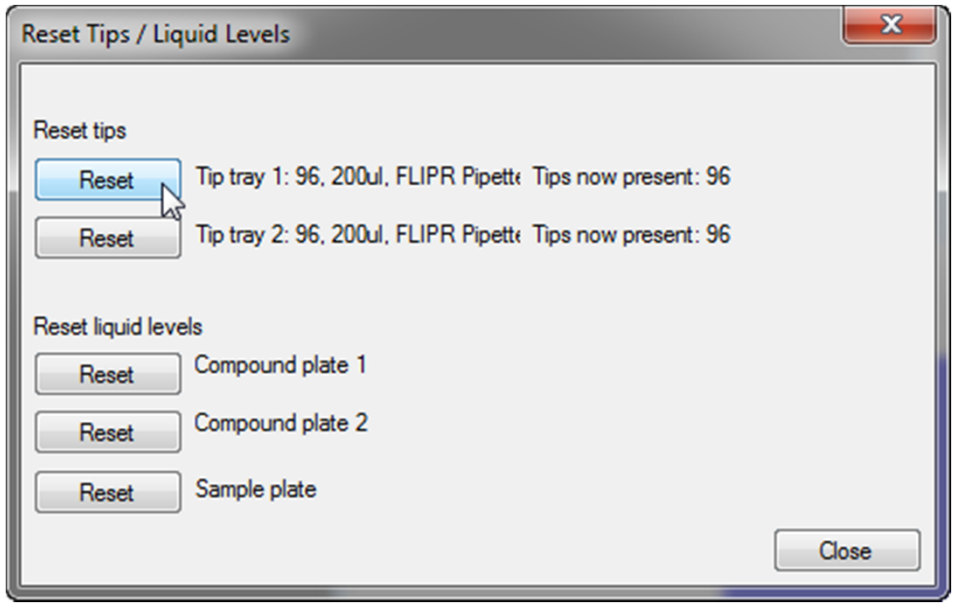
To configure this dialog:

- Select **Journal** under the **Event Type** section
- Click on the **Open File** icon to navigate to the location of the previously configured journal
- Select wells to apply these parameters to

Reset Tip and Liquid Levels

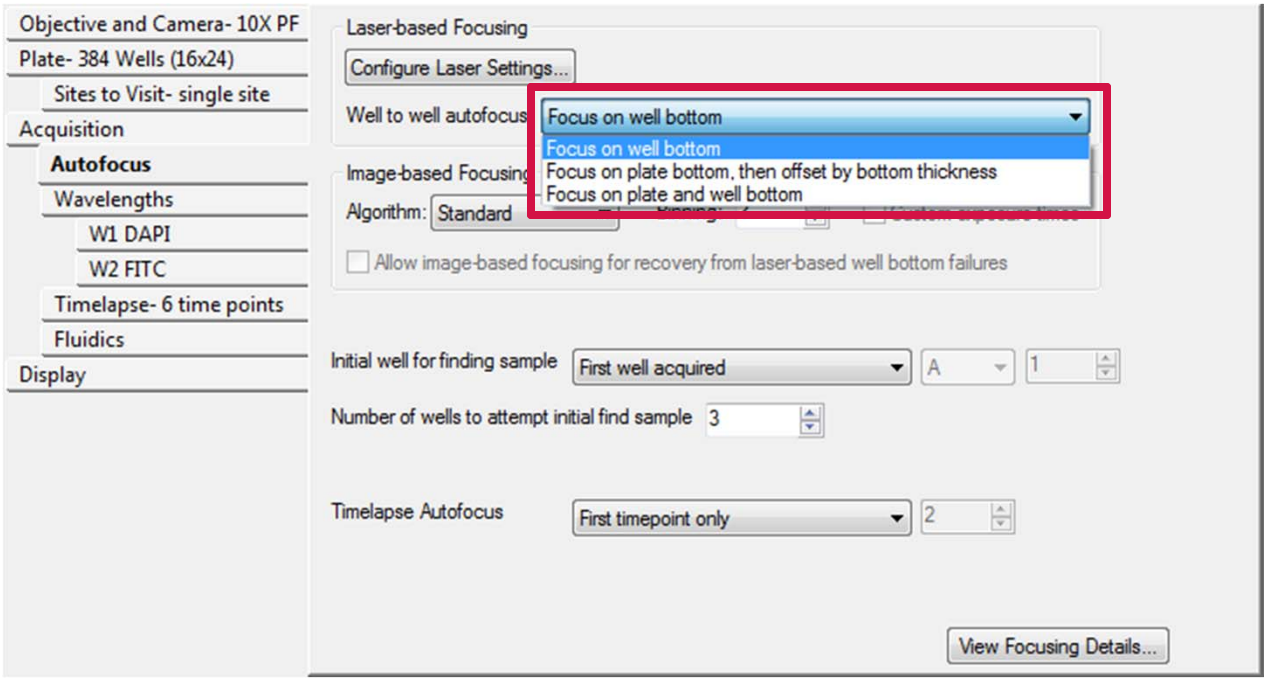
12. On the **Fluidics** tab, click on the **Reset Tip** button to
    - Reset tips after loading a full tip rack (system keeps track of tips used during testing and acquisition)
    - Reset liquid levels after loading fresh compound plates
    - Reset liquid level after inserting a fresh sample (imaging) plate

NOTE A reminder to reset tips and/or liquid levels will always appear when you press the **Acquire Plate** button



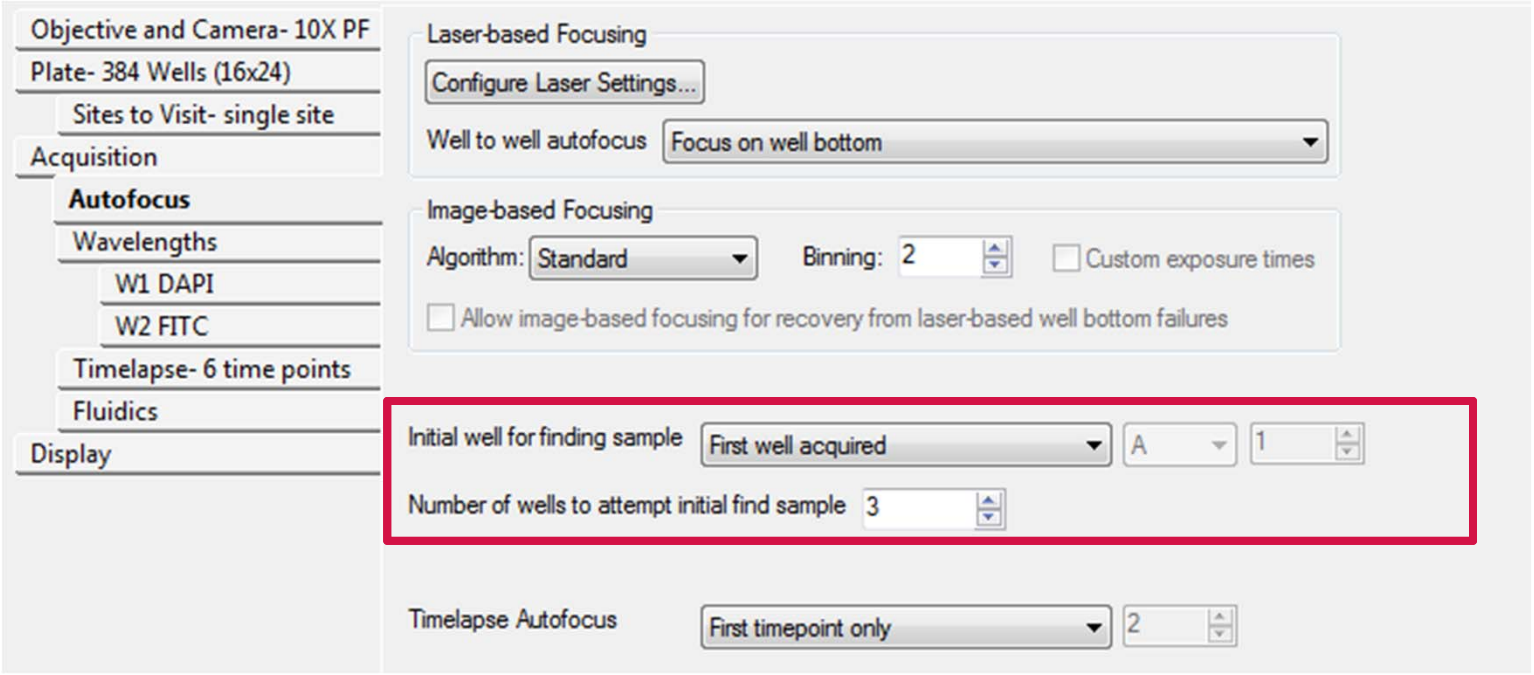
Setting Up a Timelapse Acquisition with Fluidics

13. Click on the **Autofocus** tab
    - Select the appropriate option for **Well to well autofocus**:
      - **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)



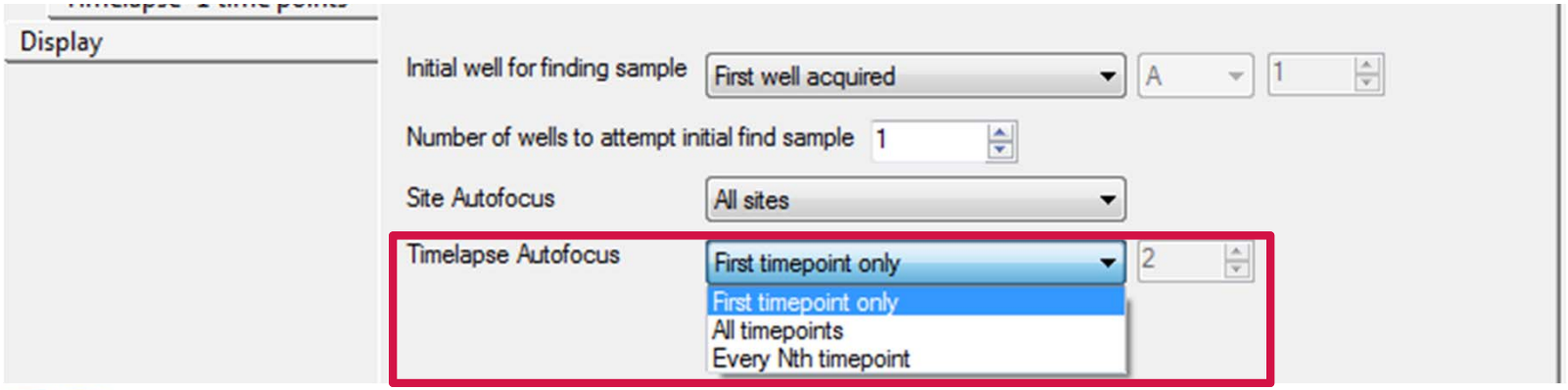
Setting Up a Timelapse Acquisition with Fluidics

13. 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 with Fluidics

13. 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.

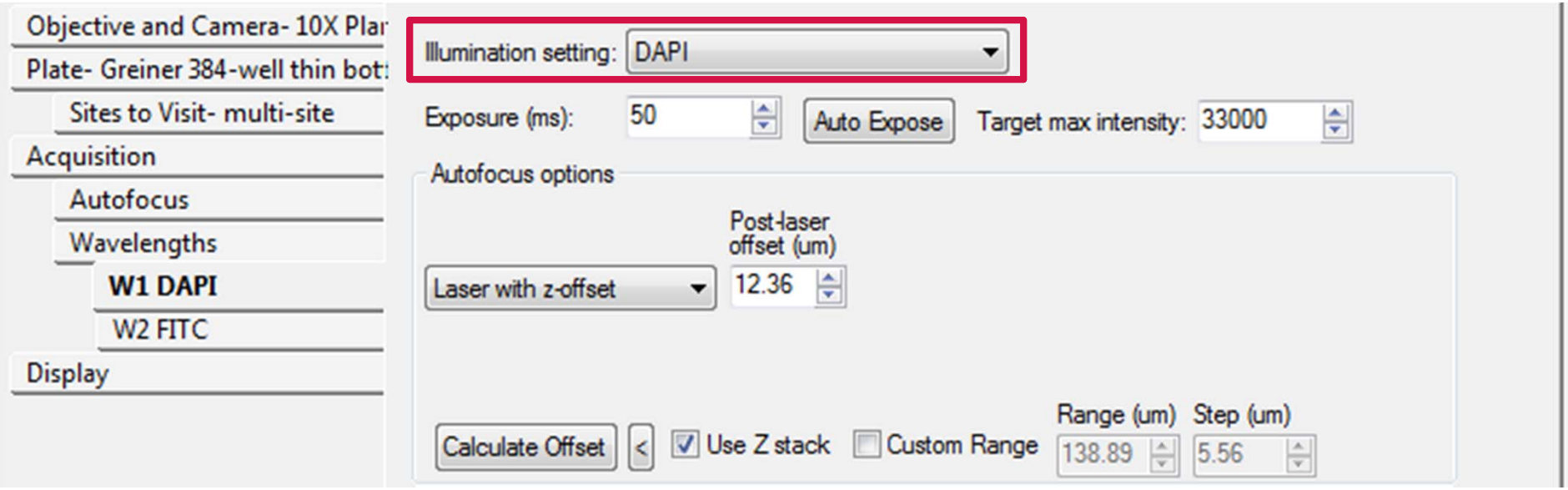


Setting Up a Timelapse Acquisition with Fluidics

14. Select the **Wavelengths** tab
    - Select 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 select up to 8 wavelengths

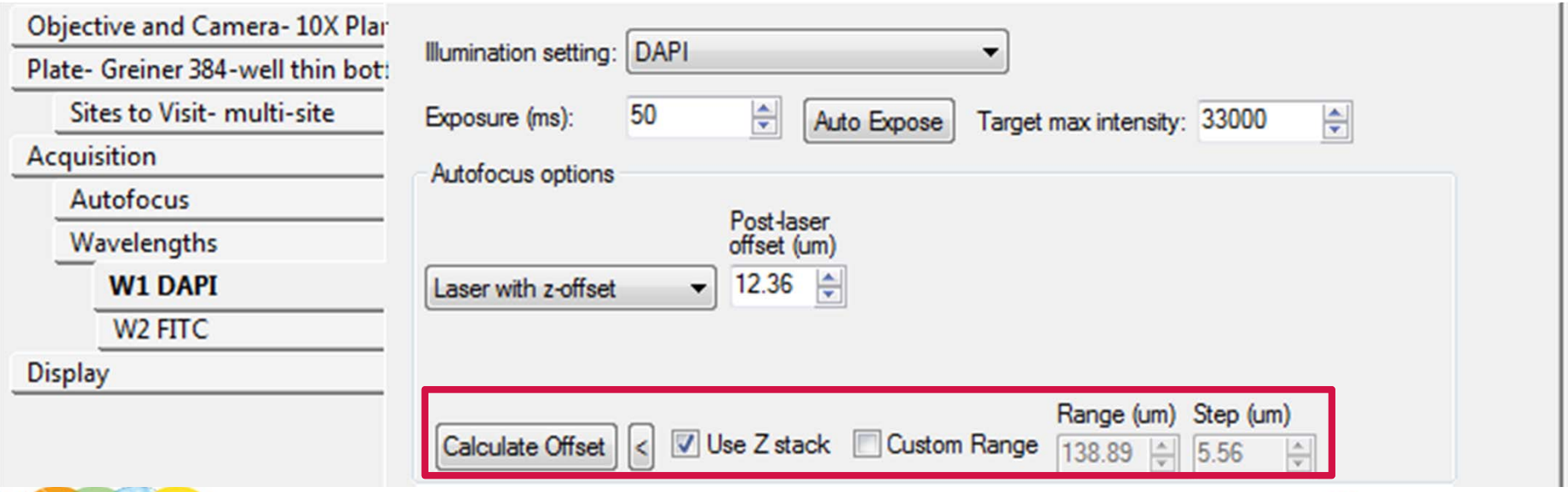
Setting Up a Timelapse Acquisition with Fluidics

15. Select the **W1** tab
    - Select correct **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

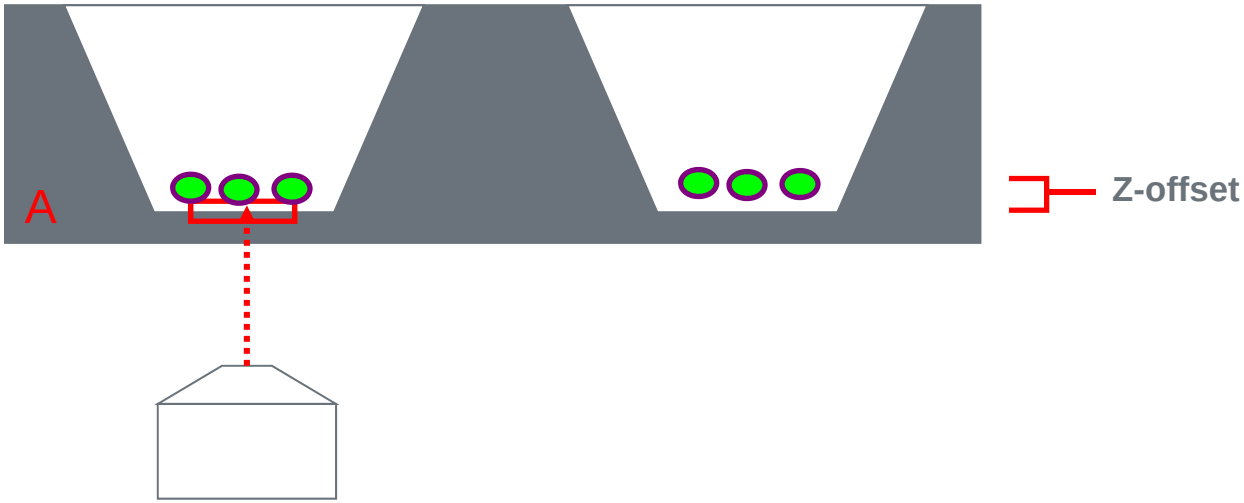


Setting Up a Timelapse Acquisition with Fluidics

15. 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?



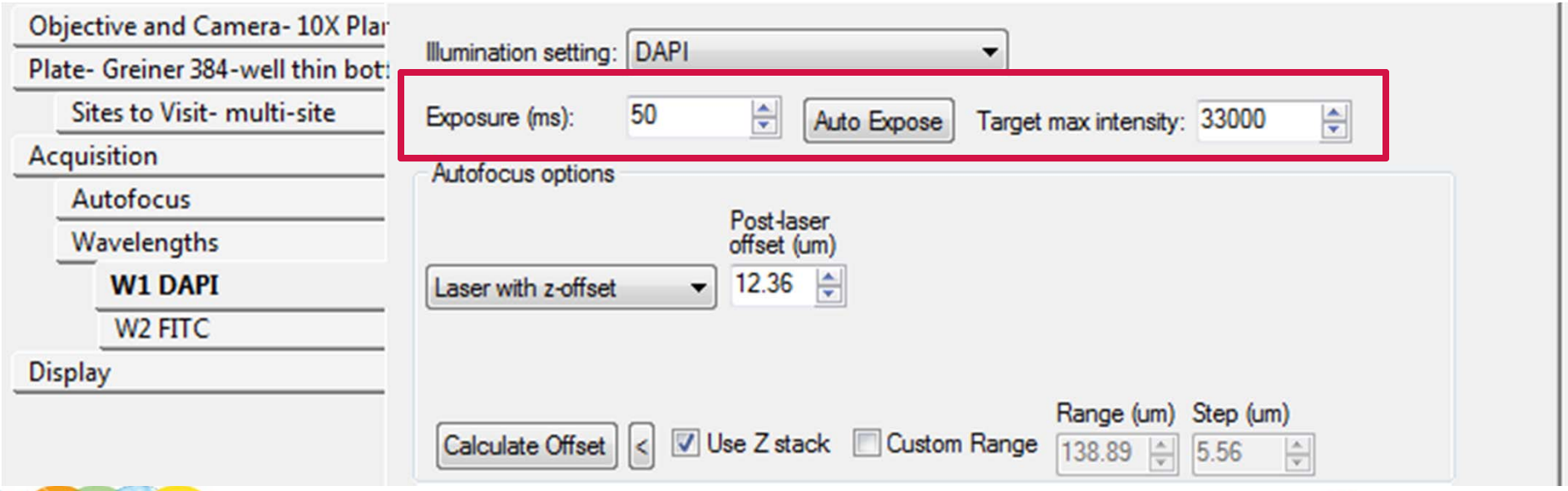
Post-laser offset is the Z distance between the bottom of the well and the sample

- 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
- It is recommend to checking multiple wells for consistency

Setting Up a Timelapse Acquisition with Fluidics

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

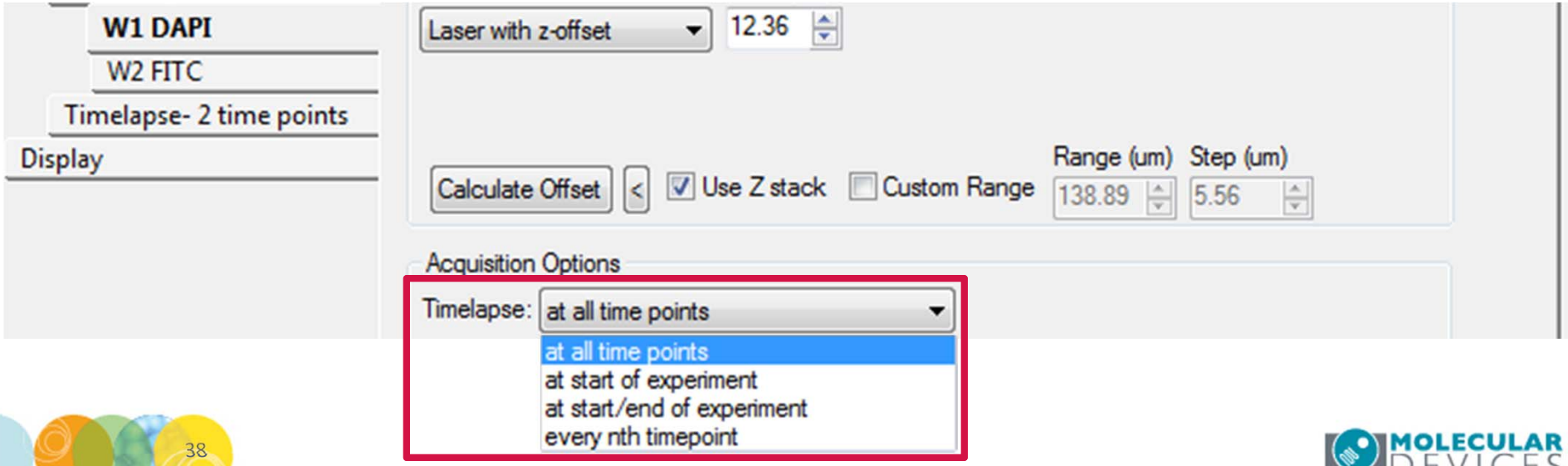
      - Evaluate the image for pixel intensity range (bit range)
      - For fluidics experiments you may need to decrease/increase exposure time according to expected results. For example, if an increase in intensity is expected after the fluidics event, set the exposure so that the first time point is between 10-25% of the bit range of the camera. It may be necessary to run test wells in order to determine the optimal exposure time for the entire experiment.



Setting Up a Timelapse Acquisition with Fluidics

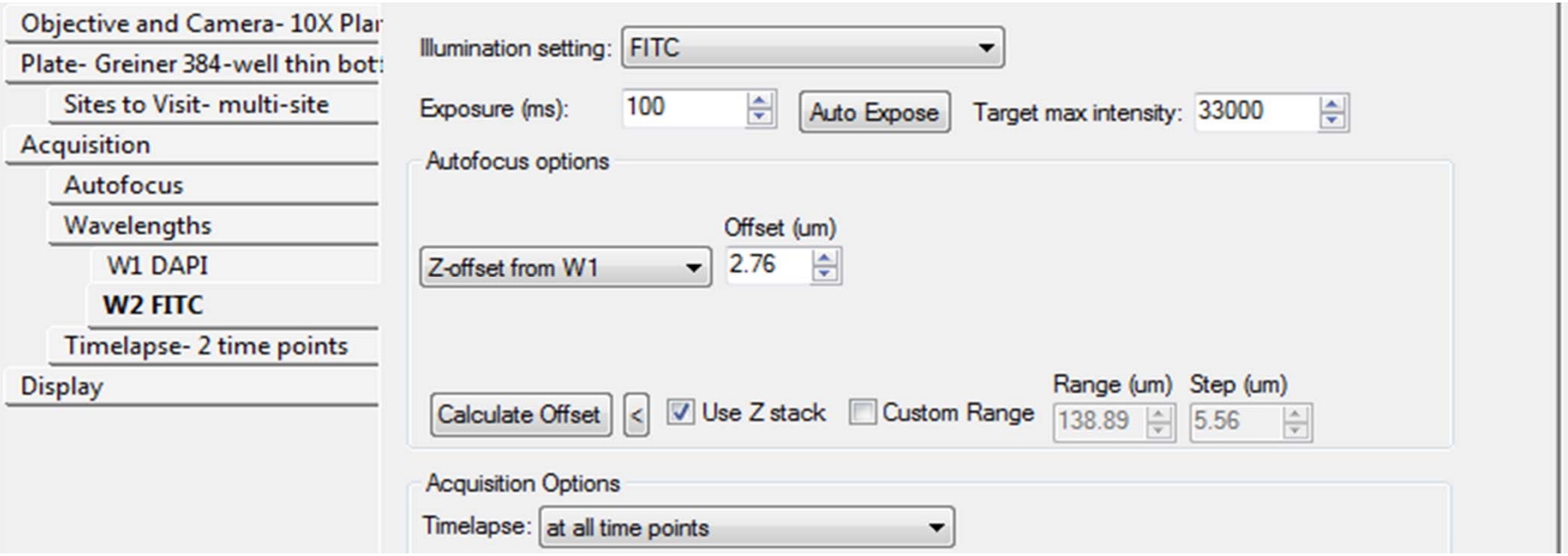
15. On the **W1** tab
    - Select the appropriate option from the **Acquisition Options** drop-down menu to specify how often to collect the selected wavelength
        - **At all time points** acquires this wavelength at each time point
        - **At start of experiment** acquires this wavelength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

NOTE This option is only available when your acquisition protocol contains more than one wavelength.



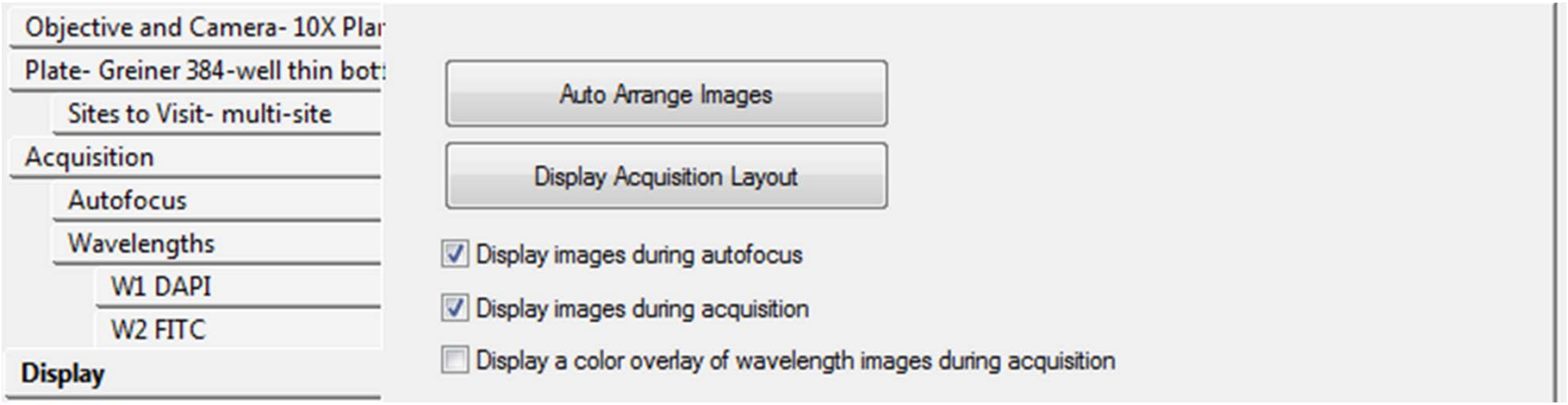
Setting Up a Timelapse Acquisition with Fluidics

16. Click on the **W2** tab (and subsequent W tabs)
    - Select 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 appropriate **Acquisition Options** for Timelapse



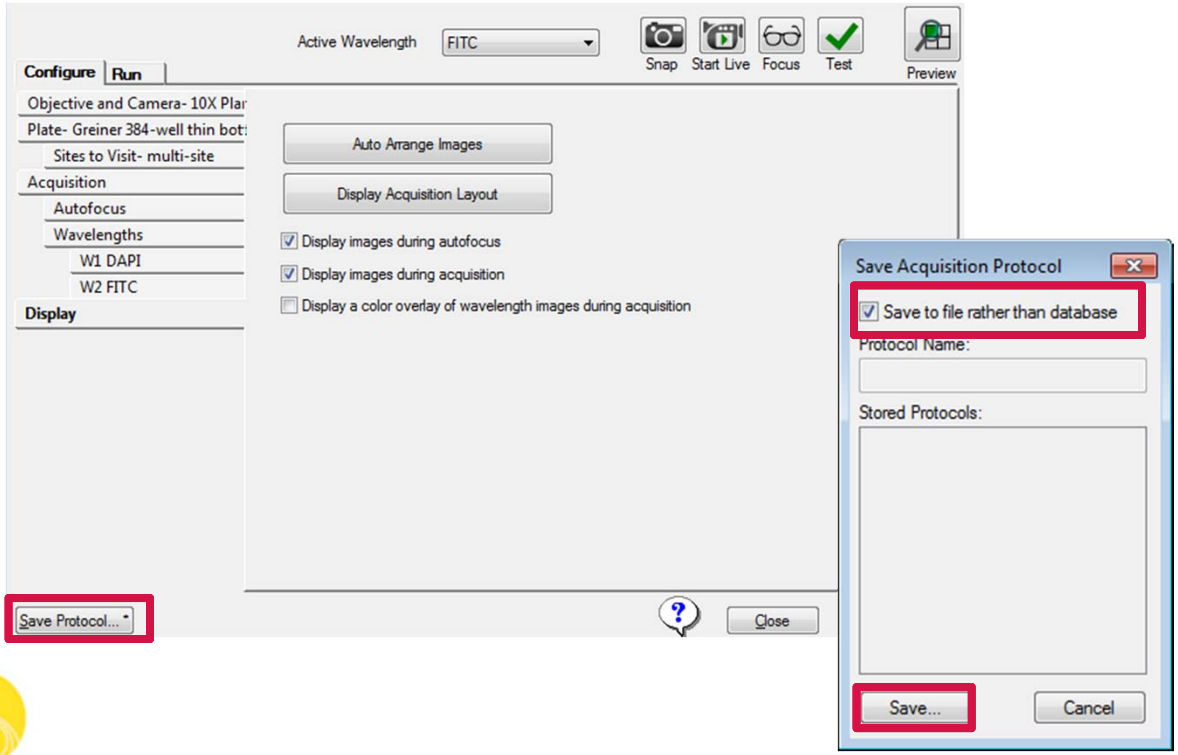
Setting Up a Timelapse Acquisition with Fluidics

17. Click on 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.



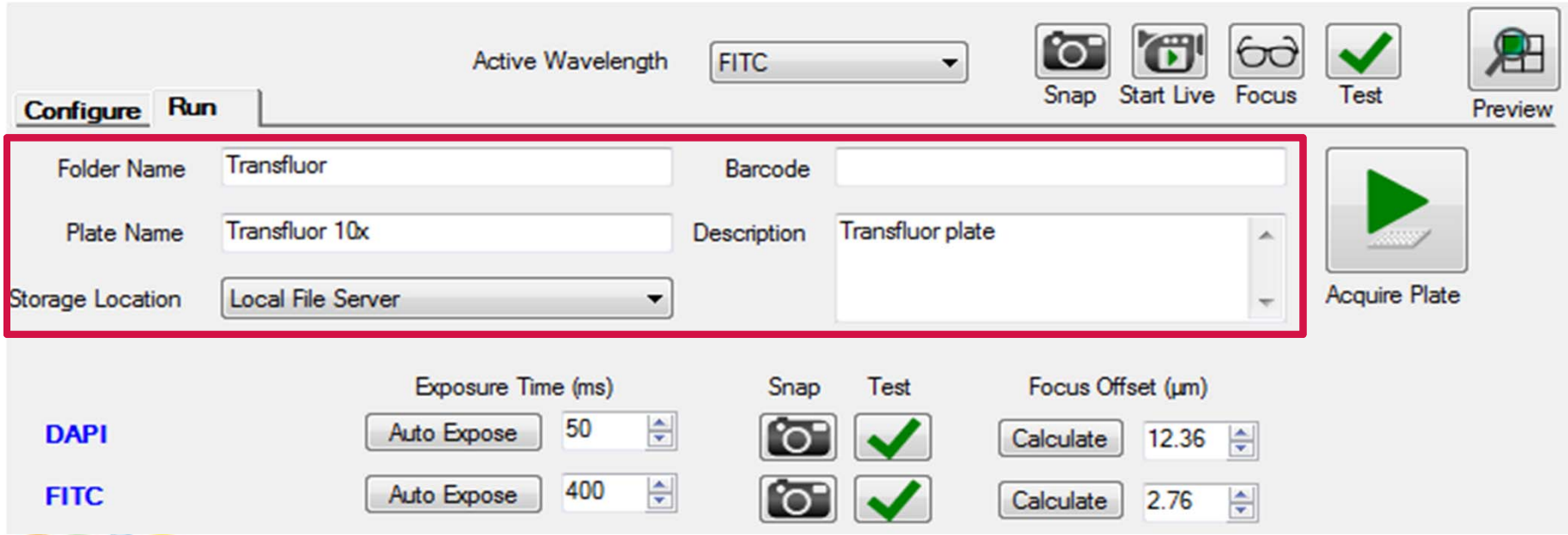
Setting Up a Timelapse Acquisition with Fluidics

18. 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
    - It is recommended to save your settings to a file, rather the database
    - Click on **Save** , name the protocol, and navigate through windows to save the file (.hts)



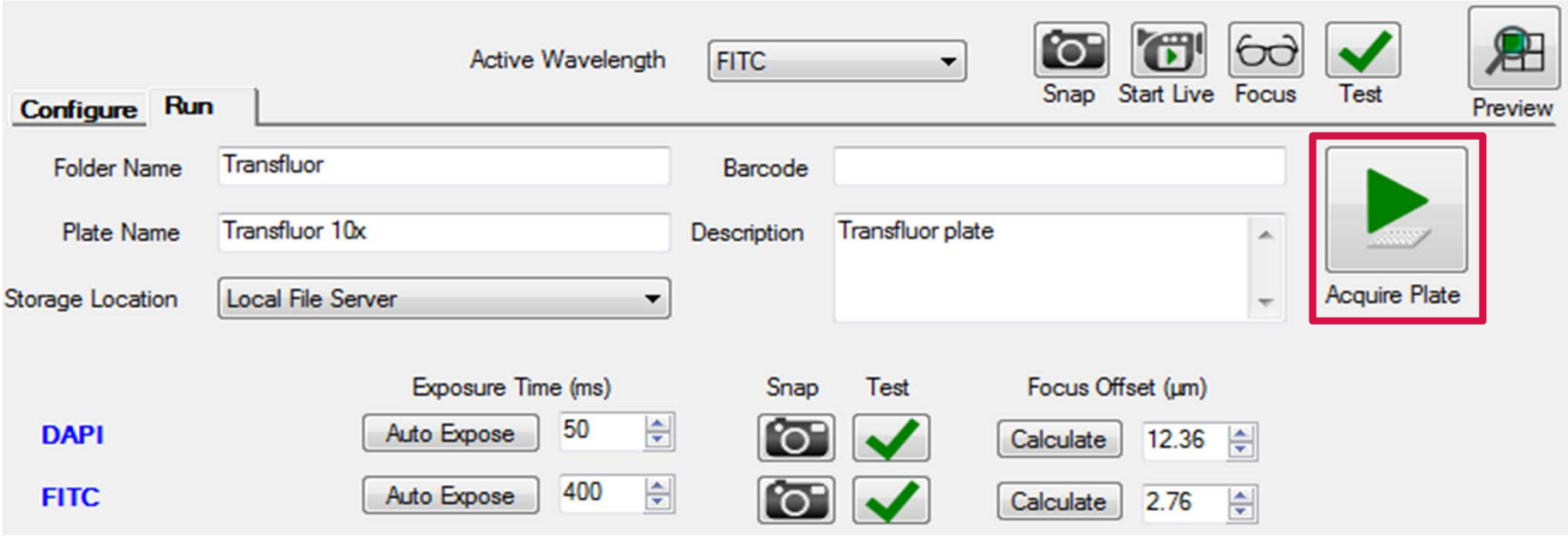
Setting Up a Timelapse Acquisition with Fluidics

19. Click on 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 with Fluidics

20. 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