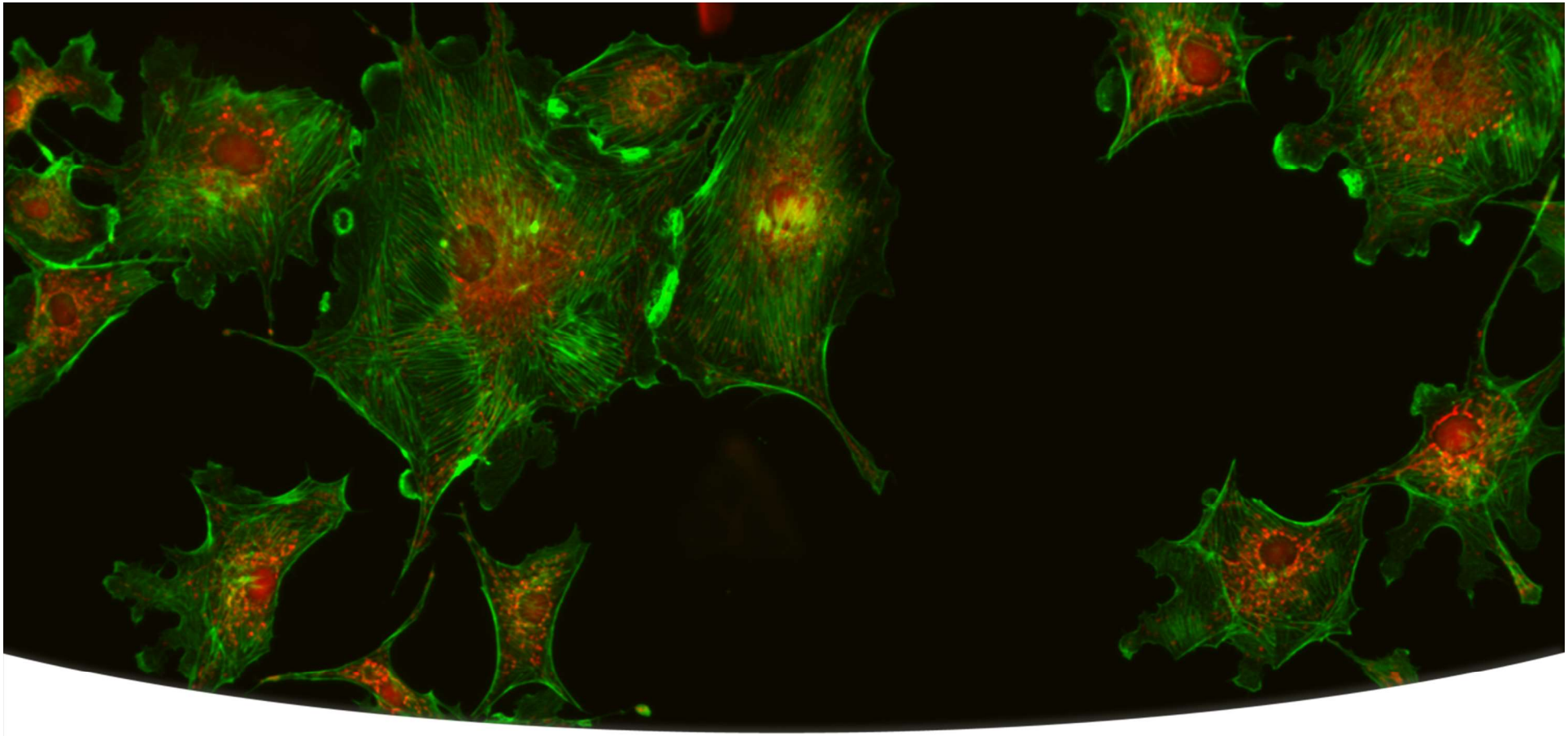


ImageXpress® Micro Standard and MetaXpress® 5.3 Acquisition Guide

Molecular Devices

Revision A

ImageXpress® Micro

Hardware Features

Together through life sciences.

Molecular Devices

The ImageXpress® Micro Imaging System

Benchtop Automated Widefield Microscope

High Speed Laser Autofocus

4 Objectives

5 Filter Cubes

Linear Encoded Stage

Sample Flexibility

Slide to 1536-Well Plates

12 Bit Images

Cooled CCD Camera

Molecular Devices

ImageXpress Micro Standard Camera and Light Source

ImageXpress Micro Standard features:

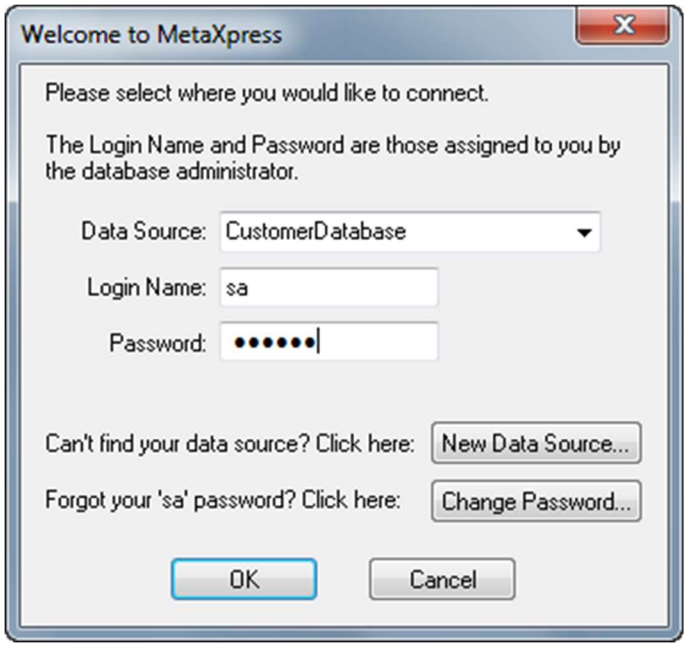
- 1.3 Megapixel CCD Camera
- 12-bit range
- 300 Watt Xenon Lamp

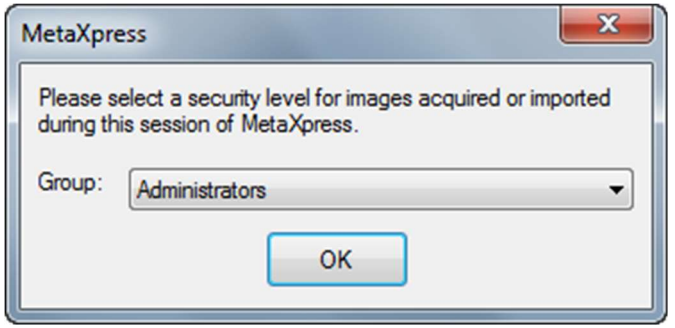
MetaXpress® 5.3

Acquisition

Starting up the ImageXpress® Micro System

- Turn on options controller
- Turn on light source (if not already on)
- Turn on power supply
- Turn on computer
- Turn on monitor
- Log in to Windows
- Start up MetaXpress® Software
- Log in to database
- Select database Group



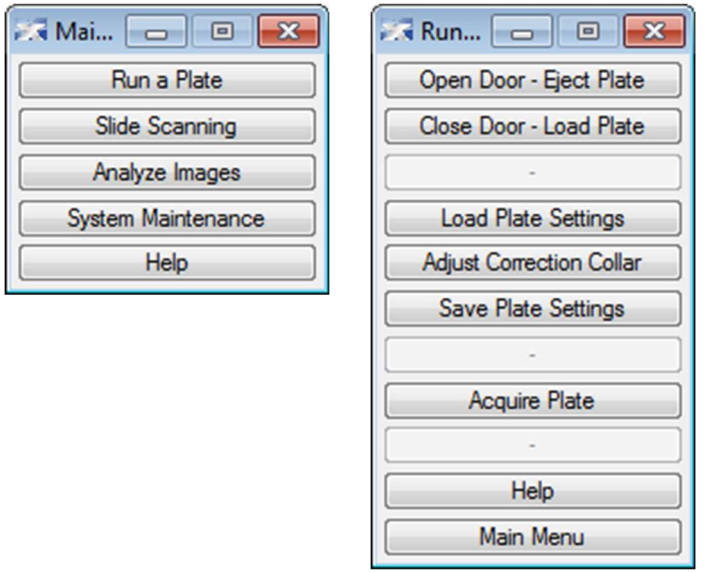


Molecular Devices

Loading the Main Taskbar

In MetaXpress, on the main toolbar:

- Click on **Journal → Taskbars → Load Taskbar**
- Navigate to C:\MX5.x\Taskbars
- Load **Main Taskbar.JTB**

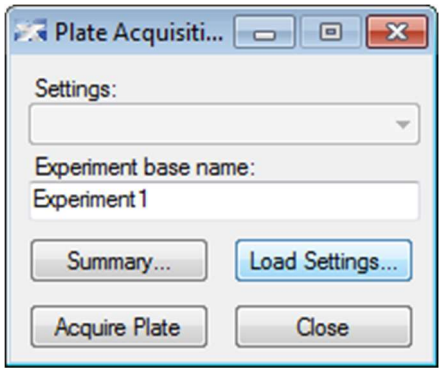


Molecular Devices

Loading an Existing Protocol – Method 1

In MetaXpress, on the main toolbar:

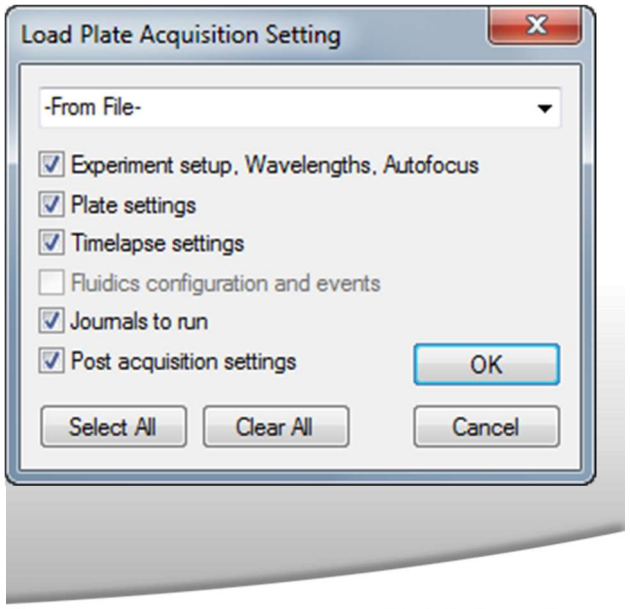
- Click on **Screening → Plate Acquisition**



- Click on **Load Settings**
  - Select **-From File-** from the drop-down menu and click on **OK**

OR

  - Select settings from the drop-down menu



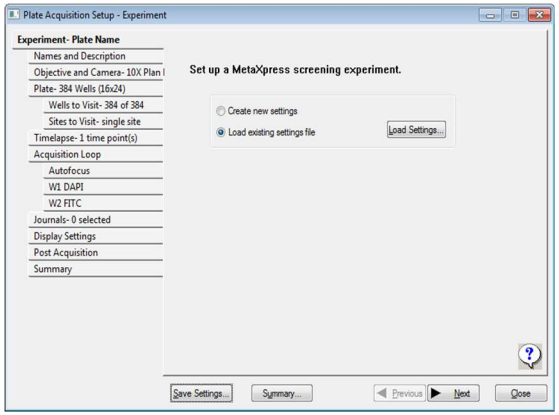
- Enter a name in the **Experiment base name field**
- Make sure there is a plate in the system
- Click **Acquire Plate**

Molecular Devices

Loading an Existing Protocol – Method 2

In MetaXpress, on the main toolbar:

- Click on **Screening → Plate Acquisition Setup**



- On the **Experiment** tab
  - Select **Load existing settings file**
  - Click on **Load Settings**
    - Select **-From File-** from the drop-down menu and click on **OK**

OR

    - Select settings from the drop-down menu

- Make sure there is a plate in the system

- On the **Summary** tab, click **Acquire Plate**

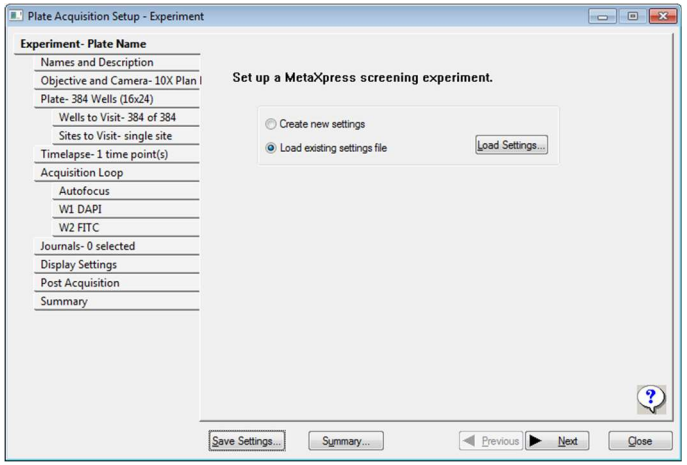
Together through life sciences.

Molecular Devices

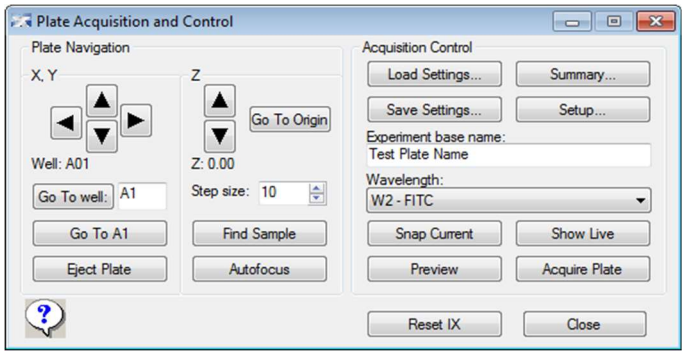
Create New Protocol Settings

In MetaXpress, on the main toolbar click on:

- **Screening → Plate Acquisition Setup**

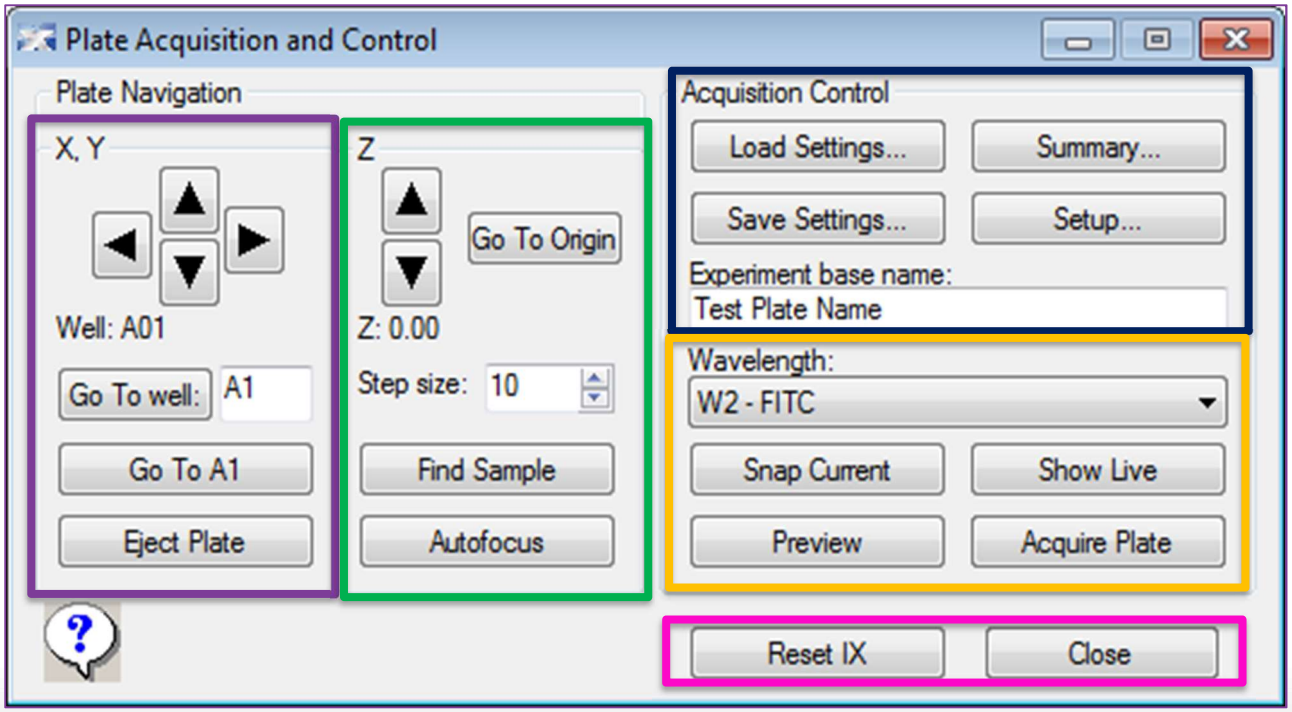


- **Screening → Plate Acquisition and Control**



Molecular Devices

Plate Acquisition and Control



- ✓ Stage Control to move well-to-well
- ✓ **Eject Plate/ Load Plate** will open/close the stage door

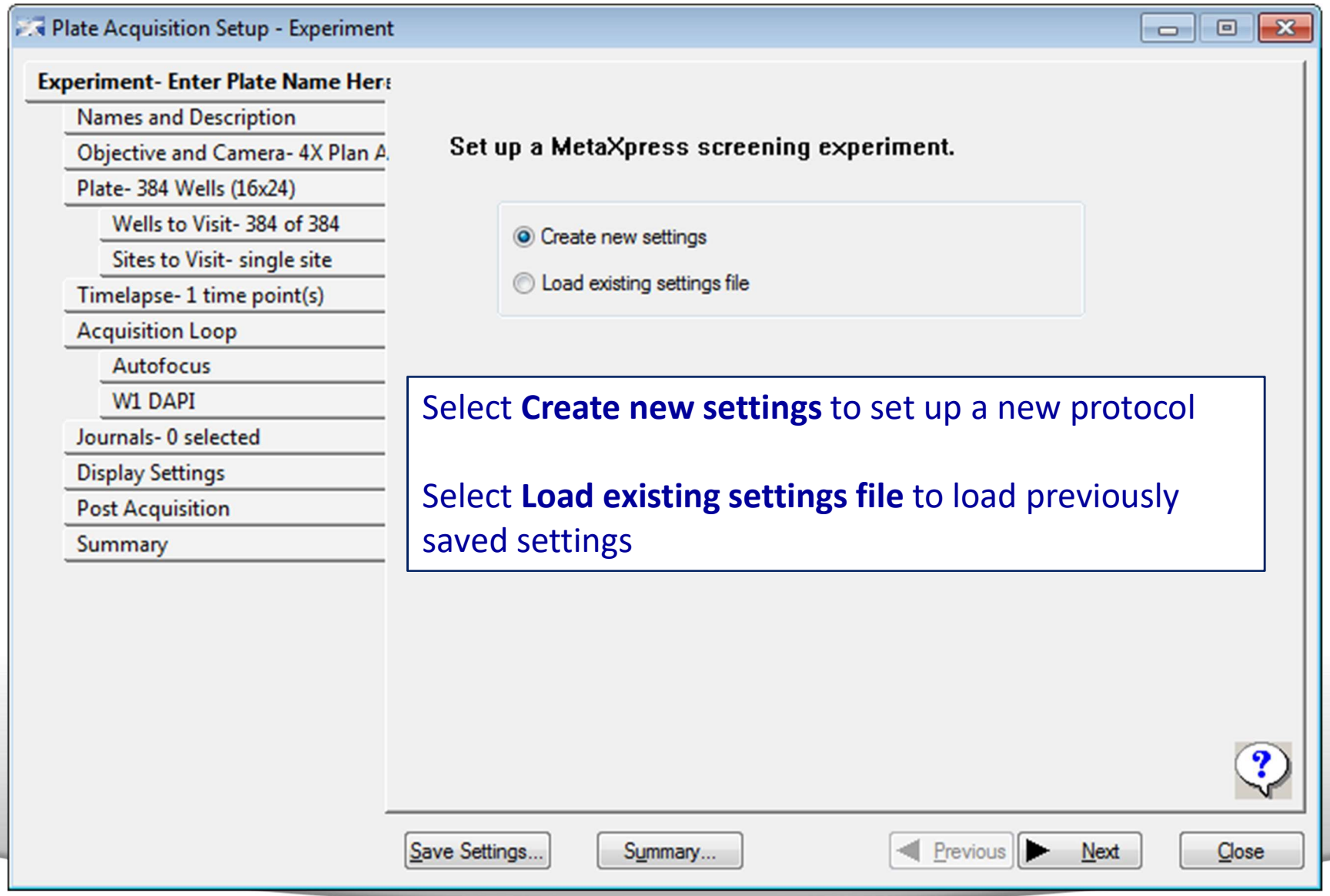
- ✓ **Find Sample:**
- ✓ **Autofocus:**

- ✓ **Summary**: open a window giving with a summary of your plate settings
- ✓ **Setup**: open the Plate Acquisition dialog box

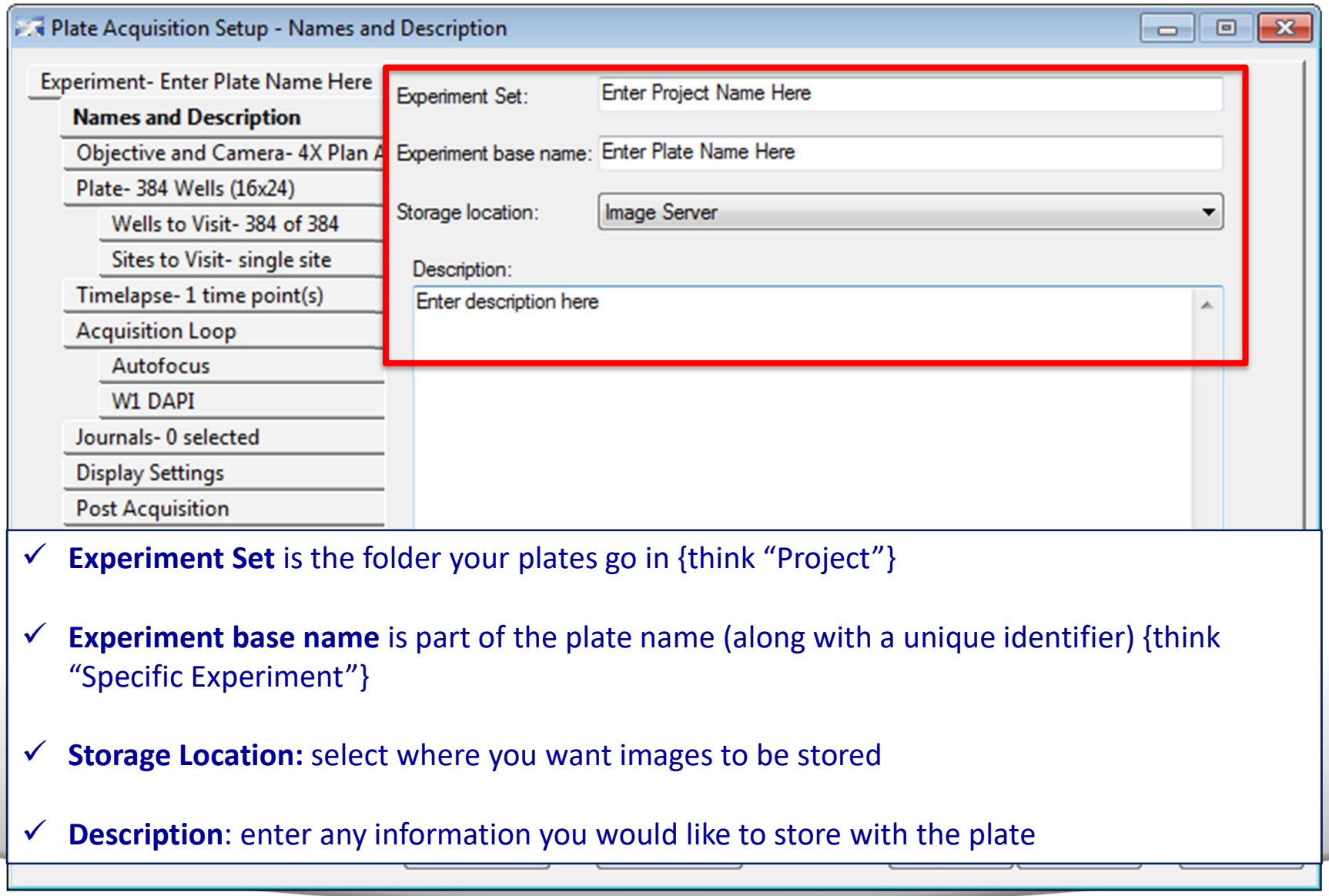
- ✓ **Snap Current**: will snap an image at current X,Y,Z position
- ✓ **Show Live:**
- ✓ **Preview**: set up MetaXpress during acquisition

- ✓ **Reset IX**: reinitializes the system
- ✓ **Close:** close Plate Acquisition and Control dialog box

Create New Protocol Settings: EXPERIMENT tab

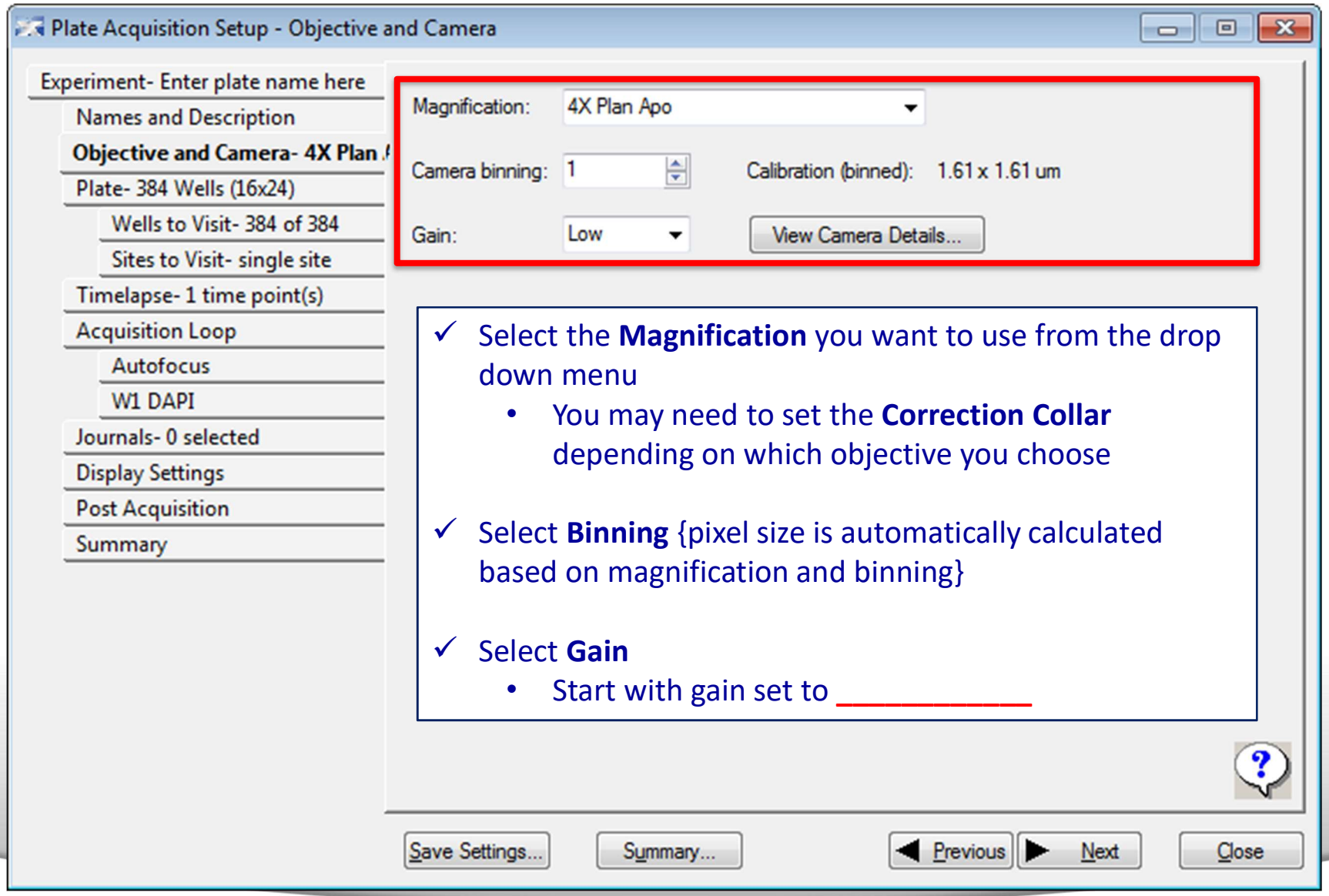


Create New Protocol Settings: NAMES AND DESCRIPTION tab



- ✓ **Experiment Set** is the folder your plates go in {think "Project"}
- ✓ **Experiment base name** is part of the plate name (along with a unique identifier) {think "Specific Experiment"}
- ✓ **Storage Location:** select where you want images to be stored
- ✓ **Description**: enter any information you would like to store with the plate

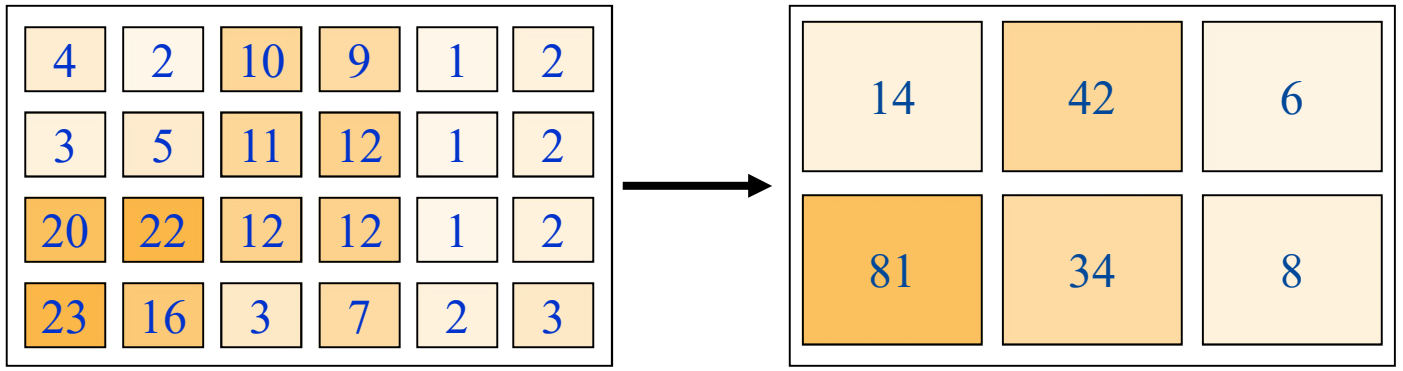
Create New Protocol Settings: OBJECTIVE AND CAMERA tab



What is Binning?

Combining groups of pixels into a single pixel during image acquisition

2x2 Binning

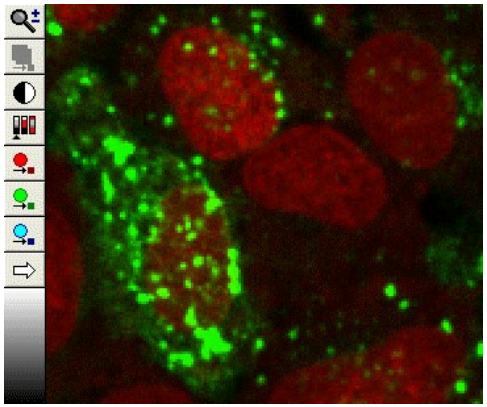


What the camera sees

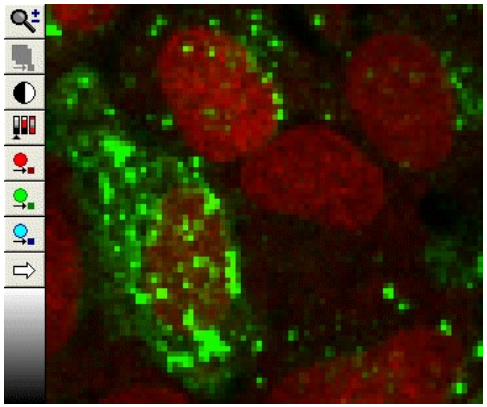
Pixels are summed

Won’t Binning Affect Resolution?

Binned images are lower resolution than unbinned images

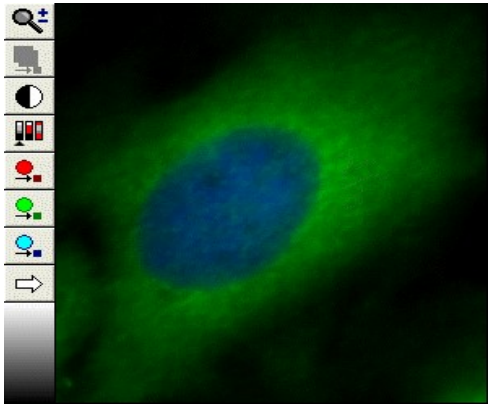


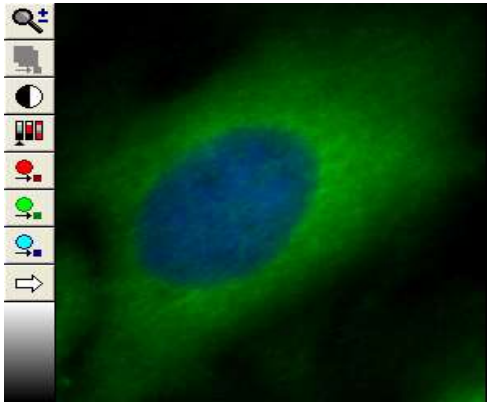
Unbinned



2x2 binned

Not all assays require unbinned images





Why Bin?

There are many advantages:

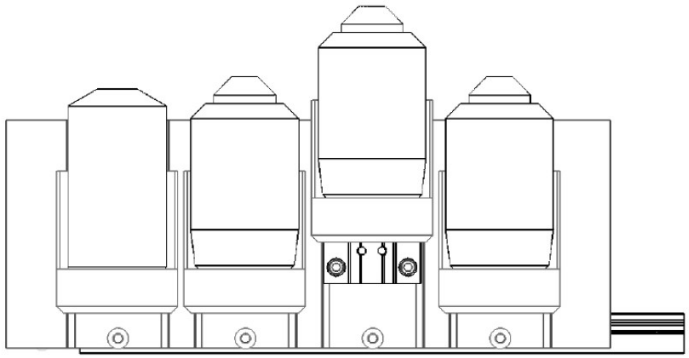
- Save Space
  - 2x2 binning reduces file size 4-fold
- Increase Speed
  - Faster image transfer from MetaXpress to database
  - Faster image analysis

Adjusting Correction Collars

- Correction collar should match plate

- Physical thickness
  - Set correction collar to: Optical Thickness * Refractive Index
  - **Optical thickness** is the same as plate bottom thickness as determined by Laser Autofocus Wizard
  - RI = 1.59 (Plastic)
  - RI = 1.52 (Glass)
  - Or specs from manufacturer

- Open side panels to access objectives or use Taskbar



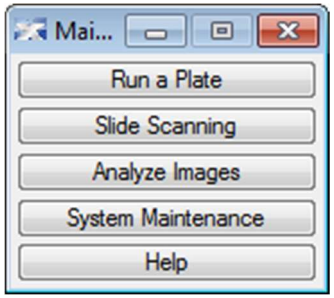
***HINT* Where can you find the value of plate bottom thickness?**

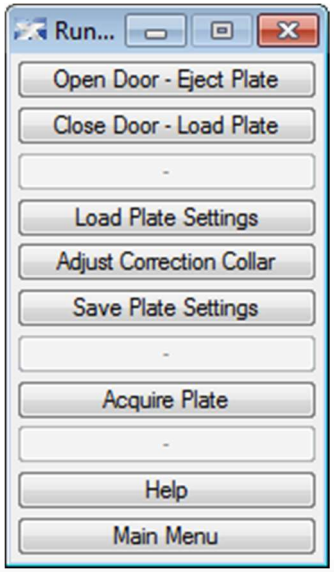
_____

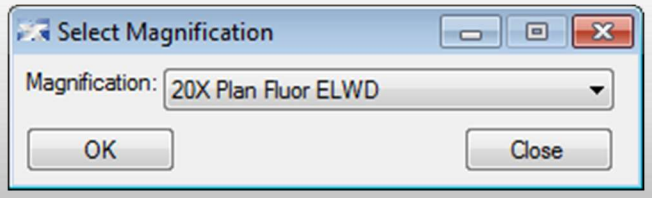
_____

_____

Adjusting Correction Collars using Taskbar

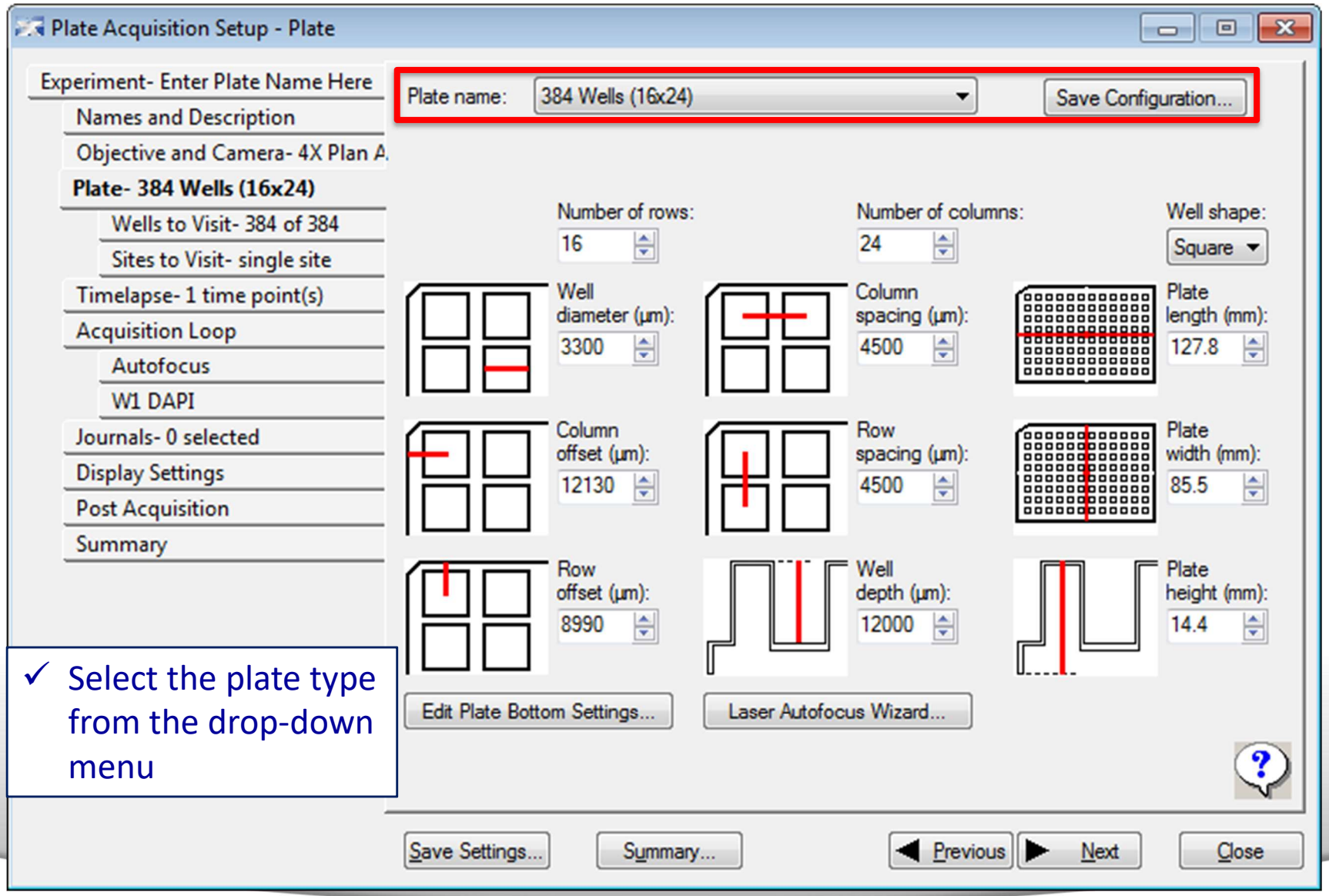






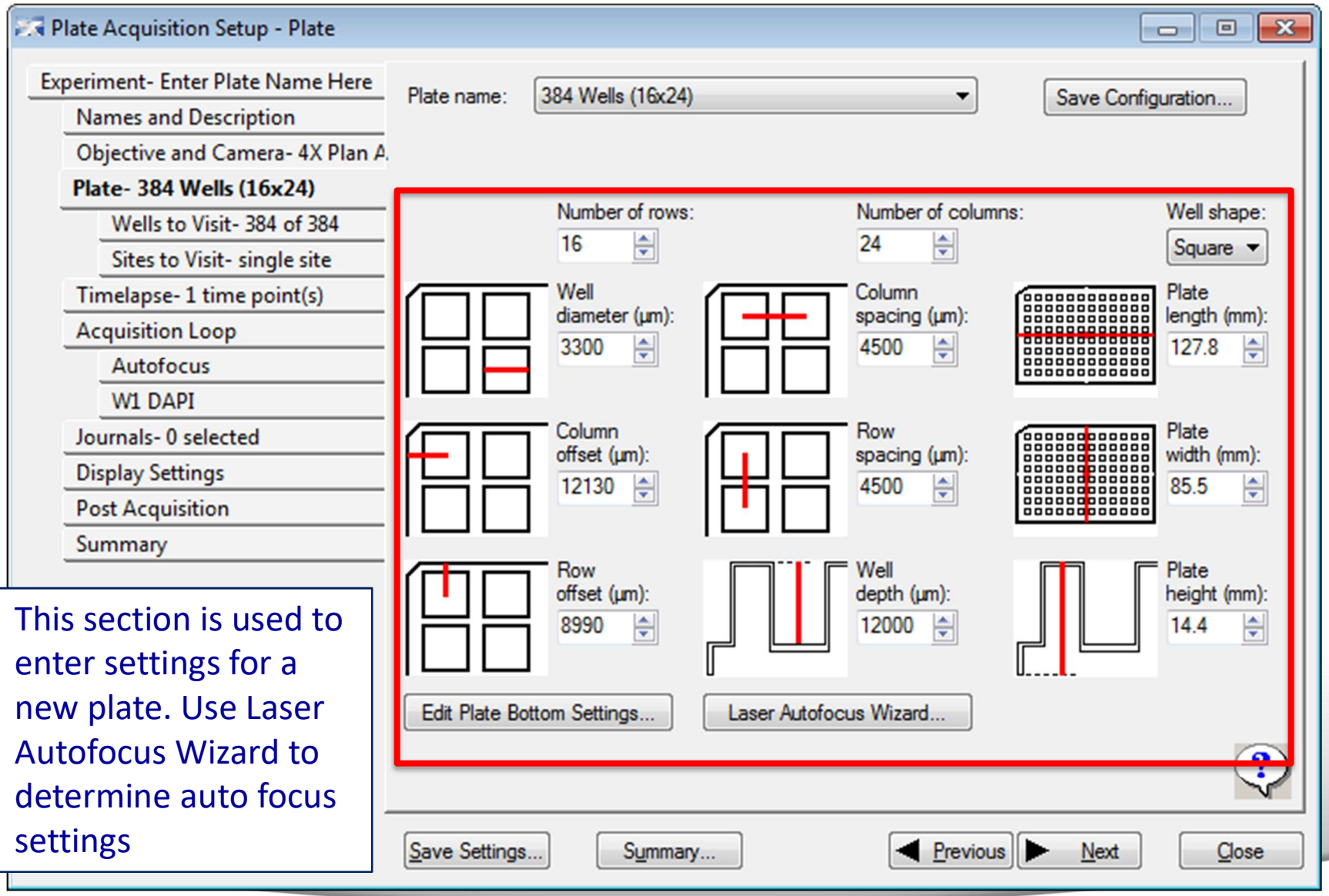
- On the Main Taskbar, click on **Run a Plate**
- Click on **Adjust Correction Collar**
- Choose the objective for which you want to change the correction collar from the drop-down menu
- Choose how you will access the objective
- Follow the on-screen prompts

Create New Protocol Settings: PLATE tab



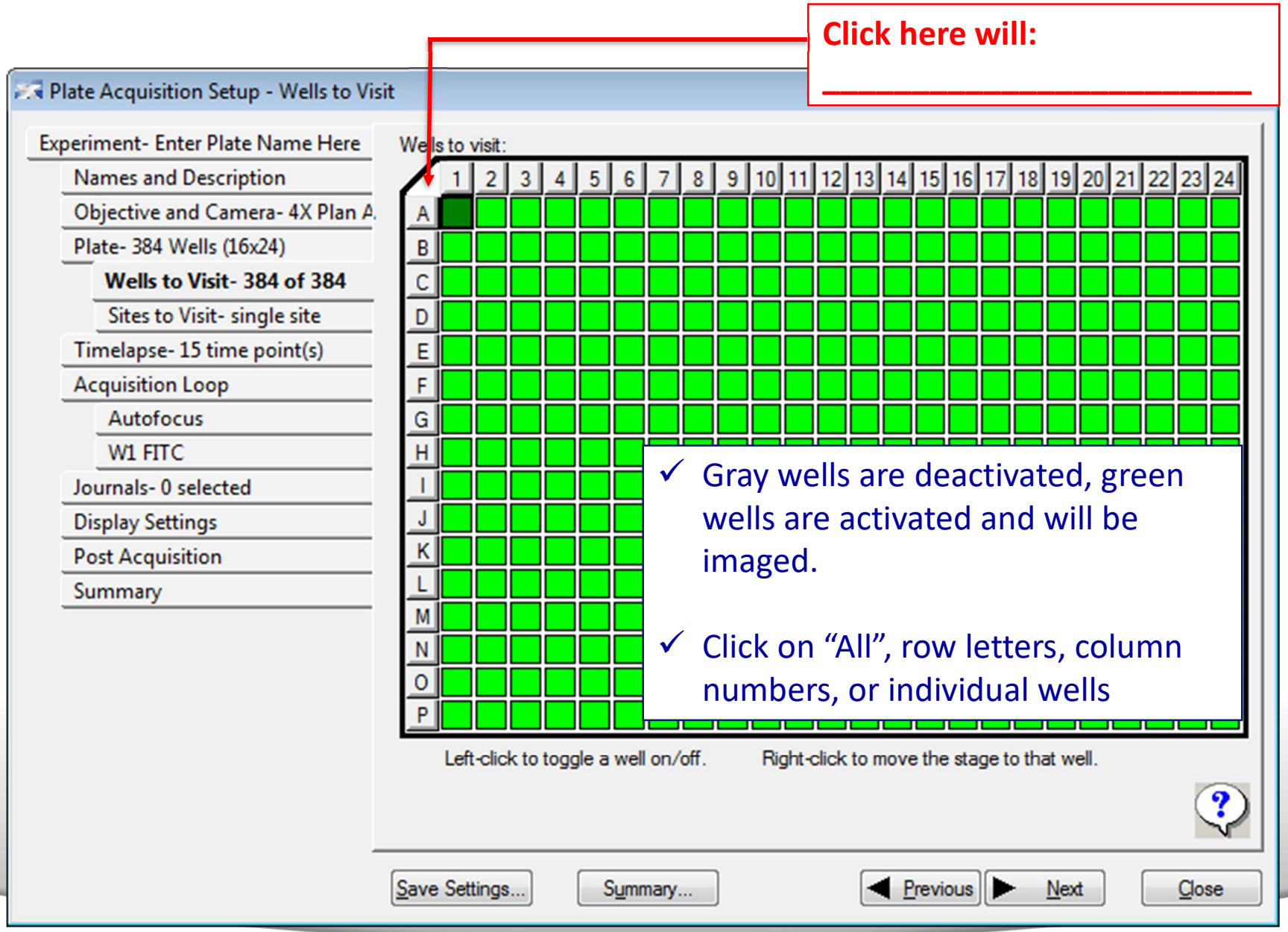
✓ Select the plate type from the drop-down menu

Create New Protocol Settings: PLATE tab

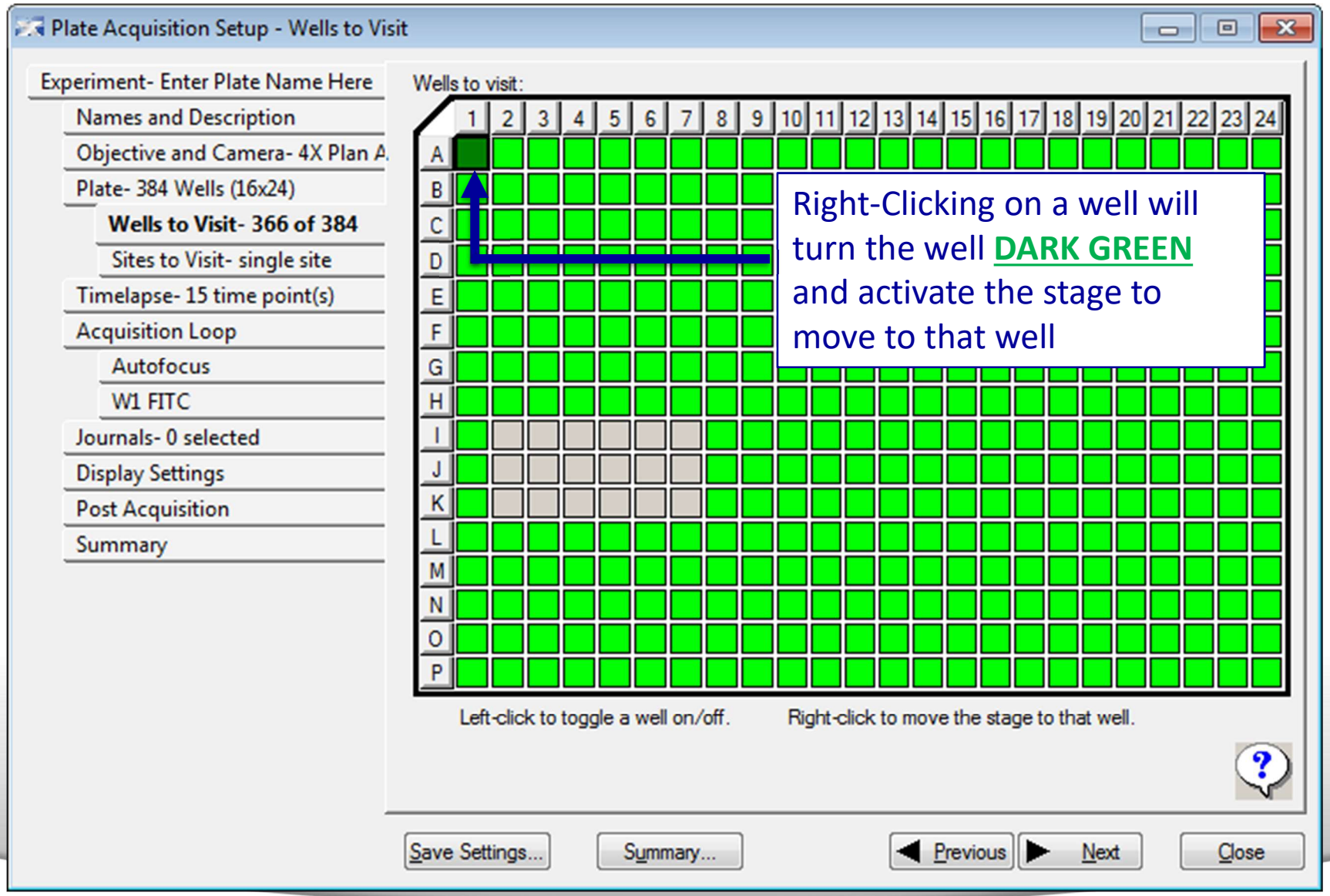


✓ This section is used to enter settings for a new plate. Use Laser Autofocus Wizard to determine auto focus settings

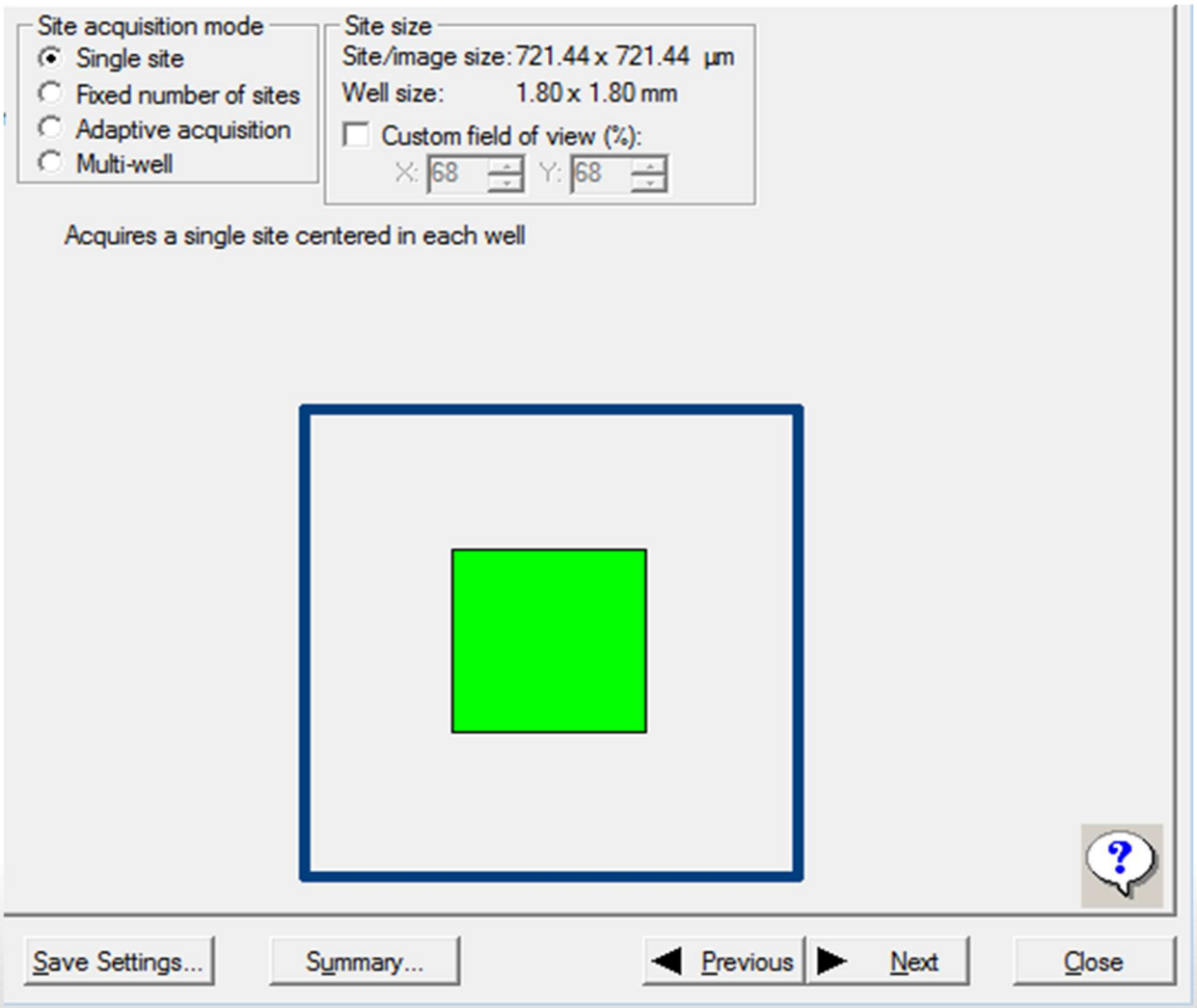
Create New Protocol Settings: WELLS TO VISIT tab



Create New Protocol Settings: WELLS TO VISIT tab



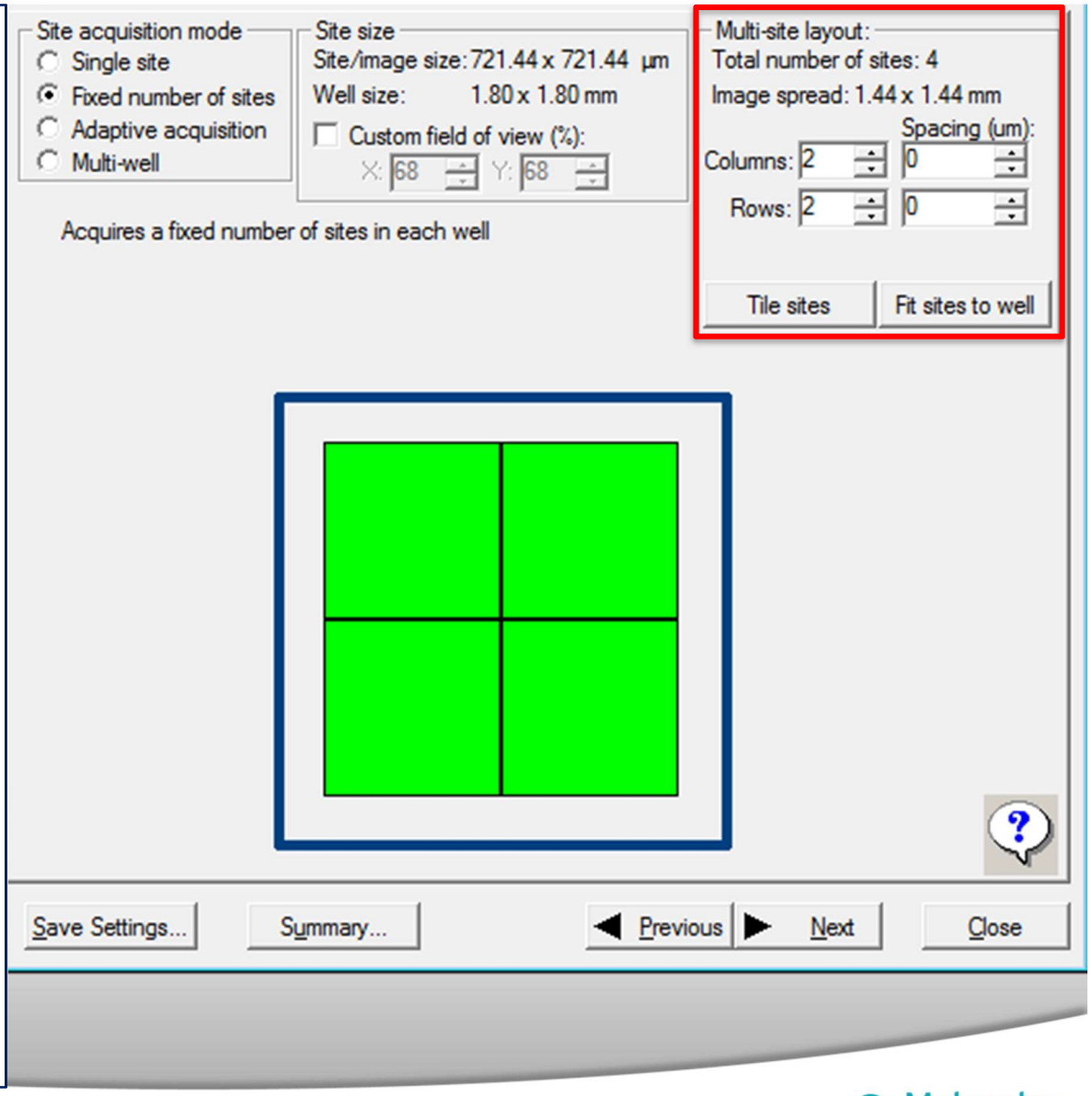
Create New Protocol Settings: SITES TO VISIT tab



✓ Single Site acquires one site in the middle of the well

Create New Protocol Settings: SITES TO VISIT tab

- ✓ Activate **Fixed number of sites** to image more than one site in a well
- ✓ Left-click sites on and off:
  - ✓ _____ is imaged
  - ✓ _____ is not
- ✓ Right-click on a site turns it **Dark Green** and the stage moves to that position
- ✓ Spacing defines the x-y spacing between sites. Negative spacing results in overlapped images. Each click of the down-arrow is 10% overlap
- ✓ **Tile sites**: _____
  _____
- ✓ **Fit sites to well**: _____
  _____



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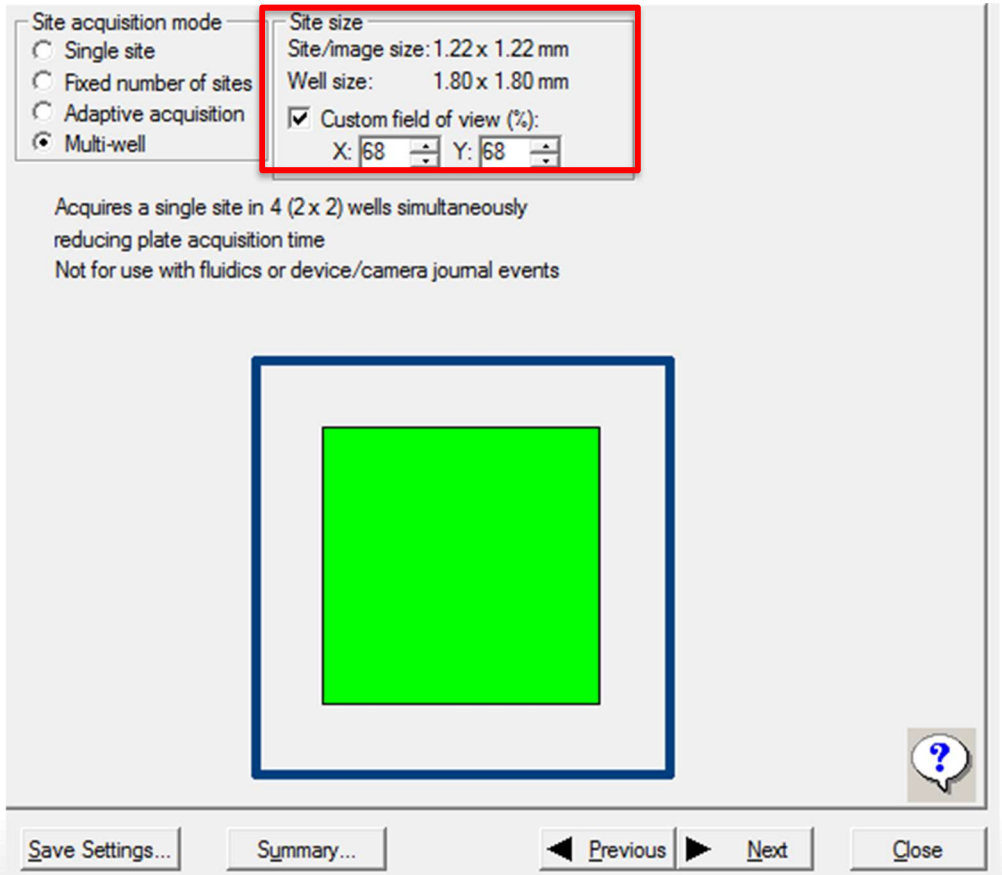
Create New Protocol Settings: SITES TO VISIT tab



✓ **Custom Field of View** Section allows you to : _____

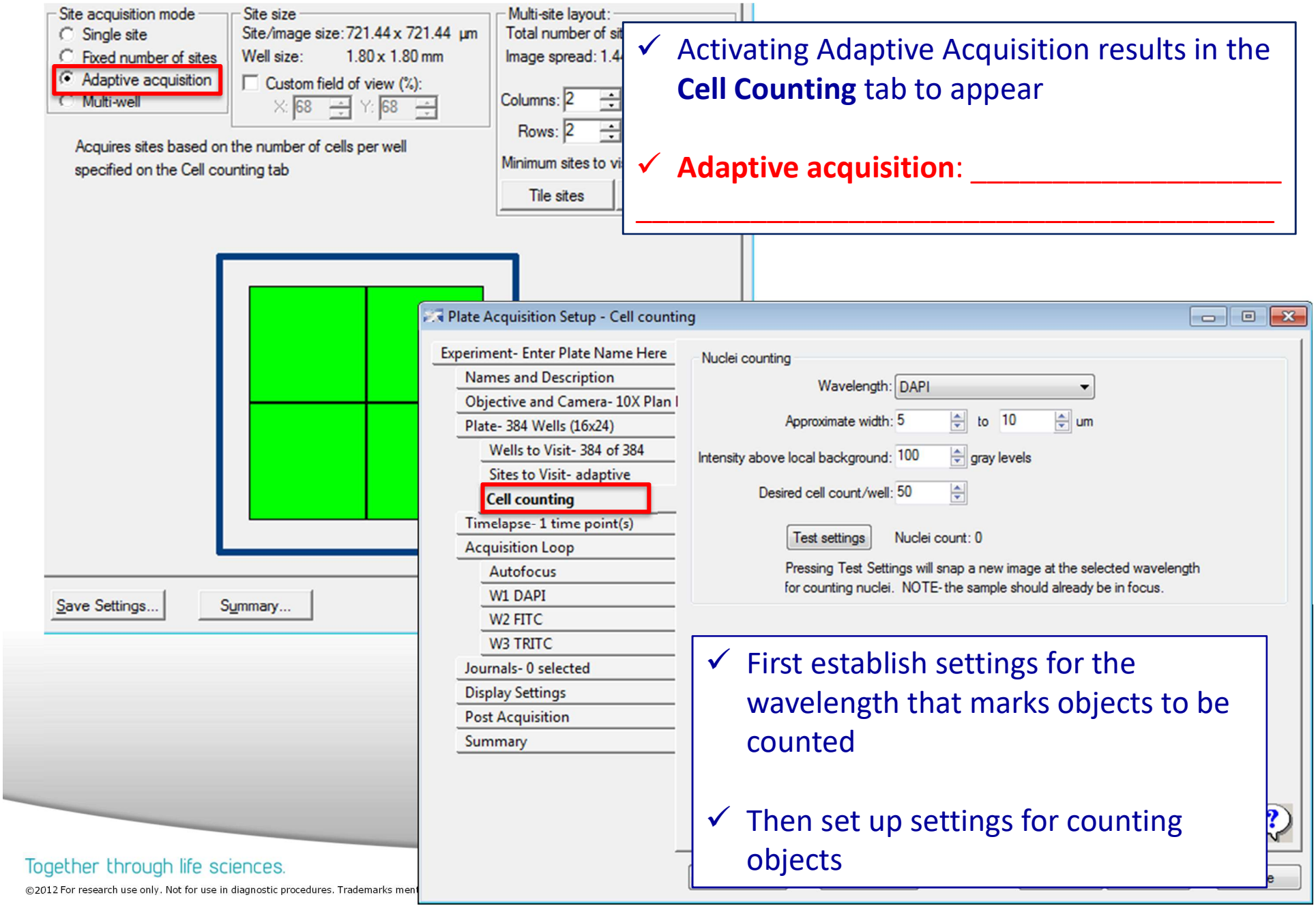
_____

_____

_____

_____

Create New Protocol Settings: SITES TO VISIT tab



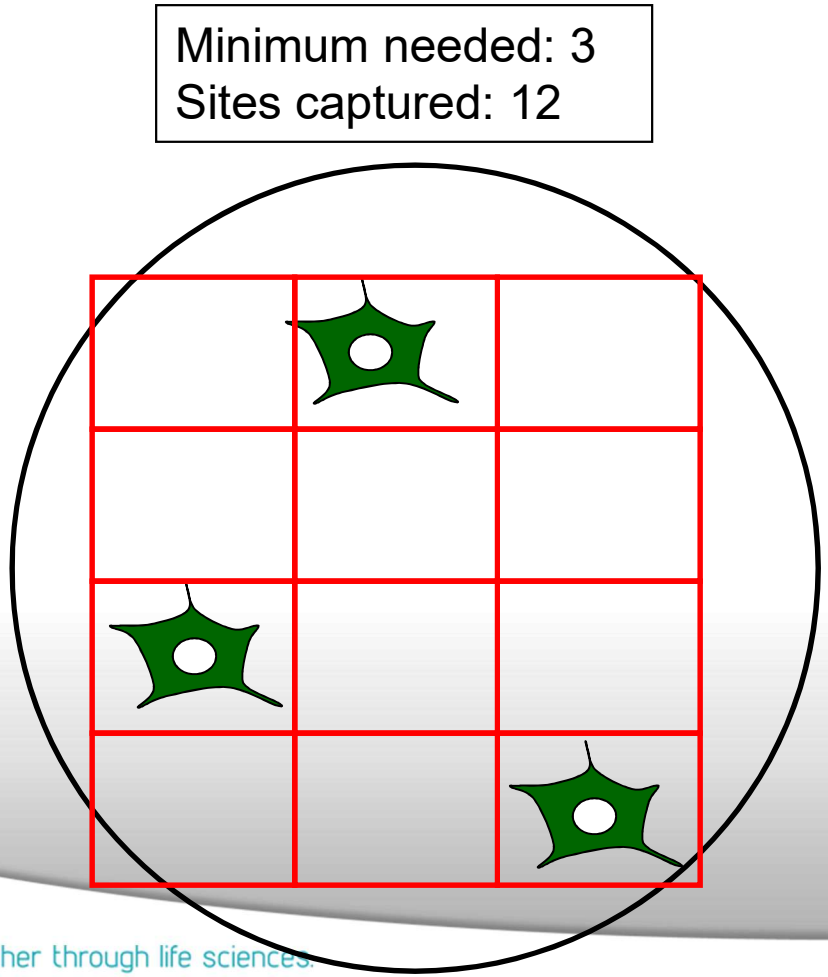
- ✓ Activating Adaptive Acquisition results in the **Cell Counting** tab to appear
- ✓ **Adaptive acquisition**: _____

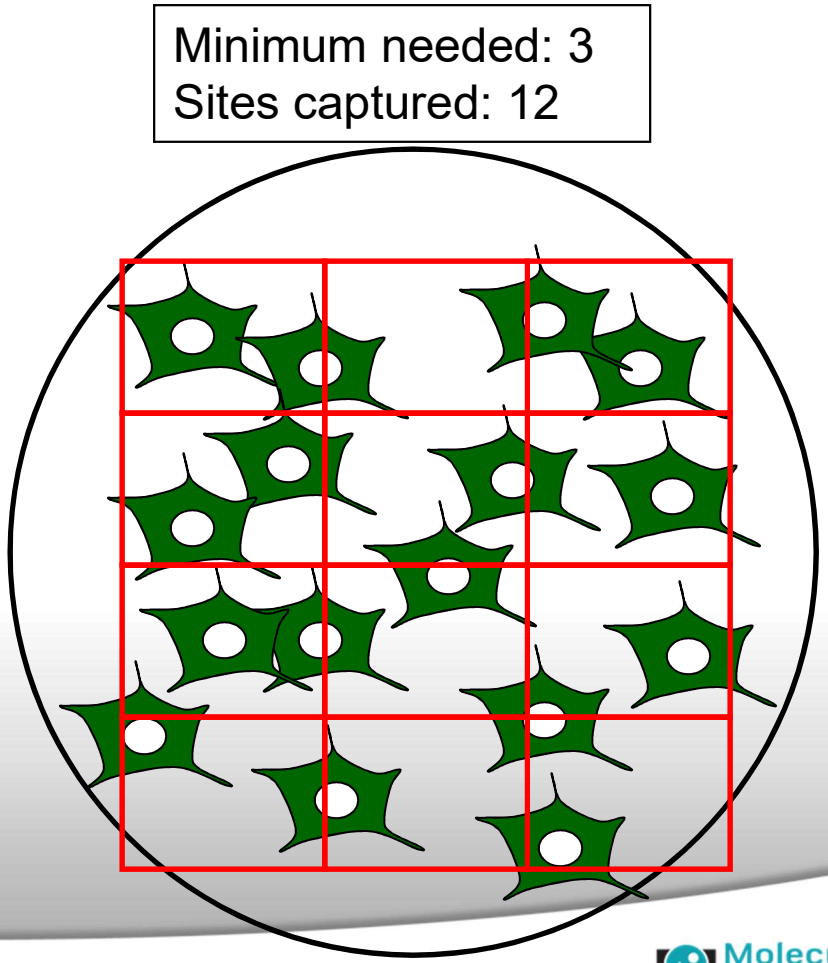
_____

- ✓ First establish settings for the wavelength that marks objects to be counted
- ✓ Then set up settings for counting objects

Adaptive Acquisition™: fast multi-site imaging

- Assays with variable cells in a field
- Traditionally capture the same number of sites per well





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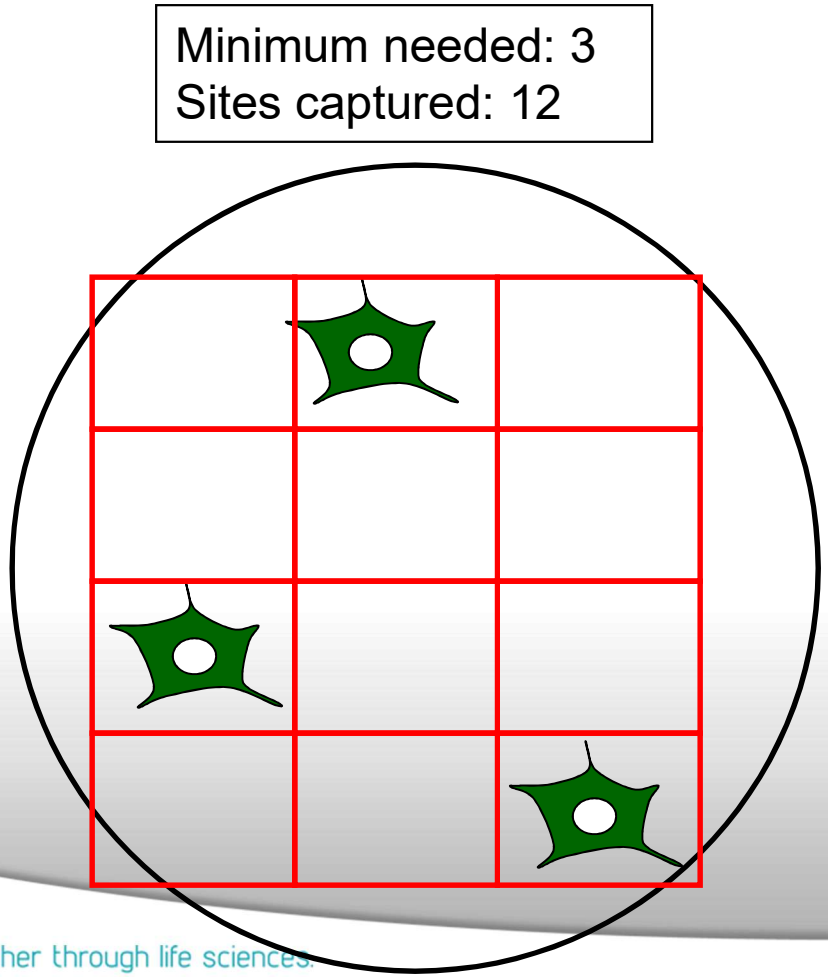
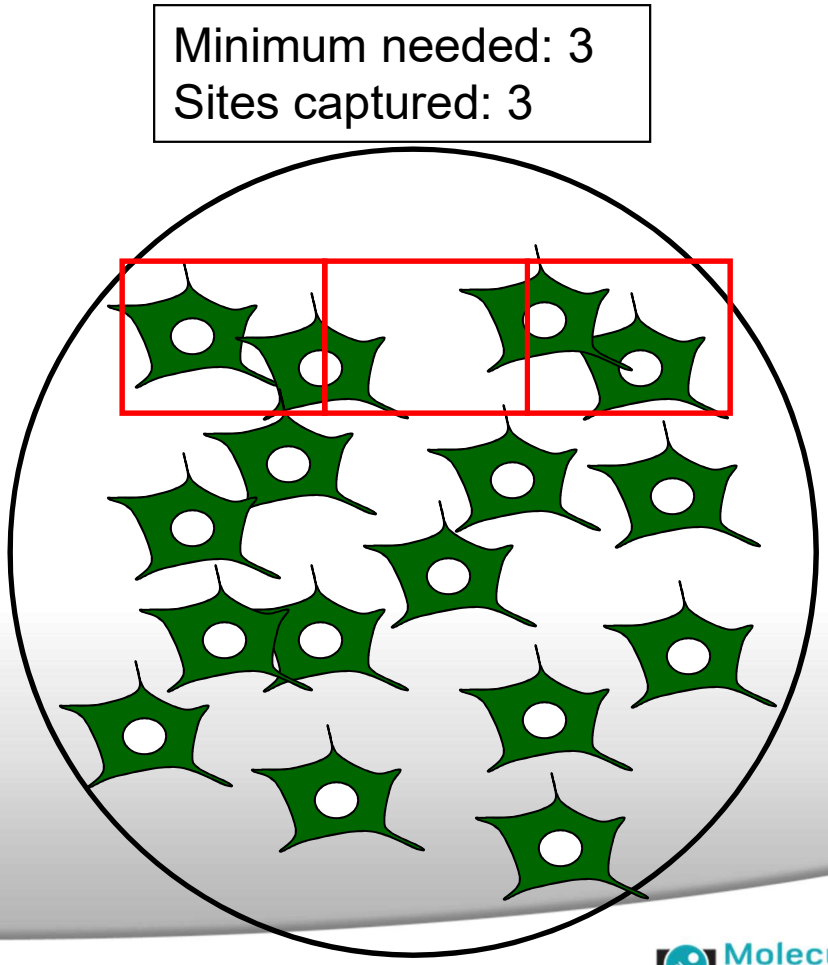
Adaptive Acquisition™: fast multi-site imaging

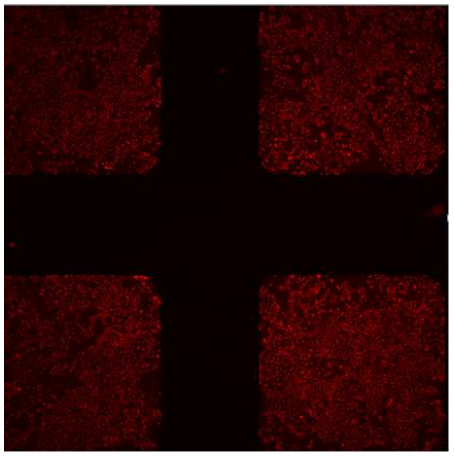
- Option to set a minimum number of cells per well
- System only collects as many sites as necessary
- Can reduce acquisition time 5-10 fold

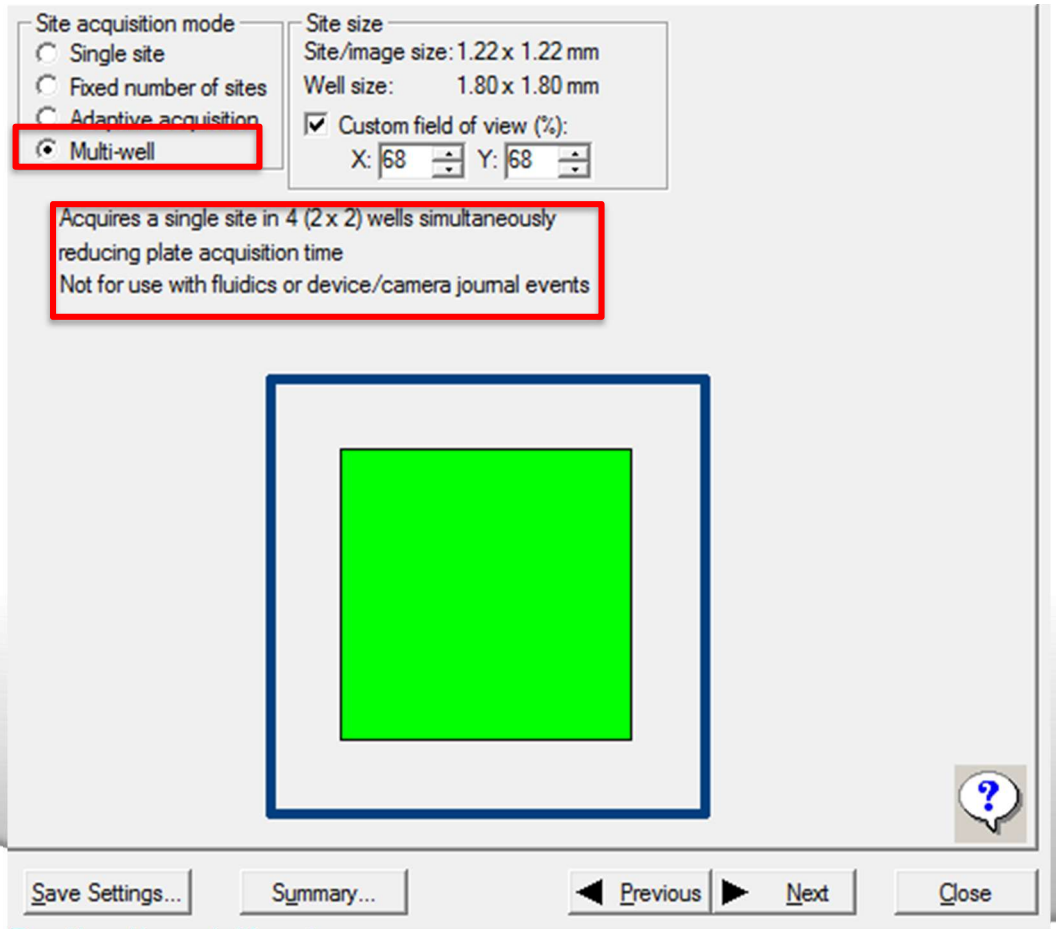




Create New Protocol Settings: SITES TO VISIT tab

If this feature is not possible with your camera and objective, the software will give you an error in yellow.

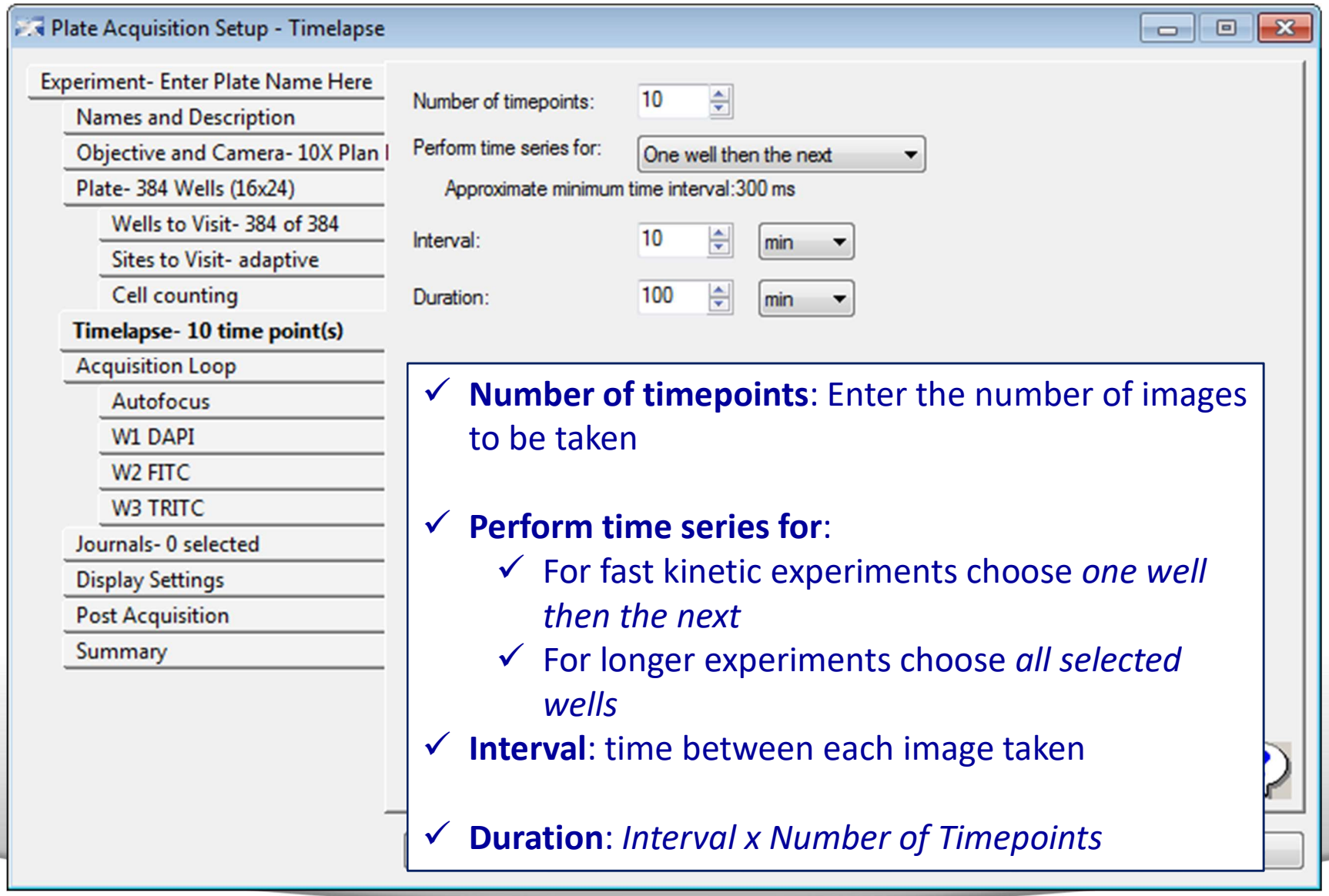




- ✓ Activating Multi-well allows capture of multiple wells in a single field of view and automated parsing out to individual images.
- ✓ Used when you capture multiple fields of view in one images such as 1536 well plate at 4X or 384 well plate at 2X
- ✓ Use *focus on plate bottom, then offset by thickness* with Multi-well acquisition protocol

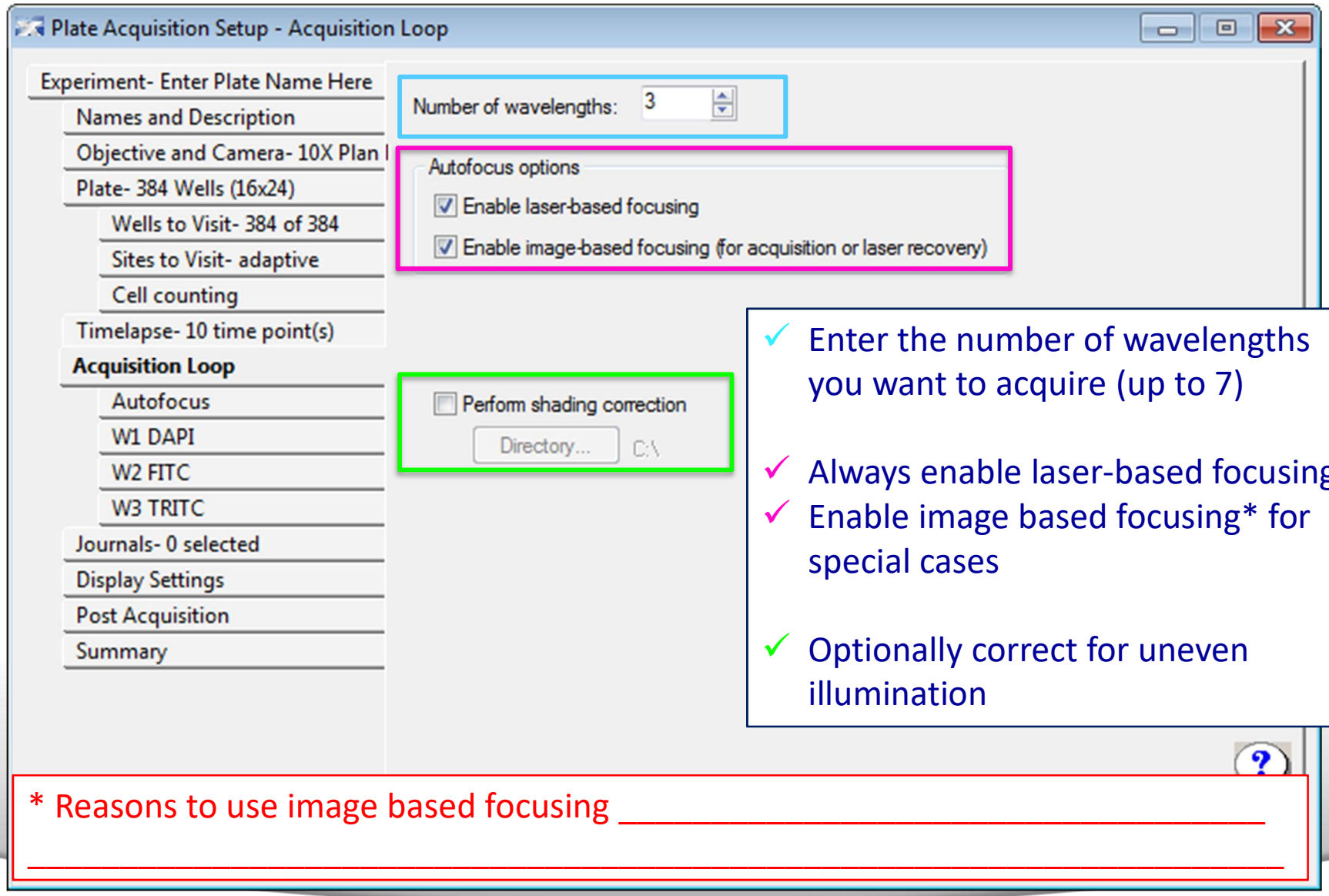
Molecular Devices

Create New Protocol Settings: TIMELAPSE tab



- ✓ **Number of timepoints**: Enter the number of images to be taken
- ✓ **Perform time series for**:
  - ✓ For fast kinetic experiments choose *one well then the next*
  - ✓ For longer experiments choose *all selected wells*
- ✓ **Interval**: time between each image taken
- ✓ **Duration**: *Interval x Number of Timepoints*

Create New Protocol Settings: ACQUISITION LOOP tab

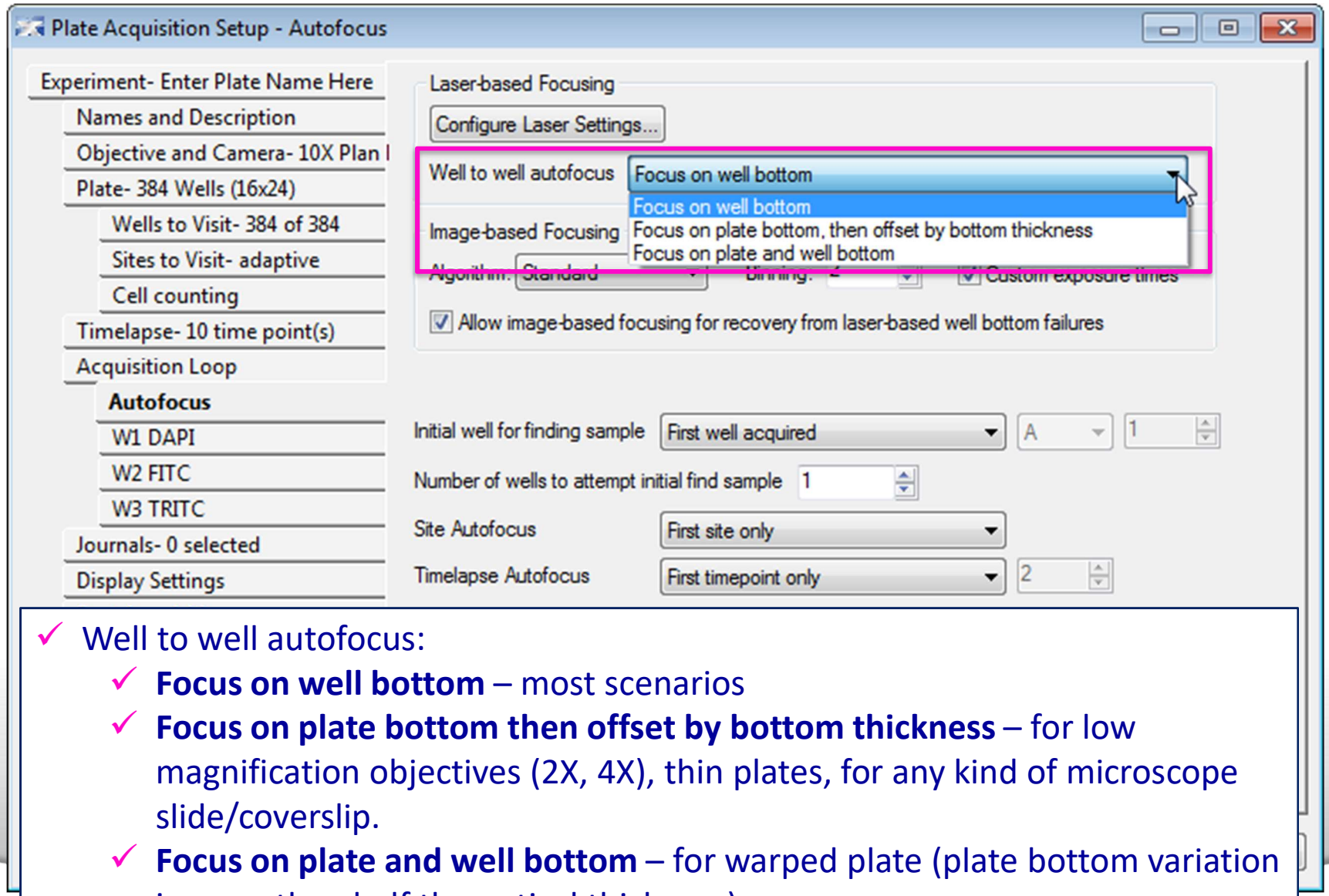


- ✓ Enter the number of wavelengths you want to acquire (up to 7)
- ✓ Always enable laser-based focusing
- ✓ Enable image based focusing* for special cases
- ✓ Optionally correct for uneven illumination

* Reasons to use image based focusing _____

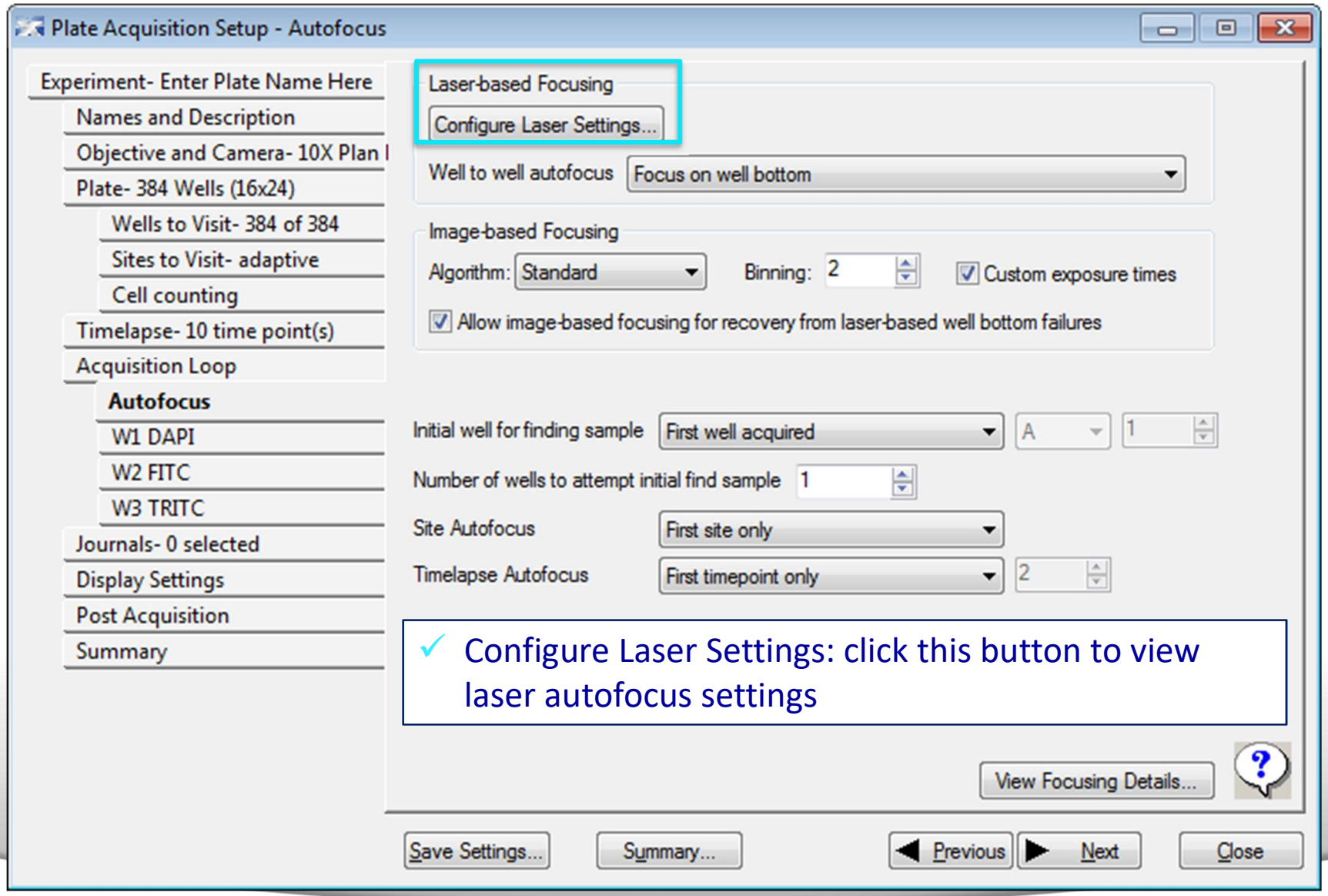
_____

Create New Protocol Settings: AUTOFOCUS tab



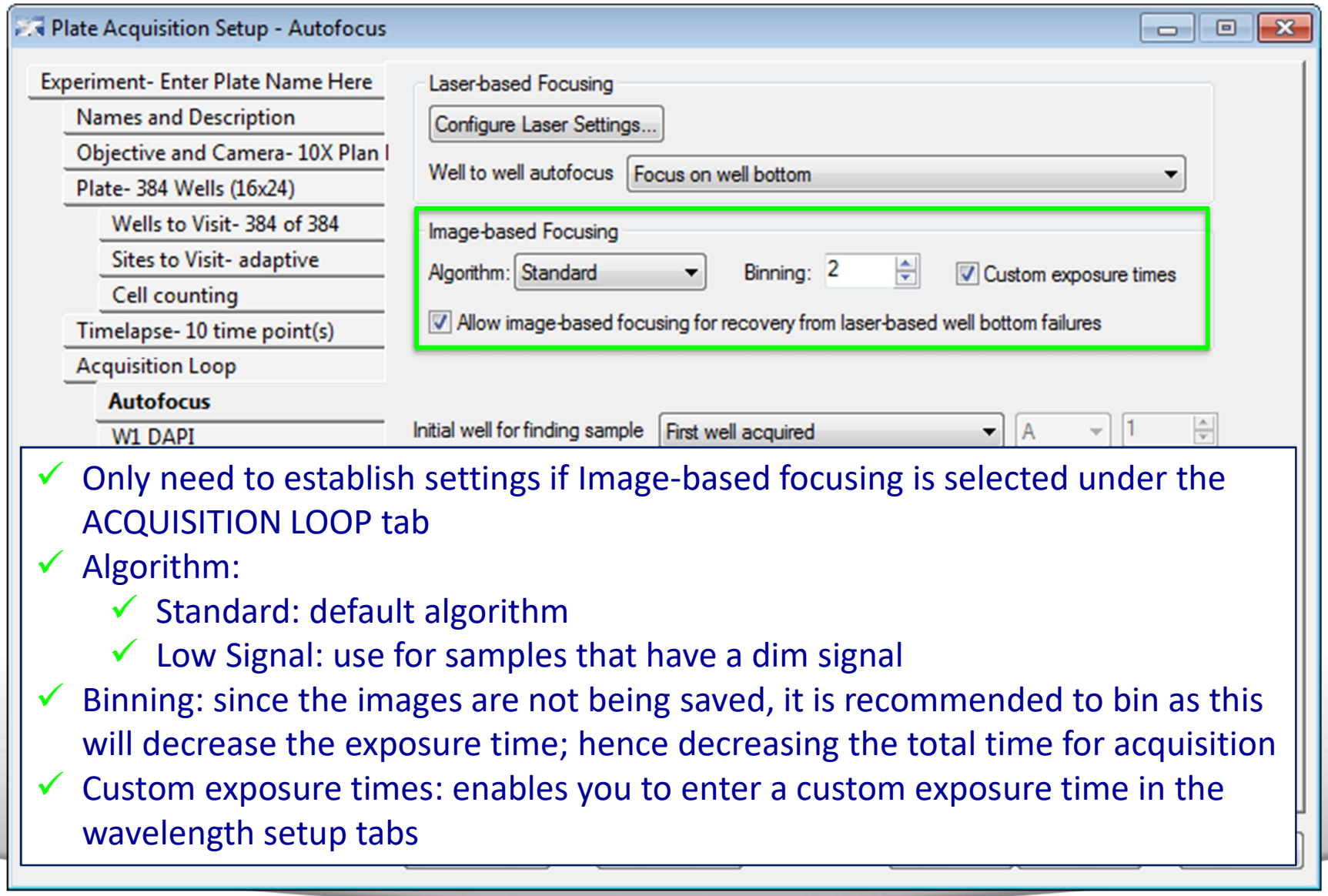
- ✓ Well to well autofocus:
  - ✓ **Focus on well bottom** – most scenarios
  - ✓ **Focus on plate bottom then offset by bottom thickness** – for low magnification objectives (2X, 4X), thin plates, for any kind of microscope slide/coverslip.
  - ✓ **Focus on plate and well bottom** – for warped plate (plate bottom variation is more than half the optical thickness)

Create New Protocol Settings: AUTOFOCUS tab



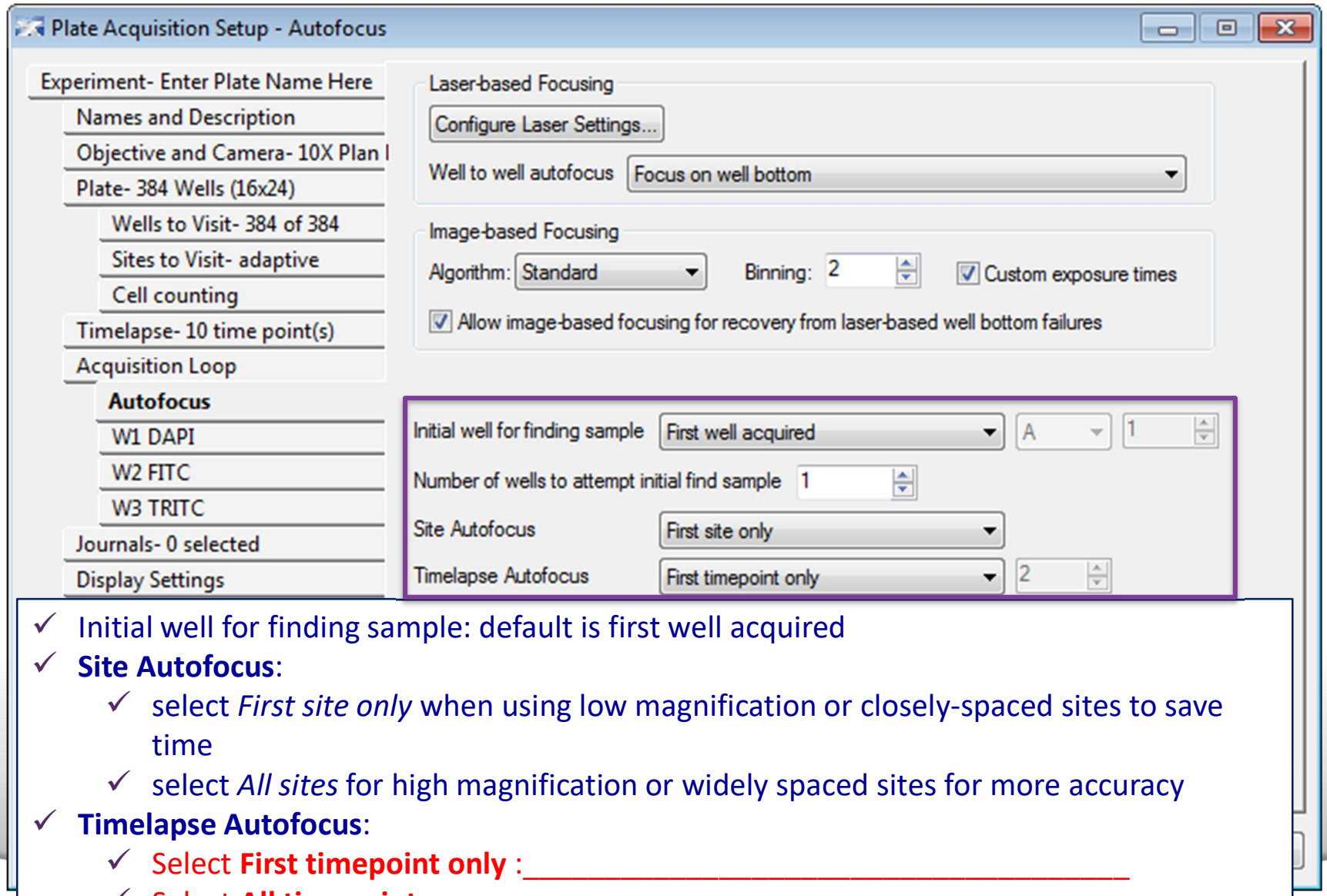
Molecular Devices

Create New Protocol Settings: AUTOFOCUS tab



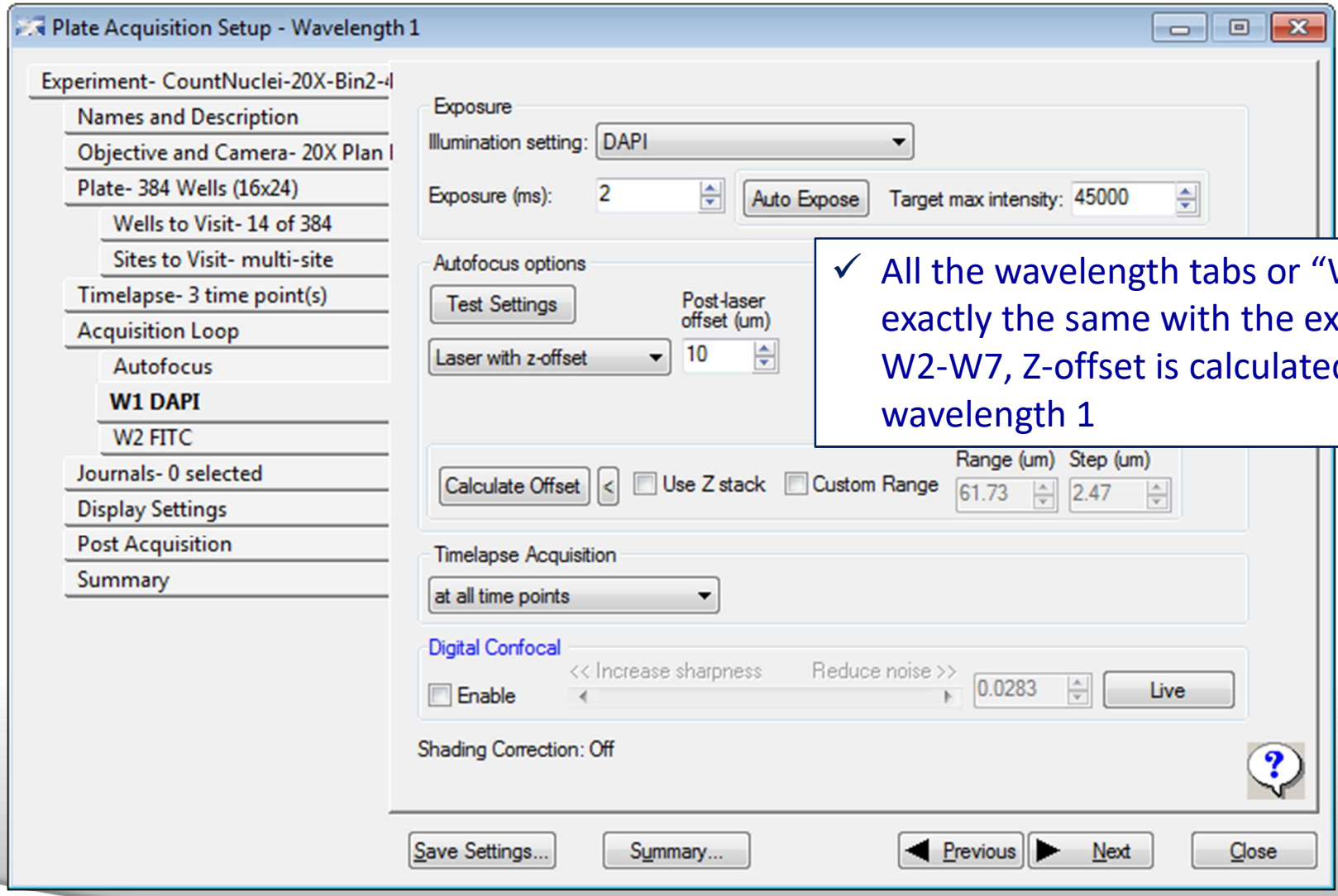
- ✓ Only need to establish settings if Image-based focusing is selected under the ACQUISITION LOOP tab
- ✓ Algorithm:
  - ✓ Standard: default algorithm
  - ✓ Low Signal: use for samples that have a dim signal
- ✓ Binning: since the images are not being saved, it is recommended to bin as this will decrease the exposure time; hence decreasing the total time for acquisition
- ✓ Custom exposure times: enables you to enter a custom exposure time in the wavelength setup tabs

Create New Protocol Settings: AUTOFOCUS tab



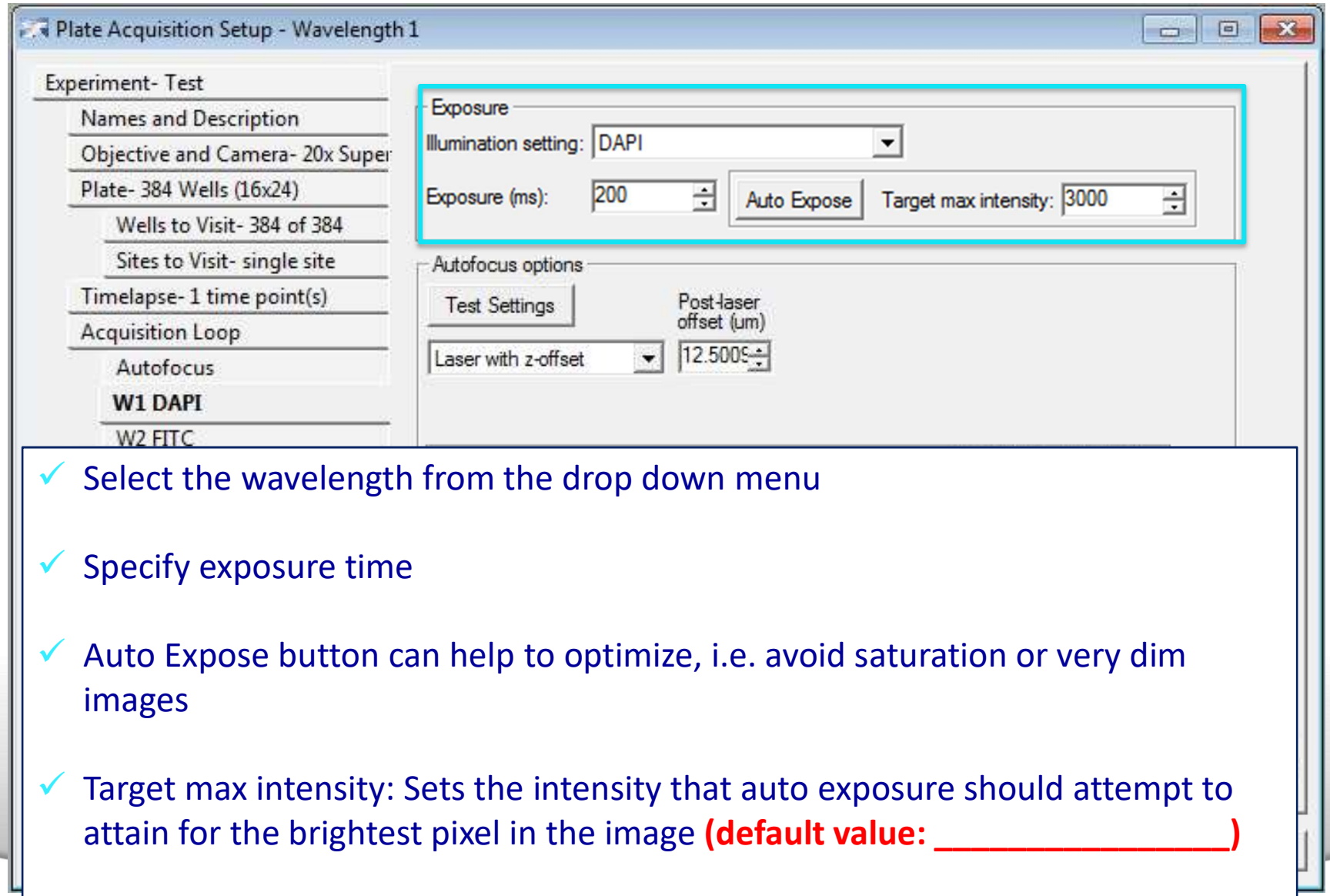
- ✓ Initial well for finding sample: default is first well acquired
- ✓ **Site Autofocus**:
  - ✓ select *First site only* when using low magnification or closely-spaced sites to save time
  - ✓ select *All sites* for high magnification or widely spaced sites for more accuracy
- ✓ **Timelapse Autofocus**:
  - ✓ Select **First timepoint only** :_____
  - ✓ Select **All timepoints**: _____
  - ✓ Select **Every Nth timepoint**: _____

Create New Protocol Settings: Wavelength tabs (W1, W2, W3 …)



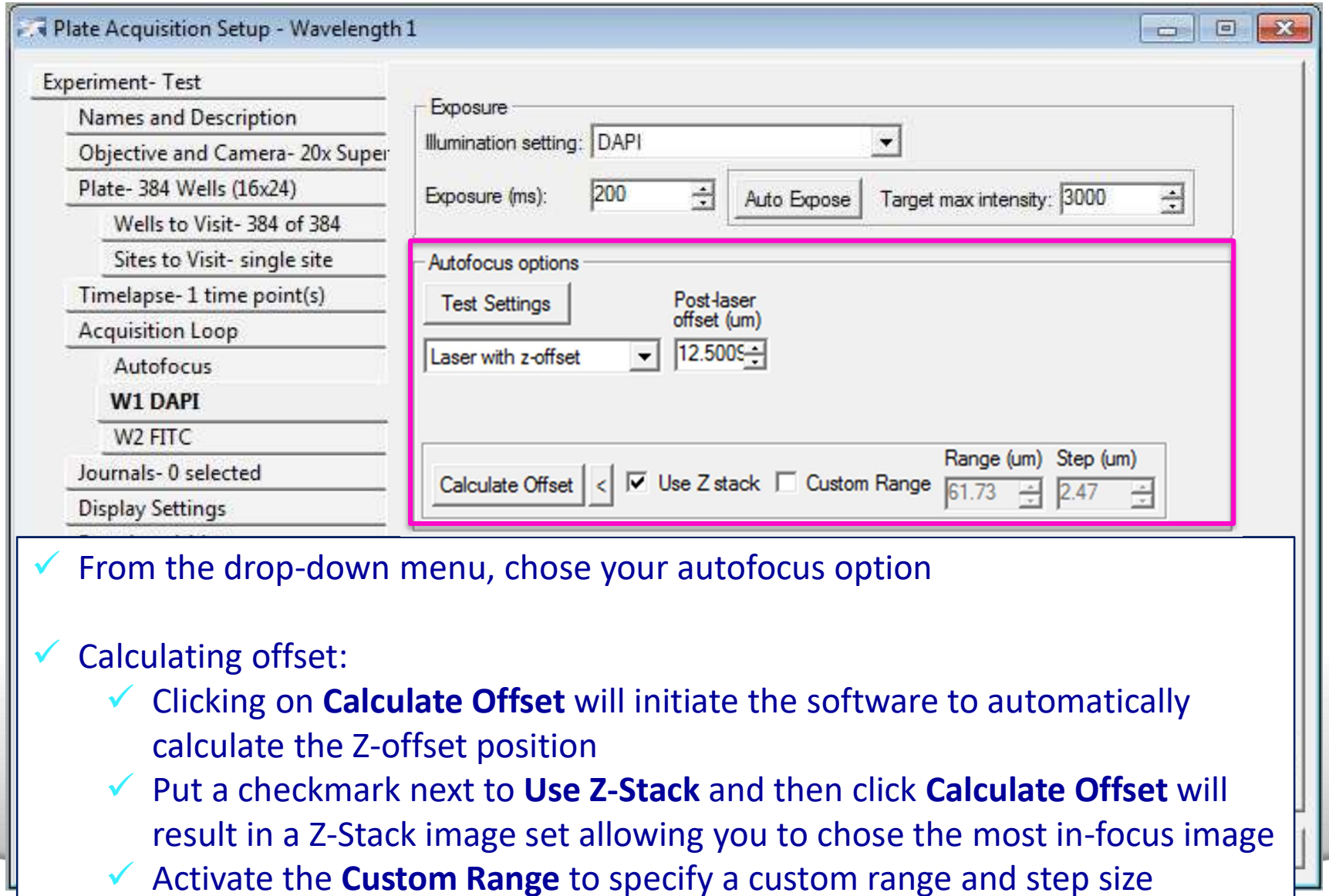
- ✓ All the wavelength tabs or “W” tabs are exactly the same with the exception in W2-W7, Z-offset is calculated from wavelength 1

Create New Protocol Settings: W1 (wavelength) tab



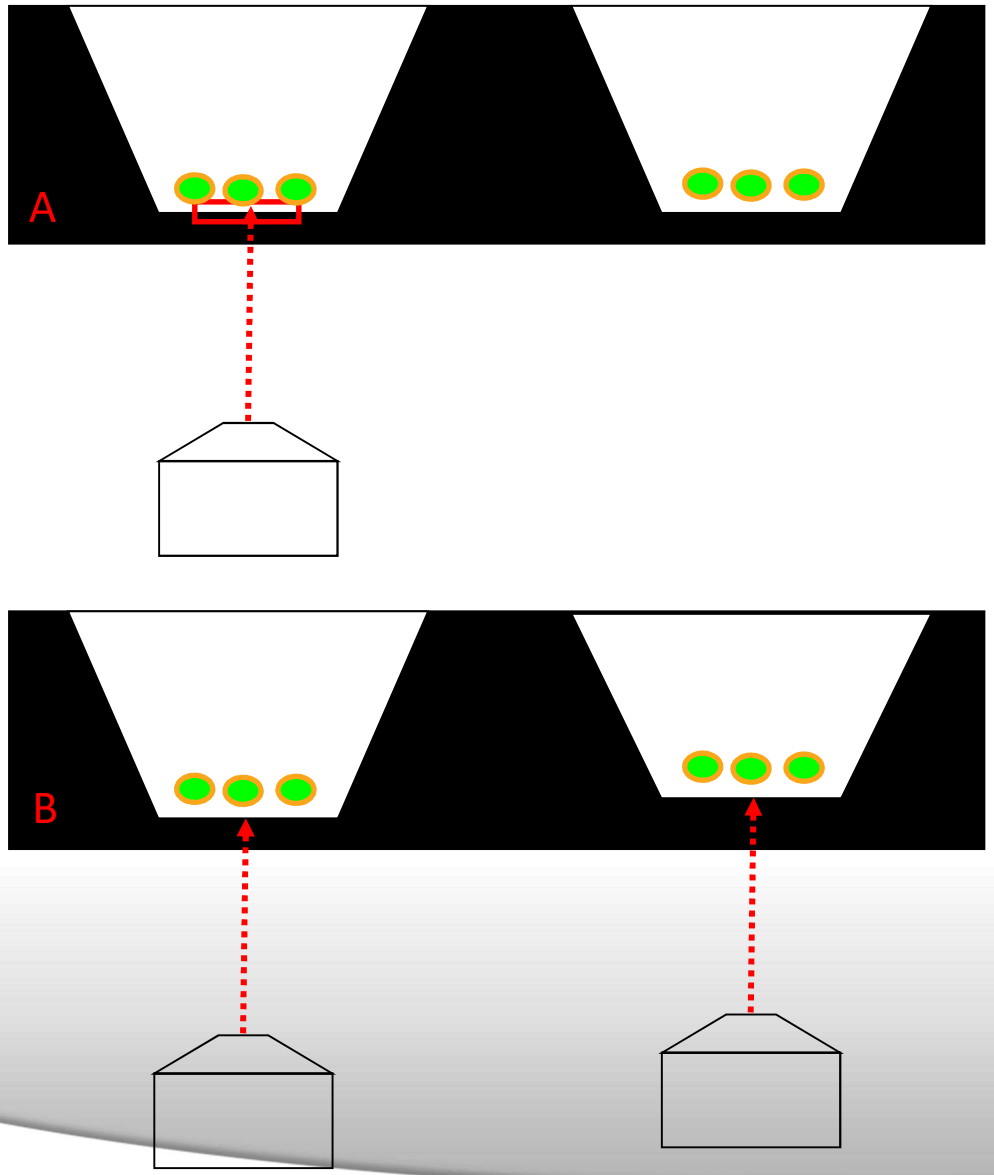
- ✓ Select the wavelength from the drop down menu
- ✓ Specify exposure time
- ✓ Auto Expose button can help to optimize, i.e. avoid saturation or very dim images
- ✓ Target max intensity: Sets the intensity that auto exposure should attempt to attain for the brightest pixel in the image **(default value: _____)**
- ✓ Check exposure times for positive and negative controls

Create New Protocol Settings: W1 (wavelength) tab



- ✓ From the drop-down menu, chose your autofocus option
- ✓ Calculating offset:
  - ✓ Clicking on **Calculate Offset** will initiate the software to automatically calculate the Z-offset position
  - ✓ Put a checkmark next to **Use Z-Stack** and then click **Calculate Offset** will result in a Z-Stack image set allowing you to chose the most in-focus image
  - ✓ Activate the **Custom Range** to specify a custom range and step size

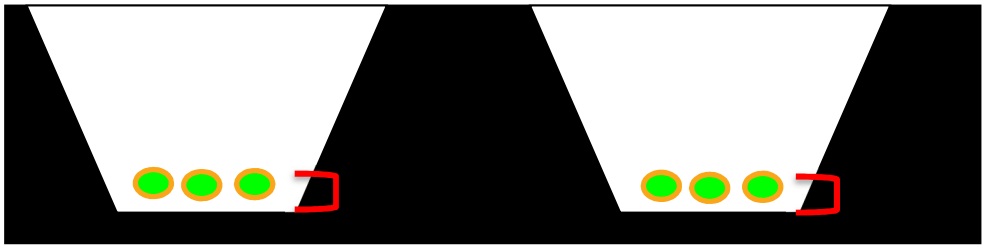
What is a Z-offset?



Z-offset

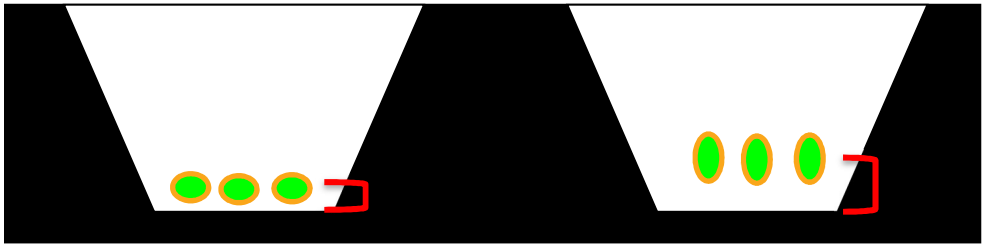
- Autofocus only puts you at the apparent well bottom NOT the biological sample of interest
- You may need an empirically determined "offset" to put you at the target
- Very wavelength dependent (chromatic aberration)
- Laser autofocus should take into account variations in the well bottom (see B).
- Can be +/-

When Do You Add in Image Based Focus?

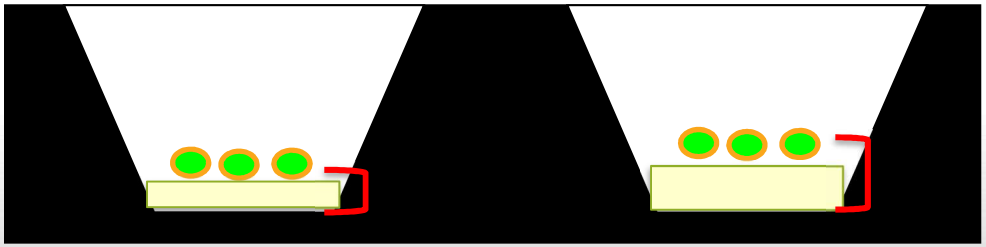


Typically all wells have the same relative offset from the laser focus.

Cases where adding in a narrow range image based focus may help:

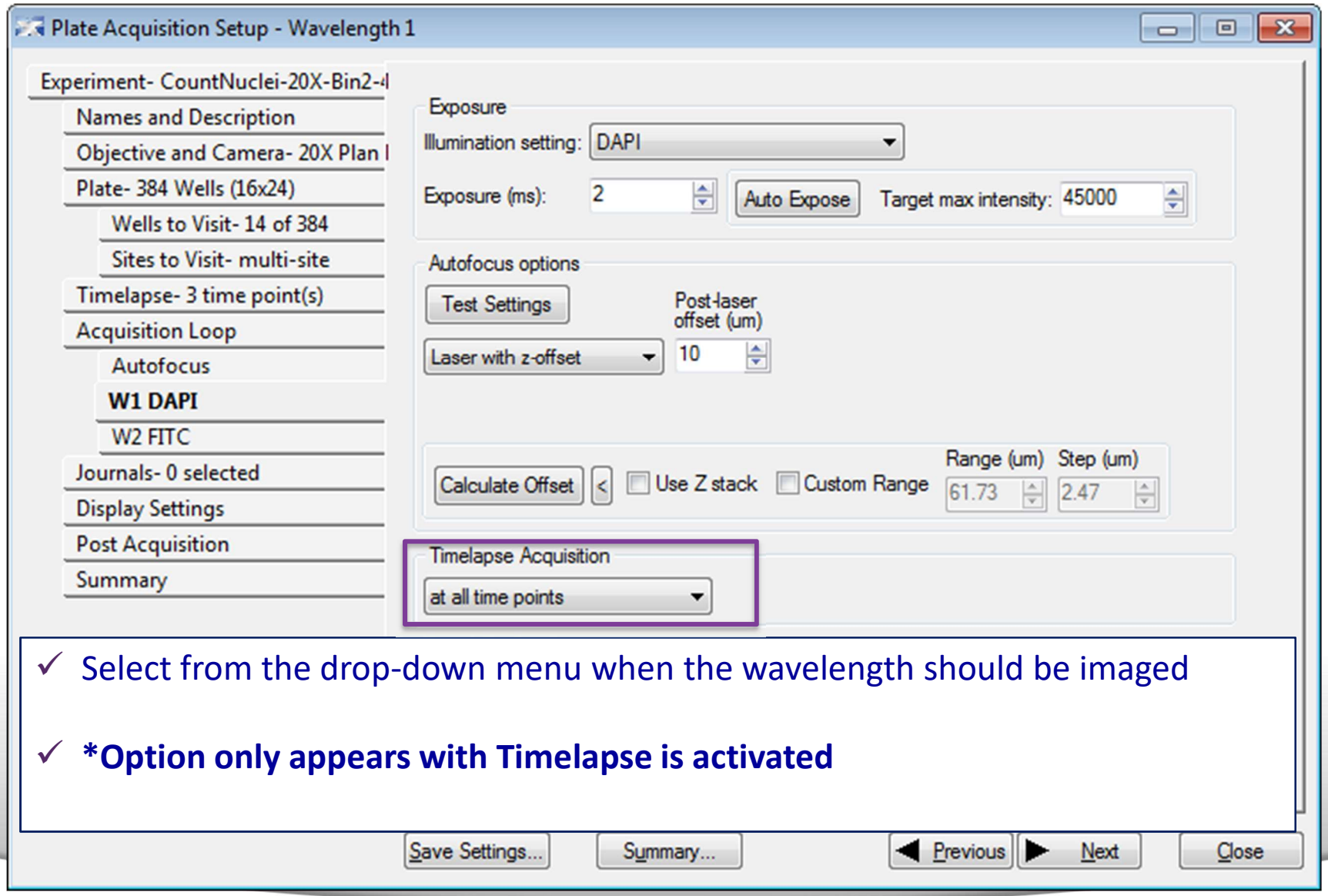


If the cell phenotype changes, such as in cases where cell goes from flat to round, the offset may vary per well.



If cells are growing on or in a surface such as a gel or coating and the amount of gel varies, the offset may vary per well.

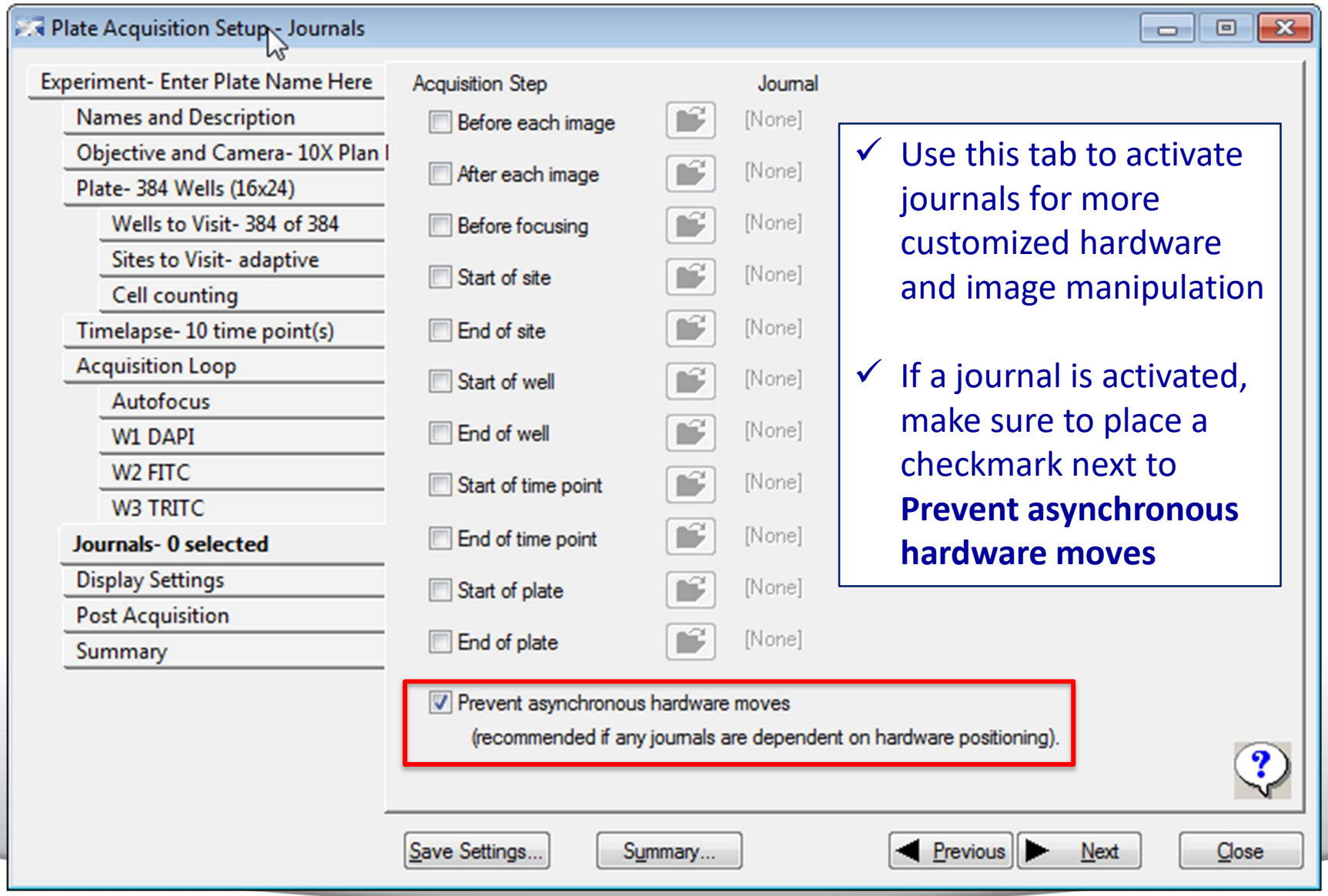
Create New Protocol Settings: W1 (wavelength) tab



- ✓ Select from the drop-down menu when the wavelength should be imaged
- ✓ ***Option only appears with Timelapse is activated**

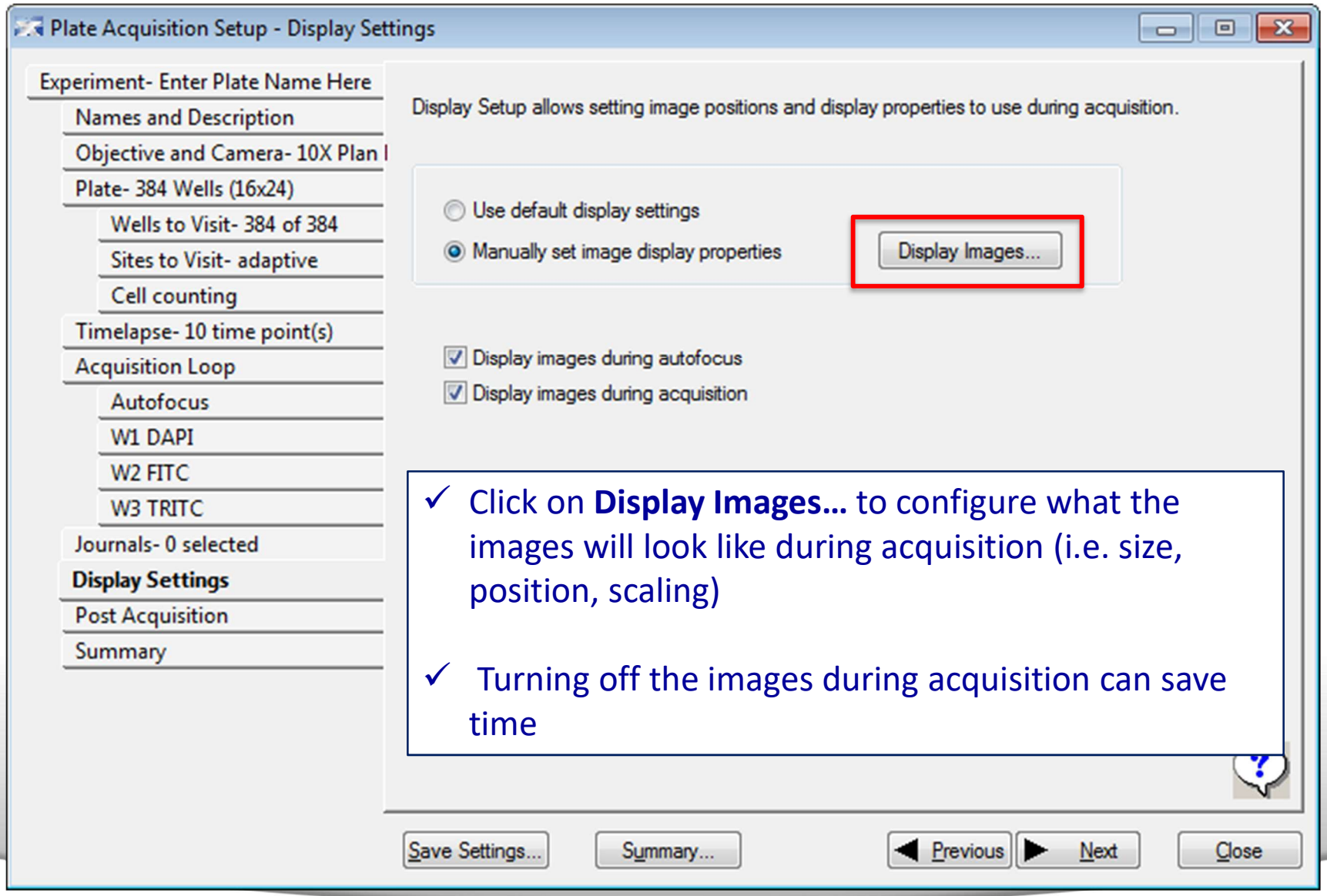
Molecular Devices

Create New Protocol Settings: JOURNAL tab

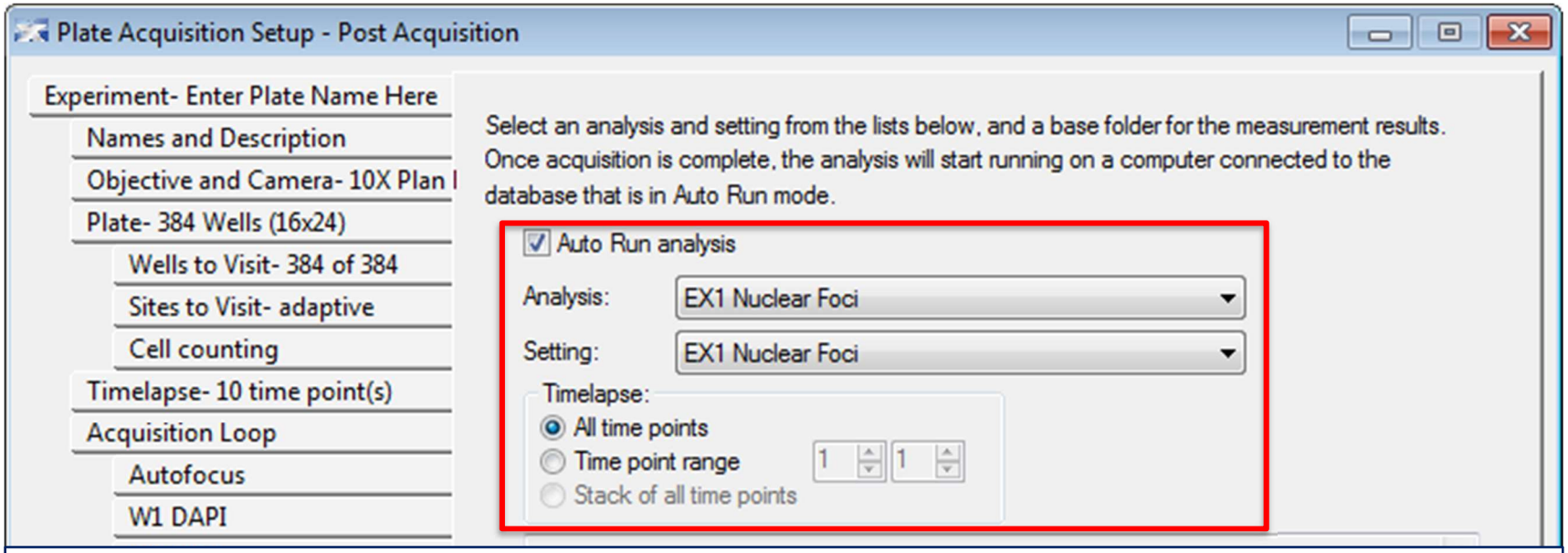


- ✓ Use this tab to activate journals for more customized hardware and image manipulation
- ✓ If a journal is activated, make sure to place a checkmark next to **Prevent asynchronous hardware moves**

Create New Protocol Settings: DISPLAY SETTINGS tab

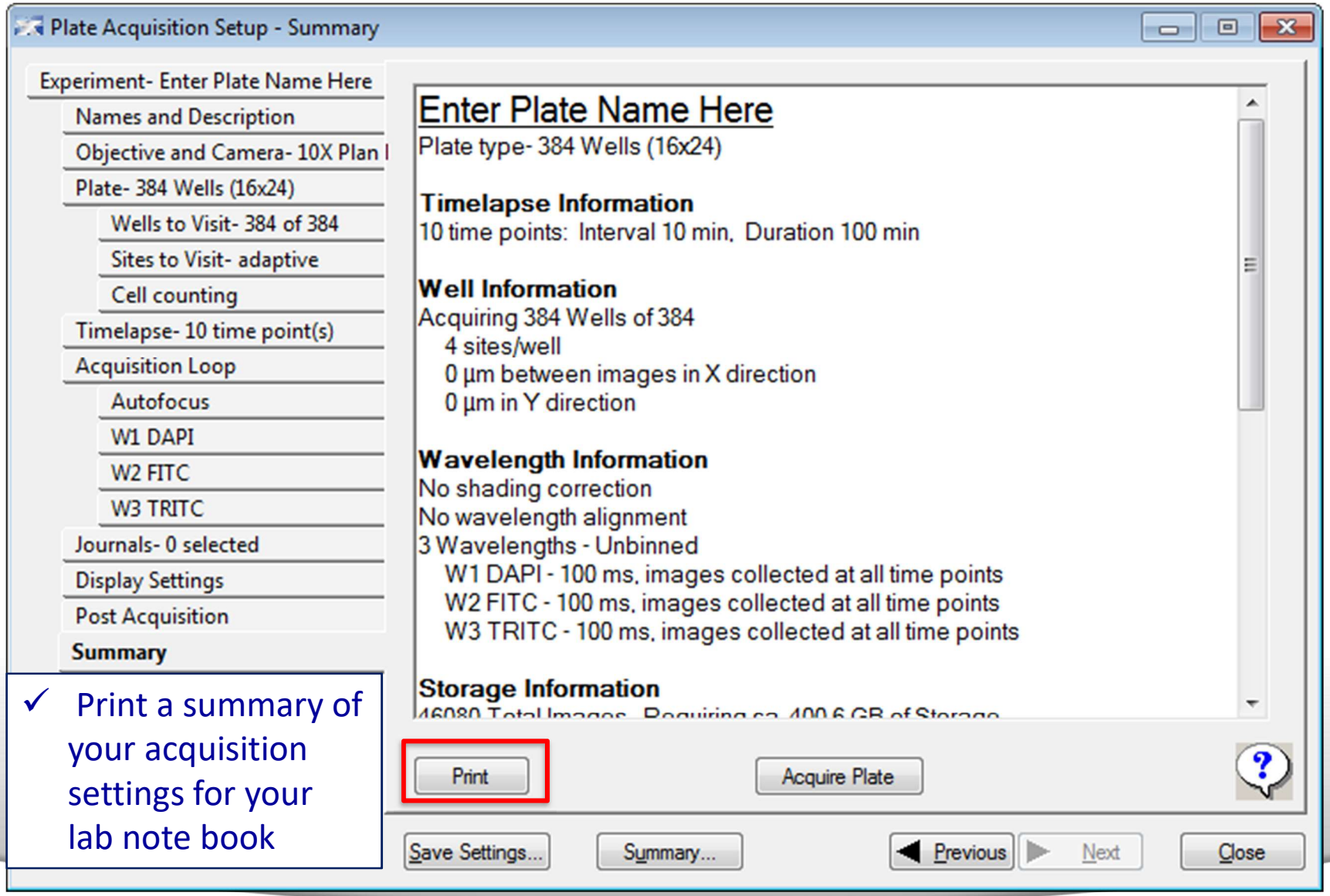


Create New Protocol Settings: POST ACQUISITION tab



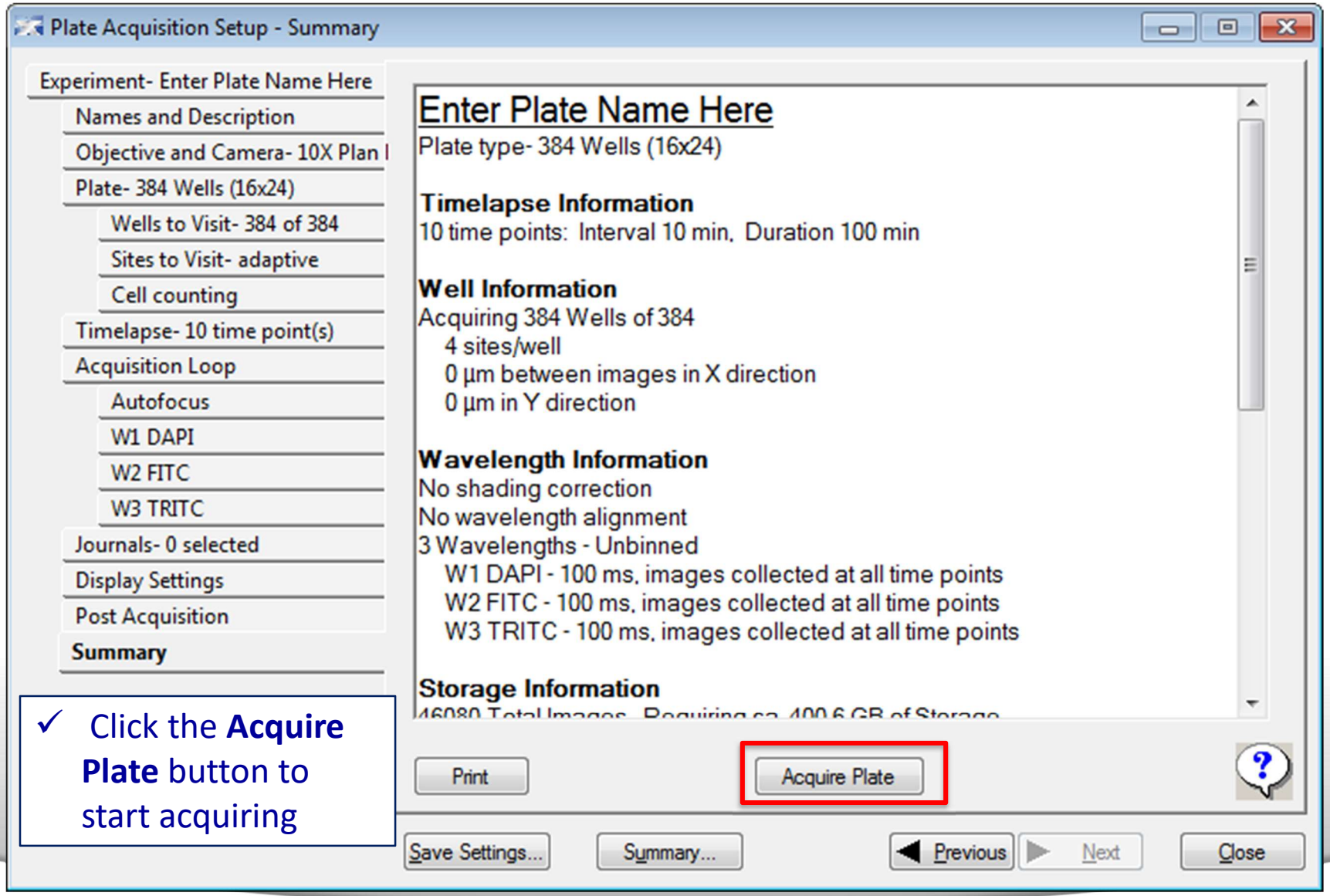
- ✓ You will first need to optimize settings before activating this feature
- ✓ Select application module/custom module/journal and settings from drop-down menu
- ✓ If acquiring timelapse data, select time points for analysis
  - ✓ **All time points**: all time points acquired will be analyzed
  - ✓ **Time point range**: single time point or range can be selected
  - ✓ **Stack of all time points**: if using a timelapse journal which analyzes the planes in a stack as separate time point
- ✓ Use this tab to mark plates for analysis in the database

Create New Protocol Settings: SUMMARY tab



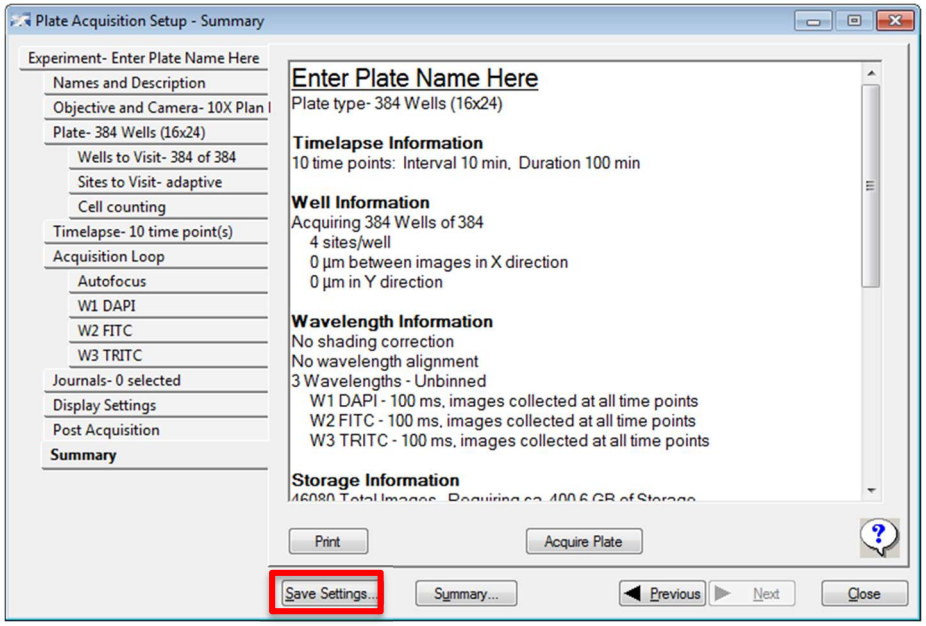
✓ Print a summary of your acquisition settings for your lab note book

Create New Protocol Settings: SUMMARY tab

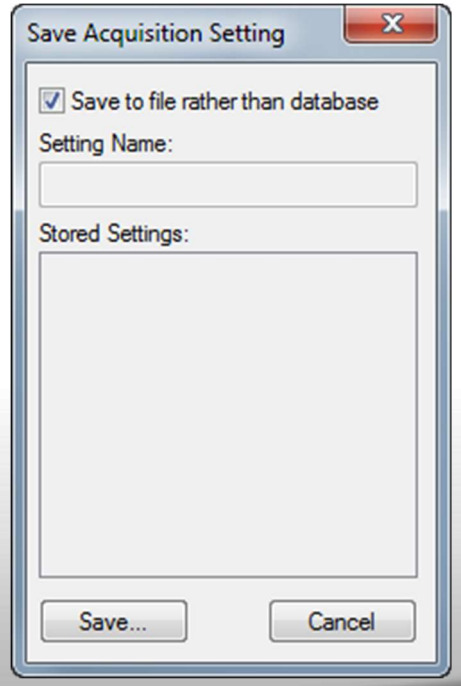


✓ Click the **Acquire Plate** button to start acquiring

Save Acquisition Settings



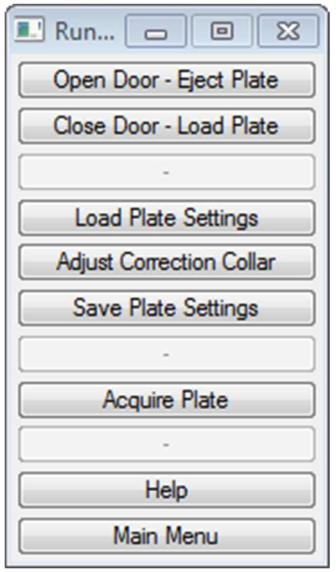
- ✓ Click the **Save Settings** button to save your acquisition settings
- ✓ It is recommended to save your settings to a file, rather the database.



Molecular Devices

Shutting down the ImageXpress® Micro System

- Remove the plate from the system
- Exit out of MetaXpress
- Turn off the lamp
- Turn off the power supply
- Turn off options controller
- Turn off computer (optional)
- Turn off monitor (optional)



Support Resources

- F1 / HELP within MetaXpress® Software
- Support and Knowledge Base: http://mdc.custhelp.com/app/home
- Email support@moldev.com
- 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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