

# ImageXpress<sup>®</sup> Micro XL and MetaXpress<sup>®</sup> 5.3 Acquisition Guide



Revision C

# ImageXpress® Micro XLS

## Hardware Features

# The ImageXpress® Micro XLS 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**

**16 Bit Images  
sCMOS Camera**

Together through life sciences.

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# ImageXpress Micro Standard and Micro XLS

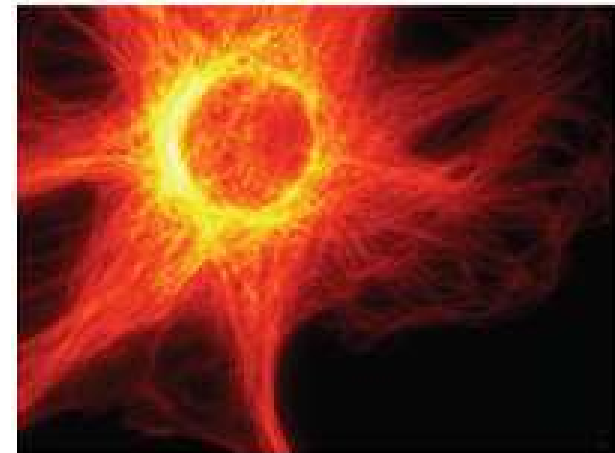


## ImageXpress Micro XLS features:

- 5.5 Megapixel sCMOS Camera
- 16-bit range
- Solid State Light Source

## ImageXpress Micro Standard features:

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



# ImageXpress Micro XLS Light Source

- 10,000 hour life span
- No parts exchange required
  - No bulb exchange every 1,000 hours
  - No shading correction after bulb exchange

## Solid State Light Source

- On-demand illumination
  - Maintains life-span
  - No warm-up time
- <1 ms electronic shutter
- Eliminates failures with mechanical shutters

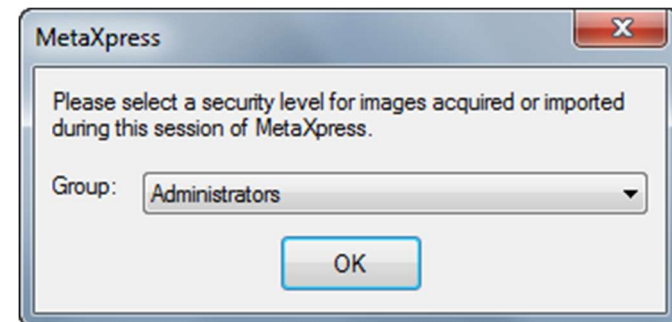
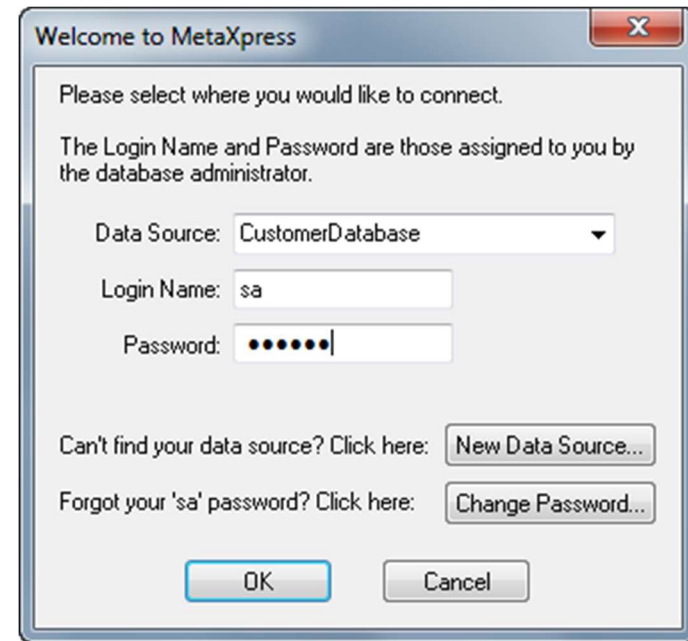
Minimizes Support Requirements

# 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

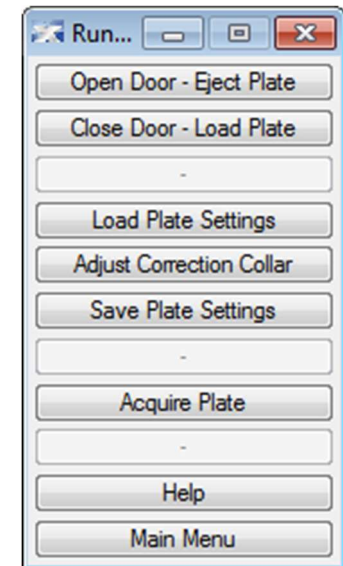
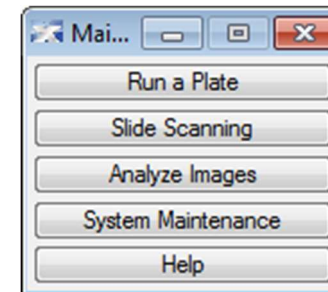


# Loading the Main Taskbar

Normally the Main Taskbar will load automatically.  
If you do not see it, press F4 on your keyboard.

If this still does not load the taskbar, then:

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

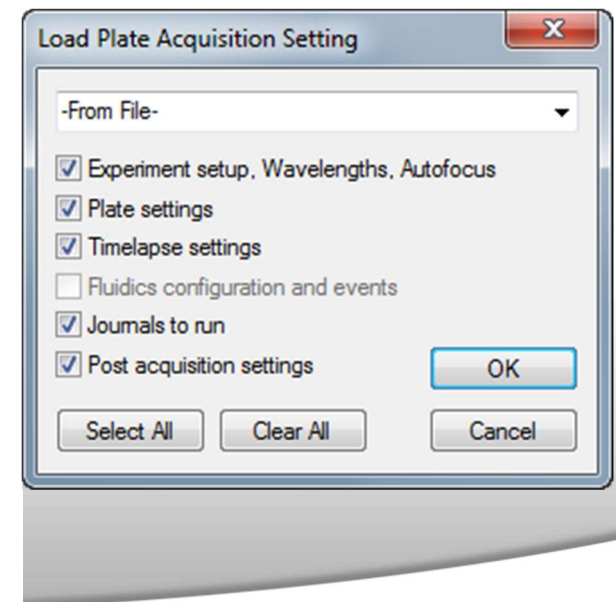
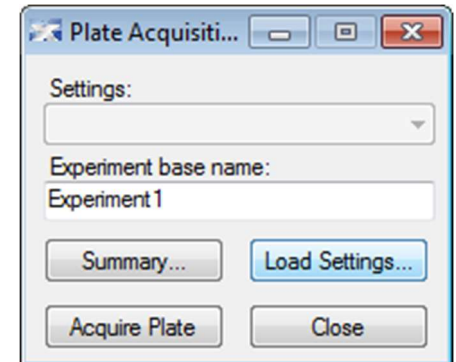




# Loading an Existing Protocol – Method 1

In MetaXpress, on the main toolbar:

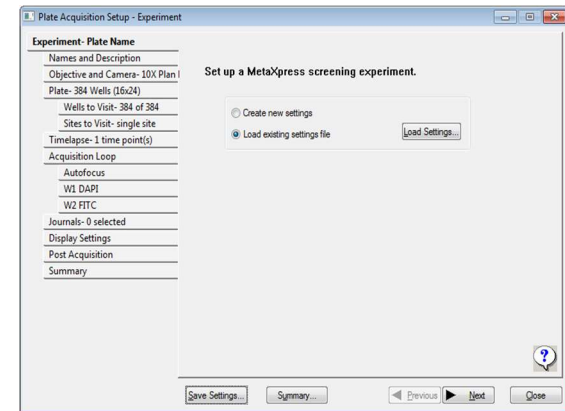
- Click on **Screening** → **Plate Acquisition**
- Click on **Load Settings**
  - Select **-From File-** from the drop-down menu and click on **OK**
- OR
- Select settings from the drop-down menu
- Enter a name in the **Experiment base name field**
- Make sure there is a plate in the system
- Click **Acquire Plate**



## Loading an Existing Protocol – Method 2

In MetaXpress, on the main toolbar:

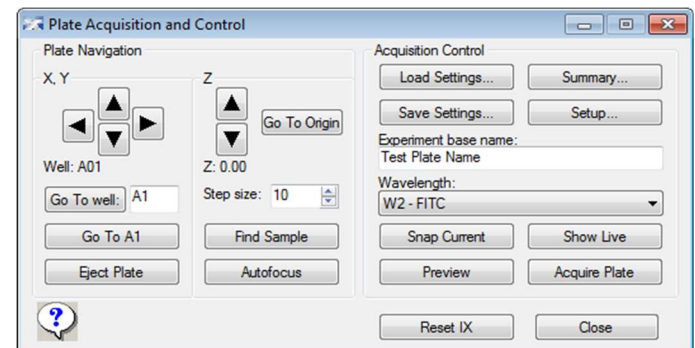
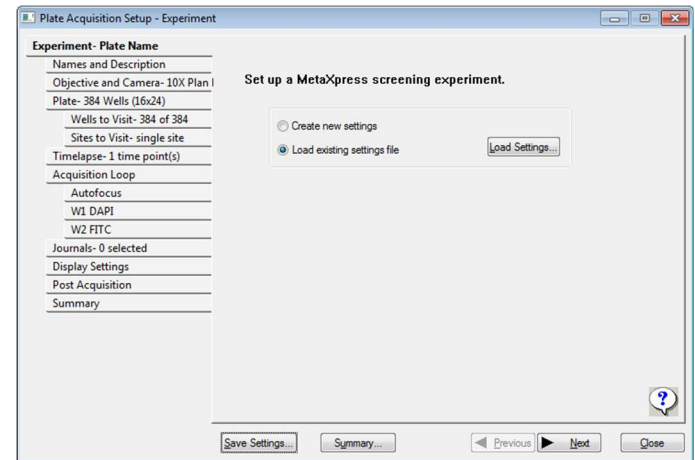
- Click on **Screening** → **Plate Acquisition Setup**
  - On the **Experiment** tab
    - Select **Load existing settings file**
    - Click on **Load Settings**
      - Select **-From File-** from the drop-down menu and click on **OK**
- OR
- Select settings from the drop-down menu
- 
- Make sure there is a plate in the system
  - On the **Summary** tab, click **Acquire Plate**



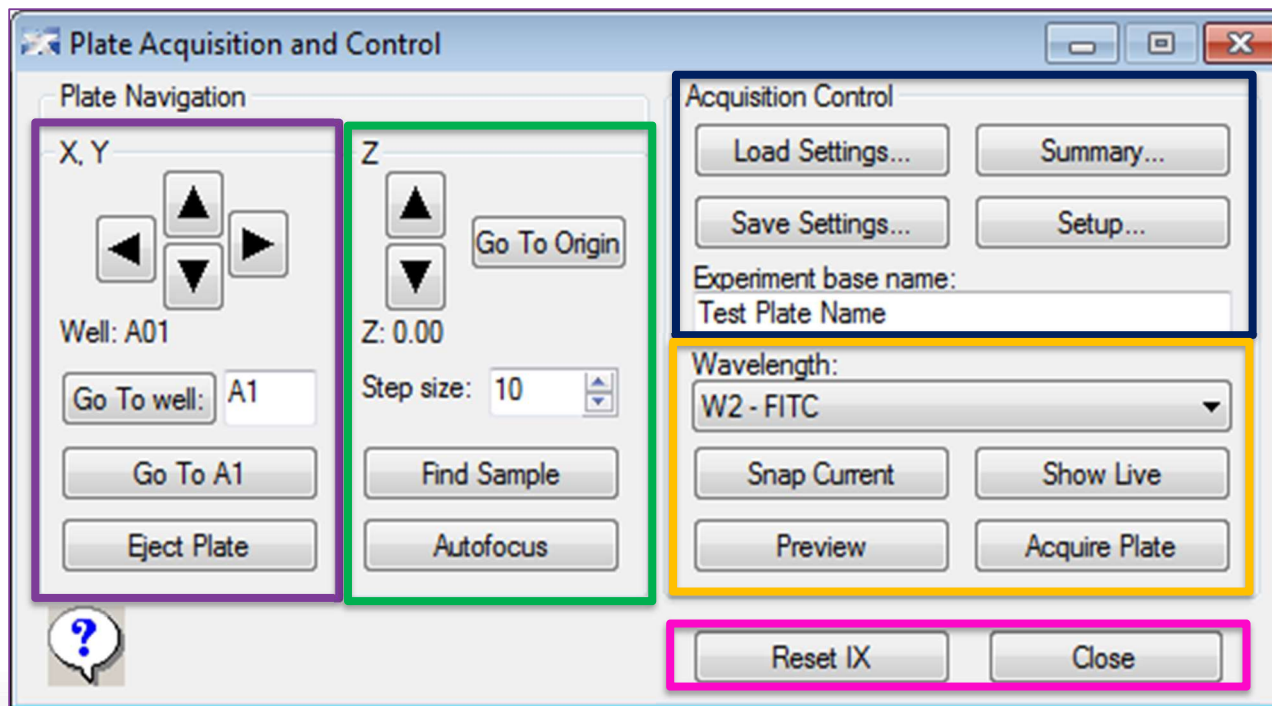
# Create New Protocol Settings

In MetaXpress, on the main toolbar click on:

- **Screening → Plate Acquisition Setup**
- **Screening → Plate Acquisition and Control**



# Plate Acquisition and Control



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

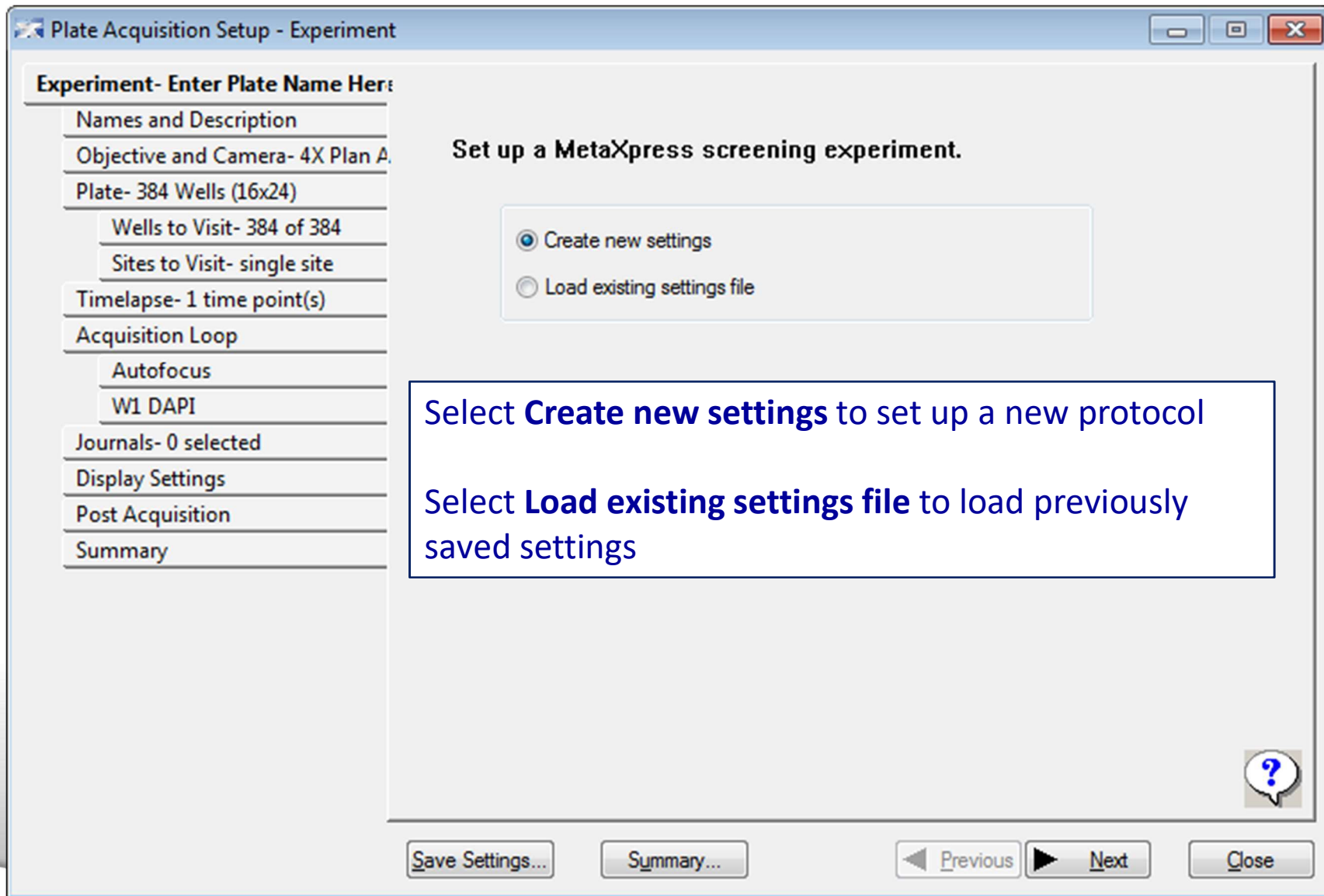
- ✓ **Find Sample:** Performs a large-range autofocus in the current XY location. If focus is found, it will then snap an image with the current wavelength.
- ✓ **Autofocus:** Performs a short-range autofocus.

- ✓ **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:** puts camera into live mode; may photobleach sample
- ✓ **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

Plate Acquisition Setup - Names and Description

Experiment- Enter Plate Name Here

**Names and Description**

Objective and Camera- 4X Plan A

Plate- 384 Wells (16x24)

Wells to Visit- 384 of 384

Sites to Visit- single site

Timelapse- 1 time point(s)

Acquisition Loop

Autofocus

W1 DAPI

Journals- 0 selected

Display Settings

Post Acquisition

Experiment Set: Enter Project Name Here

Experiment base name: Enter Plate Name Here

Storage location: Image Server

Description: Enter description here

- ✓ **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

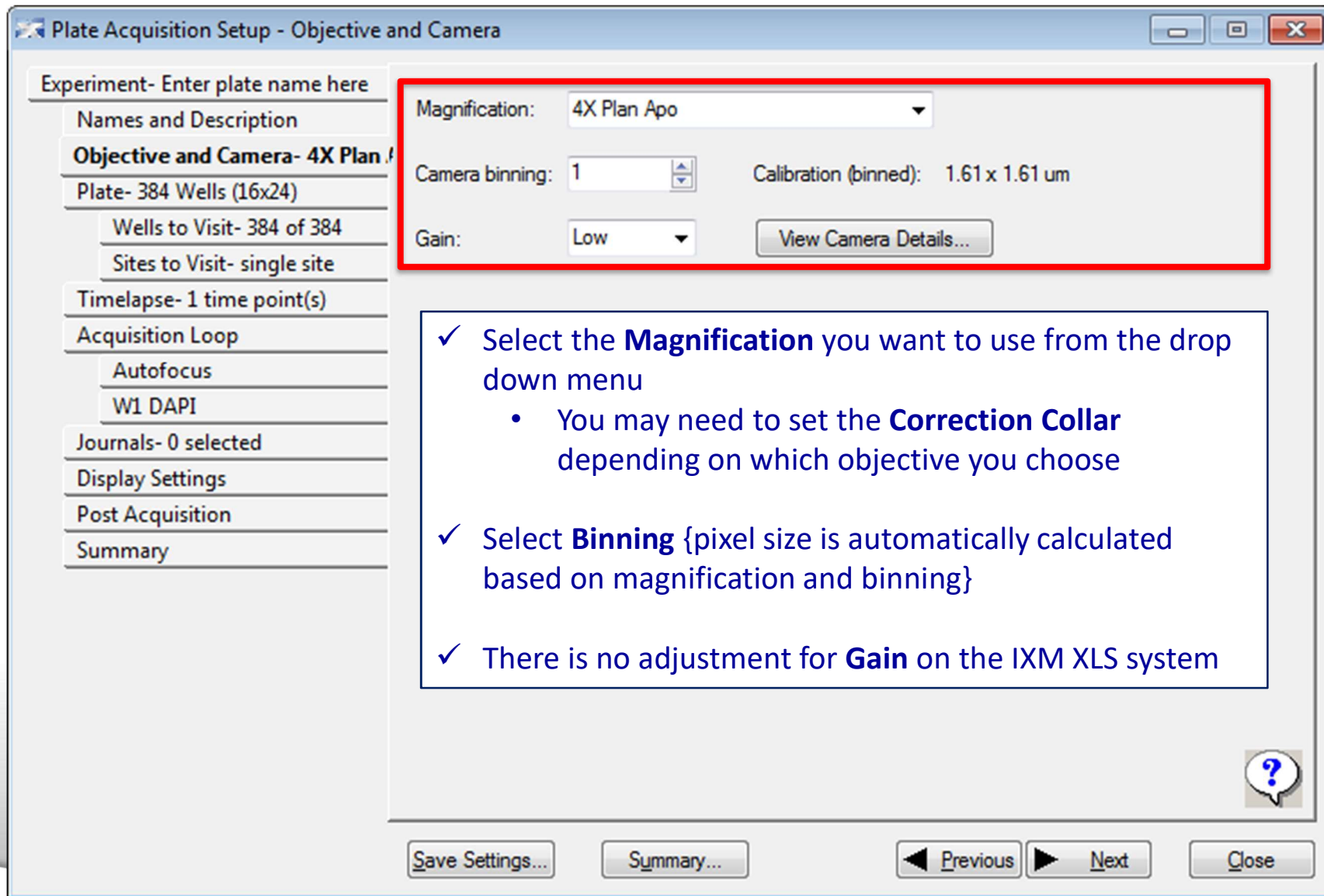


Plate Acquisition Setup - Objective and Camera

Experiment- Enter plate name here

Names and Description

**Objective and Camera- 4X Plan Apo**

Plate- 384 Wells (16x24)

Wells to Visit- 384 of 384

Sites to Visit- single site

Timelapse- 1 time point(s)

Acquisition Loop

Autofocus

W1 DAPI

Journals- 0 selected

Display Settings

Post Acquisition

Summary

Magnification: 4X Plan Apo

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

Gain: Low View Camera Details...

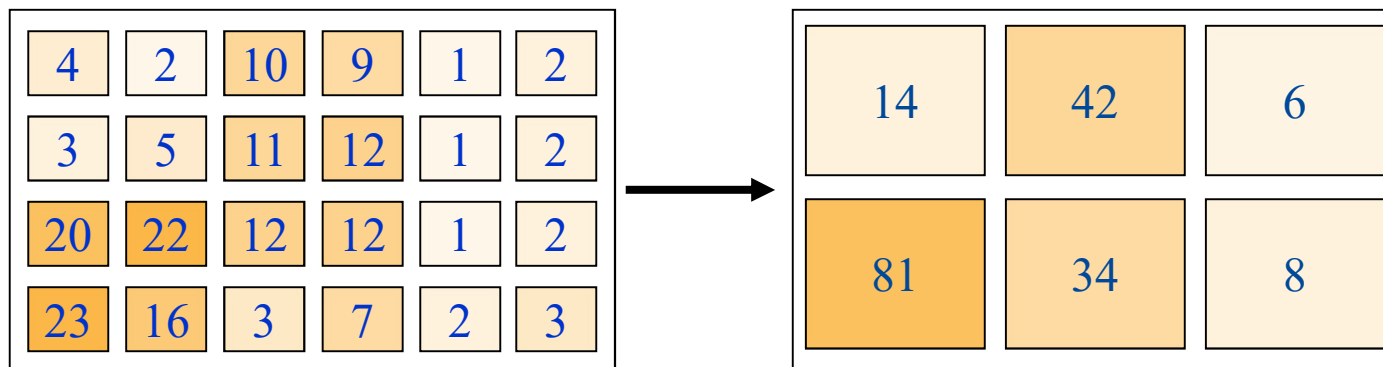
- ✓ Select the **Magnification** you want to use from the drop down menu
  - You may need to set the **Correction Collar** depending on which objective you choose
- ✓ Select **Binning** {pixel size is automatically calculated based on magnification and binning}
- ✓ There is no adjustment for **Gain** on the IXM XLS system

Save Settings... Summary... Previous Next Close

# What is Binning?

Combining groups of pixels into a single pixel during image acquisition

## 2x2 Binning



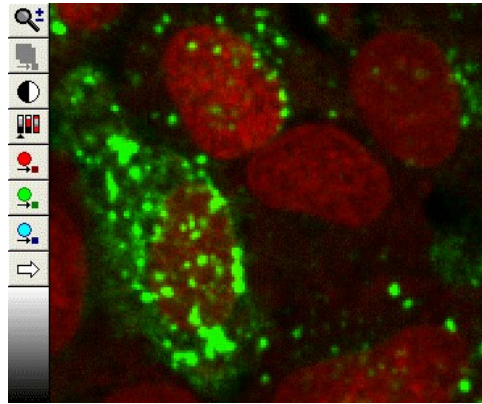
What the camera sees

Pixels are summed

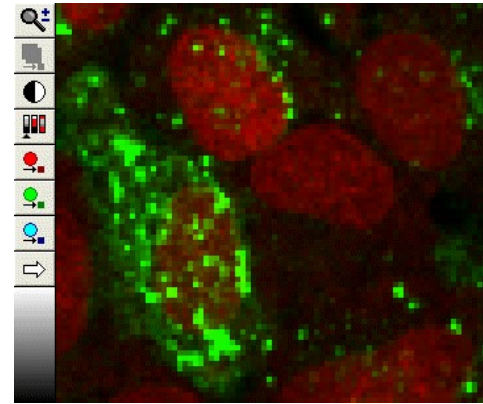


# Won't Binning Affect Resolution?

Binned images are lower resolution than unbinned images

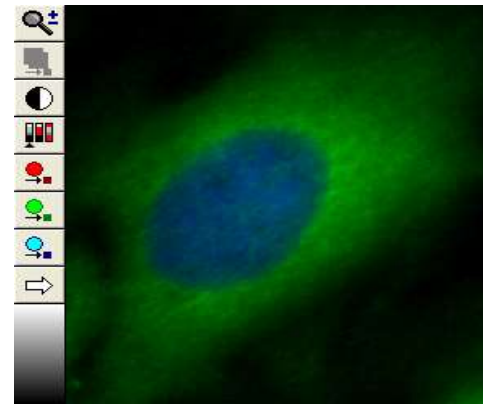
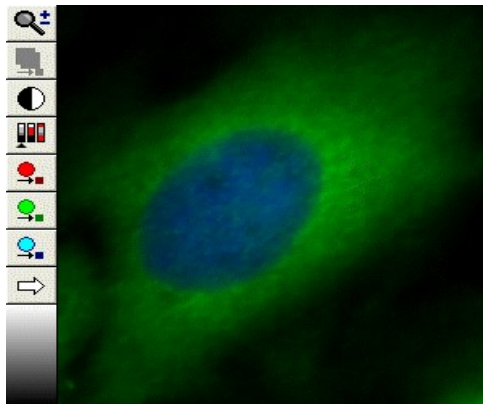


Unbinned



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

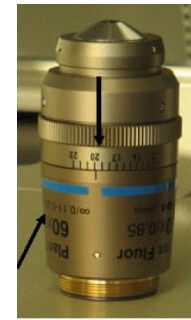
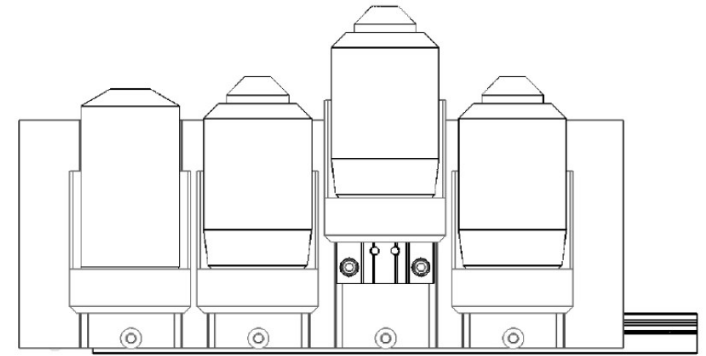
- Shorter exposure times
- Faster image transfer from camera to computer
- Faster image transfer from MetaXpress to database
- Faster image analysis

## Improve Image quality

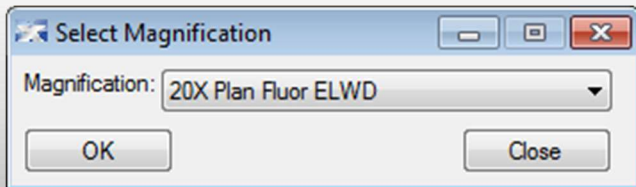
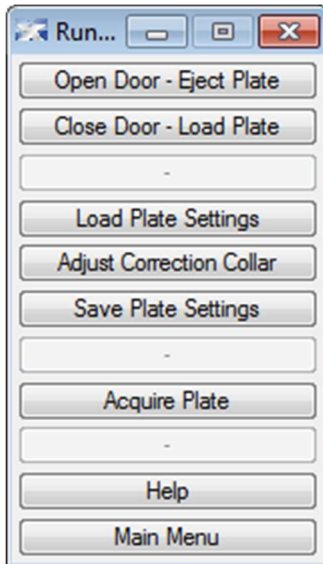
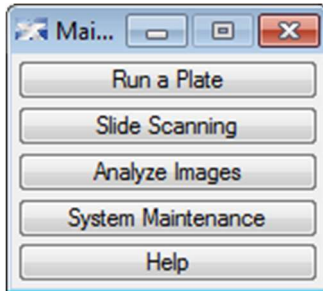
- Better signal / noise

# 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



# 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

Plate Acquisition Setup - Plate

Experiment- Enter Plate Name Here

Names and Description

Objective and Camera- 4X Plan A.

**Plate- 384 Wells (16x24)**

Wells to Visit- 384 of 384

Sites to Visit- single site

Timelapse- 1 time point(s)

Acquisition Loop

Autofocus

W1 DAPI

Journals- 0 selected

Display Settings

Post Acquisition

Summary

Plate name: 384 Wells (16x24) Save Configuration...

Number of rows: 16

Number of columns: 24

Well shape: Square

Well diameter ( $\mu\text{m}$ ): 3300

Column spacing ( $\mu\text{m}$ ): 4500

Plate length (mm): 127.8

Column offset ( $\mu\text{m}$ ): 12130

Row spacing ( $\mu\text{m}$ ): 4500

Plate width (mm): 85.5

Row offset ( $\mu\text{m}$ ): 8990

Well depth ( $\mu\text{m}$ ): 12000

Plate height (mm): 14.4

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

Save Settings... Summary... Previous Next Close

✓ Select the plate type from the drop-down menu

# Create New Protocol Settings: PLATE tab

Plate Acquisition Setup - Plate

Experiment- Enter Plate Name Here

Names and Description

Objective and Camera- 4X Plan A.

**Plate- 384 Wells (16x24)**

Wells to Visit- 384 of 384

Sites to Visit- single site

Timelapse- 1 time point(s)

Acquisition Loop

Autofocus

W1 DAPI

Journals- 0 selected

Display Settings

Post Acquisition

Summary

Plate name: 384 Wells (16x24) Save Configuration...

Number of rows: 16

Number of columns: 24

Well shape: Square

Well diameter ( $\mu\text{m}$ ): 3300

Column spacing ( $\mu\text{m}$ ): 4500

Plate length (mm): 127.8

Column offset ( $\mu\text{m}$ ): 12130

Row spacing ( $\mu\text{m}$ ): 4500

Plate width (mm): 85.5

Row offset ( $\mu\text{m}$ ): 8990

Well depth ( $\mu\text{m}$ ): 12000

Plate height (mm): 14.4

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

Save Settings... Summary... Previous Next Close

✓ 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

Plate Acquisition Setup - Wells to Visit

Experiment- Enter Plate Name Here

Names and Description

Objective and Camera- 4X Plan A.

Plate- 384 Wells (16x24)

**Wells to Visit- 384 of 384**

Sites to Visit- single site

Timelapse- 15 time point(s)

Acquisition Loop

Autofocus

W1 FITC

Journals- 0 selected

Display Settings

Post Acquisition

Summary

Wells to visit:

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A	Gray	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green
B	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green
C	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green
D	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green
E	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green
F	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green
G	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green
H	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green
I	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green
J	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green
K	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green
L	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green
M	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green
N	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green
O	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green
P	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green

Left-click to toggle a well on/off. Right-click to move the stage to that well.

Clicking here will enable/disable all wells

- ✓ Gray wells are deactivated, green wells are activated and will be imaged.
- ✓ Click on "All", row letters, column numbers, or individual wells

Save Settings... Summary... Previous Next Close

# Create New Protocol Settings: WELLS TO VISIT tab

Plate Acquisition Setup - Wells to Visit

Experiment- Enter Plate Name Here

Names and Description

Objective and Camera- 4X Plan A.

Plate- 384 Wells (16x24)

**Wells to Visit- 366 of 384**

Sites to Visit- single site

Timelapse- 15 time point(s)

Acquisition Loop

Autofocus

W1 FITC

Journals- 0 selected

Display Settings

Post Acquisition

Summary

Wells to visit:

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A																								
B																								
C																								
D																								
E																								
F																								
G																								
H																								
I																								
J																								
K																								
L																								
M																								
N																								
O																								
P																								

Right-Clicking on a well will turn the well **DARK GREEN** and activate the stage to move to that well

Left-click to toggle a well on/off. Right-click to move the stage to that well.

Save Settings... Summary... Previous Next Close



## Create New Protocol Settings: SITES TO VISIT tab

Site acquisition mode

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

Site size

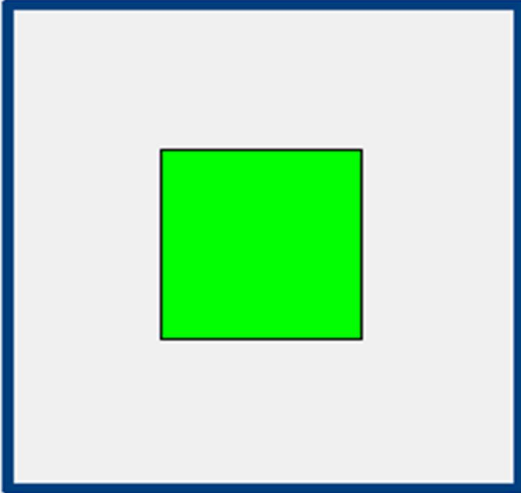
Site/image size: 721.44 x 721.44  $\mu\text{m}$

Well size: 1.80 x 1.80 mm

Custom field of view (%):

X: 68 Y: 68

Acquires a single site centered in each well



The diagram shows a large blue square representing a well. Inside it, a smaller green square represents a single site centered in the well.

Save Settings... Summary... Previous Next Close

✓ 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:
  - ✓ **Green** is imaged
  - ✓ **Gray** 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**: Set spacing to 0 in X and Y so that sites are adjacent
- ✓ **Fit sites to well**: Set spacing so that the sites are event distributed through the well

Site acquisition mode

Single site

Fixed number of sites

Adaptive acquisition

Multi-well

Site size

Site/image size: 721.44 x 721.44 µm

Well size: 1.80 x 1.80 mm

Custom field of view (%):

X: 68 Y: 68

Acquires a fixed number of sites in each well

Multi-site layout:

Total number of sites: 4

Image spread: 1.44 x 1.44 mm

Spacing (µm):

Columns: 2

Rows: 2

Tile sites

Fit sites to well

Save Settings...

Summary...

Previous

Next

Close

# Create New Protocol Settings: SITES TO VISIT tab

Site acquisition mode

Single site

Fixed number of sites

Adaptive acquisition

Multi-well

Site size

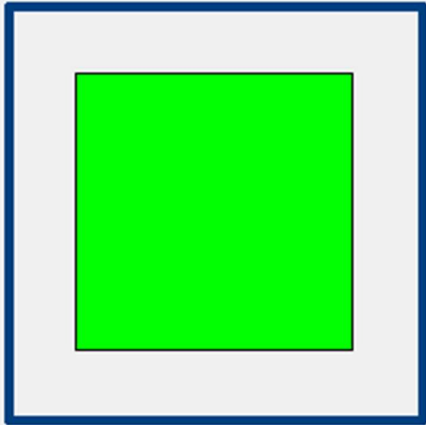
Site/image size: 1.22 x 1.22 mm

Well size: 1.80 x 1.80 mm

Custom field of view (%):

X: 68 Y: 68

Acquires a single site in 4 (2 x 2) wells simultaneously  
reducing plate acquisition time  
Not for use with fluidics or device/camera journal events



Save Settings... Summary... Previous Next Close

✓ **Custom Field of View Section** allows you to reduce the image size. This may be helpful if the image is larger than the well.

# Create New Protocol Settings: SITES TO VISIT tab

Site acquisition mode  
 Single site  
 Fixed number of sites  
 Adaptive acquisition  
 Multi-well

Site size  
Site/image size: 721.44 x 721.44  $\mu\text{m}$   
Well size: 1.80 x 1.80 mm  
 Custom field of view (%):  
X: 68 Y: 68

Multi-site layout:  
Total number of sites: 4  
Image spread: 1.44 mm  
Columns: 2  
Rows: 2  
Minimum sites to visit: 1  
Tile sites

Acquires sites based on the number of cells per well specified on the Cell counting tab

Save Settings... Summary...

- ✓ Activating Adaptive Acquisition results in the **Cell Counting** tab to appear
- ✓ **Adaptive acquisition:** Acquire sites until a specified cell count is reached, then skip to the next well.

Plate Acquisition Setup - Cell counting

Experiment- Enter Plate Name Here

Names and Description  
Objective and Camera- 10X Plan I  
Plate- 384 Wells (16x24)  
Wells to Visit- 384 of 384  
Sites to Visit- adaptive  
**Cell counting**  
Timelapse- 1 time point(s)  
Acquisition Loop  
Autofocus  
W1 DAPI  
W2 FITC  
W3 TRITC  
Journals- 0 selected  
Display Settings  
Post Acquisition  
Summary

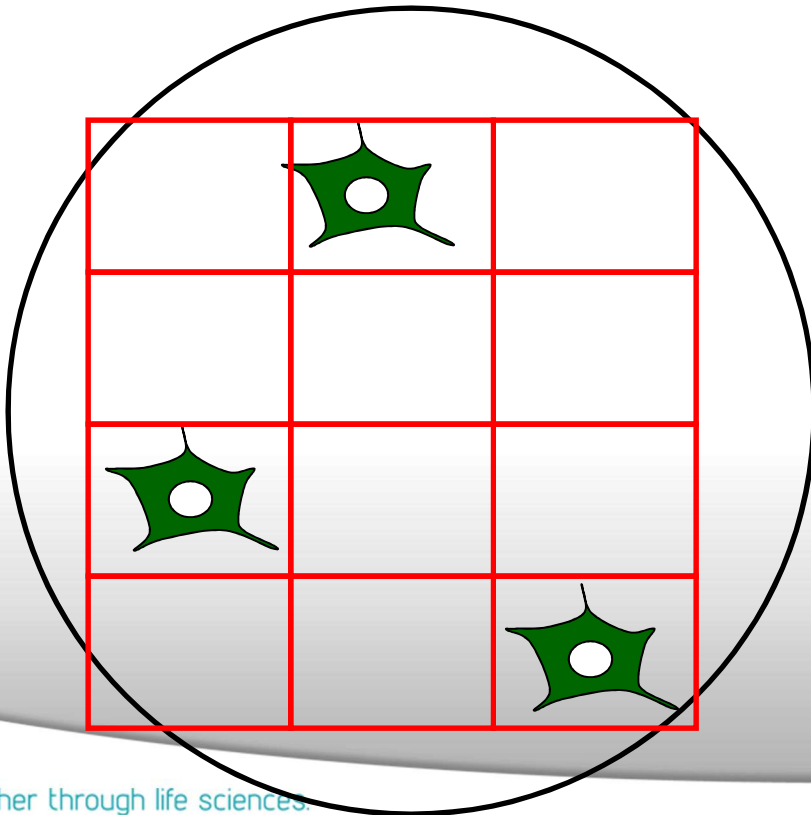
Nuclei counting  
Wavelength: DAPI  
Approximate width: 5 to 10  $\mu\text{m}$   
Intensity above local background: 100 gray levels  
Desired cell count/well: 50  
Test settings Nuclei count: 0  
Pressing Test Settings will snap a new image at the selected wavelength for counting nuclei. NOTE- the sample should already be in focus.

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

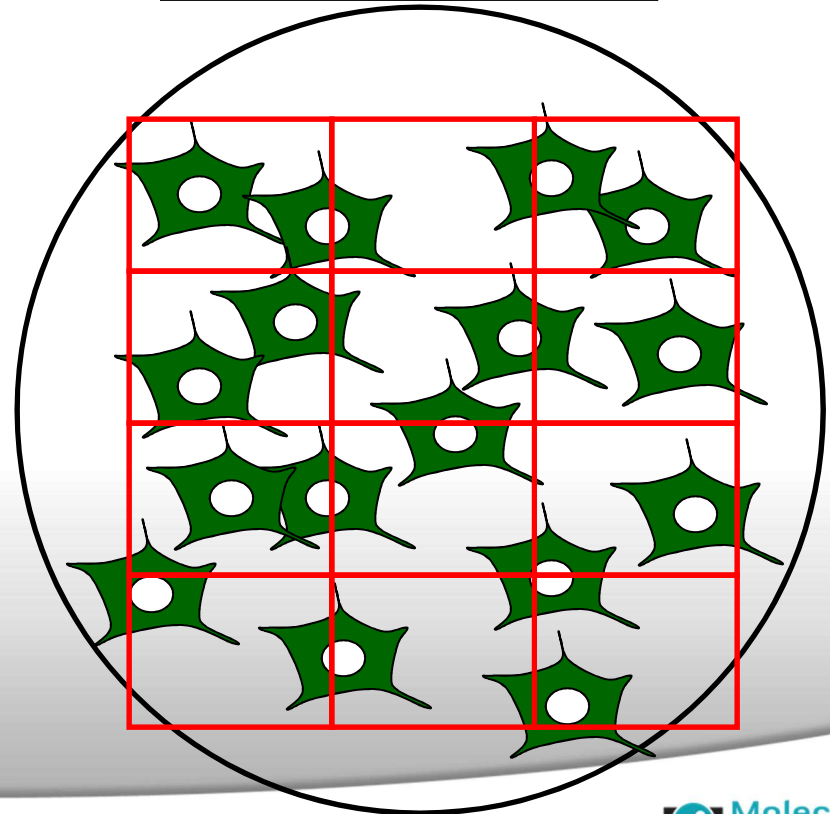
# Adaptive Acquisition™: fast multi-site imaging

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

Minimum needed: 3  
Sites captured: 12



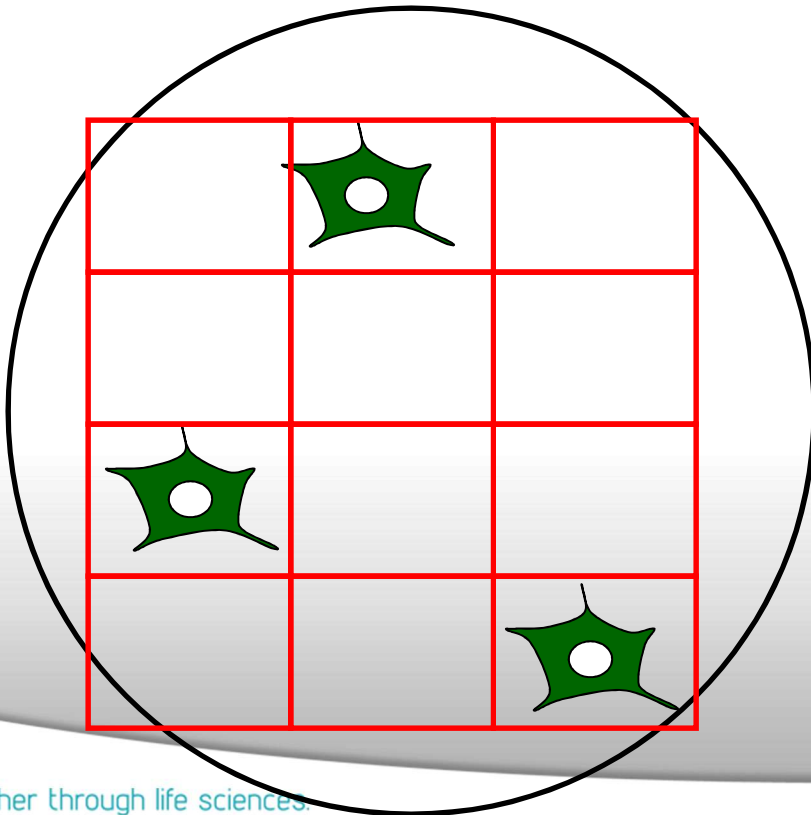
Minimum needed: 3  
Sites captured: 12



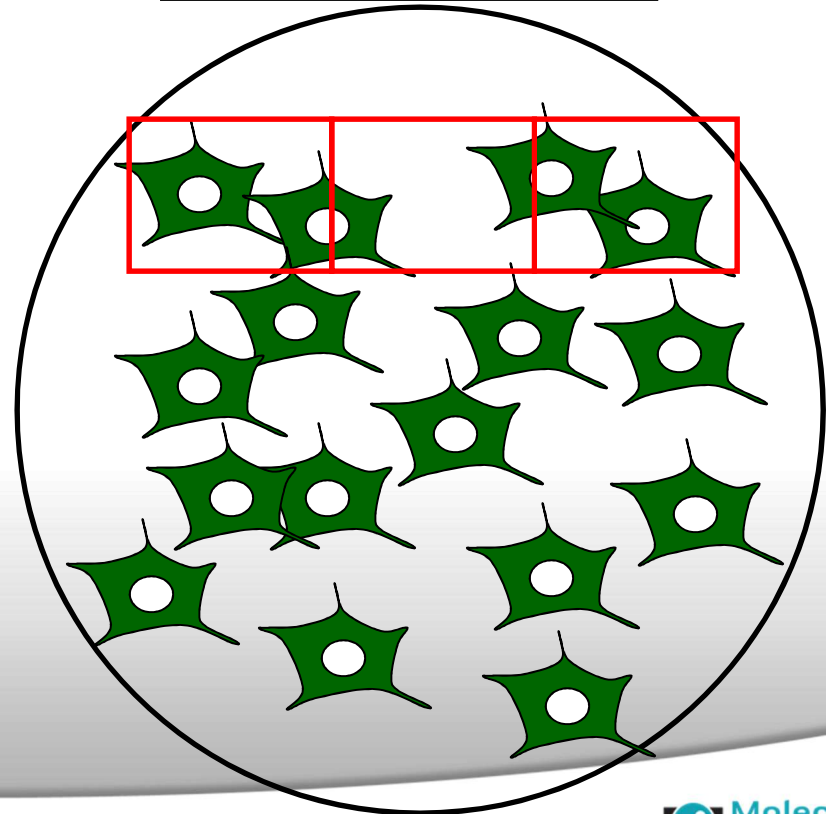
# Adaptive Acquisition™: fast multi-site imaging

- 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

Minimum needed: 3  
Sites captured: 12

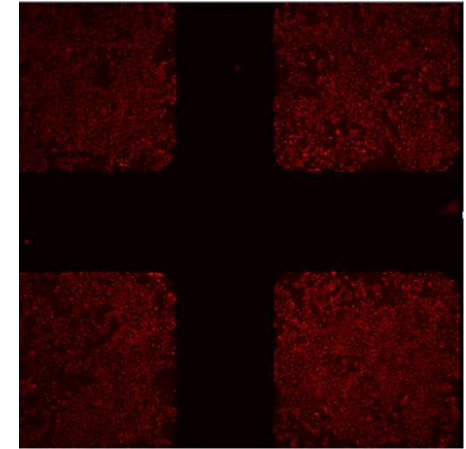


Minimum needed: 3  
Sites captured: 3



## Create New Protocol Settings: SITES TO VISIT tab

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



Site acquisition mode

Single site

Fixed number of sites

Adaptive acquisition

Multi-well

Site size

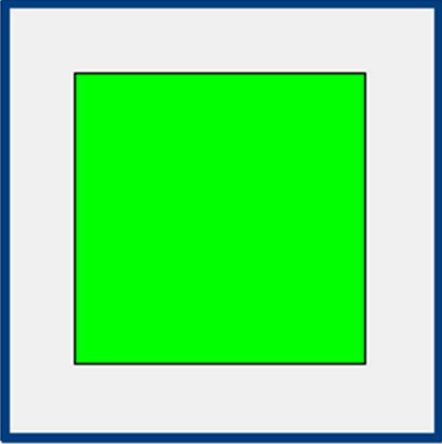
Site/image size: 1.22 x 1.22 mm

Well size: 1.80 x 1.80 mm

Custom field of view (%):

X: 68 Y: 68

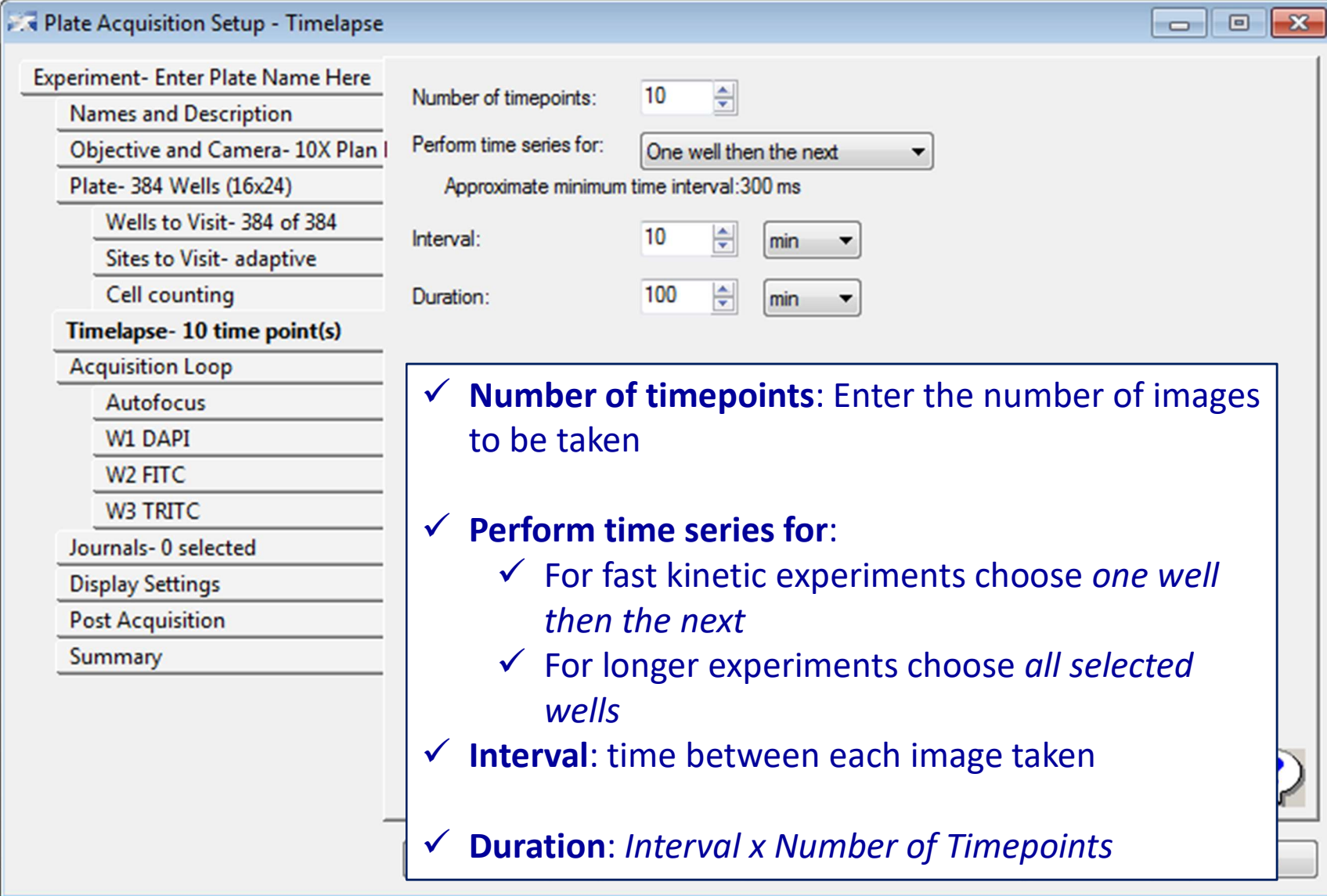
Acquires a single site in 4 (2 x 2) wells simultaneously reducing plate acquisition time  
Not for use with fluidics or device/camera journal events



Save Settings... Summary... Previous Next Close

- ✓ 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

# Create New Protocol Settings: TIMELAPSE tab



The screenshot shows the 'Plate Acquisition Setup - Timelapse' window. On the left is a sidebar with a tree view containing sections like 'Experiment- Enter Plate Name Here', 'Names and Description', 'Objective and Camera- 10X Plan', 'Plate- 384 Wells (16x24)', 'Wells to Visit- 384 of 384', 'Sites to Visit- adaptive', 'Cell counting', 'Timelapse- 10 time point(s)', 'Acquisition Loop' (with sub-items: Autofocus, W1 DAPI, W2 FITC, W3 TRITC), 'Journals- 0 selected', 'Display Settings', 'Post Acquisition', and 'Summary'. The main area contains the following settings:

- Number of timepoints: 10
- Perform time series for: One well then the next
- Approximate minimum time interval: 300 ms
- Interval: 10 min
- Duration: 100 min

A callout box on the right contains the following instructions:

- ✓ **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:**  $Interval \times Number\ of\ Timepoints$



# Create New Protocol Settings: ACQUISITION LOOP tab

Plate Acquisition Setup - Acquisition Loop

Experiment- Enter Plate Name Here

Names and Description

Objective and Camera- 10X Plan

Plate- 384 Wells (16x24)

Wells to Visit- 384 of 384

Sites to Visit- adaptive

Cell counting

Timelapse- 10 time point(s)

**Acquisition Loop**

Autofocus

W1 DAPI

W2 FITC

W3 TRITC

Journals- 0 selected

Display Settings

Post Acquisition

Summary

Number of wavelengths: 3

Autofocus options

Enable laser-based focusing

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

Perform shading correction

Directory... C:\

- ✓ 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

\* Typical reasons to use image based focusing: Suspension cells, live organisms, samples in a thick gel

# Create New Protocol Settings: AUTOFOCUS tab

Plate Acquisition Setup - Autofocus

Experiment- Enter Plate Name Here

Names and Description

Objective and Camera- 10X Plan I

Plate- 384 Wells (16x24)

Wells to Visit- 384 of 384

Sites to Visit- adaptive

Cell counting

Timelapse- 10 time point(s)

Acquisition Loop

**Autofocus**

W1 DAPI

W2 FITC

W3 TRITC

Journals- 0 selected

Display Settings

Post Acquisition

Summary

**Laser-based Focusing**

Configure Laser Settings...

Well to well autofocus: Focus on well bottom

**Image-based Focusing**

Algorithm: Standard Binning: 2  Custom exposure times

Allow image-based focusing for recovery from laser-based well bottom failures

Initial well for finding sample: First well acquired A 1

Number of wells to attempt initial find sample: 1

Site Autofocus: First site only

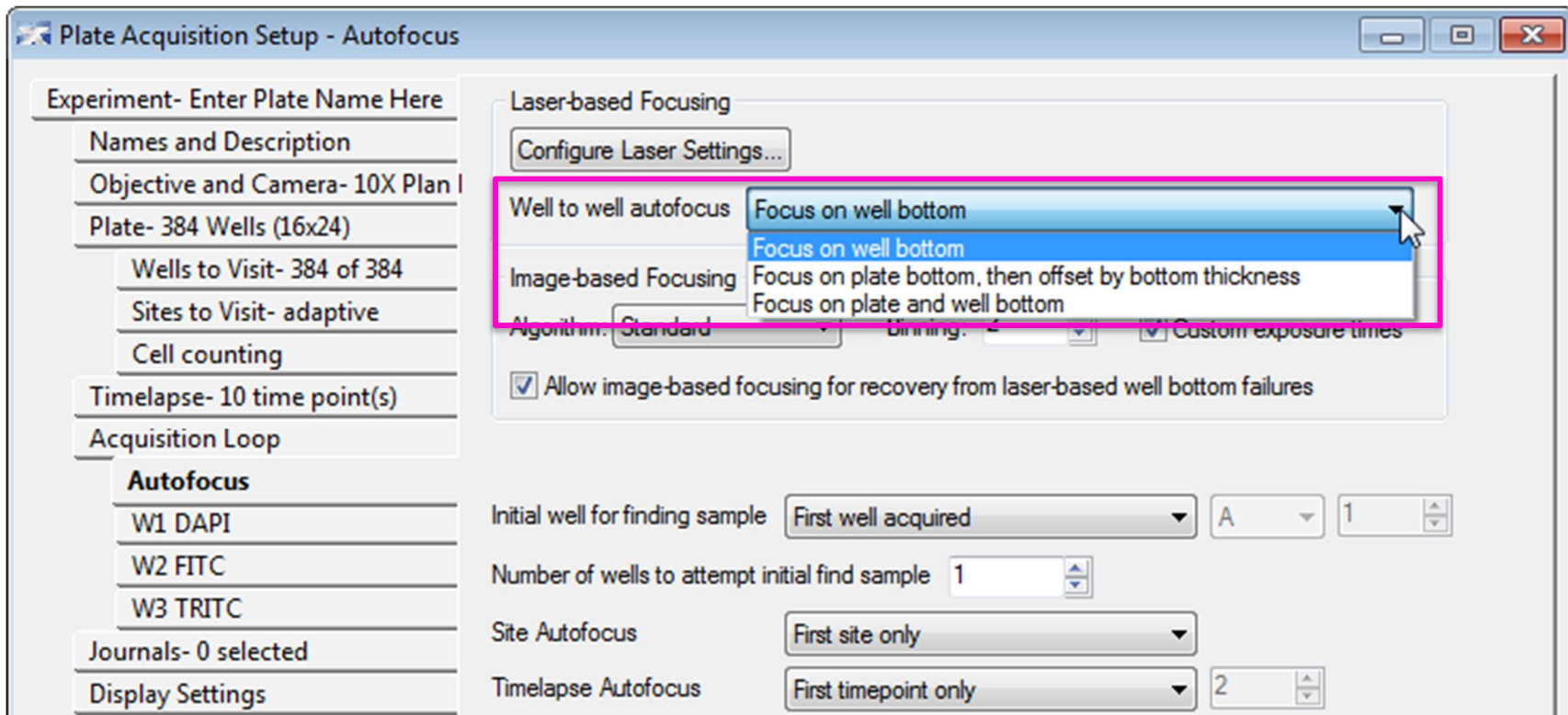
Timelapse Autofocus: First timepoint only 2

Configure Laser Settings: click this button to view laser autofocus settings

View Focusing Details... ?

Save Settings... Summary... Previous Next Close

# 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 (4X and below), or 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) – which is most thin bottom plates

# Create New Protocol Settings: AUTOFOCUS tab

Plate Acquisition Setup - Autofocus

Experiment- Enter Plate Name Here

Names and Description

Objective and Camera- 10X Plan I

Plate- 384 Wells (16x24)

Wells to Visit- 384 of 384

Sites to Visit- adaptive

Cell counting

Timelapse- 10 time point(s)

Acquisition Loop

**Autofocus**

W1 DAPI

Laser-based Focusing

Configure Laser Settings...

Well to well autofocus Focus on well bottom

Image-based Focusing

Algorithm: Standard Binning: 2  Custom exposure times

Allow image-based focusing for recovery from laser-based well bottom failures

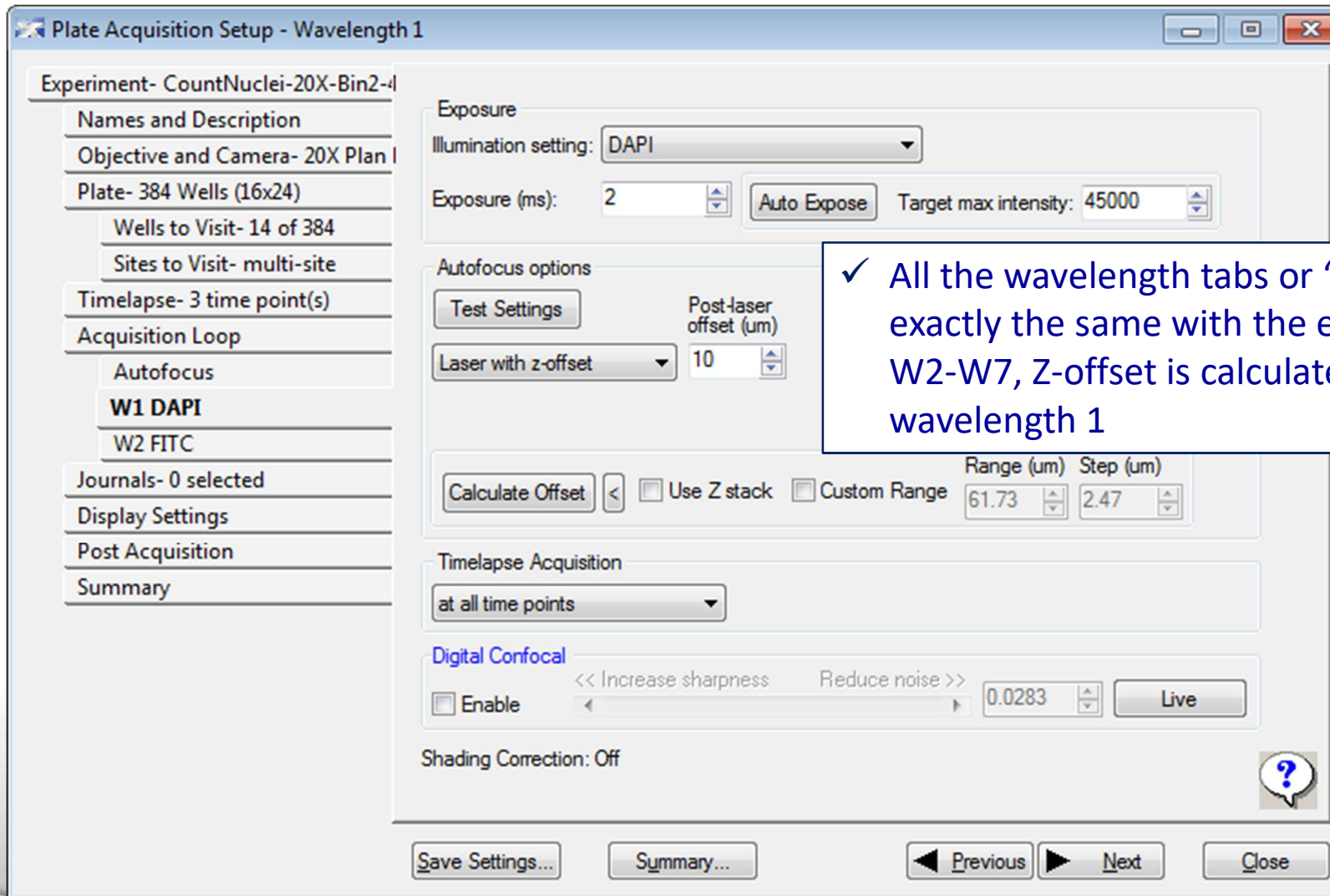
Initial well for finding sample First well acquired A 1

- ✓ 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

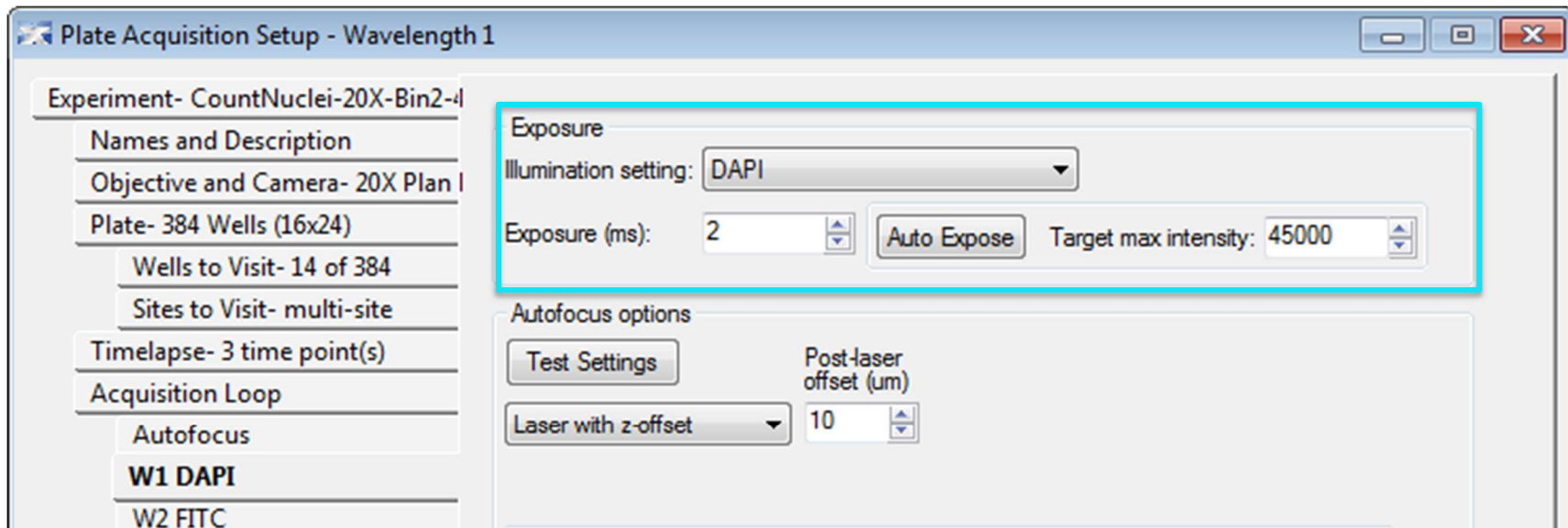
- ✓ Initial well for finding sample: default is first well acquired
- ✓ **Site Autofocus:**
  - ✓ select *First site only* when using low magnification or closely-spaced sites to save time
  - ✓ select *All sites* for high magnification or widely spaced sites for more accuracy
- ✓ **Timelapse Autofocus:**
  - ✓ Select **First timepoint only** : Fast kinetic experiments with short time intervals
  - ✓ Select **All timepoints**: Long timelapse experiments with long time intervals
  - ✓ Select **Every Nth timepoint**: Timelapse experiments with intermediate time intervals

# 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. Only use when you are in focus on a suitable sample
- ✓ Target max intensity: Sets the intensity that auto exposure should attempt to attain for the brightest pixel in the image (recommended value: 45000)
- ✓ Check exposure times for positive and negative controls

# Create New Protocol Settings: W1 (wavelength) tab

Plate Acquisition Setup - Wavelength 1

Experiment- CountNuclei-20X-Bin2-4

Names and Description

Objective and Camera- 20X Plan I

Plate- 384 Wells (16x24)

Wells to Visit- 14 of 384

Sites to Visit- multi-site

Timelapse- 3 time point(s)

Acquisition Loop

Autofocus

**W1 DAPI**

W2 FITC

Journals- 0 selected

Display Settings

Exposure

Illumination setting: DAPI

Exposure (ms): 2 Auto Expose Target max intensity: 45000

Autofocus options

Test Settings Post-laser offset (um)

Laser with z-offset 10

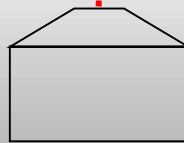
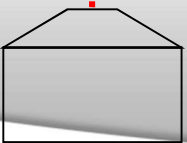
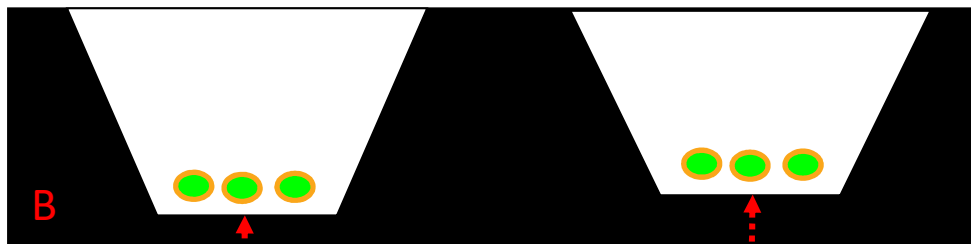
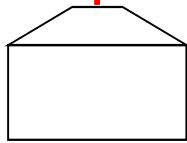
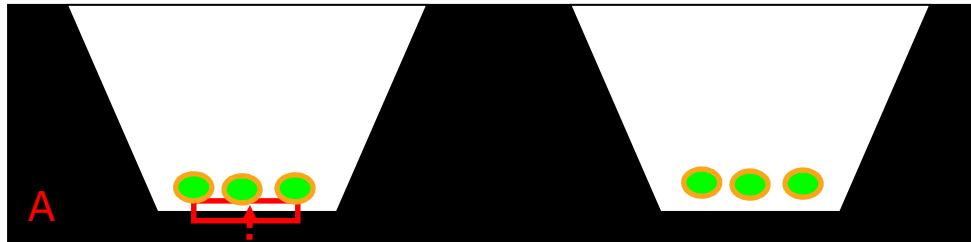
Calculate Offset <  Use Z stack  Custom Range Range (um) Step (um)

61.73 2.47

- ✓ 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



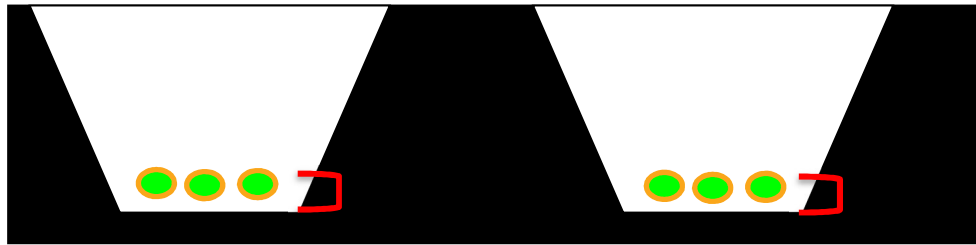
# What is a Z-offset?



 Z-offset

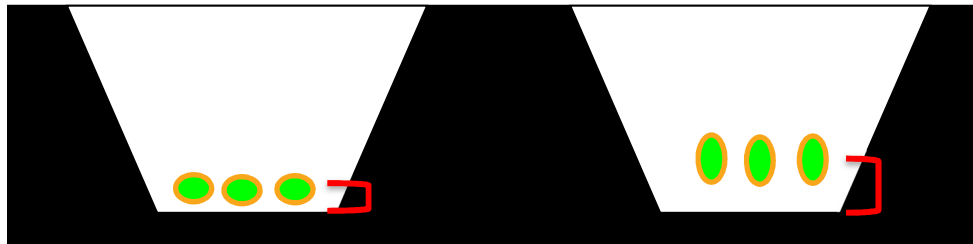
- Autofocus only puts you at the apparent well bottom NOT the biological sample of interest
- You may need an empirically determined "offset" to put you at the target
- Very wavelength dependent (chromatic aberration)
- Laser autofocus should take into account variations in the well bottom (see B).
-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

## When Do You Add in Image Based Focus?

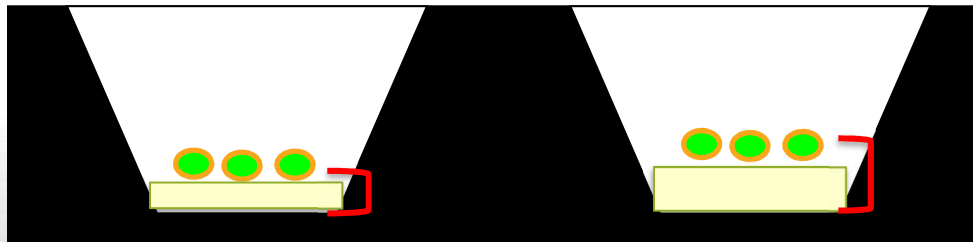


Typically all wells have the same relative offset from the laser focus.

### 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

Plate Acquisition Setup - Wavelength 1

Experiment- CountNuclei-20X-Bin2-4

Names and Description

Objective and Camera- 20X Plan I

Plate- 384 Wells (16x24)

Wells to Visit- 14 of 384

Sites to Visit- multi-site

Timelapse- 3 time point(s)

Acquisition Loop

Autofocus

**W1 DAPI**

W2 FITC

Journals- 0 selected

Display Settings

Post Acquisition

Summary

Exposure

Illumination setting: DAPI

Exposure (ms): 2 Auto Expose Target max intensity: 45000

Autofocus options

Test Settings

Post-laser offset (um) 10

Calculate Offset <  Use Z stack  Custom Range Range (um) 61.73 Step (um) 2.47

Timelapse Acquisition

at all time points

Save Settings... Summary... Previous Next Close

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

# Create New Protocol Settings: JOURNAL tab

Plate Acquisition Setup - Journals

Experiment- Enter Plate Name Here

Names and Description

Objective and Camera- 10X Plan I

Plate- 384 Wells (16x24)

Wells to Visit- 384 of 384

Sites to Visit- adaptive

Cell counting

Timelapse- 10 time point(s)

Acquisition Loop

Autofocus

W1 DAPI

W2 FITC

W3 TRITC

**Journals- 0 selected**

Display Settings

Post Acquisition

Summary

Acquisition Step	Journal
<input type="checkbox"/> Before each image	[None]
<input type="checkbox"/> After each image	[None]
<input type="checkbox"/> Before focusing	[None]
<input type="checkbox"/> Start of site	[None]
<input type="checkbox"/> End of site	[None]
<input type="checkbox"/> Start of well	[None]
<input type="checkbox"/> End of well	[None]
<input type="checkbox"/> Start of time point	[None]
<input type="checkbox"/> End of time point	[None]
<input type="checkbox"/> Start of plate	[None]
<input type="checkbox"/> End of plate	[None]

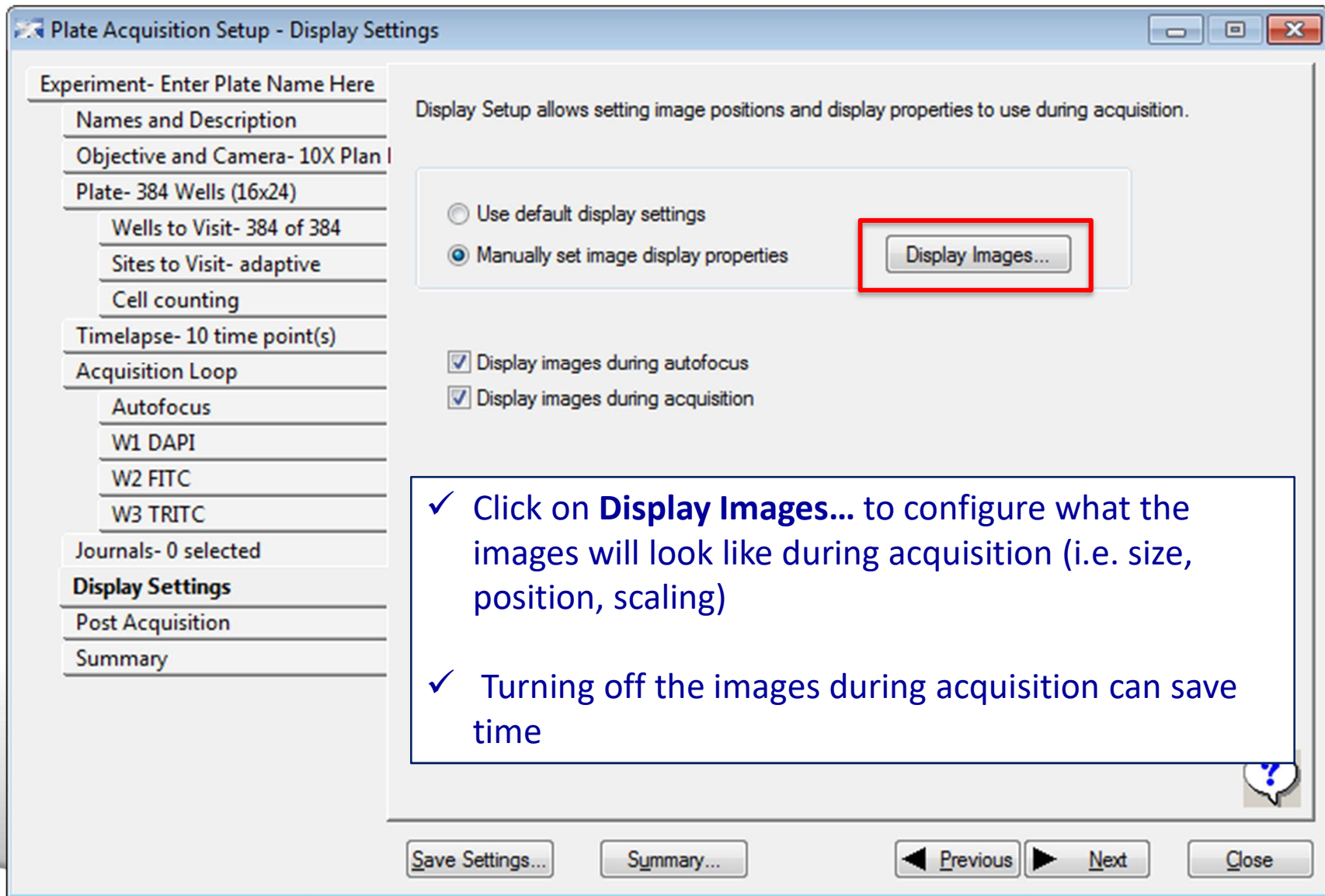
Prevent asynchronous hardware moves  
(recommended if any journals are dependent on hardware positioning).

✓ Use this tab to activate journals for more customized hardware and image manipulation

✓ With most journals, it is important to enable the option to **Prevent asynchronous hardware moves**

Save Settings... Summary... Previous Next Close

# Create New Protocol Settings: DISPLAY SETTINGS tab



# Create New Protocol Settings: POST ACQUISITION tab

Plate Acquisition Setup - Post Acquisition

Experiment- Enter Plate Name Here

Names and Description

Objective and Camera- 10X Plan I

Plate- 384 Wells (16x24)

Wells to Visit- 384 of 384

Sites to Visit- adaptive

Cell counting

Timelapse- 10 time point(s)

Acquisition Loop

Autofocus

W1 DAPI

Select an analysis and setting from the lists below, and a base folder for the measurement results. Once acquisition is complete, the analysis will start running on a computer connected to the database that is in Auto Run mode.

Auto Run analysis

Analysis: EX1 Nuclear Foci

Setting: EX1 Nuclear Foci

Timelapse:

All time points

Time point range 1 1

Stack of all time points

- ✓ 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

Plate Acquisition Setup - Summary

Experiment- Enter Plate Name Here

Names and Description

Objective and Camera- 10X Plan I

Plate- 384 Wells (16x24)

Wells to Visit- 384 of 384

Sites to Visit- adaptive

Cell counting

Timelapse- 10 time point(s)

Acquisition Loop

Autofocus

W1 DAPI

W2 FITC

W3 TRITC

Journals- 0 selected

Display Settings

Post Acquisition

**Summary**

**Enter Plate Name Here**

Plate type- 384 Wells (16x24)

**Timelapse Information**  
10 time points: Interval 10 min, Duration 100 min

**Well Information**  
Acquiring 384 Wells of 384  
4 sites/well  
0  $\mu\text{m}$  between images in X direction  
0  $\mu\text{m}$  in Y direction

**Wavelength Information**  
No shading correction  
No wavelength alignment  
3 Wavelengths - Unbinned  
W1 DAPI - 100 ms, images collected at all time points  
W2 FITC - 100 ms, images collected at all time points  
W3 TRITC - 100 ms, images collected at all time points

**Storage Information**  
16080 Total Images, Requiring ca. 100.6 GB of Storage

Print

Acquire Plate

Save Settings...

Summary...

Previous

Next

Close

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

# Create New Protocol Settings: SUMMARY tab

Plate Acquisition Setup - Summary

Experiment- Enter Plate Name Here

Names and Description

Objective and Camera- 10X Plan I

Plate- 384 Wells (16x24)

Wells to Visit- 384 of 384

Sites to Visit- adaptive

Cell counting

Timelapse- 10 time point(s)

Acquisition Loop

Autofocus

W1 DAPI

W2 FITC

W3 TRITC

Journals- 0 selected

Display Settings

Post Acquisition

**Summary**

**Enter Plate Name Here**

Plate type- 384 Wells (16x24)

**Timelapse Information**  
10 time points: Interval 10 min, Duration 100 min

**Well Information**  
Acquiring 384 Wells of 384  
4 sites/well  
0  $\mu\text{m}$  between images in X direction  
0  $\mu\text{m}$  in Y direction

**Wavelength Information**  
No shading correction  
No wavelength alignment  
3 Wavelengths - Unbinned  
W1 DAPI - 100 ms, images collected at all time points  
W2 FITC - 100 ms, images collected at all time points  
W3 TRITC - 100 ms, images collected at all time points

**Storage Information**  
16080 Total Images, Requiring ca. 400.6 GB of Storage

Print

**Acquire Plate**

Save Settings...

Summary...

Previous

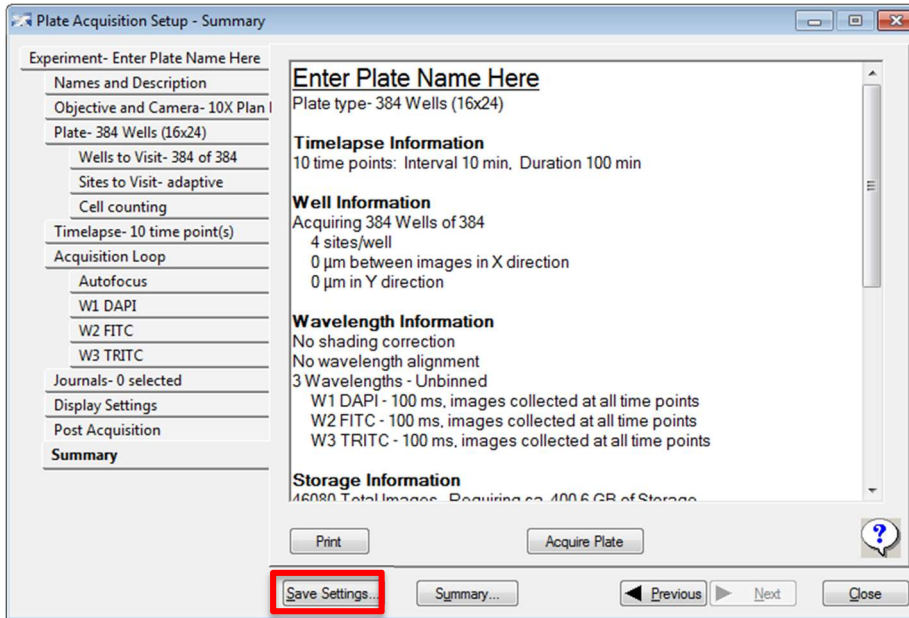
Next

Close

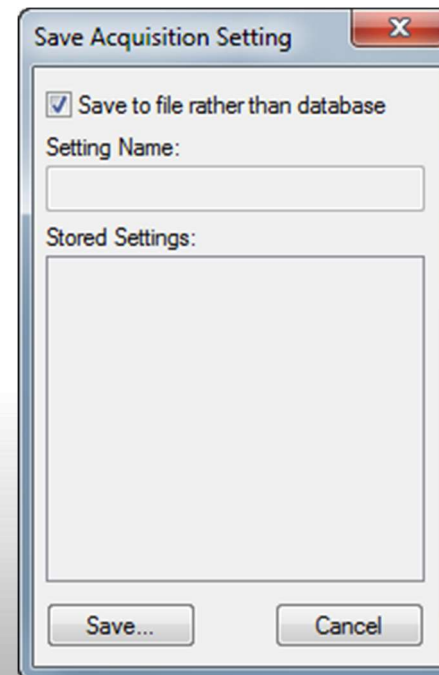
✓ Click the **Acquire Plate** button to start acquiring



# Save Acquisition Settings

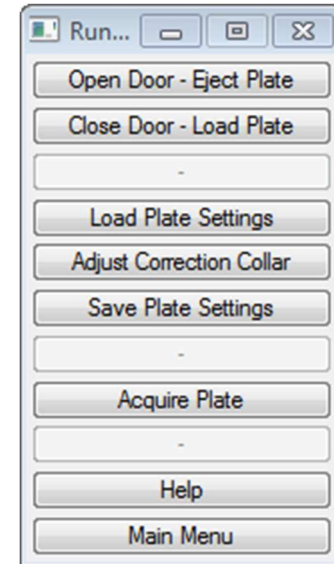


- ✓ Click the **Save Settings** button to save your acquisition settings
- ✓ It is recommended to save your settings to a file, rather the database.



# Shutting down the ImageXpress<sup>®</sup> Micro System

- Remove the plate from the system
- Exit out of MetaXpress
- 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](mailto: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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[www.moleculardevices.com](http://www.moleculardevices.com)