

MetaXpress® 6.5 Software Guide

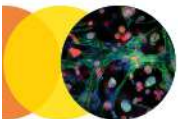
Discontinuous Timelapse Acquisition (Append Timepoint feature)

Date Revised 7/18/2019 Version B



Chapter Purpose

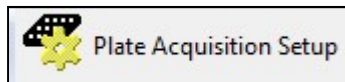
- The purpose of this chapter is to **guide the user through setting up discontinuous timelapse acquisition** using the **Append Timepoint** feature added in MetaXpress 6.5
- With discontinuous timelapse acquisition, the sample plate may be returned to the incubator in between time points, and other plates may be read in the interim.



Setting Up a Discontinuous Timelapse Acquisition

1. Open Plate Acquisition Setup

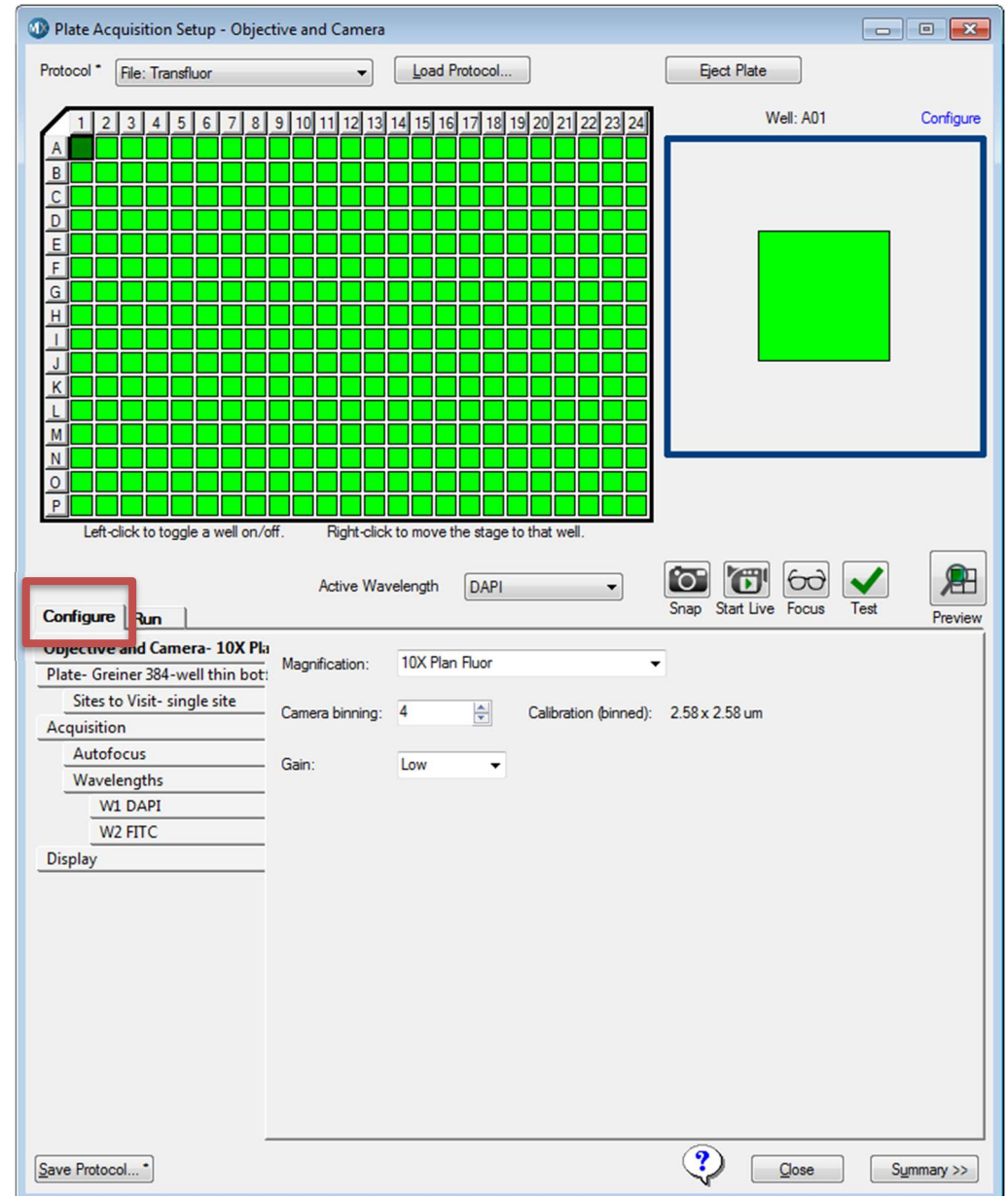
- In the main toolbar click on



OR

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

2. Select the **Configure** tab



Setting Up a Discontinuous Timelapse Acquisition

3. Configure your protocol appropriate for your assay.

Objective and Camera- 4X S Flu	Magnification:	4X S Fluor	
Plate- Corning 1536-well Black-			
Sites to Visit- multi-well			
Acquisition	Camera binning:	1	Calibration (binned): 1.61 x 1.61 um
Autofocus			
Wavelengths	Gain:	Low	
W1 DAPI			
W2 FITC			
Display			

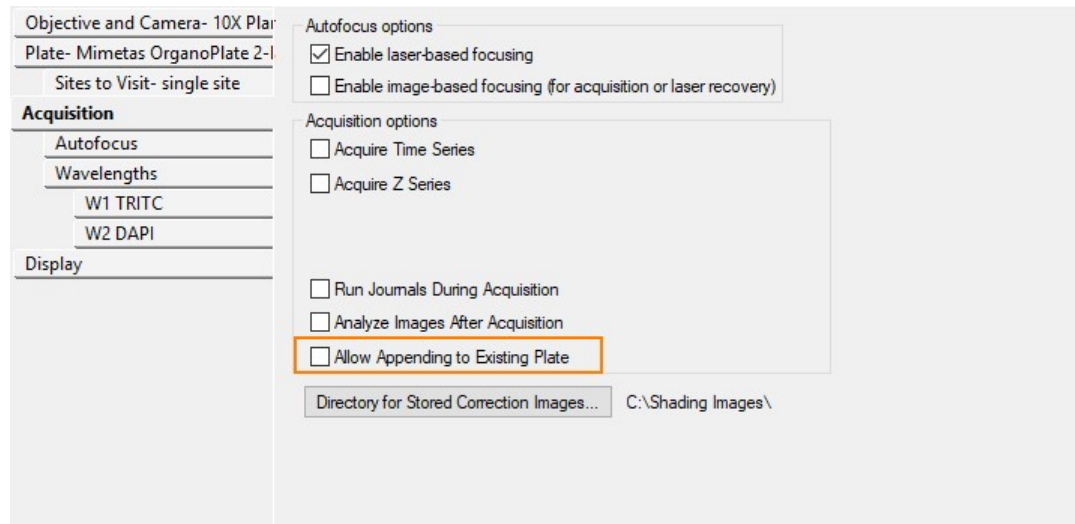


Setting Up a Discontinuous Timelapse Acquisition

4. Select the **Acquisition** tab

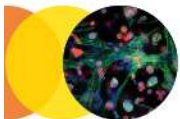
- Always **Enable laser-based focusing**
- For certain samples it may be necessary to **Enable image-based focusing**
- Optionally, enable **Acquire Time Series*** (generally not needed)
- Optionally, enable **Acquire Z Series**
- Enable **Allow Appending to Existing Plate** (in MX 6.5.3 and above)
- **Fluidics** experiments are not recommended with discontinuous acquisition

***NOTE:** The Acquire Time Series option is for continuous timelapse acquisition, for time points to be acquired with the plate left in the instrument. This can be optionally included in a discontinuous timelapse acquisition.



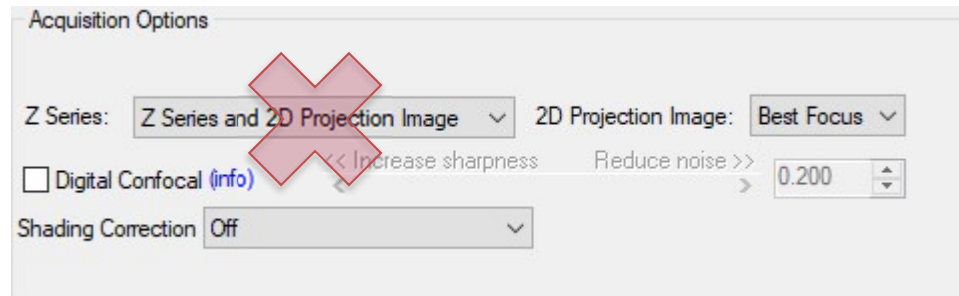
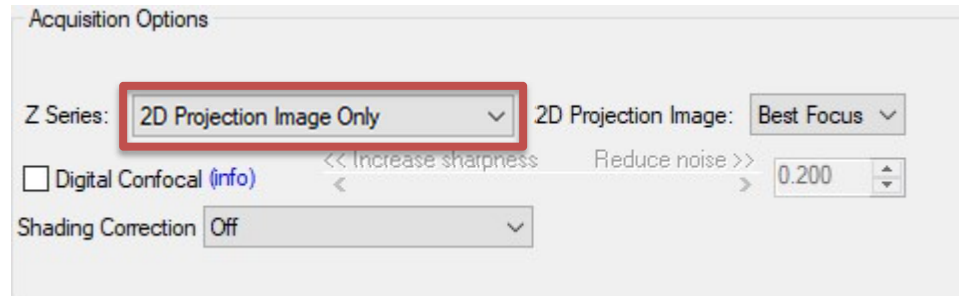
The screenshot displays the software interface for setting up a discontinuous timelapse acquisition. The 'Acquisition' tab is selected, and the 'Allow Appending to Existing Plate' checkbox is highlighted with an orange border. The interface includes sections for 'Objective and Camera', 'Plate', 'Sites to Visit', 'Autofocus options', 'Acquisition options', and 'Directory for Stored Correction Images'.

Objective and Camera- 10X Plat	Autofocus options
Plate- Mimetas OrganoPlate 2-l	<input checked="" type="checkbox"/> Enable laser-based focusing
Sites to Visit- single site	<input type="checkbox"/> Enable image-based focusing (for acquisition or laser recovery)
Acquisition	Acquisition options
Autofocus	<input type="checkbox"/> Acquire Time Series
Wavelengths	<input type="checkbox"/> Acquire Z Series
W1 TRITC	<input type="checkbox"/> Run Journals During Acquisition
W2 DAPI	<input type="checkbox"/> Analyze Images After Acquisition
Display	<input checked="" type="checkbox"/> Allow Appending to Existing Plate
	Directory for Stored Correction Images... C:\Shading Images\



Optional: Z Series Acquisition

5. If you are using Z-series acquisition, make sure that you are only storing the 2D Projection Image for each wavelength



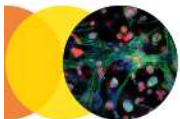
Optional: Adding continuous time points

6. If you enabled the Acquire Time Series option, select the **Timelapse** tab

- From the drop-down menu, select an option to **Perform time series for**:
 - One well then the next** for fast kinetic time lapse in a single well (or site). All time points will be collected in one well before moving to the next well.
 - All selected wells** for long-term time lapse experiments. All wells are acquired during each time point.

NOTE: Time points defined here will be acquired in a continuous acquisition. They can be appended to a discontinuous timelapse experiment.

The screenshot shows the software configuration interface for a timelapse experiment. On the left, there are several tabs: 'Objective and Camera- 10X Plan', 'Plate- Greiner 384-well thin bot:', 'Sites to Visit- multi-site', 'Acquisition', 'Autofocus', 'Wavelengths', 'W1 DAPI', 'W2 FITC', 'Timelapse- 2 time points', and 'Display'. The 'Timelapse- 2 time points' tab is selected. The main configuration area includes: 'Number of timepoints:' set to 2; 'Perform time series for:' dropdown menu with options: 'One well then the next' (selected), 'One well then the next', 'One row then the next', 'One column then the next', and 'All selected wells'; 'Approximate minimum interval:'; 'Duration:' set to 10 seconds. A red box highlights the dropdown menu.



Optional: Adding continuous time points

7. If you enabled the Acquire Time Series option, 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

NOTE: Time points defined here will be acquired in a continuous acquisition. They can be appended to a discontinuous timelapse experiment.

Objective and Camera- 10X Plat

Plate- Greiner 384-well thin bot:

Sites to Visit- multi-site

Acquisition

Autofocus

Wavelengths

W1 DAPI

W2 FITC

Timelapse- 2 time points

Display

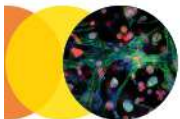
Number of timepoints: 2

Perform time series for: One well then the next

Approximate minimum time interval: 2.6 sec

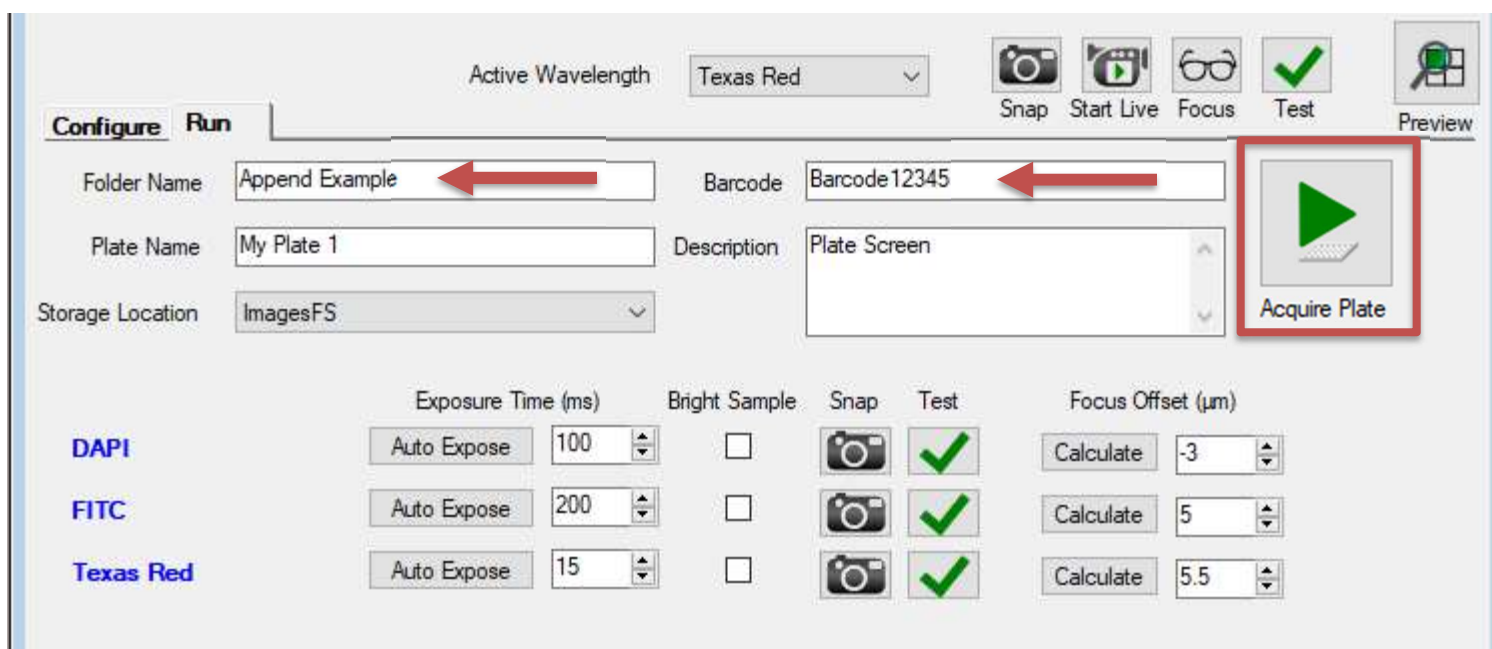
Interval: 10 sec

Duration: 10 sec



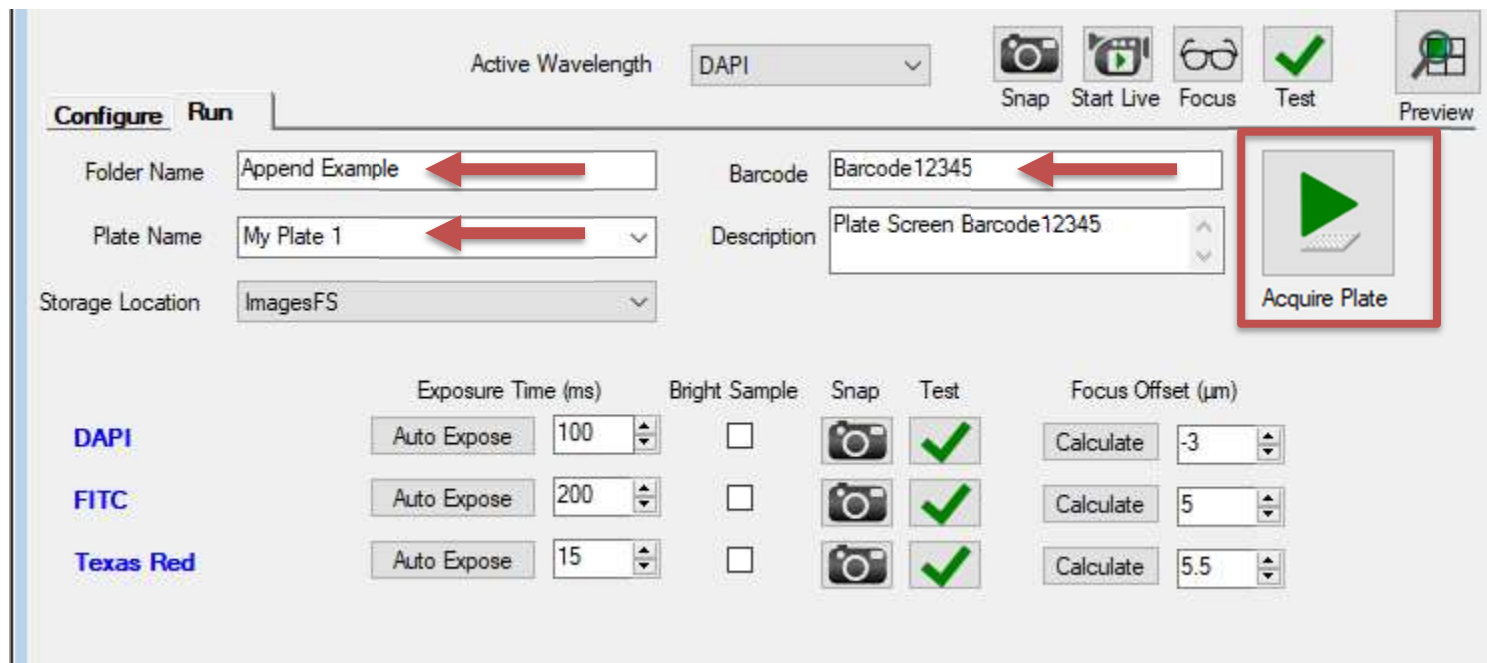
MX 6.5.0 – 6.5.2: Configuring Protocol

8. Add an appropriate **Folder Name** and **Barcode**.
9. Click **Acquire Plate** to begin acquisition of the plate



MX 6.5.3 and above: Configuring Protocol

8. Add an appropriate **Folder Name**, **Plate Name**, and **Barcode**.
9. Click **Acquire Plate** to begin acquisition of the plate



MX 6.5.0 – 6.5.2: Configuring Protocol

10. The next time you acquire the same plate to the same database with the same protocol, match the **Folder Name** and **Barcode**. The software will automatically append it to the existing plate as a new time point.

Active Wavelength: Texas Red

Snap Start Live Focus Test Preview

Configure Run

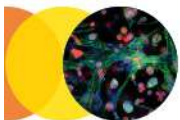
Folder Name: Append Example Barcode: Barcode12345

Plate Name: My Plate 1 Description: Plate Screen

Storage Location: ImagesFS

A plate matching this barcode and folder exists, Plate 22 will be appended

	Exposure Time (ms)	Bright Sample	Snap	Test	Focus Offset (µm)
DAPI	Auto Expose 100	<input type="checkbox"/>			Calculate -3
FITC	Auto Expose 200	<input type="checkbox"/>			Calculate 5
Texas Red	Auto Expose 15	<input type="checkbox"/>			Calculate 5.5



MX 6.5.3 and above: Configuring Protocol

- The next time you acquire the same plate to the same database with the same protocol, match the **Folder Name**, **Plate Name**, and **Barcode**. The software will automatically append it to the existing plate as a new time point, if the “Allow Appending” option is enabled.

Active Wavelength: DAPI

Snap Start Live Focus Test Preview

Folder Name: Append Example

Barcode: Barcode 12345

Plate Name: My Plate 1

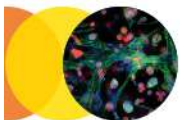
Description: Plate Screen Barcode12345

Storage Location: ImagesFS

Acquire Plate

This acquisition will append to Plate 22 as a new time point because it has the same Barcode, Folder, and Plate Names. [Configure](#) [Load Protocol](#)

	Exposure Time (ms)	Bright Sample	Snap	Test	Focus Offset (μm)
DAPI	Auto Expose 100	<input type="checkbox"/>			Calculate -3
FITC	Auto Expose 200	<input type="checkbox"/>			Calculate 5
Texas Red	Auto Expose 15	<input type="checkbox"/>			Calculate 5.5



MX 6.5.0 – 6.5.2: Configuring Protocol

11. If either the Folder Name or the Barcode does not match, the plate will be acquired as a separate, new plate.

Active Wavelength: Texas Red

Snap Start Live Focus Test Preview

Configure Run

Folder Name: Append Example Barcode:

Plate Name: My Plate 1 Description: Plate Screen

Storage Location: ImagesFS

Acquire Plate

Active Wavelength: Texas Red

Snap Start Live Focus Test Preview

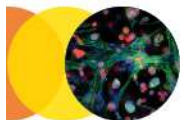
Configure Run

Folder Name: Append Example 2 Barcode: Barcode12345

Plate Name: My Plate 1 Description: Plate Screen

Storage Location: ImagesFS

Acquire Plate



MX 6.5.3 and above: Configuring Protocol

11. If the Plate Name, Folder Name, or Barcode does not match, the plate will be acquired as a separate, new plate.

Different Folder Name:

Active Wavelength: DAPI

Snap Start Live Focus Test Preview

Folder Name: Append Example 3 Barcode: Barcode12345

Plate Name: My Plate 1 Description: Plate Screen Barcode12345

Storage Location: ImagesFS

Acquire Plate

Different or Missing Barcode:

Active Wavelength: DAPI

Snap Start Live Focus Test Preview

Folder Name: Append Example Barcode:

Plate Name: My Plate 1 Description: Plate Screen Barcode12345

Storage Location: ImagesFS

Acquire Plate

Different Plate Name:

Active Wavelength: DAPI

Snap Start Live Focus Test Preview

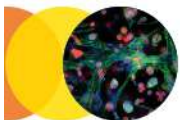
Folder Name: Append Example Barcode: Barcode12345

Plate Name: My Plate 3 Description: Plate Screen Barcode12345

Storage Location: ImagesFS

Acquire Plate

This acquisition will acquire a new plate. Select one of the existing Plate Names to append it as a new time point. [Configure](#)



Entering Barcodes

The Barcode can be entered manually or with a barcode scanner. Only alphanumeric characters should be used. There is no length requirement.

Note: The Barcode field is cleared after every acquisition. If you are reusing a manually entered barcode, keep a copy of it in the Description field (which is retained with the protocol) so that you can easily copy it to the Barcode field.

The screenshot shows a software interface with the following elements:

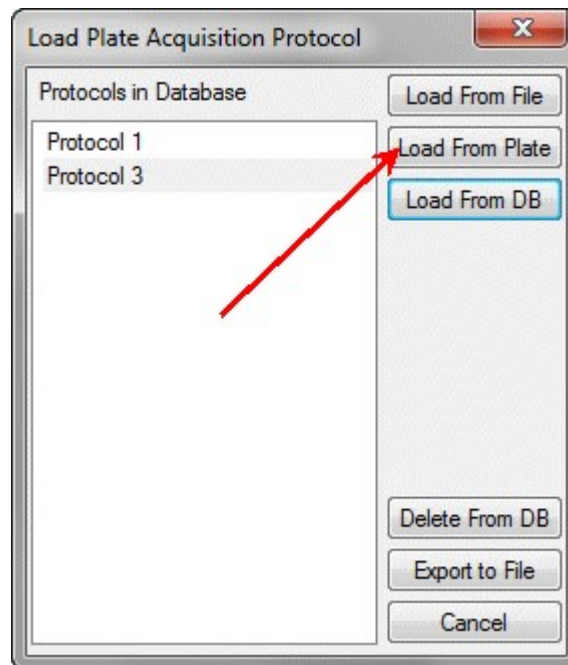
- Active Wavelength:** Texas Red (dropdown menu)
- Buttons:** Snap, Start Live, Focus, Test, Preview
- Configuration Fields:**
 - Folder Name: Append Example
 - Barcode: Barcode12345
 - Plate Name: My Plate 1
 - Description: Barcode12345, My experiment details
 - Storage Location: ImagesFS
- Acquire Plate:** A large green play button icon.
- Message:** A plate matching this barcode and folder exists, Plate 22 will be appended
- Acquisition Parameters Table:**

	Exposure Time (ms)	Bright Sample	Snap	Test	Focus Offset (μm)
DAPI	Auto Expose 100	<input type="checkbox"/>			Calculate -3
FITC	Auto Expose 200	<input type="checkbox"/>			Calculate 5
Texas Red	Auto Expose 15	<input type="checkbox"/>			Calculate 5.5



Locating the Protocol

- The Protocol should match previous protocols used for the same plate.
- If you did not save your previous protocol, use the option to **Load From Plate**.



Error Handling

- If an inappropriate protocol is selected, an error is displayed and you cannot append to the existing plate.

Site selection has changed:

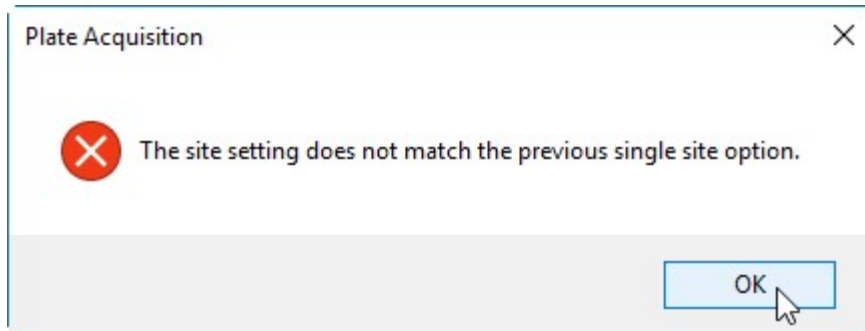
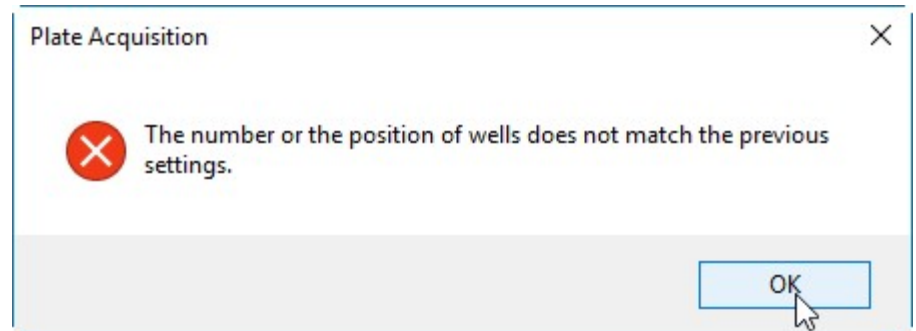
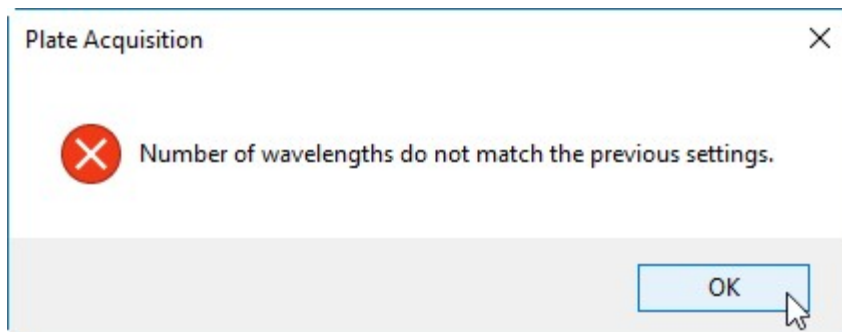


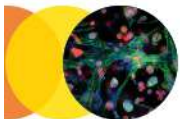
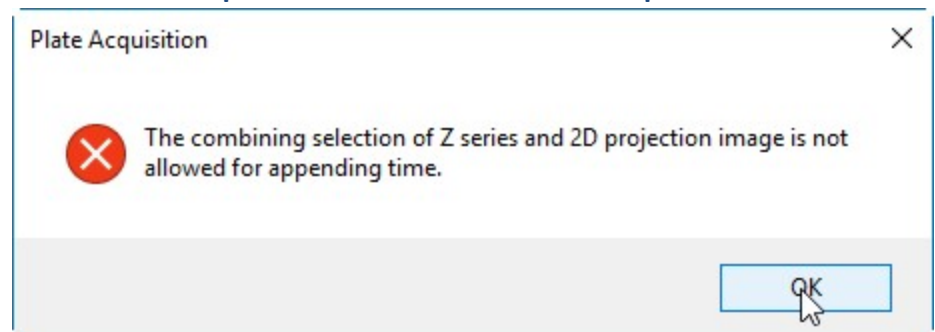
Plate type and/or well selection changed:



Wavelength selection has changed:



Z-Series option set to save all Z planes:



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

