



# MetaXpress<sup>®</sup> 6 Software Guide

Enabling Asynchronous Dispense  
in a Fluidics – Timelapse Acquisition

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# Chapter Purpose

The purpose of this chapter to guide the user through configuring a fluidics - timelapse acquisition with the **Asynchronous Dispense** option enabled. This option allows continuous acquisition of images while compound is dispensed into the well.

This chapter only describes the dialogs associated with **Asynchronous Dispense**. For more details on setting up an acquisition, please refer to the chapter on acquiring a Timelapse with Fluidics.



# A Note About Asynchronous Dispense

- Improper setup can result in a tip-shutter crash
- Improper setup can result in damage from the stage moving with the tip in the well
- Not compatible with brightfield imaging
- Not compatible with multi-site, multi-well, or adaptive acquisition
- Possible environmental leak with the shutter open
- Possible image artifacts from background room light (can cover fluidics top with a black cloth)
- Please consult with Molecular Devices before using any journals with **Asynchronous Dispense** experiment

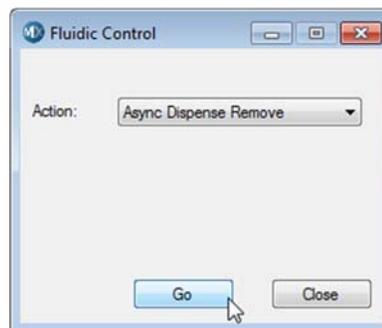


# A Note About Asynchronous Dispense Continued

- Assay optimization should be done before running critical experiments
- Pipetting may occur over multiple time points
- Only the first image is annotated with the event (**Alt+I** for Image Info)

Property Name	Property Value
Plane Refractive Index	1
Temperature	602.4
Co2 Pressure Status	Low Pressure
Async Dispense Cleanup Event1 Description	Cleanup from async dispense, all wells,
Async Dispense Cleanup Event1 Time	101
Async Dispense Event1	1
Async Dispense Event1 Description	Async dispense, all wells, 40 ul,
Async Dispense Event1 Time	11
Async Dispense Setup Event1 Description	Setup async dispense, all wells, 40 ul,
Async Dispense Setup Event1 Time	1

- If an experiment is aborted with the pipettor in the well, perform an **Async Dispense Remove** step to remove the pipettor
  - In the main menu, select **Control > ImageXpress > Fluidic Control**



For research use only. Not for use in diagnostic procedures.



# Different Fluidics Dispense Methods

- Baseline image
- Post-compound image
- Time point delay
- Fluidics operation

Note: Diagram is not to scale

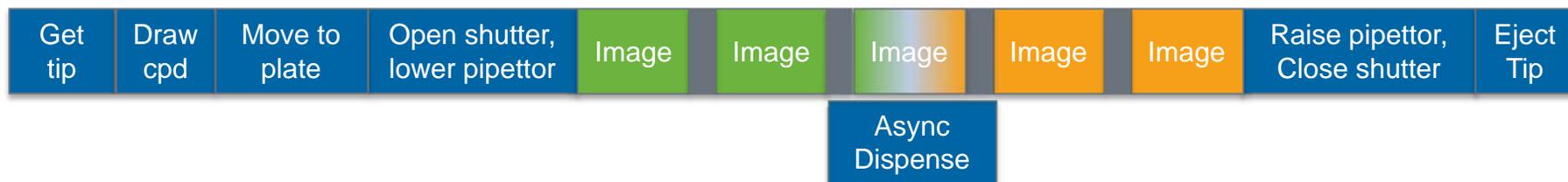
## Normal Compound Addition Event



## Compound Addition with Journals



## Asynchronous Dispense



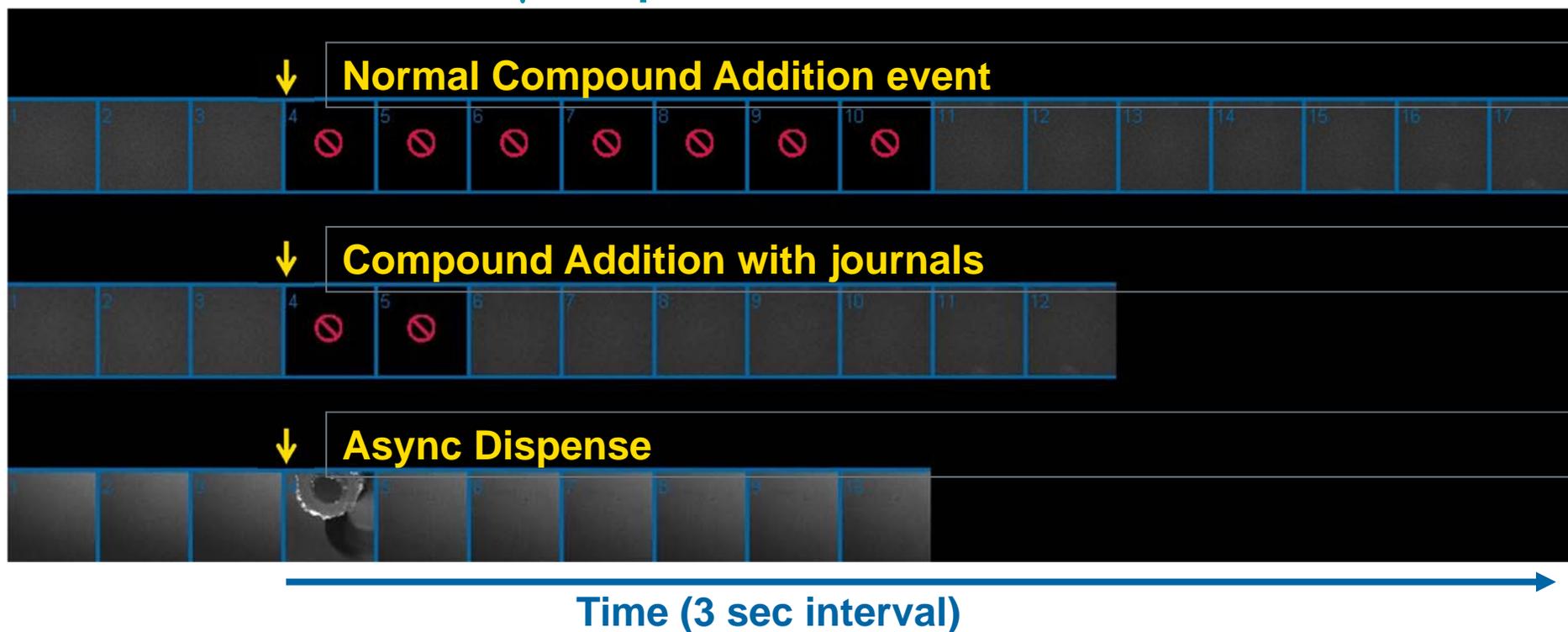
Time →



# Timing of Different Fluidics Dispense Methods

- ~22 second gap between images using normal Compound Addition event
- ~4 second gap between images using journals
- No gap between images with asynchronous dispense

## 100 $\mu$ l Dispense After Time Point 3



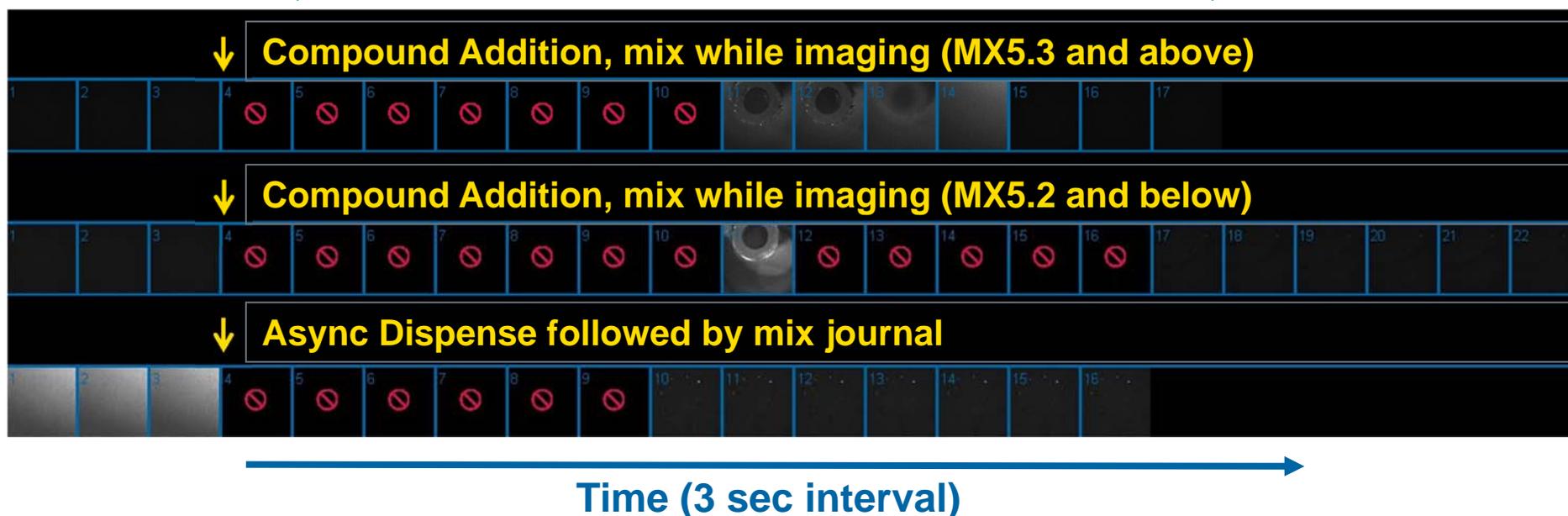
Note: Pipette tip only visible with custom reflectance cube



# Timing of Fluidics Dispense Methods with Mix

- Mix while imaging is done asynchronously in MX 5.3 and above
- Mixing in a journal adds several seconds delay
- For fastest operation, add sufficient volume so that mixing is unnecessary

## 100 $\mu$ l Dispense After Time Point 3 and Two 40 $\mu$ l Mixes



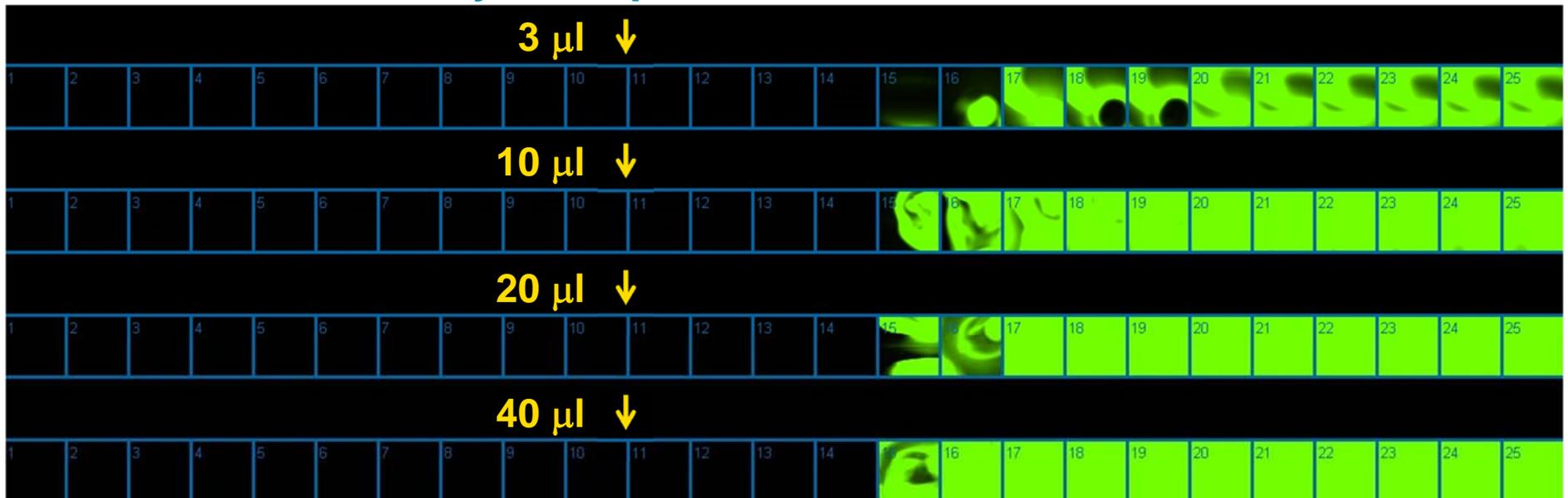
Note: Pipette tip only visible with custom reflectance cube



# Asynchronous Dispense Example with Fluorescein

- Varying volumes of fluorescein solution were added to the 96-well sample plate containing 100  $\mu\text{L}$  of PBS per well
- A 250  $\mu\text{L}/\text{sec}$  dispense rate was used in all experiments

## Async Dispense After Time Point 10

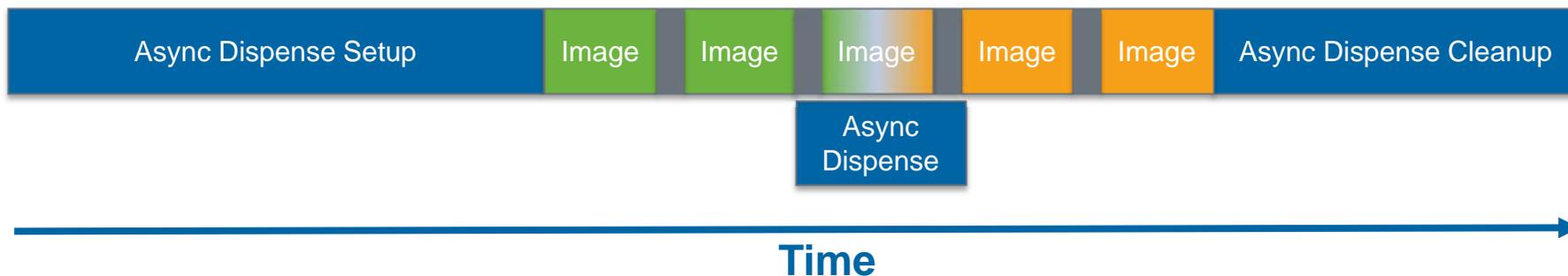


Time (0.5 sec interval)



# Asynchronous Dispense Acquisition Setup

1. When setting up an acquisition with **Asynchronous Dispense**, make sure to select the following in the **Plate Acquisition Setup** dialog
  - On the **Sites to visit** tab:
    - Select **Single site**
  - On the **Timelapse** tab:
    - Select **One well then the next** from the **Perform time series for** drop-down menu
    - Set the appropriate number of time points
  - On the **Fluidics** tab:
    - Add an **Async Dispense Setup** event before time point 1
    - Add an **Async Dispense** event after baseline time points
    - Add an **Async Dispense Cleanup** event after the last time point



# Setting Up a Timelapse Acquisition with Fluidics

1. On the **Fluidics** tab you will need to add at least 3 events:
  - Async Dispense Setup
  - Async Dispense
  - Async Dispense Cleanup

The screenshot displays the Fluidics software interface. On the left, a sidebar contains several tabs: 'Objective and Camera- 10X PF', 'Plate- 384 Wells (16x24)', 'Sites to Visit- single site', 'Acquisition', 'Autofocus', 'Wavelengths', 'W1 DAPI', 'W2 FITC', 'Timelapse- 6 time points', 'Fluidics', and 'Display'. The 'Fluidics' tab is currently selected. The main area is titled 'Scheduled Events:' and features a table with two columns: 'Time' and 'Event'. The table is currently empty. To the right of the table is a 'Configure Stations...' button. At the bottom of the interface, there are four buttons: 'Reset Tips...', 'Add new Event...', 'Delete Event...', and 'Edit Event...'. The 'Add new Event...' button is highlighted with a red rectangular box.



# Fluidic Event Dialog

Select time point for the scheduled fluidic event to occur

Select if fluidic 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 can be configured on the **Plate** tab of **Plate Acquisition Setup** dialog

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

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

Fluidic Event

Time point: 1

Before imaging  After imaging

Event Type:

Compound addition  Async Dispense Setup

Washout  Async Dispense

Journal  Async Dispense Cleanup

Compound plate: Plate 1

Tip: 96, 200ul, FLIPR

Volume (ul): 20

Wells Affected:

All wells  Selected wells

Select Wells...

OK Test at A1 Cancel

**Test A1** button will apply this fluidic event settings to well A1, but does not schedule the event. Use this option to perform an initial test of the settings that you made.



# Async Dispense Setup Event

This event occurs before the first image is acquired. At Time point 1:

- i. Pipettor picks up a tip
- ii. Aspirates volume from the compound plate
- iii. Pipettor moves to the sample plate
- iv. Shutter is left open, tip is in the well, liquid has not been dispensed yet
- v. Imaging begins

## To configure this dialog

- Enter **1** in the **Time point** spin box and select **Before imaging**
- In the **Event Type** section, select **Async Dispense Setup**
- Enter the **Volume** to be drawn from the compound plate (this must be the same or more than the total volume to be dispensed into a well)

Schedule this event  
before time point 1

Fluidic Event

Time point: 1  Before imaging  After imaging

Event Type:

Compound addition  Async Dispense Setup

Washout  Async Dispense

Journal  Async Dispense Cleanup

Compound plate: Plate 1

Tip: 96, 200ul, FLIPR

Volume (ul): 20

Wells Affected:

All wells  Selected wells



# Async Dispense Event

This event occurs after baseline images have been acquired. At the designated Time point:

- i. Imaging continues while pipettor dispenses volume into sample plate
- ii. Imaging continues after all volume has been dispensed
- iii. If multiple dispenses have been scheduled, the pipettor will dispense the specified volume for the current event

## To configure this dialog

- Enter the desired **Time point** in the spin box and select **After Imaging**
- In the **Event Type** section, select **Async Dispense**
- Enter the **Volume** to be dispensed into the sample plate

Schedule this event after the desired baseline images have been acquired

The screenshot shows the 'Fluidic Event' dialog box with the following configuration:

- Time point:** 10
- Timing:**  Before imaging,  After imaging
- Event Type:**  Compound addition,  Async Dispense Setup,  Async Dispense,  Washout,  Async Dispense Cleanup,  Journal
- Volume (ul):** 20
- Wells Affected:**  All wells,  Selected wells (with 'Select Wells...' button)
- Buttons:** OK, Test at A1, Cancel



# Async Dispense Cleanup Event

Schedule this event after the final time point

This event occurs after all images have been acquired. After the last time point:

- i. Pipettor moves out of the well
- ii. Shutter is closed
- iii. Tip is ejected

## To configure this dialog

- Set **Time point** in the spin box to last time point and select **After Imaging**
- In the **Event Type** section, select **Async Dispense Cleanup**

Fluidic Event

Time point: 50  Before imaging  After imaging

Event Type:

Compound addition  Async Dispense Setup  
 Washout  Async Dispense  
 Journal  Async Dispense Cleanup

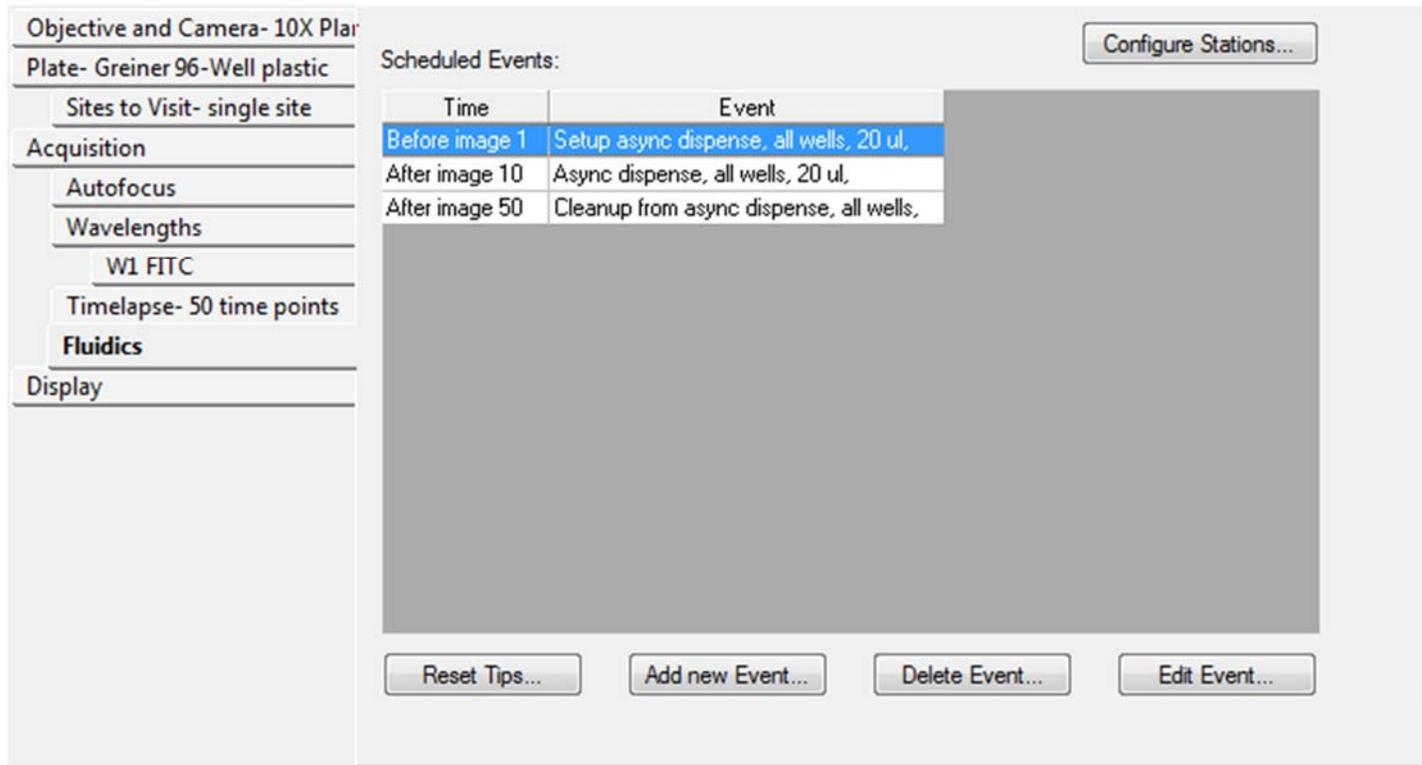
Wells Affected:

All wells  Selected wells



# Example: Asynchronous Workflow 1

- **Async Dispense Setup** event scheduled before time point 1
- **Single Async Dispense** event after time point 10
- **Async Dispense Cleanup** event scheduled after last time point (50)



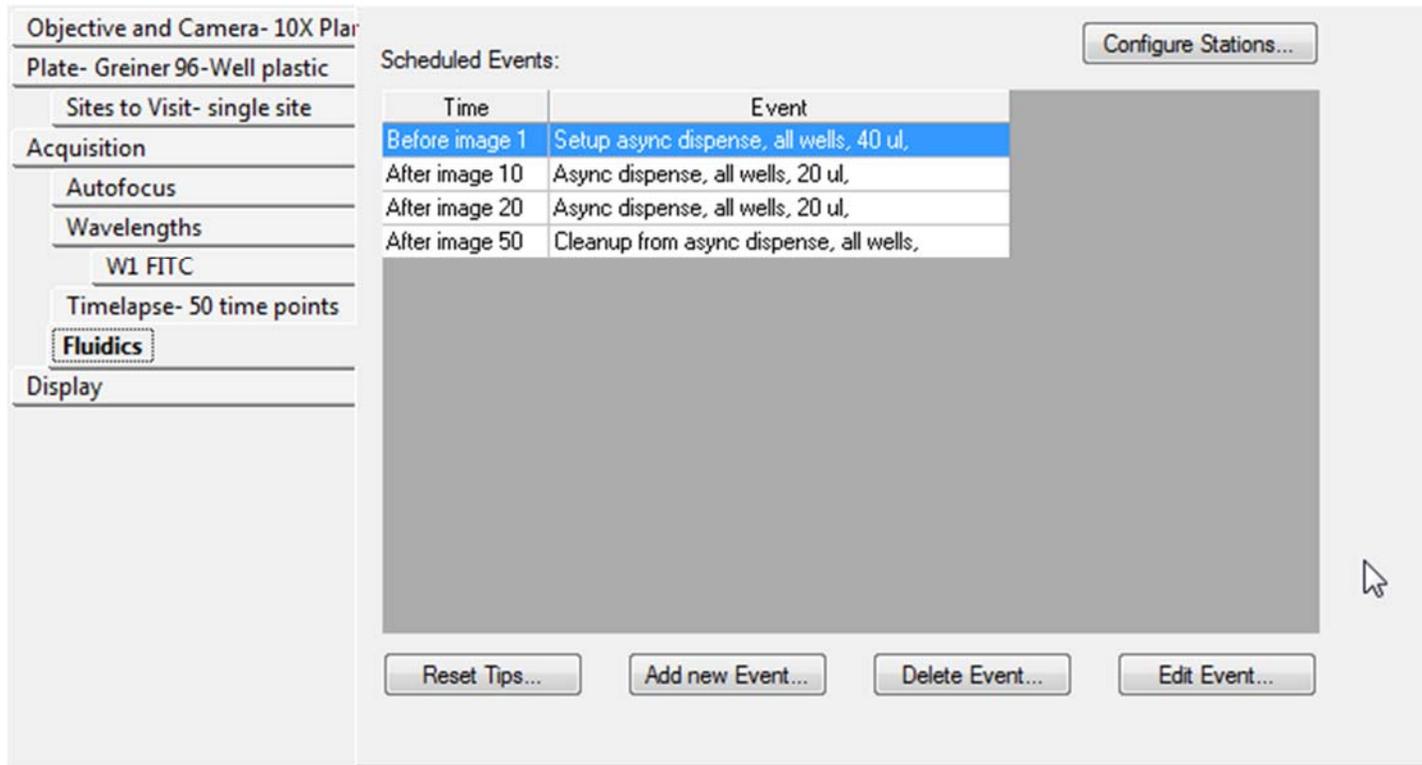
The screenshot displays the software interface for configuring a workflow. On the left, a sidebar lists various settings categories: Objective and Camera- 10X Plan, Plate- Greiner 96-Well plastic, Sites to Visit- single site, Acquisition, Autofocus, Wavelengths, W1 FITC, Timelapse- 50 time points, Fluidics, and Display. The main area is titled 'Scheduled Events:' and contains a table with two columns: 'Time' and 'Event'. The table lists three events: 'Before image 1' (Setup async dispense, all wells, 20 ul), 'After image 10' (Async dispense, all wells, 20 ul), and 'After image 50' (Cleanup from async dispense, all wells,). Below the table are four buttons: 'Reset Tips...', 'Add new Event...', 'Delete Event...', and 'Edit Event...'. A 'Configure Stations...' button is located in the top right corner of the main area.

Time	Event
Before image 1	Setup async dispense, all wells, 20 ul,
After image 10	Async dispense, all wells, 20 ul,
After image 50	Cleanup from async dispense, all wells,



# Example: Asynchronous Workflow 2

- **Async Dispense Setup** event (40  $\mu$ l) scheduled before time point 1
- **Two Async Dispense** events (each 20  $\mu$ l) at different time points
- **Async Dispense Cleanup** event scheduled after last time point



The screenshot displays the 'Fluidics' configuration window. On the left, a sidebar lists various settings: Objective and Camera- 10X Plan, Plate- Greiner 96-Well plastic, Sites to Visit- single site, Acquisition (Autofocus, Wavelengths, W1 FITC), Timelapse- 50 time points, **Fluidics** (highlighted), and Display. The main area is titled 'Scheduled Events:' and contains a table with two columns: 'Time' and 'Event'. The table lists four events: 'Before image 1' (Setup async dispense, all wells, 40 ul), 'After image 10' (Async dispense, all wells, 20 ul), 'After image 20' (Async dispense, all wells, 20 ul), and 'After image 50' (Cleanup from async dispense, all wells,). Below the table are four buttons: 'Reset Tips...', 'Add new Event...', 'Delete Event...', and 'Edit Event...'. A 'Configure Stations...' button is located in the top right corner of the main area.

Time	Event
Before image 1	Setup async dispense, all wells, 40 ul,
After image 10	Async dispense, all wells, 20 ul,
After image 20	Async dispense, all wells, 20 ul,
After image 50	Cleanup from async dispense, all wells,



# Support Resources

- F1 / HELP within MetaXpress® Software
- Support and Knowledge Base: <http://mdc.custhelp.com/>
- User Forum: <http://metamorph.moleculardevices.com/forum/>
- Request Support: <http://mdc.custhelp.com/app/ask>
- Technical Support can also be reached by telephone:
  - 1 (800) 635-5577
  - Select options for Tech Support → Cellular Imaging Products → ImageXpress Instruments





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