MetaXpress® 6 Software Guide

Enabling Asynchronous Dispense in a Fluidics – Timelapse Acquisition

Date Revised 11/30/2016 Version C

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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

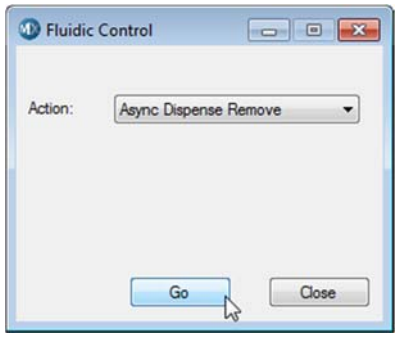
- Improper setup can result in a tip-shutter crash
- Improper setup can result in damage from the stage moving with the tip in the well
- Not compatible with brightfield imaging
- Not compatible with multi-site, multi-well, or adaptive acquisition
- Possible environmental leak with the shutter open
- 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 Descript	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**



Different Fluidics Dispense Methods

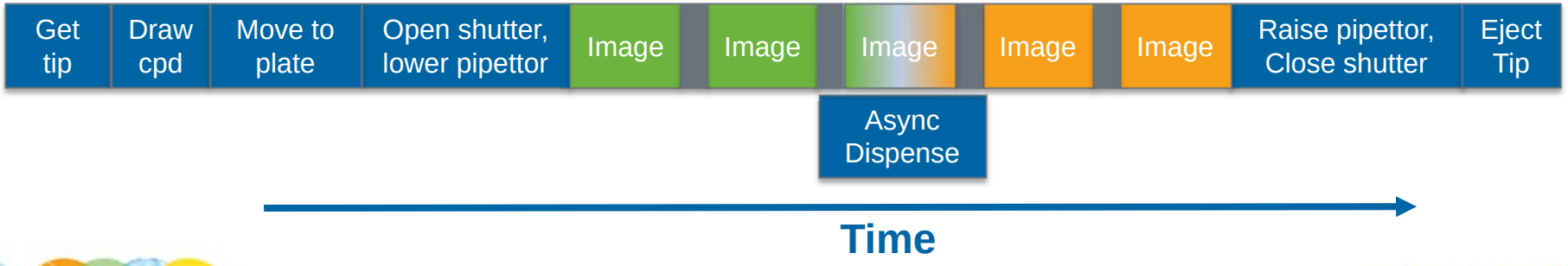
- Baseline image
- Post-compound image
- Time point delay
- Fluidics operation

Note: Diagram is not to scale

Normal Compound Addition Event

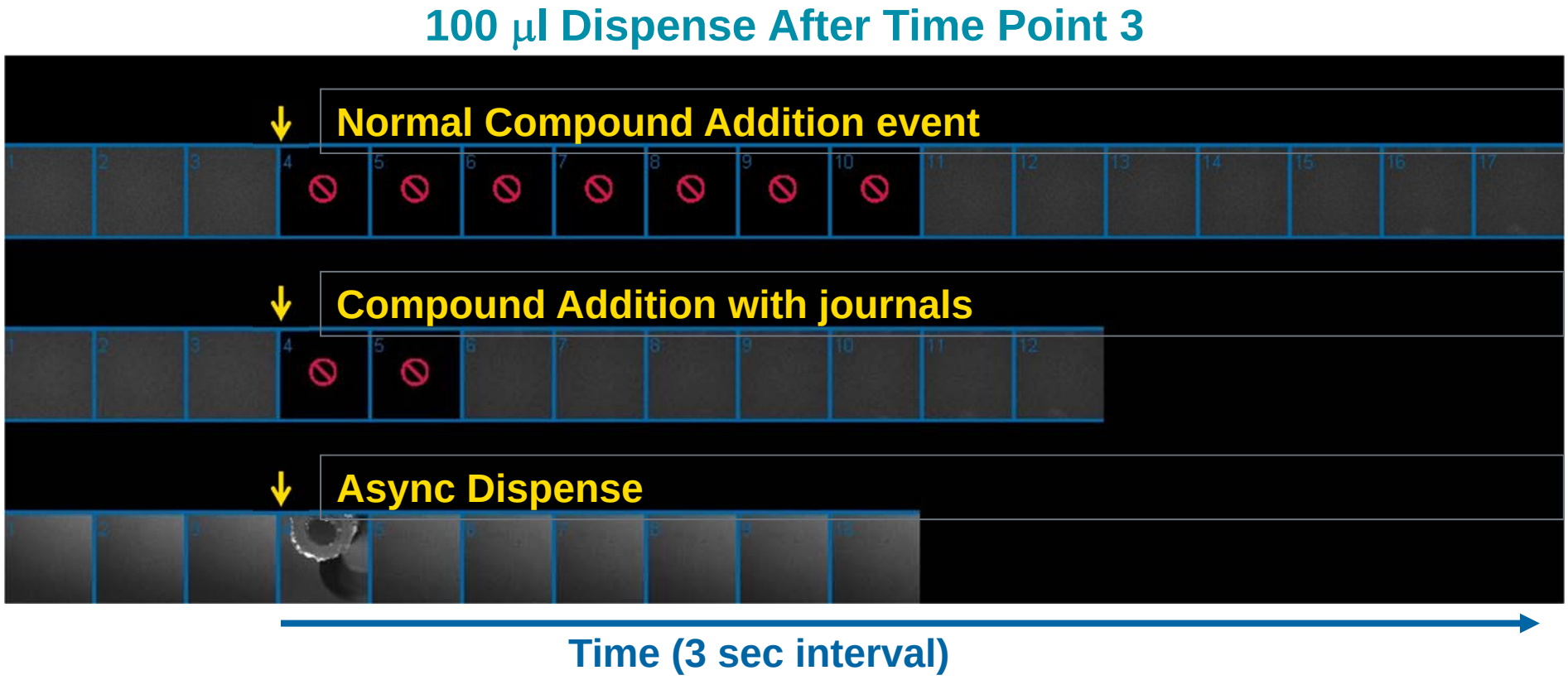
Compound Addition with Journals

Asynchronous Dispense



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

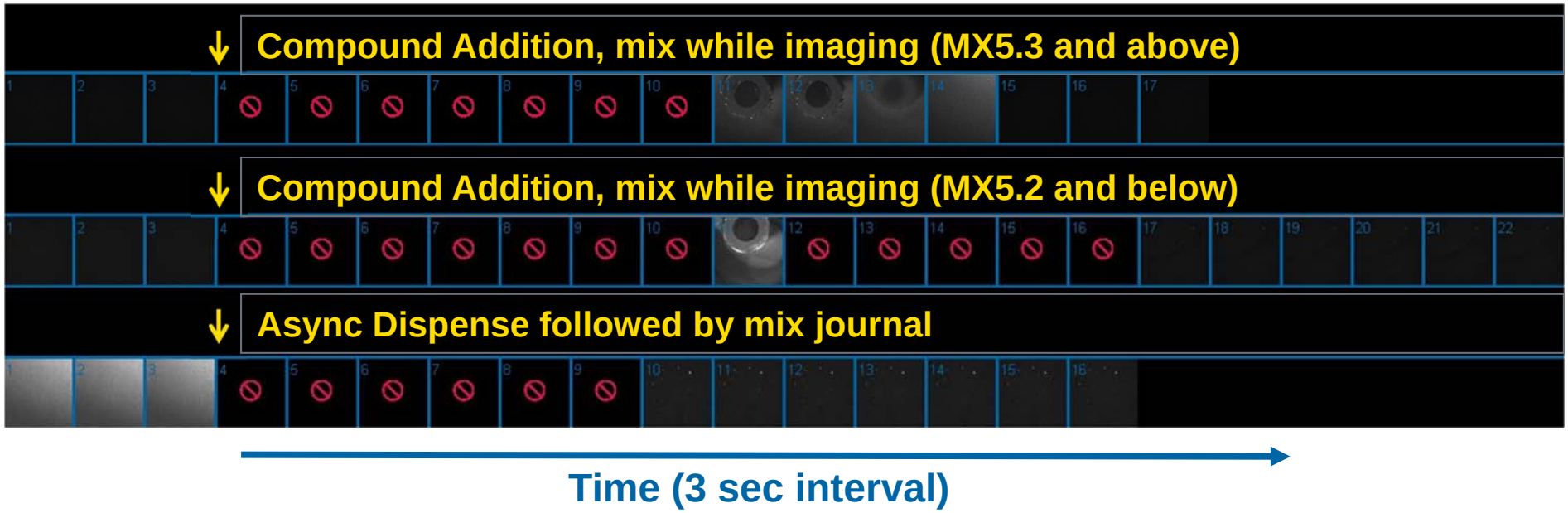


Note: Pipette tip only visible with custom reflectance cube

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 µl Dispense After Time Point 3 and Two 40 µl Mixes

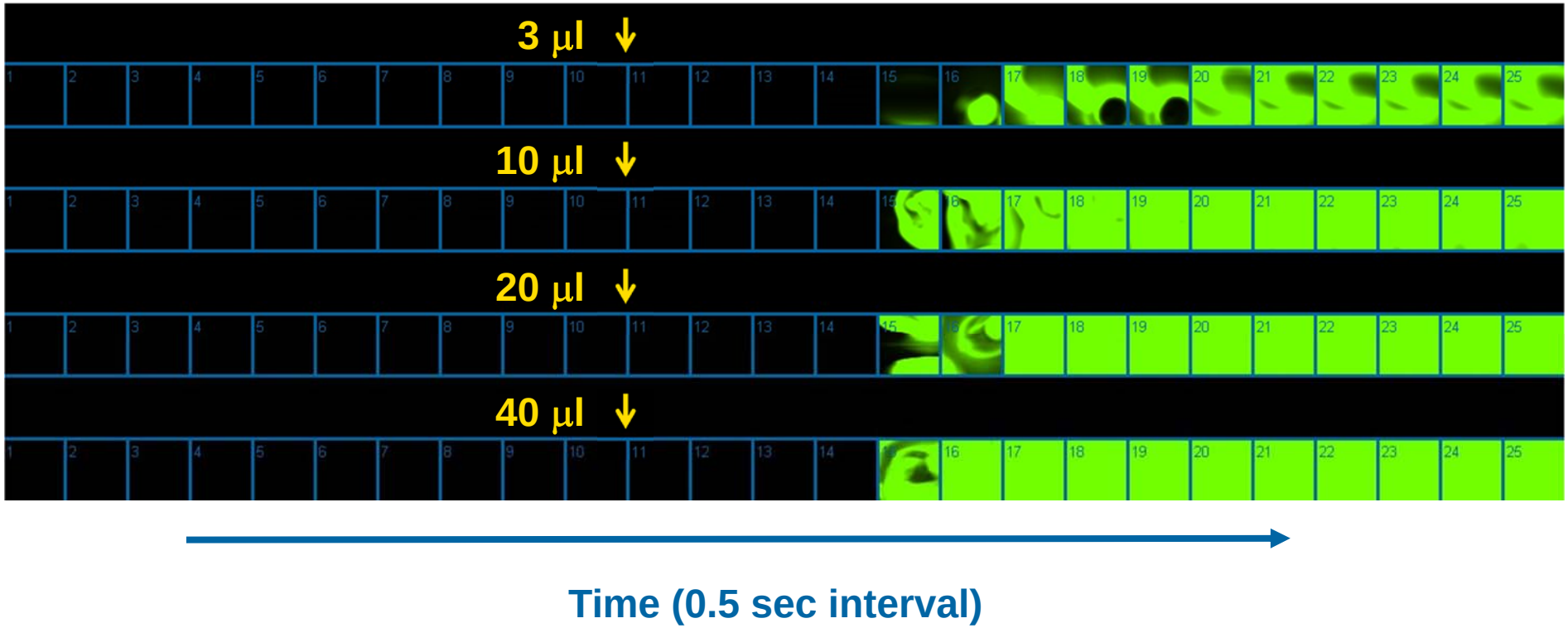


Note: Pipette tip only visible with custom reflectance cube

Asynchronous Dispense Example with Fluorescein

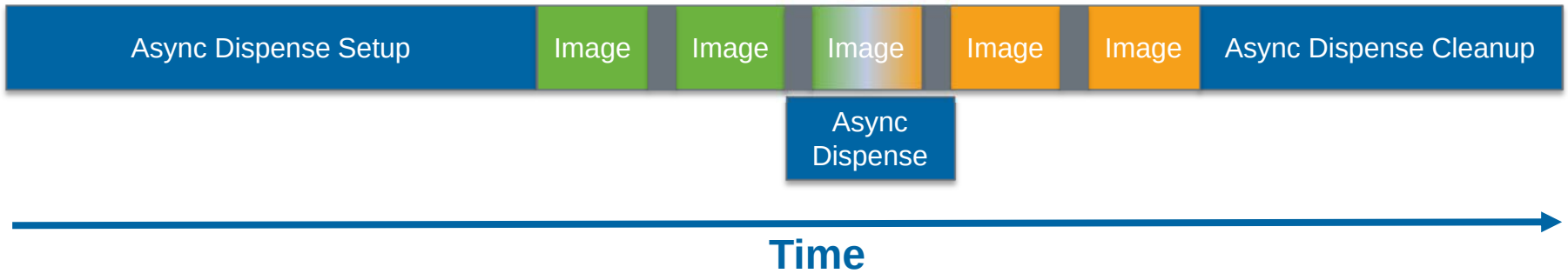
- Varying volumes of fluorescein solution were added to the 96-well sample plate containing 100 µL of PBS per well
- A 250 µl/sec dispense rate was used in all experiments

Async Dispense After Time Point 10



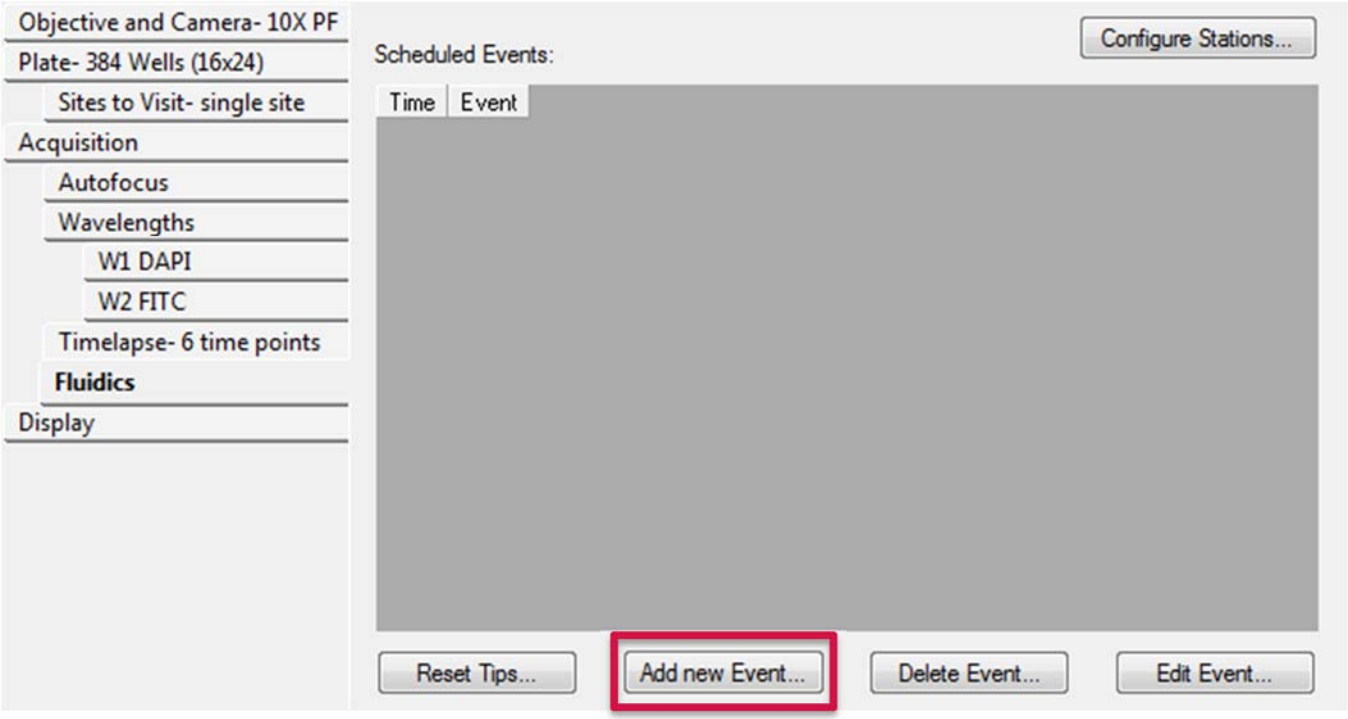
Asynchronous Dispense Acquisition Setup

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

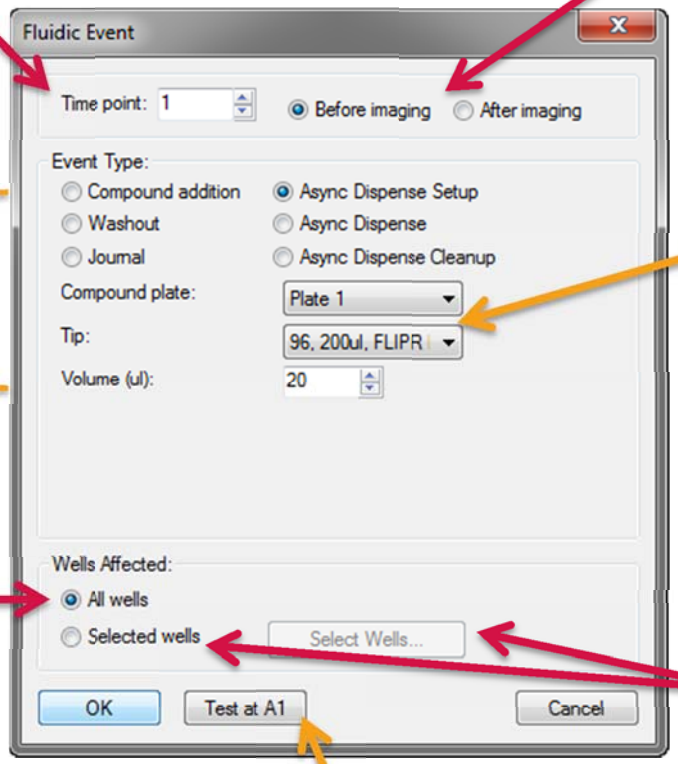
1. On the **Fluidics** tab you will need to add at least 3 events:
   - **Async Dispense Setup**
   - **Async Dispense**
   - **Async Dispense Cleanup**



Fluidic Event Dialog

Select time point for the scheduled fluidic even 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

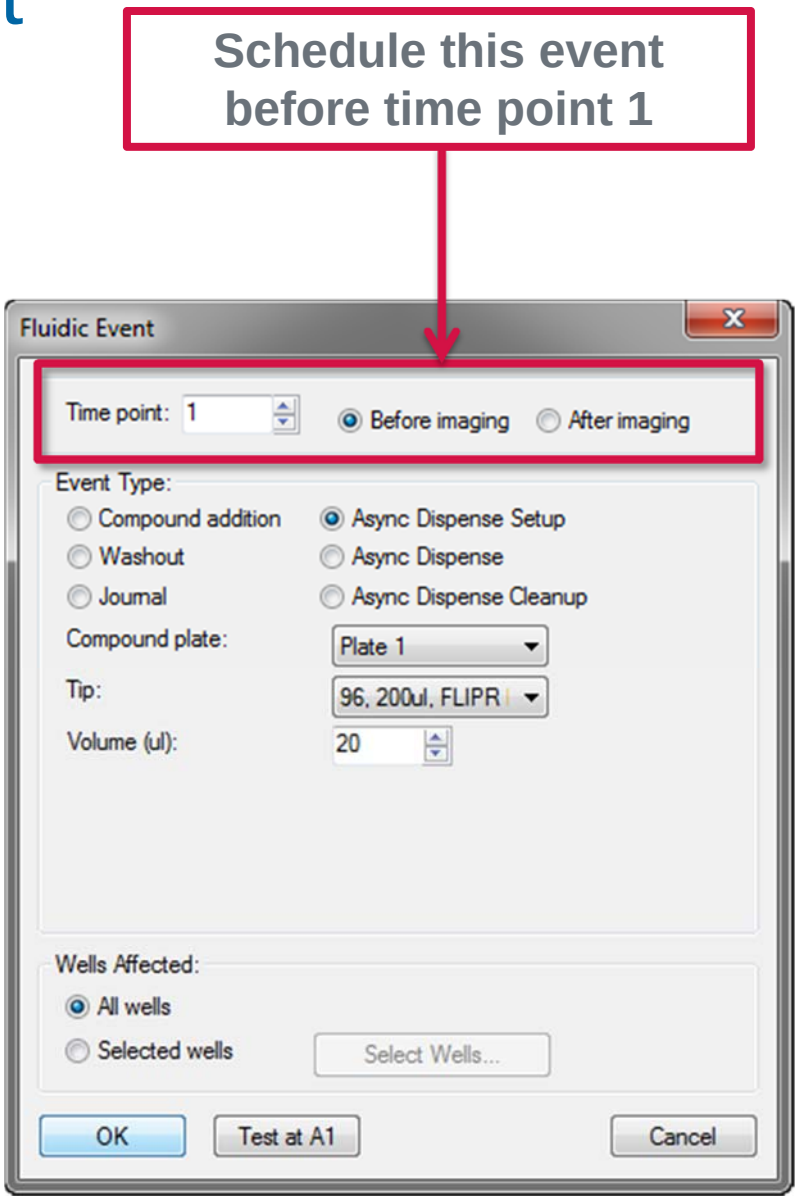
To apply the fluidic even to particular wells, select **Selected wells** and then click on the **Selected wells…** button to highlight the desired well(s) in the plate map.

Test A1 button will apply 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)

Async Dispense Event

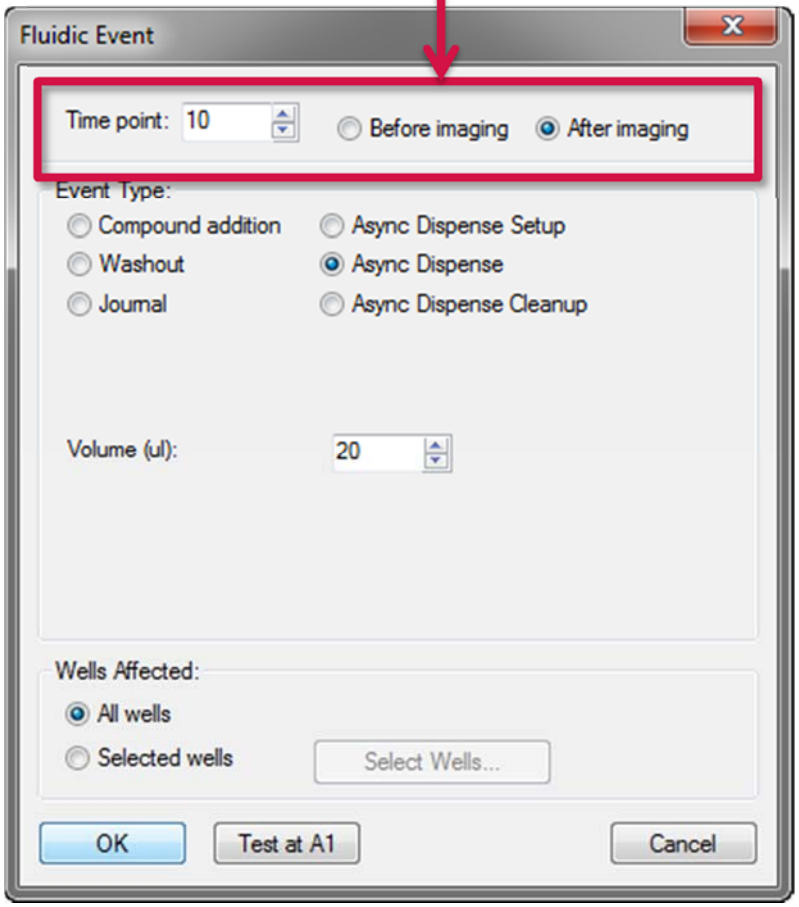
This event occurs after baseline images have been acquired. At the designated Time point:

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



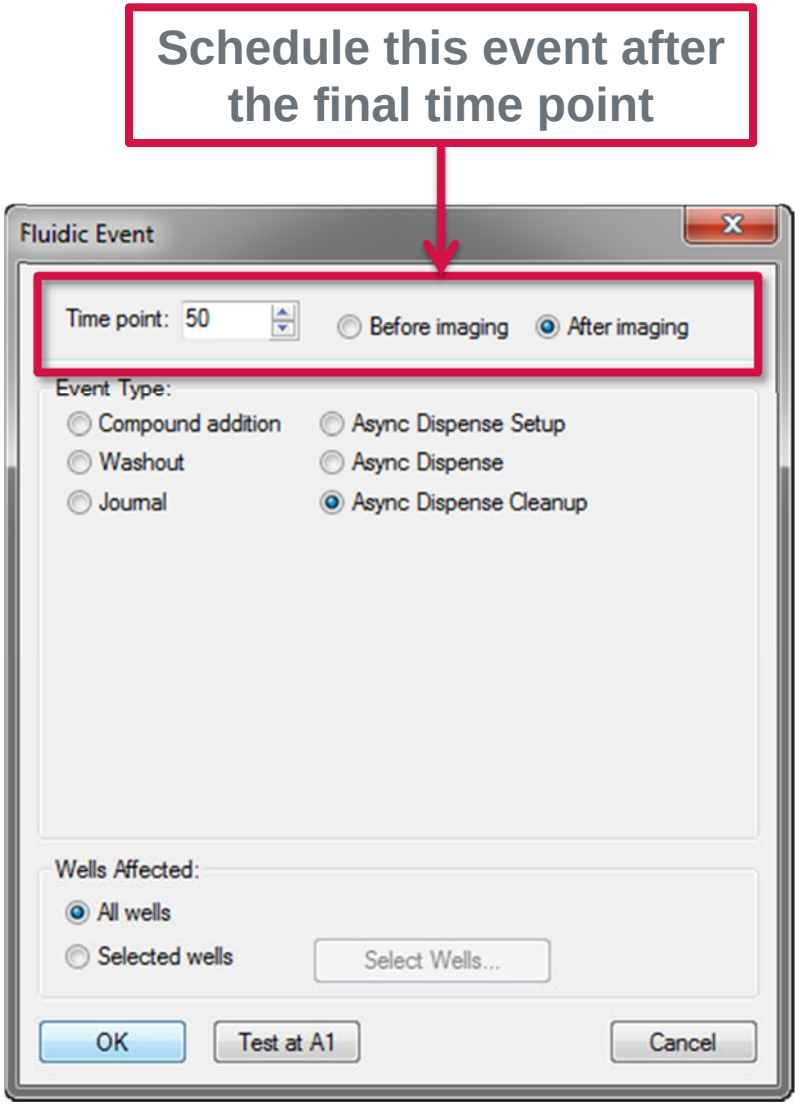
Async Dispense Cleanup Event

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

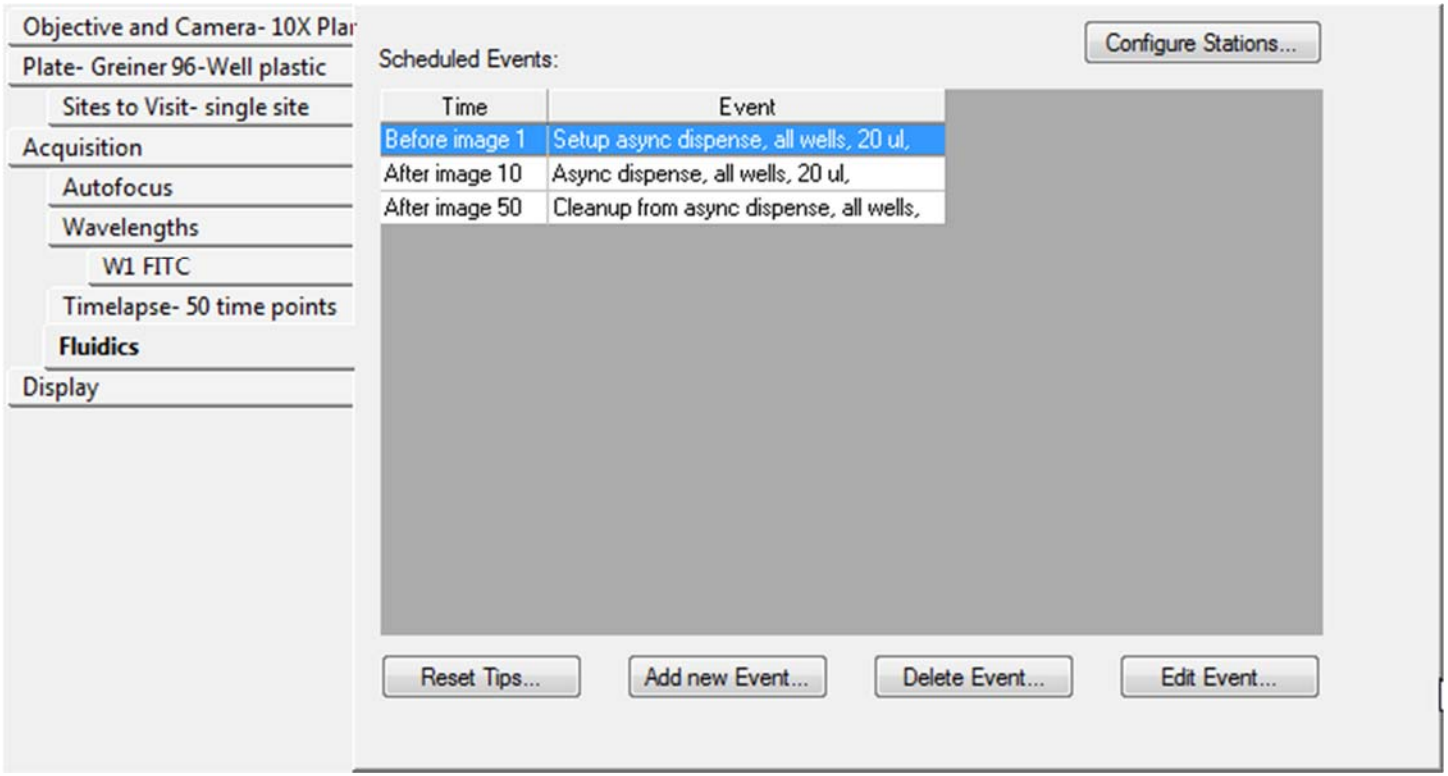
To configure this dialog

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



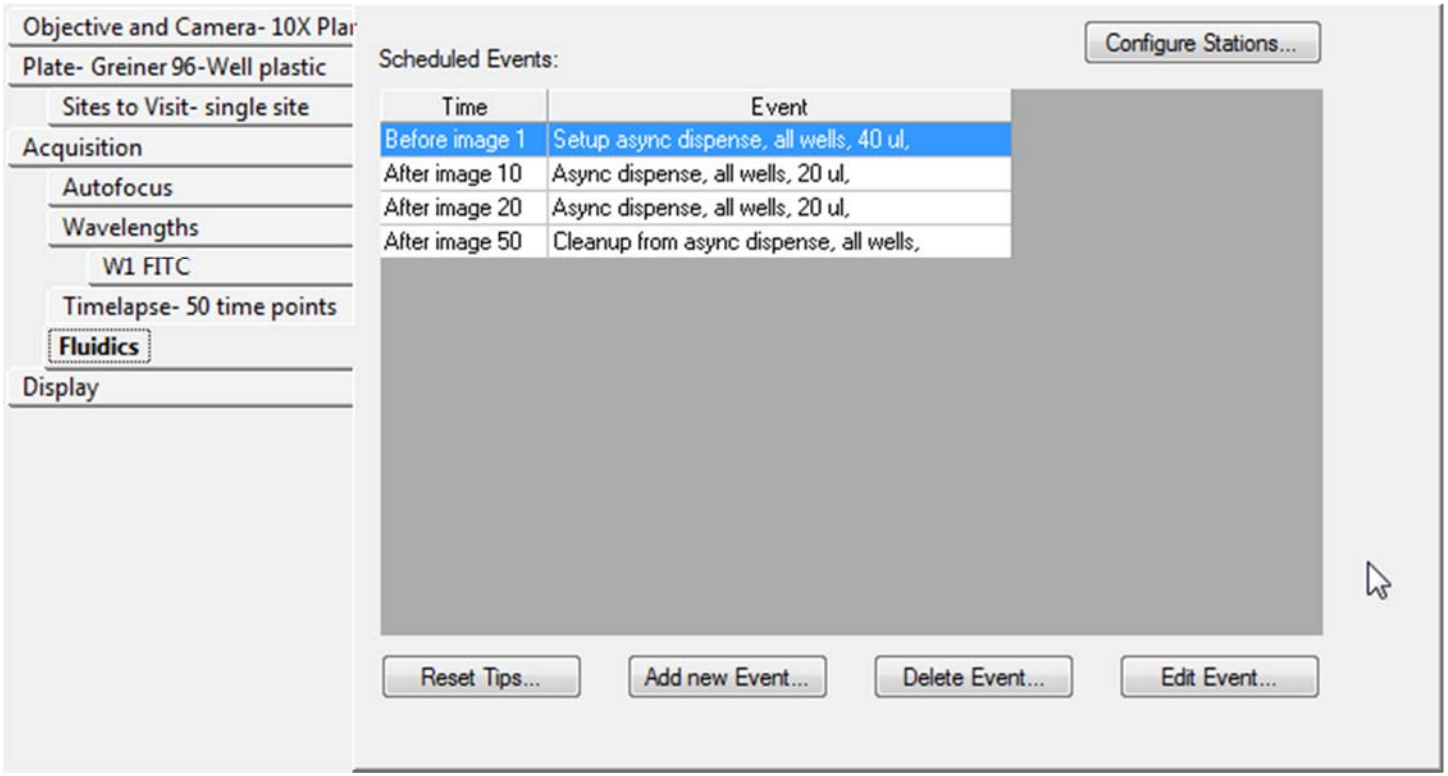
Example: Asynchronous Workflow 1

- **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)



Example: Asynchronous Workflow 2

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



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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