DocuMorph Documentation

Release

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Contents

The missing MetaMorph handbook ™

Welcome to DocuMorph! This resource allows research core facility managers of Molecular Devices' *MetaMorph* software to instruct both, new and savvy users, in making the most of their complex imaging microscopes.

This reference aspires to be the "missing handbook" replacing handwritten lab notes and vendor e-mail exchange threads. Scientists using the software are encouraged to submit existing reference material for inclusion, make suggestions, and also to directly edit this handbook.

Experiments

Streaming with FRAPPA (using Live Replay)

Introduction

Don't use Multidimensional Acqusition, except for Z

Multidimensional Acquisition is often not suitable for FRAPPA streaming experiments, because MDA does not allow running journals (and thus using the FRAPPA) during a stream. MDA only allows journals to be run at the beginning or end.

If pre-bleach images are required with minimum delay between FRAP, we can instead accomplish this type of experiment using the Live Replay.

Fig. 1.1: Resulting time stack using "Live Replay" tab in the "Acquire" window. There is fast diffusion around thin rectangle bleach region.

Note: MetaMorph 7.8.2 will support running the FRAPPA as a native triggered device and will eliminate the need to use Live Replay.
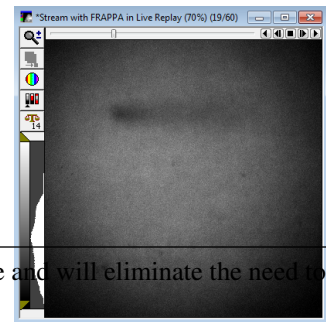
What is Live Replay

MetaMorph's "Live Replay" feature is analogous to iQ's "Loop Protocol". One is waiting for an event to occur at an unknown time point and data must be collected before and after that event.

Live Replay fills a circular buffer with as many images as set in "Before the capture point".

> **Warning:** Timestamps are inaccurate using "Live Replay"; running the same experiment can generate different time intervals in the metadata.

Steps to run an experiment

1. Select your objective in the "Mag:" drop-down (this is essential for the correct FRAPPA calibration to be used)

2. Review / change your FRAP settings with the "Change Frappa Settings" Taskbar button. If you don't see this button, click on the "FRAPPA" button to switch to the FRAPPA Taskbar.

3. Draw and save your FRAPPA region(s):

1. Snap an image and draw your region(s).

2. Save your regions by clicking "Save ROI(s) for FRAPPA" in the Taskbar.

3. If your regions are large or you have set a long dwell time in the FRAP Settings check how long the bleach time is by opening the "FRAPPA Targeted Illumination" window from the Acquire menu. The bleach "Approximate duration" is toward the bottom under the "Location" tab.

4. Open the Acquire > Acquire window.

5. Make sure "Camera State" is Overlapped under the Special tab (it will also work Non-Overlapped, but won't be as fast).

6. Click on the "Live Replay" tab.

7. Make sure "Enable Live Replay" is checked.

8. Review / change your acquisition settings under the "Live Replay" tab:

1. The value of "Before the capture point" should contain enough images to capture BOTH the pre-bleach baseline images as well as the bleaching event itself (the duration you found in step 3c above).

9. Start Live.

10. Press "Stream+FRAP in Live Replay" taskbar button.

Streaming with Mosaic (using Stream Acquisition)

To watch bleaching, we use the "Trigger component positions at specific frames" function of Acquire > Stream Acquisition...

MetaMorph's uber powerful Multidimensional Acquisition (MDA) module is does not allow running journals (and thus using the Mosaic) during a stream; only at he beginning or end.

1. Select your objective in the "Mag:" drop-down, and the select the matching Mosaic calibration in the Targeted Illumination Window "Location" tab.

2. Draw and upload your Mosaic mask:

1. Draw your region(s).

2. Save your regions by clicking "Active Region" in the Location tab of the Targeted Illumination window, and then click "Update Device Mask.

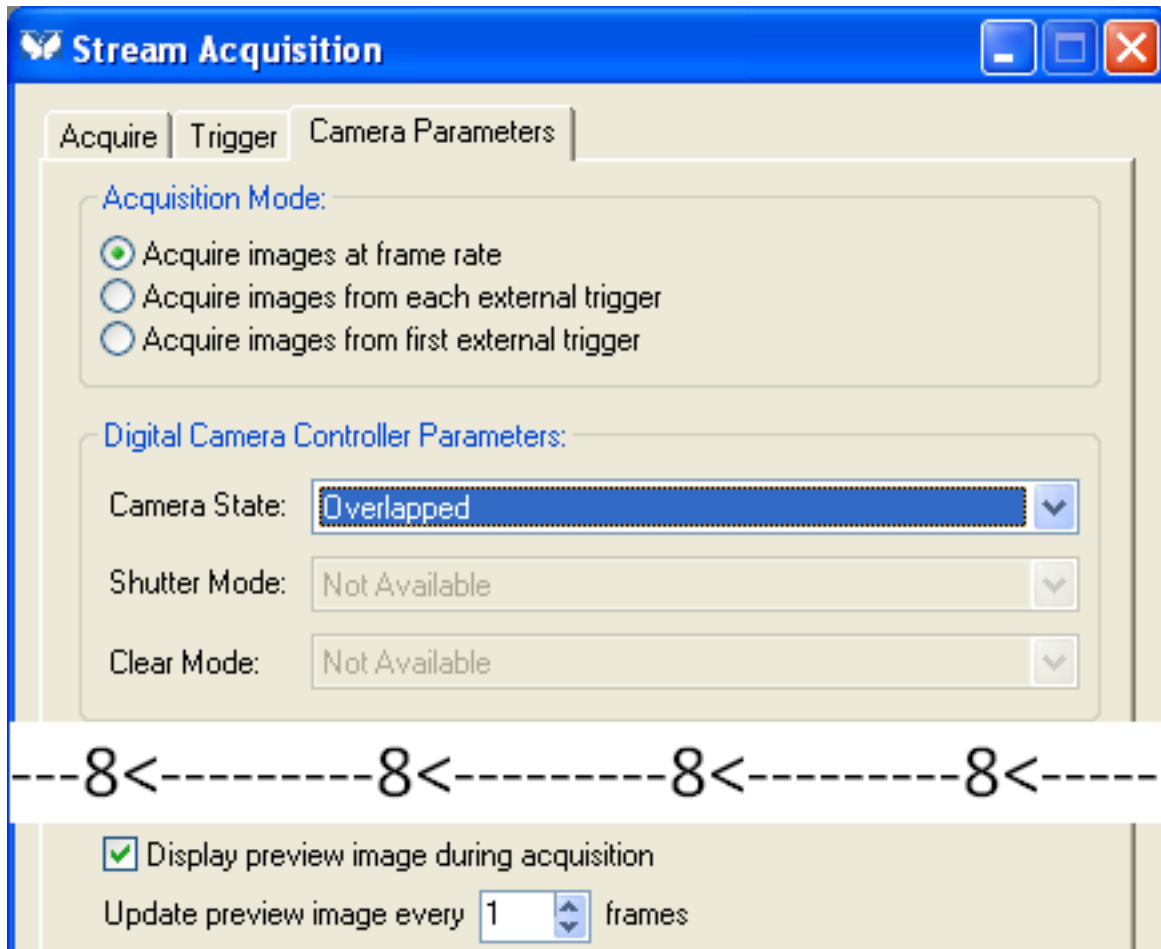3. Click "LatestMask" to complete programming the Mosaic.
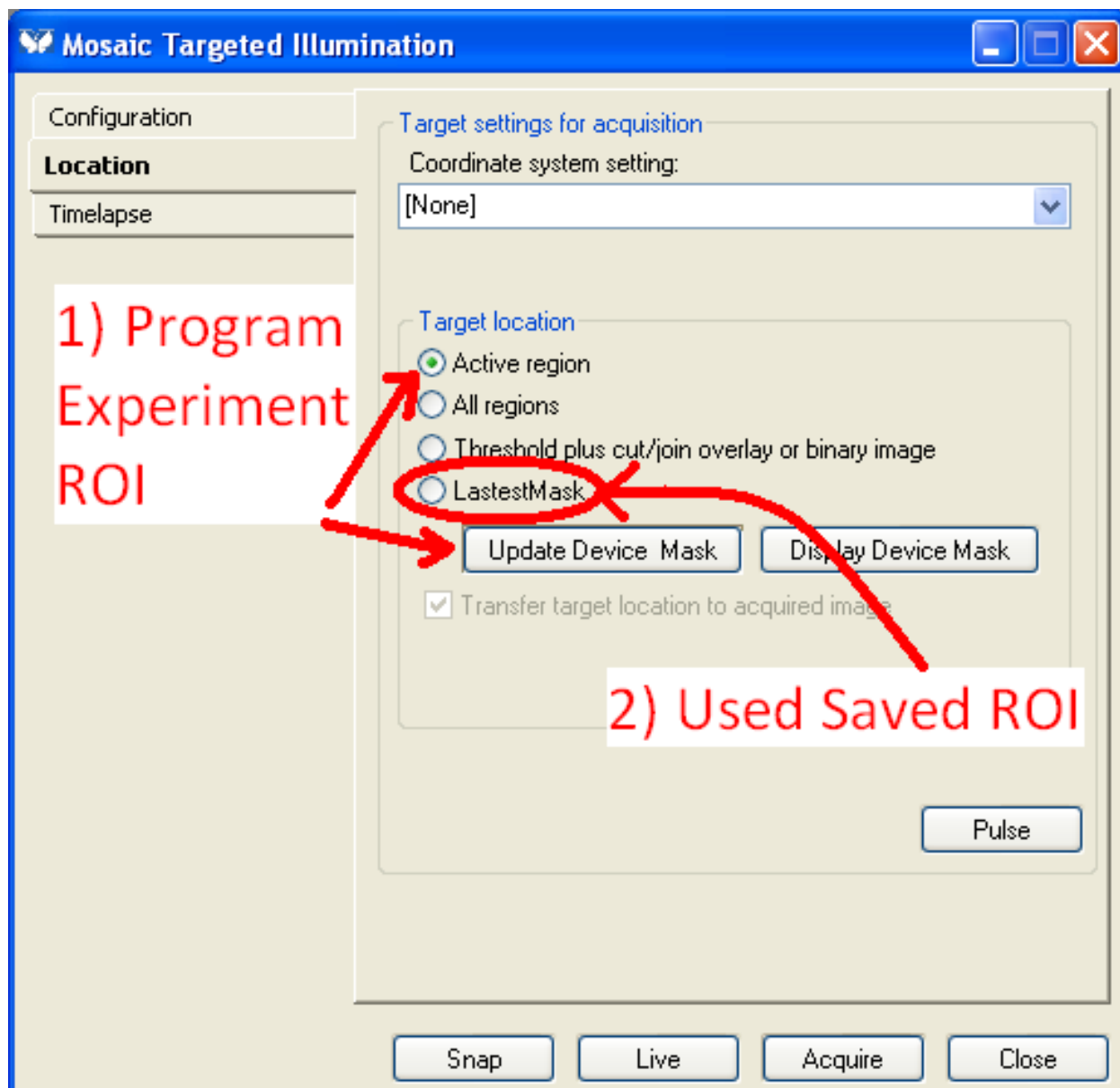
3. Open the Acquire > Stream Acquisition window. Make sure "Trigger components at specific frames" is checked.
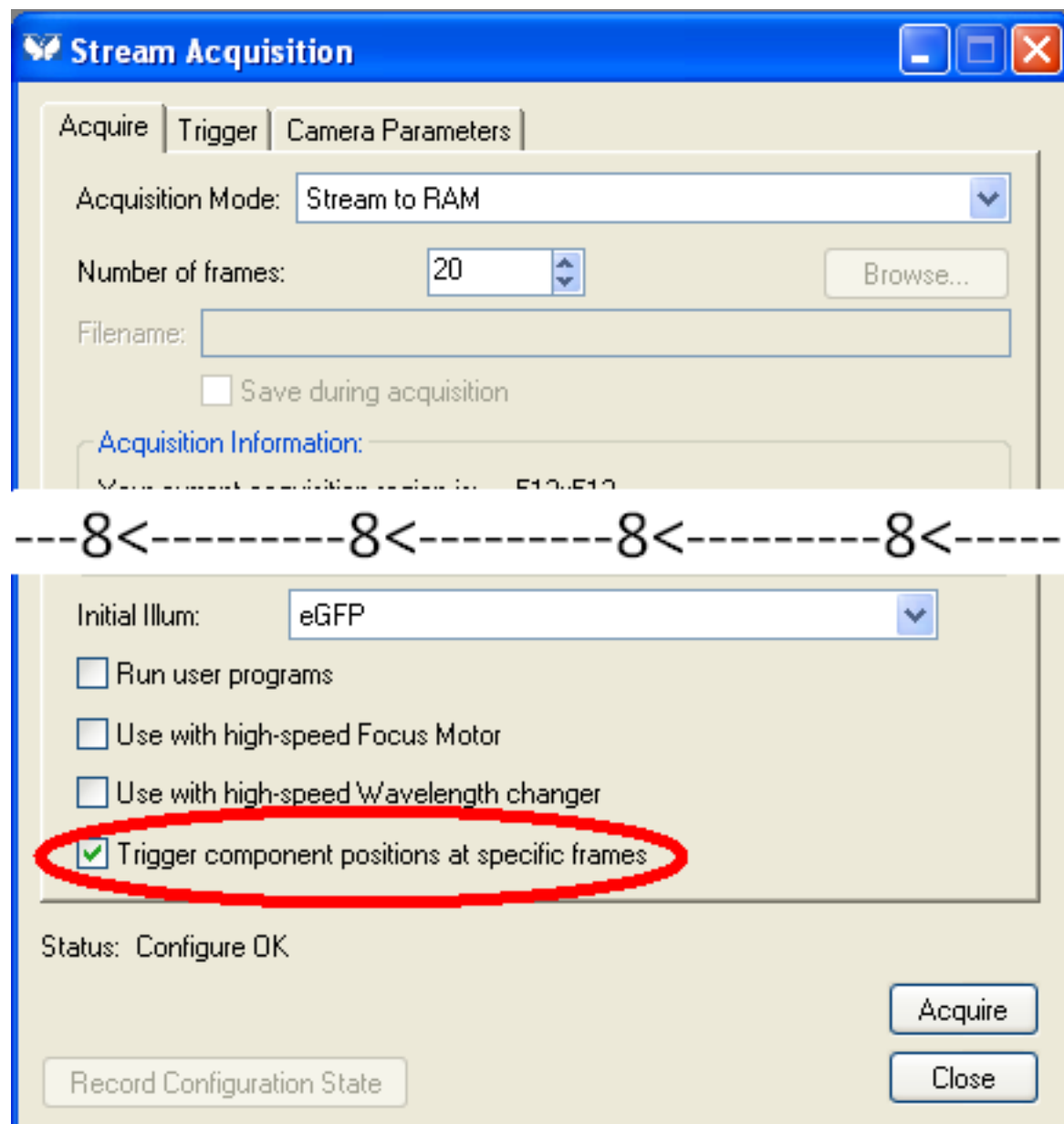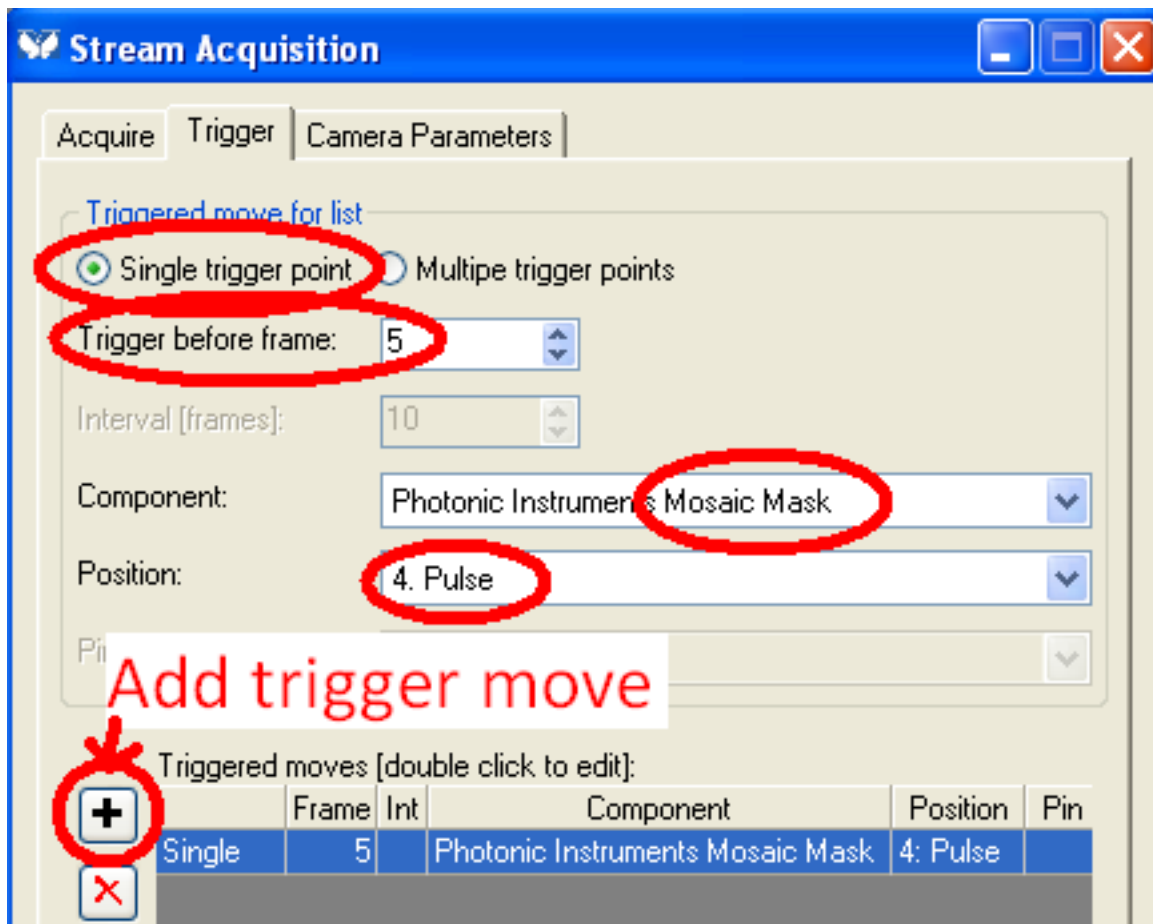
4. Make sure "Camera State" is Overlapped under the Camear Parameters tab (it will also work Non-Overlapped, but won't be as fast).

5. Click on the Trigger tab:

1. Make sure the Component is set to "Photonic Instruments Mosaic Mask" and the Position to "Pulse".

2. Set the trigger time point to pulse the Mosaic and add the "Trigger move".

6. Click "Acquire".

System Setup

FRAPPA setup

Contents

- *FRAPPA setup*
    - *Uniform fluorescent slide*
    - *First time hardware setup*
    - *Steps to calibrate*
   - *Customizing the interface*

Uniform fluorescent slide

1. Focus on the Ethidium Bromide calibration slide with the eyepieces using transmitted light. The liquid layer is thin and can require refocusing every few minutes.

2. Use a camera exposure of 200-500ms for imaging. As with most image processing calibration algorithms, a fair amount of signal is necessary for an accurate image registration.

First time hardware setup

Warning: These steps are only necessary when mechanically aligning the FRAPPA galvo system with it detached from the microscope.
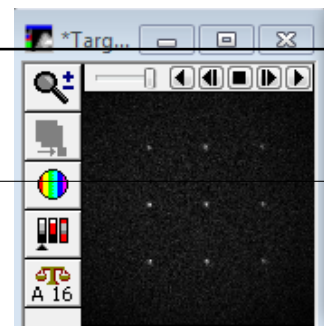
1. Select the "Illumination setting (during pulse)" and "Illumination setting (during imaging)" in the Configuration tab.

2. Adjust the "Rest Position" angle in the Setup tab to center illumination on the C-mount target on the FRAPPA excitation aperture.
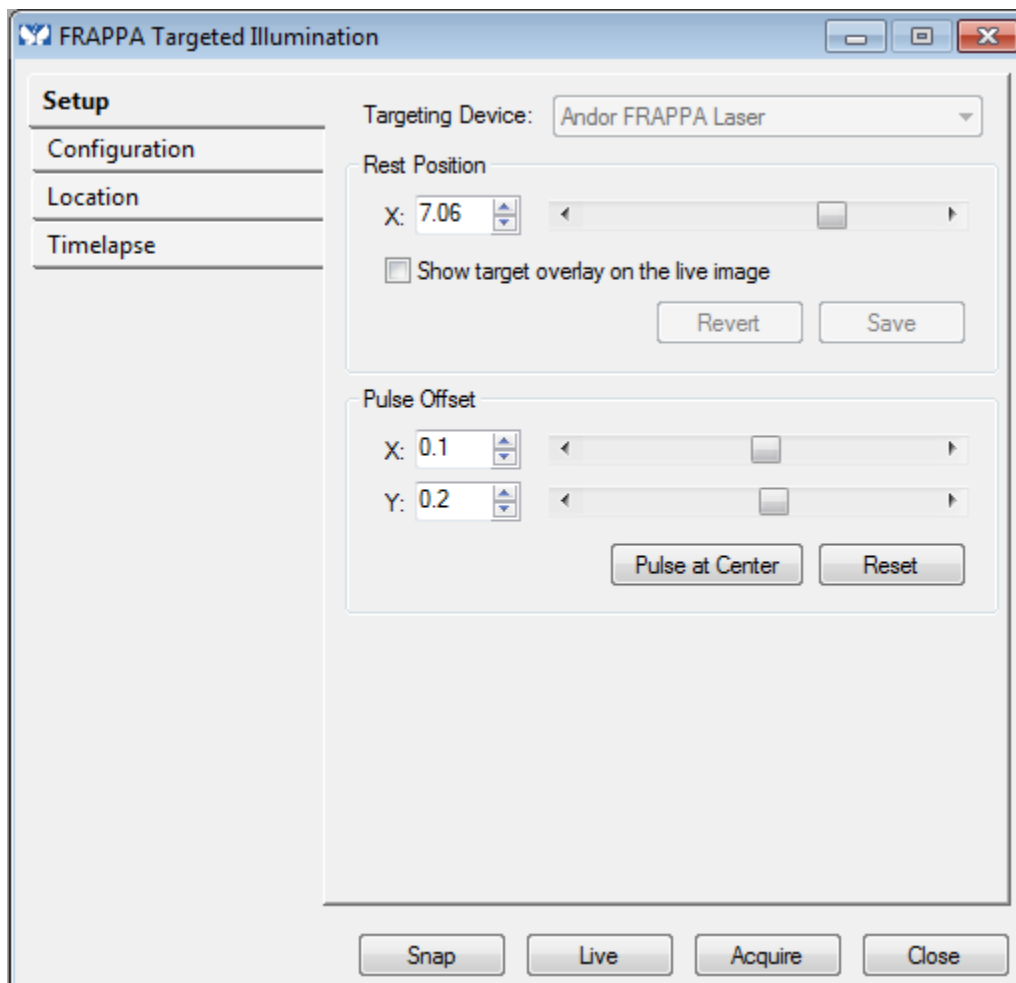

3. Click the "Save" button when you are happy with the bypass position.

4. In the Configuration tab, set the "Dwell time [us]" to 10,000 (without the comma) for an EMCCD, or 100,000 for other cameras.

5. Click "Pulse" back in the Setup tab and adjust the offsets (by 0.1 increments) to center the laser spot on the center of the image.

6. Cycle MetaMorph so that these setup values are written into the `mmproc.cfg` file, just in case the software crashes. The more convenient way of using File > (holding Shift key) + Exit unfortunately does not save FRAPPA settings.

Steps to calibrate

Note: On multi-user MetaMorph systems, it is recommended to create a special user to use "Targeted Illumination" hardware, like the FRAPPA, Mosaic and Micropoint.

The "Targeted Illumination" calibration settings are stored in each user's `mmproc.cfg`, and there are no "Backup..." or "Restore..." buttons (or journal functions) to make it possible to to keep calibrations up-to-date for every user.

1. A safe value for "Range for calibration point" for EMCCDs is 20% to 30%

2. Enable "Display images during calibration". This allows you to see the difference images as the software is doing the automated calibration, providing useful diagnostic information.

3. There should never be any need to use "Manually click on calibration points" with the FRAPPA since we have good control over the laser settings.

4. Enable autoscaling in the Acquire window's Display tab.
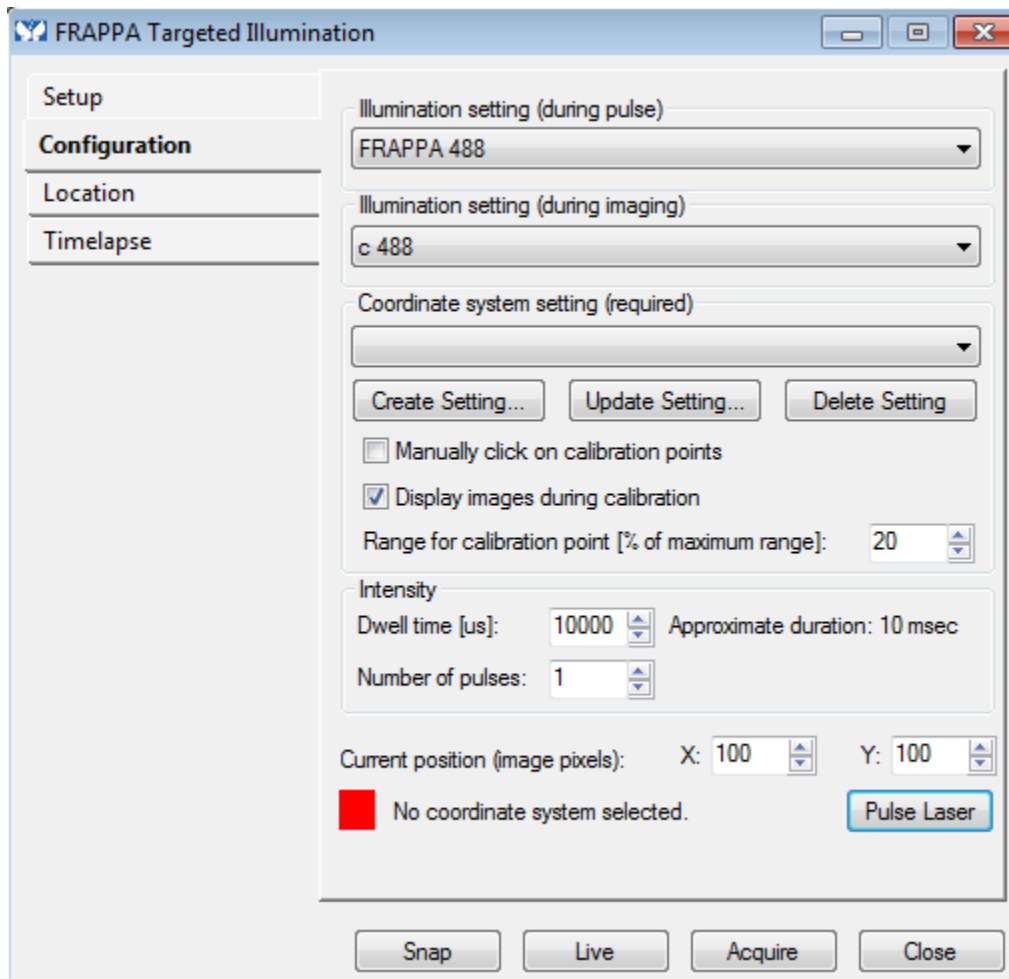
5. Select the "Mag:" setting for the current objective + camera combination in use.

Note: MetaMorph ties together the name of the Magnification setting to the Calibration setting; i.e. the Calibration name is the same as the Magnification setting. When using multiple cameras with the Targeted Illumination device

(such as a multi-camera Confocal or back-port FRAPPA system), create a Magnification setting for each camera like "100x EMCCD" and "100x sCMOS"

6. Finally, click "Create Setting...". During calibration you will see each point pulsed in a 3x3 grid.
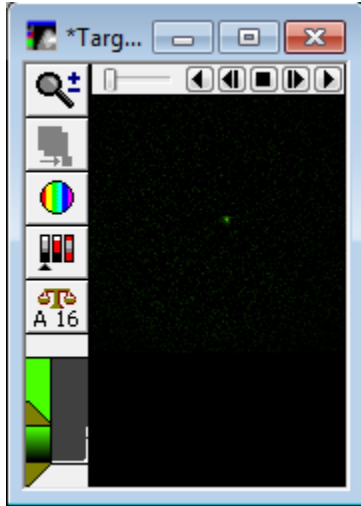
7. The new calibration setting appears in the "Coordinate system setting" dropdown list, if successful.

8. Repeat steps 5 and 6 for all objectives with sufficient NA.

9. **Important**: Change the "Dwell time [us]" back to 100, otherwise trying to FRAP 2D regions will take several minutes instead of the low order of seconds!

10. Optionally, you can draw a small region of interest and click "Pulse" to test the calibration. There are 2 different "Pulse" buttons: one under the Configuration tab and one under the Location tab. Use the "Pulse" button in the Location tab.

Customizing the interface

Create

Contribute

Development

In keeping with the open process, this project's GitHub issue tracker is the primary communication medium for enhancing content.

The full websource is available on GitHub. Best practise would be to fork the project on GitHub, test your changes with Sphinx, and then create a pull request. Although this requires knowing/learning a little about git, python and reStructuredText, it is well worth getting familiar with these tools.

Adding Content

The easiest way to contribute would be sending files containing your instructional material (.odt, .doc) containing images (.png), and associated journals (.jzp, .jnl). In keeping with the open nature of this project, raise a new issue on GitHub, otherwise send an e-mail to omsai@member.fsf.org

Requests and Fixes

As above, raise a new issue on GitHub, otherwise send an e-mail to omsai@member.fsf.org

Credits

- The infrastructure of hosting this content, using Sphinx, reStructuredText and readthedocs was suggested by David Malcom on #bostonpython.

- Homepage graphics created by Gabriel Mascarenhas de Souza hosted on openclipart.org

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