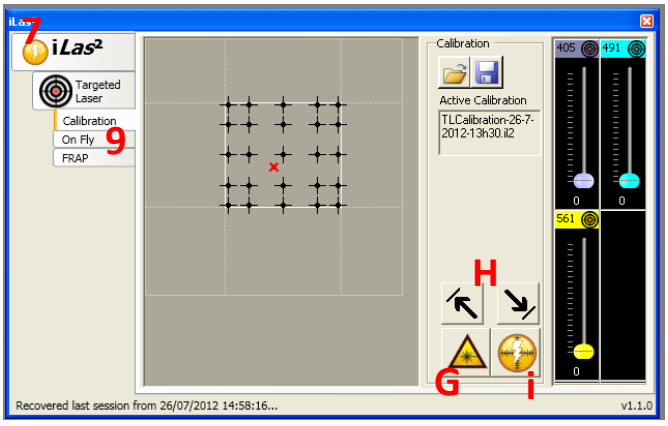
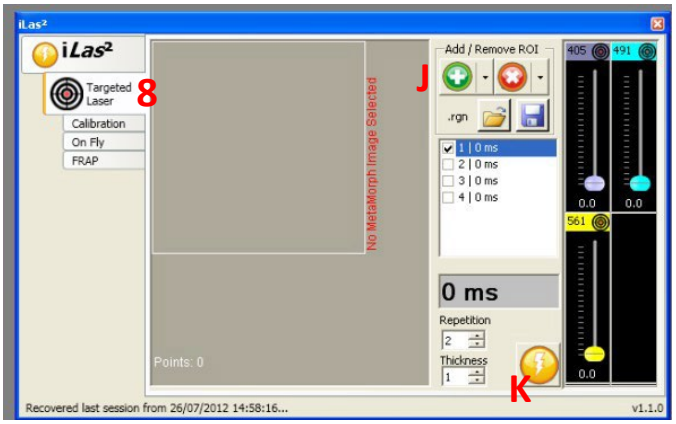
FRAP on the Fly Calibration

Before you can use FRAP the FRAP unit must be calibrated:





- Choose illumination setting suited for FRAP, e.g. “SPD_FRAP G”
- Find an empty piece of glass without cells/fluorescence
- Set the exposure time around 100 - 200ms
- Open ILAS2 with button [F]
- Open calibration tab [7] and put the power of selected laser to 100% (in ILAS2 window)
- Activate the laser [G] and press “ Show Live”
- Place laser in upper left corner by moving the red cross, press [H]
- Repeat for lower right corner
    - If the spot isn’t visible or too big and bright change power and/or exposure time
    - view is rotated, so move laser in other direction
    - keep in mind to move red cross into left and right corners of the calibration screen
- Calibrate with button [i]

- To check if your calibration was successful open “ on the fly” tab
- Change time to 3000
- Increase the power of your laser and press “ Show Live”
- Click in the image and check if laser pulse in at the position of your mouse pointer

FRAP with ROI

- Start Live mode or acquire one image
- Draw a ROI with the desired dimensions on the image
    - ROI drawing elements in top bar of Metamorph
    - Settings are available for standardizing the dimensions of your ROI
- Open tab targeted laser [8] and add the ROI with the green "+" button
    - Setup the desired iterations and check if the time it take to bleach corresponds with your desired experiment setup
    - Set the desired laser power for the bleach pulse

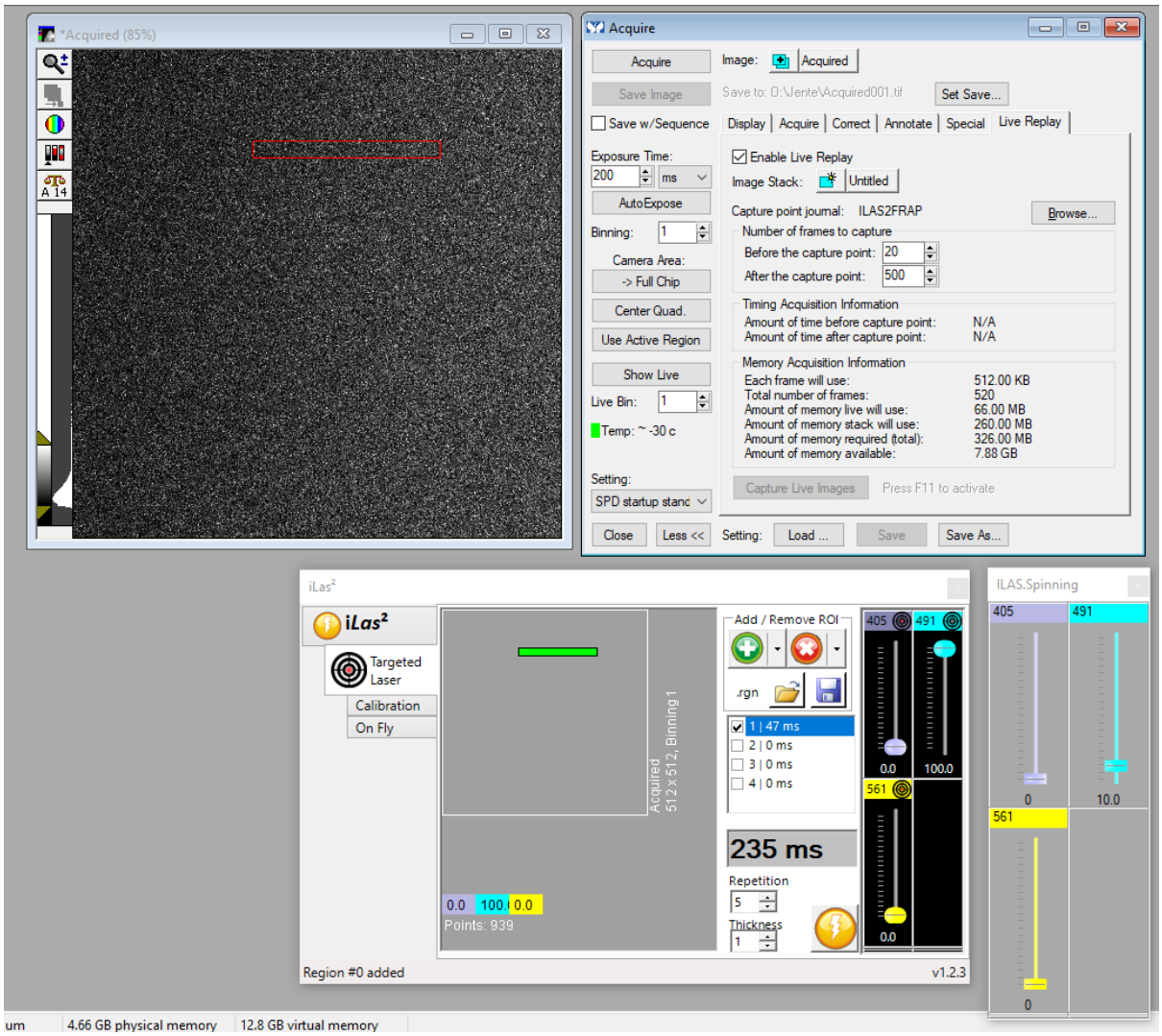
For slower intervals like >500ms continue here:

- Go to the tab FRAP in Ilas2 window and setup the actual FRAP experiment
    - Choose number of pre- & post-bleach frames
- For the actual recording
    - Click on setup MDA
    - Check the MDA if the correct components are selected
        - Timelapse selected, correct save directory and base name & correct filter set.
    - Press acquire button in the MDA window
- When you repeat the experiment, you can click on the setup MDA and acquire button in ILAS2

For intervals shorter than 500ms follow steps on next page:

For intervals shorter than 500ms continue here:

- Open the acquire window, tab special
  - Choose journal Ilas2FRAP.jnl
  - Set desired pre and post bleach frames
- Start live or acquire one image
  - Wait for buffer (pre bleach frames) to be filled (countdown visible in acquire/special tab)
  - Press F11 button to start recording and very quickly thereafter click in your image to bleach the desired positions.
- Wait for the recording to finish
  - If your bleach failed, press the cancel button as fast as possible in the lower right corner of the Metamorph window. Waiting longer can cause Metamorph to crash
- Save the acquired stack before closing the window.



FRAP live spot bleach / “on the Fly”

- Open ILAS2 with button [F]
- Got to tab “On Fly” [9]
    - Set duration to desired units (3000 is ~1sec)
    - Set desired laser power
- Open the acquire window, tab special
    - Choose journal FRAP2Ilas.jnl
    - Set desired pre and post bleach frames
- Start live or acquire one image
    - Wait for buffer (pre bleach frames) to be filled (countdown visible in acquire/special tab)
    - Press F11 button to start recording and very quickly thereafter click in your image to bleach the desired positions.
- Wait for the recording to finish
    - If your bleach failed, press the cancel button as fast as possible in the lower right corner of the Metamorph window. Waiting longer can cause Metamorph to crash
- Save the acquired stack before closing the window.

Troubleshooting - FRAP

<u>FRAP calibration error</u>

- Notification: Calibration canceled

(exceeded 15 bits) Possible errors

- Emitting fluorophore or auto fluorescence
    - Ensure that the area of the slide really empty
- Too much laser power or exposure time
    - Reduce laser power in the ILAS2 window [7] and/or exposure time [C]