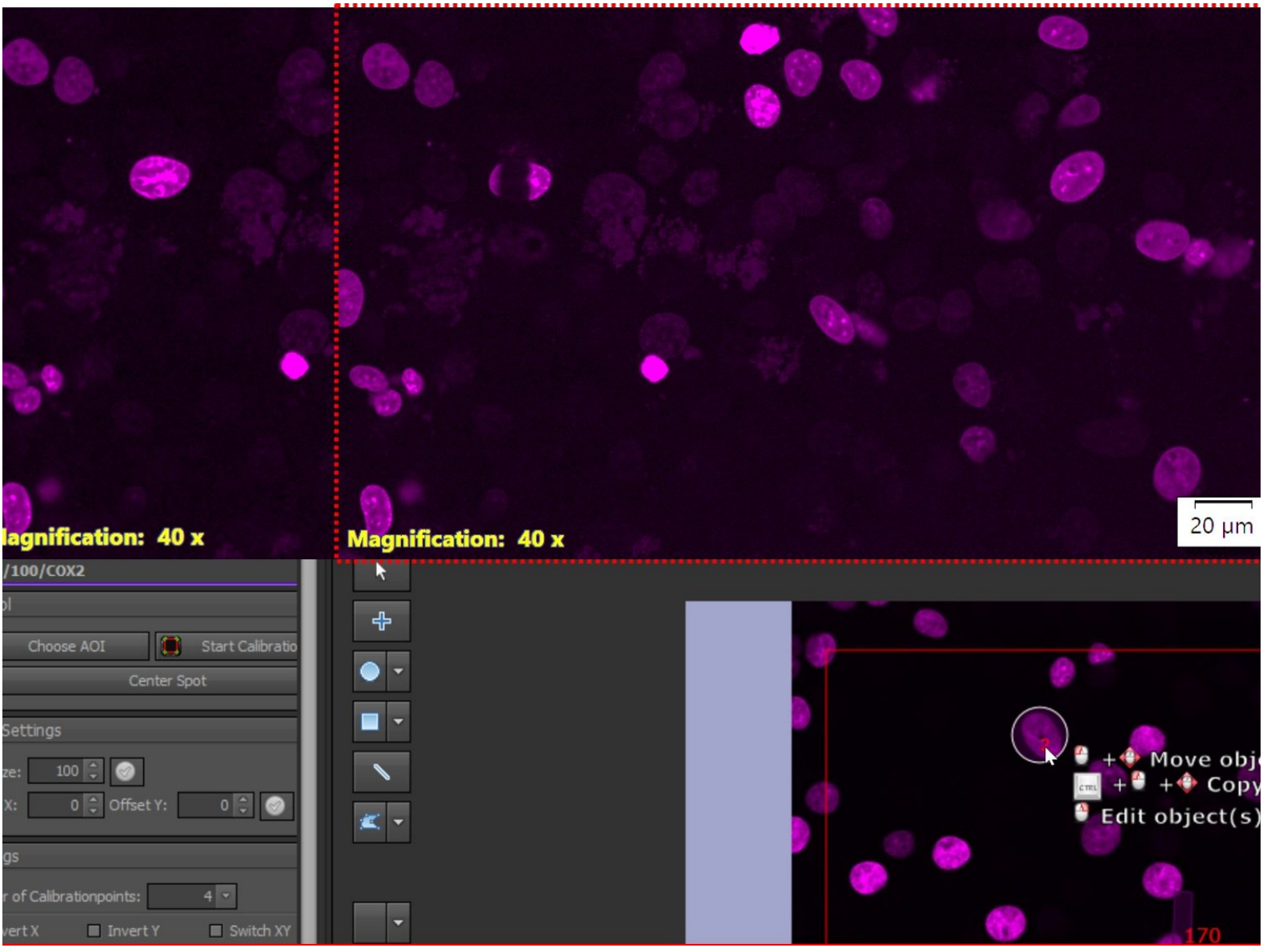
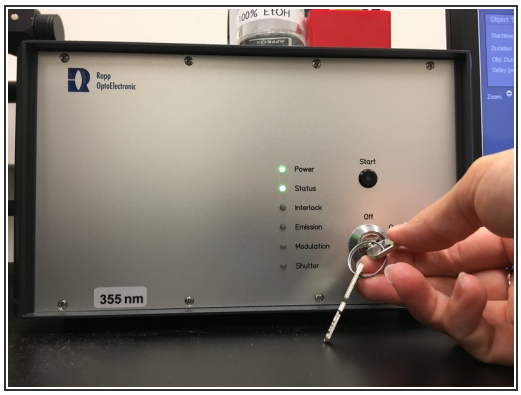
Olympus Spinning Disk - Photomanipulation unit

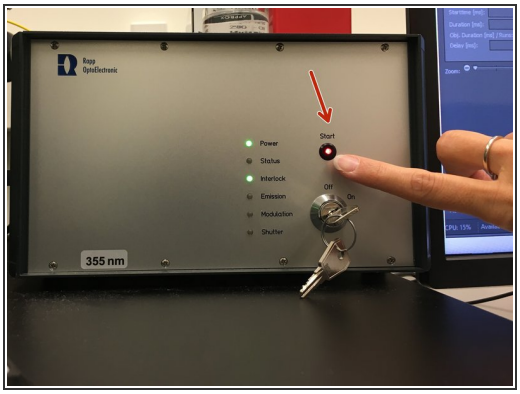
How to start and set up an experiment including photomanipulation using the RappOpto Unit (355nm, 405nm and 473nm lasers).

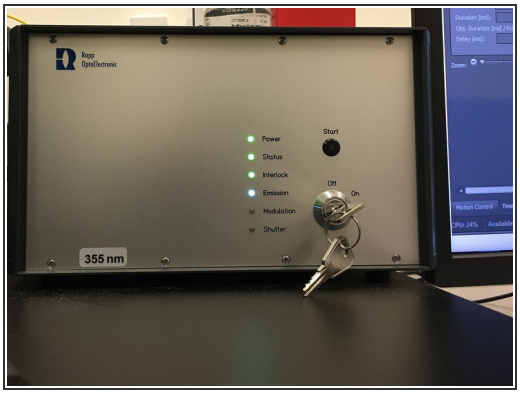
Written By: Joana Raquel Delgado Martins



Step 1 — Start the laser(s)





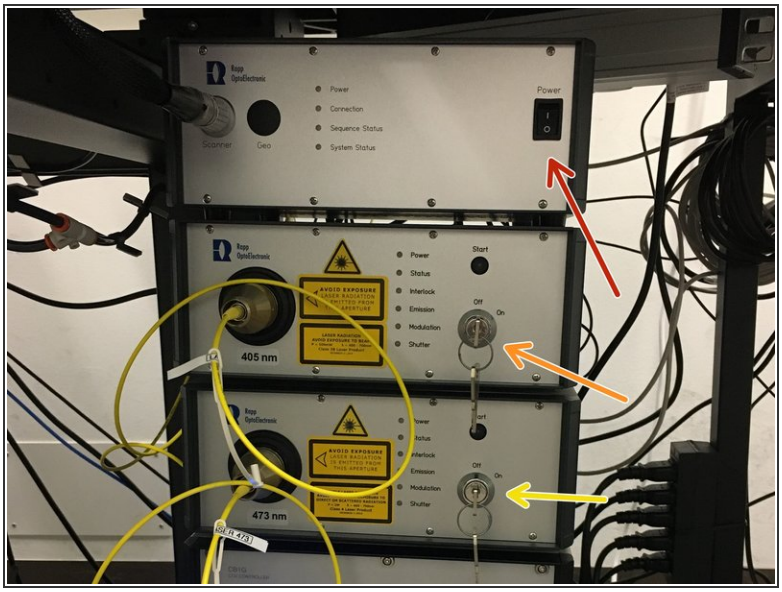


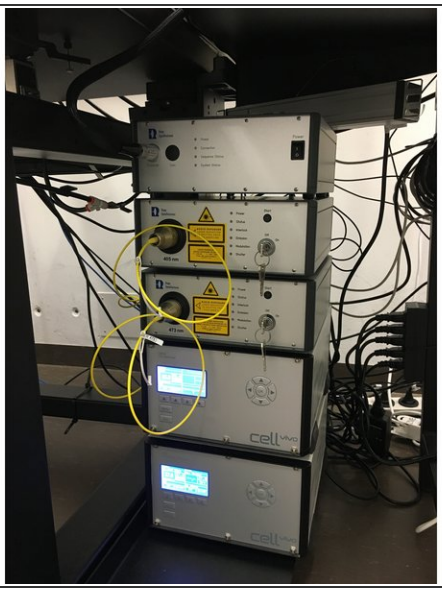
- Start the laser(s) you would like to use by turning the key to "ON".

ⓘ The 355nm laser is next to the monitor. While the others are bellow the microscope table.

- Press the "Start" button when it turns red.

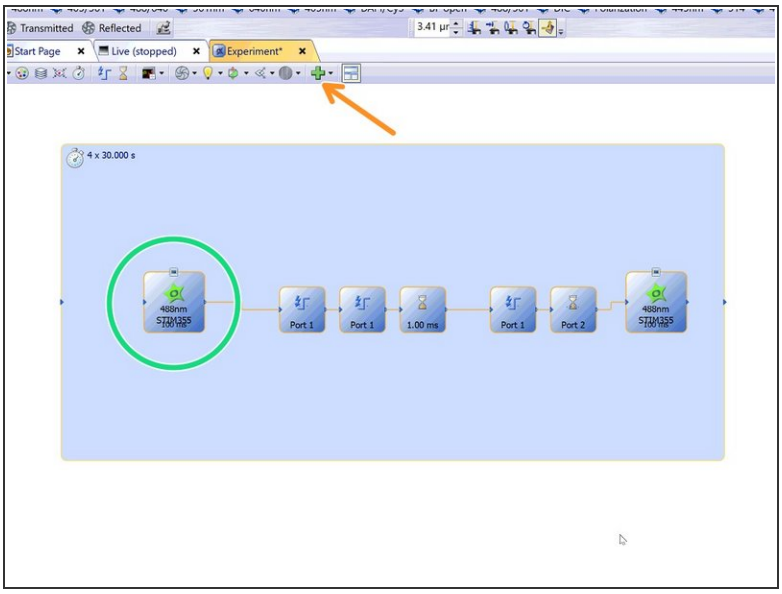
Step 2 — Start the controller box and other laser(s)

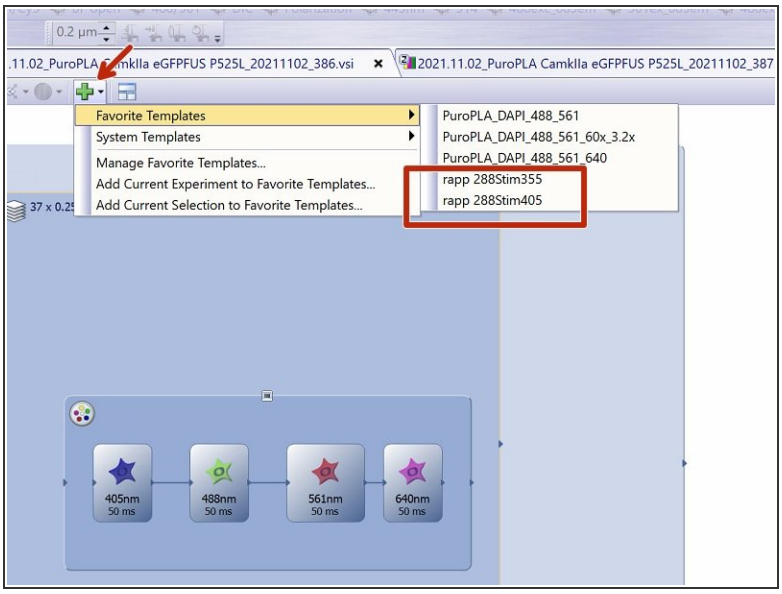




- Switch on the controller box under the microscope table.
- Optional: switch on the 405nm laser line by turning the key. Press the start button when it turns red.
- Optional: switch on the 473nm laser lines by turning the key. Press the start button when it turns red.

Step 3 — Start and set up an experiment in CellSense

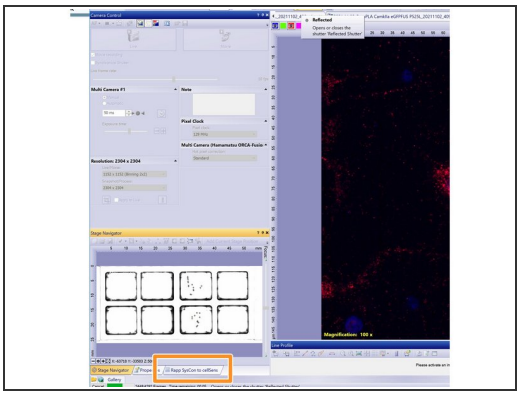


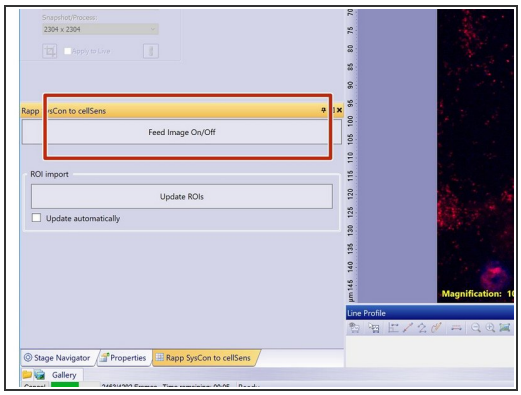


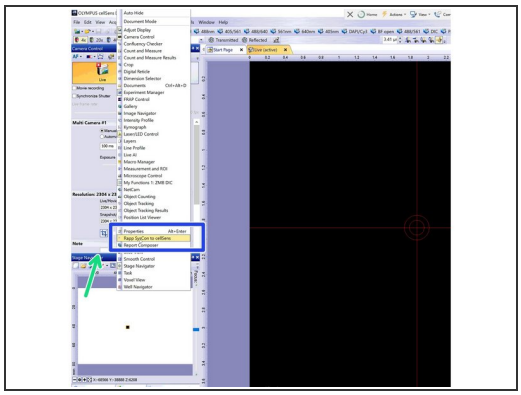
- Start CellSense
- Optional: For a triggered experiment use a template from "Favorite Templates" --> eg. 488 Stim 355.
- Otherwise choose and activate the appropriate observation method that includes "STIM" (e. 488nm STIM405).

ⓘ In this example an image is taken before stimulation and one after.

Step 4 — Rapp SysCon to CellSense

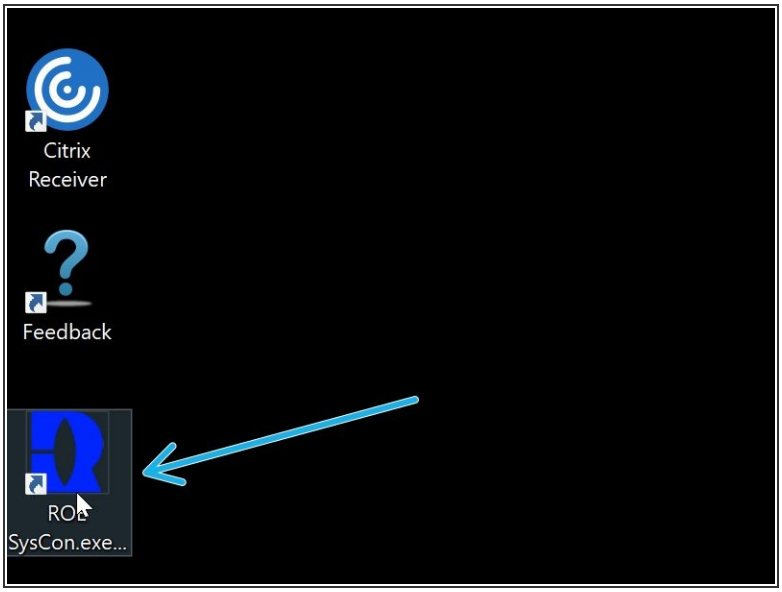


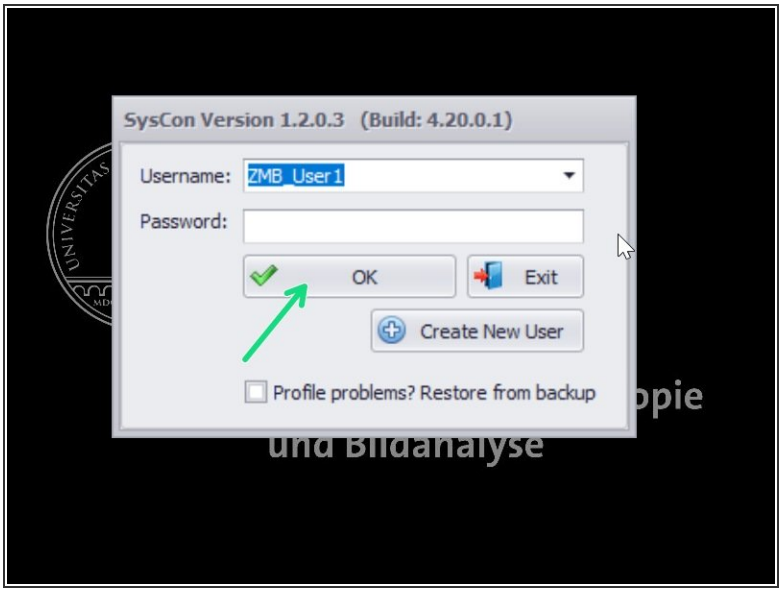




- Localize and select the "Rapp SysCon to CellSens" tab.
- "Rapp SysCon to CellSens"
  - Here you can activate the image feed to the SysCon software once it is opened.
- If the tab is not available in your profile, right click on the top of the "Stage Navigator" window.
- Select the appropriate option - "Rapp SysCon to CellSens".

Step 5 — Start SysCon software

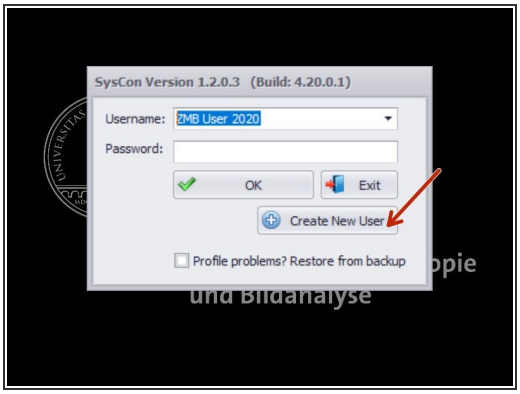


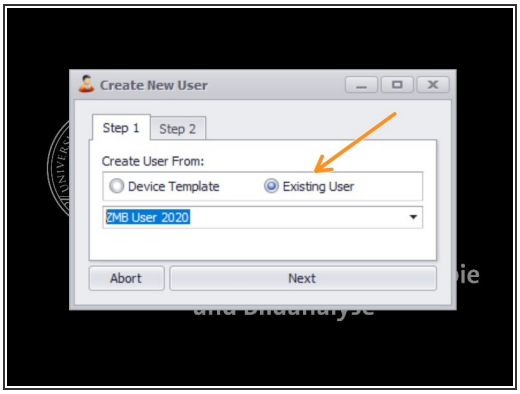


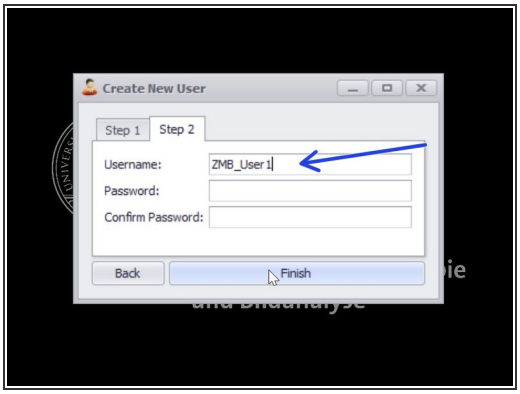
- Start SysCon software.
- Log in using your profile.

ⓘ If you haven't created a profile yet, follow the next step.

Step 6 — Optional: Create a new user (only first log in)

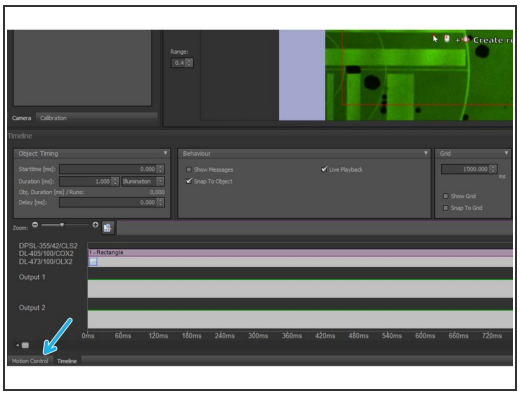


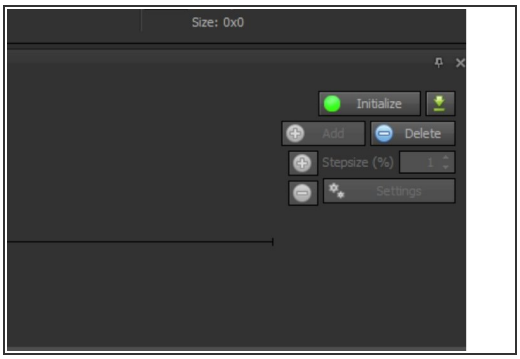


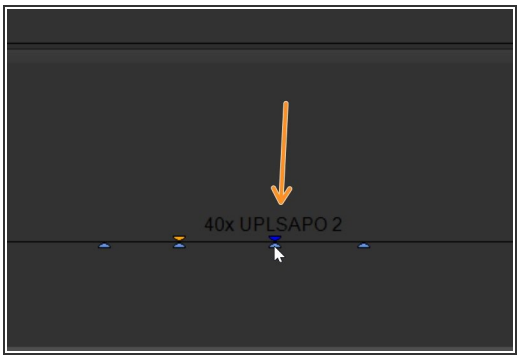


- If this is your first log in, create your own user profile.
- Choose "Existing user" - "ZMB User 2021".
- Create a new user name.

Step 7 — Optional: Only for 355nm laser - Calibrate



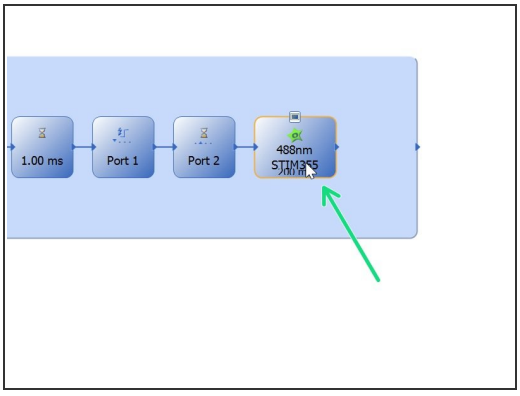


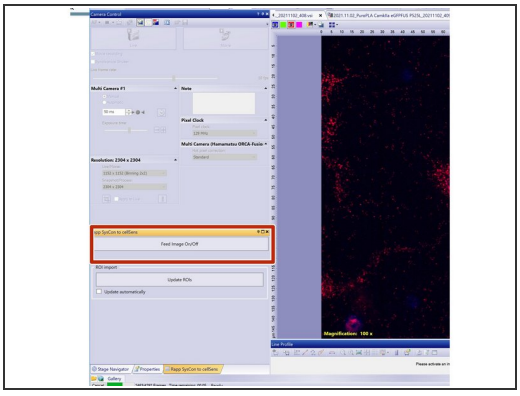


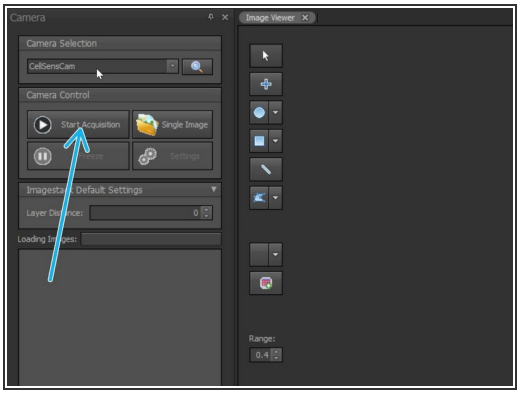
⚠ In SysCon: If you are using the 355nm laser you will need to calibrate the system.

- Choose the "Motion Control" option on the lower panel.
- Press "Calibrate" on the right side of the Motion Control panel.
- Once the Calibration is finished it will turn green.
- Choose the appropriate objective by hovering over it to display the description. Click on the appropriate one (on the image e.g 40x).

Step 8 — Camera Selection and Live Acquisition

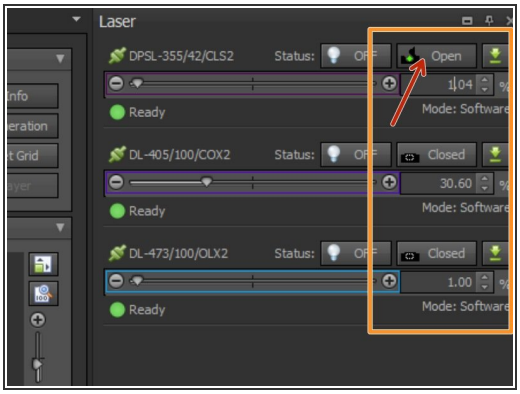


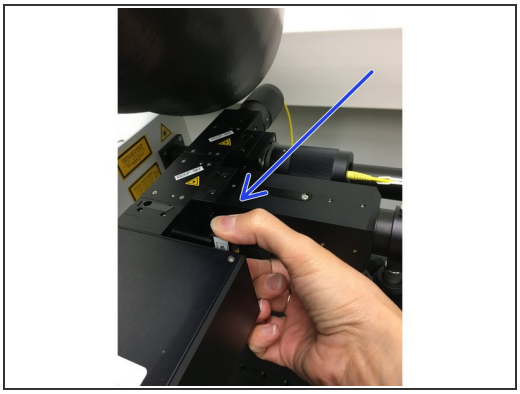


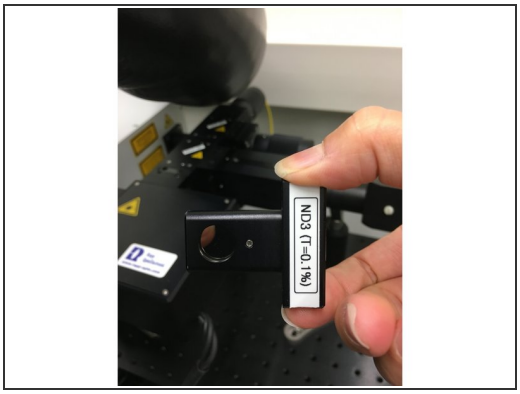


- In CellSense: Start Live mode using the appropriate Observation method e.g. "488 Stim 355".
- In CellSense: start "Feed Image".
- InSysCon: Start Acquisition.

Step 9 — ND filters and opening laser shutter(s).



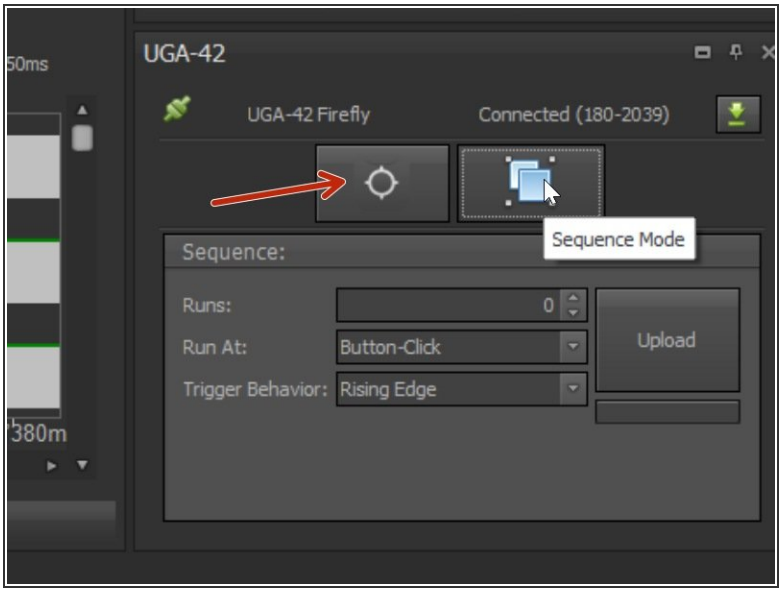


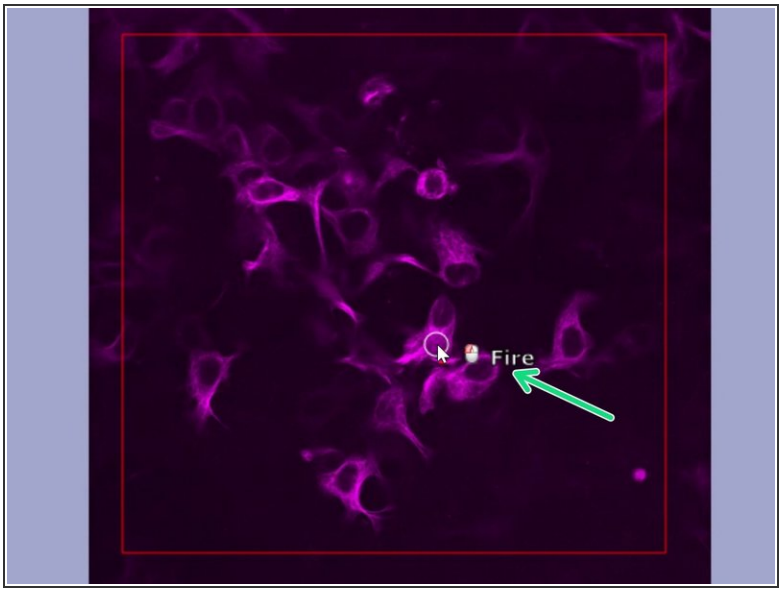


- You can control laser intensities here. The 355nm laser line will be controlled in this panel, while the 405nm and 473nm can be modulated within the experiment/sequence.
- Additionally you can add / exchange or remove the ND-2 and ND-3 filters. Please make sure you check which filter is in position depending on the power outputs you need.
  - ⓘ e.g. for ablation you should start with ND-2 inserted and remove it if you need higher powers to induce damage.
- SysCon top right: Open the shutter of your laser of choice.

⚠ KEEP LASER STATUS OFF. This will be controlled by your experiment. Otherwise you will already start frying your sample!

Step 10 — "Click and Fire" Mode



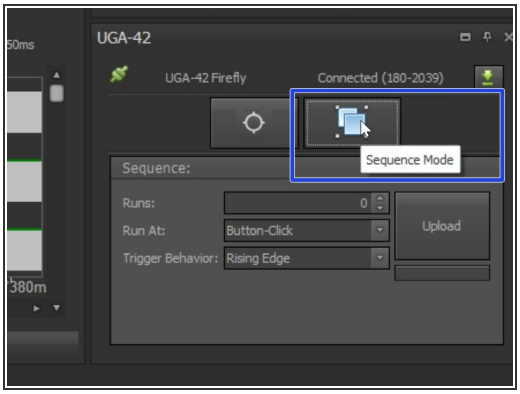


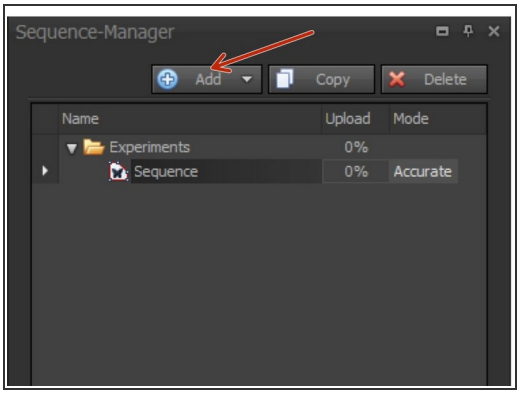
- Use the "Click And Fire" mode to test your settings or start your experiment.
- Choose it under the UG 42 panel (lower right).
- Choose the duration of the stimulation and appropriate light source.

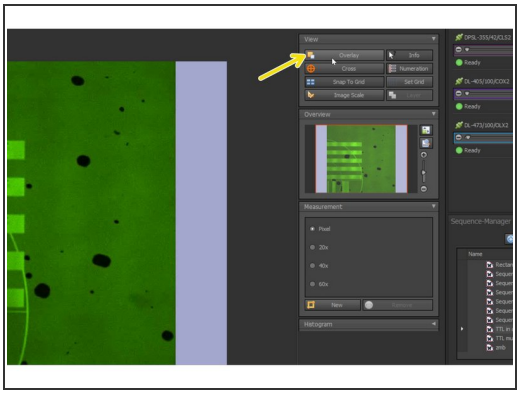
ⓘ 355nm laser intensity has to be modulated under the Laser window or by adding/removing the ND filters (see previous step).

- In the editor "Point" is pre-selected and appears as a circle in the "Live acquisition" image.
- Aim at your region of interest and press left mouse button to "Fire".

Step 11 — Sequence Mode

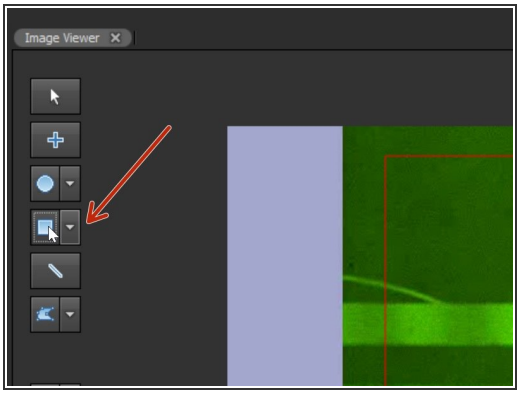


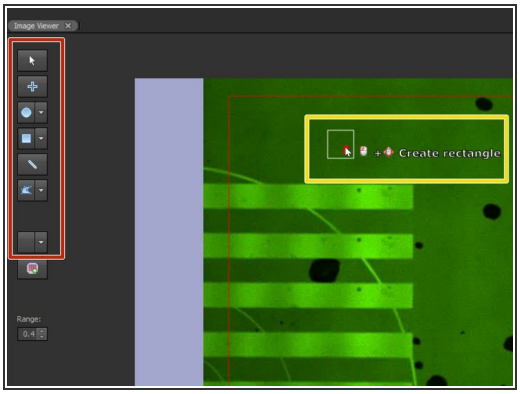


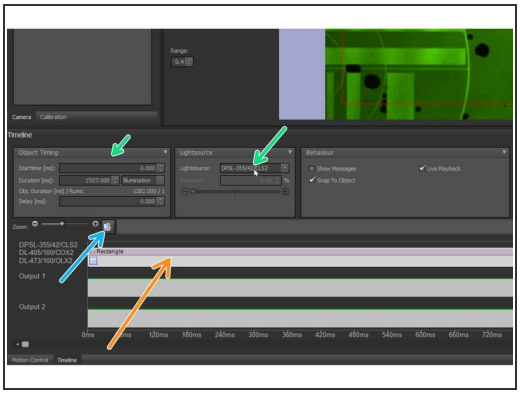


- If you want to choose different shapes/patterns or define a (triggered) sequence choose "Sequence Mode" under the UGA-42 panel.
- To create a new sequence choose "Add".
- Make sure you have "Overlay" selected so you can visualize the inserted objects.

Step 12 — Adding geometrical objects

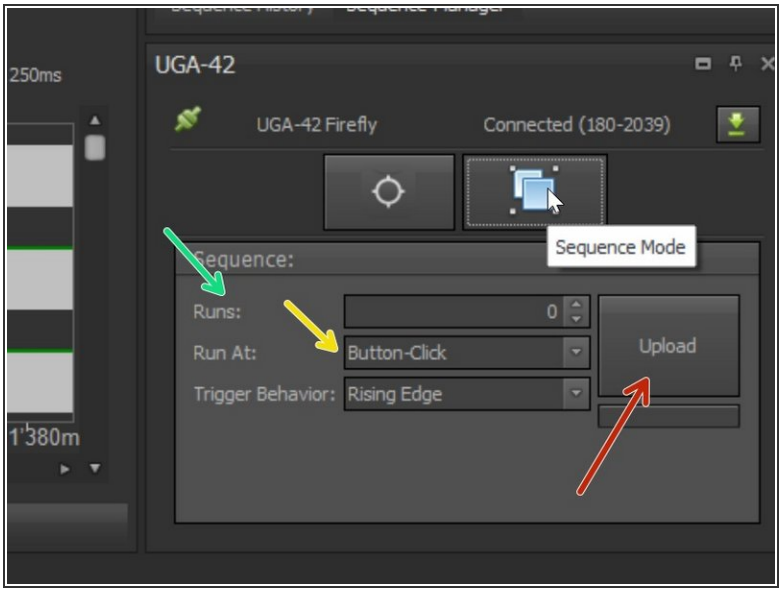


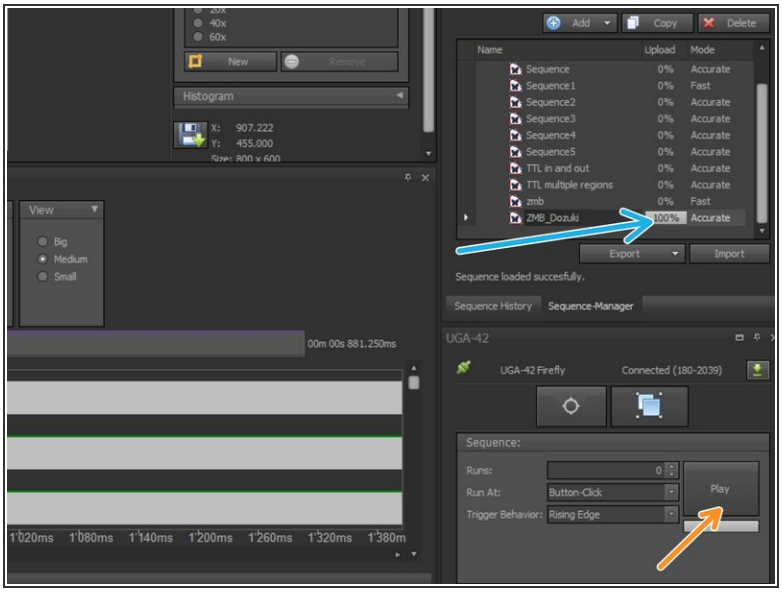




- Select the desired geometrical object from the panel.
- Click into the camera image to position the square. Keep the mouse button pressed to adjust the size.
- A corresponding item will appear in the timeline as a bar, after releasing the mouse button.
- Use this button to fit the whole sequence into the timeline.
- Select the object on the timeline to adjust the appropriate light source and exposure settings.
- To create a new object repeat the previous points.
- Further objects will appear in the timeline and can be moved as desired.

Step 13 — Upload and Play

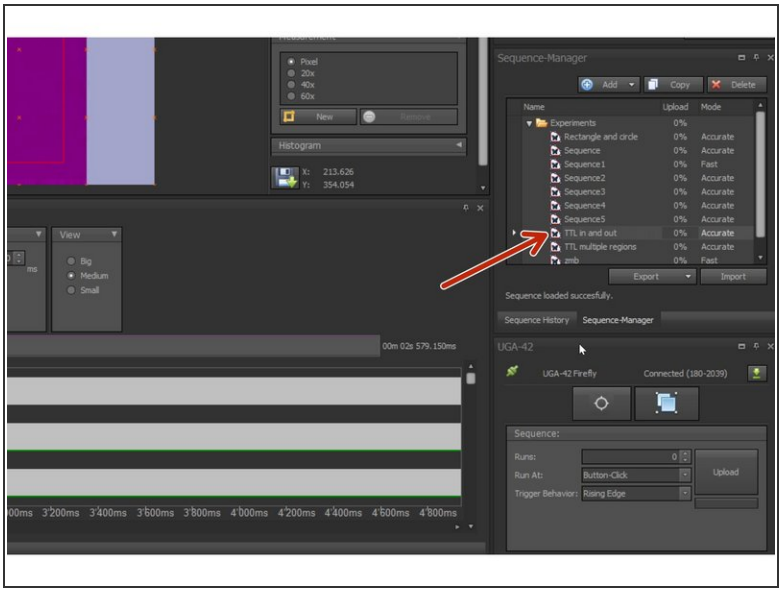


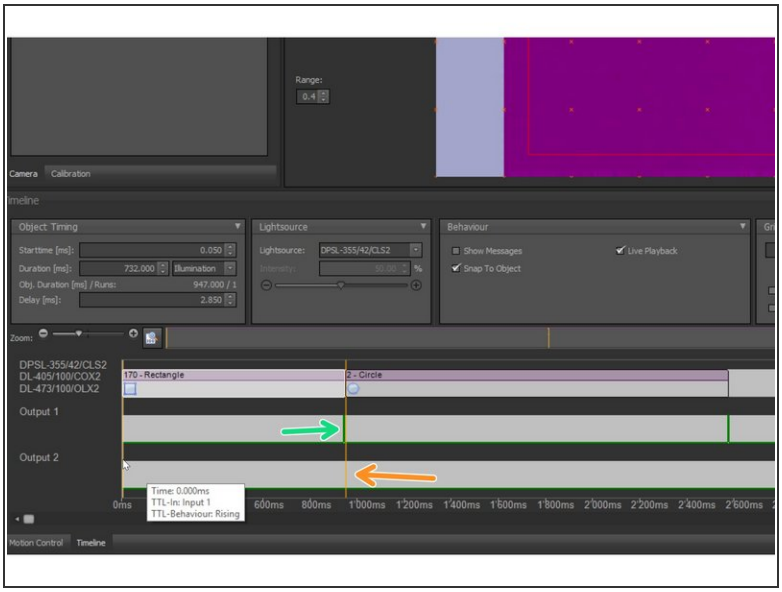


⚠ Make sure you have selected the apropriate observation method in CellSense and that the laser shutter is open.

- Upload the sequence.
- You will see the progress bar now at "100%" in the sequence.
- To play immediately your sequence choose "Button-Click".
- Select the number of sequence cycles (Runs). If 0 it will loop until you click the stop button.
- Play your sequence.

Step 14 — Using triggered sequences (TTL signals)





- Define your sequence in CellSense including a TTL pulse .
- In SysCon: You can choose one of the available sequence templates as a starting point (e.g. TTL in and out) .
- In SysCon: It is possible to create TTL pulses by right-clicking into one ’Output’ line on the timeline and choosing "Add TTL Pulse" from the context menu.
- In SysCon: It is also possible to add TTL breakpoints via ’Add Breakpoint’ in the context menu.

ⓘ These breakpoints can be positioned between objects in the timeline and appear as yellow vertical bars drawn over all timelines. The sequence will stop at each breakpoint until a TTL pulse is recognized at ’Input 1’ of the UGA 42 firefly.

- In SysCon: Upload and run your sequence.
- In CellSense: Start your experiment including a TTL pulse and wait option.
- For more details please ask ou staff.