

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".
- (i) 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

	אין
	.11.02_PuroPLA Camklia eGFPFUS P525L_20211102_386.vsi 🗙 🖓 2021.11.02_PuroPLA Camklia eGFPFUS P525L_20211102_387
	< · · · + · -
	Favorite Templates PuroPLA_DAPI_488_561
	System Templates PuroPLA_DAPI_488_561_60x_3.2x
3 4 x 30.000 s	Manage Favorite Templates PuroPLA_DAPI_488_561_640
	Add Current Experiment to Favorite Templates rapp 288Stim355
	37 x 0.2: Add Current Selection to Favorite Templates rapp 288Stim405
ASSIM TZUNZS Porti Porti Porti Porti Porti Porti Porti Porti	
a	405nm 488nm 50 ms 50 ms

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

Step 4 — Rapp SysCon to CellSense

Segnituditeses P. 239x x 204 P. The segnituditeses P. The segniteses <	Benchman calming Strate Number of parts Number of parts </th
Rapp SConto celSens • IX S Feed Image On/Off	Network Standam Standam <t< td=""></t<>
ROI import	The second secon
9 9 9 9 9 9 9 9 9 9 9 9 9 9 9 9 9 9 9	
Stage Navigator / ∄Properties	

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

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

SysCon Version 1.2.0.3 (Build: 4.20.0.1) Username: 2019 Voltage 2020 Password: Create New User	-	Create New User Step 1 Step 2 Create User From: O Device Template ZMB User 2020 Content of the second			Step 1 Step 2 Username: ZMB_User 1 Password: Confirm Password:	
Una Bilaanaiyse	pie	Abort	Next	ie	Back	Finish
		ини	Bhaanaryse			

- 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

Leven "Gibros:	Size: 0x0 p ×	
Baltimory Baltimory Baltimory Baltimory Dird Odd V Object Tarling 1.000 B 5.000 B 1.000 B 0.000 B 0.	 Initialize Add Delete Stepsize (%) Stepsize 	
OLATINGOUS Output 1 Desput 2 the films them them them them them them them them		

▲ 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.
 - (i) 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

50ms	UGA-42 VGA-42 Firefly Connected (180-2039)	□ + × 2	Solar and
	Sequence:		Spo o
	Runs: 0 C Run At: Button-Click Vuploa	ad	R # Fire
'380m ► ▼			· A.

- 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.
- (i) 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

50ms	UGA-42 = + ×	Sequence-Manager Add Copy Delete	Norm # One, NUMADIA Sec. Sec. <
	Sequence: Runs:	Name Upload Mode ▼ ≥ Experiments 0% ™ Sequence 0% Accurate	
'380m ►	Run At: Button-Click Upload Trigger Behavior: Rising Edge		Segurar Margar

- 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

250ms	UGA-42	□ • ×	Ans A	Add Image: Copy Name Upload Capy Office Sequence Office Capy Office	Mode Accurate
1'380m ▶ ▼	UGA-42 Firefly Connected (180-2039)	2 ad	Histogram Y: 907.222 Y: 455.000 Sove: and x and P X View Big Middum Small 00m 00s 881.250ms 1'b20ms 1'b80ms 1'140ms 1'200ms 1'250ms 1'320ms 1'380m *	Augustick 1, Ors Sequence2 0% Sequence2 0% Sequence3 0% Sequence4 0% The nulliple regions 0% The nulliple regions 0% The nulliple regions 0% The nulliple regions 0% The Donal 00000 Sequence loaded succesfully. Sequence History Sequence Manager UGA-42 UGA-42 Firefly Connected (Sequence History Sequence Manager UGA-42 Sequence History Sequence Manager UGA-42 Sequence History Sequence Manager UGA-42 Note that the sequence Manager Note that the sequence M	Accurate Accurate Accurate Accurate Accurate Fast

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)

Pred Sequence-Manager	Range: 6.4 🗄
Image: Segment in the segment is segment in the segment in the segment in the segment is segment in the segment in the segment in the segment is segment in the segment is segment in the segment in the segment is segment in the segment in the segment is segment in the segment is segment in the segment is segment in the segmen	Cabration
View View Sequence 5 0% Accurate Object Tr Big Big Thit mind out 0% Accurate Difference Difference <td< td=""><td>Trining Lightsource Behaviour Gri le (ms) 0.050 (2) Lightsource: DPS-355/42/LS2 (2) Driver Messages Clive Rayback: Driver Rayback: Dri</td></td<>	Trining Lightsource Behaviour Gri le (ms) 0.050 (2) Lightsource: DPS-355/42/LS2 (2) Driver Messages Clive Rayback: Driver Rayback: Dri
Om 02 579.150ms UGA-42 0 + C DP51.25 UGA-42 0 + C 0 + C DP51.25 UGA-42 + C + C DP51.25 UGA-42 + C + C DP51.25 D100 - C - C - C 00ms 3*200ms 3*500ms 3*500ms 4*500ms 4*500ms 4*500ms + C - C	35544/cl.52 170-Retangle 2-Circle 35540/cl.52 170-Retangle 2-Circle 11 1 1 12 1 1 12 1 1 11 1 1 12 1 1 11 1 1 12 1 1 11 1 1 11 1 1 11 1 1 11 1 1 11 1 1 11 1 1 11 1 1 11 1 1 11 1 1 11 1 1 11 1 1 11 1 1 11 1 1 11 1 1 11 1 1 11 1 1 11 1 1

- 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.