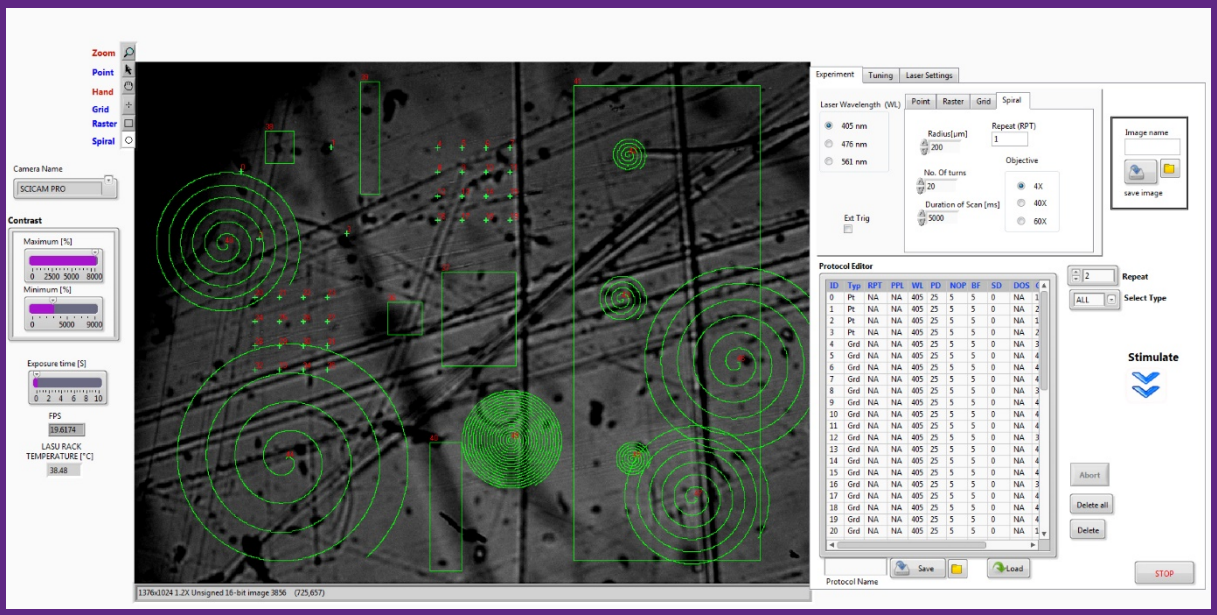
Scientifica

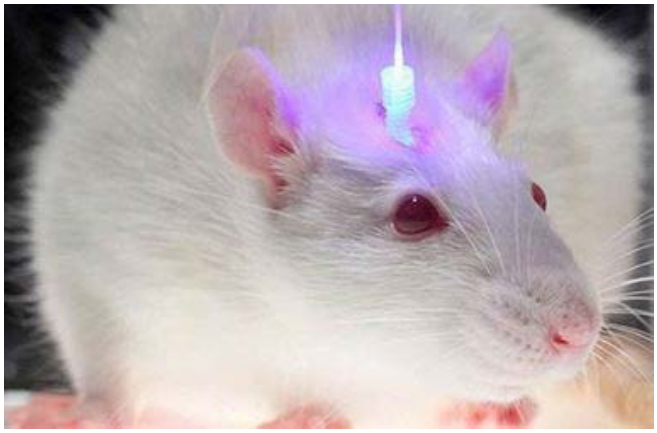
LASU

Software Operation Manual



LASU Software Operation Manual

Introduction to Photostimulation and Optogenetics



Neuronal activity has been studied historically using **pharmacological stimulation.** This is achieved through the application of drugs or **electrical stimulation** such as applying voltage or current via a micro electrode. **Photostimulation** appeared more recently and involves the activation of biological compounds using light. There are two main types of photostimulation: laser uncaging and photo activation.

Laser uncaging involves injecting or loading the sample with a compound in a caged, or inactive. form. Light is then applied to the loaded cells to break the covalent bonds of the cage, releasing the active compound. The effect can then be measured using electrophysiology. This can be carried out using caged neurotransmitters such as glutamate, serotonin, GABA etc. and with molecules such as calcium or ATP.

Photo activation utilizes opsins. These are ion channel proteins from green algae that open in response to light, letting ions into the cell. This enables the algae to respond to light; it moves towards the light. Using genetic methods, researchers can modify model animals so that their neurons express these light sensitive ion channel proteins. When light hits these ion channels, they open and ions enter the cells, changing the membrane polarity. The two first opsins to be used for photostimulation were channelrhodopsin-2 and Halorhodopsin. Both are activated by different wavelengths, therefore can be used in the same animal, and they also have two opposite effects. When channelrhodopsin-2 is stimulated it activates the neurons that express it, whereas activation of Halorhodopsin inhibits the neurons expressing it. Used in combination, these two opsins can be used to decipher maps of interactions in large areas of the brain.

The use of laser or LED light sources coupled with the microscope objective allows precise spatio-temporal photostimulation, giving insight into the role of specific neurons or molecules within a network of neurons or on the role of molecules within

a single cell. The approach limits the impact of electrode insertion, gives access to a larger area than classic approaches and is in principle easier to set up.

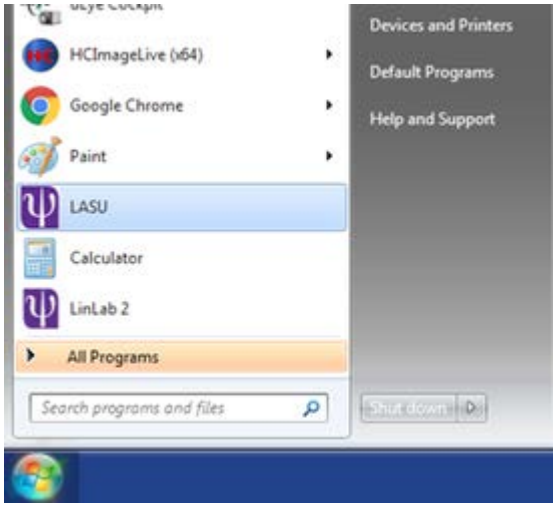
1. Installation procedure

Install the LASU software by clicking on the setup files and following the step by step instructions on the installation window.

At the end of the installation tick the check box to activate the vision run time. Type in your license number and then click finish.

The executable file contains all the drivers to run the LASU system, including camera software and dll to control the laser.

Once the installation is successful, make sure the wiring and hardware connections are secure (refer to the wiring diagram schematic). The LASU software can be accessed from the startup menu (please refer to the screenshot below).



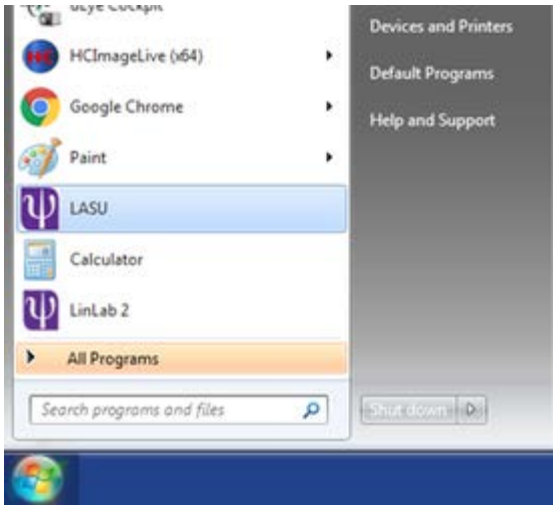
2. Starting LASU

2.1. Starting the hardware

Insert the laser interlock and laser LASU key(s) into the back of each laser unit. Plug in the DC power supply. Once this has been plugged in the green light on the top of the laser unit will flash, indicating that the internal laser temperature is stabilising. Once the temperature has stabilized, the light will turn solid blue, indicating that the laser is ready to be switched on. Rotate the laser key clockwise to the ON position. Once this is carried out, the LED on the back of the laser unit will turn green to indicate that the laser emission has been enabled.

Figure A: Laser interlock is connected, and the laser key has been inserted and turned clockwise to enable emission. Green light indicates emission enabled.

2.2. Launching the program



Make sure you have admin rights and attributes and that read only is unchecked for all the folders in the program files\Lasu

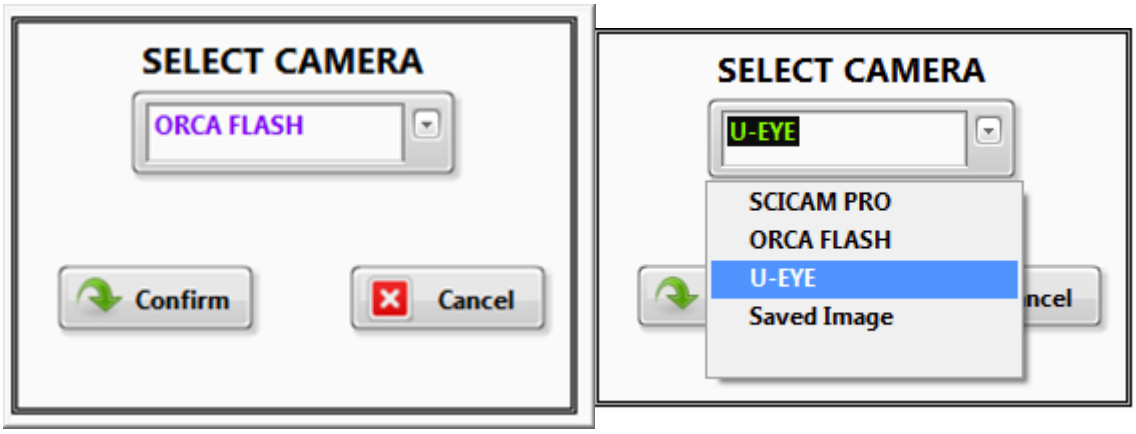
The software can be accessed from the startup menu.

2.3. Camera Selection window:

When the user launches the LASU software from the startup menu, the camera selection window is displayed. LASU 2.0 software supports two different cameras and allows a stimulation mode (Saved image).

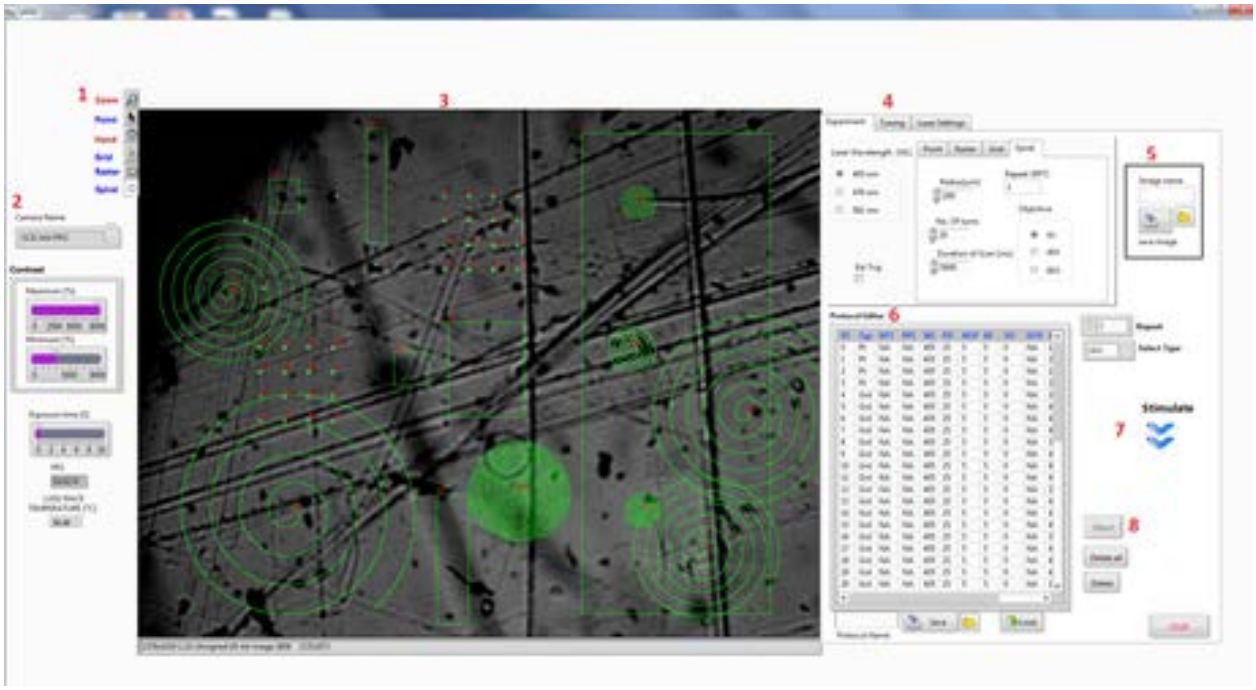
- SciCam pro
- Ueye
- Orca flash 4 cameras.
- Saved image

Select the camera you are using, then click confirm. Depending on the camera selected the available adjustment settings will be pre-loaded in the GUI that opens afterwards.



3. GUI walk through

After selecting the camera type, the main window will be displayed. For a quick reference, numbers on the screenshot have been added for each group of controls, which we describe below.



Label	Name	Function
1	*Tool bar menu*	Allows selection of a tool to perform actions on the image: ▪ ***Zoom tool:*** Zoom in/out. To zoom in click the left mouse button. To zoom out press control while clicking on the left mouse button within the live camera feed. ▪ ***Hand tool:*** *S*croll the image when the zoom in is greater than 2x. ▪ ***Point tool:*** Select points for free hand sequential stimulation. ▪ ***Raster tool:*** Mark a ROI in the live camera feed. The parameters for this tool can be defined on the raster tab. See #16. ▪ ***Grid tool:*** Select the location where the grid will be generated. The parameters for this tool are defined on the grid tab. See #16.
2	*Camera panel*	Panel displaying camera settings.

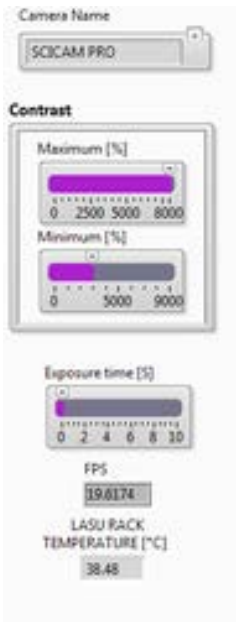
3	*Image display*	The image displayed will be live or static depending on the camera selection. A cross will indicate the position of a photostimulation point while a rectangle will mark the position of raster photostimulation.
4	*Basic settings*	Contain tabs to set the parameters of the basic functions of the LASU software: ▪ Experiment ▪ Tuning ▪ Laser
5	*Image panel*	Set parameters to save an image of the display, with and without the ROI.
6	**Protocol Editor**	After marking the Stim points, raster or grid on the display window, the data will be displayed on the protocol editor. The parameters that are not valid for a specific ROI "Type" are displayed as not applicable "NA".
7	*Stimulate*	This button allows the user to start the stimulation protocol, as displayed in the protocol editor.
8	*Abort*	The photostimulation process can be cancelled at any time by pressing this button.

4. LASU software functionalities

4.1. Camera panel

This panel contains information about the camera as well as allowing access to the different settings to adjust the visualization.

The panel interface depends on the camera selected:

<table>
<tr><th colspan="2">SciCam Pro</th><th colspan="2">U-EYE</th></tr>
<tr>
<td> 
</td>
<td>

Camera Name: Displays the selected camera name.
Contrast: Sliders allow the user to set the maximum and minimum parameters for adjusting the illumination conditions of the sample.
Exposure Time [S]: The user can employ the slider to adjust the camera for the sample emission conditions

</td>
<td>  </td>
<td>

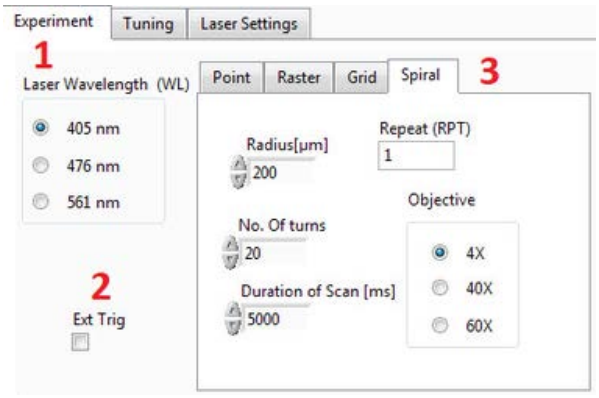
Camera Name: Displays the selected camera name.
Contrast: Sliders allow the user to set the maximum and minimum parameters for adjusting the illumination conditions of the sample.
Exposure Time [s]: The user can employ the slider to adjust the camera for the sample emission conditions
Indicator of the frame rate (FPS)
Pixel clock
Gain

</td>
</tr>
</table>

4.2. Basic settings

4.2.1. Experiment

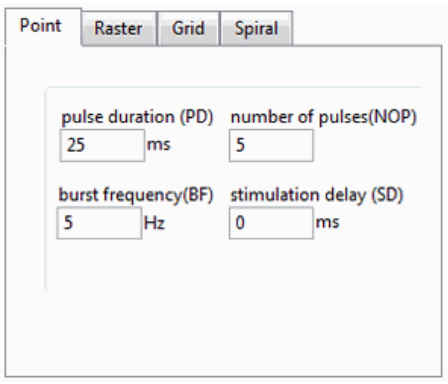
This tab allowing the design of the experiments

	Label	Name	Function
	1	Laser Selector	Select the laser needed for the stimulation protocol
	2	Ext. Trig	Selector of external triggering
	3	Protocol selector	Tab allowing to select the type of stimulation protocol • Point • Raster • Grid • Spiral

There are three photostimulation methods. They can be used independently or combined in a protocol.

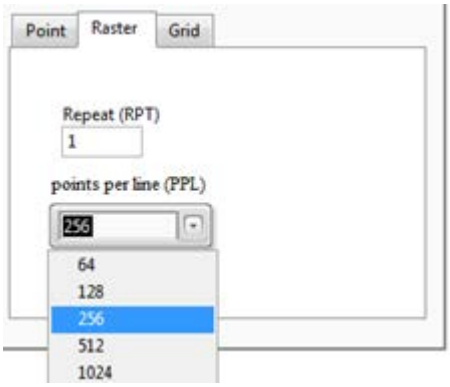
a. Points stimulation:

One can define the parameters for the point stimulation i.e. pulse duration [pd], number of pulses [NOP] per location, burst frequency [BF] and stimulation delay [SD] between points.

	For each point selected on the live image, one can adjust: • Pulse duration (ms) • Number of pulses • Burst frequency (Hz) • Stimulation delay (ms)

b. Raster

Raster scan module generates a waveform so that the laser can be swept across the selected region of interest. The user can select the number of points and lines which are linked with the maximum/minimum speed achieved by the Galvos. For example, 64 points/line will achieve faster sweep rates than 1024 points/line as they are more sparsely distributed within the selected region of interest.

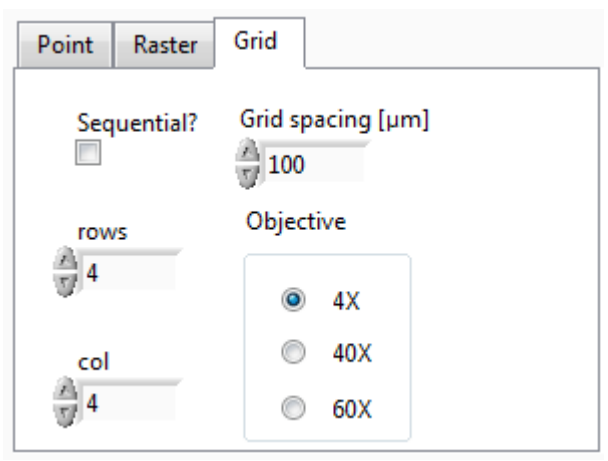
	One can select here: • Number of repeats • Number of points per line

c. Grid

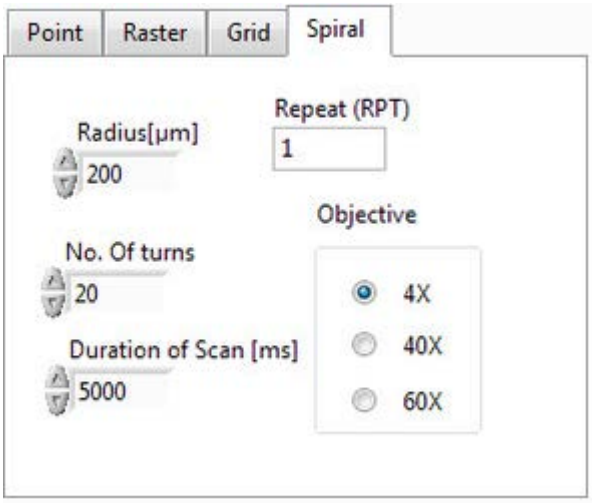
Before generating the grid on the live image camera feed the user will be required to specify the parameters for the experiment.

The number of rows and columns, the order of the points to be stimulated (Sequential/Random) and the spacing between poststimulation locations need to be selected. Please note that the system has been calibrated for the camera pixel size and Olympus LUMPLANFLN 4X 0.13NA, 40X 0.8NA and 60X 1.0NA which is linked to grid spacing control in microns.

Rows and columns are limited to 10*10 for performance reasons.

	For each grid on the live image, one can adjust: • Stimulation order (Sequential or Random) • Number of points in rows and columns • Grid spacing (um) • Objective

d. Spiral:

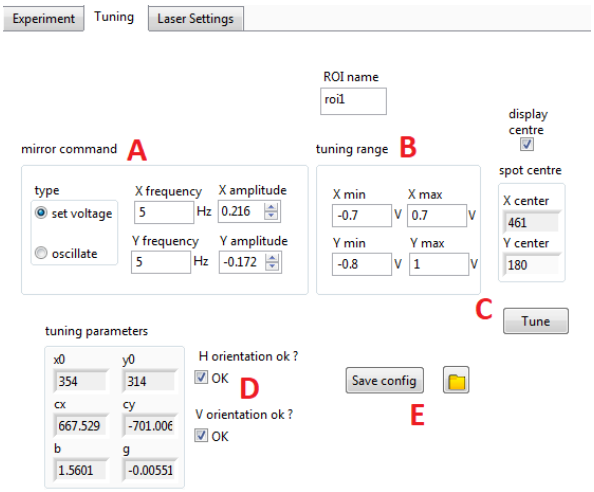


Before generating a spiral on the live image camera feed the user will be required to specify the following parameters for the experiment:

- Radius (um)
- Number of turns
- Duration of Scan (ms)
- Repeats

Please note that the system has been calibrated for the camera pixel size and Olympus LUMPLANFLN 4X 0.13NA, 40X 0.8NA and 60X 1.0NA which is linked to grid spacing control in microns.

4.2.2. Tuning



The tuning procedure allows mapping of the camera field of view with the voltage ranges of the X and Y galvo mirrors.

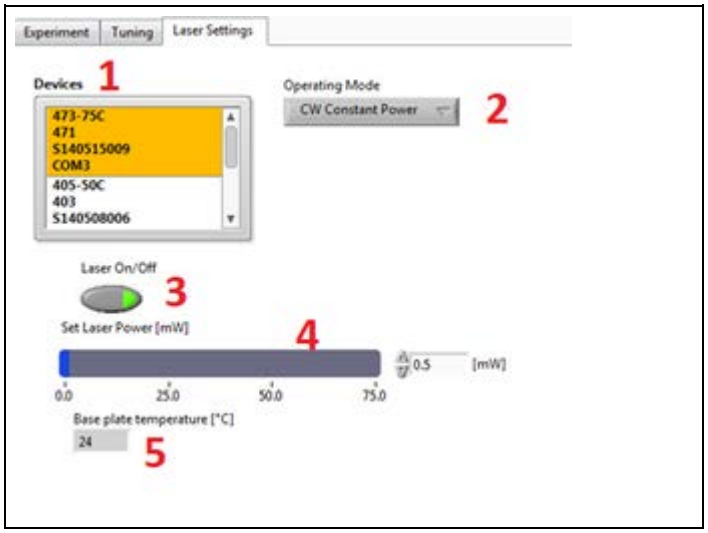
A. Mirror commands to drive the galvos
B. Voltage range of the mirrors
C. Tuning button
D. Adjustment of the axis direction
E. Save configuration

Ensure that your transmitted illumination source is switched off during the calibration process. With the fluorescent slide in place, the fluorescent spot should be visible in the centre of the camera image. If it is hard to see the laser spot you will need to adjust how the camera sees the fluorescent spot by adjusting the pixel clock, exposure time and master gain settings for the camera.

Select the Tuning tab within the LASU software. A green ring will appear around the centre of the fluorescent spot. Select Tune to move the laser through a series of movements. This process will take less than a minute. Make sure that the green ring follows the centre of the laser spot as it moves through these steps. Once the tuning process is complete, verify

correct tuning has occurred by selecting the Experiment tab and clicking on a few points on the camera image to select some stimulation points. It is recommended to select points within different areas of the image i.e. the four corners. Select Stimulate and check that the spot moves correctly to the selected stimulation points. If the spot moves accurately to the selected points, return to the Tuning tab and save the settings. If the laser spot moves in a mirror image of the selected spots, return to the Tuning tab and tick/untick the H (horizontal) and V (vertical) orientation boxes. After this, re-run the tuning process until the spot moves correctly, then save the configuration within the Tuning tab. If asked, press Continue Evaluation. If the laser spot does not move during the process the camera may need to be realigned. This will show on the Tuning tab as an X and Y amplitude of NaN. If this occurs, please re-complete the camera calibration procedure.

4.2.3. Laser Settings

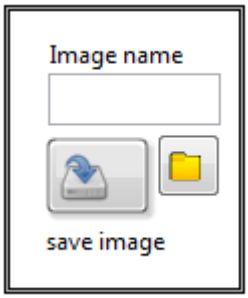


Label	Name	Function
1	**Devices**	Select the laser you want to adjust. The list includes all lasers of the system and the associated parameters. There could be up to 3 different lasers on LASU hardware • Laser model • Emission wavelength • Serial number • COM port used to communicate with the workstation

2	**Operating Mode**	Allows selection of the operation mode • CW for constant power (used for calibration) • Pulsed digital for performing an experiment
3	**Laser On/Off**	Switch to turn the laser On or Off
4	**Set Laser Power**	Slider to adjust the laser power
5	**Base plate temperature**	Indicator displaying the current base plate temperature

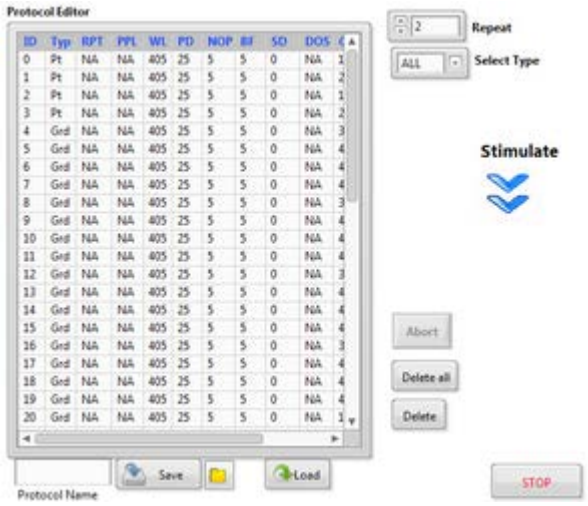
4.2.4. Saving Image

One can specify a file name of the image to be saved. By clicking the save image icon, the software saves the image with and without ROI's adding a timestamp. The image can be accessed by clicking the folder icon.

	• Image name • Save Image (the software saves the image with and without ROI's adding a time-stamp) • Access to the folder where the image is saved

4.2.5. Protocol Editor

Once the stimulation (Points, Raster or Grid) have been set up on the display window, the data will be displayed on the protocol editor.



The table contains all parameters for each point including:

- **ID** - Point number in the sequence
- **Type** - Pt, Squ or Grd (Point, Raster or Grid)
- **RPT** - Number of repetitions (Raster)
- **PPL** - Points Per Line (Raster)
- **WL** - Laser to use
- **PD** - Pulse duration (Point or Grid mode)
- **NOP** - Number of pulses (Point or Grid mode)
- **BF** - Burst Frequency (Point and Grid)
- **SD** - Stimulation delay (ms)

Some parameters are not applicable for certain ROI types. In this case, the value displayed is NA (Not Applicable).

It is possible to edit the parameters for each photostimulation point by selecting and entering a new value in the protocol editor directly. Please note that in case of a syntax error, the software will automatically mark the inadequate value in red and disable the stimulate button. Once the parameter has been set to a correct value the protocol can be executed.

Label	Name	Function
1	**Repeat**	This control box allows the photostimulation protocol to repeated the specified number of times.
2	**Select Type**	One can select a type of ROI to execute in the protocol (i.e. Point, Grid, Raster).
3	**Stimulate**	Allows the stimulation process to be started.
4	**Delete all and Delete**	Allows all of the ROIs to be deleted at once. Select one or more ROI's (using the CTRL key) and click the delete button to delete a selection of ROIs.

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