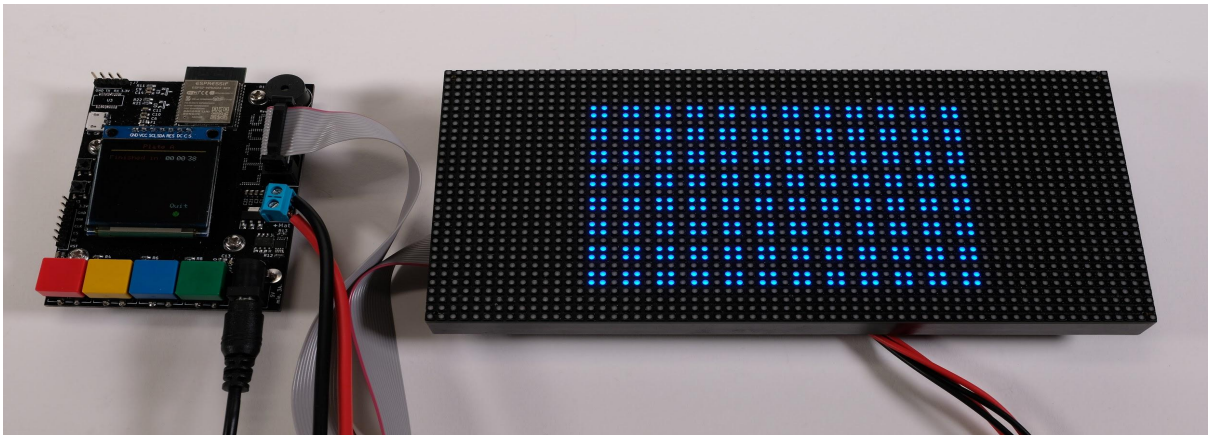
LITOS

The **L**ed **I**llumination **T**ool for **O**ptogenetic **S**timulation (LITOS) can be used for the stimulation of cells containing an optogenetic system. A commercially available 32x64 RGB LED matrix is used to illuminate cells placed in various microplate formats (6 well up to 96 wells), petri dishes or T75 flasks. The control unit for the LED matrix is a custom-built printed circuit board (PCB) containing a screen which shows relevant information during the illumination process.

During LITOS development, we focused heavily on user-friendliness. LITOS can be ordered pre-built, thus no complicated assembly process is required. Individual stimulation patterns can be first created in a spreadsheet application, like Microsoft Excel, then they will be loaded to LITOS over an easy-to-use interface, accessible as a website hosted on the device itself. Thus, no knowledge of a programming language is needed, and the device can even be operated by nontechnical orientated users.



You can find our publication here:

How to get one?

Order the material

LITOS (PCB manufacturer)
RGB LED Matrix 32x64 3 mm , from aliexpress
Power adapter (>=3A, 5V), 2.54mm
Micro USB cable for initial flashing (same cable as for old Smartphones)

Optional
To connect LITOS (the PCB) with the LED matrix, a 16-pin flat ribbon cable and a power cable are needed. Often those are included in the delivery of the 32x64 RGB LED matrix. However, to place LITOS, as recommended, outside the incubator (with the LED matrix inside the incubator), a longer flat ribbon cable and power cable must be purchased (about 1m).

Longer Power cable
Flat ribbon cable

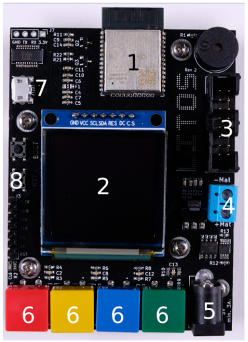
To place LITOS on a metallic surface, PCB spacers which include little magnets, can be used. The corresponding screws (4x M3, 6 mm) must be purchased separately.

LITOS - user manual

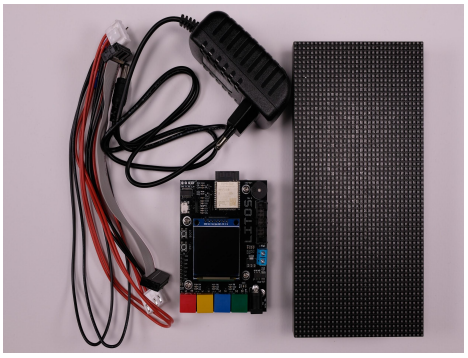
Important notes

Due to the high humidity, we recommend placing only the LED matrix with the microplates inside the incubator. LITOS's PCB with the wall power adapter should be placed outside. In order to buy the needed longer flat ribbon and power cable to connect LITOS with the LED matrix, please consult the ordering section on www.pertzlab.net/litos-ordering.

First steps: Setting LITOS up



1) ESP32 (Wi-FI)
2) OLED Display
3) Data conection to matrix
4) Power conection to matrix
5) Power conection of the PCB
6) Buttons
7) USB conection
8) Reset



1) LED matrix
2) PCB
3) Power adapter
4) Flat ribbon cable
5) Power cable

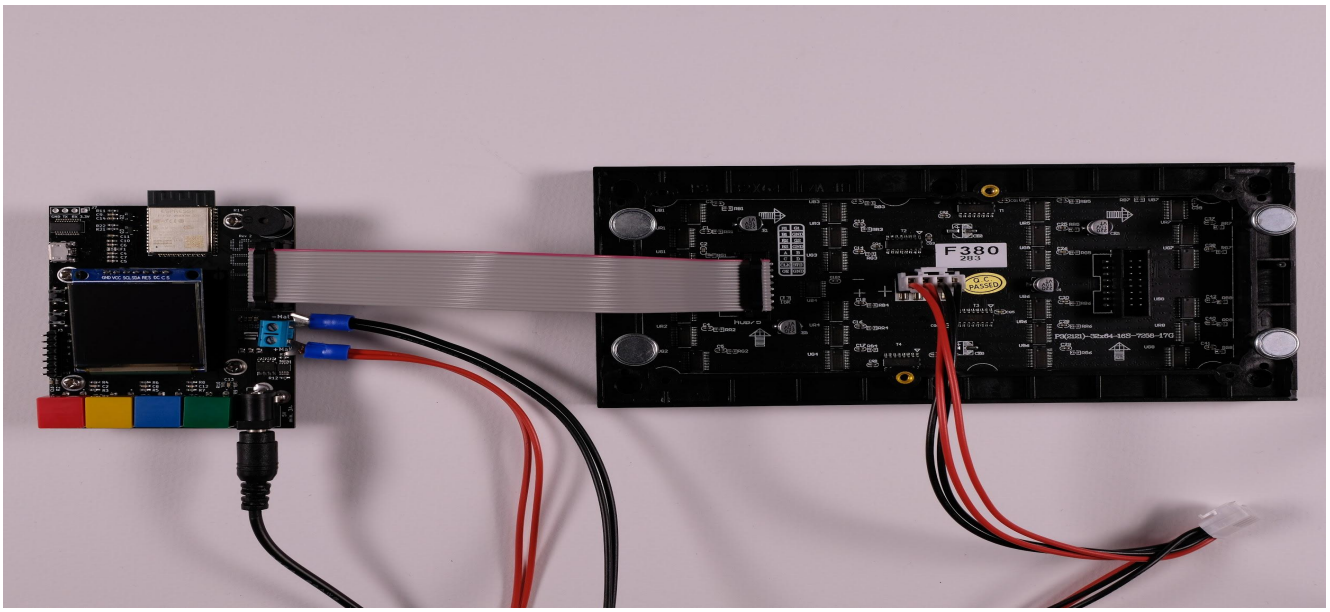
The LITOS PCB can be ordered pre-assembled. However, before the first use, you need to load the LITOS software on the PCB and connect the PCB with the LED matrix:

1. In rare cases the display might not be already attached to PCB. In this event, please **plug the display** into the headers in the middle of the PCB.
2. Connect LITOS to a computer with a micro USB cable (not included, this is the same cable as you may have used some smartphones). Then run the "litos_flash.exe" script and follow the on-screen instructions to **flash LITOS**. After the flashing process, please remove the USB cable.
3. **Connect the flat ribbon cable (16 pins)** to LITOS. On the LED matrix there are two connectors for the flat ribbon cable. Please connect the cable to the input connector. It is

located on the left side if the arrows on the back of the LED matrix point to the right and/or to the top.

4. Next, **connect the power cable** to the matrix and the PCB (cable with red / black wire). In order to insert it into the screw terminal on the PCB, we recommend that you cut off the metal fork at the end of the cable and then strip the two wires a bit. Alternative you can also bend the metal fork teeth. The red cable end belongs to “+Mat“, whereas the black cable end is inserted into the side labelled with “-Mat” on the screw terminal.

5. Start LITOS by connecting it with the wall power adapter.
6. Connect your notebook to the access point LITOS has created (like if you would connect to a W-Lan network). The name of the access point (SSID) is displayed on LITO’s screen (default name is “LITOS”). Open next a web browser (Firefox, Google Chrome, Safari, Edge) and enter 192.168.1.1 in the address bar to access the user interface. If you have problems establishing a connection to LITOS’s web interface, please restart LITOS by pressing the RST (Reset) button on the PCB.
7. LITOS can be customized on the setting page in the user interface.



Setting page

Connection settings

LITOS configuration website is either accessible over the created access point (AP mode) or over a configured W-Lan network (WPA or eduroam mode). WPA mode can be used for all networks which use the default authentication over a network name and a key (WPA2 passphrase). To connect to LITOS in WPA or eduroam mode, the displayed IP address (on LITOS’s screen) can be entered in your web browser when you are in the same network. Please pay attention that the support for eduroam networks (WPA enterprise, authentication over username and password) is still experimental and may not work depending on your university.

When LITOS is set to WPA or eduroam mode, but a connection to the desired network can not be established, AP mode will be activated after 30 s.
Depending on the connection mode, there are different settings that can be modified. For example, when LITOS is in AP mode, the name of the access point (AP SSID) can be changed (important when multiple LITOS are used simultaneously). Additionally, the access point can be optionally secured with a password.

Matrix offset

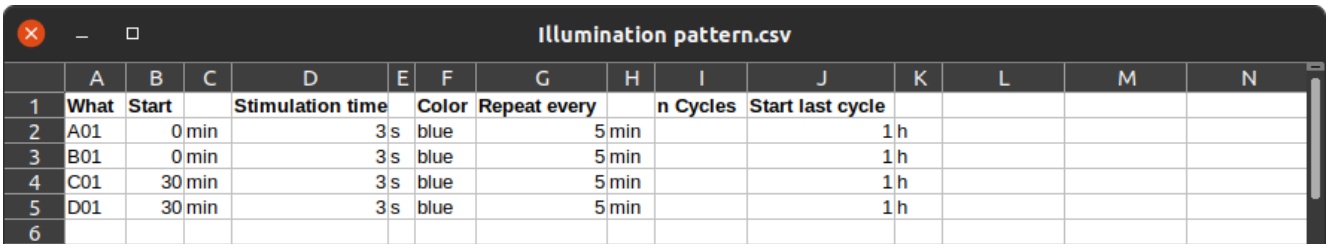
In some rare cases the illumination on the LED matrix is off by one or multiple LEDs. In other words, when the LED with the coordinates 1,1 should be illuminated, the LED with the coordinates 2,3 is powered on. With the x / y offset this can be corrected, thus in this case the x offset would be -1 and the y offset -2.

Upload and run stimulation pattern

Stimulation patterns, describing which well should be illuminated at which time point during the experiment, are stored in a pattern file. These files are comma separated value files (.csv), where one row represents an illumination pattern. With the help of the provided template, the patterns can easily be created in any spreadsheet application, like Microsoft Excel or LibreOffice Calc.
Besides storing the stimulation patterns, the pattern file can also contain custom messages which will be shown on LITOS's screen during the run of the program.
After an upload on the LITOS configuration webpage, they can be loaded and started with the press of a button. LITOS can save up to 15 different files, so there is no need to always re-upload frequently used patterns.

Designing stimulation patterns

The pattern file consists of seven main columns with four helping columns providing the corresponding units.


Illumination pattern.csv

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1	**What**	**Start**		**Stimulation time**		**Color**	**Repeat every**		**n Cycles**	**Start last cycle**				
2	A01	0	min	3	s	blue	5	min		1	h			
3	B01	0	min	3	s	blue	5	min		1	h			
4	C01	30	min	3	s	blue	5	min		1	h			
5	D01	30	min	3	s	blue	5	min		1	h			
6														

Columns:

A: The **What** column describes which part of the microtiter plate will be illuminated. Different keywords can thereby be used:

What	**Format**	**Example**	**Description**
Well	*Letter followed by Number*	B02 or B2	Well "B02"
Row	*Letter*	B	Entire row "B"
Column	*Number*	2	Entire column "2"
Matrix	Whole or W	Whole or W	Whole LED matrix
Microplate	Plate or P	Plate or P	All wells of the given microplate
LED	LED_*x_y*	LED_02_15	LED with the coordinates x = 02 and y = 15
Circle	O_*x_y_size*	O_02_15_03	Circle at x/y coordinates 02/15 and a radius of 3
Rectangle	Rec_*start_end*	Rec_02_05_ 10_15	Rectangle from coordinates 02/05 to 10/15

Messages	M_*Message*	M_Add Drug	Shows the message “Add Drug” and a countdown on the display. The duration of the countdown specified by the entry in the “stimulation time” column. With the time present in the “Start” column its endpoint is determined. Note: *A maximum of 10 messages per pattern can be stored. Further only two countdowns can be displayed at the same time. One minute after the end of a countdown, the corresponding message will be removed from the screen.*

B: The **Start** column determines the time of the (first) illumination cycle. 0 means that the illumination for this entry starts at the beginning, thus when the whole pattern is started. Other values, e.g. 10 (with s in C), indicate that the illumination (or the first cycle) is delayed by x (for this example 10s) by the rest of the illumination pattern.

C: Unit column for the start column. Indicate the temporal unit of the value present in the start column (B). Possible values are seconds (s, sec, second), **minutes** (m, min, minute, minutes), hours (h, hour, hours) and days (d, day, days). Milliseconds are not possible since LITOS only supports seconds as a minimal time unit.

D: The column **Stimulation time** defines the exposure time of a well. In other words, **the** entered number describes how long a well will be illuminated. When cycles are programmed (see below), it represents the exposure time during one illumination cycle.

E: See C
Warning*: Long sustained stimulation may lead to a heating up of the LED matrix.*

F: The illumination color is defined by a keyword or an RGB code placed in the **Color** column. Possible keywords are white, red, green, blue, yellow, pink, white and black. Black thereby means that no illumination takes place, this can be used in situations where it is desired to artificially extend the experiment duration for an incubation step after the last stimulation.
The RGB code consists of three values between 0 and 255, separated by a white space or a dash (“/”). The first of the three values represent the red part of the color, the second the green and the last value the blue part. By choosing RGB values below 255, it is possible to regulate the brightness of the LEDs, e.g. “0 0 120” (or “0/0/120”) corresponds to a faint blue.

By using the last three columns, with their corresponding units columns, loops can be generated. Thereby long and complex experiments with repeating stimulation patterns can be easily performed.

G: The column **Repeat every** defines the length of a loop. For example, a value of 1 h (the “h” must be set in column H) leads to the repetition of the illumination pattern (as defined in the other columns) once per hour.
Note: The time of the stimulation must be shorter than the time in this column.

H: Unit column for the “Repeat every” column. Indicates the temporal unit of the Repeat every column. As for the other unit columns, possible values are seconds (s, sec, second), minutes (m, min, minute, minutes), hours (h, hour, hours) and days (d, day, days).

I: The column **n Cycles** defines the number of repeating cycles, thus how many times the cycle is repeated. A value of 10 means that the stimulation is done 10 times in total, while the value 1 would have the same effect as leaving the last three columns empty. By providing a defined amount of repeating cycles, the following column, “Start last cycle”, must remain empty.

J: In the **Start last cycle** column the time, at which the last cycle of a repeating pattern should start, is specified. Taking as an example an entry with a Stimulation time equals to 10 s, “Repeated every” equals 1 hour, and “Start last cycle column” equals 6 hours. In this case, there is a 10 s stimulation every hour, and there is a 10 s stimulation every hour, and the last stimulation would start 6 hours after the beginning of the experiments. The experiment would be finished after 6 hours and 10 seconds. Please pay attention that the *Start last cycle* column can only be used, when the previous column, n cycle, does not contain any value.

K: Unit column for the “Start last cycle” column. For more information, please refer to H.

Uploading of patterns / starting experiments

1. Connect yourself to your LITOS device. Depending on the selected connection mode, the needed information is shown on LITOS’s screen located on the PCB.
    a. Access Point mode (AP mode): The name of the access point can be found on the screen. Connect your Wi-Fi capable device (notebook, smartphone) to LITOS’s access point and use a web browser to open the user interface located at 192.168.1.1
    b. WPA mode / eduroam mode: First ensure that your Wi-Fi capable device is in the same network as LITOS. Then enter the IP address shown on LITOS’s screen in the address bar of your web browser.
2. On LITOS’s user interface go to the “File manager” section. On this site, the patterns currently saved in LITOS’s internal storage are displayed. Additionally, it is possible to upload new patterns from your computer or smartphone.
   When uploading new patterns, please remember that the maximal lengths of file names cannot be longer than 30 characters.
3. To change the current pattern, go to “Load configuration”. You can either choose to illuminate a single plate (center / corner) by selecting “One Microplate” or two plates (with the help of the optional mask) by choosing “Two Microplates”. Please remember, that when you illuminate two plates at once, the first and the last column cannot be illuminated.

    a. One Microplate: Choose a stimulation pattern and state which type of Microplate you are using. In the same menu you can specify if you either place the microplate in the middle of the LED matrix (using the mask or the outline lights) or at the upper left corner of the plate.
    b. With the option “Define illumination range” certain rows and/or columns can be excluded from illumination if whole rows and / or columns are defined for illumination. This means, if for example in the pattern row B is set for illumination and the illumination range is defined from 1 to 11, Well B12 will not be illuminated. By using this option, control groups in which rows/columns are not illuminated, can be easily

created.
Note: If a well is specifically defined in a new row (e.g. B12) it will be still illuminated even if it is outside the illuminations range.

4. Click upload and follow the instructions on the display of LITOS (start of the pattern with a press on the appropriate button).
   Note: It is recommended to restart LITOS (by pressing the restart button) after loading a pattern (especially for long patterns with a lot of entries).

Note: When using patterns only consisting of circles, rectangles or pixels, every microplate can be chosen.
Note: It is not recommended to upload new patterns if a program is currently running on LITOS. Also we suggest that you avoid accessing LITOS's configuration interface when a pattern is running.

Downloads

Program code

You can find the code of LITOS on GitHub.

Template for illumination pattern

Demo1.csv
Demo2.csv

Masks for easier alignment

For the proper alignment of the microtiter plate on the LED matrix the blueprints (for building them with a 3D printer or a CNC machine) for two different masks (one / two microplates) are provided. The mask for one microplate allows its placement in the middle of the LED matrix, whereas the mask for two plates stocks them horizontally. Please note that when using the mask for two plates, the first and last row cannot be illuminated.

Mask for one microtiter plate
Blueprints for CNC machine or for building manually. file (open e.g. with LibreCAD)
Files for a 3D printer:

Mask for two microtiter plates
Blueprints for CNC machine or for building manually. file (open e.g. with LibreCAD)
Files for a 3D printer: