Supplementary Information for

The OptoGenBox - a device for long-term optogenetics in *C. elegans*

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The set-up of the OptoGenBox

Thermal control of cells

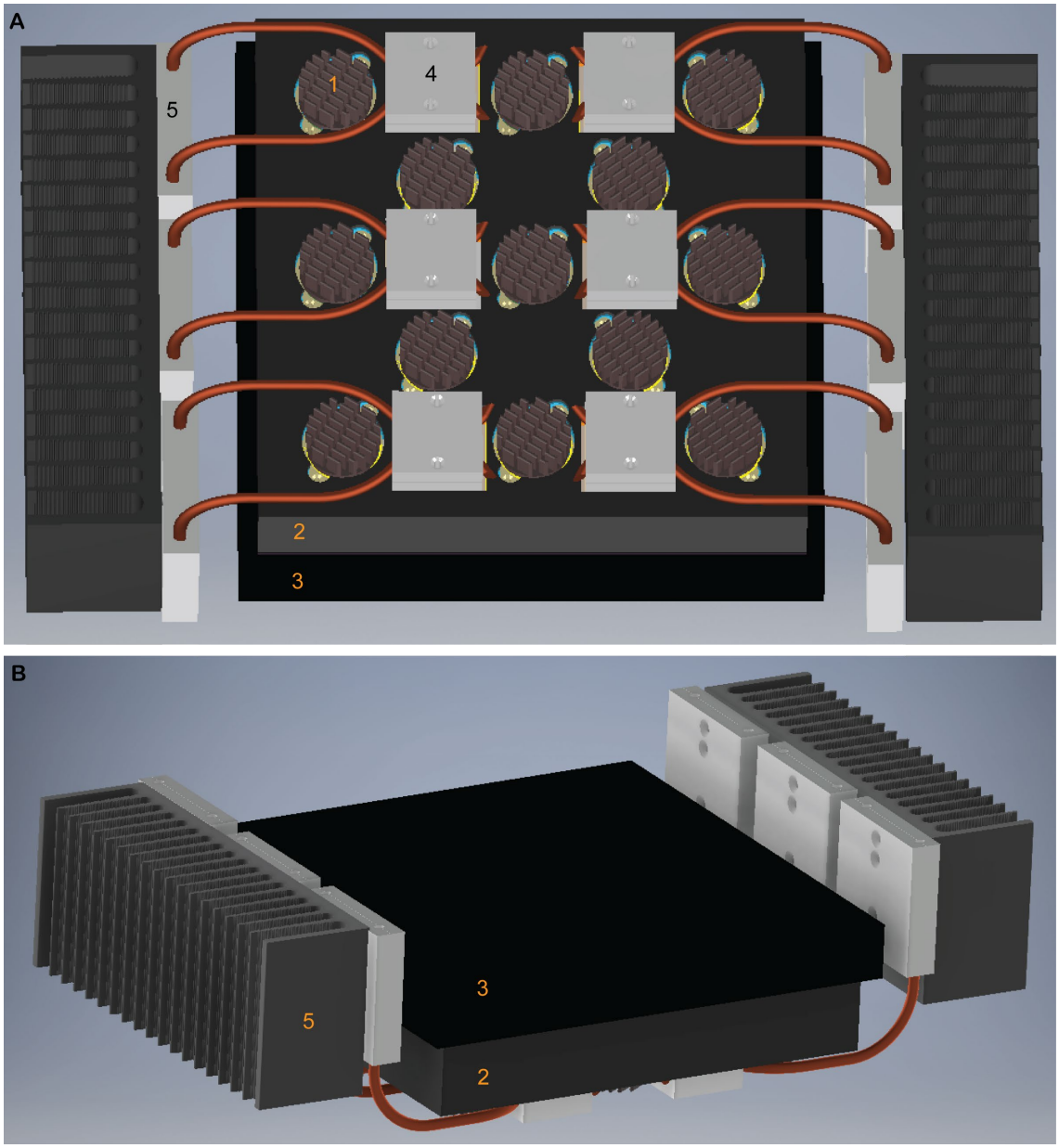


Figure S1. The incubator.

Bottom (A) and top side (B) view of the incubator. The (1) 13 LED modules are spread throughout (2) an incubator chamber and covered by (3) a lid. Six Peltier devices are covered with (4) heat spreaders. On the site are (5) heat sinks.

Software of the OptoGenBox

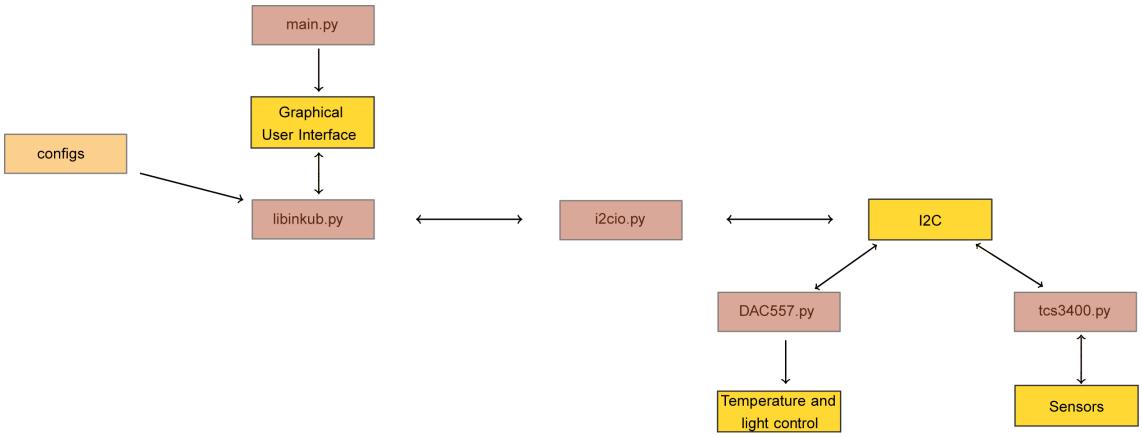


Figure S2. Scheme for the code of the OptoGenBox.

The software was written in python. The python library i2cio reads and writes commands via the device I²C bus. The python library tcs3400 controls the lightsensor TCS3400. The digital-to-analogue converter DAC5571 is needed for the temperature control and controlled by the DAC5571 python library. The main library starts the graphical user interface. The libinkub library manages the abstraction of cells and group of cells, light cycles and sensor readings. The configs file hosts all configurations for the OptoGenBox such as the users, the temperature and light intensity steps and how thoroughly the sensors get calibrated (how many calibration points get initiated). The code is published on github (https://gitlab.gwdg.de/psapir/inkubator).

Graphical User Interface

The OptoGenBox program starts by running the main script in the inkub directory in the terminal with the command lines:

```
cd inkub

./main.py
```

The program initiates by calibrating the environmental sensors. This might take a few minutes.

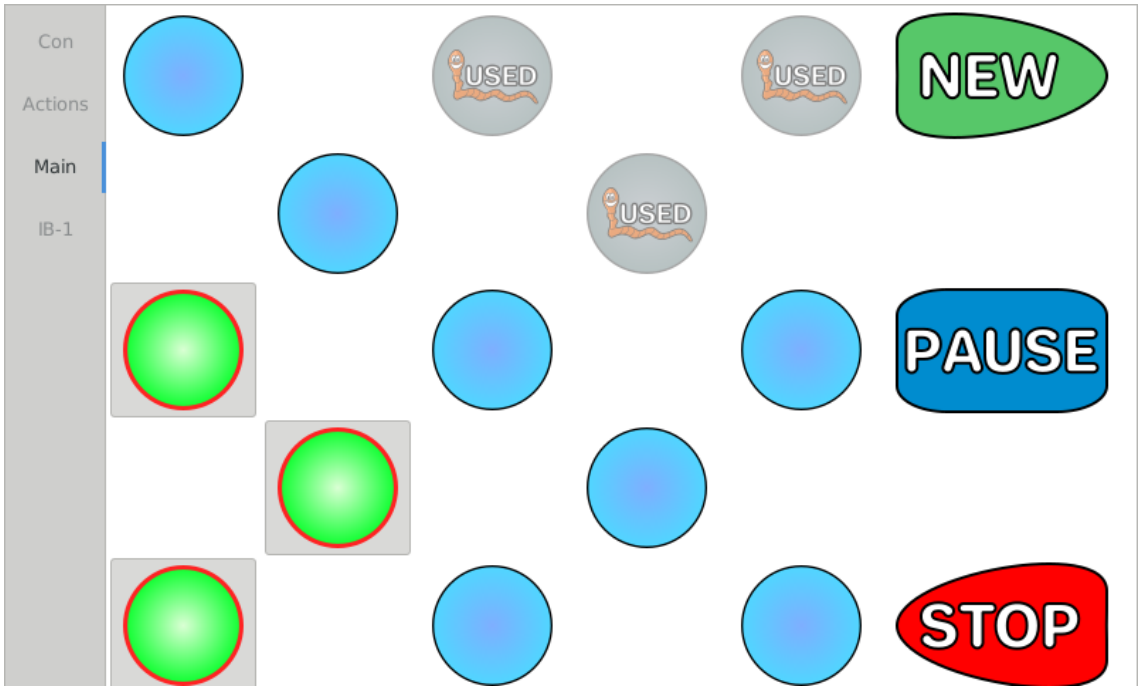


Figure S3. Main menu.

The graphical user interface allows for an intuitive usage of the OptoGenBox. The researcher first selects the cells for one experiment via the touch screen in the main menu and presses *NEW*. Once an optogenetic protocol (light and dark cycles) is defined for selected cells, these cells are displayed as used in the main menu.

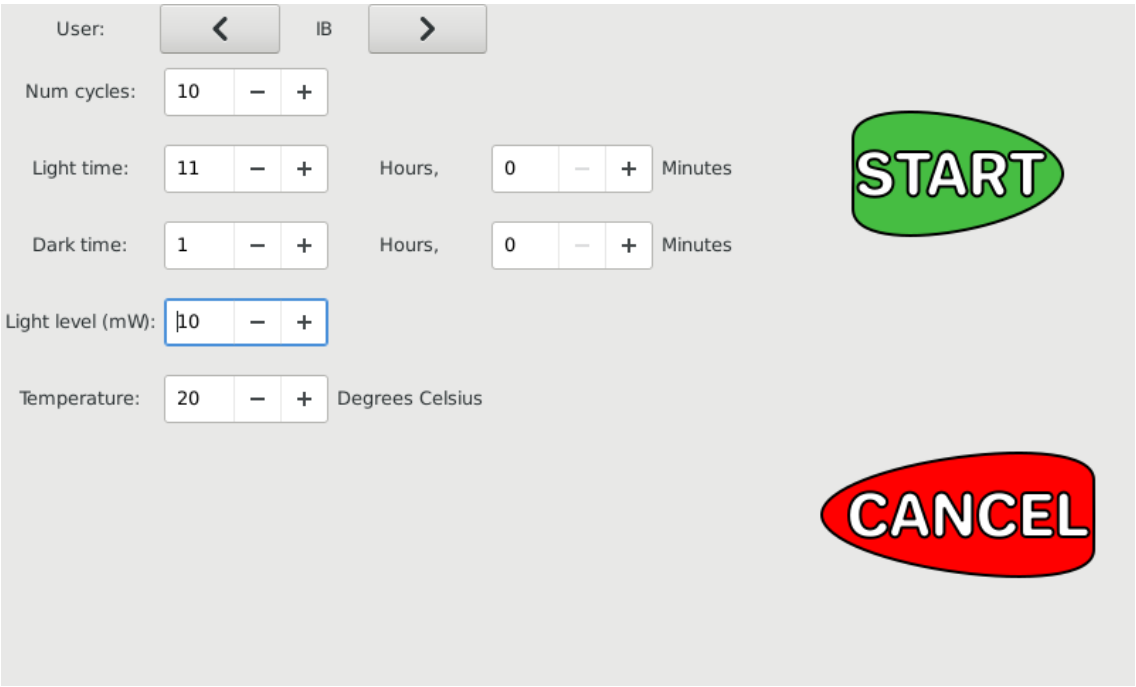


Figure S4. Optogenetic Protocol.

A new window opens and the researcher can select the user and the number of cycles. Furthermore, the researcher can define the time of light and darkness and the level of light. The temperature of the entire incubator can be chosen whenever no other experiments are running. Once all parameters are defined, the protocol gets started by pressing *START* on the touch screen.

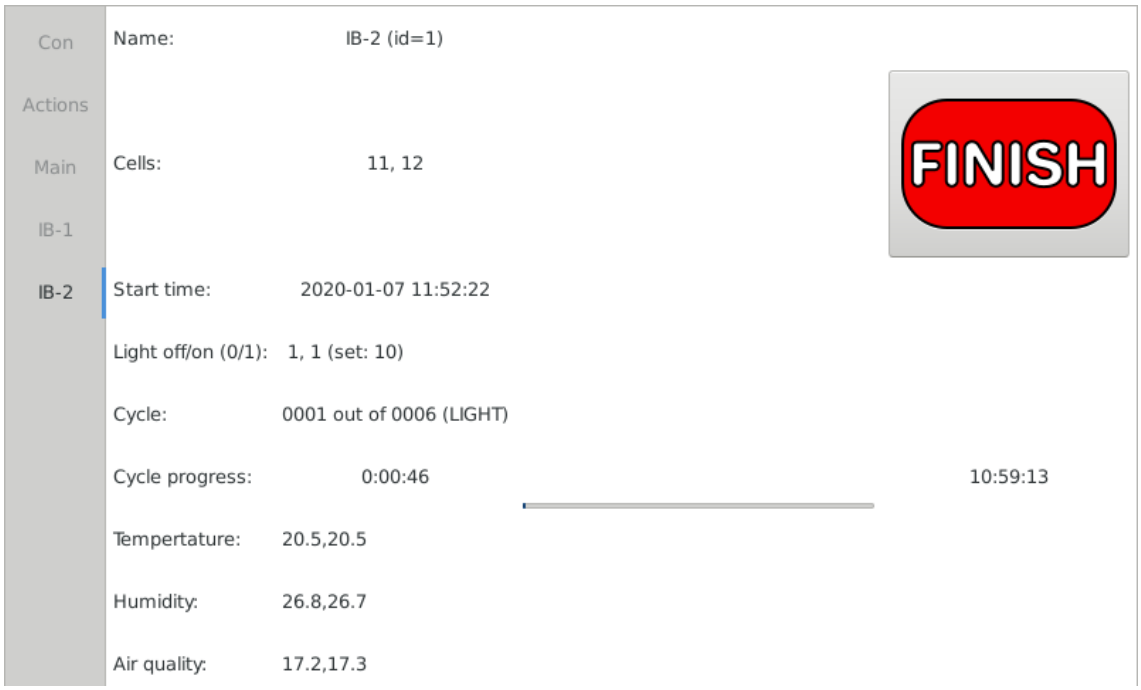
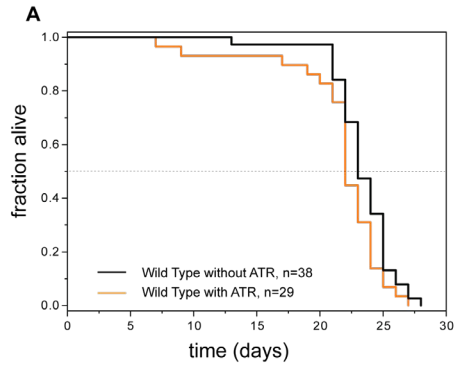


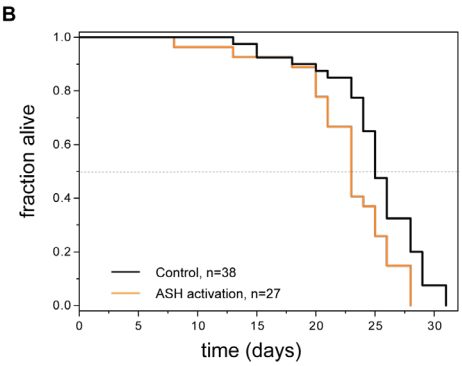
Figure S5. Status of the running protocol.

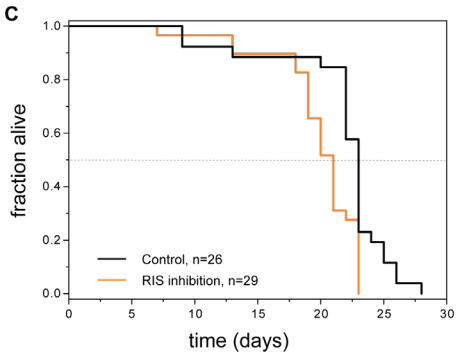
Running protocols are listed in the left panel below *Main.* To check for the current status of the protocol, one can select it via the touch screen. Information about the name of the experiment, the selected cells and the start time is portrayed. Furthermore, one can see if the light is on or off for each cell and what intensity is set. The next line gives the number of the current cycle. Below that one can see the exact timing in the current cycle. The measured temperature, humidity and air quality is additionally given for each cell. To prematurely quit the experiment one can click the *FINISH* button.

Figure S6. Finishing a running experiment.

Clicking the *FINISH* button on the running protocol screen does not automatically end the experiment. The user has to confirm again that the protocol should be stopped.







D

comparison	wild type ATR(+) vs. wild type ATR(-)	control vs. ASH activation	control vs. RIS inhibition
Figure panel	A	B	C
day 10	0.189	0.409	0.5908
day 17	0.5779	1	1
day 24	0.1335	0.0044	0.0069
day 26	0.453	0.0461	0.09

Figure S7. Second batch of lifespan experiments.

A) All-trans retinal (ATR) did not affect survival of wild-type *C. elegans* in L1 arrest.

B) ASH activation causes a reduction in lifespan. compared to control animals without the addition of ATR.

C) RIS inhibition causes a reduction in lifespan compared to control animals without the addition of ATR.

D) p-values of a statistical analysis of lifespans from A-C. Fisher's Exact Test was conducted at different time points. The p-value of dark green shaded time points is below 0.05 and therefore statistically significant. The p-value of light green shaded time points is above 0.05 but below 0.1 and hence not statistically significant but close to significance. ASH activation and RIS inhibition causes a significantly decreased lifespan in the later phases of the lifespan.

Figure S8. Phobya WaCoolT Cube 2 Watercase was modified to serve as external case for the OptoGenBox. This figure was reproduced with permission from Performance PCs.

description	manufacturer	manufacturer number	#	price, total $
mechanical parts				
case	Phobya	WaCool IT 2	1	$146
heat pipes	QuickCool	QY-SHP-D6-250SA	12	$127
heat sink	Fischer elektronics	SK580 200 SA	2	$78
dust filter	InLine	33378A	3	$7
LED-mounting Ring	MPI-bpc ES	inhouse design	13	$130
mounting material		miscellaneous		$336
material: percision mechanics & optics		inhouse design		$538
computer elements and accessories				
raspberry pi	Raspberry Pi	Raspberry Pi 2 Model B	1	$22
touchscreen	Raspberry Pi	7“, 800x400Pixel	1	$78
usb connectors		miscellaneous	2	$34
RJ-45 connector	Harting	Harting: 09454521561	1	$17
fan 80mm	Be quiet	BQT BL044	5	$34
fan 120mm	Be quiet	BQT BL046	4	$32
power sources				
Power Adapter AC/DC 330W	Mean Well	HRPG-300-15	2	$203
Power Adapter AC/DC 200W	Mean Well	SP-240-24	1	$63
Power Adapter DC/DC	Traco Power	TEL3-2022	1	$29
Power Apdater DC/DC	Traco Power	TSR3-24150	1	$38
Power Adapter DC/DC	TDK Lambda	I6A-240-14A-033V/001	2	$105
PCB (supply) & small parts	MPI-bpc ES	inhouse design	1	$61
LED module				
PCB	MPI-bpc ES	inhouse design	13	$131
LED: LCY-CLBP	Osram opto Semicondutcors	LCY-CLBP KXKZ-5F5G	78	$131
connectors	ERNI	Erni MiniBridge,2-pin	13	$22
heatsink	Fischer elektronics	ICK_LED R33x16,5G	13	$121
LED-Control-Unit				
PCB	MPI-bpc ES	inhouse design	2	$157
Controller IC [I²C]	NXP Semiconductors	PCA9685	4	$9

constant current source	ON Semiconductors	CAT4101	14	$39
other electronical parts		miscellaneous	158	$49
Heating & cooling				
Peltier devices 100W	True Components	HP-127120-40x40	6	$134
Power Amplifiers	Texas Instruments	OPA541AP	3	$61
PCB	MPI-bpc ES	inhouse design	1	$67
temperature sensor	RS Pro	PT100, 8x2mm	1	$11
other electronical parts		miscellaneous	38	$56
Main control-unit				
PCB control	MPI-bpc ES	inhouse design	1	$78
other electronical parts		miscellaneous	85	$45
Lid-&Sensor Unit				
PCB	MPI-bpc ES	inhouse design	1	$146
Light-Sensors	AMS	TCS3400	13	$36
Environment Sensors	Bosch Sensortec	BME680	13	$110
Multiplexer [I²C]	NXP Semiconductors	PCA9548A	2	$3
other electronical parts		miscellaneous	40	$11
				$3,496

Table S1. Material list for the OptoGenBox.

Abbreviations

ATR - all-trans retinal

DAC - digital-to-analogue converter

I^2C – inter-integrated circuit

IMS – insulated metal substrate

LED – light-emitting diode

NGM- nematode growths medium

PCB – printed circuit board

PWM – pulse width modulation