LEAP Rig Opto

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Contents:

Materials

All data and code available at https://github.com/kylethieringer/leap_rig_opto.git

Green LEDs:

Name: Luxeon Green (**530nm**) Rebel LED on a SinkPAD-II 20mm Tri-Star Base - 489 lumens @ 700mA
Wavelength Range: 520nm to 540nm
Recommended Operating Current: 700mA
Typical Forward Voltage: 8.7 Vf
Max Forward Voltage: 10.53 Vf

Red LEDs:

Name: Luxeon Red (**627nm**) Rebel LED on a SinkPAD-II 20mm Tri-Star Base - 138 lumens @ 350mA
Wavelength Range: 620nm to 645nm
Recommended Operating Current: 350mA
Typical Forward Voltage: 8.7 Vf
Max Forward Voltage: 10.53 Vf

Optics:

Name: Carclo 27° Frosted 20 mm Circular Beam Optic - Integrated Legs
Beam Angle: 27°
Efficiency: 85%

ThorLabs T-Cube LED Driver:

Interfaces with the computer so we can control the voltages and trigger of the led lights. Mode must be set to “MOD” for this function to work. The Current Limit was set according to the specifications of the LEDs and then fine tuned to calibrate rigs to emit relatively similar power with red leds at a given voltage.

Luxeon FlexBlock LED Driver:

These LED drivers are designed to effectively boost the forward voltage of a power source inline with the leds. They are specifically made to handle LED loads that are higher than the supplied voltage of the given power source (in our case the Thorlabs T-Cube). I do not understand the physics of this, but without it, LEDs flicker at best.

LED Rings:

Manufactured out of aluminum by the physics workshop. Designed to support 6 LEDs (alternating 3 red and 3 green). (Fig. 1a)

Wiring Diagram:

Fig. 1b illustrates the circuit in one individual chamber. Due to power limits, the user can only use one led circuit at a time. If both circuits are needed simultaneously, one simple option would be to plug the red circuit into chamber 1 flexblock and the green circuit into chamber 2 flexblock. This would mean that chamber 2 would not have any opto during the experiment but would be the controller for one of the led colors in chamber 1.

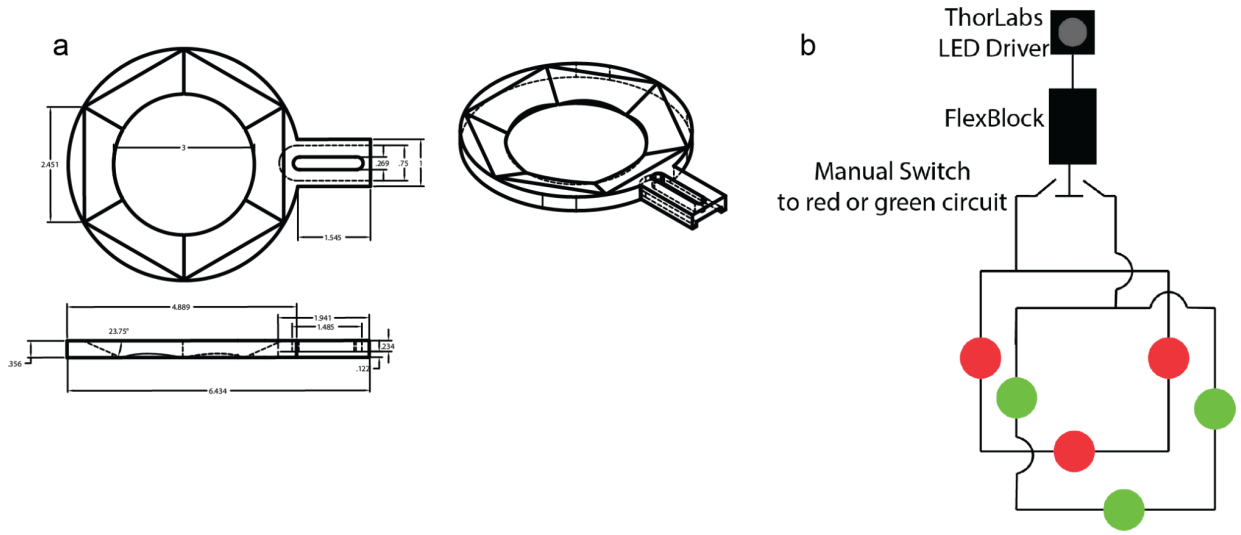

Figure 1 experimental setup

Results

Power Density Readings:

The current limits of each ThorLabs T-Cube LED Driver was adjusted by measuring power from 2.5V in the red channel. This was done because the red channel is more commonly used by members in the lab. LED intensity was measured using thorlabs PM100D. The wavelength was set to 627 nm or 530 nm for red and green leds respectively.

For linearity tests, a calibration stimulus was used for each rig consisting of 10 seconds each of: 0V, .2V, .25V, .5V, .7V, 1V, 2V, 3V, 4V, 5V. The power meter sensor (Thorlabs S130VC) was placed centered on the mic array without a coverslip then light intensity values were measured for each power step. After taking measurements on two separate days, the average was taken and plotted for all rigs (Fig. 2a,b) (Tables 1,3). Despite human error in placing the sensor or other unaccounted for sources of variability, the LED intensity was very consistent across days (Fig. 2e,f) (Tables 1,3). All rigs produced tightly

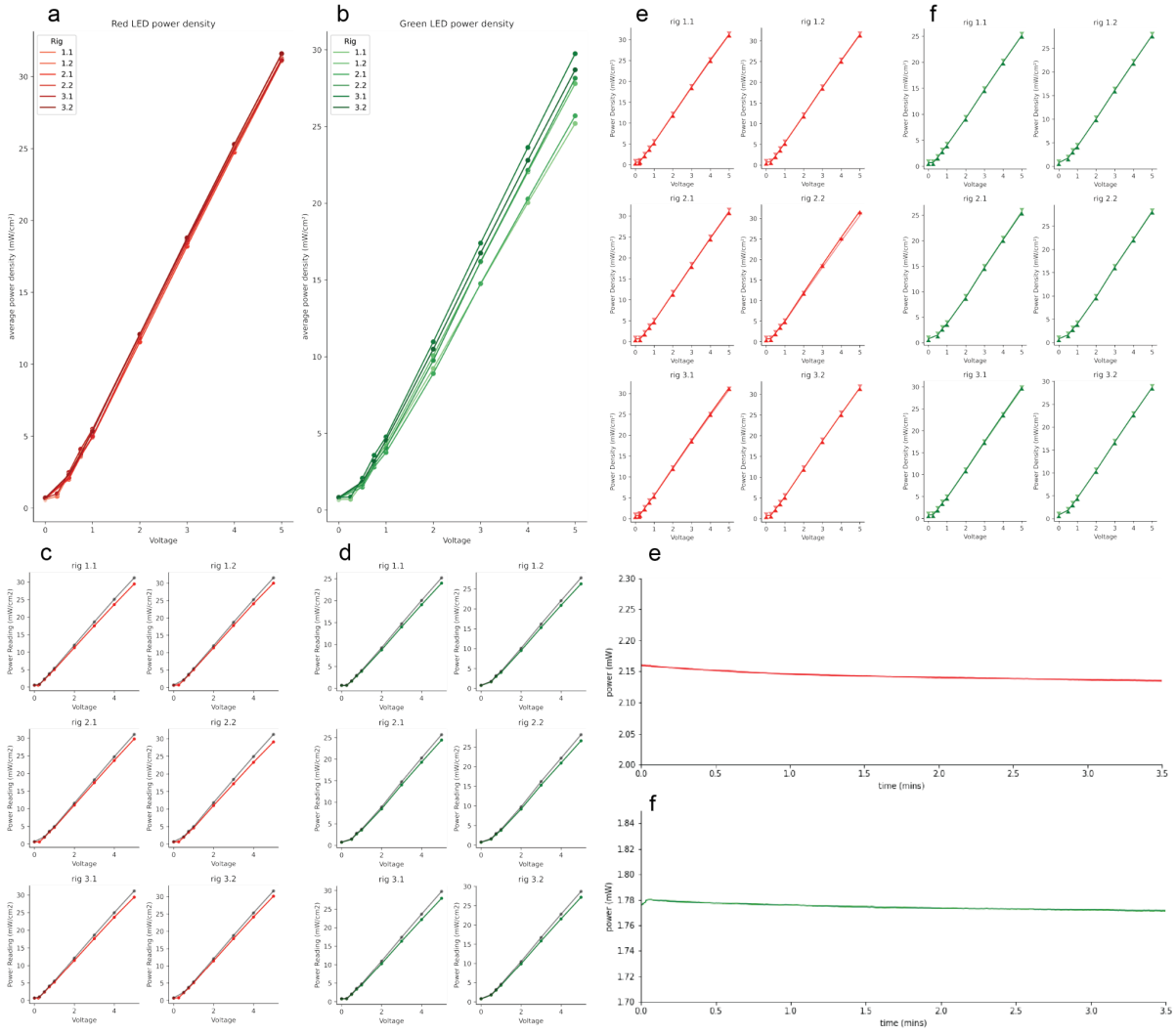

Figure 2 Power Linearity and Loss

distributed red led power densities due to calibration. While the power densities produced by green leds varied between rigs (Fig. 2b), each individual chamber was consistent across days (Fig. 2f). This allows for the user to confidently create stimuli but the voltages supplied to each chamber's leds will vary more than what will be required in the red channel. One point to note is that there is a minimum power requirement that is slightly different between rigs. Blank values in the Tables indicate when insufficient power was supplied and thus no LED power was measured. A general rule of thumb could be to use >.25V for red LEDs (Table 1) and >.5V for green LEDs (Table 3).

To determine the effects of the coverslip on power intensity, and to account for variability from light refraction, the coverslip was placed over the power meter sensor and propped up to remain level. Again the power meter sensor was placed in the center of the chamber. The same calibration stim was used and compared to the average values found without the coverslip (Fig. 2c,d) (Tables 2,4). The coverslip reduced the measured power, but never more than 3mW/cm^2 for any chamber (Tables 1-4). Then the power loss over time was determined through constant power detection by the power meter over multiple minutes. The LEDs used in the setups are high powered and emit heat as well as light. Due to the nature of thermodynamics, as the LEDs heat they are becoming less efficient at producing light so it becomes crucial to draw the heat away. The LEDs are mounted on an aluminum ring for this purpose and there is minimal power loss over time (Fig2. e,f).

The homogeneity of light around the chamber was measured by placing the sensor above each of the 9 mics to remain consistent across all chambers (Fig. 3a,c). 9 measurements were taken for each chamber given 2.5V. Mics are numbered 1-9 from back to front and left to right. The difference between the max and min values in each chamber did not surpass 10% for the Red LEDs or 11% for the green LEDs(Fig. 3b, d) (Tables 5,6). The variabilities in power around the chamber come from differences in individual LEDs or human error in wiring and/or mounting the LEDs. These values may further change with the addition of the coverslip, but were not measured due to the

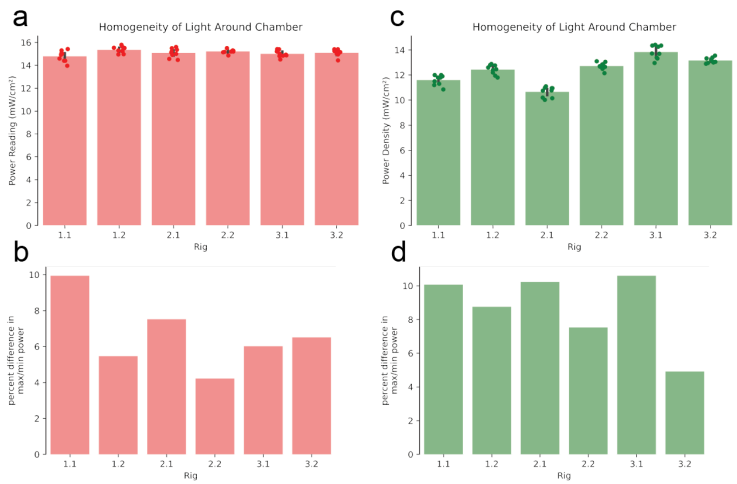

Figure 3 Power Homogeneity

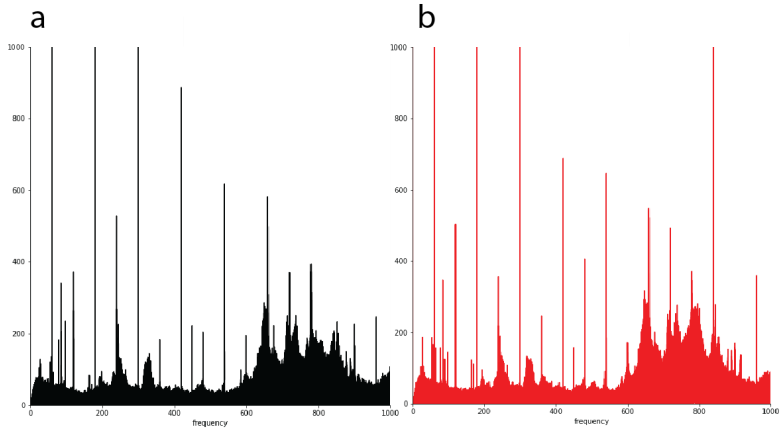

Figure 4 Power Homogeneity

technical and laborious challenge of balancing the coverslip over the power sensor for 9 different locations in the chamber for each chamber.

The microphones were checked for any sound interference that may come from the LEDs. The same calibration stimulus pattern was used, ranging from 0V to 5V for 10 seconds each. Figure 4 illustrates a representative rig with all 9 microphones transformed with a fourfier transformation to visual the amplitude of frequencies. If there was noise coming from the LEDs at any voltage, there would be a peak in the red (with lights on) but not the black (control) trace. There were no notable peaks in the range of frequencies we are mostly concerned with (0-1000hz) nor a broader range of frequencies.

Setting Up Experiments

1. Follow all setup that you typically do in the sleap rigs to get ready for experiments, including but not limited to:
    - Sigmacote coverslips
    - Fill humidifiers
    - Turn on IR lights
    - Double check your experimental script for the correct duration, and stimulus files
2. Open Motif as you normally would and connect cameras.
    - Run Baseline recordings and plot audio to check that all mics are on and working and the IR lights are on but NOT flashing
    - Currently we do not have a way to view recordings live with the opto script as it stands
3. Check opto LED drivers (black Thorlabs box with knob)
    - To check which LED light is plugged in, turn switch to ‘CW’ and turn the knob.
    - To switch LEDs find the labeled black and red wires and switch the connection
    - IMPORTANT: for opto to work with the script, the driver must be switched to ‘MOD’ and the knob must be turned to any value other than off.
4. Load flies into chambers
    - Ensure that the coverslip and its holder are aligned properly. Failure to do so will leave gaps between the coverslip and the mic array. Flies are escape artists and will crawl where you don’t want them. Avoid this headache!
    - I suggest loading female and male together
5. Place plastic ring on top of IR lights
    - These have the simple purpose as to block the IR lights from shining up to the opto LEDs. The objectives on the optoLEDs will reflect light back onto the coverslip which will show up in the video. Using the rings also has the added benefit of increasing contrast between the black background and the illuminated flies.
    - If you forget to place the ring on top of the lights you can either quit out of the experiment with ctrl-c and restart with the rings on or you can ignore it

Reminder: the opto script does not have a place to input experimental metadata such as fly genotypes, dates of birth, or manipulations. So make sure you take these notes somewhere.

Running Experiments

1. Open cmder and type these commands:
    cd C:\code\leap_rigs
    activate leap_rigs
    - Terminal should say this:
        C:\code\leap_rigs (main -> origin)

(leap_rigs) λ

- Run Experiment with this:
  python your_experiment_script.py

2. Experiments will be saved to “D:\Motif”
   - Copy over to your personal drive somewhere
   - DON’T leave files on rig computers, these are not meant for long term storage

NOTE on Canceling an Experiment: if you ctrl-c during the experiment, it will stop recording and will not save the files. However, this will not reset the daq or motif. Before running another experiment, run a short 10 second script. This will throw up an error when trying to save BUT will fully shut down everything and you will be good to go now. Better to have the error with this file than your precious data!!

Creating your experiment script

1. Copy the file pilot_opto_v2.py from the folder “C:\code\leap_rigs” on the rig computers.
   - You will need to create a new script on each computer.
2. Update your experiment parameters including:
   - **Experiment duration** (in minutes)
   - **record_left_camera** = True or False
   - **record_right_camera** = True or False
   - **opto_stim_left** = “opto_stims\example_stim_1.mat”
   - **opto_stim_right** = “opto_stims\example_stim_2.mat”
3. Save file in C:\code\leap_rigs

NOTE: If you only want opto in the right chamber you cannot set **opto_stim_left** = None if **record_left_camera** = True. You must pass a stim of all zeros or set left camera to false

Closed Loop Opto: see this script for Talmo’s implementation of real time sleap tracking https://github.com/murthylab/leap_rigs/blob/114341d92675bae8376f3d611b94e1debf2d8c5f/leap_rigs/tracking.py

And the DaqController class in this file for more info on how the opto stim is used by the daq controller

https://github.com/murthylab/leap_rigs/blob/114341d92675bae8376f3d611b94e1debf2d8c5f/leap_rigs/daq.py

Creating an opto stim

Relevant code and documentation: https://github.com/kylethieringer/leap_rig_opto.git

1. The experiment script takes .mat files as input to the daq to trigger the lights. Therefore, the .mat files should be vectors of shape (samples, 1). The number of samples indicates

the length of the stimulus—the daq frequency is 10,000 samples/sec, so a vector of shape (10000,1) will last one second.

2. A simple stimulus of one second on and one second off can be created with the following code in MATLAB:

   *stim = [ones(10000,1) * 3; zeros(10000,1)]; save('example_stim.mat', 'stim');*

3. IMPORTANT: The variable must be saved as 'stim' (all lowercase).
4. Voltage can be any double type
5. If the length of the stim does not equal the length of the recording, the stimulus will repeat until the end of the recording.
6. Save the stim to "C:\code\opto_stims" on each computer