*PyPlr*: A versatile, integrated system of hardware and software for researching the human pupillary light reflex

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Abstract

We introduce *PyPlr –* a versatile, integrated system of hardware and software to support a broad spectrum of research applications concerning the human pupillary light reflex. PyPlr is a custom Python library for integrating a research-grade video-based eye-tracker system with a light source and streamlining such processes as the design, optimisation and delivery of stimuli, synchronisation of devices, and extraction, cleaning, and analysis of pupil data. We additionally describe how full-field, homogenous stimulation of the retina can be realised with a low-cost integrating sphere that serves as an alternative to a Maxwellian setup. Users can integrate their own light source, but we provide full native software support for a high-end, research-grade 10-primary light engine which offers advanced control over the temporal and spectral properties of light stimuli. Here, we describe the hardware and software in detail and demonstrate its capabilities with two example applications: 1) pupillometer-style measurement and parametrisation of the pupil flash response, and 2) comparing the post-illumination pupil response (PIPR) to long and short-wavelength light. The system holds promise for researchers who would favour a flexible approach to studying the pupillary light reflex and the ability to employ a wide range of temporally and spectrally varying stimuli, including simple narrowband stimuli.

Keywords: Pupillometry, instrumentation, pupillary light reflex, ganzfeld, melanopsin

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**Introduction**

The pupillary light reflex (PLR) is the constriction of the pupil in response to changing light levels. Though its precise biological purpose is unclear, the PLR optimises retinal image quality by regulating the ammount and trajectory of light that strikes the retina (Hirata et al., 2003; McDougal & Gamlin, 2015) and it may also help to protect photoreceptors from dangerous levels of light (Laughlin, 1992; Woodhouse & Campbell, 1975). Importantly, as the PLR can be observed directly, it serves as a valuable tool for gaining insight into the integrity and activity of the autonomic nervous system (Girkin, 2003). Indeed, subjective visual assessments of the PLR, such as the swinging flashlight test (Levatin, 1959; Thompson, 1966), are still used routinely in clinical investigations to unmask afferent pupillary defects and give clues as to a patient’s neurological state. Though useful in critical care, such techniques are less suited to research due to their limited sensitivity and specificity, and to the poor inter and intraobserver reliability that exists even among specialists (Litvan et al., 2000; Meeker et al., 2005). The advent and commercial availability of video-based pupillometric techniques in the 1970s enabled researchers and clinical practitioners to base their investigations on repeatable and precise quantitative measurements of pupil size. As a consequence, the behavior of the pupil in response to light is now well characterised in both health and disease (Loewenfeld, 1993).

The aperture of the pupil at any given time is determined by the tone of the *dilator* and *sphincter pupillae*—the two opponent smooth muscles of the iris. The iris sphincter receives parasympathetic innervation and is responsible for the constriction of the pupil that follows an increase in retinal illumination (McDougal & Gamlin, 2015). When light strikes the retina, photons are absorbed by photoreceptors and the neural signal traverses a short reflex arc comprising the photoreceptor, bipolar and ganglion cells of the retina, the olivary pretectal nucleus of the midbrain and the Edinger-Westphal nucleus, which projects to the iris sphincter muscle via the ciliary ganglion (Hall & Chilcott, 2018). Following a sudden flash of white light, a normal pupil will begin to constrict after around 200-300 milliseconds and, after reaching peak constriction, will enter a redilation phase and return to baseline. Redilation of the pupil upon light cessation depends on two integrated processes: relaxation of the sphincter muscle due to parasympathetic inhibition, and contraction of the dilator muscle following excitation in the sympathetic pathway (Szabadi, 2018). The pupil flash response is parametrised in terms of latency, amplitude, velocity and acceleration. Its dynamics are affected by normal ageing (Bitsios et al., 1996; Winston et al., 2019), but degrees of abnormality exist in a broad range of psychiatric, neurological and ophthalmic conditions (Chen et al., 2011; Girkin, 2003; Van Stavern et al., 2019), making the pupil flash response an important tool in research and diagnostics (Hall & Chilcott, 2018; Troiani, 2020).

It was once assumed that the PLR was controlled entirely by the integration of signals from rod and cone photoreceptors, but we now know that steady-state pupil size is largely under the influence of intrinsically photosensitive retinal ganglion cells (ipRGCs)—a subpopulation of retinal ganglion cells which express the photopigment melanopsin in their axons and soma (Clarke et al., 2003a; Provencio et al., 2000). ipRGCs are sensitive to high intensity, short-wavelength (blue) light and control non-visual functions, such as circadian photoentrainment and pupil size (Spitschan, 2019), via direct projections to the suprachiasmatic nucleus of the hypothalamus and the olivary pretectal nucleus, respectively (Do, 2019). The post-illumination pupil response (PIPR) describes the sustained constriction of the pupil following exposure to short-wavelength light, usually relative to long-wavelength light, and is assumed to be a unique non-invasive signature of melanopsin processing in the human retina (Adhikari et al., 2015; Clarke et al., 2003b; Kankipati et al., 2010). Like the flash response to white light, the PIPR has been researched extensively for its potential as a biomarker in various ocular and neurodegenerative diseases (Chougule et al., 2019; Feigl & Zele, 2014; Kankipati et al., 2011).

To research the PLR requires a system for illuminating the retina and measuring pupil size. For patient monitoring in critical care, hand-held pupillometers offer an attractive all-in-one solution as they are portable, reliable and easy to use (Meeker et al., 2005; Taylor et al., 2003). These ‘point-and-shoot’ devices are aimed at the eye to deliver a light stimulus and use infrared illumination, video recording and internal algorithms to provide an instantaneous readout of the pupil response and its parameters. The downsides of automated pupillometers are that they are expensive and inflexible, usually offering minimal control over stimulus parameters (e.g., duration, wavelength, intensity) and in some cases no access to the raw data—limitations which make them less suited for research. Converesely, video-based eye trackers usually measure pupil diameter or area as part of their gaze estimation pipeline and are often favoured in research for their versatility. But video-based eye trackers and similar recording devices must be integrated with a system for administering light stimuli. This task may not be too difficult for basic experiments where a standard computer screen will suffice, but when the research calls for advanced control over the spatial extent and spectral properties of a light stimulus, a bespoke steup is needed. One solution is to use a Maxwellian view pupillometry system (e.g., Adhikari et al., 2015; Cao et al., 2015), where a light stimulus is focused onto an aperture placed in front of the eye, or in the entrance plane of a pharmacologically dilated pupil, and the consensual pupil response is measured from the other eye. An alternative which does not require complex optical engineering, pharmacological dilation of the pupil, or strict fxation control, is to use a full-field, or ‘Ganzfeld’, illumination system (e.g., Bonmati-Carrion et al., 2018; Kardon et al., 2009); but commercial solutions for this mode of stimulation are prohibitively expensive.

Here we describe *PyPlr*—a novel, versatile, integrated system of hardware and software which supports a broad spectrum of research applications concerning the pupillary light reflex. Fundamentally, *PyPlr* is a custom Python software for integrating a research-grade eye tracker with a light source and for streamlining such processes as stimulus design, optimisation and delivery, communication with respect to timing, and extraction, cleaning, and analysis of the pupil data. We additionally describe how full-field, homogenous stimulation of the retina can be realised with a low-cost integrating sphere that serves as an alternative to the more-complicated Maxwellian view pupillometry setup. We describe the hardware and software in detail before illustrating its application with two examples: 1) pupillometer-style measurement and parametrisation of the pupil flash response, and 2) comparing the post-illumination pupil response (PIPR) to long and short-wavelength light.

# System overview

## Eye tracker

## *PyPlr* was developed against Pupil Core (Pupil Labs, GmbH)—a versatile, open-source and relatively inexpensive eye-tracking ecosystem that affords real-time access to data, high sampling rates, and precise model-based 3D estimation of pupil size (see Kassner et al., 2014, for a detailed overview of the system). A Pupil Core headset is the only hardware dependency for our system. Users can easily integrate their own light source and stimulus geometry, as we leverage the forward-facing world camerea to timestamp light stimuli with good temporal accuracy. The Pupil Labs Software Pupil Capture and the Network API are required for interfacing with the Pupil Core system.

## *pupil.py.* All of the code for communicating with Pupil Core via Pupil Capture is contained within the *pupil.py* module—a thin, (mostly) object-oriented wrapper for the Pupil Labs Network API, which uses the ZeroMQ (<https://zeromq.org/>) messaging library and MessagePack (like JSON, but faster and more efficient: <https://msgpack.org/index.html>) for fast and reliable communication. The three most important tools, described below, are *PupilCore()*, *LightStamper(),* and *PupilGrabber().* A minimal example demonstrating the use of these tools is given in Figure X.

## *PupilCore().* The *PupilCore()* classsimplifies connecting to the eye tracker and controlling basic functionality, such as starting and stopping recording, sending event markers and accessing the current time.

## *LightStamper().* The *LightStamper()* isthe basis for integrating with a light source and creating labelled timestamps that can be used to extract the pupil data relative to the onset of a light stimulus. It is subclass of threading.Thread which has its ‘.run’ method overridden with code for detecting the onset of a light. Once initiated, the LightStamper() keeps track of the two most recent frames from the Pupil Core world camera, which must be aimed at the light source. When the average RGB difference of the two frames exceeds a given threshold, an ‘annotation’ is sent to Pupil Capture to mark the time of light onset. Figure 2 shows a minimal example of how to use the pupil module to measure the light reflex with any light source (e.g., a light switch in a dark room). Given a suitable geometry and appropriately tuned parameters, the *LightStamper()* flawlessly detects the first frame where the onset of a light stimulus is noticeable, thereby supporting seamless integration with any light source.

## The accuracy of the LightStamper() is limited by the degree to which Pupil Core’s cameras are synchronised. In our own testing, we found this to be suboptimal, meaning that latency measures are affected. More here.

Graphical user interface, text, application, email

Description automatically generated

Figure 1. Example code showing how to use PyPlr’s pupil.py module to record a pupil response to any light stimulus. The result is a Pupil Labs recording with an annotation marking the time at which the light was presented.

A picture containing wall, indoor, person

Description automatically generated A picture containing diagram

Description automatically generated

Figure 2. Showing the physical setup (left) and software architecture (right).

**Light source**

While users are free to integrate their own light source and stimulus geometry, we provide full native support for the Spectra Tune Lab (STLAB: LEDMOTIVE technologies, LLC)—a high-end, research-grade, 10-LED channel light engine which allows for advanced control over the temporal and spectral properties of light stimuli. The STLAB connects via a network cable to a small computer calleed the LIGHT HUB (a Beaglebone running Linux), which in turn connects to a computer via USB. All functionality for interfacing with the STLAB is contained in the *stlab.py* module, the main features of which are outlined below. The intensity of each LED is specified with a value between 0-4095 (12-bit resolution), corresponding to the minimum and maximum output.

**stlab.py.**

*SpectraTuneLab(…).*A class for the STLAB device which uses the Python requests library to wrap the entire RESTFUL\_API.

***CalibrationContext(…).*** A class for working with calibration data.

***Device timing.*** The STLAB operates in *synchronous* mode by default, meaning that all commands sent by the LIGHT HUB must be acknowledged before a new instruction can be processed. According to the device manual, response times in this mode of operation are on the order of around 250 milliseconds. We verify this with our own testing, but also observed that on rare occasions, perhaps when the LIGHT HUB is busy processing other tasks, the response time can be up to 5 s. Clearly this is not suitable for administering light stimuli with exact timing. To do this, we leverage the STLAB’s *asynchronous* mode of operation, which allows for real-time spectral streaming with a spectral switching time of less than 10 milliseconds (i.e. 1 spectrum every 10 milliseconds). This mode of operation requires the advanced preparation of *video files*, which are then played using the play\_video\_file(…) function.

***Video files.*** STLABvideo files—idiosyncratically referred to as *dynamic sequence files* and given a .DSF file extension—are JSON files with a particular structure. The core input for making a video file are a time vector to specify the spectral switching times and separate vectors to specify the intensity of each LED channel at that time point. The *make\_video\_file(…)* function will convert a pandas data frame into the required JSON format. Alternatively, we provide convenience functions for making simple stimuli (e.g., timed pulses of light).

Text

Description automatically generated with medium confidence

Figure 3. Internal structure of a video file.

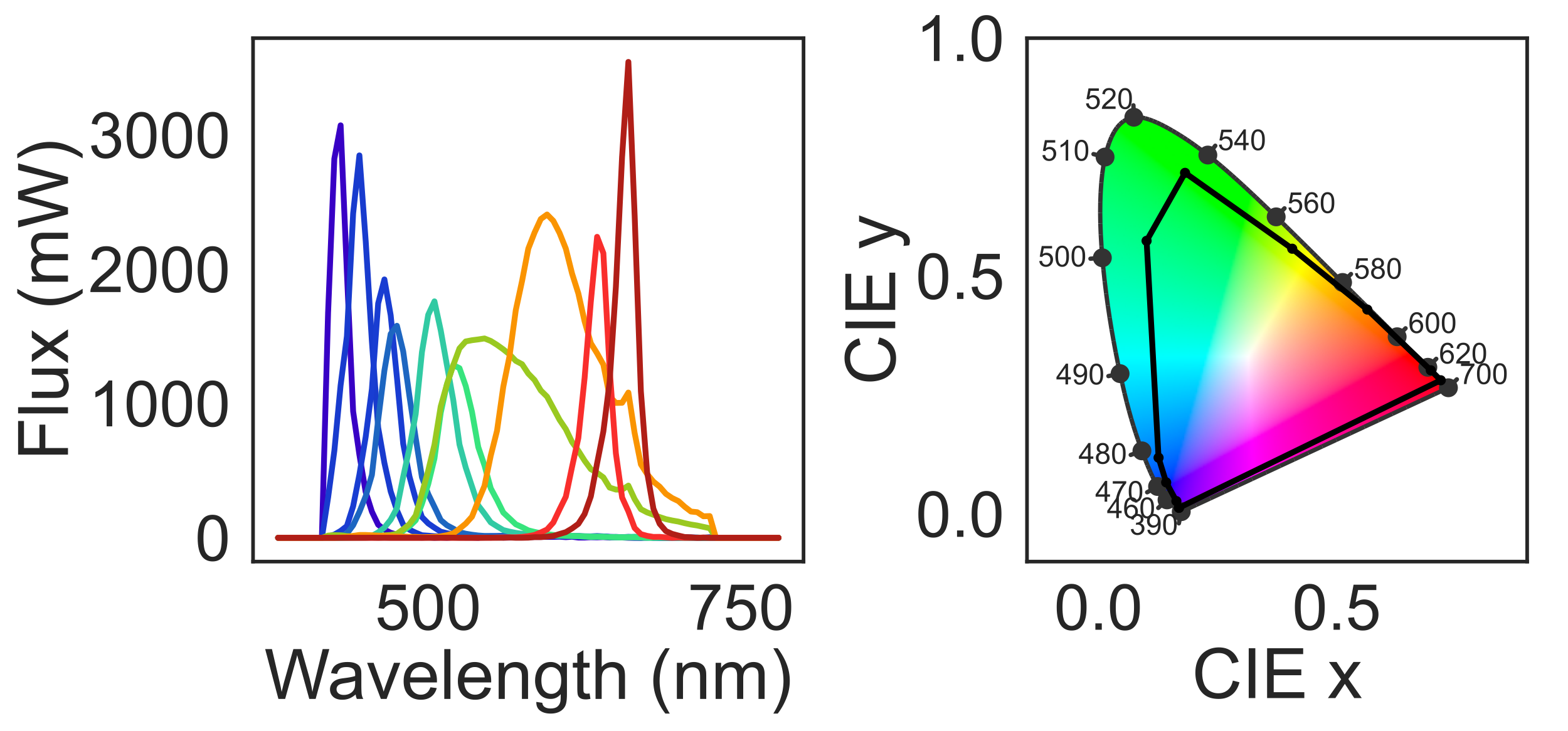


Figure 4.Spectral power distributions (left) and CIE color coordinates (right) of the 10 STLAB led channels.

**Integrating sphere**

An integrating sphere was constructed from two 45-cm diameter flanged acrylic half-domes (Project Plastics Ltd), each sprayed on the inside with multiple coats of Avian-B high reflectance paint (Avian Technologies LLC). A 28 cm opening in one of the domes serves as a viewing port to a full field, ‘Ganzfeld’, stimulus, and an additional 7 cm (~9° from the plane of the viewing port) opening opposite the viewing port was included to allow for secondary stimuli (e.g., a fixation target) or to afford exclusion of the foveal macular pigment from stimulation. On the same half of the sphere as the viewing port, a 30 mm entry port for the light source was cut at an angle of 22.5-deg from the top, such that it could not be seen directly when looking straight ahead. The sphere was housed on a stabilised wooden fixing plate.

**Spectrometer**

Whatever the light source, a spectrometer is needed to calibrate the system we used an OceanOptics spectrometer to obtain measurements of calibrated radiance at the viewing port. It is supported in our software, but the code can easily be modified to add support for another spectrometer.

**Data analysis**

**pipeline.py.** After exporting data from Pupil Player, the pipeline.py module can be used to load, clean, and extract the pupil data.

**PLR.py.** A module to assist with plotting and parametrisation of the PLR and PIPR.

# Examples

**Pupil flash response**

Here we show how our system can be used to measure and parametrise the pupil flash response, and we compare the results with a NeurOptics PLR-3000.

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Figure 5. Pupillometer-style measurement and parametrisation of the flash response (not the final script). Produces a plot of flash response and saves parameters in CSV file. This script uses the PupilGrabber() class to work with real-time pupil data and therefore bypasses the Pupil Player software.

**PIPR**

Here we describe an experiment that measures the PIPR.

**Discussion**

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