The 100€ lab: A 3-D printable open source platform for fluorescence microscopy, optogenetics and accurate temperature control during behaviour of zebrafish, Drosophila and C. elegans.

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ABSTRACT

To do...

INTRO

The advent of protein engineering has brought about a plethora of genetically encoded actuators and sensors that have revolutionised neuroscience as we knew it but a mere decade ago. On the back of an ever expanding array of genetically accessible model organisms, these highly sophisticated molecular machines have allowed researchers to both monitor and manipulate molecular and neuronal processes at unprecedented breadth (e.g.: [1]–[3]). In parallel, developments in consumer-oriented manufacturing techniques such as 3-D printing as well as low-cost and user-friendly microelectronic circuits have brought about a silent revolution in the way that individual researchers may customise their lab-equipment or build entire setups from scratch (reviewed in: [4]–[6]). Similarly, already ultra-low cost light emitting diodes (LEDs), when collimated, now provide sufficient power to photo-activate most iterations of Channelrhodopsins or excite fluorescent proteins for optical imaging, while a small Peltier-element suffices to thermo-activate heat-sensitive proteins [7], [8]. In tandem, falling prices of high-performance charge-coupled device (CCD) chips and optical components such as lenses and spectral filters mean that today already a most basic webcam, in combination with coloured transparent plastic, may suffice to perform sophisticated optical measurements. Taken together,
modern biosciences today stand at a precipice of technological possibilities, where an entire functional laboratory set-up capable of delivering high-quality data over a wide range of experimental scenarios can be built from scratch for a mere fraction of the cost traditionally required to purchase any one of its individual components. Here, we present such as design.

Assembled exclusively from readily available off-the-shelf electronic, mechanical and optical components, the “FlyPi” provides a modular solution for basic light- and fluorescence-microscopy as well as time-precise opto- and thermogenetic stimulation during behavioural monitoring of small, genetically tractable model species such as zebrafish (Danio rerio), fruit flies (Drosophila Melanogaster) or nematodes (Caenorhabditis elegans). The system is based on an Arduino microcontroller [9] and a Raspberry Pi 2 single board computer (RPi; [10]), which also provides sufficient computing power for basic data analysis, word processing and web-access using a range of fully open source software solutions that are pre-installed on the SD-card image provided. The mechanical chassis is 3-D printed and all source code is fully open, such that the design and future modifications can be readily distributed electronically to enable rapid sharing across research labs and institutes of science education. This not only facilitates reproducibility of experimental results across labs, but promotes rapid iteration and prototyping of novel modifications to adapt the basic design for a wide range of specialised applications. More generally, it presents a key step towards a true democratisation of scientific research and education, independent of financial backing [4].

Here, we first present the basic mode of operation including options for micropositioning of samples and electrodes and demonstrate the FlyPi’s suitability for light microscopy and use as a basic medical diagnostic tool. Second, we present its fluorescence capability including basic calcium imaging using GCaMP5 [1]. Third, we survey the FlyPi’s suitability for behavioural tracking of Drosophila and C. elegans. Fourth, we demonstrate optogenetic activation of Channelrhodopsin 2 [3] and Chrimson [11] in transgenic larval zebrafish as well as Drosophila larvae and adults. Fifth, we evaluate performance of the FlyPi’s Peltier-thermistor control loop for thermogenetic activation of Trp-A [12] in larval Drosophila. Sixth, we briefly describe our efforts to introduce this tool for university research and teaching in sub-Saharan Africa [4], [13].

RESULTS

Overview and bill of materials.

The basic FlyPi consists of the 3-D printed mainframe (Fig. 1A,B), one RPi2 computer with camera and off-the-shelf objective lens, one Arduino-Nano microcontroller as well as a custom printed circuit board (PCB) for flexible attachment of a wide range of actuators and sensors (Fig. 1C). If required, all high-power actuators can be powered independently. The mainframe allows modular placement of additional components into the camera-path such as holders for petri-dishes or microscope slides. This basic build, including power adapters and cables, can be assembled for ~100 € (Table 1; Fig. 1D,E). Additional modules e.g. for lighting and optogenetic
stimulation (~10 €), fluorescence imaging (~15 €) or temperature control (~35 €) can be added as required (below). For a full bill of materials (BOM), see Table 1.

**Imaging.** The objective focal distance can be gradually adjusted between ~2 mm (peak zoom) and infinity (panoramic), while the camera delivers 5 megapixel Bayer-filtered RGB images at 15 Hz. Spatial binning allows framerates up to 42 Hz (x2) or 90 Hz (x4). Alternatively, a monochrome but higher sensitivity infrared-capable sensor can be used [REF]. Objective focus can be set manually, or via a software-controlled continuous-rotation micro servo motor (Fig. 1F).

**Lighting:** The FlyPi can control Adafruit Neopixel LED rings and bricks [14] comprising between 8 and 24 high-power RGB-LEDs. These LED arrays can be used for flexible brightness and wavelength lighting during microscopy or behavioural experiments, and offer sufficient power to photo-activate a wide range of genetically encoded light-sensitive proteins such as Channelrhodopsin 2 (ChR2), red-shifted Channelrhodopsin (ReaChr) or Chrimson [3], [11] Alternatively, an Adafruit 8x8 high-power single wavelength LED matrix [14] can be attached for spatially selective optogenetic or visual stimulation [Ref Prieto Godino (in prep)]. Next, general purpose ports (GPP) allow flexible software-control of additional custom LEDs, e.g. to serve as excitation light sources for fluorescence microscopy or as further options for optogenetic stimulation. A series of ultra-low-cost theatre-lighting filters can be added for efficient spectral filtering, as required for fluorescence microscopy and to block stimulus artifacts from LED activation during optogenetic stimulation. Simple white weighing boats serve as effective diffusors (Fig 1, cf. Fig 2A). Brightness is controlled though pulse width modulation (PWM) or an open Python library for GPPs and the LED ring, respectively. Aliasing can be dynamically offset by either tweaking PWM rates and/or the camera framerate directly through the custom graphical user interface (GUI). Finally, a low-power RGB LED can be added as a flexibly programmable event-marker (Module X, 2 €).

**Temperature.** A 4x4 cm Peltier element coupled to a thermistor can be integrated into the base of the FlyPi chassis for accurate feedback control of temperature between ~10-40 °C with a rise time of ~1°C/s and steady-temperature accuracy of <1°C. A CPU fan and heat sieve below the Peltier element dissipate excess heat (SFig. X).

**Micropositioning.** Up to three 3-D printed micromanipulators [4] can be attached to the free faces of the FlyPi (Fig. 1D, I). The manipulators can be configured to hold probes such as electrodes or stimulation devices, or alternatively are fitted with a microscope slide mount. Like the camera objective, manipulators can be optionally fitted with continuous-rotation servo motors to provide electronic control of movement in 3 axes. Servos can be either software controlled, or via a stand-alone joystick-unit based on a separate Arduino-Uno microcontroller and a Sparkfun Joystick shield (cf. Table 1). Depending on print quality and manipulator configuration, precision can reach the order of tens of microns.
Software. All controls and displays are integrated into a modular Python3-based Graphical User Interface (GUI) (Fig. 1X), which also comprises a simple-to-use panel to define and execute looping protocols that address any of the attached peripherals. All 3-D printed parts were designed in OpenSCAD [15] which allows parametrisation of all dimension labels. Accordingly, modifications to the 3-D model are readily achieved through changing high-level variables in the OpenSCAD code. The complete source codes for the 3-D model, GUI and Arduino as well as the custom PCB layout are fully open (Supplementary Data X), and kept deliberately simple to allow for easy modification by non-experts.

Basic camera operation and microscopy

The RPi platform [10] offers a range of different camera modules, all of which are compatible with the FlyPi. Here, we use the “adjustable focus RPi RGB camera” (Table 1) as it includes a powerful objective lens embedded in a standard 12 mm micro-video thread. These low-cost objectives are readily available with a wide range of specifications. Alternatively, the RPi camera chip can be directly fitted above any other objective with minimal mechanical adjustments. Fitted with the chosen objective, the FlyPi allows behavioural tracking e.g. in a Petri dish at low zoom, while resolving structures beyond ~10 µm at high zoom (cf. Figs. 2-6). We used the Python based PiCamera library [16] to gain easy high-level access to framerates, sensitivity, contrast, white balance and which pixels are being read out (e.g. for binning or digital zoom). These key parameters are directly accessed through the GUI (cf. Fig. 1X), while others can be easily added as required. By default, photo (static and time-lapse) and video data is stored in jpeg and h264 formats, respectively. In addition, the preinstalled converter MP4Box [17] allows direct storage of video data in more widely used mp4 format which is easily converted to image-sequence data readable by open analysis packages such as Fiji [18]. Install of the other converter for straight Avi.

The camera can be mounted in two main configurations: upright or inverted (Fig. 2A,B). While the former may be primarily used for resolving larger objects such as adult Drosophila (Fig. 3C) or for behavioural tracking (cf. Fig. 5), the latter may be preferred for higher-zoom (Figs. 3D-K) and fluorescence microscopy (cf. Fig. 4), or if easy access to the top of a sample is required. Here, the image quality is easily sufficient to monitor basic physiological processes such as the heartbeat or blood-flow in live zebrafish (Fig 2D, SVid. 1). The LED ring with all LEDs active simultaneously can be used to add “white” incident or transmission illumination (e.g. Fig. 3A), while behavioural tracking may be performed under red light (cf. Fig. 5A). A series of white weighing boats mounted on top of the ring can be used as diffusors (Fig. 3A). Long-term time-lapse imaging, for example to monitor developmental processes or bacterial growth, can be performed in any configuration.

The implementation of a cost-effective option for digital microscopy also opens up possibilities for basic medical diagnosis, such as the detection of small parasitic nematodes such as Brugia malayi or Wuchereria bancrofti in human lymph tissue samples (Fig. 3G, H) or Schistosoma
eggs in urine (Fig. 2I). Similarly, the image is sufficient to detect and identify counterstained types of blood cells in an infected smear (here: *Mansonella perstans*; Fig. J,K).

**Fluorescence microscopy**

Next, we implemented fluorescence capability based on a 350 mA 410 nm LED attached to a reflective collimator as well as ultra-low cost theatre-lighting filters. The excitation and emission light was limited by a low-pass and a notch filter, respectively (ROSCO “Baldassari Blue”, and “Straw-Yellow”; Table 1; Fig. 3A, D). Imperfect emission filter efficiency for blocking direct excitation light necessitated that the source was positioned at 45° relative to the objective plane, thereby preventing direct excitation bleed-through into the camera path (Figs. 3A,B). Many commonly used fluorescent proteins and synthetic probes exhibit multiple excitation peaks. For example, Green Fluorescent Protein (GFP) is traditionally excited around 488 nm, however there is a second and larger excitation peak in the near UV [19] (Fig. 3D). We made use of this short-wavelength peak by stimulating at 410 nm to improve spectral separation of excitation and emission light despite the suboptimal emission filter. Figure 3C shows the fluorescence image recorded in a typical green/red fluorescence test-slide. The RGB camera chip allowed simultaneous visualisation of both green and red emission. If required, the red channel could be limited either through image processing, or by addition of an appropriate short-pass filter. Next, using green fluorescent beads (100 nm, Methods) we measured the point spread function (psf) of the objective as 5.4 µm (SD) at full zoom (Fig. 3E, F). This is approximately five times broader than that of a typical state-of-the art confocal or 2-photon system [20], though without optical sectioning. It also meant that the system is limited by the objective optics rather than the resolution of the camera chip. Accordingly, the use of a higher numerical aperture objective is expected to yield a further substantial improvement in spatial resolution.

Next, we tested the FlyPi’s performance during fluorescence imaging on live animals, here demonstrated using a transgenic zebrafish larva (5 dpf) expressing the GFP-based calcium sensor GCaMP6f in all neurons (Ubi:nls-GCaMP6f; Fig. G,H). At lower magnification, the live image quality was sufficient for basic fluorescence detection as required e.g. during fish sorting (Fig. 3I, SVid. 2). Similarly, the system also provided sufficient signal-to-noise dynamic images for basic calcium imaging, here demonstrated for using larval *Drosophila*. For example, GCaMP5 in muscles (Mef2-Gal4; UAS-myr::GCaMP5) revealed clear calcium signals associated with peristaltic waves as the animal freely crawled on a microscope slide (Fig. 6E-H; see also SVid. 3). Further fluorescence videos are provided in the supplementary materials (SVids. 4,5).

**Behavioural tracking**

All mankind can do is move. [...] [Sherrington]. Sherrington’s thoughts on the ultimate role of any animal’s nervous system till echoes today, where despite decades (bio)technological advances, behavioural experiments are still amongst the most powerful means for understanding neuronal function and organisation. Typically, individual or groups of animals are placed in a
controlled environment and monitored over prolonged periods of time using a camera system. Here, the FlyPi’s colour camera with adjustable zoom offers a wide range of video-monitoring options, while the RGB LED ring provides for easily adjusted wavelength and intensity lighting (Fig. 4A) including dim red light, which is largely invisible to many invertebrates including C. Elegans (Fig. 4B, SVid. 6) and Drosophila. A series of mounting adapters for petri-dishes (Sfig. X) as well as a custom chamber consisting of a 3-D printed chassis and two glass microscope slides for adult Drosophila (Fig. 4C) can be used as behavioural arenas. Following data acquisition, videos are typically fed through a series of tracking and annotation routines to note the spatial position, orientation or behavioural patterns of each animal. Today, a vast range of open behavioural analysis packages is available, including many that run directly on the RPi2 such as CTrax [21], here used to track the movements of adult Drosophila in a 10 s video (Fig. 4D; SVid. 7).

**Optogenetics and Thermogenetics**

One key advantage of using genetically tractable model organisms is the ability to selectively express proteins in select populations of cells whose state can be precisely controlled using external physical stimuli such as light (Optogenetic actuators, e.g. [22]) or heat (Thermogenetic actuators, e.g. [12]). Accordingly, the function of individual or sets of neurons can be readily studied in behavioural experiments. To date, a plethora of both light- and heat-sensitive proteins are available, with new variants being continuously developed. Many of these constructs exhibit sufficient sensitivity for activation by collimated high-power LEDs, rather than having to rely on much more costly light sources like a Xenon lamp or a laser. Similarly, temperature variation over few degrees Celsius, as achieved by an off-the-shelf Peltier element with adequate heat dissipation, is sufficient to activate or inactivate a range of temperature-sensitive proteins. We therefore implemented both opto- and thermogenetic stimulation capability in the FlyPi.

**Optogenetics.** The main stimulator for optogenetic activation is the LED ring (Fig. 5A), whose spectrum and power are appropriate for use with both ChR2 (single LED ‘blue’ Pwr<sub>460</sub>: 14.2 mW) as well as ReaChr and Chrimson (‘red’ Pwr<sub>628</sub>: 7.2 mW; ‘green’ Pwr<sub>518</sub>: 7.5 mW) (Fig. 5B) [3], [11], [23]. For demonstration, a zebrafish larva (5 dpf) expressing ChR2 in all neurons (line) was suspended in a drop of E3 on top of a microscope slide, which was in turn held above the inverted objective using the micromanipulator (Fig. 5A, C). The LED ring was positioned face-down ~2 cm above the animal, outside of the centrally positioned camera’s field of view. Concurrent maximal activation of all 12 ‘blue’ LEDs (Pwr<sub>460</sub>: ~4.9 mW cm<sup>−2</sup> at the level of the specimen) reliably elicited basic motor patterns for stimuli exceeding 500 ms, here illustrated by pectoral fin swimming bouts (Fig. 5C,D, SVid. 8). Substantially shorter stimuli did not elicit the behaviour (e.g. 3<sup>rd</sup> trial: ~150 ms), nor did activation of the other wavelength LEDs (not shown). This strongly indicated that motor networks were activated through ChR2 rather than innate escape reflexes in response to the light (cf. [24]). Notably, while in the example shown the stimulus artefact was used as a timing marker, excitation light could be blocked (>95% attenuation) using an appropriate filter (Fig. 5A dark red trace, Table 1) without substantially
affecting image quality, while timing could be verified using the flexibly programmable low-power RGB LED.

We also tested ChR2 activation in *Drosophila* larvae (elav-GAL4/+; UAS-shibrets, UAS-ChR2 / +; UAS-ChR2 / +). Animals were left to freely crawl on ink-stained agarose with both the LED ring and camera positioned above. Activation of all 12 blue LEDs reliably triggered body contractions for the duration of the 1s stimulus, followed by rapid recovery (Fig. 7D, right). Finally, full-power activation of the red ring LEDs reliably triggered the proboscis extension reflex (PER) in adult *Drosophila* expressing Chrimson in the gustatory circuit (Fig. 5G,H, line kindly provided by Olivia Schwartz and Jan Pielage).

**Thermogenetics.** Owing to their remarkable ability to tolerate a wide range of ambient temperatures, many invertebrate model species including *Drosophila* and *C. elegans* also lend themselves to thermogenetic manipulation. Through the select expression of proteins such as Trp-A or shibire1s [12], [25], sets of neurons can be readily activated or have their synaptic drive blocked by raising the ambient temperature over a narrow threshold of X and Y °C, respectively. Here, the FlyPi offers the possibility to accurately control temperature of the upper surface of a 4x4cm Peltier element embedded in its base, while a thermistor logically connected to the h-bridge that controls current-flow through the Peltier provides for immediate feedback. As a result, temperatures +/- 15-20 °C around ambient temperature are readily reached within seconds and held over many minutes (Fig. 6A).

- Quantify Peltier performance
- Some thermogenetics experiment

**DISCUSSION**

- Link to other open scopes (incl. foldscope).
- Cool open source projects with similar ethos that conceptually “fit”
- Note on Open source
- A Modular platform that can be adapted for almost anything..
- Better control – we provide the basic platform

**Potential for further development**

Clearly, the current FlyPi only scratches the surface of possible applications. Further development is expected to take place as researchers and educators integrate aspects of our design into their laboratory routines. To explicitly encourage re-sharing of such designs with the community we maintain and curate a centralised official project repository ([http://open-labware.net/projects/flypi/](http://open-labware.net/projects/flypi/)) linked to an open GitHub (implement).
Currently, one obvious limit is spatial resolution. For example, the system readily resolves individual red blood cells (Fig. 2K), but narrowly fails to resolve malaria parasites within (not shown). Here, the limit is optical rather than related to the camera chip, meaning that use of a higher N.A. and magnification objective lens should yield substantial improvements. This development might come in hand with additional improvements in the micromanipulator’s Z-axis stability to facilitate focussing at higher magnification. Similarly, photon catch efficiency of the CCD sensor could be improved by use of an unfiltered (monochrome) chip. Other alleys of development may include, but are not restricted to (i) the addition of further options for fluorescence microscopy to work over a wider range of wavelengths, likely through use of other excitation LEDs and spectral filters. (ii) The FlyPi could be tested for stimulating photoconversion of genetically encoded proteins such as CamPari, Kaede or photoconvertible GFP [26]–[28]. (iii) Auto-focussing could be implemented by iteratively rotating the servo-assisted focus while evaluating changes in the spatial autocorrelation function of the live image. (iv) A motorised manipulator could be set-up for stage-automation through a simple software routine. (v) One or several FlyPis could be networked wirelessly or through the integrated ethernet port to allow centralised access and control, thereby removing need for dedicated peripherals. Taken together, by providing all source code and designs under an open source license, together with an expandable online repository, we aim to provide a flexible, modular platform upon which enthusiastic colleagues may build and exchange modifications in time.

**Classroom teaching and laboratory improvisation**

In large parts of the world, funding restrictions hamper the widespread implementation of practical science education - a problem that is pervasive across both schools and universities [29], [30]. Oftentimes, limitations include broken or complete lack of even basic equipment such as low power light microscopes or computing resources. Here, the low cost and robustness of the FlyPi may offer a viable solution. If only one unit can be made available for an entire classroom, the teacher can project the display output of the RPi2 to the wall such that many students can follow demonstrated experiments. Already a low amount of funding may furnish an entire classroom with FlyPis, allowing students in pairs of two or three to work and maintain on their own unit. The relative ease of assembly also means that building the FlyPi itself could be integrated into part of the syllabus. In this way, a basic technical education in soldering or basic 3-D printing could be conveyed in parallel. As an additional advantage, each student could build their own equipment which brings about further benefits in equipment maintenance and long-term use beyond the classroom.

To survey to what extent FlyPi assembly and use may be beneficial in a classroom scenario, we introduced the equipment to African biomedical MSc and PhD students as well as senior members of faculty during a series of multi-day workshops at Universities in sub-Saharan Africa, including the University of KwaZulu Natal (Durban, SA), the International Centre of Insect Physiology and Ecology (icipe, Nairobi, Kenya) and Kampala International University (Dar es Salaam, Tanzania and Ishaka Bushyeni, Uganda). In one workshop, we only provided the 3-D
printed parts and unadulterated off-the-shelf electronics and took students though the entire process of assembly and installation (Fig. 7A). Having had no previous experience with basic electronics, soldering or the use of simple hand-tools such as a Dremel or cable-strippers, all students successfully assembled a working unit. Towards the end of the training, students used their own FlyPi to perform basic Neurogenetics experiments with *Drosophila*, including heat activation of larvae expressing shibire<sup>ts</sup> in all neurons (elav-GAL4/+ ; UAS-shibire<sup>ts</sup>,UAS-ChR2 / + ; UAS-ChR2 / +) and optogenetic activation of ChR2 to elicit a range of behaviours in both adults and larvae. Following the training, students took their assembled FlyPis home for their own research and teaching purposes. In other courses, we brought pre-assembled FlyPis with a range of different modules. Students learnt to operate the equipment within minutes and subsequently used them for a range of experiments and microscopy tasks, including several novel configurations not formally introduced by the faculty (Figs. 7B,C). Several experiments presented in this work were actually performed during these training courses. Finally, we used individual FlyPi modules to improvise workarounds for incomplete commercial lab equipment. For example, the RPi camera with focus drive and live image-processing options served as an excellent replacement for a missing Gel-doc camera (Fig. 7D). Similarly, we used the FlyPi as a replacement camera for odour evoked calcium imaging in *Drosophila* antennas on a commercial upright fluorescence microscope or for dissection demonstrations under a stereoscope that also utilised the LED rings for illumination. Moreover, the FlyPi’s programmable GPPs and LEDs were used to drive time-precise light-stimulus series, e.g. for independently recorded *Drosophila* electroretinograms (ERGs) while the Peltier-feedback circuit is adequate to maintain developing zebrafish embryos at a controlled temperature during prolonged experiments, or to reversibly block action potential propagation in long nerves through local cooling. Clearly, beyond its use as a self-standing piece of equipment and teaching tool, the low cost and modular nature of the FlyPi also renders it versatile to support or take over a large range of additional functions in the lab.

**METHODS**

3D Modelling and printing. All 3-D modelling was performed in OpenSCAD [15] and are provided as both scad and surface tessellation lattice files (stl). All parts were printed in polylactic acid (PLA) on an Ultimaker 2 3-D printer (Ultimaker, Geldermalsen, Netherlands) in six pre-arranged plates using the following parameters: infill 30%, no supports, 5 mm brim, layer height 0.1 mm, print speed 60 mm/s, travel speed: 200 mm/s. Total printing time of a single FlyPi, including all presented modules, was about 50 hours. Notably, this time can be substantially reduced by using faster print settings and a larger nozzle. For example, using a well-calibrated delta Rep-Rap (www.reprap.org) printing at full speed, the entire system can be printed at sufficient precision in less than 20 hours.

*PCB design and printing.* →Andre
The Graphical User Interface (GUI). The GUI was written in python 3 and configured to be slave to the more time-precise Arduino output. 

Arduino. We used an ATmega...

Spectral and power measurements. We used a commercial photo-spectrometer (USB2000+VIS-NIR, Ocean Optics, Ostfildern, Germany) and custom written software in Igor-Pro 7 (Wavemetrics) to record and analyse spectra of LEDs and filters. Peak LED power was determined using a Powermeter (Model 818, 200-1800 nm, Newport). Fluorescent beads used for estimating the psf: PS-Speck TM Microscope Point Source Kit P-7220, ThermoFisher.

Video and image acquisition: All static image data was obtained as full resolution RGB images (19xx19xx pixels) and saved as jpeg. All video data was obtained as RGB at 42 Hz (x2 binning), yielding image stack of 942x942 pixels, and saved as h264. Video data was converted to mp4 format using: MP4Box –add name.h264 name.mp4 (MP4Box, GPAC). If required, mp4 files could be further converted into avi using MPEG Streamclip (www.Squared5.com). Add the other converter! All further data analysis was performed in Image-J (NIH) and Igor-Pro 7 (Wavemetrics). Figures were prepared in Canvas 15 (ACD Systems).

Calcium imaging in Drosophila larval muscles. Second instar larvae (Mef2-Gal4; UAS-myr::GCaMP5) were left to freely crawl between a microscope slide and cover slip loosely suspended with tap water. For analysis, x2 binned video data (x*y, 42 Hz) was down-sampled by a factor of 2 in the image plane and a factor or 4 in time. Only the green channel was analysed. Following background subtraction, regions of interest were placed as indicated (Fig. 3J). Next, from each image frame we subtracted the mean image of 4 preceding frames to generated a “running average time-differential” stack – shown as the space-time plot in Fig. 3L with the original x-axis collapsed. Individual non-collapsed frames of this stack, separated by 100 ms intervals, are shown in Fig. 3M.

Zebrafish ChR2 activation. A 5 dpf zebrafish larva (line) was suspended in a drop of E3 (recipe) on top of a microscope slide and placed immediately above the inverted camera objective. The NeoPixel 12 LED ring was placed about 2 cm above the specimen, facing down. Concurrent maximal activation of all 12 blue LEDs for more than 500 ms reliably elicited pectoral fin swimming bouts. Shorter stimuli were not effective. RGB image data was obtained at 42 Hz, downsampled by a factor of 4 in time and visualised by tracking the mean brightness of two regions of interest placed onto the pectoral fins.

Drosophila larva ChR2 activation. Xth instar Drosophila larvae (elav-GAL4/+; UAS-shibireT; UAS-ChR2/+; UAS-ChR2+/+) were placed on ink-stained agarose (recipe) within the lid of a 50 ml falcon tube and left to freely crawl. The camera and NeoPixel LED ring was placed about 3 cm above the surface. Concurrent activation of all 12 blue LEDs for 1 s at a time reliably triggered larval contractions. Image data acquired at 42 Hz and saved as 8 bit greyscale. Larval
length was quantified manually in ImageJ by measuring the distance between head and tail along the body axis at 3 time-points: t = -1, 0.5 and 5 s relative to the flash (t = 0-1 s). n = 12 responses from 3 animals, error bars in SD.

*Drosophila adult Chrimson activation.* Adult *Drosophila* (line) were fixed to a cover slide by gluing their wings with nail varnish, with all other limbs moving freely. The NeoPixel 12 LED ring was positioned around the camera objective about 2 cm above the fly pointing down. Concurrent maximal activation of all 12 red LEDs for 1 s, separated by 2 s intervals, reliably elicited the proboscis extension reflex. RGB image data was obtained at 42 Hz (x2 binning). The image stack was converted to 8 bit greyscale and background over time was subtracted from the entire image stack to limit the excitation light artefact. To calculate proboscis position over time, we plot image brightness over time within a region of interest placed at the tip of the fully extended proboscis.

Thermogenetics… need to write
FIGURE LEGENDS

Figure 1 – Overview. A, The 3D model, colour coded by core structure (black), mounting adapters (blue) and micromanipulator (green). B, Printed parts and electronics, part-assembled. C, Wiring diagram and summary of electronics. Full bill of materials (BOM) in Table 1. D, The assembled FlyPi with single micromanipulator and LED-ring module, diffusor and Petri-dish adapter mounted in the bottom. E, Filter wheel mounted above the inverted camera objective. F, Peltier Element and thermistor embedded into the base. G, Automatic focus drive. H, Petri-dish mounting adapters. I, A Second micromanipulator mounted to the left face of the FlyPi holding a probe (here : 200 µl pipette tip) above the microscope slide mounted by the right micromanipulator.

Figure 2 – Basic Light Microscopy. A,B, The camera and objective can be mounted in upright (A) or inverted mode (B). In each case, the micromanipulator allows accurate positioning of a microscope slide in the image plane, while the LED ring coupled to a series of diffusors provides for flexible spectrum and brightness illumination (shown in A). C, At low zoom, the magnification is appropriate to provide high-resolution colour images of several animals at once (here: D. melanogaster fed with different food dyes). D,E, When the objective is fully extended, magnification is sufficient to resolve large neurons of the mouse brain, while different positions of the LED ring permit to highlight different structures in the tissue. F, The system is also appropriate to provide high-resolution imagery of zebrafish larvae (D. rerio) with only room-lighting (cf. SVid. 1). G,H, Brugia malayi (G) and Wuchereria bankrofti (H) in human lymph tissue biopsy (staining). I, Schistosoma eggs in human urine. J, Mansonella perstans in human blood smear and K, magnification of bottom right image section (staining).

Figure 3 – Fluorescence Microscopy. A, A collimated 410 nm LED angled at 45° and two ultra-low-cost theatre-lighting filters provide for fluorescence capability. B, Photo of the fluorescence setup. C, Fluorescence test-slide. D, Spectra of excitation LED and filters superimposed on GFP excitation and emission spectra. E,F, Point-spread function (psf) measured using green fluorescent beads (Methods): SD ~5.4 μm. G,H, Zebrafish larva expressing GCaMP6f in neurons (Ubi:nls-GCaMP6f) in transmission (G) and fluorescence mode (H). I, At low zoom the system can be used for fish-sorting (cf. SVid 2). J-M. Calcium Imaging in Drosophila larva expressing GCaMP5 in muscles (Mef2-Gal4; UAS-myr::GCaMP5). J,K, Three regions of interest (ROIs) placed across the raw image-stack of a freely crawling larva (J) reveal period bouts of increased fluorescence as peristaltic waves drive up calcium in muscles along the body (K). Arrowheads in J indicate positions of peaks in calcium wave. L, A space-time plot of the time-differentiated image stack, averaged across the short body axis, reveals regular peristaltic waves. Warm colours indicate high positive rates of change in local image brightness. M, A single peristaltic wave (as indicated in L) in 12 image planes separated by 100 ms intervals. See also SVid. 5.
Figure 4 – Behavioural Tracking. A,B, Red-light illumination from the LED ring can be used to illuminate animals during behavioural tracking – here showing C. elegans on an Agar plate (B). C, A behavioural chamber based on two microscope slides and a 3D printed chassis is adequate for behavioural monitoring of adult Drosophila. D, Animals tracked using the Ctrax [REF].

Figure 5 – Optogenetics. A, Experimental configuration suitable for optogenetic stimulation of an individual zebrafish larva suspended in a drop of E3. B, Spectrum and peak power of the three LEDs embedded at each ring position. Spectral filters can be used to limit excitation light reaching the camera (Rosco Supergel No. 19, “Fire”). C, zebrafish larva (5 dpf) expressing ChR2 in all neurons (line). D, The animal exhibits pectoral fin bursts motor patterns upon activation of blue LEDs (cf. SVid X). E,F, Drosophila larvae expressing ChR2 in all neurons (elav-GAL4/+; UAS-shibreα; UAS-ChR2/+; UAS-ChR2/+ crawling on ink-stained agar reliably contract when blue LEDs are active. G,H, Proboscis extension reflex (PER) in adult Drosophila expressing Crimson in the gustatory circuit (courtesy of Olivia Schwarz and Jan Pielage, Friedrich Miescher Institute for Biomedical Research, Basel, unpublished data) is reliably elicited by activation of red LEDs.

Figure 6 – Thermogenetics. A, performance of the Peltier-thermistor feedback loop. Command 15 and 35°C indicated by blue and red shading; room temperature 19°C (no shading)…. 

Figure 7 – Classroom teaching and equipment improvisation. A, Graduate students from different African Universities building FlyPis during a workshop held in Durban, South Africa in March 2015. B,C African graduate students and faculty in Dar es Salaam, Tanzania, using FlyPis for optogenetics experiments on proboscis extension reflex as readout. D, FlyPi with adjustable focus module mounted on top of a Gel-Doc replaces missing commercial camera.
SUPPLEMENTARY METHODS: ASSEMBLY (to do)

Mainframe

PCB

LED Ring

RGB LED

Fluorescence Module

Peltier Module

Camera with focus drive

Micromanipulator

Motorising the manipulator

SUPPLEMENTARY VIDEOS

1) Zebrafish larva circulation (Related to Fig. 2F)
2) Zebrafish sorting (related to Fig. 3I)
3) Zebrafish larva expressing GFP in the heart (related to Fig. 3)
4) Zebrafish eggs expressing GCaMP5 in all neurons (related to Fig. 3)
5) Drosophila larva calcium imaging (related to Fig. 3J-M)
6) C. elegans crawling about (related to Fig. 4B)
7) Drosophila walking about (related to Fig. 4D)
8) Zebrafish ChR2 (related to Fig. 5C,D)
9) Drosophila larval ChR2 (related to Fig. 5E,F)
10) Drosophila adult Chrimson PER (related to Fig. 5G,H)
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LINES (notes for self)

- Ubi:nls-GCaMP6f (wie schon mal benutzt), schwach grün fluoreszierend

- E{E1b;Gal4}s1101t; Tg(UAS:Kaede)s199t; Tg(mnx1:tag-RFP-t), stark grün exprimierend und stark rot in Motoneuronen.

- Muscles in flies from Matthias: The line was made by Will Constance in Darren Williams’ lab at Kings College London, a recombinant on the third chromosome of published reagents: Mef2-Gal4 (drives expression in muscles), UAS-myr::GCamp5 (membrane targeted GCamp5 from Jan Melom, then in Troy Littleton’s lab - see attached paper).

- "Chrimson expressed in neurons of the Drosophila gustatory circuit (courtesy of Olivia Schwarz and Jan Pielage, Friedrich Miescher Institute for Biomedical Research, Basel, unpublished data)"