

Organic Light-Emitting Diodes for Optogenetic Stimulation of *Drosophila* Larvae

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Abstract: Optogenetics is an emerging method in biology that enables controlling neurons with light. We use organic light-emitting diodes to stimulate neurons in *Drosophila* larvae and investigate subsequent behavioral changes at different light intensities.

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1. Introduction

Optogenetics is an emerging new method in biology for the non-invasive and timely control of neuronal behaviour with light [1]. Currently, however, only a small number of neurons can be controlled individually so that stimulation of neurons with light still lacks precision compared to the complexity of the brain. To overcome these limitations, we propose to develop a novel light source based on organic light-emitting diodes (OLEDs) that provides unprecedented high-resolution optogenetic control of thousands of neurons at once.

OLEDs are plastic-type organic semiconductors that are sandwiched between two thin-film electrodes (Fig. 1). Compared to conventional light sources, OLEDs can in principle address single neurons and quickly switch between targeted cells by using microscopic patterning of the device. Recently, OLED microdisplays were used to stimulate and study locomotion of light-sensitive algae cells [2] and for optogenetic stimulation of HEK cells [3]. These cells can be stimulated at rather low brightness illumination ($\approx 1 \mu\text{W}/\text{cm}^2$) which is easily achieved in simple OLED structures. The stimulation of neurons, however, requires considerably higher light intensities in the range of $0.1 - 10 \text{ mW}/\text{mm}^2$ [4], a brightness at which OLED efficiency typically decreases [5].

In this contribution, we use an efficient fluorescent emitter and doped charge transport layers [6] in order to achieve very high brightness at low voltages. Concurrently, we demonstrate that these high-brightness OLEDs can be used to robustly excite neurons in living *Drosophila* (fruit fly) larvae.

2. High-brightness OLEDs

The structure of the OLEDs used in this study is shown in Fig. 2a. OLEDs are prepared by thermal evaporation in a UHV chamber (Angstrom Engineering) at a base pressure of 10^{-7} mbar on pre-cleaned glass substrates that were pre-patterned with ITO. The materials involved are 2,2',7,7'-tetrakis(N,N'-di-p-methylphenylamino)-9,9'-spirobifluorene (Spiro-TTB) p-doped with 2,2'-(perfluoronaphthalene-2,6-diyldiene)dimalononitrile (F6-TCNNQ) (2 wt%) as hole transport layer, N,N'-di(naphthalene-1-yl)-N,N'-diphenylbenzidine (NPB) as electron blocking layer, the fluorescent emitter 2,5,8,11-tetra-tert-butylperylene (TBPe) doped with 1.5 wt% into the host 2-methyl-9,10-bis(naphthalen-2-yl)anthracene (MADN) as emission layer, bis-(2-methyl-8-chinolinolato)-(4-phenyl-phenolato)-aluminium(III) (BAIq2) as hole blocking layer, and 4,7-diphenyl-1,10-phenanthroline (BPhen) doped with cesium (2 wt%) as n-doped electron transport layer, finished by an aluminum cathode. Samples are encapsulated in nitrogen atmosphere with a glass lid and moisture getter directly after fabrication. The active area of the device is 16.1 mm^2 .

Figure 2b shows the current density and optical power density of the device as a function of the voltage. Due to efficient doping of the charge transport layers, current densities above $1 \text{ A}/\text{cm}^2$ are reached at 12 V. We measured the power density using a calibrated power meter (Gentec-EO) with a photodiode (PH100-Si) placed in direct contact with the OLED, while the OLED was driven in steady state (voltage pulse length $> 1 \text{ s}$). A maximum brightness of $0.85 \text{ mW}/\text{mm}^2$ was reached at 14 V, which is sufficient to trigger action potentials in neurons that are transfected with relatively light-sensitive ion channels (e.g. ChR2 C128S [7], CsChrimson [8]). Optimizing the sample layout further, we believe that power densities above $1 \text{ mW}/\text{mm}^2$ can be reached even below 10 V by reducing the voltage loss in ITO contact lines.

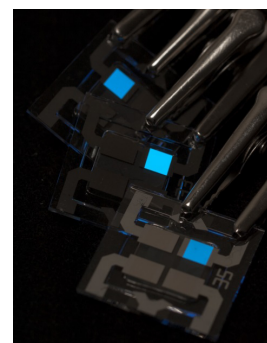


Fig. 1. Photograph of blue OLEDs from our lab

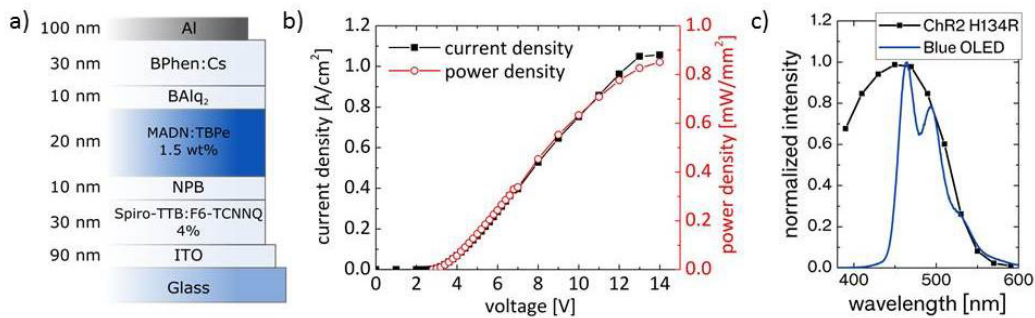


Fig. 2. a) Structure of the OLED. b) Current density and optical power density of the OLED as a function of the voltage. c) OLED emission spectrum and activation spectrum of the channelrhodopsin ChR2 H134R [9].

A key requirement to enable optogenetic activation is a good overlap of the emission spectrum of the light source with the activation spectrum of the used channelrhodopsin. Figure 2c shows the emission spectrum of the OLED in forward direction. The emission shows two peaks at 465 nm and 493 nm and the maximum of the spectrum is located at the maximum of the activation spectrum of the channelrhodopsin ChR2 H134R [9]. In addition, the relatively broad spectrum of the OLED overlaps very well with the activation spectrum of the ChR making the OLED highly suitable for optogenetic activation.

3. Stimulating neurons in live animals

In order to test that the brightness of our OLEDs is sufficient to trigger action potentials in neurons, we used the devices to stimulate living *Drosophila* larvae that were expressing the channelrhodopsin ChR2 H134R in motor neurons. Stimulation of the larvae with blue light thus leads to a firing of action potentials in motor neurons, which causes a visible contraction and immobilization of the larva [10,11].

OK371-GAL4/UAS-H134R-ChR2 flies were raised on solid cornmeal-based medium supplemented with 1mM all-trans-retinal. We mounted the OLED underneath an upright microscope (Nikon Eclipse Ni) with 4x/0.13 NA objective and studied the behavior of third instar larvae by confining them in a silicone chamber filled with sucrose solution (5% w/v), which was located on top of the OLED. The sample was illuminated by a fibre-coupled mercury light source attenuated with ND filters to $< 1 \text{ mW/mm}^2$ and long pass filtered ($> 600 \text{ nm}$) in order to avoid activation of the ChR. The OLED was driven with voltage pulses of 5 s duration and 50% duty cycle (0.1 Hz frequency). The driving voltage of the OLED was varied in order to study the larval response to different brightness levels. The larval behavior was measured with an attached sCMOS camera (Andor Neo) over the course of 60 s at a speed of 10 frames/s.

Exemplary images of the larva on top of the OLED are shown in Fig. 3a. The larva was relaxed if the OLED was turned off but showed a strong contraction when the OLED was turned on. In order to quantify the larval response, we tracked the positions of head and tail in each frame of the recorded traces using the software ImageJ and the plugin MtrackJ. From those positions, we calculated the distance between head and tail, which indicates whether the larva is relaxed or contracted. OLED illumination timing was tracked from the videos as well. As an example, Figure 3b shows head-tail distance traces for two OLED power densities, marking the OLED on-time by blue shaded areas. Upon OLED turn-on, a robust contraction of the larva is observed, while the larva returns to its relaxed state within less than 1 s after the OLED is turned off. This behavior is achieved at power densities of $10 \text{ } \mu\text{W/mm}^2$ or above, whereas lower power densities caused no visible contraction.

In order to verify that the contractions are caused by the OLED light source, we calculated the Fourier transform of the head-tail distance traces. The result is shown in Fig. 3c. For power densities from $10 \text{ } \mu\text{W/mm}^2$, a clear peak in amplitude is observed at 0.1 Hz, the frequency used for switching the OLED. In contrast, lower stimulation brightness causes no observable peak in the Fourier transform, confirming that the light from the OLED did not lead to action potential firing.

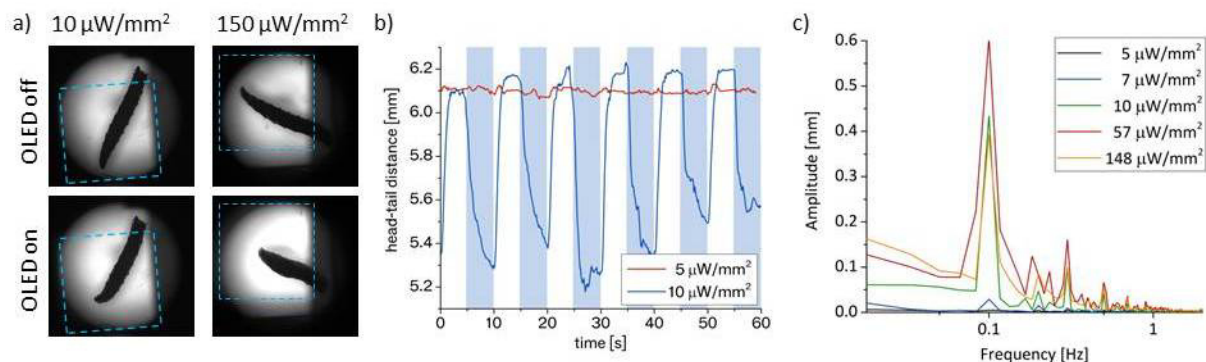


Fig. 3. Larval response upon stimulation with 5 s long light pulses at different optical powers. a) Image of the larva on top of the OLED. Top: OLED off. Bottom: OLED on. Blue dashed lines indicate the OLED pixel area. b) Head-tail distance traces. Blue shaded areas mark when the OLED was turned on. A robust contraction of the larva is detected for power densities from $10 \mu\text{W}/\text{mm}^2$. c) Fourier-transform of the head-tail distance traces marking a peak response at the stimulation frequency of 0.1 Hz.

4. Conclusions

In this contribution, we demonstrated that OLEDs are capable of stimulating neurons in living animals. In particular, we have proven that OLEDs can achieve high enough brightness to trigger robust action potential firing. We envisage that the brightness of our OLEDs can be further increased by reducing resistive losses in the ITO contacts and by reducing the pixel area in order to decrease losses due to Joule heating.

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