Review of: "An economical and highly adaptable optogenetics system for individual and population-level manipulation of Caenorhabditis elegans"

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This work presents a low cost LED illumination system that can be used in a variety of contexts. While many LED illumination systems for optogenetics exist (Bugaj and Lim, 2019; Crawford and San-Miguel, 2020; Gerhardt et al., 2016; Kawazoe et al., 2013; Kyriakakis et al., 2021; Pokala and Glater, 2018; Repina et al., 2020; Robbins et al., 2021), some are complicated to build and/or are more costly. The assembly of the system with some optional configurations is described, including manual and automated optical stimulation versions. It is also helpful that some of the theory and reasoning for the design decisions are discussed, as well as some limitations of the system. The authors extensively validate the system by stimulating Channel Rhodopsin 2 (ChR2) in *C. Elegans* and used it to show the illumination system's utility in discriminating specific light-induced behaviors in *C. Elegans*.

While there are many options for Do It Yourself "DIY" LED illumination systems, they vary in the details of the instructions, costs, automation, validation, the flexibility for different contexts and the intended purpose. For example, the "OptoGenie" can be battery powered, meaning it can be placed almost anywhere and has some level of automation (Robbins et al., 2021). Our work (Kyriakakis et al., 2021) has software and hardware for controlling several LEDs, but does not have the lenses for focusing the light enough to use with ChR2 from a distance as "Optogenie" and the LED system presented in this work. Other systems are specifically designed for use with multi-well plates (Bugaj and Lim, 2019; Gerhardt et al., 2016; Repina et al., 2020). In short, they all have different niches they can fill. This work describes a system with a satisfactory level of detail for non-expert in electronics to build and still allows for automation at a low cost. The one part I am not sure is easily achievable (or at least at a low cost) is preparing the lens holder from a lathe. Perhaps if this was 3D printed, sharing the 3D print file would make it easier for others to produce.

One important characterization done in this work is the for light intensity and light distribution. The authors use the system with different lenses and characterize it by shining light at different angles. This is important because these model of LEDs are commonly used, and this work describes how to apply them with enough power to fully activate ChR2, which is the most light optogenetic tool currently used. To

determine how much the system changes as it warms up, the authors measure the light intensity after the LED has been on for several minutes. One measurement that may have been useful is a longer term measurement after the LED has been on for an hour or more, assuming it has reached a temperature equilibrium. A minor point, but it would have been simple to record the room temp and leave the LED on for an extended period.

The system was extensively characterized in worms that ChR2 expressed by promoters that express it in different cell types (excitatory and inhibitory neurons). The authors validated this tool could elicit specific behaviours that are comparable to using a fluorescent microscope. However, unlike the microscope that is limited to a single worm at a time, this system can stimulate multiple worms at a time. The authors additionally developed an ImageJ plugin for tracking the multiple worms' behaviour. This was done by mounting a simple smartphone onto the stereoscope and taking videos to be analyzed with the plugin. Using a smartphone makes for a simple and inexpensive setup for these types of experiments. The authors use this setup to show that it is sensitive enough to distinguish between behaviours when using the LED to activate excitatory versus inhibitory neurons.

Overall, this system is a nice addition to the toolbox of inexpensive LED illumination systems, as well as for imaging and quantifying optogenetically modulated behaviours in worms. The authors note that this system could also be used in Drosophila, as they require only a fraction of the light intensity for activation that this system provides (Meloni et al., 2020; Pulver et al., 2009). Because of the power of the system, it could be used to stimulate many other optogenetic systems or ChR2 in other systems, such as cell cultures. The combination of detailed assembly instructions, characterization of the LED performance in different arrangements, software for controlling the LED, software for quantifying the worms behaviour, and the validation *in vivo* provides users a complete setup without the need to develop any parts of the system on their own.

Bibliography

Bugaj, L.J., and Lim, W.A. (2019). High-throughput multicolor optogenetics in microwell plates. Nat. Protoc. *14*, 2205–2228.

Crawford, Z., and San-Miguel, A. (2020). An inexpensive programmable optogenetic platform for controlled neuronal activation regimens in C. elegans. APL Bioengineering *4*, 016101.

Gerhardt, K.P., Olson, E.J., Castillo-Hair, S.M., Hartsough, L.A., Landry, B.P., Ekness, F., Yokoo, R., Gomez, E.J., Ramakrishnan, P., Suh, J., et al. (2016). An open-hardware platform for optogenetics and photobiology. Sci. Rep. *6*, 35363.

Kawazoe, Y., Yawo, H., and Kimura, K.D. (2013). A simple optogenetic system for behavioral analysis of freely moving small animals. Neurosci. Res. *75*, 65–68.

Kyriakakis, P., Fernandez de Cossio, L., Howard, P.W., Kouv, S., Catanho, M., Hu, V.J., Kyriakakis, R., Allen,

M.E., Ma, Y., Aguilar-Rivera, M., et al. (2021). Building a simple and versatile illumination system for optogenetic experiments. J. Vis. Exp.

Meloni, I., Sachidanandan, D., Thum, A.S., Kittel, R.J., and Murawski, C. (2020). Controlling the behaviour of Drosophila melanogaster via smartphone optogenetics. Sci. Rep. *10*, 17614.

Pokala, N., and Glater, E.E. (2018). Using Optogenetics to Understand Neuronal Mechanisms Underlying Behavior in C. elegans. J. Undergrad. Neurosci. Educ. *16*, A152–A158.

Pulver, S.R., Pashkovski, S.L., Hornstein, N.J., Garrity, P.A., and Griffith, L.C. (2009). Temporal dynamics of neuronal activation by Channelrhodopsin-2 and TRPA1 determine behavioral output in Drosophila larvae. J. Neurophysiol. *101*, 3075–3088.

Repina, N.A., McClave, T., Johnson, H.J., Bao, X., Kane, R.S., and Schaffer, D.V. (2020). Engineered illumination devices for optogenetic control of cellular signaling dynamics. Cell Rep. *31*, 107737.

Robbins, M., Siddiqui, O., Fuchsberger, T., Goodfellow, G., Paulsen, O., Kaminski, C.F., Euser, T., and Schierle, G.S.K. (2021). OptoGenie: an open-source device for the optogenetic stimulation of cells. Journal of Open Hardware *5*.