

# Automated calibration of optoPlate LEDs to reduce light dose variation in optogenetic experiments

Edvard OS Grødem<sup>1</sup> , Kieran Sweeney<sup>1</sup>  & Megan N McClean<sup>\*,1,2</sup> 

<sup>1</sup>Department of Biomedical Engineering, University of Wisconsin–Madison, Madison, WI, USA; <sup>2</sup>Carbone Cancer Center, University of Wisconsin School of Medicine & Public Health, Madison, WI, USA; \*Author for correspondence: [mmcclean@wisc.edu](mailto:mmcclean@wisc.edu)

BioTechniques 69: 313–316 (October 2020) 10.2144/btn-2020-0077

First draft submitted: 29 May 2020; Accepted for publication: 13 July 2020; Published online: 29 July 2020

## ABSTRACT

Optogenetic systems use light to precisely control and investigate cellular processes. Until recently, there had been few instruments available for applying controlled light doses to cultures of cells. The optoPlate, a programmable array of 192 LEDs, was developed to meet this need. However, LED performance varies, and without calibration there are substantial brightness differences between LEDs on an optoPlate. Here we present a method for calibrating an optoPlate that uses a programmable microscope stage and optical power meter to automatically measure all 192 LEDs of an optoPlate. The resulting brightness measurements are used to calculate calibration values that tune the electrical current supplied to each optoPlate LED to reduce brightness variation in optogenetic experiments.

## METHOD SUMMARY

A programmable microscope stage and optical power meter are used to automatically measure all 192 LEDs of an optoPlate. The resulting brightness measurements are used to calculate calibration values that tune the electrical current supplied to each LED to reduce brightness variation between LEDs.

## KEYWORDS:

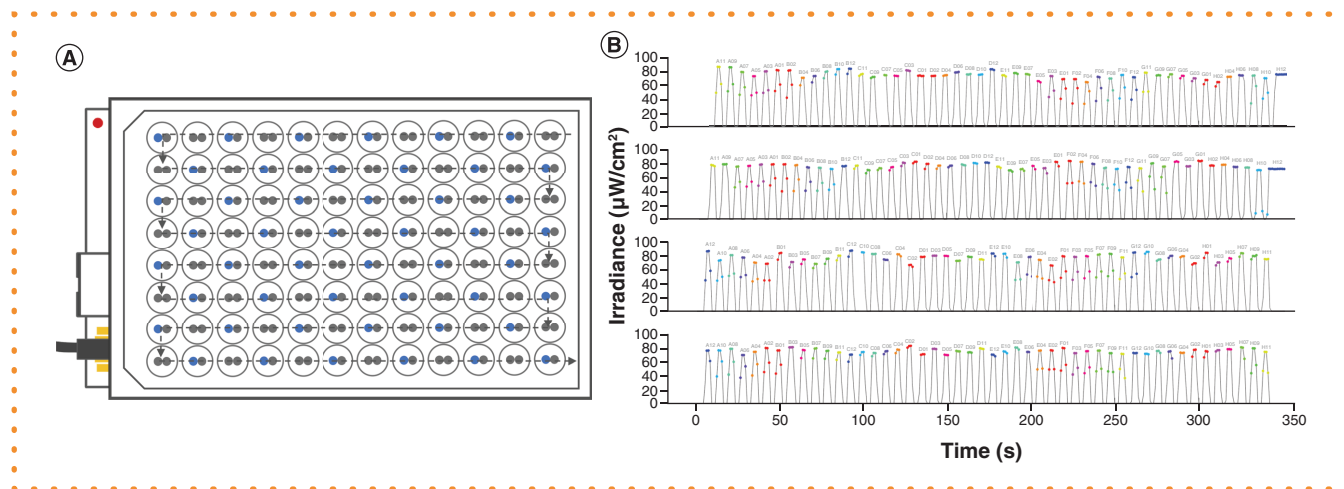
calibration • optogenetics • optoPlate

The availability and capability of optogenetic systems – genetically encoded, light-sensitive proteins that can control cellular events such as gene expression or protein localization – are ever expanding. It is important to control these optogenetic systems with precise, quantifiable and reproducible light doses, as their response is often light dose dependent and excess light can be harmful to cells. The optoPlate [1], a programmable array of 192 LEDs configured to deliver a custom, time-varying light dose to each well of a 96-well plate, was developed to meet this need. However, without calibration, there are substantial brightness differences between LEDs on an optoPlate, even when each LED receives the same electrical current. With all LEDs of an optoPlate receiving a pulse width modulated 1.9-mA current at 50% duty cycle, we observed maximum and minimum LED irradiances of 113.7 and 80.1  $\mu\text{W}/\text{cm}^2$ , respectively.

This is a known problem with LEDs, the solution to which is to tune the pulse width modulation of the current supplied to each LED such that the brightest LEDs are dimmed to match the dimmest LED [2,3]. This process can involve multiple rounds of brightness measurements and tuning to get right. Here we present a fast method for calibrating an optoPlate in which an optical power sensor is automatically passed over the LEDs in a set order to acquire irradiance measurements from which we calculate a tuning value per LED. We use a programmable microscope stage to automatically move the optical power sensor, although this method could be implemented using other devices whose movement can be programmed, such as a 3D printer. We assembled the optoPlate as described by Bugaj and Lim [1] but replaced its firmware to allow calibration such that the brightness of each LED is controlled by both a calibration value and an input value as follows:

$$\text{LED intensity} \propto \frac{\text{input values}}{255} \times \frac{\text{calibration values}}{255}$$

The input value is supplied by a user to control LED brightness over the course of an experiment. The calibration value must only be set once and accounts for brightness differences between LEDs so that two LEDs set to the same input value will have the same brightness after calibration. Our optoPlate firmware was built with PlatformIO and allows users to design LED light programs and flash them to the optoPlate using MATLAB. All custom software and hardware components used are available in the supplementary materials, along with a detailed description of our protocol and all experimental data presented here.



**Figure 1. Measuring optoPlate LEDs.** (A) Our optoPlate calibration method involves a measurement process in which an optical power meter is rastered across the optoPlate over a series of four passes in which one LED is active in alternating wells. (B) This generates irradiance measurements from which it is easy to identify each LED and measure its brightness.

To calibrate an optoPlate, we first mount it and an optical power sensor to a microscope using 3D-printed adaptors (included in the Supplementary materials). We initially set all optoPlate LEDs to the maximum possible calibration value (255) and the same, constant input value. We first turn the focus knob to move the optical power sensor to a set level close to the mounted optoPlate (we use a z-position of 4000 µm), then use the microscope's programmable stage to automatically move the optical power sensor over each optoPlate LED in a set order (Figure 1A) to acquire a time series of irradiance measurements from which the brightness of each LED can be determined (Figure 1B) and used to calculate calibration values. To ensure that each LED creates a distinct peak in the irradiance signal, we do not measure all 192 LEDs in one pass. Instead, we measure the LEDs over the course of four passes in which we raster the optical power meter across the optoPlate such that every other LED encountered is inactive, leading to an irradiance signal in which measurements of inactive LEDs create clear troughs between measurements of bright LEDs. We achieve this by setting the optoPlate to deliver a checkerboard illumination pattern such that no two illuminated wells are directly above, below or beside each other in a given pass. We measure the remaining wells by setting the optoPlate to deliver the inverse checkerboard illumination pattern. Because the optoPlate has two LEDs per well of a 96-well plate, we repeat this process twice: once for the left LED and once for the right LED.

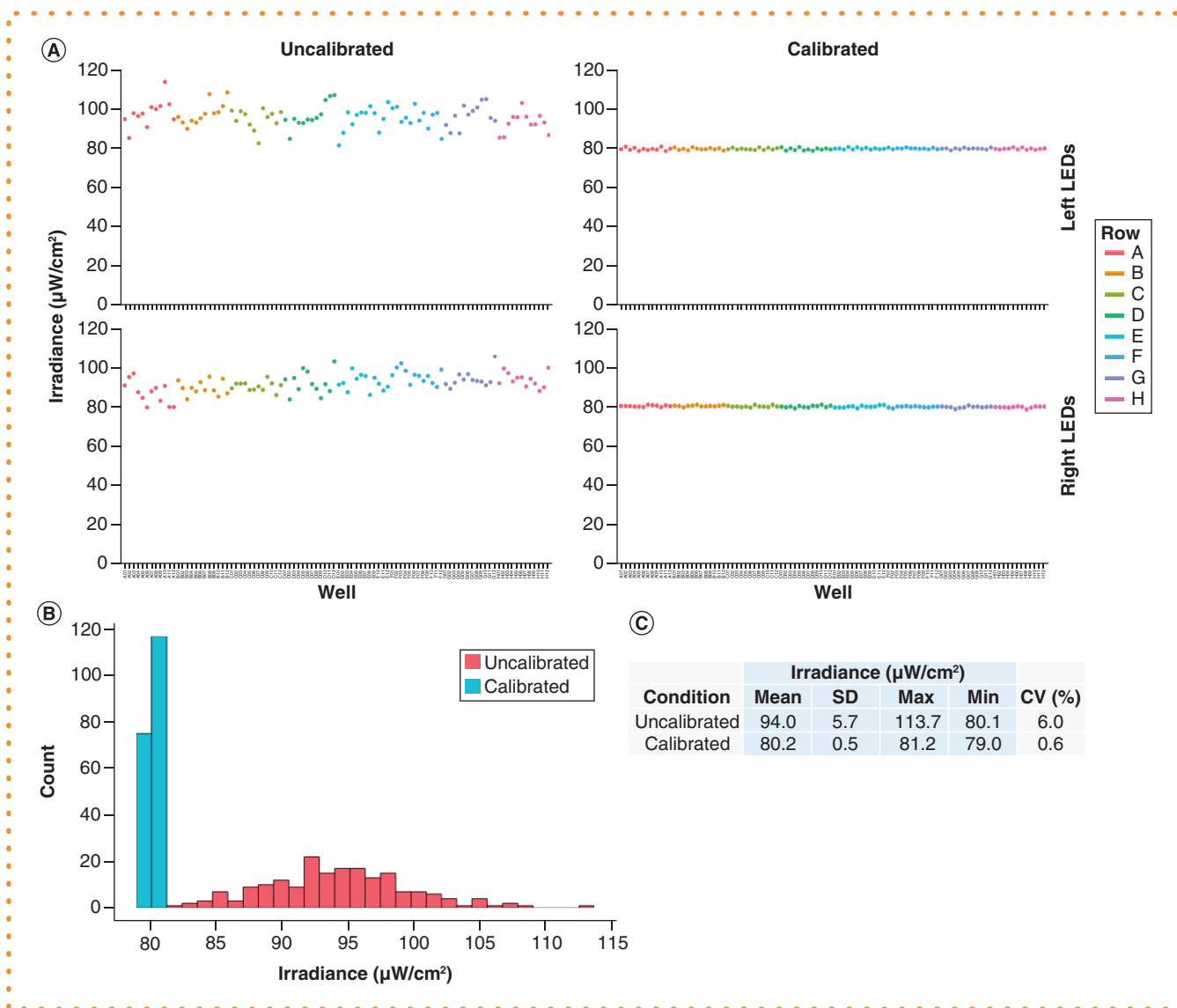
Once all optoPlate LEDs are measured, we analyze the irradiance measurements using a MATLAB script that determines the brightness of each LED (based on the known order in which they were measured) and calculates calibration values such that the brightest LEDs are dimmed to match the dimmest LED. We flash these calibration values to the optoPlate and repeat the whole process until the coefficient of variation of the LED irradiance measurements falls below a set threshold (we use 1%). Because the optoPlate calibration script calculates calibration values using a proportional controller, they are guaranteed to converge to the correct value over successive rounds of calibration. It should be noted that this is how one calibrates an optoPlate with two LEDs of the same color per well. Additional considerations for calibrating an optoPlate with up to three different-colored LEDs per well is provided in the Supplementary protocol.

To demonstrate that it works, we used this method to calibrate one of our laboratory's optoPlates (Figure 2). Before calibration, the mean irradiance of all LEDs was 94.0 µW/cm<sup>2</sup> with a CV of 6.0%. Notably, the brightest LED was 1.40-times brighter than the dimmest LED. Such a large variation in light dose could easily obscure relevant biological phenotypes in optogenetic experiments [4]. Following just one round of calibration, this variation was substantially reduced. The brightest LEDs were dimmed to match the irradiance of the dimmest LED before calibration (80.1 µW/cm<sup>2</sup>) such that the mean irradiance of all LEDs was 80.2 µW/cm<sup>2</sup> with a CV of 0.6%. After calibration, the brightest LED was just 1.01-times brighter than the dimmest LED.

Once an optoPlate is calibrated, it is useful to establish the dose–response relationship between the input values and irradiance so that it is possible to set experimental light doses per well in absolute units (rather than by input value, which is arbitrary) and consistently achieve equivalent light doses with different optoPlates. This is done by measuring irradiance for a given input value and solving the following linear equation for the slope  $m$ :

$$\text{Irradiance} = m \times \text{input value}$$

Although this can be done with a single measurement, to demonstrate that this relationship is reliably linear, we set both LEDs of a given well to the same input value and measured well irradiance for input values ranging from 10 to 240 (Figure 3A). We acquired these

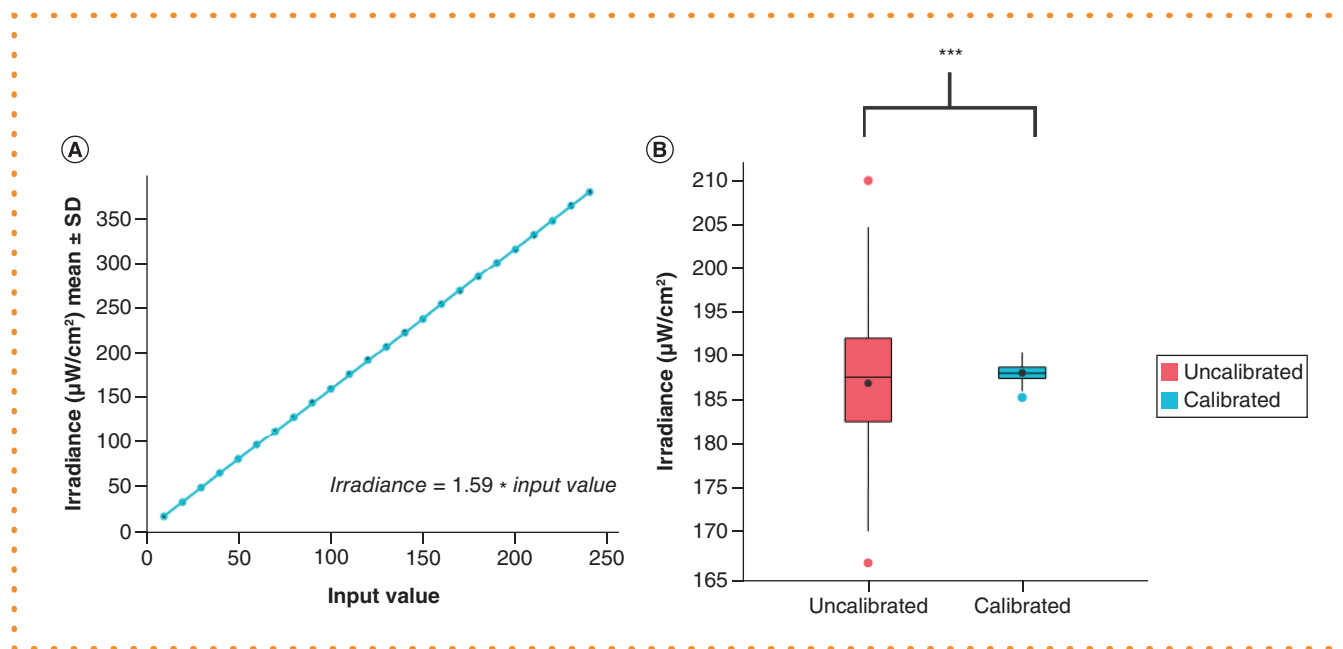


**Figure 2. Calibration reduces optoPlate LED irradiance variation.** (A) Measurements of LED irradiances before and after calibration show that calibration greatly reduces LED brightness variation. (B) A histogram depicting the same data shows that calibration reduces LED variation by dimming the brightest LEDs to match the irradiance of the dimmest LEDs. (C) Experimental light doses can vary strongly for an uncalibrated optoPlate; these differences almost disappear following calibration.

measurements quickly using the automated measurement process described for calibration and then fit these measurements to the linear equation to determine that  $m = 1.59$  for our optoPlate.

Knowing the relationship between input value and well irradiance, we next showed that the reduction in LED brightness variation achieved by calibration is not simply an artifact arising from the overall reduction in LED brightness. We set all input values of an uncalibrated optoPlate to 100 and measured the irradiance of each well. We then calibrated the optoPlate and determined that an input value of 118 was necessary to achieve the same mean irradiance as the uncalibrated optoPlate. Measurements acquired before and after calibration show that the mean irradiance per well of the calibrated optoPlate matches that of the uncalibrated optoPlate (with an approximation error of 0.64%) but with significantly reduced variation.

An uncalibrated optoPlate is a large potential source of unwanted variation in optogenetic experiments. Manually calibrating all 192 optoPlate LEDs would be laborious. By comparison, our method enables the quick and easy calibration of an optoPlate using standard equipment that most laboratories performing quantitative optogenetic experiments should have on hand. Because an optical power meter can be set to measure different wavelengths of light, our method can be used to calibrate different-colored LEDs. As more instruments for optogenetic stimulation of cells are introduced, the general approach outlined here could be adapted to speedily quantify and calibrate their light output.



**Figure 3. Calibration validation.** (A) The dose–response relationship between input value and light dose for a calibrated optoPlate. Four measurements were made per input value. Standard deviation of the measured irradiances is small and obscured by the markers. (B) Brightness variation for an optoPlate at a fixed brightness level before and after calibration. All optoPlate wells were measured for each condition. Asterisks indicate  $p < 0.001$  as calculated by Levene’s test for equality of variances.

## Protocol

The protocol for this article is available at [dx.doi.org/10.17504/protocols.io.bivmke46](https://doi.org/10.17504/protocols.io.bivmke46)

## Author contributions

E Grødem and K Sweeney contributed equally to the conception of the method; hardware and software design; acquisition, analysis and interpretation of data; drafting of the article and critical revision. MN McClean contributed to the conception of the method; acquisition, analysis and interpretation of data; drafting of the article; critical revision and general supervision.

## Financial & competing interests disclosure

This work was supported by the National Institute of Allergy and Infectious Diseases (R21AI135166) and the National Institute of General Medical Sciences (R35GM128873). K Sweeney is supported by a Genomic Sciences Training Program NHGRI Training Grant (5T32HG002760). MN McClean holds a Career Award at the Scientific Interface from the Burroughs Wellcome Fund. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

## Open access

This work is licensed under the Attribution-NonCommercial-NoDerivatives 4.0 Unported License. To view a copy of this license, visit <http://creativecommons.org/licenses/by-nc-nd/4.0/>

## References

1. Bugaj LJ, Lim WA. High-throughput multicolor optogenetics in microwell plates. *Nat. Protoc.* 14(7), 2205–2228 (2019).
2. Gerhardt KP, Olson EJ, Castillo-Hair SM *et al.* An open-hardware platform for optogenetics and photobiology. *Sci. Rep.* 6, 1–13 (2016).
3. Sweeney K, Moreno Morales N, Burmeister Z, Nimunkar AJ, McClean MN. Easy calibration of the Light Plate Apparatus for optogenetic experiments. *MethodsX* 6, 1480–1488 (2019).
4. Johnson HE, Goyal Y, Pannucci NL, Schüpbach T, Shvartsman SY, Toettcher JE. The spatiotemporal limits of developmental Erk signaling. *Dev. Cell.* 40(2), 185–192 (2017).