HIGH-THROUGHPUT MULTICOLOR OPTOGENETICS FOR THE SYSTEMATIC MANIPULATION OF CELL BEHAVIOR

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Cellular optogenetics present a powerful approach to control biochemical reactions within living cells and tissues. In principle, optogenetic control offers data-rich experiments with precise perturbations and multiple channels of control (multiple stimulation colors). Further, because light patterns are programmable and light can be applied remotely, optogenetic experiments could be readily scaled up for high-throughput manipulation without the need for expensive robotics, bespoke microfluidics platforms, and perturbative media exchanges. However, there is a lack of methods to perform optogenetic stimulation in a high-throughput microwell format. Here, we describe the optoPlate-96, an LED-based device for independent control of 96 3-color optogenetic experiments in 96- or 384-well plates. The optoPlate-96 is compatible with long-term illumination both in cell culture incubators, as well as in live-cell microscopy. We will first demonstrate how the optoPlates enable more rapid, robust, and reproducible optogenetic experiments. Then, we will briefly detail two studies that highlight the utility of optoPlate experiments. In the first, we used the optoPlate to systematically assess how cancer cells process Ras-Erk signal dynamics differently from normal cells. In the second study, we demonstrated how 3-color (blue, red, far-red) optoPlate-96 illumination enables orthogonal control of red- and blue-responsive optogenetic proteins within the same cell. We used multicolor probing to study signal integration between Ras- and PI3K signaling and uncovered new synergies between these two well-studied pathways. We anticipate that the optoPlate-96 will find broad utility and will help realize the potential of optogenetics for the quantitative dissection and manipulation of living systems.

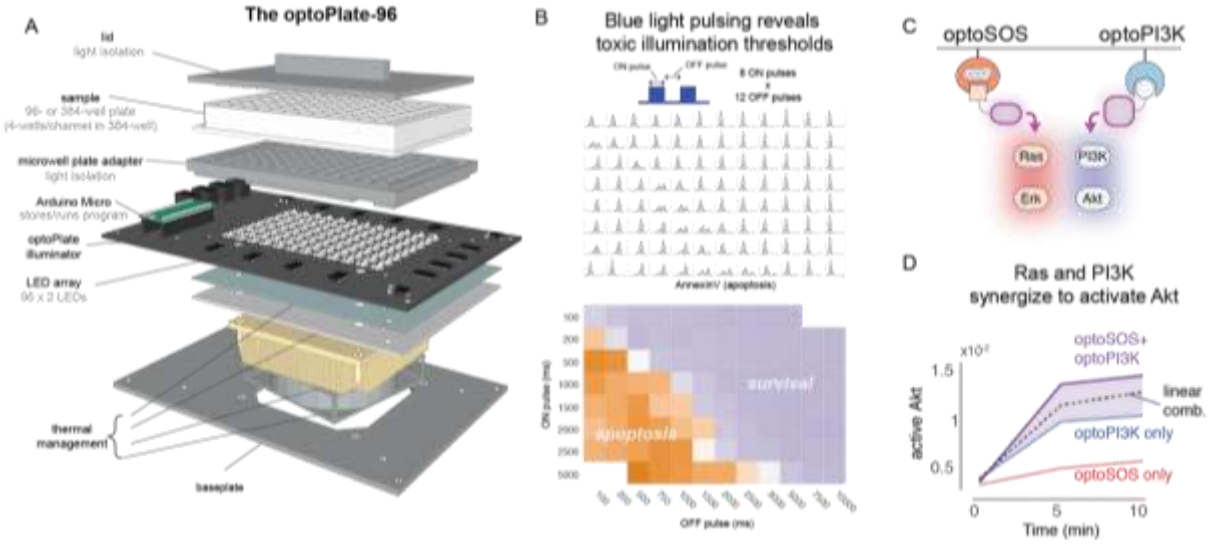

Figure 1 – A) The optoPlate-96 (exploded schematic shown) is an LED-based device for high-throughput microwell optogenetic experiments. B) Rapid scanning of blue light illumination patterns reveals toxic and non-toxic stimuli. C) Multiplexing red-and blue-responsive optogenetic probes uncovers synergy between Ras and PI3K activation of Akt.