Optogenetic control of calcium signaling over individual cells with a micro-LED array

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Abstract: We report a 16 μm-pitched micro-LED array that enables single-cell optogenetics with *in vitro* calcium imaging. Our LEDs can output bright, localized light to optogenetically address cells that are sub-10 μm apart with low crosstalk.
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Optogenetics is a powerful tool to interrogate specific cell types in complex tissues by optically altering cell activity for research at the levels of cells, circuits and behavior. To extend optogenetics to emerging single-cell studies, high-density light sources are needed to selectively address individual cells in dense populations, since it is technically challenging to achieve single-cell optogenetics via biology assays. Among available light sources, micro-sized light-emitting diode arrays (i.e. micro-LED) are suitable for high-precision optogenetic control for their scalability, good lifetime in biological environments, and medium power dissipation for *in vivo* use. To date, micro-LED arrays have been largely focused on studying action potentials or photocurrent in LED-illuminated cells [1-3]. While these early studies showcased single-cell optogenetics using micro-LEDs, studying cell activity in the electrical domain only is insufficient to fully understand cellular network dynamics. In fact, biochemical signals such as intracellular calcium concentration play an essential role in the regulation of muscle contraction, neurotransmitter release, and gene expression [4]. To this end, here we employ a high-density GaN-based micro-LED array to demonstrate optogenetic control of intracellular Ca^{2+} dynamics in single cells that are sub-10 μm apart with low crosstalk.

To conduct Ca^{2+} imaging under optogenetic stimulus, we chose a genetically coded Ca^{2+} indicator *jRCaMP1a* [5] to pair with the optogenetic actuator *ChR2* [6]. This way we can minimize the optical crosstalk between the excitation light (~575 nm from the microscope for *jRCaMP1a* imaging) and the activation light (~460 nm from the LEDs for optogenetic control via *ChR2*). Human embryonic kidney 293 cells (i.e. HEK 293) co-expressed with *jRCaMP1a* and *ChR2* were cultured on a polydimethylsiloxane (PDMS) piece and flipped upside down to face the LED array (Fig. 1a). On the device side, we fabricated a cross-bar structured, GaN-based micro-LED array that can output 462/19 nm light using standard lithography and dry etching steps [7]. The 4-by-4 pixels were patterned in 6.5 μm-by-6.5 μm sizes and placed in a 16-μm pitch, which is close to the typical diameter of a HEK 293 cell. To allow device usage in the cell medium, the array was then encapsulated with a cross-linked SU8 layer on top, and wire-bonded onto a printed circuit board (PCB). To enable optogenetic control at the single-cell level, micro-LEDs are required to output bright and localized light. To this end, we measured the optical power density (P_{light}) and the spatial profile of the illumination spot (I_{light}) of each LED pixel, respectively (Figs. 1b, 1c). When biased at injection currents (I_{LED}) ranging from 0.1 to 2.0 μA, all 16 pixels show high brightness with P_{light} ~ 0.1-1.0 mW/mm^2 (sufficient for HEK cells) and small light spots with the full width at half maximum (FWHM) < 10 μm at the array surface. Such bright and localized pixel output is encouraging for addressing single HEK cells close to the array surface.

During Ca^{2+} imaging experiments, we pulsed 575/25 nm excitation light using a microscope (Nikon FN1) with 0.5 frame per second and 100 ms exposure time per frame to alleviate the photo-bleaching effect (Fig. 2a). Meanwhile, the cell of interest was optogenetically stimulated by LED pixels in three consecutive recording periods (realigned in Fig. 2b). In each period, we illuminated the select LED pixel 20 s after the Ca^{2+} signal of the cell reached to the steady state, with the excitation light being shut off at the same time. Accordingly, we first studied the response of a single HEK cell sequentially illuminated by each pixel of the array. We found that the mean $\Delta F/F_0$ values right after the stimulation were larger in LED pixels that are closer to the cell, showing a hot spot in the $\Delta F/F_0$ map from all 16 pixels (Fig. 3c). This result suggests that our array can be used to study how each portion of the cell contribute to the overall cell activity. Next, we examined if our array can address densely packed individual cells. To achieve this, one pair of cells that are sub-10 μm apart was separately stimulated by pixel 13 and pixel 8 in the array (Fig. 3a). It is found that each LED pixel indeed evoked more Ca^{2+} signals (mean $\Delta F/F_0$ values) in the cell that is spatially closer to the pixel (e.g. pixel 13 evoked more signals in cell 1, see Fig. 3b), with the selectivity between two cells, defined as the ratio of their corresponding $\Delta F/F_0$ values, being larger than 3 (Fig. 3c) These results tell us that our LED pixel is indeed bright enough to optogenetically trigger Ca^{2+} influx to the illuminated cell. These data suggest that our array can indeed address individual cells that are sub-10 μm apart with low crosstalk; pixels 13 and 8 can provide such precise optogenetic control over cell 1 and cell 2, respectively.

In sum, we demonstrated single-cell optogenetic control of calcium signaling with a 16 μm pitched GaN micro-LED array, which can address single cells that are sub-10 μm apart with low crosstalk. We expect that such device can be broadly employed to study other cell types (e.g. neurons) and other cellular signals (e.g. potassium concentration).

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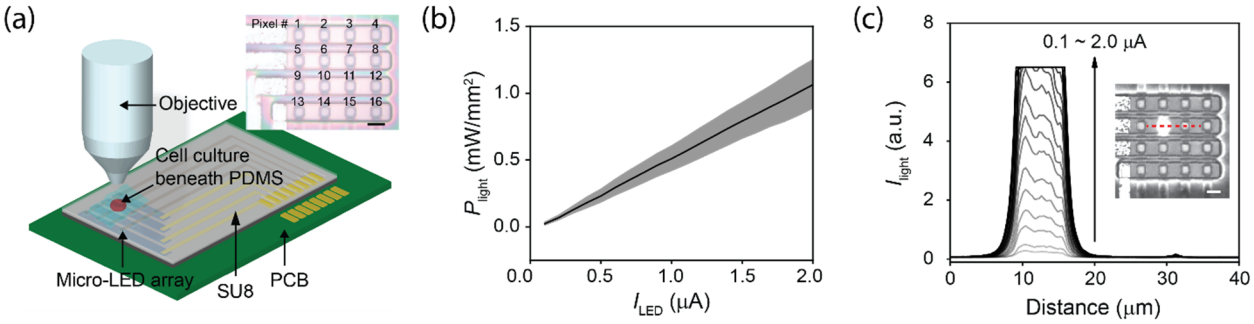

Figure 1. Device characterization. (a) Illustration of the experimental setup using a 4-by-4 micro-LED array. Scale bar, 10 μm. (b) P_{light} versus I_{LED} for all 16 pixels. (c) Spatial profile of the pixel output at the array surface with I_{LED} ranging from 0.1 to 2.0 μA. Inset shows the array with one pixel being illuminated. The dash line was employed to analyze the spatial profile of the pixel output. Scale bar, 10 μm.

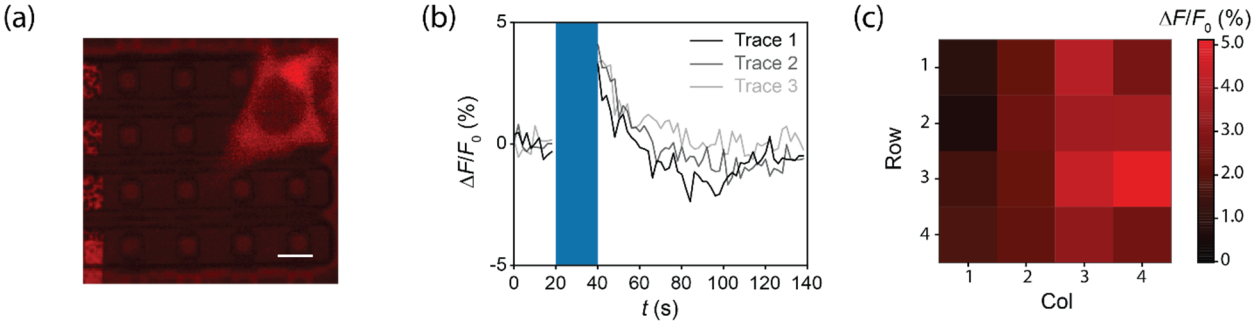

Figure 2. Single-cell experiment. (a) One cell cultured on the LED array (co-expressed with *jRCaMP1a* and *ChR2*). Scale bar, 10 μm. (b) re-aligned $\Delta F/F_0$ traces under 20-s optogenetic stimulus by pixel 8 (see Fig. 1a) in three consecutive recording periods. The blue window represents the period of optogenetic stimulus. (c) Mapping of the mean $\Delta F/F_0$ values right after the optogenetic stimulus from all 16 pixels.

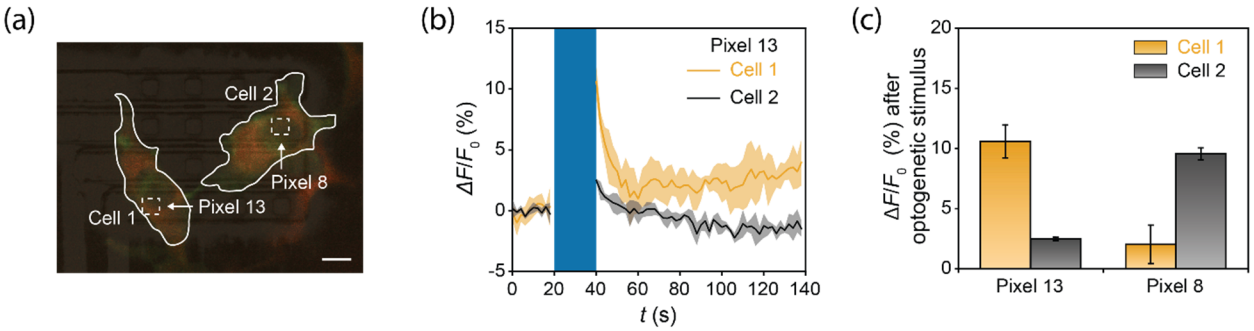

Figure 3. Two-cell experiment. (a) One pair of cells (outlined, overlapped with pixel 13 and pixel 8) that were sub-10 μm apart. Scale bar, 10 μm. (b) $\Delta F/F_0$ traces of cell 1 and cell 2 stimulated by pixels 13. Solid lines represent the mean values from three consecutive recording periods; shaded areas represent ±1 s.d. The blue window represents the period of optogenetic stimulus. (c) Analysis of the cell selectivity for both pixel 13 and pixel 8. Error bars represent ±1 s.d. (n = 3 recording periods).

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