

FAST TARGET-SELECTIVE CHEMICAL & OPTICAL STIMULATION BASED ON HIGH-THROUGHPUT MULTI-CHANNEL IMAGING DEVICE

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ABSTRACT

Caenorhabditis elegans (*C. elegans*) has been widely used in genetic and neurobiological studies because of its simplicity, similarity with humans, and optical accessibility. However, its small size and mobility make it challenging for researchers to conduct experiments on these microorganisms. The development of microfluidic technology enables easy manipulation of *C. elegans* with spatial and temporal precision; however, most current ones remain cumbersome and complicated. To overcome these limitations, we have developed a multi-channel microdevice for rapid loading, trapping, stimulating and imaging worms. The device is easy to use based on passive regulation, simple to make even for non-experts, and increase the parallel experiment numbers.

KEYWORDS

C. elegans, high-throughput, multi-channel array, selective stimulation, KillerRed

INTRODUCTION

C. elegans, a free-living nematode, has been extensively used for characterizing phenotypes and performing visual screens to identify various biological processes [1, 2]. It is a powerful model because of many conserved biological mechanisms, known wiring diagrams, sequenced genome, and transparent body. Recently, microfluidics has enabled easy manipulation and fast measurement [3]. However, most of the microsystems designed for *C. elegans* require large and expensive off-chip components as well as complex fabrication. Moreover, high-density array platforms are difficult to promptly and selectively stimulate targets without active elements. To overcome these limitations, we developed a single-layer multi-channel device for worm imaging with the capability of high-throughput target-selective stimulation.

EXPERIMENT

The PDMS device includes 133 individual channels to trap worms, readily for imaging (Fig. 1). Through a serpentine channel, worms or reagents can be delivered and worms are individually loaded to imaging channels. Restriction channels prevent worms from escaping and enable continuous flow to deliver flow and chemicals. Additionally, flow across the restriction channel is fast enough to position worms in relatively identical locations. Another key feature is the engineered resistance channels between imaging and serpentine channels to make the bulk of the flows along the serpentine channel. These features enable efficient worm loading within a few minutes without any active elements on the device (Fig. 2a-c); the device operation is very simple and does not require any complex off-chip components.

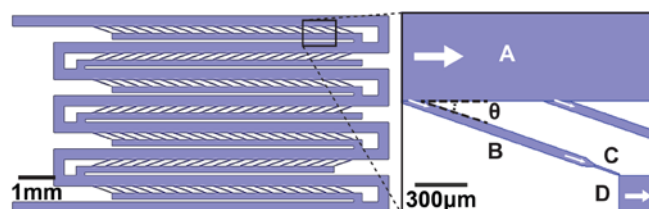


Figure 1. The schematics of multi-channel imaging device which consists of a serpentine channel (A), trapping channels for imaging individual worms (B), restriction channels (C), and resistance channels (D). White arrows represent the direction of flow which can carry worms and chemicals

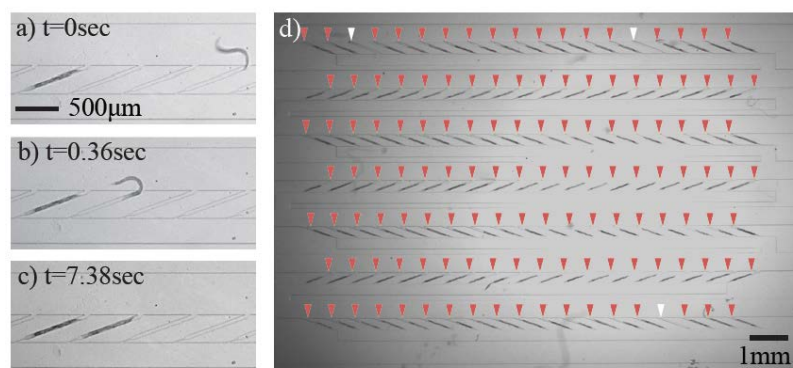


Figure 2. Optical photographs showing *C. elegans* loading in the imaging channels. a)-d) Sequential images showing the single worm trapping in an imaging channel in a few seconds. e) Optical photographs of the whole chip loaded with *C. elegans* (130 out of 133 total). Red triangles point at traps successfully loaded with single worms; white triangles at empty channels.

On average, loading efficiency is higher than 85% (Fig. 2d). The high loading efficiency induces several benefits: 1) applying the same homogenous chemical stimulation on high density of worms loaded in the device, 2) fast imaging and 3) allowing for repeated characterization of specific cellular phenotypes because of the regularly arrayed samples. Experimentally, the angle between the loading channel and the serpentine channel is optimized as 20° for high loading efficiency. Based on COMSOL simulation, the ratio of flow rates is characterized as a function of key features in the loading efficiency (Fig. 3). The more slanted the imaging channel, the higher the ratio of flow rates, which increases loading efficiency. In the case of 10° device, worms tend to be trapped in a short period time but they easily escape from the channels.

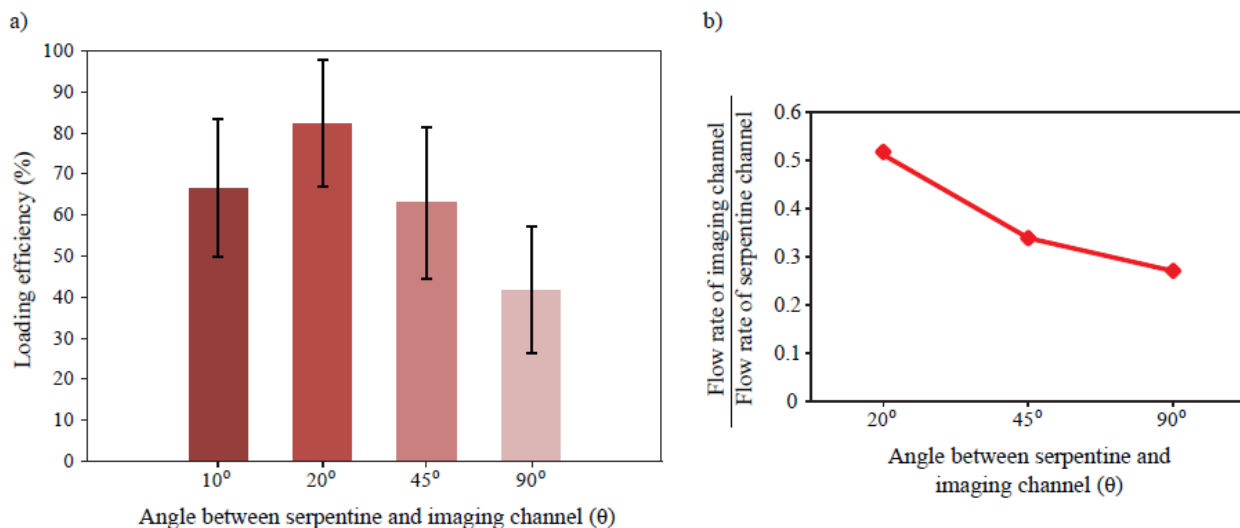


Figure 3. Device characterization. a) The loading efficiency was analyzed experimentally ($n=3$) with *C. elegans* in 1mM tetrazizole to consider channel geometry, particularly angle between serpentine and imaging channel. b) the flow ratio between serpentine and imaging channel was characterized as a function of angle using COMSOL simulation. Error bars represent SEM.

Our microdevice can be applied to observe the phenotypic changes in response to chemical stimulation useful for drug and chemical screening. Chemicals can be easily delivered to each worm in the imaging channel and switched within a few seconds (less than 10sec) in a controlled manner (Fig. 4), which could be useful as a chemical pulses for a temporal stimulation. These compound pulses can be generated using a 4-way valve and applied to track the dynamic responses such as calcium transients in the neurons. The delivery of chemicals is also very reproducible in the device from channel to channel (Fig. 4a). Besides, for a spatial stimulation, two different stimulants can be selectively applied to alternative rows by tuning flow rates from two inlets; alternatively, a gradient of concentration can be applied to each row in the whole device (Fig4b).

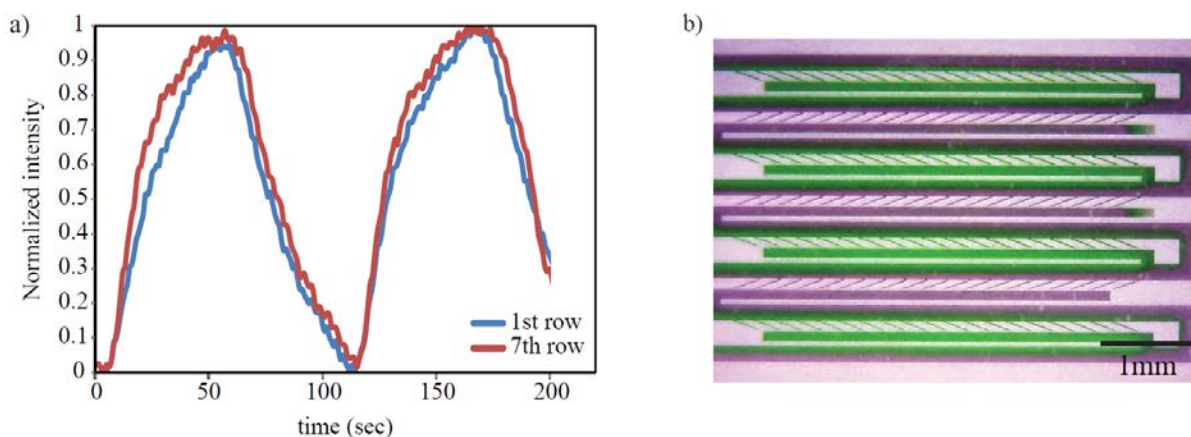


Figure 4. The spatio-temporal selection for the localized stimulation a) Plot showing saw-tooth shaped chemical delivery in one channel in the first row and the other channel in the last (7th) row as a function of time. Both channels were occupied with worms. b) Optical micrograph showing the spatial-selective stimulation caused by tuning of the flow rates from two purple and green stimulants.

Finally, to demonstrate the ability of optical selective stimulation, worms with KillerRed expressed in the mechanosensory neurons and control worms with dsRed-expressing sensory neurons were trapped, optically stimulated, and observed in the array device. Genetically encoded KillerRed produces a cell-toxic effect by reactive oxygen species induced by light-activation [4]. Because the worms were arranged in predictable orientations and locations in the array device, we could project specific light patterns to photoactivate or to image. We observed photo-ablation of ALM and PLM neurons (after 30min irradiation) and no damage in control neurons that only

express fluorescent markers (Fig. 5). We envision this multiple array device will be useful for repeatable fast imaging and selective stimulation, and will greatly facilitate a broad range of developmental and functional studies in neuroscience.

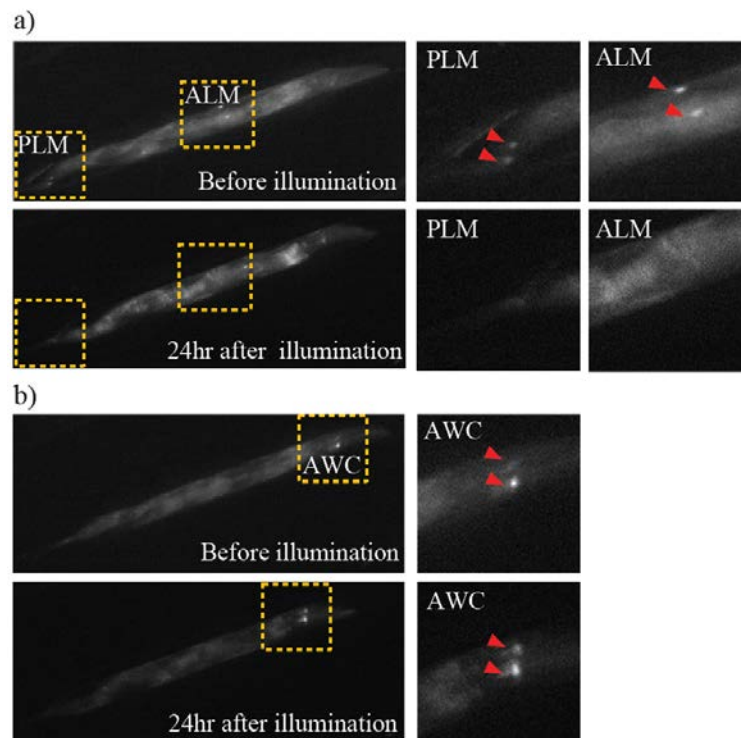


Figure 5. Fluorescent images of a KillerRed-integrated worm. Green light induces killing of mechanosensory neurons within a live *C. elegans*. Time course of a) targeted mechanosensory neurons (ALM and PLM) and b) control chemosensory neurons (AWC) before and after irradiation. Green-light irradiation ($\times 5$, 540-580 nm excitation filter, $4\text{mW}/\text{cm}^2$) for 30min let to cell death within a day. On the contrary, no effect on dsRed markers of green light was detected in control neurons even after 24hr later.

CONCLUSION

Here, we present a simple multi-channel device for fast worm imaging with the ability of high-throughput target-selective stimulation in dense arrays. Our device provides up to 97% loading efficiency without any active controls, which renders the fabrication simple and its operation easy. Using this multi-channel device, we were able to measure chemical delivery to a large number of individual worms. Furthermore, we showed the results of a selective stimulation using the phototoxic protein-integrated worms on this multi-channel device. We envision this multiple array device will be useful for repeatable fast imaging and selective stimulation, and will greatly facilitate a broad range of developmental and functional studies in neuroscience.

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