High-throughput multichannel array device for selective stimulation and long-term *in vivo* imaging in *C. elegans*

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Caenorhabditis elegans (*C. elegans*), a small nematode, has been extensively used to study biological processes because it has many conserved biological mechanisms, well-known neural networks, sequenced genome, and transparent body. Recently, microfluidics has enabled automated manipulation and fast assay by manipulating *C. elegans*. However, most of the microsystems still require complex fabrication for multi-layer and expensive off-chip components for control of active elements. 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 using PDMS and standard soft lithography procedures. The device includes a serpentine main channel, 133 individual trapping channels, restriction channels, resistance channels, and slated protrusion for efficient worm trapping, selective stimulation, and fast recovery. Through the serpentine channel, worms or reagents can be delivered and worms are individually loaded to trapping channels. Restriction channels prevent worms from escaping and enable continuous flow. To make the bulk of the flows along the serpentine channel, the engineered resistance channels were added between trapping and serpentine channels. Additionally, the slated protrusion prevents worm from swimming into resistance channel during recovery of worms by applying flow in the reverse direction.

On average, loading efficiency is higher than 85% and the head-first orientation is strongly biased (88%). The ratio of flow rates is characterized as a function of key features in the loading efficiency. As the trapping channel is slanted more, the flow in trapping channels is getting faster, which increase loading efficiency. The controlled orientation also induce several benefits: 1) applying localized stimulation, 2) fast imaging and 3) allowing for repeated characterization of specific cellular phenotypes because of the regularly arrayed samples. The space between neighboring trapping channels are minimized ($\sim$70 μm in a column) so that we achieve a density of 2.42 traps/mm^2. To demonstrate the ability for selective stimulation, worms which have KillerRed(photosensitizer)-encoded sensory neurons in the middle body and tail were trapped. The worms were then optically stimulated and observed in the device. Because the worms were arranged in predictable orientations and locations in the array device, we could project specific light patterns to photo-activate or to image. We observed photo-ablation of on the target neurons in the middle of body after 30 min irradiation (543-593 nm excitation filter, 52 mW/cm^2), while no damage in the control neurons in the tail. Based on the results, we expect 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.