OPTOGENETIC MANIPULATION OF FREELY MOVING *C. ELEGANS* IN AN ELASTOMERIC ENVIRONMENT-MIMICKING AND FORCE-MEASURING CHIP

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ABSTRACT

This paper presents integration of optogenetic illumination of *C. elegans* neural circuits and muscular force measurement in a structured microfluidic chip mimicking the *C. elegans* soil habitat. The integrated system deploys two optical pathways in an inverted microscope. One is for bright-field illumination to extract the worm body contour for real-time closed-loop tracking, the other is for optogenetic illumination to project structured light patterns with specific wavelength onto the worm body segments of interest. The behaviors of a freely moving worm in the chip under optogenetic manipulation can be recorded for off-line analysis which mainly collects the contact force between the worm body muscle and its surrounding environment. This enabling platform allows stimulating or inhibiting worm neurons and simultaneously measuring its thrust force, which offers a new insight into the correlation between neurons and locomotion behaviors of the nematode. Using wild-type *C. elegans*, we demonstrated the capability and potential of the system, and found no significant difference in thrust forces of wild-type worm under optogenetic illumination or not.

KEYWORDS: *C. elegans*, Optogenetics, Neurons, Thrust Force

INTRODUCTION

Investigating mechanosensation and locomotive behavior of *C. elegans* is a fundamental topic in model biology and has been attracting much attention. For further scientific research, controlling neural activity in freely moving organisms is an issue of crucial importance in neuroscience studies for the purpose of better understanding how external sensory signals are processed by complex internal circuits to generate different locomotion patterns. To this end, optogenetics has been accelerating progress by manipulating the neural system of *C. elegans* [1-5]. A majority of research designs customized optical control systems and illuminates freely moving transgenic *C. elegans* to evoke or inhibit certain neurons for the control of neurons and muscles [1], particular circuits [2], motor neurons [3], and chemotactic behavior [4], while few work restrain *C. elegans*' head to study its brain [5]. However, in most research, *C. elegans* is left free on an open substrate covered by agar or medium. The open and smooth substrate surface is far different from the soil which nematodes naturally inhabit, leaving a lot of opportunity to study how *C. elegans* responds to its surroundings via neural circuits. Previously, we [6] have demonstrated an elastomeric micro-pillar array with varying structures to mimic the living environment of *C. elegans*, and simultaneously to measure the multi-point forces reflecting the interaction between the worm body muscles and contacted pillars. Incorporating optogenetic illumination into the environment-mimicking and force-measuring platform would allow us to bridge up the gap among *C. elegans* neural circuits, muscular forces and complex environment, which is our ultimate goal.

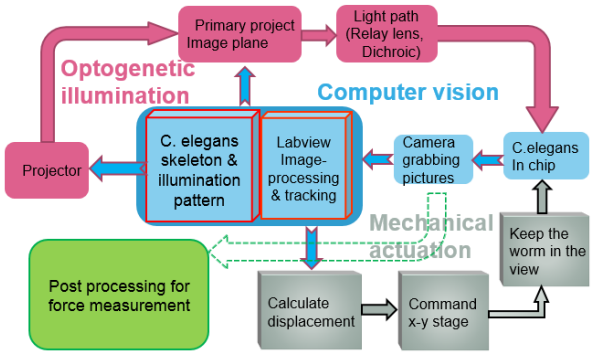

Figure 1. *The platform incorporating the optogenetic illustration into the biochip, including four key*

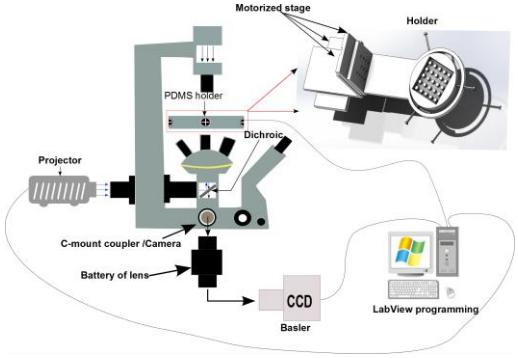

Figure 2. *Schematic experiment setup for the whole platform. Note the microscope condenser provides*

components: optogenetic illumination for neural manipulation, computer vision for illumination pattern, mechanical actuation for C. elegans tracking, and post processing for offline force measurement.

bright-field light, the projector provides optogenetic light, and the stage actuates tracking of C. elegans.

EXPERIMENTAL

To integrate the optogenetic illustration, we used the same imaging and control architecture (Fig. 1) from [1], but different off-the-shelf components for the system due to the availability. These are used to project different colored and shaped patterns on certain body segments of the nematode. In addition, video clips of *C. elegans* movement under optogenetic illumination patterns were post-processed offline to measure nematode forces, as shown in the green block of Fig. 1. Figure 2 illustrates the experimental setup. Figure 3 shows the fabricated micro-pillar PDMS array (~1cm x 1cm) device, as described before [6], within which *C. elegans* crawls. Since a more complex pillar-structured array was deployed to mimic the worm's environment, the image processing algorithm in [1, 2] for extracting *C. elegans* from uniform and simple background (e.g., agar surface) cannot be used unchanged. Therefore, we propose a customized algorithm (Fig. 4b) to isolate the worm body from its surrounding pillars. Pivotal to optogenetic manipulation is how to extract nematode skeleton automatically (Fig. 4c), based on which patterned illumination is generated and projected onto different body segments of a worm (Fig. 4d).

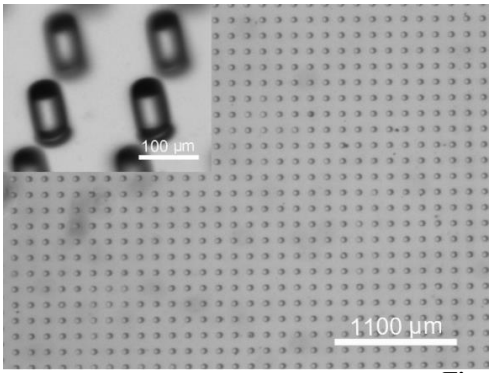

Figure 3. *Top view of the PDMS chip under optical microscope. Inset: zoom-in view of the micro-pillars.*

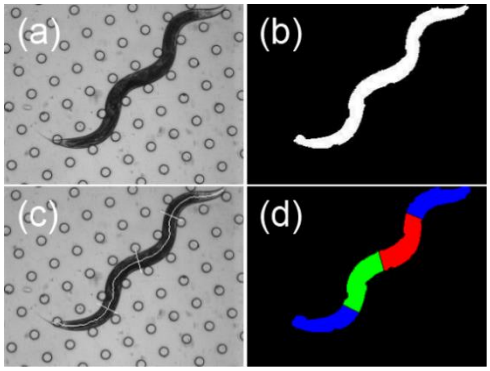

Figure 4. *Main steps for our customized image processing algorithm. (a) The original image. (b) Isolated worm body after proper thresholding and morphology operations. (c) Extracted and smoothed skeleton of the worm body, which is then divided into certain (e.g., four) segments. (d) Generated optogenetic illustration patterns using information in (b) and (c). Note the color for each segment can be varied or switched on/off at will.*

Next, the optogenetic illumination test of free behaving *C. elegans* was carried out. We placed the *C. elegans*-dwelling chip on a holder which was mounted on an x-y stage (Fig. 2) during experiment. The moving worm was tracked automatically via our customized algorithm, and projected simultaneously with bright-field light via microscope condenser and optogenetic light via an external projector. With this setup we can obtain ~30 Hz for illumination refresh and ~30 μm for spatial resolution, both comparable to literature [1, 2]. During operation, we obtain a video clip recording the locomotion behavior of the worm under multi-mode illumination in the structured micro-pillar array, utilizing a Nikon Eclipse Ti-U fluorescence microscope under 4x magnification. With regards to force measuring, we processed offline the recorded video sequences to obtain the force magnitude and direction by calculating deformation of the pillars using a customized MATLAB script previously described in [6].

RESULTS AND DISCUSSION

As demonstration of force measurement for a wild-type worm, a false-colored image frame illustrating the wild-type *C. elegans* which moves in the micro-pillar array under blue light projected on its head segment is shown in Fig. 5a. We plotted four circles representing four example pillars that exhibit greatest deformation during the imaging time, and superimpose the forces with magnitude and direction on top of the contact point. The time-elapsed force magnitude for the four pillars is shown in Fig. 5b. The maximum force value is below 30 μN in the experiment, and all force values are within the reported range for this lattice configuration structure [6]. This holds true both, in the condition of no optogenetic illumination, as well as similarity in the maximum force

occurring when the mid-body of the worm contacts with the sensing pillar. The wild-type worm behaves normally, indicating its low sensitivity to illuminated light in terms of thrust force.

Meanwhile, we also observed nematode locomotion under different modes of illumination, including projecting different colors to different worm body segments using various light intensity. Generally, wild-type worms did not behave very differently under a variety of illumination conditions, with the exception of white light leading to faster movement due to lighting burns.

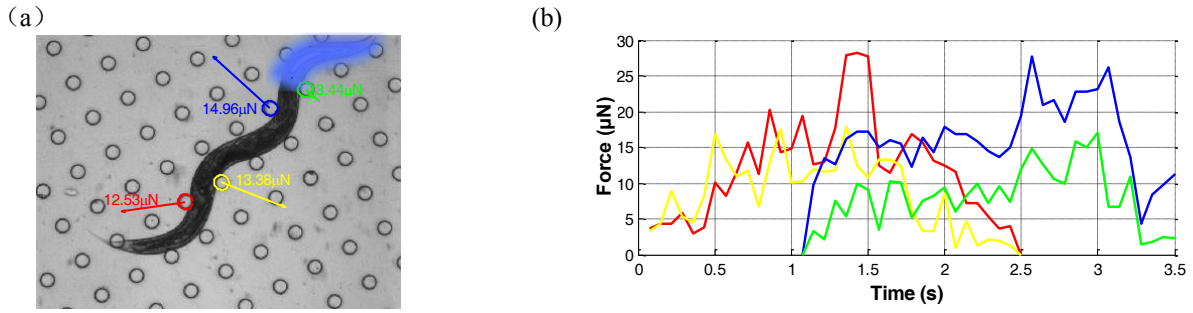

Figure 5. *A wild-type C. elegans which moves in the micro-pillar array under blue light projected on its anterior body segment. (a) The blue false-color represents blue light projected on the worm head area. Different color-outlined circles are in contact with different worm body segments exhibiting greatest forces. The arrows pointing from the circle centers plot the force magnitude and direction. (b) Time-elapsed force measurement of the four selected pillars (identified by color) for a period of 3.5 seconds during which blue light was projected on the worm's head.*

CONCLUSION

We have proposed an integrated illumination system and demonstrated a novel nematode neuron circuits and locomotion assay by combining optogenetic manipulation with force sensing in an environment-mimicking micro-device. This integrated platform can serve as a powerful enabling tool for interrogation of neuron activities when *C. elegans* moves in its natural habitat-like environment.

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REFERENCES

[1] J. N. Stirman, M. M. Crane, S. J. Husson, S. Wabnig, C. Schultheis, A. Gottschalk, and H. Lu, "Real-time multimodal optical control of neurons and muscles in freely behaving Caenorhabditis elegans," *Nat Methods*, 8, 153-8, 2011.

[2] A. M. Leifer, C. Fang-Yen, M. Gershow, M. J. Alkema, and A. D. Samuel, "Optogenetic manipulation of neural activity in freely moving Caenorhabditis elegans," *Nat Methods*, 8, 147-52, 2011.

[3] Q. Wen, M. D. Po, E. Hulme, S. Chen, X. Liu, S. W. Kwok, M. Gershow, A. M. Leifer, V. Butler, C. Fang-Yen, T. Kawano, W. R. Schafer, G. Whitesides, M. Wyart, D. B. Chklovskii, M. Zhen, and A. D. Samuel, "Proprioceptive coupling within motor neurons drives C. elegans forward locomotion," *Neuron*, 76, 750-61, 2012.

[4] A. Kocabas, C. H. Shen, Z. V. Guo, and S. Ramanathan,"Controlling interneuron activity in Caenorhabditis elegans to evoke chemotactic behaviour," *Nature*, 490, 273-7, 2012.

[5] T. Schrodel, R. Prevedel, K. Aumayr, M. Zimmer, and A. Vaziri, "Brain-wide 3D imaging of neuronal activity in Caenorhabditis elegans with sculpted light," *Nat Methods*, 10, 1013-20, 2013.

[6] S. Johari, V. Nock, M. M. Alkaisi, and W. Wang, "On-chip analysis of C. elegans muscular forces and locomotion patterns in microstructured environments," *Lab Chip*, 13, 1699-707, 2013.

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