ELASTOMERIC PILLAR ARRAYS FOR INTEGRATED MEASUREMENT OF *C. ELEGANS* LOCOMOTION FORCES

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ABSTRACT

Concurrent measurement of force and locomotion metrics (such as speed, amplitude) of *Caernorhabditis elegans* (*C. elegans*) has important biological implications. This paper reports the multi-point force measurement of *C. elegans* in motion and its integration with the amplitude and velocity measurement in a single locomotion assay by using transparent and elastomeric PDMS micropillars as force sensing elements. The experimental results demonstrate the efficacy of force measurement, leading to two preliminary but interesting findings on force patterns related to locomotion, namely, the maximum force level turns to be irrelevant to the pillar spacing, and the mid-body of a worm generates the maximum force level as predicted by theoretical analysis. Simultaneous measurement of force and locomotion metrics enabled by image processing has also been demonstrated.

KEYWORDS: *C. elegans*, PDMS, Force Measurement, Locomotion

INTRODUCTION

Genetically modified *C. elegans* mutants with different numbers of muscle arms have been an attractive model organism used to investigate the correlation between muscle arms and the nematode motion patterns. Since the muscle arms act as pathways for the muscles to receive stimulation from the nerve, their quantity was found to affect the nematode locomotion patterns. To further characterize it, we have recently demonstrated the measurement of nematode locomotion forces using a novel elastomeric micropillar-based device [1]. In this approach each individual pillar of an array in a channel is used as a single force sensor. A nematode using a pillar for locomotion deflects the free end of this pillar. This deflection is recorded via an imaging system and used to obtain a force value via a custom image-processing algorithm. Meanwhile, other researchers have used a matrix of microstructured agar pillars to enhance *C. elegans* locomotion and provide behavioral screening for mechanosensory and uncoordinated mutants [2]. While the underlying biophysics of this effect are not yet well understood, worm body wall muscles pushing against the microstructure are likely to play a significant role. Since elastomeric PDMS pillars allow force measurement, in this paper we aim to collect data that may be used to explain in terms of force how the geometry of the pillars enhances the locomotion. To this end, we fabricated a matrix (Fig. 1, rather than an array in [1]) of PDMS pillars with varying pillar spacing, which is a key geometric parameter. We also took advantage of transparency of the PDMS device to get the velocity and amplitude of crawling *C. elegans* to achieve integrated locomotion assay.

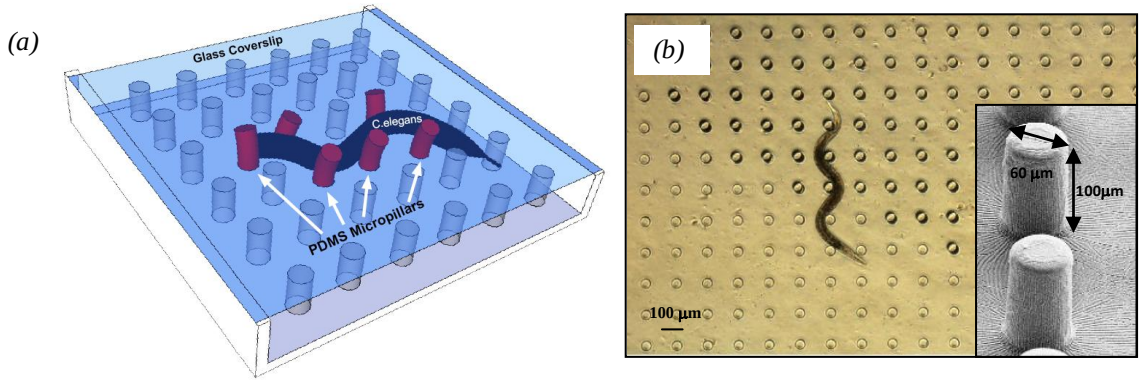

Figure 1. (a)Schematic of the C. elegans movement deflecting the micropillars in the PDMS device. (b) Top view of the PDMS micropillar matrix used for combined force measurement and locomotion studies. A C. elegans crawling inside the device in a sinusoidal movement pattern. Inset: SEM micrograph of the pillars.

FORCE SENSING PRINCIPLE

When moving inside the device, the worm was observed to touch the micropillars at half its height. The worm-pillar contact force is illustrated in Fig. 2 and modeled as a linear spring force-deflection relation [1]:

$$f = \frac{\Delta}{\left(\frac{l^3}{3EI} + \frac{d^2(1+\gamma)l}{4EI}\right) + \frac{l^2}{2EI}(h-l)},$$

where l is the pillar length from the load to the support, I the moment of inertia, E the PDMS Young's modulus, and

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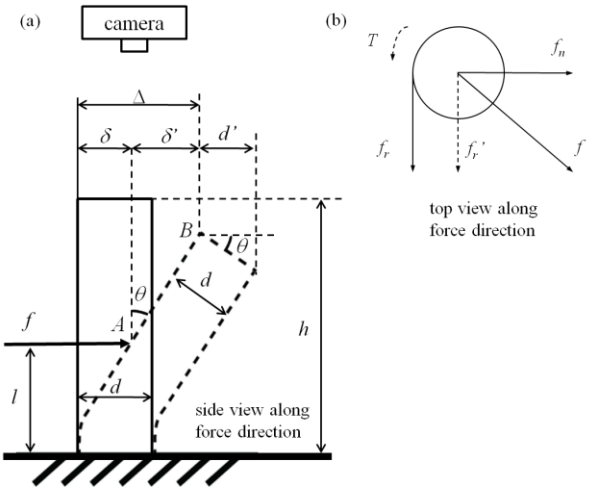

Figure 2. Schematic of the deflecting pillar model used for force analysis. (a) Measurement setup and pillar parameters. (b) Worm-pillar contact force is a composed force, i.e. $f = f_n + f_r$.

γ the Poisson's ratio. The deflection Δ of the free end of the pillar is recorded via the microscopy imaging system. SEM imaging can be used to validate pillar's diameter *d* and height *h*.

EXPERIMENTAL

The device has a 60 × 60 matrix of PDMS pillars. Two side walls are 20 µm higher than the pillar, supporting a glass coverslip that protects the micropillars, worms, and channels from potential contamination. The other two sides are open and flat area serving as worm loading/unloading zone. Worm movement through the pillar matrix was imaged using a Nikon Eclipse 80i fluorescence microscope under 10× in bright-field mode and the videos obtained at 8 Hz were processed offline using a custom image-processing algorithm. Before loading a new worm sample, the device was washed thoroughly to avoid cross-contamination. Eight wild type (N2) L4 stages worm samples were used for experiments, with each sample recorded for 33-45 frames during which the worm moved in a sinusoidal manner.

RESULTS AND DISCUSSION

Results were presented below for single-worm measurement and all worm samples. For single-worm, firstly, Fig. 3 plots the force measurement data for a worm sample. It shows that continuous force is generated by the worm, which is comparable with the natural anatomy of *C. elegans*. This continuity of force levels was found previously in arrayed-pillars [1], indicating the number of pillars in the device does not affect force generation. Secondly, looking into the continuous multi-point force measurements, we observed that force variations across sensing pillars and frames can be directly related to various body parts of the locomotion sequence. As illustrated in Fig. 4, specific locomotion events such as making contact/breaking contact, sudden acceleration as well as bending and continuous propulsion can be distinguished from the recorded force plot. For example, the sudden increase of force value on pillar #2 in the middle (~3s) of the recorded video clip was related to the change of the forward motion, which started from the worm's head and went through the main body that has the major chunk of body wall muscles.

For all worm samples, three to four pillars that had most deflections over the imaging time period were processed to obtain the force levels, as shown in Fig. 5. The maximum force level observed in the experiment was 62 µN and the average maximum force level was 34.62 µN. Force values recorded in this work were found to be several times greater than [3] as their work focused only on short contact events for a series of brief worm nose touching and body wrapping movements around the SU-8 pillar free end, with each contact occur less than 500 millisecond. On the contrary, the force that we measured was during the continuous movement of the worm body in contact with the micropillars for about 5 seconds.

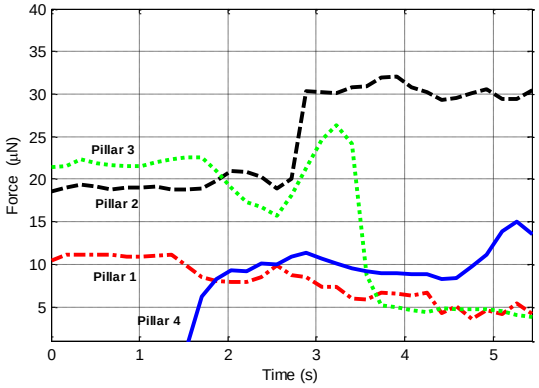

Figure 3. Plot of the force magnitude measured on four pillars for a sample worm crawling through the micropillar matrix for a period of 5 seconds. Some representative image frames corresponding to this force plot are shown in Fig. 4.

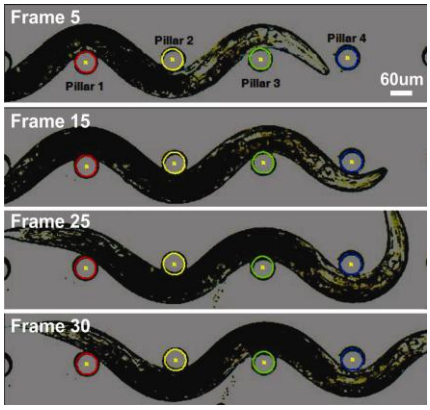

Figure 4. Micrograph sequence illustrating nematode body contact with the measurement pillars. Changes in magnitude in the force plot can be attributed to certain parts of the locomotion sequence, such as contact (Frame 5 to 15) and head bending (Frame 15-25).

There are two preliminary but interesting findings. Firstly, variation in the pillar spacing did not significantly affect the maximum force levels (Table 1). This is a little surprising and we will collect more data for conclusive result. Secondly, the maximum force was found to occur when the mid-part of the body, mainly in the intestine region between dorsal-ventral (Fig. 4, pillar #2), interacted with the sensing pillar. This finding agrees with theoretical analysis [4], which states when crawling *C. elegans* concentrates most of its bending force around the middle part of its body.

We have also demonstrated concurrent measurement of nematode velocity and amplitude with the device. As shown in Fig. 6, addition of force measurement to the microstructure did not seem to affect the locomotion phenotype.

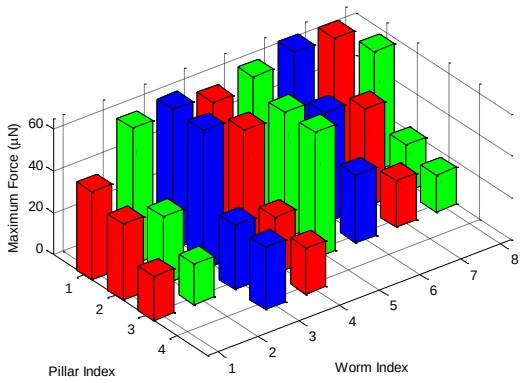

Figure 5. Maximum forces measured for eight C. elegans. Up to six pillars were in contact with each sample but three to four pillars bearing most forces are shown here.

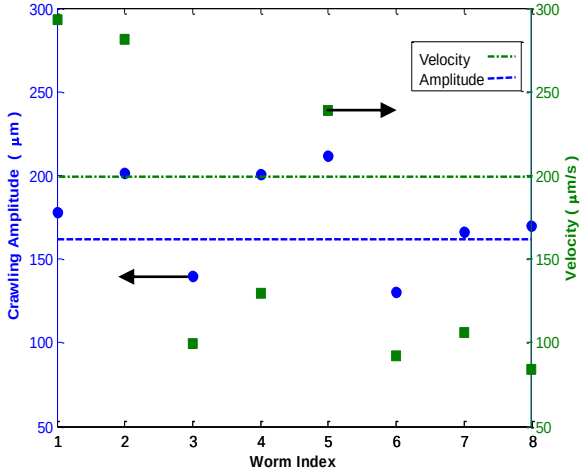

Figure 6. Plot of locomotion amplitude and velocity for eight C. elegans samples. Values indicate nematode crawling behavior and demonstrate multi-parameter sensing capability of the device. Plotted lines indicate the average amplitude and velocity comparable to the reported for smooth crawling in [1, 5-6]. This evidenced that the worm can exhibit smooth crawling behavior even in the presence of the microstructured sensor arrays, similar to conventional agar plates.

Table 1. Pillar matrix spacing and maximum measured forces for eight different nematodes.

Worm Index	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Pillar Spacing (μm)	**110 × 110**			**120 × 120**		**130 × 130**		
Max. Force (μN)	**55**	**47**	**57**	**52**	**57**	**62**	**32**	**61**

CONCLUSION

We have demonstrated a novel nematode locomotion assay with elastomeric PDMS micropillars as force sensing elements and applied the device to simultaneously characterize *C. elegans* crawling velocity, amplitude and force magnitude. Our findings suggest that *C. elegans* generated greater force from their middle body section during locomotion, regardless of the pillar spacing. With the integrated assay capability for force, velocity, and amplitude, the presented platform can find immediate application in explaining *C. elegans* locomotion enhancement from the perspective of force.

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