Development of an Image-Based Algorithm for the Motility Characterizations of the Nematode *Caenorhabditis Elegans*

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Abstract— **The nematode *Caenorhabditis (C.) elegans* has been widely used as a model animal for fundamental biological research. In order to investigate the effect of exercise on degenerative behaviors of *C. elegans*, such as physiological (lifespan, progeny) and biomechanical (propulsion, total power and swimming gait) properties, we developed a flow visualization technique to characterize the motility of *C. elegans*.**

Quantifying the motility of micro-organisms is always essential in understanding their biomechanical properties. Up to date, however, the direct measurement of the motility of *C. elegans* remains a big challenge due to lack of proper tools. Therefore, a simple image-based algorithm using a micro-particle image velocimetry (μPIV) for deriving the kinetic power and propulsive force of the nematode *C. elegans* was developed in this study. For the measurement, each worm was confined in a 0.5 μL droplet which was sandwiched between two glass slides separated by two tapes. The motility of the confined worm was derived from the fluid motion according to the law of conservation of energy. The experimental result showed that our measured values appear to be in good agreements with the prior data. The image-based algorithm was proven to be a simple and automated measure for characterizing the dynamic motility of micro-swimmers. The study will eventually provide valuable information for treating and preventing degenerative diseases in higher animals.

Keywords— **μPIV, *C. elegans*, propulsion, power, motility.**

I. INTRODUCTION

Evidence has shown that regularly physical exercise is effective in prevention of neurodegenerative diseases. It is also well known that exhaustive exercise causes muscle fatique or cell damage [1]. Therefore, characterizing the relationship between biomechanical properties and exercise is key to understanding the effectiveness of exercise.

Among all model animals, *C. elegans* is particularly useful in the genetic and neuronal research. Currently, however, biomechanical characterization of micro-sized organisms remains a big challenge due to lack of proper tools. It is difficult to achieve the direct measurements of nematode body because the worm's body is too small and adapt for environmental changes [2-3]. Alternatively, indirect measures are used to analyze the worms. Most commonly, the worm swimming gait was assumed to be a perfect sinusoidal waveform to obtain a theoretical model. Although the model simplified the calculation, the prediction actually deviated from the real situation [4]. After consistantly studying the neurophysiological and the behavior of worm , we found that *C. elegans* will adjust its body to adapt different environments. Therefore, some research used granular medium to determine the locomotion of *C. elegans* [5]; others used the deformation of mechanical structures, such as polydimethylsiloxane (PDMS) pillars, to sense the worm's forces [6-7]. However, these methods are too complicated to make the experiments and they have some unavoidable disadvantages.

To simplify the measurement process, we developed an image-based algorithm using a μPIV system to derive the kinetic power and propulsive force of *C. elegans*. These measurements eventually provide quantitative clues of the worm's physiological changes in response to exercise and can be potentially applied for higher animals.

II. MATERIALS AND METHODS

A. Strains of Worms and Culture Conditions

In this study, Bristol strain N2 and the mutant, KG532 *[kin-2(ce179) X]* were used. N2 is a wild-type strain representing reference behavior in this research. KG532 is a hyperactive strain carrying defective gene *kin-2*. The worm of N2 (n=4) and KG532 (n=4) are placed in the NGM buffer. The other group (n=4) is the wild-type worms in high viscosity medium which mixing dextran with the NGM buffer to achieve a 6% dextran solution. All worms were grown and maintained on nematode growth medium agar plates at 20℃ incubator and fed the *Escherichia coli (E. coli)* strain OP50 [9]. To obtain young adult worms for measurements, L4 stage worms were picked and transferred to a new agar plate spread with a lawn of *E. coli*. The isolated worms were then allowed to grow for 8 h in an incubator until turned to the stage of one-day-old adult.

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B. Derivation of Kinetic Power and Propulsive Force

The motility of *C. elegans* herein is derived from velocity fields based on micro particle image velocimetry (μPIV). μPIV is a flow visualization technique used for velocity measurements in microfluidics. More detailed information of PIV can be referred to the past literature [10]. The fluid is seeded with tracer particles in the flow field and is visualized by an epifluorescent microscope. A digital camera is used to capture the flow fields. Each pairs of particle images are divided into numerous interrogation windows and process with the spatial cross-correlation algorithm [11]. When the velocity and direction of the flow are determined, an acceleration field is obtained simultaneously (Fig. 1). The acceleration hence can be used for the derivation of the biomechanical properties, such as kinetic power and propulsive forces.

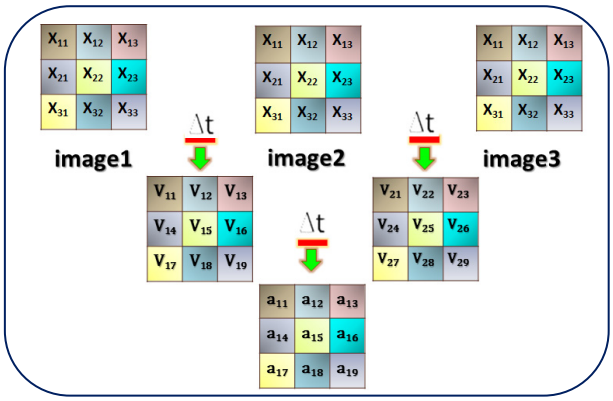

Fig. 1 Derivation of acceleration from displacements.

To implement the assessment of the motility, a worm is confined in an aqueous droplet. Base on the conservation of energy, the total force from the fluid is equivalent to the force exerted by the worm in the isolated droplet. As shown in Fig. 2, the energy of worm is obtained from the inner product of the total force and the displacement of interrogation window. In addition, the propulsive force is obtained from the inner product of the total force and the unit displacement of worm.

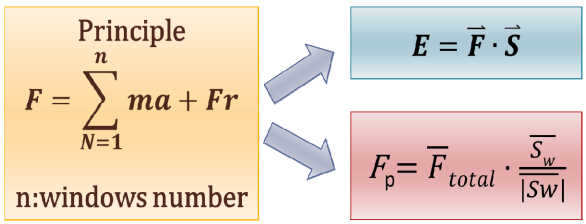

Fig. 2 Derivation of the kinetic power and propulsion.

C. Method of Measurement

A self-developed μPIV system was used to measure the velocity field induced by a worm in the droplet as shown in Fig. 3. An inverted epifluorescent microscope (IX71, Olympus) equipped with a 10× objective was used for visualizing the fluid motion in a droplet. In the microchip, a 0.5-μL aqueous droplet containing *C. elegans* and tracer particles (d_p =3.2 μm, Thermo Fisher Scientific) was sandwiched between two glass slides separated by a spacer of 110 μm. A high speed camera (GX3, NAC) was used to capture consecutive particle images induced by the worm's locomotion. The inset in the lower right of Fig. 3 is a contour of an instantaneous acceleration field from a swimming N2 worm.

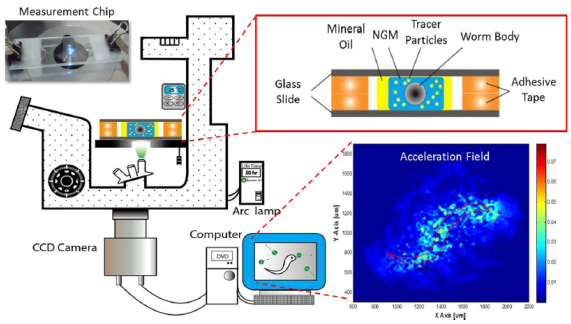

Fig. 3 Schematic of the experimental setup. The upper right corner shows measurement chip.

III. Results and Discussion

A. Measurements of Kinetic Power and Propulsion

The movements of N2 worms over three cycles based on the proposed algorithm were analyzed. In Fig. 4, the black solid line represents a trajectory projected by the centroid of the wild-type worm over a swimming cycle. Five postures in different time phases (i), (ii), (iii), (iv) and (v) show corresponding changes thereof.

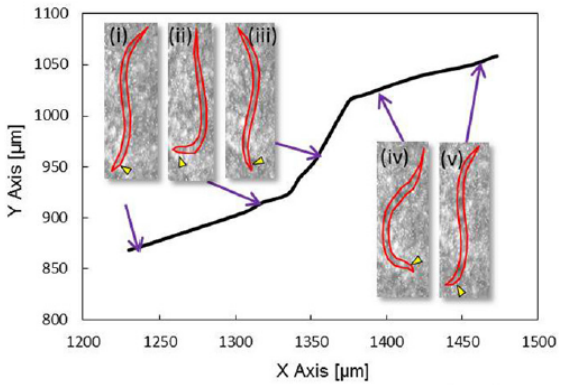

Fig. 4 Trajectory of the worm's body centroid over a swimming cycle. (i) ~ (v) show five postures in different time phases.

According to Fig. 5, the output power is high in phases (i), (iii), and (v), because the worm elongates its body; whereas the power decreases in phases (ii) and (iv) when the worm experiences a bending posture. To reflect the periodic behavior during swimming, the trend of the kinetic power (blue line) looks like a letter "W" while that of the propulsion looks like a letter "M". Noted that the opposite peaks of the power and propulsion is because the negative propulsion is designated as forward movement herein. Nevertheless, both the power and propulsive force becomes significant in the phase (i), (iii), and (v) but decreases in the phase (ii) and (iv). The results suggest that the worm body will store energy (phases ii and iv) and then releases the energy for locomotion by stretching its body (phases i, iii, v).

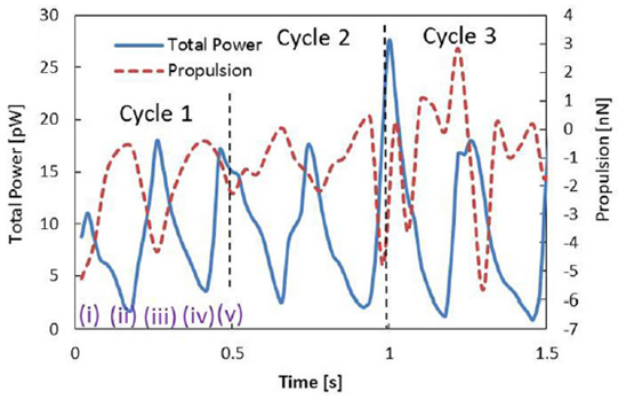

Fig. 5. Variations of power and propulsion over three cycles.

To avoid size effect, the power and propulsion were normalized with each worm's body length to form unit power and unit propulsion. For the N2 worm, the unit propulsion and power are 0.6 ± 0.5 nN/mm and 3.9 ± 2.2 pW/mm. Fig. 6 shows our measured values compared with the prior studies [12-15]. The reported power and propulsion range from 1.3 pW to 3 pW and from 1.7 to 7.6 nN, respectively. Our measured motility data appear to show good agreements and on the same order with the prior data.

However, the hardware limitation prohibited (*i.e.*, a high resolution camera for small tracer particles is required) the number of interrogation window and the measurement uncertainties between worms, there are the large standard deviations. Moreover, the number of the worm in each group (n = 4) is too small as well. The other groups have the same trend of power and propulsion. The unit propulsion and power of the KG532 worm are 1.0 ± 0.4 nN/mm and 12.3 ± 6.2 pW/mm, and the high viscosity group are 4.1 ± 3.2 nN/mm and 55.1 ± 35.4 pW/mm. These results showed the variations between the different strains of worms and the worms under different circumstances, our measurements value were with the predictions accurately.

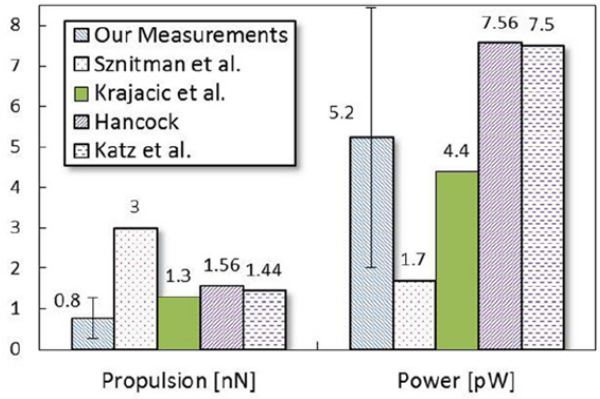

Fig. 6. Comparsions of our measurements and other studies.

B. *Comparisons of Strains and Environmental Conditions*

Comparisons of the motility performances between the N2 and the *kin-2* mutant are shown in Fig. 7. The *kin-2* worms have higher power and propulsion than N2 worms, and they are significant differences.

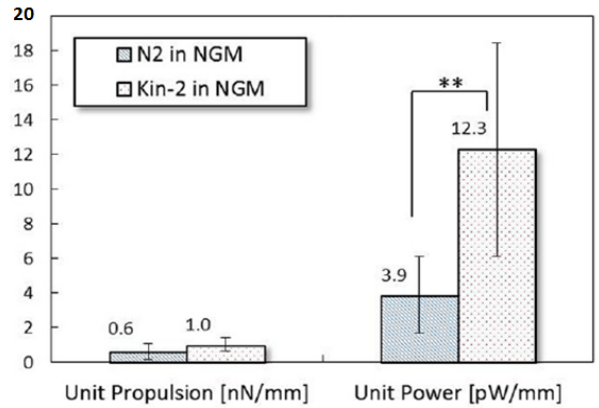

Fig. 7. Comparsions of N2 and the *kin-2* worm. ** denotes p < 0.1 based on Mann-Whitney U test.

Comparisons of the motility performances between the N2 in the NGM buffer and in the high viscosity medium are shown in Fig. 8. According to the results, the propulsion of N2 worm in the high viscosity solution is four time larger than that in the NGM. The power of N2 worm in the viscosity solution is almost fourteen times than in the NGM. In other words, the power is positively proportional to the increased viscosity because the N2 worm's power is fully contributed to its forward moving. Also, the propulsive force is stronger than the N2 in the NGM buffer.

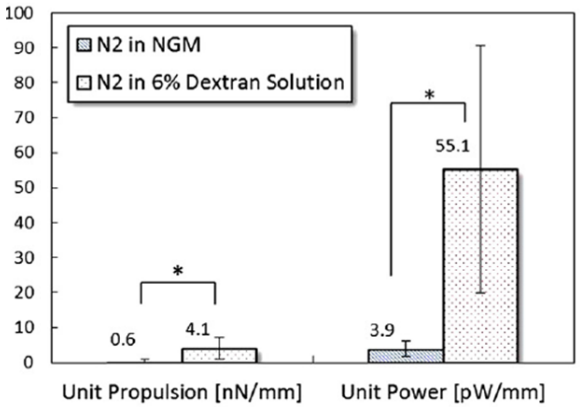

Fig. 8. Comparsions of N2 and the high viscosity group. ** denotes $p < 0.005$ based on Mann-Whitney U test.

IV. Conclusions

By incorporating the powerful tool, μPIV, into the microfluidic analysis with a self-developed image-based algorithm, the motility of *C. elegans* can be characterized in a simple way. Our study measured the different types of worms and the worms in different environments. According to the results, the N2 worms in the NGM buffer yielded a time-averaged power of 5.2 ± 3.1 pW and a time-averaged propulsive force of 1.0 ± 0.8 nN. Compared with the prior literature, our measurement results are in a reasonable range. And compared with the different groups is also in good agreements as expected. Therefore, the image-based algorithm provides a simple and automated measure for the dynamic motility of different micro-swimmers.

Acknowledgment

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