Galvanotactic and phototactic control of *Tetrahymena pyriformis* as a microfluidic workhorse

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A eukaryotic ciliate, *Tetrahymena pyriformis*, has been controlled using galvanotaxis and phototaxis in a low Reynolds number fluidic environment. A cell-tracking algorithm demonstrates the controllability of *Tetrahymena pyriformis* under two types of external stimuli. Electrical stimulation, in the form of a direct current electric field through the containing fluid, causes a change in swimming direction toward the cathode. Photostimulation, by high intensity broadband light, results in a rotational motion of the cells. The motivation of this work is to progress further with biological microfluidic actuators and sensors for use in engineered systems. © *2009 American Institute of Physics*. [DOI: 10.1063/1.3123254]

Recently, as the field of engineered micronanoscale robotics matures, a need for control and measurement of miniaturized systems has emerged. This reduction in length scale brings several limitations, such as supplying power and wireless control to the robotic structures. The biomolecular motors embedded in the cell bodies of microorganisms allow for simple power generation at limited cost. In ciliate protozoa, hundreds of cilia can coordinate and self-organize to produce a collective thrust.[1] The ciliary coordinating mechanisms may be used to provide power to microscale actuators and sensors for robotic applications.

Tetrahymena pyriformis (*T. pyriformis*) swim using cilia, a common locomotive appendage in eukaryotes. There are approximately 600 cilia, both oral and locomotive, covering the cell body. The locomotive cilia of *T. pyriformis* allow cells to develop a characteristic swimming behavior that is highly dependent upon physical stimuli. These cilia are also useful to engineers because of the diverse signaling pathways available for control.[2] Each cilium's motion is three-dimensional, with the capability for a variety of power and recovery strokes depending upon external stimuli. Cilia coordination, and by consequence swimming direction, is controlled by electrochemical signaling across the permeable cell membrane. Electrochemical signaling within the cell and cilia motion is dependent upon many cell-signaling pathways. However, simple control mechanisms exist that may directly influence this cilia motion. The most common of these control mechanisms are taxes, where organisms will show a directional motion due to a stimulus. These taxes come in many varieties, such as chemotaxis, phototaxis, aerotaxis, galvanotaxis, and gravitaxis, each correlating to chemical gradients, light, air, electric fields, and gravity, respectively. In this work, galvanotaxis and phototaxis are applied as control mechanisms for *T. pyriformis*.

There have been several studies on control of eukaryotic and prokaryotic microorganisms to produce useful work. On/off and electrokinetic directional control of microstructure movement using docked *Serratia marcescens* has been demonstrated by controlled exposure to ultraviolet light and electric fields.[3,4] Phototactic control of *Euglena* work groups has also been demonstrated for assembling microstructures.[5] Galvanotactic control and a physical model for galvanotactic motion of *Paramecium* have also been demonstrated.[6,7]

T. pyriformis is often used as an analog to other less motile eukaryotic cells such as monocytes. As signaling mechanisms of *T. pyriformis* have high homology to higher ranked eukaryotes, it is frequently used as a model cell in biochemistry and cell biology.[8] When compared with prokaryotes such as bacteria, eukaryotic cells such as *T. pyriformis* are genetically and structurally more complex. This complexity correlates with a greater number of signaling mechanisms and a hierarchy is present for cellular responses. For these reasons, *T. pyriformis* is a good candidate for control using galvanotaxis and phototaxis.

T. pyriformis cells are cultured using standard medium containing 0.1% yeast extract and 1% tryptone (Difco, Michigan, USA) solved in distilled water.[9] The cell cultures are generated by inoculating 10 ml of fresh medium with 200 μl of conditioned cells (cell density 10^4 cells ml^{-1}). The cells quickly transition to a logarithmic growth phase that can be sustained for several days. The medium is cultured for 18 h at 28 °C to a cell density of approximately 10^4 cells ml^{-1}.

A tracking algorithm for *T. pyriformis* is achieved by detecting and determining the correspondence of a cell's center of mass from one video frame to another. Consecutive images describing motions of *T. pyriformis* are acquired, digitized, and imported into MATLAB for analysis. First, images of all frames are binarized to transform the cells into a solid white color on a black background. Second, the center of mass and orientation of each cell is calculated and recorded to establish their positions at each frame within the video sequence. The center of mass is determined by

$$x_c = \frac{\sum A_i x_i}{A_{\text{tot}}}, \quad y_c = \frac{\sum A_i y_i}{A_{\text{tot}}},$$

where x_c and y_c represent centroid coordinates and A represents area.

We may assume that two cells are the same cell if they are located closely to one another in two consecutive frames.

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If there are two or more cells that may be the same closest cell at a given frame interval, the closest cell is set as a new entity and the orphaned cell is discarded. This situation may happen when cells undergo close contact or move out from the field of view. The velocity of each cell between two frames is determined by

$$v_n = \frac{(x_{n+1} - x_n)i + (y_{n+1} - y_n)j}{\Delta t},$$

where (x, y) is the cell's coordinate in the frame n and Δt is the time interval between the consecutive frames. The velocity vector v is confined to the i and j directions.

The experiments for both taxes were performed in the standard culture medium. Conductivity of the medium is suitable for galvanotaxis and the optical transmission of light through the medium is suitable for phototaxis. The cells remain motile in the conditioned culture medium and using this solution alleviates the need for further processing prior to use, such as centrifugation and resuspension. Cells were transferred from the stock culture to a modified 35 mm petri dish acting as galvanotaxis/phototaxis assay.

The one-dimensional galvanotaxis assay is fabricated by accepted techniques[4,6] using parallel carbon electrodes connected to a dc power supply (Xantrex, XHR 150–7). This simple configuration limits the amount of electrolysis that may result due to the applied voltage. The carbon electrodes allow the *T. pyriformis* cells to stay motile for an extended period of time without cell poisoning.

We have accumulated data for *T. pyriformis* during 30 s of motion using a Leica DMIRB inverted phase-contrast microscope and vision system (Redlake Motion Pro X-3). Evaluation of the registered individual swimming characters of ciliates verified that active directional responses were registered. Swimming trajectories for cells are calculated using the proposed tracking algorithm and accumulated data. For galvanotaxis, a 5 V/cm potential field is applied between the two electrodes orienting the *T. pyriformis* in an anterior-facing cathode configuration due to a self-aligning torque caused by the collective cilia motion. As shown in Fig. 1(b), most of the *T. pyriformis* that moved randomly in Fig. 1(a) go toward the cathode when the electric field is applied. To analyze galvanotaxis of *T. pyriformis*, we define a representative velocity v_r, which is the sum of all velocity vectors divided by the number of cells in the field of view

$$v_r = \frac{\sum v_k}{m},$$

where v_k is the velocity of the kth cell and m is the number of cells in one frame. The direction of the representative velocity at each instance in time is shown in Fig. 2(a). We initially applied an electric field and reversed the polarities at 12 and 23 s causing the cells to swim back and forth. The direction of v_r is closely aligned at 180° from 3–12 s and from 24–30 s, then closely aligned at 0° between 13 and 23 s, showing motion toward the cathode at all times. In Fig. 2(b), the magnitude of v_r is shown. After changing the field polarity, the magnitude of v_r reduced to zero and proceeded to increase. Galvanotactic control of *T. pyriformis* is very repeatable, controllable, and with a change in polarity the cells will quickly turn and reverse their swimming direction. In Fig. 2(c), a probability density function of velocity directions between 15 and 20 s is shown. Most cells' velocity directions are closely aligned at 0° verifying that the cells are moving toward the cathode.

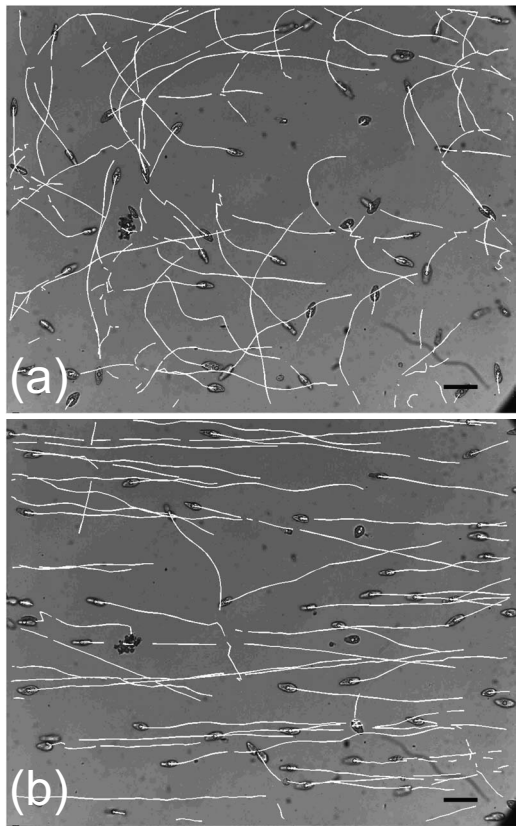

FIG. 1. Galvanotaxis motion of *T. pyriformis* (a) normal status (b) when electric field (5.0 V/cm) is applied. The scale bars are 100 μm.

In galvanotaxis, current flow direction determines cilia motion handedness and speed.[10] The swimming state of a peritrich ciliate—affected by the electric stimuli of the environment—is the resultant of two opposite ciliary forces: ciliary augmentation produced by hyperpolarization and activation of voltage-dependent Ca^{2+} channels on the anodal end and ciliary reversal produced by depolarization and activation of voltage-dependent K^+ channels of the cell.[7] As electrical current crosses from the cell body out into the surrounding fluid, the cilia reverse direction and slow their beating rate. When current flows into the cell body from the surrounding fluid, the cilia speed up in the normal wave motion direction. This creates the self-aligning torque and straight swimming associated with many ciliates under electric fields.[2]

Phototaxis is performed using unfiltered radiation from a mercury vapor lamp. The mercury light source emits broadband white light, with wavelengths from 250 to 700 nm being present at varying intensities. Several spectral peaks exist across entire range, many of which show phototactic effects in other organisms.[3,5,11] The exposed area of light upon the free swimming cells is controlled by a small adjustable iris located between the lamp and the area of interest. The light is projected through the petri dish using a microscope objective. We have accumulated data for many typical *T. pyriformis* responses when exposed to mercury vapor light. One typical example is illustrated in Fig. 3(a) where a *T. pyriformis* rotates counterclockwise at 1.28 Hz for 4.6 s and escapes from the lit area. Figure 3(b) shows the orientation of *T. pyriformis*. The angular position is established by determin-

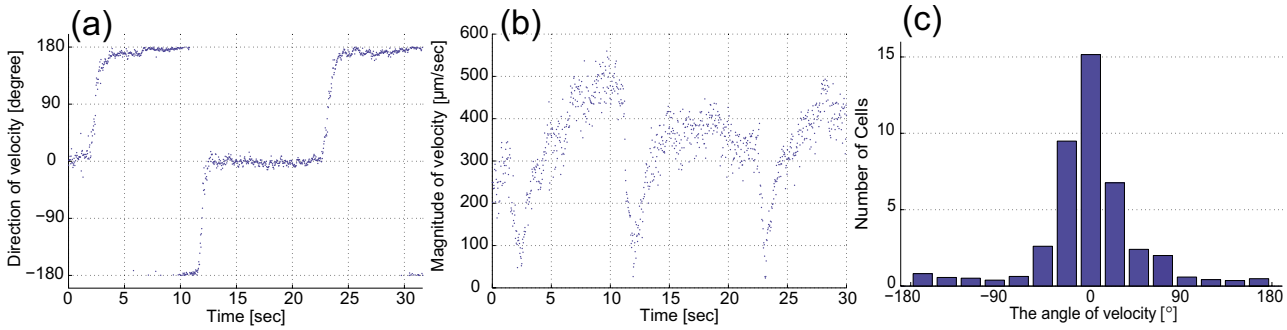

FIG. 2. (Color online) Representative velocity v_r, which is the sum of all velocity vectors divided by the number of *T. pyriformis* while switching polarities (a) direction of v_r (b) magnitude of v_r (c) probability density function of velocity directions between 15 and 20 s.

ing the long axis of the cell body from the centroid position.

The mechanism for phototaxis in the *T. pyriformis* cells of this experiment is not precisely known. Most phototactic eukaryotes have a photoreceptor structure within the cell body, such as eyespots of *Euglena* or colored pigments seen in *Blepharisma*.[12] The phenomenon shown by *T. pyriformis* exposed to high intensity mercury vapor light cannot be described accurately as the uncoordinated motion seen in dying cells. Retained swimming ability, one of the most significant physiological reference indices, proved that if the exposure time is kept under 10 s, the *T. pyriformis* are not terminally damaged by the near UV wavelengths of the broadband light.[13] The *T. pyriformis* display in-plane rotational motion when exposed to the light, where the cells rotate counterclockwise and after a short period of time move out of plane, continuing in a corkscrew motion. Oddly, *T. pyriformis* do not possess any direct photoreceptor molecules in the cell body. Phototaxis of *T. thermophila*, also lacking photoreceptors, has been shown at 600 nm but it is not known if the same signaling mechanism is present in *T. pyriformis*.[11] No response is observed when exposed wavelengths are over 570 nm using a long pass filter and in the 450–550 nm range using both blue and green dichroic filters. This does not verify that UV light causes phototaxis, but it is likely. There is the possibility of phospholipid damage taking place due to the presence of UV light. However, the time scales for that type of damage are substantially longer.[14] It is also possible that the cells experience some level of heating due to overall radiation, with total energy that cannot be equaled when specific wavelengths are excluded. It is likely that *T. pyriformis* have the ability to detect potentially dangerous UV wavelengths and perform a search and escape maneuver.

In this paper, we demonstrate that *T. pyriformis* is controllable using galvanotaxis and phototaxis. *T. pyriformis* move rapidly toward the cathode when an electric field is applied. Also, *T. pyriformis* are captured in an unfiltered light beam for a period of time and escape, showing negative phototaxis by moving away from the high intensity light. The galvanotactic and phototactic controls of *T. pyriformis* may be utilized directly as transport mechanism for engineered microstructures.

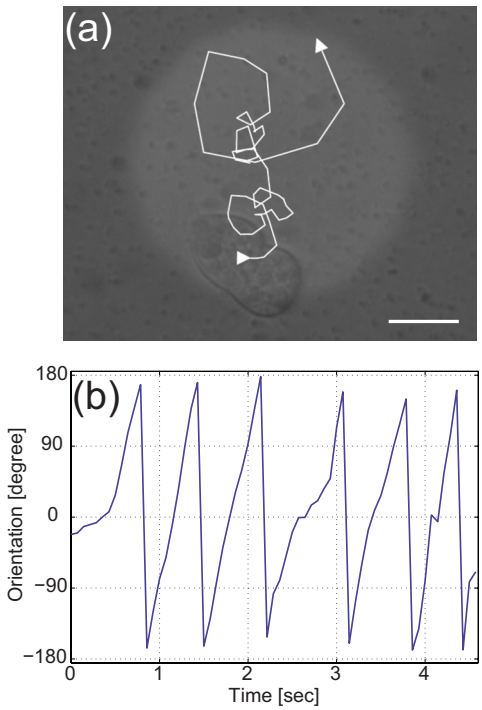

FIG. 3. (Color online) Phototactic motion of *T. pyriformis* in the light circle (a) path in the light (b) orientation of *T. pyriformis* in the light. The scale bars are 25 μm.

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