Image analysis for recording cell locomotion

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Abstract: Time-lapse videomicroscopy was used in conjunction with digital image processing to analyze cellular movement of *Tetrahymena pyriformis* as the test organism. Sequential images were acquired with a CCD video camera and processed with a computer to obtain swim tracks of cells over a known period of time. By examining the tracks of *Tetrahymena*, we have calculated swimming velocities and turning frequencies. Additionally, this method was applied to obtain swim patterns in the presence of stimulating agents, and suggested its applicability for a wide range of motile cells.

Key words:cell motility, image processing, protozoa

INTRODUCTION

Several types of motile cells are able to detect chemical stimulants and to react to these stimuli with locomotory changes. This phenomenon, known as chemosensory transduction is a feature of many cells, ranging from microorganisms such as bacteria[1] and protozoa to leukocytes[2]. The ciliated protozoa offer an interesting opportunity to study the relationship between chemoreception, signal transduction and cell behavior. As in higher organisms, ciliates possess highly evolved cytoarchitecture as well as an intracellular messenger system regulating various cell function[3,4]. Furthermore, recent advances in genetic engineering have made it possible to manipulate functional genes and introduce them into the ciliates such as *Tetrahymena* and *Paramecium*[5]. Since these ciliates are easy to grow and handle in the laboratory, they can be considered as excellent model cells. This means that correlations between cell behavior, cell structure and molecular events can be studied experimentally more easily than in higher cells.

Many studies of the chemosensory behavior of *Tetrahymena* have shown that this ciliate is chemoattracted to a range of different peptides, amino acids and several signal compounds[6,7,8,9]. *Tetrahymena* detect soluble chemicals in their environment and accumulate in some chemicals and disperse from others. They accomplish this behavior not by orienting and swimming toward or away from the chemical's source, but rather by modulating frequency of turning and speed of swimming[10]. Thus, recording of turning frequency and swimming rate is necessary to elucidate chemosensory response.

In this report, we describe a time-lapse

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imaging method for obtaining swim tracks of the ciliated protozoan *Tetrahymena*. We have adapted this imaging technique to determine turning frequency and swimming rate; this should be useful as a basis for analyses of locomotory behaviors of motile microorganisms.

MATERIALS AND METHODS

Cell Culture.

Tetrahymena pyriformis GL was grown at 28℃ in 1 % proteose peptone containing 1 % yeast extract and 0.87 % glucose. Cells were collected by centrifugation, washed and resuspended in 10 mM Mops /Tris, pH 7.2.

Digital image processing.

The measurement of motility pattern was initiated by transferring 50 μl aliquot of the cell suspension into a plastic petri dish (ϕ 22mm) containing 0.4 ml of 10 mM Mops/Tris (pH 7.2) solution with and without test substance. After rapid mixing of the medium, time-lapse images were obtained with a C5985 video camera (Hamamatsu Photonics, Japan) in darkfield illumination using an inverted microscope with a x4 objective lens[10]. Video signals were digitized by a personal computer with an LG-3 frame grabber (Scion, USA) and converted to TIFF format. The subsequent image processing was carried out on the computer using the public domain NIH Image program (developed at the U.S. National Institutes of Health and available on the Internet at http://rsb.info.nih.gov/nih-image/).

RESULTS AND DISCUSSION

Swimming behavior of ciliated protozoa is exclusively dependent on cilia that function as motile organelles. Figure 1 shows a phase-contrast micrograph of *Tetrahymena pyriformis* used in the present observations. As can be seen from Figure 1, many cilia are attached to the surface of *Tetrahymena* cell. By regulating the frequency and direction of ciliary beat, *Tetrahymena* changes swimming patterns in response to various environmental stimuli.

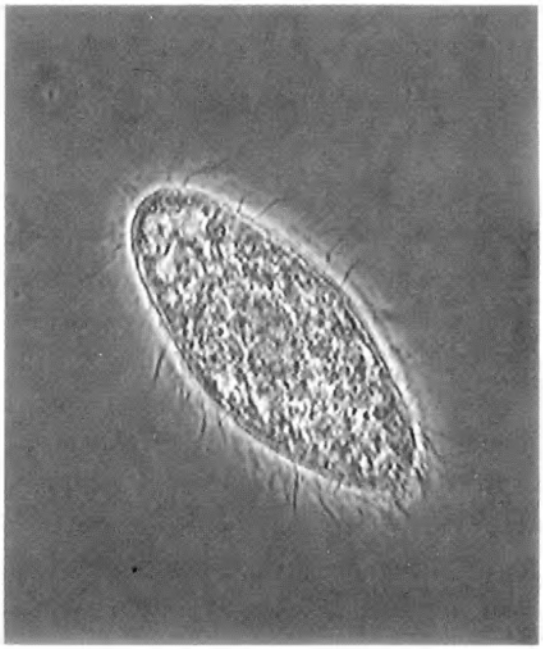

Figure 1. Phase-contrast image of *Tetrahymena pyriformis*.
Many cilium can be seen on the cell surface. Cells were fixed in formaldehyde solution

When observing the motility pattern of *Tetrahymena* in darkfield illumination with a microscope, it consists of smooth tracks(runs) interrupted randomly by a turn which alters the direction of motion[10]. To quantitatively examine the pattern of

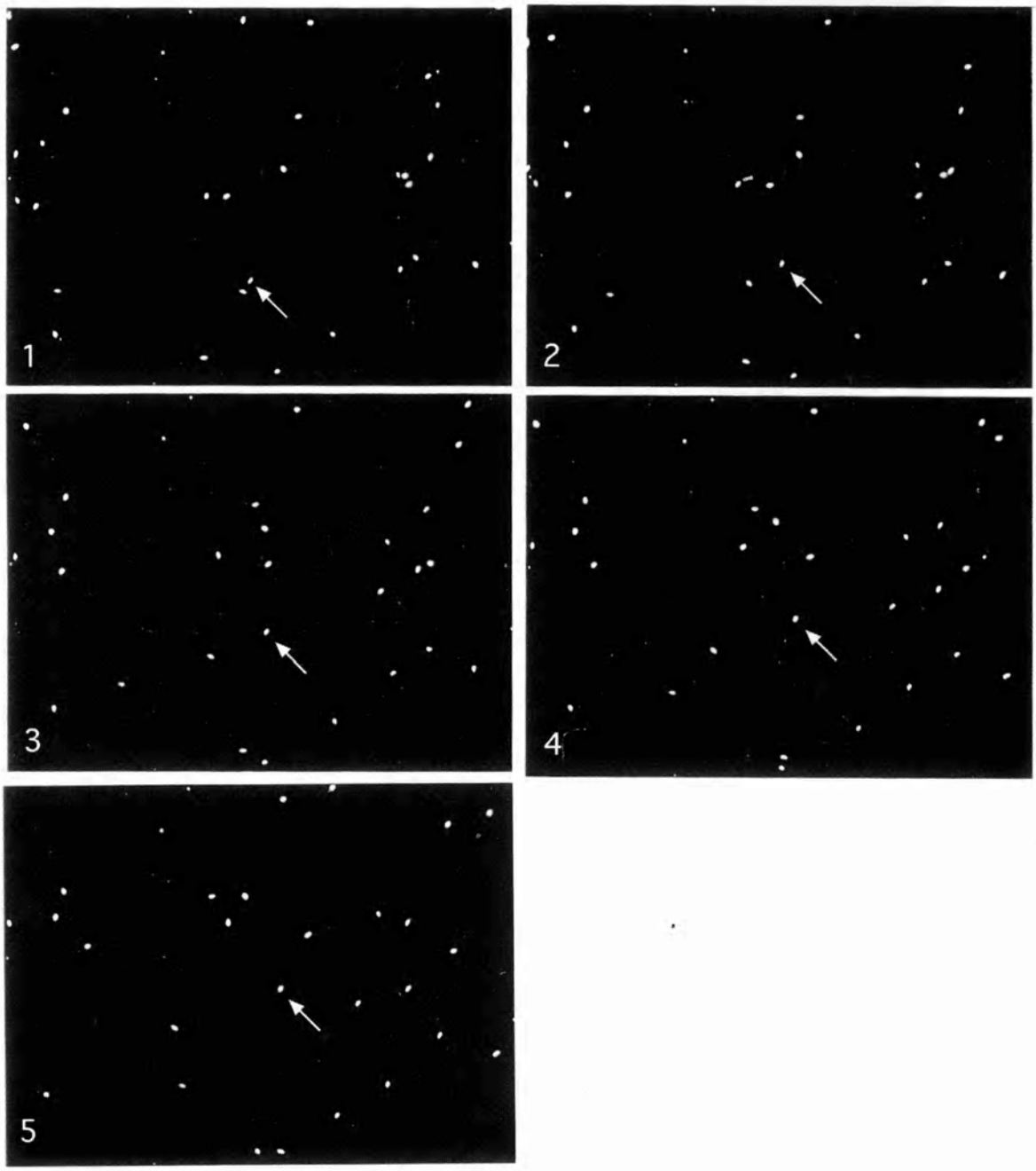

Figure 2. Time-lapse images of *Tetrahymena* cells moving in buffer solution.
All figures presented are darkfield images captured during a period of 1 minute. There was a 0.2 second interval between consecutive frames. The positions of one representative cell which swam in a straight line are indicated by arrows.

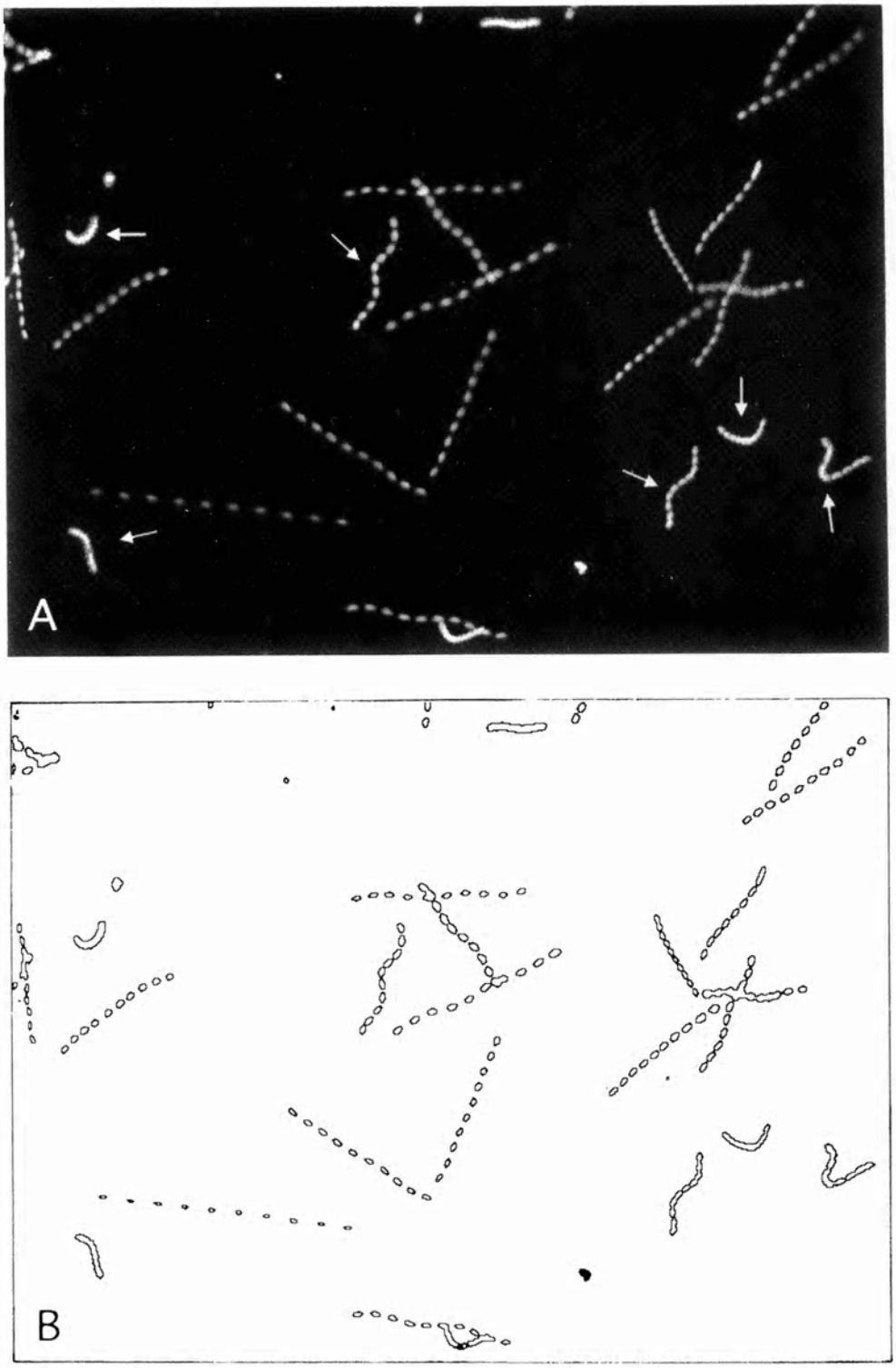

Figure 3. Swim tracks and their respective schematic representation.
The swim tracks of *Tetrahymena* cells in darkfield illumination (A) and their schematic representation (B). Ten successive images partially shown in Figure 2, each acquired with a 0.1 second delay, were added to produce one image. Arrows indicate turning cells.

concentration, KCl depolarizes the cell membrane[11] and induces the characteristic avoidance reaction observed in these ciliates[12]. *Tetrahymena* in the KCl containing solution showed circular patterns of swimming suggesting that they underwent partial ciliary reversal (Figure 4). This observation indicates that the present method could be used to identify distinctly different locomotor behaviors after cells were treated with certain stimulating agents.

Analyses of normal swimming behaviors of motile cells and changes that occur in response to a variety of stimuli were initially done by descriptive observations using light microscopy. Refinement of analyses came mainly with a number of photographic techniques such as time-lapse photography[13,14,15,16]. Although these techniques added greater precision and a quantitative aspect to analyses of locomotory behaviors, most require a delicate process of photography followed by transfer of the information from film, by printing, on to paper prior to the analysis of swim tracks. In this report, we have investigated an image processing method for obtaining swim tracks of the ciliated protozoan *Tetrahymena*. This method allows one to generate a large amount of data in a short period of time, since the direct recording of video images onto computer memory eliminates the several step process used in photographic methods. In addition, partial automation of the image processing is possible using computer programming, making this technique more useful.

Finally, by changing the objective lens of a microscope, this method would be applicable to other motile cells of different sizes. The flexibility of the interval for image acquisition in the present method could also permit recording of cellular tracks for different speeds.

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和文抄録： 真核単細胞生物である*Tetrahymena pyriformis*の細胞運動を，顕微鏡に接続したビデオカメラとディジタル画像処理法により解析した。ビデオカメラより得られた映像信号は経時的にコンピュータを用いてディジタル化した後，各静止画像を統合することにより細胞の遊泳軌跡を得た。この軌跡を解析することにより，*Tetrahymena*の走化性の指標となる遊泳速度と方向転換頻度を算出することが可能であった。

キーワード：細胞運動，画像処理，原生動物