Abstract

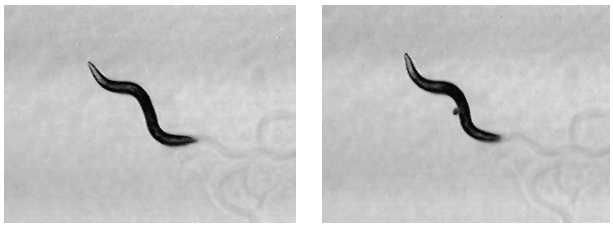
Correlating animal behavior with cellular and molecular phenomena is a new frontier of neural biology research. Rapid progress in this exciting new area requires extensive use of information processing technology. For example, the egg-laying patterns of the nematode *C. elegans* are frequently the object of time-intensive genetic and neuroscience studies, creating a demand for some automated remedy. Fortunately, implementing a real-time video processing system to analyze and quantify the detailed behavior of such animals is converging to a tractable problem due to advances in microprocessor and video technology. Computer engineering researchers at the Georgia Institute of Technology (GIT), in collaboration with neurobiologists at the University of California at San Diego (UCSD), have already implemented a computer vision worm-tracking algorithm utilizing a video camera, a Pentium 3 PC, and a computer controlled microscope stage. The goal of this research has been to extend the capabilities of the worm-tracking system to identify and quantify egg-laying events under real-time constraints. I developed an egg detection algorithm based on morphological signal processing principles in C and integrated it into the existing program. The fully automated egg detection system can now achieve consistent and objective egg-laying quantification. When the researchers at UCSD incorporate the algorithms into their new tracking system the required time and costs for these experiments will be significantly reduced.

Introduction

A nematode worm was the topic of over 1000 speeches at last year's 13th International *Caenorhabditis elegans* Meeting, and was used by the winners of this year's Nobel Prize in Physiology. Scientists are focusing so much attention on the worm because its genome and nervous system are complex enough to provide insight into those of larger organisms, yet simple enough to understand at the molecular and cellular level. The worm's genome is sequenced, and the location and synaptic connectivity of its 302 neurons are known. It is believed that the study of the nematode's neurons and genes will lead to an increased understanding of the role that human neurons and genes play in development and disease. In the majority of the experiments performed, the researcher tries to relate molecular or cellular changes in the organism to changes in the worm's observable behavior, a procedure known as phenotyping.

In a typical experiment, the biologist will directly alter the nematode or its environment and study the resulting changes in its behavior. A principle component of the worm's behavior is its egg-laying behavior (See figure 1, below). It has been shown that the egg-laying behavior can be statistically characterized as a combination of two Poisson processes, which helps to quantify the behavior with a few parameters. The eggs are about 50 micrometers in diameter, and are expelled from the vulva near the center of the worm's body. At the present time, to perform an egg-laying experiment, the 1 mm long nematode must be followed under high magnification for several hours. This process has been greatly simplified by a program recently developed at GIT and UCSD, which automates the tracking process. A stream of images is sent from the microscope to the video capture card. The computer then analyzes the images and communicates with the automated stage controller to maintain the worm in the center of the field of view.

The microscope view is recorded throughout the process, and the tape is played back by a researcher who visually searches for egg-laying events and records the times from a VCR counter. This approach is undesirable for several reasons. First, the task involves monitoring a relatively uninteresting scene for long periods of time. Second, the VCR counter is imprecise. Third, the human observer is prone to error, so results from two observers may conflict. Finally, the information must be manually entered into a database for statistical analysis, presenting more opportunity for error and consuming more valuable time.



(a) Before the egg has been laid. (b) After the egg has been laid.

Figure 1. Ideal egg-laying event: minimal noise and no complications.

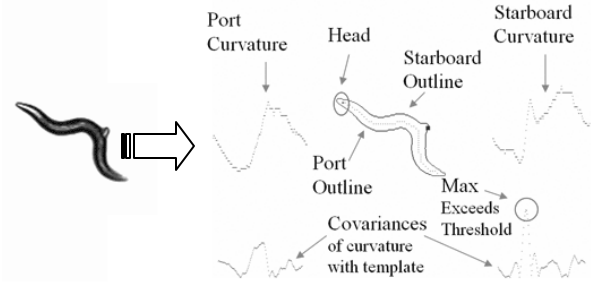


Figure 2. Extracted grayscale worm with egg and annotated screen shot of fully analyzed worm.

Methodology

In fall of 2000, I became familiar with the existing tracking program and began writing new software for egg detection. Since visual tracking is the first step in the process, the new software was integrated into the existing tracking program written in C++. The fast prototyping capabilities of MATLAB have been exploited throughout the development process. I wrote the rest of the functions for analyzing the images of the worms myself using standard C only (no DSP libraries).

Phase 1 – Obtaining noise free images. I improved the data collection process resulting in significant noise reduction and, therefore, less complex images. First, I reduced shadowing effects by iteratively adjusting the angle and position of the light source until the angle of incidence of the light was precisely orthogonal to the Petri dish. Next, I prescribed a method for applying a thin and consistent bacterial lawn to the auger in the Petri dish to further reduce noise. Finally, I developed a method for manually adjusting lighting intensity and camera gain, achieving significant increases in image quality.

Phase 2 – Isolating binary nematode images and their backbones and outlines. Each incoming image is a grayscale image, which undergoes a series of transformations. The first step I took was to isolate a binary image of the worm. The incoming image was downsampled to an appropriate size to decrease processing time. Then, the binary threshold of the image was taken at a level that was variable with the statistical properties of the grayscale image. Next, the binary image was labeled, and the worm was isolated from the background noise by properties such as area, circumscribing rectangle, and current center of mass relative to the last image. Holes in the remaining blob were floodfilled, and new image borders were defined using the circumscribing rectangle of the extracted blob. From the new extracted image, the thinned skeleton and the 1 pixel wide outline were obtained using standard methods.

Phase 3 – Implementing the Egg Detection Analysis Tools. The thinned skeleton and outline needed to be traversed and analyzed, so I have written many functions for this purpose. The endpoints of the skeleton were identified by their single neighbor properties, and a maximum distance between endpoints signified an approximate head or tail of the worm. The center third of the skeleton was then located and traversed for branch points, or pixels with at least three neighbors. This approach gave rise to a graph theory model of the worm, where branch points and endpoints were nodes and the pixels connecting them were edges. This approach also produced a representation of the worm as a sampled curve, which has proven to be very useful in classifying various genetic mutations realized in the worms' behavior, also known as phenotyping. The presence of a node in the center third of the skeleton indicated a great change in slope along the outline of the worm in the corresponding region. An egg appeared as a bump on the worm's binary image, which would cause such great changes in the outline's slope. This image was then flagged for further analysis (See figure 2, previous page).

The worm's outline in the flagged region was then traversed, and the change in angle (curvature) was correlated (with the mean removed) with a template known to be an egg. When the covariance crossed an experimentally determined threshold, an egg was detected. Since the curvature information was 1 dimensional, the computational complexity of this most important classification phase was significantly reduced compared to previous attempts. Another big advantage to analyzing the change of the angle of the worm's outline is that it is independent of translation or rotation between images.

Conclusion

After 2 years of working on this research project, the egg detection system is functional and automated. During 4 hours of testing the algorithm on 3 separate worms (one at a time), there were 10 eggs laid in the center third of the worm which were detected. There were 3 eggs laid outside the center third of the worm that went undetected, and there were 7 false positives. The algorithm averaged 5 frames per second. One year ago, Intel Microprocessor Research Labs gave me an undergraduate grant of $2,000 towards working on the project. Five months ago, I won 2nd place out of 17 finalists in the National Intel Undergraduate Research Contest. I worked on the vast majority of the egg detection portion of the project by myself. I collaborated with another undergraduate for a short period of time to explore potentially useful transforms and found it very rewarding. I was advised by Dr. Ron Schafer.

As a byproduct of trying to detect the eggs, several useful models for the worm's body structure have been developed, which contributed to the classification work being done at UCSD. The UCSD researchers also found the image preparation algorithms useful. Due to these contributions, I was a co-author on this paper recently published in the Journal of Neuroscience Methods 118 (2002) 9-21: "Using machine vision to analyze and classify *Caenorhabditis elegans* behavioral phenotypes quantitatively."