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Automated detection and analysis of foraging behavior in *Caenorhabditis elegans*

Kuang-Man Huang [a,*], Pamela Cosman [a], William R. Schafer [b]

[a] *Department of Electrical and Computer Engineering, University of California at San Diego, La Jolla, San Diego, CA 92093-0407, USA*
[b] *Cell Biology Division, MRC Laboratory of Molecular Biology, Cambridge CB2 0QH, UK*

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Abstract

Foraging is a rapid, side-to-side movement of the nose generated by *Caenorhabditis elegans* as it explores its environment. In this paper, we present an automated method to detect and analyze foraging behavior of *C. elegans* in a video sequence. Several morphological image-processing methods are used to locate the precise nose position of the worm in each image. Then foraging events are detected by measuring the bending angle of the nose and investigating the overall bending curve using periodograms. We measure foraging-related parameters which have not previously been studied. The algorithm has applications in classifying and characterizing genetic mutations associated with this behavior.

Keywords: *Caenorhabditis elegans*; Foraging; Image processing; Automated detection; Periodogram

1. Introduction

The nematode *Caenorhabditis elegans* is widely used in studies of nervous system function and behavior. *C. elegans* is particularly useful as a neurobiological model because of its completely sequenced genome and its amenability to classical and molecular genetics. In addition, it has a compact and well-defined nervous system, in which each neuron (of 302 in total) has been individually identified and characterized at the level of synaptic connectivity (White et al., 1986). In principle, it is possible to identify mutants with specific behavioral abnormalities and understand mechanistically how individual gene products act within the context of the neural circuitry to produce these behavioral phenotypes.

Despite its anatomically simple nervous system, *C. elegans* is capable of surprisingly diverse patterns of behaviors. While some of these, such as feeding, egg-laying, and defecation, are mechanically simple (Avery and Thomas, 1997; Schafer, 2006), other behaviors involve complex motor programs requiring intricate coordination of muscle groups. These include locomotor behaviors such as backward and forward crawling, swimming, and copulation (Barr and Garcia, 2006). Recently, there has been increasing interest in quantitatively characterizing and modeling these more complex motor programs (Chen et al., 2006; Karbowski et al., 2007); however, significant questions remain regarding the nature of these behaviors and how they are generated by the nervous system.

A *C. elegans* behavior that has received comparatively little attention is foraging. Foraging is a term used to describe rapid, side-to-side movements of the nose generated by the worm as it explores its environment. Several neurons, including the OLQ and IL1 sensory neurons and the RMG motorneurons, have been shown to be required for this behavior (Driscoll and Kaplan, 1997; Kaplan and Horvitz, 1993). Various genes conferring a foraging abnormal ("Fab") phenotype have also been identified; for example, the AMPA-type glutamate receptor gene *glr-1* is required for foraging (Hart et al., 1995), and the G-protein alpha-subunit gene *goa-1* as well as other genes in the Go/Gq signaling pathway affect the rate of foraging (Alkema et al., 2005; Segalat et al., 1995). However, the precise nature of the foraging movements in wild type and mutant strains has not been characterized.

Studies of foraging behavior have relied exclusively on the time-consuming analysis of video recordings by human observers. For this reason, we use an automated system (Baek et al., 2002; Cronin et al., 2005; Feng et al., 2004; Geng et al., 2004; Hoy et al., 1996, 1997) consisting of a tracking microscope and image-processing software to follow and analyze the

* Corresponding author.
E-mail address: houston21@hotmail.com (K.-M. Huang).

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movements of individual animals at high magnification. Using video data collected using an automated tracking system, we have been able to reliably detect foraging events and provide the first quantitative description of foraging movements in *C. elegans*. Fourier analysis of these data identifies characteristic frequencies that can be used to parameterize foraging patterns. These analyses provide more precise methods for defining the effects of specific genes and neurons on *C. elegans* behavior. This paper is organized as follows. In Section 2, we describe the foraging detection algorithm, including image acquisition and pre-processing. In Section 3, we evaluate the algorithm by testing it on a variety of videos of mutant worms, and verifying the results with manual observations. We also describe how to extract foraging-related parameters, and we combine these parameters with Fourier analysis to analyze foraging behavior. Discussion and conclusions appear in Sections 4 and 5.

2. Materials and methods

2.1. Strains and culture methods

C. elegans strains were maintained as described (Brenner, 1974). For all assays, 4-day-old young adults were used; fourth-stage larvae were picked the evening before the experiment and tracked the following morning. Plates used for tracking were prepared by spreading one drop of a saturated LB (Luria broth) culture of *Escherichia coli* strain OP50 onto NGM (nematode growth medium) agar plates. Experimental animals were allowed to acclimate for 5 min before tracking. We used wild type worms and the following mutants: *dgk-1(nu62); glr-1(n2361); goa-1(n1143); trpa-1(ok999)*.

2.2. Acquisition of image data

C. elegans locomotion was tracked with a Zeiss Stemi 2000-C microscope mounted with a Cohu High Performance CCD video camera essentially as described (Geng et al., 2004). The microscope was outfitted for brightfield illumination from a 12 V 20 W halogen bulb reflected from a flat mirror positioned at an angle of approximately 45°. A tracker controlled by a Dell 1.0 GHz Pentium-III desktop computer was used to maintain the worms in the center of the optical field of the microscope during observation. To record the locomotion of an animal, image frames of the animal were captured at a frequency of 30 Hz and then saved as AVI video files (encoded with mpeg4v2) for at least 1 min ($30 \times 60 = 1800$ images per video). Next, we binarized the image using an adaptive threshold (the average value minus three times the standard deviation) and found the connected component with the largest area. The original image was then trimmed to the smallest axis-aligned rectangle that contained this component, and saved as eight-bit grayscale data. The dimensions of each image, and the coordinates of the upper left corner of the rectangle box containing the worm body in the tracker field were also recorded simultaneously. The microscope was fixed to its largest magnification (50×) during observation. The number of pixels per millimeter was fixed at 312.5 pixel/mm for all worms.

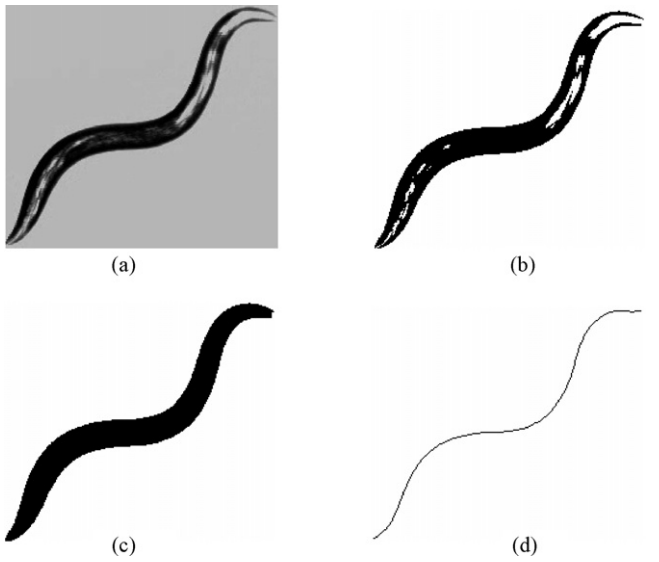

Fig. 1. (a) Gray level image acquired from a video sequence, (b) corresponding binary image after thresholding, (c) binary image after hole filling and closing operator and (d) skeleton after skeletonizing and pruning algorithm.

2.3. Image pre-processing

To facilitate analysis, the grayscale images were subjected to preliminary image processing to generate a simplified representation of the body (Geng et al., 2004). First any images which were snapped when the stage was moving (the current coordinate of the stage was different from the previous coordinate) were discarded because these images were usually blurry. Then for each good image frame (Fig. 1a), an adaptive local thresholding algorithm (Fig. 1b) followed by a morphological closing operator (binary dilation followed by erosion) was used. As described in (Geng et al., 2004), a corresponding reference binary image was also generated by filling holes inside the worm body based on image content information. The difference between these two binary images provided a good indication of which image areas are worm body and which are background (Fig. 1c). Following binarization, a morphological skeleton was obtained (Fig. 1d) (Geng et al., 2004; Gonzalez and Woods, 2002).

2.4. Locating the worm nose

After a morphological skeleton is obtained, 25 evenly spaced skeleton points are extracted. The two end points on the skeleton represent the head and tail positions (Fig. 2a). Using the

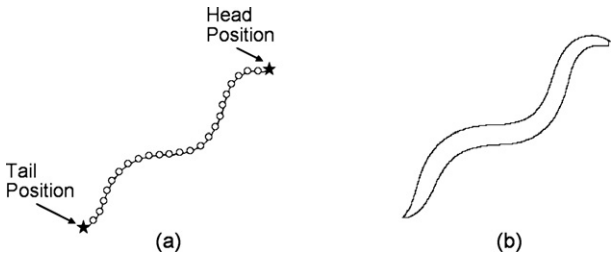

Fig. 2. (a) Skeleton with 25 sampled skeleton points and (b) the exterior contour of the worm body.