Abstract

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1 Introduction

2 Background

Dr. Kim at RFUMS wishes to know which neurons affect the food related behavior of *C. elegans*.

Due to the ineffectiveness of humans to observe and record worm activity with precision over either long or short time scales, it has become almost standard to record freely moving *C. elegans* either one at a time or in a group. These videos are then processed to segment the worm and provide intermediate representations of the worm's body (binary image, skeleton and sampled skeleton points). Features are then extracted from the intermediate representations and further mined for patterns to quantify phenotypes as in [1] or support hypotheses various hypotheses linking genetic manipulation to observed locomotory behavior.

Some recording implementations ([2, 3]) focus on one area of the dish, negating the need to recenter the worm(s) under study. Quantitative methods developed for this type of system are used to track the centroid of multiple worms only in that area of the assay and do not require a motorized tracker. Other implementations ([4]) used either a motorized stage (usually when using a microscope providing this functionality) or a motorized X-Y translation stage upon which a camera is affixed. Moving the camera over the stage (containing the assay and thus animal under study) is preferred to minimize the possible effects of the mechanical motion on the nematode's locomotory behavior.

We wish to record one worm at a time and are interested in comparing his locomotory behavior off-food to candidate mutant worms on food. It has been suggested in [] that wild-type *C. elegans'* off-food behavior is remarkably different from an on-food environment. Worms under this condition can travel longer distances in more frenetic behavioral patterns (ie: fast), requiring a larger dish. Other research groups use the *E. coli* bacterial lawn to restrict the area in which the animal will visit, an minimizing its maximal speed as explained in [], minimizing the worm's speed. This highlights the importance of developing a system for which a minimal amount of parameters need to be tweaked for appropriate motorized tracking is minimal for any type of worm under any experimental condition. For this system to be useful to Dr. Kim, the motorized tracking parameters need to be generalizable to any mutant- or wild-type of worm regardless of speed and activity.

2.1 Acquiring video for analysis

A critical first step in analyzing and quantifying worm behaviors involves acquiring video. In order to capture subtle differences in individual animal behavior, it is of high importance to capture worm movement at appropriate time and imaging resolution under imaging conditions favoring accurate extraction of intemediate representations of the worm's per-frame posture.

A high and accurate frame rate will provide time scales suitable for motion analysis. It will also provide more image data from which to extract features, allowing data scientists to throw out video frames for which segmentation and skeletionizing is ineffective, in which the image has become corrupt, or the nematode has been blurred by the motion of the camera. Traditionally challenging postures to segment or skeletonize are looped or coiled. The practice of throwing out these frames is slightly misguided, however, as often the frames being thrown out are those of high importance for locomotory analysis.

In order to provide and advantage to the segmentation process, optimizing the image quality is important. This can be accomplished by provided evenand uniform illumination at time of capture, facilitating the process of finding intensity thresholds that best separate a worm from its background. In order to minimize unwanted image artifacts effecting the selection of only worm pixels during segmentation, it is recommended to ensure proper carein the pouring of the

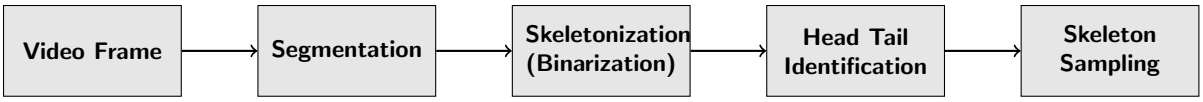

Figure 1: Standard Image Processing Sequence

agar (substrate upon which the worm is crawling), and imaging the plate with few fingerprints on the dish (trivial, I know, but important!) Also, the dish should be imaged with the agar facing up, allowing possible condensation observed over long recording periods to travel downwards, minimizing its impact on the image quality.

Most trackers available are written in MATLAB/C++ and require the Image Analysis Toolbox in order to acquire frames from a camera.

2.2 Quantifying worm behavior through video

Though slightly outside the scope of our current investigation, it is important to note some of the standards for extracting features. [4] proposes a decomposition approach to extracting features from the videos.

In general 4 "intermediate" representations are calculated using the sequence from 1. Decompositional features for phenotyping.

Using unsupervised learning techniques, [5] uses eigenworm representations of the worm postures developed in [6] to do really cool stuff. Their approach clusters worm mutants together. We would extend this approach, focusing on a smaller, more focused subset of mutants, instead of a wide variety of mutants they profile in [1].