Nemo: Machine Vision Quantification of Caenorhabditis Elegans Behavior

SUYING JIN, Ahis Shrestha, Joe Thatcher, Javier Carmona, Steve Mendoza and Katsushi Arisaka

UCLA, *Elegant Mind Club* @ Department of Physics and Astronomy

Elegant Mind Club
UCLA
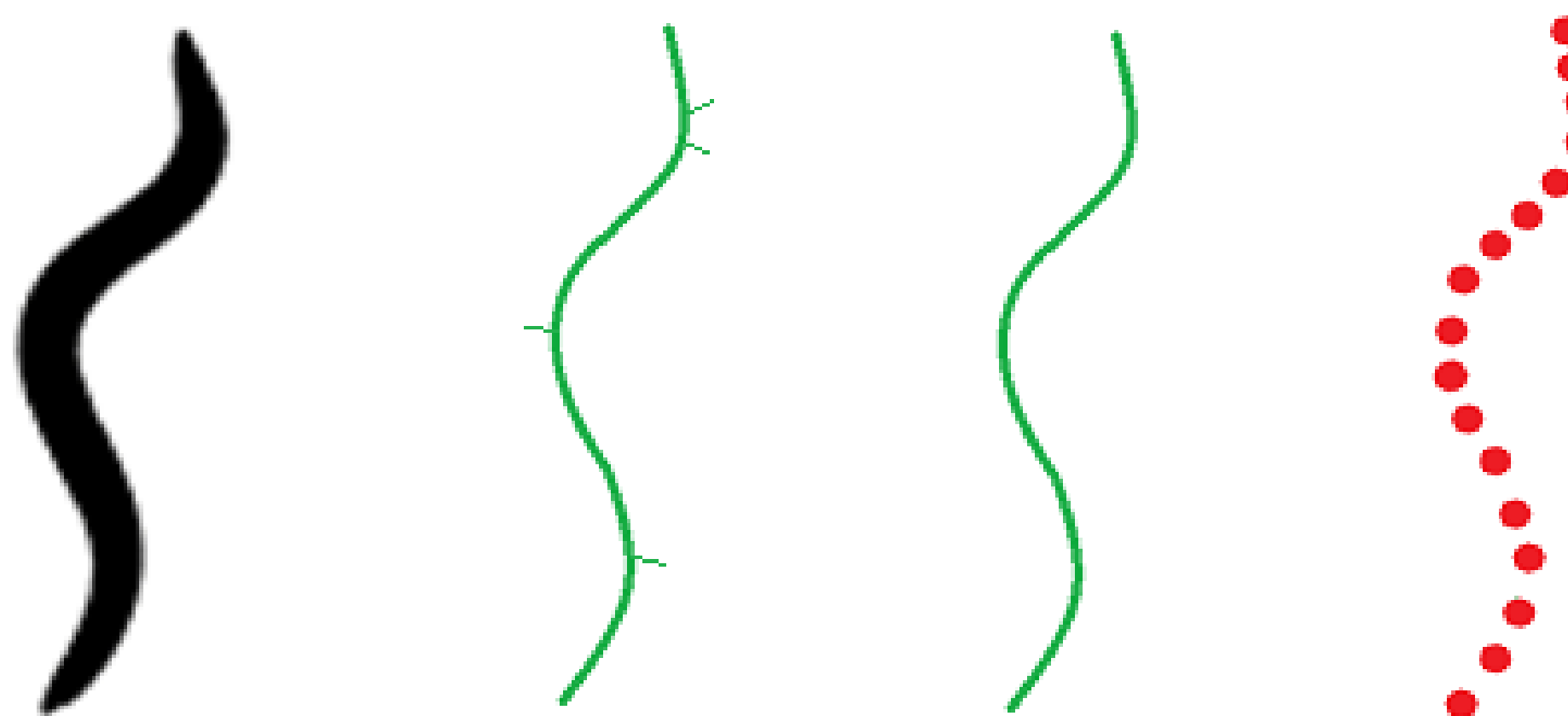
http://www.elegantmind.org

UCLA Science Poster Day on May 24, 2016

INTRODUCTION

- Caenorhabditis elegans is truly a model organism for neurophysics research, with only 302 neurons and a completed connectome

- With the goal of truly understanding the phenomenon of animal intelligence, the simple model "Input"→"Brain"→"Output" is probed in detail for C. elegans.

- "Inputs" are easily manipulated through a broad array of controlled behavioral experiments e.g., Electrotaxis, Phototaxis, Thermotaxis, Magnetotaxis.

- Accordingly, it is desirable to quantify these "outputs" through behavioral analysis software employing machine vision, rather than settling for qualitative, observational data

ALGORITHMIC DESIGN

Preprocessing of raw videos
- Brightness and contrast tweaked
- Thresholded to binary video
- Morphological operations: area opening, erosion and dilation

Extracting image mask
- Largest 8-connected object (the worm) is stored as a binary mask
- Smaller pixel area objects are thus thrown out (e.g. dust, air bubble, laser glare)

Ω Cutting (2D)
Determines where to "cut" the mask of worms in turns where head and tail touch. Ring->Rope

Projection (3D)
Three camera views (xy, xz, yz) are combined to form binary volume

Skeletonization
- Boundary pixels (voxels) removed iteratively
- Produces a tree-like skeleton rather than the desired single pixel (voxel) curve
- Two worm endpoints are chosen from the multiple endpoint, other branches discarded

Head/Tail Orientation
- User is prompted to choose head and tail for the beginning frame
- Head/tail identification done automatically throughout remaining frames

Behavioral Phenotype Detection
- The worm's behavior in each frame is indentified (forward, reversal, omega turn, pause)
- New states in (3D)? Broader array of behavioral phenotypes may need to be defined

Segmentation
- Skeleton is split into N (user specified) segments
- Stage data is incorporated for global position
- Space and time coordinates of center of mass and nodes connecting segments are exported to excel file
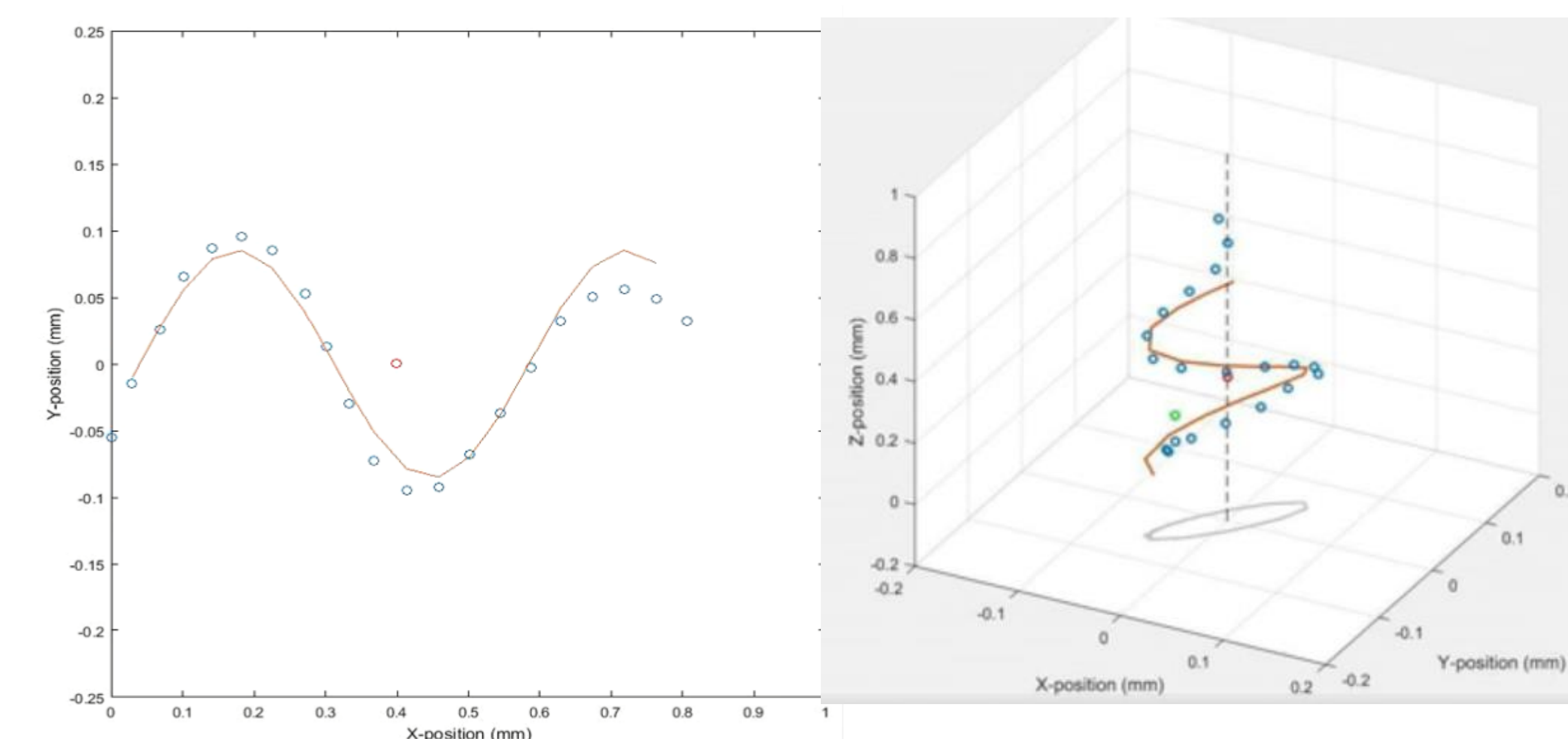
SKELETONIZATION AND SEGMENTATION

- Worm body is first reduced to a skeleton by iteratively removing the outermost layer of pixels (or voxels in 3D)
- Branched skeleton is corrected by assuming head and tail are endpoints with maximum mutual distance and removing all but those branches
- Final data stored as N (user specified) evenly spaced coordinates along smooth skeleton

OMEGA CUTTING

- Masks with a non-empty interior edge (orange in diagram) are set aside for correction prior to skeletonization
- Omega cutting is performed on these masks by traversing the interior and exterior edges and measuring local angles to determine where to "cut"
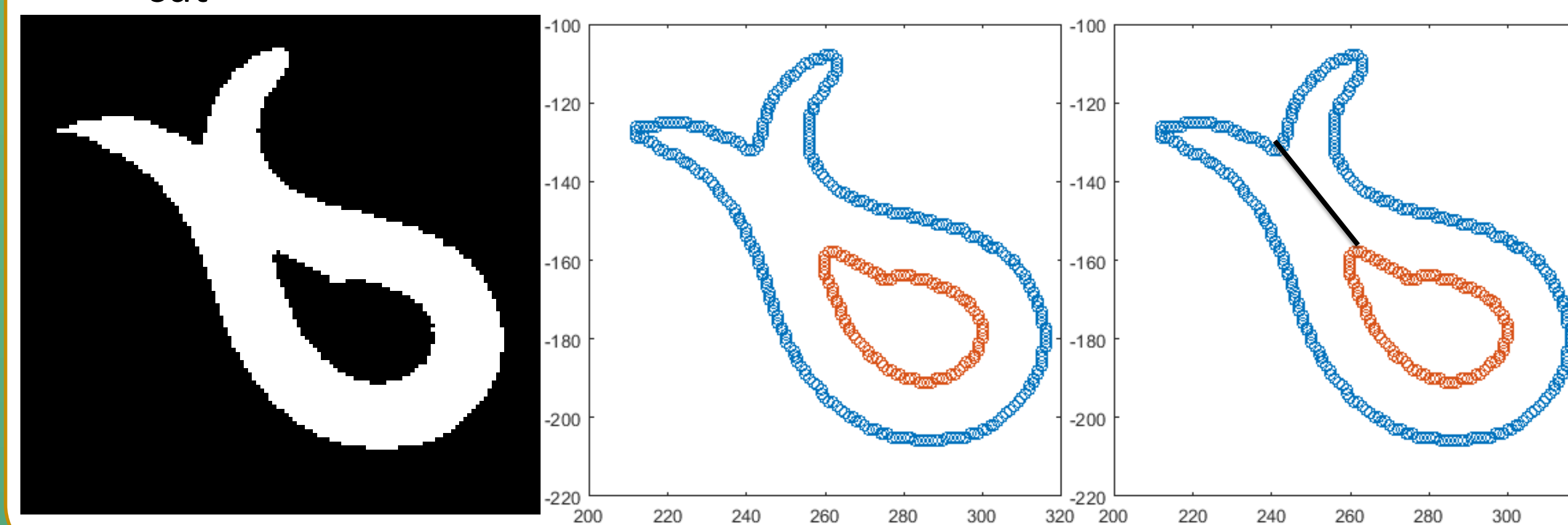
3D IMAGING METHOD

- Supplementing Nemo with the 3-d volume reconstruction method introduced in two papers by Namseop Kwon et. al, NEMO3D has almost identical functionality
- Detection of phases unique to 3D and potential 3D omega cutting yet to be implemented

BEHAVIORAL PHENOTYPE DETECTION
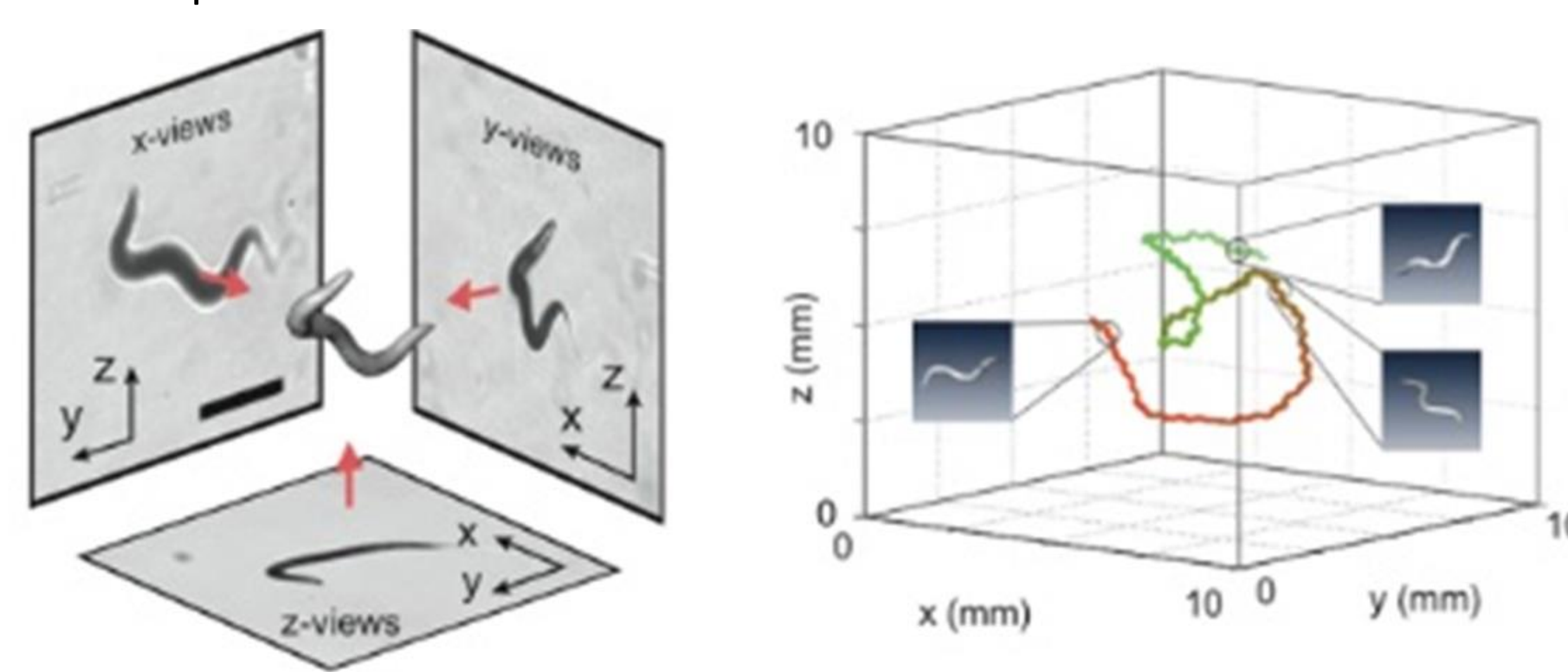
FORWARD   PAUSE   BACKWARDS   TURN
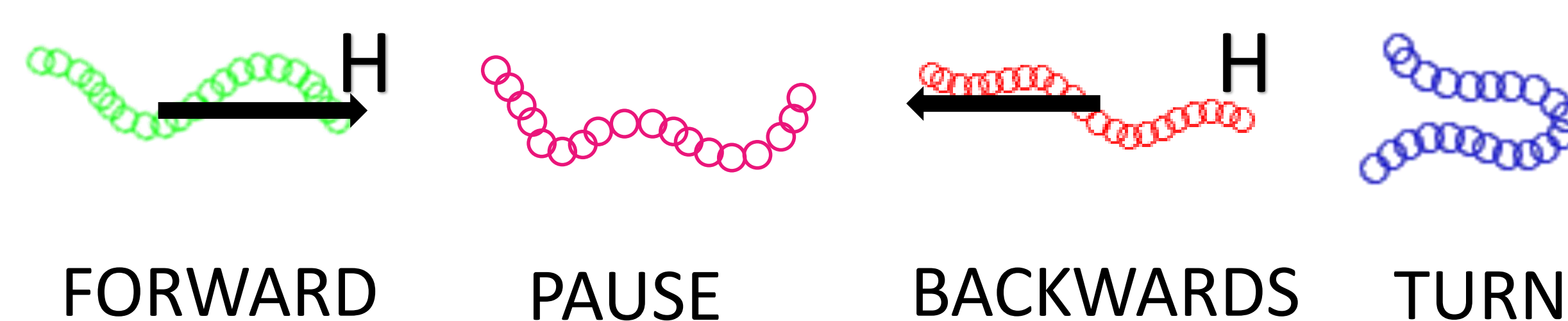
- To detect forward motion, backwards motion, or pause, a motion vector is defined based on the worm's center of mass location in a previous frame to the current COM location

- Turns are detected separately based on the ratio of distance between the head and tail points to the worm's overall body length

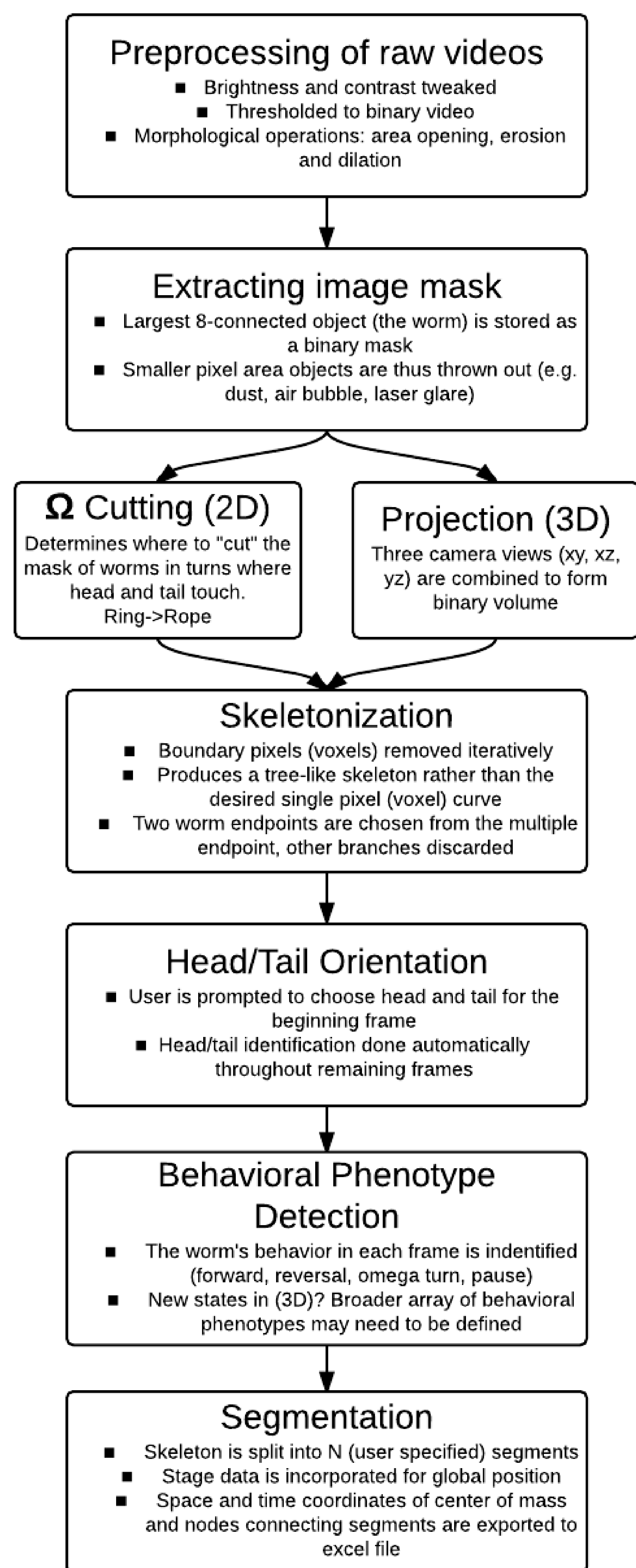- New motion states may be defined in 3D

FURTHER ANALYSES FROM NEMO OUTPUT

- Extracting coordinates from each frame allows for fitting of the worm's body data points to a sine function (2D) or helix (3D)

- Fitting to sine function provides measurements of wave speed to compare with translational speed measurements using center of mass only

- 3D fitting provides a measurement of helicity

- Fitting allows one to find new relationships among the variables available

- Phototaxis group showed that wave speed and strength of avoidance reflex are functions of gelatin concentration while translational speed is not

- Electrotaxis group showed bearing angle and damping parameter to be functions of electric field strength

CONCLUSIONS

- Nemo reduces the information stored in a video of worm behavior into a set of N coordinates along the body for each frame, thus efficiently preserving the results of any C. elegans behavioral experiment

- Automated behavioral analysis eliminates bias and provides reproducibility

- While there are no further analyses to be immediately performed on video data, sets of coordinates allow for new conclusions to be drawn which would have been previously unavailable e.g. wave speed, damping parameter

- Ultimate goal is to combine this behavioral analysis software with 302-neuron observation and pattern extraction software

RESOURCES

- George Tsibidis, Nektarios Tavernarakis (2007). Nemo: a computational tool for analyzing nematode locomotion, BMC Neuroscience
- Namseop Kwon, Jaeyeon Pyo, Seung-Jae Lee, Jung Ho Je (2013). 3-D worm tracker for freely moving C. elegans, PLoS ONE 8(2): e57484 (Source of 3D Imaging Method figure)
- MATLAB and Statistics Toolbox Release 2012b, The MathWorks, Inc., Natick, Massachusetts, United States.