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11/24/2024
Capstone Final

Quantifying Biological Rhythms:

An FFT-Based Approach to Visualization of Nematode Feeding Frequencies

Abstract

This paper describes a novel application employing the Fast Fourier Transform (FFT) in analyzing microscopy video data of nematodes, particularly focusing on their rhythmic feeding motions. By extending FFT into the temporal dimension of video clips, our methodology generates a stack of intensity maps, each representing varying frequency amplitudes. This approach allows for the visualization of spatial frequency distributions within the nematode, effectively highlighting areas of significant harmonic motion. Key to this process is the video preprocessing phase, which includes motion stabilization, an essential step for accurate FFT analysis. This is achieved through a center of mass stabilization technique based on the nematode's moments, ensuring the subject remains consistently positioned within the video frame.

The GUI facilitates users in importing videos, selecting, and cropping regions of interest, and choosing from a suite of preprocessing options, including convolution, background subtraction, and sharpening. Post-stabilization, the tool performs FFT on the processed video, resulting in a frequency-specific intensity map series. Users can interactively explore these maps, thresholding amplitudes to paint them onto the video, thus illustrating the precise locations and strengths of specific frequencies. This application not only dramatically accelerates the analysis of nematode harmonic motions but also provides clear insights into the biological origins of observed frequencies. The tool's capabilities are pivotal for researchers aiming to understand the genetic and neurochemical underpinnings of nematode responses to external stimuli, with broader implications in biological and pharmacological research.

Introduction

Caenorhabditis elegans is a model organism [1] for exploring complex biochemical mechanisms and behavior. The hermaphrodite nematode, C. elegans is ~1mm long, with a transparent body, and has exactly 302 neurons, and a largely mapped out neural synaptic connectome. Although one of the simplest organisms with a nervous system, it shares many neurochemical similarities with humans, exhibits robust behavior, and offers a simpler and more ethical alternative for invasive biological research to understand fundamental neuroscience questions [2].

This study developed a software tool to enable researchers to quantify changes in the feeding or “pumping” rate, as might be observed following administration of pharmaceuticals and biochemicals (such as serotonin.) The tool will also explore how genetic modifications affect responses to chemicals, offering insights into which genes influence responses to such substances. Compounds like serotonin have been shown to stimulate pharyngeal pumping [3]. This could be useful in assessing 1) functions of novel genes, and 2) characterize pharmacological compounds as potential therapies for neuropsychiatric, neurodegenerative, and neuromuscular disorders [4].

We describe here the development and features of a semi-automated video processing tool, written in Matlab. It applies to nematode videos, modern image and signal processing algorithms, in time and frequency domains, under graphical control by a biologist. The user selects a video file to analyze, upon which it is loaded and displayed. The user then graphically selects a

worm to analyze, wherein the software attempts to stabilize large scale motions and register all frames, and then computes the Fourier transform of every pixel in a user-defined region of interest (ROI) and displays temporal frequency spectrum superimposed on the worm.

Method

Software organization

In our Matlab-based software, the Graphical User Interface (GUI) is meticulously constructed using individual uicontrols, eschewing the standard GUI builder app for a more tailored approach. This decision results in an interface that is not only efficient but also intuitively navigable, thanks to its streamlined design of a single window divided into three distinct columns, facilitating a natural, left-to-right workflow. The first column, the video panel, is where users upload and manipulate raw microscopy videos—enabling play, cutting, and clipping to adjust size and length. The center column, the capture video panel, serves as the operational core where users can select preprocessing options such as convolution and define the Region of Interest (ROI). Upon pressing the 'Analyze' button, the selected preprocessing is applied, and the Fast Fourier Transform (FFT) is executed. The outcomes of this process, including pseudo-colored intensity maps representing various frequencies, are displayed in the same panel, allowing users to scroll through and observe the frequency distributions across the nematode's body. The rightmost column, the data panel, complements this analysis with an amplitude spectrum display. It features a moving vertical line that syncs with the frequency selected in the capture panel. This interactive element is designed to enhance the user's understanding by simultaneously displaying the highlighted frequency on a copy of the captured video, thus visually accentuating the spatial distribution of the amplitude and frequency chosen. The GUI's singular, consolidated figure approach ensures maximum understandability and efficiency, embodying a seamless blend of simplicity and functionality in its design.

Motion Correction

A key challenge in analyzing nematode videos is distinguishing the worm’s overall movement from the specific movements of its pharynx. To address this, we developed a motion correction algorithm based on the calculated moments of the worm in each video frame. This process involves segmenting each frame to isolate the nematode, creating a binary image where the nematode is distinct from the background. From this binary image, we compute the moments of inertia, offering a quantitative measure of the nematodes mass distribution within each frame. This method effectively separates the worm’s general movement from the targeted pharyngeal activity, enabling more precise analysis.

The Center of Mass is determined using the formulae:

$$M_x = \frac{\sum(x \cdot I)}{M}, M_y = \frac{\sum(y \cdot I)}{M}$$

Where x, y are the pixel coordinates, and I is the intensity at each pixel and M is the total mass. The summation is conducted over all pixels that constitute the nematode in the binary segmented image. This calculation of M_x and M_y provides the x and y coordinates of the COM, allowing us to track and correct for the worm’s movement in the capture video frames.

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Fast Fourier Transform (FFT) Processing

The FFT [5] was applied to the time dimension of the stabilized worm video data, transforming the temporal information into the frequency domain. This transformation is performed for every pixel within the ROI across all stacked frames in the clip. The spectral amplitude is represented as pseudo-color intensity maps, each frame of which representing spectral energy at different frequencies. The frames of $(x, y, frequency)$ data are scrollable. Their spatial average is also presented as a line graph of energy vs. frequency, immediately suggesting which frequencies have greatest intensities. A cubic polynomial least squares fit is applied to estimate the most probable peak pumping frequency.

Results and Conclusion

Our software's development interestingly outpaced the readiness of its intended users, leading to limited initial testing. Despite these constraints, the preliminary tests conducted on a handful of sample videos have been promising. The application consistently aligned with established research on nematode pharynx pumping rates, corroborating the estimates provided by senior biologists. Specifically, our analysis identified that wild-type (WT) nematodes display an average pumping frequency of around 4 Hz. This frequency falls well within the documented range of 200–300 pumps per minute (3.33–5 Hz) when food is present [6] [7] (cf. Horvitz et al., 1982., Hobson et al., 2006).

Crucially, the precision of frequency extraction by our software surpasses that of expert estimations. If subsequent validation confirms its accuracy, this could mark a significant enhancement over existing methodologies, offering greater sensitivity in experimental observations. One of the standout features of our application is its ability to overlay thresholded FFT intensity data onto video frames, vividly highlighting regions within the nematode where pumping action is most intense. This visual representation not only confirms frequency data but also provides potentially valuable biological insights, particularly regarding the biological targets of mammalian targeting drugs or the importance of neuronal genes that are being studied.

The spatial visualization of pumping frequency, combined with our tool's ability to precisely quantify such activity, ushers in new possibilities for biological research. This is especially relevant in exploring the effects of drugs on neural pathways and motor control in nematodes. In conclusion, our interdisciplinary approach not only reaffirms existing scientific knowledge but also introduces a novel and more robust method for visualizing and analyzing subtle biological movements. We look forward to further user feedback and to the contributions our application will make to biological and pharmacological research.

Acknowledgements

Special thanks to Dr. Brian Rasnow for his endless technical guidance and invaluable support in developing this application. Our gratitude extends to Dr. Gareth Harris for presenting a challenge that necessitated a solution and for providing access to data crucial for the development of our application. Additionally, we appreciate Dr. William Barber's insights into digital image processing and his significant contributions to the motion correction methodology.

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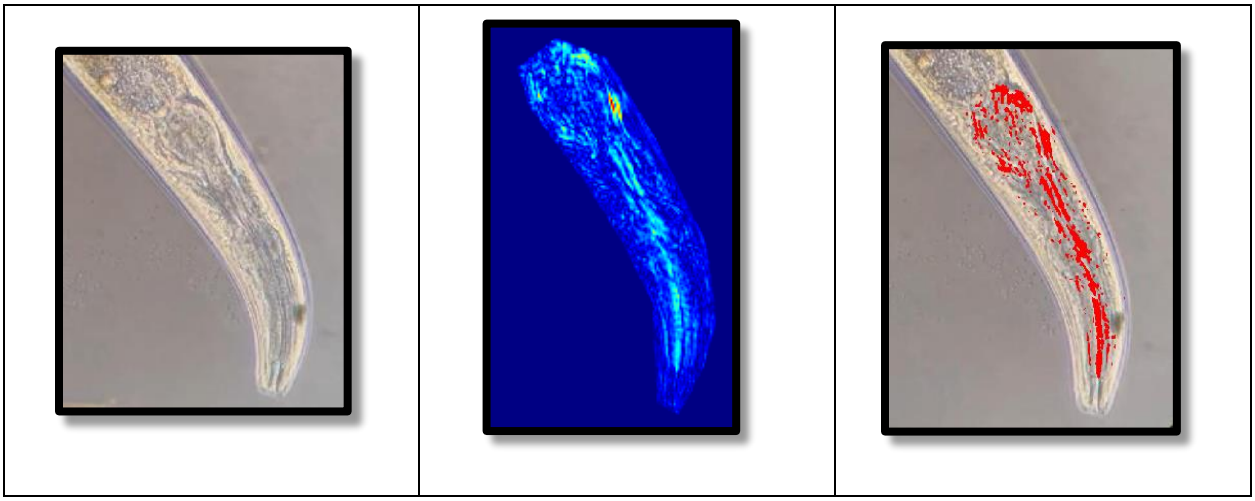

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