# OrNet: Spatiotemporal Analysis of Organelle Morphology


**Walter Avila[1], Shannon Quinn[2], Marcus Hill[2], Meekail Zain[2], Andrew Durden[2], Rachel Mattson[3], Frederick Quinn[4], S. Cahkra Cennubhotla[5]**

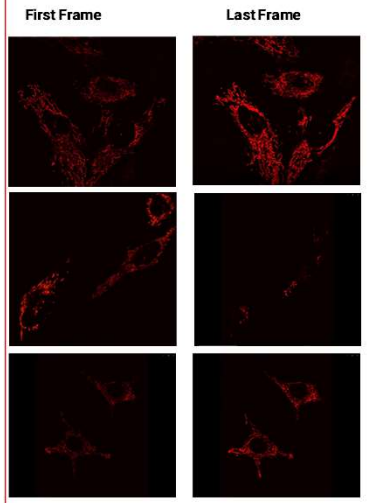
[1]Emory University , Atlanta, GA, [2]Department of Computer Science, University of Georgia, Athens, GA, [3]Department of Cognitive Science, University of Georgia, Athens, GA
[4]Department of Infectious Diseases, University of Georgia, Athens, GA, [5]Department of Computational and Systems Biology, University of Pittsburgh, Pittsburgh, PA


## Introduction

- Modeling changes in organelle morphology in response to infections is pivotal in studying pathogenic behaviors. Mitochondria are the most meaningful organelles to study because their structure transitions between fission and fusion states, especially in response to potentially fatal infections like tuberculosis, caused by *Mycobacterium tuberculosis* (Mtb). Mtb leads to about 1.5 million human deaths annually and disrupts host cell mitochondria morphology. Accurately modeling mitochondria could provide crucial information about such severe infections.
- Early approaches to studying mitochondria has consisted of labor-intensive manual inspection of fluorescent microscopy imagery of these organelles. **There is a need for techniques using quantitative measurements to more automatically model organellar dynamics to better guide the development of crucial treatments and tests.**
- The project at hand addresses this need by continuing development on OrNet. This Python framework analyzes spatiotemporal relationships of organelles via the construction of dynamic social network graphs of fluorescently tagged mitochondria in live microscopy videos. These graphs can be leveraged to quantitatively determine time points of anomalous behavior and spatial regions where organellar structures undergo significant structural changes in response to external stimuli.
- We aim to improve the Temporal Anomaly Detection (TAD) in OrNet. Detecting when morphology-altering events occur is important to understanding mitochondria and improves qualitative assessments of microscopy imagery by eliminating the need to inspect every frame manually.

## Data

- Live confocal imaging videos were taken of HeLa cells fluorescently tagged with the protein DsRed2-Mito-7. Cells were coerced into three different mitochondrial conditions to replicate mitochondrial changes in response to external stimuli.




- OrNet constructs dynamic social networks of fluorescently tagged mitochondria in live microscopy videos. The social network graph states are updated at each frame and are represented as Laplacian matrices, which are decomposed into their eigenvalues and eigenvectors to describe organelle morphology quantitatively. The eigenvalue vectors utilized for TAD are formatted as M X N NumPy arrays that correspond to each imaged cell. Thus, M is the number of video frames, and N is the number of eigenvalues.

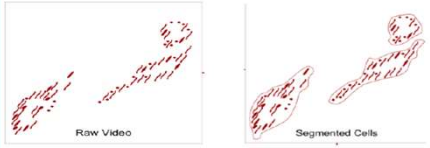
## Objective

- TAD flags frames whose averages are outliers by computing the z-score for each frame based on the average of its eigenvalue vector (row) and the mean of a few preceding averages. A fixed **"window"** parameter determines the number of preceding averages. If the z-score exceeds a **"threshold"** parameter (default two standard deviations), the frame is an outlier.
- The current TAD is sensitive to these parameters and taking the average eigenvalue per frame gives disproportionate weight to trailing eigenvalues. We wish to use different parameters and introduce a weighted average per frame into TAD to assess its behavior. We seek to ask:

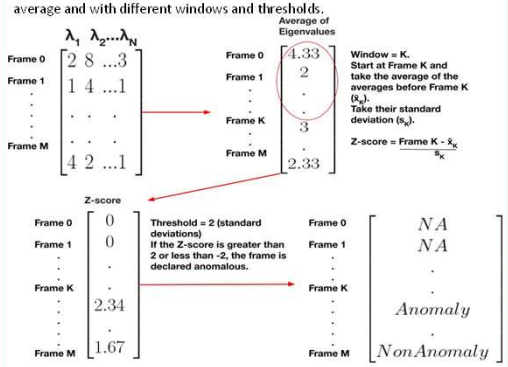
**Using three different window and threshold parameter values, 10,20,50, and 2.0,2.5, and 3.0, respectively, how does the TAD using the simple average per frame compare to the TAD using the weighted average per frame among cells in three different mitochondrial conditions?**

## Methodology

1) Raw videos of cells from each experimental group were taken. Social networks graphs were created for segmented individual cells from each video.




2) Segmented cells are ultimately represented as eigenvalue vector M X N NumPy arrays. There were 29 Control arrays, 66 LLO arrays, and 31 MDIVI arrays. Arrays correspond to each cell in a video. Conduct TAD on each array using the simple average and with different windows and thresholds.




3) For each set of window and threshold parameter values among each experimental group, calculate the proportion of frames declared anomalous by the TAD per NumPy array and average that proportion among each experimental group.

4) Repeat steps 1 to 3 but using TAD with the weighted average eigenvalue per frame.

5) Compare TAD with simple average to TAD with weighted average.

## Results

- The output by TAD is a plot containing two subplots. The top subplot visualizes the eigenvalue time-series of a cell. The bottom subplot shows the corresponding outlier signal plot where peaks represent time-points declared anomalous by TAD.

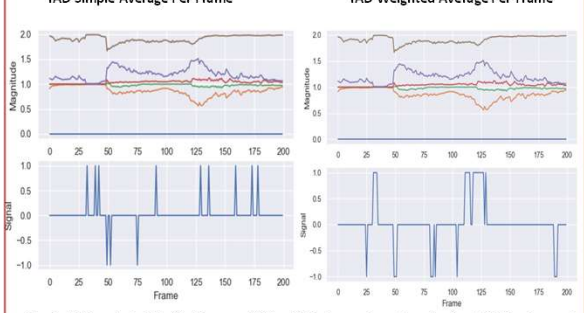



*Fig. 1: TAD output plots for the same LLO cell that experienced a mitochondrial fission event. These plots were generated using the following default parameters: Window = 20 & Threshold = 2.0 Standard Deviations .The plot on the left consists of the magnitude and signals plot generated using the simple average per frame. The plot on the right consists of the same magnitude plot but a different signals plot generated using the weighted average per frame.*

- To compare the TAD with the simple average to the TAD with the weighted average, the average proportion of frames declared anomalous for each NumPy array per experimental group for different window sizes was calculated.

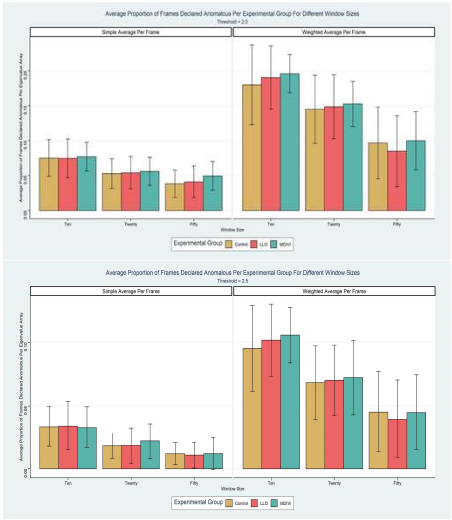


*Fig. 2: Average Proportion of Frames Declared Anomalous Per Experimental Group For Different Window Sizes for Threshold = 2.0 & 2.5. The x-axis consists of different windows with which the TAD was performed. The y-axis consists of the average proportion of frames declared anomalous per NumPy array. Error bars are standard deviations. The left subplot consists of the data generated by the TAD with simple average. The right subplot consists of the data generated by the TAD with weighted average. The same plot was created for Threshold = 3.0 and showed the same overall pattern but is not included.*

## Discussion

- On average, the weighted average TAD code declares a higher proportion of frames anomalous in each experimental group for each window size and threshold than the TAD code utilizing the simple average per timepoint.
- T-tests comparing experimental group average proportions generated by TAD with simple average and TAD with weighted average per window all yielded significant differences ( $p < 0.05$).
- Regardless of which TAD was utilized, increasing the window and threshold parameters, on average, decreased the proportion of frames declared anomalous for each experimental group.
- Within the same window size, threshold, and TAD version, the average proportions varied little between each experimental group.

## Conclusion and Future Directions

- Modeling when structural changes occur in mitochondria can aid in understanding pathogen infection behaviors and how cells respond to pathogens.
- Our goal is to implement OrNet to conduct large-scale genomic screens of genetic mutants of Mtb to better understand how these pathogens invade cells and induce cell death at the genetic level.
- Learning that the TAD using the weighted average, on average, declares a higher proportion of frames per imaged cell in different mitochondrial conditions anomalous than the TAD using the simple average will guide how OrNet is implemented in the future and is another important step in achieving our goal.
- The original videos need to be rigorously revisited to link phenotypic changes to anomalous frames and to compare anomalous frames from both TAD versions.
- Further comparisons will include determining differences in how frequent certain sections of the videos for each mitochondrial condition are declared anomalous by both TAD versions.

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