Quantification of Mitochondrial Dynamics in High Invasive and Low Invasive Prostate Cancer Cells

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3:00 PM EST

Research Project Details

Project Duration

September 2015 - Present

Advisor

Dr. Andrew Cohen

THE WISTAR INSTITUTE

Collaborators

Dr. M. Cecilia Caino

Dr. Dario C. Altieri

Drexel UNIVERSITY

Hosting Institute

Drexel University

What Happens during Cell Metastasis?

Previous Work: M. Cecilia Caino and Dario Altieri (August 2015)[1]

Mitochondria are transported to the periphery of the cells

- Mitochondria organelles migrate toward the focal adhesion points of the cell in high invasive cancer cells
- Transported along microtubules and actin tracks

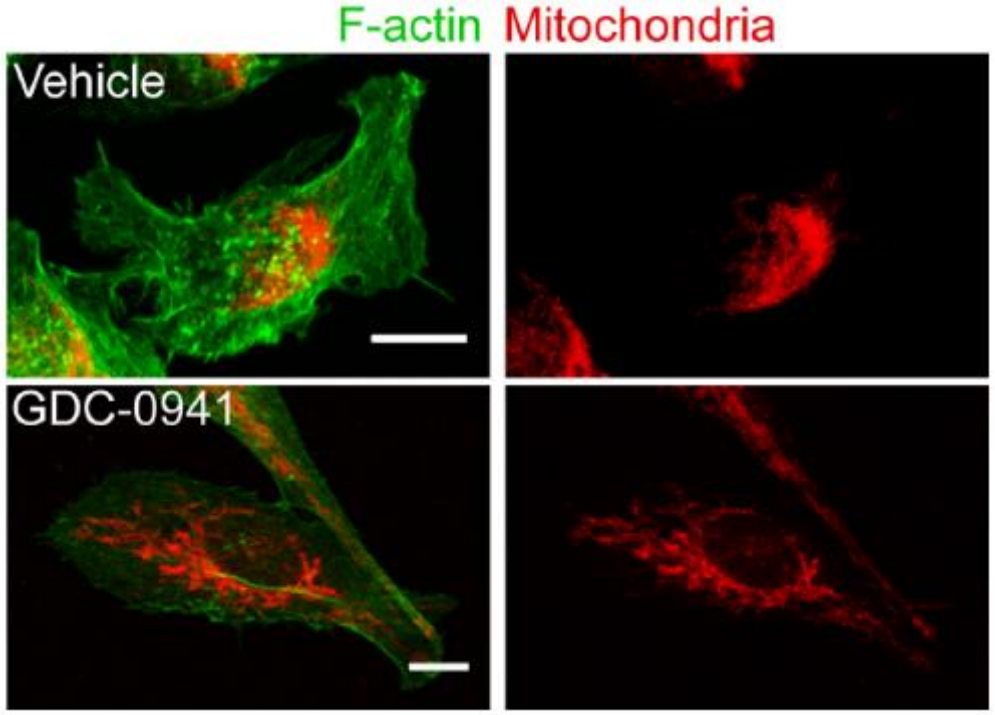


Figure 1. Confocal microscopy image of tumor cells treated with vehicle (control) and GDC-0941 (PI3K antagonist). Maximum projection of 3D stack. Scale bar = 50um.[1]

[1] Caino, M. Cecilia, and Dario C. Altieri. "Disabling Mitochondrial Reprogramming in Cancer." Pharmacological Research 102 (2015): 42-45. Web.

Why Mitochondria?

Previous Work: M. Cecilia Caino and Dario Altieri (November 2015)[2]

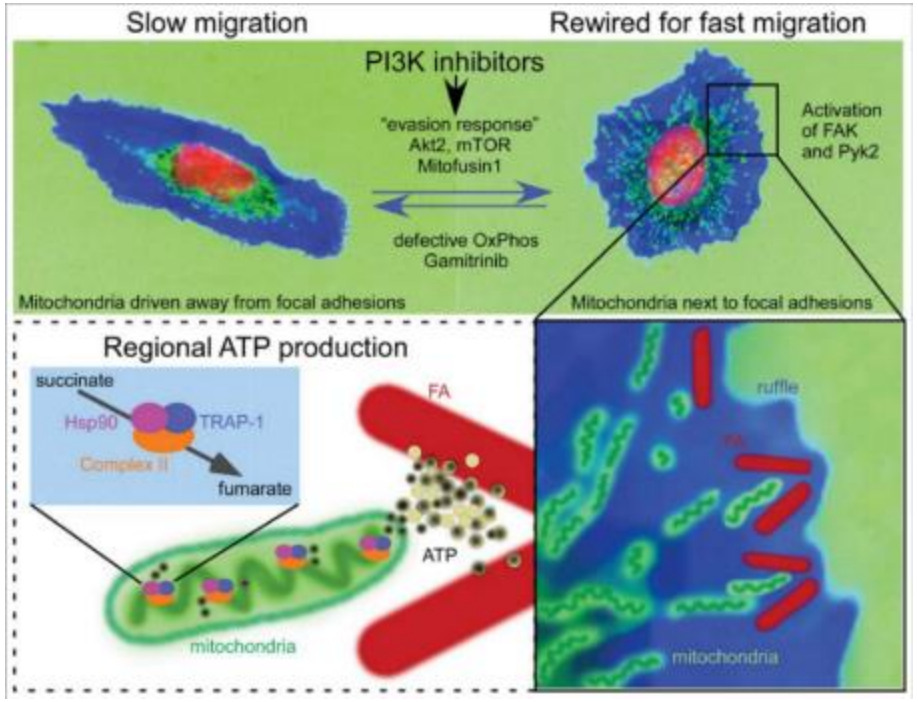


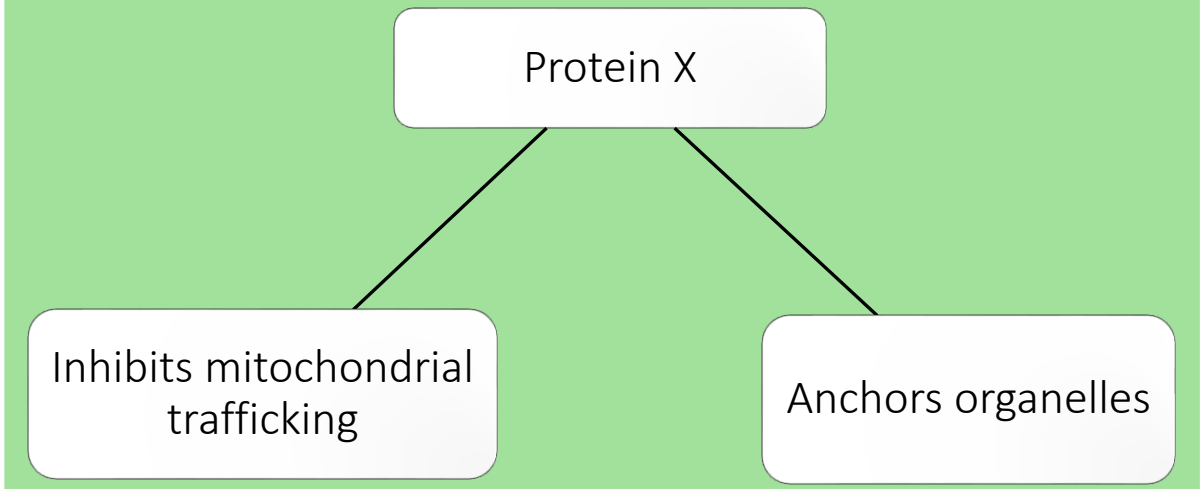
Figure 2. Schematic representation of mitochondria localization in the presence of PI3K inhibitors. This promotes regional ATP production near the focal adhesion points. [2]

- In healthy cells, mitochondria are transported based on the demand for energy production
- During metastasis, external mobilizing processing are in need of high levels of macromolecules
- ATP is delivered to focal adhesion points

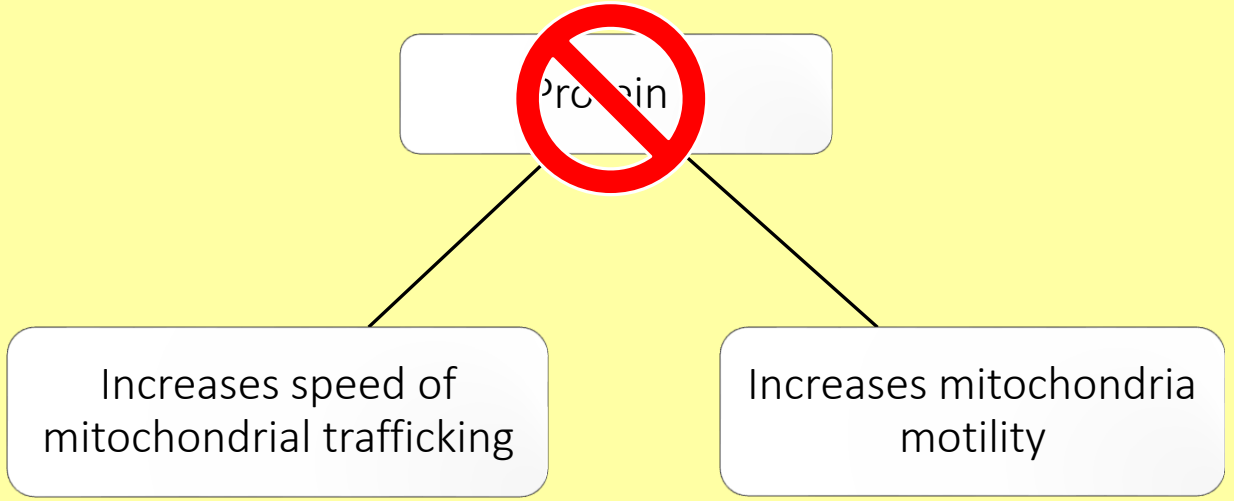
[2] Caino, M. Cecilia, and Dario C. Altieri. "Cancer Cells Exploit Adaptive Mitochondrial Dynamics to Increase Tumor Cell Invasion." *Cell Cycle*14.20 (2015): 3242-247.

Mechanism Related to Mitochondria Mobility

In non-invasive cells



In highly-invasive cells



Objectives/Hypotheses Tested

Three major detectable morphologies or behaviors are expected to occur in the absence of Protein X:

1) Increased number of mitochondrial fission and fusion events in high invasive cell (HI) versus control (CTRL)

2) Increased speed of mitochondria in high invasive cell (HI) versus control (CTRL)

3) Mitochondria closer to the boundary in high invasive cell (HI) versus control (CTRL)

Experiment Parameters

- Cells underwent gene therapy to suppress the expression of Protein X → High-invasive cells
- Prostate cancer cells
- 30 cells total
  - 15 Low-invasive cells (Control)
  - 15 High-invasive cells
- Mitochondria marked with Red Flourescent Protein (RFP)
- Time-lapse confocal microscopy images of each cell
  - 101 frame taken, 10 seconds apart

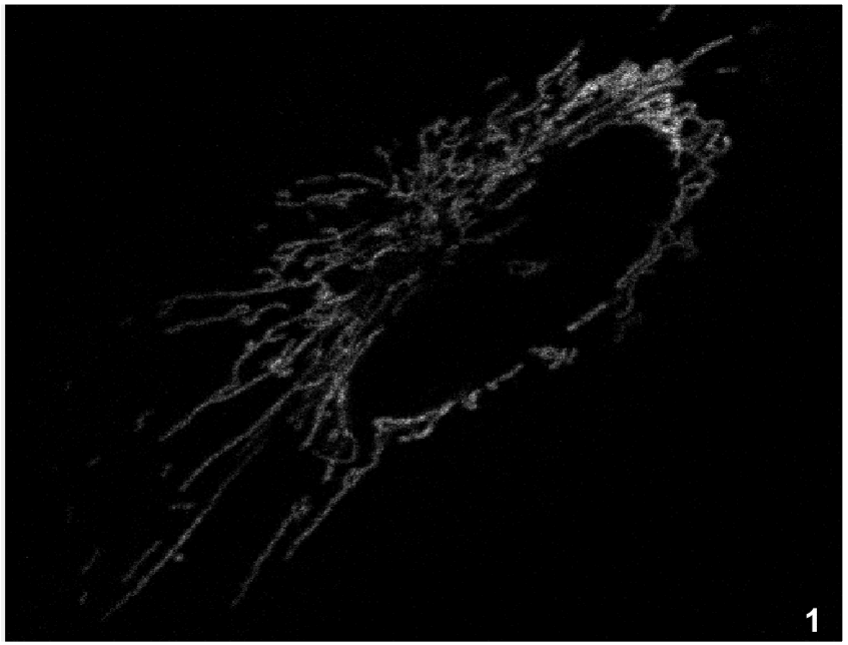


Figure 3. Time-lapse confocal images of mitochondria in a control cell before image processing.

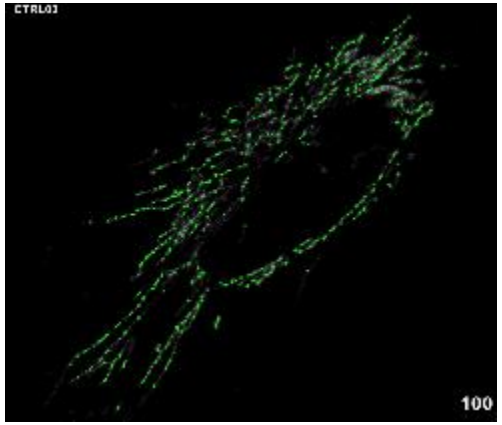
Computational Analysis

Pre-Processing

Filter Image

- **Iterative Gaussian[3]**
- **Median Filter**

Segmentation



Tracking

Assign Tracks IDs (Hungarian Algorithm[4])

Global Track Analysis

Preliminary Database

ID
Start Frame
End Frame
Lifetime
Centroid (per Frame)
Size (per Frame)

[3] Michel, René, Ralf Steinmeyer, Michael Falk, and Gregory S. Harms. "A New Detection Algorithm for Image Analysis of Single, Fluorescence-labeled Proteins in Living Cells." *Microscopy Research and Technique Microsc. Res. Tech.* 70.9 (2007): 763-70. Web.

[4] Munkres, James. "Algorithms for the Assignment and Transportation Problems." *Journal of the Society for Industrial and Applied Mathematics* 5.1 (1957): 32-38. Web.

Fission & Fusion

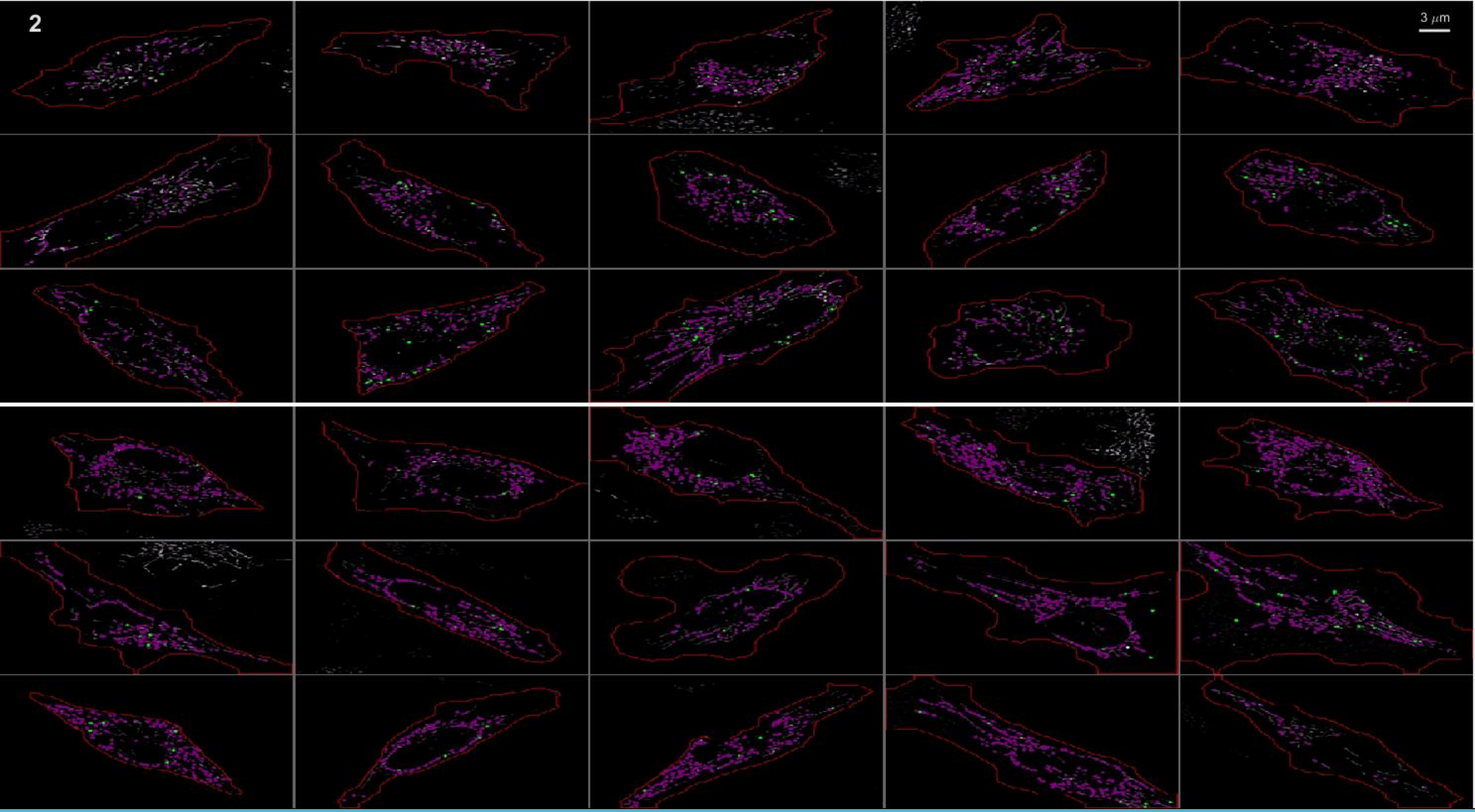
- Use start frame and end frame of a track
- Identifies its ‘nearest self’

Velocity

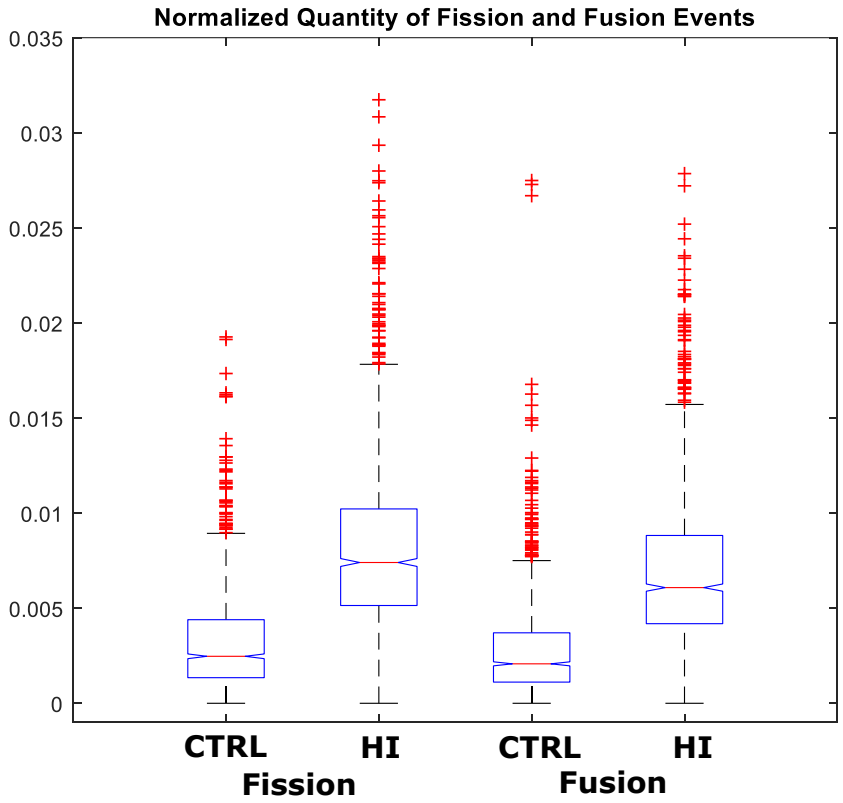
- Distance centroid traveled every frame

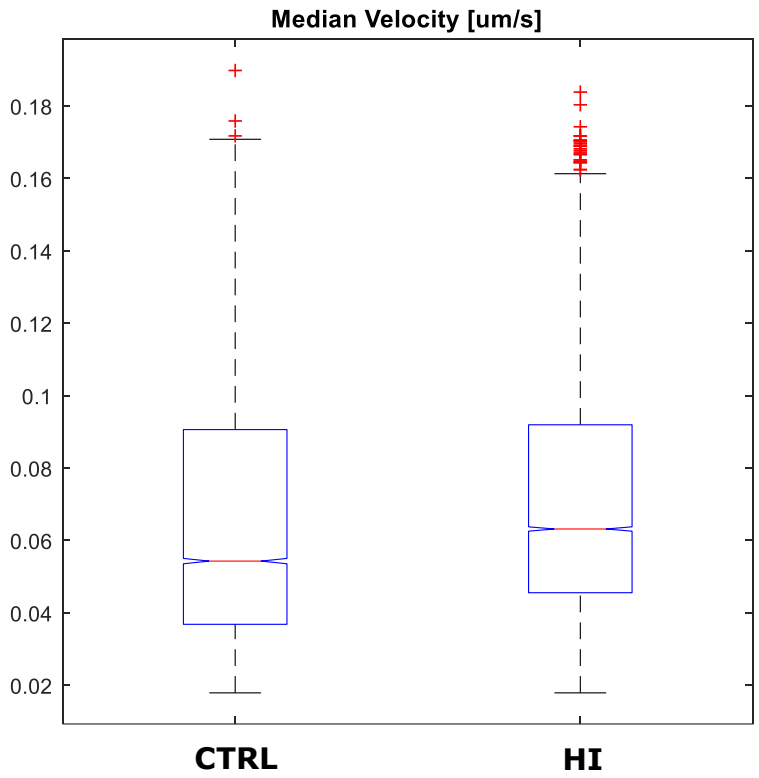
Spatiotemporal Distribution

- Distance transform of cell computed
- Obtains distance from boundary for each mitochondria centroid


2
3 μm

Results





Group	Wilcoxon Rank-Sum Test (p-value)
Fission (CTRL v HI)	4e-219
Fusion (CTRL v HI)	9e-197
Velocity (CTRL v HI)	3e-96

3D Movie Demo

Project Reflection

ROLES & RESPONSIBILITIES

Software developer for a mitochondria analysis package containing the following features:

- *Segmentation and tracking* of mitochondria
- *Quantification of fission and fusion* events
- Calculation of *mitochondria migration velocity*
- Calculation of *spatial distribution* of the mitochondria
- Automatic output of *plots, movies, and annotated images*

This software was developed in MATLAB and MEX (C, C++)

Project Reflection

SKILLS LEARNED

- Developed good coding practices
- Greatly improved my skills in programming algorithms
- Learned how to construct visuals that best ‘tell your story’
- Contributed to a research publication end-to-end
- Learned how to assess if results ‘make sense’
- Learned how to combat frustration and failure

Thank you for listening! Questions?

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Drexel University, 2017

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