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LARGE-SCALE ANALYSIS OF SPATIOTEMPORAL ORGANELLAR NETWORK EVOLUTION

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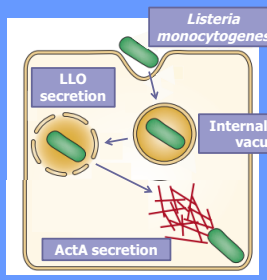
Introduction

The goal of the presented research is to develop and use a screening system to study the interactions between host organelles and secreted bacterial virulence factors. The screening system employs live imaging of infected cells in combination with a post-acquisition image processing software package called OrNet. This will allow for analysis of minute changes in organelle shape, distribution, and mass over the course of infection. By examining changes in organelle dynamics during infections with bacterial secretion mutants, we propose to determine which bacterial proteins affect organelle processes, and use that information to better understand bacterial virulence mechanisms and host cell control of organelle function.

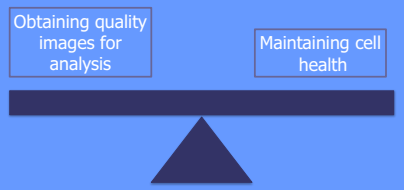
We will begin by studying changes in mitochondrial morphology during *Listeria monocytogenes* infection. Recently, it was discovered that the intracellular pathogen *L. monocytogenes* alters the dynamics of mitochondria in HeLa cells, causing mitochondrial fragmentation. While the direct cause of fragmentation remains unclear, the presence of the *L. monocytogenes* pore-forming toxin listeriolysin O (LLO) was found to be necessary for its occurrence(1, 2). Our goal is to further characterize mitochondrial fragmentation during *L. monocytogenes* infection by live imaging cells infected with fluorescently labeled candidate mutants and analyze the resulting videos with the bioimaging toolkit OrNet.



During *Listeria monocytogenes* infection in a variety of cell lines, LLO is known to assist in escape of the internalization vacuole. *L. monocytogenes* mediated polymerization of actin (shown in red) is a means of propulsion through host cytoplasm. Modified from Hamon et al (3).

I. Determine the best method of imaging mitochondria in live human cells over extended time with high resolution

Mitochondria are dynamic organelles and rounds of fission and fusion are essential for maintenance of function(4). Thus, it is necessary to image with relative frequency in order to capture the morphological changes. Unfortunately, prolonged exposure to light is damaging to cells, as free radicals are produced as a byproduct of excitation(5-7) Therefore, it is necessary to find a balance between imaging and keeping cells healthy on the microscope.



One equipment choice made to reach this balance is to use the resonance scanning head on the Nikon A1R confocal. The Nikon A1R confocal possesses two distinct scanning heads, which provide two different methods of acquiring an image. The galvano scanning head is primarily used when imaging fixed samples. This head scans the field relatively slowly and captures a very high quality image. The resonance head, however, scans the field more quickly. The shorter image acquisition prolongs the life of the fluorophore and still provides a quality of image that can be examined by our image processing tools (https://www.nikoninstruments.com/Products/Confocal-Microscopes/A1R).

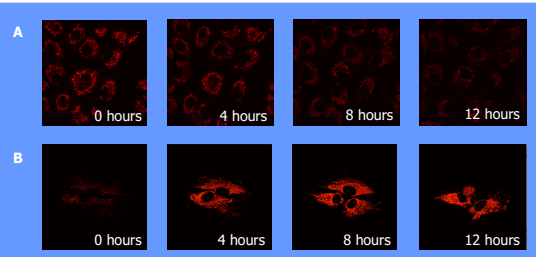


Fig. 1. Cells stained with MitoTracker CMXRos photobleach during live imaging, but DsRed2-COX8A$_L$ transfected cells do not. An image was taken once every 10 seconds for 12 hours (A) A549 cells labeled with 100nm CMXros for 30 mins and imaged. (B) HeLa cells transfected with DsRed2-COX8A$_L$. Cells become brighter throughout imaging likely because of an increase in expression of the fluorescent protein.

II. Establish experimental parameters of mitochondrial structure and mass in HeLa cells

Mitochondria can exhibit a spectrum of morphologies, from fragmented to hyperfused. Thus, before examining bacterial effects on mitochondrial morphology, we will identify experimental parameters by live imaging cells under defined conditions.

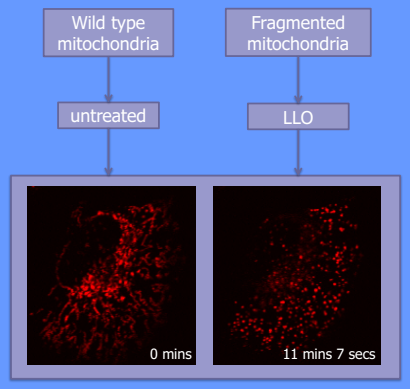
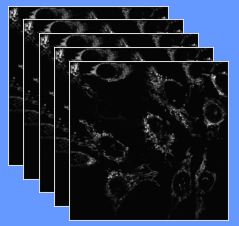


Fig. 2. Establishment of experimental parameters through exposure to 6nM recombinant LLO.

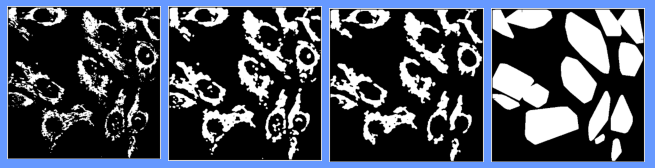
III. Use live cell imaging of *L. monocytogenes* infected HeLa cells to refine OrNet's functionality

We can use data gathered from time-lapse live imaging to observe changes in the mitochondria (or other fluorescently-tagged organelle of interest) over space and time. We are attempting to model mitochondria as **interconnected nodes in social networks**, hypothesizing that observed changes in these social networks (connectivity, centrality, size, etc) will provide additional insights into the changing spatiotemporal patterns of the mitochondria in response to *L. monocytogenes* infection.



Objectives

1. Segment out mitochondria associated with each cell (completed)
2. Quantify social network phenomena over time for each cell: quantity, shape, and spatial distribution (future)



1. Threshold each image using Ridley-Calvard algorithm to create image mask.
2. Dilate image mask and further smooth with median filter.
3. Drop all objects under specific size threshold.
4. Compute convex hull of all remaining objects.

The result from step 4 is computed for sliding windows of consecutive frames, ultimately providing a series of image masks that contain the mitochondrial networks for specific cells. This isolates the mitochondria belonging to a single cell and will allow us to identify network dynamics on a cell-by-cell basis in our continuing work.

Table 1. Known functions of candidate mutants

Strain	Known function of deleted gene
Δhly	Cholesterol-dependent pore-forming toxin
ΔsecA2	Homolog of SecA1 of the secretory pathway
ΔactA	Polymerizes host cell actin
ΔprfA	Positive transcriptional regulator that controls expression for multiple genes involved in virulence

References

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