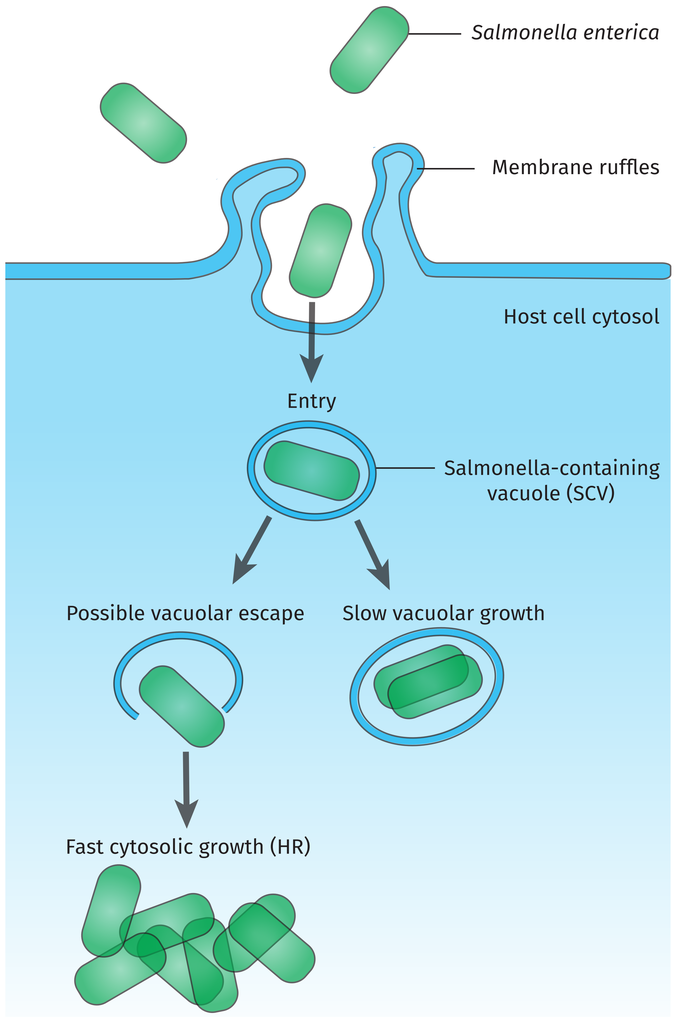
Modelling Growth in Host-Pathogen Dynamics

Taylor Dunn (taylor.dunn@dal.ca) and Andrew Rutenberg
Department of Physics & Atmospheric Science, Dalhousie University

The Invasion Process

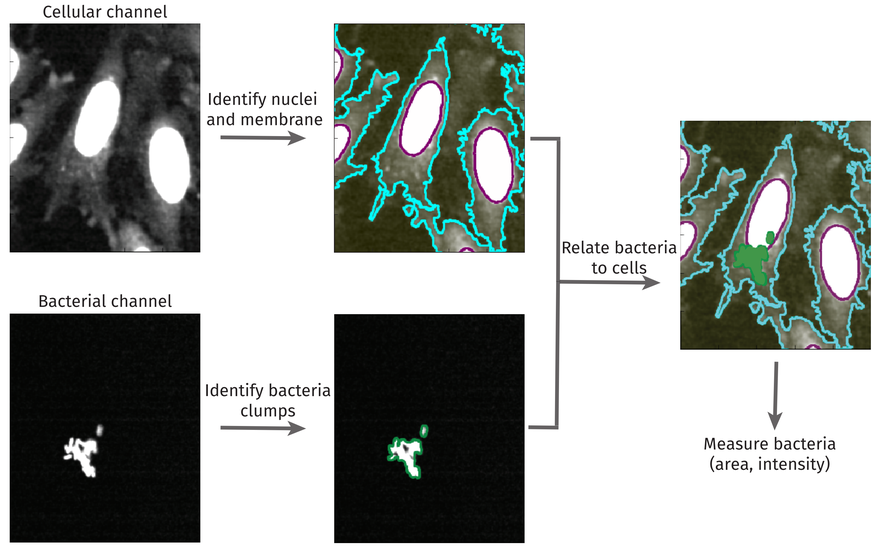
The dynamics of invasion of cells by pathogenic bacteria is a stochastic process, consisting of several stages including attachment, entry by endocytosis, possible vacuolar escape, replication and transmission to other cells [1]. To date, there has been little quantitative modelling of these laboratory invasion systems.

Despite being traditionally categorized as a vacuolar pathogen, the bacterium *Salmonella enterica* has recently been shown to hyper-replicate (HR) within the cytoplasm of some epithelial cells, while remaining slow growing within the vacuole of others [2]. The factors which influence the bacteria to exit or remain inside the vacuole remain mysterious. A key question in this regard is whether or not single host cells can have both slow and fast-growing bacteria simultaneously, and whether distinct growth rates can be extracted from experimental data. Additionally, how does the multiplicity of infection (the initial ratio of bacteria to cells) affect growth and our capacity to characterize it?

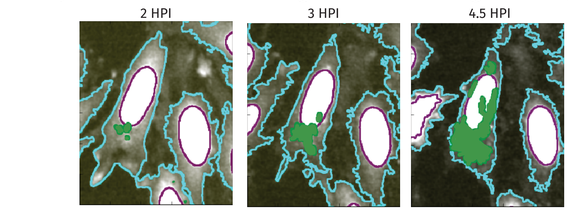


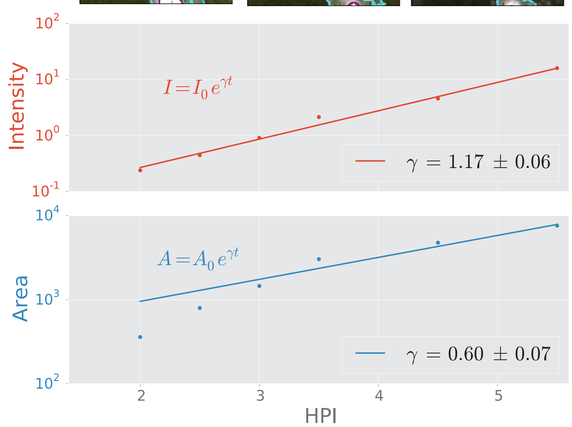
Timelapse Image Analysis

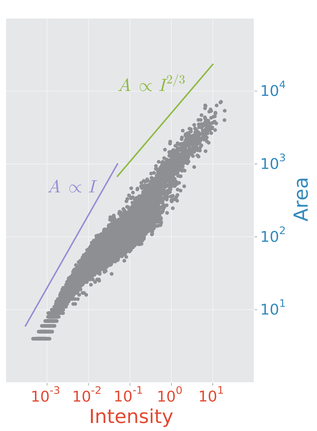
Fluorescence microscopy images of wildtype *Salmonella* invading HeLa cells were taken at various time points post infection, and at three different multiplicites of infection (MOI), by our experimental collaborators at the Institut Pasteur (lab of Jost Enninga). Using the CellProfiler image analysis software [3], we segmented these images by identifying nuclei, cellular membranes and bacteria in their respective fluorescent channels.



The bacteria were measured in terms of area (in pixels) and integrated intensity, and then assigned to cells. The bacterial load per cell was calculated at each time point, up to 5.5 hours post infection (HPI) and this growth was fit to an exponential to determine growth rates γ. To determine which growth rate (area or intensity) is a better representation of number of bacteria (and therefore the real growth), measured area was plotted versus intensity. This revealed a scaling of $A \sim I^{2/3}$ for large values, indicating that intensity is capturing the third dimension of these bacterial clumps. We therefore conclude that intensity is the superior proxy for number of bacteria over area, as it is only a measure of two dimensional size.

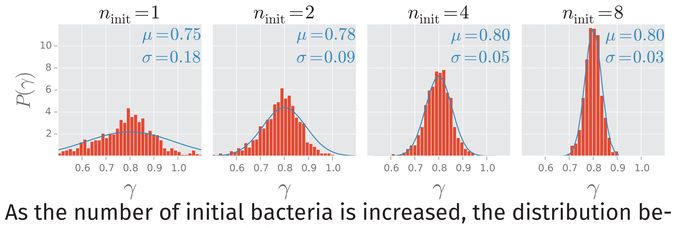






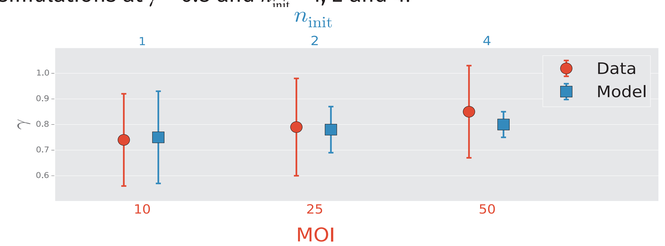
Growth Model

To examine growth behaviour at the single-cell level, stochastic simulations based on the Gillespie algorithm [4] were performed. This involved finding the division time τ of each bacterium by drawing random numbers from the exponential probability density function $P(\tau) = \gamma\, e^{-\gamma\tau}$. 10000 cells with hyper-replicating bacteria were simulated for up to 5.5 hours post infection. The average growth rate γ was kept constant at 0.8/hour, while the initial number of bacteria n_{init} was varied. Growth rates per cell were measured and the probability distributions are plotted below.



As the number of initial bacteria is increased, the distribution becomes more narrow and the mean approaches the actual value of γ - the expected behaviour for cells with unimodal bacterial growth. A mixed growth, however, would result in a lower apparent growth rate and wider distribution.

A higher MOI leads to a higher number of invading bacteria per cell, or n_{init}. From our timelapse images, cells which appeared to have hyper-replicating bacteria were identified and carefully segmented to achieve good accuracy. Intensity growth rates were extracted at MOI's of 10, 25 and 50 and the means with standard deviation bars are plotted below. Also plotted for comparison are the mean and standard deviation from 10000 stochastic growth simulations at γ = 0.8 and n_{init} = 1, 2 and 4.



For unimodal growth, we would have expected the variance in the measured growth rates to decrease with MOI as the model showed. This was clearly not the case, suggesting that cells which appear to be hyper-replicative may have a mix of slow and fast-growing bacteria. Consequently, naive fitting of single-cell growth at large MOI may not be a reliable method of extracting growth rates. We will continue to improve the growth model and analysis techniques to characterize the bimodal growth of *Salmonella*.

References

[1] Ray K et al. (2009) Life on the inside: the intracellular lifestyle of cytosolic bacteria. *Nature Rev Microbiol* **7**: 333-340.
[2] Knodler LA et al. (2014) Quantitative assessment of cytosolic *Salmonella* in epithelial cells. *PLoS ONE* **9**: e84681.
[3] Carpenter AE et al. (2006) CellProfiler: image analysis software for identifying and quantifying cell phenotypes. *Genome Biol* **7**: R100.
[4] Gillespie, DT (1977) Exact stochastic simulation of coupled reactions. *J Phys Chem* **81**: 2340-2381.