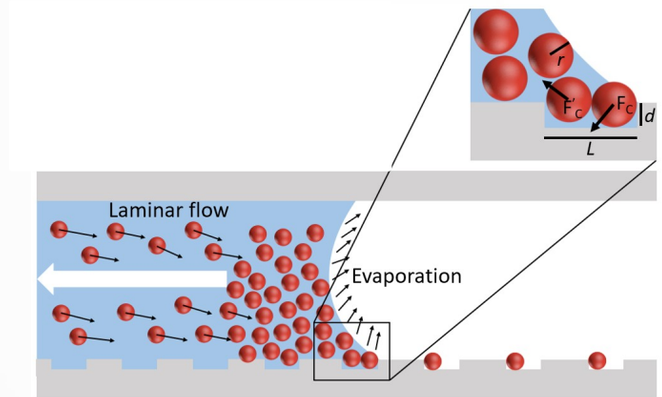
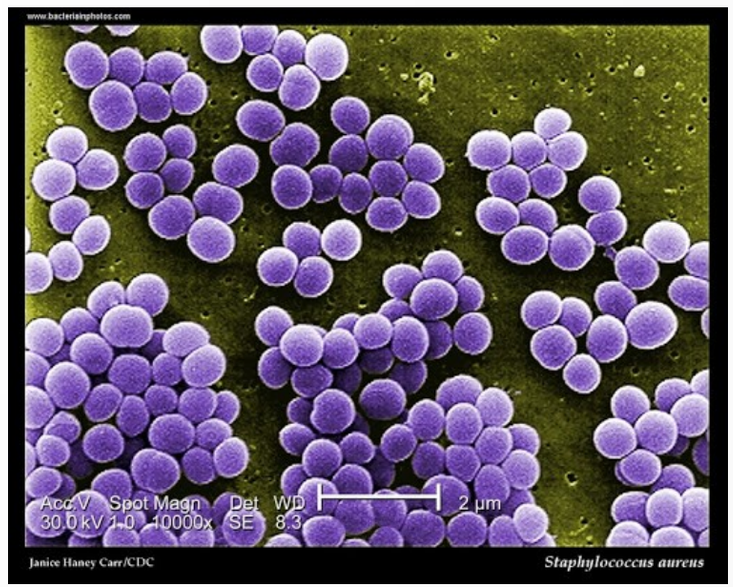
Analysing Bacteria Patterned on Surfaces

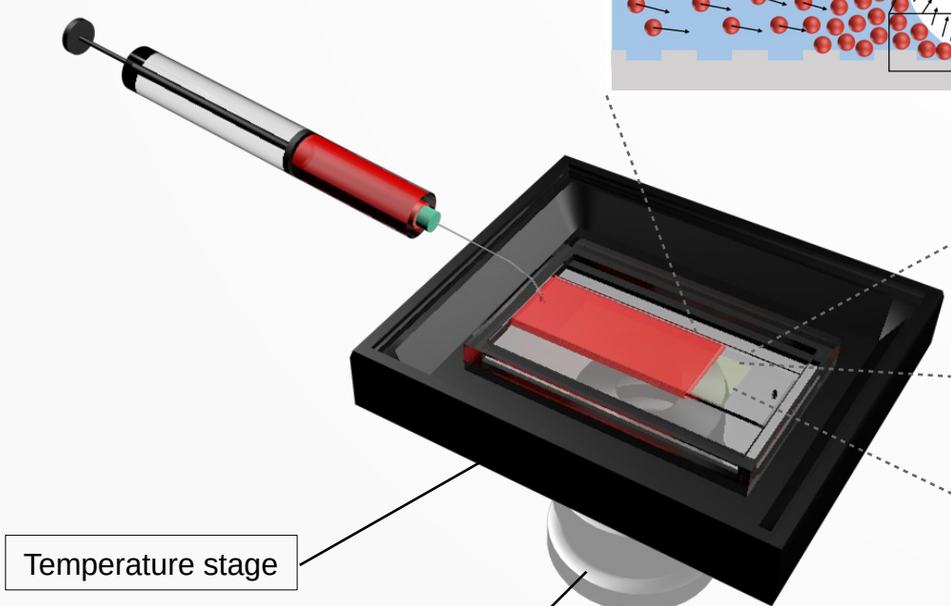
Part of my PhD research

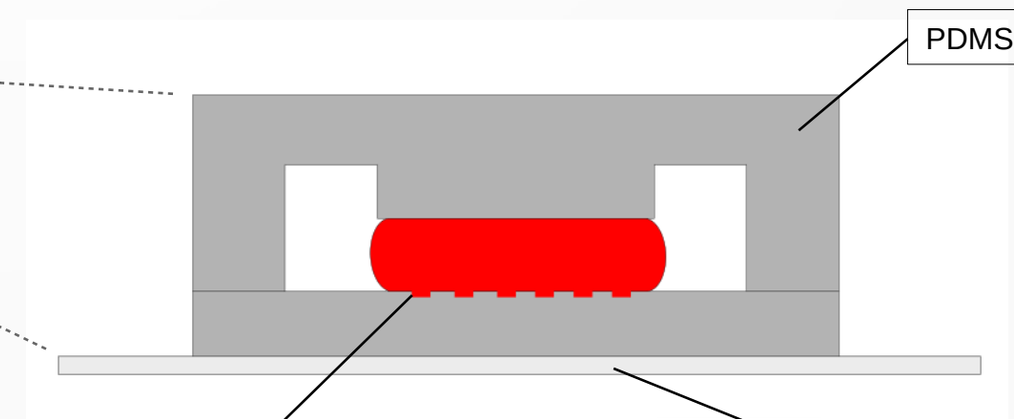
Cameron Boggon

How does it work?

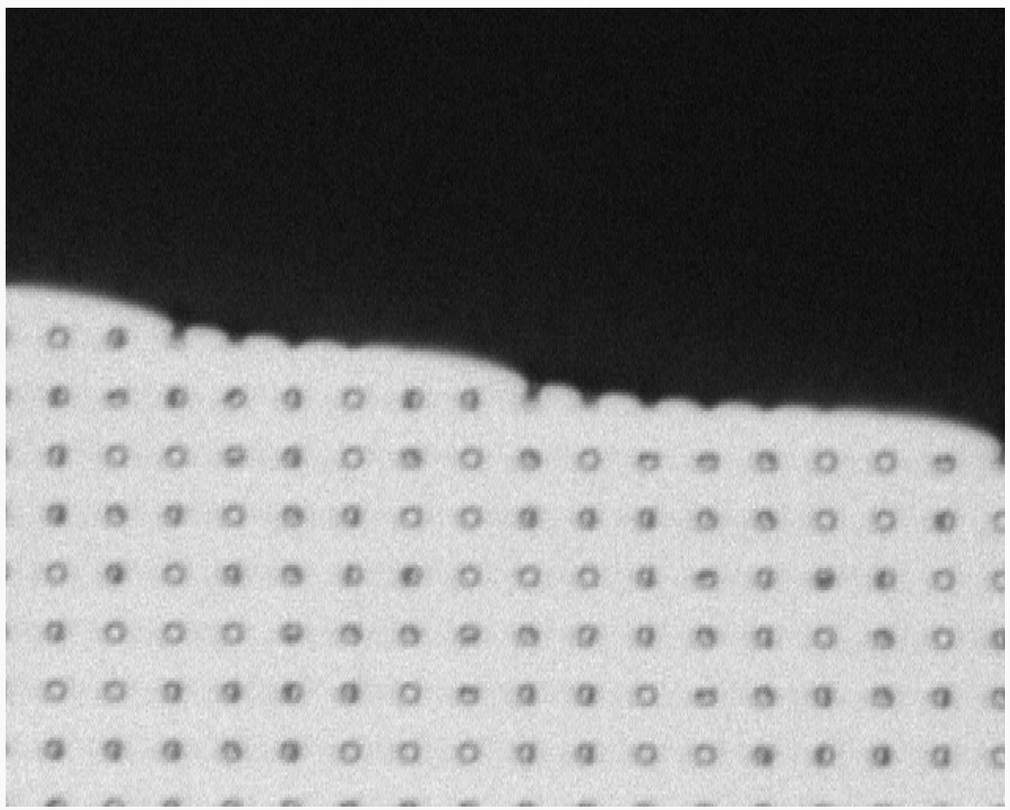




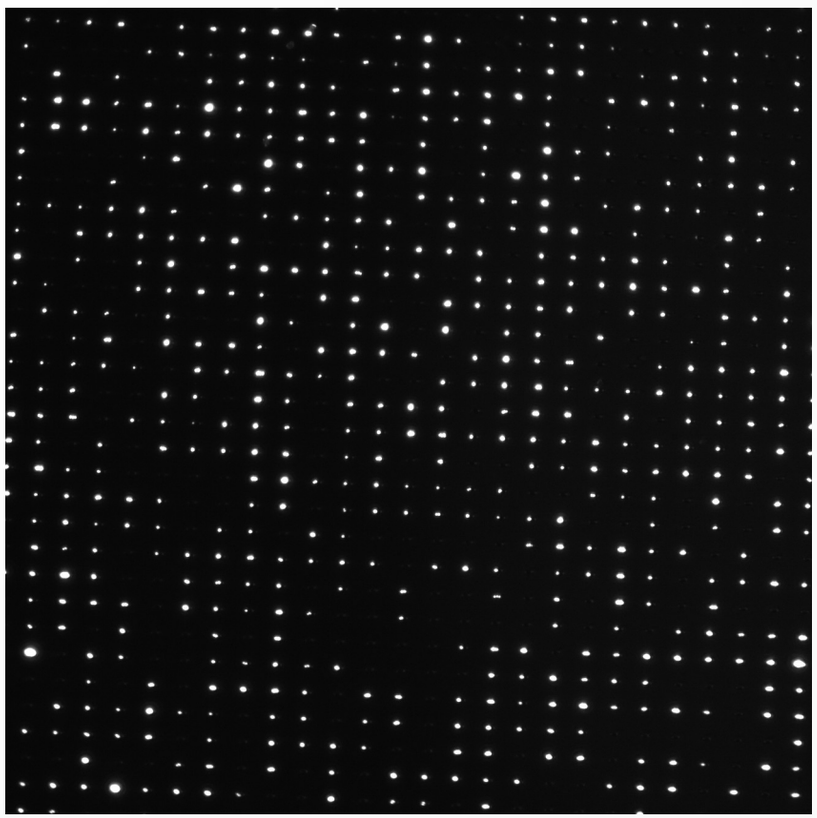




Depositing Bacteria



Phase Contrast video of bacteria deposited into 2x2 um traps.



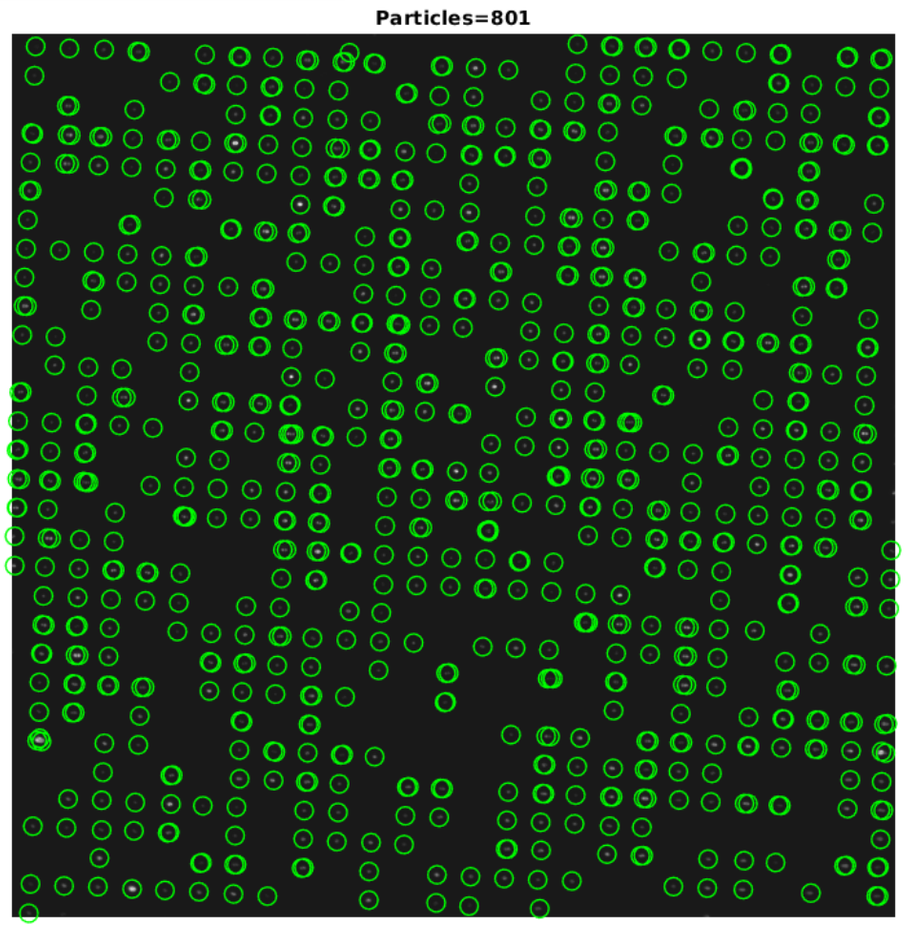
Fluorescence Image of bacteria patterned on PDMS template

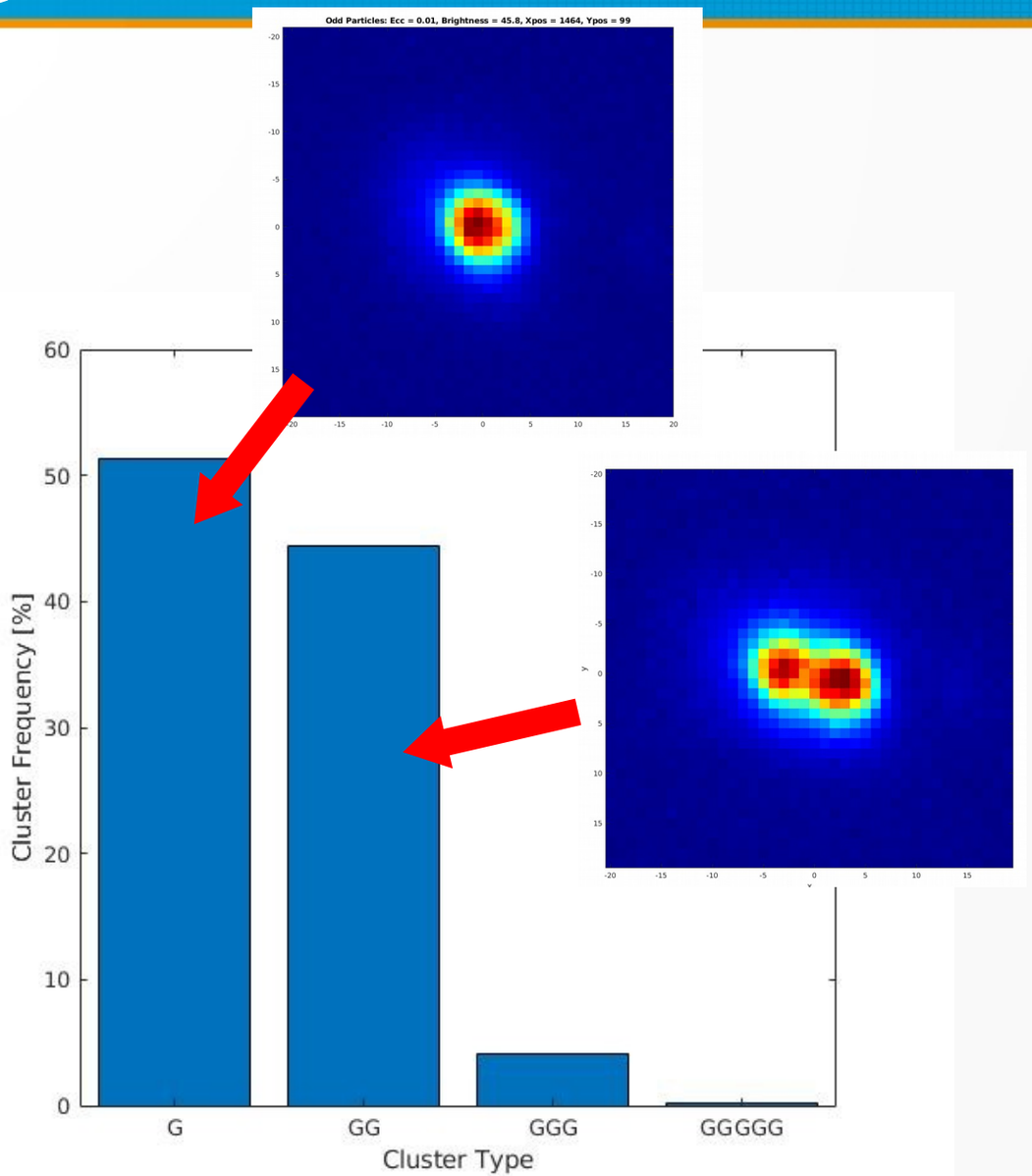
Finding Centre of Bacterium

1) Smooth image – Gaussian then box car kernel
2) Threshold – Brightest 30% pixels
3) Brightest pixel in local area (5x5 pixels)
4) Find centroid by Fourier transform (3x3 pixel window)
5) Allocate particles to clusters (10x10 pixels)

Crocker, John C., and David G. Grier. Journal of colloid and interface science 179.1 (1996)

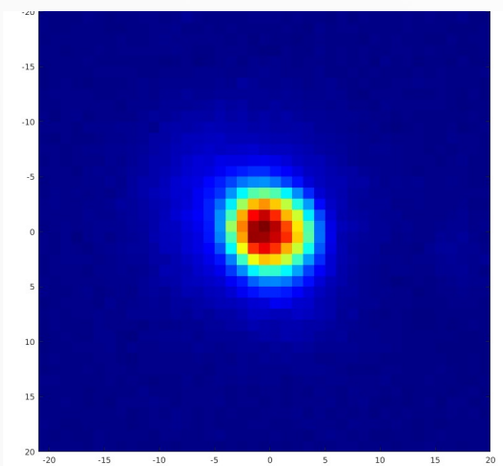
Analysing images

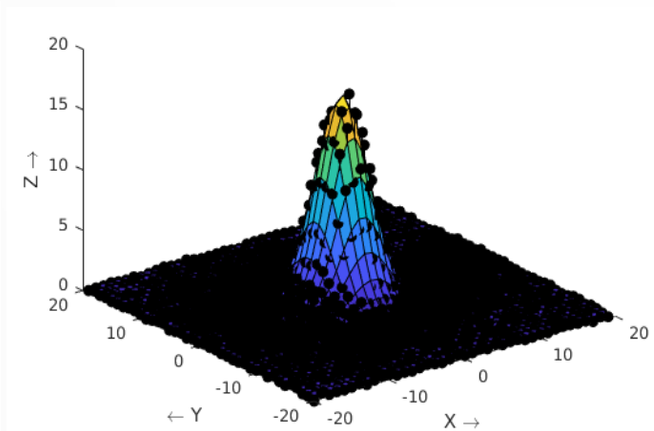


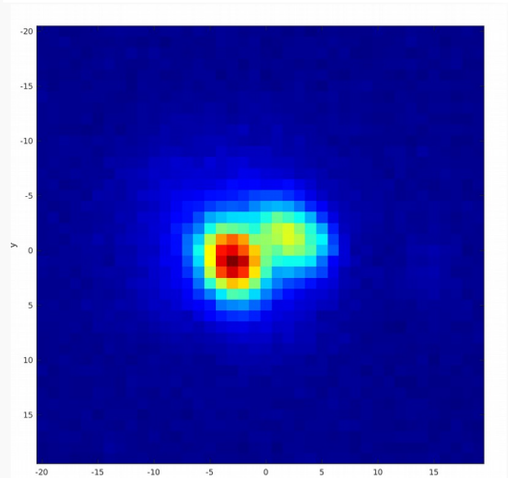


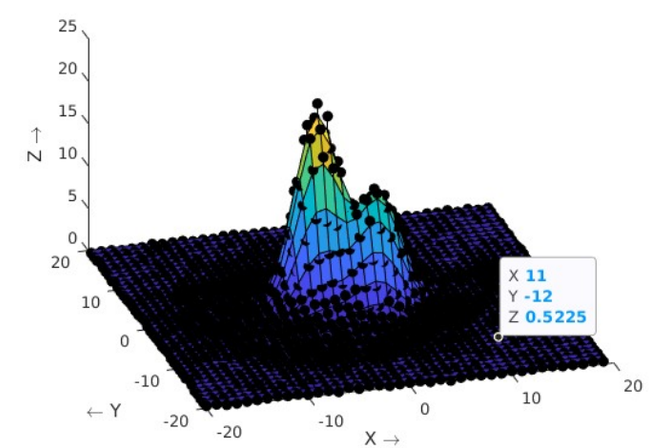
Fit 2D Gaussian to Bacteria

Equation of fit: $$f(x,y) = A + \sum_i B_i \exp\left(\frac{-(x_i-\mu_{x_i})^2}{2\sigma_{x_i}} + \frac{-(y_i-\mu_{y_i})^2}{2\sigma_{y_i}}\right)$$





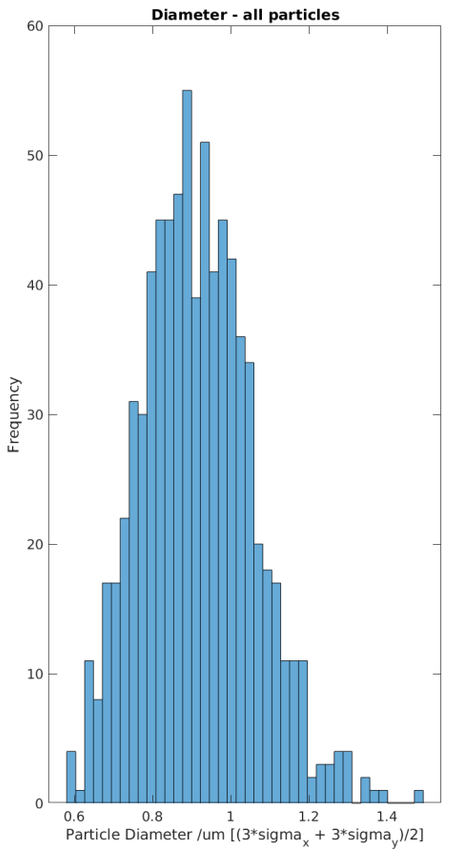


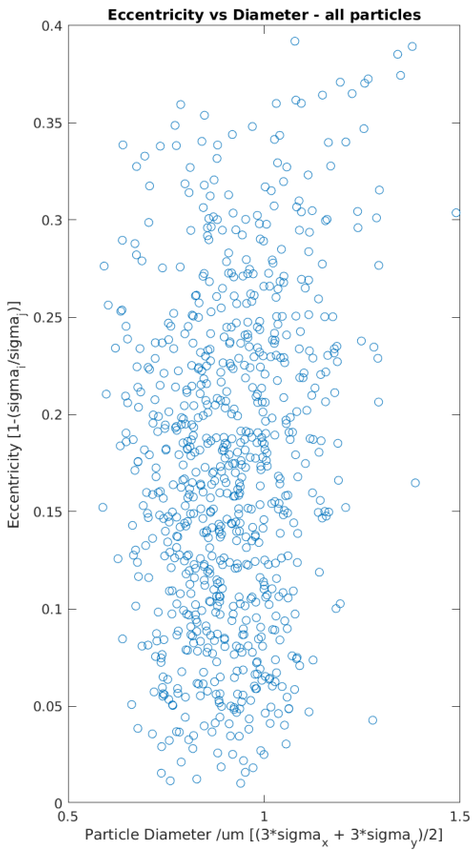


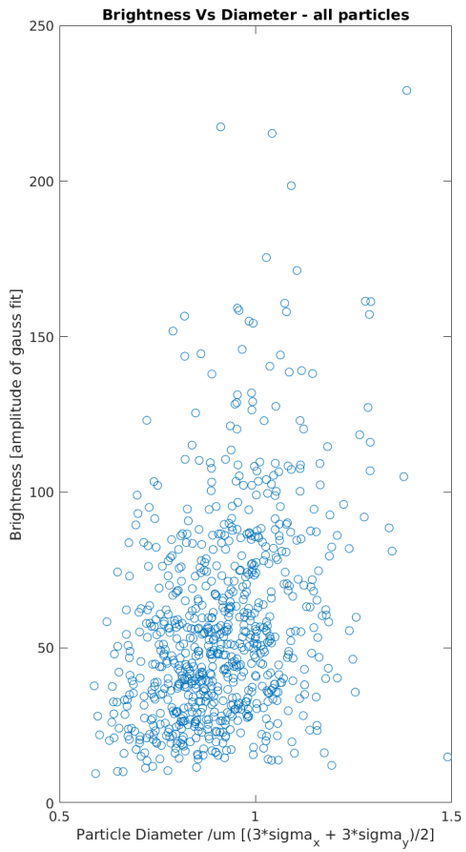
Useful Metrics:

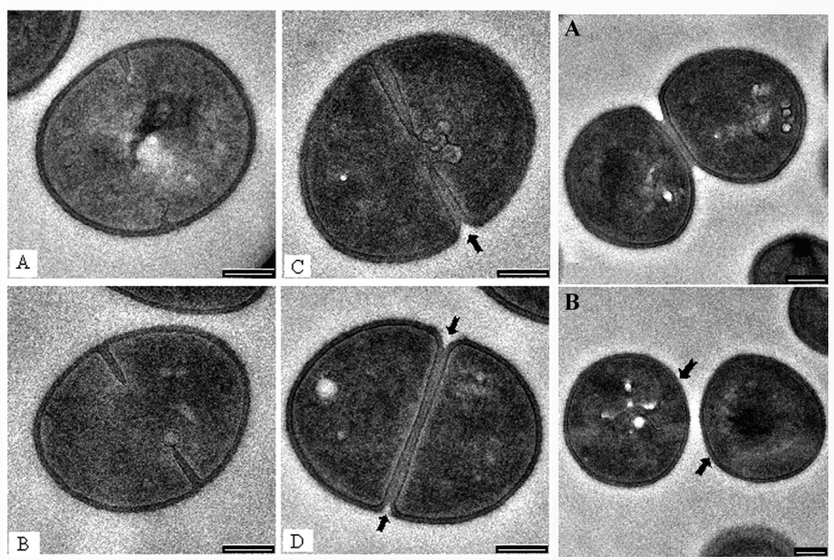
- $Eccentricity = 1 - \frac{\sigma_i}{\sigma_j}, \sigma_i < \sigma_j$
- $Particle\ Diameter = \frac{\sigma_i + \sigma_j}{2}$
- $Brightness\ of\ Bacterium = B$

Distribution in Bacteria Size





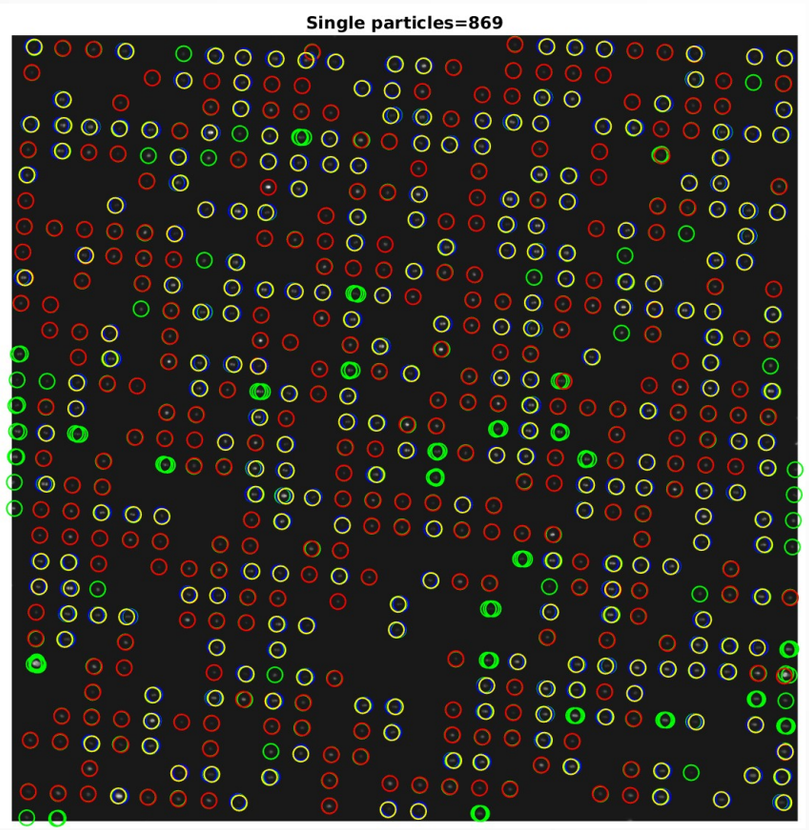


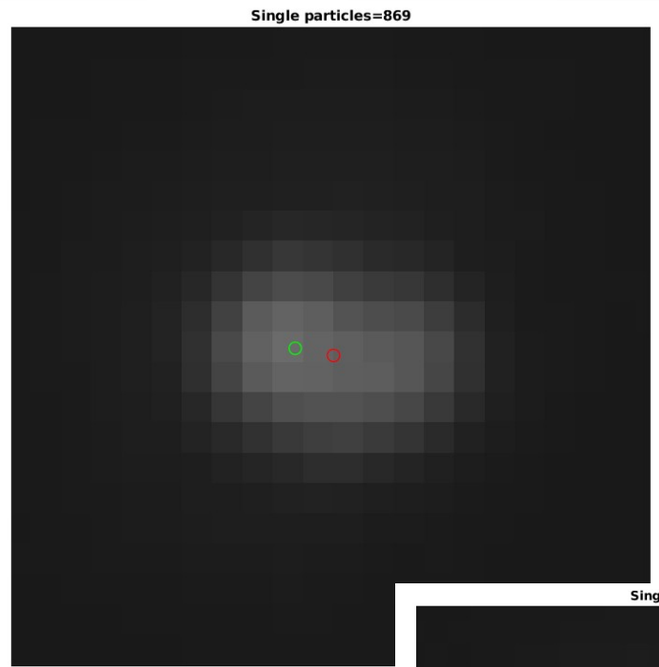


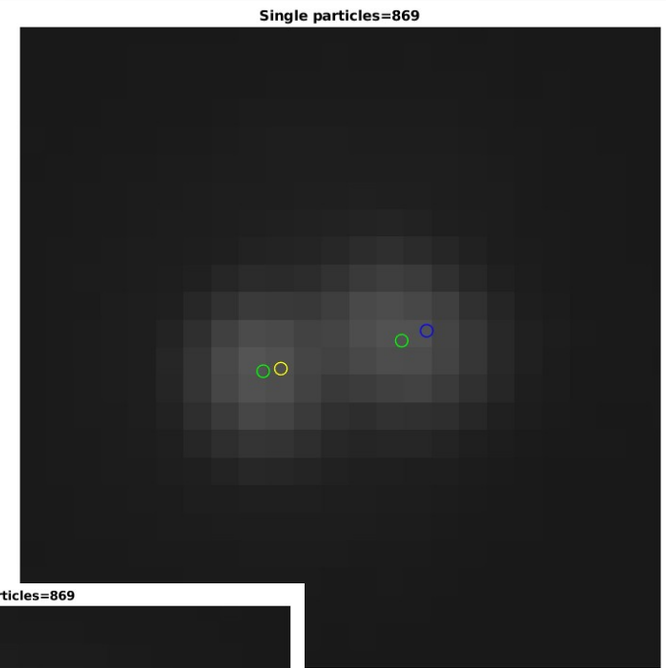
TEM images of single cell doubling.
Scale bar = 200nm

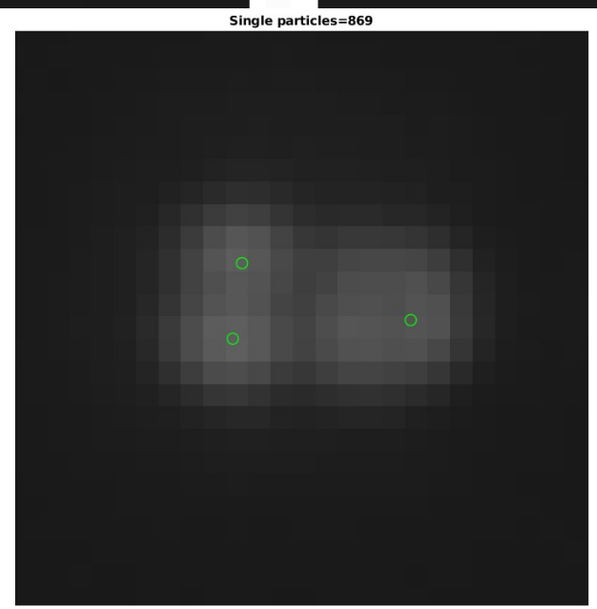
Touhami, Ahmed, Manfred H. Jericho, and Terry J. Beveridge. Journal of bacteriology (2004).

Testing Accuracy by Eye

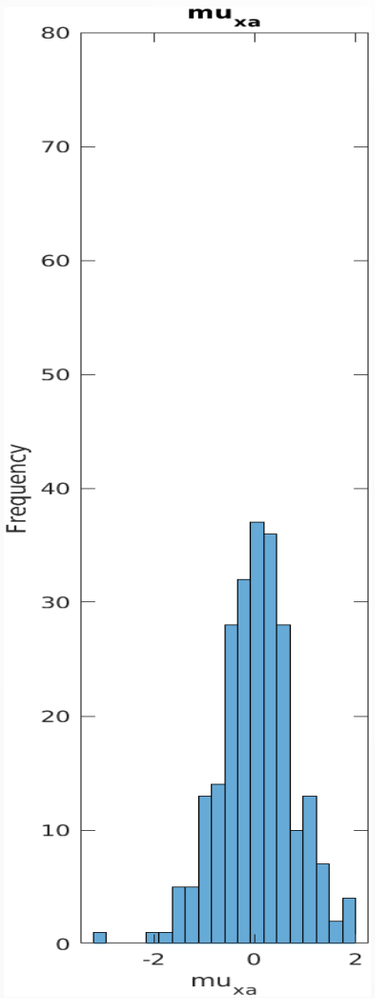


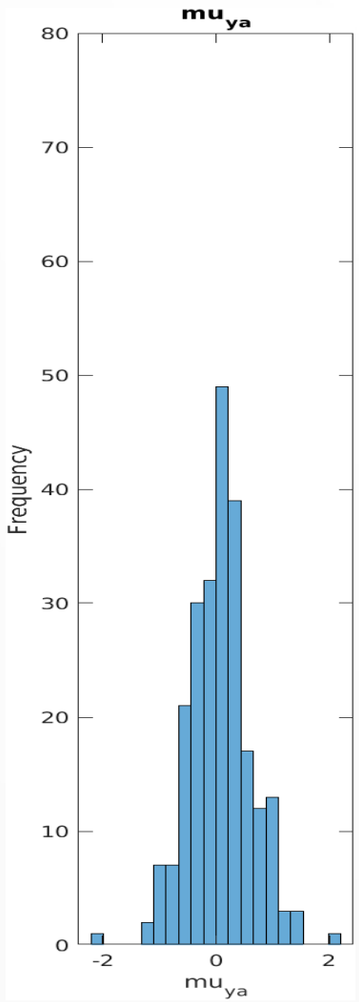


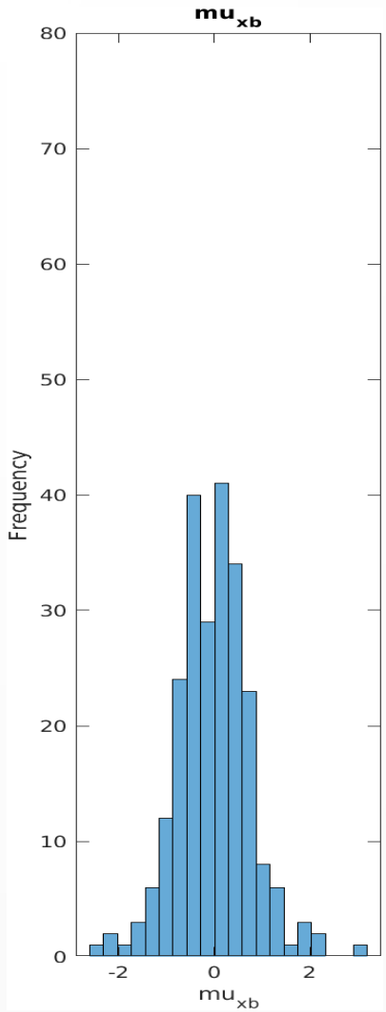


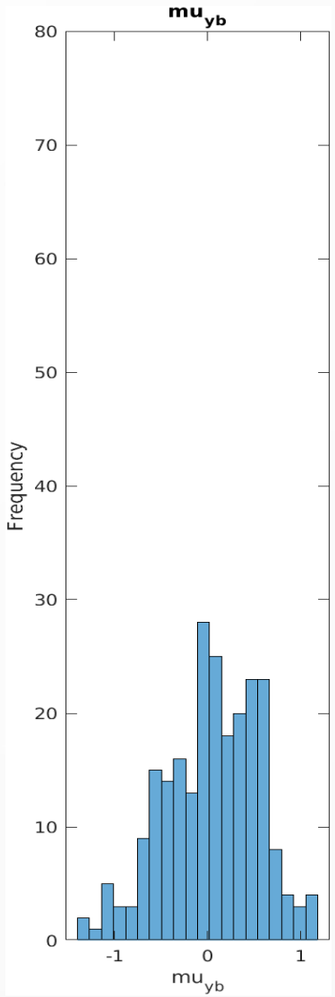


How to Test More Quantitatively?







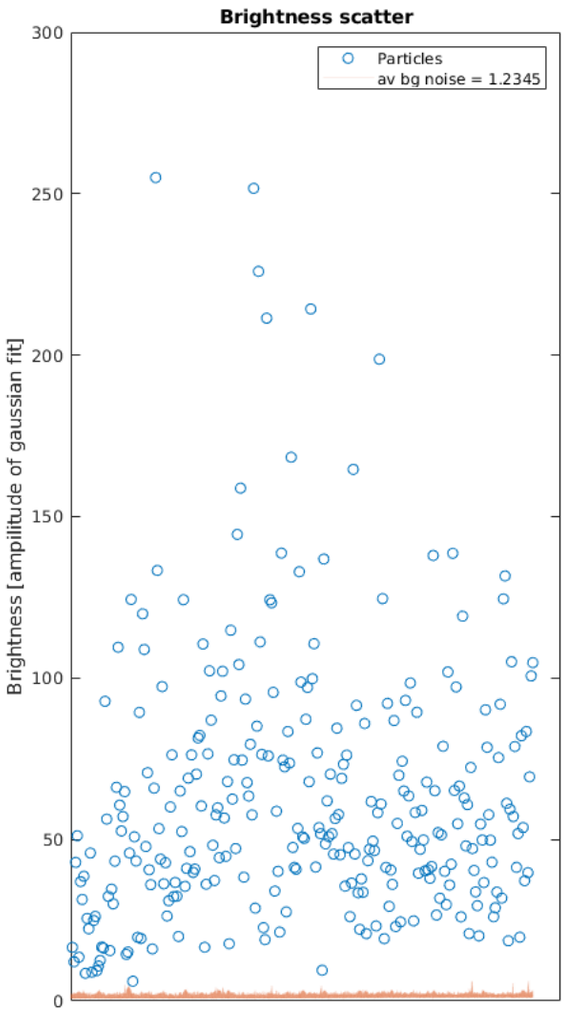


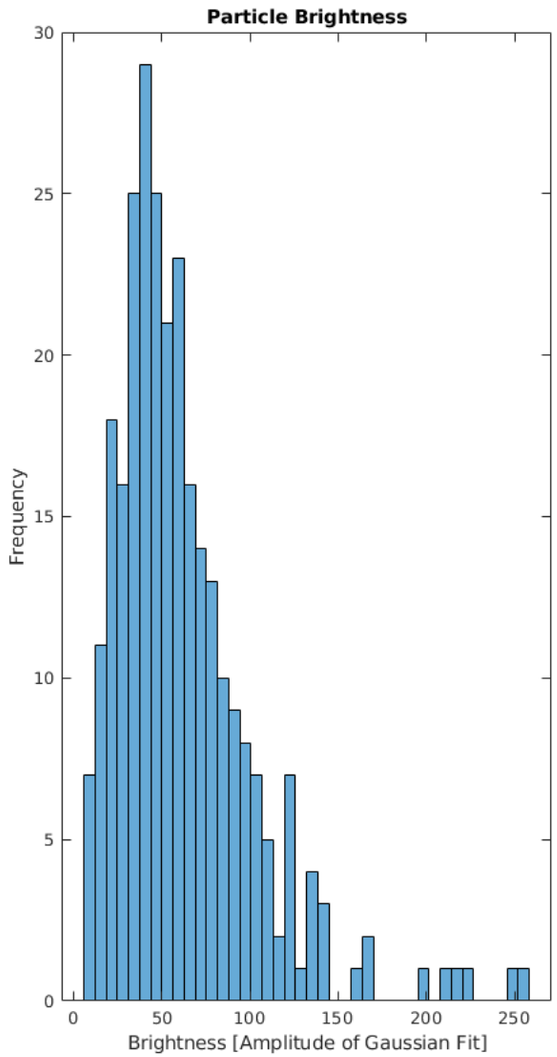
What next?

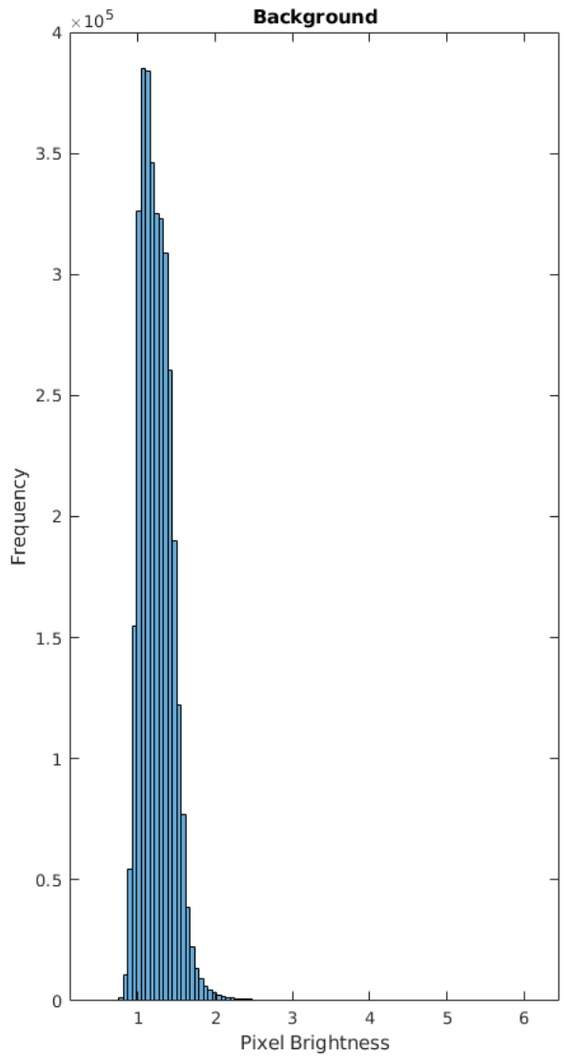
- Temporal parameters from time-lapse videos
- Simulated Data for error analysis
- What to do when we have many bacteria in 1 trap?

Questions?

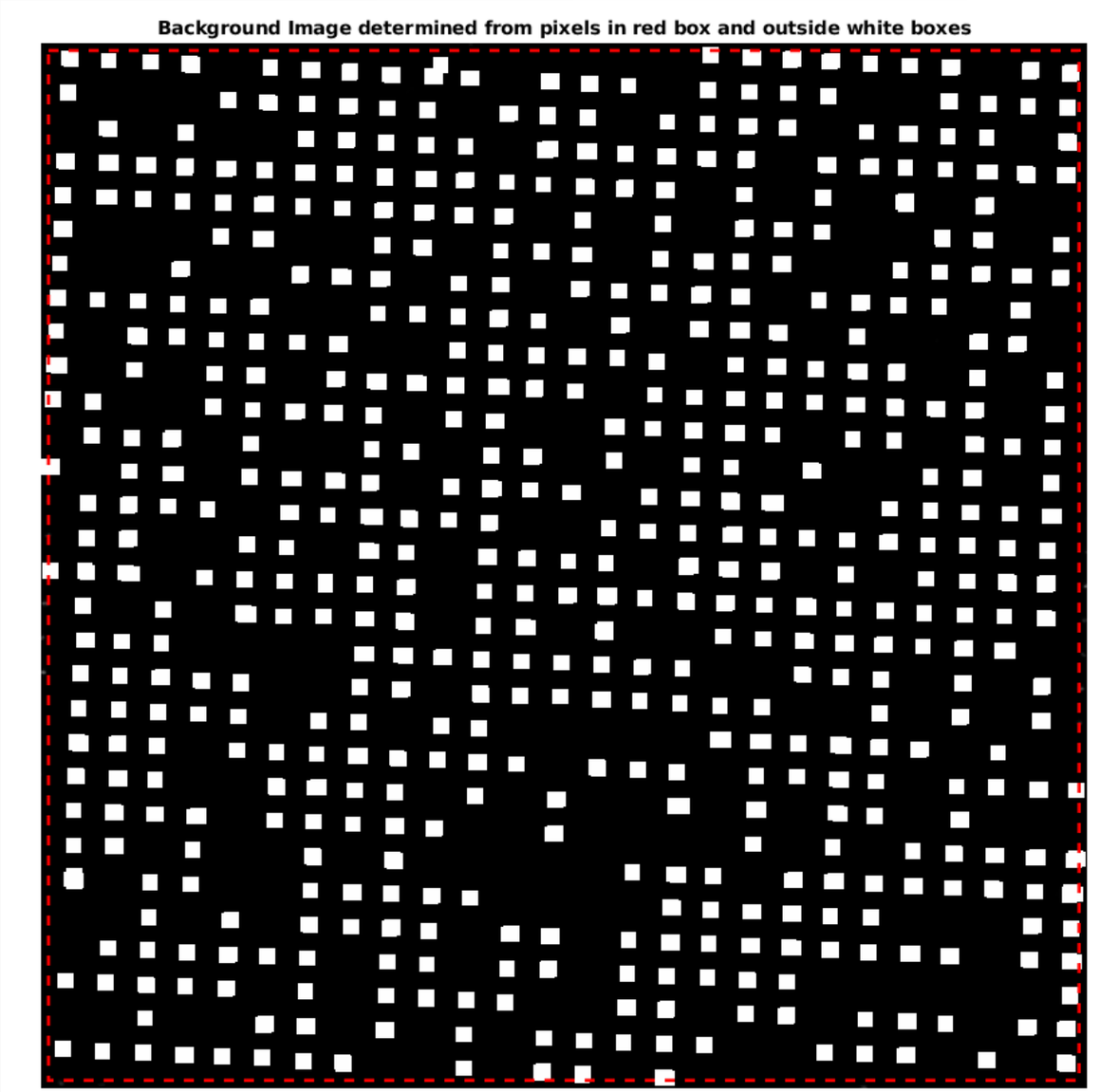
Brightness vs Background







How to Calculate Background



- Remove area around found particles. Square is 30x30 pixels.
- Discount data outside of red box as too close to boundary

Watershed Algorithm