An Ultrasensitive Bacterial Motor Revealed by Monitoring Signaling Proteins in Single Cells

Paper authored by: P. Cluzel, M. Surette, S.Leibler

Presented by: Jonathan Williams

Outline

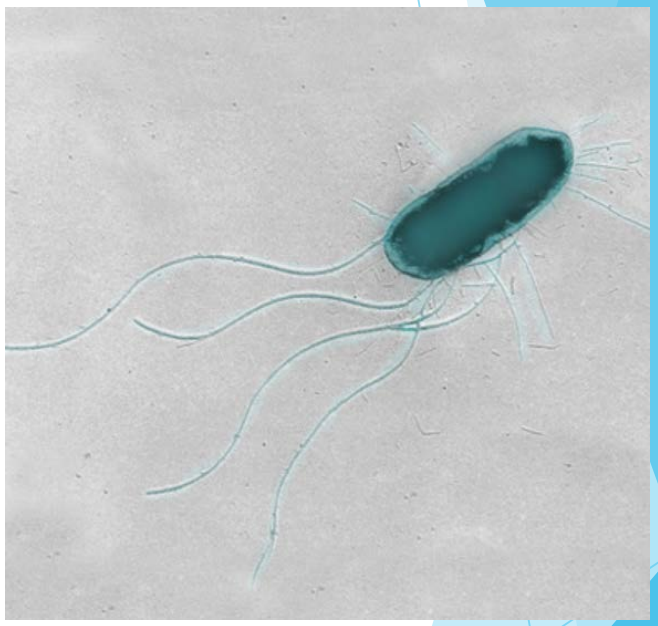
Introduce the Bacterial Motor/Signaling protein

Breakdown Fluorescence Correlation Spectroscopy

Compare Results and Discuss Implications

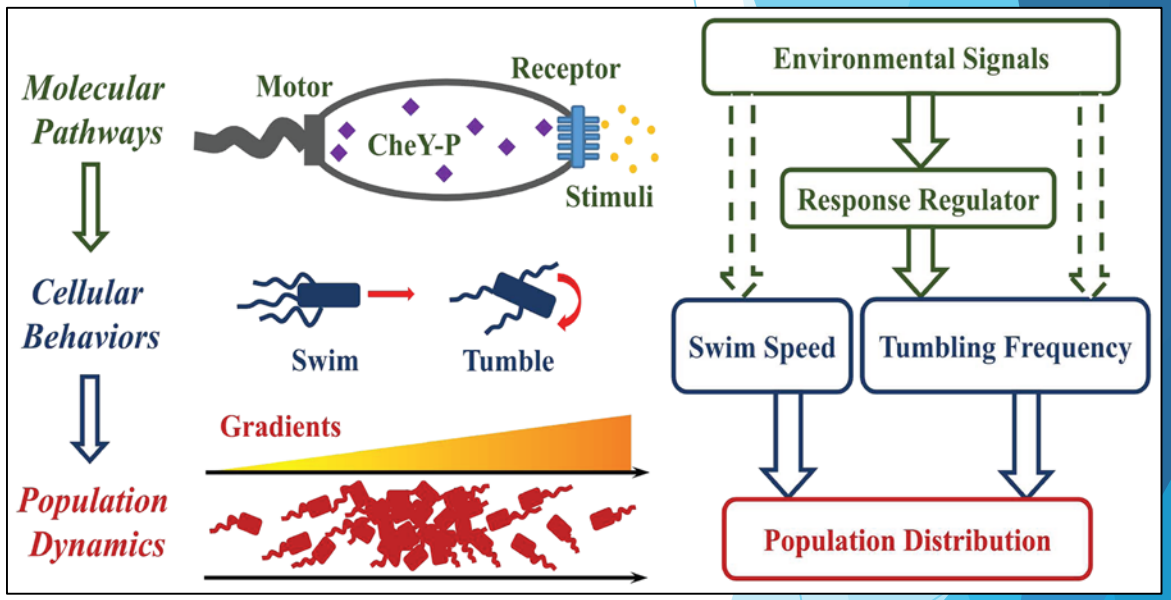
Introduction to Escherichia Coli (E. Coli)

- Flagella can either rotate clockwise (CW) or counterclockwise (CCW)
  - CW = tumbling, CCW = swimming smoothly
- Chemotactic signaling protein CheY-P is produced in response to outside stimuli
  - This results in E. coli tumbling, randomizing it's direction
- Resulting motion leads E. coli away from danger



Why care?

- Biochemical networks are the CPU's of cell life
- Current understanding of these networks relies mainly on data collected from cell populations
- This study presents an experimental method to study such biochemical networks at the single-cell level



https://journals.plos.org/ploscompbiol/article?id=10.1371/journal.pcbi.1003672

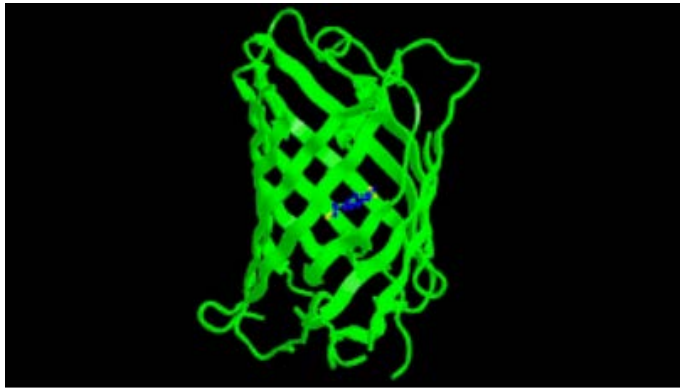
Objective and Methods

- Observe the input-output relation between CheY-P and flagellar motion in a single E. coli
  - Record CW versus CCW motion bias
- Control and measure CheY-P Concentration
  - Done with Fluorescence Correlation Spectroscopy (FCS)
- Compare these results to studies involving cell populations
  - Previously, CheY-P was ruled out as the signaling protein of CW bias due to weak correlation

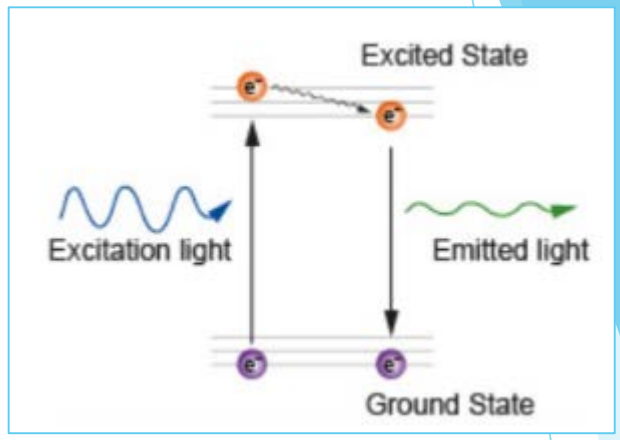
FCS - Fluorescence

- Fluorescence is the emission of light by a substance that has absorbed EM radiation

- Green Fluorescent Protein (GFP) is used as our source of fluorescence



https://proteopedia.org/wiki/index.php/Green_Fluorescent_Protein



The Nobel Prize in Chemistry 2008
Osamu Shimomura, Martin Chalfie, Roger Y. Tsien

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The Nobel Prize in Chemistry 2008


Photo: U. Montan
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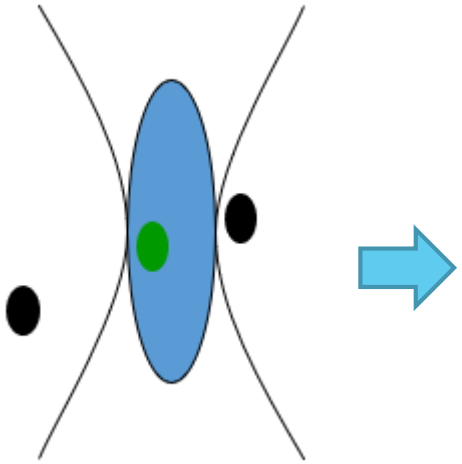
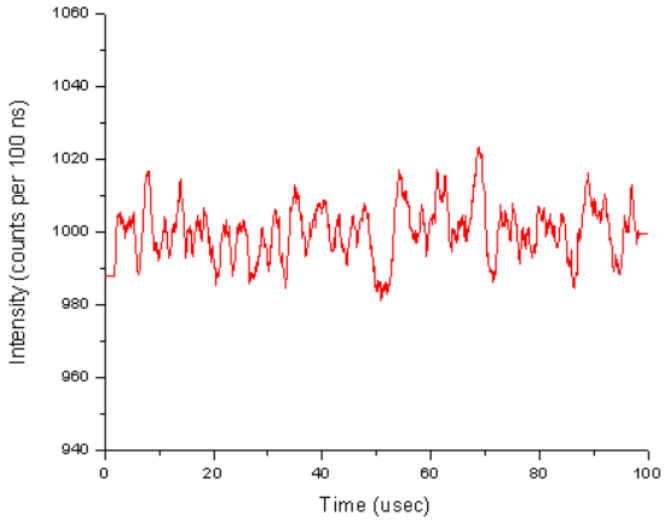
The Nobel Prize in Chemistry 2008 was awarded jointly to Osamu Shimomura, Martin Chalfie and Roger Y. Tsien *"for the discovery and development of the green fluorescent protein, GFP"*.

Fluorescence Correlation Spectroscopy

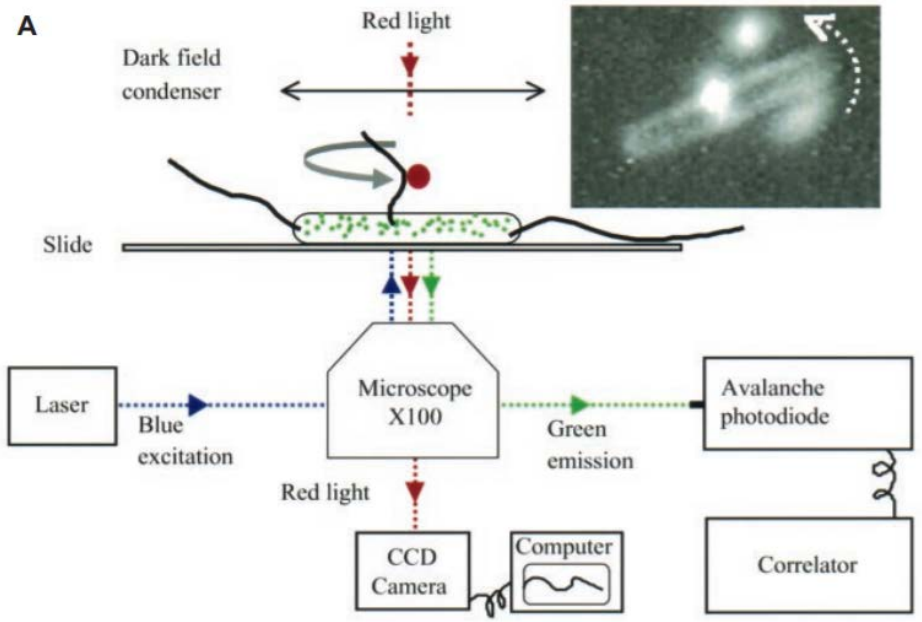




- Note that fluctuations do not appear perfectly random
- The widths of the peaks and valleys favor a characteristic time scale

Animation from https://www.zeiss.com/content/dam/Microscopy

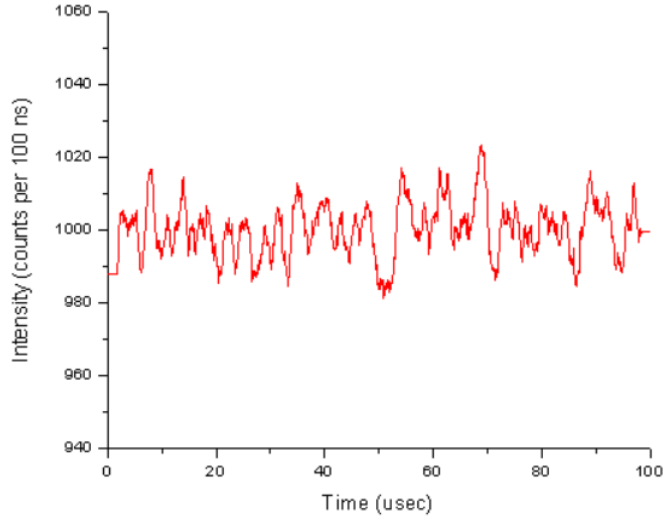
Experimental Setup



- Green Fluorescent Protein needs to fuse with CheY-P
- Start with a strain of E. Coli lacking the *CheY-P* gene entirely (100% CCW motion)
  - A promoter plasmid was introduced to give a CheY-GFP expressing gene
- Concentration of CheY-GFP is observed at the same time as flagella rotation bias
  - An inducer was used to promote Chey-GFP production

Plasmid: pMGS98 (CMR)
Inducer: isopropyl-ß-D-thigalactoside (IPTG)

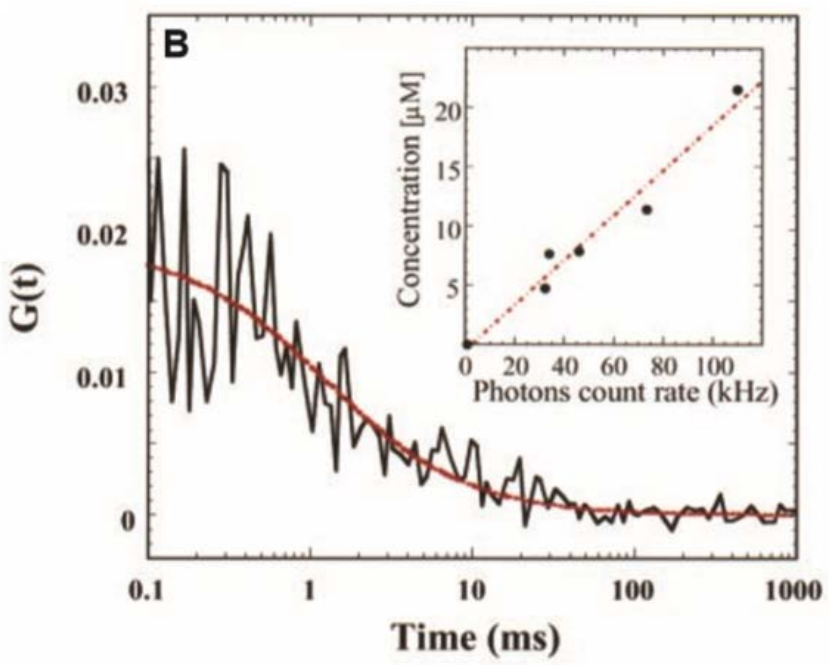
FCS – Autocorrelation



- G(t) represents the ***fluctuation*** of intensity, not intensity itself

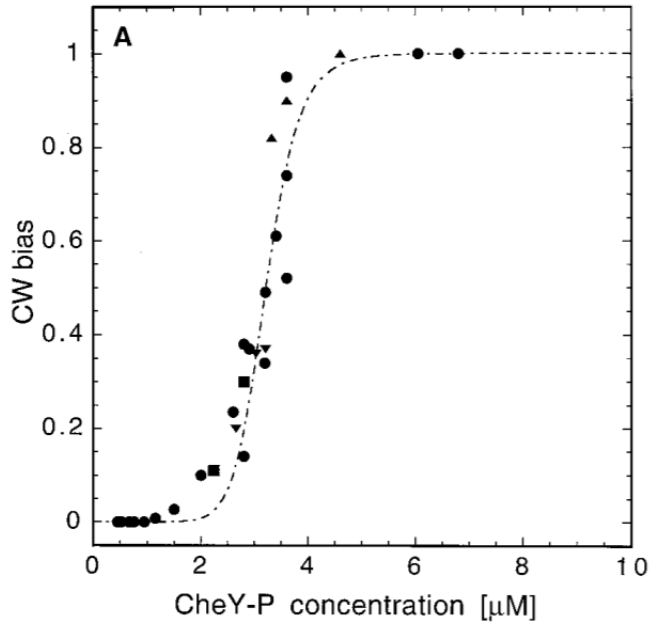
- $G(t) = \frac{1}{N}[1 + \frac{4Dt}{\omega^2}]$
  - N is the number of molecules of GFP
  - The correlation curve amplitude is inversely proportional to the particle concentration

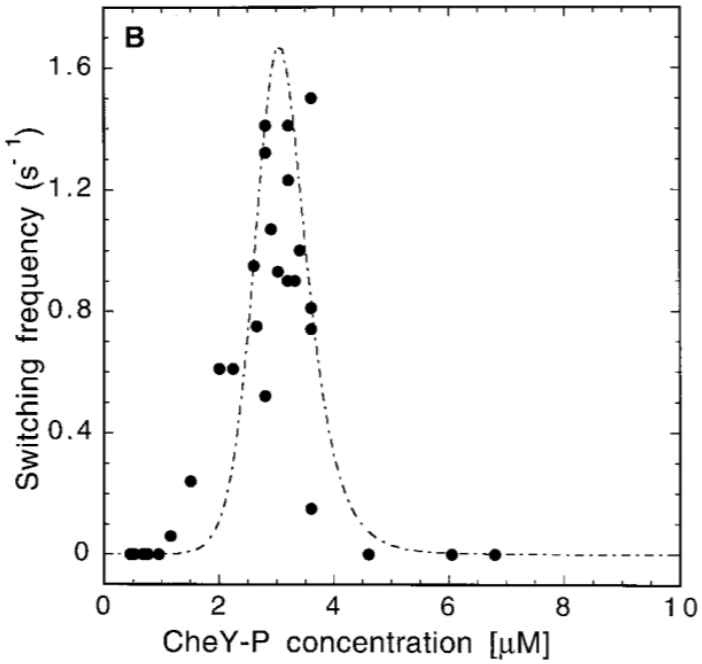
- The experiment induces constant production of CheY-P
  - Thus as time goes on, G(t) goes to 0



Results

- Strong correlation between CW bias and CheY-P concentration
- Hill coefficient of 10.3 ± 1.1
  - Previous studies found to have Hill coefficient between 3.5 – 5.5

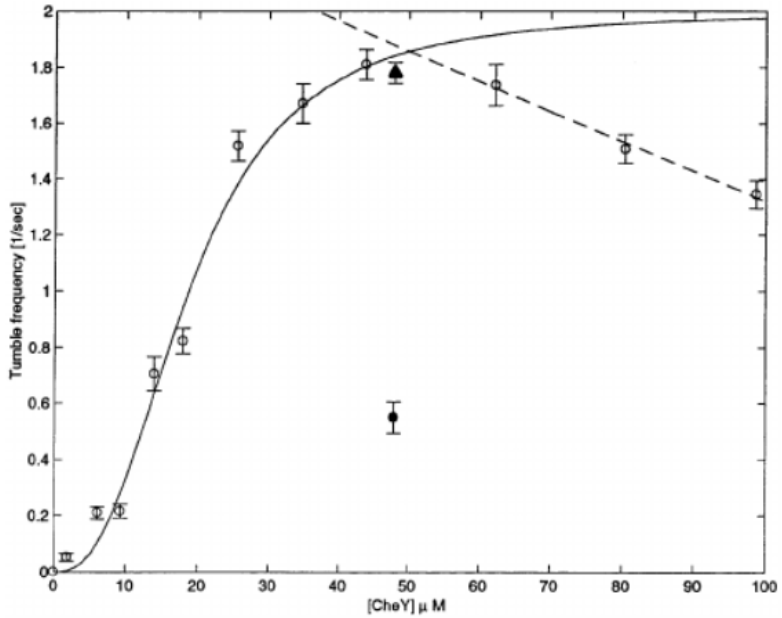


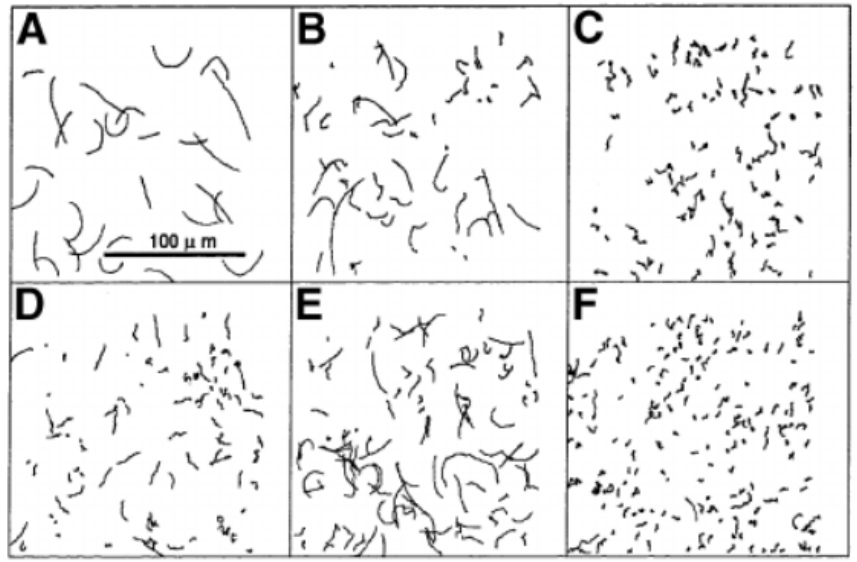


Where do other studies fall short?

- Population based studies use immunoblotting
  - In methods requiring immunoblotting, the output characteristic of flagellar motors is convoluted with CheY-P distributions
  - Bacteria diversity causes distortions of data

Alon *et al.*





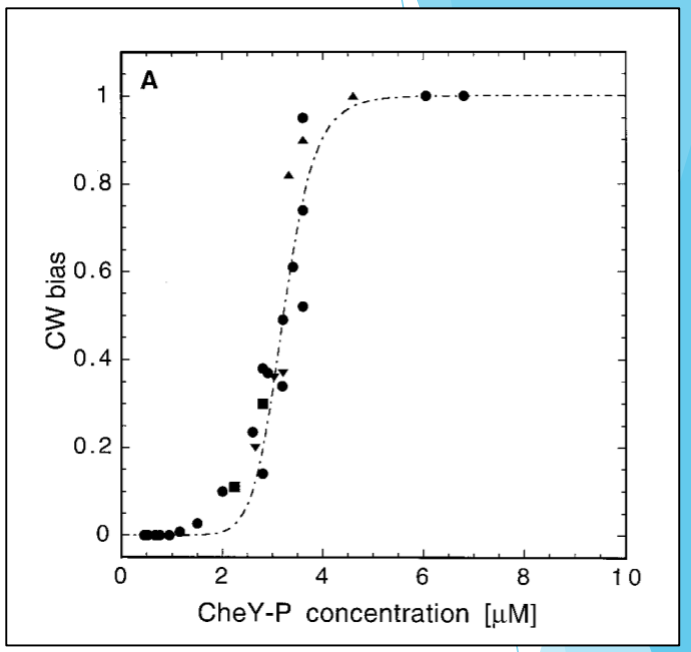
Images taken from U. Alon et al., EMBO J. 17, 4238 (1998).

Conclusion

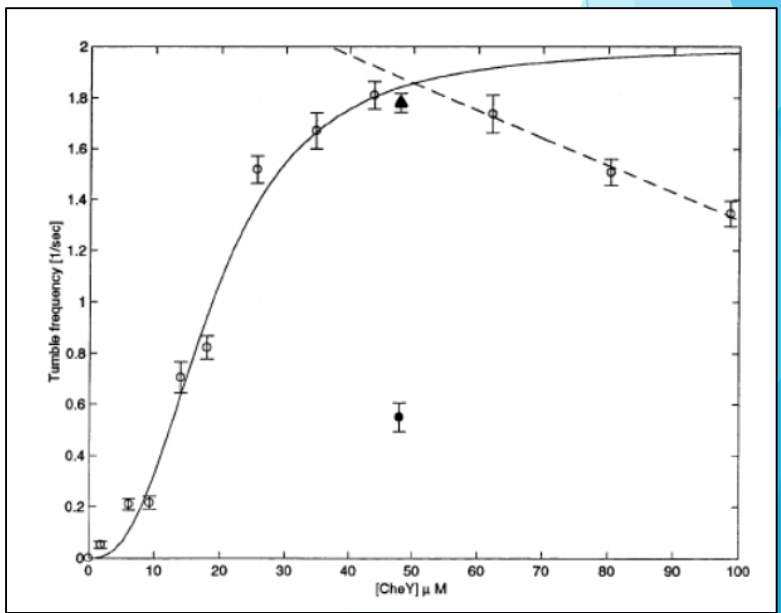
- Cluzel finds a very high Hill coefficient
  - This indicates a stronger correlation between motion and CheY-P than previous studies
  - This cements CheY-P as the main chemotactic signaling protein of E. coli motor bias

- This study demonstrates the indispensable value of single-cell measurements

Cluzel *et al.*



Alon *et al.*



References

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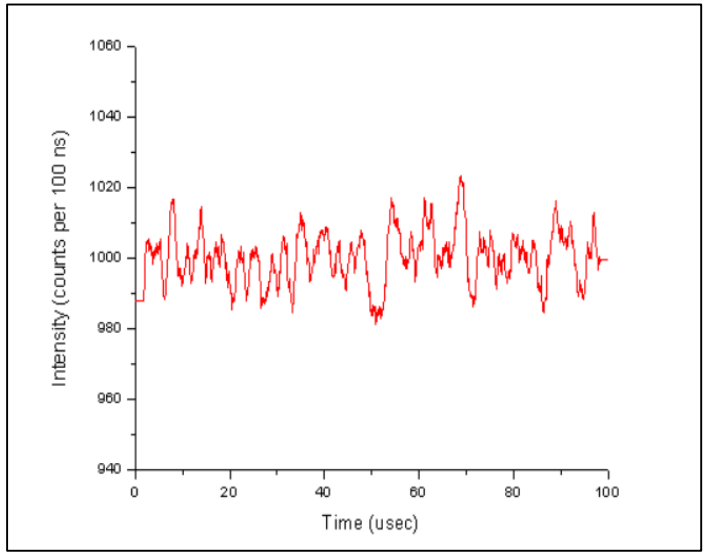
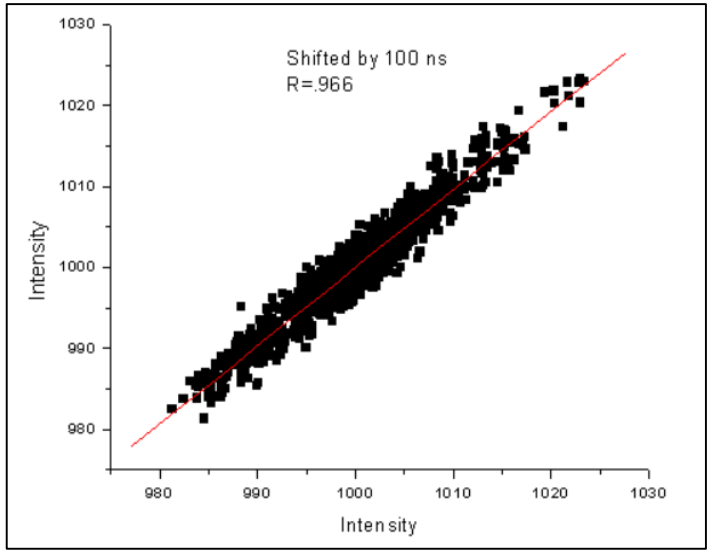
- U. Alon et al., EMBO J. 17, 4238 (1998).
  - https://www.embopress.org/doi/full/10.1093/emboj/17.15.4238
- Bo Hu,Yuhai Tu, IBM T. J. Watson Research Center, Yorktown Heights, New York, United States of America
  - https://doi.org/10.1371/journal.pcbi.1003672

FCS – Correlation

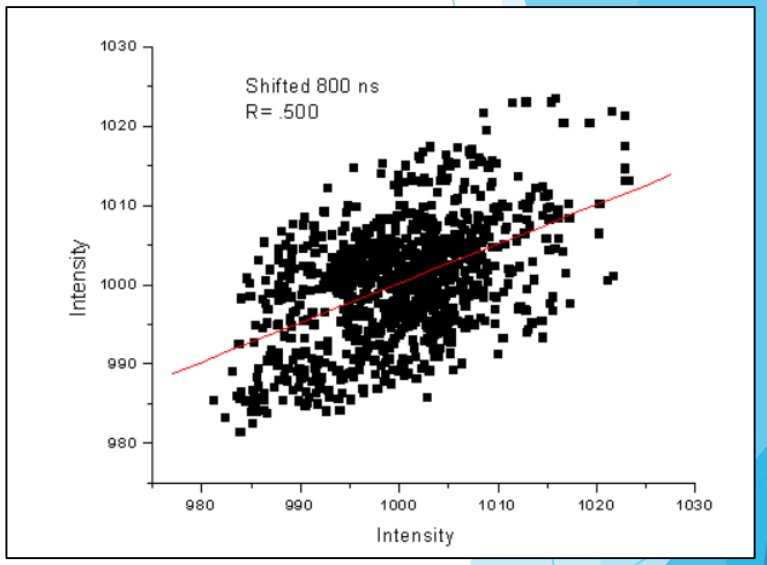
1) Fluorescence Intensity Data vs Time



2) Fluorescence Intensity Data vs Intensity shifted by 1 interval



3) Fluorescence Intensity Data vs Intensity shifted by 8 intervals



- Data taken over 100 ns intervals
- Note that fluctuations do not appear perfectly random. The widths of the peaks and valleys favor a characteristic time scale.

- R = Correlation Coefficient
  - R gets closer to 0 the larger the shift in time

Images from http://www.fcsxpert.com/classroom/theory

FCS – Autocorrelation

- R(Δt) represents the probability that the intensity will still be rising or falling at some time, Δt, later.
- R(Δt) is an autocorrelation function. It expresses the ***correlation between the fluctuation from the mean intensity*** at time 0 with the fluctuation from the mean intensity at later times.
- By dividing R(Δt) by the mean square of intensity, we acquire G(t)

Correlation of Intensity with Itself Shown as a Function of Shift in ns