"High-resolution, long-term characterization of bacterial motility using optical tweezers."

Taejin I. Min, Patrick J. Mears, Ion M. Chubiz, Christopher V. Rao, Ido Golding, Yann R. Chemla.

Nature Methods, 6, 831 (2009)

Billy, Anirbit, Andrew and Ian

Grad Orientation Course Fall 2011

Overview

- Motivation
- Experimental Setup
- Results
- Discussion and Conclusions

Motivation

Structure and motion of bacteria

- Although the anatomy of bacteria is known, the motion of bacteria is not fully understood.

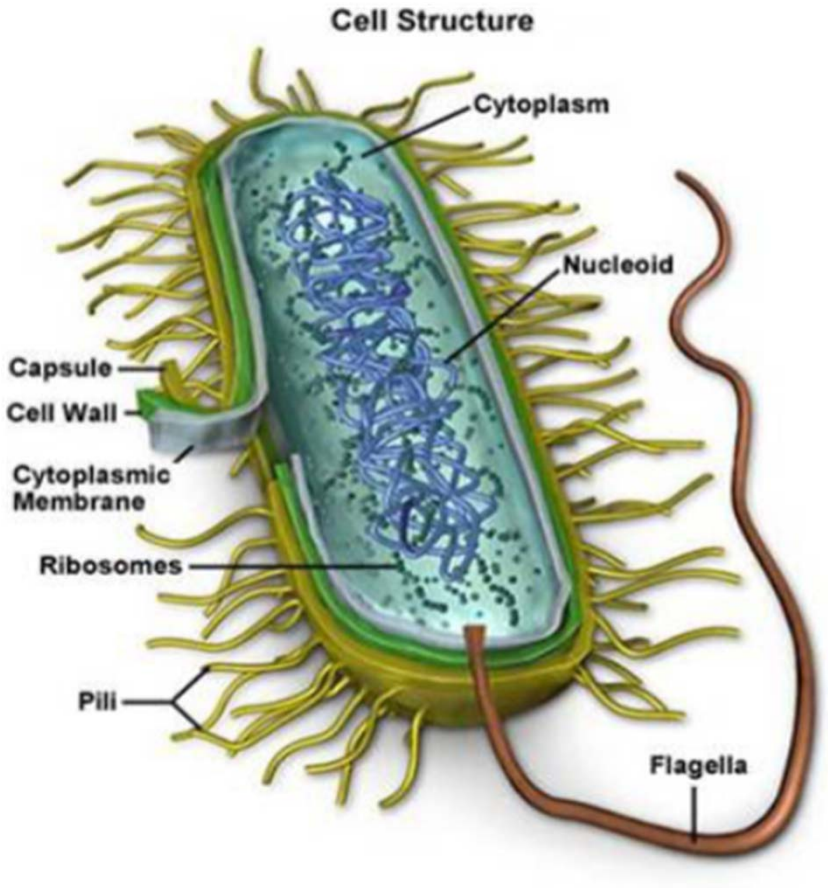


Image from

http://www.eerc.wsu.edu/SWEET/modules/docs/2009/stuck_on_bacteria.pdf

Video from the Chemla Lab at UIUC

Simple dynamics of bacterial motion

- Flagella control bacterial motion
- Counter-clockwise flagellar rotation induces constant direction swimming. This is known as a ***run***.

- Clockwise rotation causes flagella to break off, resulting in abrupt changes in cell motion known as ***tumbling***

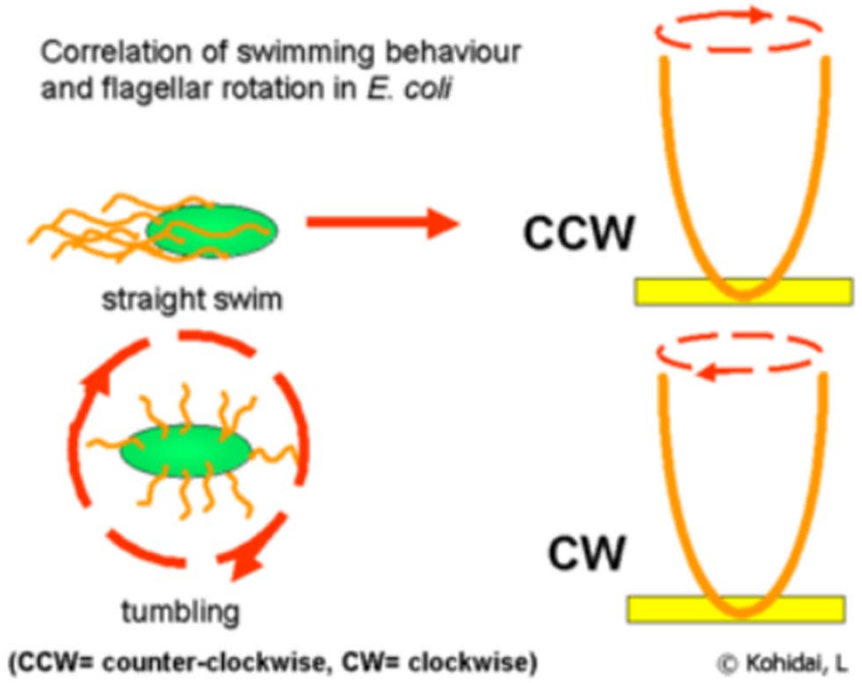


Image from Wikipedia.
Video from the Chemla Lab at UIUC.

Some definitions

- Chemotaxis is the effect of both quantity and type of chemicals in environment on the motion of cells.
- Motility is the realization of spontaneous and active motion via consumption of energy.
- Assay is testing of the activity induced by a drug or chemical on a cell.

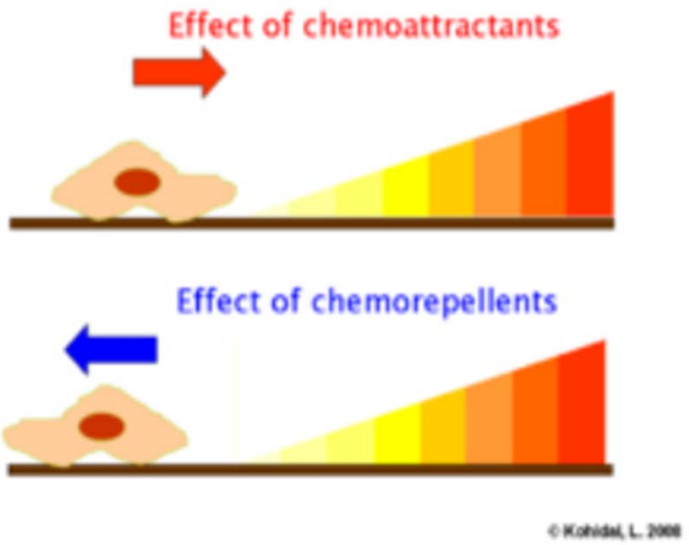


Image from:
http://en.wikipedia.org/wiki/Chemotaxis

Dynamics in response to external stimuli

Cells that encounter an increase in attractants or repellents in their environment will exhibit a greater degree of deterministic motion.

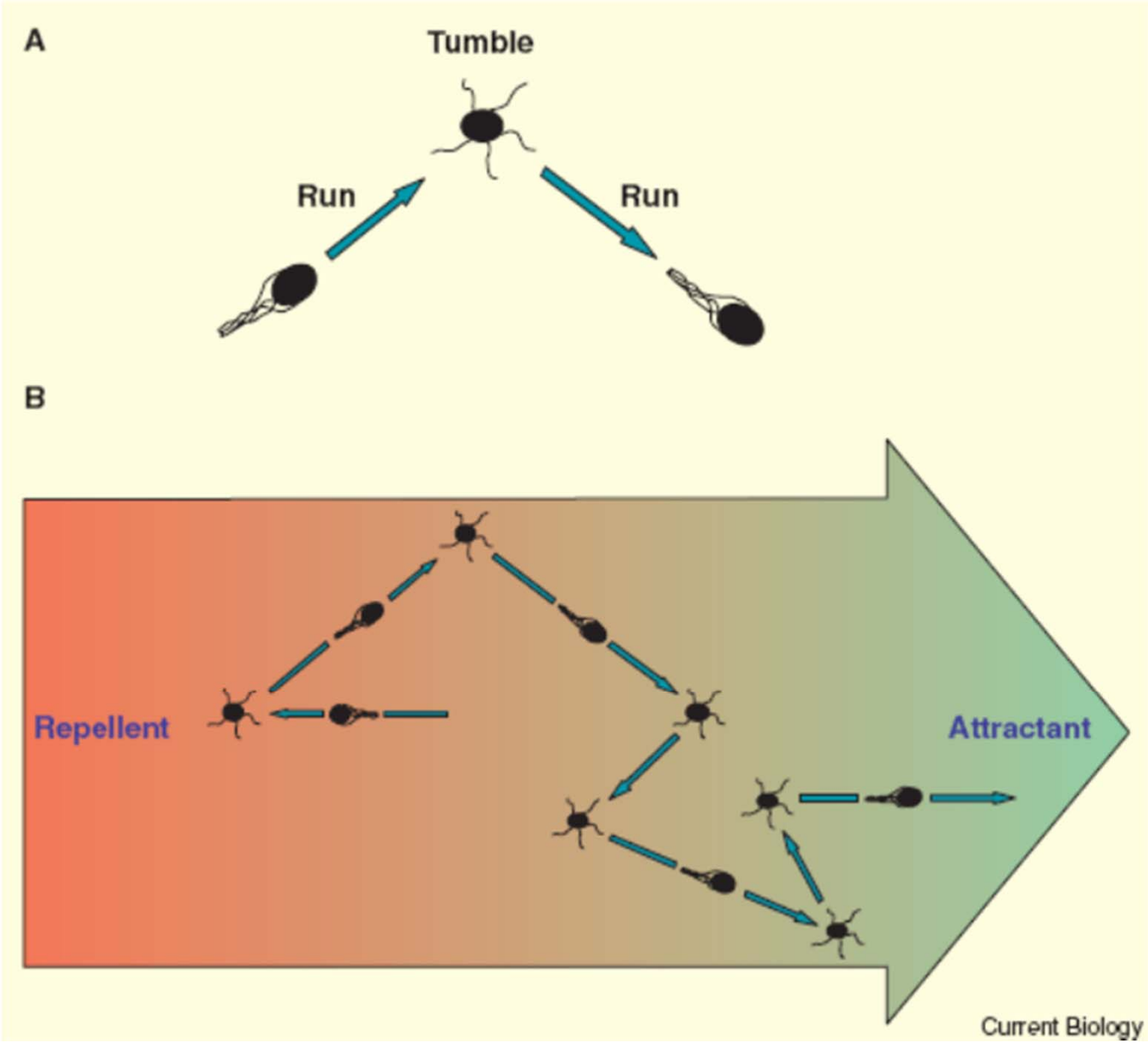


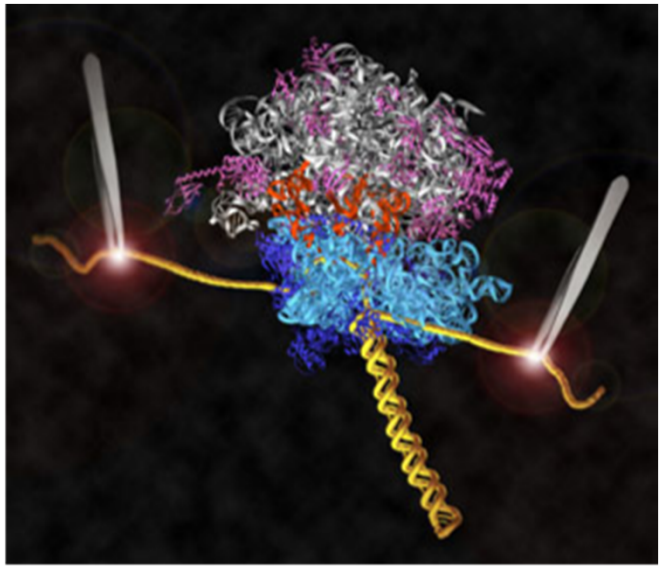
Image from the Ubaldo M. Córdova-Figueroa Research Group.

Getting a better picture of bacterial motion

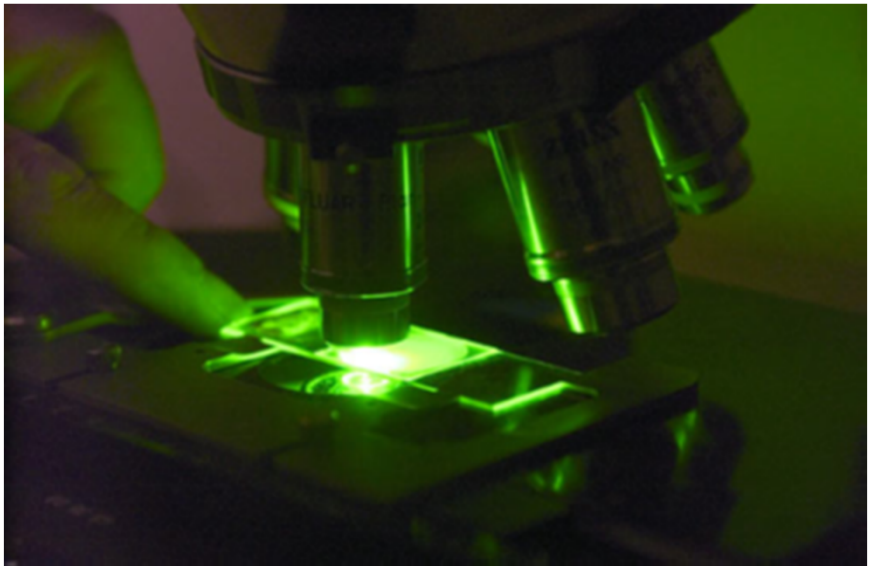
Studying complex dynamics in bacterial motion requires:

- observing bacterial motion over longer periods.
- acquiring data with frequencies exceeding those of the flagella oscillations (>100 Hz).

The use of optical tweezers and fluorescent microscopy provide these capabilities.



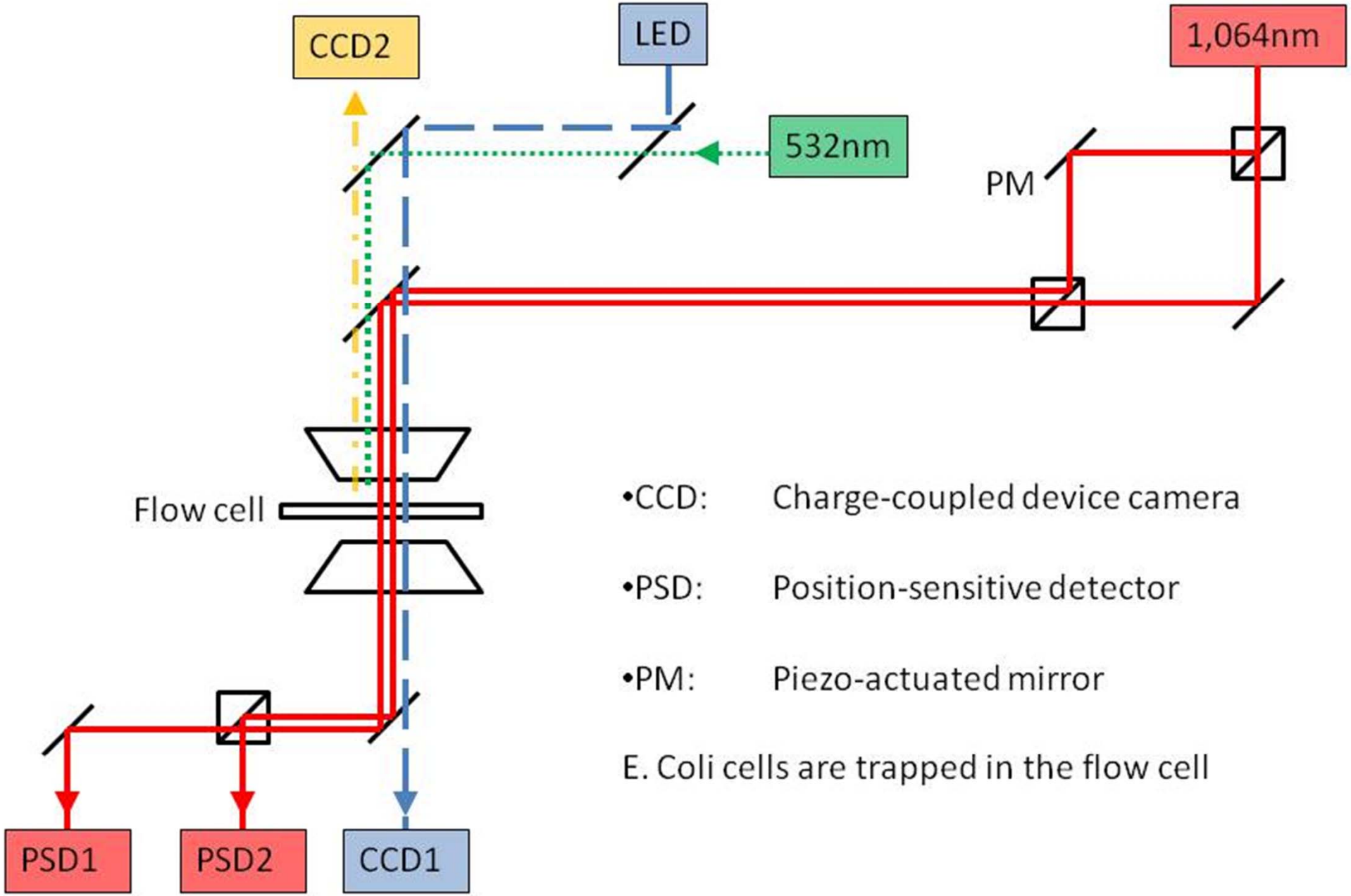
http://www.soe.ucsc.edu/news/article/1393



http://www.whoi.edu/oceanus/viewPhotoGallery.do?gType=1&gallery=true&clid=2416&mainid=12029

Experimental setup

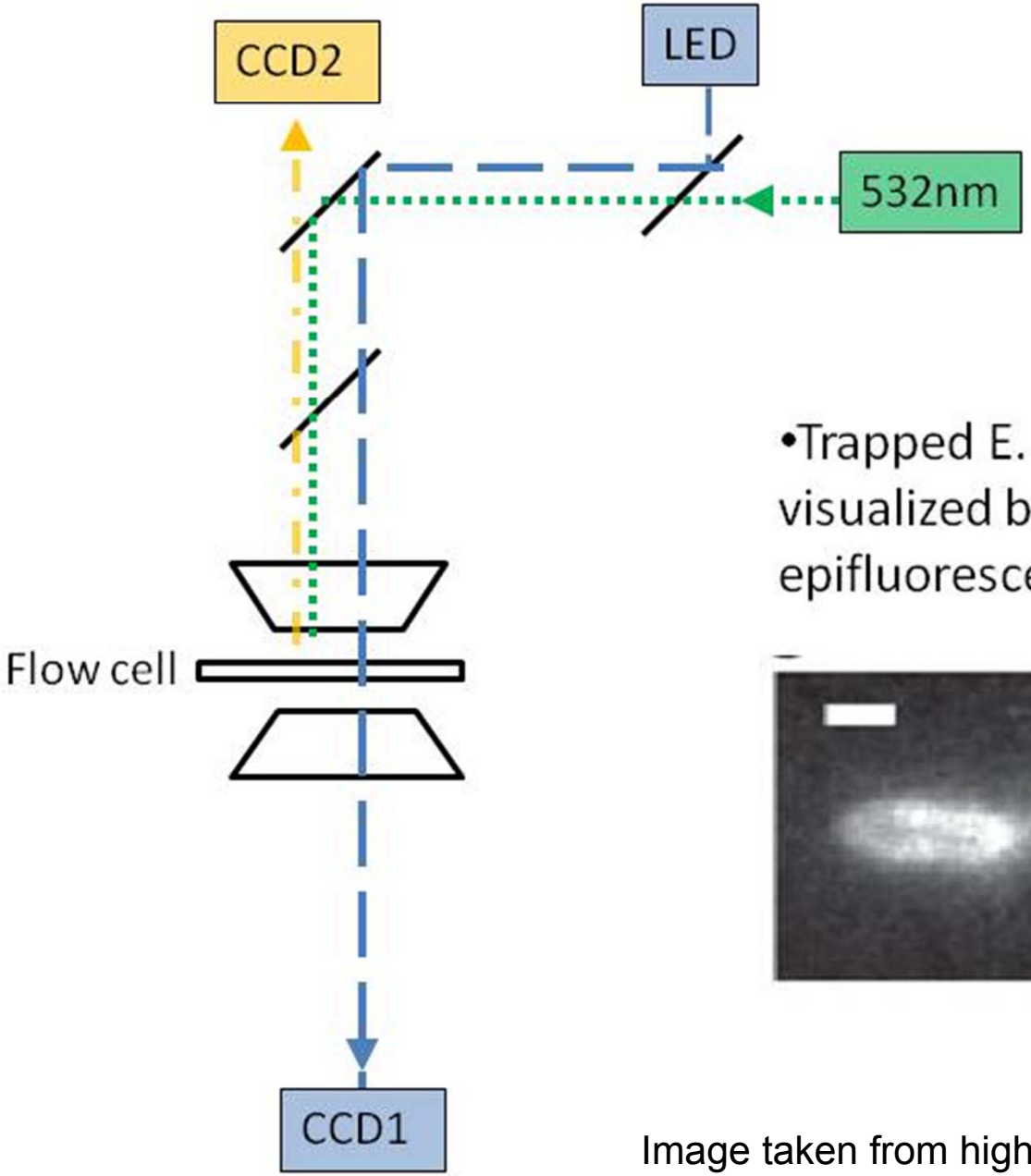
Experimental Setup



- CCD: Charge-coupled device camera
- PSD: Position-sensitive detector
- PM: Piezo-actuated mirror

E. Coli cells are trapped in the flow cell

Experimental Setup: Visualization of Trapped E. Coli Cells



•Trapped E. Coli cells were visualized by brightfield or epifluorescence microscopy

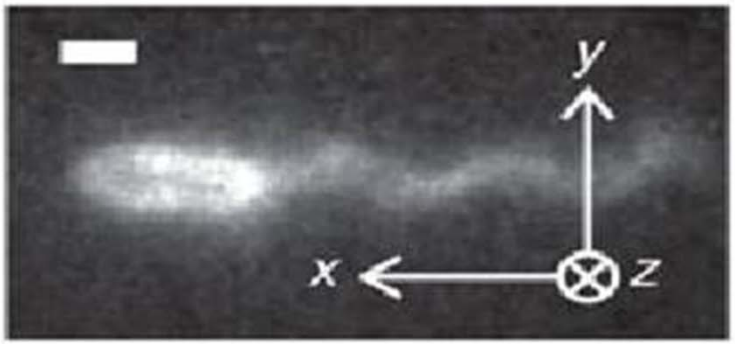
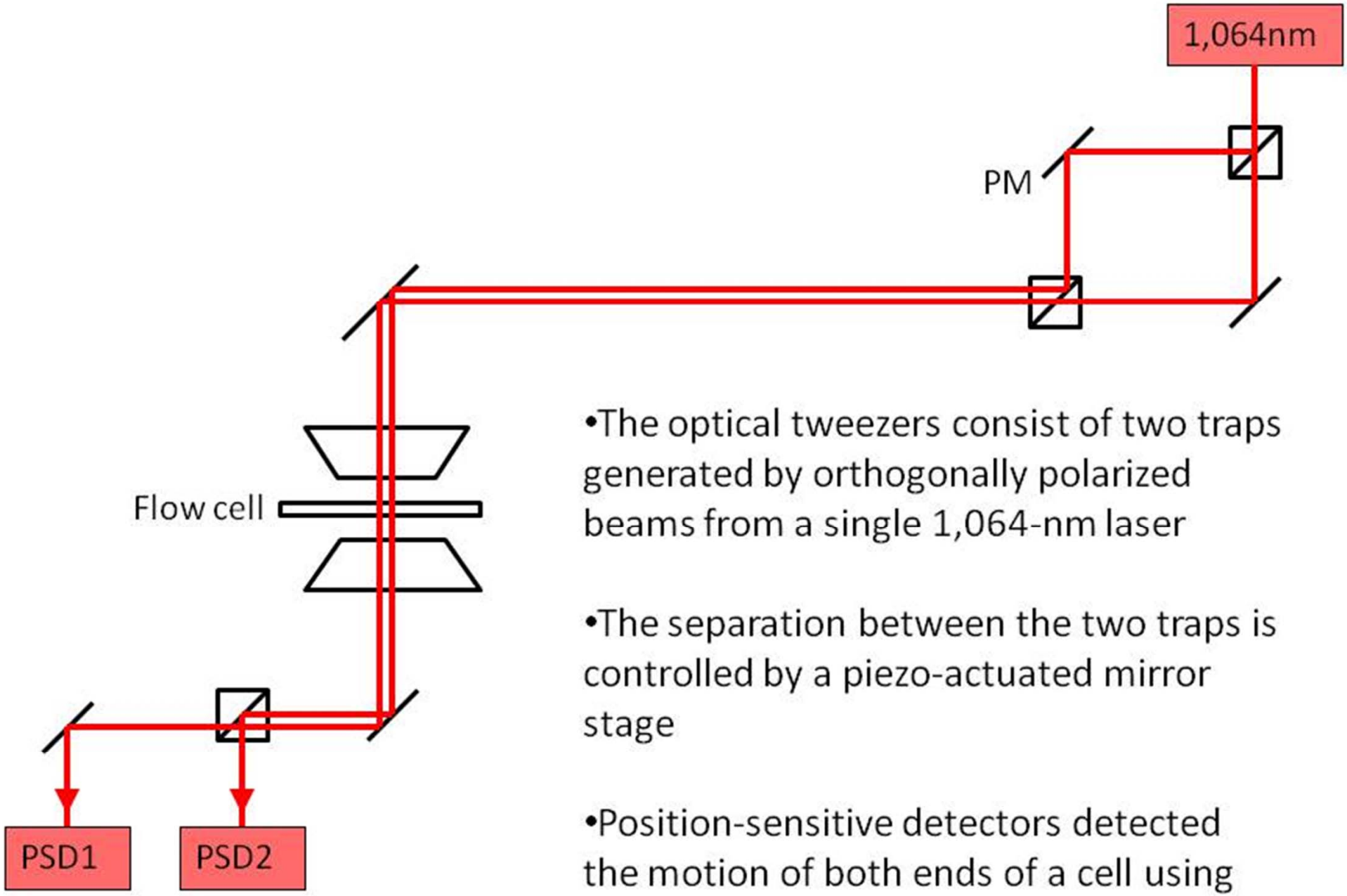


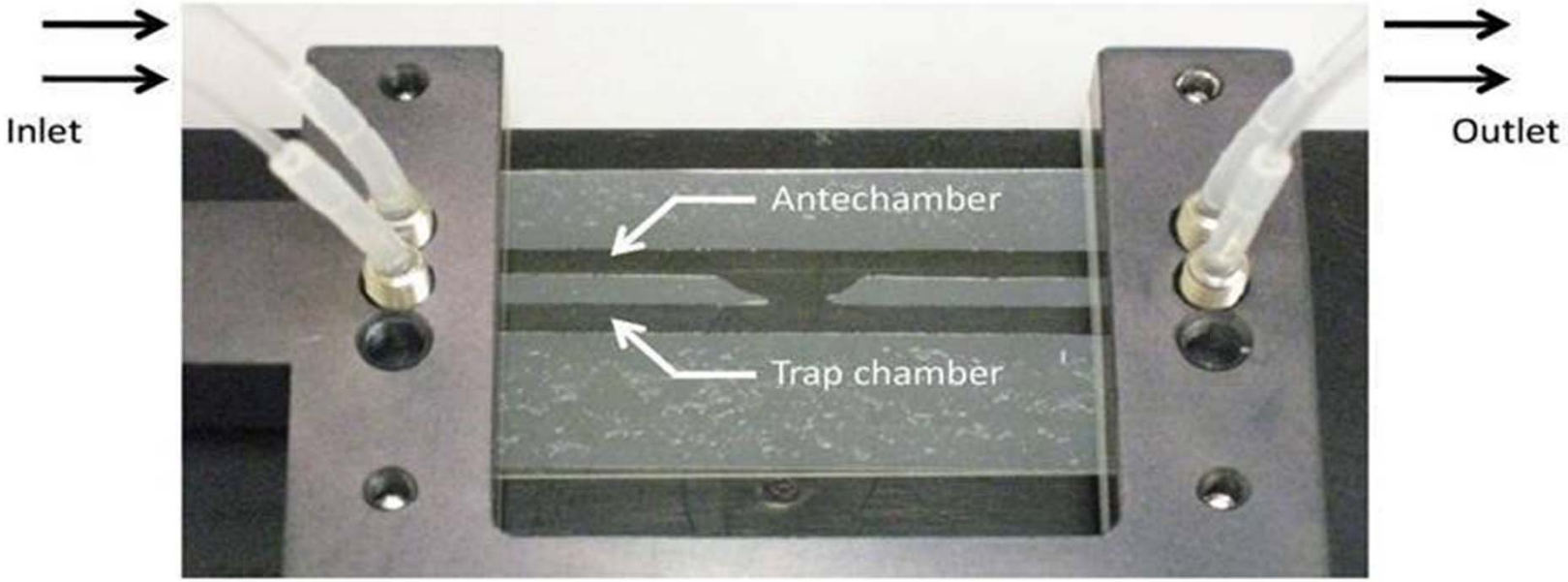
Image taken from high-resolution, long term characterization of bacterial motility using optical tweezers

Experimental Setup: Optical Trap



- The optical tweezers consist of two traps generated by orthogonally polarized beams from a single 1,064-nm laser
- The separation between the two traps is controlled by a piezo-actuated mirror stage
- Position-sensitive detectors detected the motion of both ends of a cell using back-focal plane interferometry

Experimental Setup: Flow Cell



•E. Coli cells were flowed into the antechamber, and then through a narrow inlet into the trap chamber

•Once a cell was trapped in the trap chamber, it was moved far from the connecting region to prevent possible interruptions by other cells

Image taken from high-resolution, long term characterization of bacterial motility using optical tweezers

Experimental Setup: Trapped E. Coli Cell

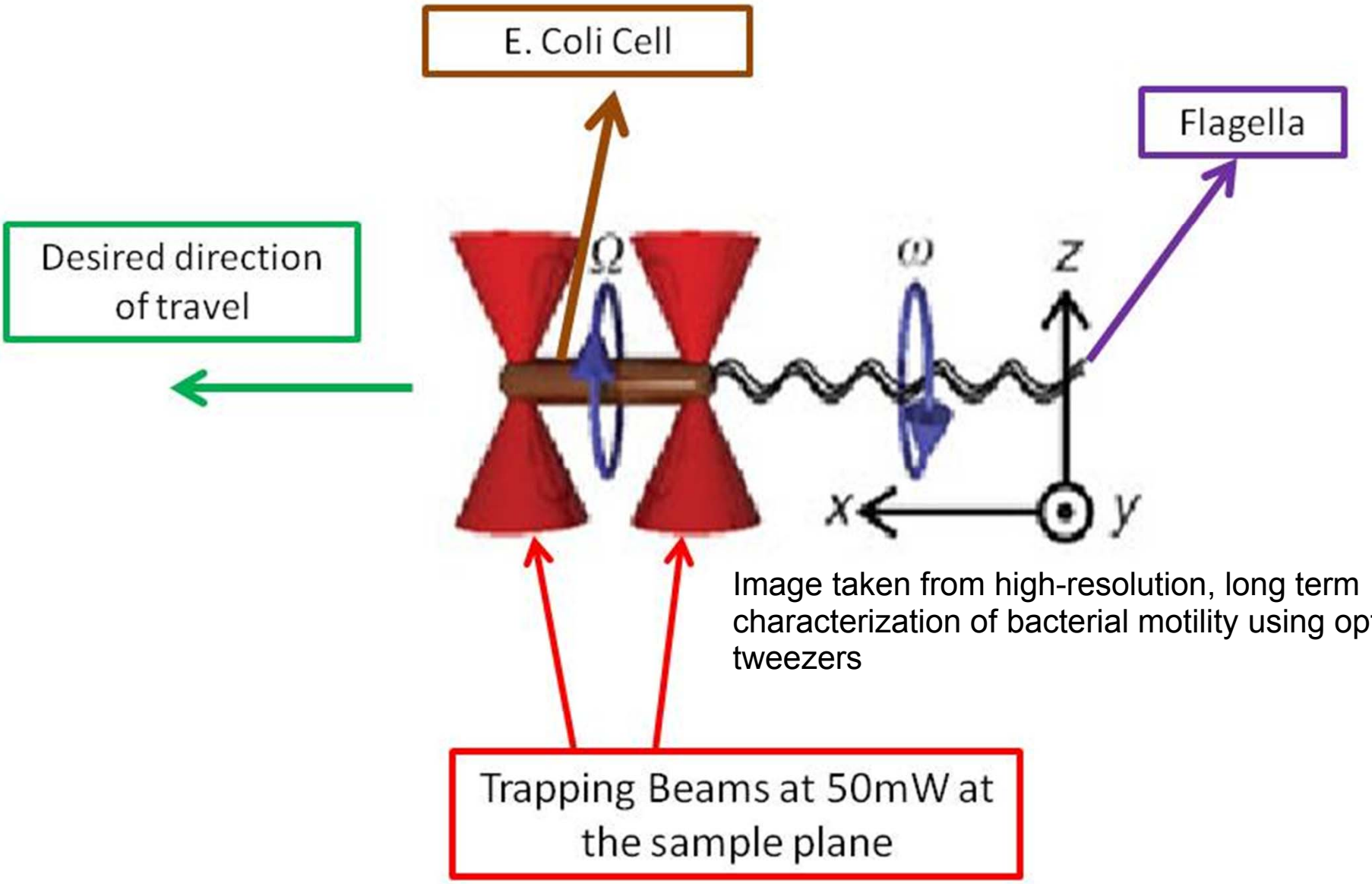
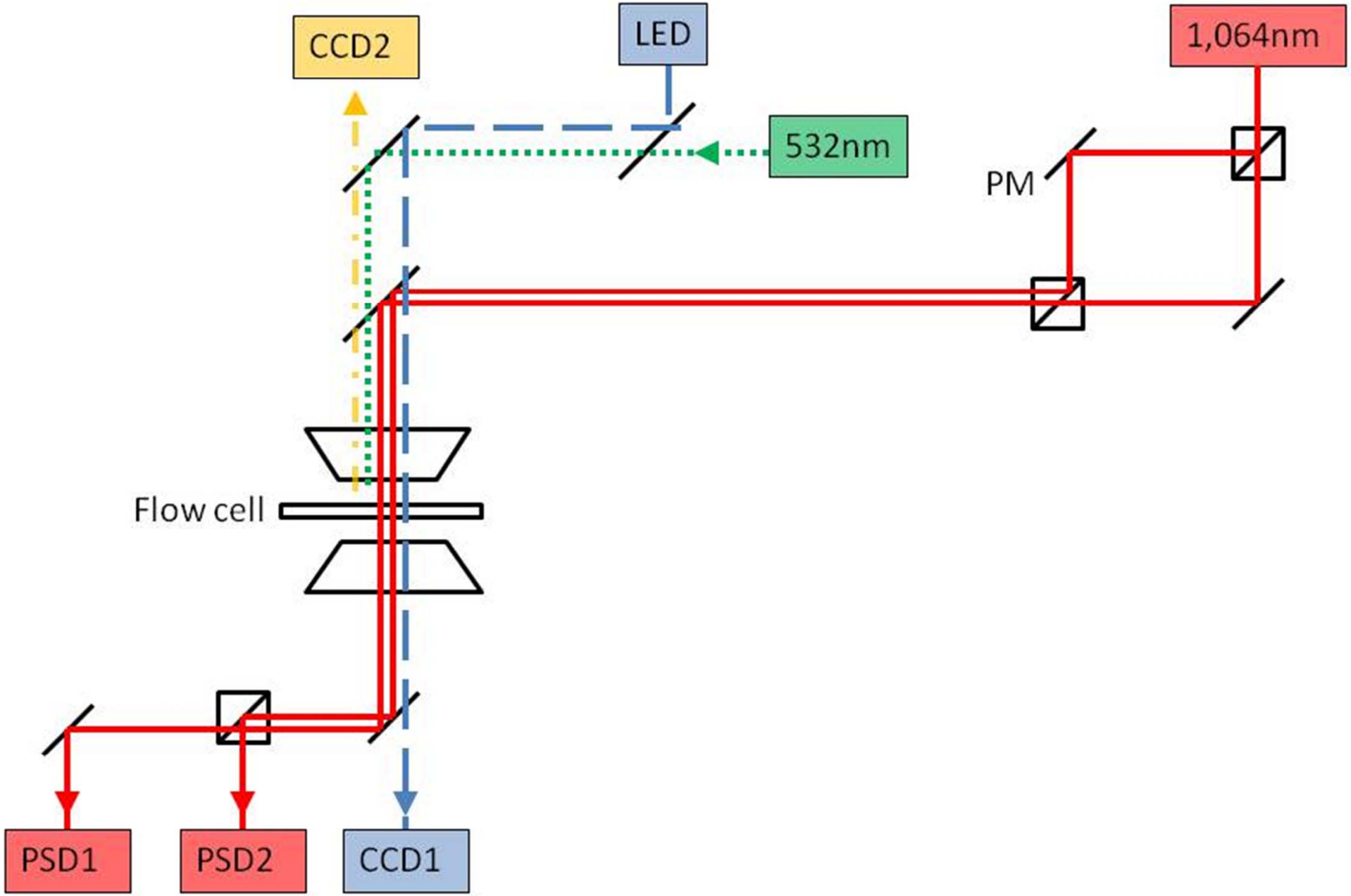


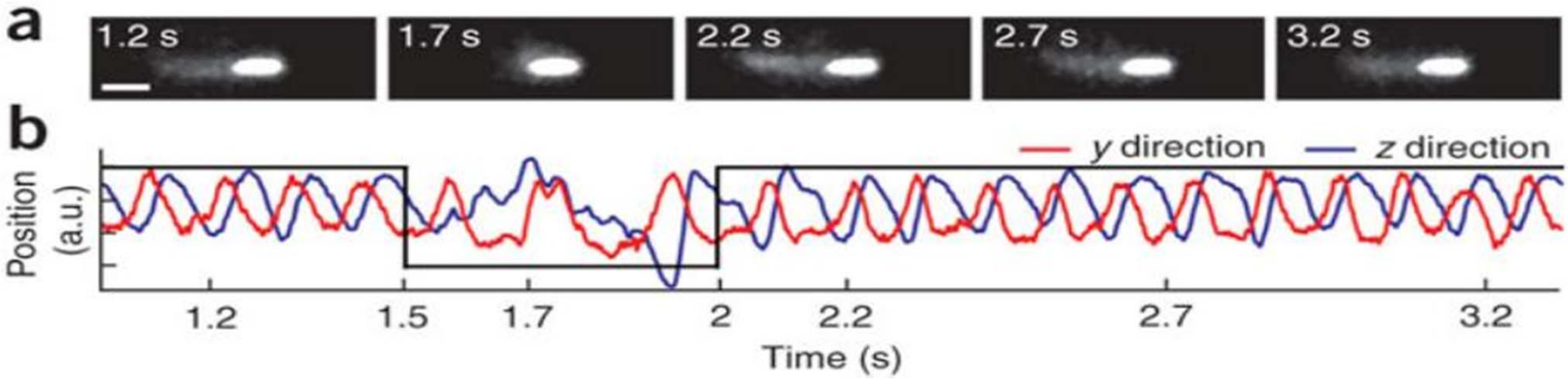
Image taken from high-resolution, long term characterization of bacterial motility using optical tweezers

Experimental Setup



Results

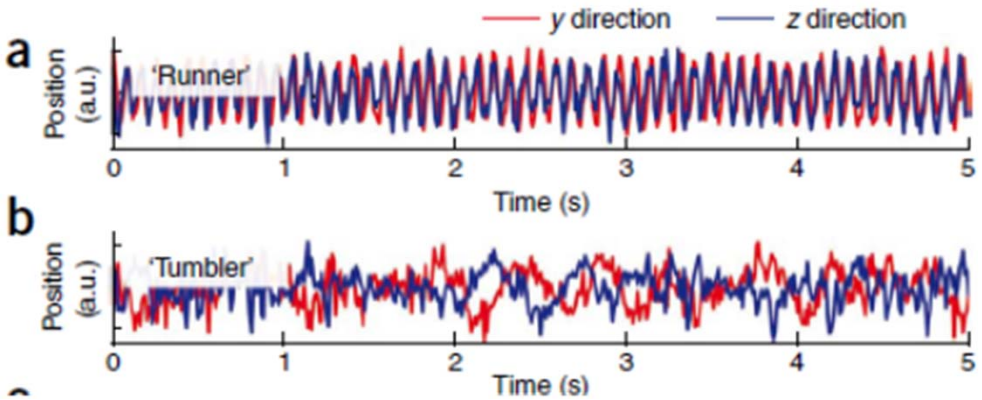
Single cell studies provide information that is hard to obtain in studies of cell populations



- The single cell data above shows that there are interesting differences in the projection of cell motion along the two directions (y and z) orthogonal to the propagation direction (x).

Comparison to the control data

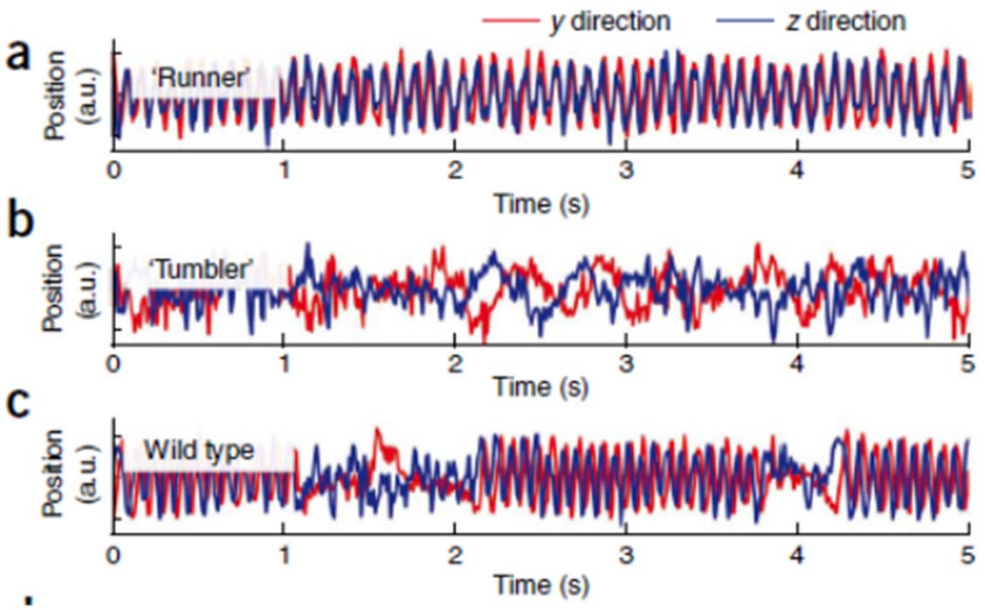
“Runner" mutants produce more periodic signals than the "tumblers”



Comparison to the control data

"Runner" mutants produce more periodic signals than the "tumblers"

"Wild-types" have a bias to run with intermittent tumbling

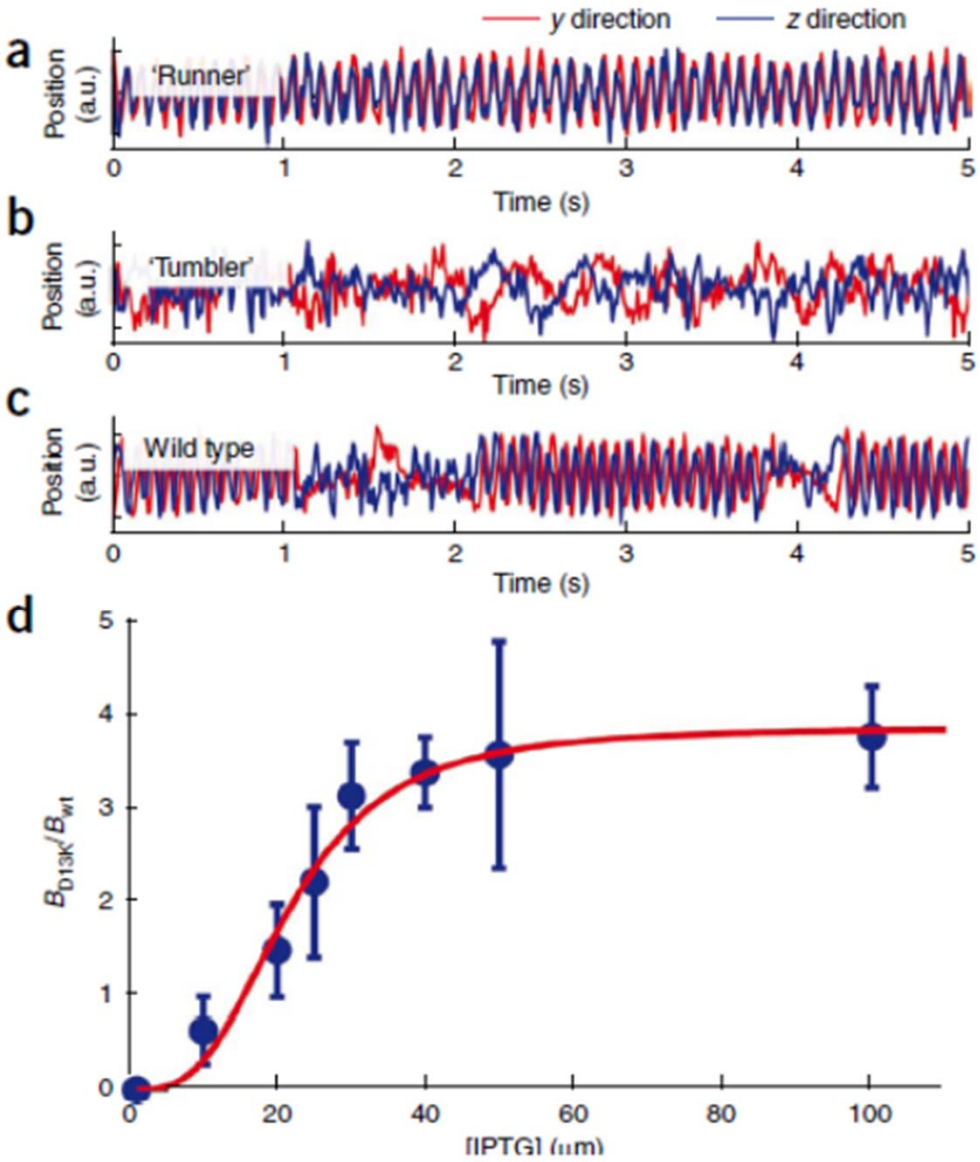


Comparison to the control data

"Runner" mutants produce more periodic signals than the "tumblers"

"Wild-types" have a bias to run with intermittent tumbling

The tumbling bias saturates as the inducer (IPTG) concentration is increased



What are the "higher-order" effects one is looking for?

Longer duration studies allow more precise tests of the "run-tumble" picture of cell motion
These studies can provide information about:

- Changes in cell velocity before and after a tumble
- Reversal of swimming direction when the flagellar bundle changes its orientation

- Changes in motor and swimming velocity as a function of multiple physiological and mechanical factors

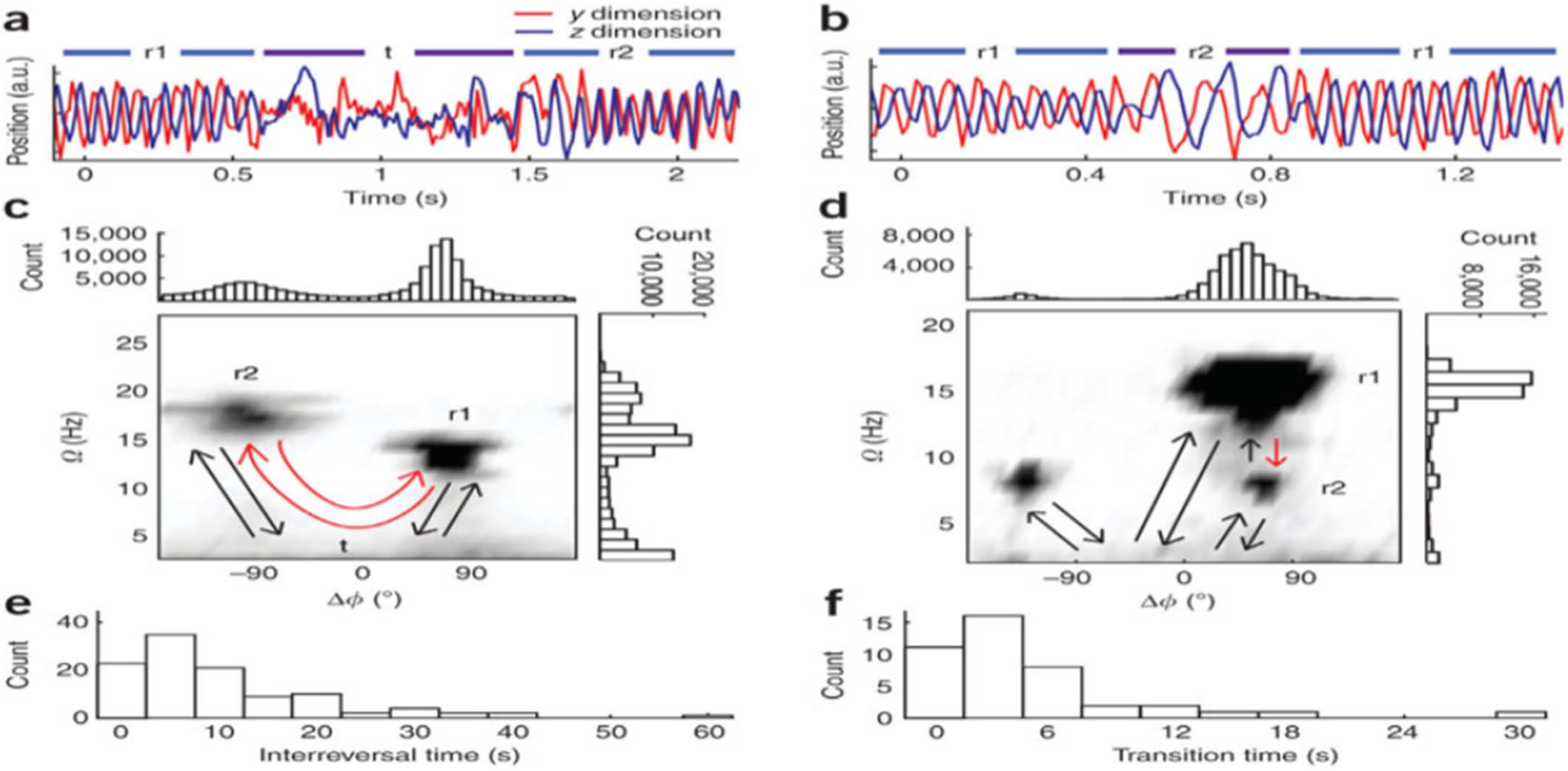
What are the experimental signatures of these finer effects?

- Reversals in phase difference between *y* and *z* signals ($\Delta\Phi$) indicate reversals in swimming direction

- Changes in oscillation frequency correspond to changes in swimming speed

The correlation between the body-roll angular velocity (Ω) and the phase difference $\Delta\Phi$ depends on the direction of motion!

Correlation between body angular velocity and y-z phase difference.



Although cell populations do not show a preferred swimming direction, single cells seem to show a bias!

"Higher-order" effects in swimming interpreted from the data

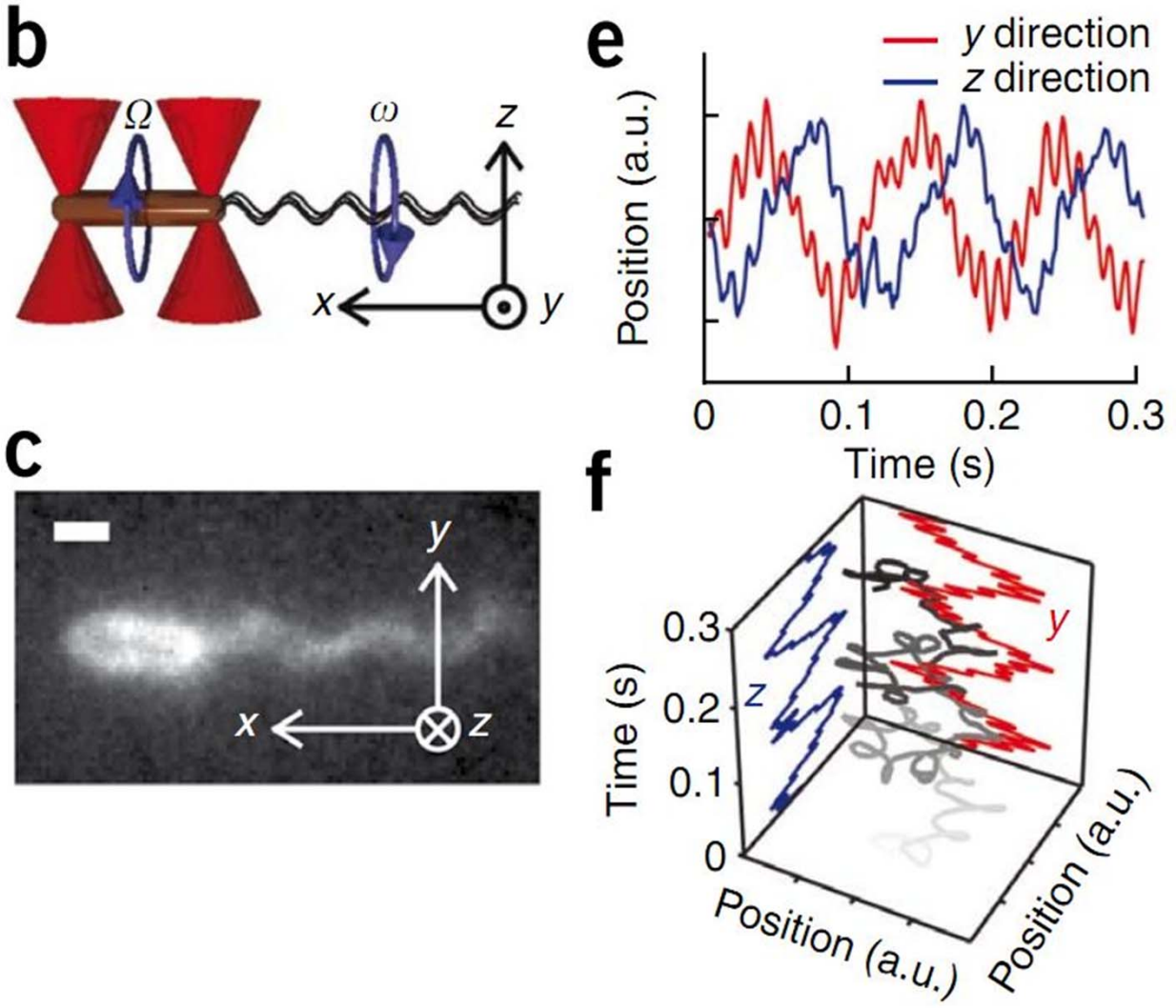
- On average, single cells tumble once every 21.3 ± 1.1 s
- Reversal of swimming direction is accompanied by changes in body roll angular velocity, but no changes in flagellar bundle rotation.
- There are occasional abrupt changes in the body roll angular velocity with no change in swimming direction.
- Changes in speed occur spontaneously without tumbling (69.5%) or after a tumble (30.5%).

Although the angular velocity of flagellar bundle rotation is unaffected by swimming features, its conformation may be affected by speed.

Discussion

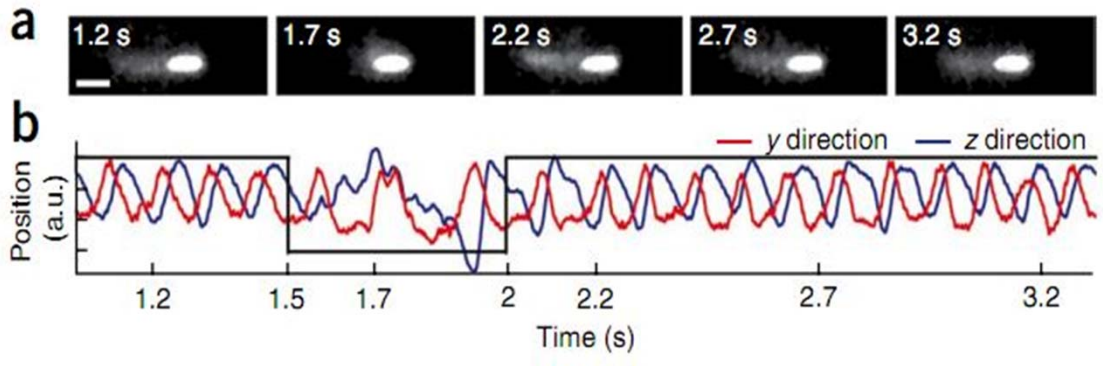
Summary of the paper goals:

Improved time-resolved motility detection



...uncovers underlying details...

Monitoring longer times...



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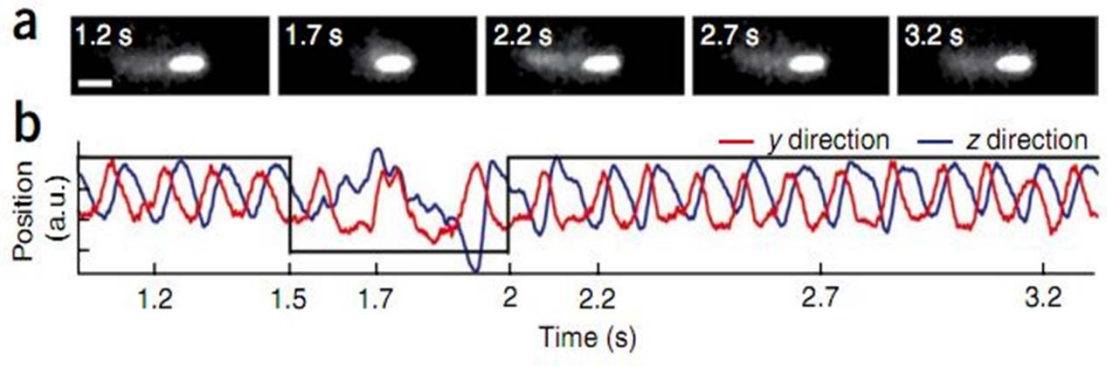
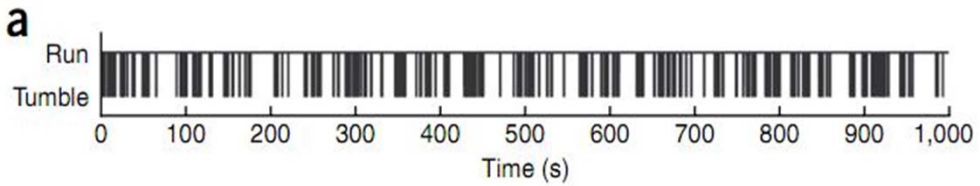
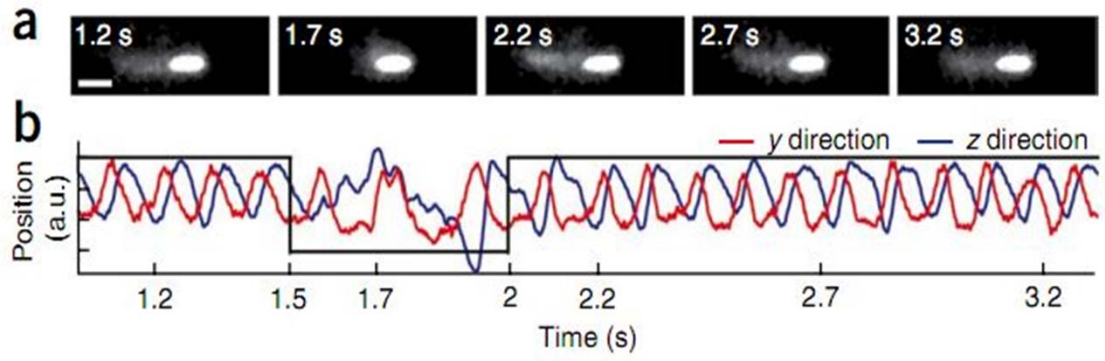


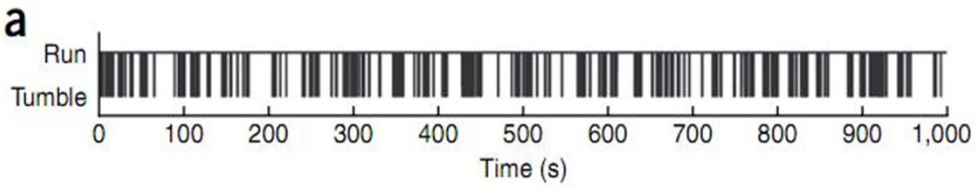


…provide a view beyond the run-tumble picture...

…uncovers underlying details...

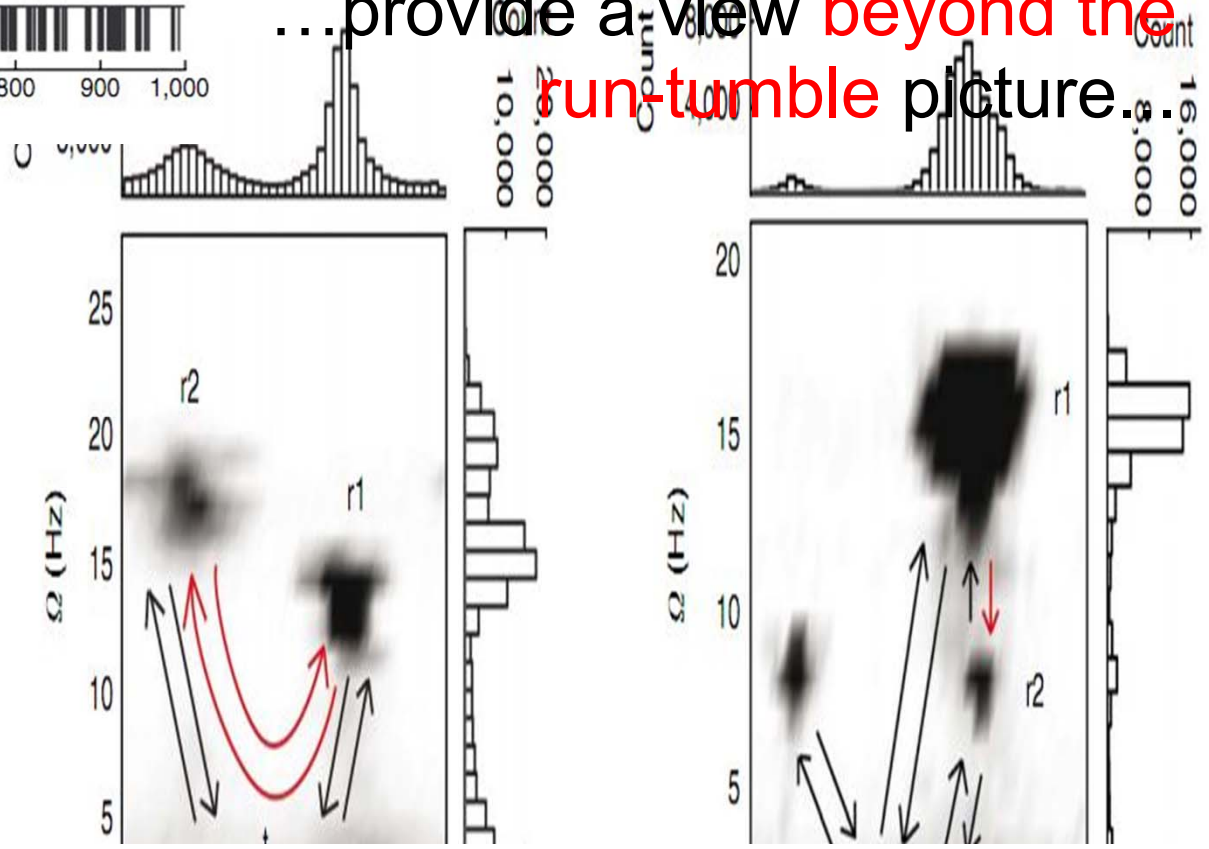
Monitoring longer times...





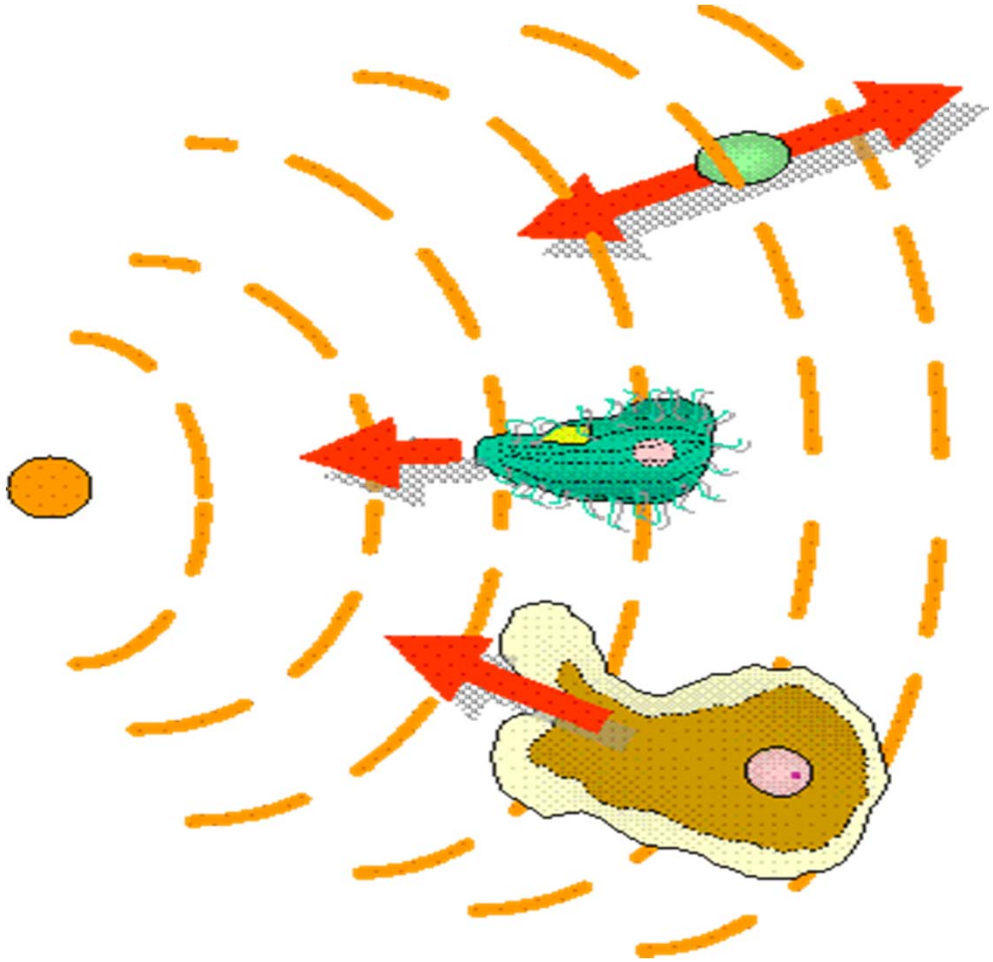
…provide a view beyond the run-tumble picture…

...with better sample statistics



Prospect:

Monitoring chemotaxis processes



Taken from
http://www.chemotaxis.usn.hu/

Expected technical improvements

- Better fluorescence readout
- Use of high-speed video acquisition
- Suppression of the deteriorating effects of optical traps

Citations: 7 in two years

2011

Title: **Non-genetic individuality in Escherichia coli motor switching**
Author(s): Mora Thierry; Bai Fan; Che Yong-Suk; et al.
Source: PHYSICAL BIOLOGY Volume: **8** Issue: **2** Article Number: **024001**
Times Cited: **0** (from All Databases)

Title: **Directional persistence of chemotactic bacteria in a traveling concentration wave**
Author(s): Saragosti J.; Calvez V.; Bournaveas N.; et al.
Source: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA
Times Cited: **0** (from All Databases)

2010

Title: **Full 3D translational and rotational optical control of multiple rod-shaped bacteria**
Author(s): Hoerner Florian; Woerdemann Mike; Mueller Stephanie; et al.
Source: JOURNAL OF BIOPHOTONICS Volume: **3** Issue: **7** Pages: **468-475** DOI: **10.1002/jbio.201000033** Published: **JUL 2010**
Times Cited: 5 (from All Databases)

Title: **Dual-trap Raman tweezers for probing dynamics and heterogeneity of interacting microbial cells**
Author(s): Li Yan; Wang Guiwen; Yao Hui-lu; et al.
Source: JOURNAL OF BIOMEDICAL OPTICS Volume: **15** Issue: **6** Article Number: **067008** DOI: **10.1117/1.3526357** Published: **NOV-DEC 2010**
Times Cited: **0** (from All Databases)

Critical review

Positive notes:

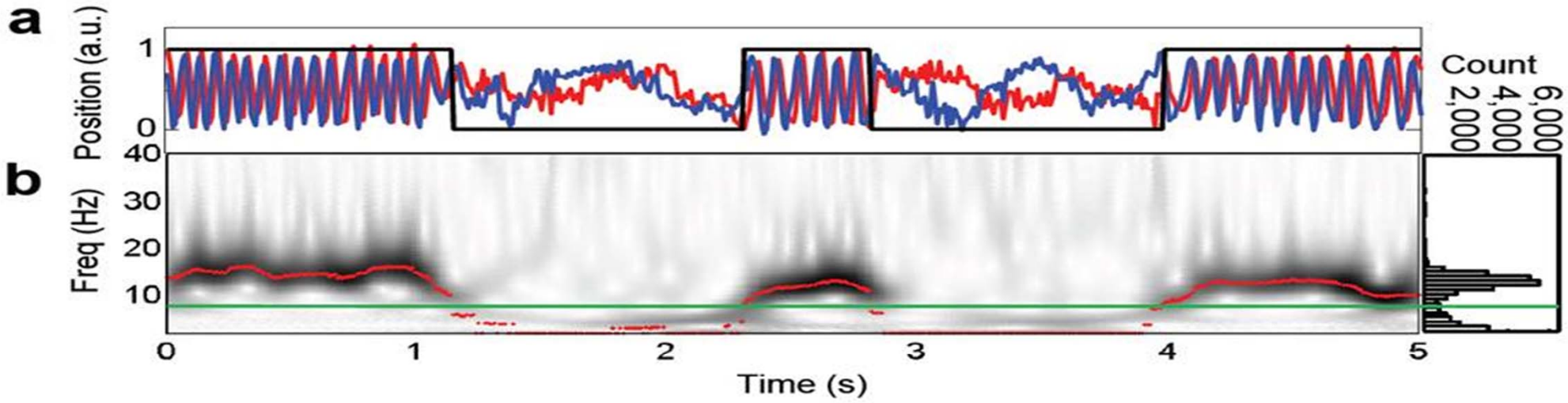
- *Clearly organized*
- *Makes effort to explain pros and cons of the technique*
- *Makes thorough use of supplementary material*

Not clear from the paper:

- *The control experiments verify that the average behavior is not altered, but they don't address changes in fluctuations possibly induced by the invasive nature of the tweezers.*
- *Monitoring of single cells, perhaps, will not reveal some generic features of motility (~ 40 cells used!).*
- *Pinning of cell could alter conclusions about motility.*

Thank you for your attention

Supplementary Info: Run-Tumble Analysis



Supplementary Figure 3 | Run-tumble analysis of optical trap data. (**a**) Swimming signal in *y* (red) and *z* (blue) directions from an optically trapped cell, and the binary signal (black) indicating regions of runs (1) and tumbles (0). (**b**) Continuous wavelet transform of the *y* signal in the frequency range 2 – 40 Hz. Red dots indicate the peak frequency component at each time point. Shown on the right is the histogram of peak frequencies from the entire time trace. The frequency value at the local minimum of the histogram is chosen as the threshold (green line), which is used to distinguish runs from tumbles.

Taken from High-Resolution, long term characterization of bacterial motility using optical tweezers, Supplementary Figure 3.