UC San Diego
JACOBS SCHOOL OF ENGINEERING
Electrical and Computer Engineering

Optical Tweezers Applications

(Part 2)

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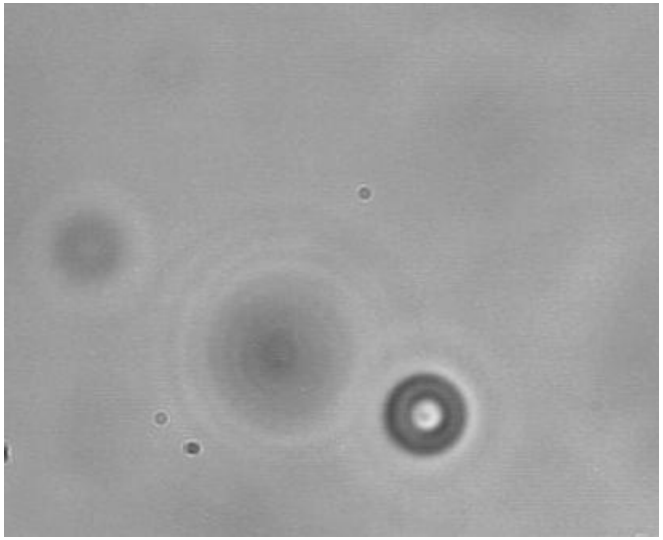
Outline

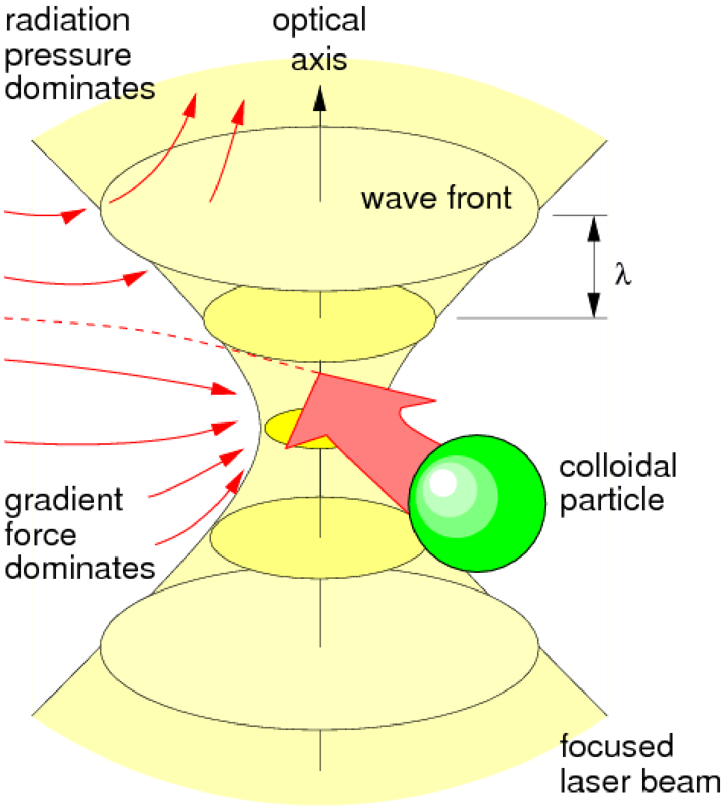
- **Optical Tweezers**
- **Application in medicine and biology**
  - **Subcellular manipulation**
  - **Cancer study**
  - **DNA stretching**
  - **Plasmonic optical tweezers**
- **Tug-of-War optical tweezers to study rod-shaped bacteria and biofilms**

How optical tweezers work

Optical Tweezers use radiation pressure from a focused laser beam to attract particle to to the the center of the beam (the highest intensity).

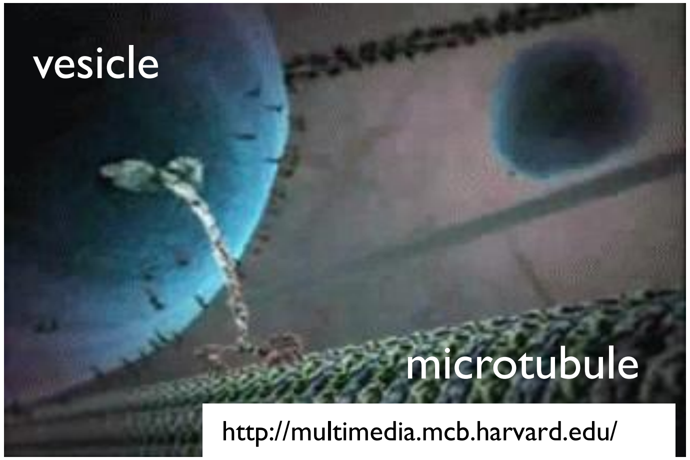


University of Cambridge: Joanne Gornall's group



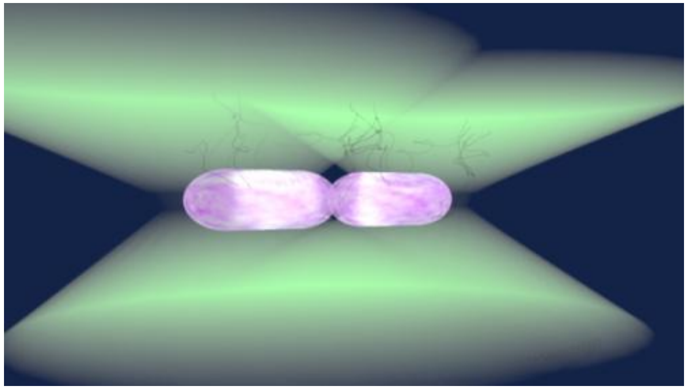
http://www.physics.nyu.edu/~dg86/figures/tweezer.png

With optical tweezers we can do …

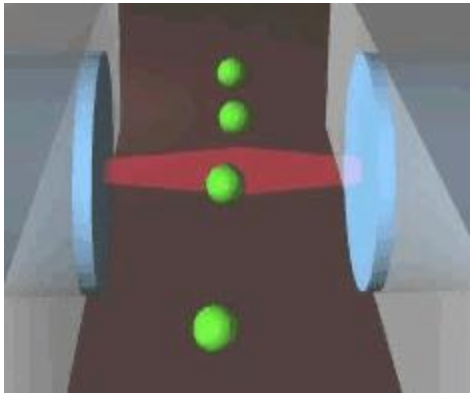


Force estimation for Kinesin motors and other molecular motors

Microsurgery and manipulation of cells
DNA injection and/or incorporation



Physical properties of microorganism
Bacteria- drug interaction



J. Guck et al, *Biophys. J.* 88(5):3689(2005)

Detect cancer cell by stretching

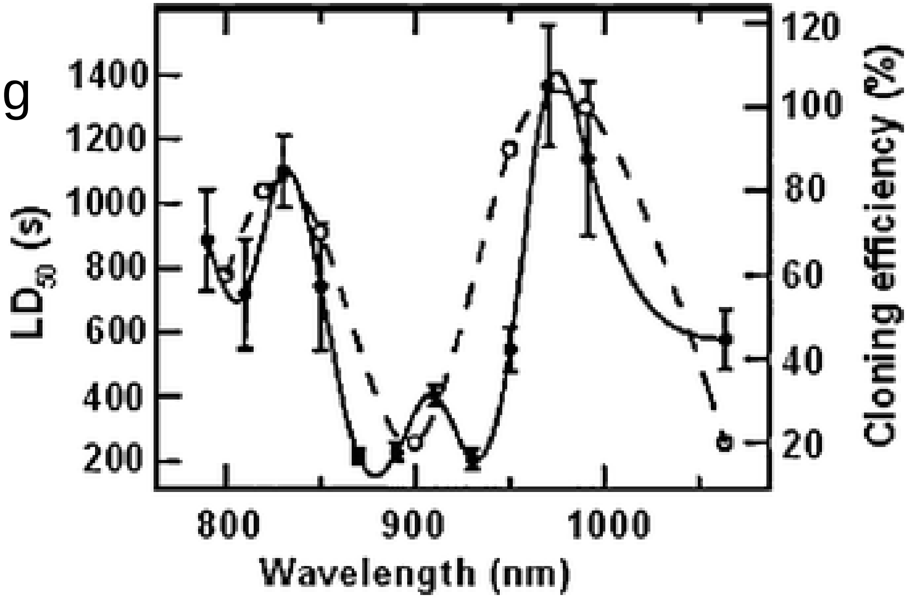
Laser wavelength for biological samples

- For trapping living microorganisms need to use 750-1200nm IR laser light to minimize damage & heating

  (due to absorption by either protein or water in cell)

- The most harmful wavelength are 850-950nm, safest are on a side

- Most common trapping wavelengths: 1064nm and 830nm



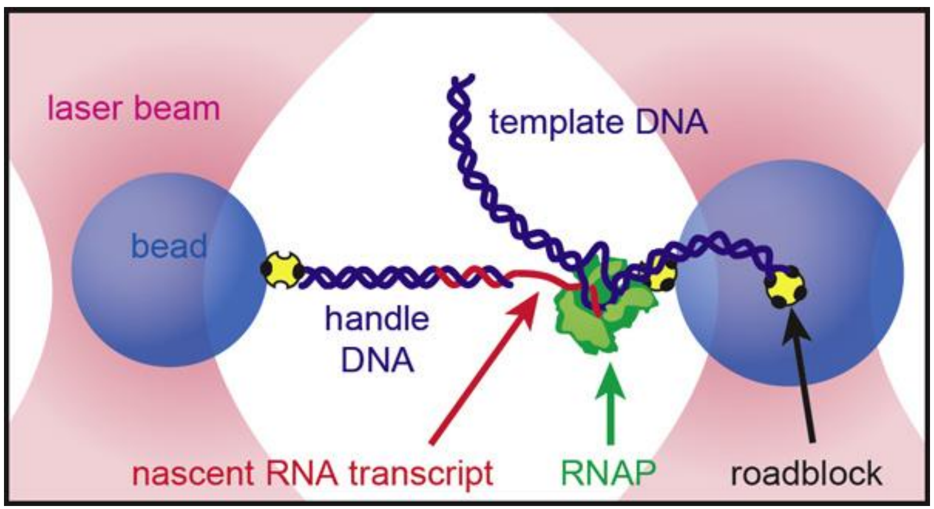
Relationship between wavelength and cell photo damage for *E. coli* (solid line and left axis) and chinese hamster ovary cells (dashed line and right axis). The higher the LD_{50} and % cloning the less damage the laser causes for a given wave number.

K. Neuman, *Biophys. J.* **77**, 2856 (1999)

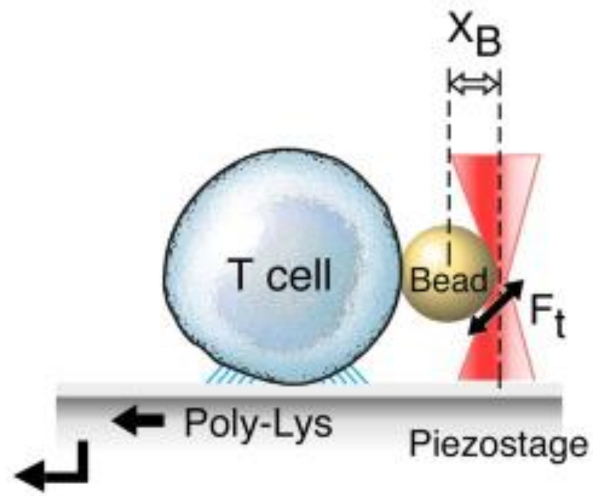
Use beads to trap biological samples

Many biological samples cannot be directly trapped due to size, shape, and adherent properties.

-> attach a micro or nano-particle onto the sample to use as a trapping "anchor" to hold onto the sample with

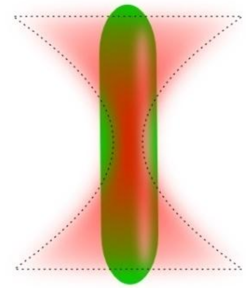


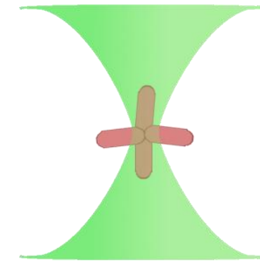
S. Block website, Stanford University



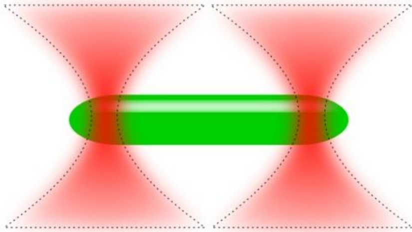
S.T. Kim, *Front. Immunol.* 3(76), 1(2012)

Multiple traps to fit complex shape



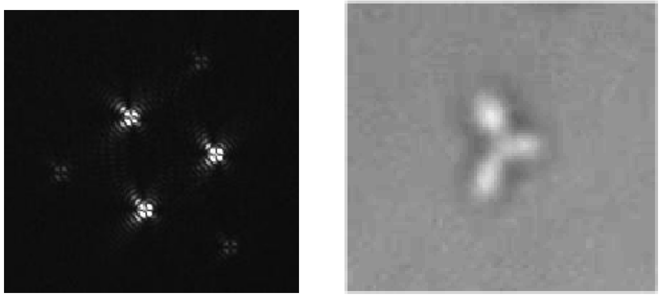


- Bacteria prefer to be aligned along the of a trapping beam.
- Dual optical tweezers (a beam splits in two and focuses into plane)
- Time- shared optical tweezers (a beam quickly ran over multiple points)

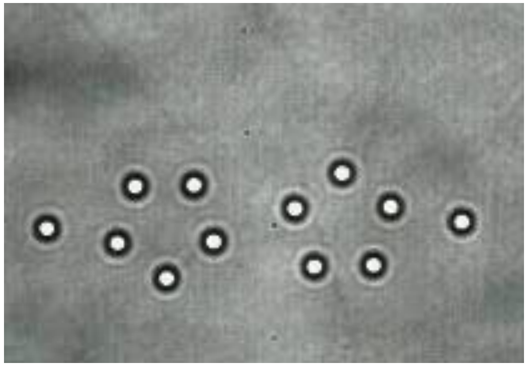


- Holographic optical tweezers (use spatial light modulator to create multiple “clusters” of traps)

3 spots applied to a mutant *S.Meliloti*



Dancing beads



Y. Roichman and D. G. Grier, 2005

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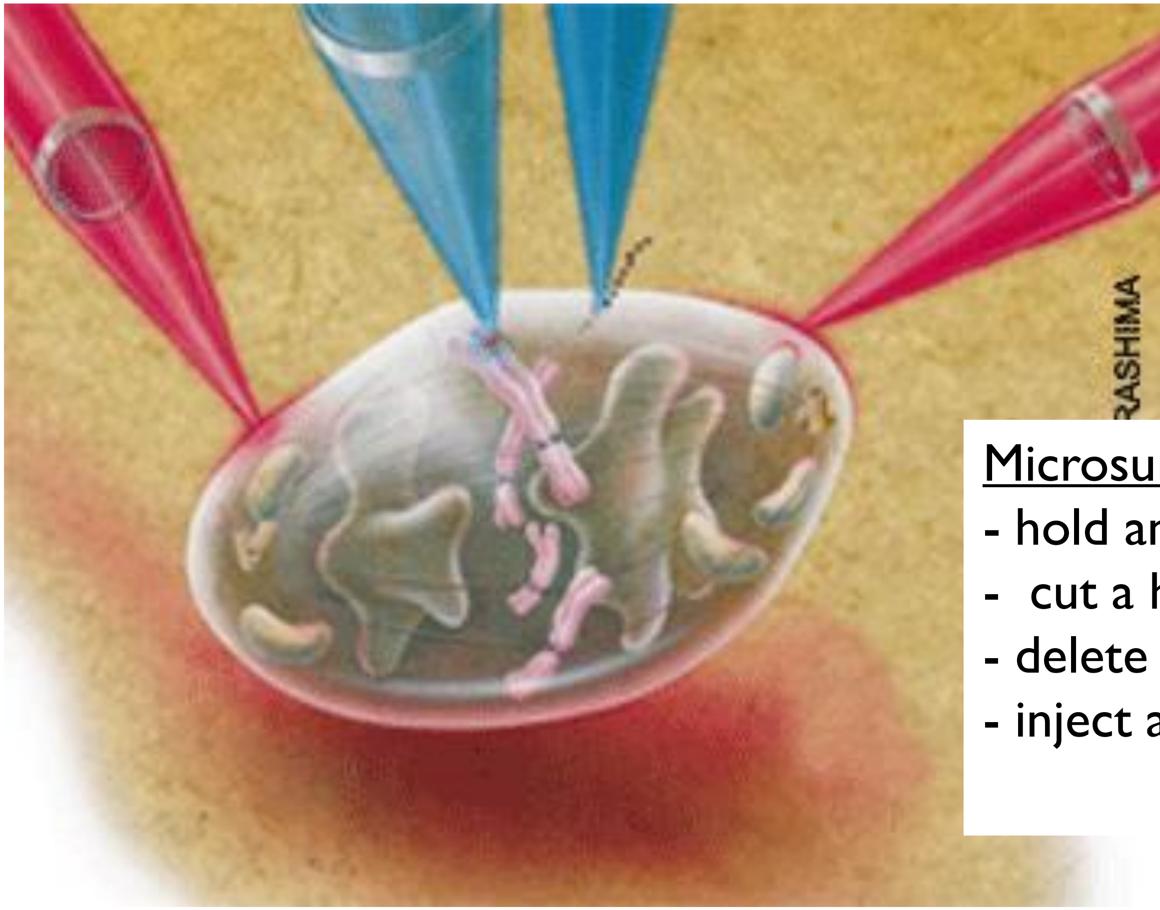
Subcellular manipulation

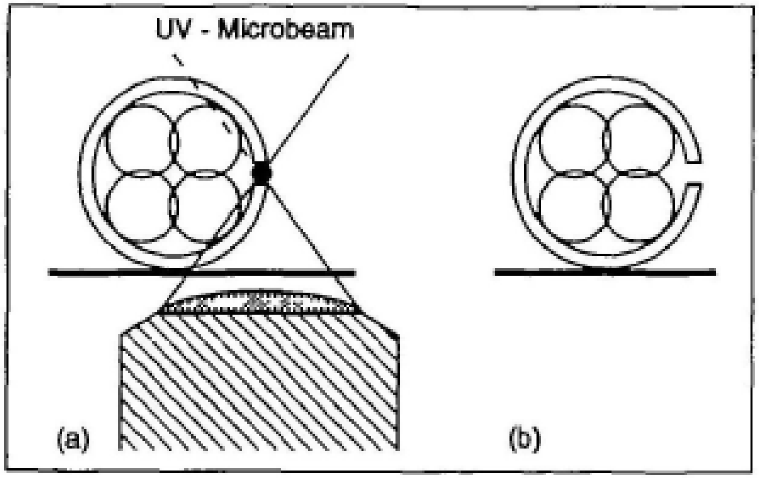


Microsurgery of cells:

- hold and manipulate a cell
- cut a hole in the cell membrane
- delete a faulty gene
- inject and incorporate new genes

Berns, MW., "Laser Scissors and Tweezers," Scientific American, Apr 1998

Human *in vitro* fertilization

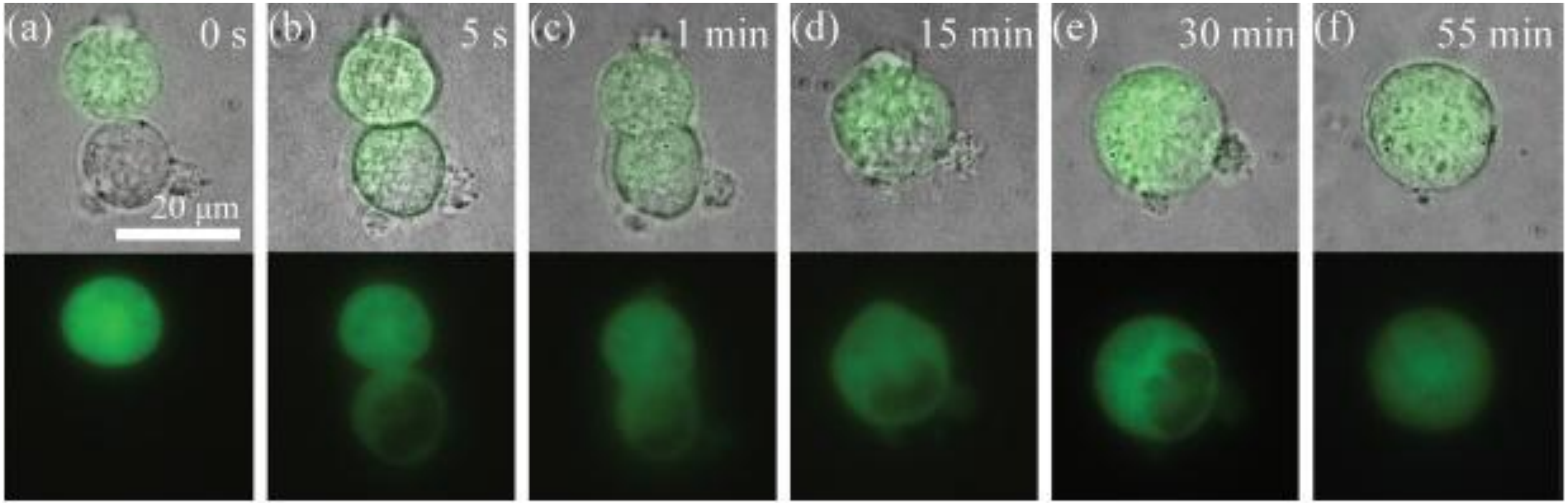


Antinori *et al.*, *Hum. Reprod.* **11**, 2488 (1996)

<u>Method:</u>

- the zona pellucida is perforated with pulse laser
- With optical tweezers the spermatozoon is transported
- Fertilization of the egg cell is allowed

Laser induced fusion of cells

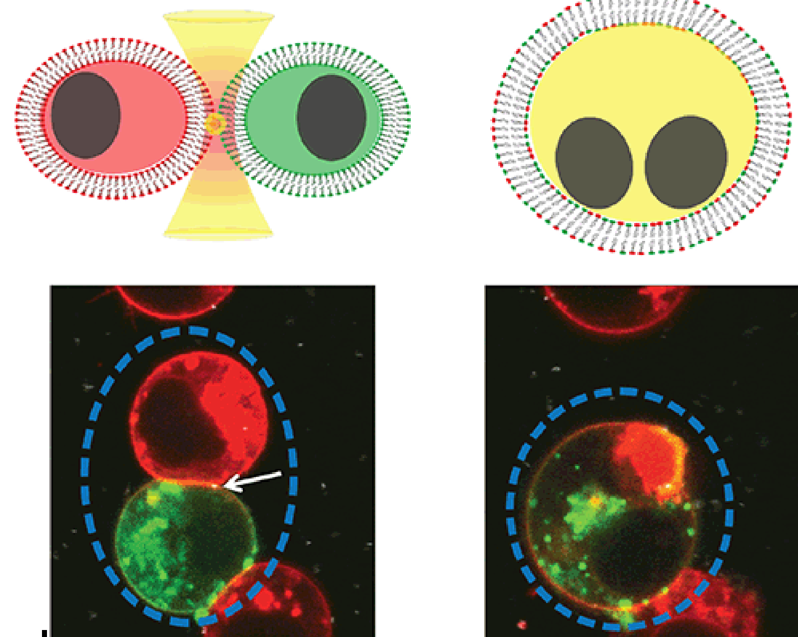


S. Chen et al, *Apl. Phys. Let.* 103, 033701 (2013)

- Laser-induced fusion of two human embryonic stem cells
- <u>Method</u>:
  - two cells are brought together by optical tweezers
  - cells' membrane are being treated with a short series of UV laser pulses
  - cells are fused together

Laser induced fusion of cells

- Hot-particle-mediated fusion between membranes



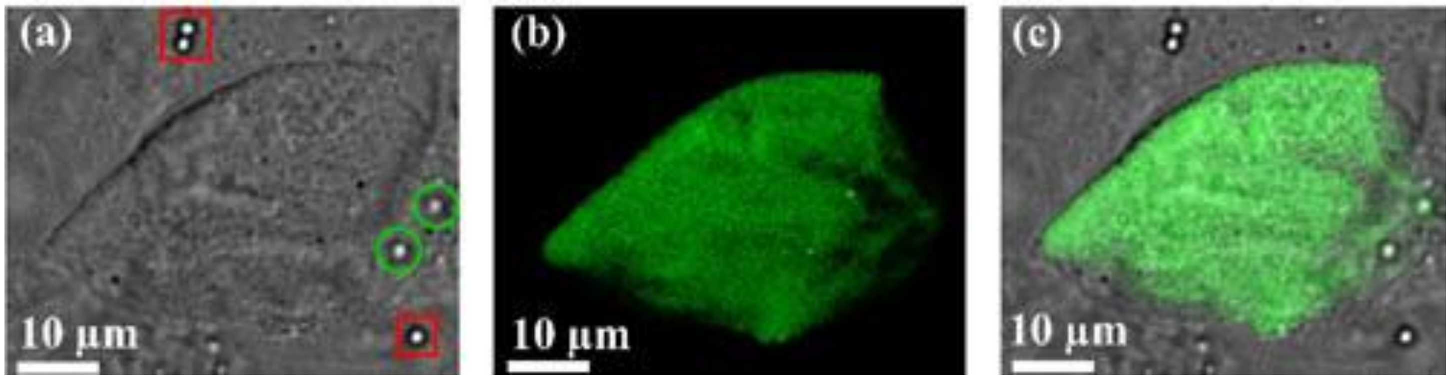
- Method: plasmonic heating induced by irradiating metallic nanoparticles
- Result: a new hybrid cell with an intact cell membrane (the cell shows signs of viability)
- Technique can be used:
  - Targeted drug delivery at the single-cell level
  - Create hybrid cells with inherited genetic properties from both original cells
  - Reprogram cells, control cellular reactions or gene expression

A. Bahadori, L.B. Oddershede P.M.Bendix, Nano Res. 6, 2034(2017)

Introduce new genes into cells

- Deliver DNA inside cancer cells

MCF-7 cancer cell



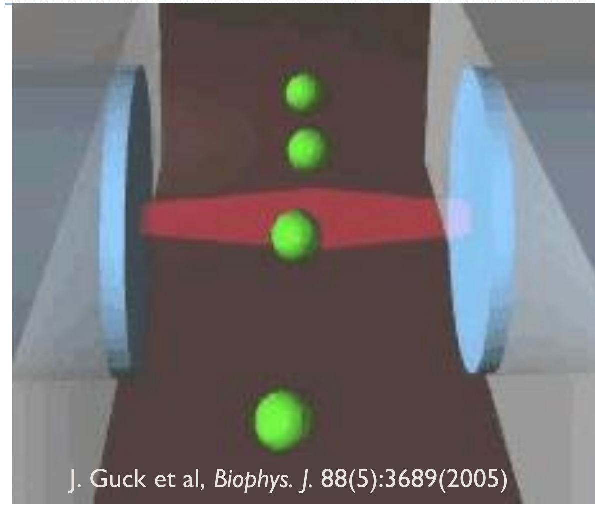
M. Waleed et al., *Biomed.Opt.Express* **4**, 1533 (2013).

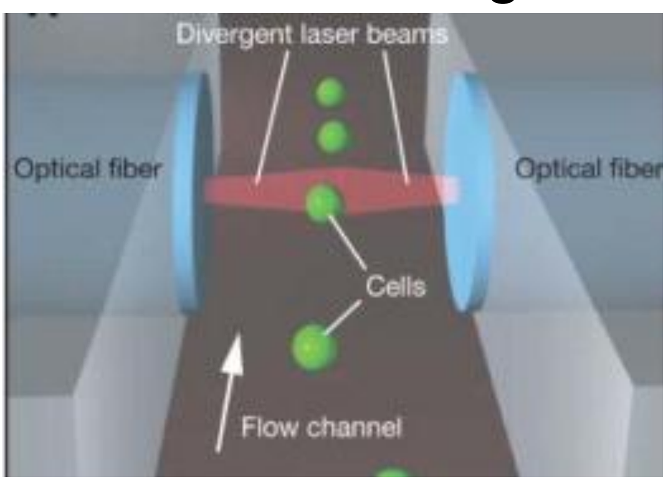
- <u>Method:</u>
  - Use a trapped microparticle to focus the NIR femtosecond laser pulse precisely at a single point on the cell membrane and to puncture it.
  - Introduced an external gene in the cell by trapping and inserting the same plasmid coated microparticle into the optoporated cell.

Cell stretching

Optical stretcher can measure accurately cell elasticity
-> differentiate cell types or between normal and unhealthy cells

Malignant (infected) cells generally are easier to stretch and show a lower elastic strength


J. Guck et al, *Biophys. J.* 88(5):3689(2005)



J. Guck et al, *Biophys. J.* 88(5):3689(2005)

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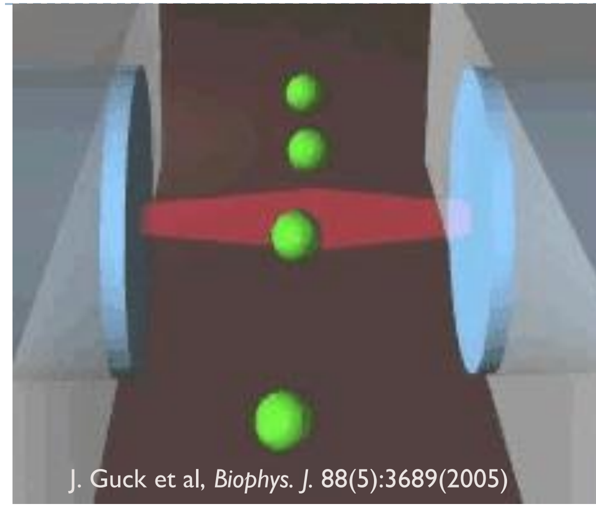
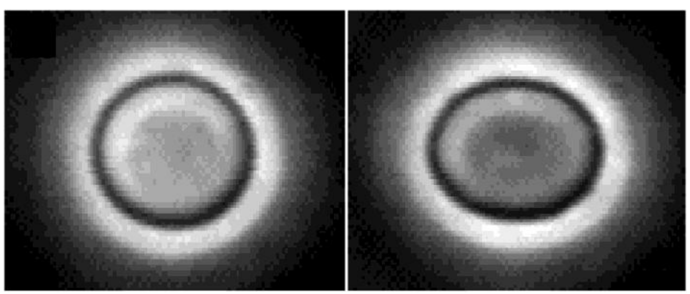


J. Guck et al, *Biophys. J.* 88(5):3689(2005)

Red blood cells



J. Guck et al, *Phys. Rev. Let.* 84: 5451(2000)

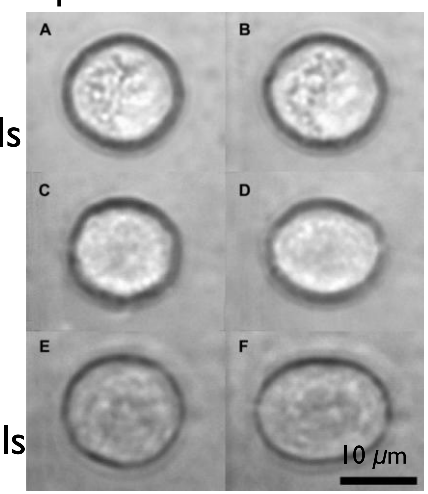
<u>breast epithelial cells:</u>

upstretched stretched

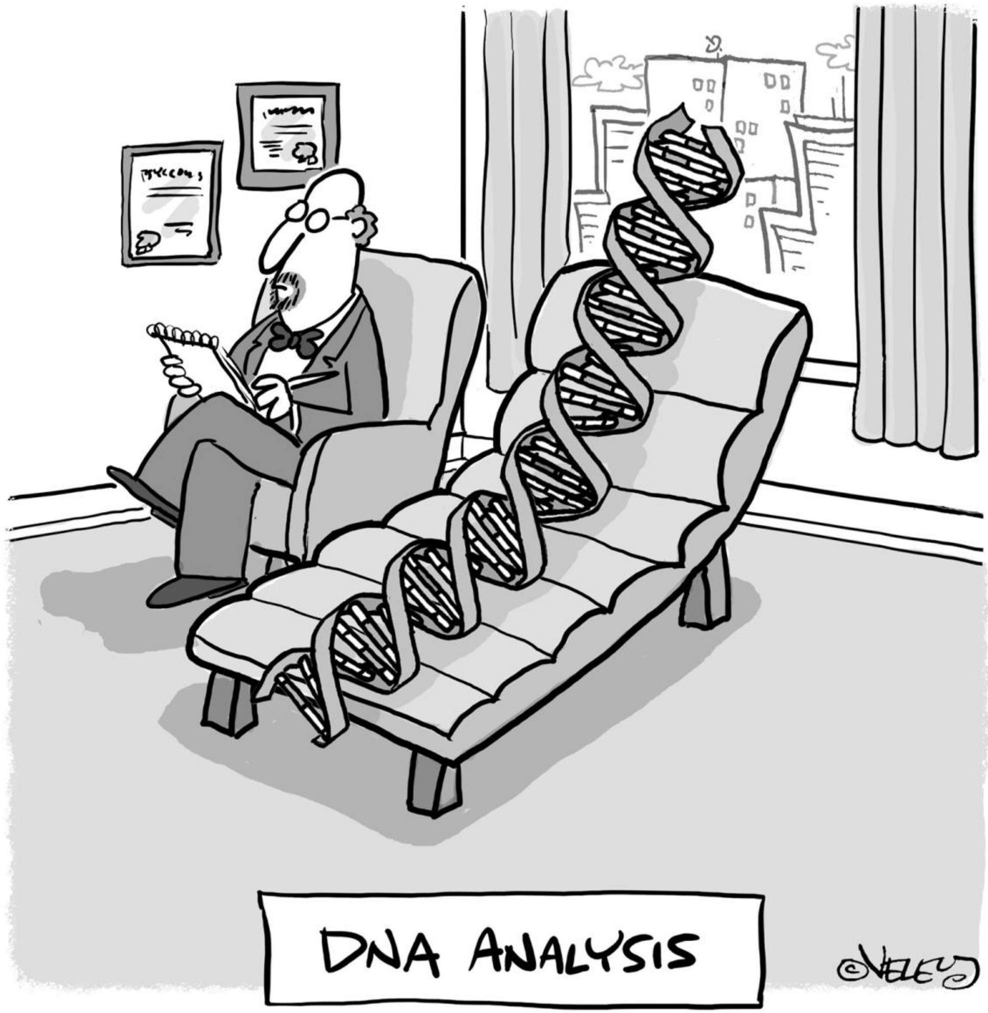
nonmalignant MCF-10 cells

cancerous MCF-7 cells

metastatic modMCF-7 cells

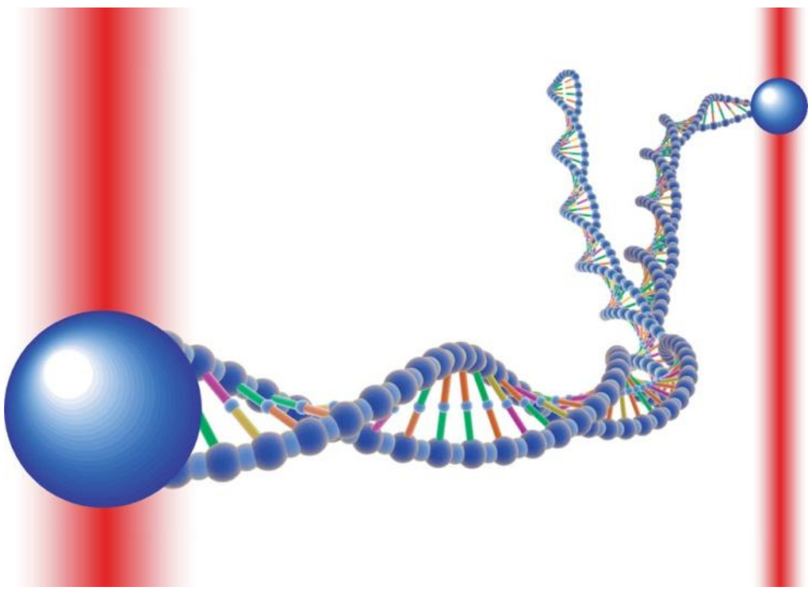


J. Guck et al, *Biophys. J.* 88(5):3689(2005)

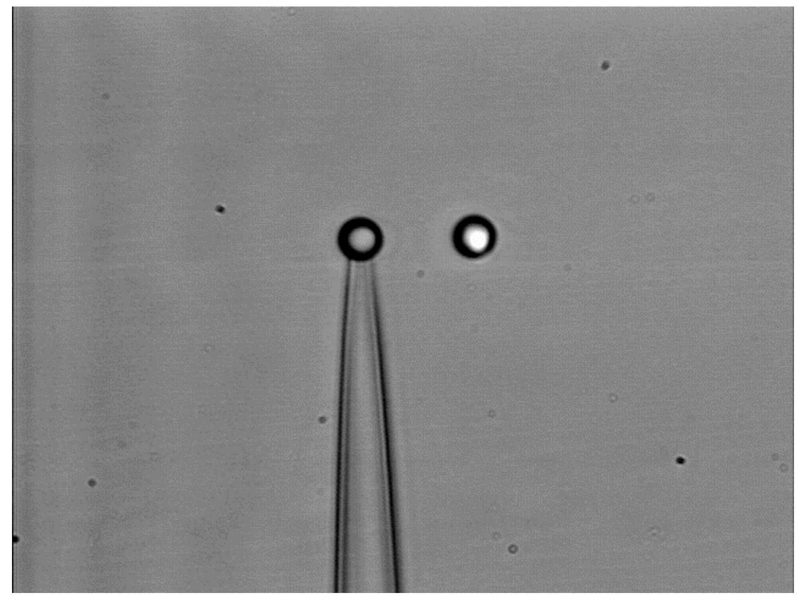

DNA ANALYSIS

Mechanical properties of DNA

DNA properties can be measured:
- length and elasticity of the DNA
-forces during phase transition or to break DNA



C. Jarzynski, Nature Physics 7, 591–592 (2011)



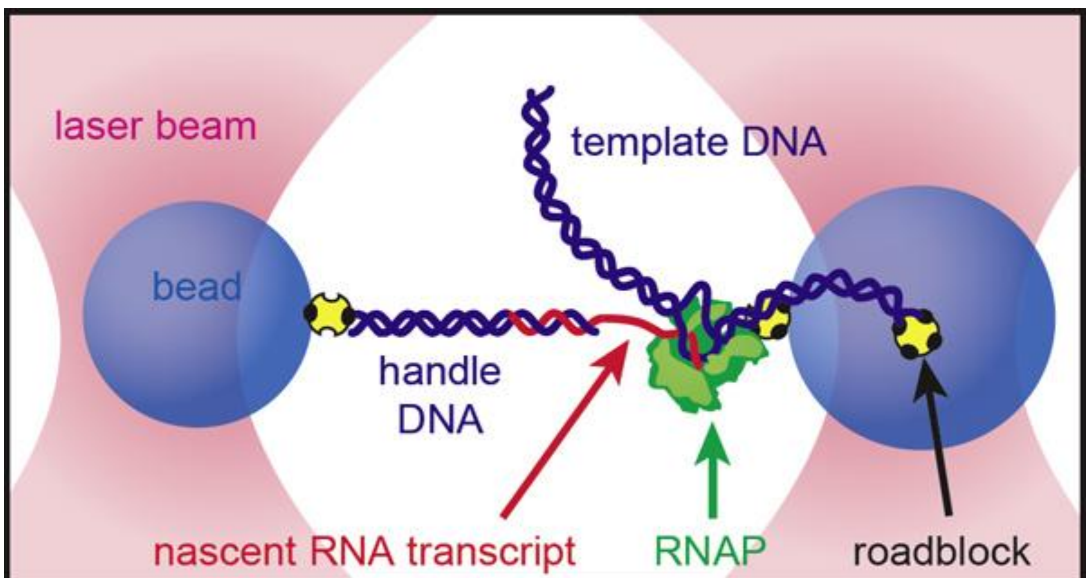
Northeastern University: Mark C. Williams group

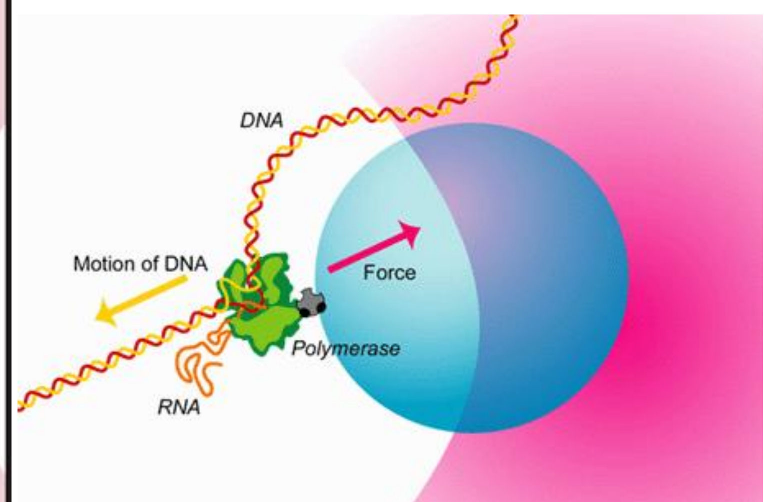
<u>Method:</u> Micron-sized glass beads are biochemically attached to the ends of DNA, optically trapped and moved away to stretch DNA

Molecular motors -RNAP

- RNAP (RNA polymerase) transcripts DNA
  - can move along the DNA at speeds 10 nucleotides per second and can support forces ~20pN

    (Yin et al. *Science*, 1995)
- <u>Method</u>: attach a single molecular motor (kinesin, myosin, RNA polymerase etc.) to a bead, probe motor properties

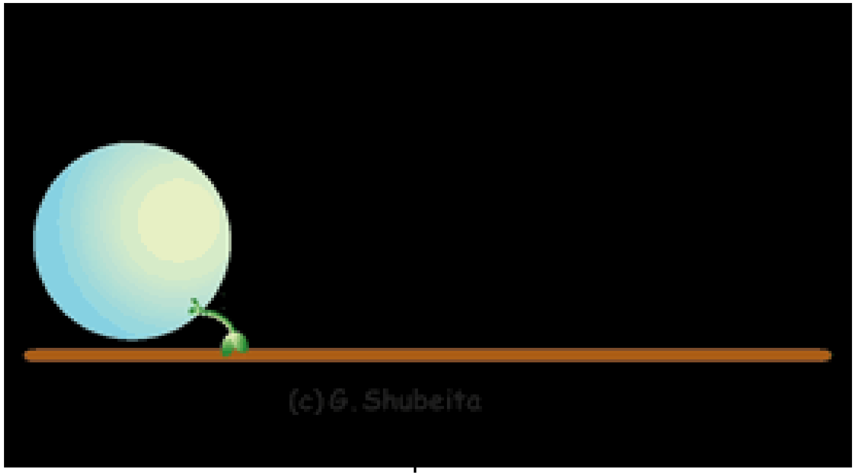


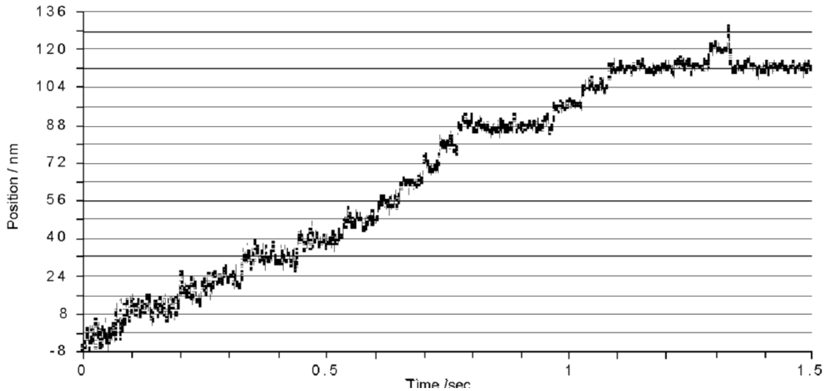


Stanford University: Block's group

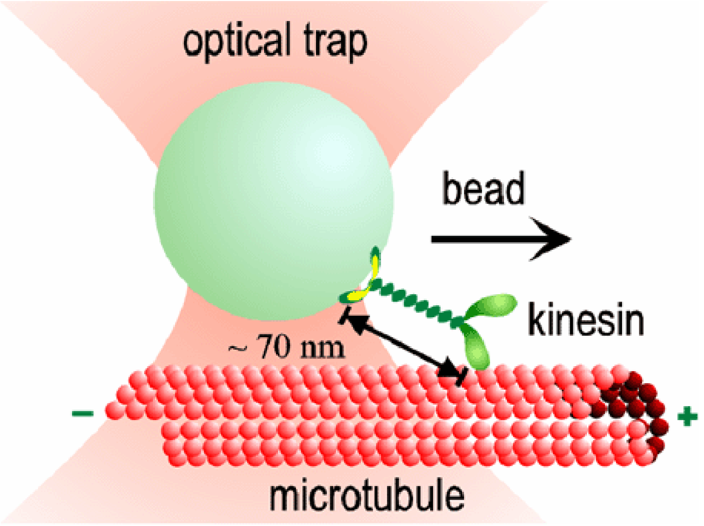
Molecular motors -Kinesin

- A kinesin motor protein carries a vesicle along a microtubule
- Kinesin walks along the microtubule with 8 nm steps, which corresponds to ~6pN force





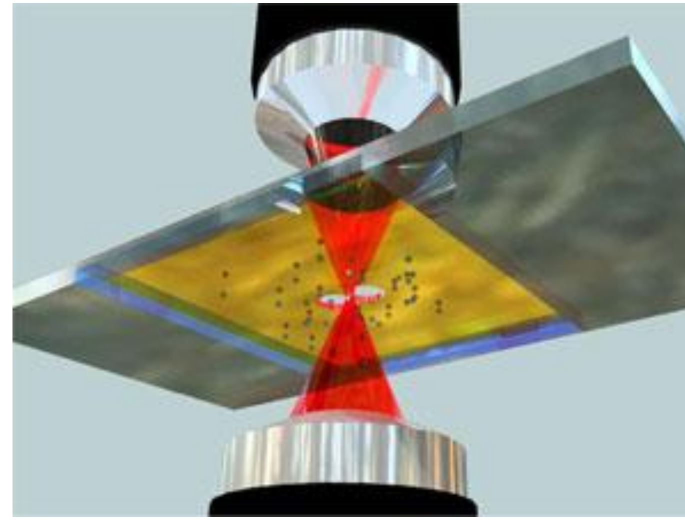
J. Molloy and M. J. Padgett, Contemp. Phys. 43, 241 (2002).
Block et al. *PNAS*, 2003.



Stanford University: Block's group

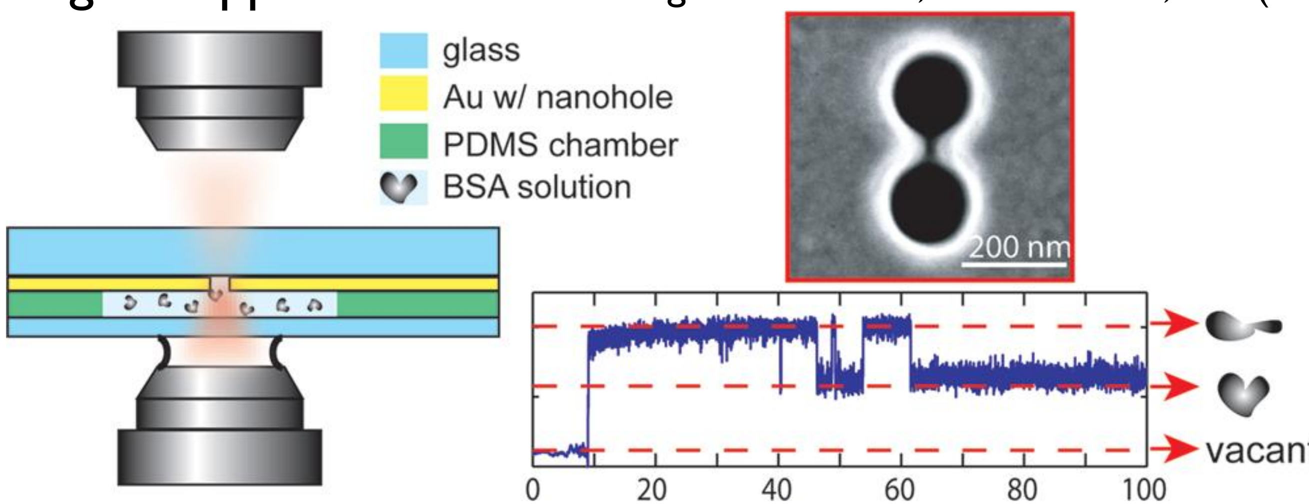
Nano-optical tweezers

- Nano-optical tweezers able to capture and analysis individual proteins, single-protein interactions with small-molecule drugs and DNA

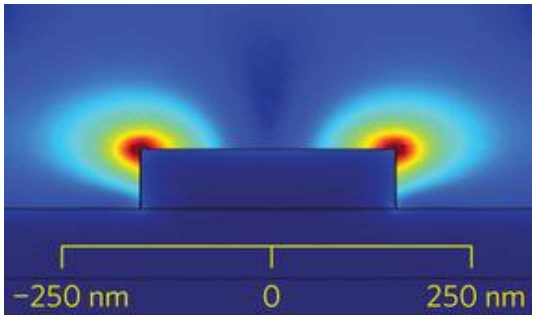


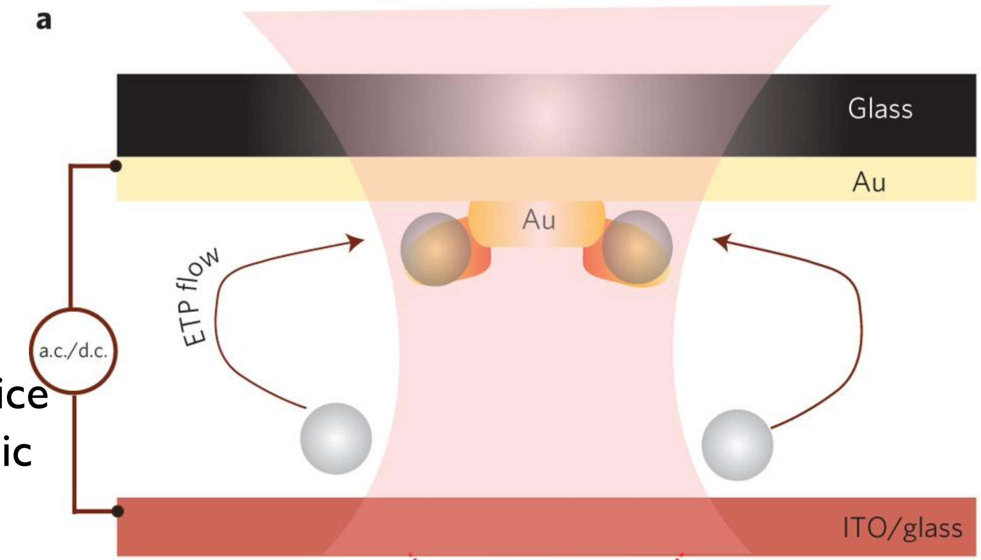
Y. Pang & R. Gordon, *Nano Lett* 12, 402 (2012)

- <u>Method:</u>
  - use double nanohole in a gold film
  - intense local field created at the tips
  - diffused molecule get trapped when it comes close

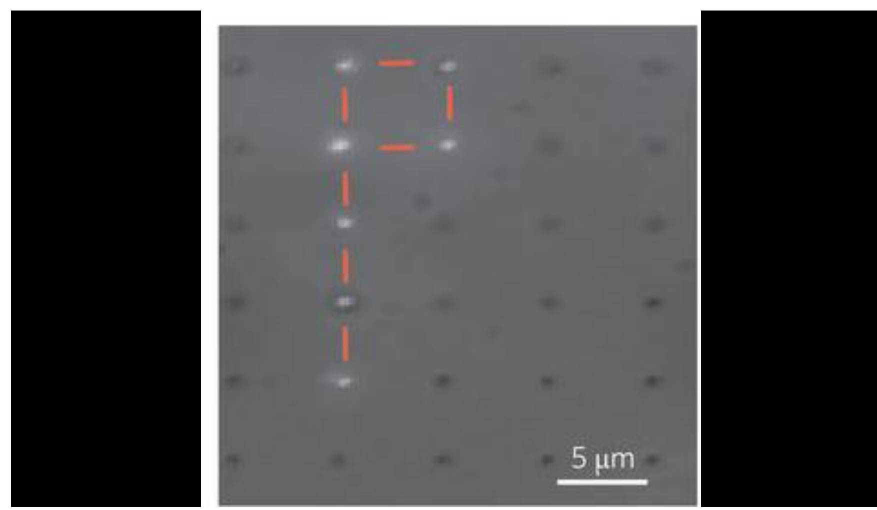


Plasmonic optical tweezers





- The electrothermoplasmonic (ETP) device can trap nano-objects to specific plasmonic nanoantennas
- The photo-induced heating of a nanoantenna in conjunction with an applied a.c. electric field can initiate rapid microscale fluid motion and particle transport.
- By applying d.c. field, the nanoobjects can be immobilized into plasmonic hotspots.

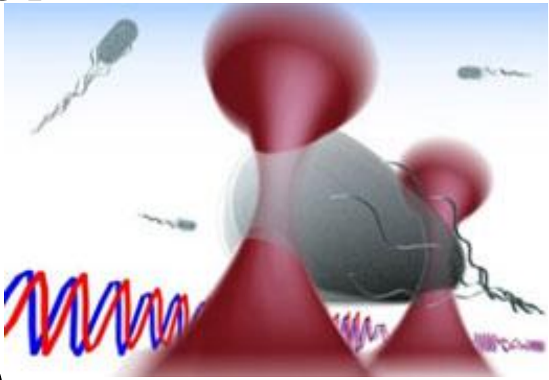


J. C. Ndukaife, et. al., *Nature Nanotech.* **11**, 53-59 (2016)
Y. Tsuboi, *Nature Nanotech.* **11**, 5-6 (2016)

Bacteria-Drug Interaction

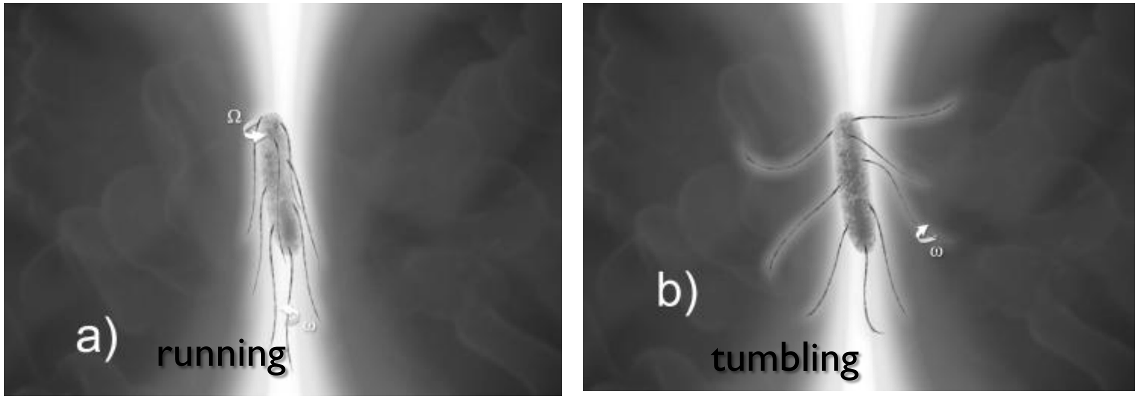
- Each year in the US, 2 million people become infected with bacteria that are resistant to antibiotics
- Need: find quickly proper drug for bacteria

- Chemotaxis - the movement of an organisms in respond to a chemical stimular (running & tumbling)



T. L. Min et al, *Nature Methods* **6**, 831 (2009)

When a bacterium senses an attractant gradient, its runs become longer as the number of tumbles decreases, such that the cell migrates up the gradient



I.A. Martinez et al, *Plos One* **8** (2013)

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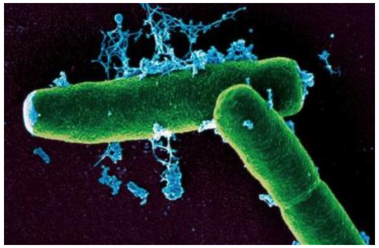
- **Tug-of-War optical tweezers to study rod-shaped bacteria and biofilms**

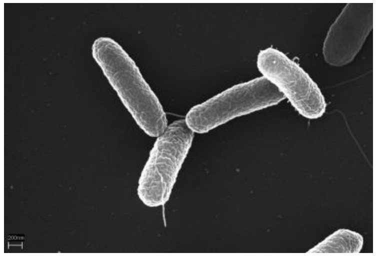
Motivation

- CDC report: 6 out of 10 of the most viral bacterial infections mediated by rod-shaped bateria[1]
- Mechanism of bacteria-drug interaction is not well understood



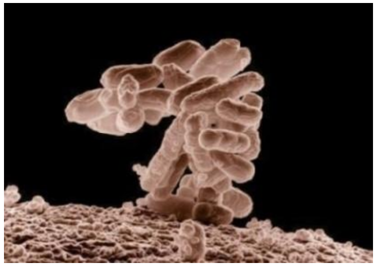
Bacillus anthracis[2]

Need easy methods for stable in-plane trapping of a *single* bacterium



Salmonella[3]

- Bacteria have tendency to aggregate and form biofilm
- Clustered bacteria have higher tolerance to antibiotics

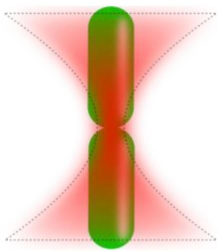


E. coli[4]

Need new methods to understand biofilm formation and strength

1 *Antibiotic resistance threats in the United States, 2013* http://www.cdc.gov/drugresistance/threat-report-2013
2 Kenyon College
3 Volker Brinkmann, Max Planck Institute for Infection Biology, Berlin, Germany
4 Courtesy of USDA-Agricultural Research Service

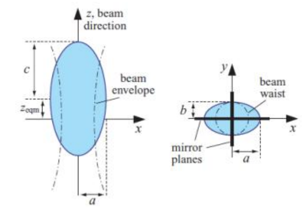
Observing complex shaped bacteria



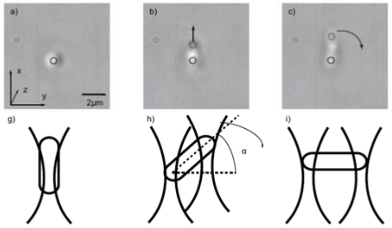
- In 1987 Ashkin introduced optical tweezers to trap and manipulate bacteria

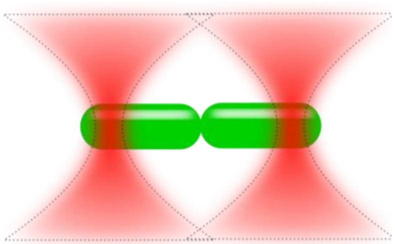
Ashkin A. , *Science* **235** , 1517 (1987)

- Bacteria prefer to be aligned along the axis of a trapping beam



S. Simpson, Phys. Rev.A 84,053808 (2011)

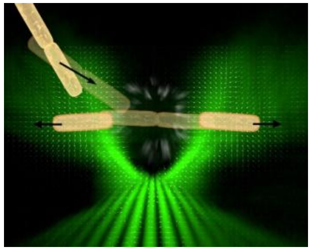


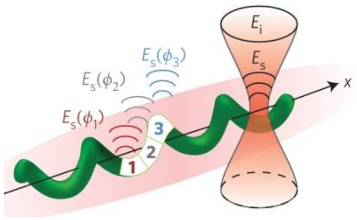


- Use multi-beam trapping: bacteria can be reorientated in space, but needs independent control

Horner F., J. Biophoton. 3, 468 (2010)

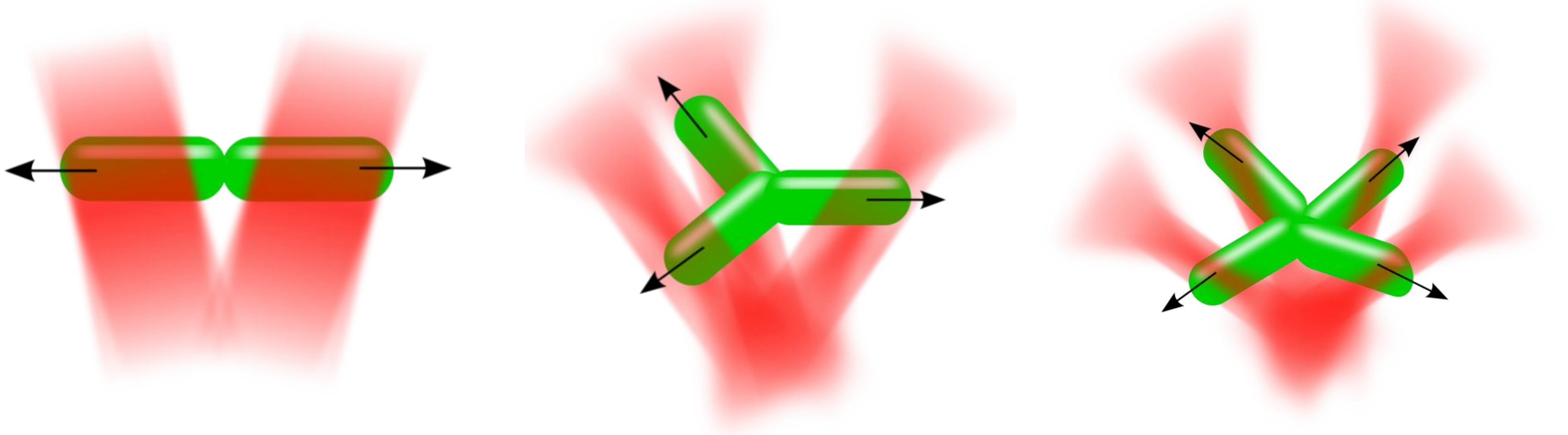
- Create object-adapted optical potentials for stable trapping and orientation of anisotropic samples





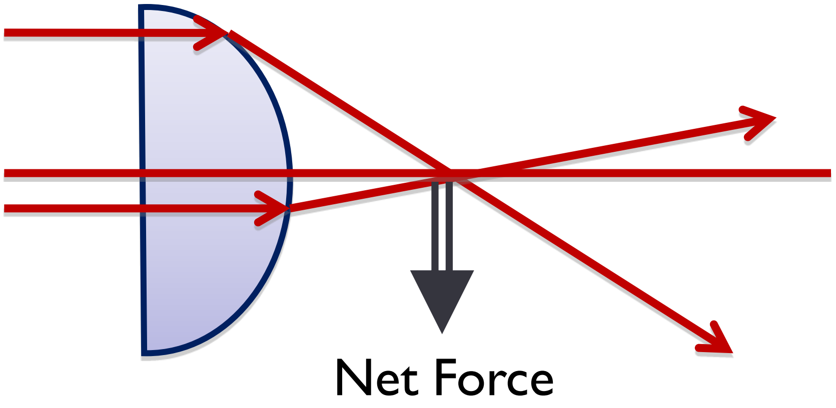
Bezryadina A., Light: Science & Applications 5, e16158 (2016)
Lamstein J. Chin. Opt. Lett. 15, 030010(2017)
Koch M. Nat. Photonics 6, 680(2012).

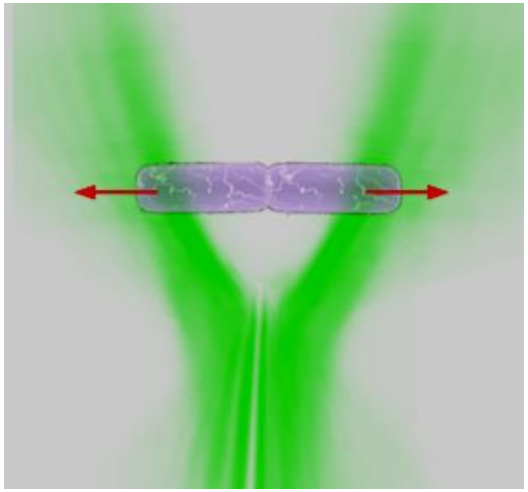
We have designed optical tweezers with lateral pulling forces to control rod-shaped and complex geometric shapes biological samples



Basic idea

By offsetting the lens from the beam path, the beam shape changes, resulting in net transverse momentum

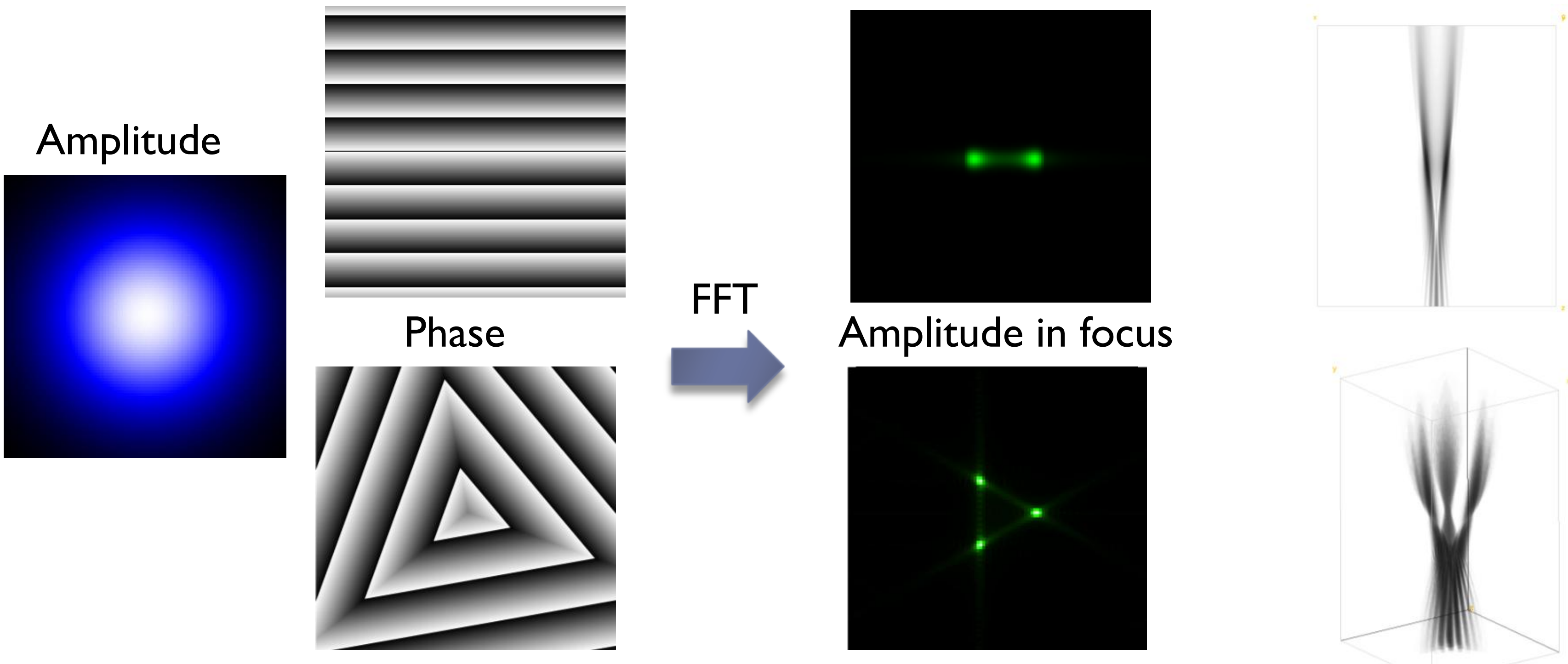




By creating a pair or multiple beams with opposite transverse momenta, rod-shaped or complex-shaped objects can be held and stretched

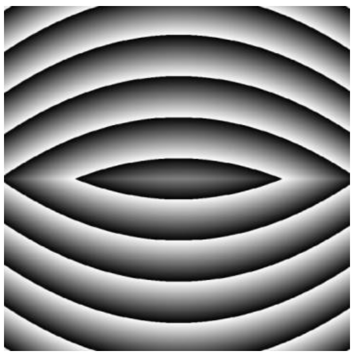
Beam design

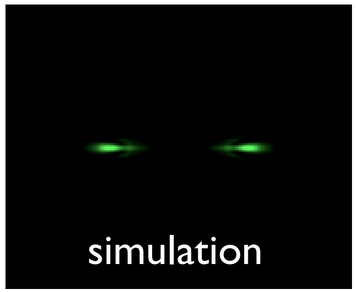
- Beam can be split into 2, 3 or more lobs with SLM



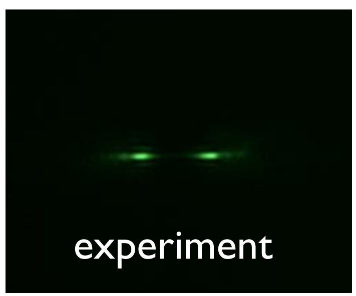
- In order to achieve strong transverse momenta and strong intensity gradients we require beams with parabolic trajectory.

Dual Tug-of-War beam

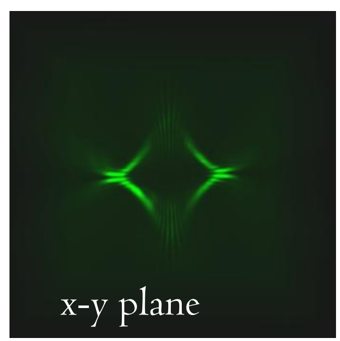


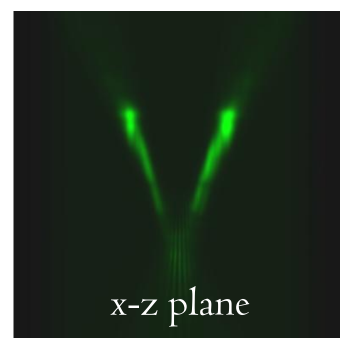


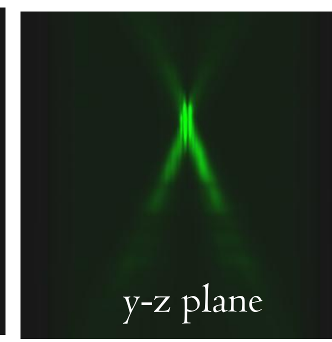
Amplitude in focus



3D volumetric beam construction from experimental data

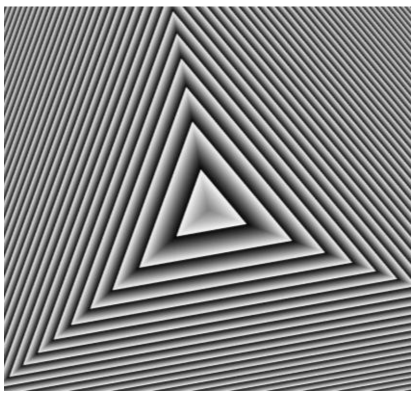


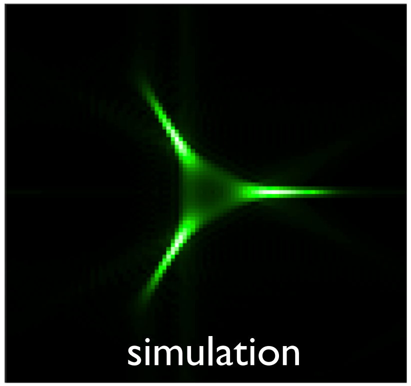




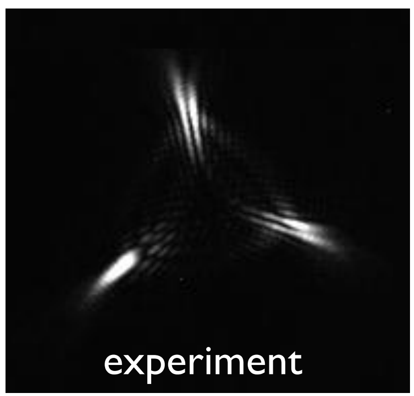
Lamstein J. Chin. Opt. Lett. 15, 030010 (2017)

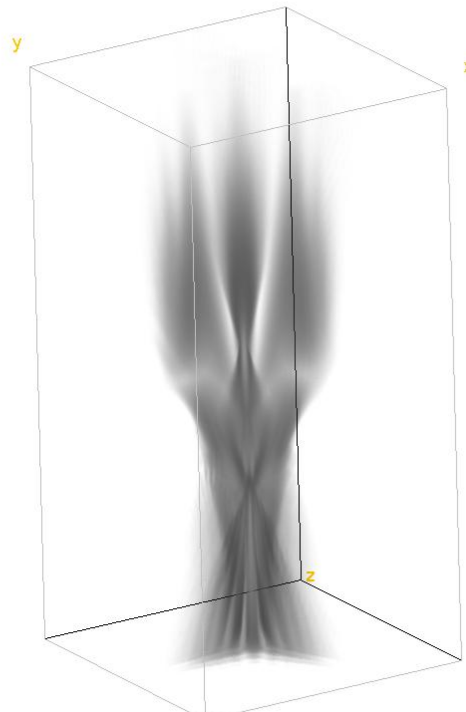
Triangular Tug-of-War beam





Amplitude in focus





Volumetric image calculated via beam propagation method

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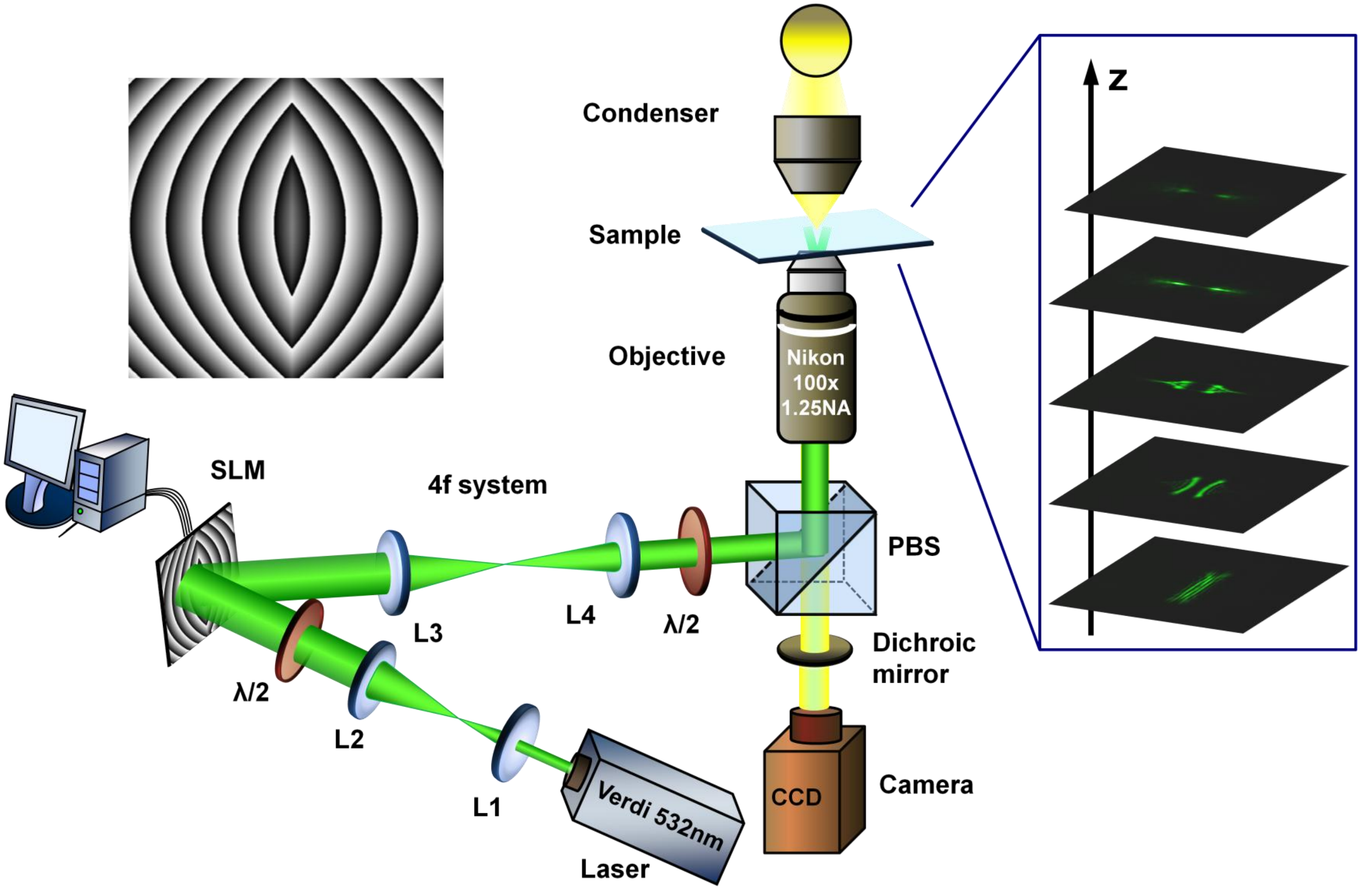
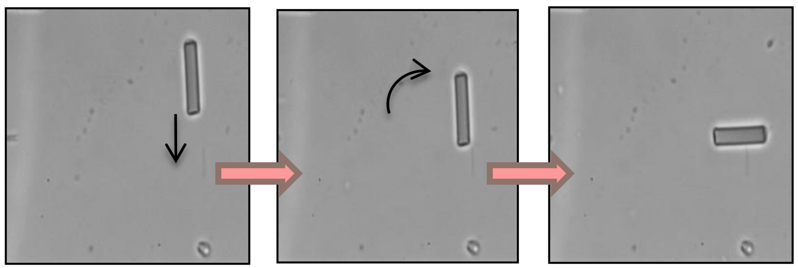
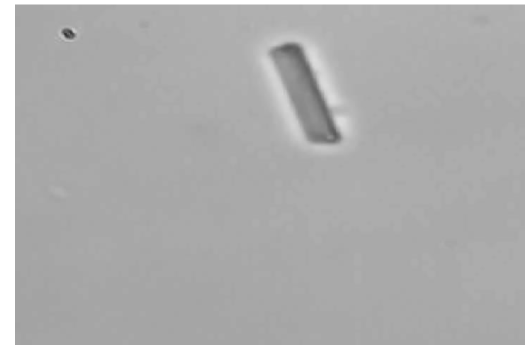
Optical tweezers setup



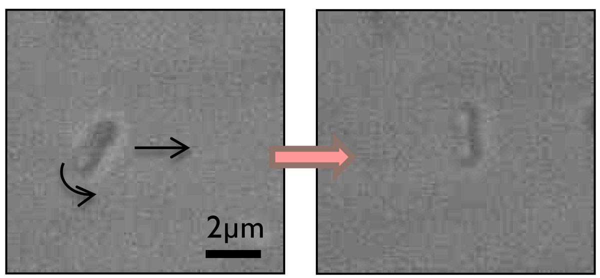
Dual TOW beam: Manipulation of different size and morphology objects

Silica rod
(size ~2μm)

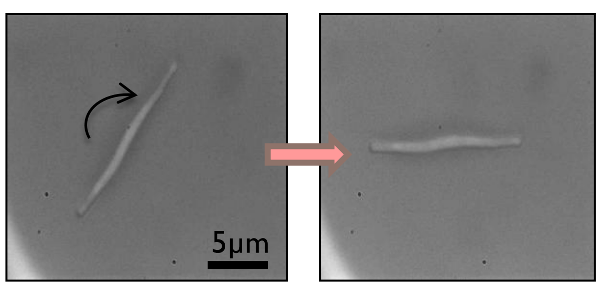


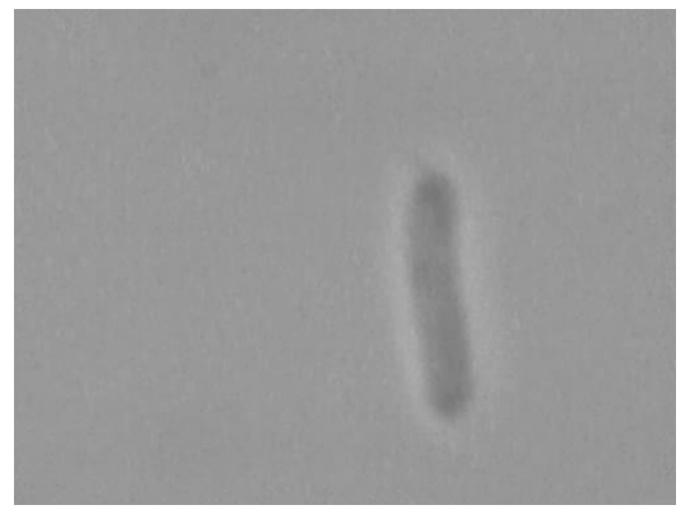


E. coli
(size ~2μm)



Long *Bacillus thuringiensis*
(size ~15μm)





Bacillus thuringiensis
(size ~5μm)

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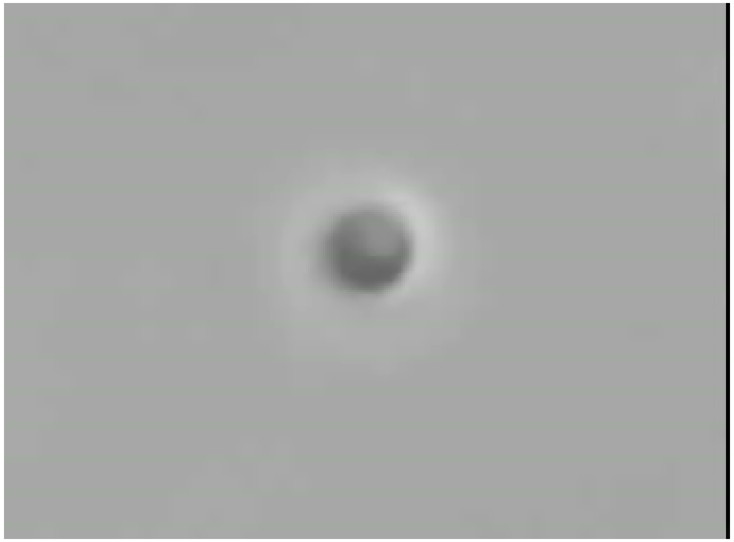
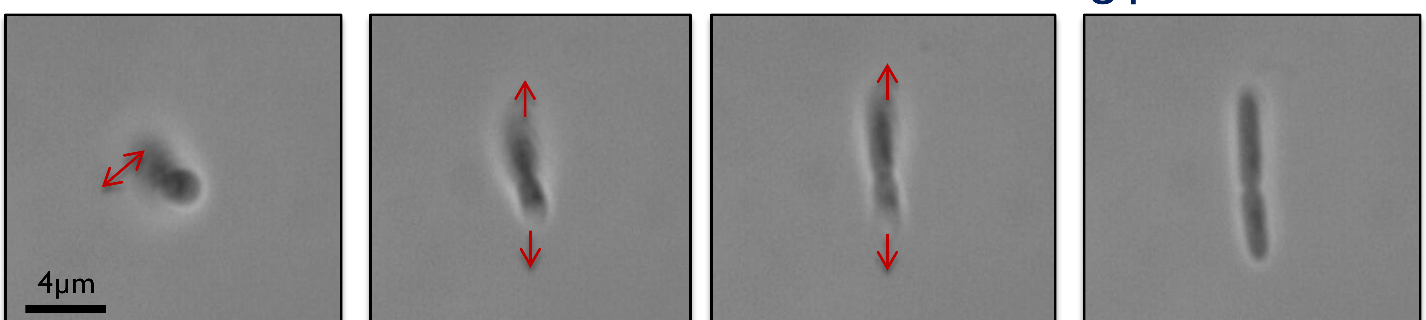
Dual Tug-of-War beam: In-plane trapping and stretching



Bacillus thuringiensis
at 7mW laser power

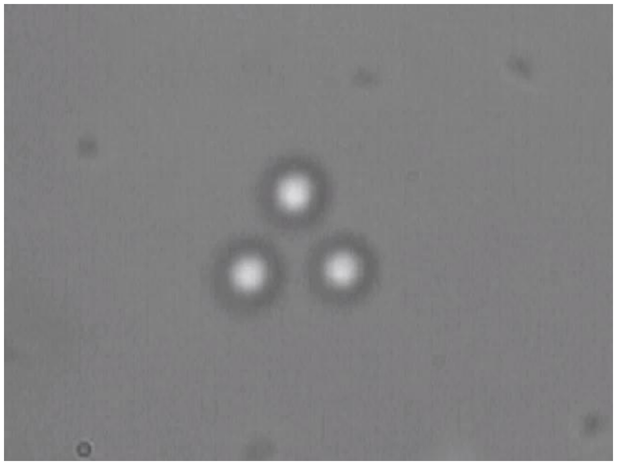
- A bacterium is trapped at one end, then self-reorients to be trapped from both ends
- After trapping, a bacterium is stretched
- The stretching forces are controlled by
  - Diverging angle between two stripes
  - Changing length/width of stripes
  - Increasing power of the beam



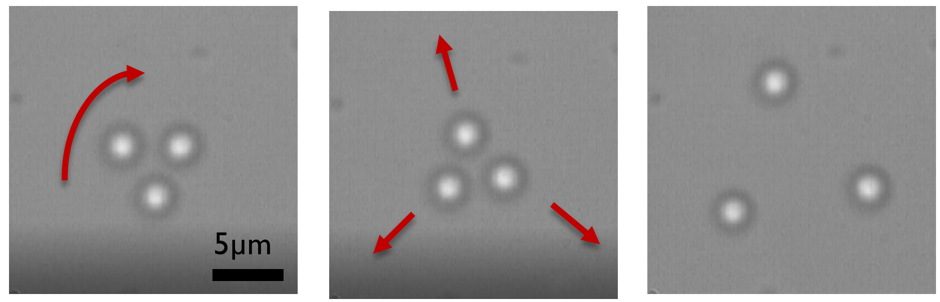
Bezryadina A., Light: Science & Applications 5, e16158 (2016)

Triangular Tug-of-War beam: Trapping with 3-fold symmetry

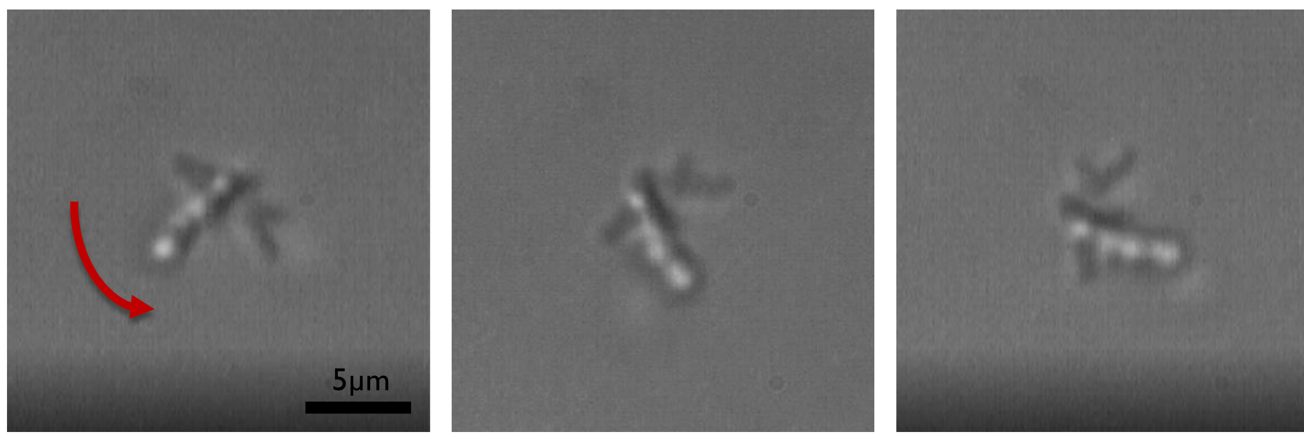
- 3 polystyrene spheres trapped by each lobe of the tweezers
- Can be rotated and moved radially



Polystyrene beads
(size ~3μm)

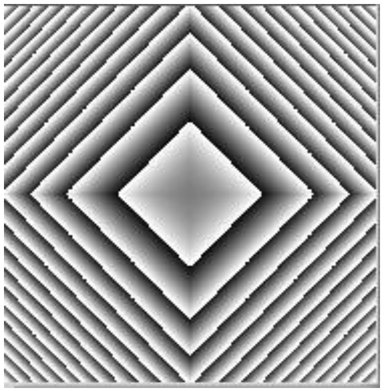


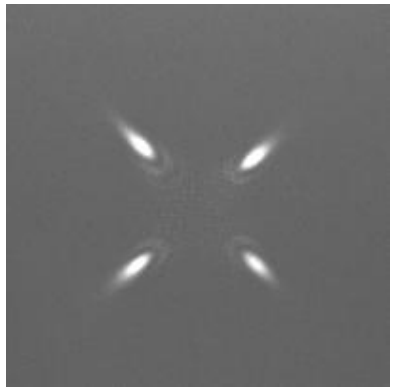
Mutant multipronged
S. meliloti bacterial cell

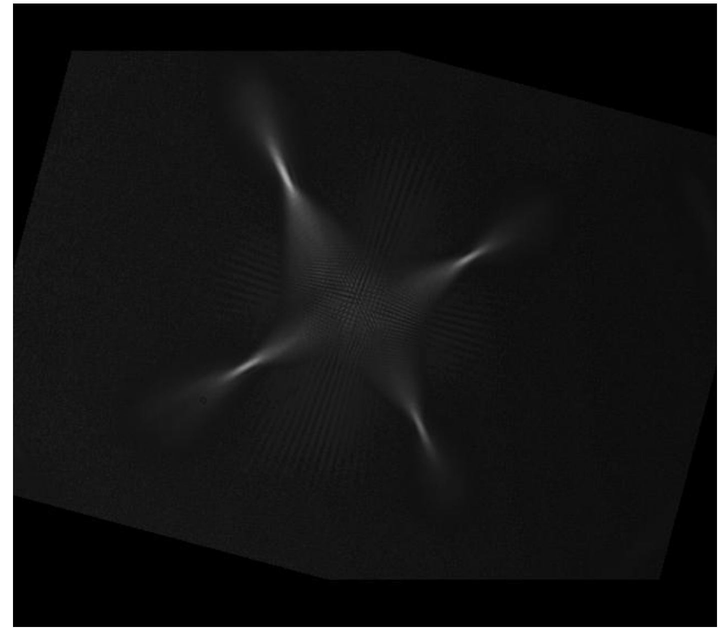


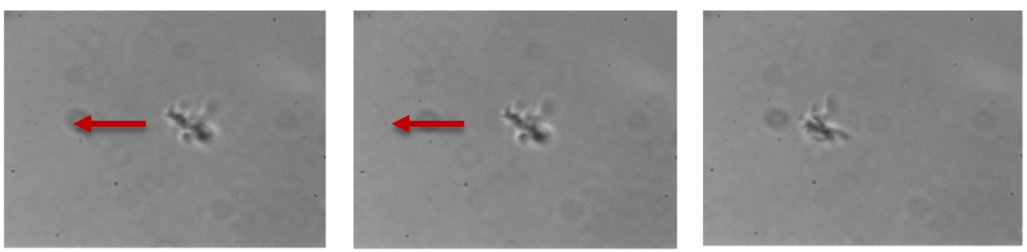
Lamstein J. Chin. Opt. Lett. 15, 030010 (2017)

Square Tug-of-War beam:



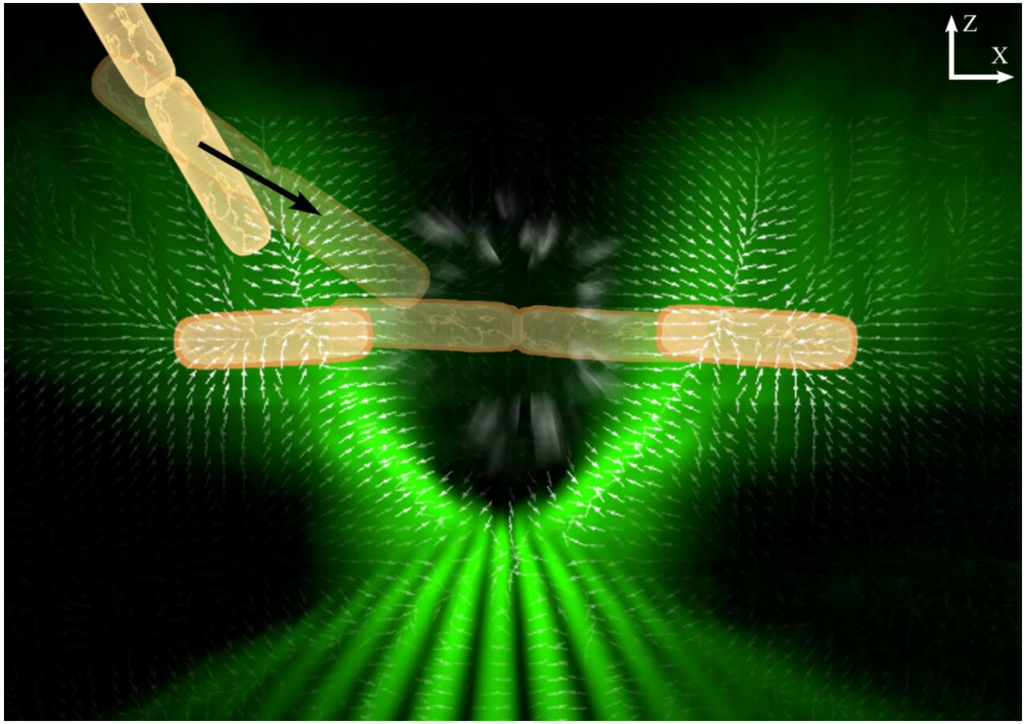






Mutant multipronged
S. meliloti bacterial cell

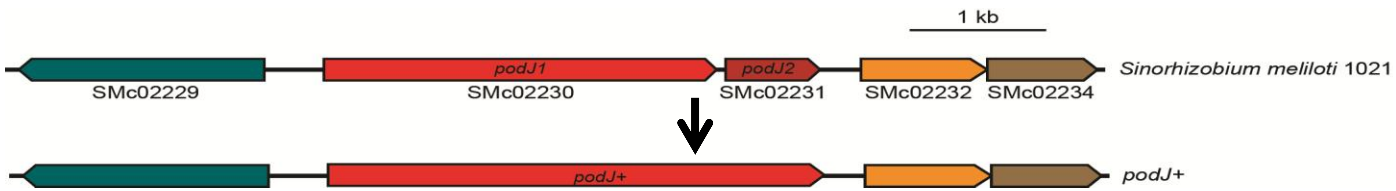
Could we stretch bacteria and break apart bacterial clusters with "Tug-of-War"



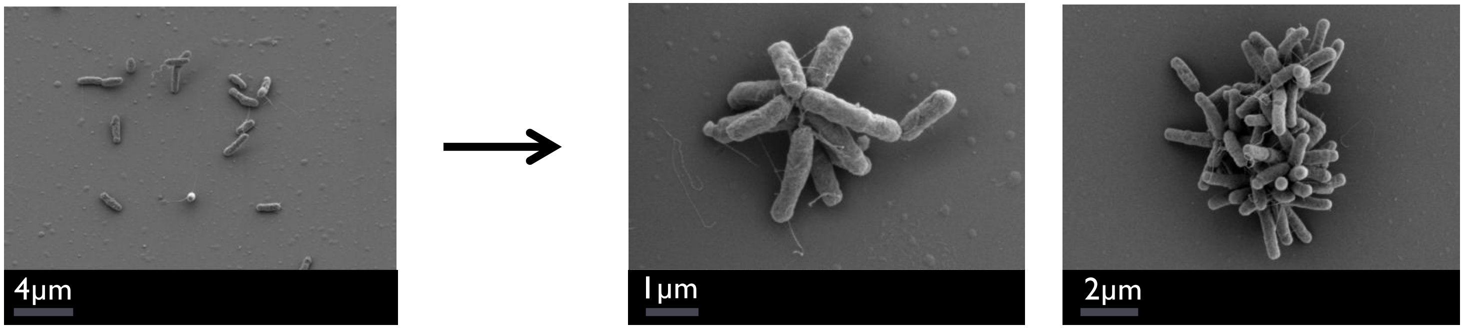
A. Bezryadina, *Opt. & Photonics News*, Dec. 2016

S. meliloti bacterial clusters

- podJ^{+} gene modification in *S. meliloti* causes biofilm formation.

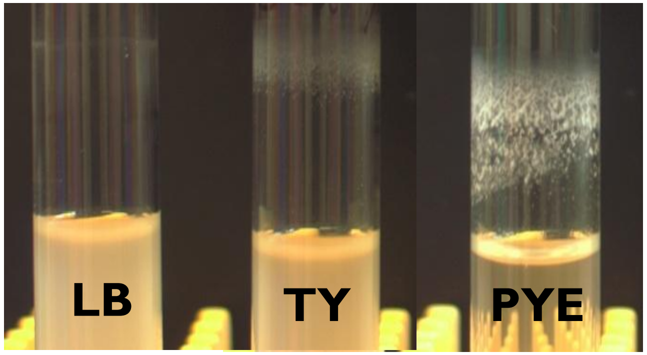


- podJ^{+} gene causes production of "glue" on one side of the bacteria.



- This biofilm formation depends on media (nutrition and growing environment).

- Tug-of-War tweezers can help to quantify strength in different media.



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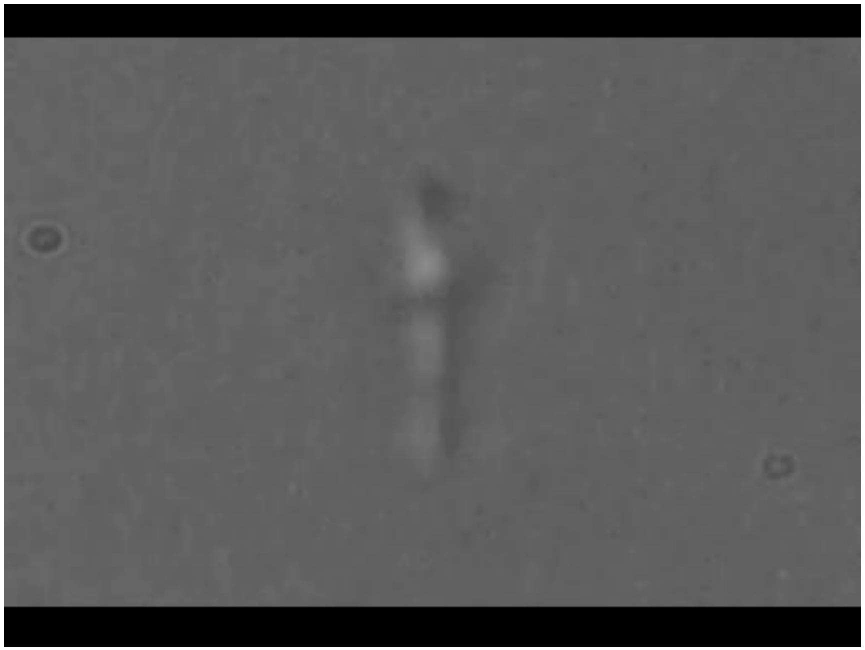
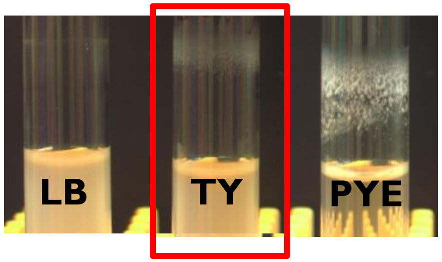
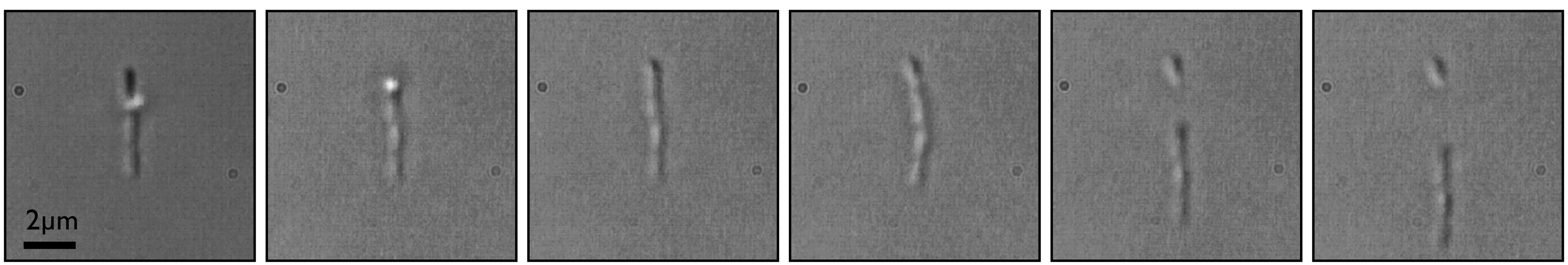
Dual Tug-of-War beam: *S. meliloti* clusters in TY media





- "Tug-of-War" tweezers generates a constant pulling force from two ends of a trapped bacterial cluster
- The *S. meliloti* cluster is stretched and eventually broken apart



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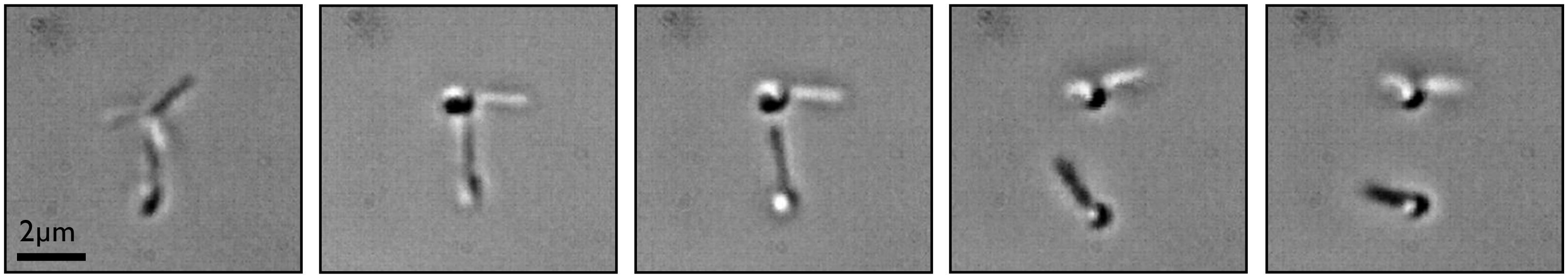
Multi-branched *S. meliloti* clusters in TY media



- "Tug-of-War" can be used on a broad range of cluster shapes
- 15-20mW laser power is applied to break apart bacterial clusters in TY media

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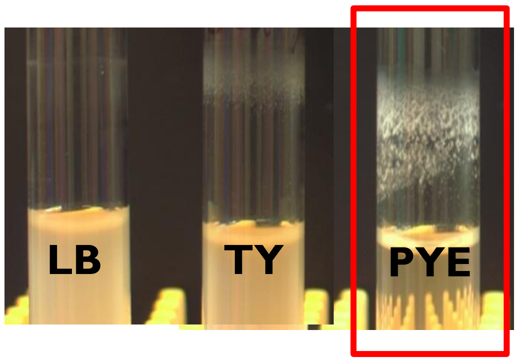
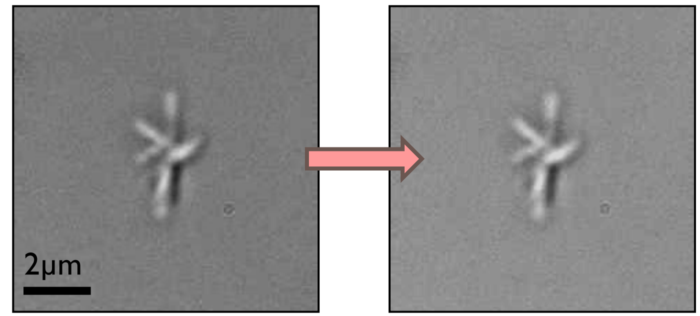
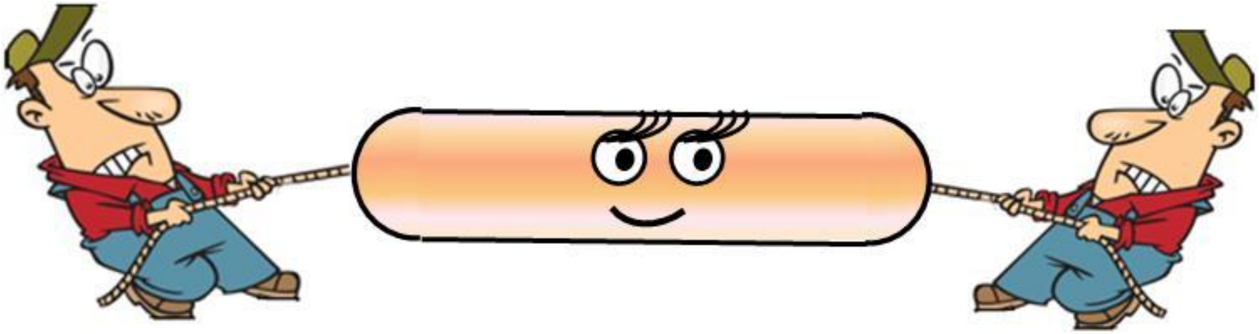
Dual Tug-of-War:
S. meliloti clusters in PYE media

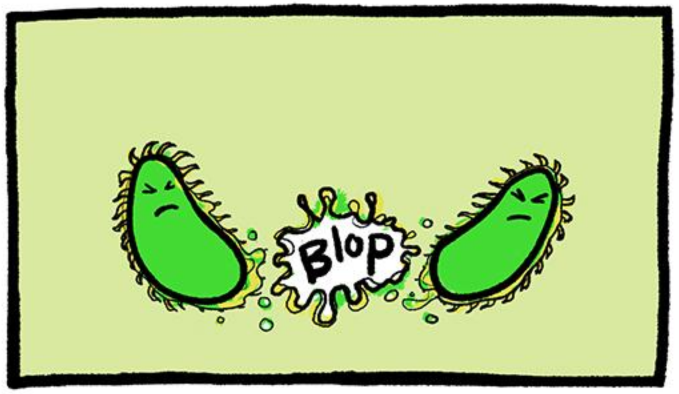




- Glue produced in PYE media is too strong for optical tweezers
- Consistent with strength of biofilm formation in PYE and TY media

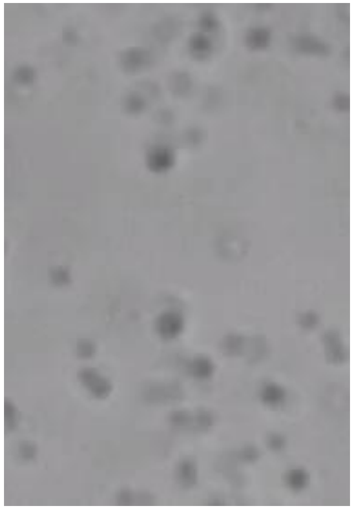


What are the pulling forces from "Tug-of-War"

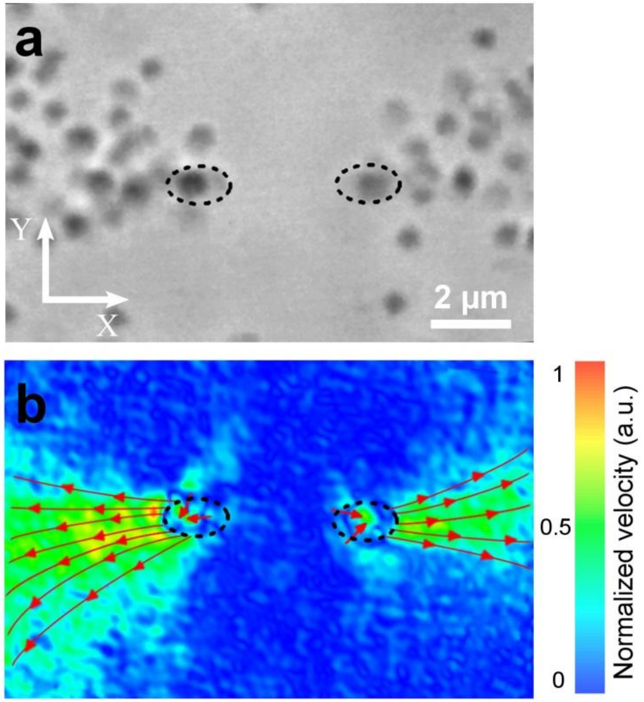


http://alaskarobotics.com/2013/04/01/bacteria-breakups/

Particle flow method: magnitude and direction of momentum



- Use very thin sample with high concentration 500nm polystyrene beads
- Particles are pushed in two opposite directions away from the center due to the pulling force



- Use particle flow dynamics analysis to estimate forces:

The time averaged velocity of the particle flow illustrates the mean momentum distribution

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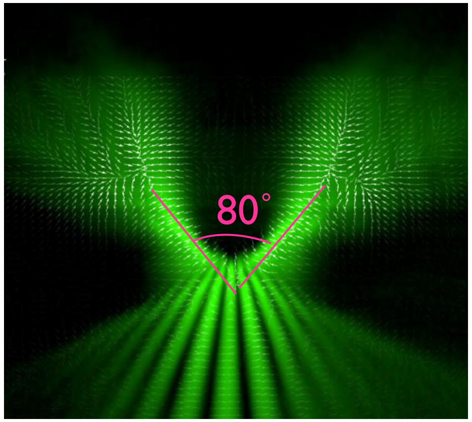
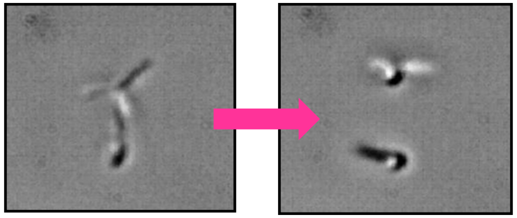
Transverse optical forces

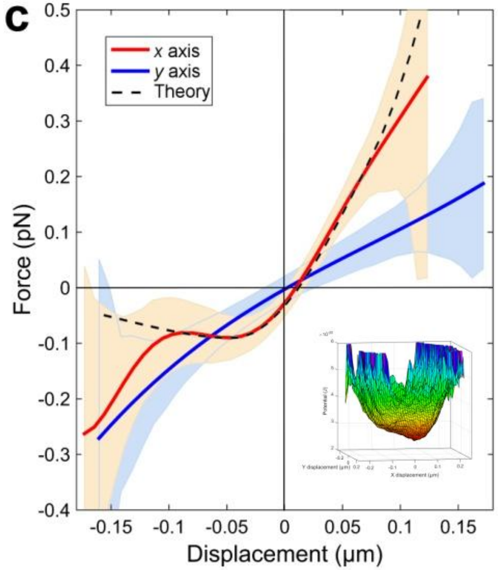


2 beams propagate at angle
→ transverse optical forces



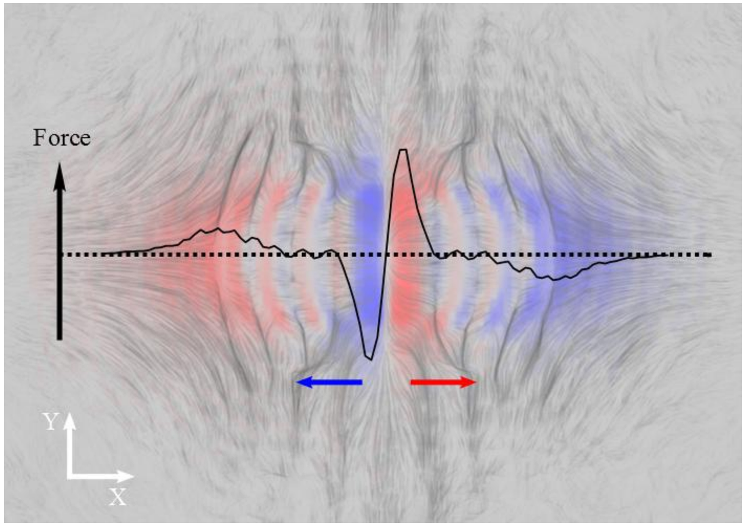
Scattering and gradient forces work together.

Theoretical force profile: created via generalized Lorenz-Mie theory for 1-μm 'bacterium-like' particle



Experimental trapping force resulting from only one arm of the TOW tweezers (use optical potential analysis)

The peak force for a ~ 1.5μm rod-shaped cell is estimated to be at least 5 pN



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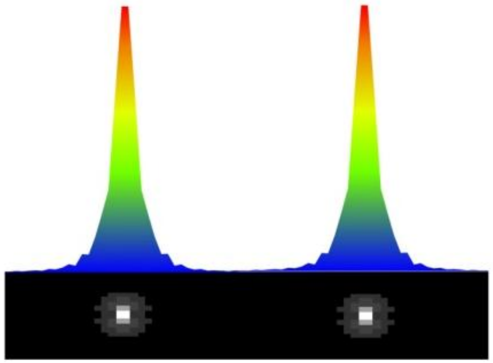
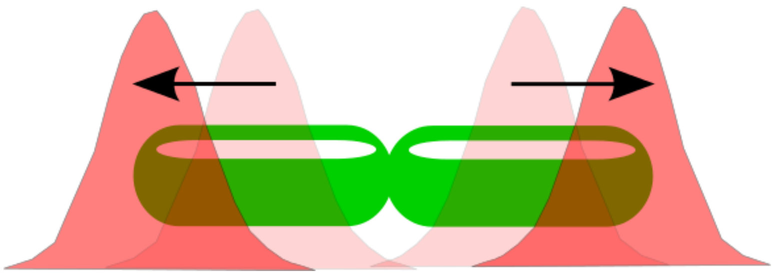
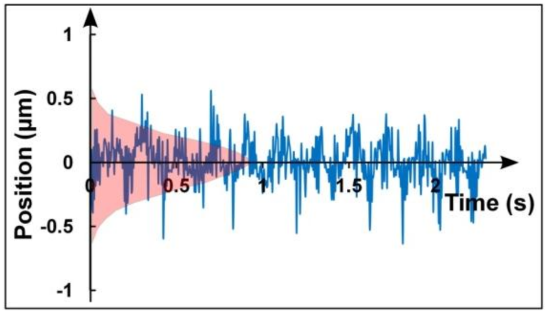
Comparison:
Separate Gaussian beams vs Tug-of-War

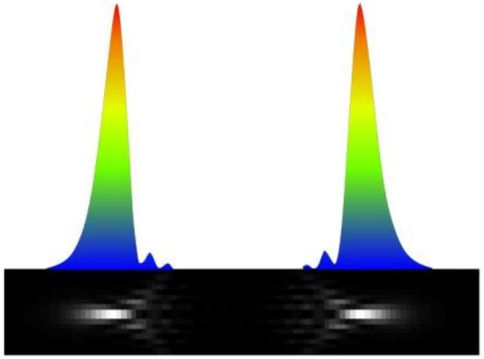
Two Gaussian beams

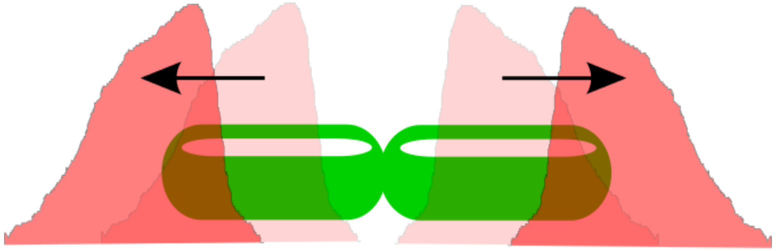


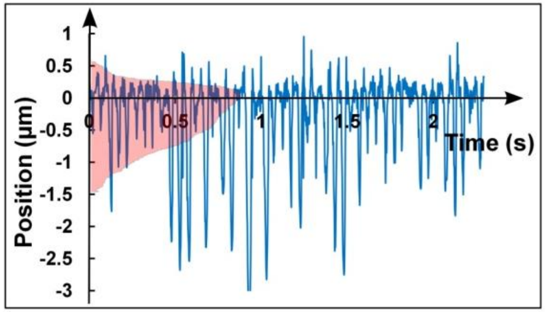




"Tug-of-War" beam







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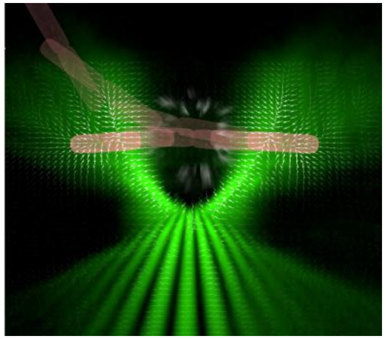
Intensity profile sharpness on bacteria side

→ **strong stiffness**

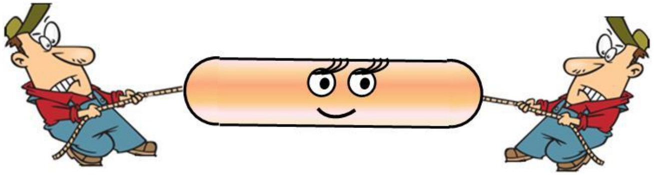
→ **better stability in "Tug-of-War" beam**

"Tug-of-War" Summary:

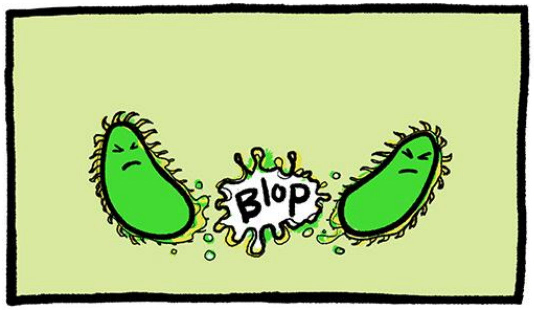


A Tug-of-war tweezers can

- Hold and self-align rod-shaped bacteria in plane
- Use low laser power
- Stretch bacteria with gentle forces without attaching beads
- Apply controllable lateral forces
- Break apart clusters of bacteria

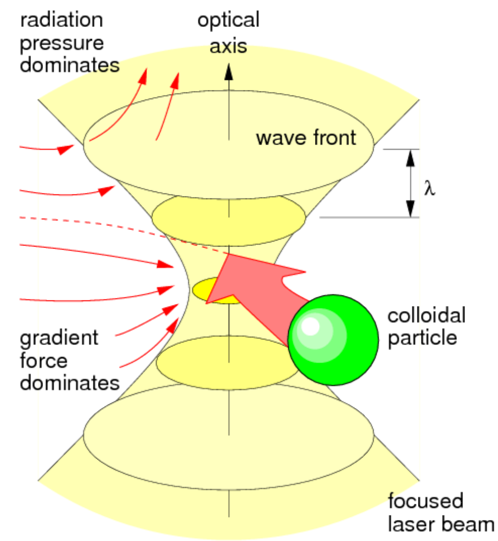


This method can be applied for other biological samples



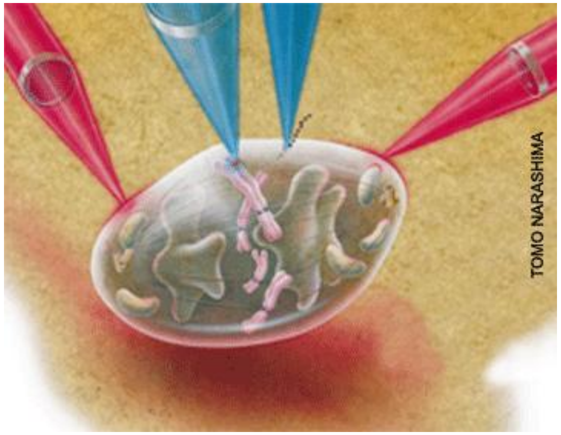
Optical Tweezers Summary:

- Optical Tweezers use radiation pressure from a focused laser beam to manipulate microscopic objects as small as a single atom.



- It can trap and control objects in size 10nm-100mm, detect and apply forces in the range of 0.1 – 100pN, measure displacements in nm range.

- Widely used in biology and medicine to understand physical properties and responds of microorganisms.



Thank you!

OSA Foundation

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