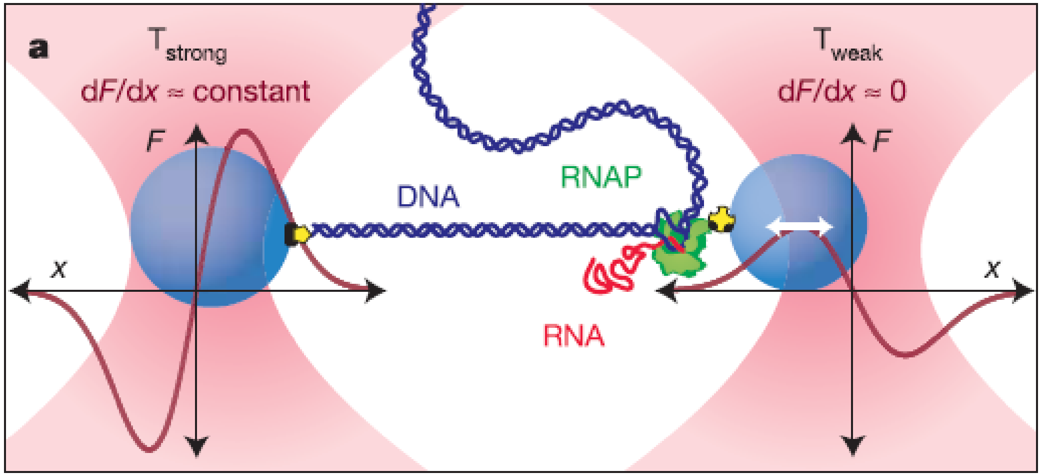
Optical Tweezers

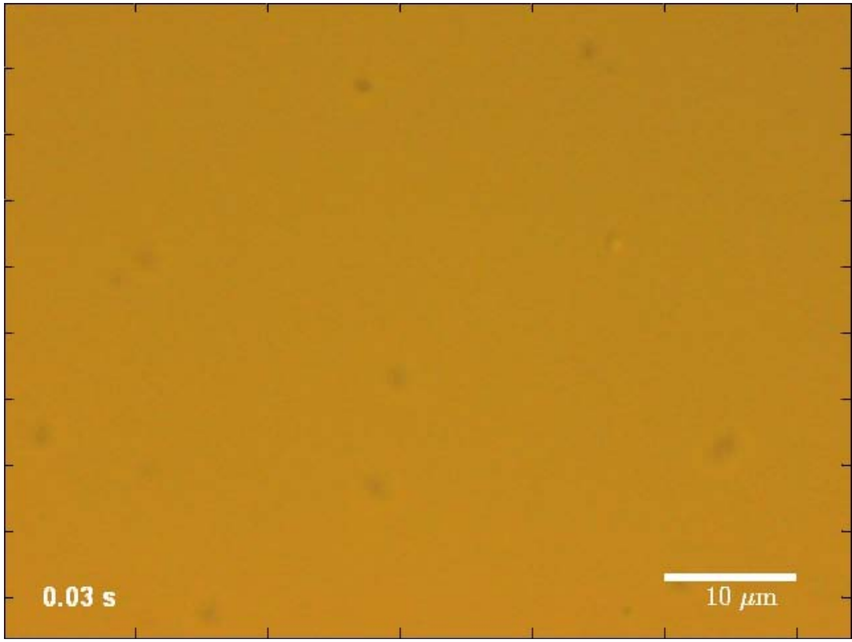
(aka. Optical traps, laser tweezers, photonic force microscope, etc.)

- Trapping and applications
- Principles
- Design
  - Layout
  - Trapping laser
  - Objective
- Position control
  - Stage motion
  - Mirrors / AODs / Holograms
- Position detection
- Calibration
  - Position calibration
  - Force calibration
- Examples

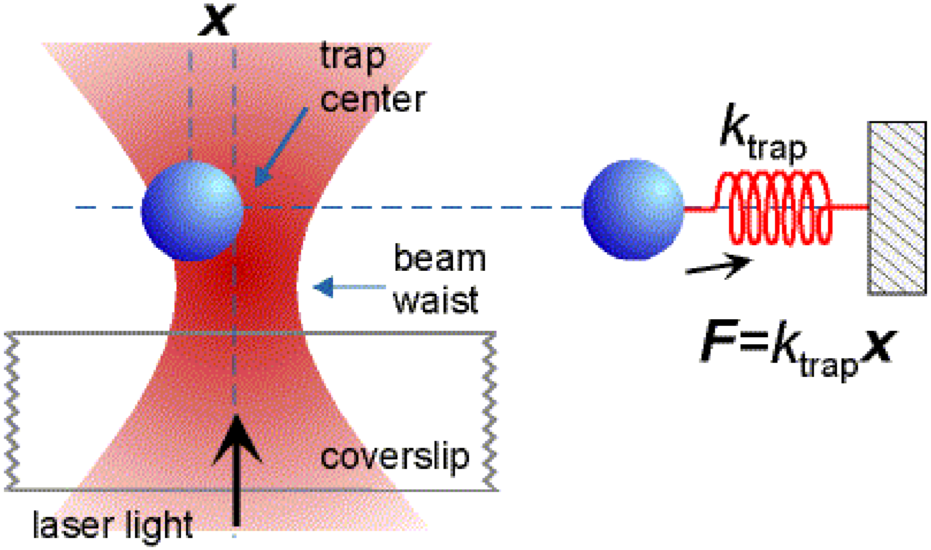


What are Optical Tweezers(OT) ?

- Optical Tweezers = Focused Laserbeam
- OT works by transfer of momentum
- Particles with higher n than surrounding medium are trapped in an approximately harmonic potential

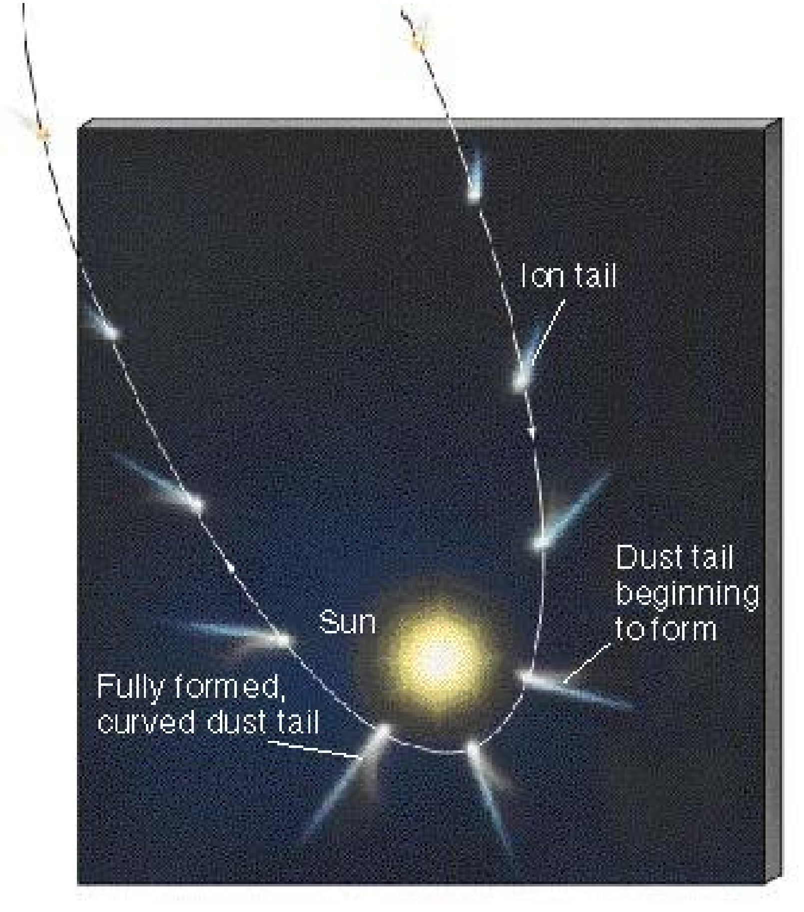


$$k \approx 0{,}001 - 0{,}1\,\mathrm{pN/nm}$$



Principle

- Photons carry momentum $p = h/\lambda$
- Change in momentum corresponds to force $F = dp/dt$
- Sunlight on earth 0.5 nN/cm^2
- Laser pointer ~10 pN
- Comet tail:



History

- Arthur Ashkin at Bell labs (Steve Chu nobel prize in 1997)
- Theory 1970 (PRL 1970 **24**), Experiment in 1986 (Optics letters **11** 1986)
- 3D optical spring
- Trap objects of 10 nm to 10 um

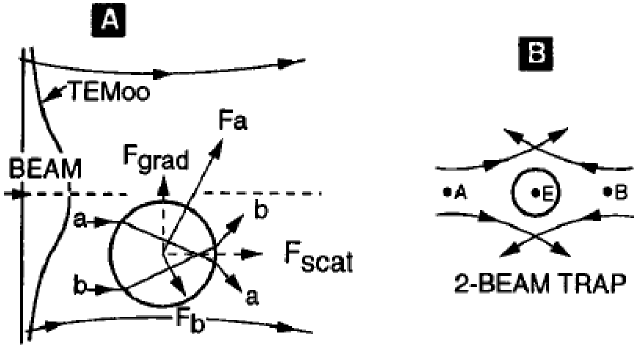


FIG. 1. (*A*) Origin of F_{scat} and F_{grad} for high index sphere displaced from TEM_{00} beam axis. (*B*) Geometry of 2-beam trap.

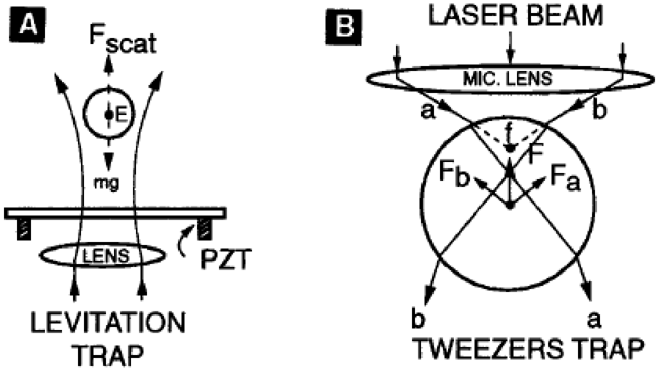
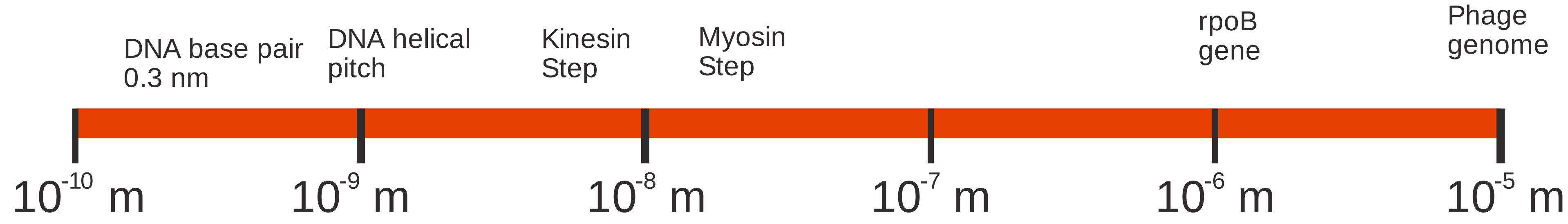


FIG. 2. (*A*) Geometry of levitation trap. (*B*) Origin of backward restoring force F for sphere located below tweezers focus *f*.

Length scales

Biological length scales



DNA base pair
0.3 nm

DNA helical
pitch

Kinesin
Step

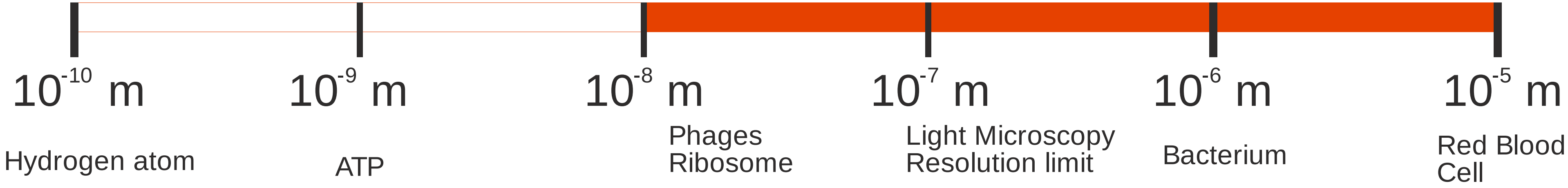
Myosin
Step

rpoB
gene

Phage
genome

10^{-10} m 10^{-9} m 10^{-8} m 10^{-7} m 10^{-6} m 10^{-5} m

Trapped particles



10^{-10} m 10^{-9} m 10^{-8} m 10^{-7} m 10^{-6} m 10^{-5} m

Hydrogen atom

ATP

Phages
Ribosome

Light Microscopy
Resolution limit

Bacterium

Red Blood
Cell

Time and force scales

Time

protein domain motions

DNA Helix unwinding

Enzymatic reactions

Protein synthesis

cell division

Force (pN)

DNA BS transition

Weight of 3um bead

Myosin V stall

Kinesin stall

RNAP stall

Phage packaging motor stall

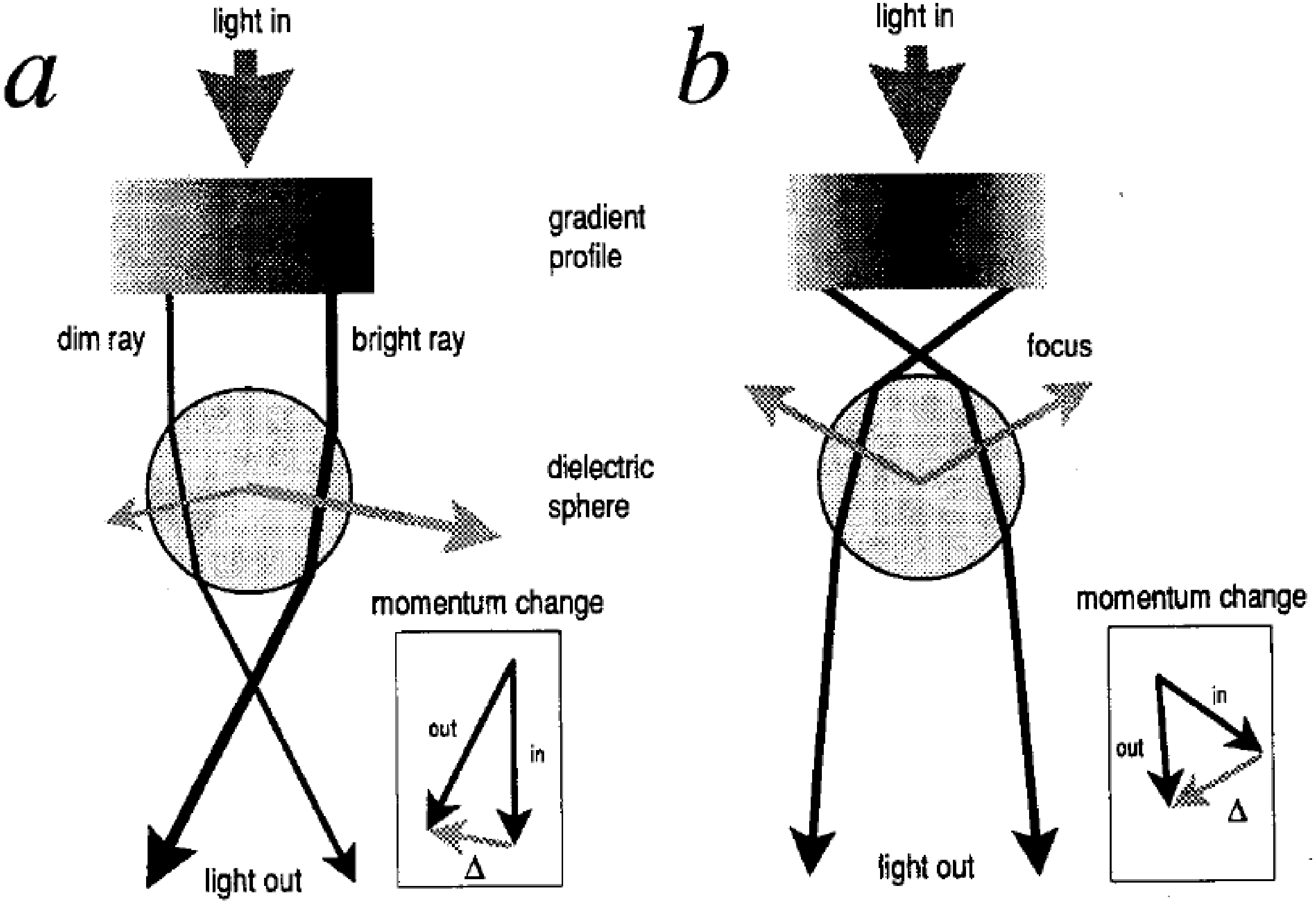
antibody antigen interaction

covalent bond

Theory
Ray-optics

- Ray-tracing to determine change in momentum

- Web-demo!



Theory
Rayleigh regime

- Particle size a << λ
- Dipole induced by optical field
- Induced dipole interacts with inhomogenous E-field near focus
- 1. Gradient force
  - Force depends on cube of radius
  - Ratio of indexes of refractions
- 2. Scattering force

Theory
Intermediate regime a ~ lambda

- Most interesting trapped particles are ca 0.1lambda – 10 lambda
- Neither point dipole nor ray optics approach give good results
- More complete solutions
  - Generalized Lorenz-Mie theory (GLMT): Barton etl al. 1989
  - Second order Born scattering: Rohrbach & Stelzer, JOSA-A (2001) **18**

$$F = \frac{Q n_m P}{c}$$

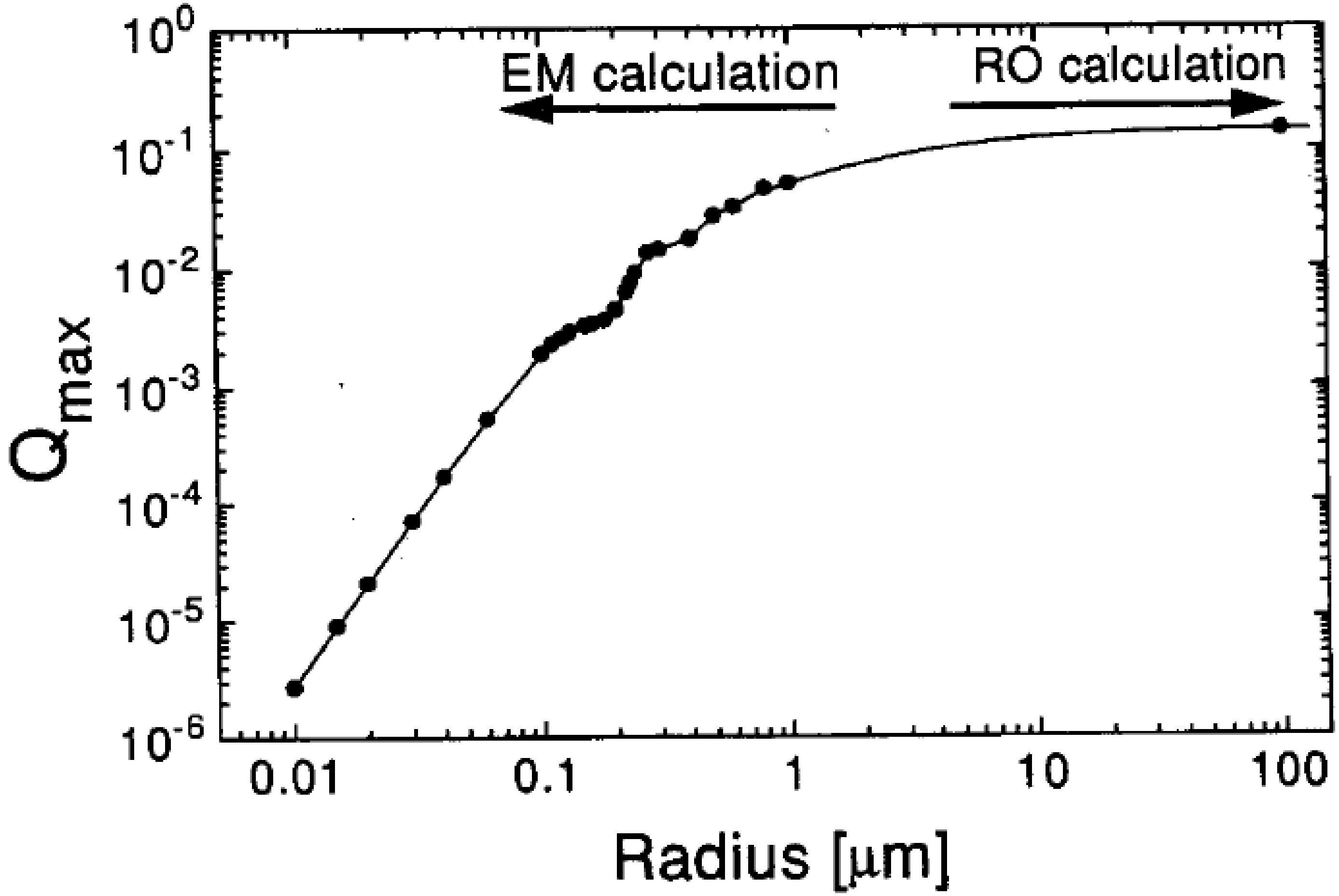
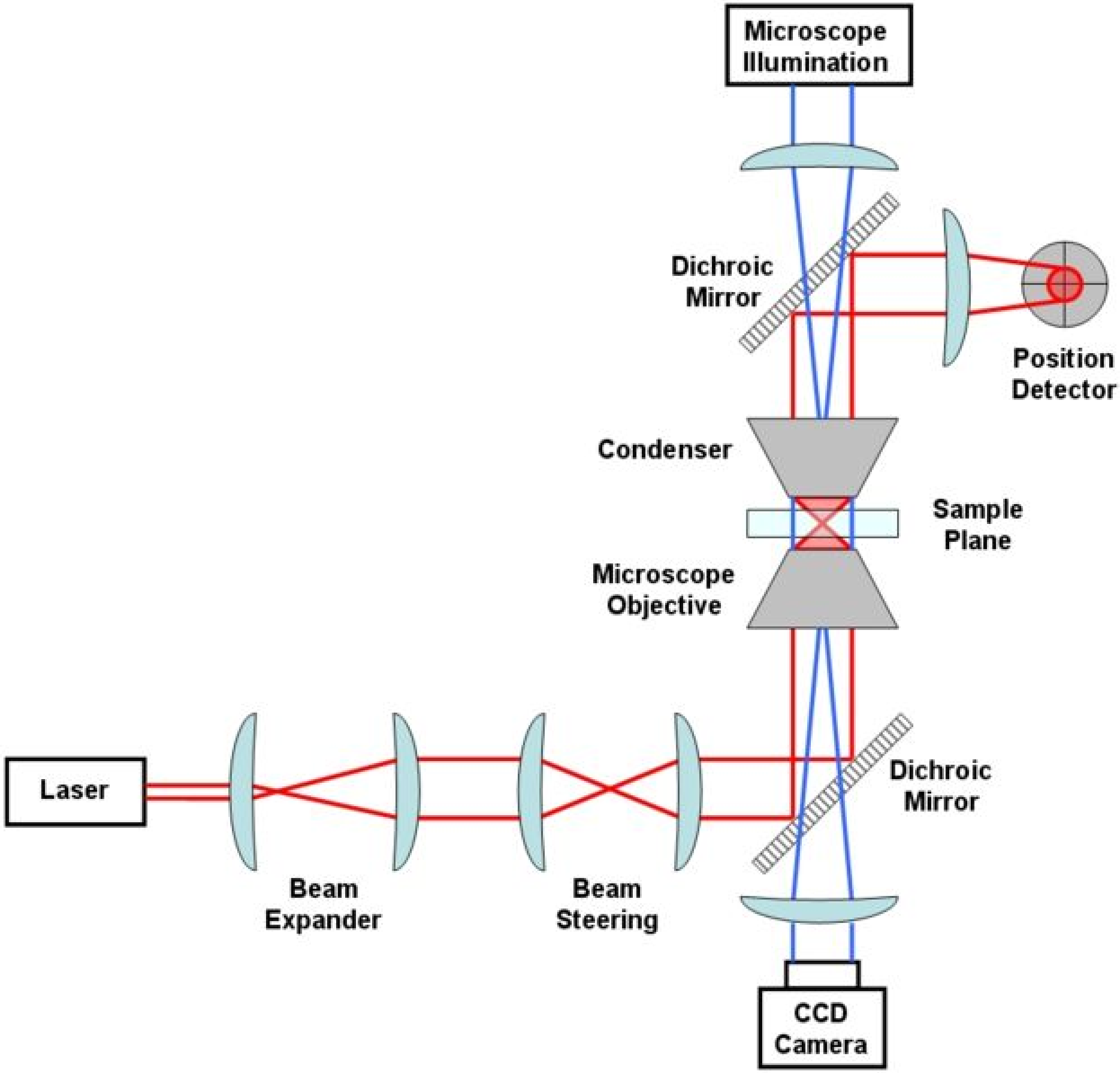


Figure 5 Computed maximum axial trapping efficiency (Q_{max}) as a function of sphere radius (redrawn from Ref. 90). Parameters were $n = 1.57$, $n_m = 1.33$, $\lambda = 1064$ nm. For the EM calculation, the spot size was 0.4 μm. For the RO calculation, the maximal cone half-angle was 60°.

Trap design

- Laser
- Beam Steering
- Beam Expander
  - Overfill objective
- 1:1 telescope
- Microscope objective
- Condensor

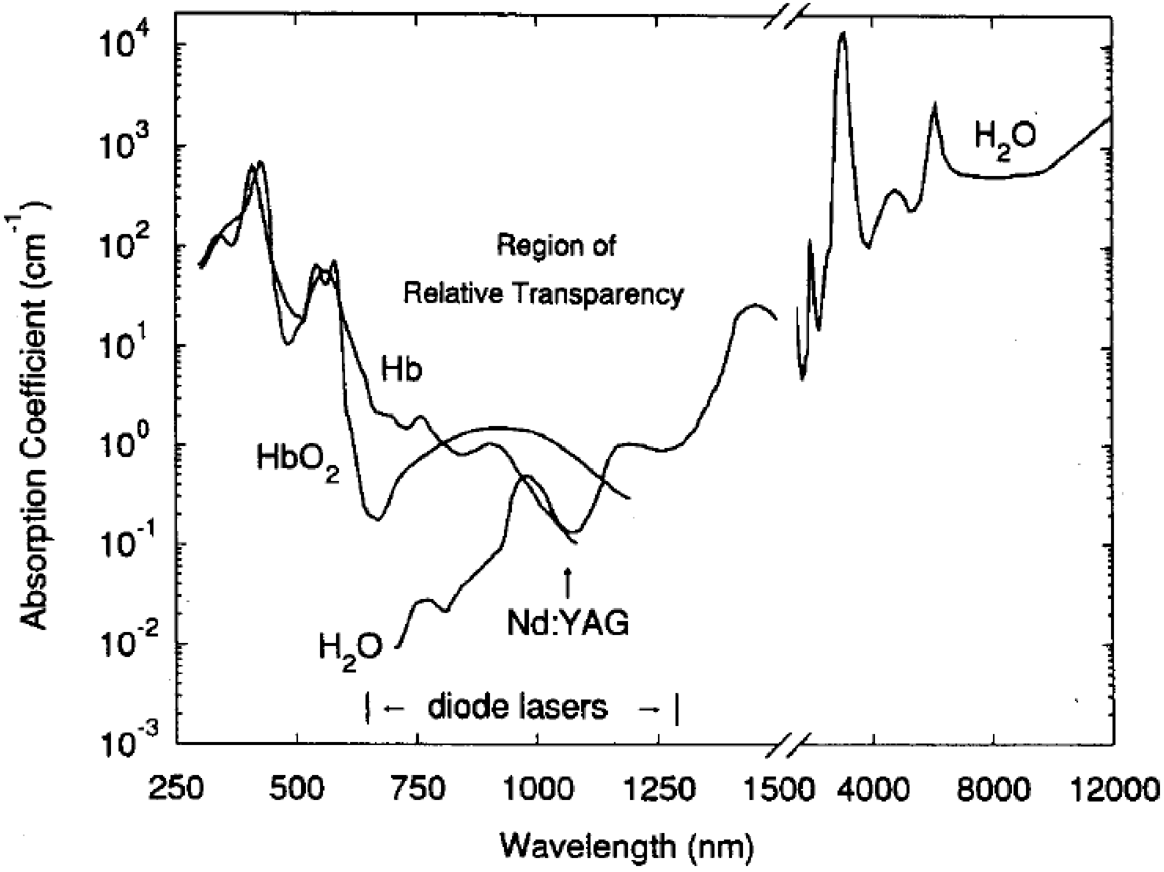

Microscope Illumination
Dichroic Mirror
Position Detector
Condenser
Sample Plane
Microscope Objective
Laser
Beam Expander
Beam Steering
Dichroic Mirror
CCD Camera

Trapping laser

- Single mode (TEM00 Guassian) output
- Power and pointing stability
  - Power fluctuation lead to stiffness fluctuations
  - Pointing instability leads to movement of trap
- Output power
  - Ca 1pN force per 10mW in specimen plane
  - Stiffness 0.15 pN/nm per W in specimen plane
  - In practive 1mW to 1 W in specimen plane
- Wavelength
  - Optical damage to biological specimen
  - Microscope objective transmission
  - Available power

Optical damate

- Biological specimens are relatively transparent in the near infrared (750 – 1200 nm)
- Damage minimum 830 and 970 nm



3D trap positioning

- Move laser focus by moving first lens in telescope
- Beam rotates around back-aperture, which corresponds to translation of focus point
- Move lens in axial direction -> change focus position along optical axis

Microscope objective

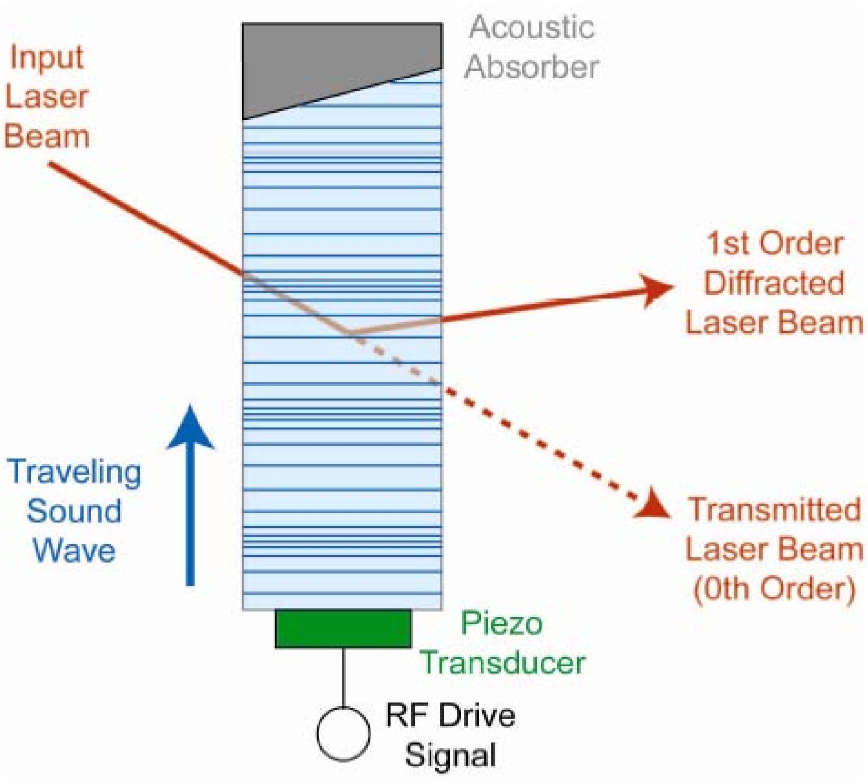
- High numerical aperture objective (NA = 1.2 – 1.4)
- High NA through Oil or water immersion
  - Spherical aberration degrades performance
  - Water immersion objectives are better
- Transmission at trapping wavelength
  - NIR transmission
  - Dual-objective method to measure transmission

Setup

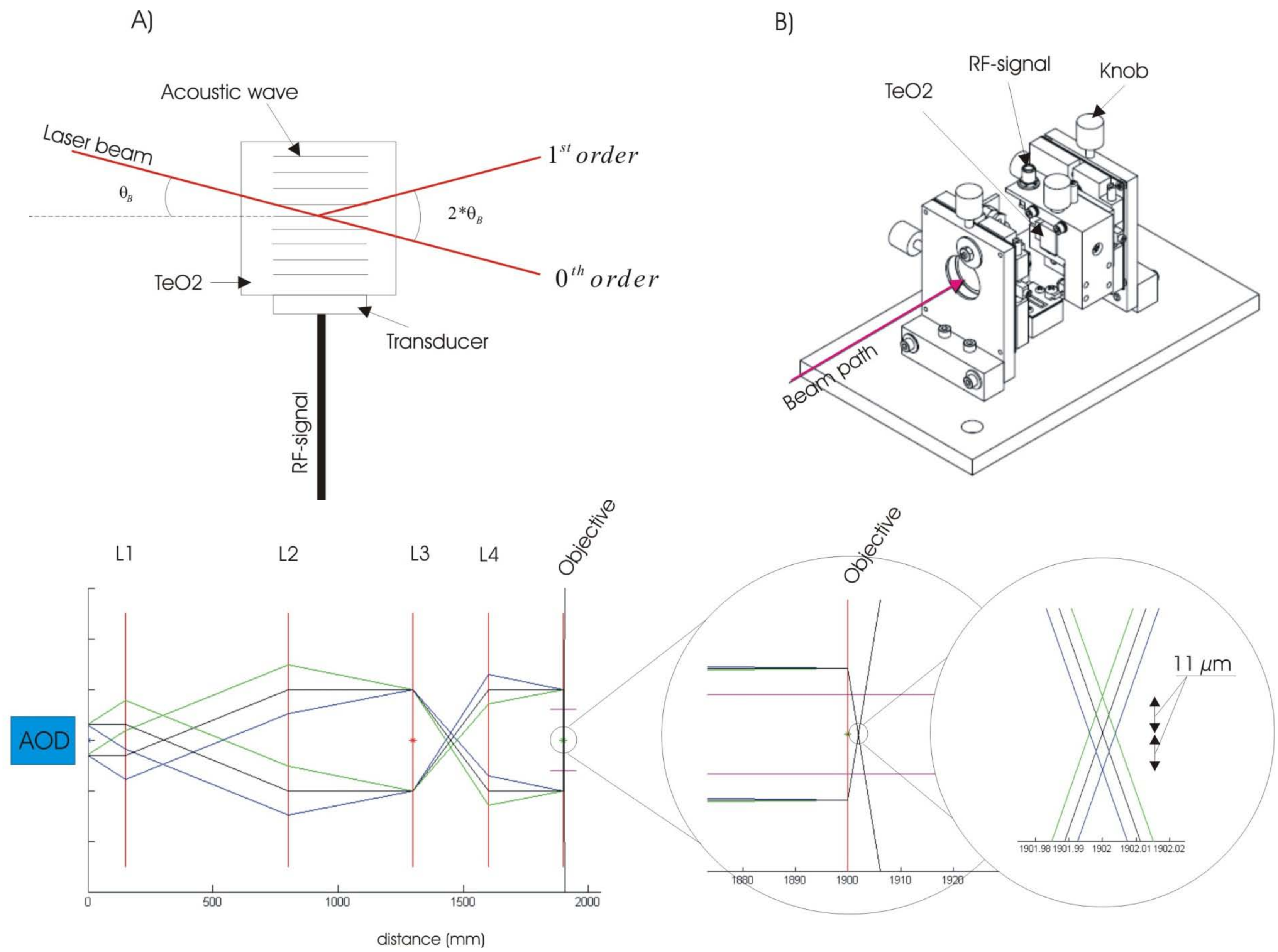
- Temperature gradients
- Acoustic vibration
  - Powersupplies etc. outside room
  - Music and voices easily coupled to trap
- Mechanical vibration
  - Short optical path
  - Damped table
- Air currents

Dynamic position control

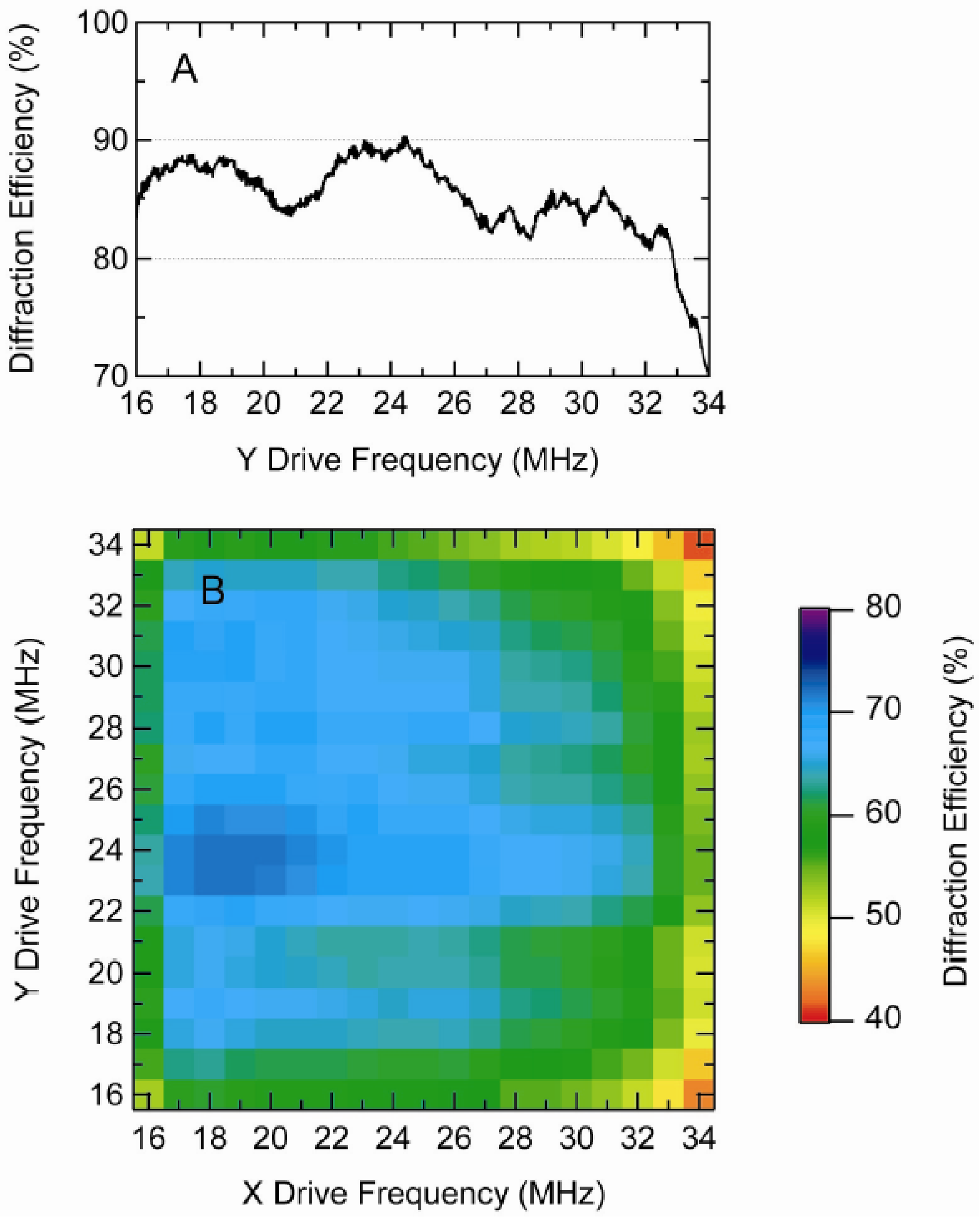
- Scanning mirror
  - Low losses
  - Large range
  - Slow (1-2 kHz)
  - Lower resolution
- Acousto-Optical Deflection (AOD)
  - Fast (100 kHz)
  - High losses
  - Non-uniform diffraction
  - High-resolution



- Acousto Optic Deflectors for Trap Steering



- Diffraction efficiency of one AOD is at most ca 80-90%
- If we use two AODs in series for X and Y deflection we get 0,8*0,8 = 0,64 throughput



- Time-shared traps with AODs

- Examples:
  - Four-trap video
  - Tetris game
  - Particle sorting

Holographic Optical Tweezers

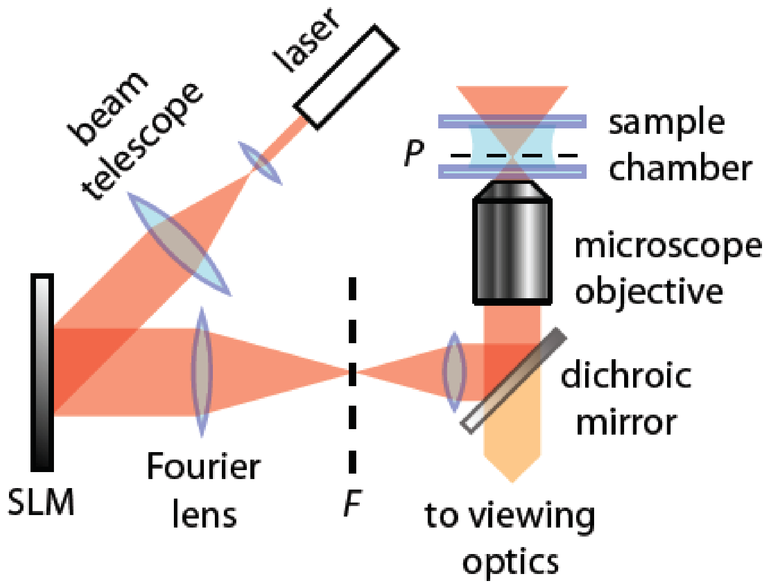


Figure 1. Simplified standard holographic optical tweezers (HOT) setup. The beam from a laser is widened in a beam telescope and illuminates a spatial light modulator (SLM). The first-order diffracted beam is collected by the Fourier lens; as the SLM is positioned in the front focal plane of the Fourier lens, the complex amplitude in the back focal plane, *F*, is the Fourier transform of the complex amplitude in the SLM plane. The remaining combination of lenses, usually including a microscope objective, images the beam in the Fourier plane into the central trapping plane, *P*, which is usually chosen to be in a liquid-filled sample chamber.

Example video!

Position detection

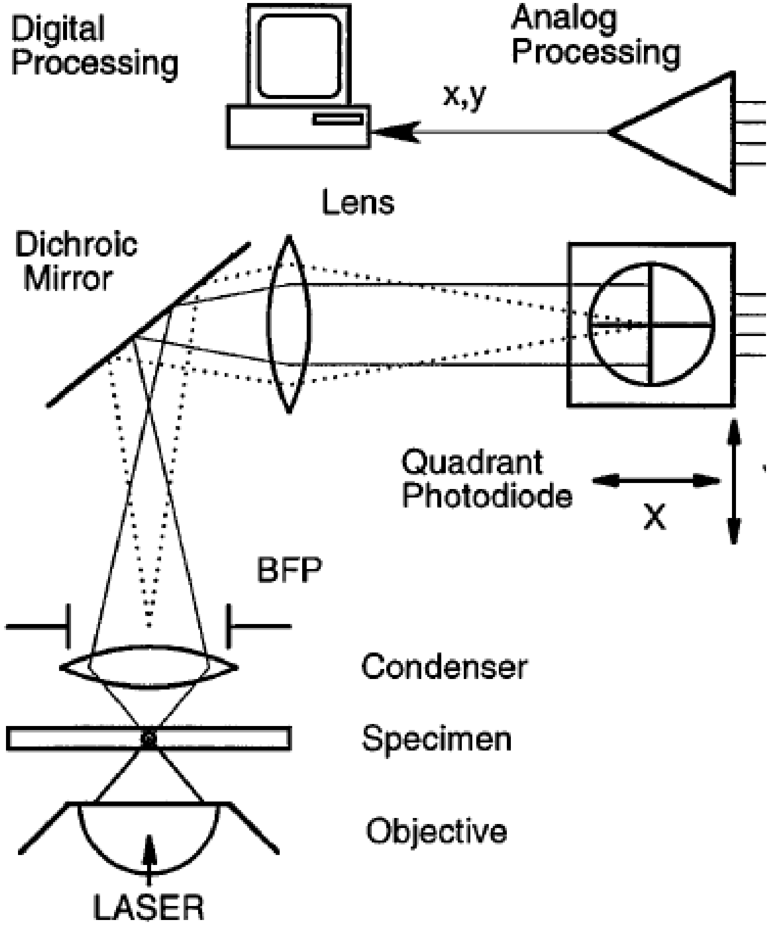
- Video tracking
  - Slow (30-120Hz)
  - Absolute position with 1-5 nm position
- Laser based Back-focal-plane detection
  - Fast (100 kHz)
  - Relative position (bead – focus)
  - 1nm or better resolution

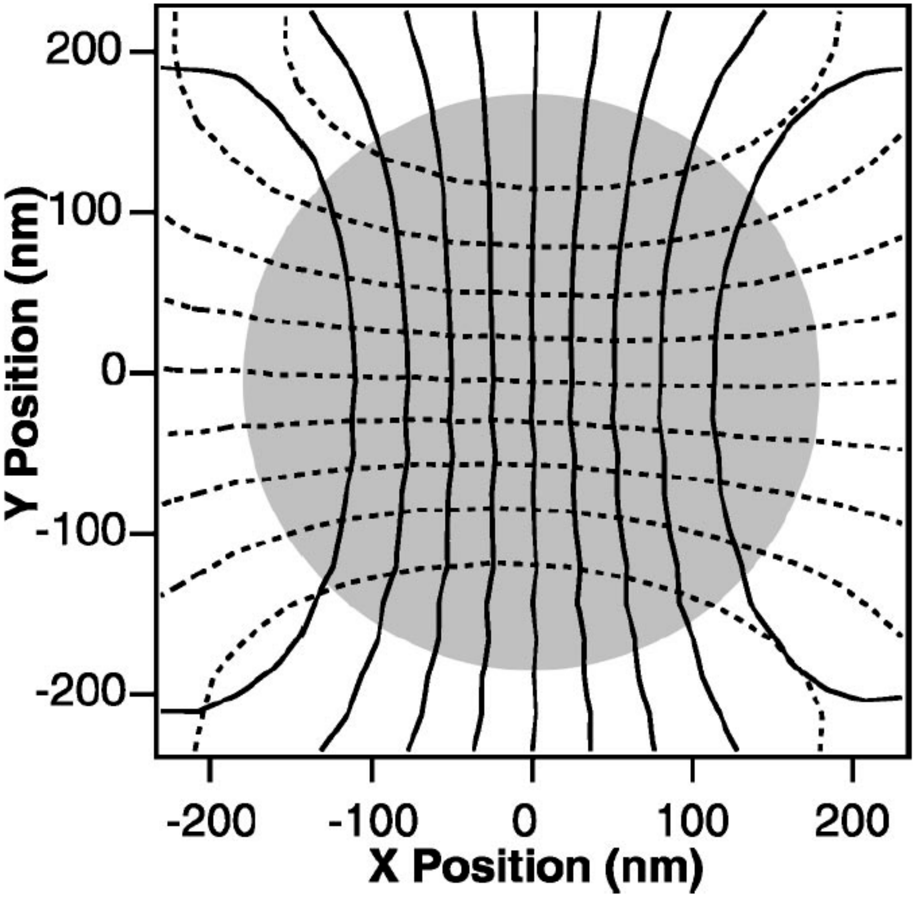
Back focal plane detection

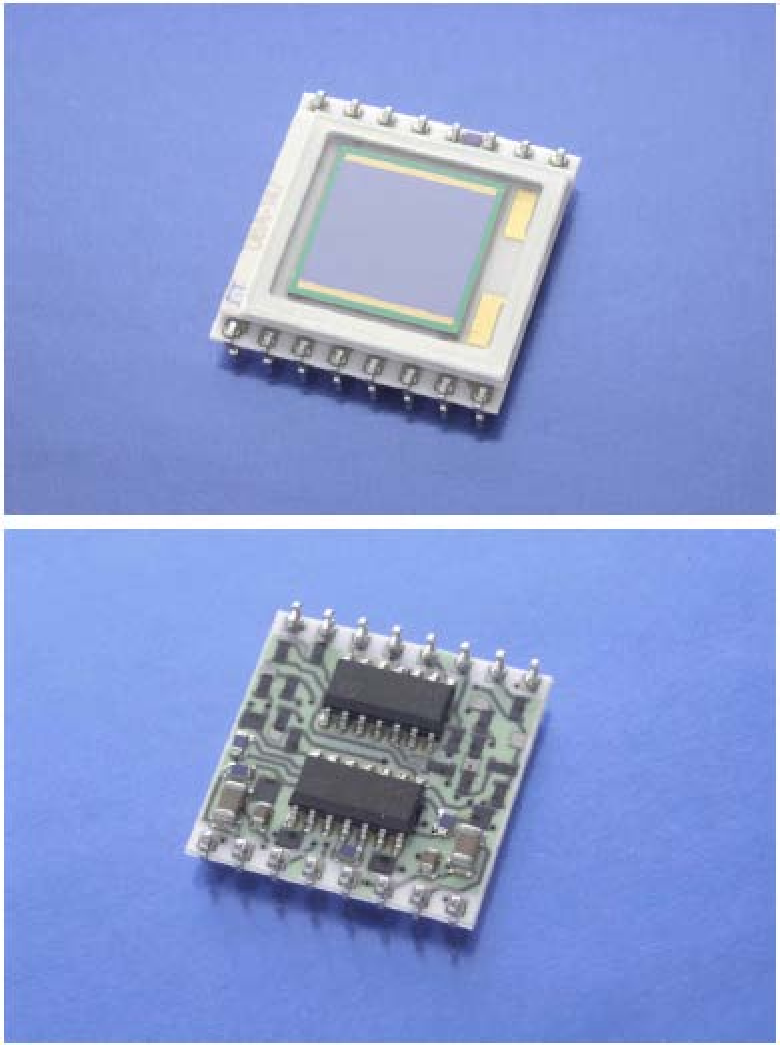
- Focus a laser on the bead
- Collect light on condensor side.
- Detect interference between unscattered and scattered light
- Image back-focal plane onto a position sensitive detector.

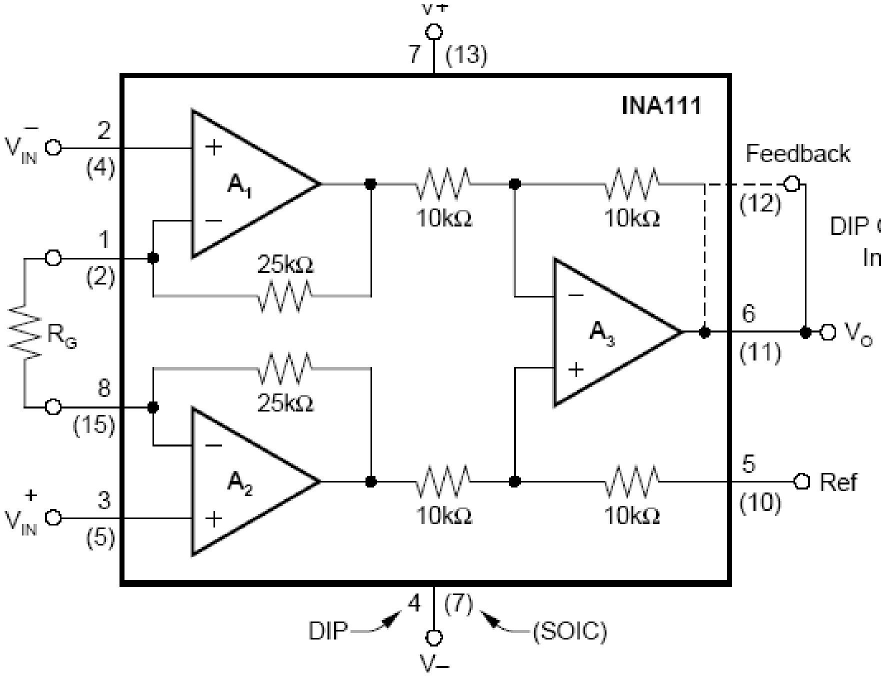
Detection

- Resolution of video microscopy limited to ca 10nm
- Interferometric detection has









$$G = 1 + \frac{50\text{k}\Omega}{R_G}$$

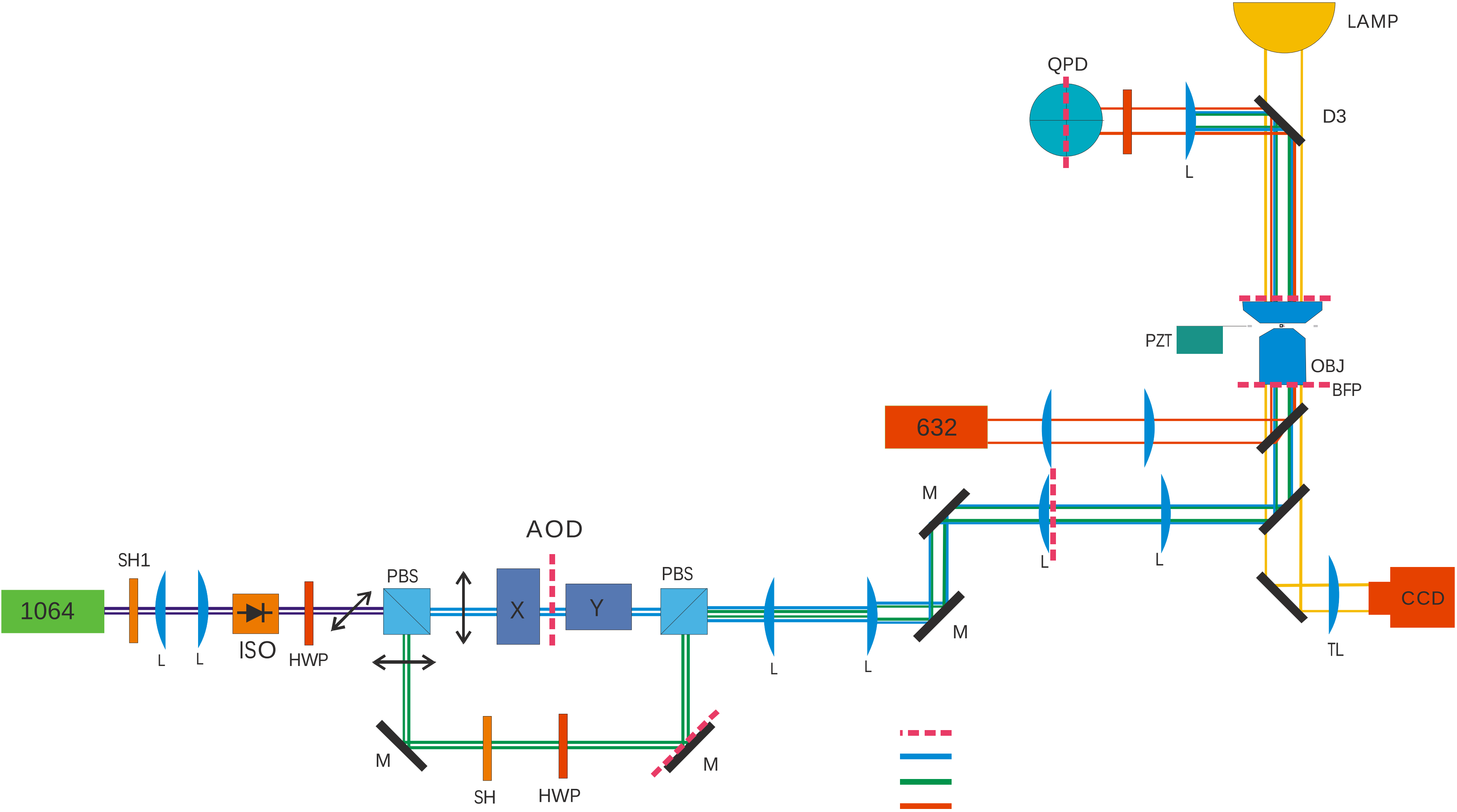
Variable gain: 1-1000

100 kOhm transimpedance resistors

(10x higher than previous detectors)

400 kHz BW quoted by manufacturer

Optical setup

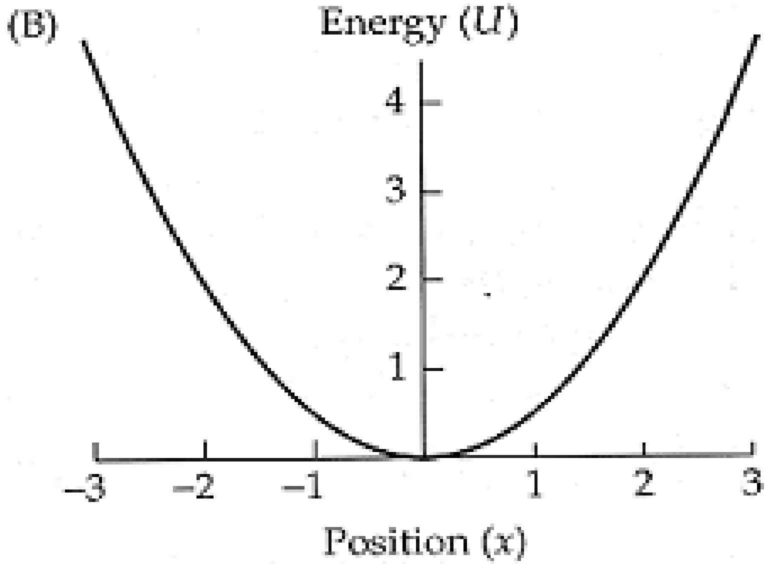


Calibration

Position Calibration

- Position
  - Stage micrometer
  - Calibrated piezoelectric stage
- Move bead through detection range
  - Scan bead with PZT stage
  - Trap a bead and move it with AODs or mirrors

The Tweezer potential is Harmonic



- Force (F) is proportional to displacement (x)

-Detected voltage(V) is proportional to displacement(x) of bead from beam focus.

→Two calibration parameters:

$$F = -kx$$

$$x = \beta V$$

Calibration: Theoretical Power Spectrum

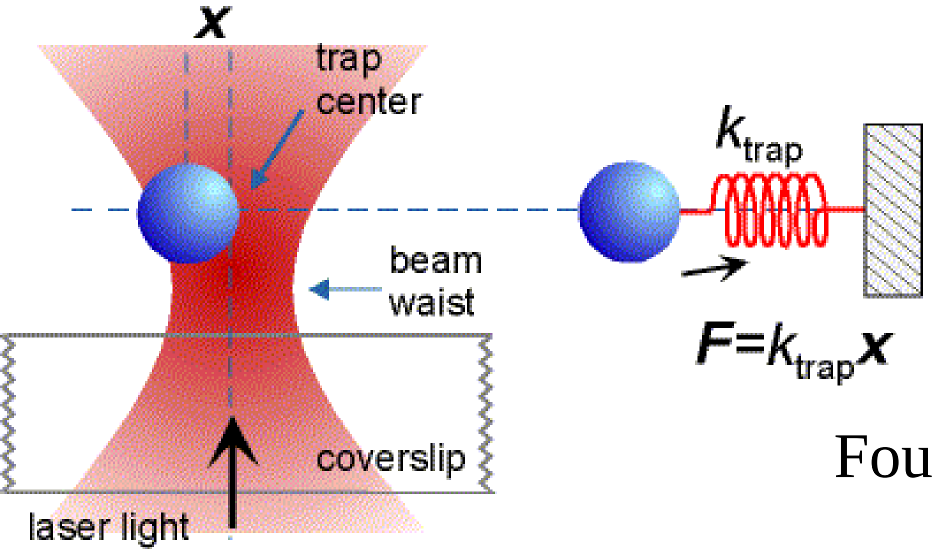
- Eq. Of motion for a Brownian particle in a harmonic potential:

$$\gamma\dot{x} + kx = F(t)$$

$$\gamma = 6\pi r\eta = \text{Stokes drag}$$

$$k = \text{trap stiffness}$$

$$F(t) = \text{random thermal force}$$

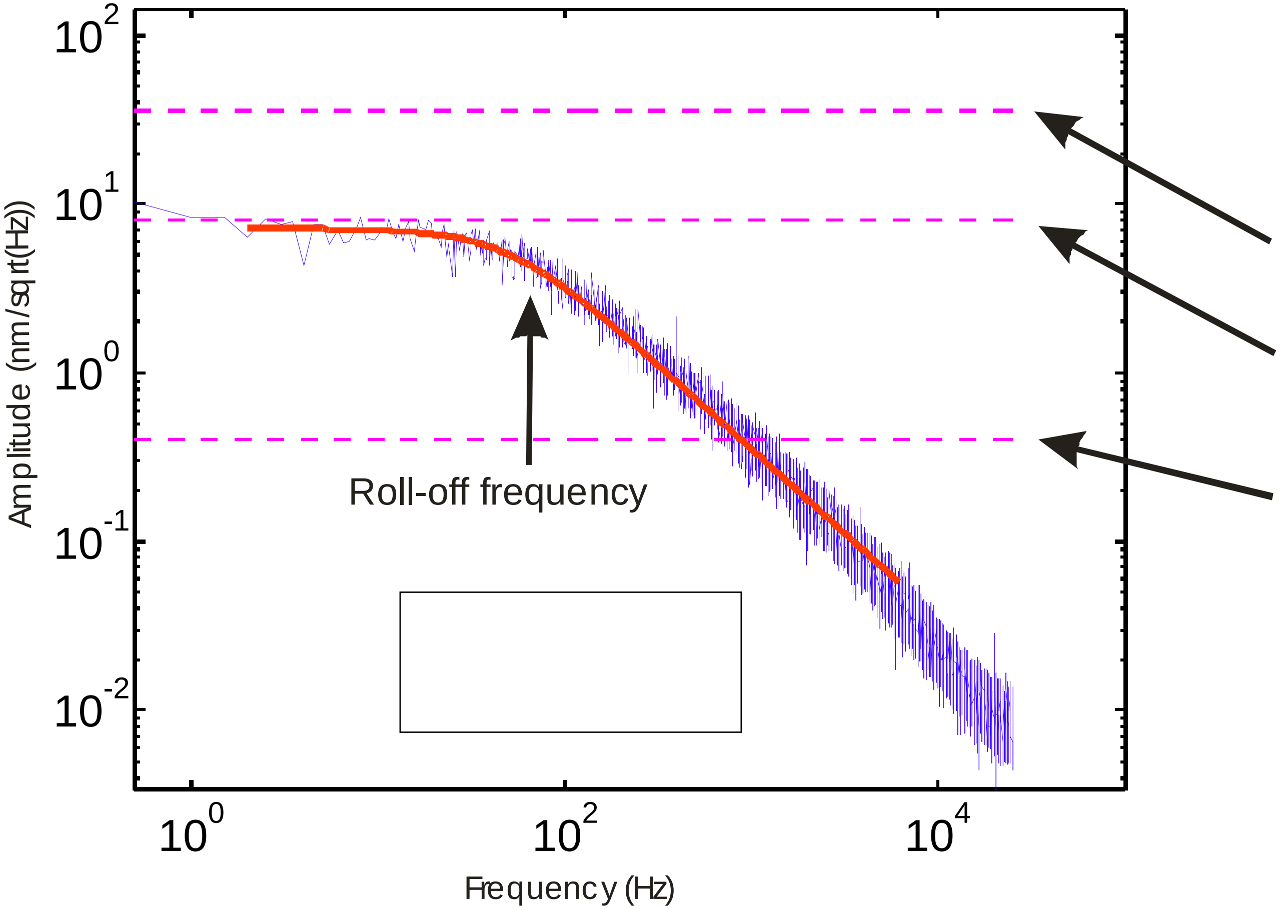


Fourier transform gives Power Spectrum :

$$S_{xx}(f) = \frac{k_B T}{2\pi^3\gamma(f_0^2 + f^2)}$$

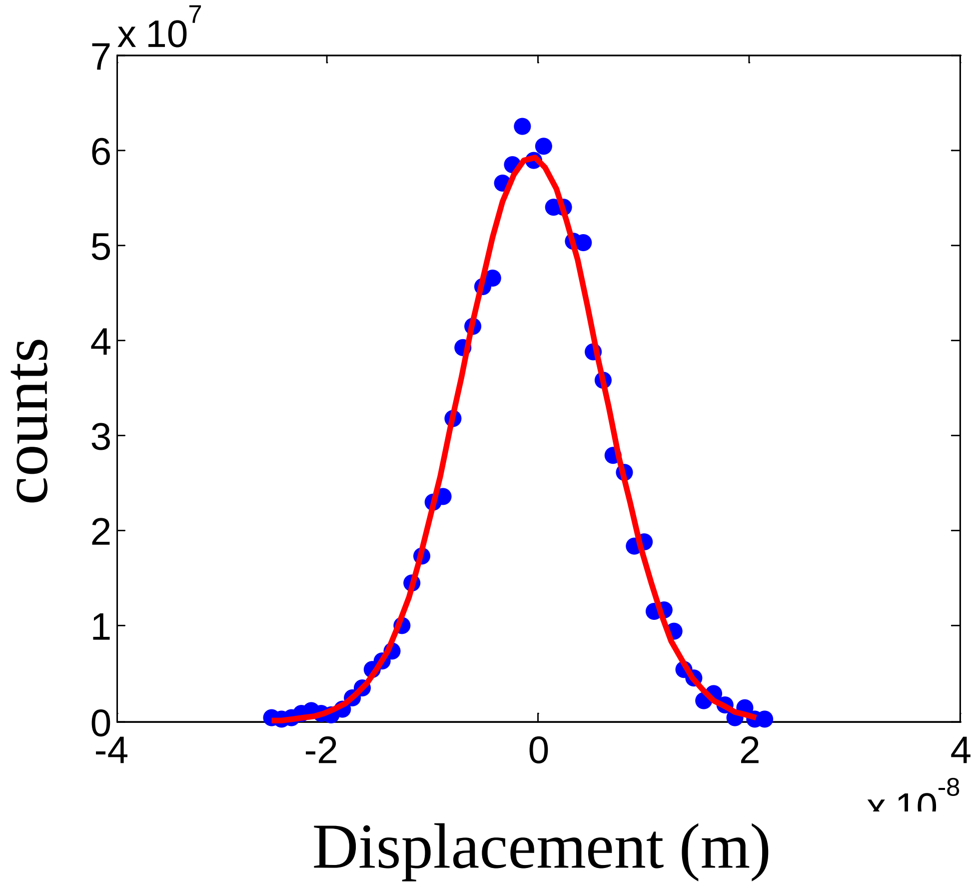
$$f_0 = \frac{k}{2\pi\gamma} = \text{corner frequency}$$

Calibration: Power Spectrum method



Calibration: Equipartition Theorem

- Equipartition Theorem: $< x^2 >= \frac{k_B T}{k}$



Force calibration problems

- Detection bandwidth
- Unintended signal filtering
- Anti-aliasing
- Drag coefficient (Faxens law)
  - Stokes law OK only when we are infinitely far away from surfaces

Force calibration, drag-force

- Drag-force method
  - Move stage or create flow to push bead out of trap
  - Triangle-waveform stage motion
  - Check previous calibration, check for nonlinearity
  - Proximity to surfaces is a problem -> Faxens law instead of Stokes law

Optical Tweezers in biology

- First success in biology: studying kinesin, myosin (conventional molecular motors)
- Nucleic acid enzymes
  - RNAP (Block lab)
- Trapping whole cells (Goksör, Enger, Hanstorp)
- Lipid membrane manipulation PRL: force barriers for membrane tube formation