Fundamentals in Biophotonics

Optical tweezers

Optogentics –manipulating objects, mind, movement and more….

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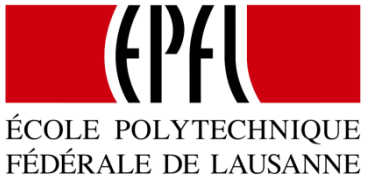

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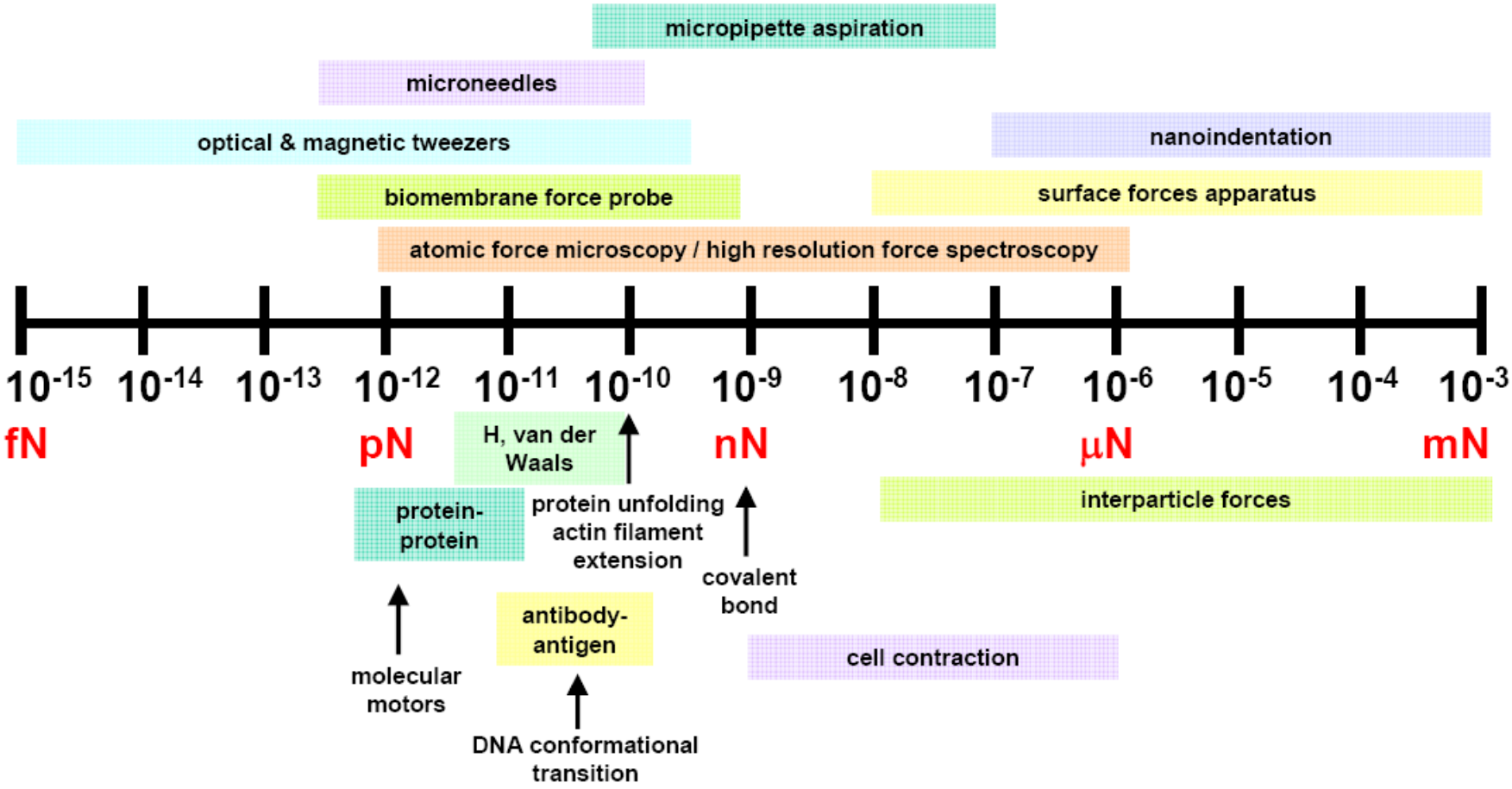
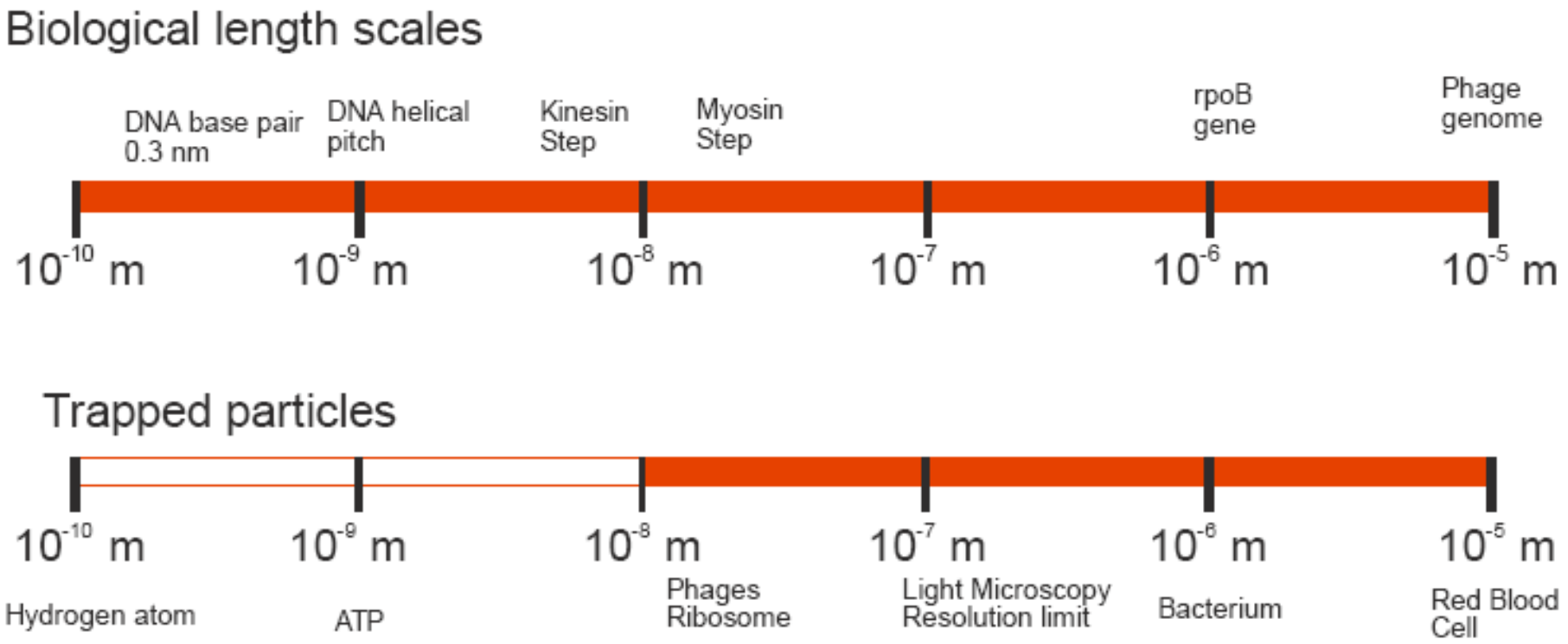
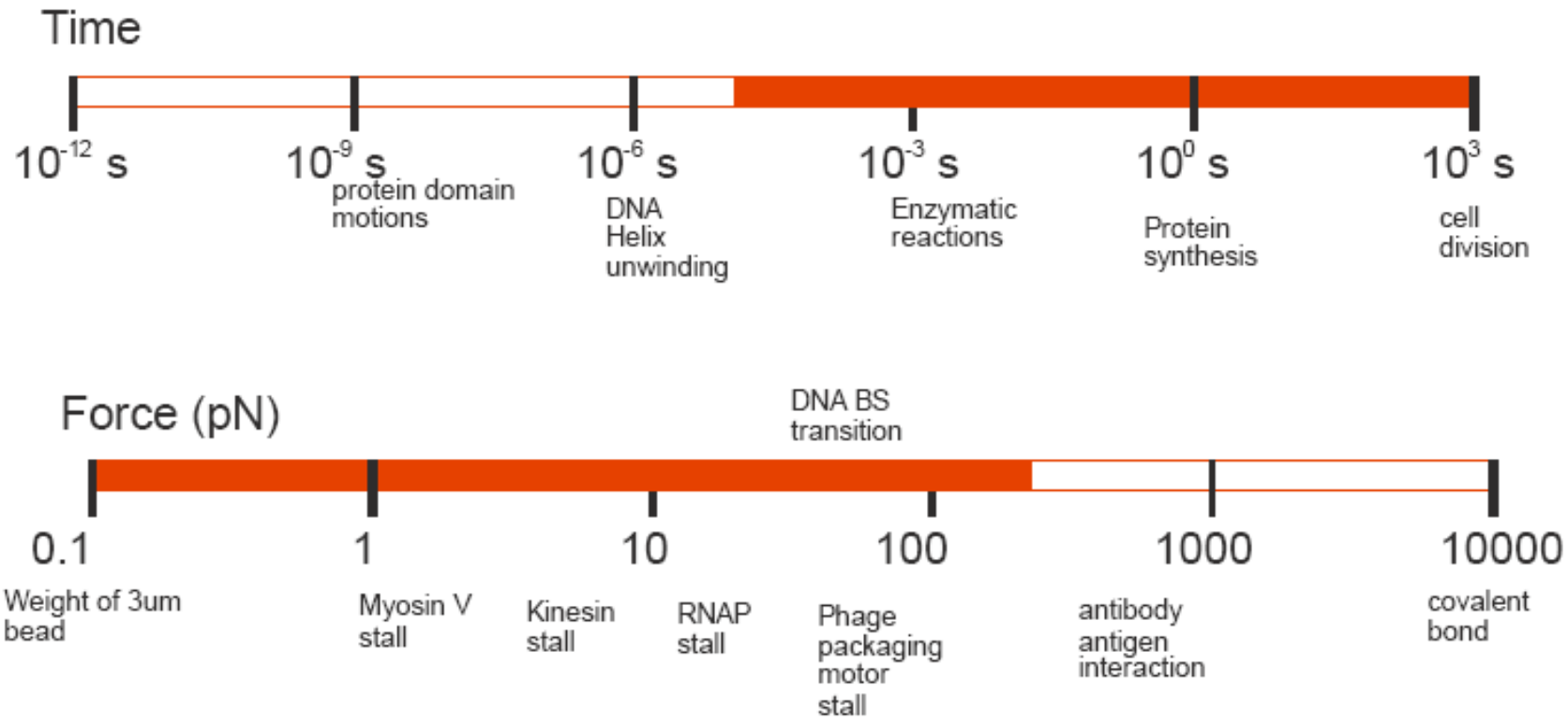
Force range for various nanomechanical instruments



Length scales



Time and force scales



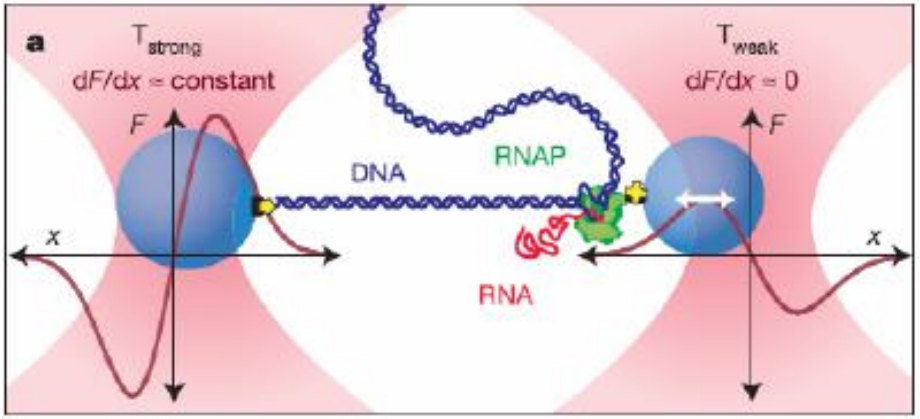
1 picoNewton (10^{-12} N) is roughly equal to...

– ...the gravitational attraction between you and a book at arms length
– …the radiation pressure on a penny from a flashlight 1 yard away
– ...1 millionth the weight of a grain of salt

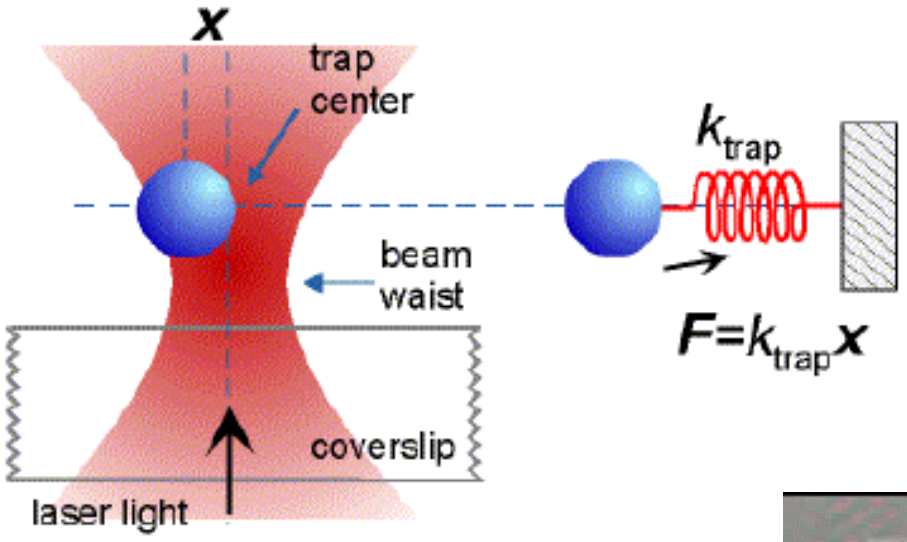
Optical tweezers

(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 Laser beam

- OT works by transfer of momentum

- Particles with higher n than surrounding medium are trapped in an approximately harmonic potential

k ≈ 0,001– 0,1 pN/nm

Principle

- Radiation pressure is the force per unit area on a object due to change in light momentum
- Photons carry momentum $$\left|\vec{p}\right| = \frac{h}{\lambda}$$
- **Change in momentum corresponds** to the **force** and it can be calculated by the difference is **momentum flux S** between entering and leaving a object

$$\vec{F} = \frac{n}{c}\iint\left(\vec{S}_{in} - \vec{S}_{out}\right)dA$$

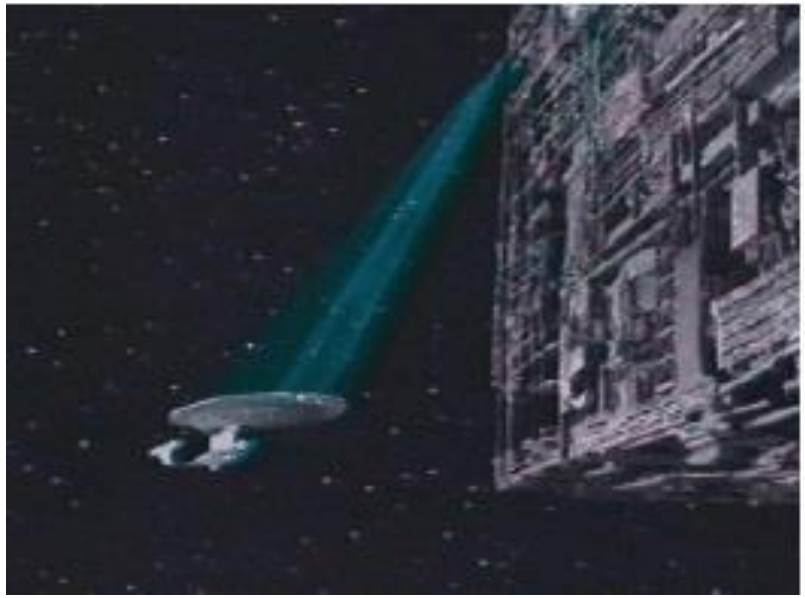
- Applying this formula to a 100% reflecting mirror reflecting a 60W lamp gives a pressure of:

$$\vec{F} = 2\frac{n}{c}\iint\left(\vec{S}_{in}\right)dA$$

$$\vec{F} = 2\frac{n}{c}W = 4\times10^{-7}\,N$$

Principle

- Gravity pulls on a 1 kg mirror with 9.8 N so the force of the photons is negligible.
- However, if the same light is reflected by a object of 1 µg it can't be ignored!
- Using a laser on a microscopic particle will realize this situation.
- Sunlight on earth 0.5 nN/cm^2
- Laser pointer ~10 pN



Physics of optical trapping

- The physics of the trapping mechanism is based on optical gradient and scattering forces arising from the interaction of strongly focused laser light with matter

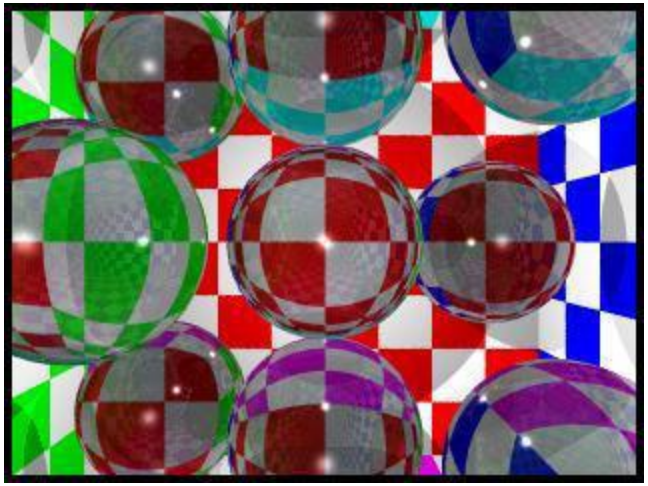
- Simple models that explain optical trapping behavior can be applied in the **Mie scattering ($d >> \lambda$)** and the **Rayleigh scattering ($d << \lambda$)** regimes depending on the size of the particle relative to the wavelength of laser light

- A real optical tweezers typically works in the intermediate ($d \approx \lambda$) regime, requiring a rigorous application of complicated approaches such as Generalized Lorentz-Mie Scattering or T-Matrix theory (beyond the scope of this lecture!)

- However, insight into the trapping mechanism can be gained from studying limiting cases

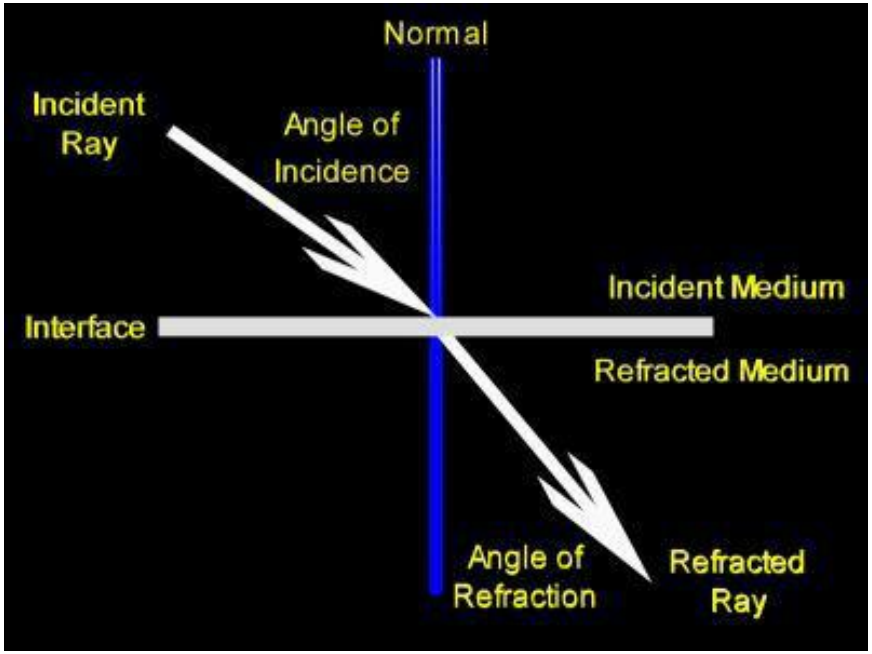
Basic ray optics Snell law

- The amount that the substance bends light is its **Refraction** (or Refractive) Index or RI.

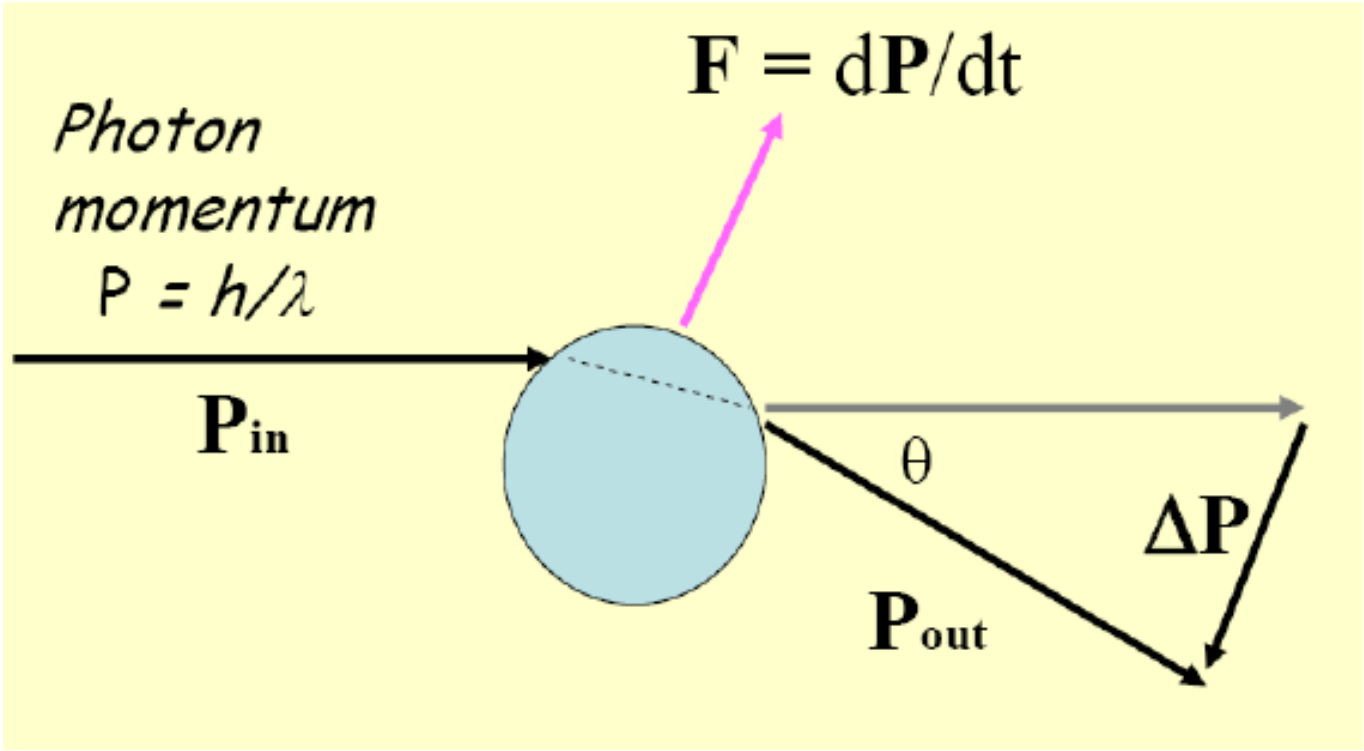


$$\frac{\sin\vartheta_1}{\sin\vartheta_2} = \frac{v_1}{v_2} = \frac{n_2}{n_1}$$

Snell's Law



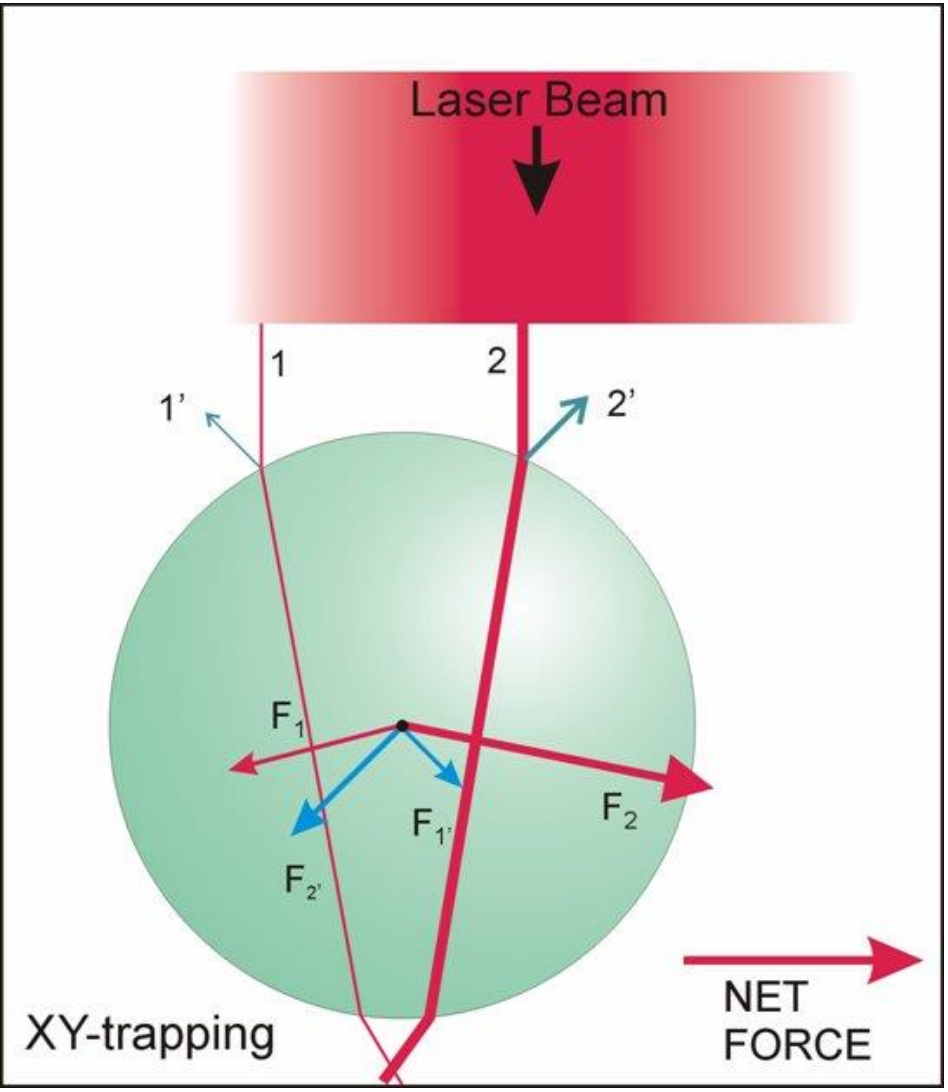
Photon meets refracting object



For every action there exists an equal but opposite reaction

Sir Isaac Newton (3rd law of motion)

Theory Mie regime d >> λ



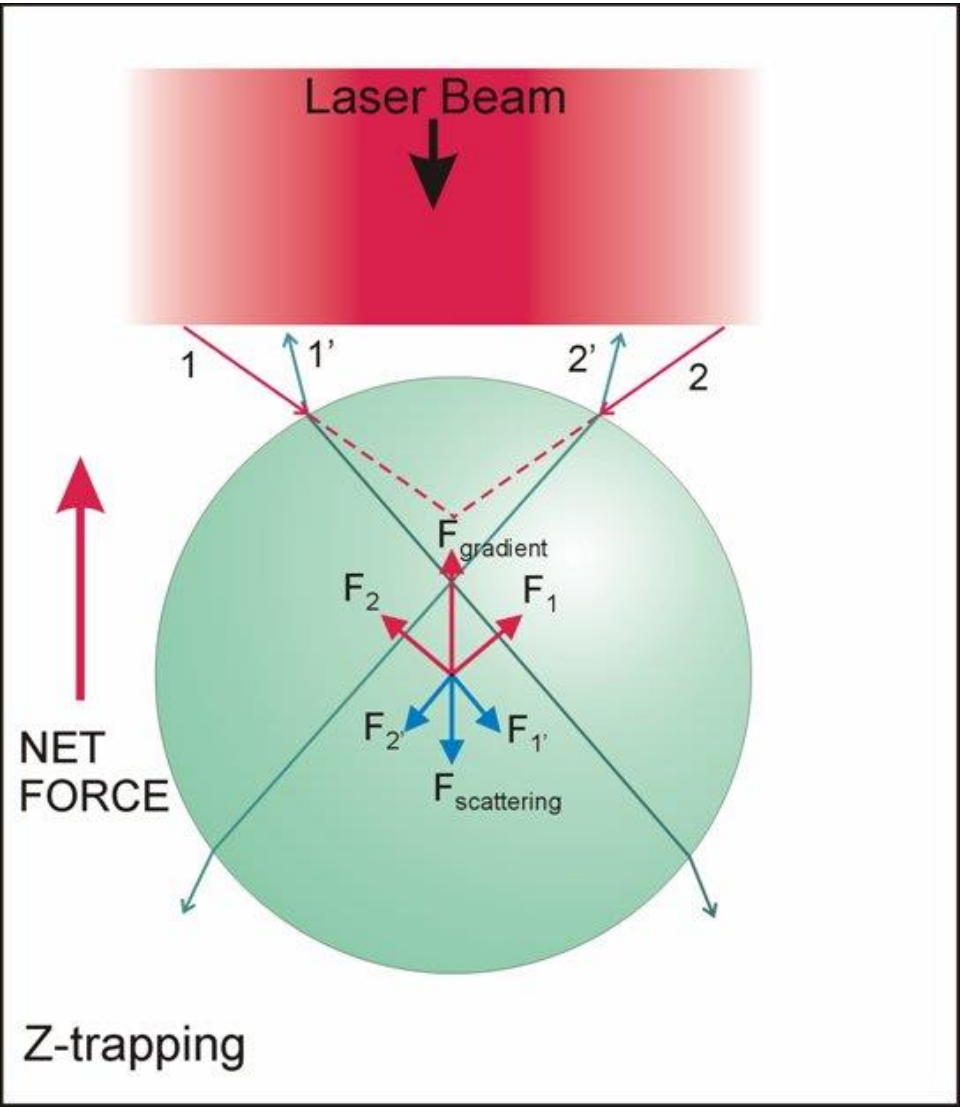
Applied in the Mie regime d >> λ, so that we can consider 'rays' of light being refracted at the interface between dielectric media

For simplicity consider a spherical particle of refractive index n, suspended in water of refractive index nw.

Refraction of the 'ray' as it crosses the sphere implies a transfer of momentum from the sphere to the 'ray', and hence an equal and opposite transfer of momentum from the 'ray' to the sphere

The gradient in intensity (number of 'rays') across the sphere produces a net transverse force towards the beam axis – an optical gradient force

Theory Mie regime d >> λ



To achieve trapping in the axial (z-) direction requires focusing of the beam where a similar argument for refraction providing an optical gradient force towards the focus can be made

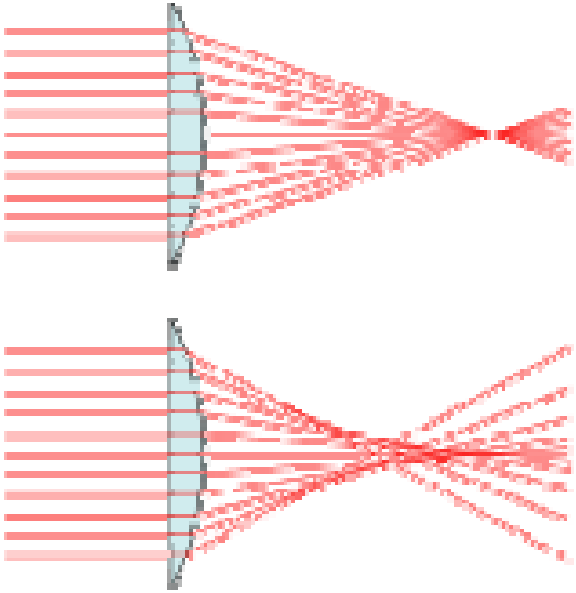
Axial trapping must also overcome the 'pushing' effect of the small reflection at the sphere-water interface due to the mismatch in refractive indices – the optical scattering force

Stable 3D trapping requires that the **gradient force** exceeds the **scattering force**, which may be achieved with strong (high numerical aperture) focusing

Choosing microscope objective properly

- 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* (less n mismatch; longer WD)

- Transmission at trapping wavelength
  – *NIR transmission (we'll discuss why we need NIR later)*
  – *Dual-objective method to measure transmission*

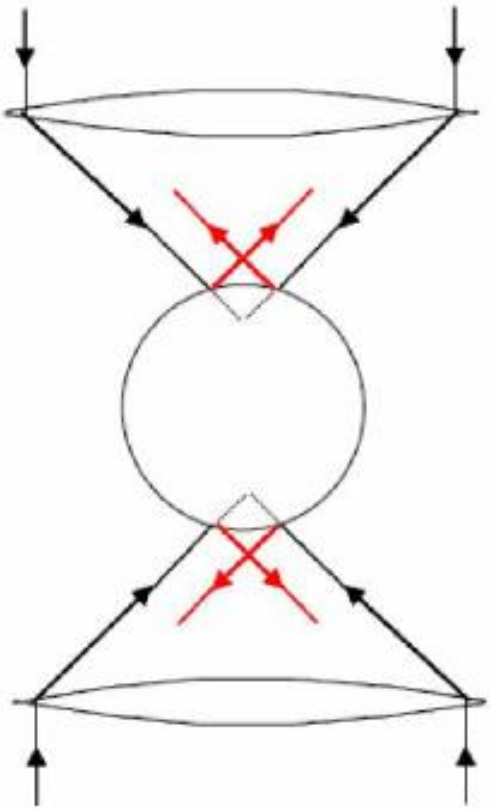


Solution 2 **cancel it out**

- We can also use two laser beams to trap the sphere

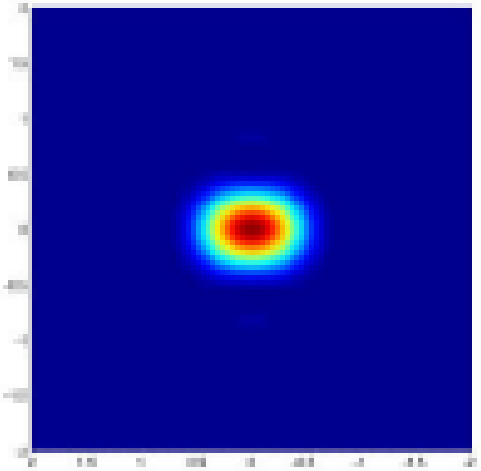
- Reflective forces cancel
- Low NA objectives

- The design is complex and difficult to keep both lasers aligned (at least twice the equipment investment)

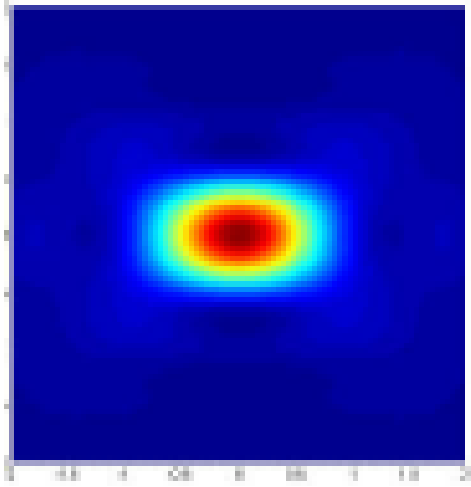


Theory Rayleigh regime d << λ,

x-y plane



x-z plane



- Applied in the Rayleigh regime d << λ, so that we can consider an electric dipole that is polarized by the application of an electric field
- A separation of charge (electric dipole) is induced in the dielectric by the applied field:

$$\vec{p} = \alpha \cdot \vec{E}$$

- The interaction energy of the dipole is given by

$$U_{dip} = -\vec{p} \cdot \vec{E} = -\alpha \cdot \vec{E} \cdot \vec{E} \propto I(r)$$

- Remembering that the intensity distribution is gaussian in the transverse plane we see that for small displacements from the axis we have

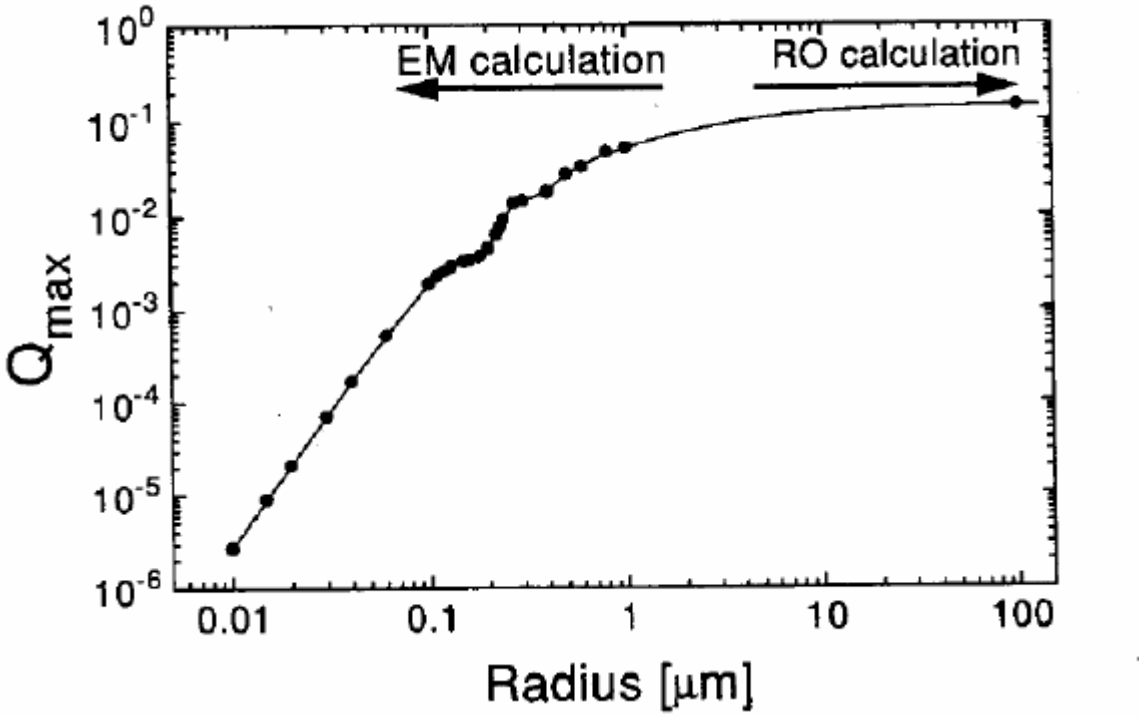
$$\vec{F}_{grad} = -\nabla U_{dip} \propto \nabla I(r) = -k_i x_i$$

- i.e. a force proportional to the gradient in intensity
- Strong confinement is therefore achieved by strong focusing

Theory intermediate regime d~λ

- Most interesting trapped particles are ca 0.1n λ– 10 λ
- 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



General remarks

- Both models for limiting cases give similar behavior for the forces in optical tweezers
- A particle is trapped close to the focus of the laser beam (in fact the equilibrium position is just beyond the focus due to the scattering force=
- For small displacements from equilibrium the restoring force on the particle is proportional to the displacement and directed towards the equilibrium position, i.e. it behaves as a mass-spring oscillator with **spring constant κ.**
- The spring constant is **proportional to the trap intensity**
- The spring constant in the axial (z-) direction is different from (and **weaker** than) the transverse (x- and y-) directions (in fact for nanoparticles the spring constants in x- and y- are also different from each other due to **polarization induces symmetry breaking)**

First trapping realized

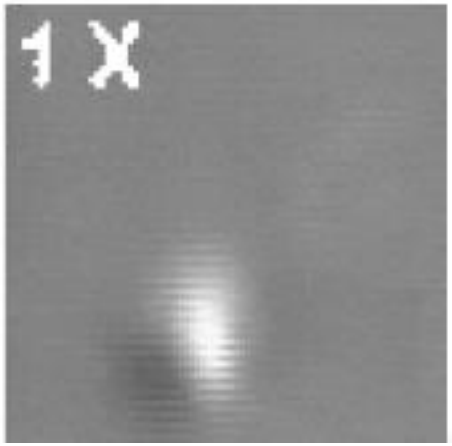
288 OPTICS LETTERS / Vol. 11, No. 5 / May 1986

Observation of a single-beam gradient force optical trap for dielectric particles

A. Ashkin, J. M. Dziedzic, J. E. Bjorkholm, and Steven Chu
AT&T Bell Laboratories, Holmdel, New Jersey 07733

- a stable single-beam trap

Anti-scattering force:
Forward momentum is increased by lens -focusing effect.



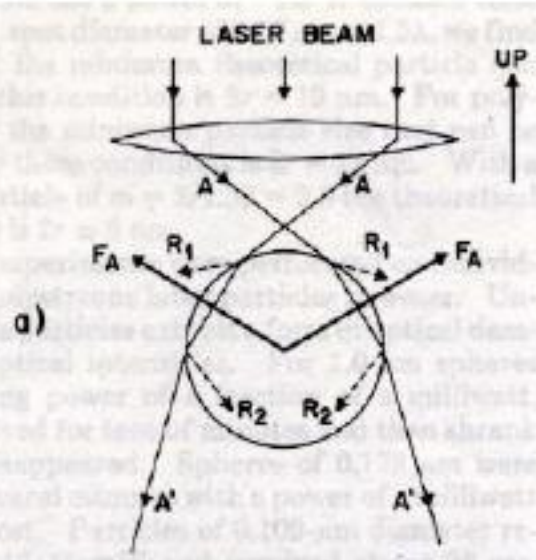
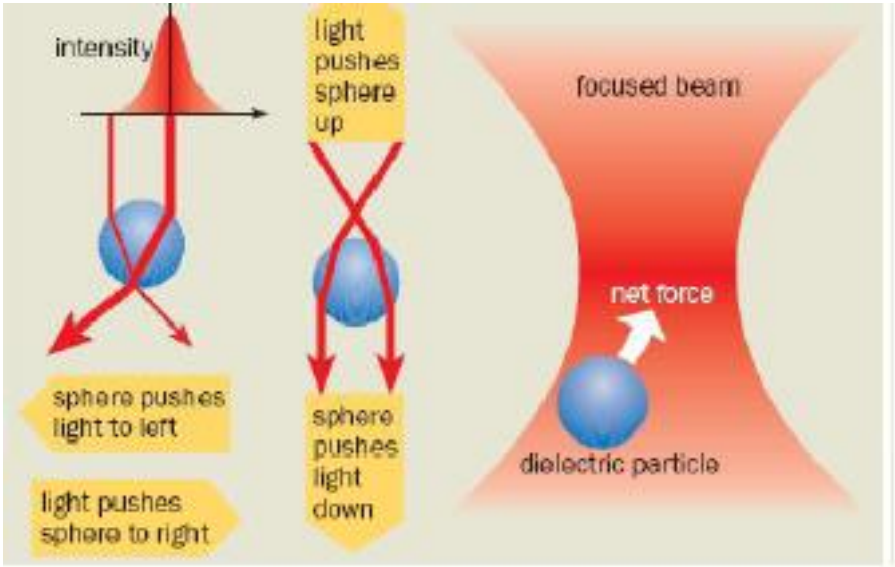
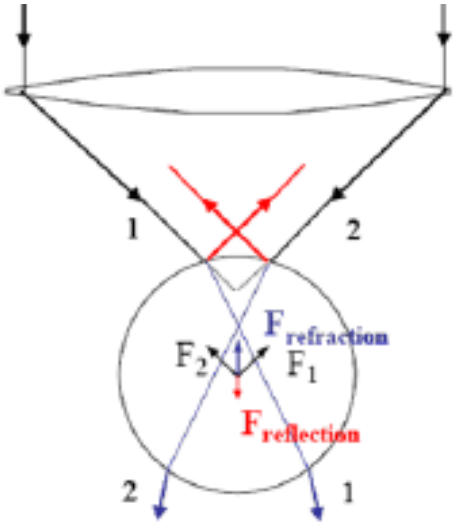


Fig. 1. a) Diagram showing the ray optics of a spherical Mie particle trapped in water by the highly convergent light of a single-beam gradient force trap. b) Photograph, taken in fluorescence, of a 10-μm sphere trapped in water, showing the paths of the incident and scattered light rays.

Basic Physics Summary

- Index refraction mismatch deflect the light
- Momentum variation push sphere towards high intensity region
- High NA or dual beam overcome the reflection





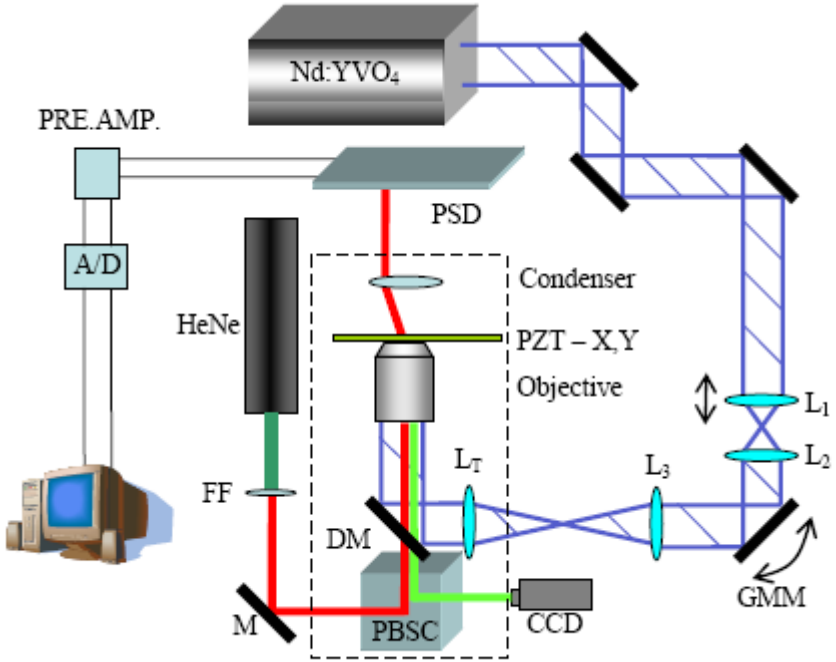
- Trap manipulation
- Position detection
- Force & trap stiffness calibration

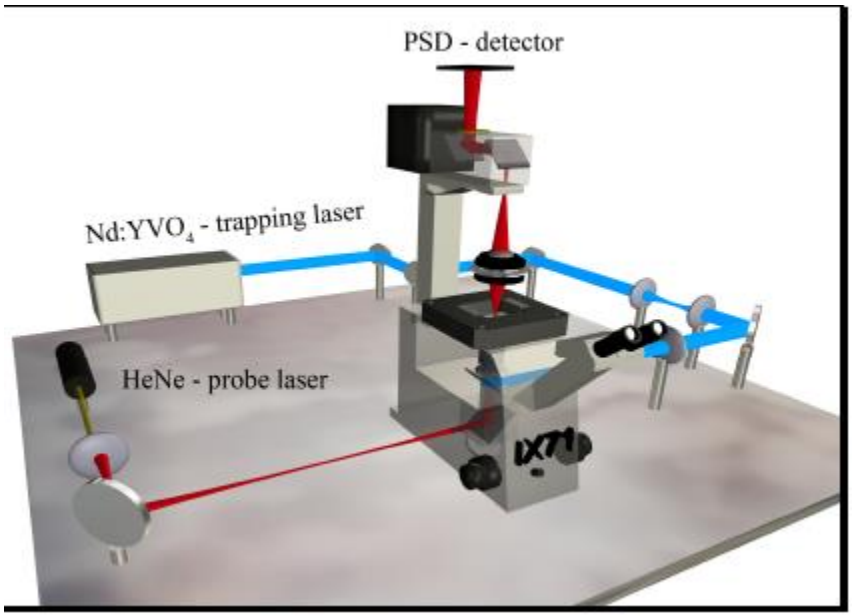
SOME TECHNICAL ISSUES

Trap design

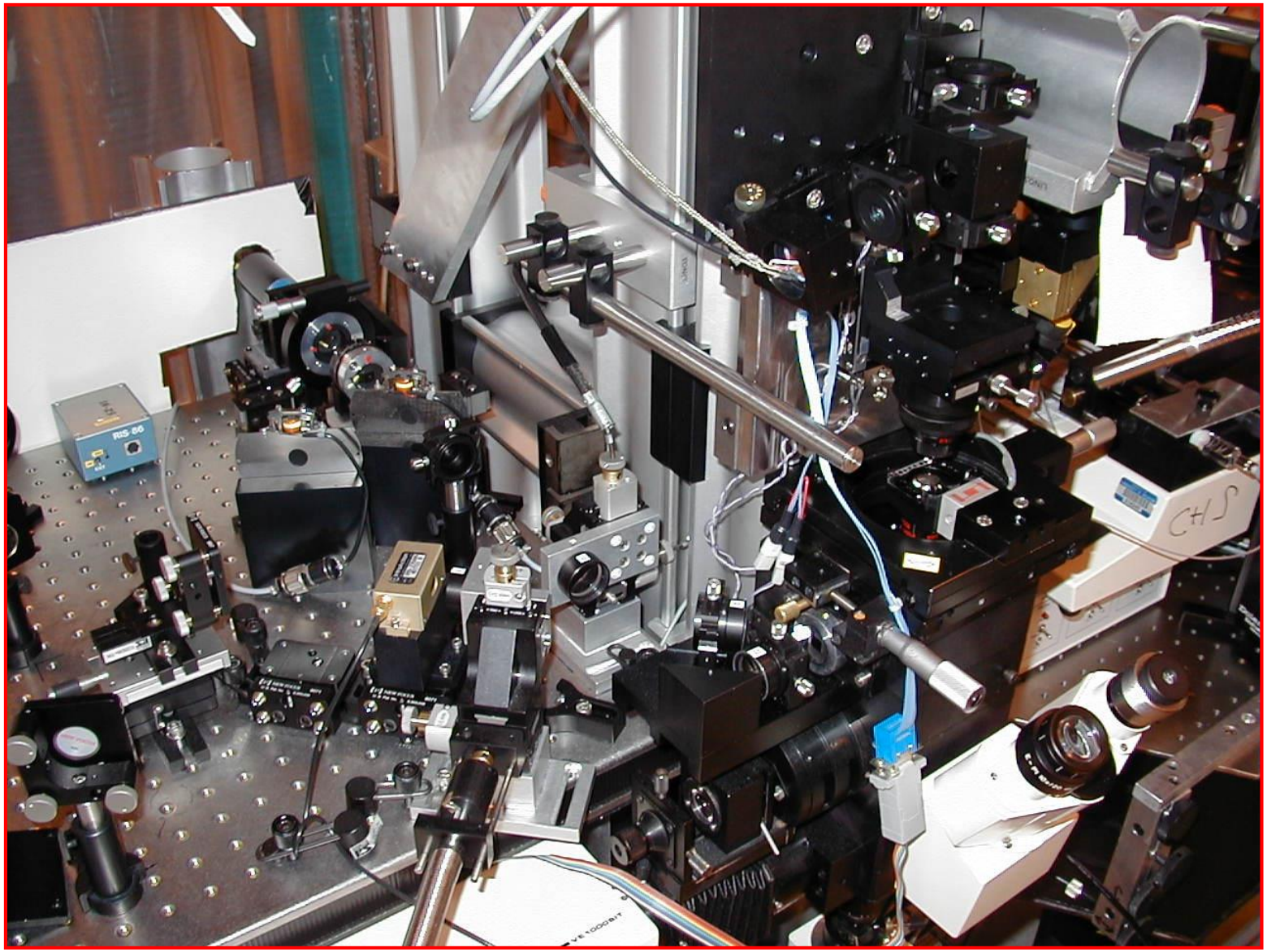
- Laser
- Beam/Sample steering
  - AOD
  - Steerable mirror
  - nano-positioning stages
- Beam Expander
  - overfill back focal plane
- Microscope objective
- Condensor
- Positions sensitive detectors photodiodes

Trap design SINGLE BEAM

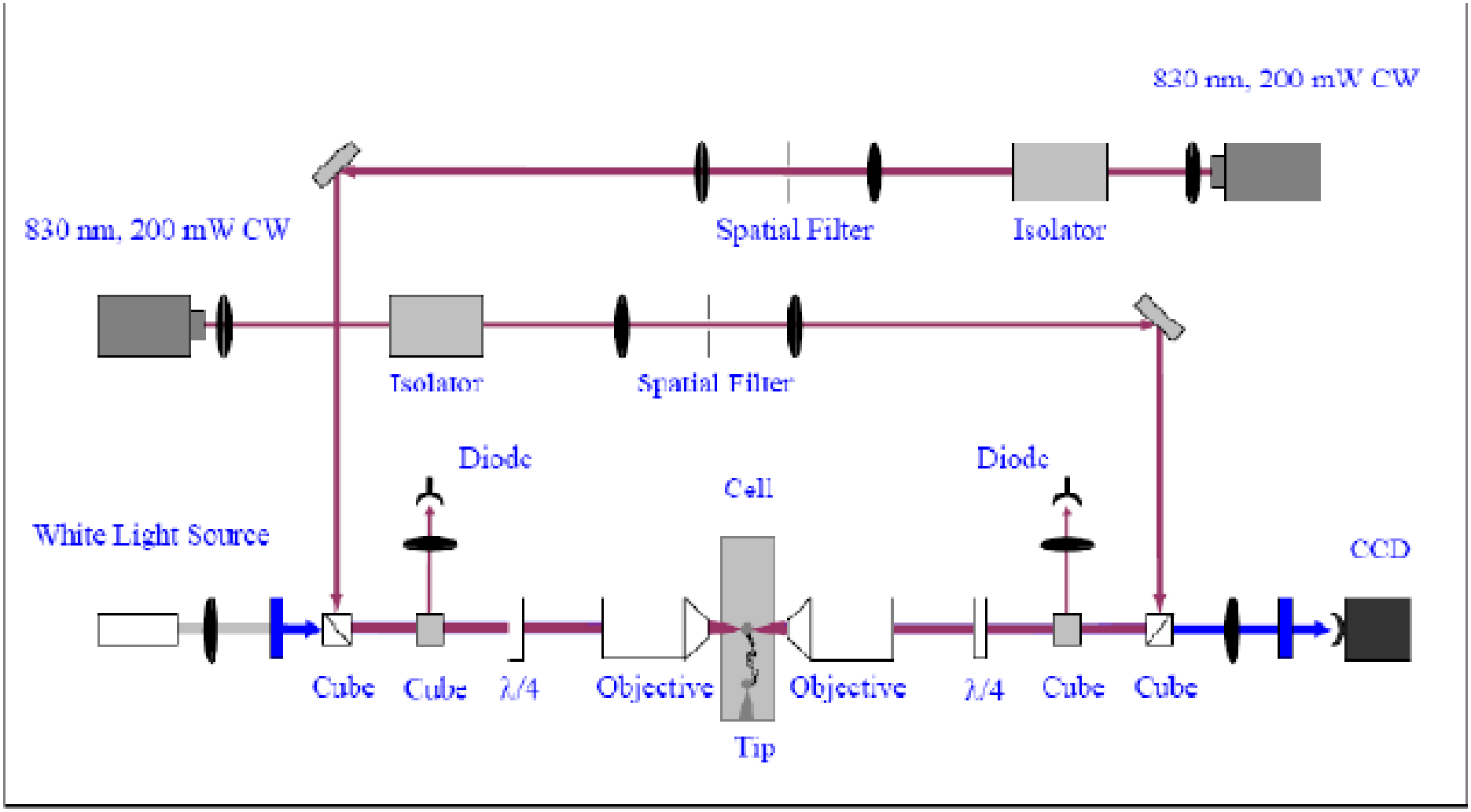




…and how it looks in real life



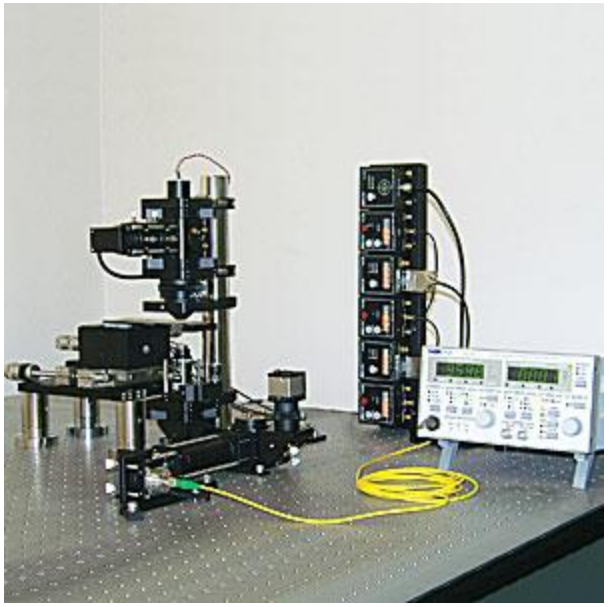
Trap design DUAL BEAM



Commercially available systems

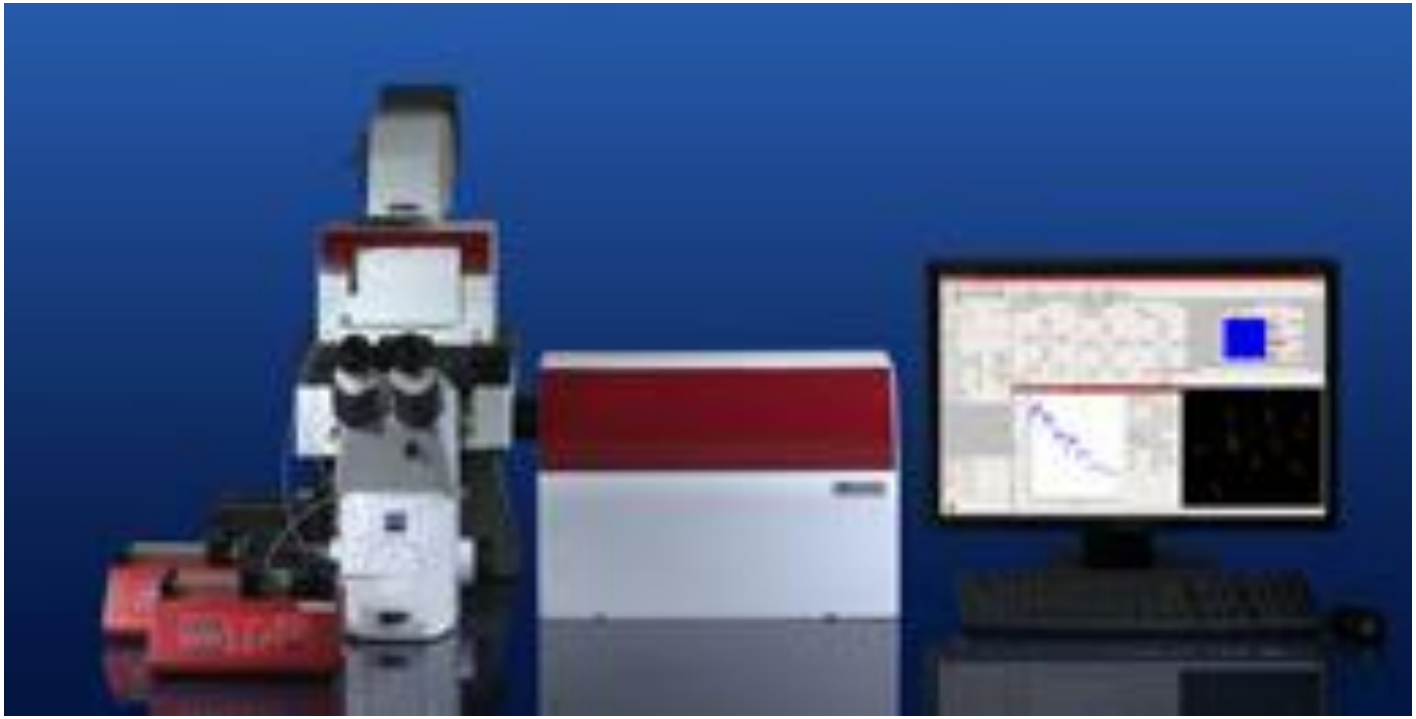
- Thorlabs 20 000 Euro

“Trapping Kit is a carefully selected collection of components with which an optical trap can be constructed. The advantage to purchasing and assembling this kit is the flexibility it provides over optical trap systems built using a traditional microscope. Since the optical trap system is built using standard Thorlabs' lens tube and cage components, it is easy to modify or upgrade the system using other standard components



Commercially available systems

- JPK Nanotracker 400 000 euro



Trapping laser

- Single mode (TEM00 Gaussian) 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 practice 1mW to 1 W in specimen plane

- Wavelength
  - – Optical damage to biological specimen
  - – Microscope objective transmission
  - – Available power

Trapping laser

Continuous-wave (CW) diode-pumped Nd:YAG laser (1064 nm) or its close relatives, Nd:YLF (1047 nm) and Nd:YVO4 (1064) nm). These represent the most economical choices to achieve the requisite power (1-10 W) and output stability.

But other sources suitable for optical trapping exist. Recent years have seen the emergence of high intensity, single-mode diode lasers, available in the wavelength region from 700-1500 nm, with powers up to ~1 W. Diode lasers possess exceptional amplitude stability, and are more economical than Nd-based lasers.

` Another option is the CW Ti:sapphire laser, which affords continuous tuning through much of the near infrared region (700-1000 nm) along with high output power. However, it requires a separate pump source, typically suffers reduced amplitude stability, and is far-and-away more costly than the alternatives

For now, Nd-based lasers continue to dominate the optical trapping field, but sources at other wavelengths may represent more advantageous

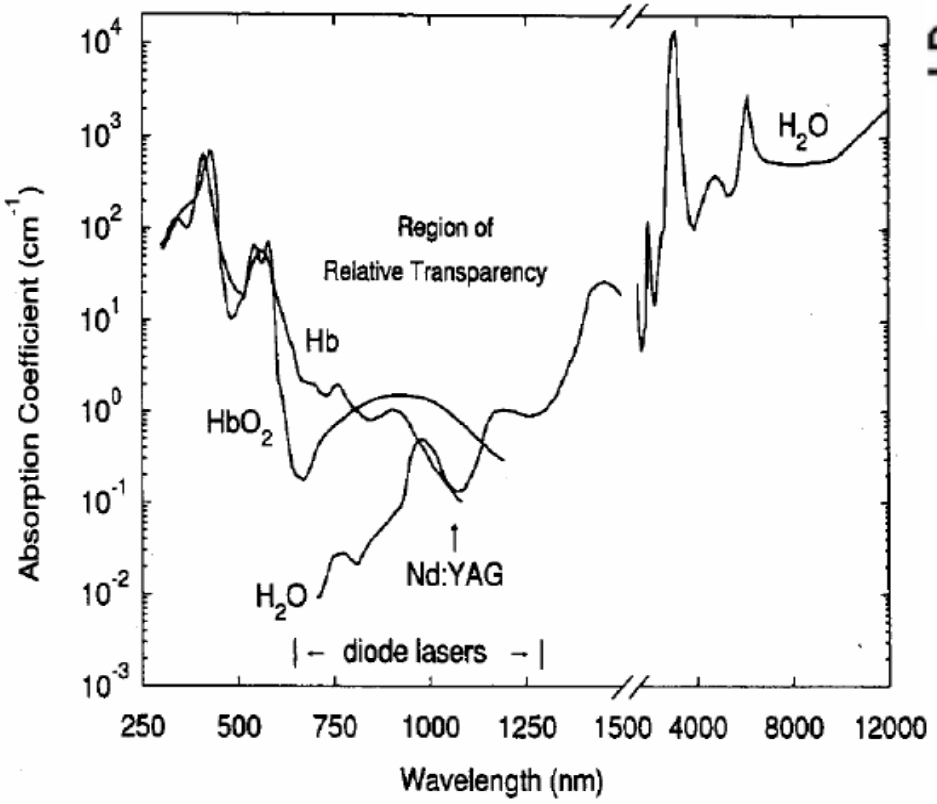
- choices for reducing photodamage

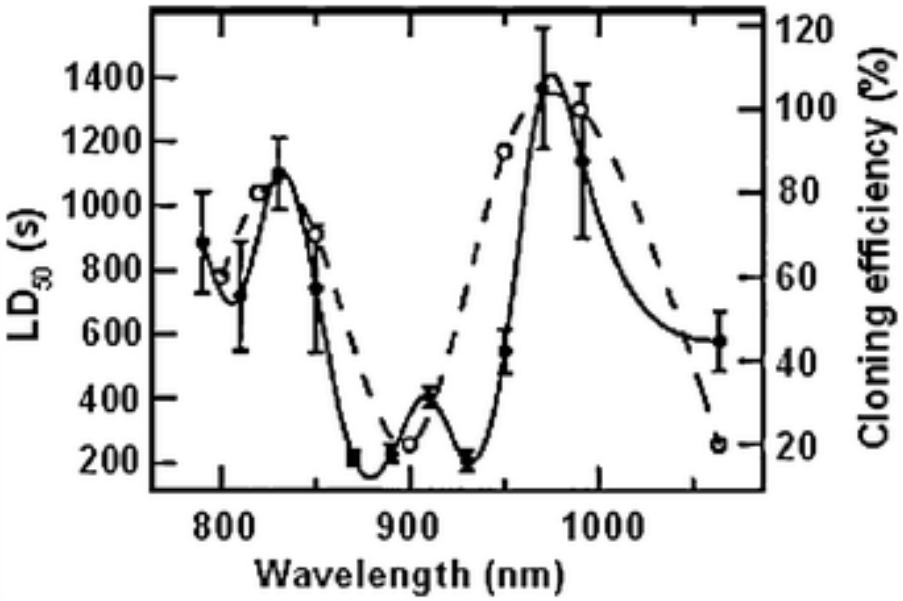
neodymium-doped yttrium aluminium garnet Nd:$Y_3Al_5O_{12}$

Optical damage

- Biological specimens are relatively transparent in the near infrared
- (750 –1200 nm)

- Damage minimum 830 and 970 nm





Relationship between wavelength and cell photodamage for *E. coli* (solid line and left axis) and CHO cells (dashed line and right axis). The higher the LD_{50} and % cloning the less damage the laser causes for a given wavenumber..

Microscope objective

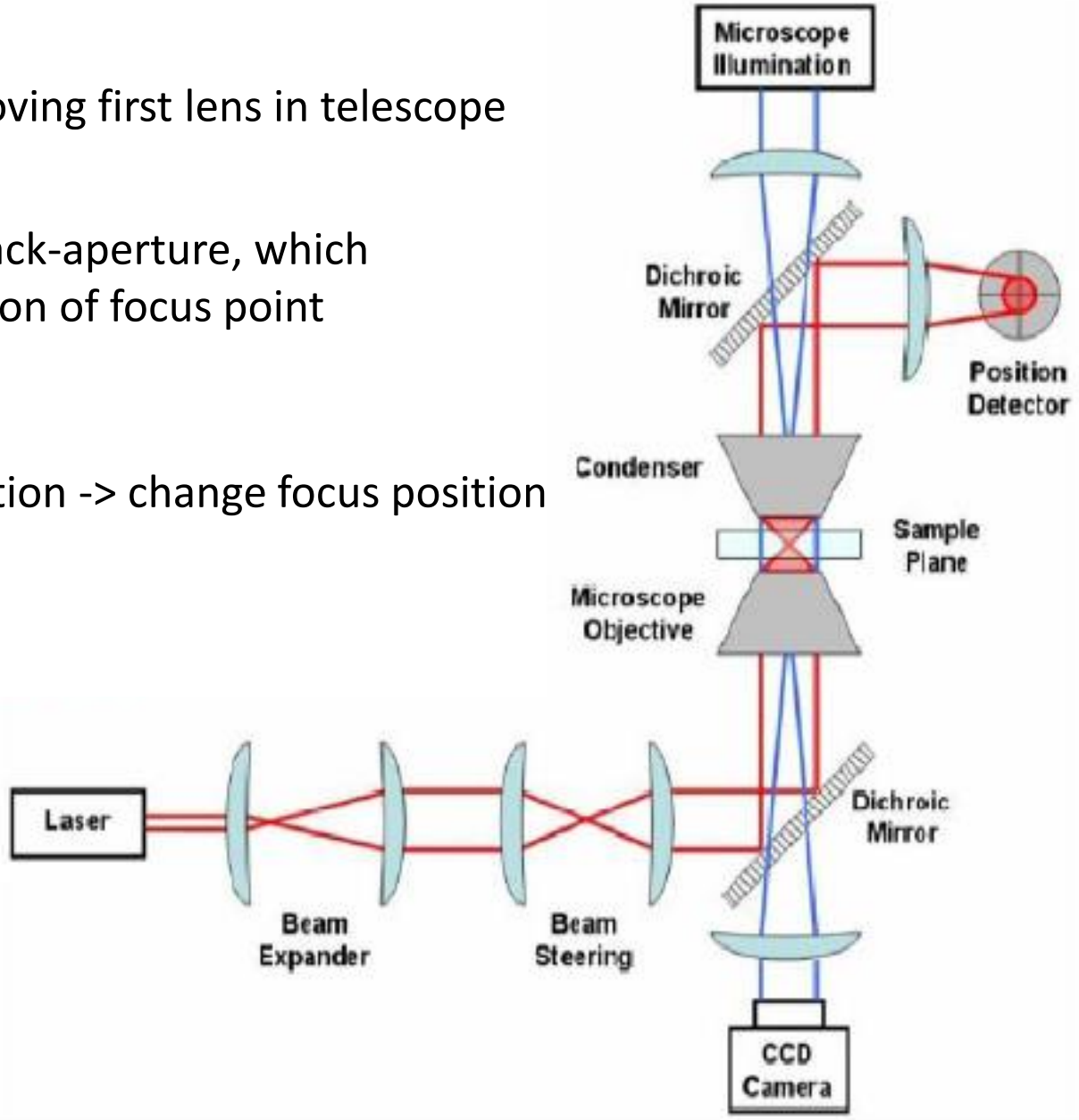
- High numerical aperture objective (NA =1.2 – 1.4)
- High NA through Oil or water immersion
  - Spherical aberration degrades performance
  - Water immersion objectives are better
- Transmission at trapping wavelength
  - NIR transmission
  - Dual-objective method to measure transmission

Objectives

Part number	*Manufacturer*	*Magnification/ Tube length (mm)/ Numerical aperture*	*Type designation*	*Transmission (±5%)*			
				830 nm	*850 nm*	*990 nm*	*1064 nm*
461832	Zeiss	63/160/1.2 Water	Plan NeoFluar	66	65	64	64
506038	Leica	100/∞/1.4-0.7 Oil	Plan Apo	58	56	54	53
85020	Nikon	60/160/1.4 Oil	Plan Apo	54	51	17	40
93108	Nikon	60/∞/1.4 Oil	Plan Apo CFI	59	54	13	39
93110	Nikon	100/∞/1.4 Oil	Plan Apo CFI	50	47	35	32
93110IR	Nikon	100/∞/1.4 Oil	Plan Apo IR CFI	61	60	59	59
93144	Nikon	100/∞/1.3 Oil	Plan Fluor CFI	61	59	-	61

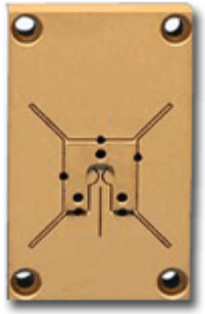
3D trap positioning

- 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



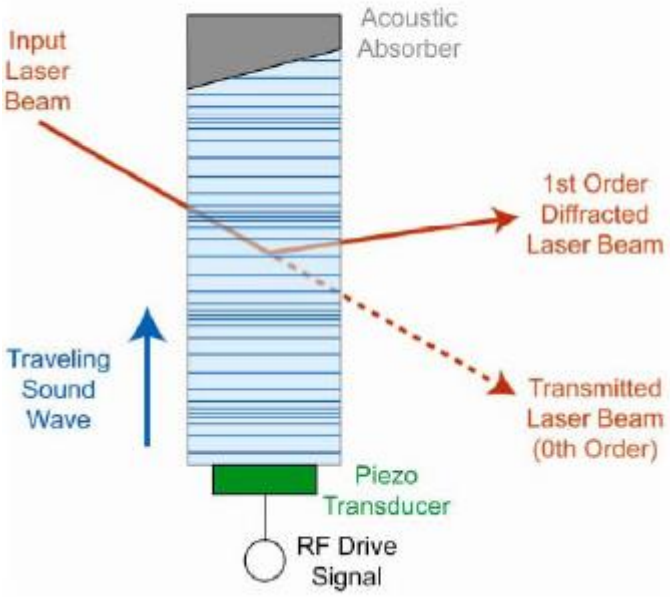
3D trap positioning

- 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

The laser beam is deflected by sound waves in the TeO_2– crystal inside the AOD.



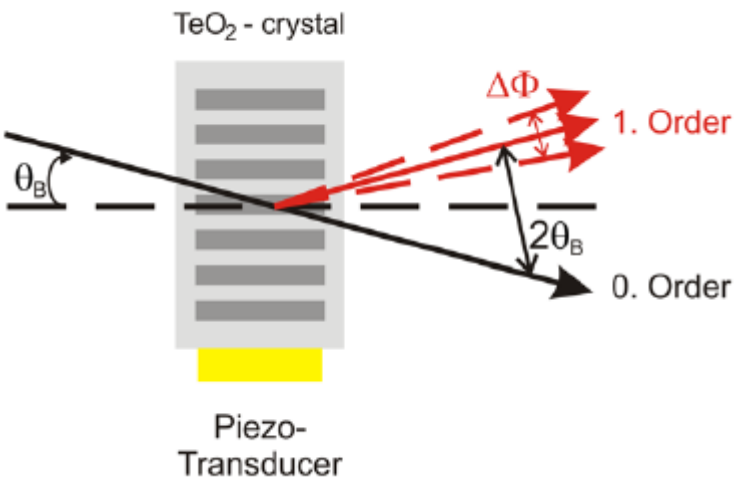
AOD operation

- An **acousto-optic deflector** AOD spatially controls the optical beam. In the operation of an acousto-optic deflector the **power driving** the acoustic transducer is kept on, at **a constant level**, while the **acoustic frequency** is **varied** to deflect the beam to different angular positions. The acousto-optic deflector makes use of the acoustic frequency dependent diffraction angle, where a change in the angle $\Delta\theta_d$ as a function of the change in frequency Δf given as

$$(12)\ \Delta\theta_d = \frac{\lambda}{\nu}\Delta f$$

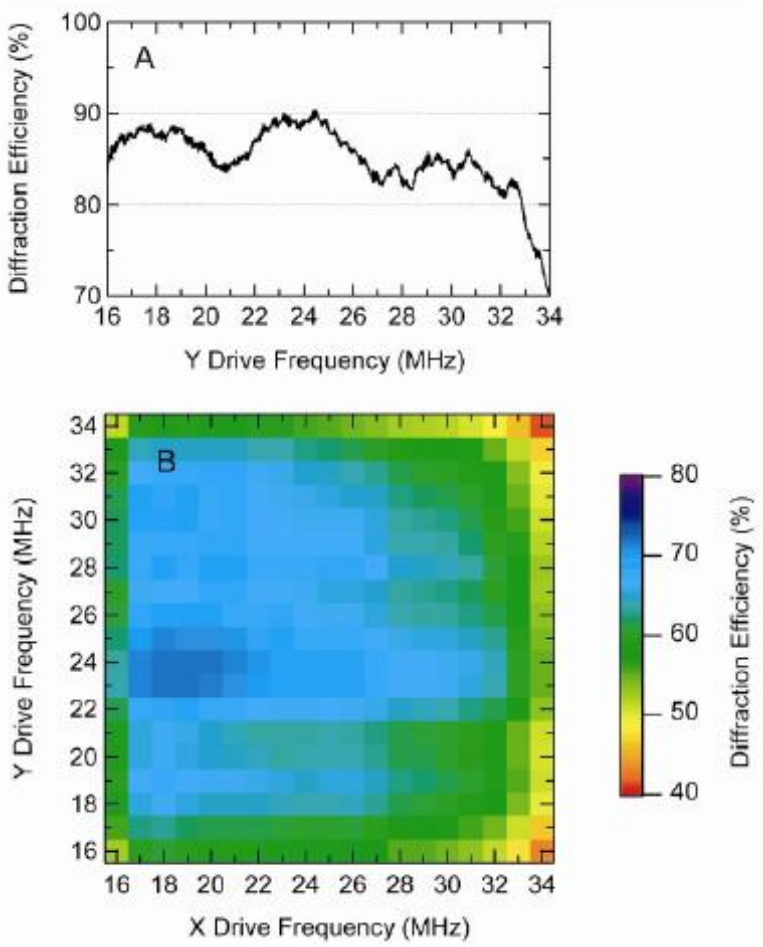
where λ is the optical wavelength and v is the velocity of the acoustic wave.

In AOM modulator power is varied while acoustic frequency is kept constant



- 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



Bragg-condition $\sin \vartheta_B = \frac{\lambda}{\Delta}$

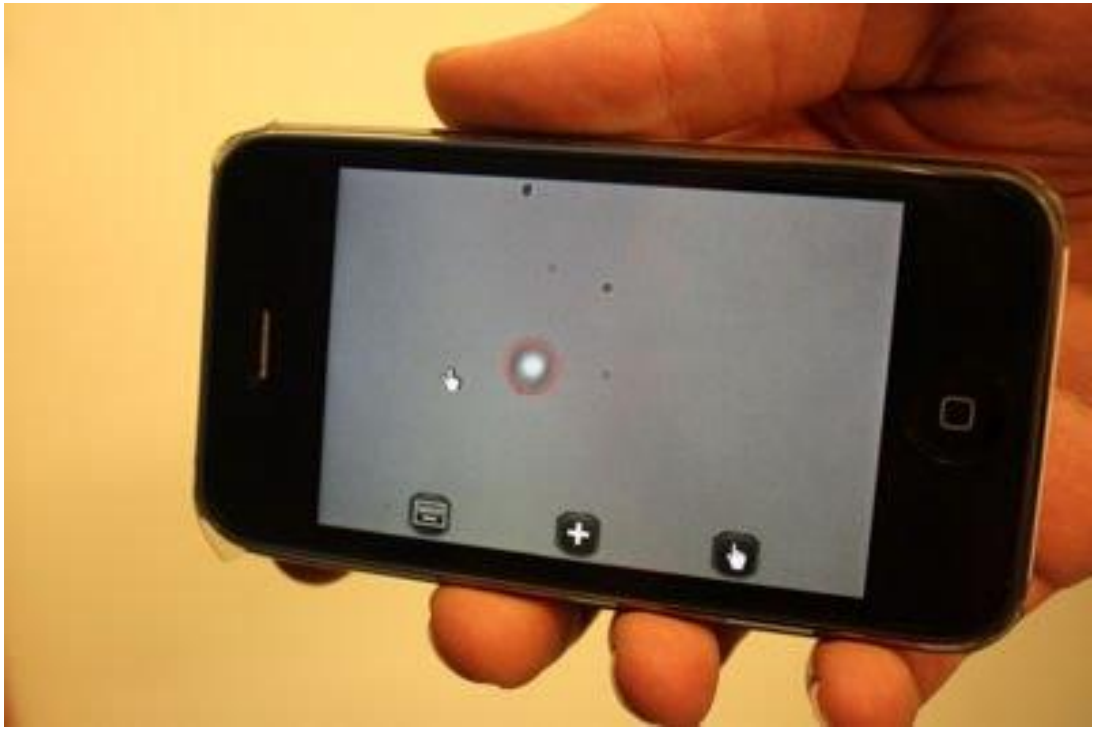
If the laser wavelength matches the Bragg-condition the first order of the incoming laser beam with wavelength λ is deflected with angle $2\Theta_B$

a change of a materials permittivity, ε,

due to a mechanical strain

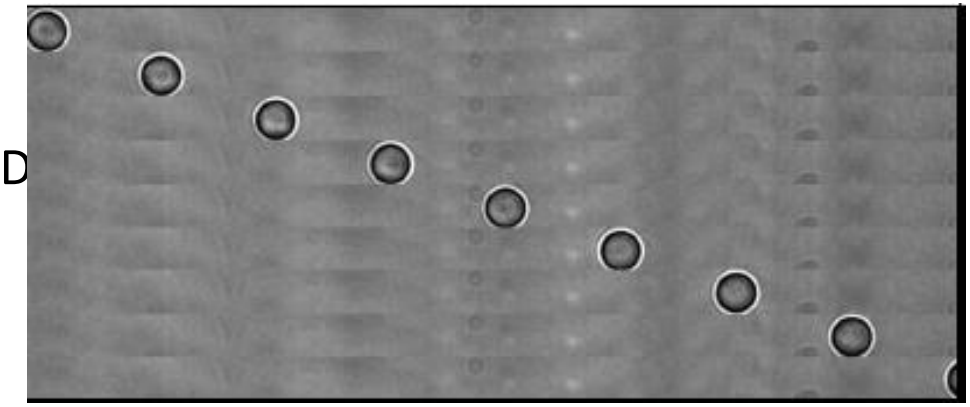
Time-shared traps with AODs

Holo-assembler

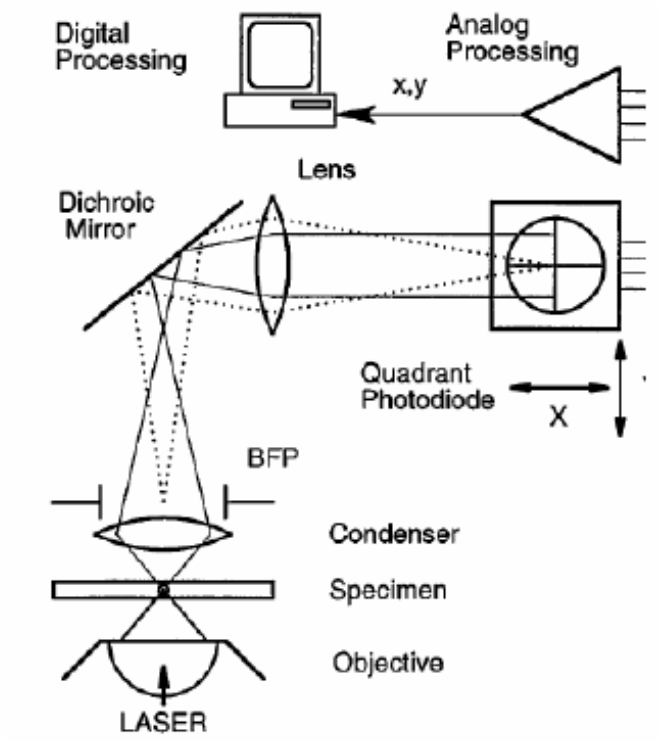


Position detection

- Video tracking
  – Slow
  -Data bandwidth needed for a 1kx1k CCD
  -1000x1000x8bit + processing
  -30-120Hz limited by video rate)
  - Absolute position with
  -10 nm position



- Laser based Back-focal-plane detection
  -Fast (100 kHz)
  -Relative position (bead – focus)
  -1nm or better resolution

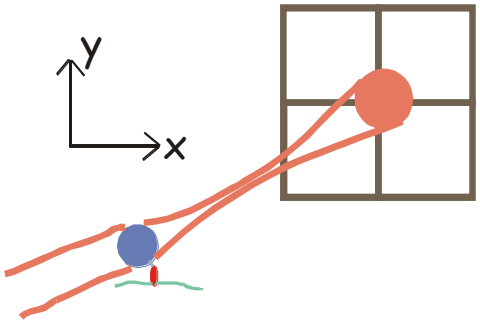


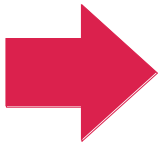
Laser based Back-focal-plane detection

- 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.

- Laser light (slightly) focused by trapped bead. Changes in position of bead gives rise to changes in light intensity impinging on the quadrant photodiode. Changes in position in plane perpendicular to direction of propagation of laser light: Appropriate sums and differences of the signal from the four quadrants.

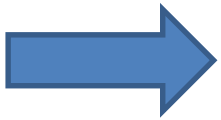
- Changes in position in direction of propagation of laser light:
- Sum of signal in all four quadrants (also used to normalize x and y signal).





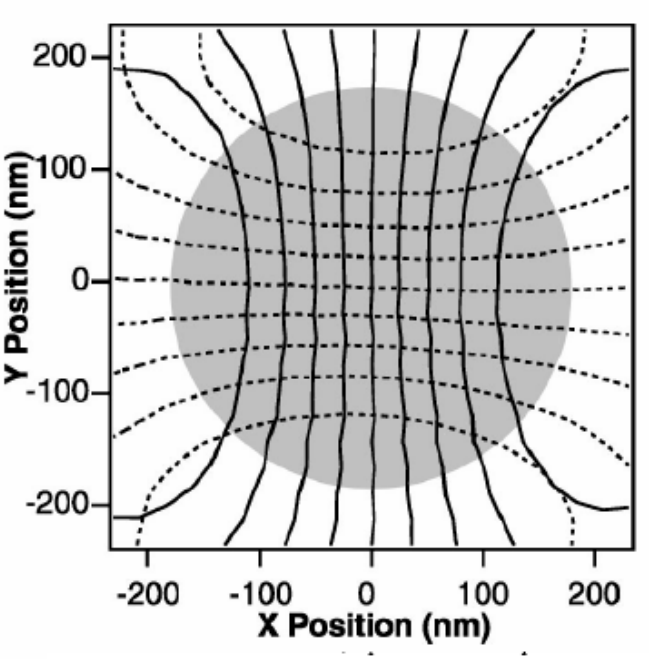
$(x(t), y(t))$

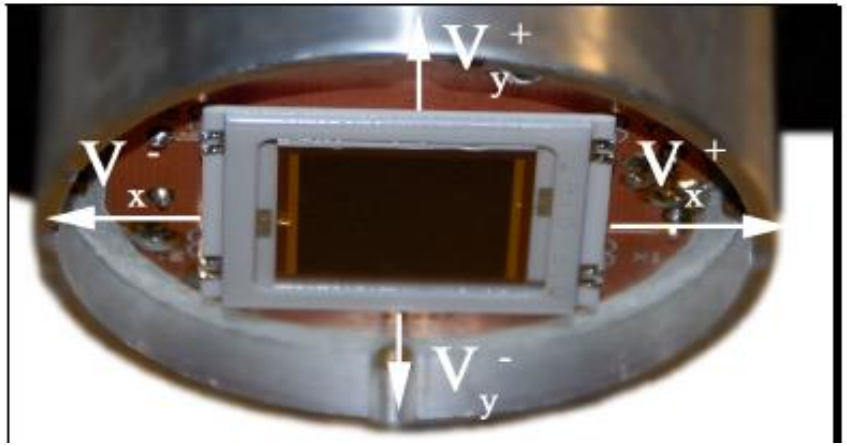
Precise position measurement

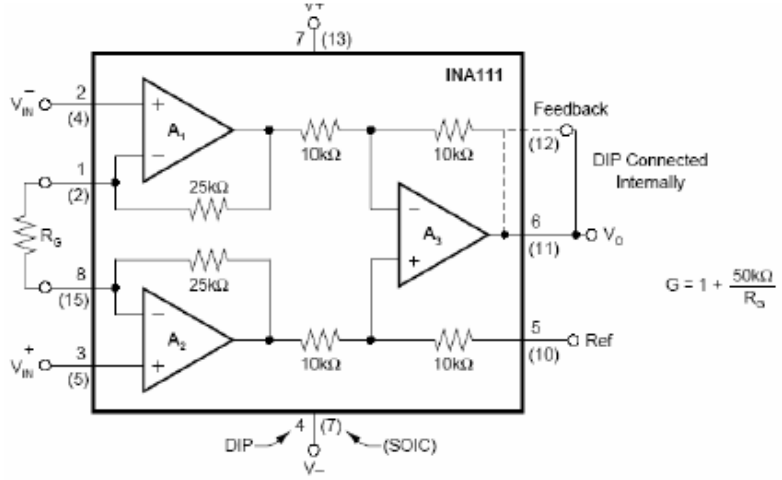


force measurement

Laser based Back-focal-plane detection





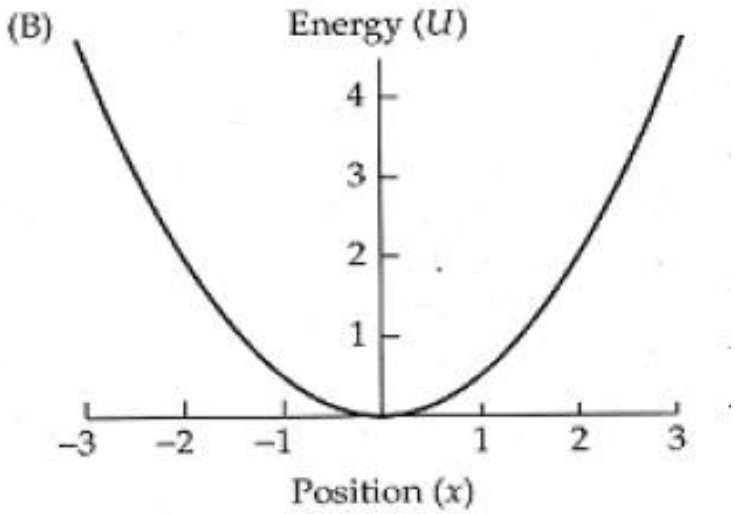


Variable gain: 1-1000

- PSD converts incoming light to four continuous photocurrents, two for each lateral direction (X and Y), that are converted to voltages via electronic converters.
- It is thereby possible to measure the two lateral directions simultaneously, X(+ -Vx,Vx) and Y(+ -Vy,Vy)

Calibration Position Calibration

- The Tweezers potential is Harmonic



- Force (F) is proportional to displacement
- Detected voltage (V) is proportional to displacement (x) of bead from beam focus
- Two calibration parameters:

$$F = kx$$

$$x = \beta V$$

Force Calibration: Theoretical Power Spectrum

Eq. of motion for a Brownian particle in harmonic potential

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

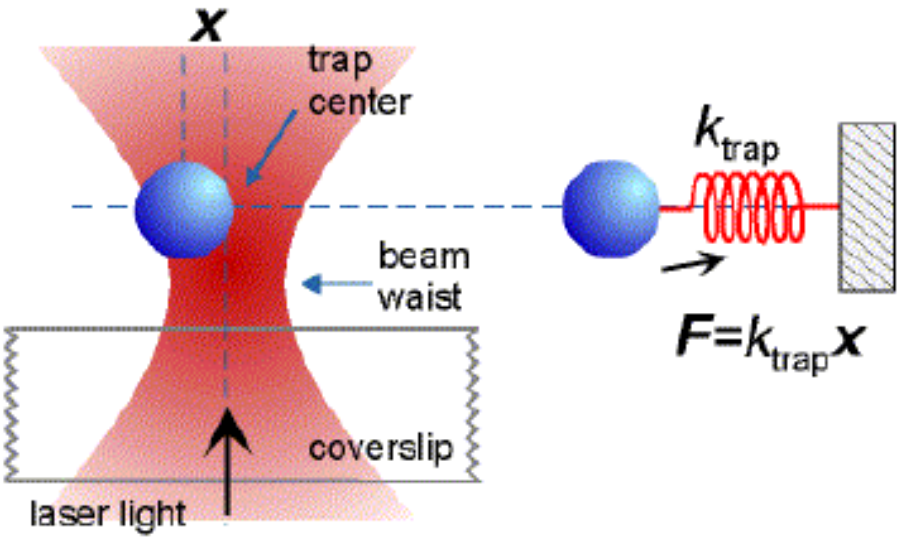
Low Reynolds number (10^{-3}): inertia of bead may be neglected

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

$\gamma = 6\pi r\eta =$ Stokes drag

$k =$ trap stiffness

$F(t) =$ random thermal forces



Fourier transform gives Power Spectrum

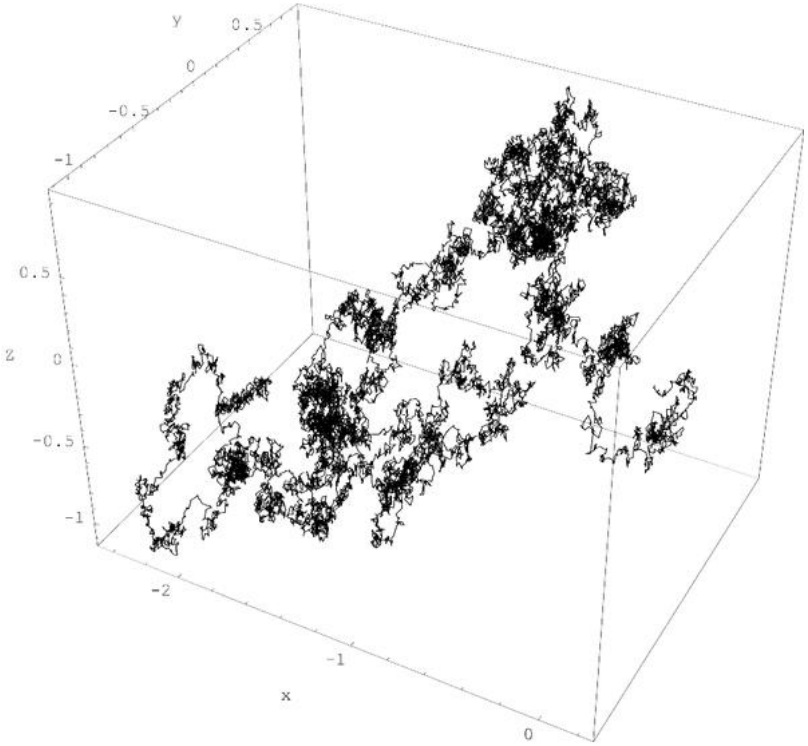
$$0 = -2\pi i\gamma f\tilde{x}(f) - \kappa\tilde{x}(f) + \tilde{F}(f)$$

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

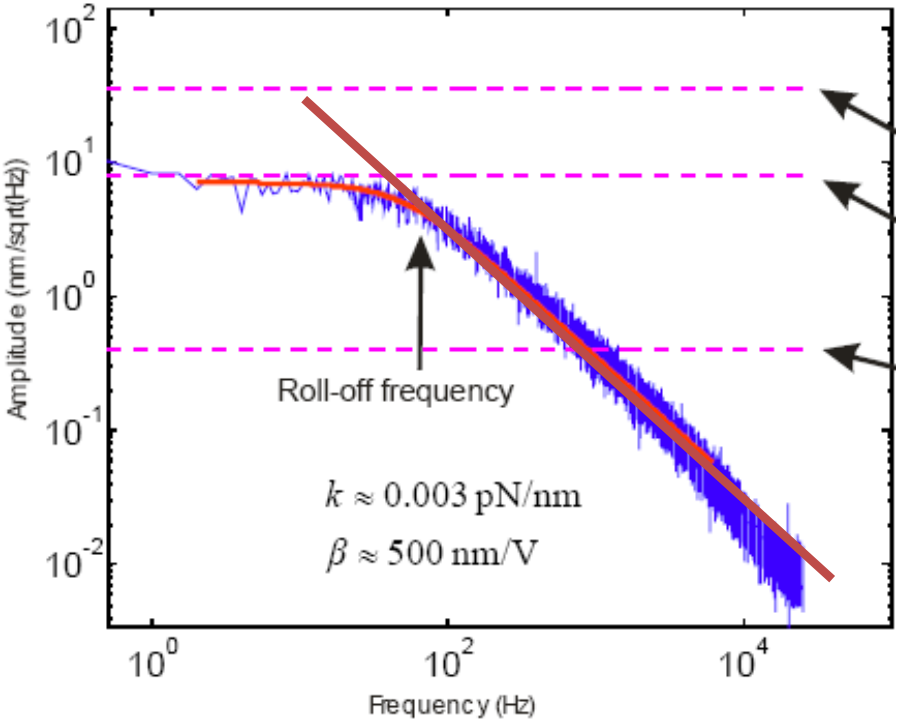
$$S_{xx}(f) \equiv \left\langle \left|\tilde{x}(f)\right|^2 \right\rangle = \frac{\left\langle \left|\tilde{F}(f)\right|^2 \right\rangle}{(2\pi\gamma)^2\left(f^2 + f_c^2\right)} \propto \frac{1}{f^2 + f_c^2}$$

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

How does the Power Spectrum of a trapped bead look like?



- Brownian motion



3 D Brownian motion

$S_{xx}(f) =$ falls of as $\frac{1}{f^2}$

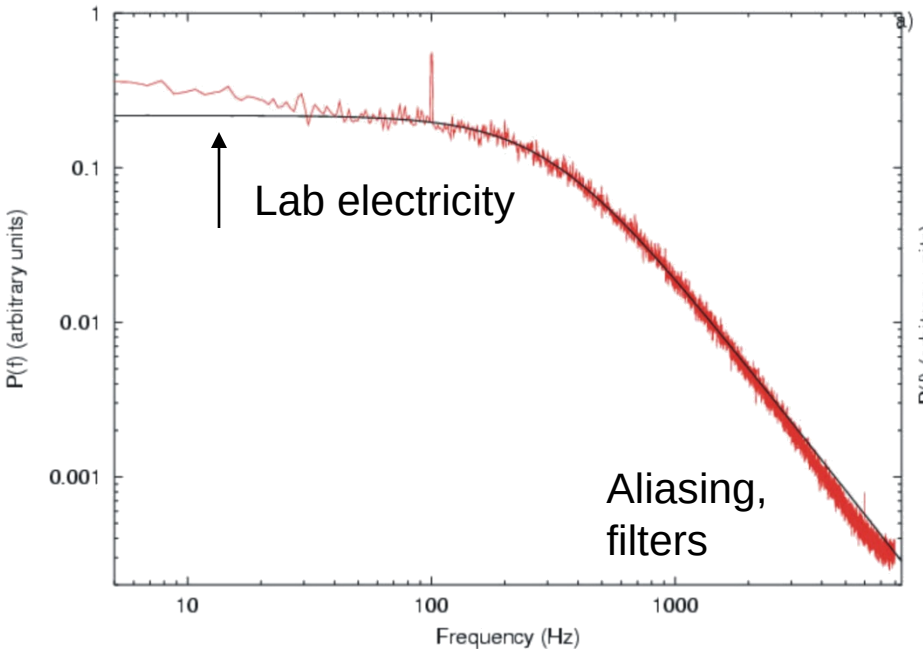
Fit, and find corner frequency, $f_c = \kappa/(2\pi\gamma)$, to determine κ

Harmonic trapping potential: Gaussian distribution of positions.

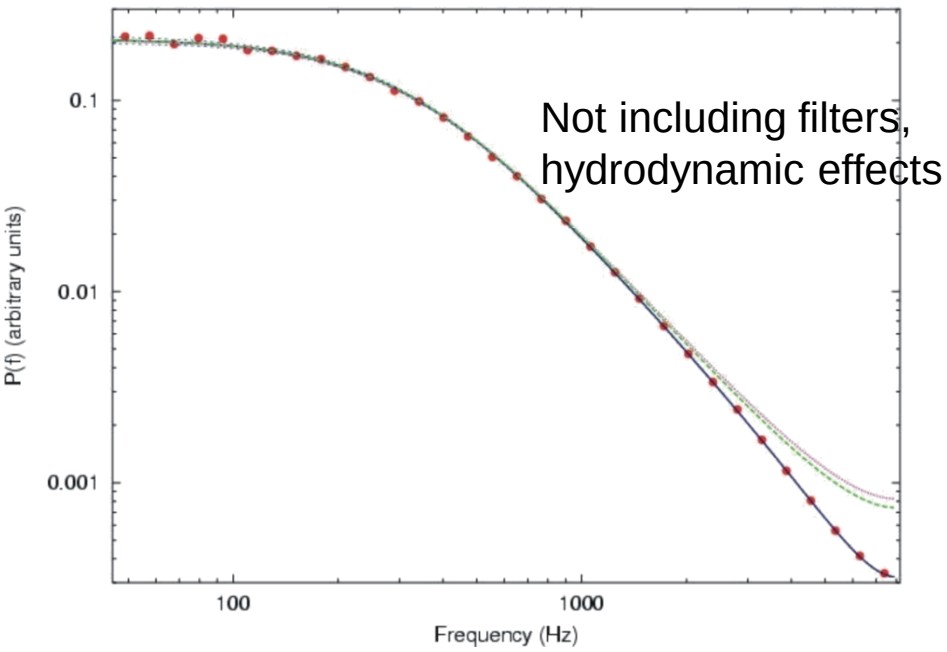
Allows for absolute calibration of photodiode (mV $\leftrightarrow$ nm).

Force Calibration: In practice

Without, f_c = 309 Hz

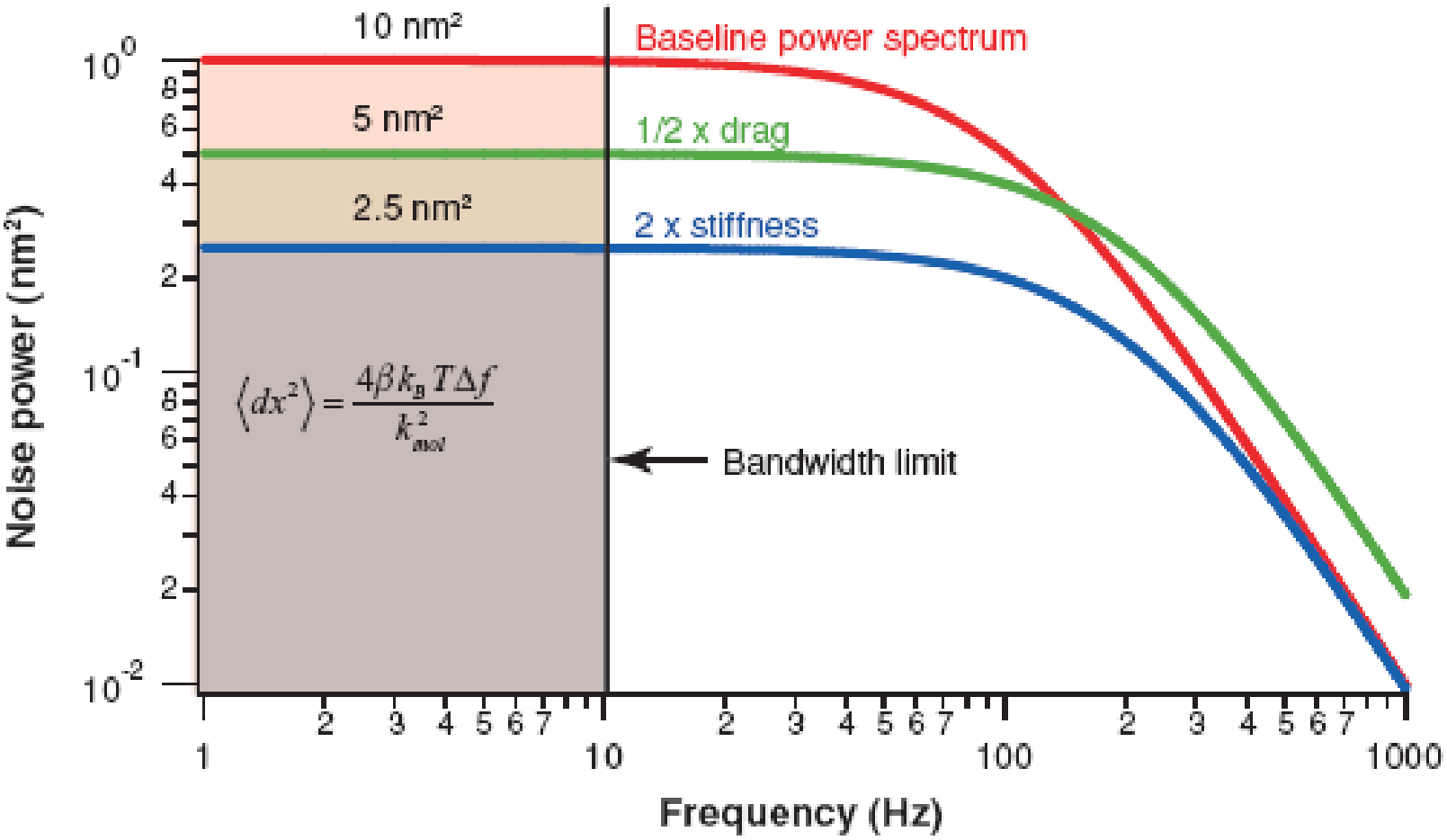


With, f_c = 320.4 ±1.6 Hz



When a simple Lorentzian fit is made the various errors might cancel

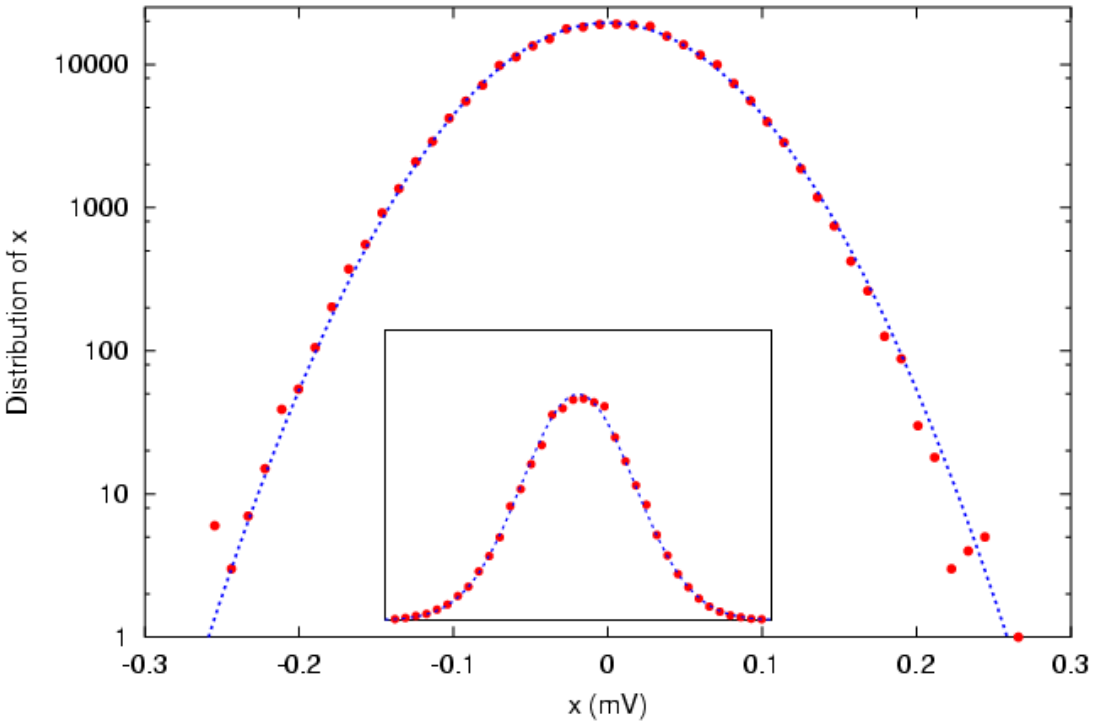
Position Power Spectra



Calibration: Equipartition Theorem

- Position histogram
- Model independent data analysis!
- Equipartition Theorem:

$$\left\langle x^2 \right\rangle = \frac{k_B T}{k}$$



In any physical system in thermal equilibrium, every particle has exactly the same average kinetic energy, (3/2)kBT .
The Ek is shared equally among all of its independent parts, on the average, once the system has reached thermal equilibrium.

Histogram: Gaussian shape, consistent with harmonic potential.

Force calibration problems and other technical issues

- Detection bandwidth
- Unintended signal filtering
- Anti-aliasing
- Drag coefficient (Faxens law)

  – Stokes law OK only when we are infinitely far away from surfaces

Temperature gradients

- Acoustic vibration
  – *Power supplies etc. outside room*
  – *Music and voices easily coupled to trap*
- Mechanical vibration
  – *Short optical path*
  – *Damped table*
- Air currents

Setting up *"good enough" optical* tweezers is not that trivial

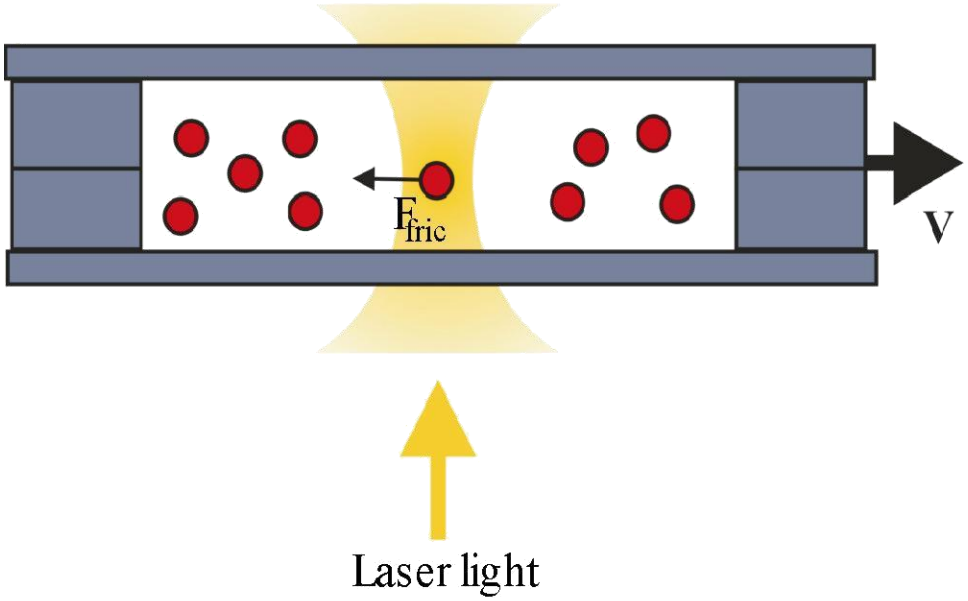
Force calibration, drag-force

- Chamber moved at a given velocity, v. Frictional force, Ffric known (Stoke's law):

$$\vec{F}_{fric} = \gamma \vec{v}$$

$$\gamma = 6\pi r\eta$$

- Observe at which velocity the bead escapes from the trapping laser light. This is the highest force felt by the bead from the trap.

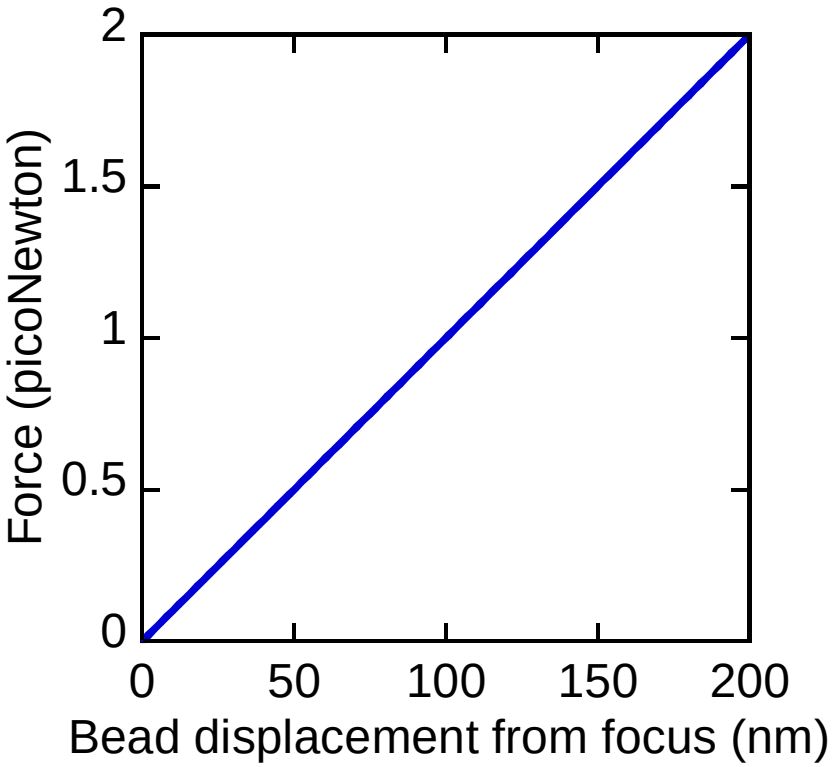


Drag-force method
– Move stage or create flow to push bead out of trap
– Triangle-waveform stage motion

– Check previous calibration, check for Nonlinearity

– Proximity to surfaces is a problem -> Faxens law instead of Stokes law

Characteristics of optical traps



- Forces are linearly related to the object displacement.
- The slope of the force-displacement curve is called the stiffness of the optical trap (in N/m).
- The stiffness dependence on the bead size and shape and the laser power.

Micrometer sized glass or polystyrene beads are commonly used as attachment handles of the materials under investigation.

The advantage of this approach is the clear and uniform interaction between the beads and the laser beam.

Characteristics of optical traps

Typical stiffness: 100pN/micrometer

Typical displacements: 1-500 nm

Typical forces: 0.1-100 pN

Measurable speeds: ~1 kHz

Comparison of forces with other techniques and biological processes:

Optical traps	10^{-13}-10^{-10} N
Electric fields (electrophoresis)	0-10^{-12} N
AFM	10^{-11}-10^{-7} N

Kinesin step	3-5 pN
RNA polymerase stalling	15-30 pN
Virus motor stalling	~50 pN
DNA conformational change	~65 pN
Biotin-streptavidin binding	300-400 pN

Optical traps for Biophysics

- **Pro's:**

  Remote manipulation of biomolecules

  Measurable forces and distances are well suited for enzyme dynamics and molecular motors

  They work in normal buffer conditions

- **Con's:**

  Radiation damages of samples

  Slow throughput

  need for handle design

Suggested further reading

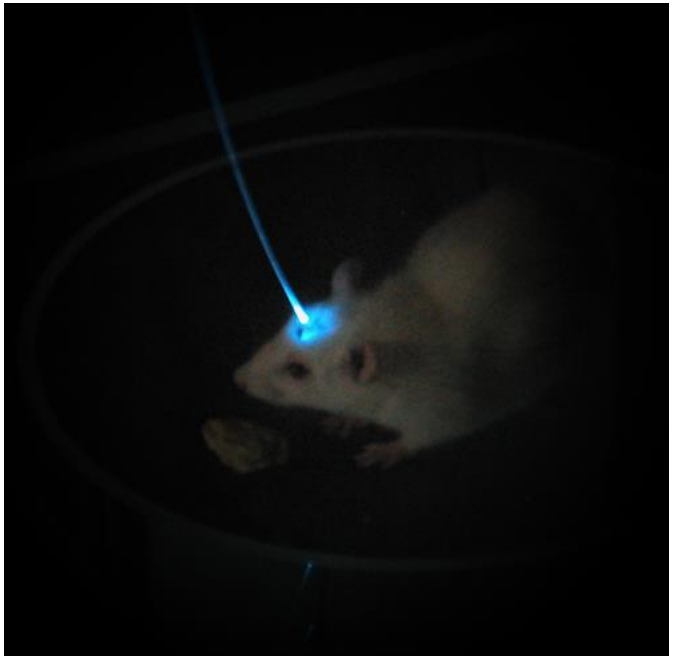
- ‘Optical trapping’, K. C. Neuman & S. M. Block. Rev. Sci. Instrum. 75(9) 2787-2809 (2004)
- ‘Lights, action: optical tweezers’, J. E. Molloy & M. J. Padgett. Contemp. Phys. 43(4) 241-258 (2002)
- ‘Signals and noise in micromechanical measurements’, F. Gittes & C. F. Schmidt. Methods in Cell Biology 55 129-156 (1998)
- The ‘Holoassembler’: www.holoassembler.com. State-of-the art micro and nanomanipulation with fingertip control!

Optogenetics

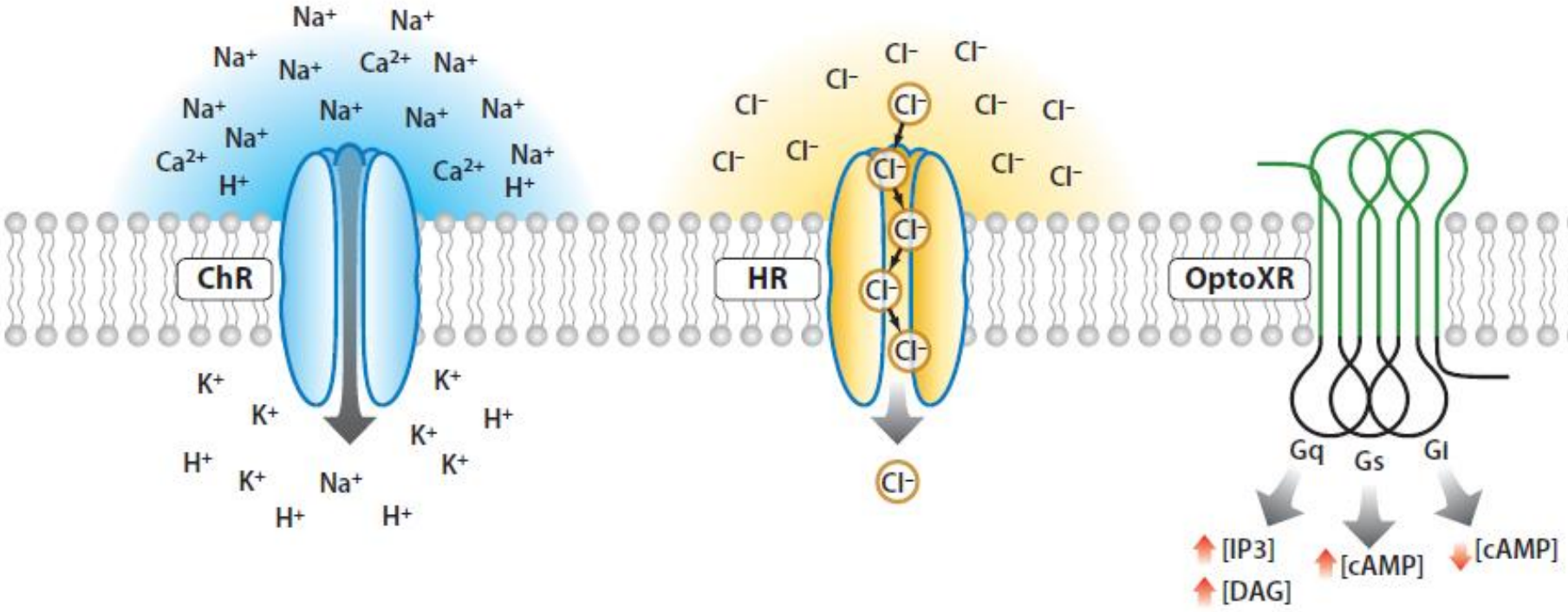
- *¨A method by which all neurons of just one type could be inactivated, leaving the others more or less unaltered ¨*
- *Francis Crick 1979*

- Refers to the integration of optics and genetics to achieve gain- or los-of-function of well-defined events within specific cells of living tissue
- Allows optical control of a particular type of neuron - can express light activated ion channels (or receptors) under a specified promoter.

- Fast excitation: Channelrhodopsin2
- Fast inhibition: Holorhodopsin
- Bi-stable modulation: step opsins
- Control of intracellular signalling: OptoXRs



Optogenetic tool families

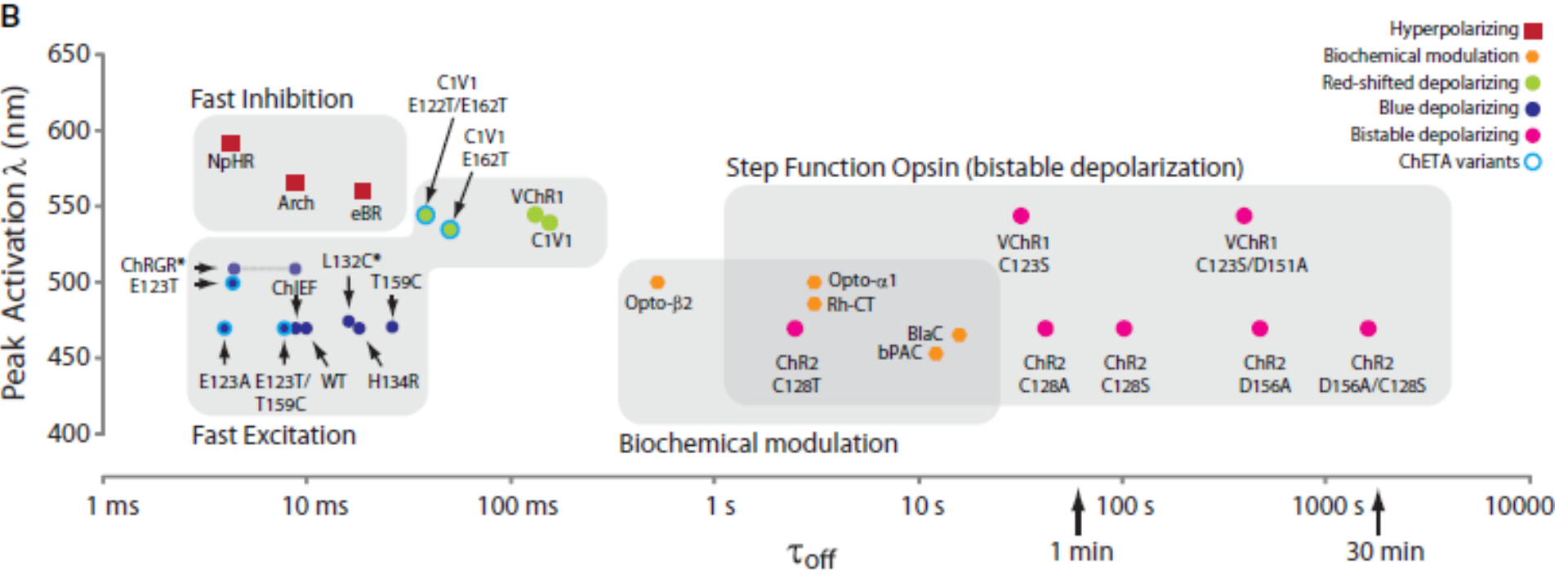


Channelrhodopsins conduct cations and depolarize neurons upon illumination
Halorhodopsins conduct chloride ions into the cytoplasm upon yellow light illuminatio
(*center*).

OptoXRs are rhodopsin-GPCR (G protein–coupled receptor) chimeras that respond to green (500 nm) light with activation of the biological functions dictated by the intracellular loops used in the hybrid

Optogenetics requires

- Engineered control tools that can be targeted to specific cells
- Technology for light delivery
- Methods for integrating optical control with compatible readouts (such as fluorescent organic or genetically encoded activity indicators, electrical recording, fMRI signals, or quantitative behavioural design).
- high-temporal and cellular precision within intact mammalian neural tissue

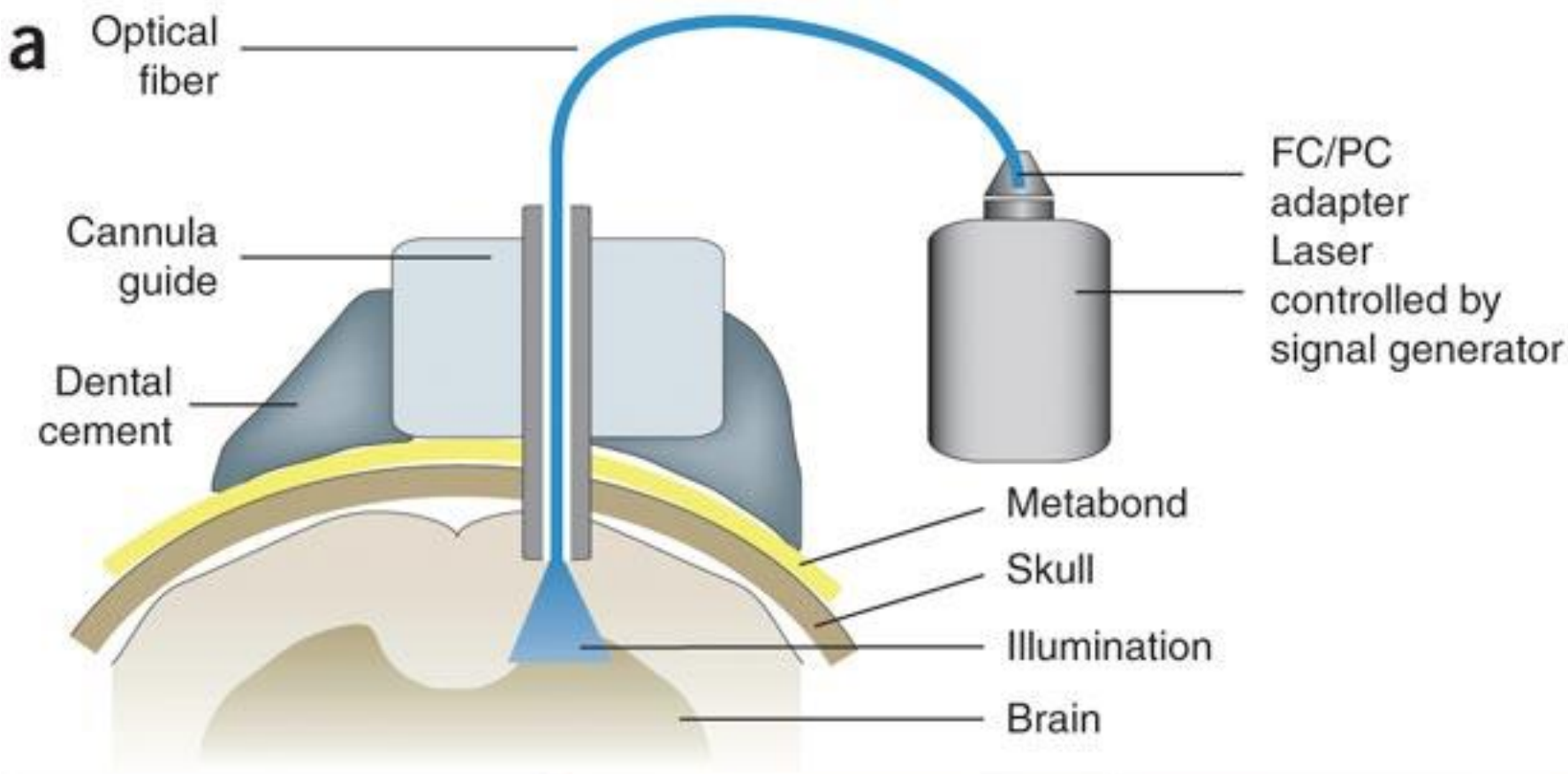


Advantages

- Precisely control one cell type while leaving the others unaltered (Genetically targeted to a specific group of neurons)
- Select activation of neuronal pathways (as opposed to electrical stimulation which activates many neuronal pathways)
- Fast temporal resolution: Millisecond scale precision to keep pace with the known dynamics of the targeted neural events such as action potentials and synaptic currents
- Can be operative within intact systems including freely moving animals. Can directly correlate neural activity in vitro with behaviour

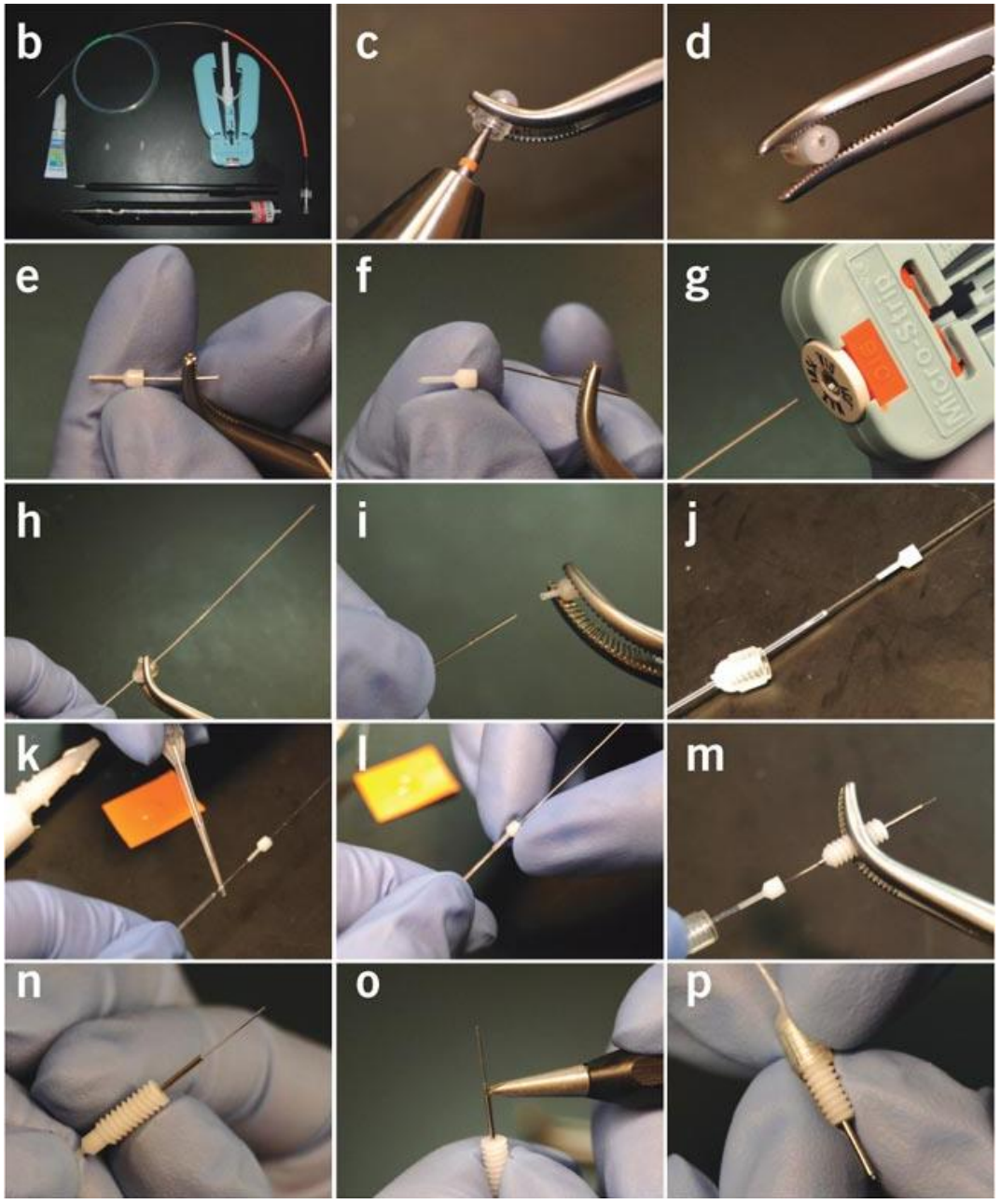
Preparation of the optical fiber for in vivo neural control in mammals

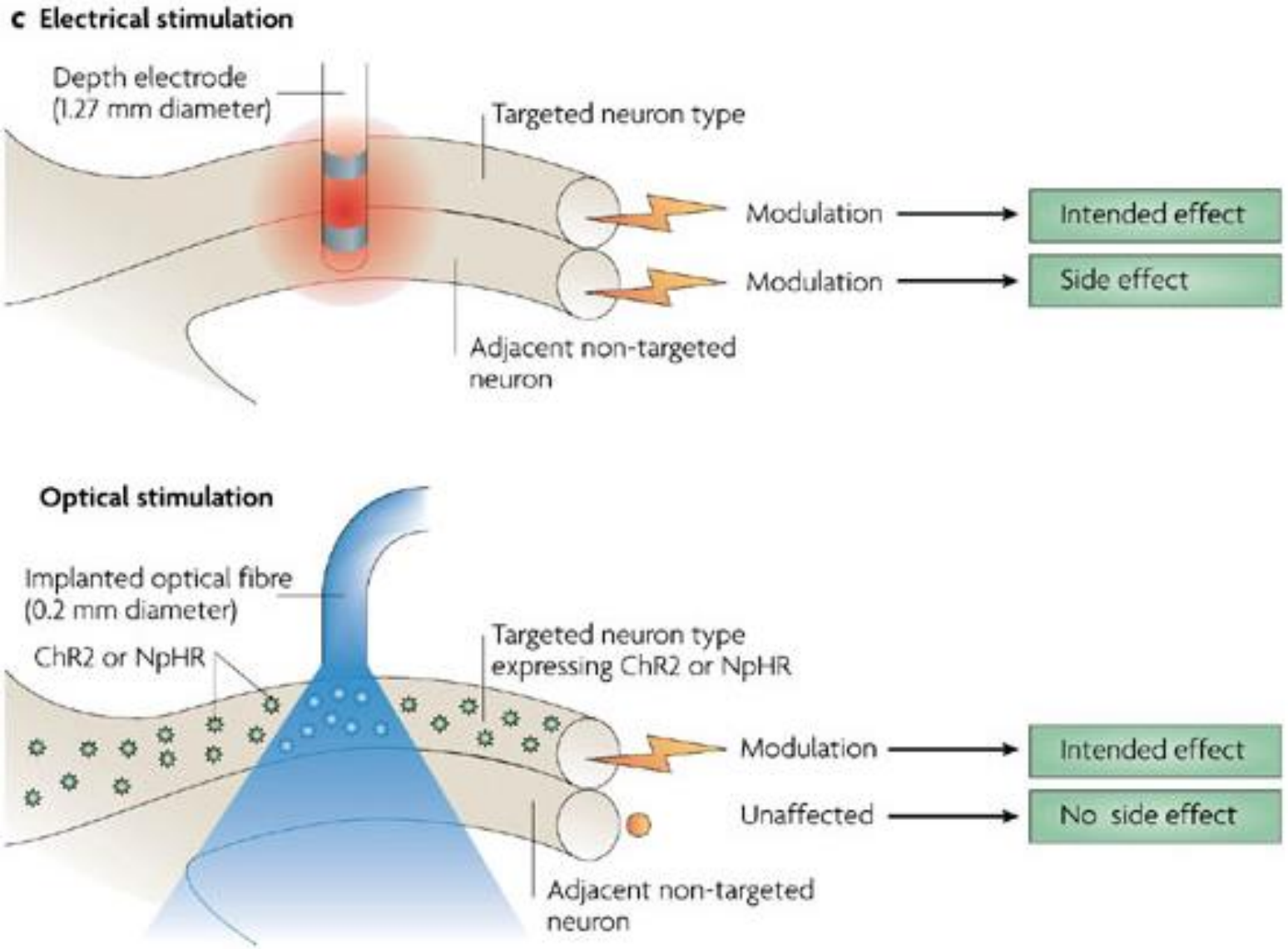
- Diagram of the optical neural interface (ONI), consisting of a stereotactically implanted cannula, an optical fiber, and a solid state laser controlled by a signal generator. The fiber is prepared with the appropriate length to illuminate the target brain region



Preparation of the optical fiber for in vivo neural control in mammals

- Stereotactically implanted cannula guide not only to deliver virus but also to direct an optical fiber to the same brain area of interest (In this approach, the use of a single cannula guide for both viral vector delivery and optical fiber targeting ensures the co-registration of transduced brain area and light illumination.
- The cannula guide is chronically implanted onto the skull of each experimental subject. A dummy cannula (stylet and screw cap) is temporarily inserted into the cannula guide between experiments to prevent clogging and infection.



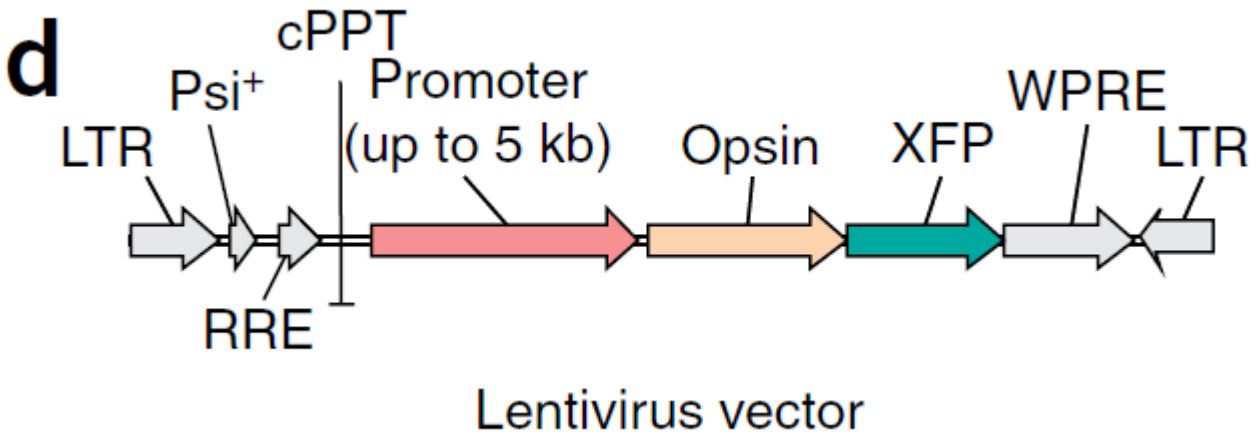

c Electrical stimulation
Depth electrode
(1.27 mm diameter)
Targeted neuron type
Modulation
Intended effect
Modulation
Side effect
Adjacent non-targeted
neuron
Optical stimulation
Implanted optical fibre
(0.2 mm diameter)
ChR2 or NpHR
Targeted neuron type
expressing ChR2 or NpHR
Modulation
Intended effect
Unaffected
No side effect
Adjacent non-targeted
neuron

How do you express opsins in neurons?

- Viral Vectors – a common tool to deliver genetic material to cells
- **Important features:**
- Safety – modified to be replication deficient
- Low toxicity – won't kill the cell after infection
- Stable – won't rearrange its genome
- Cell type specificity – a specific promoter is used to infect a selective group of cells
- Identification – contain some sort of marker to indicate which cells have been infected (GFP

Lentivirus construct:	**Adenovirus construct:**
Good infection rate, expression persist for years (encorporates into genome) Long transfection time (~ 2 weeks) Larger packaging capacity (< 10 kb total length, promoter up to 5 kb Biosafety requirements (PPE, BSL2+ lab)	adenoviral DNA does not integrate into the genome and is not replicated during cell division Lower infection rate, shorter expression time (few weeks) Lower packaging capacity (up to 5 kb total length) Biosafe

Lentivirus Opsin construct



Example:

Promoter = hypocretin promoter (3.086 kb)

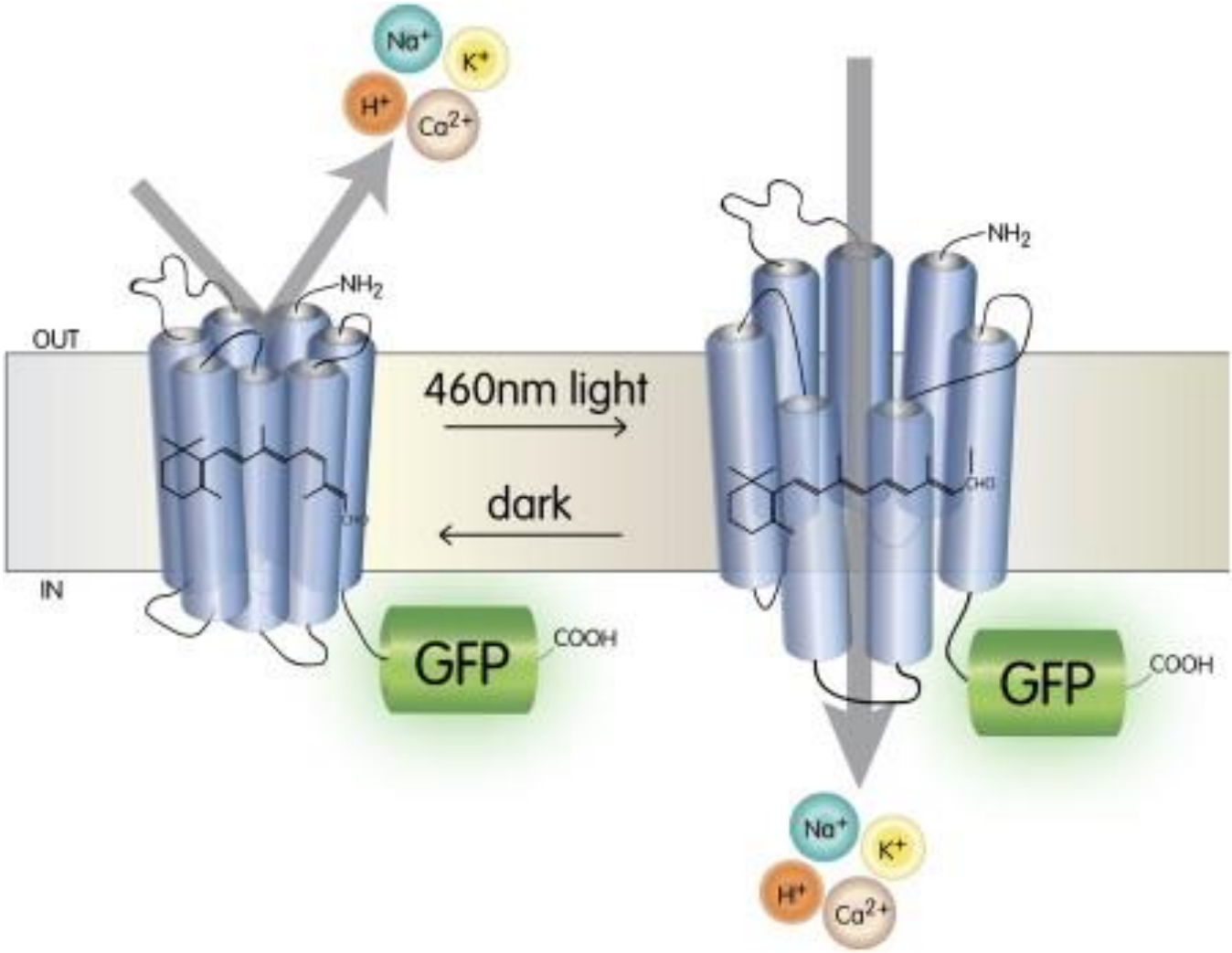
Fluorophore = mCherry (red)

Opsin = ChR2

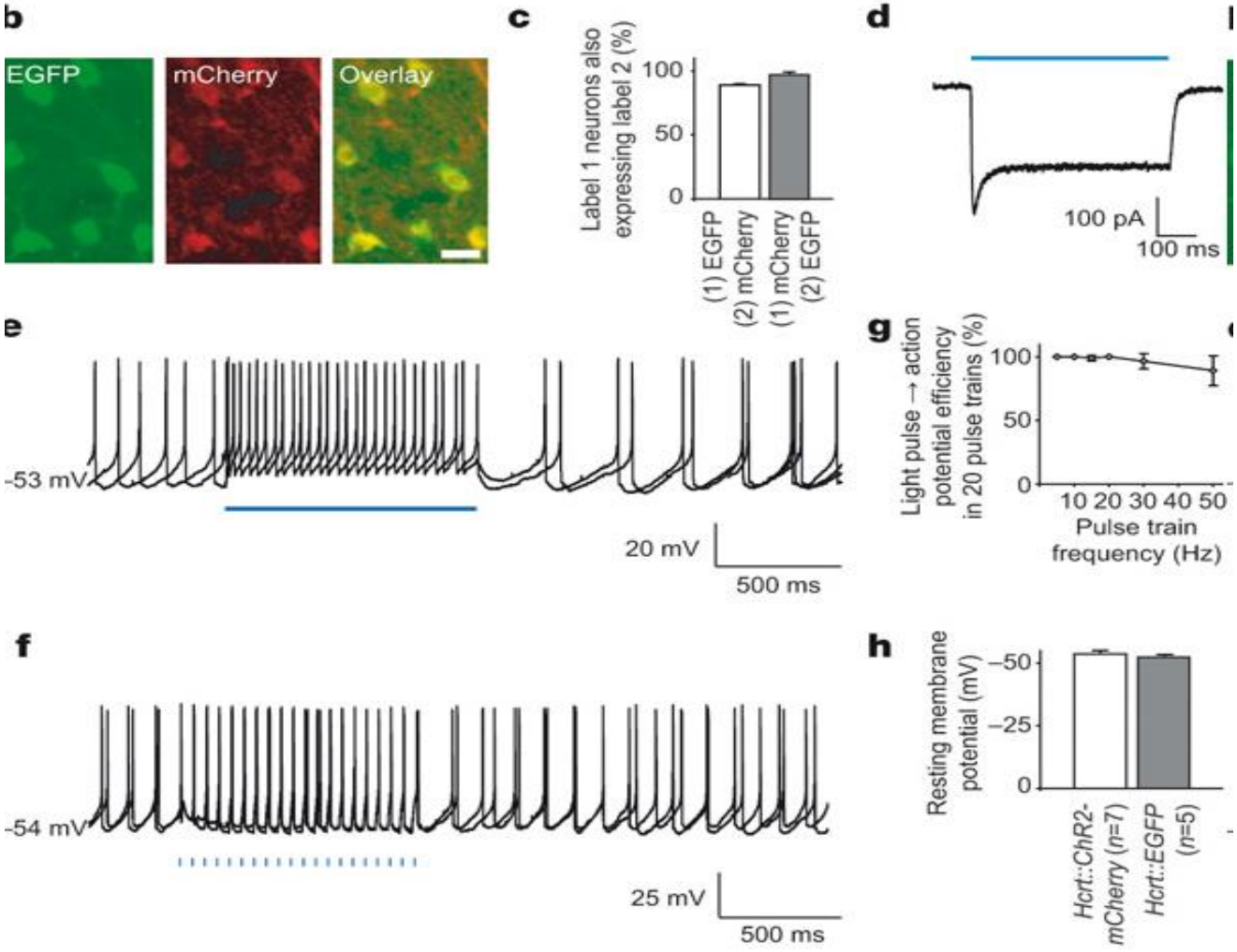
Rapid on/off, precise activation of neurons on the millisecond timescale

Fast excitation: Channelrhodopsin2

- Light activated ion channel isolated from green algae Chlamydomonas reinhardtii
- unspecific cation channels, conducting H+, Na+, K+, and Ca2+ ions.
- Blue light opens ChR2 (absorbs 480 nm)
- Neuron depolarizes



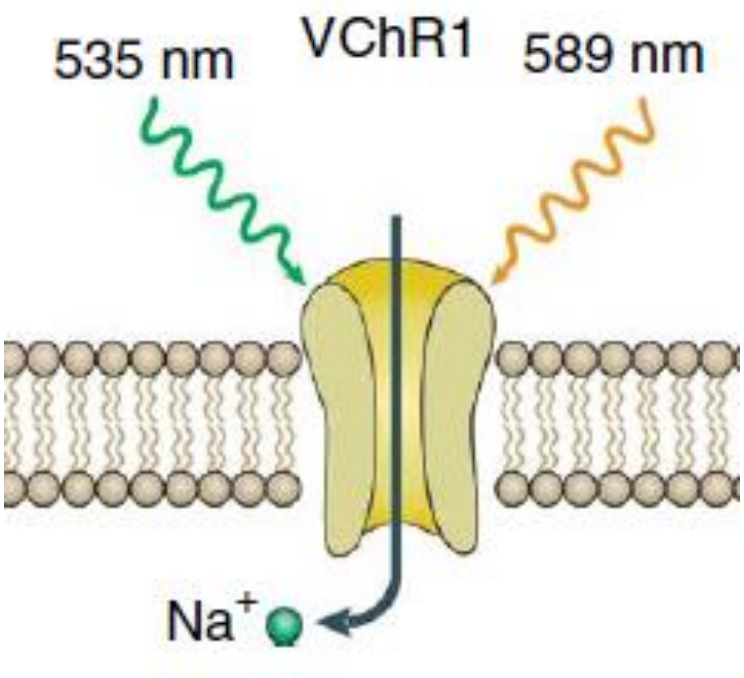
Selective expression of ChR2 in hypocretin neurons



- http://www.nature.com/nature/journal/v450/n7168/extref/nature06310-s2.mov

Bistable opsins/Step Function Opsins

- Mutate ChR to significantly prolong photocycle.
- Conductance of wild type ChR2 deactivates ~10 ms upon light sensation.
- Mutations to ChR change the time constants of deactivation from 2, 42 to ~100 s (can stay on for long periods of time)
- SFOs can be switched on and off with blue and green light pulses, respectively
- effectively responsive to light at orders of magnitude lower intensity than wild-type channelrhodopsins



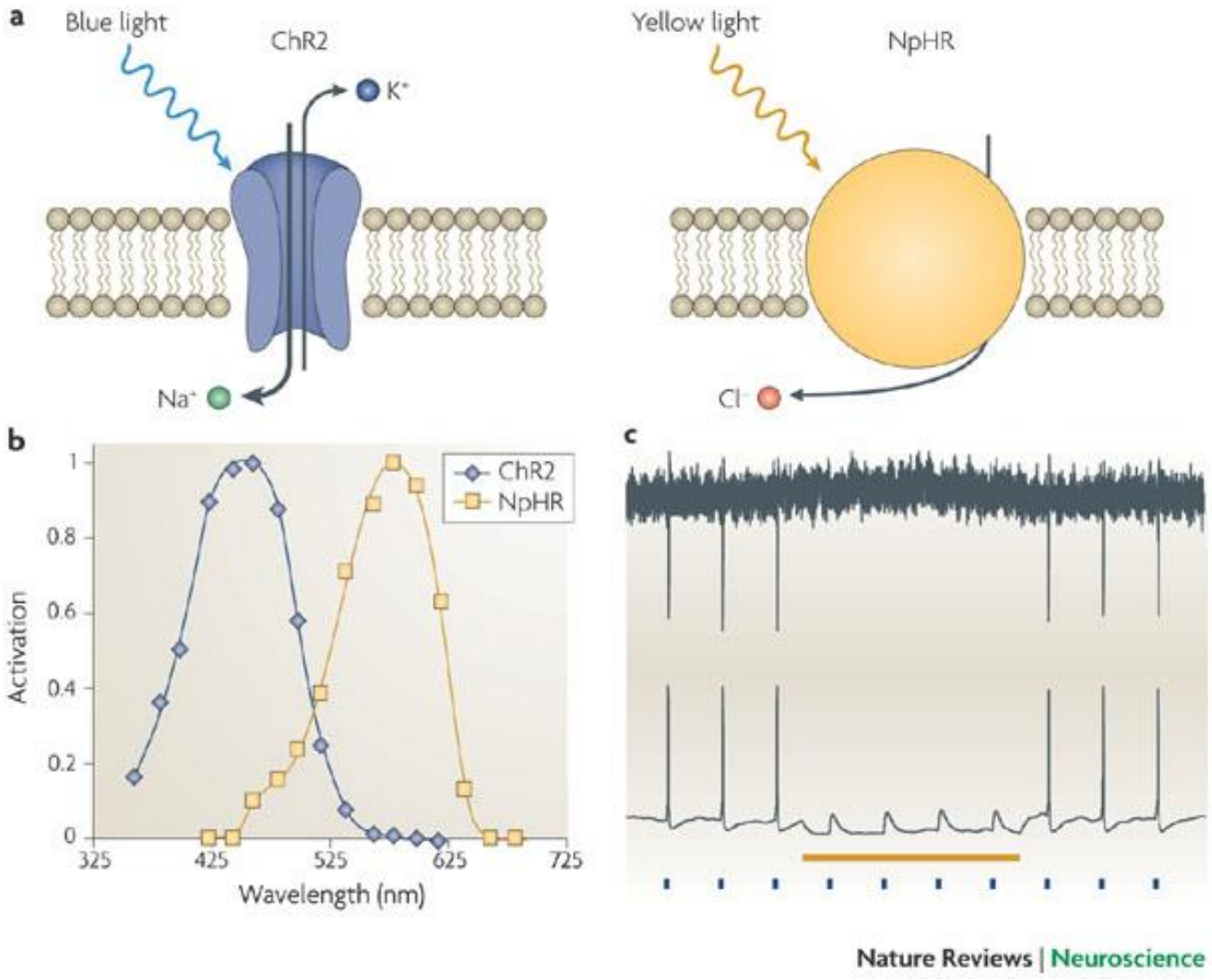
Useful for many long–time scale, neuromodulatory, developmental

chemical cofactor independence in mammalian brains. (ie not activating GPCRs – can maintain selectivity

Halorhodopsin

- Channelrhodopsin2

Halorhodopsin

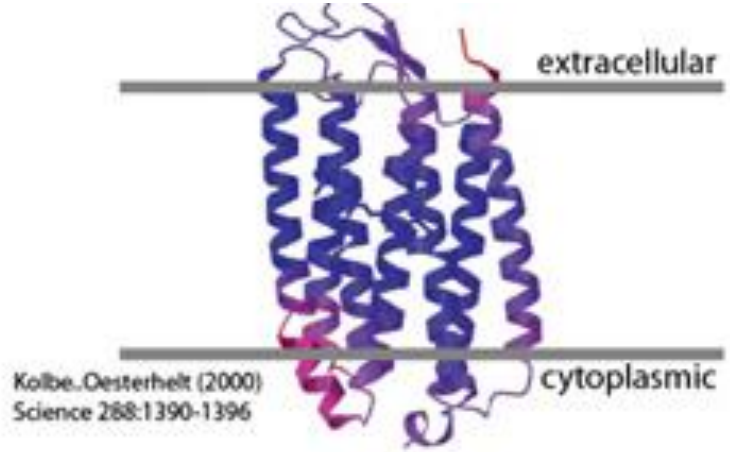


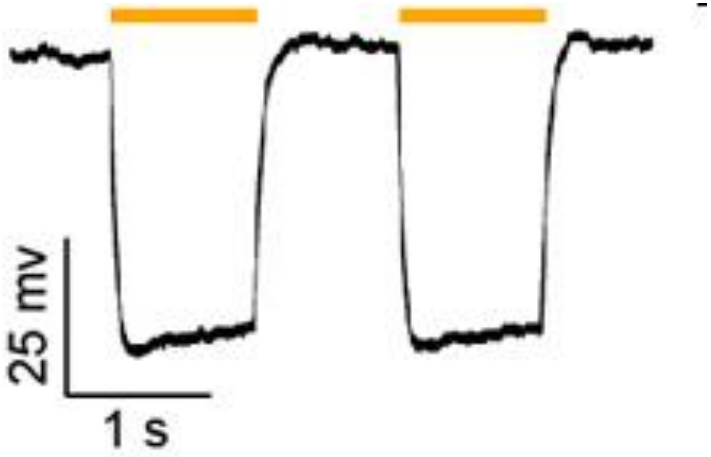
Halorhodopsin uses green/yellow light to move chloride ions into the cell, overcoming the membrane potential.

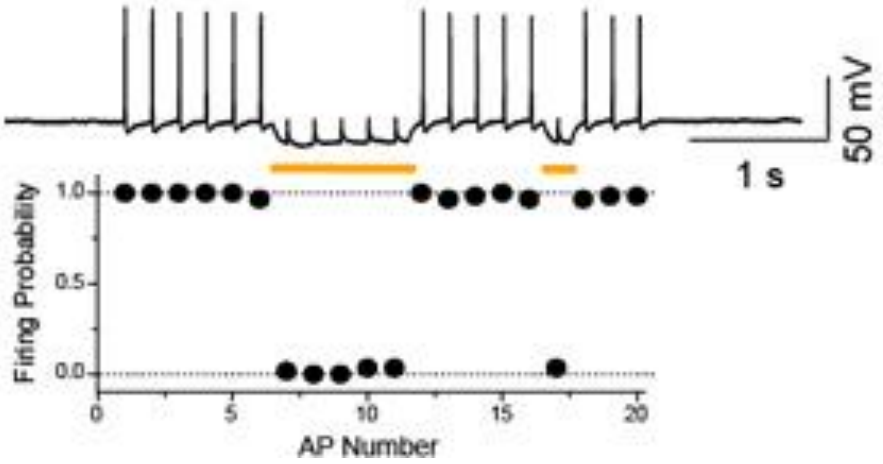
Yellow light opens chloride conductance to hyperpolarize the cell

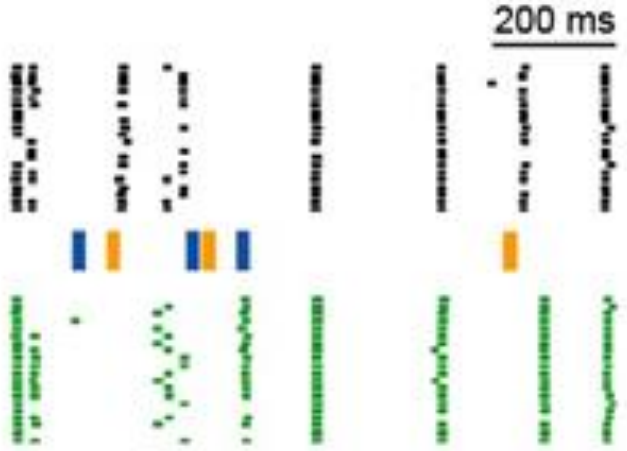
Halorhodopsin can be used alone to silence neurons or in conjunction with Channelrhodopsin2 to activate, silence and desynchronize neural tissue Useful in mapping functional circuits

Halorhodopsin silences neurons

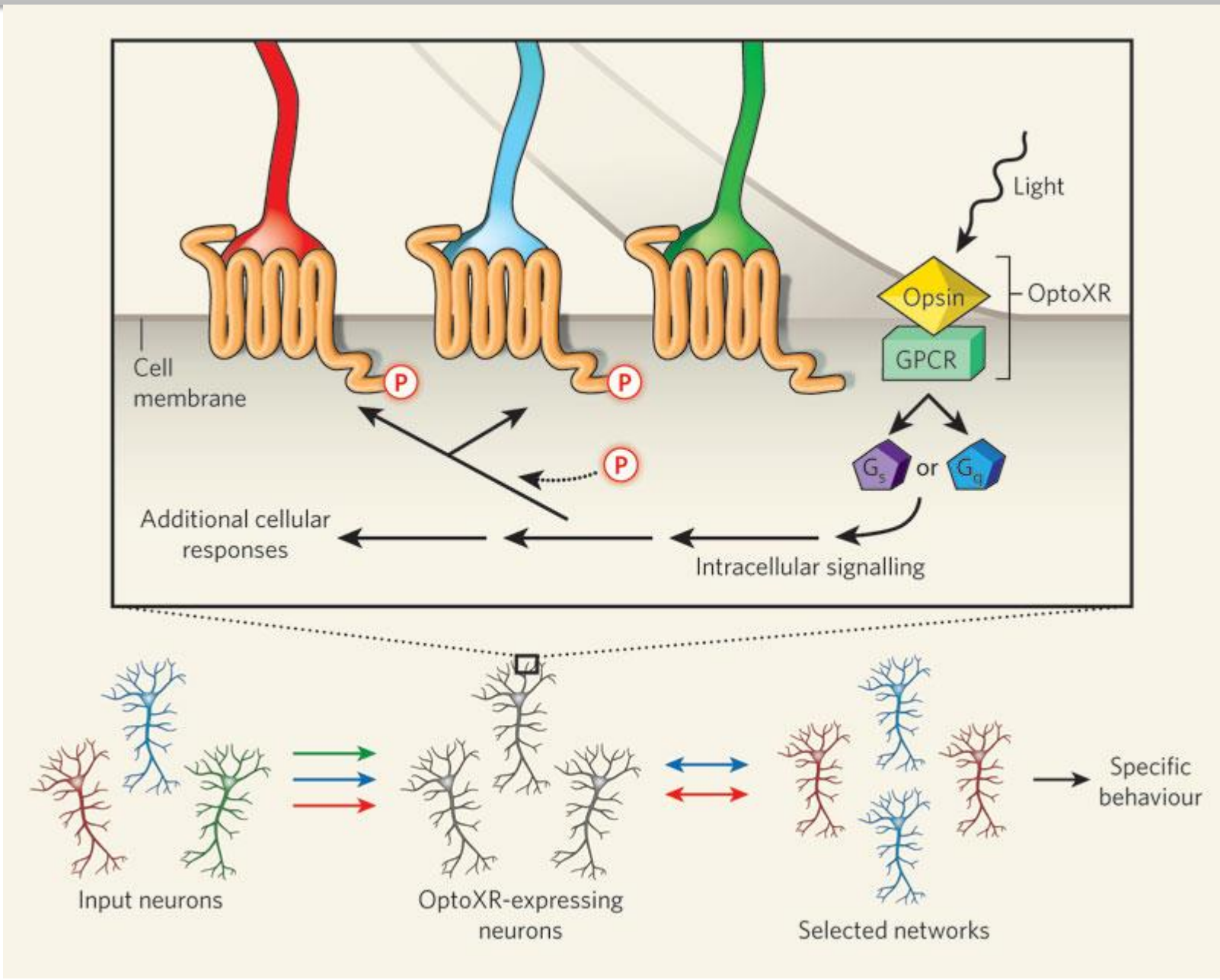




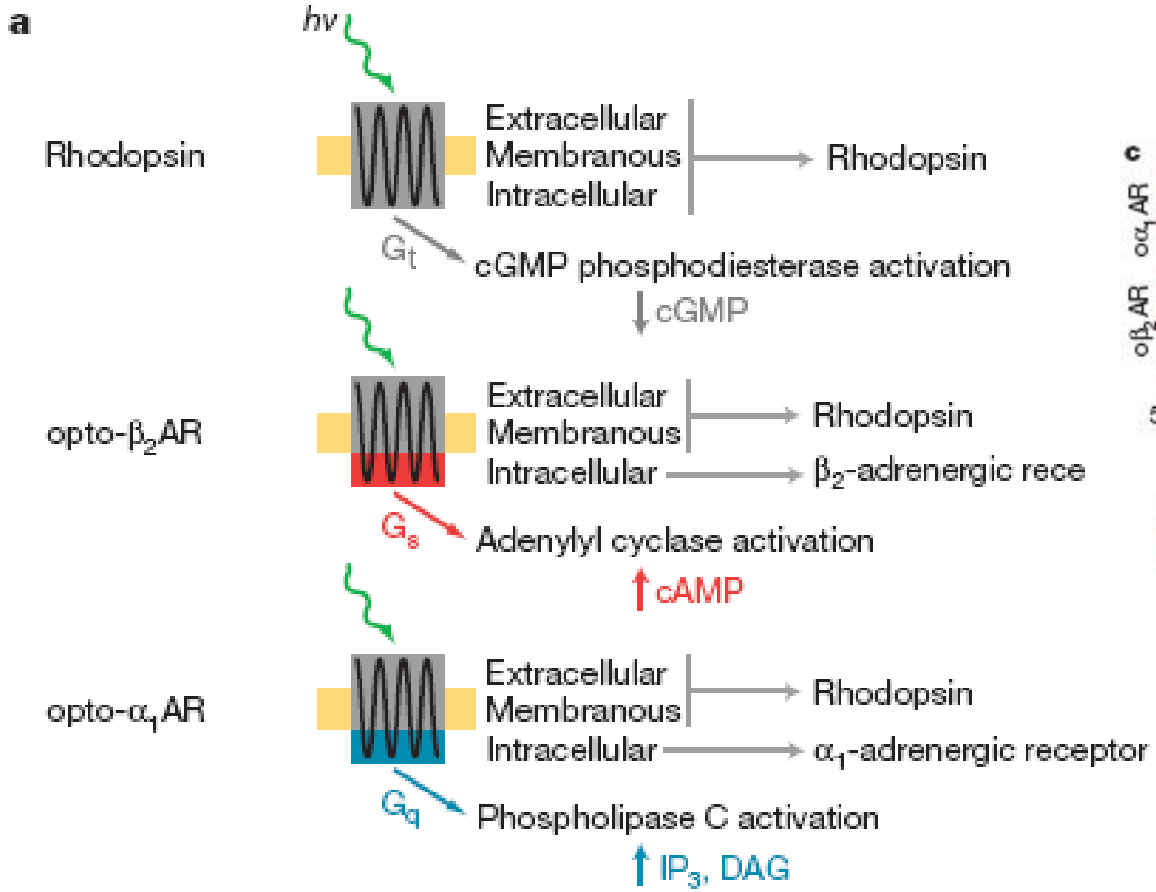


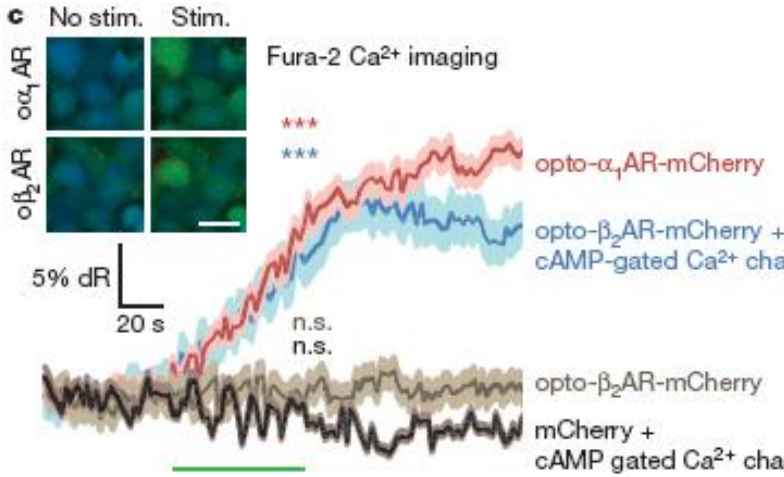


Light stimulation, intracellular signalling and regulation of function in neural networks



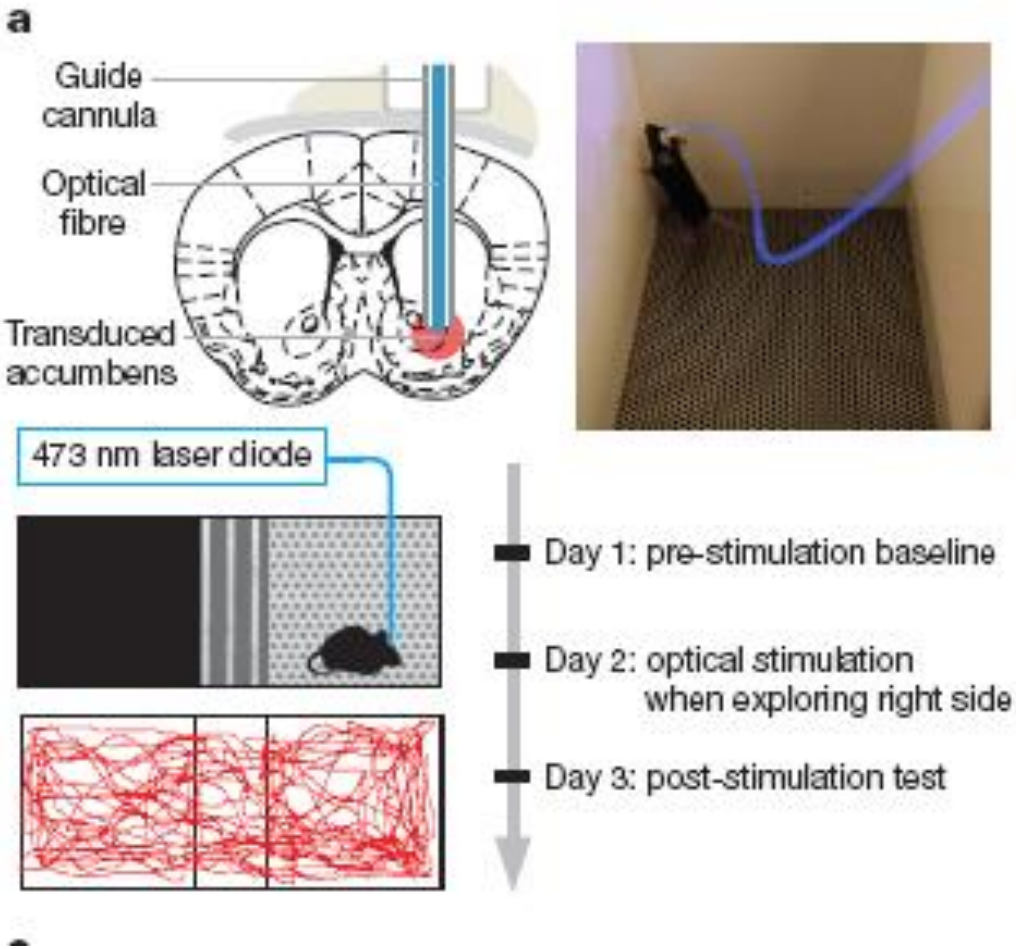
OptoXR- optogenetic control of intracellular signalling

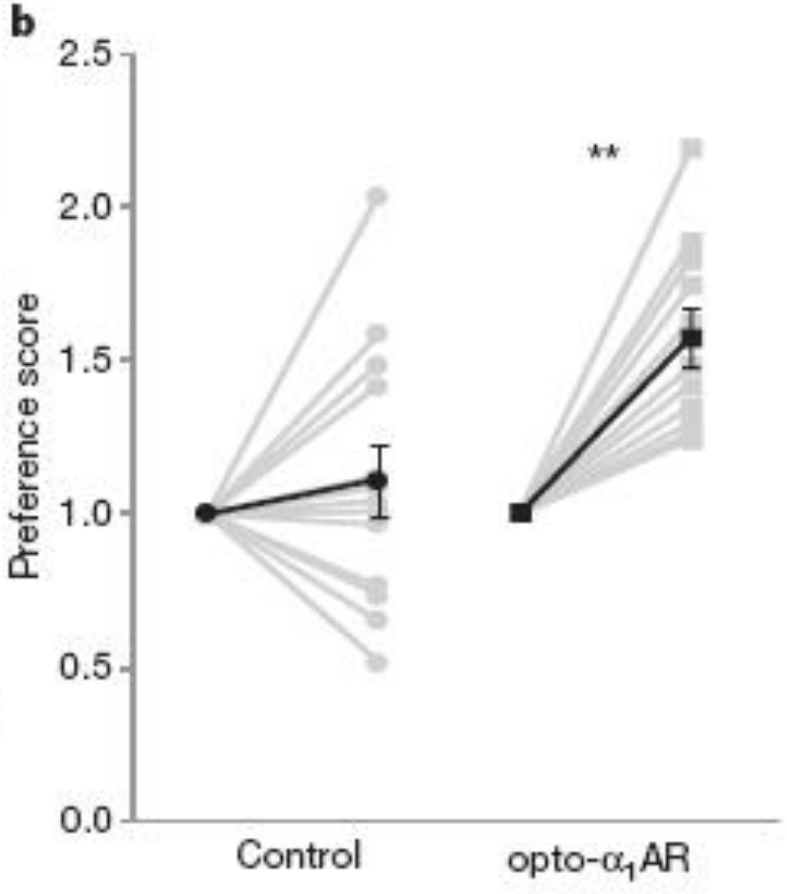




Optical activation of α1ARs in the Nucleus Accumbens

- Optical activation of α1ARs in the Nucleus Accumbens can induce rewarding behavior





Summary

- Optogenetics is a way to selectively control a specific cell-type or neuronal circuit
- Channelrhodopsin (ChR2) opens a cation conductance with blue light. Depolarizes the Cell
- Step function opsins open a delayed cation conductance with blue light and is turned off with yellow light
- Halorhodopsin opens a Cl- pump, hyperpolarizes the cell
- OptoXRs activate GPCRs with light to control intracellular signalling.