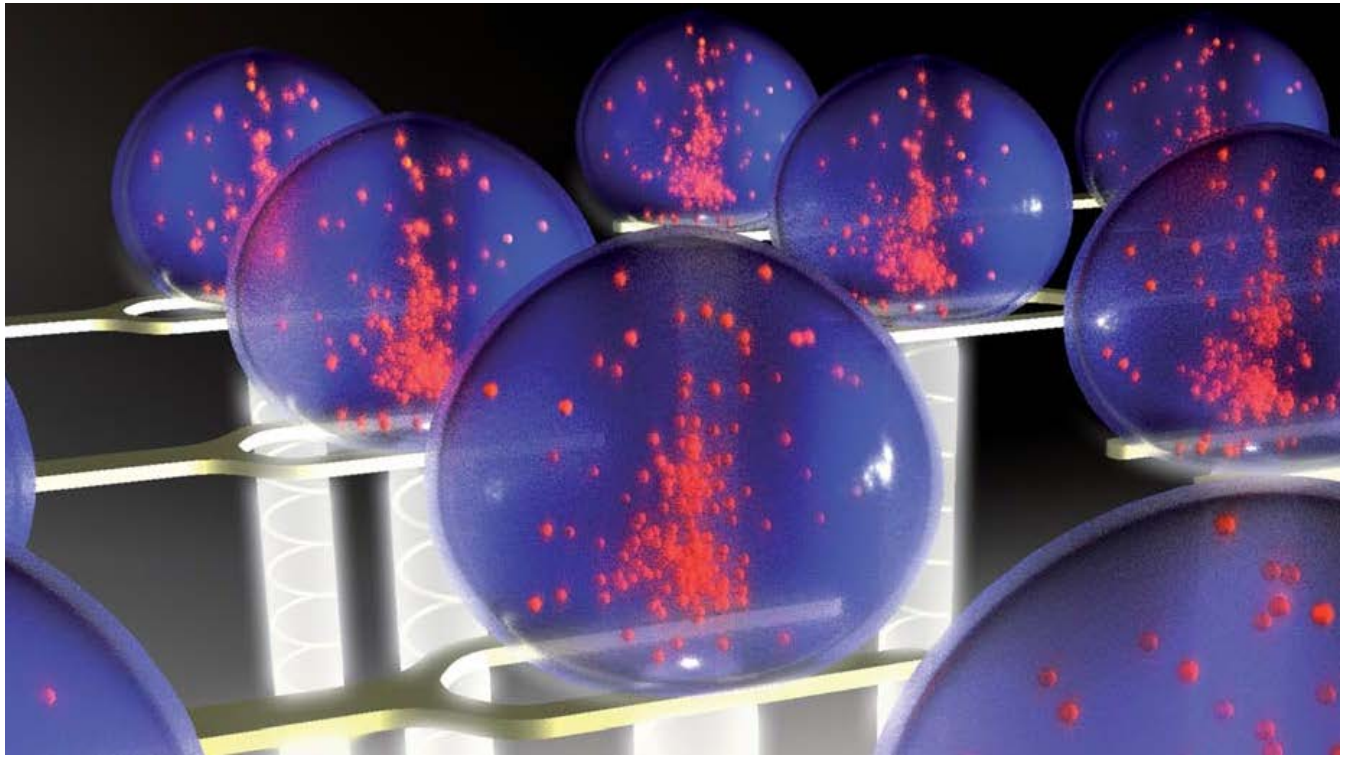
Optoelectrical Microfluidics

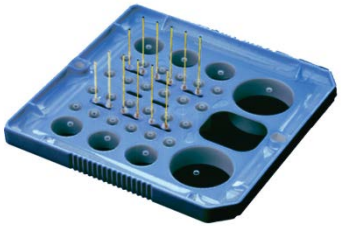


Despoina Goniotaki

Technical Journal Club

12th August 2014

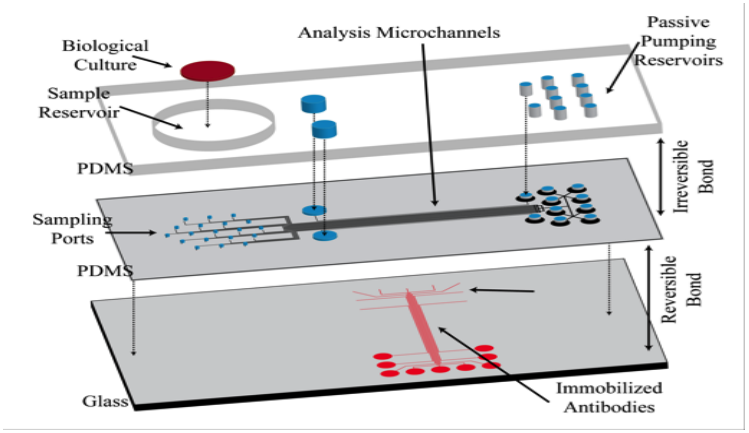
Background



- Microfluidics:
  fluidic applications at the submillimetre scale
  based on modern microfabrication methods

Substantially reduce: - sample volume
- consumption of reagents & samples
- time spent on experiment

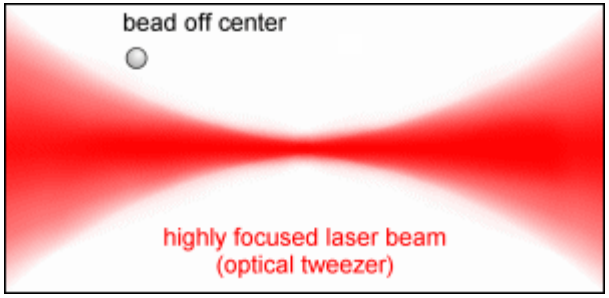
Substantially improve: - sample throughput
- accuracy & reliability
- sensitivity of experiments

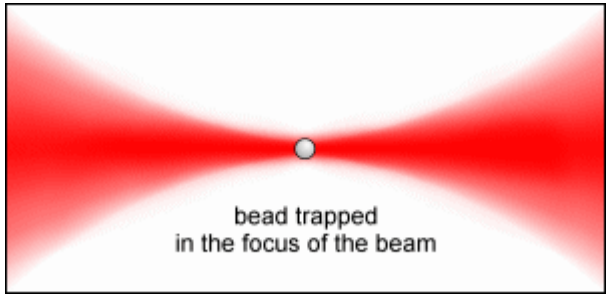


Optical Tweezers

In 1970, Arthur Ashkin demonstrated that dielectric particles can be accelerated and trapped by radiation pressure .

Sixteen years later he produced the so called *Optical Tweezers:* *a set up that traps particles (single atoms-100* µm) *using a single,highly focused laser beam*, without mechanical contact.



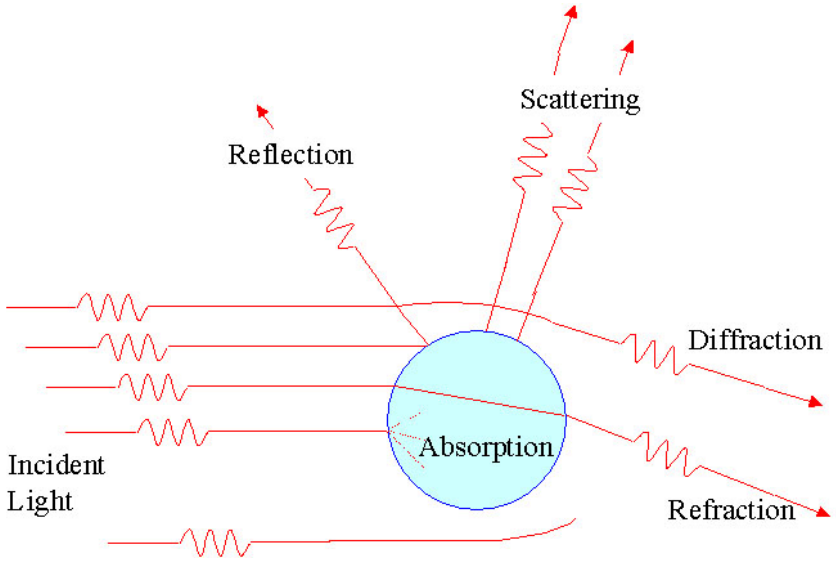


Theoretical Description

Scattering: the interaction of light with an object.

Can be divided into:

a. RADIATION pattern: Reflection & Refraction - surface of the particle - depends on the particle refractive index



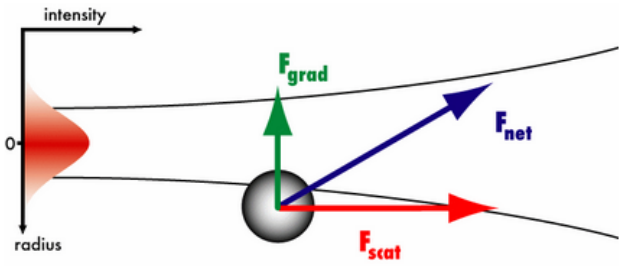
b. DIFFRACTION pattern: due to rearrangements of the wavefront after interaction with the particle – depends on particle geometry

Theoretical Description

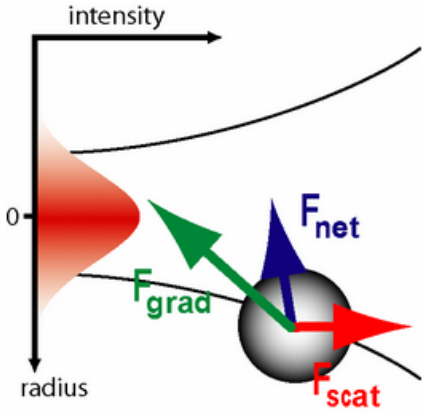
Rayleigh Regime (D<< λ)

- In the Rayleigh regime, the particle is very small compared to the wavelength (***D* << *λ***).
- The distinction between the components of reflection, refraction and diffraction can be ignored.
- The particle can be viewed as an induced dipole behaving according to simple electromagnetic laws.

Slightly diverging laser beam.



Tightly focused laser beam (Optical Tweezers)



Scattering Force

$$F_{scat} = n_m \frac{\sigma \langle S \rangle}{c}$$

Gradient Force

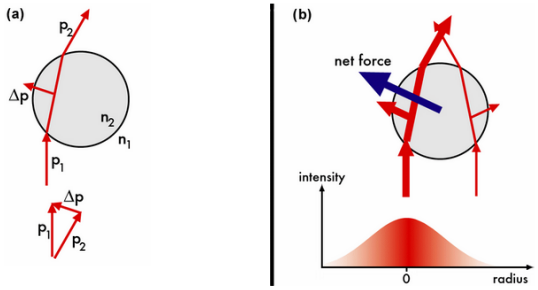
$$\vec{F}_{grad}(\vec{r}) = \frac{1}{2 n_m \varepsilon_0 c} \alpha \nabla I(\vec{r})$$

where n_m is the refractive index of the surrounding medium, **<*S*>** is the time averaged Poynting vector, ***c*** is the speed of light, and ***σ*** is the particle's cross section

Theoretical Description

Ray Optics Regime ($D >> \lambda$)

- In the ray optics regime, the size of the object is much larger than the wavelength of the light ($D >> \lambda$).
- A single beam can be tracked throughout the particle.
- If the ratio of the refractive index of the particle to that of the surrounding medium is not close to one but sufficiently large, diffraction effects can be neglected.
- This situation is for example given when whole cells, which are microns or tens of microns in size, are trapped using infrared light while suspended in solution.



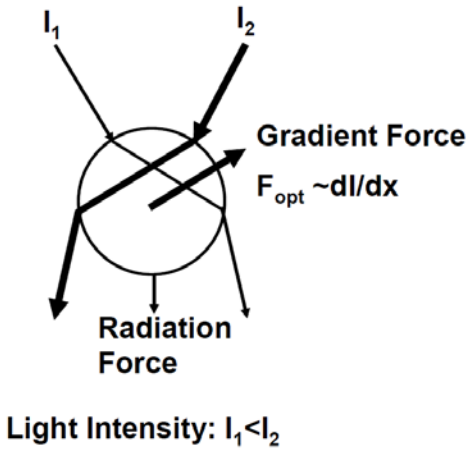


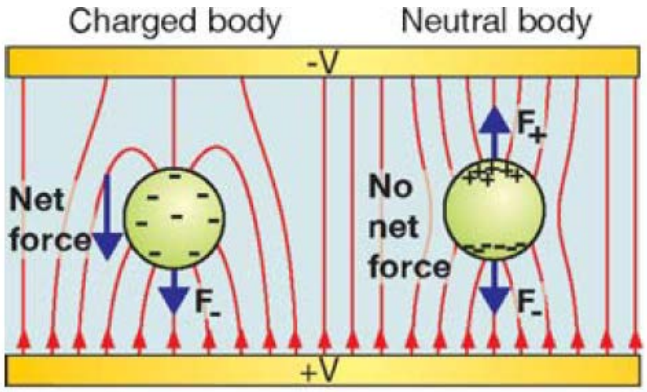
Fig. 3: The momentum (red arrows) of (a) one ray and (b) two rays with different intensities propagating through a sphere. The blue arrow indicates the restoring net force.

Microscale Cell Manipulation

- Electrical Forces to physically manipulate cells

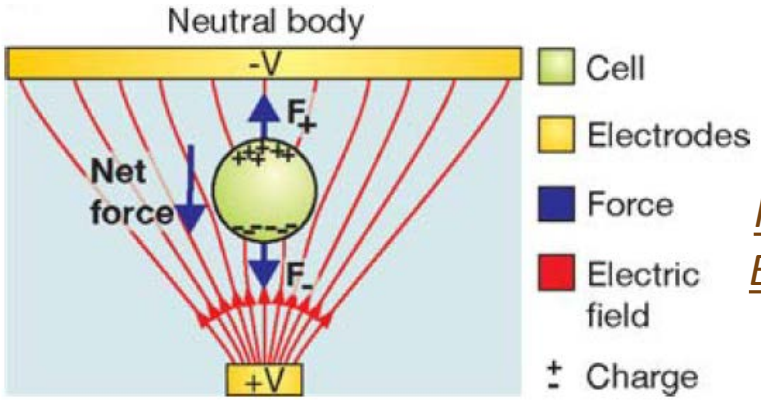
AC electrokinetics:

Electrophoresis (EP): the force on a charged particle in an electric field



Uniform Electric Field

Dielectrophoresis (DEP): the force on a polarizable particle—such as a cell—in a spatially nonuniform electric field



Nonuniform Electric Field

Particle Manipulation

a. Optical tweezers

→ Lack resolution,high laser intensity, low throughput

b. Alternating Current (AC) electrokinetics

→ high throughput, microfabrication

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c. Optoelectrofluidics

(optics + electrokinetics)

- high resolution
- high throughput
- programmable manipulation

✓ *simultaneously trap & sort thousands of particles.*

✓ *automated individual addressing of particles.*

Introduction of the technology

nature Vol 436|21 July 2005|doi:10.1038/nature03831

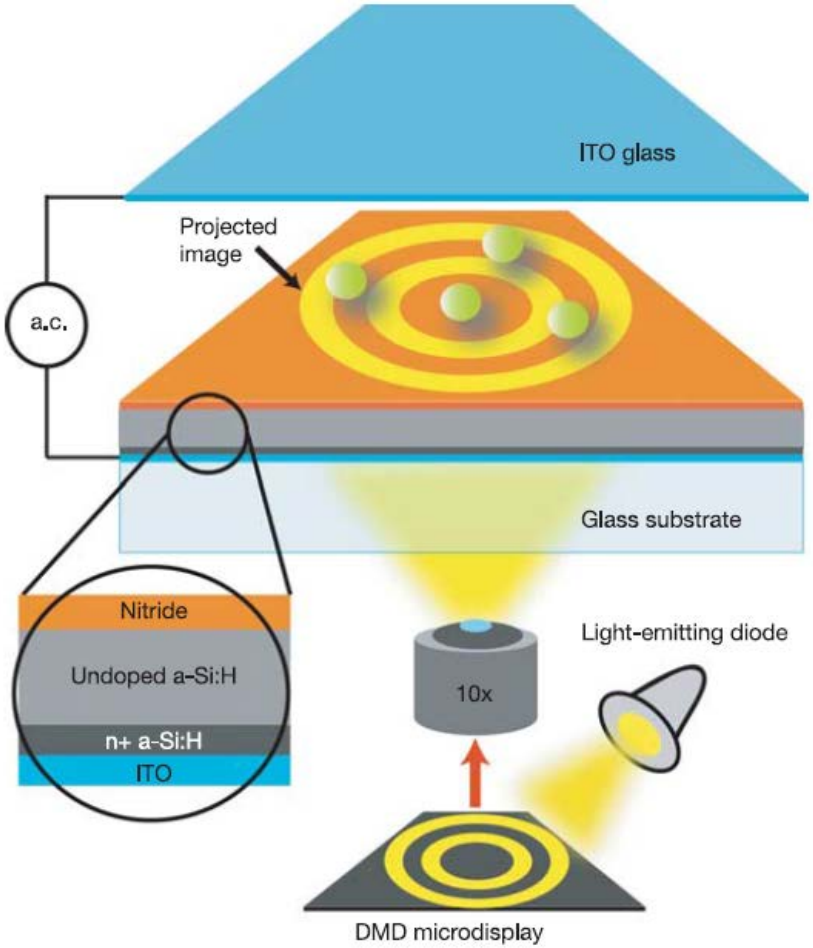
LETTERS

Massively parallel manipulation of single cells and microparticles using optical images

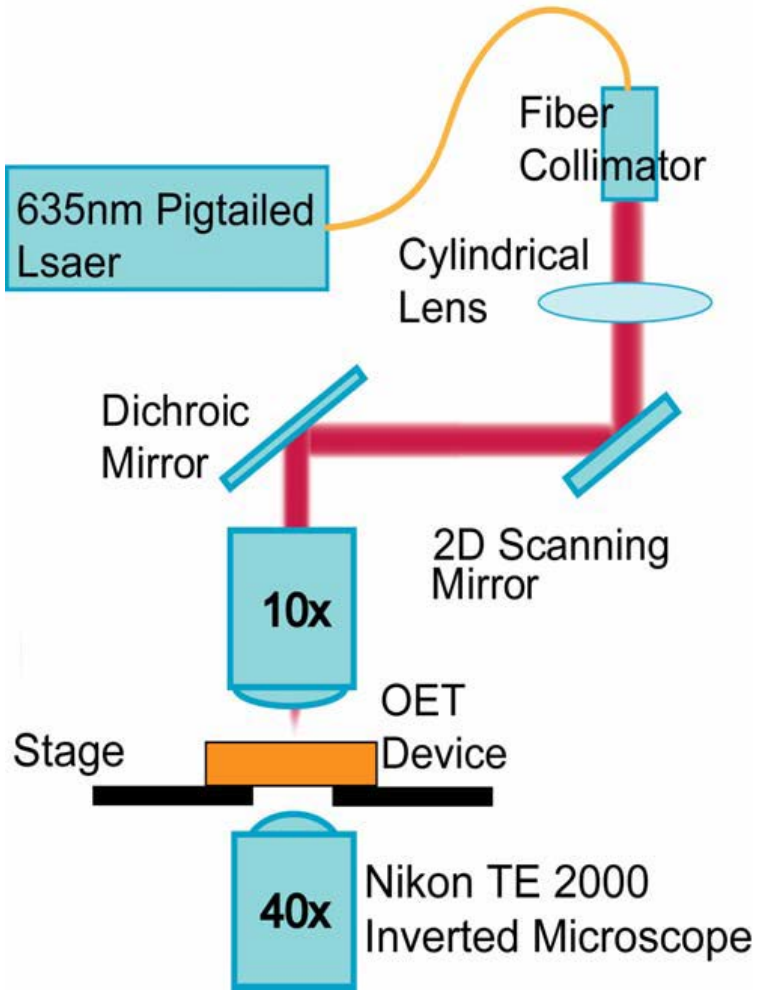
Pei Yu Chiou[1], Aaron T. Ohta[1] & Ming C. Wu[1]

Introduction

- Optoelectronic Tweezers (OETs) – ITO platform



Optical Sorting – experimental set-up



✓ *optical manipulation with extremely low optical power*

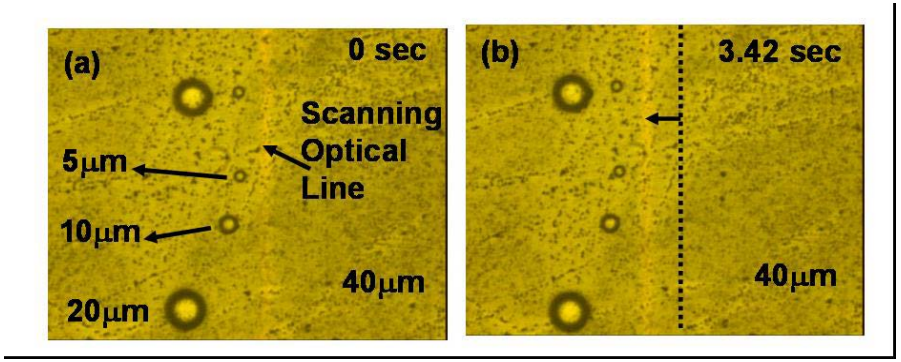
minimum light intensity: 10 nW/μm2, 5 times less than optical tweezers

✓ *tightly-focused light is not necessary in OET manipulation.*

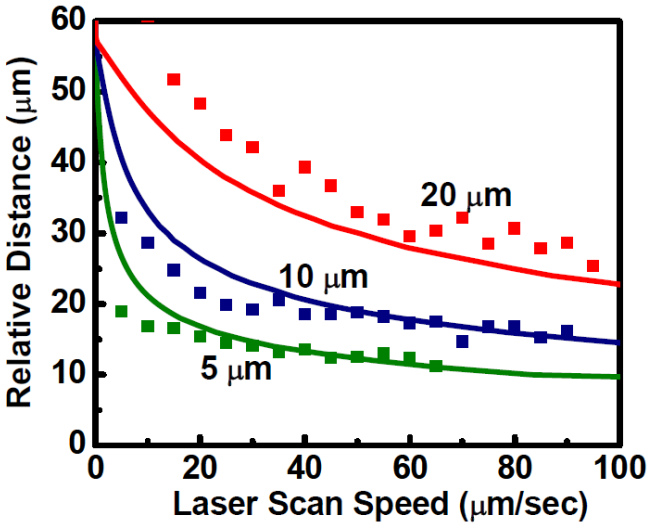
This increases OET's effective manipulation area and enables massively parallel manipulation of single particles or cells

Optical Sorting of different particle sizes

Relative distances of microparticles from the scanning beam center

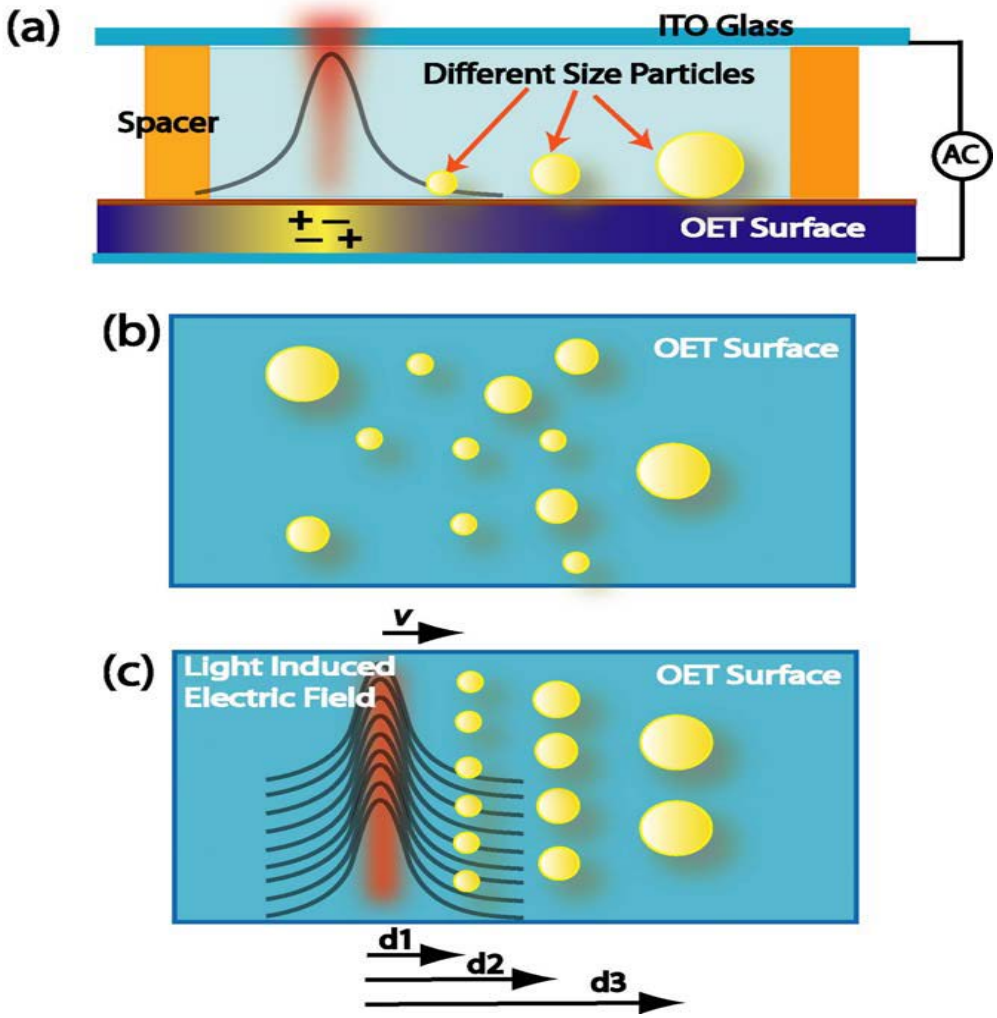


Particle size



Scanning speed

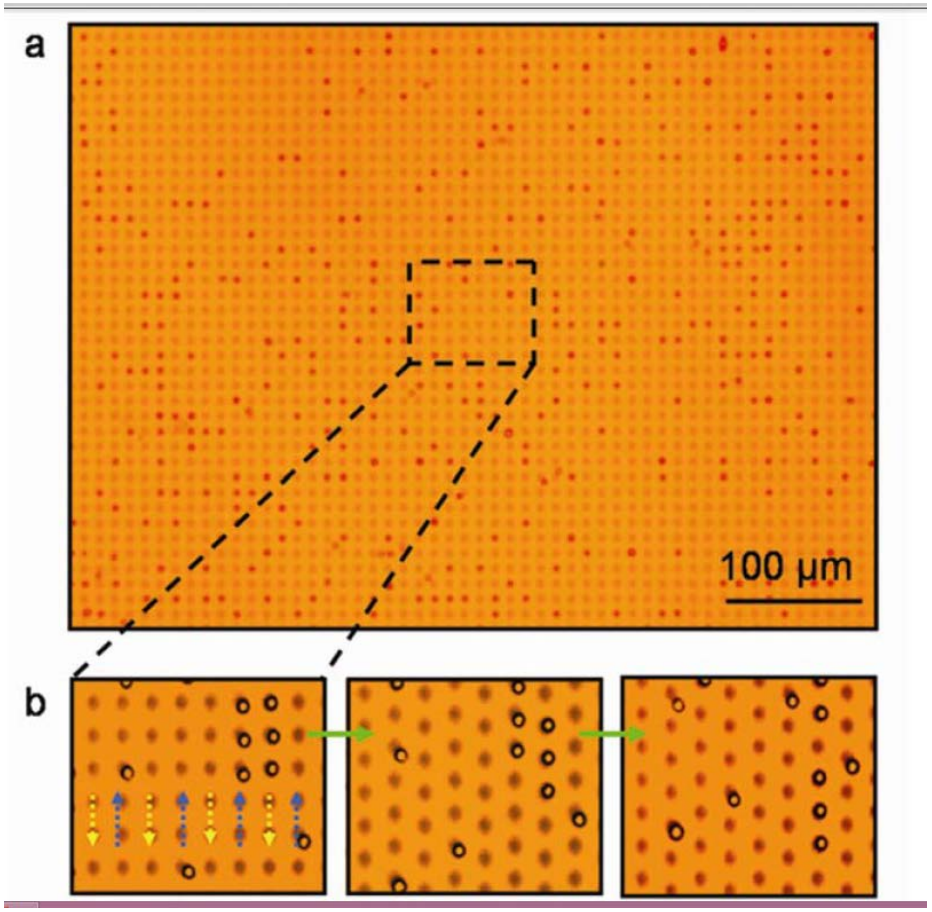
Particle Sorting



Particles are sorted according to their sizes by a lineshaped scanning laser beam across the OET surface.

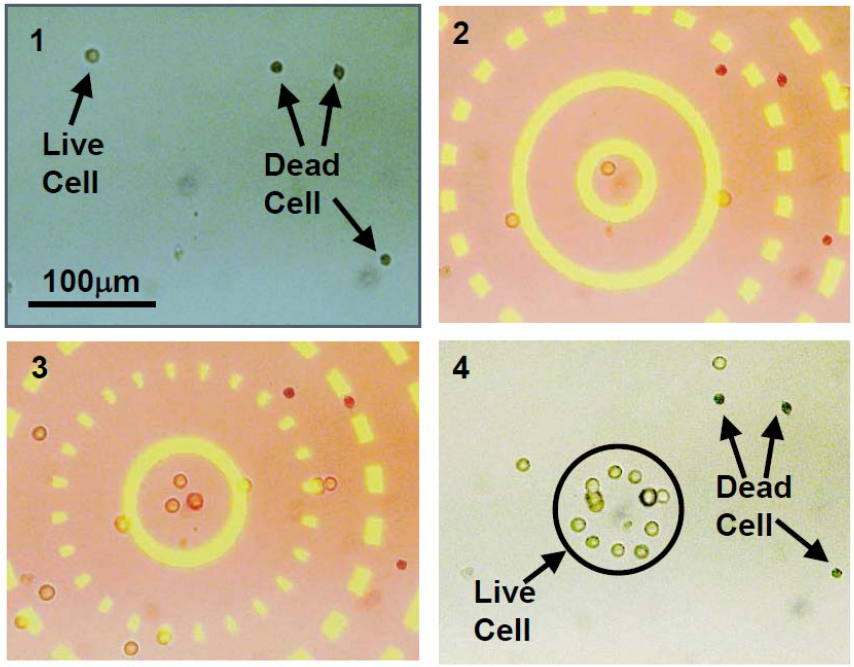
Massive parallel optical manipulation of particles

- 15,000 DEP traps across an area of 1.3 × 1.0 mm2



The particles are trapped in the dark area by the induced negative DEP forces, which push the beads into the non-illuminated regions, where the electric field is weaker.

Selective concentration of live white blood cells



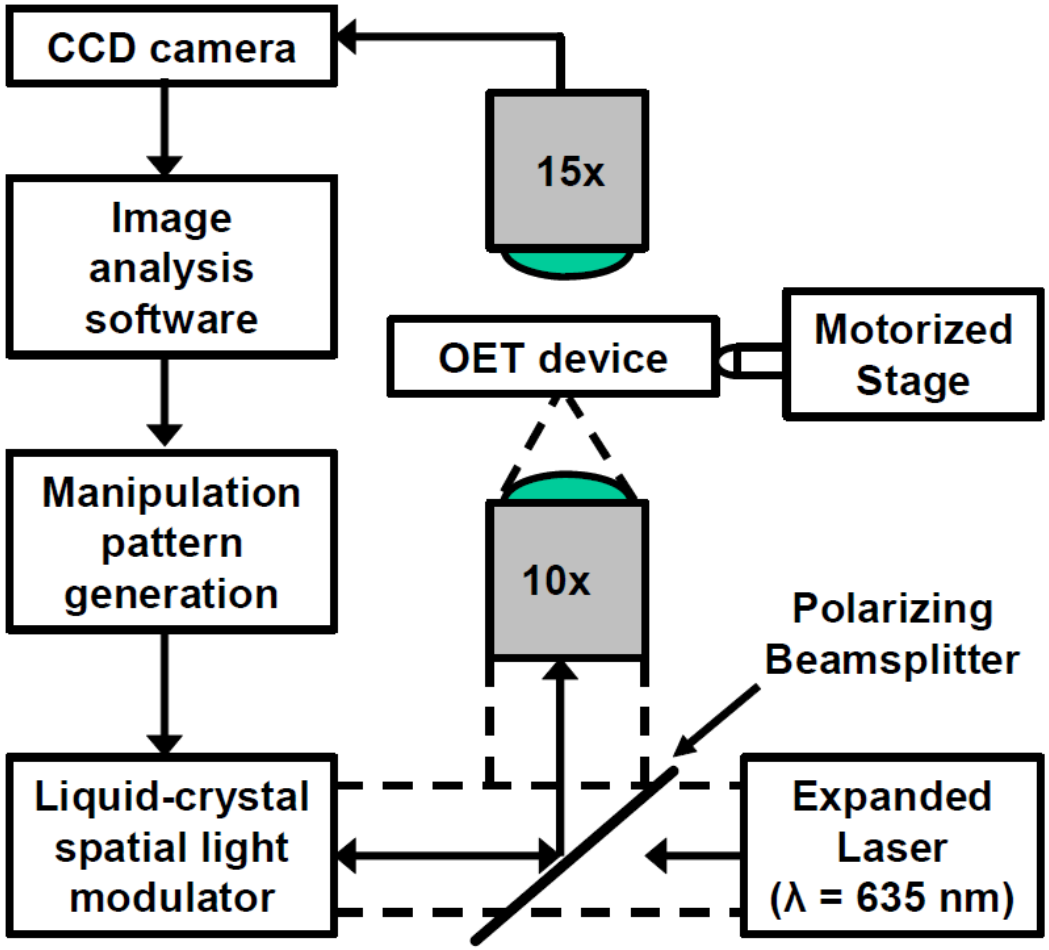
cells are suspended in an isotonic buffer medium of 8.5% sucrose and 0.3% dextrose, mixed with a solution of 0.4% Trypan blue dye to check the cell viability.

Cell conductivity: 10mS/m

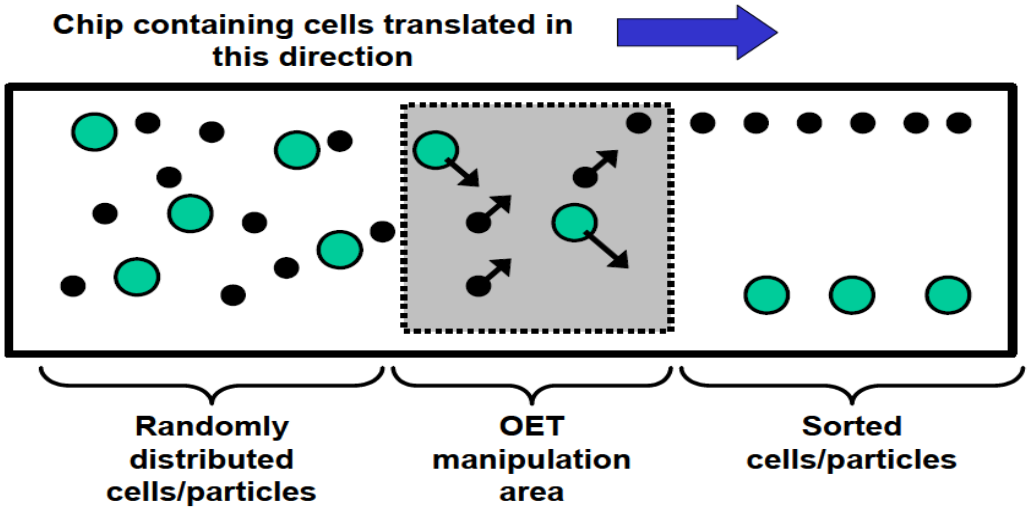
Applied ac signal: 14Vpp

Frequency: 120kHz

Continuous cell sorting set-up

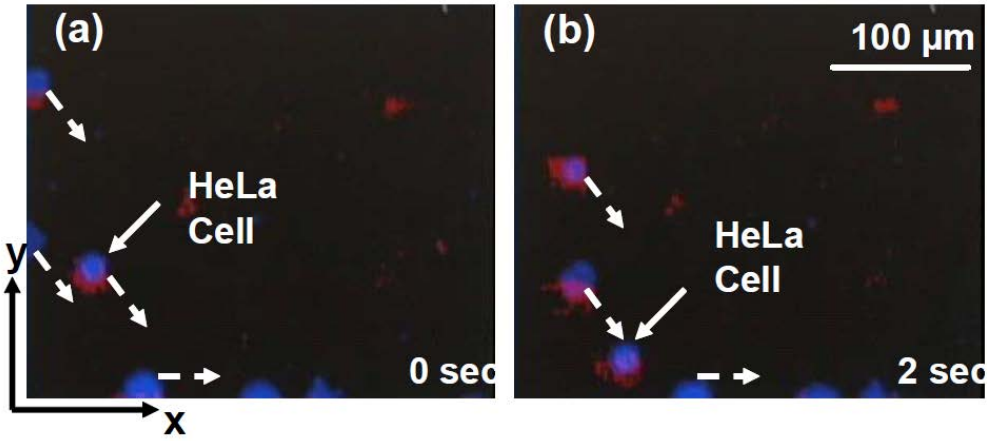


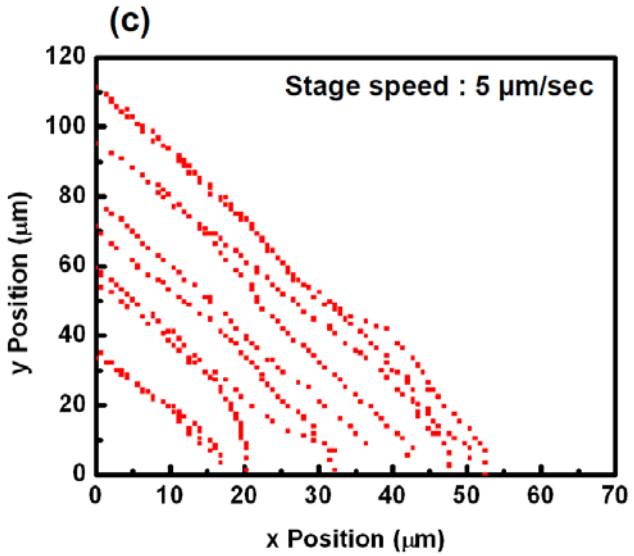
Continuous cell sorting set-up



The OET chip is positioned on a motorized stage, allowing the manipulation pattern to be rastered across the entire OET chip.

The sorting of HeLa cells based on color





(a), (b) HeLa cells concentrated at the bottom of the images.
(c) Trajectories of the HeLa cells entering the active area from the left side of the image.

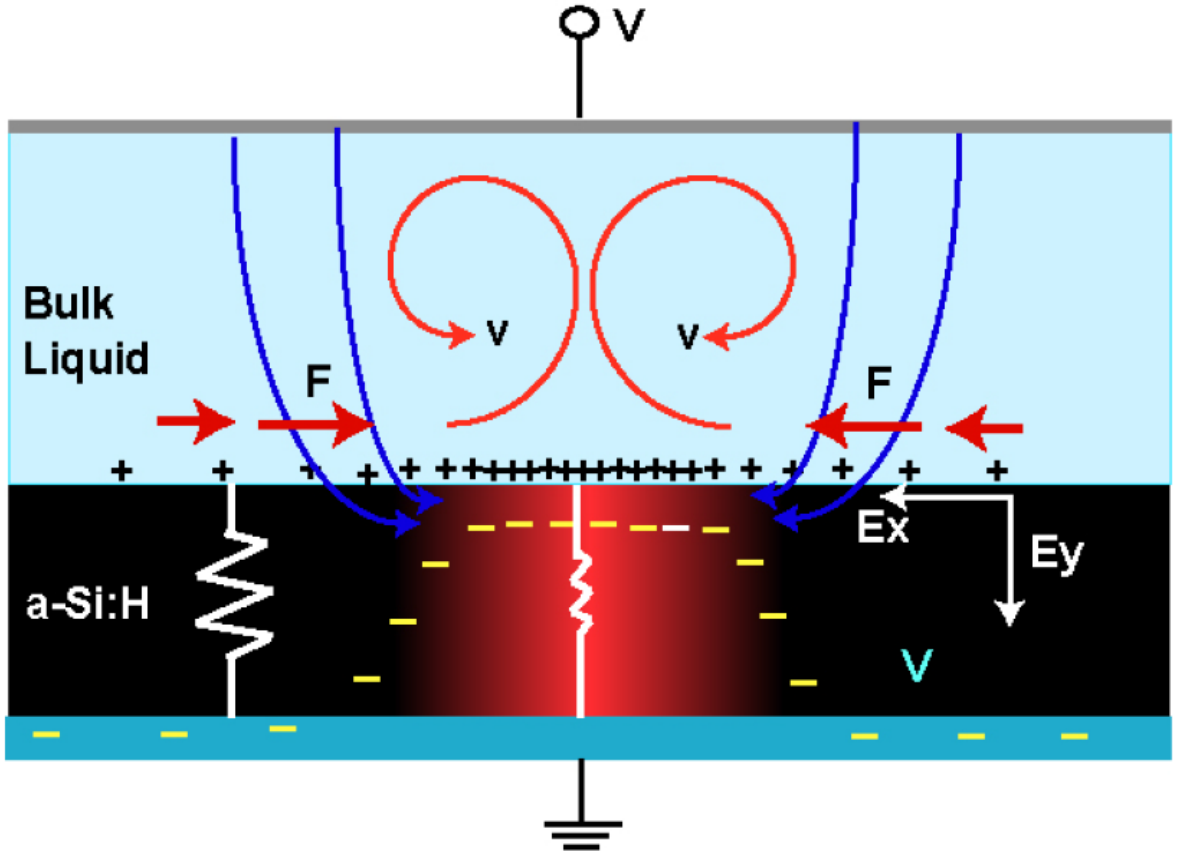
How to trap nanoparticles?

Particle trapping using DEP forces is effective when the size of the particles is large.

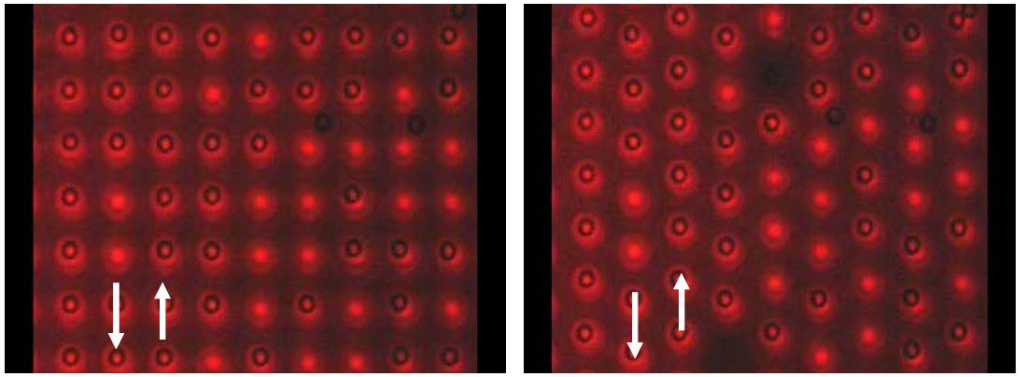
→ Small particles require a strong electric field gradient to compensate for the reduced particle volume.

Consider:
-spacing between upper & bottom ITO surfaces
-position/distance of electrodes
-frequency,ac signal

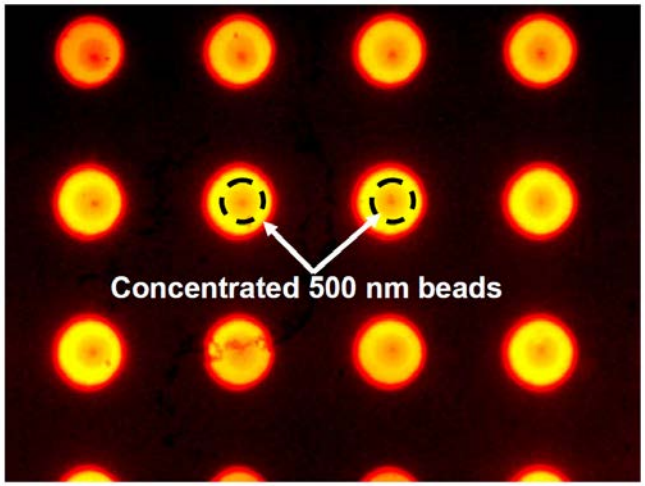
Optically-induced dielectrophoretic force (ODEP)



Optically-induced dielectrophoretic force (ODEP) for trapping



Parallel trapping & transportation of nanobeads on OET device



Concentration of 500nm polysterene beads at the centers of electrodes

Innovations

- Production of optoelectronic tweezers (OET)

  optical manipulation of single cells, micro-particles, and nano-particles.

- Potential to use cheap, incoherent light sources (e.g. LEDs)

- Optically-induced dielectrophoretic force (ODEP)

Applications: *Manipulate nanoscopic particles, e.g. quantum dots, carbon nanotubes nanowires, and biomolecules such as DNAs and proteins.*

Application 1

PAPER

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Cite this: *Lab Chip*, 2013, 13, 1371

High-purity and label-free isolation of circulating tumor cells (CTCs) in a microfluidic platform by using optically-induced-dielectrophoretic (ODEP) force†

Song-Bin Huang,‡[a] Min-Hsien Wu,‡[a] Yen-Heng Lin,[bc] Chia-Hsun Hsieh,[de] Chih-Liang Yang,[a] Hung-Chih Lin,[f] Ching-Ping Tseng*[g] and Gwo-Bin Lee*[h]

Introduction

Circulating Tumor Cells (CTCs)

- Rare cell species present in the peripheral blood.

- Crucial biomarker for the indication of cancer progression, metastasis & selection of proper therapeutics.

  Common CTC isolation scheme:

  Positive selection: Immunomagnetic beads, surface-coated with antibody recognizing specific CTC surface antigens to bind & separate CTCs from the leukocyte (WBC) background by application of a magnetic field

- Limitations:

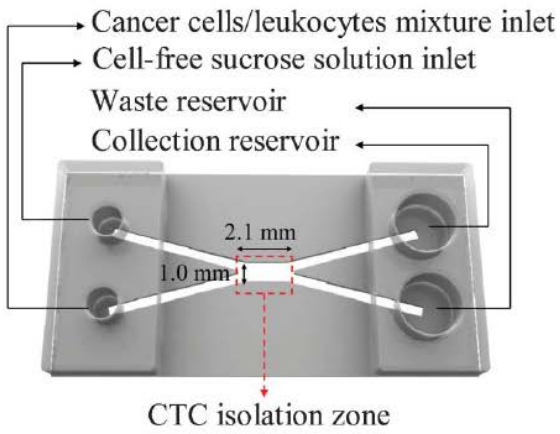
-epithelial surface markers (EpCAMs,CKs) are notexpressed in all tumors (sarcomas,melanomas)
-highly invasive CTCs can undergo epithelial tomesenchymal transmition

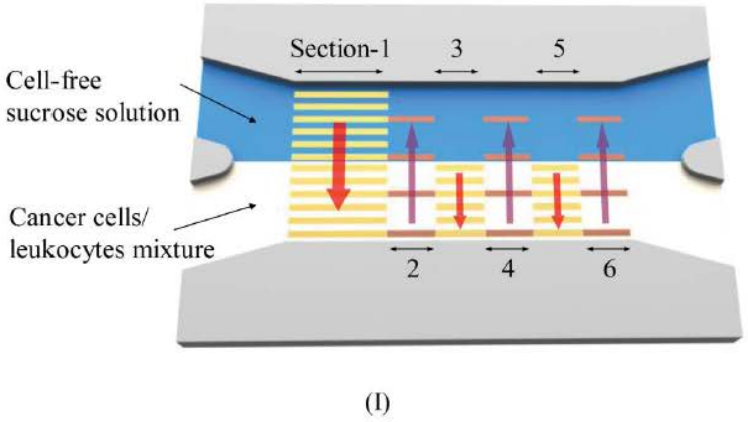
Alternative: harvest CTCs in a label-free manner (negative selection-based CTC isolation) → low purity

Optically induced dielectrophoretic (ODEP) force in a microfluidic platform to further purify the CTCs from the pre –isolated cell mixture.

Working principle:
fine tuning of the operating conditions of ODEP force, permit isolation of CTCs from the blood cell background in an effective manner.

SET UP:



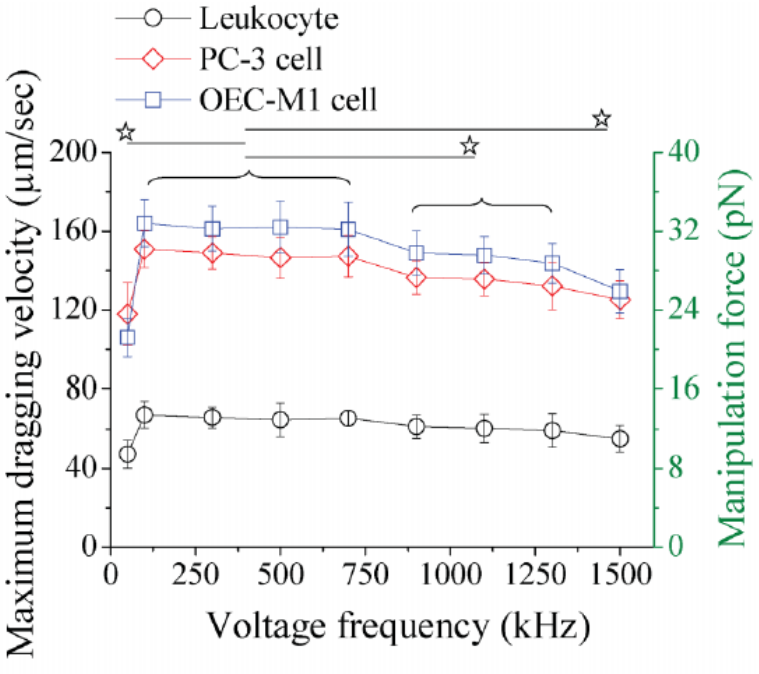


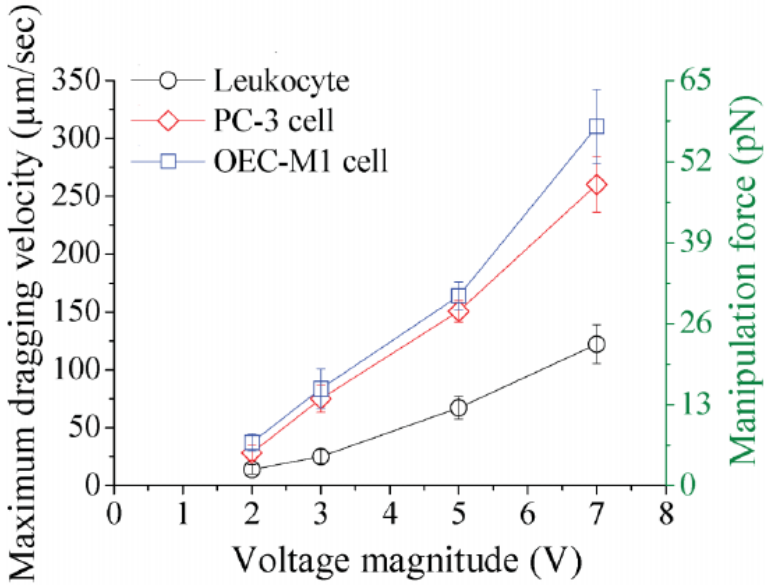
(I)

Characterization of operating conditions of ODEP for cancer isolation

ODEP is dependent on:

- size and inherent nature(e.g. conductivity or permittivity) of the cells
- the voltage conditions (magnitude, frequency) of the surrounding solution.



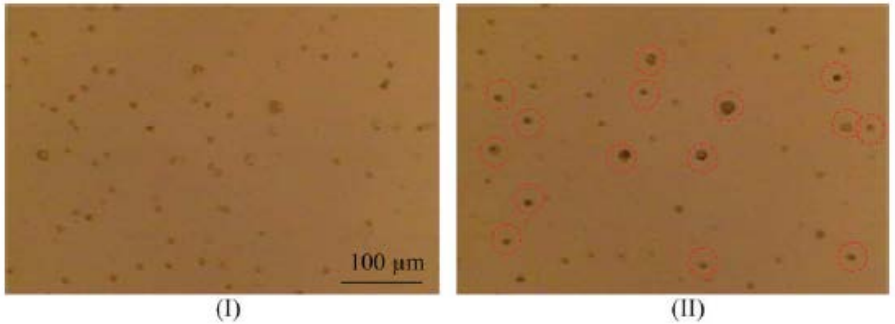


Characterization of operating conditions of ODEP for cancer isolation

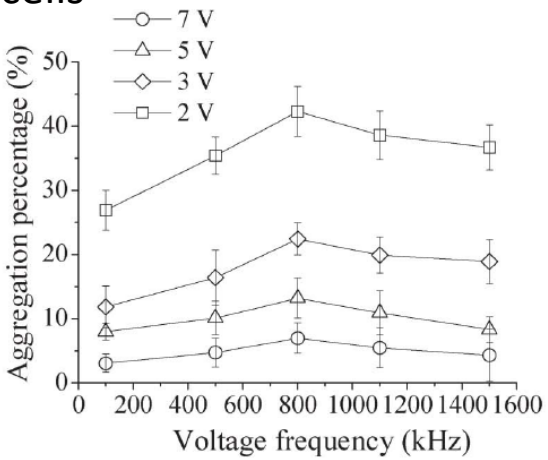
- In ODEP-based cell manipulation, an AC voltage is applied to produce an alternating electric field.

Such electric fields could force a biological cell to form an oscillating dipole.

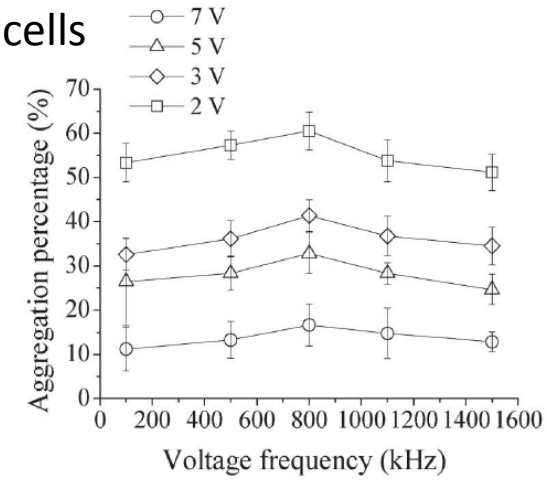
Problem: Cell Aggregation



PC3 cells



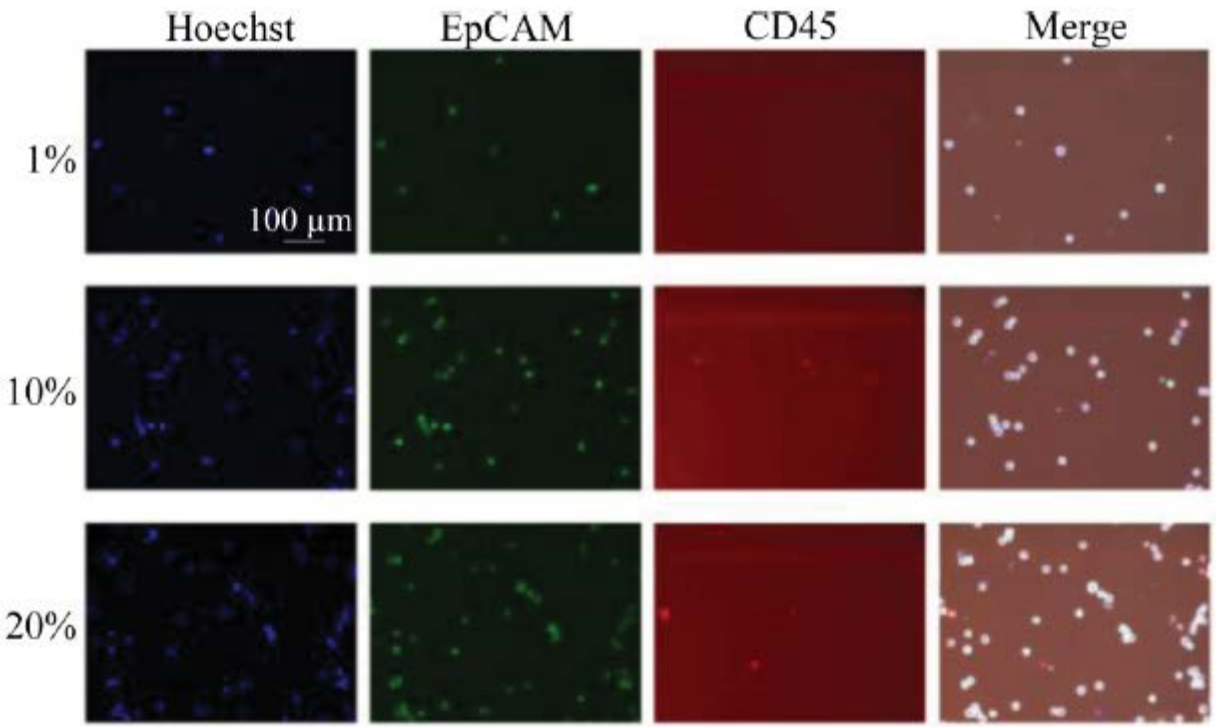
OEC-M1 cells



Isolation of CTCs

- *Chosen conditions:*
  - the AC voltage of 5 V & at 100 kHz

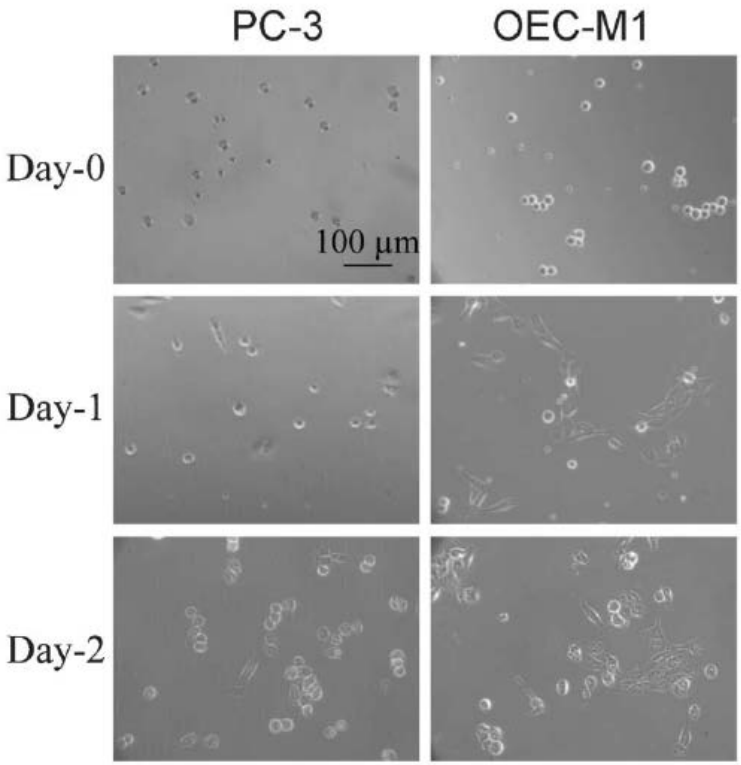
PC3 cell Isolation & Immunostaining

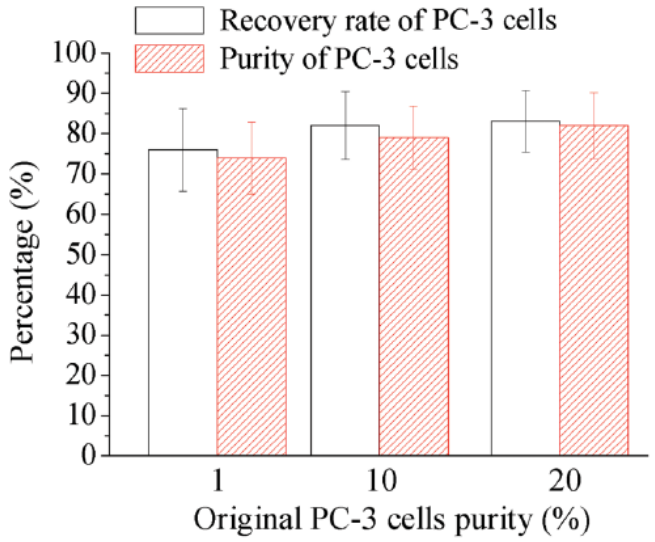


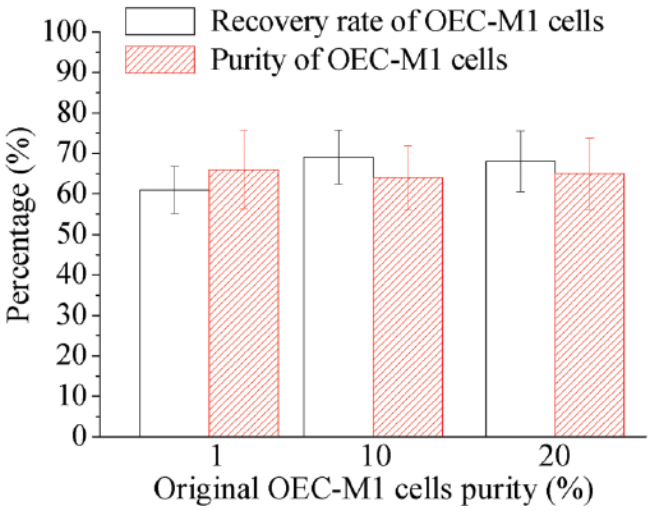
Isolation of CTCs

- *Chosen conditions:*
  - the AC voltage of 5 V & at 100 kHz

Evaluation of cell viability & proliferative capability







Innovations

- Harvest of untreated,viable & all positive CTCs

  High recovery rate, high purity

- Cancer cells can be isolated in a continuous, tunable, effective, efficient & cell-friendly manner

- Achieved significantly reduced cell aggregation

Applications: *CTC isolation for the subsequent cell based or biochemical assays.*

Application 2

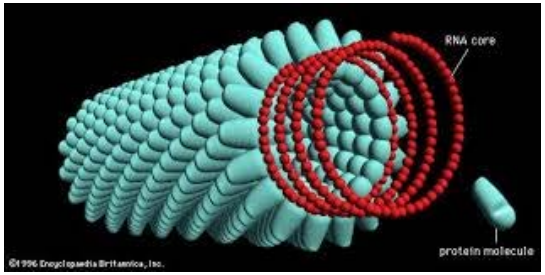
ARTICLES
PUBLISHED ONLINE: 20 JULY 2014 | DOI: 10.1038/NNANO.2014.140
nature nanotechnology

Optical trapping of individual human immunodeficiency viruses in culture fluid reveals heterogeneity with single-molecule resolution

Yuanjie Pang, Hanna Song, Jin H. Kim, Ximiao Hou† and Wei Cheng*

Optical Traping of viruses

- Optical trapping of tobacco mosaic virus (TMV) was demonstrated in 1987.



- However, TMV posseses a couple of special features that facilitate trapping by optical tweezers:

- Is 300 nm long and 15 nm in diameter
- It has a long cylindrical structure
- It has a permanent dipole moment

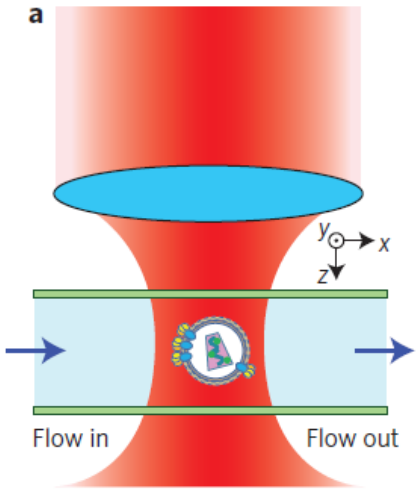
its trapping by optical tweezers.

Optical Traping of viruses - Limitations

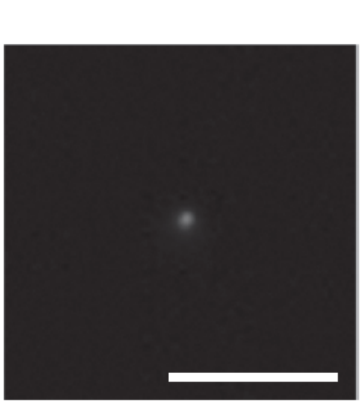
Most animal viruses

- are small, spherical & with small dipole moments.
- According to Rayleigh scattering principles: the smaller the particle, the more difficult is to trap & manipulate by light.
- Refractive indices of animal viruses are unknown.

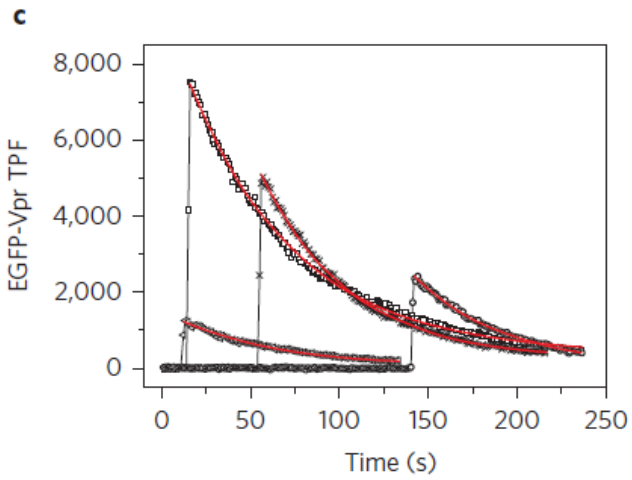
Optical trapping of single HIV-1 in culture fluid



trapping of a virion by the 830 nm infrared laser focused at the centre of the chamber



Two-photon fluorescence (TPF) image of a trapped HIV virion

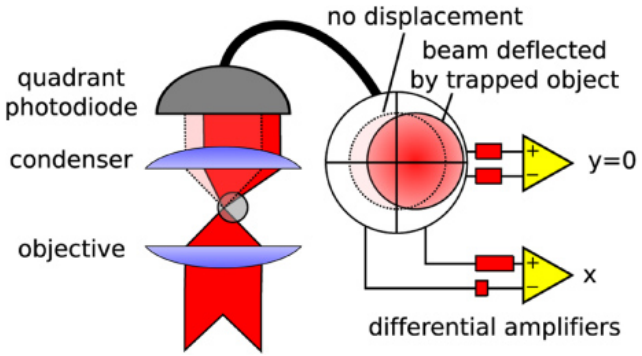


Representative TPF time courses from individually trapped HIV virions. All traces are fit with single exponential decay (red), with time constants for each trace as follows: 60.7 s (squares), 49.5 s (crosses), 51.5 s (circles) and 54.1 s (triangles).

- HIV-1 virions derived from the X4-tropic NL4-3 provirus clone tagged internally with enhanced green fluorescent protein (EGFP) fused to Vpr
- Live virus stock was diluted in the complete media and injected into a microfluidic chamber (no fixation)

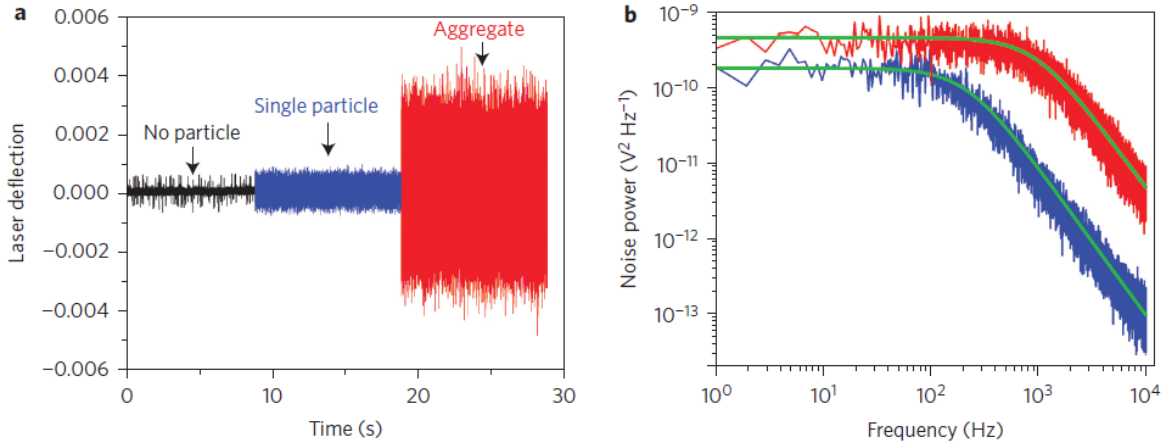
How do we know that the trapped particle corresponds to a single HIV-1 virion?

Optimized back-focal-plane interferometry



directly measures forces of optically trapped particles

Power spectra



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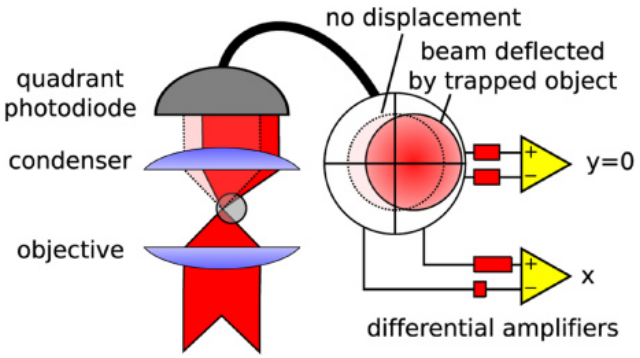
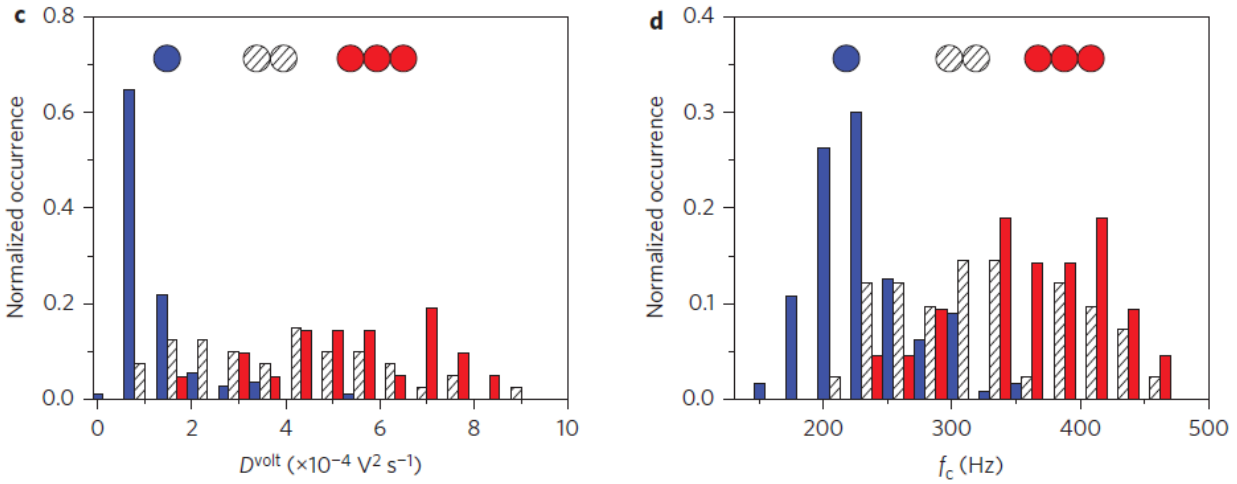
Optimized back-focal-plane interferometry

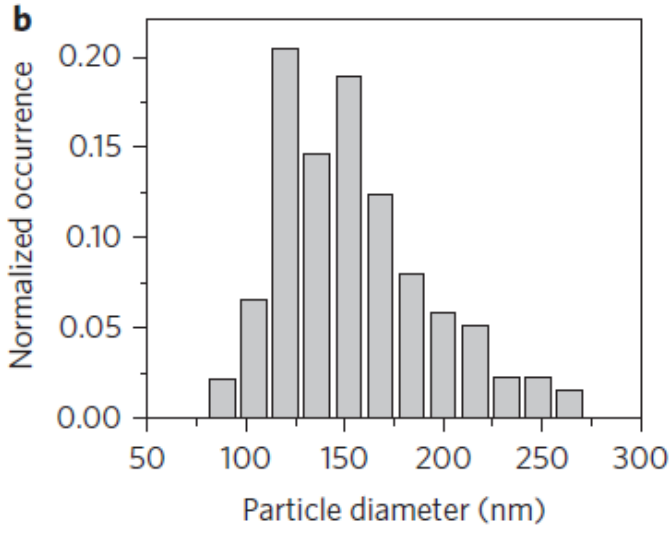


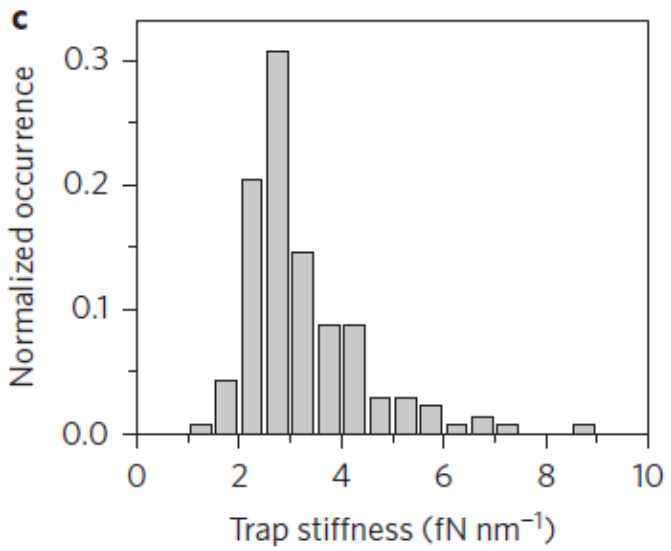
directly measures forces of optically trapped particles



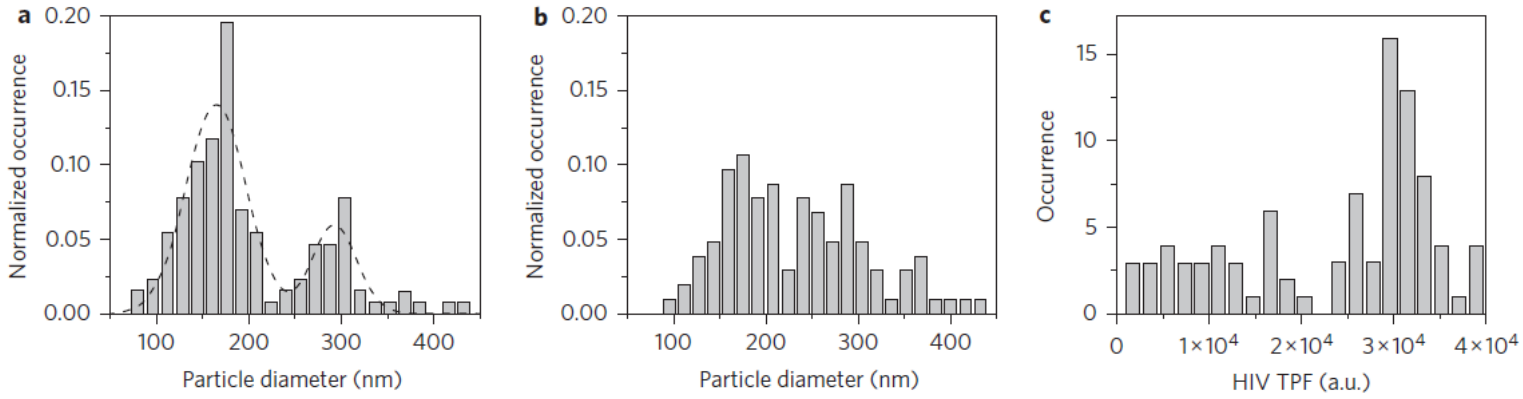
Histograms for Dvolt (c) and fc (d), derived from Lorentzian fitting parameters for one (blue, N = 110), two (hashed, N = 41) and three (red, N = 21) particles trapped

Virion characteristics

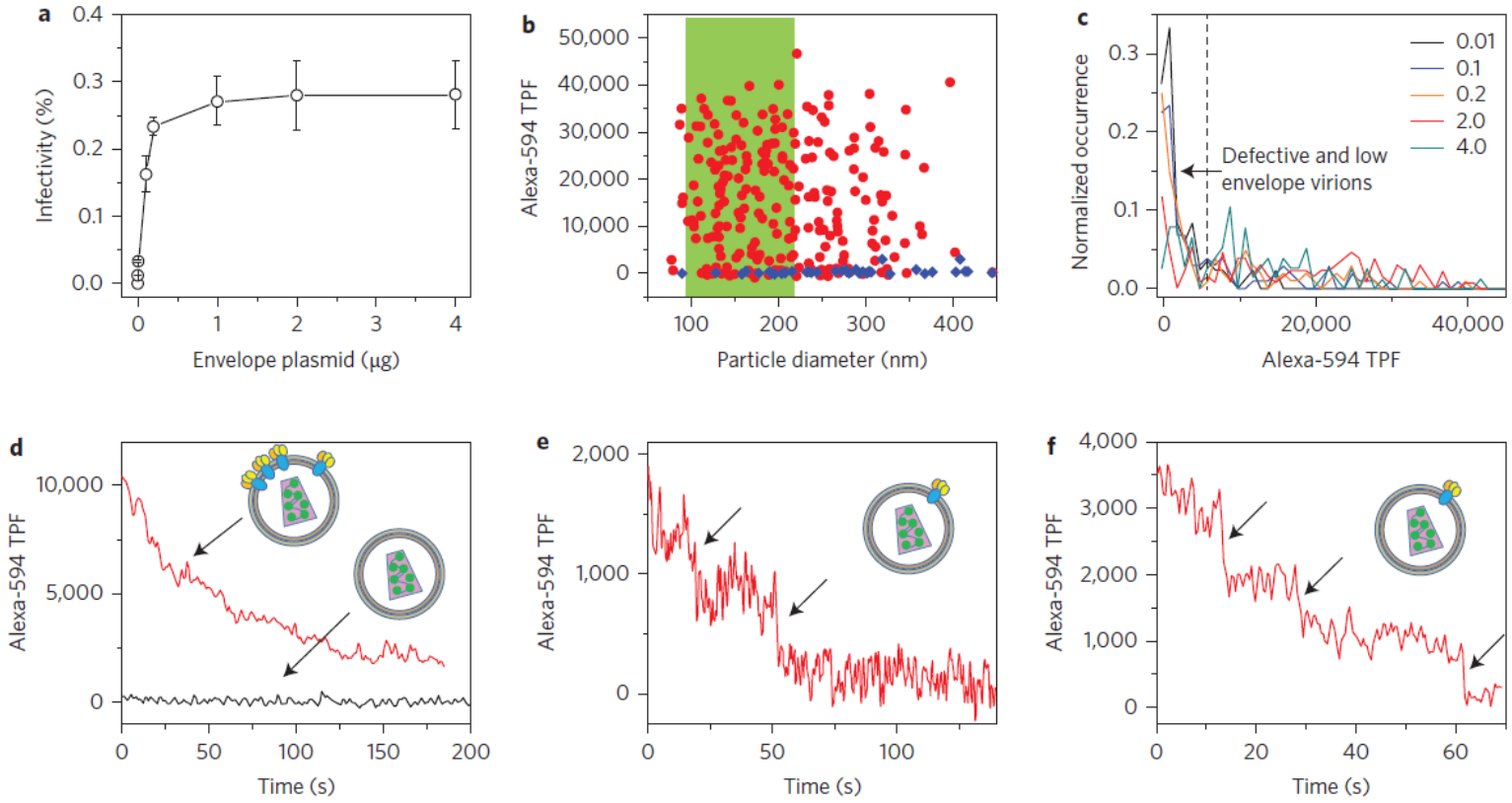




Aggregation of HIV at high virion concentration



Optical trapping virometry



Infectivity of HIV-1 virions as a function of pEnv in the presence of 20 μg ml−1 DEAE–dextran. Error bars are standard deviations from three independent replicates

TPF intensity histograms for Alexa-594 from single HIV-1 particles, with black, blue, orange, red and olive curves for virions from 0.01 (N = 84), 0.1 (N = 102), 0.2 (N = 100), 2.0 (N = 128) and 4.0 μg (N=76) pEnv, respectively.

Innovations

- Study virion transmission and infection at the single-particle level.

  individual HIV-1 differs in the numbers of envelope glycoproteins by more than one order of magnitude

- Heterogeneity of HIV1 virions

- Analogous to flow cytometry – ultrasensitive detection,multi-parameter analysis & sorting

Applications:

viruses and other nanoparticles in biological fluid with single-molecule resolution.

Outlook

- high resolution
- high throughput
- programmable manipulation

The technology is still in search of a ‘killer application’

“Applications where it is the result that is the value, not the device”

Thank you!