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Winter College on Micro and Nano Photonics for Life Sciences

11 - 22 February 2008

Optical Trapping and Structured Light III : applications for the biosciences

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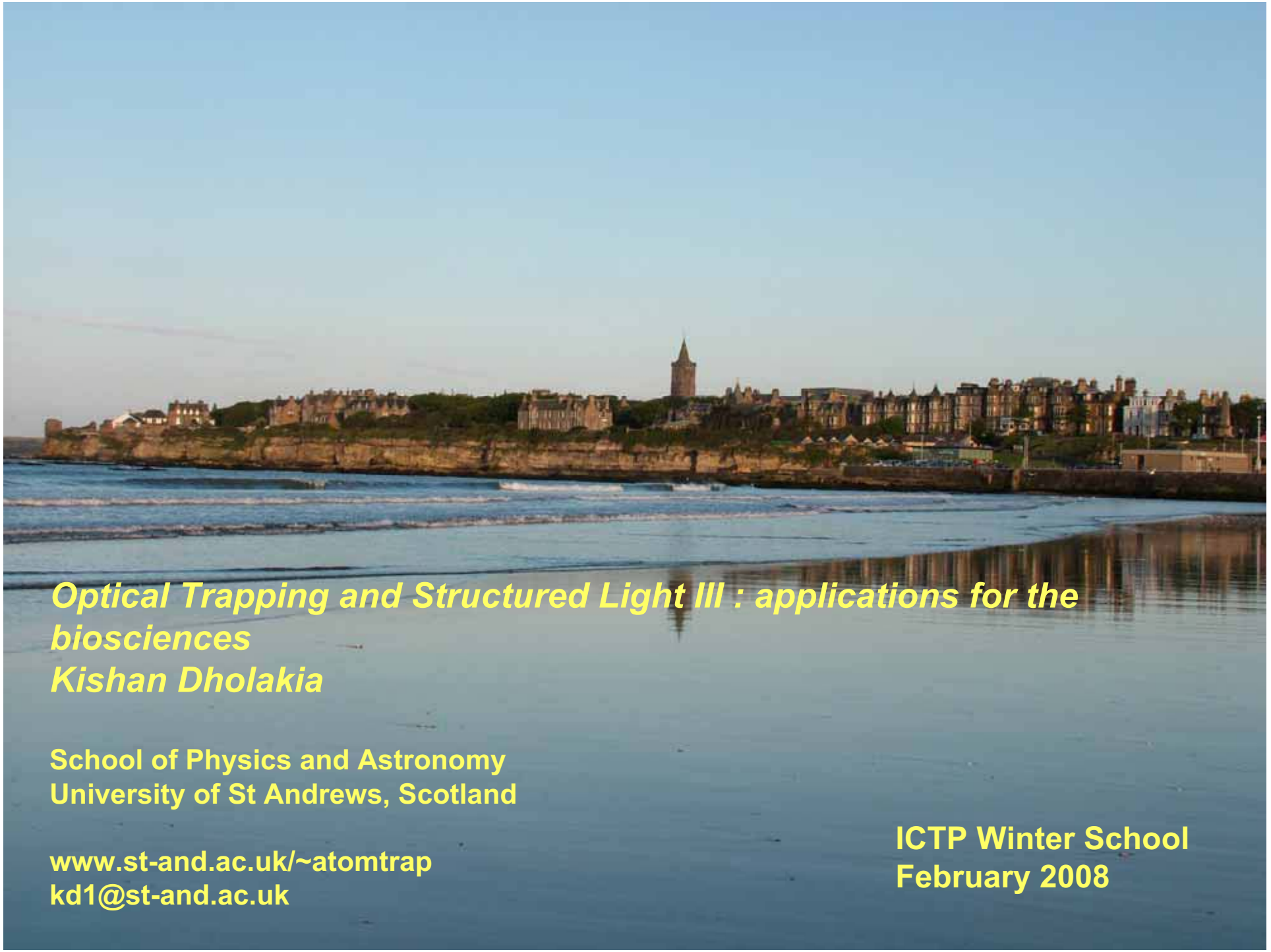

Optical Trapping and Structured Light III : applications for the biosciences

Kishan Dholakia

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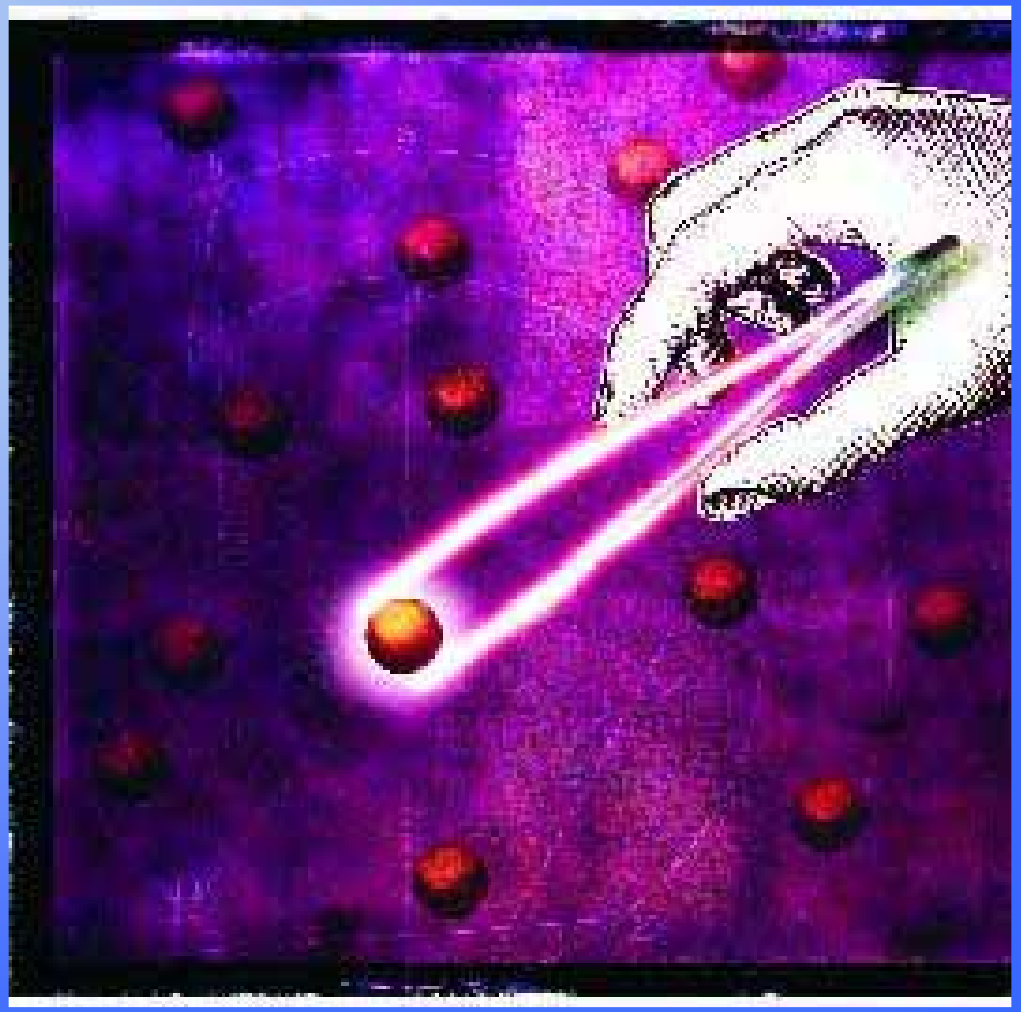
ICTP Winter School
February 2008

This lecture

- structured light for bioscience

Cell sorting
Photoporation
Raman



Microfluidics

The laws of microfluidics have been known for over 100 years

Unusual fluidic phenomena may occur under unusual conditions

- Very small channels/high velocities
- Surface treatments
- High electric fields
- Non-ideal fluids (such as blood)

Ref:

T. M. Squires and S. R. Quake: Microfluidics: *Fluid physics at the nanoliter scale*
Rev. Mod. Phys., Vol. 77, No. 3, July 2005 ..and references therein (50 pages)

cf. the automation in electronics. Big difference: the physics has not changed there!

Fluid dynamics makes life a bit unpredictable!

Viscous flow regime

At small sizes, fluid flow is dominated by viscous flow. Inertial flow is almost non-existent. This means the flow is “Laminar” with no turbulence.

Viscous flow is like flowing syrup

This also means we cannot rely on turbulence for mixing. Diffusion dominates

Inertial vs. viscous flow

Reynolds number (Re)

For Re < 2300, no turbulence (Laminar flow)

For Re > 4000, turbulence (mixing)

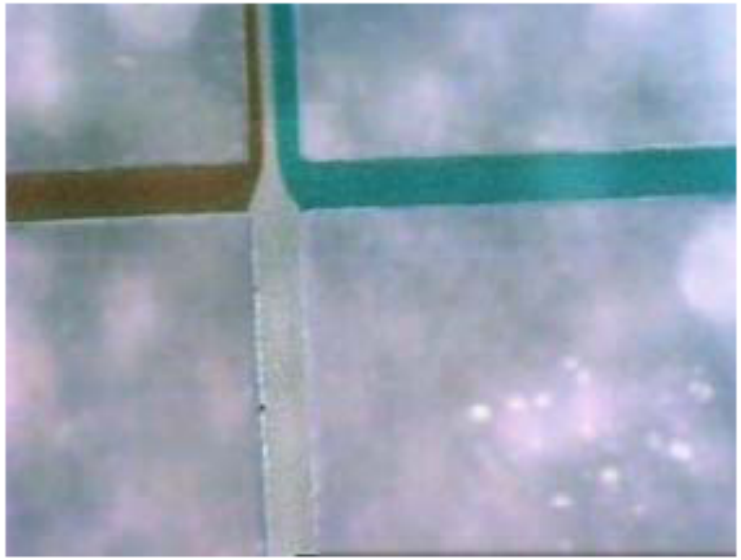
$$R_e = \frac{\rho U L}{\eta}$$

ρ = density of fluid

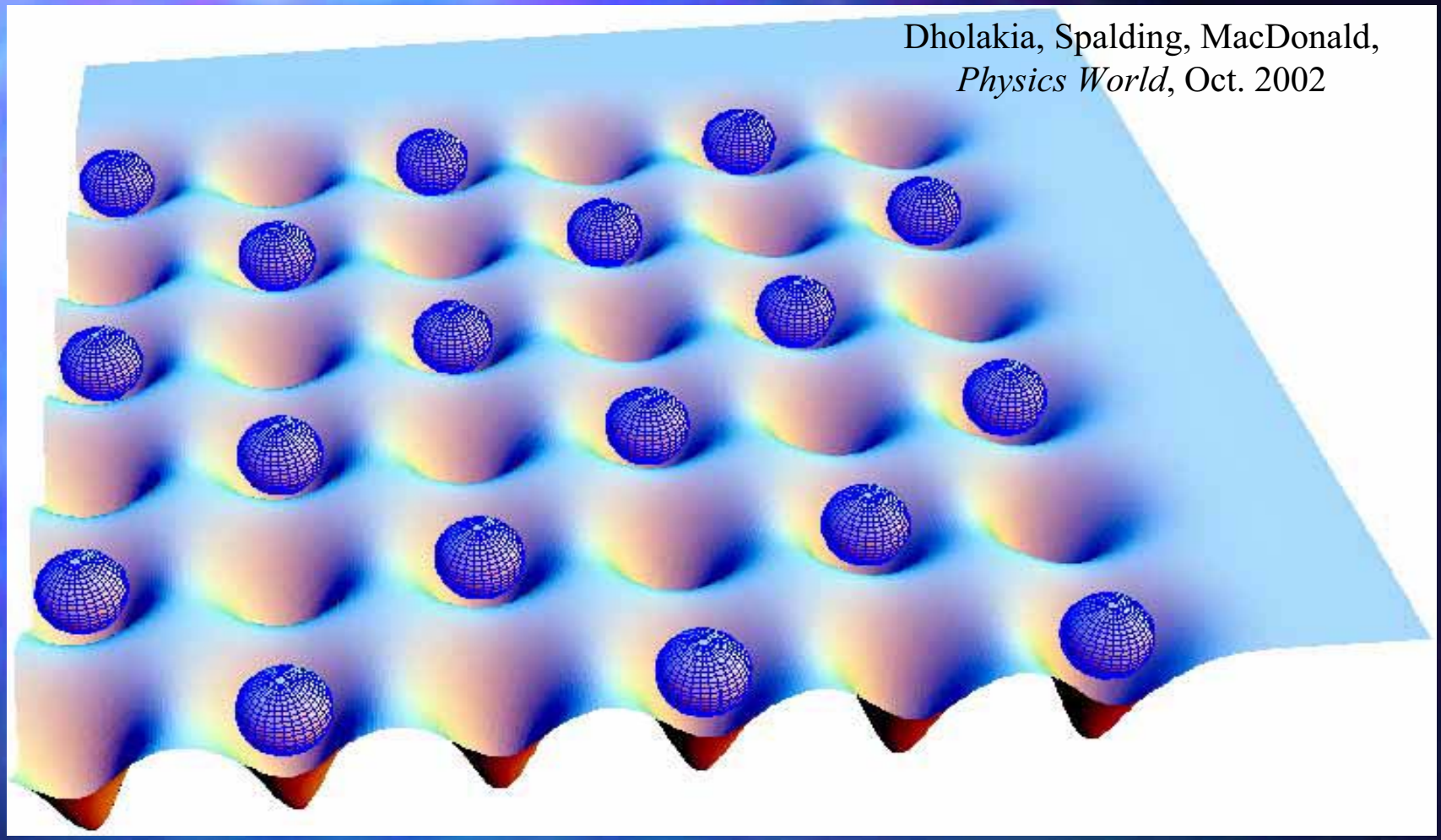
η = viscosity of fluid

U = velocity of fluid

L = characteristic length (size)



"Characteristic lengths" of micro-fluidics is 10-100 microns. Typical Re < 1.



Dholakia, Spalding, MacDonald,
Physics World, Oct. 2002

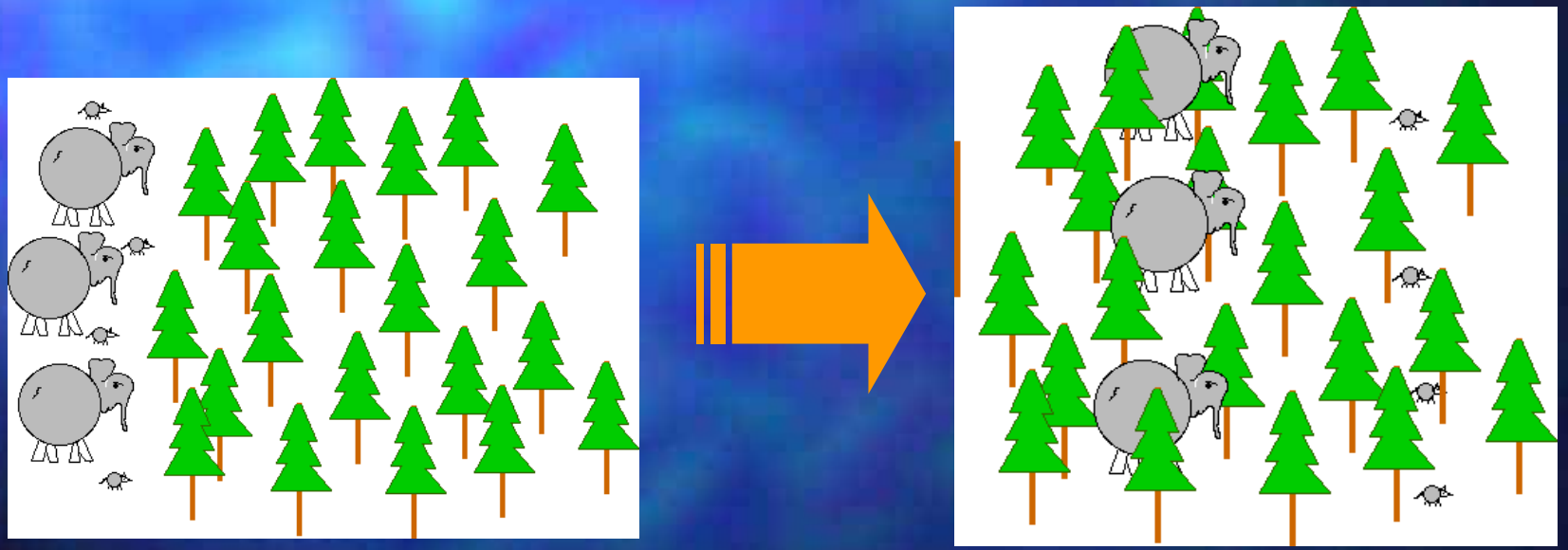
Holographic /interferometric/time sharing can create 2D/3D arrays of trap sites: useful for optical sorting/guiding and other studies

Separation is a major issue at the nanoscale

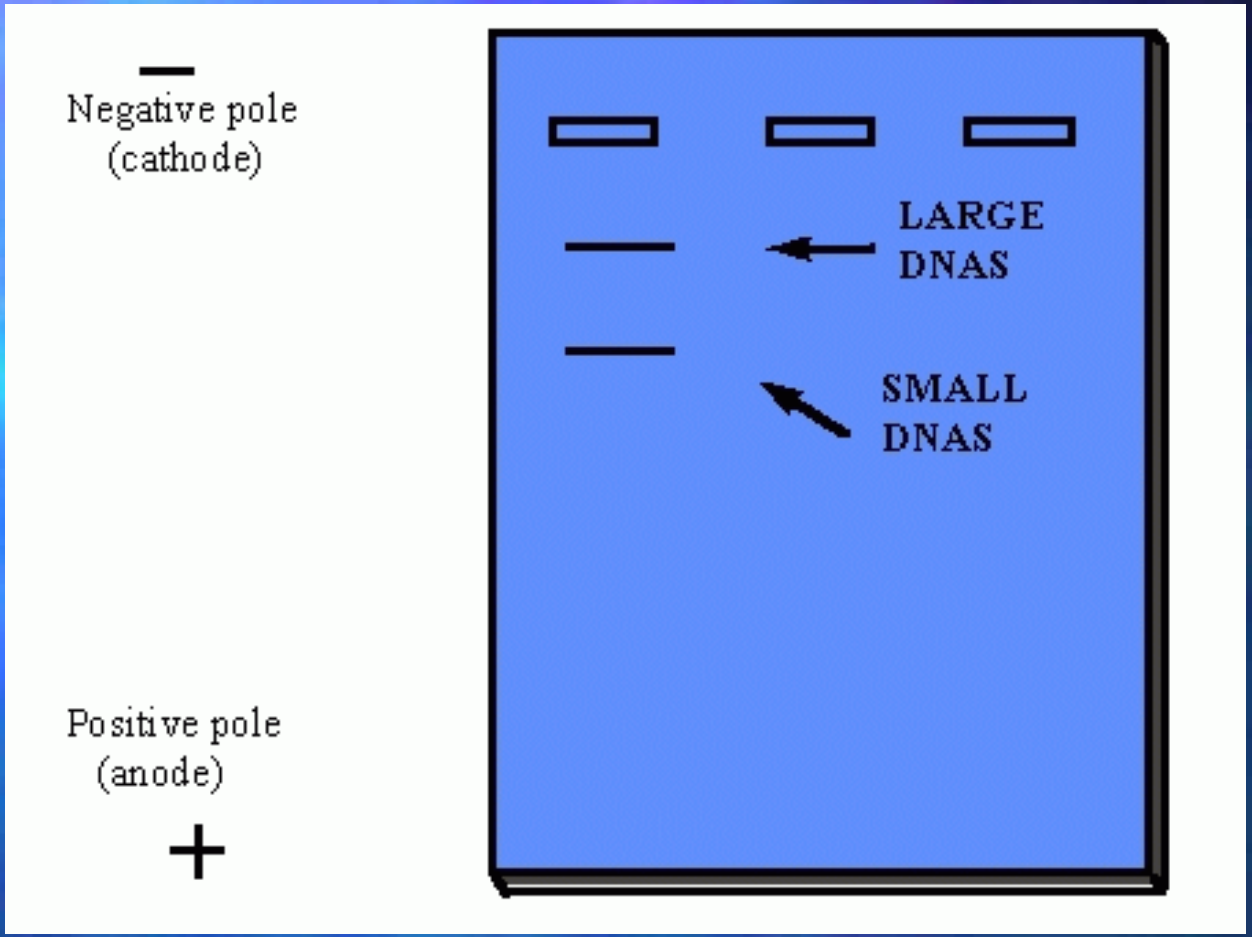
Individual DNA fragments may be isolated by electrophoresis in polyacrylamide or agarose gels

-separation of DNA fragments by size.

-During gel electrophoresis, small molecules migrate faster through the pores of the gel matrix than larger molecules.



See http://www.uic.edu/classes/phar/phar331/lecture6/



Agarose gel (made from seaweed)

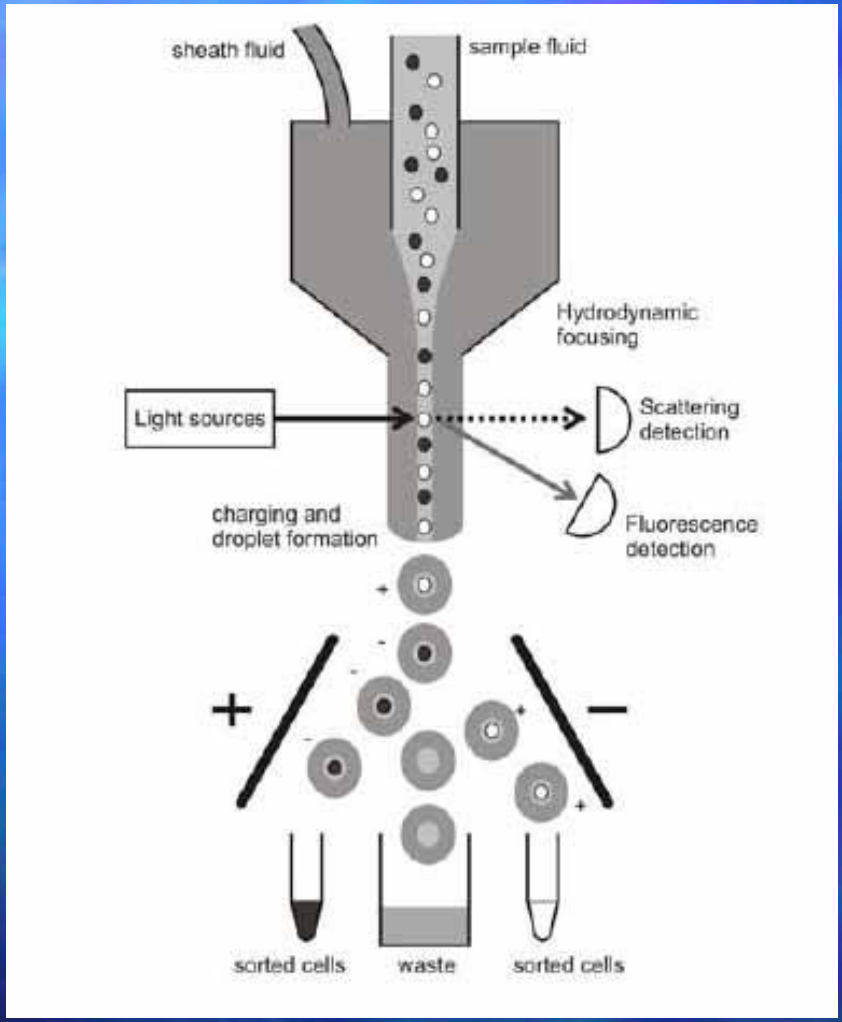
Compare with molecular weight markers

Obtain estimate of size of DNA fragments

Gel electrophoresis

- Separation of macromolecules based on size, charge
- Gel is “solid” colloid.
- Electrophoresis is the migration of charged objects in the presence of E field
- Activated electrodes at either end of the gel create a force
- Used to separate nucleic acids and proteins

Fluorescence activated Cell sorting: FACS

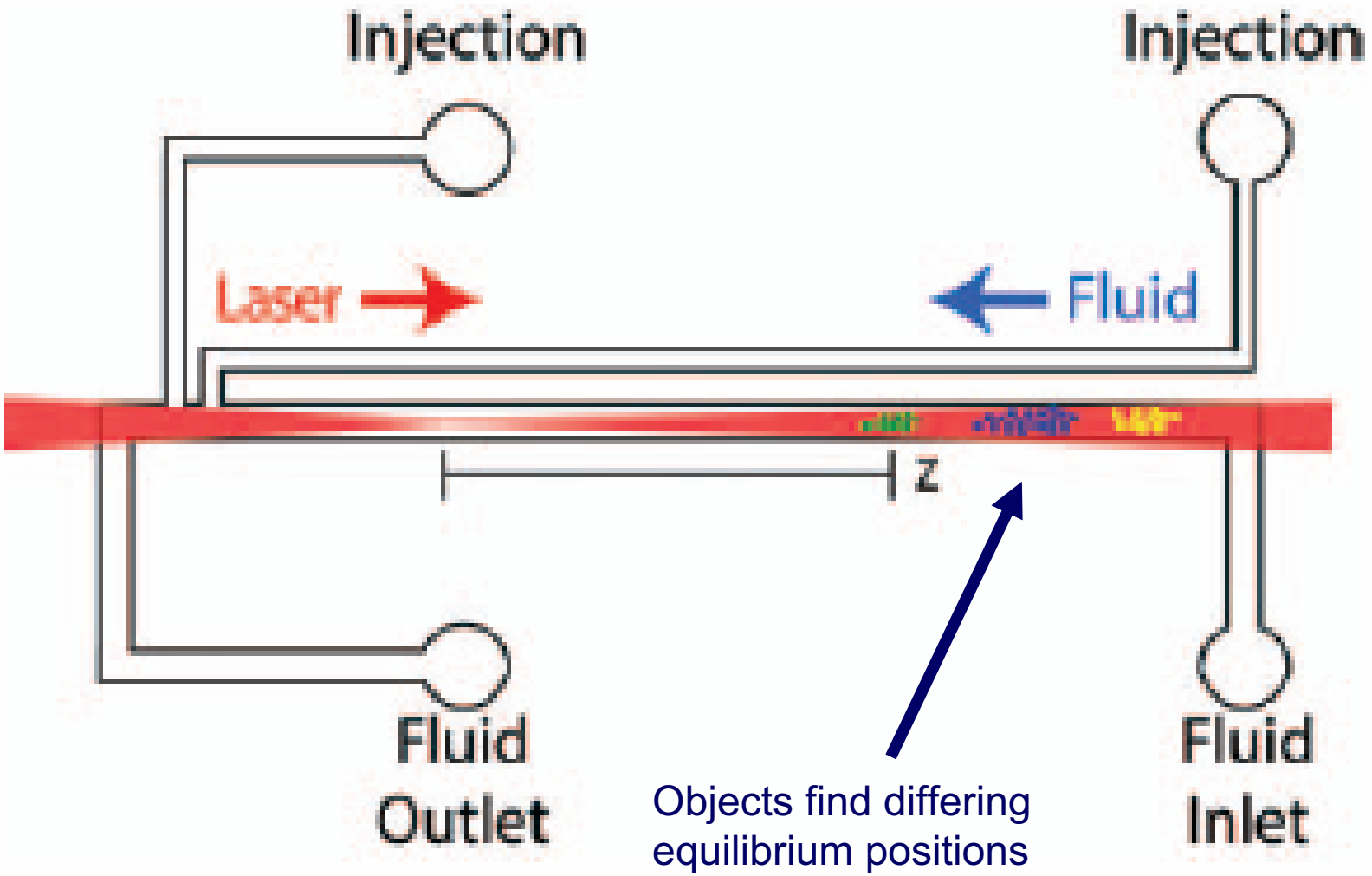


Other methods such as magnetically activated cell sorting exist too..

super-paramagnetic beads coated with suitable antibodies attach to cells of choice

But this is hard for small cell samples, microfluidic environments or small volumes of analyte

Optical chromatography for microfluidics…



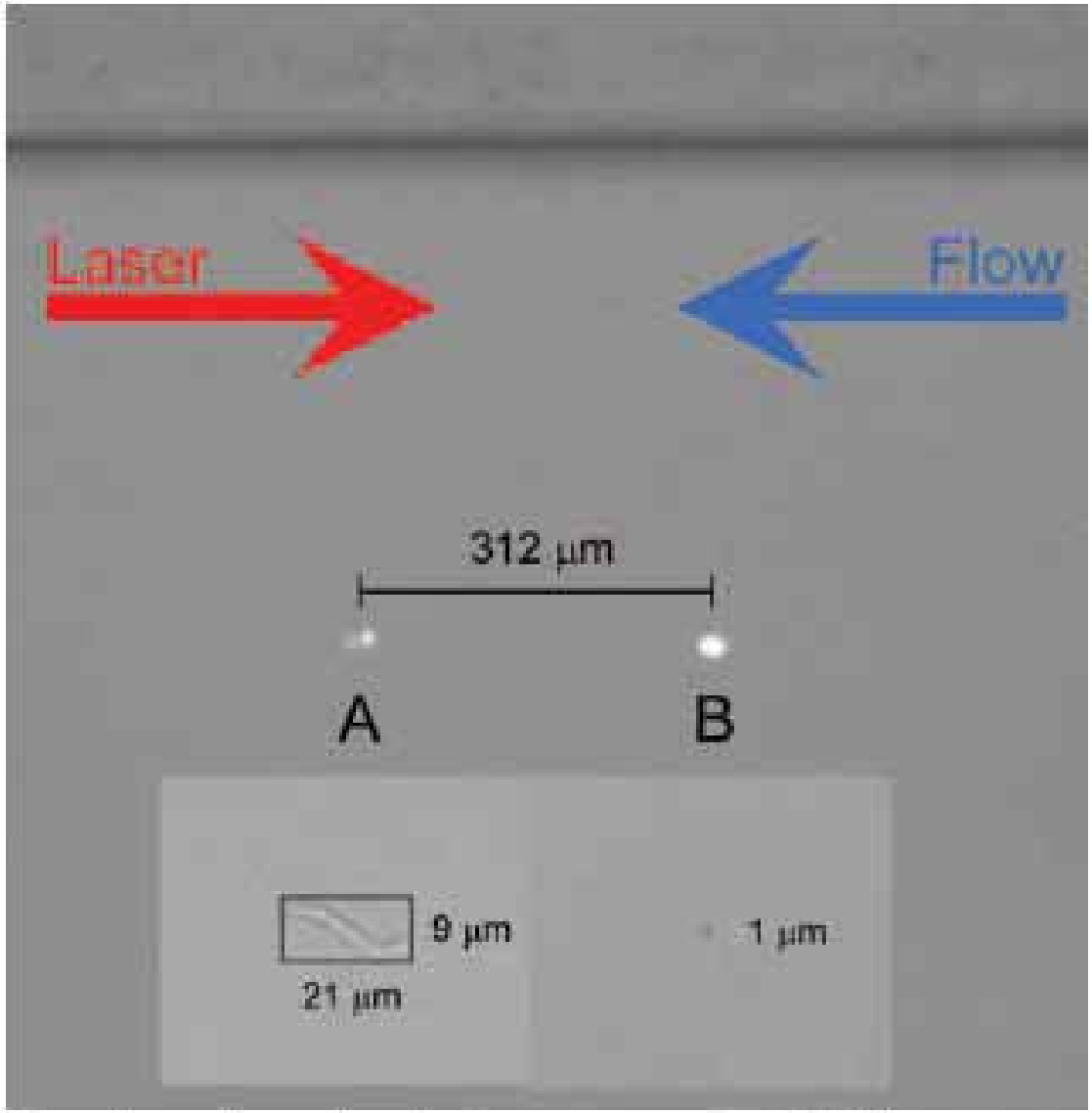


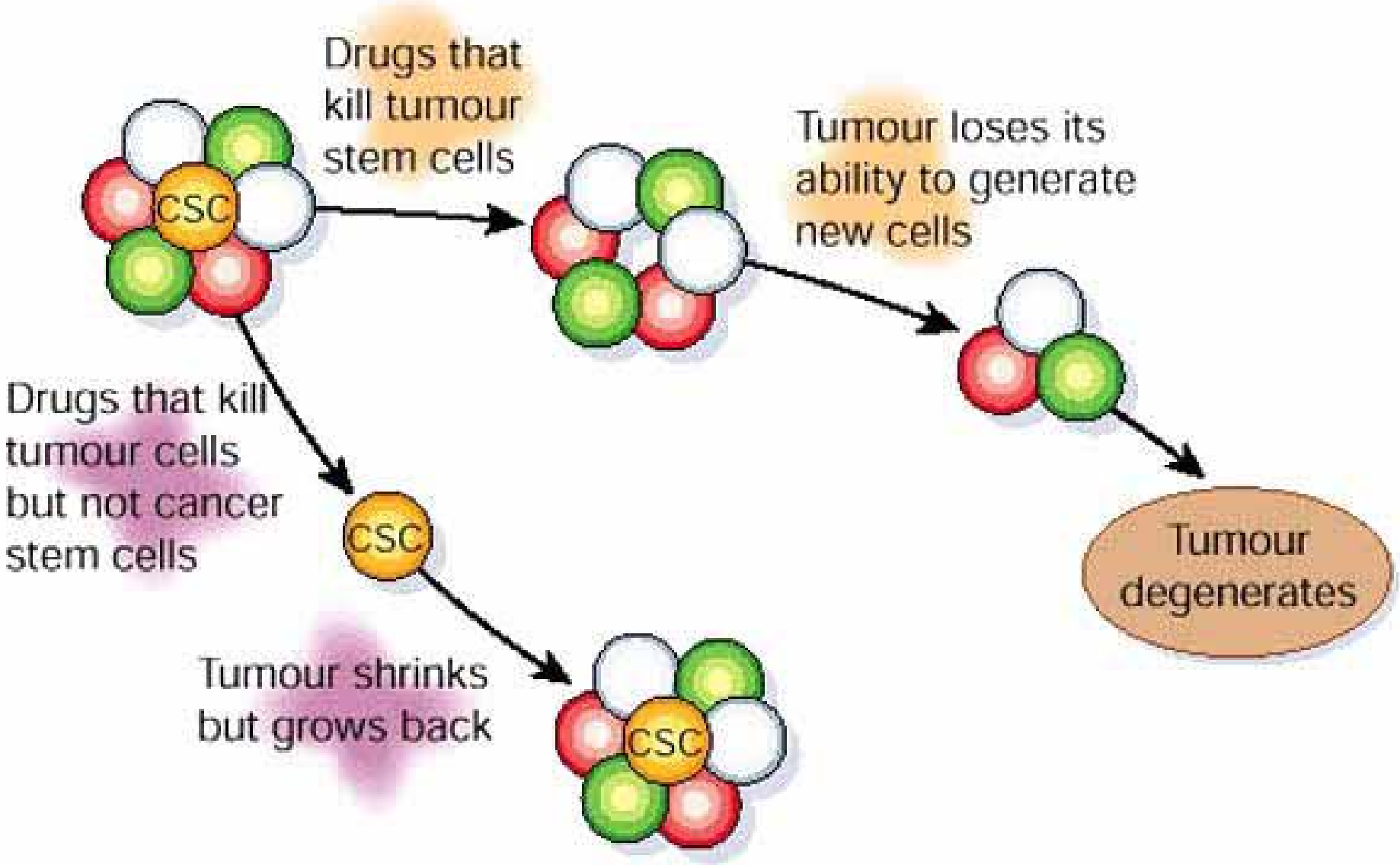
Figure 4 *Image data showing a representative optical chromatographic separation of A) Bacillus anthracis (Sterne strain 34F2) cell (left) and B) spore (right) in laser light scatter at 40× total magnification. Insets are brightfield images collected at 400× total magnification. Conditions: 0.75 W laser power using an 850-nm fiber laser; 28-µm/sec fluid velocity.*

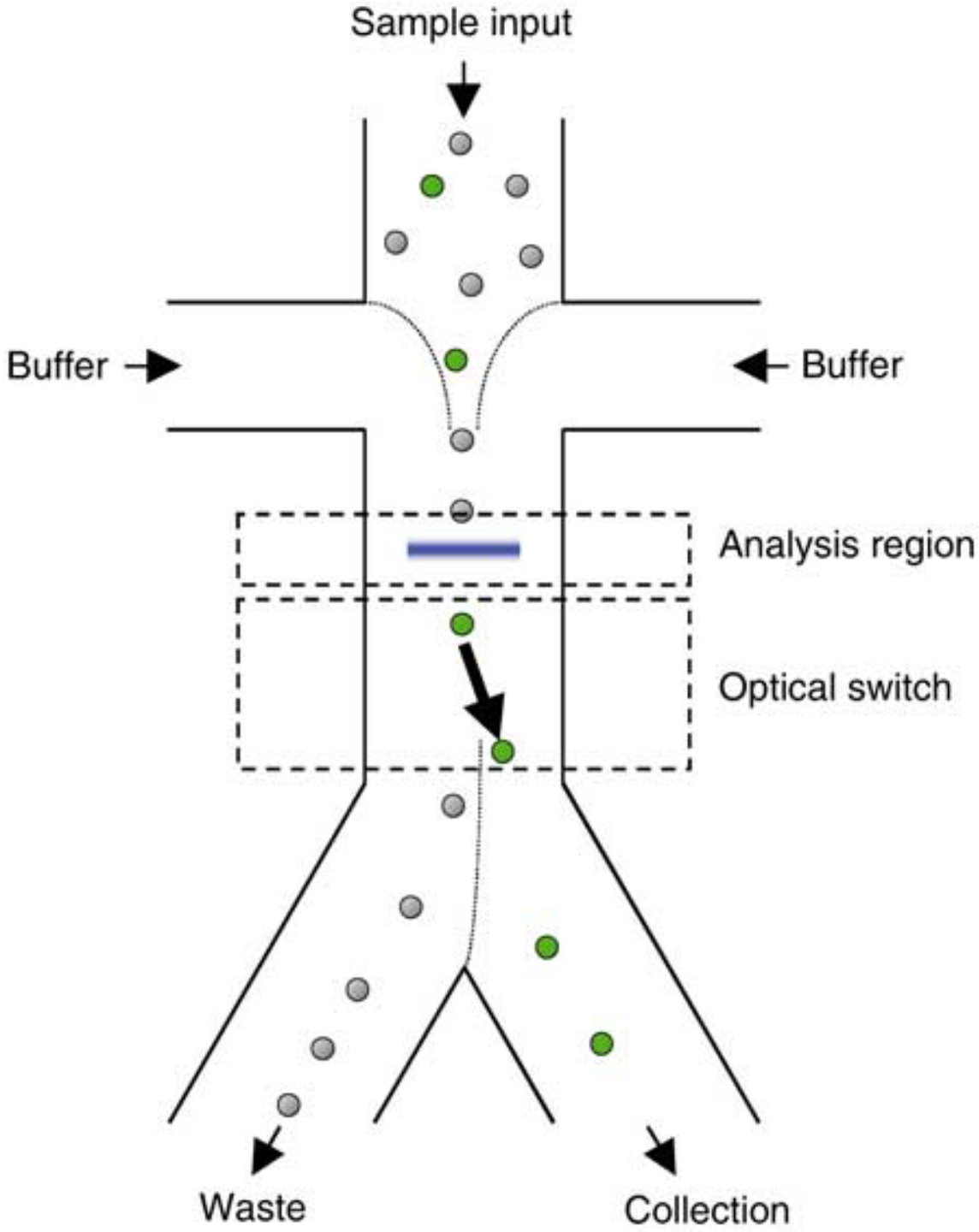
Sean Hart/Alex Terray group

Optical Chromatography

Balance the optical scattering force vs the linear Stokes drag in the fluid: this will depend therefore on the refractive index of the particle

Cell sorting





ACTIVE SORTING

Nature Biotechnology 23, 83 - 87 (2004)
Published online: 19 December 2004; |
doi:10.1038/nbt1050 Microfluidic sorting of mammalian cells by optical force switching
Mark M Wang, Eugene Tu, Daniel E Raymond, Joon Mo Yang, Haichuan Zhang, Norbert Hagen, Bob Dees, Elinore M Mercer, Anita H Forster, Ilona Kariv, Philippe J Marchand & William F Butler

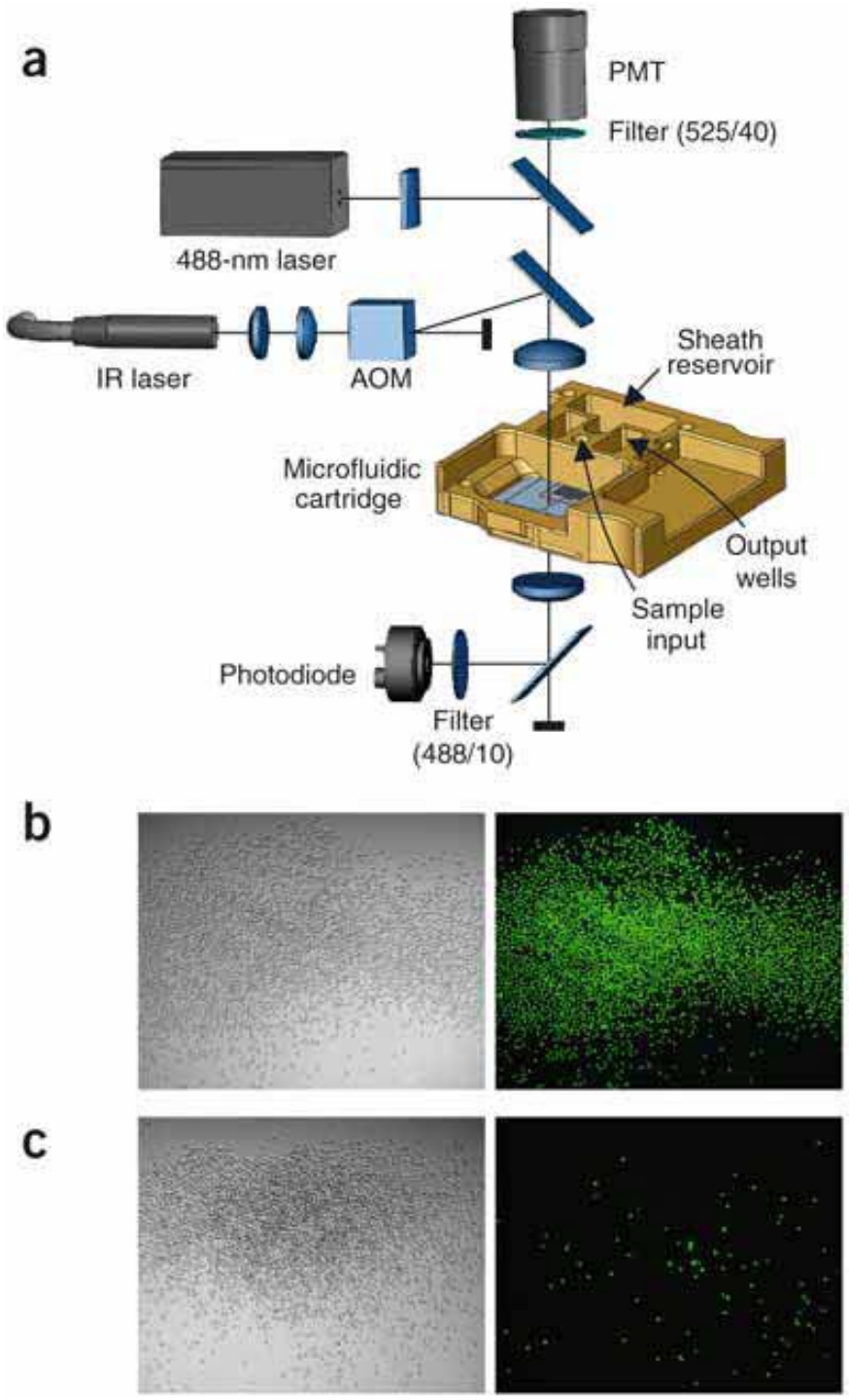


Figure 2. Cell sorting with an optically switched microfluidic fluorescence-activated cell sorter. **(a) Schematic of the cell sorter instrument and the microfluidic cartridge. The near-infrared laser and 488-nm laser are focused through the same lens onto the microfluidic chip. The presence of a cell in the analysis region is detected by a photodiode and the fluorescence of that cell is measured by the PMT. Based on a gating of the fluorescence signal the AOM is triggered to optically switch the cell (GFP-positive cells are switched to the collection well). Sample is loaded directly onto and off the cartridge by pipette. Windows on the bottom of the collection wells permit viewing of the sorted populations. IR, infrared. (b,c) Brightfield (left) and fluorescence (right) images of the resulting cell populations in the collection well (b) and waste well (c) are shown for a typical sort of the GFP-expressing HeLa cells.**

Recovered populations were verified to be both viable and unstressed by evaluation of the transcriptional expression of two genes, *HSPA6* and *FOS*, known indicators of cellular stress.

Potential Landscapes

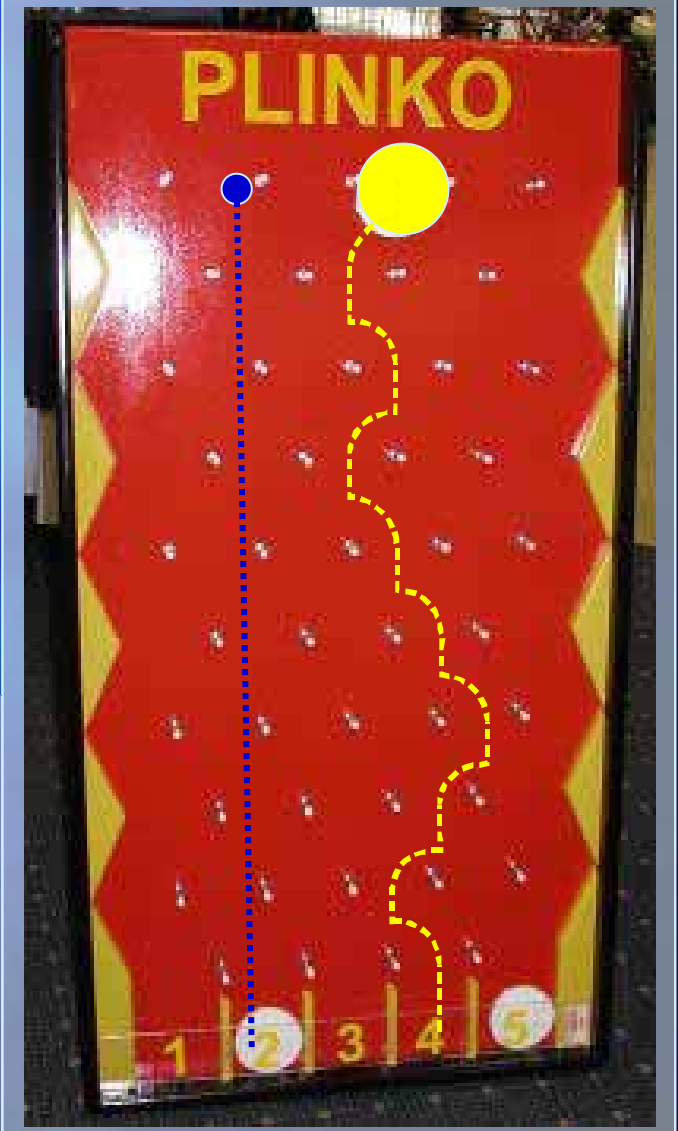
Eggs inhabit the lowest energy positions in an egg-box

In “Plinko” the path followed by a counter can be either

deterministic (----)

or

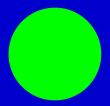
statistical (----)



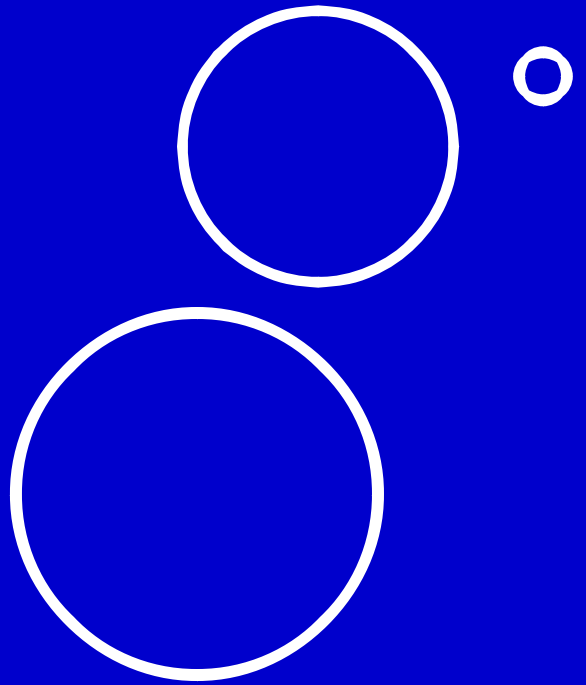
Particle placement

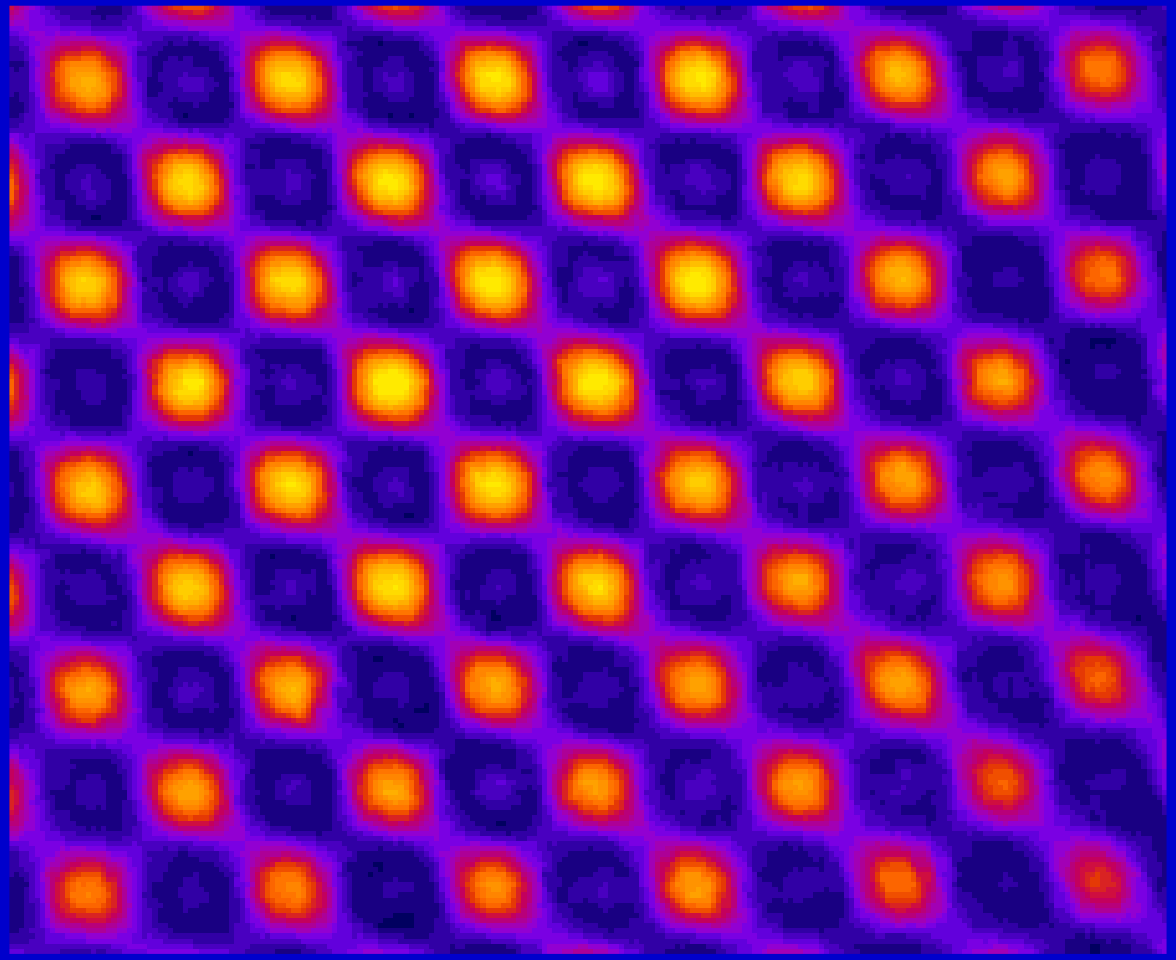
Like in any landscape, the lowest energy position for a particle is a function not only of the landscape but also of the particles size and density.

$n_{sphere} < n_{medium}$



$n_{sphere} > n_{medium}$



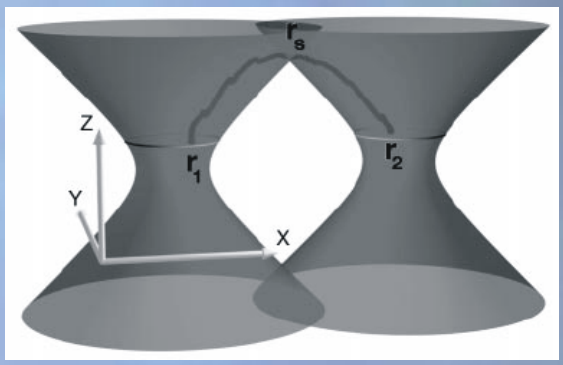


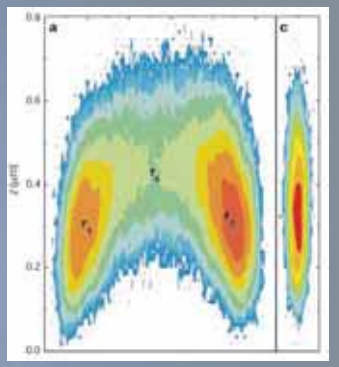
Particle Escape

How do particles get from one location in a landscape to another?

A particle can escape unaided from a local well due to thermal activation.

e.g. Kramer's rates
McCann et Al. Nature Dec. 1999

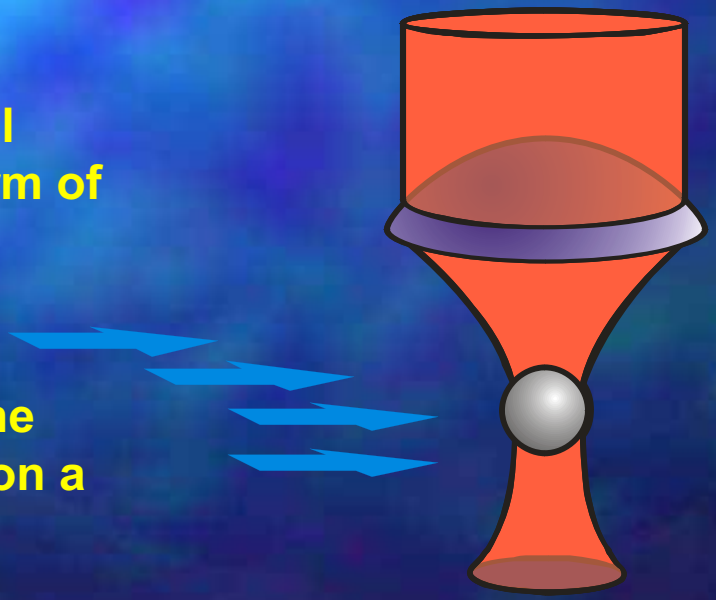




Larger particles like cells are unlikely to escape due to thermal activation alone.

Above 5 μm particles will usually require some form of external activation.

A common example is the Stoke's drag exerted upon a particle by a fluid flow.



gradient force

$$\propto \frac{\varepsilon - \varepsilon_0}{\varepsilon_0} \int \eta \, dV$$

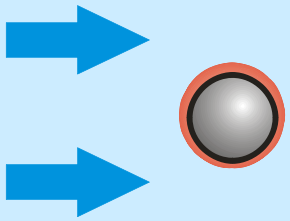
viscous drag

$$F = 6\pi\sigma r v$$

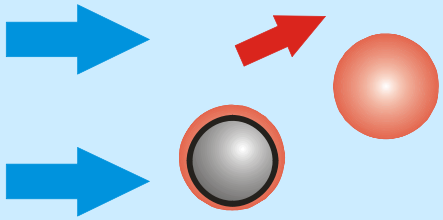
σ = *viscosity*
v = *velocity*
r = *radius*

Particle Activation – *Fluid Flow*

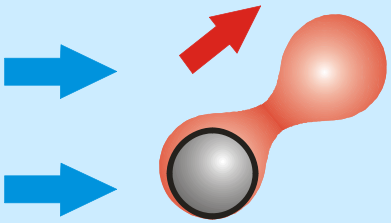
In a single trap the particle escapes in line with the drag force



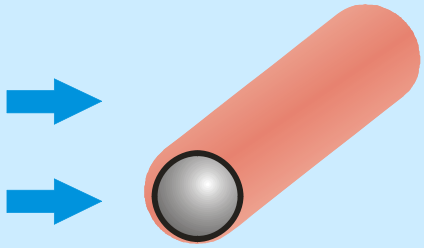
Where a second trap is present the trajectory of the particle is affected



If the traps are linked then there is a preferred route of escape



In the extreme case we can consider what happens in a line trap



gradient force

$$\propto \frac{\varepsilon - \varepsilon_0}{\varepsilon_0} \int \eta \, dV$$

viscous drag

$$F = 6\pi\eta r v$$

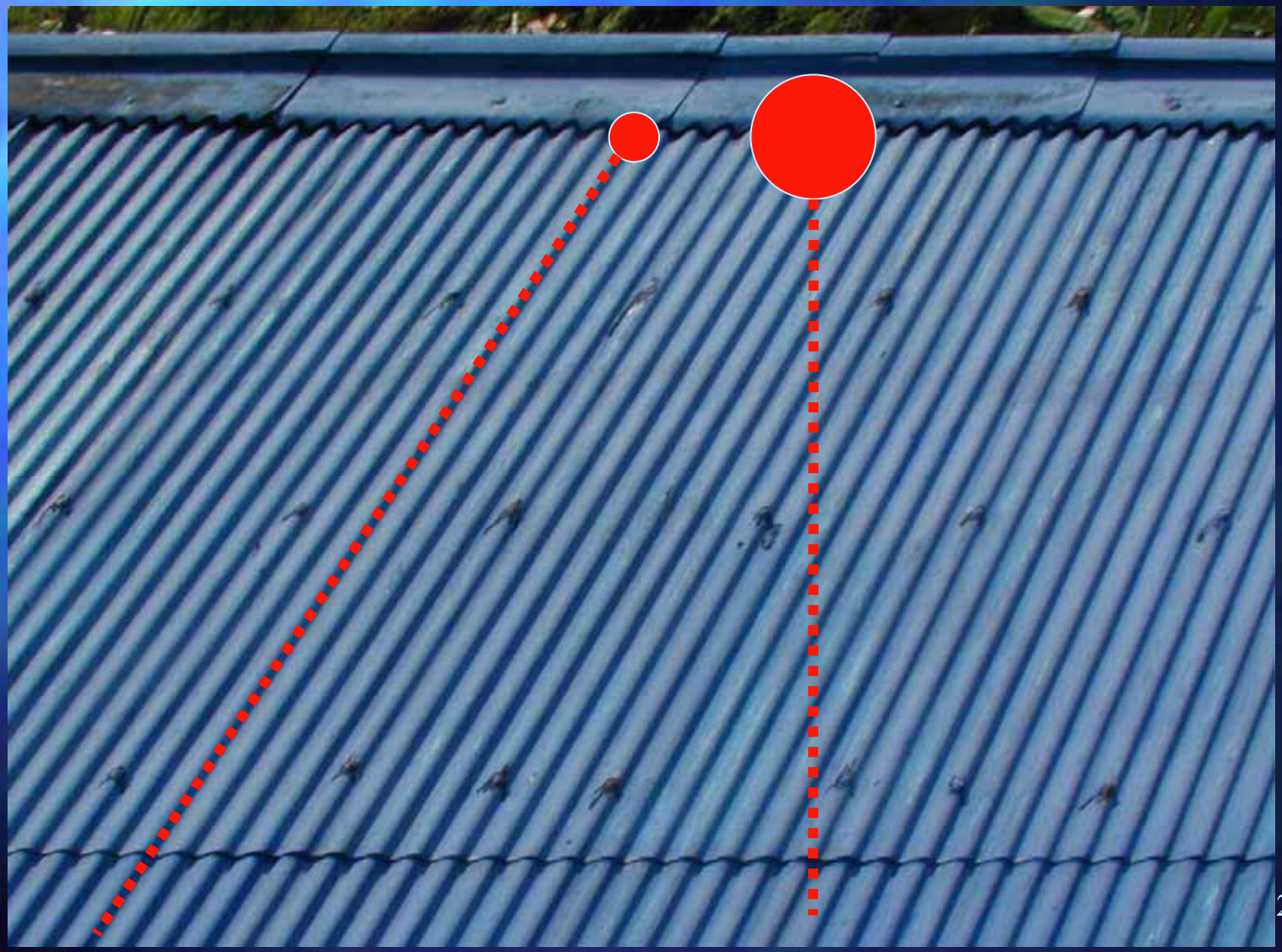
σ = *viscosity*
v = *velocity*
r = *radius*

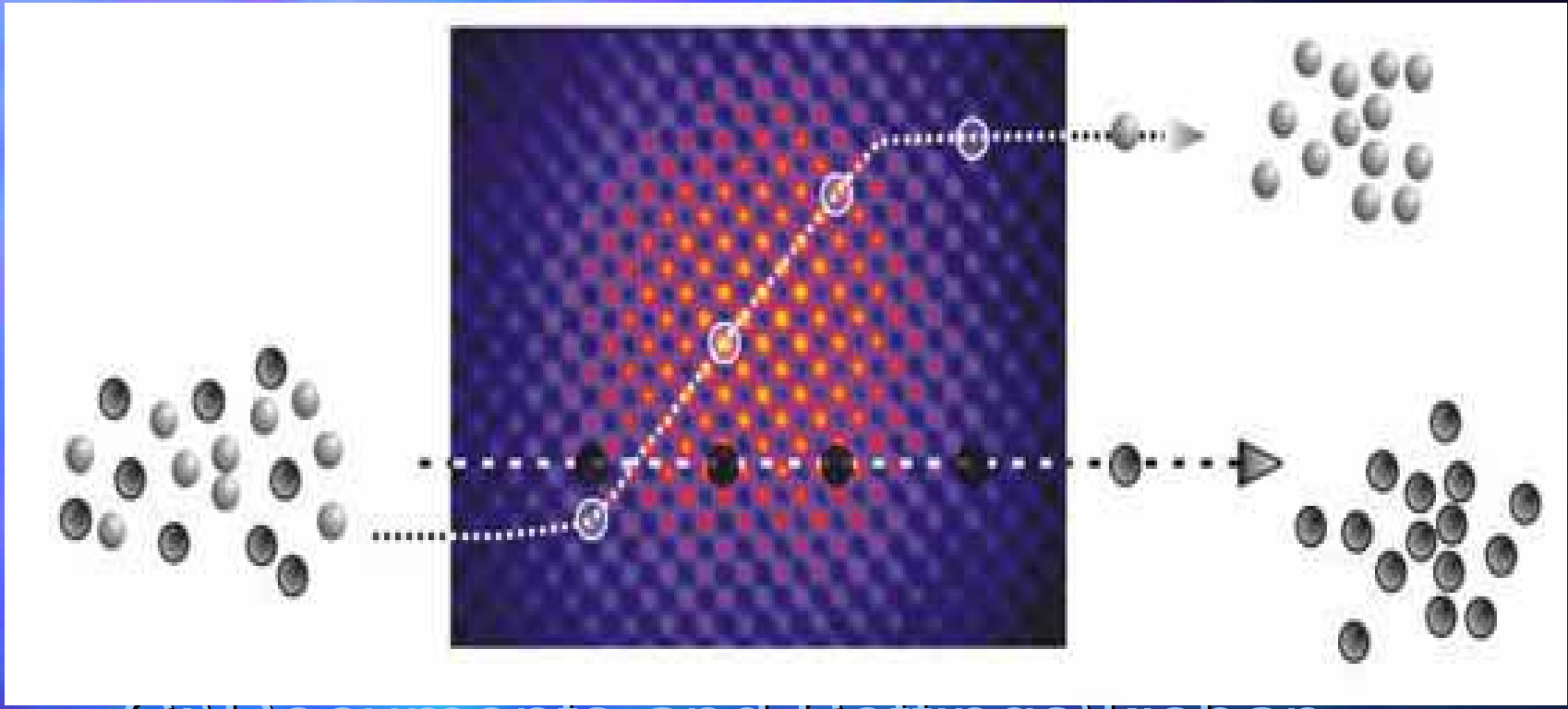
Potential Landscape Plus Driving Force

The landscape can also comprise of a set of parallel ridges like this roof

Sorting in a Potential Landscape

But it is more interesting when the ridges go across the fall-line

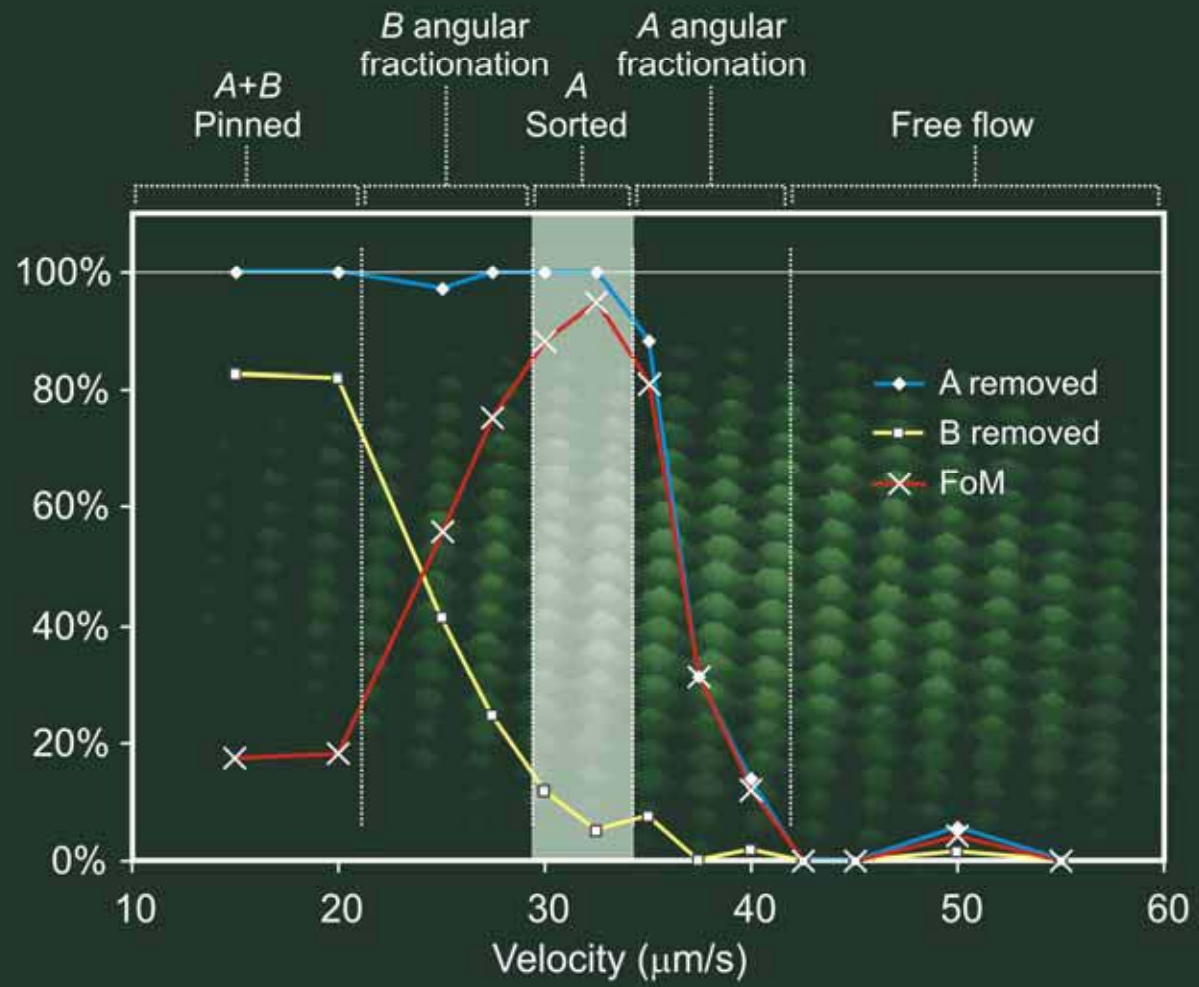




- C:\Documents and Settings\kishan dholakia\My Documents\INAUGURAL\Kishan\Movie 03 Channels EW Deflection Mix 135 Microns.avi

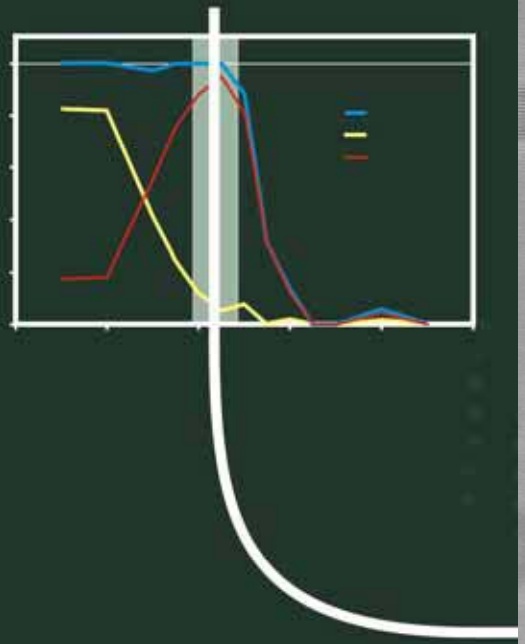
Dynamic phases of optical sorting

- As the power in the lattice or the flow velocity is changed we see a range of dynamic phases from trapping to hopping or guiding to free flow.

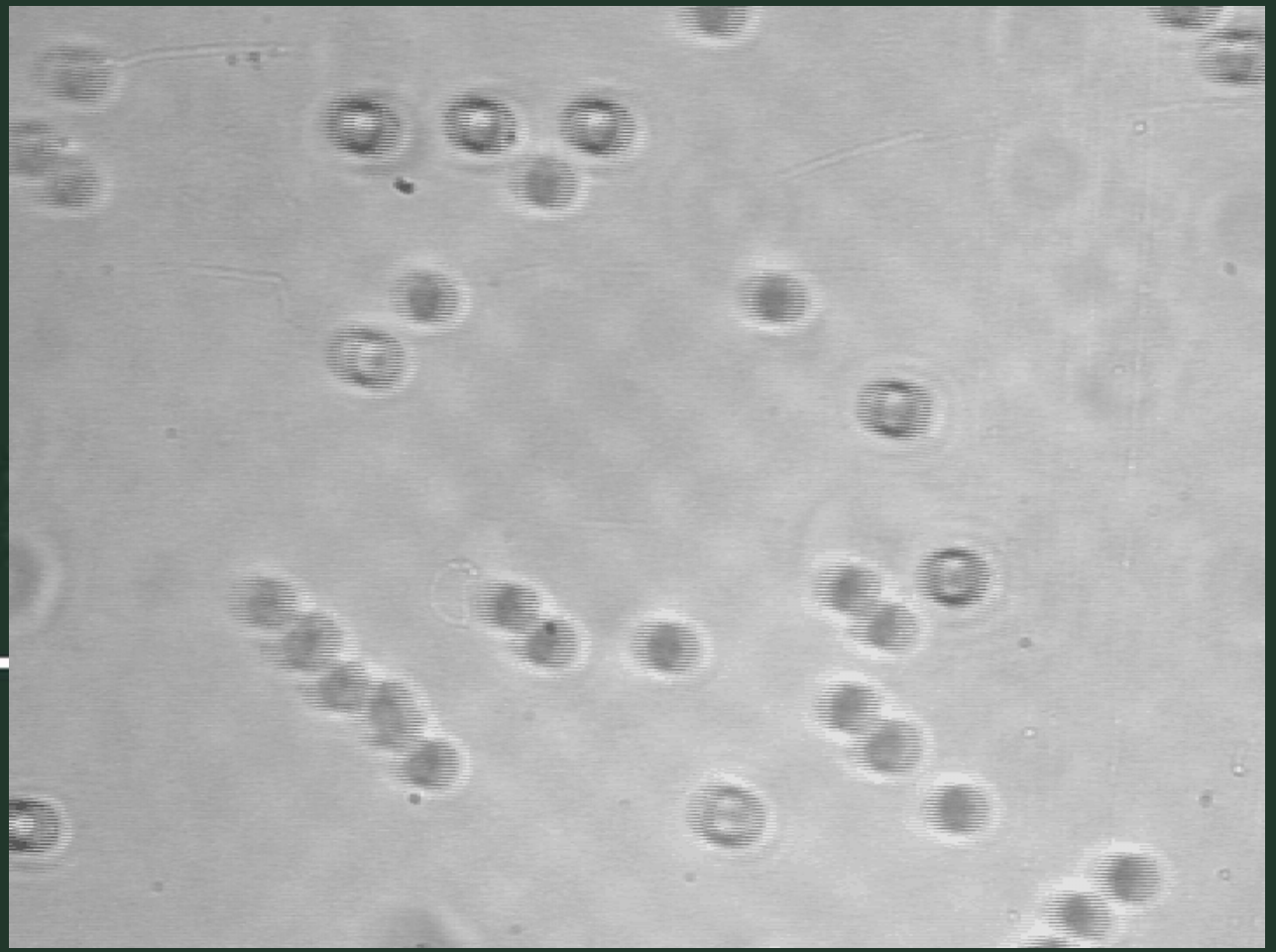


Sorting

- All of the strongly interacting species are deflected without jamming
- The vast majority of the weakly interacting species go straight through

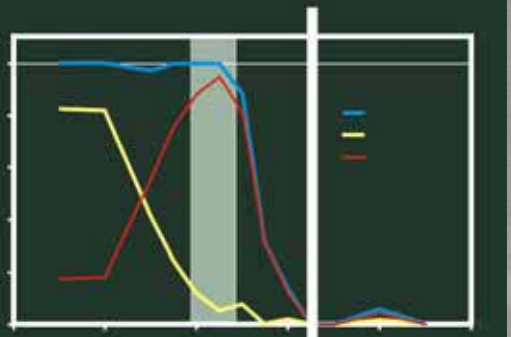


1W total power at sample

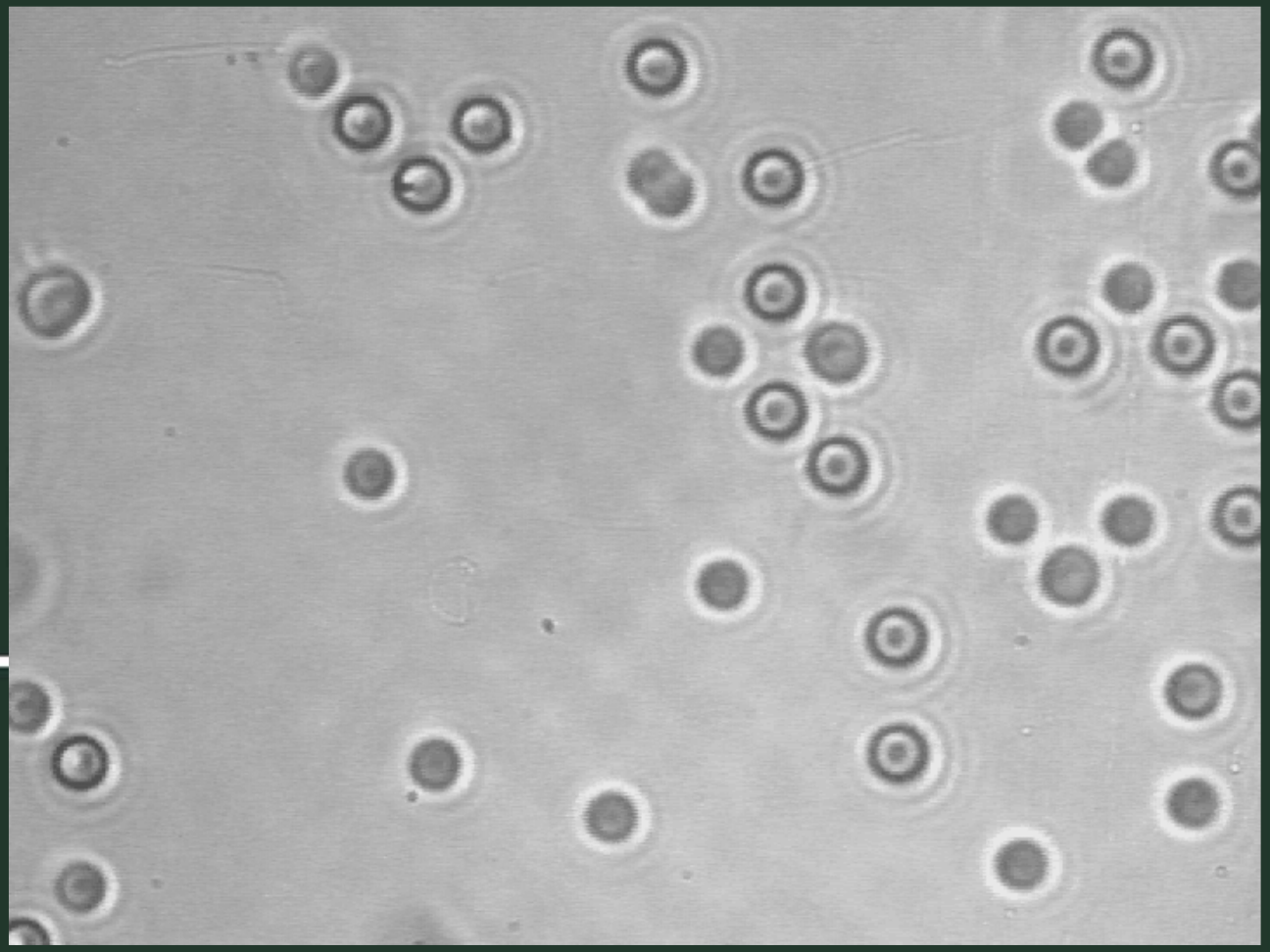


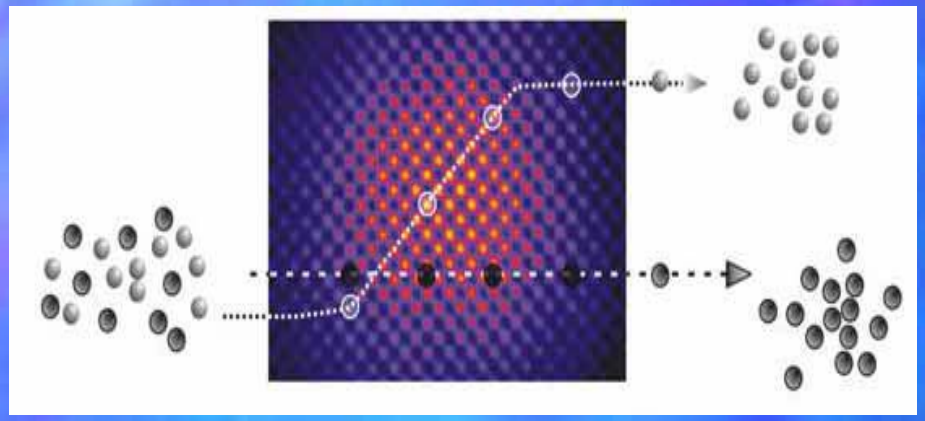
Straight-through flow

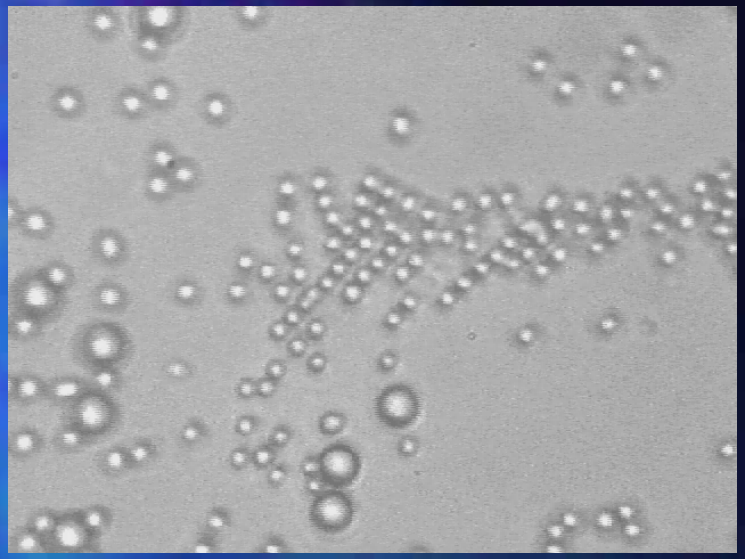
- All both species are swept straight through: no jamming no sorting

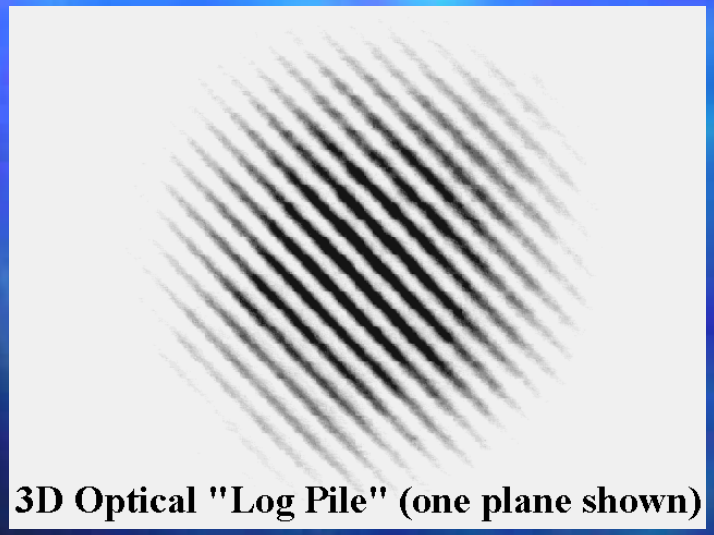


1W total power at sample

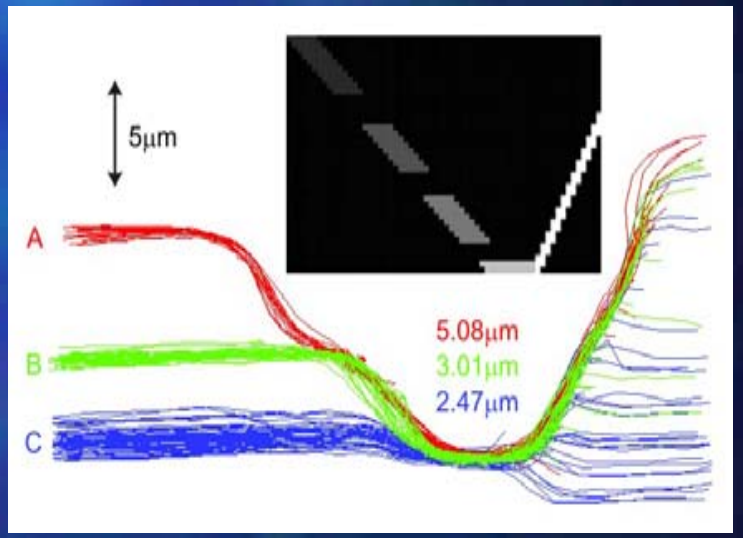








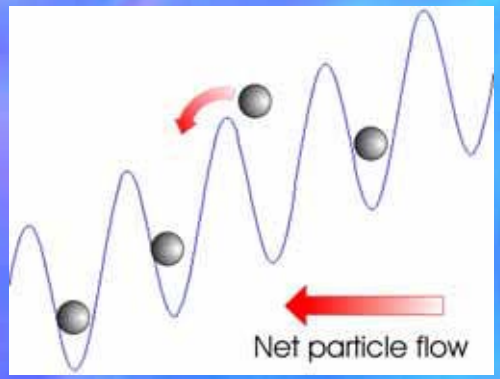
3D Optical "Log Pile" (one plane shown)



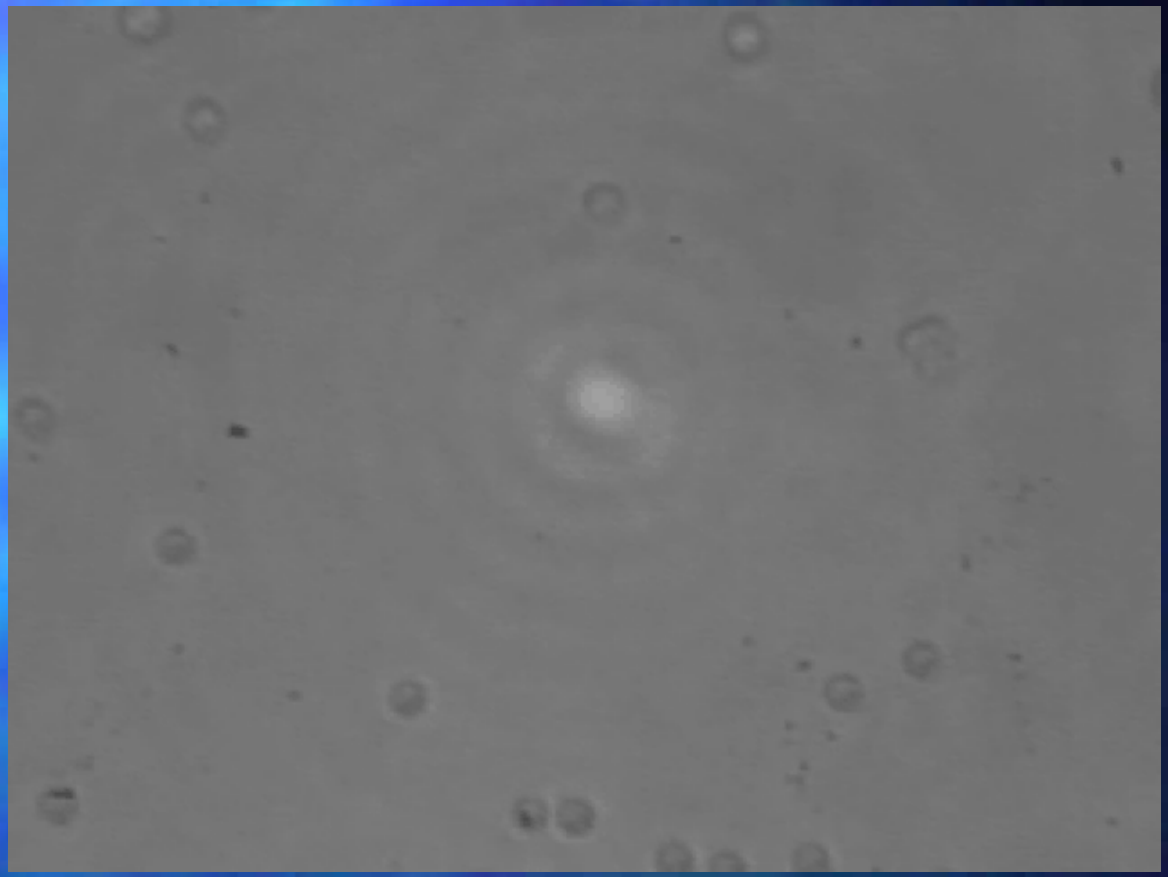
Nature 426, 421 (2003); Optics Letters 32, 1144 (2007)

Review of microfluidic sorting with light: IEEE Journal of Selected Topics in Quantum Electronics 13 1646 - 1654 (2007)

Hopping Silica Spheres: aim to sort without flow



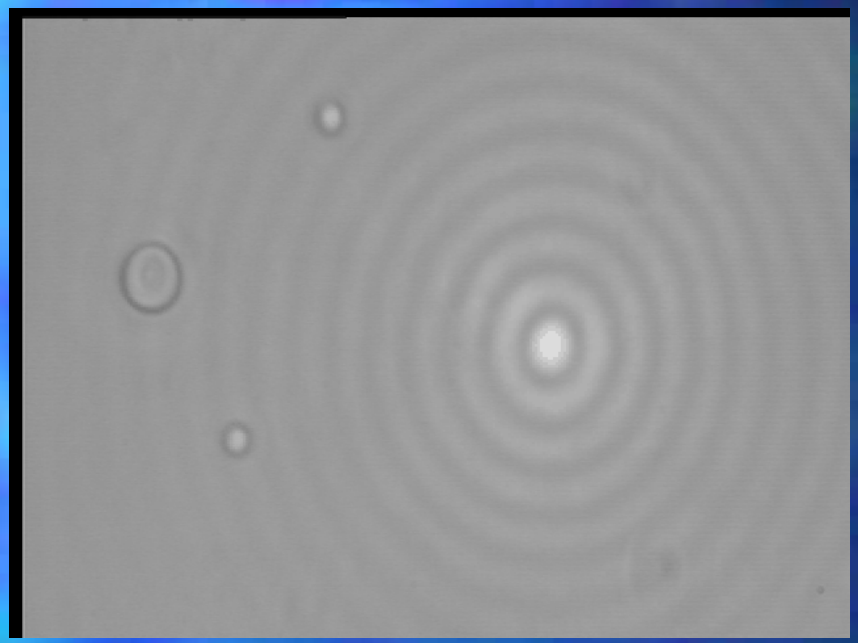
5μm



2.3μm silica spheres ‘hopping’ into the centre of a Bessel beam

Video is speeded up - real process 1/40 of the speed shown here
(normal video length about 20 minutes)

Size-dependence of particle behaviour

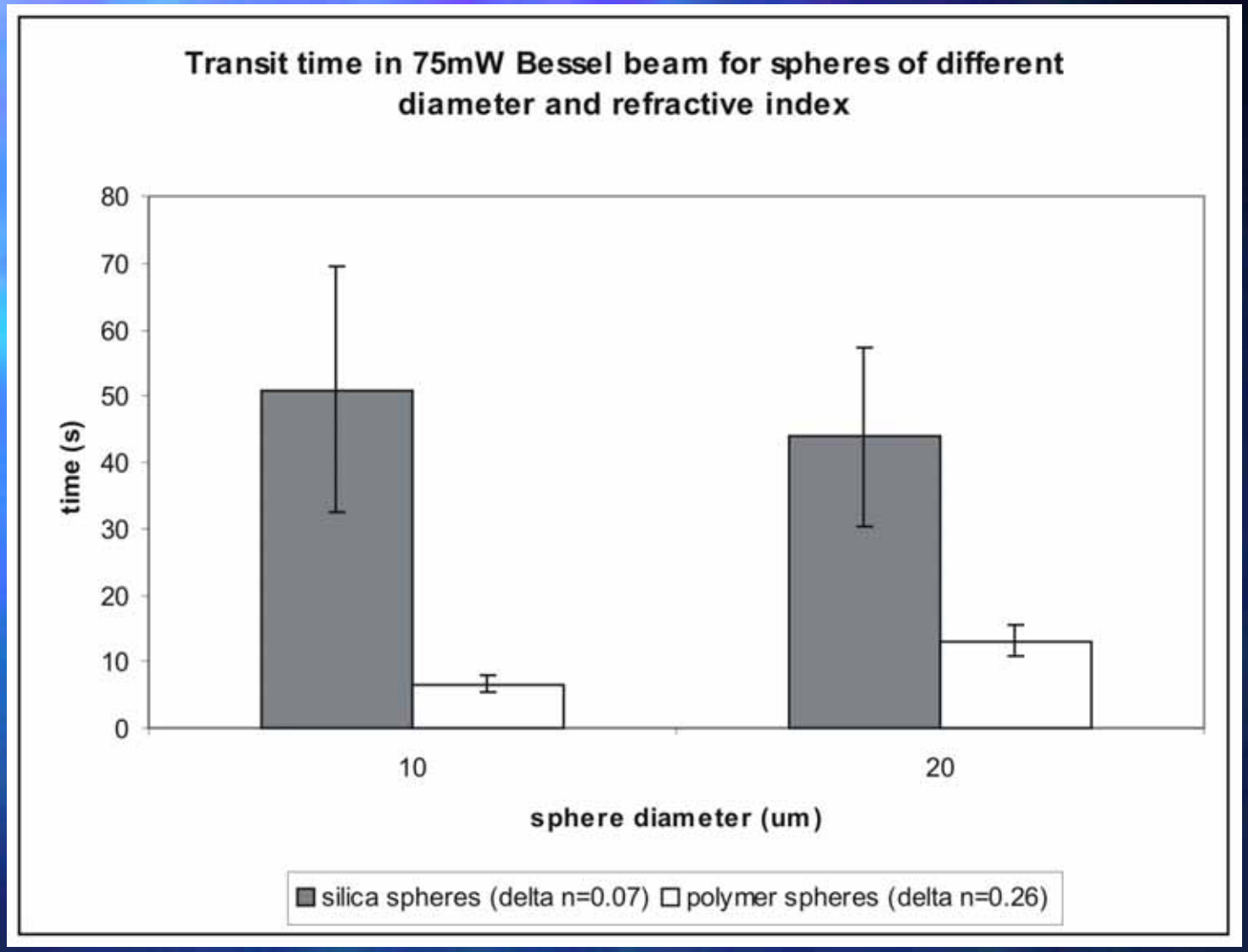


2.3µm and 5µm silica spheres behaving differently because of their size.

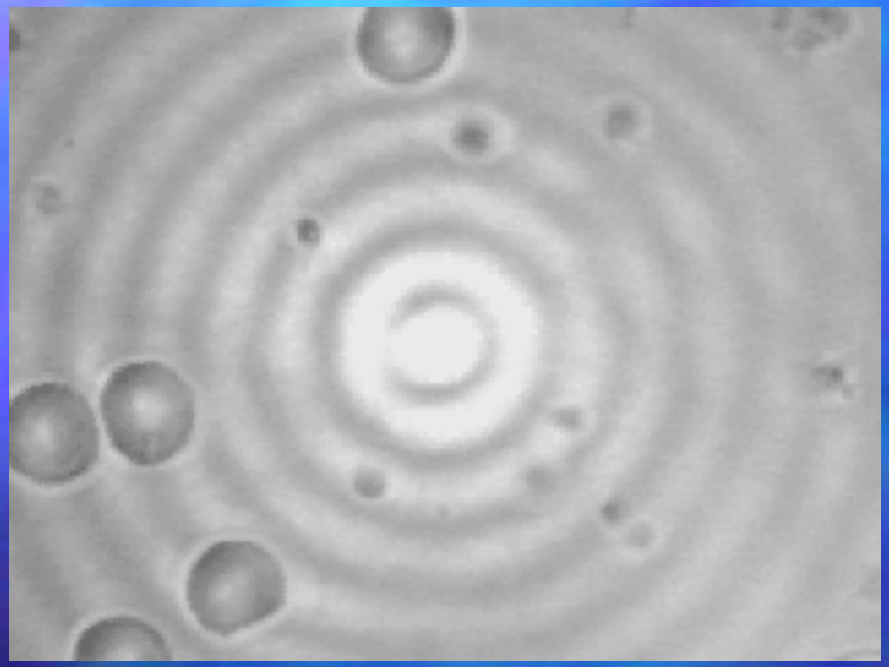
• The larger spheres move into the core of the Bessel beam more quickly because they effectively see the overall potential profile.

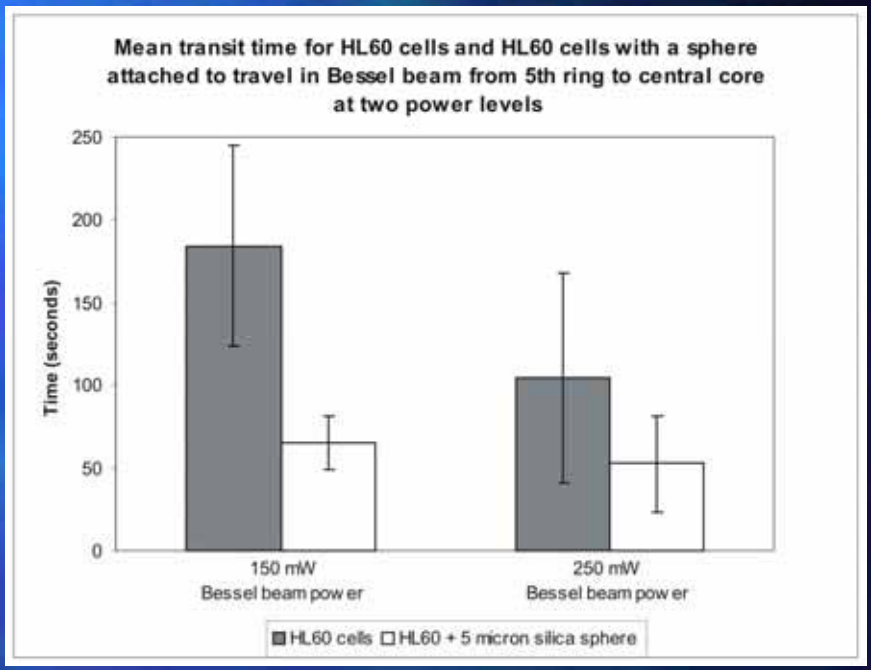
• If power is increased high enough hopping ceases and smaller spheres are "locked in" within the rings.

Theoretical predictions: K. Volke-Sepulveda et al. JOSA B **21**, 1749 (Sept 2004)


Transit time in 75mW Bessel beam for spheres of different diameter and refractive index
80
70
60
50
40
30
20
10
0
time (s)
10
20
sphere diameter (um)
silica spheres (delta n=0.07)
polymer spheres (delta n=0.26)

Cell sorting with a Bessel Beam





Cell types tagged with spheres (anitbody-anitgen binding) works well for small samples

L. Paterson et al., Appl Phys Lett 87, 123901 (2005);

J Biomed. Optics (2007)

Cell transfection

Transfection: The transfer of exogenous DNA into a cell.

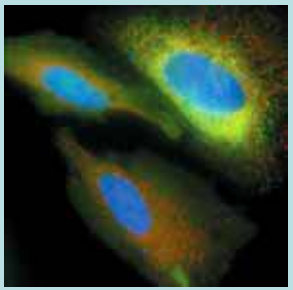
www.nature.com

How to transfect ?

dragon.zoo.utoronto.ca

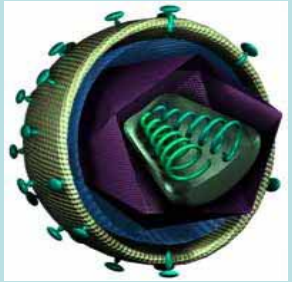
Electro/ Sonoporation: Cells exposed to pulses of high electrical voltage or to acoustic waves

Invitrogen



Chemical transfection: *Lipofectamine* or *calcium phosphate.* Cells take up DNA by phagocytosis or membrane fusion

waisman.wisc.edu

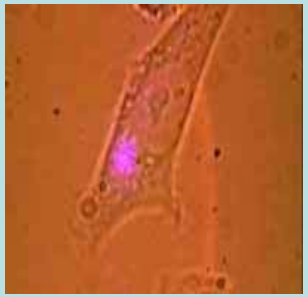


Viral vectors: Use of viruses to transport genomes inside cells they infect.

ohsu.edu

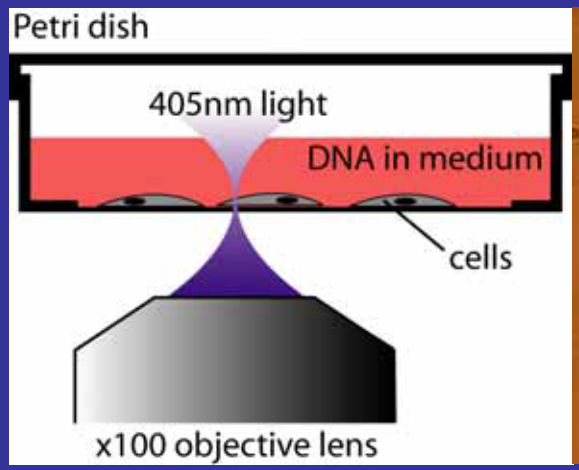
Microinjection: Plasmid injected directly into the cell nucleus.

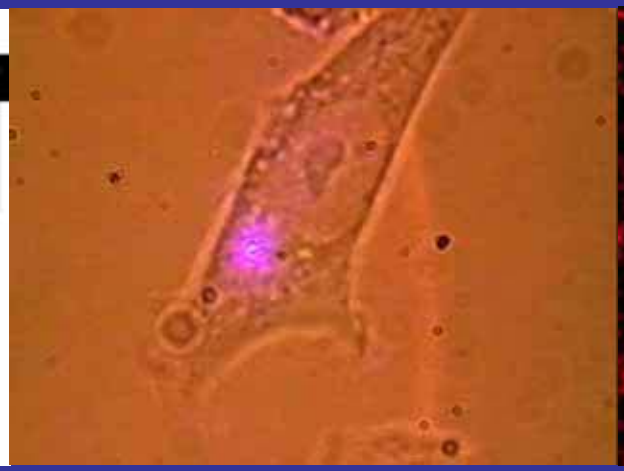
Univ. St Andrews

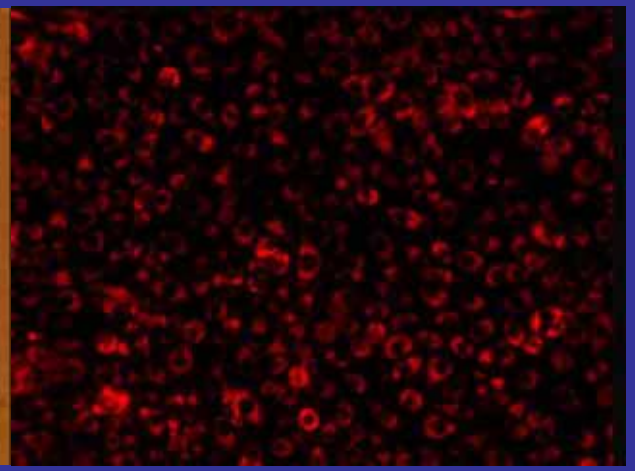


Optical: Focussed laser opens transient pores in cell membrane

CW - Diode Based Approach



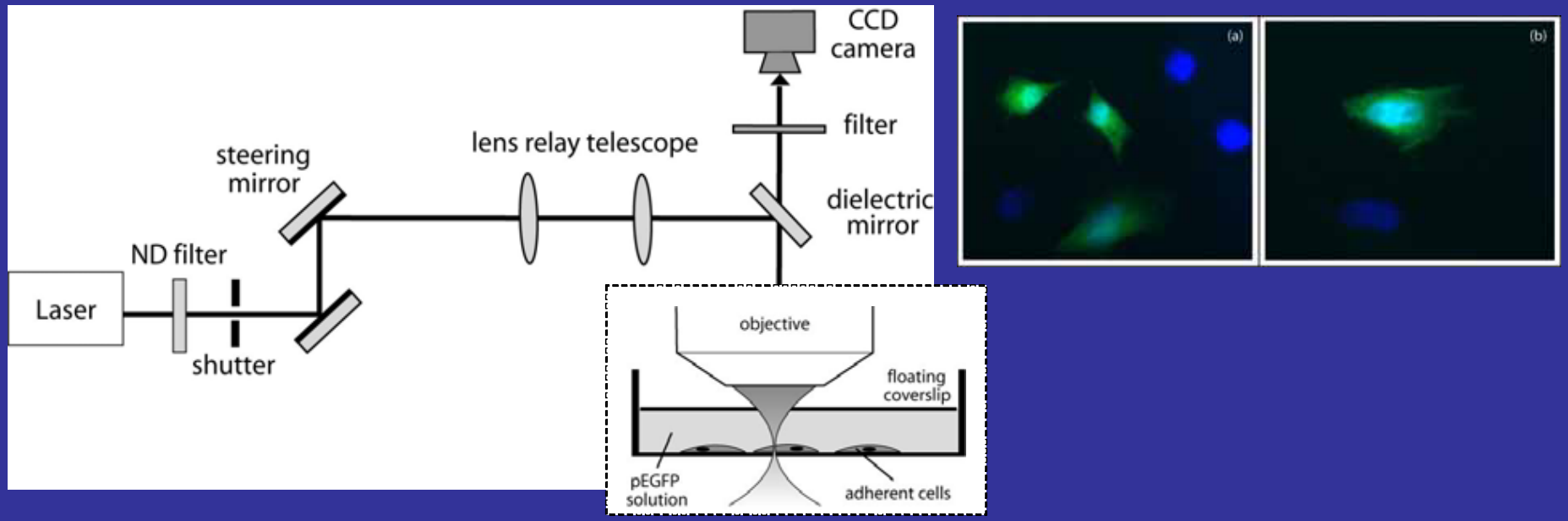




- inexpensve approach
- Low power requirement ~1mW for 40ms
- Stable colonies of antibiotic resistant RFP / GFP expressing cells formed.

L. Paterson et al, Optics Express 13, p595 (2005)
Femtosecond laser nanosurgery: Vogel et al, Appl. Phys B vol81, 1015 (2005)

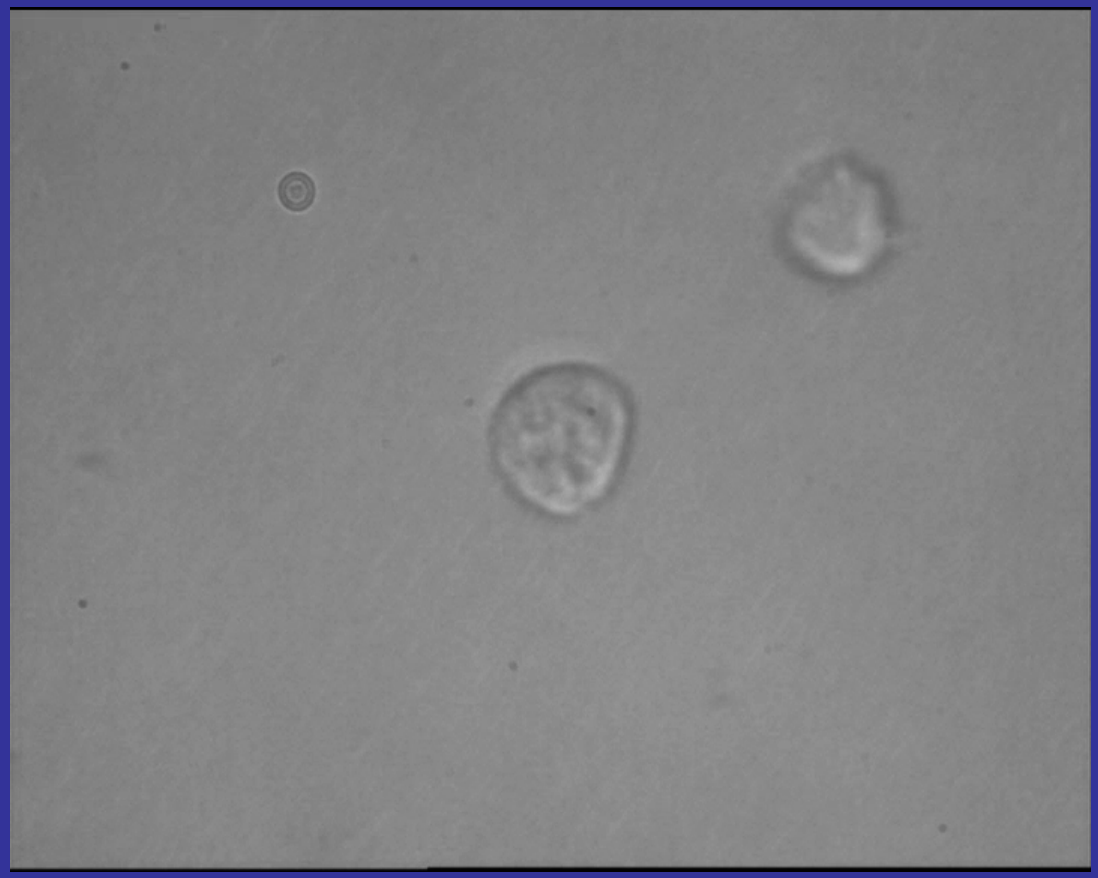
Femtosecond approach



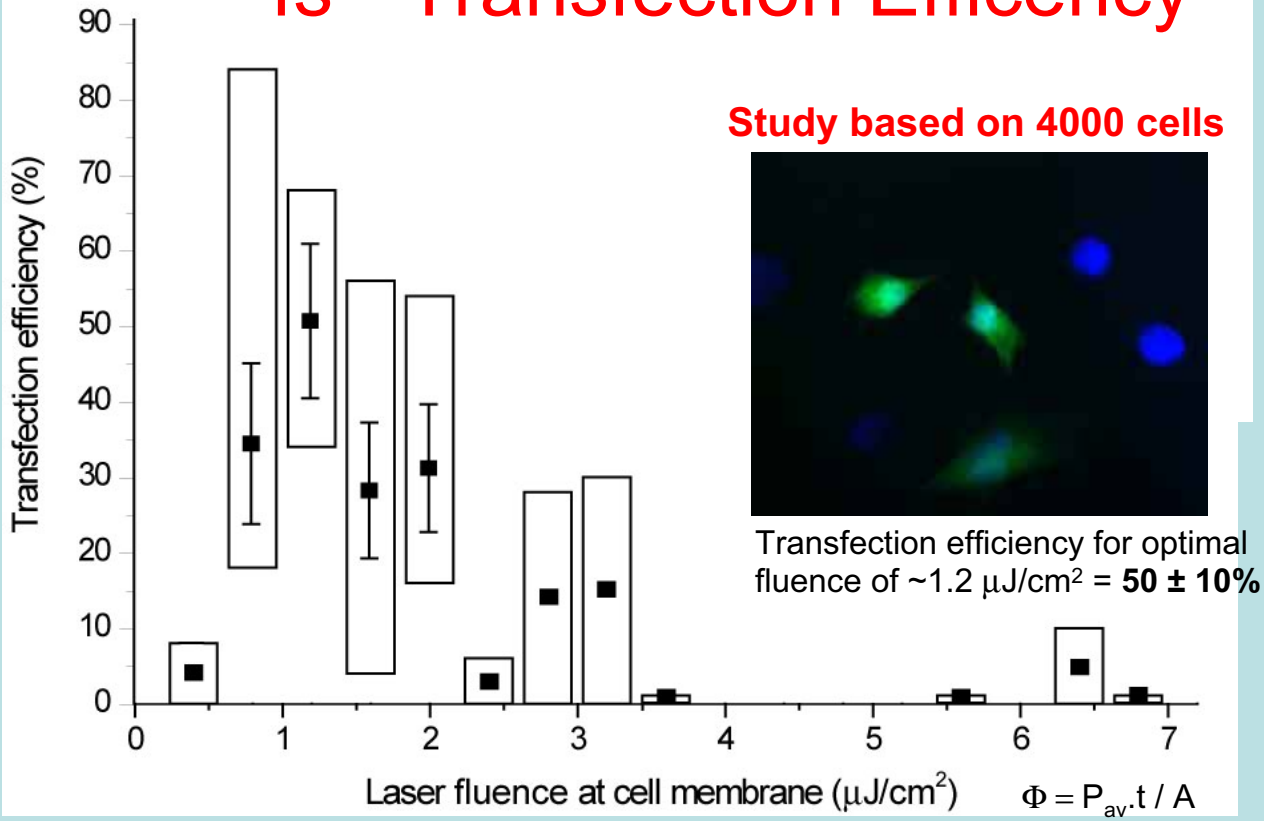
- First demonstrated by Tirlapur et al (Nature, **418**, p. 290, 2002); other groups have used other short pulsed sources. J.S. Soughayer et al., Anal. Chem. 72, 1342 (2000)
- Compatible with multiphoton microscopes
- Non-linear process - high intensity. I ~ 10 W/cm

This study: D. Stevenson et al, Optics Express 14, p7125 (2006)

Tweeze and porate with the same laser



fs - Transfection Efficency



Study based on 4000 cells

Transfection efficiency for optimal fluence of ~1.2 μJ/cm² = **50 ± 10%**

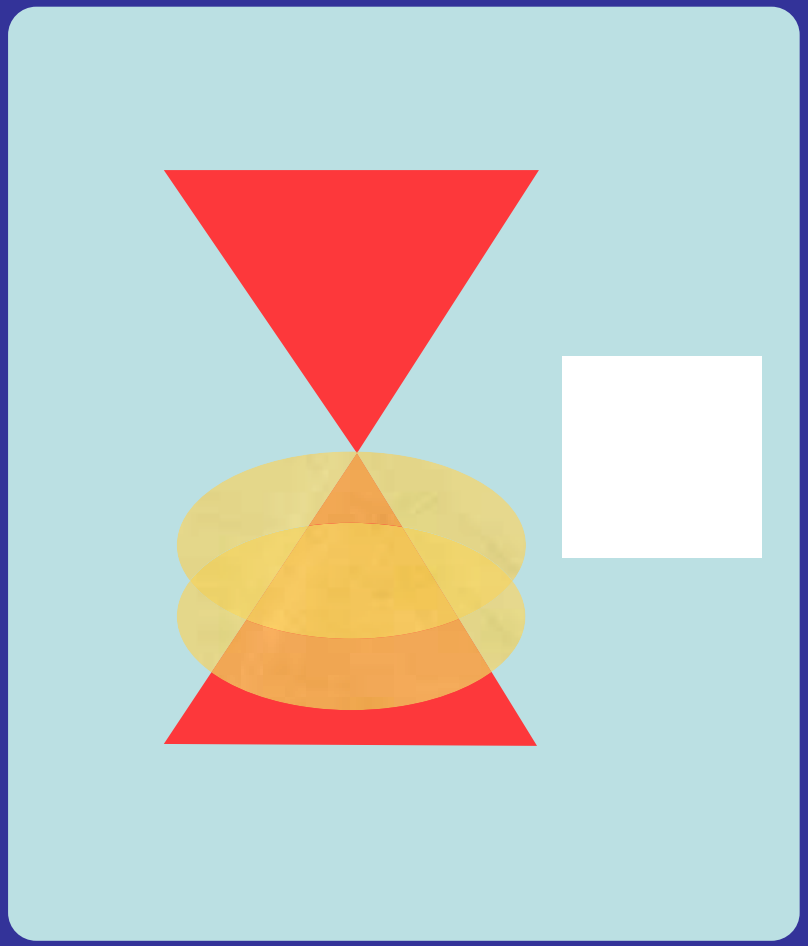
Factors compromising transfection efficiency

- Translocation, transcription, translation and viability

Data includes cells lost by laser action

D. Stevenson et al, Optics Express 14, p7125 (2006)

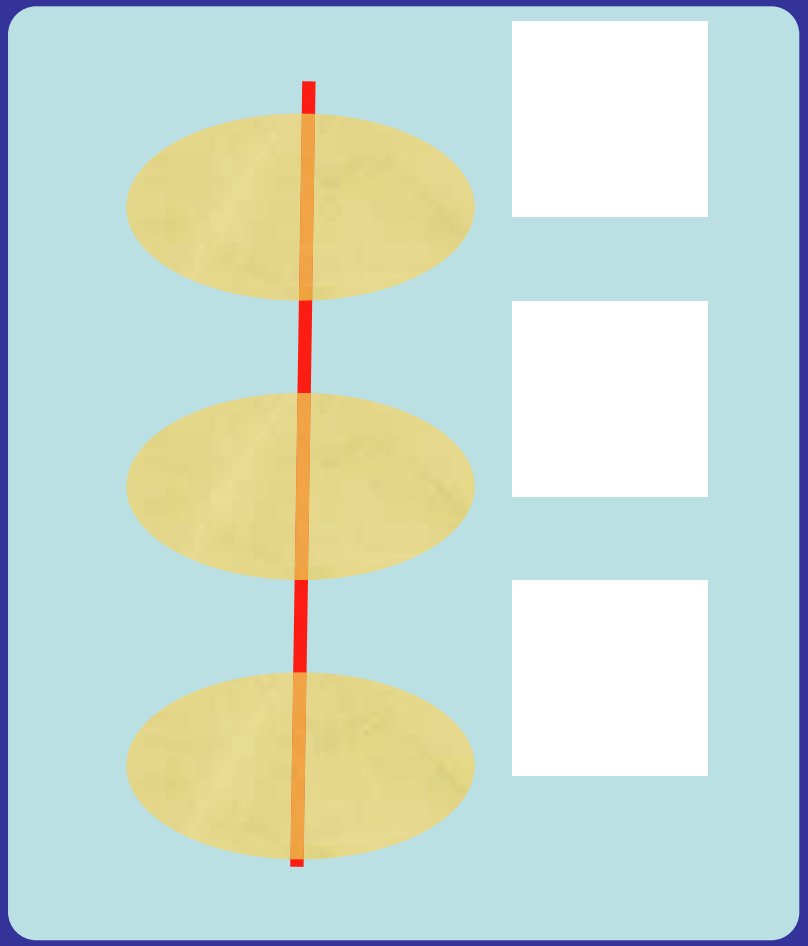
A different approach?



- Multiphoton process requires exact placement of cell membrane.
- Error of a few μm can prevent transfection.

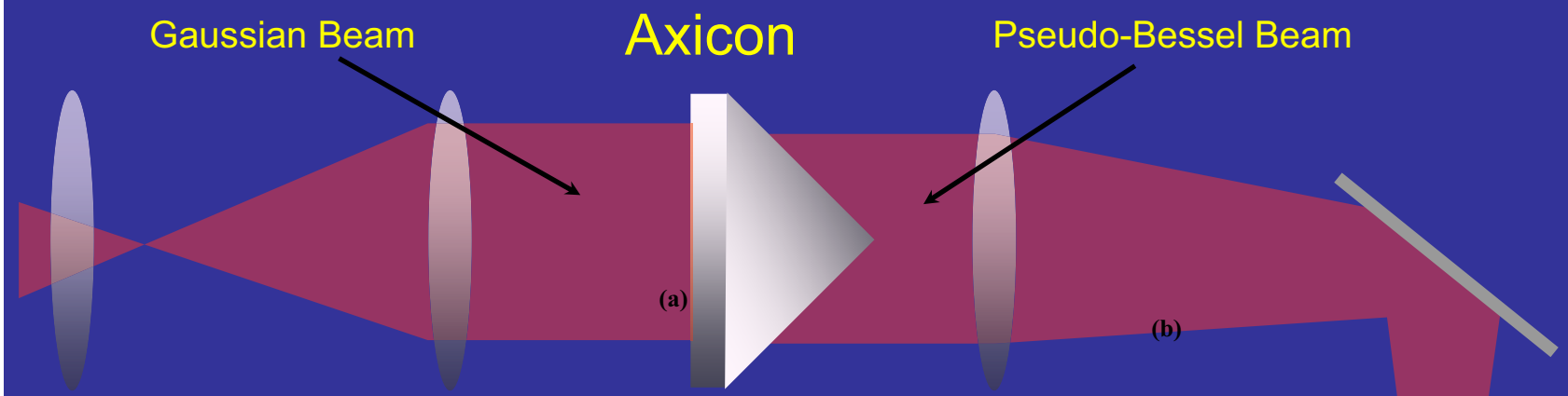
Bessel Photoporation

(The Optical Syringe)

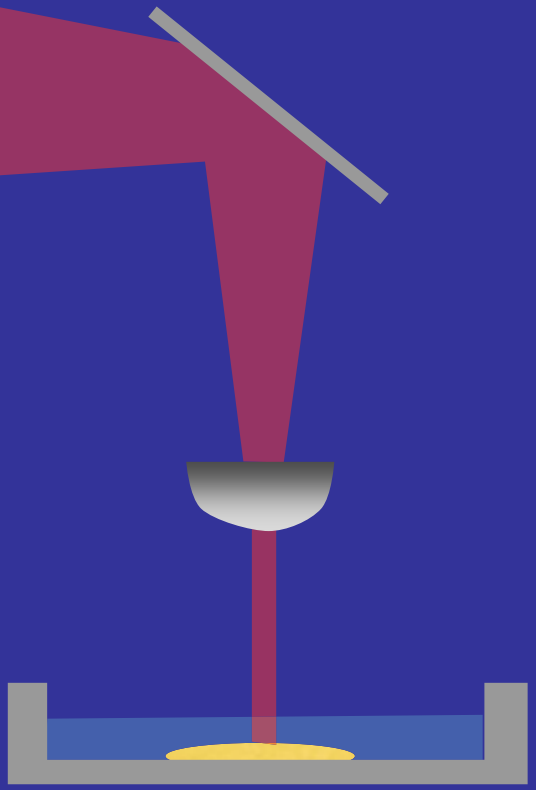


- Non-gaussian light beams behave differently.
- The bessel beam preserves a non-diffracting central core over long distances.
- Can perform fs-poration over a much greater '*depth of focus.*'
- Beam also *'self-heals'* - is this a further advantage?

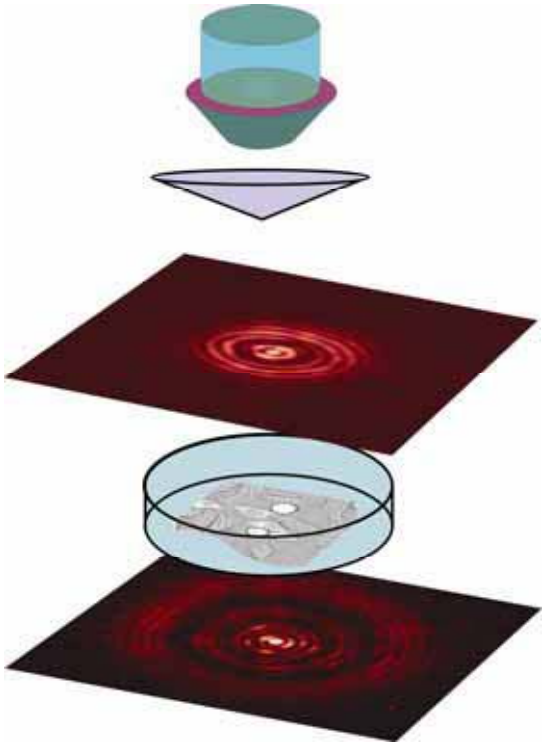
Practical Implementation

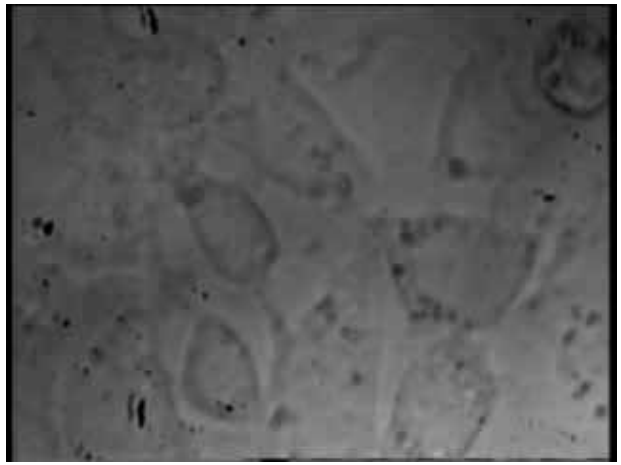


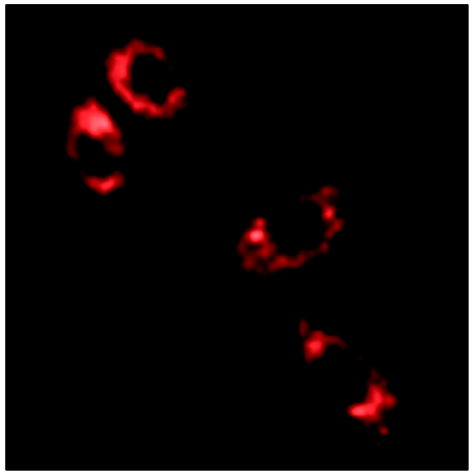
- In practice use axicon to form Bessel beam.
- Beam is then telescoped to give desired core size.
- Aspheric lens used to focus beam on sample.
- Non-diffracting core for fixed distance.
- Smaller the core, shorter propagation length.

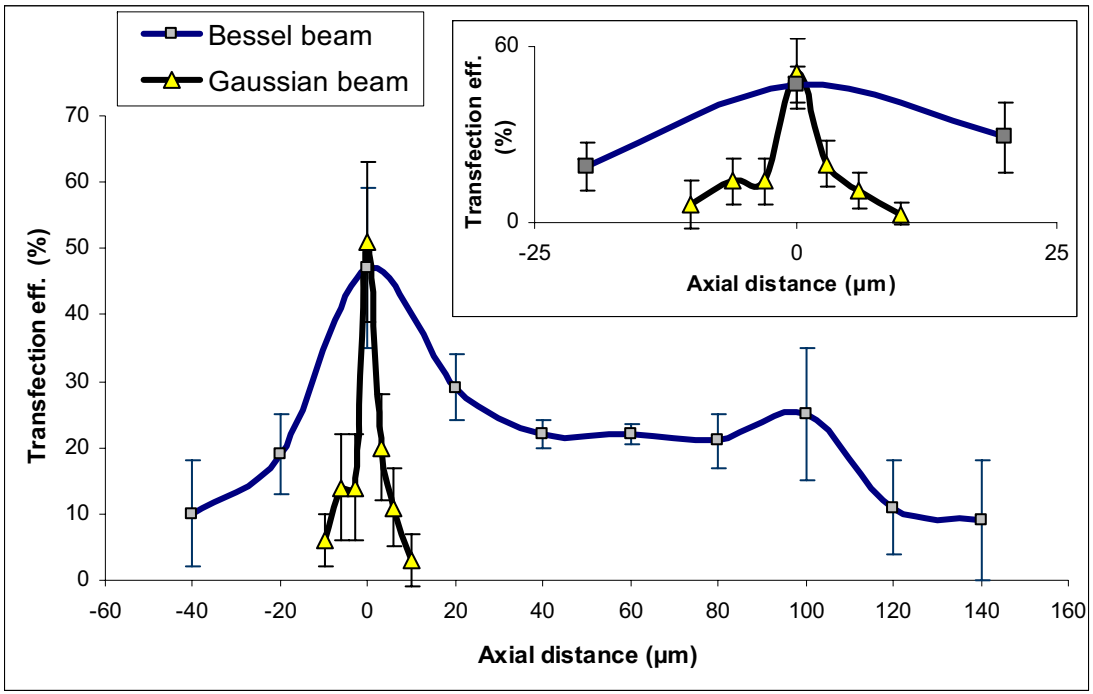


Photonics solves real biological issues..

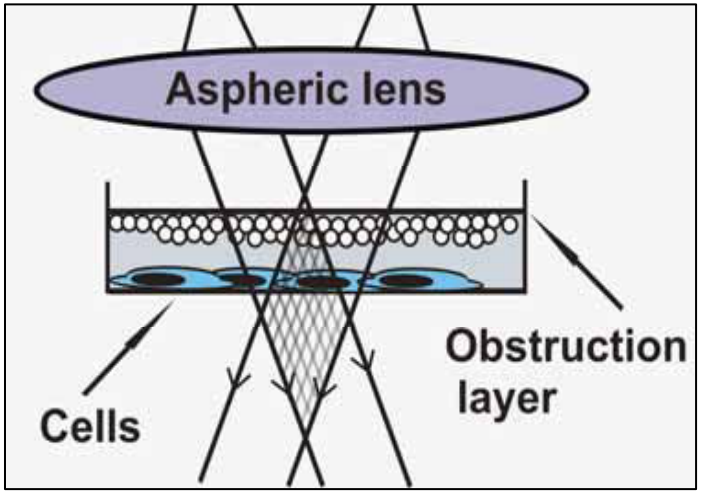


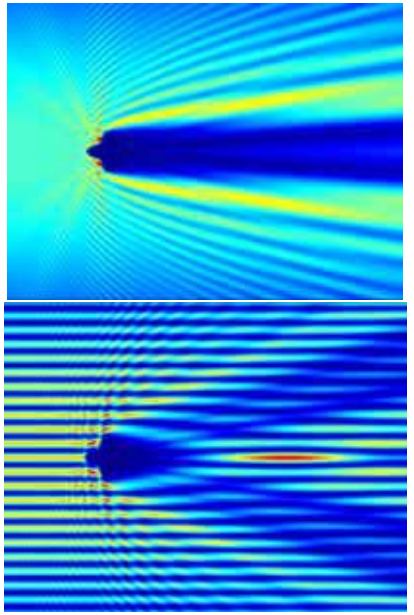


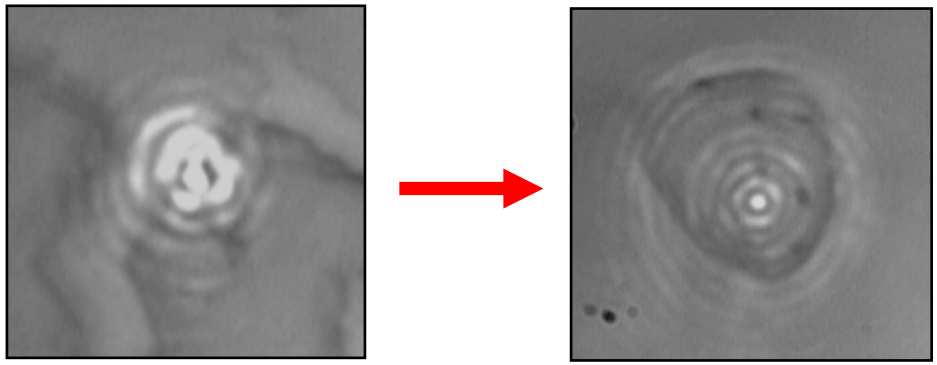




Self-healing of the Bessel beam shows poration beyond obstacles

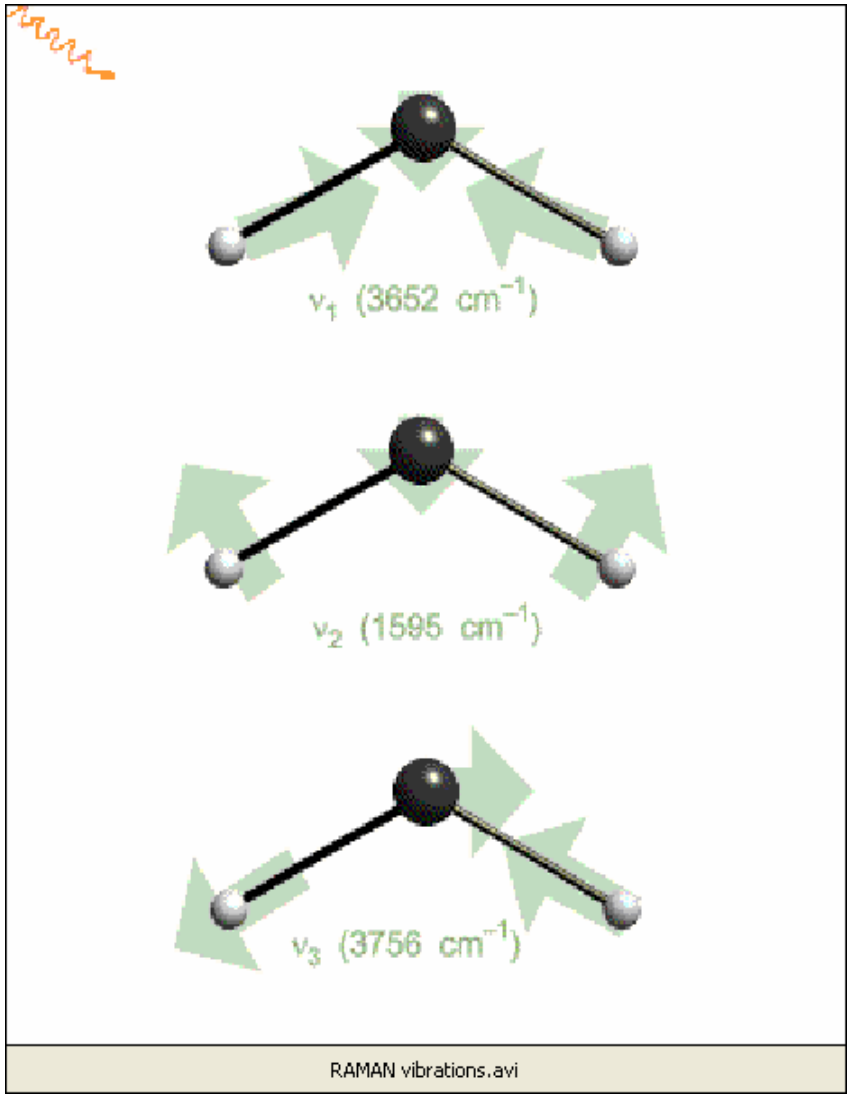


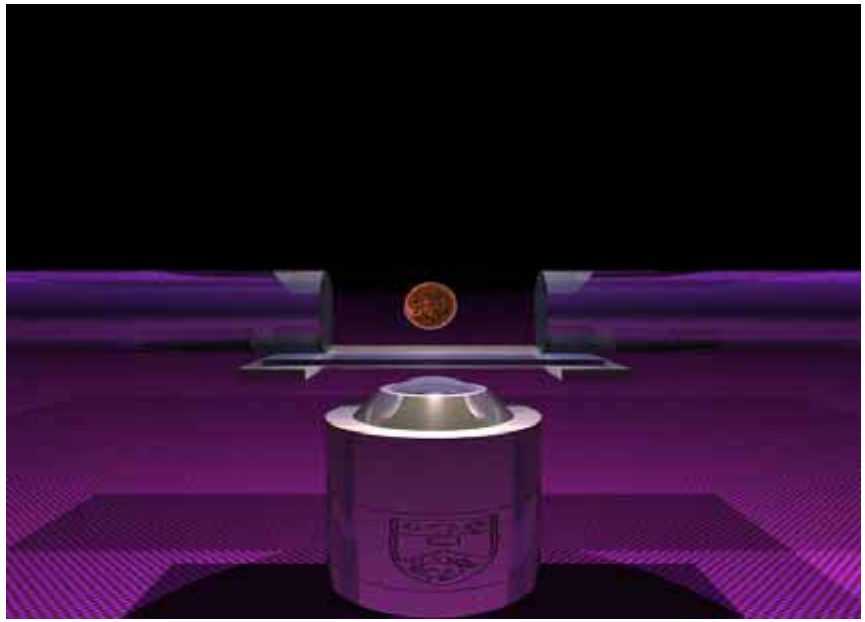




Self-healing in optical traps: Nature 419, 145 (2002)

Raman spectroscopy





The scattered light carries information on the molecular constituents of the cell: identify cell abnormalities

Fibre optic trap

NO HIGH-N.A. OPTICS

Compatible with microfluidics

Advantages for holding and analysing large cells: lower power density, compatible with microfluidics, no intercellular trapping/deformation

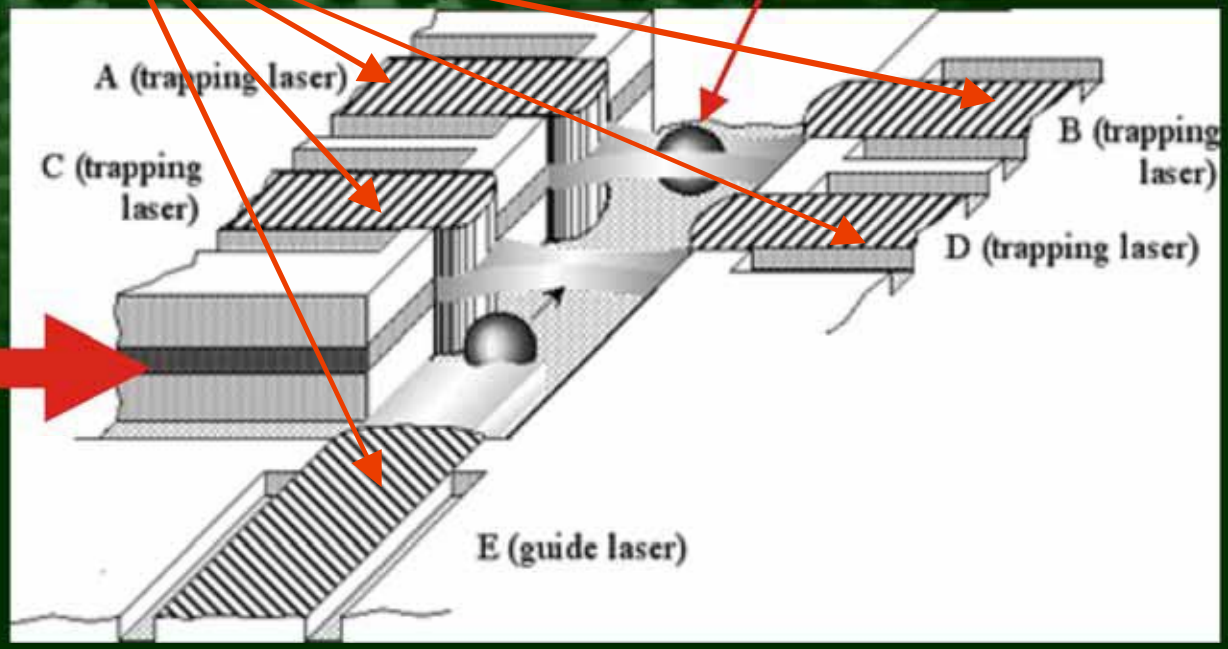
Integrated optical tweezers

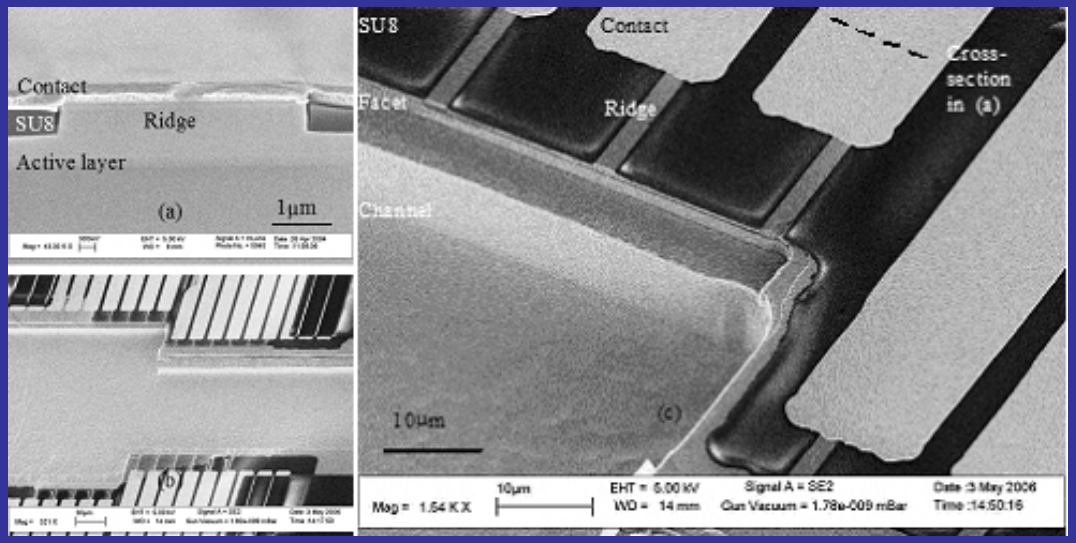
By integrating the lasers for optical trapping into the system it is possible to massively simplify the system, opening up the possibility of mass production.

Integrated lasers

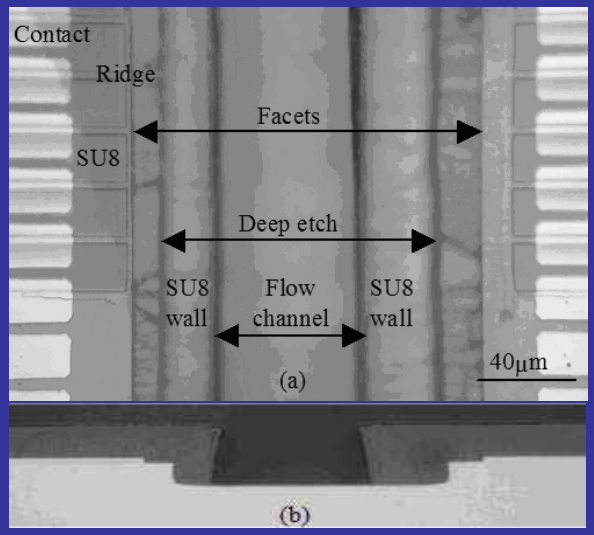
Dual beam optical traps

Microfluidic channel

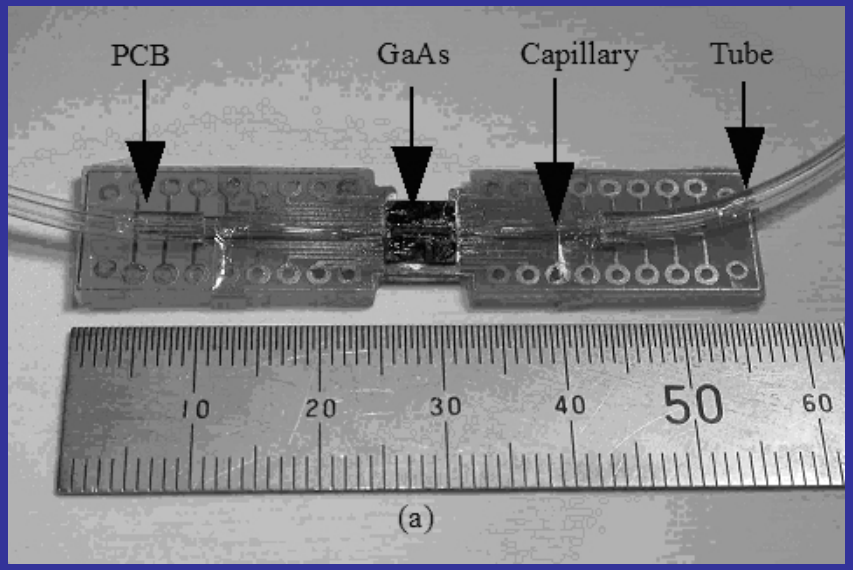


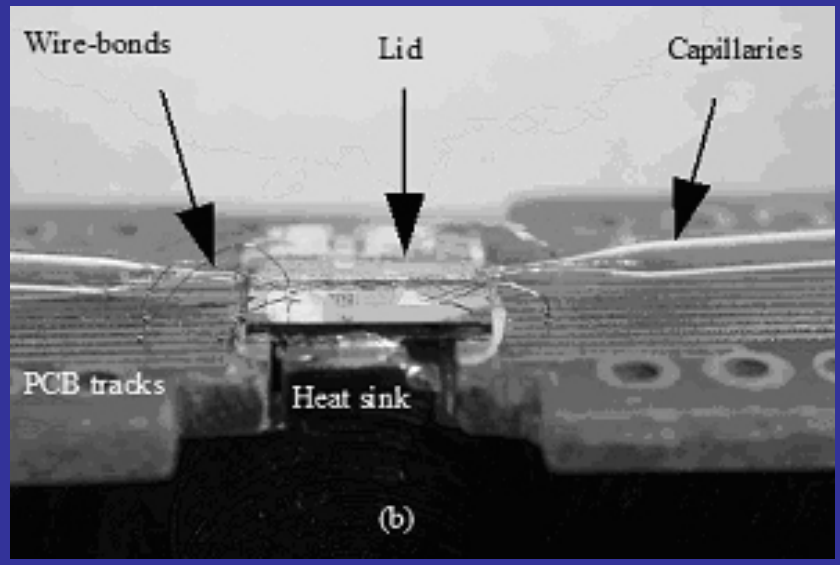


SEM images of lasers: (a) cross-section through a laser; (b) lasers facing one another across the channel; (c) close-up of facets and channel wall. Note that the channel insulation has not yet been added.

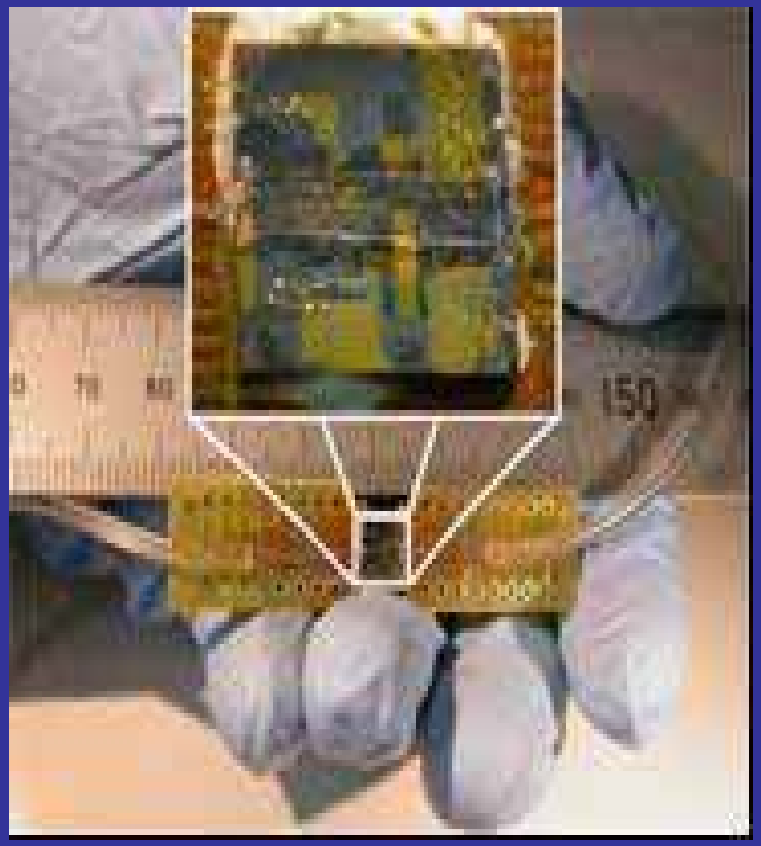


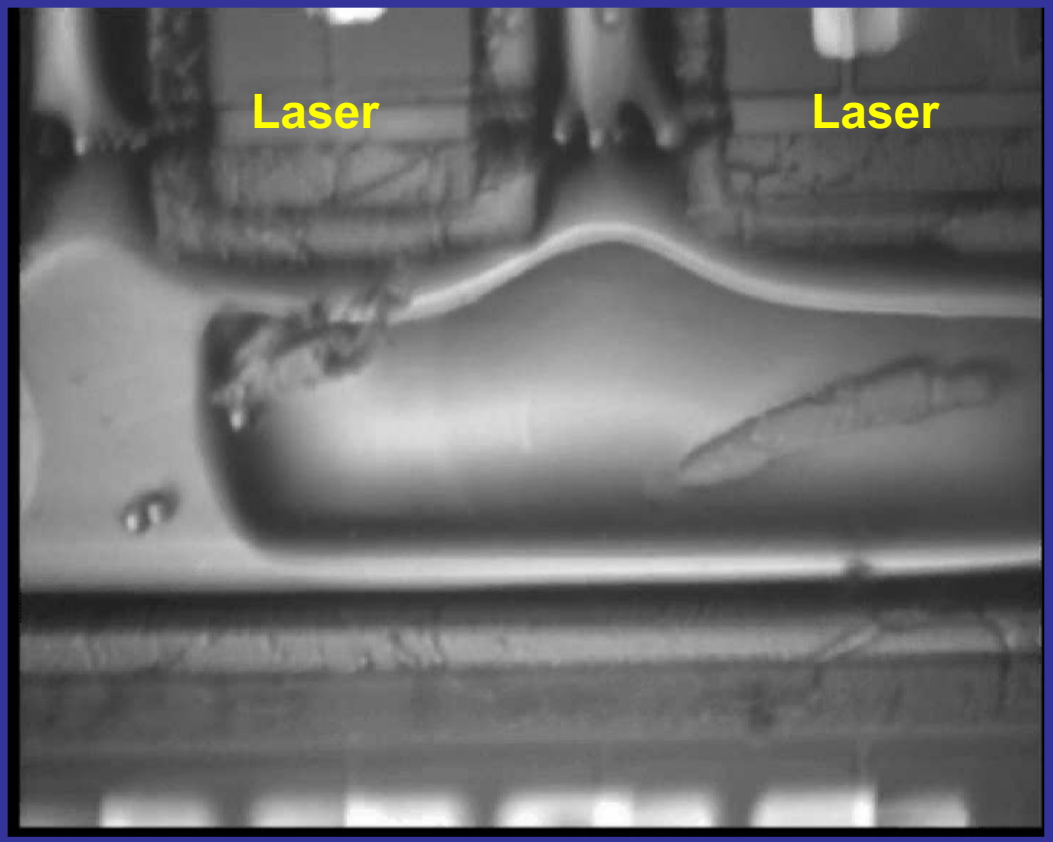
Photographs of channel with insulation: (a) plan view; (b) cross-sectional view





Photographs of device: (a) mounted on PCB, with tubes leading to pump; (b) fine capillaries feed fluid beneath the glass lid, sealed with NOA-71, and electrical power is provided via wire-bonds from the circuit board





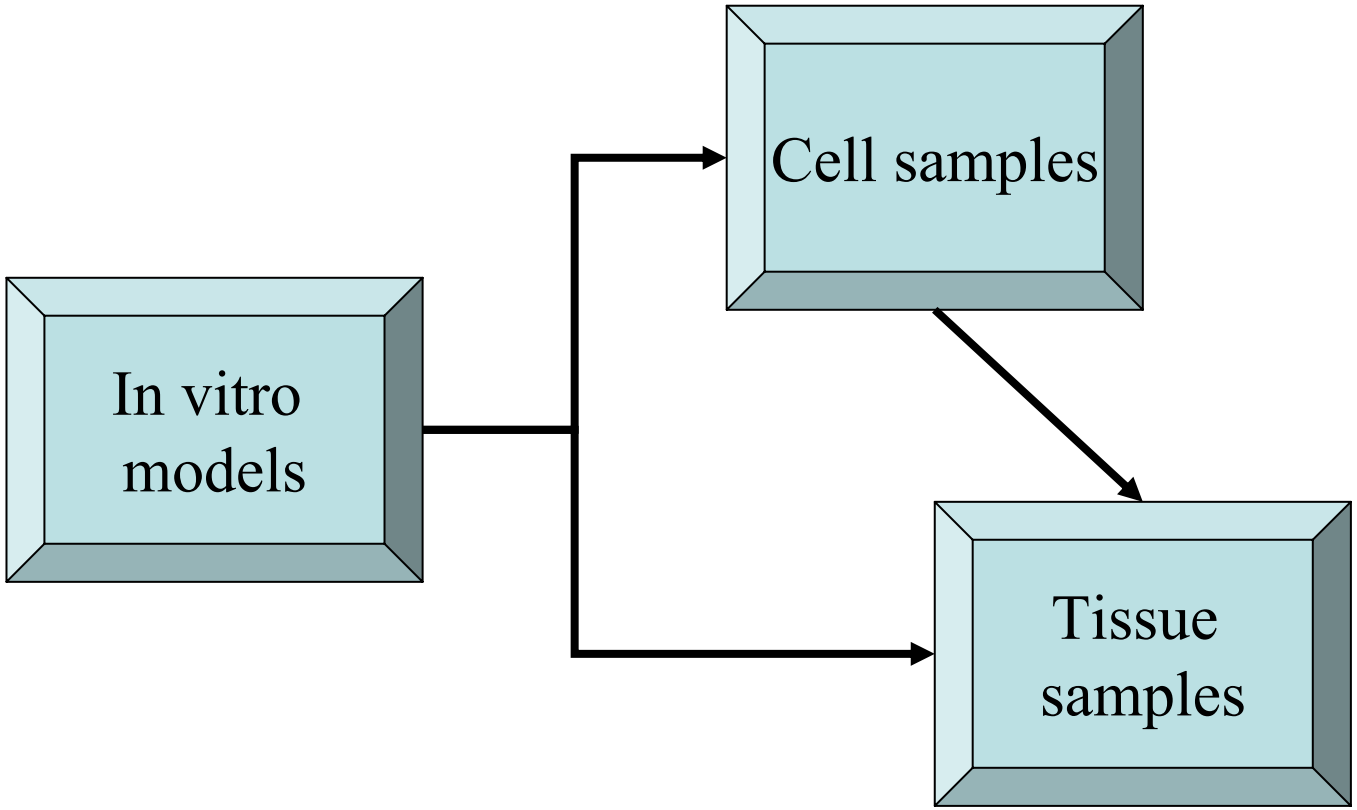
Laser Laser Laser

Integrated optical micromanipulation

S. Cran-McGreehin et al., Lab-on-a-chip 6, 1122 (2006),

S. Cran-McGreehin at al., Optics Express 14, 7723-7729 (2006)

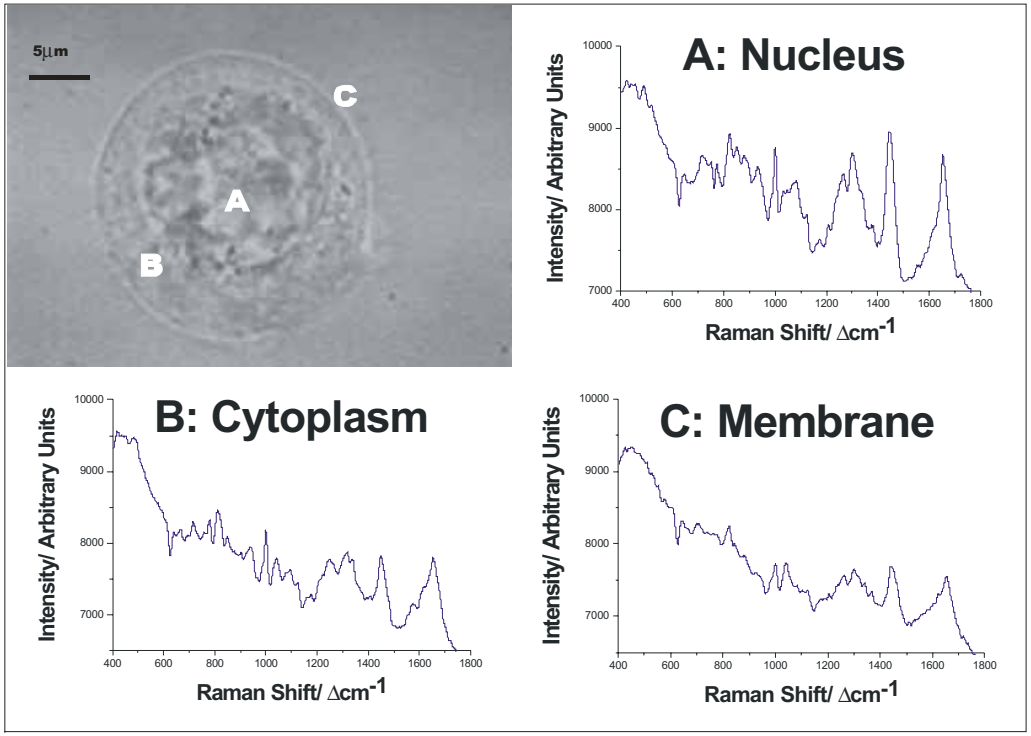
Pathway



In vitro Models

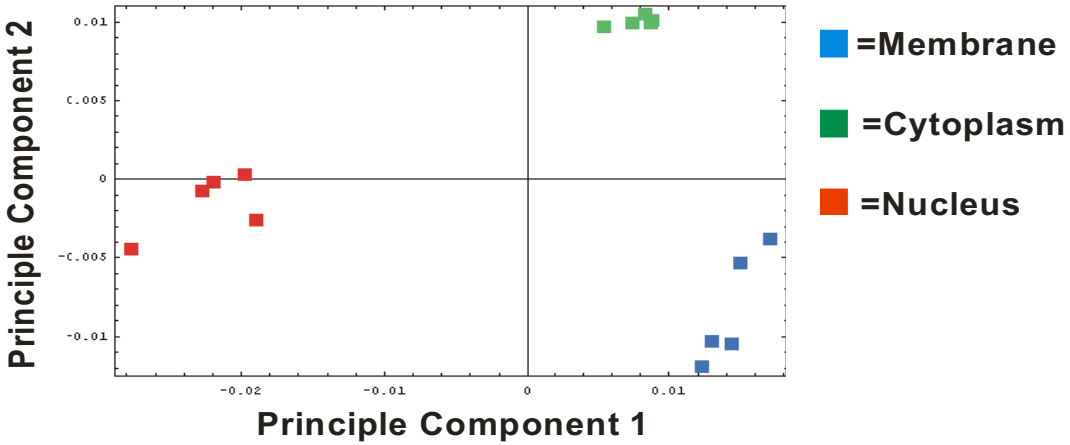
- Cell systems
  - Tumour cells: well-characterised cell lines
    - Lymphoid cells – lymphoma, leukaemia
    - Epithelial cells – cervical, prostate, breast cancer
  - Normal cells
  - Engineered cells representing malignant pathways
    - Primary keratinocytes expressing papillomavirus genes
    - Primary bronchial epithelial cells expressing telomerase, CDK4 etc

A single human keratinocyte cell held in an optical trap

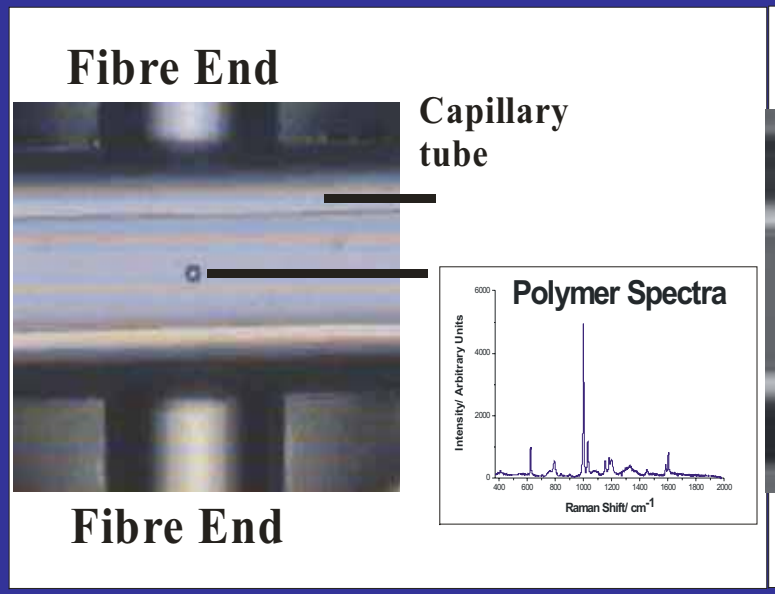


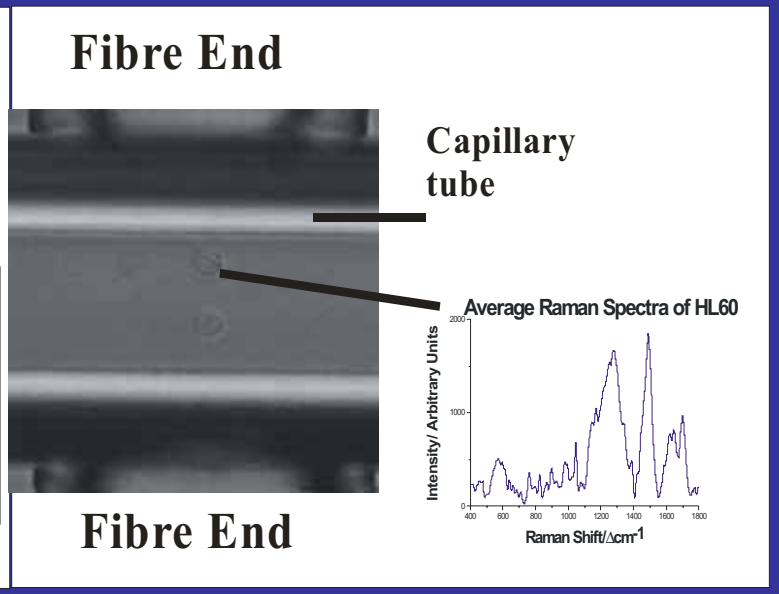
P Jess et al. Optics Express **14**, 5779 (2006)

Review of Raman in traps: D V Petrov 2007 *J. Opt. A: Pure Appl. Opt.* **9** S139-S156



Microfluidic Raman: on demand single cell analysis





Raman acquisition time is ~minutes so this is low throughput at the moment……

Acknowledgements

Integrated Trap: S McGreehin, T Krauss, R Marchington and P Reece

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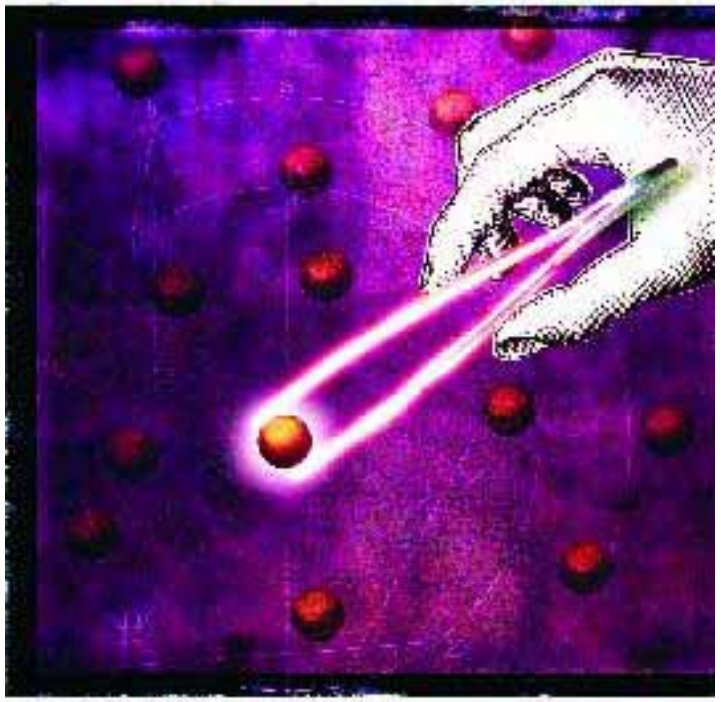
Photoporation: X Tsampoula, V Garces-Chavez, D Stevenson, B Agate, CTA Brown, W Sibbett

Raman: P Jess, I Cormack, M Mazilu



Biology/Medicine co-Investigators

F Gunn-Moore (neuroscience)

A Riches (cancer biology)

CS Herrington (pathology)

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