Nano-Bio-Sensing Summer School @ EPFL
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Optoelectronic Tweezers – A New Parallel Optical Manipulation Tool

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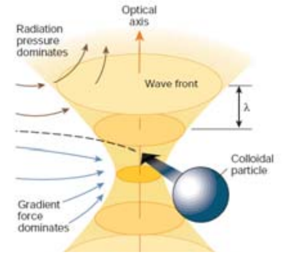
OUTLINE

- Introduction
- Principle of optoelectronic tweezers (OET)
- Recent developments and current projects
  - Bio-compatible OET
  - Sorting of mature neuron cells
  - Single-cell electroporation
  - Manipulation of nanowires
  - Micro-assembly of nanowires, microdisks
  - Nano-Pen
- Summary

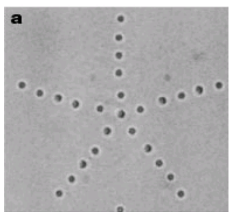
Micro/Nano Particle Manipulation

Optical Tweezers (OT)
(Ashkin 1986)



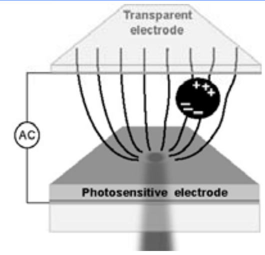
Grier, *Nature*, 2003

Holographic OT



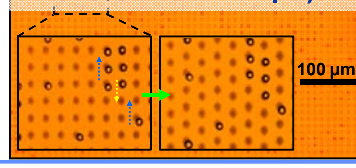
- High optical power
  - ~ mW / trap
- Small manipulation area
  - ~ 100um x 100um

Optoelectronic Tweezers (OET)



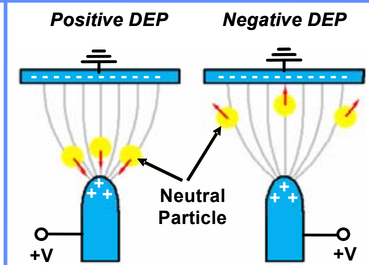
Chiou, Ohta, Wu, *Nature* 2005

Direct Optical Image (15,000 individually addressable traps)

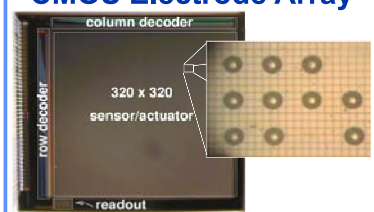


- Low optical power, does not require laser
- Actuation by optical image
- Large area (> mm)

Dielectrophoresis (DEP)



CMOS Electrode Array



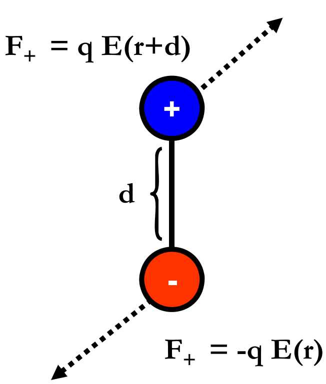
www.siliconbiosystems.com

- Parallel, programmable
- Pitch limited by circuit area
- Higher cost (CMOS)

Dielectrophoresis (DEP)

Fixed Dipole in Non-uniform Electric Field

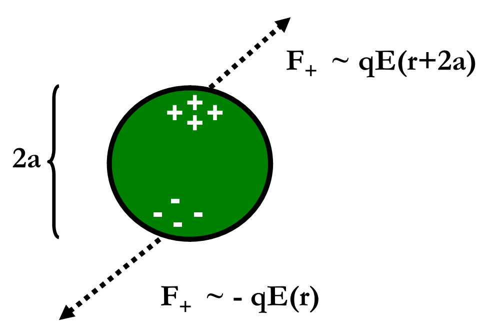
$$F_d = q\left(E(r+d) - E(r)\right)$$
$$= qd\nabla E = \mu \nabla E$$
$$\mu = qd \quad : \text{electrical dipole}$$



Induced Dipole in Non-uniform Electric Field

$$F_d = \mu_{eff} \nabla E$$
$$= 2\pi a^3 \varepsilon_m \mathrm{Re}[K^*] \nabla E^2$$

CM-Factor: Attractive or Repulsive Force



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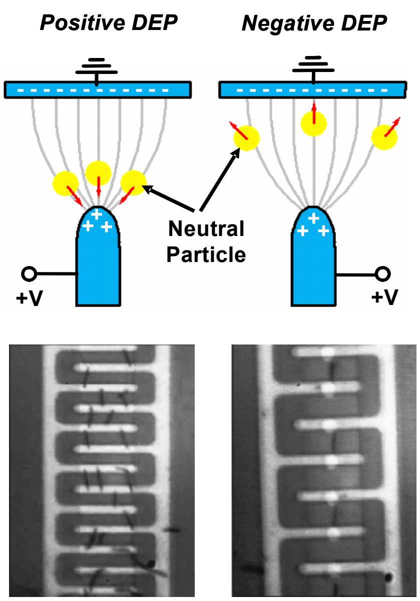
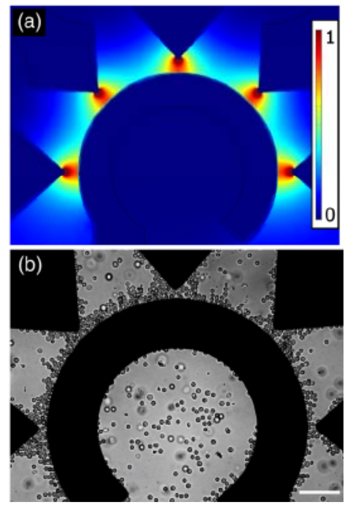
Electrode-Based DEP



Smith *et al.*, *Appl. Phys. Letters*, 2000.



MacQueen *et al. Bioelectrochemistry,* 72, 2008.

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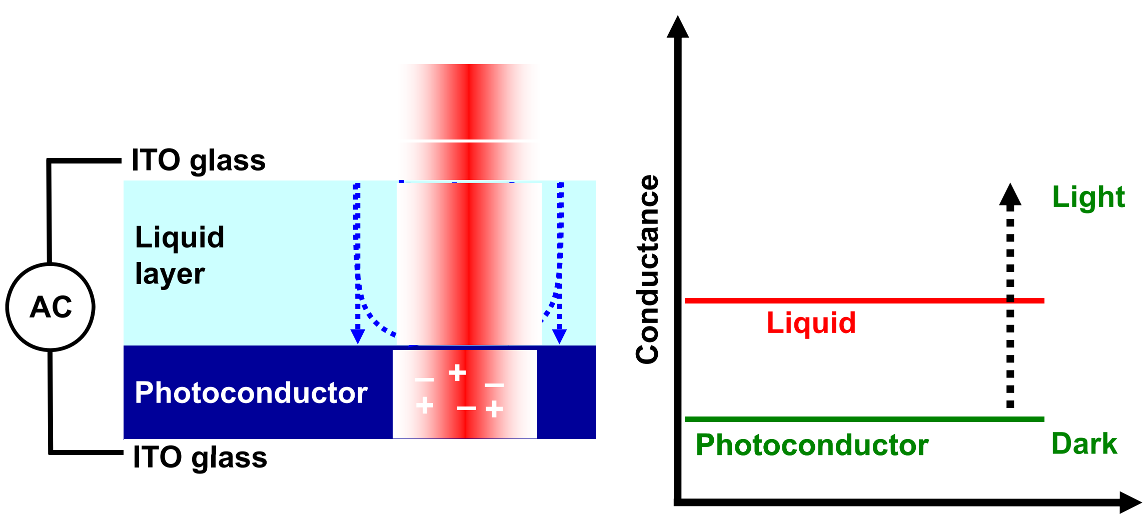
Light-induced Dielectrophoresis



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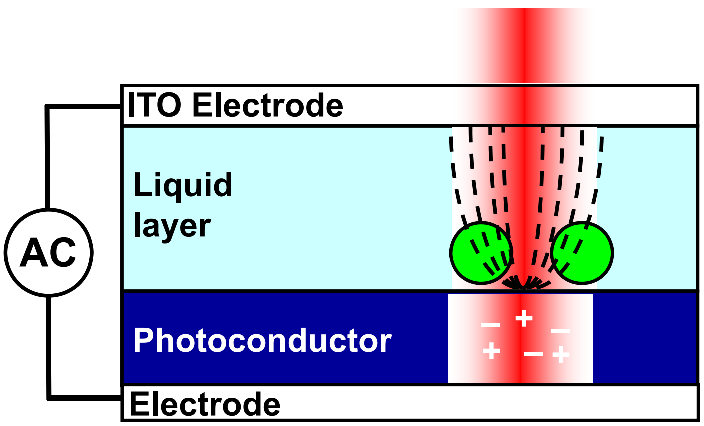
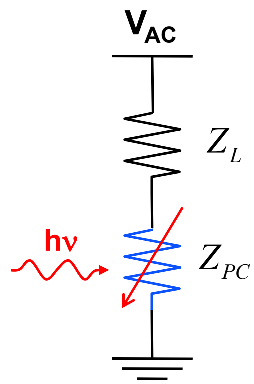
Optoelectronic Tweezers Principle

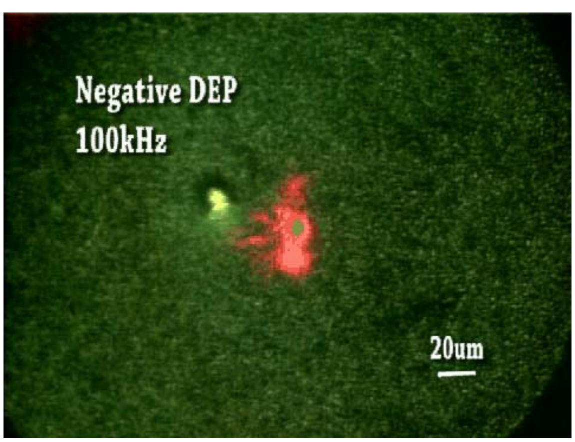
- **Based on light-induced dielectrophoresis (DEP)**
  - **Also called LDEP, ODEP (optically-induced DEP)**
- **Illumination creates virtual electrodes**





$Z_L > Z_{PC}$

Positive and Negative OET



$$F_{dep} = 2\pi a^3 \varepsilon_m \mathrm{Re}[K^*(\omega)]\nabla(E^2)$$

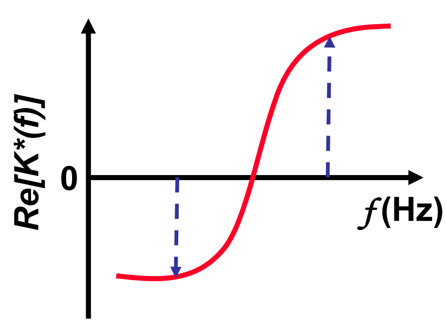
$$K^*(\omega) = \frac{\varepsilon_p^* - \varepsilon_m^*}{\varepsilon_p^* + 2\varepsilon_m^*}, \ \varepsilon^* = \varepsilon + \frac{\sigma}{j\omega}$$

ε_p^* : Dielectric Constant of Particles (usually a function of frequency)

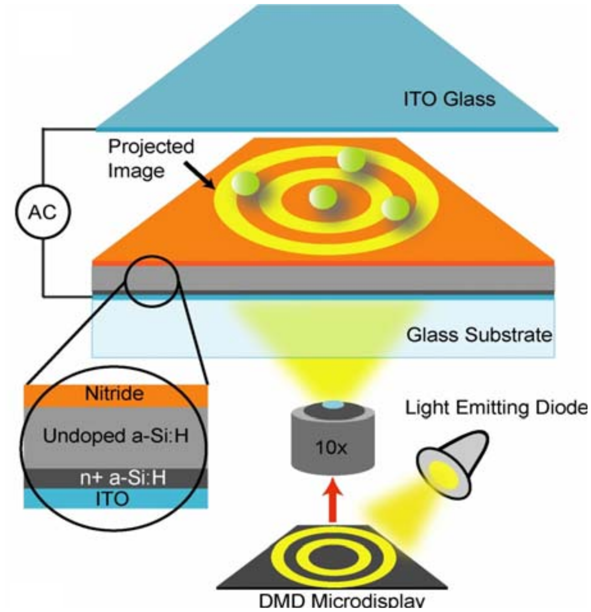
ε_m^* : Dielectric Constant of Media

Frequency Response

- **Dielectric signature**
- **Depends on size, composition, surface charge**

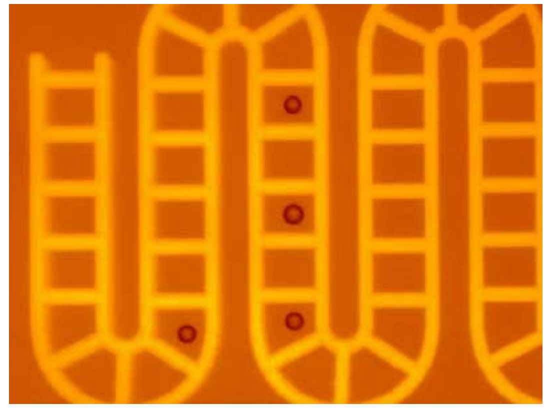


Dynamic OET Trap Using Digital Micromirror Projector



P.Y. Chiou, A.T. Ohta, M.C. Wu, *Nature,* 2005

Optical Conveyor Belt



- **Lower optical intensity: 10,000x lower than conventional optical tweezers**
- **Programmable: Trapping and manipulation using a digital projector**
- **Massively parallel: 15,000 individually controlled traps**

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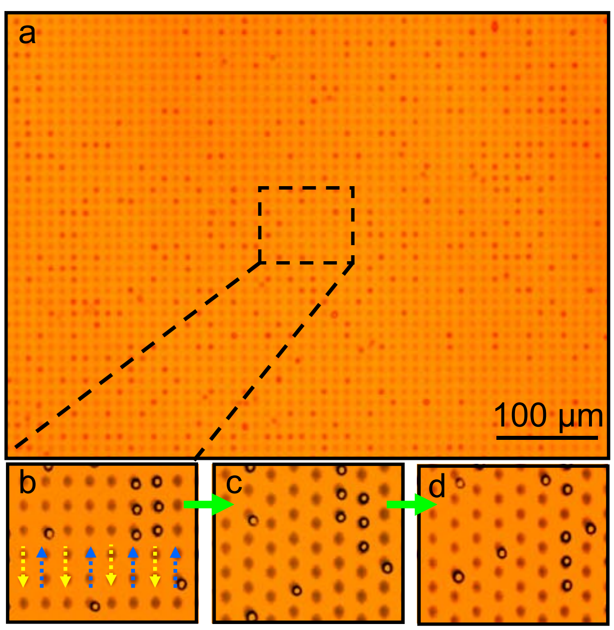
Massively Parallel Manipulation



15,000 individually addressable traps

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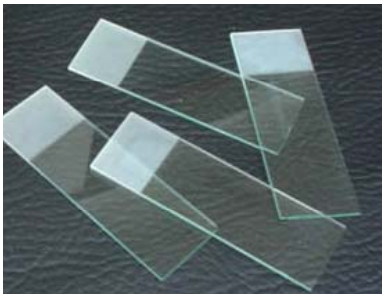
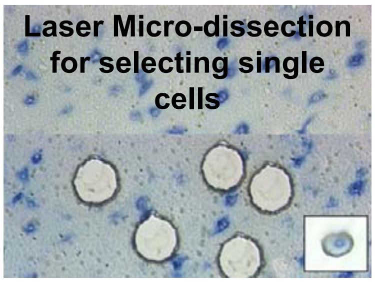
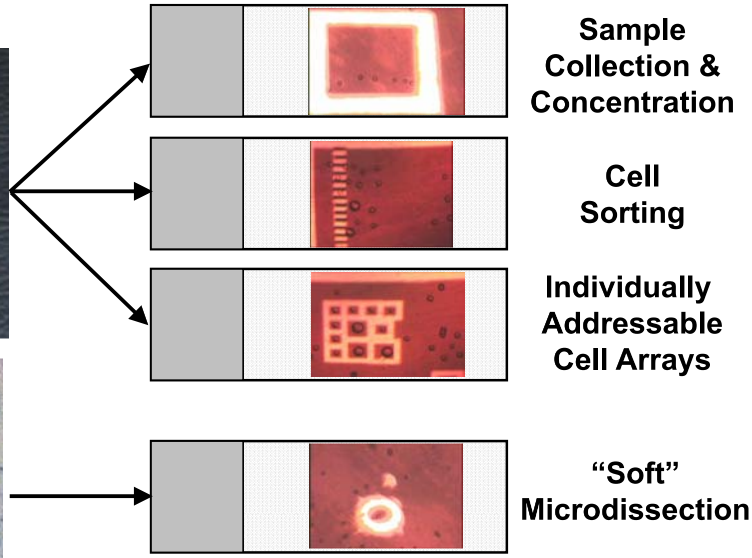
Smart Slides

Microscope Slides



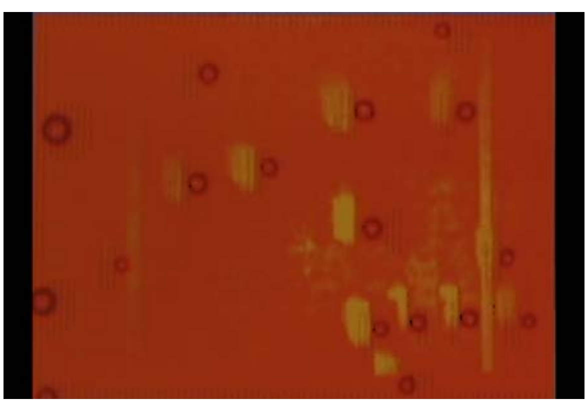




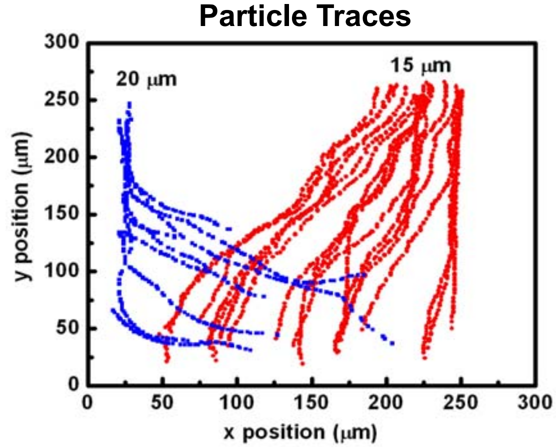
Cell screening and single cell selection

A. T. Ohta, P. Y. Chiou, T. H. Han, J. C. Liao, U. Bhardwaj, E. R. B. McCabe, F. Yu, R. Sun, and M. C. Wu, "Dynamic Cell and Microparticle Control via Optoelectronic Tweezers," J. Microelectromechanical Systems, vol. 16, pp. 491-499, 2007.

Automatic Sorting by Video Images



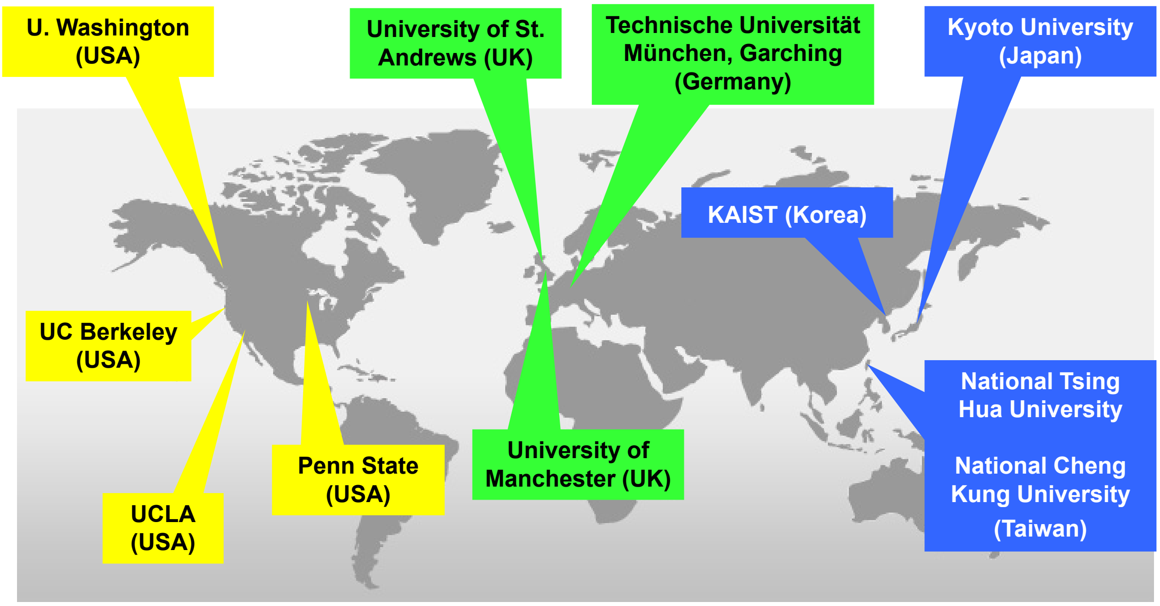
Particle Traces



- Sorting by analyzing particle sizes in video images in real time
- Can be extended to sort by other visual attributes such as texture, florescence, etc.

- Stage moving speed : 10 µm/sec
- Cells with different DEP response show different slopes of traces.

OET Around the World



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Proliferation of OET

- **Photoconductive materials**
  - **Amorphous Si** (2003)
  - **Phototransistor for operation in culture media** (2007)
  - **CdS** (2008, Kyoto Univ., Japan)
  - **Plasmonic nanoparticles** (2007, U. Washington; 2008, Manchester, UK)
  - **Polymer** (2009, National Cheng Kung Univ. Taiwan)
- **Optics**
  - **Scanning lasers** (2003)
  - **DMD** (2005)
  - **LCD flat panel display** (2007, KAIST, Korea)
- **Mode of operation**
  - **DEP** (2003)
  - **Electroosmosis** (2000, Princeton, on ITO)
  - **Electrothermal** (1995, Toyohashi U, Japan, on glass)

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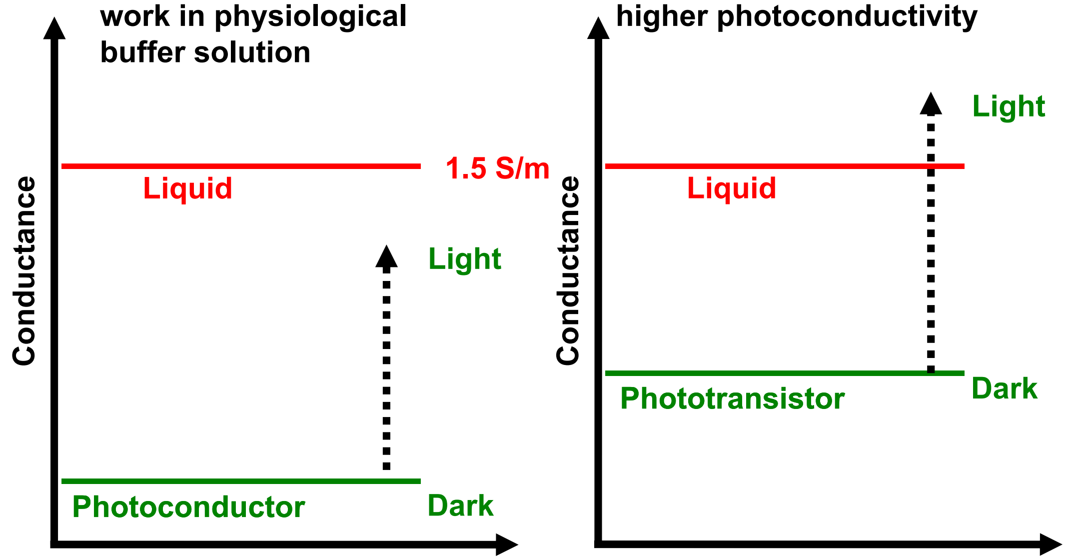
Biocompatible OET

Normal OET with a-Si photoconductor does not work in physiological buffer solution

Biocompatible OET needs a new photoconductor with much higher photoconductivity



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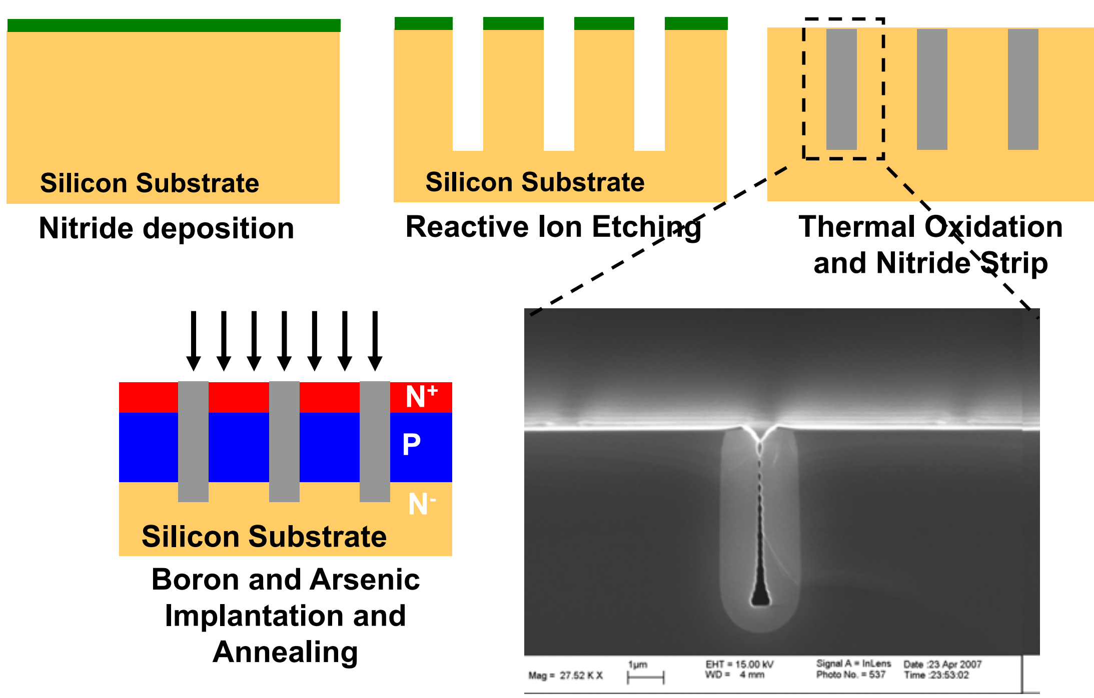
PhOET Fabrication



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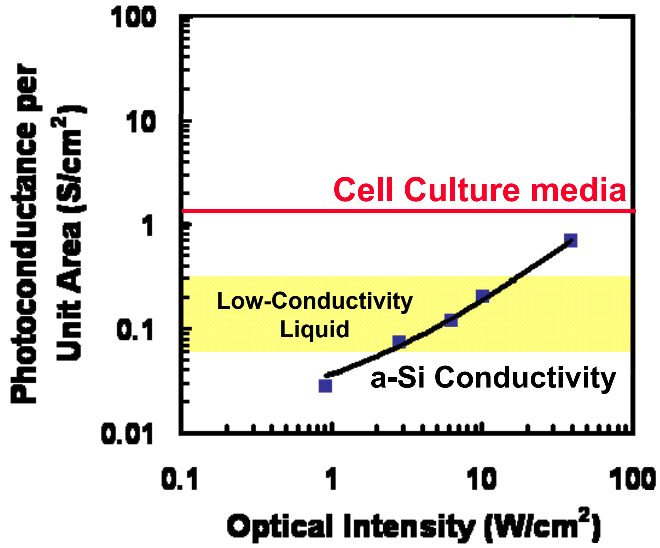
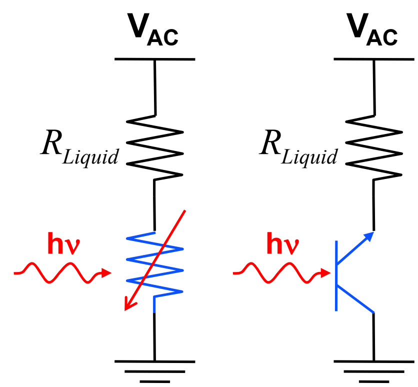
Phototransistor OET (Ph-OET) for Operation in Cell Culture Media



Equivalent Circuit:



a-Si OET

Photo-transistor OET

- **500x higher photoconductivity** than a-Si at 1 W/cm^2
- Require < 1 W/cm^2 to operate in cell culture media

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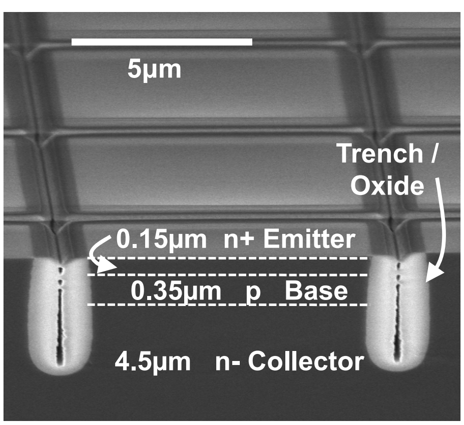
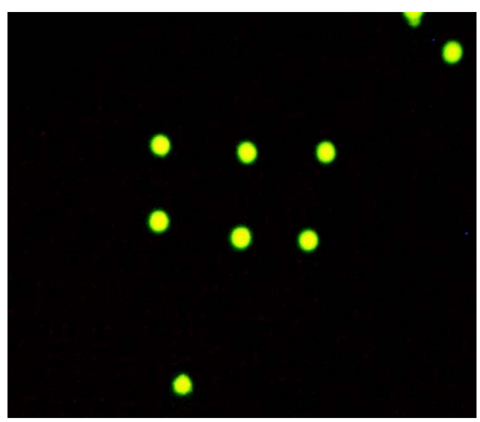
Phototransistor-OET



Single Crystalline Si
Bipolar Junction Transistor

2x3 Array of HeLa Cells
Trapped by Ph-OET
in PBS solution (1.5 S/m)



Cell viability tested by
Calcium AM dye

H. Y. Hsu, A. T. Ohta, P. Y. Chiou, A. Jamshidi, and M. C. Wu, "Phototransistor-based optoelectronic tweezers for cell manipulation in highly conductive solution," *TRANSDUCERS, 2007.*

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Applications of OET

- **Bio:**
  - Cell sorting
  - Sorting of differentiated neuron cells
  - Cell fate study
  - Cell-cell interaction
  - Light-induced electroporation
- **Nano:**
  - Trapping, patterning of nanoparticles
  - Self-assembled nanoparticles for SERS

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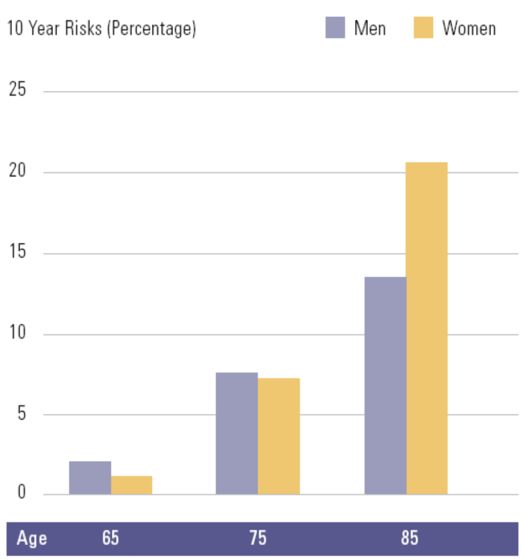
Neural Degenerative Diseases

Risk by age group



Seshadri et al., Stroke 2006

- **Neural degenerative diseases in U.S.**
  - **Alzheimer's disease: 5.3 million**
  - **Parkinson's disease: 0.5 million**
- **Limitation of conventional treatment**
  - **Medication → Drug resistance**
  - **Surgery → Risk of brain surgery**
  - **Cell replacement therapy → limited source.**
- **Stem cell technology has the potential to make cell therapy clinically competitive**

Lindvall et al., Science, 1990

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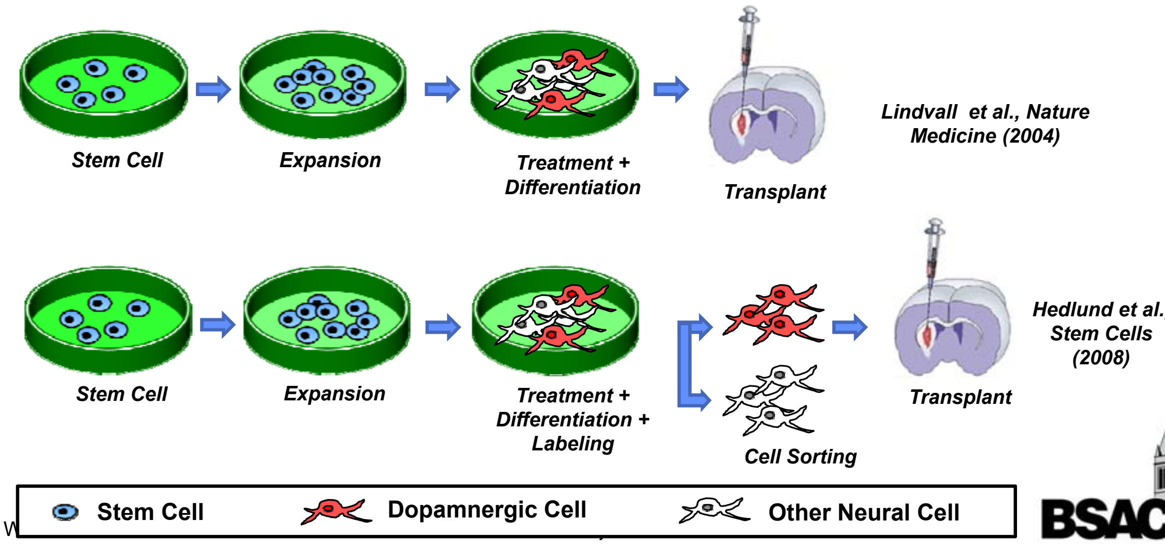
Stem Cell Based Replacement Therapy

- **Specific condition require injection of specific types of Neuron (Parkison's diseases → Dopaminergic neurons)**
  - **Less precise (~50 %) : Drug treatment determine cell fate**
  - **More precise (~90 %) : Cell sorting based on markers**



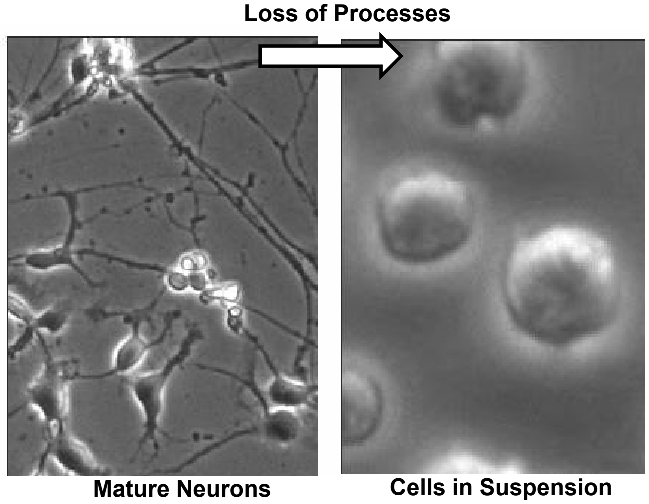
Conventional Cell Sorting Techniques

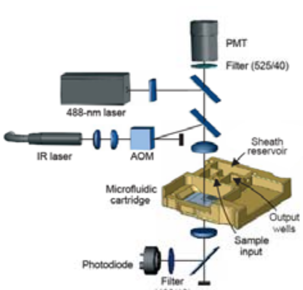
FACS Cytometer

Current technologies focus on cells suspension

- **Loss of cell viability**
- **Render the sorted neurons immature prior to injection**
- **Digestion of the surface maker**

Loss of Processes





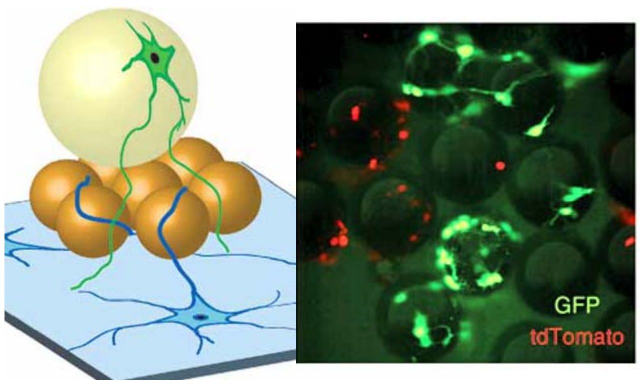
Microfluidic-based Sorters

Wang et al, NAT. Biotech. (2008)

Colloids as Growth Support

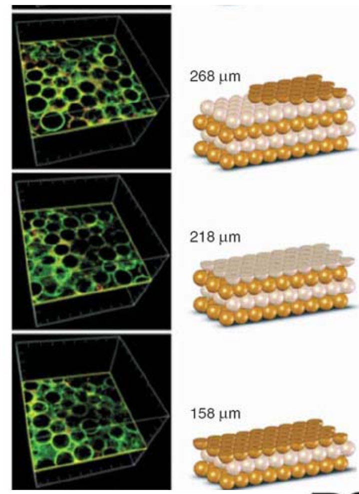
- Colloids (>45μm) as neural growth support
  - Physical manipulation of neuron without dissociation from surface
  - Colloid material: glass, biodegradable polymer (poly(N-sopropylacrylamide))
  - Allow treatment and genetic modification

Neuron Growth on Glass Beads

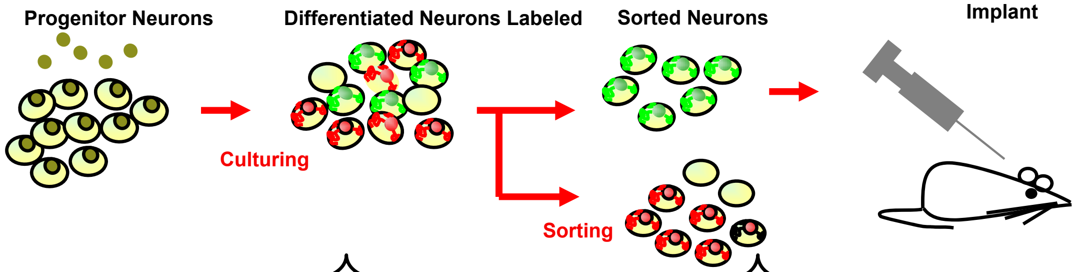


Pautot et al, NAT. METHODS (2008)

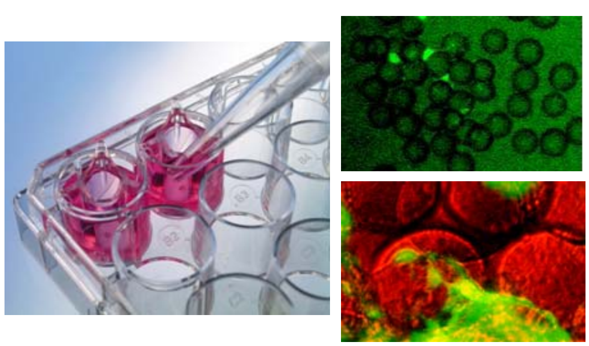
Layered Assembly of 3-D Neuron Network



Sorting of Differentiated Neurons

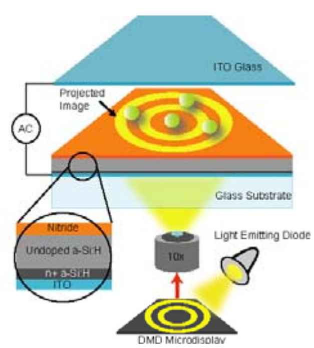


Cells on Colloid Beads



Pautot et al, *Nat. Methods (2008)*

Optoelectronic Tweezers

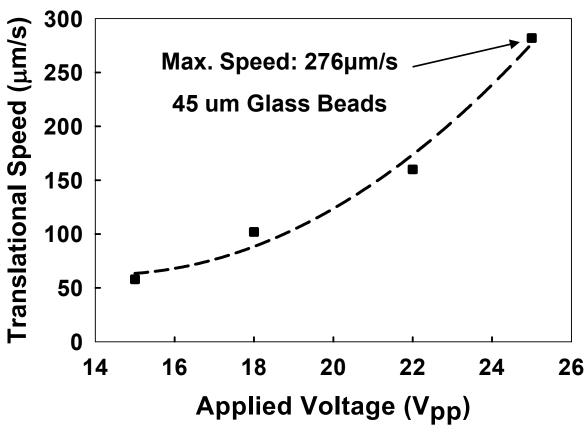


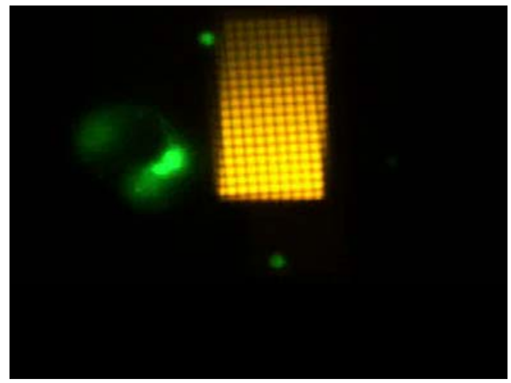
Chiou et al., *Nature*, 2005

DEP Force for Large Particles

$$F_{DEP} = 2\pi\varepsilon_m r^3 \,\mathrm{Re}(f_{cm}) \left|E_{RMS}\right|^2$$

Large Force with Large Particles





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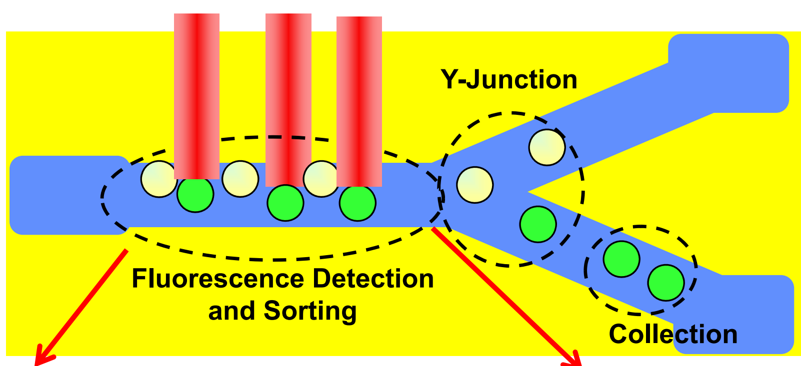
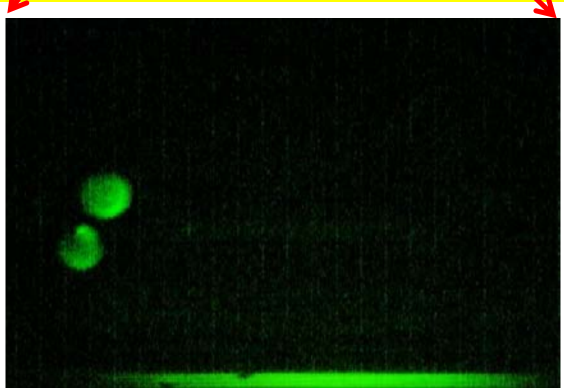
Microfluidics for Collecting Sorted Neurons





Fluorescent neural beads are detected and automatically switched by OET to lower part of the channel (video 3x speed)

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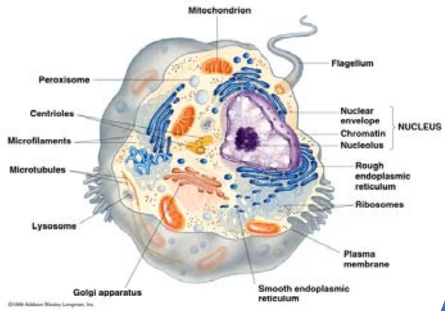
Throughput

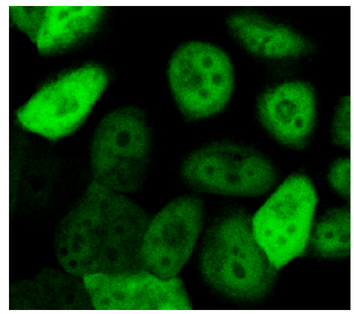
- **Number of neurons needed for cell replacement therapy: ~ 5,000**
- **Throughput demonstrated: 1800 neurons/hour**
- **Further increase of throughput:**
  - **100x by increased flow rate**
  - **2x by decreased channel width**
- **Potentially, a throughput of 3.6×10^5 neurons/hour can be achieved**
  - **Sorting can be achieved within minutes**

Light-Induced Electroporation

Cellular Poration



kensbiorefs.com/NewCellStr.html



cas.muohio.edu/micro/People/carlin.html

- **Delivery of:**
  - **DNA**
  - **Drugs**
  - **Biomarkers**

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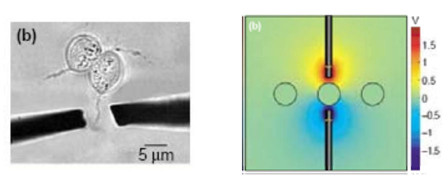
Conventional Techniques

Bulk Electroporation

Pros: High Throughput
Cons: No selectivity

celltransfection.com
btxonline.com

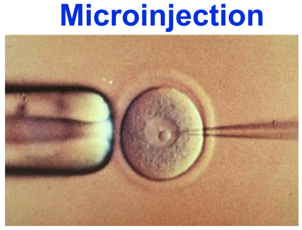
Single Cell Electroporation



Pros: High Selectivity
Cons: Low Throughput

Olofsson *et al. Current Opinion in BioTech*, 14, 2003.

Microinjection



Pros: High Selectivity & Dosing Control
Cons: Very Low Throughput

research.uci.edu/tmf/dnaMicro.htm

Liposomal Delivery

Pros: High Viability & Dosing Control
Cons: Low efficacy & High Variability

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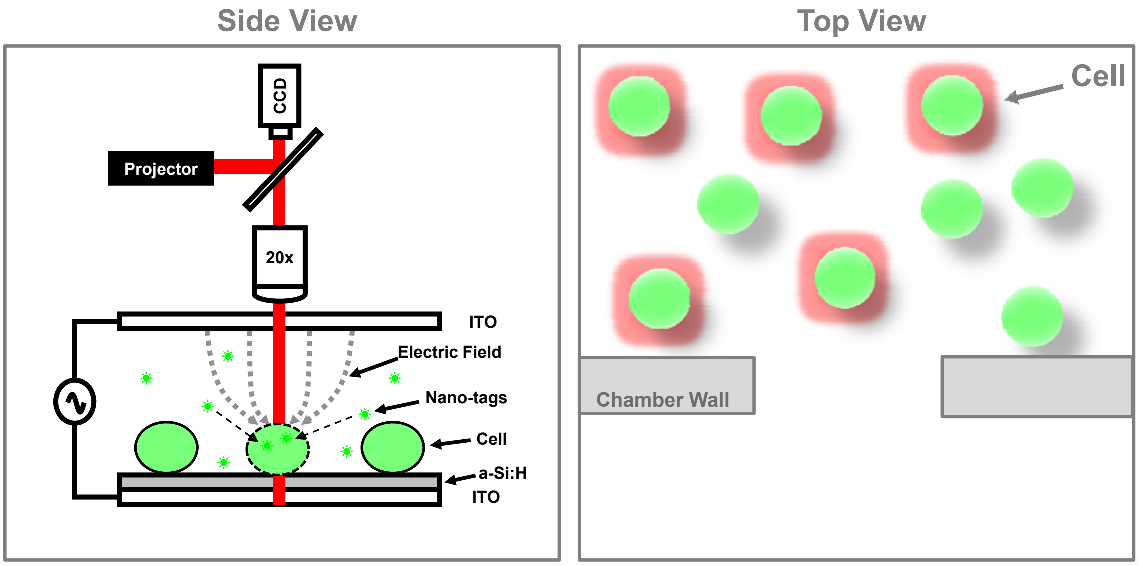
Light-Induced Electroporation Using OET

- Selectivity, throughput, viability



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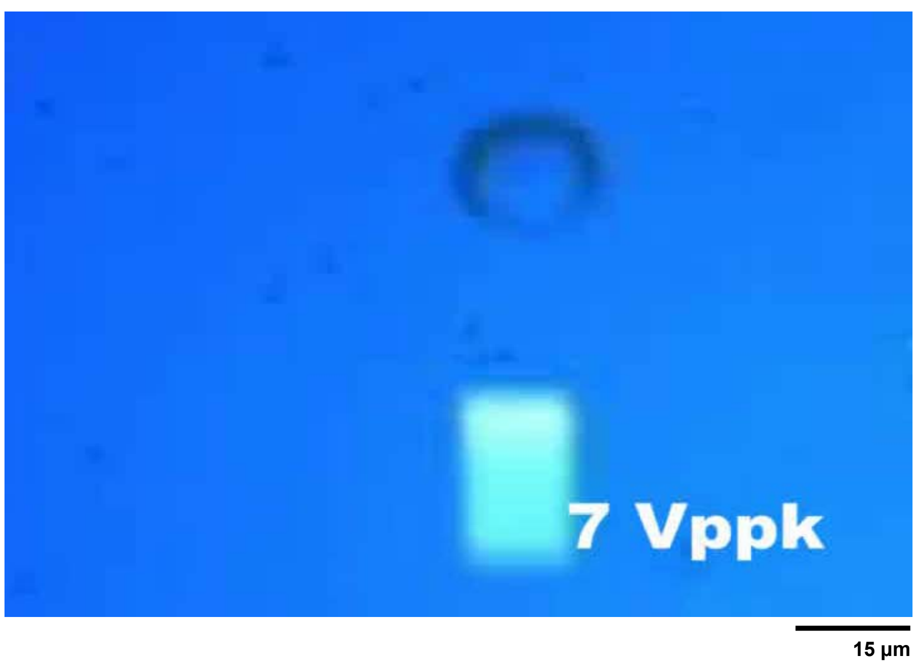
Electroporation & DEP Response



HeLa Cells

Cytopulse® Cytoporation Medium: 10 mS/m

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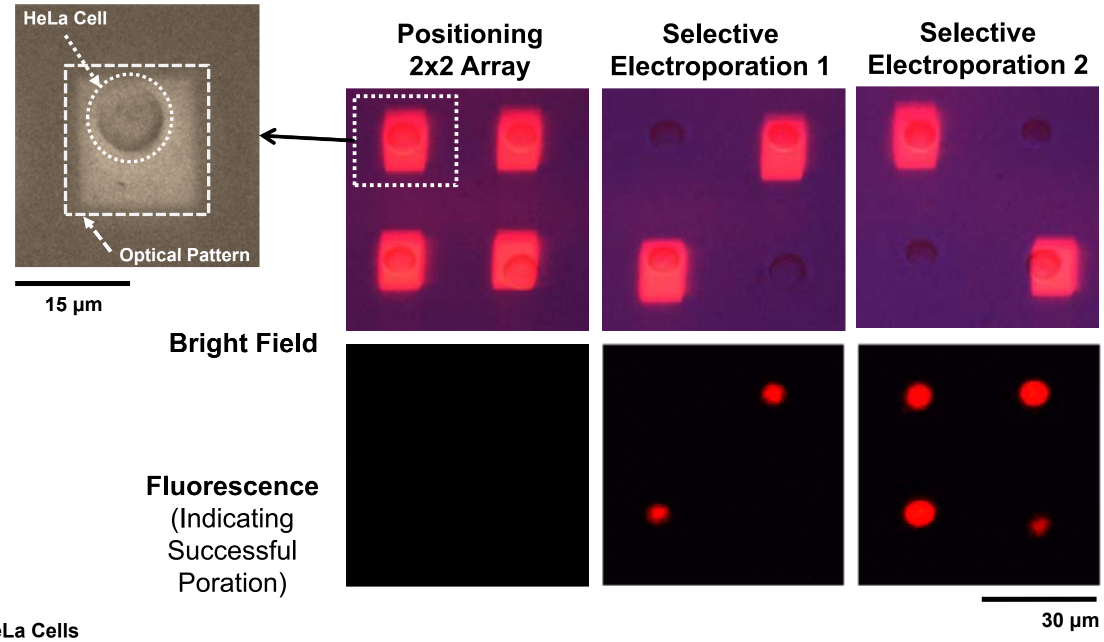
Cell Trapping and Electroporation

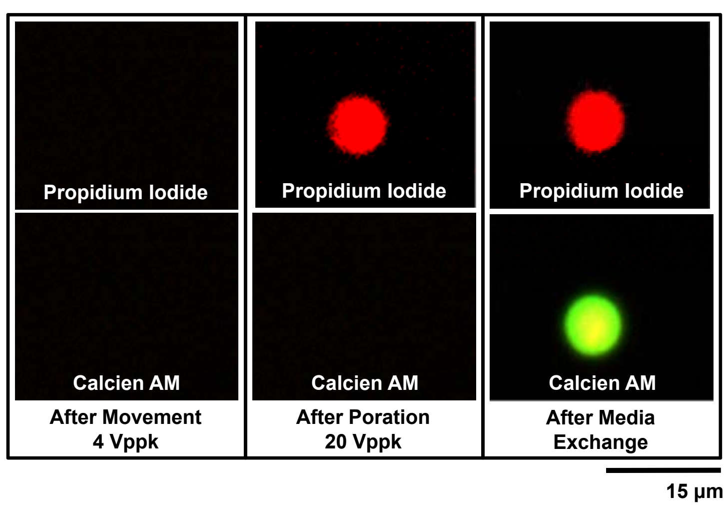


HeLa Cells
Cytopulse® Cytoporation Medium: 10 mS/m
Invitrogen® Propidium Iodide

Cell remains viable (verified by Calcein AM dye)

Viability Analysis

- **Electroporate cells in presence of Propidium Iodide.**
  - **Successfully electroporated cells fluoresce red.**
- **Exchange cell environment with Calcein AM dye containing media.**
  - **Cells with intact membranes and proper enzymes fluoresce green.**

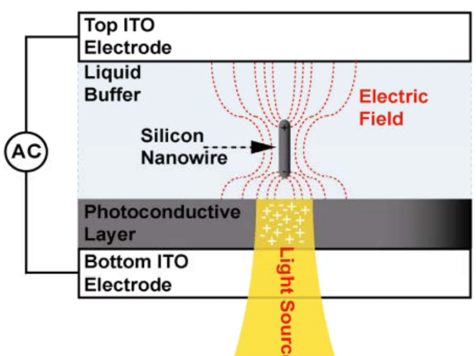


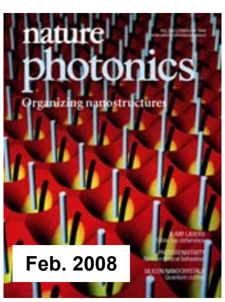
HeLa Cells
Cytopulse® Cytoporation Medium: 10 mS/m
Invitrogen® Propidium Iodide
Invitrogen® Calcein AM

Valley *et al. MEMS*, 2009.

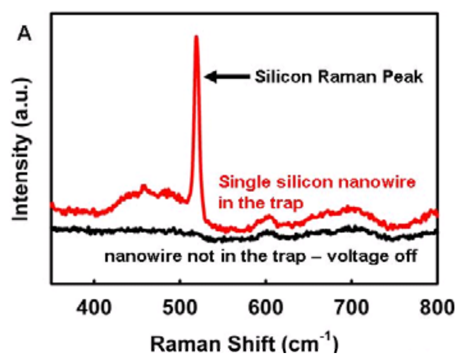
Trapping, Patterning, Assembly of Nanowires and Nanoparticles

OET Trapping of Nanowires



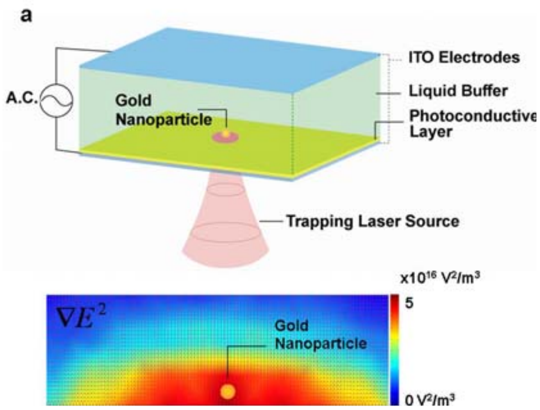


In-situ Raman of Trapped NW

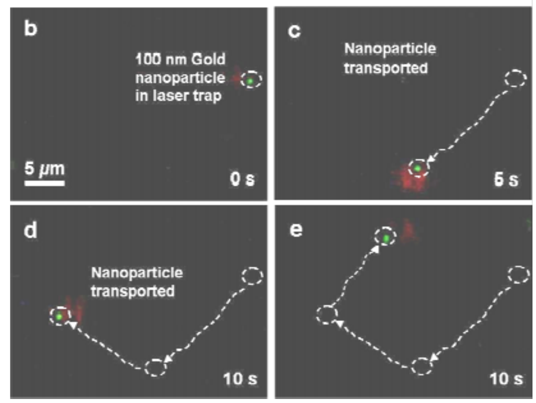


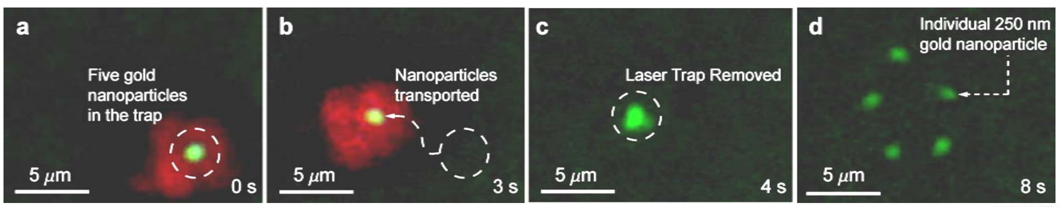
A. Jamshidi, P.J. Pauzauskie, P.J. Schuck, A.T. Ohta, P.-Y. Chiou, J. Chou, P. Yang, M. C. Wu, "Dynamic manipulation and separation of individual semiconducting and metallic nanowires," *Nature Photonics, vol. 2, pp. 86-89, 2008.*

Trapping of Single Gold Nanoparticles



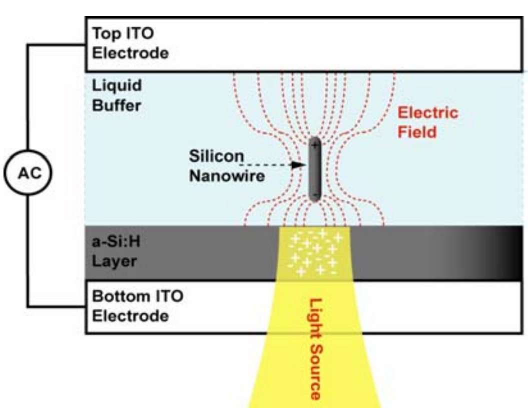
100 nm Au Nanoparticle





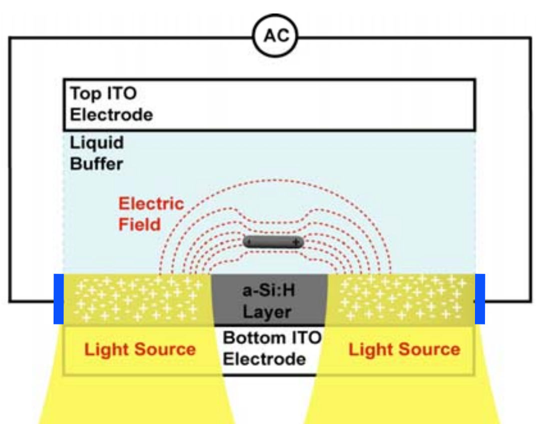
Vertical vs. Lateral OET

Vertical-Field OET



A. Jamshidi, et al., *MEMS 2007.*
A. Jamshidi, et al, *Nature Photonics 2008*

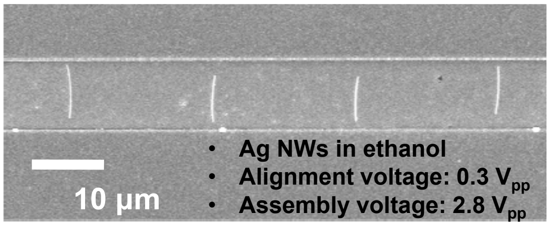
Lateral-Field OET

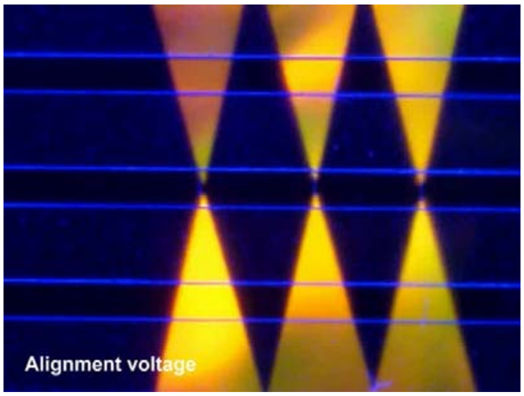


A. T. Ohta, A. Jamshidi, P. J. Pauzauskie, H.-Y. Hsu, P. Yang, and M. C. Wu, "Trapping and transport of silicon nanowires using lateral-field optoelectronic tweezers," *CLEO 2007.*

Micro-Assembly Using LOET

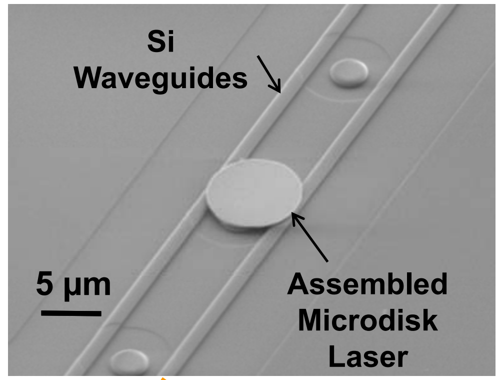
Parallel Assembly of Ag NWs





Ohta, et al., IEEE/LEOS Optical MEMS and Nanophotonics, 2008

Micro-Assembly of Microdisk Laser on Si Photonic Waveguides



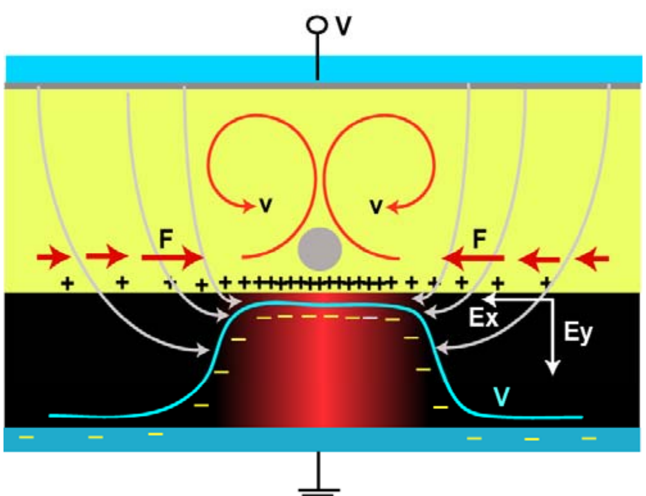
Near-Field Image of Output Light from Si Waveguide

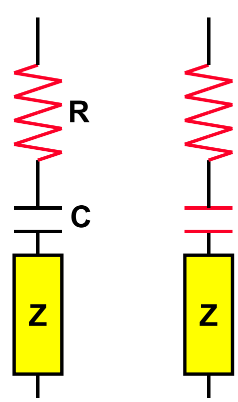
- 0.6 mW threshold pump
- 7% Q.E. in Si waveguides

Tien, et al., OFC, 2009

BSAC

Light Induced AC Electroosmosis

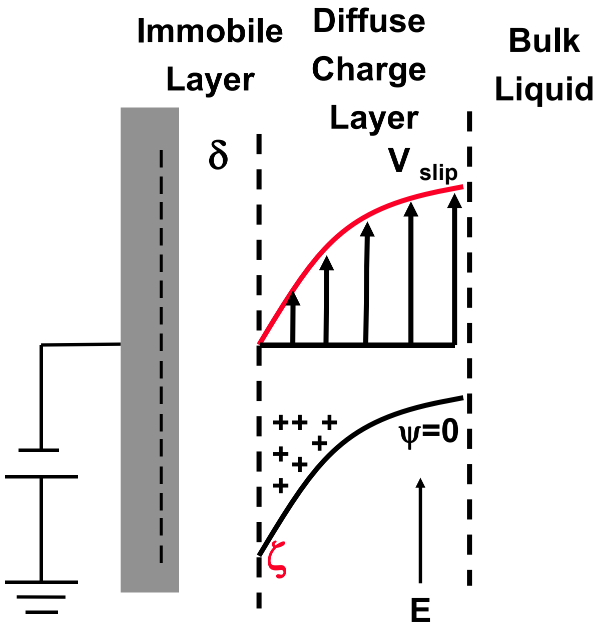


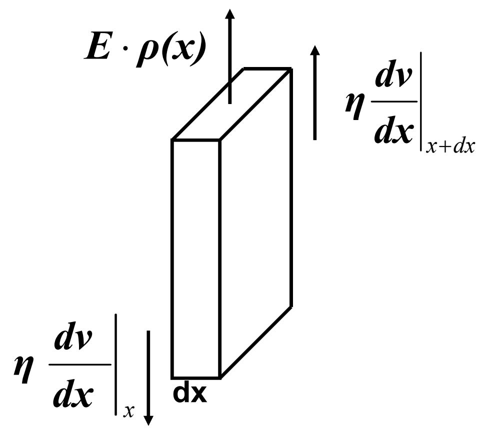


- Double layer charges build up at low ac frequency (1kHz ~ 10 kHz)
- Surface charge driven by tangential electric field as a driving force for fluid flow : Electroosmosis

R. C. Hayward, et al. (Princeton) Nature (2000)
P. Y. Chiou et al. (UCB/UCLA) JMEMS (2008)
S. J. Williams, et al. (Purdue), Lab Chip (2008)
H. Hwang et al. (KAIST) Lab Chip (2009)
H. Hwang et al. (KAIST) Langmuir (2009)

Principle of Electroosmosis

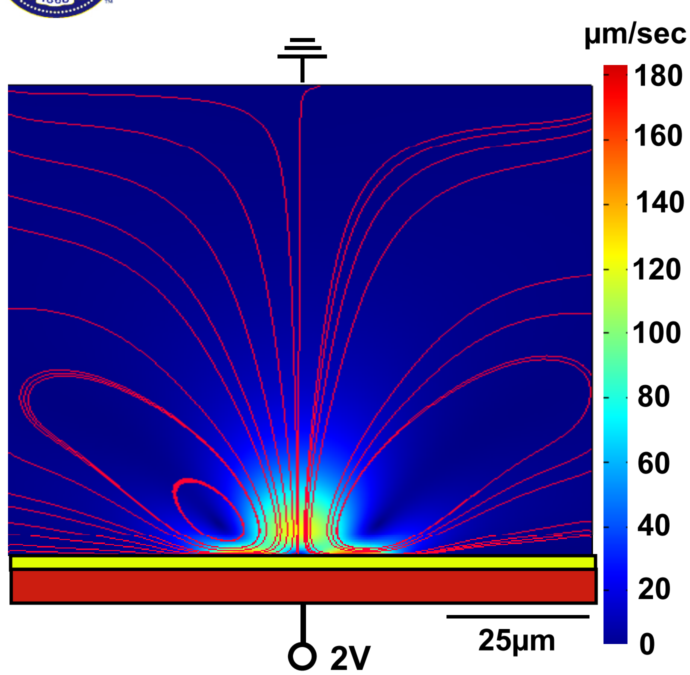




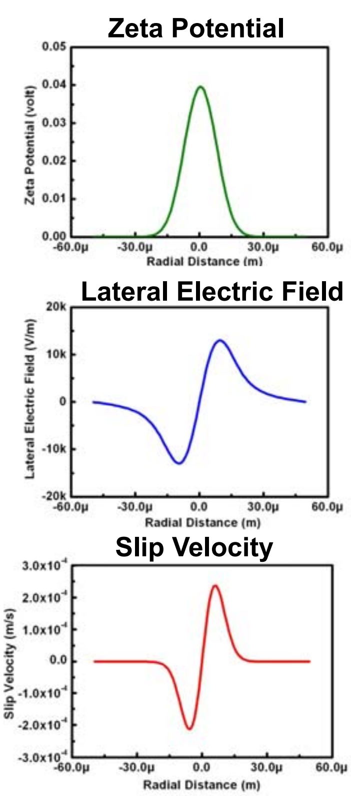
$$-E \cdot \varepsilon \frac{d^2\psi}{dx^2} dx = \eta \frac{d^2 v}{dx^2} dx$$

$$V_{slip} = -\frac{\varepsilon \zeta E}{\eta}$$

Simulated AC Electroosmosis Flow



Flow Pattern near Virtual Electrode



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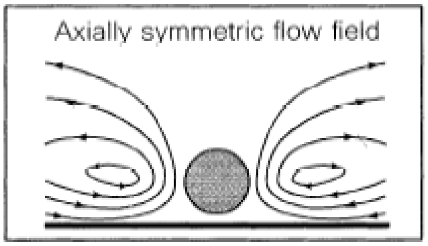
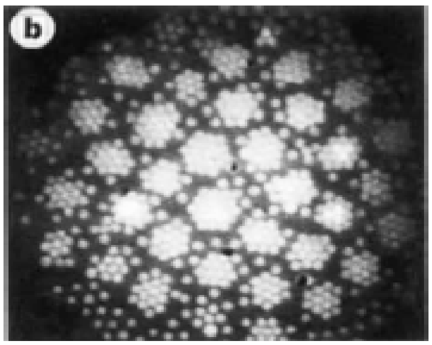
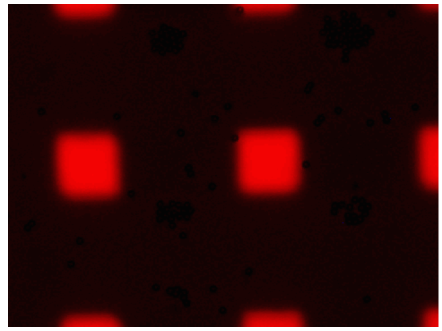
Optically Guided Formation of Crystalline Structure

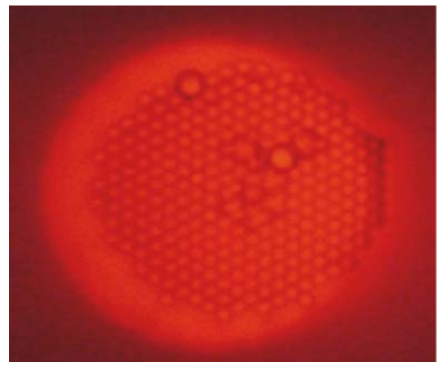




Yeh, Nature, 1997

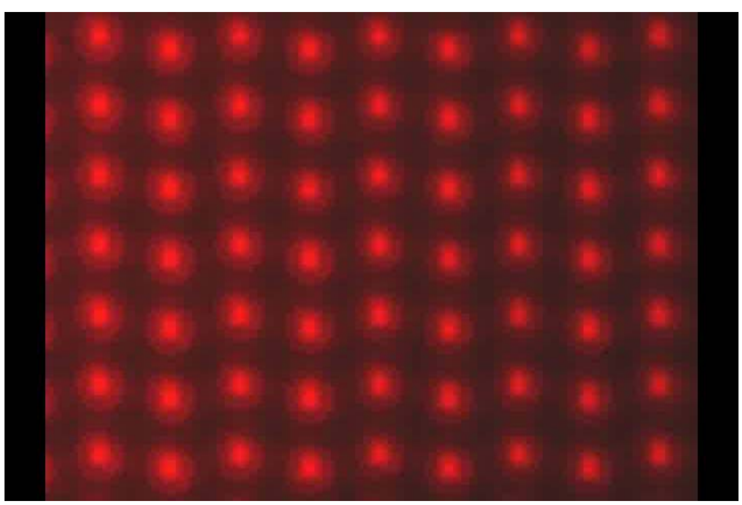
- Particle induced ac electroosmosis flow





Monolayer crystalline structure

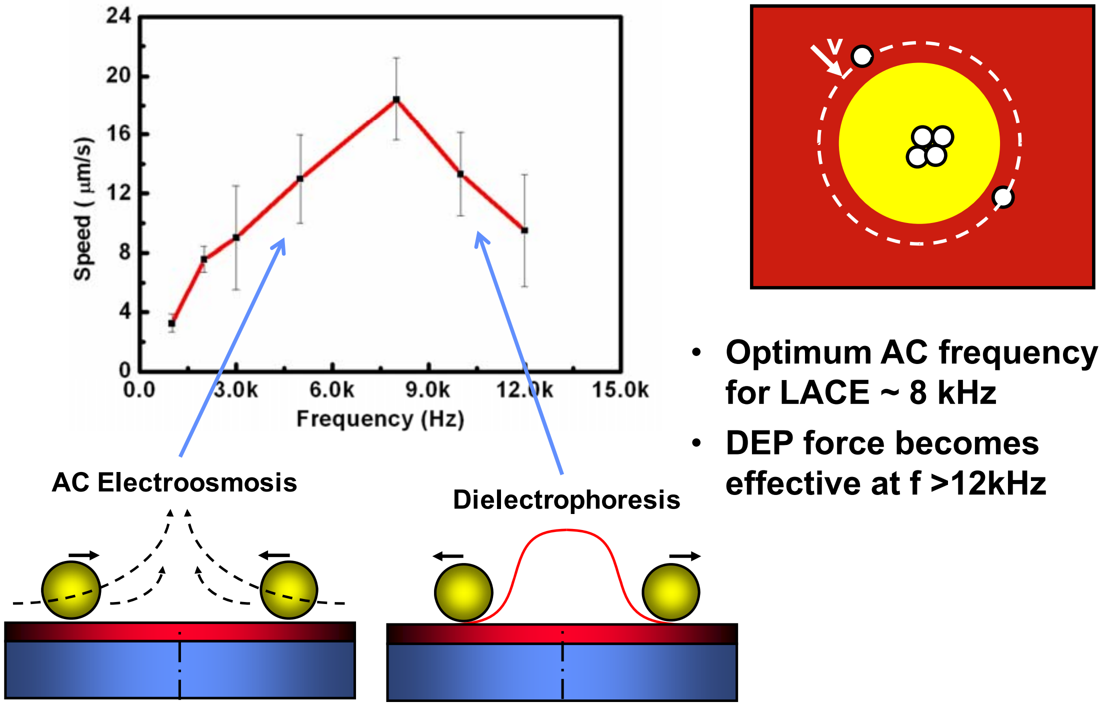
31,000 Single Particle Traps -- One Particle per Vortex --



Each trap uses a single DMD pixel !

2 µm latex bead

Frequency Response of Light Induced AC Electroosmosis



- Optimum AC frequency for LACE ~ 8 kHz
- DEP force becomes effective at f >12kHz

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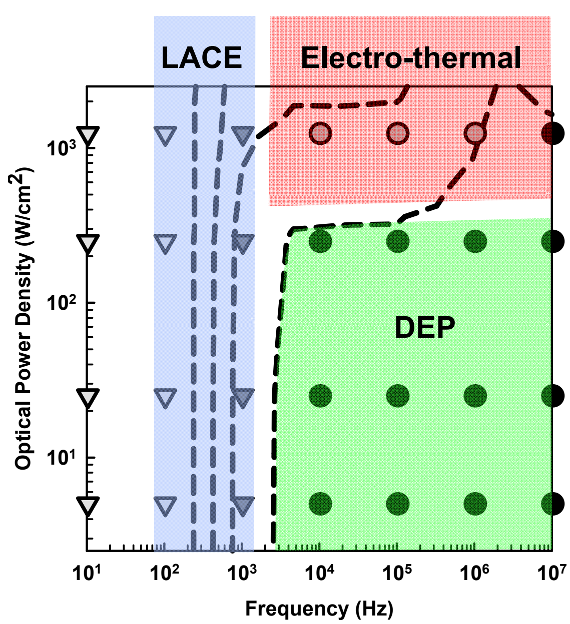
Parameter Ranges for Various Electrokinetic Effects in OET

- Multiple electrokinetic effects present in OET
  - DEP
  - LACE
  - Electro-thermal
- Key parameters:
  - AC frequency
  - Optical power density



Valley *et al.* ***JMEMS***, 17, 2007.

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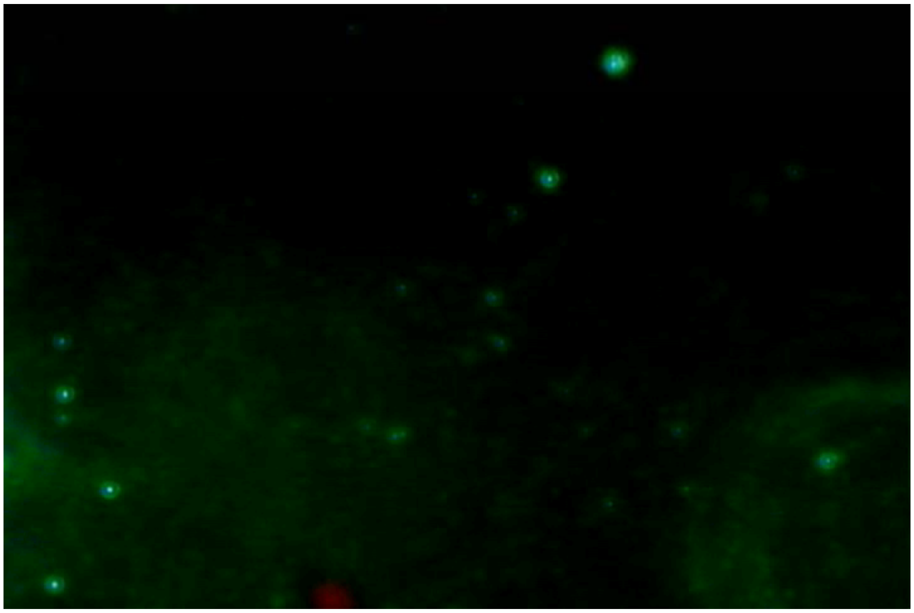
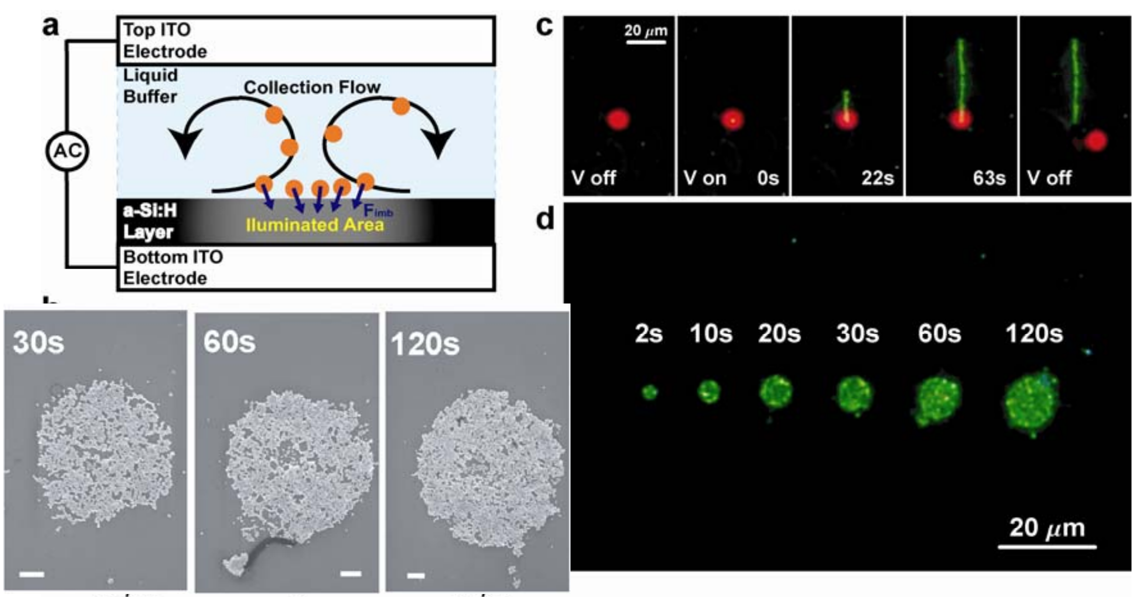
Nano-Pen: Trapping and Immobilization of Gold Nanoparticles



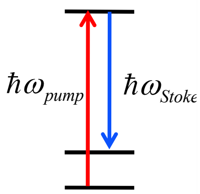
Immobilization of Nanoparticles: NanoPen

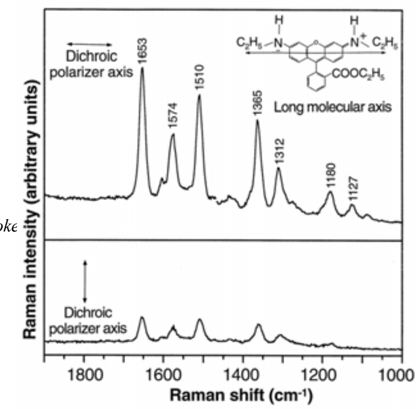
- Collection of nanoparticles over long range through LACE mechanism
- Immobilization of nanoparticles through strong DEP force



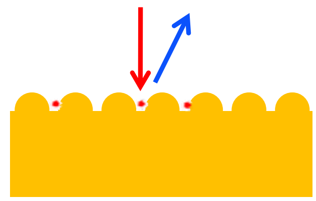
Arash, et al, submitted to Nano Letters

Surface-Enhanced Raman Spectroscopy (SERS)

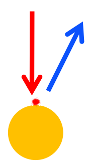




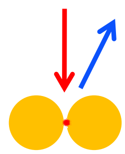
Rough Metal Surface



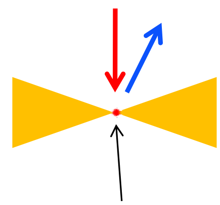
Single Particle



Dimer



Optical Antenna



Molecule

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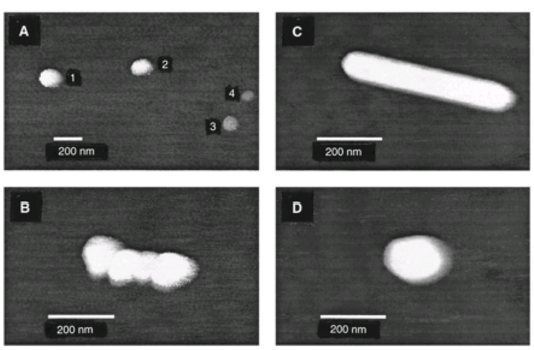
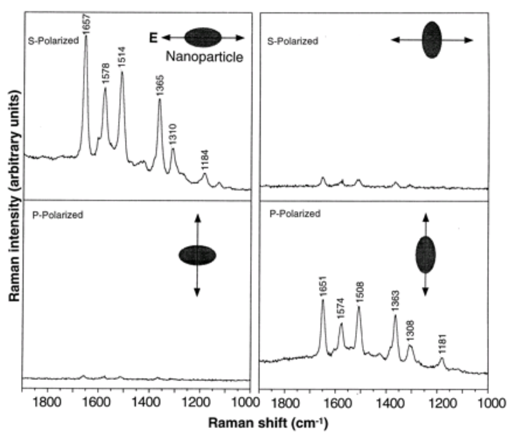
Single Molecule Detection

Enhancement = 10^{14} ~ 10^{15}

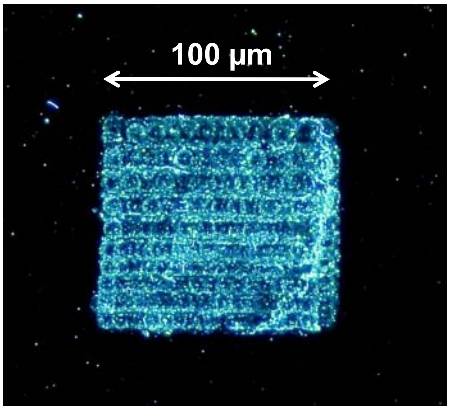




S. Nie and S. R. Emory, "Probing Single Molecules and Single Nanoparticles by Surface-Enhanced Raman Scattering," Science, vol. 275, pp. 1102-1106, 1997

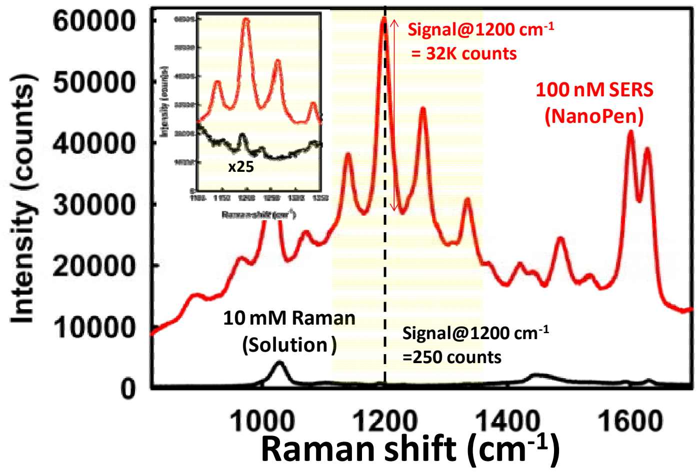
K. Kneipp, Y. Wang, H. Kneipp, L. T. Perelman, I. Itzkan, R. R. Dasari, and M. S. Feld, "Single Molecule Detection Using Surface-Enhanced Raman Scattering (SERS)," Physical Review Letters, vol. 78, p. 1667, 1997

Au Nanoparticle Patch Patterned by NanoPen for SERS



Dark Field Image of
Dried Au Nanoparticle Patch
Created by NanoPen

Surface-Enhanced Raman Spectrum



SERS Enhancement > 10^7

Summary

- Optoelectronic tweezers (OET)
  - A new, image-based optical manipulation technique
  - Parallel manipulation, real-time reconfiguration
- There is a growing OET community worldwide
- Key advances from UCB:
  - Bio-compatible OET in cell culture media
  - Sorting of mature neuron cells on beads
  - Selective single cell poration
  - Trap single nanowire (d=20nm, l~ um) and single Au nanoparticle (d= 60nm)
  - NanoPen: immobilization of trapped Au nanoparticles
    - > 10^7 enhancement factor for surface-enhanced Raman spectroscopy (SERS)

Acknowledgments

- Current OET Group
  - Arash Jamshidi, Hsan-yin Hsu, Justin Valley, Steven Neale
- Former members
  - Prof. Pei-Yu Chiou (UCLA)
  - Prof. Aaron Ohta (Univ. Hawaii)
- Nanowire collaboration
  - Peidong Yang Group @ UCB
  - Chang-Hasnain Group @UCB
  - Ali Javey Group @ UCB
- Raman characterization
  - Jim Schuck, MF, LBL
- Sorting differentiated neurons
  - Ehud Isacoff Group @ UCB, H. Lee,
  - S. Pautot
- Project support
  - CCC (NIH Nanomedicine)
  - CMISE (NASA URETI)
  - DARPA Seedling
  - Applied Biosystems

Thank you !