

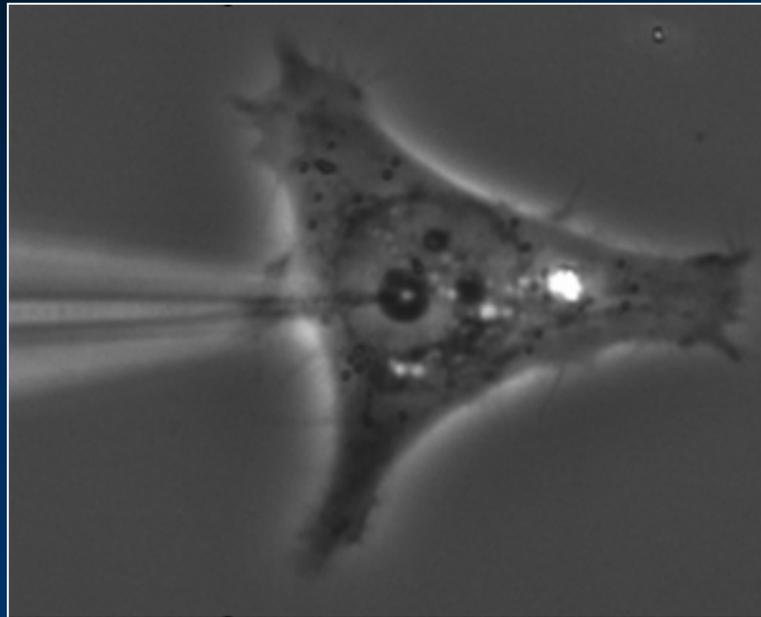
Carbon Nanopipettes

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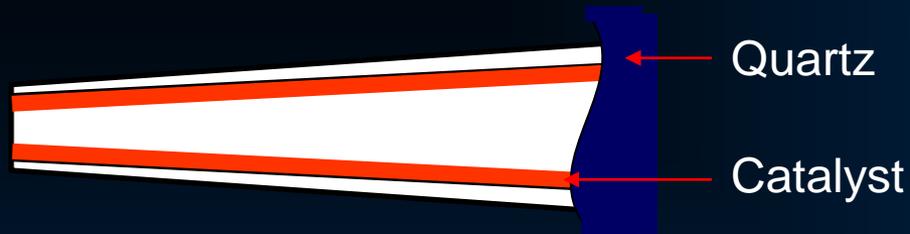
bau@seas.upenn.edu



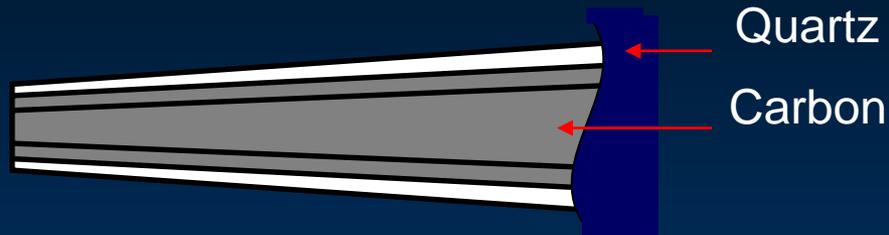
Challenge: how do you integrate a nanoscopic structure with a macroscopic handle?

Solution: integrated manufacturing

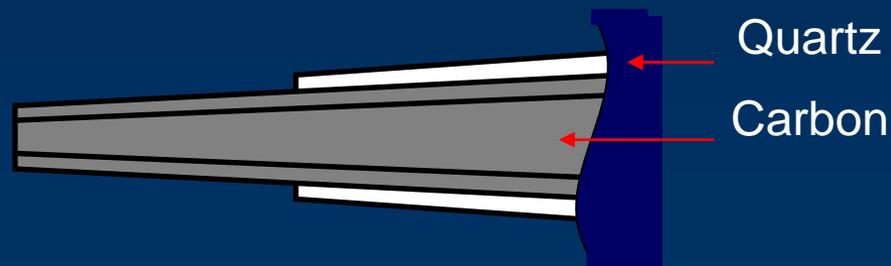
Pull Quartz Micropipettes



Deposit Carbon Film by CVD



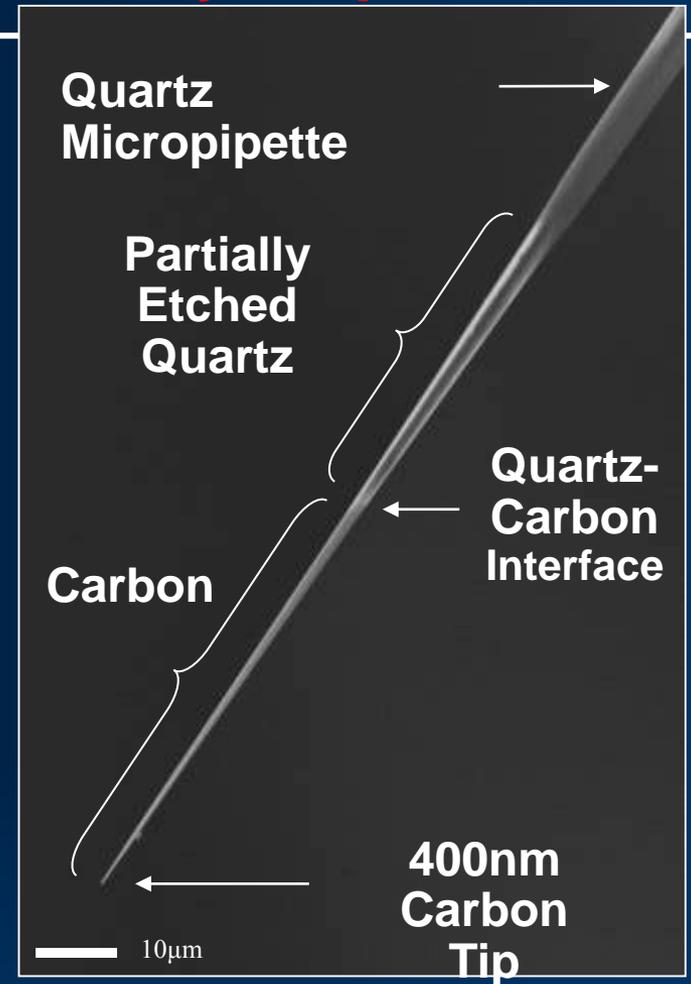
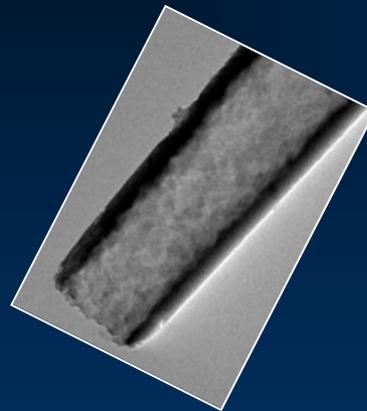
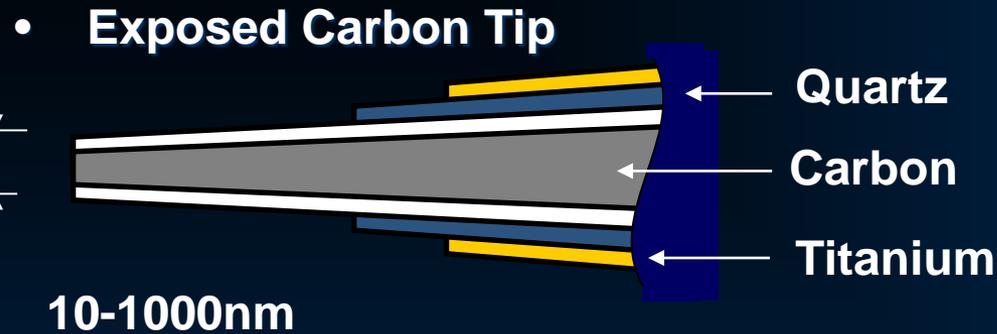
Expose Carbon Tip with BHF



Kim, B. M, Murray, T., and Bau, H. H., 2005, The Fabrication of Integrated Carbon Pipes with Sub Micron Diameters, Nanotechnology, 16, 1317-1320.

B. M. Kim and H. H. Bau, Nano and Micro Scale Structures: Methods, Devices and Applications Thereof, Patent application 2006/0116971

Carbon NanoPipette (CNP) - No Assembly Required

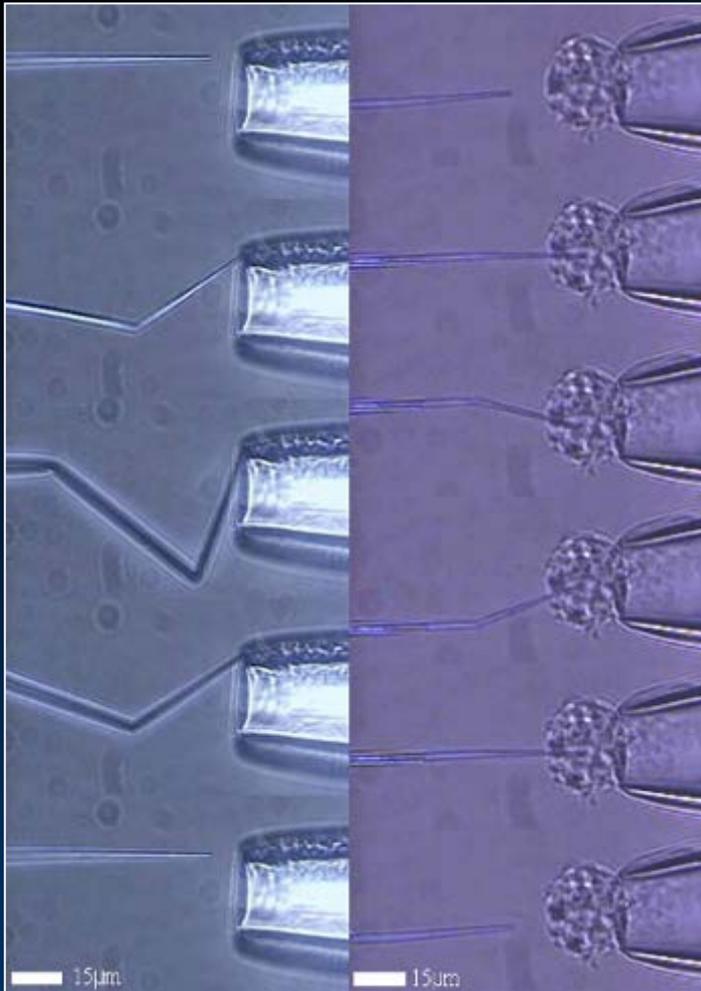


Glass

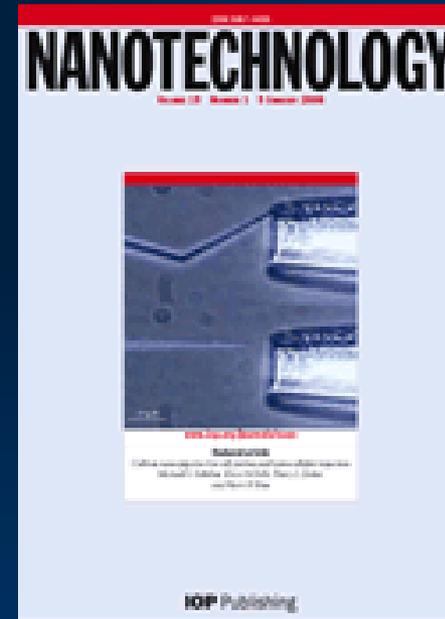
2µm

Carbon Nanopipe

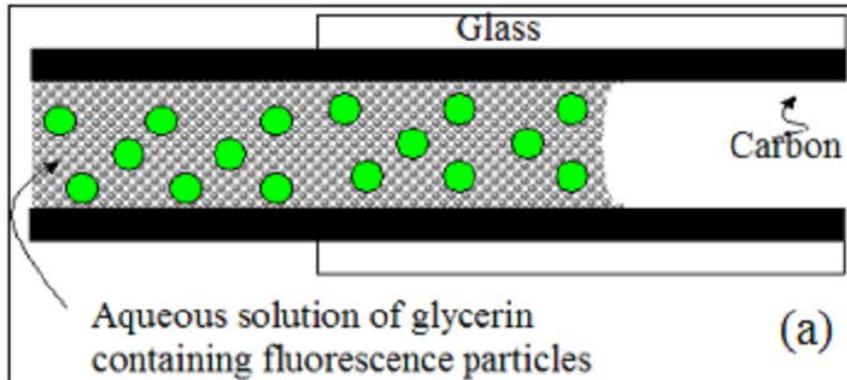
CNP's Mechanical Characteristics



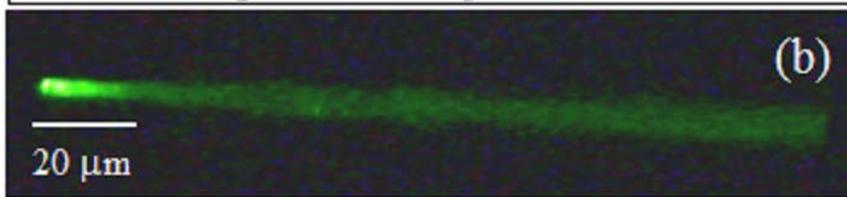
When pushed against hard surface, the CNP's tip buckles without breaking (left) yet the CNP is stiff enough to penetrate into a biological cell (right)



CNP: Optical Properties



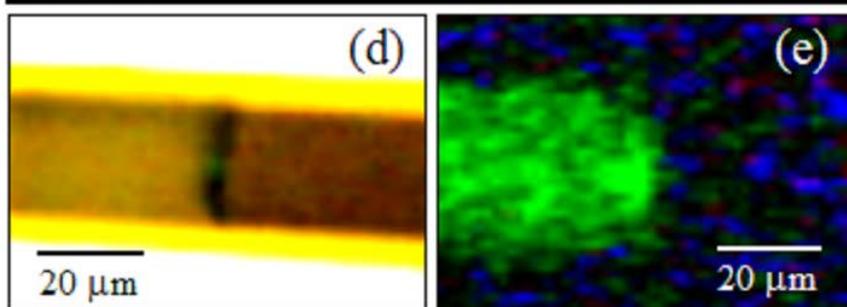
(a) A schematic of a carbon pipette partially filled with a suspension of 50nm fluorescent particles in water-glycerin blend.



(b) A fluorescent image of a portion of the tip filled with a suspension.



(c) An optical image complementing the fluorescent image of b.



(d) An optical image of the suspension-air interface inside the carbon pipette.

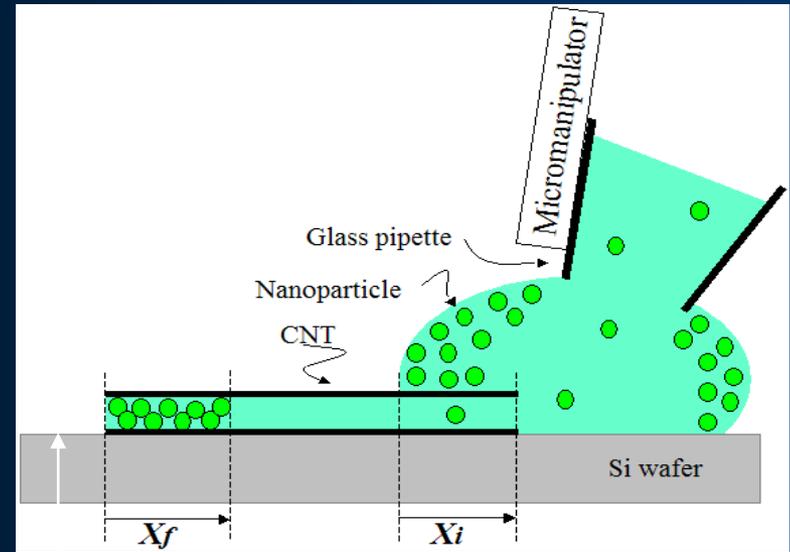
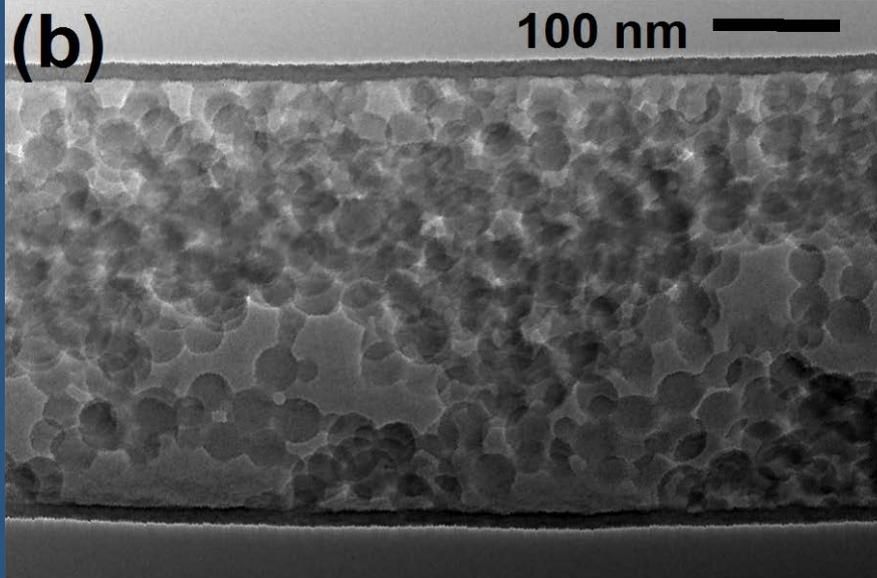
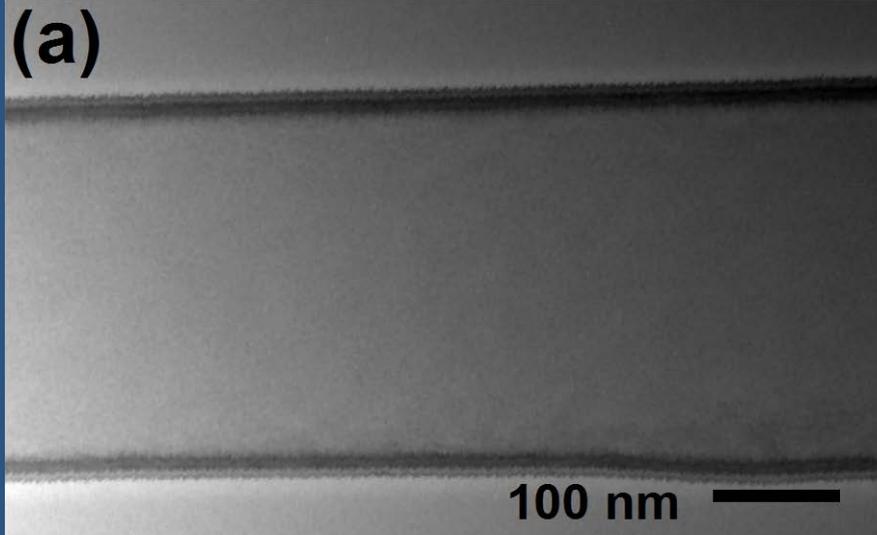


(e) A fluorescent image complementing the optical image of Fig. d. The liquid part of the suspension is a water-glycerin mixture.

Carbon NanoPipes are transparent to: Light, electrons, and X-rays

Kim B. M, Murray, T., and Bau, H. H., 2005, The Fabrication of Integrated Carbon Pipes with Sub Micron Diameters, *Nanotechnology*, 16, 1317-1320.

Sample Holders for Electron Microscope Imaging



Carbon Nanopipe Diameter:
~500nm

Particles ~40nm, Fluorescent
Polystyrene

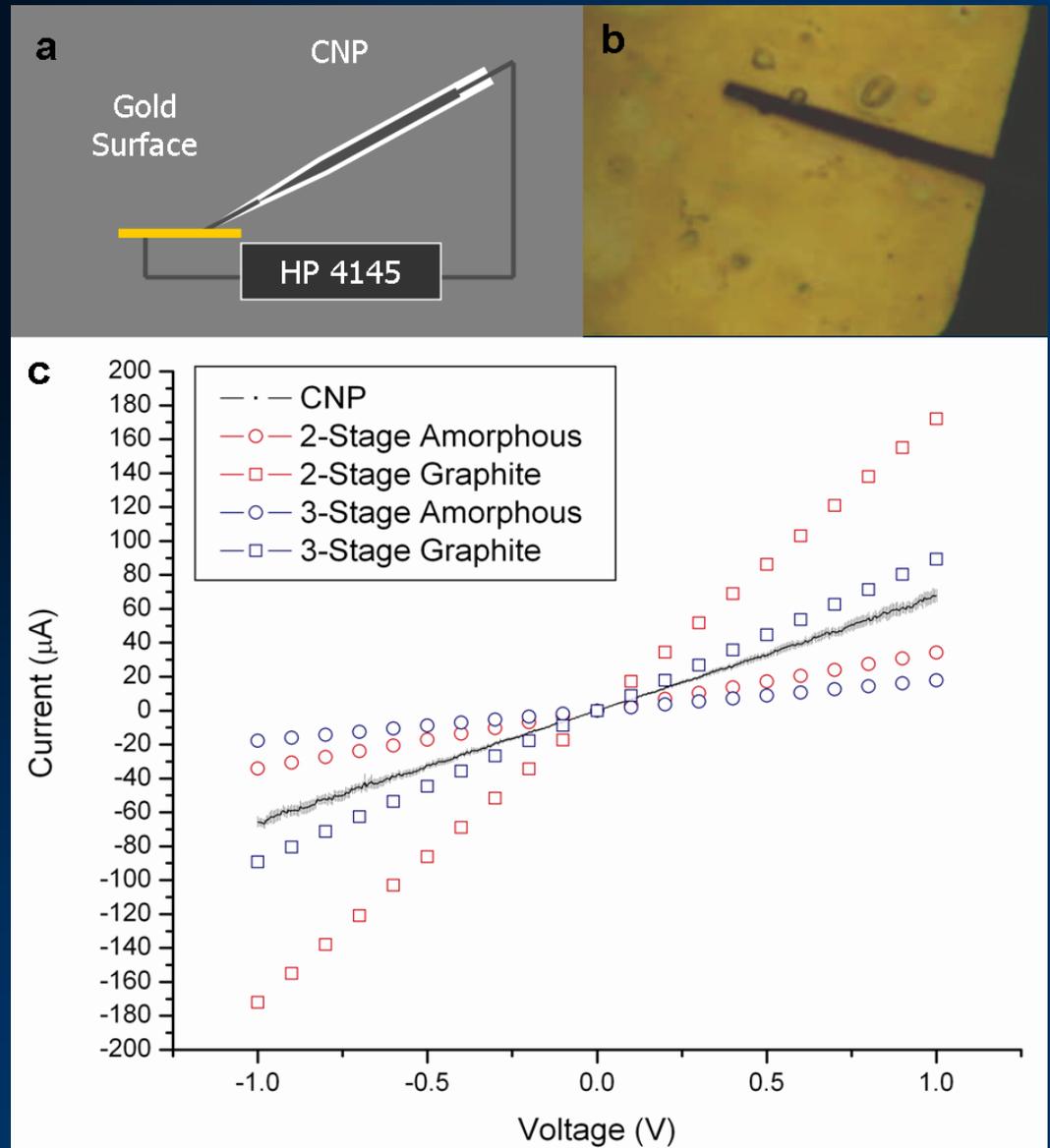
Kim, B. M., Qian, S., and Bau, H., H., 2005,
Filling Carbon Nanotubes with Particles, Nano
Letters, 5 (5) 873 – 878

CNPs' Electrical Properties

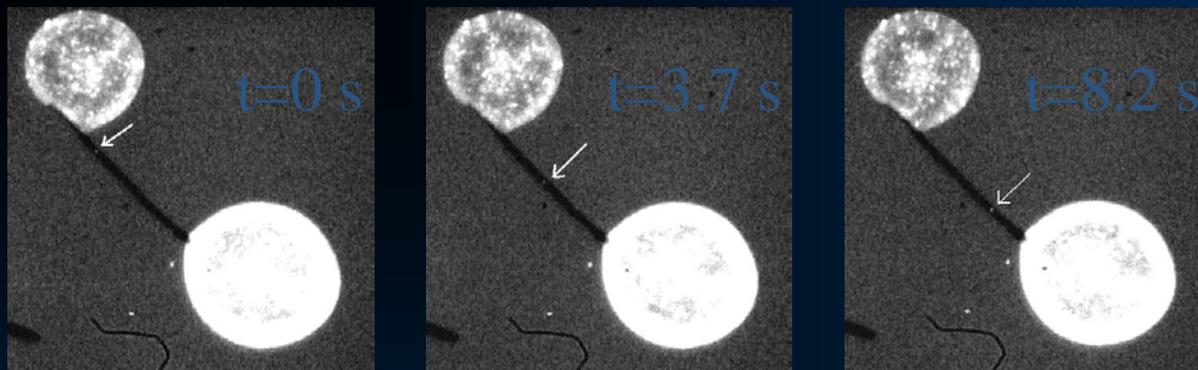
Ohmic Resistance

Electrical resistance of CNPs : the current is depicted as a function of the potential difference. Solid lines (theory). Symbols (experiments).

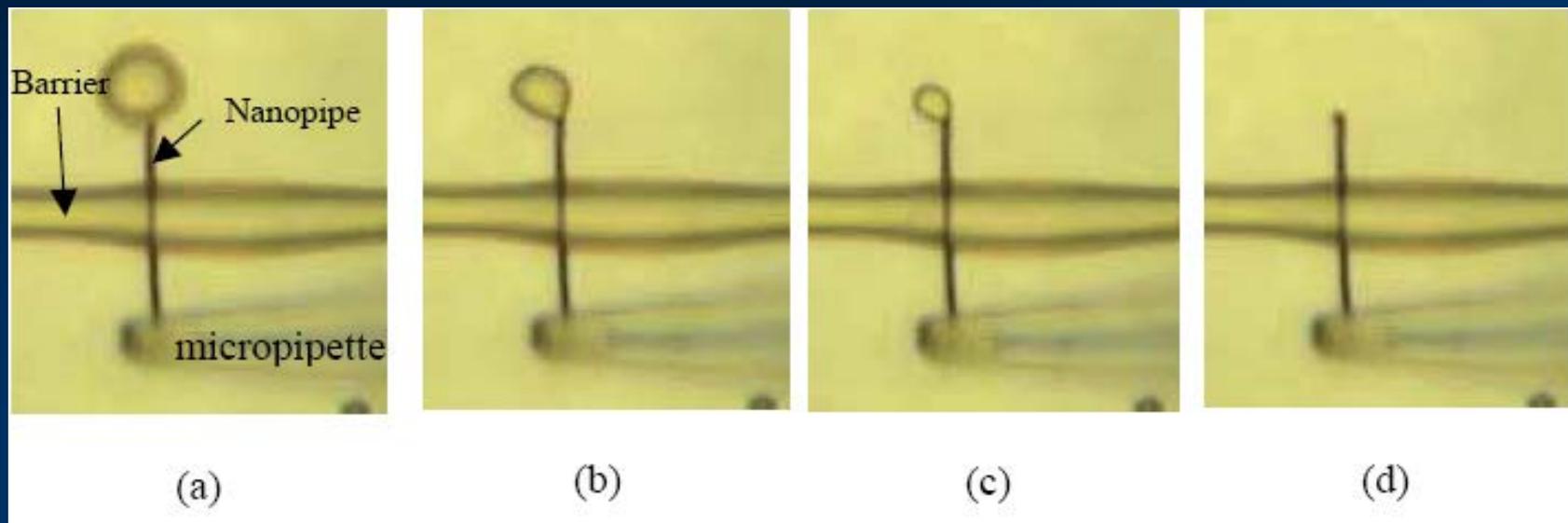
Typical resistance $\sim 15\text{k}\Omega$



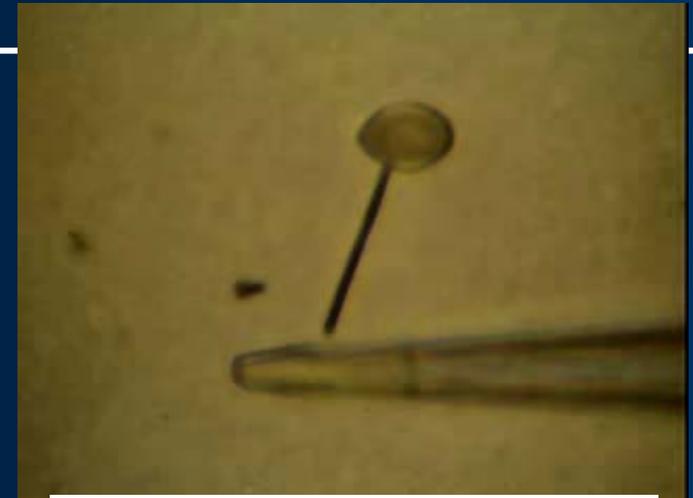
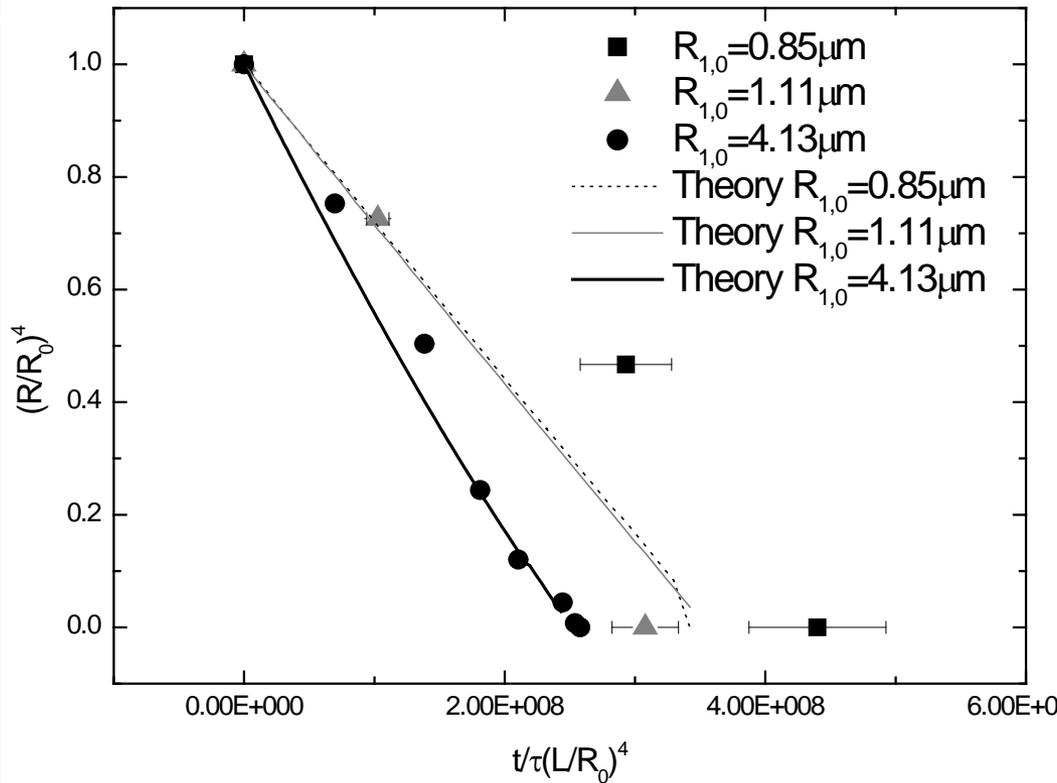
Flow Characteristics of the Carbon Nanopipes



The position of the fluorescent particle inside the nanotube as liquid flows from the smaller drop to the bigger drop. The arrow indicates the location of the particle.



MEASURING FLOW RATES at ATTOLITER PER SECOND



$$\left(\frac{R}{R_0}\right)^4 = 1 - \frac{2\sigma \sin \theta}{\chi \eta L} \left(\frac{a}{R_0}\right)^4 t$$

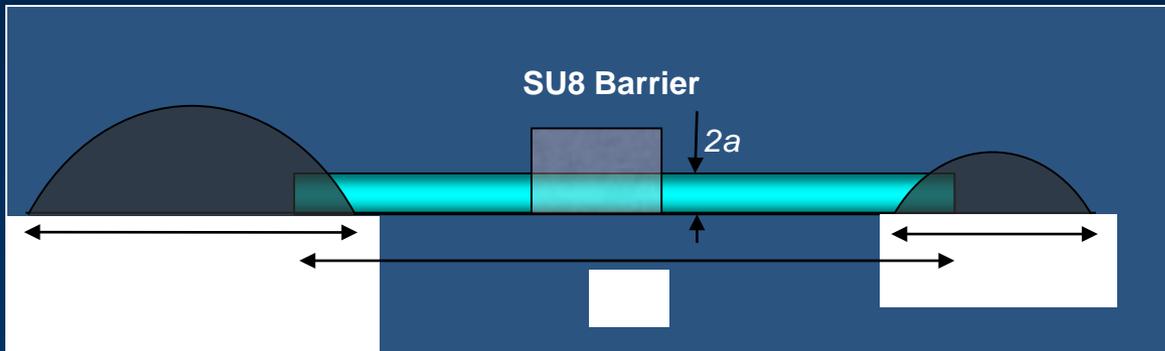
σ =surface tension

η =viscosity

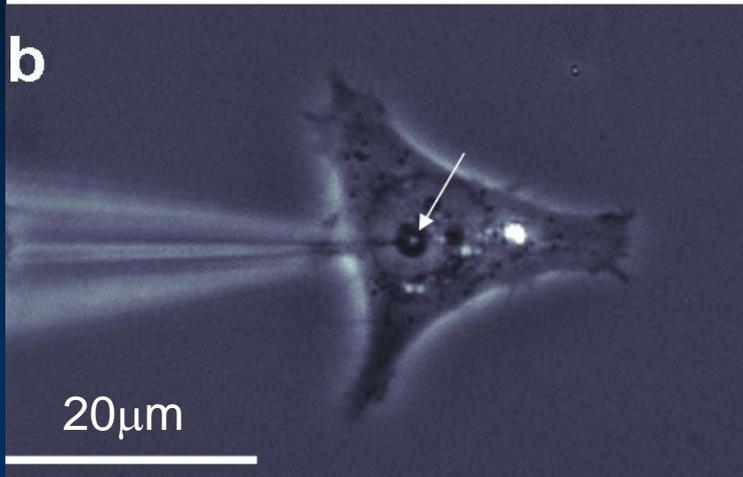
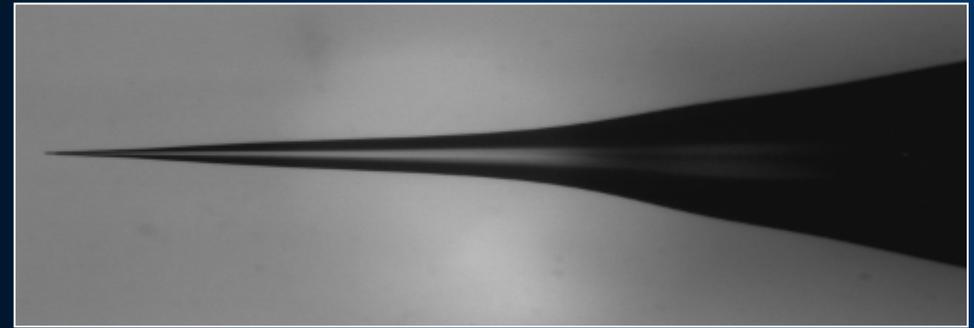
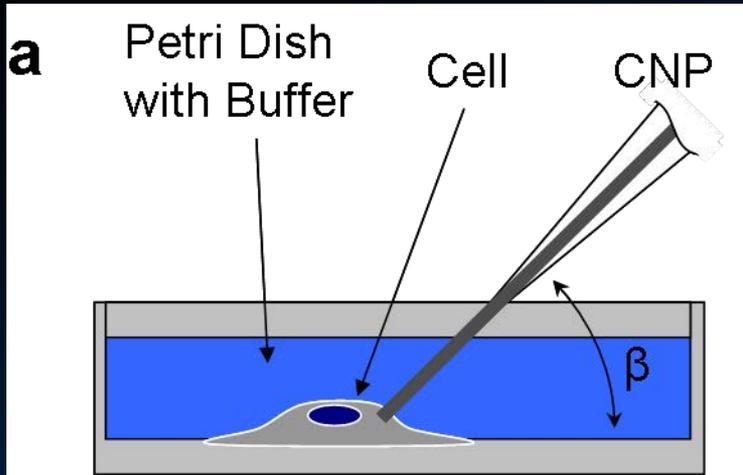
θ =contact angle

a = tube radius

$$\chi = \left(3 + \left(\frac{1 - \cos \theta}{\sin \theta} \right)^2 \right) \left(\frac{1 - \cos \theta}{\sin \theta} \right)$$

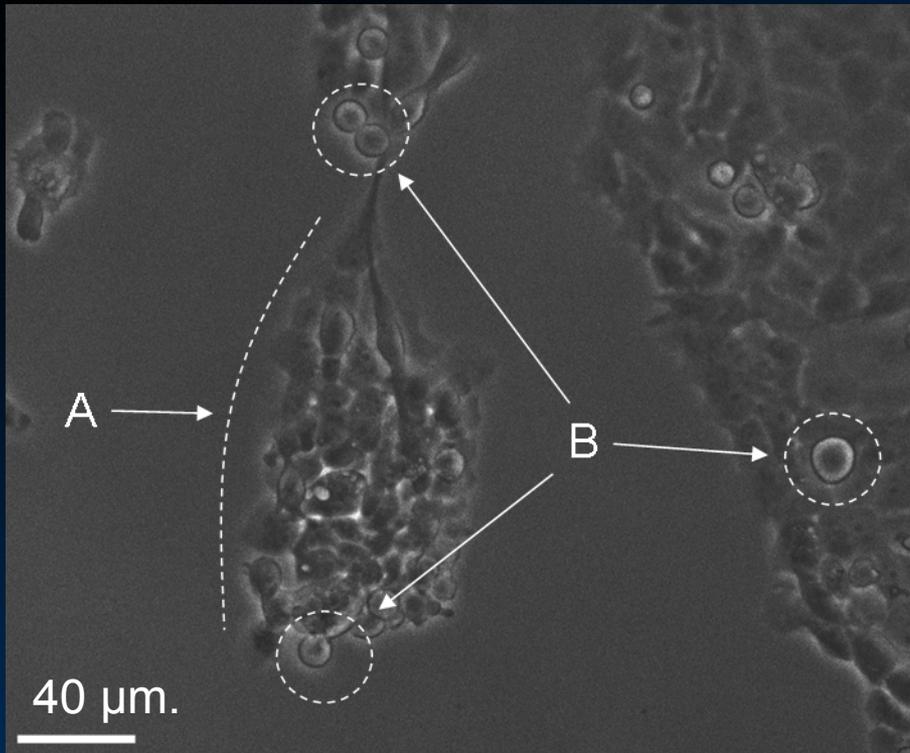


Probing Cells



Probing Cells Under an Inverted Microscope. (a) Schematic depicting a CNP probing a plated adherent cell in a Petri dish. (b) Phase contrast optical image showing a CNP probing an adherent OSCC (10 μ m).

Cell Viability upon Probing with CNPs

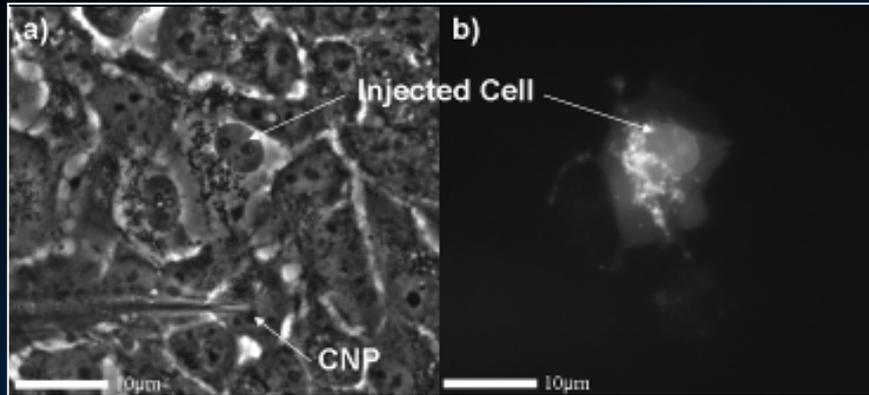


Trypan Blue Exclusion Test

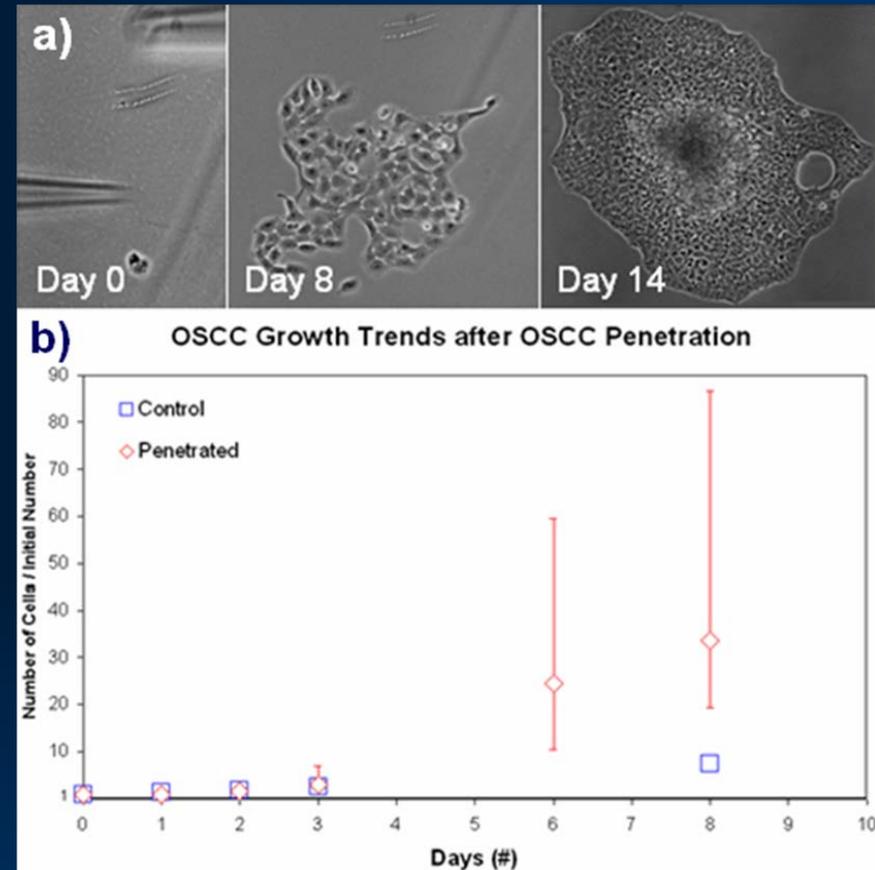
The cells along the edge were probed for short times with CNPs (A, dotted line). When Trypan Blue was added to the extracellular solution, the probed cells remained colorless (viable) while dying or dead cells turned blue (B, dotted circles).

CNPs' Cell Toxicity

Cells Remain Viable After CNP Probing

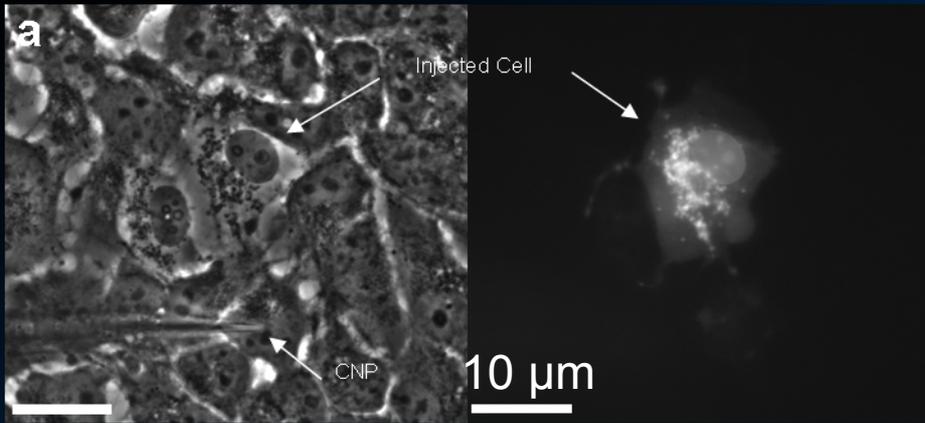


(a) OSCC prior to injection. (b) Same OSCC subsequent to dye injection (observation with fluorescence microscopy)

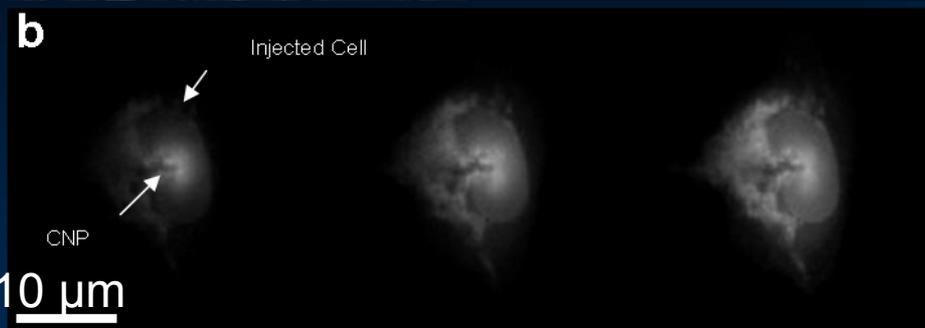


(a) Right to left: proliferation of speared OSCC observed over two weeks. (b) Average normalized number of speared (circle) and un-speared (square) OSCC as a function of time.

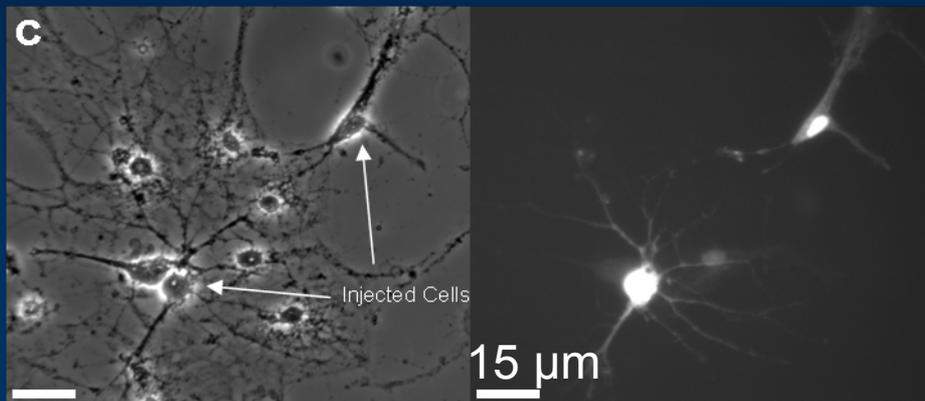
Injection of Fluorescent Dye into Cells



(a) Left to right: OSCC before and after fluorescent dye injection with a CNP.



(b) Left to right: Fluorescent images show an OSCC being injected three times with a CNP. Scale bar, 10 μm.



(c) Left to right: Fluorescent images shows two neuron cells remain viable 1 week after being injected with fluorescent dye by a CNP.

Calcium Signaling: Secondary Messenger Injection

Intracellular Ca^{+2} regulates processes by activating or inhibiting signaling pathways

Short Term

- Secretion
- Contraction
- Synaptic transmission
- Metabolism

Long Term

- Gene expression
- Cell cycles
- Growth
- Division
- Apoptosis

Secondary messengers transduce membrane signals to release calcium from intracellular calcium stores

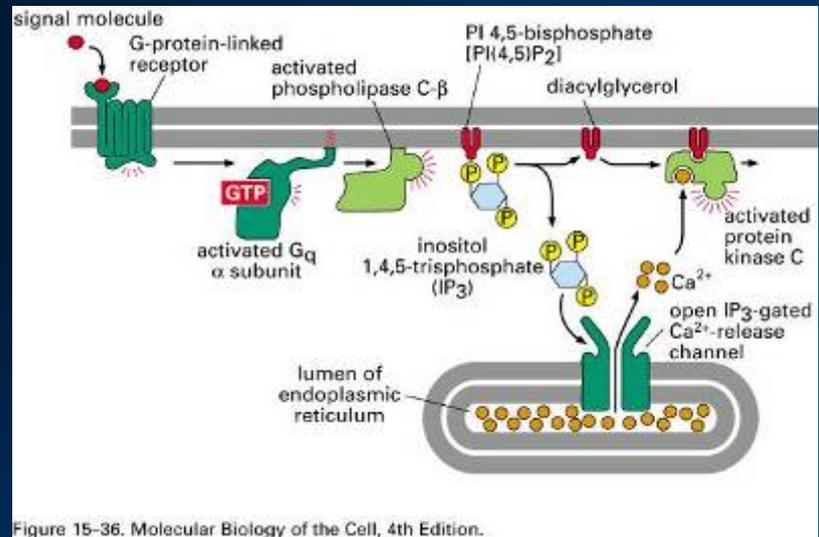
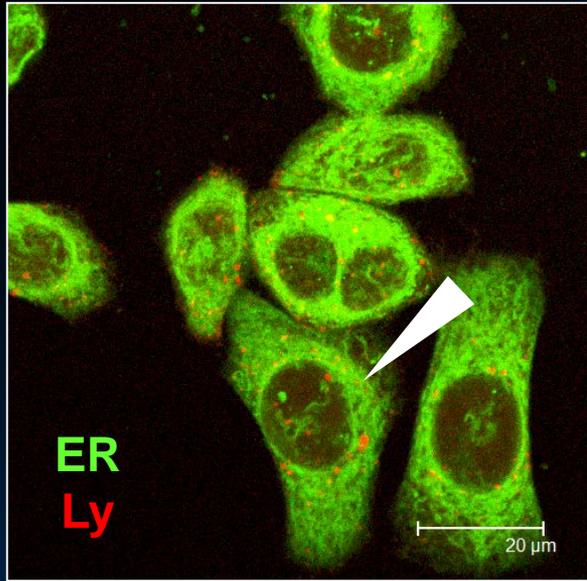


Figure 15-36. Molecular Biology of the Cell, 4th Edition.

Unregulated calcium release implicated in cancer
[Monteith et al, Nat Rev Cancer, 2007]

Second Messenger Injection using CNPs



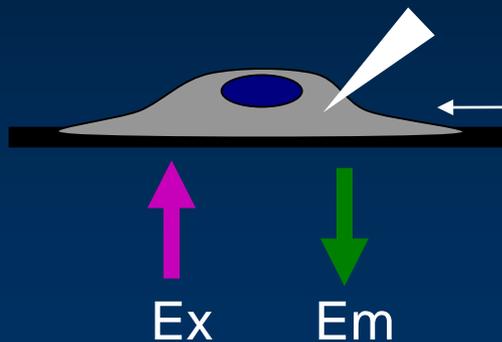
Confocal by Brailoiu GC, Temple

Second Messengers:

- IP_3 – Inositol trisphosphate
- $cADPr$ – Cyclic adenosine diphosphate ribose
- $NAADP$ – Nicotinic acid adenine dinucleotide phosphate

Calcium Stores:

- *Endoplasmic Reticulum* (ER) – sensitive to IP_3 and $cADPr$ (in some cells)
- *Lysosomes* (Ly) – sensitive to $NAADP$ (controversial)

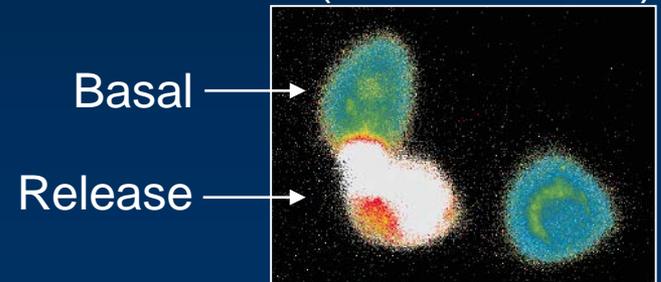


Breast cancer cells (SKBR3) loaded with Fura-2AM

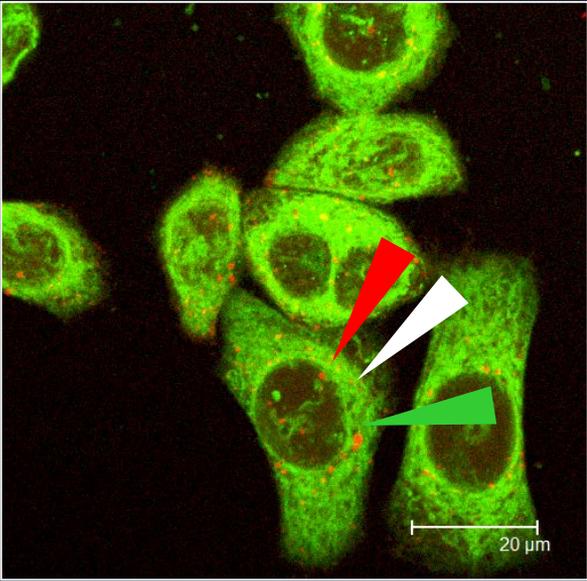
Ex: 340, 380 nm

Em: 540 nm

Fluorescent Images (340nm/380nm)



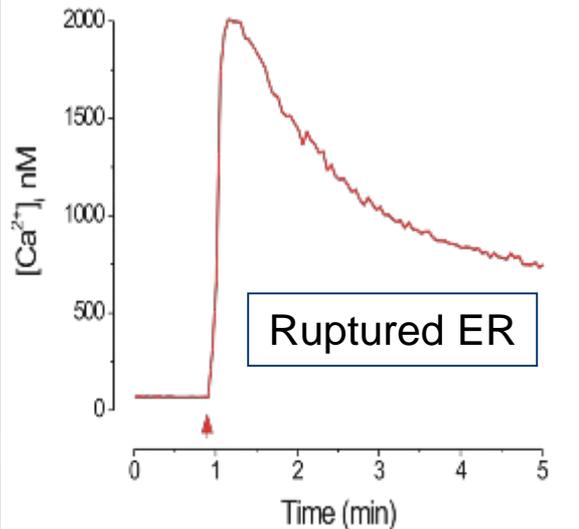
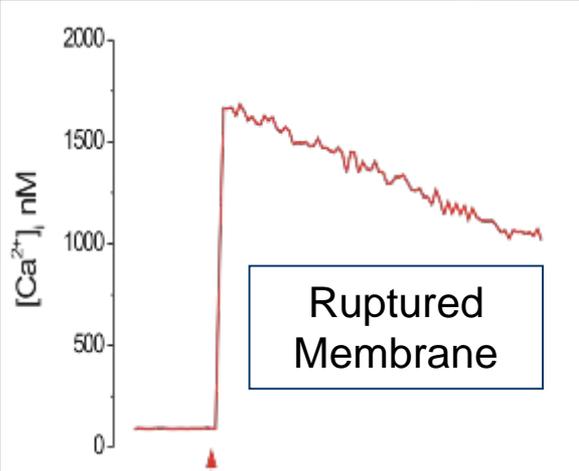
Calcium Signaling via Second Messenger Injection



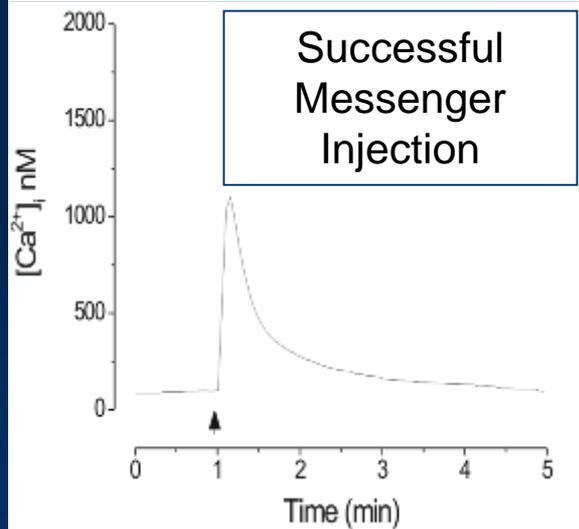
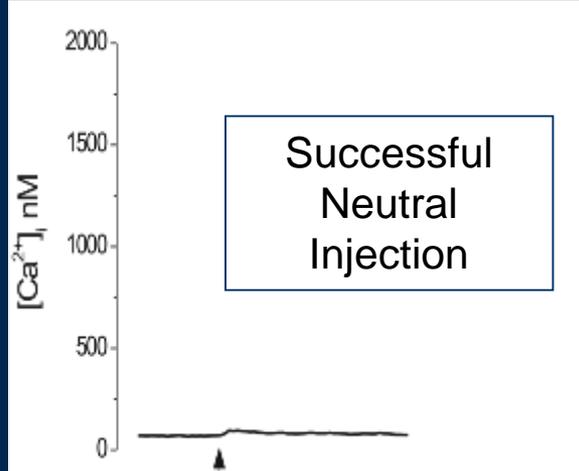
Confocal by Brailoiu GC, Temple

- ✓ Proper cell probing techniques drastically reduce damage to cells and increase injection efficiency

Damaging Injection



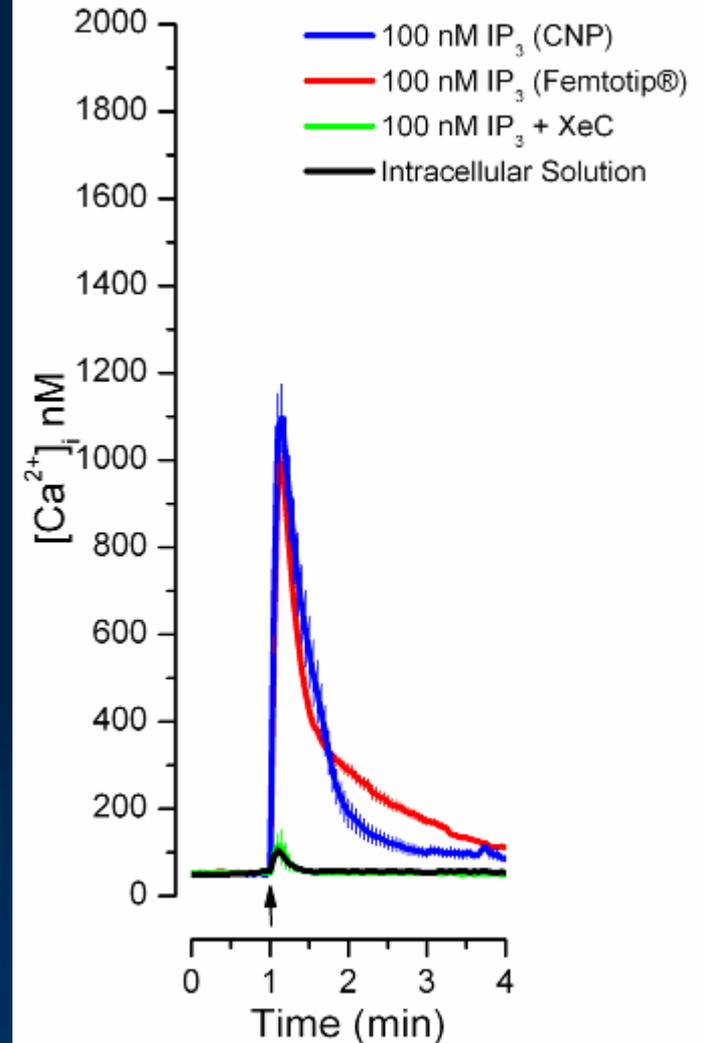
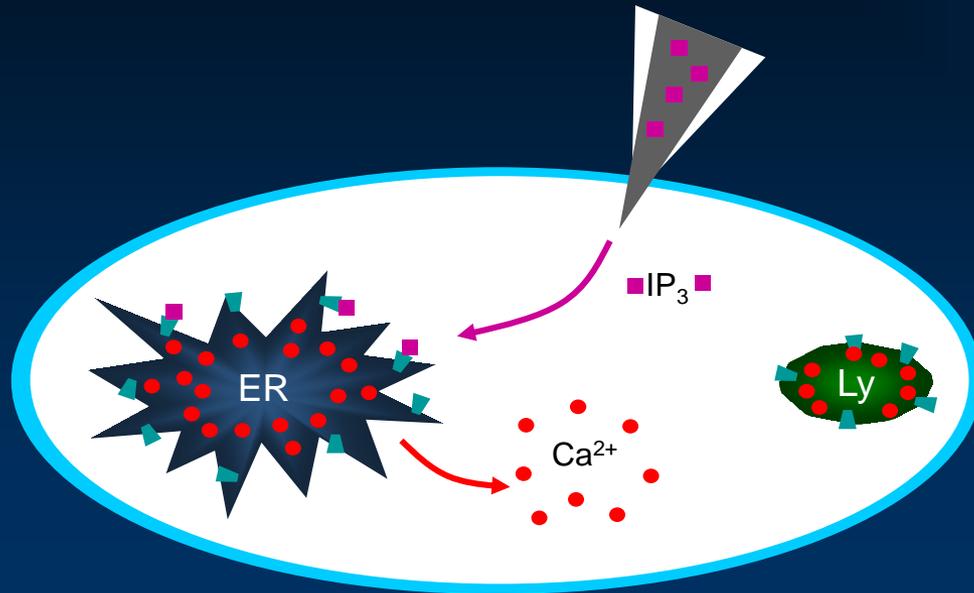
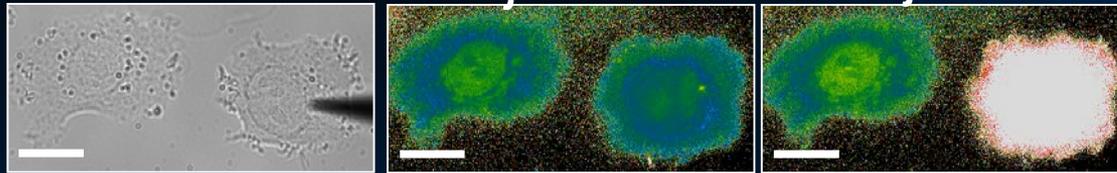
Successful Injection



IP₃-Induced Ca²⁺ Release in Breast Cancer Cells

IP₃ – inositol triphosphate

Targeting Before injection After injection

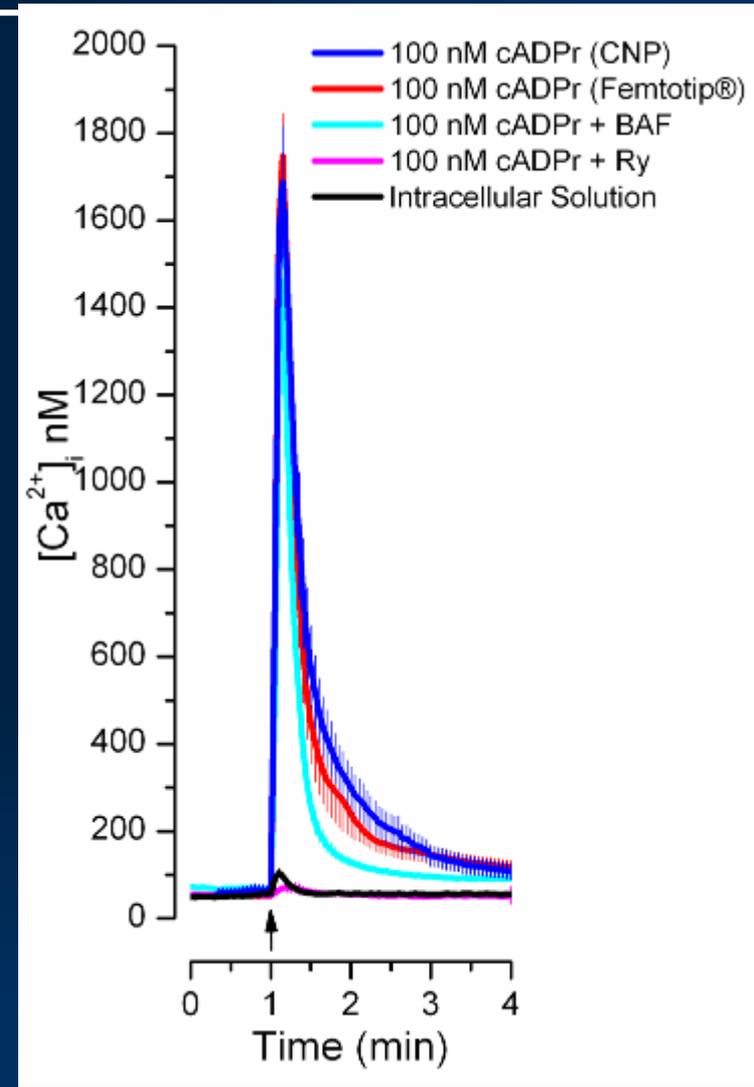
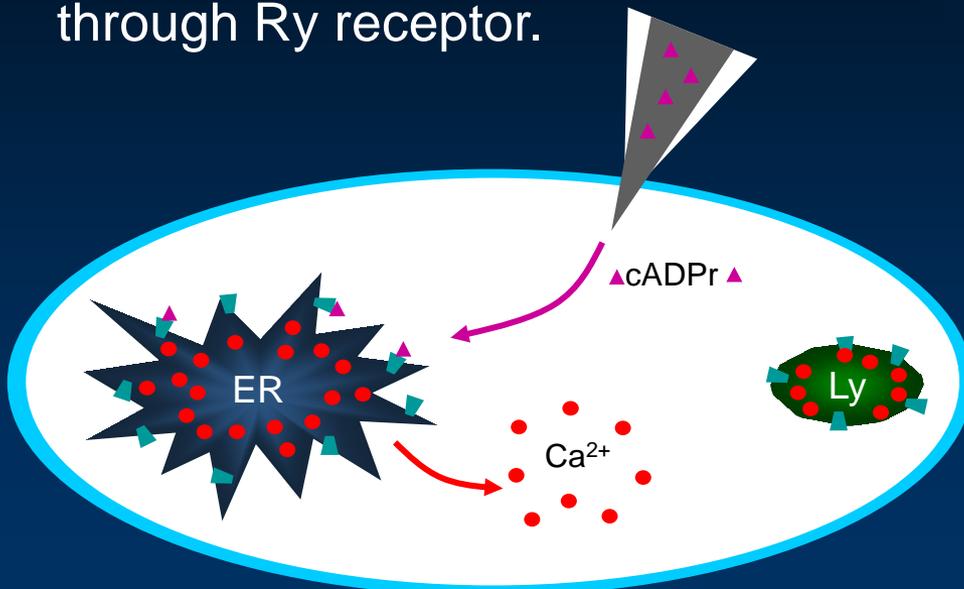


Traces = average 6 cells +/- s.e.m

cADPr-Induced Ca^{2+} Release in Breast Cancer Cells

cADPr – cyclic adenosine diphosphate ribose

- Calcium released by cADPr when acidic calcium stores are depleted.
- No calcium released when Ry receptor is blocked.
- Conclusion \rightarrow ER is sensitive to cADPr through Ry receptor.

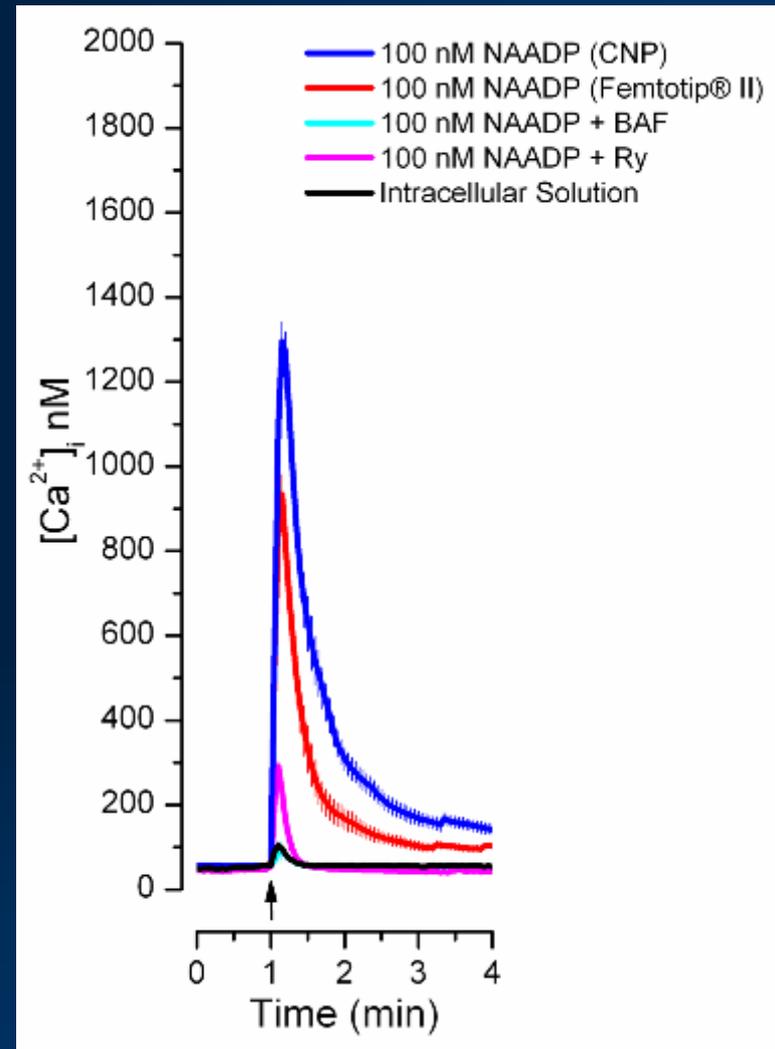
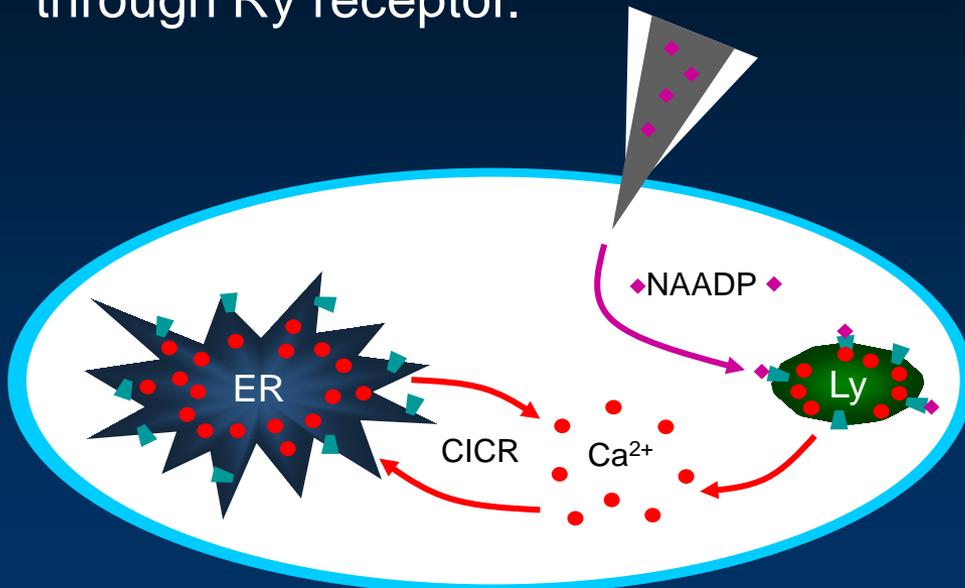


Traces = average 6 cells \pm s.e.m

NAADP-Induced Ca^{2+} Release in Breast Cancer Cells

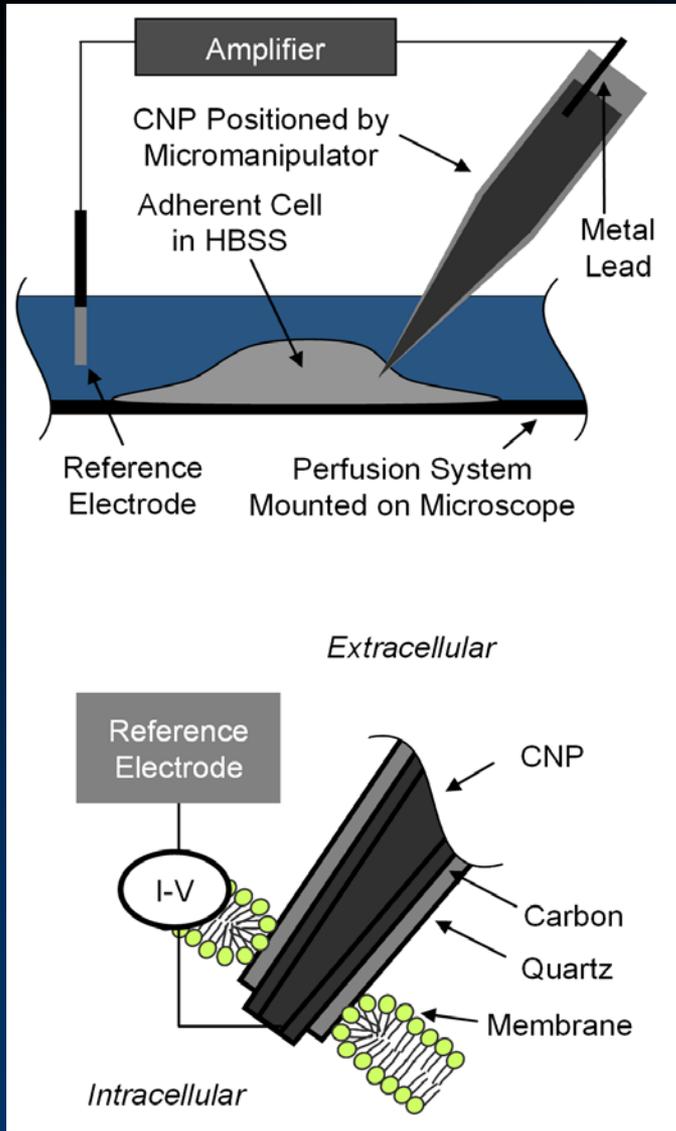
NAADP - nicotinic acid adenine dinucleotide phosphate

- No calcium released when acidic calcium stores are depleted.
- Partial release when Ry receptor is blocked.
- Conclusion \rightarrow Ly is sensitive to NAADP. Calcium-induced calcium release from ER through Ry receptor.

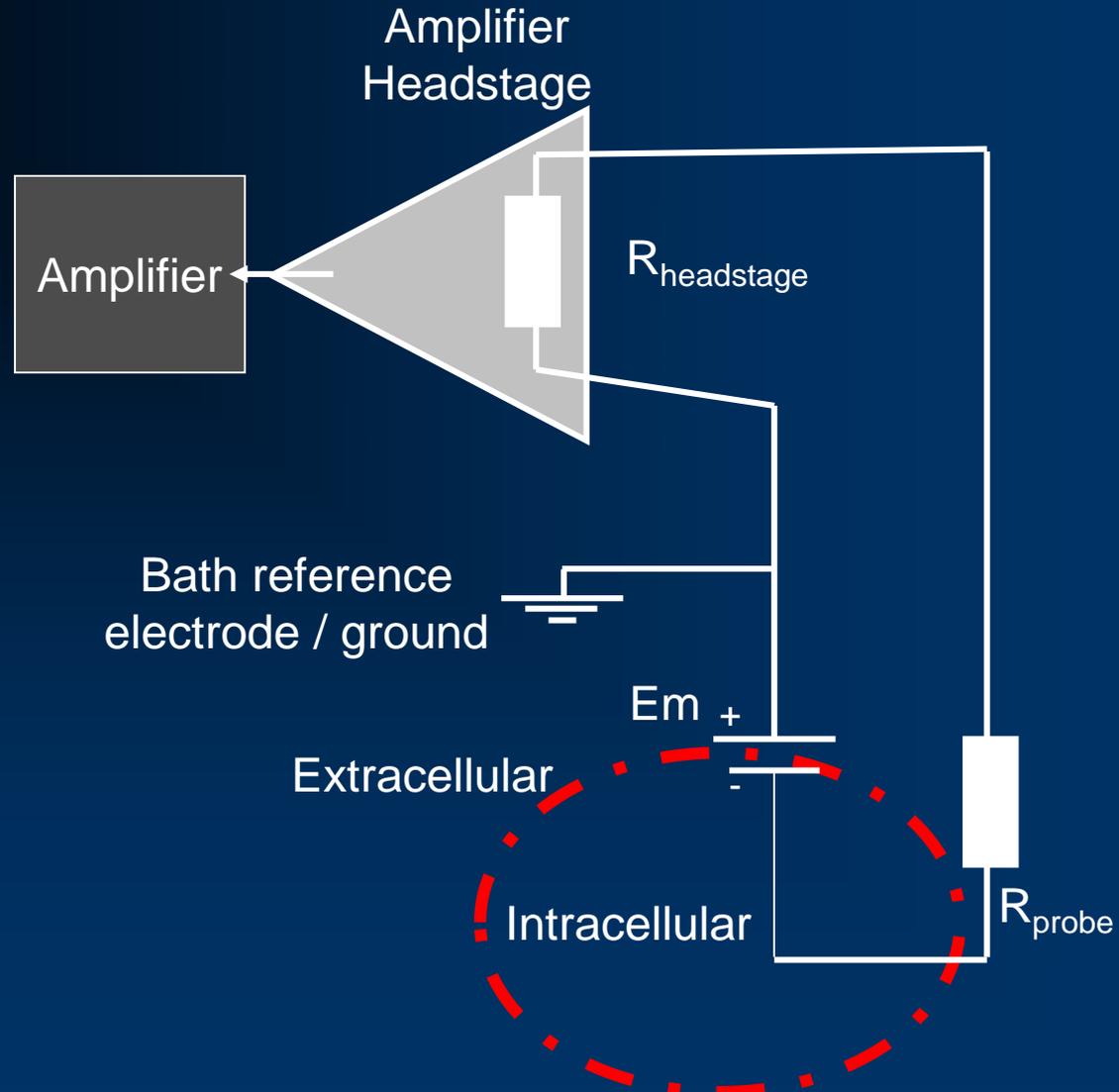


Traces = average 6 cells +/- s.e.m

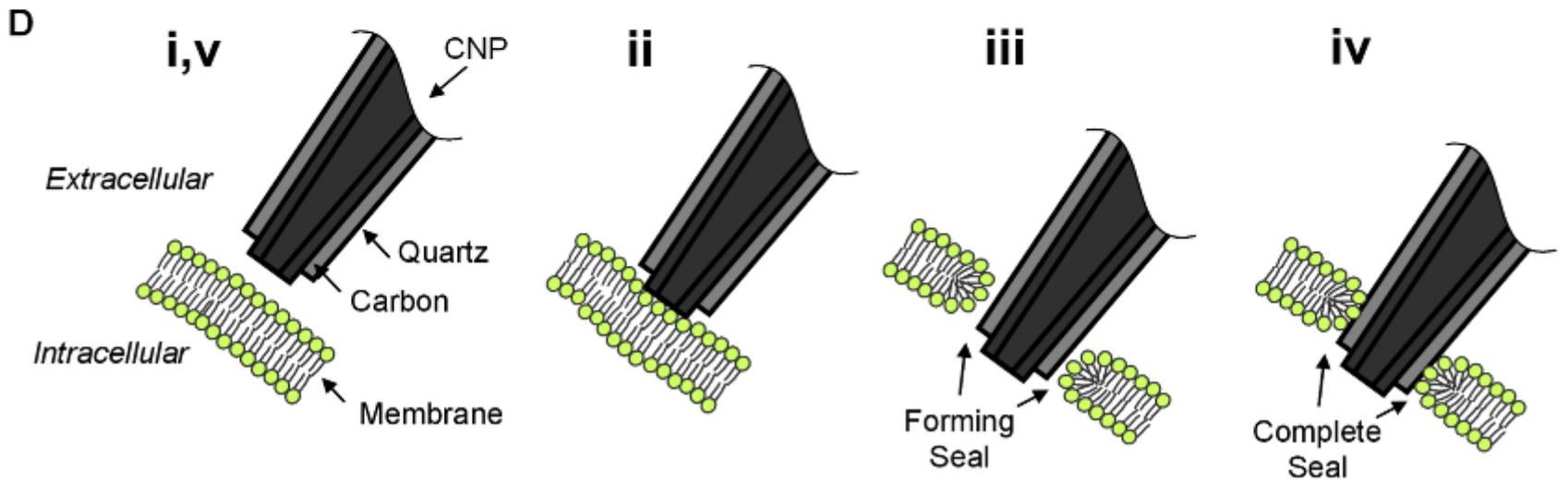
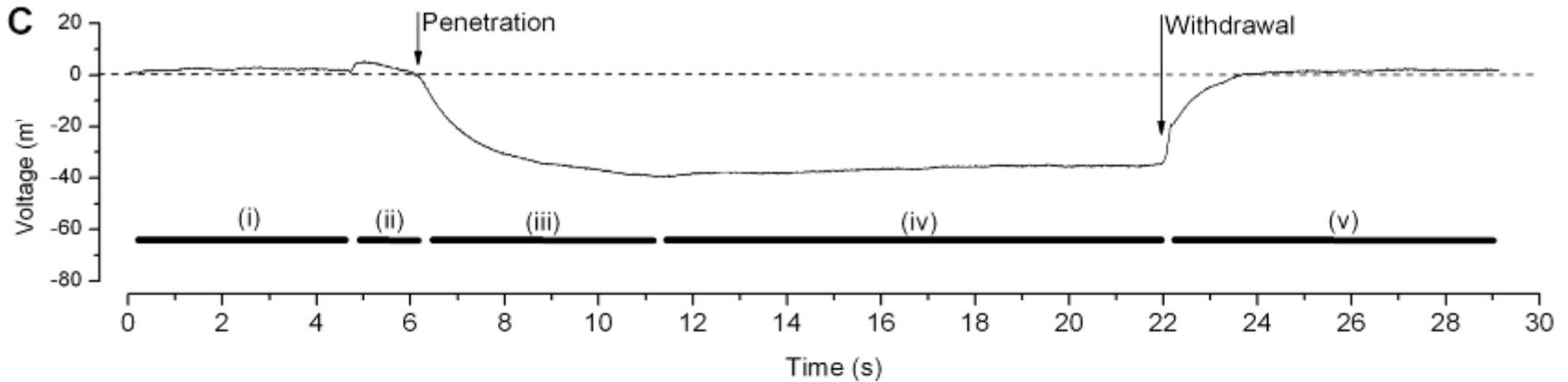
Carbon Nanopipettes for Cell Electrophysiology



Experimental Set-up



Cell Membrane Potential

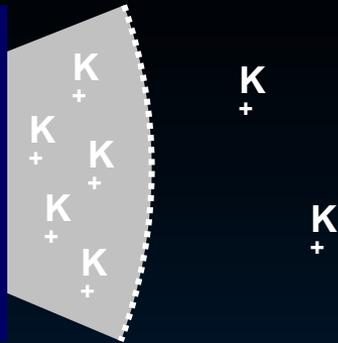


Probing events are electrically recorded with CNPs

Schrlau, M., Dun, N., and Bau, H. H., 2009, Cell Electrophysiology with Carbon Nanopipettes, ACS Nano 3 (3), 563-568

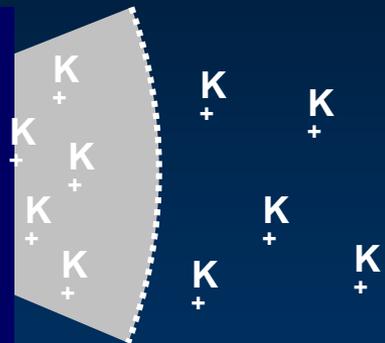
Measuring Cell Response to Chemical Stimuli

Normal



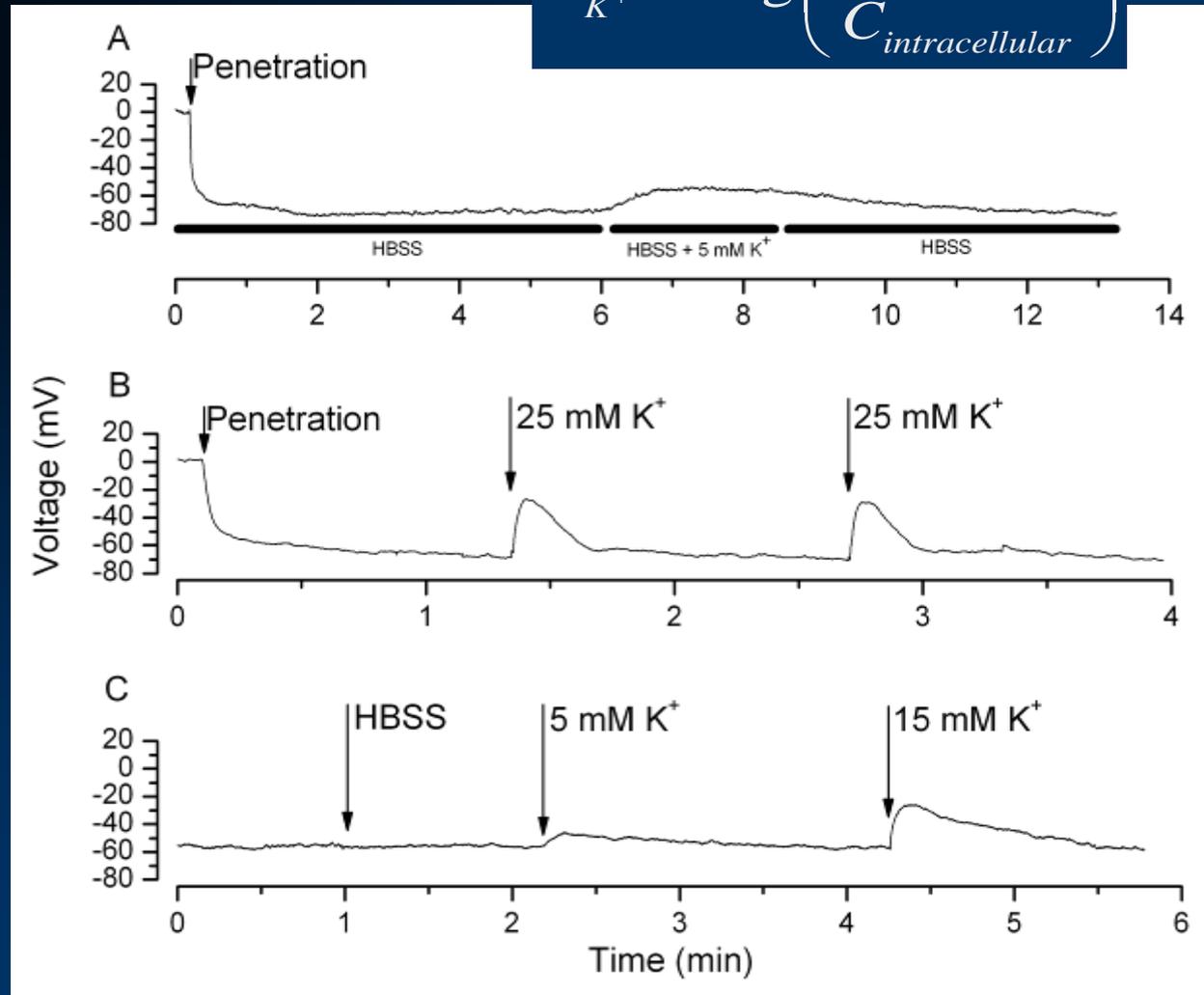
Resting
Membrane
Potential

Increasing $[K^+]_{\text{extracellular}}$

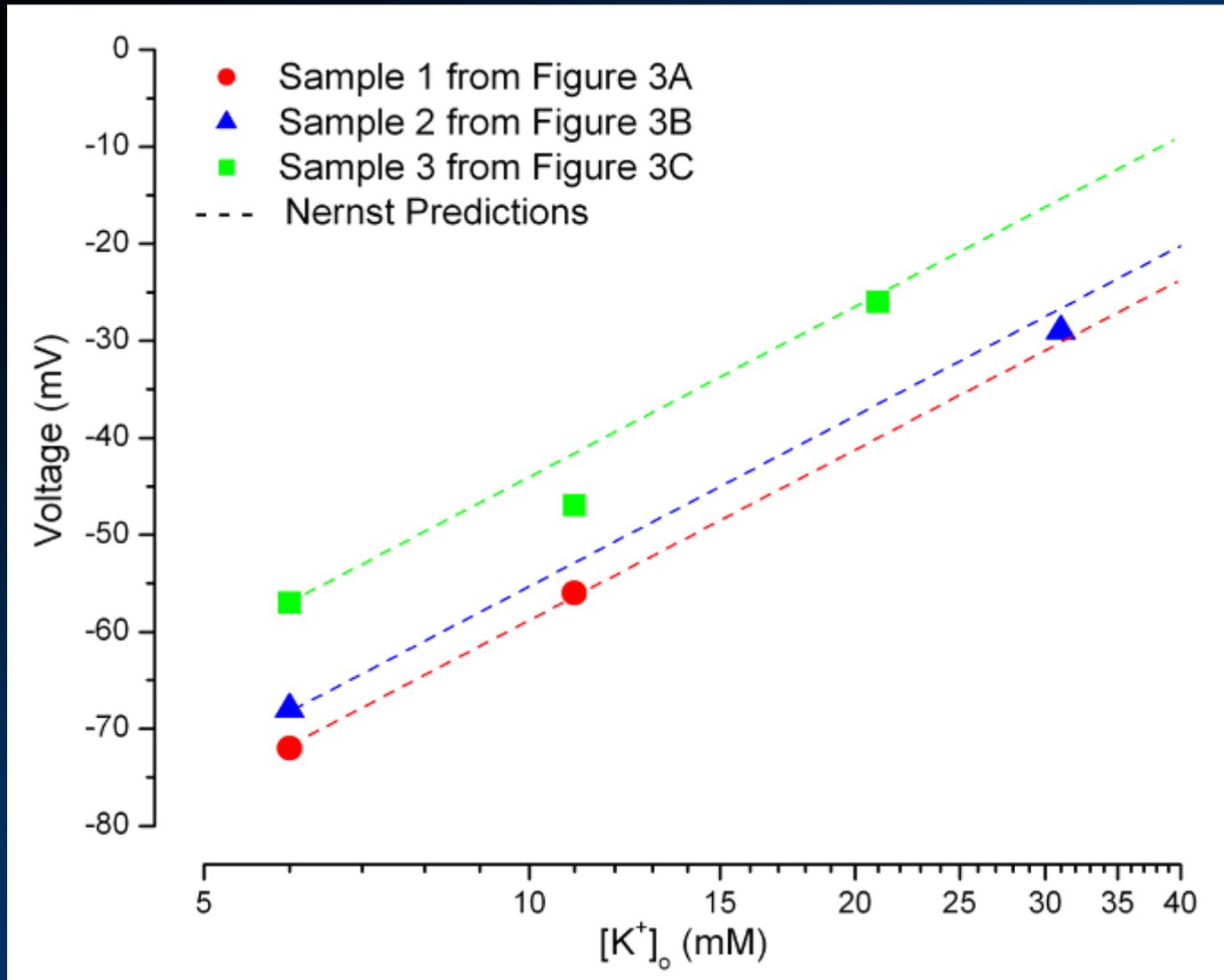


Depolarization

$$E_{K^+} \propto \log \left(\frac{C_{\text{extracellular}}}{C_{\text{intracellular}}} \right)$$

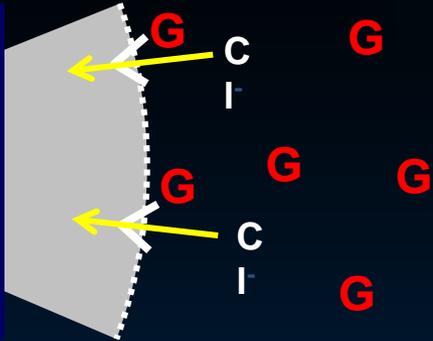


Experimental Results compared with Nernst Predictions



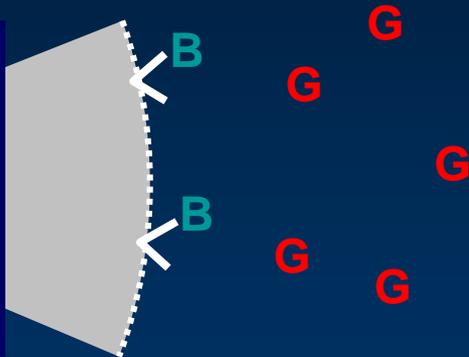
Measuring Response to Pharmacological Stimuli

HBSS then **GABA**

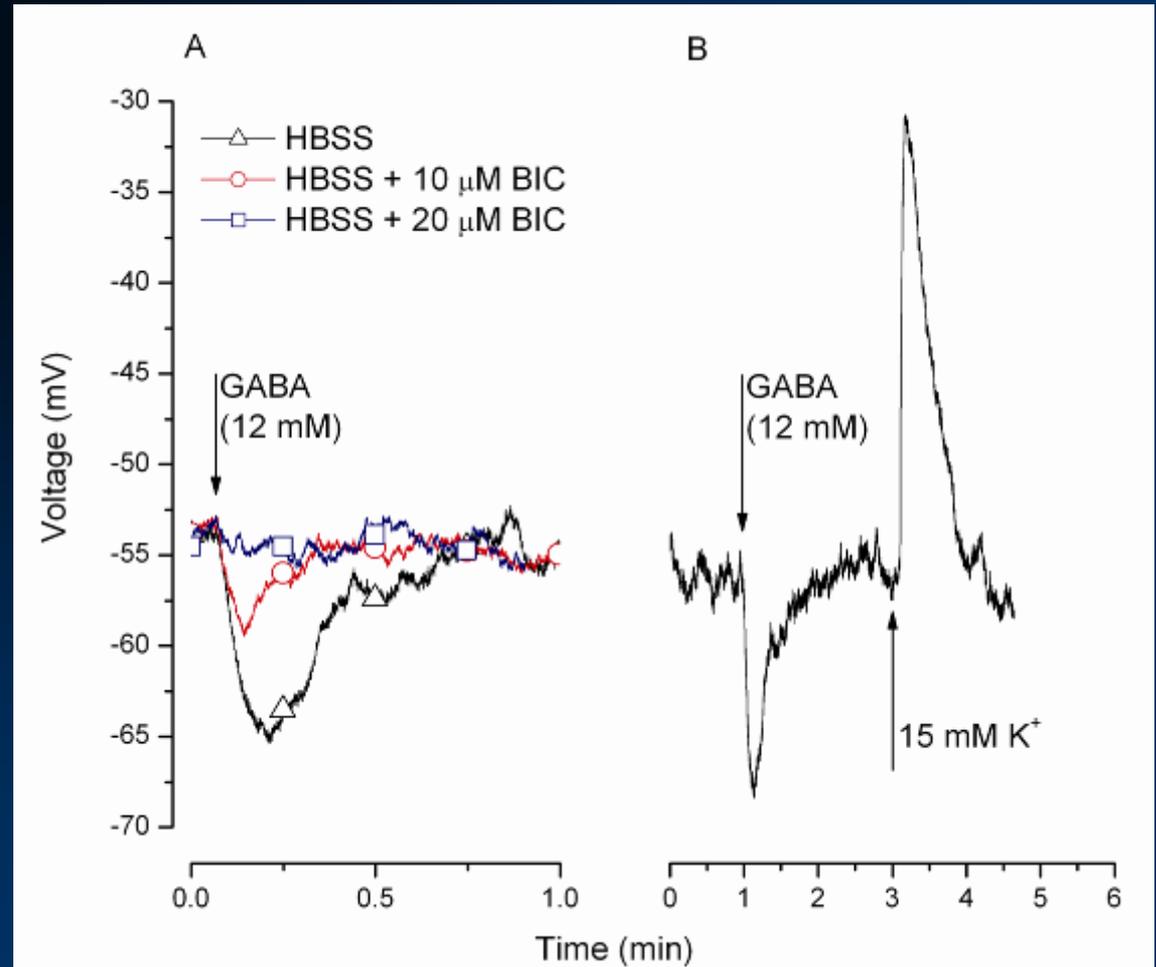


Hyperpolarization

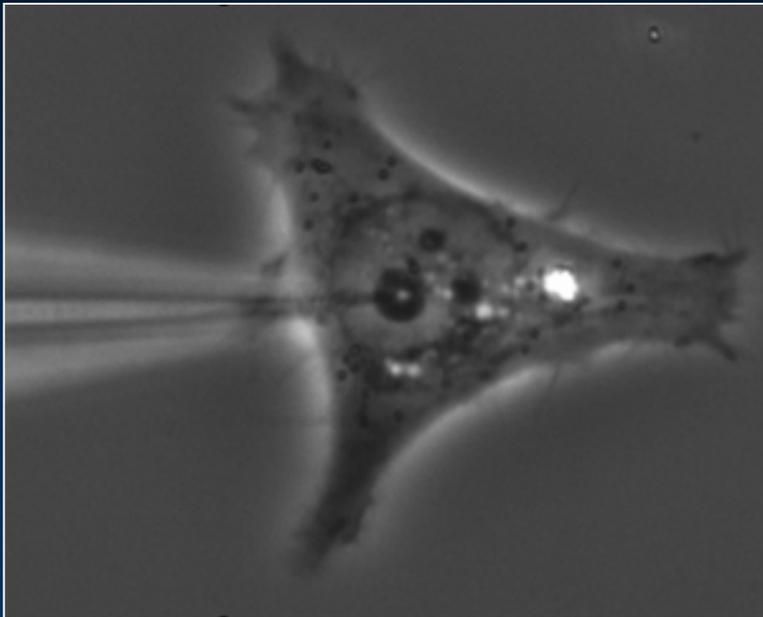
HBSS + **BIC** then **GABA**



No change



Carbon Nanopipettes (CNPs) for automated microinjection & the study subcellular tRNA dynamics



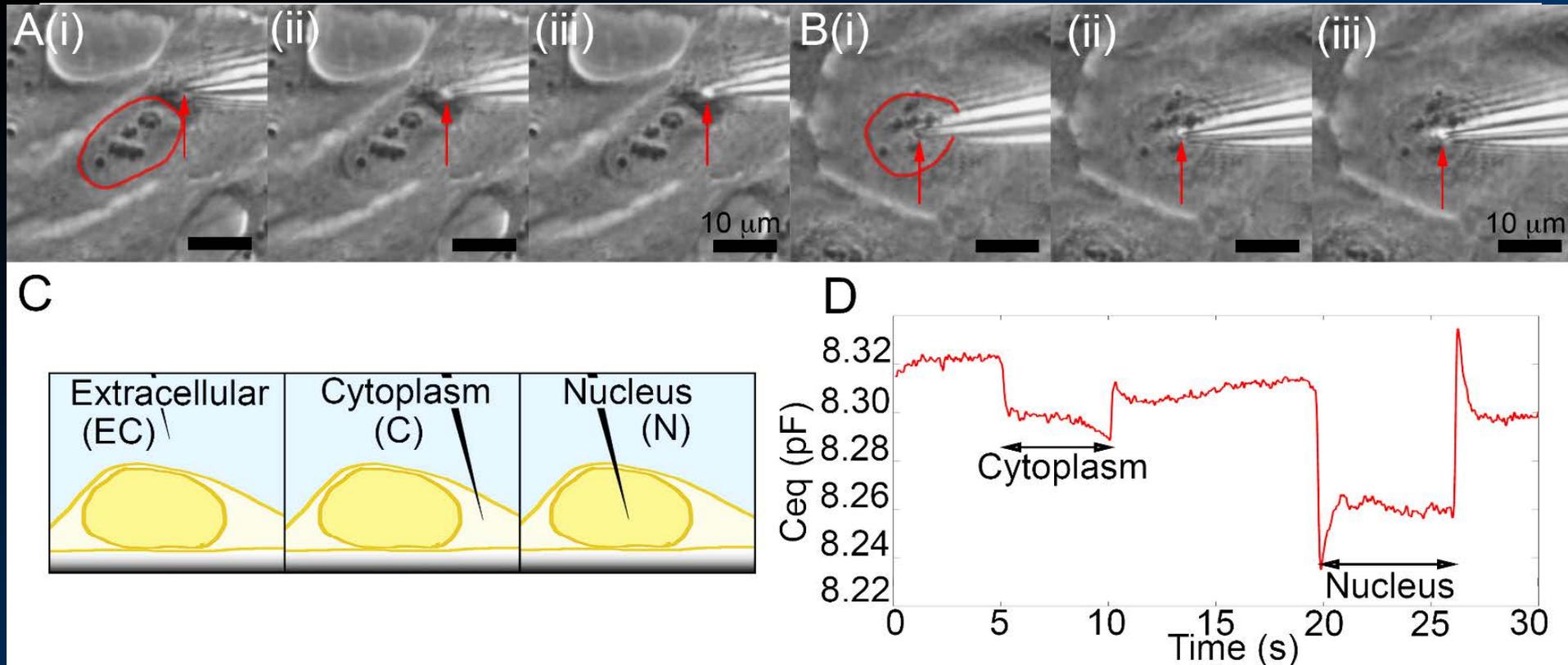
Motivation

Microinjection provides about the only means to controllably introduce reagents with known compositions at a known time into cells to enable dynamic studies of cell functions

Conventional microinjection is a low throughput, tedious process that requires a great amount of skill

Our objectives are to improve injection tools, automate the injection process, and use our system to carry out various studies in cell biology such as alternations in tRNA intracellular distribution, resulting from stressors

Detection of Cell Penetration



Anderson, S., and Bau, H. H., 2014, Electrical Detection of Cellular Penetration during Microinjection with Carbon Nanopipettes, *Nanotechnology* 25, 245102

Anderson, S., and Bau, H. H., 2015, Carbon Nanoelectrodes for Single-Cell Probing, *Nanotechnology* 26, 185101

Upenn semi-automated injection system

The screenshot displays the 'nk2gui2' software interface, titled 'Auto Injection GUI'. The interface is organized into several functional panels:

- Panel A (Camera):** Contains controls for camera operation, including 'Start / Contin.', 'End', and 'Reset' buttons. It also features input fields for 'ExposureTime' (0.01), 'Gain' (8), 'Offset' (74), 'Contrast Gain' (26), and 'ContrastOffset' (130). There are also 'Adjust Type' radio buttons for 'Manual' and 'Auto', and 'Photo' buttons for 'Raw pic save' and 'Pic save (not raw)'. 'Video' settings include 'Raw Record', 'Record', 'Frame' (10 per), and 'Video Time' (30 Sec).
- Panel B:** A large microscopy image showing a field of cells, with several small white markers indicating the desired injection points.
- Panel C (Injection):** Contains parameters for the injection process: 'save current location' button, 'Target Ref Z position' (-29120 ms OR -1138 um), 'Inject angle (cnp and z-plane)' (45, >3 deg), 'Injection speed (z-v) (um/s)' (5), 'Move Speed (x,y,z) (um/s)' (100), and 'pull back delay (s)' (0.5). It also includes 'Units' (um selected, micro step), 'Injection' and '3 Point Inject' buttons, and a note: 'Injection setting is based on left Panel'.
- Panel D (Femtojet Control):** Features an 'Update Setting from Device' button, 'Compensation P (hPa)' (10), 'Time (0.1s)' (1), 'Inject P (hPa)' (50), and a 'Clean' button. It also has a '5 (0.1s)' button and a scale from 15 to 500.
- Panel E:** Includes an 'Info. Board' dropdown menu, a 'Parameters & Settings' button, and a note: '1 um ~ 25.6 microsteps' and 'lower value of Z means farther away from substrate'.

Operator marks desired injection points on the computer screen. The system calculates an optimal path for the injection probe

Computation of Microinjector's Trajectory

The screenshot displays the 'nk2gui2' software interface, which is used for controlling a microinjector. The main window is titled 'Auto Injection GUI' and contains several panels and controls:

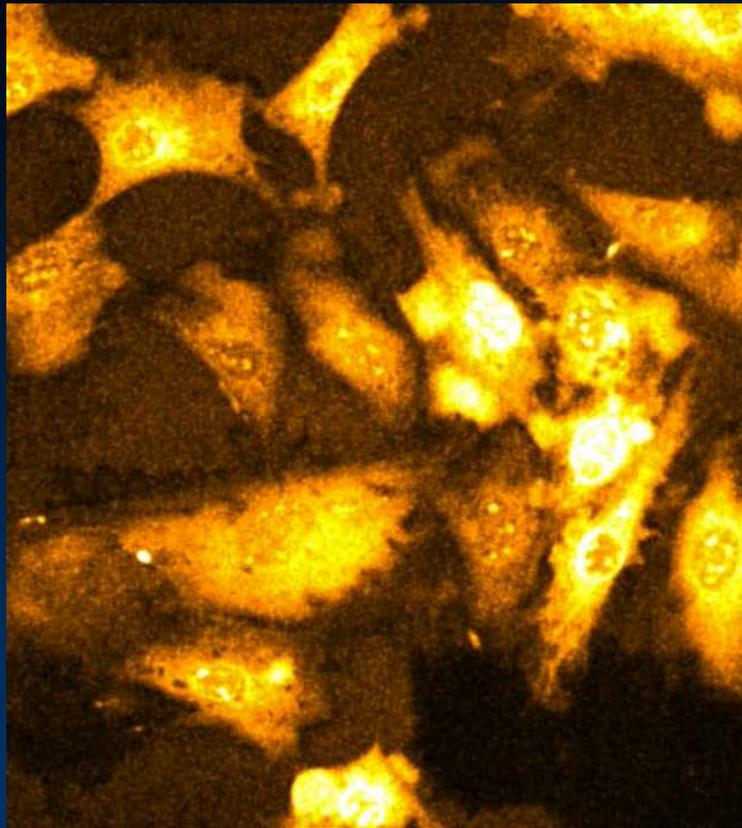
- Camera Panel:** Includes a 'Start / Continue' button and a 'Gain 50' control.
- Sub-GUI Window:** A smaller window overlaid on the main interface, titled 'Sub-GUI Window'. It features a central grayscale image of a cell. Below the image are several numerical controls: 'ExposureTime' (0.05), 'Gain 50', 'Offset 30', 'Contrast Gain 255', and 'Contrast Offset 130'. The window has tabs for 'Calibration', 'Inject(um) Selection', 'Inject(ms) Selection', and 'Mouse Injection'.
- Photo Panel:** Contains buttons for 'Raw pic save' and 'Pic save (not raw)'.
- Video Panel:** Includes 'Raw Record' and 'Record' buttons, along with 'Frame' (10 per Sec) and 'Video Time' (30 Sec) controls.
- Injection Parameters:** A section with a 'save current location' button and input fields for '262437' (ms OR) and '10250' (um). Below these are fields for 'z-plane' (45), 'v (um/s)' (100), 'v (um/s)' (100), and 'v (s)' (0.5). A note states: 'Due to the feedback error the larger current position get more accuracy injection'. An 'injection' button is located below these fields.
- Graphic Injection Panel:** Contains a 'Z-location and injection angle based on left Panel' section and a 'Magnification' control set to '40'. An 'Inject' button is at the bottom.
- Info Broad:** A yellow highlighted area with a dropdown arrow.
- Parameters & Settings:** A button at the bottom of the main interface.
- Footer:** A text box at the bottom right stating '1 um ~ 25.6 microsteps' and 'lower value of Z means farther away from substrate'.

Semi-automated Injection with Electrical Feedback

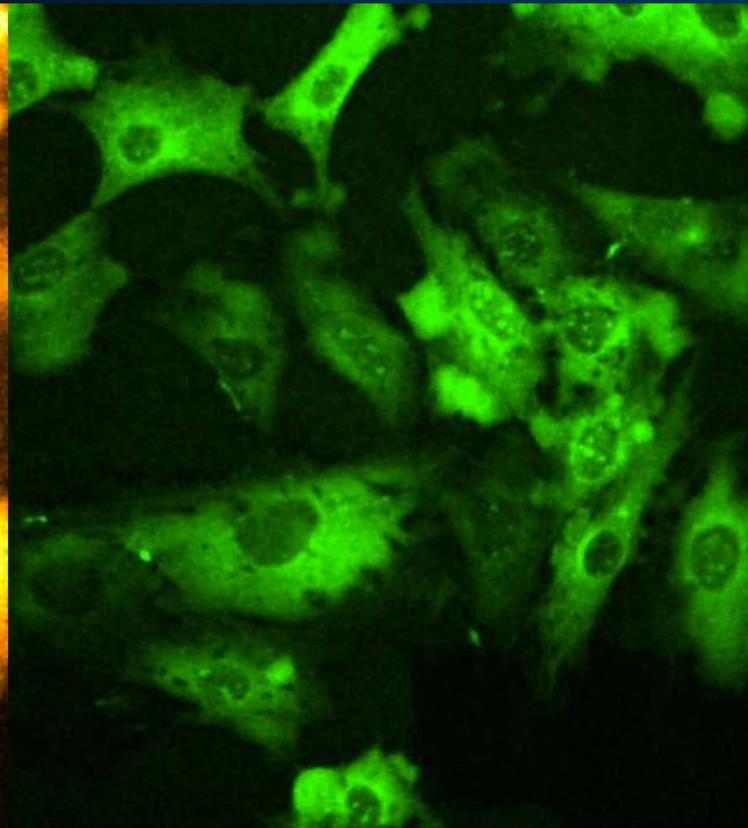


tRNA – more than protein synthesis

Stressor-Induced tRNA translocation Amino Acid Deprivation

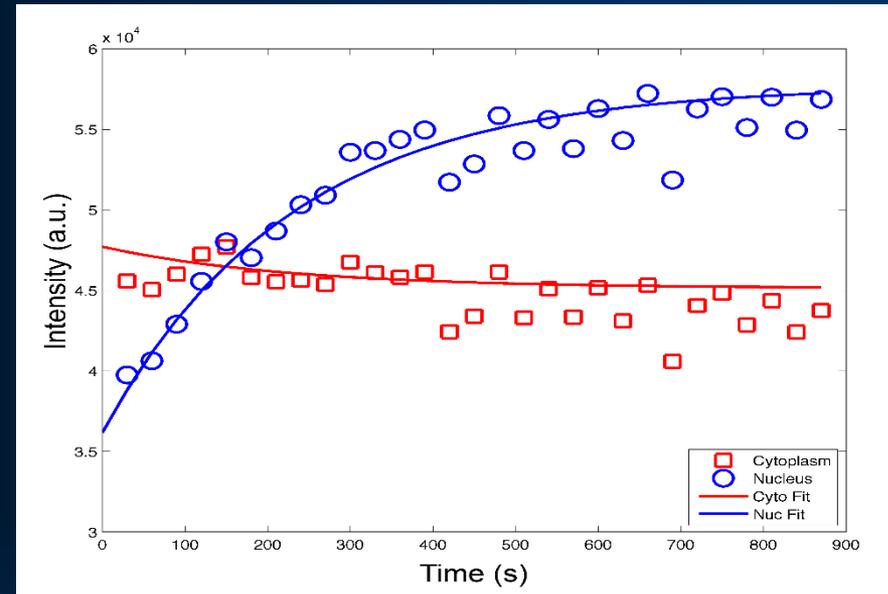
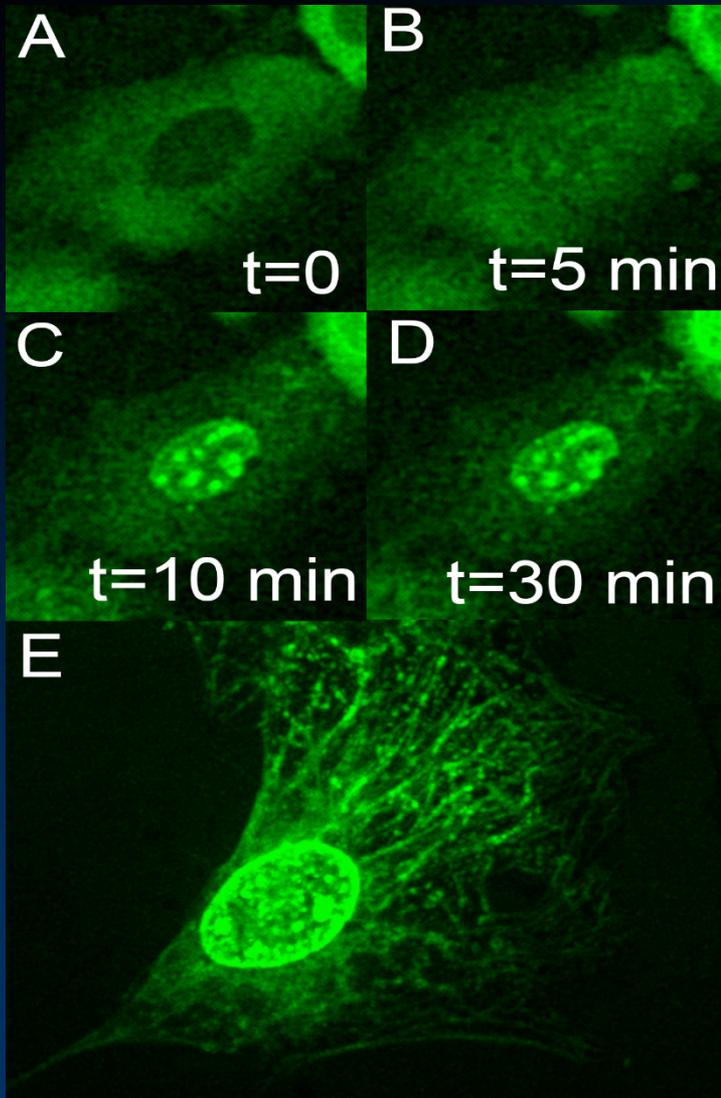


Cy3-labeled bulk tRNA



Rhodamine-labeled bulk tRNA

tRNA Trafficking Dynamics – Amino Acid Deprivation



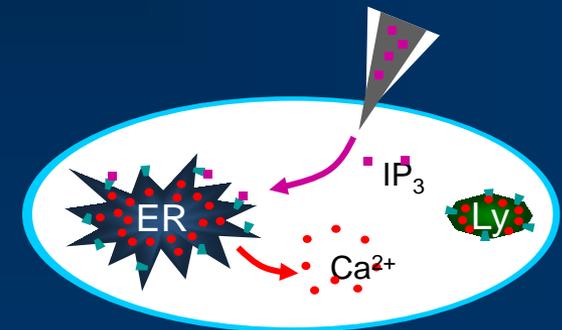
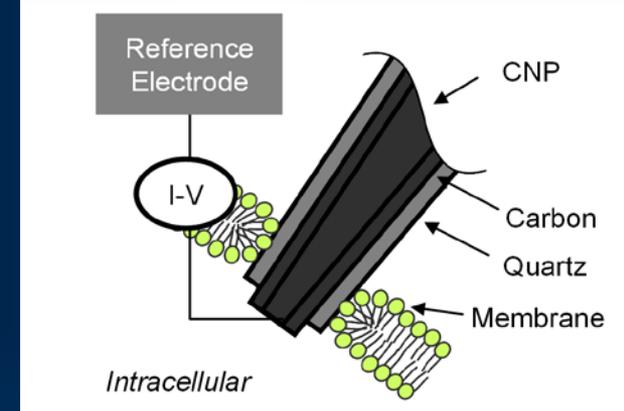
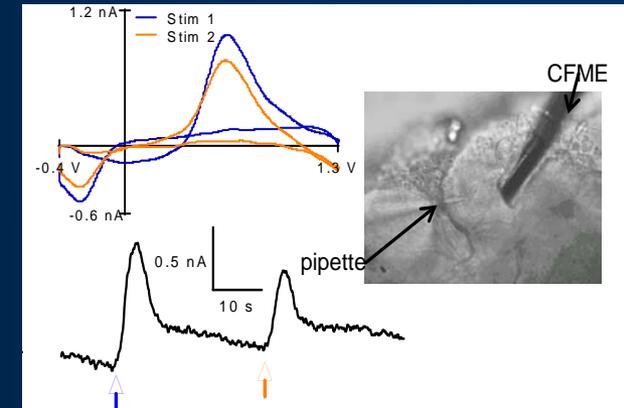
$$V_N \frac{dC_N}{dt} = AK_C C_C - AK_N C_N.$$

$$V_C \frac{dC_C}{dt} = AK_N C_N - AK_C C_C.$$

$$\frac{C_N(t)}{C_C(t)} = \frac{(1 - e^{-(k_N + k_C \varphi)t})}{\left(\frac{k_N}{k_C} + \varphi e^{-(k_N + k_C \varphi)t}\right)}$$

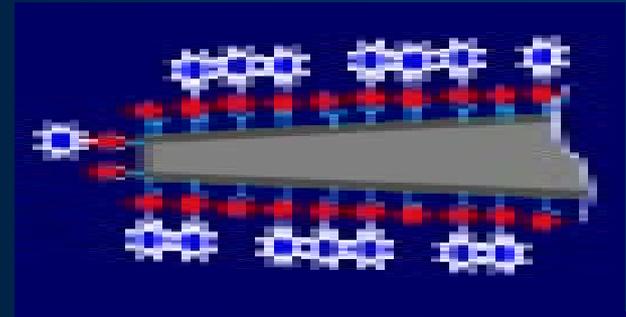
Other Applications of CNPs

- Monitoring neurotransmitters concentrations in the *Drosophila* Brain.
- H. R. Rees, S. E. Anderson, E. Privman, H. H. Bau, & B. J. Venton, 2015, Carbon nanopipette electrodes for dopamine detection in *Drosophila*. *Analytical Chemistry* 87 (7), 3849-3855
- Monitoring function of ion channel blockers through cell electrophysiology
- Schlau, M., Dun, N., and Bau, H. H., 2009, Cell Electrophysiology with Carbon Nanopipettes, *ACS Nano* 3 (3), 563-568
- Studying the role of secondary messengers in calcium release in the cell
- Schlau M, Brailoiu, E., Patel, S., Gogotsi, Y., Dun, N., and Bau, H. H., 2008, Carbon Nanopipettes Characterize Calcium Release Pathways in Breast Cancer Cells, *Nanotechnology* 19, 325102

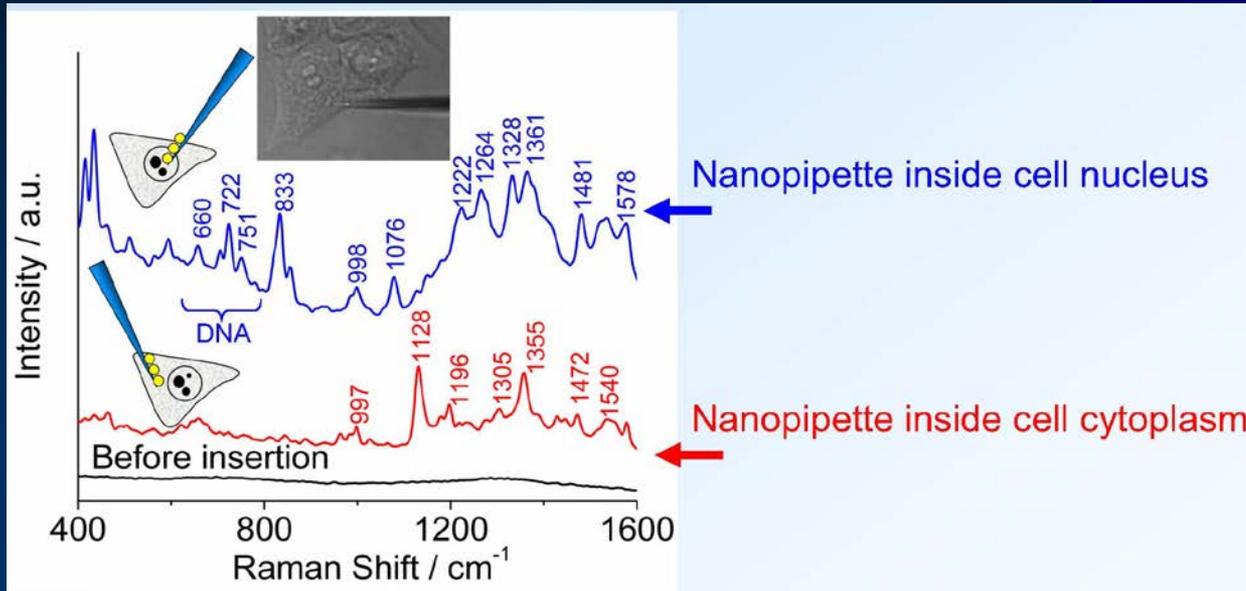
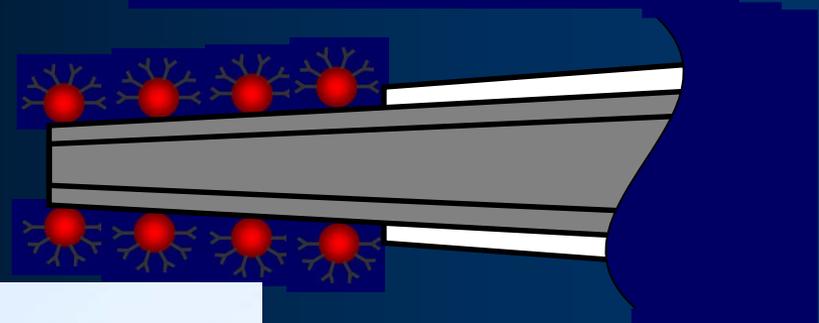


Biosensors

Direct functionalization of the CNP's tip



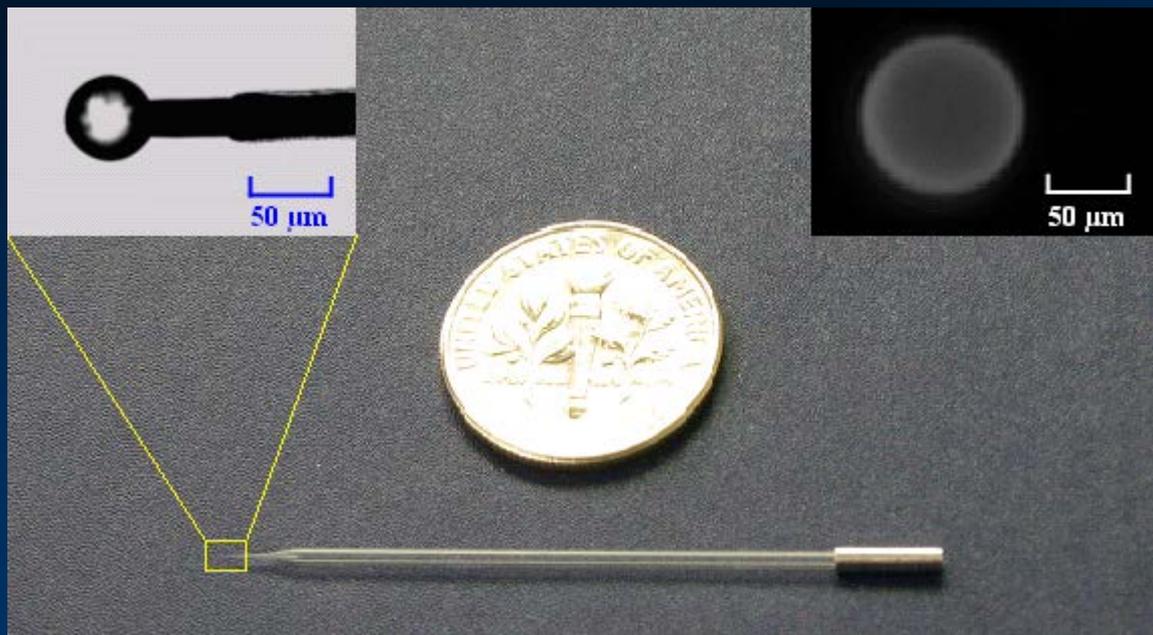
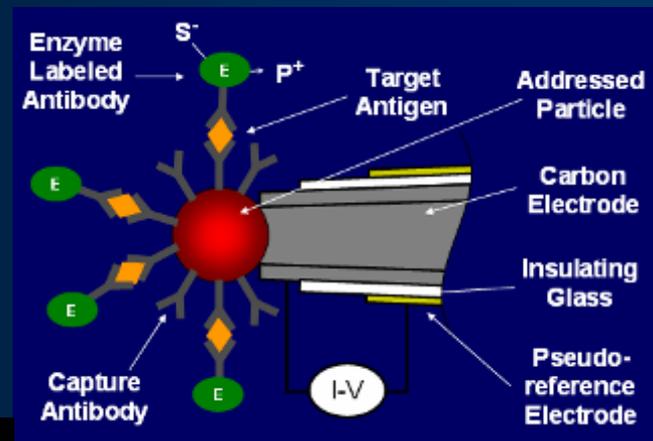
Attachment of nanoparticles to the CNP tip



Raman Shift inside cells
(Gogotsi Group
Drexel)

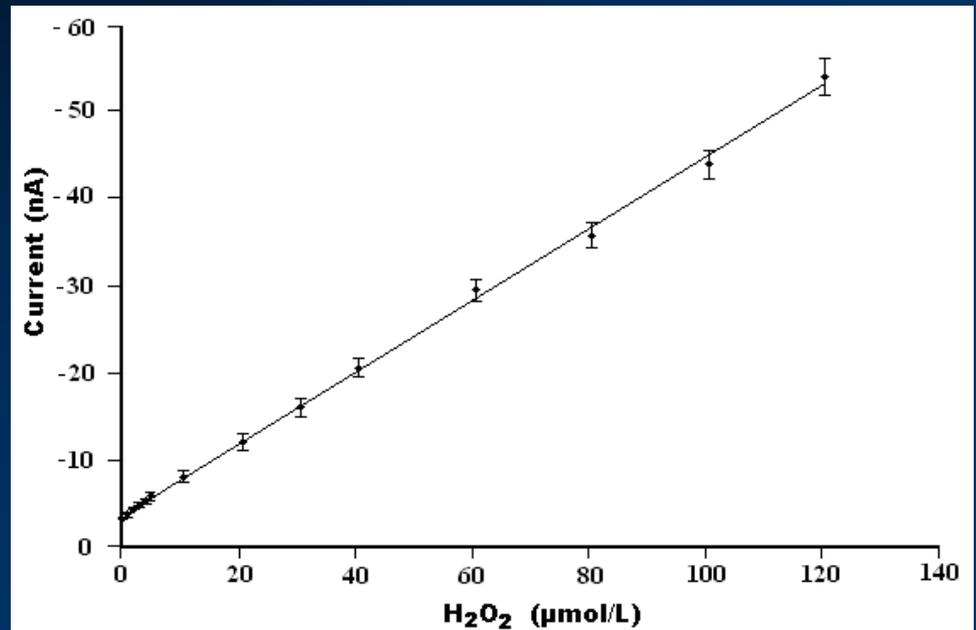
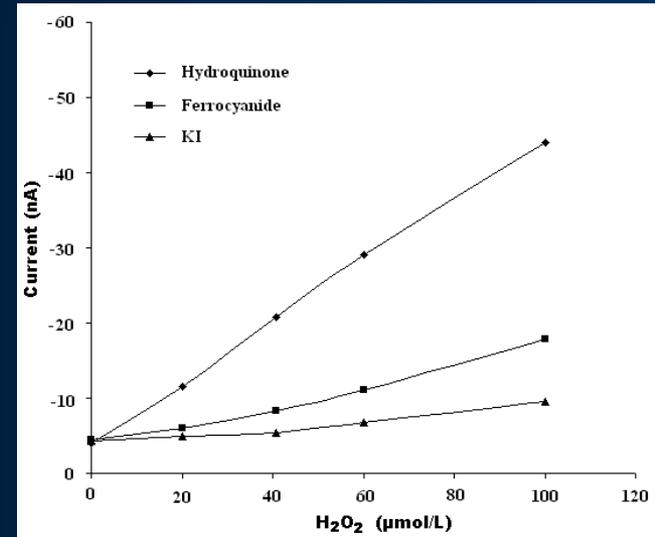
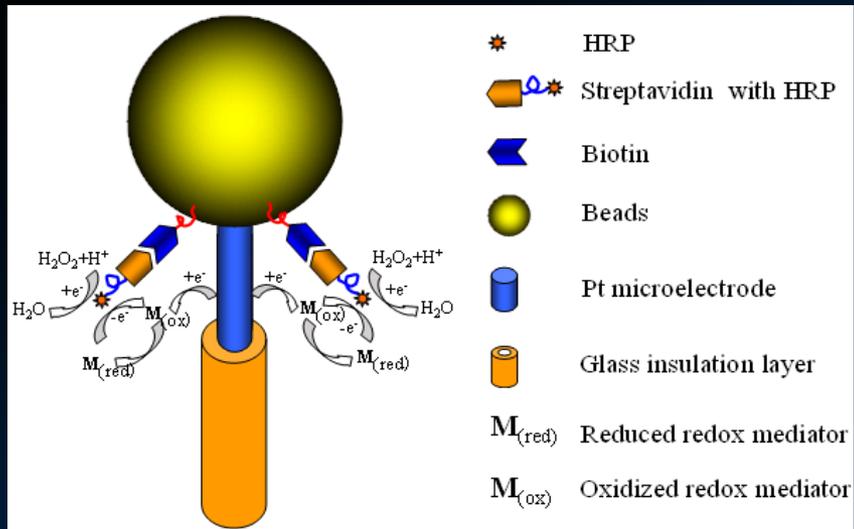
Single Bead-Based Electrochemical Sensor

Attachment of a single functionalized bead

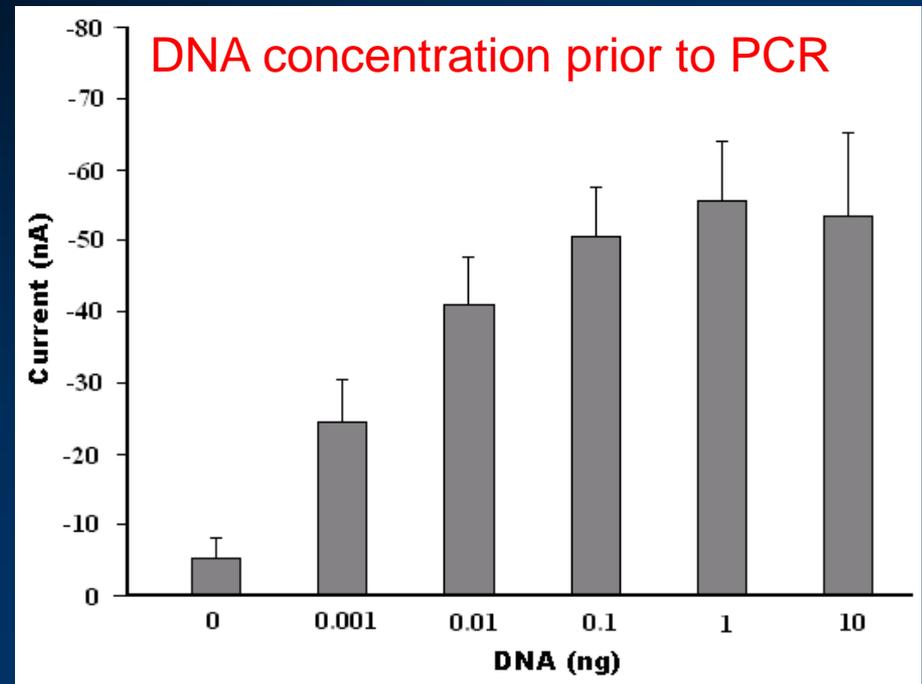
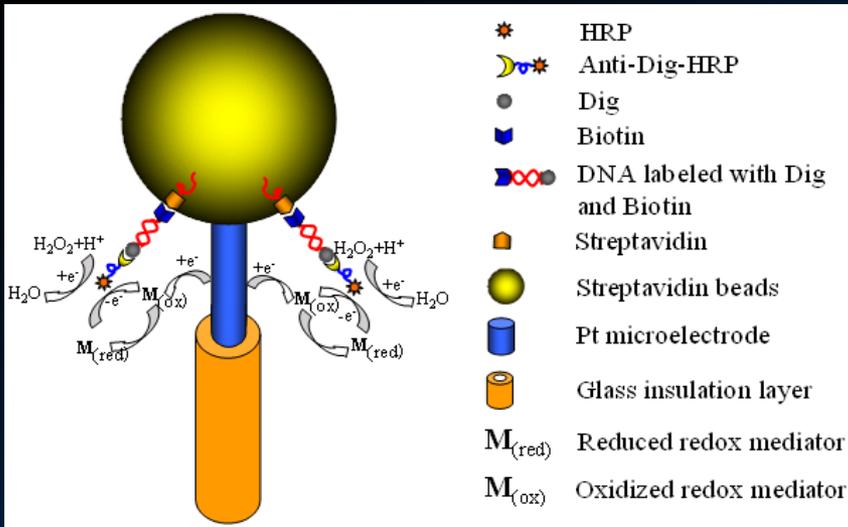


Liu, C., Schlau, M., Bau, H. H., 2009, Single bead-based electrochemical biosensor, to appear in Biosensors and Bioelectronics.

Detection of H_2O_2

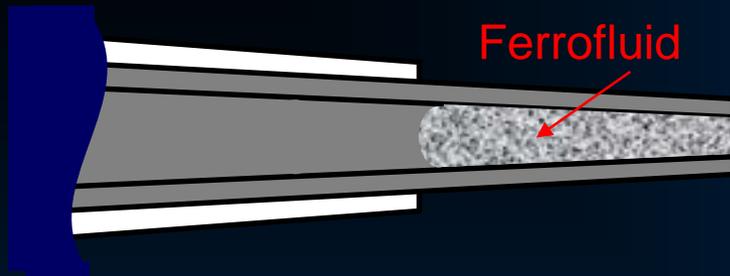


Detection of DNA

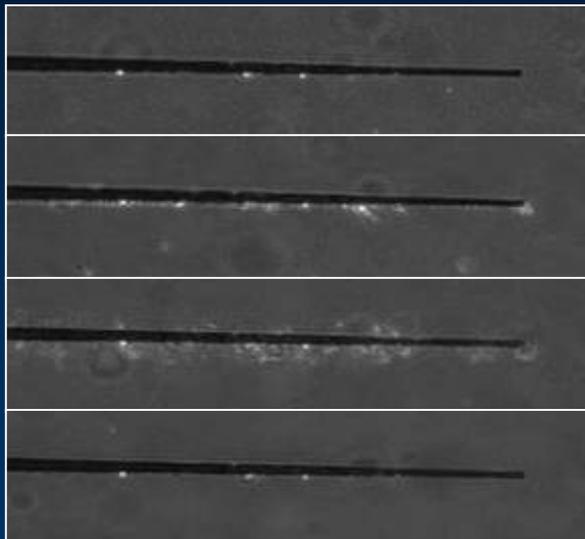


CNPs as Magnetic Manipulators

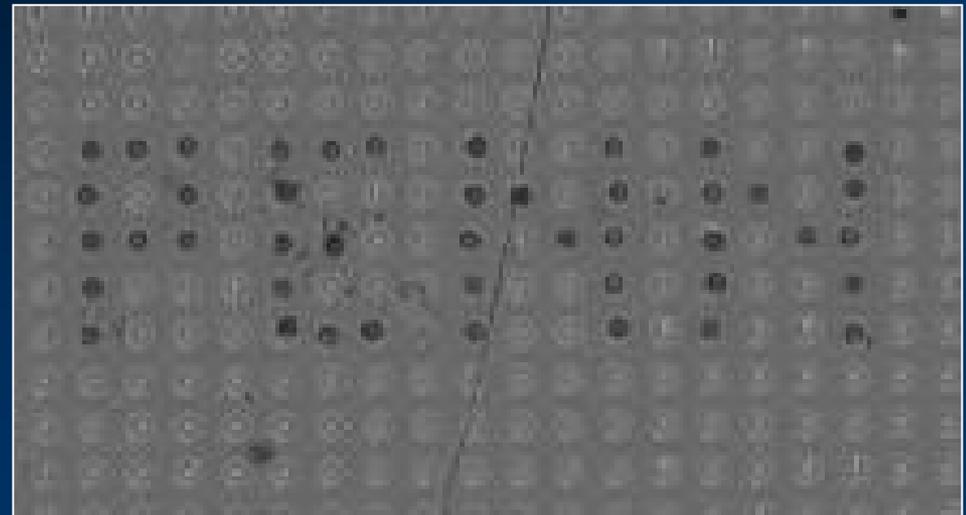
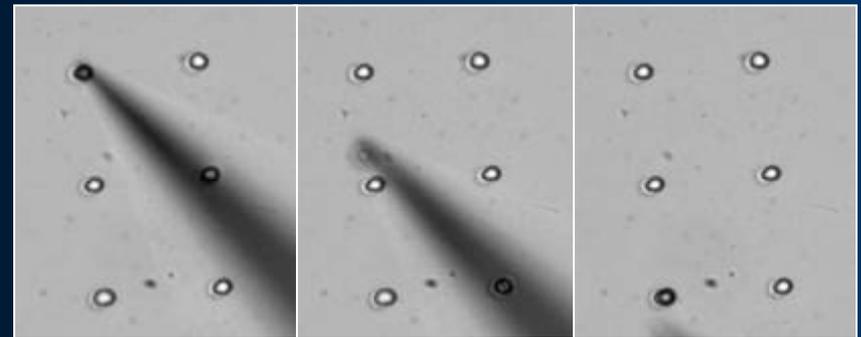
Superparamagnetic (SPM) CNP*



Capturing SPM Nanoparticles (150nm dia.)

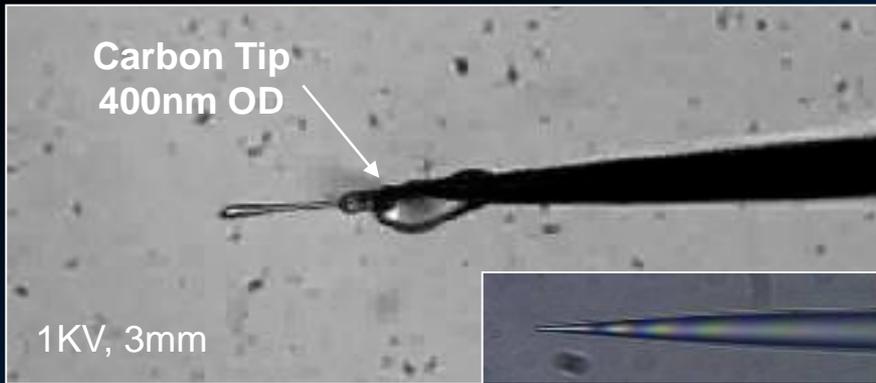


Capturing & Manipulating Microparticles & Arrays

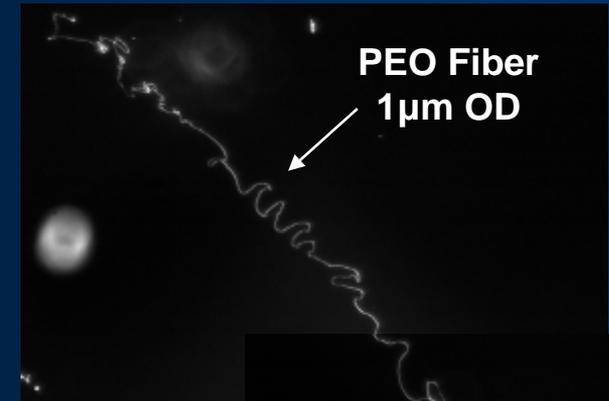


Electrospray Applications

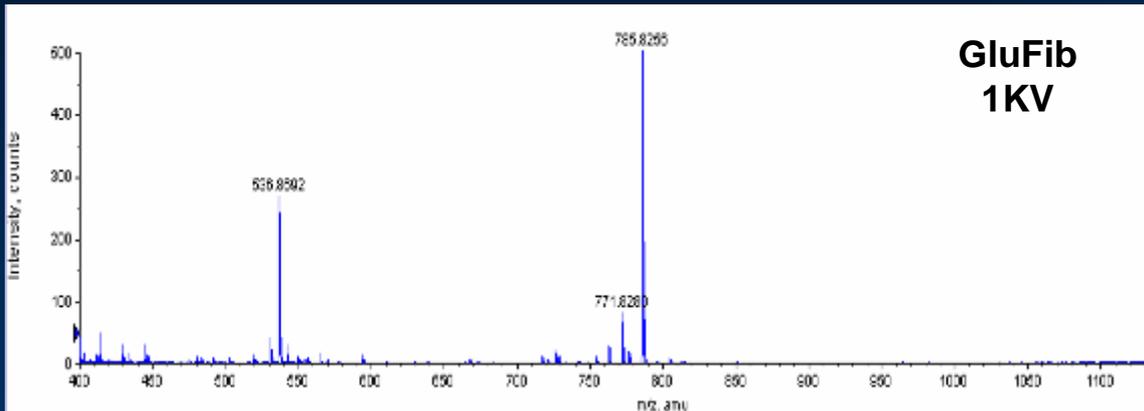
Electrodripping†



Electrospinning †‡



Electrospray Ionization ‡*

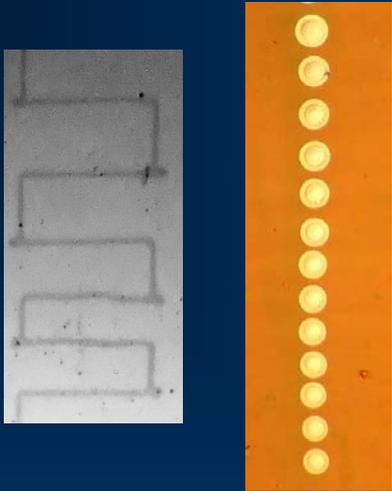
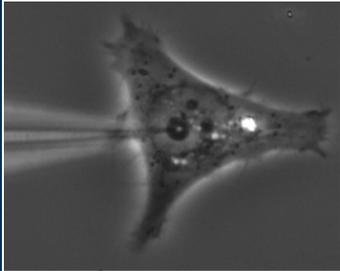


† J. Santiago-Aviles, Electrical & Systems Engineering, University of Pennsylvania, Philadelphia, USA

‡ D. Byun, Aerospace & Information Engineering, Konkuk University, Seoul, Korea

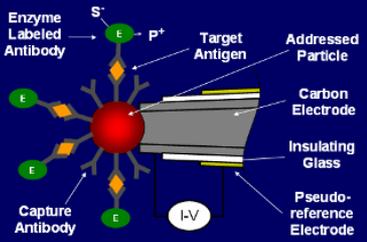
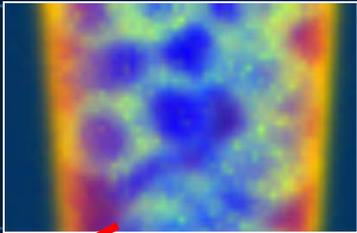
*C-X Yuan, Proteomics Core Facility, University of Pennsylvania, Philadelphia, USA

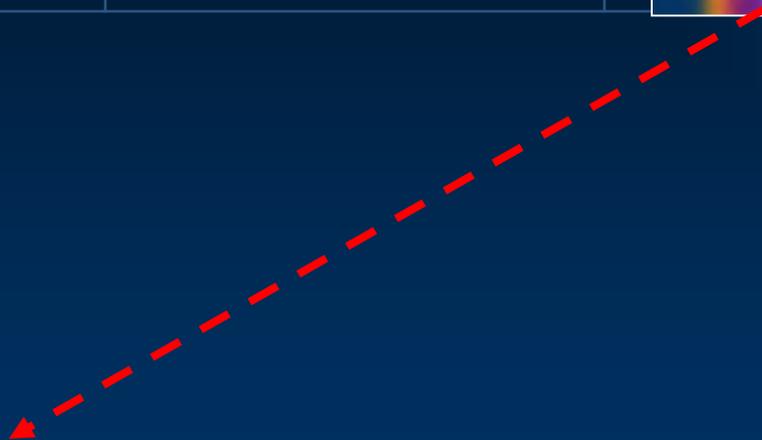
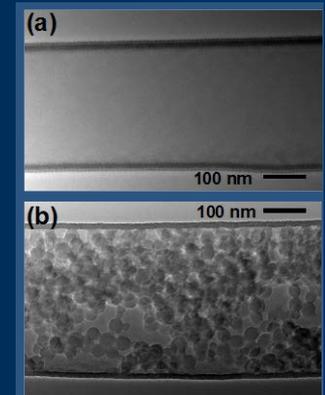
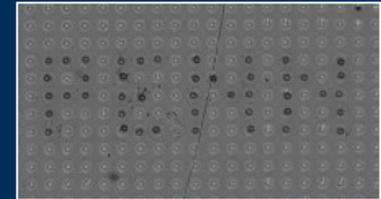
POTENTIAL APPLICATIONS OF CNPs

Application	Description	
Nanoelectrodes	Electrochemistry	
Nozzles (injectors)	Nanofabrication Printing Protein / Oligo Arrays Electro-spinning Mass spectroscopy	
Cellular Probes	Cell sensing & modifications	

**D. Byun,
Aerospace &
Information
Engineering,
Konkuk
University, Seoul,
Korea**

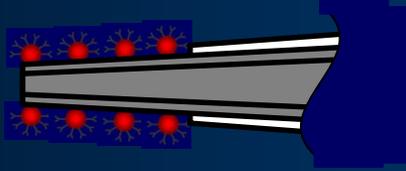
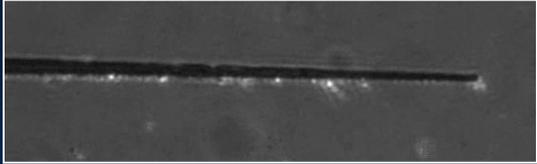
POTENTIAL APPLICATIONS (CONTINUED)

Application	Description	
Particle manipulators Bio-sensors	Bead arrays Bio-sensors	
Sample holders	X ray spectroscopy TEM of viruses & bacteria	



* Hitchcock, A.P., Johansson, G.A., Mitchell, G.E., Keefe, M.H., and Tyliszczak, T., 2007, 3-d chemical imaging using angle-scan tomography in a soft X-ray scanning transmission X-ray microscope, 15th Vacuum Ultraviolet Radiation Physics Conference, Berlin, August 1, 2007. Accepted for publication in Appl. Phys. A.

POTENTIAL APPLICATIONS (CONTINUED)

Injection	Sensing, Cell function modification	Calcium messengers; Transcription alternations
Sensor	Functionalized surface SERS	
Actuator	Magnetic probe	
Cell- physiology	Cell potential measurements Automated injection	