Carbon Nanopipettes

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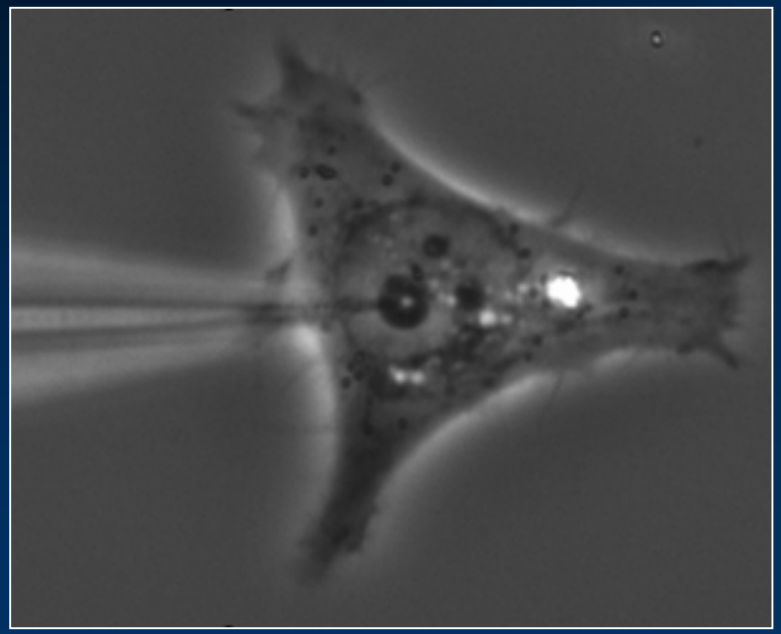
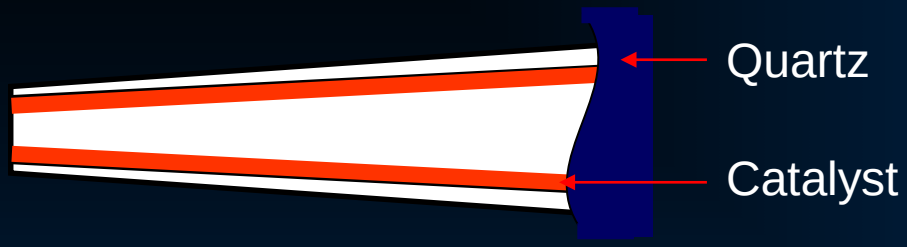


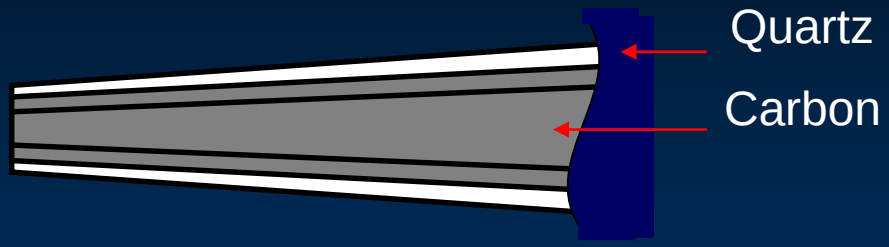
Challenge: how do you integrate a nanoscopic structure with a macroscopic handle?

Solution: integrated manufacturing

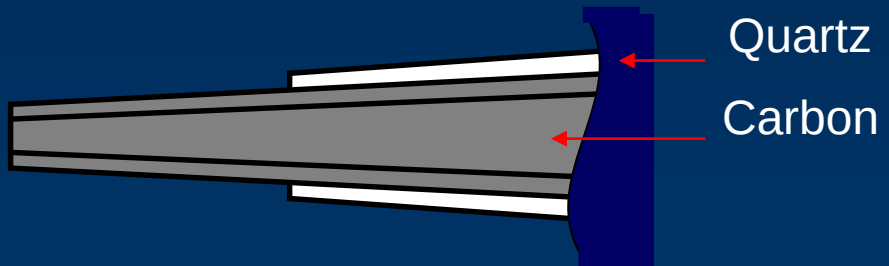
Pull Quartz Micropipettes



Deposit Carbon Film by CVD



Expose Carbon Tip with BHF

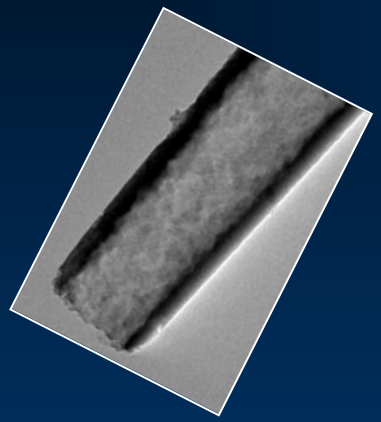


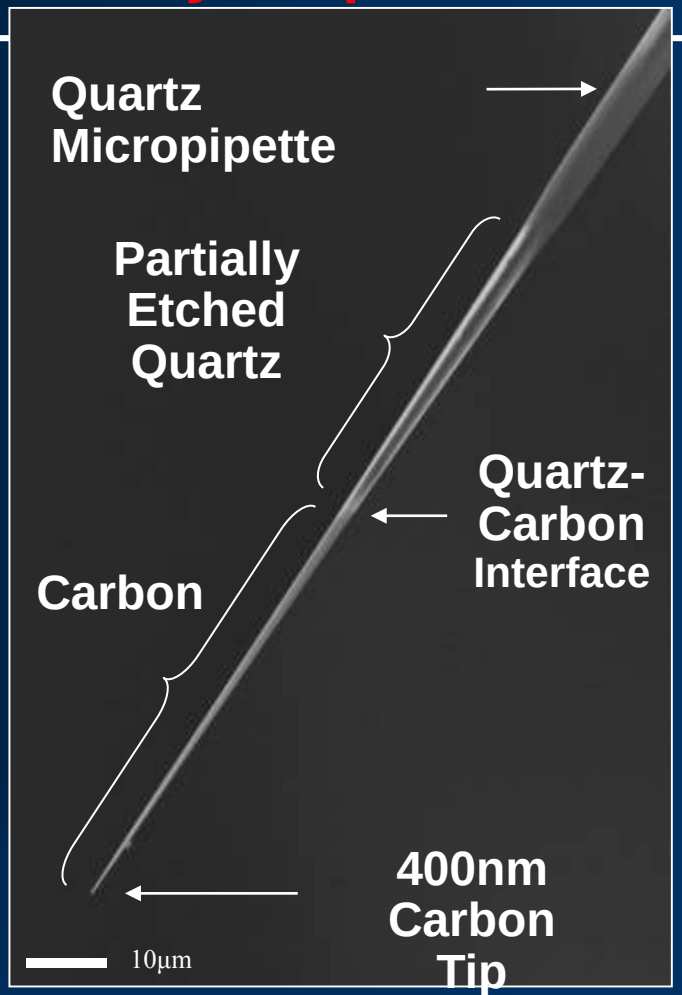
Kim, B. M, Murray, T., and Bau, H. H., 2005, The Fabrication of Integrated Carbon Pipes with Sub Micron Diameters, Nanotechnology, 16, 1317-1320.

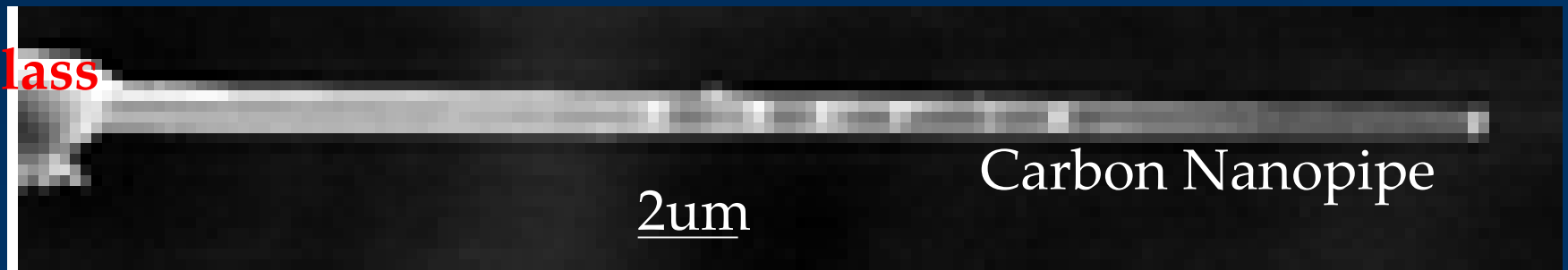
B. M. Kim and H. H. Bau, Nano and Micros Scale Structures: Methods, Devices and Applications Thereof, Patent application 2006/0116971

Carbon NanoPipette (CNP) - No Assembly Required

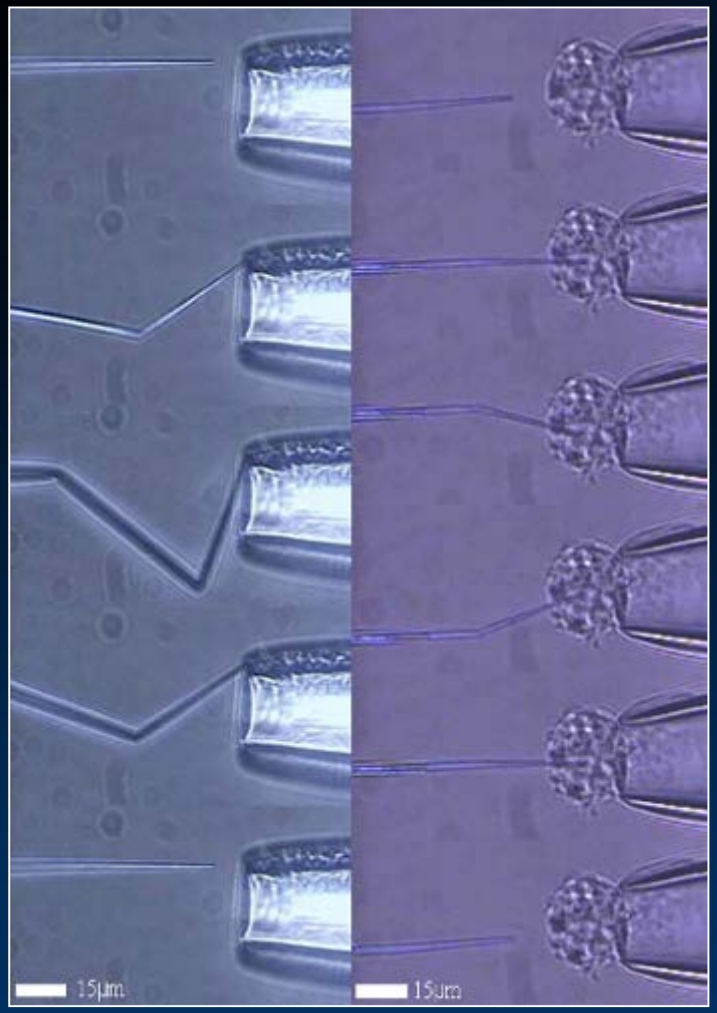
- Exposed Carbon Tip







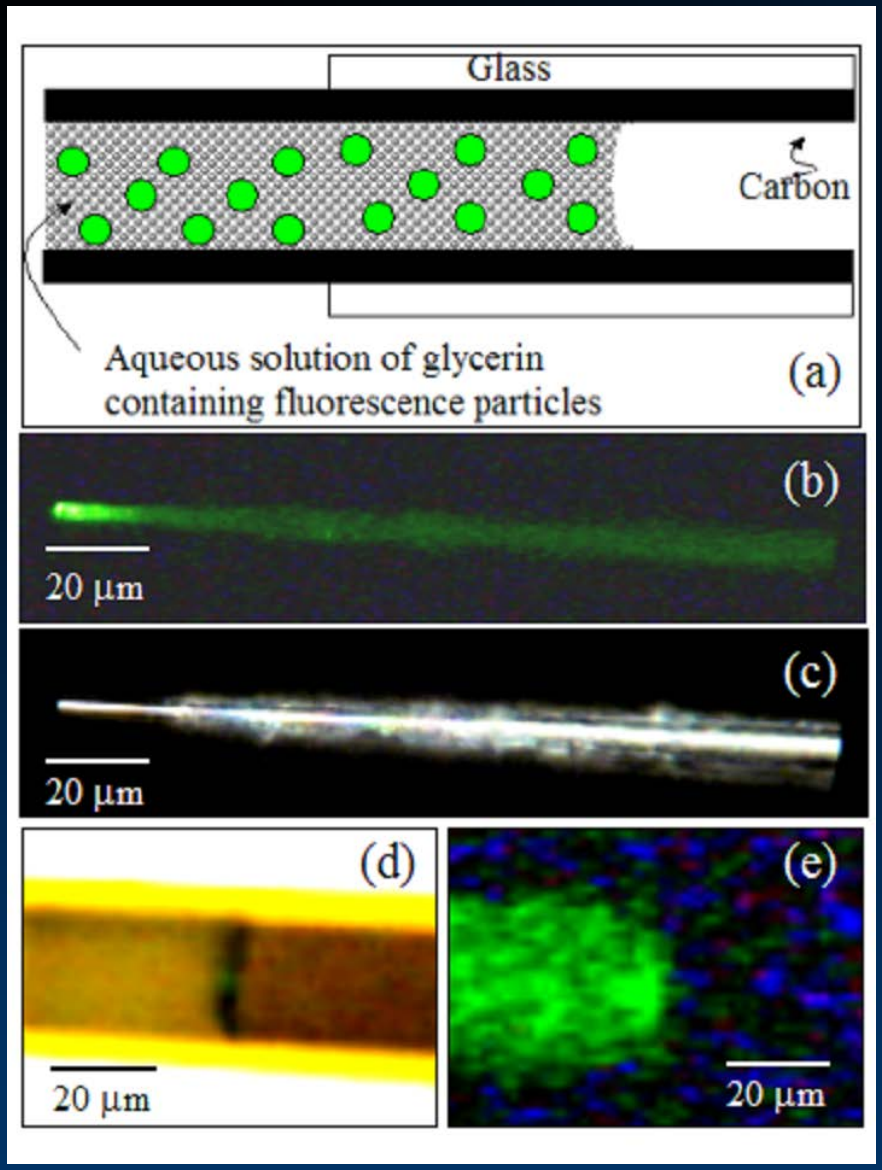
CNP's Mechanical Characteristics



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)

Schrlau, M. G.; Falls, E. M.; Ziober, B. L.; Bau, H. H., *Nanotechnology*, **2008**

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.

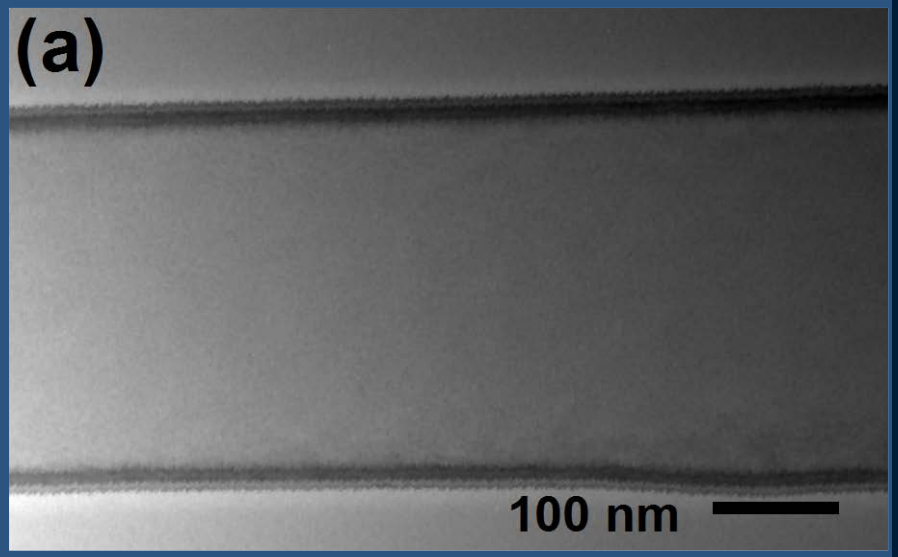
(d) An optical image of the suspension-air interface inside the carbon pipette.

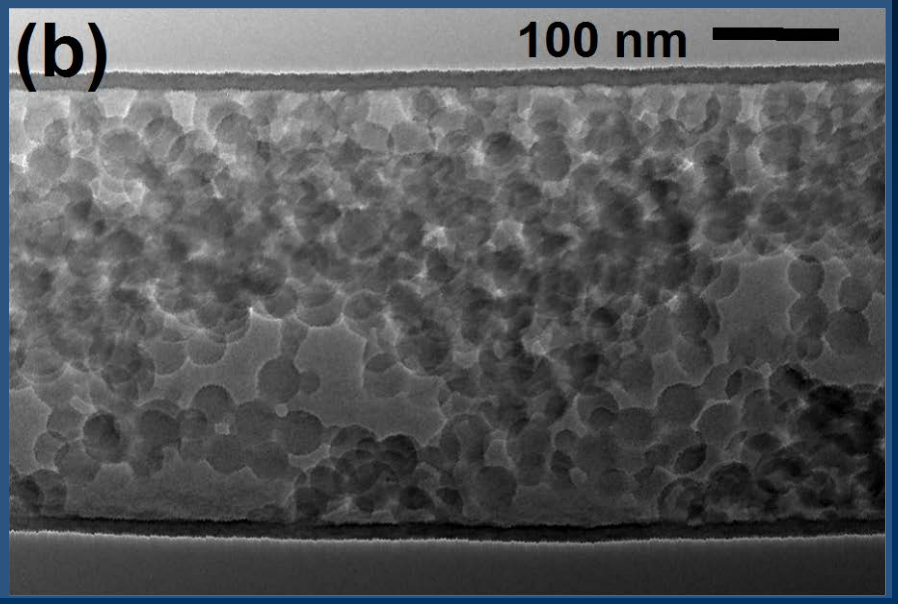
(e) A florescent image complementing the optical image of Fig. d. The liquid part of the suspension is a water-glycerin mixture.

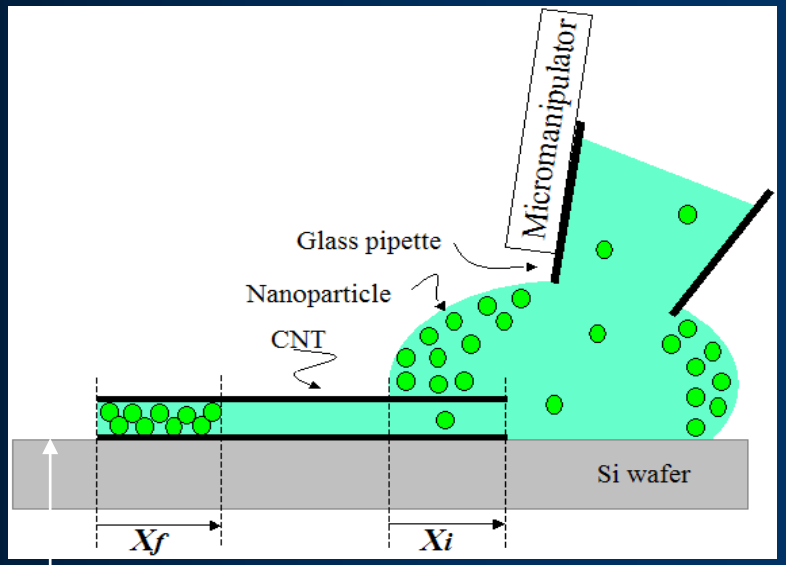
Carbon NanoPipes are transparent to: Light, electrons, and X-rays

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

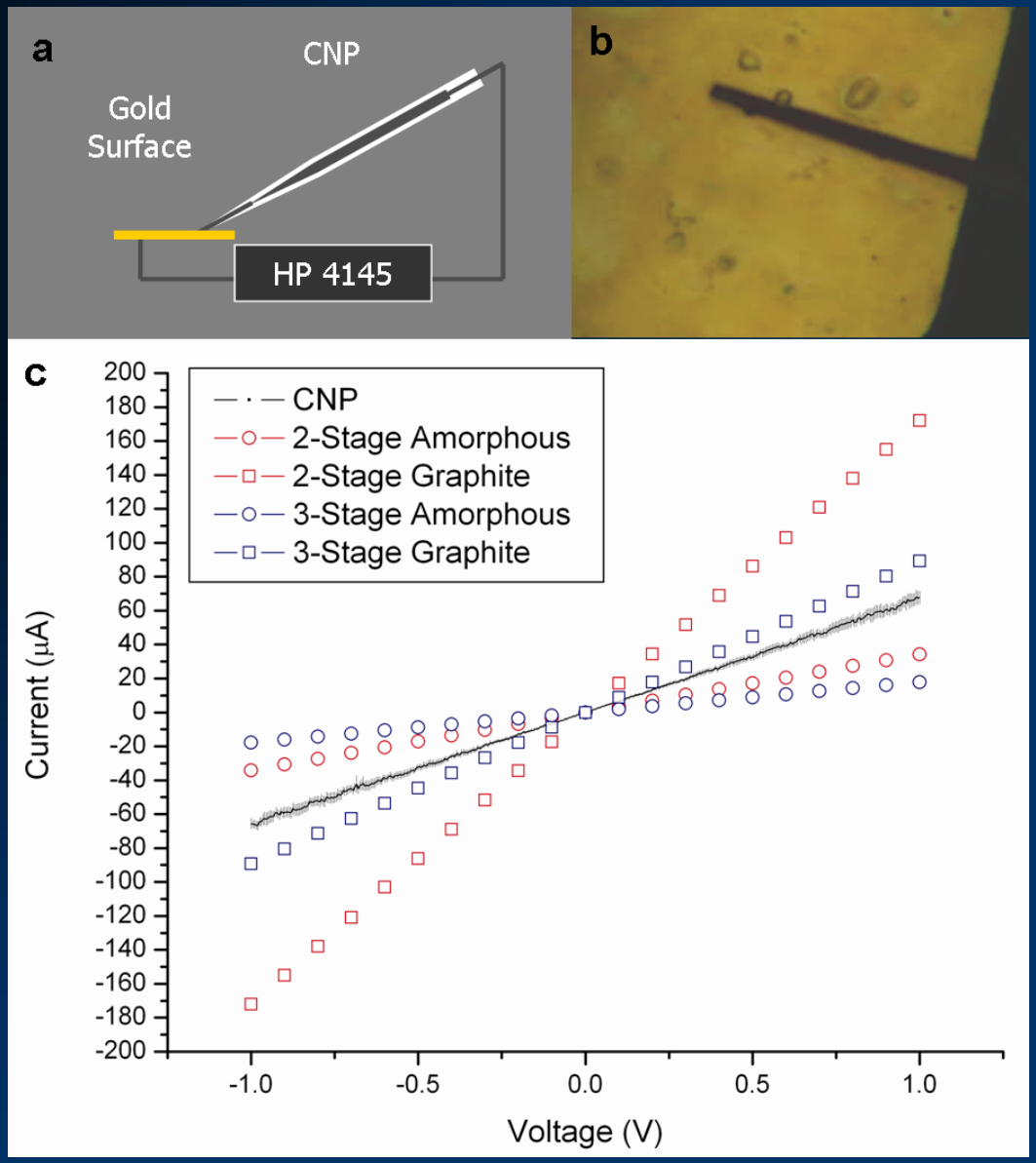
Kim, B. M., Qian, S., and Bau, H., H., 2005, Filling Carbon Nanotubes with Particles, Nano Letters, *5* (5) 873 – 878

CNPs' Electrical Properties

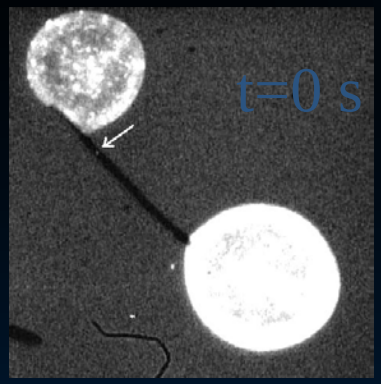
Ohmic Resistance

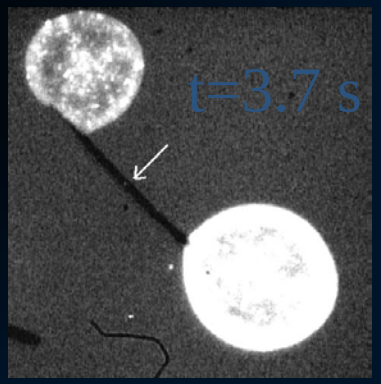
Electrical resistance of CNPs : the current is depicted as a function of the potential difference. Solid lines (theory). Symbols (experiments).

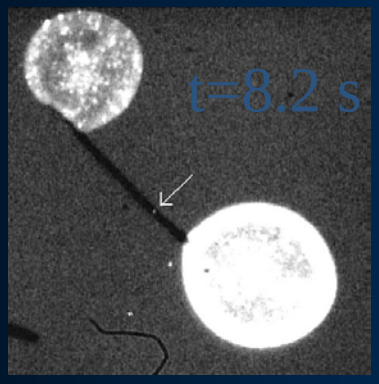
Typical resistance ~15kΩ

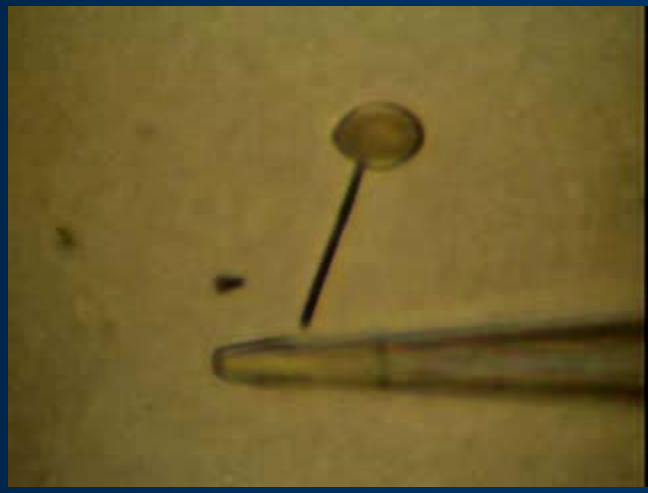


Flow Characteristics of the Carbon Nanopipes

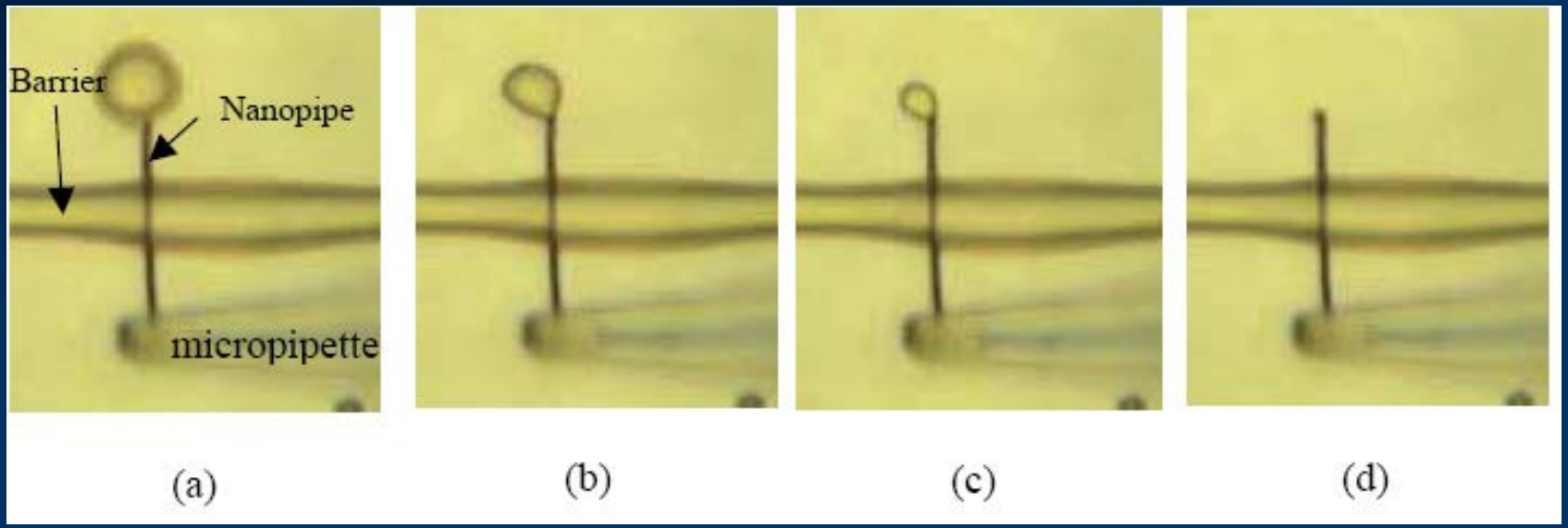




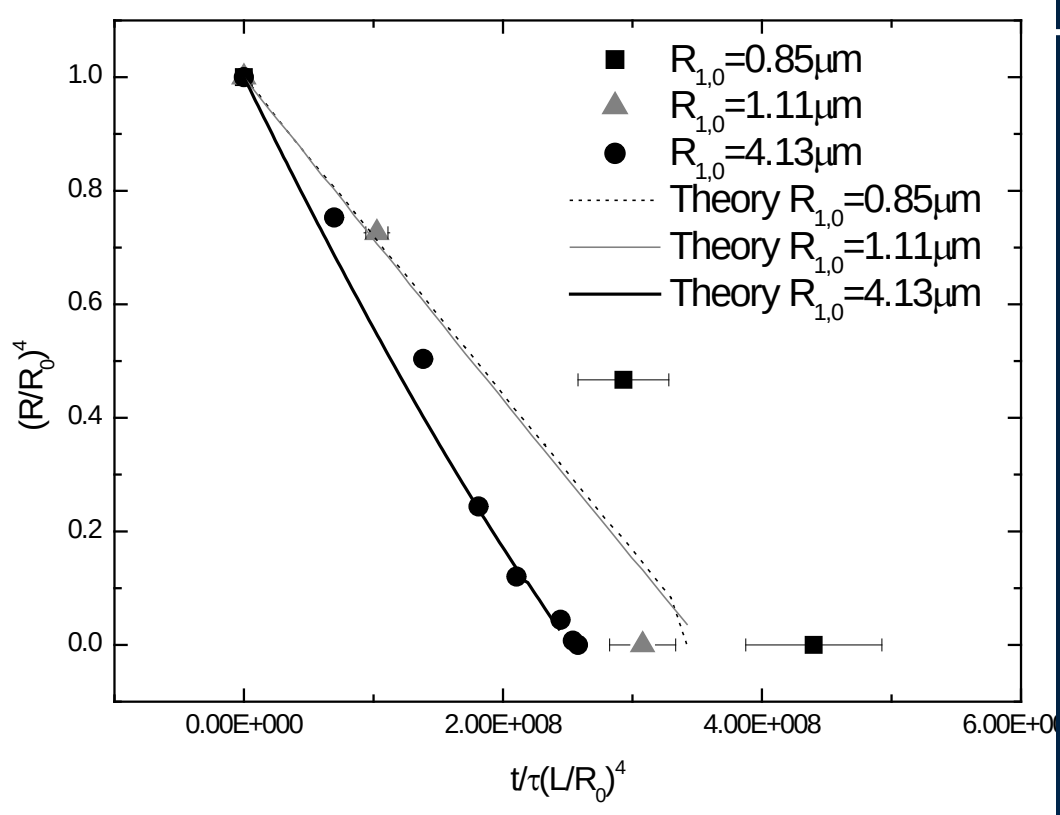


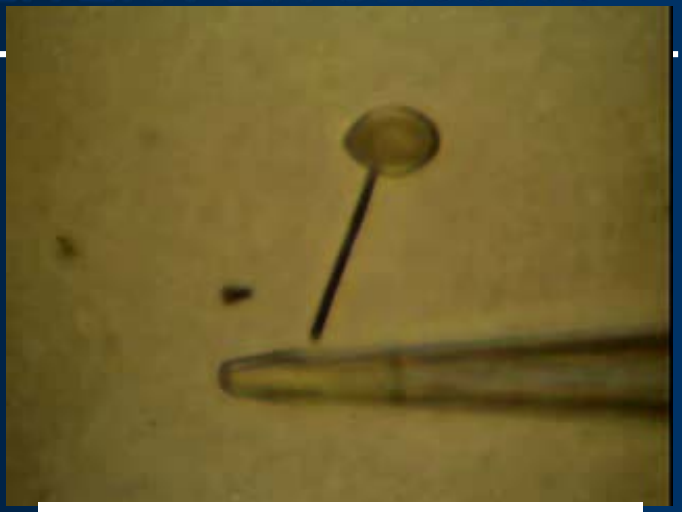


The position of the fluorescent particle inside the nanotube as liquid flows from the smaller drop to the bigger drop. The arrow indicates the location of the particle.



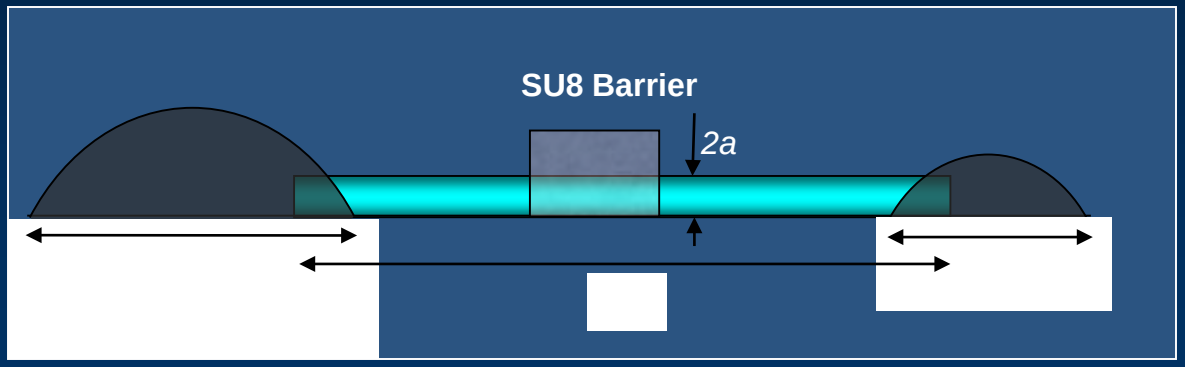
MEASURING FLOW RATES at ATTOLITER PER SECOND





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

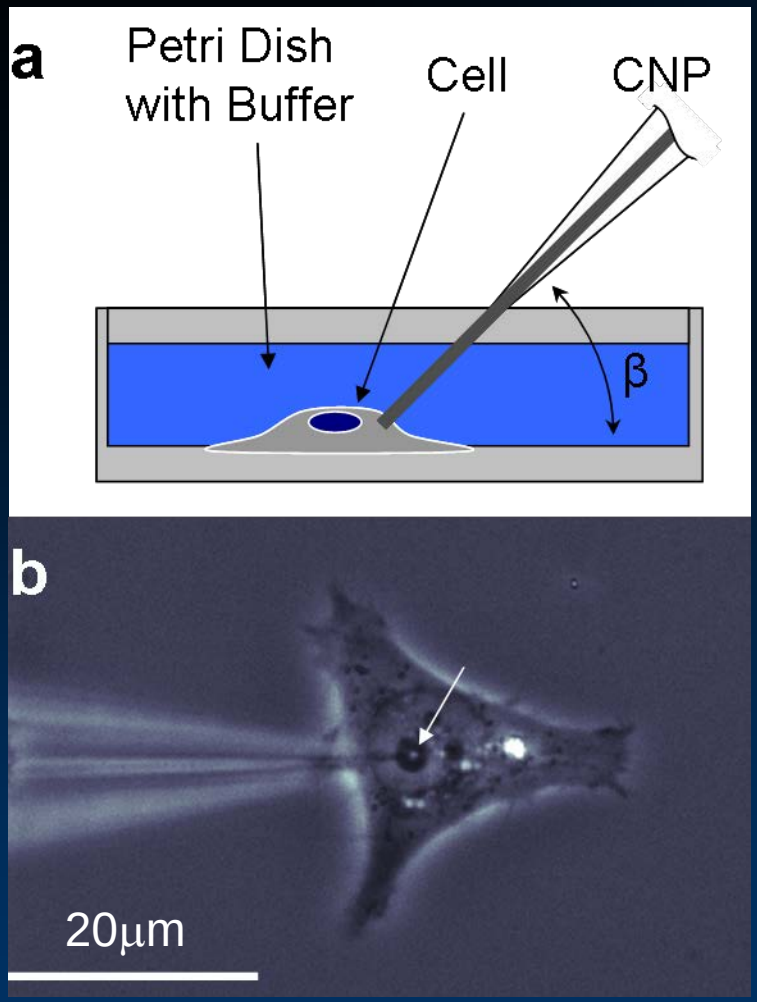
σ=surface tension
η=viscosity
θ=contact angle
a = tube radius

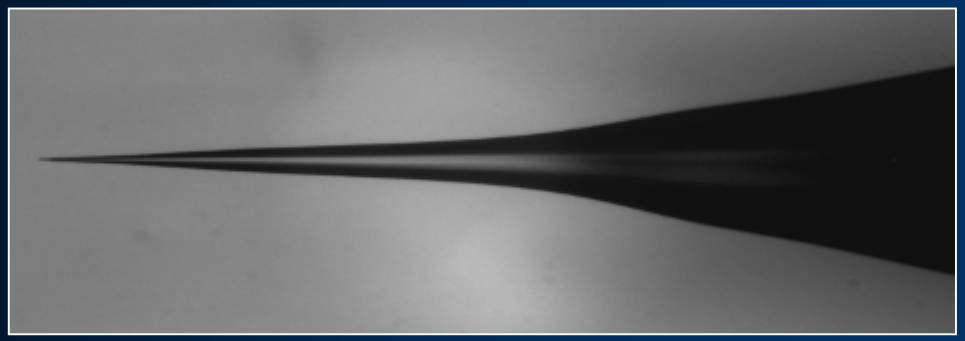


$$\chi = \left(3 + \left(\frac{1-\cos\theta}{\sin\theta}\right)^2\right)\left(\frac{1-\cos\theta}{\sin\theta}\right)$$

Sinha, S., Rossi, M., Mattia, D., Gogotsi, Y., and **Bau, H. H.,** 2007, Induction and Measurement of Minute Flow Rates through Nanopipes, *Physics of Fluids* 19, 013603

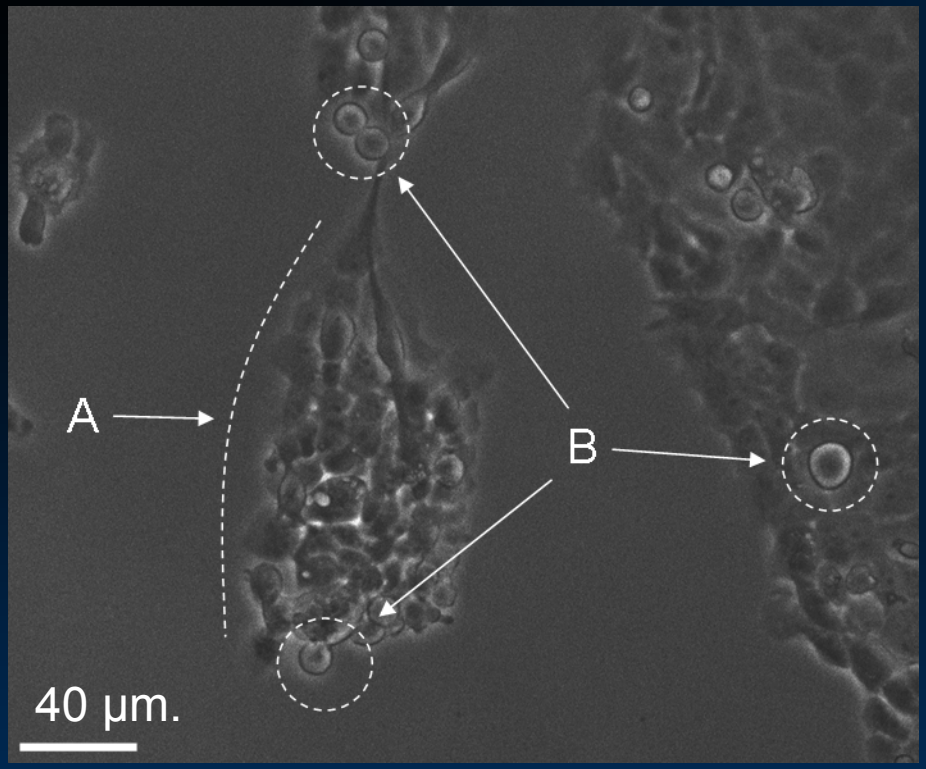
Probing Cells





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 um).

Cell Viability upon Probing with CNPs

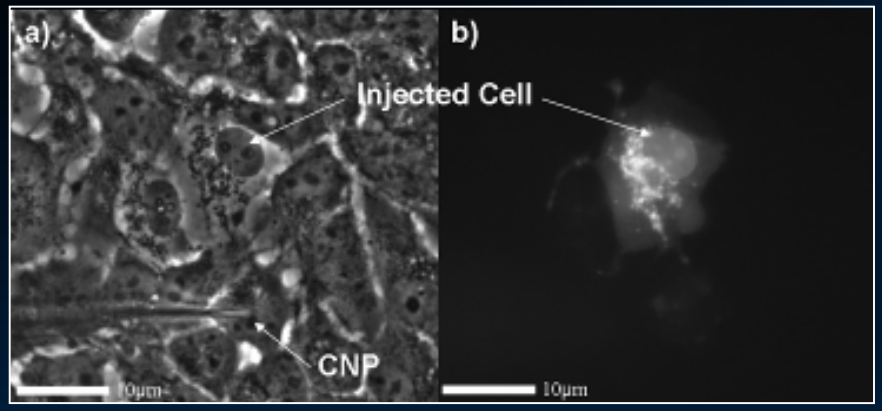


Trypan Blue Exclusion Test

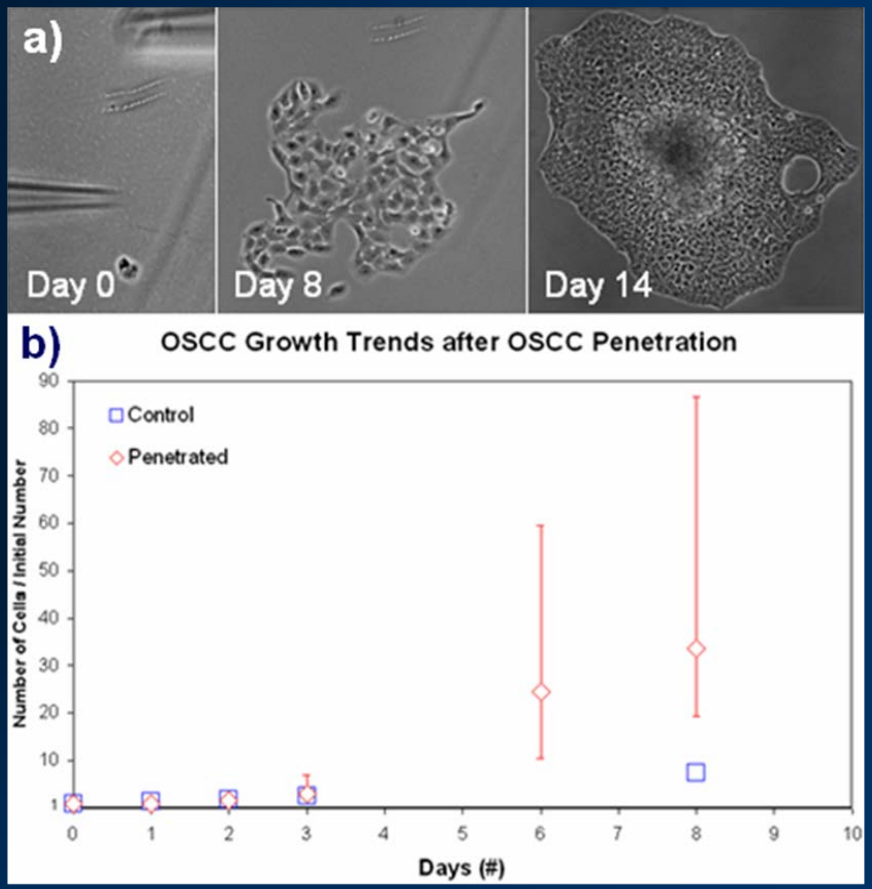
The cells along the edge were probed for short times with CNPs (A, dotted line). When Trypan Blue was added to the extracellular solution, the probed cells remained colorless (viable) while dying or dead cells turned blue (B, dotted circles).

CNPs' Cell Toxicity

Cells Remain Viable After CNP Probing



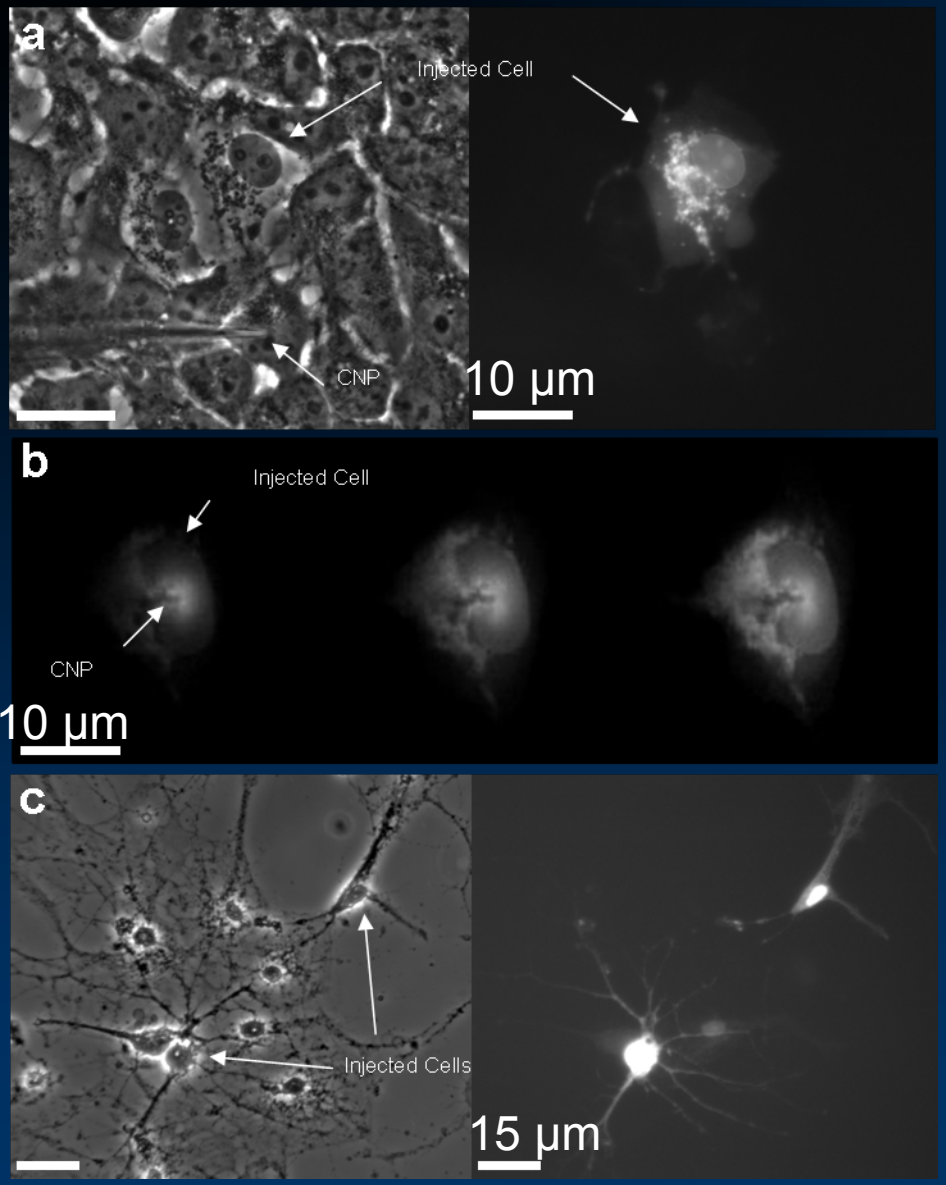
(a) OSCC prior to injection. (b) Same OSCC subsequent to dye injection (observation with fluorescence microscopy)



(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.

Schrlau, M. G.; Falls, E. M.; Ziober, B. L.; Bau, H. H., *Nanotechnology*, **2008**

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

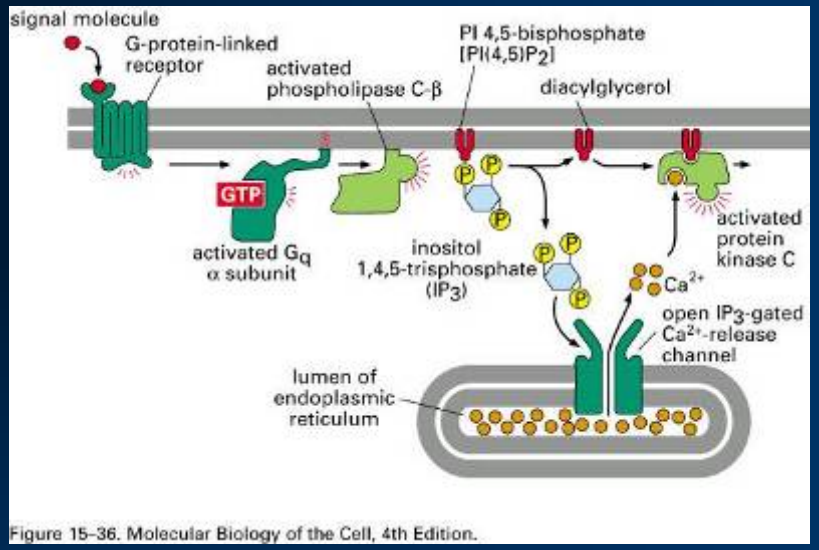
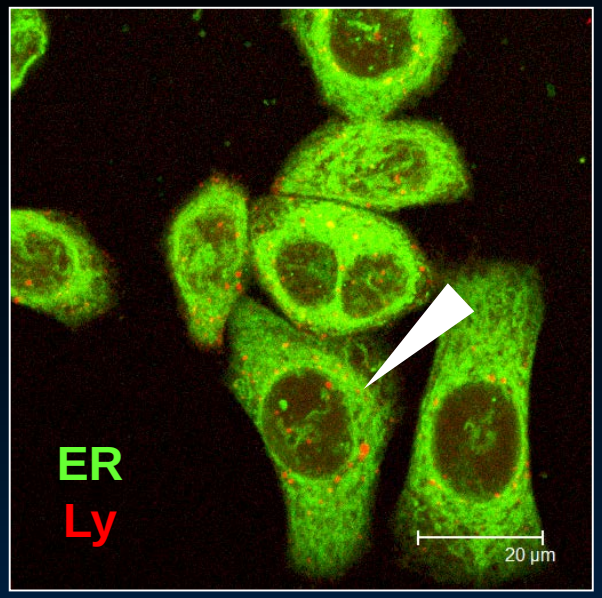


Figure 15-36. Molecular Biology of the Cell, 4th Edition.

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

Second Messenger Injection using CNPs



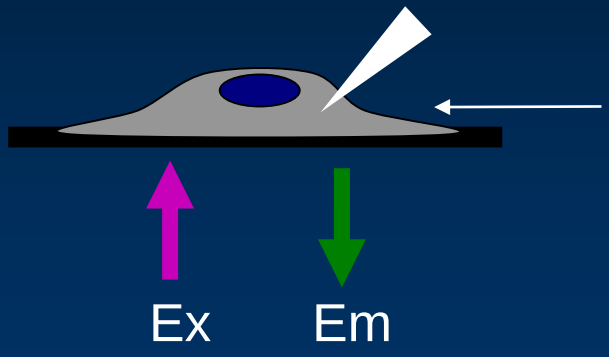
Confocal by Brailoiu GC, Temple

Second Messengers:

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

Calcium Stores:

- *Endoplasmic Reticulum* (ER) – sensitive to IP3 and cADPr (in some cells)
- *Lysosomes* (Ly) – sensitive to NAADP (controversial)

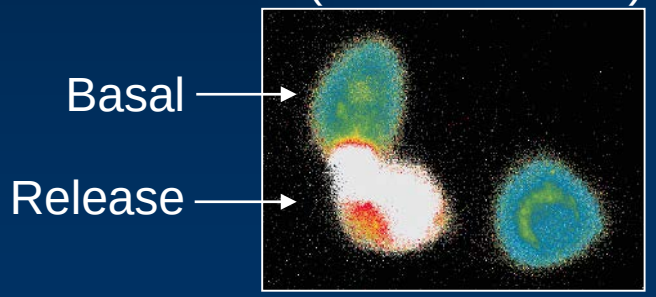


Breast cancer cells (SKBR3) loaded with Fura-2AM

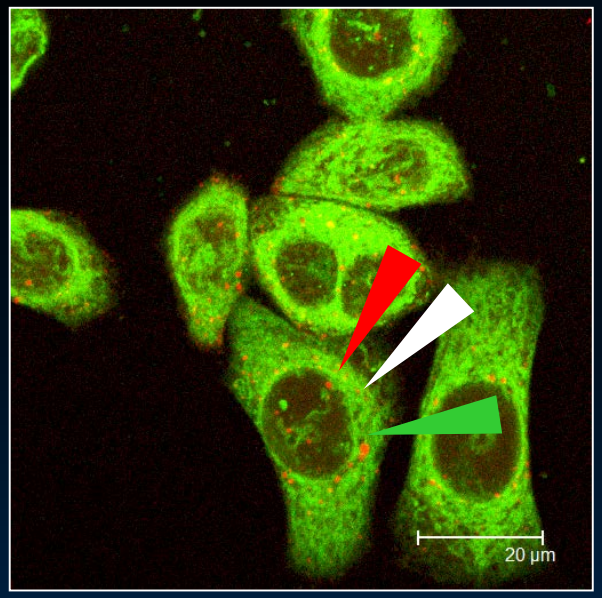
Ex: 340, 380 nm

Em: 540 nm

Fluorescent Images (340nm/380nm)



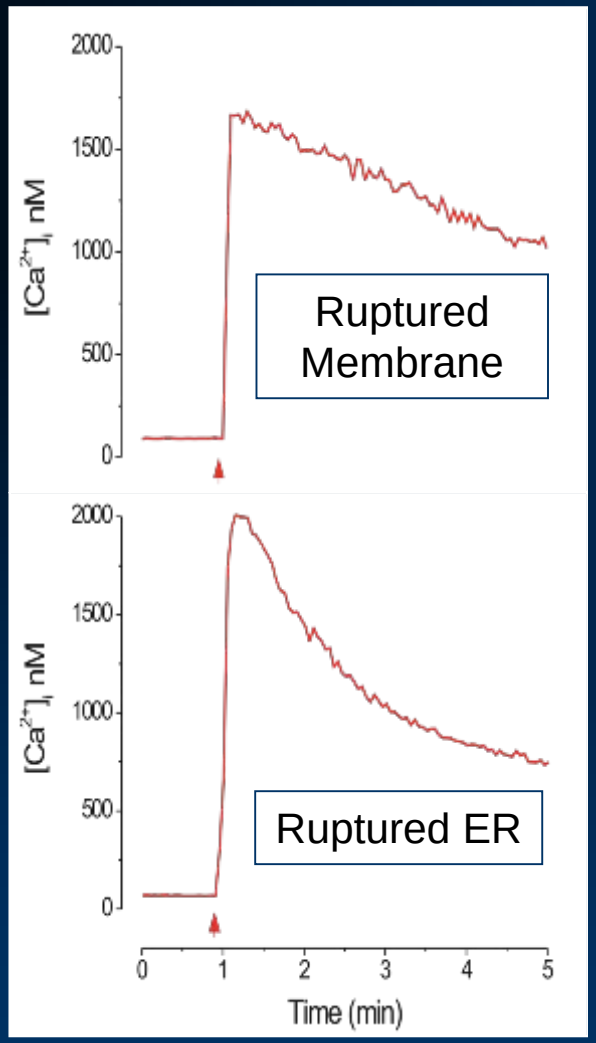
Calcium Signaling via Second Messenger Injection



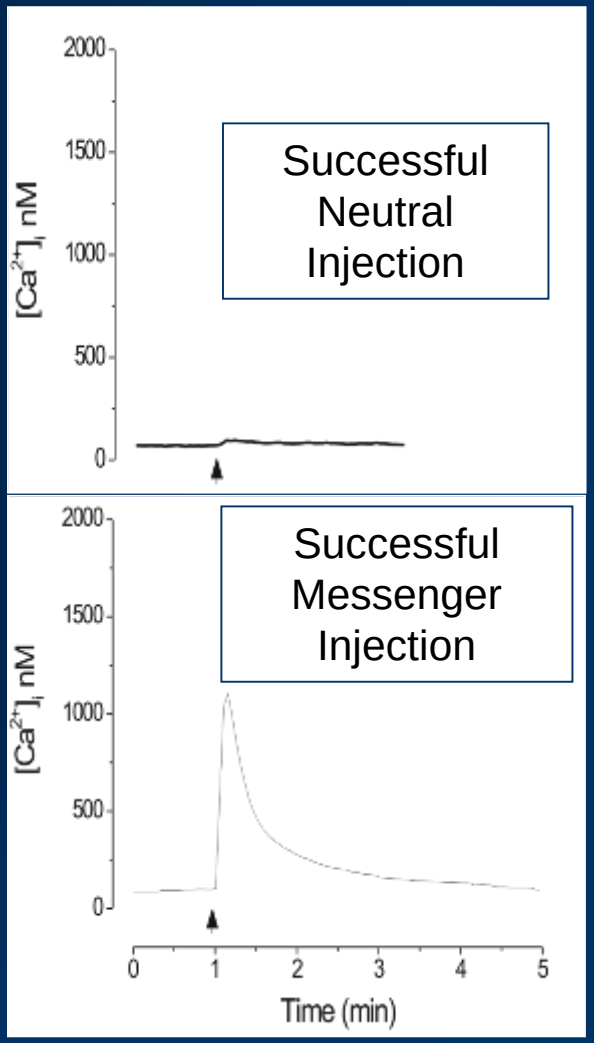
Confocal by Brailoiu GC, Temple

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

Damaging Injection



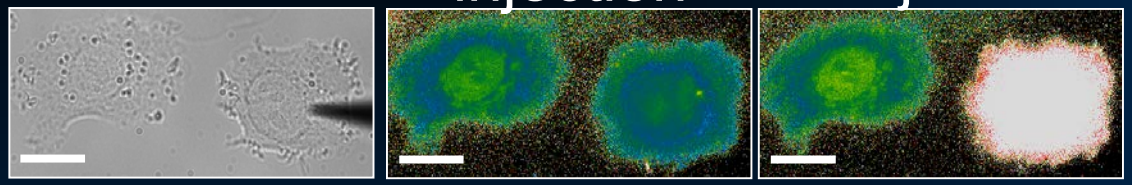
Successful Injection

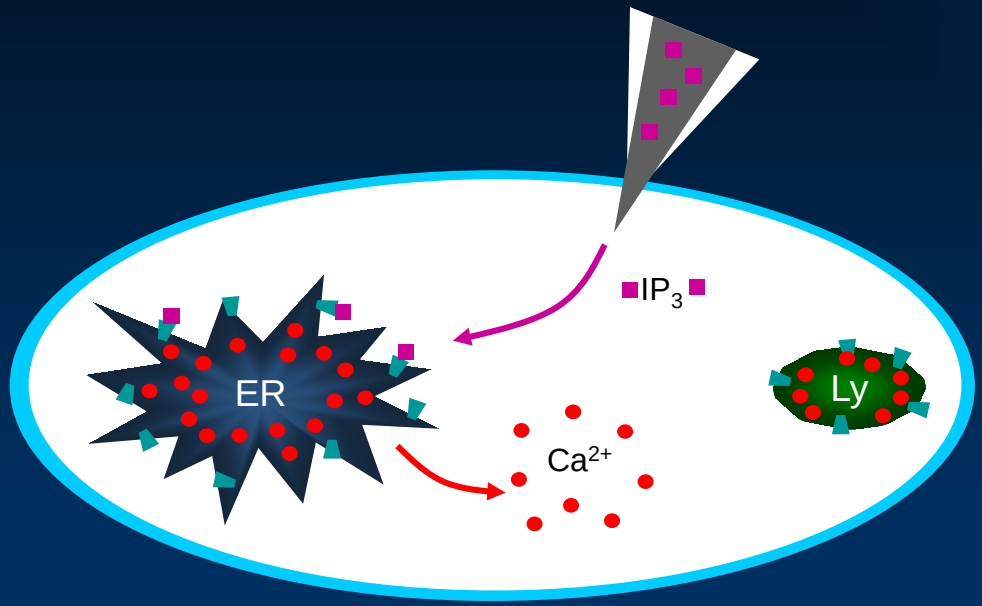


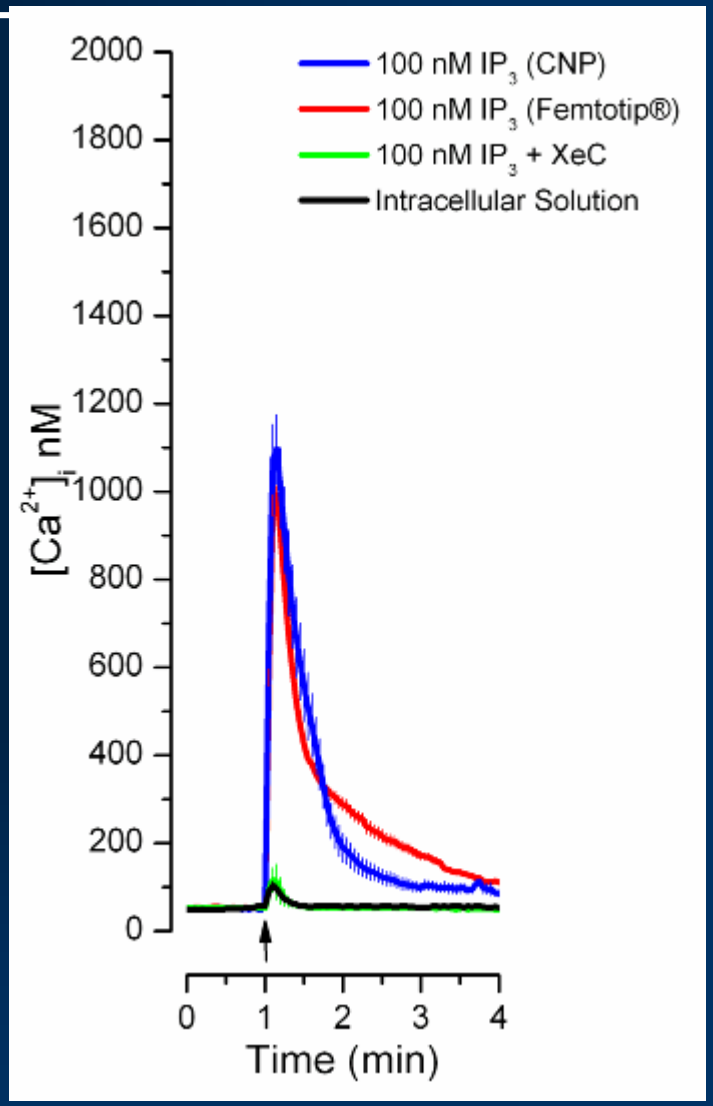
IP_3-Induced Ca^{+2} Release in Breast Cancer Cells

IP_3 – inositol triphosphate

Targeting | Before injection | After injection







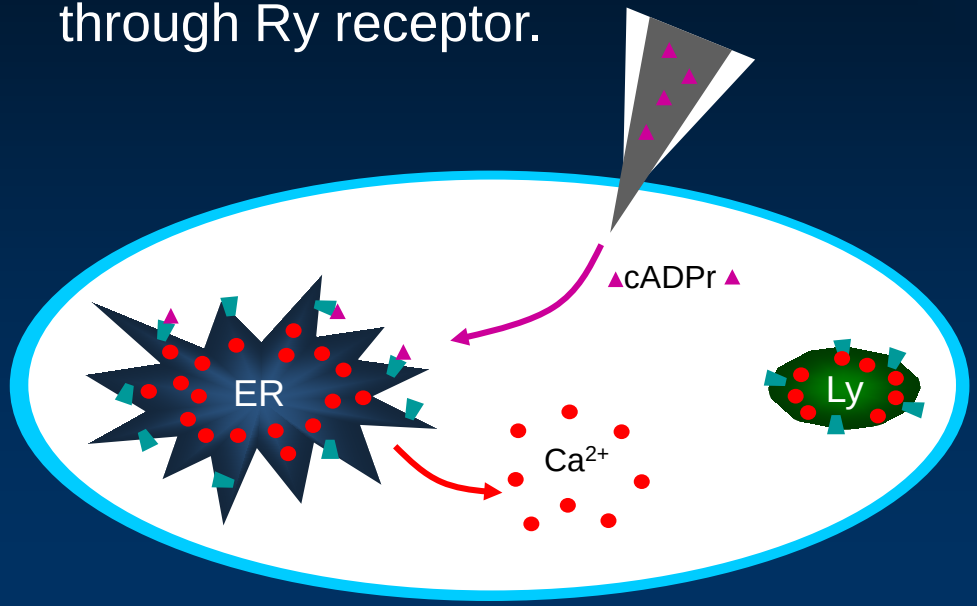
Traces = average 6 cells +/- s.e.m

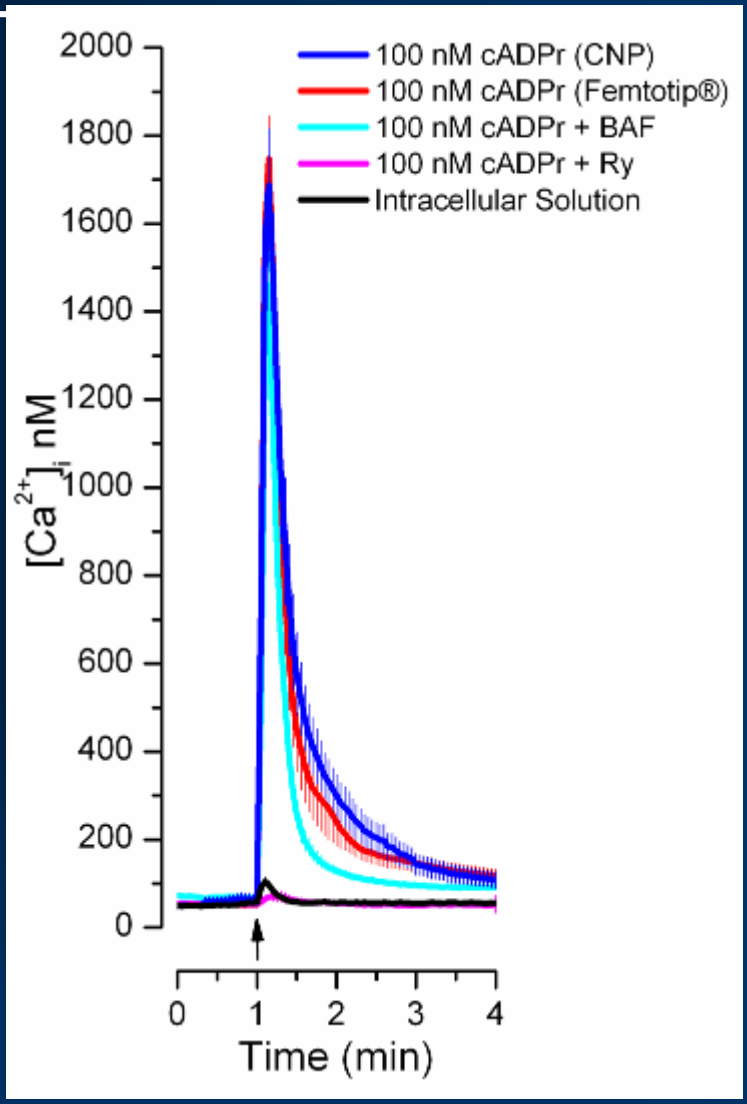
Schrlau M, Brailoiu, E., Patel, S., Gogotsi, Y., Dun, N., and Bau, H. H., 2008, Carbon Nanopipettes Characterize Calcium Release Pathways in Breast Cancer Cells, Nanotechnology 19, 325102

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 → ER is sensitive to cADPr through Ry receptor.





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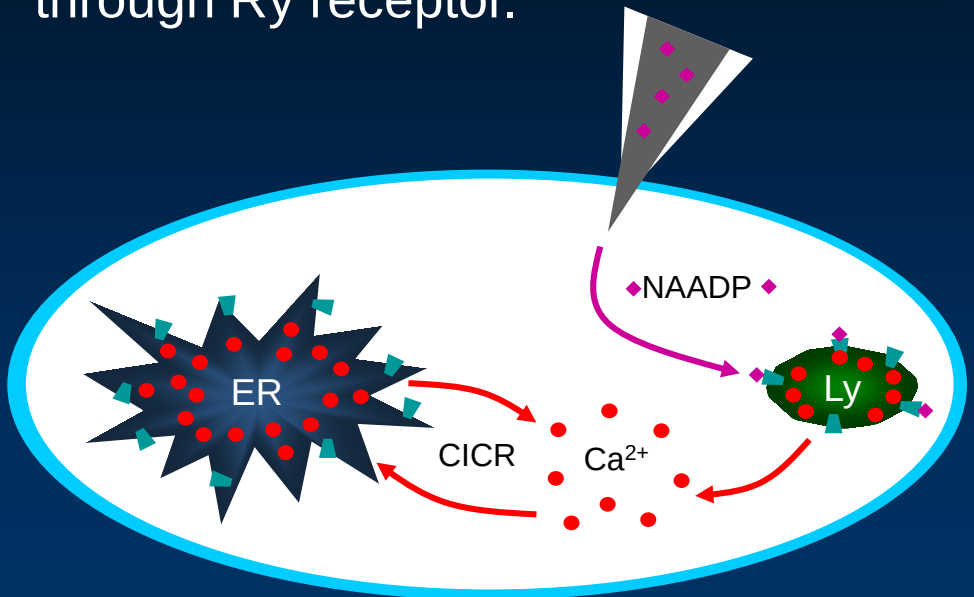
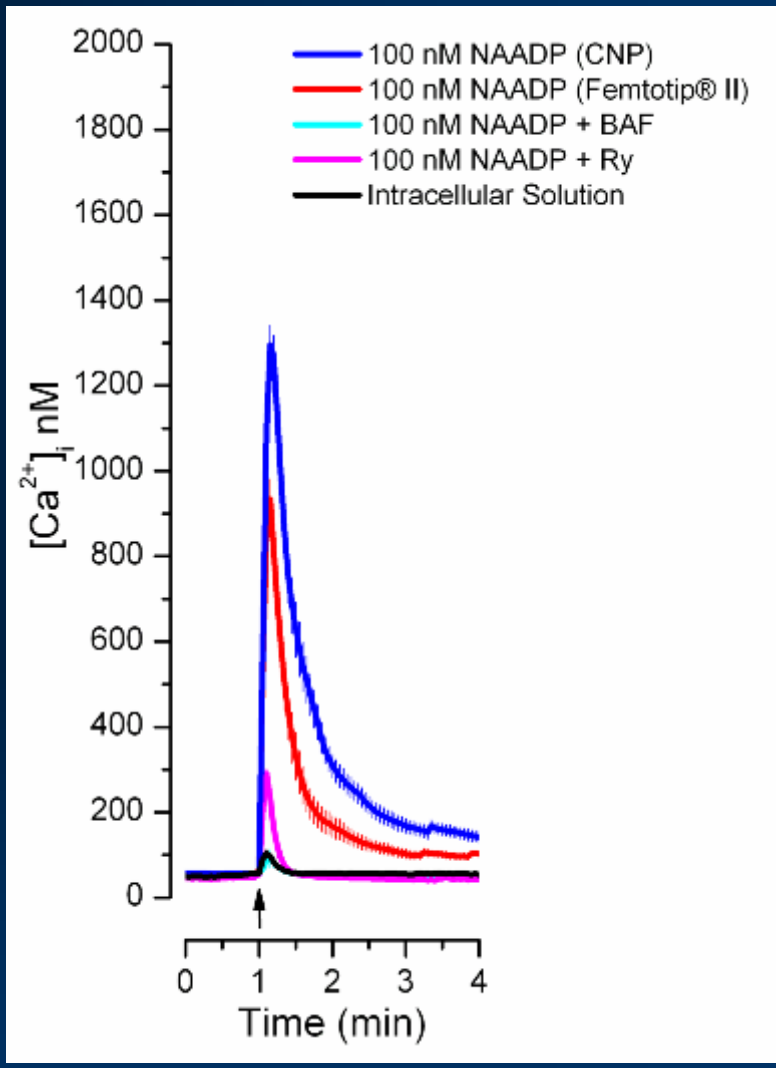
Schrlau M, Brailoiu, E., Patel, S., Gogotsi, Y., Dun, N., and Bau, H. H., 2008, Carbon Nanopipettes Characterize Calcium Release Pathways in Breast Cancer Cells, Nanotechnology 19, 325102

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 → Ly is sensitive to NAADP. Calcium-induced calcium release from ER through Ry receptor.

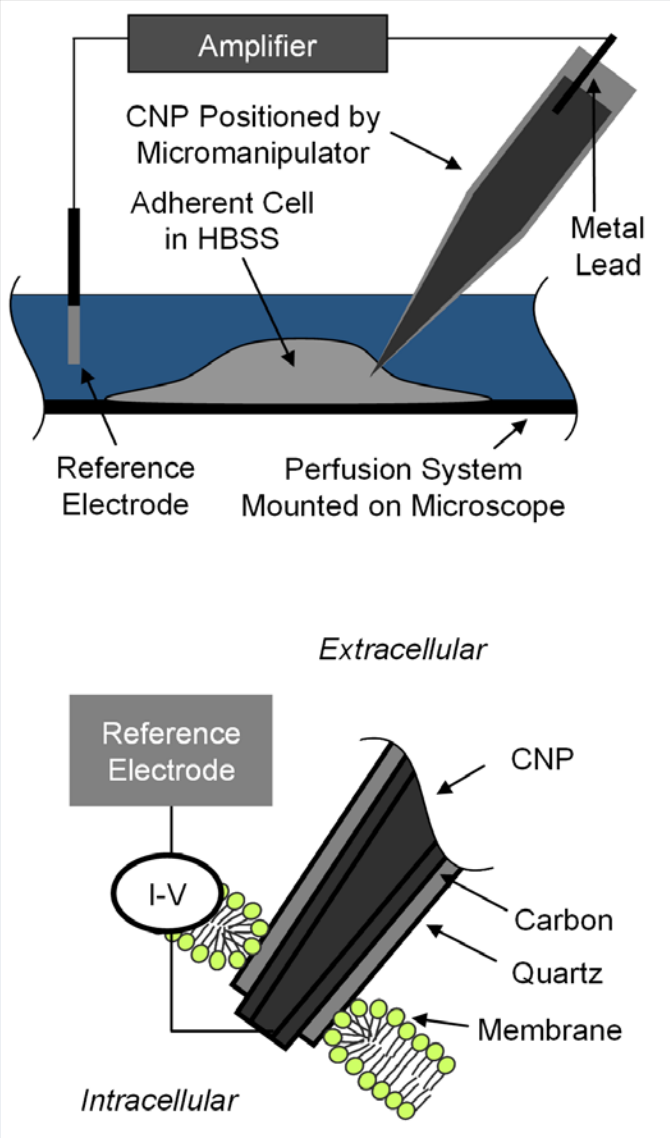




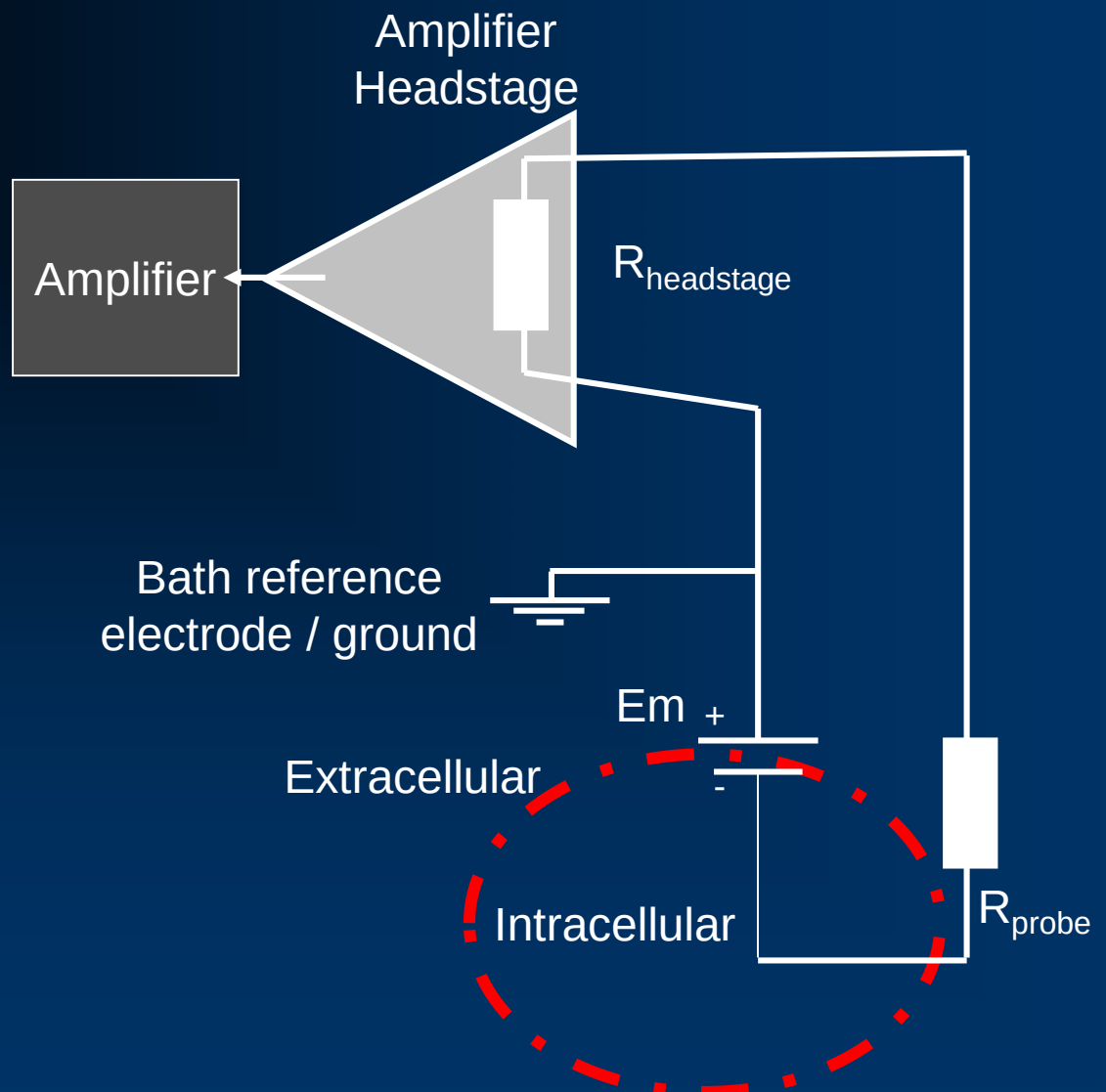
Traces = average 6 cells +/- s.e.m

Schrlau 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

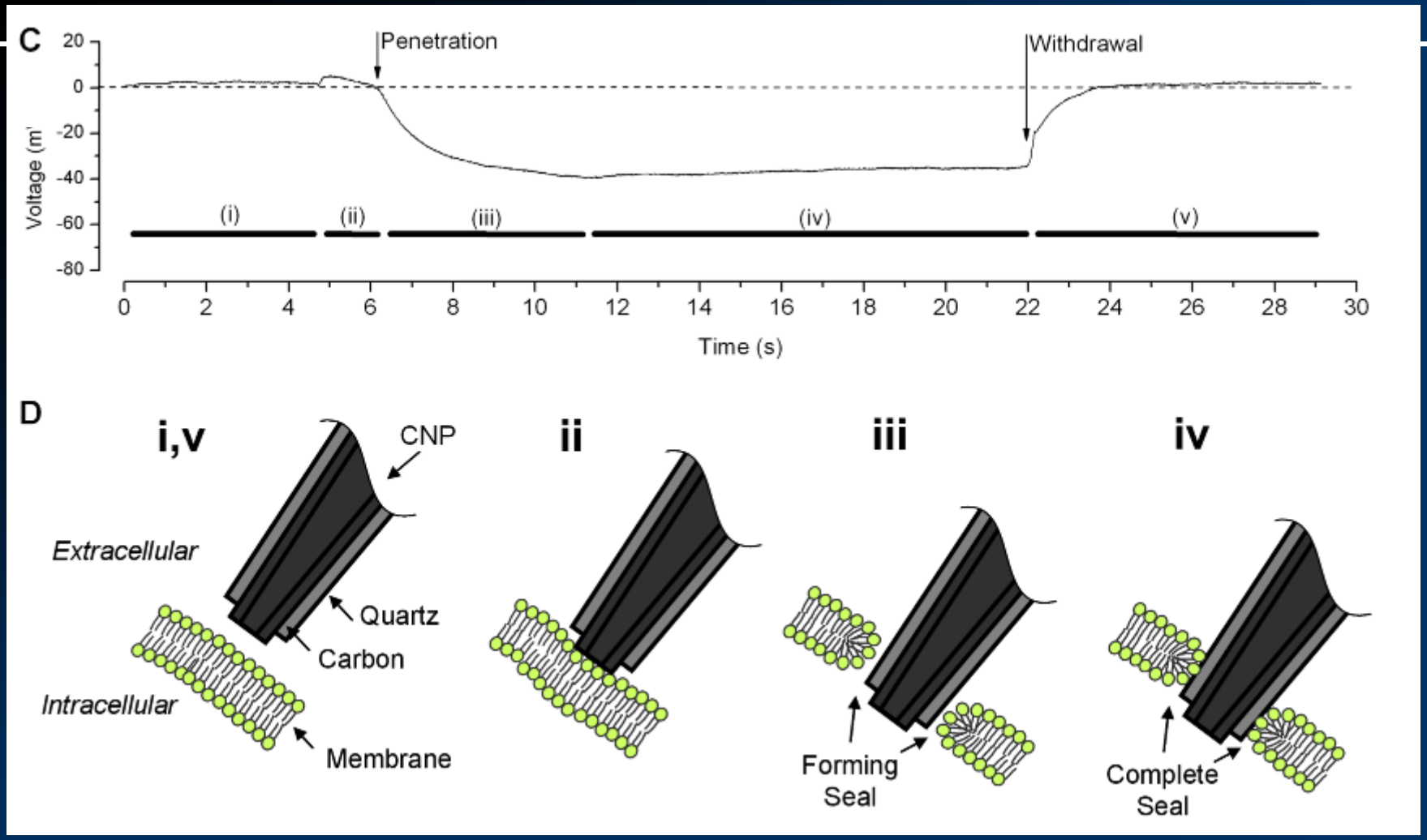
Carbon Nanopipettes for Cell Electrophysiology



Experimental Set-up



Cell Membrane Potential

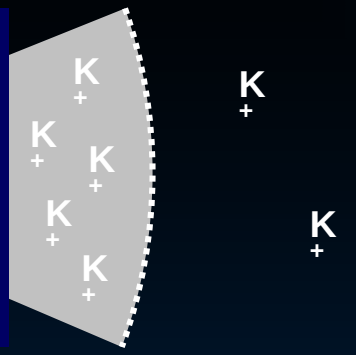


Probing events are electrically recorded with CNPs

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

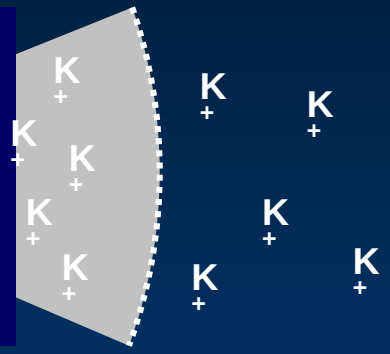
Measuring Cell Response to Chemical Stimuli

Normal



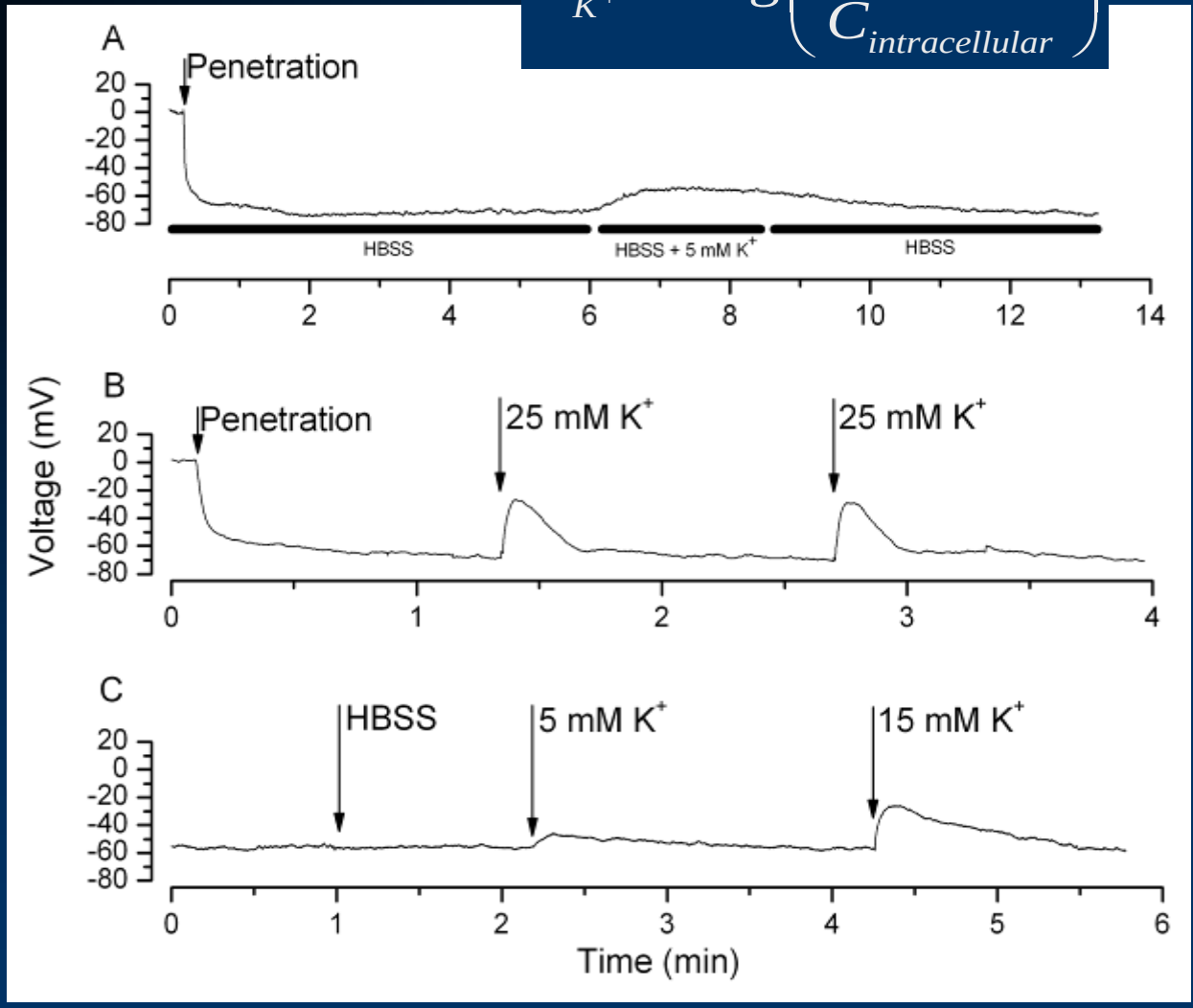
Resting Membrane Potential

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

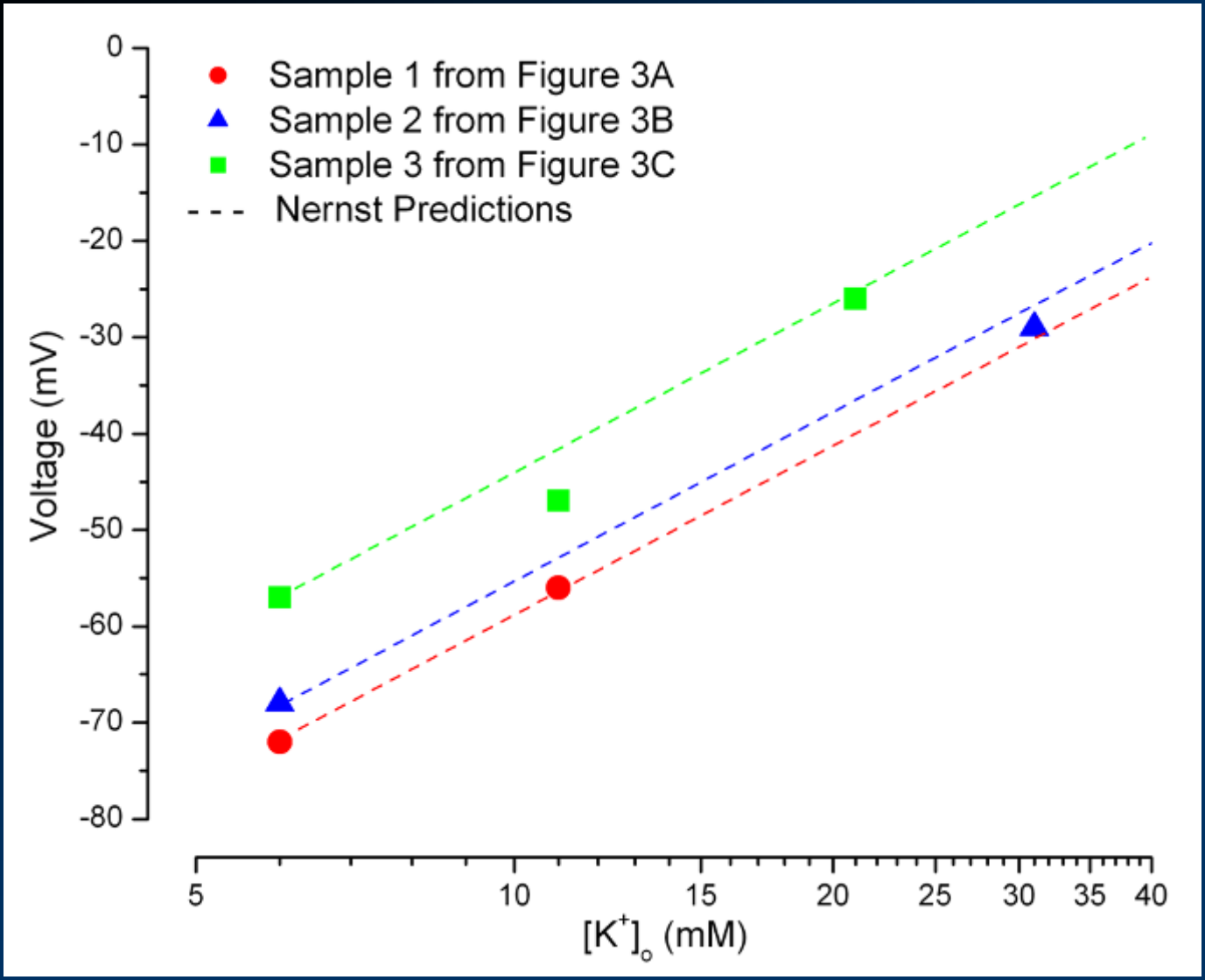


Depolarization

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

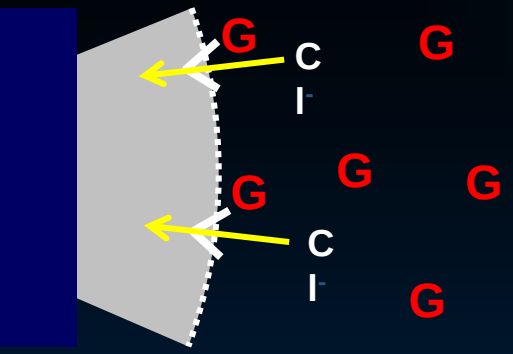


Experimental Results compared with Nernst Predictions



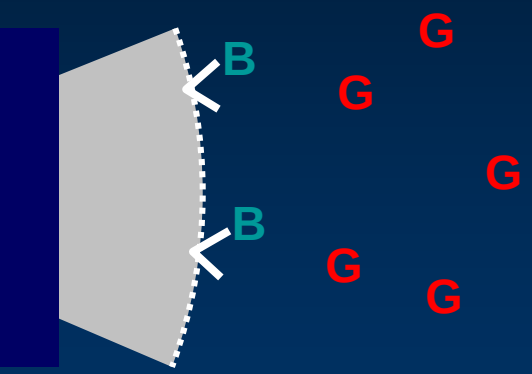
Measuring Response to Pharmacological Stimuli

HBSS then GABA

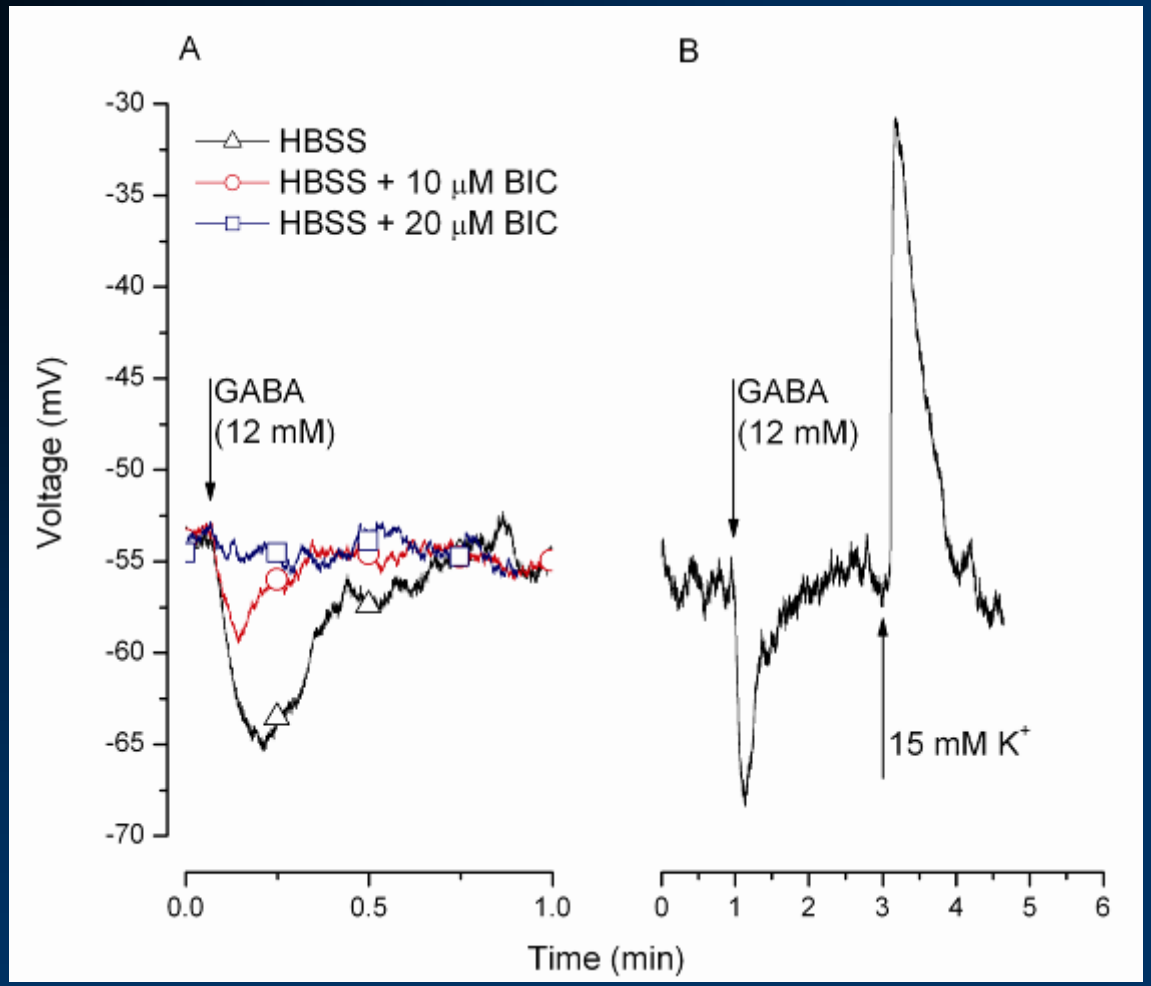


Hyperpolarization

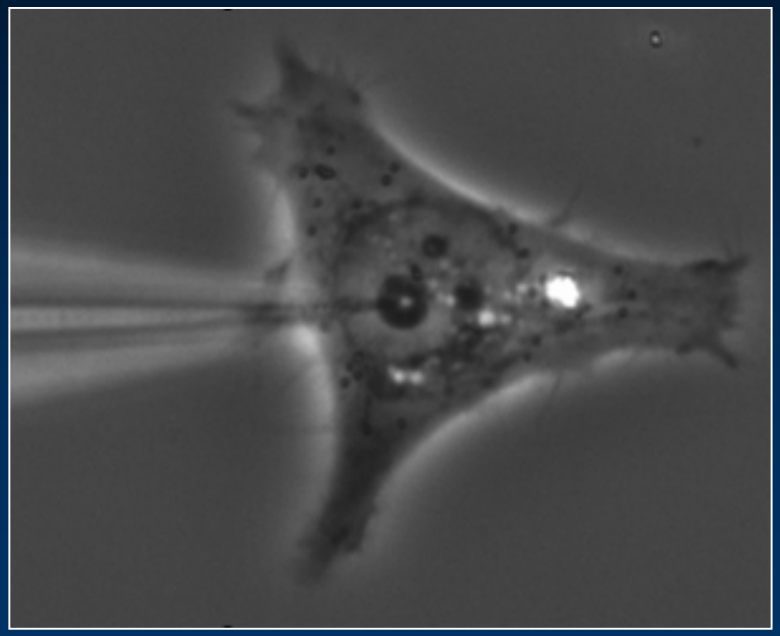
HBSS + BIC then GABA

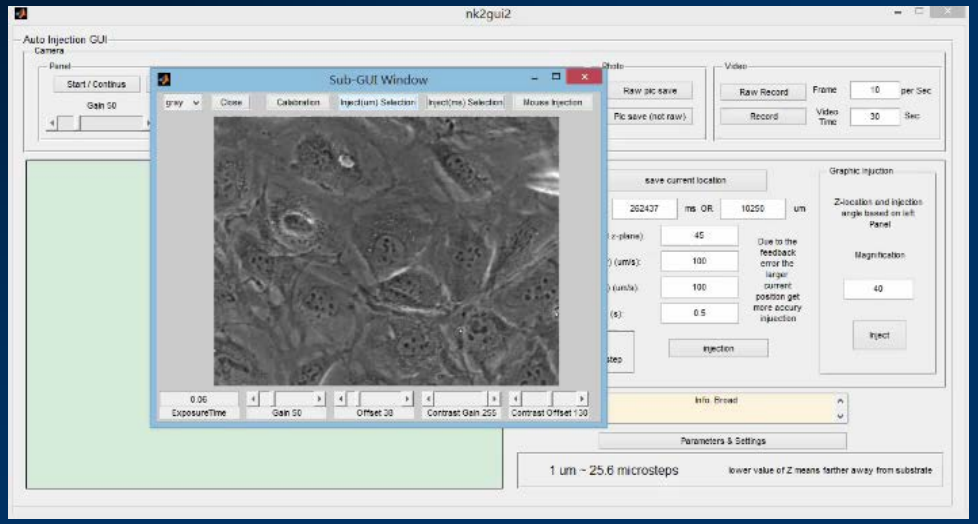


No change



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





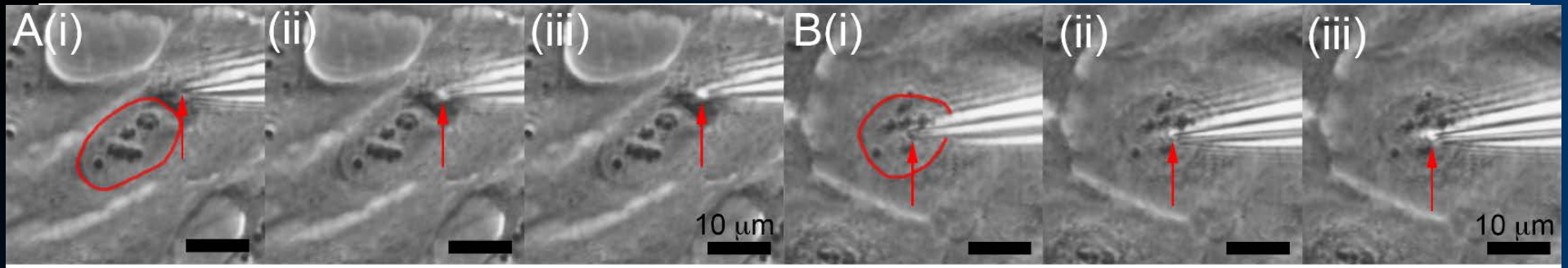
Motivation

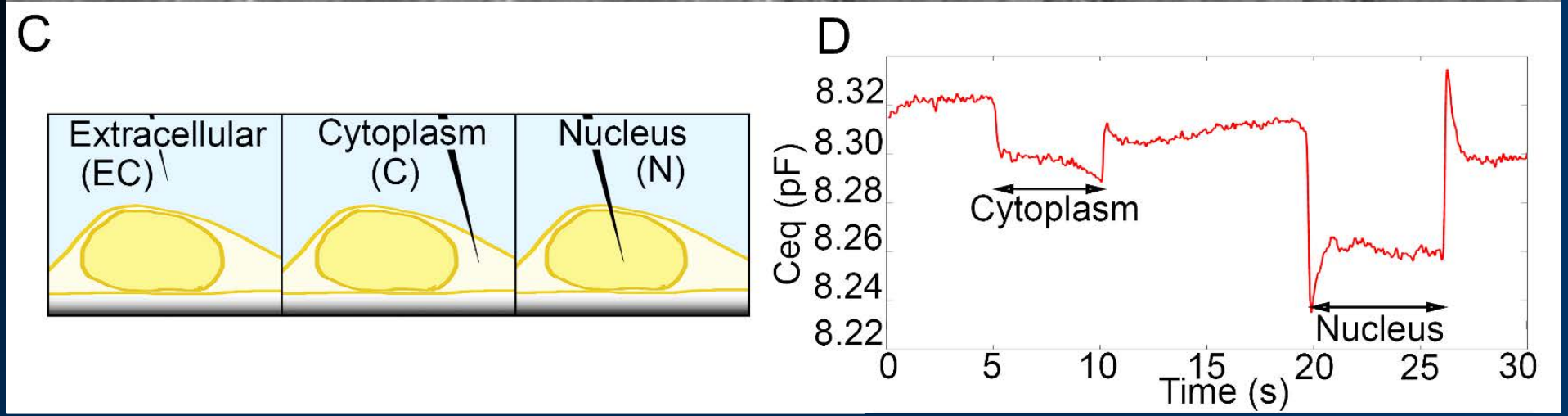
Microinjection provides about the only means to controllably introduce reagents with known compositions at a known time into cells to enable dynamic studies of cell functions

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

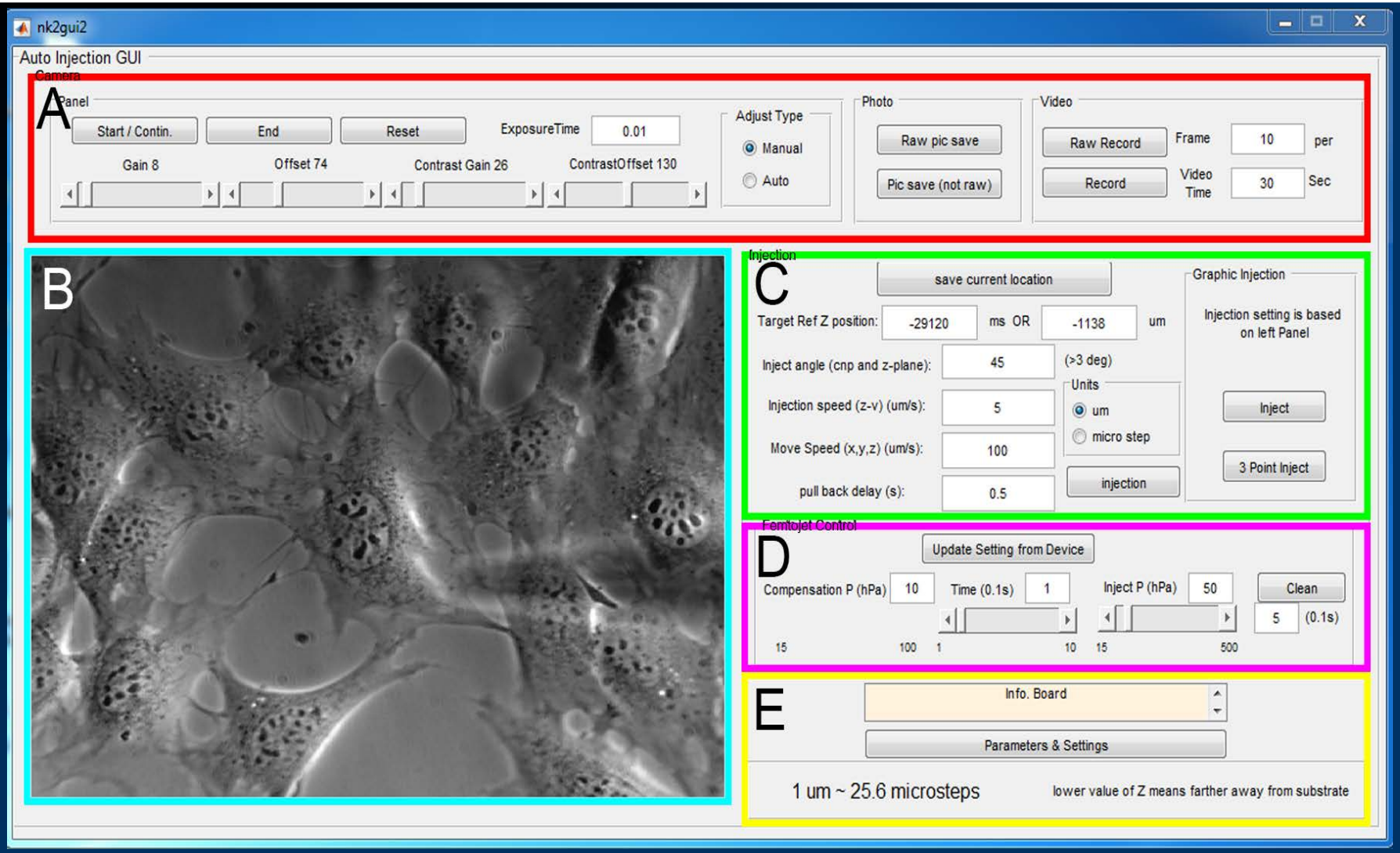




Anderson, S., and Bau, H. H., 2014, Electrical Detection of Cellular Penetration during Microinjection with Carbon Nanopipettes, Nanotechnology 25, 245102

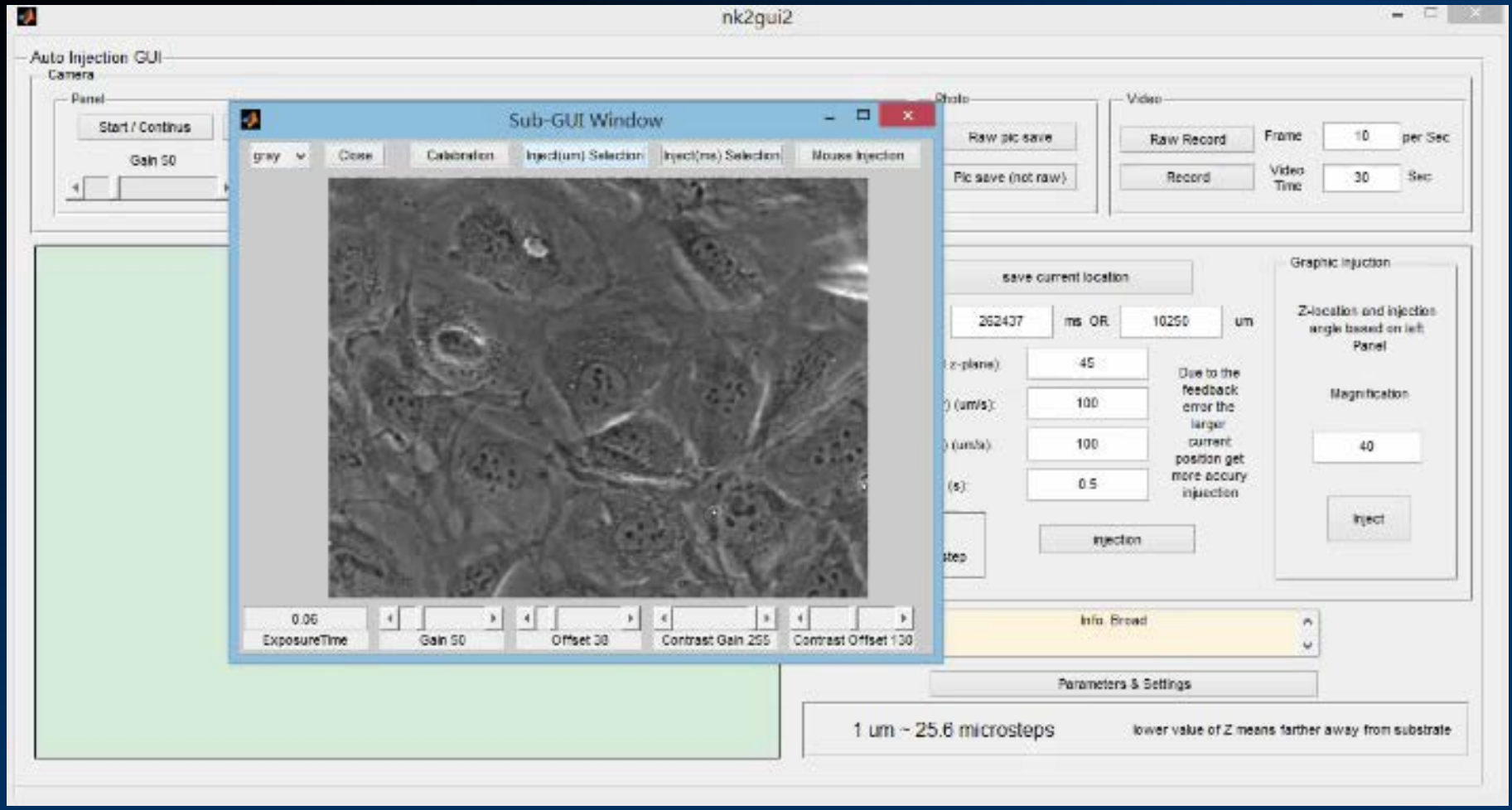
Anderson, S., and Bau, H. H., 2015, Carbon Nanoelectrodes for Single-Cell Probing, Nanotechnology **26**, 185101

Upenn semi-automated injection system

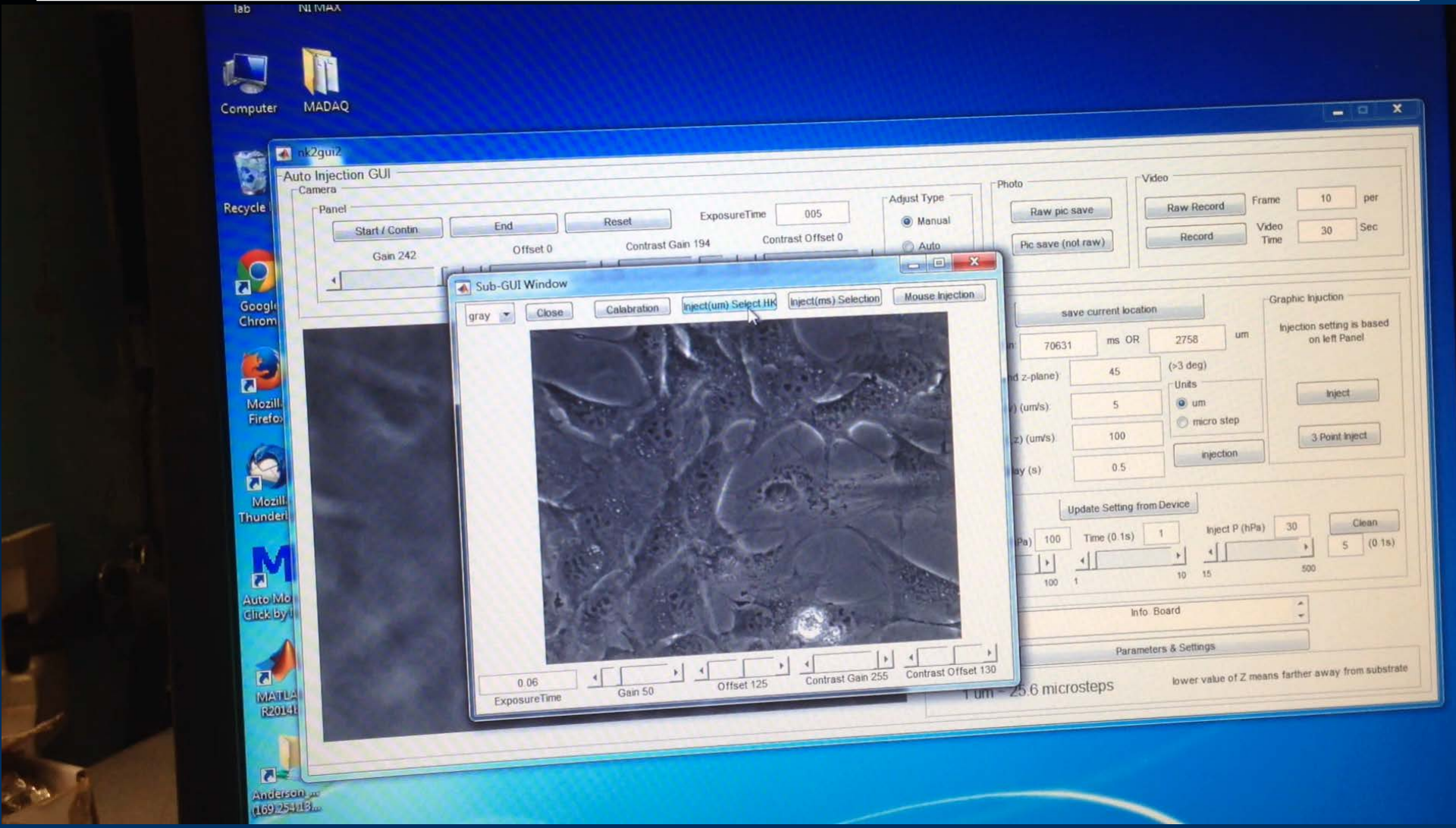


Operator marks desired injection points on the computer screen. The system calculates an optimal path for the injection probe

Computation of Microinjector's Trajectory



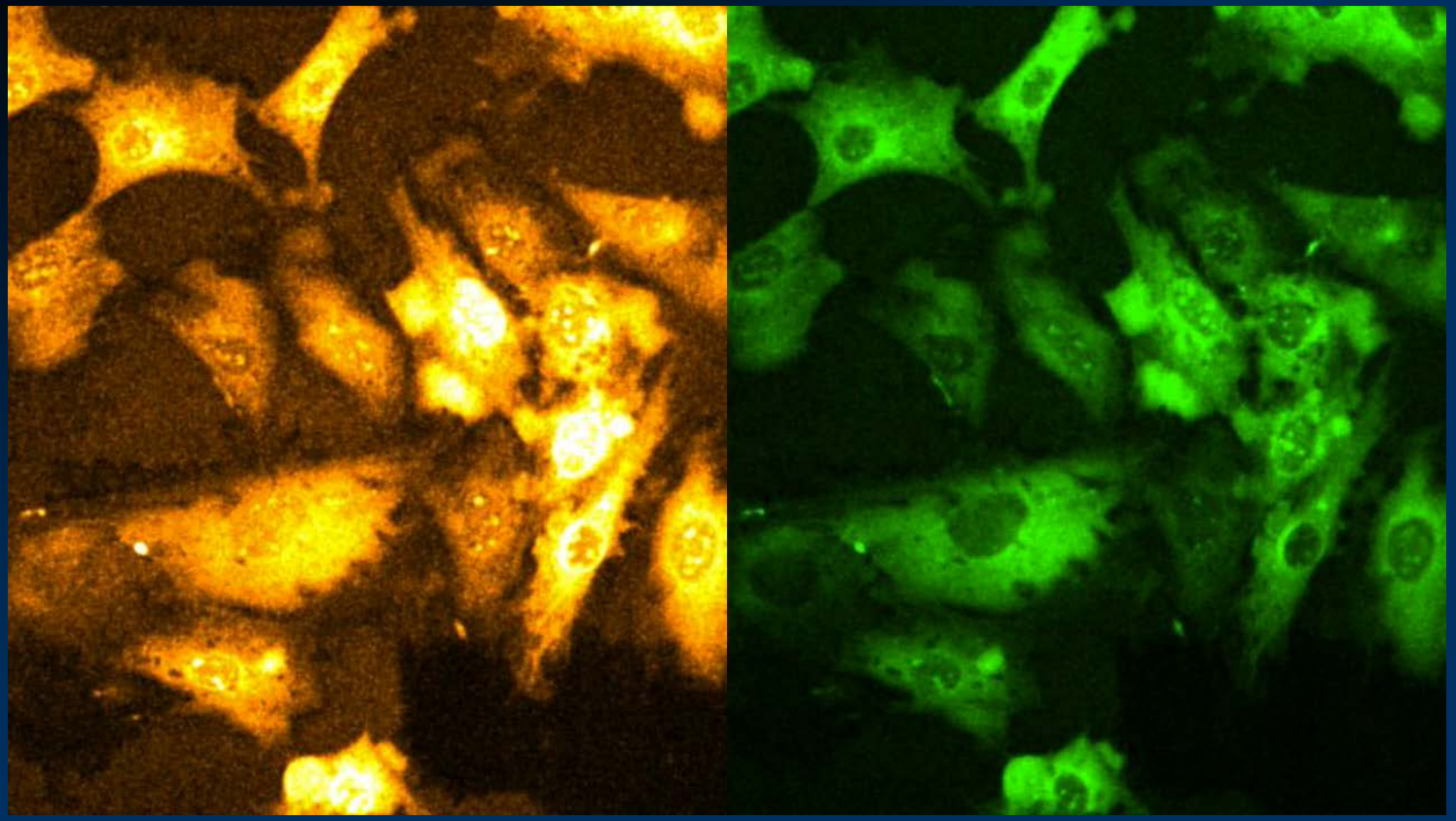
Semi-automated Injection with Electrical Feedback



tRNA – more than protein synthesis

Stressor-Induced tRNA translocation

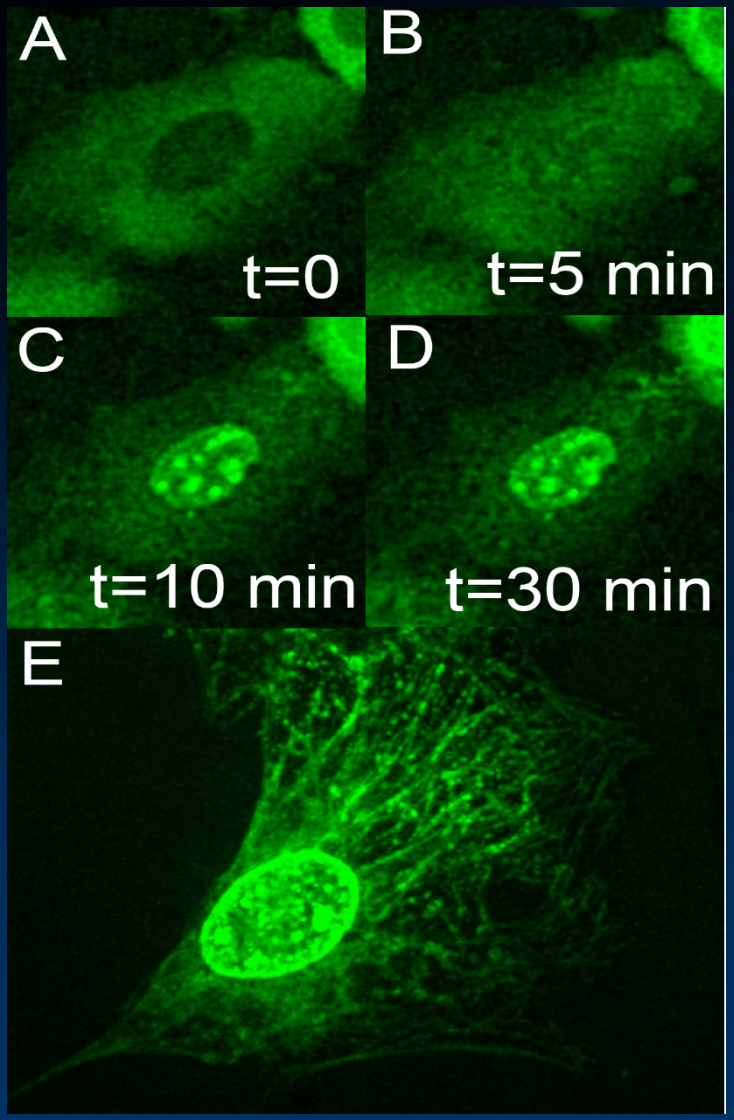
Amino Acid Deprivation

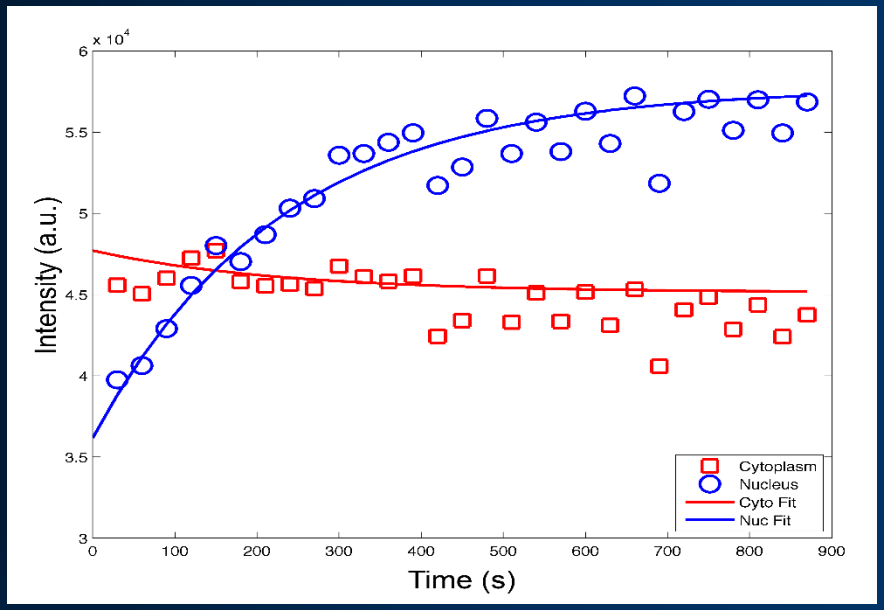


Cy3-labeled bulk tRNA

Rhodamine -labeled bulk tRNA

tRNA Trafficking Dynamics – Amino Acid Deprivation





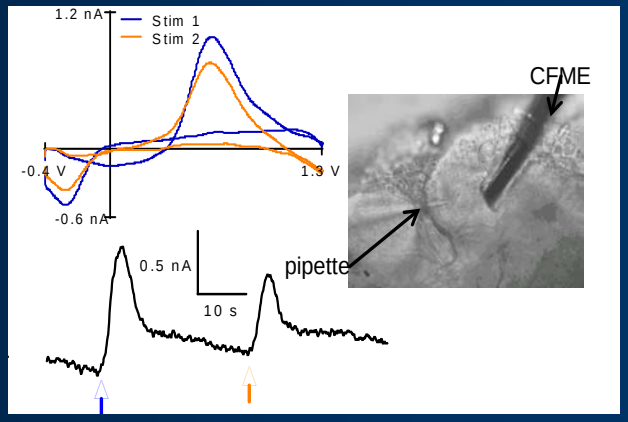
$$V_N \frac{dC_N}{dt} = AK_C C_C - AK_N C_N.$$

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

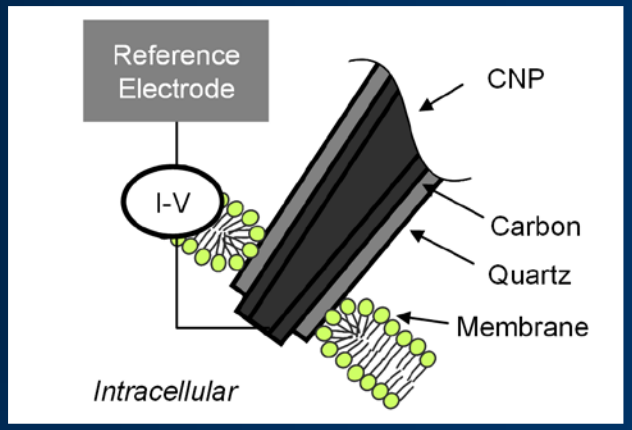
$$\frac{C_N(t)}{C_C(t)} = \frac{\left(1-e^{-(k_N+k_C\varphi)t}\right)}{\left({}^{k_N}/_{k_C}+\varphi e^{-(k_N+k_C\varphi)t}\right)}$$

Other Applications of CNPs

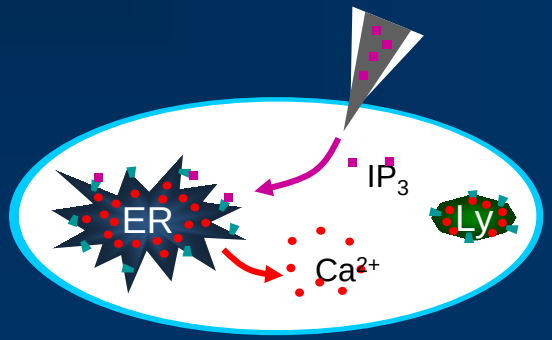
- Monitoring neurotransmitters concentrations in the *Drosophila* Brain.
- H. R. Rees, S. E. Anderson, E. Privman, H. H. Bau, & B. J. Venton, 2015, Carbon nanopipette electrodes for dopamine detection in Drosophila. Analytical Chemistry *87* (7), 3849-3855



- Monitoring function of ion channel blockers through cell electrophysiology
- Schrlau, M., Dun, N., and **Bau, H. H.**, 2009, Cell Electrophysiology with Carbon Nanopipettes, ACS Nano 3 (3), 563-568

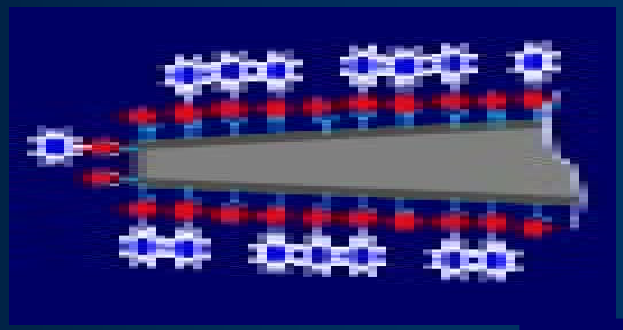


- Studying the role of secondary messengers in calcium release in the cell
- Schrlau M, Brailoiu, E., Patel, S., Gogotsi, Y., Dun, N., and Bau, H. H., 2008, Carbon Nanopipettes Characterize Calcium Release Pathways in Breast Cancer Cells, Nanotechnology 19, 325102

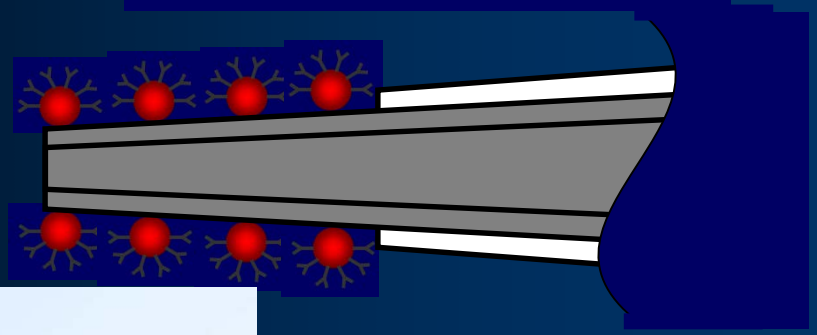


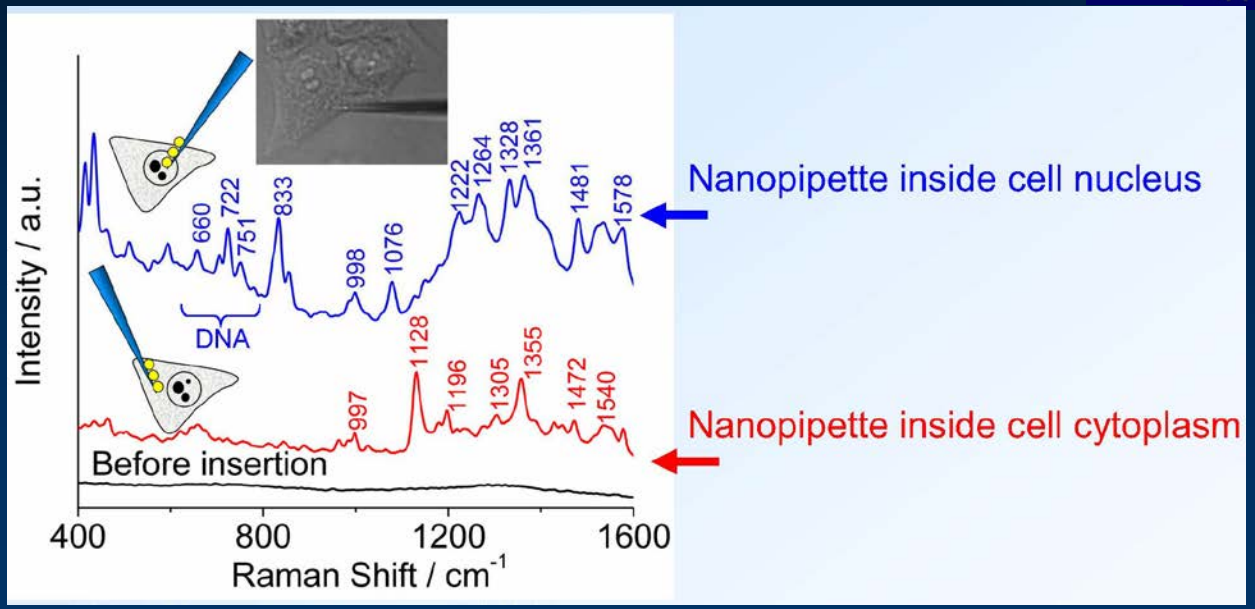
Biosensors

Direct functionalization of the CNP's tip



Attachment of nanopraticles to the CNP tip

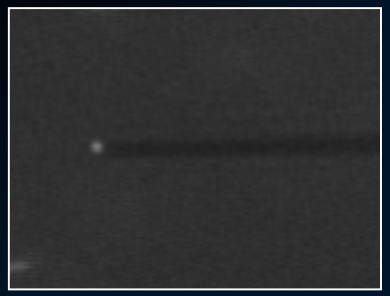


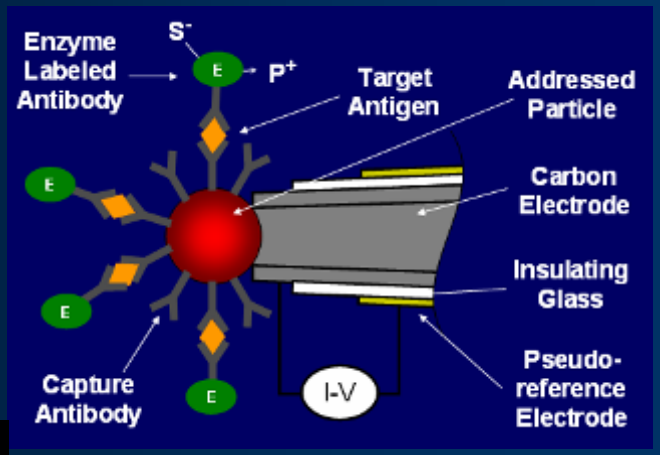


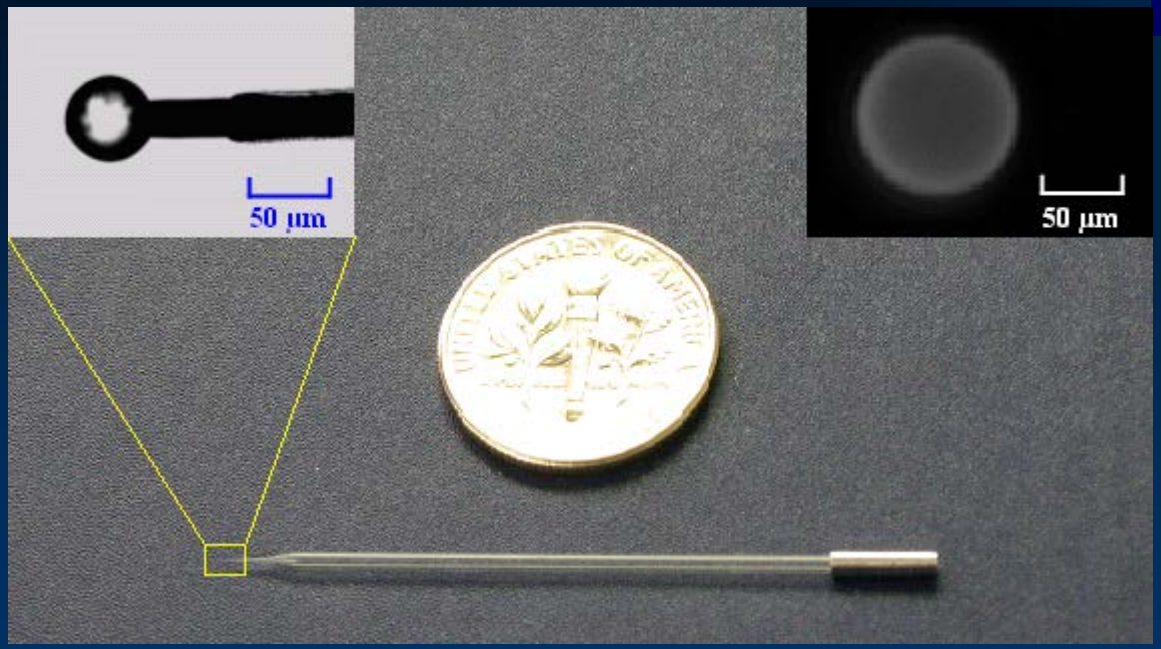
Raman Shift inside cells (Gogotsi Group Drexel)

Single Bead-Based Electrochemical Sensor

Attachment of a single functionalized bead

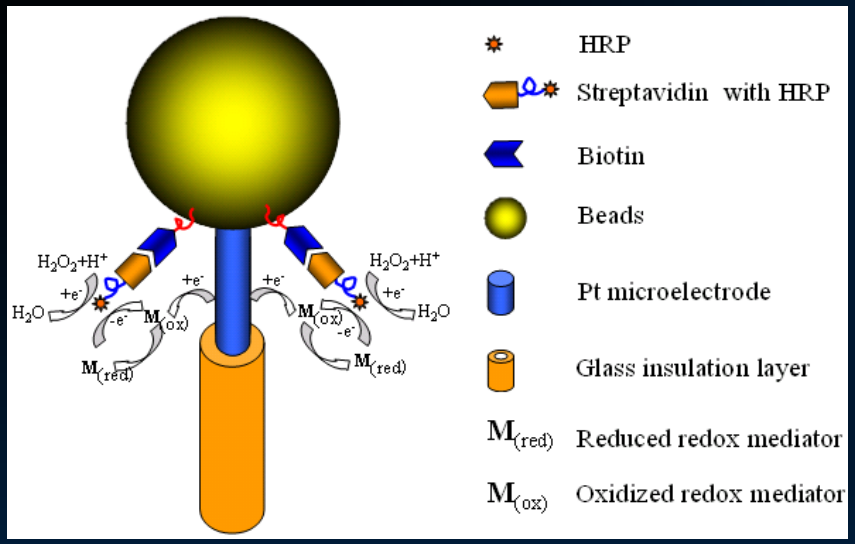


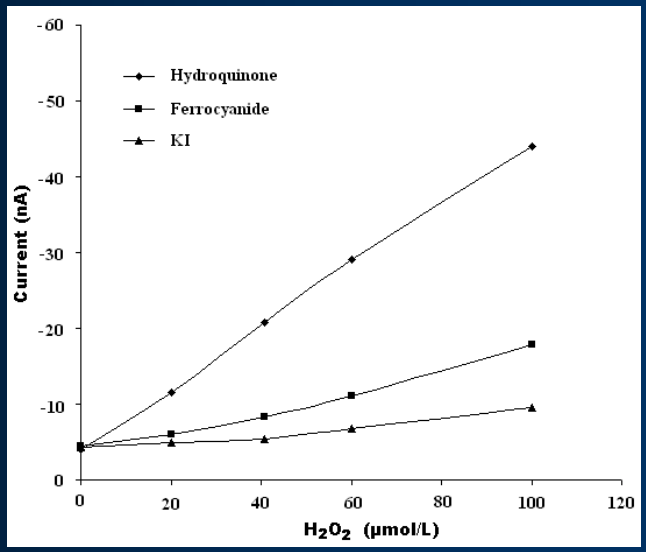


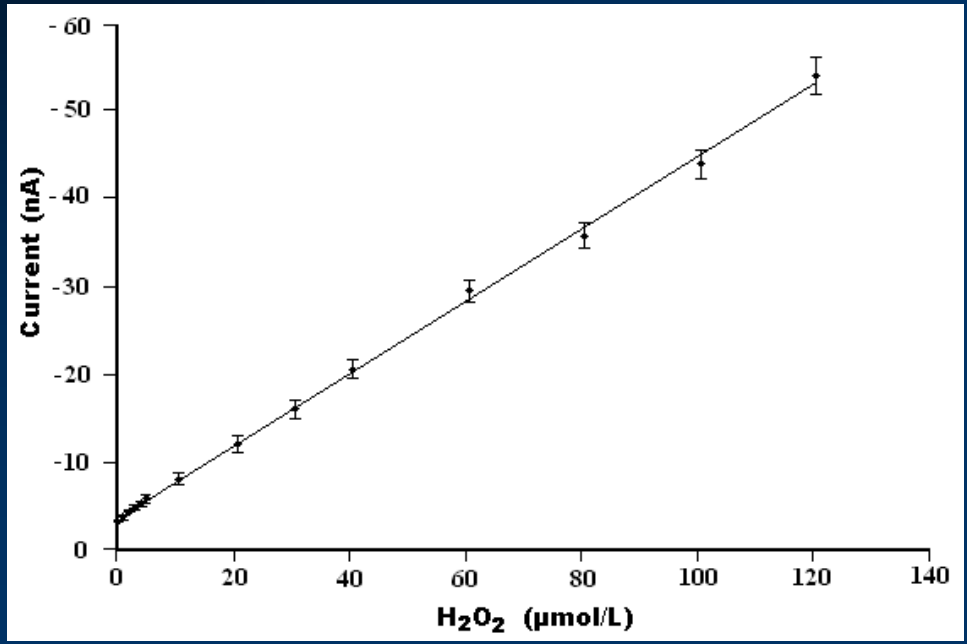


Liu, C., Schrlau, M., Bau, H. H., 2009, Single bead-based electrochemical biosensor, to appear in Biosensors and Bioelectronics.

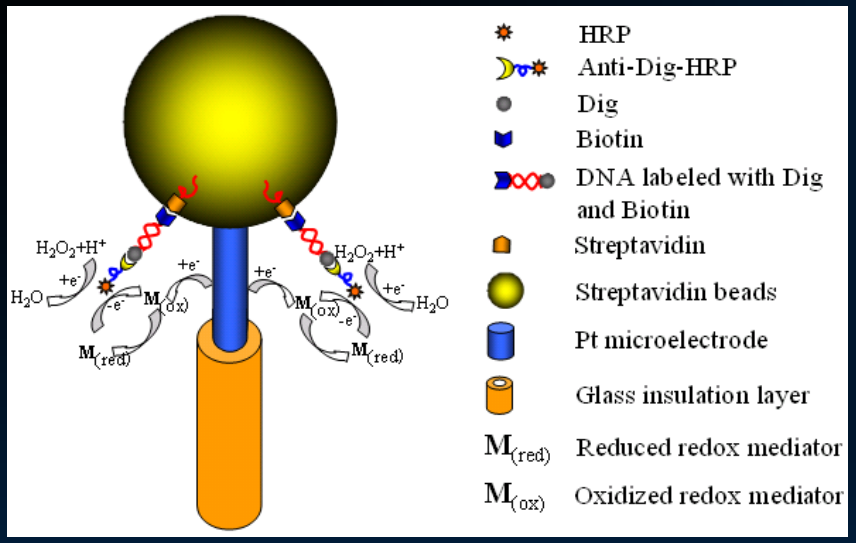
Detection of H_2O_2

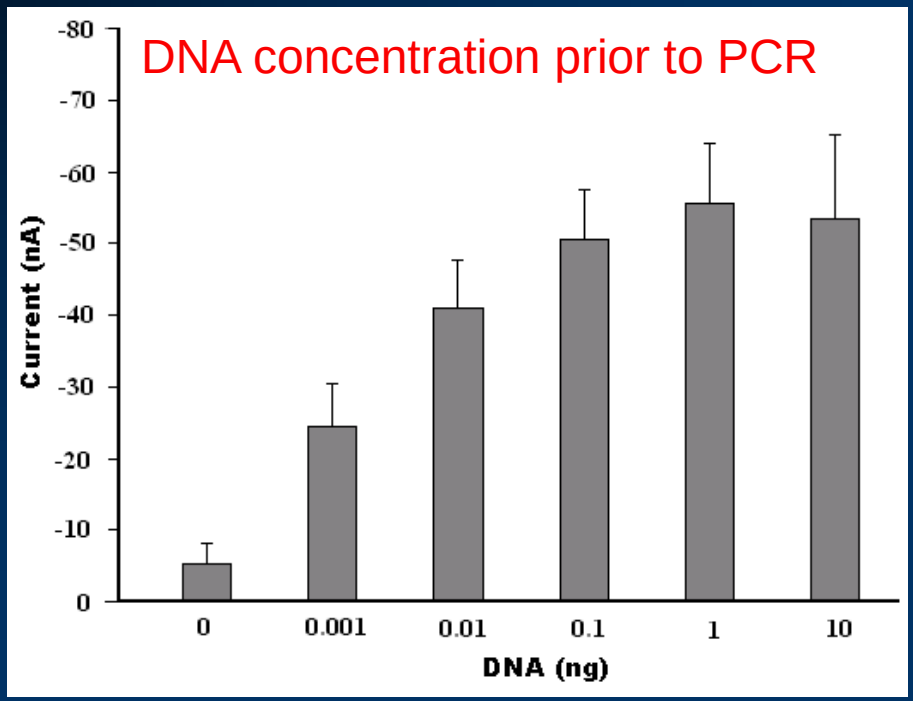






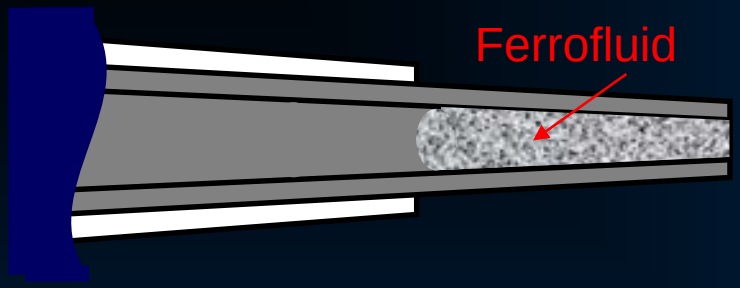
Detection of DNA



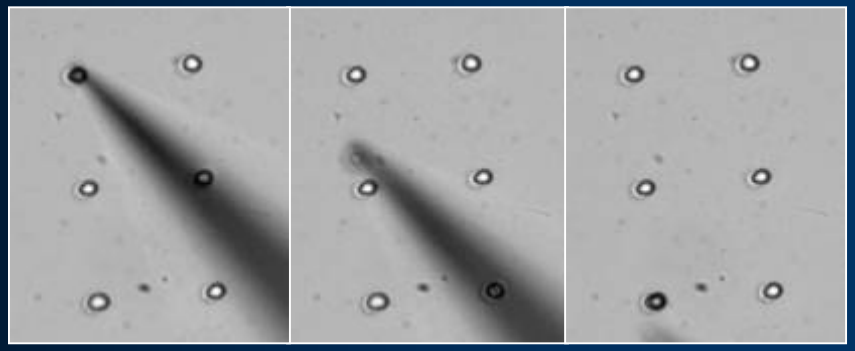


CNPs as Magnetic Manipulators

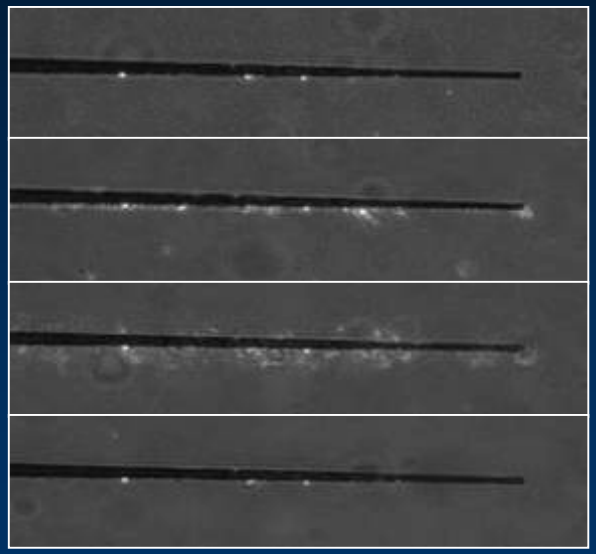
Superparamagnetic (SPM) CNP*

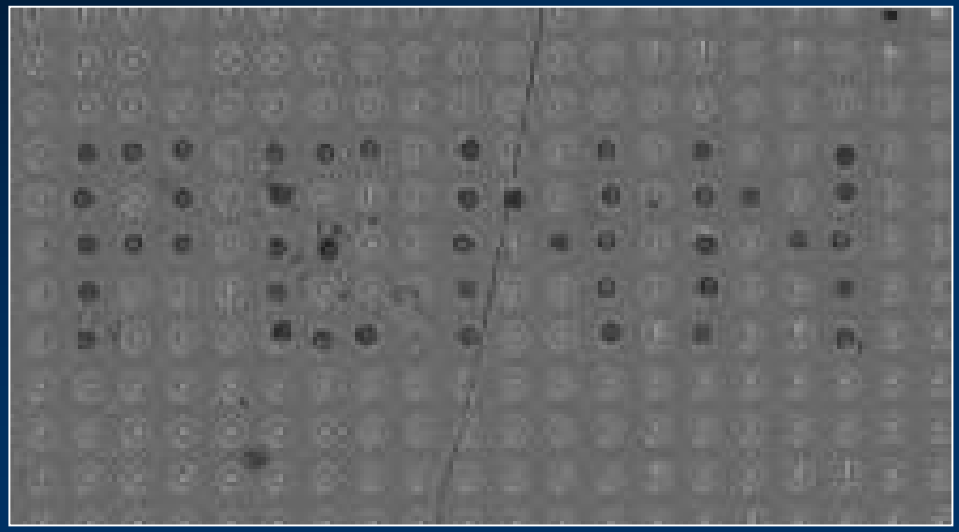


Capturing & Manipulating Microparticles & Arrays



Capturing SPM Nanoparticles (150nm dia.)

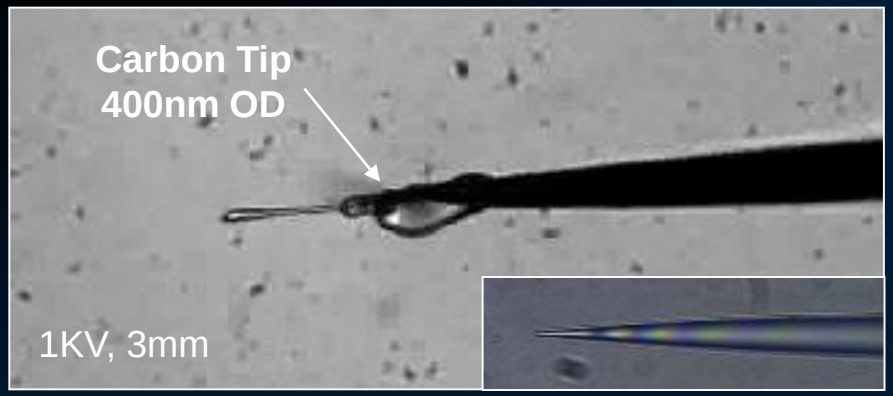




**B. Polyak, Surgery, Drexel University College of Medicine, Philadelphia, USA*

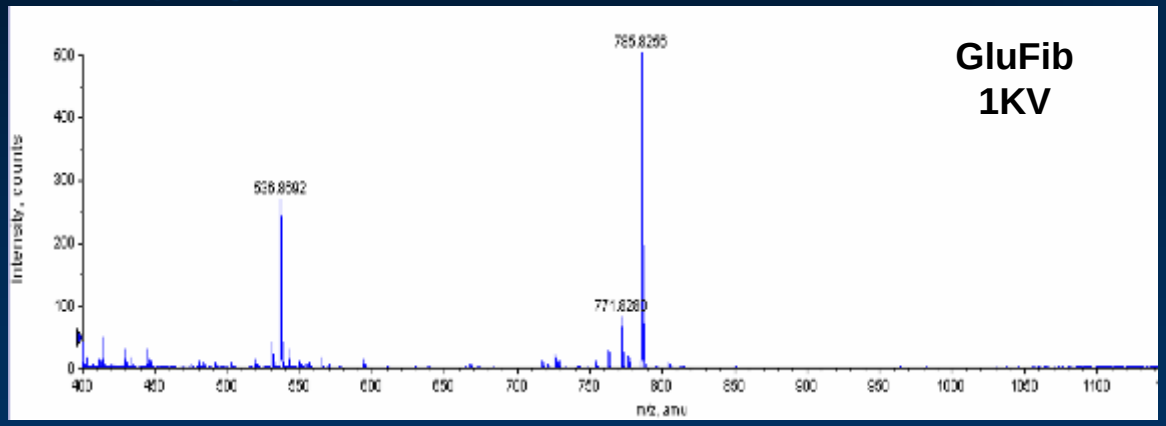
Electrospray Applications

Electrodripping†



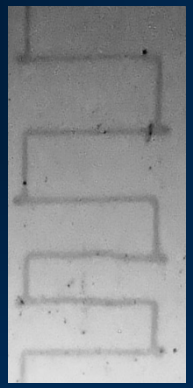
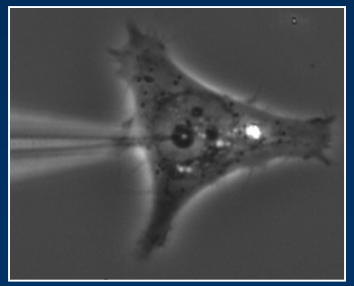
Electrospinning †‡

Electrospray Ionization ‡*



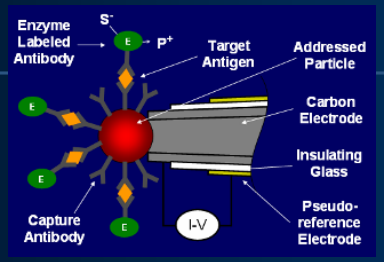
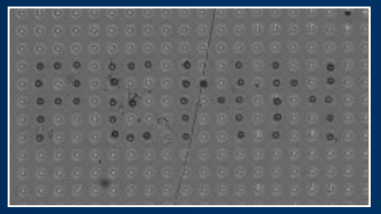
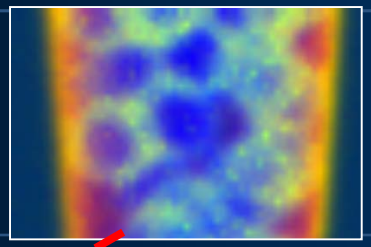
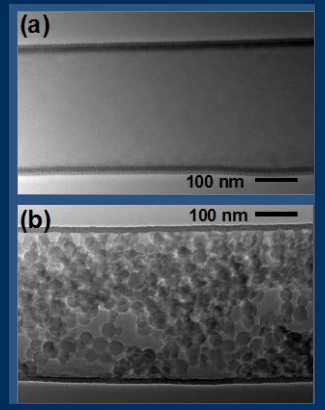
† J. Santiago-Aviles, Electrical & Systems Engineering, University of Pennsylvania, Philadelphia, USA
‡ D. Byun, Aerospace & Information Engineering, Konkuk University, Seoul, Korea
**C-X Yuan, Proteomics Core Facility, University of Pennsylvania, Philadelphia, USA*

POTENTIAL APPLICATIONS OF CNPs

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

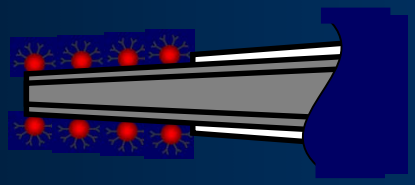
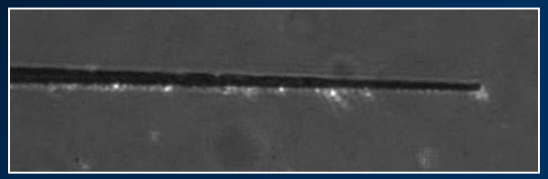
D. Byun, Aerospace & Information Engineering, Konkuk University, Seoul, Korea

POTENTIAL APPLICATIONS (CONTINUED)

Application	Description	
Particle manipulators	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 Tyliszcak, 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