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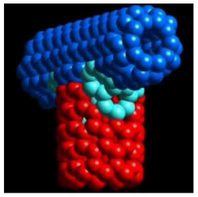
Nanotechnology in Biomedical Applications



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Acknowledgement: Hua Chen, Prabhu Arumugam, Jun Li, Russell Andrews, Jing Li, Y. Lu, David Loftus, Pho Nguyen

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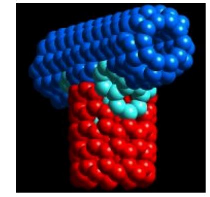
Outline



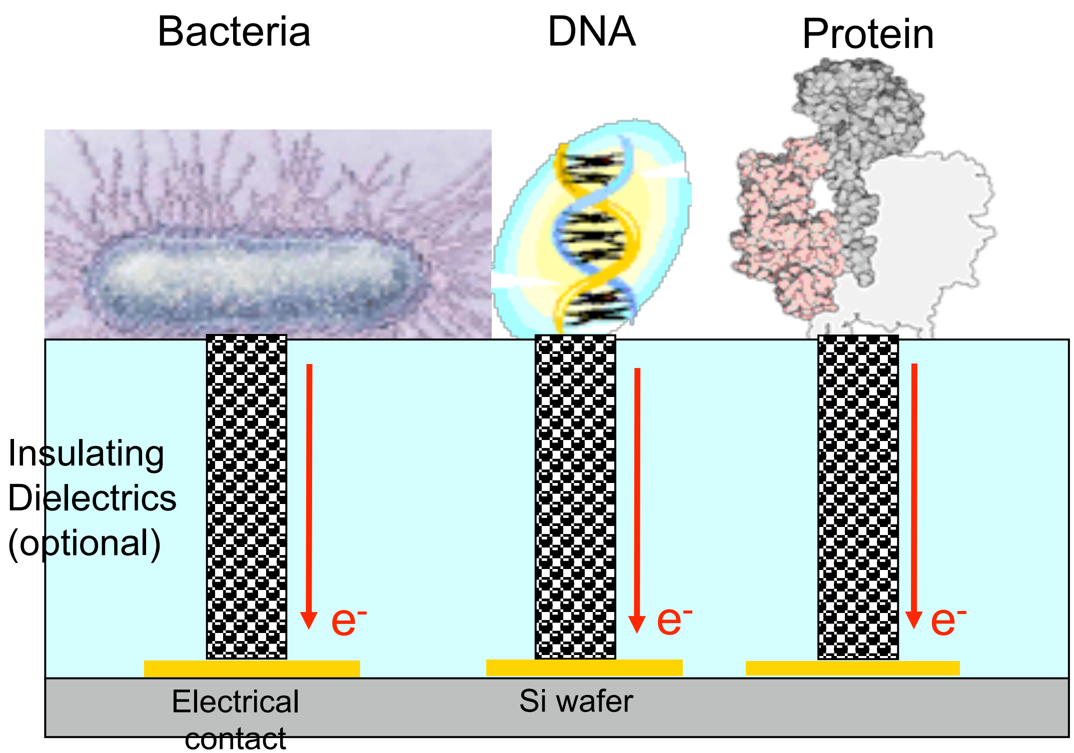
- Carbon Nanofiber (CNF) Nanoelectrode Array for Biosensors

- CNF Nanoelectrode Array for Deep Brain Stimulation

- Gas/Vapor Sensors for Medical Diagnosis

- CNT in Ophthalmological Applications

Nanoelectrode Array for Biosensors



Directly interface solid-state electronics with DNAs, RNAs, proteins, and microbes in a miniaturized multiplex chip for quick detection (Lock and Key approach)

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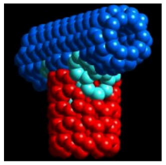
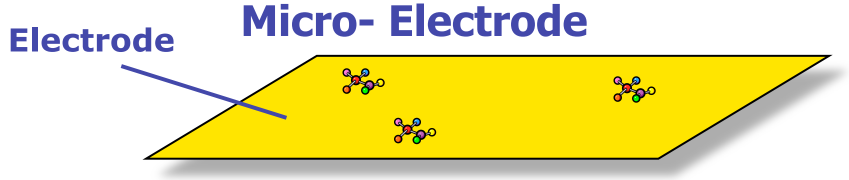
Nanoelectrode for Sensors

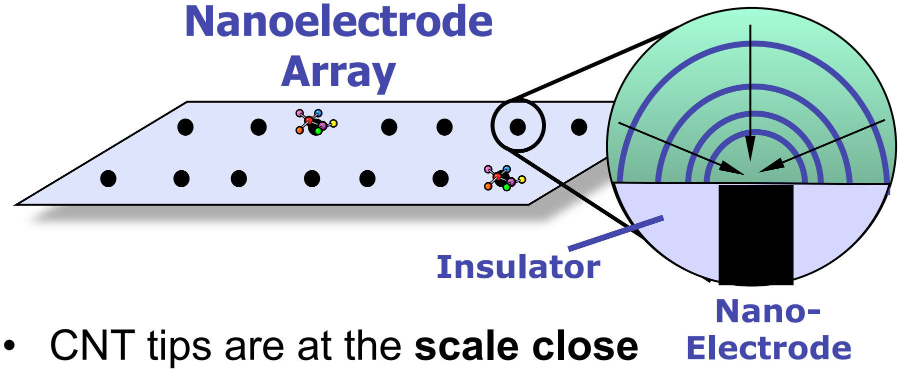


Nanoscale electrodes create a dramatic improvement in signal detection over traditional electrodes

Traditional Macro- or Micro- Electrode



Nanoelectrode Array



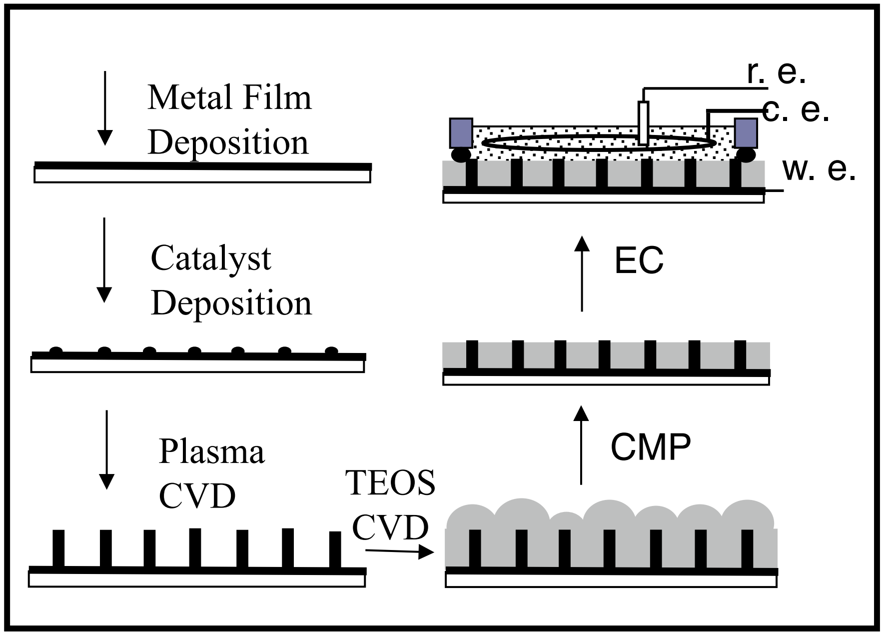
- **Scale difference** between macro-/ micro- electrodes and molecules is tremendous
- **Background noise** on electrode surface is therefore significant
- **Significant amount** of target molecules required

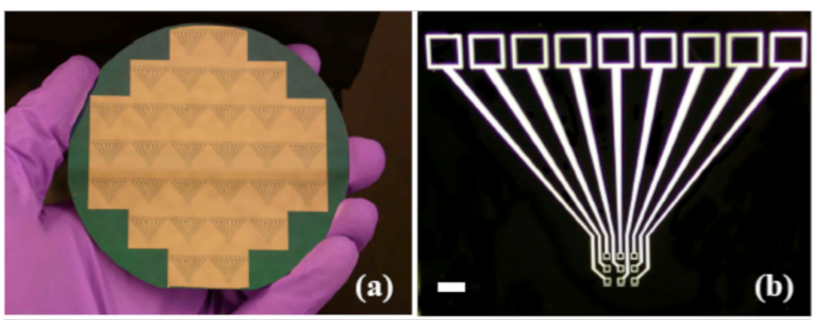
- CNT tips are at the **scale close to** molecules
- Dramatically **reduced background noise**
- Multiple electrodes result in **magnified signal** and **desired redundance** for statistical reliability.

Candidates: ~~SWNTs~~, ~~MWNTs~~, Vertical CNFs or Vertical SiNWs

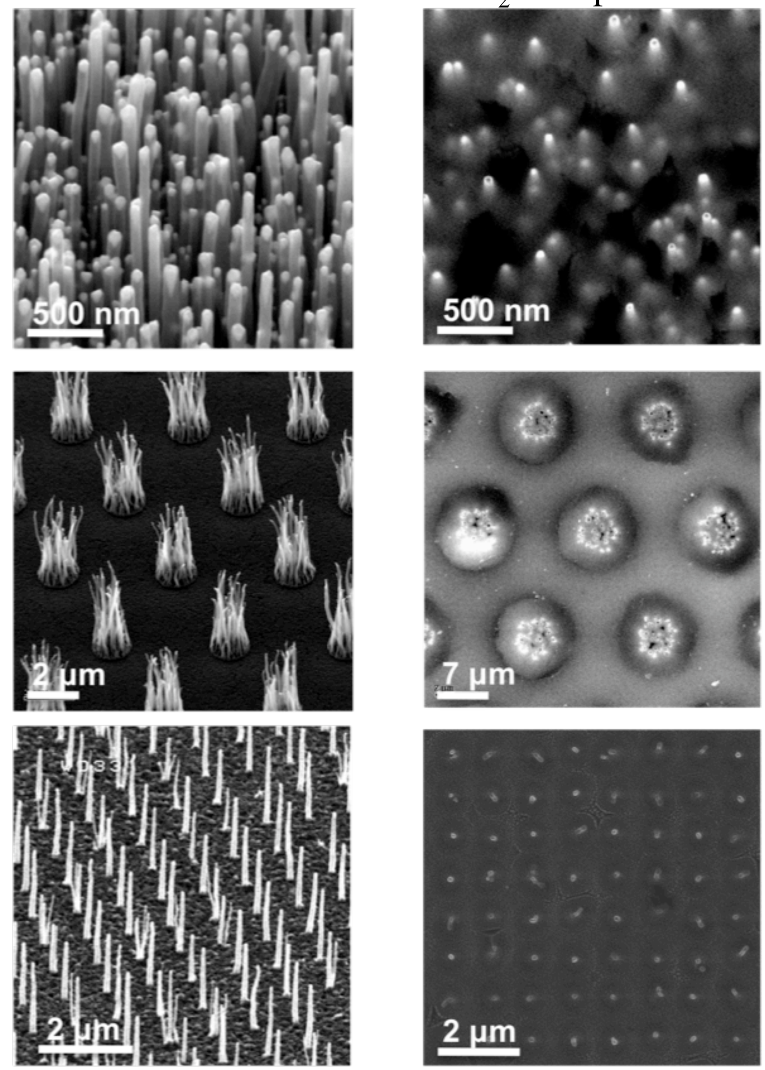
Source: Jun Li

Nanoelectrode Array Fabrication





As Grown CNFs | SiO_2 Encapsulated



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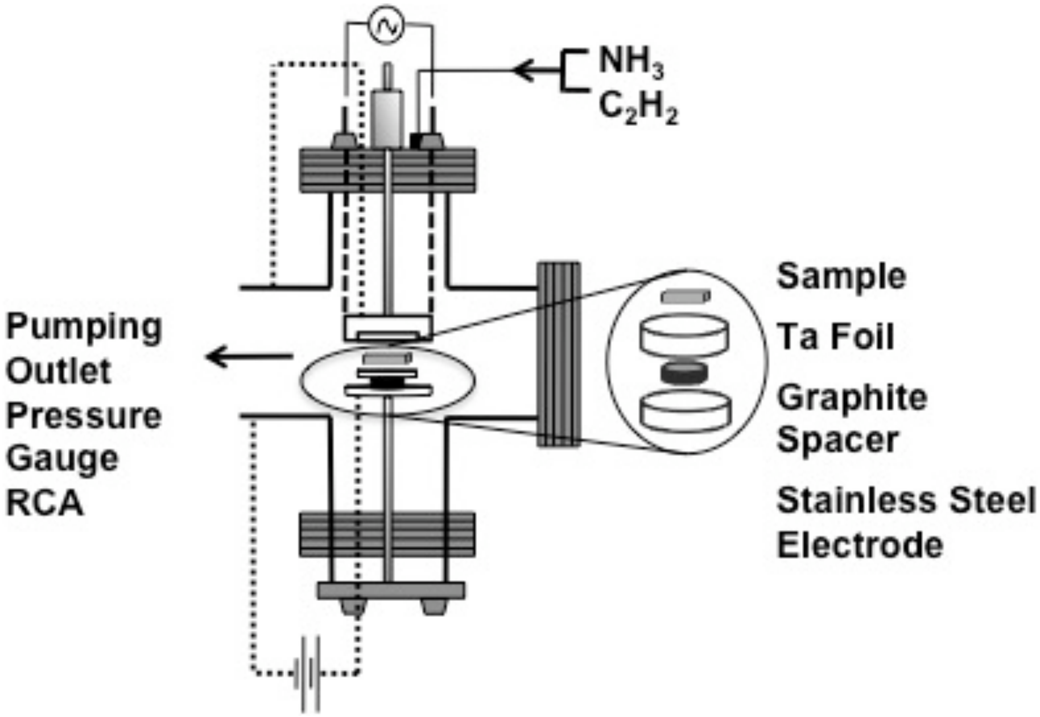
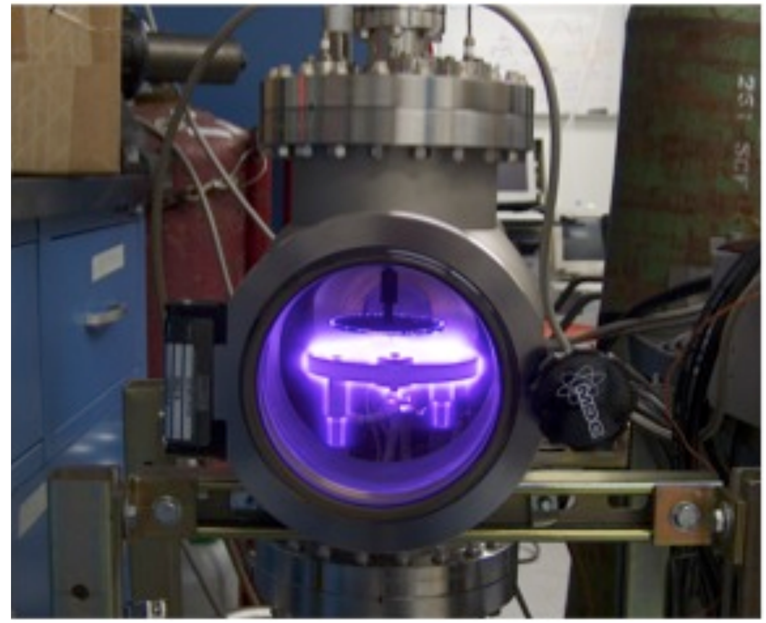
CNF Growth by
Plasma Enhanced Chemical Vapor Deposition (PECVD)

PECVD Reactor Schematic



Custom Built PECVD Reactor



<u>Growth Process</u>

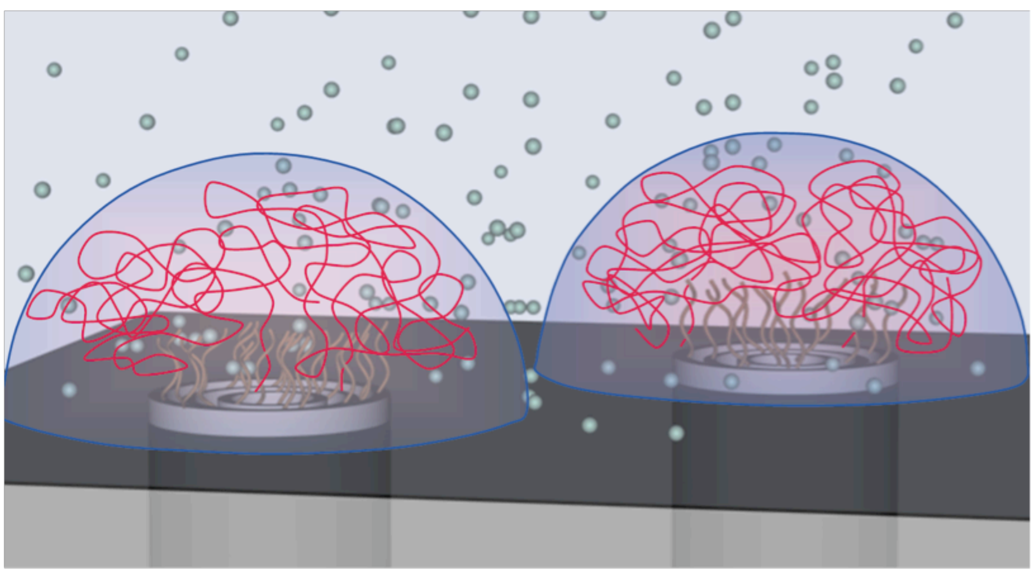
- Heated to 650 C
- Plasma discharge 500 W, 530 V, 0.97 A
- 150 sccm NH_3/50 sccm C_2H_2, 5-6 torr
- Growth rate- 1000 nm/min
- Quality is good, alignment is good

Cruden, B., et al., *Appl. Phys. Lett.* **2003**, 94, 4070-4078.

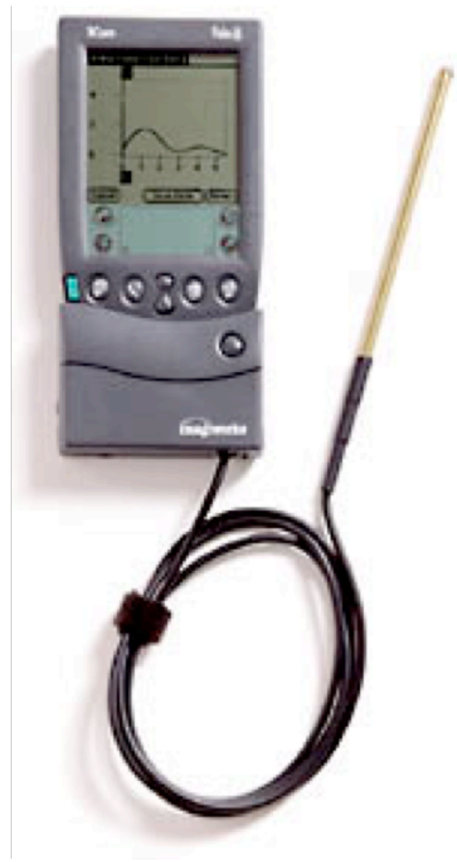
Potential for Cancer Diagnostics

- Probe molecule that would serve as signature for specific cancer cells to be attached to CNF ends

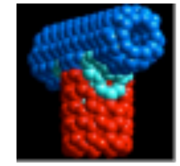
- Current flow upon hybridization through CNF electrode to signal processing IC chip.



CNF-based biosensor for cancer diagnostics

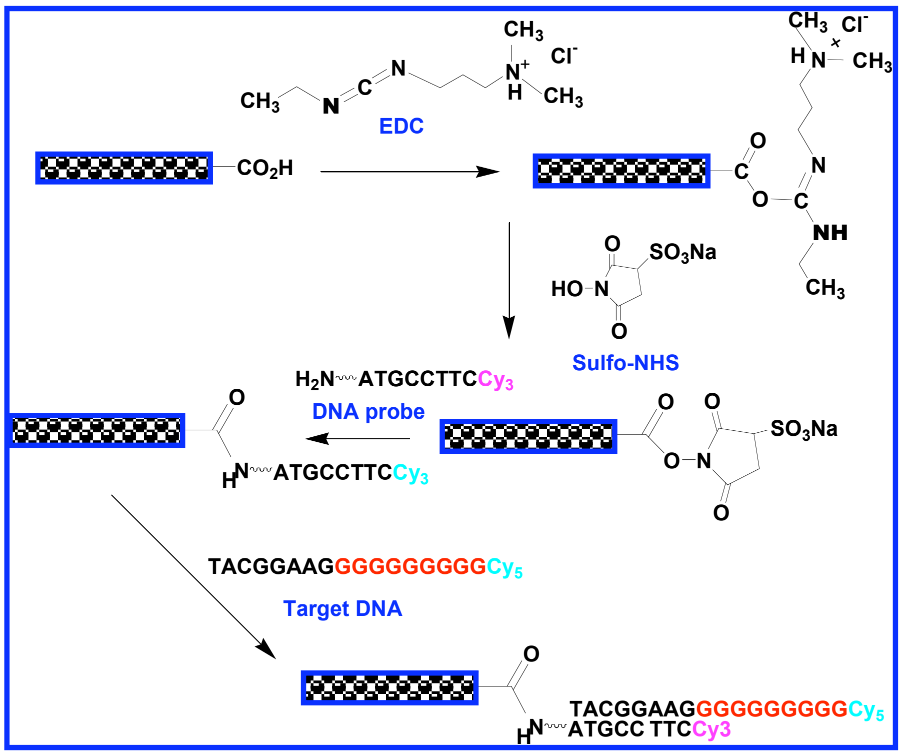


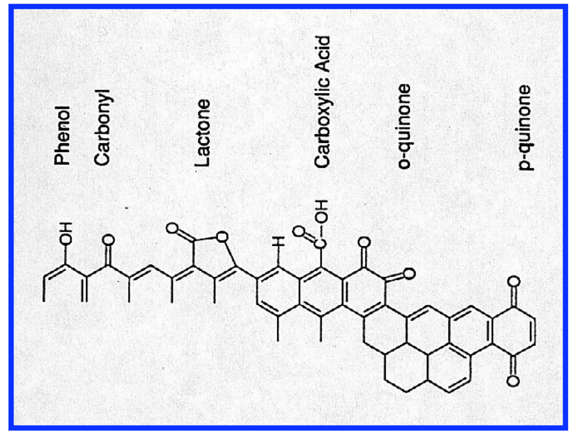
CNF Functionalization

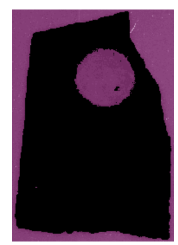


Electrochemically produce carboxylic acid groups on the surface in 1M NaOH (1.5 V 60 sec etch)

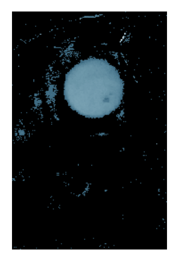
Highly selective reaction of primary amine with surface carboxylic acid group







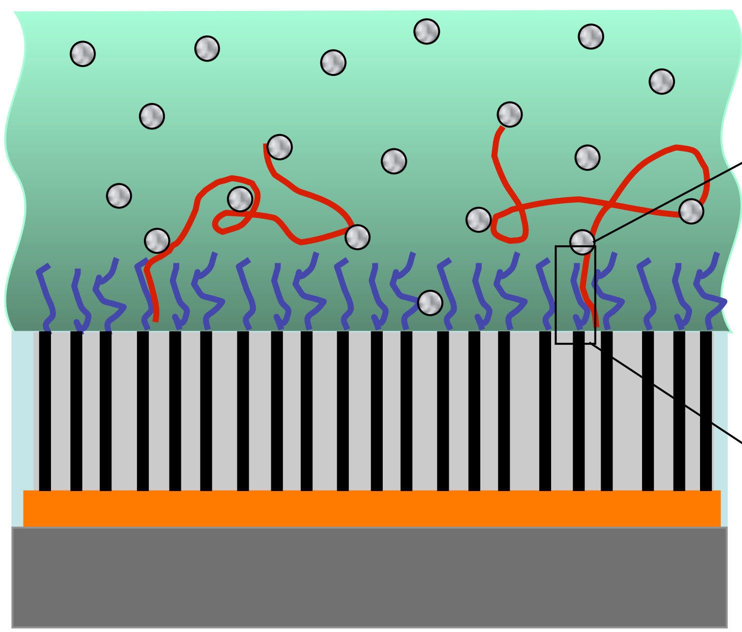
Cy3 Scan: Probe DNA

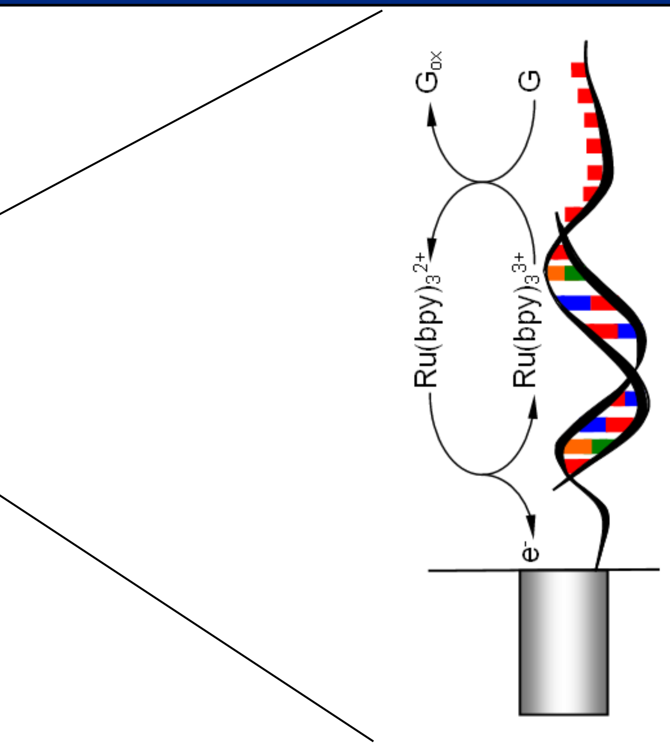


Cy5 Scan: Target DNA

C. Nguyen et al, Nanolett., 2002, 2, 1079.

Electrochemical Detection Methodology





- **CNF array electrode functionalized with DNA probe as an ultrasensitive sensor for detecting the hybridization of target DNA from the sample.**
  - Signal from redox bases (Guanine) in the excess DNA single strands
- **The signal can be amplified with metal ion mediator.**

Electrochemical Detection of DNA Hybridization By AC Voltammetry

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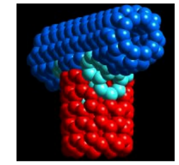
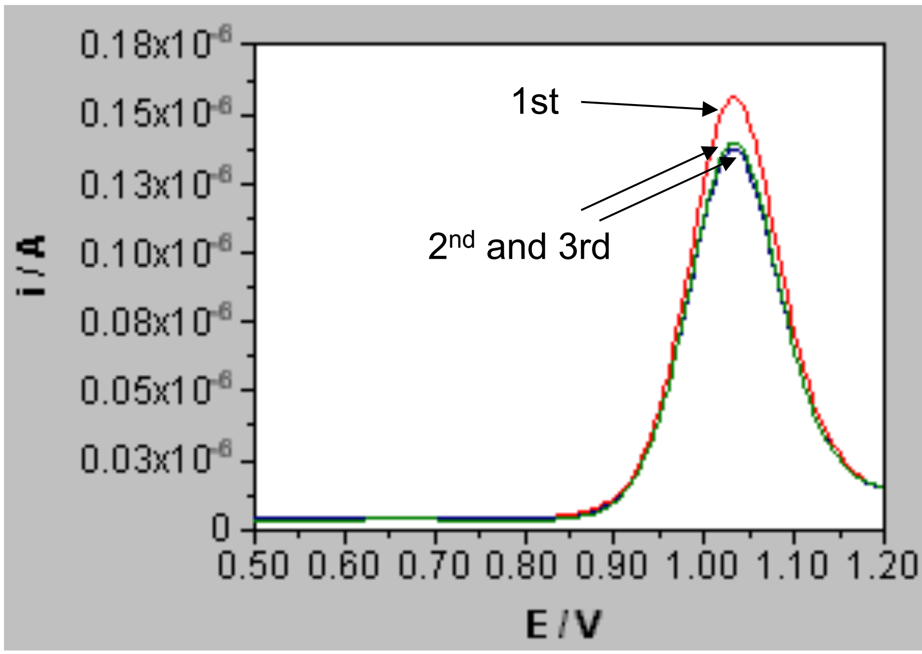
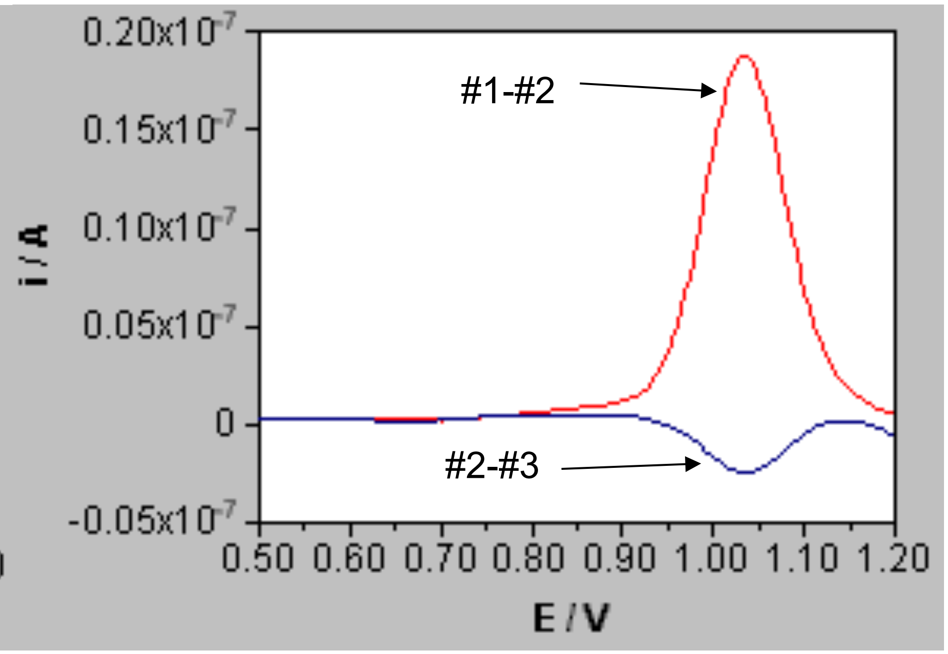






1st, 2nd, and 3rd scan in AC voltammetry

1st – 2nd scan: mainly DNA signal

2nd – 3rd scan: Background

Lower CNF Density ⇒ Lower Detection Limit

J. Li, H.T. Ng, A. Cassell, W. Fan, H. Chen, J. Koehne, J. Han, M. Meyyappan, NanoLetters, 2003, Vol. 3, p. 597.

Deep Brain Stimulation

Medtronic

Deep Brain Stimulation (DBS)
Has been demonstrated to be an effective neurosurgical treatment for several pathologies including:

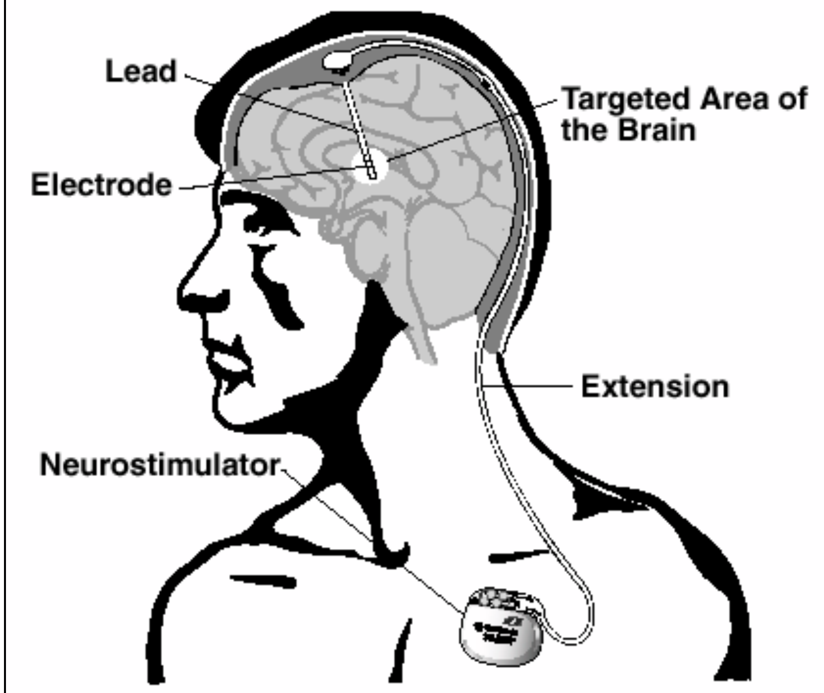
- Parkinson's disease (30,000-40,000 patients)
- chronic pain (1500-2000 patients)
- tremor (500-1000 patients)
- epilepsy (20-50 patients)
- depression (20-50 patients)
- Tourette syndrome (10-50 patients)

Expert Rev Med Devices 4:591-603, 2007

How: Four Interrelated Hypotheses
Paradox of similar effects to lesioning of target structure is explained by the following:

- Depolarization Blockage
- Synaptic Inhibition
- Synaptic Depression
- Stimulation Induced Modulation of Pathways

Current Techniques for Deep Brain Stimulation



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PROBLEMS: Indiscriminate Activation

• Stimulation indiscriminately affects all tissue around the electrode (size: 1.27mm diameter with four 1.5mm contacts)

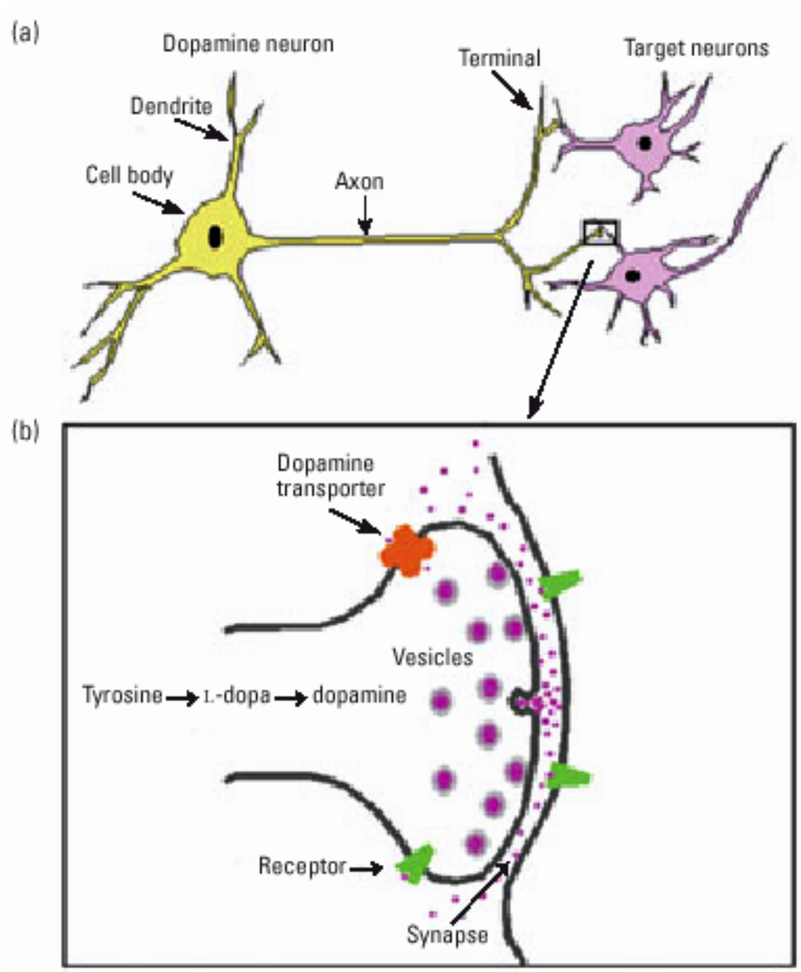
• Crude method without feedback

IMPROVEMENTS:

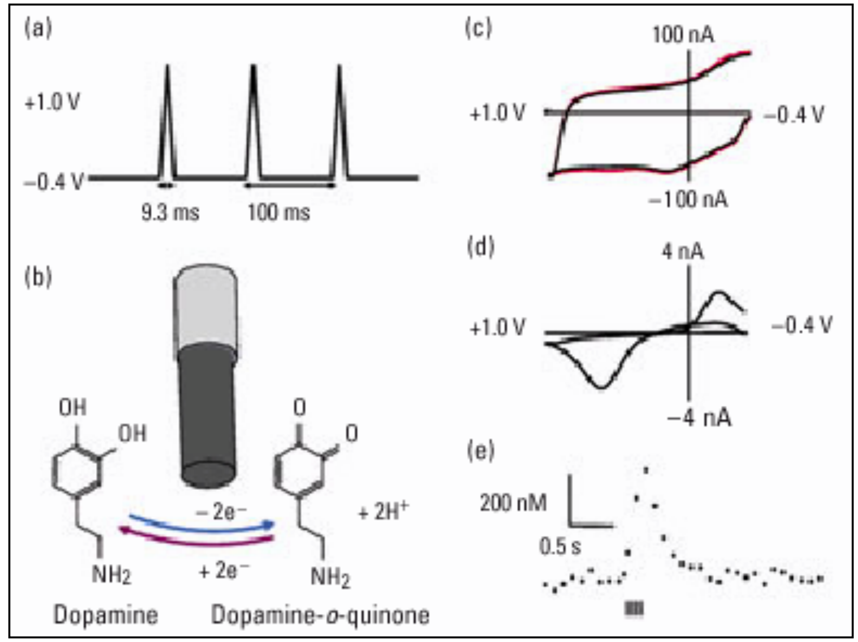
Targeted Activation to specific location down to sub mm scale

Obtain feedback information – such as neurotransmitter levels

Current Techniques for Electrochemical Monitoring of Neurotransmitters with Carbon Fiber Electrodes



R.Mark Wightman, *Analytical Chemistry*, **414A** (Oct. 2003).



HOW: Cyclic Voltammetry (CV)

Carbon fiber micro-electrodes (10um dia.)

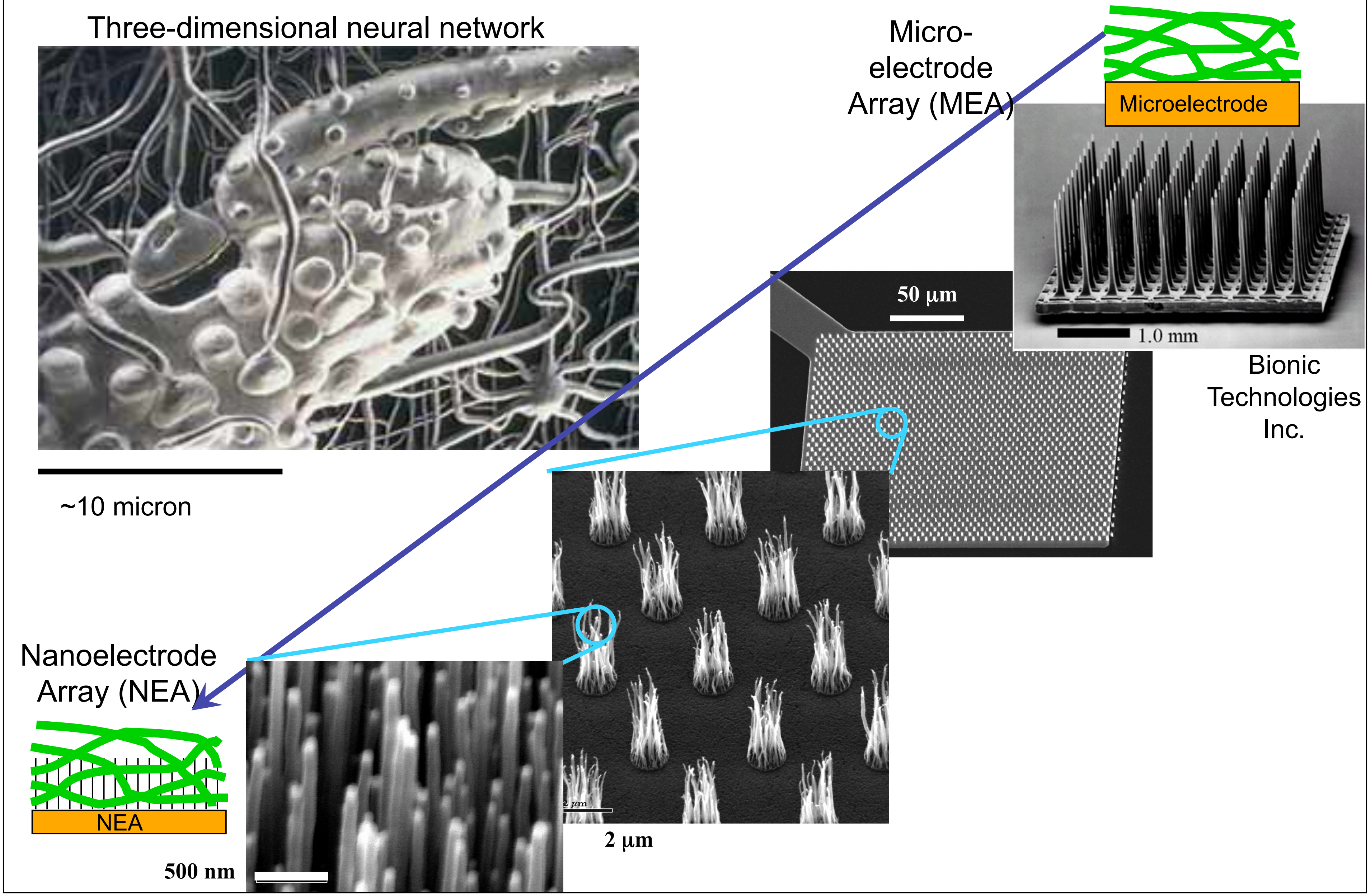
Best detection is 500nM with temporal resolution of tens of milliseconds

Most neurotransmitters are electrochemically active (i.e. dopamine & glutamate)

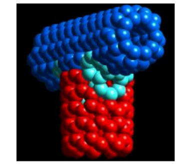
IMPROVEMENTS: Requirements for Electrodes

(1) Ultrahigh sensitivity: ~ 1 nM
(2) Fast speed: ~ 10 ms resolution
(3) Good for long-term implantation

Vertical Aligned CNF Array: A Novel Electrical Neural Interface



Goal: To Develop an Integrated Multiplex Chip as an Implantable Device for DBS and Electrochemical Recording



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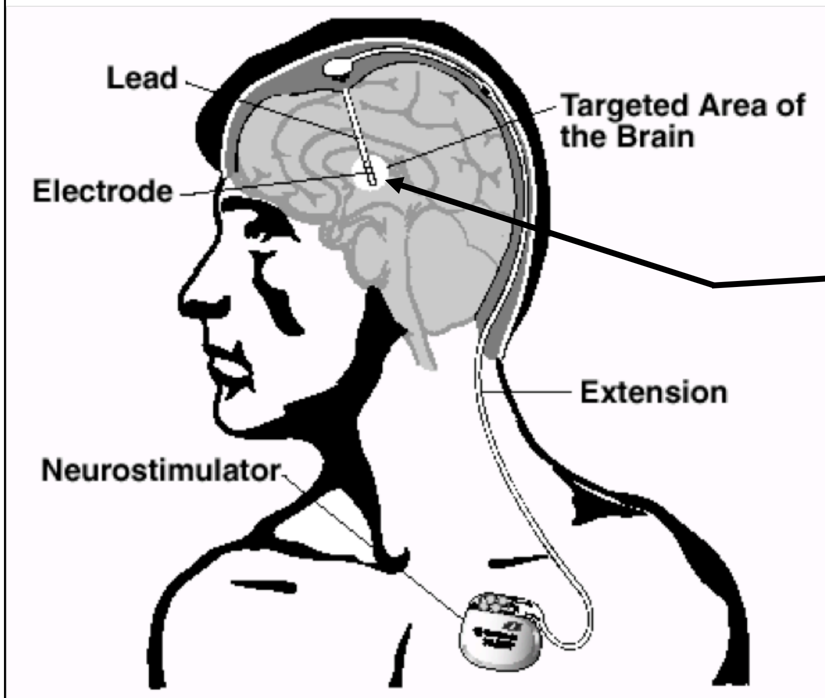
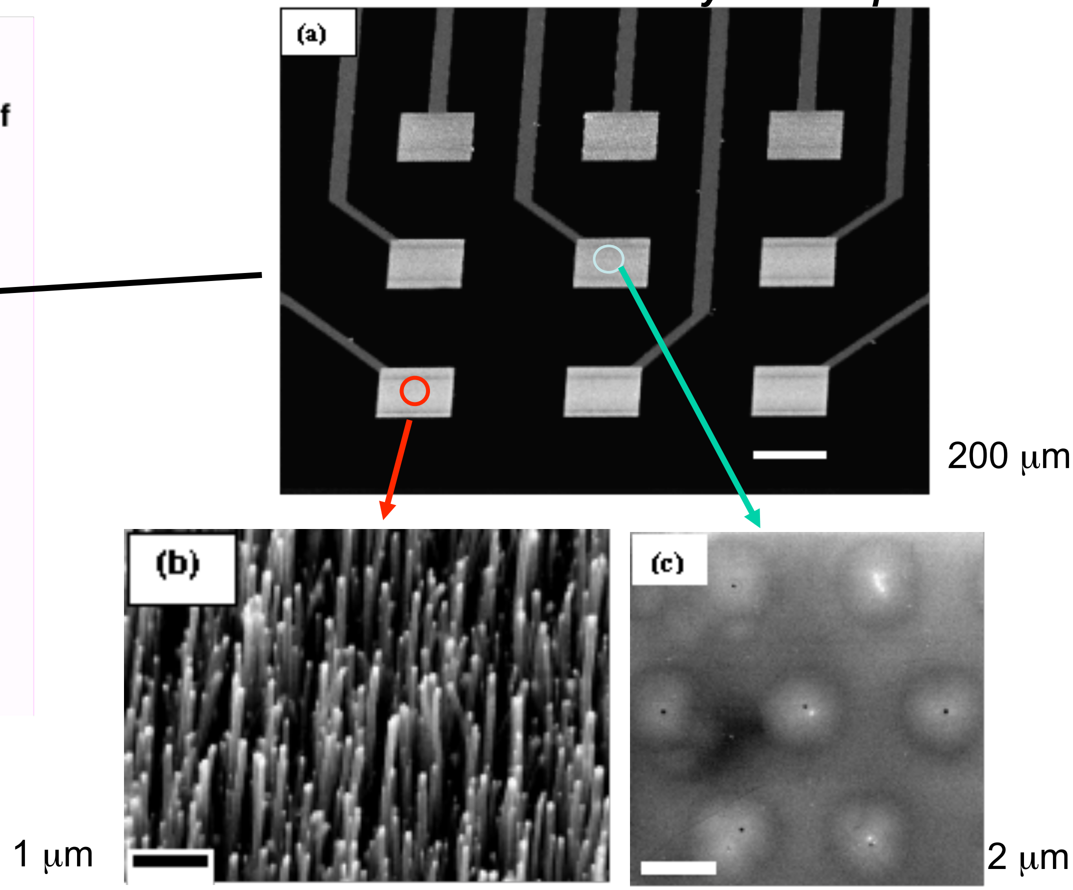
Active Electrode Array at the Tip



Medtronic

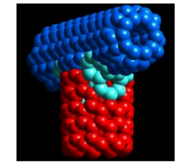


Goal:
Create a "smart" device based on a multiplexed CNF array for localized and efficient stimulation and neurochemical recording

Stimulating Electrode: uncoated CNFs with large surface area

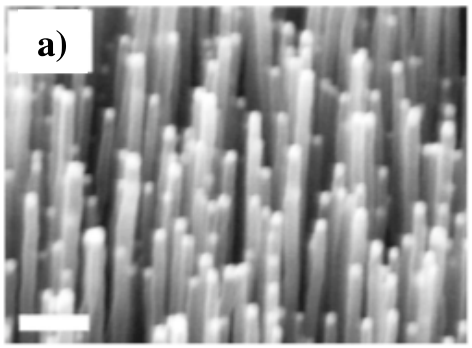
Recording Electrode: embedded in SiO_2 with ultrahigh sensitivity

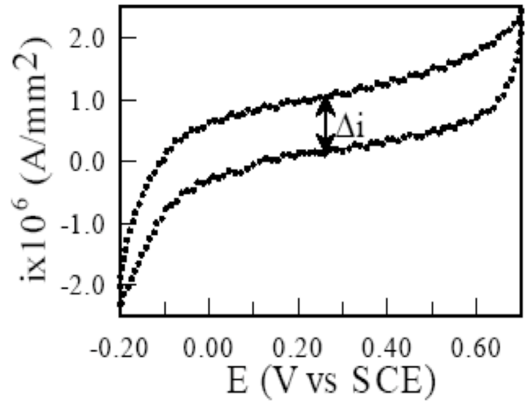
Polypyrrole Coated Vertically Aligned CNF Array for Neurostimulation

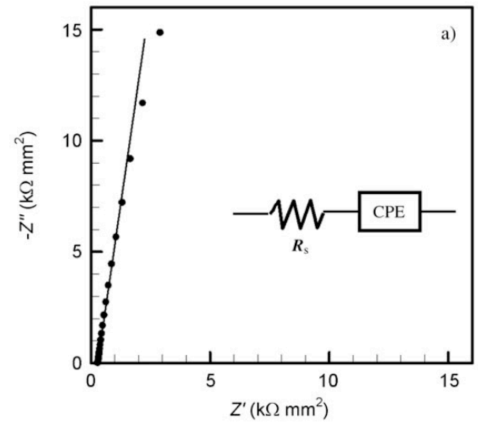


Polypyrrole coating applied to increase the capacitance and decrease the impedance

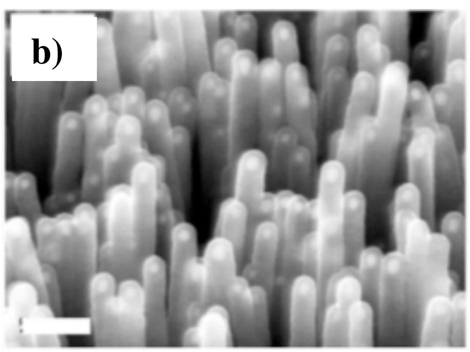
As-grown CNF Array

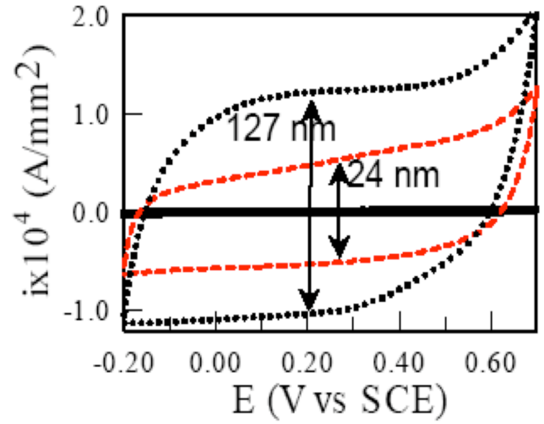


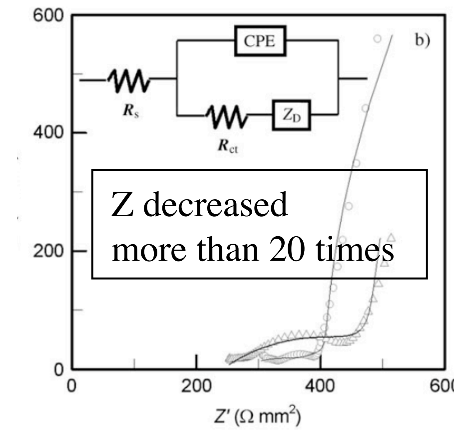




Polypyrrole Coated CNF Array







High Capacitance ($C_0 = \Delta i/2\nu$)
Noble metal ~ 20 μF/cm^2
As-grown CNF array: 0.4 mF/cm^2
Ppy-coated CNF array: 40 to 100 mF/cm^2

Low Impedance
At 1 kHz, the impedance is negligible compared to the solution resistance

Nguyen-Vu, B. T. D., et al. *Small* **2006**, 2, 89-94.

Biocompatability of Polypyrrole Coated Vertically Aligned CNF Array

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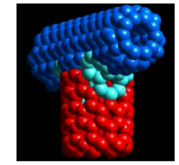
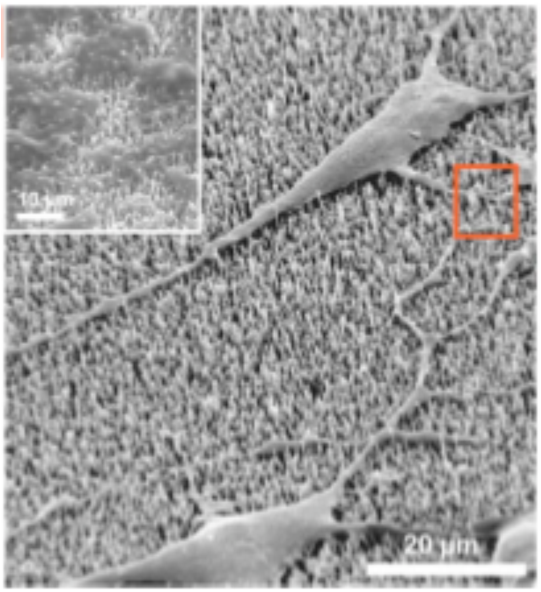
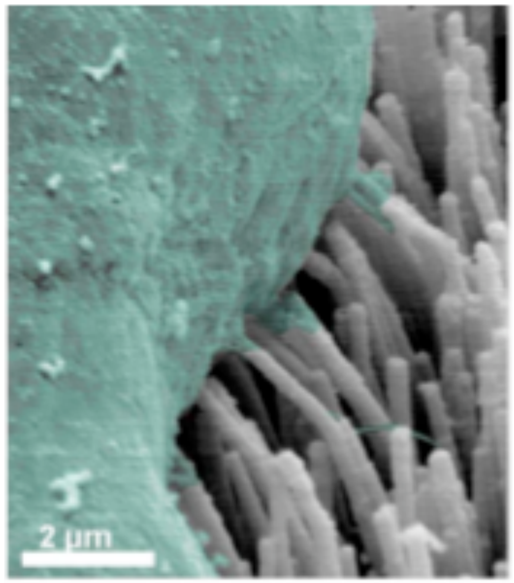
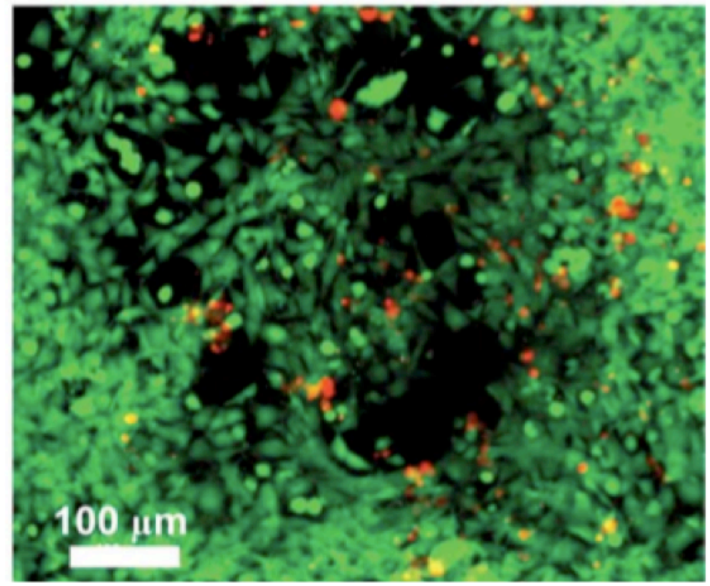


PC12 Cell on Polypyrrole Coated CNFs







- Brush-like polypyrrole coated CNFs make intimate physical contact with PC12 cells
- PC12 cells observed to spread and differentiate on CNF array

- Polypyrrole coated CNFs support cell growth and proliferation

Nguyen-Vu, B. T. D., et al. *IEEE Trans*. *Biomed*. *Eng*. **2007**, 54, 1121-1128.

Stimulation of Rat Hippocampal Slice by Polypyrrole Coated Vertically Aligned CNF Array

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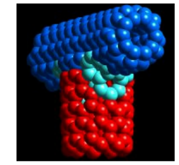
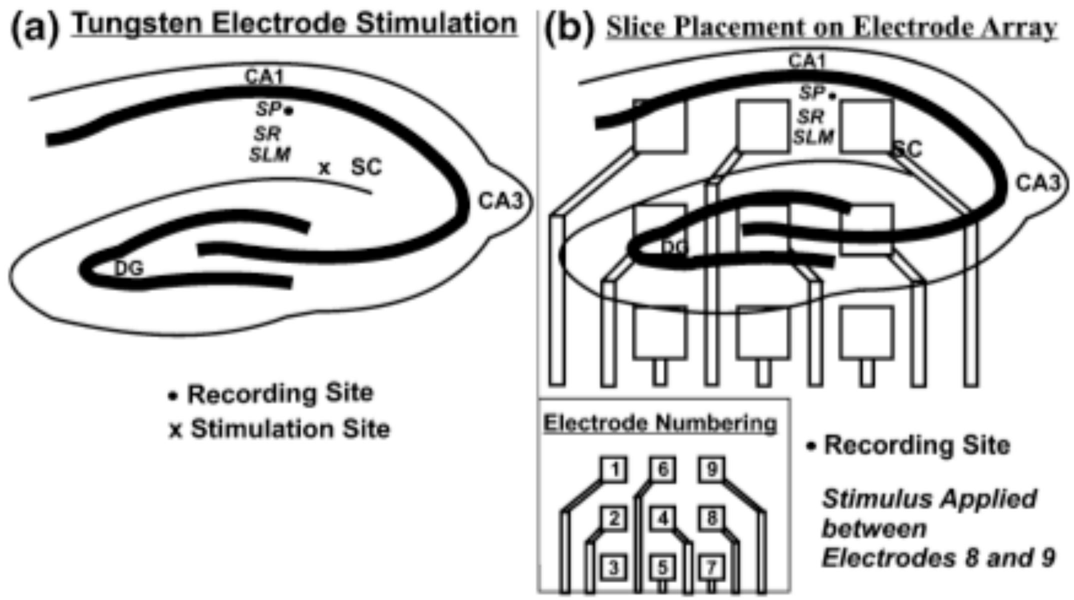
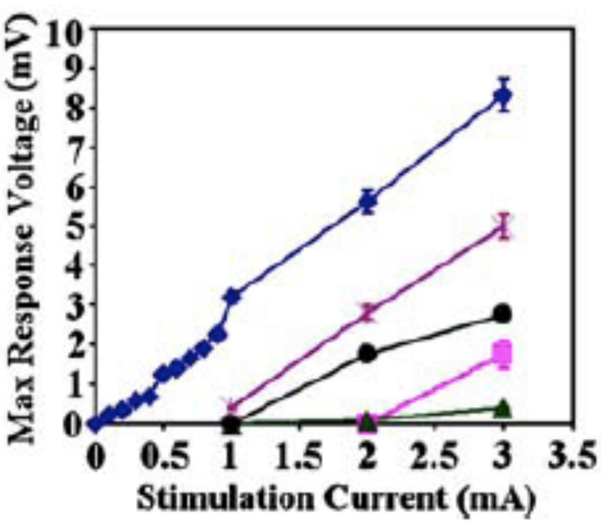


Experiment: Measure voltage for a given stimulation current

Stimulation by:
- •W wire
- ◆Pt Microelectrode
- •CNFs
- ◆PPy coated CNFs

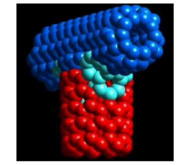




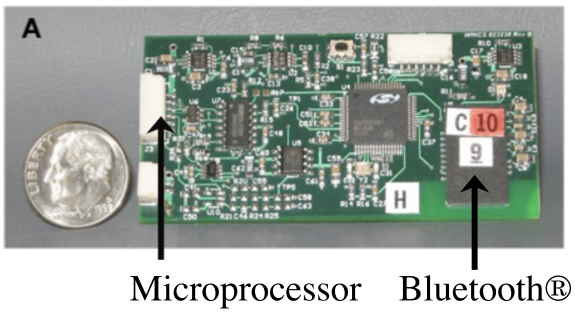
1) Only PPy coated CNFs were able to stimulate tissue under 1 mA stimulation current.
2) Only PPy coated CNFs did not induce the electrolysis of water (less than 1 mA and 1V)

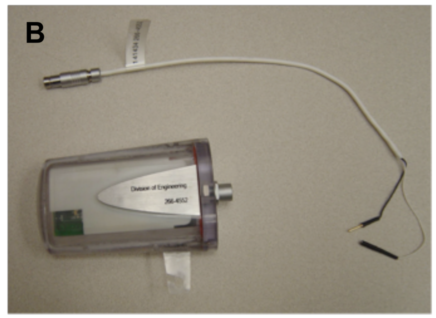
de Asis, E. D., et al. *Biomed. Microdevices* **2009**, 11, 801-808.

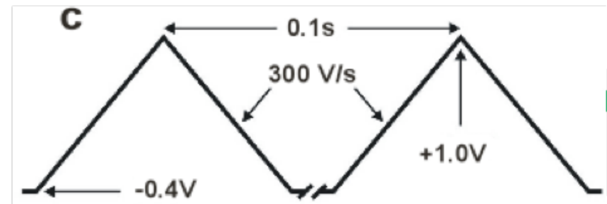
Dopamine Detection by CNF Array and Wireless Instantaneous Neurotransmitter Concentration Sensor (WINCS)

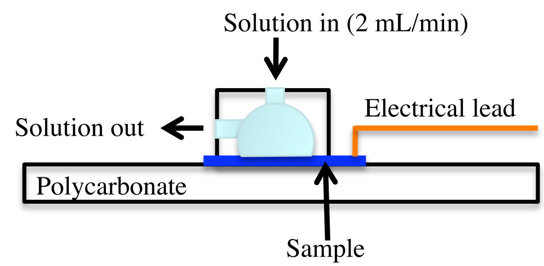


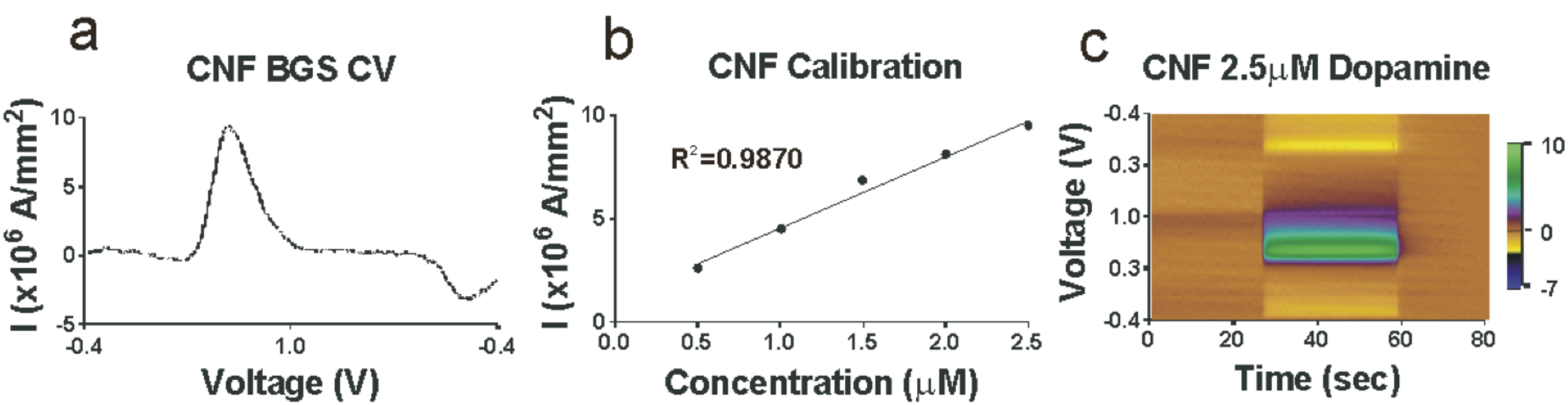
Mayo Clinic's Sterilizable WINCS Unit











Koehne, J. E., et al. *Analyst*, **2011**, 136, 1802-1805.

Gas/Vapor Sensors in Biomedical Applications

- Some diseases have specific markers which show up in excess concentration in the breath of sick people relative to normal people.

Examples: Acetone in diabetes patients
NO in asthma patients

- In these cases, simple chemical sensors with pattern recognition can be valuable.

Why Nanomaterials/Nanosensors?

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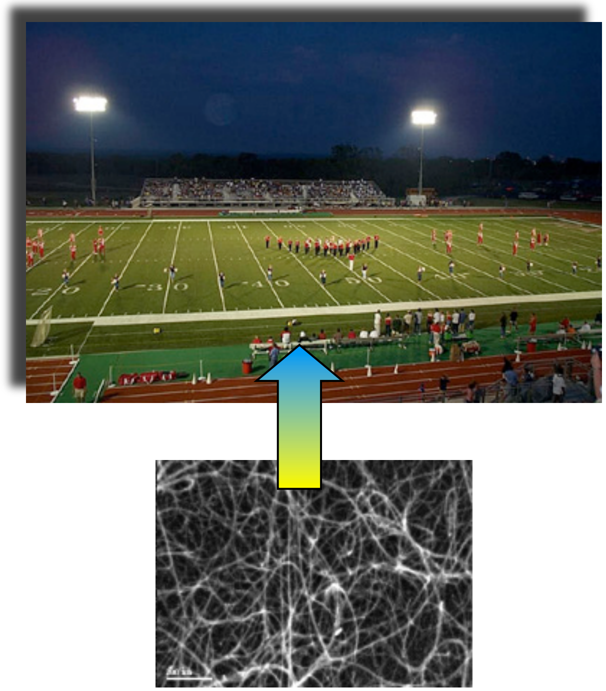
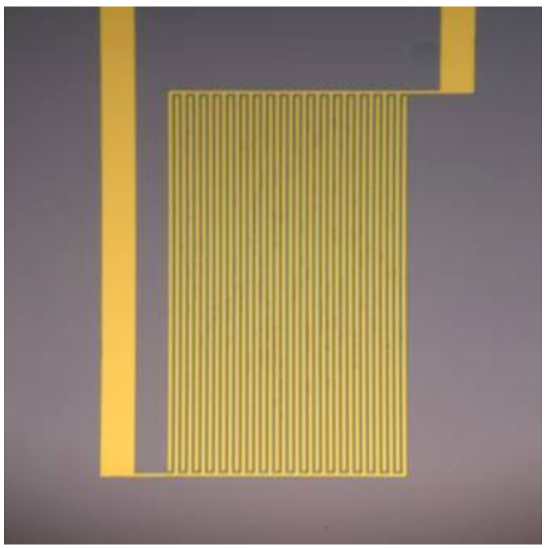
- Compared to existing systems, potential exists to improve sensitivity limits, and certainly size and power needs
- Why? Nanomaterials have a large surface area. Example: SWCNTs have a surface area ~1600 m^2/gm which translates to the size of a football field for only 4 gm.
- Large surface area → large adsorption rates for gases and vapors → changes some measurable properties of the nanomaterial → basis for sensing
    - Dielectric
    - Capacitance
    - Conductance
    - Deflection of a cantilever

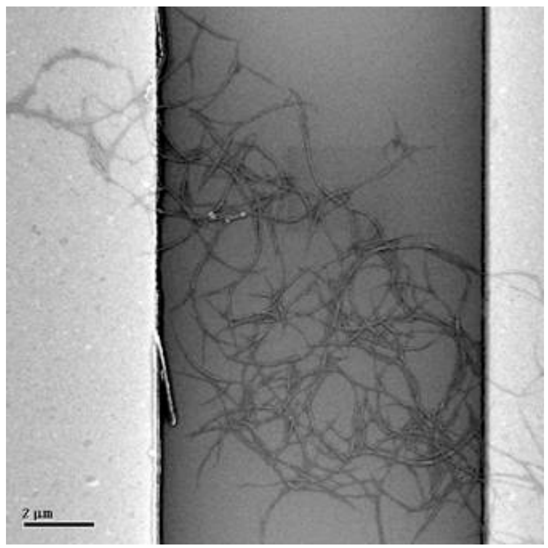


4 grams

SWCNT Chemiresistor

- Easy production using simple microfabrication
- 2 Terminal I-V measurement
- Low energy barrier - Room temperature sensing
- Low power consumption: 50-100 μW/sensor





Processing Steps

1. Interdigited microscale electrode device fabrication
2. Disperse purified nanotubes in DMF (dimethyl formamide)
3. Solution casting of CNTs across the electrodes

Jing Li et al., Nano Lett., **3**, 929 (2003)

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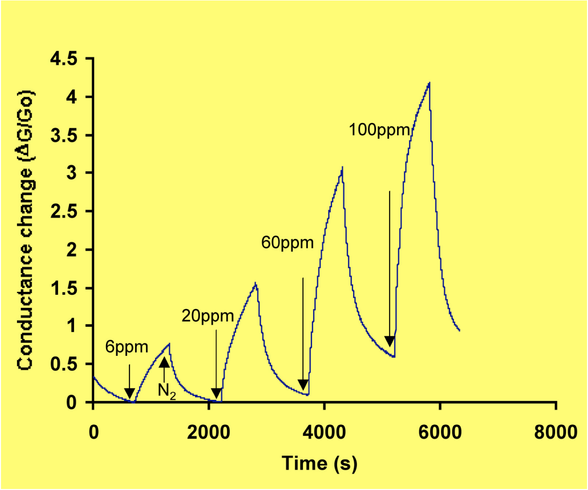
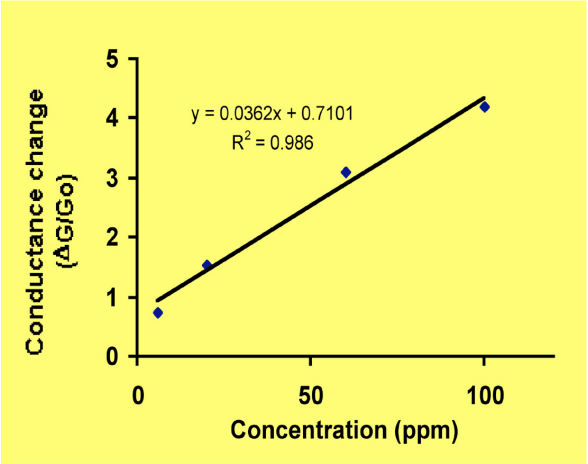
SWCNT Sensor Testing





Detection limit for NO_2 is 4 ppb.

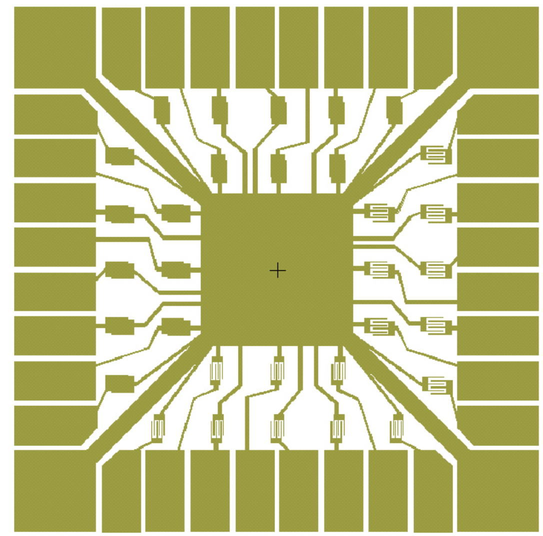
- Test condition:
  Flow rate: 400 ml/min
  Temperature: 23 °C
  Purge gas: N_2 & Carrier gas: Air

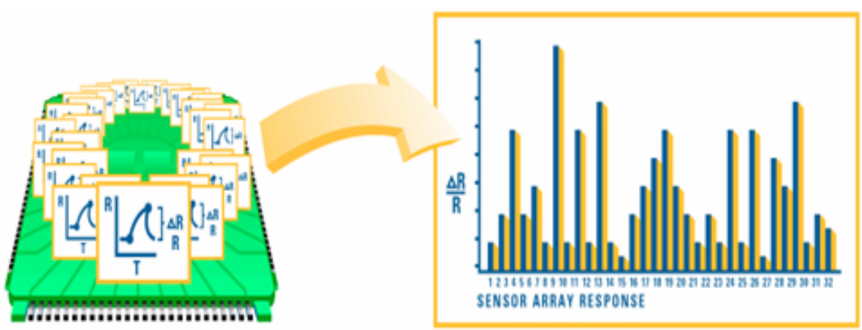
- Measure response to various concentrations, plot conductance change vs. concentration

Preliminary tests show a sensitivity of 10 ppm for acetone. Further studies are needed for interfering chemicals and pattern recognition.

Nanosensing Approach: Selectivity

- Use a sensor array
- Variations among sensors
  - physical differences
  - coating
  - doping

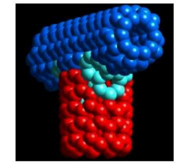




Using pattern matching algorithms, the data is converted into a unique response pattern

<u>Operation:</u>

1. The relative change of current or resistance is correlated to the concentration of analyte.
2. Array device "learns" the response pattern in the *training* mode.
3. Unknowns are then classified in the *identification* mode.
4. Sensor can be "refreshed" using UV LED, heating or purging



Retinal Cell Transplantation

- In the early stage of macular degeneration, retinal pigment epithelial (RPE) cells die, which leads to loss of photoreceptors. Solution?—replace the cells that are lost.

- RPE cells and iris pigment epithelial (IPE) cells can be harvested from the eye, grown in culture, then put back into the eye (“autologous transplantation”).

Courtesy: David Loftus

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Problems with Retinal Cell Transplantation

- Transplantation of suspensions of epithelial cells into the sub-retinal space fails to re-establish the proper architecture of the RPE layer. Instead of a sheet of uniformly oriented cells, you get a "jumble" of cells.

Solution:

- Establish the proper orientation of the epithelial cells prior to transplantation, by growing them in culture on a physical support:

Current Status, Problems and a Possible Solution

- The Obvious Strategy: Natural Substrates for Retinal Transplantation
  - Anterior Lens Capsule (*basal lamina)*
  - Descemet's Membrane (posterior cornea)

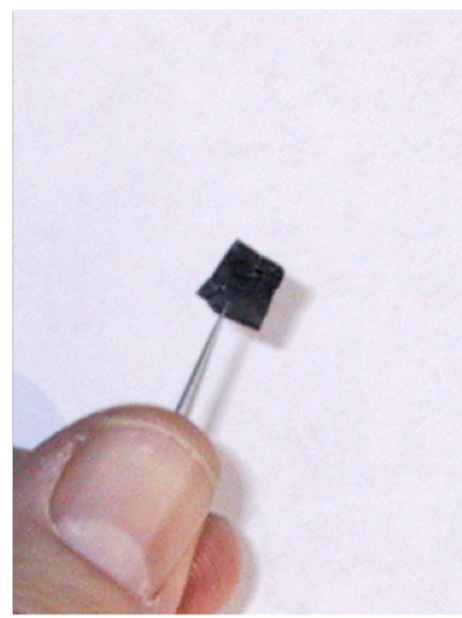
Excellent growth of retinal epithelial cells, assembly of true "epithelial architecture."

Problem!: Membranes with attached epithelial cells cannot be easily implanted into the eye, because the membranes are flimsy and tend to "curl up." They lack the mechanical properties necessary for surgical handling.

Solution:

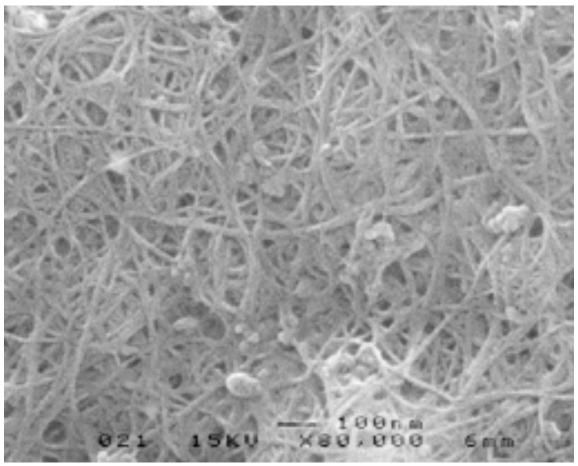
Carbon Nanotube Bucky Paper

A meshwork of carbon nanotubes formed into a paper-like structure

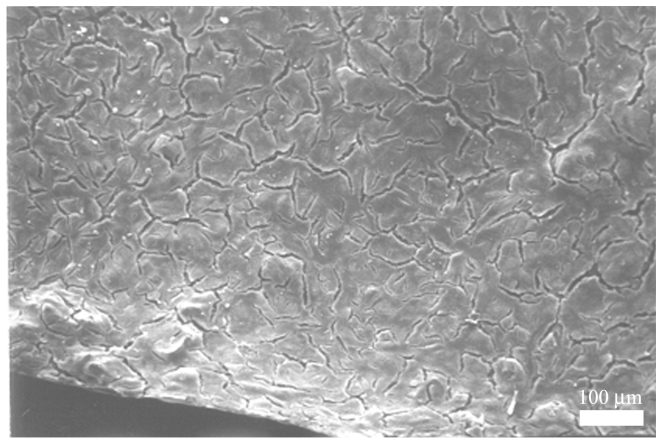


RPE cells grown on Carbon Nanotube Bucky Paper

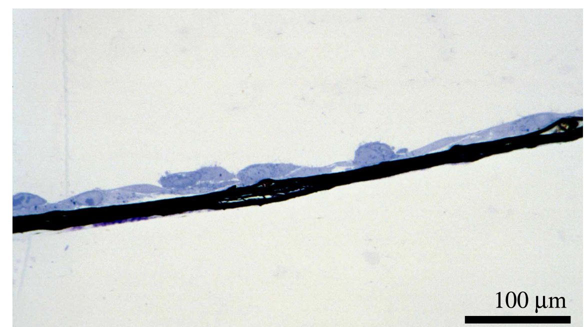
As-prepared bucky paper



SEM Image after growth of RPE results

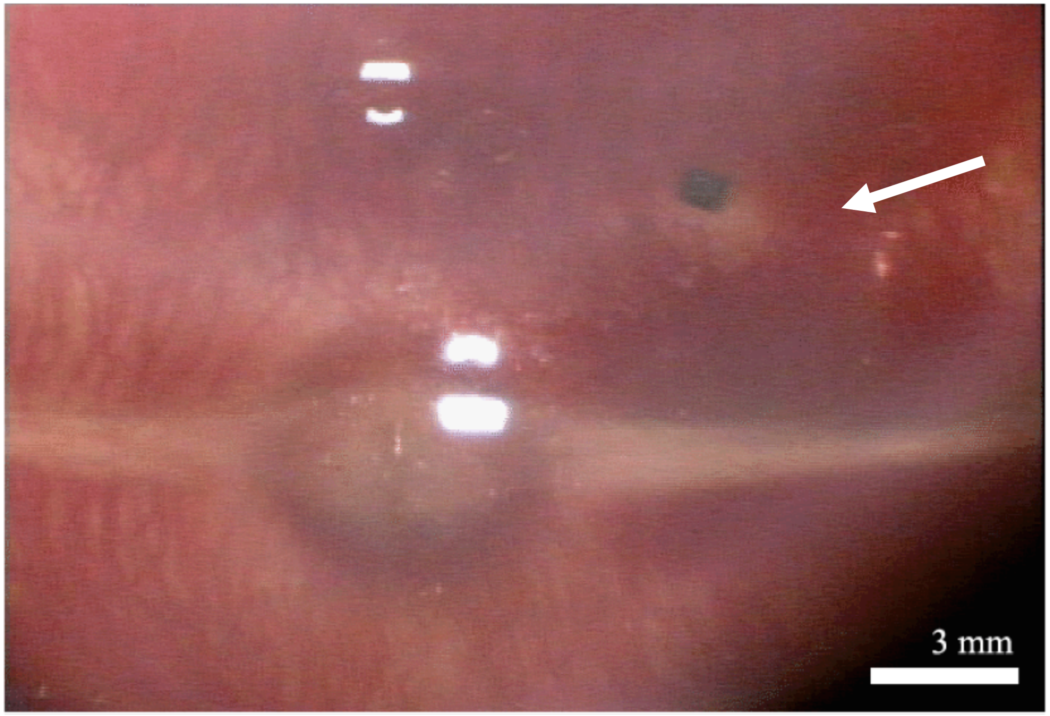


Light micrograph/histological staining of RPE grown on bucky paper



- Confluent monolayer, with uniform orientation of cells
- Excellent attachment of RPE cells to the Bucky Paper surface; confirmation of correct apical/basolateral orientation

Implantation of Carbon Nanotube Bucky Paper into the Sub-Retinal Space of an Albino Rabbit



Result: Bucky paper is easily manipulated during surgery (does not tear and stays flat), and is immunologically well-tolerated by the eye.

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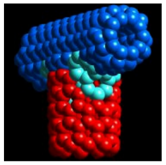
Carbon Nanotube Biocompatibility



Vision Chip

Retinal Cell Transplantation

Islet Cell Transplantation

Hemostatic Bucky Paper/ Wound Healing

Artificial Blood Vessels

Tissue Engineering

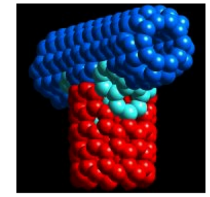
?

NEEDS:

Implantable Physiological Sensors
- Remote sensing
- Early medical intervention
- Novel medical countermeasures
  - *Cardiovascular physiology*

Long Duration Space Flight –How to deliver medical therapy?
- Acute injury
  - Hemostatic Bucly Paper
  - Bucky Paper for Wound Healing
- Cancer Therapy
  - *Adoptive Immunotherapy Delivered by Encapsulated Cells*
  - *Immune Shielded Delivery of Chemotherapy*
- Therapy for diabetes
  - *Transplantation of Islet Cells*

Summary



- Nanotechnology is an enabling technology that will impact almost all economic sectors: one of the most important and with great potential is the health/medical sector.
    - Nanomaterials for drug delivery
    - Early warning sensors
    - Implantable devices
    - Artificial parts with improved characteristics
- Carbon nanotubes and nanofibers show promise for use in sensor development, electrodes and other biomedical applications.