Carbon Nanofiber Nanoelectrodes for Biosensing Applications

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NASA Ames Research Center

- Established in 1939 as the second laboratory of the National Advisory Committee for Aeronautics (named after NACA chair, Joseph S. Ames)
- Ames is 1 of 10 NASA field centers
- Located in the heart of the silicon valley
  - High-tech companies, start-ups, biotechnology
- Ames Technical Areas
  - Nanotechnology
  - Information technology
  - Fundamental space biology
  - Biotechnology
  - Thermal protective systems
  - Human factors research

Biosensor Motivation

NASA Applications

- Astronaut health monitoring
    - Lab-on-a-chip
- Water Quality monitoring
    - Pathogen detection on ISS and long duration missions
- Planetary exploration
    - Life on other planets

Outside Applications and Customers

- Medical Diagnostics
    - NIH, DARPA
- Environmental Monitoring
    - EPA, NIH
- Biowarfare agent detection
    - DHS, DARPA
- Food Safety
    - FDA

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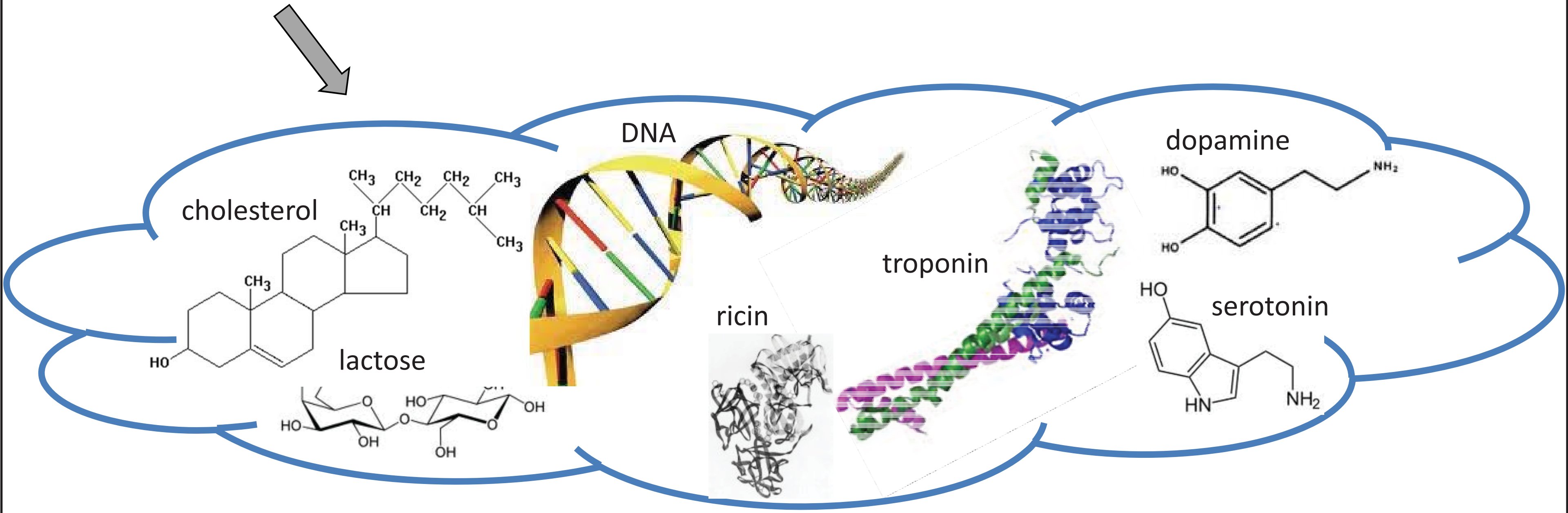
NASA

Biosensor Basics

Biomolecule + Transducer + Reader/Signal Processor



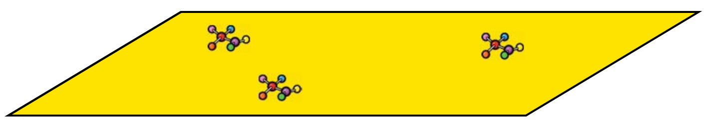
Type of Signal Transduction: optical, electrical, **electrochemical**, surface plasmon resonance, piezoelectric

Nanoelectrodes for Sensors

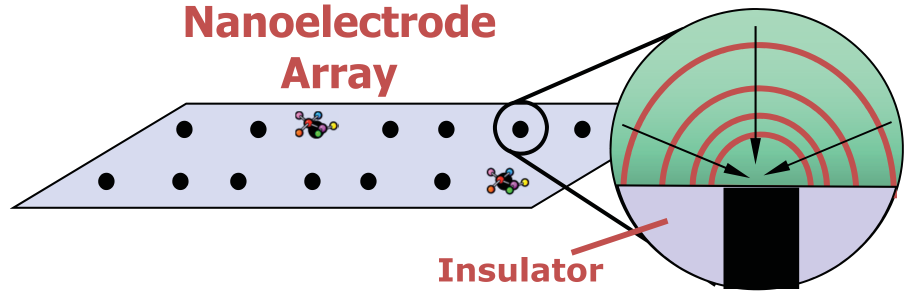
Nanoscale electrodes create a dramatic improvement in signal detection over traditional electrodes for small analyte concentrations

Background: $i_n \propto C_d^0 A$

Traditional Macroelectrode



Nanoelectrode Array



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

- Nanoelectrodes are at the **scale close to** molecules
- with dramatically **reduced background noise**
- Multiple electrodes results in **magnified signal** and **desired redundance** for statistical reliability.

Macroelectrode vs. Nanoelectrode

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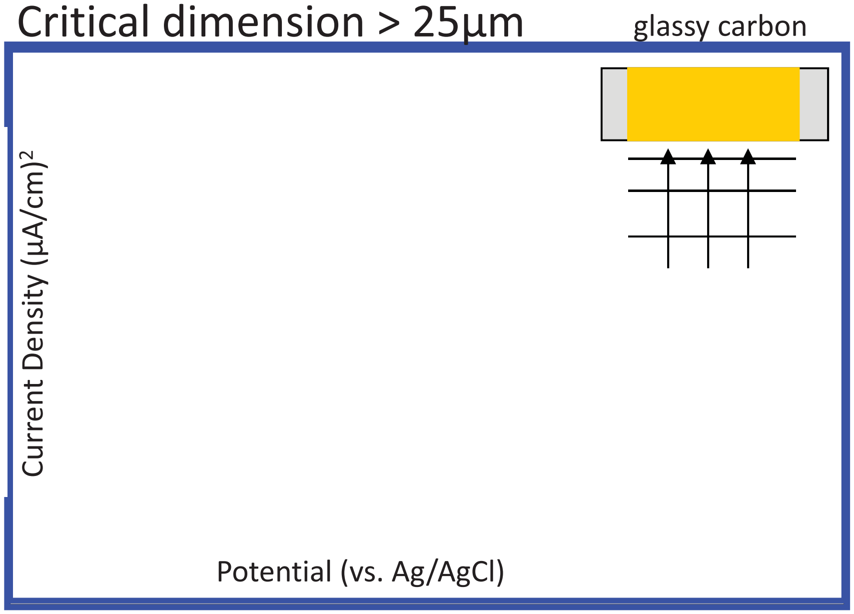
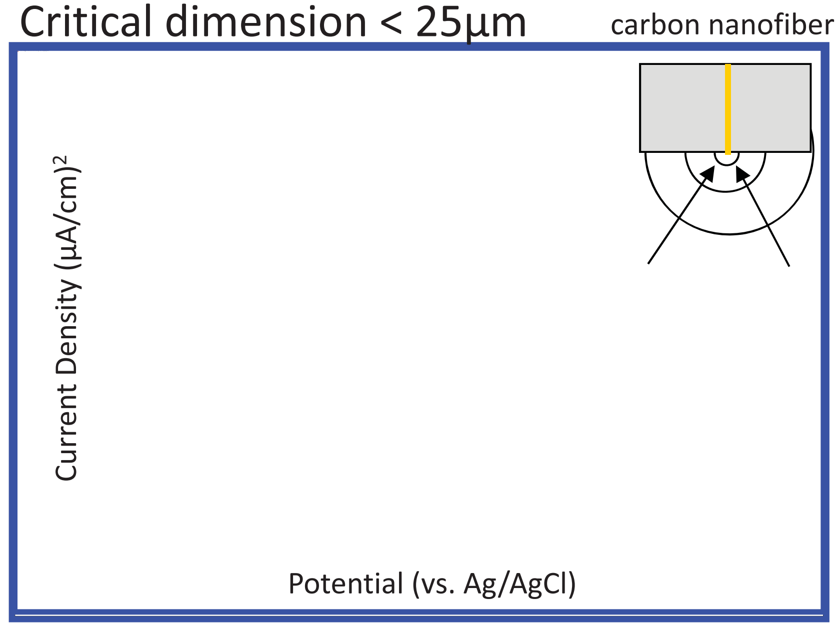
Critical dimension > 25µm



Semi-infinite planar linear diffusion

Critical dimension < 25µm



Semi-infinite hemispherical diffusion:
Current exhibits a steady state
Diffusion layer is approximately 6r

Nanostructured Ensemble or Array Electrode

- Spatial Resolution: defined by **r**
- Sensitivity: signal to noise
  - $i_s/i_n \approx nFC_0D_0/\mathbf{r}$

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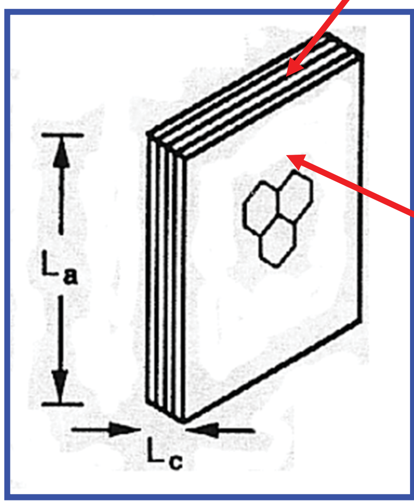
Carbon Nanofibers (CNFs)

HOPG



Edge Plane:

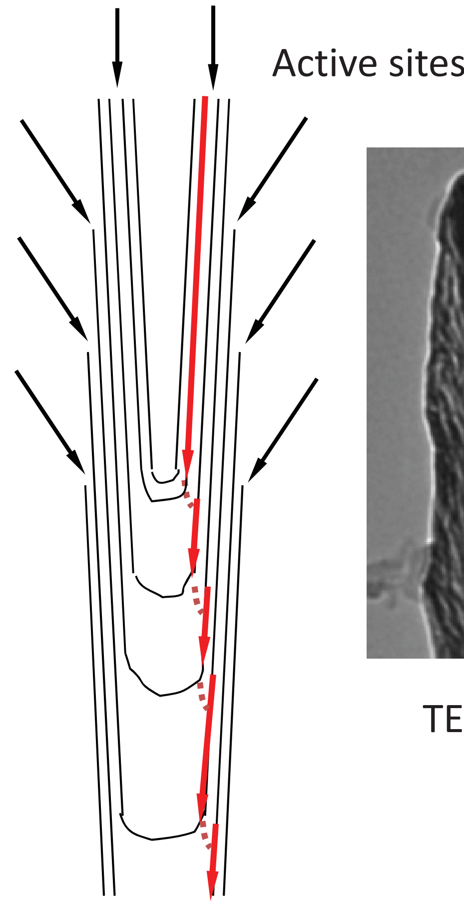
(1) High electron transfer rate (~ 0.1 cm/s)

(2) Very high specific capacitance (>60 μF/cm^2)

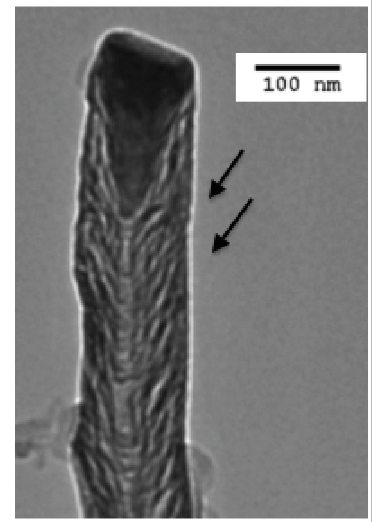
Basal Plane:

(1) Low electron transfer rate (< 10^{-7} cm/s)

(2) Anomalously low capacitance (~1.9 μF/cm^2)



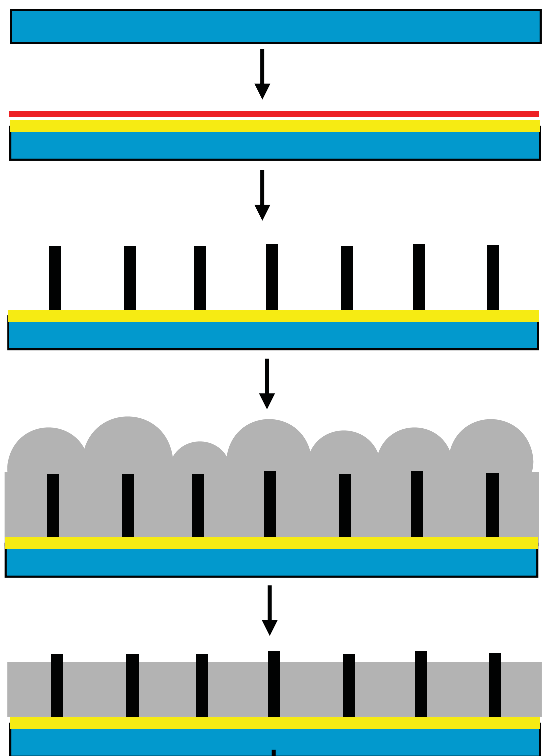
Bamboo-like CNFs



TEM of CNF

R. L. McCreery, A. J. Bard, in *Electroanalytical Chemistry*, Ed., 1991, 17, 221.

CNF Array Preparation

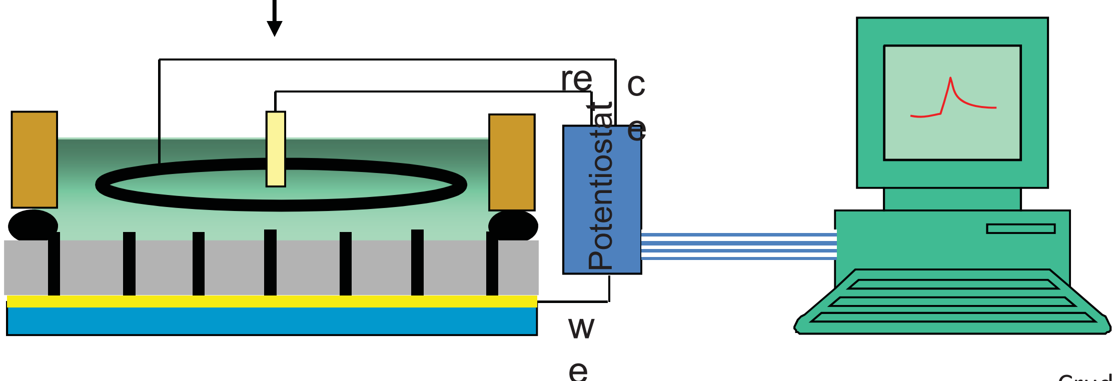


(1) Coat silicon wafer with underlying Cr metal & Ni catalyst metal

(2) Growth of Vertically Aligned CNF Array by Plasma Enhanced Chemical Vapor Deposition (PECVD)

(2) Dielectric Encapsulation of silicon dioxide by TEOS Chemical Vapor Deposition (TEOS CVD)

(3) Planarization by Chemical Mechanical Polishing (CMP)

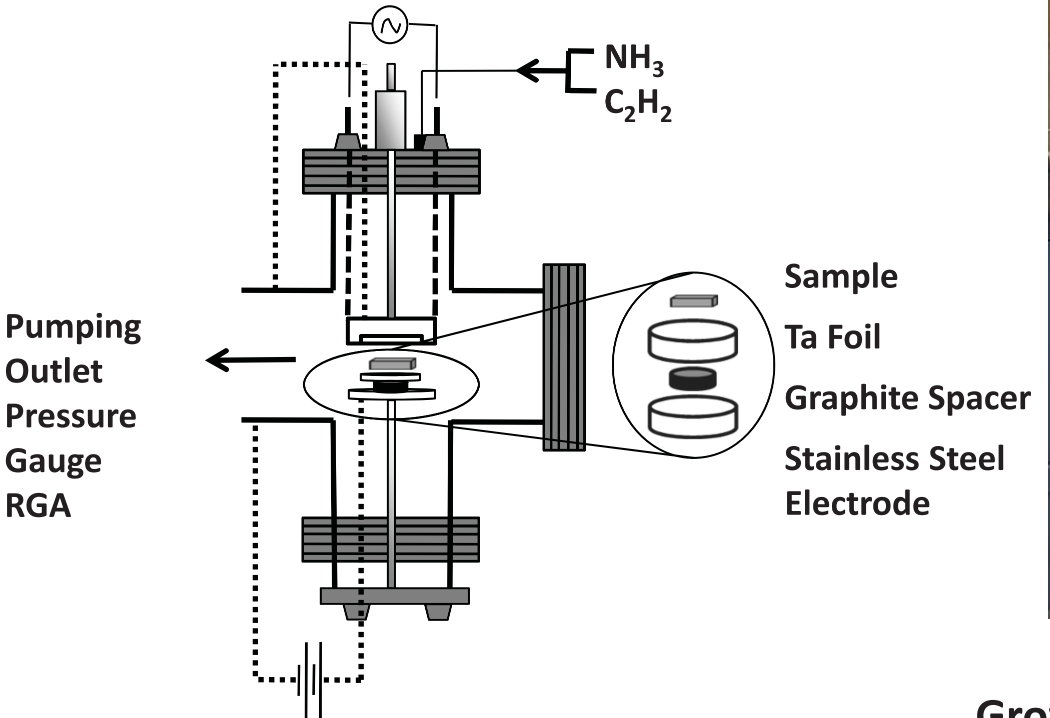


(5) Electrochemical Characterization

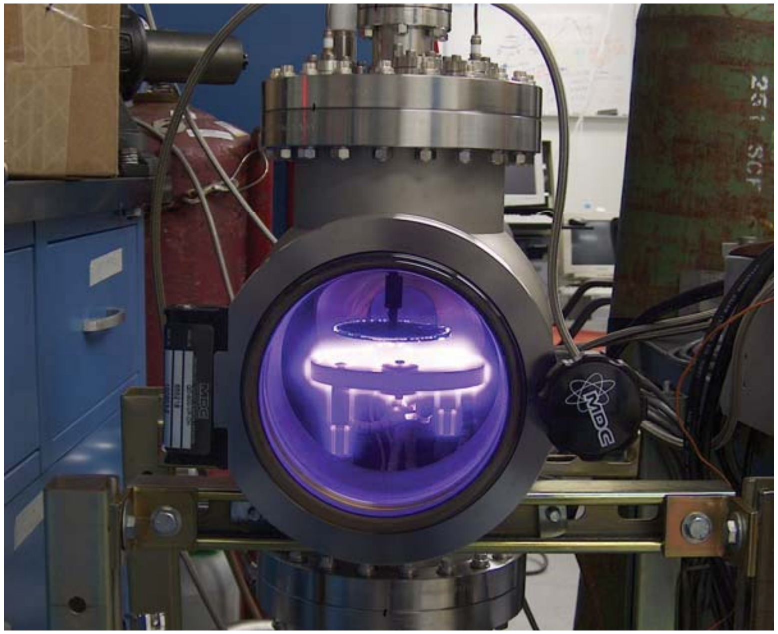
Cruden, B., et al., *Appl. Phys. Lett.* **2003**, 94, 4070-4078.

CNF Growth by Plasma Enhanced Chemical Vapor Deposition (PECVD)

PECVD Reactor Schematic



Custom Built PECVD Reactor

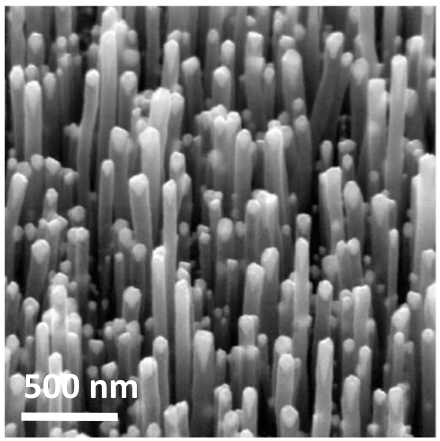
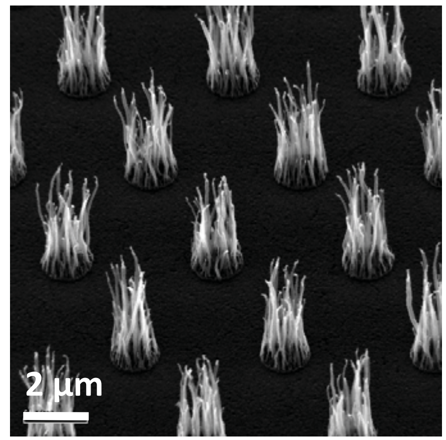
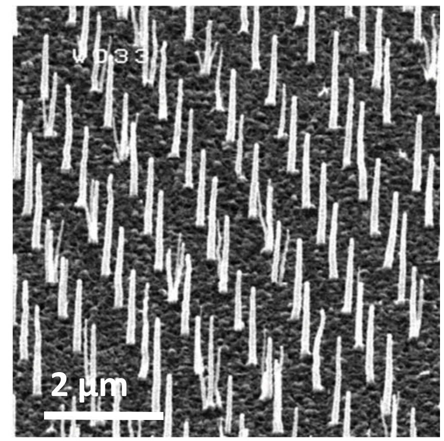
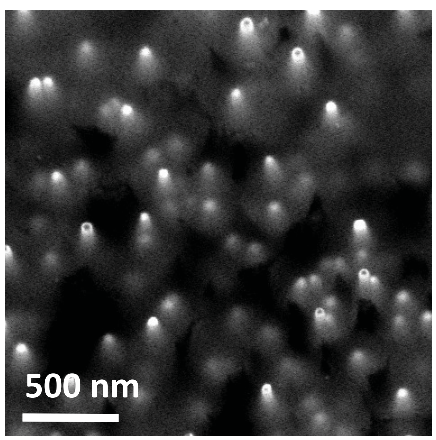
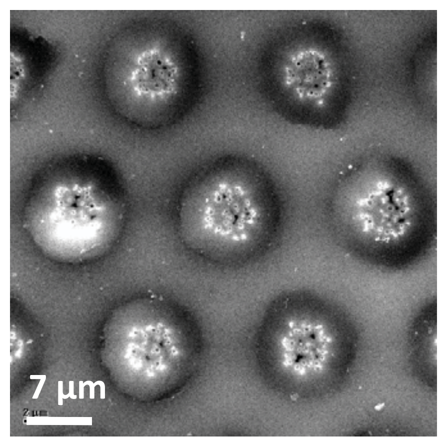
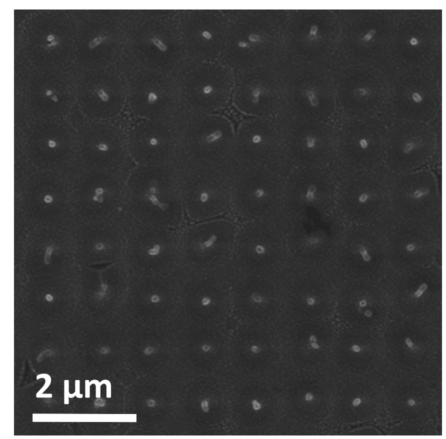


Growth Process

- 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.

Define CNF Placement by Catalyst Placement

	Continuous Layer of Catalyst	Photolithography Defined Catalyst Spots	Electron Beam Lithography Defined Catalyst Spots
As Grown CNFs			
SiO_2 Encapsulated CNFs			

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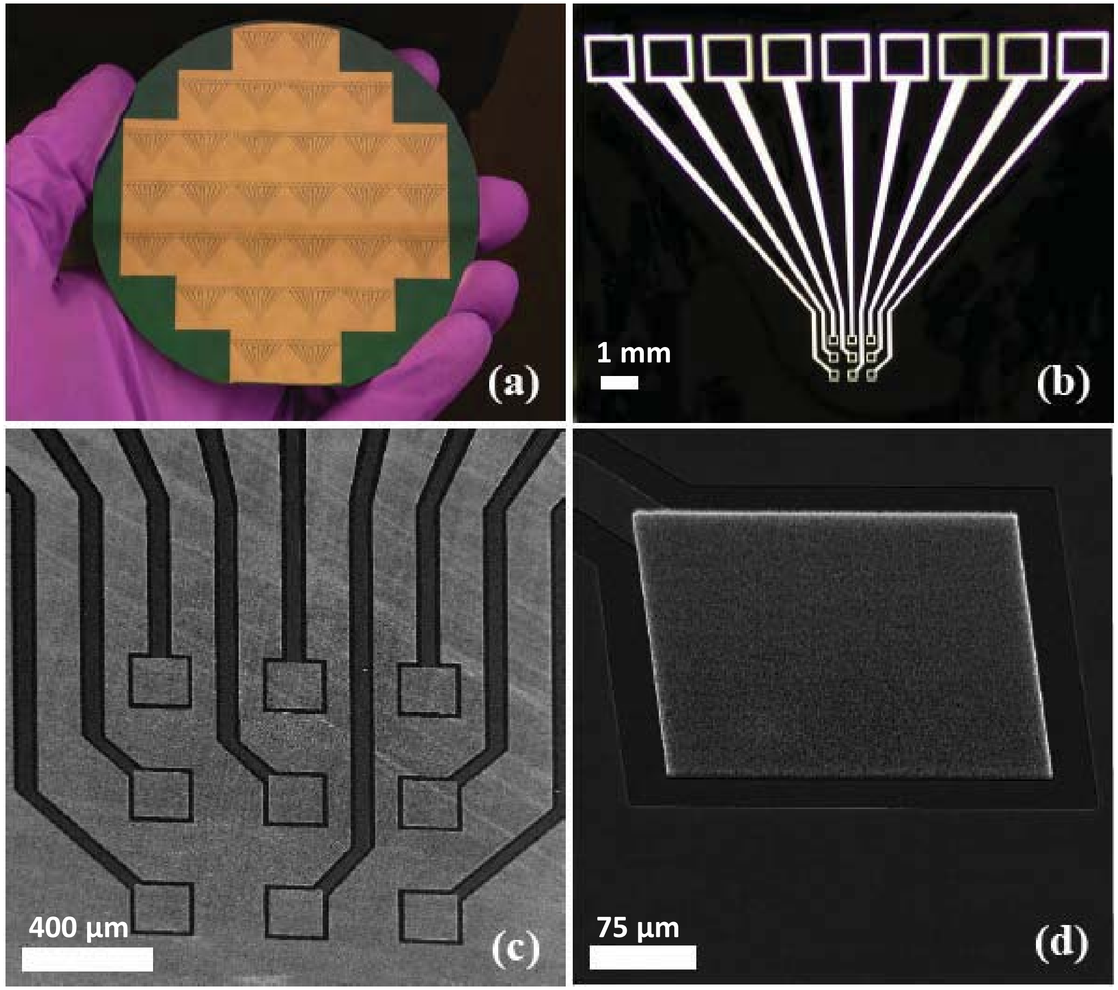
Fabrication of 3x3 Array

30 devices on a 4” Si wafer

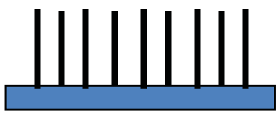


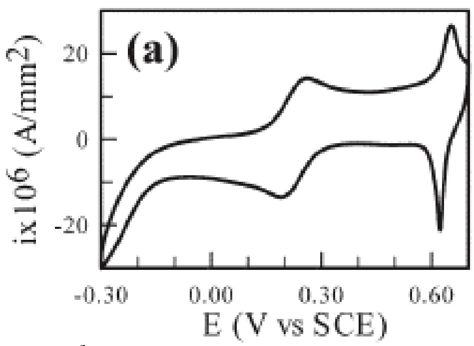
- 200 µm by 200 µm electrode dimensions
- 9 individually addressed electrodes
- potentially 9 different target molecules

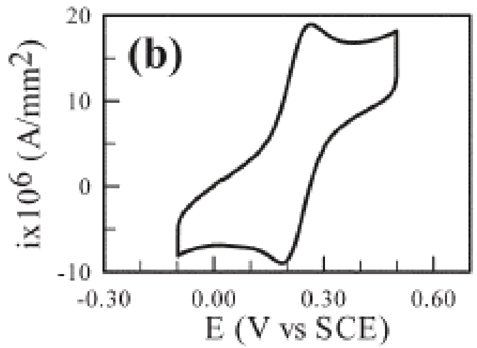
Electrochemistry of CNF Arrays

As grown CNFs

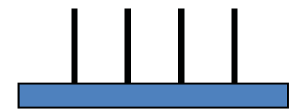
High density







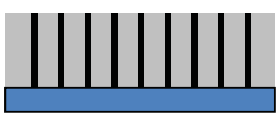
Low density

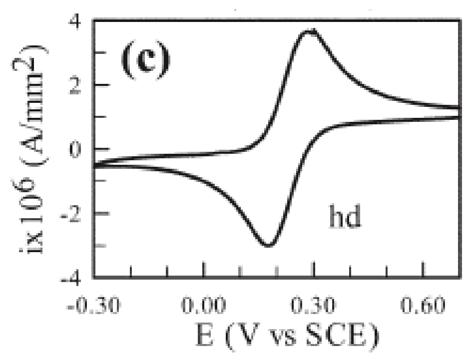


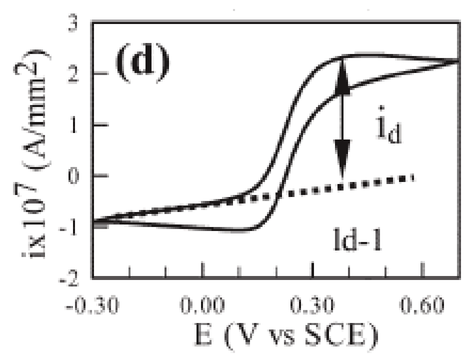
All scans performed in
1mM $K_4[Fe(CN)_6]$
in 1M KCl at 20 mV/s

Embedded CNFs

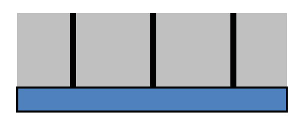
Embedded high density





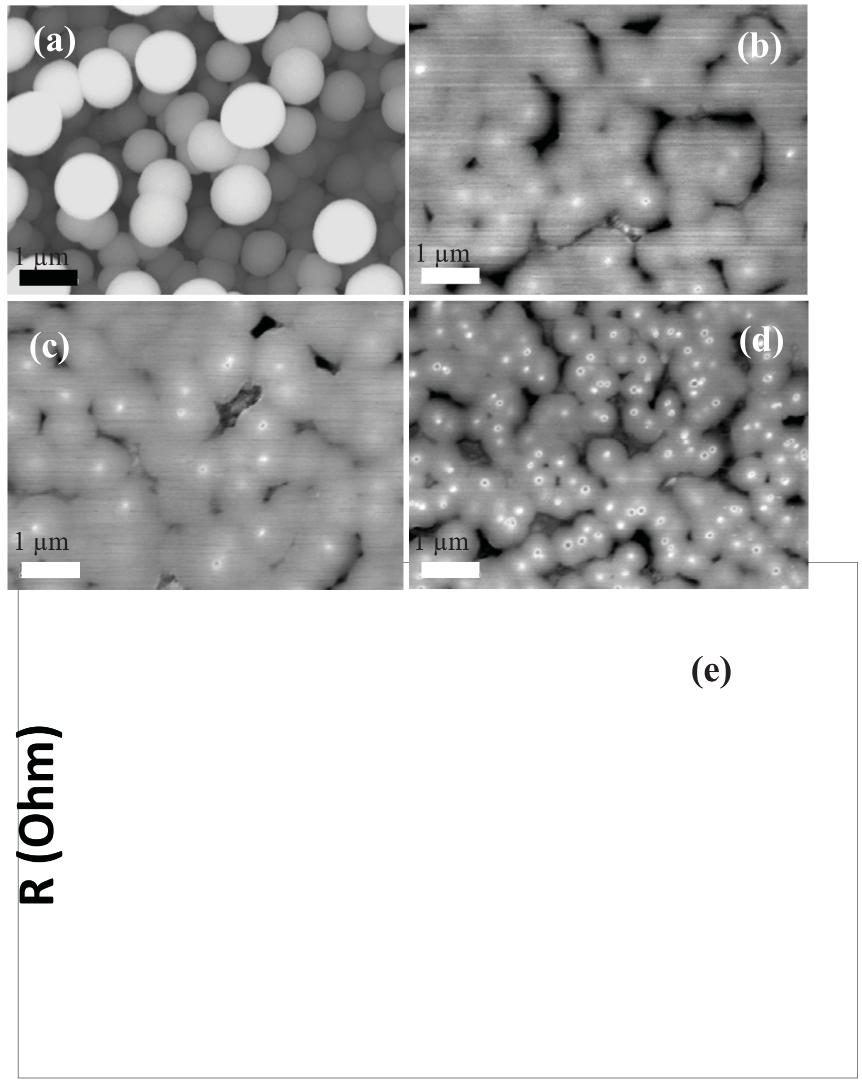


Embedded low density



Koehne, J.; Li, J.; Cassell, A.; Chen, H.; Ye, Q.; Ng, H. T.; Han, J.; Meyyappan, M. *J. Mat. Chem.*, **2004**, 14, 676-684.

Chemical Mechanical Polish to Control CNF Density

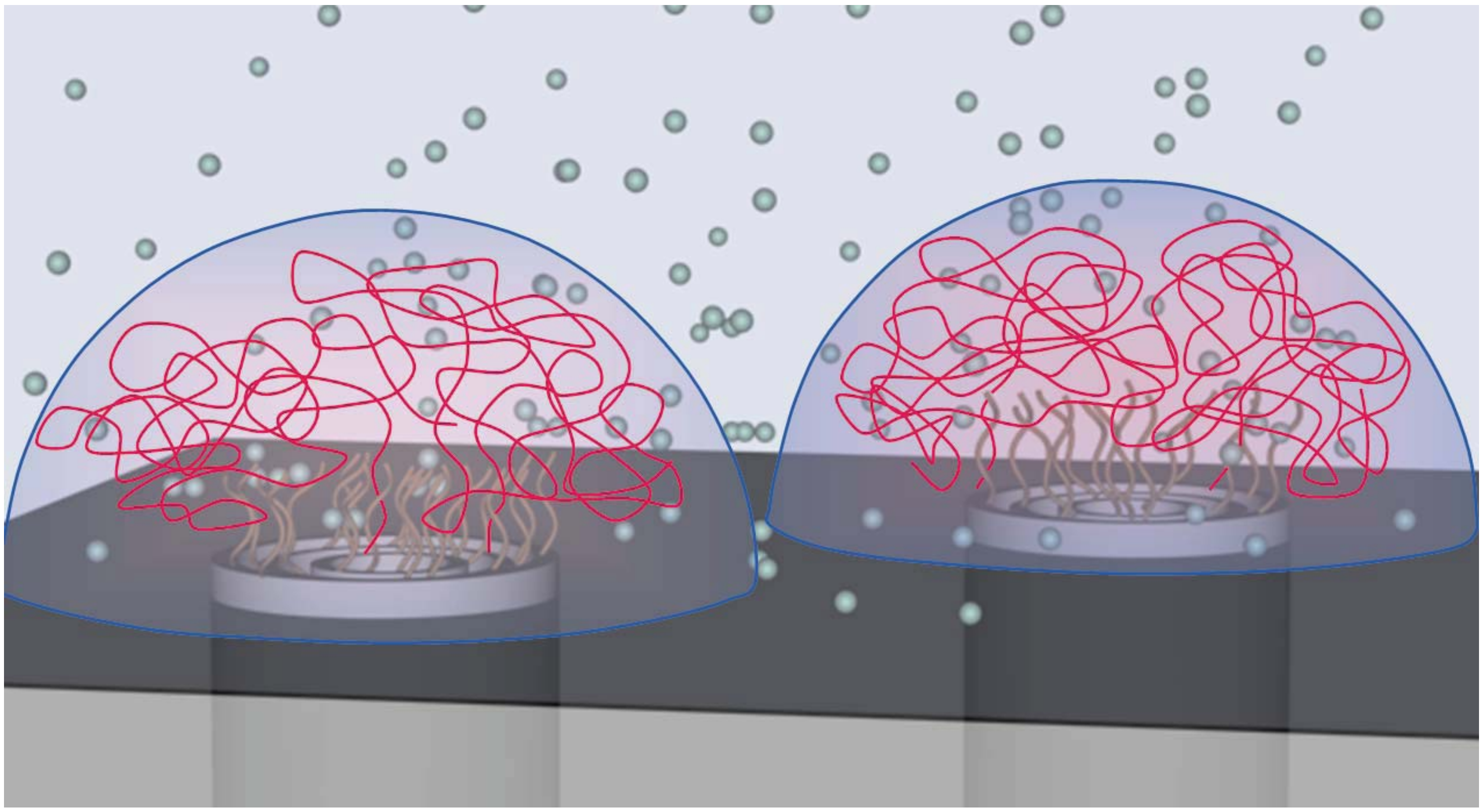


Note:
For nanoelectrode behavior, diffusion layer is approximately 6r

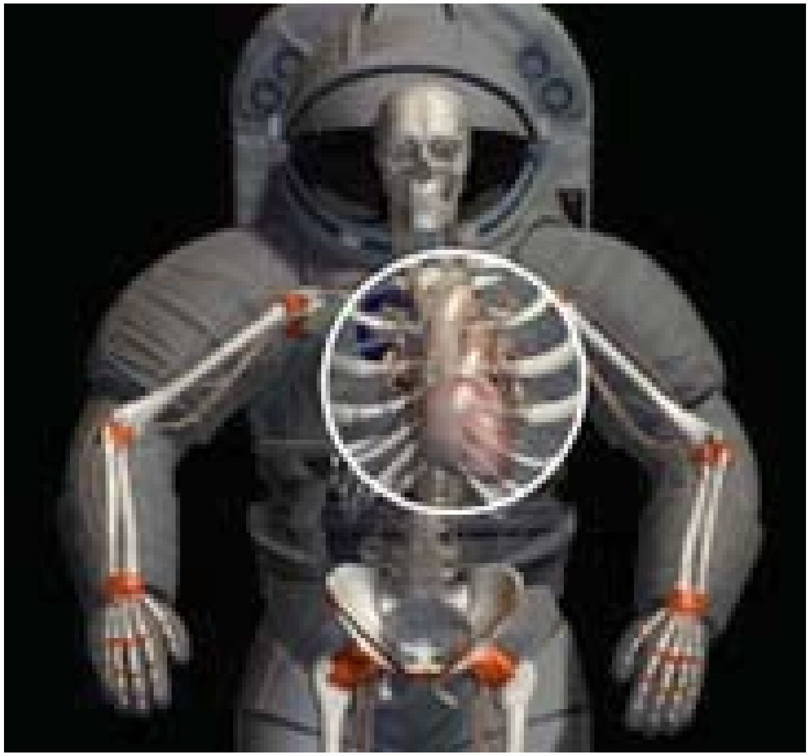
Biomolecule Detection Using CNF Array

Objective:

Test an ultrasmall biosensor for point of care diagnostics for astronaut health monitoring



Astronaut Heart Health Monitoring



Microgravity and Cardiovascular Health

- Fluid Shifts
- Changes in total blood volume
- Changes in heart beat
- Diminished aerobic activity

↓

Need for on-flight diagnostics

↓

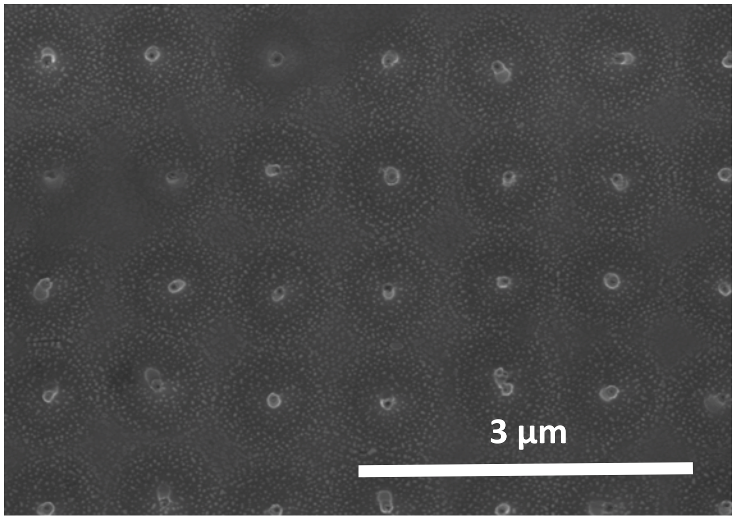
Troponin-I

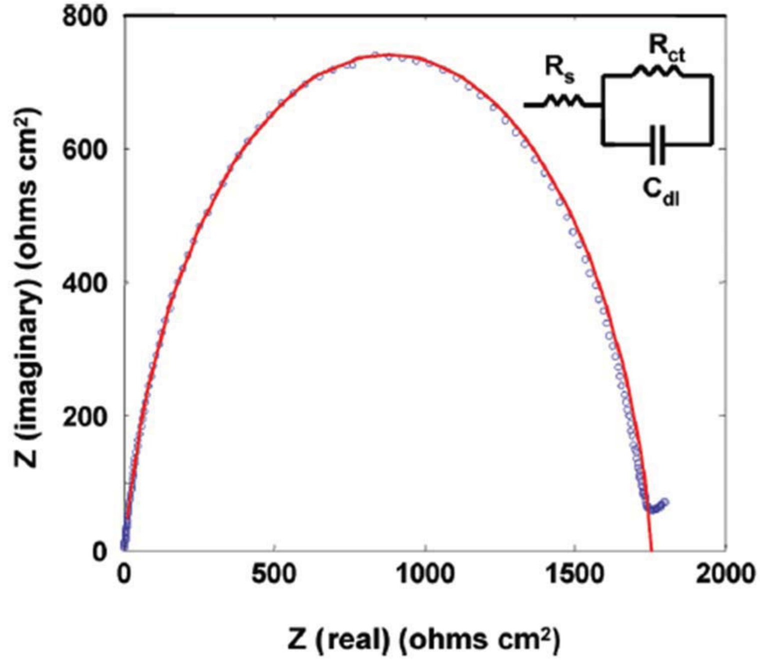
- biomarker: acute myocardial infarction
- normal levels: 0.4 ng/mL and lower
- risk of heart attack: 2.0 ng/mL and above

http://www.nasa.gov/exploration/humanresearch/areas_study/physiology/physiology_cardio.html

lectrochemical Impedance Spectroscopy of CNF Electrode

ultralow density CNF

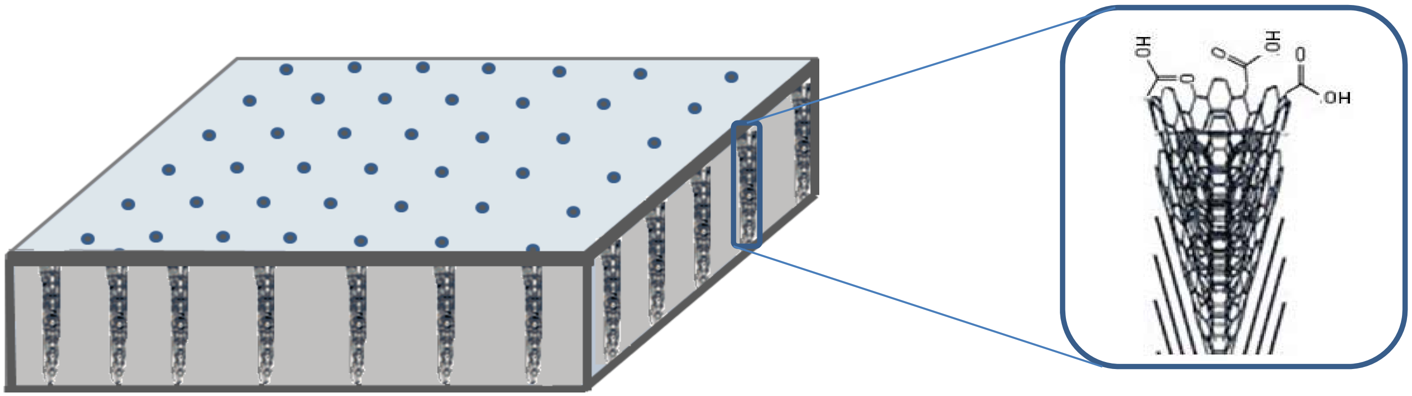


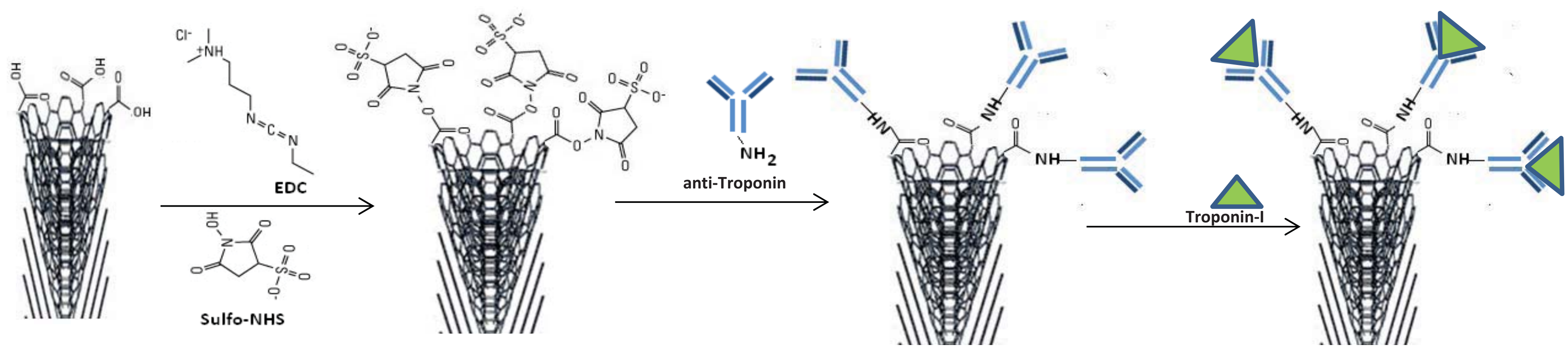


Fitting Parameters	Randomly Grown CNF	CNF (low density)	CNF (ultralow density)
I (A/mm^2)	7.1 X 10^{-6}	1.8 X 10^{-6}	2.5 X 10^{-7}
R_{ct} (KΩ)	N/A	1.8	17.3
CPE (µF)	906	3.3	2.5
n	0.79	0.89	0.91

S. Siddiqui, P. U. Arumugam, H. Chen, J. Li, M. Meyyappan, *ACS Nano,* **2010**, 4, 955-961.

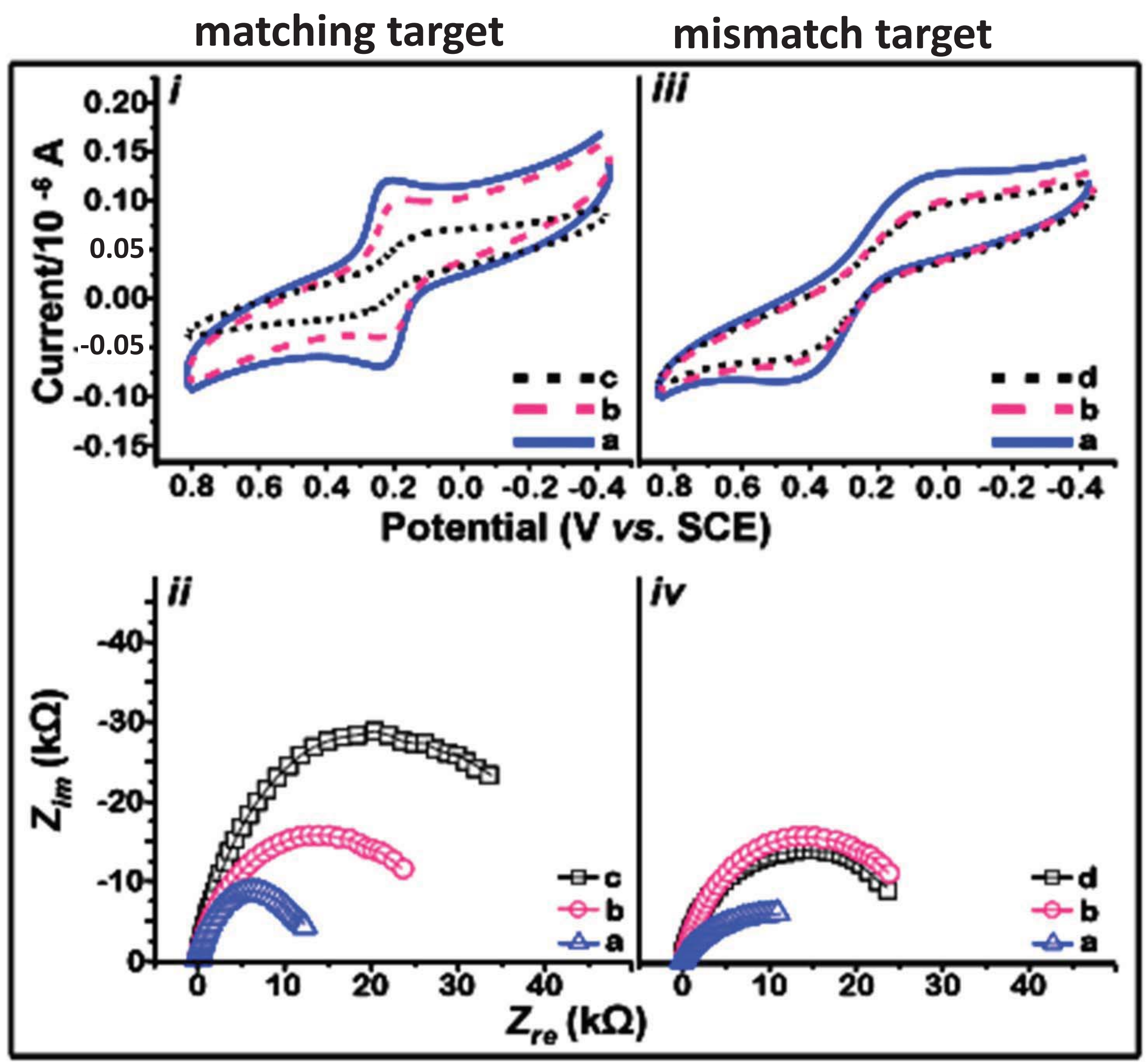
Surface Preparation of CNF Electrode





Troponin-I Detection

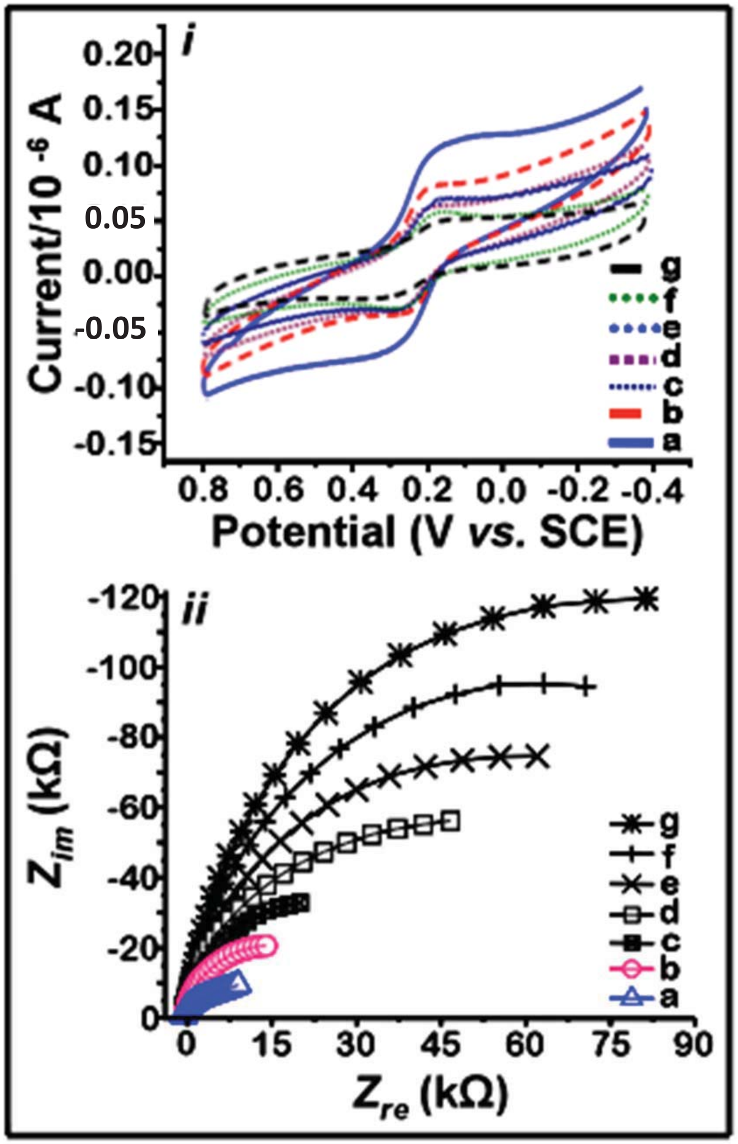
Blue: bare electrode
Pink: with anti-troponin
Black: with anti-troponin and protein

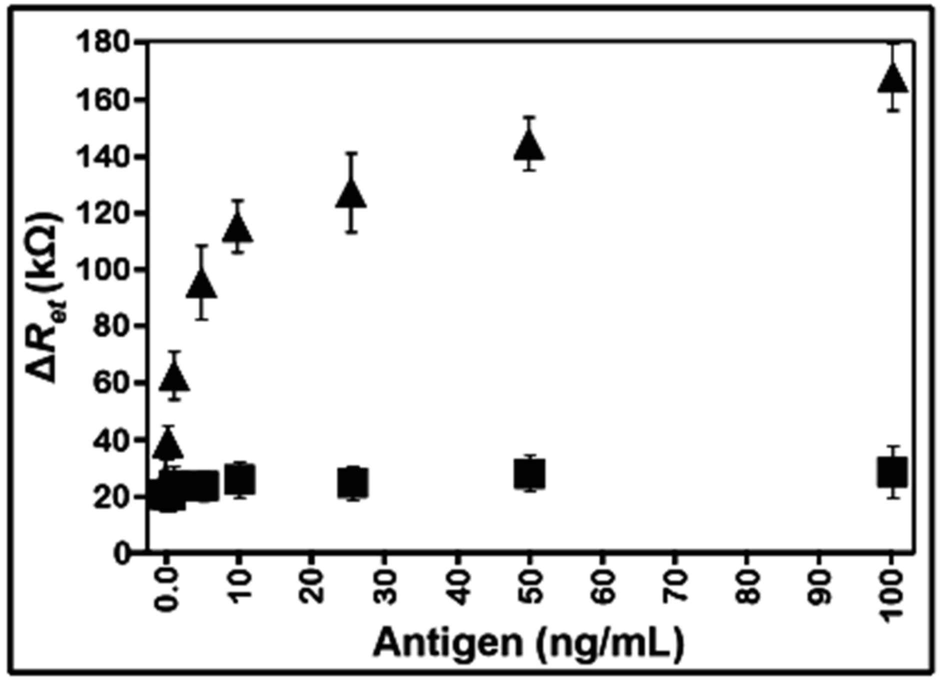


Increase in R_{ct} observed upon anti-troponin immobilization and matching protein binding

A. Periyakaruppan, R. P. Gandhiraman, M. Meyyappan, J. E. Koehne, *Anal. Chem.*, **2013**, 85, 3858-3863.

Troponin-I Concentration Study





Troponin-I concentration range: 100 ng/mL to 0.25 ng/mL
Detection down to 0.25 ng/mL

A. Periyakaruppan, R. P. Gandhiraman, M. Meyyappan, J. E. Koehne, *Anal. Chem.,* **2013**, 85, 3858-3863.

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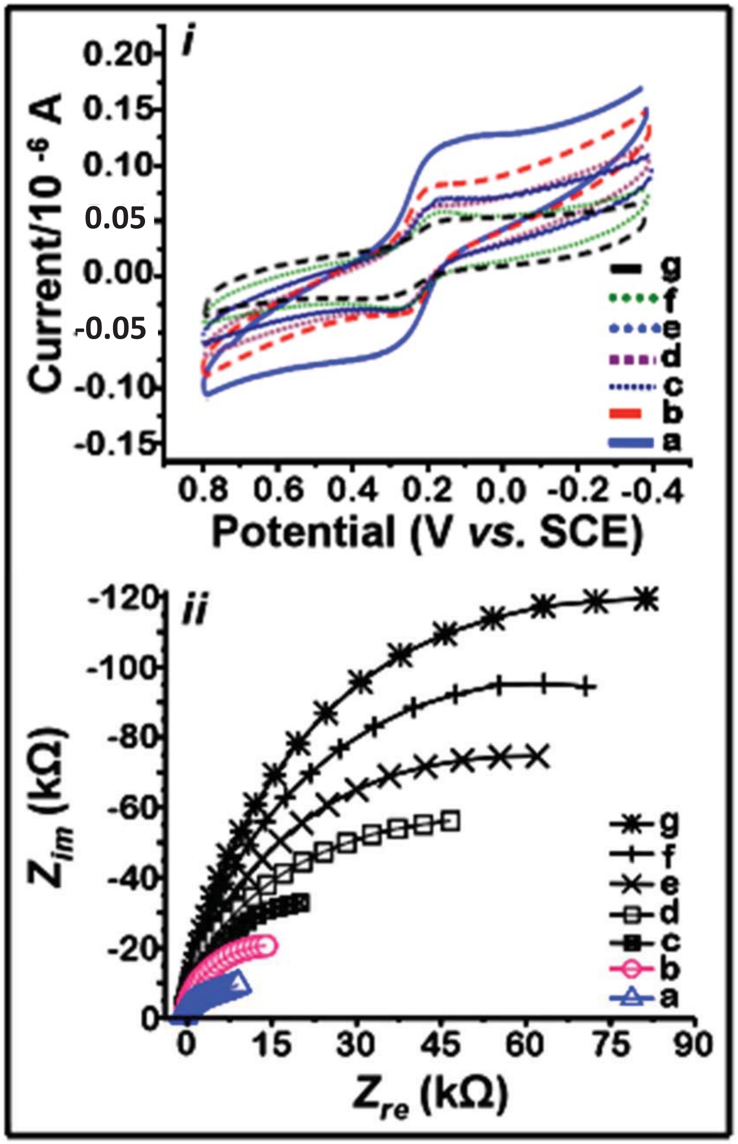

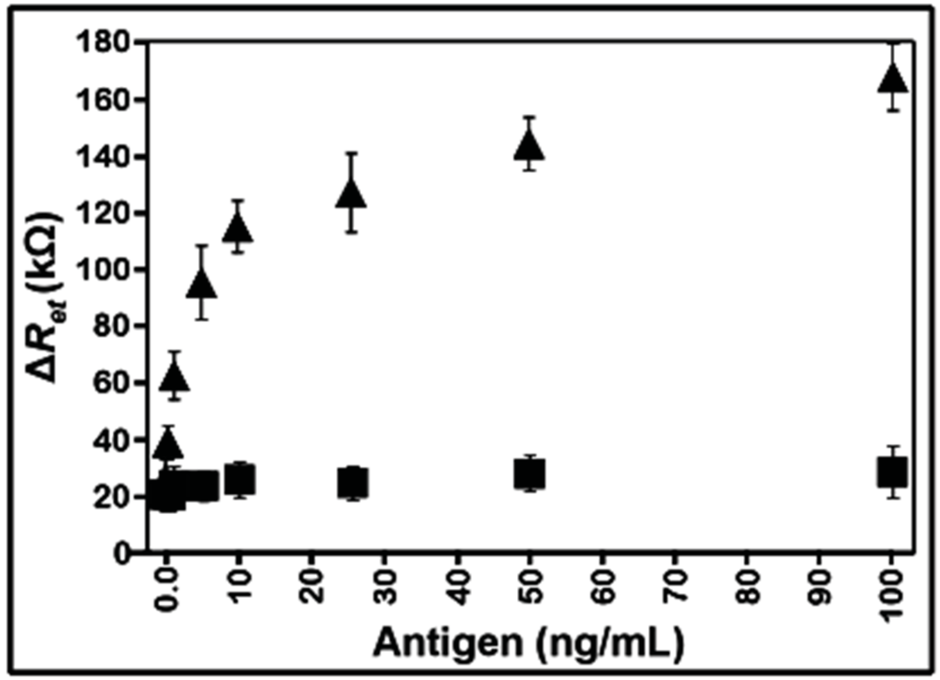

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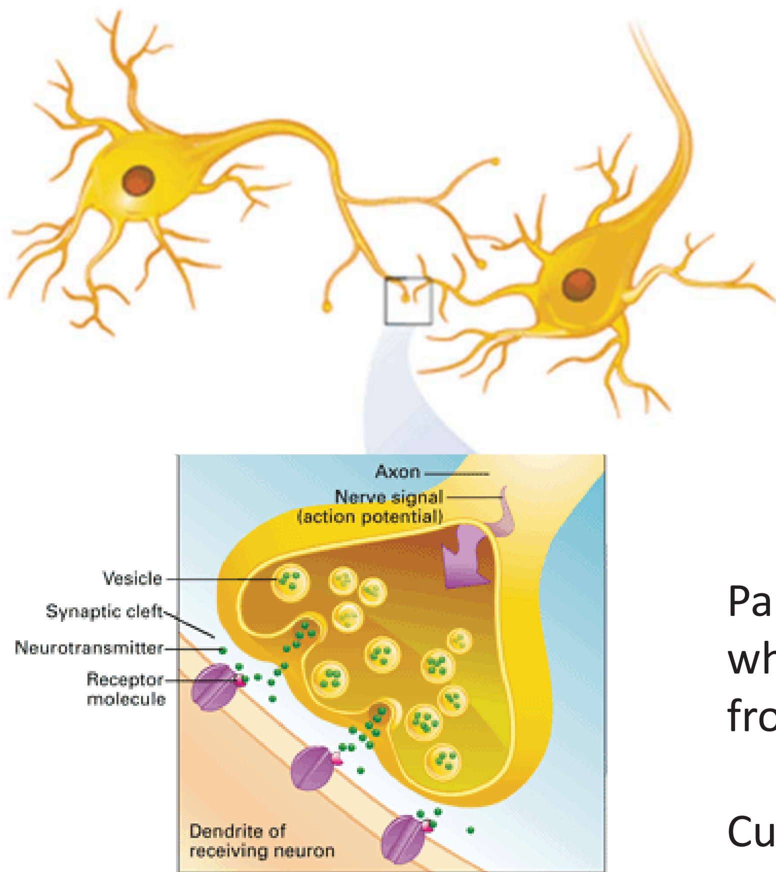
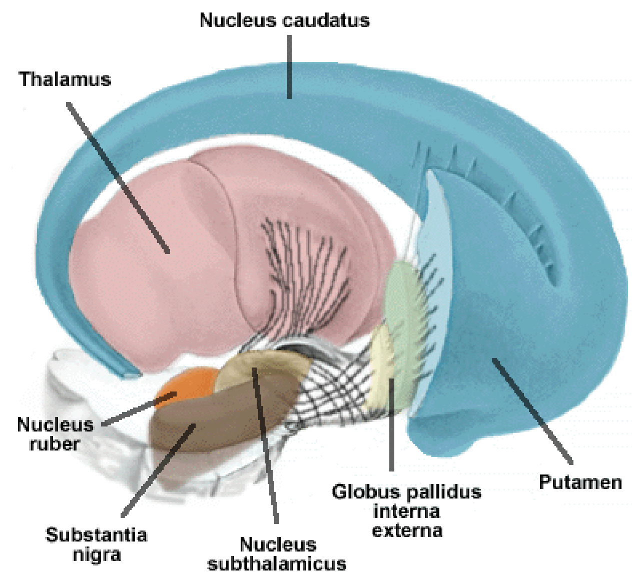
Motivation: Parkinson’s Disease





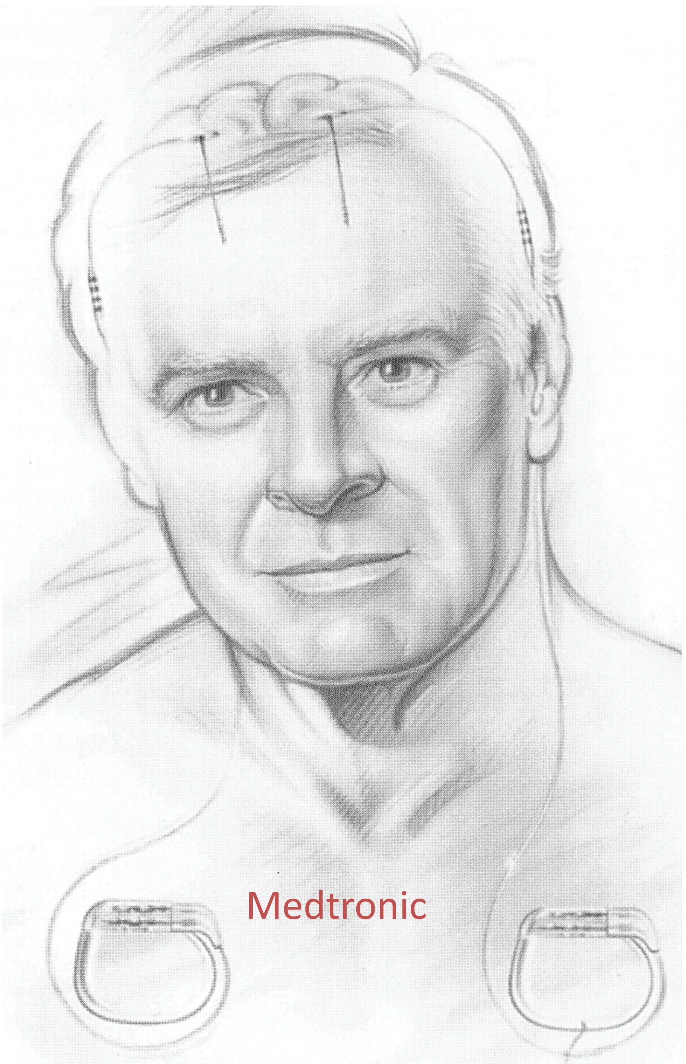
Parkinson’s disease is a neurodegenerative disorder in which patients have insufficient production of dopamine from dopaminergic cells in the substantia nigra

Current treatments include L-dopa, dopamine agonists, MAO-B inhibitors, surgery (ablation and deep brain stimulation)

http://knight.noble-hs.sad60.k12.me.us/context/exploringLife/text/chapter28/concept28.2.html

http://www.profelis.org/webpages-cn/lectures/neuroanatomy_1ns.html

Deep Brain Stimulation



Deep Brain Stimulation (DBS)

-Started in the 1960's

-Over 80,000 successful surgeries

-Has been demonstrated to be an effective neurosurgical treatment for several pathologies including:

- tremor
- epilepsy
- Parkinson's disease
- depression
- Tourette syndrome
- chronic pain

How DBS Works

- Brain pacemaker, electrical impulses to different areas of the brain
- Stimulation 24/7

Potential Improvements

-Time consuming and difficult to program without feedback

-Want real-time monitoring of the neurochemical output

-Development of chemically-guided placement of DBS electrodes *in vivo*.

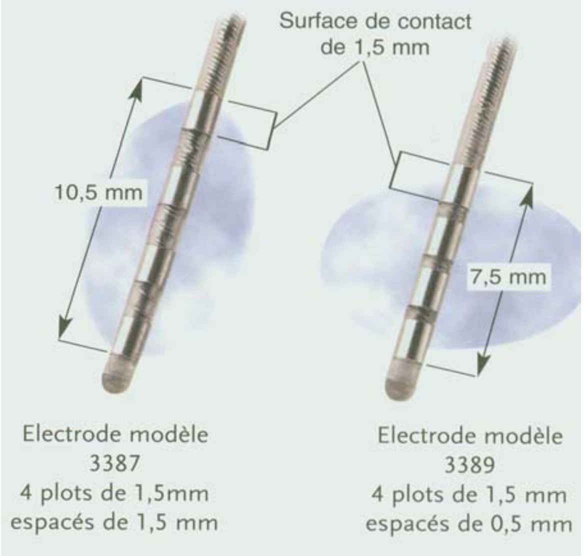
Clinical efficacy is not questioned, but mechanisms are very poorly understood

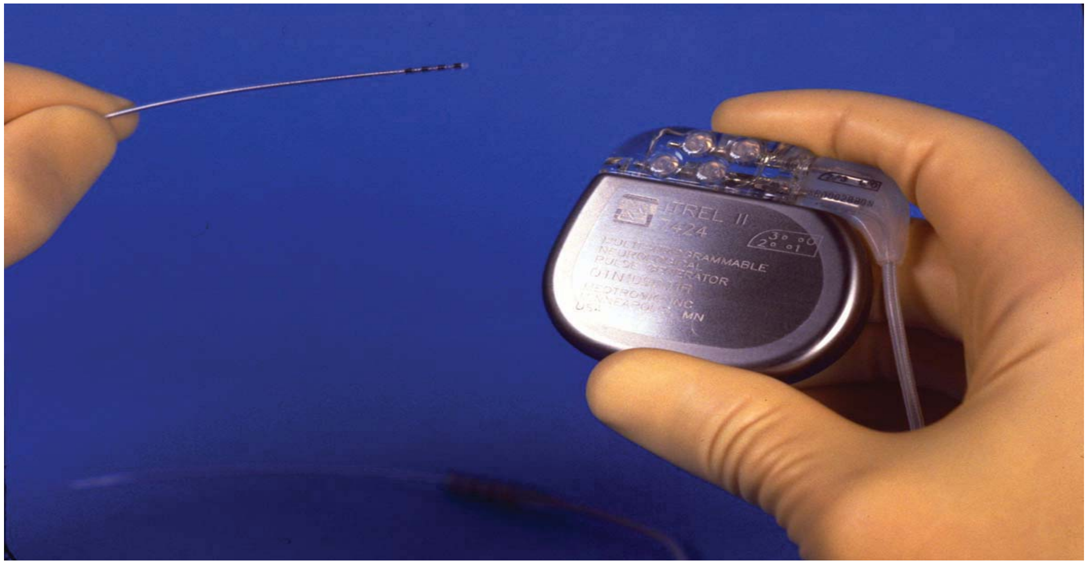
History of DBS

- DBS used for pain control since 1960s
- DBS for tremor began in Europe (1987)
- Europe: CE mark approval for
  - Activa® Tremor Control Therapy in 1993
  - Activa® Parkinson's Control Therapy in 1998
- USA: FDA approval for
  - Activa® Tremor Control Therapy in 1997
  - Activa® Parkinson's Control Therapy in 2002

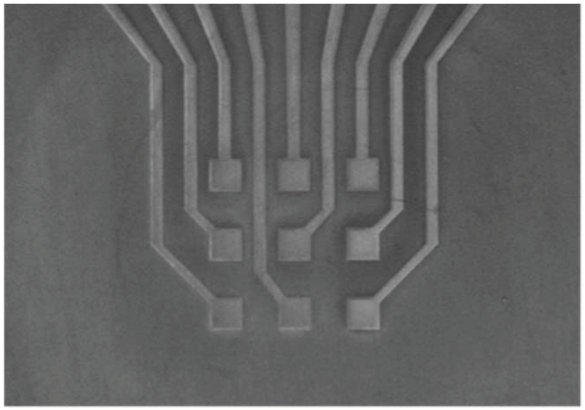
Deep Brain Stimulation Electrodes

DBS Electrodes from Medtronic





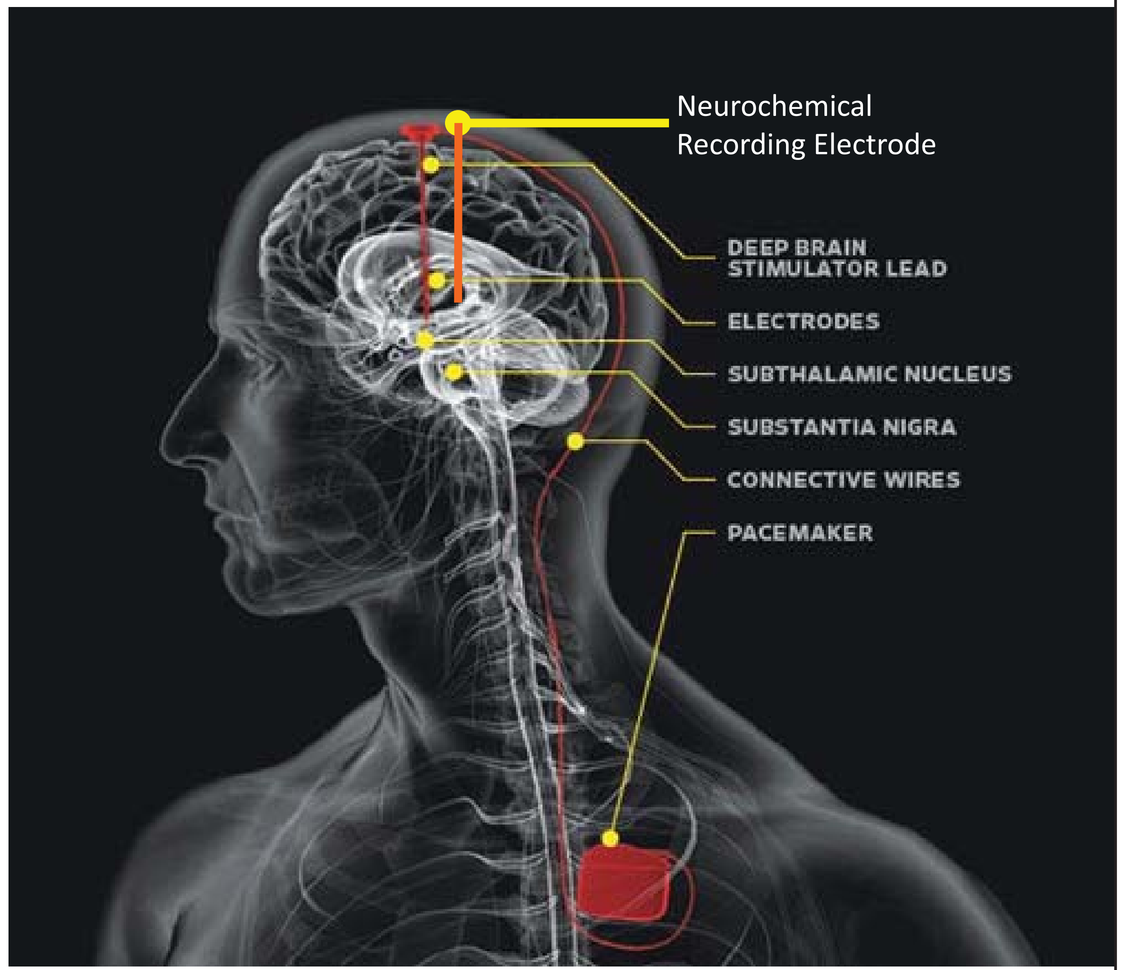
CNF Electrodes



Current 3x3 CNF device does not have an optimal geometry for implantation but can be used to preliminary in vitro investigations.

CNF Array for Applications in Deep Brain Stimulation

Stimulating Electrode



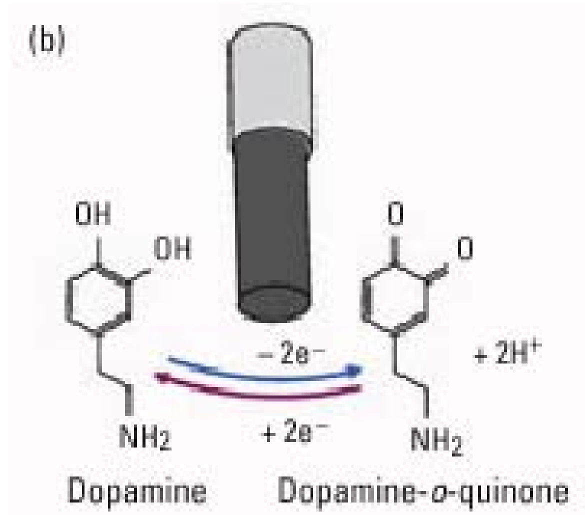
Goal:

Develop a multiplexed CNF array for localized, fast, and efficient and neurochemical recording

Electrochemical Detection of Neurotransmitters

- Molecules of Interest
  - Dopamine
    - Movement disorders, addiction
  - Serotonin
    - Depression, hunger
  - Adenosine
  - Oxygen
  - Hydrogen Ions (pH)

- Techniques
  - Differential Pulse Voltammetry
    - More sensitive
  - Fast Scan Cyclic Voltammetry
    - Better temporal resolution



Simultaneous Detection of Neurotransmitters

Glassy Carbon Electrode

Carbon Nanofiber Electrode

Ascorbic Acid Dopamine Serotonin

-CNF electrode has ability to distinguish multiple electroactive brain chemicals in a mixture!

-Detection limits 50nM for DA and 100nM for 5-HT

MAYO CLINIC

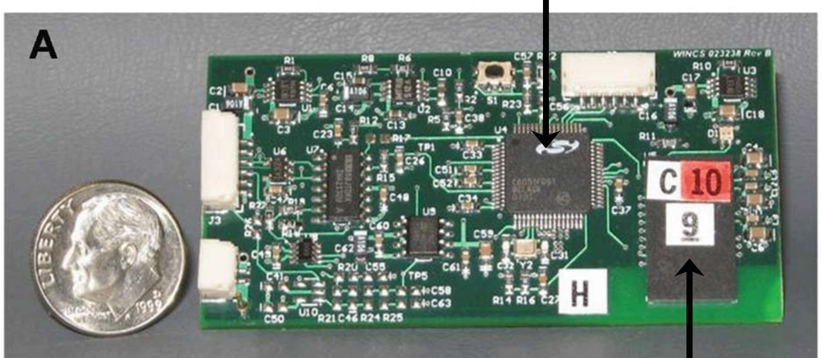
Wireless Instantaneous Neurotransmitter Concentration Sensor (WINCS)

The Mayo Clinic-developed WINCS is a microprocessor-controlled, MRI-compatible, battery-powered instrument that combines Bluetooth® digital telemetry with fast scan cyclic voltammetry and constant potential amperometry.

Standard Potentiostat

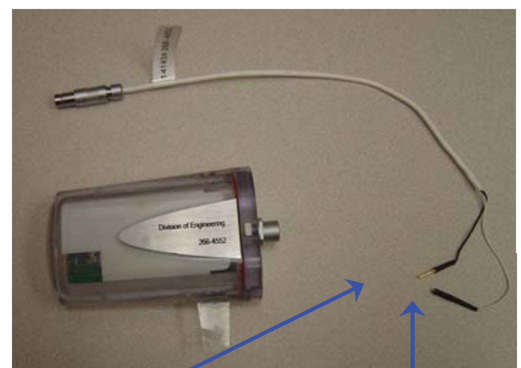
Printed Circuit Board

Microprocessor



Bluetooth®

Sterilizable WINCS Unit



Reference Electrode Lead

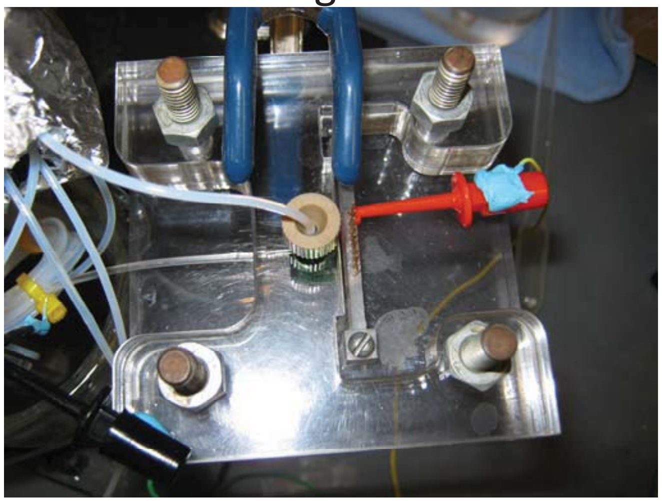
Working Electrode Lead

WINCS was designed in compliance with FDA-recognized standards for medical electrical device safety.

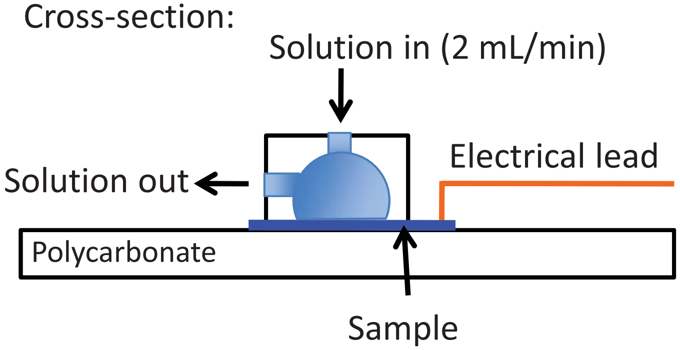
Bledsoe, J. M. et al., *J. Neurosurg*, **2010**, 11, 712-723.

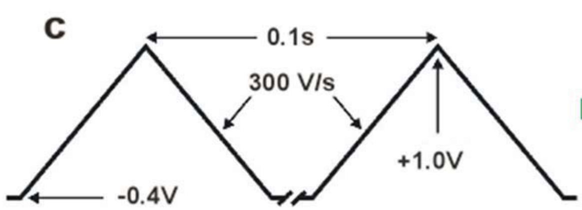
Experimental Setup

Custom-Designed Flow Cell

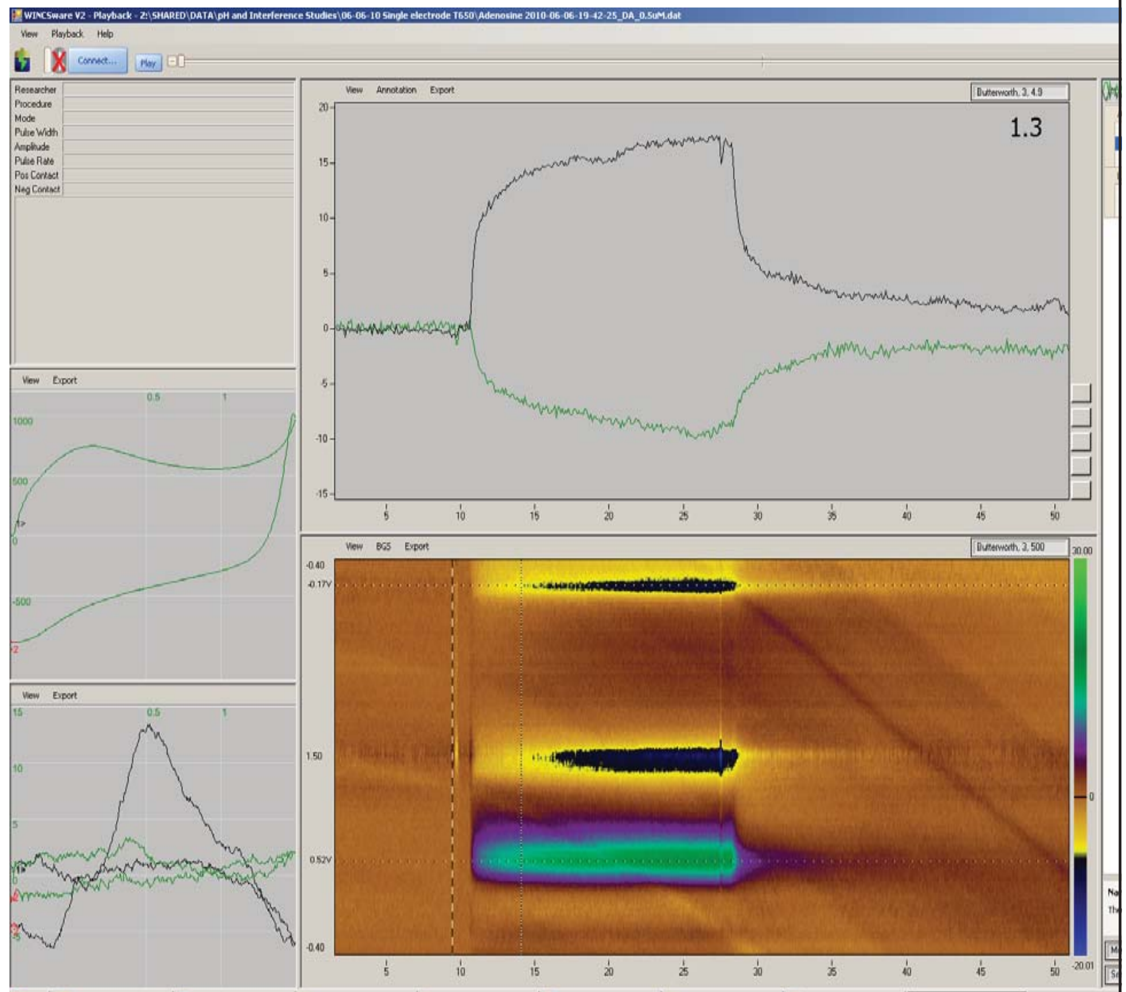


Cross-section:





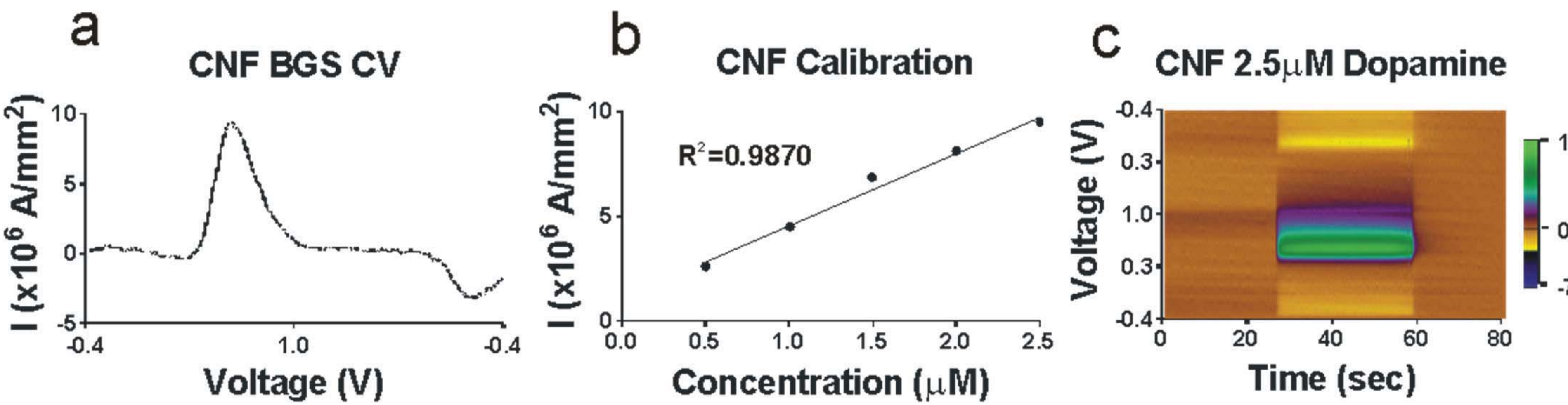
WINCSware User Interface



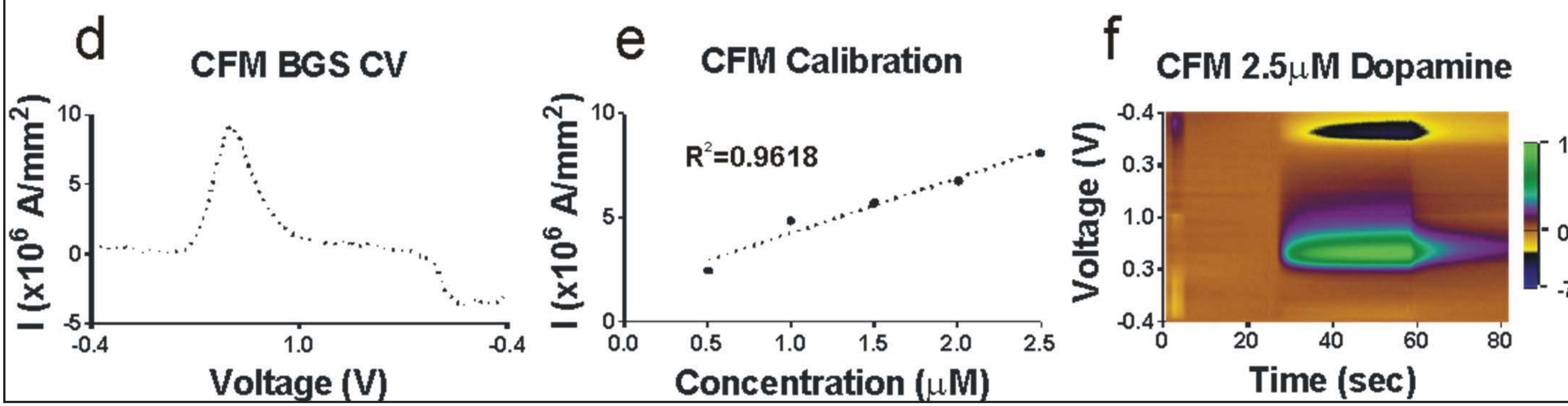
WINCSware allows viewing of the data in nearly real-time

Dopamine Detection

Carbon Nanofiber Electrode



Carbon Fiber Microelectrode

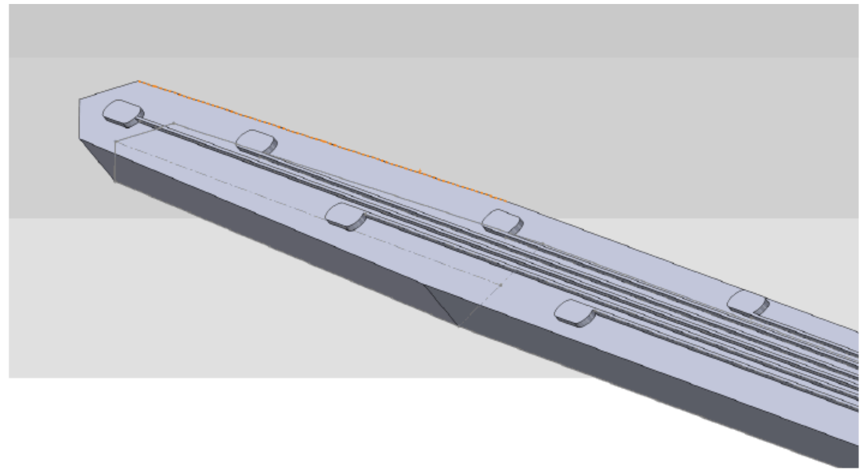


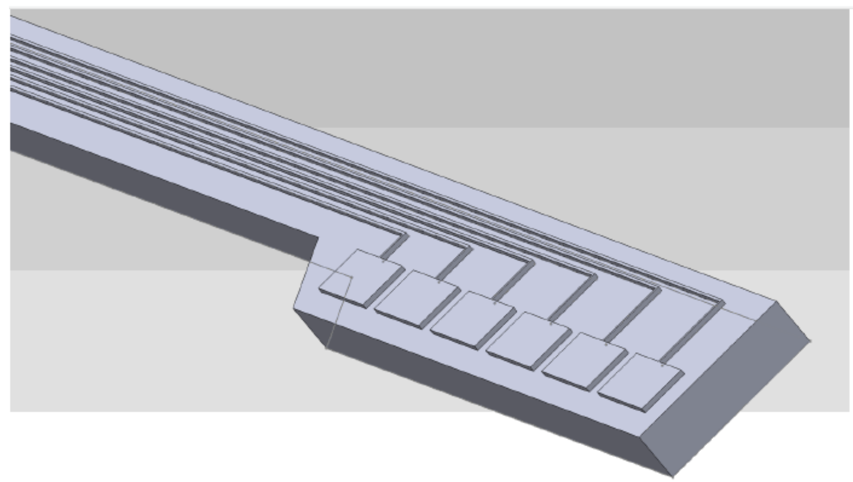
In the Works

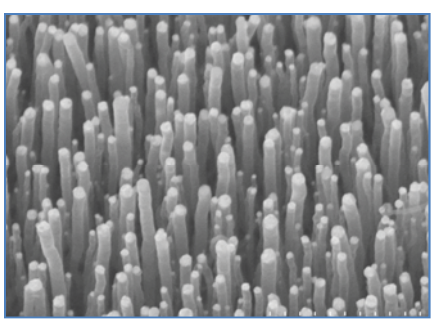
Want to Combine:

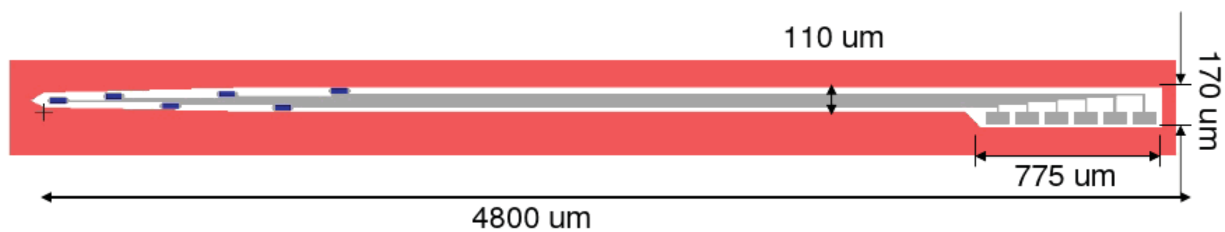
1) Penetrating multiplexed array
   - Ability to spatially resolve chemical release events

2) Array of individual carbon nanofiber nanoelectrodes
   - High sensitivity (increased signal to noise)
   - Rapid detection (increased cell time constant)
   - Wide potential window of carbon









Summary

- Carbon nanofibers can be used to as nanoscale electrodes to reduce background noise while maintaining large sampling volume
- Carbon nanofiber nanoelectrode arrays are easily fabricated using standard silicon processing
  - CNF spacing defined by photolithography, e-beam lithography or top layer dielectric polishing time
- Carbon nanofibers have been used as sensitive nanoelectrodes for cyclic voltammetry and electrochemical impedance spectroscopy investigations
- Changes in R_{ct} are measured after antibody immobilization and protein binding
- Carbon nanofiber nanoelectrode arrays have been used to detect down to 0.25 ng/mL troponin-I
- CNFs can distinguish between multiple electroactive analytes in a mixture using differential pulse voltammetry
- CNFs nanoelectrode arrays easily integrate with WINCS
- CNFs detect dopamine with similar performance to a standard carbon fiber microelectrode
- The flexible multiplexing capability of CNF devices will be used in the future for in vivo studies of neurotransmitter detection

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