Carbon nanofiber nanoelectrodes for neural stimulation and chemical detection: The era of "smart" deep brain stimulation

Jessica E. Koehne, NASA Ames Research Center, Moffett Field, CA

A sensor platform based on vertically aligned carbon nanofibers (CNFs) has been developed. Their inherent nanometer scale, high conductivity, wide potential window, good biocompatibility and well-defined surface chemistry make them ideal candidates as biosensor electrodes. Here, we report two studies using vertically aligned CNF nanoelectrodes for biomedical applications. CNF arrays are investigated as neural stimulation and neurotransmitter recording electrodes for application in deep brain stimulation (DBS). Polypyrrole coated CNF nanoelectrodes have shown great promise as stimulating electrodes due to their large surface area, low impedance, biocompatibility and capacity for highly localized stimulation. CNFs embedded in SiO_2 have been used as sensing electrodes for neurotransmitter detection. Our approach combines a multiplexed CNF electrode chip, developed at NASA Ames Research Center, with the Wireless Instantaneous Neurotransmitter Concentration Sensor (WINCS) system, developed at the Mayo Clinic. Preliminary results indicate that the CNF nanoelectrode arrays are easily integrated with WINCS for neurotransmitter detection in a multiplexed array format. In the future, combining CNF based stimulating and recording electrodes with WINCS may lay the foundation for an implantable "smart" therapeutic system that utilizes neurochemical feedback control while likely resulting in increased DBS application in various neuropsychiatric disorders. In total, our goal is to take advantage of the nanostructure of CNF arrays for biosensing studies requiring ultrahigh sensitivity, high-degree of miniaturization, and selective biofunctionalization.

Biography

Dr. Jessica E. Koehne is a research scientist at the NASA Ames Center for Nanotechnology where she leads the Nano-Biosensors Group. Her research interests include the interface between nanoscale materials, electronics and biological systems with an emphasis on biosensing. Dr. Koehne has developed carbon nanotube and nanofiber based sensor platforms for the detection of DNA, rRNA, proteins and neurotransmitters with applications ranging from point-of-care to homeland security. Dr. Koehne has published over 40 peer reviewed articles in the field of nanotechnology and has received numerous awards for technical achievement including the 2011 Presidential Early Career Award for Scientists and Engineers.

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Outline

- Biosensing motivation
- Structure and fabrication of carbon nanofibers
- Application of carbon nanofiber electrodes in deep brain stimulation therapy
- Neurochemical recording by carbon nanofiber electrodes and Wireless Instantaneous Neurotransmitter Concentrator Sensor system

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
- Some of Ames Technical Areas
  - Astrobiology
  - Thermal protective systems
  - Simulation technology
  - Atmospheric science
  - Fundamental space biology
  - Human factors research
  - Nanotechnology

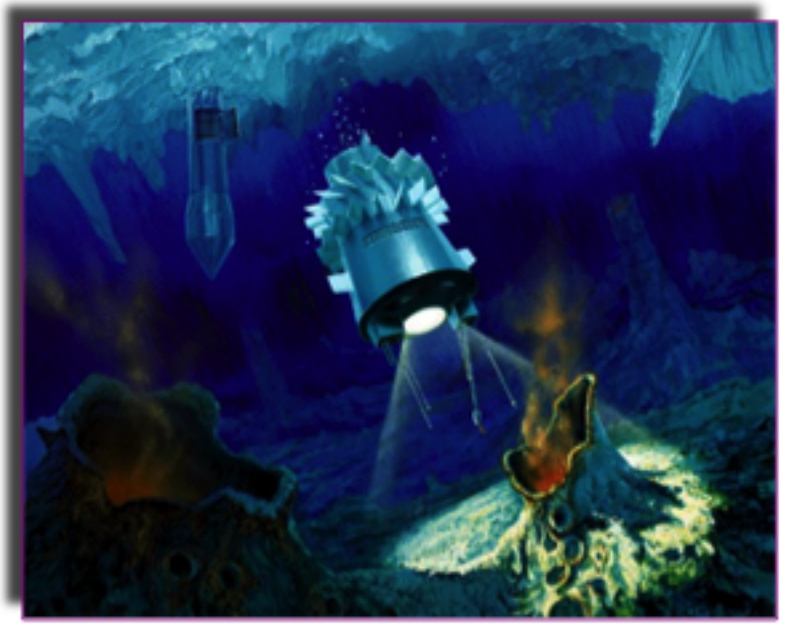
Biosensor Motivation

NASA Applications

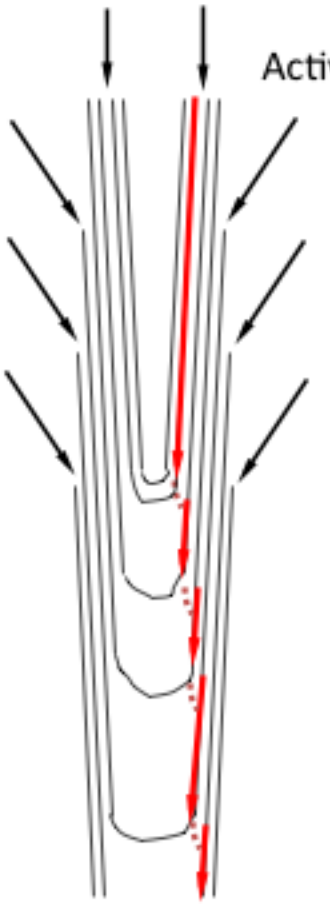
- Astronaut health monitoring
  - Lab-on-a-chip
- Water Quality monitoring
  - Pathogen detection on ISS and long duration missions
- Planetary exploration
  - Life on other planets

Outside Applications and Customers

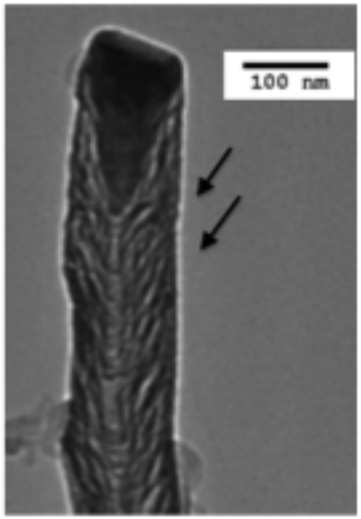
- Medical Diagnostics
  - NIH, DARPA
- Environmental Monitoring
  - EPA, NIH
- Biowarfare agent detection
  - DHS, DARPA
- Food Safety
  - FDA



What are Carbon Nanofibers (CNFs)?

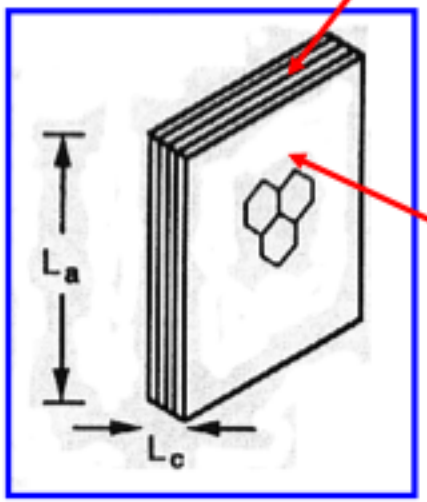


Bamboo-like CNFs



TEM of CNF

HOPG



Edge Plane:
(1) High electron transfer rate (~ 0.1 cm/s)
(2) Very high specific capacitance (>60 μF/cm²)

Basal Plane:
(1) Low electron transfer rate (< 10^{-7} cm/s)
(2) Anomalously low capacitance (~1.9 μF/cm²)

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

Why CNF as biosensor electrode material?

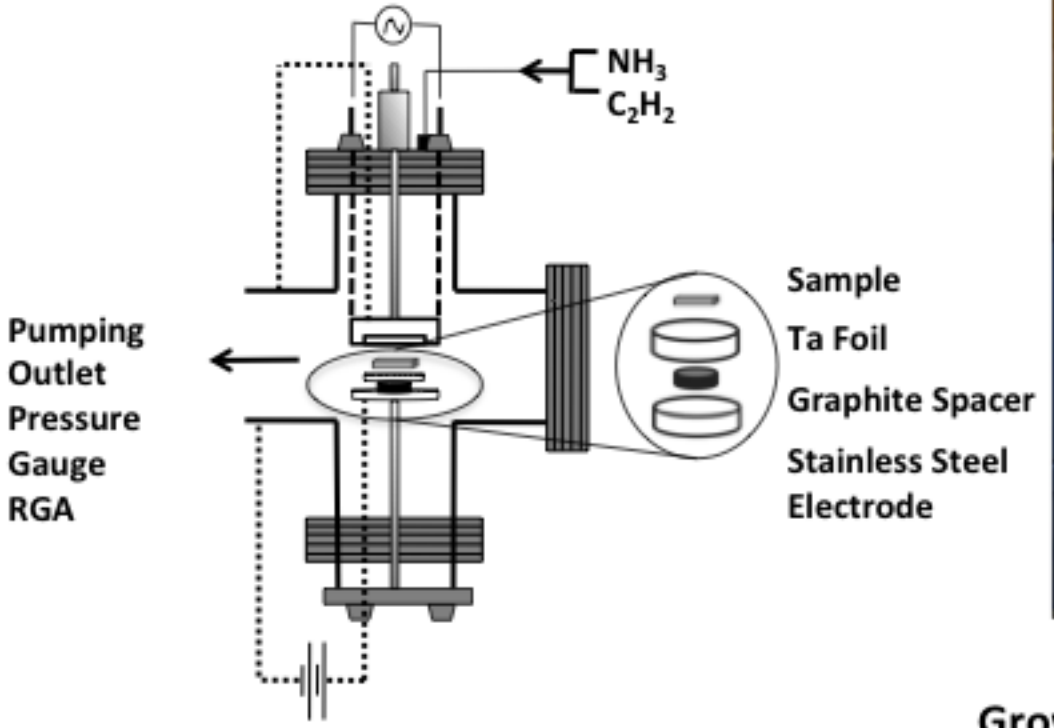
1) Good conductivity
2) Wide potential window
3) Many active sites for electron transfer
4) Easy to pattern, grow and process on silicon devices

CNF Growth by Plasma Enhanced Chemical Vapor Deposition (PECVD)

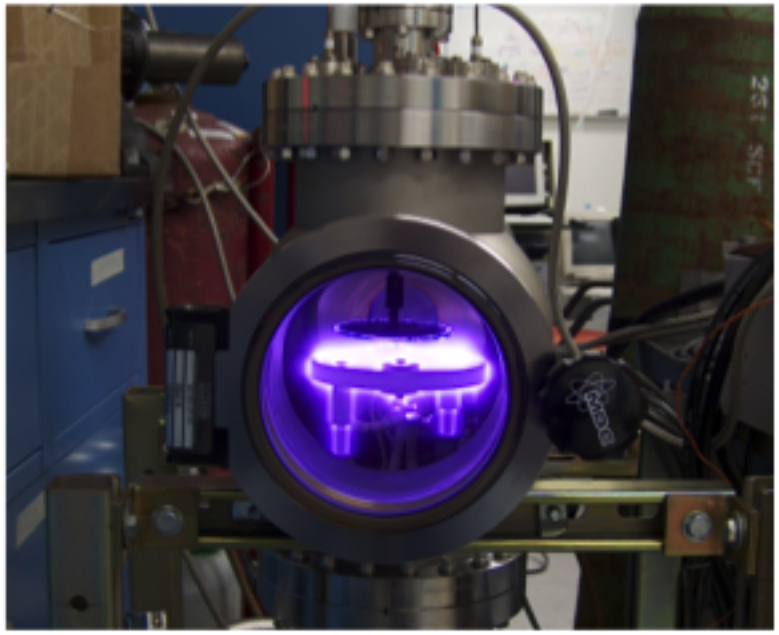
Ames Research Center

NASA

PECVD Reactor Schematic



Custom Built PECVD Reactor



Growth Process

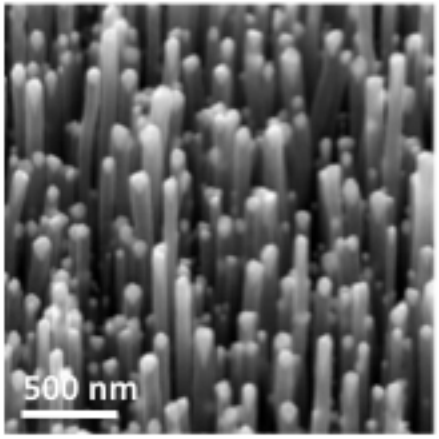
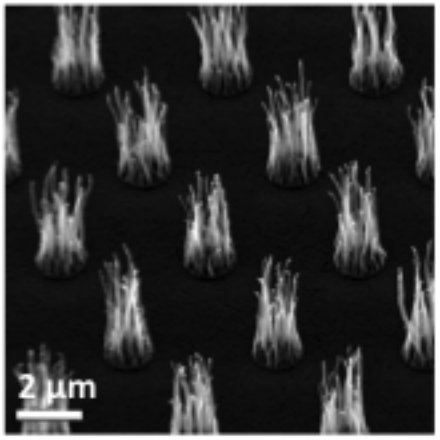
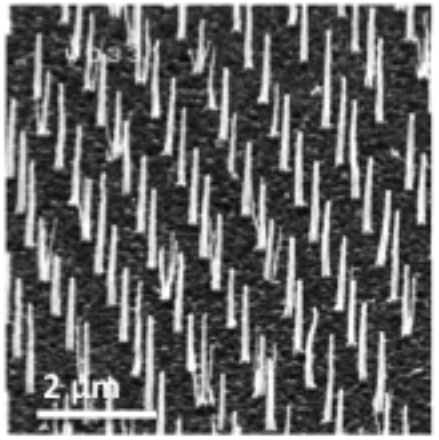
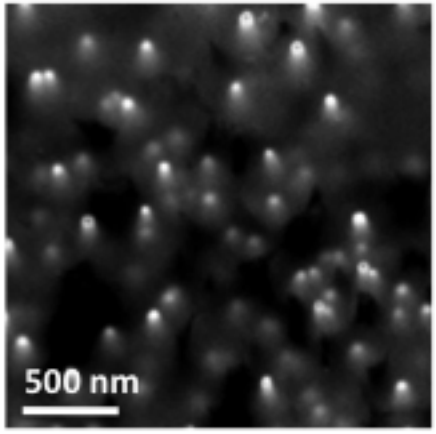
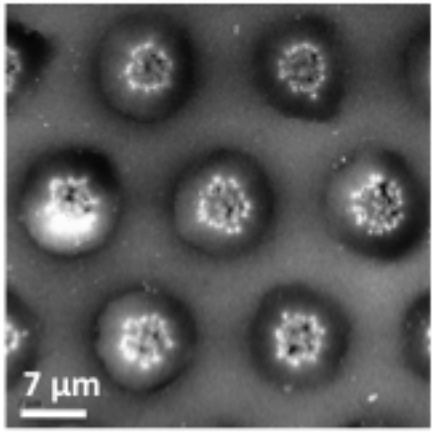
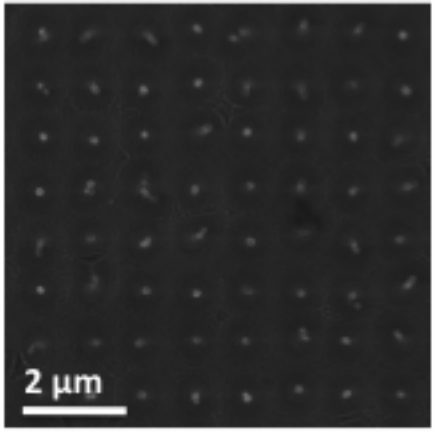
- Heated to 650 C
- Plasma discharge 500 W, 530 V, 0.97 A
- 150 sccm NH_3/50 sccm C_2H_2, 5-6 torr
- Growth rate- 1000 nm/min
- Quality is good, alignment is good

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

Ames Research Center

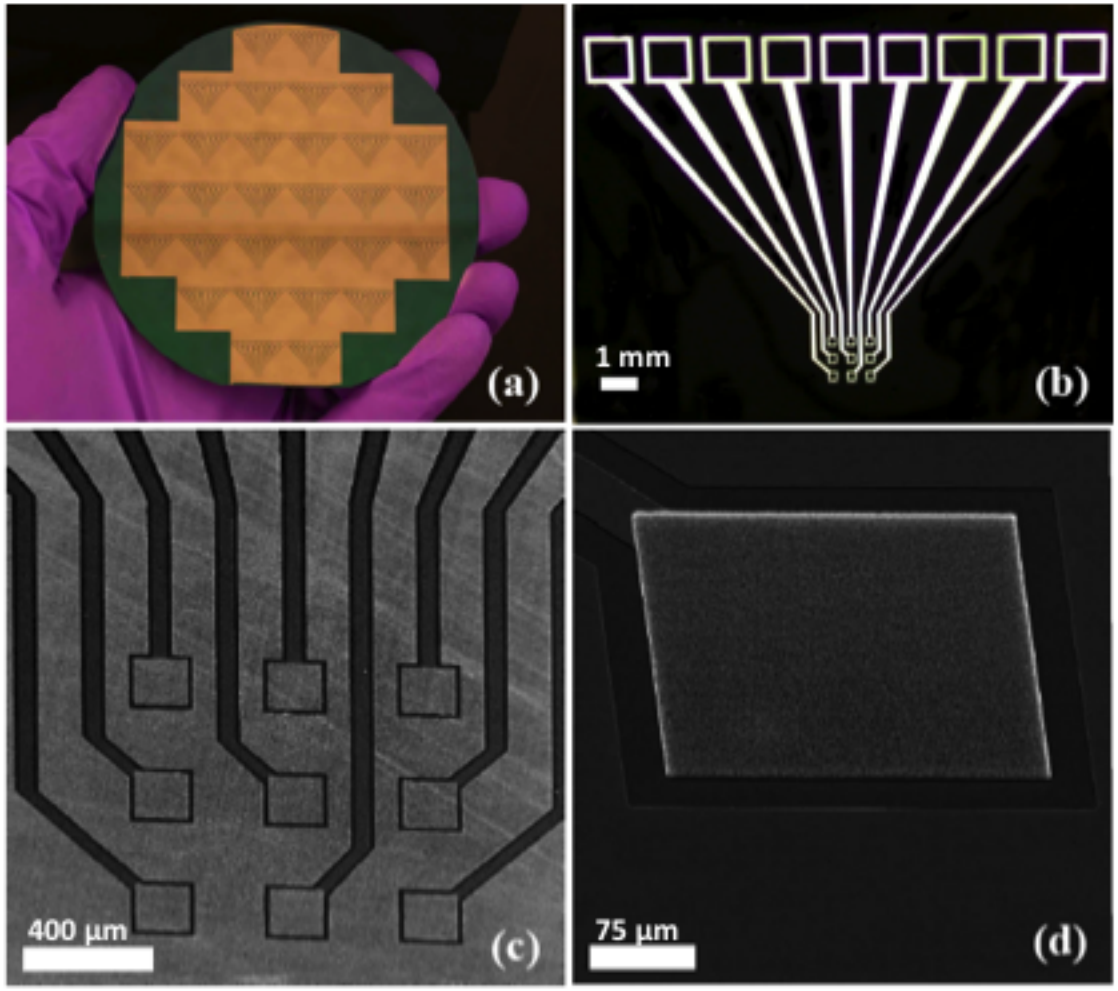
Define CNF Placement by Catalyst Placement

NASA

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

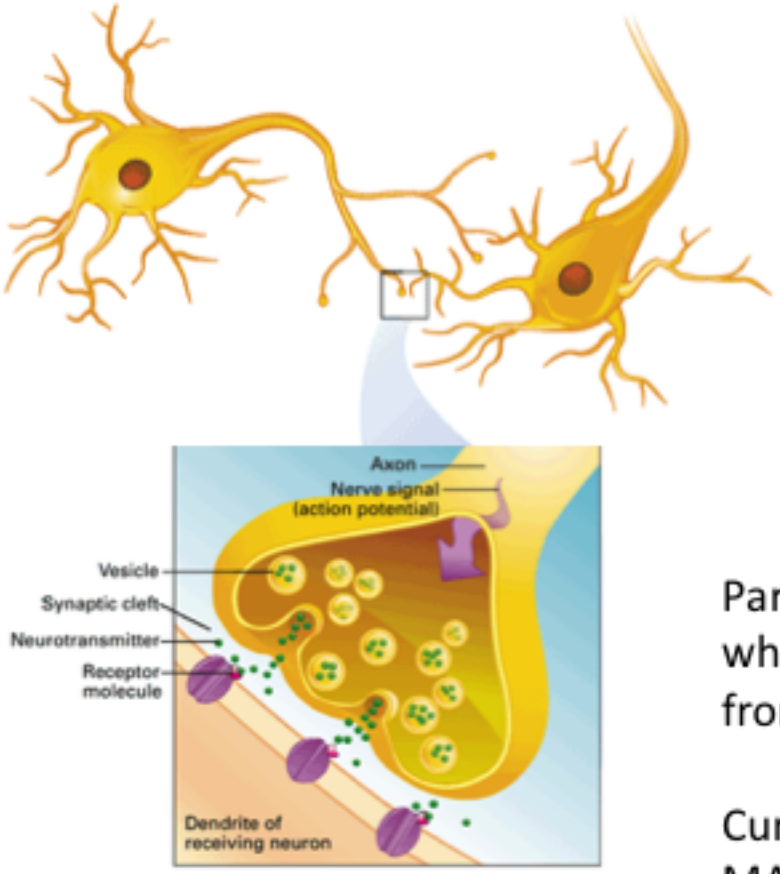
Fabrication of 3x3 Array

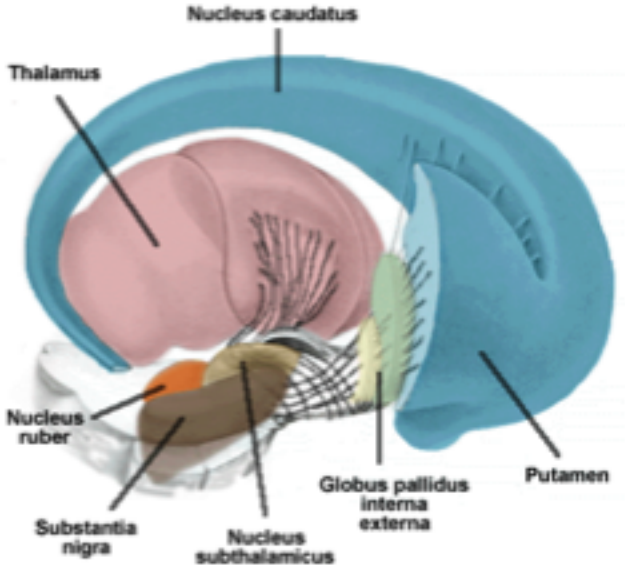
30 devices on a 4" Si wafer



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

Motivation: Parkinson's Disease





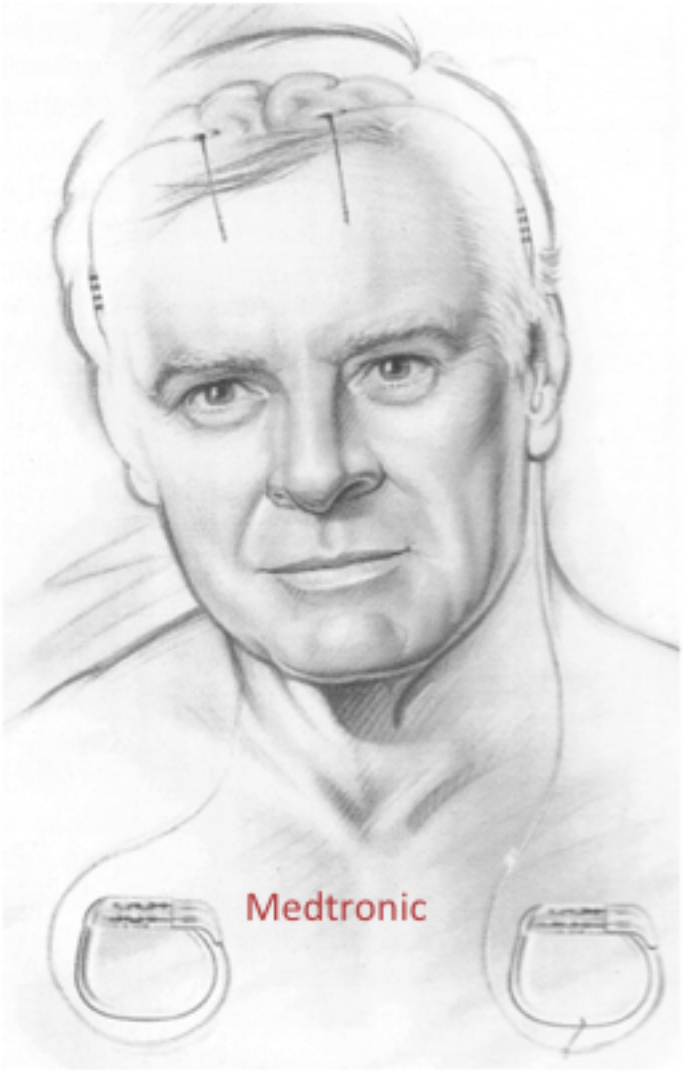
Parkinson's disease is a neurodegenerative disorder in which patients have insufficient production of dopamine from dopaminergic cells in the substantia nigra

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

http://professional.medtronic.com/therapies/deep-brain-stimulation/presentations-and-downloads/index.htm

Ames Research Center

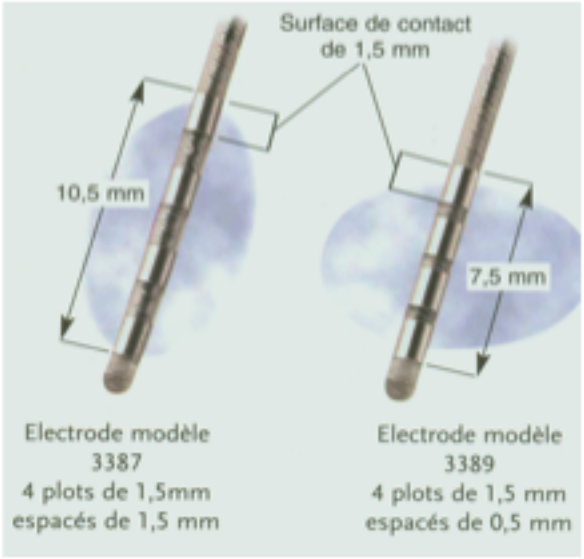
History of DBS

NASA

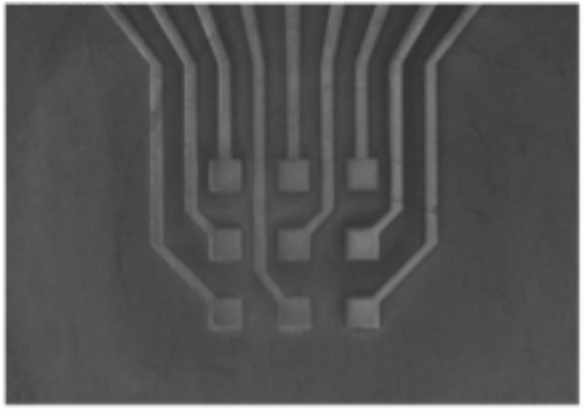
- DBS used for pain control since 1960s
- DBS for tremor began in Europe (1987)
- Europe: CE mark approval for
  - Activa® Tremor Control Therapy in 1993
  - Activa® Parkinson's Control Therapy in 1998
- USA: FDA approval for
  - Activa® Tremor Control Therapy in 1997
  - Activa® Parkinson's Control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

Deep Brain Stimulation Electrodes

DBS Electrodes from Medtronic



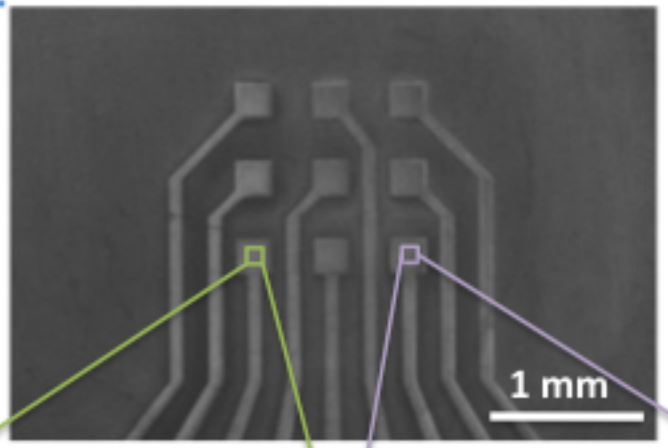
CNF Electrodes

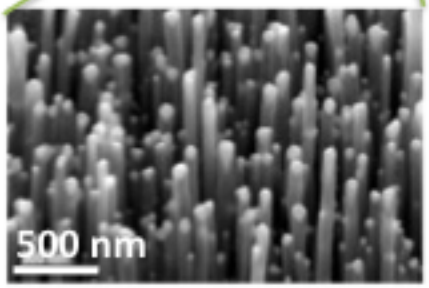


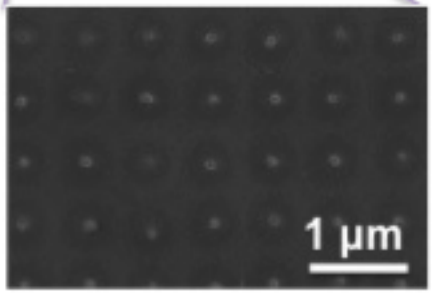
Current 3x3 CNF device does not have an optimal geometry for implantation but can be used to preliminary in vitro investigations.

CNF Array for Applications in Deep Brain Stimulation

Active Electrode Array at the Tip







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

Stimulating Electrode:
Bare CNFs with high capacitance and low impedance

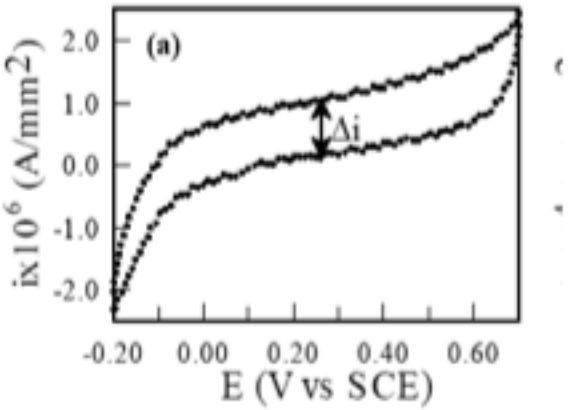
Recording Electrode:
CNFs embedded in SiO_2 with ultrahigh sensitivity

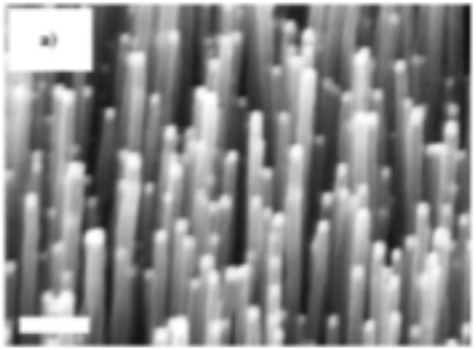
Neural Stimulation Using CNF Array

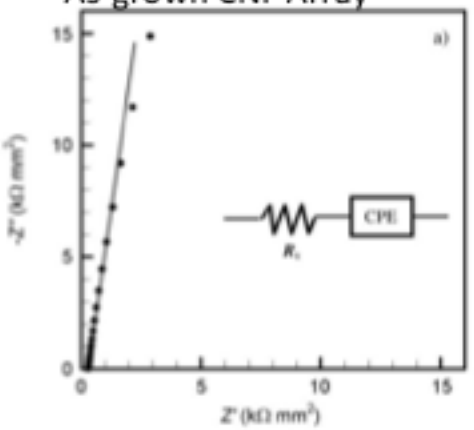
High Capacitance ($C_0 = \Delta i/2v$)
Noble metal ~ 20 μF/cm²
As-grown CNF array: 0.4 mF/cm²
Ppy-coated CNF array: 40 to 100 mF/cm²

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

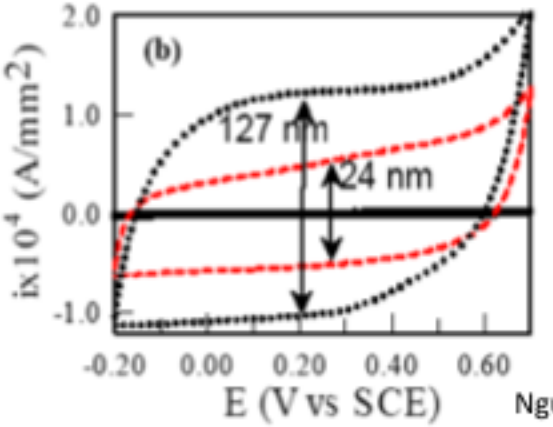
As-grown CNF Array

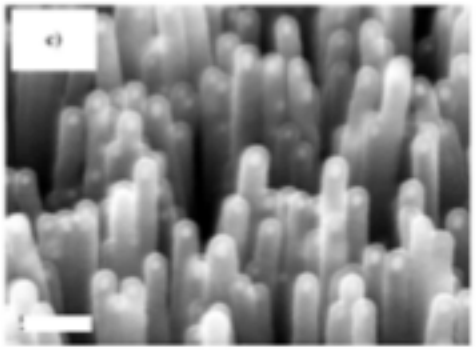




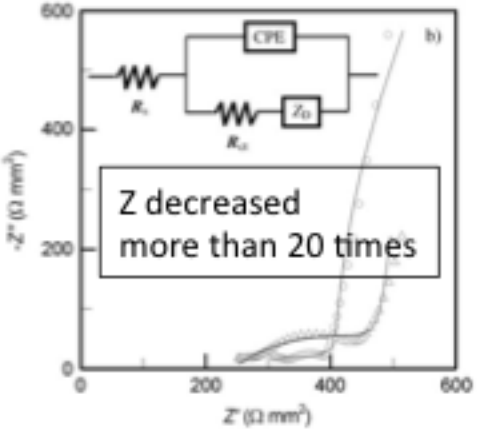


After PPy coating





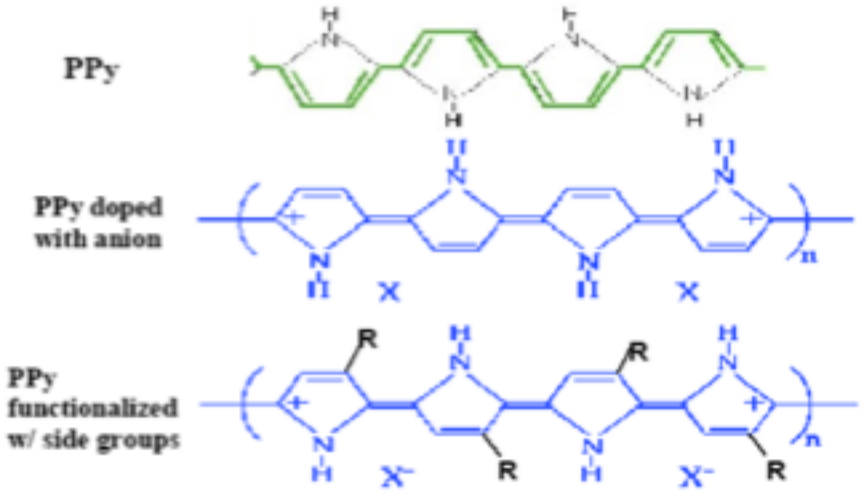
After Ppy coating



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

Biocompatibility

Optimization though chemical modification



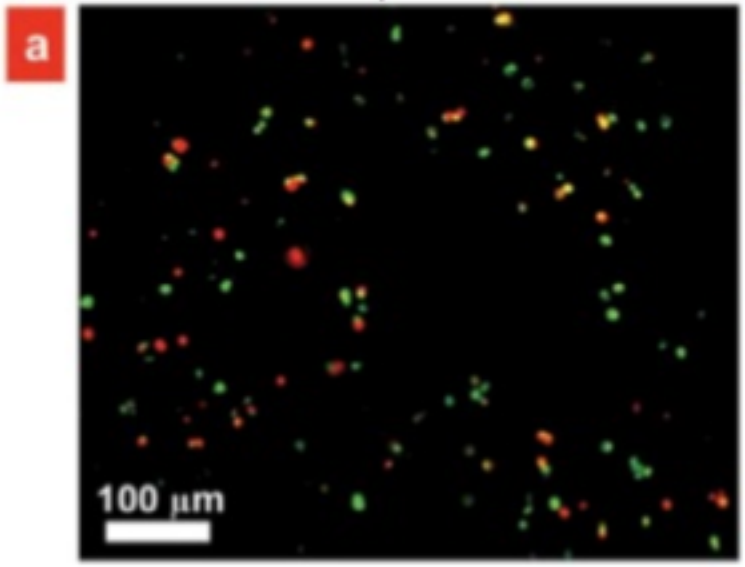
Freedom for modification:

(1) Changing anion dopants such as Cl^-, ClO_4^-, poly(styrenesulfonate) (PSS), other polyelectrolytes, etc.

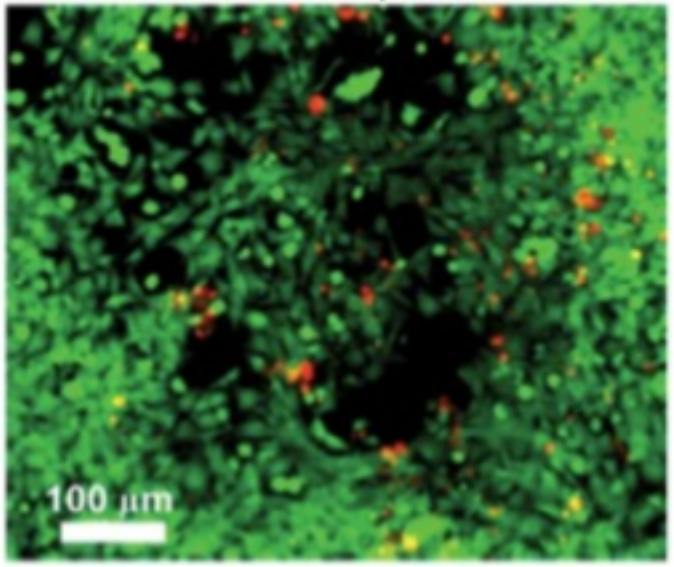
(2) Loading with drugs such as antibiotics, anti-inflammatory molecules, etc. and biomolecules such as nerve growth factors.

(3) Surface functionalization with cell adhesion molecules and specific ligands.

Cl^- dopant

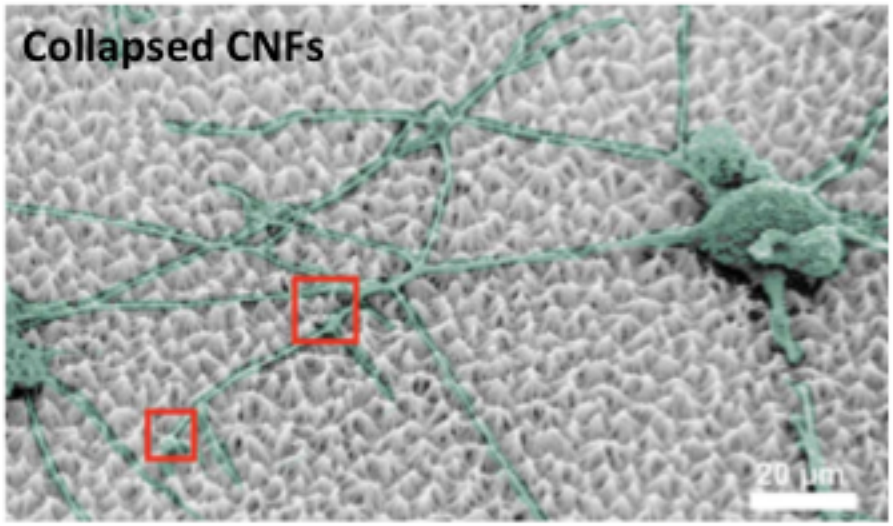


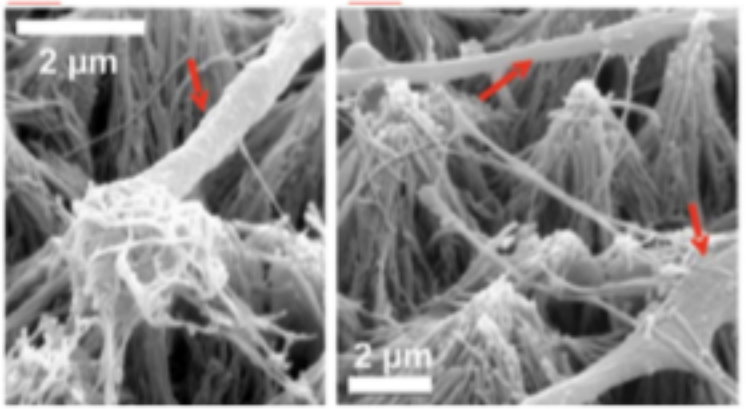
PSS dopant

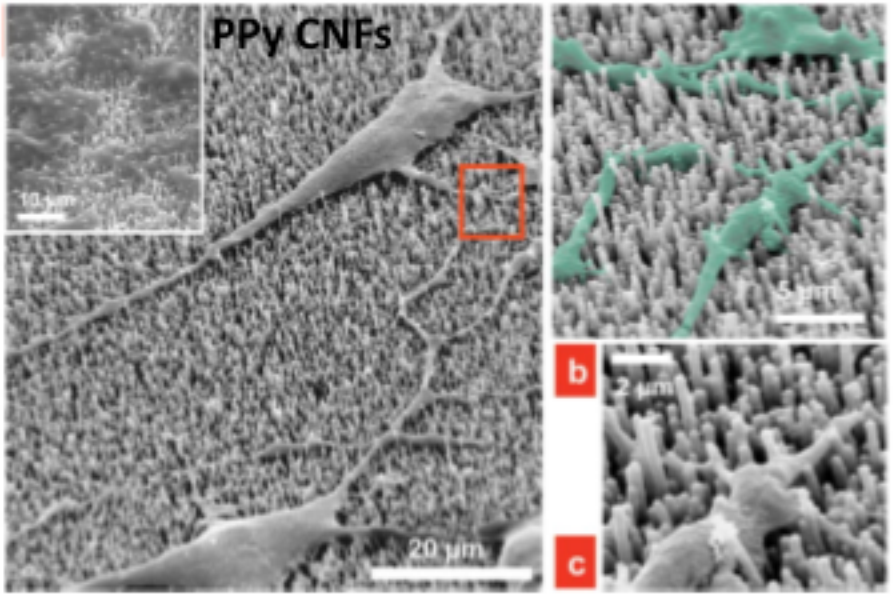


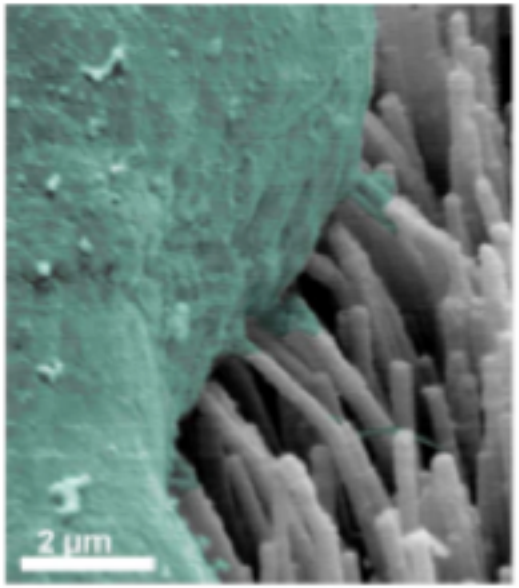
Nguyen-Vu, B. T. D., et al. IEEE Trans. Biomed. Eng. **2007**, 54, 1121-1128.

PC12 cells on PPy coated CNFs







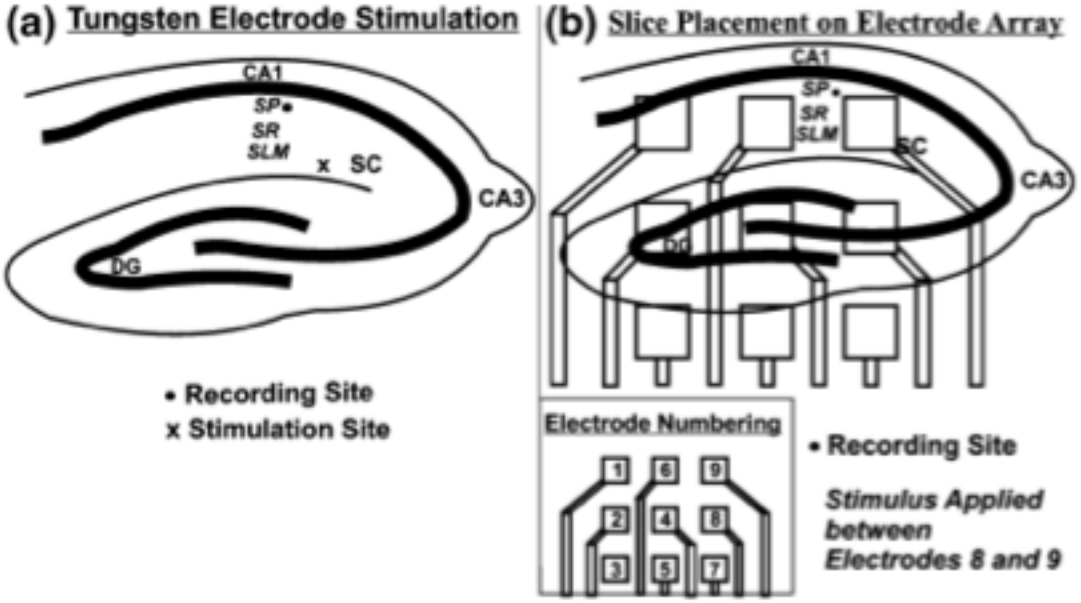


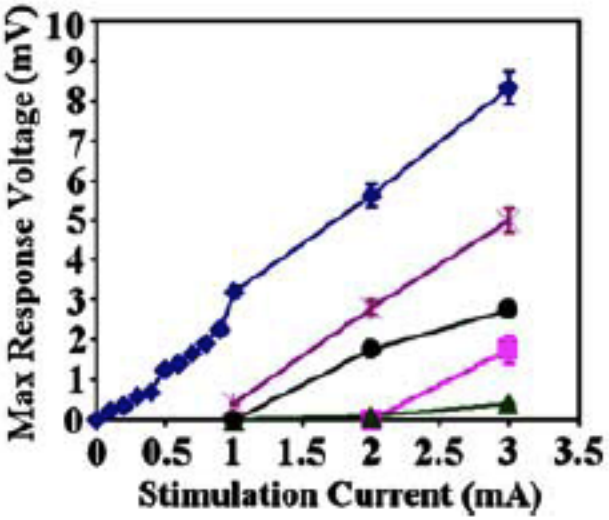
Nguyen-Vu, B. T. D., et al. IEEE Trans. Biomed. Eng. **2007**, 54, 1121-1128.

Stimulation of Rat Hippocampal Brain Slices

Experiment: Measure voltage for a given stimulation current

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



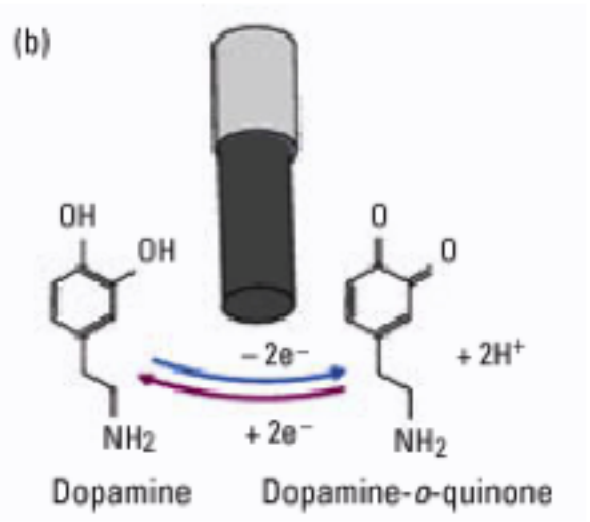


1) Only PPy coated CNFs were able to stimulate tissue under 1 mA stimulation current.
2) Only PPy coated CNFs did not induce the electrolysis of water (less than 1 mA and 1V)

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

Electrochemical Detection of Neurotransmitters

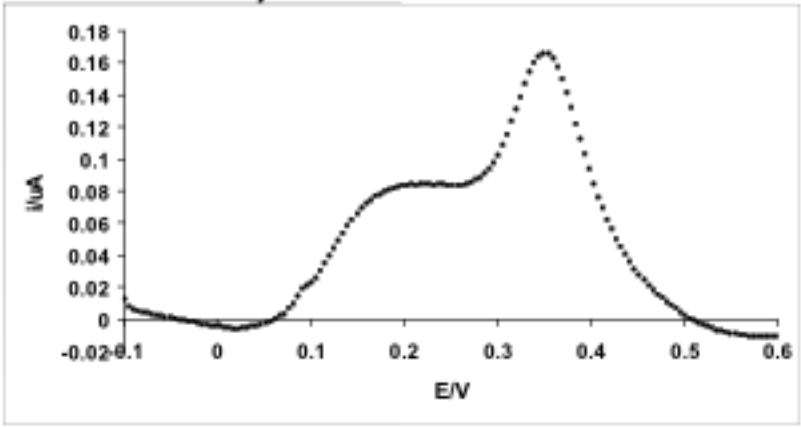
- Molecules of Interest
  - Dopamine
    - Movement disorders, addiction
  - Serotonin
    - Depression, hunger
  - Adenosine
  - Oxygen
  - Hydrogen Ions (pH)



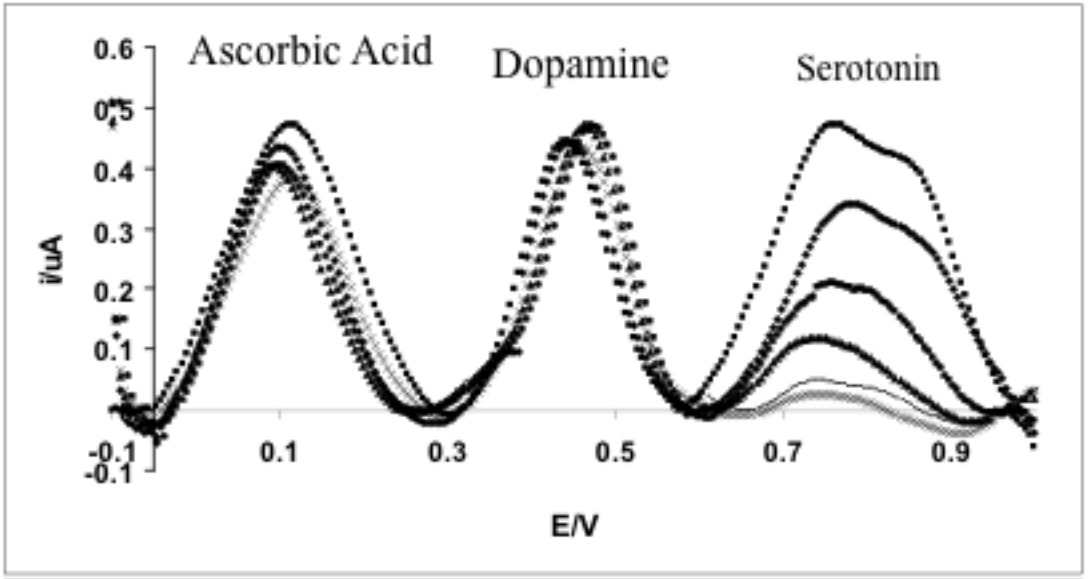
- Techniques
  - Differential Pulse Voltammetry
    - More sensitive
  - Fast Scan Cyclic Voltammetry
    - Better temporal resolution

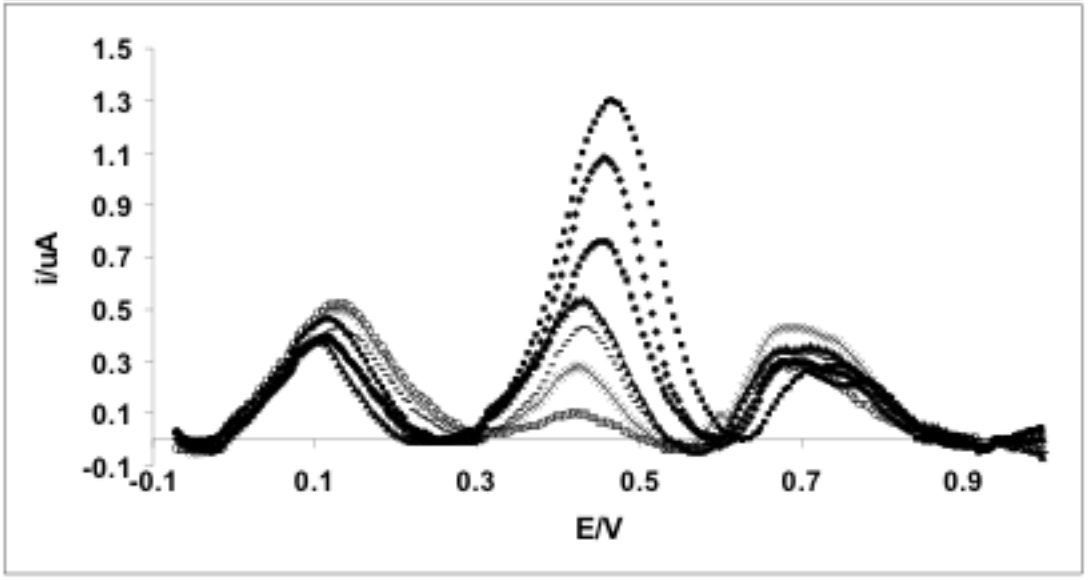
Simultaneous Detection of Neurotransmitters

Glassy Carbon Electrode



Carbon Nanofiber Electrode





-CNF electrode has ability to distinguish multiple electroactive brain chemicals in a mixture!
-Detection limits 50nM for DA and 100nM for 5-HT

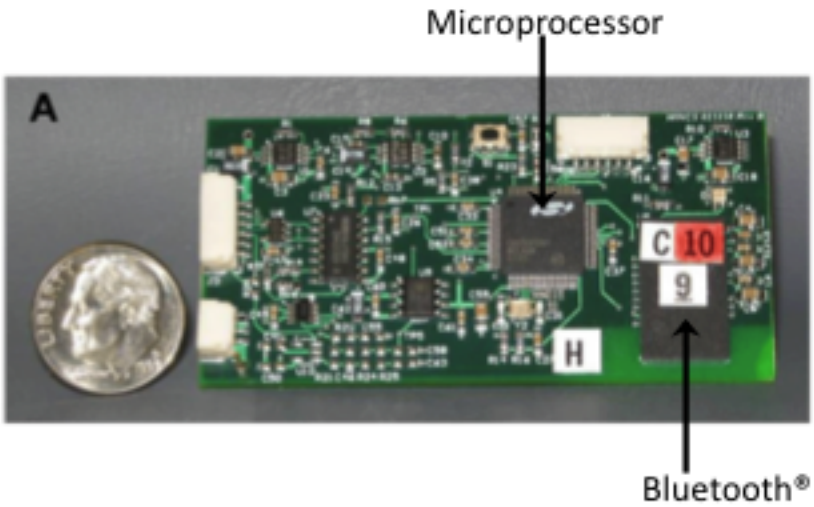
E. Rand, A. Periyakaruupan, Z. Tanaka, D. A. Zhang, M. P. Marsh, R. J. Andrews, B. Chen, M. Meyyappan, K. H. Lee, J. E. Koehne *Biosens Bioelect* **2013**, 42, 434-438.

Wireless Instantaneous Neurotransmitter Concentration Sensor (WINCS)

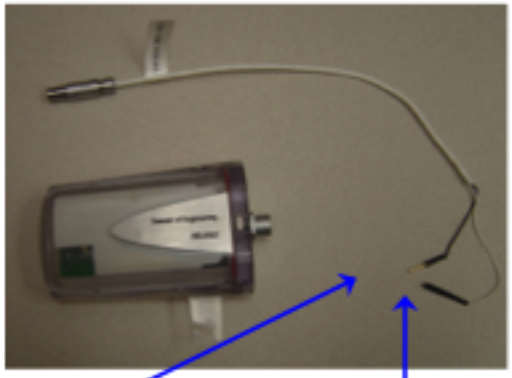
The Mayo Clinic-developed WINCS is a microprocessor-controlled, MRI-compatible, battery-powered instrument that combines Bluetooth® digital telemetry with fast scan cyclic voltammetry and constant potential amperometry.

Standard Potentiostat

Printed Circuit Board



Sterilizable WINCS Unit

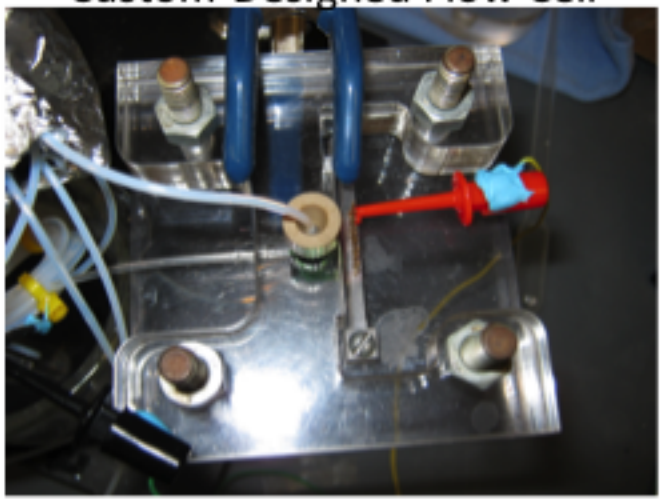


WINCS was designed in compliance with FDA-recognized standards for medical electrical device safety.

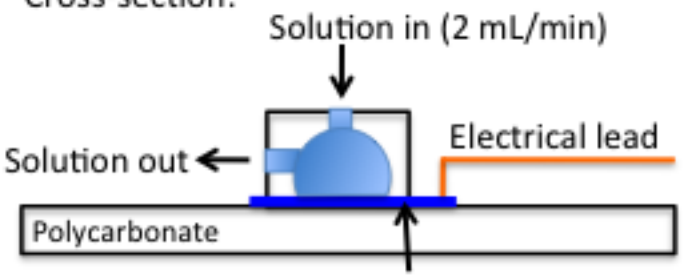
Bledsoe, J. M. et al., *J. Neurosurg*, **2010**, 11, 712-723.

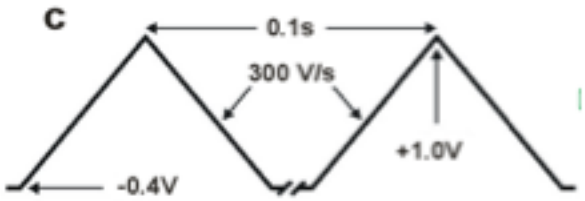
Experimental Setup

Custom-Designed Flow Cell

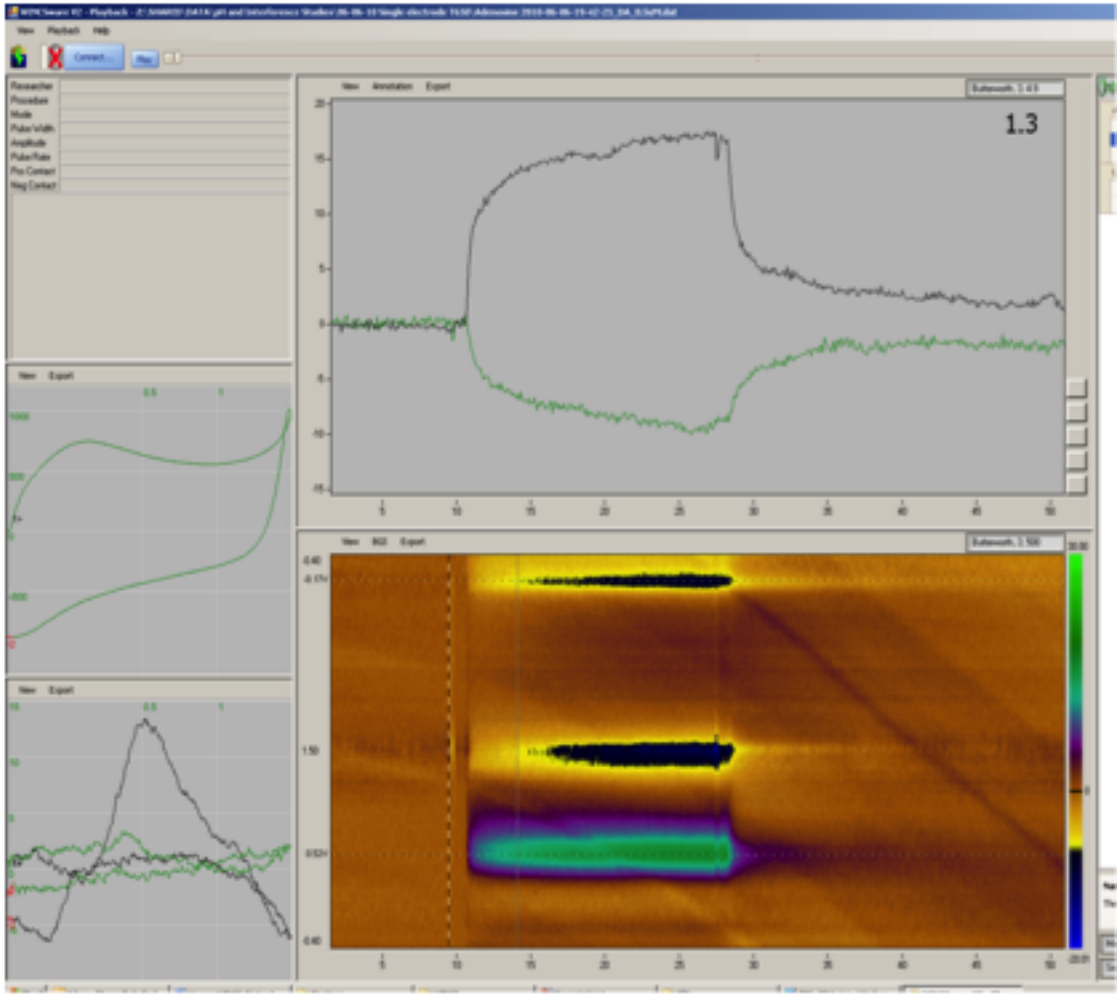


Cross-section:



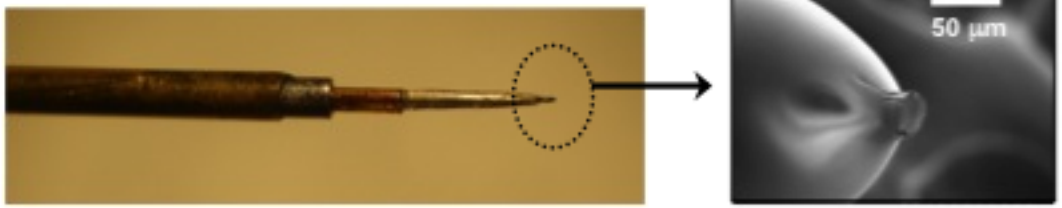


WINCSware User Interface



WINCSware allows viewing of the data in nearly real-time

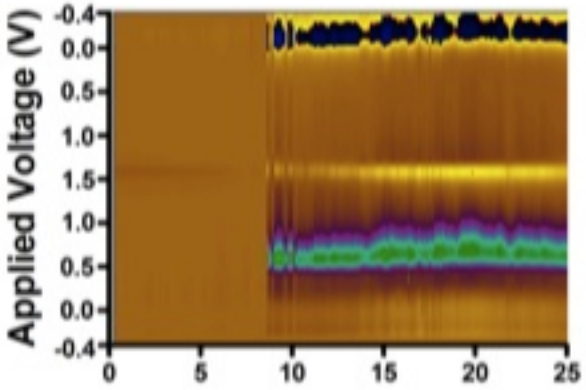
WINCStrode for the Detection of Dopamine



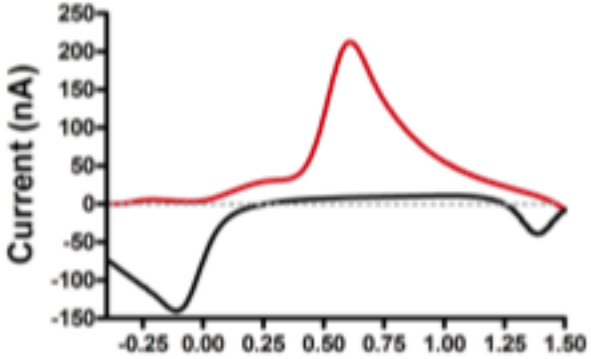
The WINCS carbon fiber electrode (WINCStrode) is based on an approved human extracellular tungsten electrophysiology electrode that was modified by the addition of a short section of carbon-fiber to enable FSCV recordings.

Dopamine Detection:

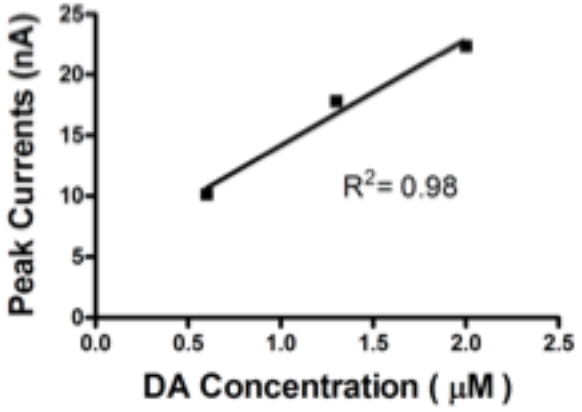
3D Color Plots



Background Subtracted Cyclic Voltammogram



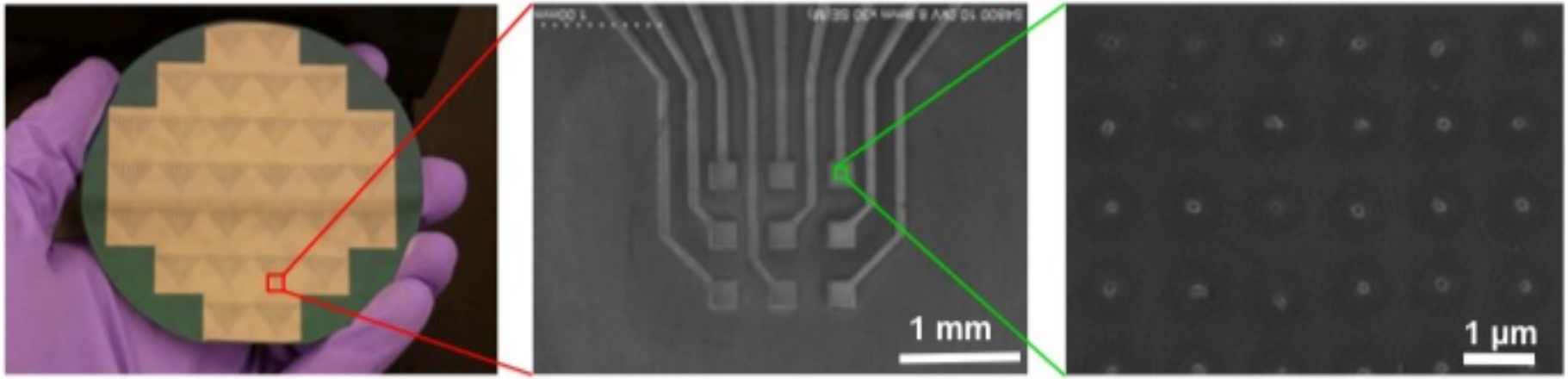
Calibration Curve



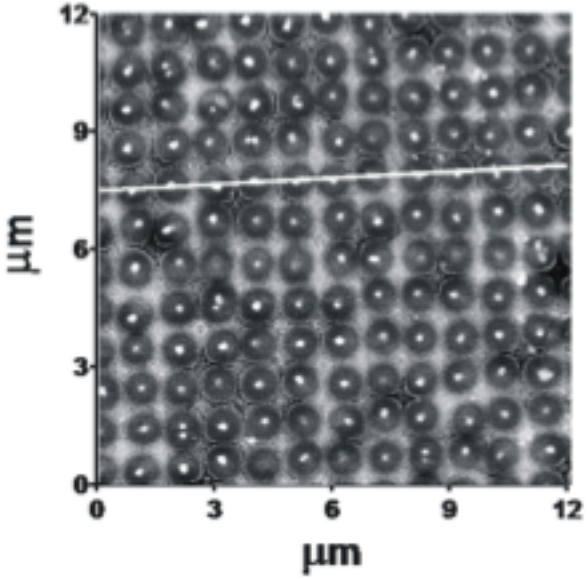
Agnesi, F. et al., *J. Neurosurg*, **2010**, 11, 701-711.

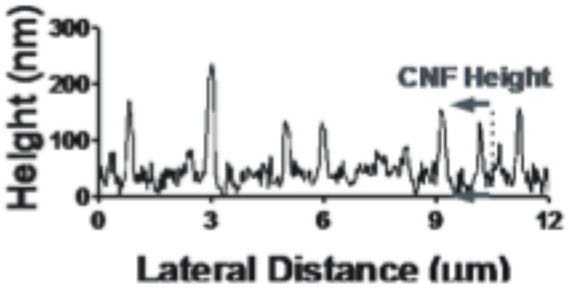
WINCS Carbon Nanofiber Electrode (WINCSnanotrode)

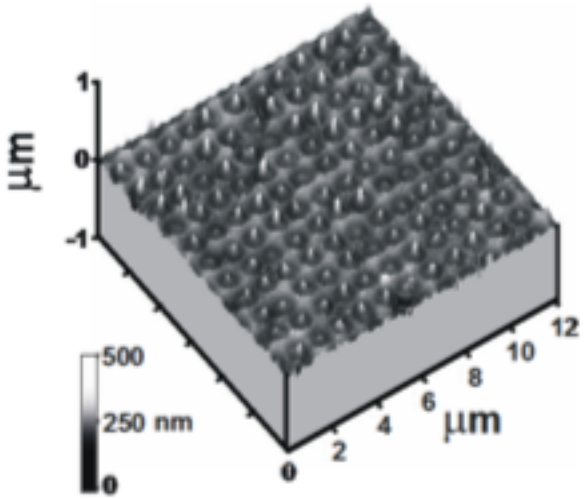
Scanning Electron Microscopy (SEM) Data:



Atomic Force Microscopy (AFM) Data:





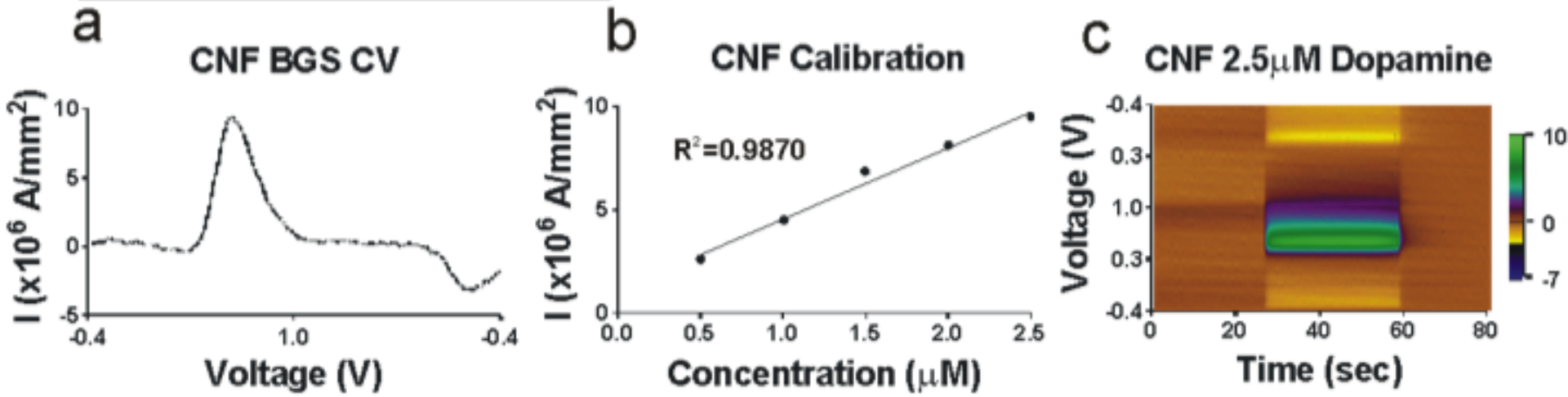


Use a combination of SEM and AFM analysis to determine total electrode surface area

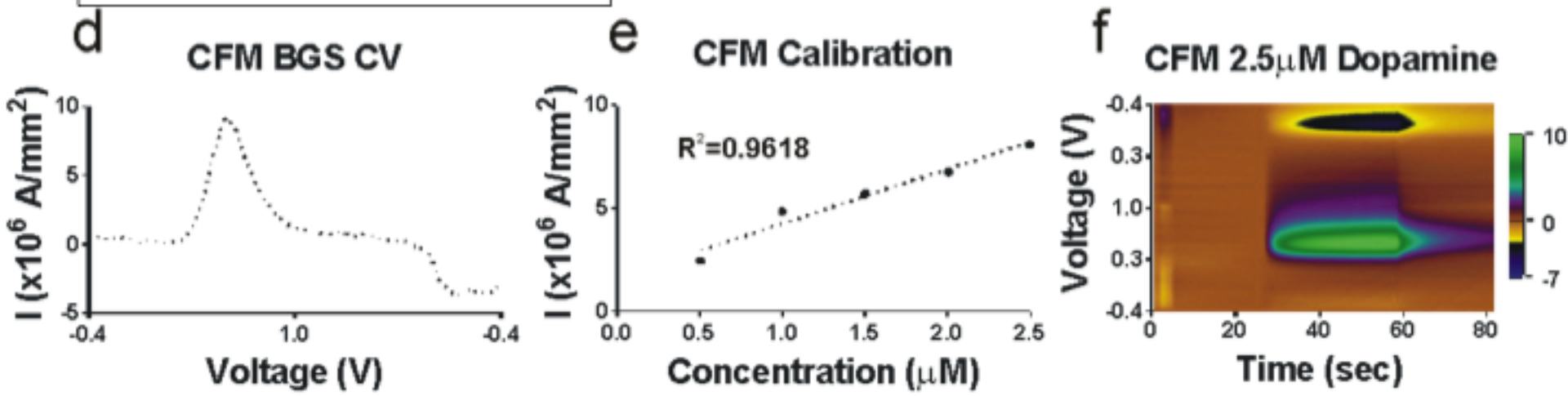
Koehne, J. E., et al. *Analyst* **2011**, 136, 1802-1805.

Dopamine Detection

Carbon Nanofiber Electrode



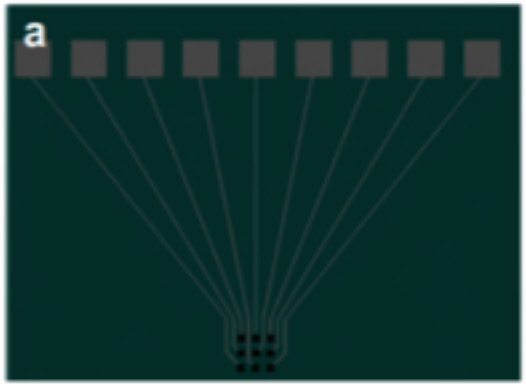
Carbon Fiber Microelectrode



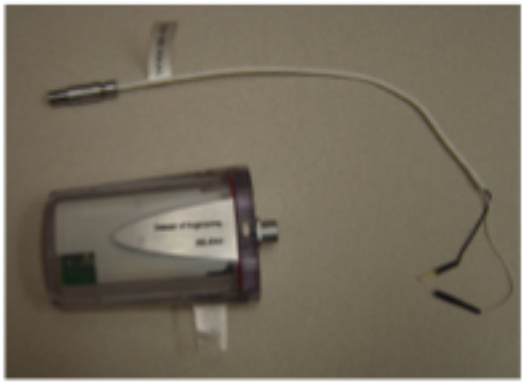
J. E. Koehne, M. Marsh, A. Boakye, B. Douglas, I. Y. Kim, S. Y. Chang, D. P. Jang, K. E. Bennet, C. Kimble, R. Andrews, M. Meyyappan, K. H. Lee *Analyst* **2011**, 136, 1802-1805.

Multichannel Recording

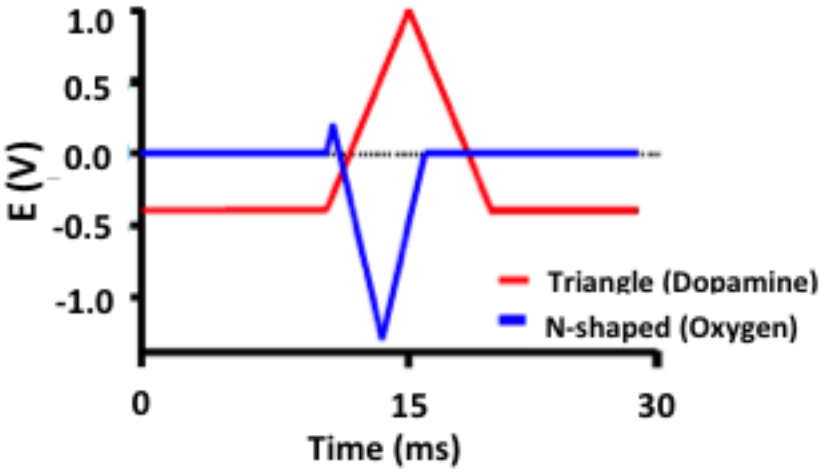
Device: 3x3 Array

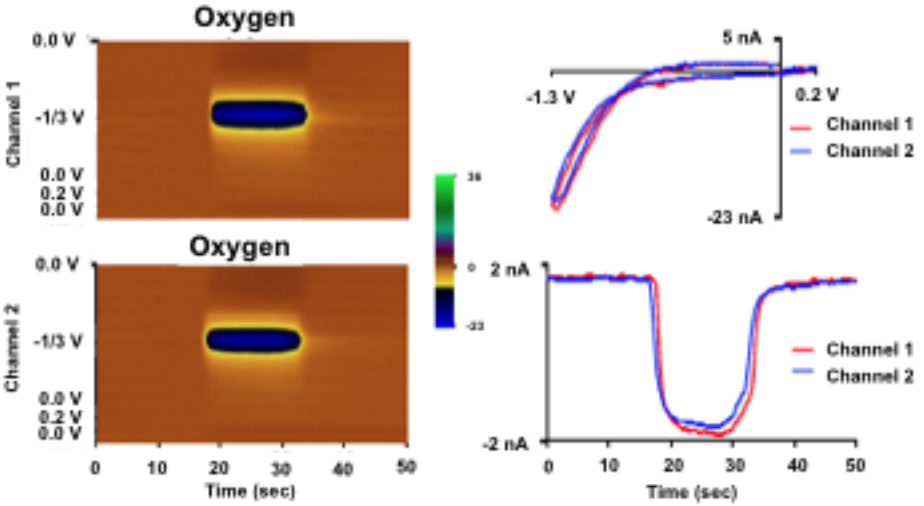


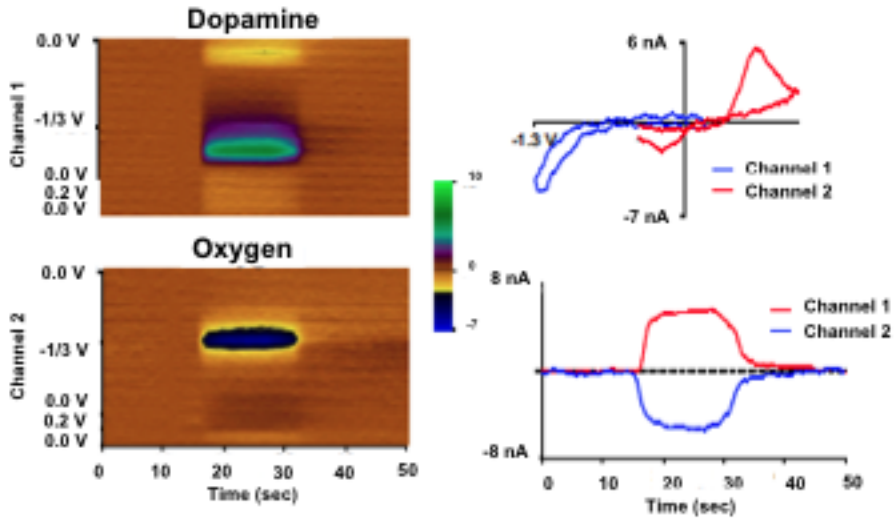
Instruments: 2 WINCS



Waveforms

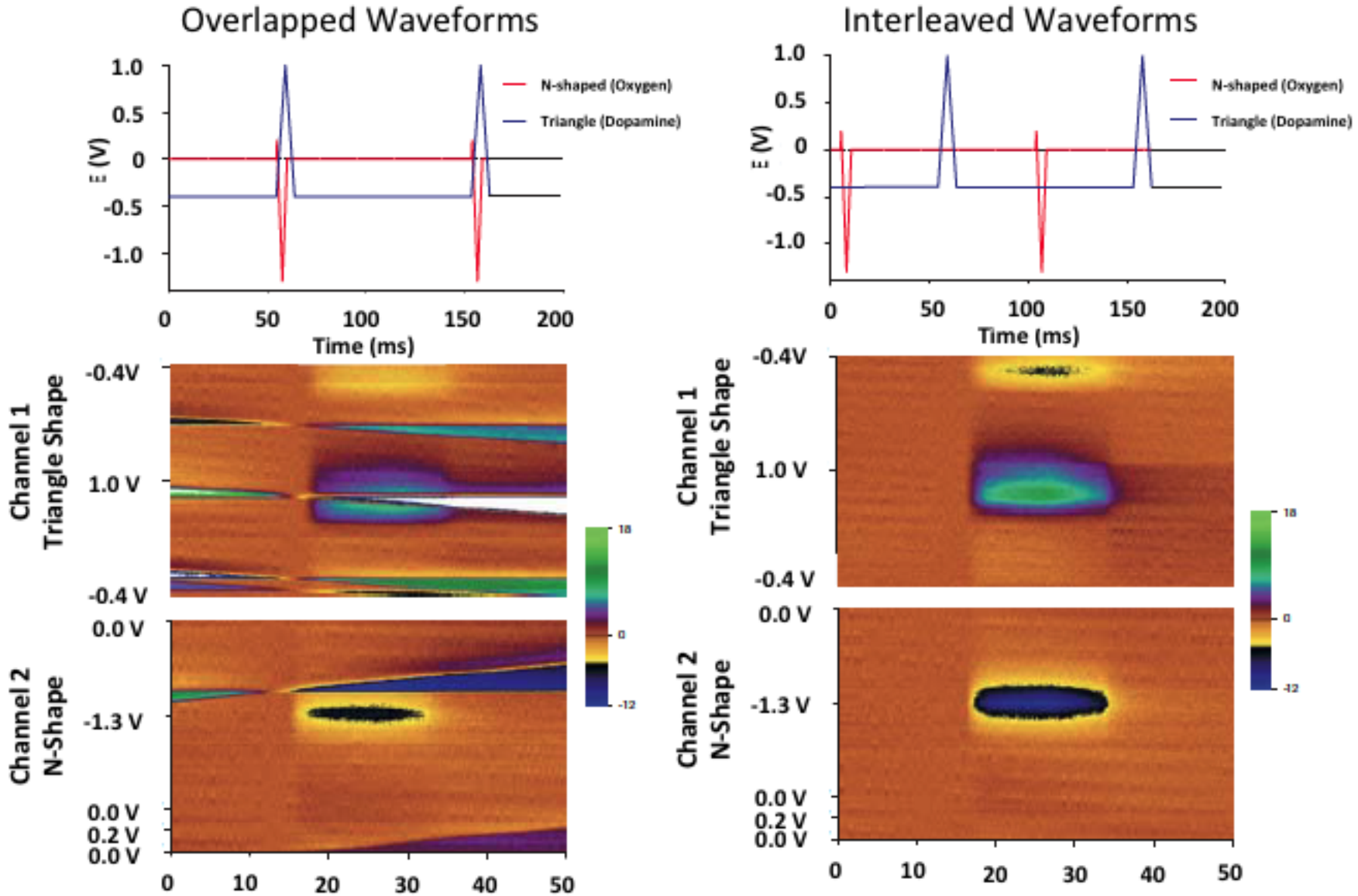






M. P. Marsh, J. E. Koehne, R. J. Andrews, M. Meyyappan, K. E. Bennet, K. H. Lee *Biomed Eng Lett,* **2012**, 2,271-277.

Multichannel Crosstalk

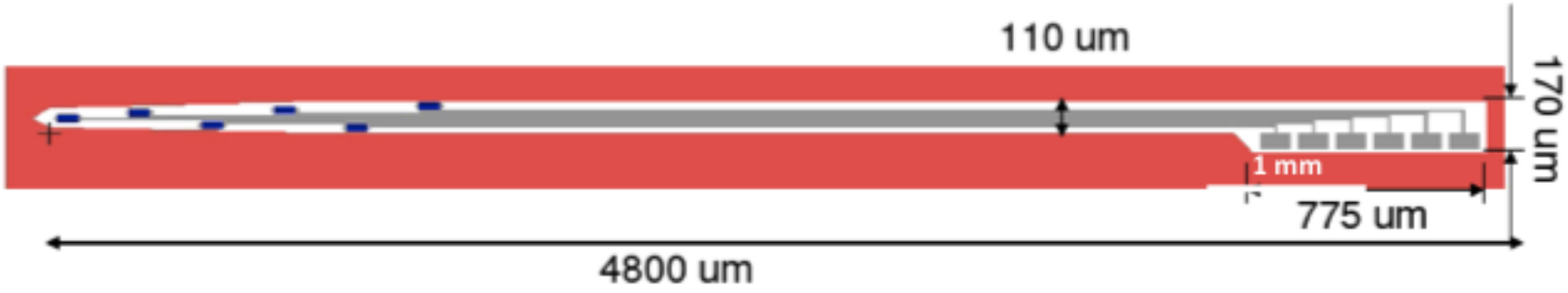


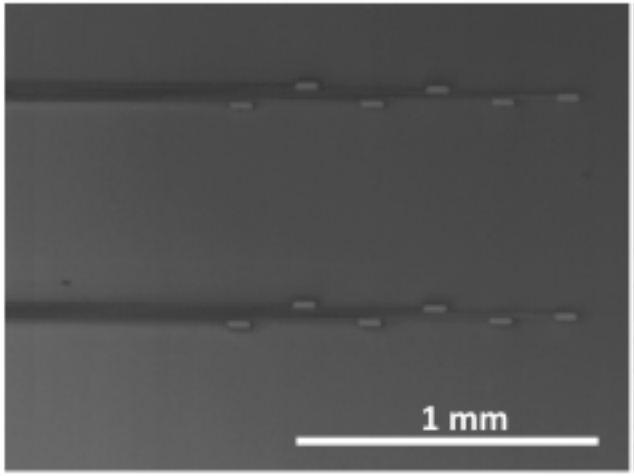
M. P. Marsh, J. E. Koehne, R. J. Andrews, M. Meyyappan, K. E. Bennet, K. H. Lee *Biomed Eng Lett*, **2012**, 2,271-277.

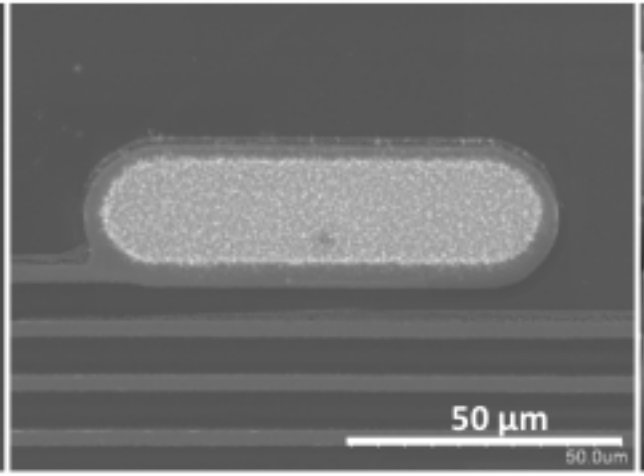
Implantable Style CNF Electrode Needle

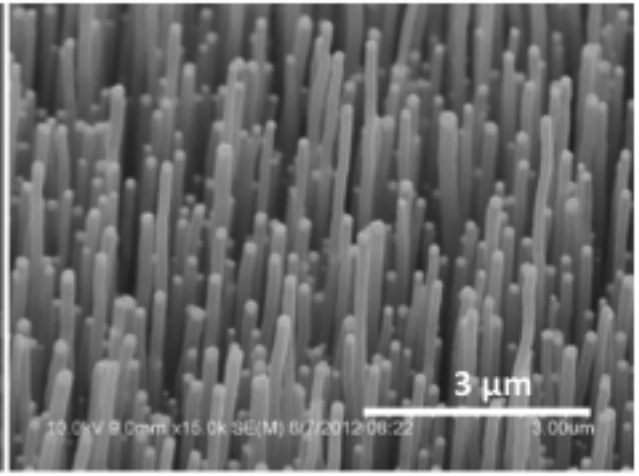
Penetrating multiplexed array

- Ability to spatially resolve



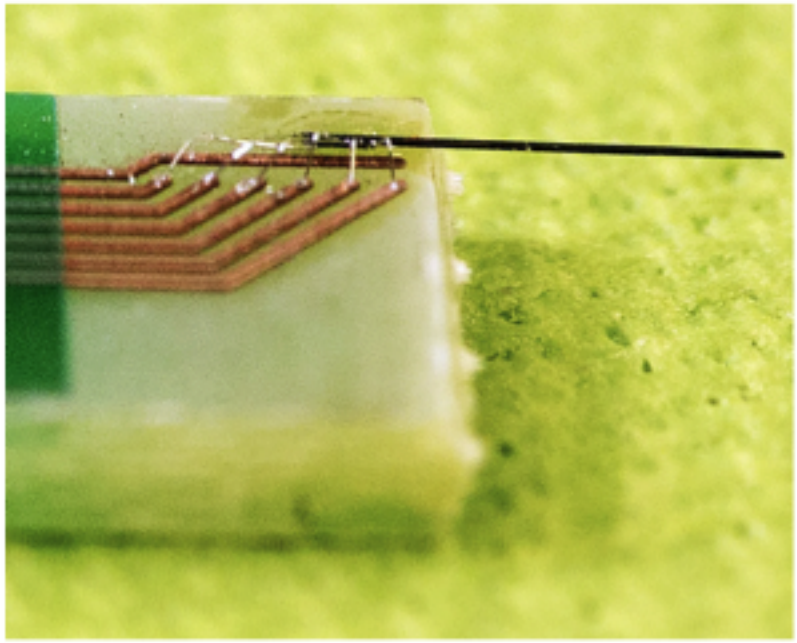


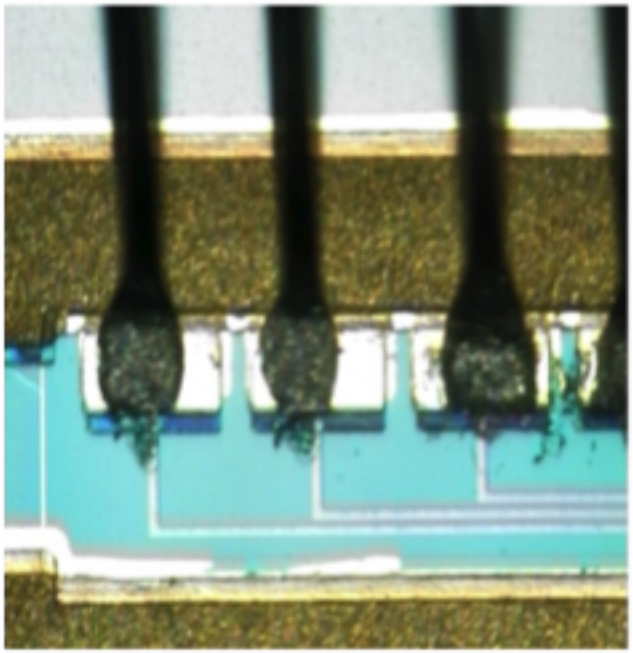




MAYO CLINIC

Needle Assembly



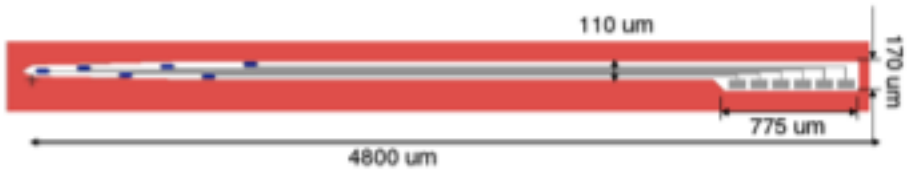


Simultaneous Multichannel Oxygen Detection

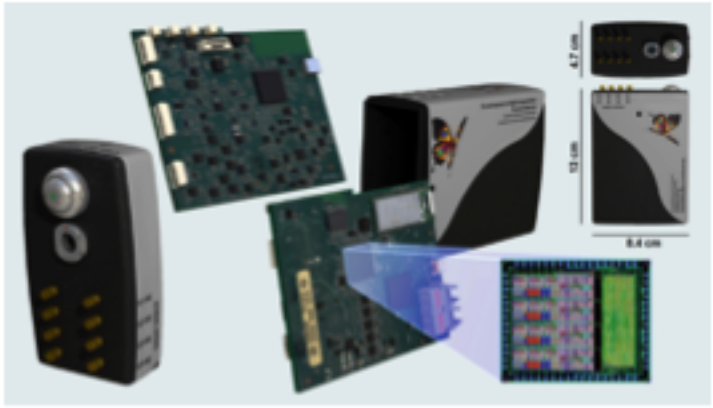
MAYO CLINIC

NASA

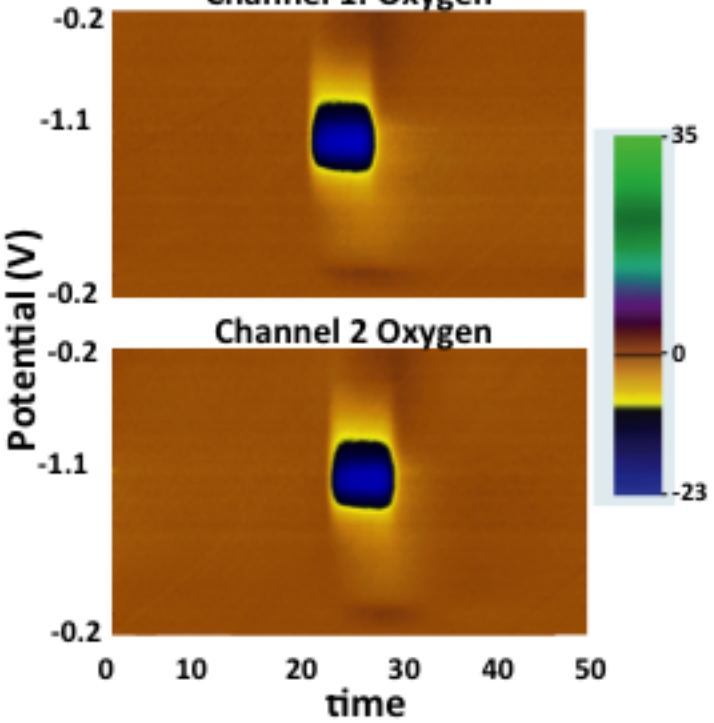
Device: Needle



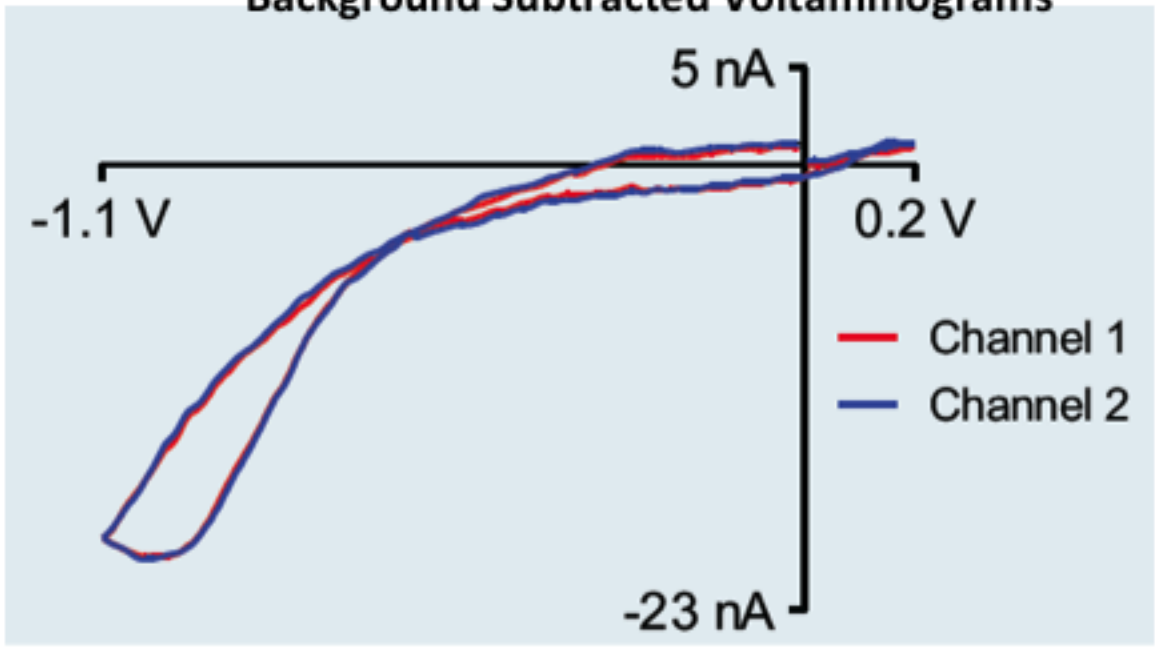
Instrument: WINCS Harmoni



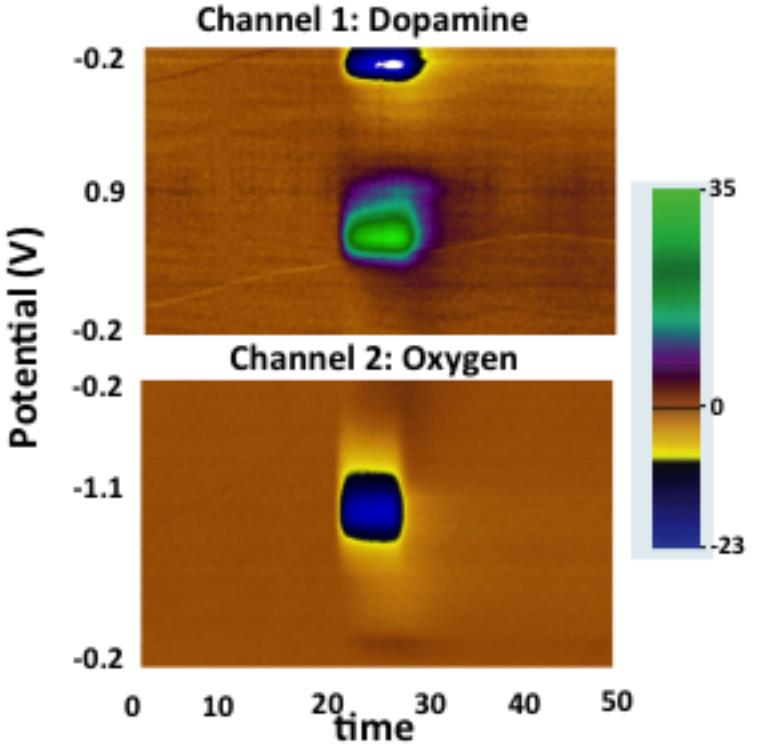
Channel 1: Oxygen

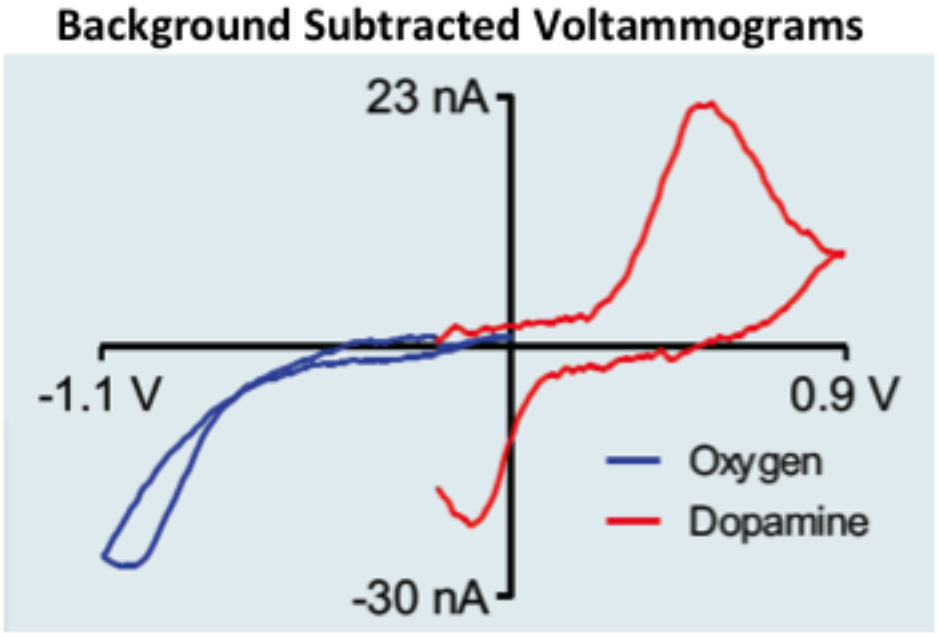


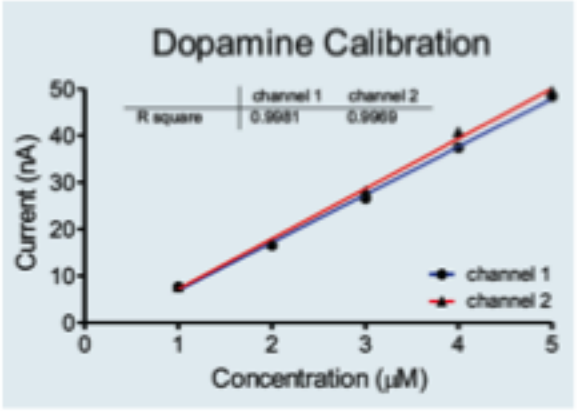
Background Subtracted Voltammograms

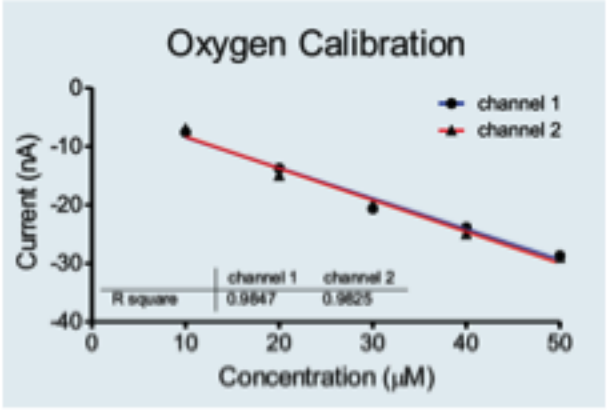


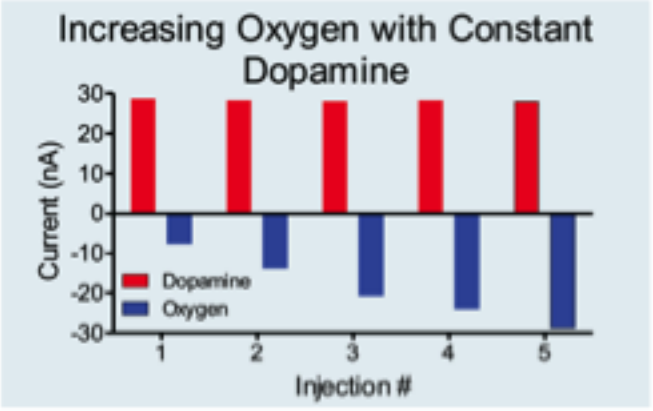
Multichannel Detection: Dopamine and Oxygen











Summary

- 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
- High surface area carbon nanofibers have been demonstrated as effective stimulation electrodes
- Conductive polymer coatings have allowed us to tune the electrical properties and biocompatability
- CNF sensors can distinguish between multiple electroactive analytes in a mixture using differential pulse voltammetry
- CNFs nanoelectrode arrays easily integrate with WINCS
- CNFs detect dopamine and oxygen with no channel cross-talk
- We have successfully fabricated a needle-like, implantable CNF electrode array for future in vivo investigations

Acknowledgements

- NASA Ames Research Center
  - **Emily Rand**
  - Adwoa Boakye
  - Brandon Douglas
  - **Russell J. Andrews**
  - **M. Meyyappan**
- Mayo Clinic
  - Department of Physiology and Bioengineering
    - **Michael Marsh**
    - Su-youne Chang
    - Inyong Kim
    - **Kendall H. Lee**
  - Department of Engineering
    - Christopher J. Kimble
    - **Kevin E. Bennet**

Funding

NASA Ames Research Center
NIH (R01 Ns75013)
Presidential Early Career Award for Scientists and Engineers (NASA)

NASA Educational Programs

- NASA Internship Programs

  http://intern.nasa.gov/

  Fall, Spring and Summer Sessions

- NASA Fellowships

  http://intern.nasa.gov/

  http://www.nasa.gov/offices/oct/early_stage_innovation/grants NSTRF.html

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