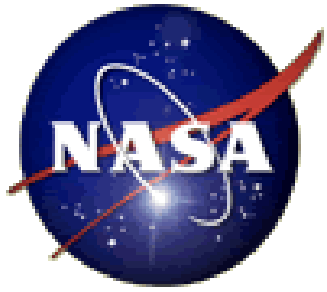


Carbon Nanomaterials for Biosensing Applications

Jessica E. Koehne

NASA Ames Research Center

Moffett Field, CA

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

Outline

- Biosensors motivation for NASA missions
  - Cardiac health in microgravity
  - Nanoelectrode array devices on silicon
  - Cardiac protein detection
- In-space manufacturing of biosensors for NASA missions
  - Atmospheric plasma jet printer and printed devices
  - Ink jet printed devices

Biosensor Motivation

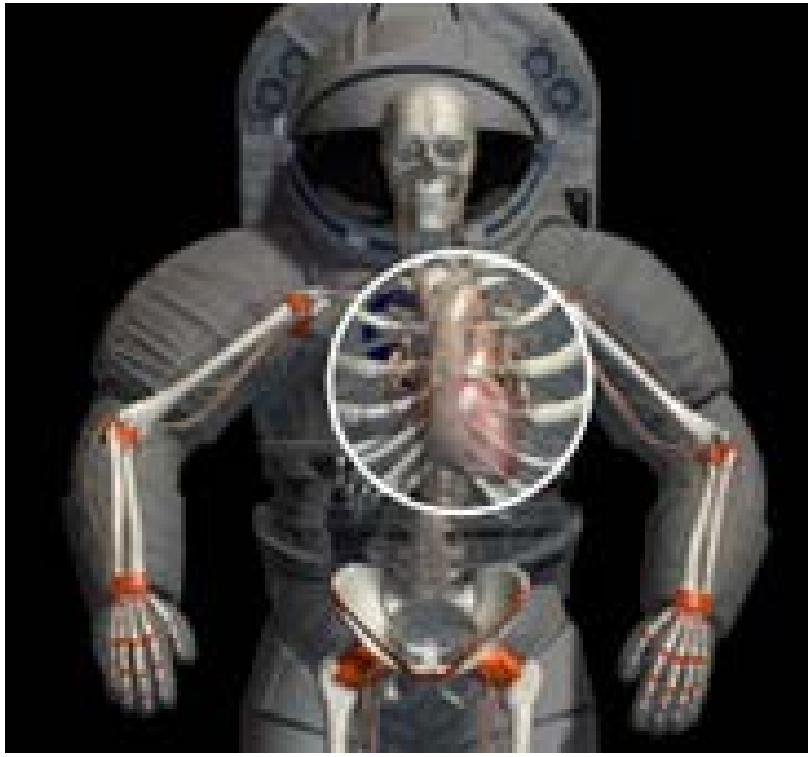
NASA Applications

- Astronaut health monitoring
  - Lab-on-a-chip (DNA, rRNA, ricin, cholesterol, dopamine, serotonin, pH)
- 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

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

Nanoelectrodes for Sensors

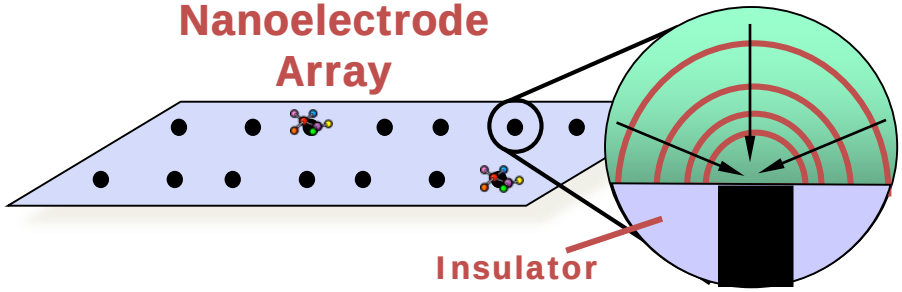
Nanoscale electrodes create a dramatic improvement in signal detection over traditional electrodes for small analyte concentrations

Background: $i_n \propto C_d^0 A$

Traditional Macroelectrode

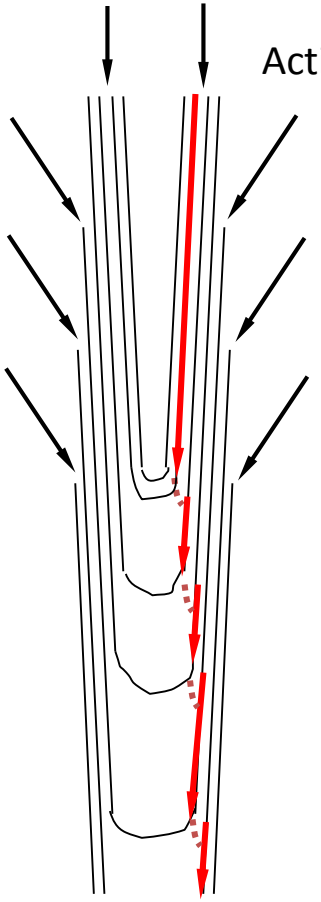
- **Scale difference** between macroelectrode and molecules is tremendous
- **Background noise** on electrode surface is therefore significant
- **Significant amount** of target molecules required

Nanoelectrode Array

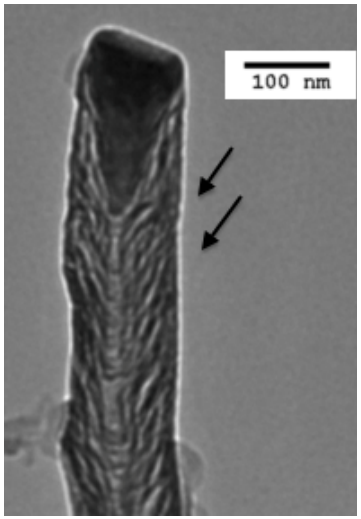


- Nanoelectrodes are at the **scale close to** molecules
- with dramatically **reduced background noise**
- Multiple electrodes results in **magnified signal** and **desired redundance** for statistical reliability.

What are Carbon Nanofibers (CNFs)?

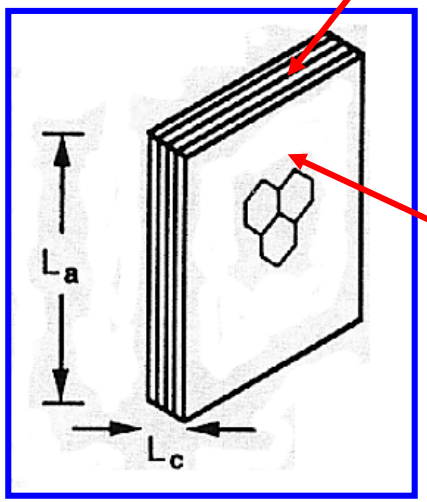


Bamboo-like CNFs



TEM of CNF

HOPG



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

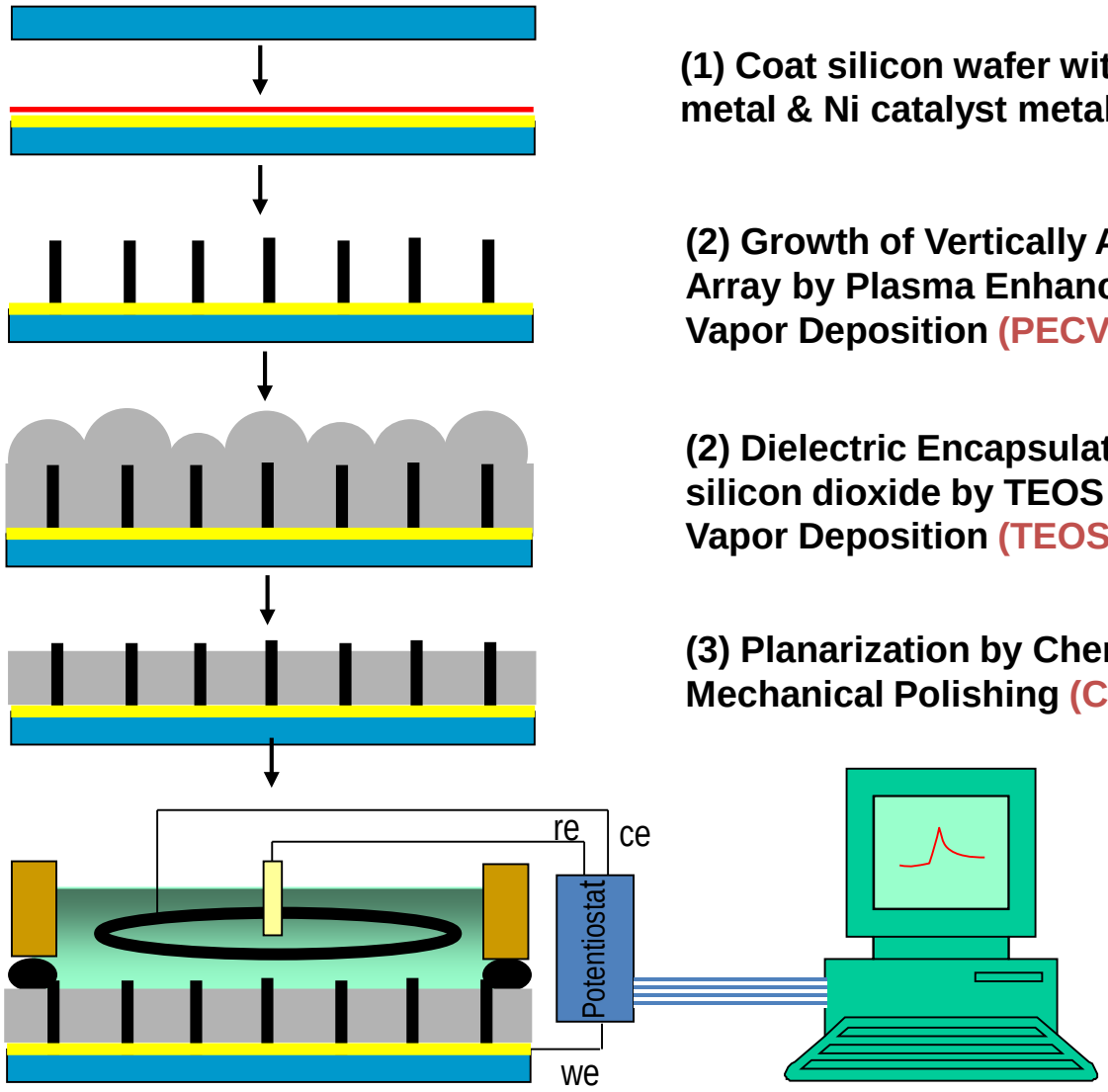
Basal Plane:
(1) Low electron transfer rate (< 10^{-7} cm/s)
(2) Anomalously low capacitance (~1.9 μF/cm^2)

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 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)

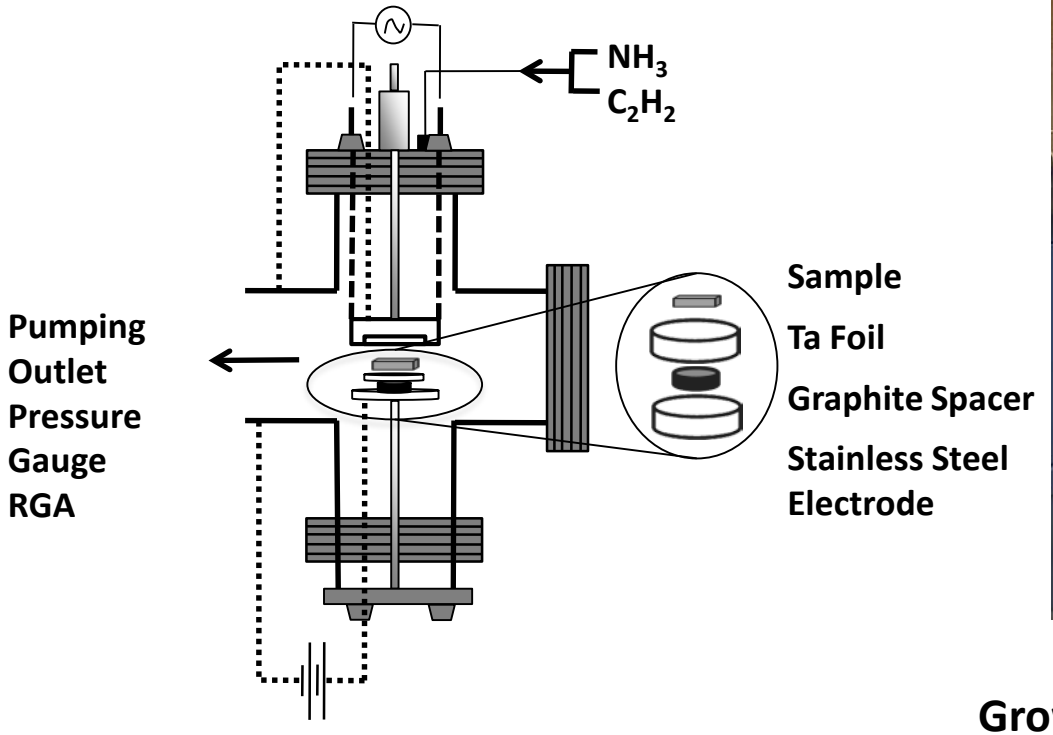
(3) Planarization by Chemical Mechanical Polishing (CMP)

(5) Electrochemical Characterization

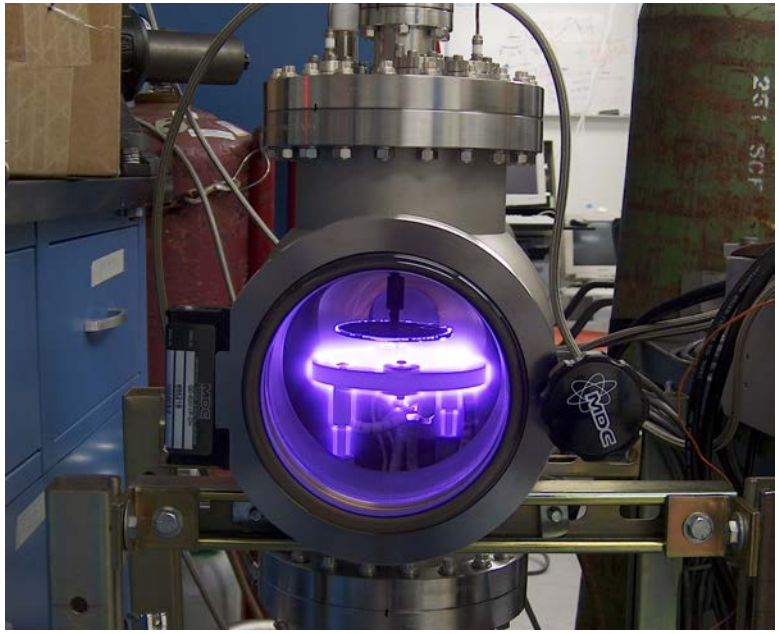
Cruden, B., et al., *Appl. Phys. Lett.* **2003**, 94, 4070-4078.

CNF Growth by Plasma Enhanced Chemical Vapor Deposition (PECVD)

PECVD Reactor Schematic



Custom Built PECVD Reactor

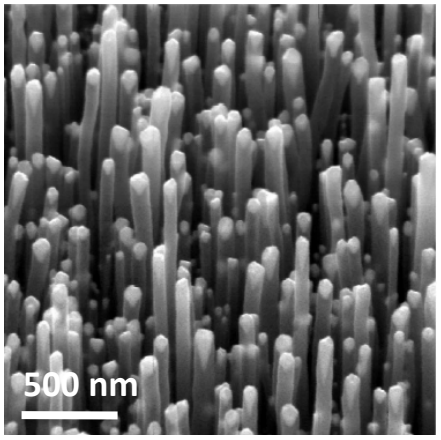
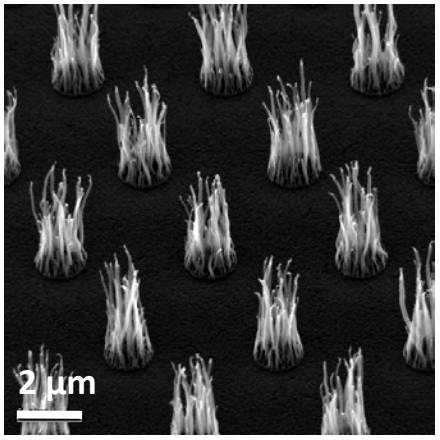
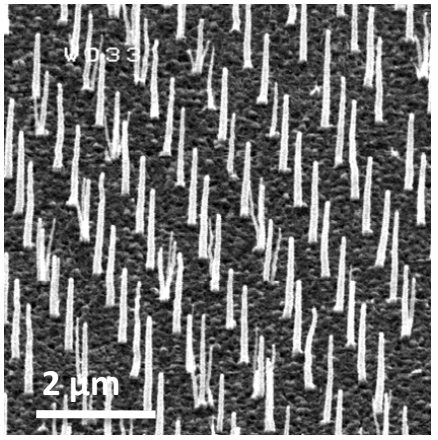
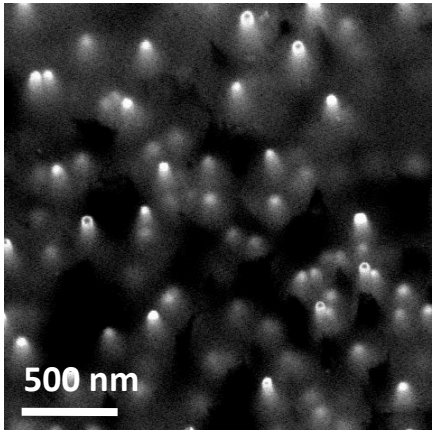
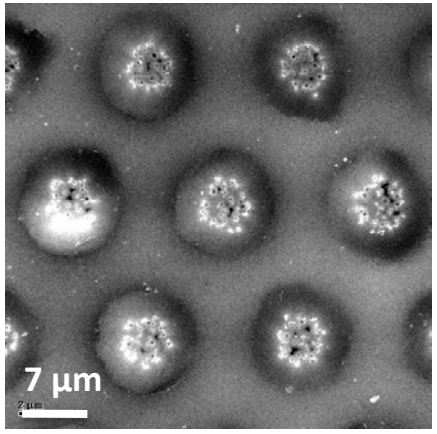
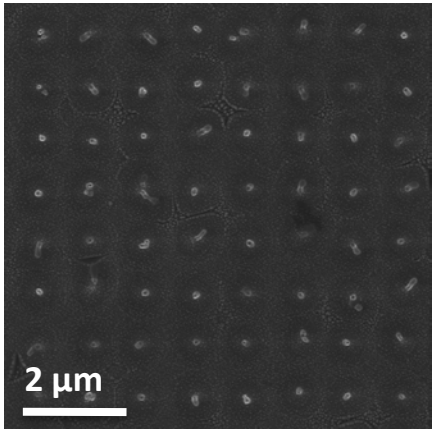


Growth Process

- 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- 100-1000 nm/min
- Quality is good, alignment is good

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

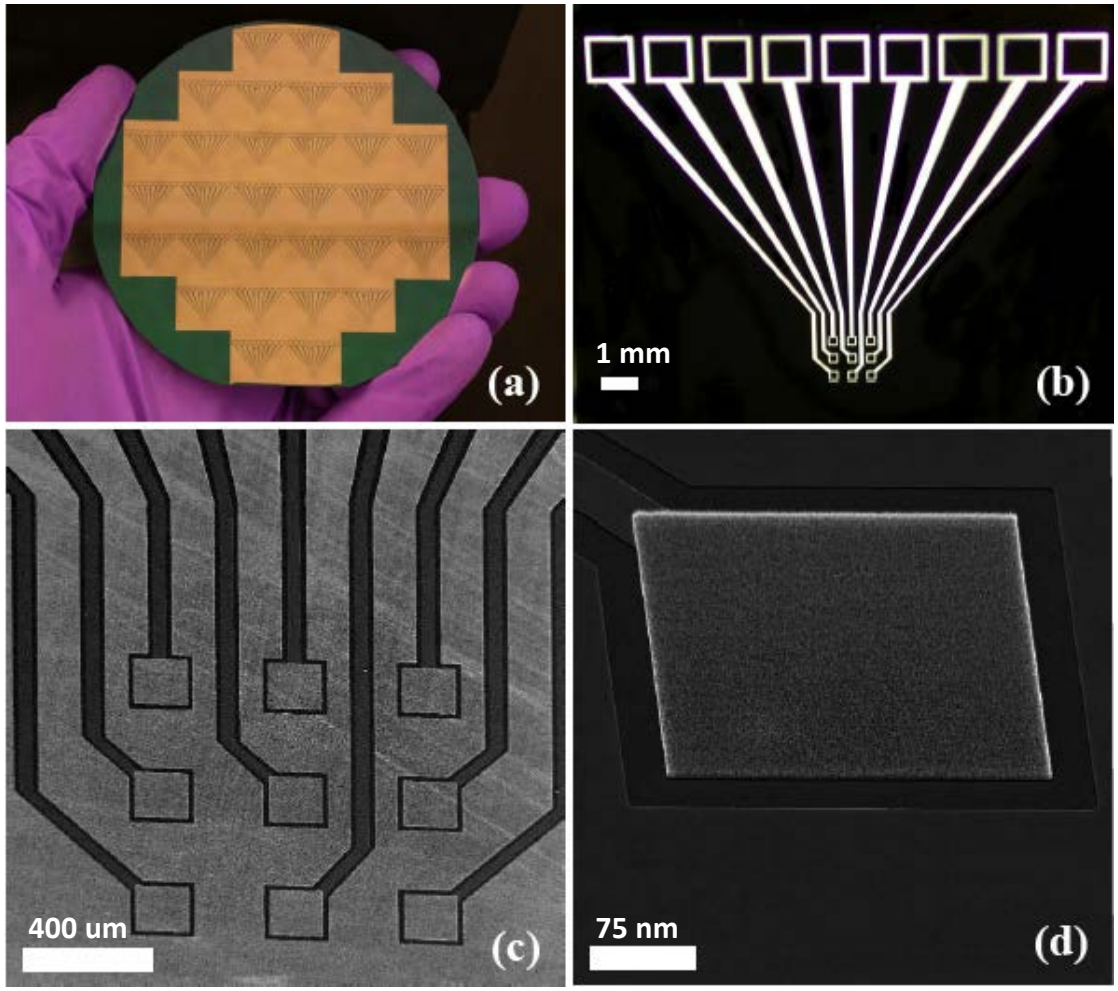
Define CNF Placement by Catalyst Placement

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

Li, J., Koehne, J. E., Cassell, A. M., Chen, H., Ng, H. T., Ye, Q., Fan, W., Han, J., Meyyappan, M. *Electroanalysis*. 2005, 17, 15-27.

Fabrication of 3x3 Array

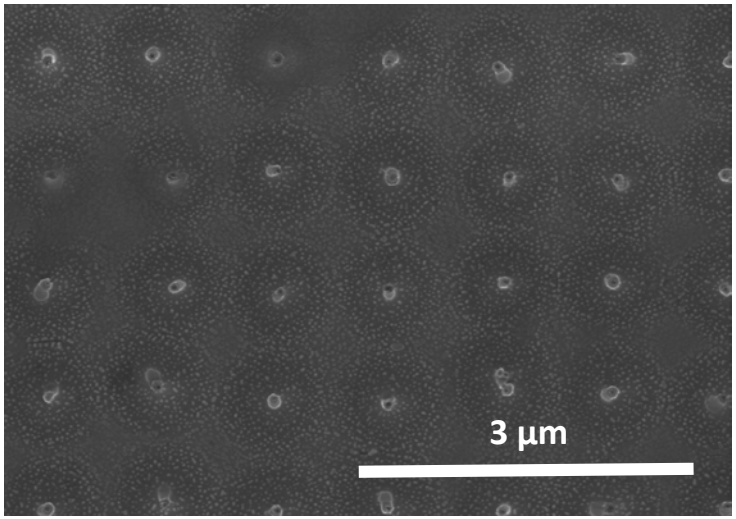
30 devices on a 4” Si wafer

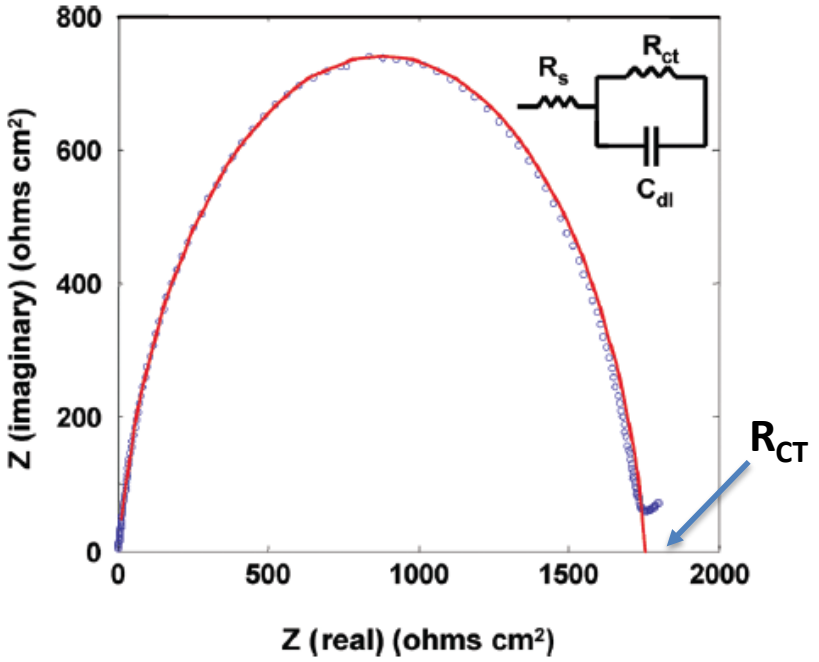


- 200 µm by 200 µm electrode dimensions
- 9 individually addressed electrodes
- potentially 9 different target molecules

Electrochemical Impedance Spectroscopy of CNF Electrode

ultralow density CNF

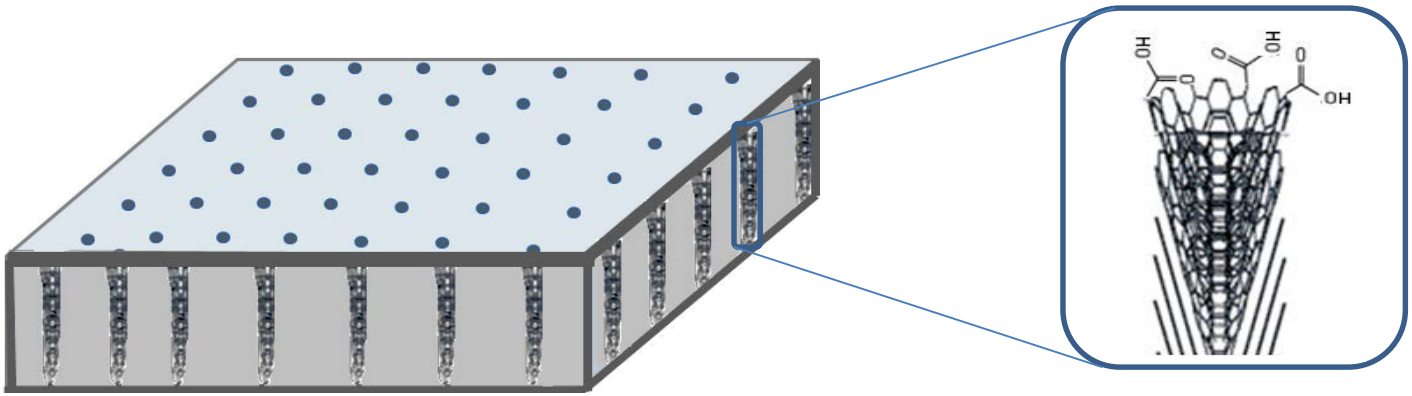


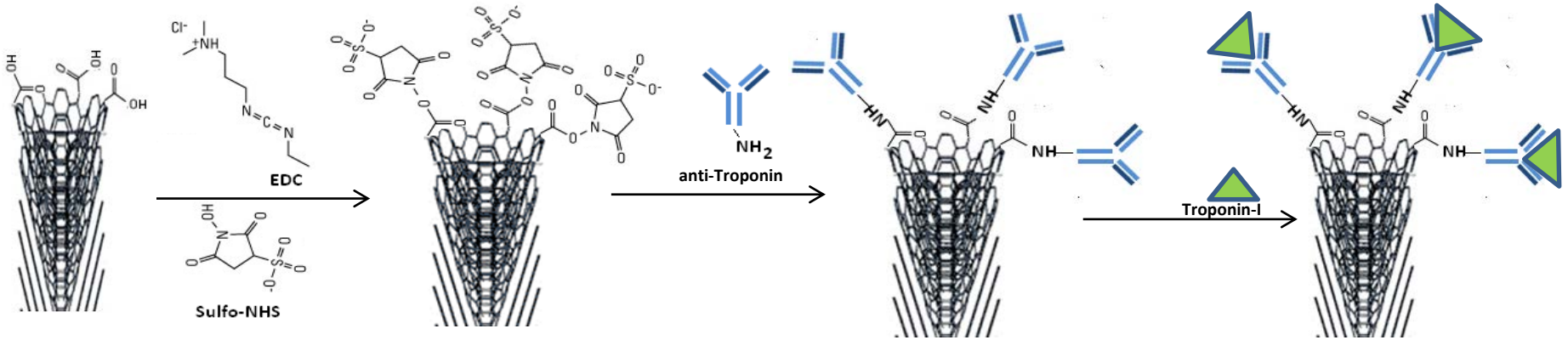


Fitting Parameters	Randomly Grown CNF	CNF (low density)	CNF (ultralow density)
I (A/mm^2)	7.1 X 10^{-6}	1.8 X 10^{-6}	2.5 X 10^{-7}
R_{ct} (KΩ)	N/A	1.8	17.3
CPE (µF)	906	3.3	2.5
n	0.79	0.89	0.91

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

Surface Preparation of CNF Electrode





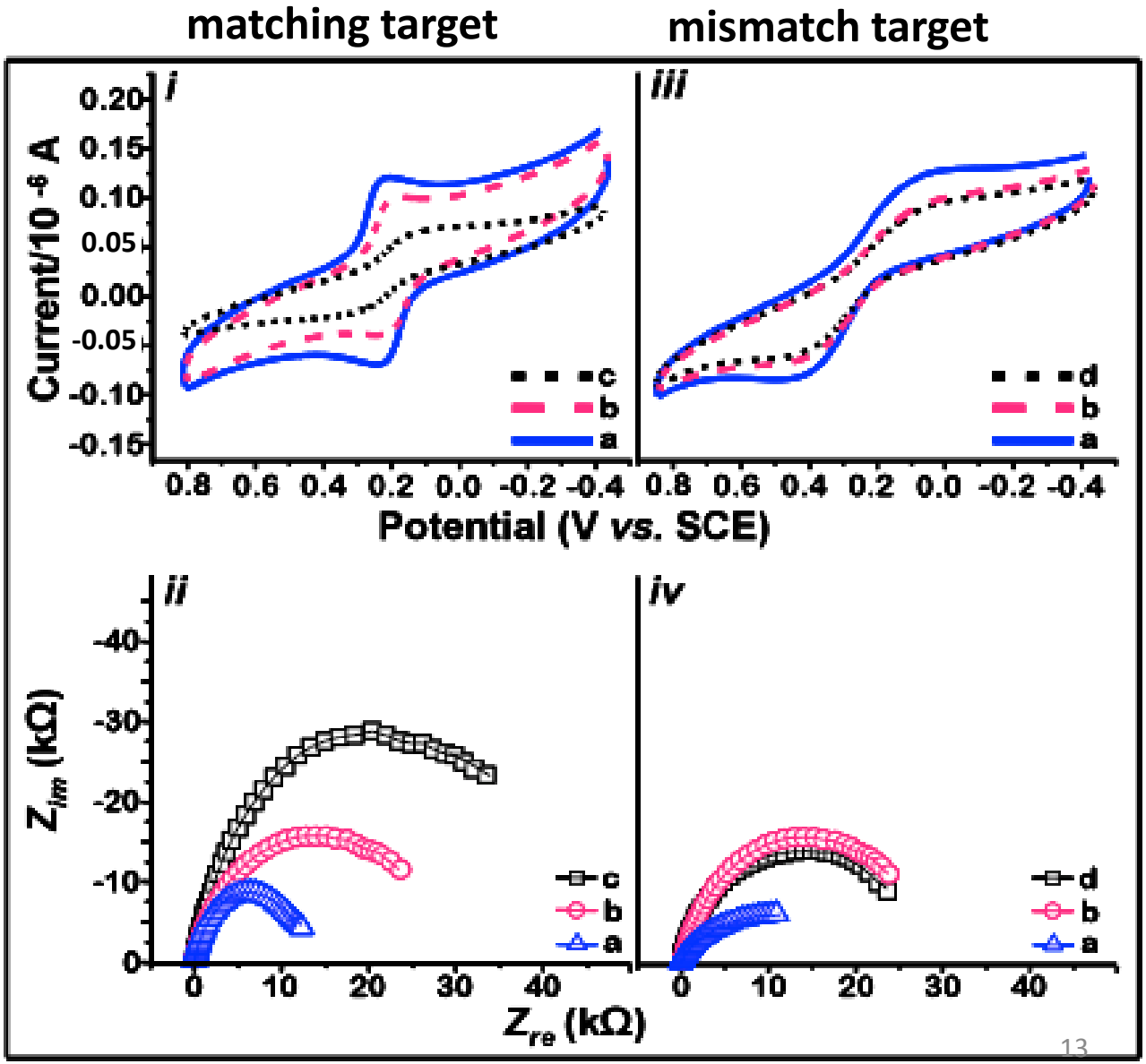
A. Periyakaruppan, R. P. Gandhiraman, M. Meyyappan, J. E. Koehne, *Anal. Chem.,* **2013**, 85, 3858-3863.

Troponin-I Detection

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

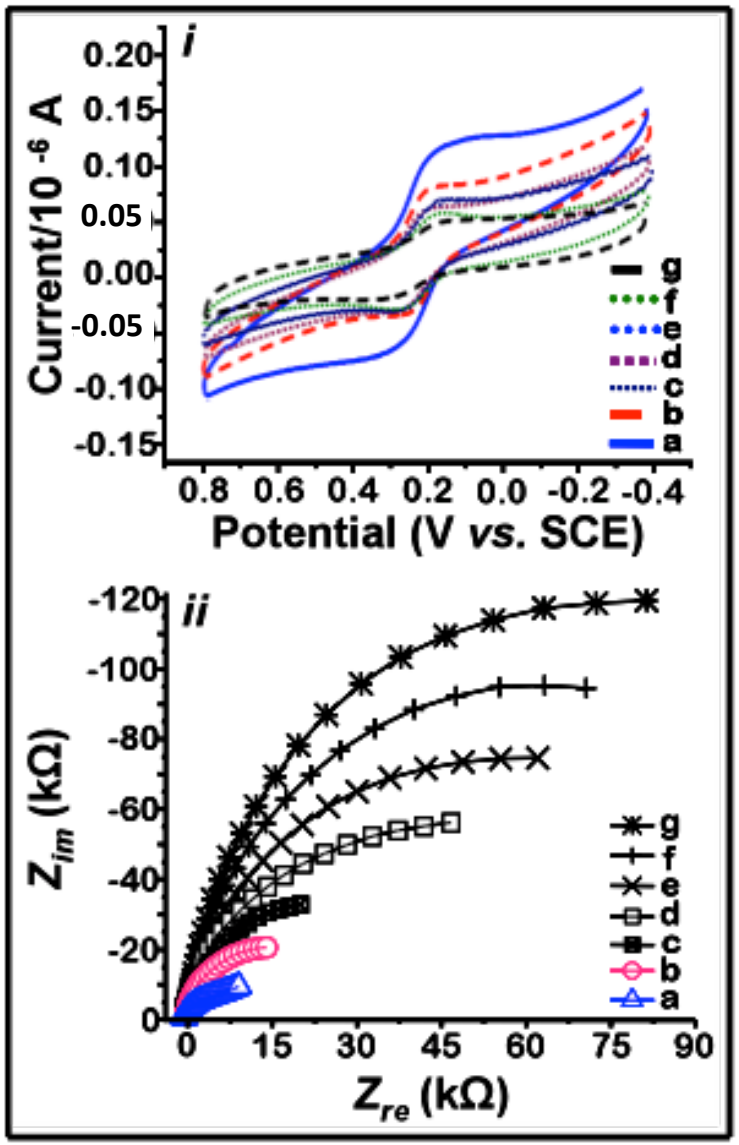
1 mM $Fe[(CN)_6]^{3-/4-}$

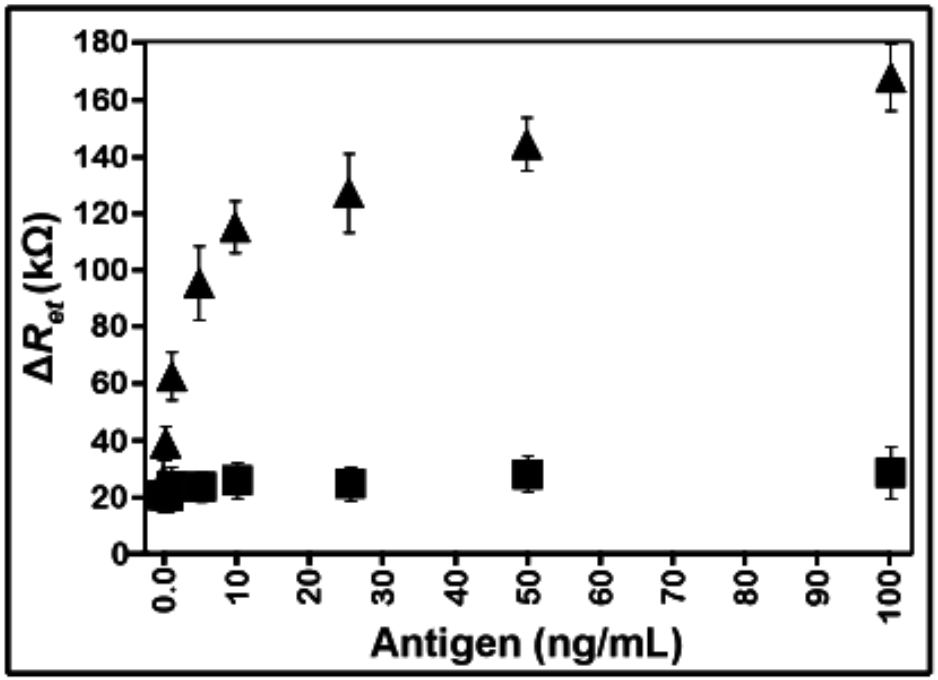
Increase in R_{ct} observed upon anti-troponin immobilization and matching protein binding



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

Troponin-I Concentration Study

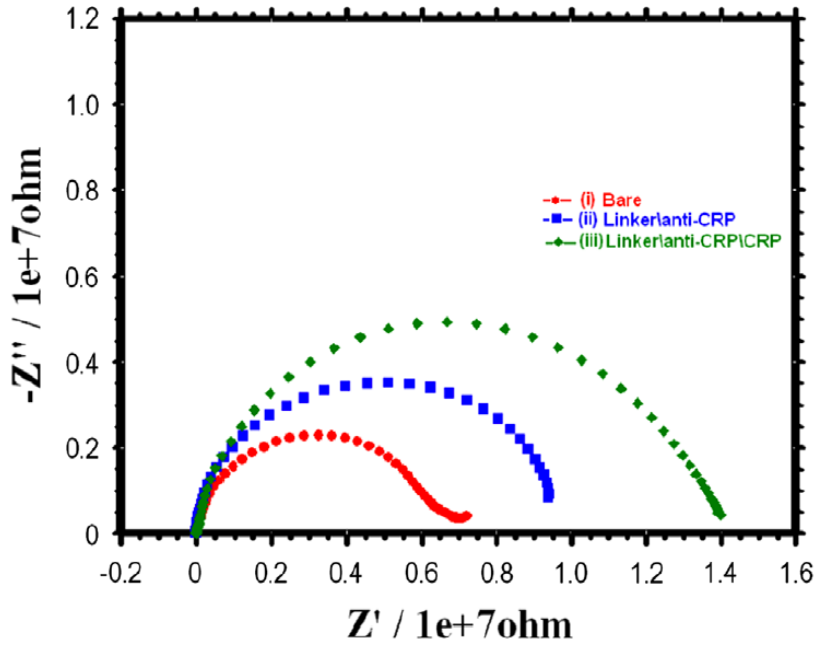


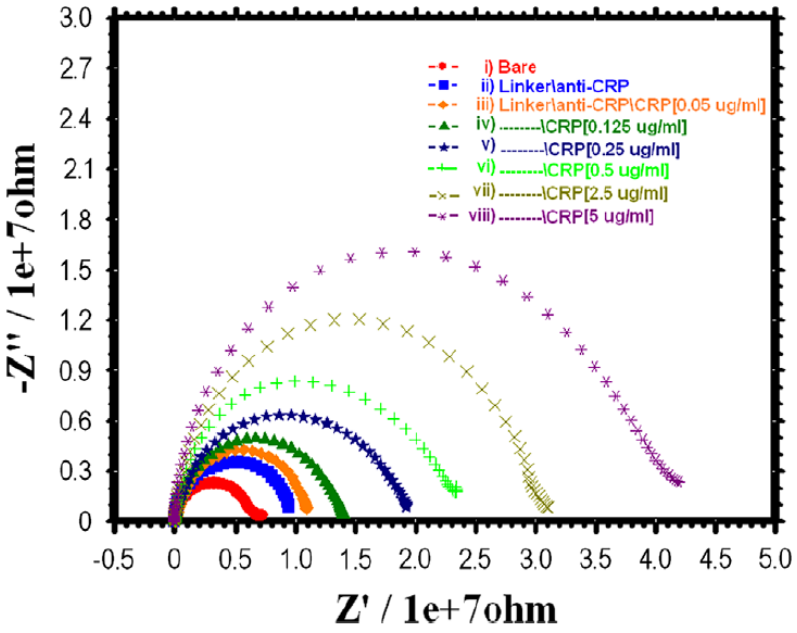


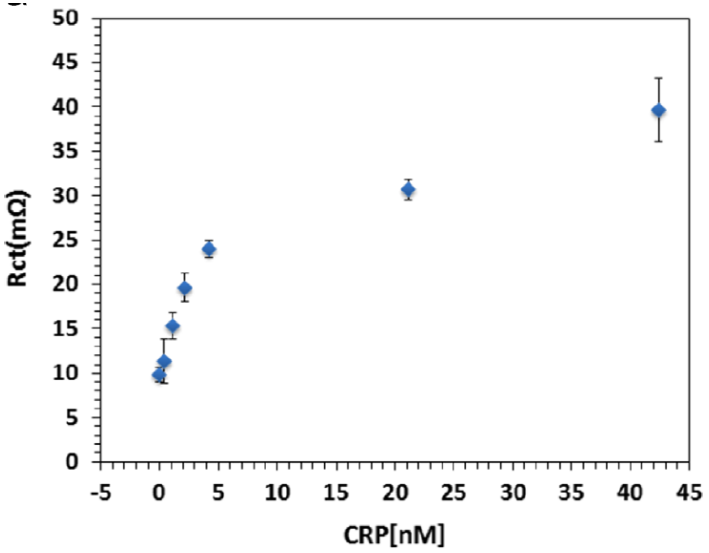
Troponin-I concentration range: 100 ng/mL to 0.25 ng/mL
Detection down to 0.25 ng/mL

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

Detection of C-Reactive Protein (CRP)



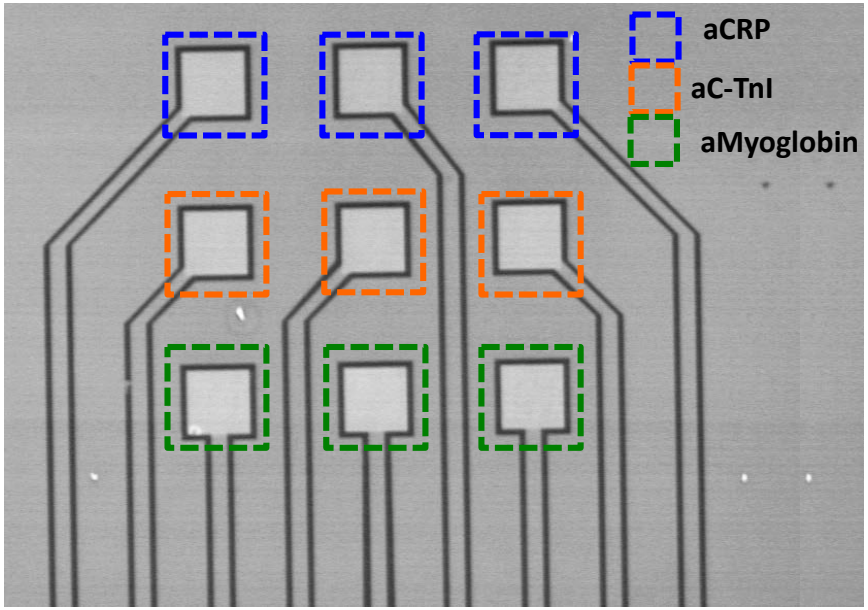


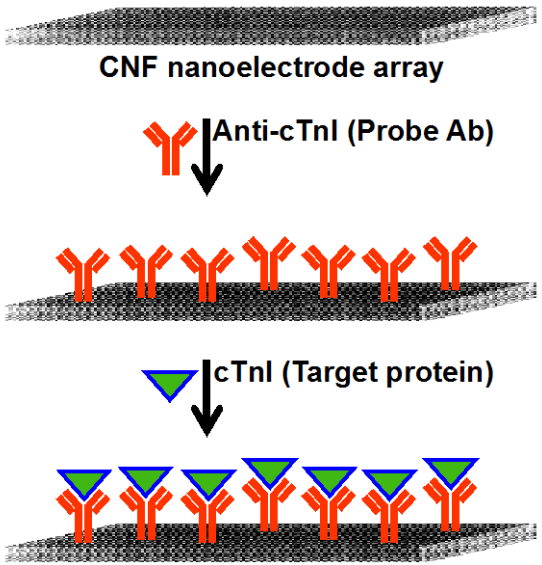


CRP Limit of Detection
11 ng/mL or 90 pM

Gupta, R. K., Periyakaruppan, Meyyappan, M., Koehne, J. E. *Biosens Bioelect* **2014**, 59, 112-119.

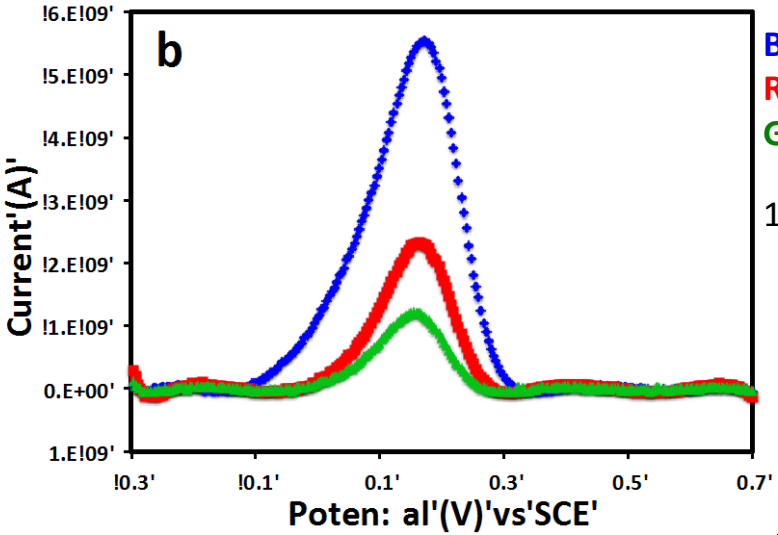
Multiplexed Sensor for Heart Disease Diagnosis





Detection of:

1) C-Reactive Protein
2) Cardiac Troponin-I
3) Myoglobin



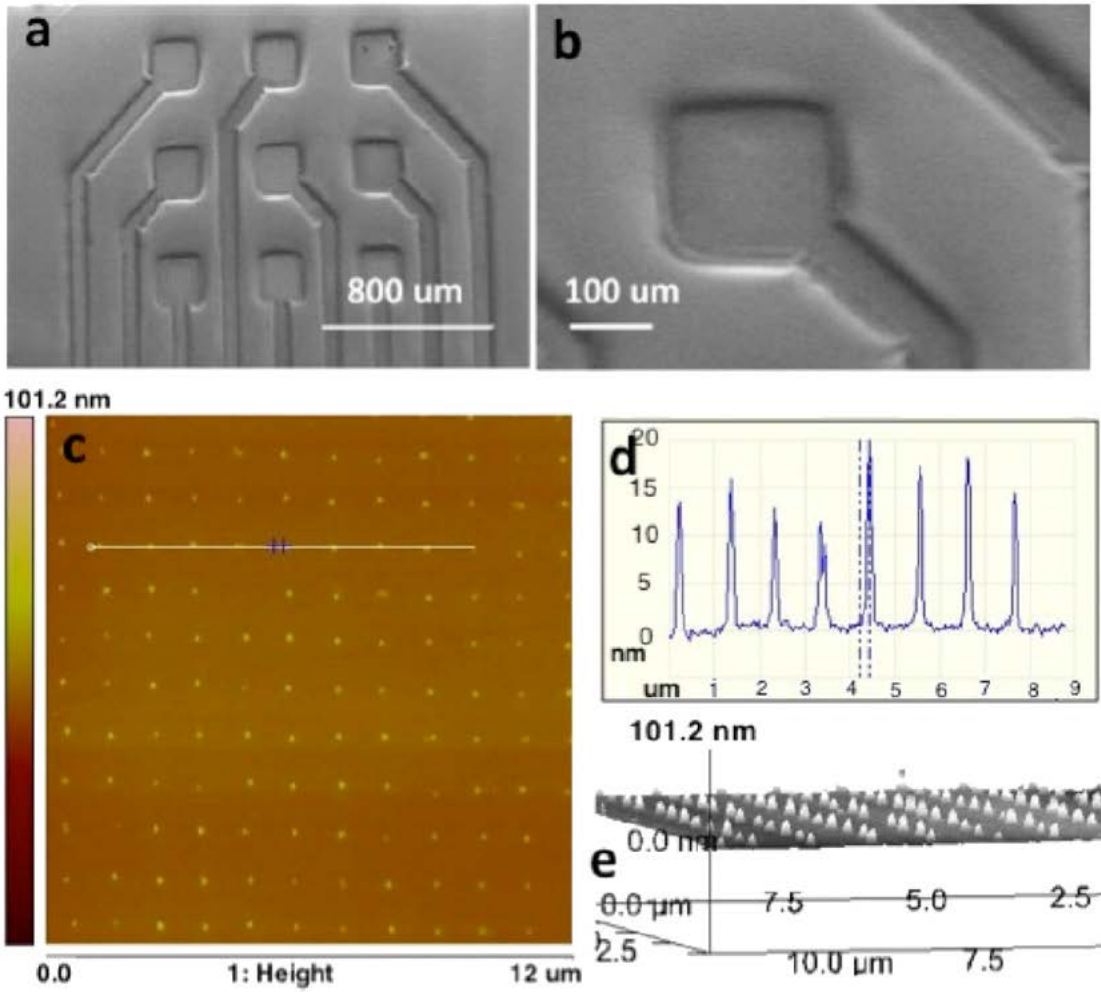
Blue: bare
Red: antibody
Green: antigen

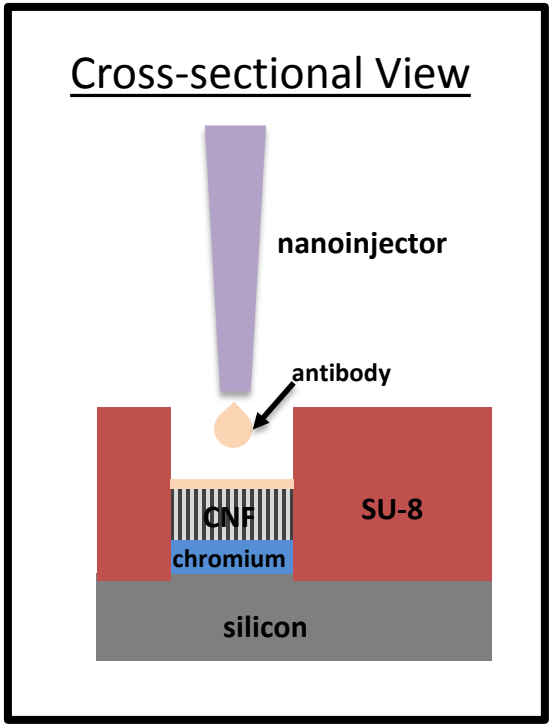
1 mM $K_4Fe(CN)_6$

Gupta, R. K., Pandya, R., Sieffert, T., Meyyappan, M., Koehne, J. E. *J Electroanal Chem*, 773 (2016) 53-62.

Preparation of Multiplexed Sensor

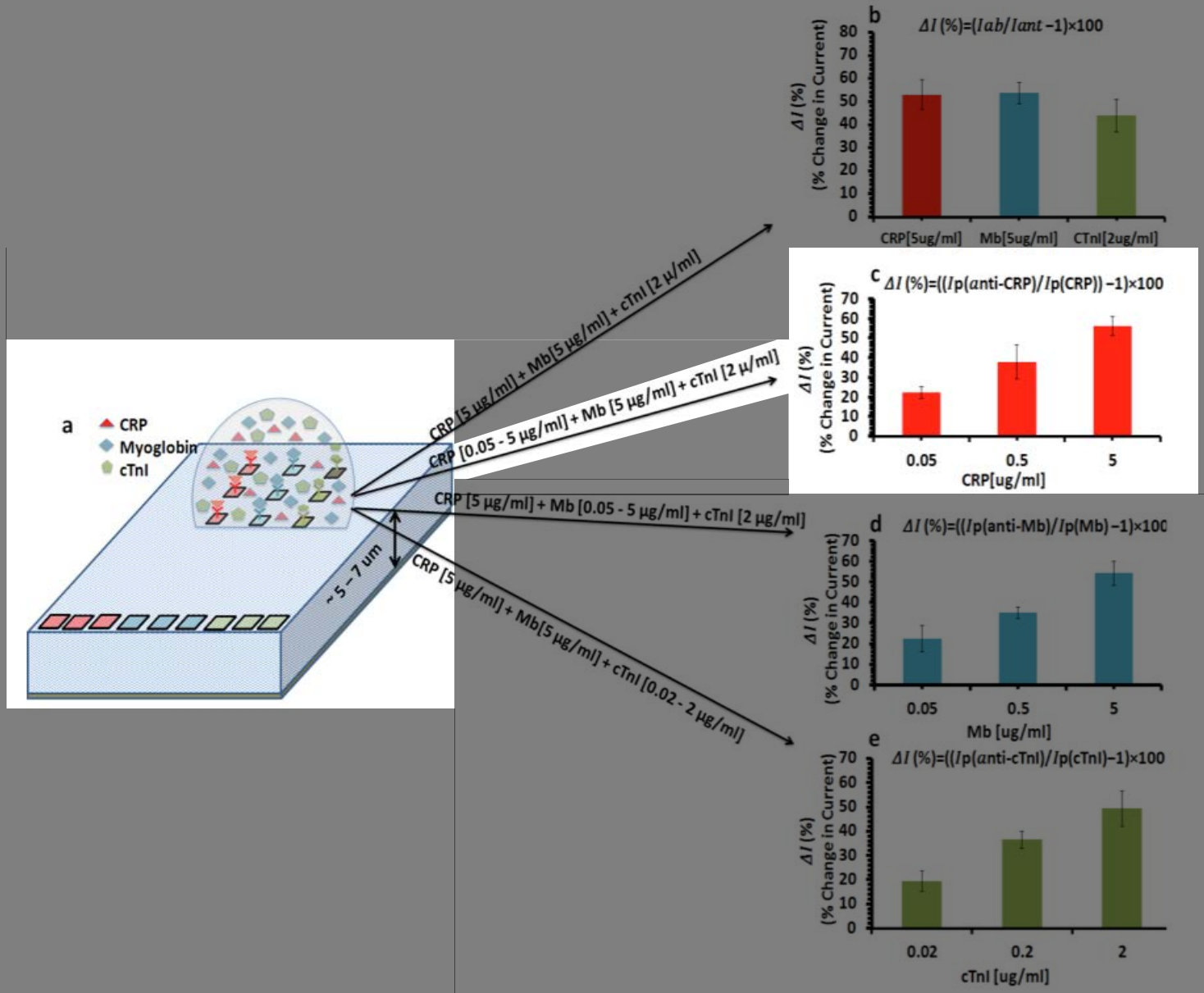
Create wells using SU-8





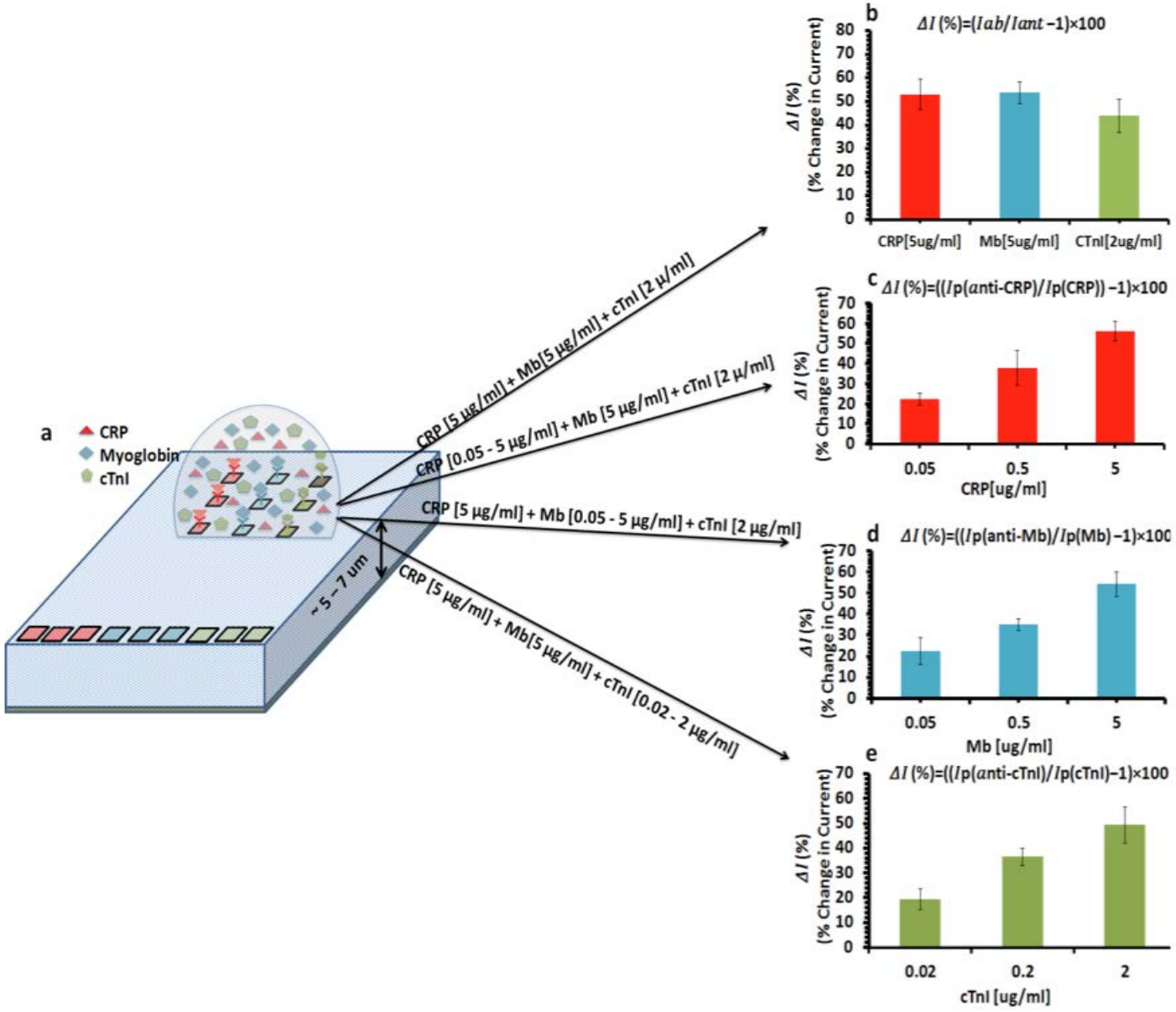
Gupta, R. K., Pandya, R., Sieffert, T., Meyyappan, M., Koehne, J. E. *J Electroanal Chem*, 773 (2016) 53-62.

Multiplexed Sensing



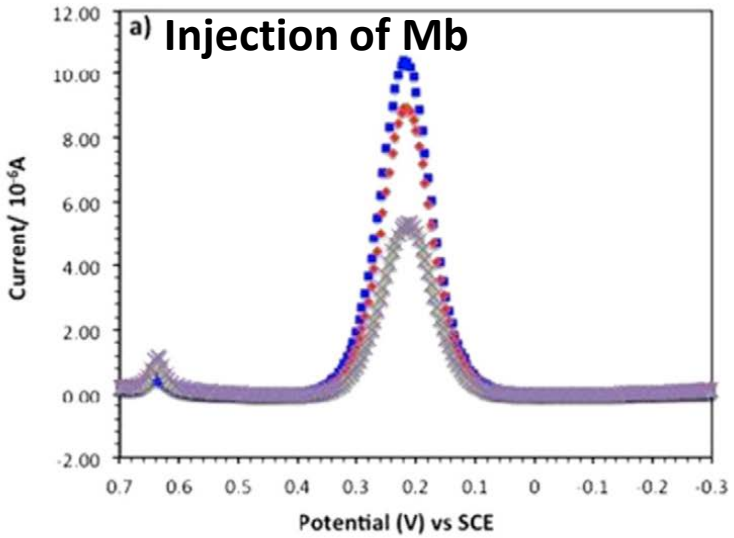
Gupta, R. K., Pandya, R., Sieffert, T., Meyyappan, M., Koehne, J. E. *J Electroanal Chem*, 773 (2016) 53-62.

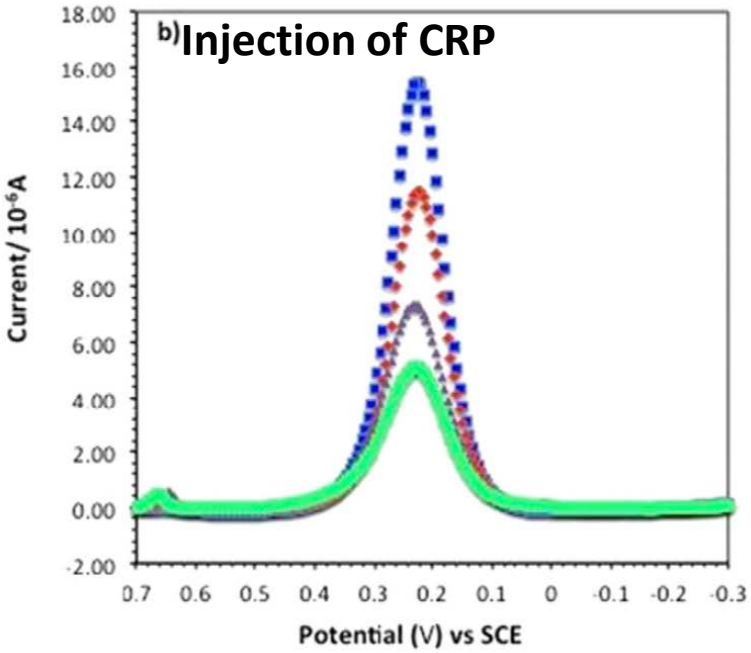
Multiplexed Sensing



Gupta, R. K., Pandya, R., Sieffert, T., Meyyappan, M., Koehne, J. E. *J Electroanal Chem*, 773 (2016) 53-62.

CRP detection in blood serum

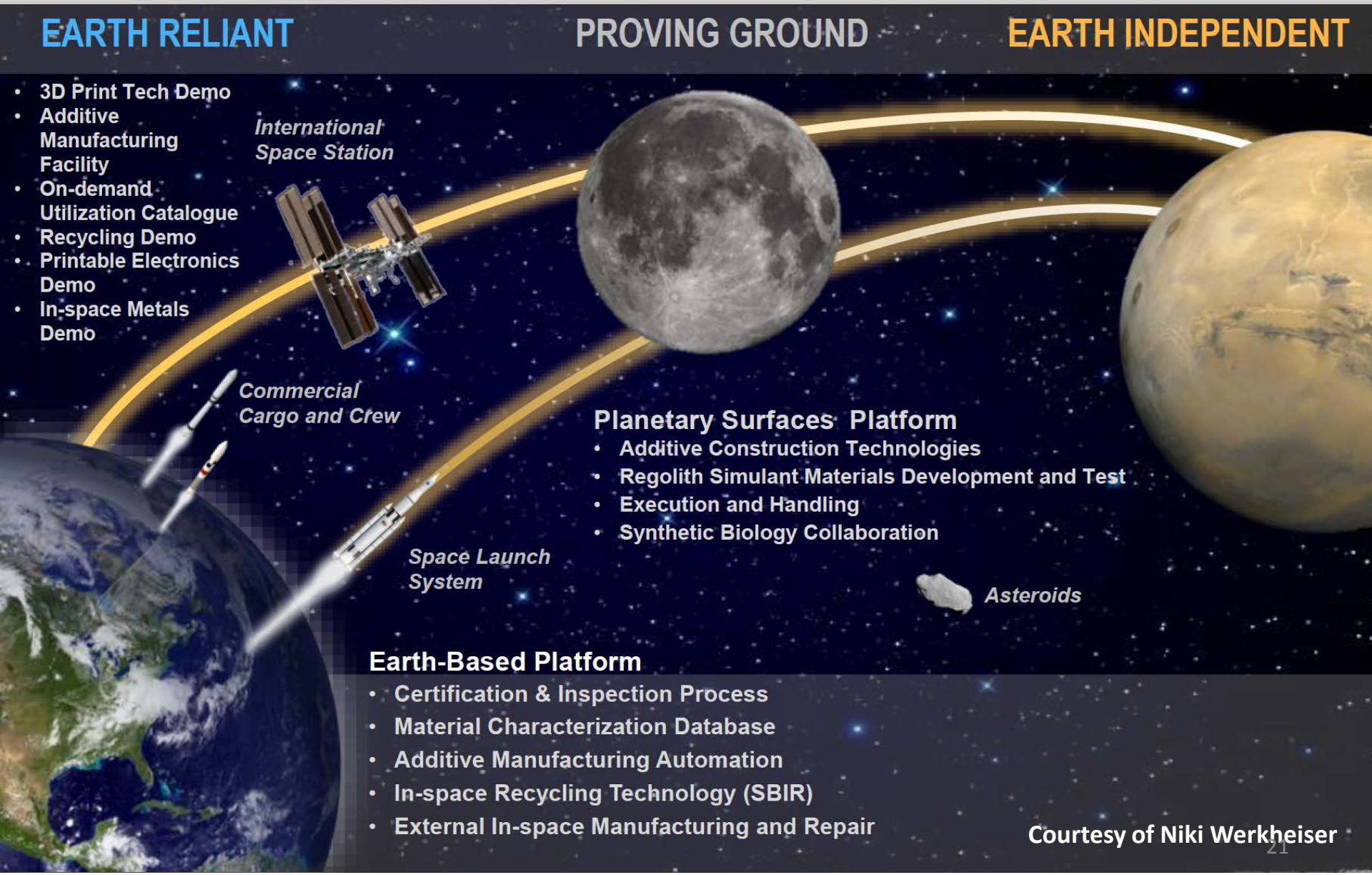




Blue: Bare electrode
Red: After anti-CRP immobilization
Purple: After skim milk passivation
Green: After blood serum injection
-CRP-free spiked with 100 ng/mL protein

Gupta, R. K., Pandya, R., Sieffert, T., Meyyappan, M., Koehne, J. E. *J Electroanal Chem*, 773 (2016) 53-62.

In-space Manufacturing Path to Exploration



Courtesy of Niki Werkheiser

In-space Manufacturing Technology Development Roadmap

Earth-based

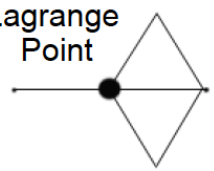
International Space Station

3D Print Tech Demo

Plastic Printing Demo
Add Mfctr. Facility
Recycler SmallSats
Printable Electronics
Metal Printing
Self-repair/ replicate
External In-space Mfctr

Exploration

Asteroids



Lagrange Point

Lunar

Mars

Pre-2012	2014	2015	2016	2017	2018	2020-25	2025	2030 - 40
Ground & Parabolic centric: • Multiple FDM Zero-G parabolic flights • Trade/System Studies for Metals • Ground-based Printable Electronics/Spacecraft • Verification & Certification Processes under development • Materials Database • Cubesat Design & Development	• **In-space:3D Print: First Plastic Printer on ISS Tech Demo** • **NIAC Contour Crafting** • **NIAC Printable Spacecraft** • **Small Sat in a Day** • **AF/NASA Space-based Additive NRC Study** • **ISRU Phase II SBIRs** • **Ionic Liquids** • **Printable Electronics**	• ***3D Print Tech Demo*** • ***Future Engineer Challenge*** • ***Utilization Catalogue*** • ***ISM Verification & Cert Process Development*** • ***Add. Mfctr. Facility (AMF)*** • ***In-space Recycler SBIR*** • ***In-space Material Database*** • ***External In-space 3D Printing*** • ***Autonomous Processes*** • ***Additive In-space Repair***		**ISS:** *Utilization/Facility Focus* • In-space Recycler Demo • Integrated Facility Systems for stronger types of extrusion materials for multiple uses including metals & various plastics • Printable Electronics Tech Demo • Synthetic Biology Demo • Metal Demo Options		*Lunar, Lagrange FabLabs* • Initial Robotic/Remote Missions • Provision some feedstock • Evolve to utilizing in situ materials (natural resources, synthetic biology) • Product: Ability to produce multiple spares, parts, tools, etc. “living off the land” • Autonomous final milling to specification	*Planetary Surfaces Points Fab* • Transport vehicle and sites would need Fab capability • Additive Construction	*Mars Multi-Material Fab Lab* • Utilize in situ resources for feedstock • Build various items from multiple types of materials (metal, plastic, composite, ceramic, etc.) • Product: Fab Lab providing self-sustainment at remote destination

ISS Technology Demonstrations are Key in ‘Bridging’ Technology Development to Full Implementation of this Critical Exploration Technology.

Courtesy of Niki Werkheiser

In-Space Manufacturing of Biosensors

Substrates

- **Paper**
- **Polyimide**

Inks

- Carbon Nanotube
- Silver Nanoparticles
- SU-8

Printers

- **Ink Jet**
- **Atmospheric Plasma Jet**

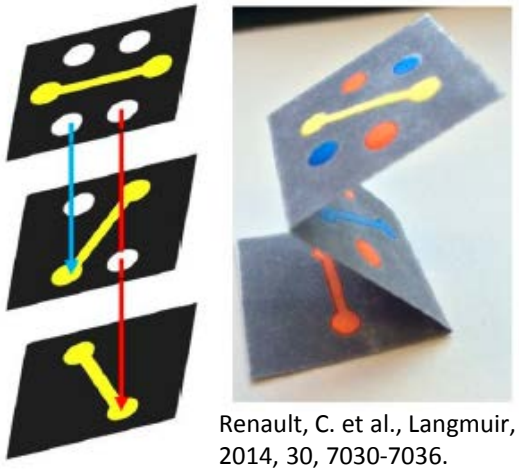
Target and Applications

- Ca^{2+} from urine
  - Bone density loss in microgravity
- Cortisol and hormones from sweat
  - Stress markers for human performance
- Troponin-I from blood
  - Cardiac health monitoring in microgravity

Substrates for In-Space Manufacturing of Biosensors

Paper (Whatman No. 4)

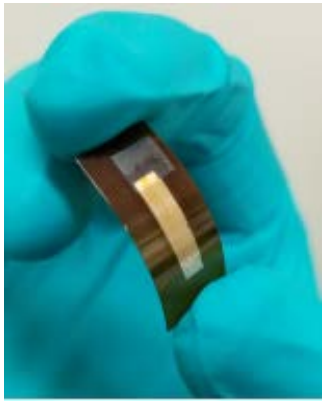
- Flexible
- Chemically inert
- Porous
  - High surface area for printed electrodes
  - Large pores for fluid movement



Renault, C. et al., Langmuir, 2014, 30, 7030-7036.

Plastic (Kapton)

- Flexible
- Chemically inert
- Flat
- Can pretreat for improved adhesion
  - Oxygen plasma
  - Oxidizing acid treatment



Pandhi, T., et al., 64th International Astronautical Congress, 2018, *in press*.

Printers for In-Space Manufacturing of Biosensors

Ink Jet

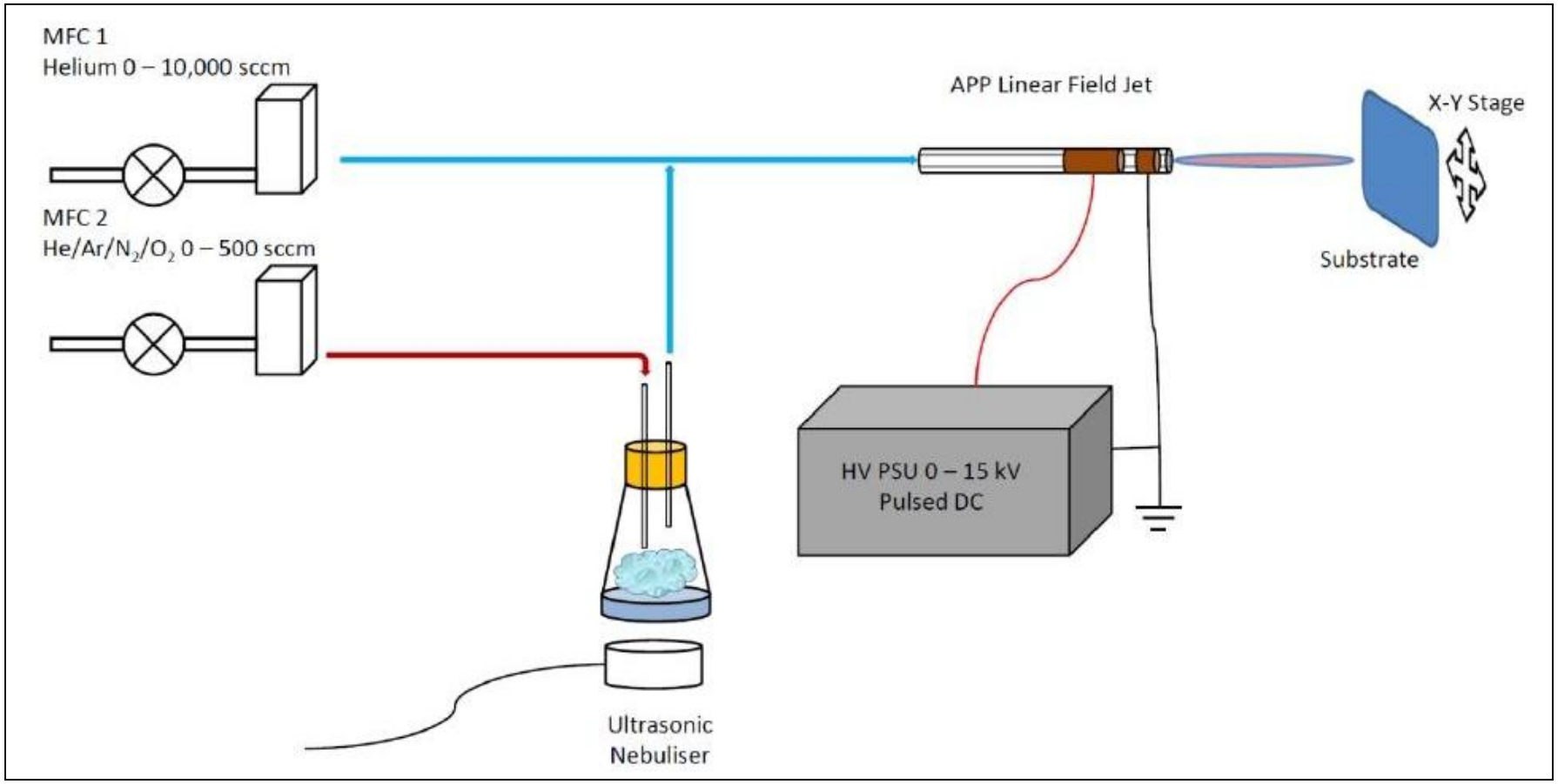
- DMP-2800 Dimatix printer
- Piezo drop-on-demand ink delivery
- Low viscosity, aqueous, nanoparticle inks
- Print on flat substrates

Atmospheric Pressure Plasma Jet

- Nebulized aerosol ink delivery
- Low viscosity, aqueous, nanoparticle inks
- Print on flat and curved substrates
- Tunable chemistry from plasma environment

Gandhiraman, R. P., *et al.*, Appl. Phys. Lett., 108, 2016, 123103-1.

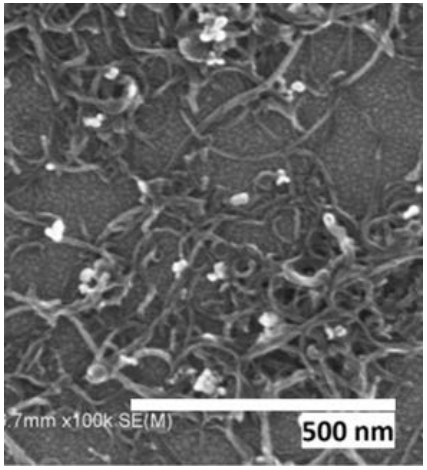
Atmospheric Pressure Plasma Printer Schematic

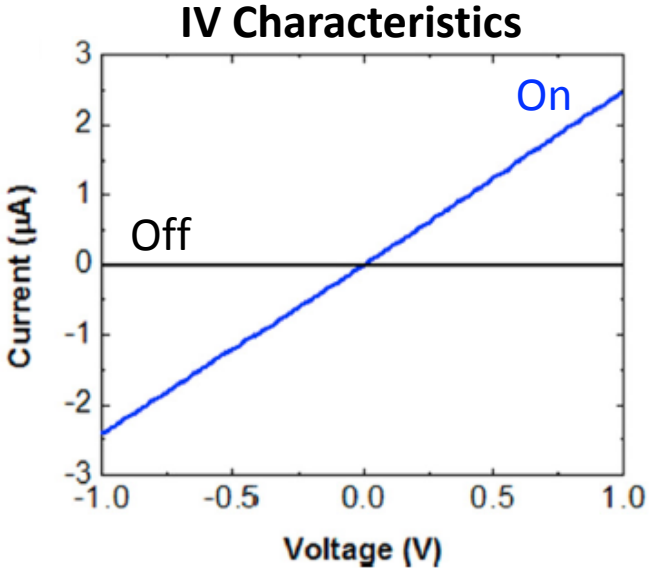


Gandhiraman, R. P., *et al.*, Appl. Phys. Lett., 108, 2016, 123103-1.

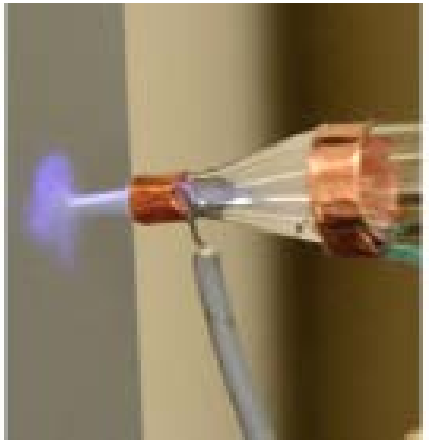
Why Add Plasma?

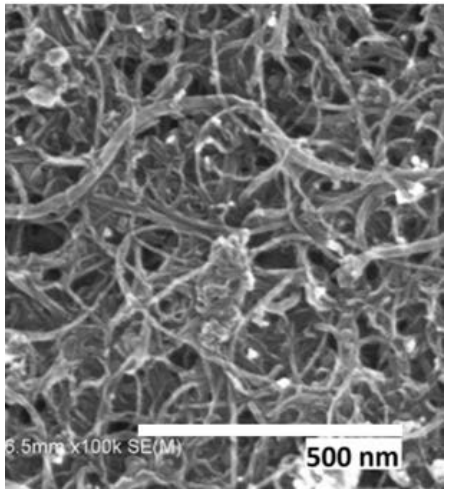
Plasma Turned Off





Plasma Turned On





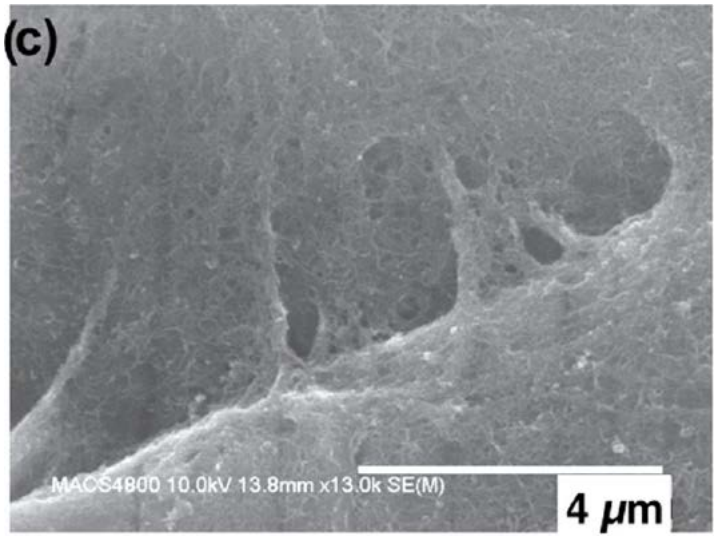
* Low abundance of printed CNTs
* Less focused materials deposition
* ~0.001 µS conductance

* Higher abundance of printed CNTs
* More focused materials deposition
* 1-2 µS conductance

Plasma Printed CNT Sensor on Paper

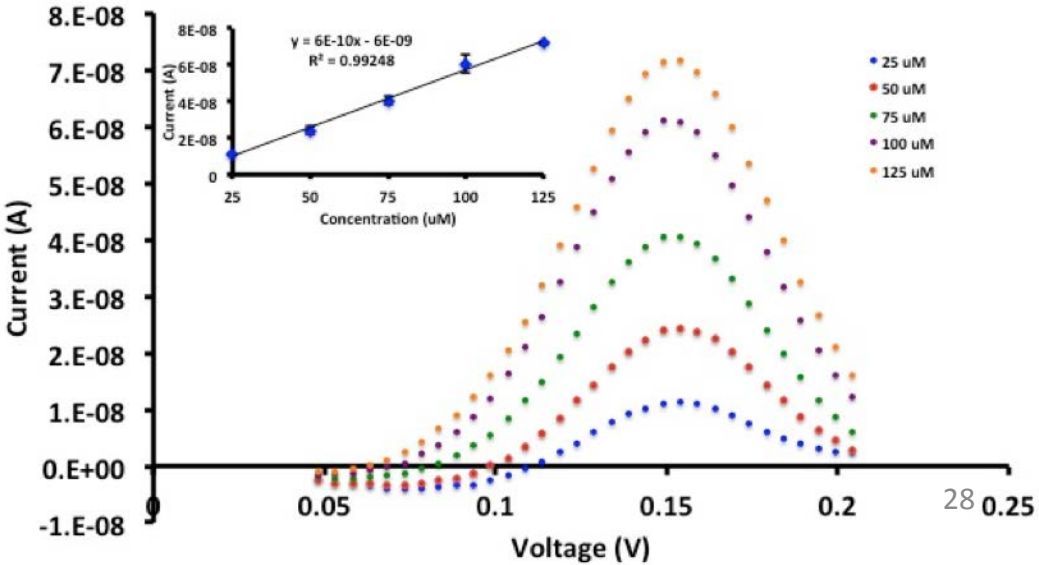
Device

- MWCNT-COOH working electrode
- Whatman No. 4 Substrate
- Pt wire counter electrode
- Ag/AgCl reference electrode



Electrochemical detection

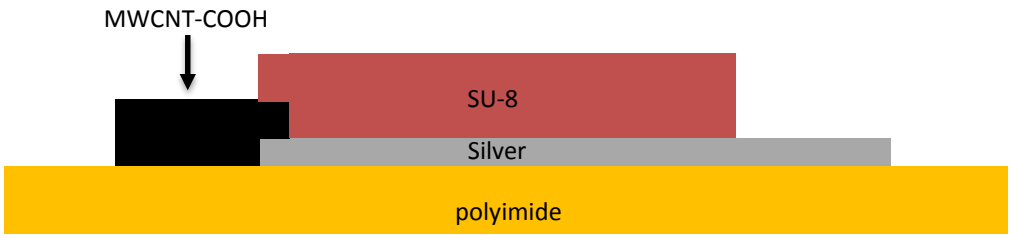
- Dopamine in PBS
- Differential pulse voltammetry
- Custom liquid cell
- Good response in the µM range



Biosensor Fabrication by Inkjet

Substrate: Polyimide
Working electrode: Carbon Nanotube
Counter electrode: Carbon Nanotube
Reference electrode: Ag/AgCl (electrochemical chlorination of Ag electrode)
Leads and Contact Pads: Silver Nanoparticles
Encapsulating Dielectric: SU-8

Cross Section



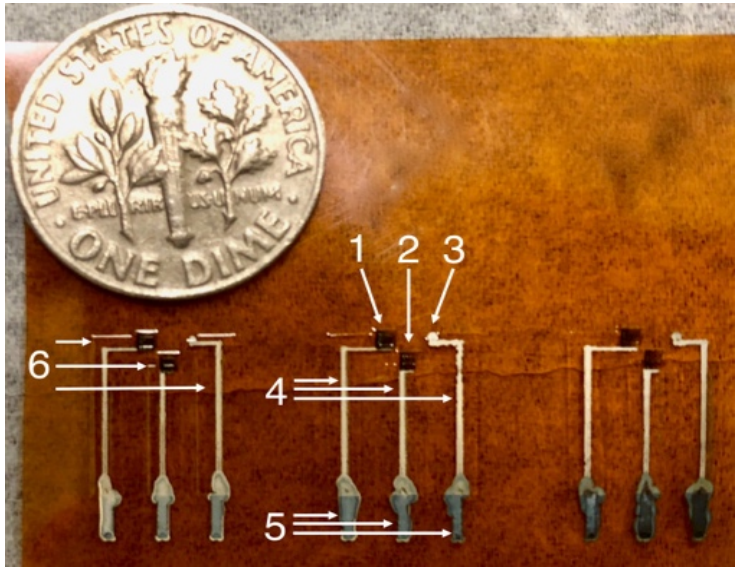
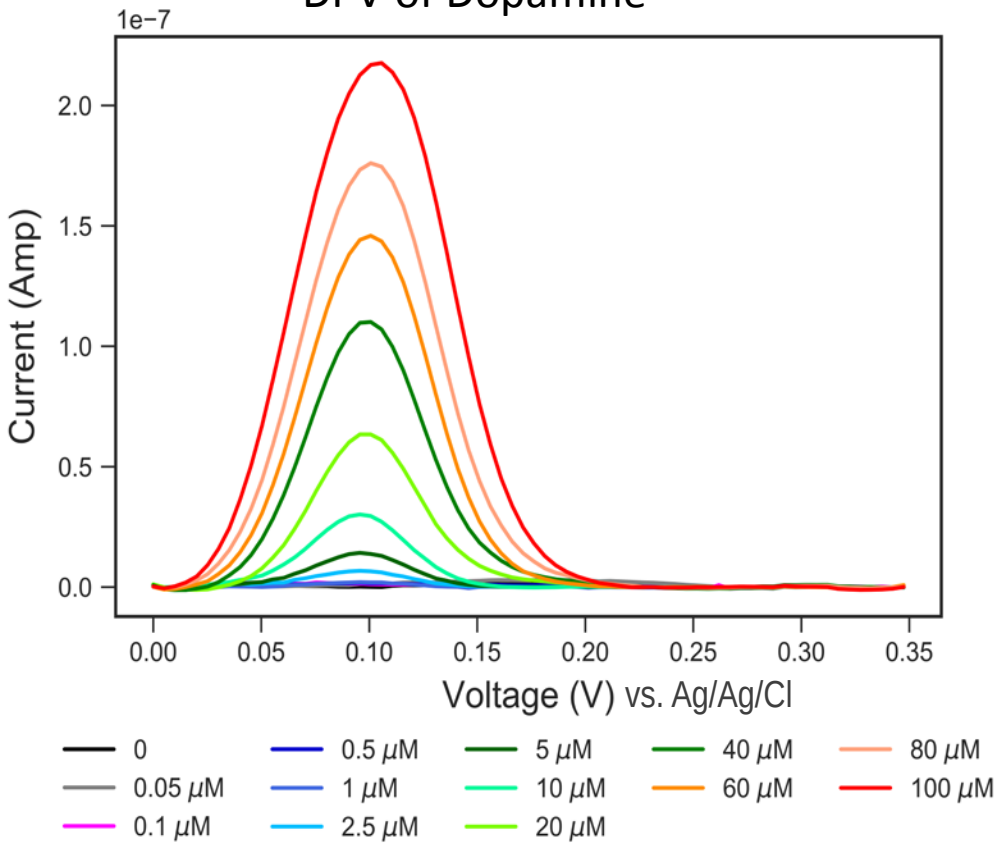


Figure 1. Printed electrochemical biosensor in polyimide substrate. 1) Counter electrode; 2) Working electrode; 3) reference electrode; 4) SU-8 layer; 5) Silver connection lead; 6) Connection pads. Dime diameter 17.89 mm.

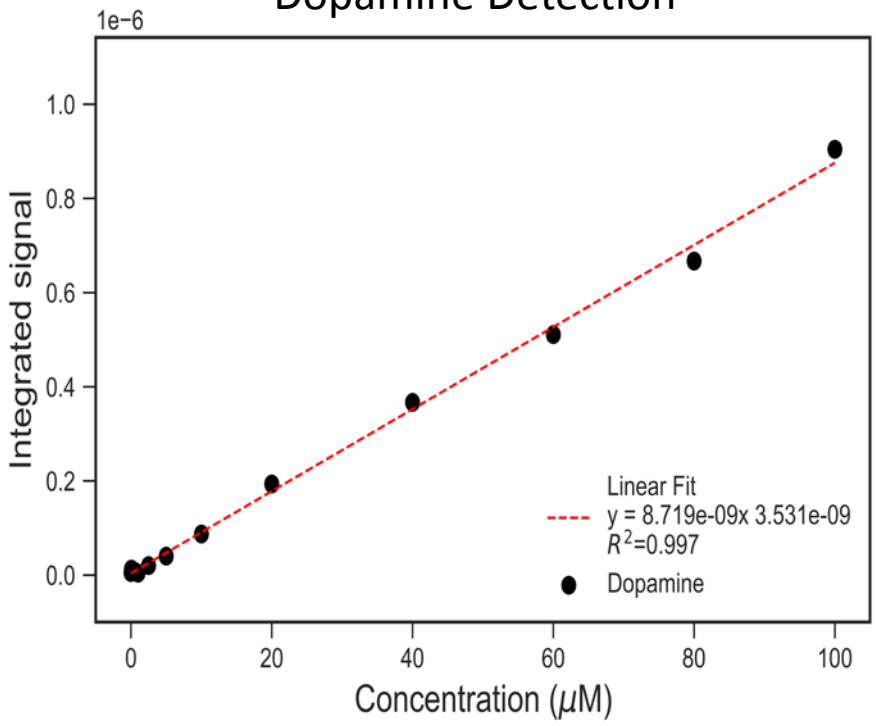
- **Hands-free manufacturing**
- **Layer-by-layer fabrication using 1 printer and 3 inks**

Biosensor Fabrication by Inkjet Electrochemical Characterization

DPV of Dopamine



Dopamine Detection



- **Detection down to 100 nM**

Summary

- Carbon nanofiber nanoelectrode arrays are easily fabricated using standard silicon processing
- Carbon nanofibers have been used as sensitive nanoelectrodes for voltammetry and electrochemical impedance spectroscopy investigations
- Carbon nanofiber nanoelectrode arrays have been used to detect down to 0.25 ng/mL troponin-I and 11 ng/mL CRP
- Multiplexing has been demonstrated for Troponin-I, CRP and myoglobin
- Fully inkjet printed prototype for in-space manufacturing of biosensors

Acknowledgements

- Rakesh Kumar
- Adaikkappan Periyakaruppan
- Ruchi Pandya



- Diana Diaz-Cartagena
- Milton Cordeiro
- Meyya Meyyappan


Funding

- NASA STMD Nanotechnology project
- NASA STMD In-Space Manufacturing project

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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