NSF Nanoscale Science and Engineering Center
for High-rate Nanomanufacturing (CHN)
www.nano.neu.edu

Directed Assembly of Nanoparticles for Biosensing Applications

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Outline

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- **Biosensor Applications**
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  - **Vision**
  - **Capabilities, Users and Needs**
- **Research**
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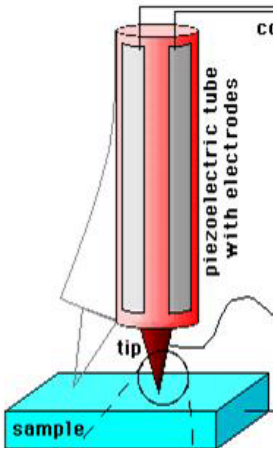
  - **ELISA assay for nanoparticles to evalute pH, stability and particle concentration**
  - **Detection results**
- **Summary**

CHN Vision
The Path from Nanoscience to Nanomanufacturing

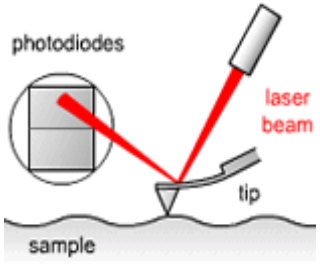
Past and Present (Nanoscience)

Manipulation of few atoms and SWNTs

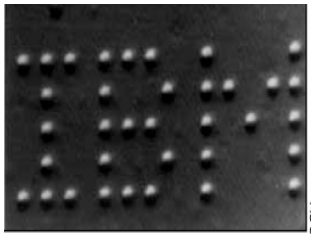
STM 1981



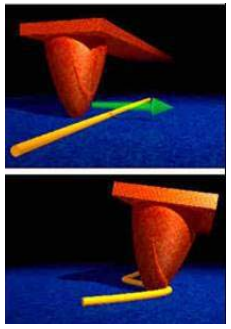
Source: IBM



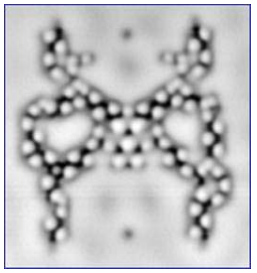
AFM 1986



STM manipulation of atoms 1989



AFM manipulation of a SWNT 1999

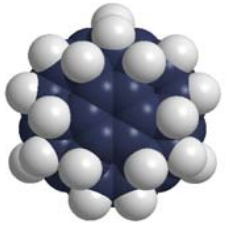


Molecular logic gate 2002

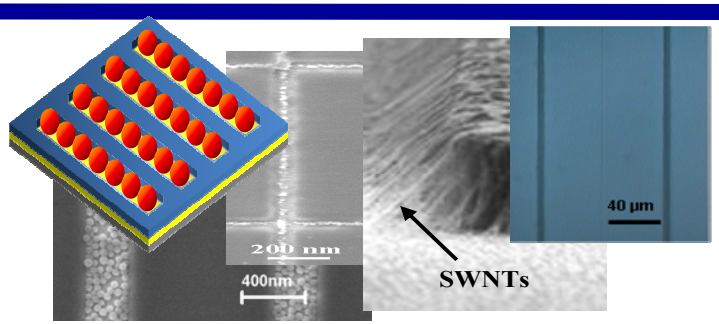
CHN Vision
The Path from Nanoscience to Nanomanufacturing

Future (Nanomanufacturing)

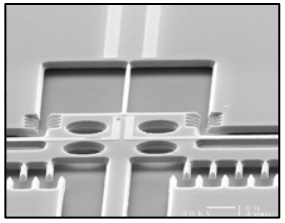
Manipulation of billions of Nanoelements



Synthesis

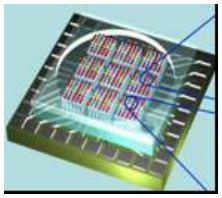


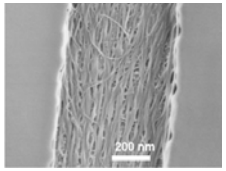
High-rate Directed Assembly



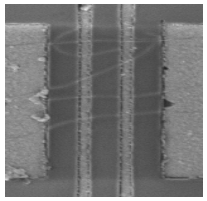
Reliability

Biosensor





Batteries and nanomaterials



CNT Memory device

EHS, Quality & Process Control

Economics and Life Cycle

Our Mission: To bridge the gap between nanoscale scientific research and the creation of nanotechnology-based commercial products

Center for High-rate Nanomanufacturing

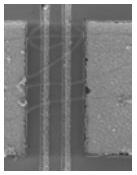
NSF Center of High-rate Nanomanufacturing Applications Road Map

Photo-Voltaic

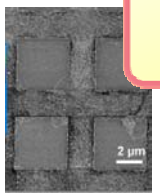
Flexible Electronics

Batteries

Memory Devices



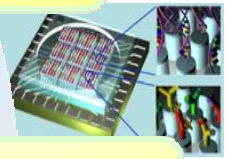
Structural



Energy

Electronics

Biosensors

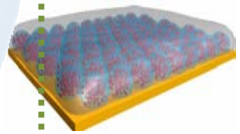


EMI Shielding

Materials

Bio/Med

Drug Delivery



Assembly & Transfer
Thrust I & II

CHN core technology

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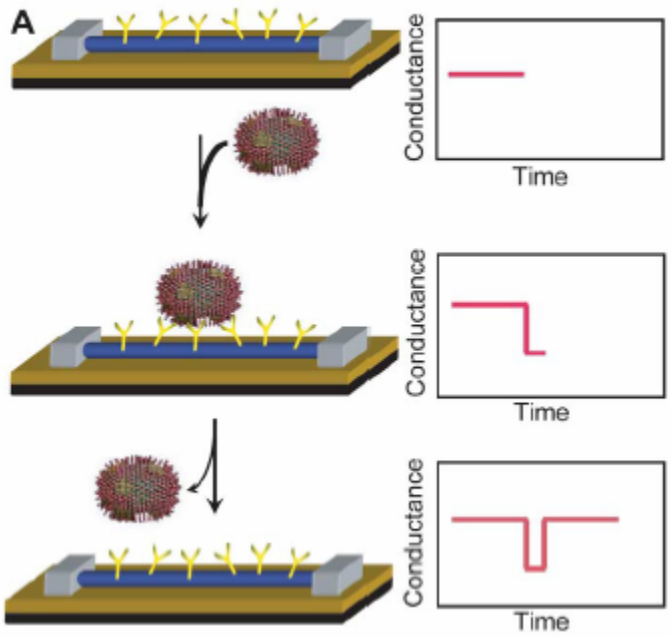
Introduction

- **Today, most diagnostic markers are single species, such as PSA. There is a need for diagnostic devices capable of quantitatively determining the level of multiple markers present in biological fluids or tissue to provide an accurate, quick and efficient diagnosis of a patient.**

- **A variety of multiplex approaches are being developed at the present time, including**
  - **Antibody arrays with fluorescence or surface plasmon resonance detection (Lee, 2006 and Woodbury, 2002),**
  - **Flow cytometry of coded nano-particles coated with capture agents (Morgan, 2004) and**
  - **Use of liquid chromatography/mass spectrometry (Rifai, 2006).**

Introduction



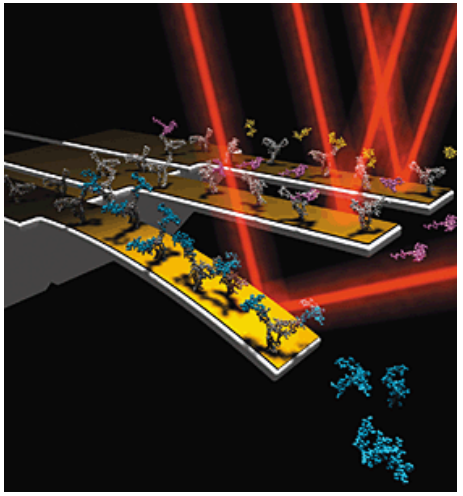
F. Patolsky and C.M. Lieber, "Nanowire nanosensors," Materials Today, 8, 20-28 (2005)

- **A silicon nanowire device was used to detect biological and chemical species (Patolsky, 2005). The device offers the simultaneous detection of up to two oppositely charged viruses*.**

*** Antibodies are not patterned (immobilized), so maximum sensitivity and density are not attained**

- **Cantilevers are coated with antibodies to PSA, When PSA binds to the antibodies, the cantilever is deflected. The cantilever motion originates from the free-energy change induced by specific biomolecular binding*.**

Wu, G.D., R.H.; Hansen, K.M.; Thundat, T.; Cote, R.J.; Majumdar, A. , Nature Biotechnology, 2001. 19(9): p. 856-860.



Current and Future Needs

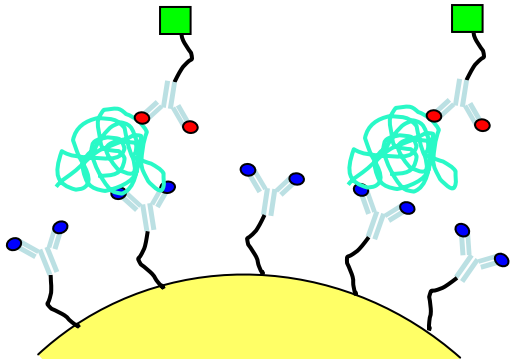
Why the Biosensor is Important?

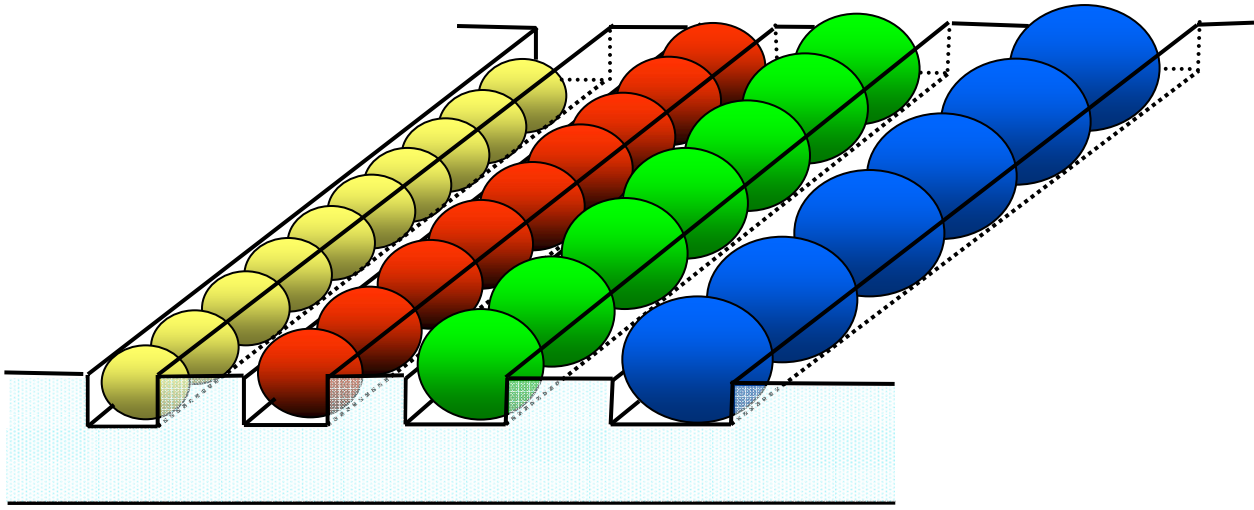
- Current and planned implantable biosensors:
  - Cannot readily detect more than two biomarkers,
  - Are relatively large in size
  - Most are not biocompatible
  - Do not have potential for combining biosensing and drug release

Current and Future Needs

- Tremendous need for detection *multiple* biomarkers present biological fluids or tissue
- Need to increase sensitivity & selectivity of detection
- Need bio- compatibility and small size suitable for implantation
- Low cost

Biosensor Vision





➢ Goals

- **Simultaneous measurement of multiple biomarkers with one device**
- **Very small size (can be as small as 100 μm x 100 μm)**
- **Can be made of all biocompatible material**
- **Low cost**
- **Future development will lead to a device where drugs are released based on the detected antigen.**
- **In-vivo measurement**
- **No issues with sample collection and storage**

Capabilities, Users and Needs

Capability

- Nano-technology based device
- Detect several cancers at the same time
- Inserted intravenously into the blood stream
- High sensitivity
- High specificity
- Early detection

End User

- Patients
- Drug Development

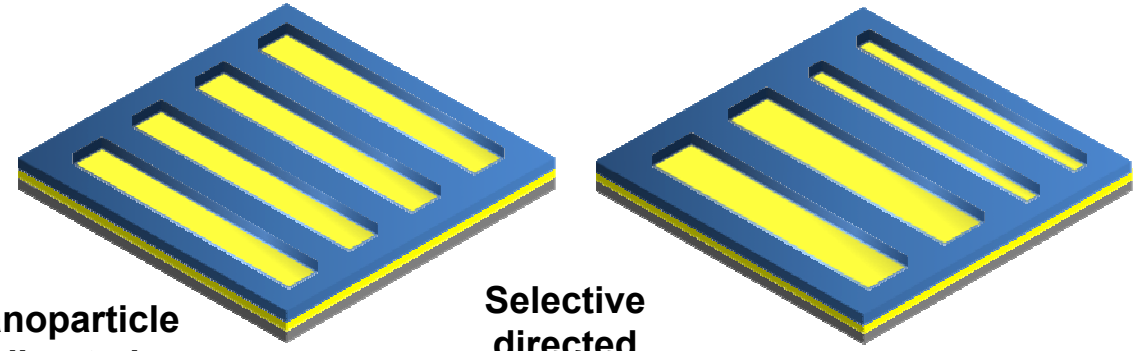
Current Needs

- Early detection of cancers
- Low-cost test
- Quick and accurate results
- Effective monitoring for patients in remission

What Users Wanted:

- ❑ High sensitivity
- ❑ High specificity
- ❑ Early detection
- ❑ Focus on other chronic diseases

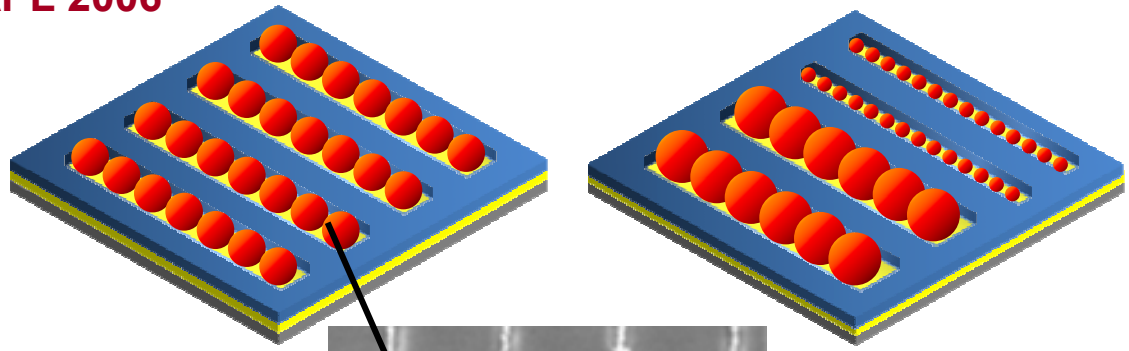
Nanotrench Template Directed Assembly Using Electrophoresis or Chemical Functionalization

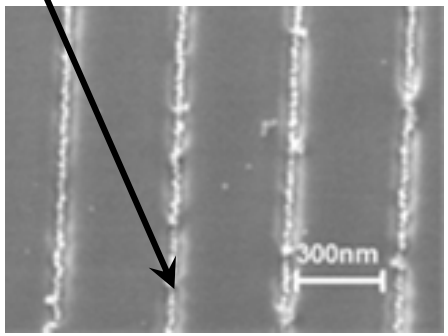


Nanoparticle directed assembly

Selective directed assembly of nanoparticles

APL 2006





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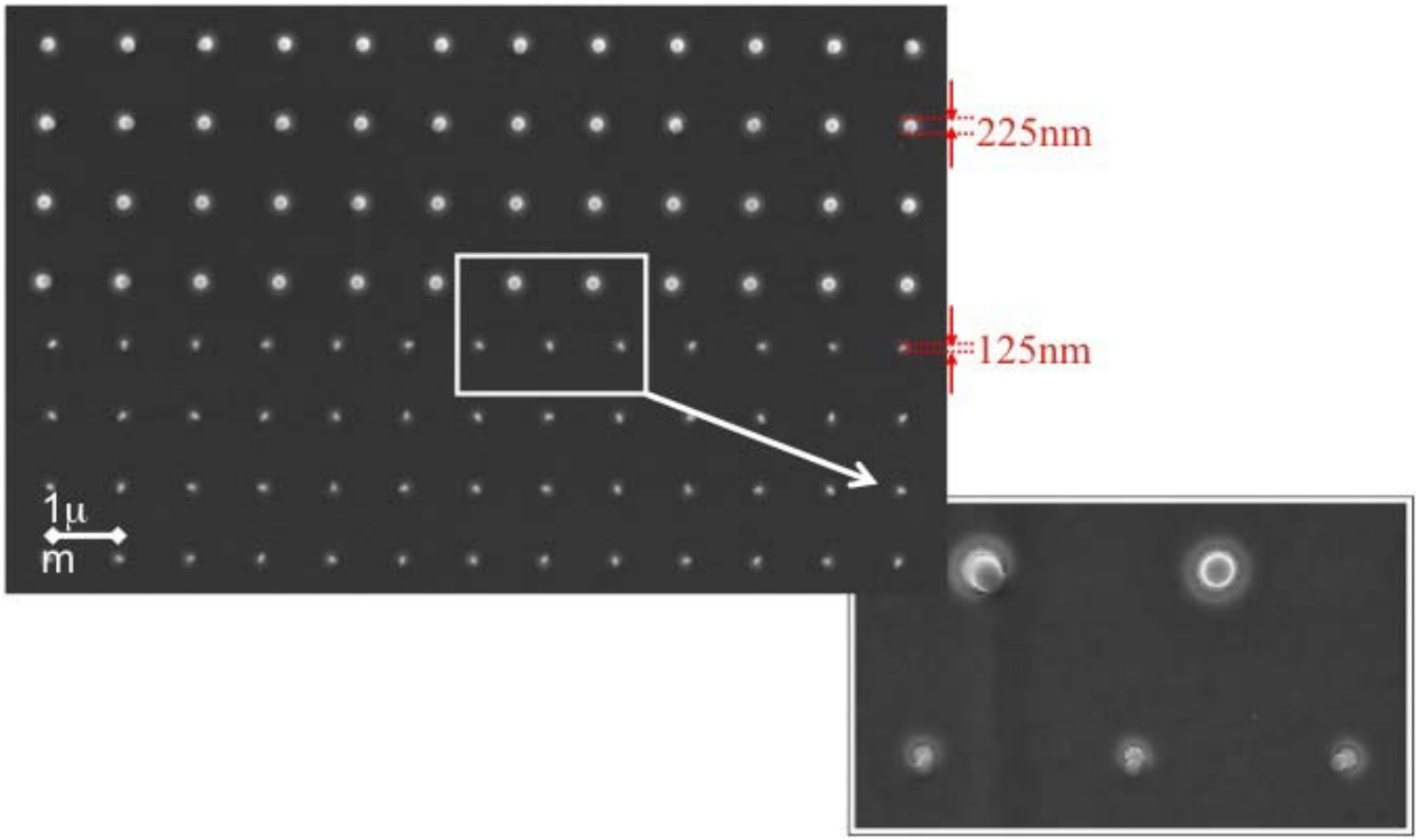
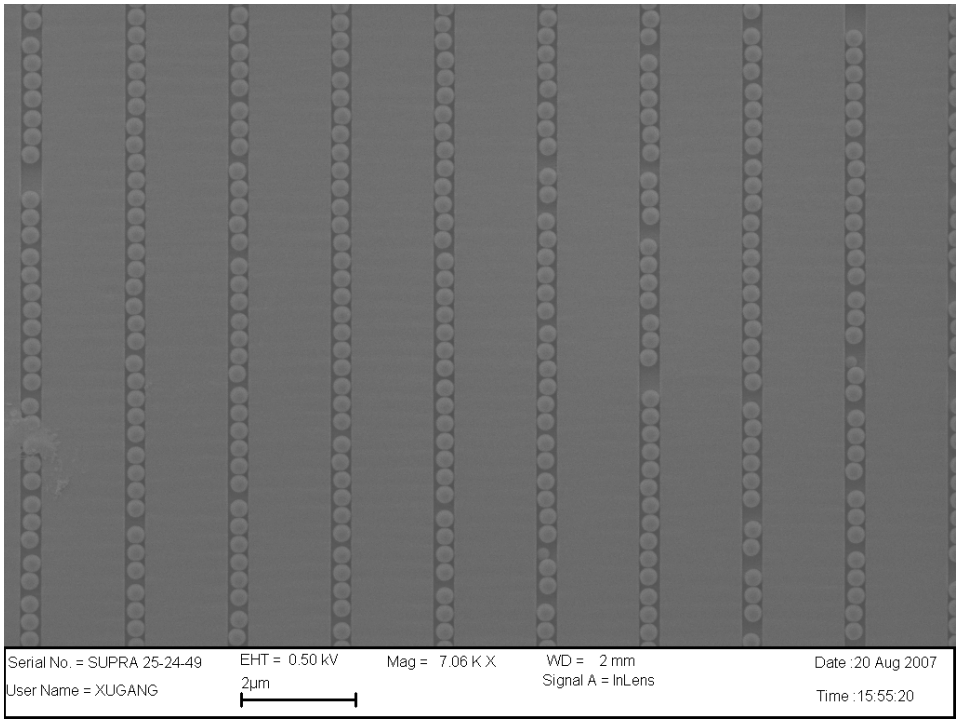
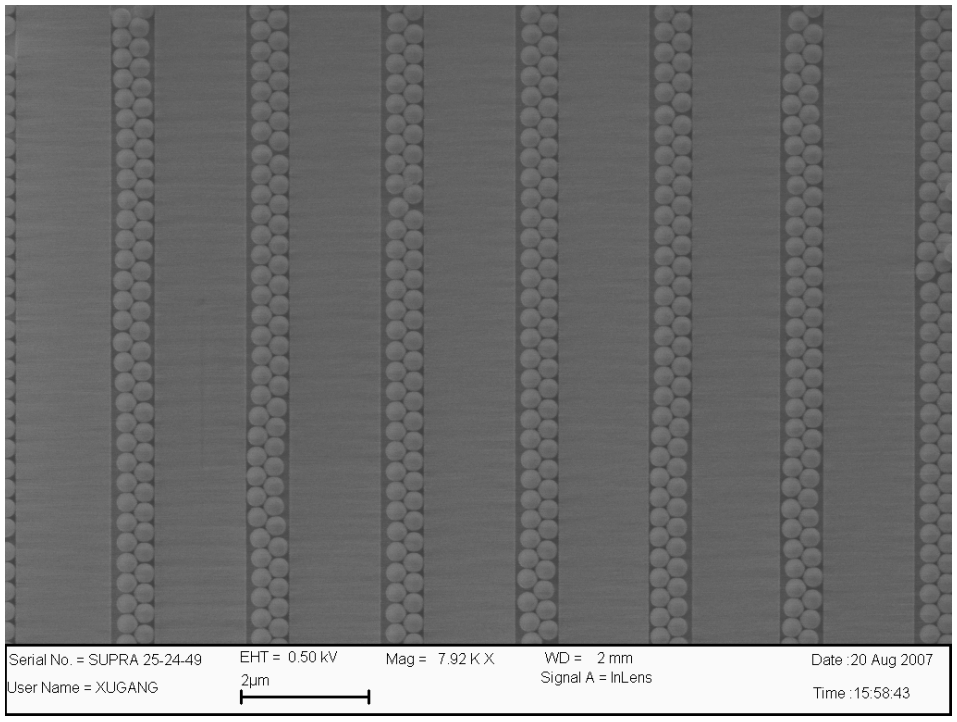
Size-selective Assembly Results



1 um spaced array of 200nm and 100 nm fluorescent PSL particles assembled in 150nm deep circular trenches with the diameter of 225nm and 125nm.

Assembly of PSA IgG Coated Particles





Assembly of 320nm PSA IgG carboxyl polystyrene particles

The process is controlled by fine tuning key parameters such as:

- **pH,**
- **Ionic strength,**
- **Particle concentration,**
- **Assembly voltage and time.**

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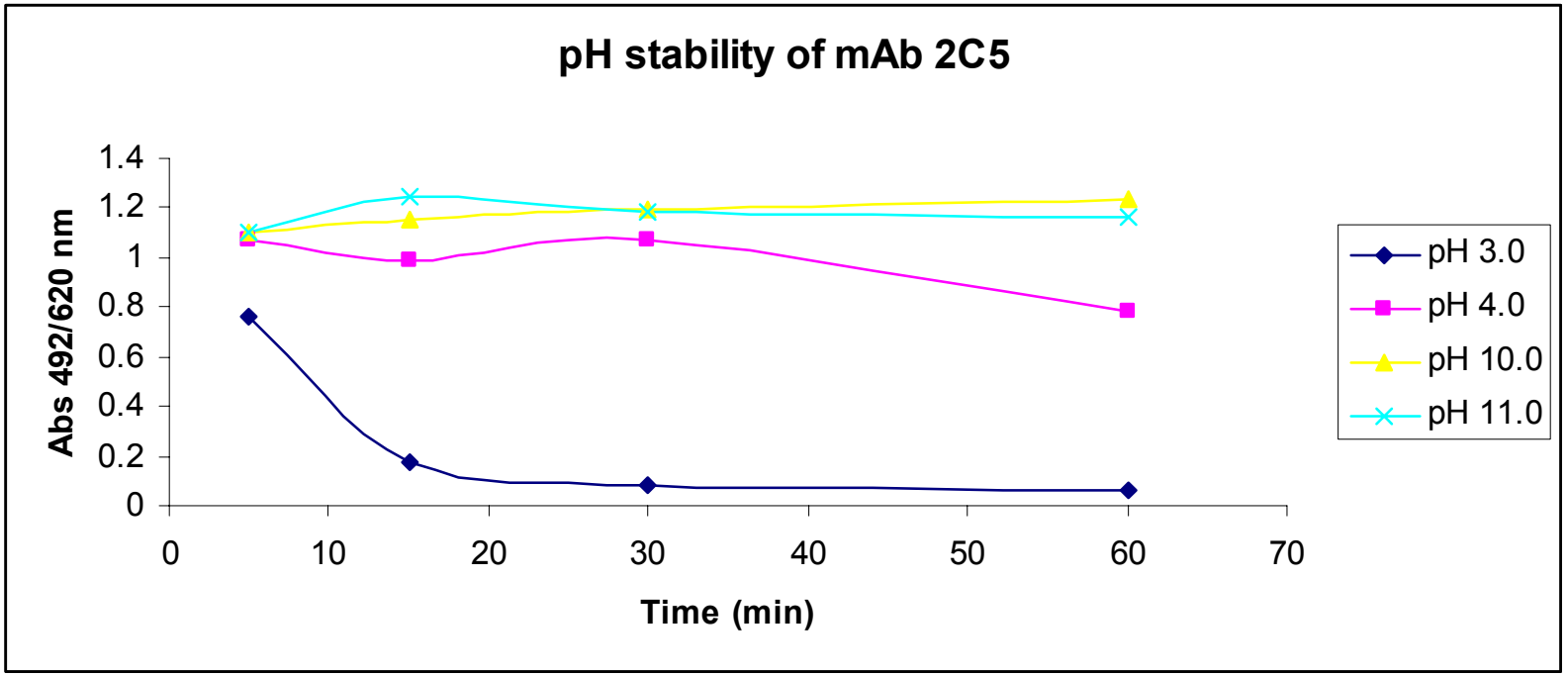
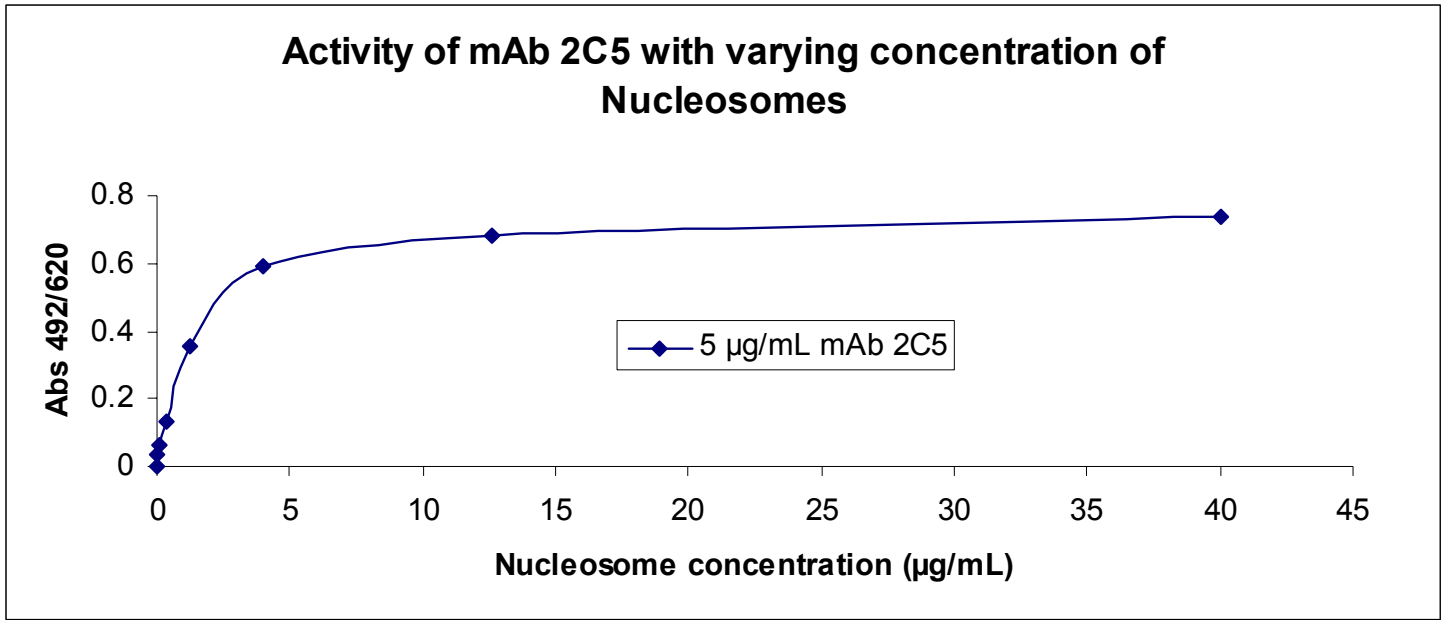
ELISA assay for PSL particles with mAb 2C5; Solution Stability



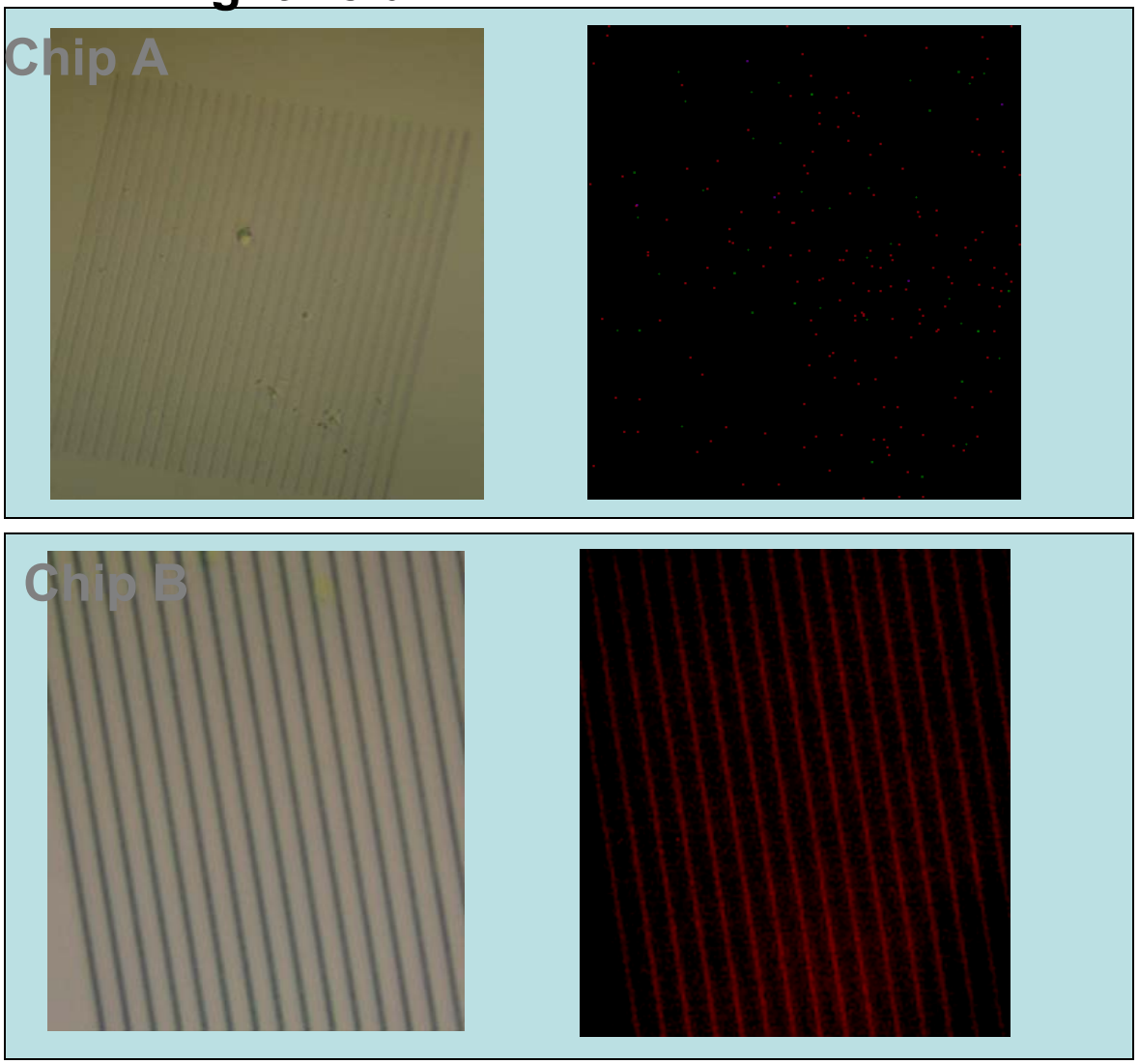
- Nucleosome antigen specific mAb 2C5 was incubated at different pH values for 5, 15, 30, 60 min and their activity was measured using ELISA method.
- The antibody maintained their specific activity at pH 10 and pH 11 for up to 60 min of incubation.
- Activity after incubation at pH 4 was good till 30 min but decreased at 60 min pH 3 Activity after incubation at pH 3 decreased within 5 min .

ELISA assay for PSL particles with mAb 2C5; Effect of Concentration



- Fixed amount of antibody mAb 2C5 (5 µg/mL) was incubated with varying concentrations (0 – 40 µg/mL) of nucleosome antigen to find amount of antigen that binds this fixed amount of antibody.
- Here it was observed that approx. 5 µg antibody can bind to approx. 4 µg antigen.

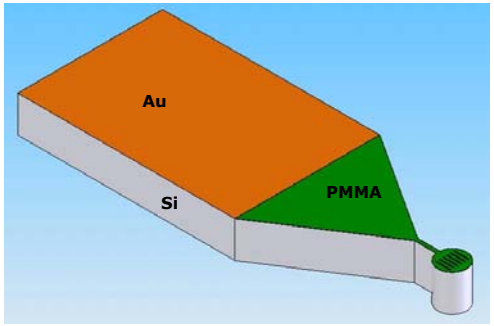
Sandwich Complex with Fluorescence Detection

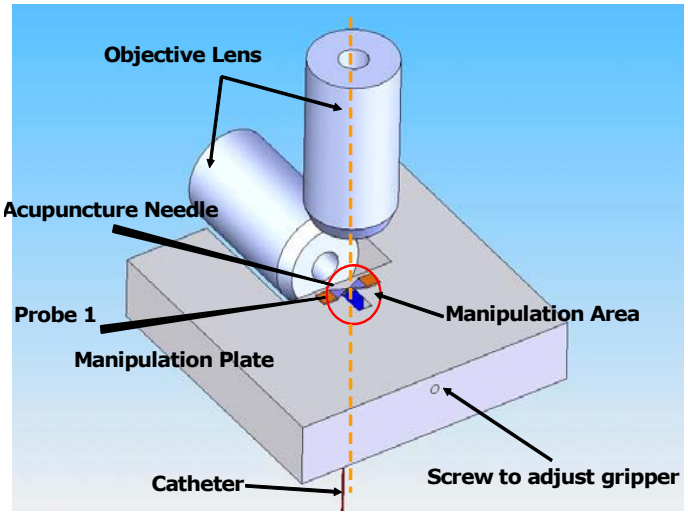


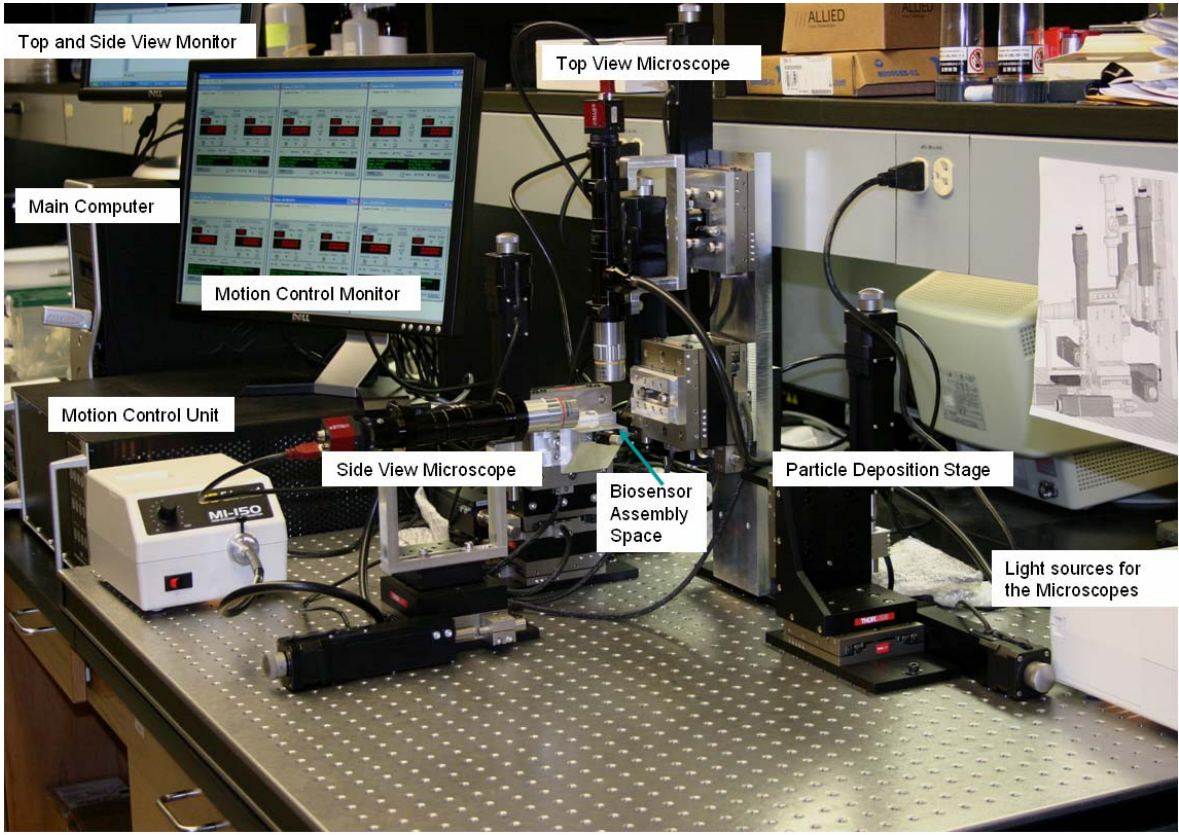
Chip A: negative control; Chip B: incubation with PSA 1 mg/mL.

- **Incubated with capture anti-PSA Ab followed by blocking with BSA. The control chip was not incubated with the detection antibody - just blocked.**
- **incubated the chip with human plasma spiked with 1 ug/mL (level typical for advanced prostate cancer).**
- **After washing incubated chips with fluorescently labeled detection antibody, we observed a strong signal for the chip with detection antibody only.**

Biosensor Assembly Setup







- Using two monitors:
- one shows the control of the stages and the other showing the top and side view of the biosensor assembly region to merge vision information with motion control.
- This allows precise manipulation of the stages and the microscopes to facilitate the an automated assembly process of the biosensor chip.

Questions?