## Subtask C-4

# Biochip and Microarrays for Rapid Assessment of New Chemicals


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- Dr. Ray Runyan: Cell Biology and Anatomy
- Matt Scholz: Cell Biology and Anatomy
- Amruta Kulkarni: Electrical and Computer Engineering
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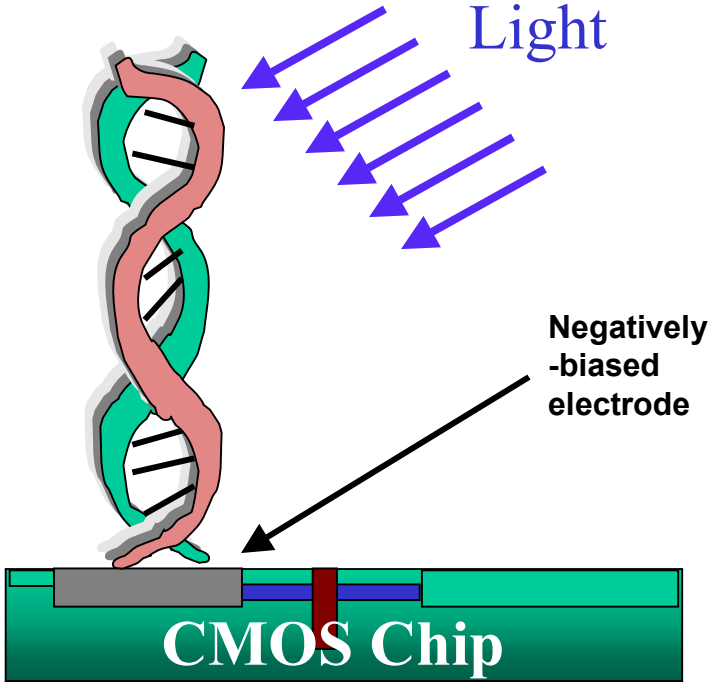
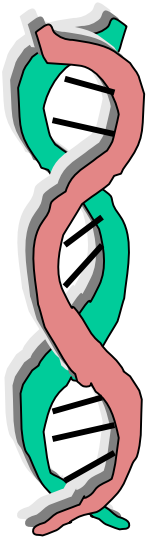
# Project Objectives and Impact

- **Rapid assessment of chemicals and process chemistries**
- **Important for both chemical suppliers (starting materials) and equipment suppliers/end users (for process-generated by-products, interactions of multiple chemicals, proprietary chemistries in R/D stage, etc.)**
- **A first step towards an on-line ESH monitor.**

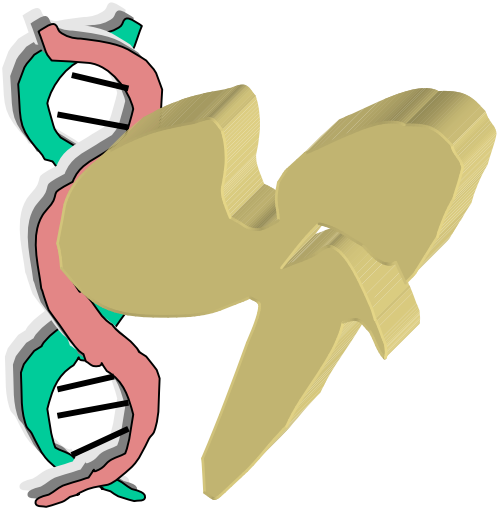



**Novel Technology**

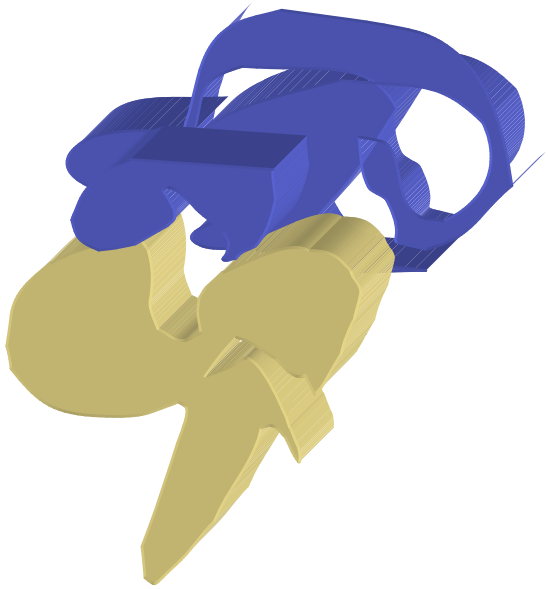
# Biological Interactions of Interest



**Interactions between nucleic acids**



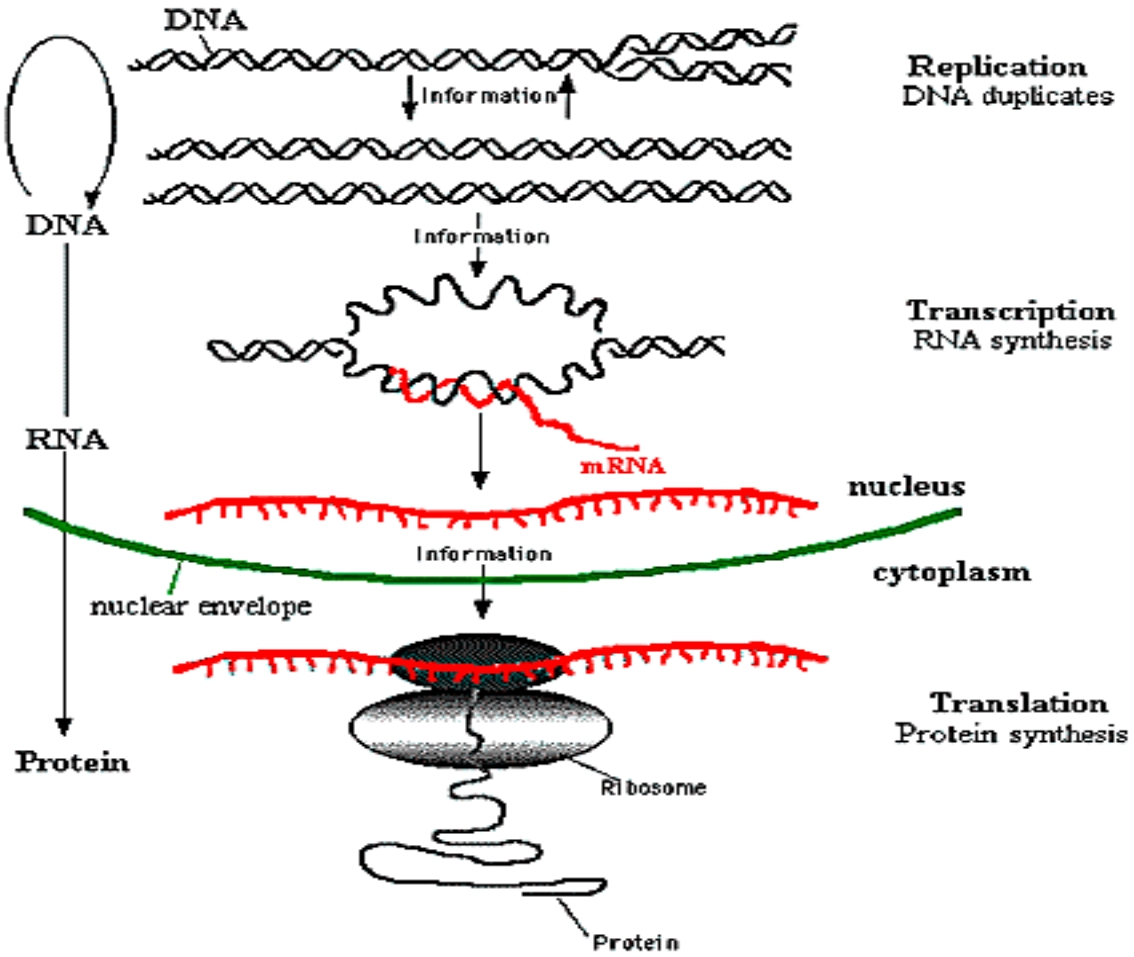
**Interactions between proteins and nucleic acids**



**Interactions between proteins**

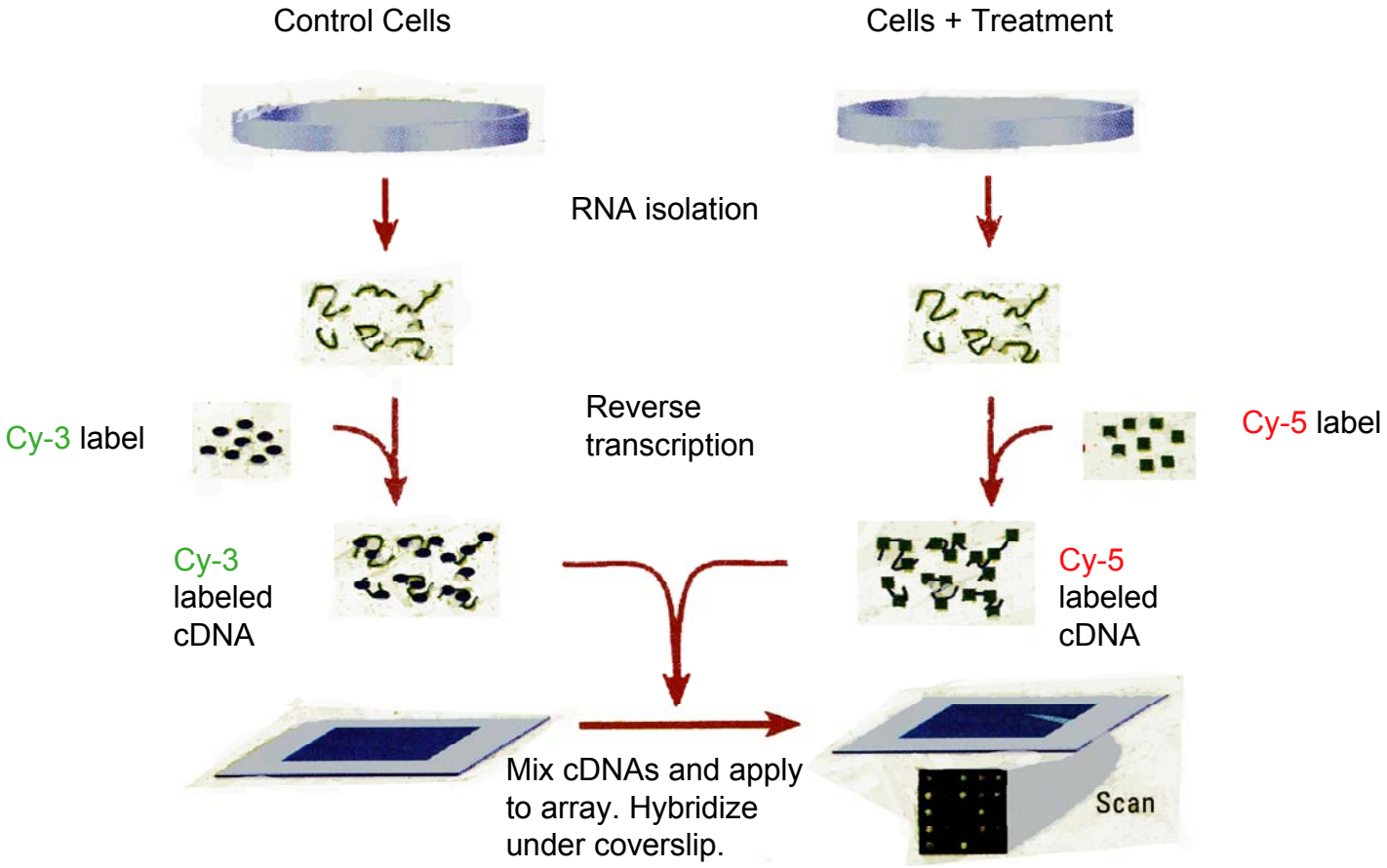
# Role of DNA

DNA forms the template for mRNA, which forms the template for proteins. Proteins carry out the metabolic processes of the cell.




**The Central Dogma of Molecular Biology**

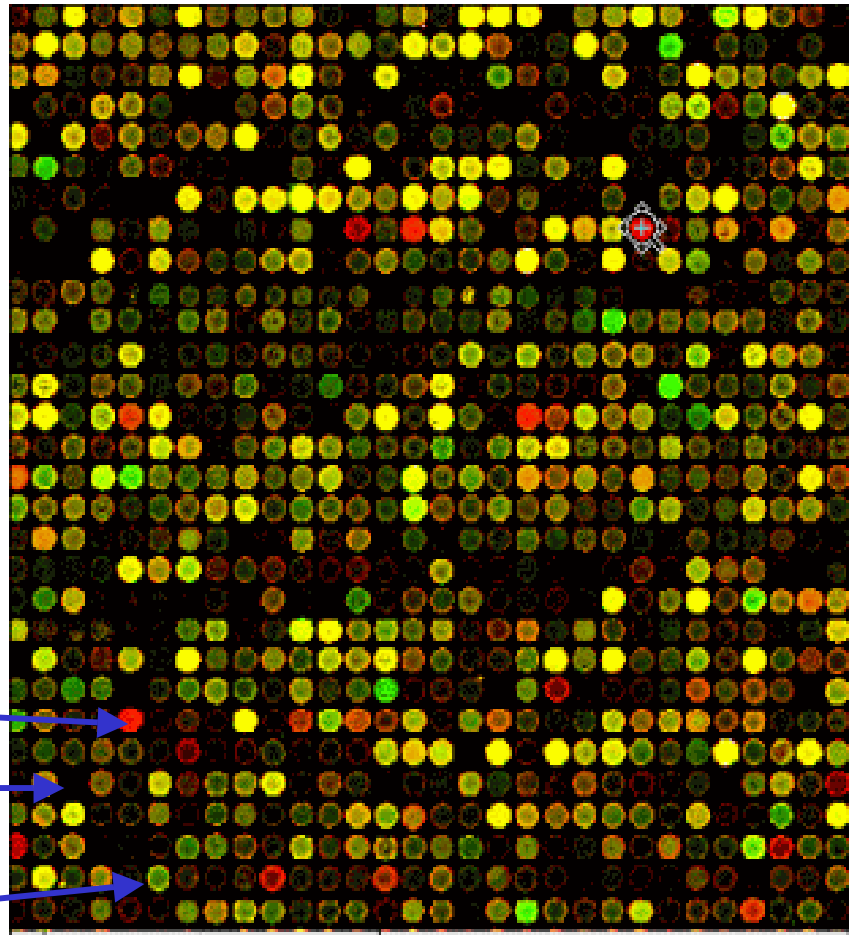
# Microarray Measurement of Differential Gene Expressions




Spots with more Cy3 are genes down-regulated by treatment
Spots with more Cy5 are genes up-regulated by treatment
Mixed spots are genes unaffected by treatment

# Power of the Approach

Obtain information about differential gene expression across diverse set of arrayed molecules in a single experiment



Red = more cy5 than cy3

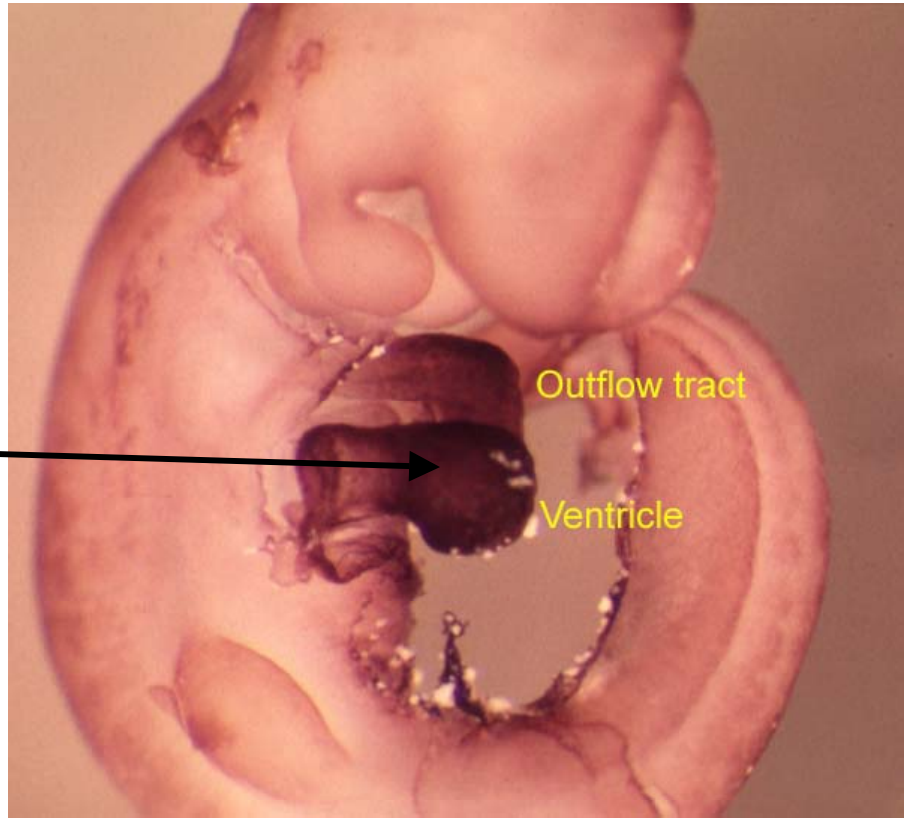
Yellow = “equal” cy5 and cy3

Green = more cy3 than cy5

# Does TCE Affect Gene Expression in the Heart?

- Pregnant rats were exposed to 110 ppm TCE in drinking water
- Rat embryos were collected at day 11 when heart valves were forming
- mRNA was extracted from treated and control embryos and converted to cDNA

P137 mRNA is labeled with chromophore

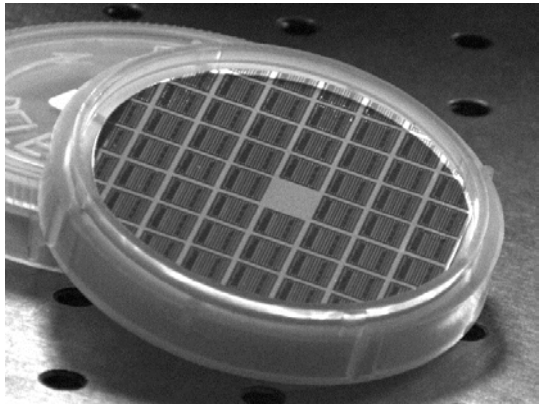



Rat embryo showing expression of p137 in heart

# CMOS Substrates

## Why CMOS ?

- Complementary Metal Oxide Semiconductor (CMOS)
- Replace “dumb” glass substrate with “smart” substrate capable of self-interrogation
- Introduce electronic control to printing, hybridization, and detection
- Couple advances in microelectronics to advances in microarrays



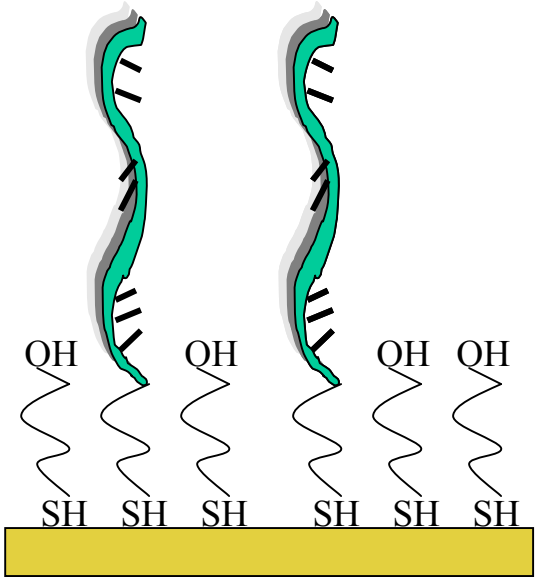
## Why Miniaturize ?

1. Reduced Cost
2. Reduced Cost
3. Reduced Cost
4. Increased Reliability
5. Increased Functionality
6. Reduced Size

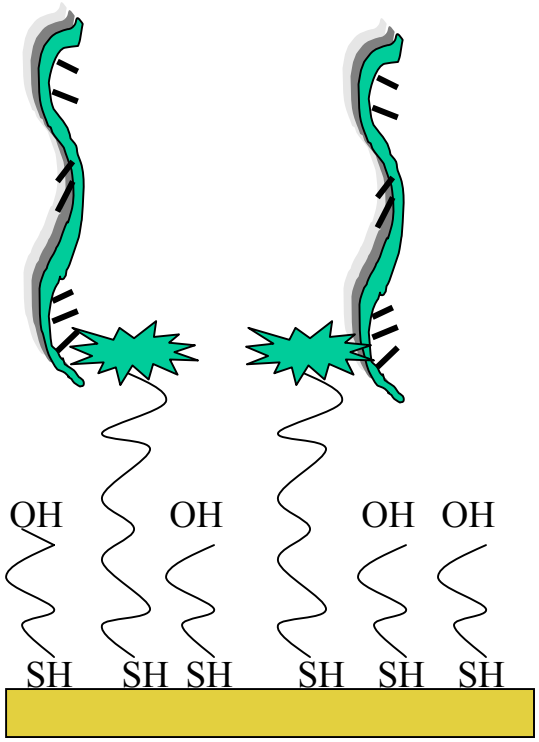
# CMOS Biochip Features

- Multiplexed array of electrodes embedded on a silicon chip
- Electric fields used to increase binding efficiency and reduce nonspecific binding
- Binding sites monitored with capacitance measurements
- Circuitry is designed to perform electrochemical analysis at each electrode
- Optics are used to detect target-to-probe binding while electrochemical methods assess surface chemistry and confirm optical data

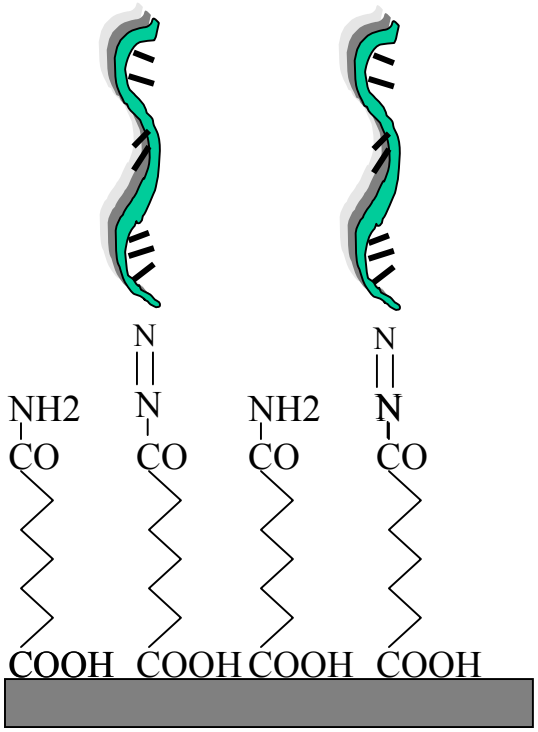
# Three Approaches to Coupling




Mixed monolayer of sulfhydrylated DNA and mercaptopropanol on gold electrodes

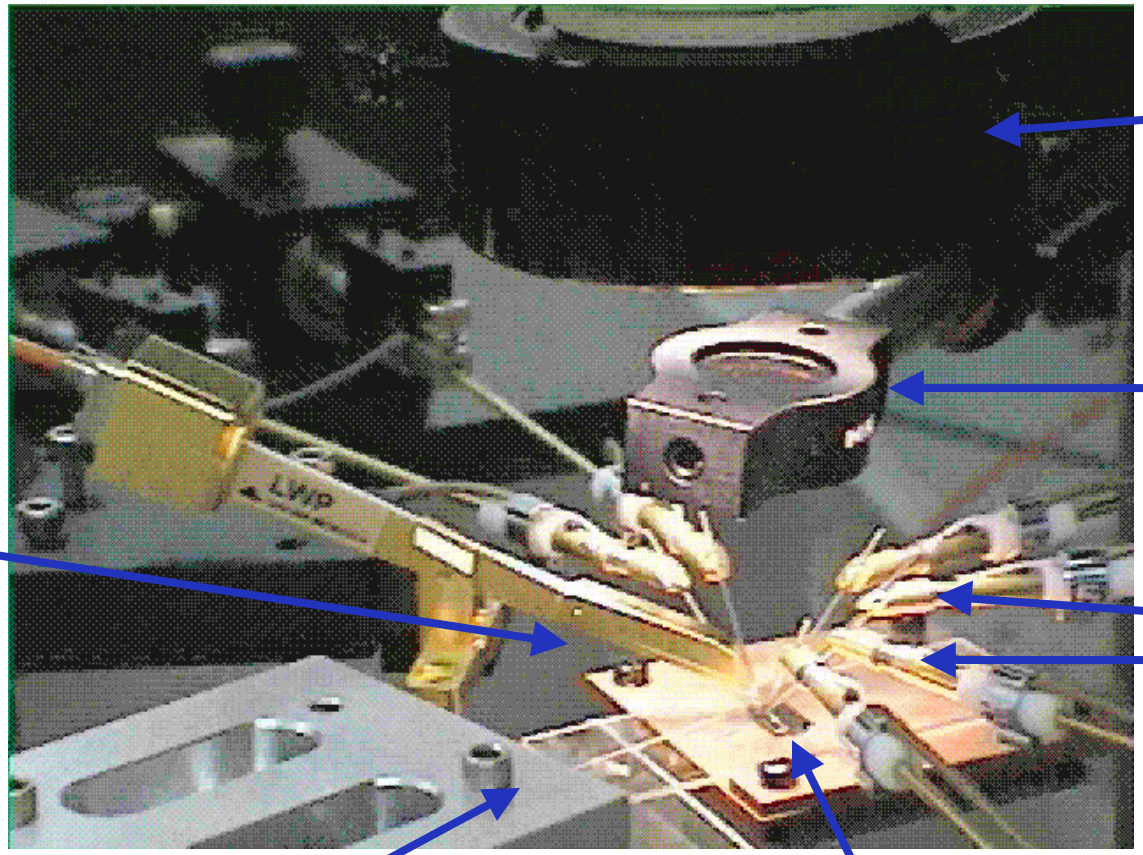



Mixed monolayer of mercaptopropanol and photoactive crosslinker on Au electrodes




SAM crosslinker links amidated DNA to ITO electrodes

# Experimental Set-Up



# Simulation of Electric Field Distributions on a Silicon Chip

## Calculate the Potential Distribution

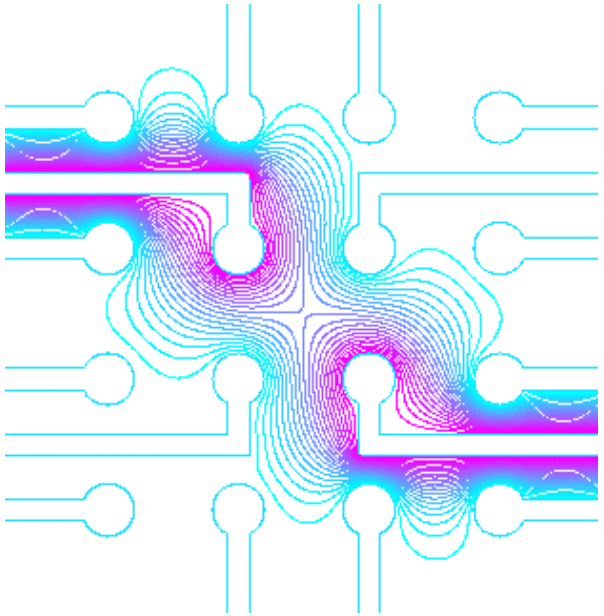
**Determine the potential distribution by solving Laplace's equation self consistently**

$$V_{i,j} = (V_{i+1,j} + V_{i,j+1} + V_{i-1,j} + V_{i,j-1}) / 4.$$

**Then calculate the electric field vectors**

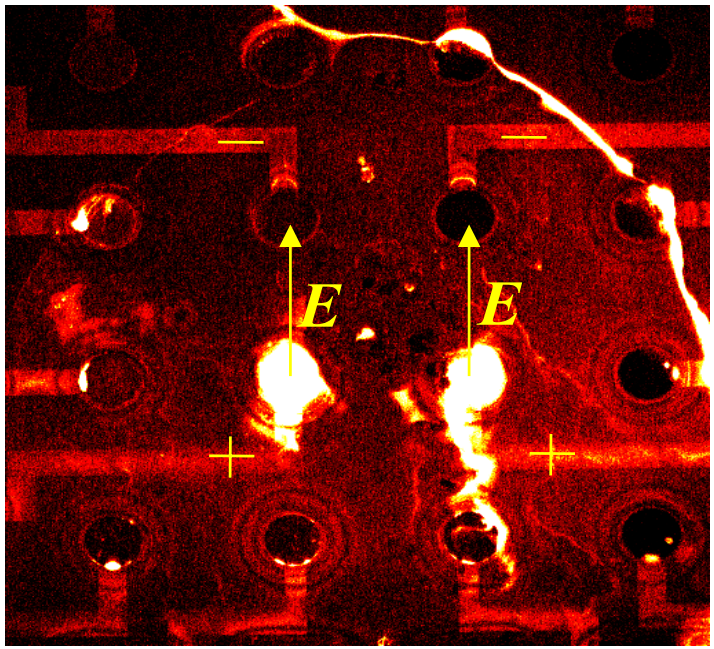
$$\vec{E}_{i,j} = (V_{i-1,j} - V_{i+1,j})/2\Delta x \ \vec{x} + (V_{i,j-1} - V_{i,j+1})/2\Delta y \ \vec{y}$$

## Potential Distribution at Microelectrode Array

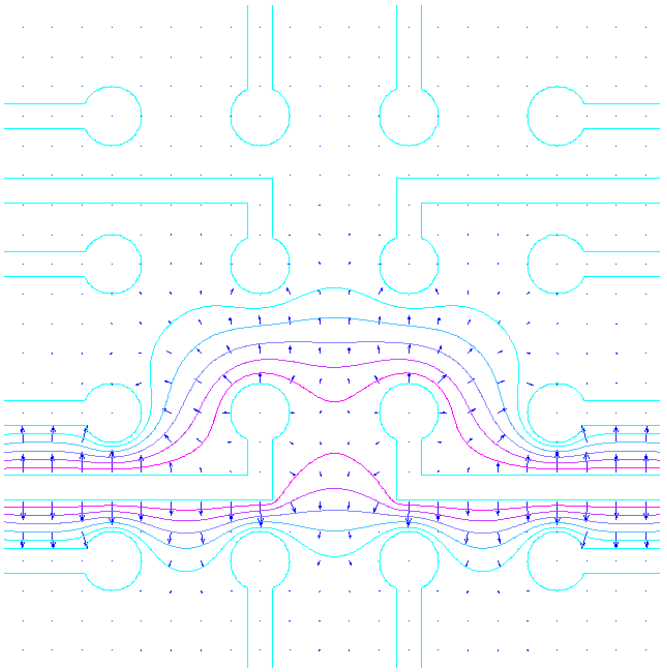


# Electric Field Directed DNA

Confocal Image
of bound DNA

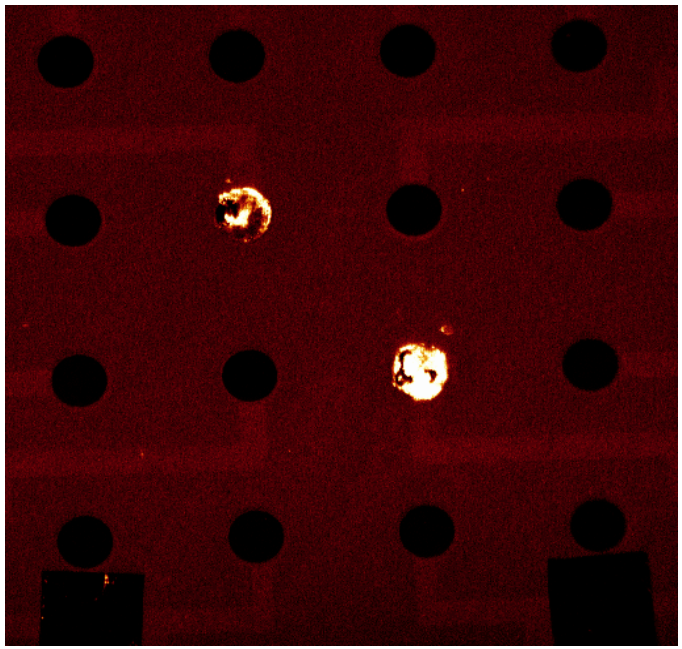



Simulated E-field

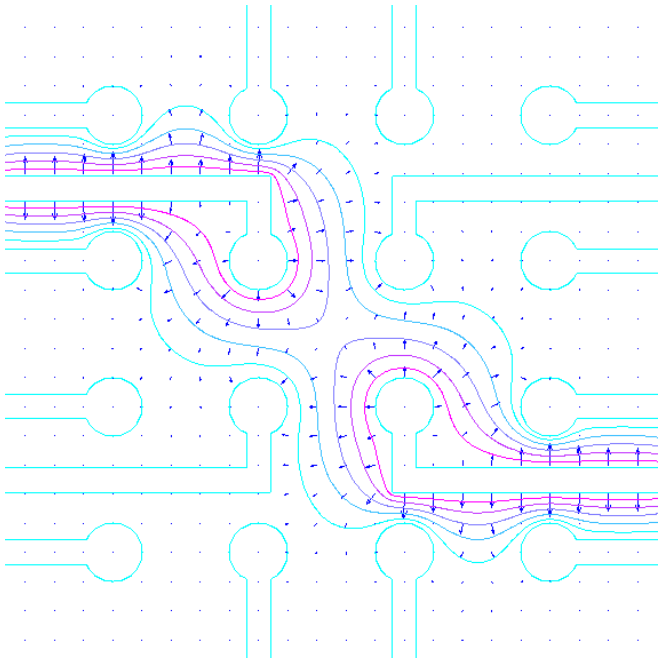


# Improved Electric Field Directed DNA

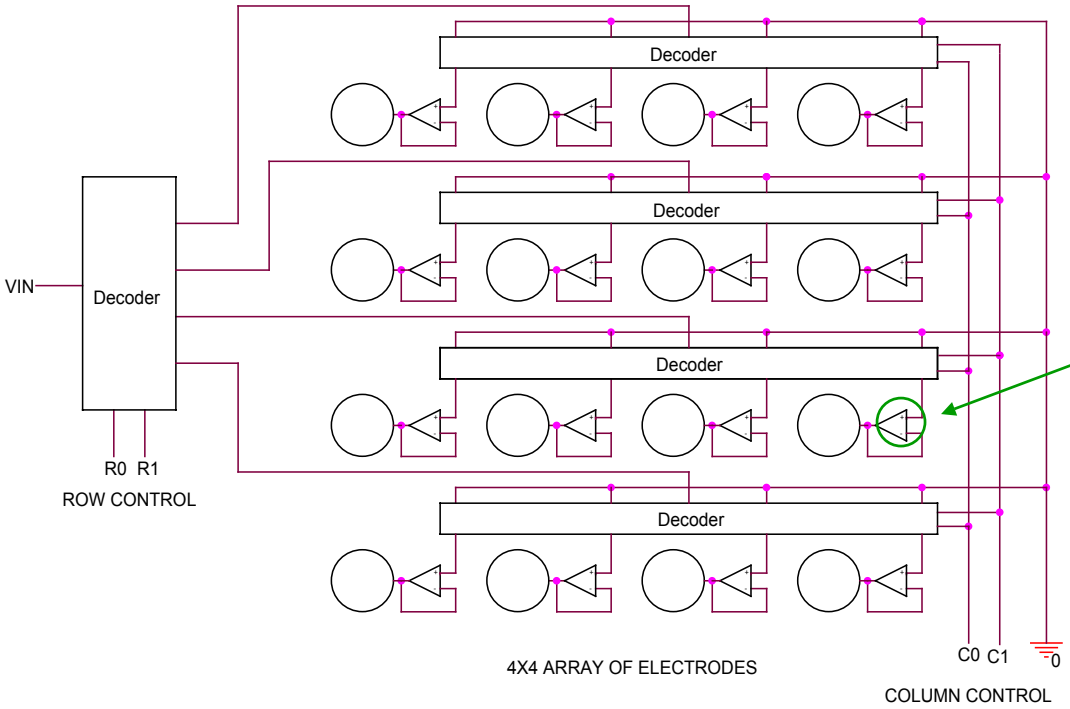
Confocal Image of bound DNA

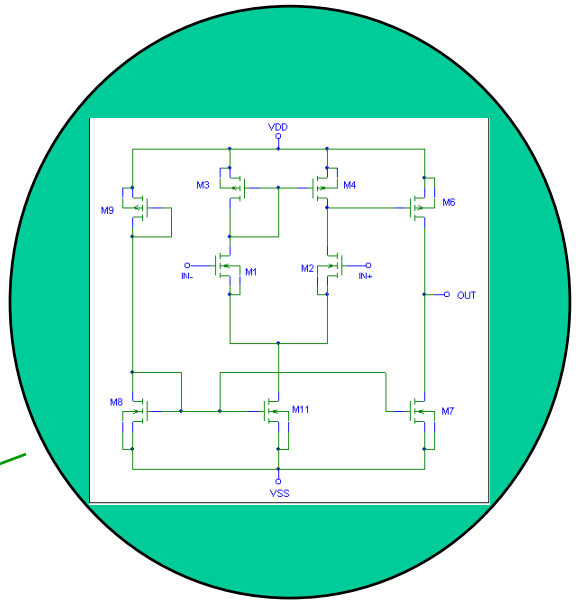


Simulated E-field



# Circuit Design for Programmable CMOS Chip



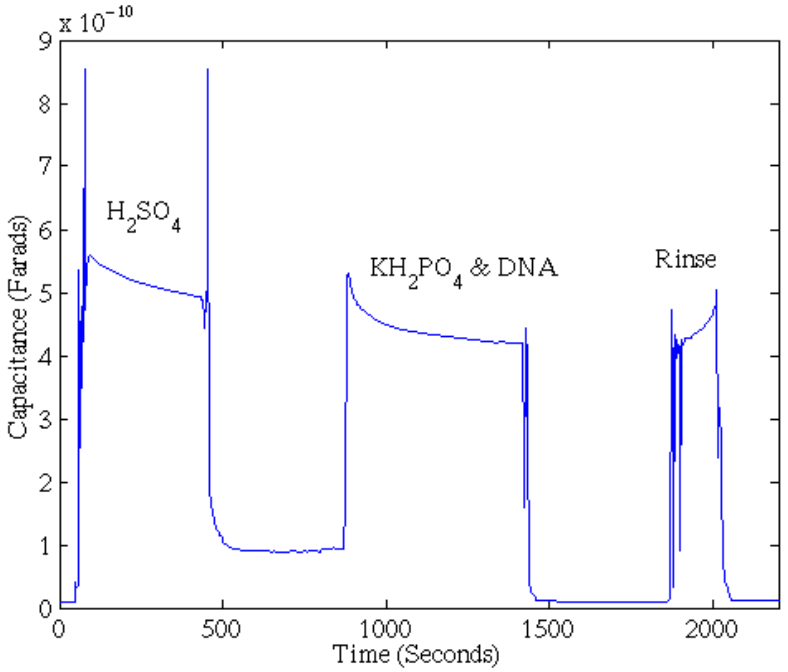

Pad Buffer Design

Simulation for 4x4 array
Actual array will be 8x8
Scalable to 1024x1024 and beyond

# Hybridization Site Preparation

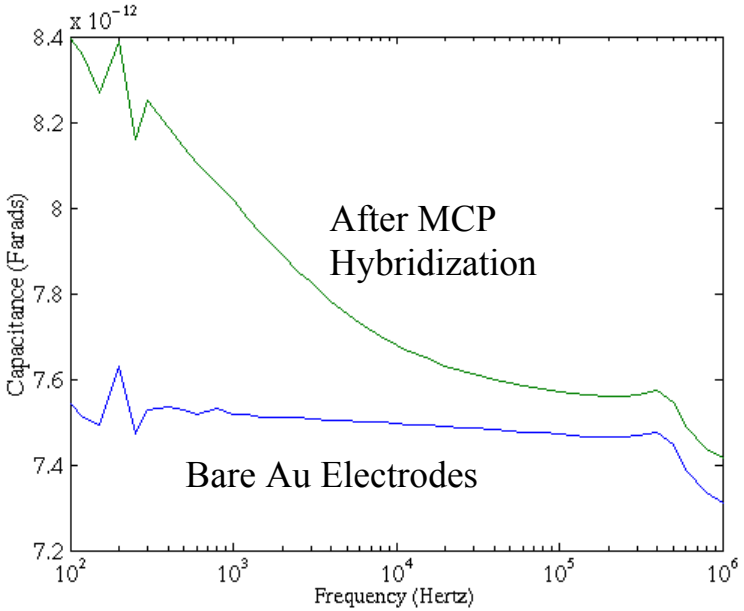
## Capacitance Measurements

- Measured capacitance is used to monitor oxide formation and surface conditions at hybridization site.
- Capacitance measurements have been used to determine surface coverage of single stranded DNA to levels of (1-10) x $10^{12}$ molecules/$cm^2$
  A. Steel, et. al. Anal. Chem. 1998 p.4670
- Human chronic gonadotropin hormone have detection limits of 15 x $10^{-15}$ M
  C. Berggren, and G. Johansson, Anal. Chem. 1997 p. 3651

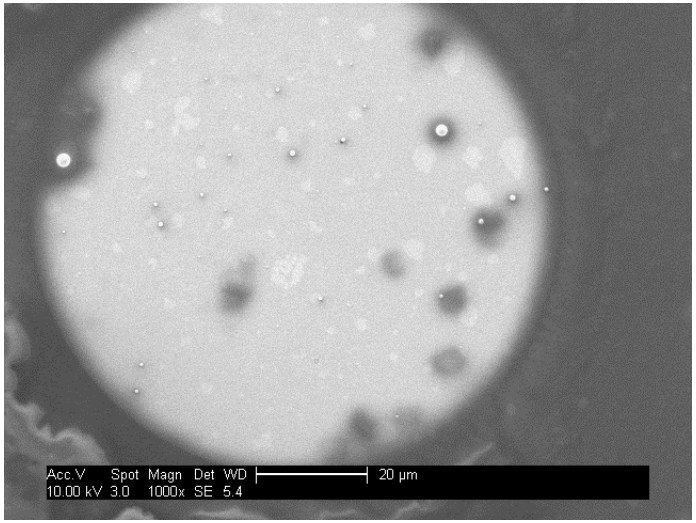


# MCP Coated Electrodes

## Capacitance Data

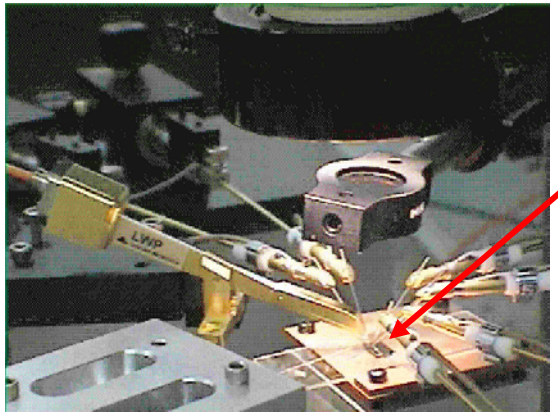



## Electron Microscope Image



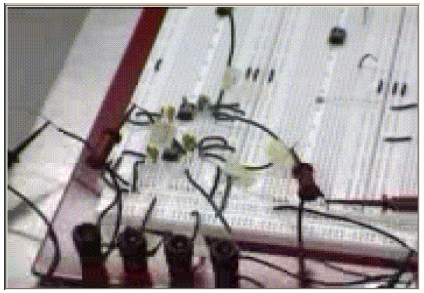
# Electro-Chemical Characterization

Silicon Test Chip Characterization

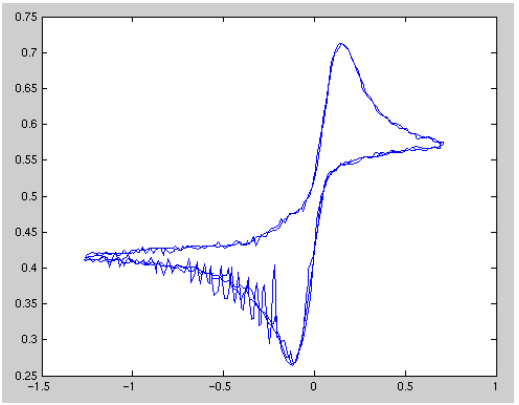


Silicon Chip

Breadboard Electronics



Cyclic Voltammetry

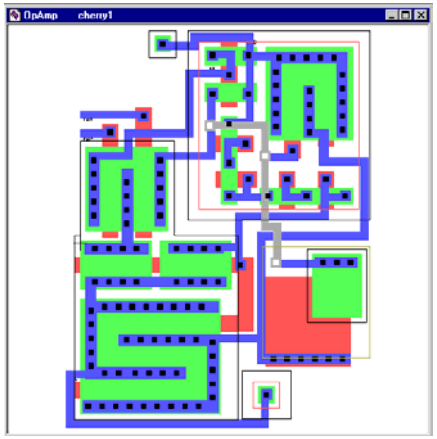


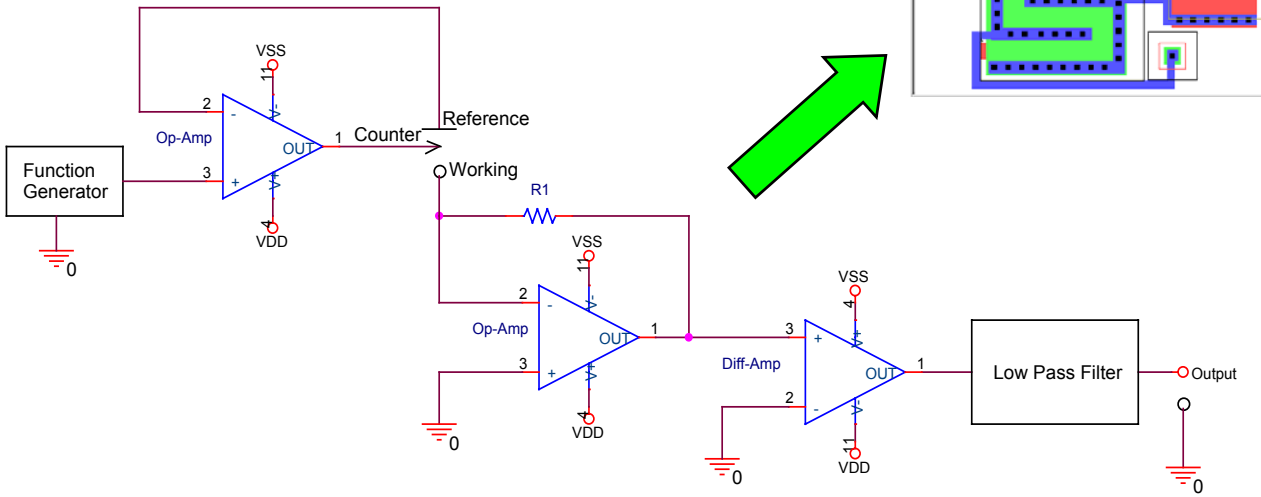
# Potentiostat on a Chip

## CMOS Circuit Design

- Cyclic Voltammetry
- Reduced Noise Due to Proximity of Electronics
- Parallel Chemical Sensors Achievable on Same Chip

## Transistor Layout

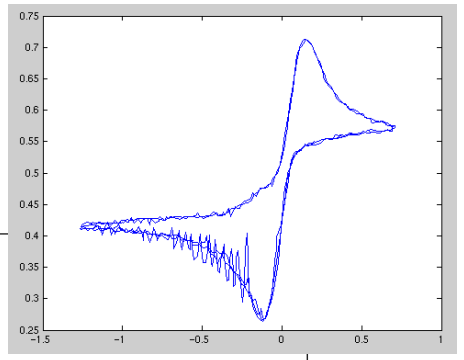


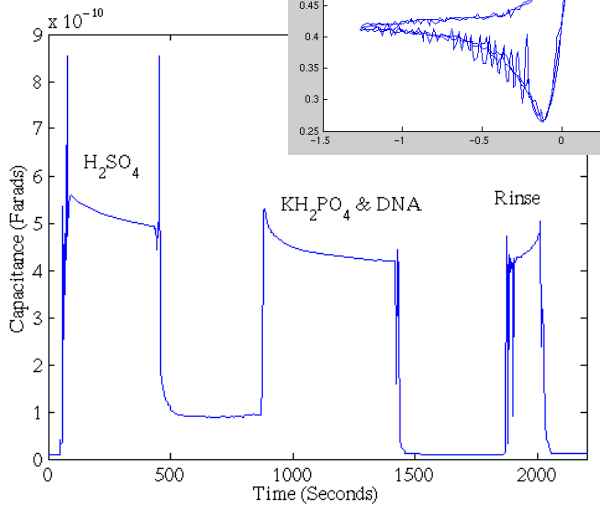



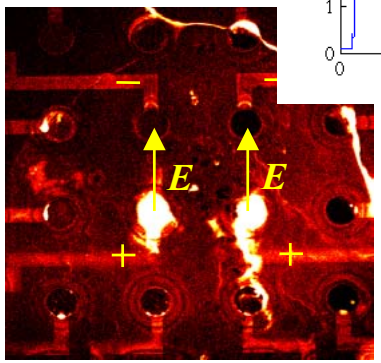
# Characterization

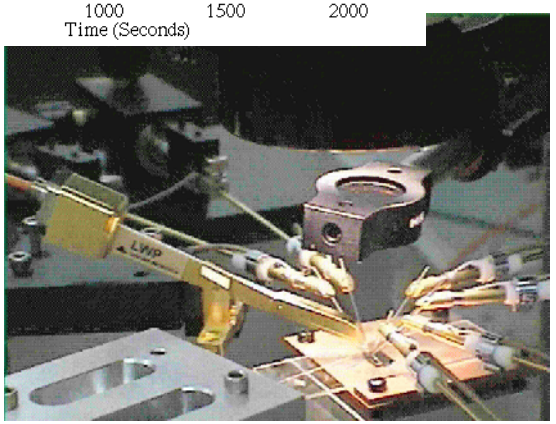
## Methods

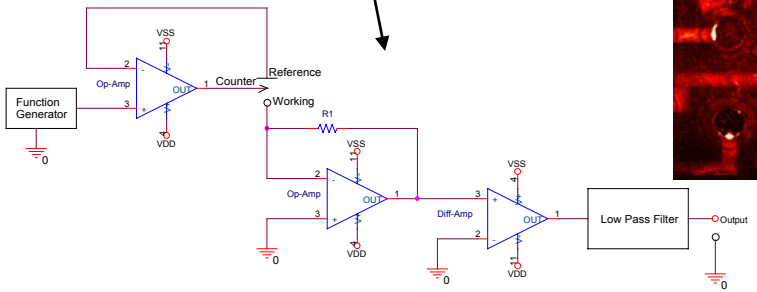
- Cyclic Voltammetry
- Capacitance Changes
- Fluorescence from Labeled DNA
- Confocal Microscopy
- Electron Microscopy
- CMOS Circuitry Design








Design of Programmable CMOS Chip

# Future Plans

**Next year plan:**

•Determine optimal chemistries and protocols for attaching DNA to electrodes

•Produce CMOS chips with circuitry for on-chip electrochemical analysis

•Quantify DNA with biochemical assays and optical and electrochemical techniques

•Continue work on electrical addressing of DNA to specified electrodes

**Long-Term Plans:**

•Low-cost sensors for use by chemical suppliers (responsible for starting feed materials ) and process engineers and ESH professionals (responsible for evaluation of new chemistries during and after the processing cycle)

•New generation of highly selective and inexpensive sensors for real-time and online monitoring in the manufacturing site.