

bioMS for Cardiac Markers

iSens: A PoC for Cardiac Markers

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for the
iSens Group, IIT Bombay

NPSM funded

Faculty Involved - IITB

- Prof. R.Lal, EE
- Prof. P.R.Apte, EE
- Prof. R.Pinto, EE
- Prof. R.O.Dusane, ME&MS (HWCVD)
- Prof. S.Mukherji, Bio-school (Immobilization)
- Prof. A. Q. Contractor, Chemistry (Conducting polymers)
- Prof. P.Gandhi, Mech. Engg (Cantilever design/characterization)
- Prof. S.Duttgupta, EE
- Prof. V.Ramgopal Rao, EE



Outline

- Motivation
- Project Goals
- Prototype
- ✓ Sensor architectures and structures
 - Affinity cantilevers
 - EIS capacitors
 - Conducting polymer devices
- Summary
- Inter-disciplinary research: our experience

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Motivation

- Inexpensive, smart, rugged sensors that can be used outside laboratories.
- Should be relevant to present ground realities in India.
- Changing lifestyles have made South Asians susceptible to cardiac dysfunctions such as Acute Myocardial Infarction.
- Damage to an area of heart muscle occurs due to inadequate supply of oxygen to that area.
- Diagnosis of AMI done through various clinical and pathological tests (e.g. ECG, CPK-MB levels, Troponin I & T levels, myoglobin, etc.).

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Project Goals

- Ultimate goal of project is to Design & Prototype iSens: A Point of Care Assay System for Molecular Markers for Myocardial Infarction
- Would involve:
 - Design & prototype of processes
 - Design & prototype of structures
 - Integration of system for quick assay of cardiac markers
 - Testing of the devices and systems at various stages of the development
 - Overall protocol testing
- Timeframe:
 - By middle of 2005

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Molecular Markers

- Enzymatic markers
 - Creatine kinase-Total (CK)
 - Creatine kinase MB (CK-MB) ✓
 - Lactate dehydrogenase (LDH)
 - Aspartate aminotransferase (AST)
 - Glycogen phosphorylase isoenzyme BB (GPBB)
- Protein molecules
 - Troponin
 - Myoglobin ✓
- Cell-free ladder DNA fragments

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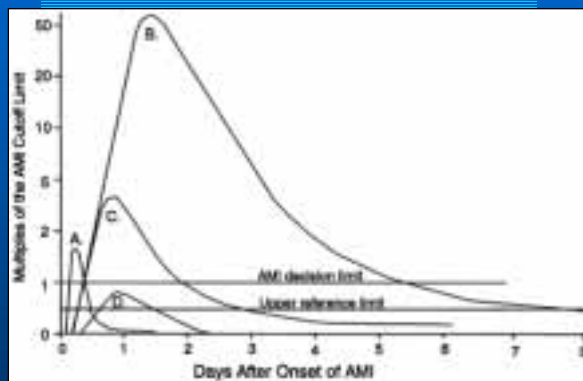
Molecular Markers: Issues

- Concentration & change of concentration across individuals
- Specificity
- Temporal response
- Ease of designing assay protocols
- Protocol robustness
 - Of the marker for the dysfunction
 - Of the sensor for the marker
 - Of the sensor system, including reagents

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Temporal Variations of Markers



From the users viewpoint:

- The marker suite should cover early and late detection
- Should permit tracking multiple attacks
- Should be acceptable to the Indian physician and yet be contemporary
- Should be affordable & easy to use & robust

A: early release of myoglobin or CKMB isoforms after AMI;
B: cardiac troponin after AMI; C: CK-MB after AMI;
D: cardiac troponin after unstable angina.

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Conventional Approaches

- Sequential assays for enzymatic activity
- Immunoassays:
 - ELISA (Enzyme Linked ImmunoSorbant Assay)
 - Radioimmunoassay
 - Immunoprecipitation
- Chromatography (!)
- Electrophoresis for separating isomers and then looking for activity (for lab (skilled labor, time consuming))

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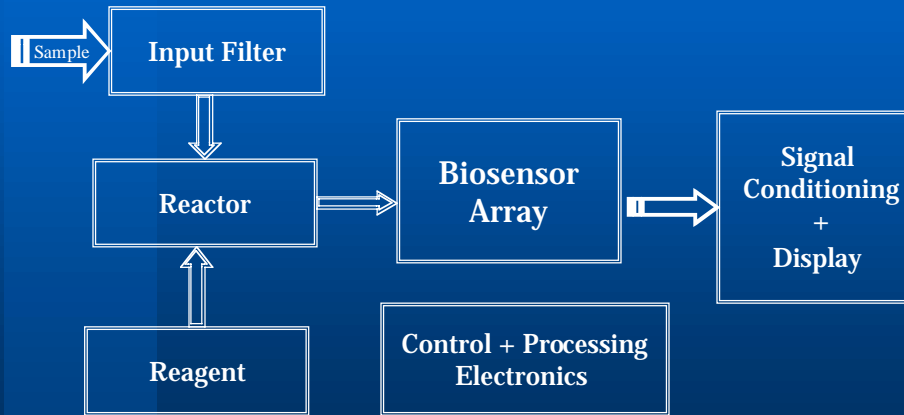
Our approach

- Biosensor array based or microfabricated sequential assay systems
- General approach is to use affinity sensors
- Use direct affinity sensors:
 - Affinity cantilevers
 - EIS capacitors
 - Conducting polymer devices

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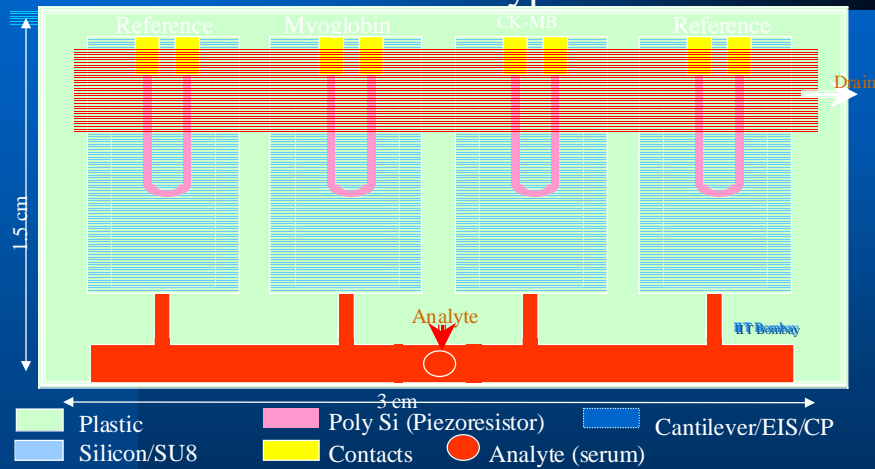
Functional Architecture



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iSens Prototype



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Biosensors to be used in our system

- Affinity micro-cantilevers
- EIS capacitors as affinity and catalytic biosensors
- Conducting polymer FETs as affinity & catalytic biosensors

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Sub-system Options: Filter

- External Filter
- Filter on cover glass
- Filter in nitride/oxide
- Filter in polysilicon
- Filter in silicon

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Biosensors to be used in our system

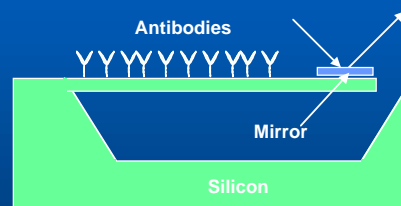
- Affinity micro-cantilevers
- EIS capacitors as affinity and catalytic biosensors
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Biosensor Array: Affinity Cantilevers-1

- Based on resonating structure Or deflection:
 - ⇒ Attachment changes mass or stiffness of resonating structure and thence resonant frequency
 - ⇒ Attachment changes the surface stress leading to a bending of the cantilever



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Biosensor Array: Affinity Cantilevers-2

- An important consideration: Asymmetric immobilization
- Silicon<!Too stiff unless SIMOX wafer used!>
 - Silicon/oxide/chrome-gold
 - Antibody on gold
- Polysilicon
 - Polysilicon/oxide/chrome-gold
 - Antibody on gold

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SU8

- **SU8 is negative, epoxy-type, near-UV photo-resist**
- **Aspect ratio larger than 1:50 (Typical 1:20)**
 - (Using MeV proton beam irradiation (instead of UV) the aspect ratio can go up to 100)
- **Possible to perform gray tone lithography**
- **Possible e-beam resist**
- **Strong Adhesion**
- **Biocompatible**
- **Strong Chemical resistance**

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Biosensor Array: Affinity Cantilevers-3

- Silicon Nitride
 - Nitride/polysilicon/nitride/chrome-gold
 - Antibody on gold
- SU-8
 - SU8/polysilicon/oxide/chrome-gold/antibody
 - SU8/ low-temp (<150°C) polysilicon/SU8/antibody*

* patented process



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Cantilever Design Issues

- Surface Forces are very small $\rightarrow \sim 2$ mN/m
 - When bio-molecules attach to antibodies on the cantilever
- Stresses result in $\Delta R/R$ of
 - 1×10^{-7} in Poly-Si cantilevers (.5 μm thick and 200 μm long)
 - 10×10^{-6} in SU-8 cantilevers (2.5 μm thick and 100 μm long)
- Deflections are of the order of
 - 10's nm in polysilicon cantilevers
 - 100's of nm in SU-8 cantilevers
- We are moving in the direction of
 - Nitride/polysilicon/nitride/chrome-gold
 - Su-8 with Poly-Si piezoresistors



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Affinity Cantilever Design Issues -2

- Simulations show that 'surface stress' is uniform – for the entire length of cantilever
 - A 'U-Bend' cantilever is preferred
 - Gives maximum $\Delta R/R$
 - Easy for making contacts to the piezoresistor

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Cantilever Layout Design

SEM microphotographs of Cr-Au on 1 μ m Oxide



U-bend Cantilevers – narrow and long



U-bend Cantilevers with mirrors for optical measurements

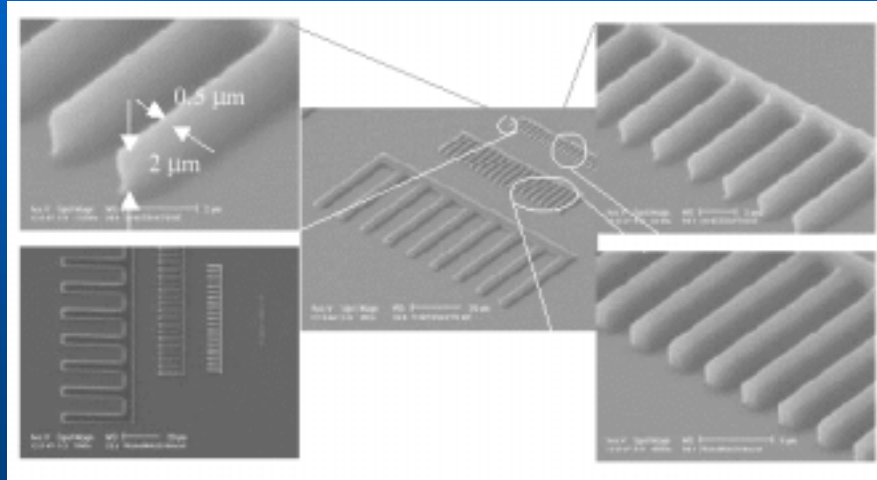


U-bend Cantilevers – wide and long

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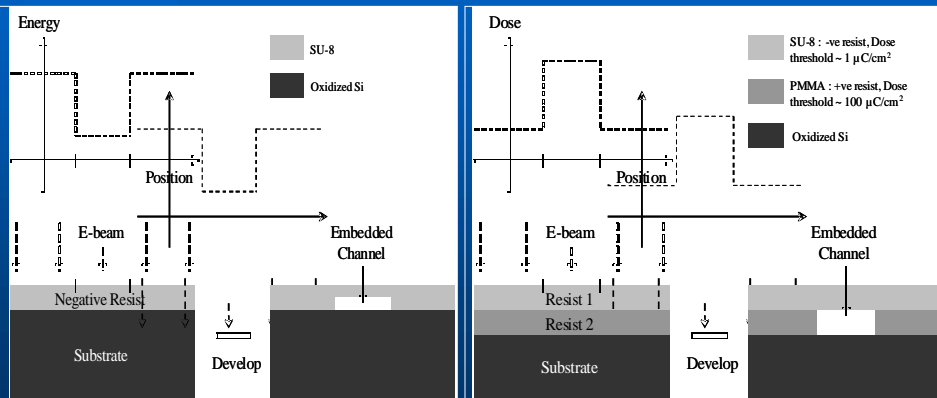
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E-beam Exposure of SU8



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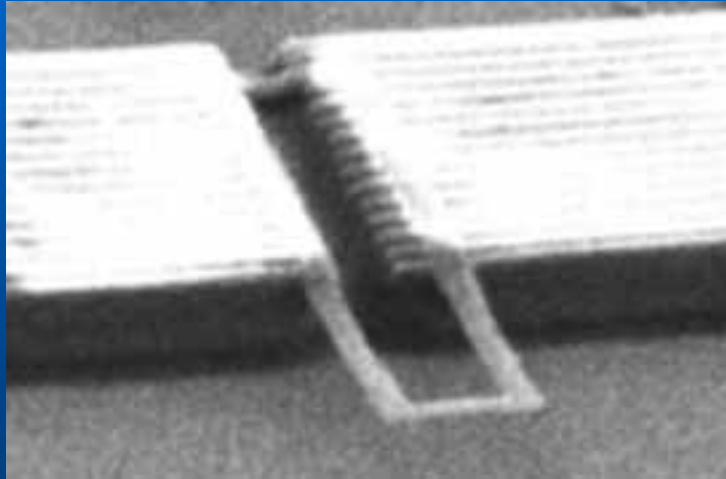
Novel approach for fabricating micro/nanofluidic systems -Electron Beam Lithography



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Cantilever: U-shaped SU-8 Structures

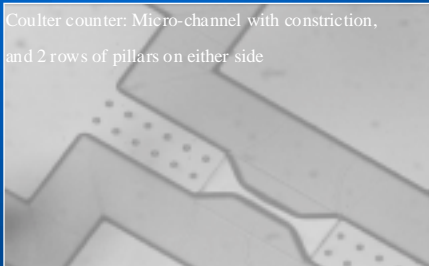


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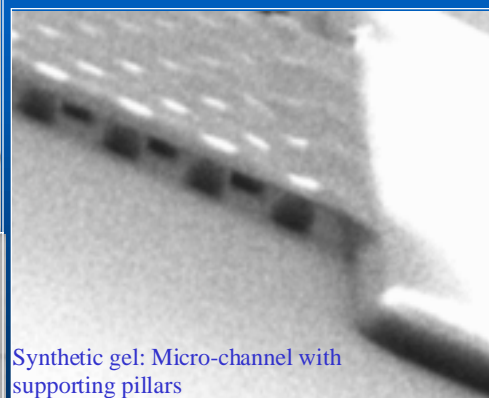
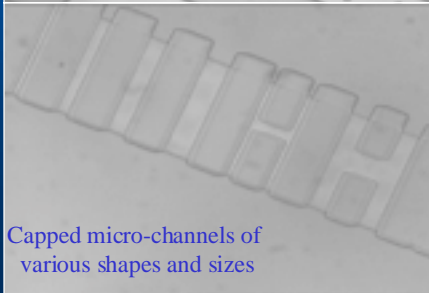
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Nanofluidic Systems - EBL

Coulter counter: Micro-channel with constriction, and 2 rows of pillars on either side



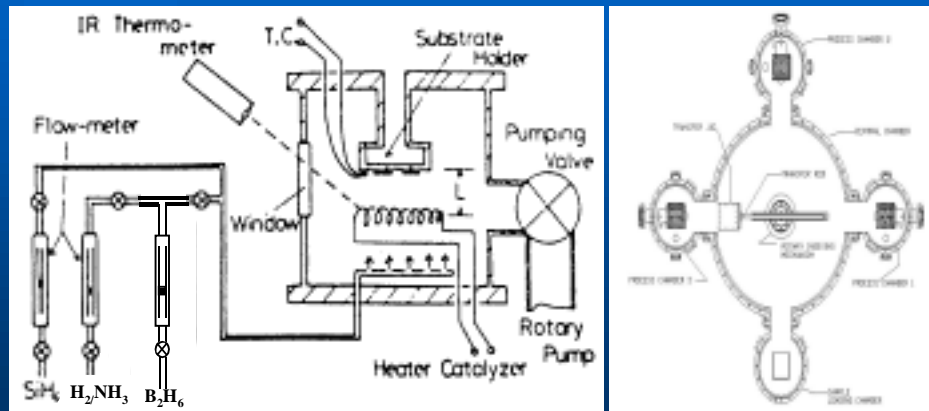
Capped micro-channels of various shapes and sizes



Synthetic gel: Micro-channel with supporting pillars

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Hot-Wire CVD for Low-Temperature poly/nitride Deposition – Cluster tool



Prof. R.O.Dusane, ME&MS

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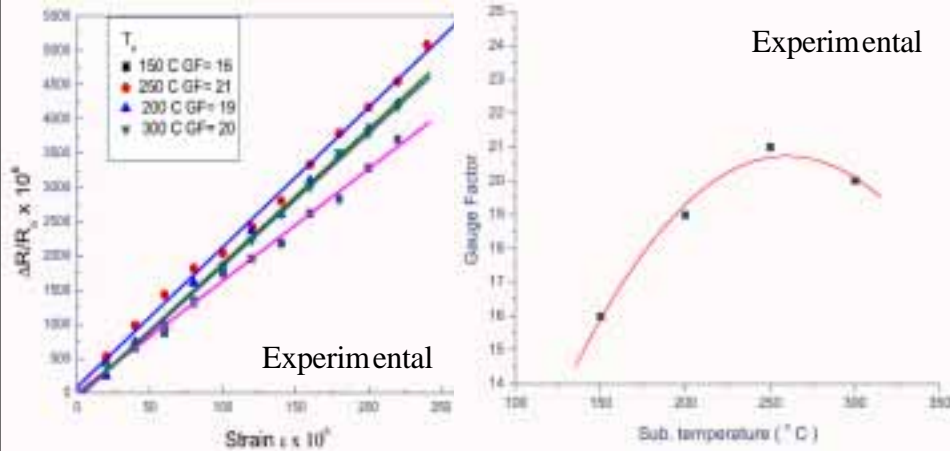
HWCVD Polysilicon as Piezoresistor

- Gauge Factor defined as
 - $G.F. = \frac{\Delta R}{R_0}$
- High gauge factor of polysilicon (K~30) as compared to that of metal (K~2)
- Gauge factor of polysilicon depends on
 - Grain size
 - Doping concentration
 - Doping type
 - Texture
- Above factors dependent upon hot-wire process parameters like substrate temperature, hydrogen dilution and boron flow

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Low-temp HWCVD piezo-poly for SU8/nitride Cantilevers



➤ HWCVD-Also for in-situ nitride-poly-nitride cantilever structures 

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Selective Immobilization – Surface Treatment

- Wet processes: eg. Silanization
 - Damage to surface and structure
 - Long duration process
- Dry processes: Plasma, UV or Gamma ray
 - Damage
 - Storage: samples undergoing UV treatment
 - Safety requirements: Gamma ray treatment
- Surface modification and SU-8

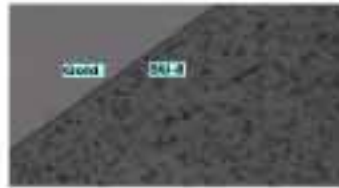


Immobilization chamber

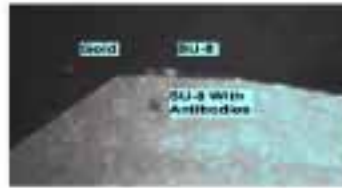
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Immobilization on SU8 after a dry surface treatment using HWCVD



Gold/SU-8 surface observed by Microscope under Halogen exposure



Gold/SU-8 surface observed by Fluorescence Microscope



Silicon/SU-8 surface observed by Microscope under Halogen exposure



Silicon/SU-8 surface observed by Fluorescence Microscope

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Biosensors to be used in system

- Affinity micro-cantilevers
- EIS capacitors as affinity and catalytic biosensors
- Conducting polymer FETs as affinity & catalytic biosensors

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Biosensor Array: ISFET & EIS Cap based

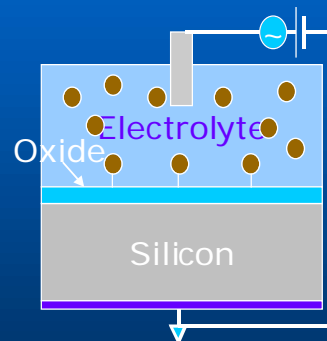
- Compatible with standard silicon fab, but for gate dielectric & immobilization
- Gate dielectric options
 - SiO₂/Si₃N₄
 - SiO₂/Al₂O₃
 - SiO₂/Si₃N₄/Tantalum oxide
 - Immobilize antibody on dielectric
- Gate Area Definition
 - Conventional Wet Oxide
 - HSQ
 - SU8

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Biosensor Array: Capacitive Affinity Sensor

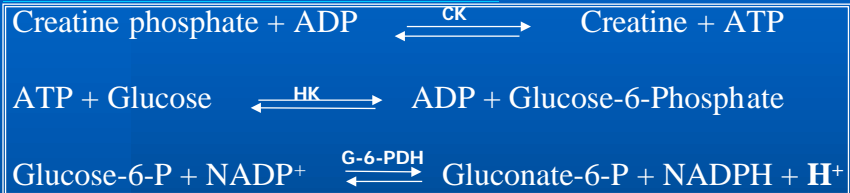
- Electrolyte-Insulator-Semiconductor (EIS) capacitive sensors most widely used
- Surface of dielectric on semiconductor modified by immobilizing molecules with affinity specific for analyte
- Attachment changes capacitance of EIS structure



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Possible assay approach

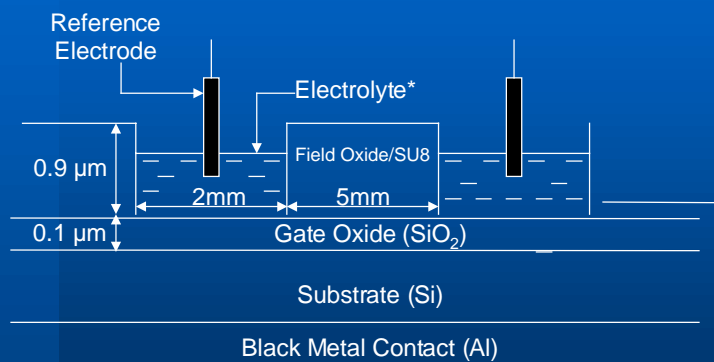


- Normally done in a buffered solution at a pH of 6.7
- Absence of buffer – EIS can be used (change in H^+)
- First time demonstration of EIS capacitors for CK-MB detection
- Enzymes are attached to the detection antibodies serving as labels
- Detection scheme is based on the variation in Capacitance of the EIS capacitor, due to the presence of Enzymes
- Change in capacitance is brought about by the products of a reaction that the enzyme catalyzes

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EIS Capacitor – Structure for test

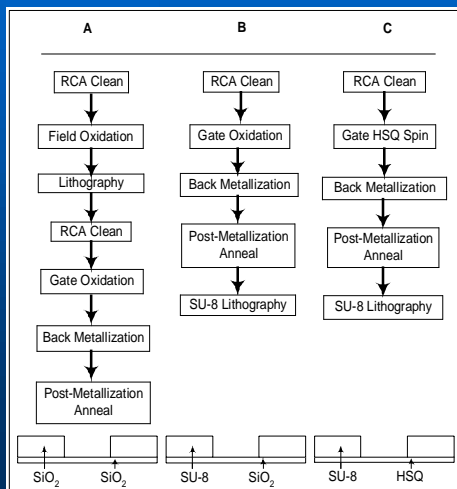


* Buffer used is PBS Sodium Chloride 137 mM, Potassium Chloride 2.7 mM and Potassium Phosphate 10mM; Antibody – 2 mg ml^{-1}

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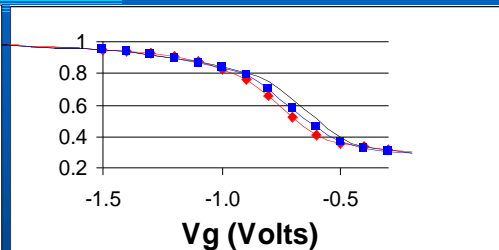
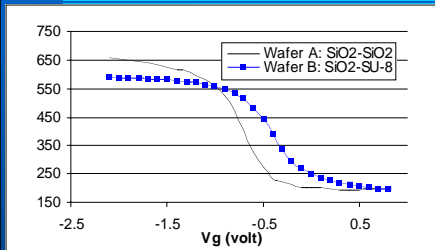
Low Temperature (Sub 200°C) EIS Process Flow for CK-MB



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EIS Capacitor Characterization



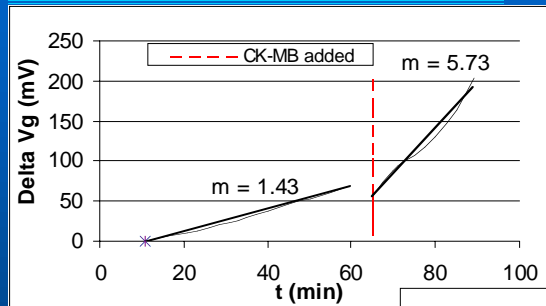
C/Cox	S(mV/pH) pH 7-6	S(mV/pH) pH 6-5	S(mV/pH) pH 5-4
0.4	182.87	52.48	65.98
0.5	185.94	44.48	71.84
0.6	187.48	47.02	70.16
0.7	194.55	44.80	68.97

Excellent pH sensitivity ~ 5 milli-pH

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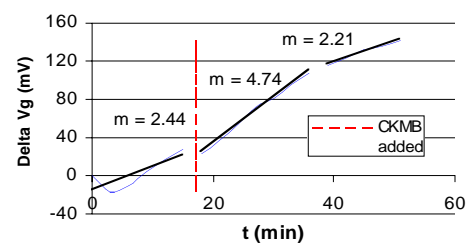
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Low temperature EIS Structures for CK-MB Detection



Change in slope before and after addition of $1\mu\text{l}$ CK-MB to $15\mu\text{l}$ of reagent (wet oxide wafer)

Change in slope before and after addition of serum, showing completion of reaction after about 20 minutes (SU8 wafer)



Biosensors to be used in our system

- Affinity micro-cantilevers
- EIS capacitors as affinity and catalytic biosensors
- Conducting polymer FETs as affinity & catalytic biosensors

BiosensorArray: Conducting polymer FETs-1

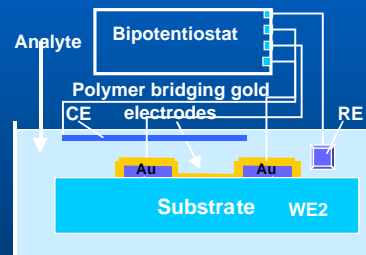
- Simplest structure: could be all made on glass or plastic
- Various conducting polymers could be used:
 - Polyaniline
 - Functionalized polymers
- Immobilize after formation of CP sensor
- Possible to have both affinity & catalytic biosensor
- Also explore an affinity switch

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BiosensorArray: Conducting polymer FETs-2

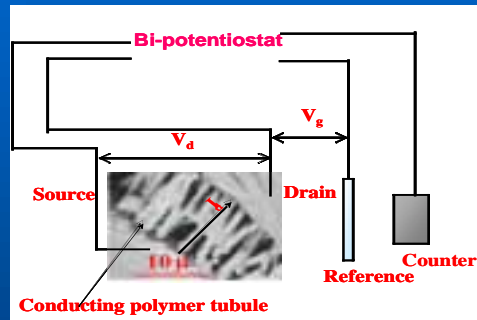
- Inter-digitated noble metal electrode covered with conducting polymer
- Conductance change occurs with change of state (such as change of pH in enzyme catalyzed or affinity reaction) that can be used for sensing



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Conducting Polymer based CK-MB Sensor



Prof. Contractor's
Lab, Chemistry

- Electrochemical deposition of Polyamine on Au coated isoporous polycarbonate membrane
- Potentiodynamic/potentiostatic immobilization of CK-MB reagent for 30 min on polyaniline
- Measurement of conductance before and after addition of CK-MB control.

S.No.	Before addition of CK-MB	After addition of CK-MB	Sensor response Ag/g _m
1.	$1.477 \times 10^{-2} \text{ S}$	$4.567 \times 10^{-2} \text{ S}$	2.10
2.	$2.279 \times 10^{-2} \text{ S}$	$4.009 \times 10^{-2} \text{ S}$	0.77

Fluidics & Pump

- External
- Hydrostatic drive
- Diaphragm pump

Will be done on plastic and/or glass; not being currently investigated.

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Processes & Systems

- Micro-fabrication
- HWCVD system for polysilicon/nitride
- Immobilization
- Characterization systems
- Sensor electronics (table-top system)

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Proposed Technology

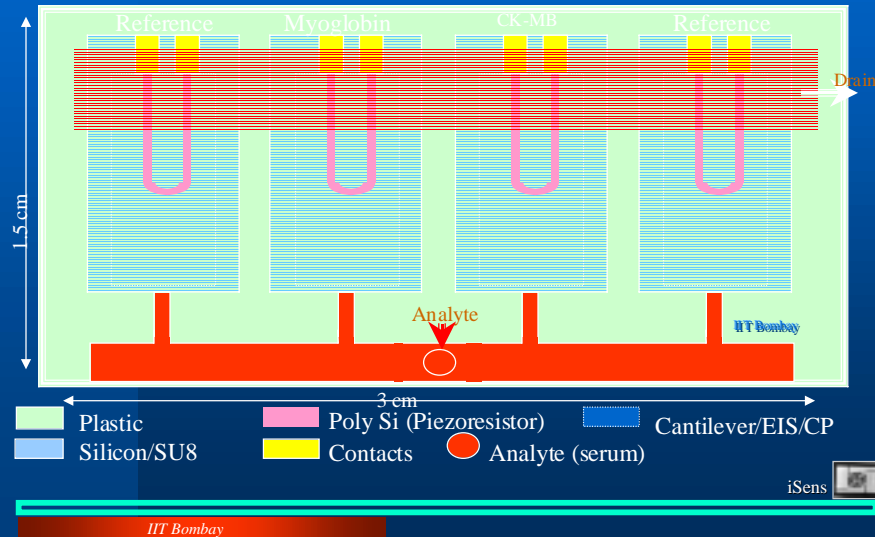
- iSens technology has the following pros & cons
 - Benefits: Simple procedure, low cost, fast response
 - Disadvantages: Unproven new technology
- New international standards being adopted have been kept in mind but need to be tried out for the Indian context
- Exciting inter-disciplinary research in EE

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iSens Prototype

Confidential



Other research activities

- Molecular Electronics – Porphyrin based
- Bio-MEMS and its integration with CMOS
- Novel device Architectures (Vertical MOSFETs, EJMOS, DTMOS)
- SH/DH MOSFETs
- Mixed signal CMOS device design for SoC applications
- Esaki Tunnel FETs
- New Materials (HWCVD Poly, High-K, nitride etc.)
- Plasma Implantation induced damage
- Technology-Circuit interaction

<http://www.ee.iitb.ac.in/~microel>

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Inter-disciplinary Research

+^s

- Students gain valuable experience, gained a mutual respect for the knowledge, skills, abilities and viewpoints of the other disciplines
- Possible to explore new areas which would have been difficult within a department - after all, nature is interdisciplinary
- Possible to build socially relevant systems with such collaborations

-^s

- Put in a lot of time building trust, especially since individuals are bringing their own jargon to the table.
- There are often differences of perceptions and approaches to problem solving that can cause problems (Science-Engineering)
- Faculty seem to feel they will not be given "due credit" for participating in interdisciplinary research programs. To a large extent it seems that senior faculty or faculty comfortable with their academic careers are willing to participate in interdisciplinary research, while junior faculty focus their work in discipline-based areas. The system does not provide a measure for interdisciplinary contributions. Peer review processes sometimes also frowned at new interface areas
- Students are unwilling to take up new areas because of lack of job markets in new areas (particularly true for India)
- The participant in interdisciplinary research must have a willingness to collaborate and be willing to give up some control.

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