Medical Diagnostics Technologies Based on BioMEMS

~ Painless One-Step Blood Testing ~

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Contents

- BioMEMS
- Silicon Microneedles and Microprobes
- Reliable Painless Sampling Devices
- Point-of-Care Testing and Optimal POCT Technique
- Biosensors of Blood Glucose, Lactate, and Alcohol
- Biochip Platforms for Measurement of Proteins and Activity of Enzymes

Kumetrix's Core Technology

- MEMS (Micro-Electro-Mechanical Systems): silicon microneedles, silicon microprobes, microfluidics-enabled chips (lab-on-a-chip)
- Bioassays: medical and toxic exposure diagnostics based on biosensors and/or biochips
- Instrumentation: electronics, device packaging, software, algorithm, data handling
- Point-of-Care Testing or Self-Testing systems

What is BioMEMS?

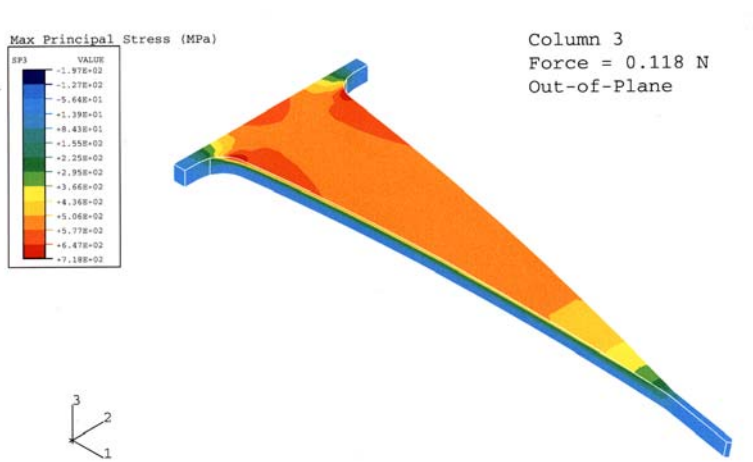
- MEMS technology used to design and fabricate medical devices (e.g., microbiosensors, biochips).
- A versatile platform to make biodiagnostic systems for performing automatic, fast, accurate, cost-effective and user-friendly assays (no skill required), particularly for point-of-care testing and self-testing.
- Integration of multidisciplinary, state-of-the-art technologies, involving physical, chemical, biological, mathematical, computational sciences, and mechanic /electronic engineering principles to study bioscientific events.

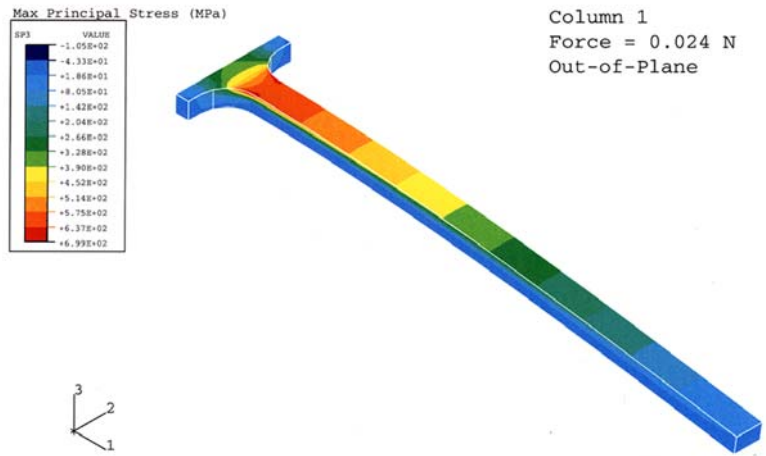
MEMS: Silicon as a Mechanical Material

Kurt. E. Petersen, Proceedings of IEEE, Vol. 70 420-457 (1982)

- Silicon is abundant, inexpensive, and of high purity and perfection
- Silicon processing is highly amenable to miniaturization
- Photolithographic patterning allows for rapid evaluation of design ideas
- Batch-fabrication results in high volume manufacturing at low unit cost
- Silicon is also a biocompatible material (essential for blood testing)

Design Consideration and Element Analysis





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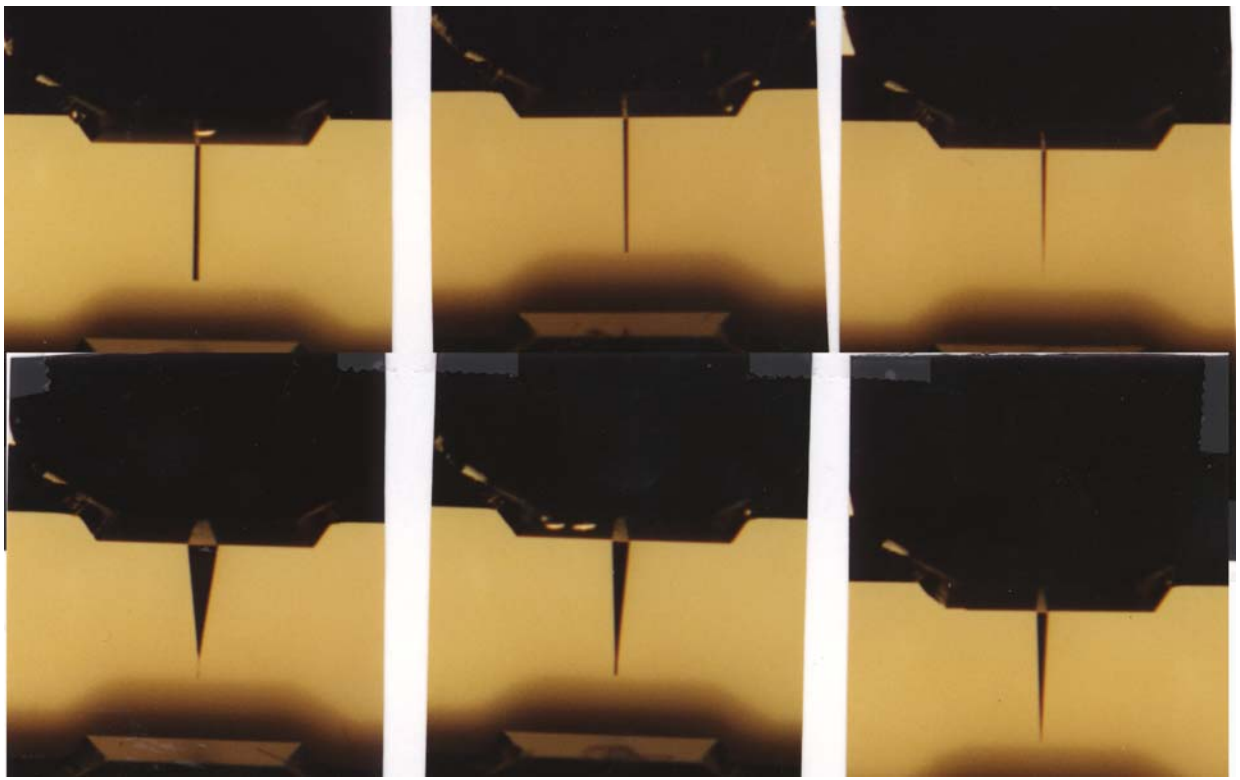
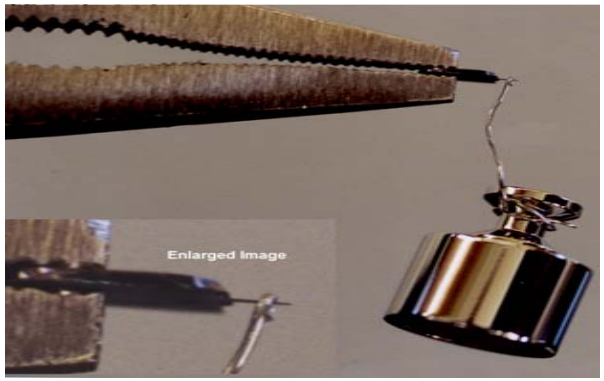
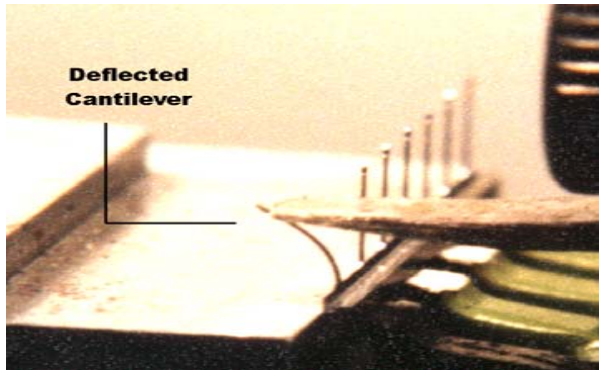
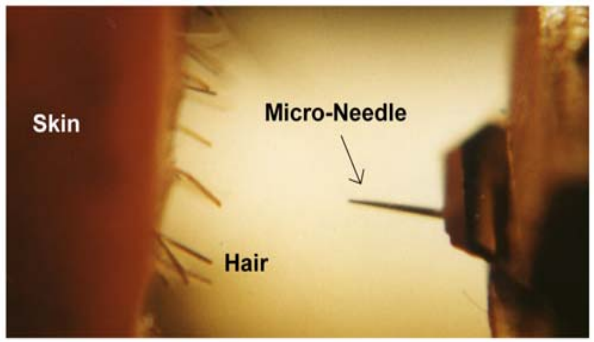
Needle Shapes and Sizes



Tough, Flexible Needles Puncture Skin Effortlessly

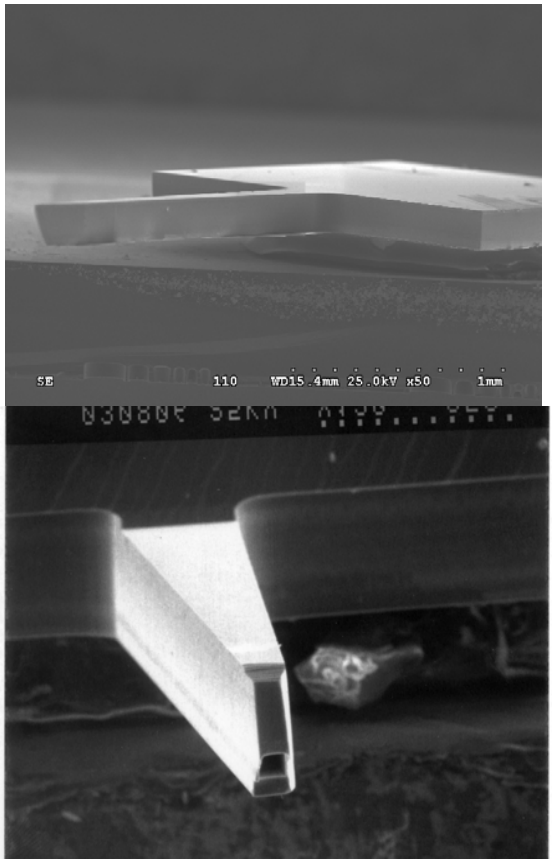






Silicon microneedles, pioneered by Kumetrix, which are comparable in cross-section to a human hair, yet strong enough to penetrate human skin without breakage.

Proven Microneedle Capability



Scanning Electron Micrographs (SEMS)

Alcatel 601-E etcher
>1 million microneedle chips annually

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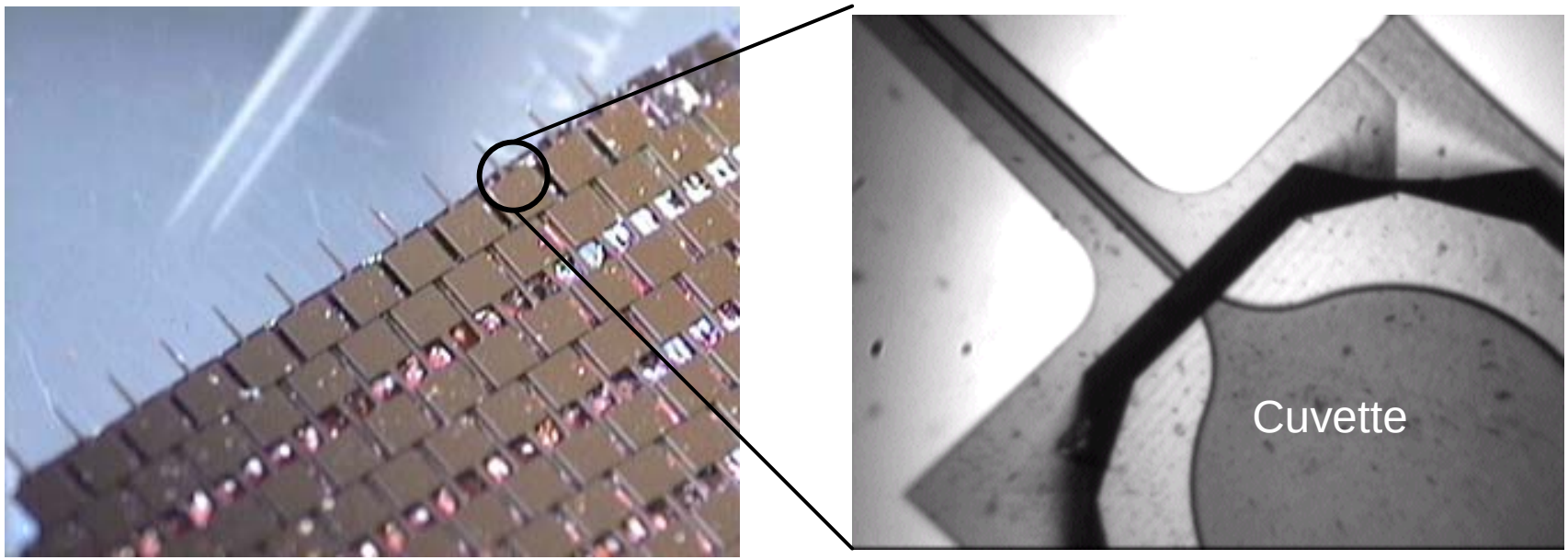
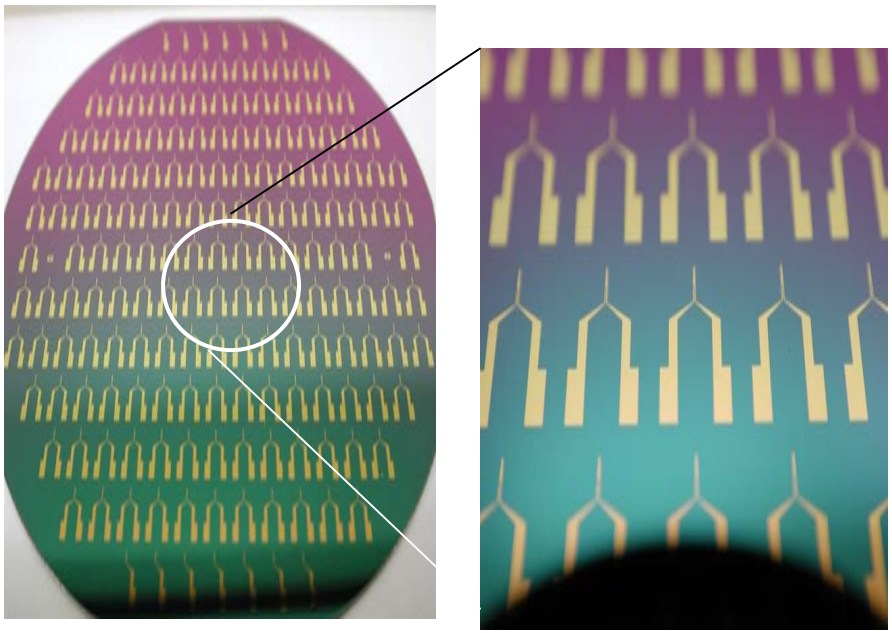
Wafer-Level Fabrication Disposables



Silicon Microprobes

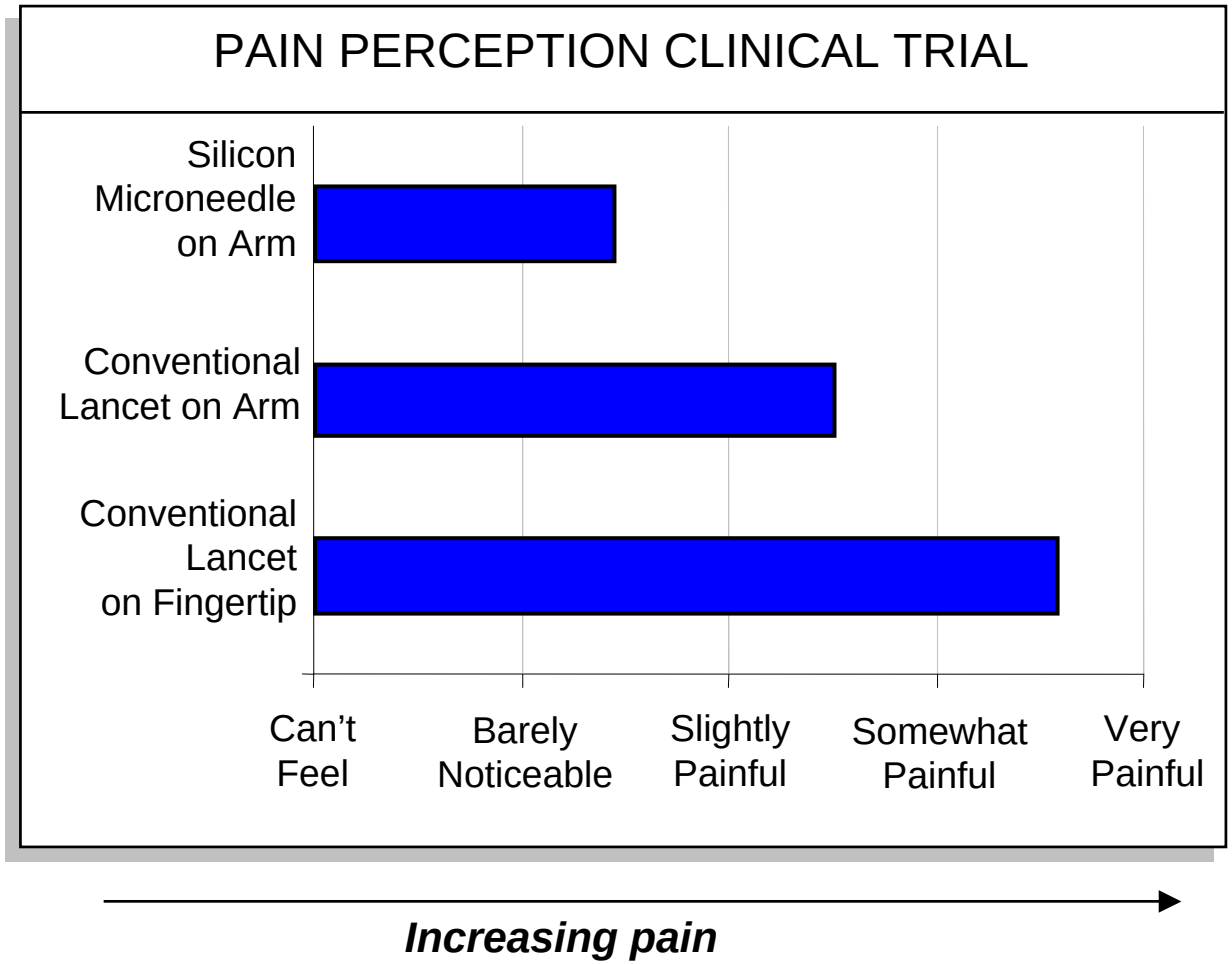


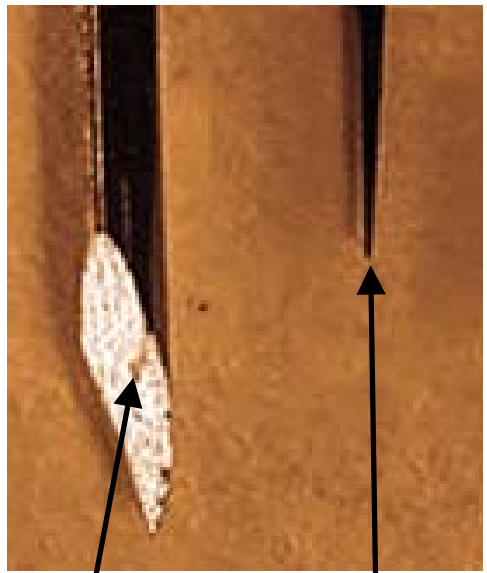
Probe-shaped electrodes at wafer-level

Finished Strip of Microprobes

Painfree Silicon Microneedles

Life saving technology: painless blood testing





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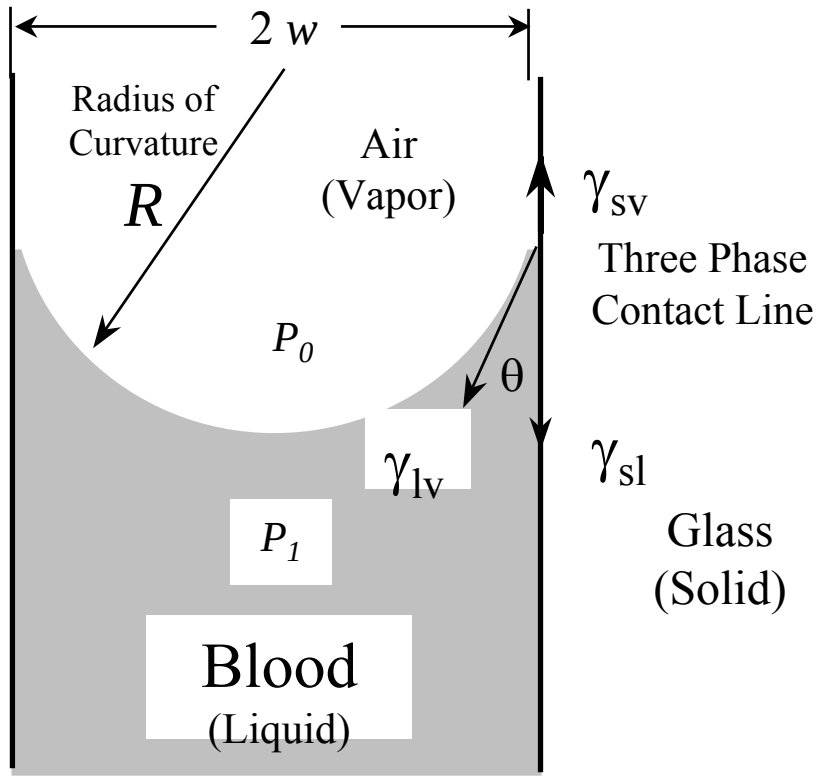
Microfluidic Design Criteria

- Completely fill cuvette
  - Uniformly distribute blood
  - Eliminate air pockets
- Use smallest required volume
- Optimize time to fill

Dimension of Circular Ducts

$$\gamma_{sv} = \gamma_{sl} + \gamma_{lv}\cos\theta$$

$$\Delta P = P_1 - P_0 = \frac{2\gamma_{lv}\cos\theta}{R}$$

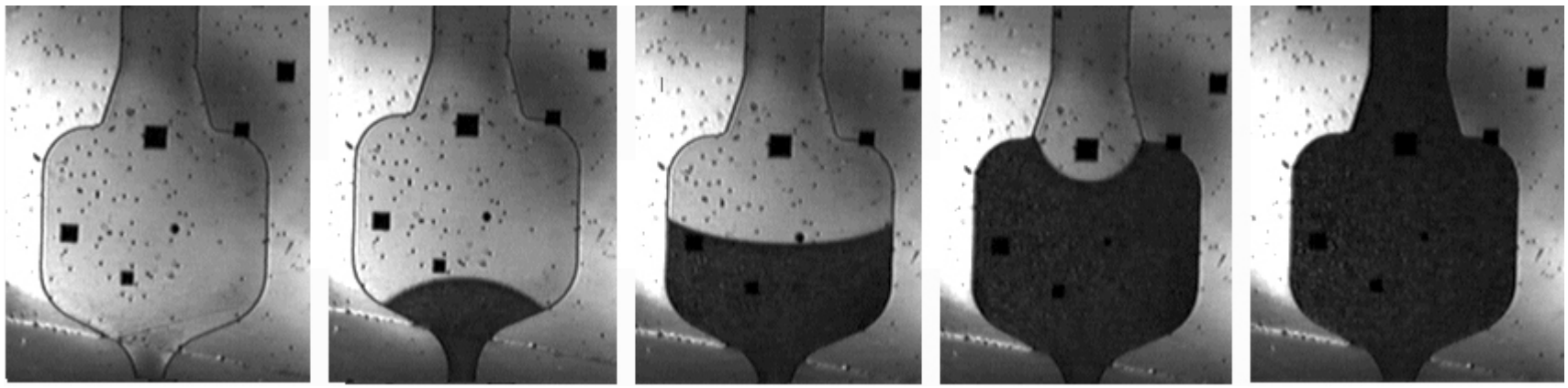


Viscous Flow Through Circular Ducts

$$Q = \frac{\Delta P \, \pi R^4}{8 \, \mu L}$$

Q	flow rate
ΔP	pressure drop
R	radius of duct
μ	fluid viscosity
L	length of duct

Microcuvette Filling with Blood in < 1 Second



200 nanoliter microcuvette

Result: Reliable Painless Sampling

- Alternative sites such as arms have fewer nerve endings per square inch than the fingertips, thus resulting in less pain. However, only submicroliter blood can be reliably drawn from these sites.
- Submicroliter blood transfer into a test strip is a big problem because of requirement for good coordination and eyesight which diabetics typically lack.
- Kumetrix's human hair-sized microneedle allows submicroliter blood to be drawn painlessly, automatically and reliably into an on-chip microcuvette where the assay performs immediately. One step, no manual blood transfer!

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Ideal for Point-of-Care Testing

- No risk of sample loss, or degradation
- Real-time results for rapid assessment of patient status
- Immediate impact on therapeutics/patient care
- Allows time-critical preparation / life-saving treatment
- Personalized medical management
- More frequent, less expensive testing - positive impact on public health
- Healthcare costs reduced via diagnostics or self-monitoring without professional involvement
- Inexpensive, portable, and no skill required testing--controlling regional epidemics and preventing national or global pandemics (e.g. avian flu)

Electrochemical Technique: Optimal for Integration with Point-of-Care

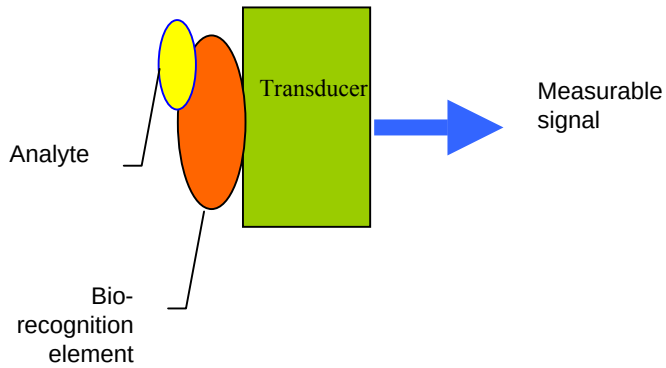
Electrochemical detection characterization:

- High sensitivity (independent of sample volume)
- Excellent selectivity via integration with biorecognition elements
- Independence from turbidity and optical path length
- Picoliter or nanoliter sample requirement (beneficial to seniors and babies or painless alternative site testing)
- Direct, fast and real-time measurement (no separation need)
- Various readout signals: current, potential (voltage), conductance, impedance
- Low cost, particularly in mass-scale fabrication -- allowing disposable consumables (e.g. blood glucose test strips)
- Inherent miniaturization allowing integration with modern microfabrication technologies (e.g.bioMEMS), and with portable readout meters (simple / inexpensive device)

Electrochemical device is superior to optical system because of higher sensitivity, lower power consumption, less sample requirement, and no alignment need; potent capabilities for rapid monitoring of various biological species (e.g. bacteria, viruses, DNA, proteins, small molecules) in the field / at office or home.

What is a Biosensor?

- A biosensor is a bioanalytical device incorporating a biological material or a biomimic (e.g. enzymes, antibodies, nucleic acids, tissue, microorganisms, organelles, cell receptors) integrated within a physicochemical transducer or transducing microsystem
- Output may be optical, electrochemical, thermometric, piezoelectric, or magnetic.

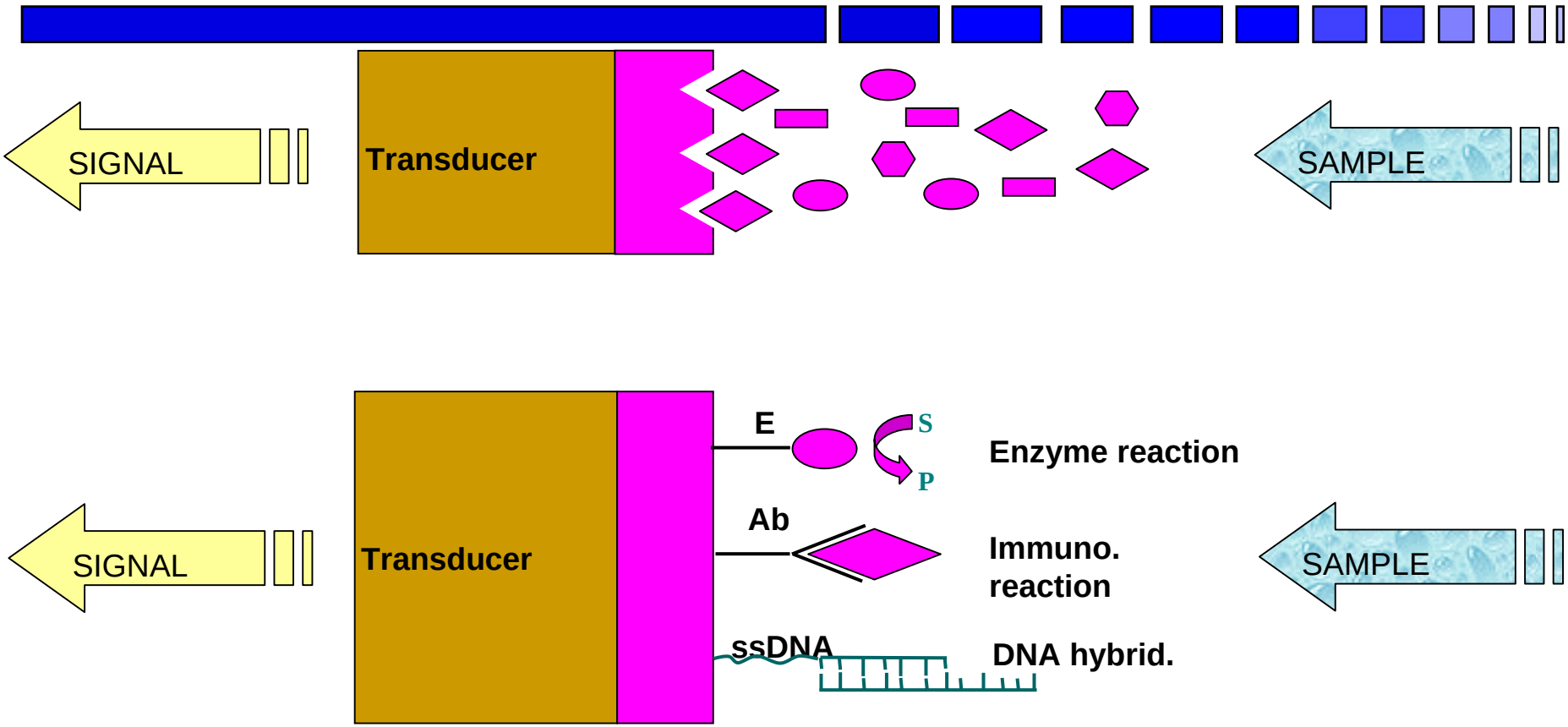


Single-element biosensor containing biorecognition element, transducer, and output

Important biosensor attributes: sensitivity, specificity, simplicity, and continuous monitoring capability

Biosensor Specificity:

Coupling Biorecognition / Transduction Subsystems



Excellent selectivity guarantees the measured signal results from the analyte of interest.
Biosensors provide the best tool for point-of-care monitoring, due to their high specificity/sensitivity, fast readout, portability, and low cost (disposable consumables).

Electrochemical Biosensor Design

- Mediated Biosensors
  - Advantages:

    Reduced interference by lowering operational potential, minimal oxygen dependence, increased signal density via mediator
  - Disadvantages:

    Mediator leakage (toxicity), long-term stability issues
- Non-Mediated Biosensor Integrated with Modified Film Catalyst

  No leakage, reduced interference due to lower applied potential, minimal oxygen dependence with advanced membrane technologies, significantly increased signal density via catalyst

Glucose Biosensor: Working Principle

The prosthetic group (FAD) of glucose oxidase (GOx, EC 1.1.3.4) is reduced by glucose yielding gluconate; the reduced form ($FADH_2$) is then reoxidized by either oxygen or an electron transfer mediator (M^+).

- The regeneration of active GOx by oxygen is shown below:

$$\text{Glucose} + \text{GOx(FAD)} \longrightarrow \text{Gluconate} + \text{GOx}(FADH_2)$$

$$\text{GOx}(FADH_2) + O_2 \longrightarrow \text{GOx(FAD)} + H_2O_2$$

$$2\,H_2O_2 \longrightarrow O_2 + 2\,H_2O + 2\,e^-$$

- The mediator-based system is exhibited:

$$\text{Glucose} + \text{GOx(FAD)} \longrightarrow \text{Gluconate} + \text{GOx}(FADH_2)$$

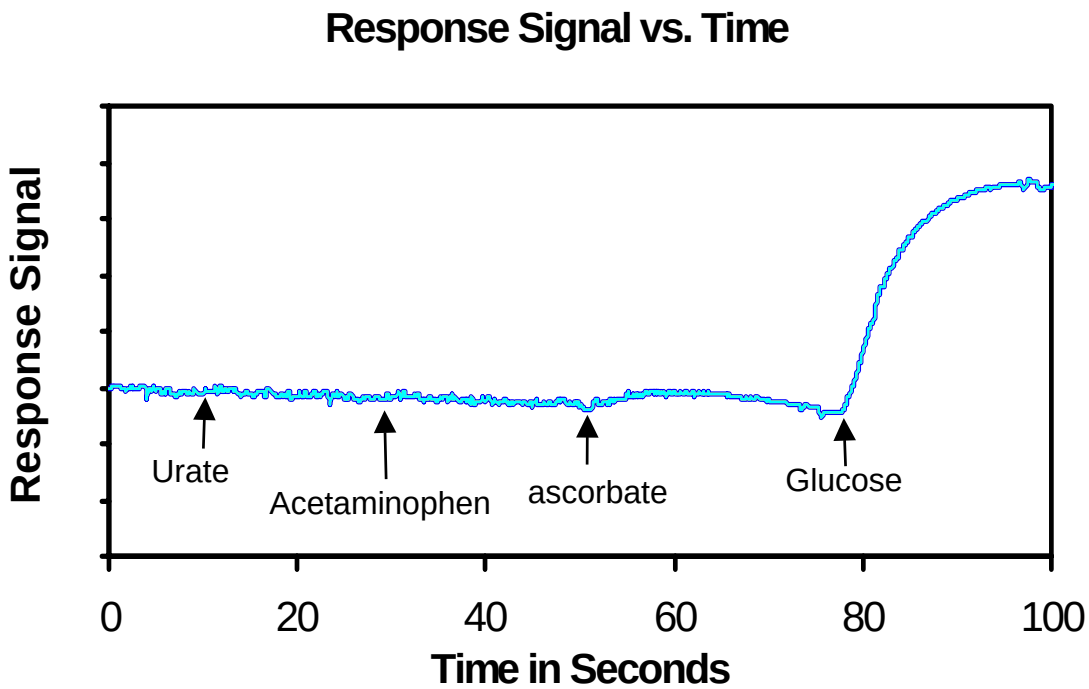
$$\text{GOx}(FADH_2) + 2M^+ \longrightarrow \text{GOx(FAD)} + 2M + 2H^+$$

$$2M \longrightarrow 2M^+ + 2\,e^-$$

Glucose Biosensor:
Trends in Glucose Self-Testing

- No Pain
- Alternative Site Testing
- Sub-Microliter Sample Volume
- No Manual Blood Transfer
- Readout in Less Than Five Seconds
- Immunity to Interferents

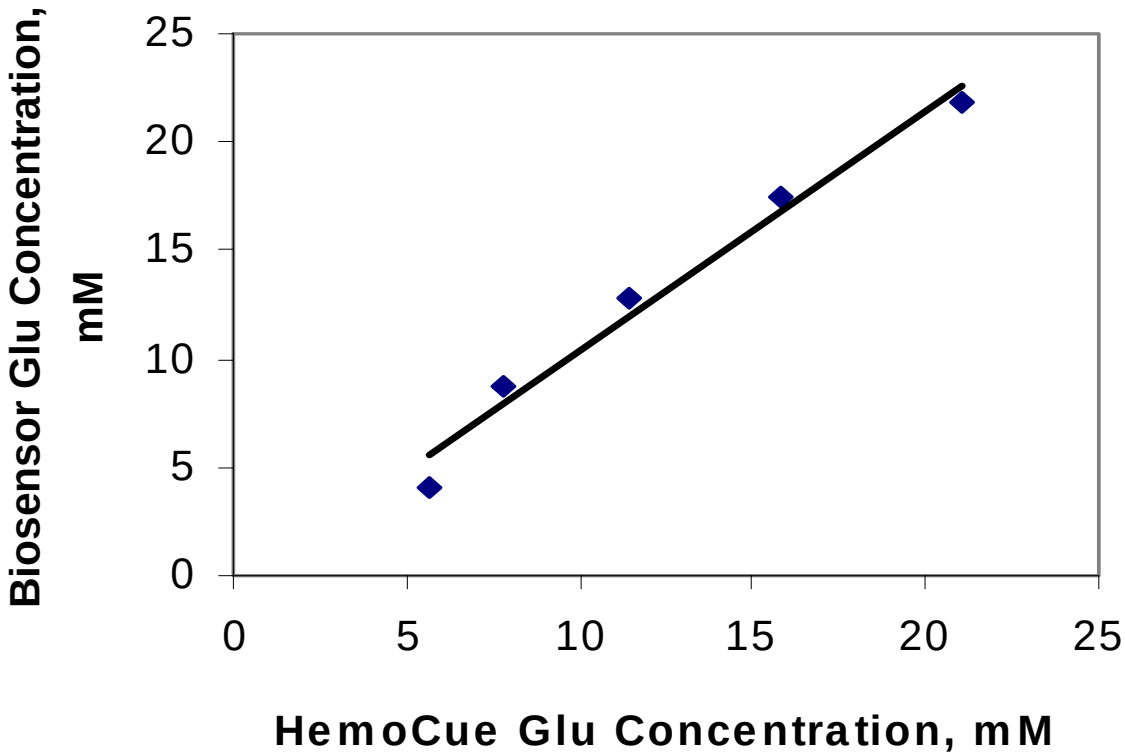
Glucose Biosensor: Specificity



Immunity to common interferents in blood: ascorbate, urate, and acetaminophen

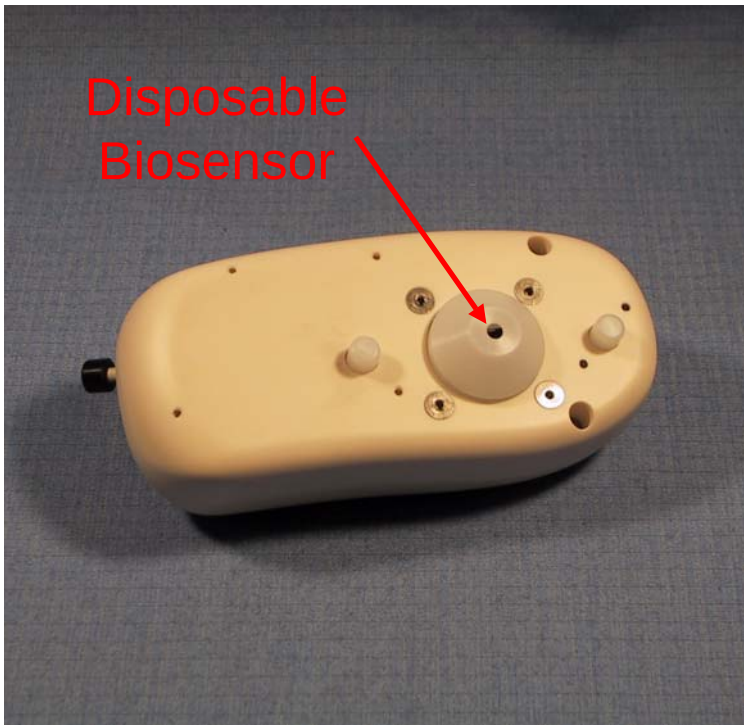
Glucose Biosensor Accuracy:

Glucose Biosensor Output vs HemoCue Output



Single-Use Biosensor

In Situ Self-Testing for Blood Analytes



Lactate Biosensor: Working Principle

- **Lactate Oxidase (LOx, EC 1.1.3.2)**

$$\text{L-lactate} + O_2 \xrightarrow{\text{LOx}} \text{Pyruvate} + H_2O_2$$

$$H_2O_2 \xrightarrow{\text{Anode}} O_2 + 2H^+ + 2e^-$$

- **Lactic Dehydrogenase (EC 1.1.2.3) (cytochrome b2)**

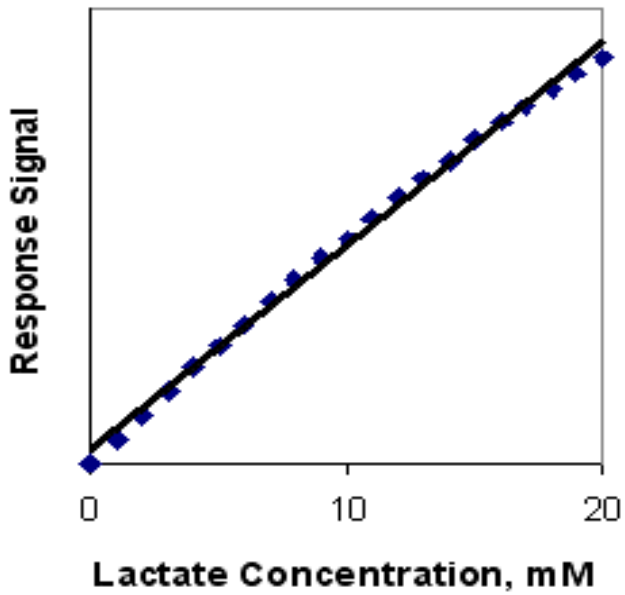
$$\text{Lactate} + 2\,M^+ \xrightarrow{\text{cytochrome b2}} \text{Pyruvate} + 2\,M + 2H^+$$

$$2\,M \xrightarrow{\text{Anode}} 2M^+ + 2e^-$$

Lactate is the most reliable indicator for resuscitation from shock and ischemia.

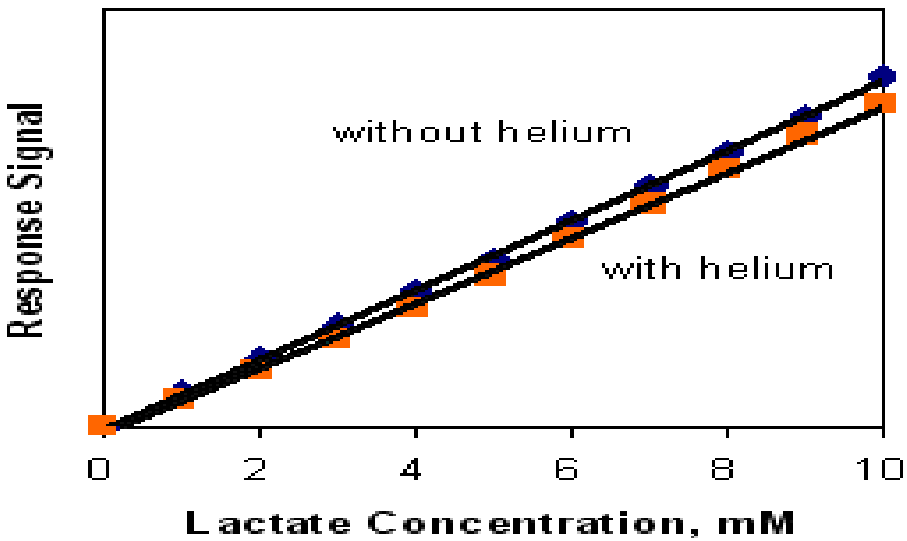
Lactate Biosensor:
Wide Linearity, High Specificity

Linear range over 0-20 mM (up to 30 mM, recently developed)



No interference from ascorbate, acetaminophen and urate interference

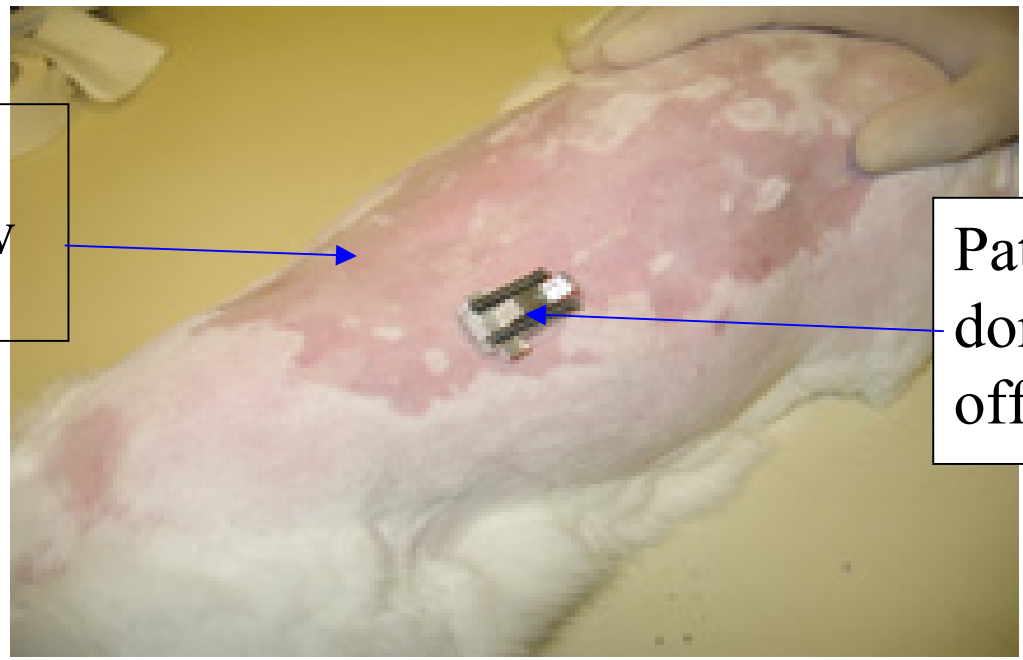
Lactate Biosensor:
Oxygen Independence



Oxygen independence is necessary to allow biosensor accurate continuous monitoring of tissue/blood lactate because body oxygen level is varied at different sites.

Continuous Biosensor
In Vivo Monitoring of Blood Analytes

Testing rabbit shaved to allow adhesion



Patch placed on dorsal surface, off-center

Lactate Biosensor:
In Vivo Continuous Monitoring of Lactate

Alcohol Biosensor:
Working Principle

Alcohol Oxidase (AOx, EC 1.1.3.13)

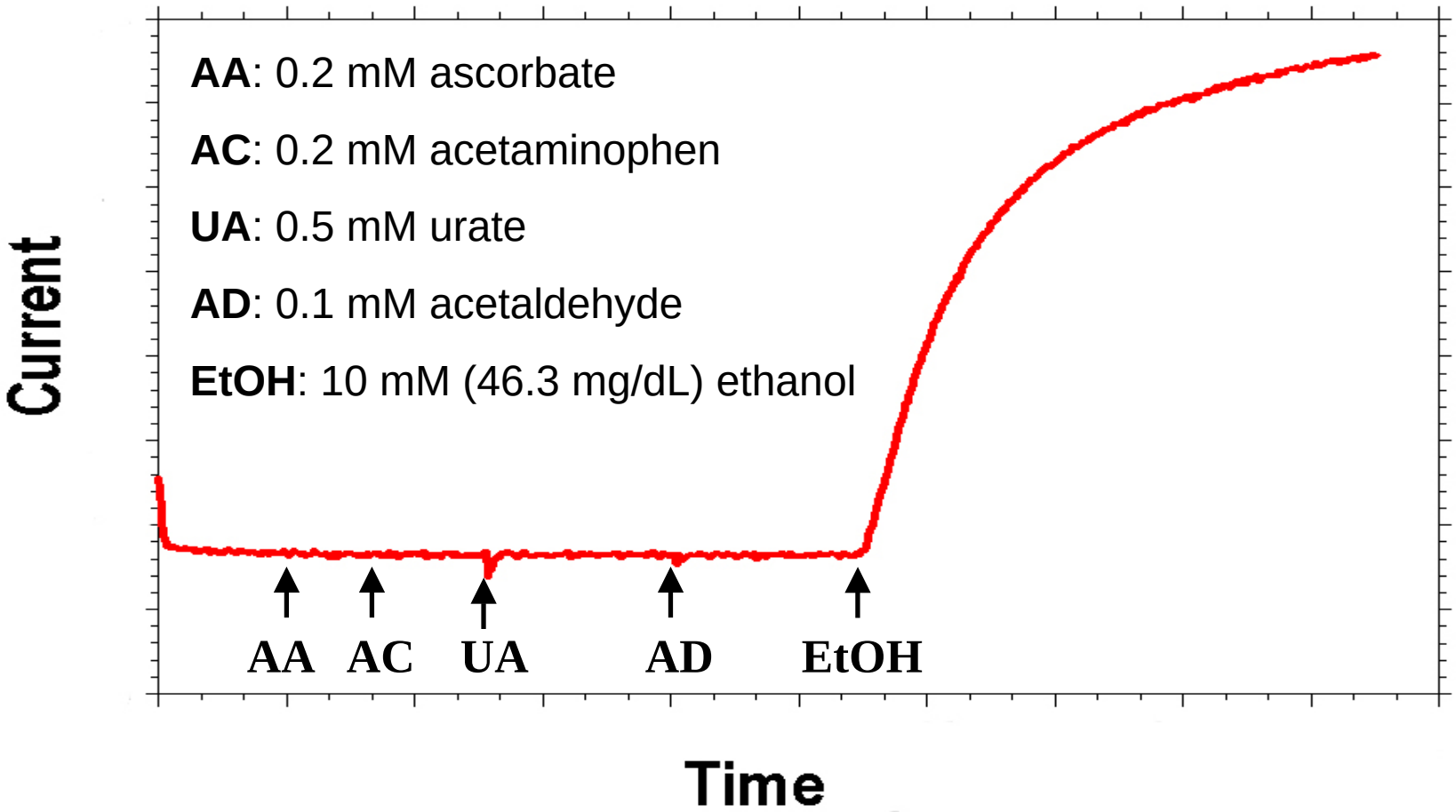
$$\text{Ethanol} + O_2 \xrightarrow{AOx} \text{Acetaldehyde} + H_2O_2$$

Anode

$$H_2O_2 \longrightarrow O_2 + 2H^+ + 2e^-$$

An alcohol breath analyzer directly measures breath alcohol and converts it into blood alcohol concentration, with undependable readings.

Alcohol Biosensor:
Interference Elimination



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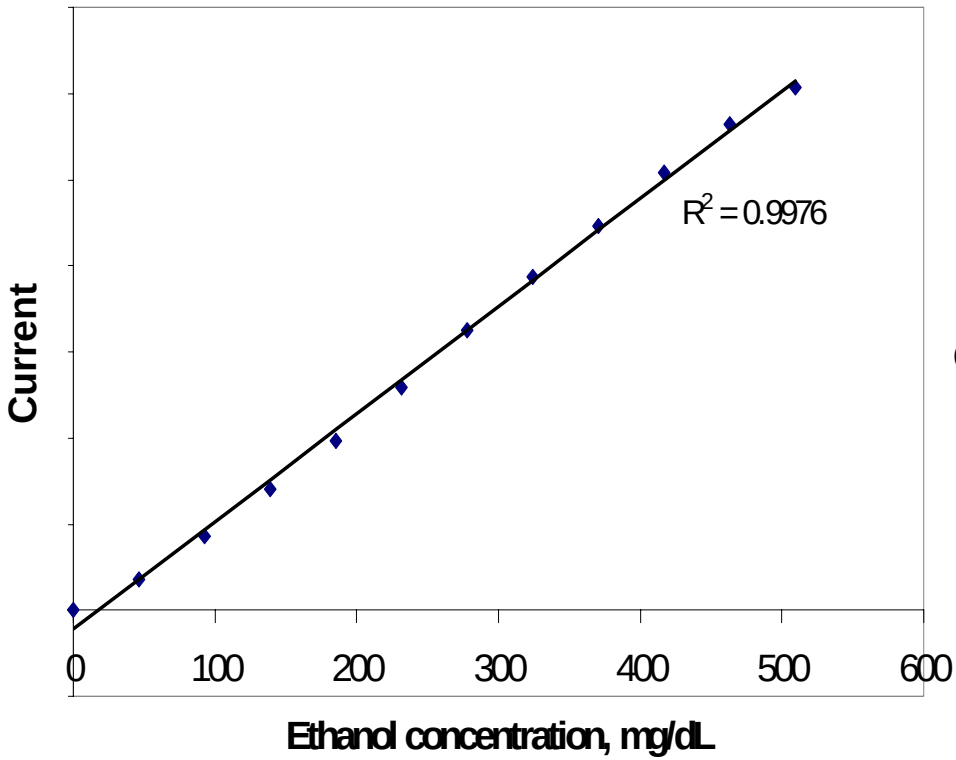
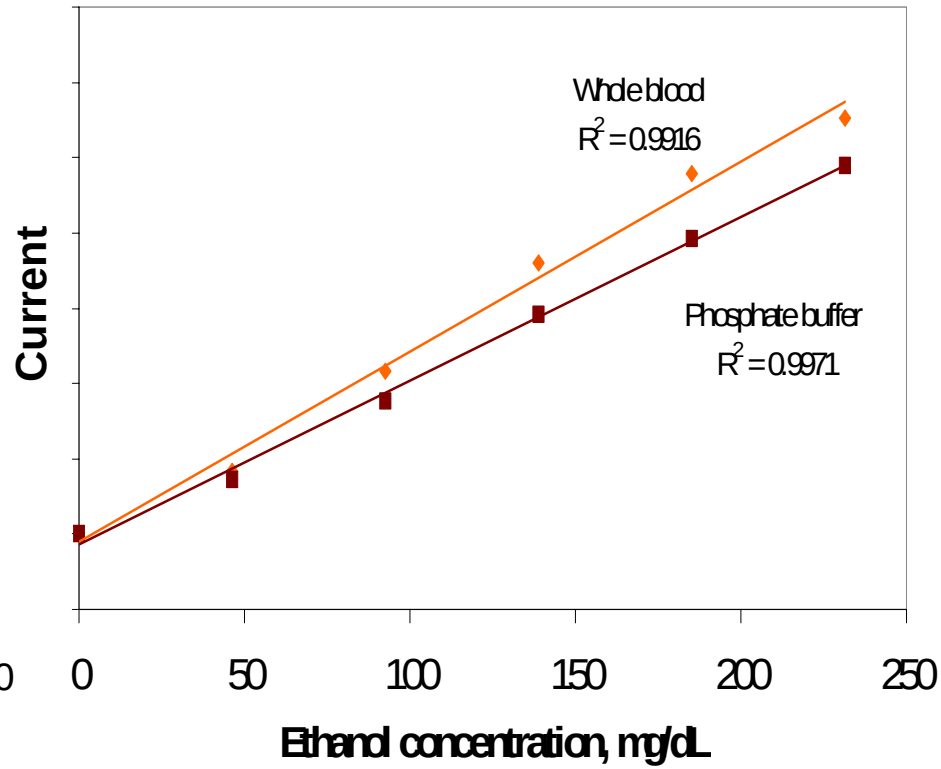
Alcohol Biosensor:
Linear Range, Biocompatibility

Linear range up to 0.5% BAC with resolution of 0.005%





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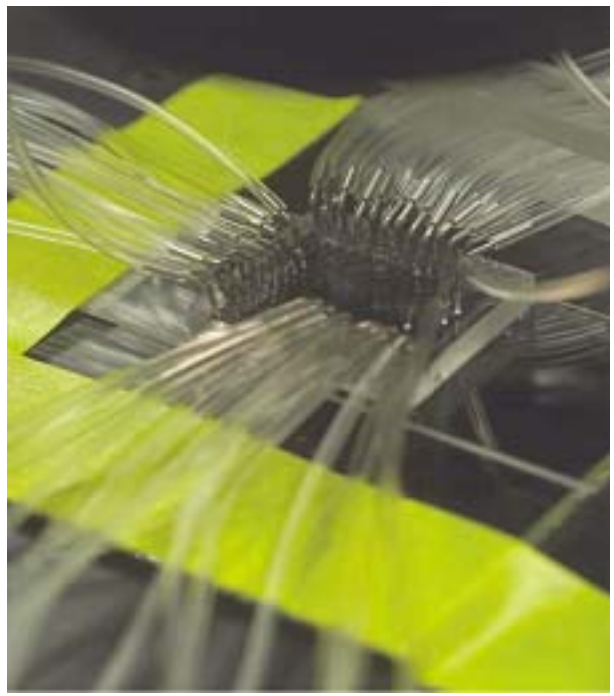
Problems with Current Biochips

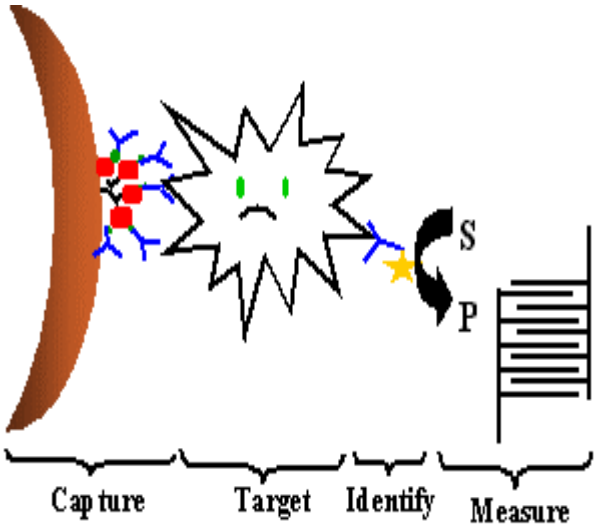
- Most of current biochip systems have many connections, producing a “Medusa-like” array of small diameter tubes connecting the chip to external liquid reservoirs, valves, and pumps. The purpose of lab-on-a-chip is defeated because of the large, power-hungry ancillary system.
- Field-use lab-on-a-chip is hindered because the overall size (including the reservoirs, pumps and power supply devices) is similar to a bench instrument.

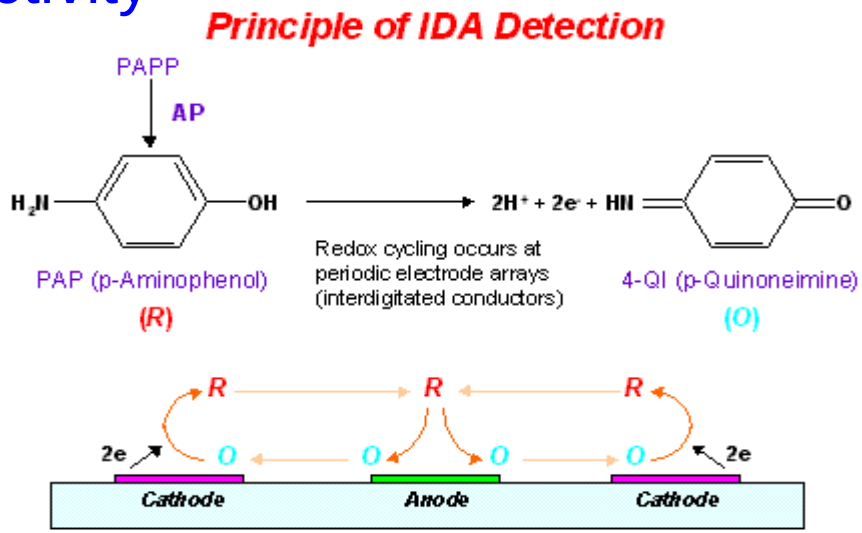
Do we really need such a biochip?



Biochips Based on Electrochemical Readout

High Sensitivity/Selectivity





Recognition element (Ab, aptamer) specifically identifies the target (protein, toxin, allergens, etc). ELISA (Enzyme-Linked ImmunoSorbent Assay) provides high sensitivity/specificity.

Incorporation of appropriate enzymatic and electrochemical reactions with ELISA : **multimillion-fold amplification** for targets has been achieved.

Integrated biochip - ideal immunoassay platform for Point-of-Care

On-Chip Immunoassay Platform
Based on ELISA and Electrochemical Readout

- On-chip electrochemical ELISA detected human insulin at its physiological level (nM) in a much cheaper and faster manner versus commercial Microtiter assays.
- High selectivity (no cross-reaction with insulin's similar compounds, C-peptide and proinsulin)
- Non Medusa type of biochip
- Other species (e.g., anthrax) can be detected using a similar biochip format

On-Chip Enzyme Activity Assay Platform
Anti-terrorism

- On-chip electrochemical assay for measurement of **blood cholinesterase** activity over 0-16,000 U/L
- Overall assay procedure (from blood sampling to readout) accomplished in less than one minute by unskilled personnel in the field
- Sufficient time for nerve agent attacked victims to inject the lifesaving antidote (attack causes death in four minutes without antidote)

Summary - BioMEMS Technologies

- Painless Silicon Microdevices (microneedles, microprobes, biochips)
- Optimal Integration of Electrochemical Techniques with Point-of Care Testing
- Glucose, Lactate, Alcohol Microbiosensors
- Biochips for Human Insulin, Blood Cholinesterase

Acknowledgement

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Thanks for Your Attention

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