Microfluidics, A Review with Selected Biological Applications

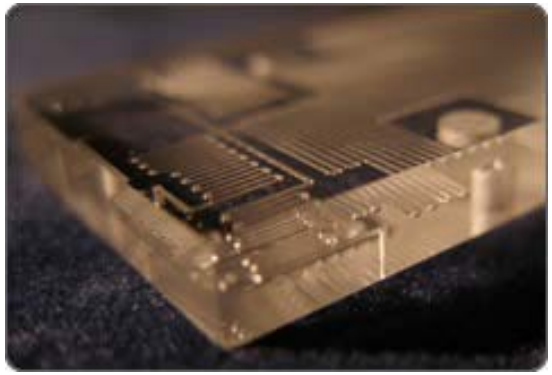
PART 2

Outline

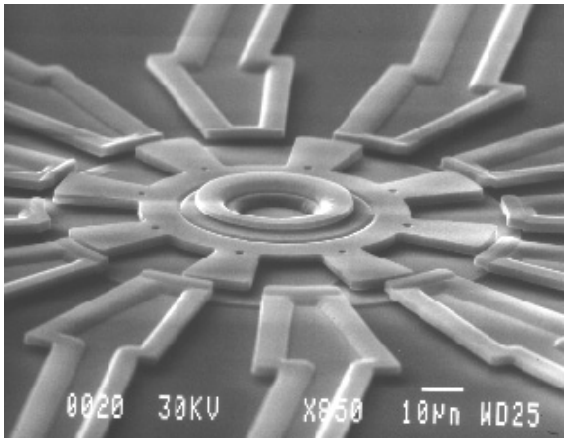
- Microfluidics Overview
- Physics of Microfluidics
- Operational Components
- Materials and Applications

Common Materials

- SiO_2 – etching and stacking
- Metals – Additive and Subtractive Processes



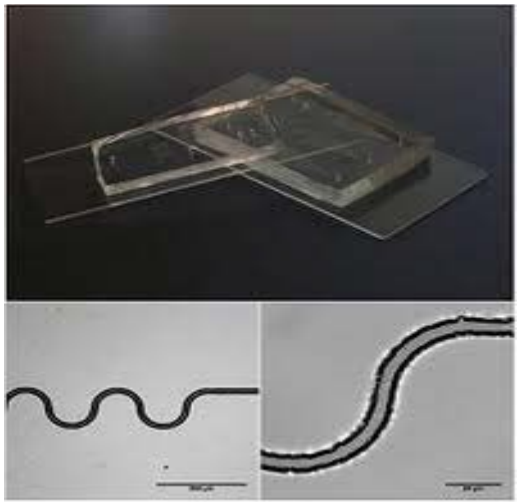
http://www.energi-tech.co.uk/microfluidics.htm



http://www.tmi.vu.lt/legacy/pfk/funkc_darinia i/nanostructures/nano_robots.htm

Common Materials

- Polymers – physical deformation and shaping
- Paper – substrate used for flow and cheap



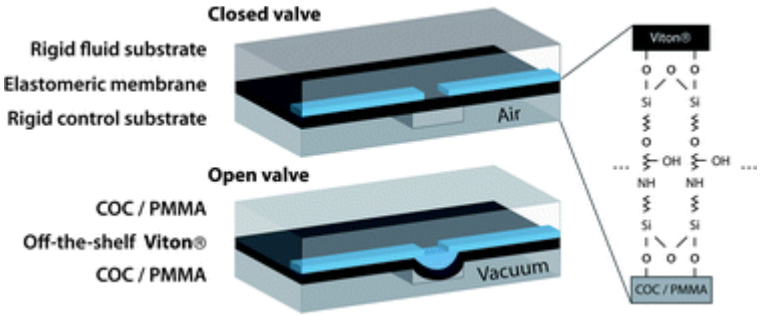
http://www.websters-online-dictionary.org/definitions/Microfluidics?cx=partner-pub-0939450753529744%3Av0qd01-tdlq&cof=FORID%3A9&ie=UTF-8&q=Microfluidics&sa=Search#922

Common Materials

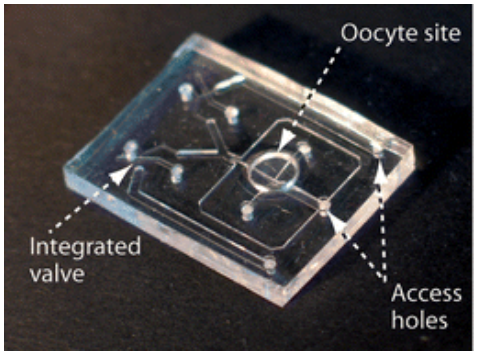
- SiO_2 – Plasma etching or chemical etching
  - Expensive, dangerous
- Metals – sacrificial layers
  - Expensive (materials wasted), much more complex
- Polymers – photopatterning, molding, embossing
  - Inexpensive, easy to mass produce
- Paper – Active or passive results
  - Inexpensive, ease of flow, generally slow

Common Polymer Materials

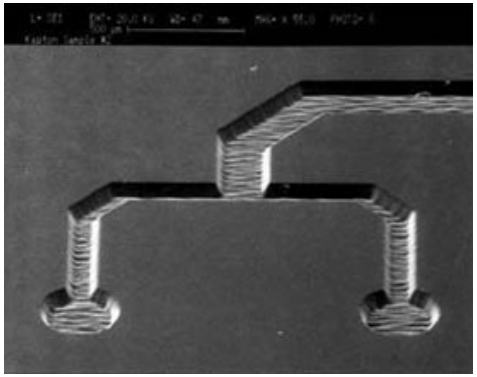
- Polymers
- PMMA, PDMS, Polyimides, SU-8



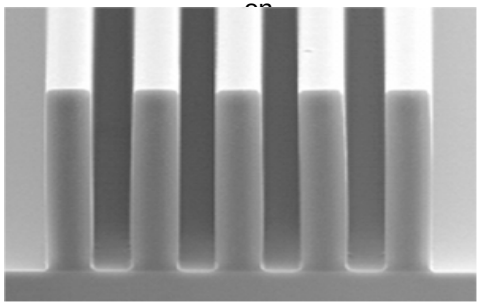
http://www.nanotechclearinghouse.com/module-NewsFeeds-view-option-viewfeed-fid-97-startnum-123.html



http://www.rsc.org/Publishing/Journals/lc/article.asp?Type=Issue&Journalcode=LC&Issue=11&SubYear=2008&Volume=8&Page=0&GA=



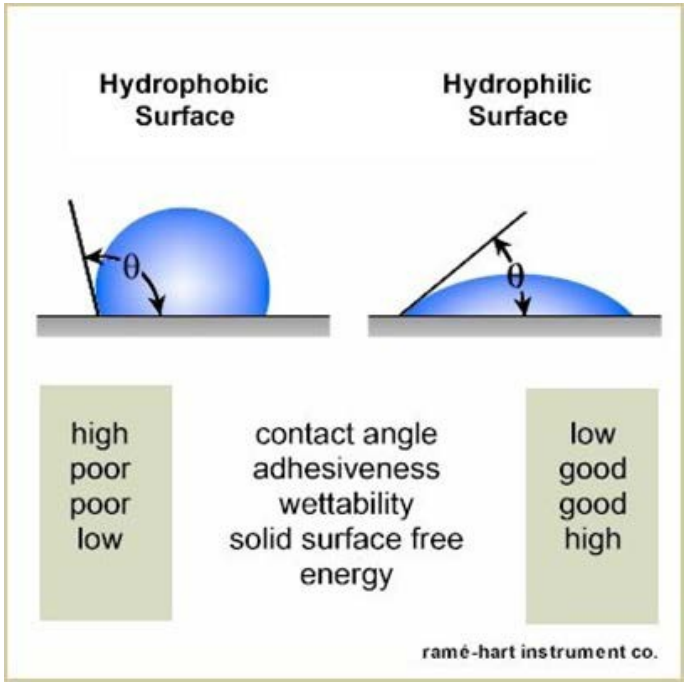
http://medicaldesign.com/engineering-prototyping/research-development/biomems-evolution-advancement-20091201/



http://www.nkc-mems.com/product_e/su8_3000.html

Common Materials

- PDMS
- Non-toxic, optically transparent, surface is easily modified
  - –Naturally hydrophobic, Oxygen plasma treatment renders it hydrophilic
  - –Useful for fluid flow and surface bonding
  - –Hyperelastic
  - –Biocompatible



http://www.ramehart.com/contactangle.htm.

Common Polymer Materials

- PMMA – thermoplastic, used for molding, opaque to UV light
- PDMS – thermoset, non-toxic, optically transparent, cured
- Polyimides – thermoset, used for MEMS, high strength, dielectric strength, heat resistance, photopatterned
- SU-8 – typically sacrificial layer, high mechanical strength, high aspect ratios, negative photoresist

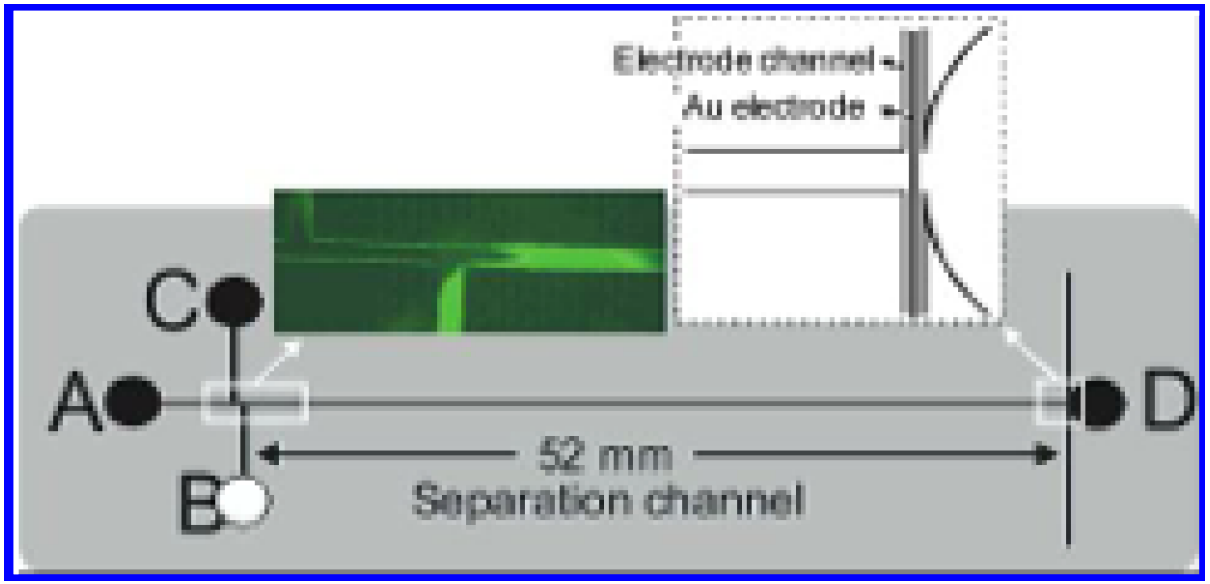
Common Polymer Materials

Compared Fields	Polymers			
	Polyimides	PMMA	PDMS	SU-8
Primary Manufacturer	HD-Microsystems	Plaskolite INC	Dow Corning	MicroChem Corp.
Brand Name	HD-4100 series	Optix Acrylite	Sylgard 184	SU-8
Approximate Cost	$2,185/kg	$2.70/kg	$16.40/kg	$500/kg
Optical Transparency	Yes	Yes (opaque to UV)	Yes	Yes (>340 nm)

Integrating the Parts

- Pump are used to move the fluid through the channels.
- Valves are used to control where and which direction the fluid flows.
- Mixers/stirrer keep fluids together and stop laminar flow.
- Many devices are made from the simple materials reviewed

PULSED AMPEROMETRIC DETECTION



Capillaryelectrophoresis chip with integrated pulsed amperometric detection. Channels: 50 µm width, 50 µm deep. Sample loop: 580 µm long. The diagram shows the electrode position at the end of the separation channel and the connections to the electrochemical analyzer.

Carlos D. Garcı aand Charles S. Henry, “Direct Determination of Carbohydrates, Amino Acids, and Antibiotics by Microchip Electrophoresis with Pulsed Amperometric Detection” Anal. Chem. 2003, 75, 4778-4783

PULSED AMPEROMETRIC DETECTORS

ELECTROCHEMICAL DETECTORS

Pulsed Amperometric Detection (PAD)

This follows established electrochemical liquid detector technology

This detector is based on the measurements of the current resulting from oxidation/reduction reaction of the analyte at a suitable electrode. Since the level of the current is directly proportional to the analyte concentration, this detector could be used for quantification.

Can be used for the separation and detection of underivatized carbohydrates, amino acids, and sulfur-containing antibiotics in an electrophoretic microchip

http://hplc.chem.shu.edu/NEW/HPLC_Book/Detectors/det_elec.html

PULSED AMPEROMETRIC DETECTORS

Direct Determination of Carbohydrates, Amino Acids, and Antibiotics by Microchip Electrophoresis with Pulsed Amperometric Detection (2003)

Lab on a chip configuration for microchip electrophoresis with pulsed amperometric detection (PAD)

The configuration consists of a layer of polydimethylsiloxane (PDMS) that contains the microfluidic channels, reservoirs, and a gold microwire, sealed to a second layer of polydimethylsiloxane.

The separation and the direct detection of underivatized carbohydrates, amino acids, and two antibiotics were achieved by optimizing the electric field, the electrolyte composition, and the PAD parameters.

The inclusion of a Au wire microelectrode was also demonstrated, showing very good stability and mass limits of detection in the femtomole range.

S. Henry, “Direct Determination of Carbohydrates, Amino Acids, and Antibiotics by Microchip Electrophoresis with Pulsed Amperometric Detection” Anal. Chem. 2003, 75,4778-4783

PULSED AMPEROMETRIC DETECTORS

Pulsed Amperometric Detection Parameters for Detection of Carbohydrates, Amino Acids and Antibiotics			
Test Material	*Clean*	*Reactivate*	*Detect*
carbohydrates	+1.4 V	-0.5 V	+0.7 V
amino acids	+1.8 V	-0.5 V	+0.7 V
antibiotics	+1.8 V	-0.5 V	+0.5 V
Cleaning, 0.05 s; reactivation, 0.025 s; and detection, 0.15 s.			

Carlos D. Garcı aand Charles S. Henry, “Direct Determination of Carbohydrates, Amino Acids, and Antibiotics by Microchip Electrophoresis with Pulsed Amperometric Detection”Anal. Chem. 2003, 75, 4778-4783

A POCKET-SIZED CONVECTIVE PCR THERMOCYCLER

Many diagnostic assays rely on the polymerase chain reaction (PCR), which requires thermocycling and conventional macro instruments that are relatively slow and consume considerable electrical power to perform repeated heating and cooling steps

Microfluidic thermocycling system that harnesses natural convection phenomena to amplify DNA rapidly by the PCR in a greatly simplified format.

A key element of this design is an architecture that allows the entire thermocycling process to be actuated pseudo-isothermally by simply maintaining a single heater at a constant temperature, thereby enabling a pocket-sized battery-powered device to be constructed at a cost of about US$10.

Agrawal, Nitin, Yassin A. Hassan, and Victor M. Ugaz. "A Pocket-Sized Convective PCR Thermocycler." *Angewandte Chemie International Edition* 46.23: 4316-4319

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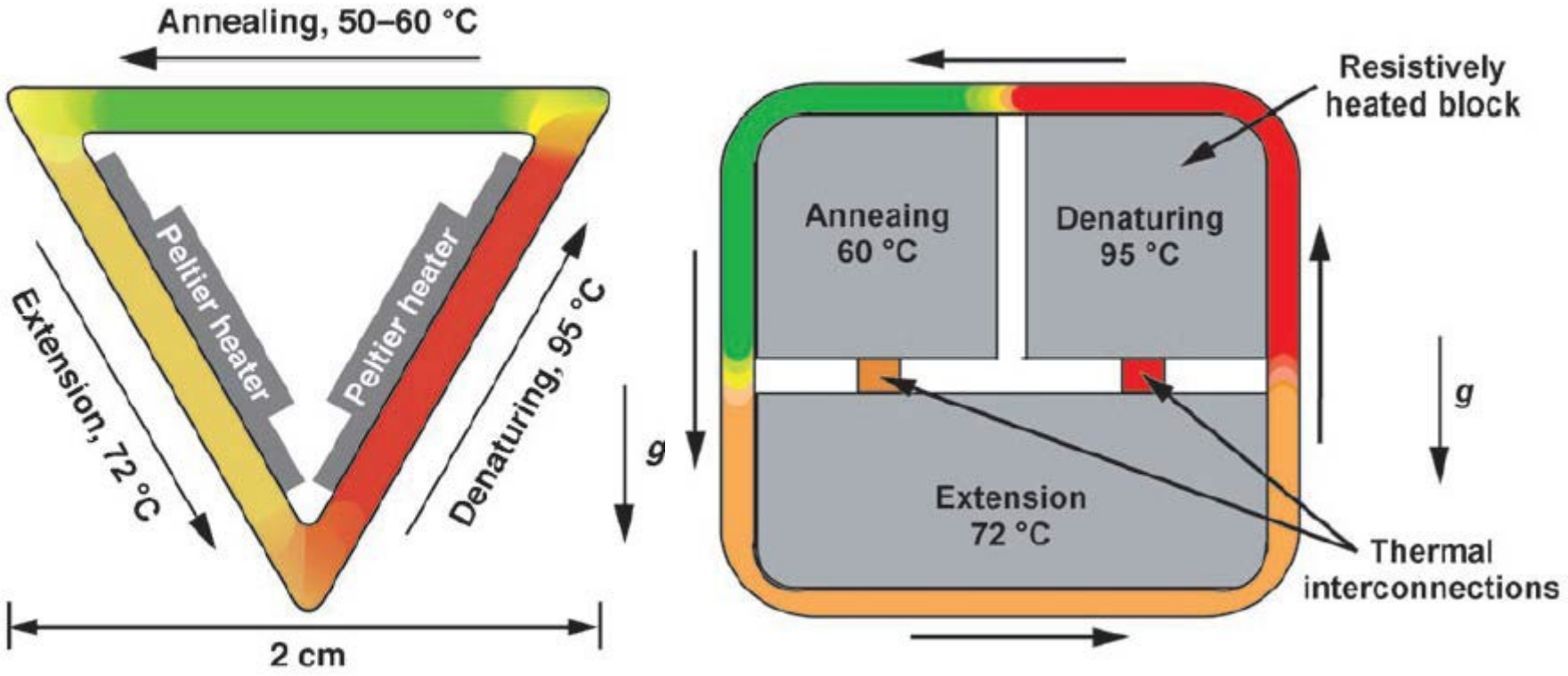


Agrawal, Nitin, Yassin A. Hassan, and Victor M. Ugaz. "A Pocket-Sized Convective PCR Thermocycler." *Angewandte Chemie International Edition* 46.23: 4316-4319

Paper based systems

- 1988 The first commercial immunoassay, Unipath launches home pregnancy test kits
- 1995 US Patent 5,409,664 describes a laminated assay device for detection of cholesterol with walls made by hydrophobic printing or cutting
- 2003 US Patent 6,573,108 presents the first example of a tangential flow device, incorporation of multiple channels, timers to show when the assay is over, and a filtration step for sample pretreatment. Walls could be made by screen printing, dipping in polymer with a template held against paper, or by a computer-controlled deposition system. Researchers proposed polymers such as heteropolysaccharides, acrylic polymers and copolymers, and silanes
- 2007 The first scientific journal publication by the Whitesides group describing a multichannel system with photoresist walls
- 2008 The first international conference on bioactive paper in Finland

Paper based systems

Colorimetric assays are conducted in the paper "wells," utilizing the interaction between species-specific enzymes and chromogenic substrates

The presence of pathogenic bacteria is indicated by a color change, a result that may be easily interpreted by the user without the need for complex instrumentation

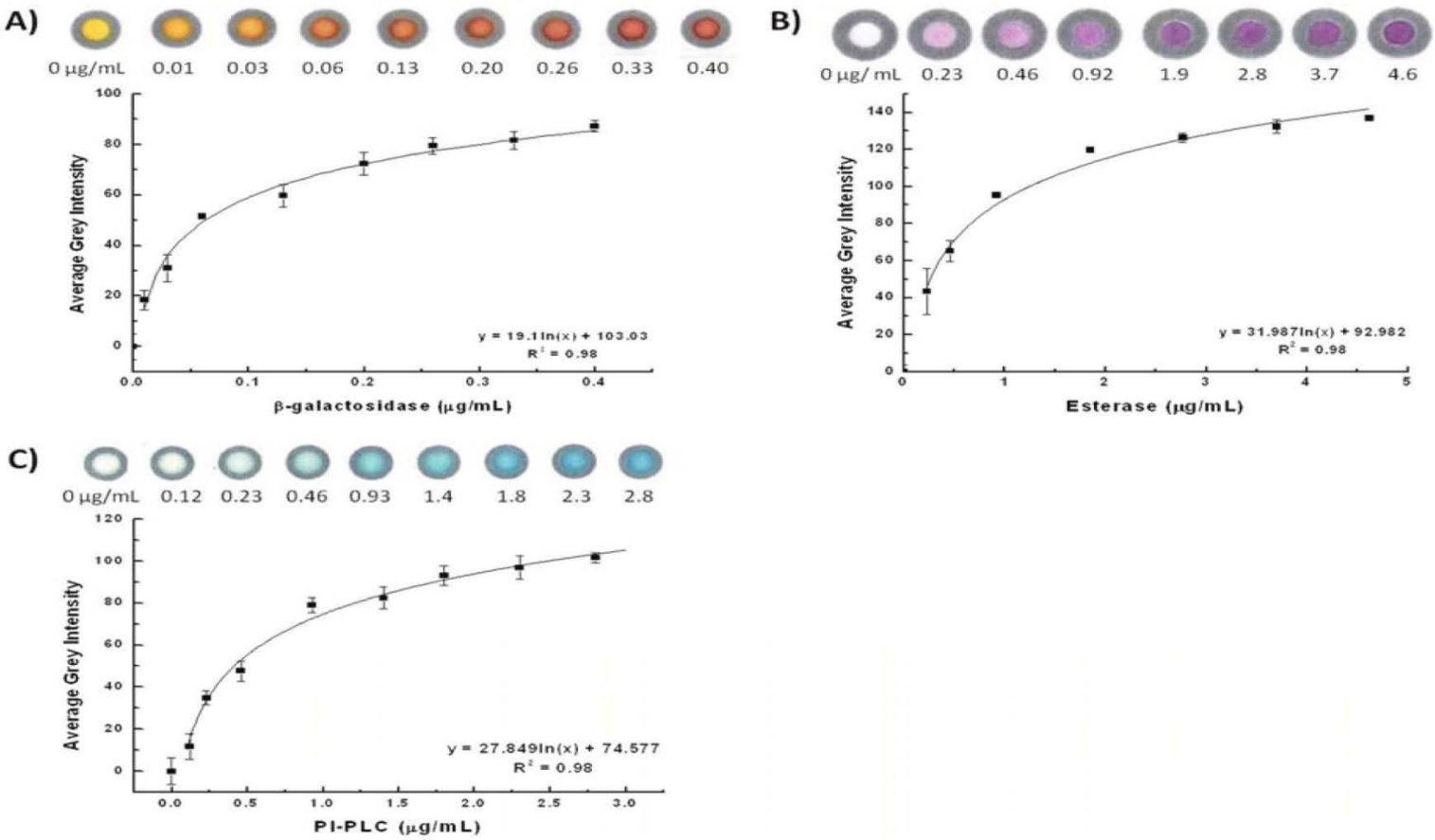
Semiquantitative analysis is performed by creating a digital image of the devices with an office scanner

Created by use of wax printing on filter paper, wax defines flow regions

Recent technology emulates conventional PDMS/Si systems

Jana C. Jokerst, Jaclyn A. Adkins, Bledar Bisha, Mallory M. Mentele, Lawrence D. Goodridge, and Charles S. Henry, "Development of a Paper-Based Analytical Device for Colorimetric Detection of Select Foodborne Pathogens', Anal. Chem. 2012, 84, **2900−2907**

Paper based systems



Determination of lowest detectable amount of (A) β-galactosidase, (B) esterase, and (C) PI-PLC enzymes via optimal substrate concentrations. Average grey intensities are plotted vs the amount of enzyme in each spot

Jana C. Jokerst, Jaclyn A. Adkins, Bledar Bisha, Mallory M. Mentele, Lawrence D. Goodridge, and Charles S. Henry, "Development of a Paper-Based Analytical Device for Colorimetric Detection of Select Foodborne Pathogens', Anal. Chem. 2012, 84, **2900−2907**

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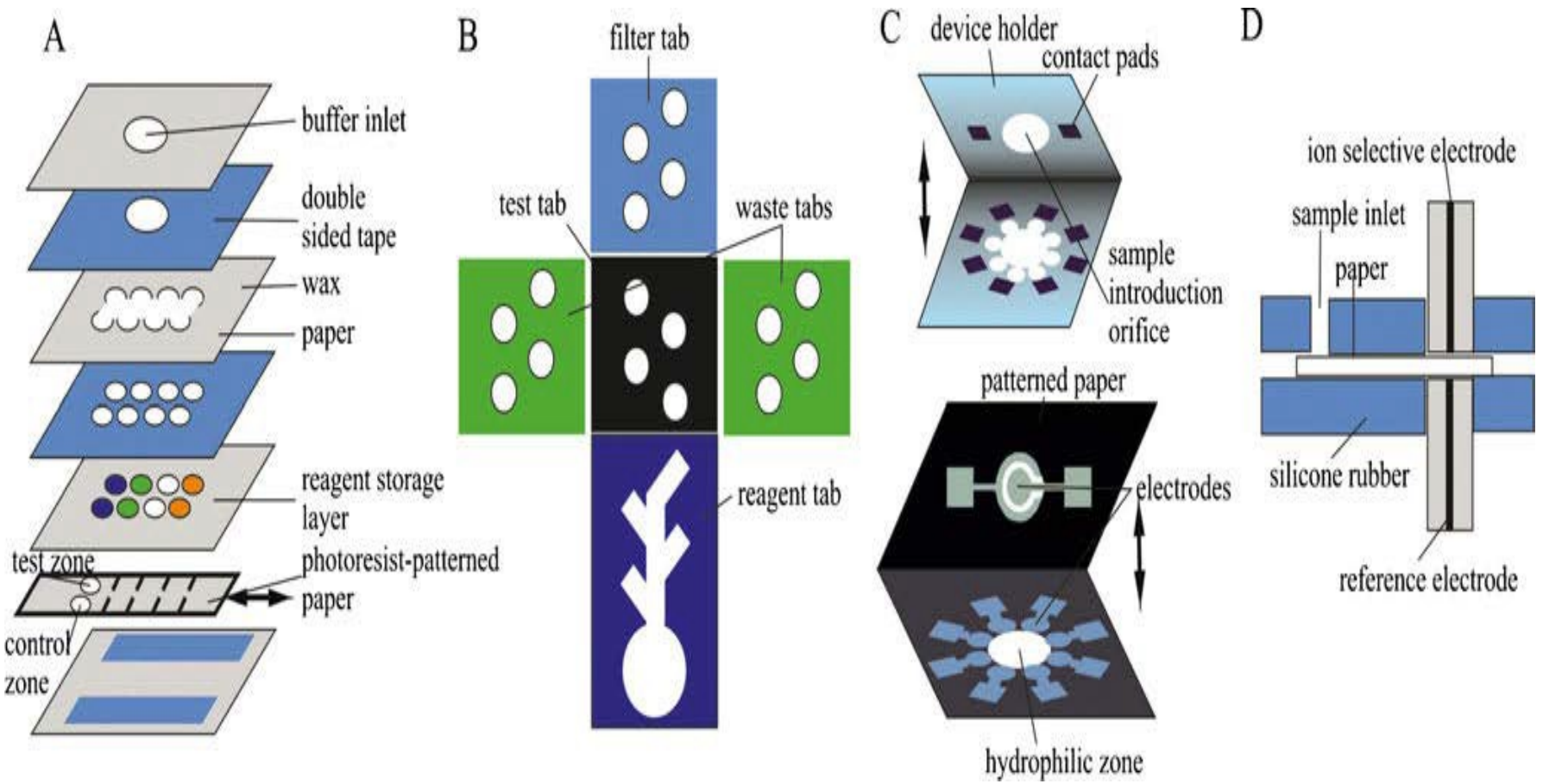


Immunoassays:
a Whitesides group assay with a movable strip, b. origami-based device for detection of tumor markers, c. electrochemiluminescence detection of tumor markers, d potentiometric immunoassay

Paper based systems

- Used for the separation and detection of underivatized carbohy- drates, amino acids, and sulfur-containing antibiotics
- This paper device determined glucose, lactate, and uric acid in biological samples using oxidase enzyme reactions (similar to previous research on a silicon/pdms system)
- These include glucose oxidase, lactate oxidase, and uricase
- These techniques are previously expressed in other technical publications by Dr. Henry in conventional devices

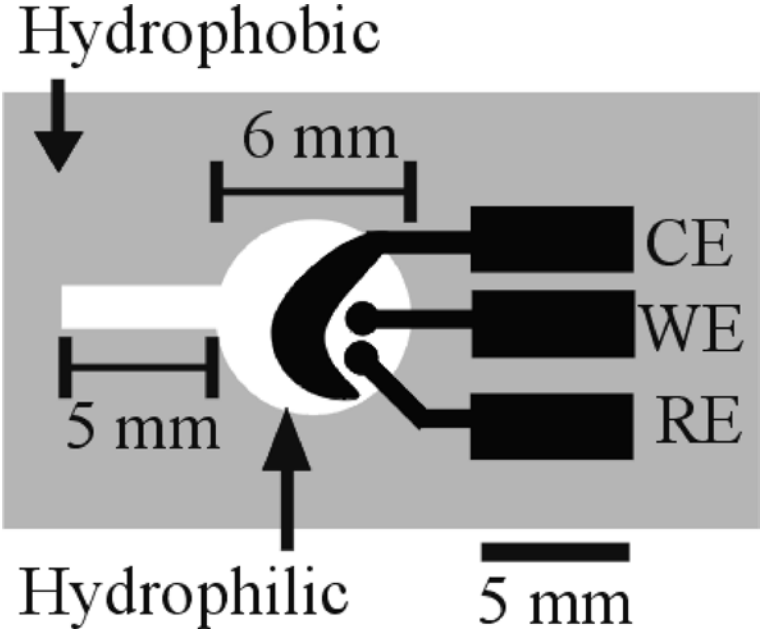
Wijitar Dungchai, Orawon Chailapakul, and Charles S. Henry ,Electrochemical Detection for Paper-Based Microfluidics *Anal. Chem.* 2009, *81,* 5821–5826

Paper based systems

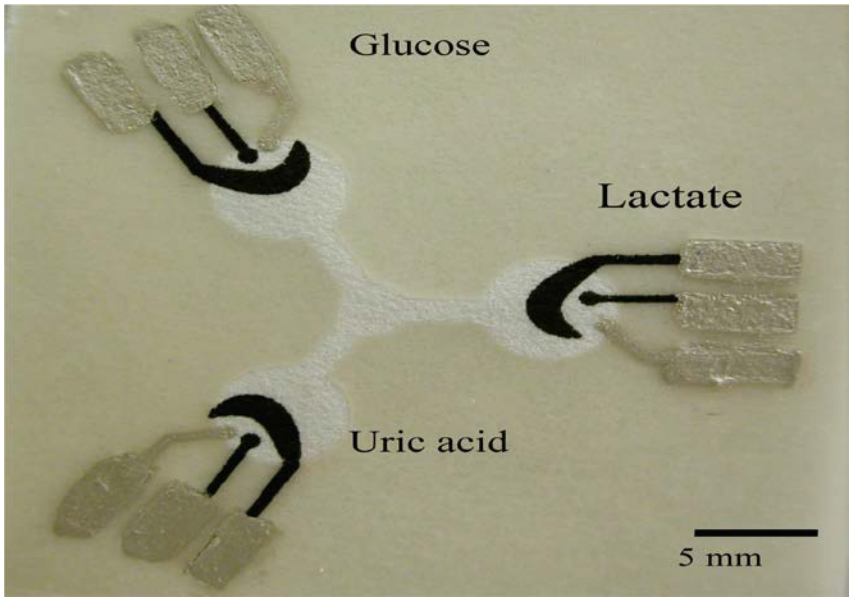
- Multi stack configuration from Dr. Henry's work.
- Layer 1: Photo- lithography was used to pattern Whatman filter paper using SU8 resist
- Layer 2: Electrochemical Detector for Paper-Based Microfluidic Devices. The electrodes were screen- printed in house using carbon ink containing PB as the working (WE) and counter electrode (CE) and silver/silver chloride ink as the reference electrode (RE) and conductive pads.

- Wijitar Dungchai, Orawon Chailapakul, and Charles S. Henry ,Electrochemical Detection for Paper-Based Microfluidics *Anal. Chem.* 2009, *81,* 5821–5826

Paper based systems



Basic design of the electrochemical detection cell for paper-based microfluidic devices.
WE, working electrode;
RE, reference electrode;
CE, counter electrode.



The hydrophilic area at the center of the device wicks sample into the three separate test zones where independent enzyme reactions occur.
The silver electrodes and contact pads are made
from Ag/AgCl paste with the black electrode portions being the Pb modified carbon electrodes.
The device size is 4 cm 4 cm.

Wijitar Dungchai, Orawon Chailapakul, and Charles S. Henry ,Electrochemical Detection for Paper-Based Microfluidics
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MicroBioLab

- This is a device that can dissect a very small amount DNA, protein, cells, or drug
- It can chemically analyze a liquid sample 10,000 times smaller than commercial analytical instruments
- This time-saving chip could be built for genetic diagnosis, DNA fingerprinting, and drug research
- Conserves test media, and reduces disposal issues

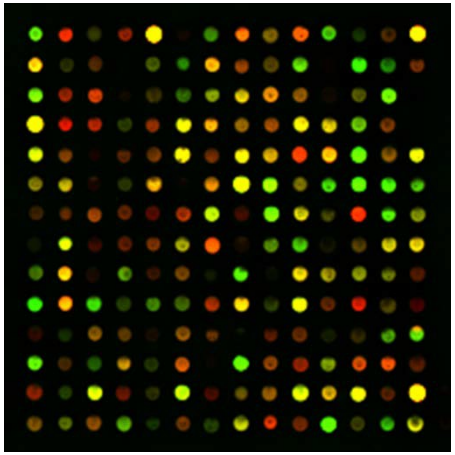
Micro Arrays

- How they work
  - Microarrays work by exploiting the ability of mRNA to bind to DNA templates.
- What they do
  - Analyzes gene expressions consisting of small membranes.
  - Can determine the expression levels of hundreds or even thousands of genes in cells by measuring the amount of mRNA in a single experiment

http://genechip.com/index.affx

Micro Arrays Uses

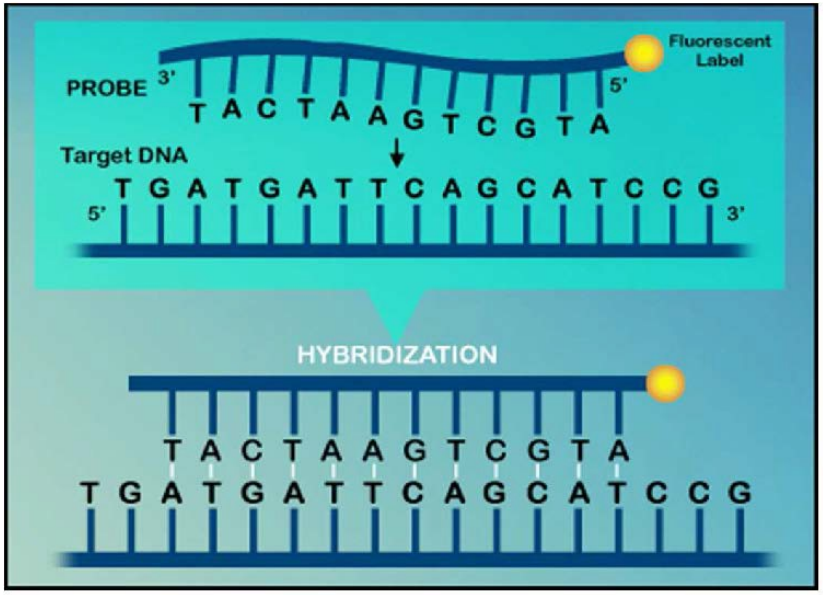
- Test structure that can be used with microfluidic technology to interrogate samples.
- Used to understand fundamental aspects of growth and development.
- Also used to identify and classify DNA sequence information and assign their function.



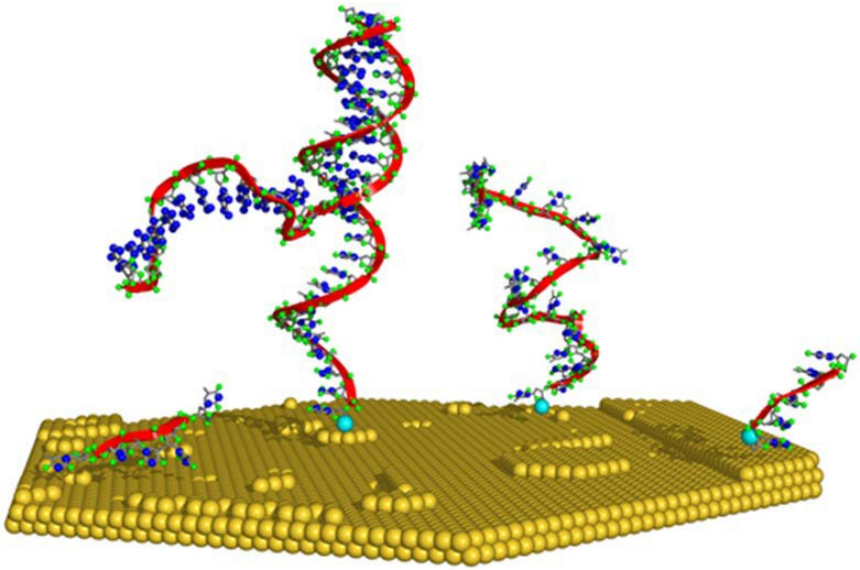
Micro Arrays

- Why they are important
  - You can survey large number of genes rather quickly.
  - Can compose and contrast cells and tissues from being healthy of diseased.
  - Show how and why certain genes can or can not work together.

Micro Arrays



http://members.cox.net/amgough/Fanconigenetics-PGD.htm



www.als.lbl.gov/als/science/sci_archive/128dna.html

Advantages/Disadvantages

- Advantages
  - Very Small
  - Disposable
  - Sealed
  - Portable
  - Very Fast response time
- Disadvantages
  - Takes place of jobs

Summary

- Microfluidics is an important emerging technology, that uses the tools taught in the NMT program
- Key points are fluid flow, components, and scaling
- Many biological applications that may provide employment