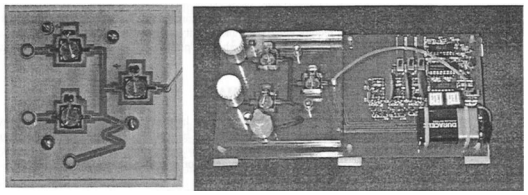
Microfluidics



Bruce K. Gale

Fundamentals of Micromachining

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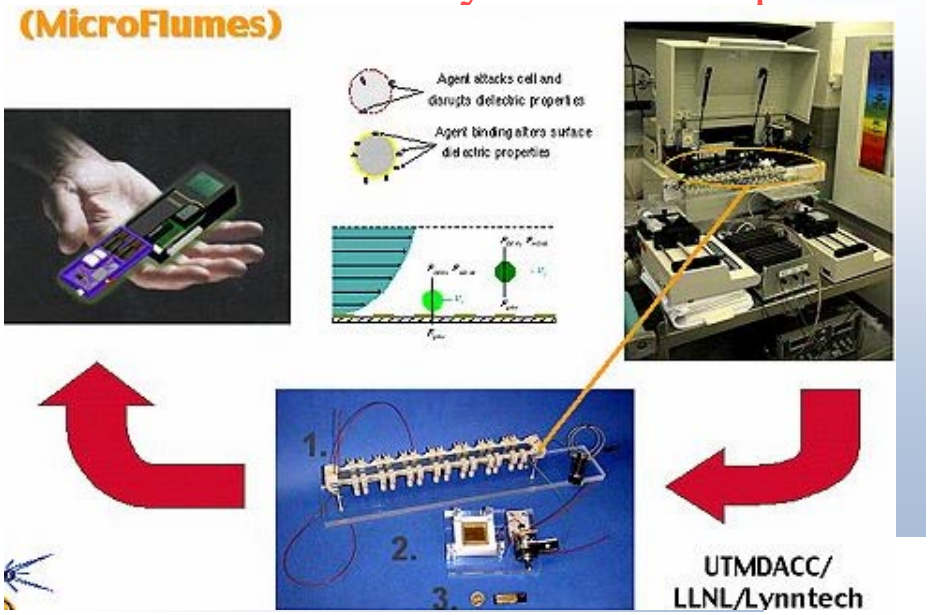
Microfluidic System Concept



Concept

- One system to provide all of the possible required analyses for a given type problem
- All processing steps are performed on the chip
- No user interaction required except for initialization
- Portable bedside systems possible

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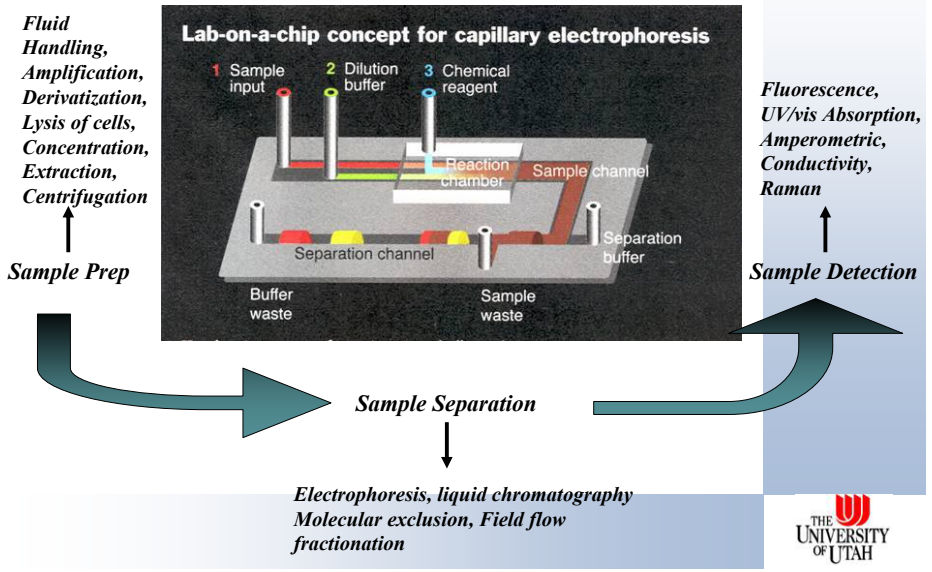
Lab-on-a-Chip
(Body Fluid In; Answer Out)



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Goals:

- Fast
- Portable
- Robust
- Easy to use
- Flexible
- Inexpensive
- Modular?

Considerations in Microscale Biomedical Analysis Systems

- Biocompatibility
  - Defined for each application and system
  - Cells, proteins, DNA, tissues all have different requirements
  - Typically low protein absorption, no leaching, "non-reactive"
- Harsh chemicals and environment
- Small sample handling
- Interfacing with macroscale world
- Pumps, valves, flow control
  - High pressures, flow rates, and volumes possible
- Sample injection
- Multimodal: Fluids, Electrical, Optical, etc.
- Interfaces with existing systems (standards)

Components

- Separation
- Mixing
- Reaction
- Sample injection
- Sample preparation
- Detection
- Pumping
- Transport (channels)

- Reservoirs
- Flow control
- Control
- Intelligence and Memory
- Power
- Display
- Other analysis
- Sample collection?

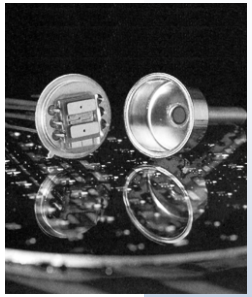
Don't forget packaging!!

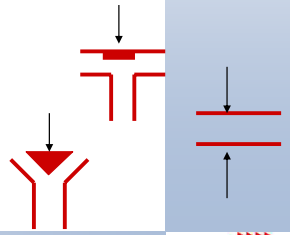
Microfluidic Scaling

- All flow is laminar (no turbulent mixing)
- Surface tension becomes significant
- No inertia effects
- Apparent viscosity increases

Fluid Control Components

- Pumps, valves, channels
  - Pumps and valves of similar design
  - No perfect pumps or valves
- Generally require mechanical actuation
- Valve types
  - A: restriction perpendicular to flow
  - B: restriction parallel to flow
  - C: combination of A and B
  - D: phase change (freezing)

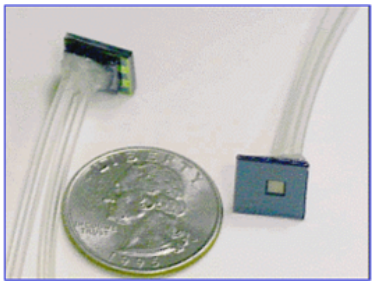




Microvalve

Thermo-Pneumatic Micro-Valve Characterization

Joanne Deval

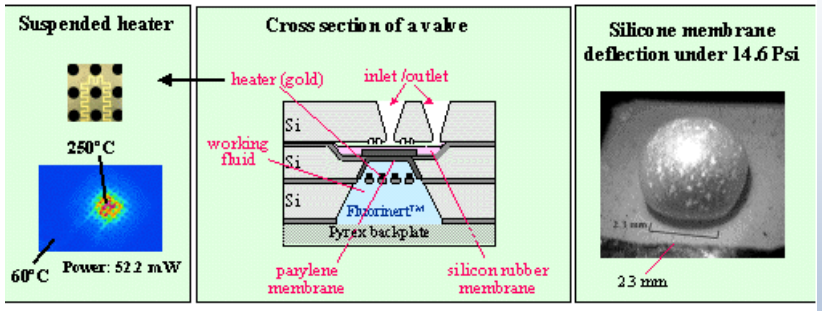


•How do they work ?

•What do we need to know about them ?

Microvalve

How do they work ?



•Fluorinert ™ was preferred to air and DI water

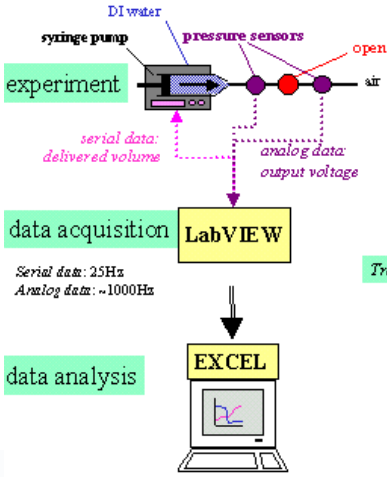
→ *Highest deflection achieved with same power input to the heater (thanks to a large thermal expansion coefficient)*

•Parylene membrane was added to prevent leakage of the working fluid

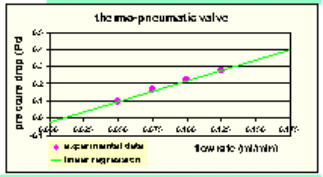
→ *Low permeability to moisture and gases*

Microvalve

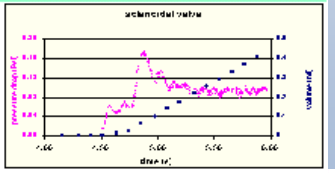
Pressure Drop Versus Flow Rate



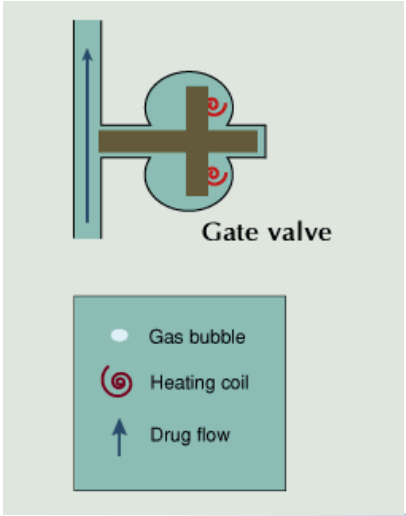
after averaging the steady values



Transient response of pressure to a change in flow rate

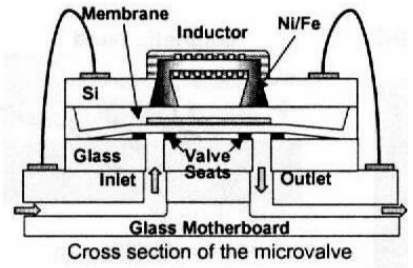


Bubble Gate Valve

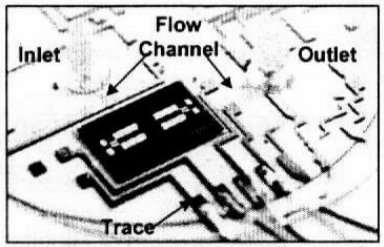


- Basic Operation
  - Current travels down platinum wires, heating the coil .
  - The coils boil water to produce bubbles
  - Bubbles push on the cross's arms and force it away from the main channel
  - Bubbles generated on the other side of the arms closes the gate valve
- Envision growing a bubble in the channel

Magnetic Valve



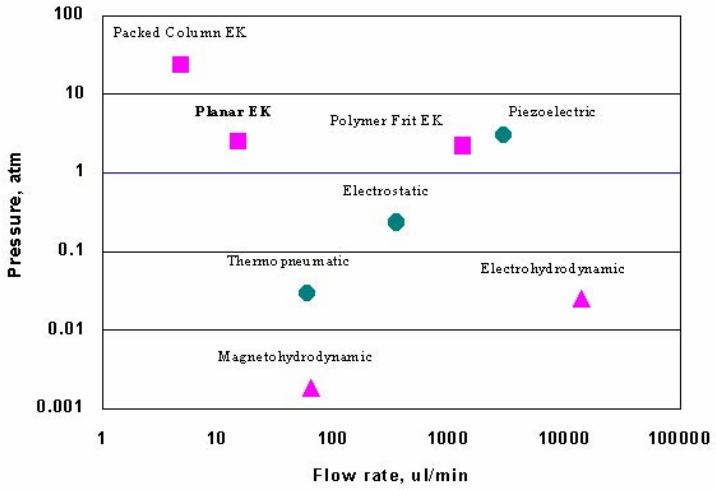
Cross section of the microvalve



Microvalve mounted on a motherboard

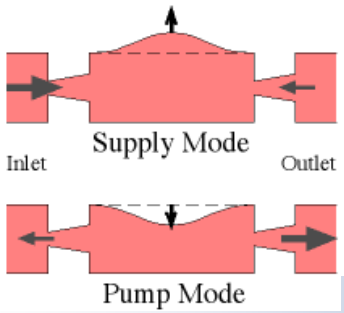
- Example of a typical mechanical valve
- Can be attached to glass motherboard
- Modular

Pumps



Pump Types

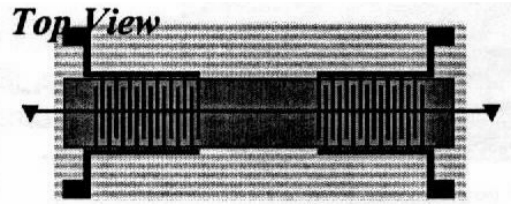
- Valved
  - Piezoelectric
  - Thermo pneumatic
  - Electrostatic
- Valveless
  - Electro hydrodynamic (EHD)
  - Diffuser
  - Electroosmotic (electrokinetic)
  - Bubble



Diffuser pump concept

Microfluidic Scaling: Pumping

- Mechanical (blister pouch)
  - L^3
  - No fluid contact
  - Generic
  - Innovation in the blister pouch solves valving
  - Difficult to further miniaturize
  - Difficult to multiplex
- Acoustic
  - L^2
  - No fluidic contact
  - R & D
  - Generic
  - Doesn't solve valving yet
  - ZnO technology still difficult to reproduce
  - Easy to further miniaturize



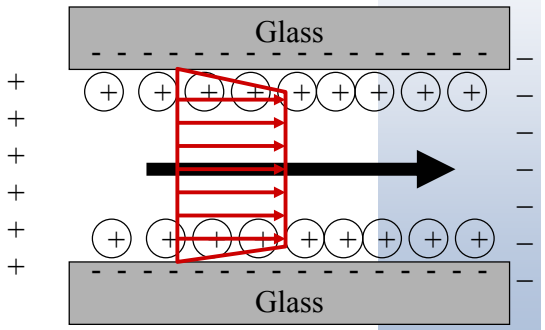
Microfluidic Scaling: Pumping

- Electroosmotic
  - I^2
  - Fluid contact
  - Development
  - Not generic
  - May solve valving
  - Mixing difficult to implement
  - Many parameters influence propulsion force
  - High voltage source is not convenient
  - Better for high-throughput screening and smaller samples

- Centrifugal
  - I^3
  - No fluid contact
  - Established
  - Generic
  - Solves valving elegantly
  - Widest dynamic range
  - Simple and inexpensive CD player for drive
  - Mixing easy to implement
  - Most functions demonstrated
  - Cell work possible
  - Sample preparation easier
  - Better for diagnostics

Electroosmotic Pumping

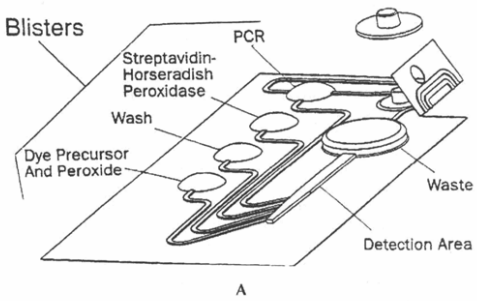
- Requires materials with surface charge
  - Preferably permanent
- Glasses and many polymers have permanent negative surface charge
- Positive charges assemble on surface
- Applied charges pull assembled charges
- Charges at surfaces drag bulk material

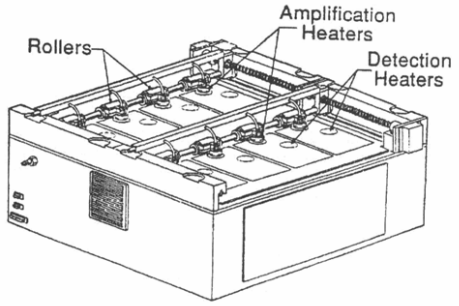


Mechanical Actuators for Pumping

- Actuation mechanisms:
  - electrostatic = electrostatic attraction of charged plates
  - thermal = expansion of solids or fluids; phase change
  - shape memory alloy = considerable change in length (TiNi)
  - pneumatic/hydraulic = fluid pressure
  - piezoelectric = electrically induced strain
  - magnetic
  - chemical (including hydrogels)
  - biological

Kodak System

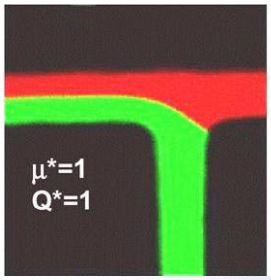


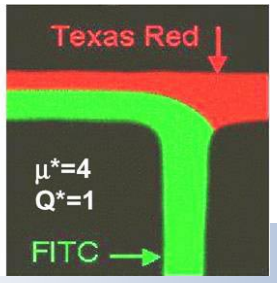


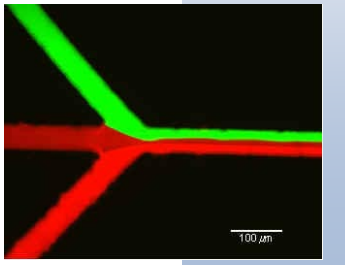
- Blister pouch used for pumping
- Commercially available
- Disposable pouch used with complex base system

Microscale Mixing

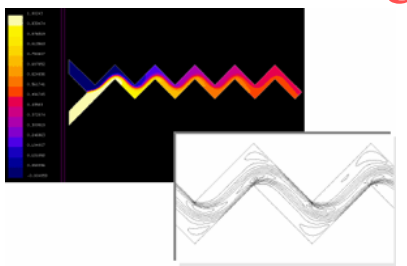
- Laminar flows make mixing very difficult
- Occurs almost exclusively through diffusion
- Goal then is to maximize surface areas for diffusion
  - High surface to volume
- Good mixing critical for many bioassays
- Recirculation mixes quickly



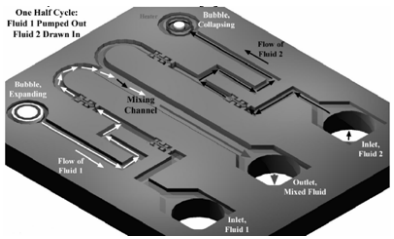




Mixing Methods

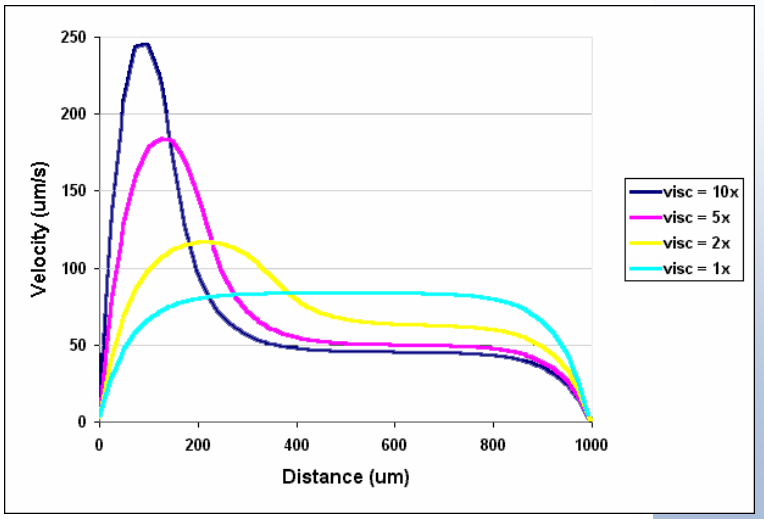


- Active mixing and pumping
  - Recirculation
- Chaotic advection
  - Flow disturbances
- Multiple flows at small dimensions



Bubble pump mixer

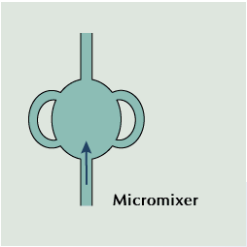
Fluid Viscosity Effects



Varying viscosities severely impact flow profile

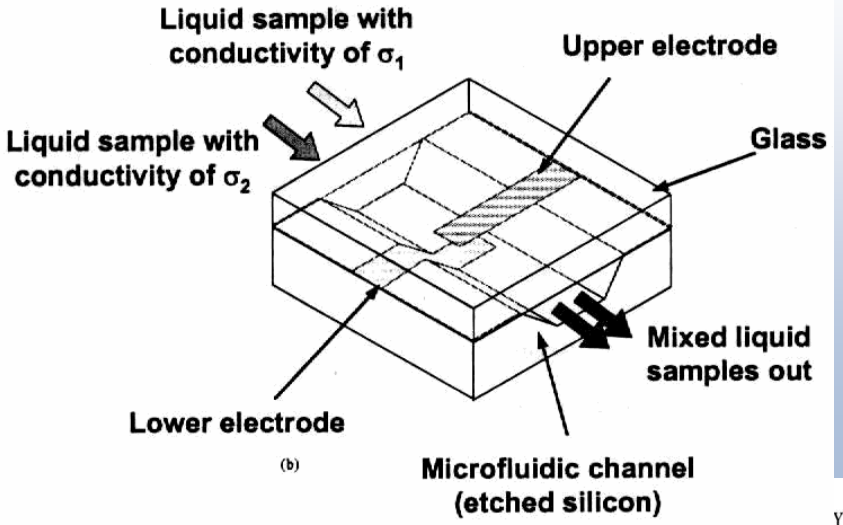
Micromixers

Active MEMS Mixer

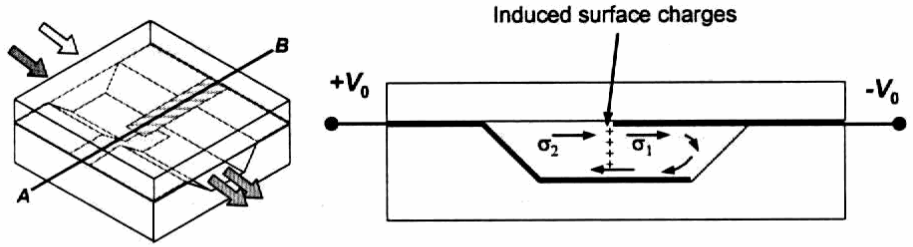


Recirculating Micromixer

Electrohydrodynamic Mixing



Electrohydrodynamic Convection

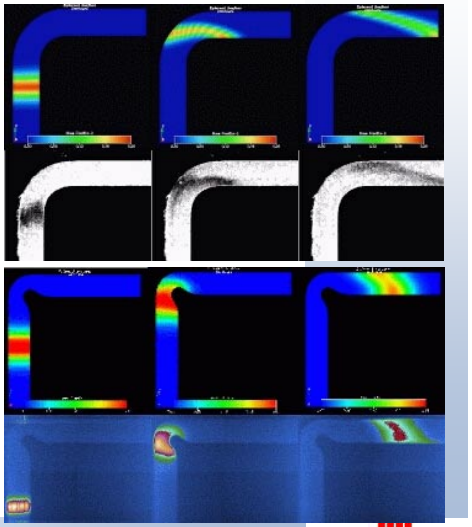


- Surface charges are induced at the interface
- External electric fields make the induced charges move
- Moving charges produce the shear force
- Liquids are moving along with the induced charges and being mixed

(b)

Channel Considerations

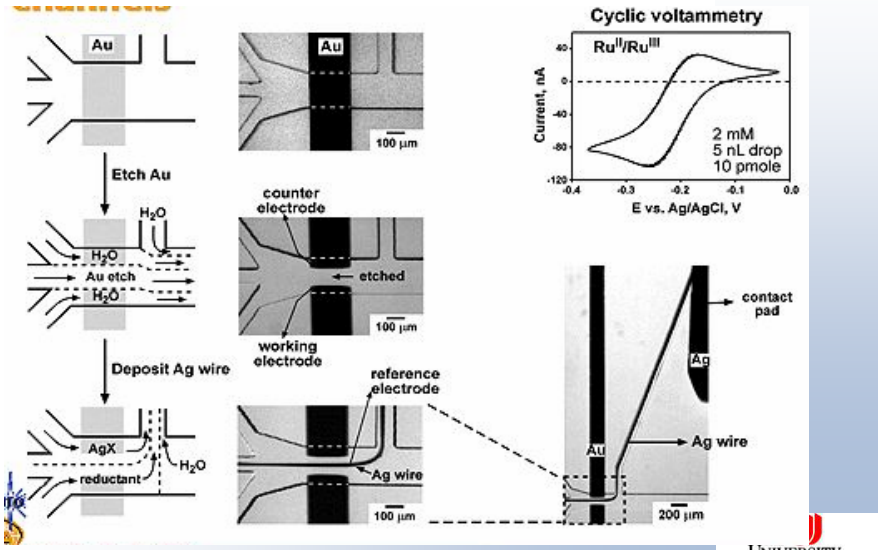
- Channel cross section
  - Hemispherical
  - Rectangular
  - Triangular
  - Trapezoidal
  - Round
- Geometry critical to reducing broadening of injected samples



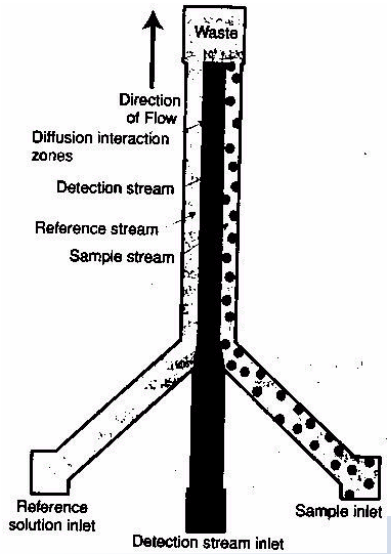
Flow Measurement

- Turbine?
- Hot wire anemometer
- Ultrasonic
- Optically
- Others?

Opportunities of Microfluidics

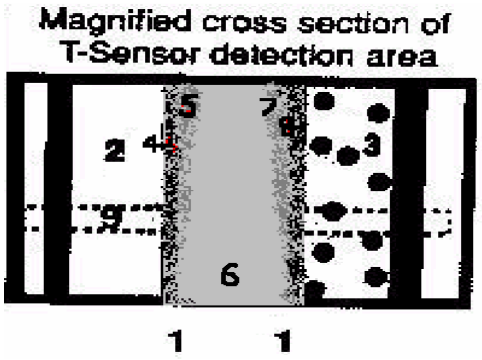


T Sensor



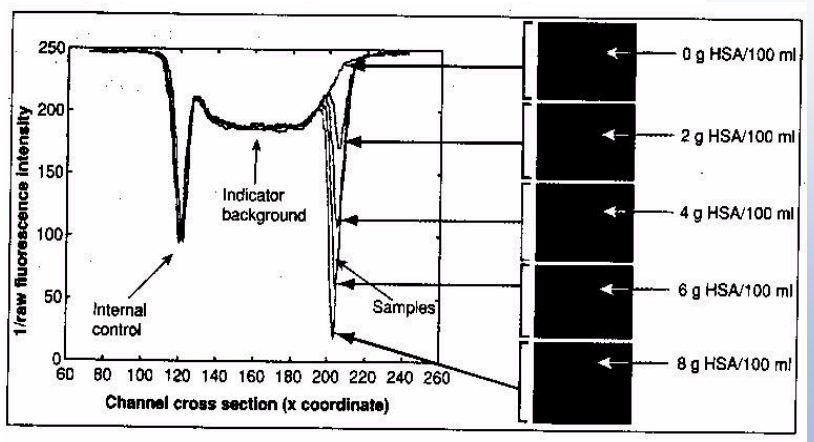
- Channel width is ~200um
- Flow is laminar
- Combines separation and detection functions
- Fluid interaction during the parallel flow
- Large particles in blood do not diffuse
- H^+,Na^+ and small molecules diffuse rapidly between streams
- Interaction zones are formed due to inter diffusion
- Indicator changes color or fluorescence intensity
- The ratio of fluorescence gives the concentration of analyte

T Sensor

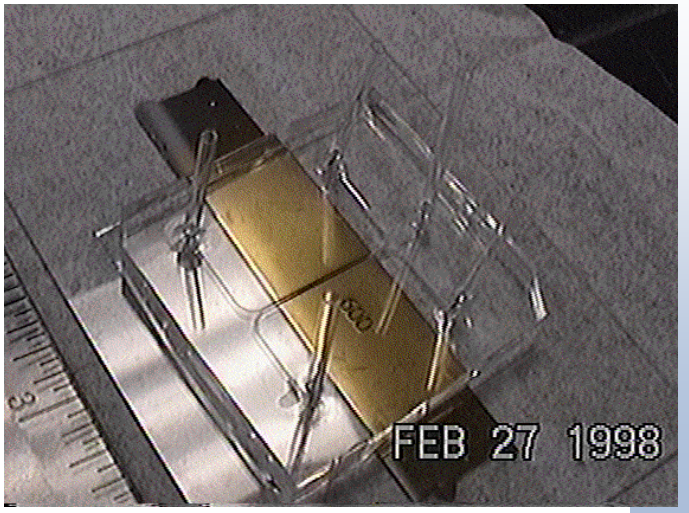


1. Original blood flow boundaries
2. Reference stream
3. Particle-laden sample stream
4. Diffusion of detector substance into reference stream
5. Diffusion of reference analyte into detection stream
6. Detection stream
7. Diffusion of sample analyte into detection stream
8. Diffusion of detector substance into sample
9. Detector cross-section (linear detector array) or imaging CCD

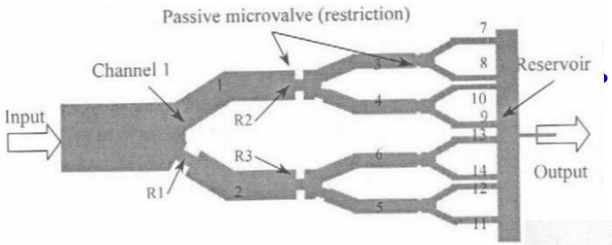
T Sensor



Packaged H-Filter

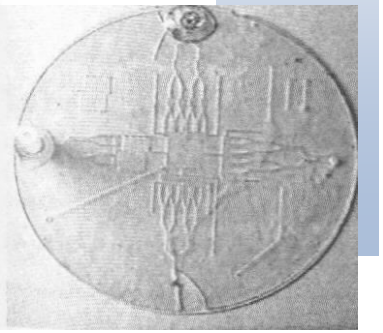


Structurally Programmable Arrays



- Chong Ahn, Univ. Cincinnati

- Valving accomplished by channel size reduction
- Program hard wired into system
- Can also be done using hydrophobic sections



Separations

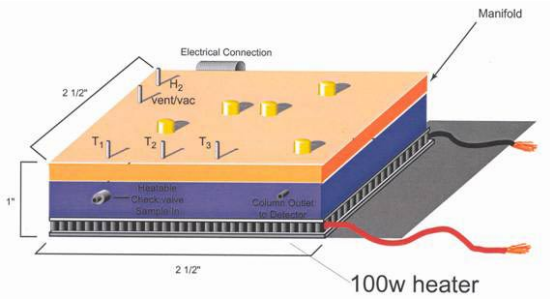
- Chromatography
- Wide variety of methods
- Issues
  - Resolution
  - Field strength
  - Analysis time
  - Contaminants
- Electrophoresis
- Field- flow fractionation
- Gas and liquid chromatography
- Blotting (Directions)
- Size exclusion
- Affinity

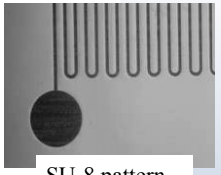
Motivation for On Chip

- Combination of chemical reactions, sample injection, and separation of reaction products in one system
- Speed up analysis times
- Reduce fluid handling
- Improve resolutions
- Reduce sample sizes
- Allow parallel processing
- Reduce costs
- Integrated signal detection and processing
- Smaller systems (portable)

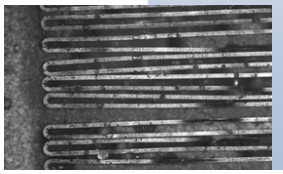
Micro Gas Chromatograph

Principle Layout





SU-8 pattern on X-ray mask



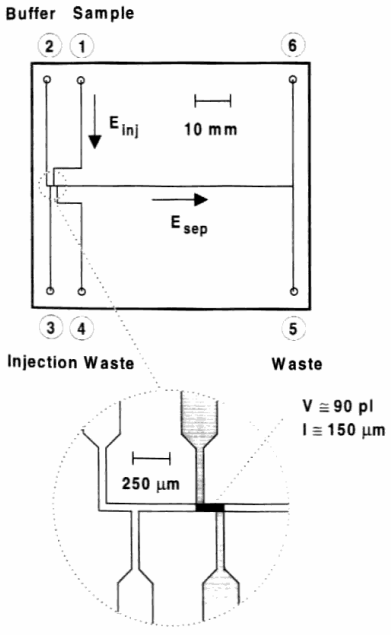
1st exposures in 1mm PMMA

3 Functional Layers
Stacked to Build a 3D MEMS Device

Courtesy Ed Overton, LSU

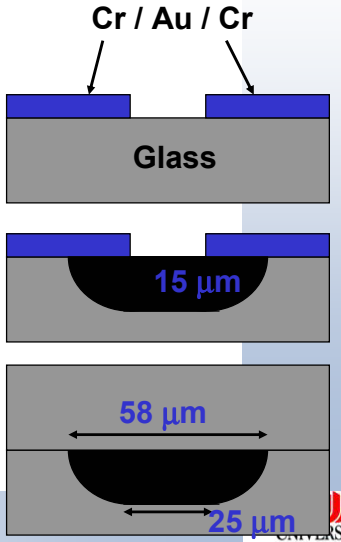
Electrophoresis

- Used to separate charged particles on basis of size and charge
- Electric fields are applied across gels which slow "large" particles moving through gel
- High resolution separations possible



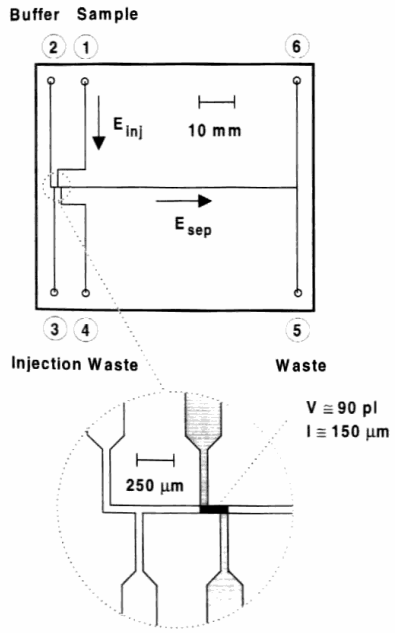
Fabrication

- Fabricated on 4 inch square glass plates
- Cr/Au/Cr mask was used to etch glass plates
- HF/HNO_3 etchant used to etch channels
- 2 mm access holes drilled in second plate
- Air squeezed out and then bonded at 440 C for 2 h

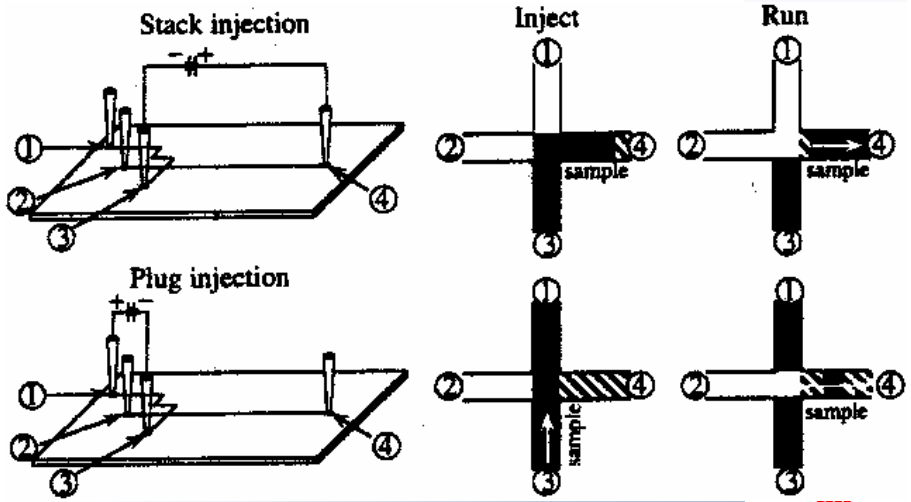


Channel Layout

- Thick lines are 240 µm across
- Double T injector used
- Injection volume ~ 100 pL
- Injection to Detection distance is ~ 5 cm



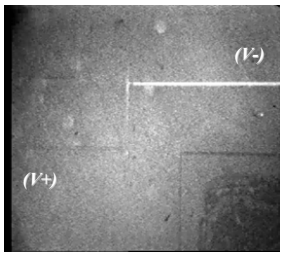
Sample Handling in T Injector

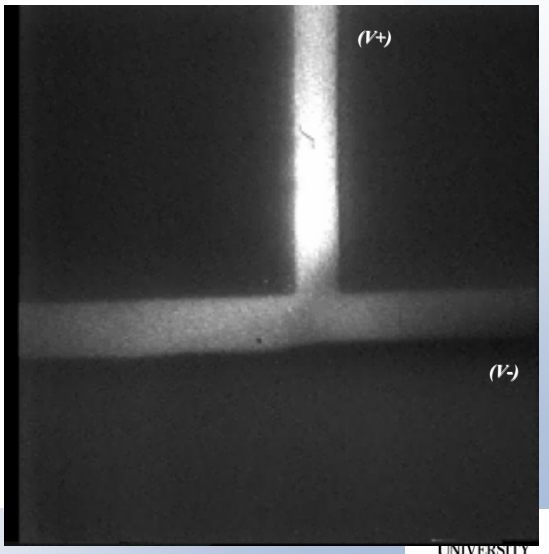


Typical Operation and Detection

- Operation
  - 10 µL to sample reservoir
  - Sample moved using 1 kV into injection area
  - Separation performed by applying 6 kV across electrophoresis channel
- Detection
  - Laser-induced fluorescence
  - 488 nm argon ion laser
  - Emission collected by PMT
  - Observes a 11 pL volume
  - Bandpass filtered signal
  - Problems w/ scattering off curved glass surfaces and bonding process
  - Detection limit 30 pM

Nanofluidics in PMMA Microdevices



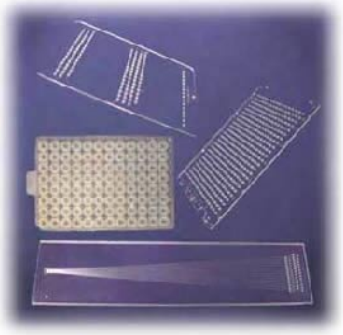


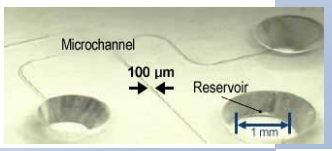
- Epi-illumination
- fluorescein seed
- Electrokinetically driven flow (reverse-polarity)
- Substrate Material: PMMA
- Channel dimensions: 20 (width) x 50 µ m

Microfluidic Chips

Electroosmotic flow for pumping

Sometimes called electrokinetic pumping





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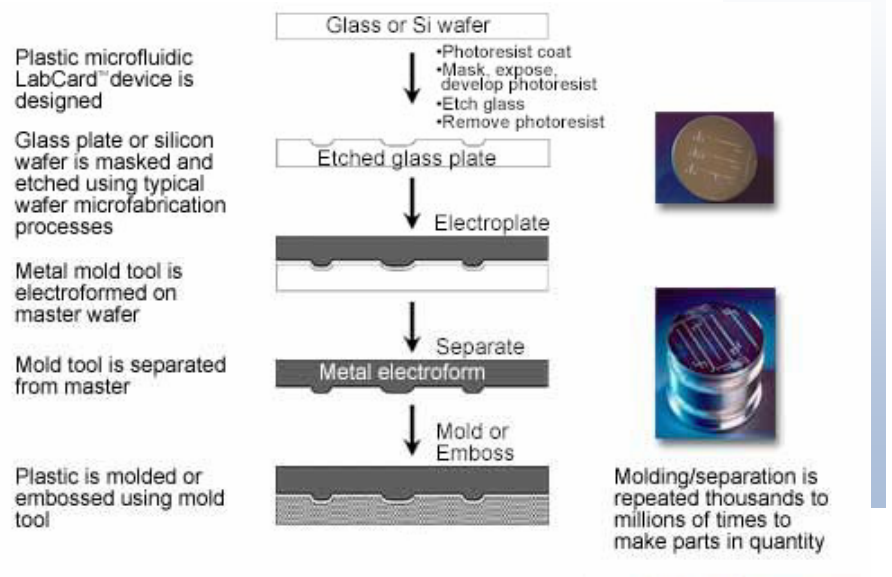
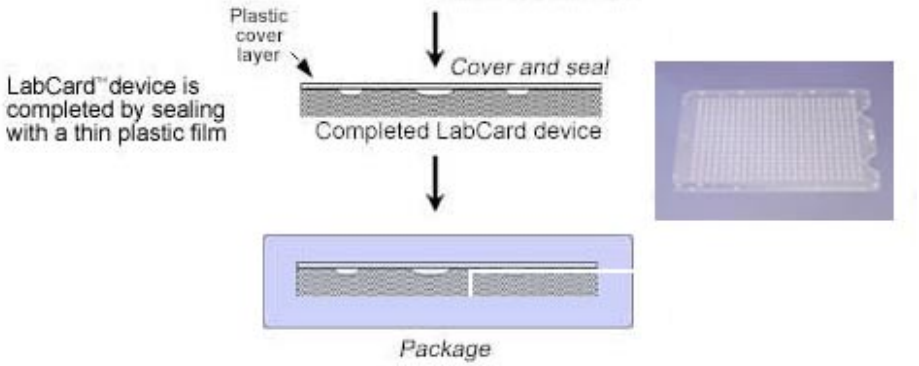
Microfluidic Chip Fabrication



Microfluidic Chip Fabrication



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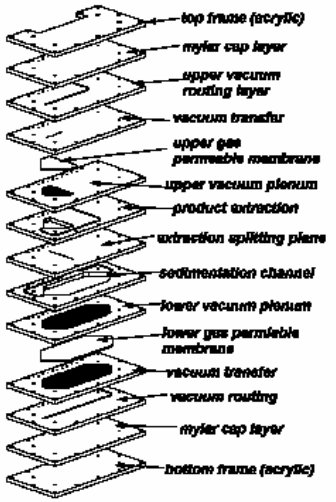
Microfluidic Chip Fabrication

- Laminate structures allow greater complexity
- Can be made using laser ablation or PDMS soft lithography



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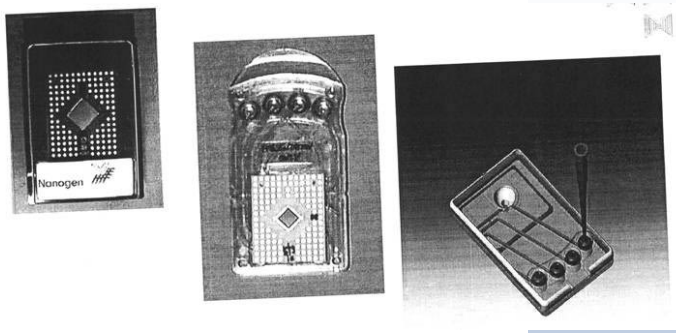
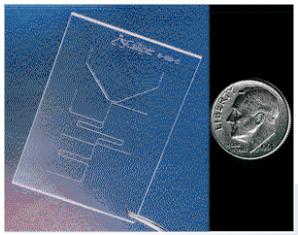
Biochips

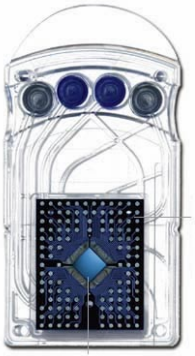
Nanogen





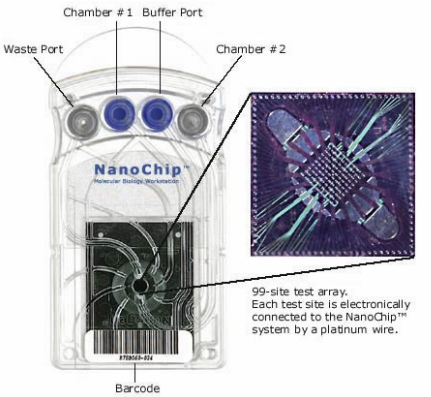
Caliper

System for Reading Chips

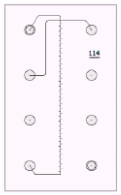


The NanoChip™ electronic chip contains platinum wires which are connected to a computer controller once the NanoChip™ is inserted into the NanoChip™ Molecular Biology Workstation.

The microchip is similar to that used in many computers and enables extremely precise control of each individual test site.

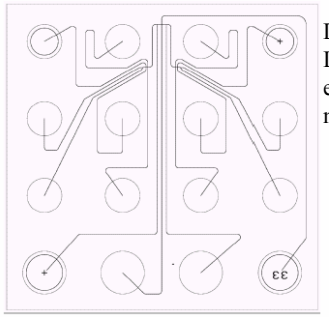


Caliper Technologies LOC- Oligonucleotide Separation



NS 114 chip (glass); separations

Figure 1: NS 114 glass chip (depth = 12 µm, width = 30 µm, and separation length from the intersection = 30 mm).



DNA LabChip™; electrophoretic mobility

Figure 2: DNA LabChip® (channels: 12 µm deep and 36 µm wide)

Oligo = man-made DNA fragment, use to initiate DNA synthesis

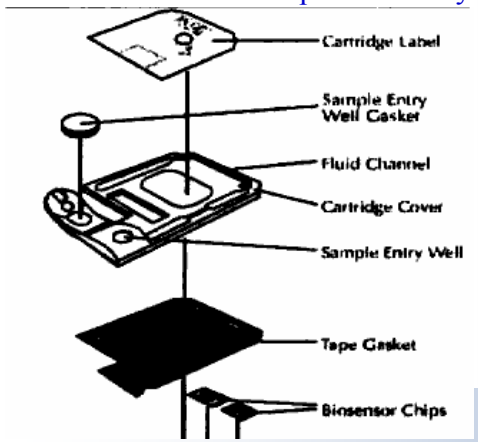
- Sequence-specific
- Large fields needed
- Long separation columns

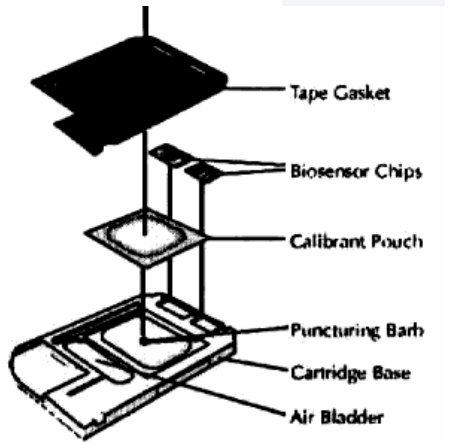
12µm X 30µm X 30 mm

12µm X 36µm X ? mm

Cartridge Concept

- In vitro sensors for multiple analytes
  - Modularity vs. integration
  - From Si to plastic and hyl





Types of Detectors

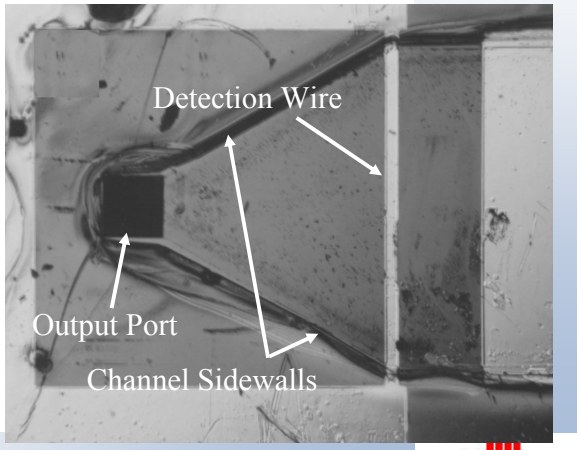
- Optical
  - Fluorescence
  - Absorption (UV, etc)
  - Light scattering
  - Refractive index
  - Radiation
- Electrochemical
  - Amperometric
  - Potentiometric
  - Conductimetric
- Mechanical
- Thermal
  - Conductivity and Flame Ionization
- Chemical
- Magnetic

Detector Issues

- Volume
- Complexity
- Sensitivity
- Selectivity
- Bulk
- Cost
- Applicability

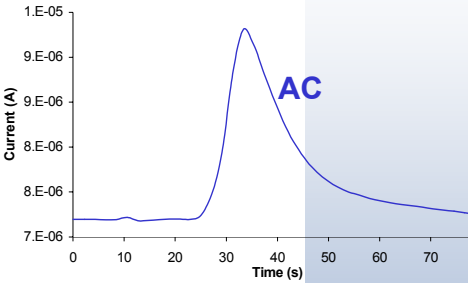
Fabrication Results

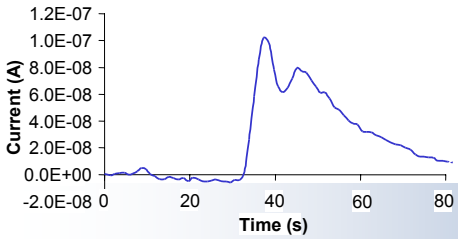
- Micrograph of detector wire across channel defined by polyimide
- Wire is 19 µm wide
- Location of wire eliminates all end effects

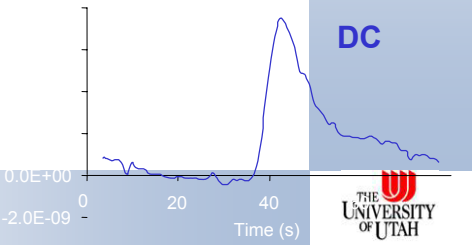


Peak Detection

- Typical measured responses shown at right
- Note high signal to noise ratio
- Sufficiently fast to detect double injection







LabCD: A Bioanalytic / µ-TAS Platform

What are the necessary platform attributes to encompass:

- Drug discovery and development
- Life science research
- Clinical and molecular diagnostics

- **Flexible fluid processing** wide range of volumes, flow rates, pressures
- **Flexibility in fluids** wide range of viscosity, pH, ionic strength, aqueous, organic solvents, biological fluids
- **Flexibility in assay** homogenous, heterogeneous, cell based
- **Detection options** Colorimetry, fluorescence, luminescence
- **Integration** World-interface + macrofluidics + microfluidics + temperature control + detection ...
- **Automation and simplicity** replace labor intensive processes

From Gamera Biosciences

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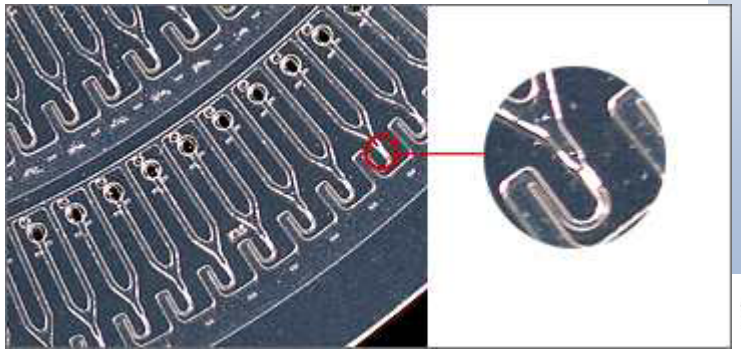
LabCD Platform

- Instrument
  - Control
  - Rotary drive
  - Detection
  - Actuation

- Disc
  - Fluidics layer
  - Electronics layer
  - Informatics layer



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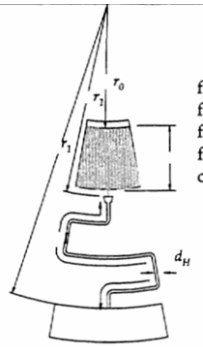
Pumping and Valving



Disc geometry/properties		Fluid properties		Rotation	
fluid channel position	r_0, r_1	density	ρ	velocity	ω
fluid channel diameter	d_H	viscosity	η	accel	α
fluid channel length	l	surface	γ		
fluid "head"	H				
contact angle	θ_C				

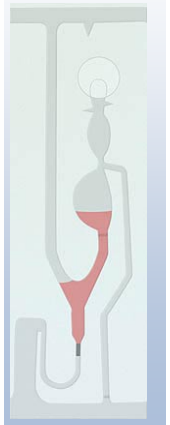
$$\Delta r = [r_1 - (r_0 - H)]$$
$$\bar{r} = [r_1 + (r_0 - H)]/2$$

Pumping

$$U = \frac{d_H^2 \rho \omega^2 \bar{r} \Delta r}{32 \eta l}$$
$$Q = AU$$

"Capillary" Valving

$$\omega_c \propto \left[\frac{\gamma \sin\theta_c}{\rho \bar{r} \Delta r d_H}\right]^{1/2}$$



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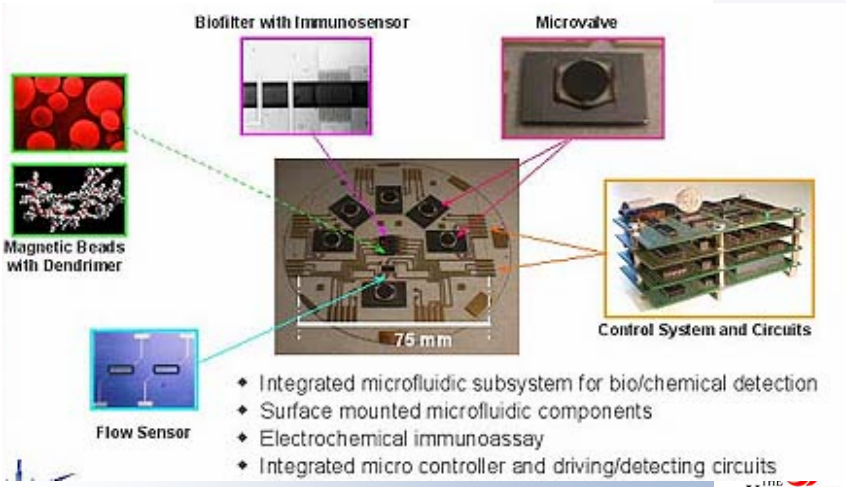
Integrated Microfluidic System



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Microfluidic Motherboard

Reservoir Level

The Mini System

Detection Level

Reservoirs on the Mother-Board

The Stacked Mother-Boards

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Challenges for Total Integration of Microfluidic Chips

- Reagent storage and reconstitution
- Integrated microvalves and micropumps
- Packaging
  - Interconnects (optimize → reduce → eliminate)
  - Filling / bubbles / dead volume
  - Leakage
- Surface functionalization
- Microflow measurement and characterization
- Control algorithms, data processing, and communications
- Integrated, ultrasensitive detection
- Heterogenous material integration
- Sensitivity limited by sample volume (front end amplifiers?)
- Low power
  - Harness energy from host or ambient
  - Low power pressure sources

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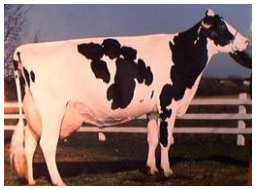

Embryo Care Systems

- Domestic animal breeding is big business, and getting bigger…
  - **IVF, transgenics, cloning**

Costs are high

IVF - $100s to a few $1000s
$1-3 million to produce a transgenic cow

Procedures very "harsh" and laborious

Courtesy of David Beebe, University of Wisconsin

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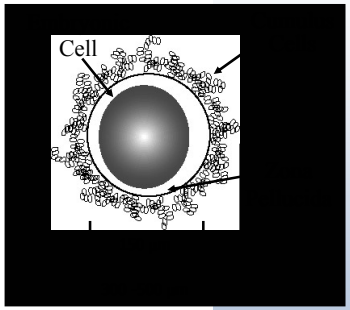
Embryo Physiology

- Embryo size
  - ~ 100μm (Mice) to 150μm (Cattle)
  - Doesn't significantly change over the culture period
- Zona Pellucida
  - 8-15 μm thick
  - Passive glycoprotein matrix
  - Acts as a porous fence around embryonic cells
- Cumulus
  - On egg when removed from oviduct
  - Protects egg from polyspermy



Embryo with Cumulus

Embryo

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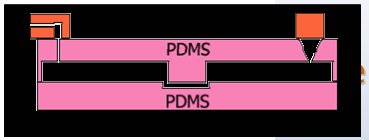
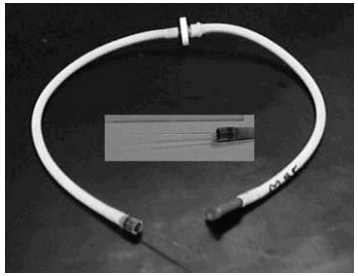
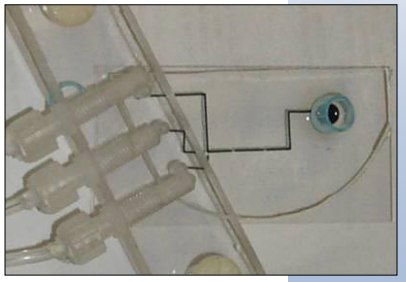
Rationale for Embryo Micro Processing Systems

Current techniques are inefficient & labor intensive







Traditional **New**

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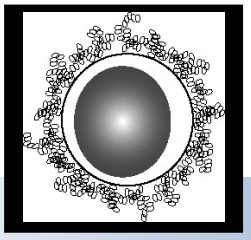
Microfluidic Removal Procedures

Zona Pellucida

- Chemical process
- Chimera formation

Cumulus Cells

- Mechanical process
- Most *in-vitro* procedures



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Zona Pellucida Removal

A Chemical Process

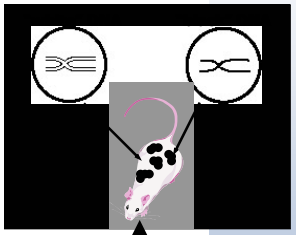
Chimera Formation

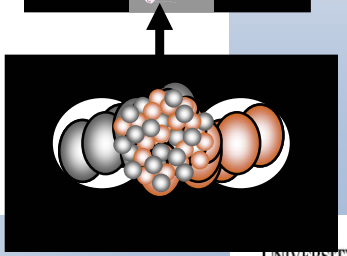
Tarkowski, *Nature* 1961

- Remove zona
- Place embryonic cells in intimate contact
- Culture

Microfluidic Advantages

- Quick media changes
  - "t" junction
- Small numbers of embryos
- Ease of placement





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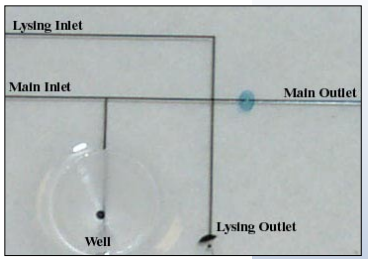
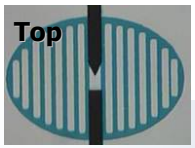
Zona Removal Device

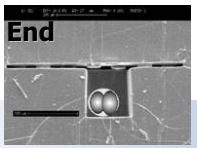
Sample Loading And Unloading
- Easy embryo introduction and retrieval

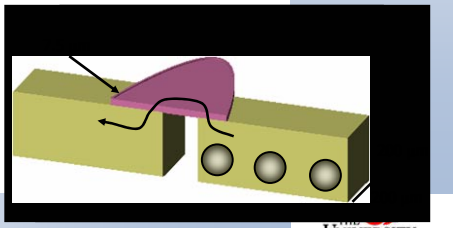
"t" junction
- Precise plug formation

"Parking" region
- Allows for quick media changes









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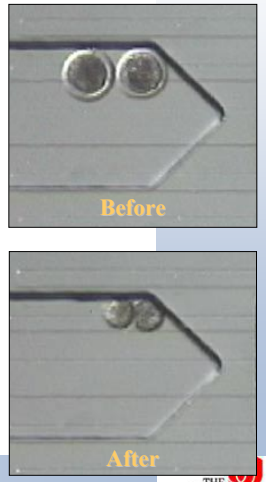
Zona Removal Results

Introduce embryos into device
Move to parking region
Flow in plug of acidic media
Flow in culture media



Cumulus Removal
A Mechanical Process

Cumulus Removal

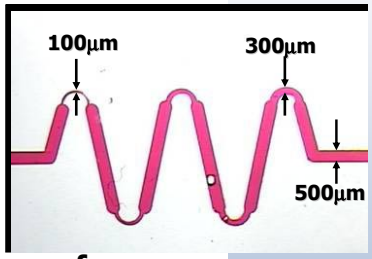
- **Useful for**
  - **Freezing**
  - **Fertilization**
- **Present Techniques**
  - **Manual and done in bulk**
  - **Requires quick and precise handling**
  - **Induce high stress**

Microfluidic Advantages

Precise fluidic control
Single embryo processing

Initial Design

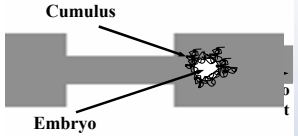
Channel with "S" curves
Parametric study
Each curve progressively narrower
50 μm decrements



Observations
Cumulus pushed to front and rear of embryo
Embryo too large to fit through final corner and cumulus removed

Second Generation

- Conditioning Regions
  - For arrangement of cumulus prior to removal



Removal Ports
Remove cumulus from embryo