Microfabricated Biochemical Analysis: Bulk Properties to Single Molecules

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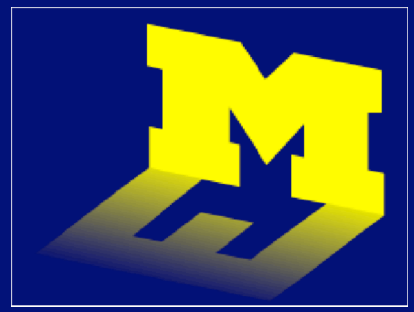
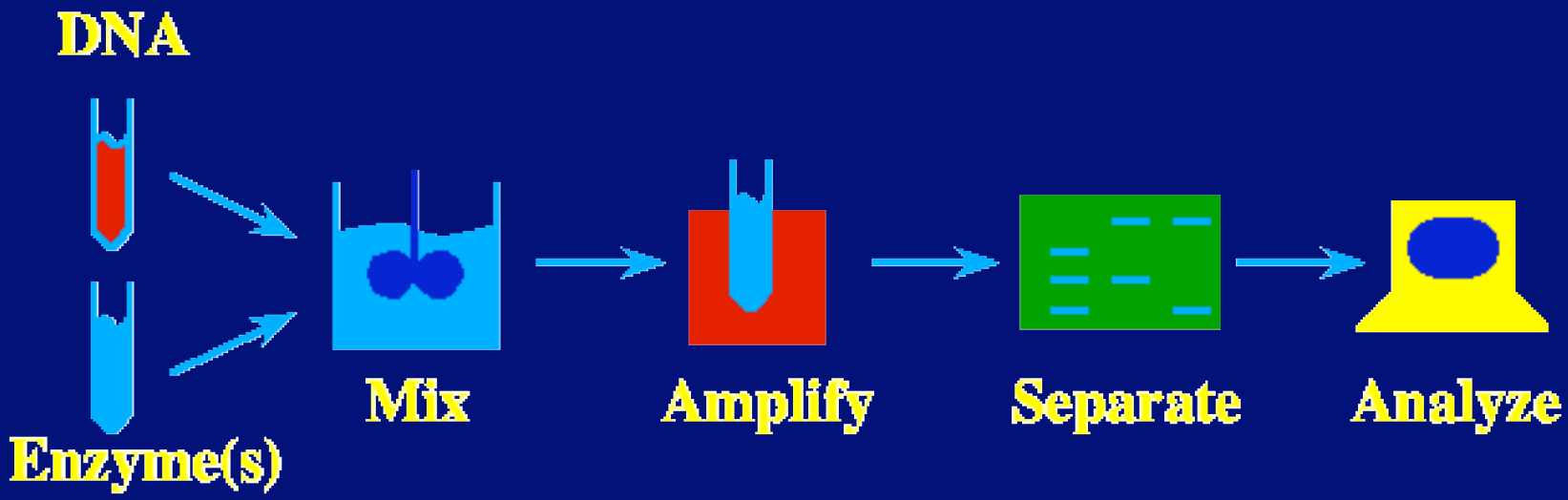
Support:

NIH (NHGRI, NIAID, NIDCR)

Becton Dickinson



Macro-scale



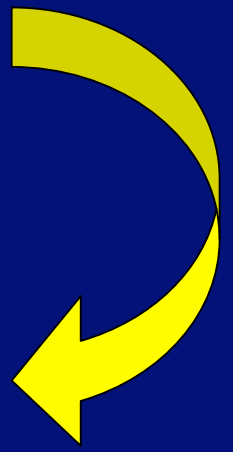
Micro-scale

Brief History of the Personal Computer

1972 The first digital personal microcomputer:
MITS 816 (no display or keyboard)

1976 Apple II
(Cray I Supercomputer)

1981 IBM PC, DOS



2002 Mac G4 Dual Processor (15 Gigaflops)

Ref: Virtual Computer History Museum
(http://virtualmuseum.dlib.vt.edu/index.html)

Brief History of the Genome Project

1953 Watson and Crick publish structure of DNA

1990 NIH/DOE present 5-year plan to Congress

1998 Celara Genomics formed
(Integrated DNA analyzer)

2000 Draft of human genome

2003 Sequencing centers (G5): DOE JGI, Baylor College,
Sanger Centre, Washington U., Whitehead Institure/MIT

Ref: NIH (NHGRI)
(http://www.ornl.gov/hgmis/project/timeline.html)

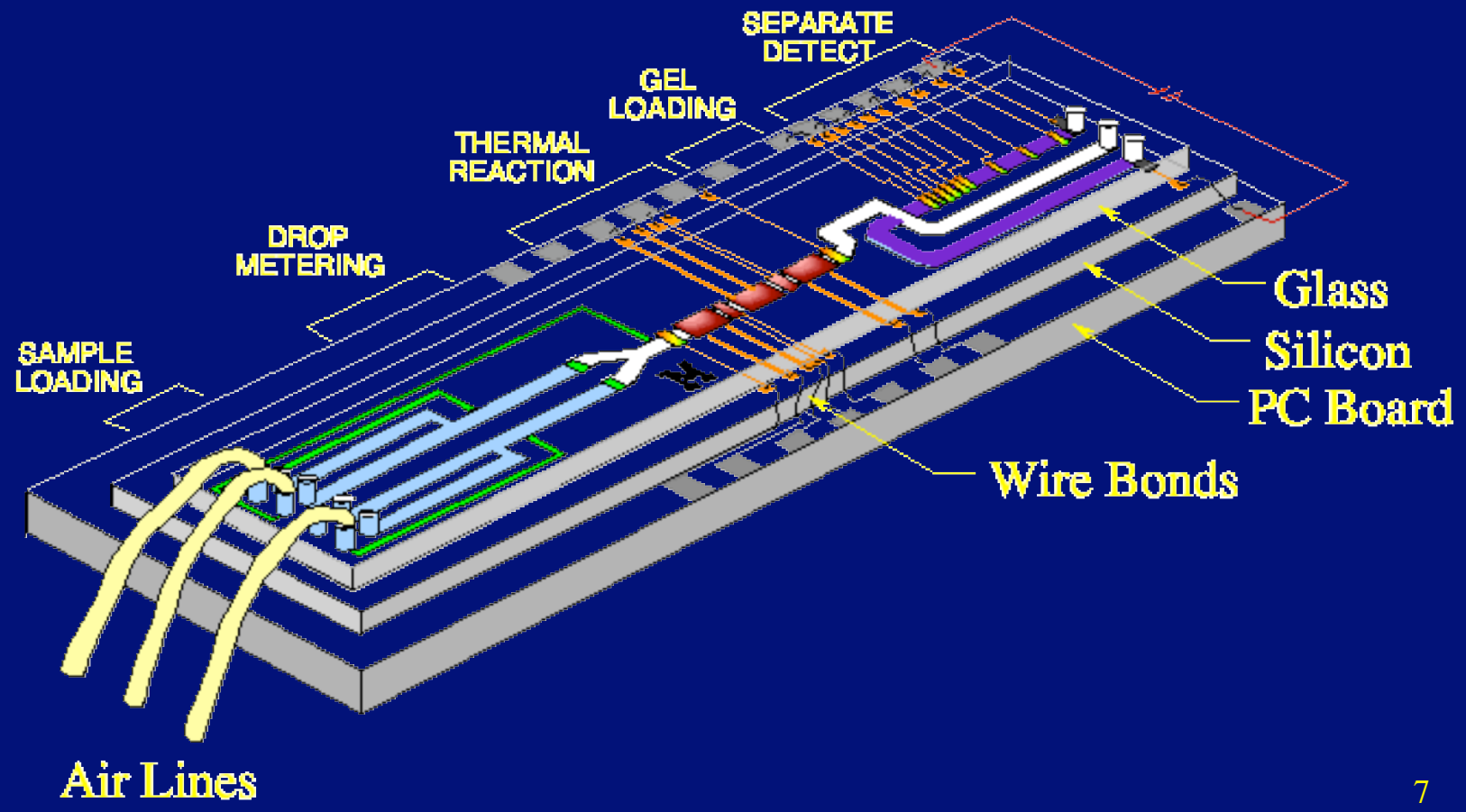
Impact of Genetic Testing

Application	units of observation	approx. calculation	Tests yr.
Medicine:			
Pathogen testing of clinical samples	pathogens X patients	$10^3 \times 10^6$	10^9
Genetic testing (simple, single gene traits)	genes X patients	$10^2 \times 10^6$	10^8
Genetic testing (complex, multi-gene traits)	genes X variants X patients	$10^3 \times 10^3 \times 10^8$	10^{14}
Agriculture:			
Plant and animal breeding	genes X species X individuals	$10^3 \times 10^2 \times 10^8$	10^{13}
Pathogen testing of field samples	pathogens X species X individuals	$10^3 \times 10^2 \times 10^8$	10^{13}
Biotechnology:			
Bacterial testing of food products	pathogens X batch	$10^2 \times 10^7$	10^9
Police forensics	samples X identity loci	$10^7 \times 10^2$	10^9
Environmental and ecological testing	samples X identity loci	$10^8 \times 10^2$	10^{10}
		TOTAL =	**$>10^{14}$**

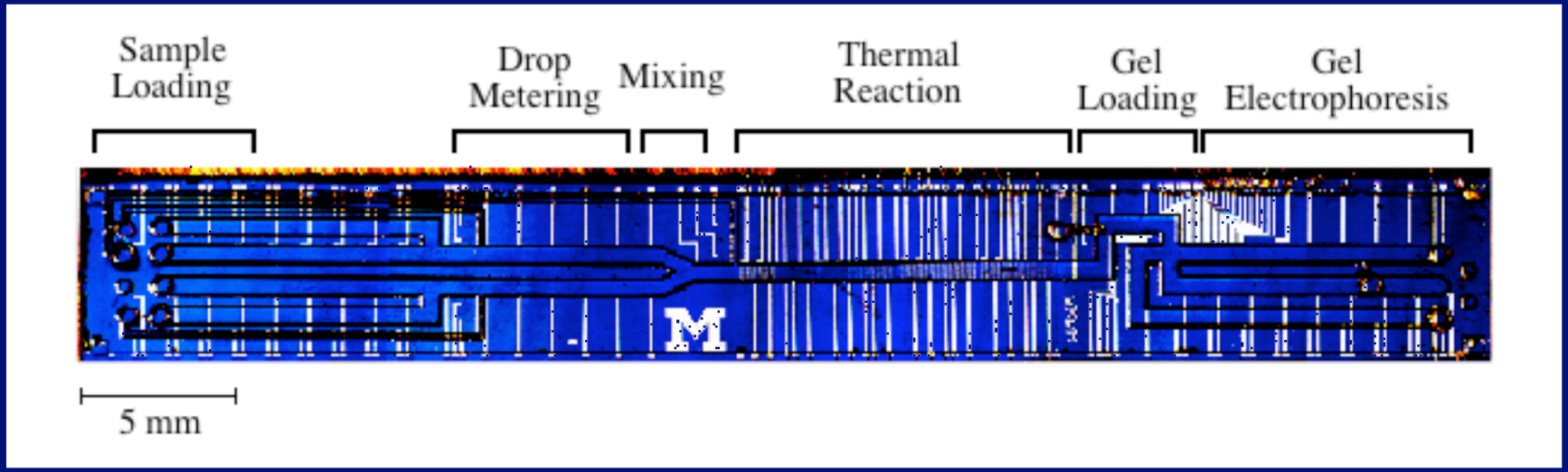
Microfabricated Biochemical Analysis

- Integrated Device
- Phase-Change Valve
- Viscometer
- Single Molecule

Microfabricated DNA Analysis: Fluidics, Reactions, and Separations

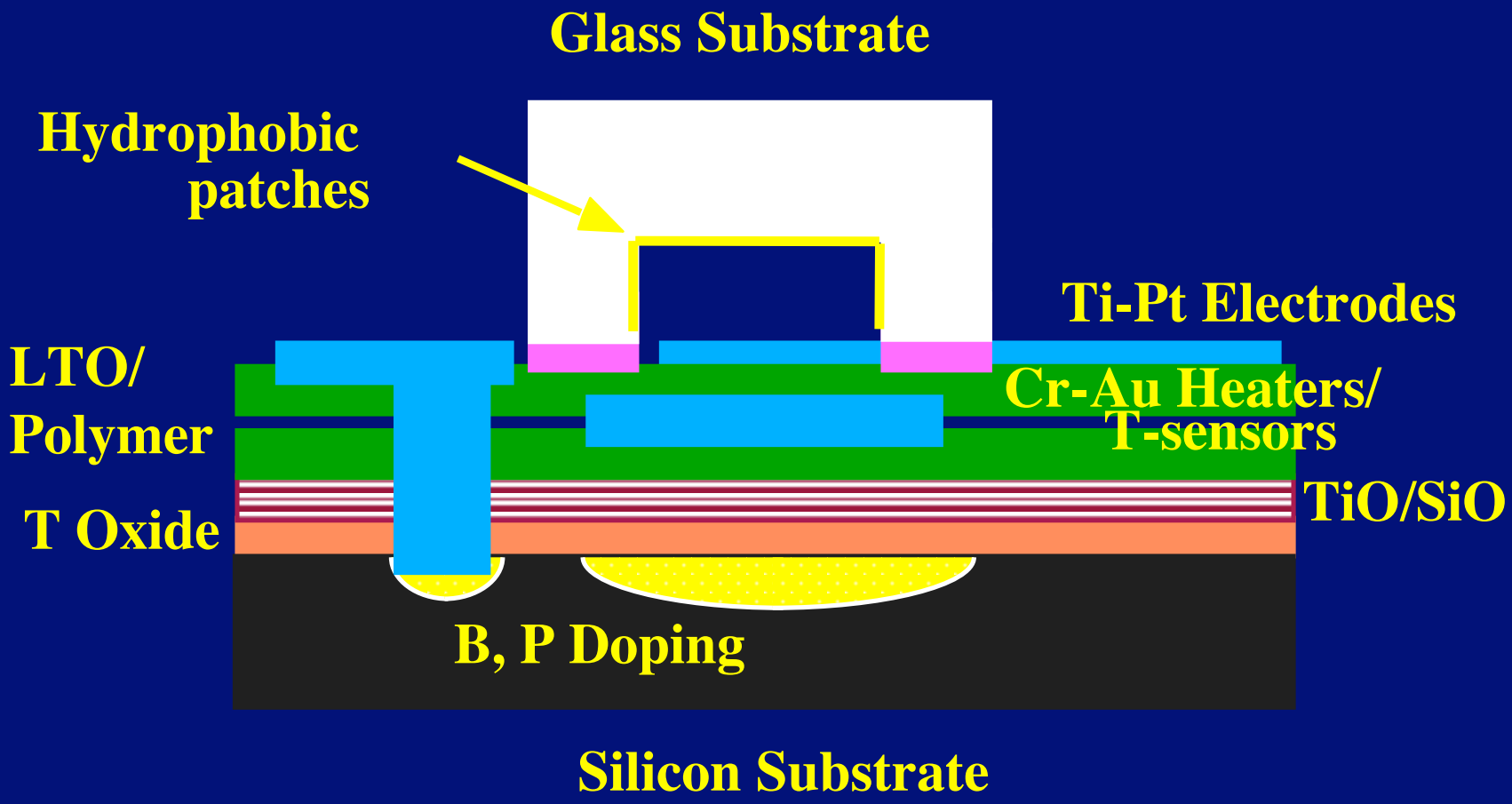


Si/Glass Device

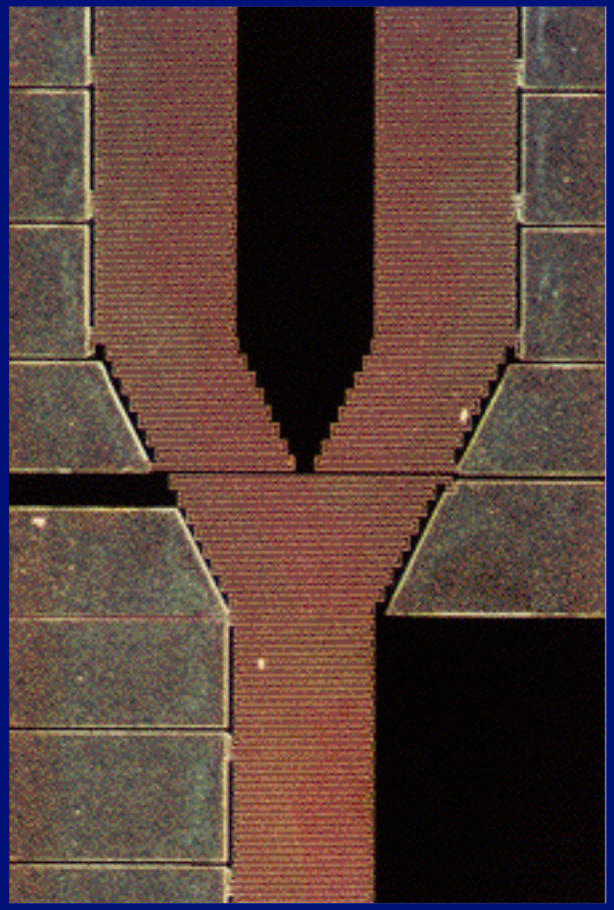


- Individual Drops (~100 nl)
- Integrated Heating (± 0.1 C)
- Crosslinked Gel (PAGE)
- Integrated Detection (1 ng/μl)

Device Construction

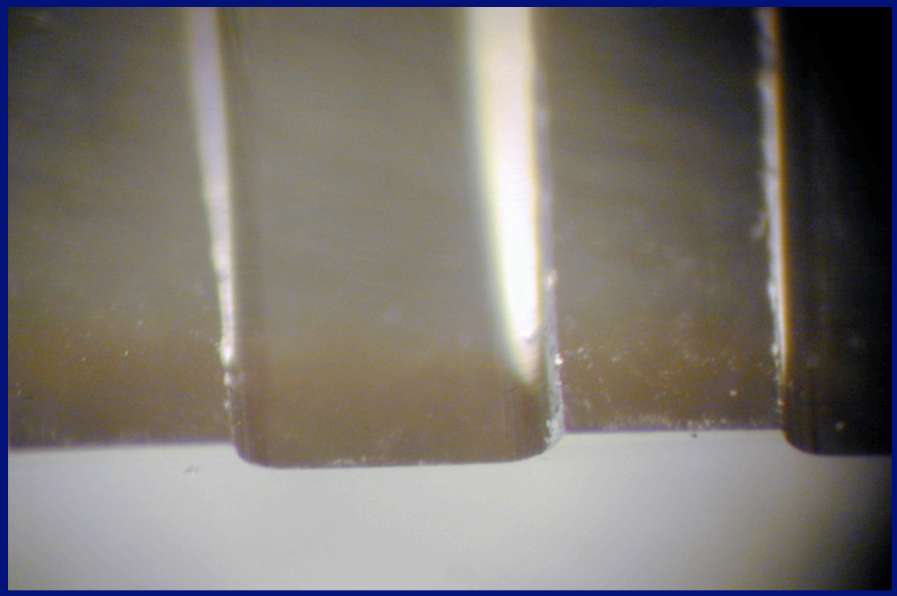


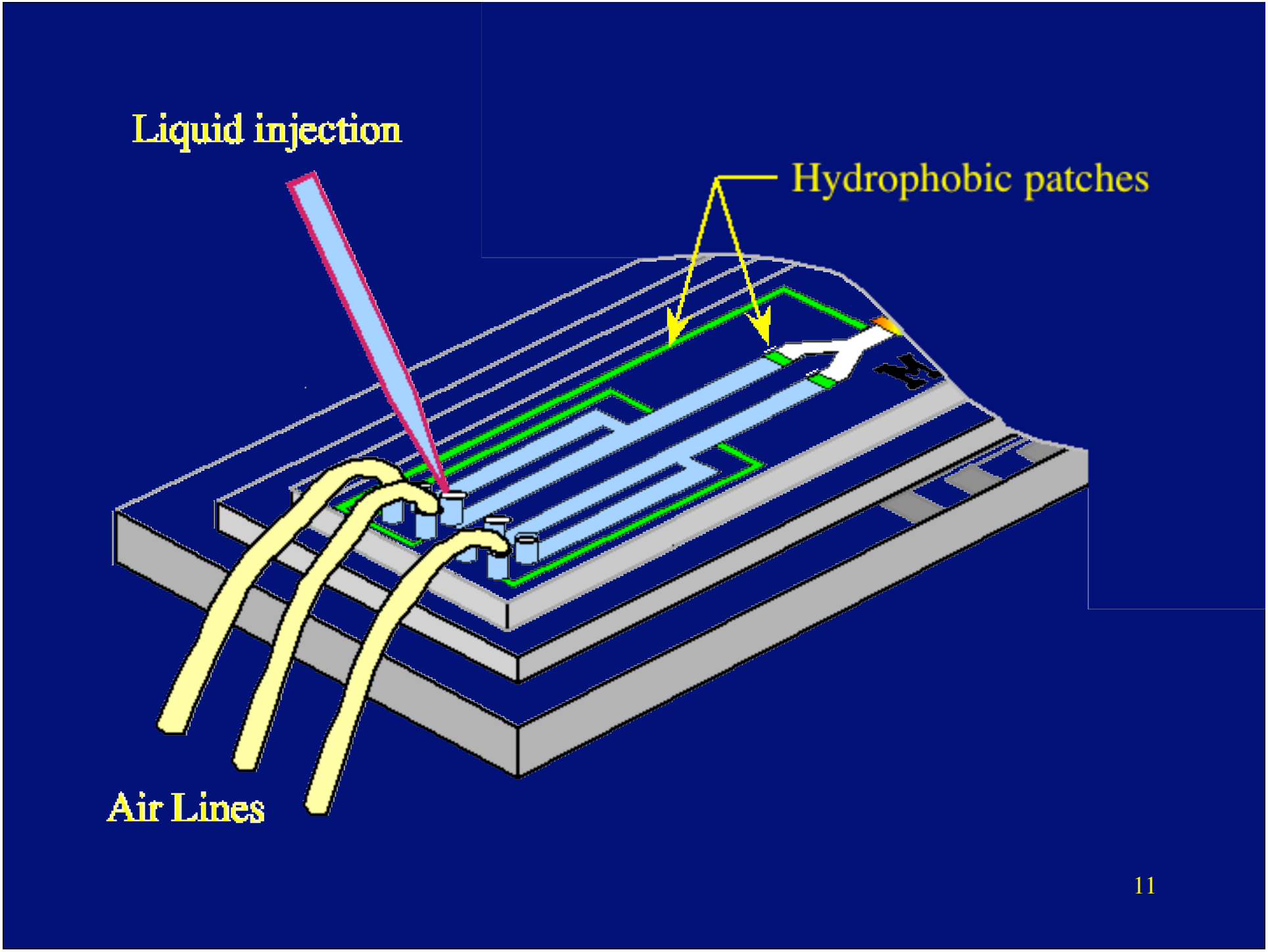
Device Construction



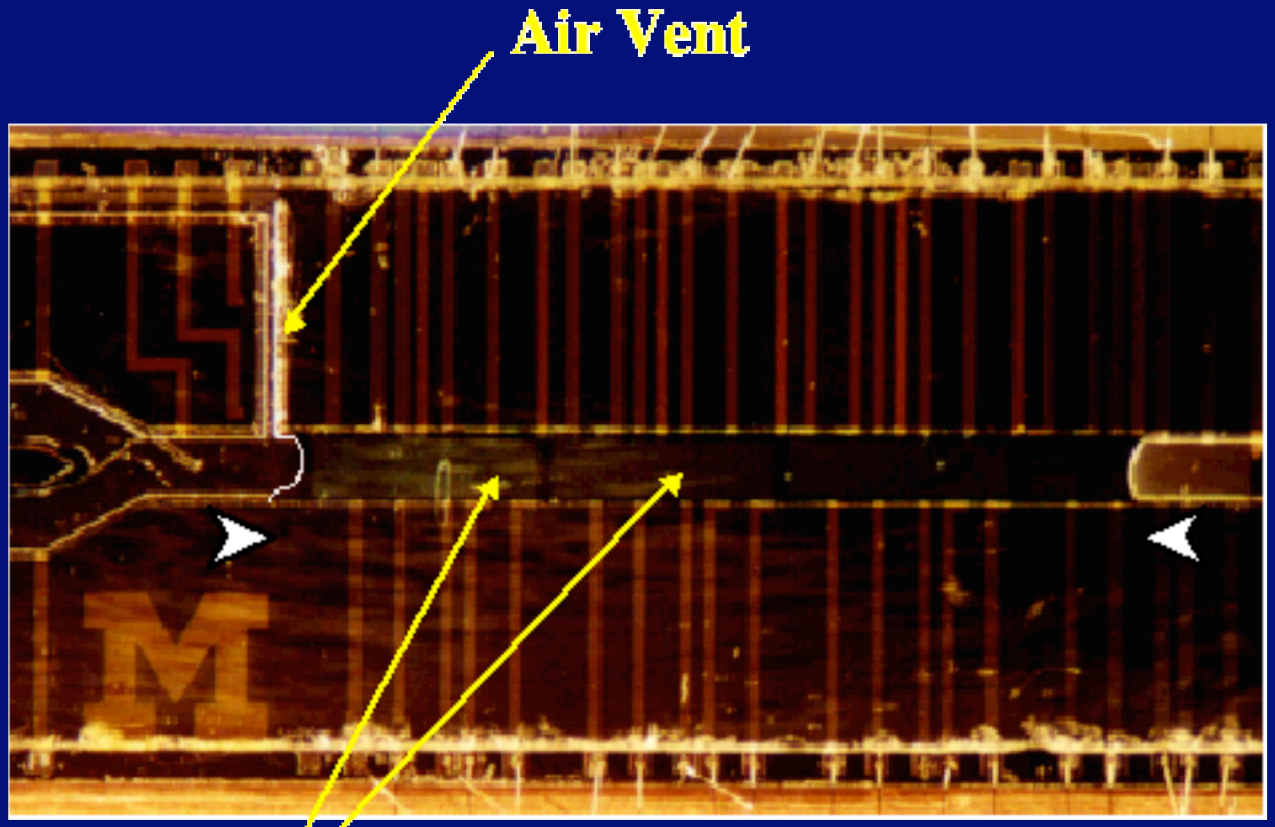
Heater/Sensor Substrate

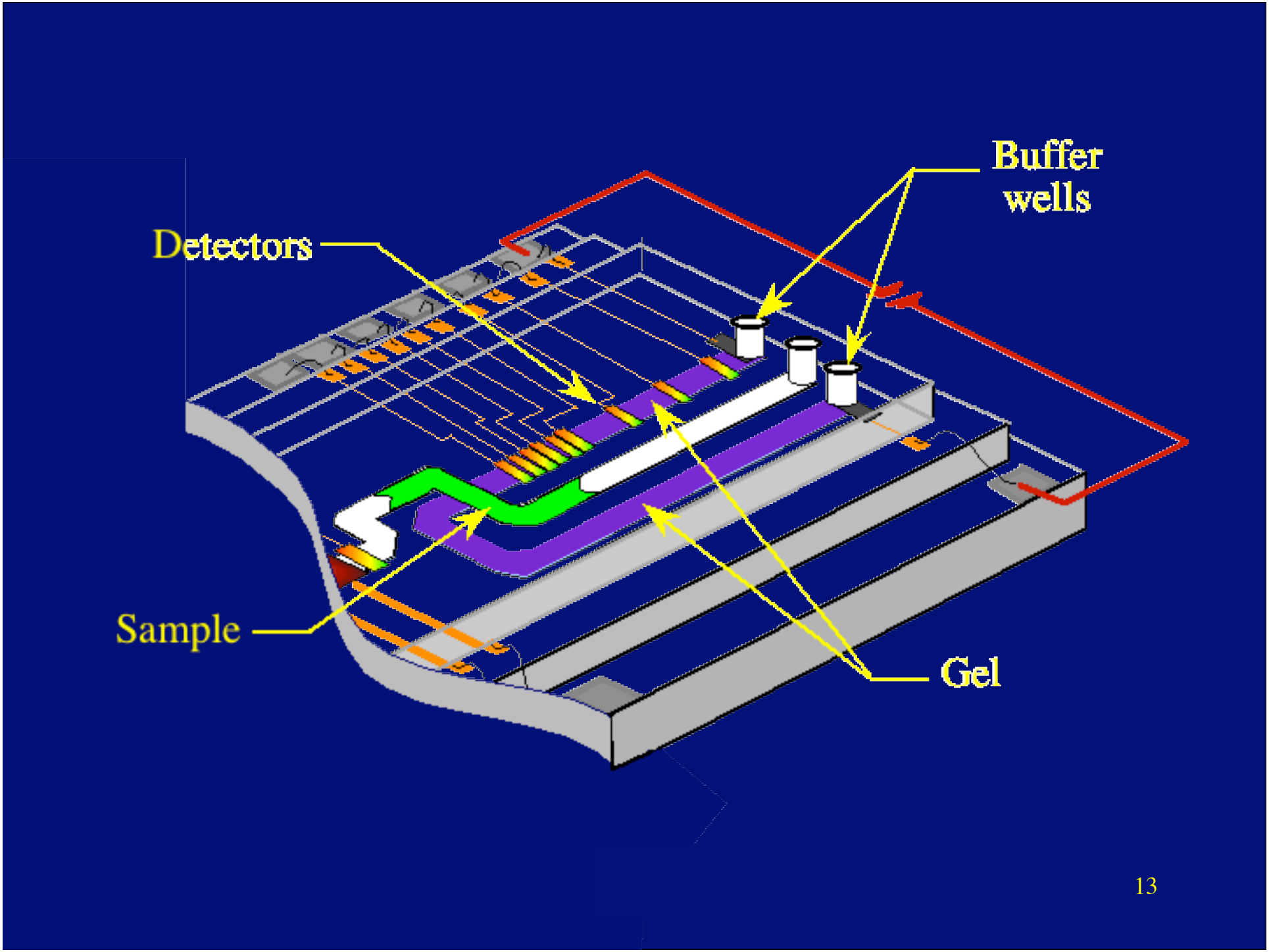
Glass Substrate




Liquid injection
Hydrophobic patches
Air Lines

240 nl Reaction Volume




Buffer wells
Detectors
Sample
Gel

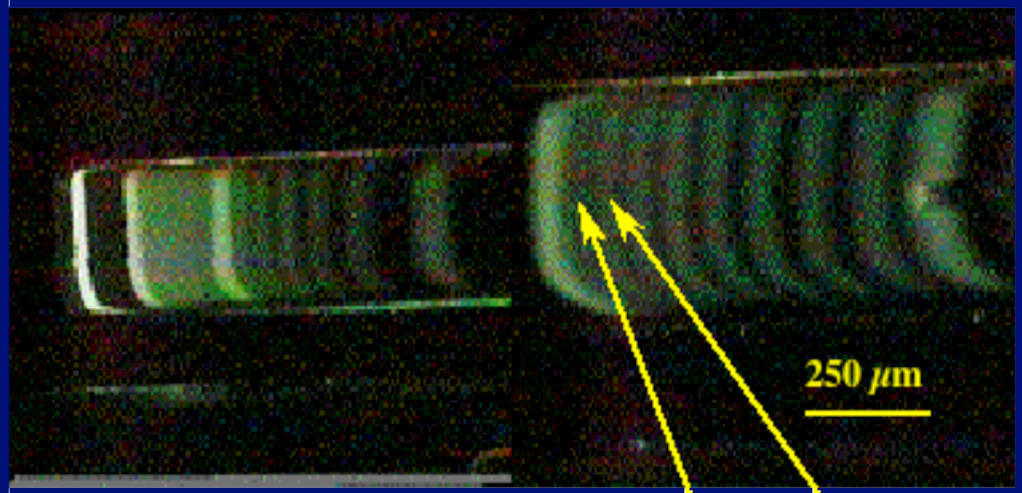
Gel Electrophoresis: Loading

Sample in intersection

10 s compaction

Remove excess

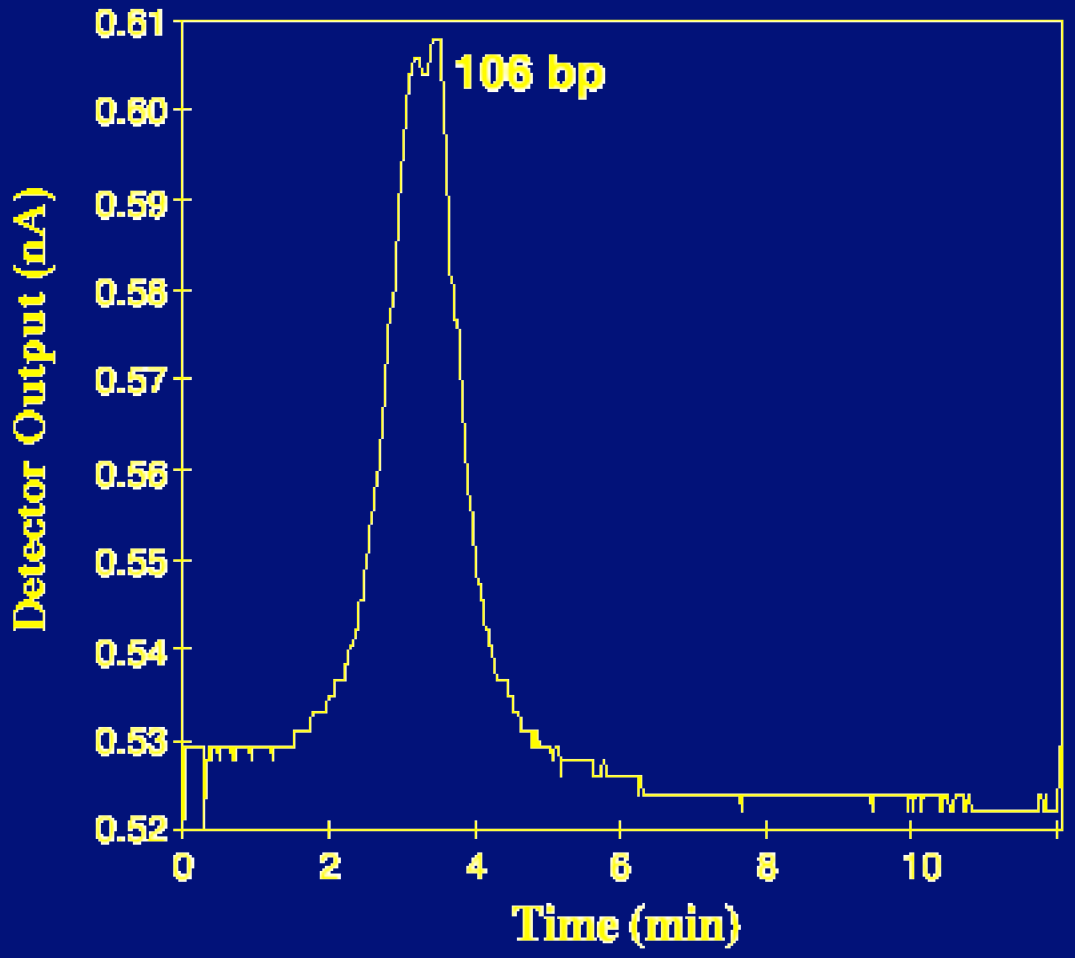
Gel Electrophoresis: Separation



Field of 6 V/cm
50 bp ladder

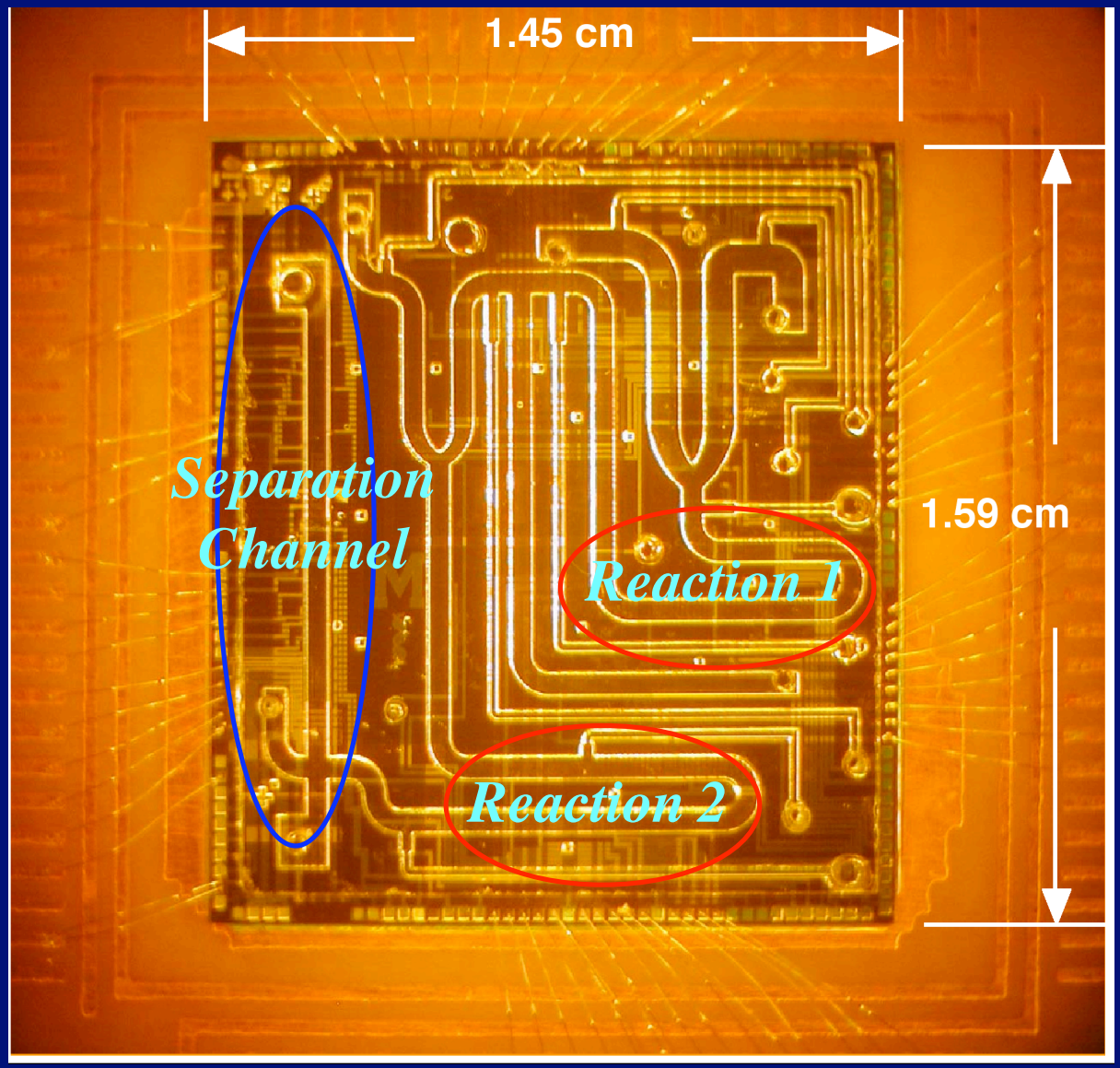
800 bp 750 bp

Integrated Run



- Injection (μl)
- Meter (120 nl)
- Mix (DNA, Enzymes)
- Amplification (SDA)
- Separation (8 V/cm)
- Detection (on-chip)

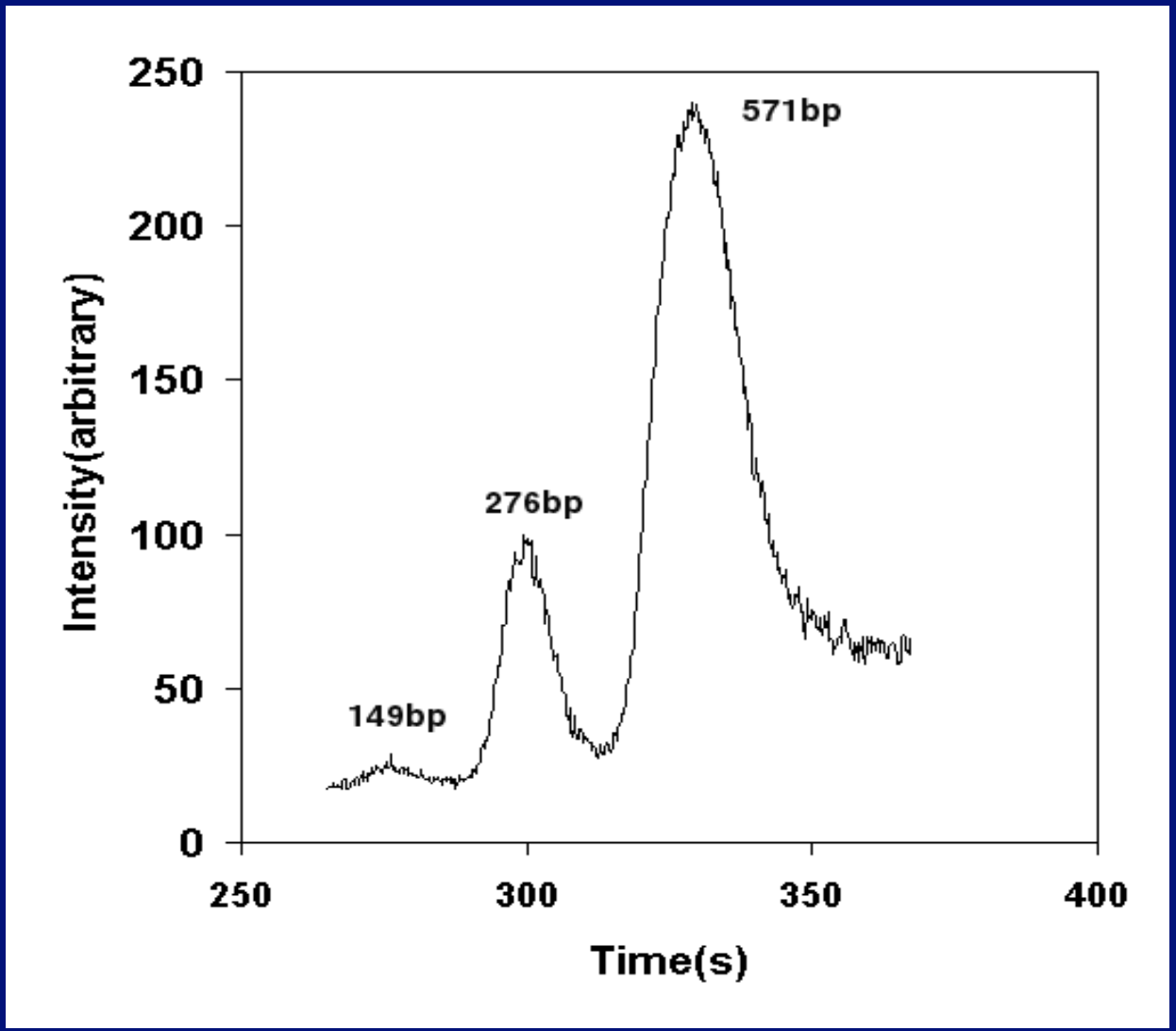
Two Reactions in Series + Separation



Reaction system

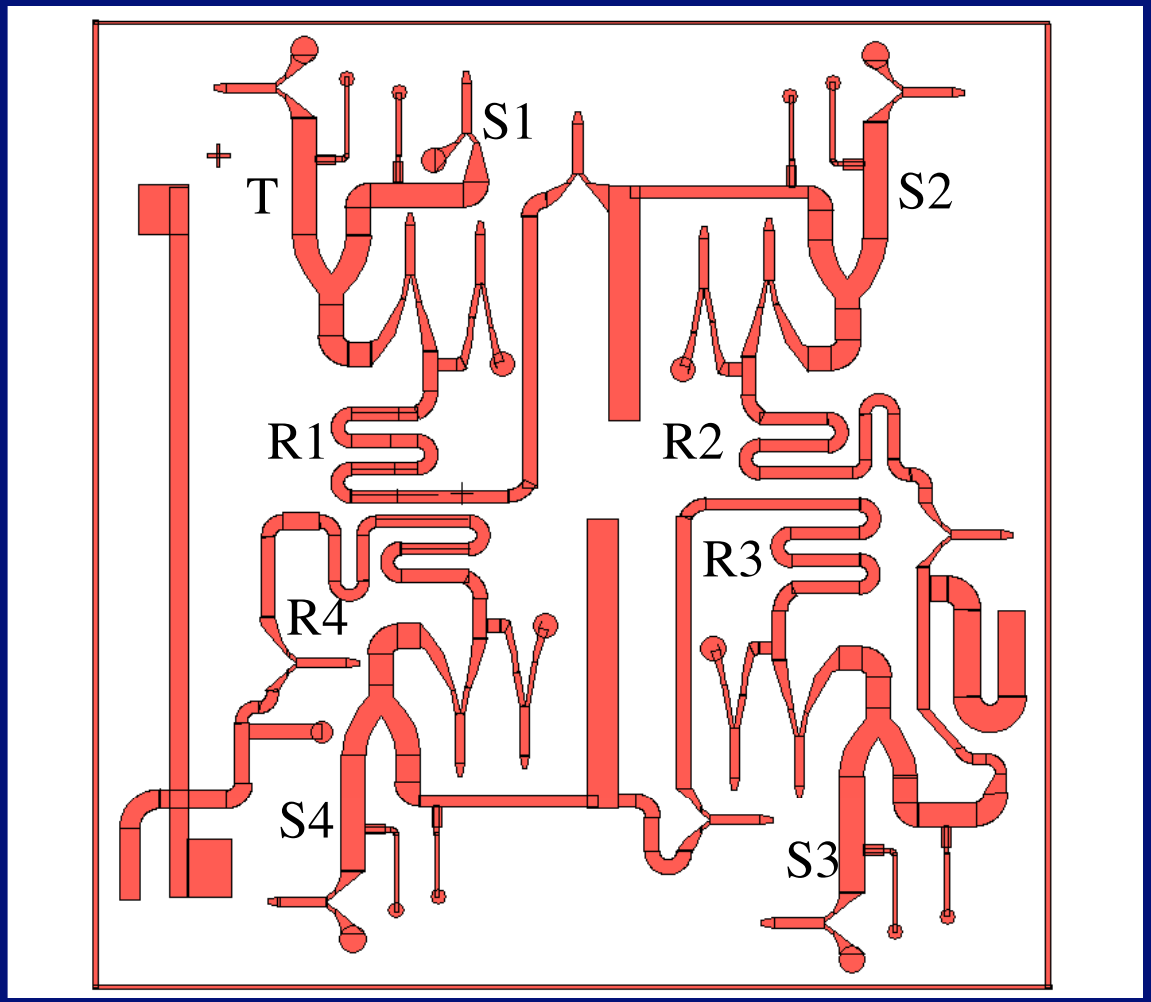
Reaction volume: 240 nl
Reaction temperature: 37°C
Reaction time: 10 minutes

Separation Result

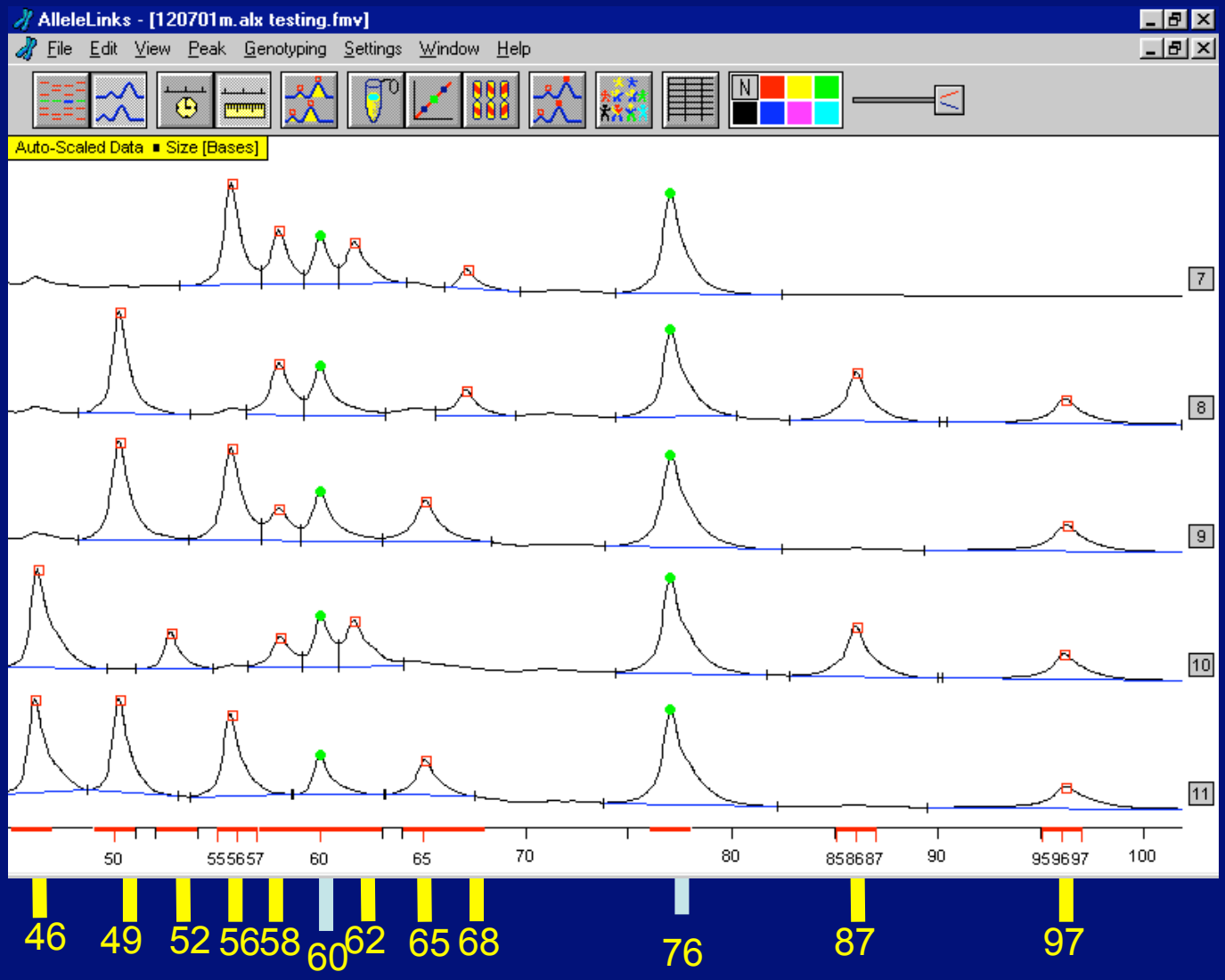


Multiple Reaction Chambers

(4 reactions w/ valves, 1 separation)



Multiplexed Genotyping
(PCR/PCR/ExoSAP/Lig (LDR)-Single tube reaction)



10-plex SNP LDR genotyping
+ 2 positive control peaks.

300 individuals typed in <3 days.

5 individuals shown on ALF

Components for Analysis

Fluidic:

- Fluidic manifold
- Phase-change (wax) valves
- Expansion chamber pumps
- Splitting/mixing
- Viscometer

Separation:

- Short cross-linked gels
- Linear gels
- Replaceable polymers
- Native/denaturing

Reaction:

- PCR amplification/ quantification
- Ligation
- Roll cell convection
- Sanger seq. reactions

Other:

- Bonding/packaging
- Liquid detection
- Reagent storage
- Single molecules

Microfabricated Biochemical Analysis

- Integrated Device
- Phase-Change Valve
- Viscometer
- Single Molecule

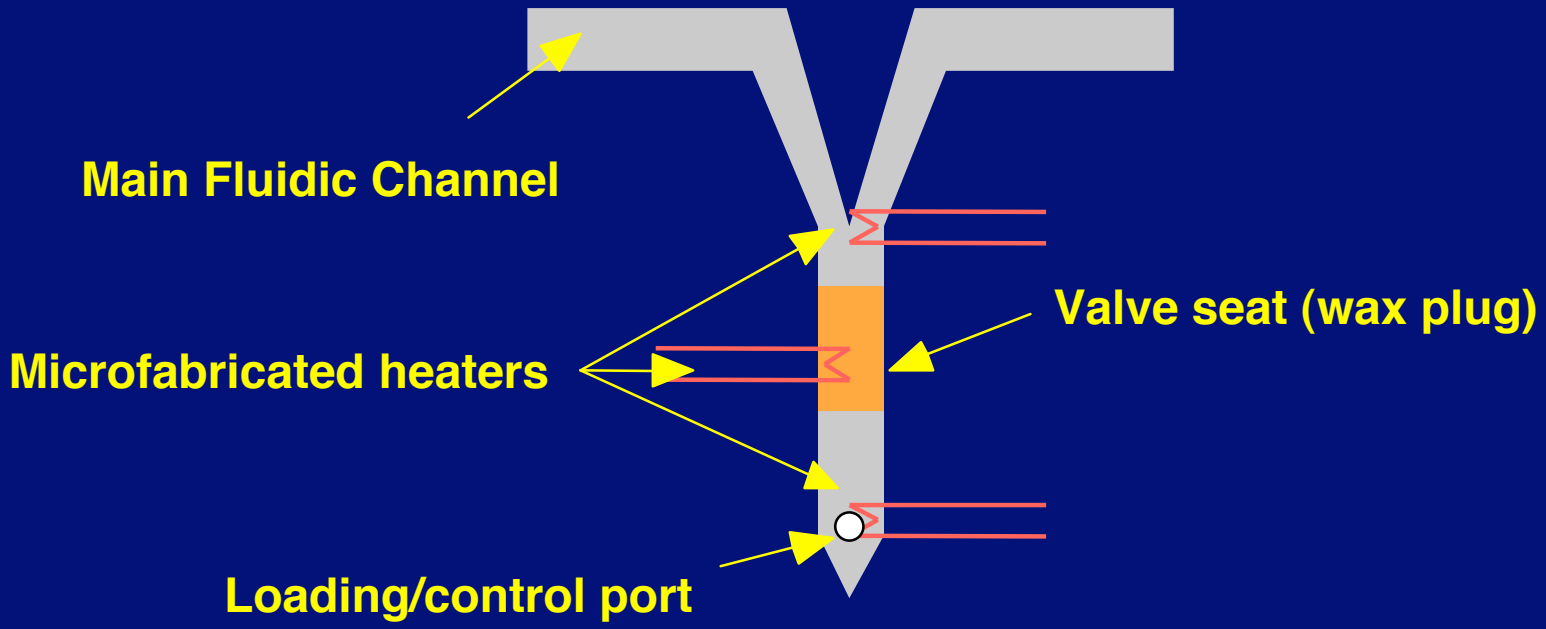
Ideal Sealing Valve

Requirements

- Biocompatible
- Simple actuation
- No new fabrication steps
- Individually addressable
- Latched Valve
- Seals both gas and liquid (T up to 95 C)

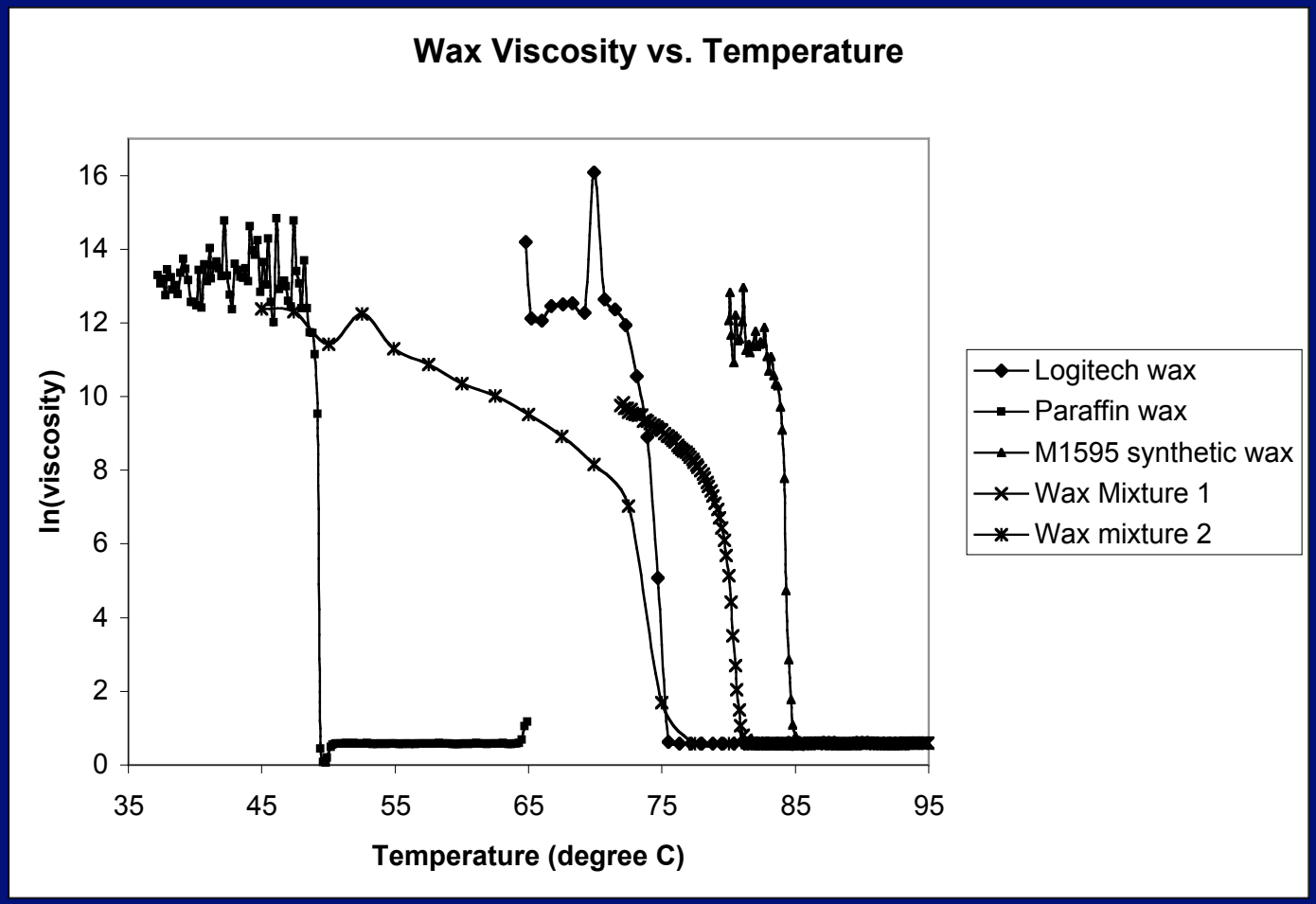
Phase-Change Valve

Concept



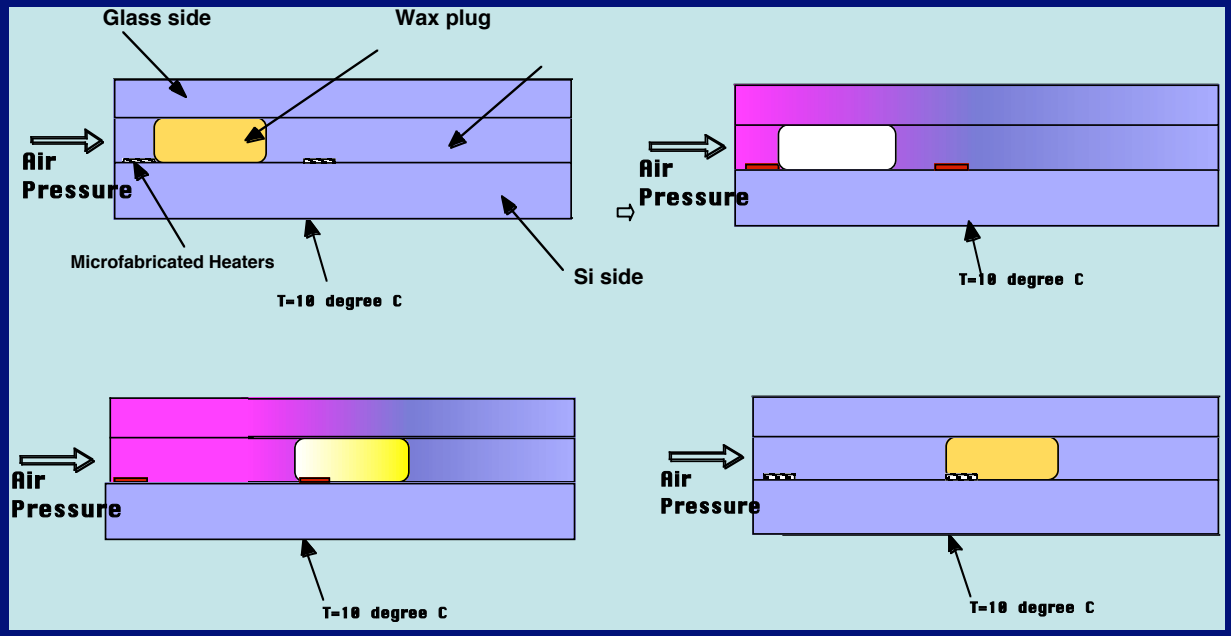
Phase Change Material

Transition



Controlled motion

Temperature gradients

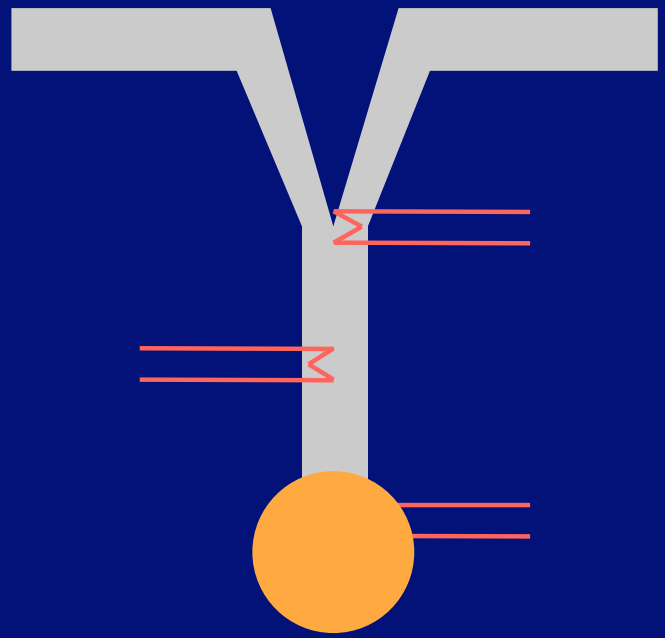


- Temporal/spatial gradient
- Front solidification controllable
- Arrays of valves possible

Phase-Change Valve

Operation

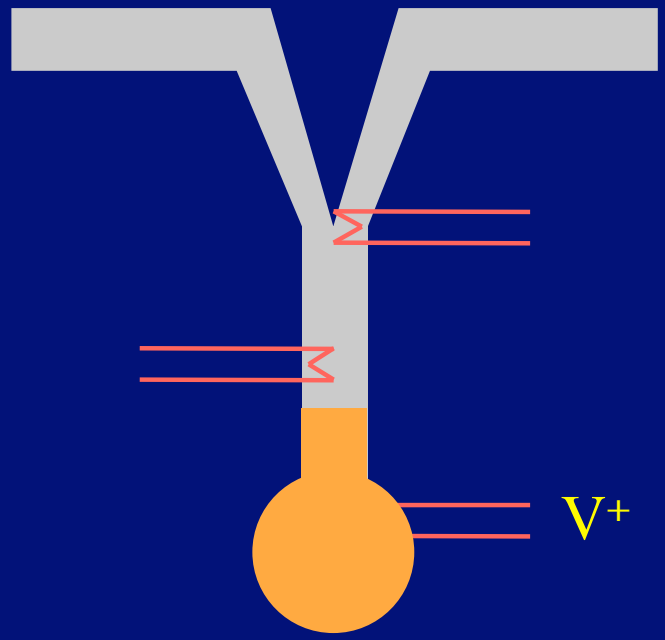
Loading



Phase-Change Valve

Operation

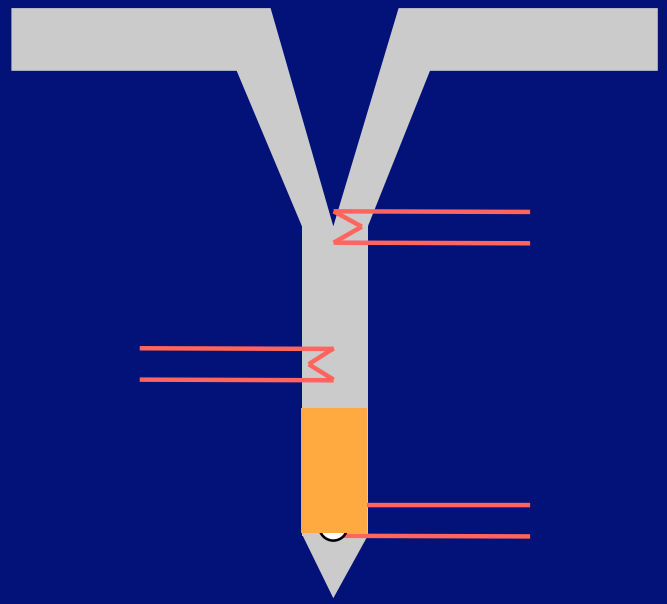
Loading



Phase-Change Valve

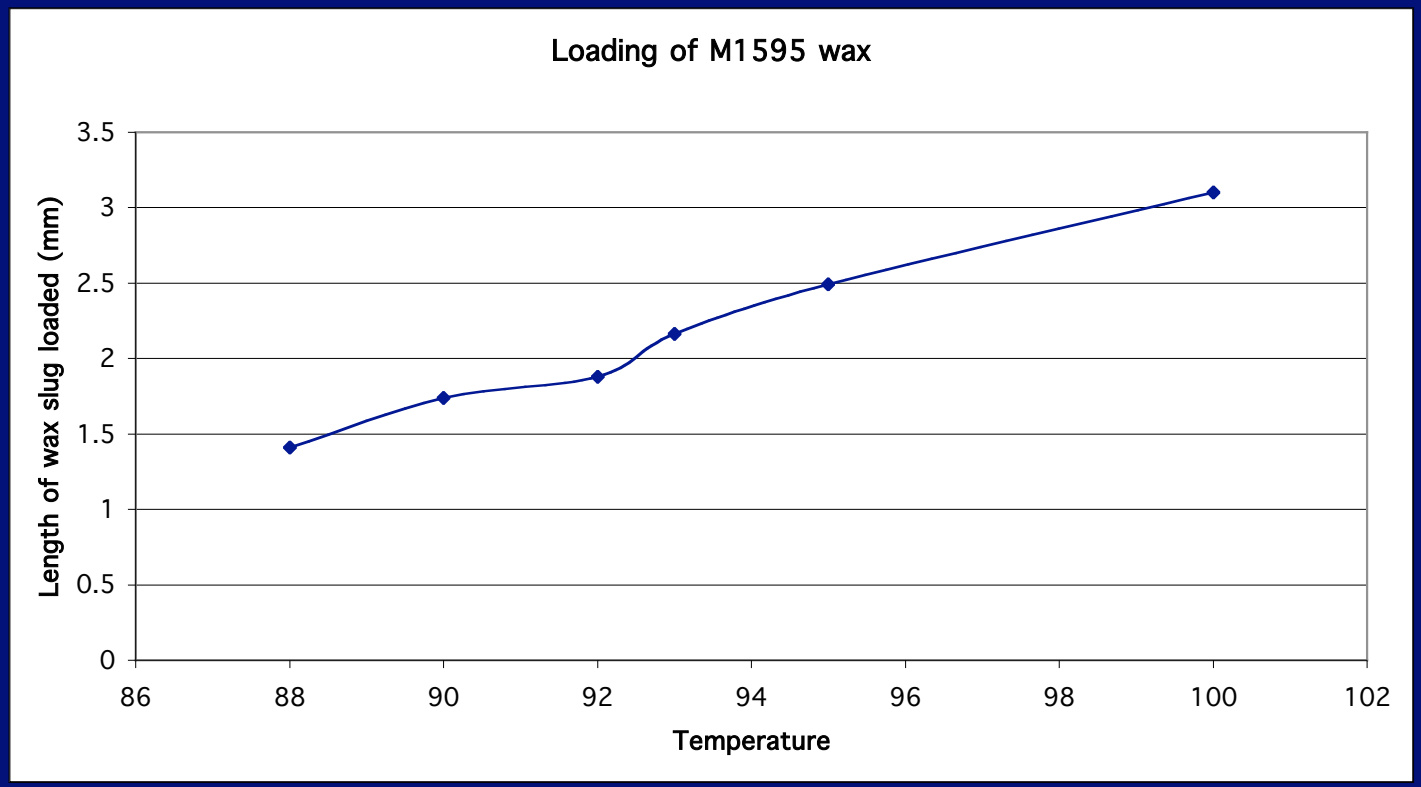
Operation

Loading



Phase-Change Valve

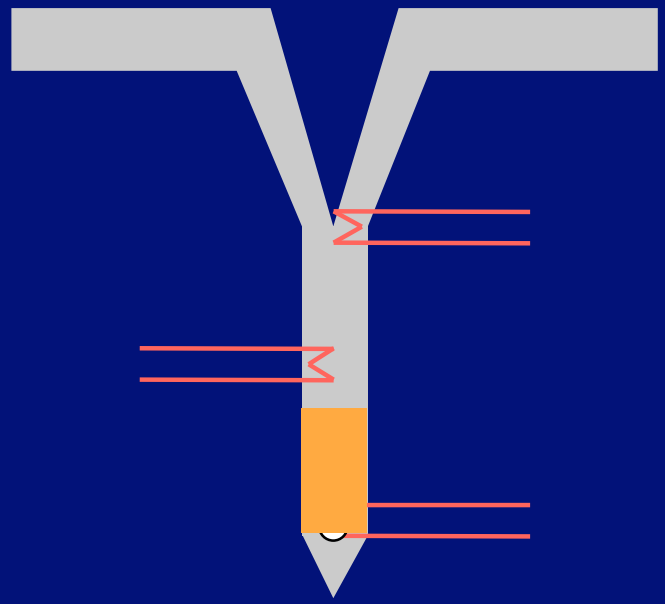
Loading wax



Phase-Change Valve

Operation

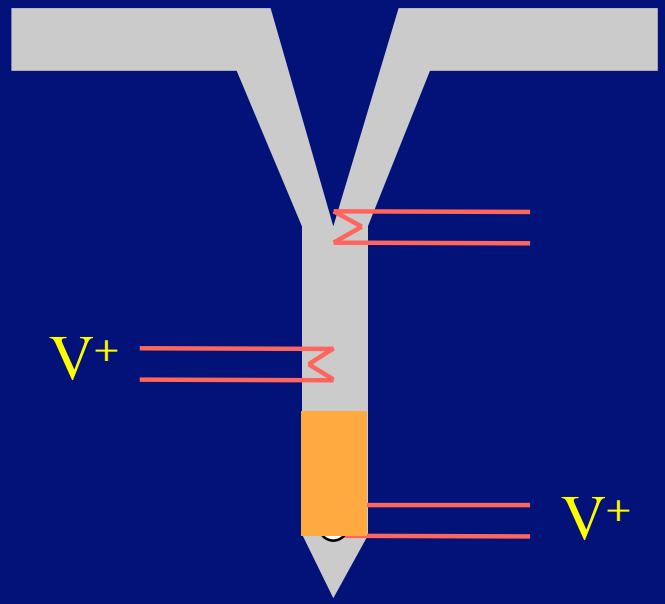
Closing



Phase-Change Valve

Operation

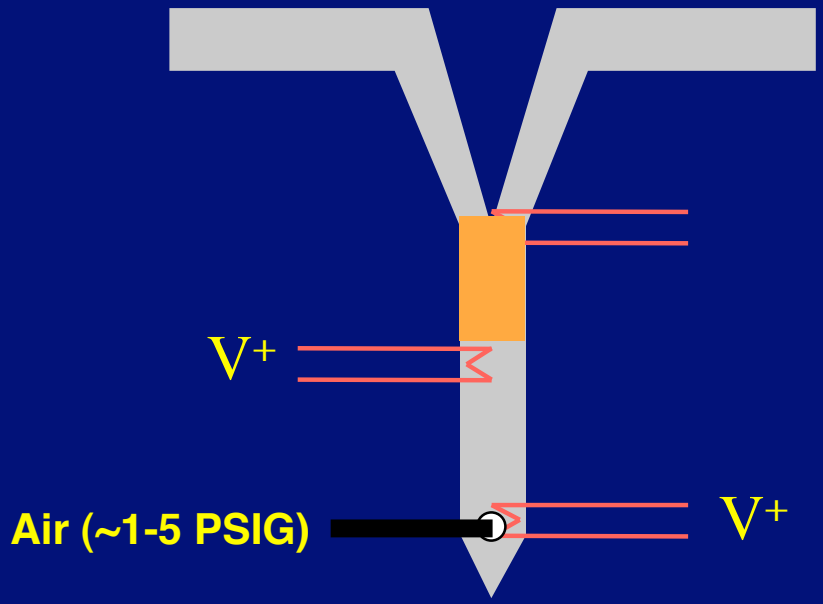
Closing



Phase-Change Valve

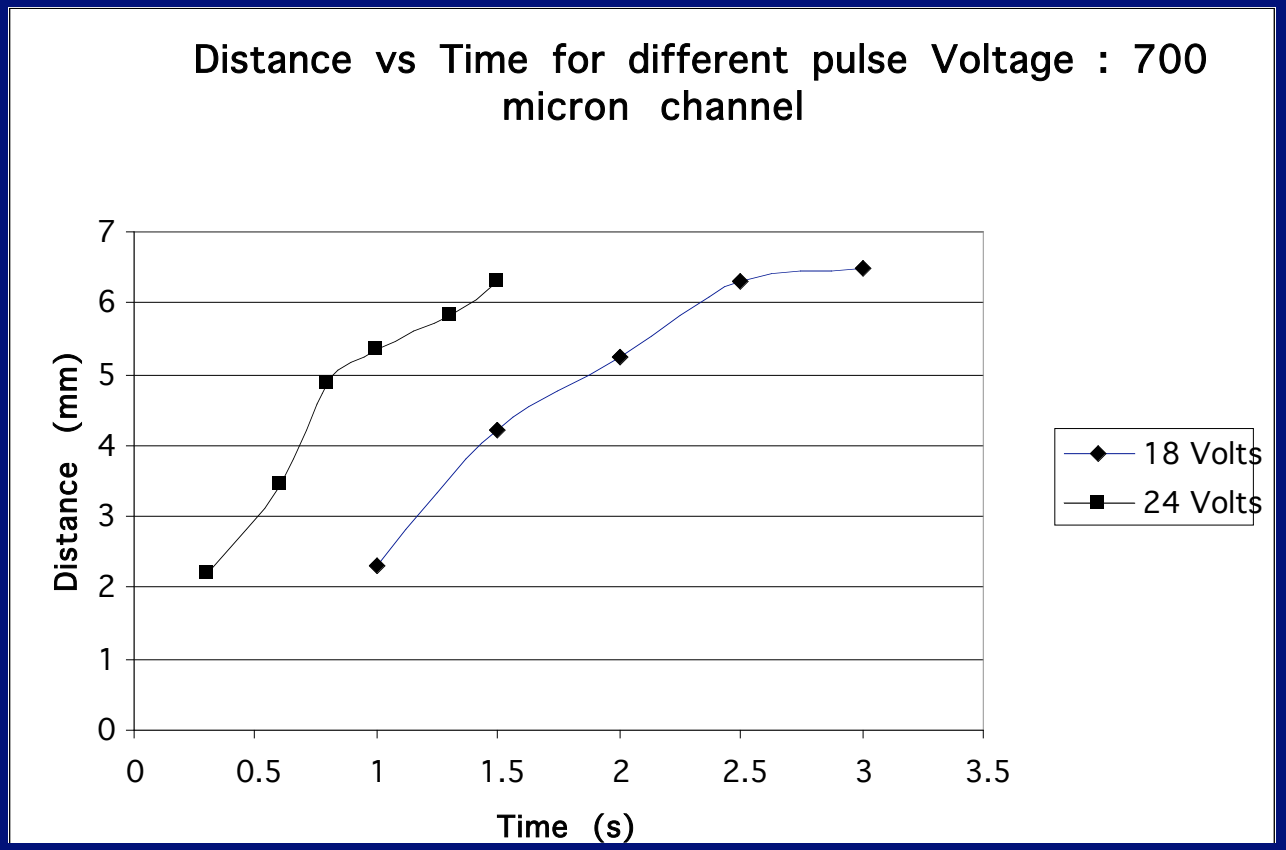
Operation

Closing



Problem Description

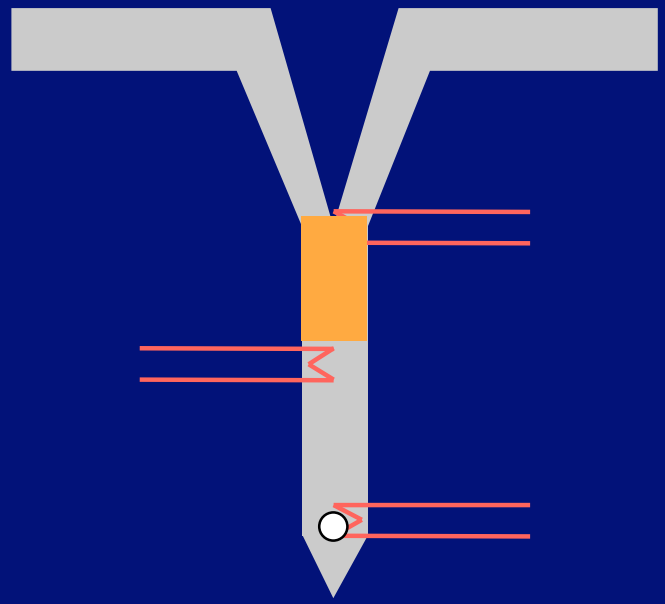
Objectives



Phase-Change Valve

Operation

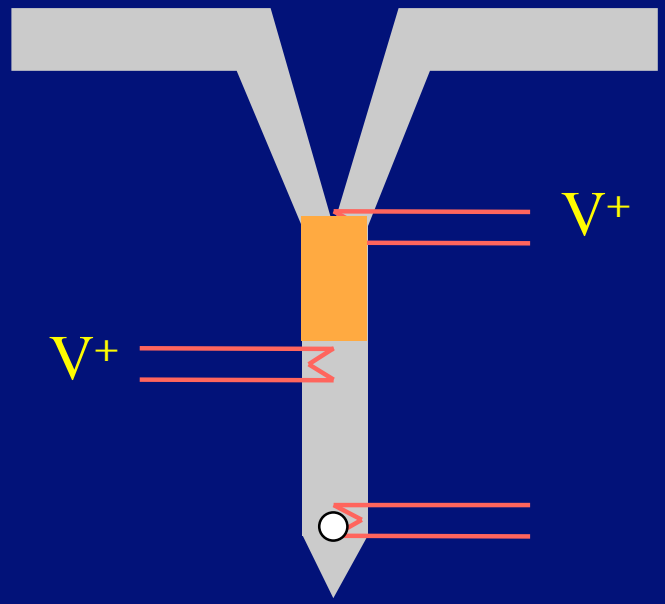
Opening



Phase-Change Valve

Operation

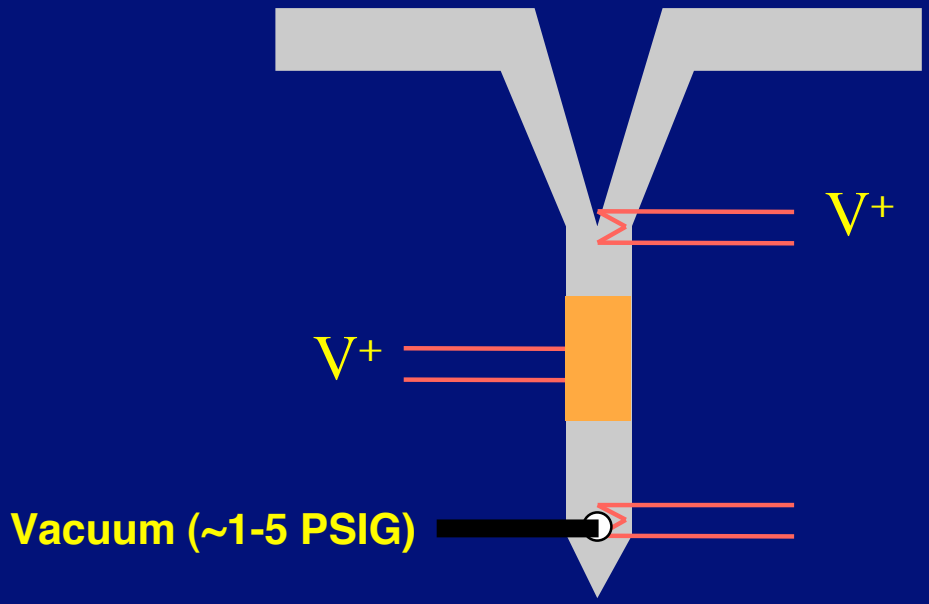
Opening



Phase-Change Valve

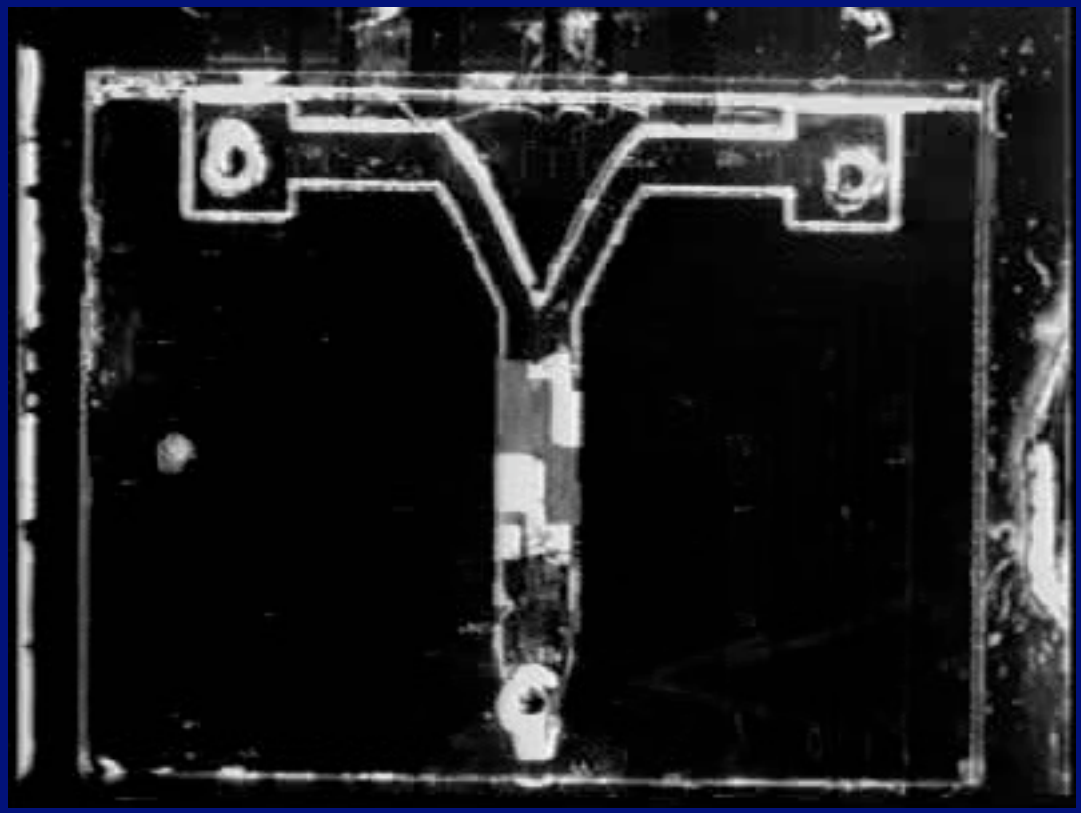
Operation

Opening



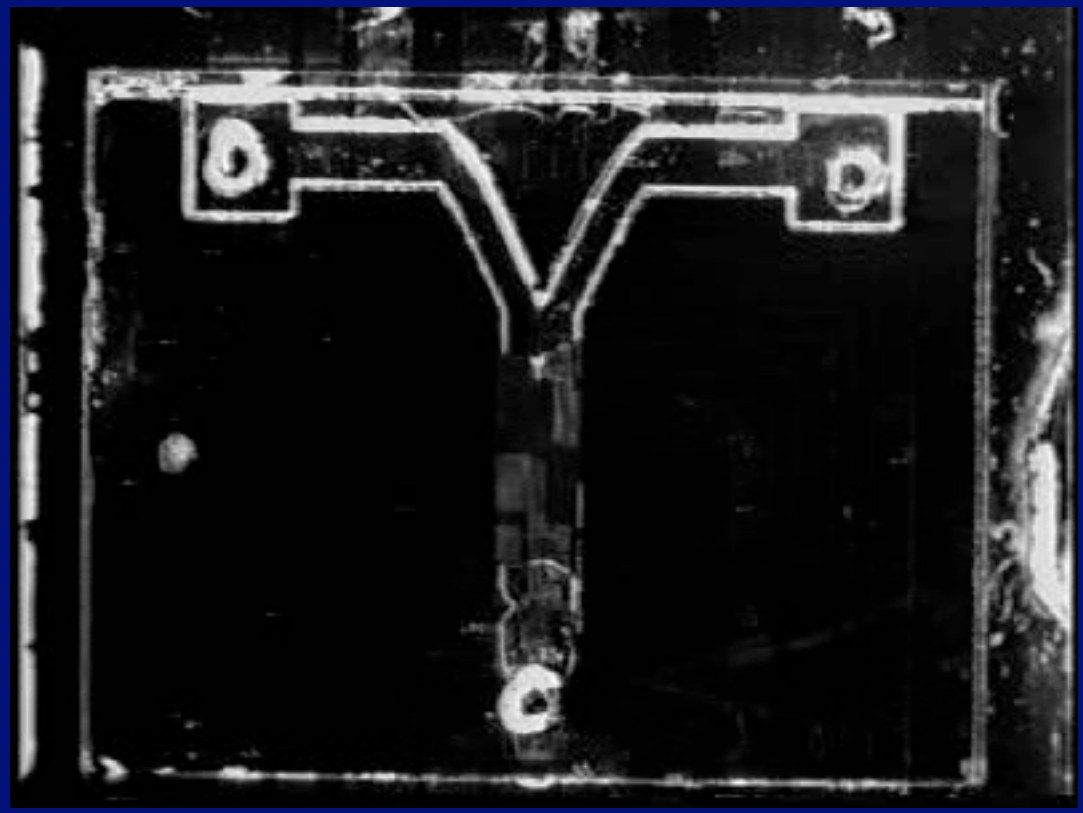
Valve Opening

Piston Material



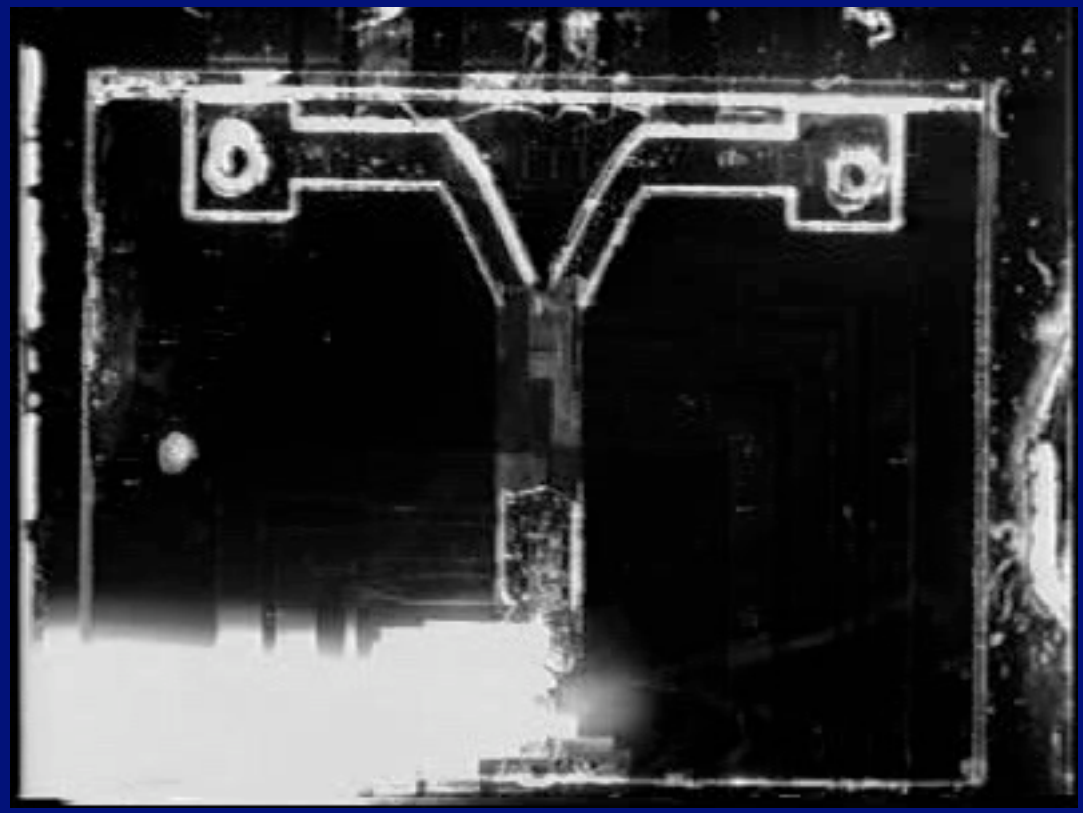
Valve Opening

Piston Material



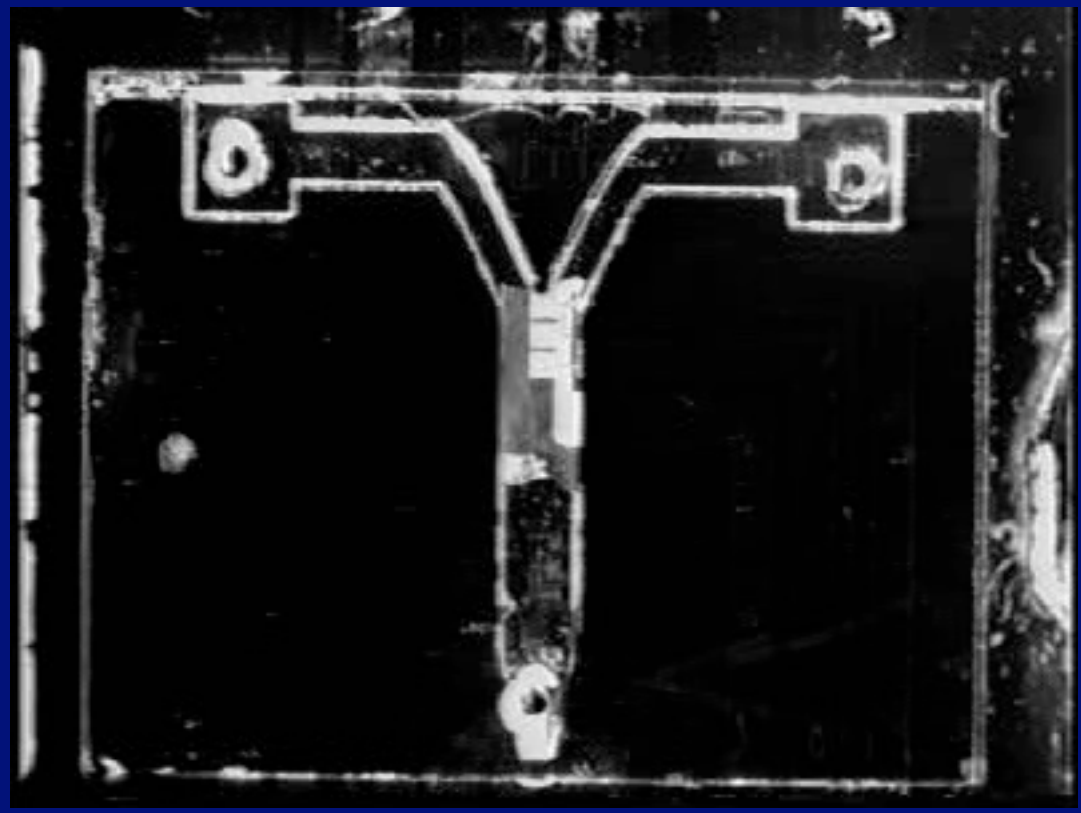
Valve Opening

Piston Material



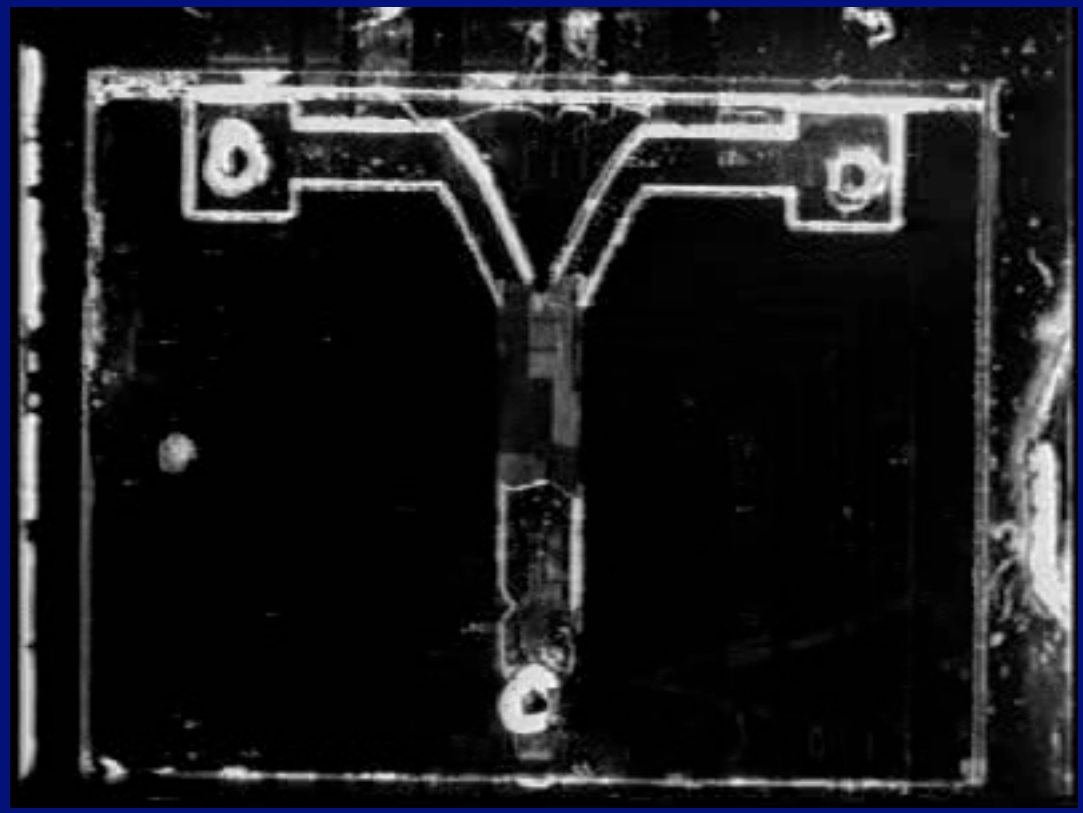
Valve Opening

Piston Material



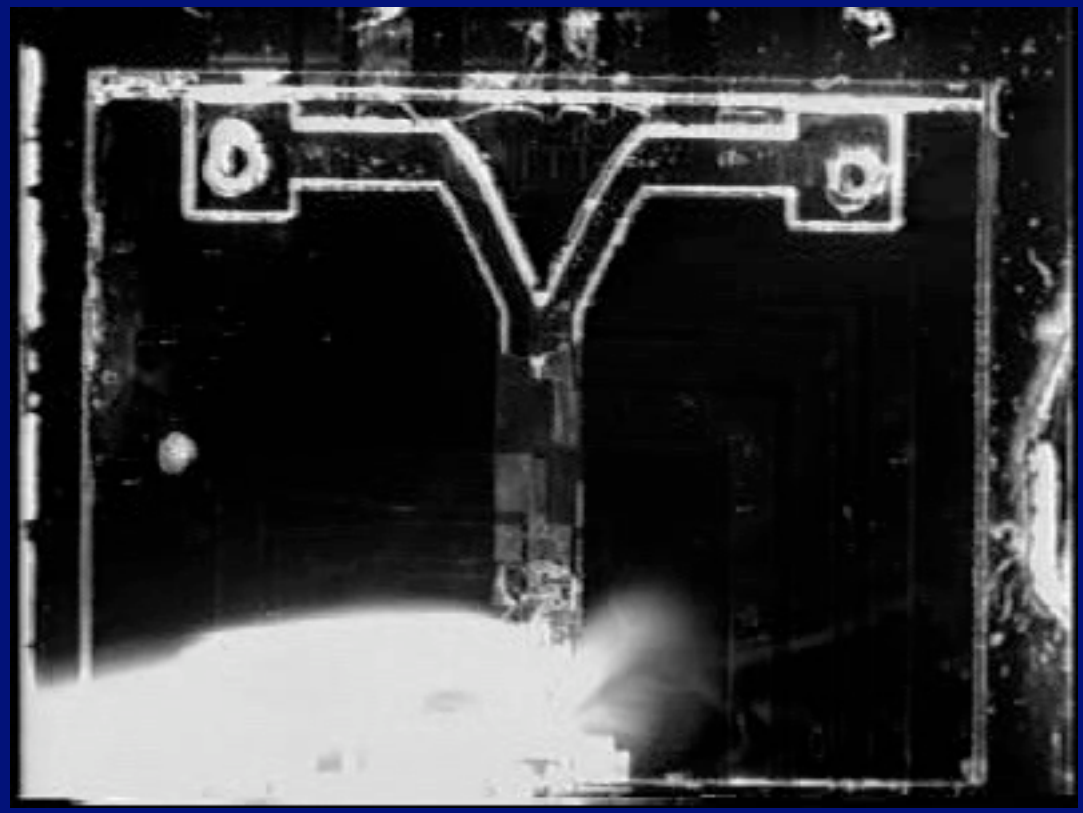
Valve Opening

Piston Material



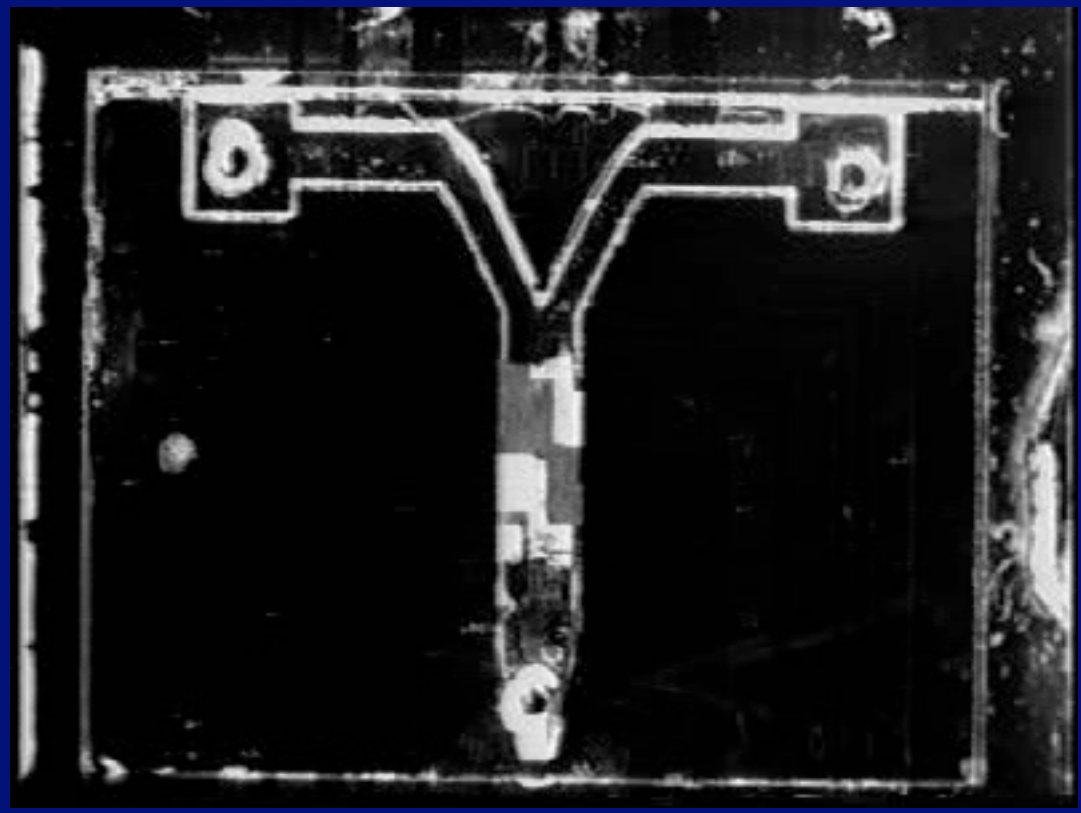
Valve Opening

Piston Material



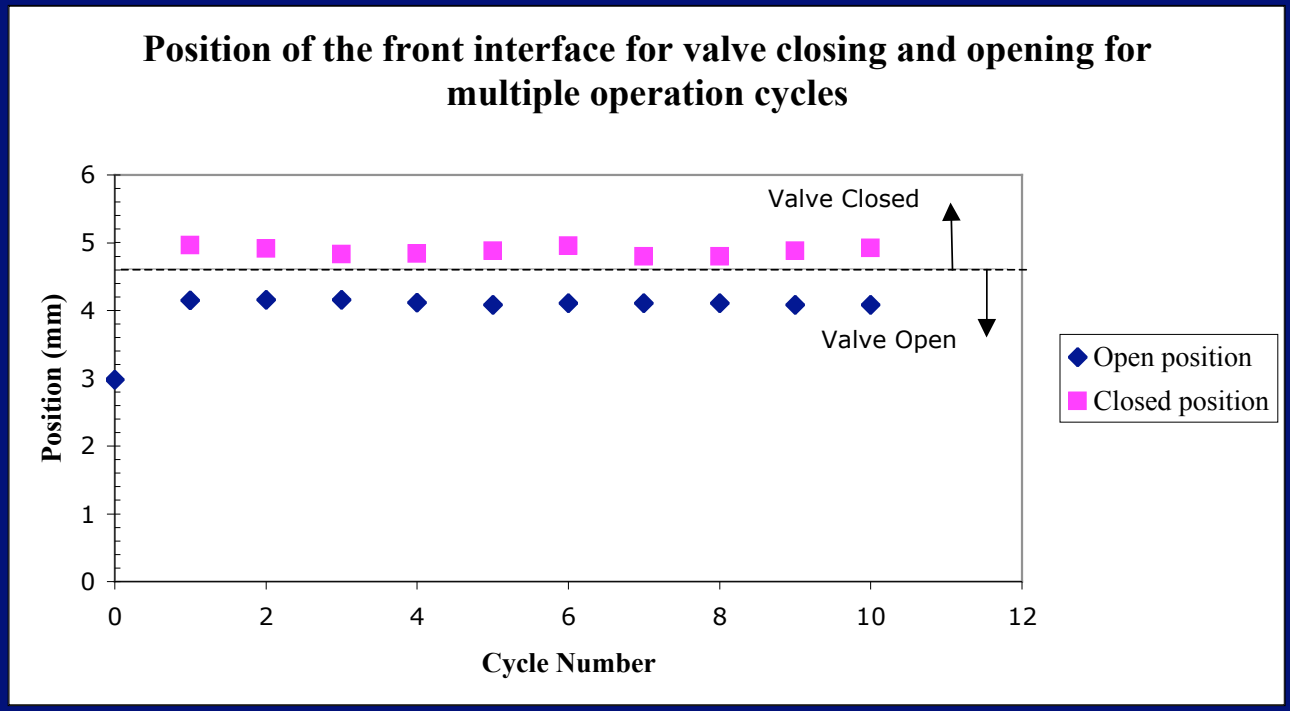
Valve Opening

Piston Material



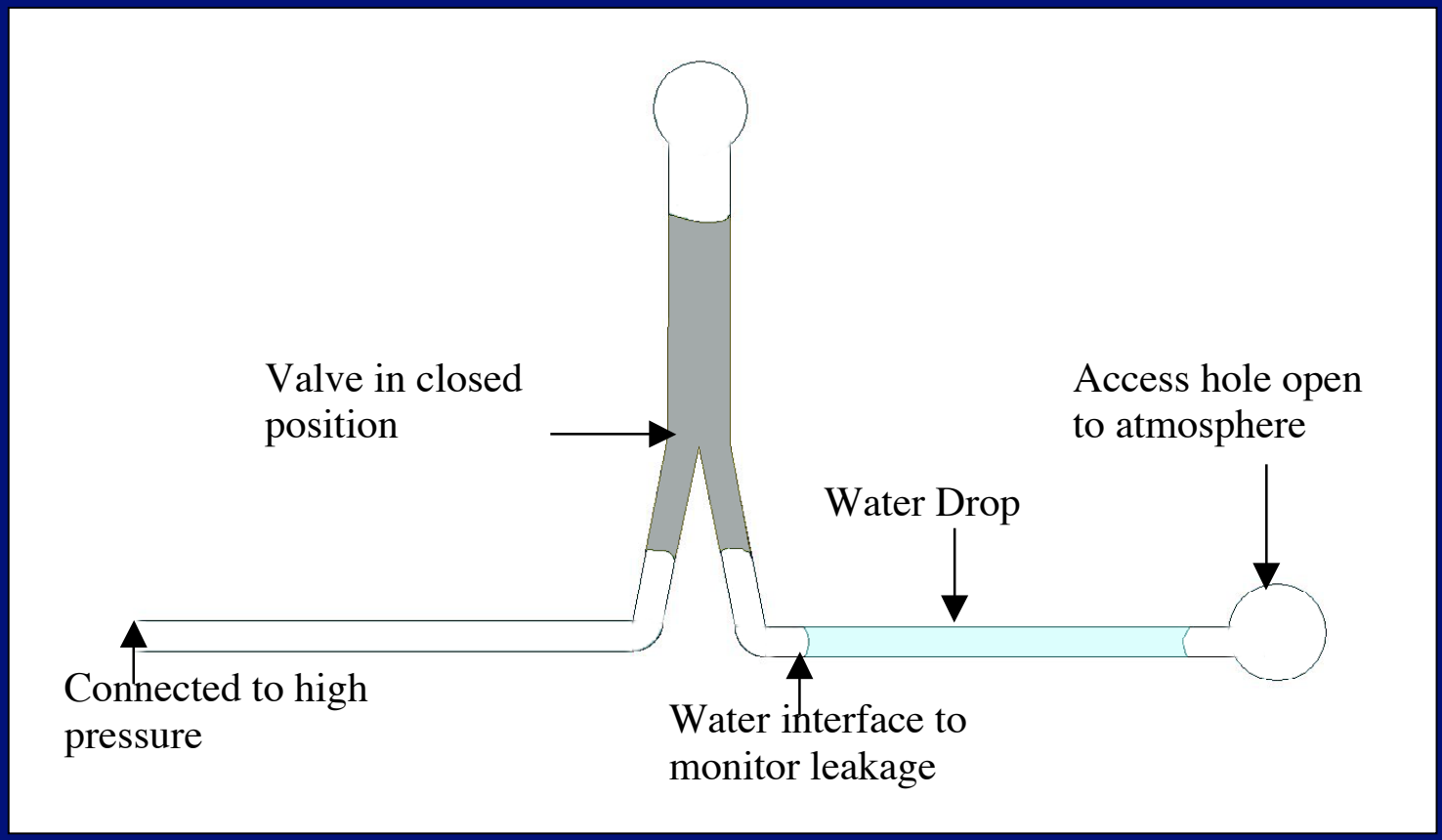
Valve Reproducibility

Cycling



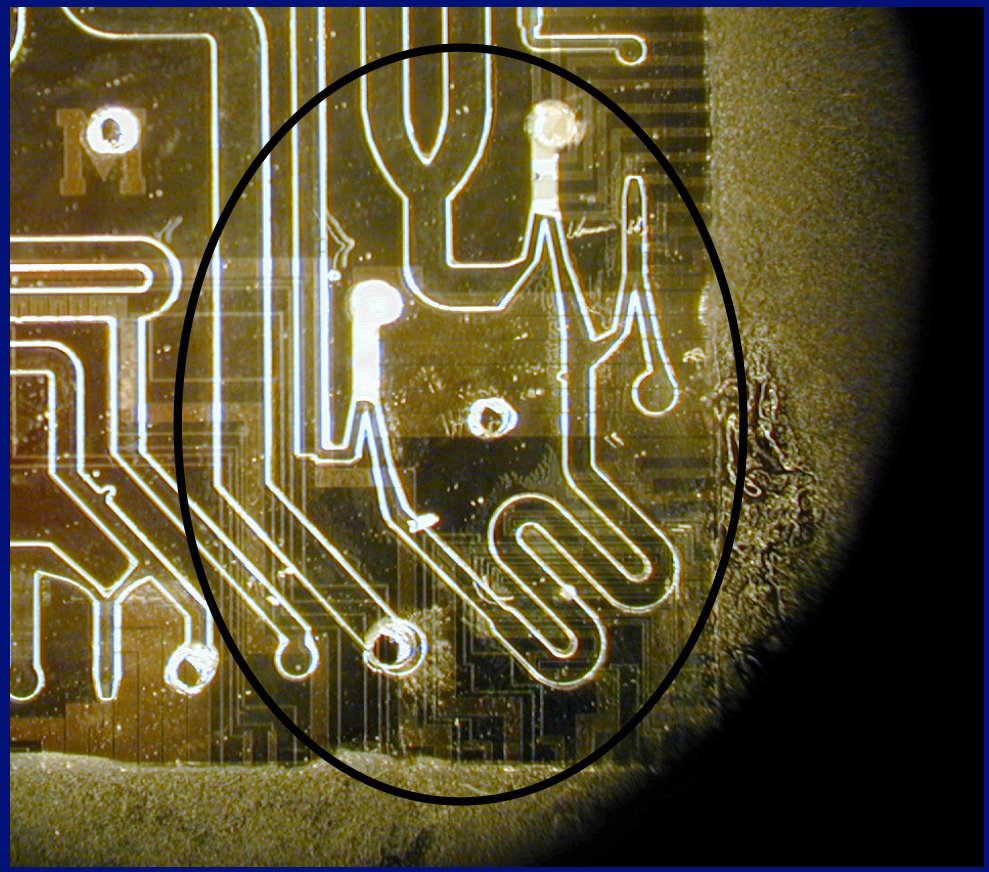
Valve Closing

Pressure Test



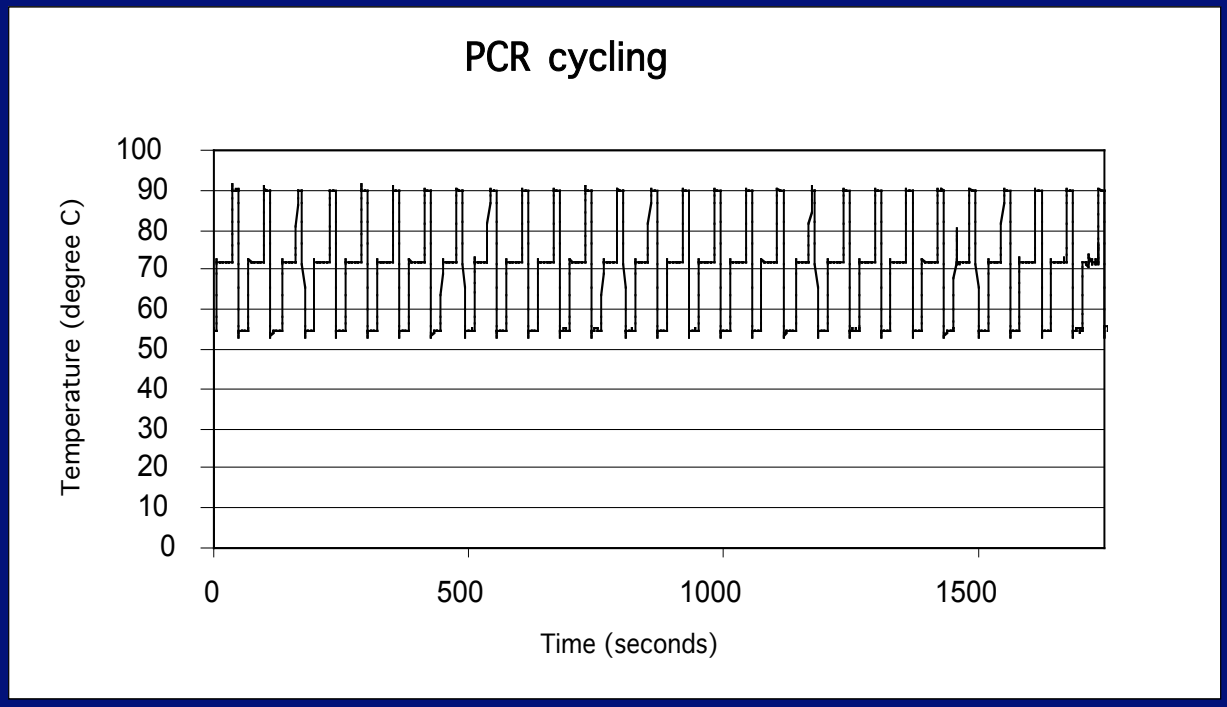
Biochemical Reaction

Device



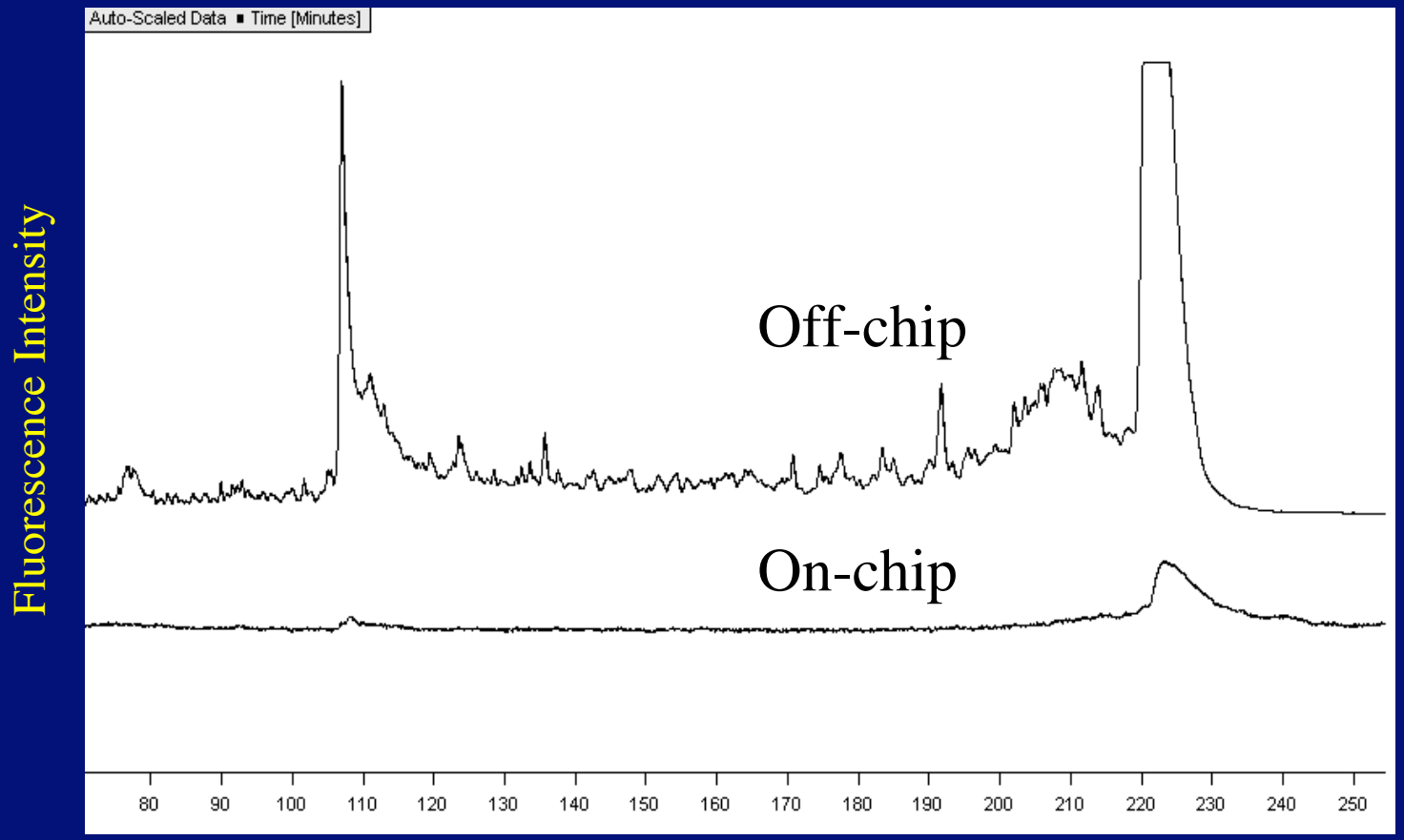
Biochemical Reaction

Cycles



Integrated Device

Result



Microfabricated Biochemical Analysis

- Integrated Device
- Phase-Change Valve
- Viscometer
- Single Molecule

Micro-scale Viscosity Measurement

- Viscosity of input sample important
- Body Fluids' viscosity diagnostic tool
- Low volume of sample
- Macroscale viscometers hard to use

Viscosity in Medicine

Predictor/ Risk factor *(Preventive)*

- *Blood and Blood Plasma* - Cardiovascular Risk factor, Ischemia, Type II Diabetes
- *Synovial Fluid* - Osteoarthritis Severity
- Mucin Asthma
- Spittle

Indicator/ Marker *(Diagnostic)*

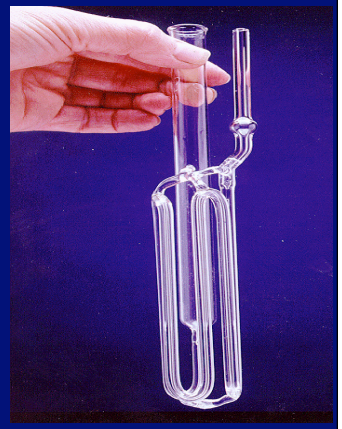
- *Blood Plasma* -
  - Hyperviscosity Syndrome (growth of Tumour etc.)
  - Coronary Heart Disease, Diabetic Retinopathy
  - Rejection in Organ Transplantation
- *Amniotic Fluid* - Fetal Lung Maturity
- *Seminal Fluid* - Sperm Motility

Motivation

Current Instrumentation

o Rotational Viscometers

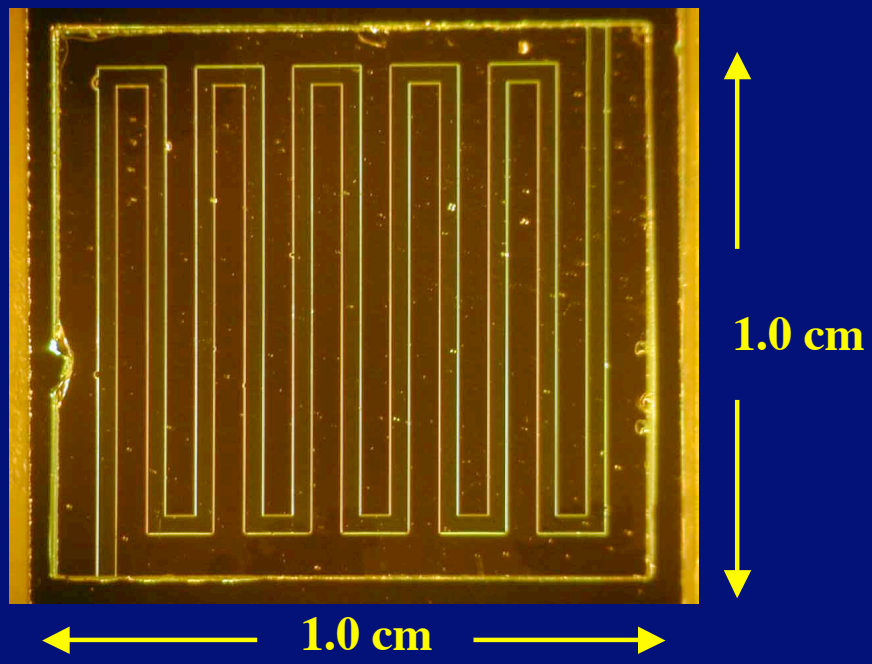
o Capillary viscometer

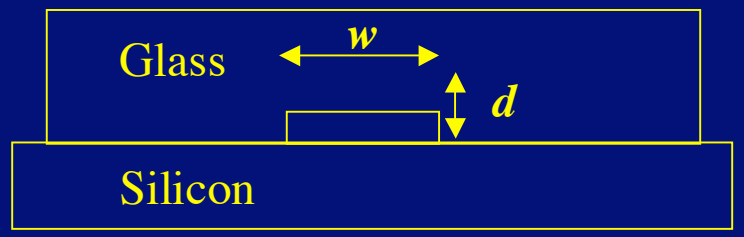


Limitations

- ✓ Allows only serial testing
- ✓ Expensive, elaborate set-up, not portable
- ✓ Not available for Personal Healthcare

Microfluidic Viscometer



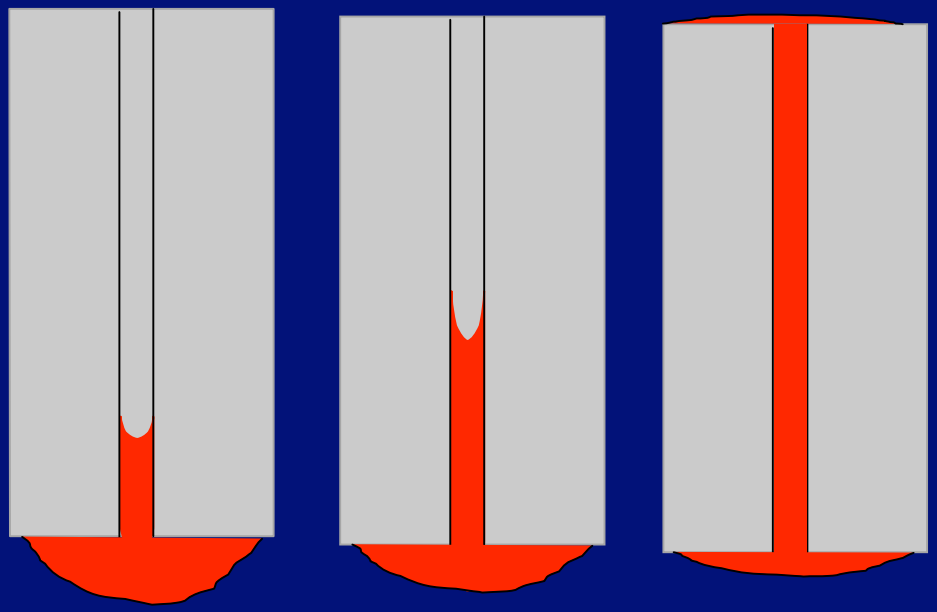


$d = 34\mu m$

$w = 340\mu m$

$L_{total} = 9cm$

Capillary Pressure Driven Flow



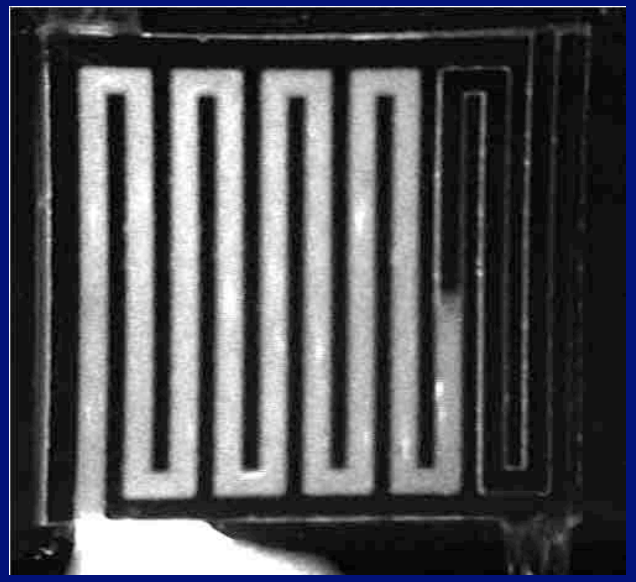
$$v = \frac{d^2}{S\mu}\ \frac{\Delta P}{L}$$

Laminar Flow

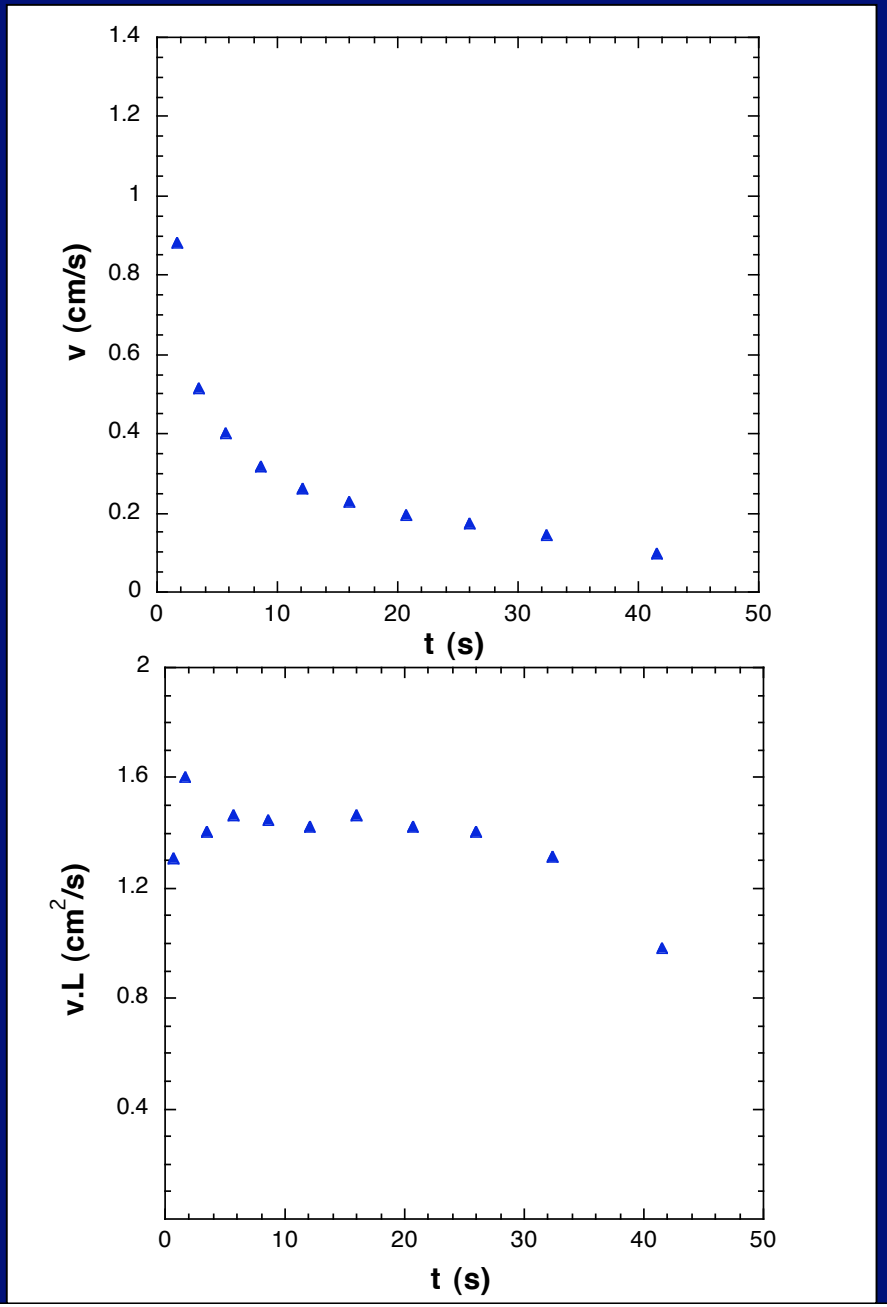
$$\Delta P = 2\sigma \cos\theta\left(\frac{1}{d} + \frac{1}{w}\right)$$

$$\mu = \frac{d^2}{S} \cdot \Delta P \cdot \frac{1}{vL}$$

Data Analysis: vL ⟶ Viscosity

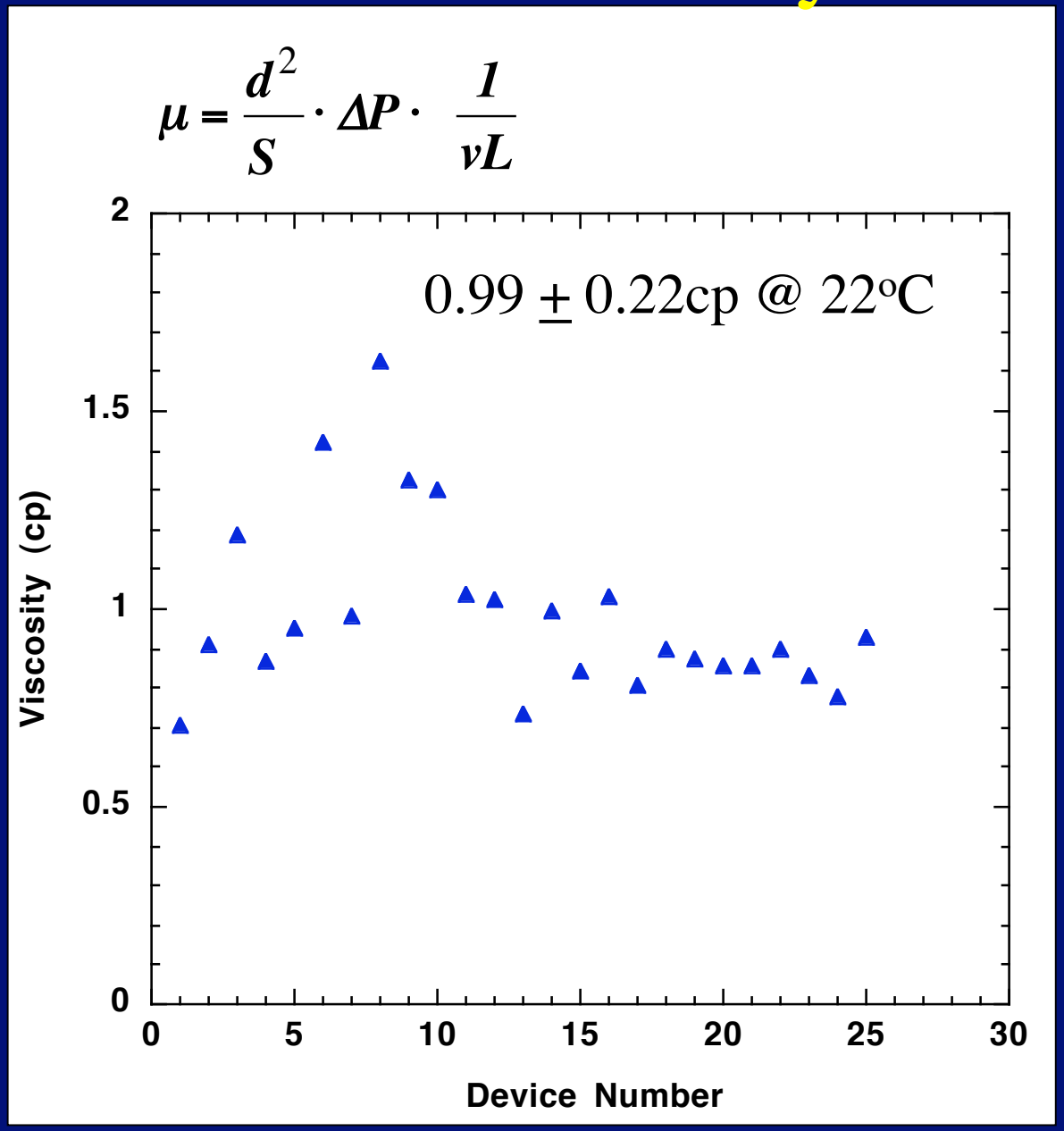


$$\mu = \frac{d^2}{S} \cdot \Delta P \cdot \frac{1}{vL}$$



Viscosity of Water

$$\mu = \frac{d^2}{S} \cdot \Delta P \cdot \frac{1}{vL}$$



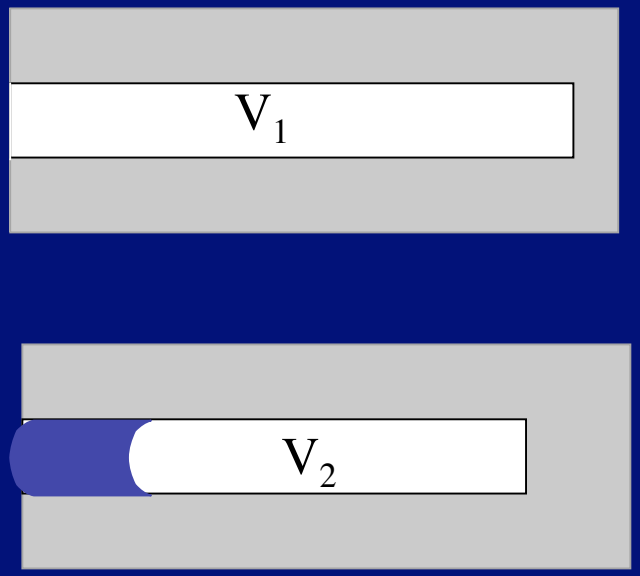
Advantages

- Quick and Easy
- Cheap - therefore disposable
- Portable
- Multiple Channels allow parallel processing

Limitations

- Need to know the geometry (d and S) accurately
- Capillary Pressure has to be calculated
- Low Precision

Microfluidic Measurement of ΔP (Capillary Pressure)



$$P_{atm}V_1 = (P_{atm} + \Delta P)V_2$$

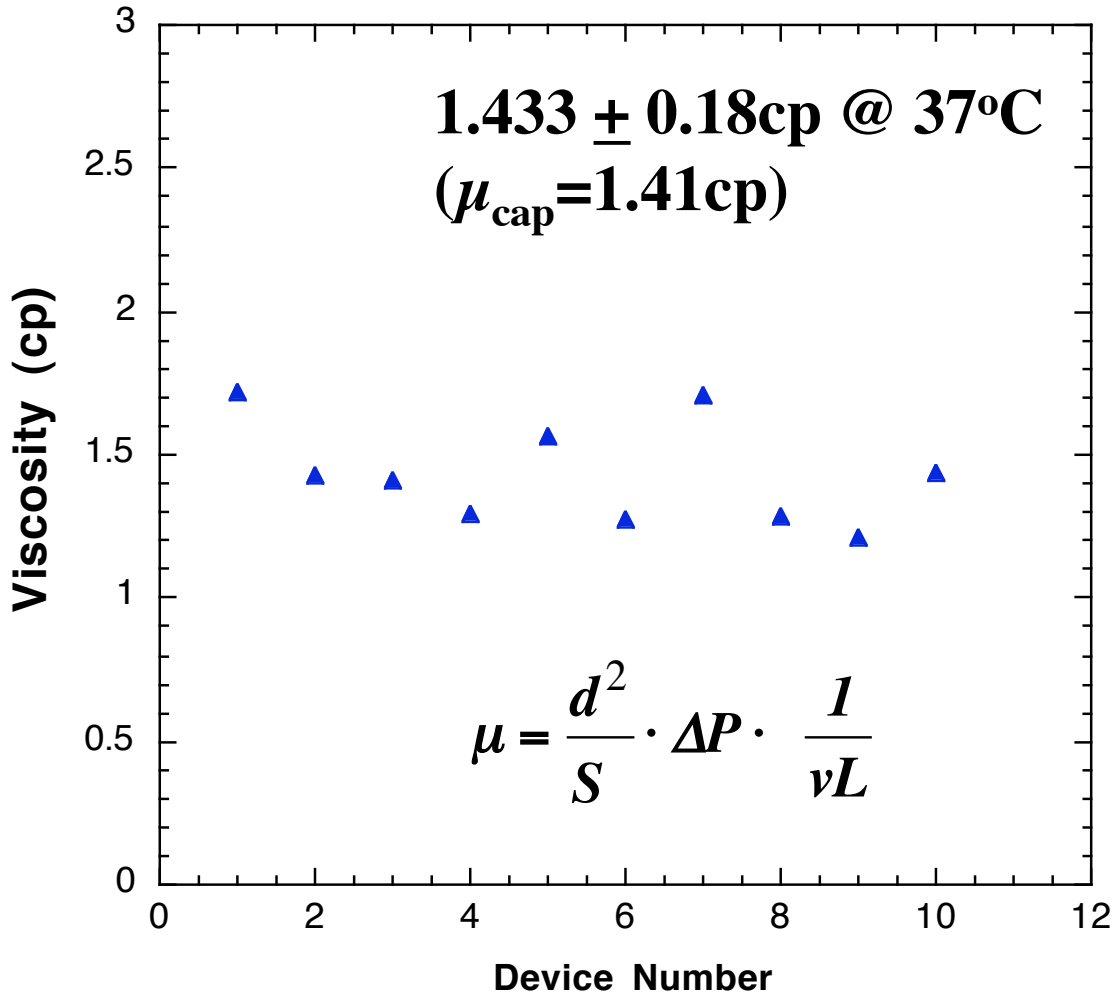
$$\Delta P = P_{atm}\left(\frac{V_1}{V_2} - 1\right)$$

$$\Delta P = 2\sigma \cos\theta\left(\frac{1}{d} + \frac{1}{w}\right)$$

$$\mu = \frac{d^2}{S} \cdot \Delta P \cdot \frac{1}{vL}$$

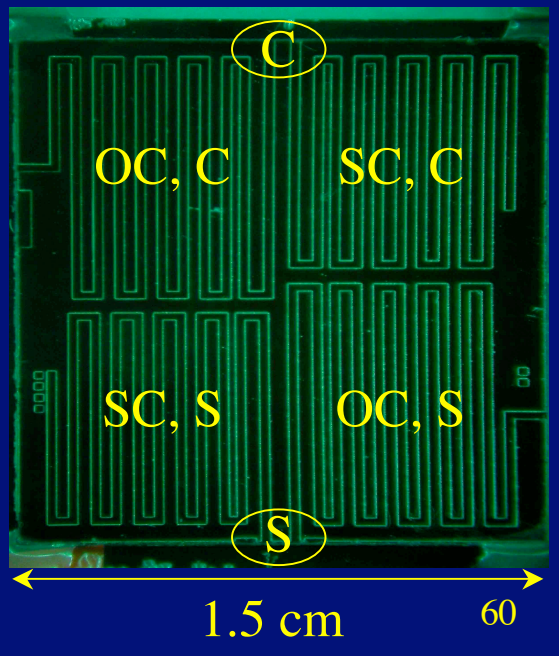
Self-Calibrating Nanoliter Viscometer

Blood Plasma Viscosity

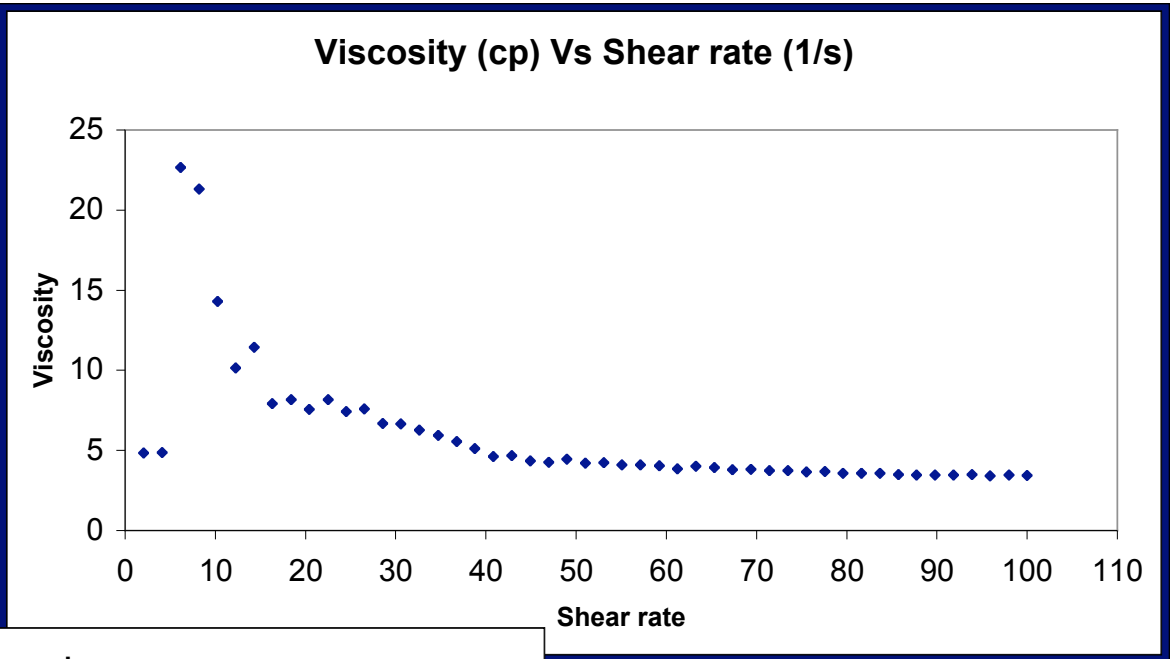


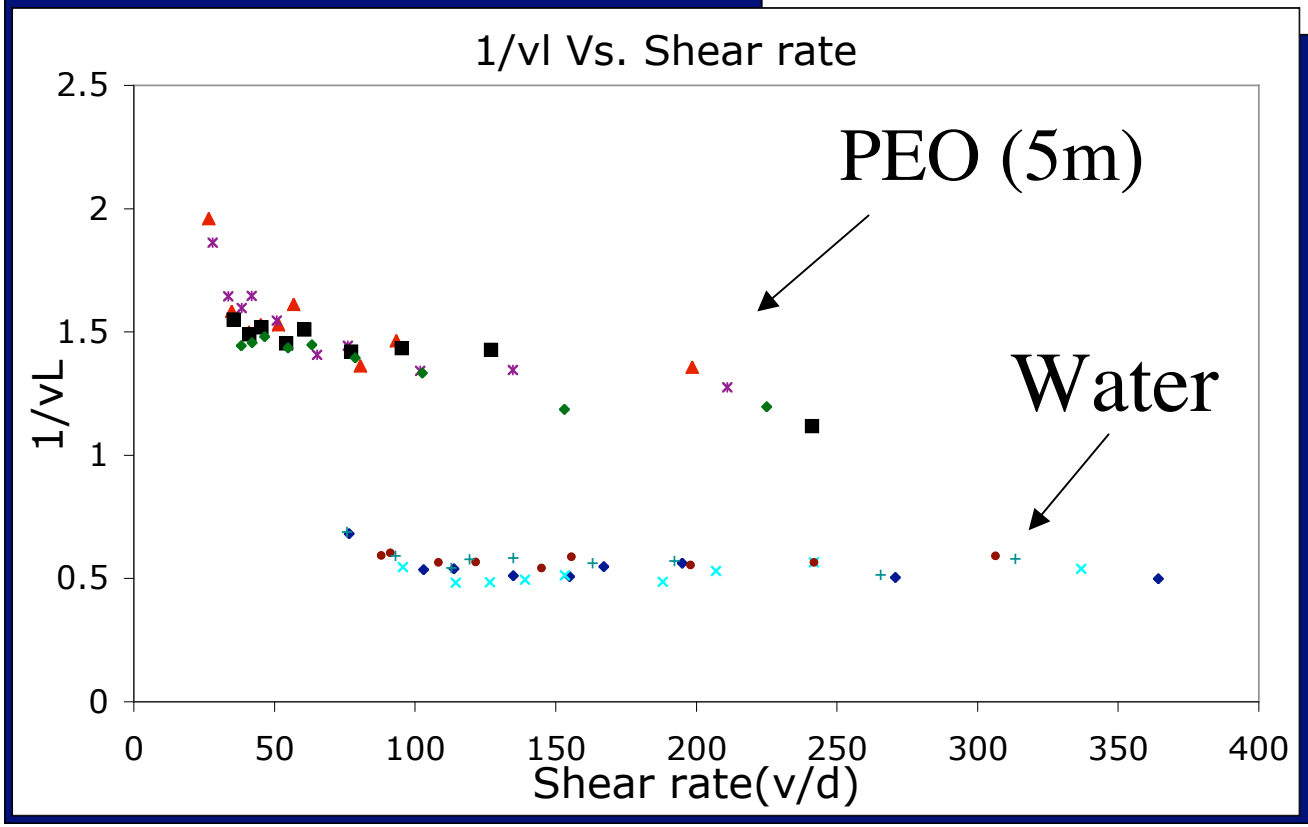
Advantages

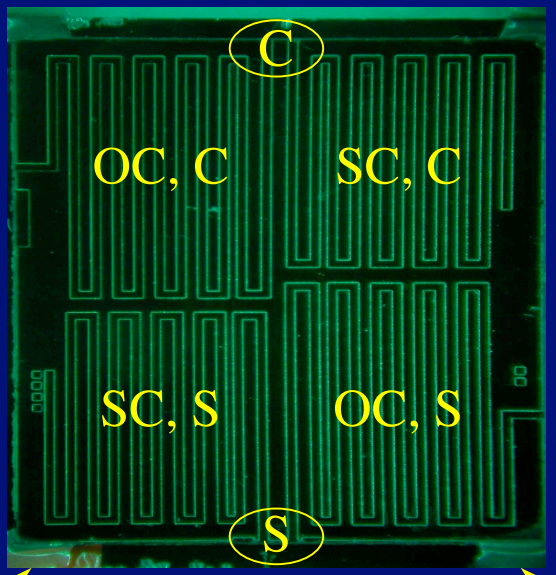
- Self Calibrating
- Better precision
- Applicable to a wider range of liquids



Non-Newtonian



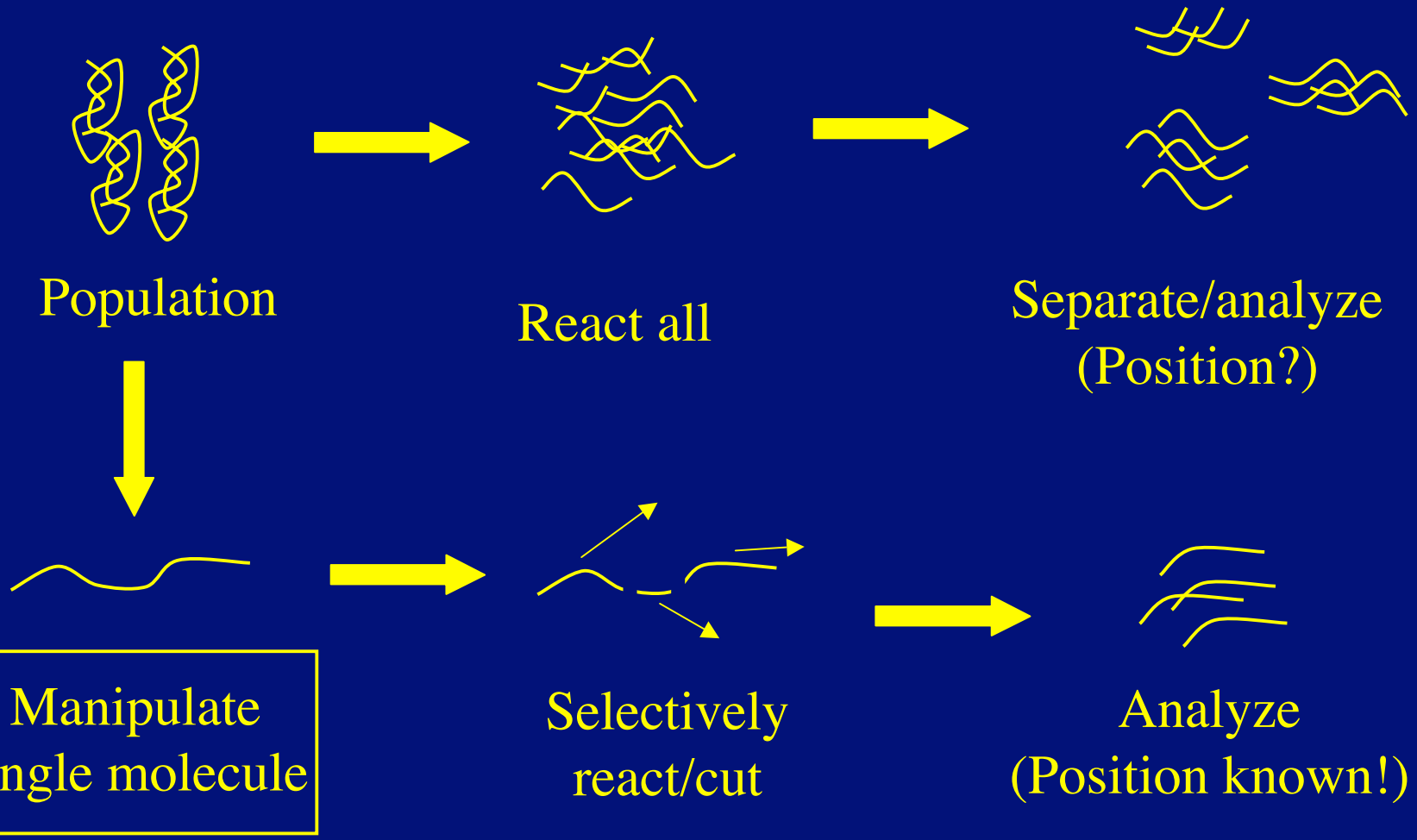




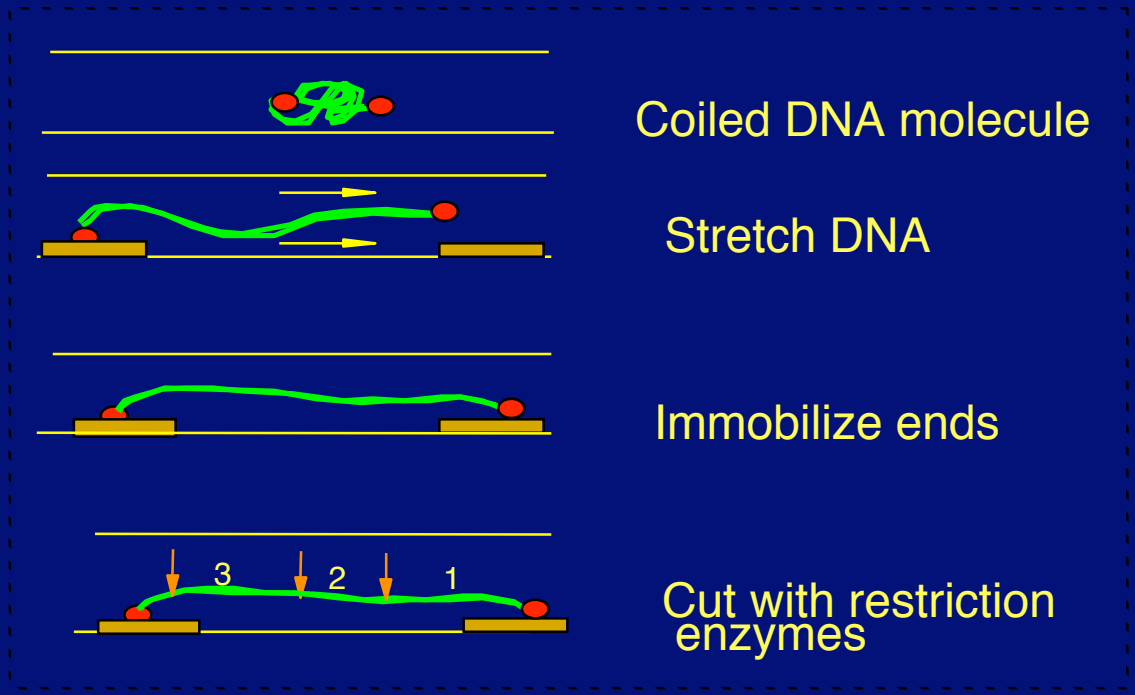
Microfabricated Biochemical Analysis

- Integrated Device
- Phase-Change Valve
- Viscometer
- Single Molecule

Advanced Component Development: Single Molecule Manipulations

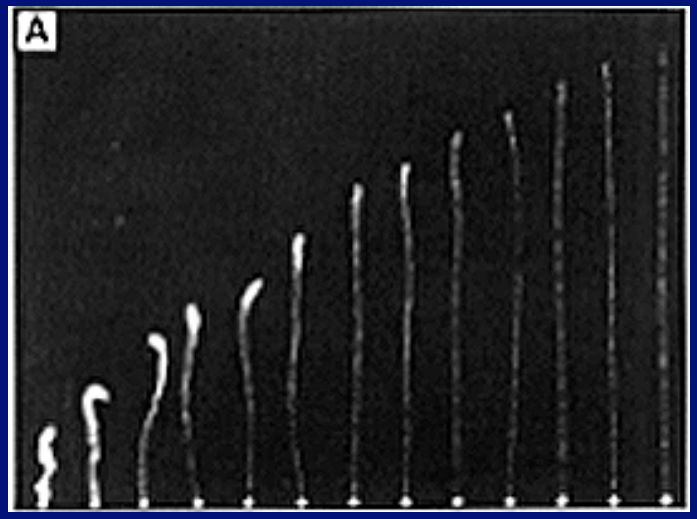


Single Molecule Analysis



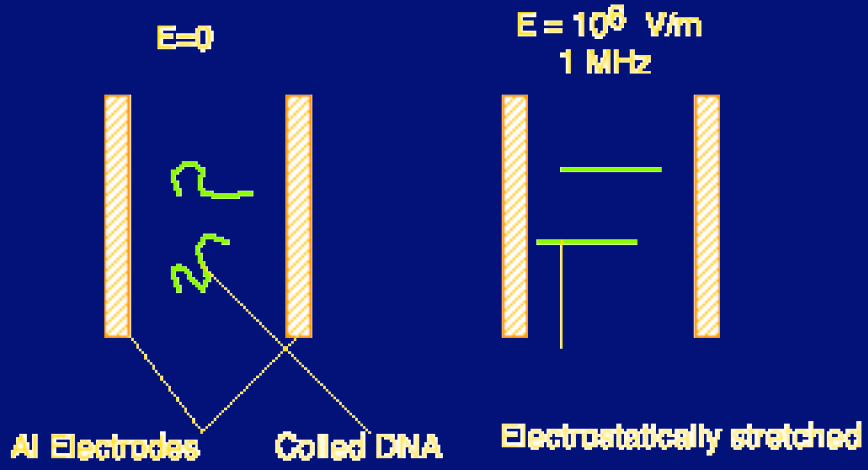
DNA Stretching

Hydrodynamic forces



Chu, Larson et. al. (1995)

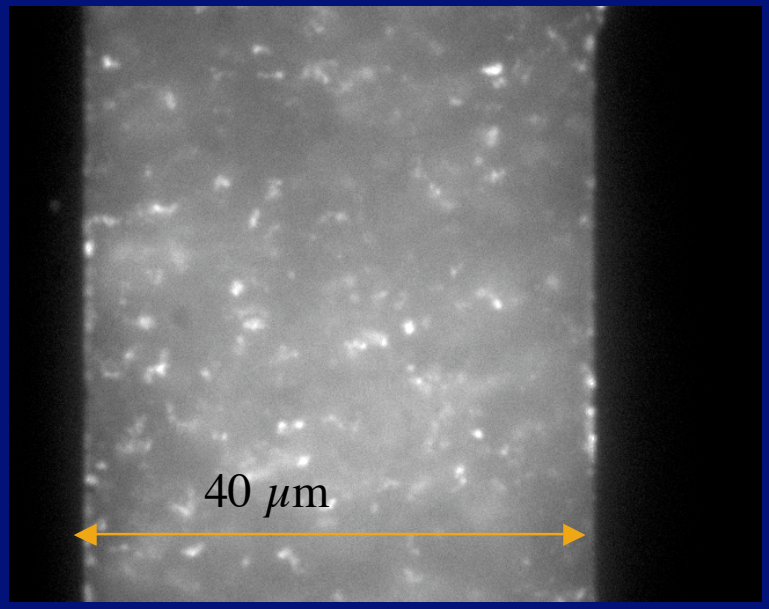
Electrostatic forces



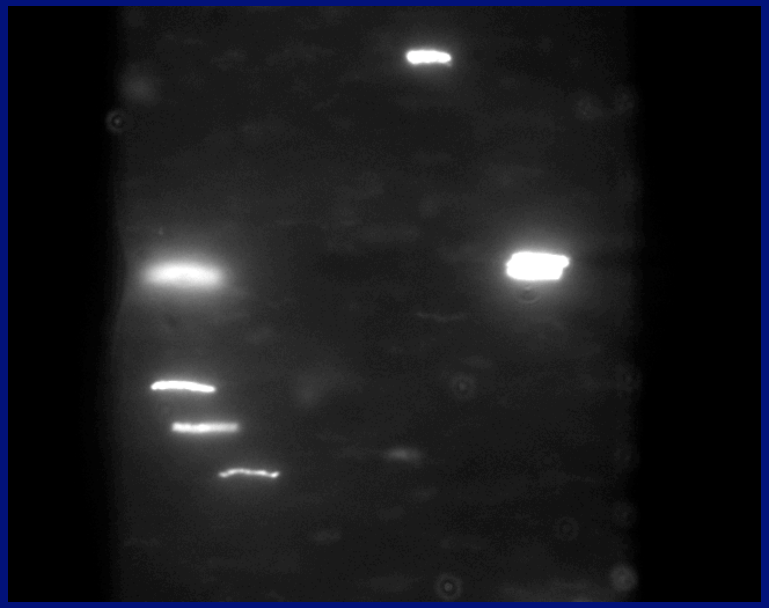
Washizu et. al. (1995)

DNA Electrostretching

Lambda DNA Stretched in Tris Hcl



Relaxed DNA Molecules

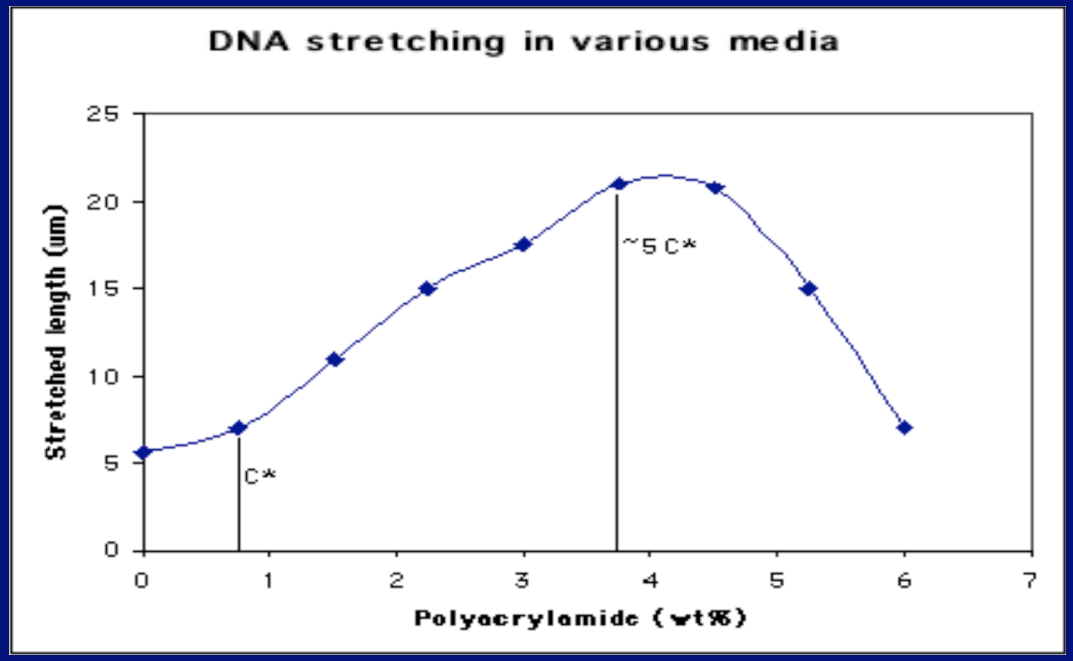


DNA partly stretched under AC field (10^6 V/m, 1MHz)

Electrostretching

Medium

DNA Stretching vs Polymer concentration



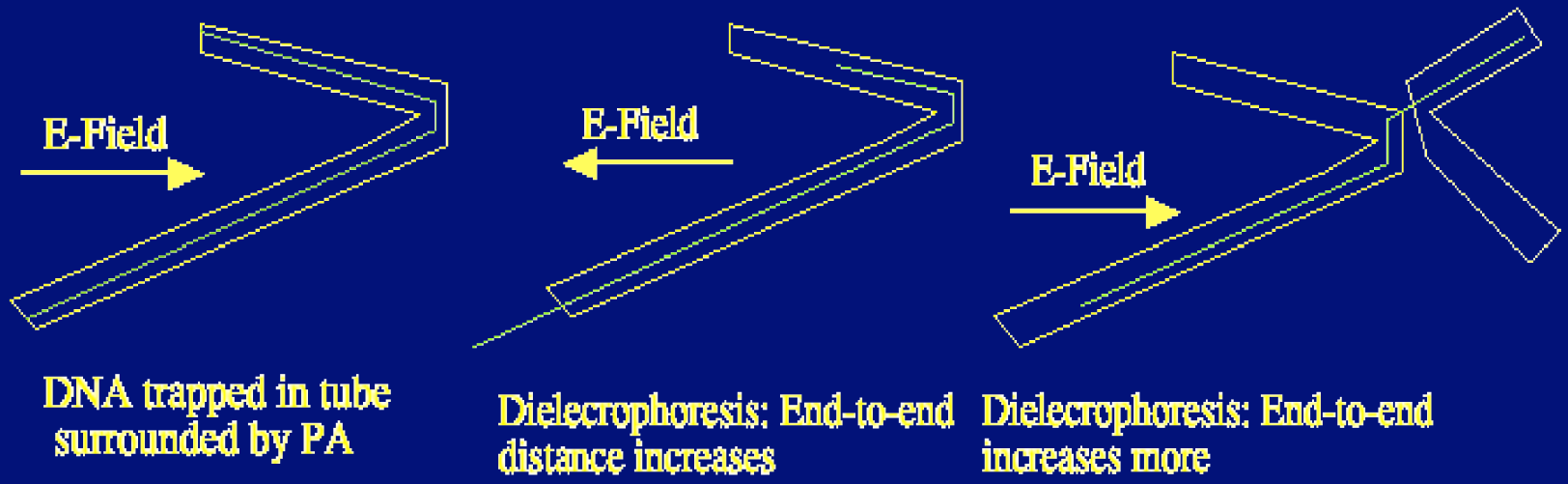
Conditions

- Lambda DNA
- Gold Electrodes
- Tris HCl with varying LPA
- AC field ($3x10^5$ V/m,1MHz)

C* : Critical entanglement concentration

5 C* : Polymer well entangled; Tube diameter close to DNA size

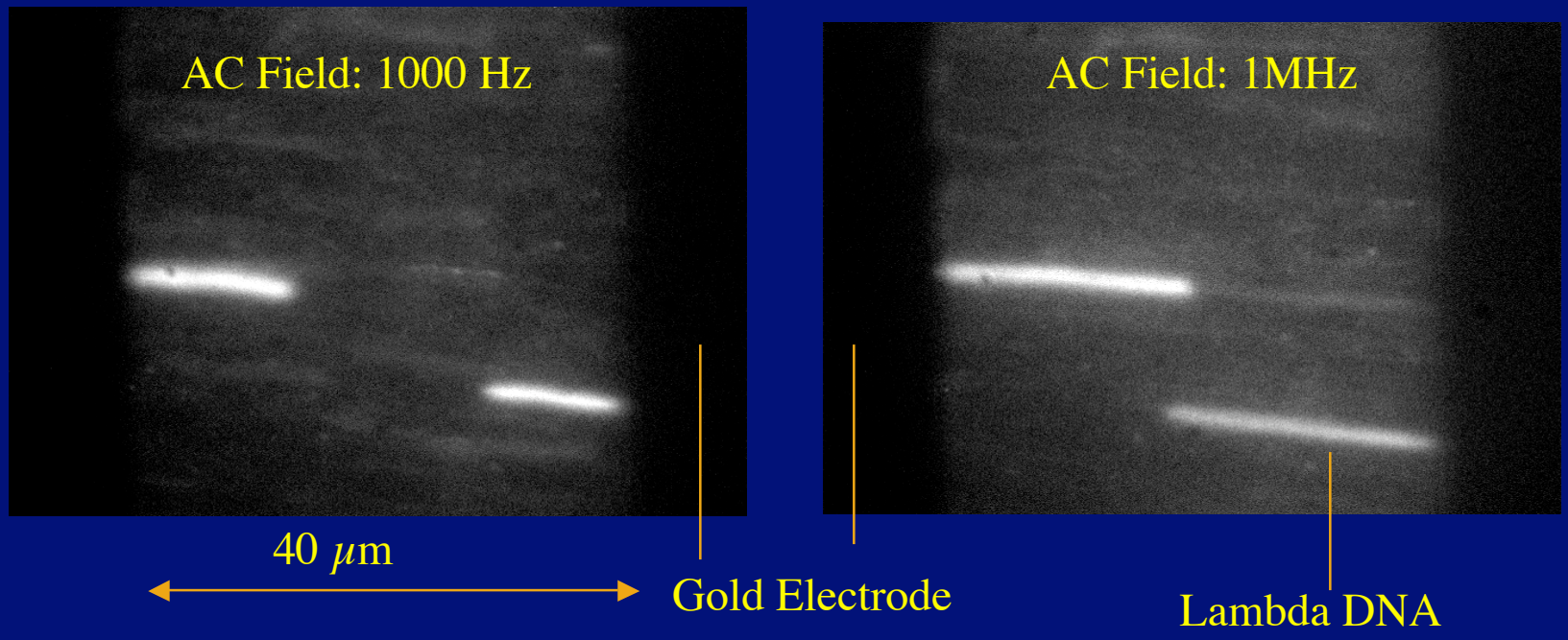
Mechanism of DNA Stretching: AC fields



- Linear Polyacryamide confines DNA molecules within tubes
- At 5 C*, Tube diameter approaches DNA diameter
- AC field helps in Dielectrophoretic stretching

DNA Immobilization and Optimization

Lambda DNA Stretched in Linear Polyacrylamide

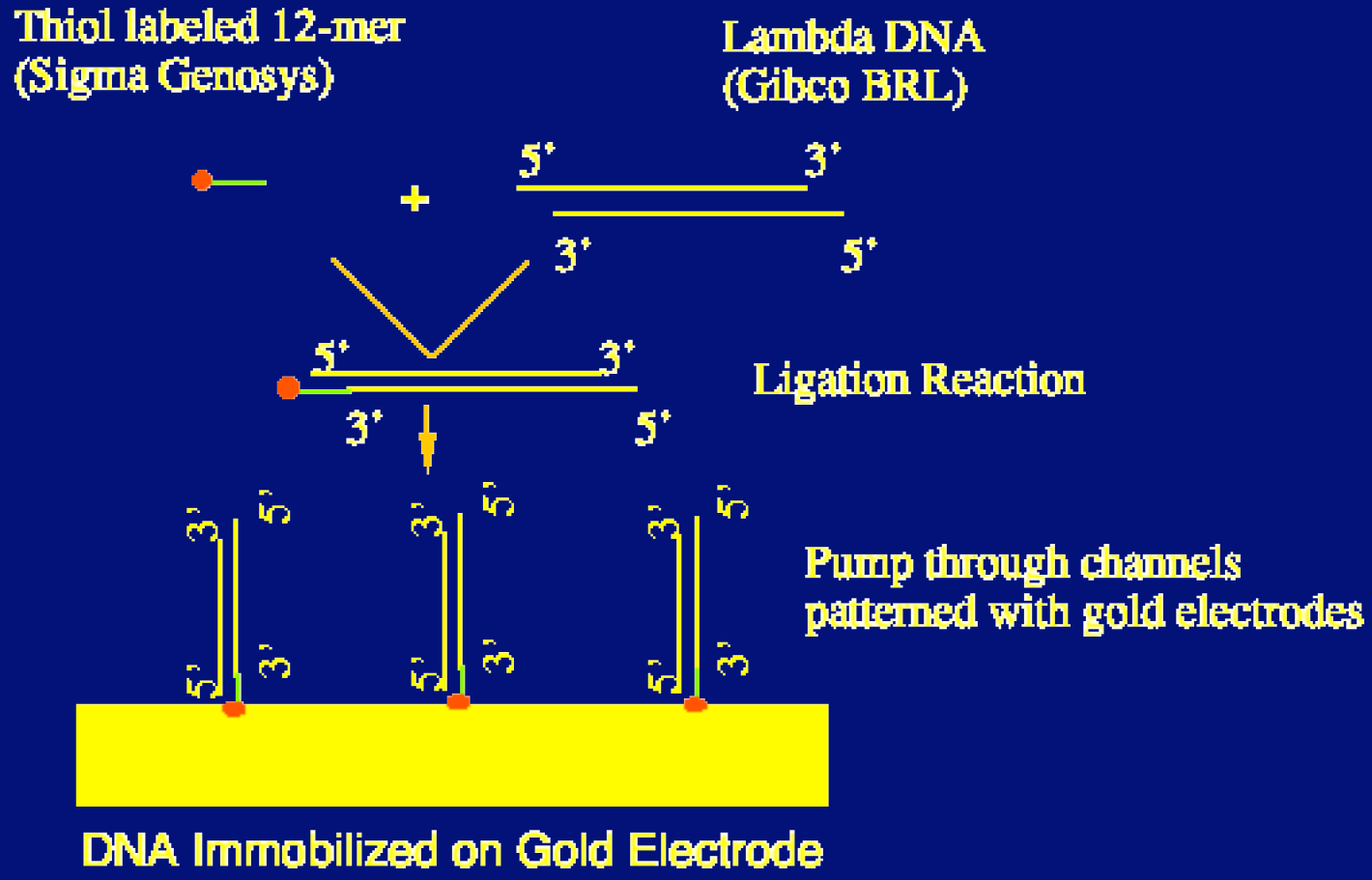


DNA immobilized at gold Electrodes by Dielectrophoresis

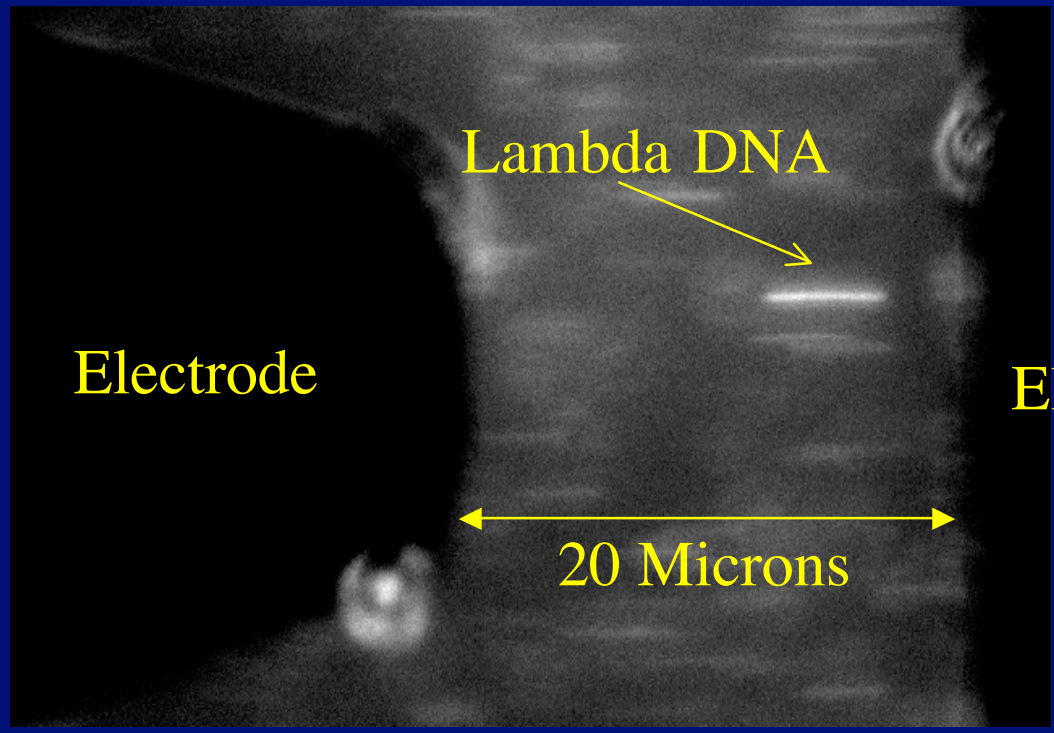
DNA fully stretched under AC field (10^6 V/m, 1MHz)

DNA Immobilization

Thiol-On -Gold

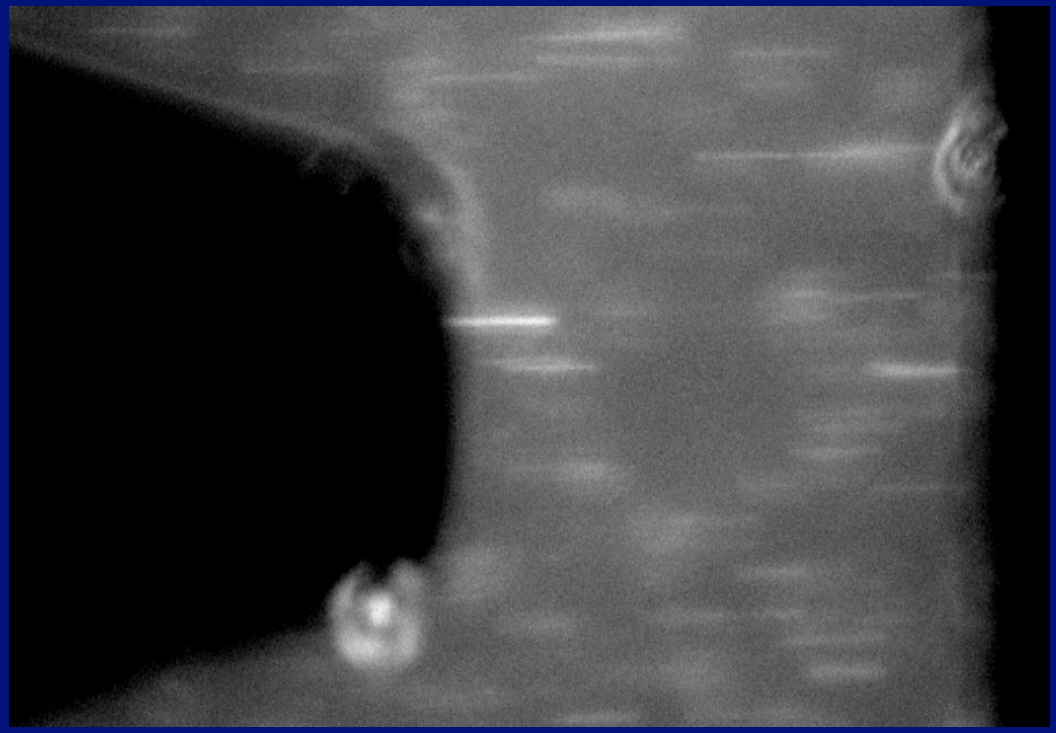


Single Molecule Manipulations



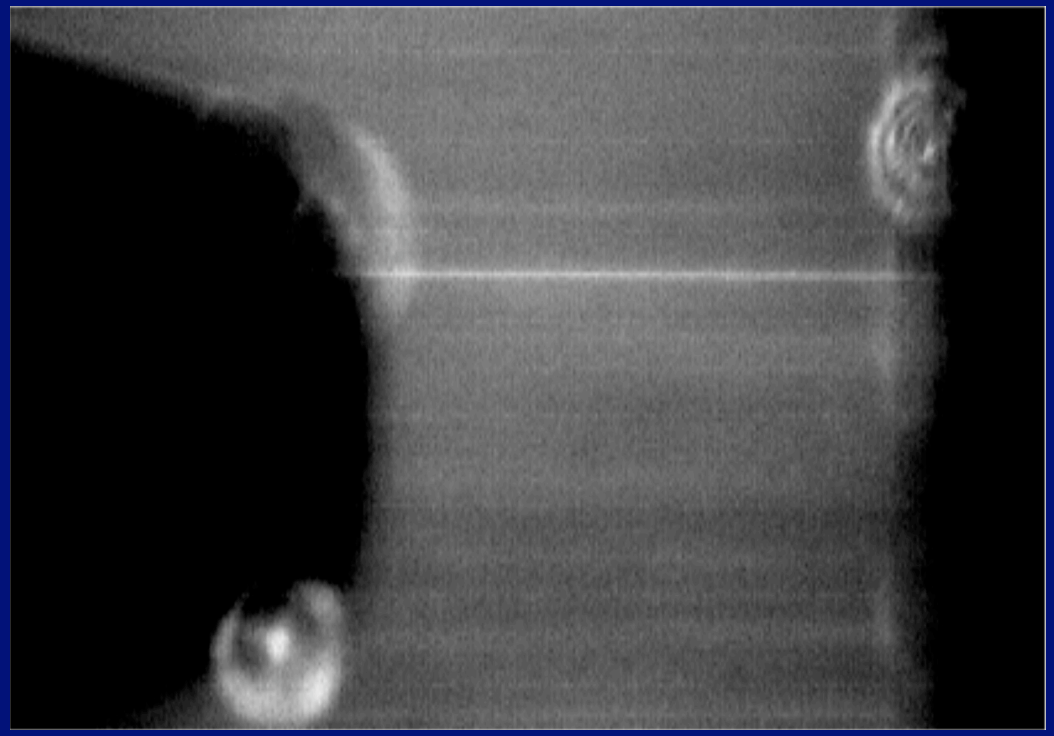
Electric field on — DNA molecule stretches…

Single Molecule Manipulations



…and moves towards pointed electrode.

Single Molecule Manipulations

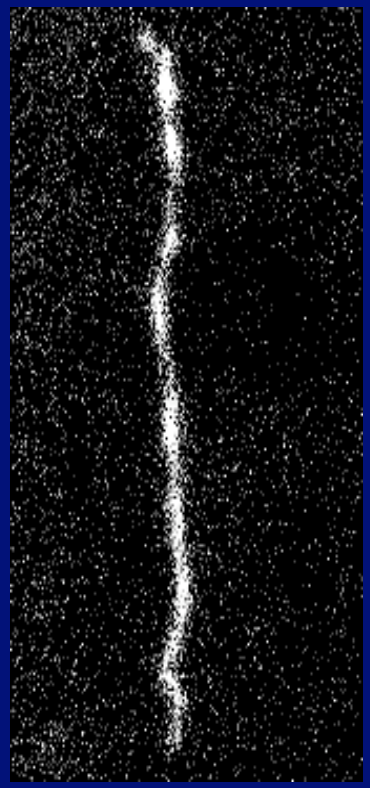


Field (frequency) increases — DNA stretches between electrodes

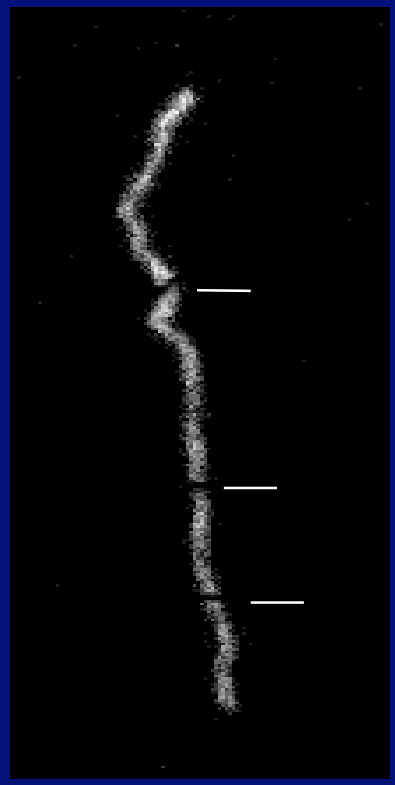
Reactions

Microscale

Stretch

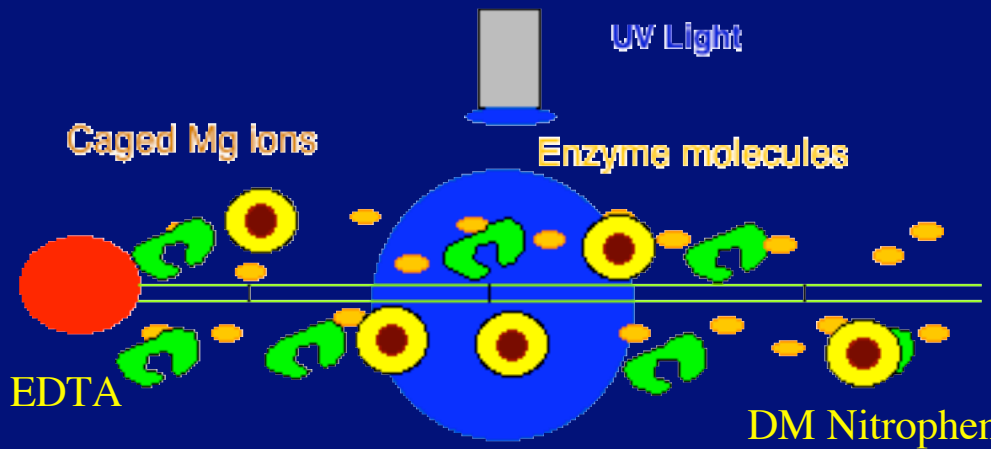


then cut

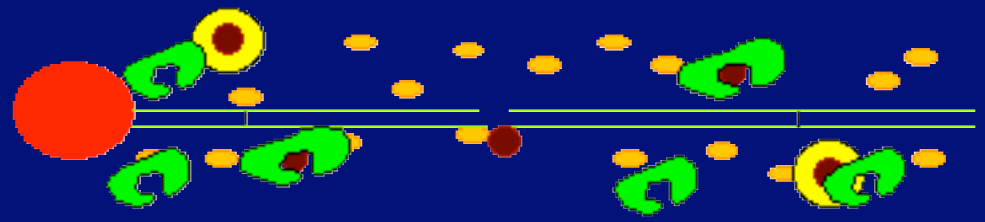


Localized Reactions

UV Activation



Photolysis of caged compound by UV illumination

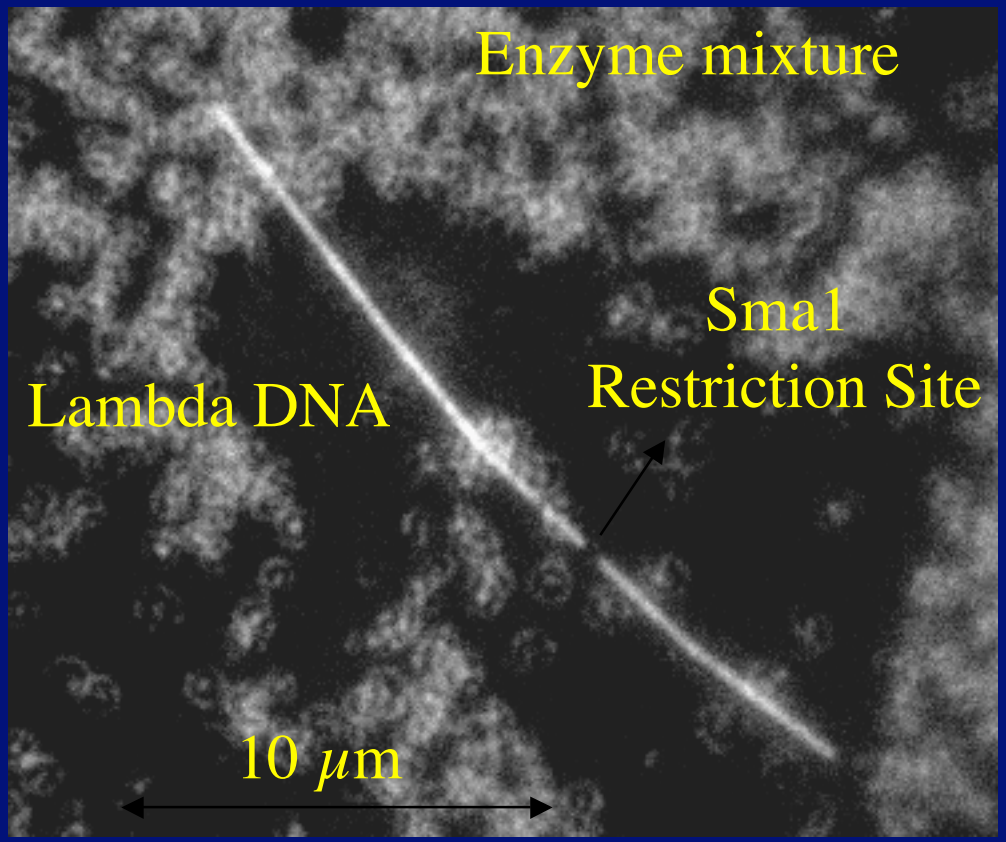


Localized restriction digestion reaction of stretched DNA

UV induced reactions

Microscale

Zone 1 illumination



Conclusions

- Microfabricated components for chemical analysis
- Integrated chemical analysis system
- Potential for inexpensive, self-contained, complex systems
- Many future applications