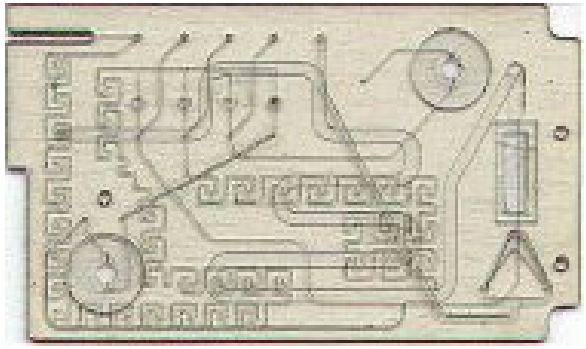
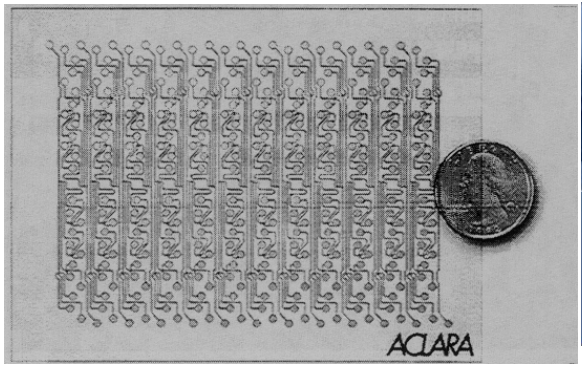
Novel Microfluidic Valving and Packaging Designs for Protein Containing Biochips

Chunmeng Lu and L. James Lee
Department of Chemical and Biomolecular Engineering
The Ohio State University

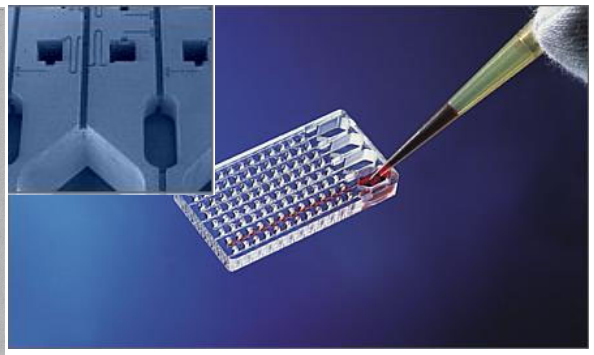
Lab-on-a-Chip



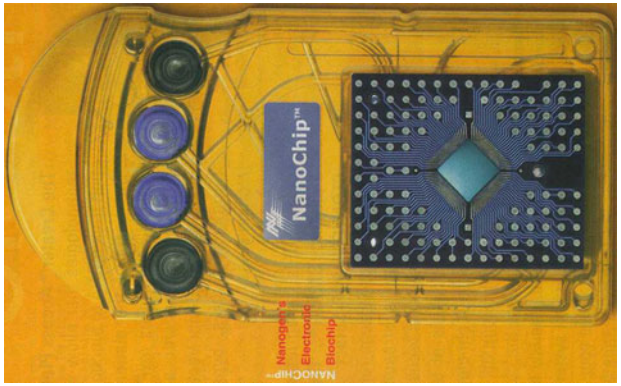
O.R.C.A microFluids
(Micronics, Inc.)



LabCard™
(ACLARA BioSciences, Inc.)



Microtiterplate Lilliput
(STEAG microParts GmbH)

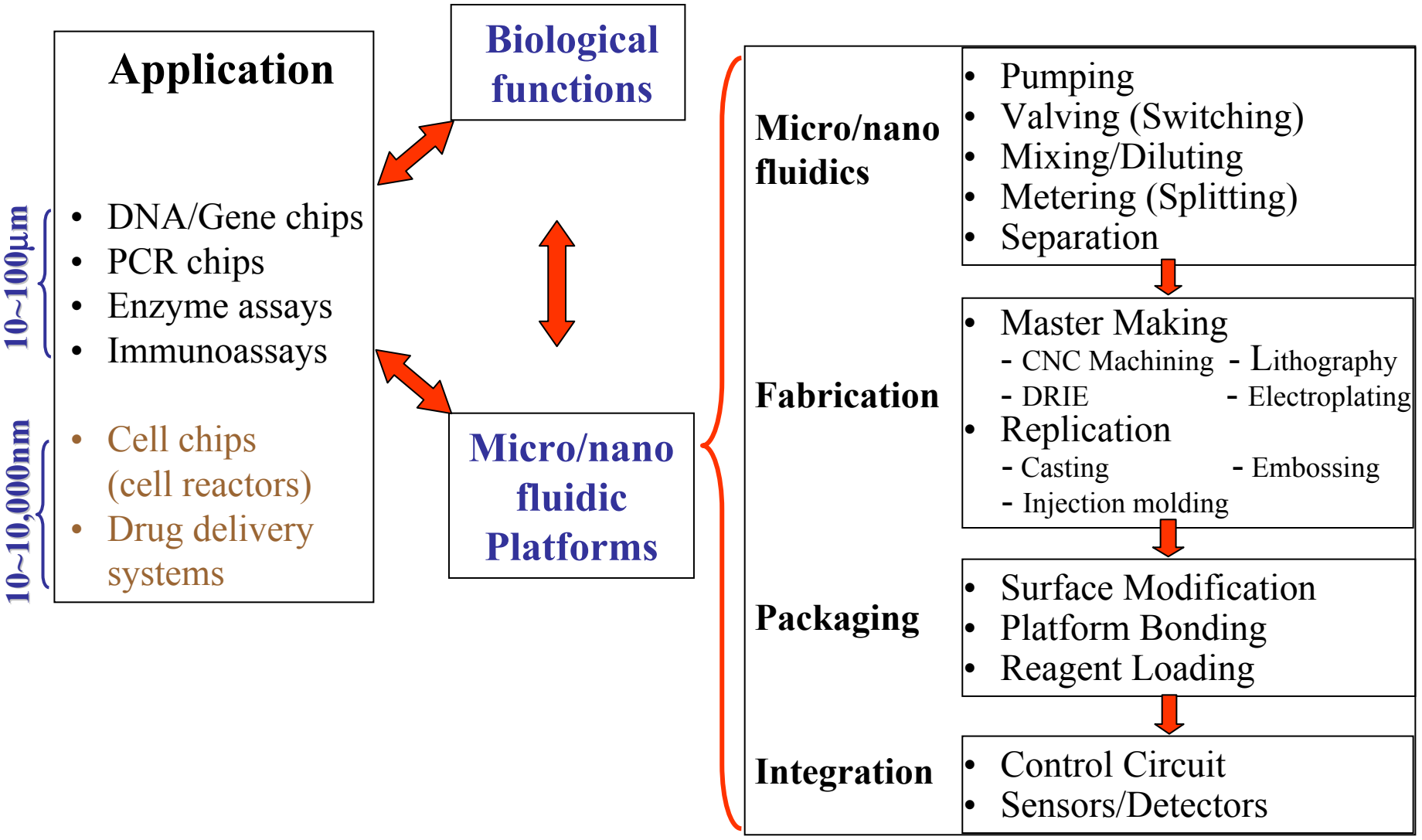


NanoChip™
(Nanogen, Inc.)

LabChip®
(Caliper, Inc.)

- Point of care -- fast response
- Small amount of sample < µl
- Parallel detection
- Information storage

Lab-on-a-Chip

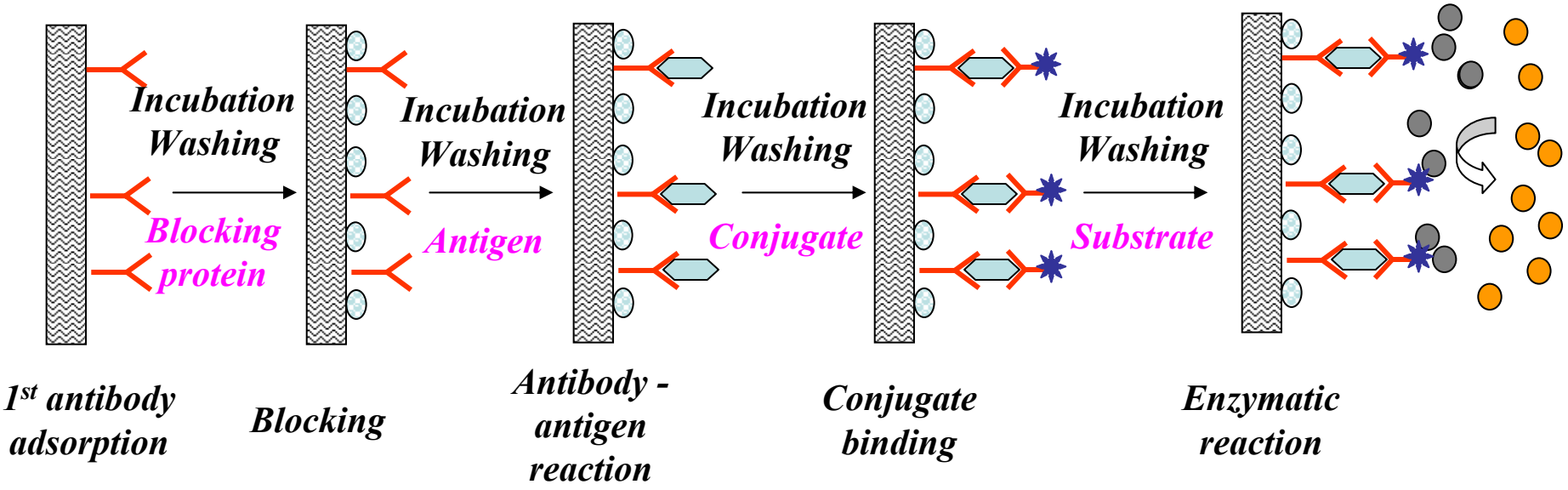

Application
10~100μm
• DNA/Gene chips
• PCR chips
• Enzyme assays
• Immunoassays
10~10,000nm
• Cell chips (cell reactors)
• Drug delivery systems
Biological functions
Micro/nano fluidic Platforms
Micro/nano fluidics
• Pumping
• Valving (Switching)
• Mixing/Diluting
• Metering (Splitting)
• Separation
Fabrication
• Master Making
- CNC Machining - Lithography
- DRIE - Electroplating
• Replication
- Casting - Embossing
- Injection molding
Packaging
• Surface Modification
• Platform Bonding
• Reagent Loading
Integration
• Control Circuit
• Sensors/Detectors

ELISA

- Enzyme-linked Immuno-Sorbent Assay
  - Bioassays in life sciences research
  - Food Safety – detection of foodborne pathogens and toxins
  - Biodiagnostics – detection of cancer and immuno diseases
  - Environmental pollutants
  - National security – detection of bio- and chemi-warfare agents

$5-10B/yr. ELISA market for cancer, HIV, food and water detection!

Schematic of ELISA



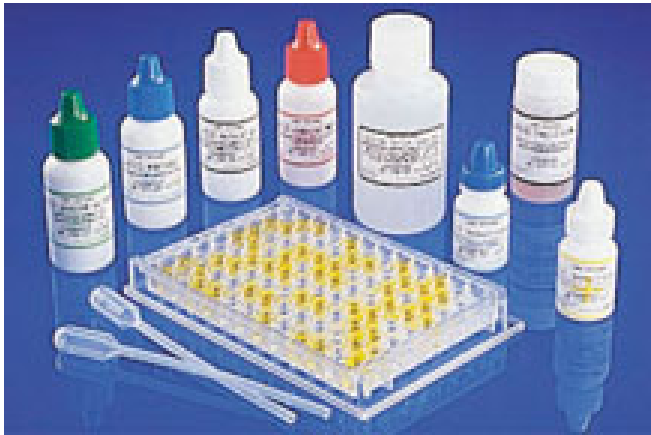
- 1st antibody
- Blocking protein
- **Antigen**
- Conjugate (2nd antibody)
- Enzyme
- Substrate
- Detectable product

High Selectivity!!

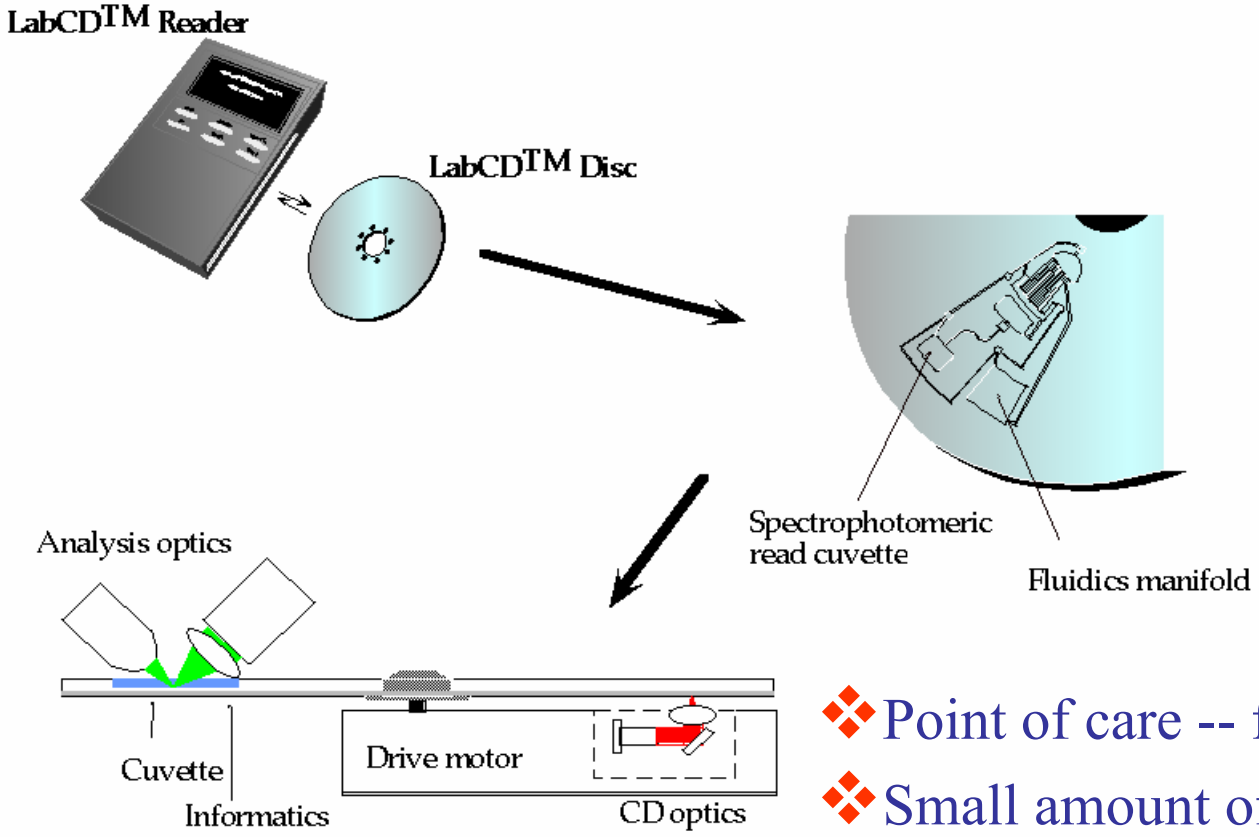
High Sensitivity!!

Conventional 96-well ELISA

- Time consuming (hours to 2~3 days)
  - Immunoreaction is diffusion controlled
  - Long incubation time (several hours)
- Relatively large reagent consumption (several hundred μl)
- Labor intensive
- Inconsistent results



CD Microfluidic Platform System

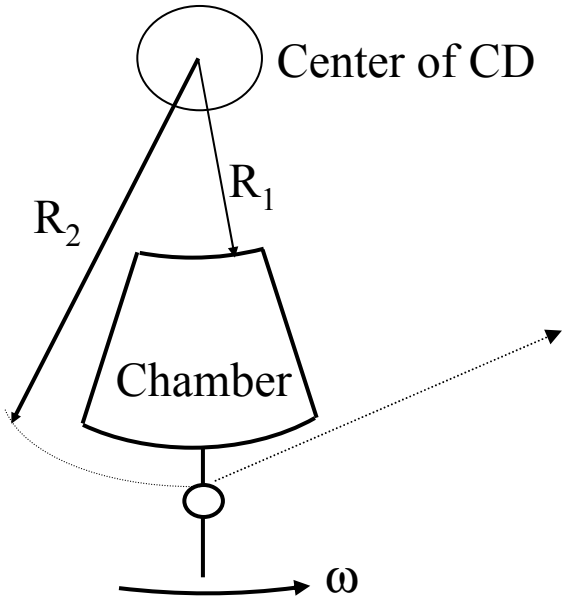


- Point of care -- fast response
- Small amount of sample -- < µl
- Parallel detection
- Information storage

Pumping and Valving

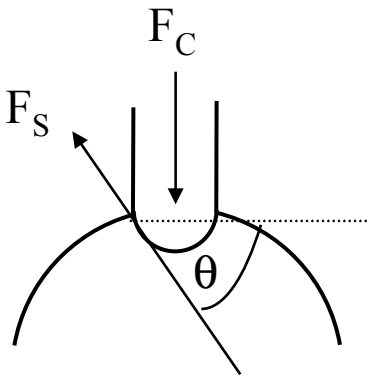
Driving Force

⇧ **Centrifugal force**



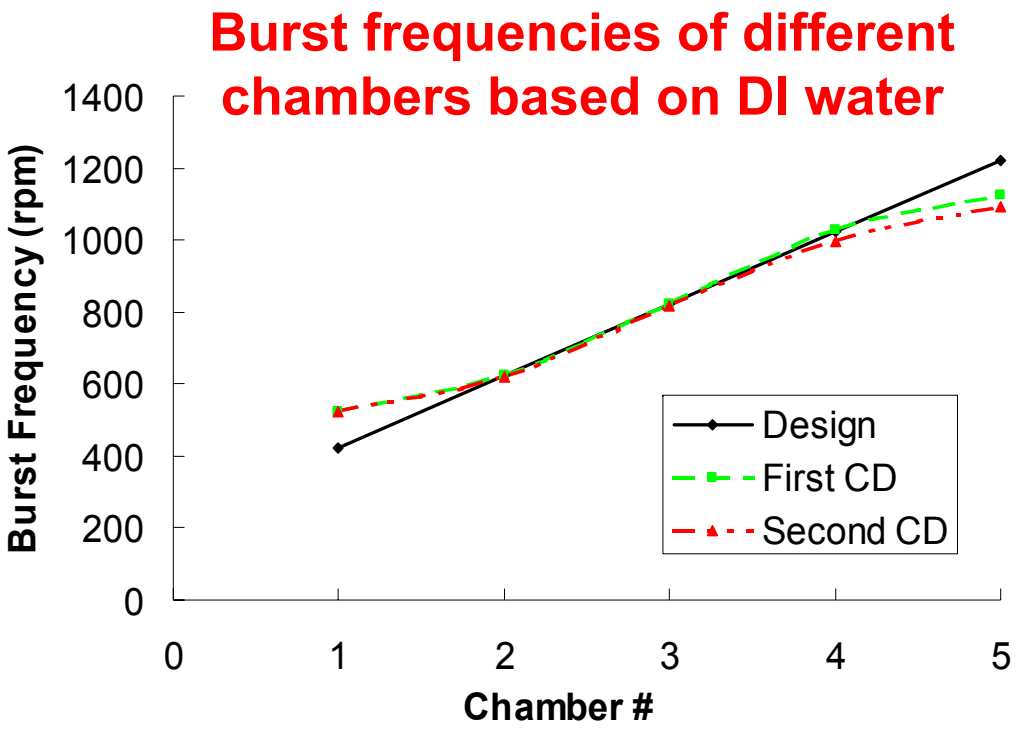
Capillary Valve

⇧ **Balance of centrifugal force and capillary force**

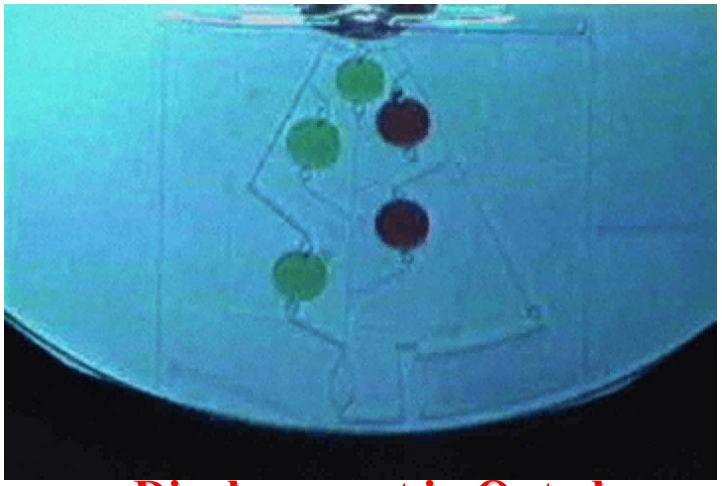


$$f_b = \sqrt{\frac{\gamma \cdot \sin\theta}{\pi^2 \cdot \rho \cdot \overline{R} \cdot \Delta R \cdot d_H}}$$

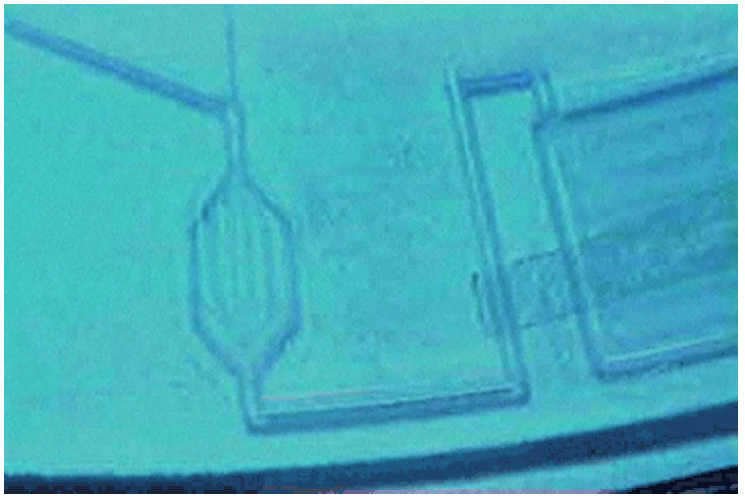
Pumping and Valving - Flow Sequencing



Flow Sequencing



Displacement in Optode

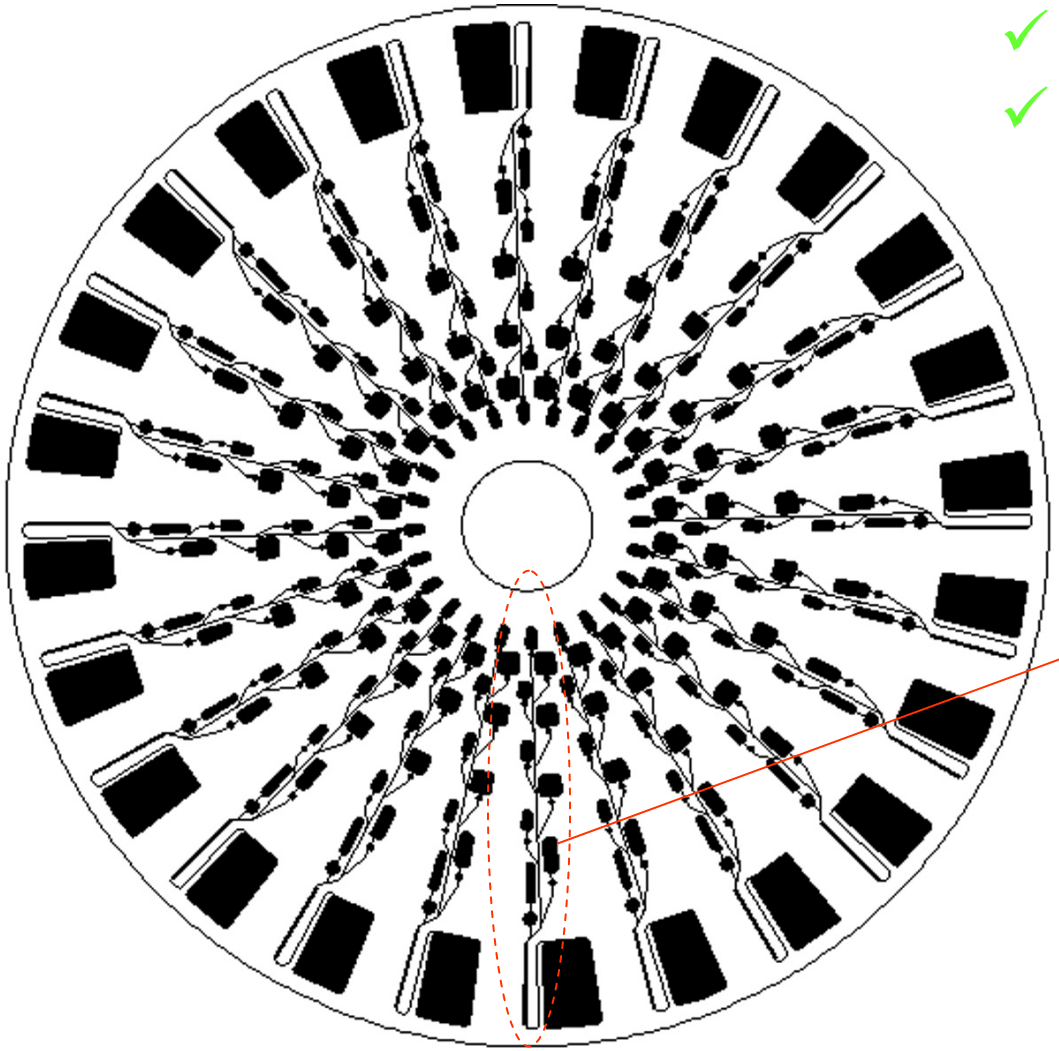


Chamber index:

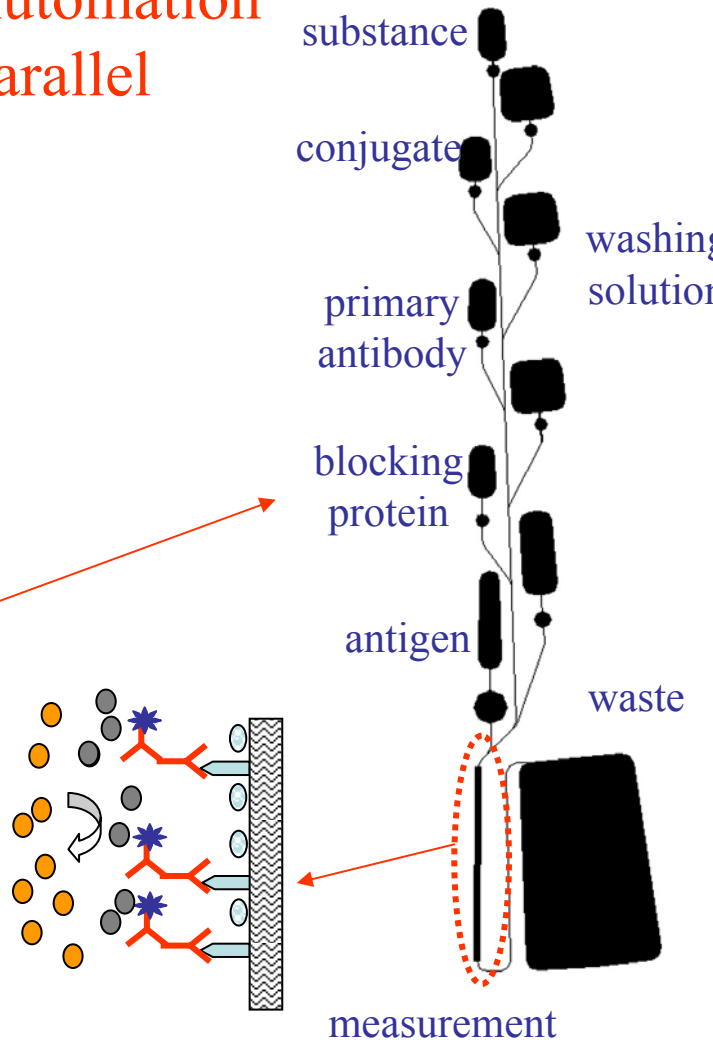
1- Calibration 1; 2- Wash 1; 3- Calibration 2; 4- Wash 2; 5- Sample

M. J. Madou, L.J. Lee, S. Daunert, K. W. Koelling, S. Lai, C-H Shih, *Biomedical Microdevices*, 2001

CD-ELISA Chip Design



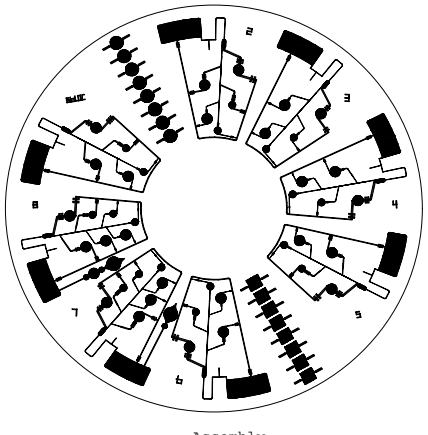
✓ Automation
✓ Parallel



Issues

- Design: dimension control; aspect ratio; multiple depth; many reservoirs
- Protein blocking: Valving
- Protein preloading: Bonding

CD Manufacturing

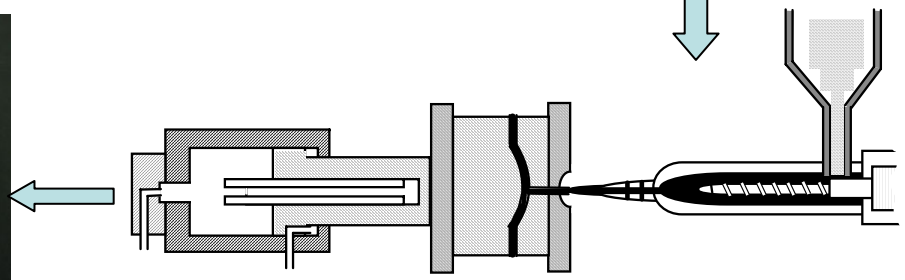


CAD design

Mask fabrication (metal mask)

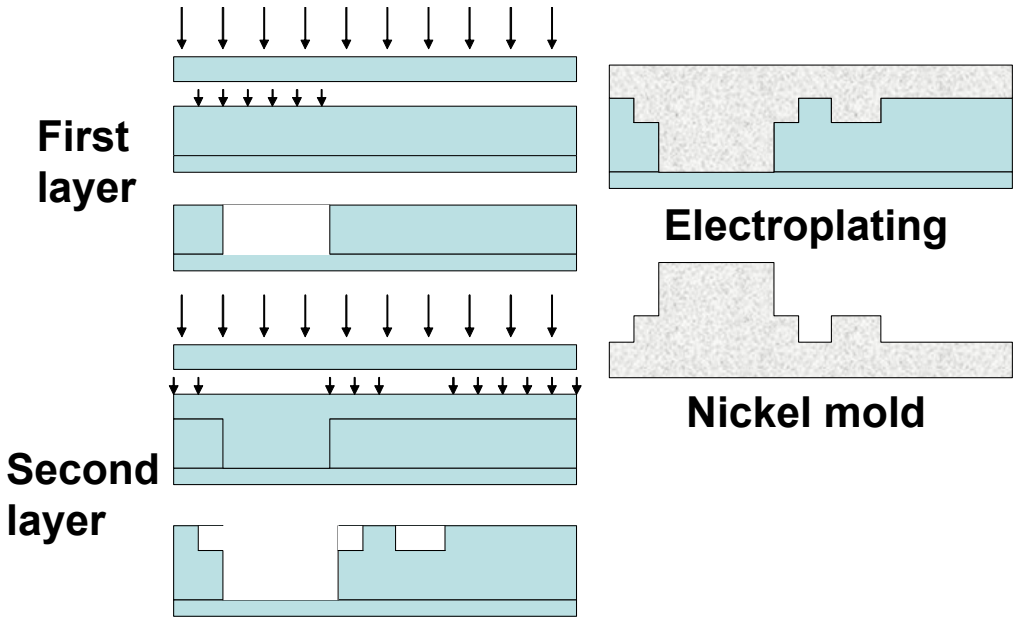
multi-layer photolithography (SU-8 mold)

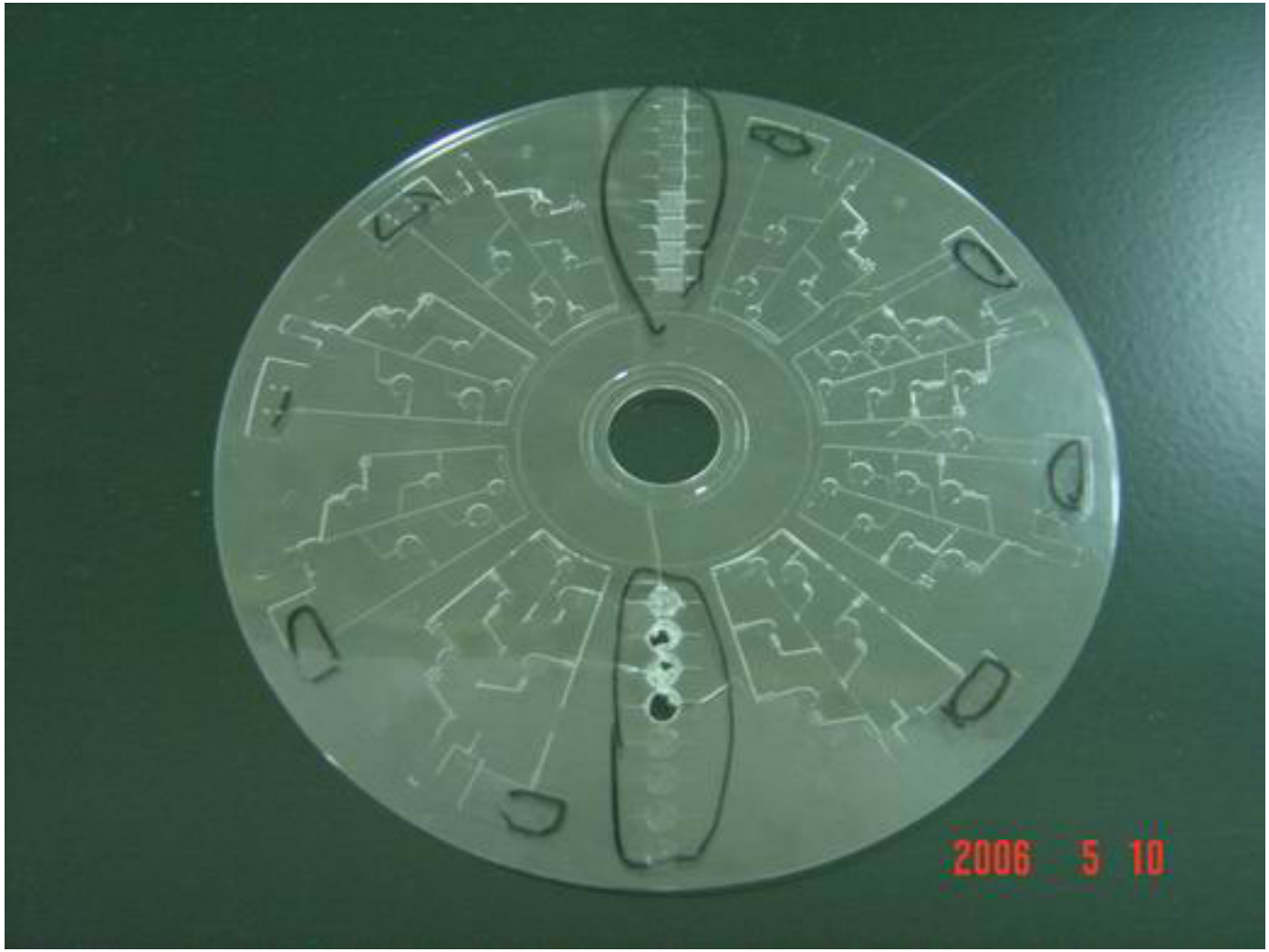
Electroplating (Nickel mold)



Design Issues

- Higher aspect ratio desirable
- Multiple depth is needed for a larger design window for 9-reservoir CD and bubble free sample loading
- Mold fabrication is more difficult
- Mold release

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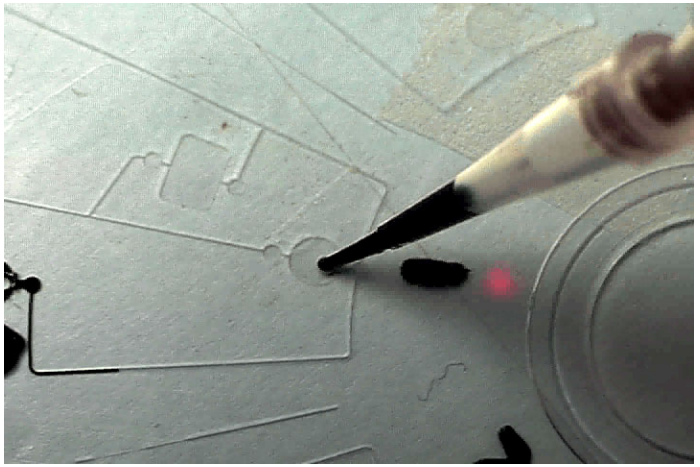
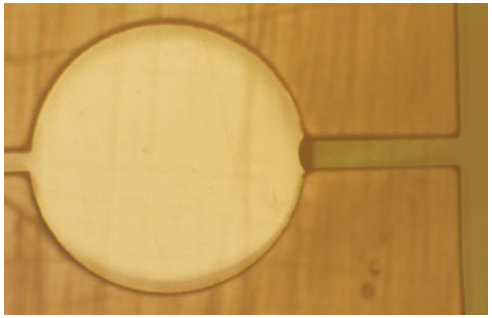
Issues

- Flow sequence: dimension; aspect ratio; multiple depth, more reservoirs
- Protein blocking: Valving
- Protein preloading: Bonding

Protein Issues in Capillary Valving

- Surface property changes after protein blocking
- The valving capacity lost or reduced





w/o protein blocking

with protein blocking

Principle of Super-hydrophobicity

- Wensel's theory

$$\cos\theta = r(\gamma_{sv} - \gamma_{sl}) / \gamma_{lv} \quad (1)$$

r=real surface area/projected surface area

(roughness factor)

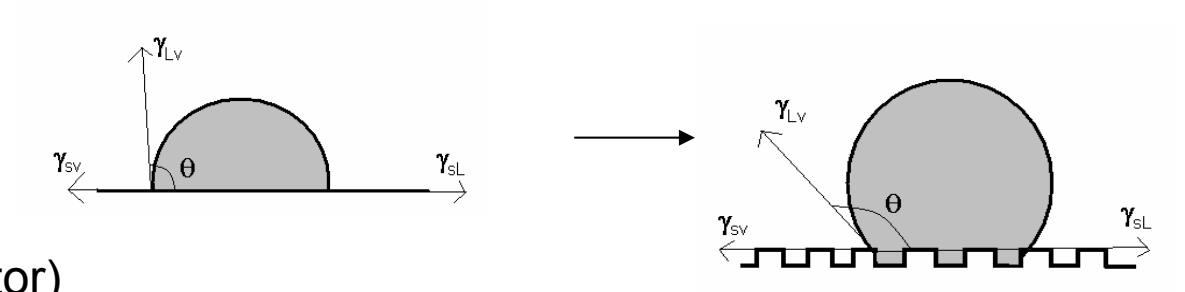
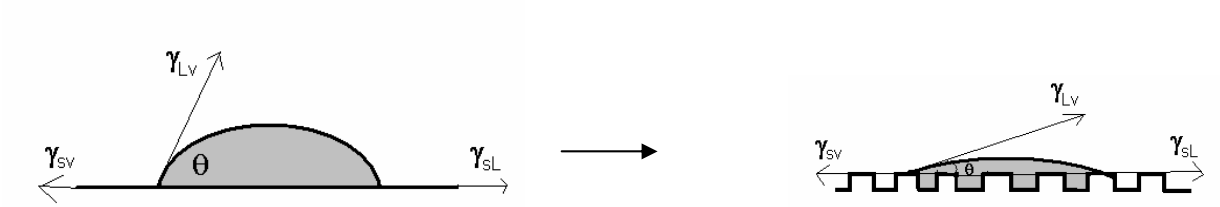


Fig.1



- Cassie's theory

$$\cos\theta^* = f\cos\theta - (1-f) \quad (2)$$

f is the solid fraction at the interface

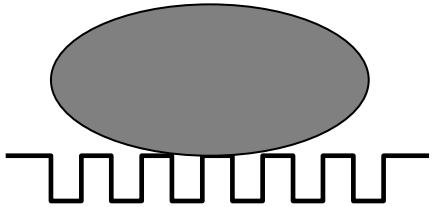
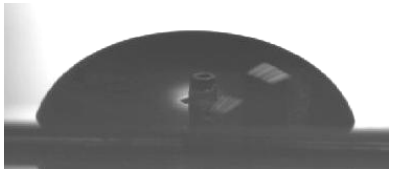


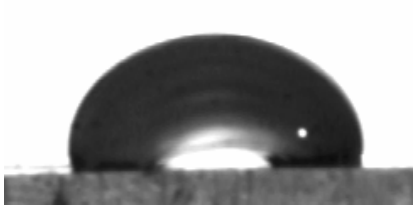
Fig.2

Plasma Treated and Surface Microstructured PMMA

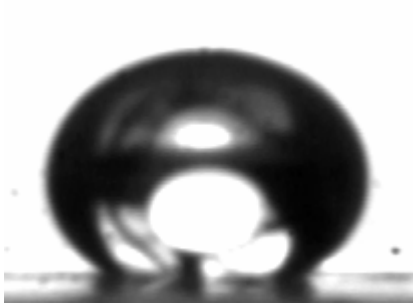
- Typical PMMA θ~ 73 º

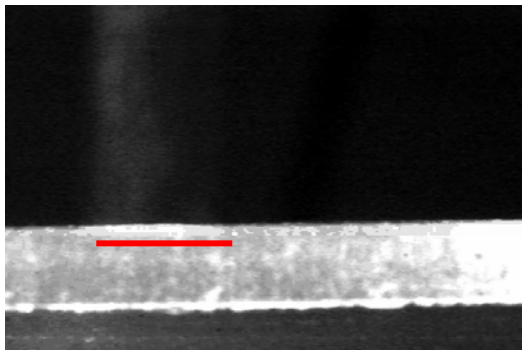


- Fluorine plasma (CHF3) treated PMMA surface θ ~ 108º

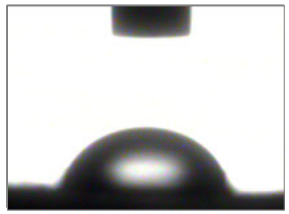


- Fluorine plasma treated micro-patterned PMMA surface θ > 160º, f=0.1

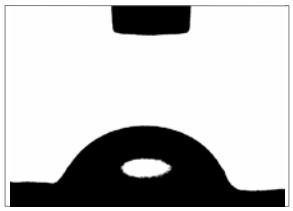




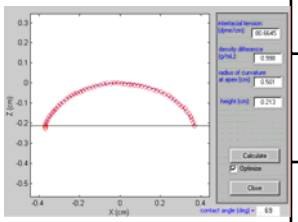
Effect of Protein Blocking on Contact Angle



Picture of Teflon-PLA

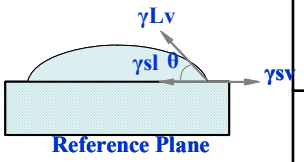


Edited image



Calculated result

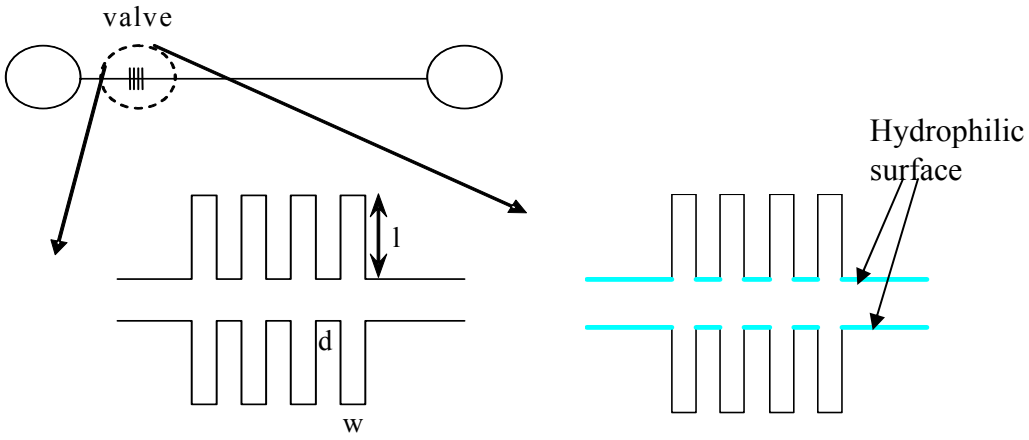
Young-Laplace Equation: $\cos\theta = \frac{\gamma_{sv} - \gamma_{sL}}{\gamma_{Lv}}$



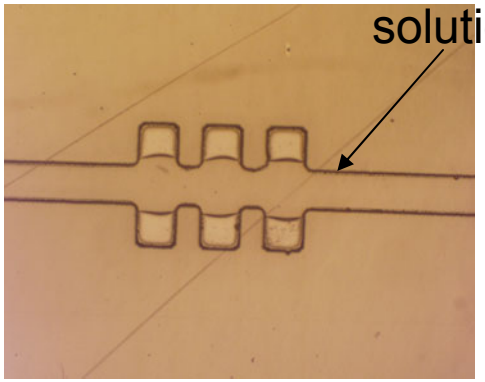
- Controlled environment
- CCD camera
- Drop profile analysis
- Treatment BSA solution=1.0%
- Testing BSA solution=0.2%

Treat method and substrate materials	0 min	5 min
No plasma without protein treatment (PMMA)	73	68
No plasma with protein treatment (PMMA)	74	42
No plasma with protein treatment (PLGA)	70	53
No plasma with protein treatment (Tape)	106	105
No plasma with protein treatment(COC)	79	58
With plasma with protein treatment (PMMA)	80	57
With plasma without protein treatment (PMMA)	108	106
With plasma with protein (PMMA with microfeaturel)	161	150

Fishbone Design Based on Superhydrophobicity

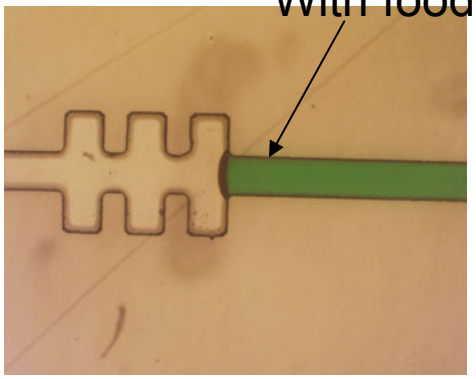


Fishbone design



Advantages:

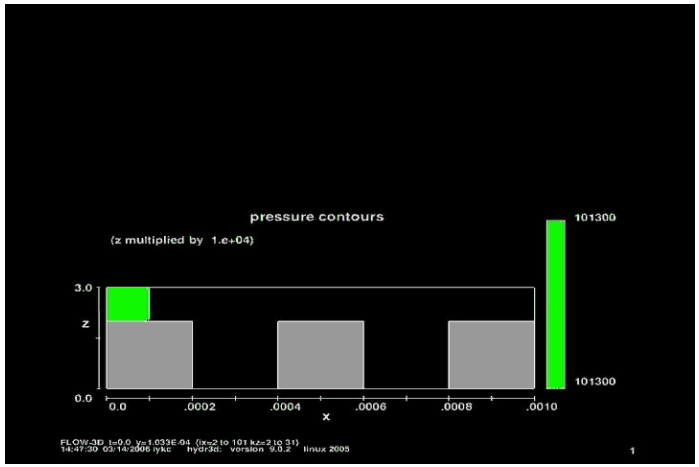
- Protein-proof with superhydrophobic property
- Easy fabrication (embossing or injection molding)
- Easy alignment of bonding

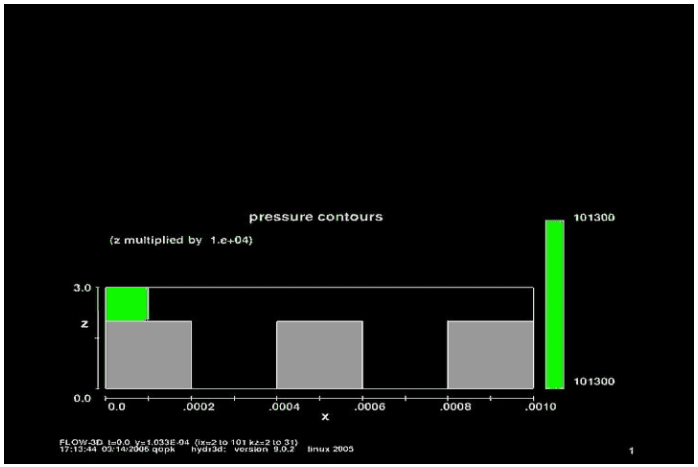


Blocking Process Simulation (Flow-3D®) and Visualization

Channel: 200μm)

Flow speed: 2mm/s

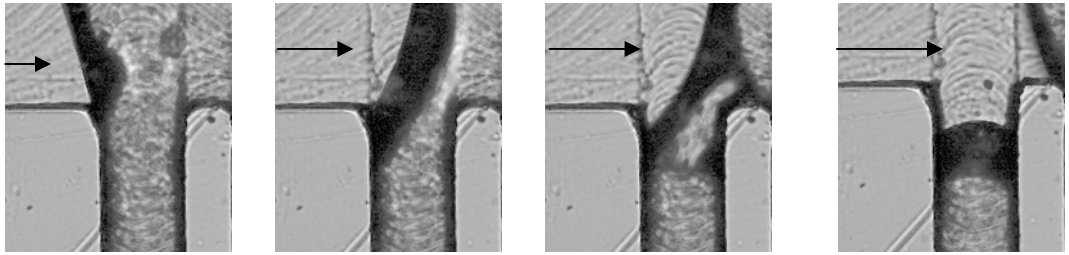




Hydrophobic

Hydrophilic

PMMA surface w/o plasma treatment



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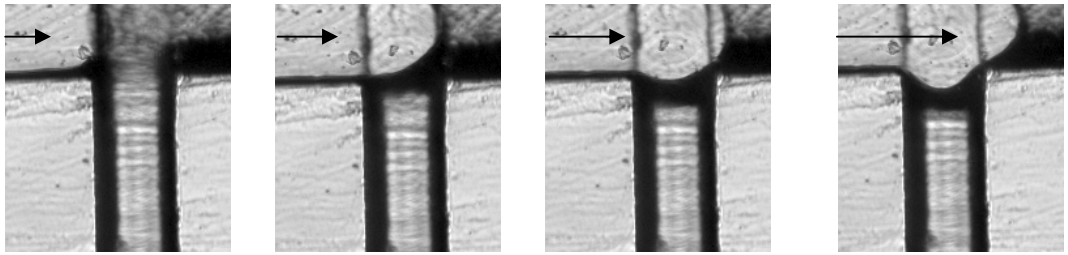
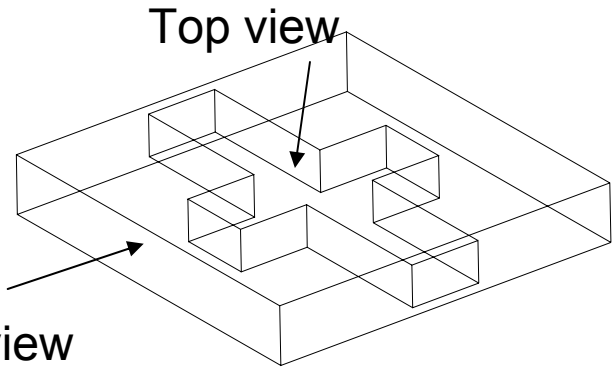
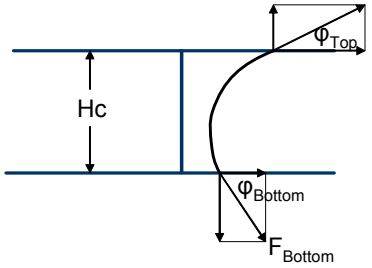
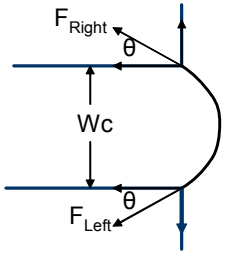
Hydrophobic surface with plasma treatment

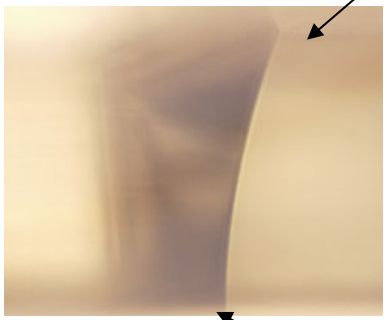


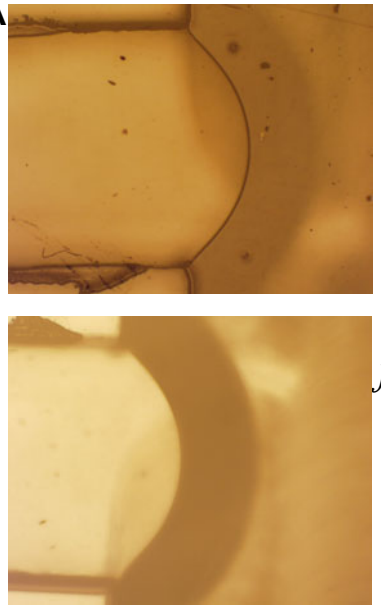
calculated burst frequency









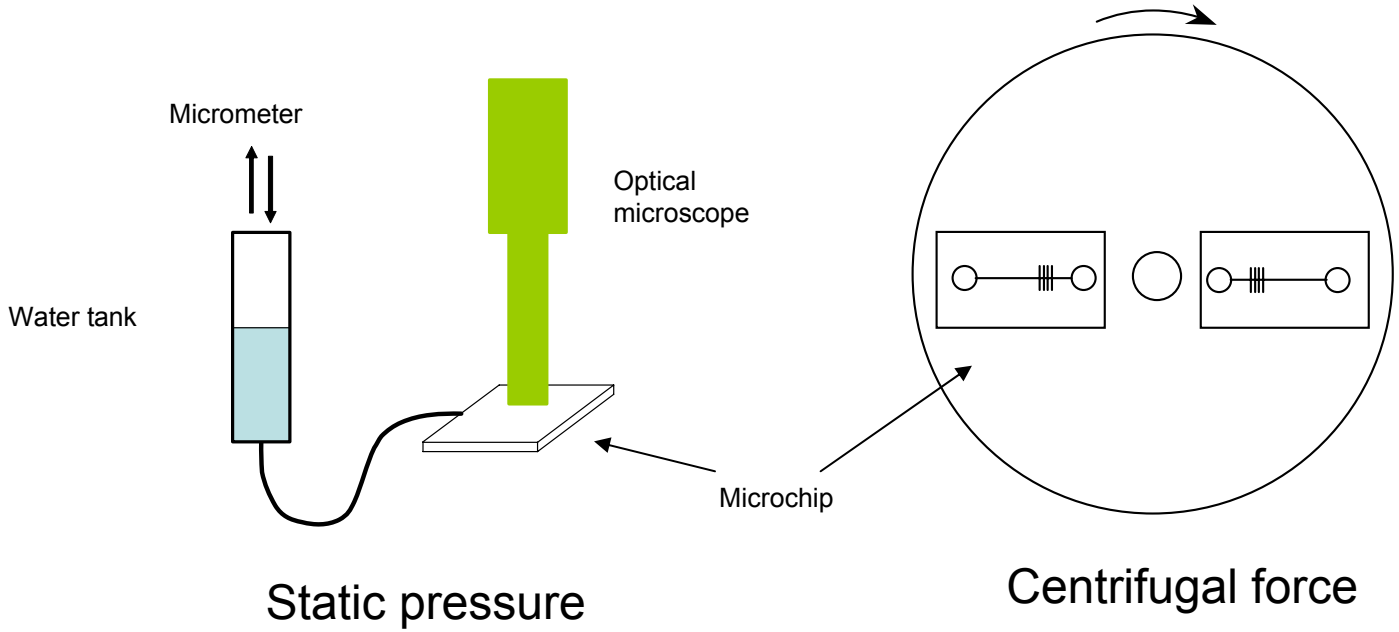


Side view

Top and bottom view

$$f = \omega / 2\pi = \left(\frac{\frac{2\gamma \sin\theta}{W_c} - \frac{\gamma(\cos\theta_{top} + \cos\theta_{bottom})}{H_c}}{4\pi^2 \rho \Delta R \bar{R}} \right)^{1/2}$$

Holding pressure test



Experimental vs. theoretical Burst frequency

- Single channel

  -Syringe injection plus vacuum removal

- CD device

  -Centrifugal force

- Why discrepant?

  -bonding defect

  -local defect

  -loading defect

Parameter	Valve 1	Valve 2	Valve3	Valve4	Valve5
Protein Treatment Type	0.1 wt% BSA soak	0.1 wt% BSA soak	0.1 wt% BSA soak	0.1 wt% BSA soak	0.1 wt% BSA soak
R1 (mm)	23.3	21.6	32.5	39.0	25.5
R2 (mm)	27.0	27.2	39.0	44.5	31.1
R_delta	3.7	5.6	6.5	5.5	5.6
Width	200	200	200	200	200
Depth(mm)	100	100	100	100	100
(mN/m)	72.9	72.9	72.9	72.9	72.9
Density	1.0	1.0	1.0	1.0	1.0
Burst Freq.	768	634	486	489	589
Exp. Burst Freq	761	705	478	541	573
Match?	YES	NO	YES	NO	YES

Issues

- Flow sequence: dimension; aspect ratio; multiple depth; more reservoirs
- Protein issues: Valving
- Protein preloading: Bonding

Platform Bonding Methods

Silicon/Glass/Metal Materials:

- Anodic bonding
- Fusion bonding
- Eutectic bonding
- Adhesive bonding

✓ Well developed in IC industry

✘ Usually high temperature, high voltage, or high pressure

✘ most not applicable to polymers

Polymeric Materials:

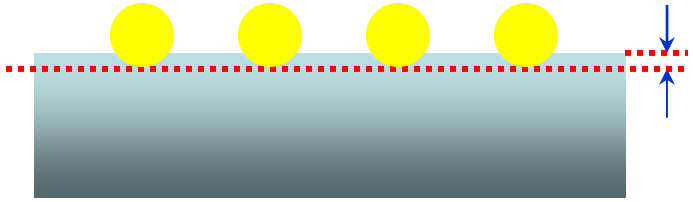
- Welding (hot plate, laser, ultrasound)
- Lamination (adhesive tape, film thermal bonding)
- Chemical (solvent) bonding

✓ Well developed in polymer industry

✘ Applicable mainly to relatively large features (several hundred microns)

Typical dimensions in BioNEMS/MEMS applications: 10 nm ~ 100 μm

Surface T_g of PS under CO_2



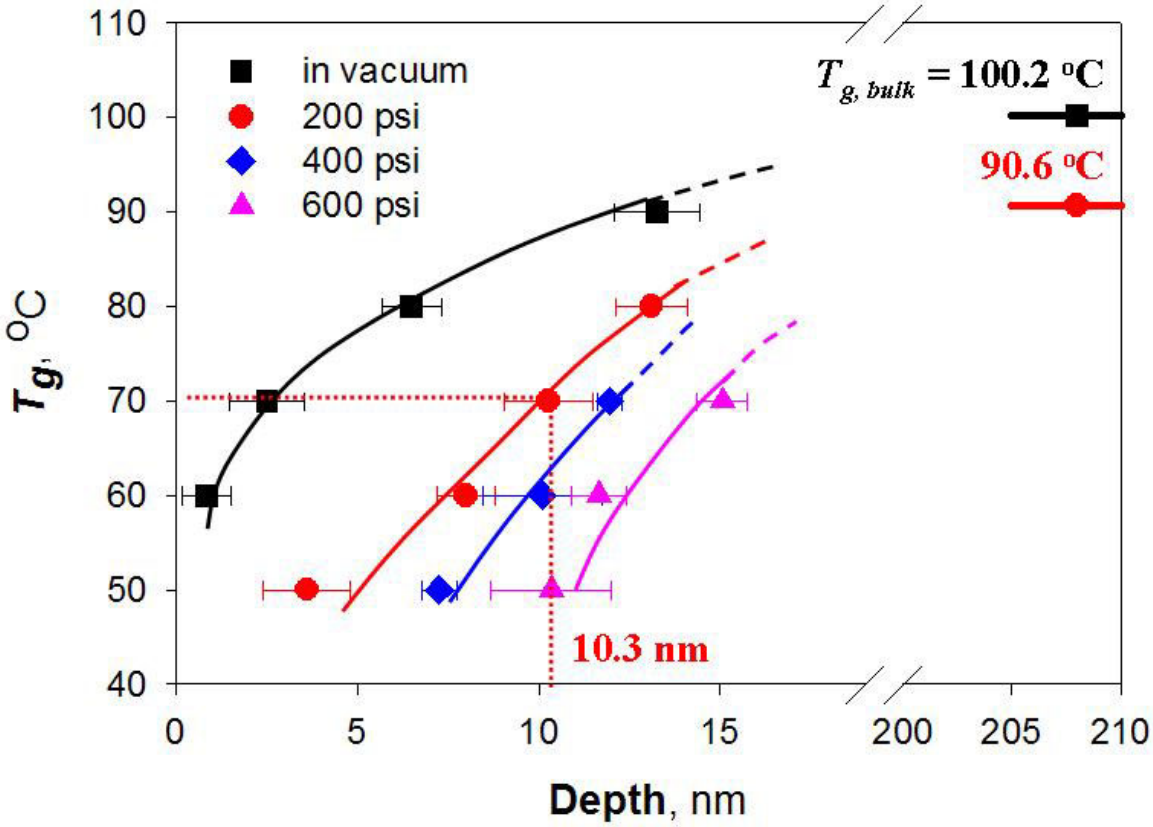
Particle Penetration Depth

↕

Surface Layer Thickness

↕

Surface T_g



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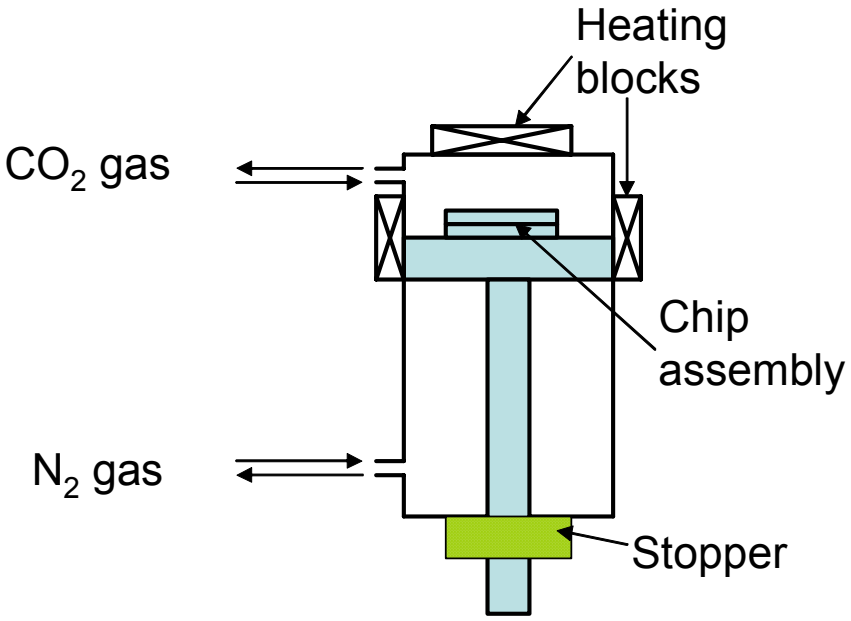
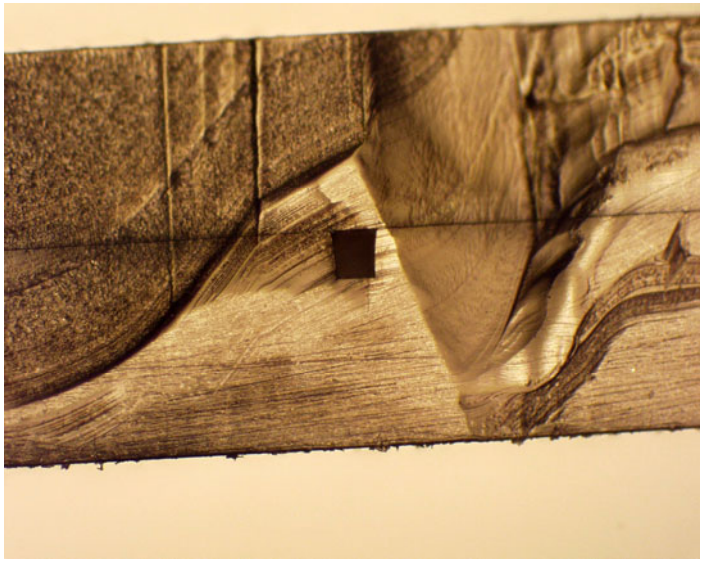
CO_2 Bonding Experimental Setup



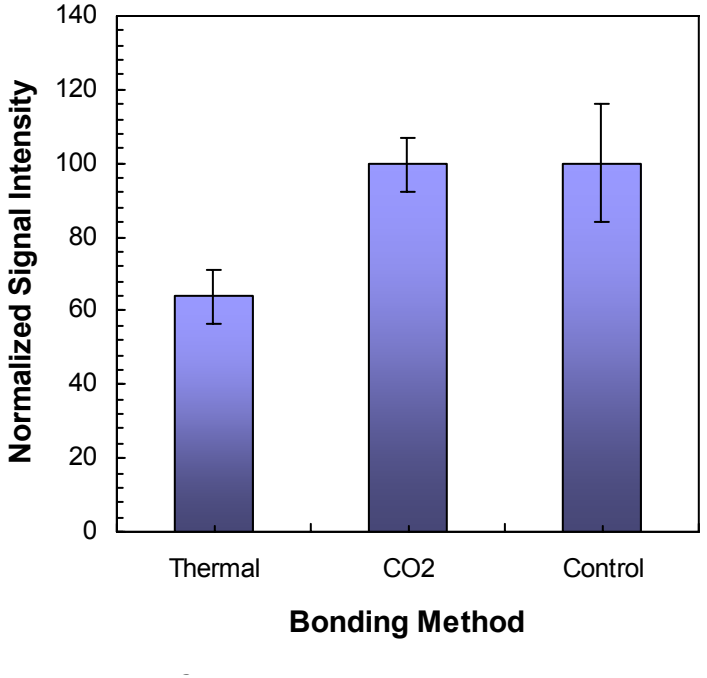
CO2 bonding

(200psi, 75psi, 1 hour)

CO_2 Bonding and Testing Results



CO2 bonding (200psi, 75psi, 1 hour, PLGA interlayer)



Thermal lamination: 140ºC, 10sec

Collaboration with Ritek and Tecan