生物微流體技術

王安邦

國立臺灣大學 應用力學研究所

Institute of Applied Mechanics

National Taiwan University

Contact: Tel: 886-2-2363-0979 ext. 405,

e-mail: abwang@spring.iam.ntu.edu.tw

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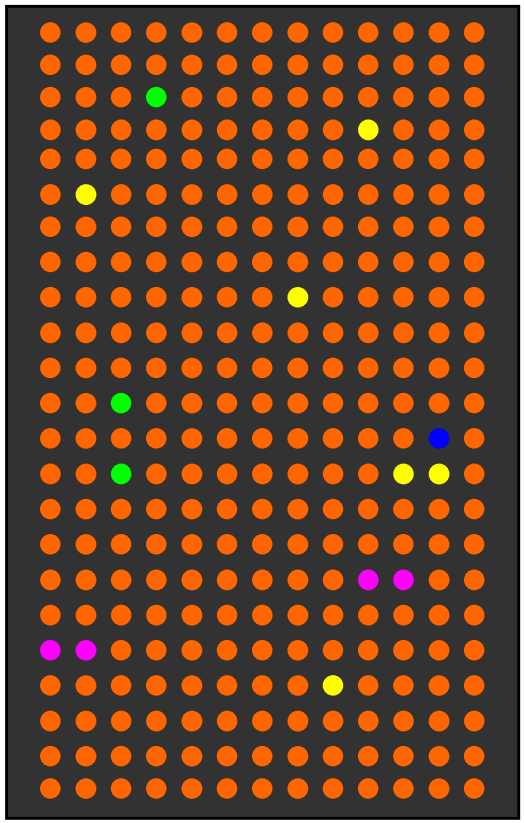
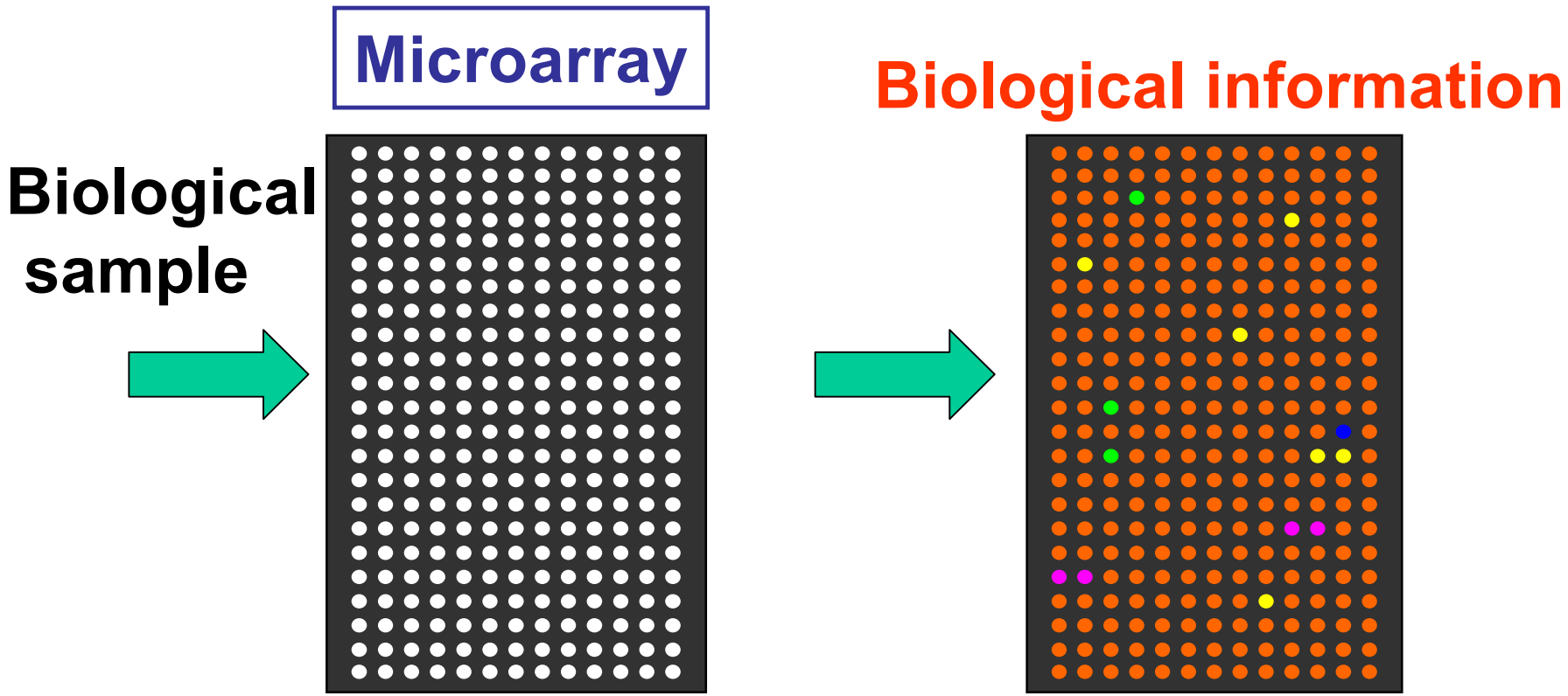


- 微陣列製程技術
  - Contact Printing
  - Non-contact printing
- 微流體系統技術

What and Why "Microarray" ?

- *What is Microarray ?*
- *Why using Microarray ?*



Classification of microarray printing techniques

Photolithography manufacturing technique

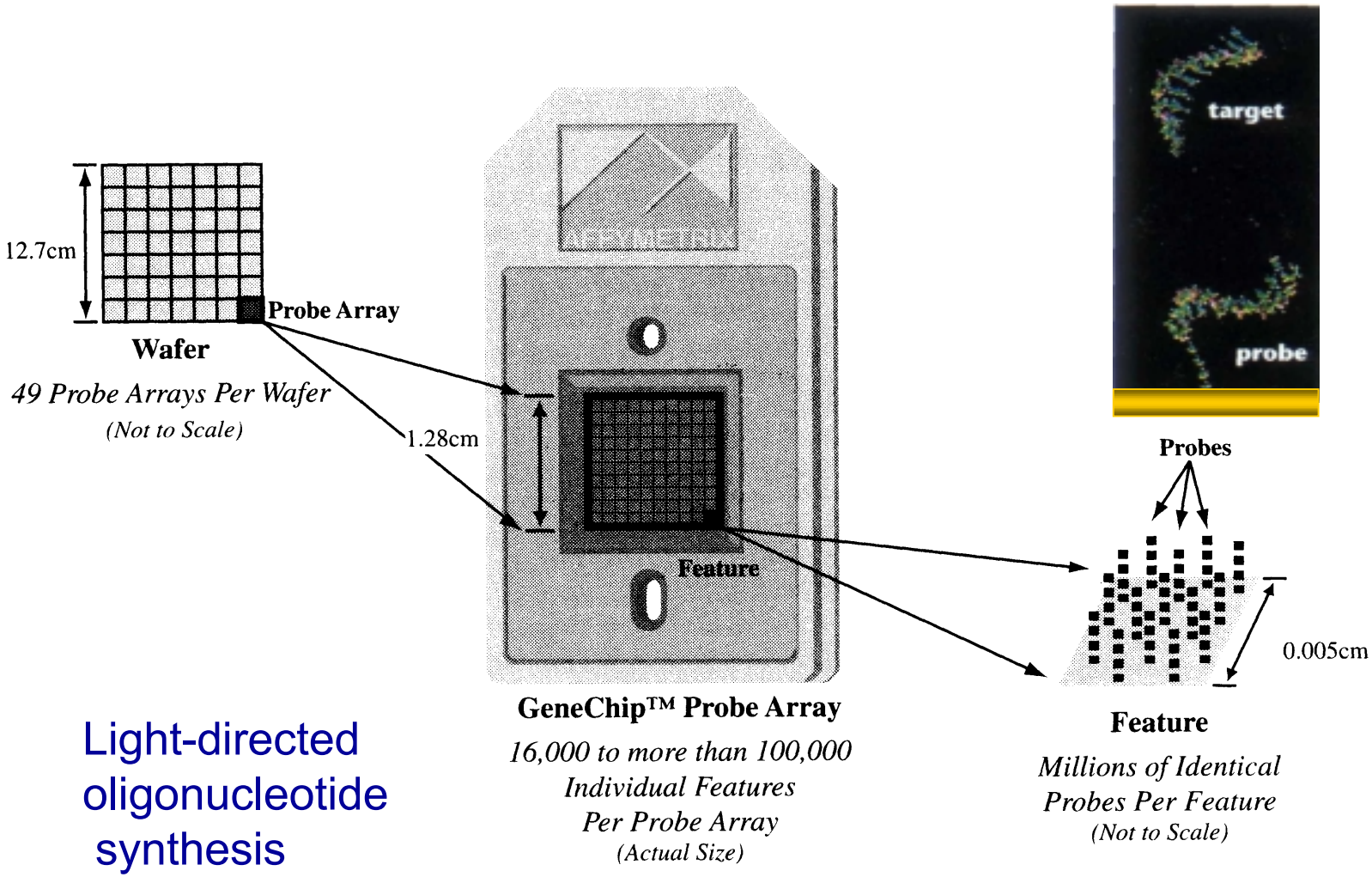
Droplet-Injection Printing **(Non-Contact method)**

- **Fluid delivery by injection onto substrate**
- **Active sample pickup**
- **Programmable print volume**

Pin-Printing **(Contact method)**

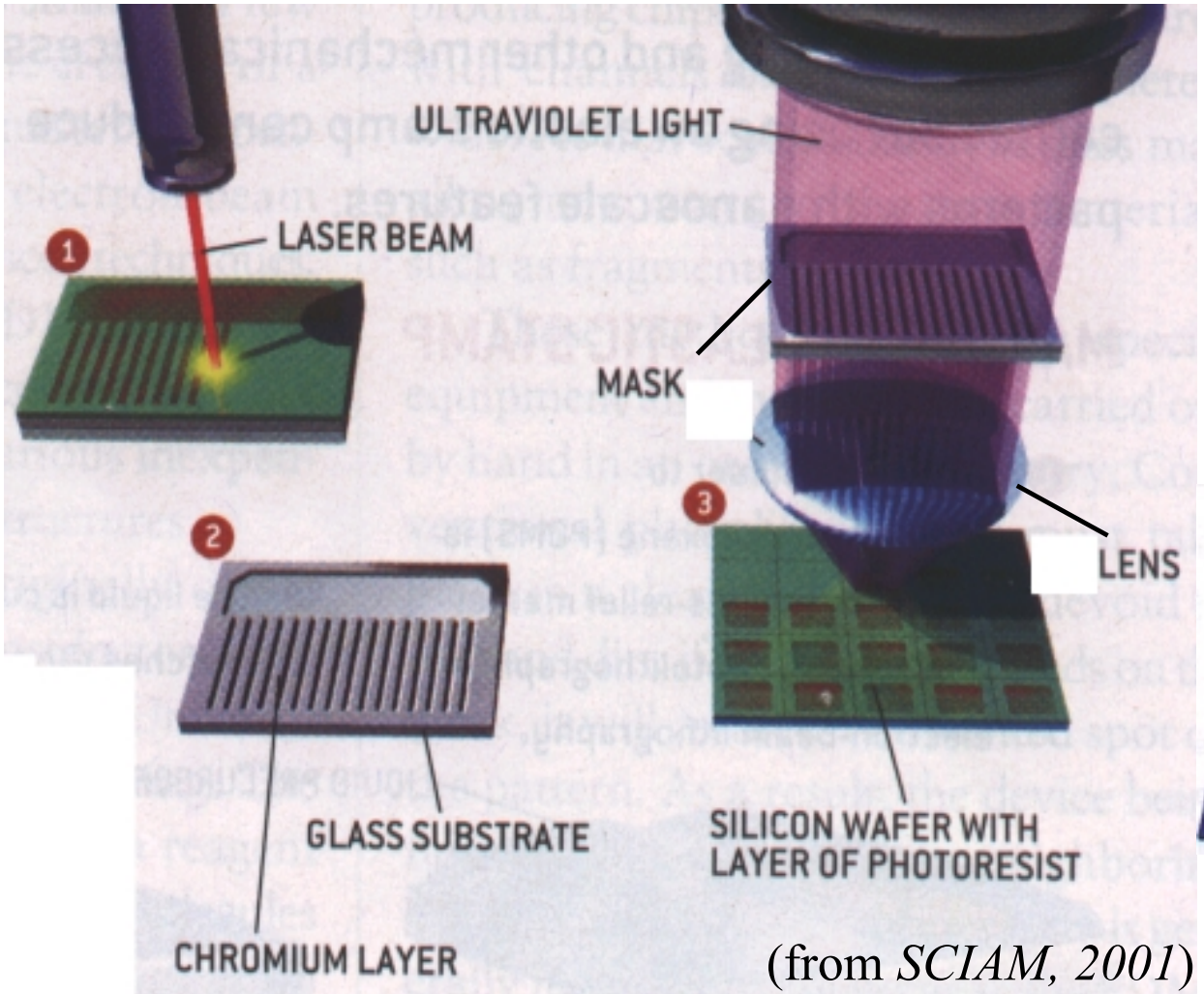
- **Fluid delivery through contact pins onto substrate**
- **Passive sample pickup (pin dipping)**
- **Fixed print volume**

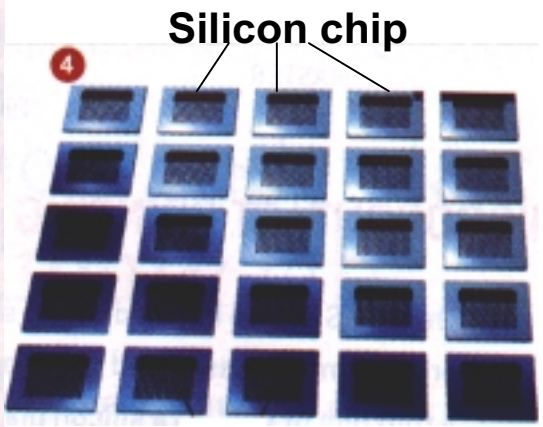
Photolithography Manufacturing Technique (I)



Light-directed oligonucleotide synthesis

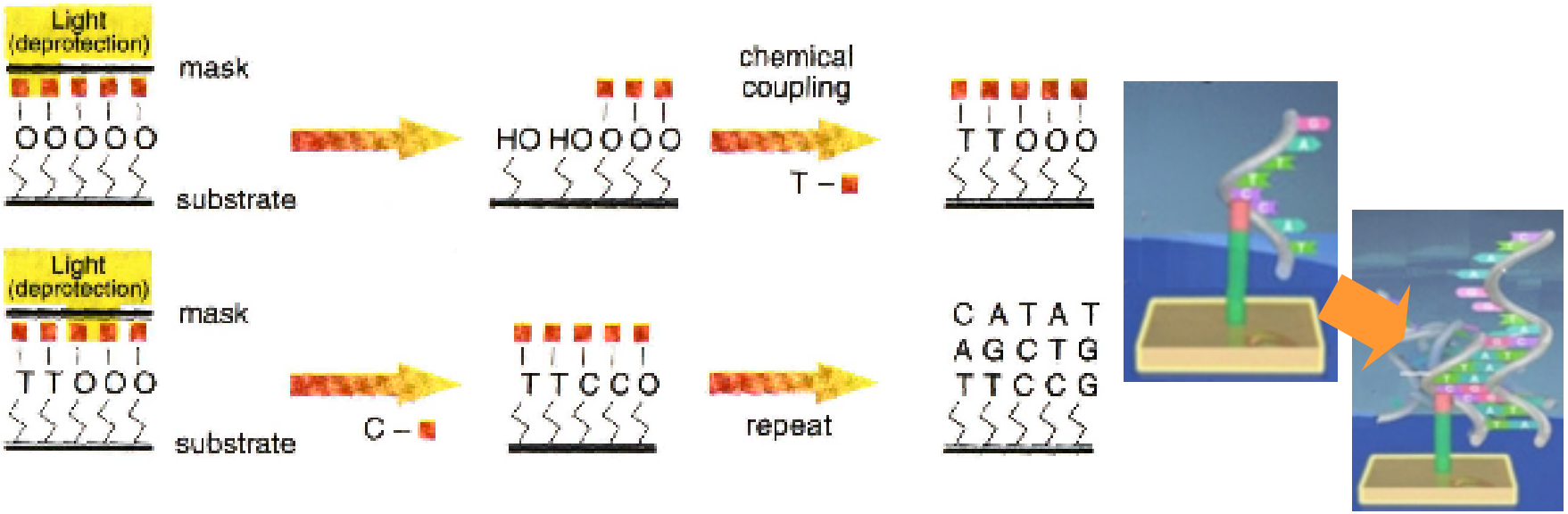
Photolithography Manufacturing Technique (II)





(from *SCIAM, 2001*)

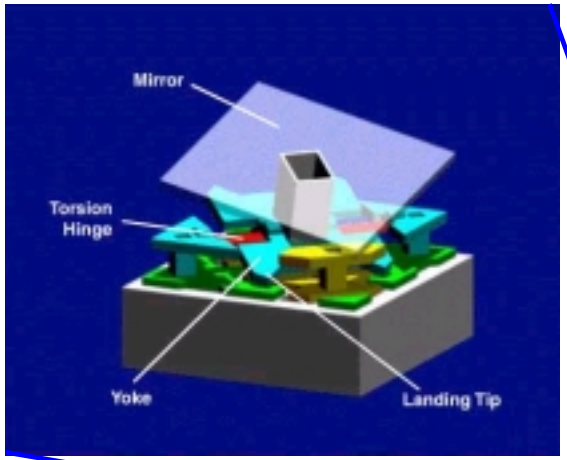
Photolithography
Manufacturing Technique (III)



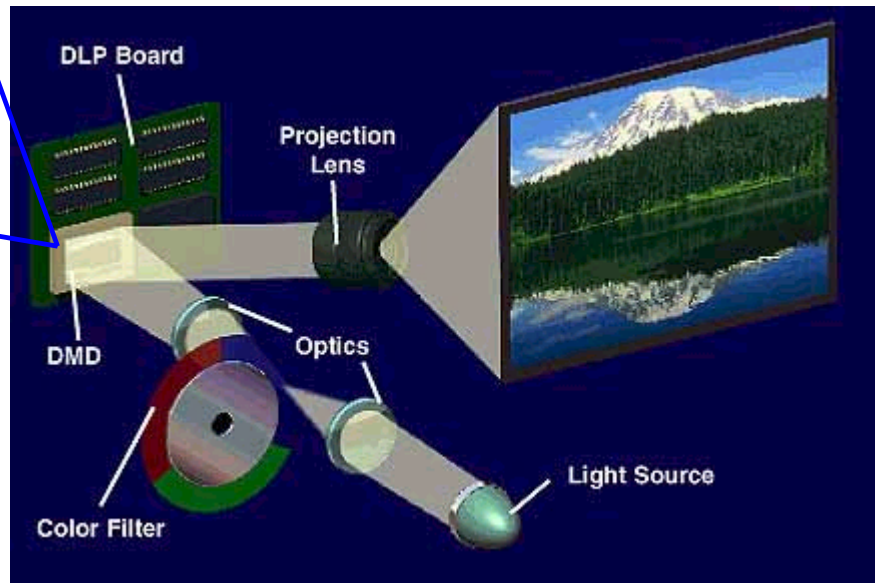
Advantages:
- no cloning, spotting and no PCR required
- high feature density

Disadvantages:
- high cost process
- lower chemical step yields
- low manufacture flexibility

Photolithography
Manufacturing Technique (IV)

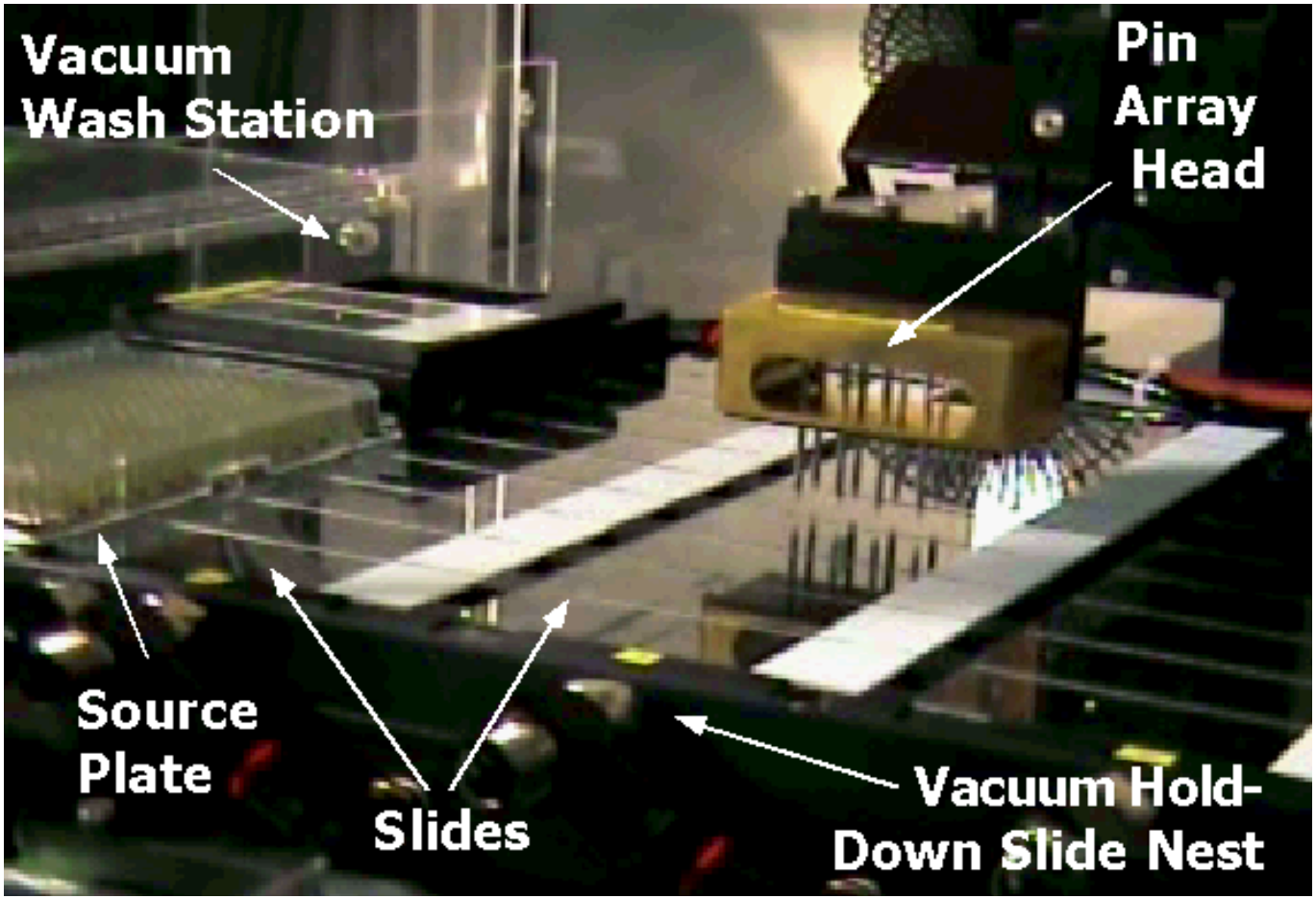


DMD, a solution for flexibility, simplicity and reduced cost



(WWW.TI.COM)

Pin-printing system



Microarray Printing System (I)

Function:

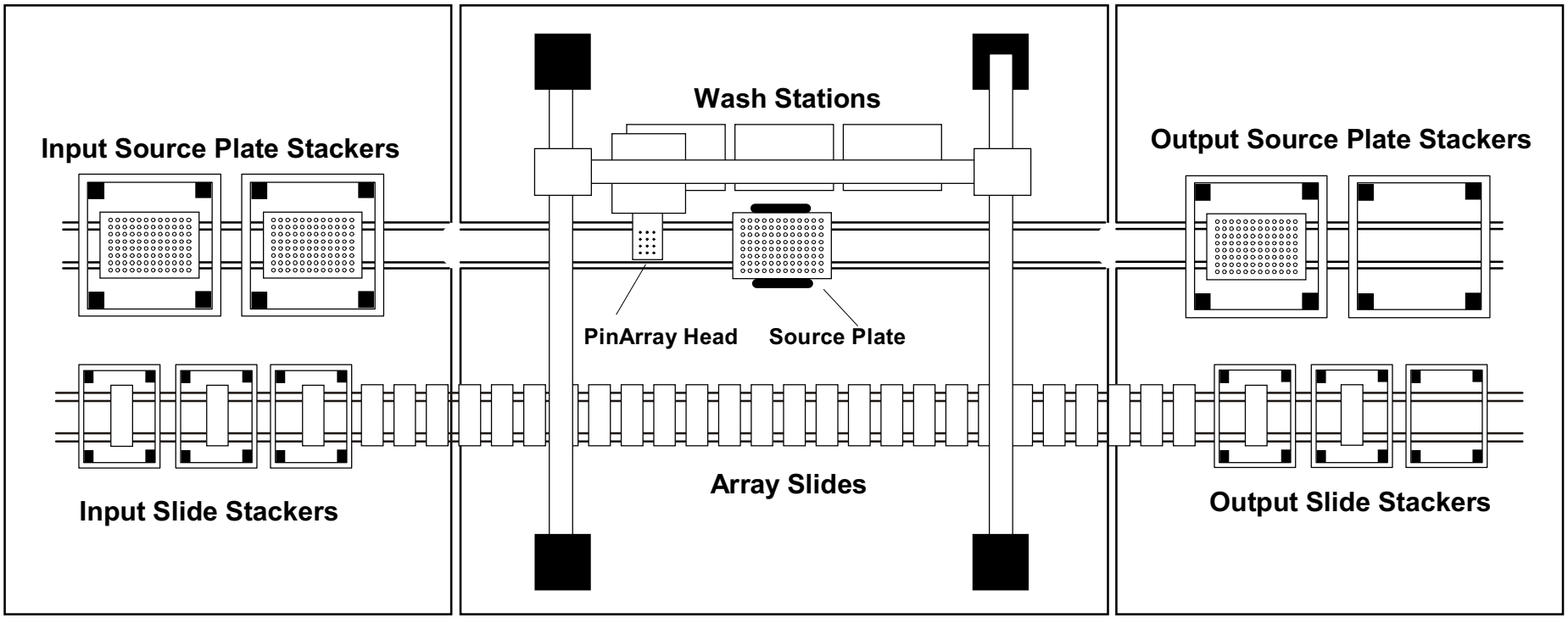
General requirements:

- robust for long-period use
- automation with little/no user intervention
- high precision
- high reproducibility

Microarray Printing System (II)

- ***Printing system includes Hardware & Software***
- ***Hardware:***
  - **print head**
  - **plate and substrate handling**
  - **XYZ-positioning stage**
  - **enviromental control system**
- ***Software:***
  - **instrument control software**
  - **sample tracking software**

Microarray Printing System (III)



Input Stacking Module

Pin Array Transfer Module
(100 source plates, 300 slides)

Output Stacking Module

XYZ-Positioning Stage

Requirements:

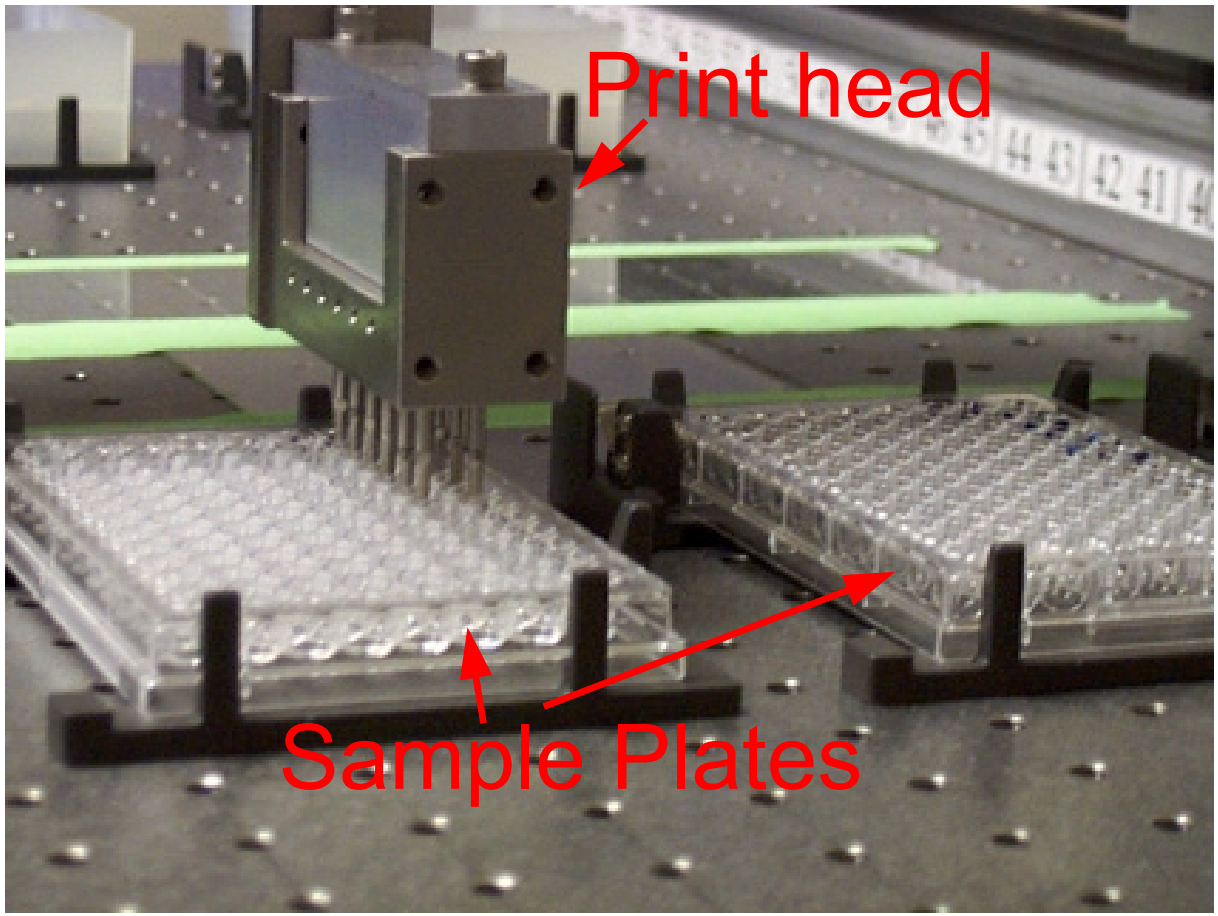
- **Repeatability**
- **Accuracy**
- **Resolution**
- **Traversing velocity**
- **Positioning feedback**

Maximum traversing velocity↗ Resolution ↘

Accuracy ↘; vice versa.

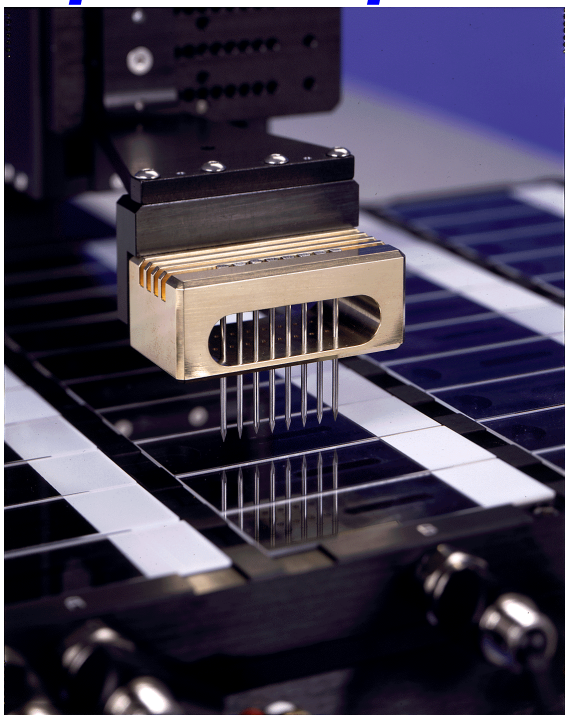
Encoded stepping motor ⇒ Resolution ↗ cost ↗

Loading samples

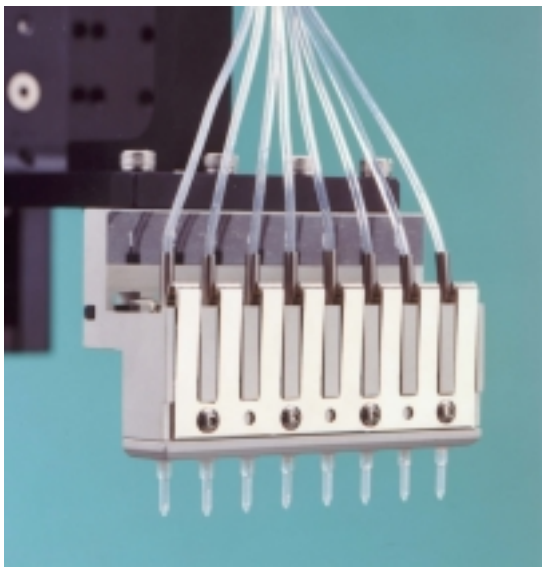


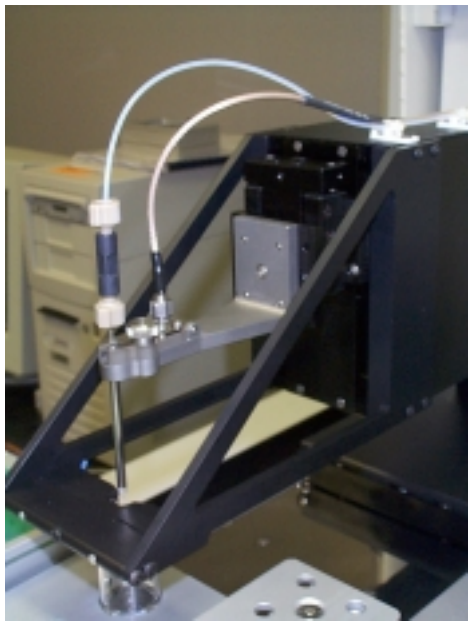
Examples of Printing Head

Up to 64 pins



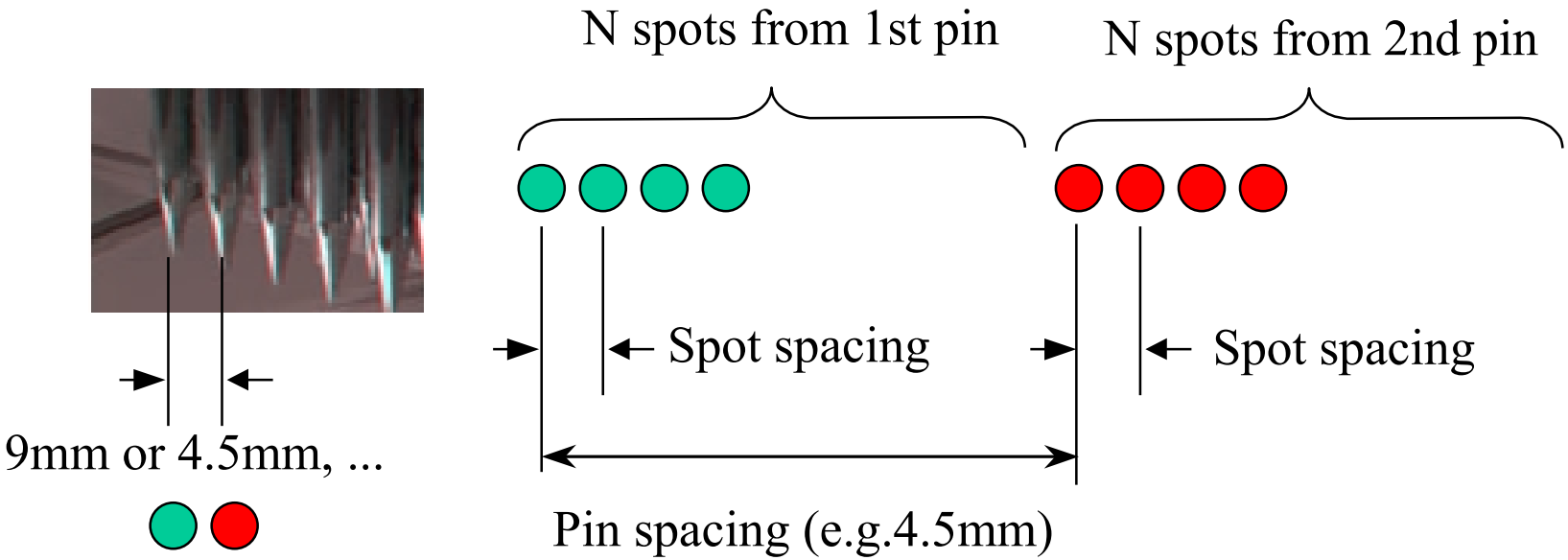
(www.cartesiantech.com)





And more …..

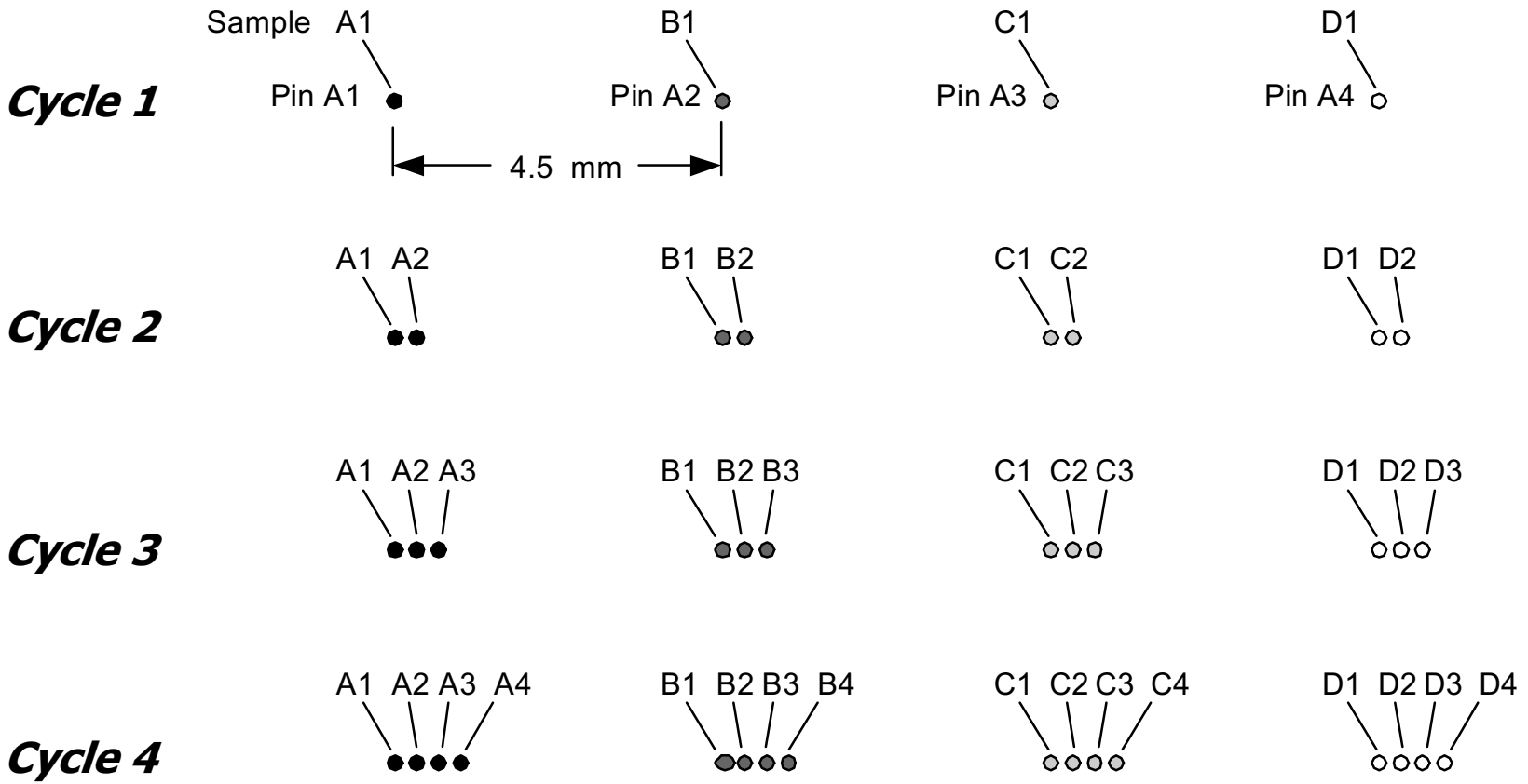
Pin Spacing & Spot spacing



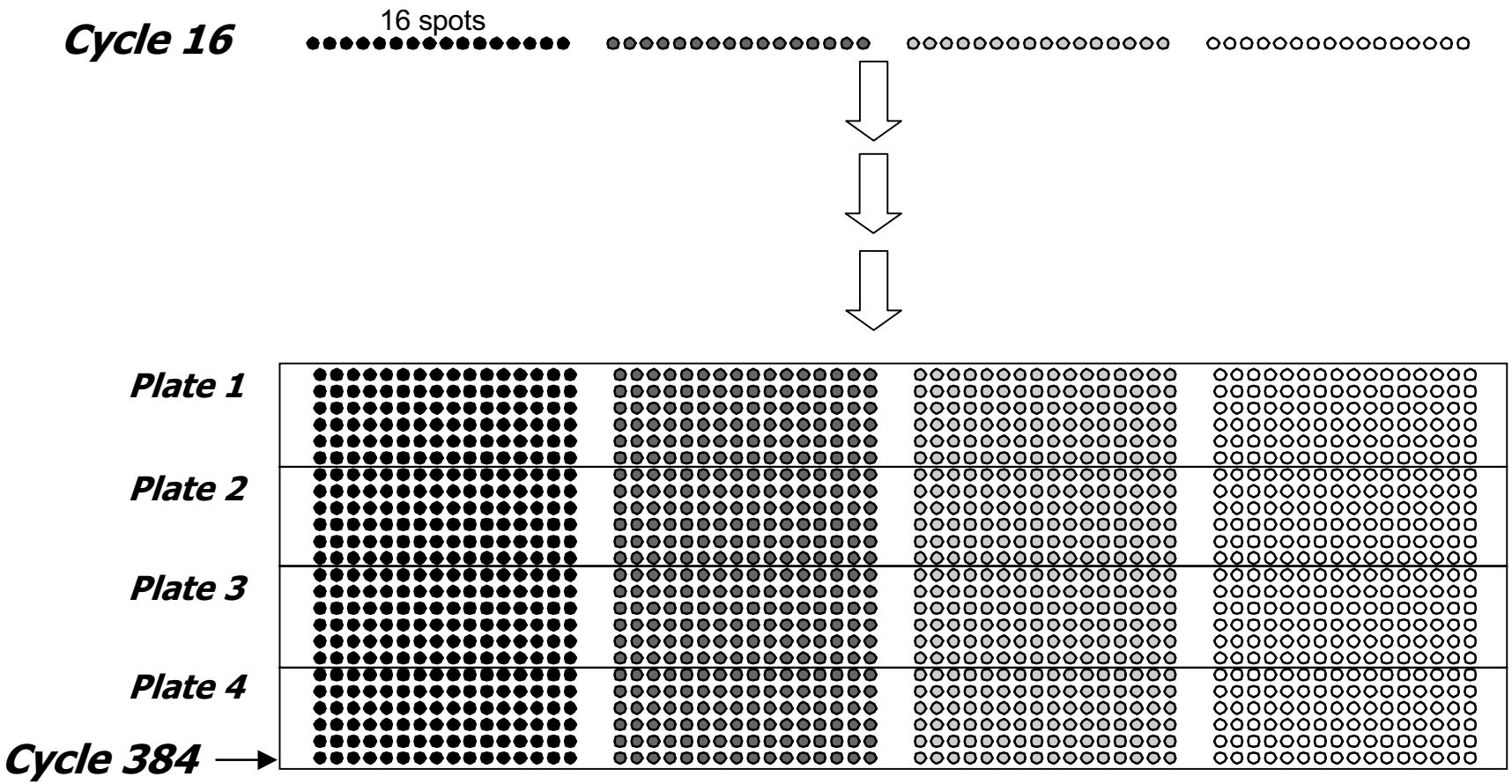
Pin spacing = spot spacing x N

N should match for the use of well plates (96 (8 x 12) or 384 (16 x 24)).

Deposition Process (I)



Deposition Process (II)

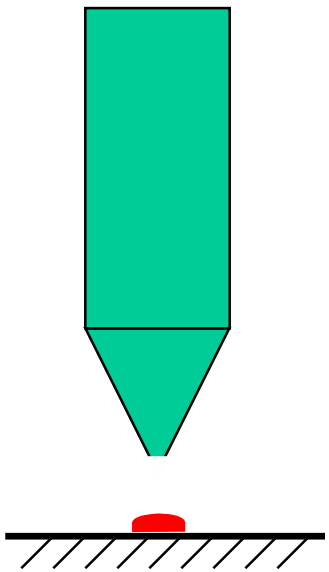


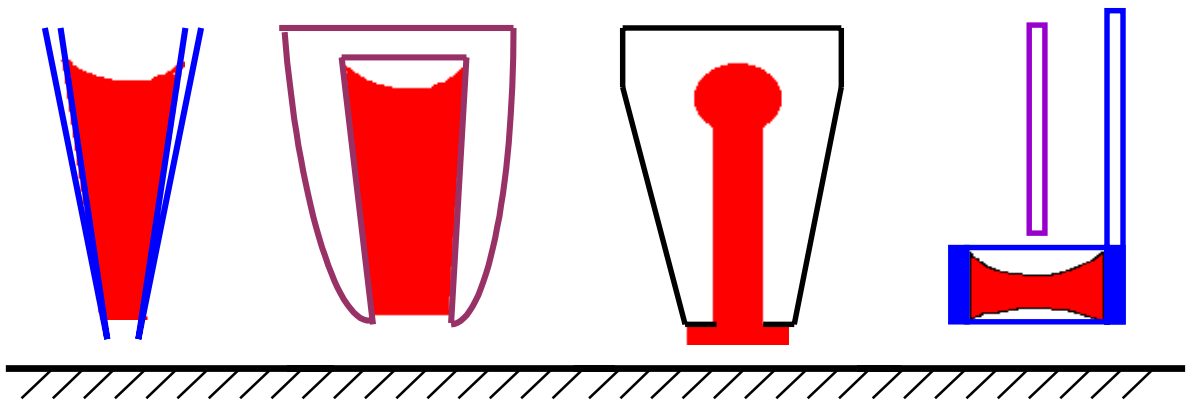
Characteristics of the pin-printing

- Pin-based transfer
- Simple and inexpensive
- Transfer process
  1. Pin dipped into sample (Volume held at tip by surface tension)
  2. Pin touched to surface to leave spot
  3. Pin washed and dried
- Fixed print volume
- Spot volume dependent on several factors
  - Pin surface energy and shape
  - Sample solution properties (viscosity, surface energy)
  - Surface properties (surface energy)

Variations of Pin-design

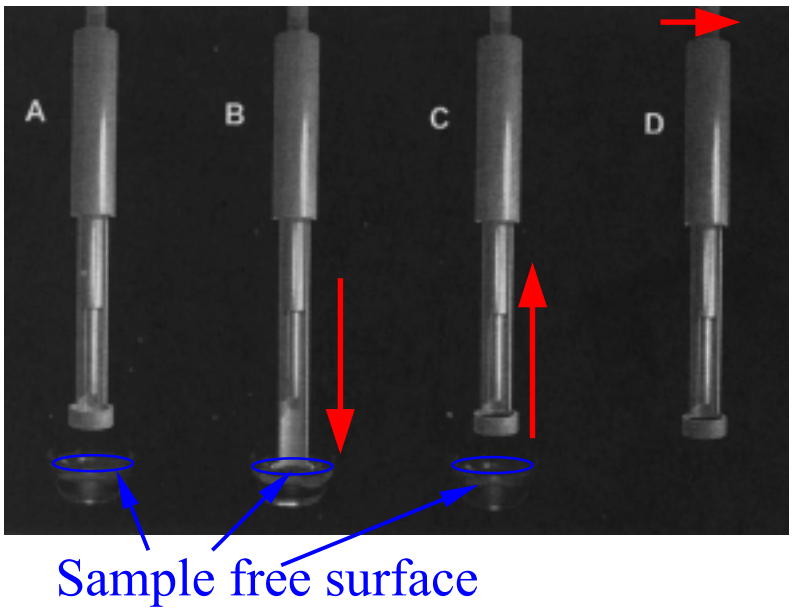
- Solid pin:
  - simple & robust
  - relatively small loading volume
  - printing process rather low
- Hollow pin:
  - hold larger sample volumes for each stroke
  - fragile & difficult to clean
  - susceptible to rust for high salt solution
  - relatively expensive



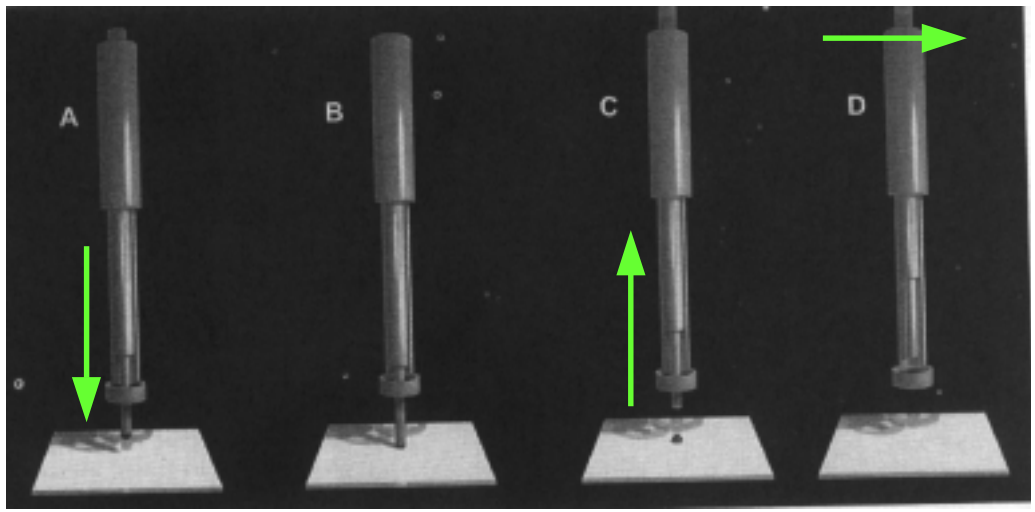


Working Process for Pin-and Ring

(a) Loading sample by Ring

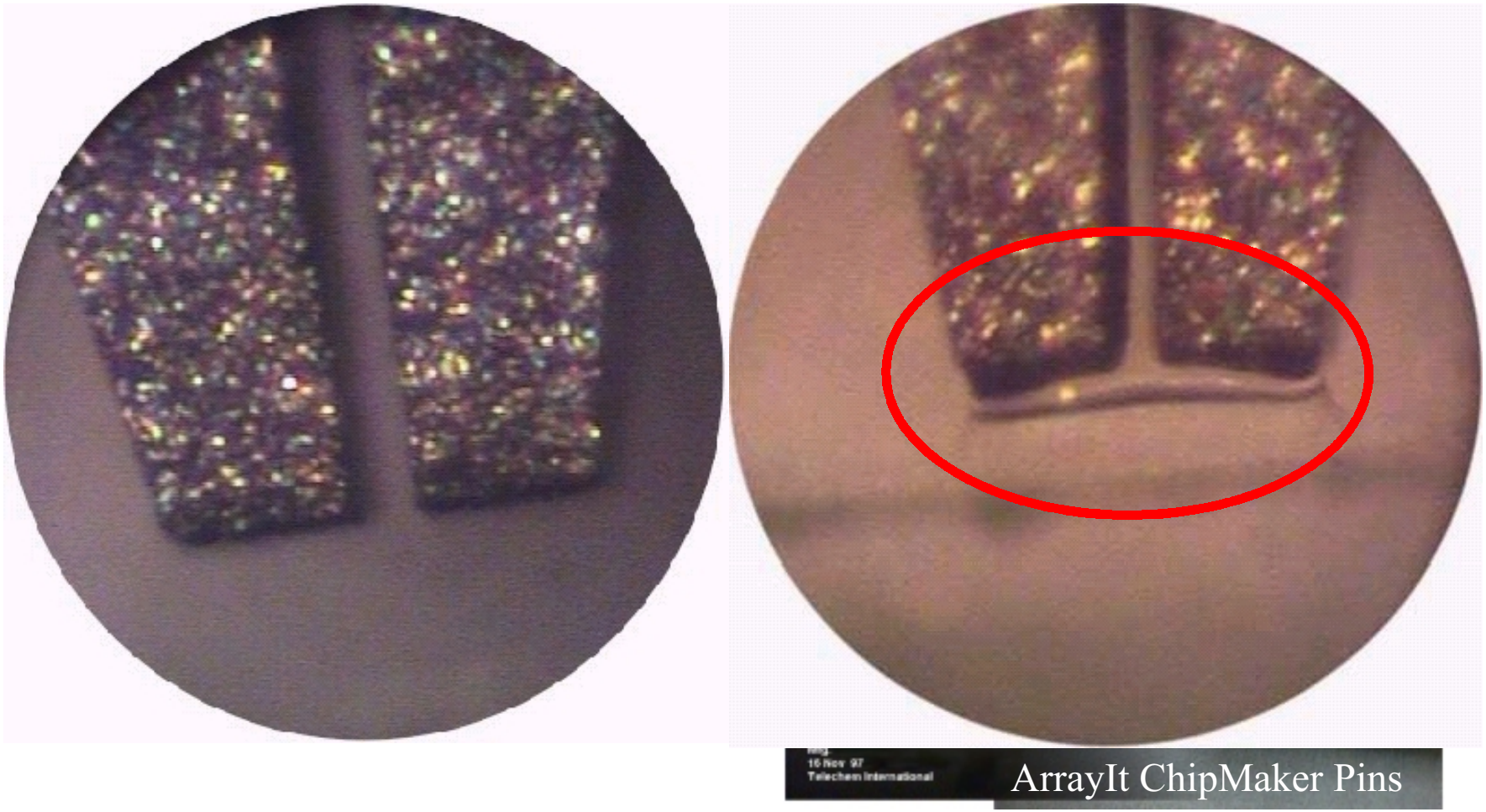


(b) Printing sample by Pin



(from Schena)

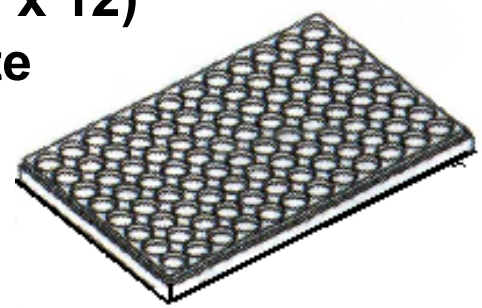
Example of Hollow Pins



Print head

- **Requirement: pin should be held, but can move freely up and down (by spring/gravitation) during contact with to the glass surface.**
- **The spacing of pins or nozzles is restricted by the geometry of source microplates.**
  96-, 384- and 1536-well microplate have center-to-center spacing 9, 4.5 and 2.25mm

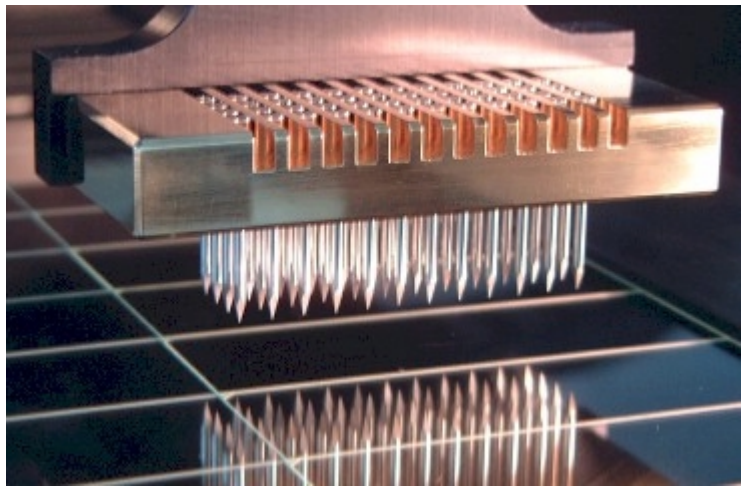
96-well (8 x 12) microplate



384-well (16 x 24) microplate

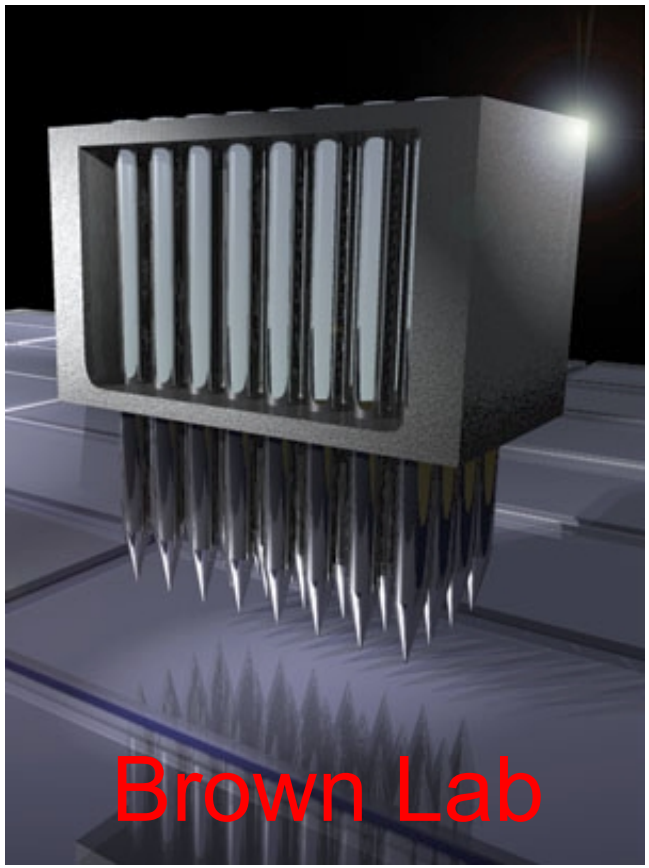
Examples of Print head Matrix

Up to 64 pins



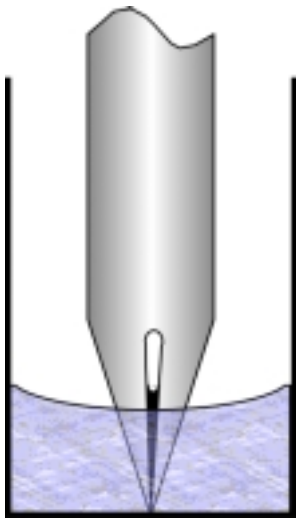
TeleChem ArrayIt™

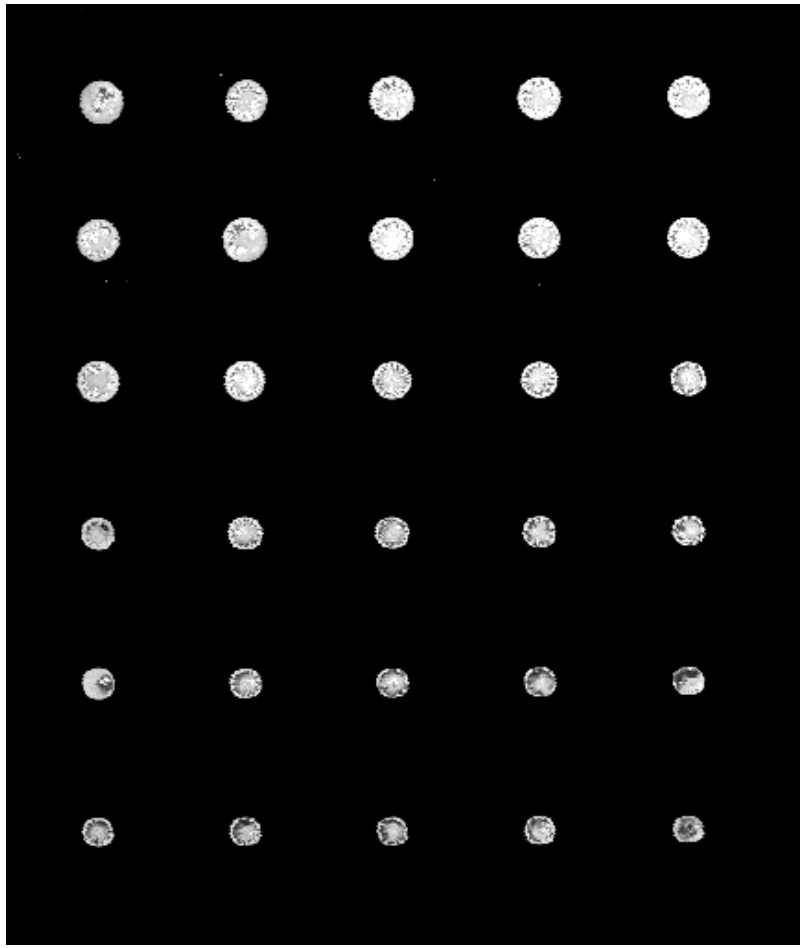
Stealth Printhead (SPH48)



Loading sample & Pre-printing

- Pin should not dip too deep into sample
- Pin can touch bottom of well
- 10 to 15 uL in 384-well plate

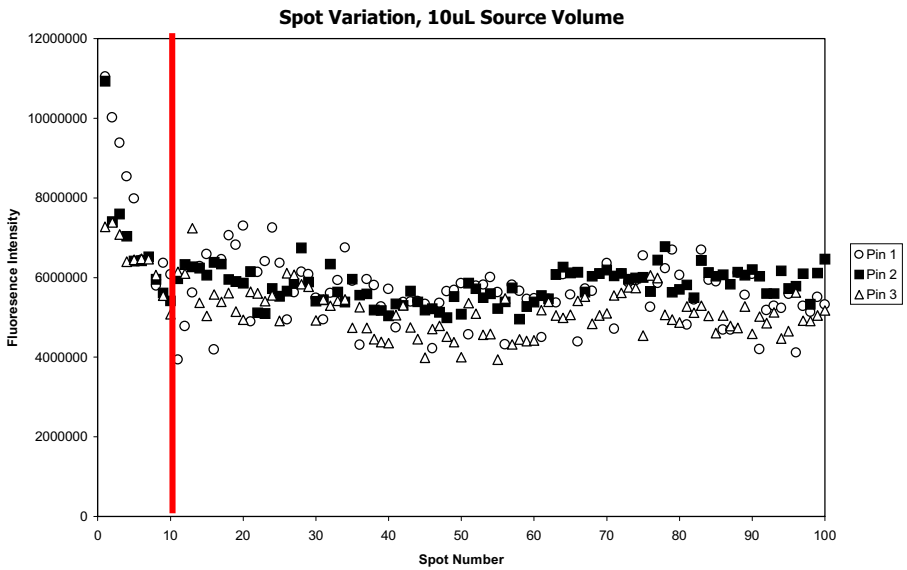


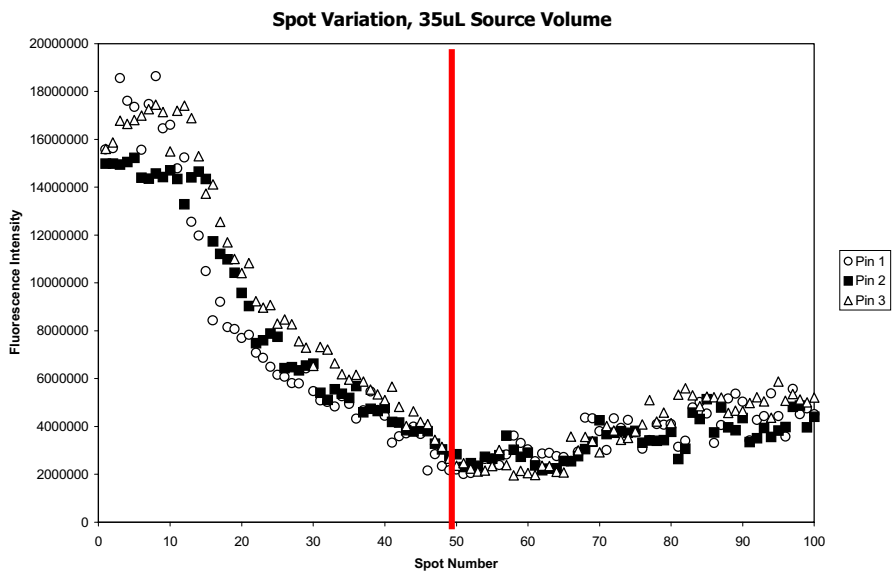


First 30 spots from one pin (Cy3-labeled oligo)

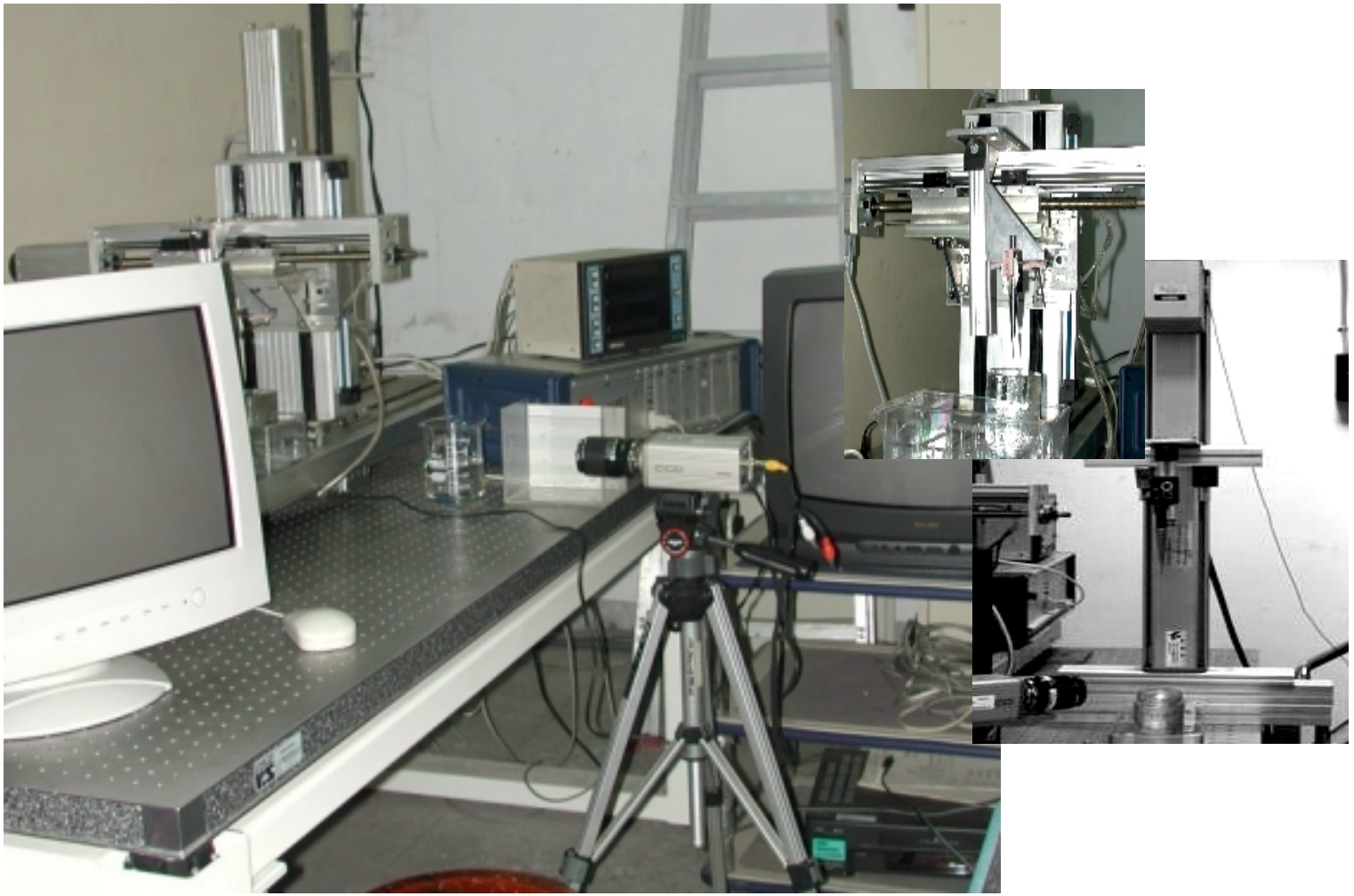
Preprinting of Sample Loading

- The preprinting is necessary to drain excess sample solution from the pin
- Empirical best results from 1mm-layer (4-6μl) on microplate

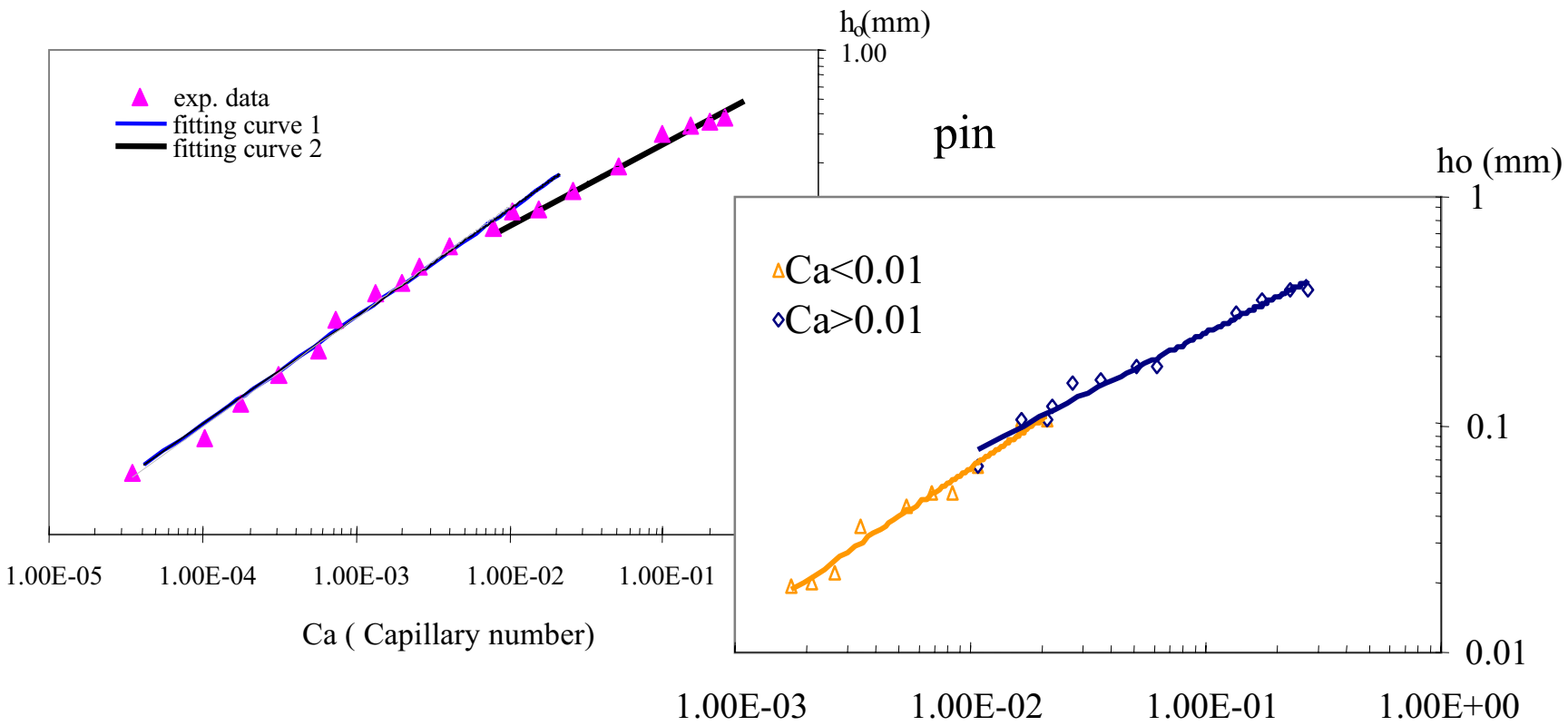




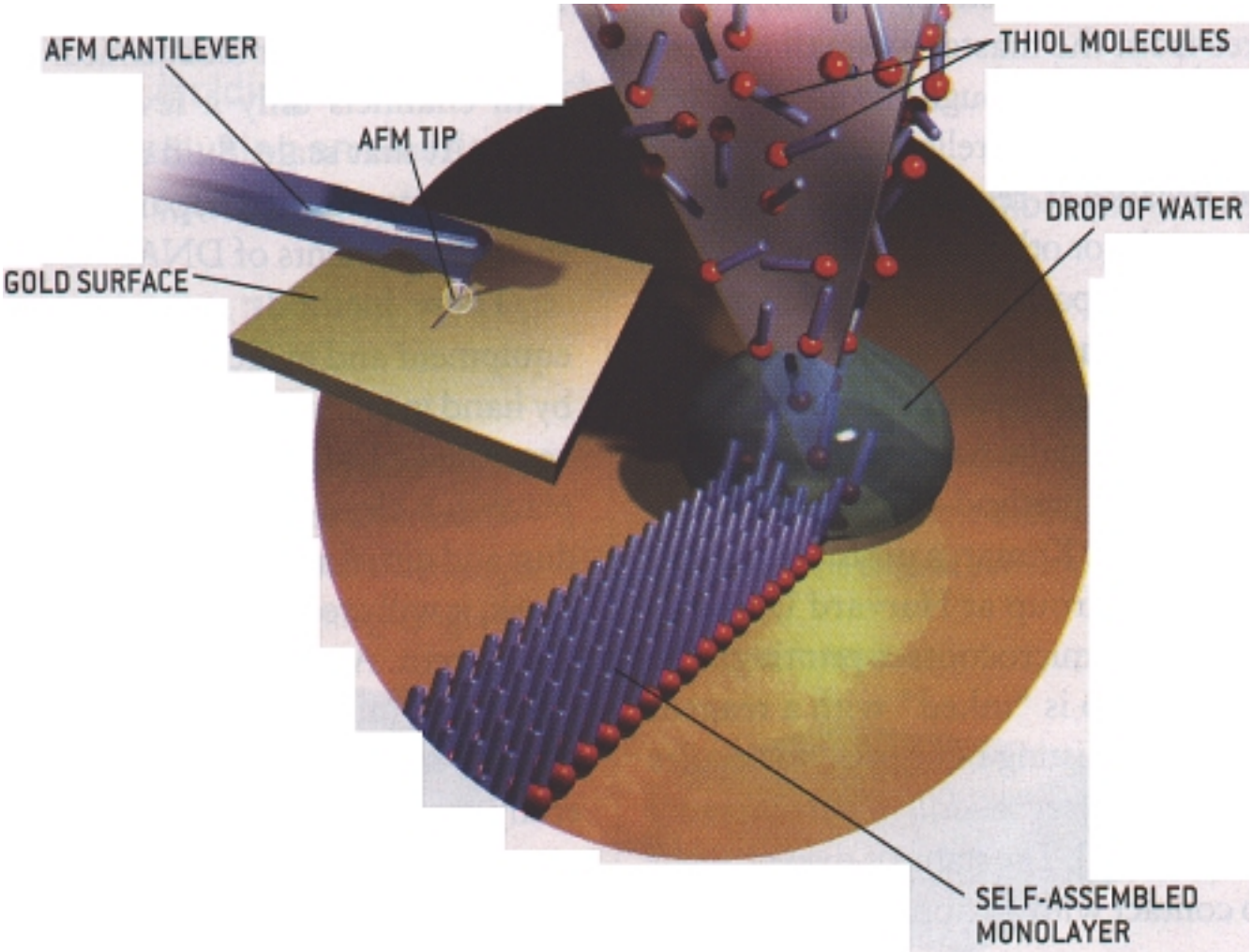
Microarrayer test rig of the NTU-BIOMEMS



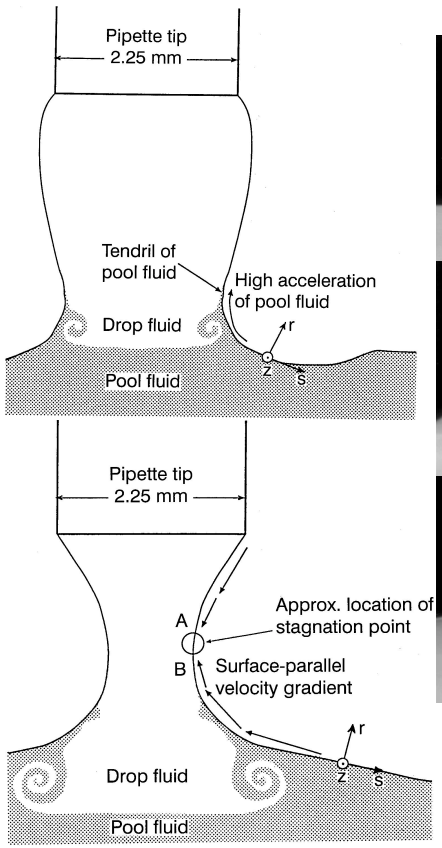
Sampling test for the Plate and Pin

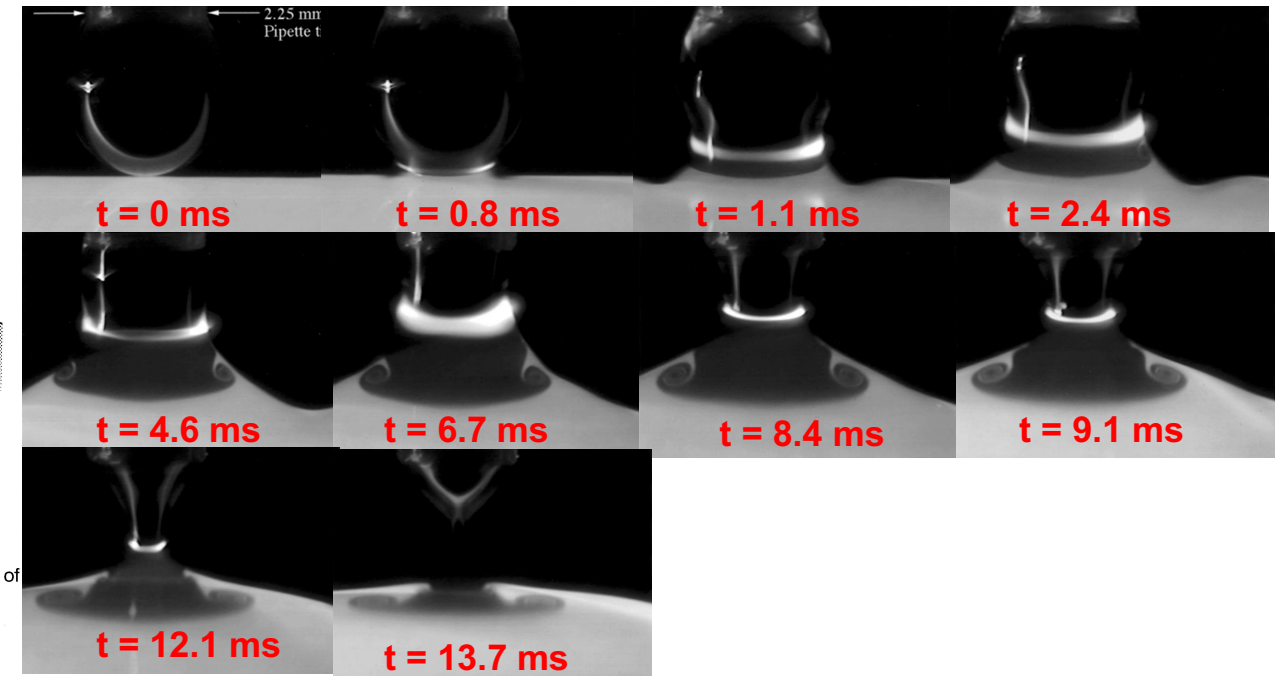


Advanced sample-loading technique



Pin Washing and Sample Carryover (I)





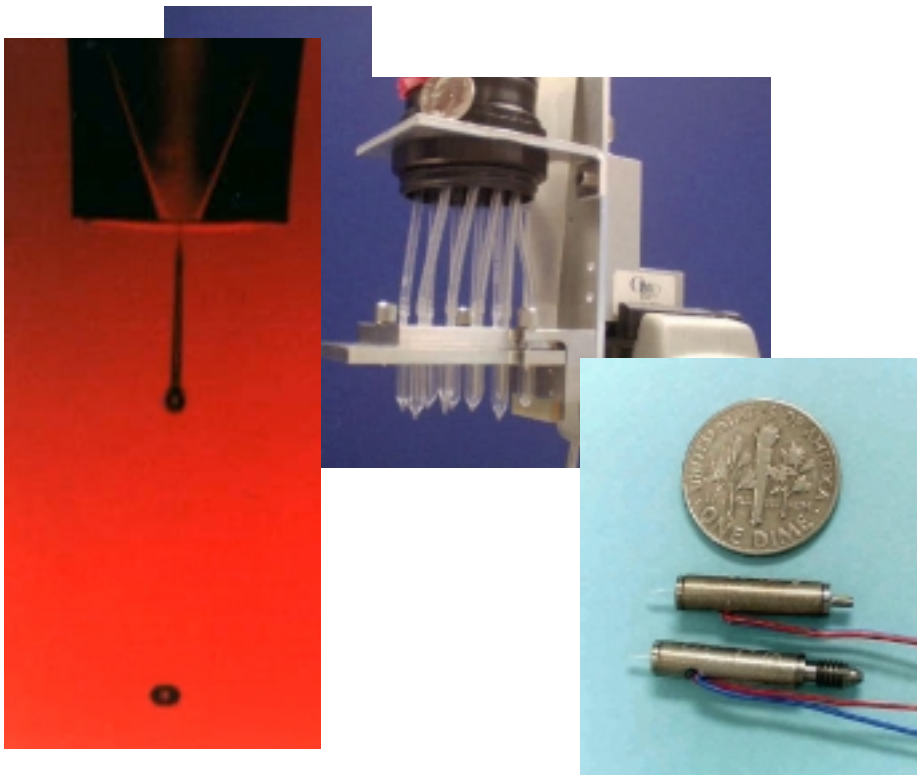
Dooley et al. (1997)

Pin Washing and Sample Carryover (II)

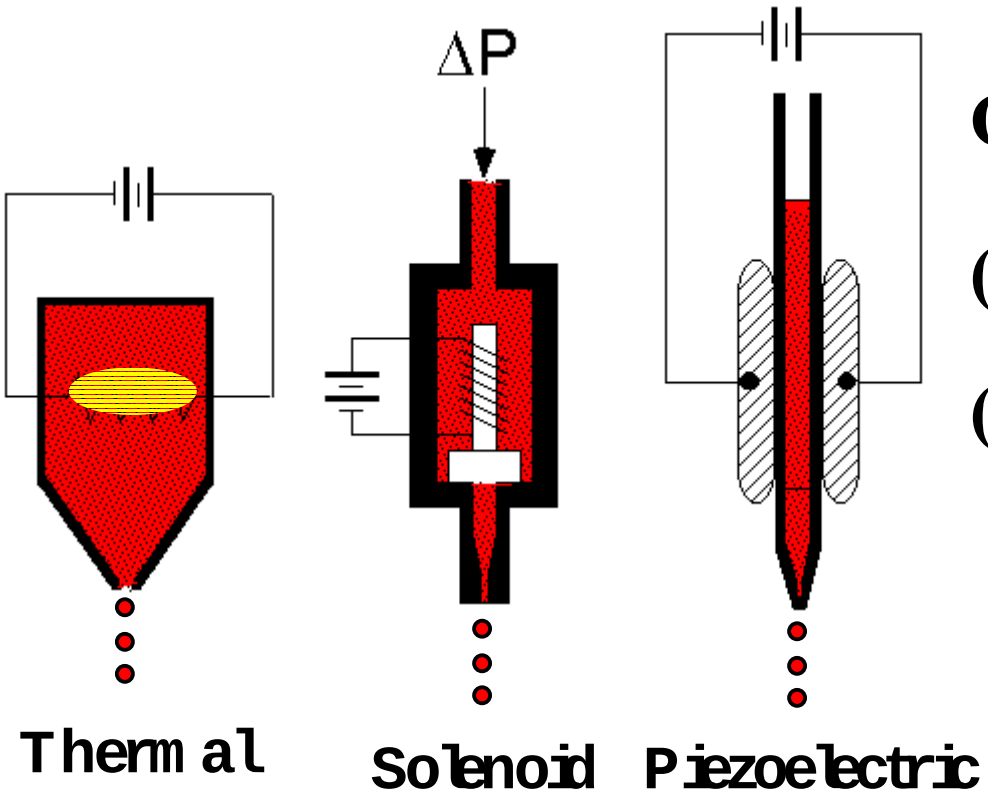
- **Purpose of pin washing:**
  prevent sample carryover that complicate the hybridization results
- **Procedure of pin washing:**
  dip the pins into distilled clean water, remove the wash water with a vacuum, repeat the two process some times till the sample carryover < 1 part per 10000, drying

Droplet-Injection Technique

Advantages: No carrayover



Types of injection Technology



Operation modes:

(a) Continuous Mode

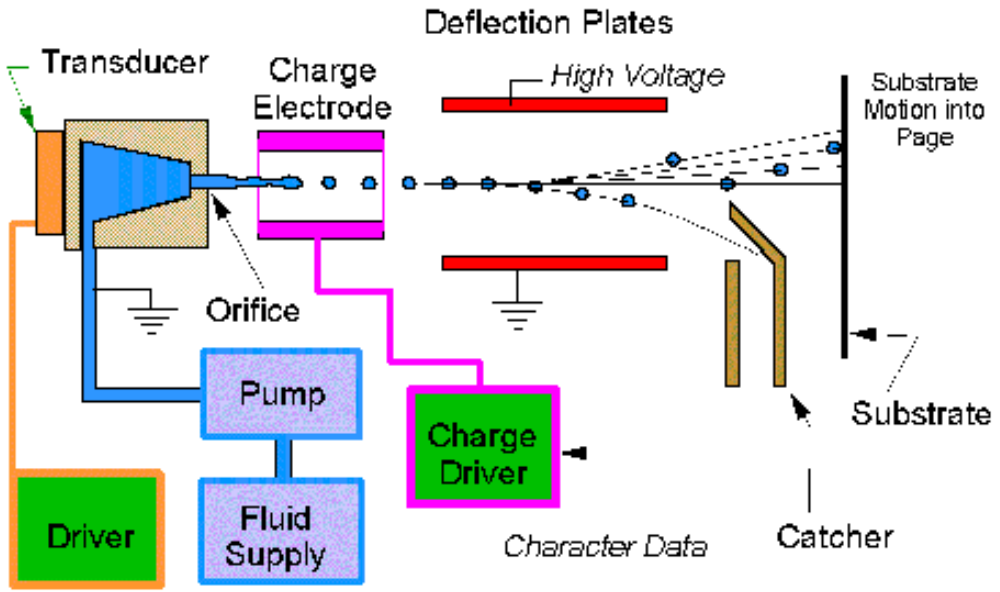
(b) Drop-on-demand Mode

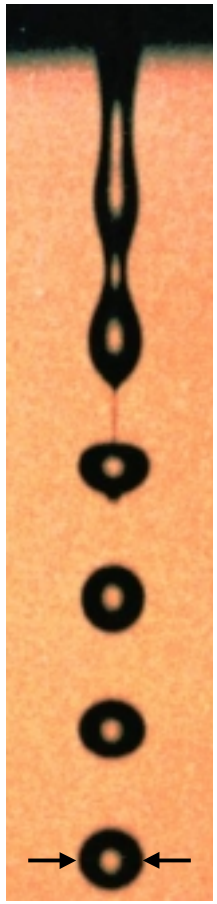
Inkjet Technology (I)

Continuous Mode:

Drop generation rate: 80k ~ 1MHz

Drop size: 20 μm ~ 1mm (typ. 150 μm)



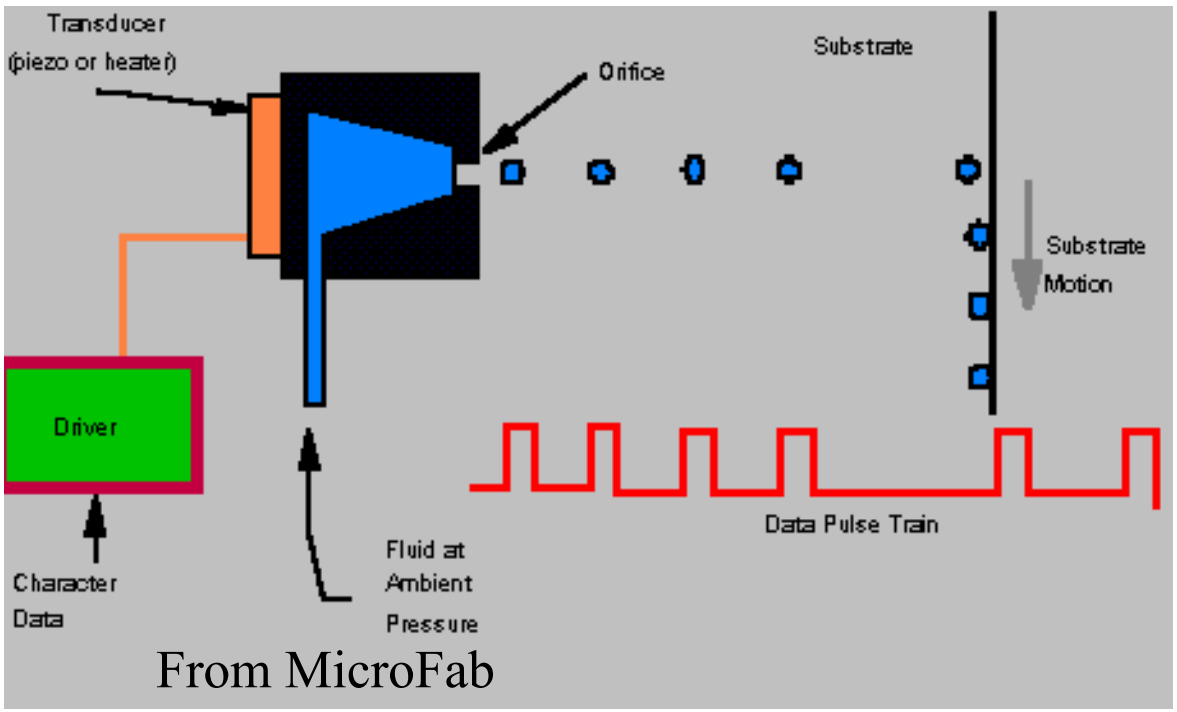


Inkjet Technology (II)

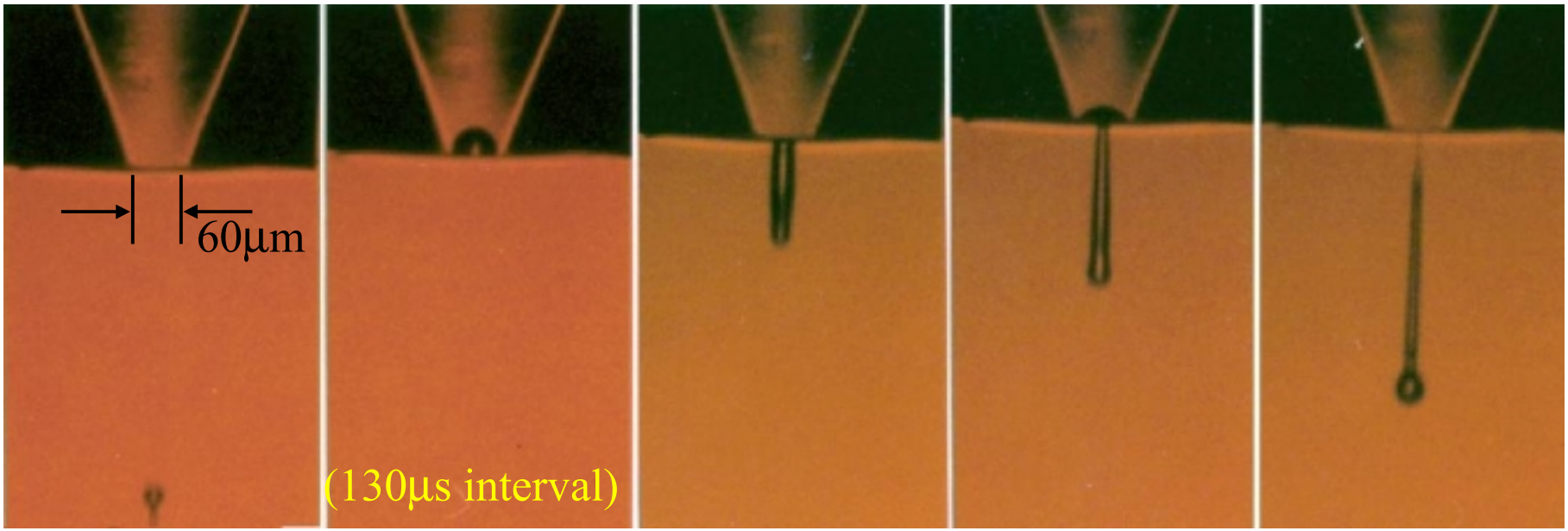
Drop-on-demand Mode:

ΔVol.⇨Δp⇨fluid velocity ⇨ drop generation

simple, greater energy needed, smaller drop



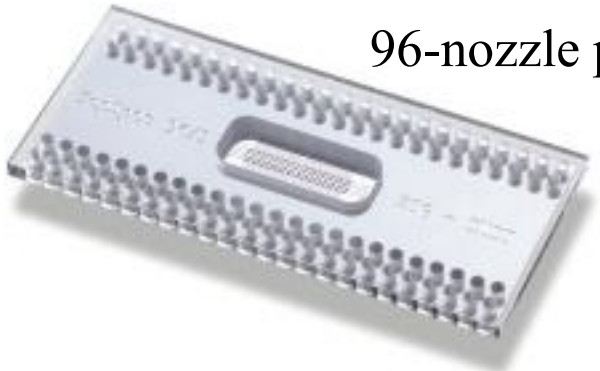
Inkjet Technology (IIa)

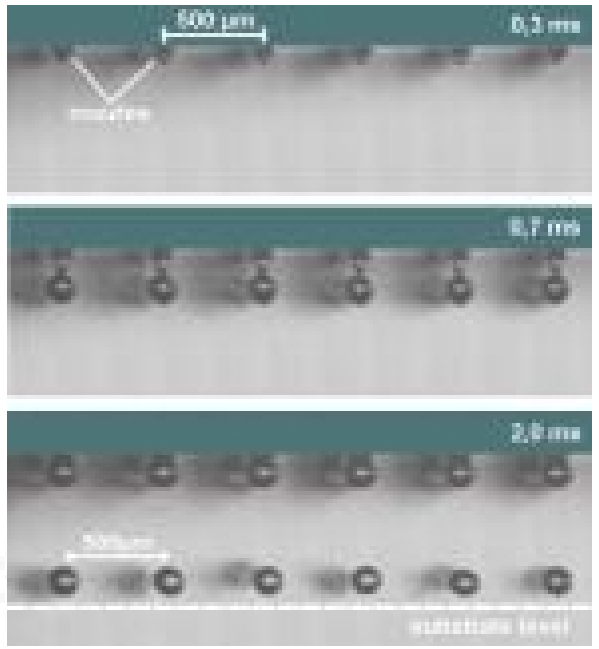


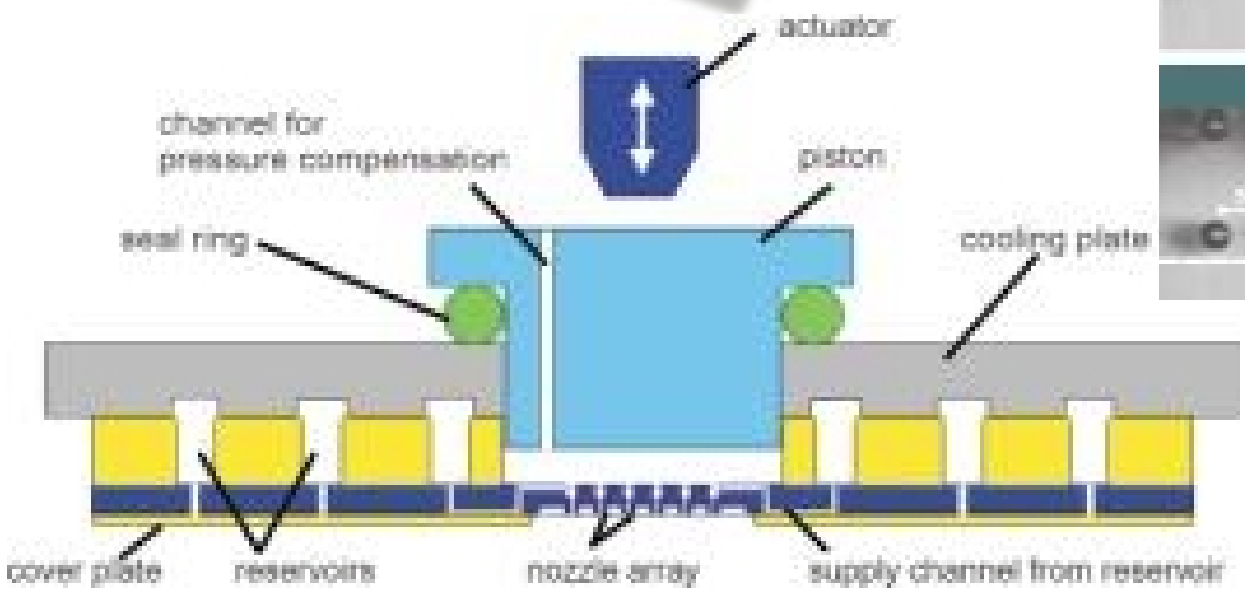
From MicroFab

Inkjet Technology (III)

96-nozzle print head







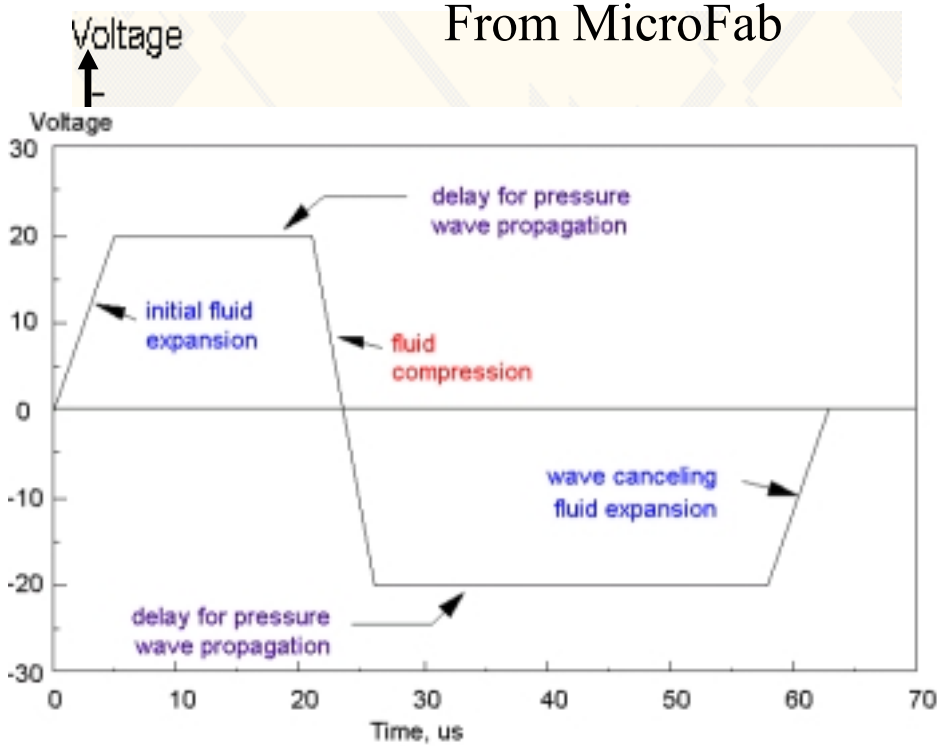
(from MST news)

Inkjet Technology (IV)

Influence Parameters of injection:

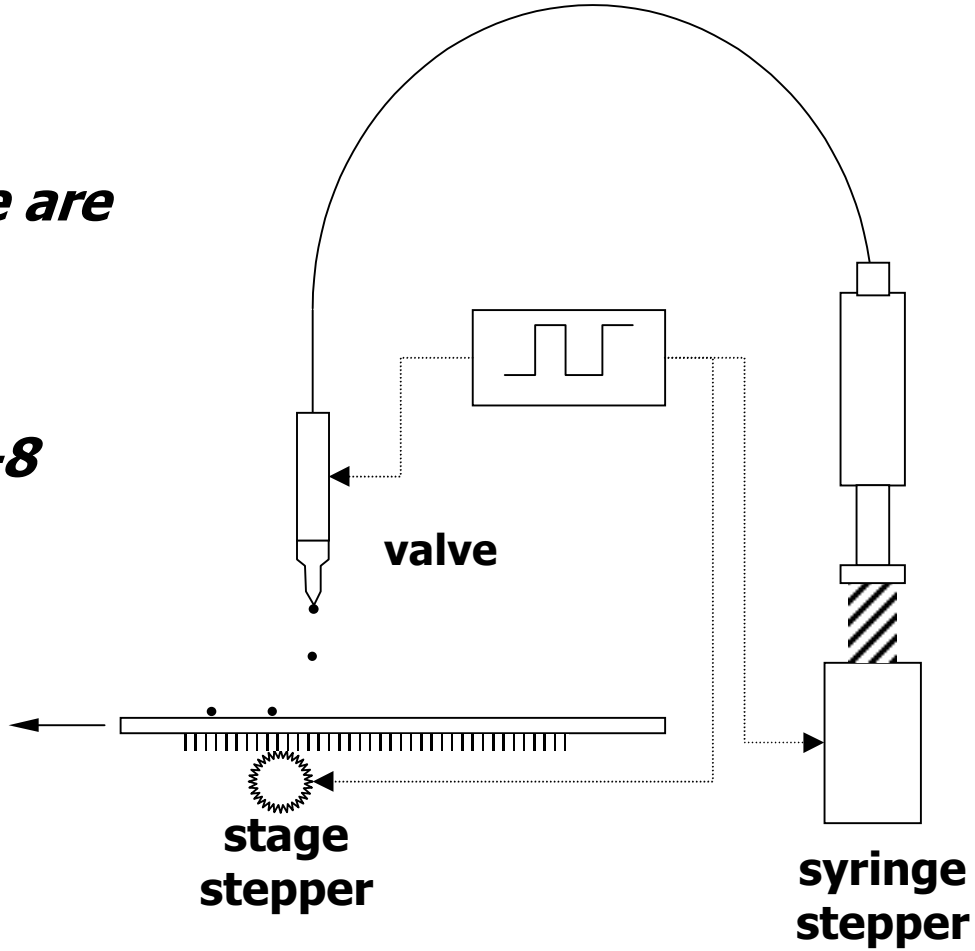
- Orifice diameter(Orifice diameter ↗ ⇨ Drop ↗)
- Pulse width effect
- Fluid properties
  (viscosity,
  density,
  surface tension.

From MicroFab



High-Speed Printing

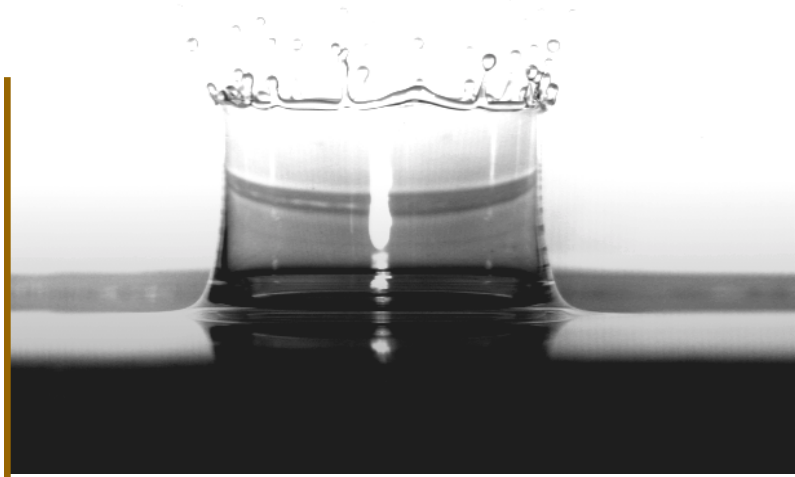
- ***Valve, syringe and stage are synchronized***
- ***"On the fly" printing***
- ***Very high print rates (4-8 slides/s)***



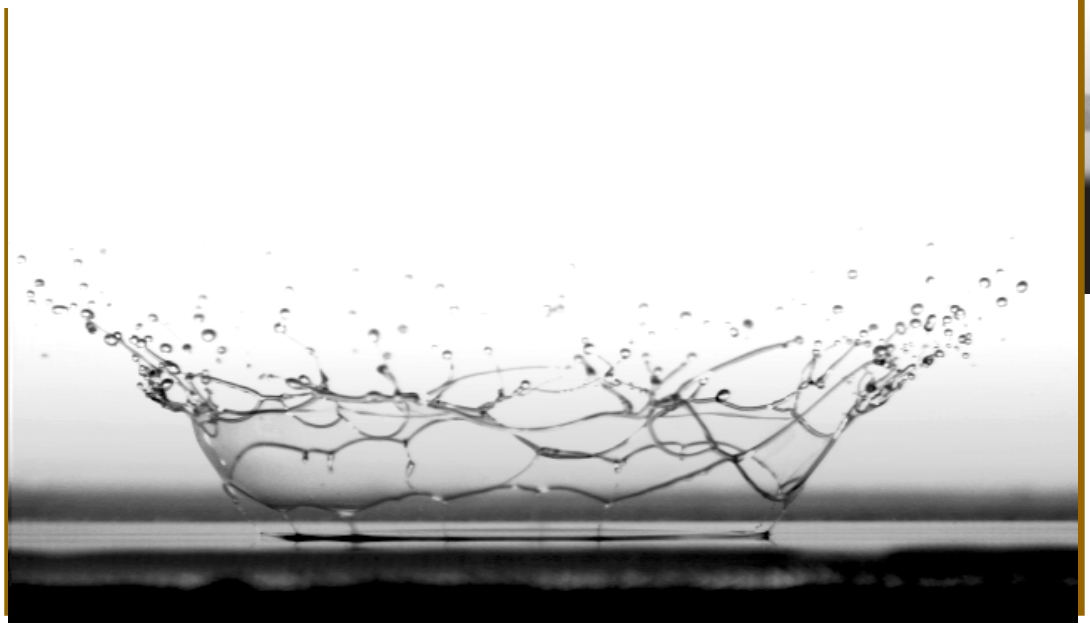
Novel Test-Rig for Drop-film Interactions

世上第一部精密液膜撞擊實驗平臺
首度發現撞擊極薄液膜特殊之特性
Physics of Fluids(2000/09)

Impact onto thick films



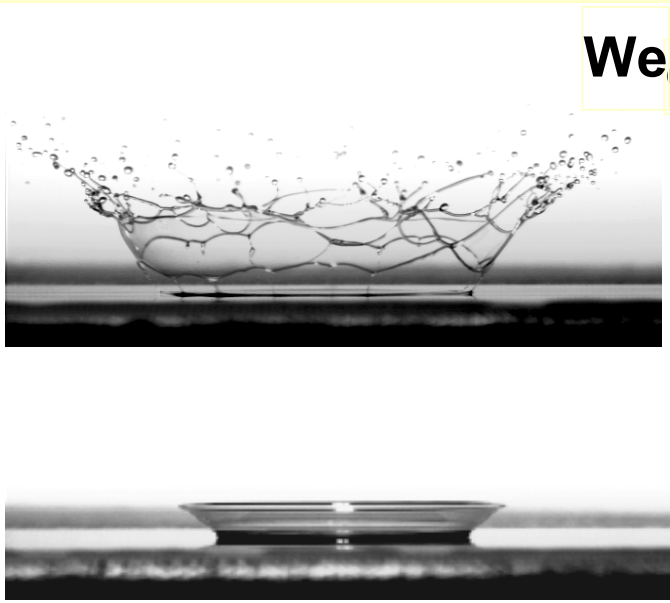
Impact onto thin films

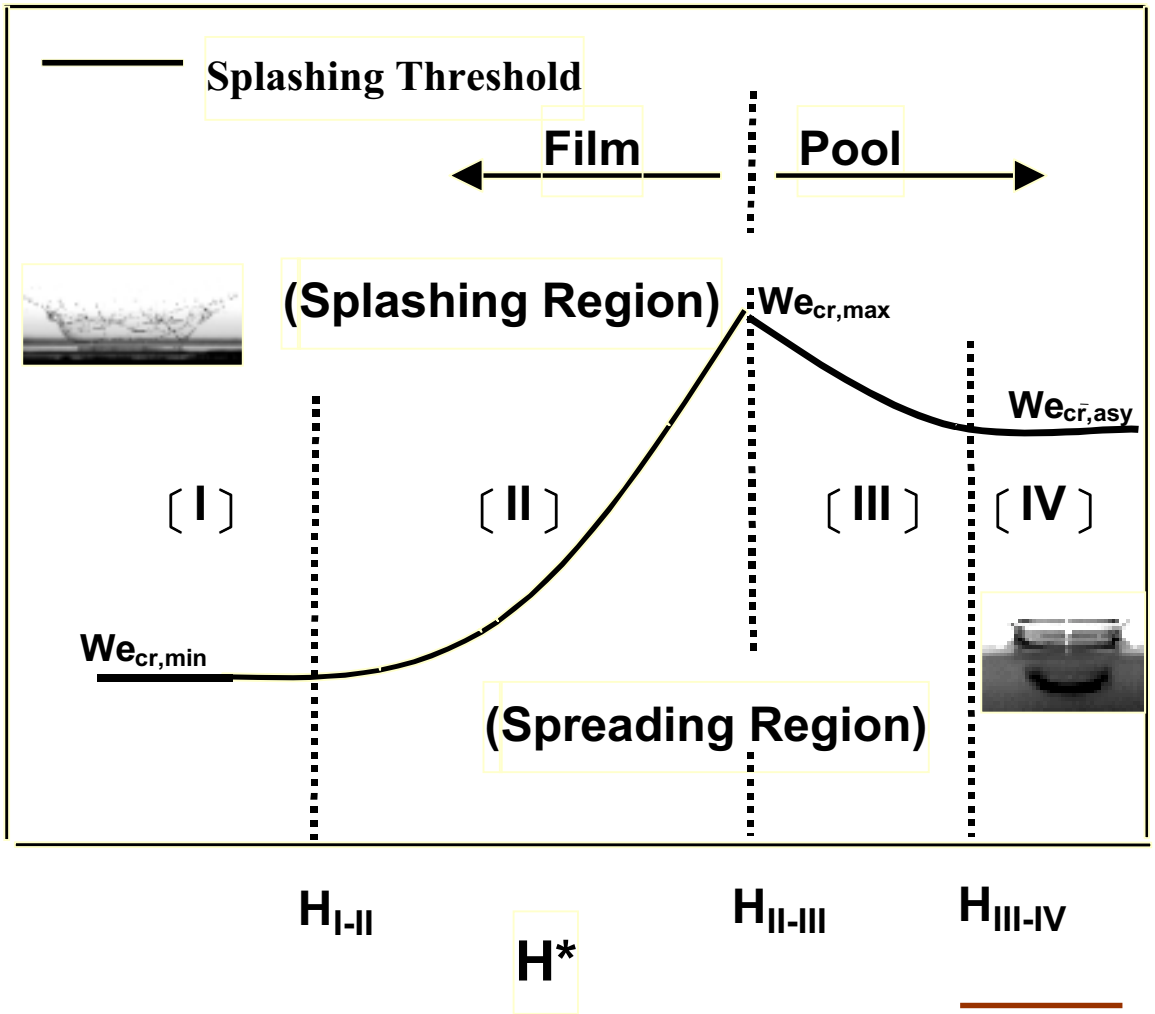


Splashing Threshold of Drop Impact

應用例: 生醫晶片製程,
噴霧設計,...

世上首次發現這些特性

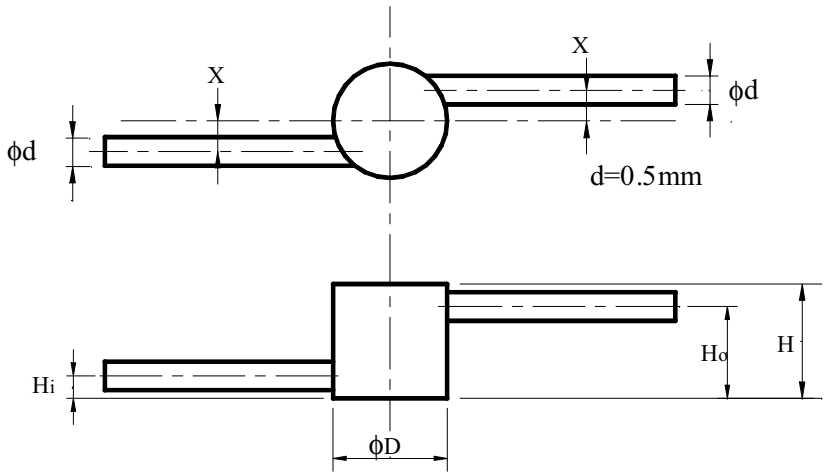




Design of Bio-fluid system

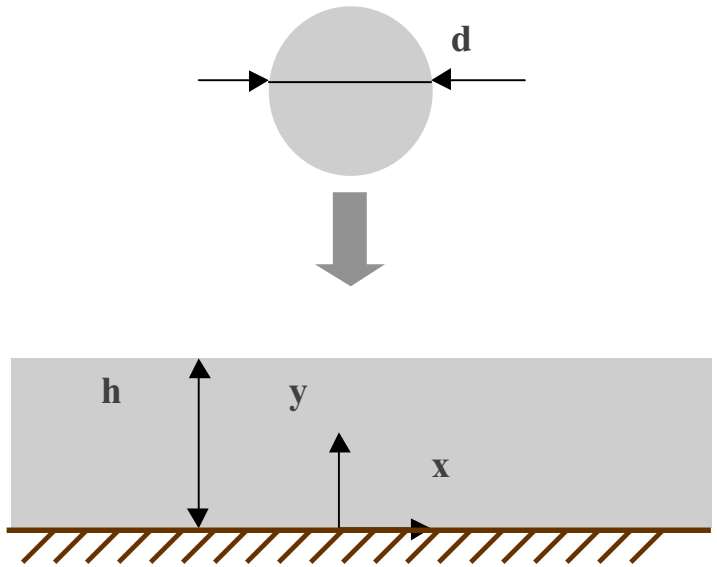
(A) 流道式沖洗法

$H_i/H=H_o/H=0.175$， e=0



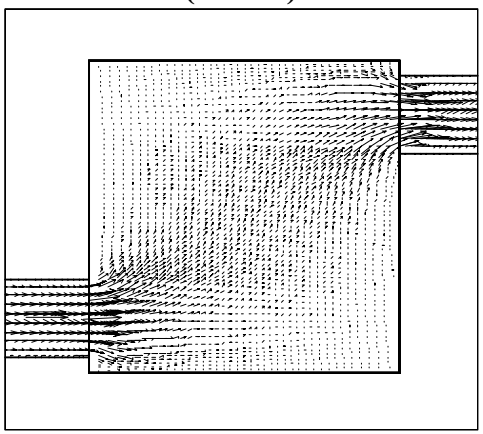
(B) 液滴式沖洗法

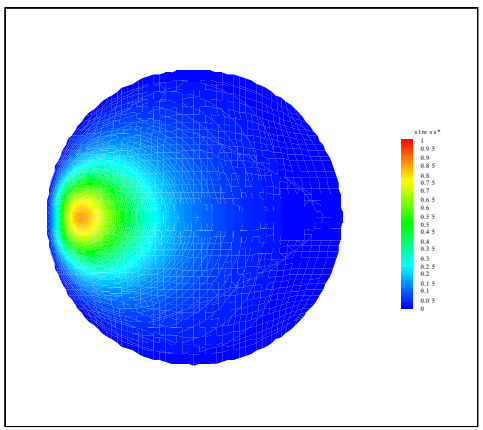
H*=h/d, r=x/d



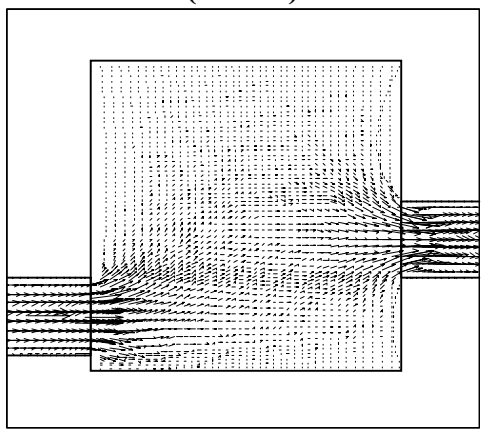
Design of Bio-fluid system

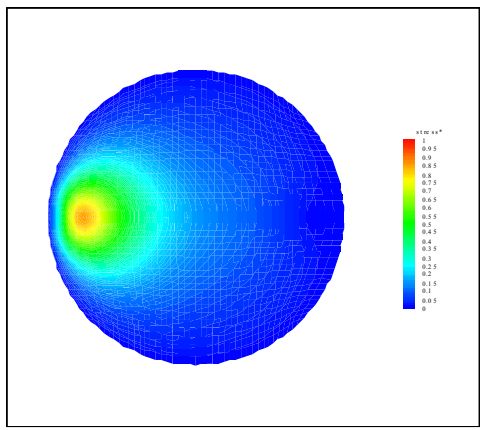
1. $H_0/H=0.825$ (case 6)



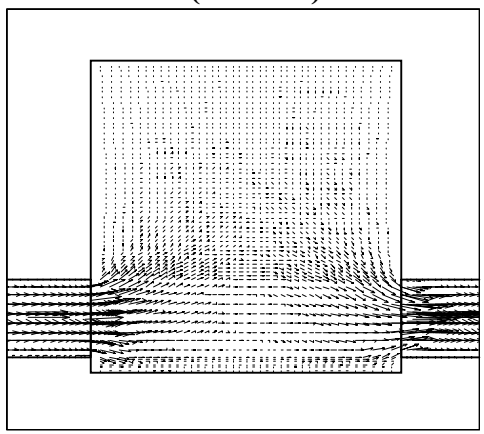


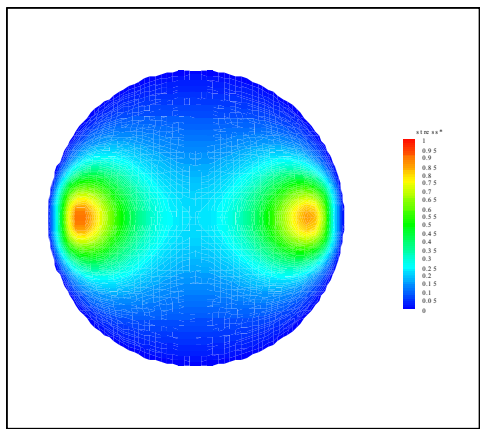
2. $H_0/H=0.425$ (case 2)



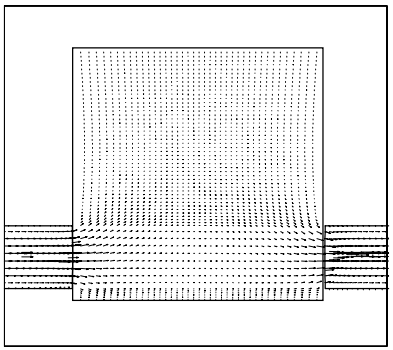
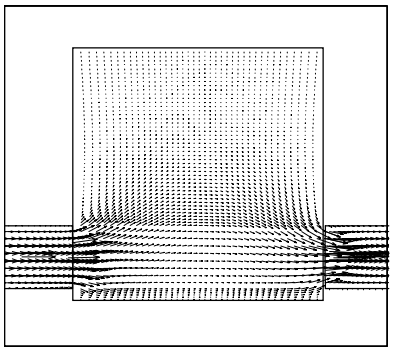
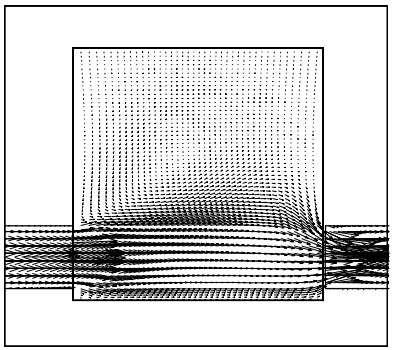
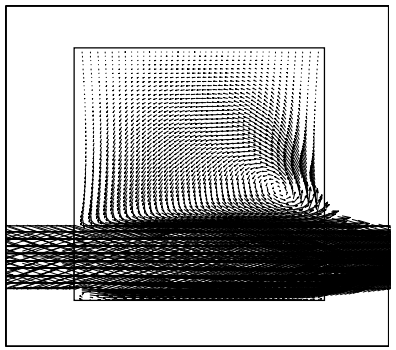
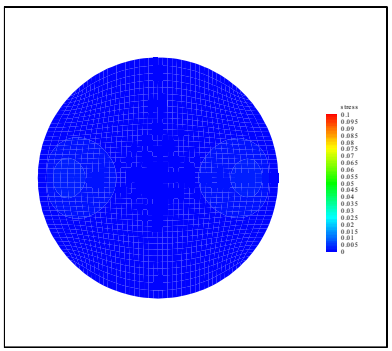
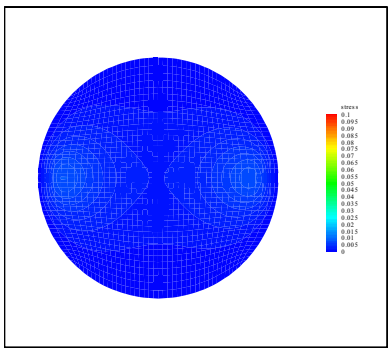
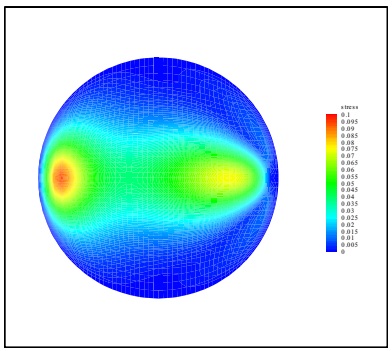
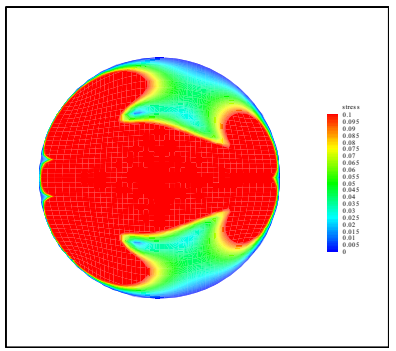


3. $H_0/H=0.175$ (case 3b)





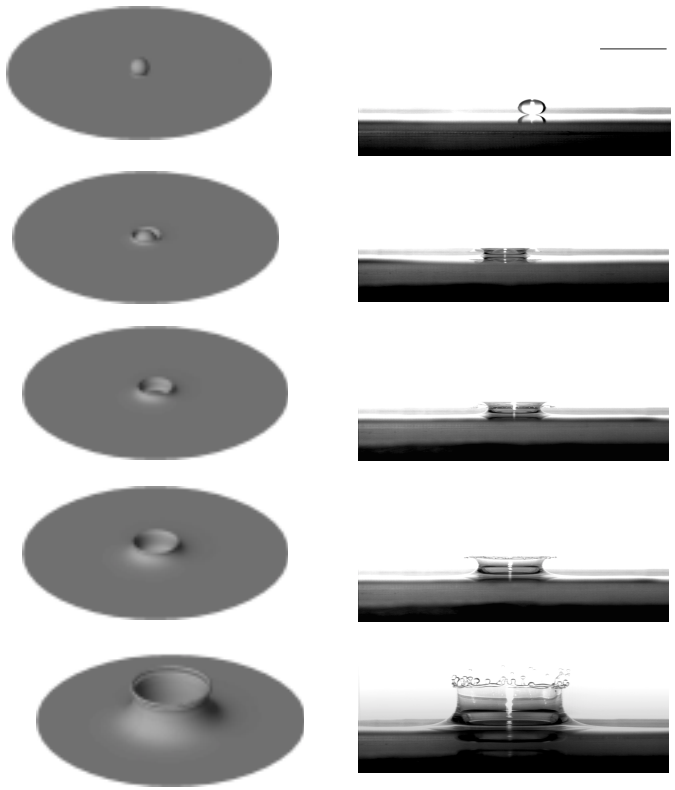
Design of Bio-fluid system

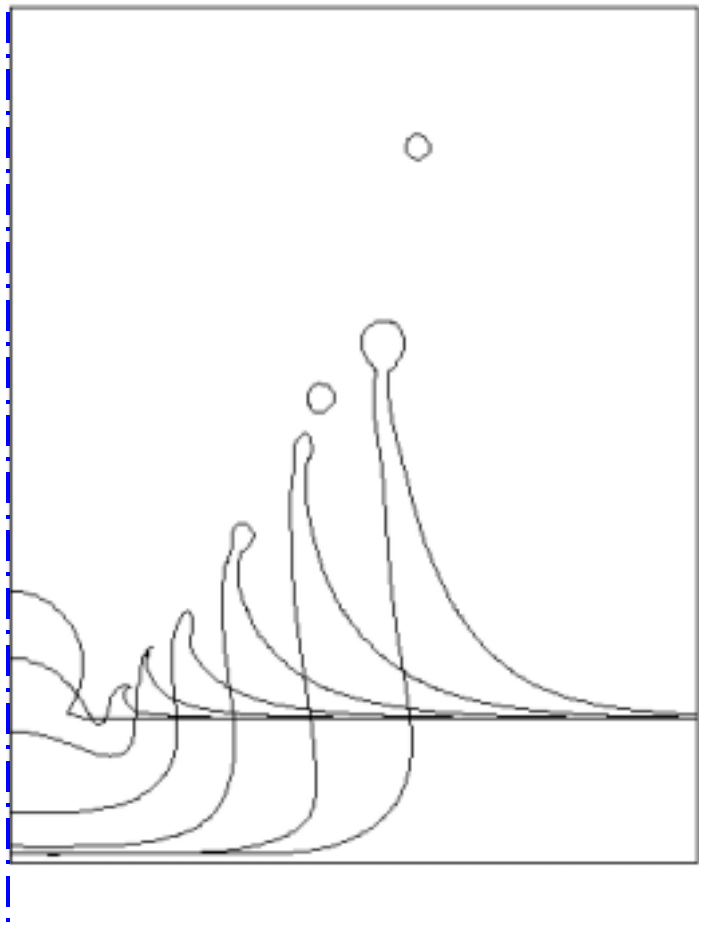
1. Re=0.622 (case3a)	2. Re=1.870 (case3b)	3. Re=18.703 (case3c)	4. Re=187.032 (case3d)
			
			

*上圖爲 x=0 截面流速分佈圖，下圖爲槽底剪應力分佈圖。

Injection Washing Technique

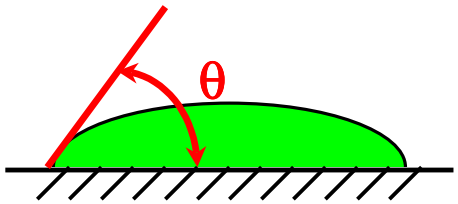
Simulation & Experiment



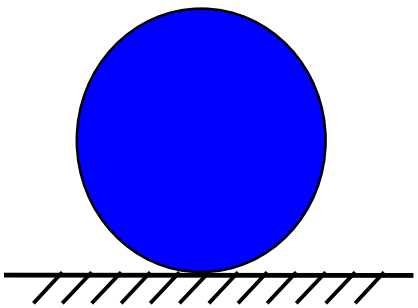


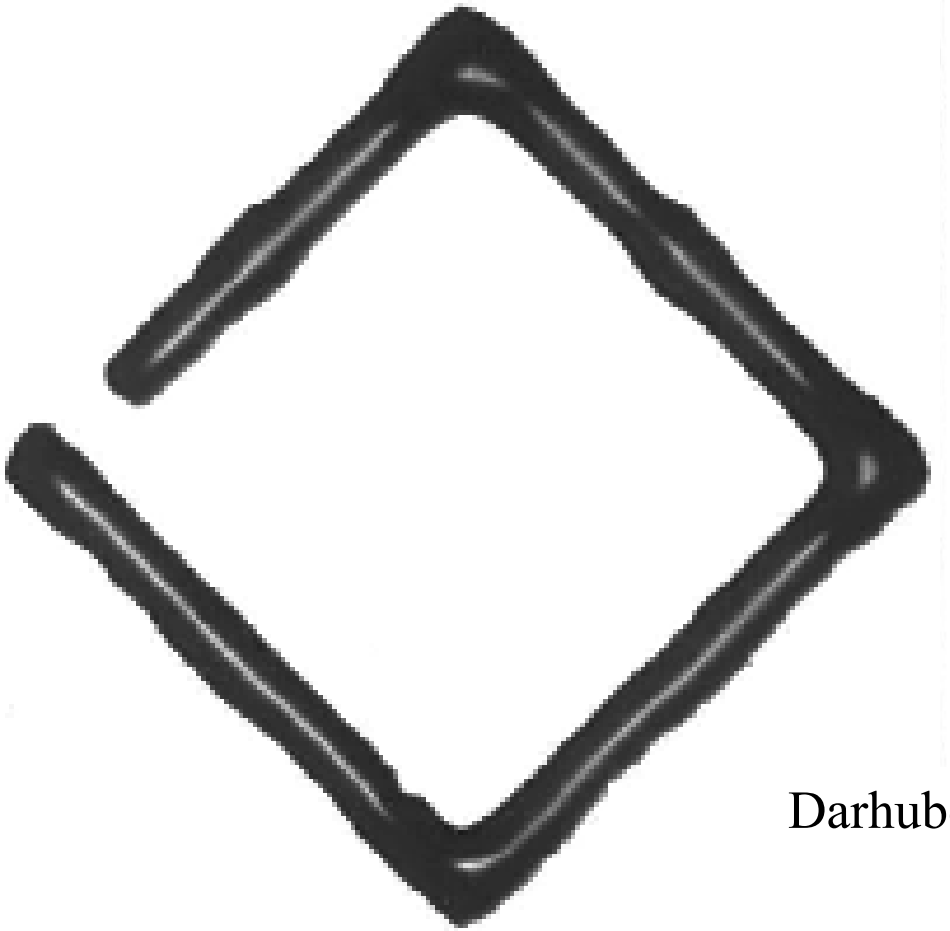
Advancing interface technology

wetting



Non-wetting





Darhuber et al. (2000)

Concluding Remarks

- Microarray is a useful tool for gathering systematic information
- The manufacturing techniques for microarray and microfluid system have showed their significant progress in the last decade and are expected to have further and faster improvement in the near future.