Research Center for Applied Sciences
Academia Sinica, Taipei, Taiwan

Application – Microfluidic Cell Culture Devices

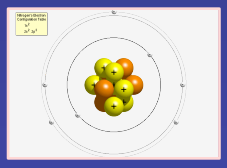
Yi-Chung Tung, Ph.D.
Assistant Research Fellow
Research Center for Applied Sciences
Academia Sinica, Taipei, Taiwan

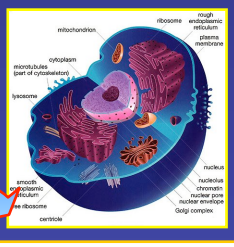
Outline

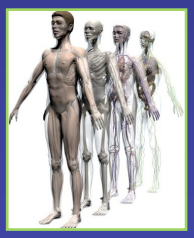
- Introduction
- Microfluidic Actuation
- Microfluidic Devices
  - Computerized microfluidic device using PDMS channels and Braille display
  - Three-dimensional cell spheroid culture in PDMS microfluidic device
  - Microfluidic flow cytometry actuated by Braille Displays
- Conclusion and Future Work
- Acknowledgement

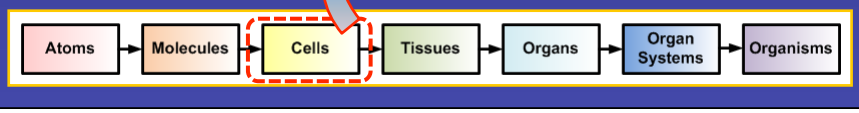
Cells

- Cells are the basic functional units of most living organisms.
- Cells sense and response to changes in their environment, and communicate with neighboring cells by releasing chemicals or by generating electrical signals.







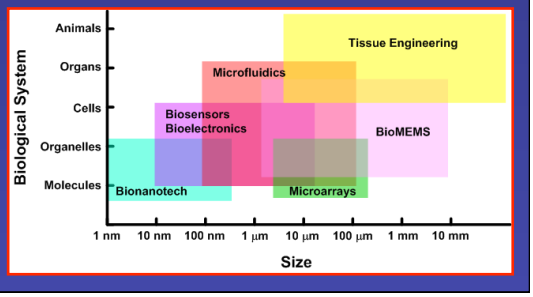


In vitro Cell Culture

- ***in vivo* Cell Study**
  - Lack of fully understanding microenvironments
  - Difficult to control all biological parameters
  - Clinical operation required
- ***in vitro* Cell Culture**
  - Well-defined microenvironments
  - Large number of samples available
  - Time-consuming for taking care of cells
    - Changing Media, Passaging etc.
  - Different from *in-vivo* environment

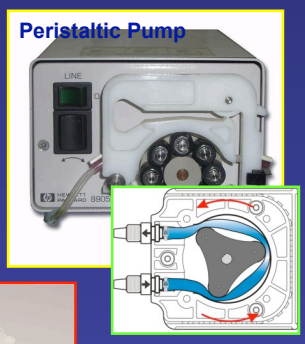
Microfluidic Devices

- Why Microfluidic Devices?
  - Unique properties (laminar flow, surface tension…)
  - Small sample volume and easy to scale up
  - Well-controlled microenvironments
  - Precise spatial and temporal control
  - Able to mimic the rich biochemical and biophysical complexity of the cellular microenvironment



Microfluidic Actuation

- Old ways…

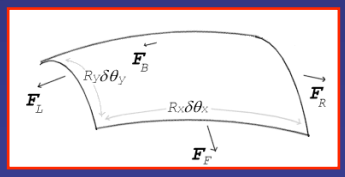


Droplet-Based Passive Pumping

- **Surface Tension Driven Flow**
- **Surface Tension (γ):**

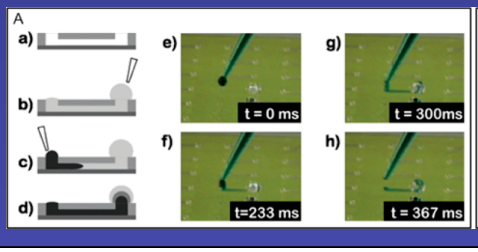
  Young-Lapalace Equation:

$$\Delta p = \gamma\left(\frac{1}{R_x} + \frac{1}{R_y}\right)$$



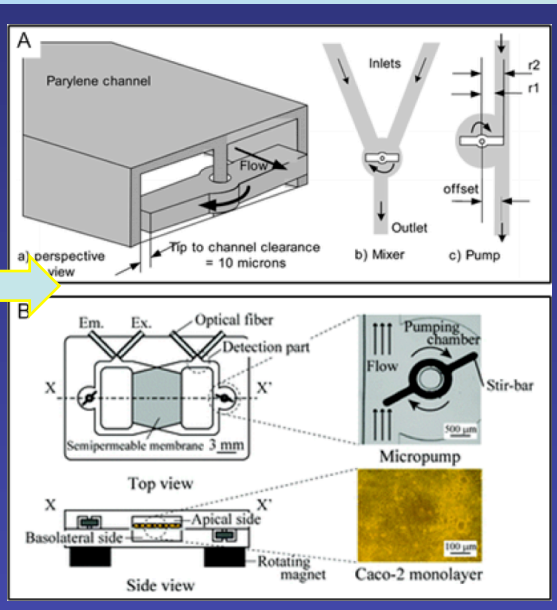
Where Δp is the pressure difference, γ is surface tension, R_x and R_y are radii of curvature in each of the axes that are parallel to the surface.

Water at 25°C, γ = 71.97 (dyn/cm)



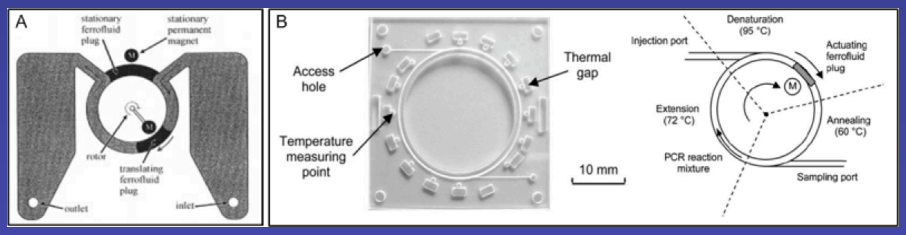
Actuation by External Magnetic Field

- **Micro-stirrer.**

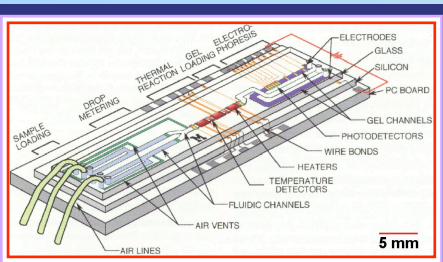


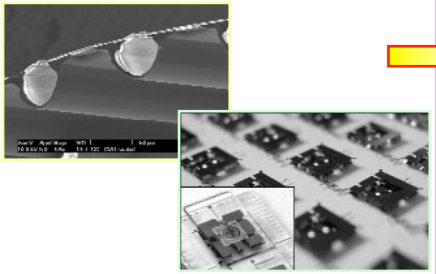
Actuation by External Magnetic Field

- **Ferrofluid -** a liquid which becomes strongly polarised in the presence of a magnetic field.

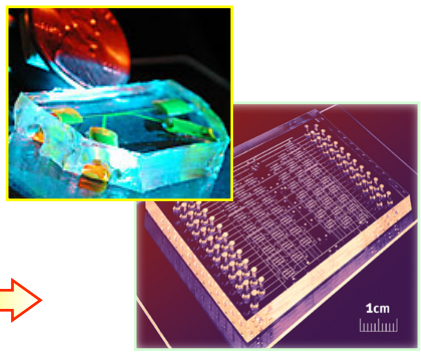


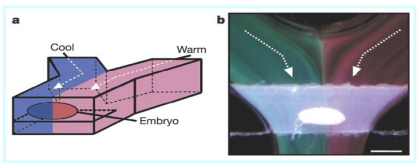
Polymer Microfluidic Devices





Silicon and Glass

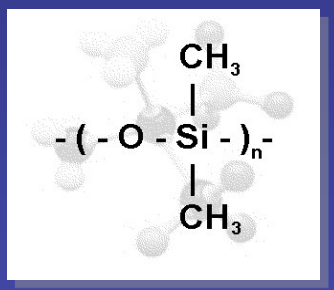




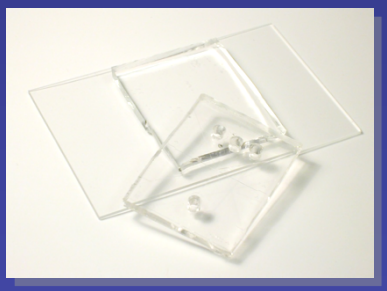
Polymer (PDMS)

Introduction - PDMS

- **PDMS (Polydimethylsiloxane)**
  - **PDMS is durable, optically transparent, and inexpensive**
  - **PDMS can be patterned by *Soft Lithography***



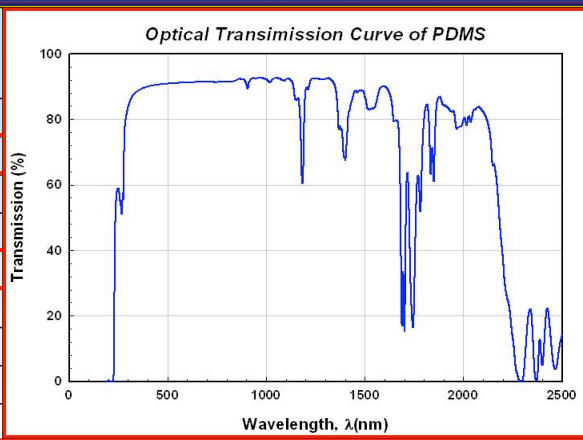
Silicone



PDMS Microfluidic Channel

PDMS Material Properties

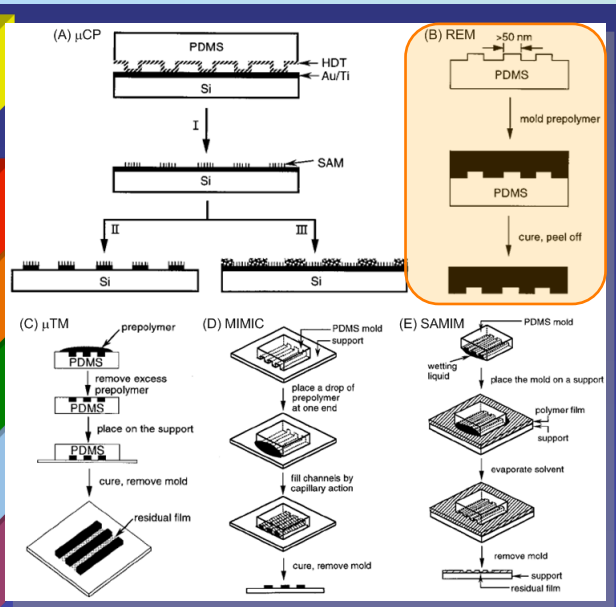
Density			
Young's Modulus			
Poisson's Ratio			
Tensile Strength			
Maximum Strain			
Thermal Expansion Ratio			
Thermal Conductivity			
Permittivity			
Resistivity			
Transparency (Visible Light)	Very Good	Excellent	Opaque



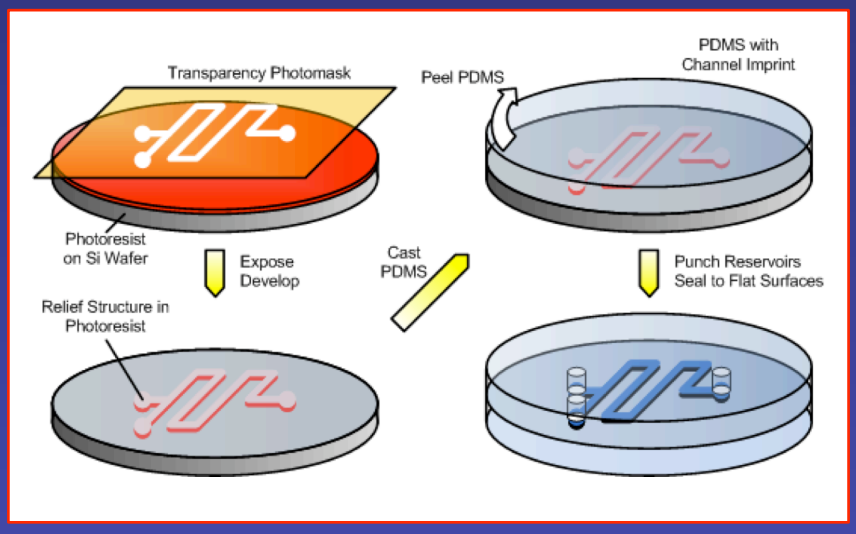
Introduction – Soft Lithography

Soft Lithography

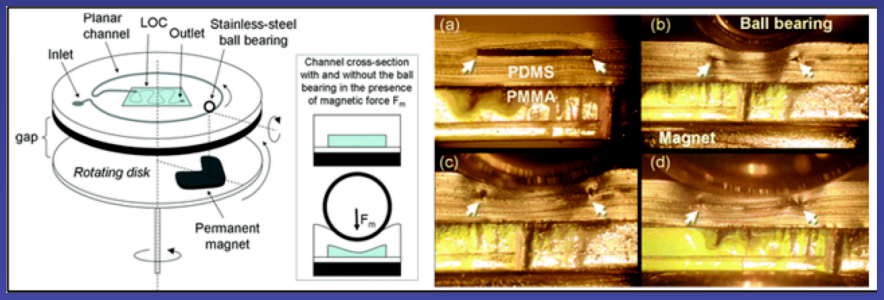
- *Microcontact Printing (μCP)*
- *Replica Molding (REM)*
- *Microtransfer Molding (μTM)*
- *Micromolding in Capillary (MIMIC)*
- *Solvent-Assisted Micromolding (SAMIM)*



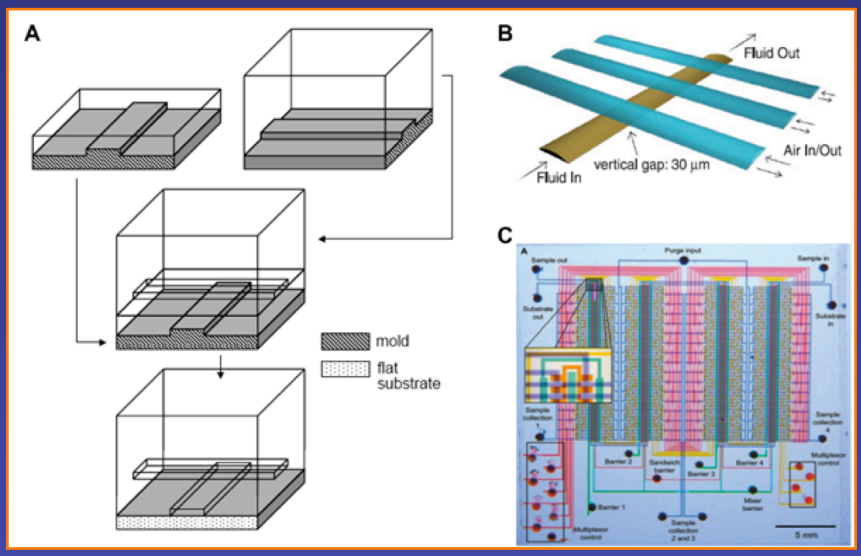
Introduction – Replica Molding (REM)



Deformation-Based Microfluidic Actuation

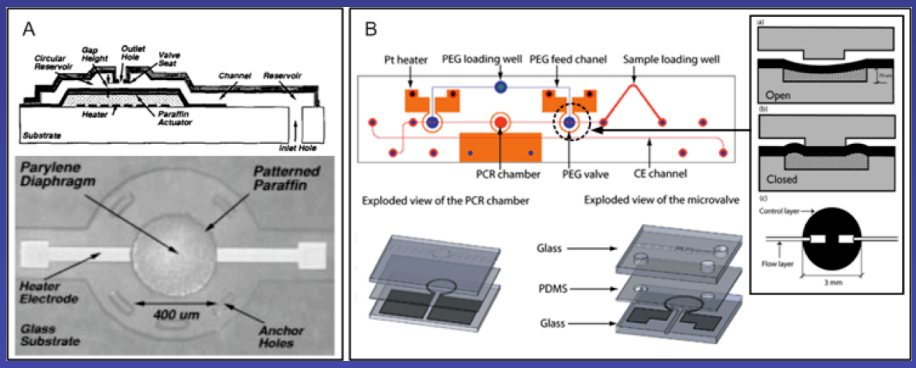


Multilayer Soft Lithography (MSL) with Pneumatic Actuation



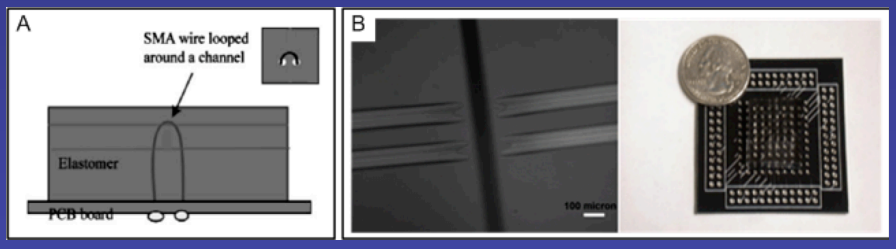
Thermally Actuated Phase-Change Microfluidic Components

- Taking advantage of materials that exhibit large volumetric change during phase change (e.g. polyethylene glycol (PEG), and paraffin), temperature regulated active microfluidic components have been developed.

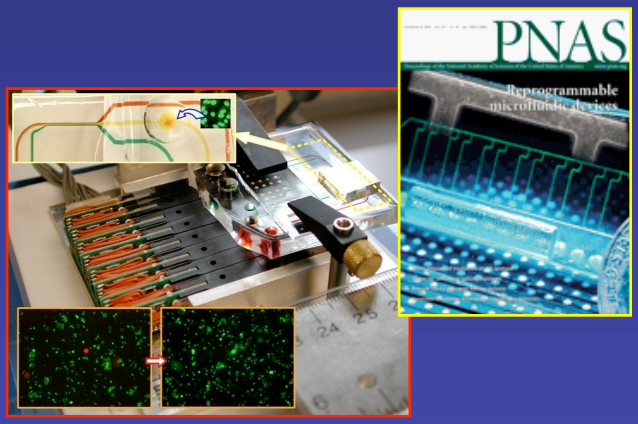


Shape Memory Alloy Actuators

- A shape memory alloy (SMA) is an alloy that "remembers" its shape, and can be returned to that shape after being deformed, by applying heat to the alloy.

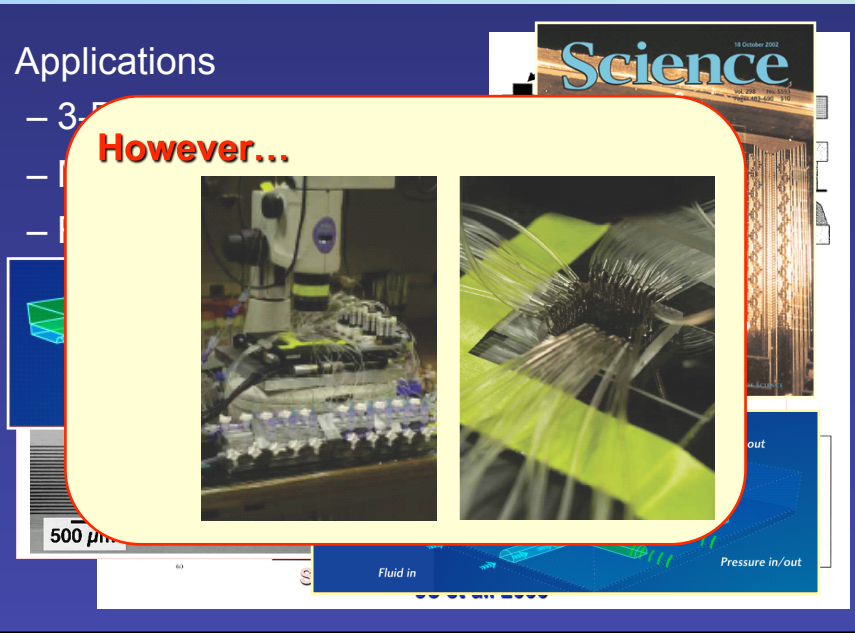


Computerized Microfluidic Device Using PDMS Channels and Braille Display



Introduction – PDMS-Based Devices

- Applications
  - 3-
  -
  -

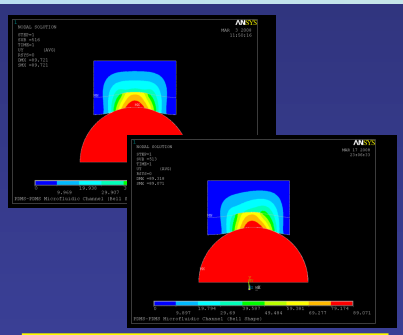


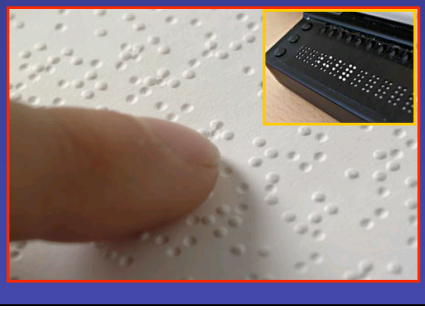
However…

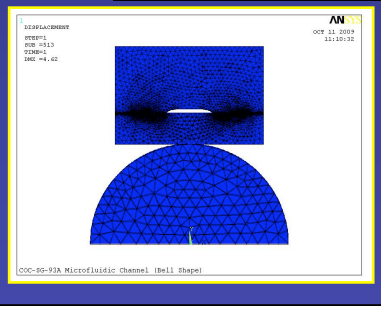
Braille Display-Based Microfluidic Systems

Braille Display

- Piezoelectric Actuation
- Computer Programmable (USB)
- Low Power Consumption
- Fast Response

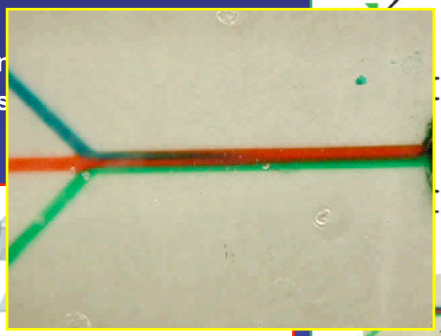


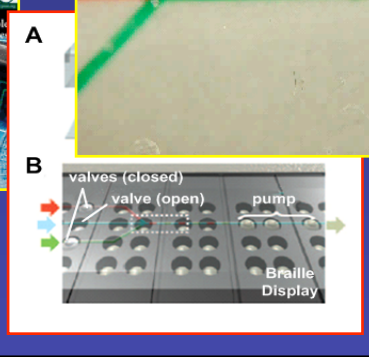


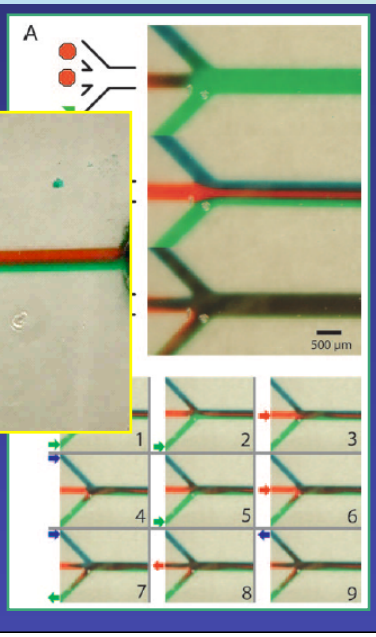


Braille Display-Based Microfluidic Systems

- **Advantages**
  - Portable
  - Versatile
  - No Fluidic Ir
  - Fully Dispos

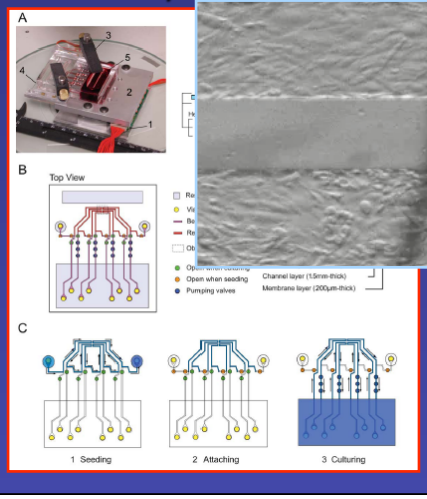


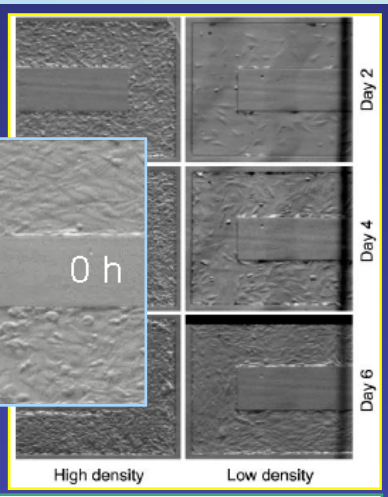


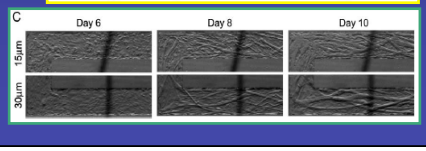


Braille Display-Based Cell Culture

- Long-term culture for C2C12 myoblast cells (out of incubators)

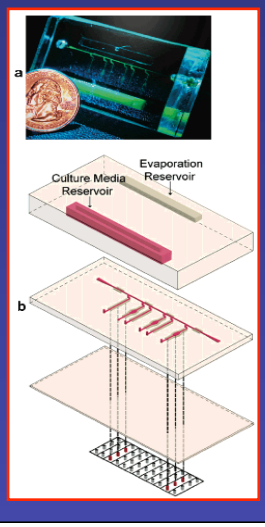


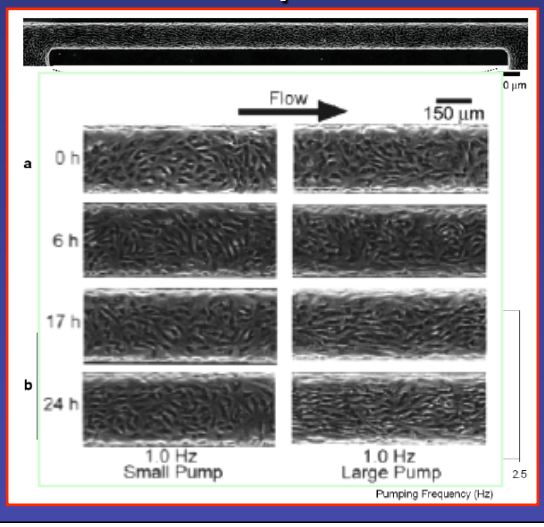




Cell Culture in Various Shear Stress Conditions

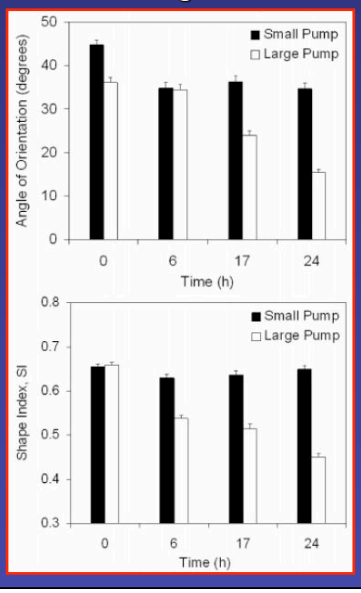
- Endothelial Cell (EC) Culture under Various Shear Stress Conditions – Temporal Patterns



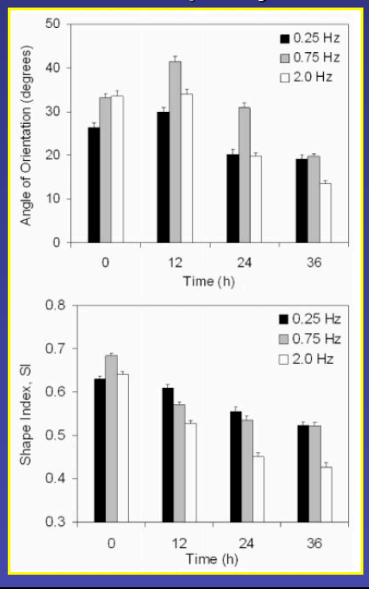


Cell Culture in Various Shear Stress Conditions

Shear Stress Magnitude:

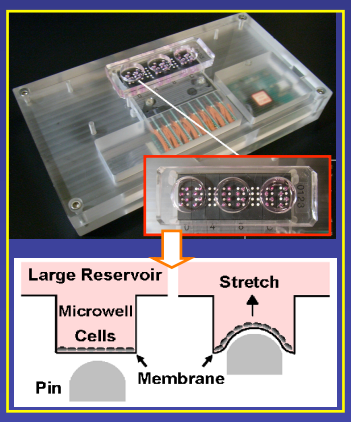


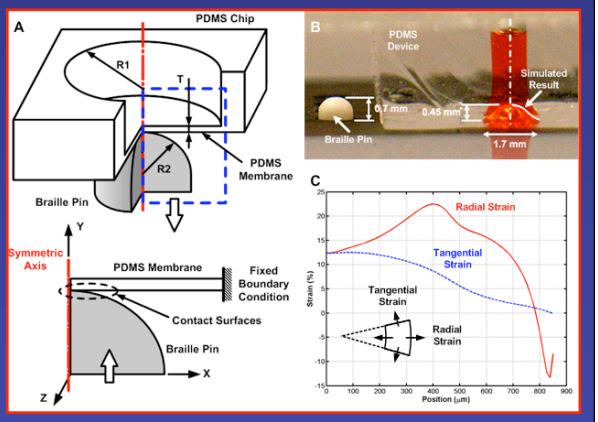
Shear Stress Frequency:



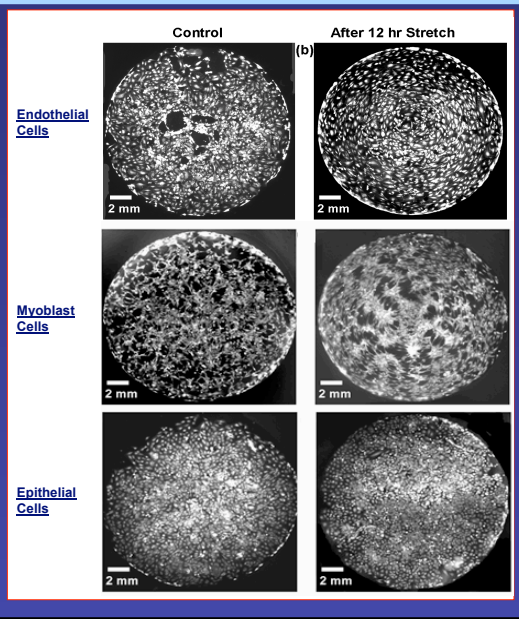
Individually Programmable Cell Stretching Microwell Arrays Actuated by a Braille Display

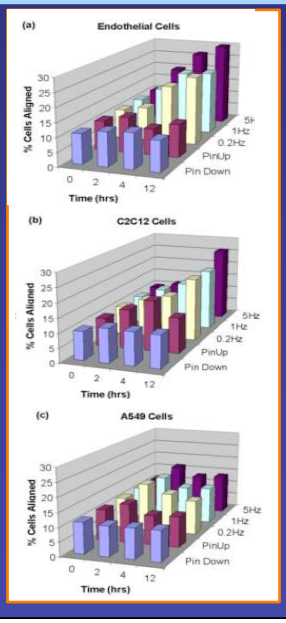
- Test various cell samples, culture medium, and stretching conditions simultaneously
- Small cell sample required



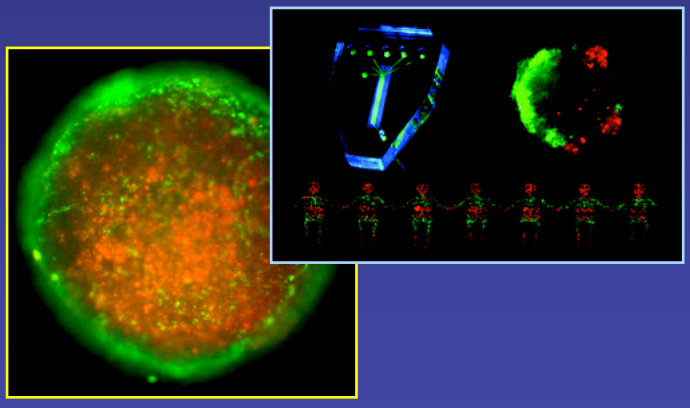


Individually Programmable Cell Stretching Microwell Arrays Actuated by a Braille Display



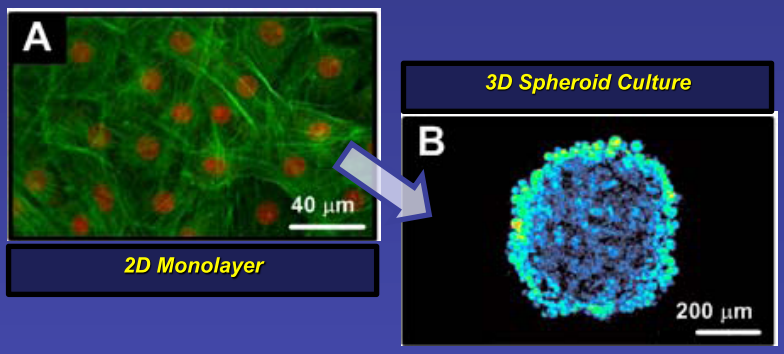


Three-Dimensional Cell Spheroid Culture in PDMS Microfluidic Device

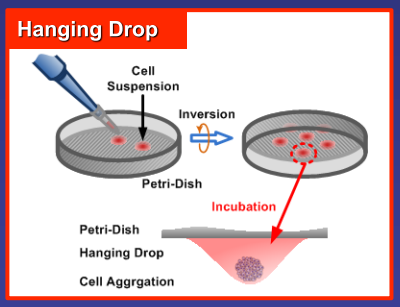


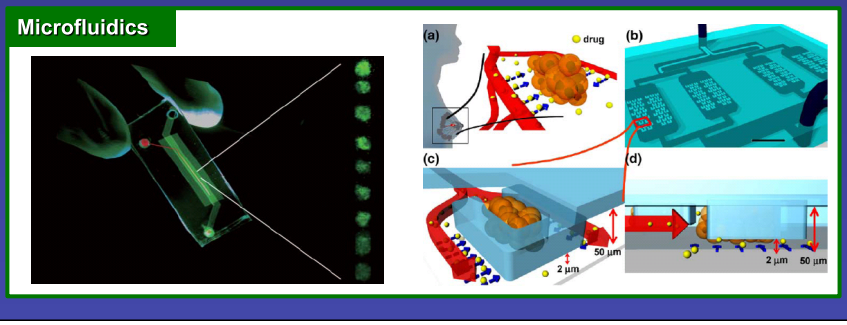
Three-Dimensional Cell Culture - Spheroid

- Moving from cell monolayers to three-dimensional (3D) cultures is motivated by the need to work with cellular models that better mimic the environment of living tissues.
- For example, tumor spheroids have been widely used as an *in vitro* 3D model to simulate the multicellular microenvironment when investigating tumor cell physiology and responses to therapeutic agents.



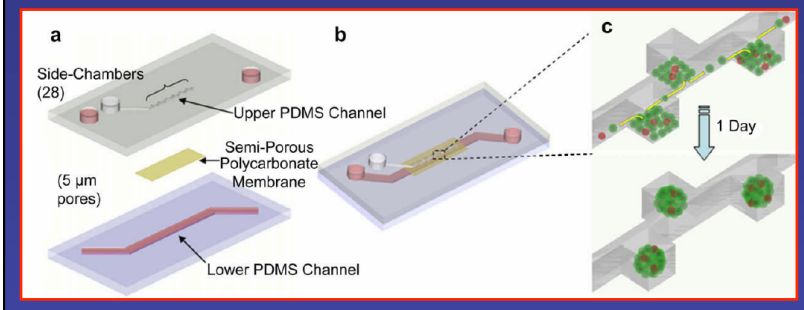
How to Form Spheroids ?



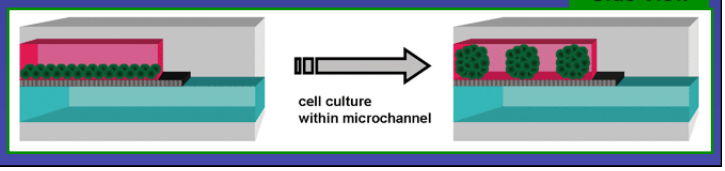


Spheroid Culture in a Microfluidic Device

- Dual-Layer Compartmentalized PDMS microfluidic Device
  - Efficient formation of uniform-sized spheroids
  - Simple yet robust operation
  - Capable of scaling up for high throughput screening

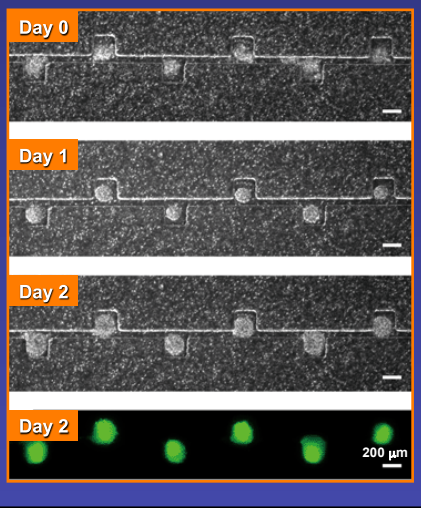


Side View

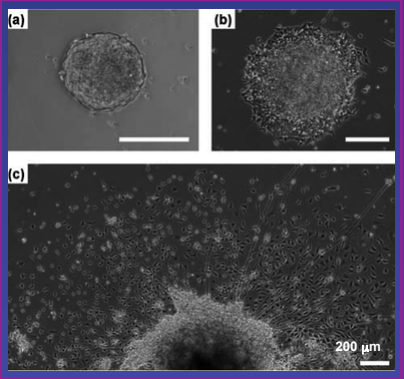


Application – Embryoid Body (EB) Culture

- Appropriate morphology and embryo size are critical for the sequential development stages of naturally conceived embryos.
- Mouse embryonic stem (ES) cells (ES-D3 cell line, ATCC)



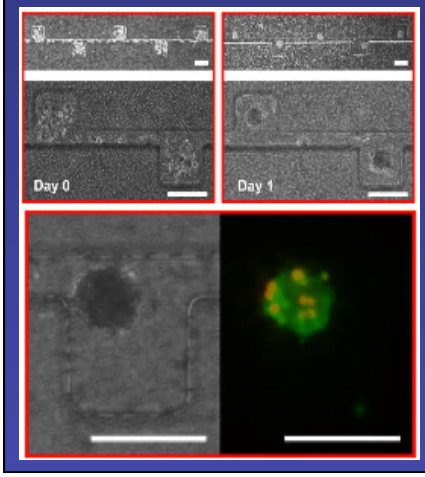
Before Exposure to the Differentiation Medium

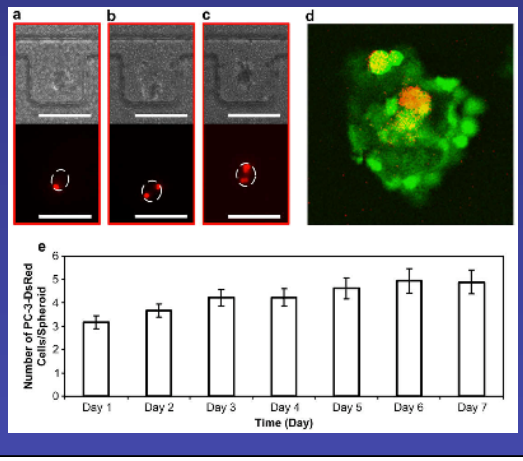


7 Days after Exposure to the Differentiation Medium

Application – Cancer Cell Co-Culture

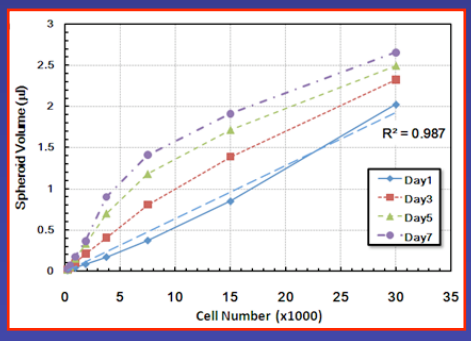
- Endothelial, osteoblast, and prostate cancer cell co-culture to mimic the *in-vivo* niche microenvironment
- Slower prostate cancer cell proliferation rate (doubling time: ~9 days)
- Endothelial cells proliferate, and osteoblast cells are quiescent

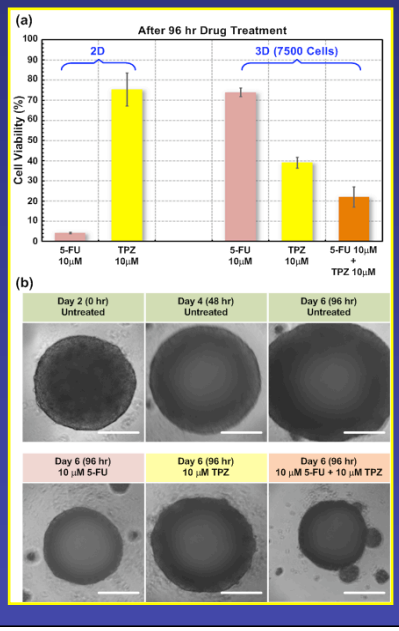




Application – Cancer Cell Drug Test

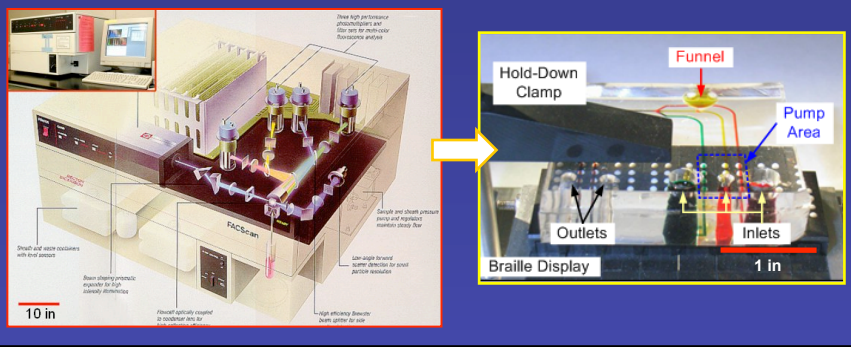
- **Human carcinoma cell that stably express mesothelin (A431.H9) cell**
- **Drug test for commonly used chemotherapy agent: Fluorouracil (5-Fu) and Tirapazamine (TPZ)**
- **Cell viability test using *Alamar blue***





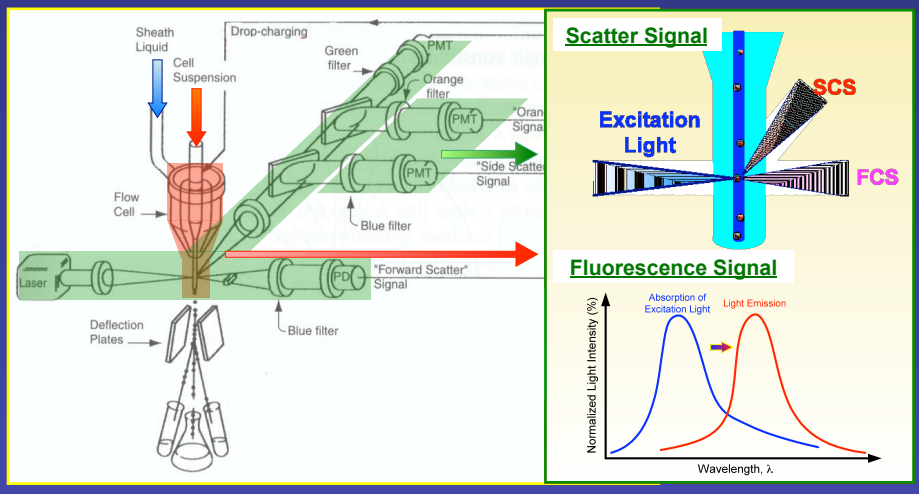
Flow Cytometry

- Rapid analysis of biological samples
  - Disease diagnosis and monitoring
  - Cell biology
  - Toxicology
  - Environmental monitoring

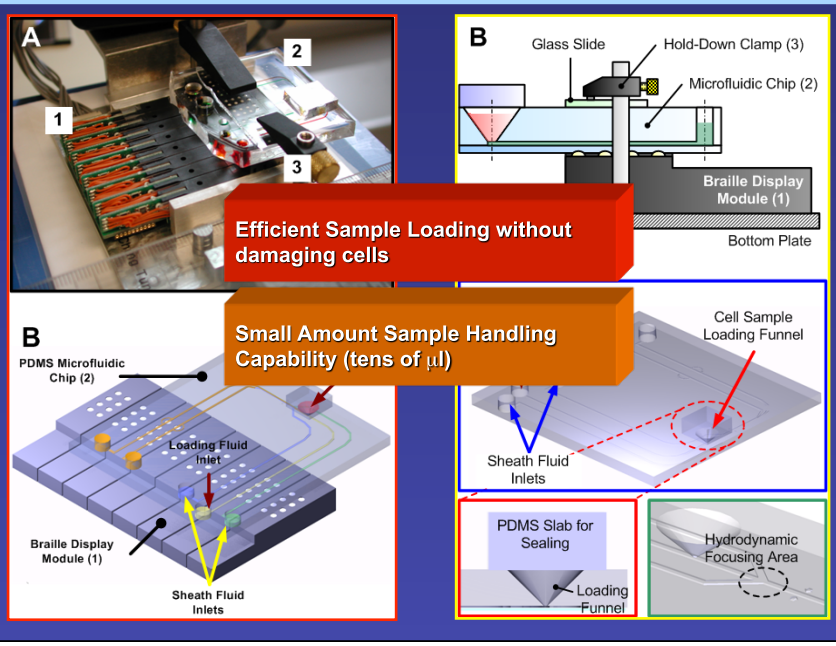


Flow Cytometry

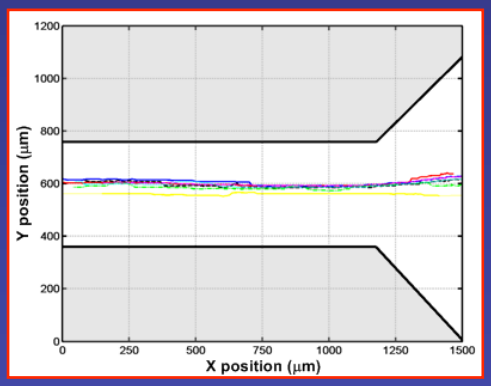
- Basic Operation of Flow Cytometer

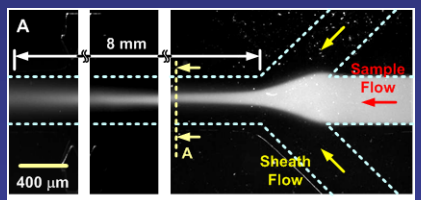


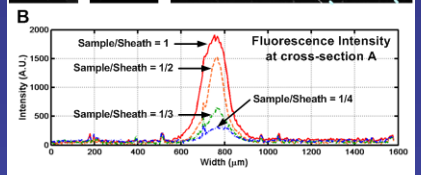
Device Design

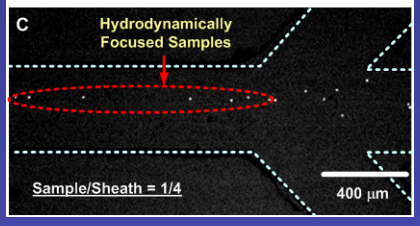


Sample Loading & Hydrodynamic Focusing



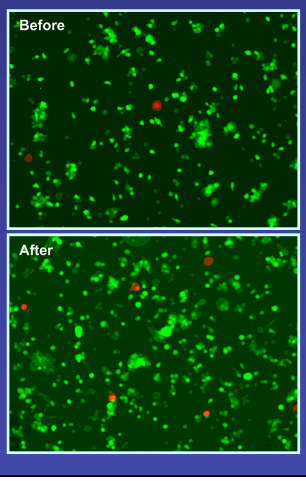


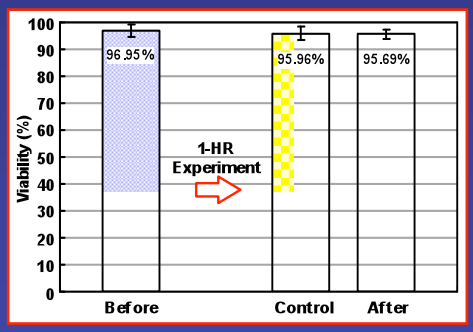




Cell Viability after Loading

- C2C12 Myoblast Cells Stained Using LIVE/DEAD Viability/ Cytotoxicity Kit
  - Calcein AM for LIVE Cell (ex/em 494 nm/517 nm)
  - Ethidium homodimer-1 (EthD-1) for DEAD Cell (ex/em 528 nm/617 nm)



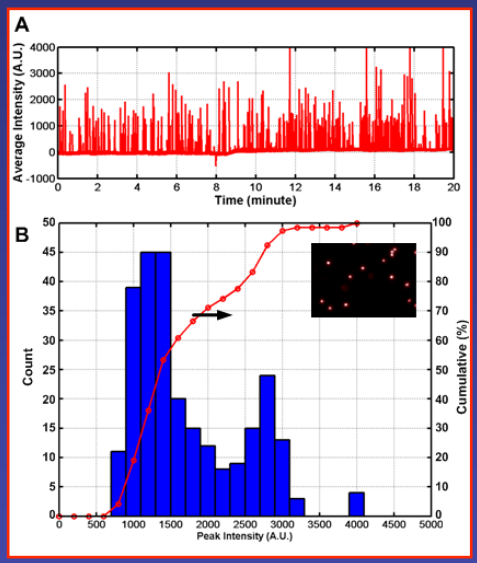


Cell Cycle Analysis

- Human promyelocytic leukemic (HL60) cells
  - Hypotonic DNA Stain:
    - Sodium citrate
    - Triton X-100
    - Propidium Iodide
    - Ribonuclease A
    - Distilled Water

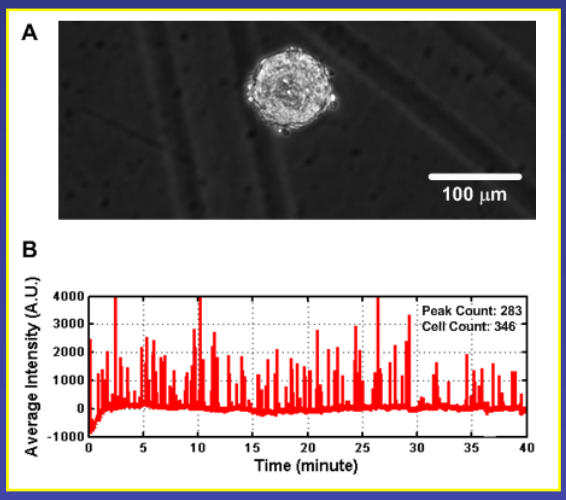
	μFC*	BD*	Ref+
G0/G1	56.4%	66.9%	60.0%
S	26.1%	15.7%	22.5%
G2/M	17.5%	17.4%	17.5%
Uncertainty	6.0%	5.4%	NA

* Cumulative Histogram Method
\+ Wolbers et al., *Electrophoresis*, 2006, 27, 5073.



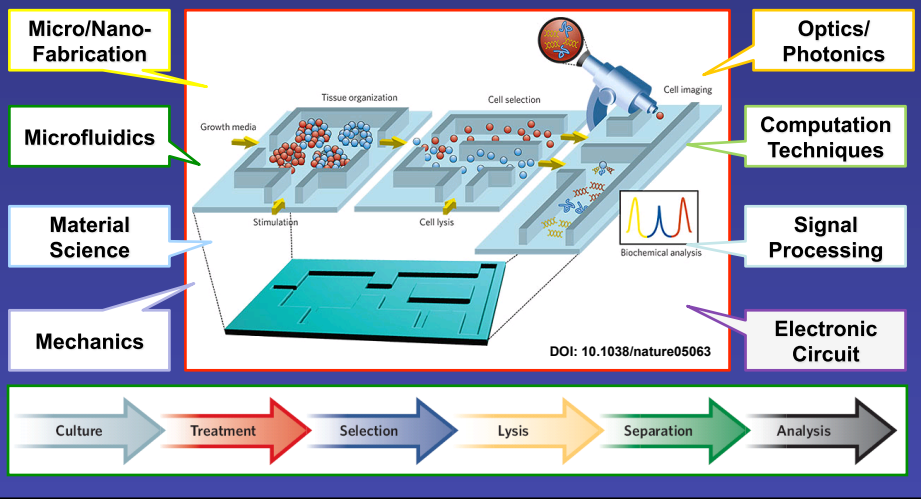
Single Embyroid Body Cell Counting

- mES Cell (ES-D3): Hanging drop cell culture to form spheroid
- Dissociated in Trypsin mixed with Syto 9 (50 μl)

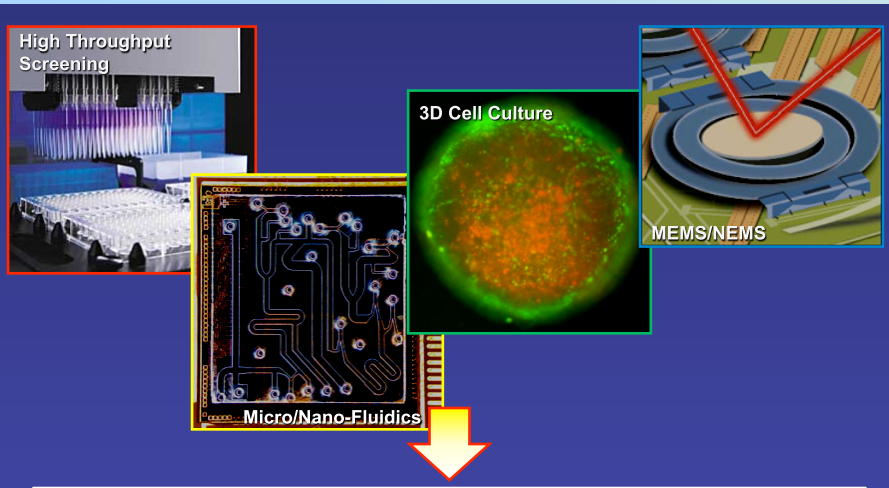


Integrated Biomedical Microdevices

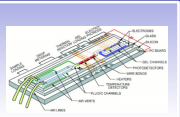
- Precisely Control or Mimic the Rich Biochemical and Biophysical Complexity of the Cellular Microenviroments.
- Cell Activity Monitoring and Observation.



Future Work



Smaller Integrated Platforms, Easier Operation, Lower Cost, Better Models *in vitro*, More Characterization Capabilities, and Higher Throughput



Questions?

http://www.rcas.sinica.edu.tw/faculty/tungy.html

E-mail: tungy@gate.sinica.edu.tw