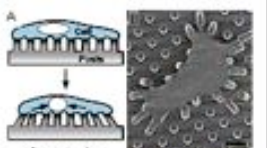
Application –
Microfluidic Cell Analysis Devices (I)

Date: 2013/05/24

Dr. Yi-Chung Tung

Mechanotransduction and the Study of Cellular Forces

- Mechanical forces play a critical role in nearly all aspects of cell biology, from cell migration to morphogenesis to cell proliferation.
- hese forces are ubiquitous to the interactions between cells and their substrates (such as shear stress in the vascular tree).
- Even in the absence of applied external forces, cells themselves apply forces against their surroundings by actively contracting their actin-myosin cytoskeletal networks.



Nature Protocol

PROTOCOL

Assaying stem cell mechanobiology on microfabricated elastomeric substrates with geometrically modulated rigidity

Michael T Yang[1,2], Jianping Fu[1–3], Yang-Kao Wang[1,2], Ravi A Desai[1] & Christopher S Chen[1]

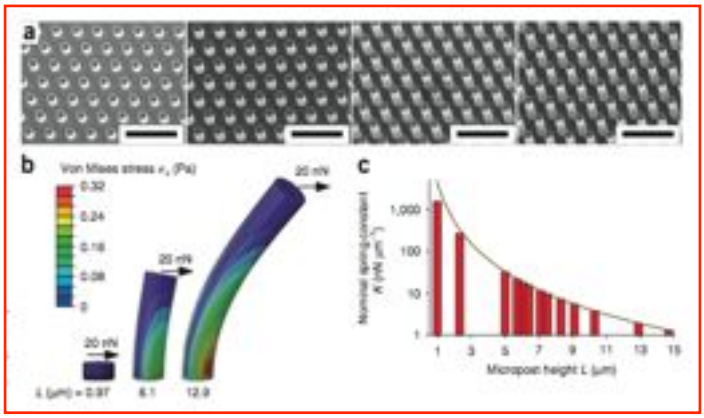
[1]Department of Bioengineering, University of Pennsylvania, Philadelphia, Pennsylvania, USA. [2]Present addresses: Department of Mechanical Engineering and Department of Biomedical Engineering, University of Michigan, Ann Arbor, Michigan, USA (J.F.); Department of Medicine, Skeleton-Joint Research Center, National Cheng Kung University, Taiwan, Taiwan (Y.-K.W.). [3]These authors contributed equally to this work. Correspondence should be addressed to C.S.C. (chrischen@seas.upenn.edu).

Published online 27 January 2011; doi:10.1038/nprot.2010.139

We describe the use of a microfabricated cell culture substrate, consisting of a uniform array of closely spaced, vertical, elastomeric microposts, to study the effects of substrate rigidity on cell function. Elastomeric micropost substrates are micromolded from silicon masters comprised of microposts of different heights to yield substrates of different rigidities. The tips of the elastomeric microposts are functionalized with extracellular matrix through microcontact printing to promote cell adhesion. These substrates, therefore, present the same topographical cues to adherent cells while varying substrate rigidity only through manipulation of micropost height. This protocol describes how to fabricate the silicon micropost array masters (~2 weeks to complete) and elastomeric substrates (3 d), as well as how to perform cell culture experiments (1–14 d), immunofluorescence imaging (2 d), traction force analysis (2 d) and stem cell differentiation assays (1 d) on these substrates in order to examine the effect of substrate rigidity on stem cell morphology, traction force generation, focal adhesion organization and differentiation.

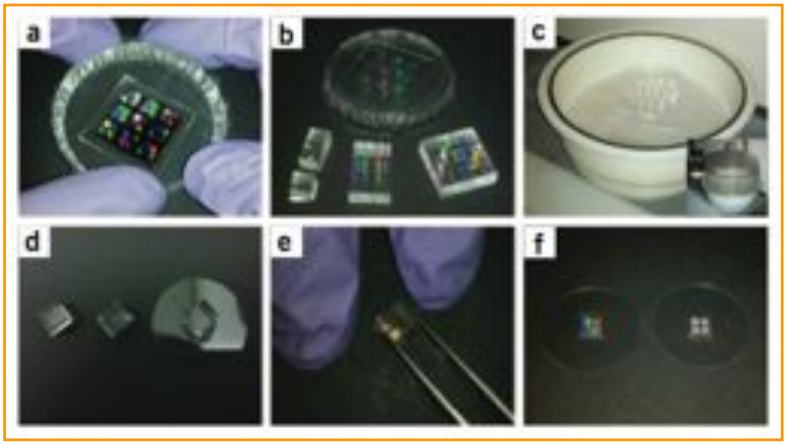
PDMS Micro-Post Array

- Characterization of micropost array.
- Deformation vs. Dimensions.



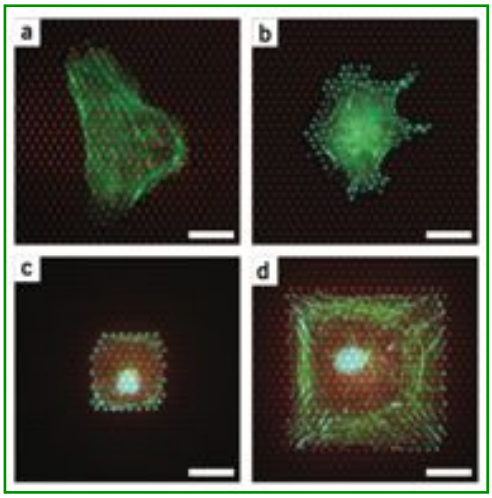
PDMS Micro-Post Array

- Replica molding of a micropost array master.
- Surface treatment and pattern transfer.



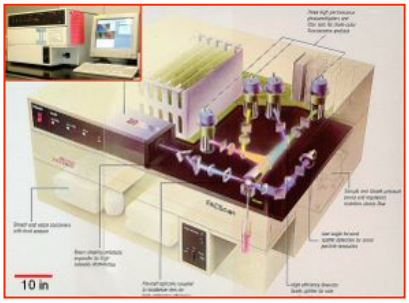
Cells on Microposts

- Force measurement and beyond.



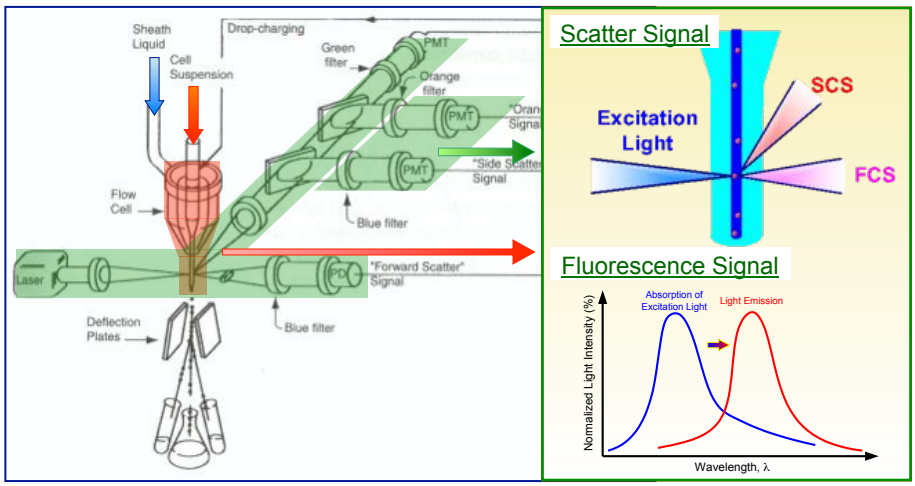
Introduction – Flow Cytometer

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

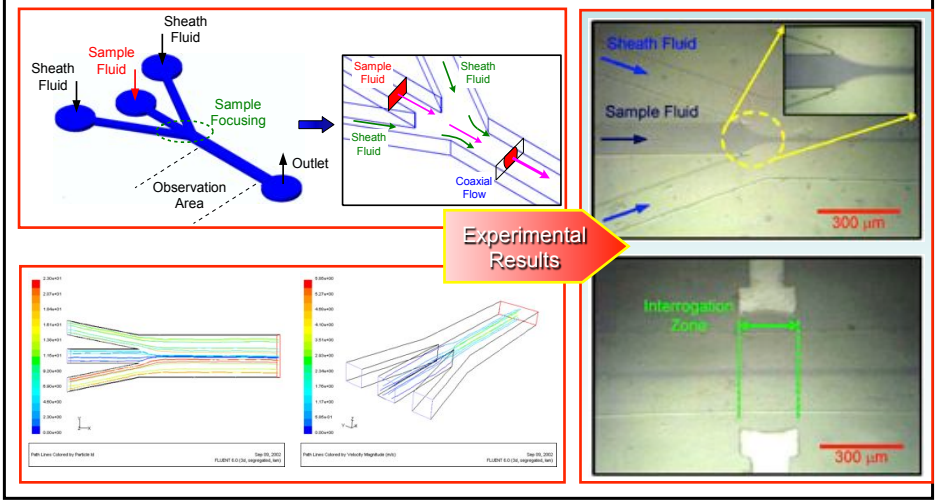


Introduction – Flow Cytometer

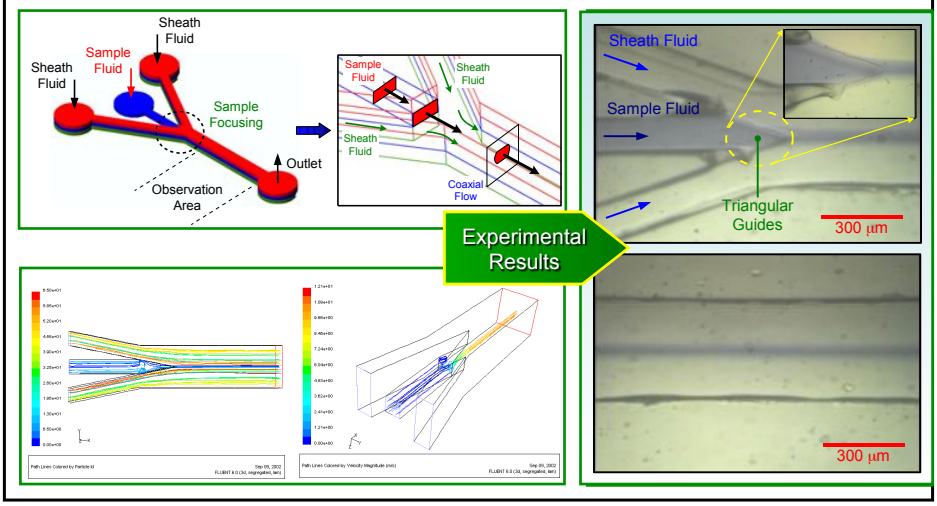
- Basic Operation of Flow Cytometer



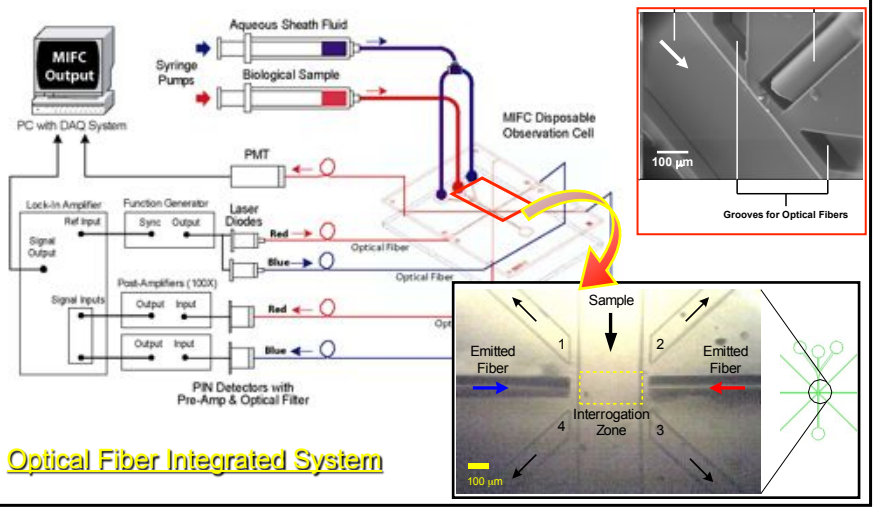
PDMS Microfluidic Channel



PDMS Microfluidic Channel

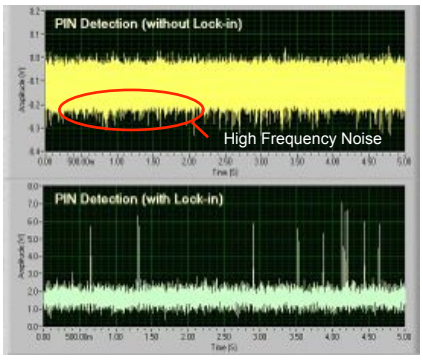


Opto-Fluidic Intergated System

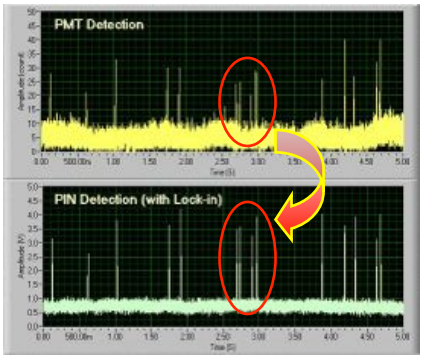


Optical Fiber Integrated System

Experimental Results (I)

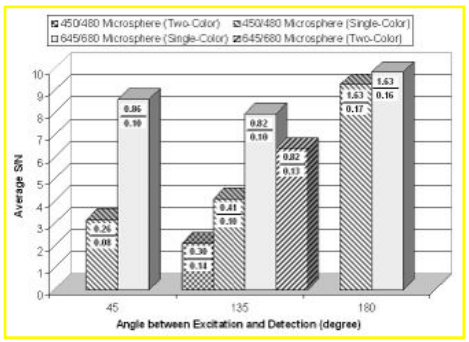


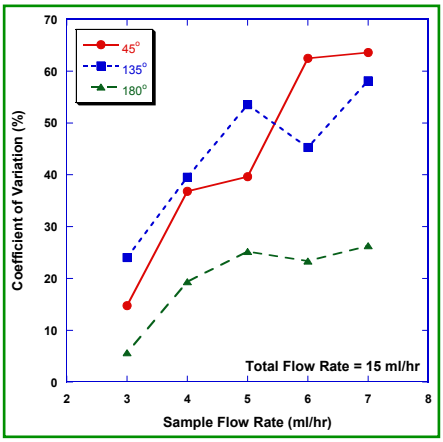
Lock-in Measurement for Fluorescence Detection Using Silicon-Based PIN Photodiode



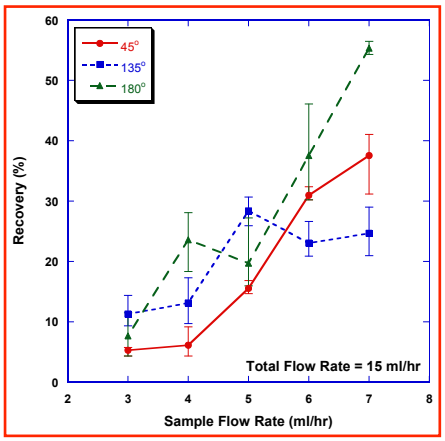
Simultaneous PMT and PIN Photodiode Detection

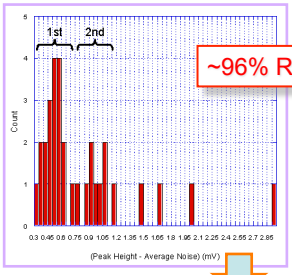
Experimental Results (II)



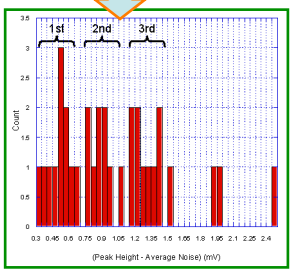


Experimental Results (III)



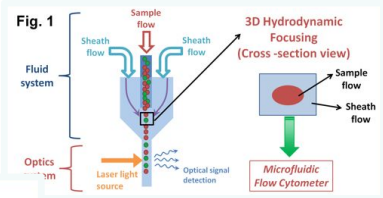


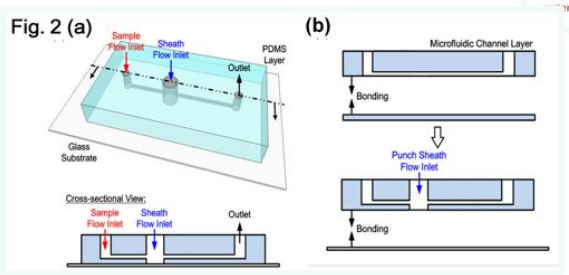
~96% Recovery



Simple 3D Hydrodynamic Focusing

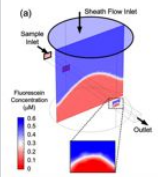
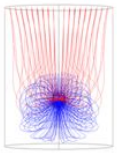
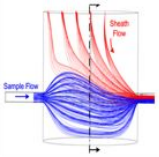
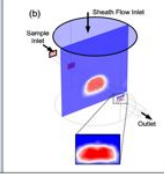
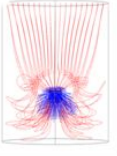
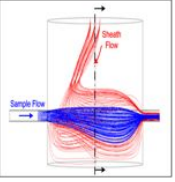
- Single Channel Layer, Minimum Interconnections: Single Sample Inlet and Single Sheath Flow Inlet





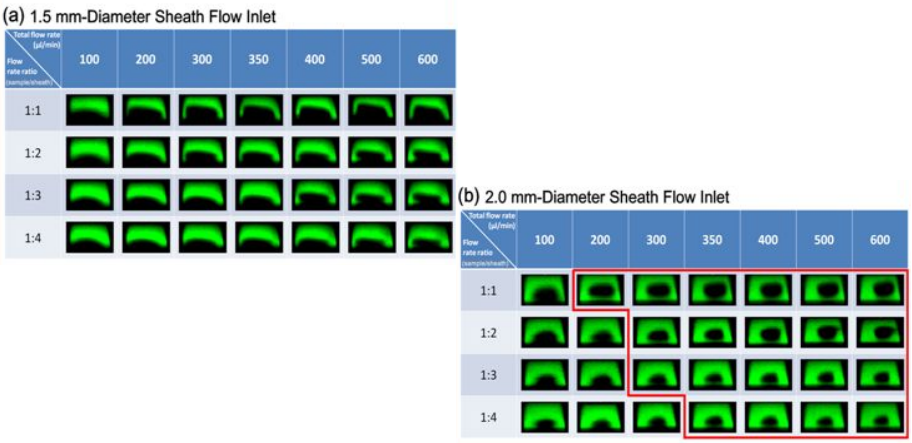
Numerical Simulation

- 3D computational fluidic dynamics (CFD) model.

Total flow rate (Flow rate ratio 1:1)	Concentration profiles	Particle tracing profiles	
		Cross section view	Side view
100 uL/min			
500 uL/min			

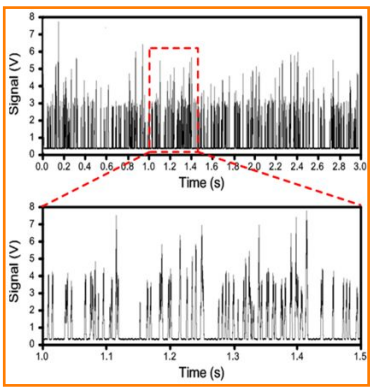
Confocal Imaging

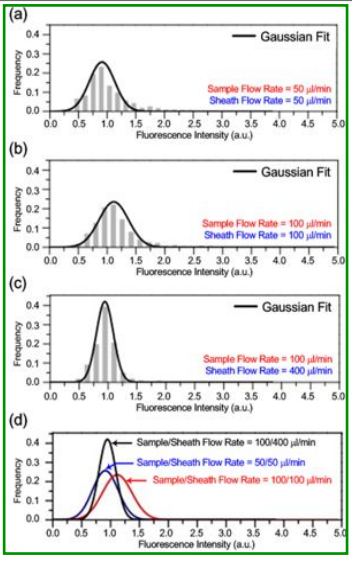
- Fluorescein and Confocal Microscopy Z-Stack Imaging.



Bead Characterization

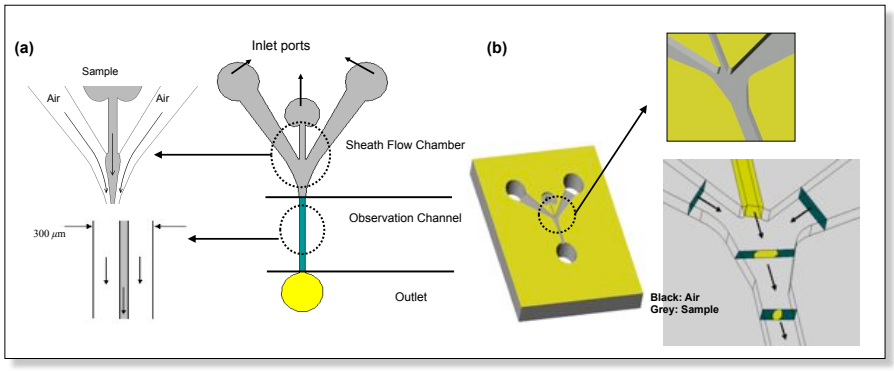
- Time-Domain Measurement.
- Histograms of Peak Fluorescence Intensities.



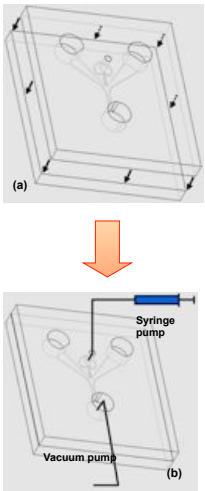


Air Sheath Flow Cytometry

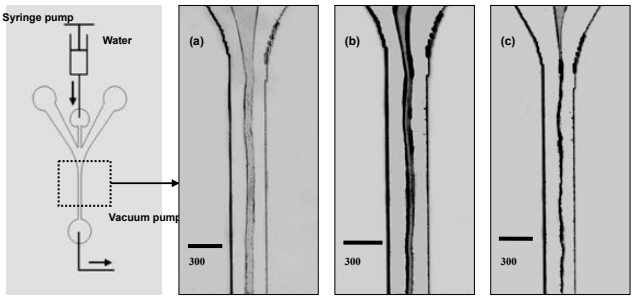
- Minimize instrument footprint
- Less contamination concern
- Promising for *Point-of-Care*



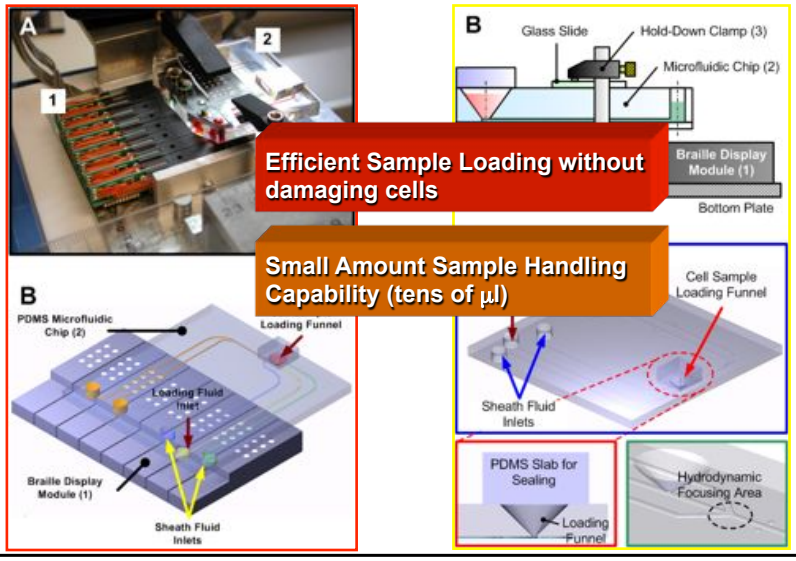
Hydrodynamic Focusing



- Stably focused aqueous sample core flow along the microfluidic channel of *μ*FC. (a) flow rate = 20 mL/h, (b) flow rate = 10 mL/h, (c) flow rate = 6 mL/h. The unit of size bar is *μ*m.



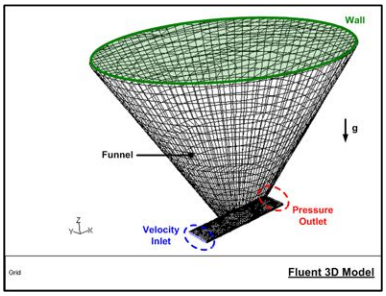
Braille Flow Cytometer



Efficient Sample Loading without damaging cells

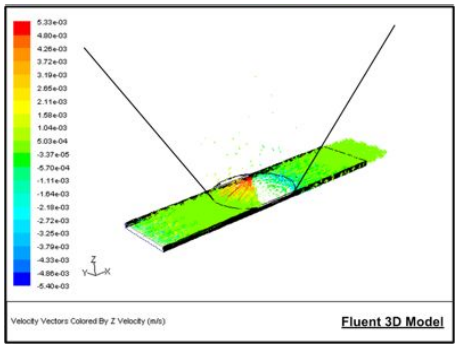
Small Amount Sample Handling Capability (tens of μl)

Flow Simulation

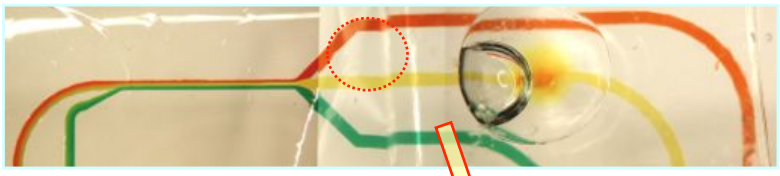


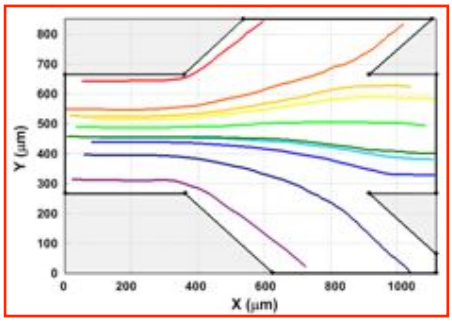
3D Laminar Model in Fluent 6.2 (Steady State)

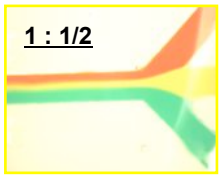
- Path Lines
- Static Pressure
- Velocity in X-Direction
- Velocity in Z-Direction

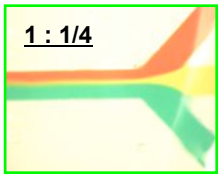


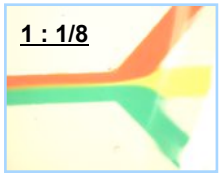
Sample Loading & Hydrodynamic Focusing



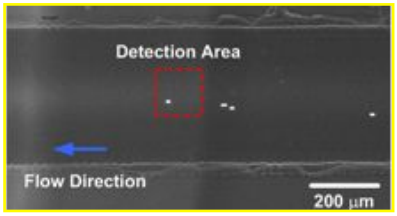


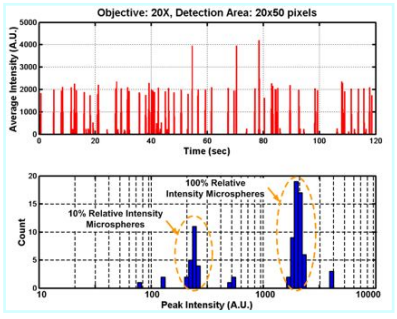


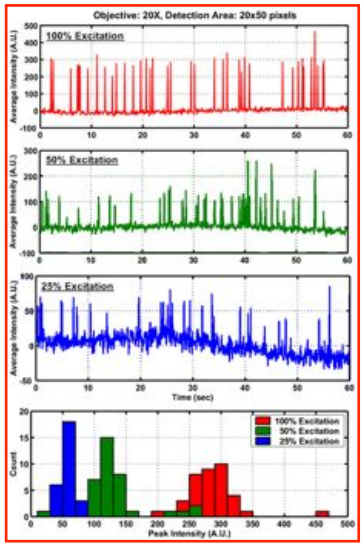




Bead Calibration

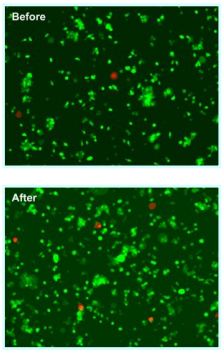


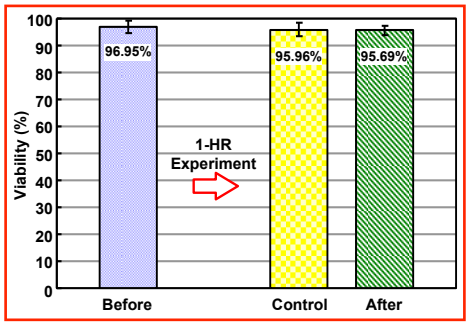




Cell Viability after Loading

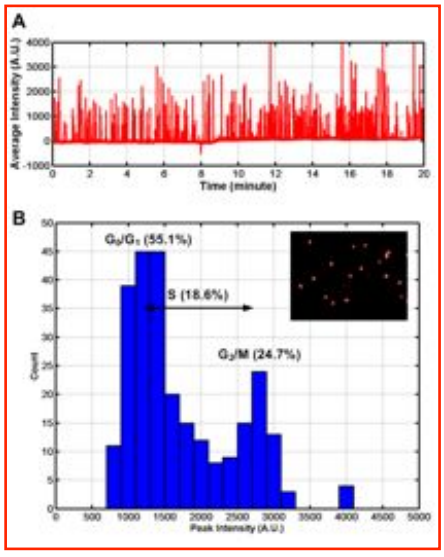
- **C2C12 Myoblast Cells Stained Using LIVE/DEAD Viability/ Cytotoxicity Kit**
  - Calcein AM for LIVE Cell (ex/em 494 nm/517 nm)
  - Ethidium homodimer-1 (EthD-1) for DEAD Cell (ex/em 528 nm/617 nm)





Cell Cycle Analysis

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



Single Embryoid Body Cell Counting

- **ES Cell: Hanging drop cell culture to form spheroid**
- **Dissociated in Trypsin mixed with Syto 9 (40 μl)**