Advanced 4
Biological Application
～Single Cell Analysis～

Prof. T. Fukuda, Prof. F. Arai
and Assistant Prof. M. Nakajima
Dept. of Micro / Nano Systems Engineering
Nagoya University

Contents

1. Introduction

2. Single cell immobilization using local photo polymerization for on-chip single cell analysis

3. Single virus infection to a specific cell by optical tweezers and

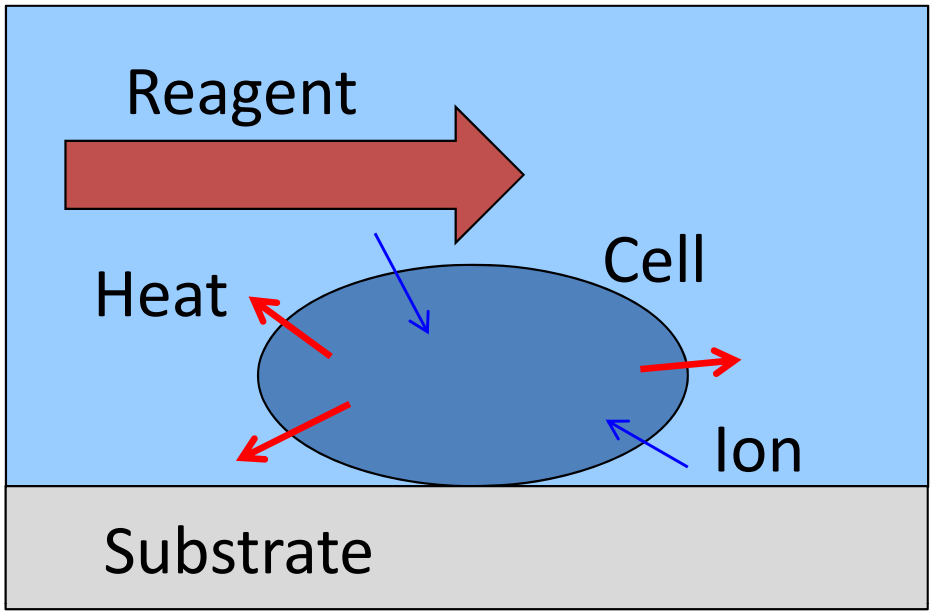
4. Summary

Background

In recent years, bio-industries employing cells have been expanding.

Ex) Drug screening (Evaluation of drug resistance)
Environmental purification (remove of Hg)

Cell measurement method



- Immobilization of cell on the substrate
- Injection of reagent with various conditions
- Measurement of cell response against stimuli (pH, virus infection, etc.)

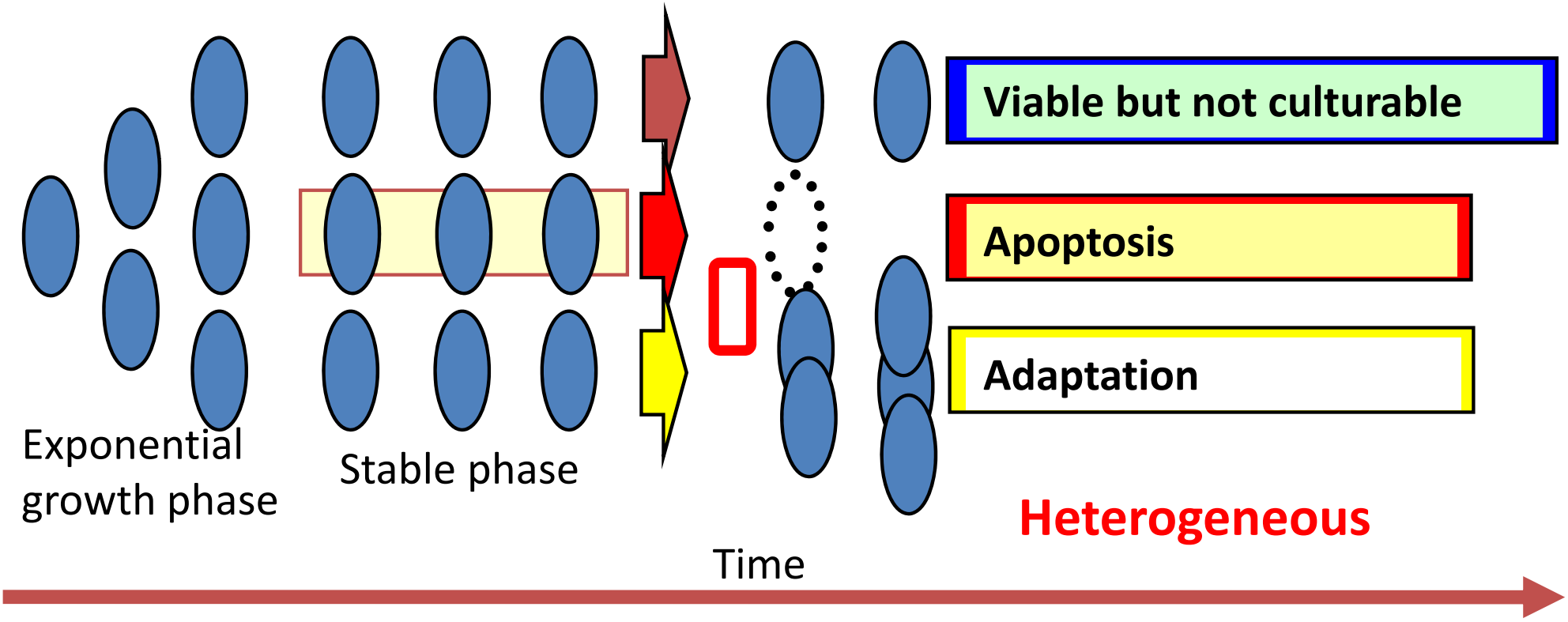
Importance of Single Cell Analysis

Group cell analysis

Available information

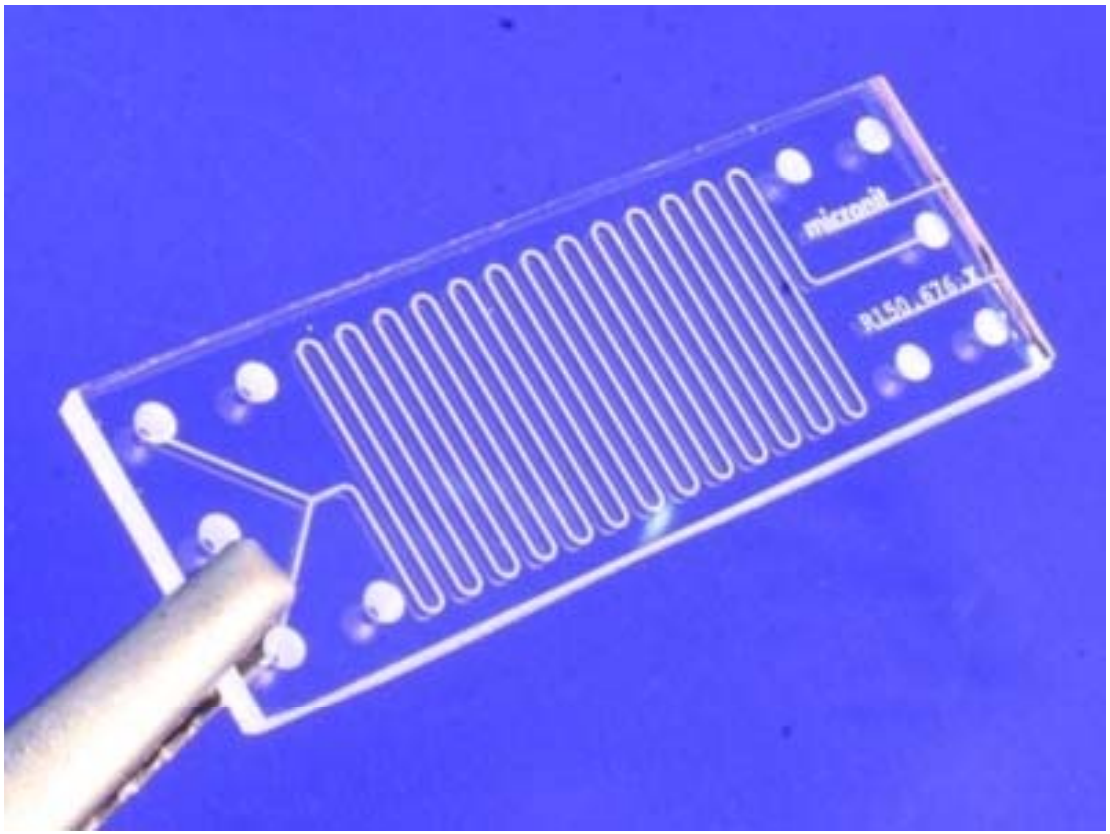
Averaged information

Microbial community



Individual cell's information is necessary

On-Chip Cell Analysis



Microreactor-chip (by Micronit Microfluidics)

Advantages of on-chip cell analysis

- Avoidance of disturbances and contaminations
- Small consumption of reagents
- Disposable

Works on a microfluidic chip

Measurement

Environment

- Flow speed
- Flow volume
- Temperature
- pH
- O_2 density

Cell

- Cell state
  - cell surface marker
  - cell cycle
  - concentration of ion
  - etc.
- Size
- Stiffness
- Impedance

Control

Manipulation

Chemical reaction

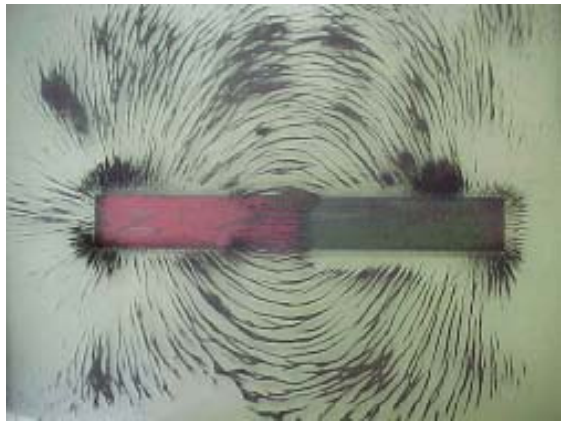
- Mix reagents
- Change environment (Stimulation)

Physical interaction

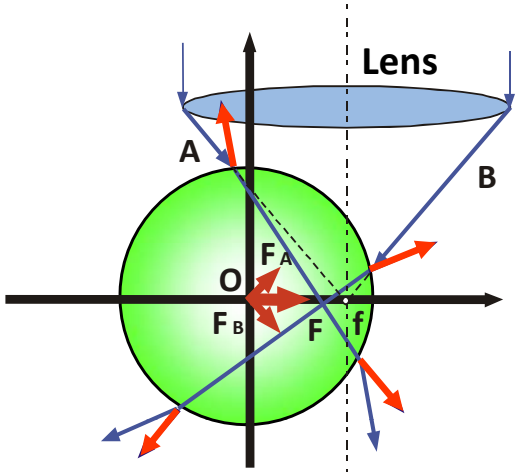
- Separate
- Arrange
- Cut
- Fuse
- Apply force
- Vibrate
- Dispense

Non-contact manipulation on a Chip

Magnetic force

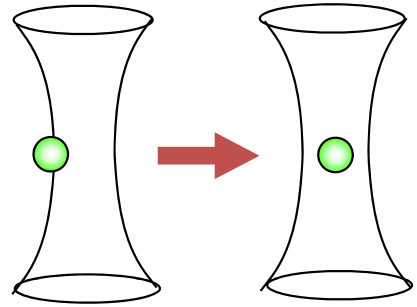


Optical force

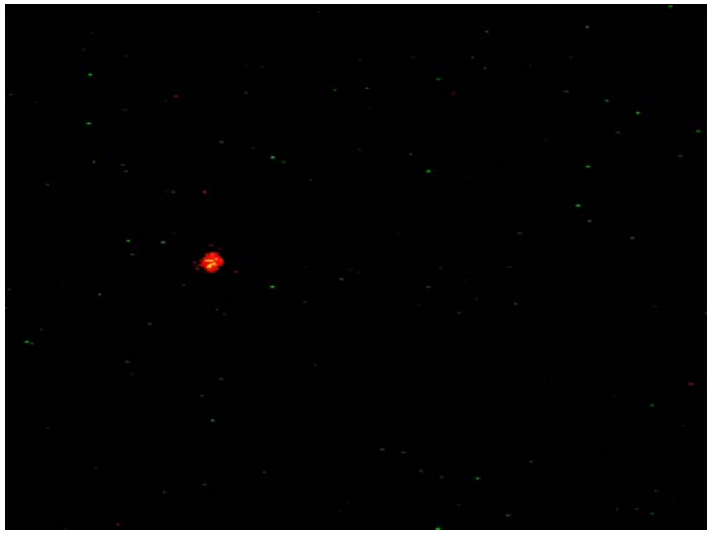


Mie particle

Focused Laser



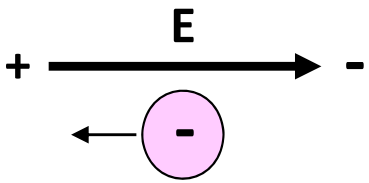
Rayleigh particle



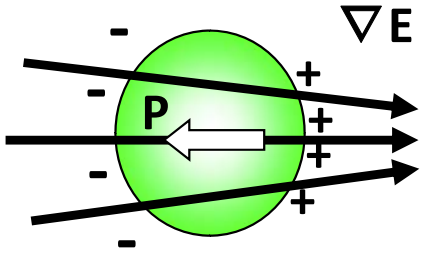
5 μm

Single virus manipulation

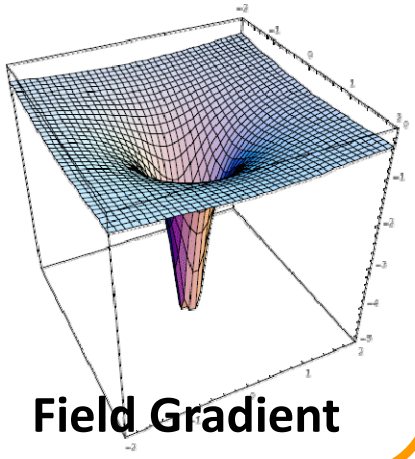
Electrostatic force



Electrophoresis



Dielectrophoresis



Field Gradient

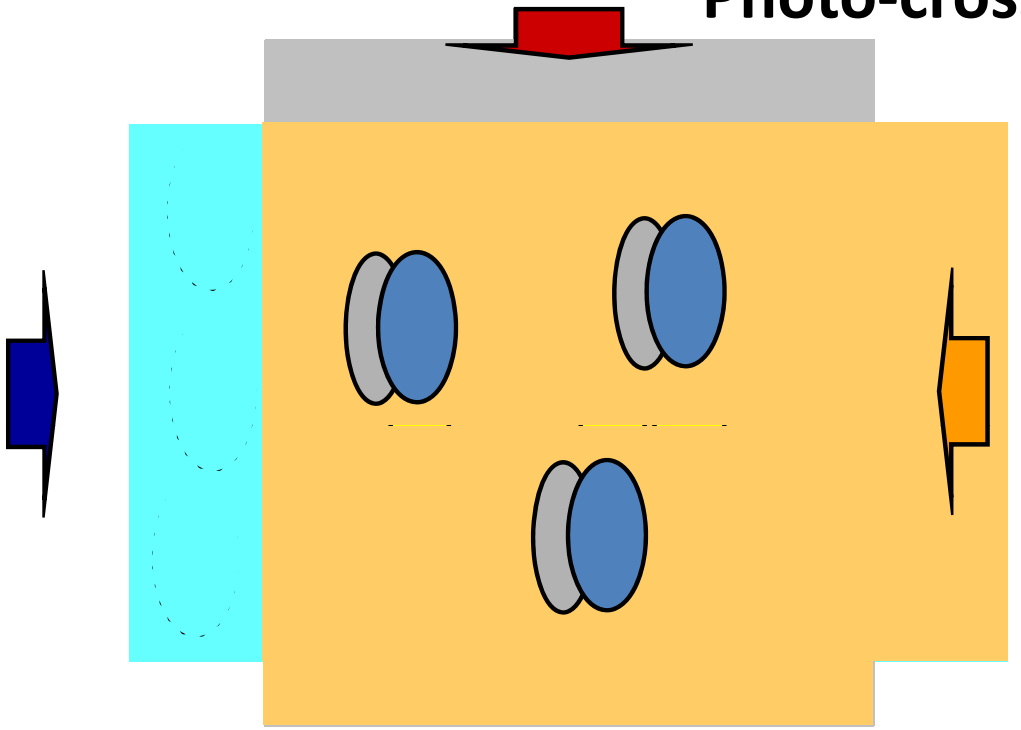
Contents

Single Cell Immobilization for Cell Analysis

Photo-crosslinkable resin

Sorted Cell

FCM
LSC
Thermal gelation method
etc.



Reagent

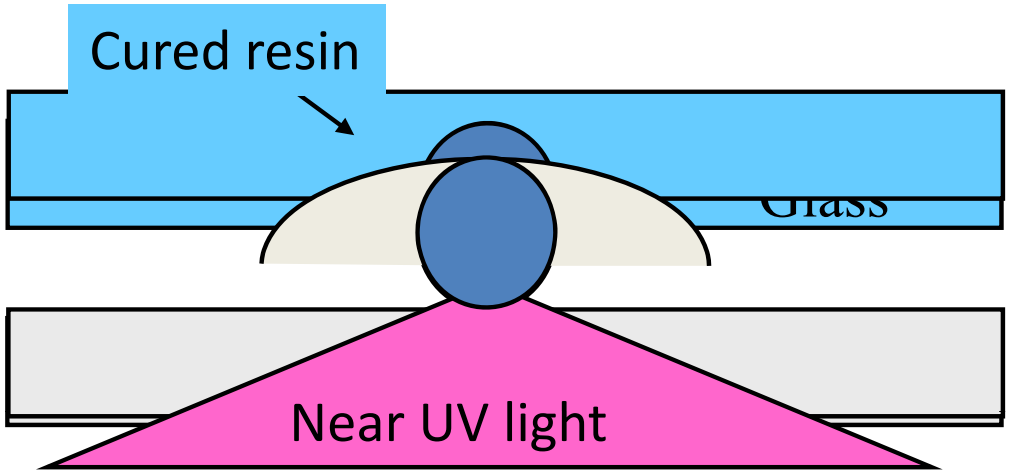
Growth medium
Fluorescent dye
etc.

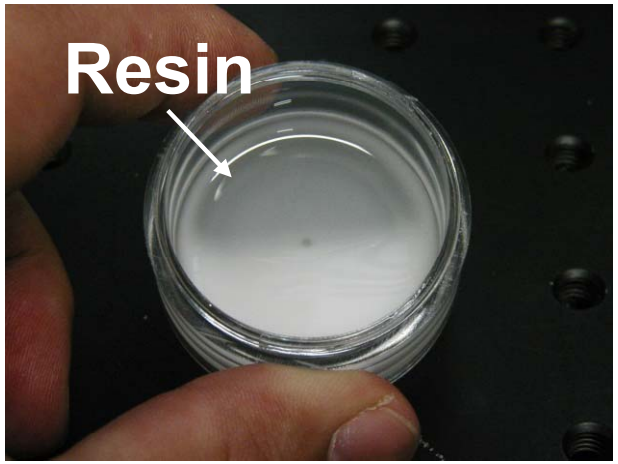
Micro-reactor

Strong immobilization by using photo-crosslinkable resin introduced locally

Cell position is controllable by optical tweezers

Immobilization using photo-crosslinkable resin

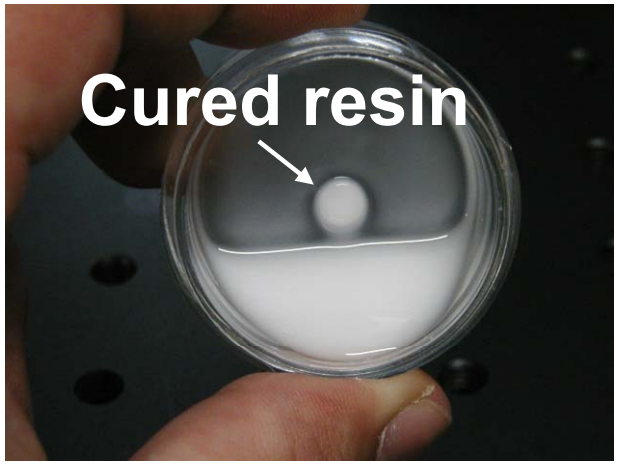




Before cure

Properties of resin

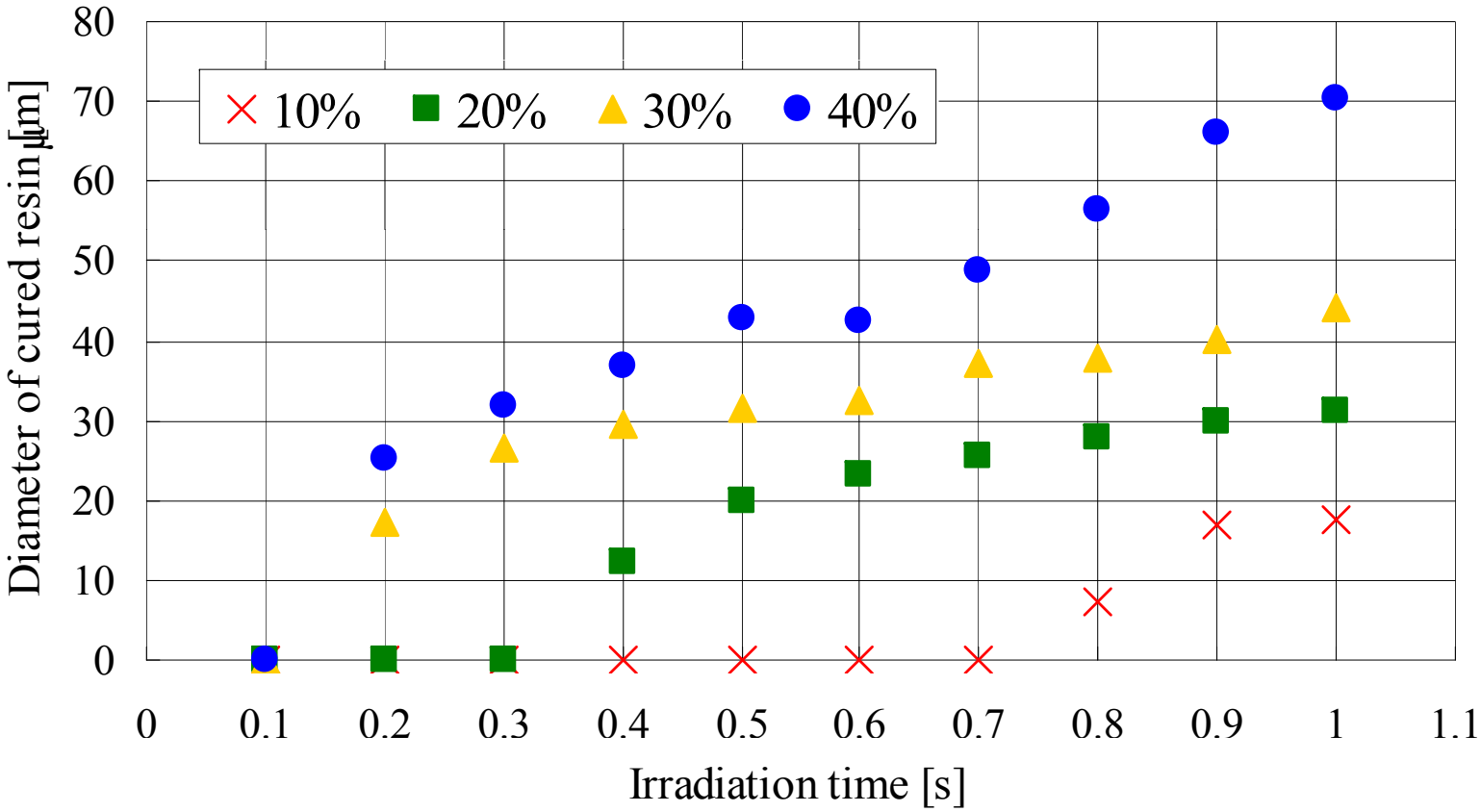
- This resin is cured by near ultraviolet light (l=300-400nm)
- Polymer length: 38nm
- Hydrophilic and hydrophobic mixture
- Harmless to the cell



After cure

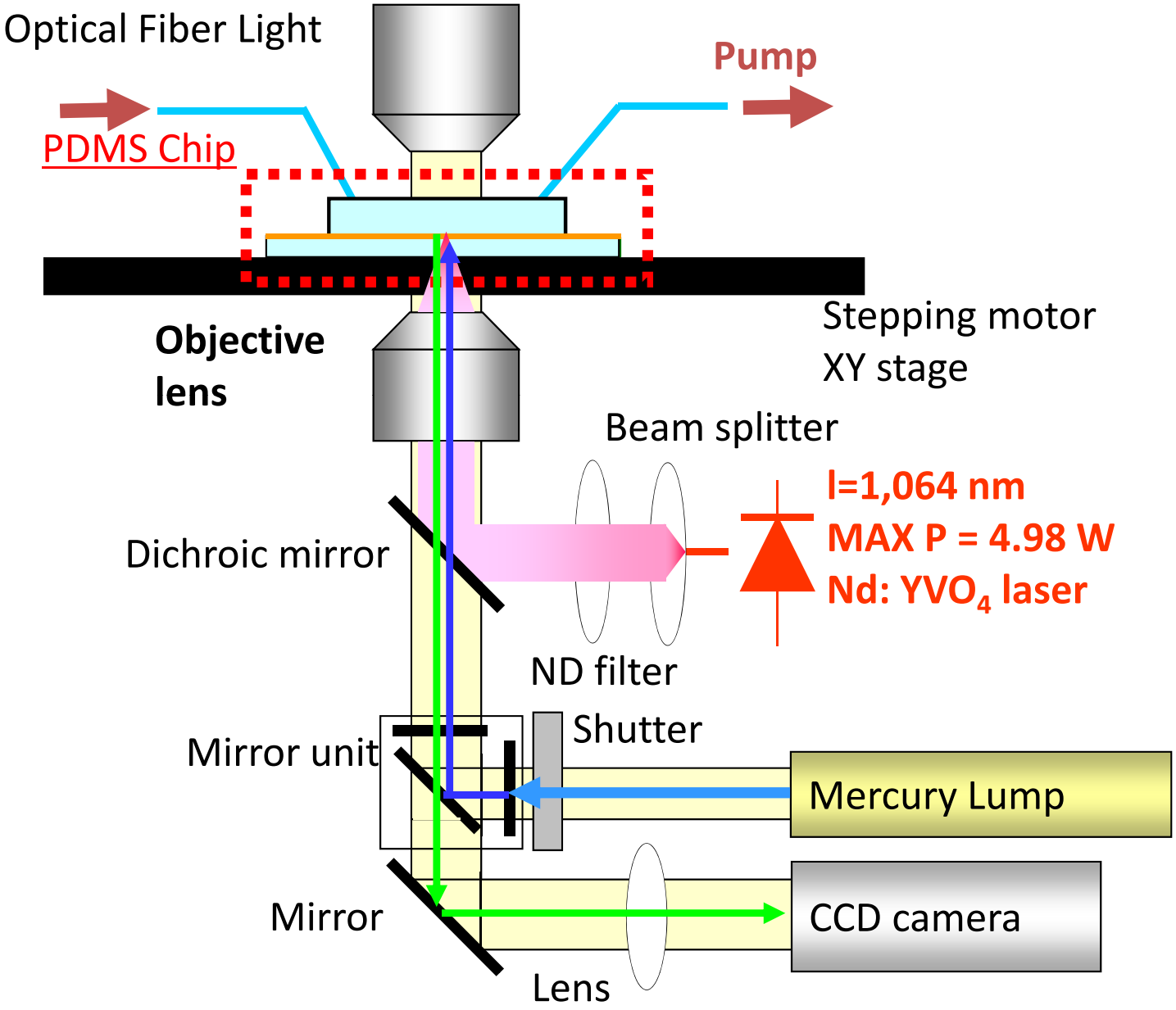
Optical Control of Polymerized Region

Wave range: 330-385nm, Optical power: 30mW, Irradiation range: ϕ30μm

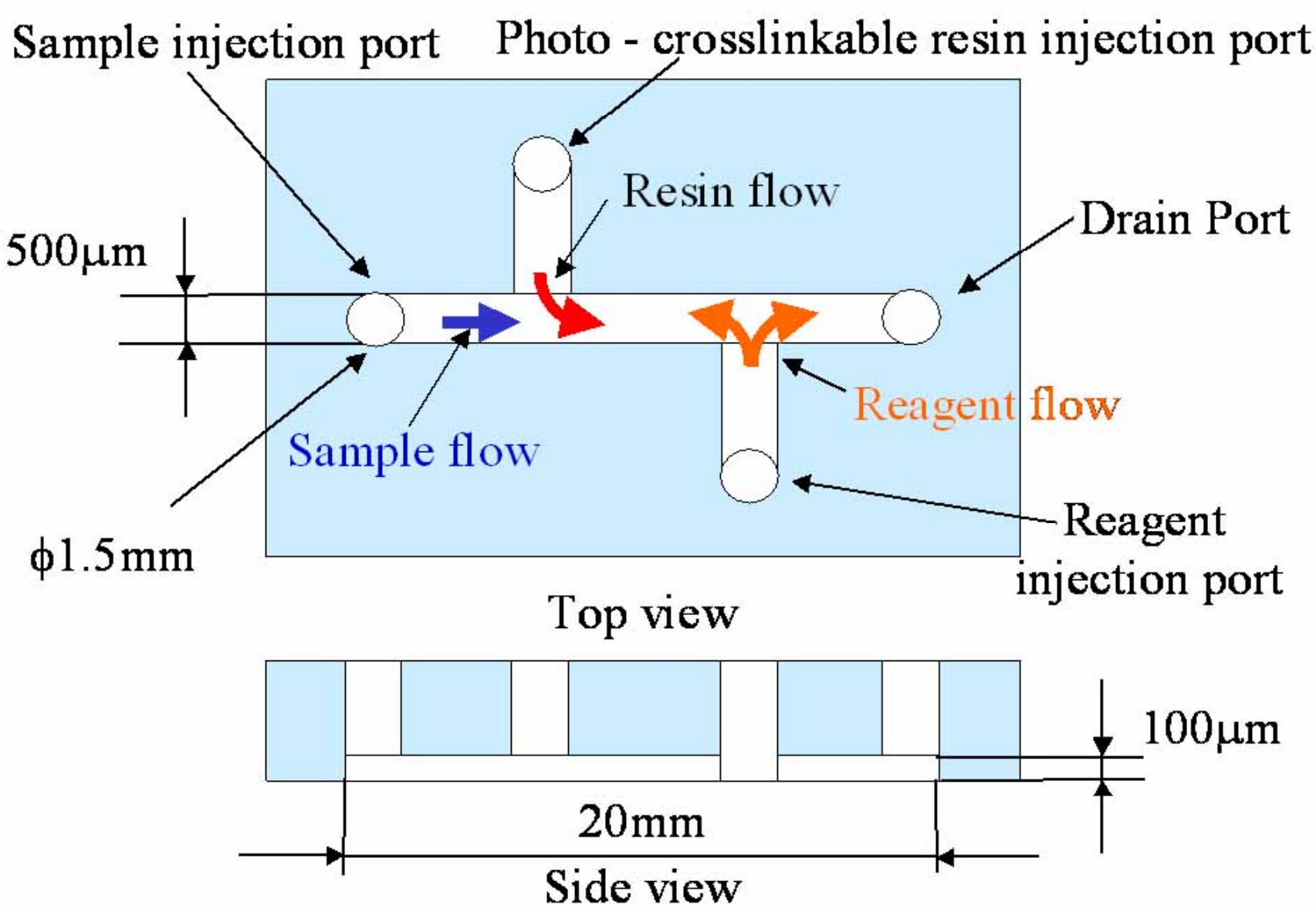


Relation between irradiation time of near UV light and cured range of the resin

Laser Manipulation System



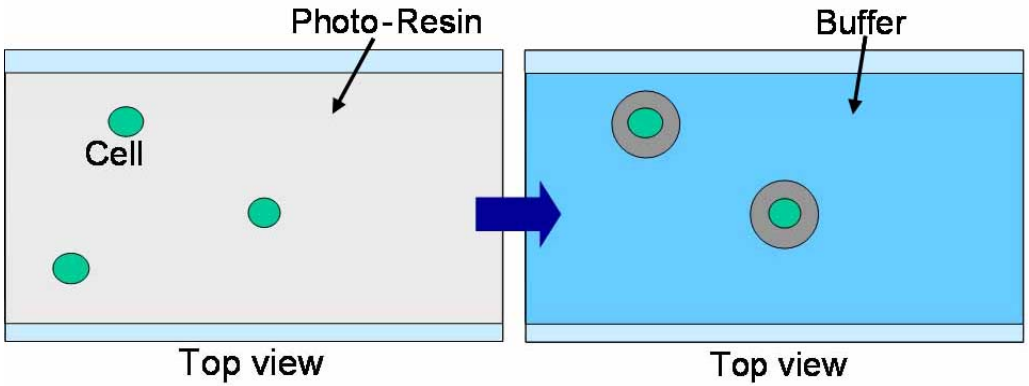
Design of Microfluidic Chip



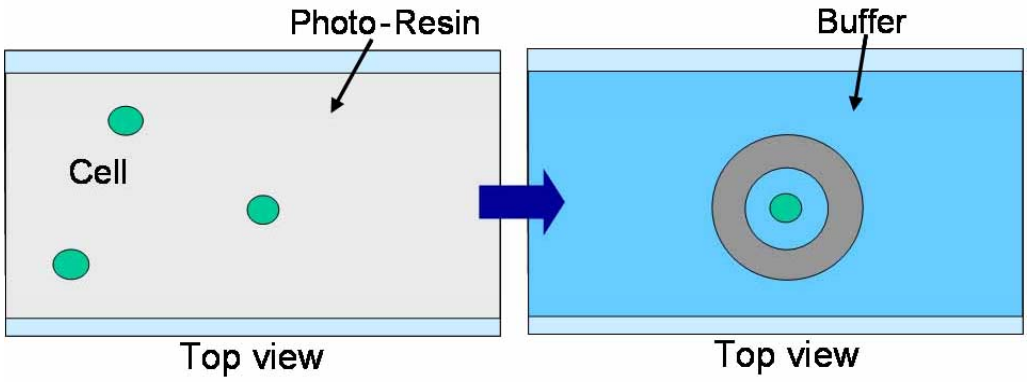
Immobilization Method

Classification of immobilization using photo-resin

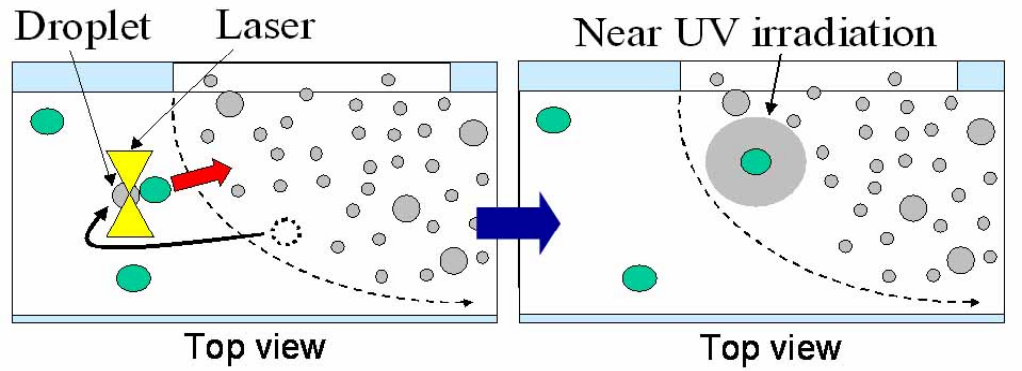
1. Direct immobilization



2. Caging method

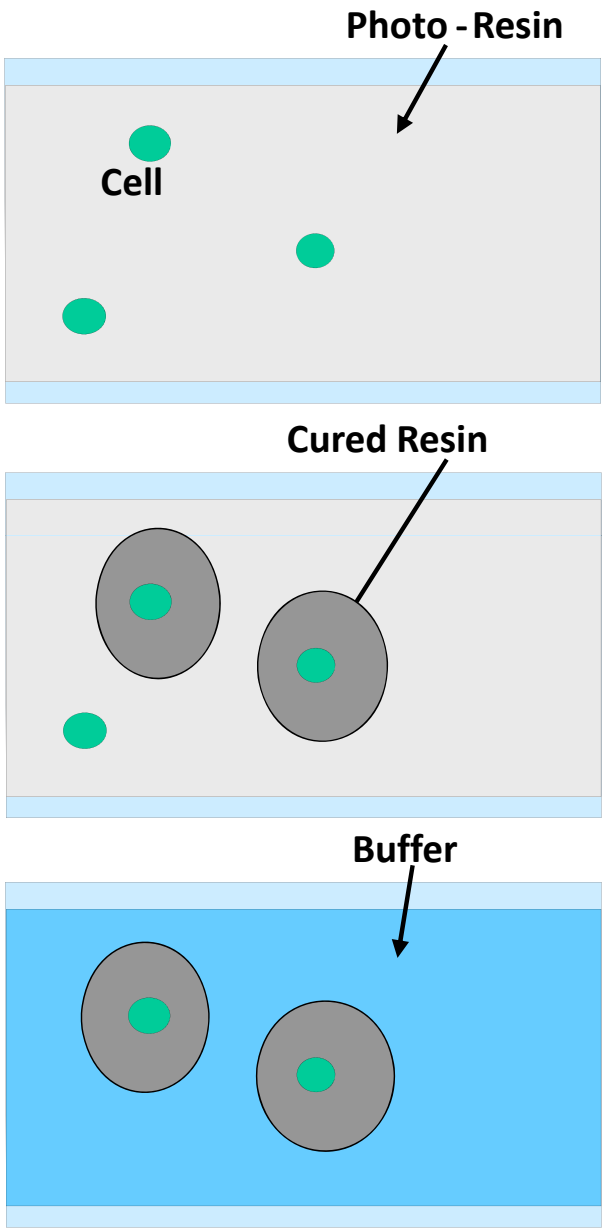


3. Position control method

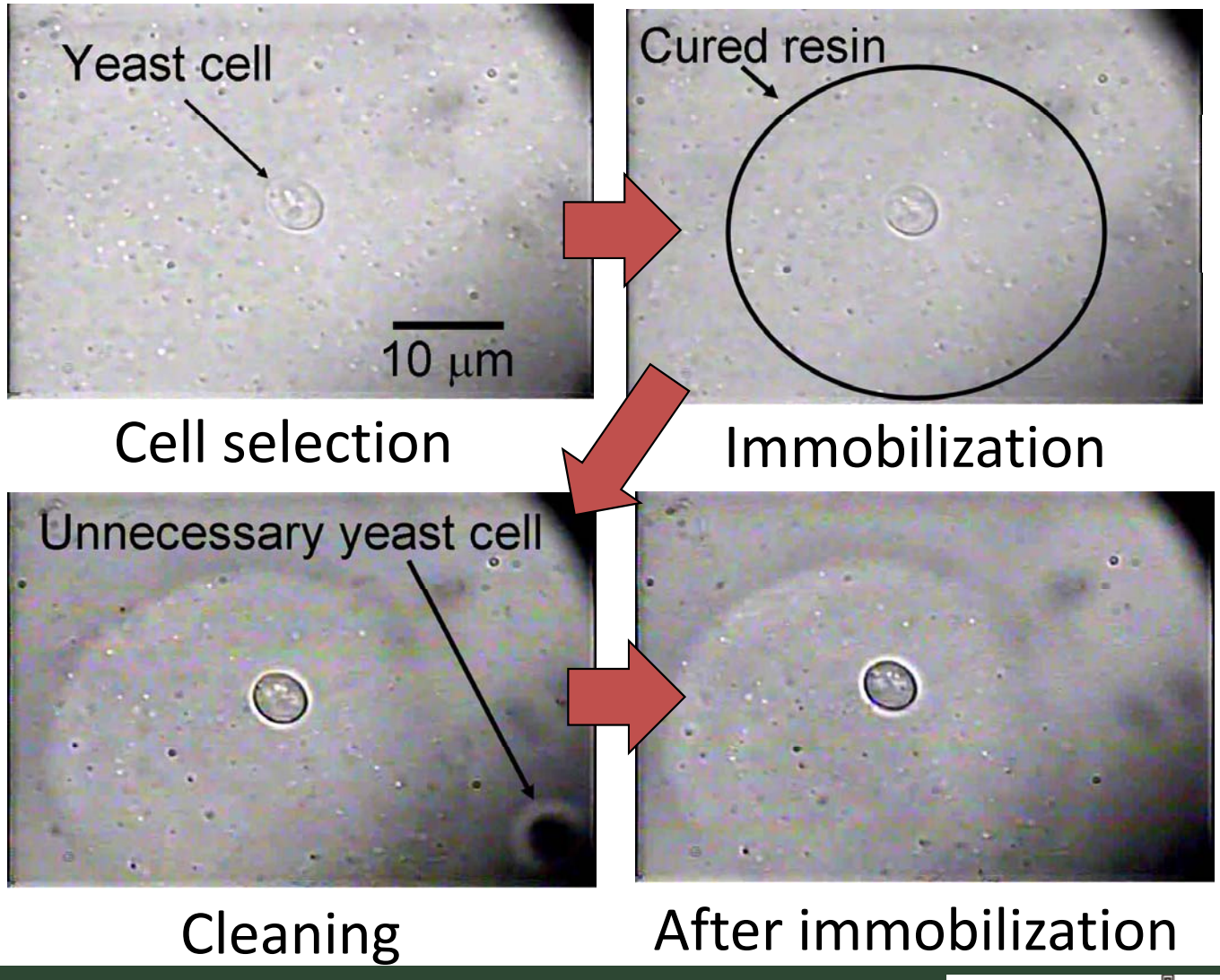


F. Arai, et. al., The Analyst, 130, pp.304-310, 2005.

Direct Immobilization Method



Target: Yeast cell, Resin: Hydrophilic
Resin content: 20%, Light source: Mercury lamp



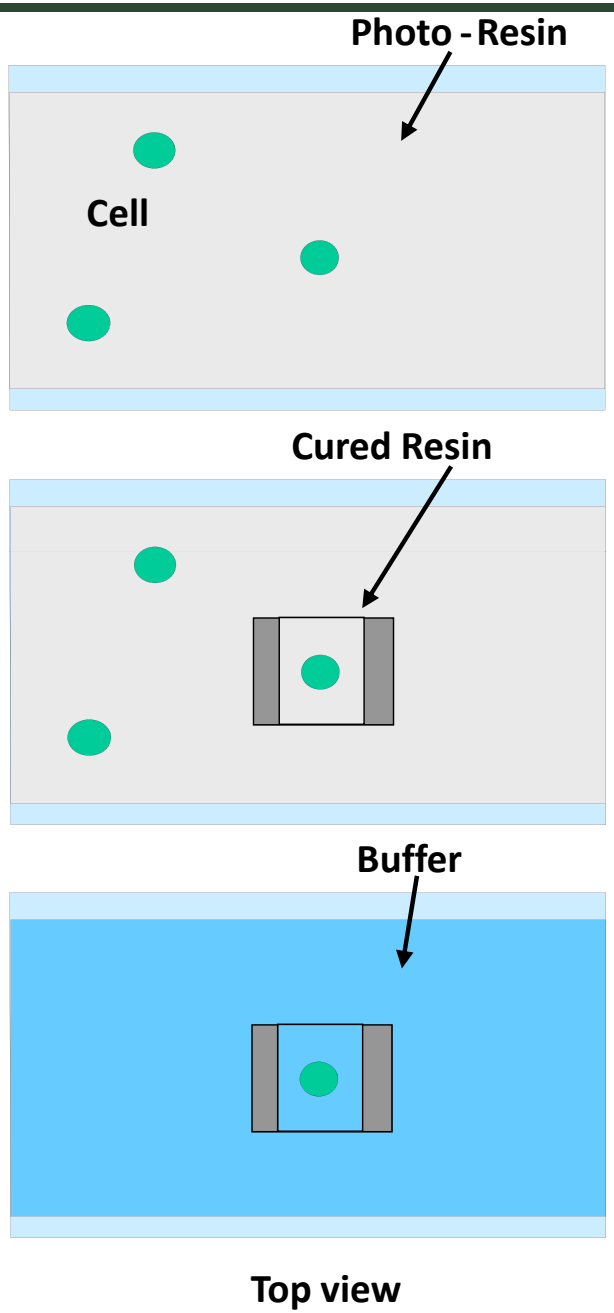
Cell selection

Immobilization

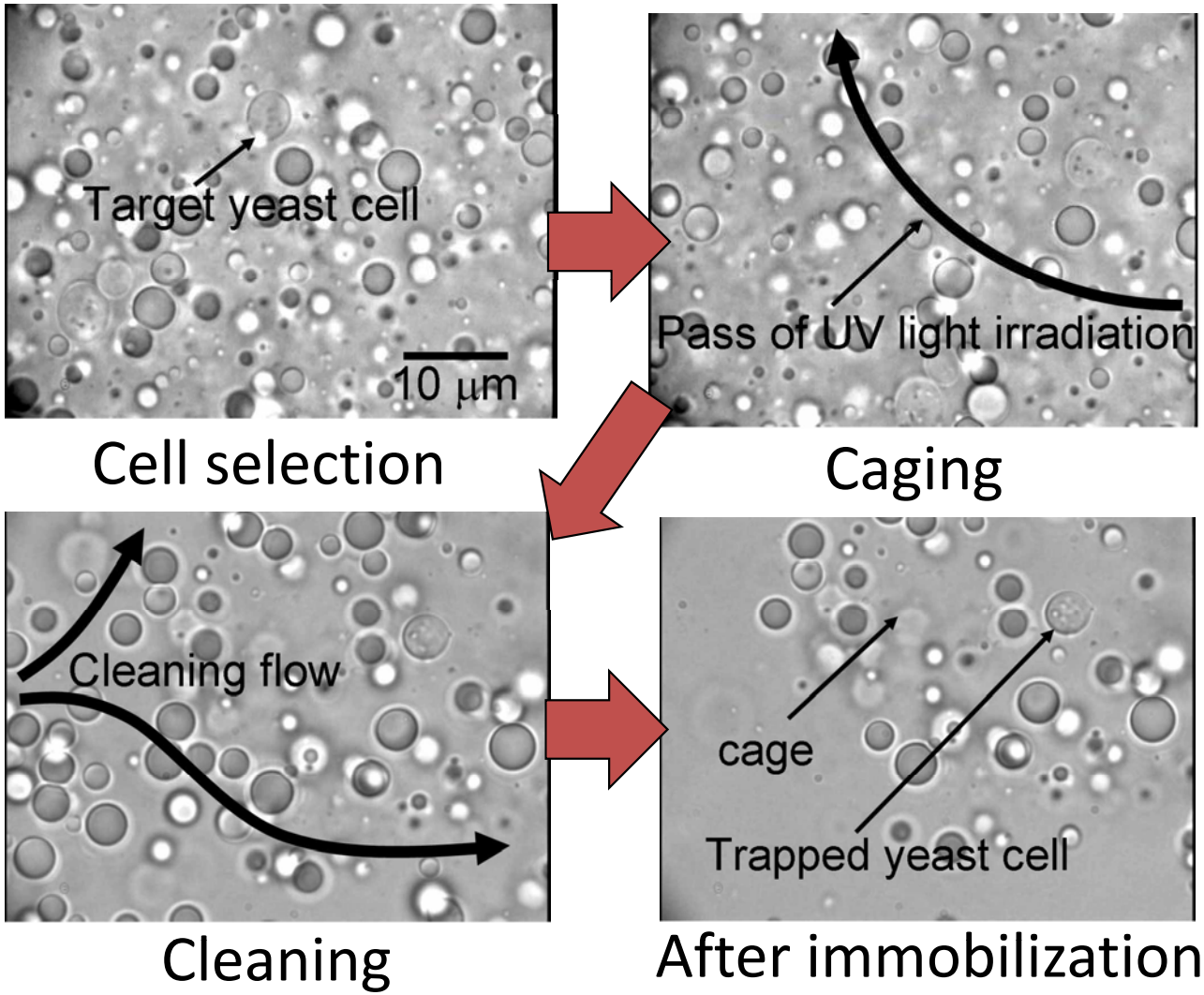
Cleaning

After immobilization

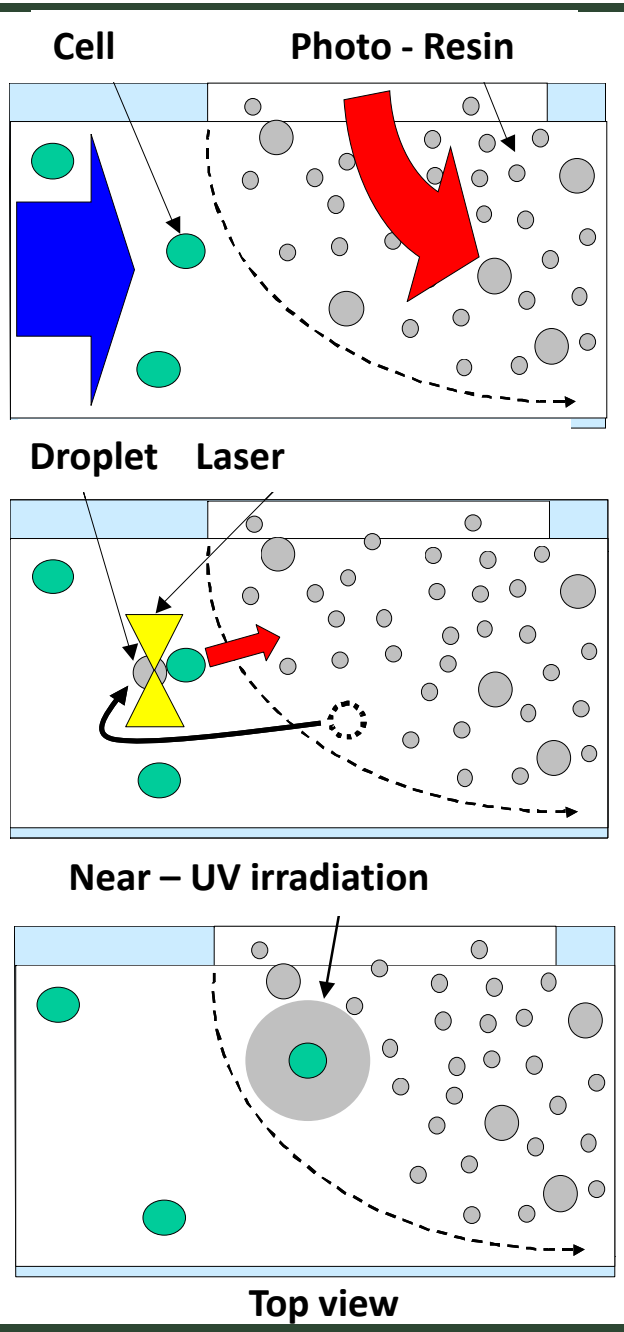
Caging Method



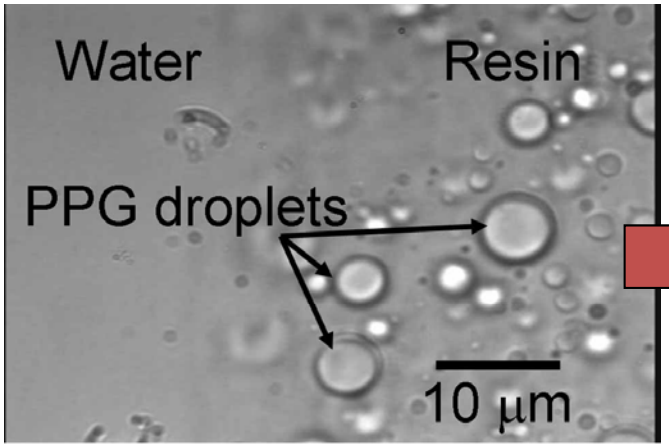
Target: Yeast cell, Resin: Hydrophilic
Resin content: 20%, Light source: Mercury lamp



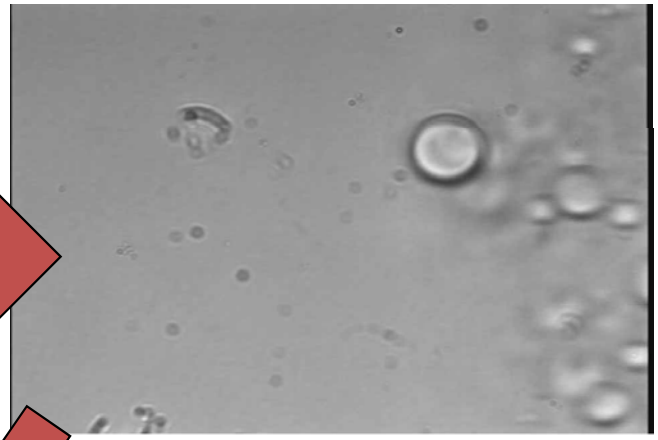
Position Control Method



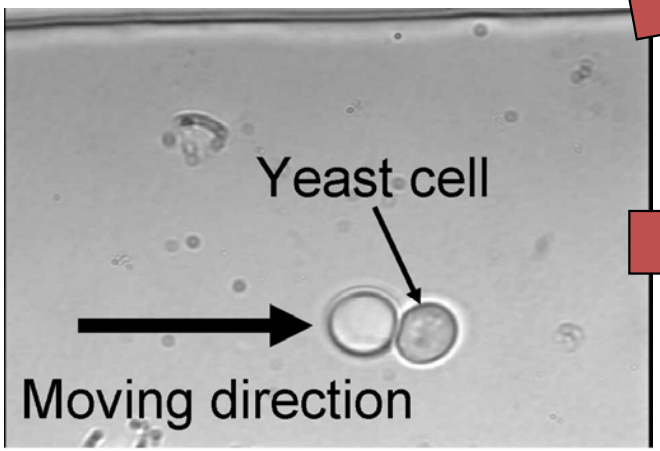
Target: Yeast cell, Resin: Hydrophilic & Hydrophobic
Resin content: 20%, Light source: Mercury lamp



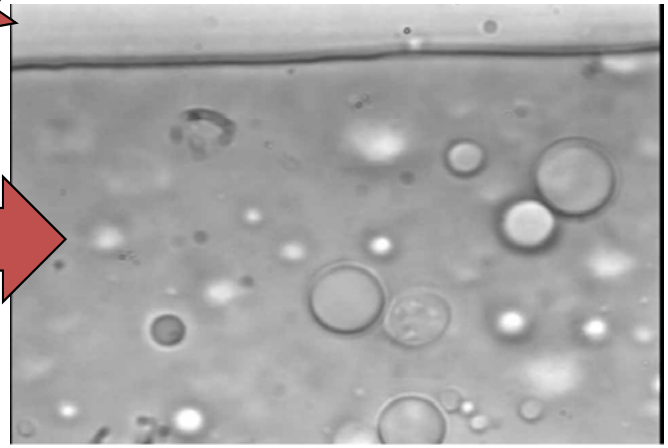
Droplet selection



Trap of droplet



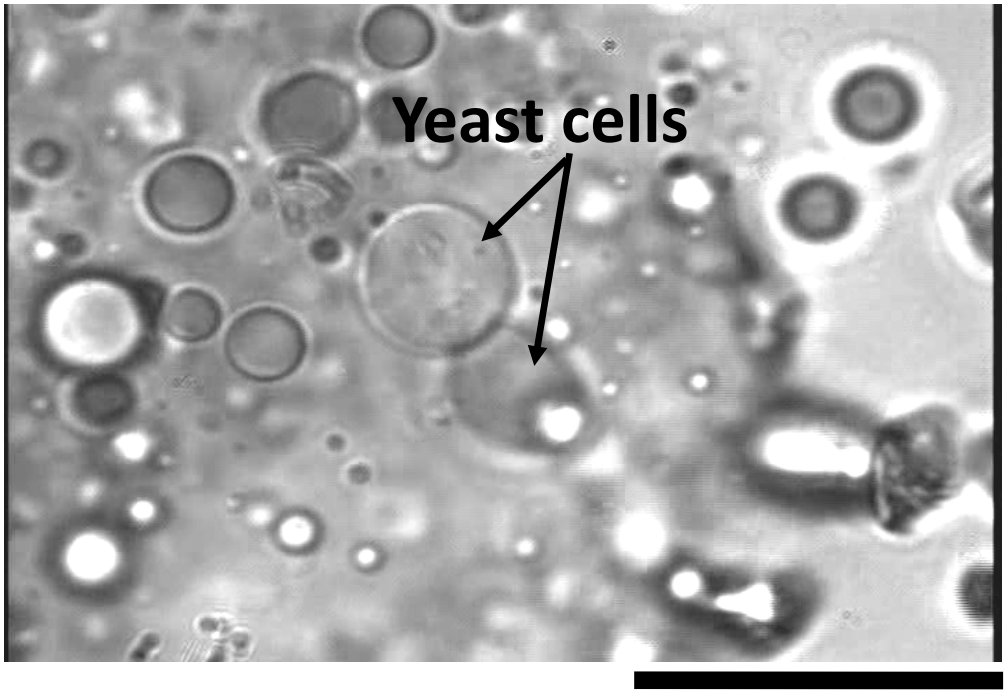
Cell transport



After immobilization

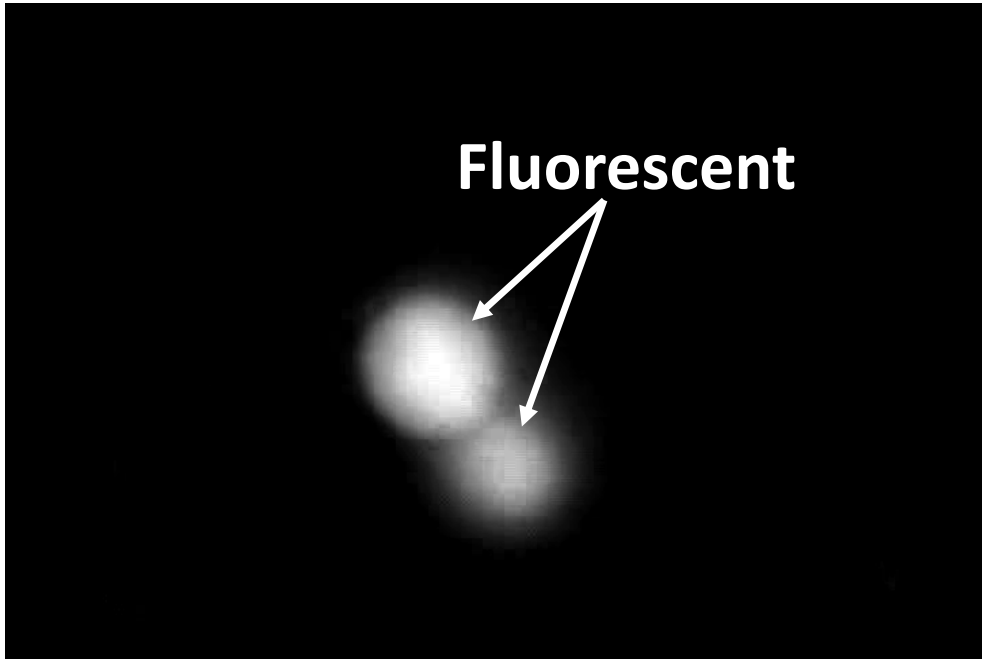
Viability Test of Immobilized Cell

Fluorescence reagent: Calcein – AM,
Density of reagent: 1mg/1ml,
Dyeing time: 30 minutes



10μm

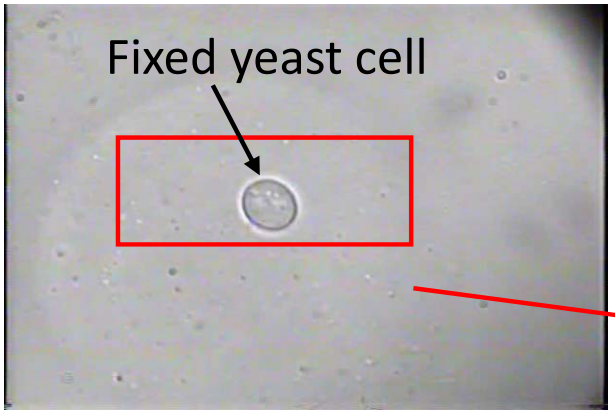
Before dyeing

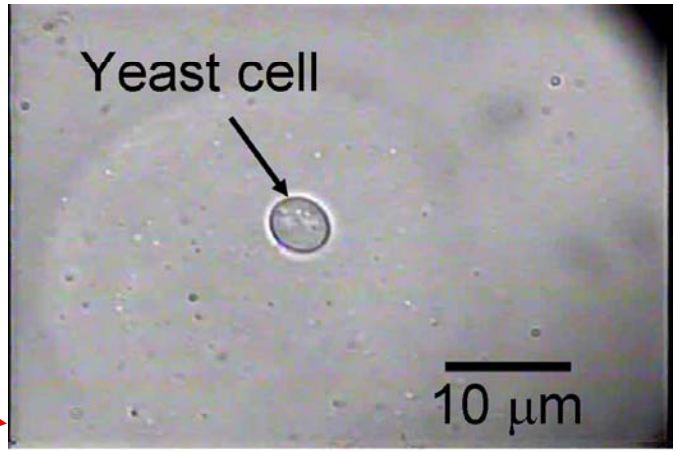


After dyeing

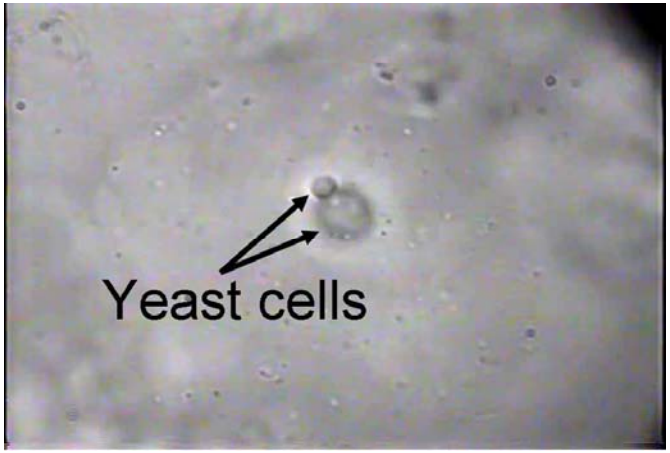
Immobilized cell was alive.

Real-time monitoring of cell culture





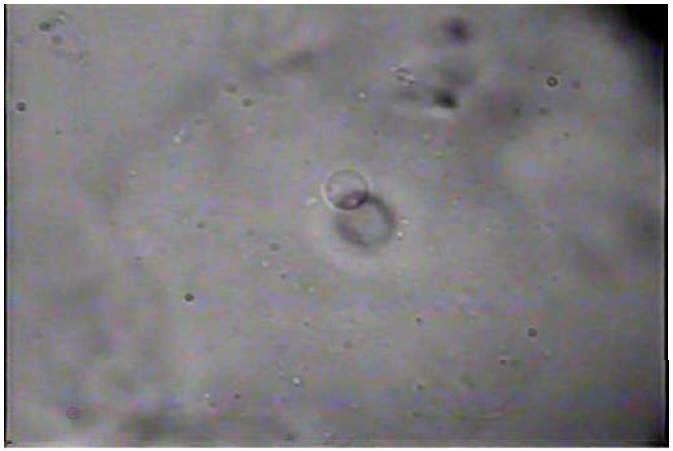
0 hour



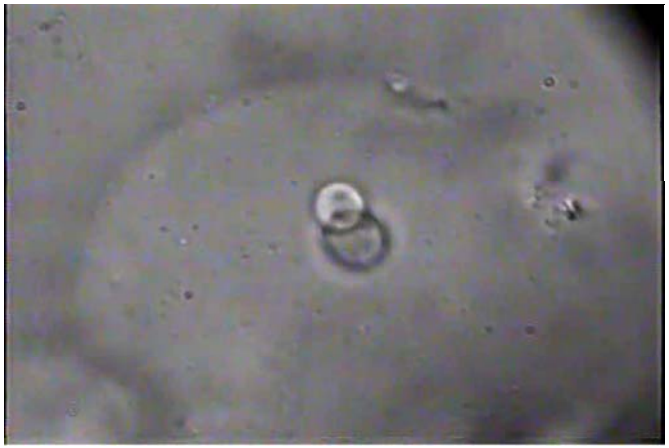
5 hours 20 min

Conditions

Culture medium :
YPDBROTH(5%)
pH : 6.5
Oxygen : 4.2ml/l
Temperarture : 30°C

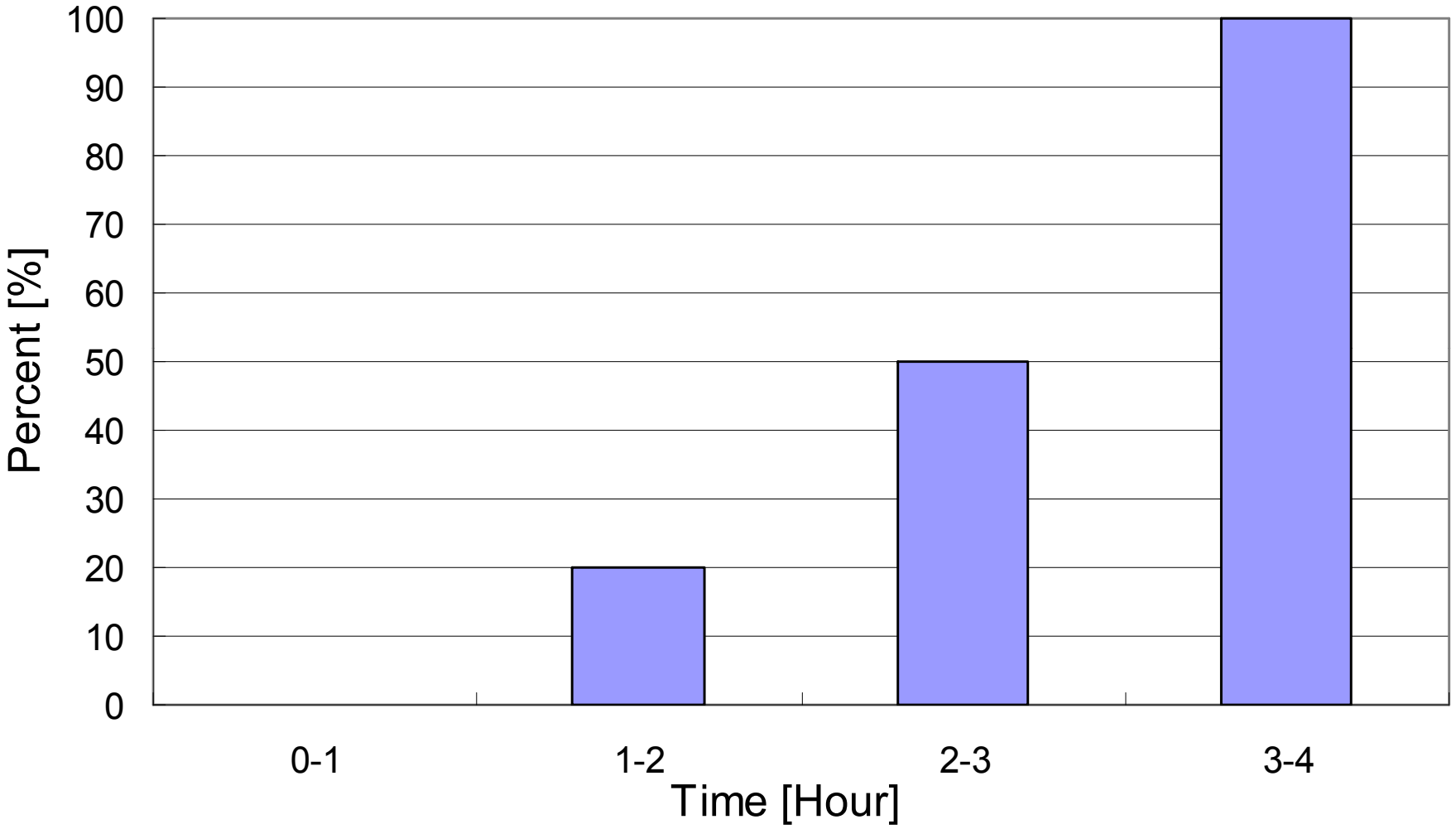


7 hours 20 min



8 hours

Culture Result



Relationship between Culture time and proliferated cell

Summary

Single cell immobilization and direct monitoring of cell activity was succeeded.

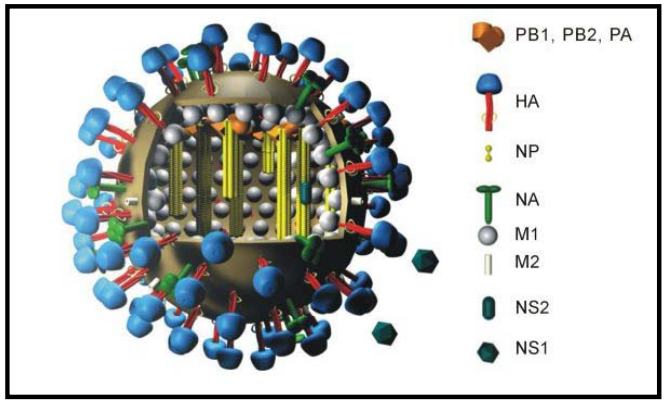
1. We confirmed the effectiveness the fixation method using locally injected photo-crosslinkable resin

2. We controlled cell position by optical tweezers

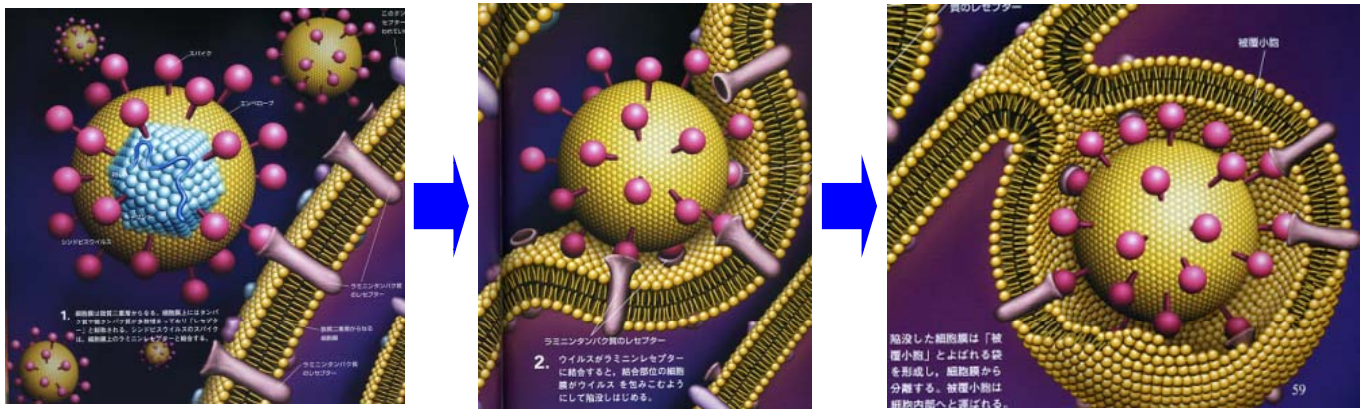
3. We observed the fluorescent dyeing and culture of fixed yeast cell

Contents

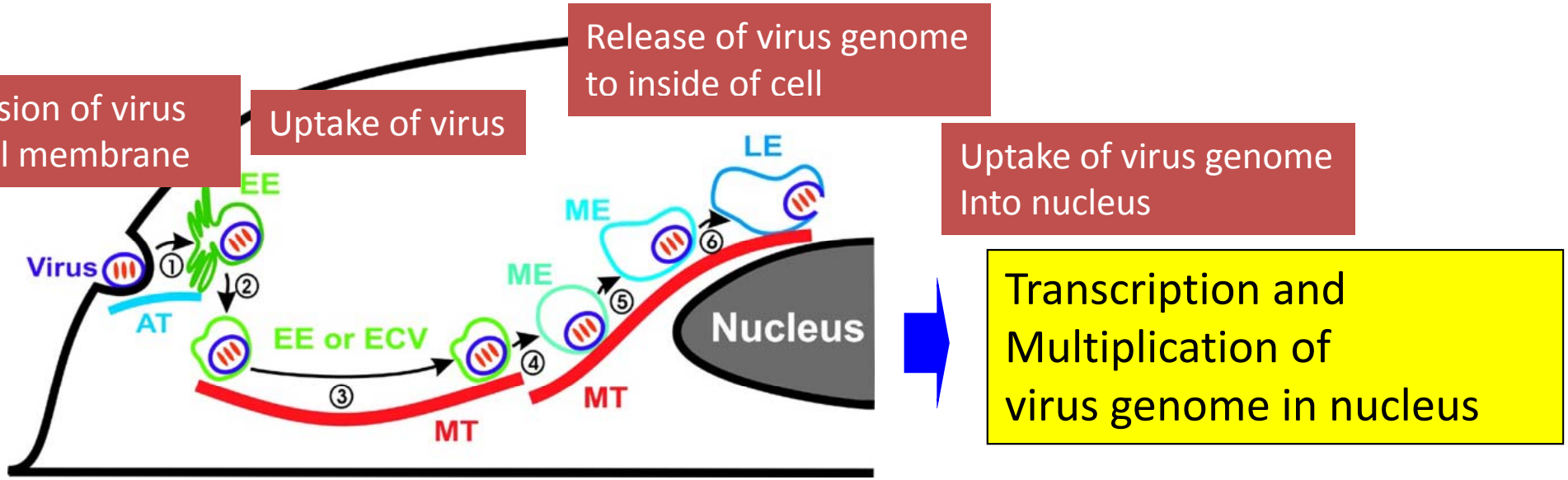
Infection Mechanism of Influenza Virus



Structure of virus particle



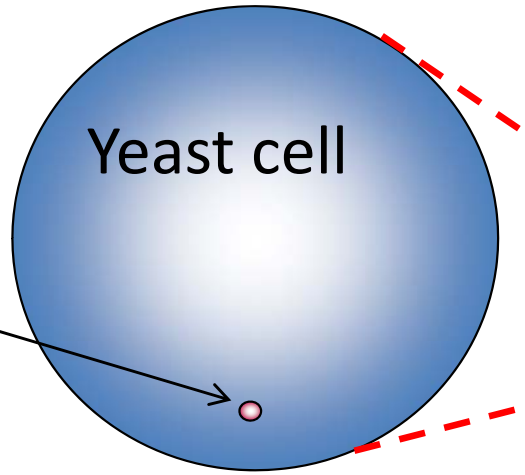
Mechanism of virus infection



Transcription and Multiplication of virus genome in nucleus

Infection mechanism of virus was not well investigated.

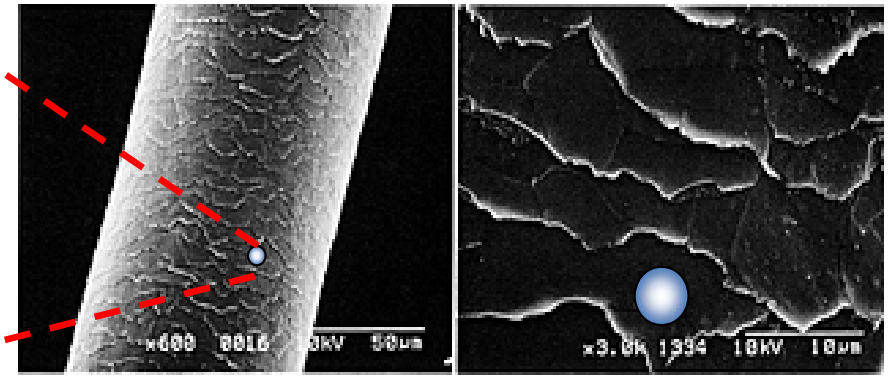
Nanomanipulation in a microfluidic chip



Influenza virus

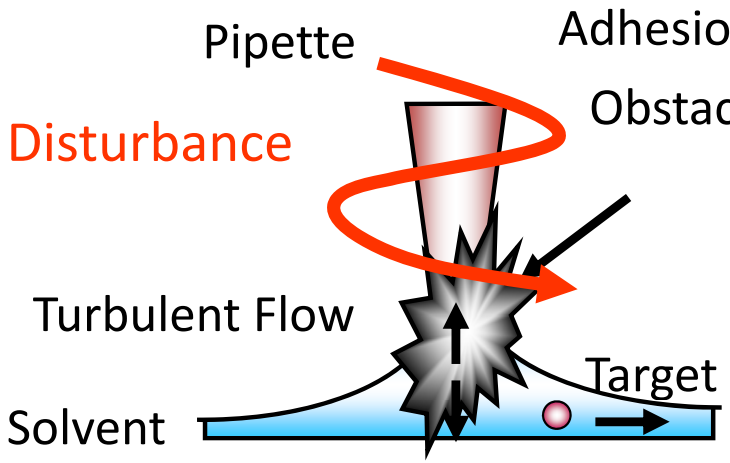
about 100 nmf

about 5 μmϕ

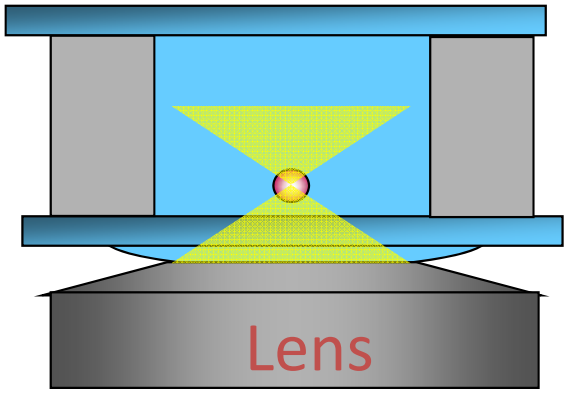


Human hair

Dish: Opened space → Microfluidic chip: Closed space

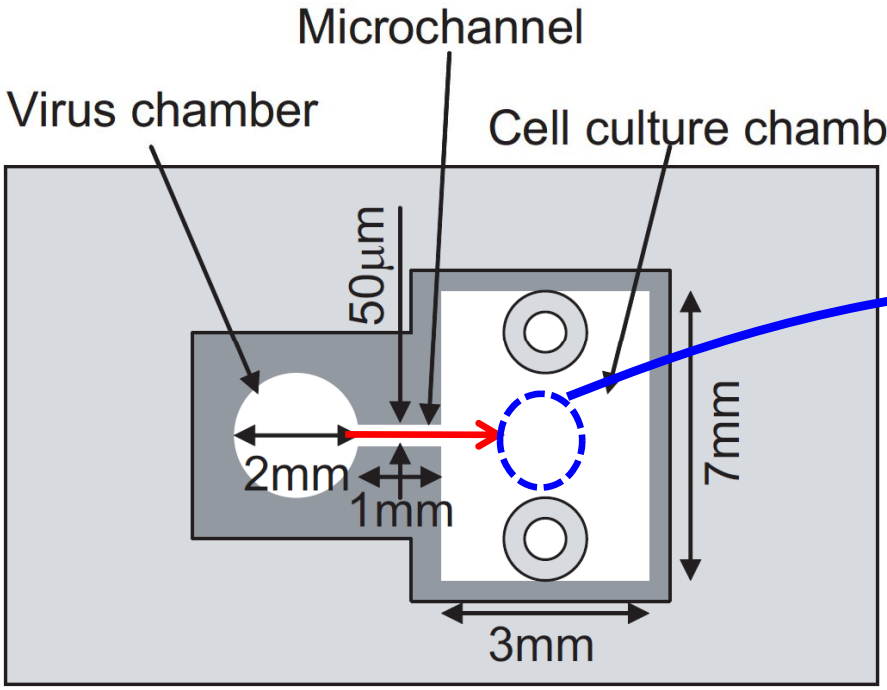


- Positioning difficulty
- Slow operation
- Contamination



Non-contact manipulation

Previous single virus manipulation

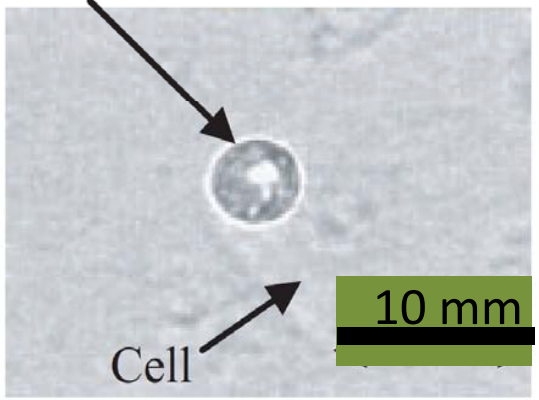


Microchip(top view)

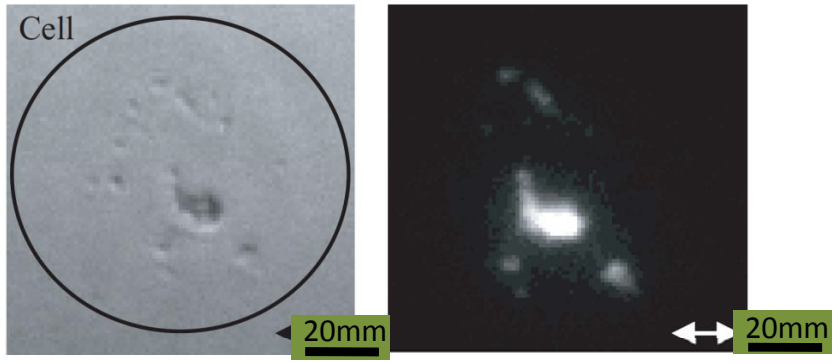
Transferring and infecting virus

Infection

Gel microtool (PNIPAAm)



Gel microtool including a virus



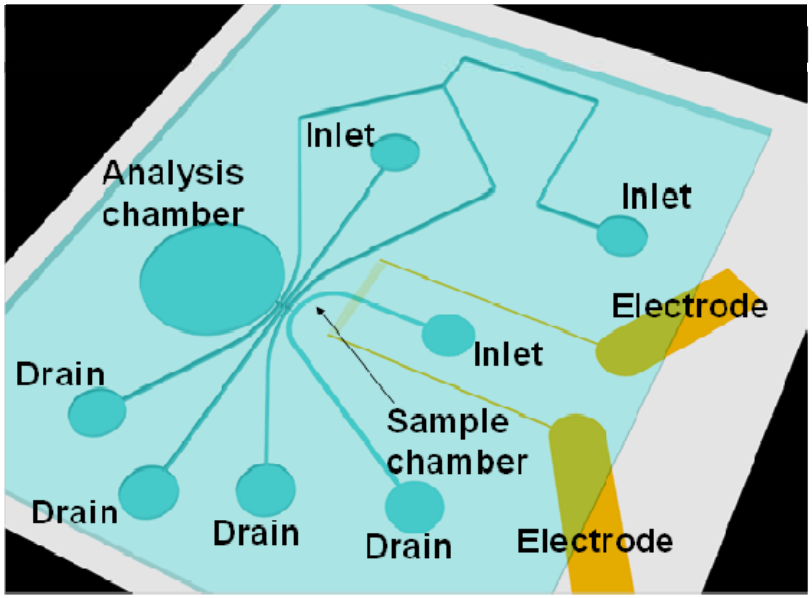
Replicated viruses in the infected cell

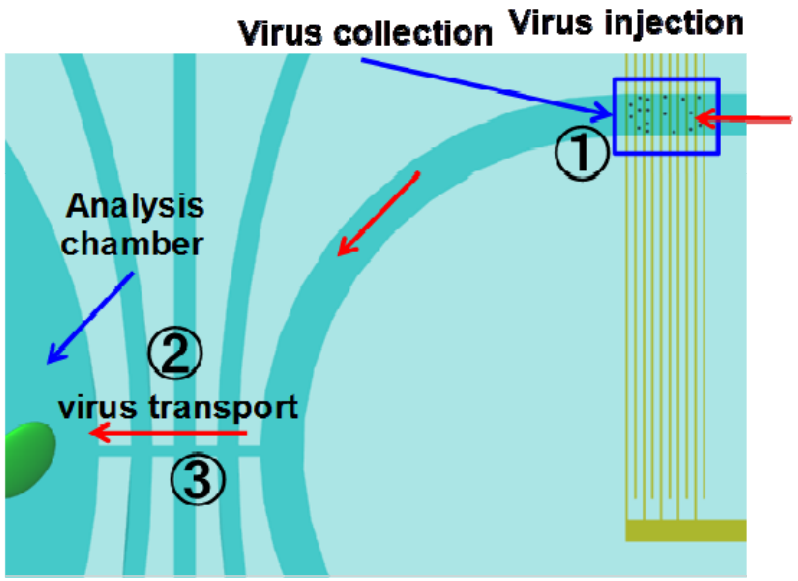
Problems : Interfusion of unnecessary virus
Difficulty of finding single influenza virus

Ichikawa, Honda, Arai et al, 2007 JRM, 19, pp. 569-576, 2007.

Issues for single virus infection system

1. To find a target virus on a chip
2. To manipulate a target virus to a specific cell
3. To avoid extra virus incursion to analysis chamber





①Concentration of viruses by dielectrophoretic (DEP) force

②Transport of single virus to a cell by optical tweezers

③Separation of chambers by *in-situ* photopolymerization

H. Maruyama, et. al., Microfluidics and nanofluidics, 2010 (im press).

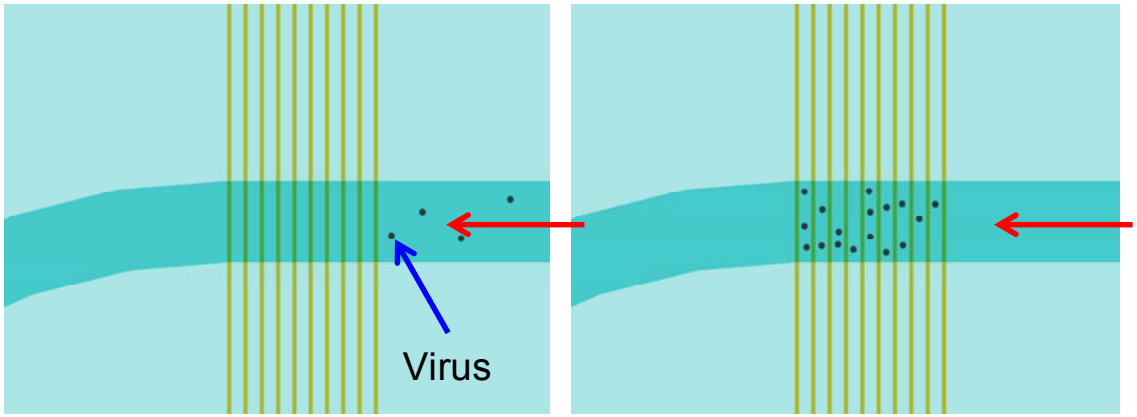
Virus concentration by DEP force

Finding virus is very difficult

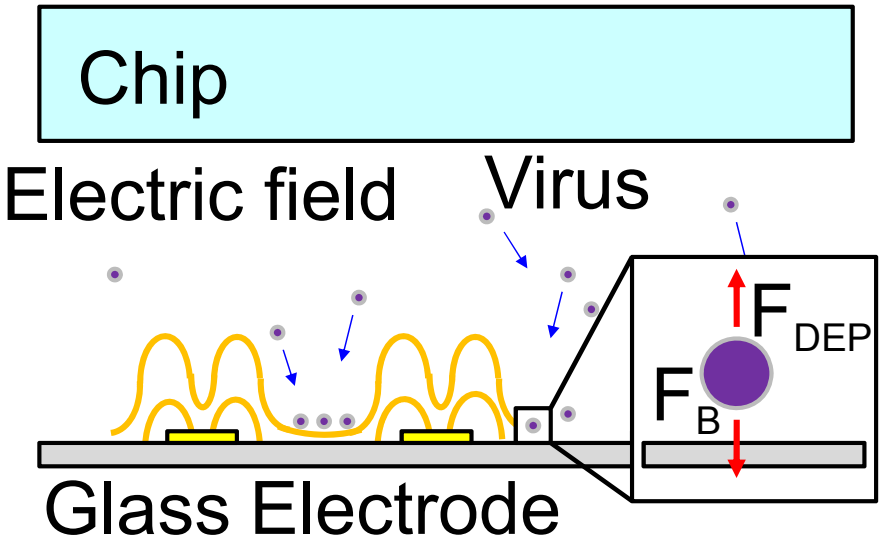
1×10^6 virus/ml

1 virus per 500 mm x 500 mm

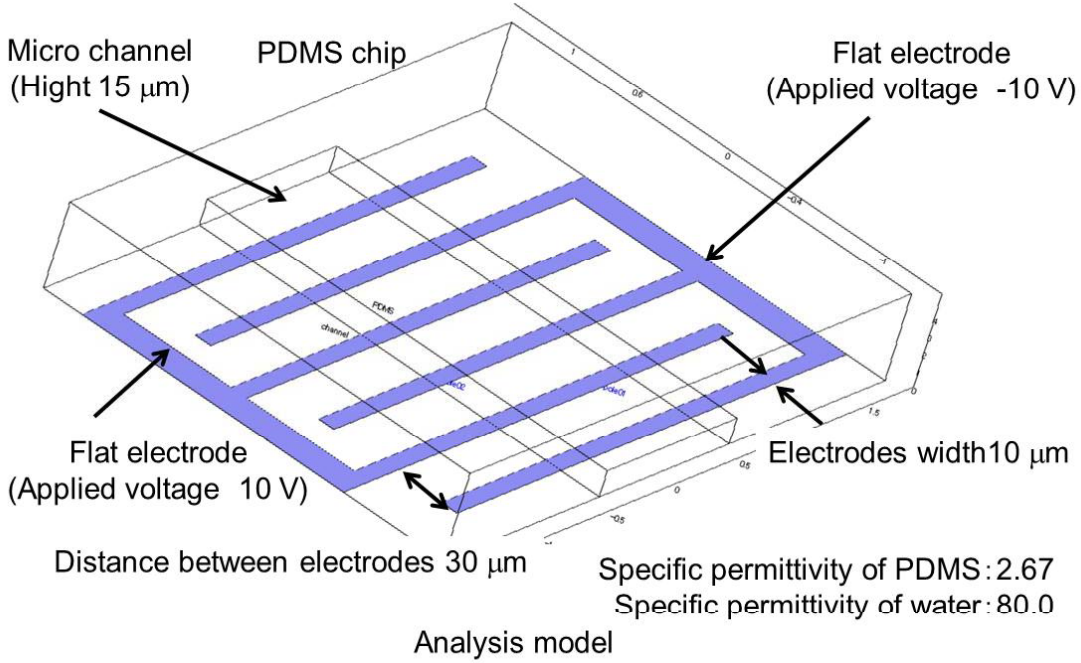
(Chamber height: 15 mm)



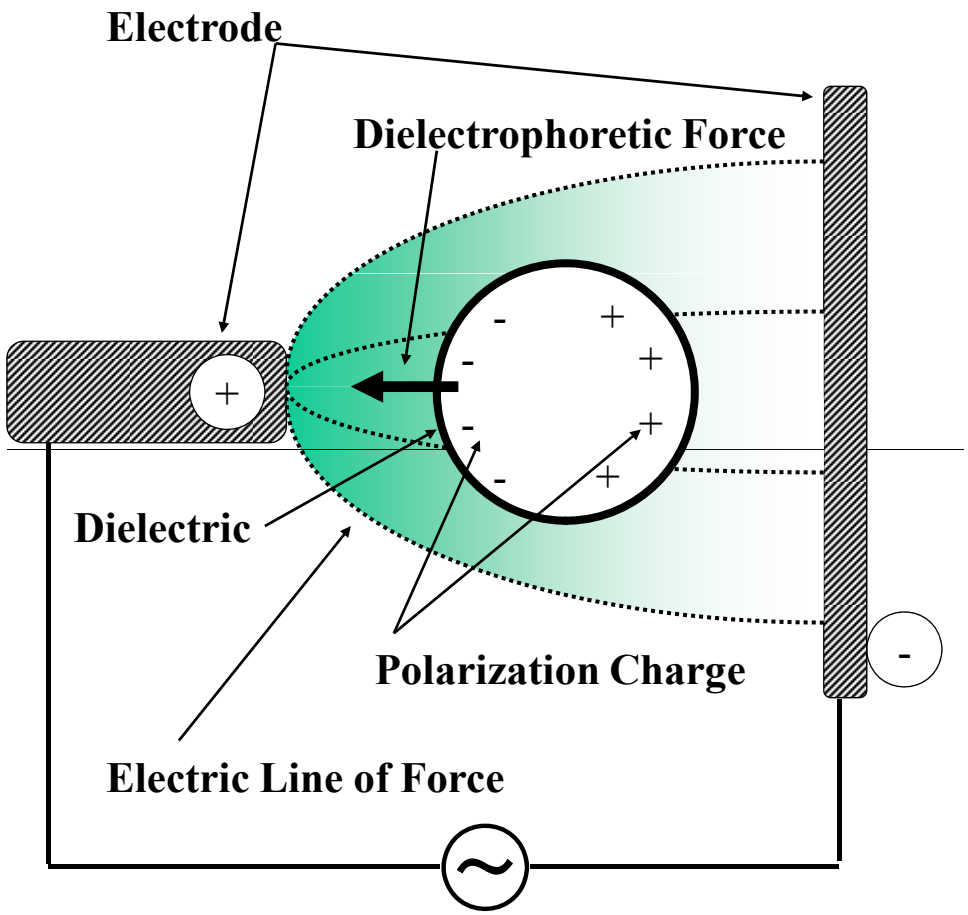
Concept of DEP concentration



Analytical model



Principle of dielectrophoresis



$$\vec{F}_{DEP} = 2\pi\varepsilon_m r^3 \mathrm{Re}\left[\frac{\varepsilon'_p - \varepsilon'_m}{\varepsilon'_p + 2\varepsilon'_m}\right]\nabla\left|\vec{E}\right|^2$$

$$\varepsilon'_p = \varepsilon_p - \frac{\sigma_p}{\omega}j \qquad \varepsilon'_m = \varepsilon_m - \frac{\sigma_m}{\omega}j$$

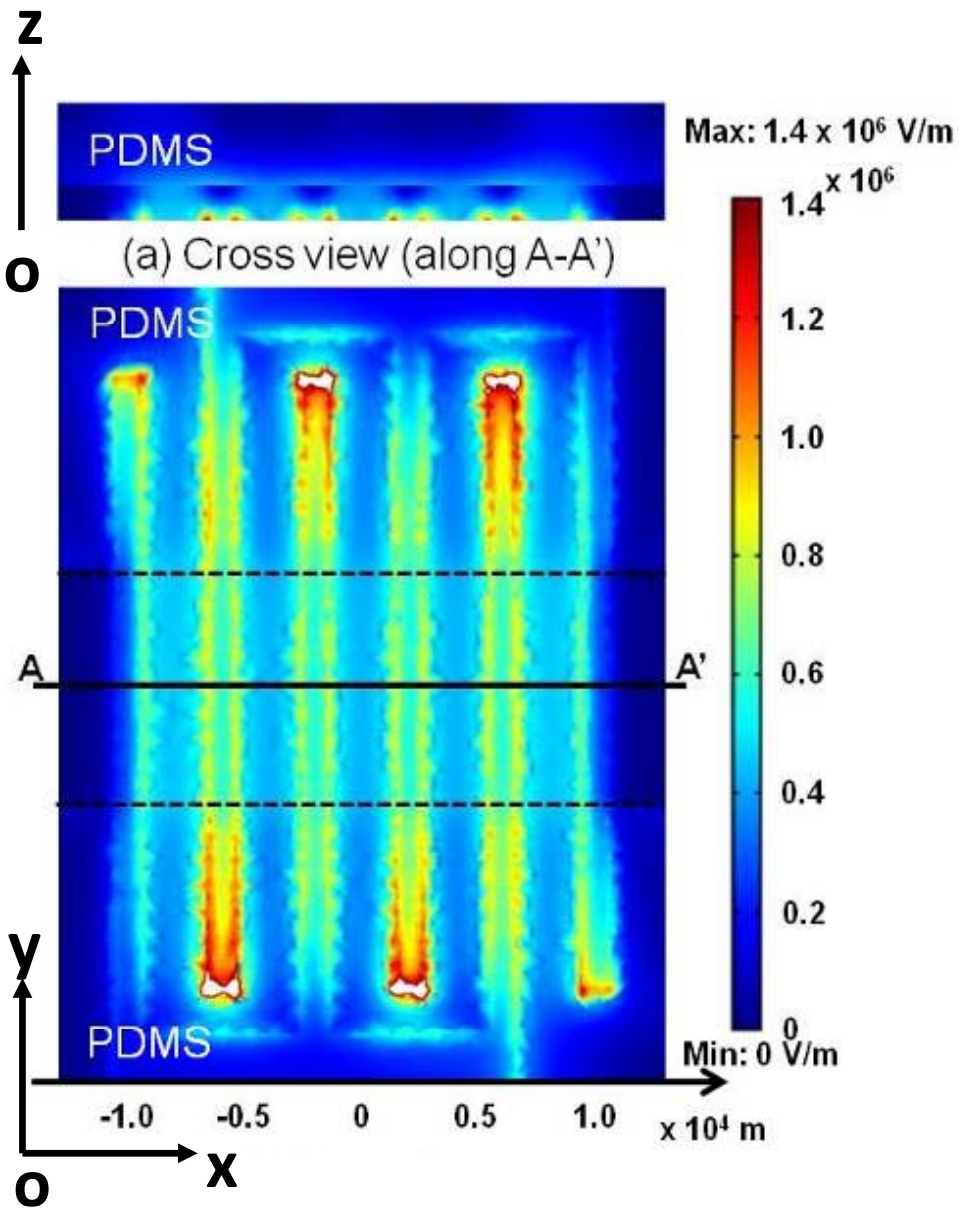
r : Radius of the particle
$\vec{E}$: Outside electric field
ω : Impressed frequency

$\varepsilon_m, \varepsilon_p$: Permittivity of solution and particle

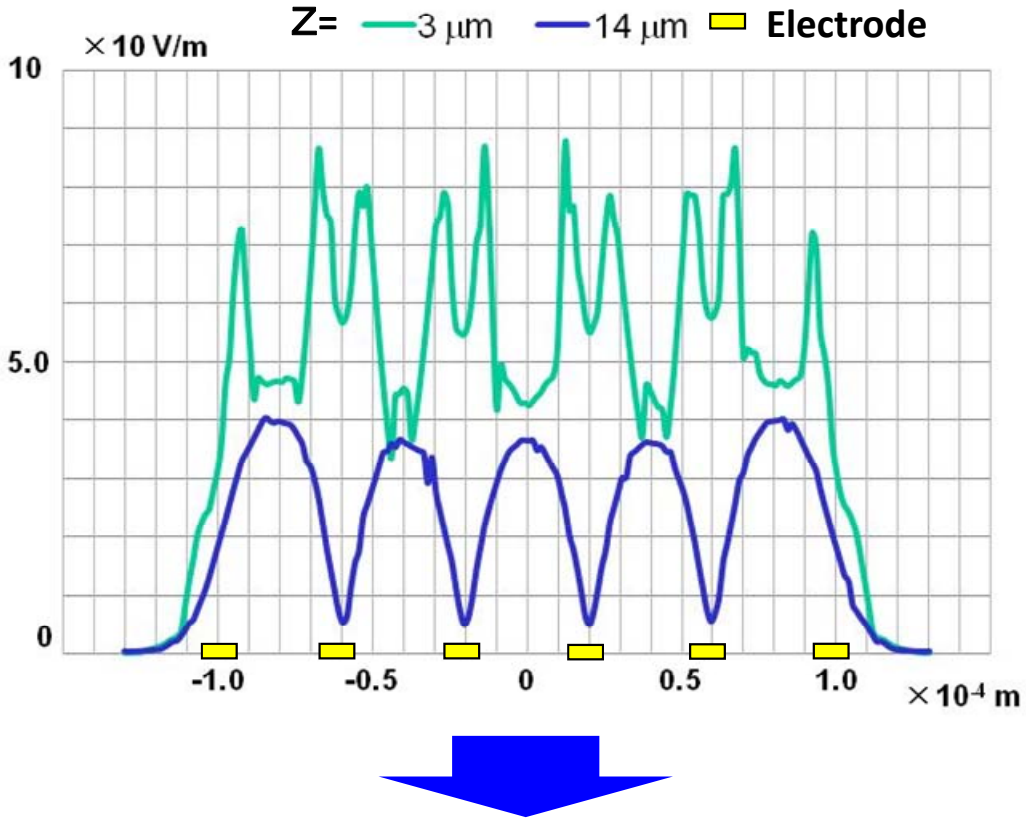
σ_m, σ_p : Conductivity of solution and particle

Virus was trapped at…
high electric field gradient part (Positive dielectrophoresis)
low electric field gradient part (Negative dielectrophoresis)

Analytical results

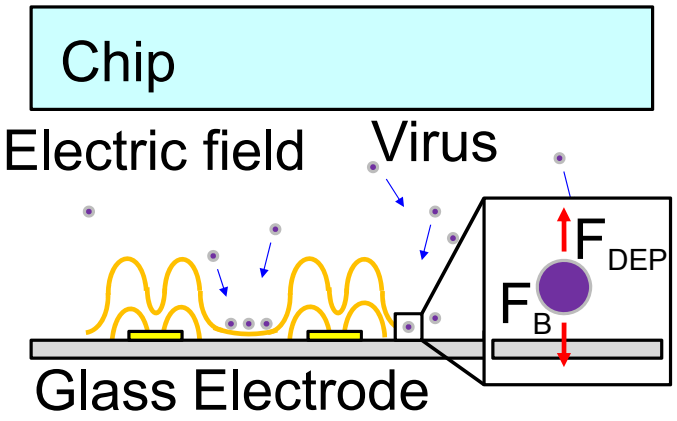


Distribution of electrical field (z = 3 mm)



Virus was trapped between electrodes by negative DEP force (frequency: 3 MHz)

Analysis of virus motion in vertical direction



Assumptions:

Diameter of virus:100 [nm], Permitivity of virus:2 [F/m],
Density of virus:1 [g/cm^3], Conductivity of virus:10^{-2}[S/m]

a=100 × $^{10-9}$ [m], r=1.0 [g/cm^3], R=8.31447[JK^{-1}mol^{-1}],
h=1.004 × $^{10-6}$[m^2/s], N_A=6.02 × $^{10-9}$, e_m=81 [F/m], e_p=2 [F/m],
s_m=81 [F/m], s_p=2 [F/m],

DEP force(upper direction: 50 nm upper from suvstratte)

$$F_{DEP} = 2\pi\varepsilon_m r^3 \,\mathrm{Re}\left[\frac{\varepsilon'_p - \varepsilon'_m}{\varepsilon'_p + 2\varepsilon'_m}\right]\nabla|E|^2 \qquad \varepsilon'_p = \varepsilon_p - \frac{\sigma_p}{\omega}j \quad \varepsilon'_m = \varepsilon_m - \frac{\sigma_m}{\omega}j$$

$$Min(F_{DEP}) = 6.69\times10^{-6}[N] > F_B$$

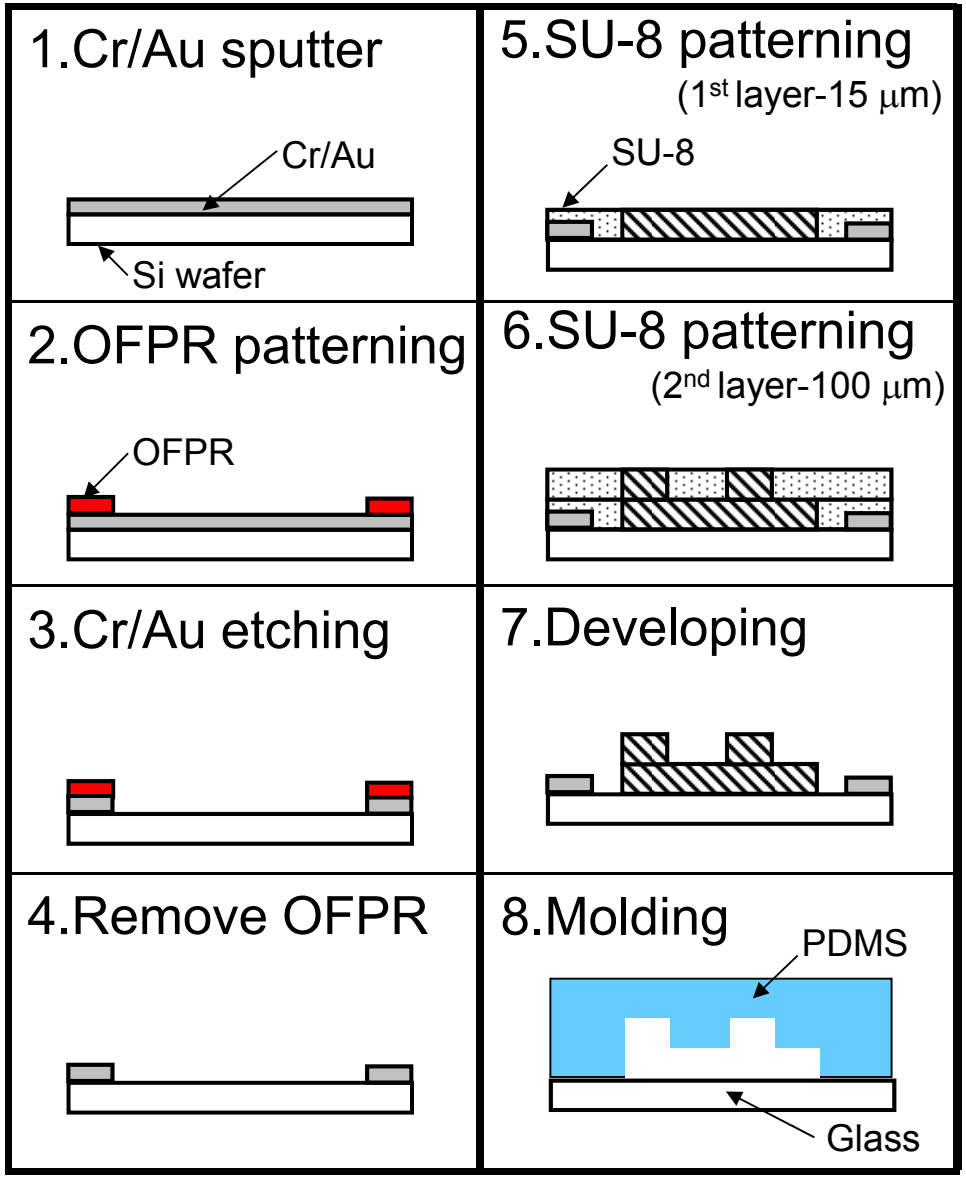
Force induced by Brownian motion (lower direction)

$$F_B = m_v \times < \ddot{x} > = \frac{4}{3}\rho\pi a^3 \times \sqrt{\frac{RT}{3\pi\eta a N_A}} = 1.54\times10^{-26}[N]$$

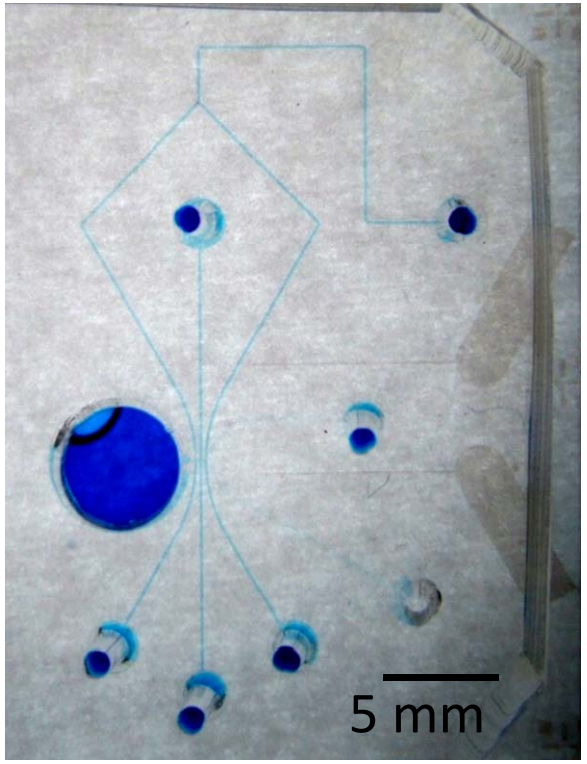
Virus does not adhere to glass surface by DEP floating.

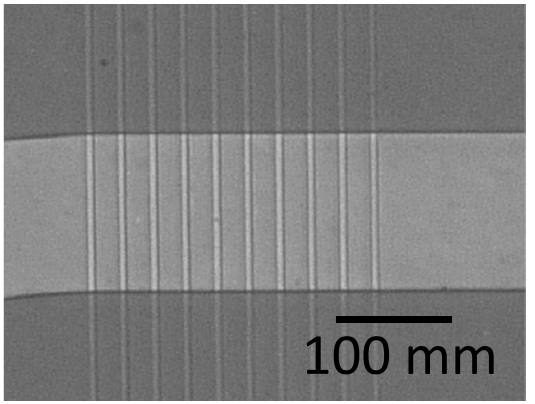
Microfluidic chip for single virus infection

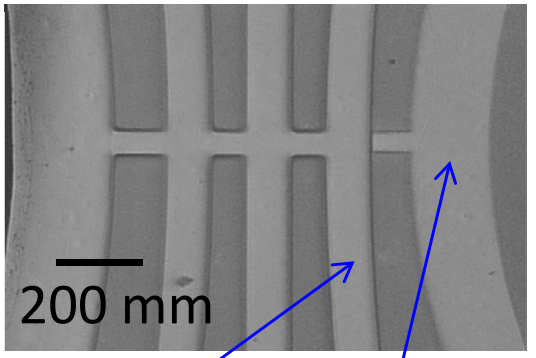
Fabrication process

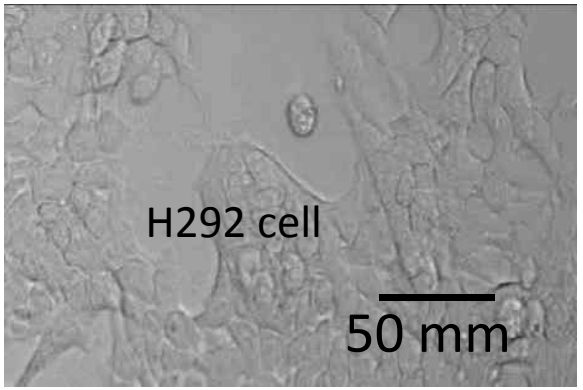


Photographs





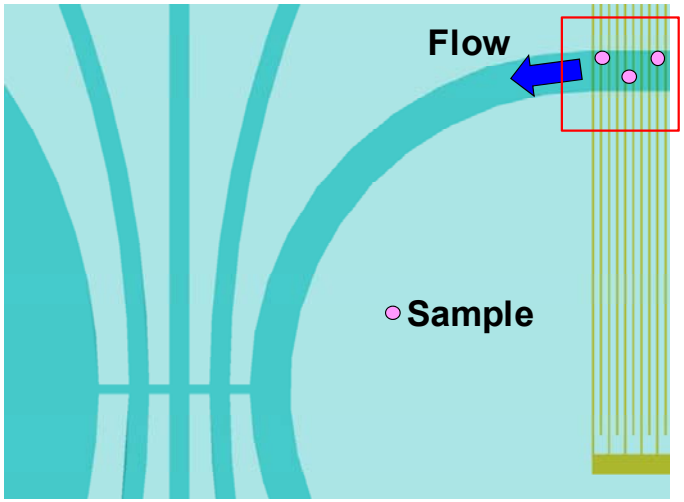




Height: 115μm Width: 100μm

Height: 15μm Width: 200μm

DEP concentration of viruses



100 mm

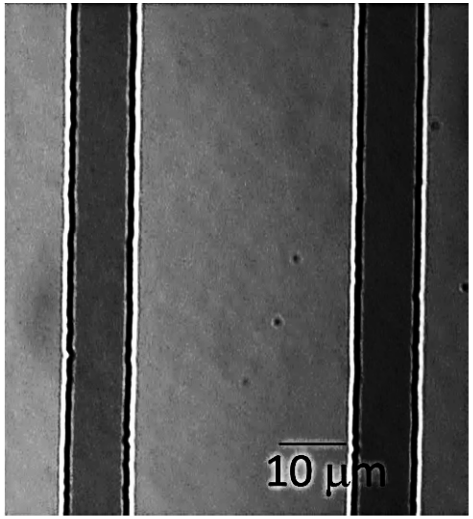
Conditions:

Applied voltage:
20Vp-p, 3MHz, square

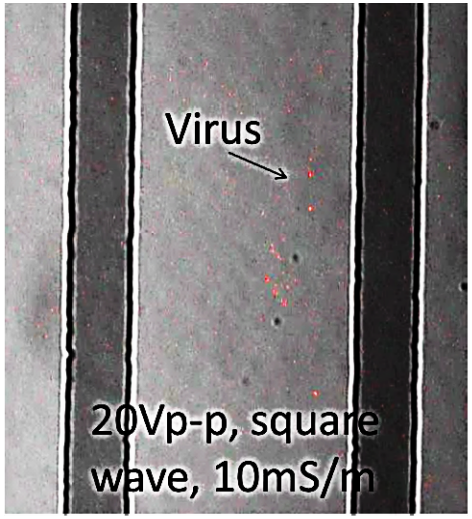
Conductivity of solution:
10 mS/m

Virus was stained by DiI

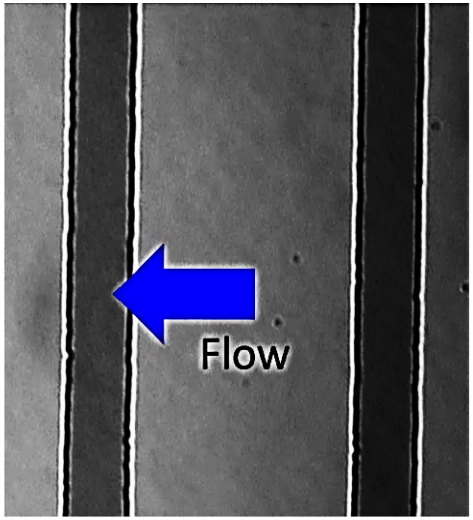
Experimental result



(d) Before virus concentration



(e) After virus concentration



(f) After virus flow

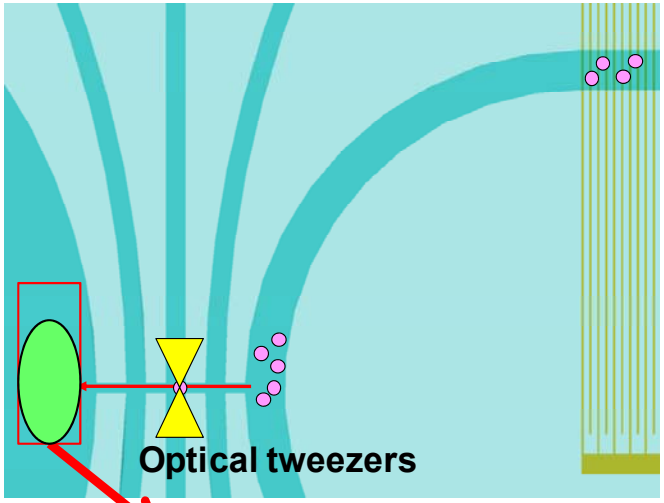
Concentration of Influenza virus

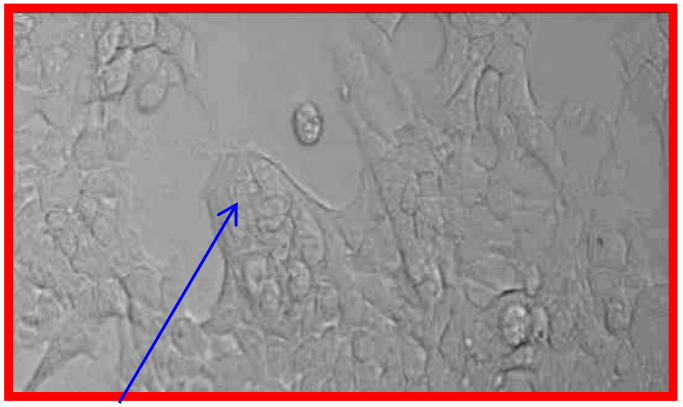
1.0×10^6 virus/ml

1.0×10^9 virus/ml

Avoid of adhesion of virus to glass

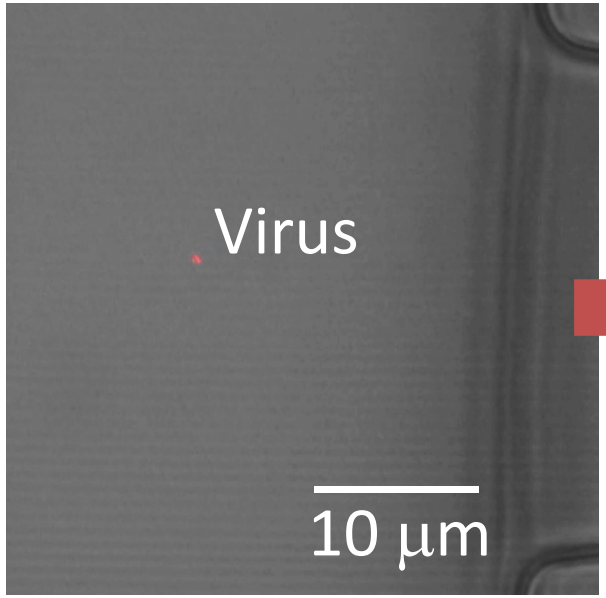
Virus transport to a specific cell

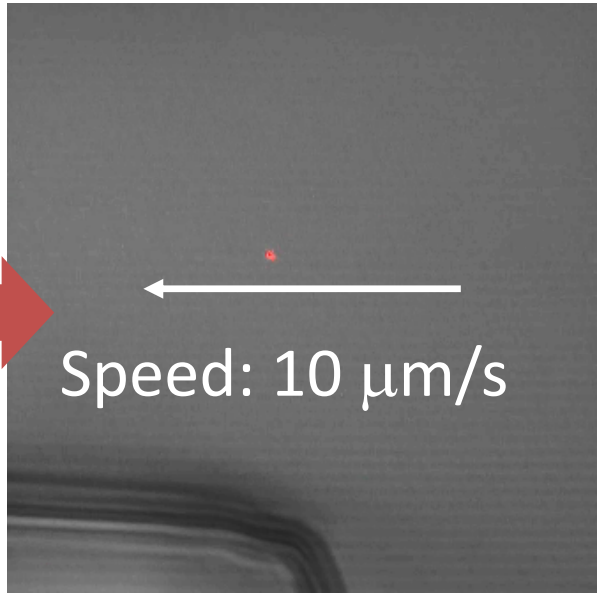


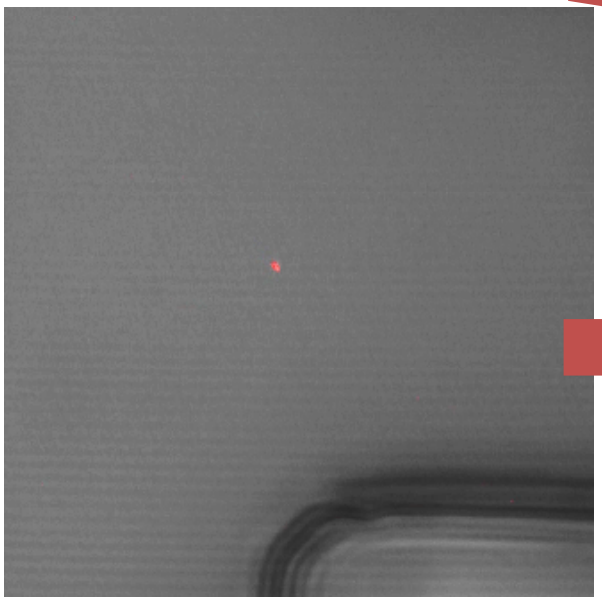


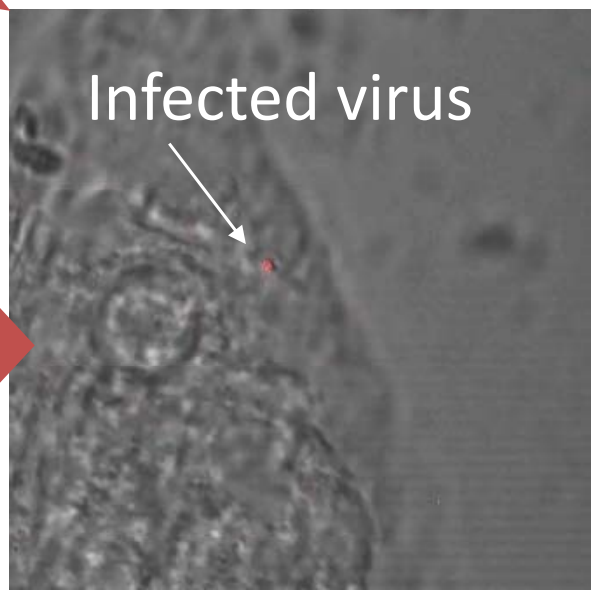
H292 cell

Transport length:
700 mm
Laser power: 1W (1064 nm)



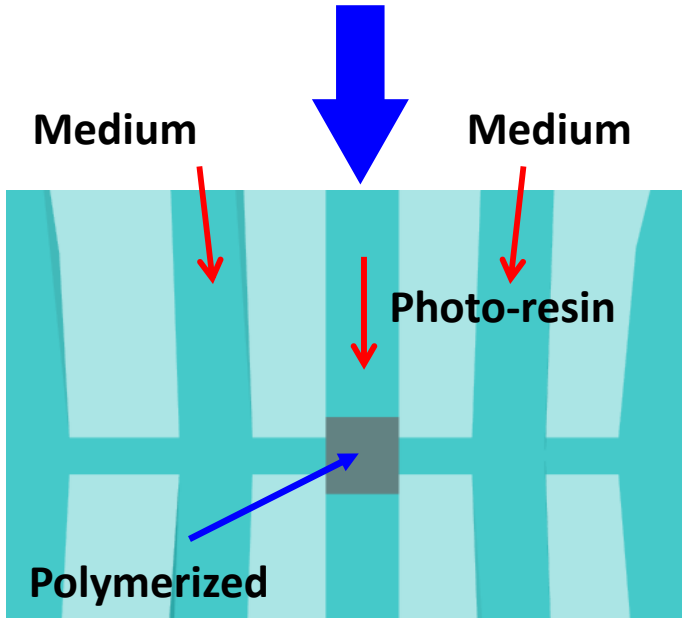






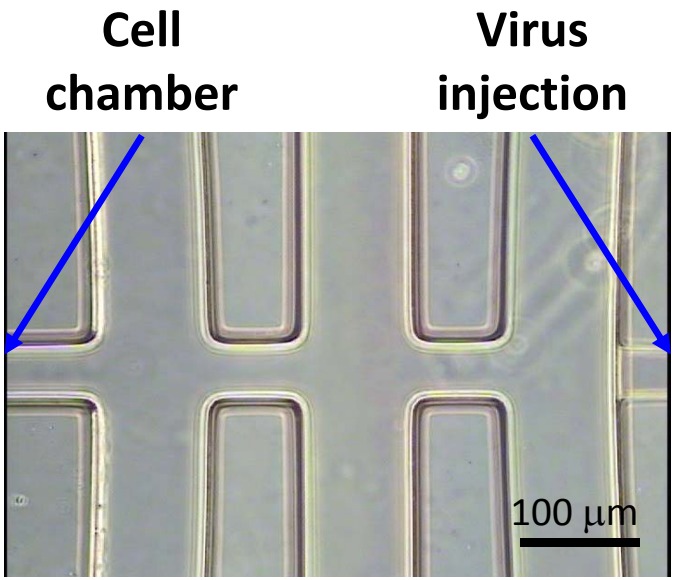
Separation of chambers by photo-resin

For avoiding incursion of the extra virus to experiment area, the area was isolated by photopolymerization.

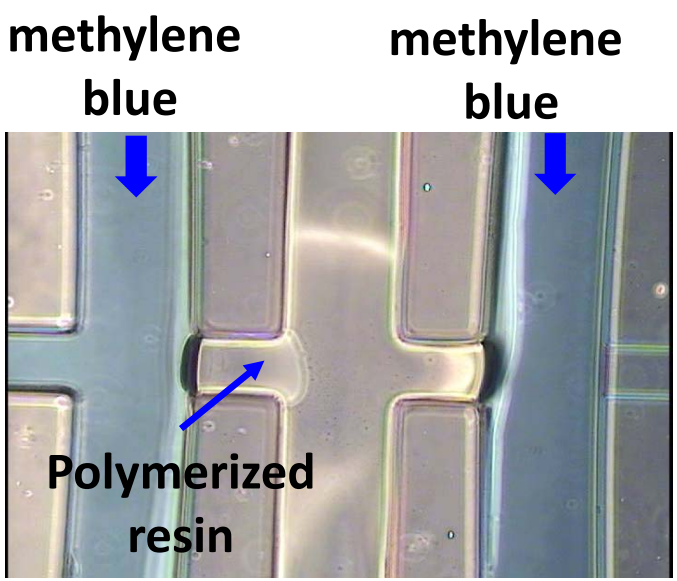


Polyethylene glycol methacrylate (200) (PEG-MA)

- Polymerization by UV illumination
- Biocompatible



Before polymerization



After polymerization

Summary

On-chip single virus infection to a specific cell was succeeded by solving these issues.

1. To find a target virus on a chip
   → DEP concentration ($10x10^6 \rightarrow 10x10^9$ virus/ml)
2. To avoid adhesion of virus to glass
   → DEP floating of virus on the glass surface
3. To manipulate a target virus to a specific cell
   → Manipulation by optical tweezers (10 μm/s)
4. To avoid extra virus incursion to cell chamber
   → Separation of chambers physically using photo-crosslinkable resin

References

1. A. Ichikawa, A. Honda, M. Ejima, T. Tanikawa, F. Arai, T. Fukuda, “In-situ formation of a gel microbead for laser micromanipulation of microorganisms, DNA, and viruses,” Journal of Robotics and Mechatronics 19, pp. 569-576, 2007.

2. H. Maruyama, F. Arai, T. Fukuda, T. Katsuragi, “Immobilization of individual cells by local photo polymerization on a chip,” The Analyst, Vol.130, No.3,(2005), pp.304-310.

3. H. Maruyama, K. Kotani, T. Masuda, A. Honda, T. Takahata, F. Arai, ” Nanomanipulation of single influenza virus using dielectrophoretic concentration and optical tweezers for single virus infection to a specific cell on a microfluidic chip,” Microfluidics and Nanofluidics, (2010). (in press)

Single Cell Analysis

Prof. T. Fukuda and Assistant Prof. M. Nakajima
Center for Micro-nano Mechatronics
Dept. of Micro / Nano Systems Engineering
Nagoya University

Contents

- Introduction
- Bio-Micro-manipulation System

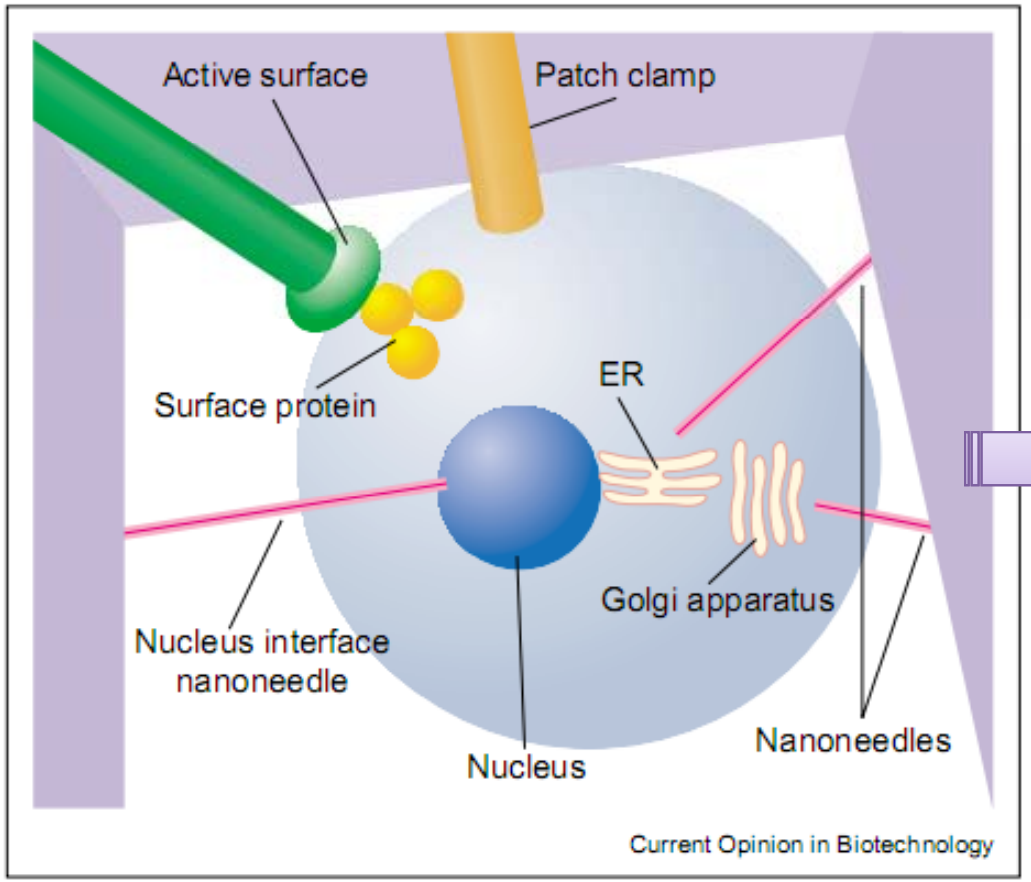
  <Laser-trapping and probe type Micromanipulation system>
  - Semi-closed microchip
  - Rapid prototyping of Micro structures inside micro-channel
  - Control of rotation of flagellar motor with dual Micro-Nano pipettes
- Bio-Nano-manipulation System

  <Environmental-SEM (E-SEM) Nanomanipulation System>
  - Single Cell Viability Evaluation (Local Stiffness/Electric Property)
  - Single Cell Manipulation depending on Sticking Condition
  - Single Cell Adhesion Measurement using Nanofork
- Conclusion and Future Works

Background -Single cell analysis-

Group cells analysis can only give average biological information

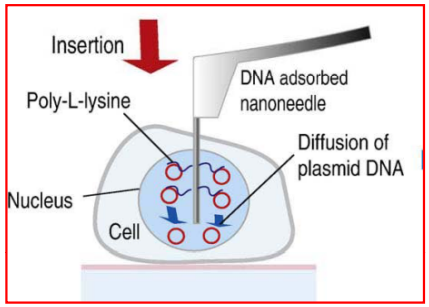
Single cell analysis have benefits of the understanding of individual cell characteristics more precisely



(H. Andersson, etc. 2004)

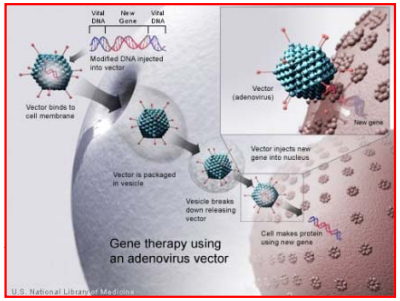
Single Cell Analysis and Treatments

Applications



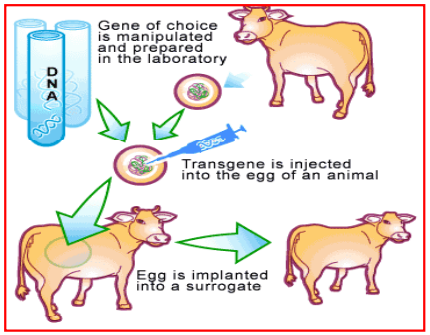
Gene Delivery

(S.W. Han et al. 2008)



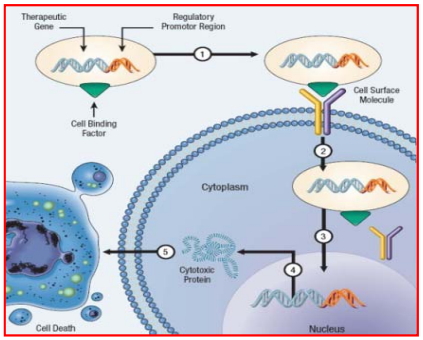
Gene Therapy

(U.S. National Library of Medicine)



Nucleus Transplantation

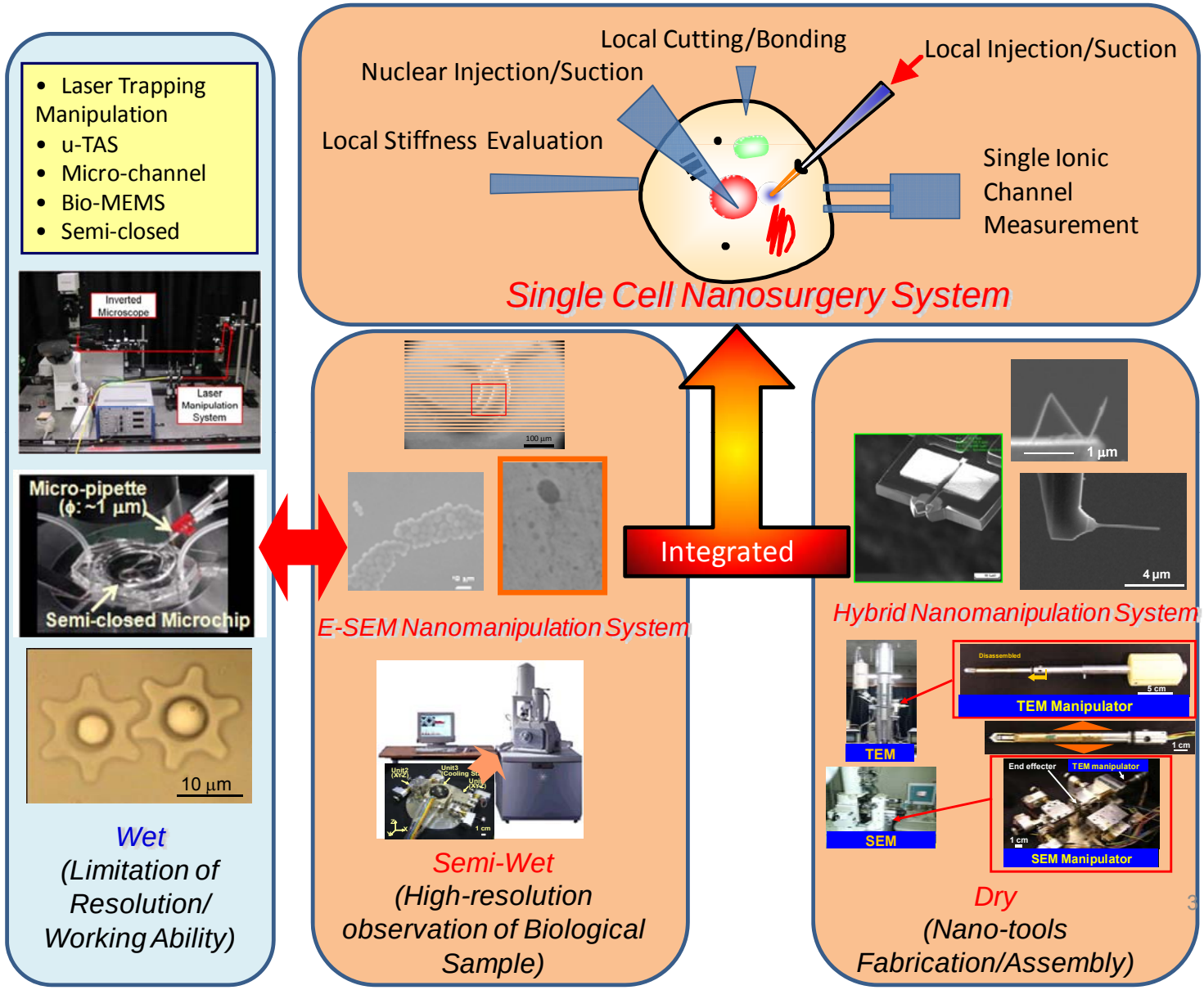
(Helmut Kae, 2003)



Suicide Therapy

(http://www.tuxenblog.com/)

Single Cell Nanosurgery System based on Single Cell Analysis



T. Fukuda et al., IEEE Industrial Electronics Magazine, Vol. 4,pp. 13-22, 2010.

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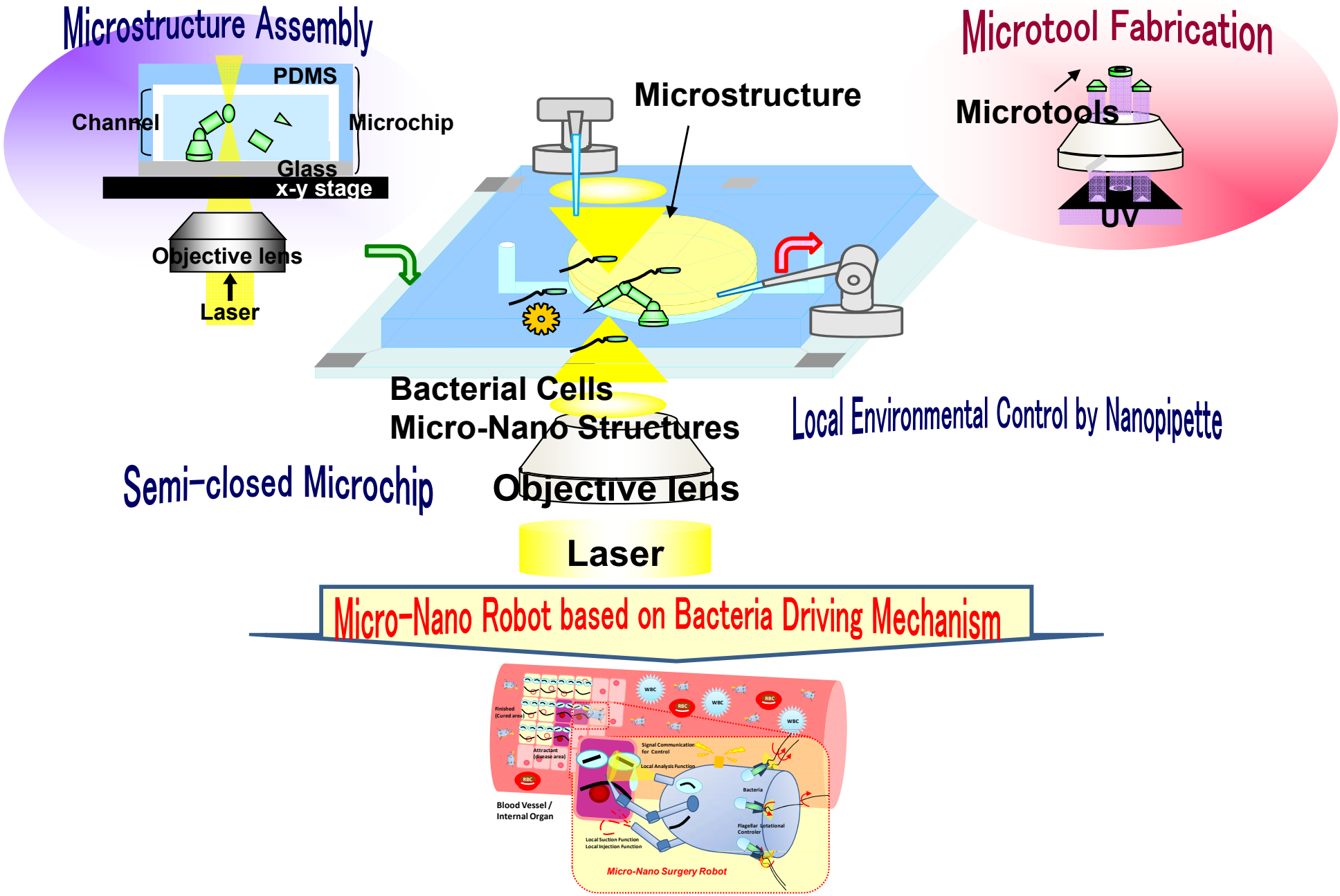
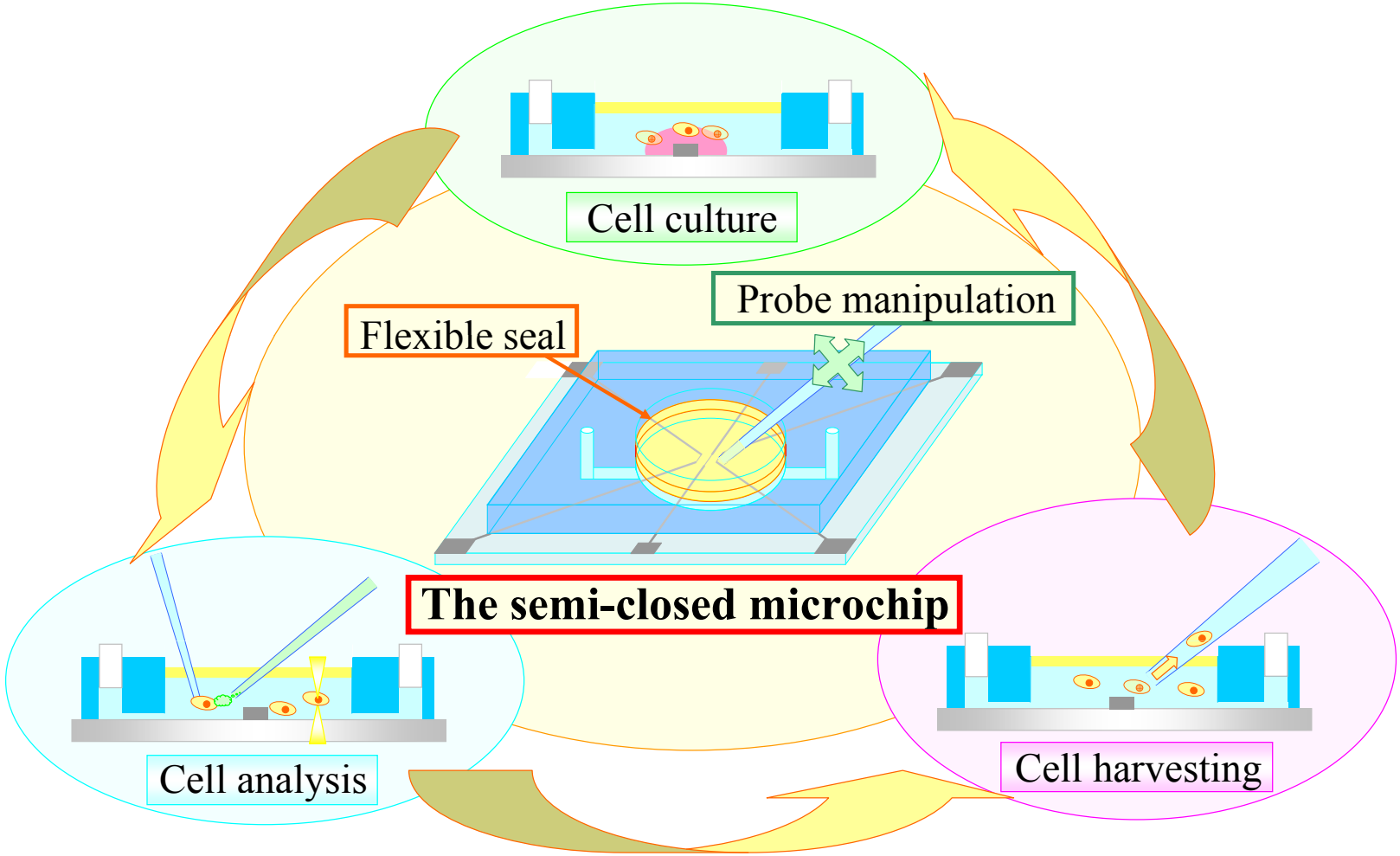
Micromanipulation System under Optical Microscope



Objective of "Semi-closed Microchip"

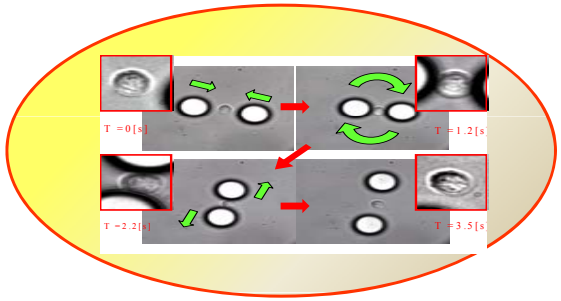
Realize probe type manipulations in microchips by flexible seal.

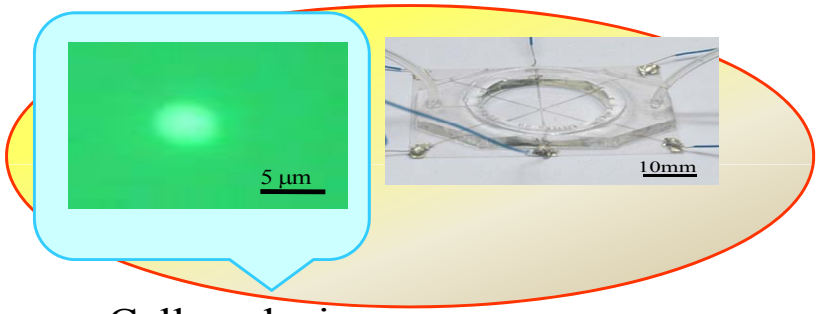


The concept of the semi-closed microchip.

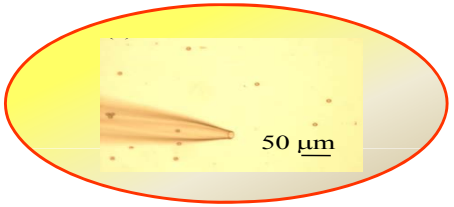
M. Takeuchi, et al. Proc of ICRA2009, 2009

Integrated Semi-closed Microchip





Cell analysis



Probe manipulation

Laser manipulation

Cell analysis

Outlet

Inlet

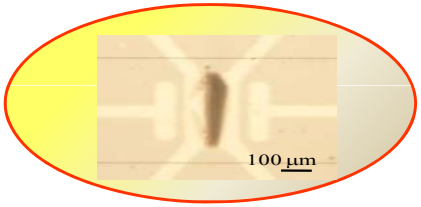
Micro valve

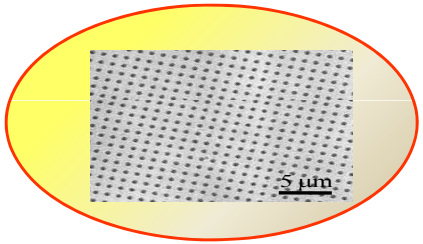
Nano scale filter

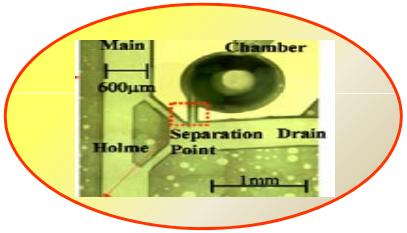
Cell sorter

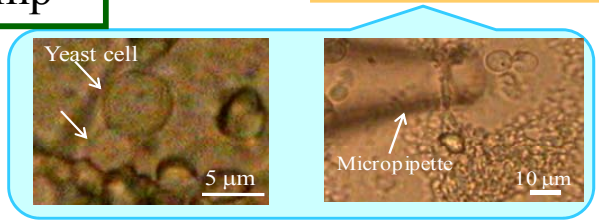
Micro pomp

Cell culture and harvesting









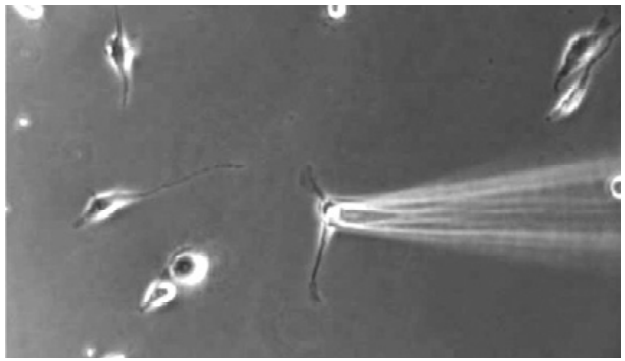
Cell culture

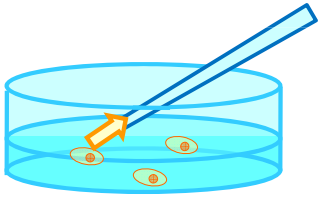
Cell harvesting

M. Takeuchi, et al. Proc of ICRA2009, 2009

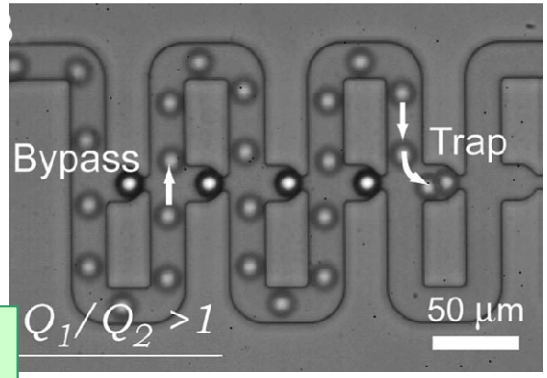
Conventional works –features of the open and closed chambers-

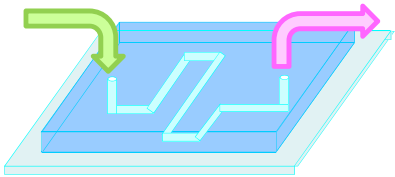
Open chambers	Closed chambers
➢Allowing the probe manipulation ▪ Arbitrary manipulation ➢Environmental change by evaporation of solution is unavoidable.	➢Non contact manipulation techniques are needed. ▪ Optical tweezers : pN order force ▪ Fluid force : complex microchannels ➢The influence of the external environment is small.





T. Matsuda et al. (2008)





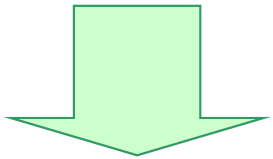
S. Takeuchi et al. (2007)

We propose "Semi-closed microchip".

M. Takeuchi, et al. Proc of ICRA2009, 2009

Semi-closed microchip

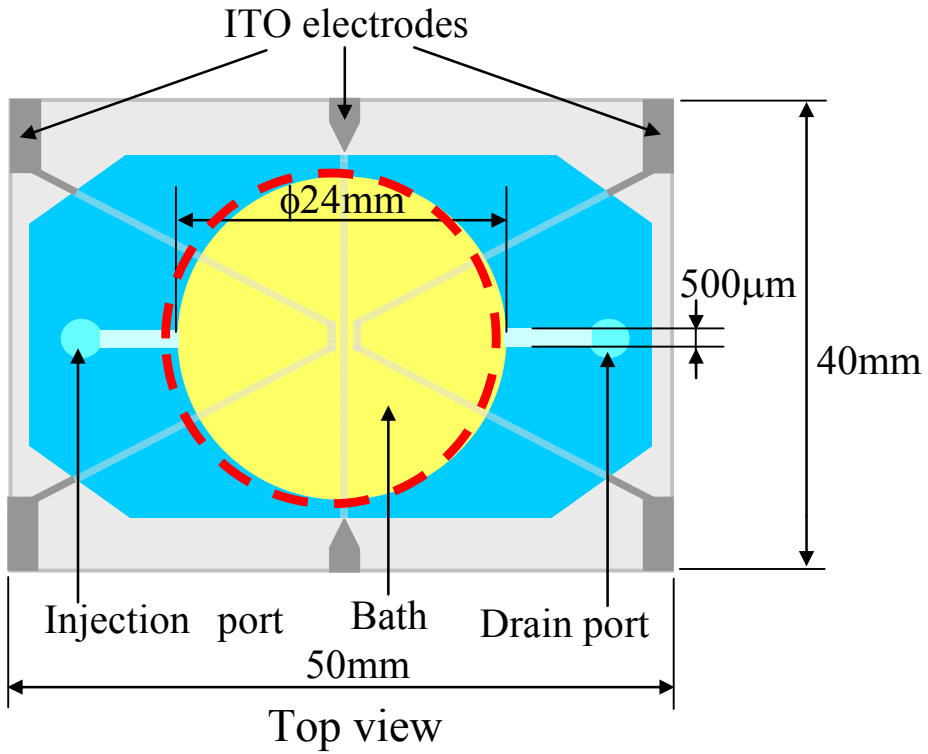
Sealing the bath by oil film.



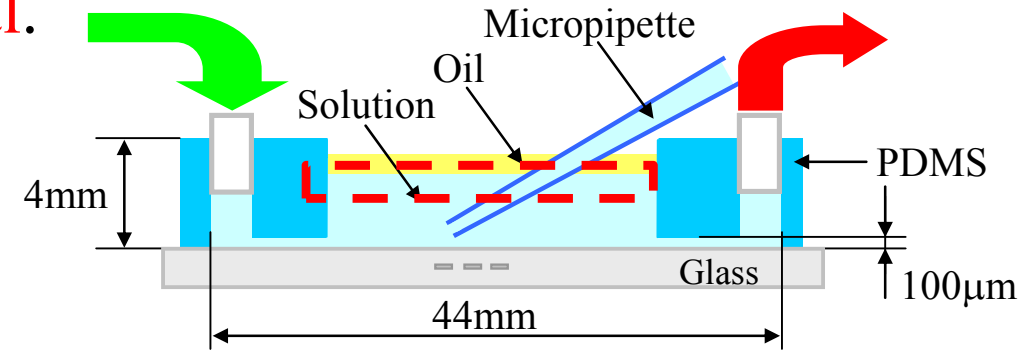
➢Preventing the loss of solution in the bath due to evaporation.

➢Realizing the insertion of micropipette with keeping the seal.

Realizing even thickness of the oil film is important.



Top view



Side view

(PDMS: poly(dimethylsiloxane))

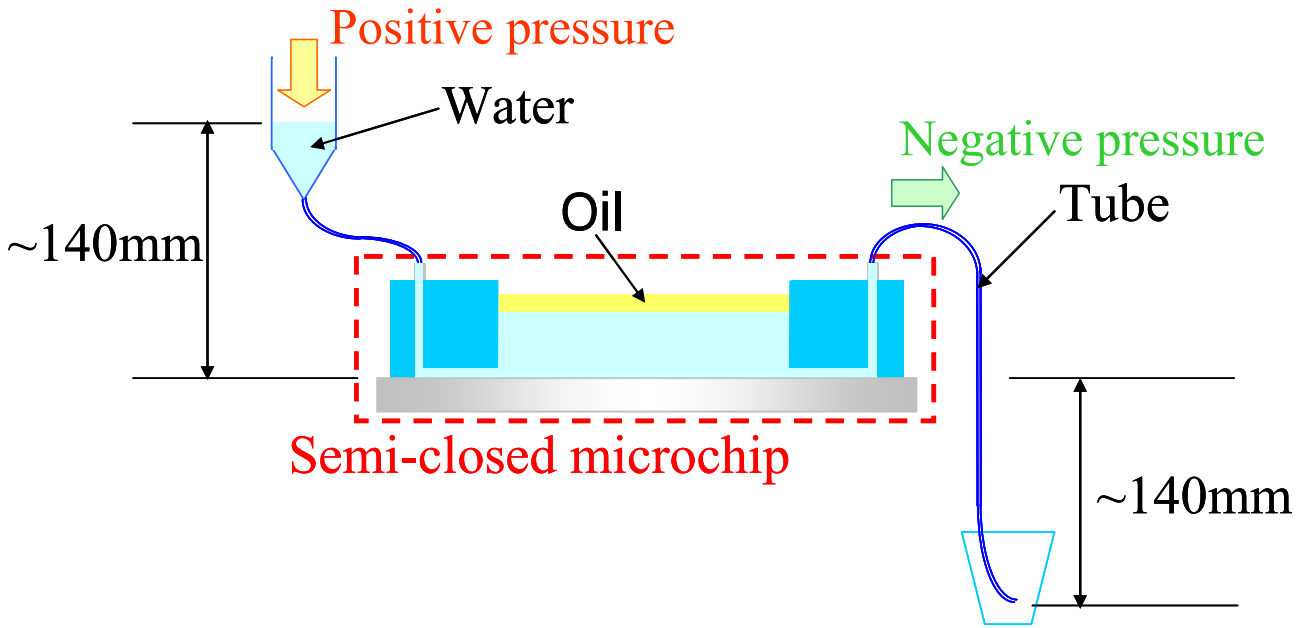
M. Takeuchi, et al. Proc of ICRA2009, 2009

Solution exchange –Experimental setup-

The exchange of the solution in the bath is needed for cell culture and cell analysis.

The exchange was conducted by applying the positive pressure to the inlet and negative pressure to the outlet.

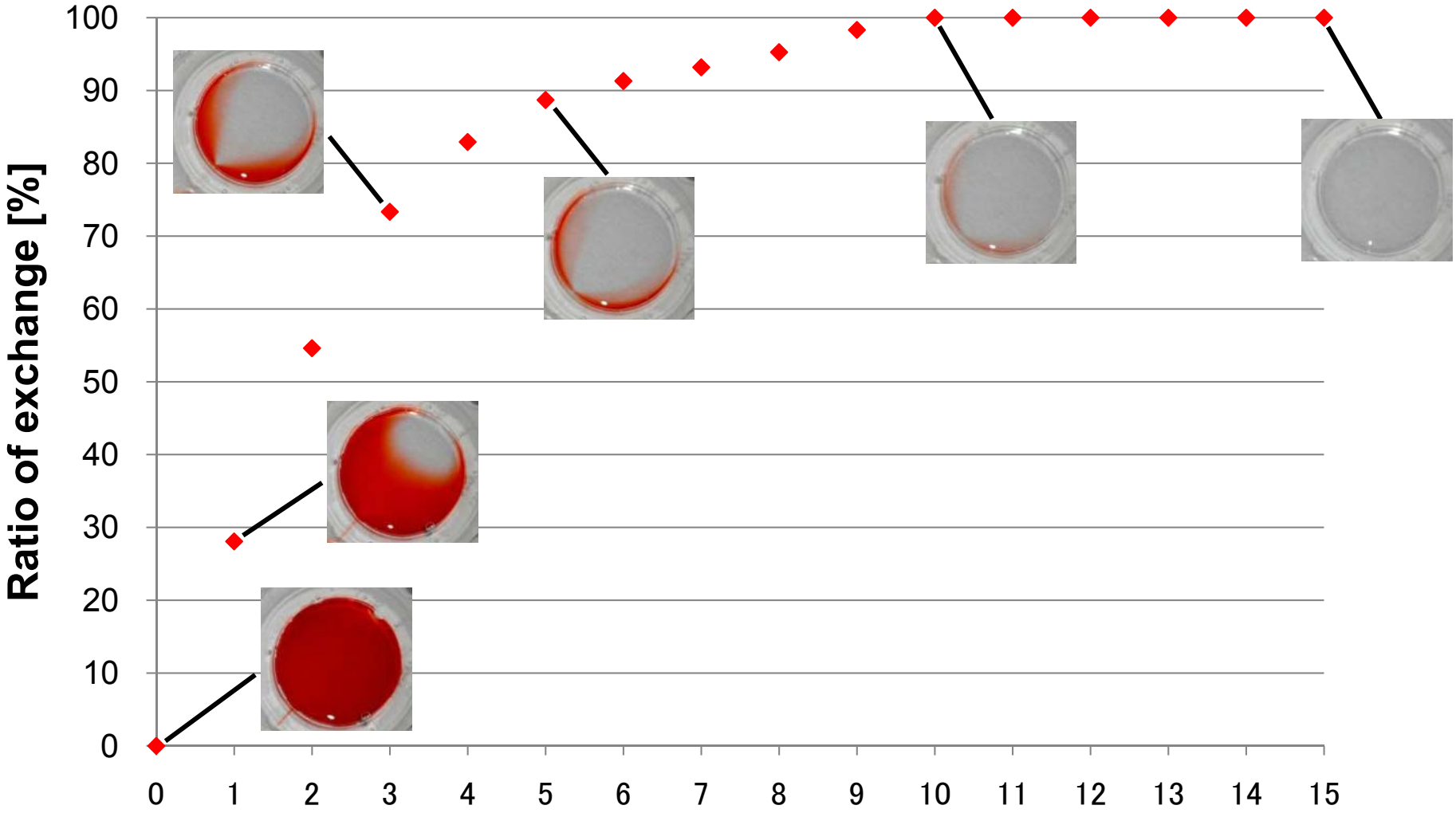
- The water in the bath was stained red before the exchange.
- The colorless water flowed into the bath.



A schematic of the experimental setup.

M. Takeuchi et al., Proc of ICRA2009, 2009

Solution exchange –Experimental results-



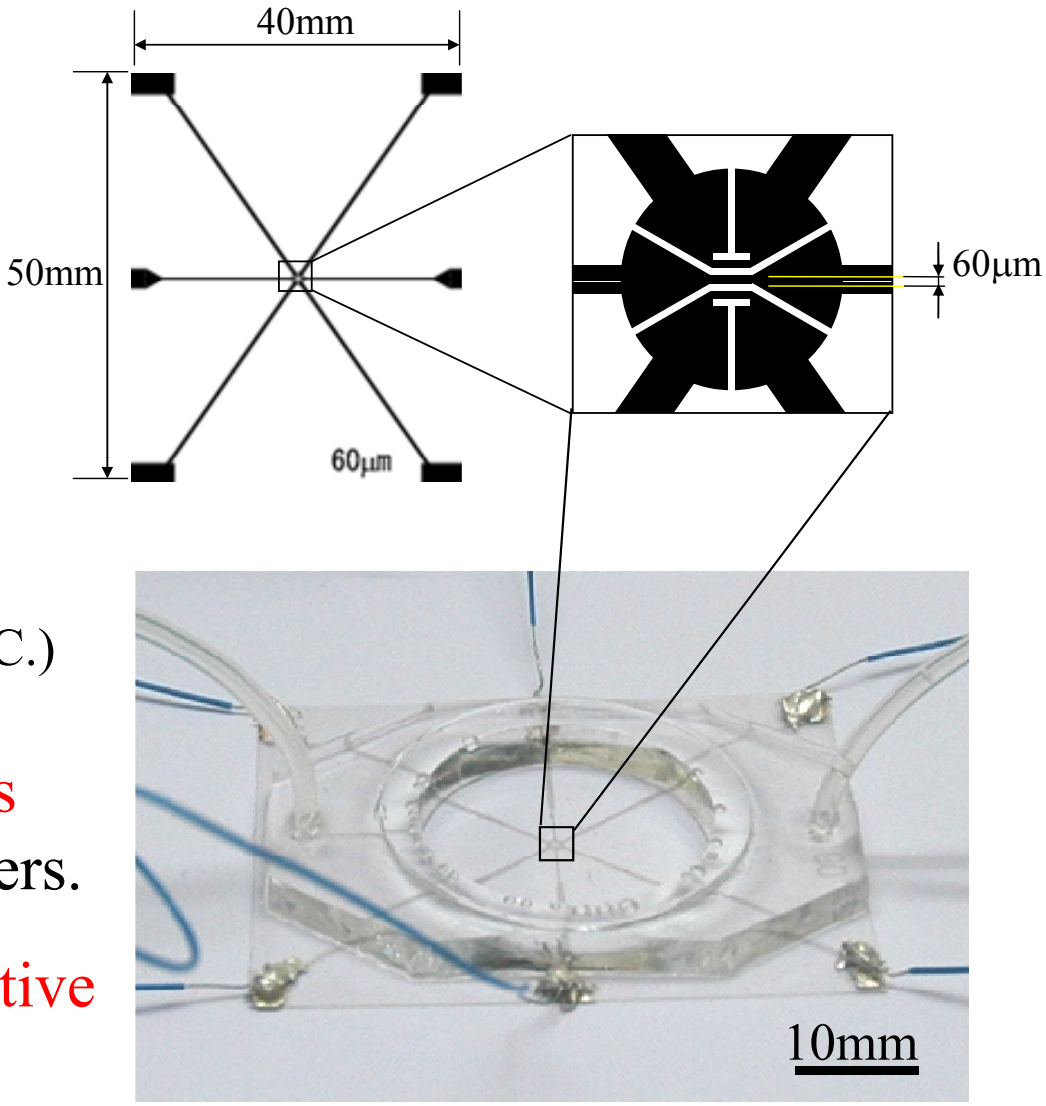
$$\left(\text{Ratio of exchange} = \frac{\text{Size of the white area}}{\text{Size of the bath}}\right)$$

(Flow volume: about 430 µl/min)

80% of the solution in the bath can be exchanged in 5 minutes.

Cell fixation in the semi-closed microchip

Fixation of the cells in the bath is needed during the exchange of the solution.



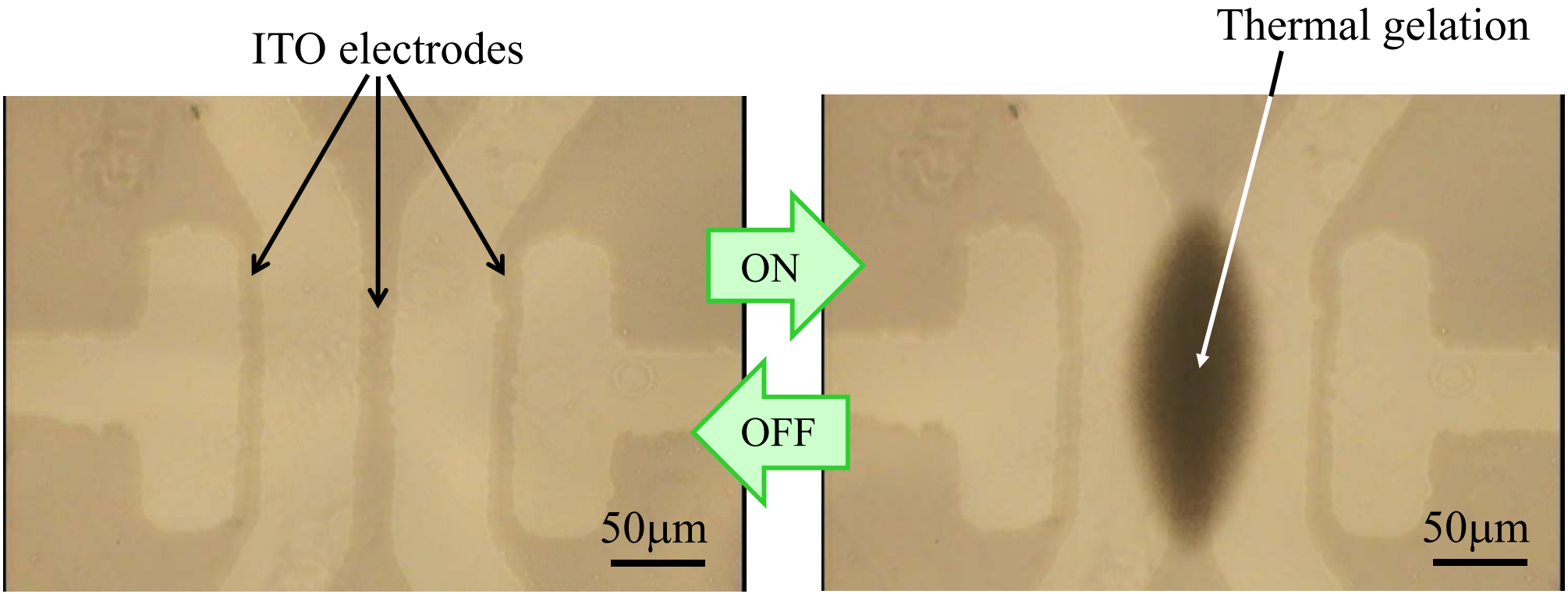
Thermo sensitive gel (PNIPAAm) was used to fix cells in the bath.

(PNIPAAm solution is gelled over 32 ° C.)

- Indium Tin Oxide (ITO) electrodes were fabricated in the bath as heaters.
- Cells can be fixed by thermo sensitive gel (PNIPAAm) when applying a voltage to the ITO electrode.

M. Takeuchi et al., Proc of ICRA 2009, 2009

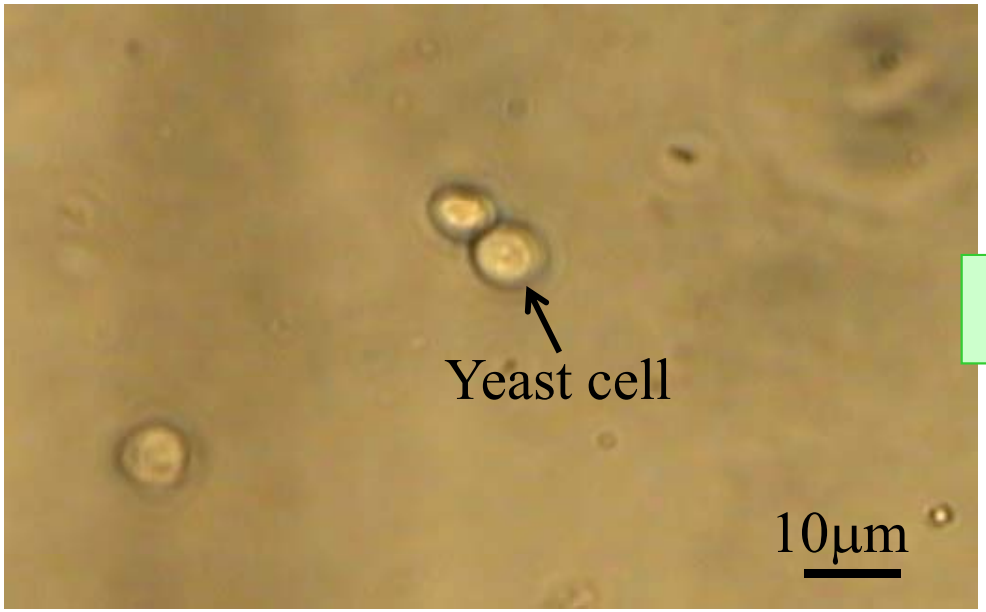
Thermal gelation of the PNIPAAm solution



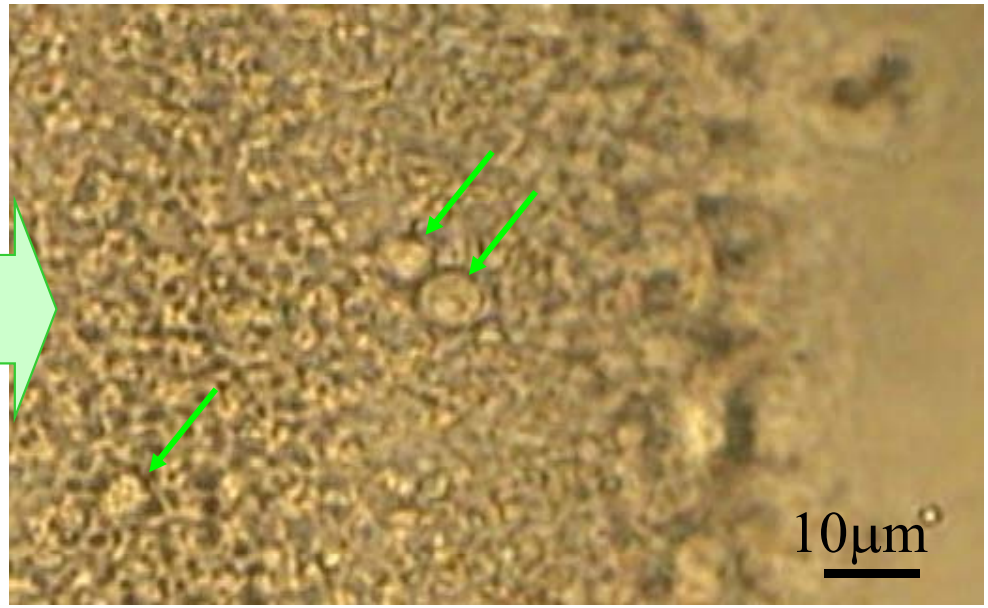
Thermo sensitive gel (PNIPAAm) solution showed reversible sol-gel change by the fabricated ITO electrode.

M. Takeuchi et al., Proc of ICRA 2009, 2009

Cell fixation in the semi-closed microchip -Experimental results-



Before the fixation



After the fixation

Cells can be fixed locally by the thermo sensitive gel when applying a voltage to the ITO electrode.

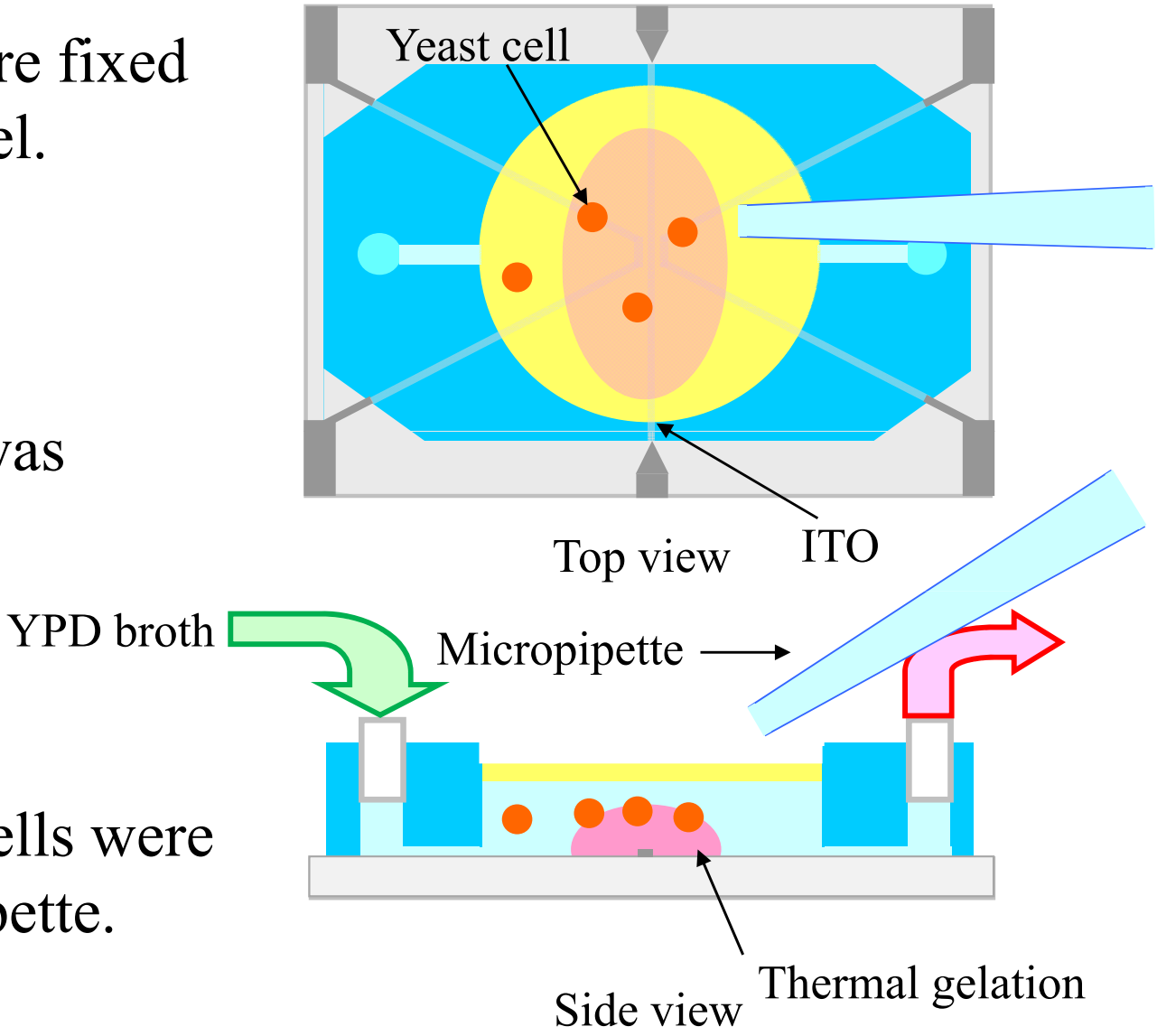
M. Takeuchi et al., Proc of ICRA 2009, 2009.

Cell harvesting -Experimental procedure-

Yeast cells in the bath were fixed by the thermo sensitive gel.

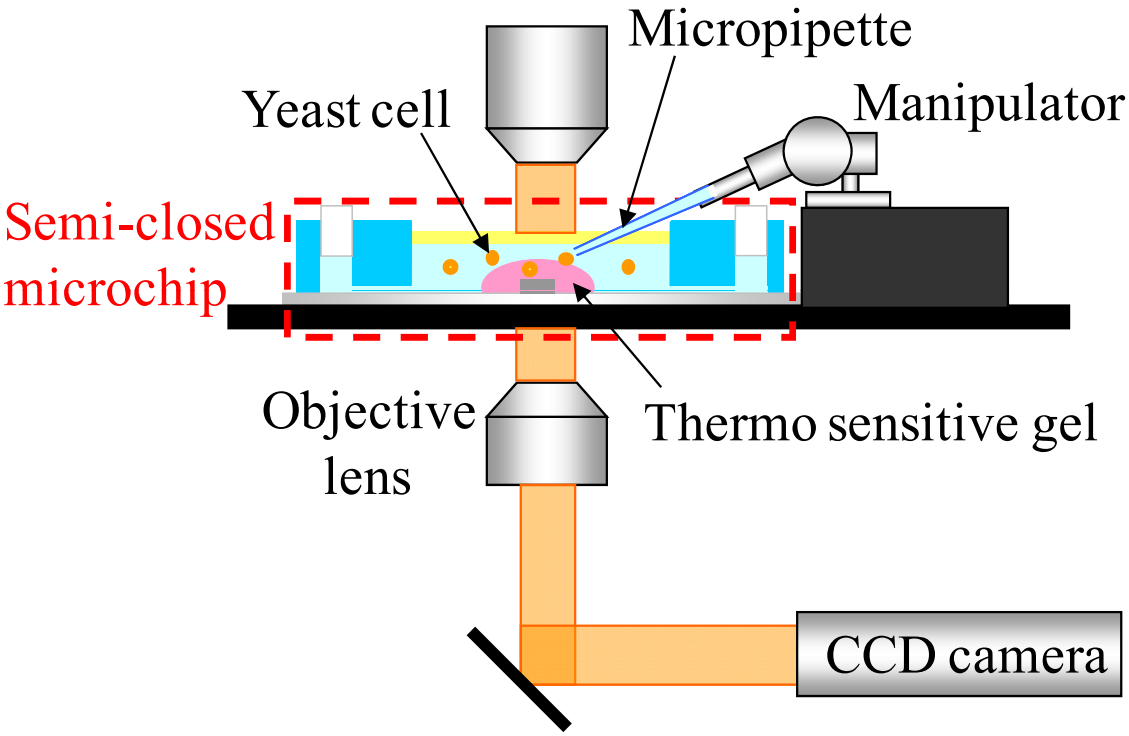
The solution in the bath was exchanged to YPD broth.

After culture, the target cells were harvested by the micropipette.



M. Takeuchi et al., Proc of ICRA2009, 2009

Cell harvesting -Experimental setup-

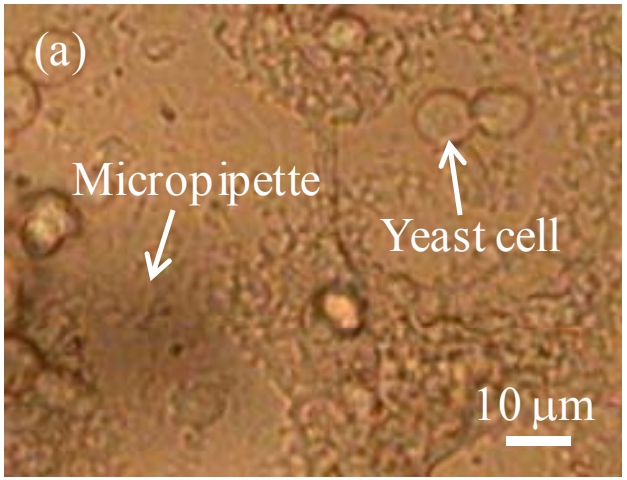


Experimental setup

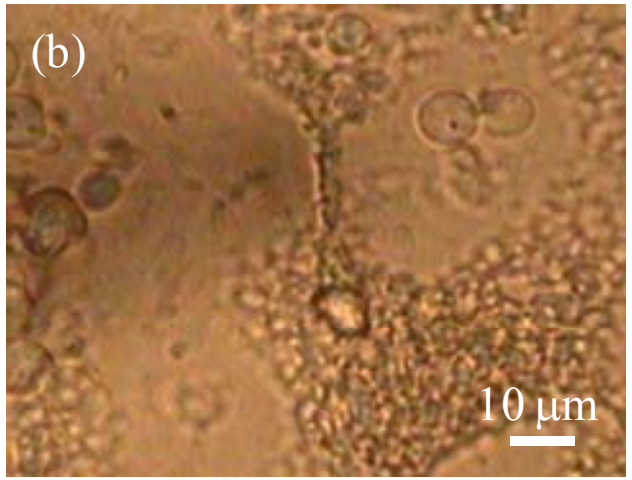
Insertion of the micropipette to the semi-closed microchip

M. Takeuchi et al., Proc of ICRA2009, 2009

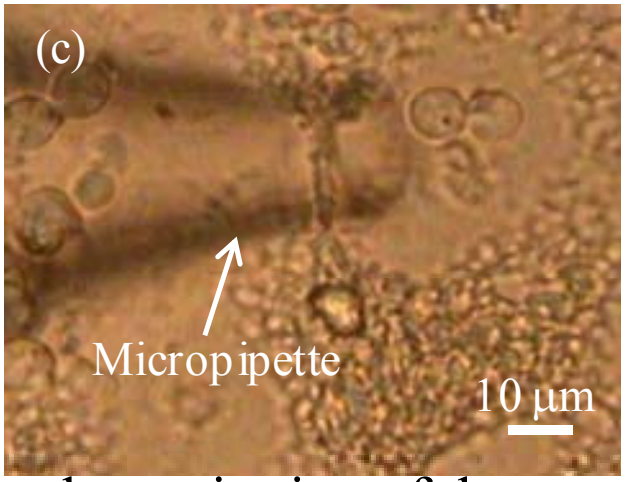
Cell harvesting –Experimental results-



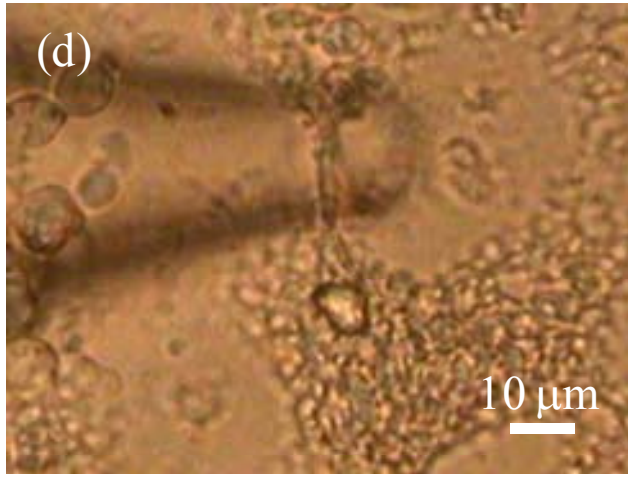
Before harvesting the cell.



Positioning of the micropipette.



During the aspiration of the target cell.



After harvesting the target cell.

The target cell can be harvested by micropipette.

Contents

- Introduction

- Bio-Micro-manipulation System
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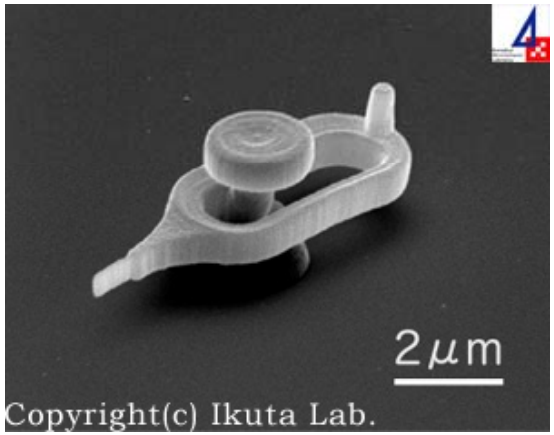
  <Environmental-SEM (E-SEM) Nanomanipulation System>
  - Single Cell Viability Evaluation (Local Stiffness/Electric Property)

- Conclusion and Future Works

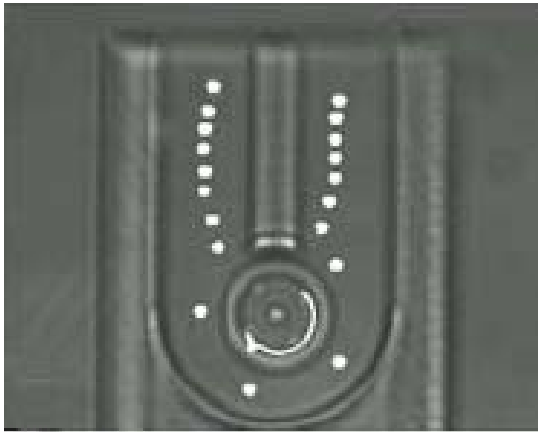
Previous research of in suit optical microfabrication

The 3D complicated shape microstructures fabricated by optical microfabrication



Stiffness measurement tool

K. Ikuta et al. (2000)



Micro -rotator

S. Maruo et al. (2007)

Issues

- It takes long time to fabricate the microstructures
- The fabrication process needs well-organized condition with expensive instruments

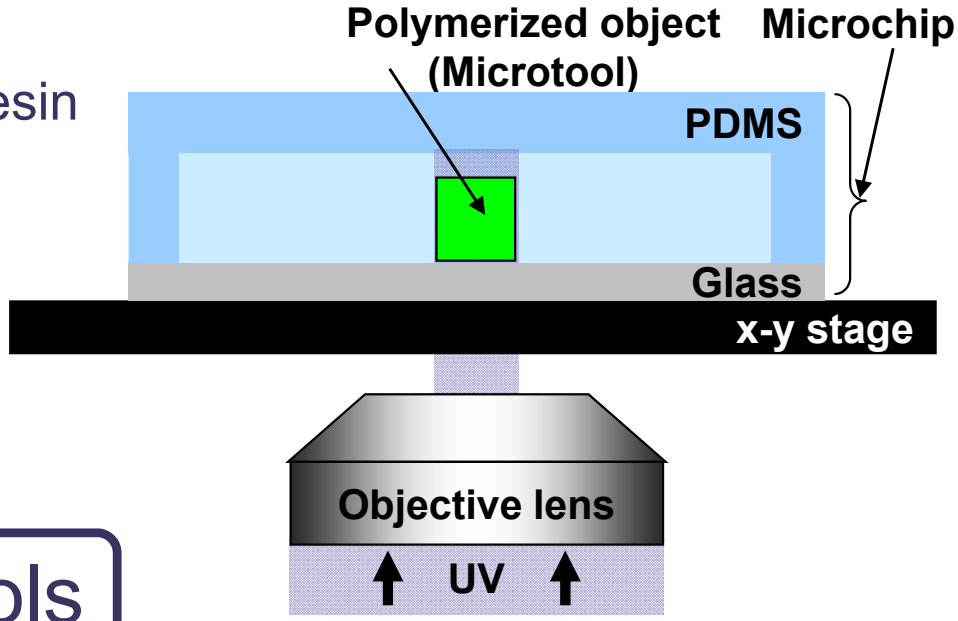
M. Takeuchi et al., Proc of ICRA2009, 2009, M. Ito et al.
Trans. of the Japan Soc. of Mech. Eng., C, Vol. 75, No. 752, pp. 194-199, 2009 (in Japanese)

Microtool fabrication with photo-crosslinkable resin

Material of microtools is photo-crosslinkable resin

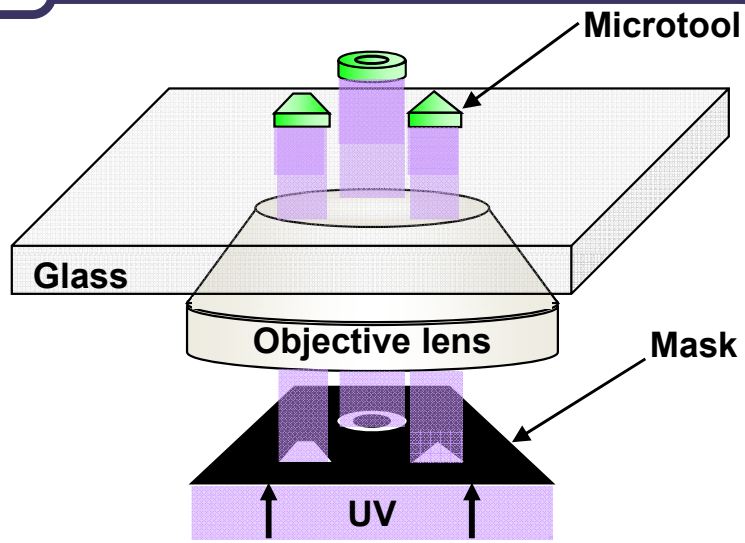
PEG-DA（poly ethylene glycol diacrylate）
& Polymeric Initiator

Low toxicity



Arbitrary shape microtools

The patterned UV-ray through the mask is illuminated to photo-crosslinkable resin

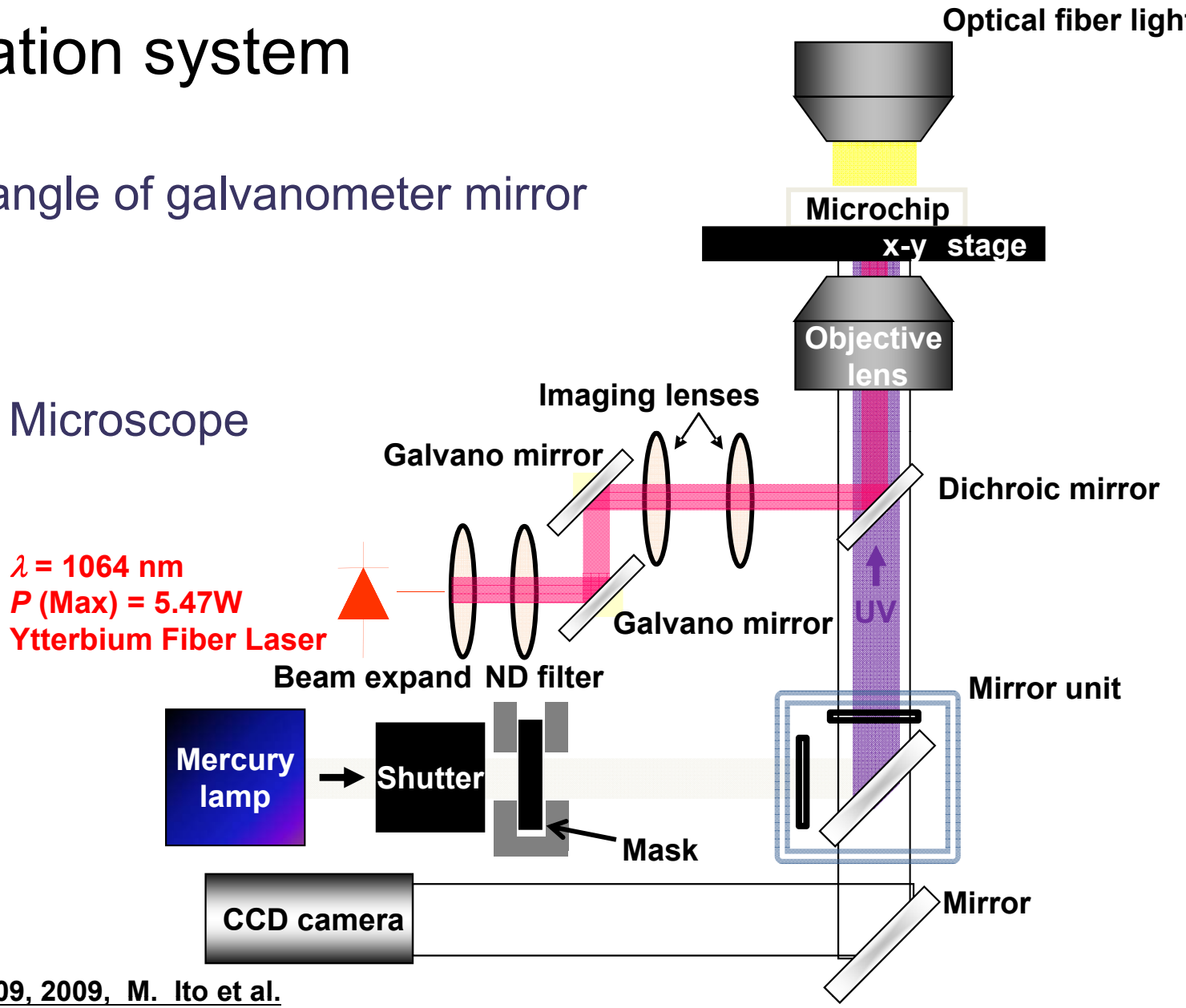


M. Takeuchi et al., Proc of ICRA2009, 2009, M. Ito et al.
Trans. of the Japan Soc. of Mech. Eng., C, Vol. 75, No. 752, pp. 194-199, 2009 (in Japanese)

Experimental setup

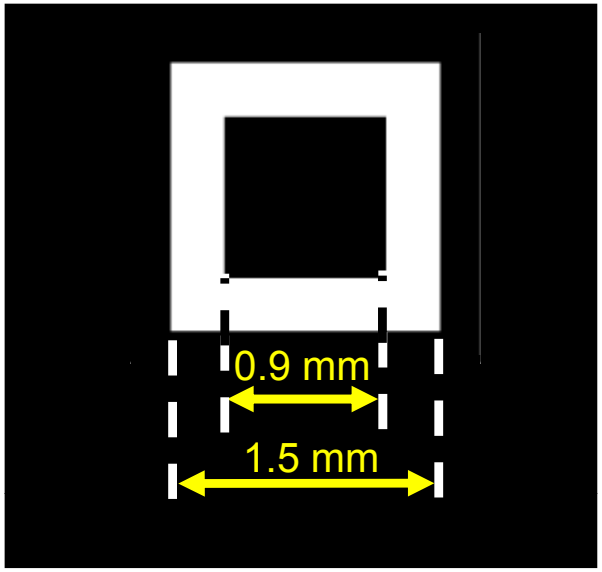
Laser manipulation system

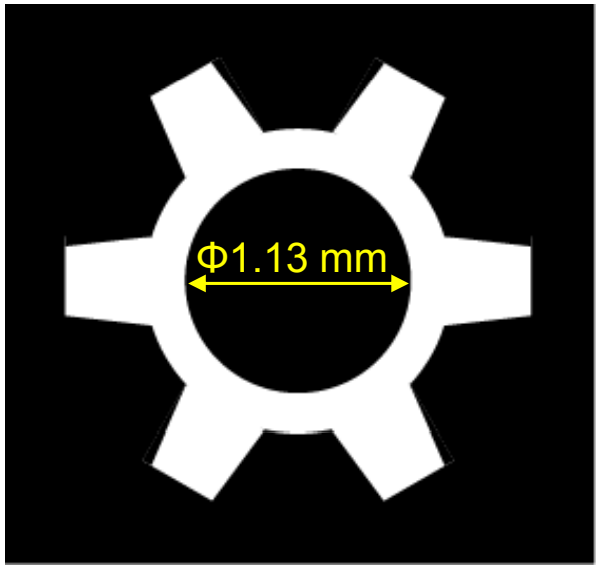
- Optical tweezers
  Controlling the angle of galvanometer mirror
- UV-ray
  Mercury lamp
- Observation
  Inverted Optical Microscope
  CCD camera



M. Takeuchi et al., Proc of ICRA2009, 2009, M. Ito et al.
Trans. of the Japan Soc. of Mech. Eng., C, Vol. 75, No. 752, pp. 194-199, 2009 (in Japanese)

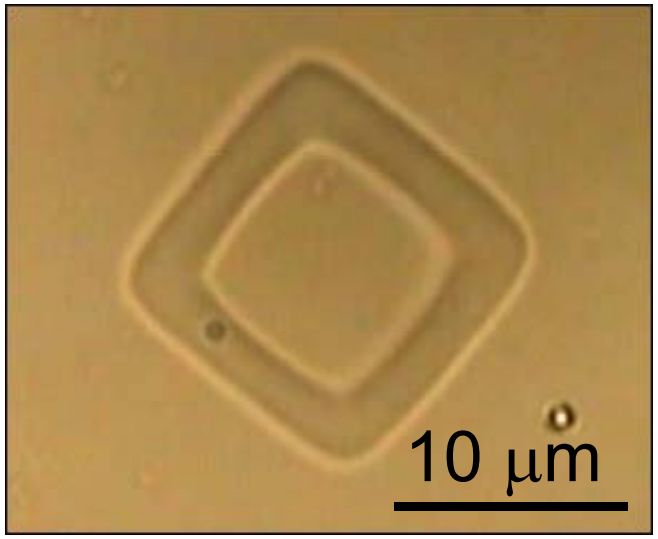
Arbitrary shape microtools

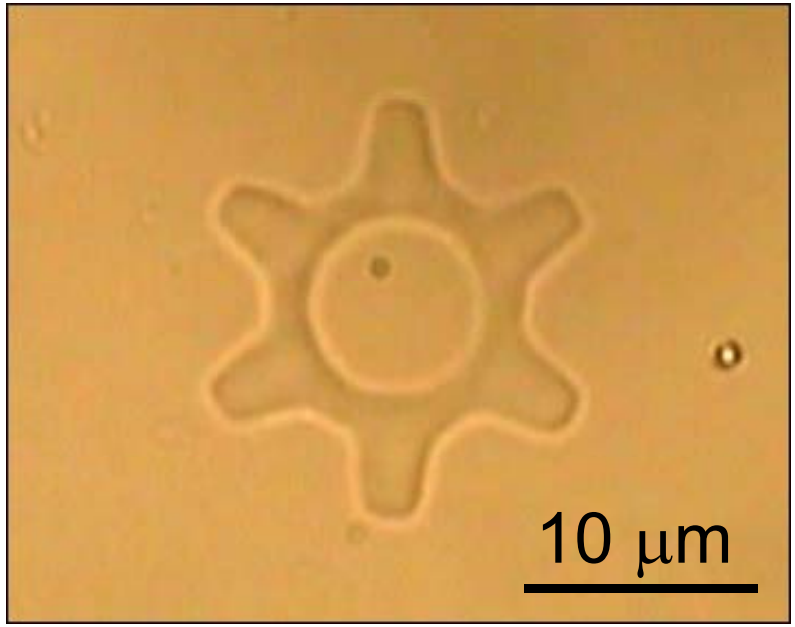




× 100 objective lens

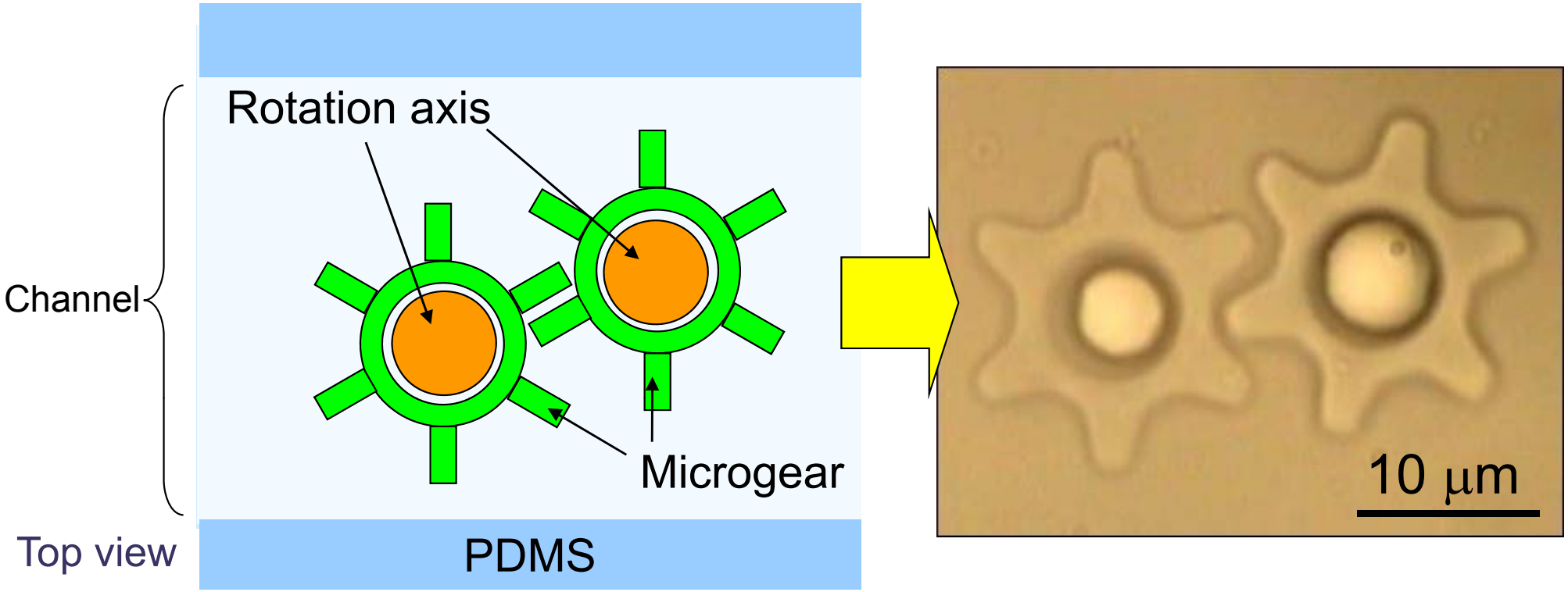
The microtool was about 1/108 size compared with the pattern of the mask





M. Takeuchi et al., Proc of ICRA2009, 2009, M. Ito et al.
Trans. of the Japan Soc. of Mech. Eng., C, Vol. 75, No. 752, pp. 194-199, 2009 (in Japanese)

Assembly methods of rotational microstructure



Assembly methods of microstructure

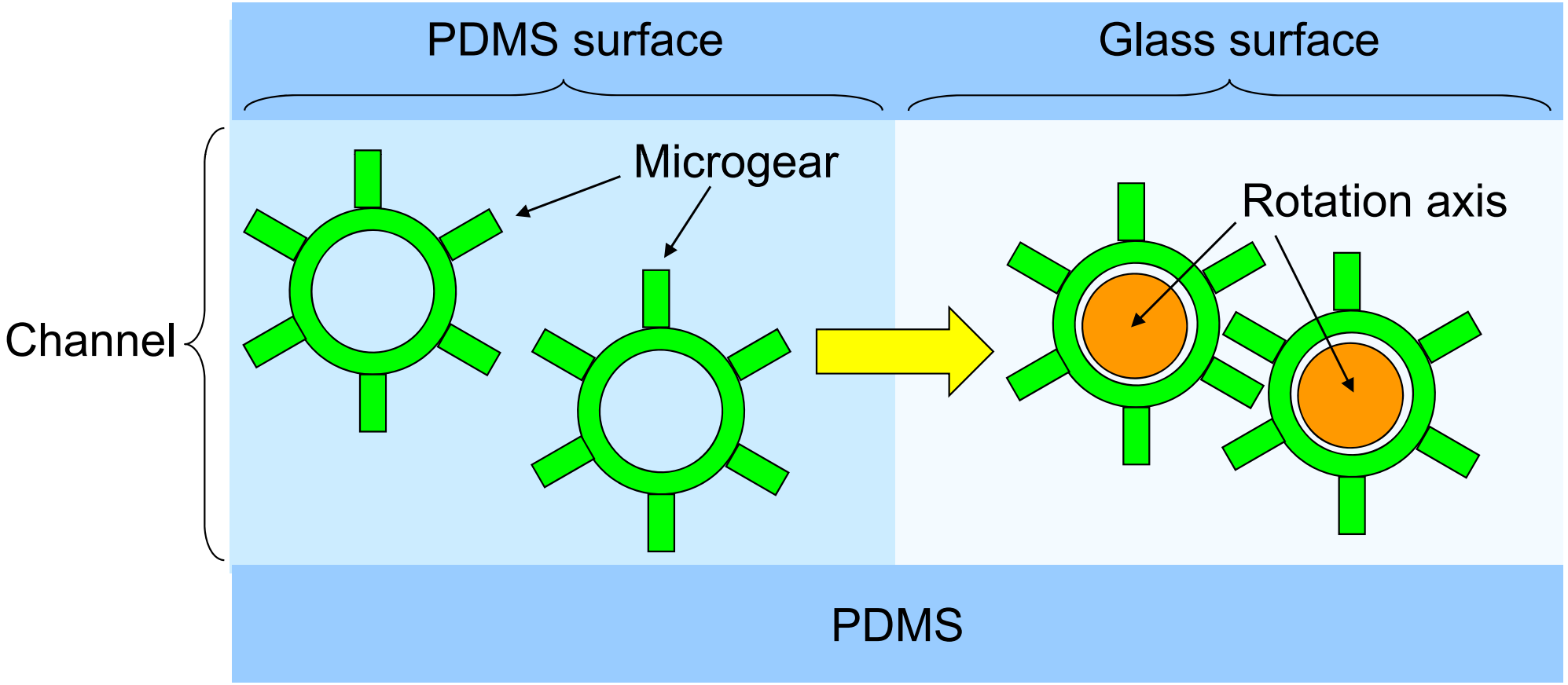
Ⅰ. Laser manipulation assembly
Ⅱ. Non-laser manipulation assembly

M. Takeuchi et al., Proc of ICRA2009, 2009, M. Ito et al.
Trans. of the Japan Soc. of Mech. Eng., C, Vol. 75, No. 752, pp. 194-199, 2009 (in Japanese)

II. Non-laser manipulation assembly

Microtools fabricated on the PDMS ➡ Moving freely

Microtools fabricated on the Glass ➡ Adhering on it



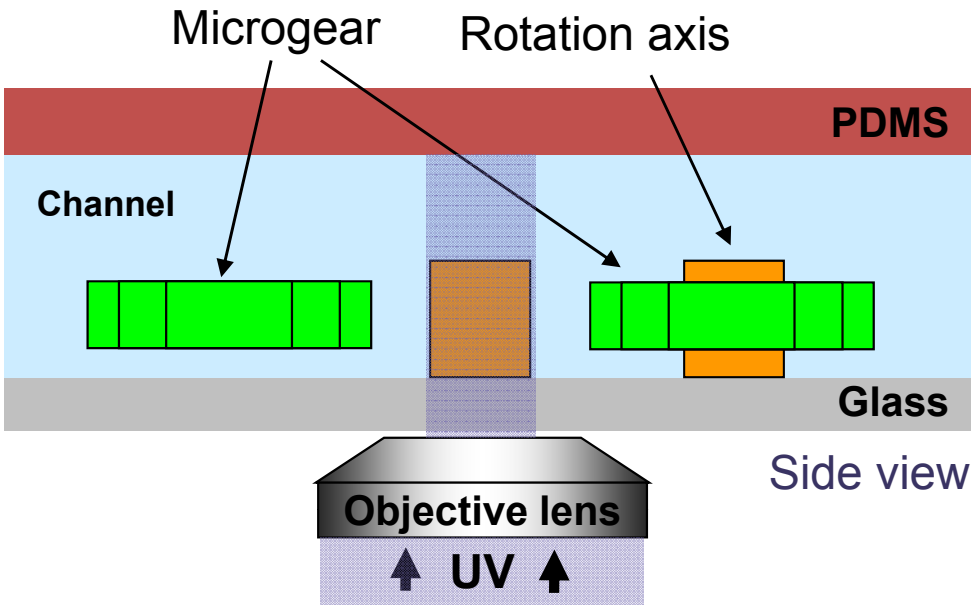
Top view

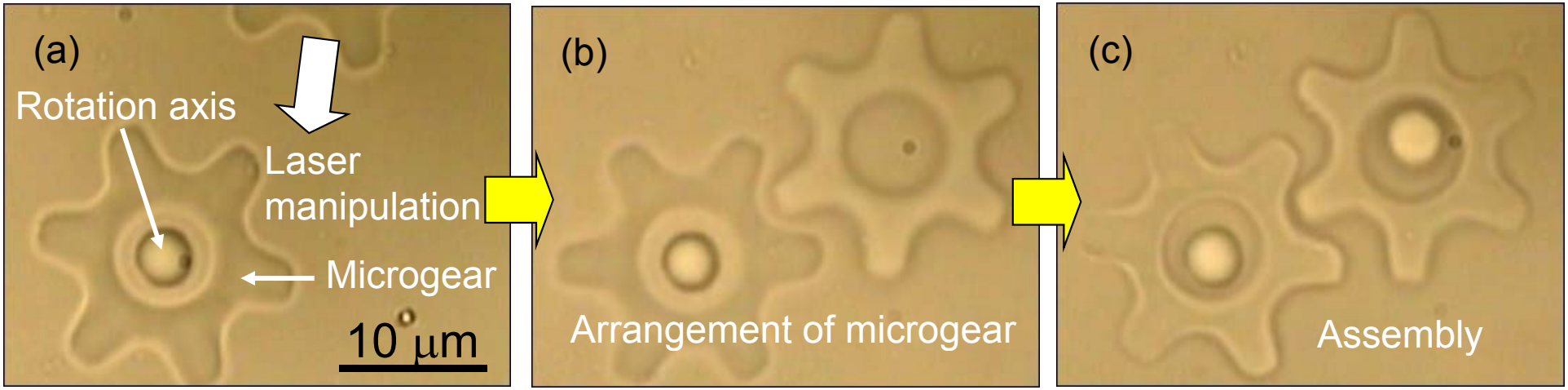
M. Takeuchi et al., Proc of ICRA2009, 2009, M. Ito et al.
Trans. of the Japan Soc. of Mech. Eng., C, Vol. 75, No. 752, pp. 194-199, 2009 (in Japanese)

II. Non-laser manipulation assembly

①Manipulating microgears to engage them

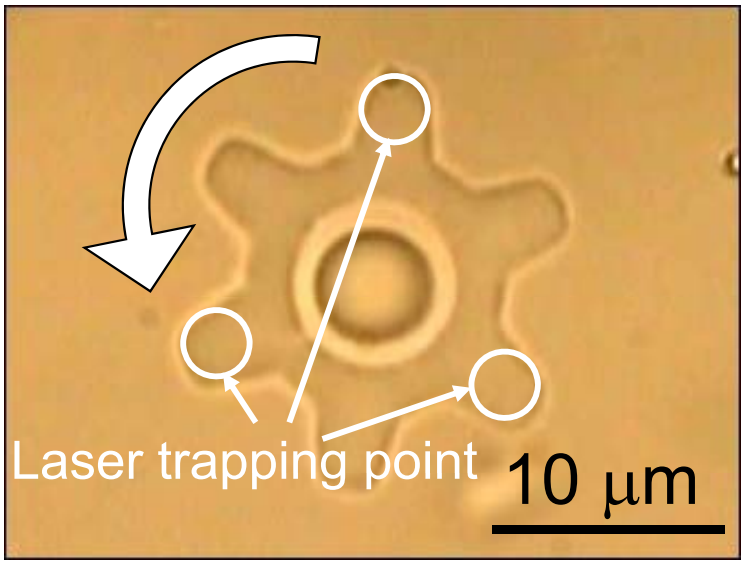
② Fabricating the rotation axis in the hole of the microgear by controlling the stage position

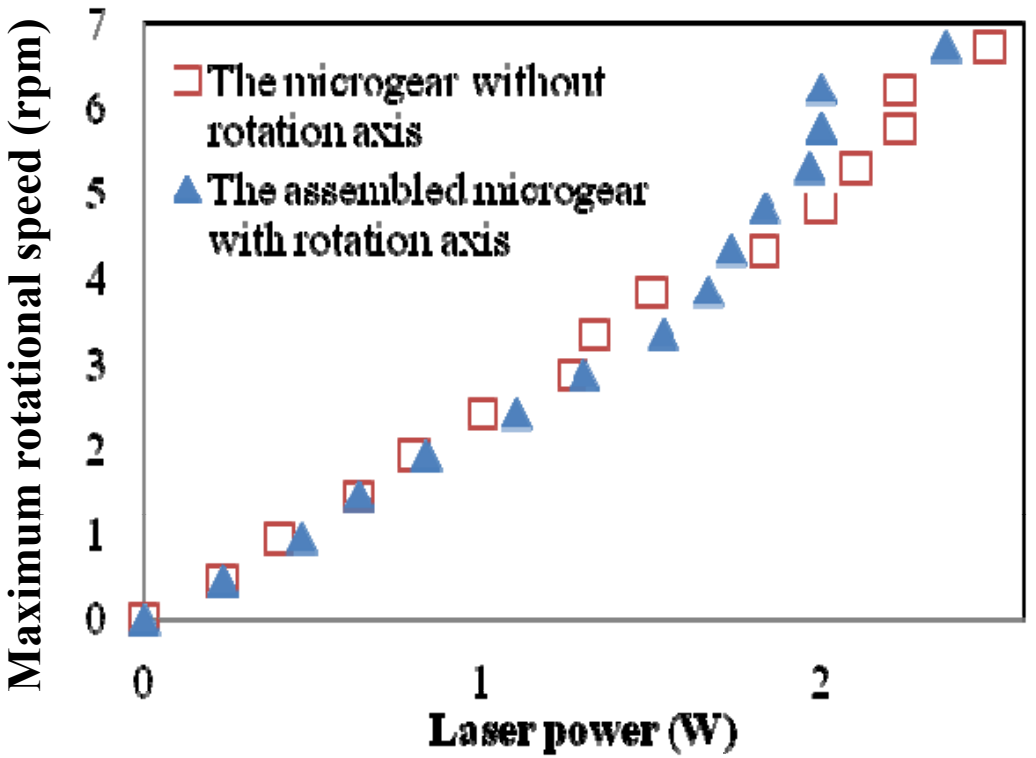




M. Takeuchi et al., Proc of ICRA2009, 2009, M. Ito et al.
Trans. of the Japan Soc. of Mech. Eng., C, Vol. 75, No. 752, pp. 194-199, 2009 (in Japanese)

Rotation speed of assembled microgear





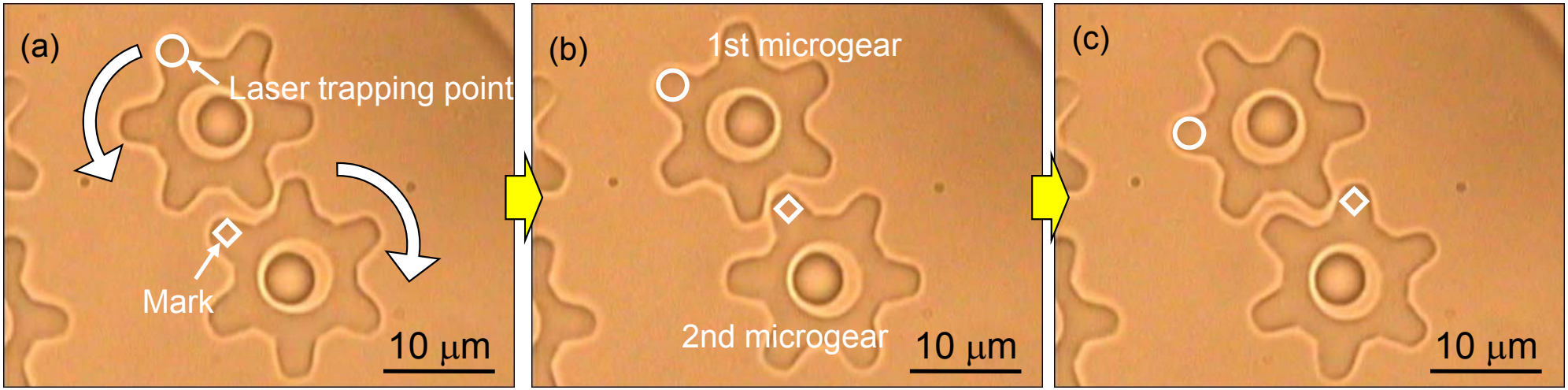
Rotation speed depending on the laser power

- The rotation speed is almost proportional to the laser power
- The rotation speeds of the microgear without rotation axis and the assembled microgear with rotation axis are almost same

M. Takeuchi et al., Proc of ICRA2009, 2009, M. Ito et al.
Trans. of the Japan Soc. of Mech. Eng., C, Vol. 75, No. 752, pp. 194-199, 2009 (in Japanese)

Driving the 2nd microgear

We succeeded in driving the 2nd microgear by rotation of 1st microgear



Using this microstructure, it is able to transmit the motion with high accuracy in the microchip

M. Takeuchi et al., Proc of ICRA2009, 2009, M. Ito et al.
Trans. of the Japan Soc. of Mech. Eng., C, Vol. 75, No. 752, pp. 194-199, 2009 (in Japanese)

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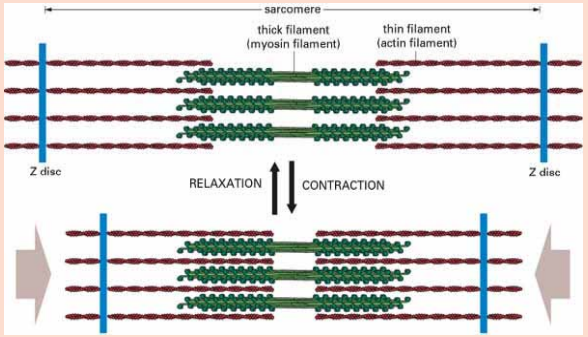
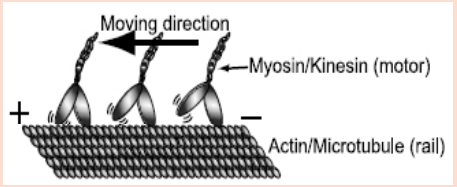
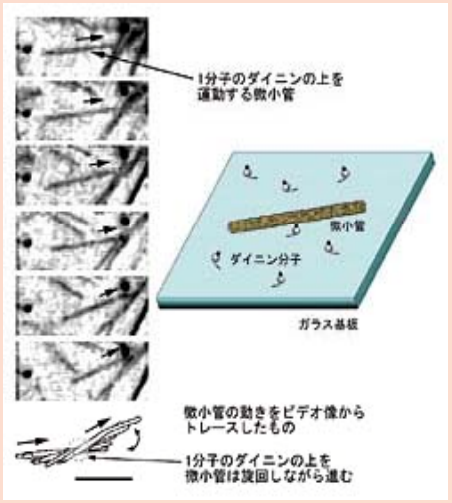
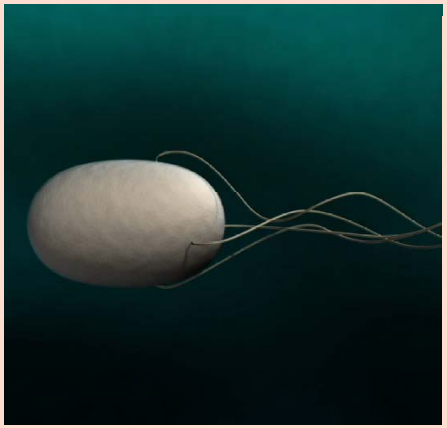
Biological Actuator

Actin-Myosin	Microtube-Kinesin	Microtube-Dynin	Flagellar Motor
			
(J. Spudich et al., Stanford Univ.)	(Yokokawa et al., Tokyo Univ.)	(K. Ooiwa, Mirai-ICT Research Center)	(Nanba et al., Osaka Univ.)

Nano-structures based on protein

- **Actin filaments: ~6-7 nm (dia.)**
- **Myosin: ~30 nm (dia.)**
- **Kinesin: ~80 nm (dia.)**
- **Flagellar motor: ~40 nm (Ring dia.)**

Energy Efficiency

Flagellar motor = ~100%

Muscle (Actin-Myosin) > 70 %

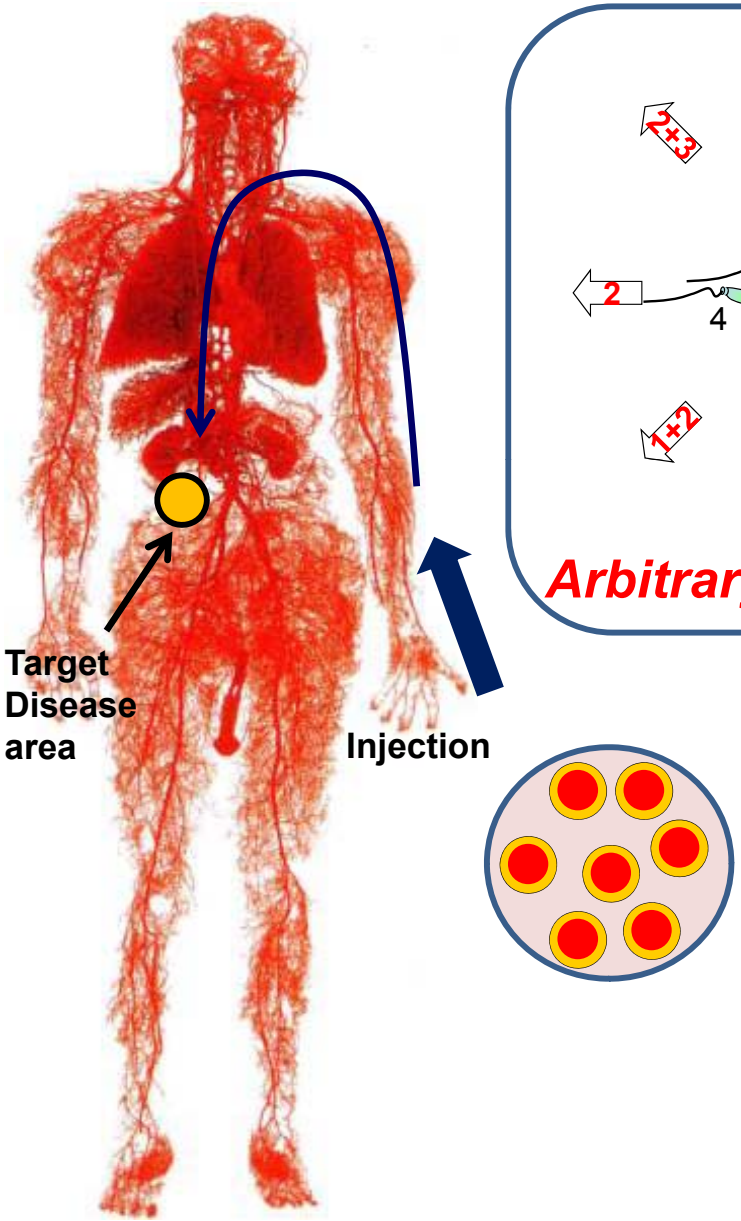
Internal-Combustion Engine < 40 %

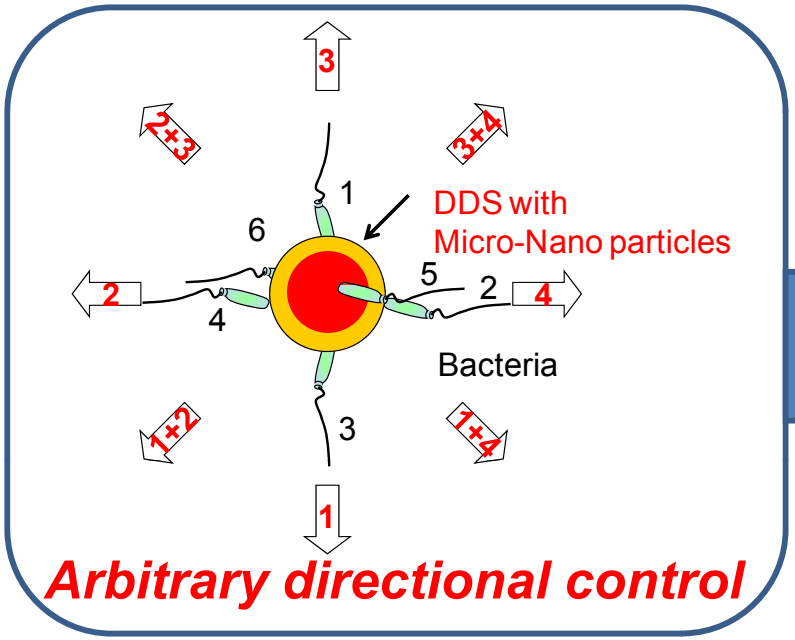
Macro world: Chemical Energy ⇒ Thermal Energy ⇒ Mechanical Energy

Nano world (Biological Actuator): Chemical Energy ⇒ Mechanical Energy

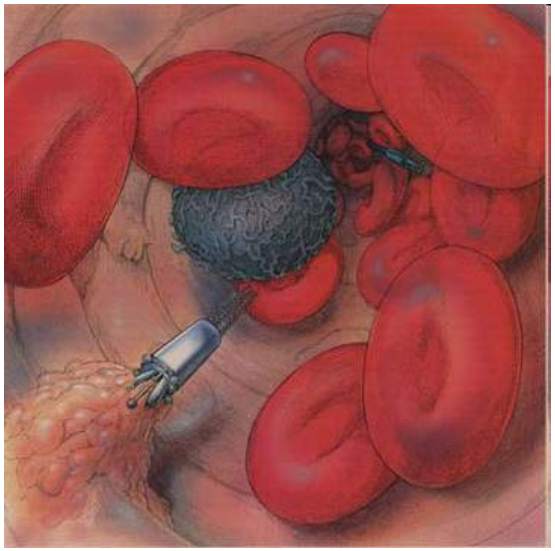
Micro-Nano System with Bacteria Driving Mechanism

Application for DDS (Drug-Delivery-System)

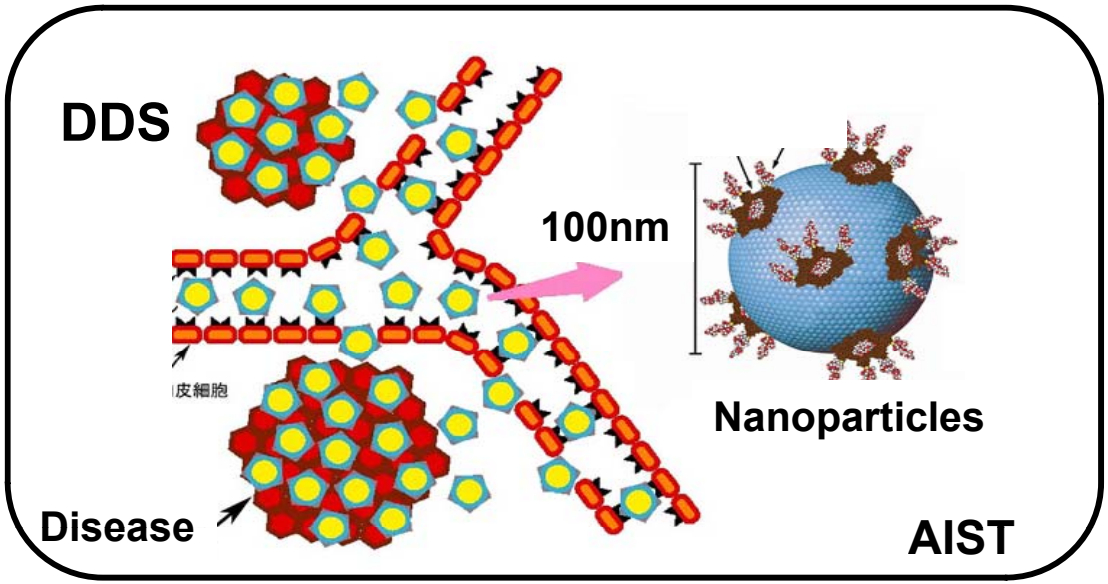




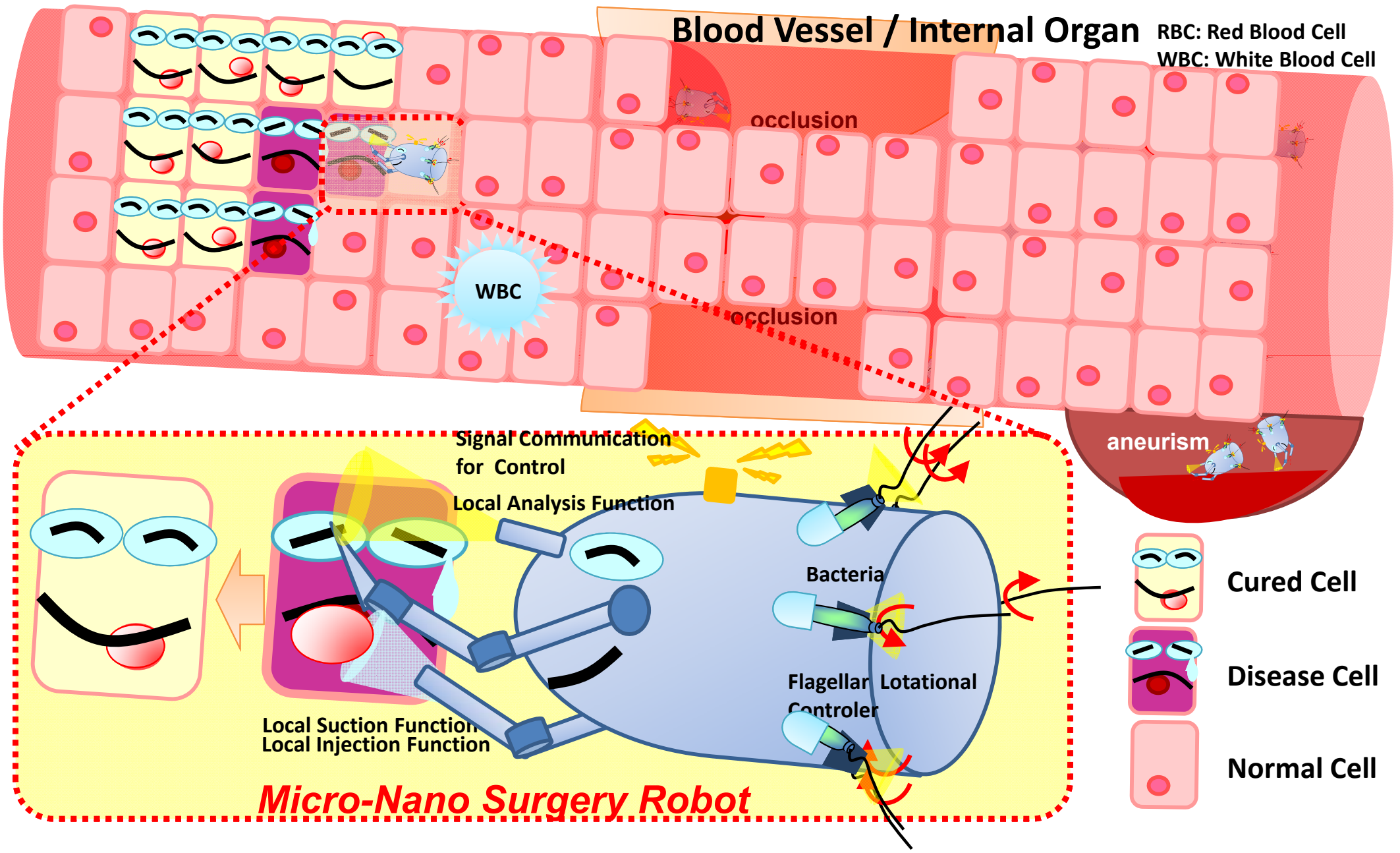
Micro-Nano machines inside blood vessel



Scientific American, 1998

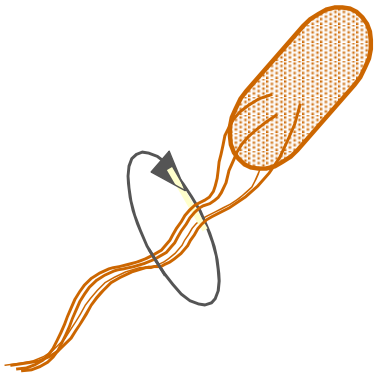


Future Micro-Nano Robot by Bacteria Driving Mechanism

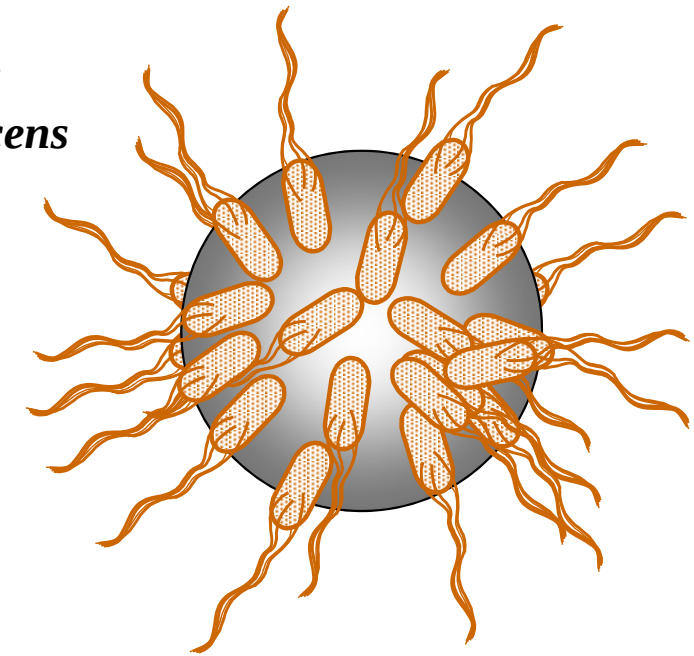


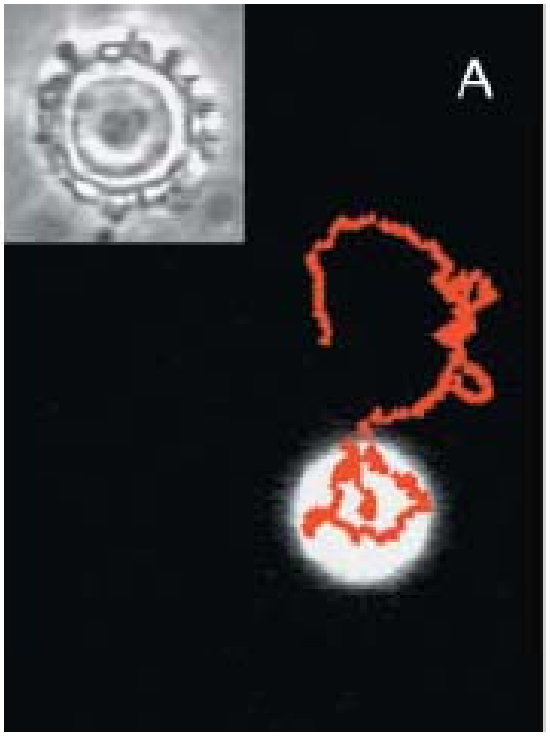
Background -Future Bacterial Driven Micro-Nano Robot-

Conventional Research



Serratia marcescens



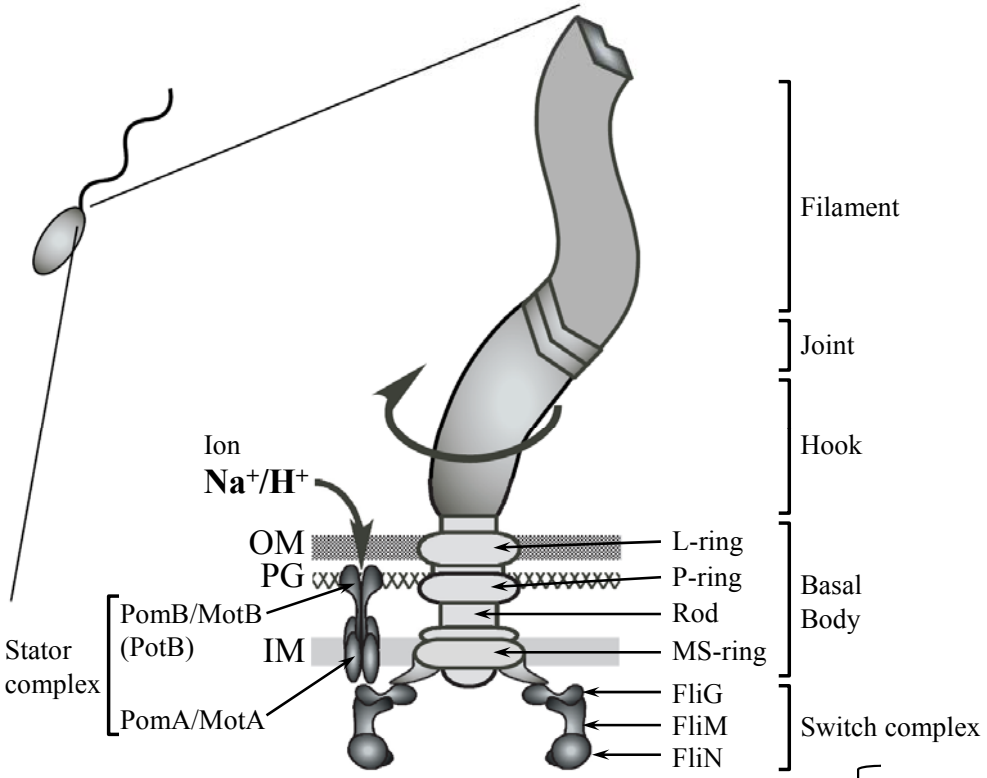


Polystyrene beads were moved by attached bacteria

Darnton et al. Biophysical J. 2004

Structure of Bacterial Flagellar Motor

Many bacteria swim in liquid with rotating their flagellar filaments by flagellar motor.



Known information

- Structure of flagellum
- Energy source is H^+/Na^+
- Flagellar stepping rotation and its step size

Energy Source: Ion motive force

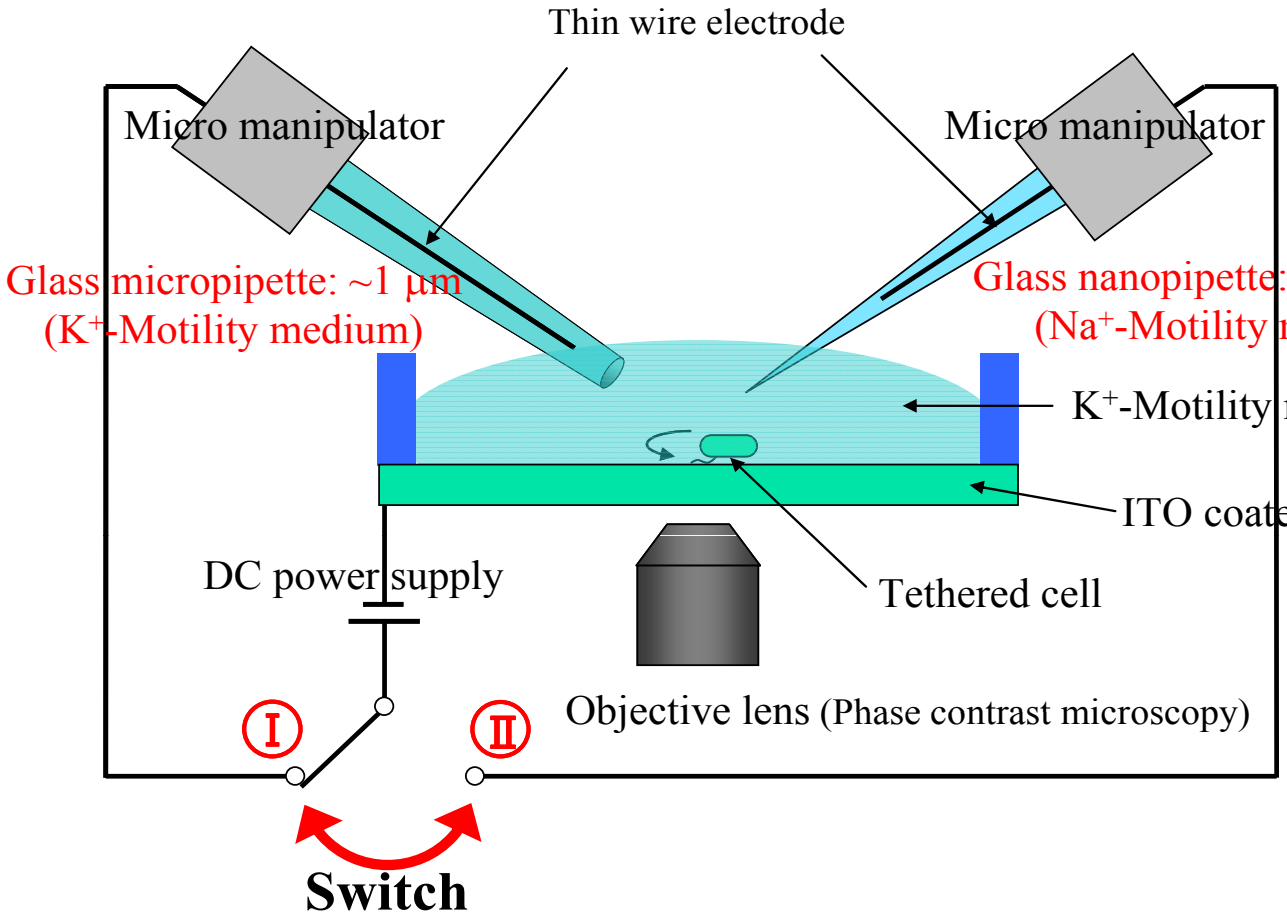
$$IMF = V_m - k_B T / q \ln(C_o / C_i)$$

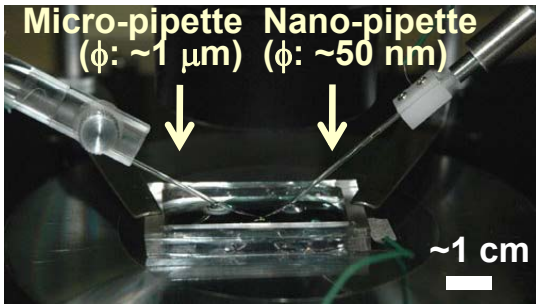
IMF: converts ion-motive force
V_m : transmembrane voltage (inside minus outside)
k_B : Boltzmann's constant,
T : absolute temperature
q : charge
C_i, C_o: concentrations of coupling ion inside and outside cell

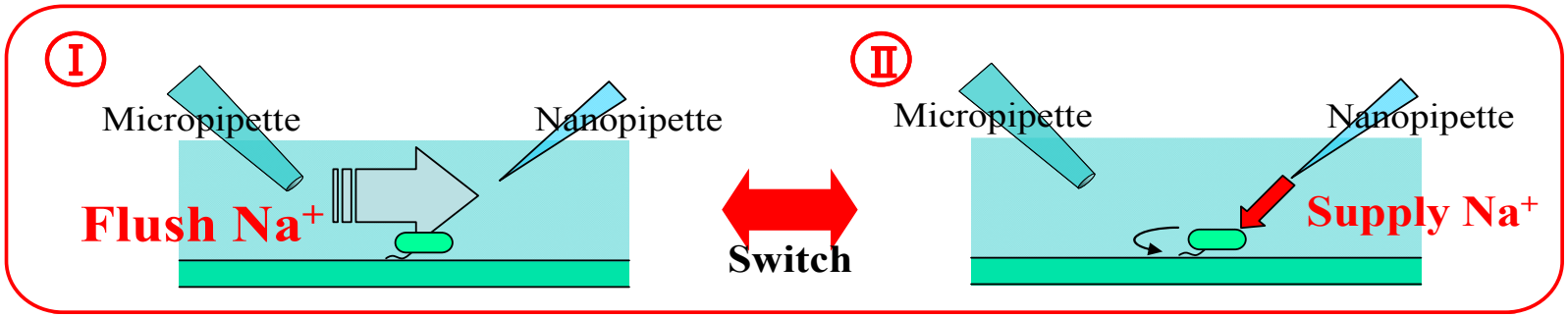
Y. Sowa, et al., Rev. Biophys., Vol. 41, No. 2, pp. 103-132, 2008.

The method to control the flagellar motor has not been established.

Switching of Spots with Dual Pipettes



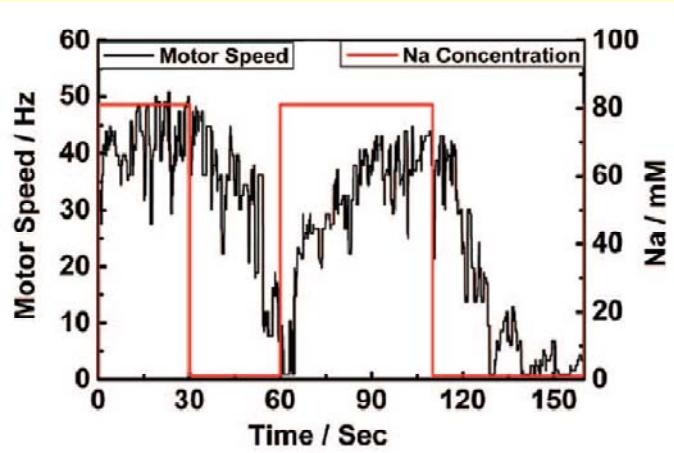




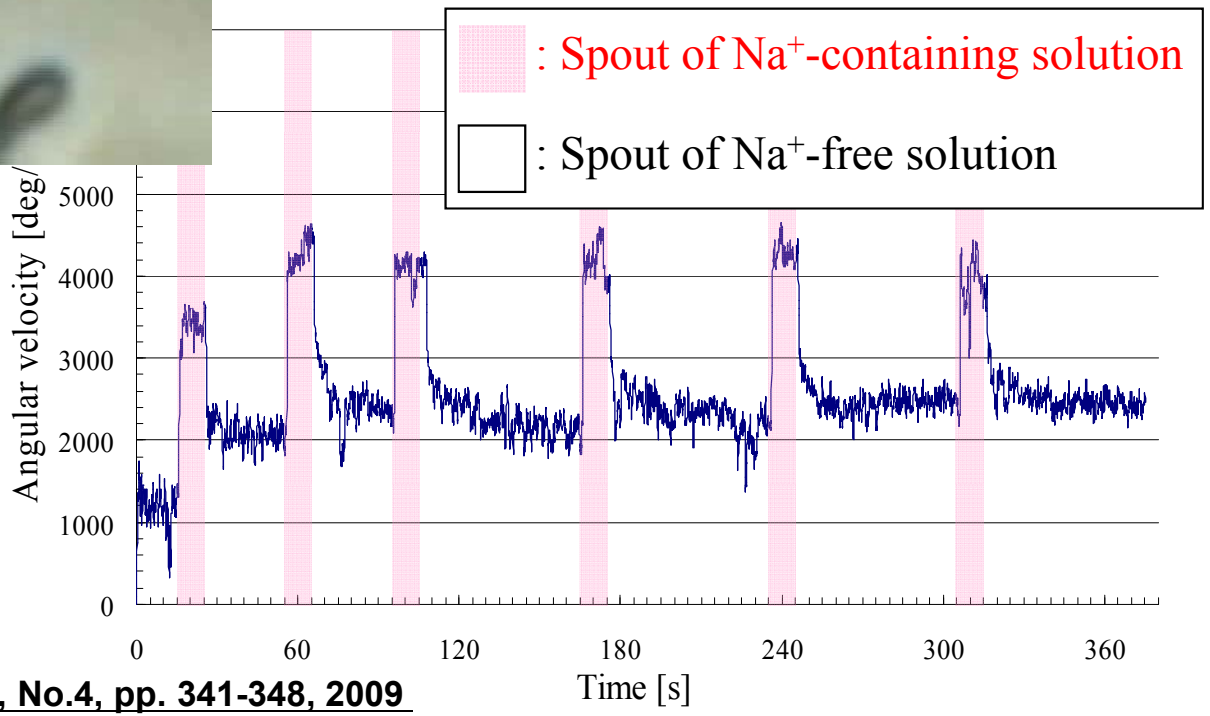
K. Nogawa et al., IEEE Trans. Nanobioscience, Vol.8, No.4, pp. 341-348, 2009

Switching of Spots with Dual Pipettes

＜Conventional research＞

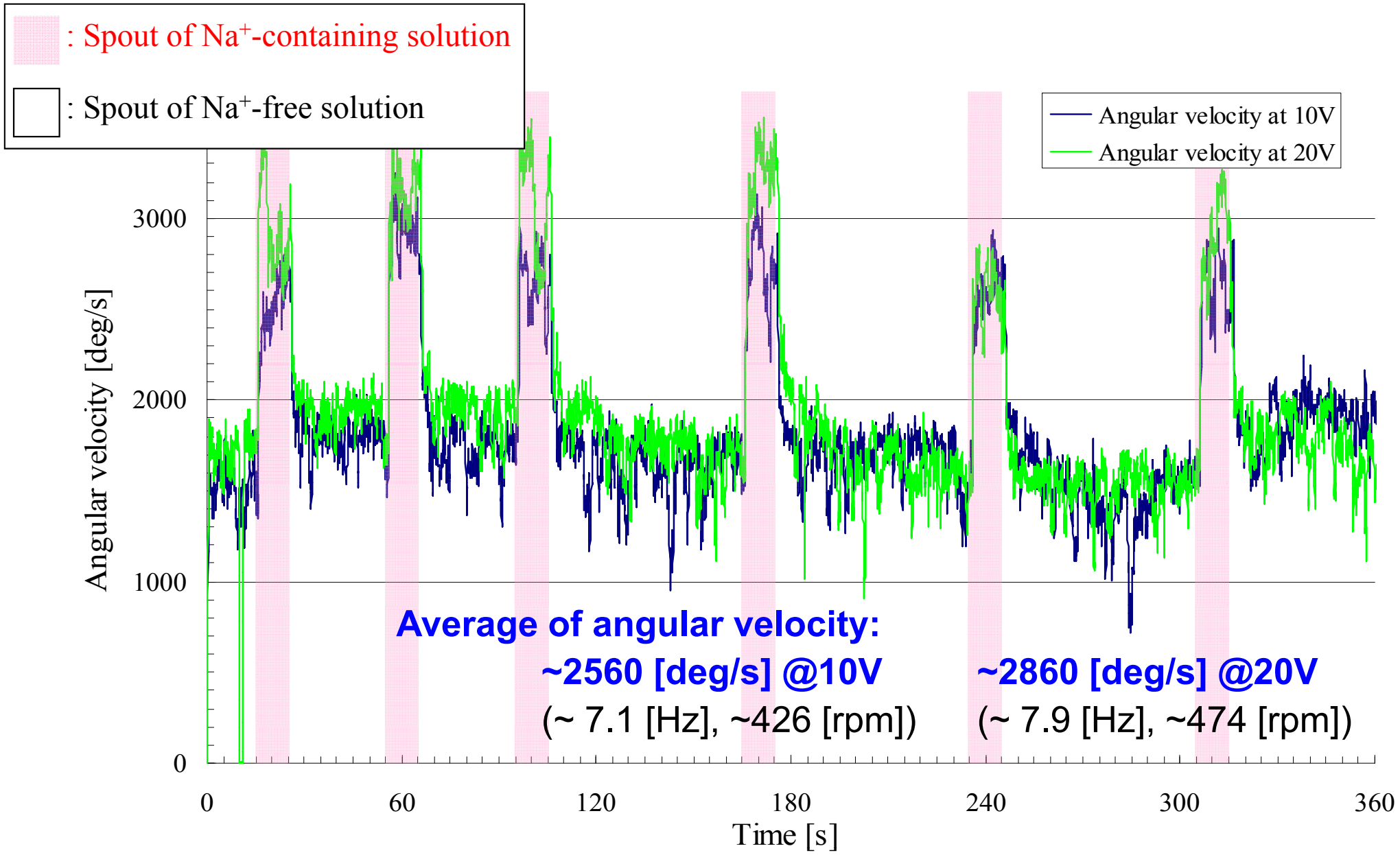


Piper *et al*, JACS, 2008, 130 (31), 10386-10393



K. Nogawa et al., IEEE Trans. Nanobioscience, Vol.8, No.4, pp. 341-348, 2009

Switching of Spots with Dual Pipettes



K. Nogawa et al., IEEE Trans. Nanobioscience, Vol.8, No.4, pp. 341-348, 2009

Contents

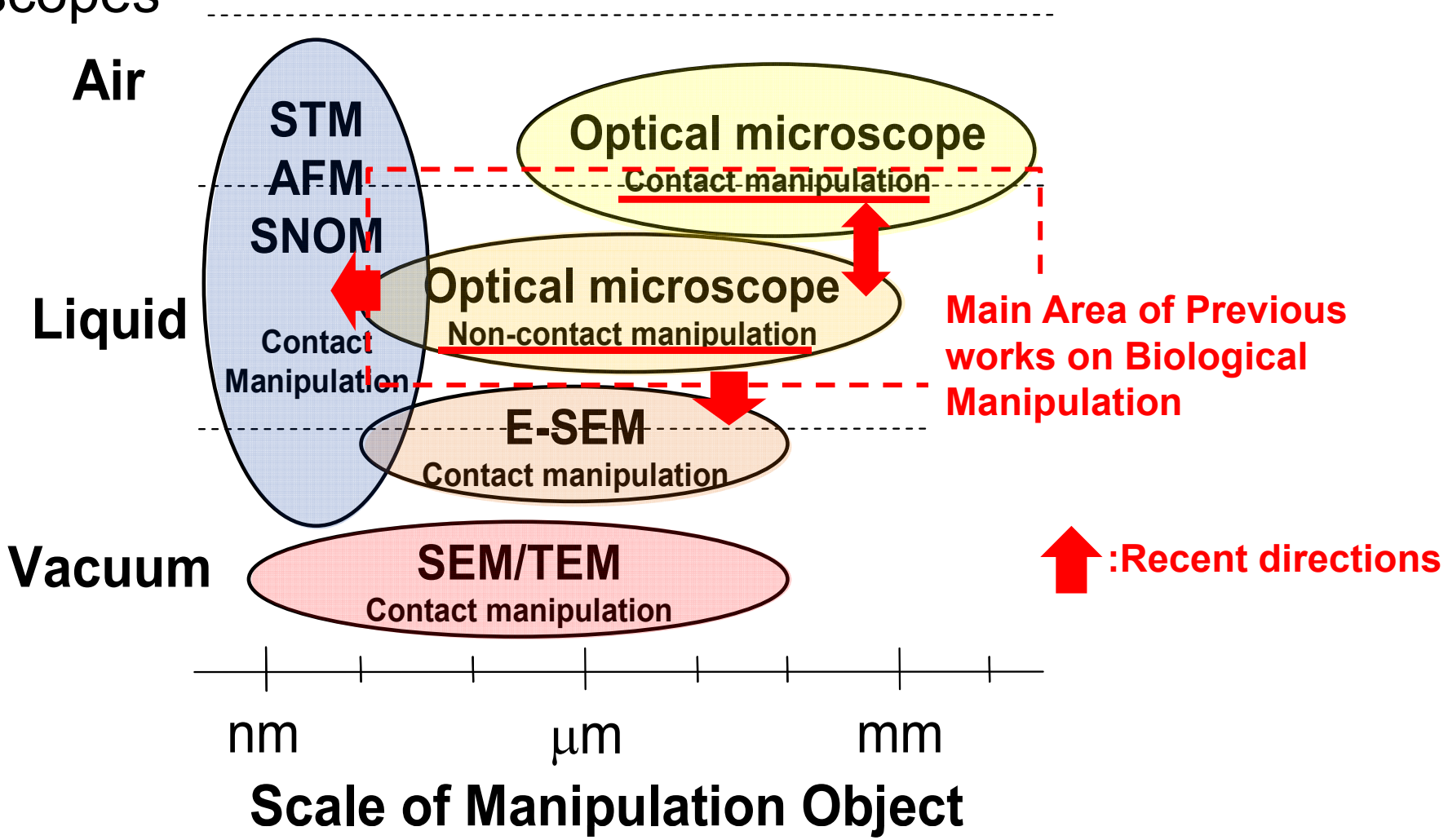
- Introduction

- Bio-Micro-manipulation System
  <Laser-trapping and probe type Micromanipulation system>
  - ➢ Semi-closed microchip
  - ➢ Rapid prototyping of Micro structures inside micro-channel
  - ➢ Control of rotation of flagellar motor with dual Micro-Nano pipettes
- Bio-Nano-manipulation System
  <Environmental-SEM (E-SEM) Nanomanipulation System>
  - ➢ Single Cell Viability Evaluation (Local Stiffness/Electric Property)

- Conclusion and Future Works

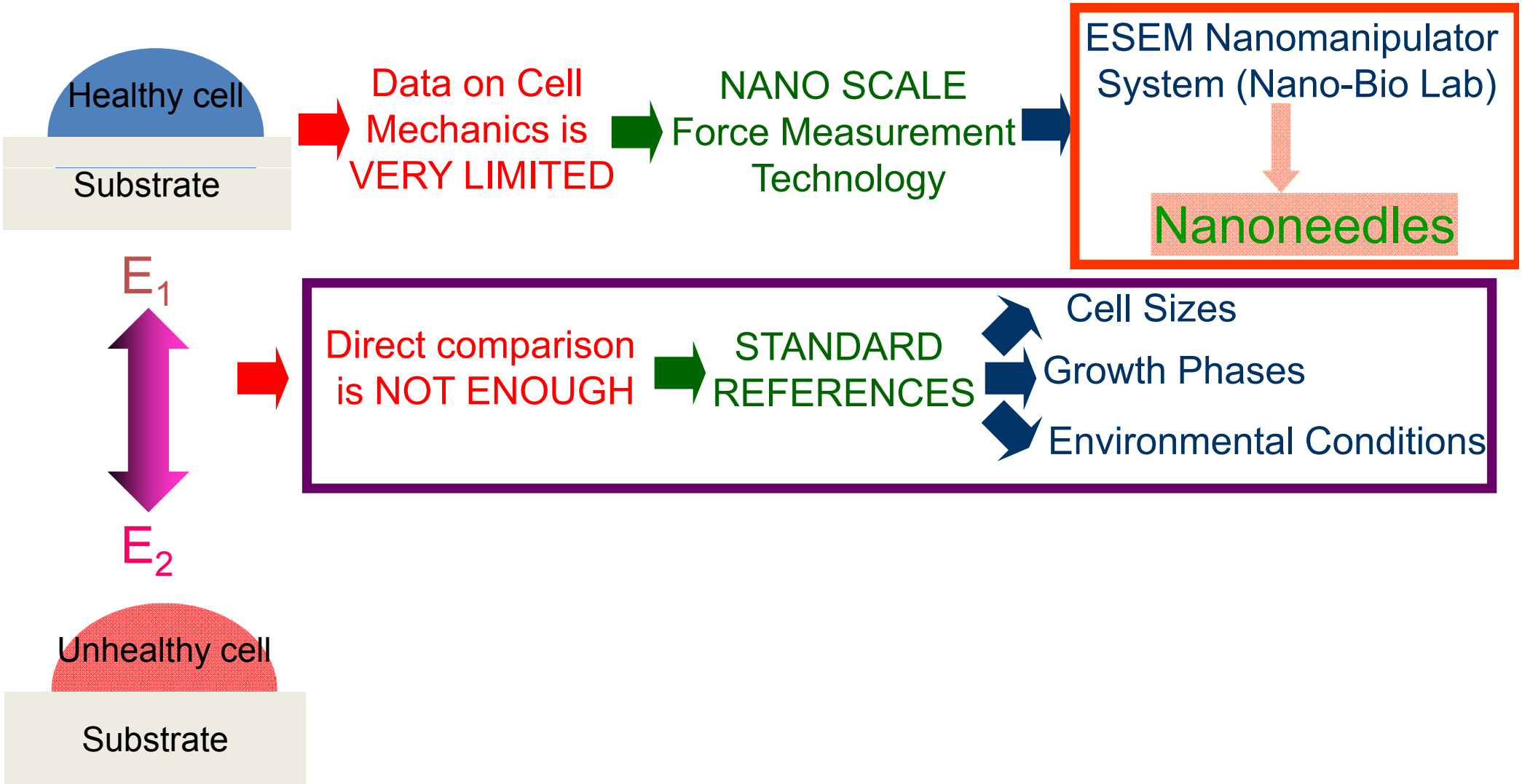
Micro-Nanorobotic Manipulation System

Micro-Nanorobotic Manipulation System under various Microscopes



Problems

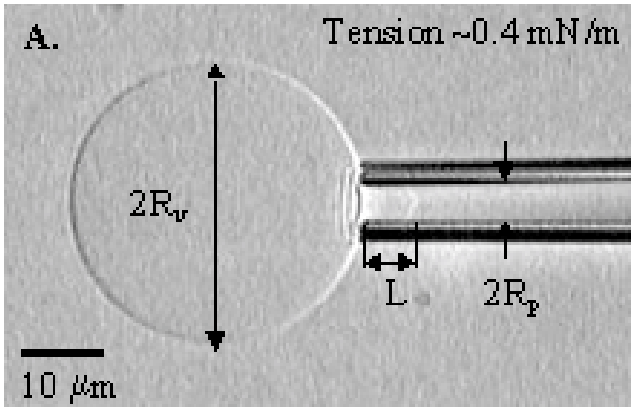
PROBLEMS	SOLUTIONS	PROPOSES

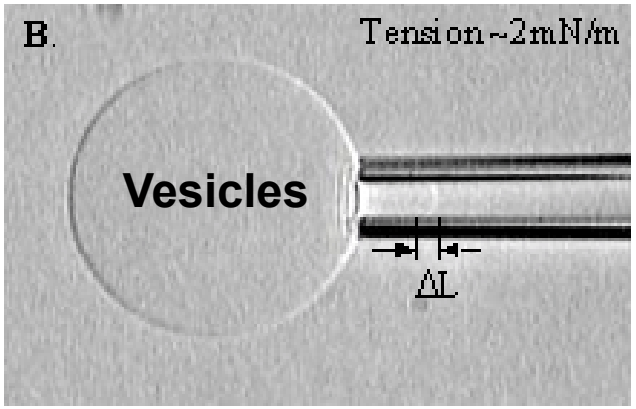


IEEE Transactions on Nanotechnology, Vol. 7 Issue 5, pp. 607-616, 2008
IEEE Transactions on Nanobioscience, Vol. 7 Issue 3, pp. 185-193, 2008

Conventional Methods for Cells Mechanical Characterizations

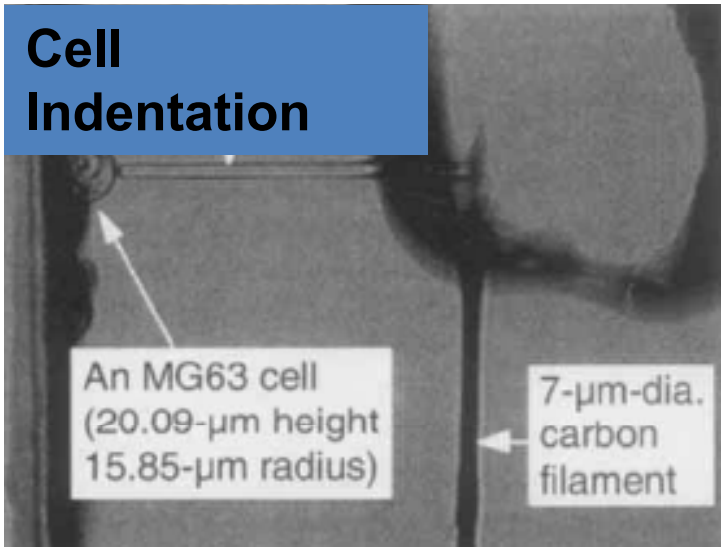
Micropipette Aspiration





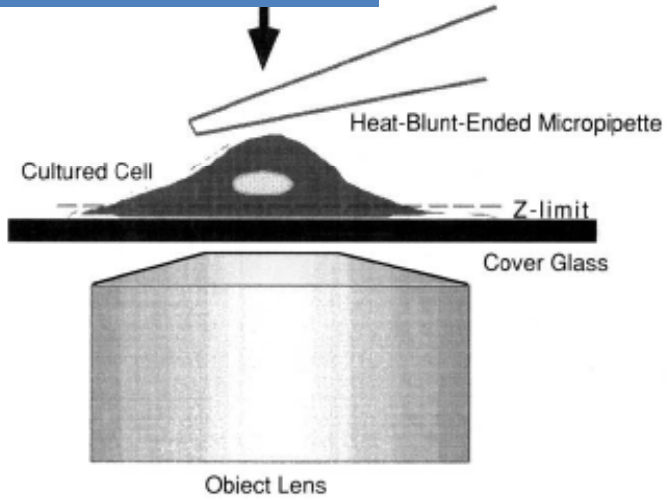
Langmuir Vol. 18 (2002)

Cell Indentation



Journal of Orthopaedic Research, Vol. 17 (1999)

Cell Poking



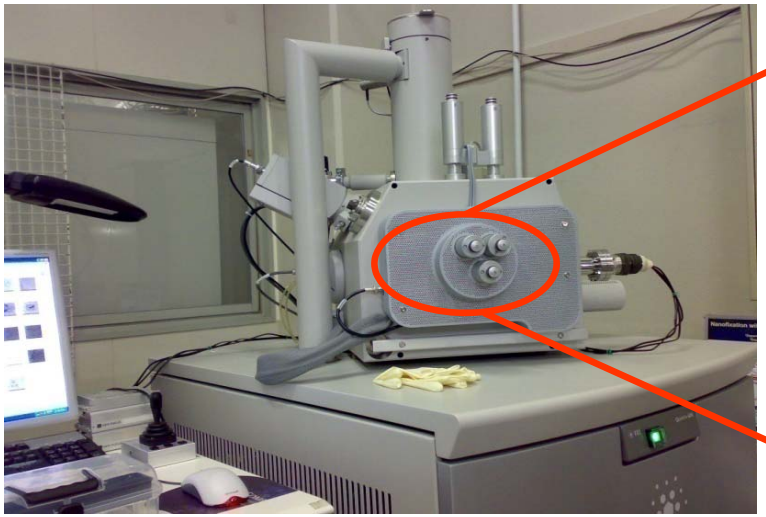
Materials Science and Engineering Vol. C17 (2001)

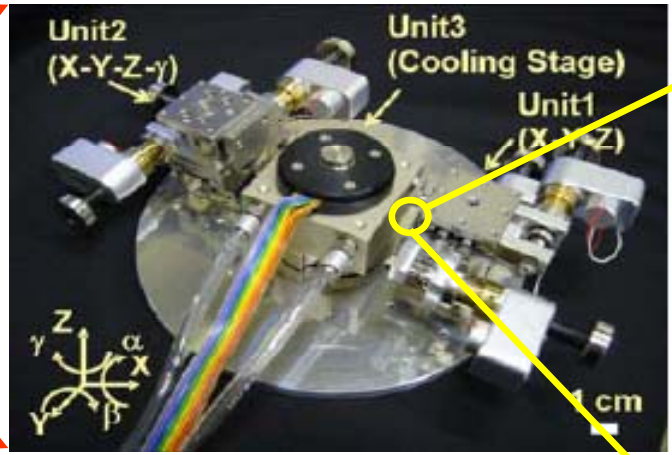
- Limited functionality
- Inflexible environment

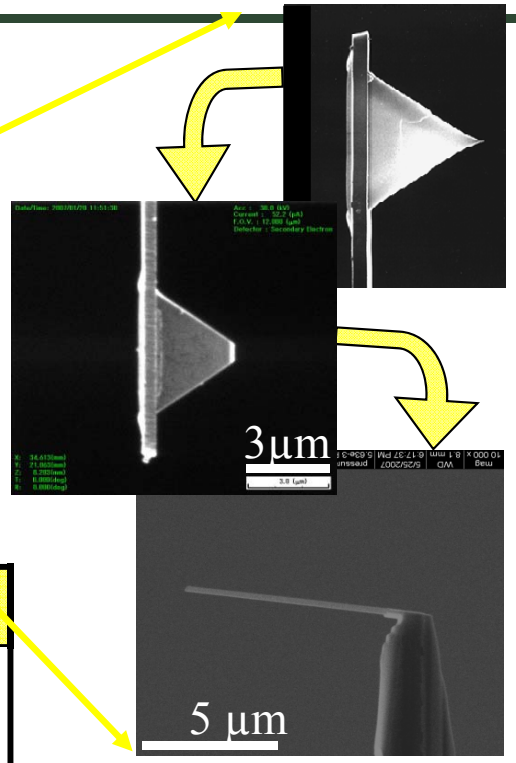
ESEM-Nanomanipulation System

ESEM-Nanomanipulation System

Materials & Methods







Environmental Scanning Electron Microscope (ESEM)	
Vacuum Mode	E-SEM Mode (10–2600 Pa) Low Vacuum Mode (10–130 Pa) High Vacuum Mode (~10^{-4} Pa)
Acc. Voltage	0.2 ~ 30 kV
Resolution	3.5 nm (E-SEM Mode) 15 nm (Low Vacuum Mode) 3.5 nm (High Vacuum Mode)
Obs. Space	150 mm × 150 mm × 65 mm
Max. Obs. Area	f0.5 mm (E-SEM Mode) f18 mm (Low and High Vacuum Mode)
Detectors	SED, RED

Nanomanipulator	
DOFs	Unit1: 4 DOFs (X-Y-Z-g), Unit2: 3 DOFs (X-Y-Z) Total: 7 DOFs
Actuators	7 Picomotors™, (Unit1, Unit2)
Work. Space	~ 16 mm × ~ 16 mm × ~12 mm × ±5°
Positioning Resolution	~ 30 nm (Unit 1, Unit2)
Cooling Stage	Unit3 (Cooling water temp. ± 20 ° C)

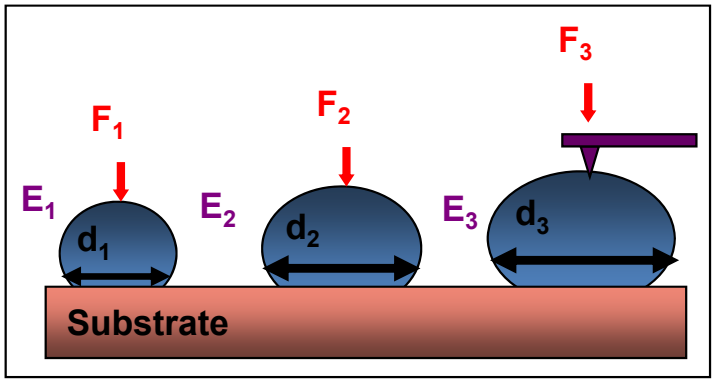
End-effector	
Multi-shapes	•Sharp Tip (0.02-2 N/m spring constants) •Flat Tip (0.2-2 N/m spring constants) •Nanoneedle (0.2, 0.8, 2 N/m spring constants) (< 200 nm diameters)

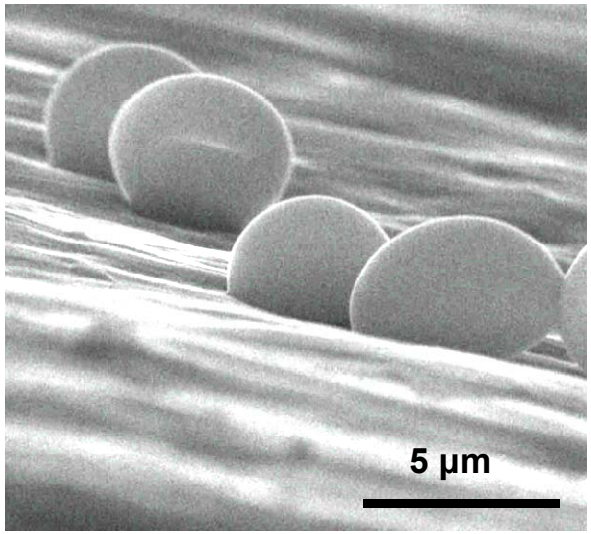
M. R. Ahmad et al., *IEEE Trans. on Nanobioscience*, 7(3), pp. 185-193, 2008.

Single Cell Elastic Characterizations

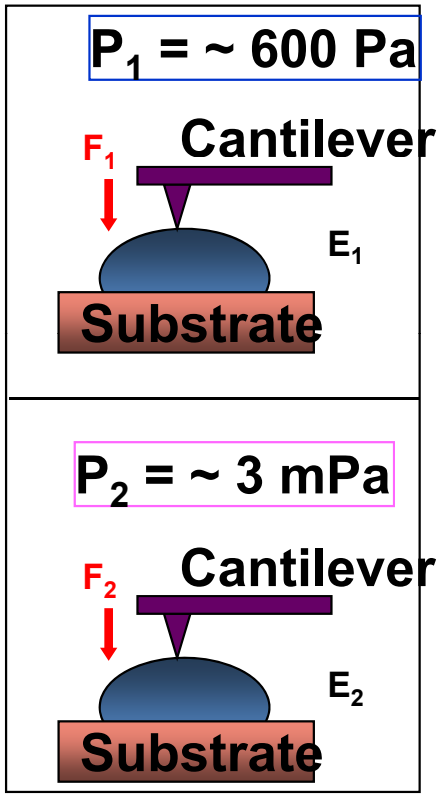
Purpose: To determine the elastic property of single cells

Different Cell Sizes





Different Environmental Conditions

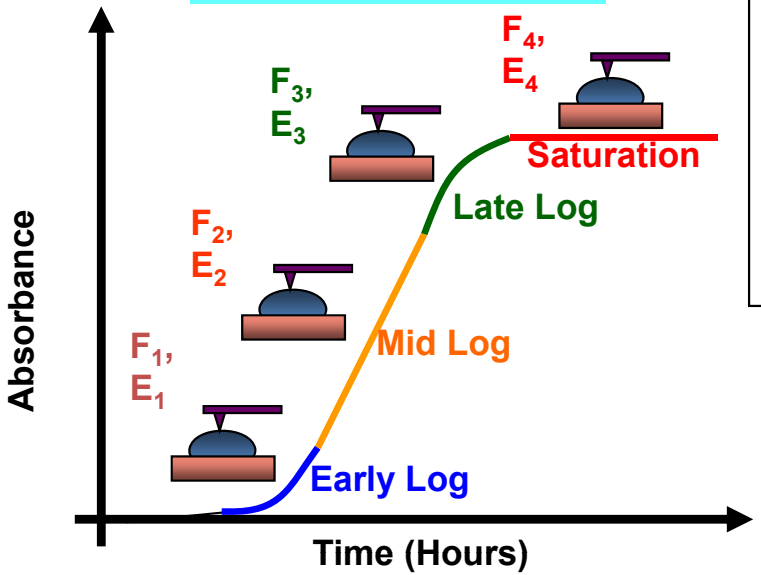


Experimental type:
Single cells penetration test

Cell type:
W303 wild-type yeast cells

Nano-probe:
Sharp tip cantilever
(*k*=0.02 - 0.09 N/m)

Different Growth Phases

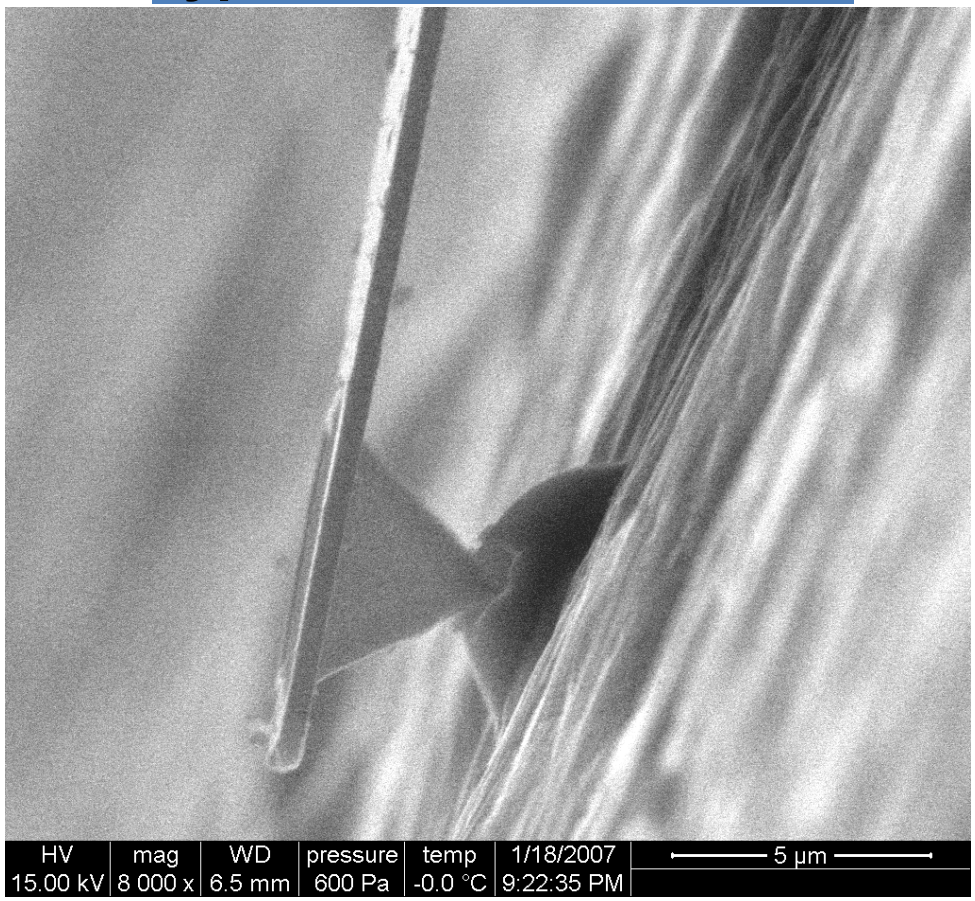


M. R. Ahmad et al., *IEEE Trans. on Nanobioscience*, 7(3), pp. 185-193, 2008.

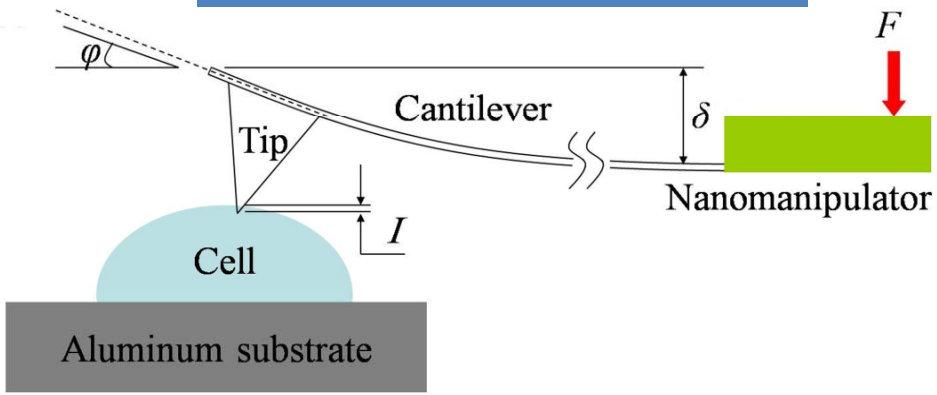
Parameters Determination

Single cells elasticity

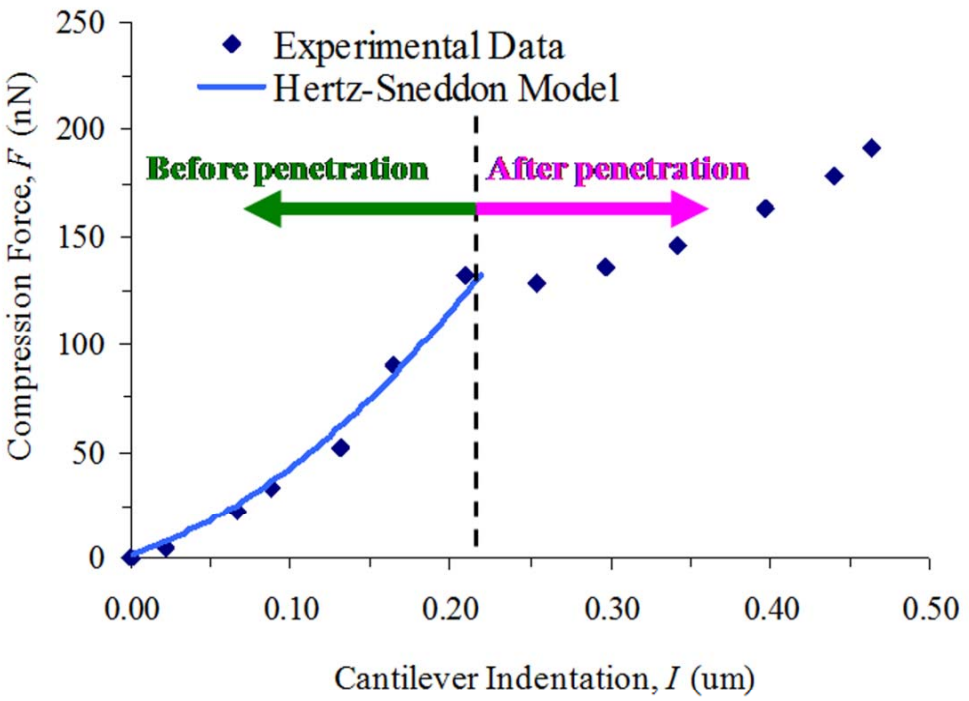
Typical Penetration Test



Hertz-Sneddon Model



Typical Force-Indentation Curve



Hooke's law:

$Force = k\delta = k[\varphi(2/3)L]$

Hertz-Sneddon model:

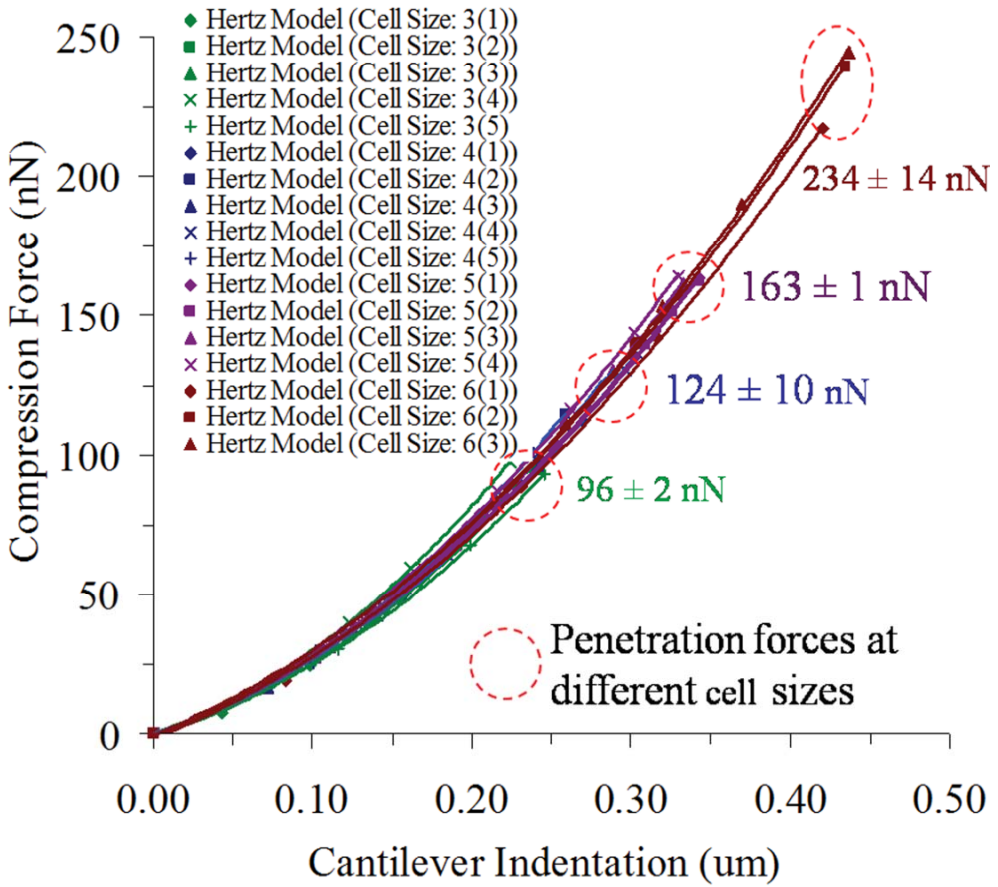
$Force_{cone} = (2/\pi)\tan\varphi(EI^2/1-v^2)$

M. R. Ahmad et al., *IEEE Trans. on Nanobioscience*, 7(3), pp. 185-193, 2008.

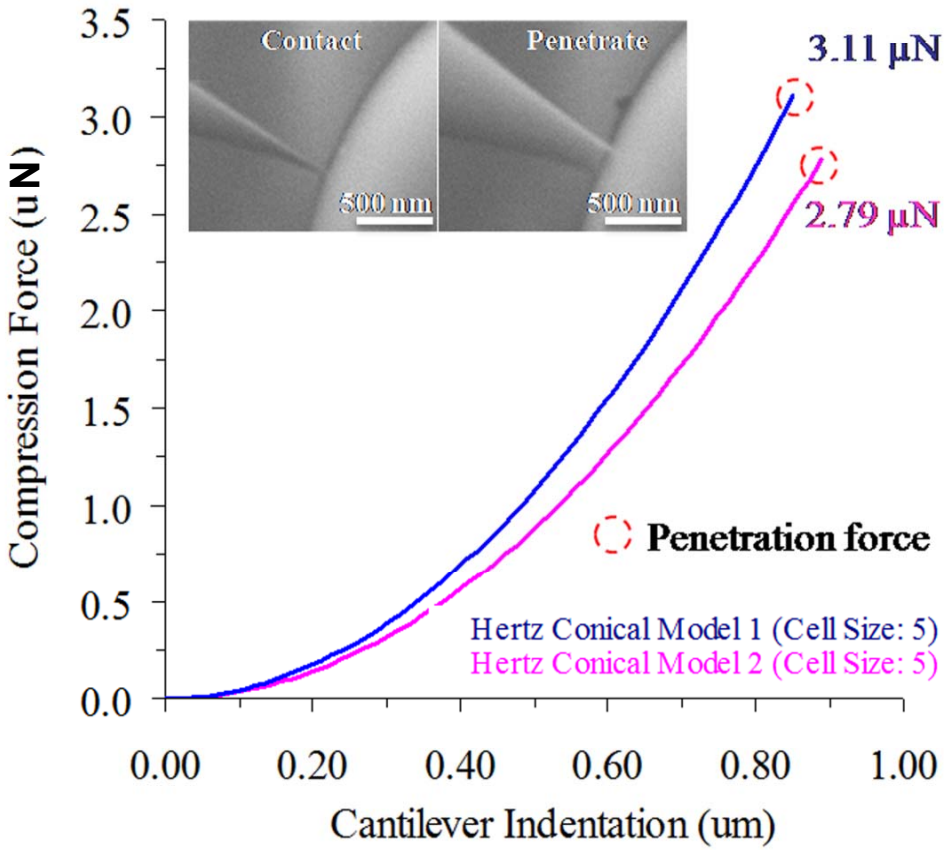
Results

Single cells elasticity

Cell Strength at Different Cell Sizes



Cell Strength at High Vacuum



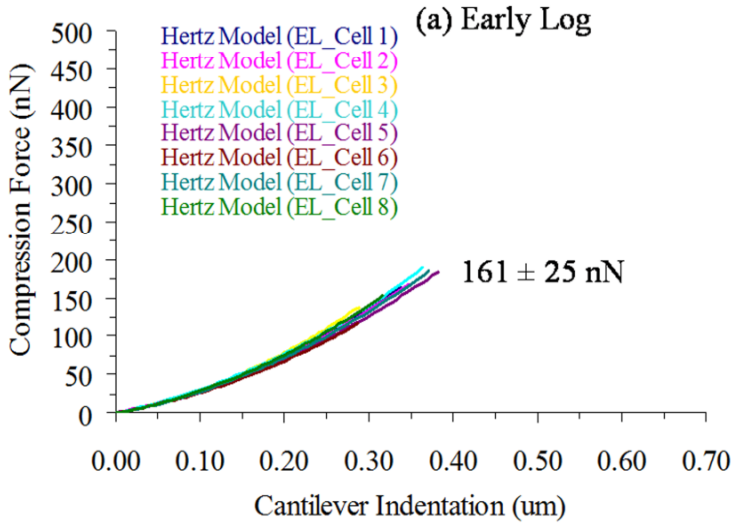
Cell stiffness increments when cell size increases

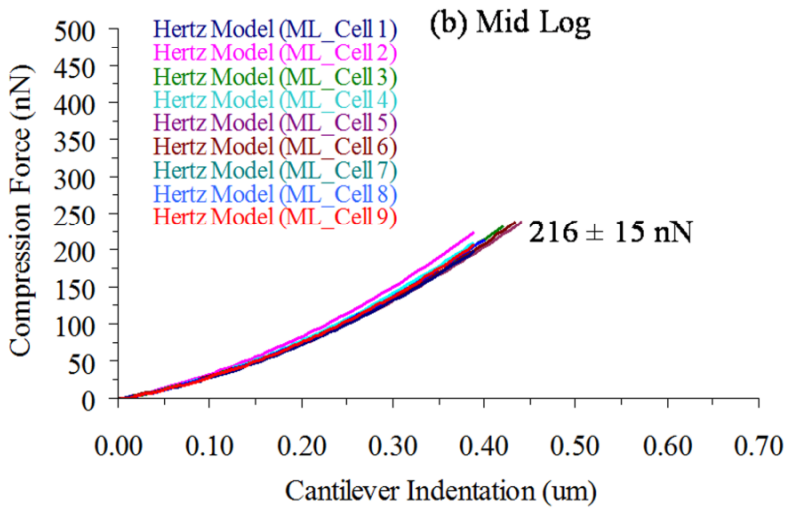
Cell stiffness significantly increases under a high vacuum condition

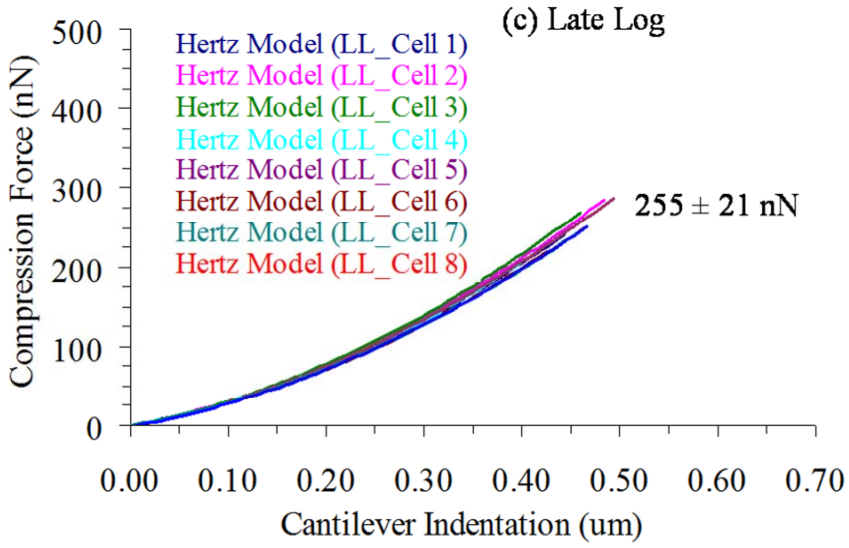
M. R. Ahmad et al., *IEEE Trans. on Nanobioscience*, 7(3), pp. 185-193, 2008.

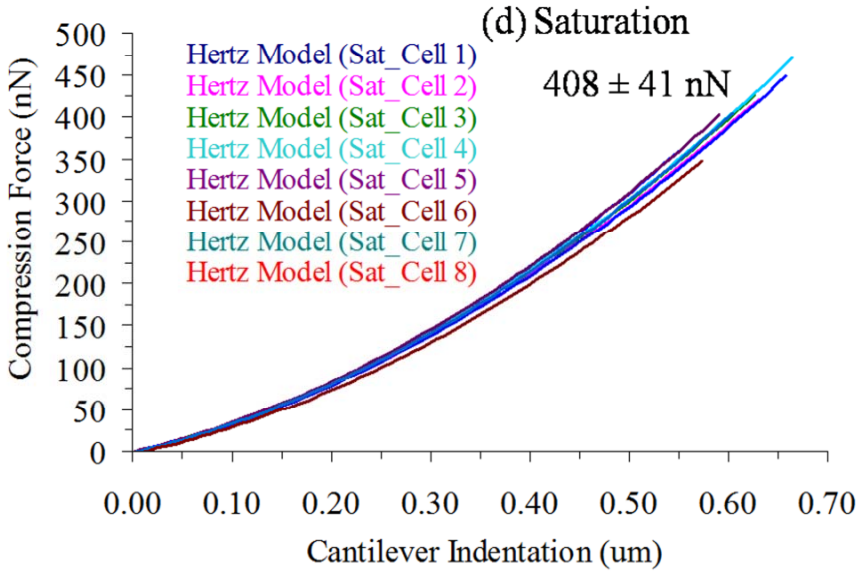
Results

Cell Strength at Different Growth Phases





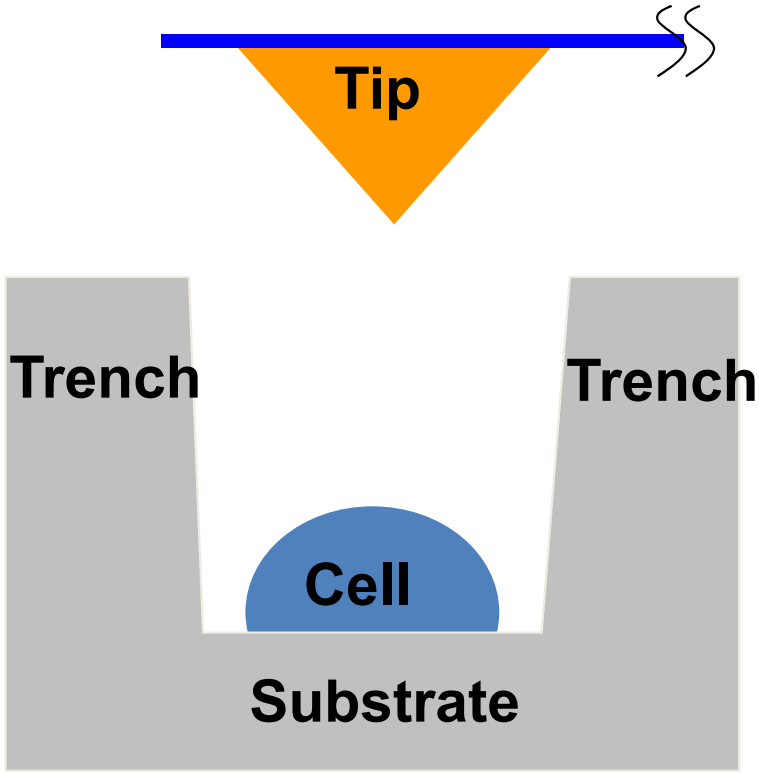




M. R. Ahmad et al., *IEEE Trans. on Nanobioscience*, 7(3), pp. 185-193, 2008.

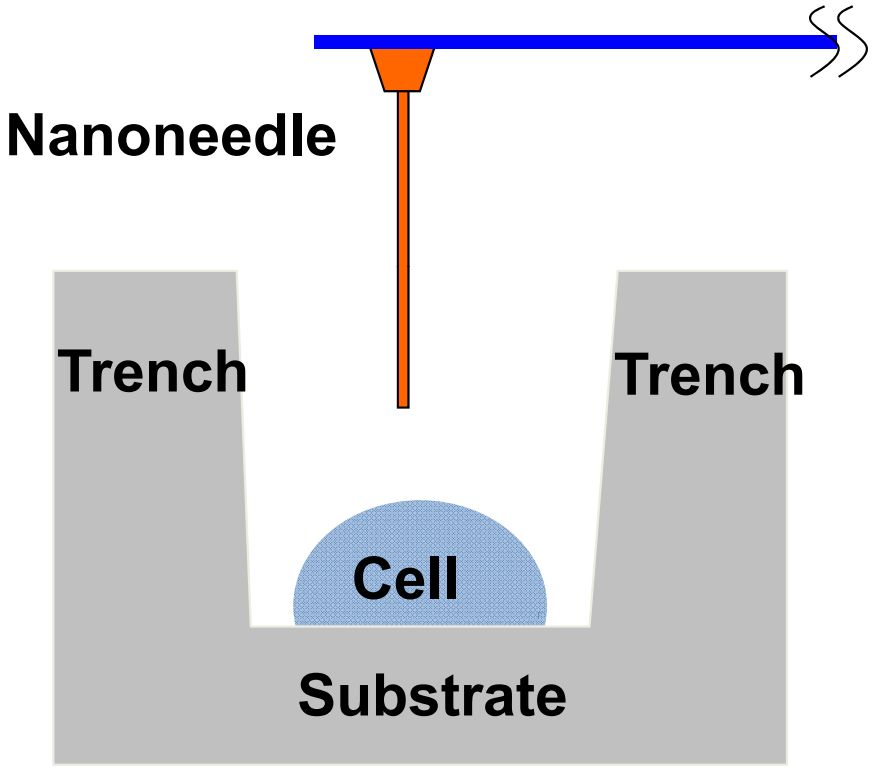
Problems

ISSUE:
Standard AFM cantilever tip has a low aspect ratio



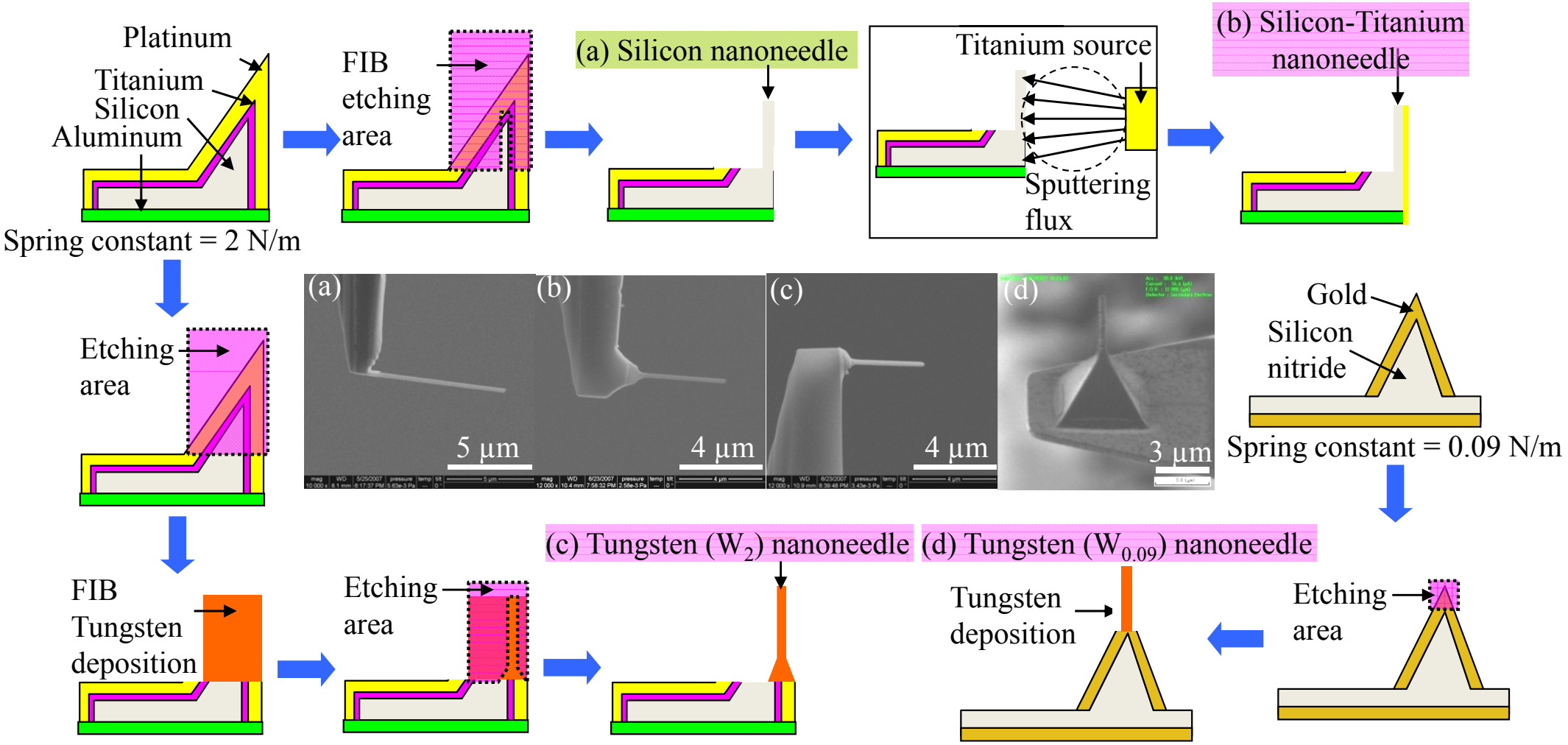
PROBLEM:
Tip can not reach sample that located under a trench

ISSUE:
Nanoneedle tip has a rigid body



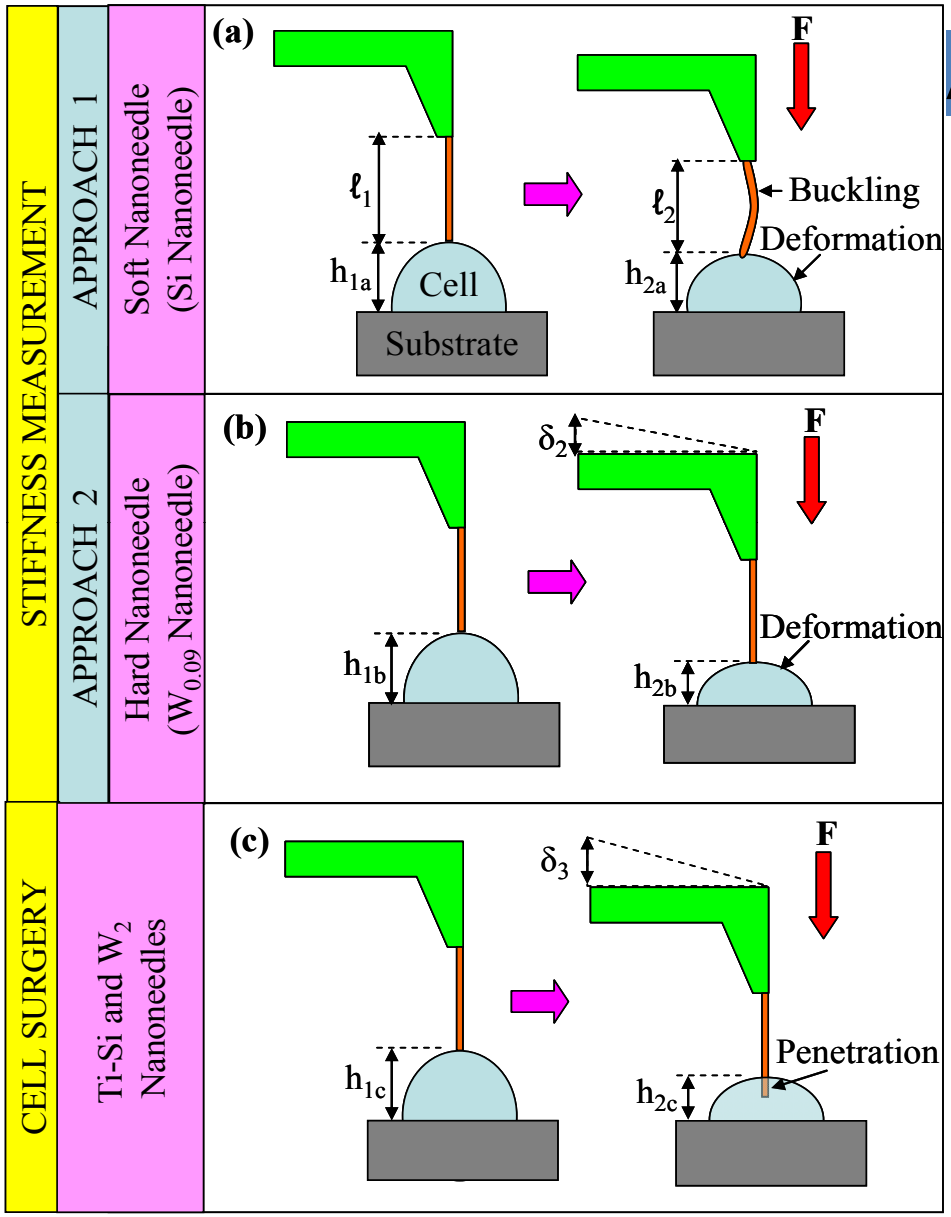
PROBLEM:
Sample may be damaged due to an excessive force

Fabrication Methods of Nanoneedles

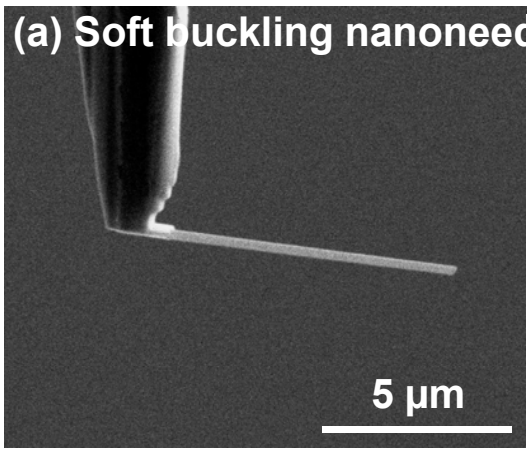


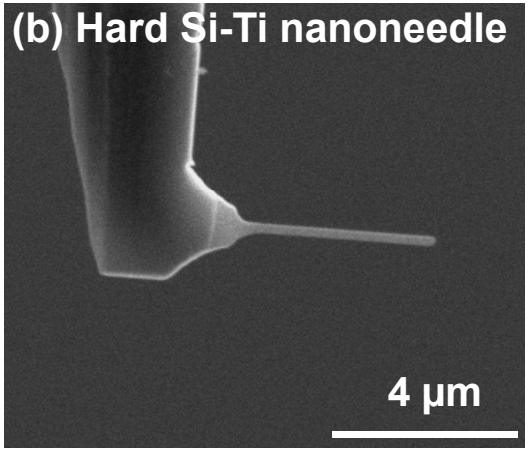
M/ R. Ahmad et al., IEEE Trans. on Nanotech, 7(5), pp. 607-616, 2008.

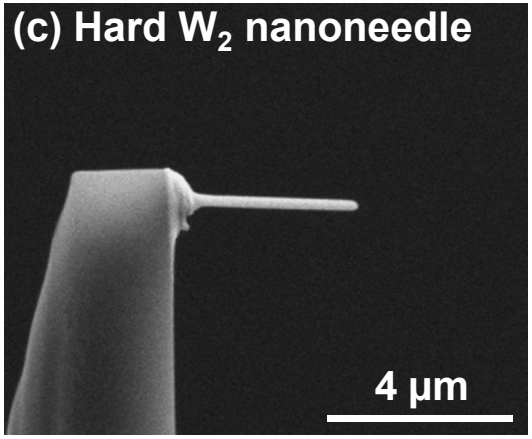
Nano-probes for Single Cells Analysis

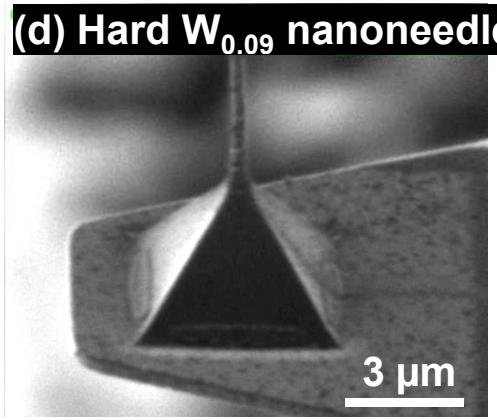


Actual images of the nano-probes







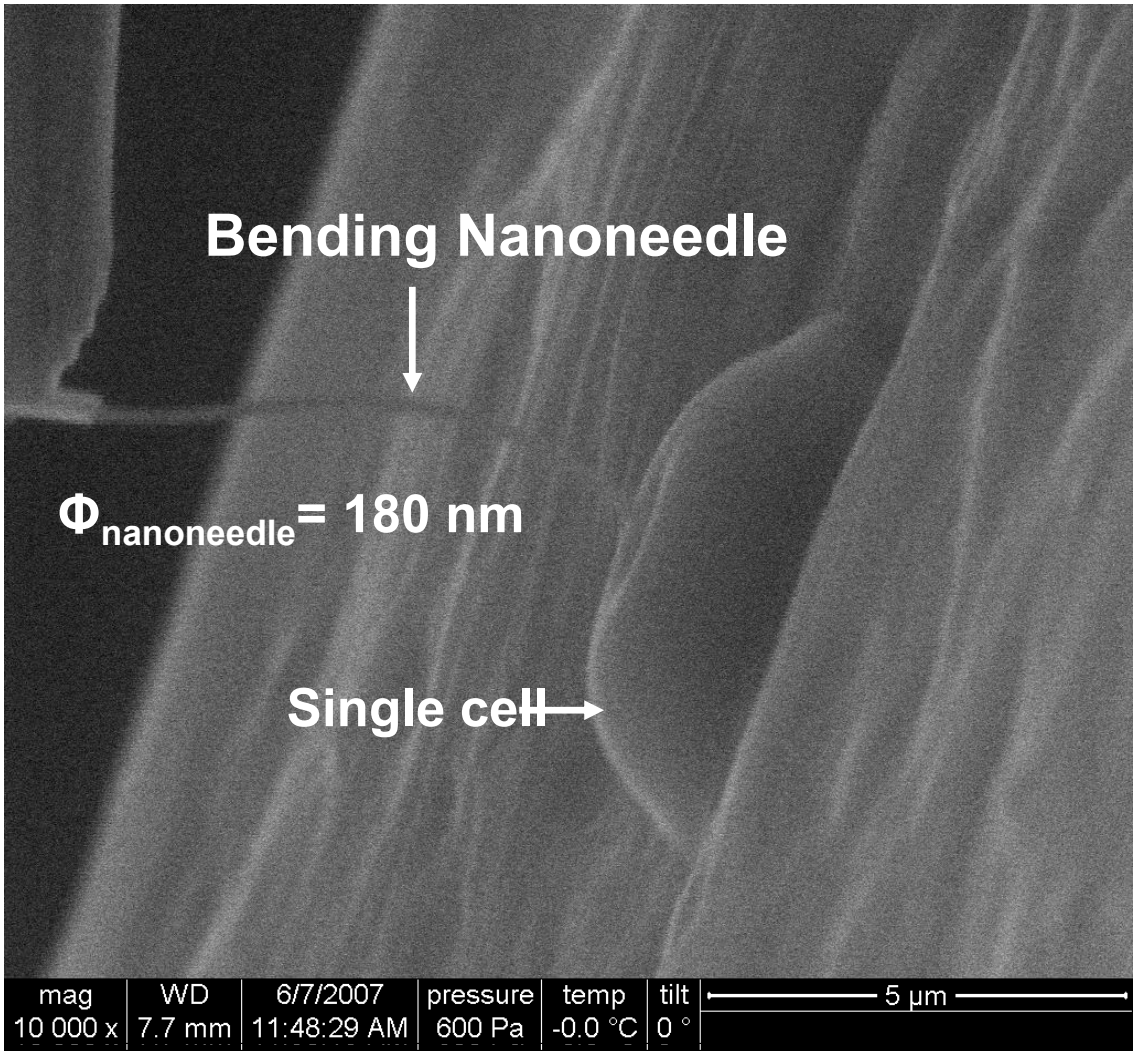


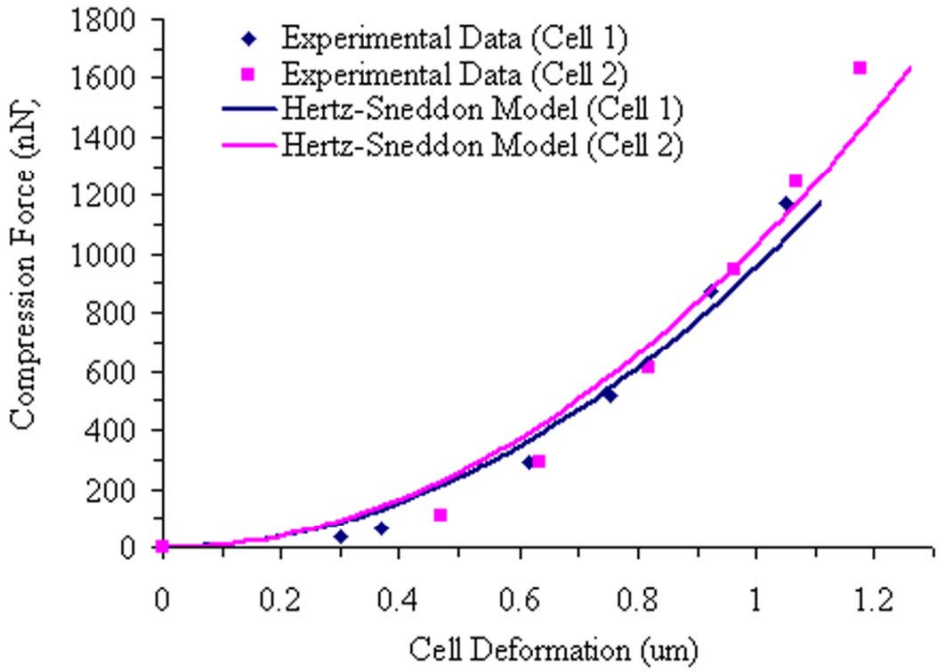
The diameter of the nano-probes is around 170 – 200 nm

M/ R. Ahmad et al., IEEE Trans. on Nanotech, 7(5), pp. 607-616, 2008.

Results

Whole Cell Stiffness Measurement by a Buckling Nanoneedle





	Cell Physical Parameters		Cell Stiffness Characteristics	
	Height (µm)	Diameter (µm)	Spring Constant (N/m)	Young Modulus (MPa)
Cell 1	2.824	6.524	0.92	3.64
Cell 2	3.062	6.417	0.95	3.92

M/ R. Ahmad et al., IEEE Trans. on Nanotech, 7(5), pp. 607-616, 2008.

Future Direction –System Cell Engineering-