Advanced 2
Micro-Nanorobotic Manipulation

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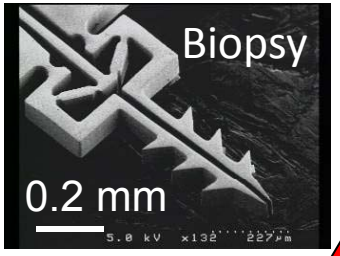
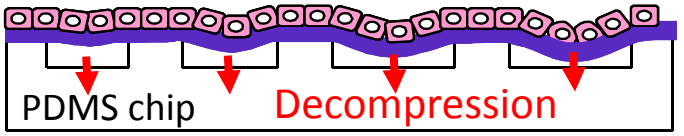
Multi-scale Bio-Manipulation

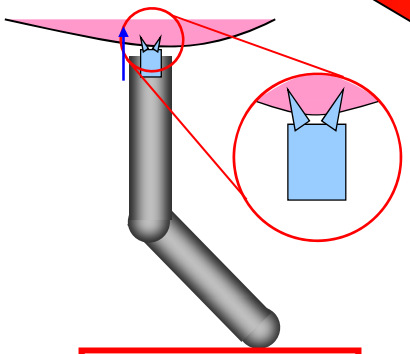


1. Tissues

Differentiation control
in tissue engineering

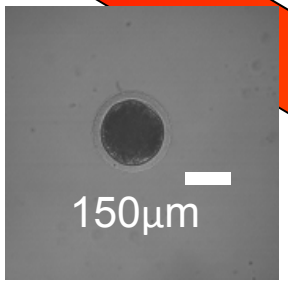


mm



Endoscope

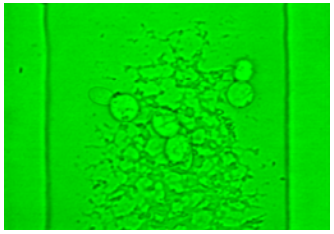
2. Oocyte



Oocyte

3. Cells

μm

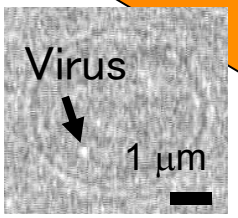


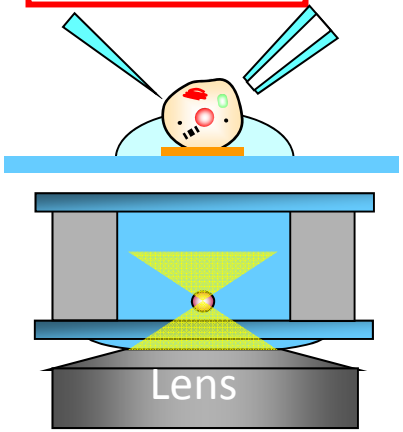
Micro-nanomanipulation

Bio-chip

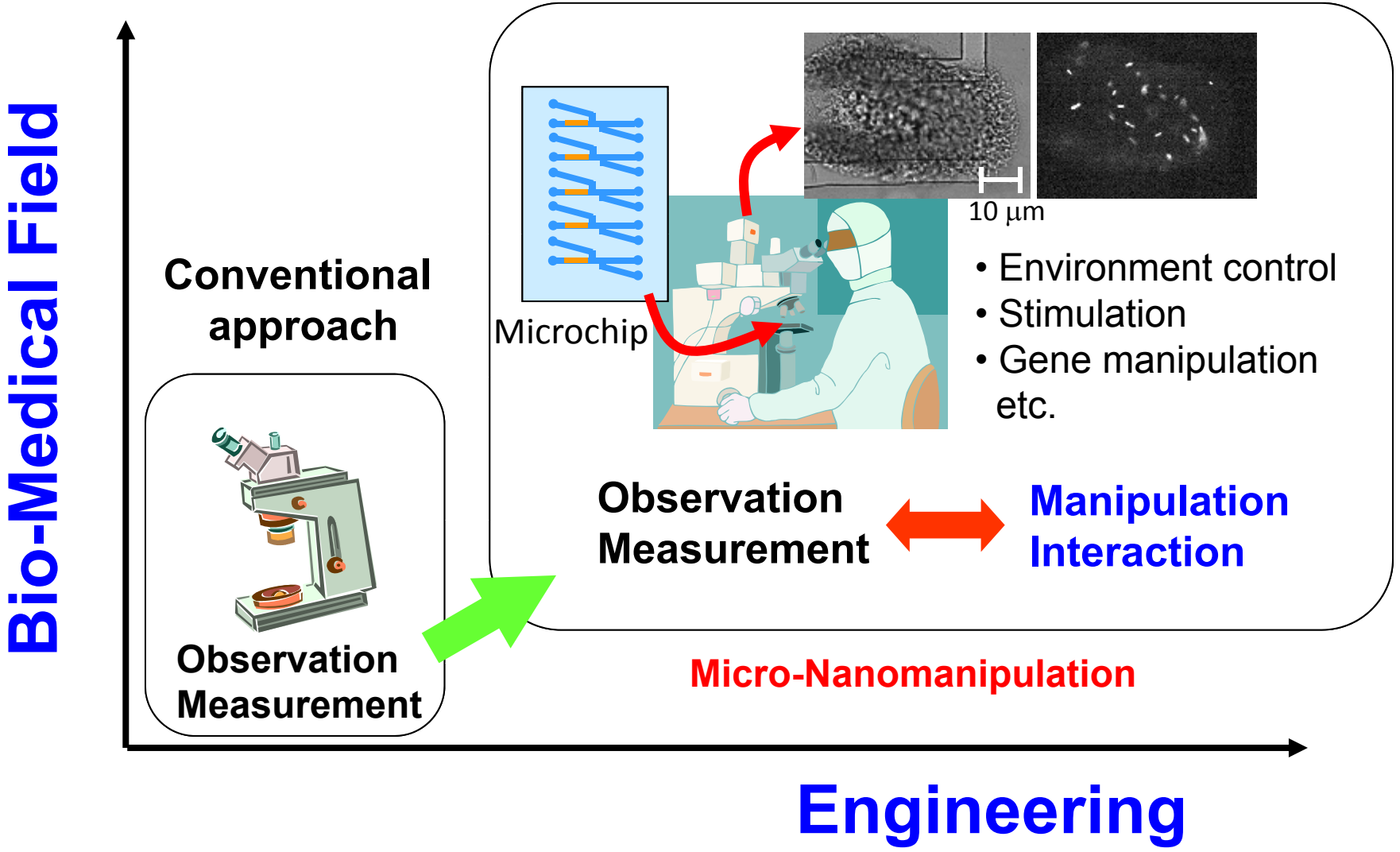
4. Molecules

nm



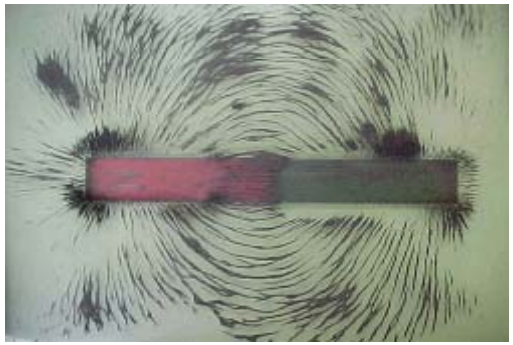


Strategy for Single Cell Analysis

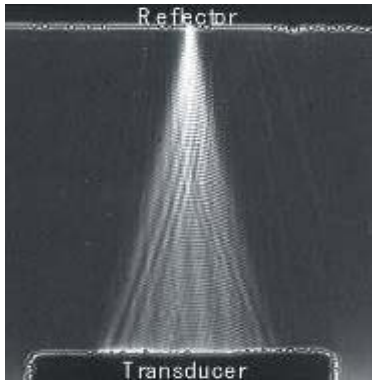


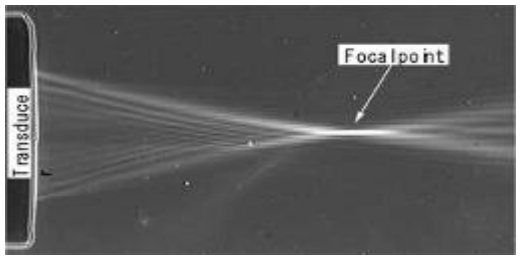
Classification of Non-contact Manipulation in Liquid

Magnetic force



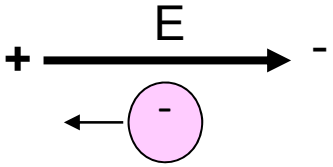
Ultra sonic force



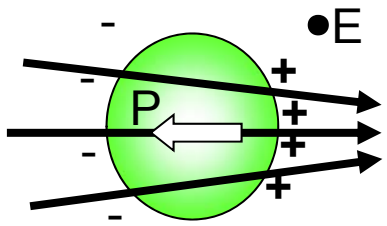


T. Kozuka, et al

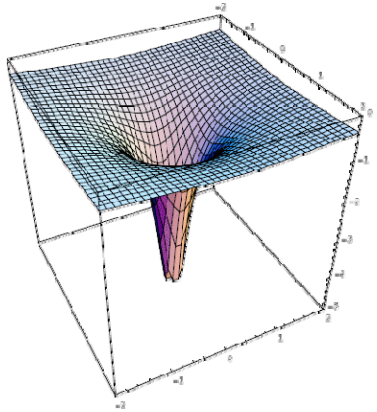
Electrostatic force



Electrophoresis

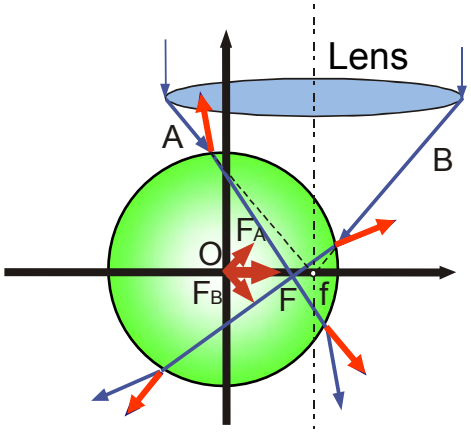


Dielectrophoresis



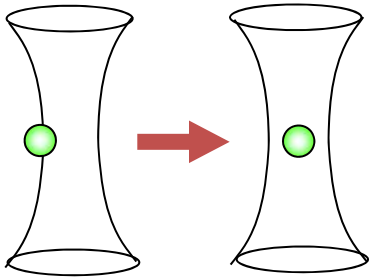
Field Gradient

Optical force



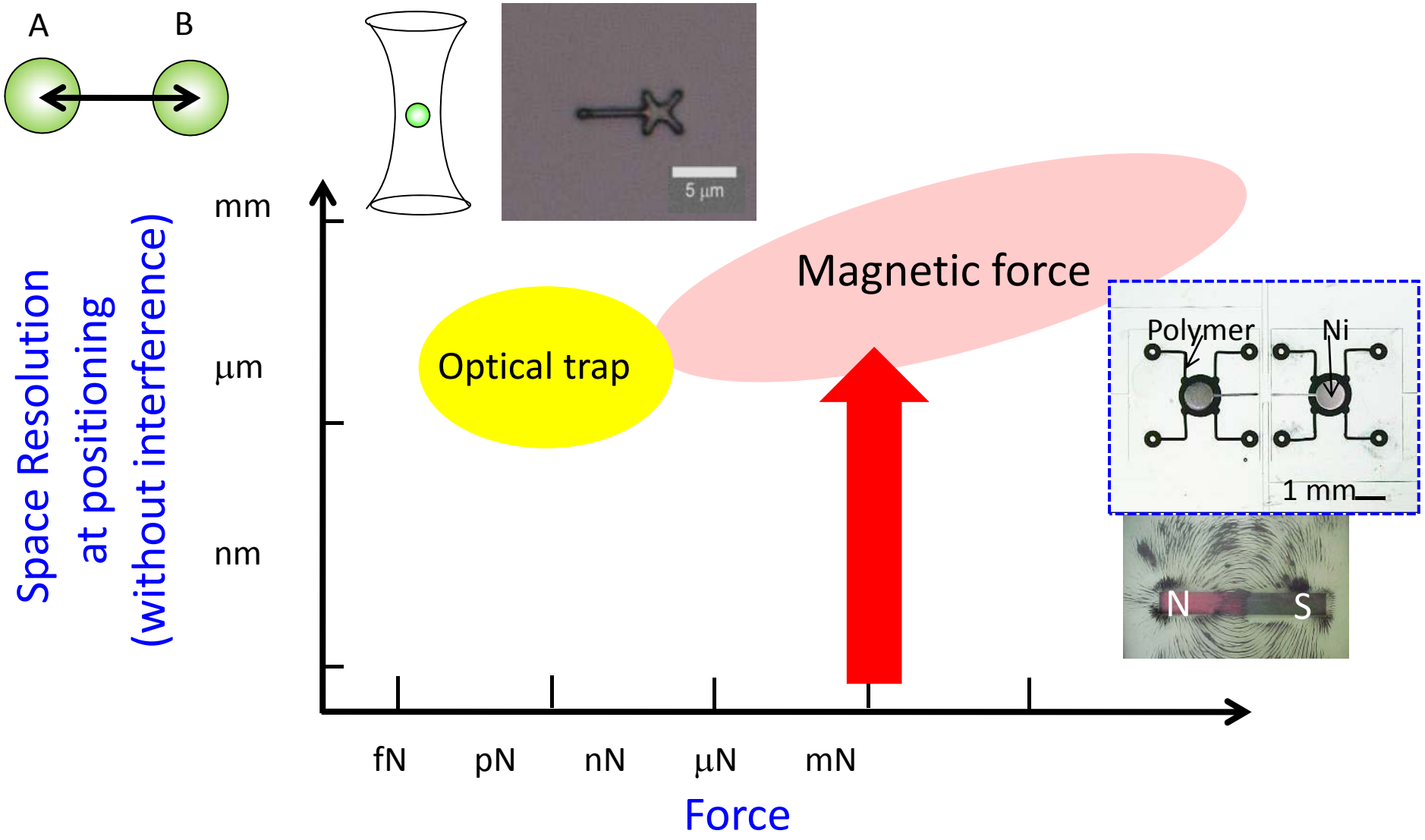
Mie particle

Focused Laser



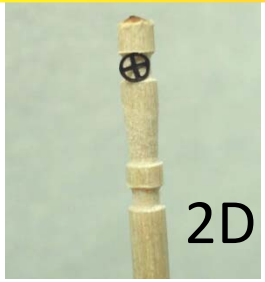
Rayleigh particle

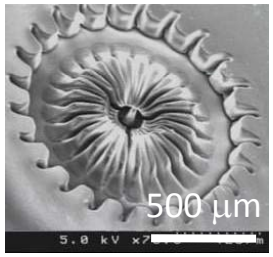
Force for Noncontact Manipulation

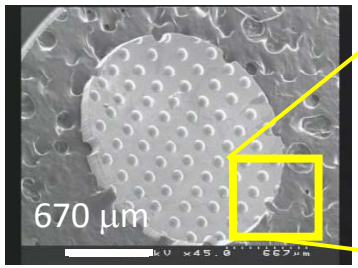


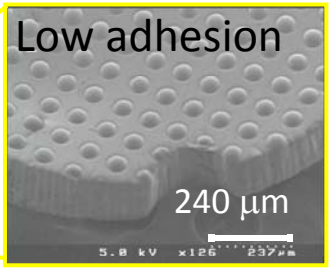
Transition of Magnetically Driven Microtools

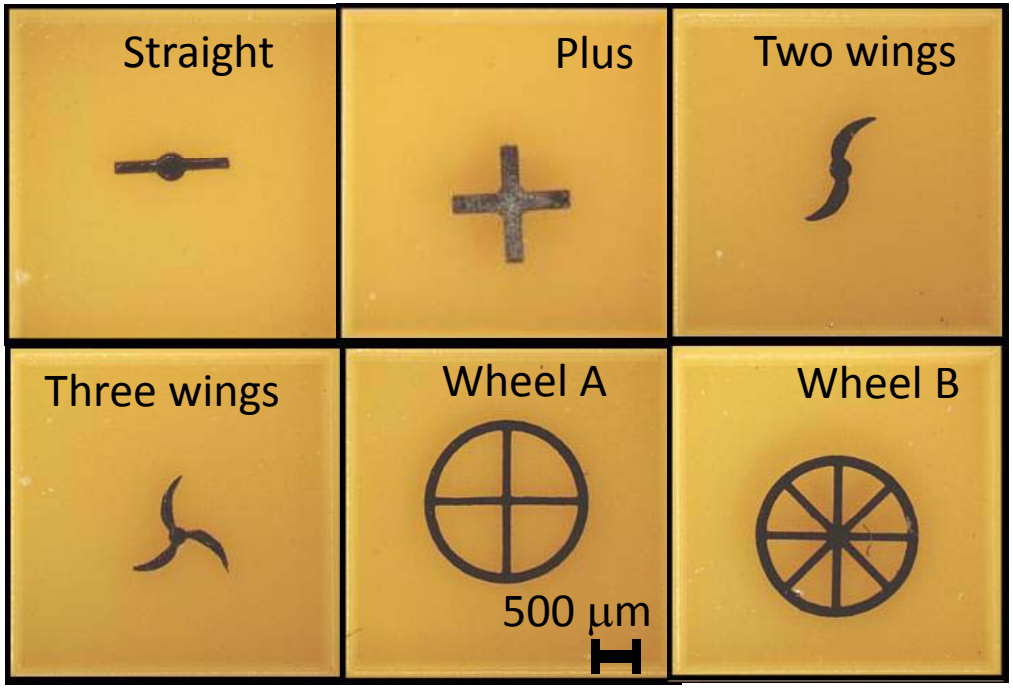
2004 2006 2008



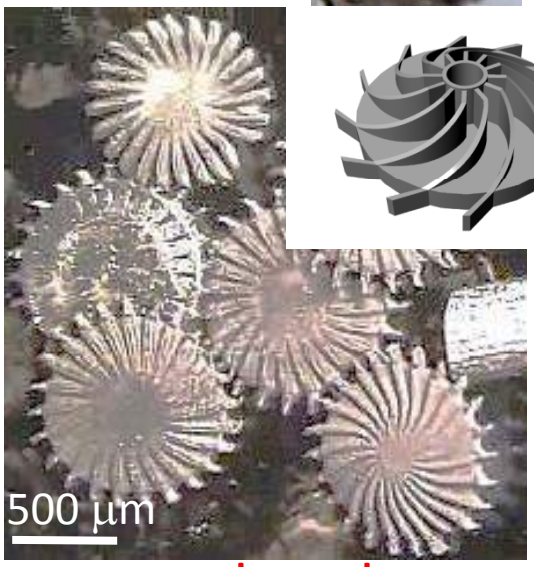








Flexible:
E= 5 MPa



3D

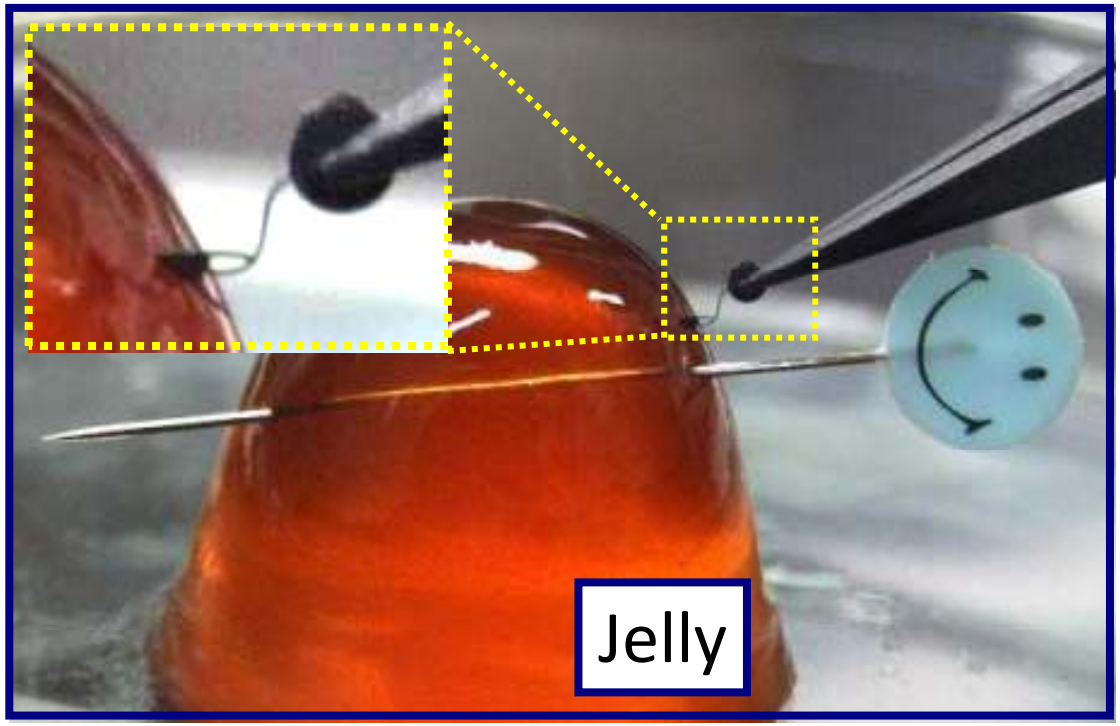
Many kinds of magnetically driven microtools are produced.

Fabrication Method of Conventional MMT

Polydimethylsiloxane (PDMS)

Magnetite

Composite (PDMS : Magnetite =1:1)

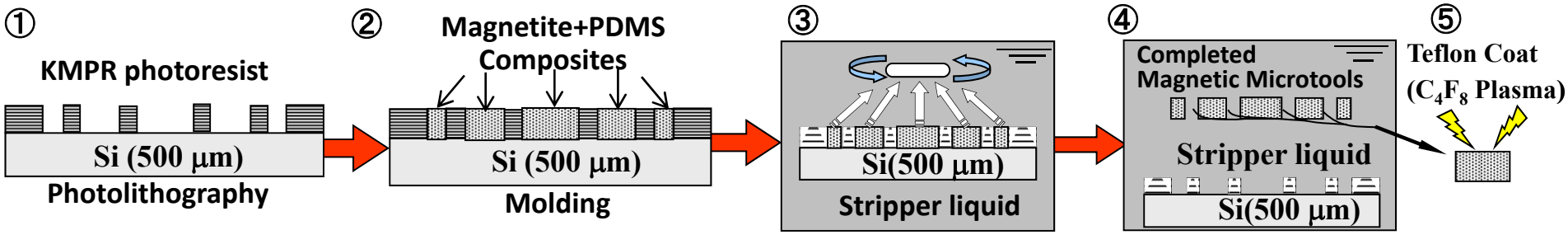


Young's Modulus ≈ 5 MPa

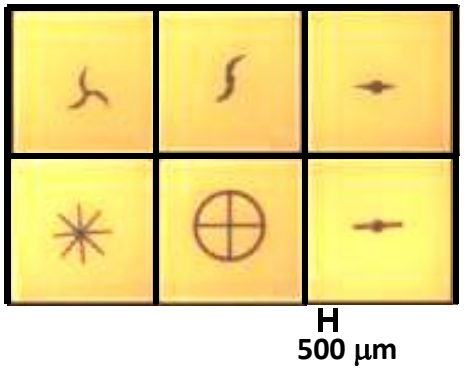
MMT is magnetic material and flexible

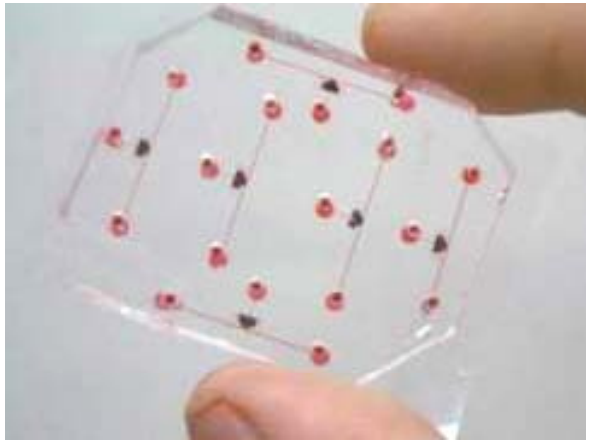
(Magnetically driven Microtool)

Conventional MMT 1

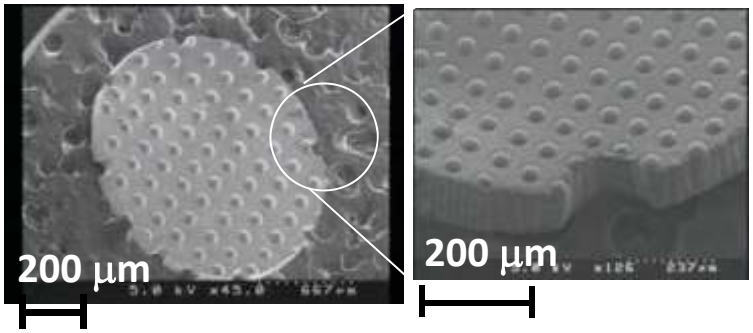


Patent No. 2006-357260, 2008-061115, 2008-127558

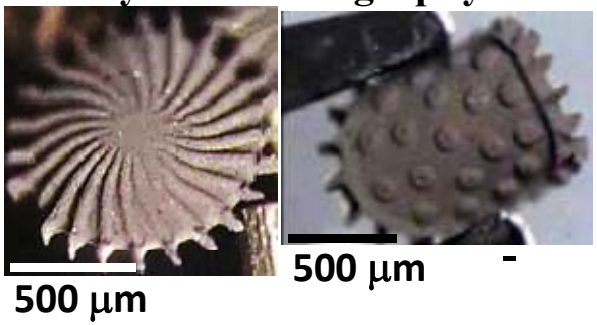




+ Reflow Process

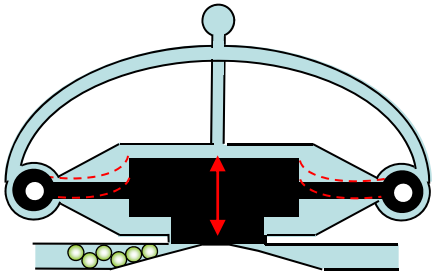


+ Grey-scale Lithography



Conventional MMT 2

Lateral & Deformation motion

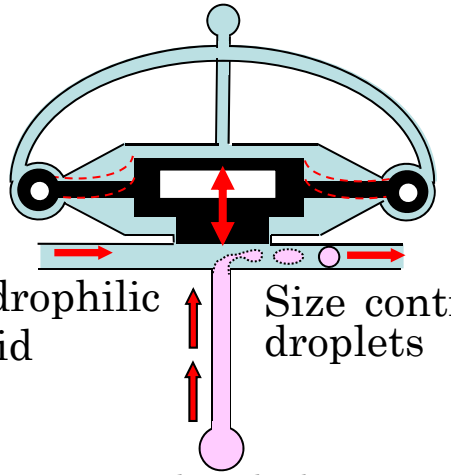


PDMS column

Oocyte

【Microvalve】

200 μm

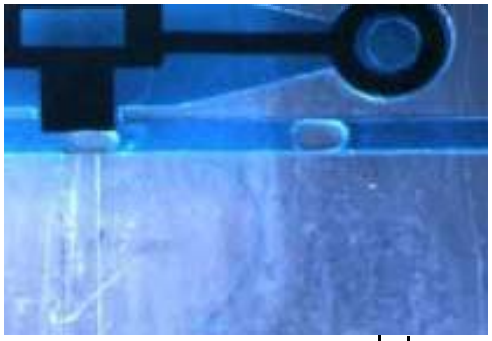


Hydrophilic Fluid

Size controlled droplets

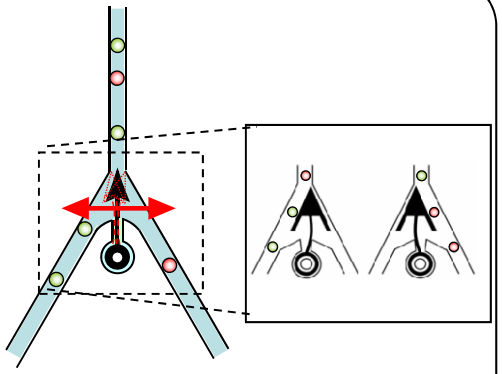
Hydrophobic Fluid

【Microdroplet Generator】



MMT (f ≦20 Hz)

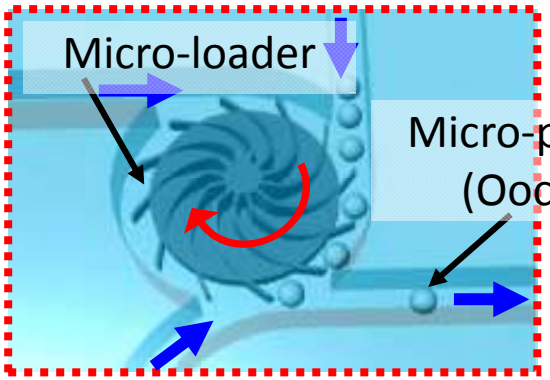
200 μm



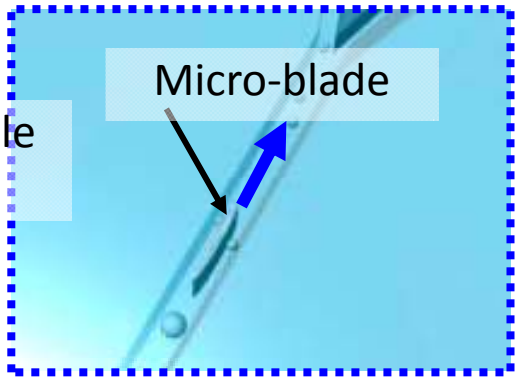
【Microsorter】

150 μm

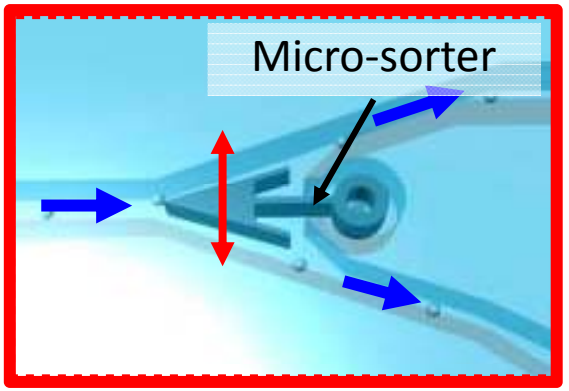
On-chip Sorting System



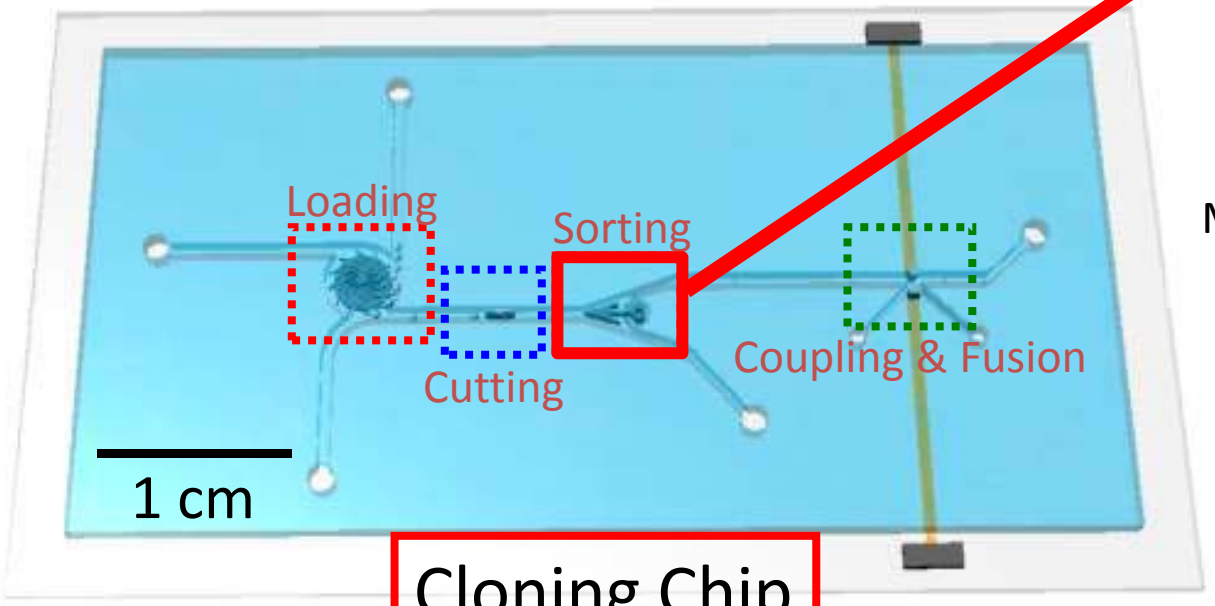
Loading



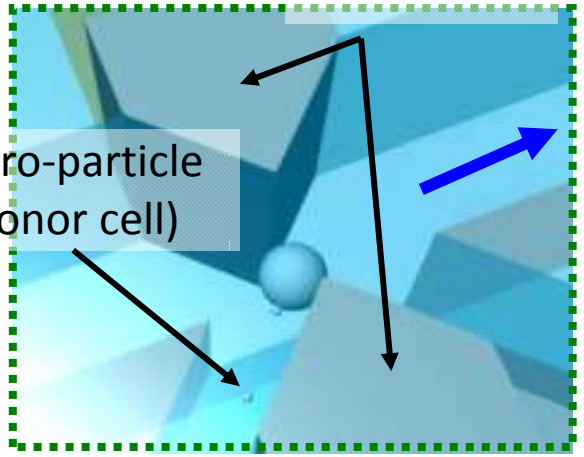
Cutting



Sorting



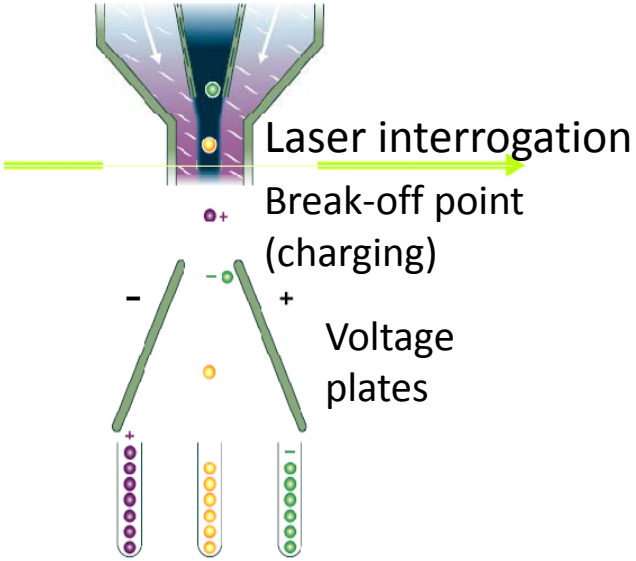
Cloning Chip



Coupling & Fusion

Background of the On-chip Sorting System

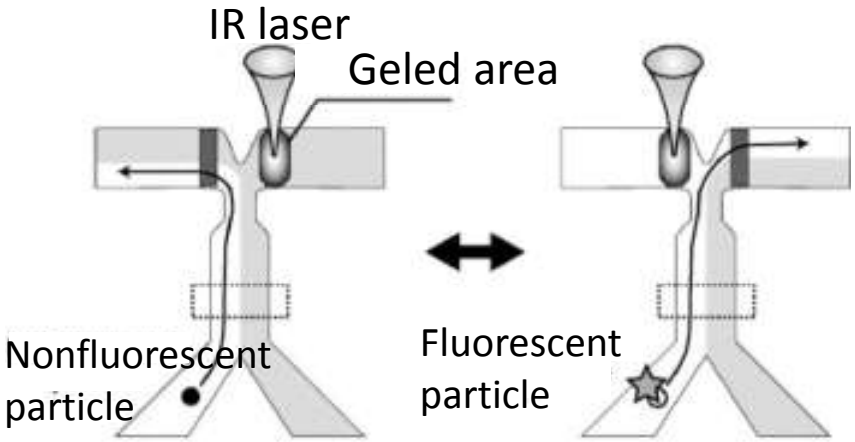
Electrostatic flow sorting



Cell sorter on flow cytometry

http://www.ab-direct.com/support/electrostatic_cell_sorting-711.html

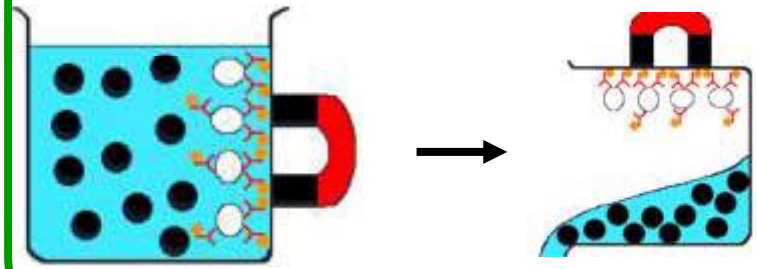
Optical sorting system



Cell sorter by IR laser and thermoreversible gelation polymer

Shoji, *et al*, IEEE J. Selected Topics in Quantum Electronics, Vol. 13, No.2, p.225, 2007

Functioned particle sorting



http://www.geocities.jp/motomchan/crypto/method/magnet.html

Superiority of the present sorting system

- No requirement of high voltage
- Low cost
- No need of special treatment

Conventional Actuation Module

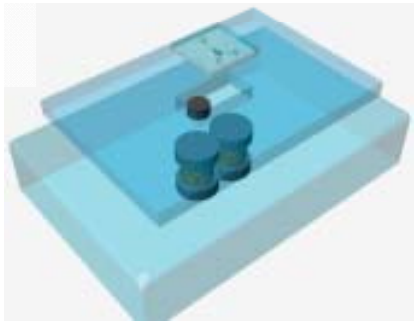
Input: 3.85V, 0.15A

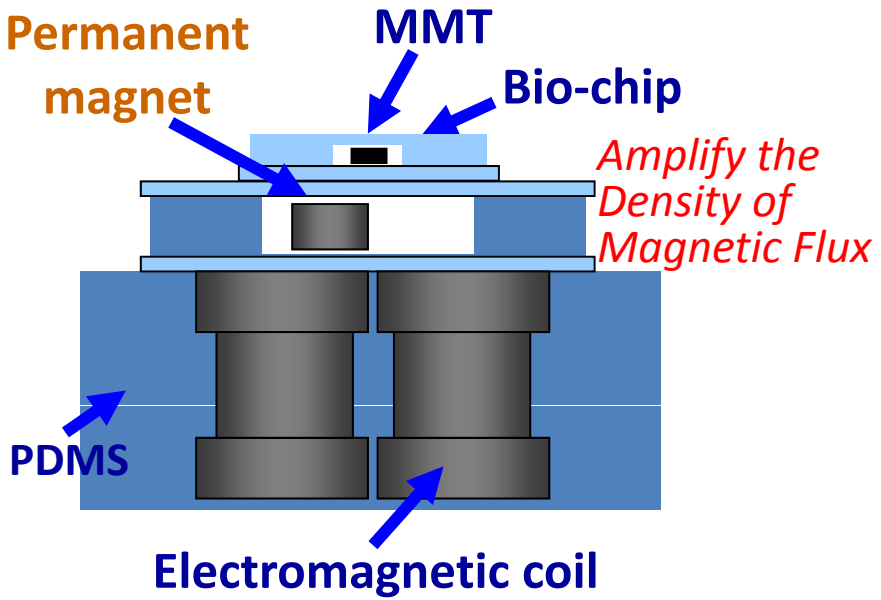
Condition

- Magnetite type MMT
- Permanent magnet unit

Frequency: about 18 Hz

Concept





System configuration

Magnetization of MMT and High speed actuation

Improvement of MMT System for Powerful Actuation

- *Improvement of MMT*

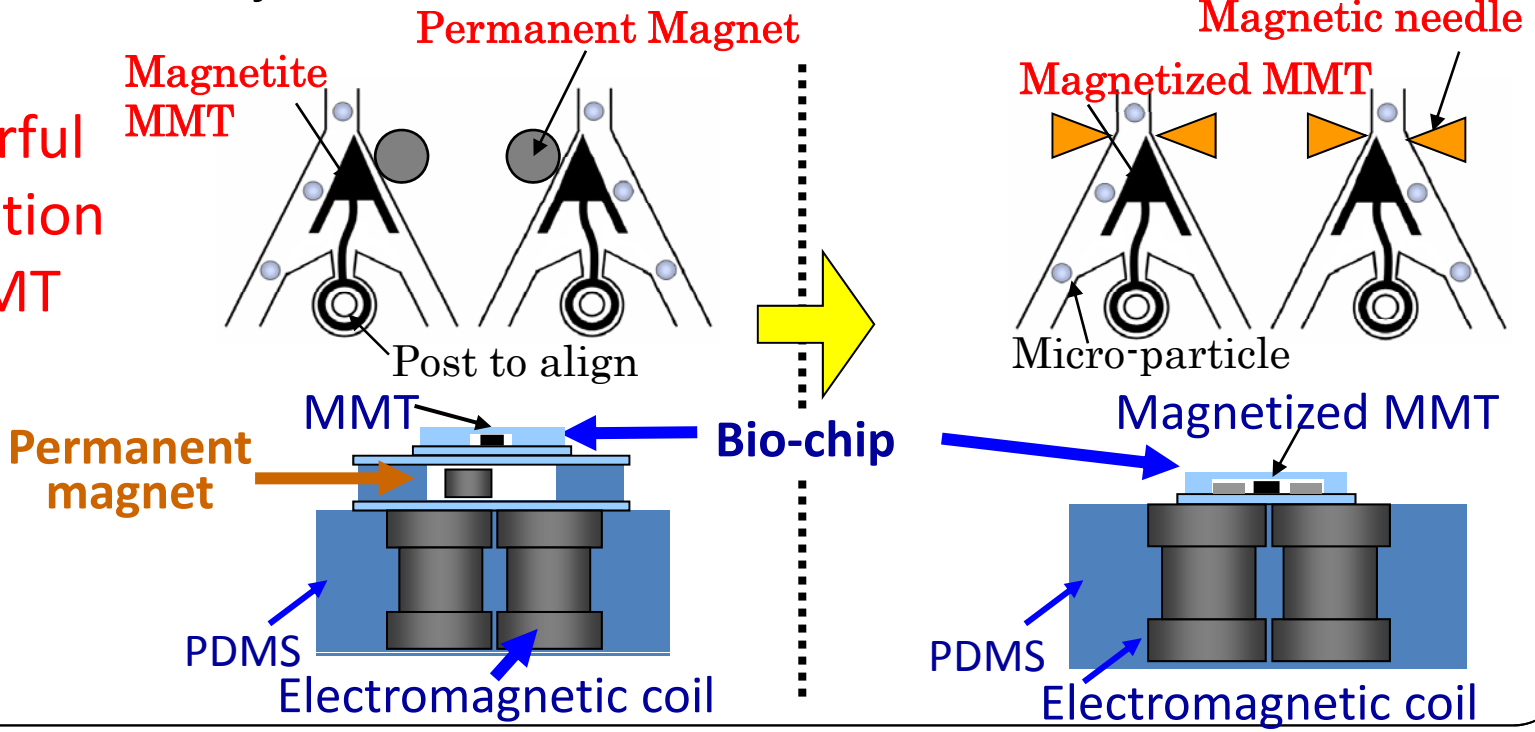
①:
Strong
Magnetization
of MMT

Magnetite type MMT

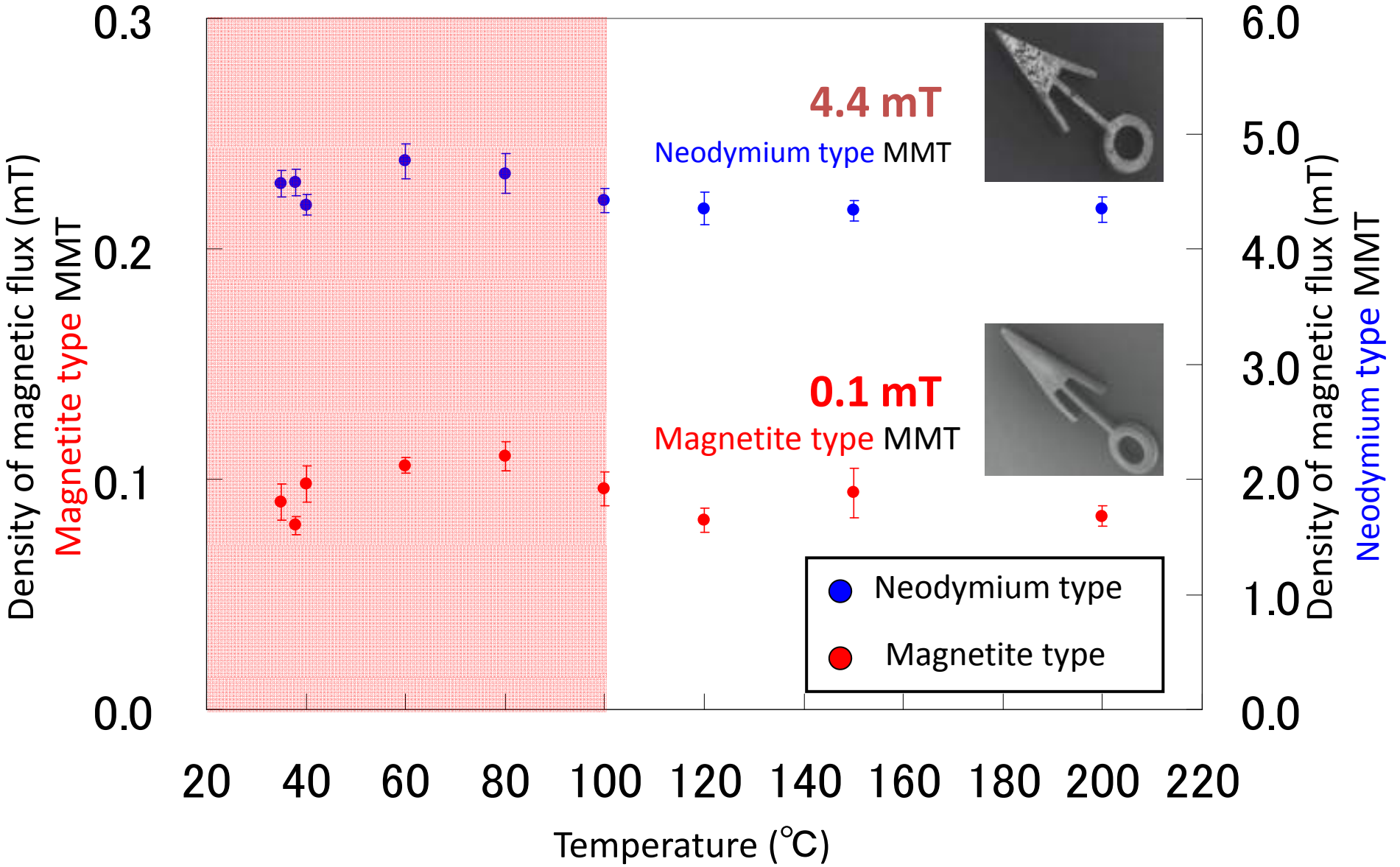
Neodymium type MMT

- *Improvement of Actuation Module*

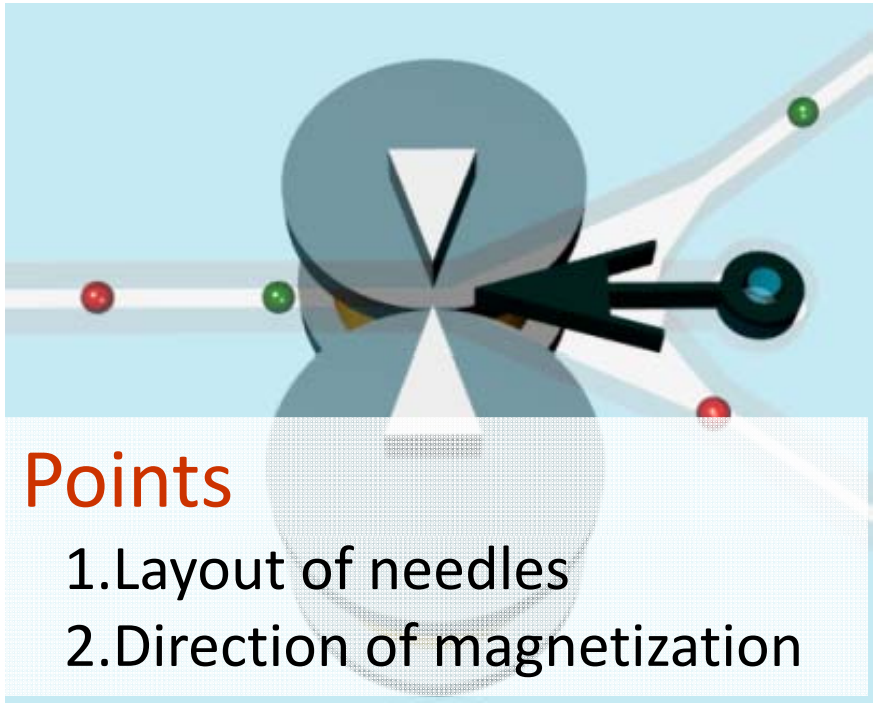
②:
Powerful
Actuation
of MMT



① Magnetization of MMT



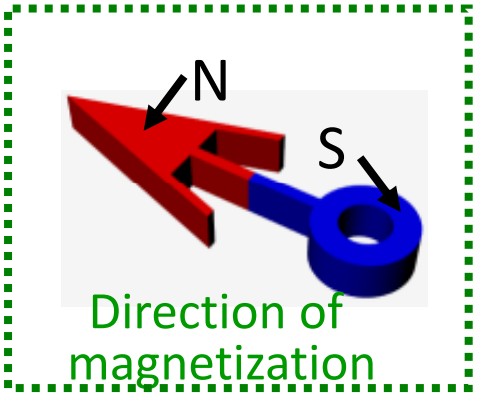
②Focusing magnetic field by On-chip metal in bio-chip



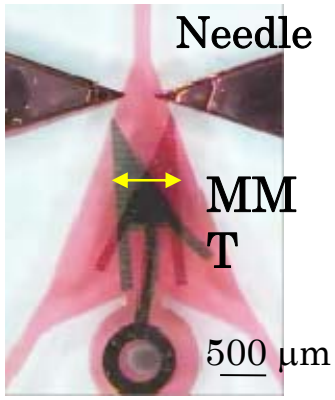
Points

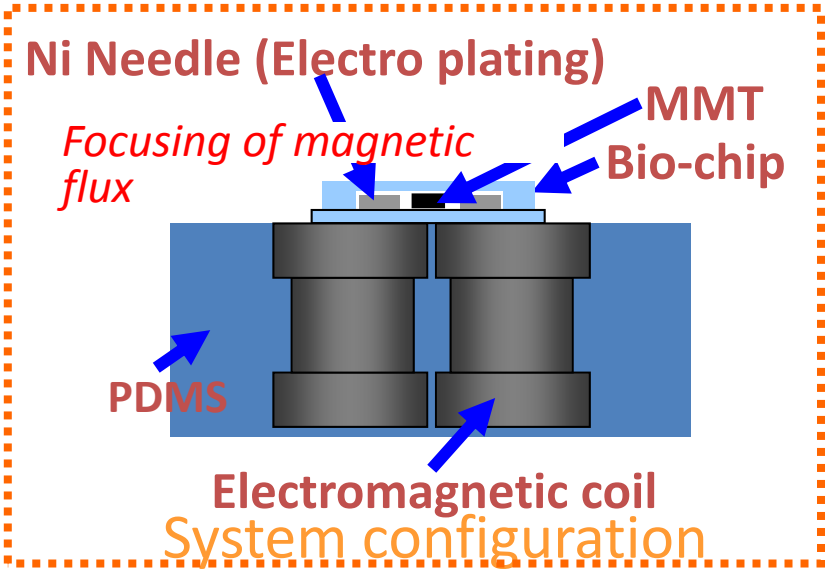
1.Layout of needles

2.Direction of magnetization

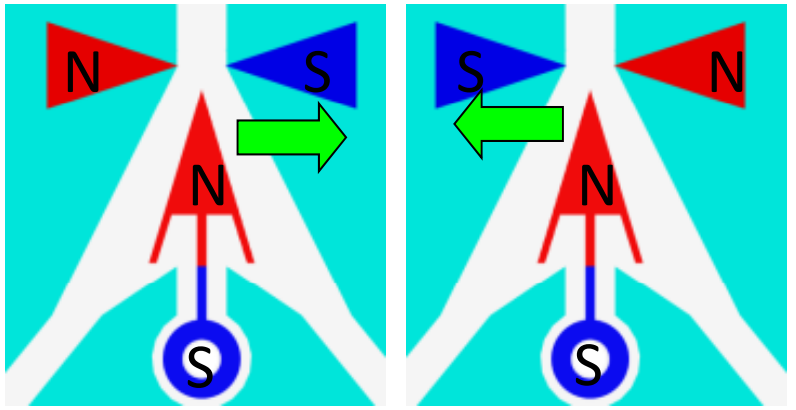


Direction of magnetization



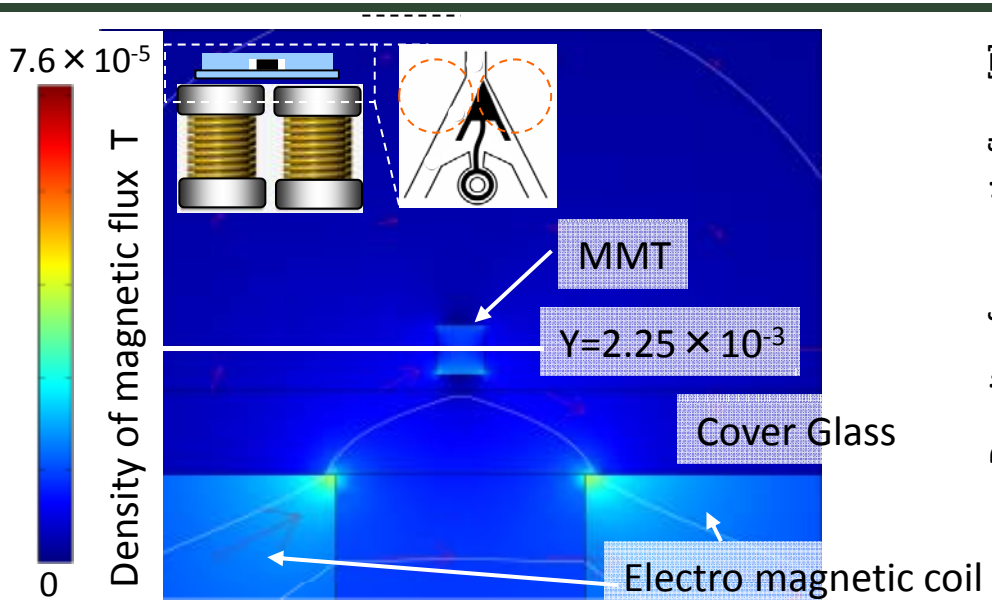


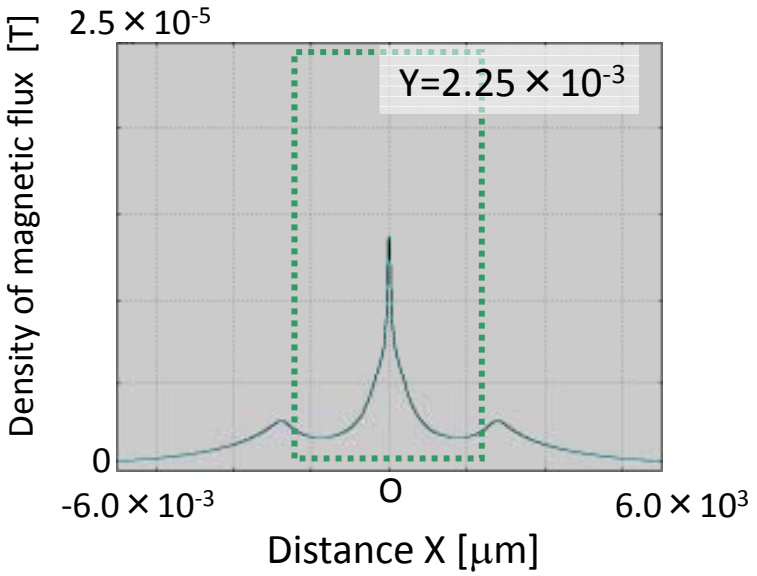
System configuration

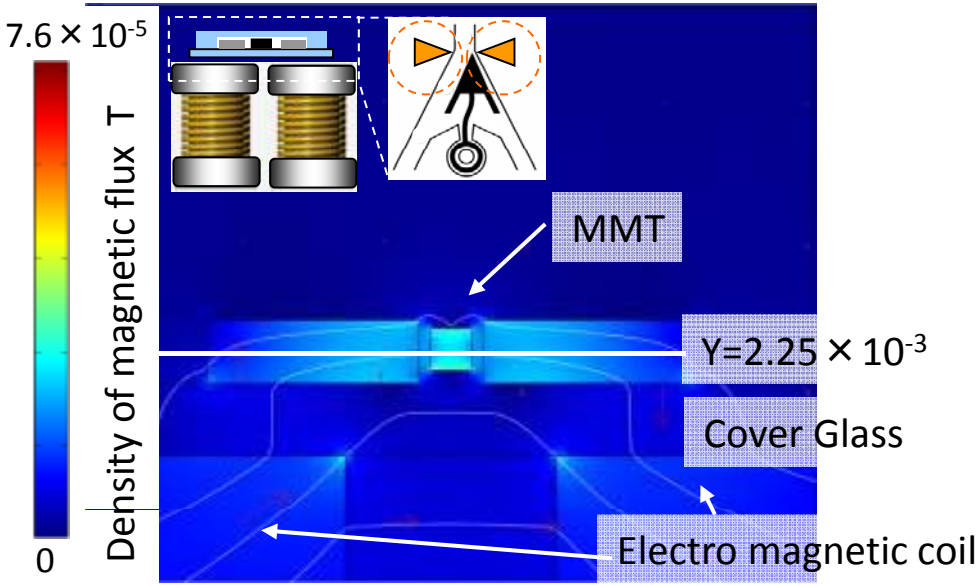


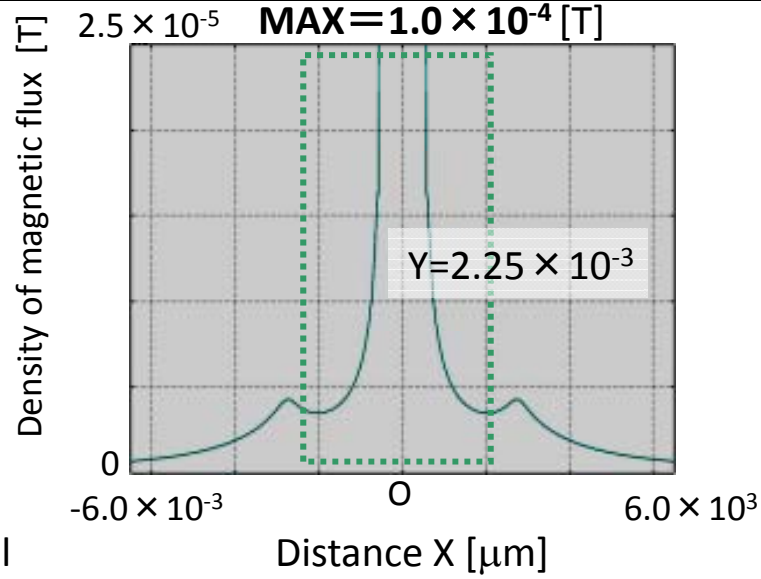
Actuation System

FEM analysis of Magnetic Flux



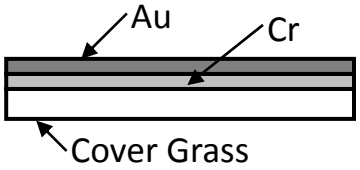




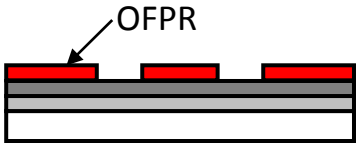


Fabrication Process of On-chip Metal for High Speed Sorting

1.Cr/Au sputter

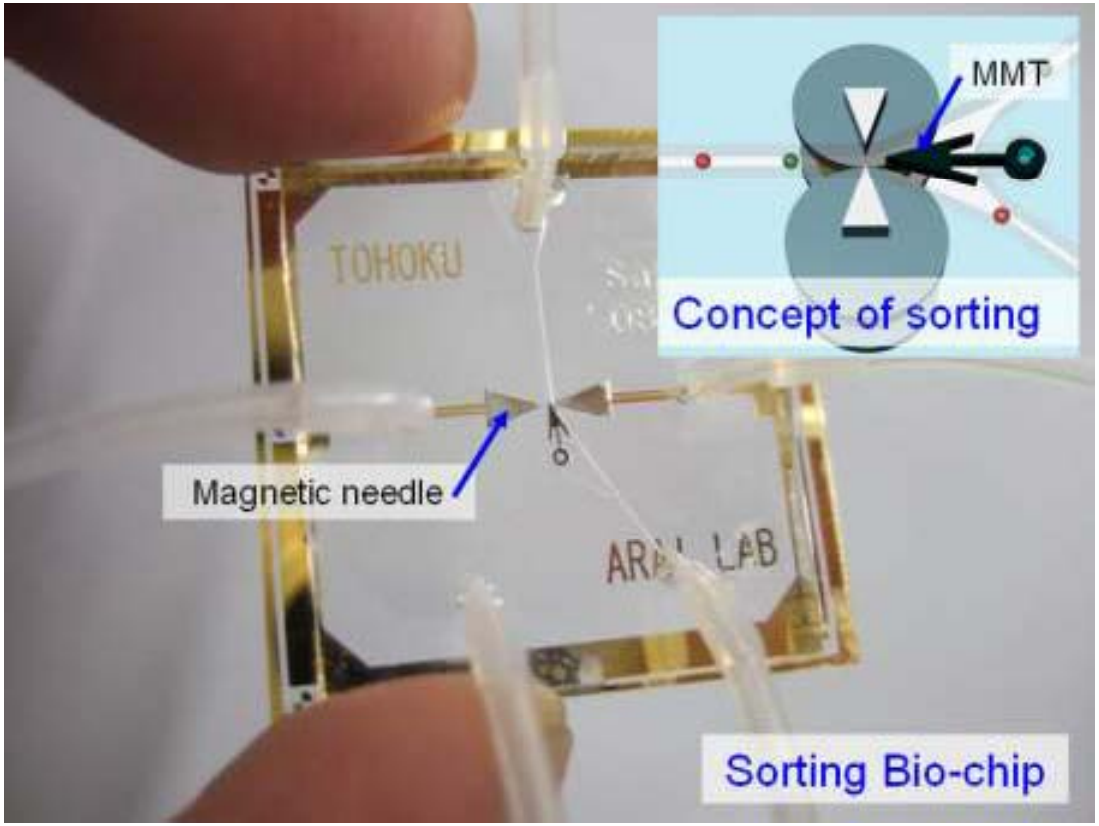


2.OFPR patterning

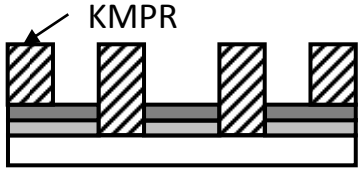


3.Cr/Au etching

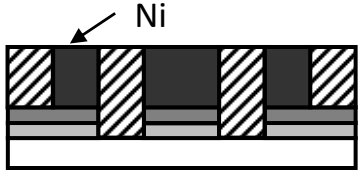
4.Remove OFPR



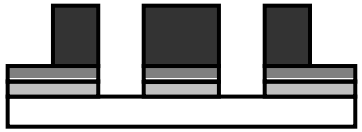
5.KMPR patterning



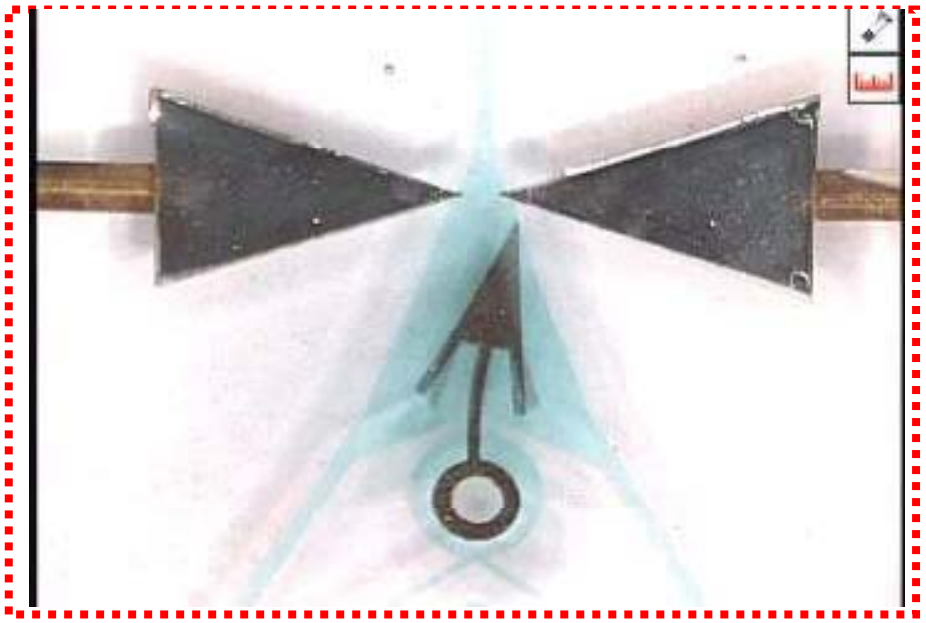
6.Ni plating



7.Remove KMPR



Focusing of Magnetic Field



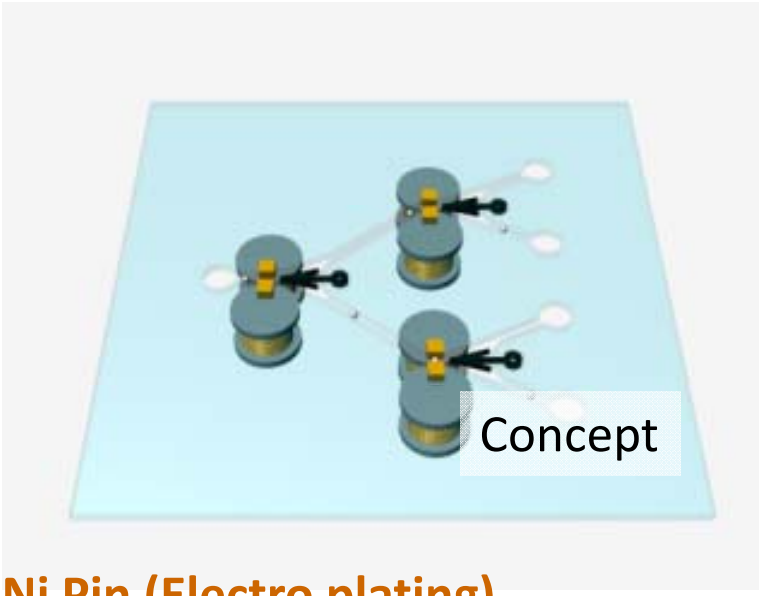
Input: 3.85 V, 0.15A

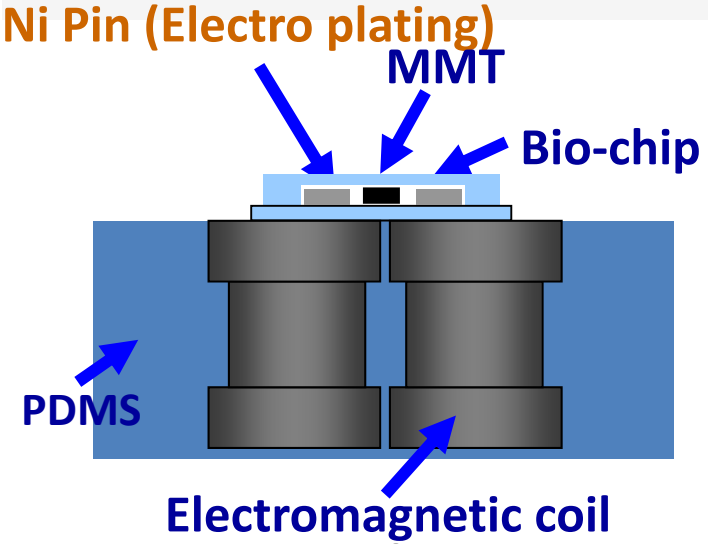
Fundamental experiment

Condition

- Neodymium type MMT
- Pin (electro plating) unit

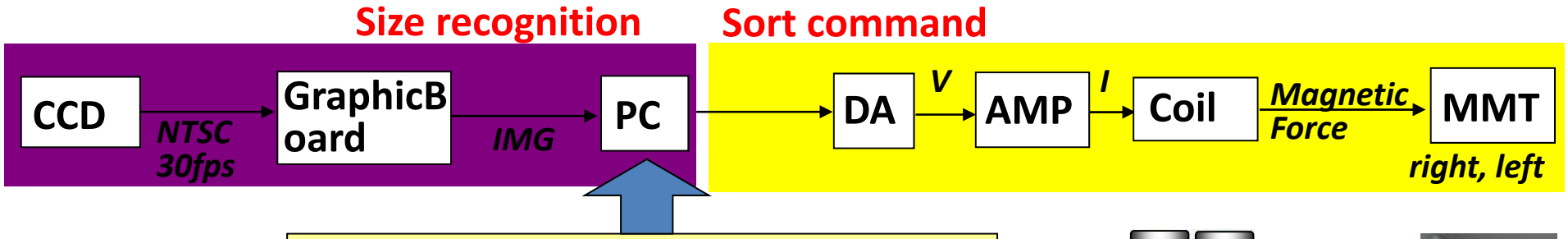
Frequency: about 180 Hz





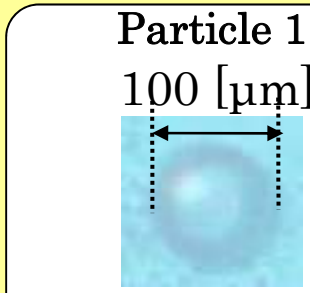
System configuration

Automation of Sorting System Diagram



Particle Recognition with Cross-correlation Image Analysis

$$R_{NCC}(a,b) = \frac{\sum_{j=0}^{H-1}\sum_{i=0}^{W-1}\left|I(a+i,b+j)T(i,j)\right|}{\sqrt{\sum_{j=0}^{H-1}\sum_{i=0}^{W-1}I(a+i,b+j)^2 \times \sum_{j=0}^{H-1}\sum_{i=0}^{W-1}T(i,j)^2}}$$



R_{NCC}: Normalized Cross-correlation Coefficient

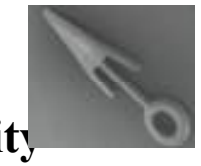
I : Input Image

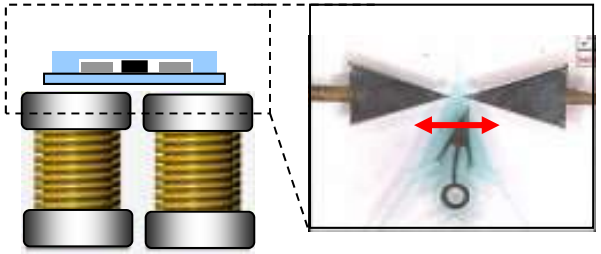
T : Template Image

a : X - axis

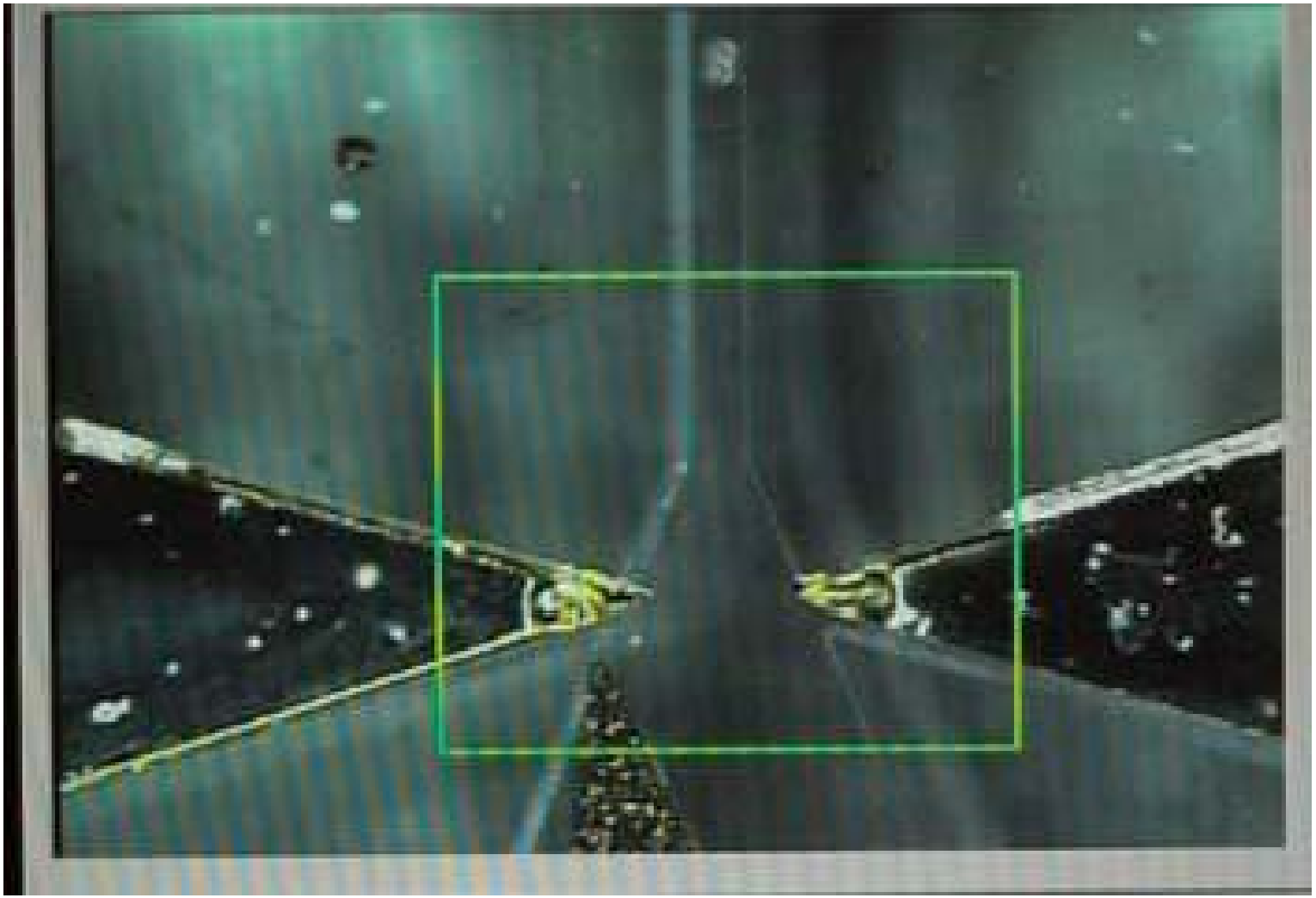
b : Y - axis

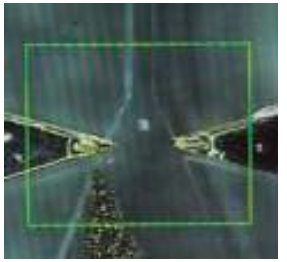
Change the Polarity





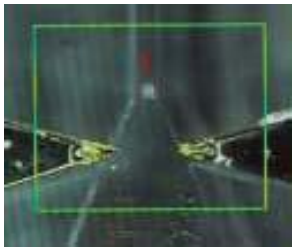
High Speed Sorting System



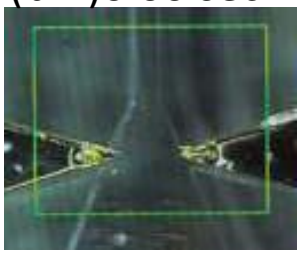


(a-1)0.00 sec

(a-2)0.12 sec



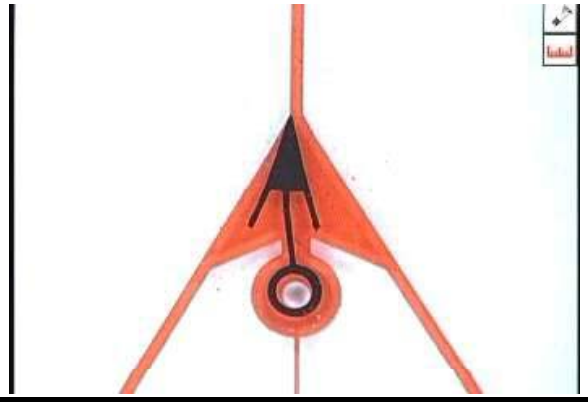
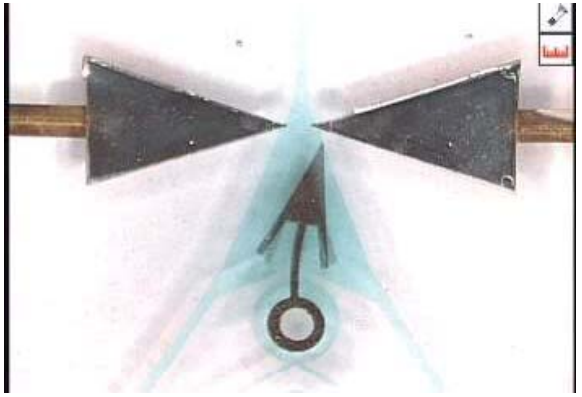
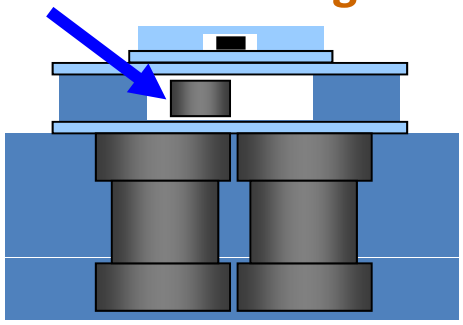
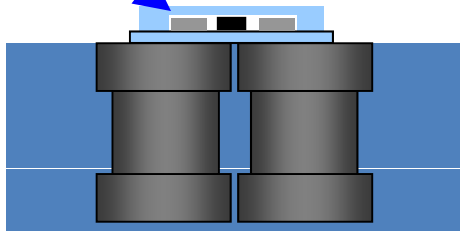
(b-1)0.00 sec



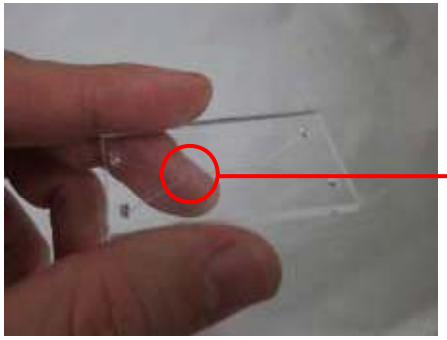
(b-2)0.34 sec

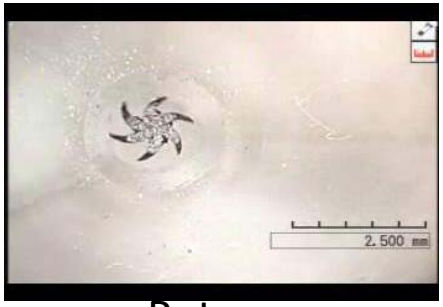
Yamanishi et. al. Biomedical Microdevices, Vol.12, p.745-752, (2010).

Development of MMT Sorting System (Summary)

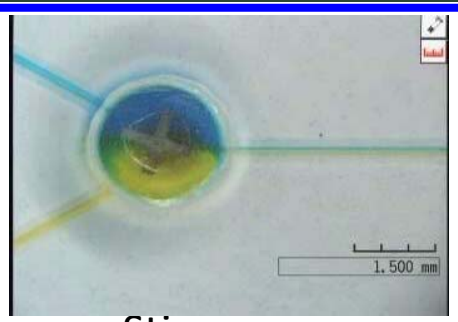
	Conventional Setting	Present Setting
		
Improved points	・Magnetite type MMT ・Permanent magnet unit	・Neodymium type MMT (Magnetised) ・Magnetic needle
Sorting Speed	Up to about 18 Hz	Up to about 180 Hz
Actuation System Size	Permanent magnet 	Magnetic needle 

Family of 1DOF MMT (rotation & lateral motion)

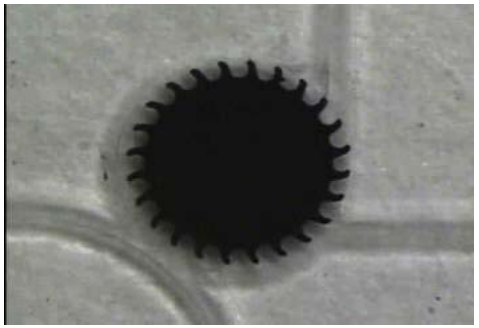




Rotor

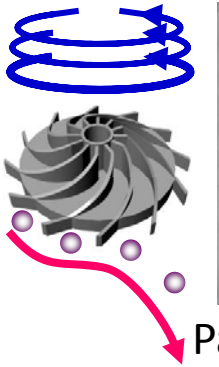


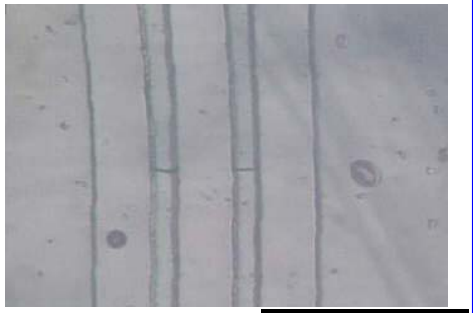
Stirrer



500 μm

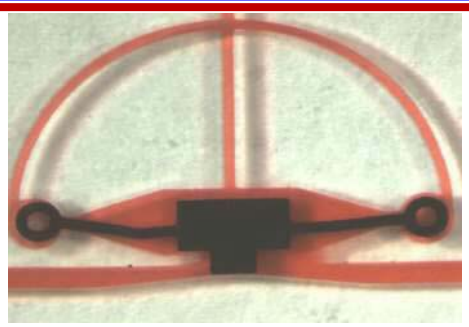
Particle loader





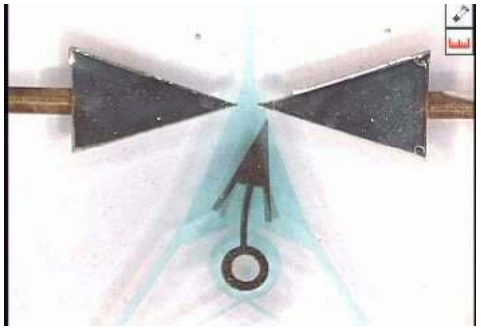
Particle Filter

MEMS2009



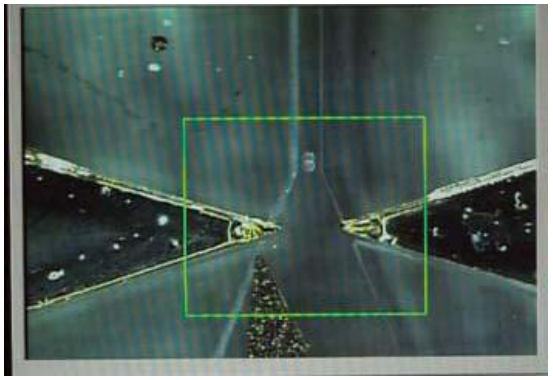
Valve

500 μm



Sortor

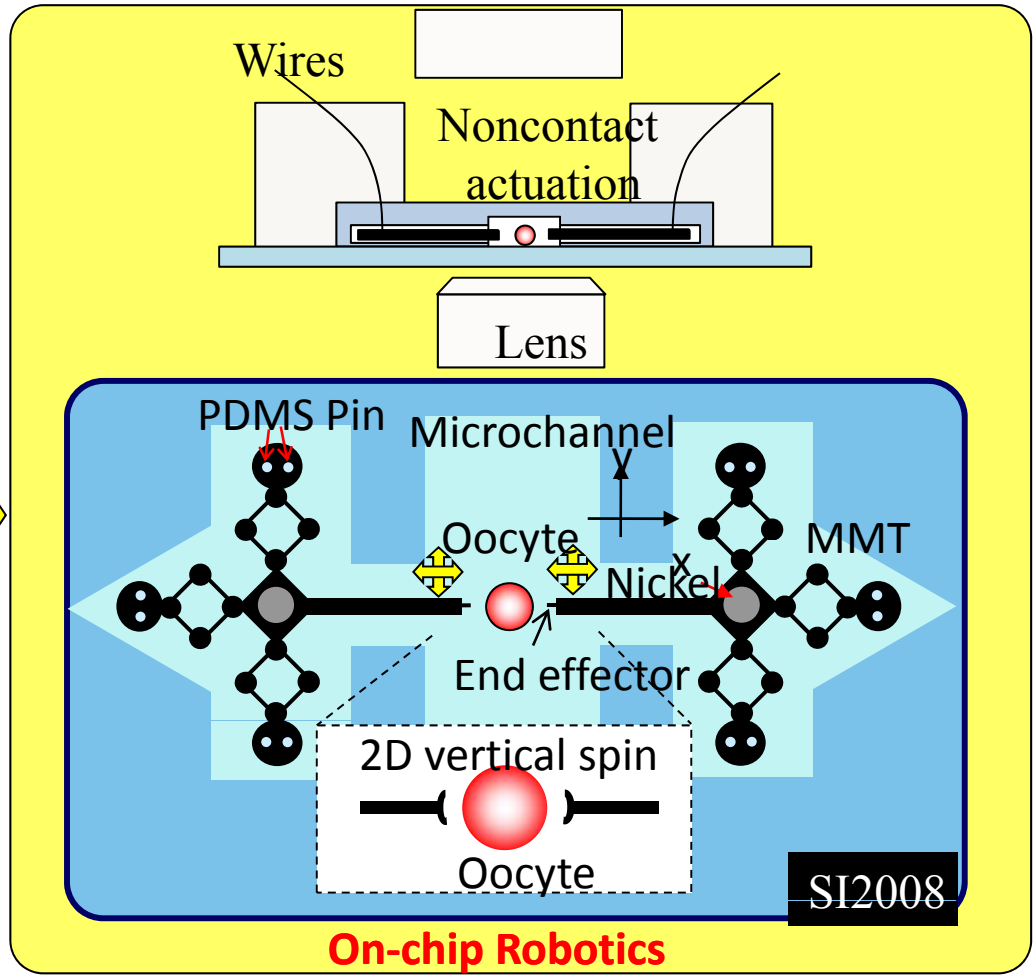
MEMS2009



2DOF-MMT

On-chip Robotics

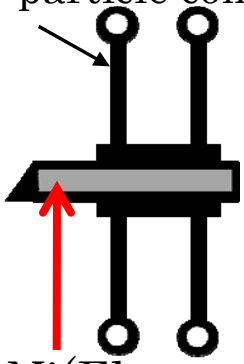
We define Robochip as a microfluidic chip in which micro-nano robots are installed, and which is targeting the single-cell based measurement, analysis, cloning and anatomical manipulation to contribute to on-chip micromanipulation such as cell sorting.



Family of Hybrid Type MMT and 2DOF MMT

Polymer-metal Hybrid MMT

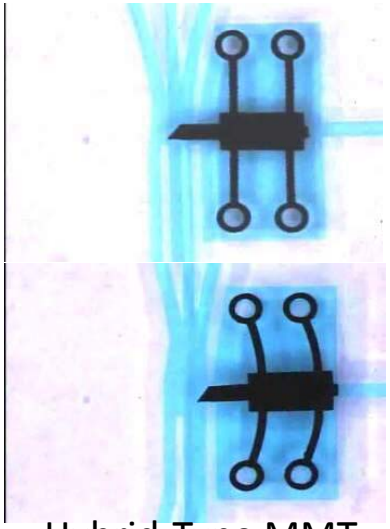
PDMS-magnetic particle composite



Ni(Electroplating)

Control
Local Rigidity
&
Magnetic Property

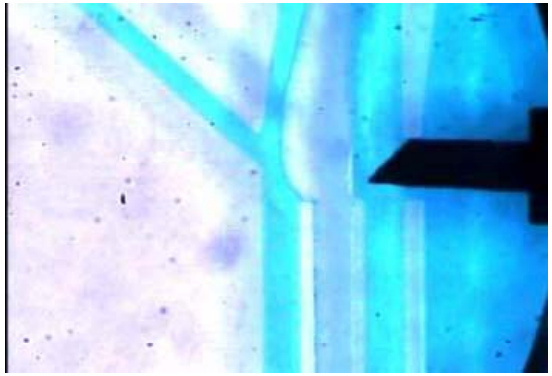
Polymer-based MMT



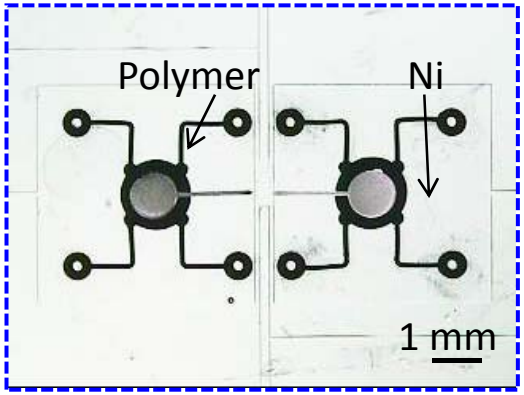
Hybrid-Type MMT

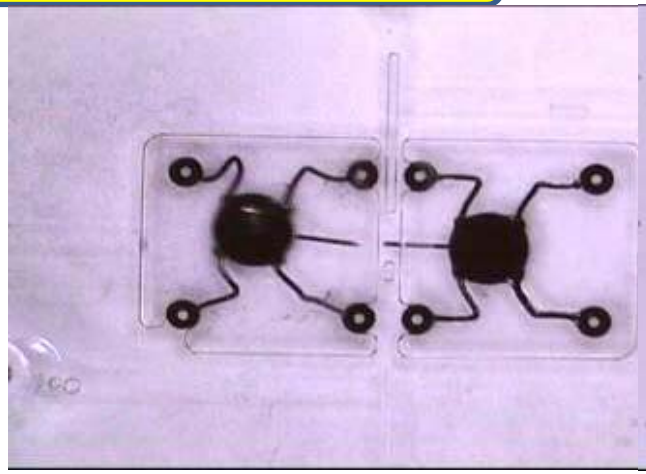
Large Displacement by Control of Magnetic Property

Chopping of Droplet

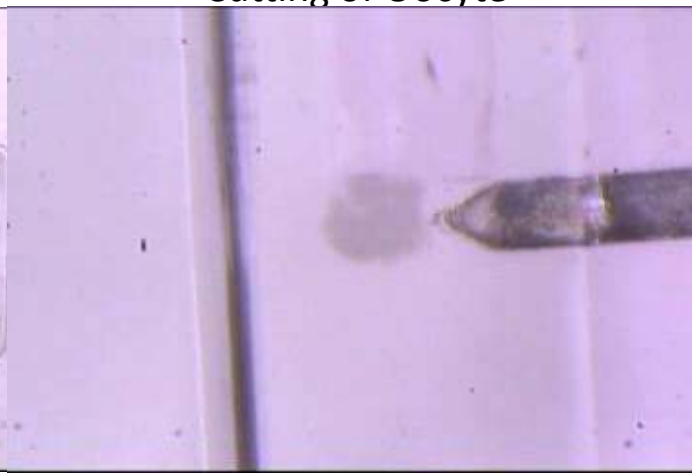


2DOF-MMT and Hybrid MMT

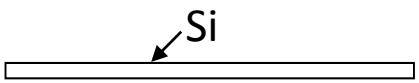
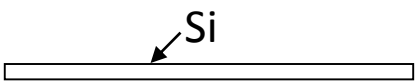
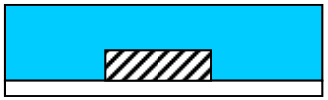
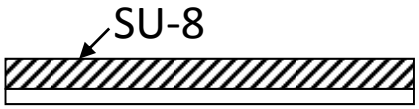
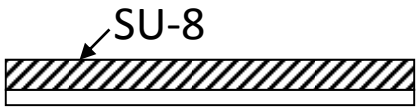
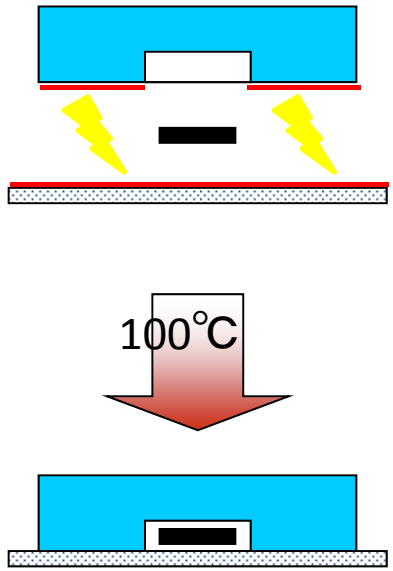
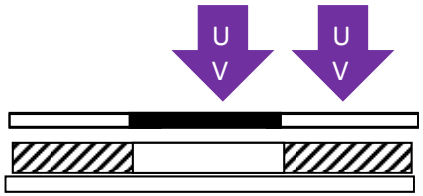
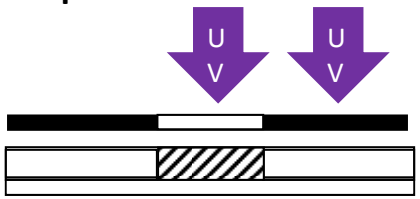
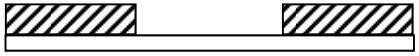
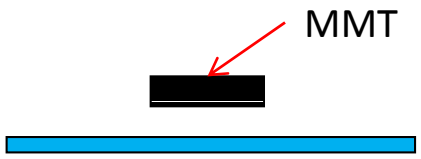




Cutting of Oocyte

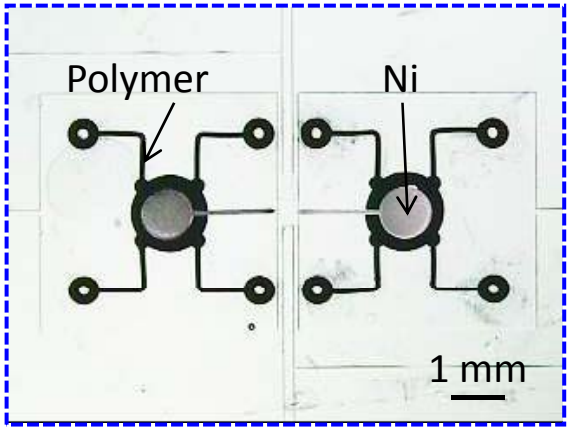


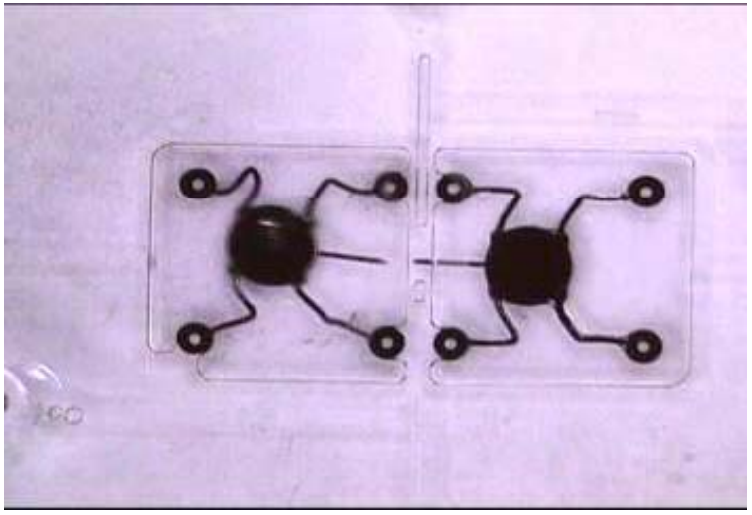
Fabrication Process of MMT

Fabrication of MMT		Fabrication of Biochip	
1.Cleaning 	**5. Spread over the Mixture of PDMS and Magnetite** 	**1.Cleaning** 	**5. Molding PDMS** 
2.Laminate Photoresist 	**6. Pour PDMS** 	**2.Laminate Photoresist** 	**6. Plasma Bonding** 
3. Exposure 	**7. Removal of PDMS** 	**3. Exposure** 	
4. Development 	**8. Release of MMT** 	**4. Development** 	

Cutting of Oocyte by Hybrid 2DOF MMT

2DOF-MMT and Hybrid MMT

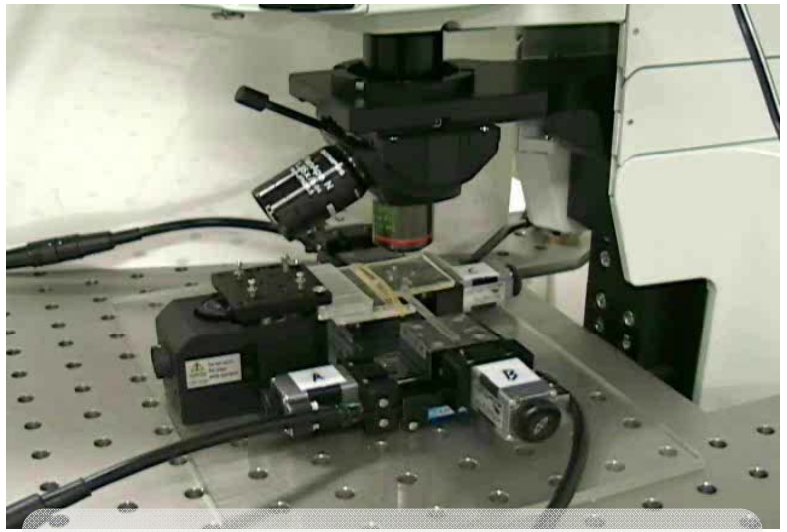




Automation & Integration

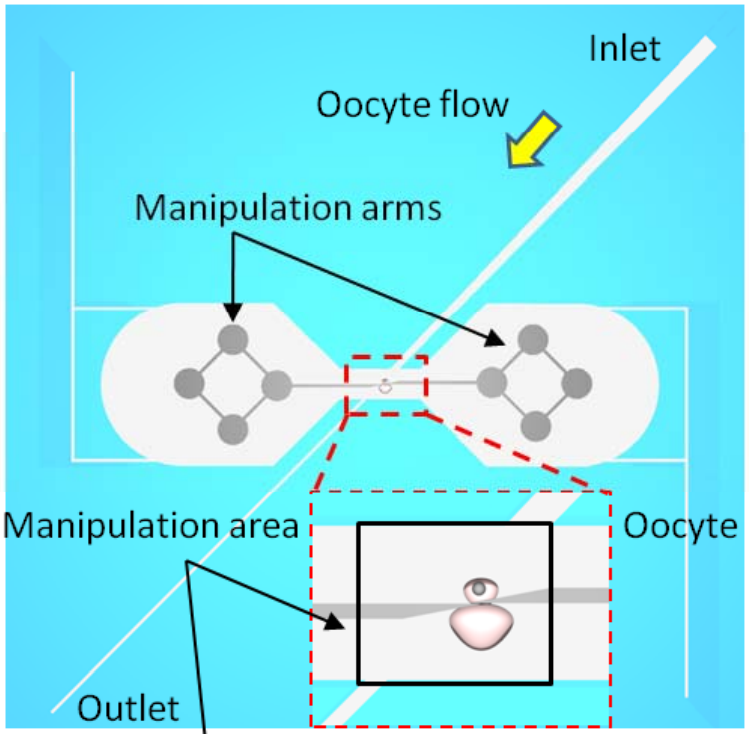
200μm

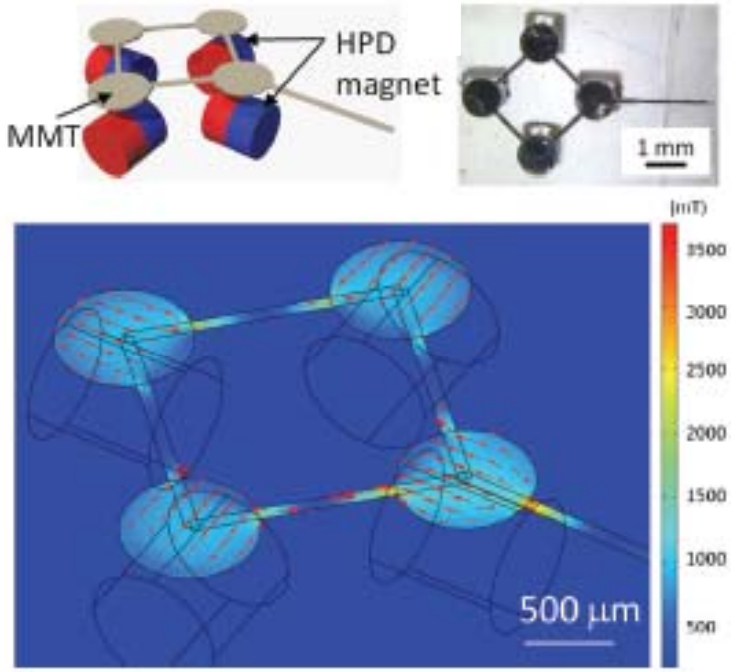
Inomata et. al. MEMS2010.

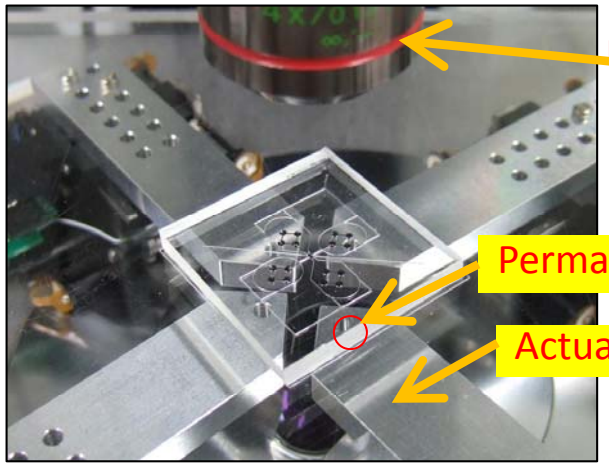


Measurement & Control Platform

Improved Magnetic Actuation







Hagiwara et al., Applied Physics Letters, Vol.97, 013701, 2010.

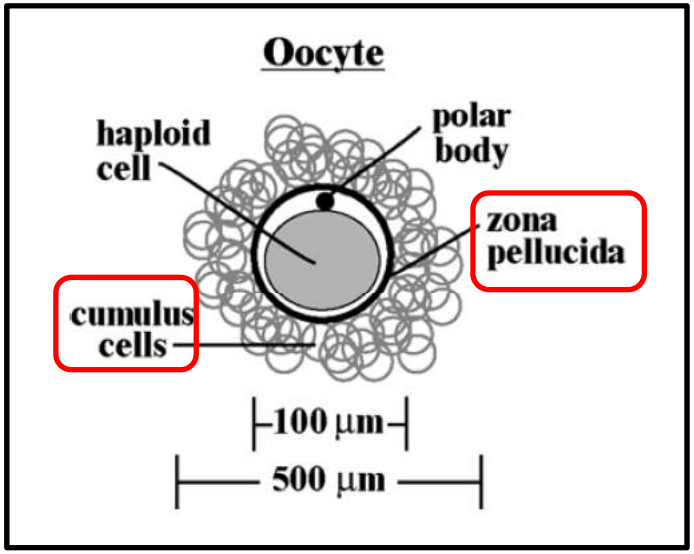
Background (Removal of Zona Pellucida)

【Zona Pellucida (Protection of Oocyte)】

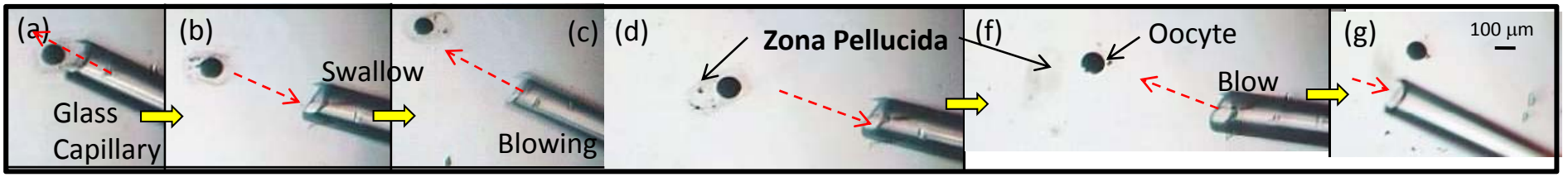
- **Advantage of Removal of Zona Pelucida**

1. **Automation of Enucleation**
   Simplify of Automation of Enucleation

2. **Fertility treatment**
   Improvement of Development Rate for Fusion Process



Beebe et al, Biomedical Mircrodevices 3,p.219-224 , (2001)



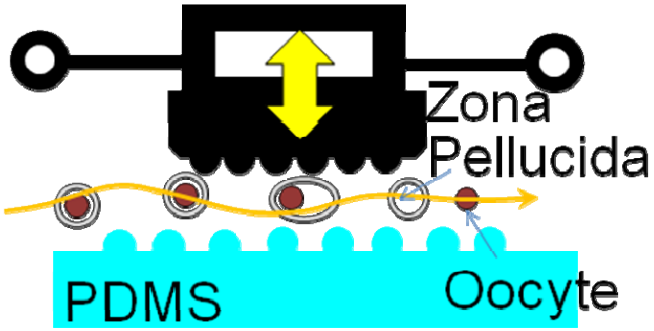
【Problem】 ・Require long time to remove zona pellucida
・Multiple treatment is difficult（Not Mass-productive）

Automation

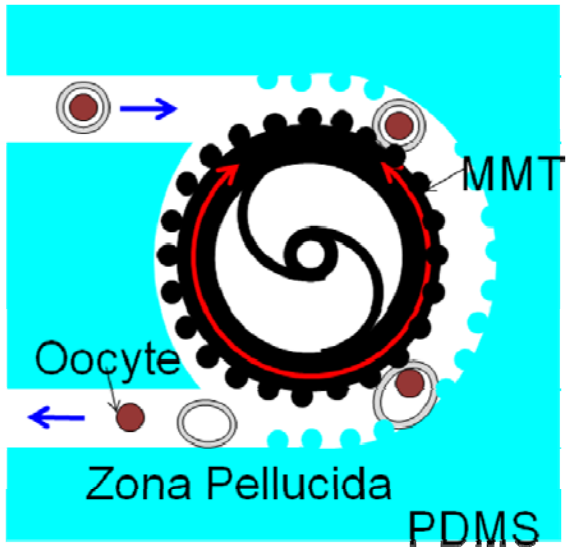
Concept of Removal of Zona Pellucida by MMT

Soft Scrubbing Off of Zona Pellucida

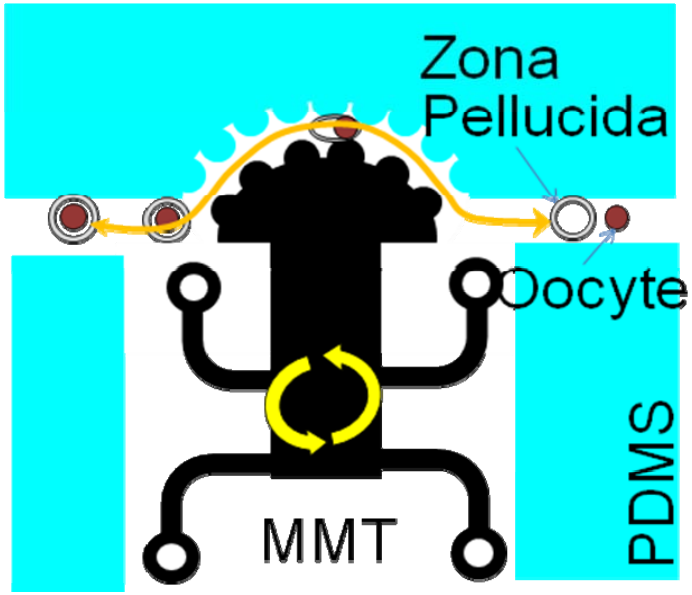
(a) 1DOF Vertical Motion



(b) 1DOF Peristaltic Motion

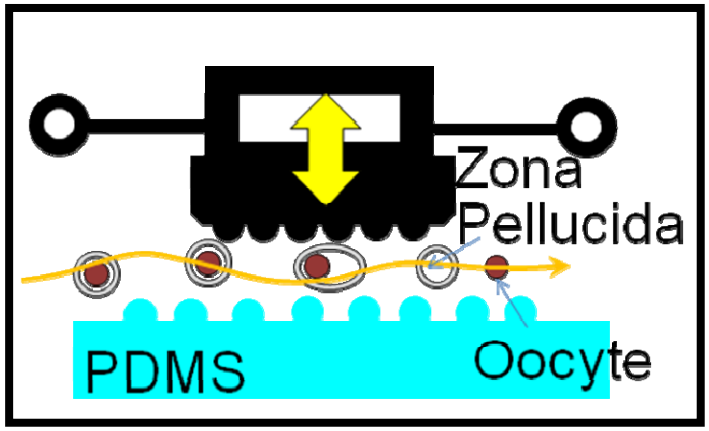


(c) 2-DOF MMT

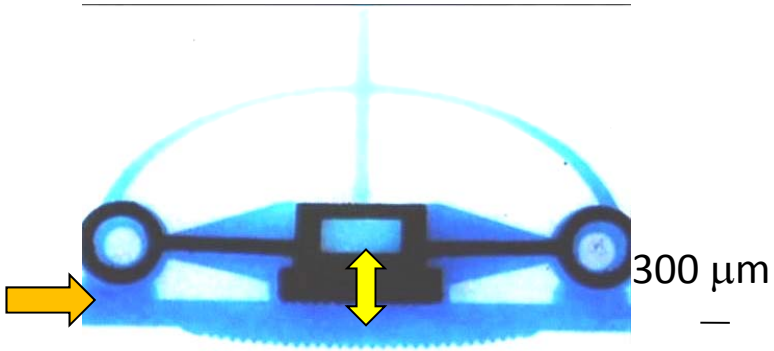


Concept and Fabrication of 1DOF-MMT

Concept



Fabricated Chip



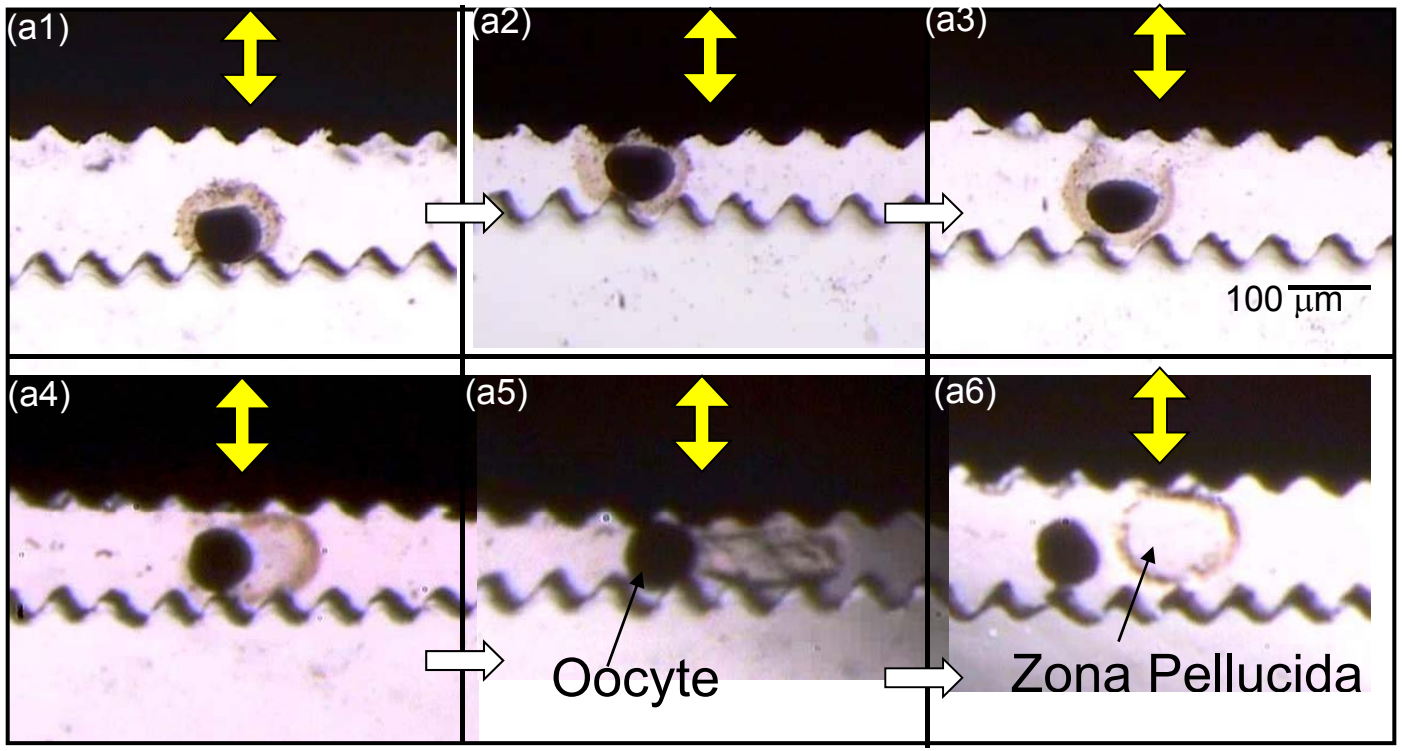
Spiked microchannel and MMT surface＋pin-supported

- **1DOF**：
  No shear stress on Oocyte
  Risk of damage of oocyte

Yamanishi et. al, Journal of Robotics and Mechatronics, Vol. 22, No. 5, pp.623-630, (2010).

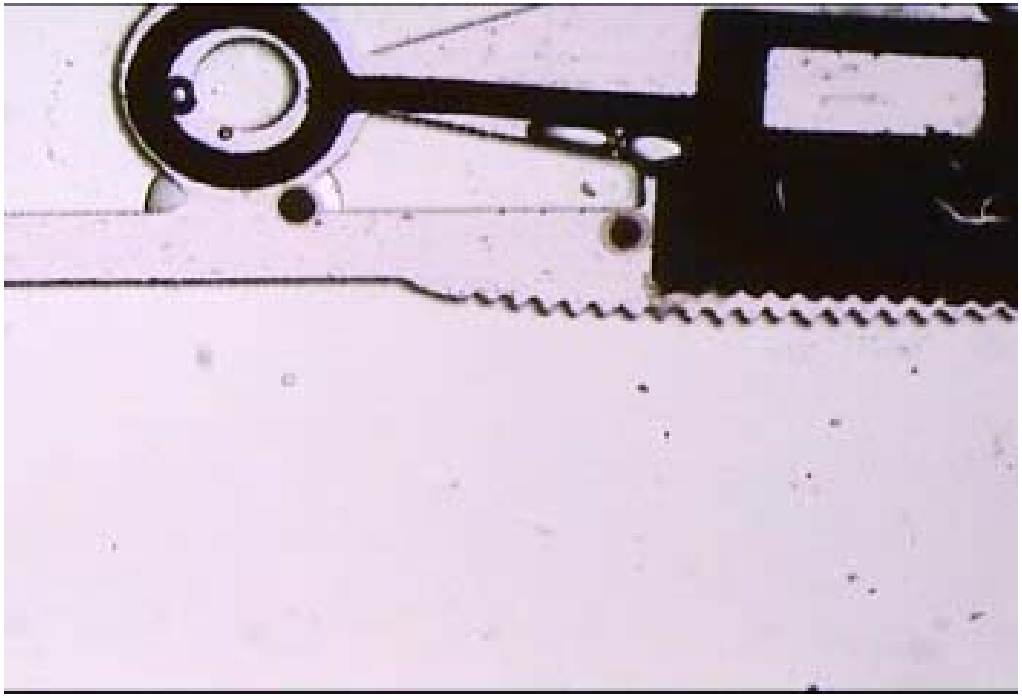
Removal of Zona Pellucida by 1DOF MMT

MMT Trajectory of Manual Operation

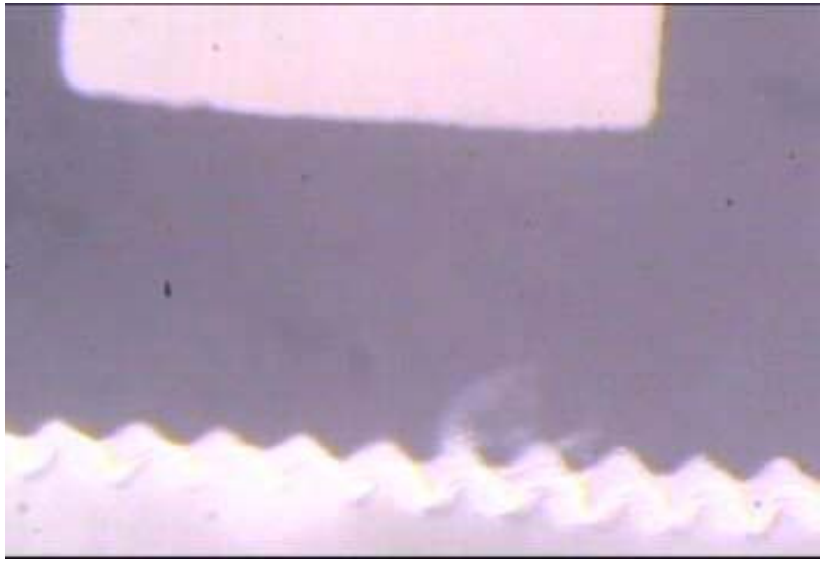


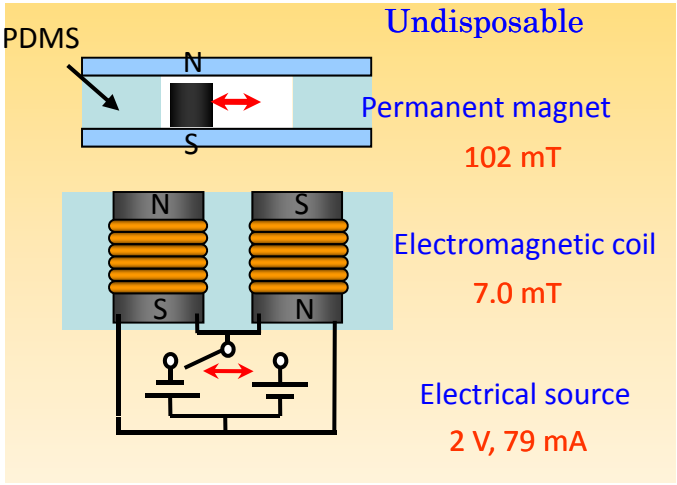
Removal of Zona Pellucida (1DOF MMT)

Successful Manual Operation



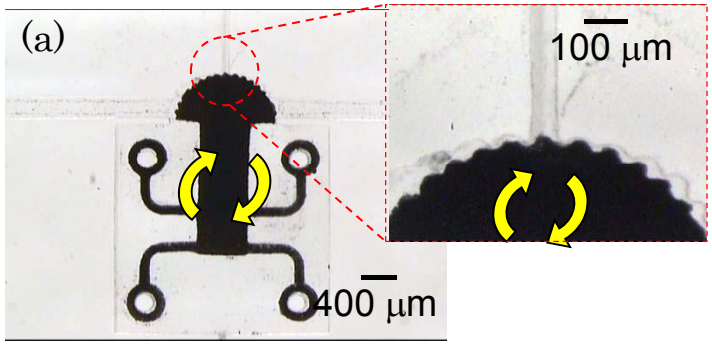
Damaged (Automation)



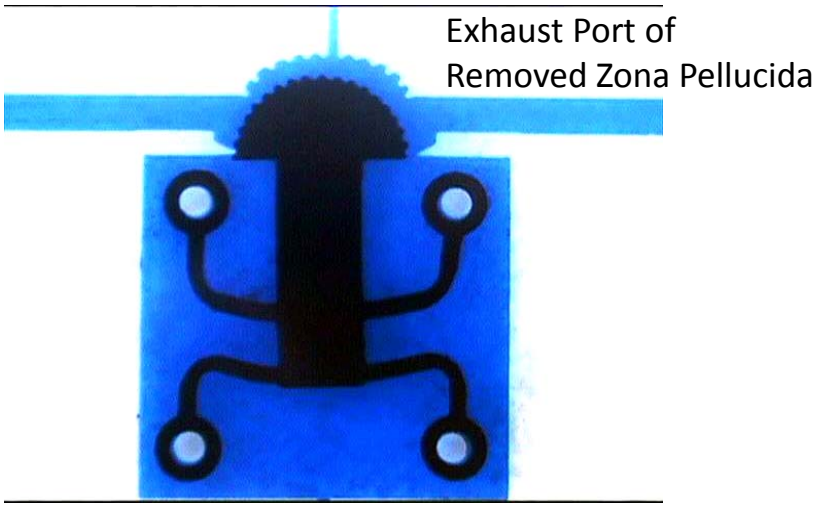


Fabrication of 2DOF-MMT

Concept



Fabricated Chip



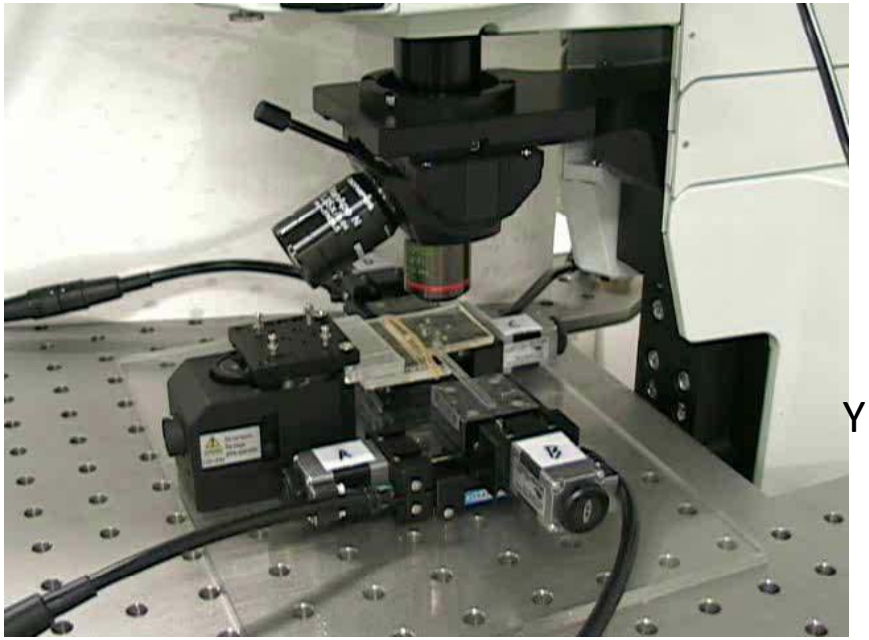
Spiked microchannel and MMT surface＋pin-supported

- **2DOF：**
  Changeable Distance between MMT and Oocyte
  Effective Shear Stress on Oocyte
  Avoid Damage of MMT by Trajectory Control

Yamanishi et. al, Journal of Robotics and Mechatronics, Vol. 22, No. 5, pp.623-630, (2010).

XY stage Controlled MMT

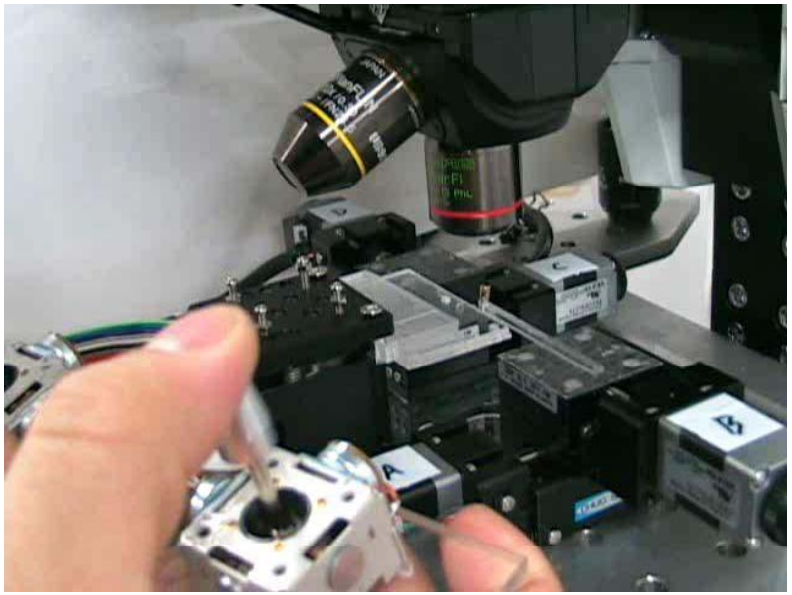
Production of Circular Orbit



X-stage $Y = B\sin\omega t$

Y-stage $Y = A\sin\omega t$

Joy-stick control



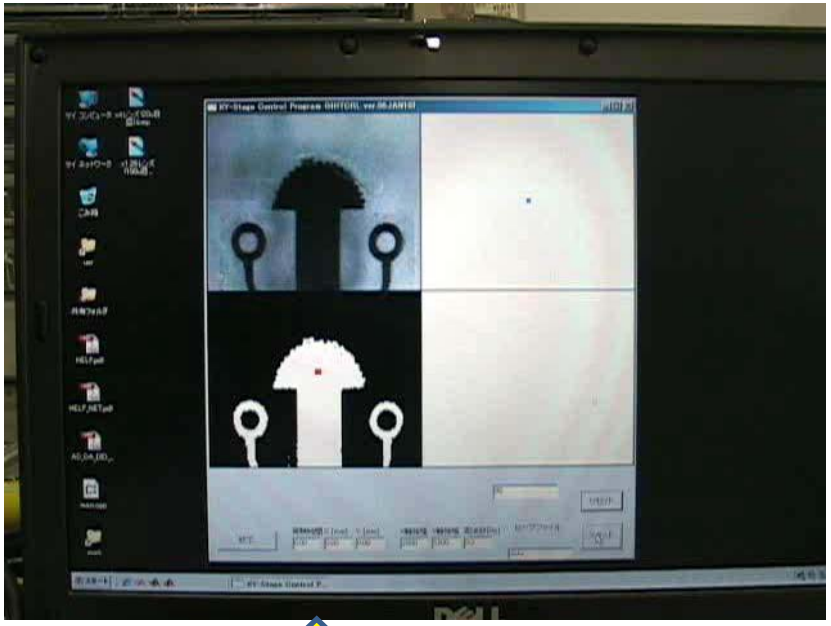
Control the Trajectory of Magnet

Raw Image

Input Trajectory of Magnet

Trajectory of Centre of Gravity

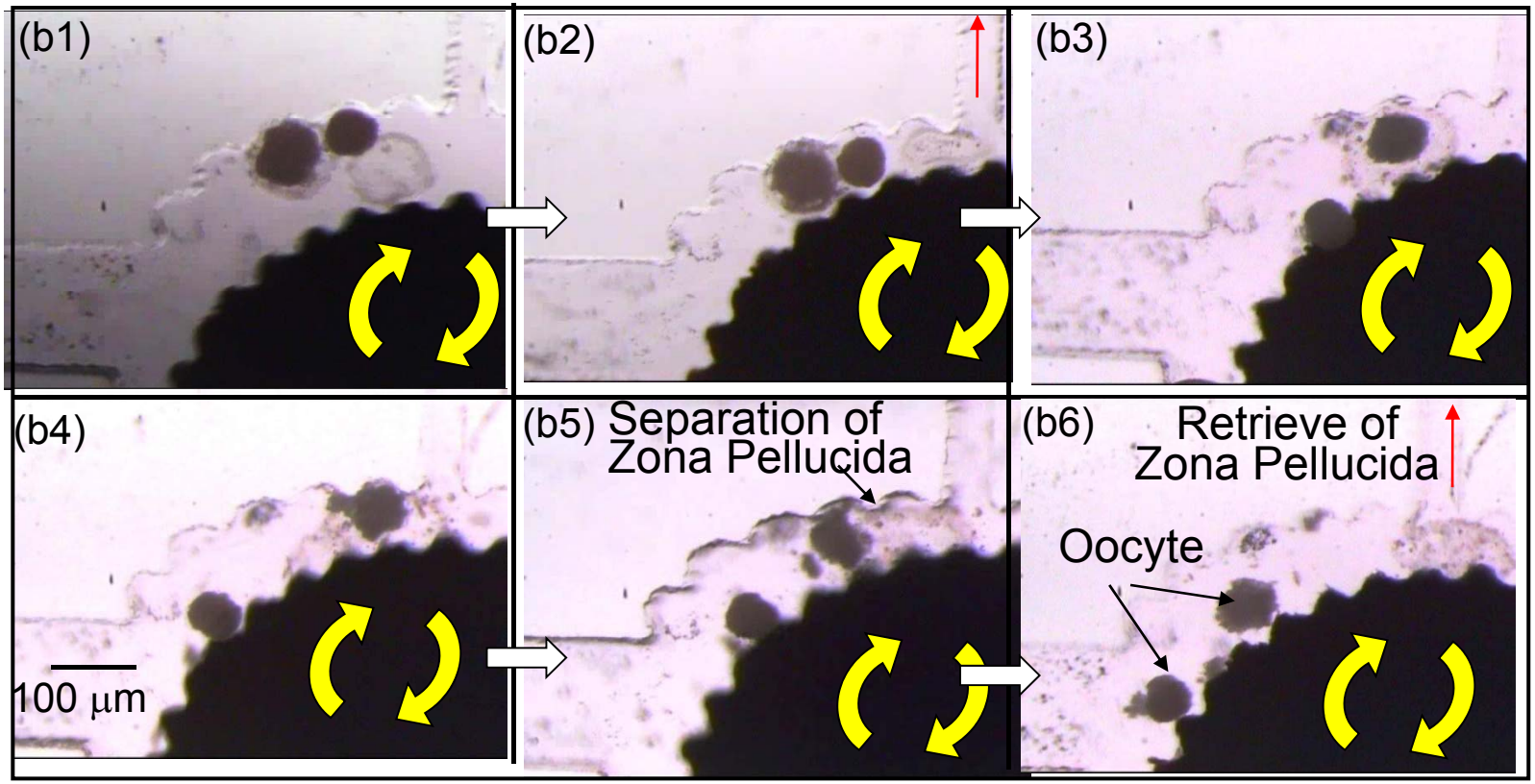
Raw Image



Trajectory of Centre of Gravity

Removal of Zona Pellucida by 2DOF MMT

- Removal of Zona Pellucida of Multiple Oocyte
- Retrieve of Zona Pellucida on a chip

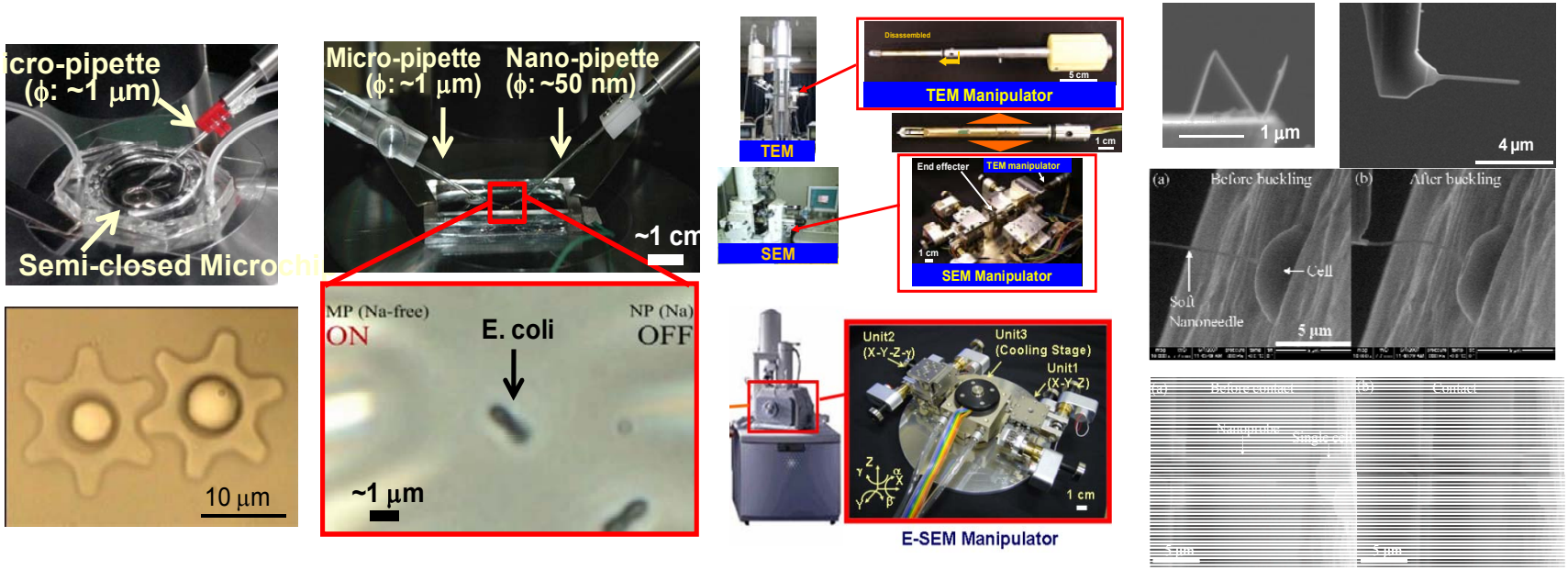


Yamanishi et. al, Journal of Robotics and Mechatronics, Vol. 22, No. 5, pp.623-630, (2010).

Classification of MMT

Classification			Characteristics
A. Control Method of Magnetic Field			
	1. Fixed Electromagnet		Magnetic field is controlled by the electromagnets
		1.1 Switching the magnetic pole	**ON/OFF type**
		1.2 Distribution of the magnetic field	Continuous type
	2. Move of Electromagnet		Control the position of the electromagnet by an actuator and control the magnetic pole and magnetic field (piezoelectric, AC/DC motor, etc.)
	3. Move of Permanent magnet		Control the position of the permanent magnet by an actuator and control the magnetic pole and magnetic field (piezoelectric, electromagnet, AC/DC motor, etc.)
B. Magnetic Characteristics of MMT			
	1. VR-type		**Use materials with a high magnetic permeability**
	2. PM-type		**Use a permanent magnet**
C. Degree of Freedom			
	1DOF x, (y, z) - lateral		Continuous, ON/OFF
	1DOF rotation		Continuous, step
	2DOF xy-lateral, lateral + rotation		Continuous, ON/OFF, step
	Multi-degree of Freedom		Continuous, ON/OFF, step

Bio-Micro/Nanorobotic Manipulation System



Center for Micro-nano Mechatronics

Department of Micro-Nano Systems Engineerings

Nagoya University, Japan

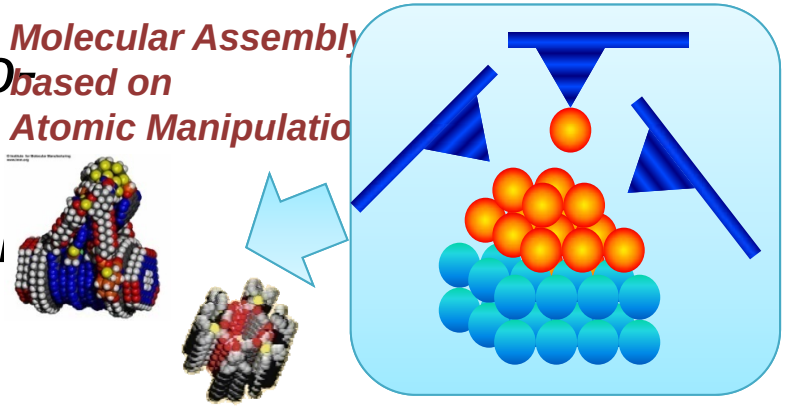
Masahiro Nakajima, Toshio Fukuda

Nanomanipulation System

Nanomanipulation :

Manipulation of individual nano-scale objects.

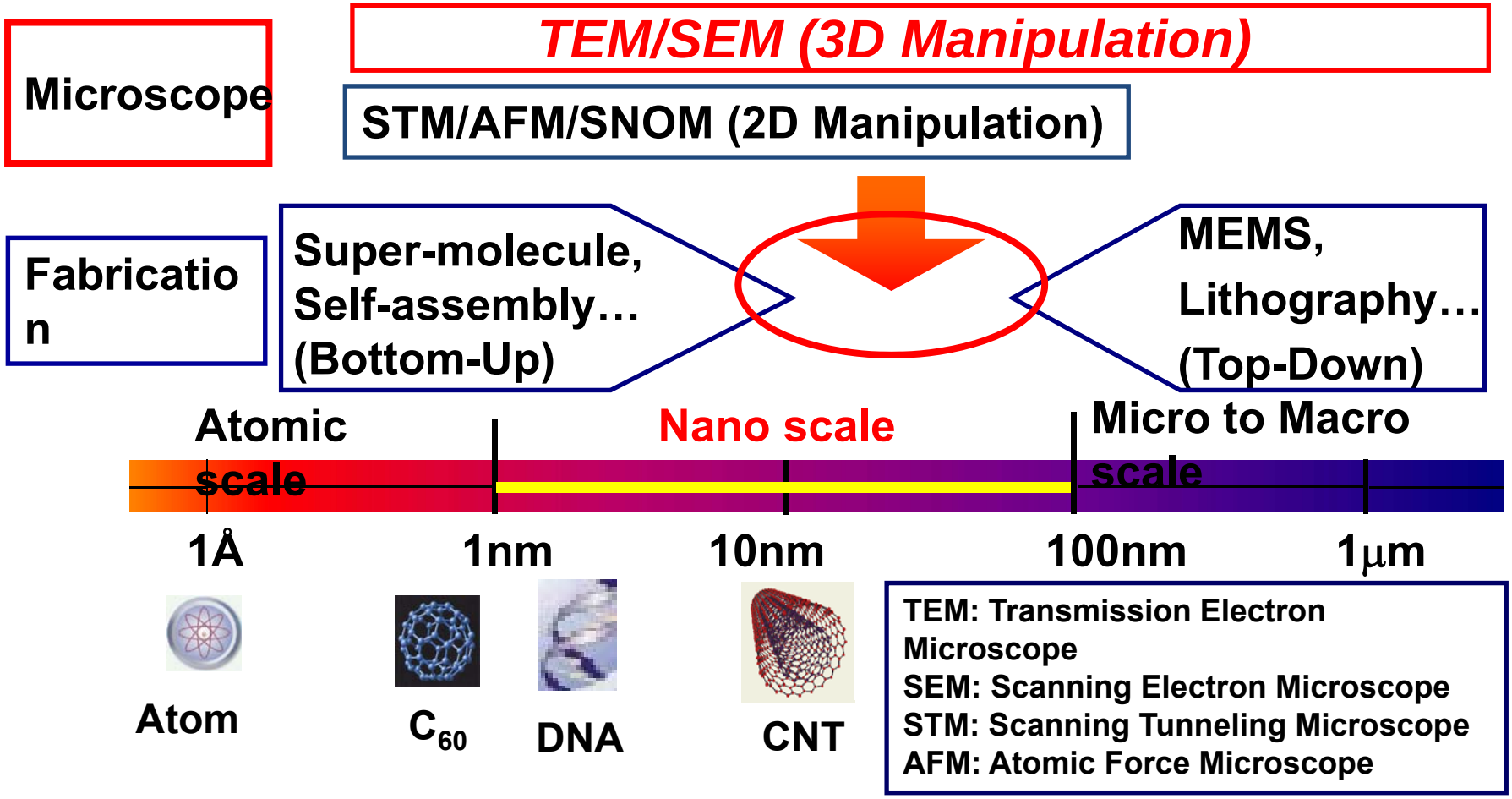
- *Property characterization of nano-materials/nanostructures*
- *Fabrication of nano-building bloc*
- *Assembly of nano-devices,…*



Basic Elements of Micro/Nanorobotic Manipulation System

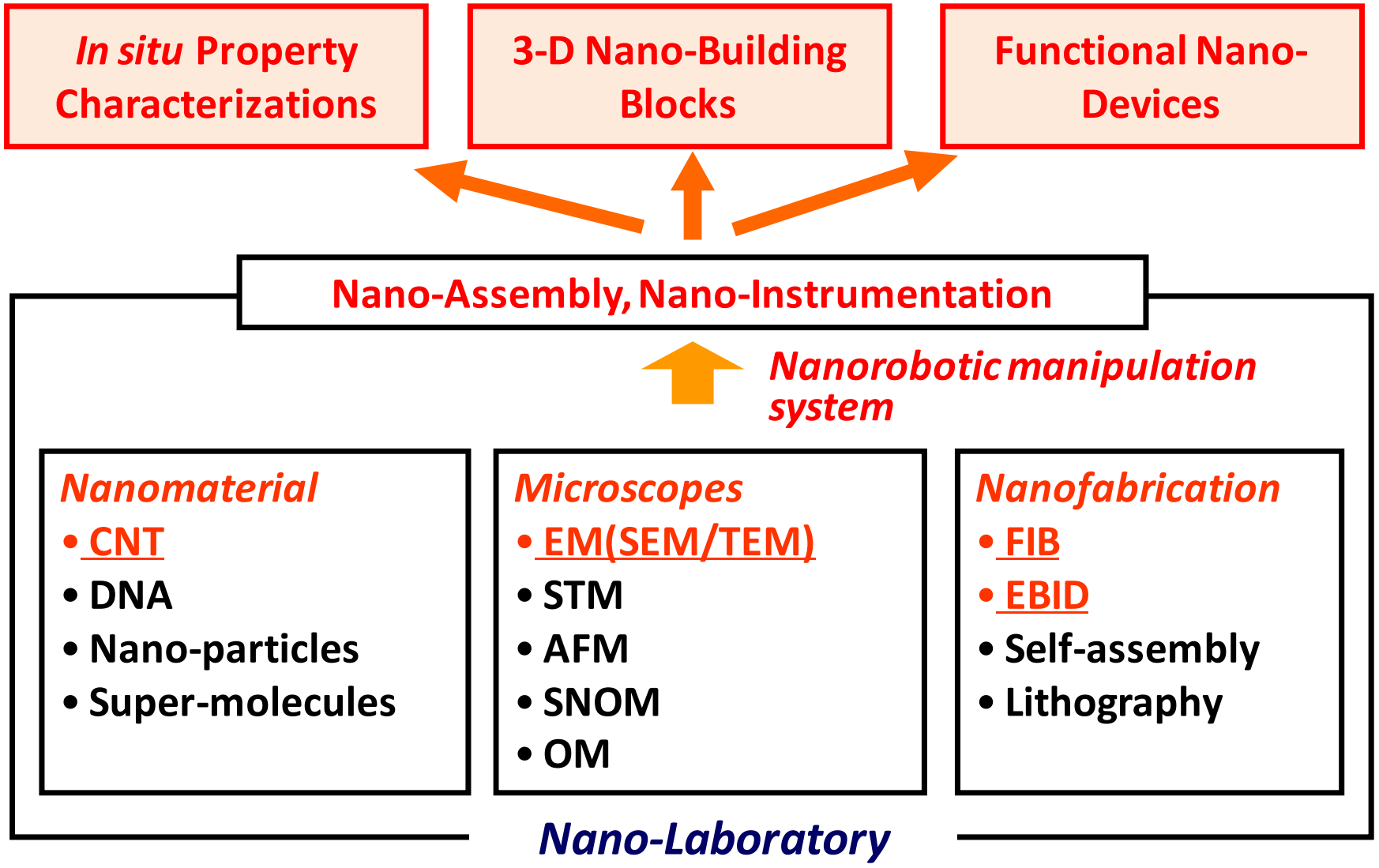
1. Observation System: Microscopes
2. Manipulators: Master-Slave System
3. Sample Chamber: Environmental Control System
4. End-effector: Grasping, Fixing, Cutting, Sensing,…

Nanomanipulation System



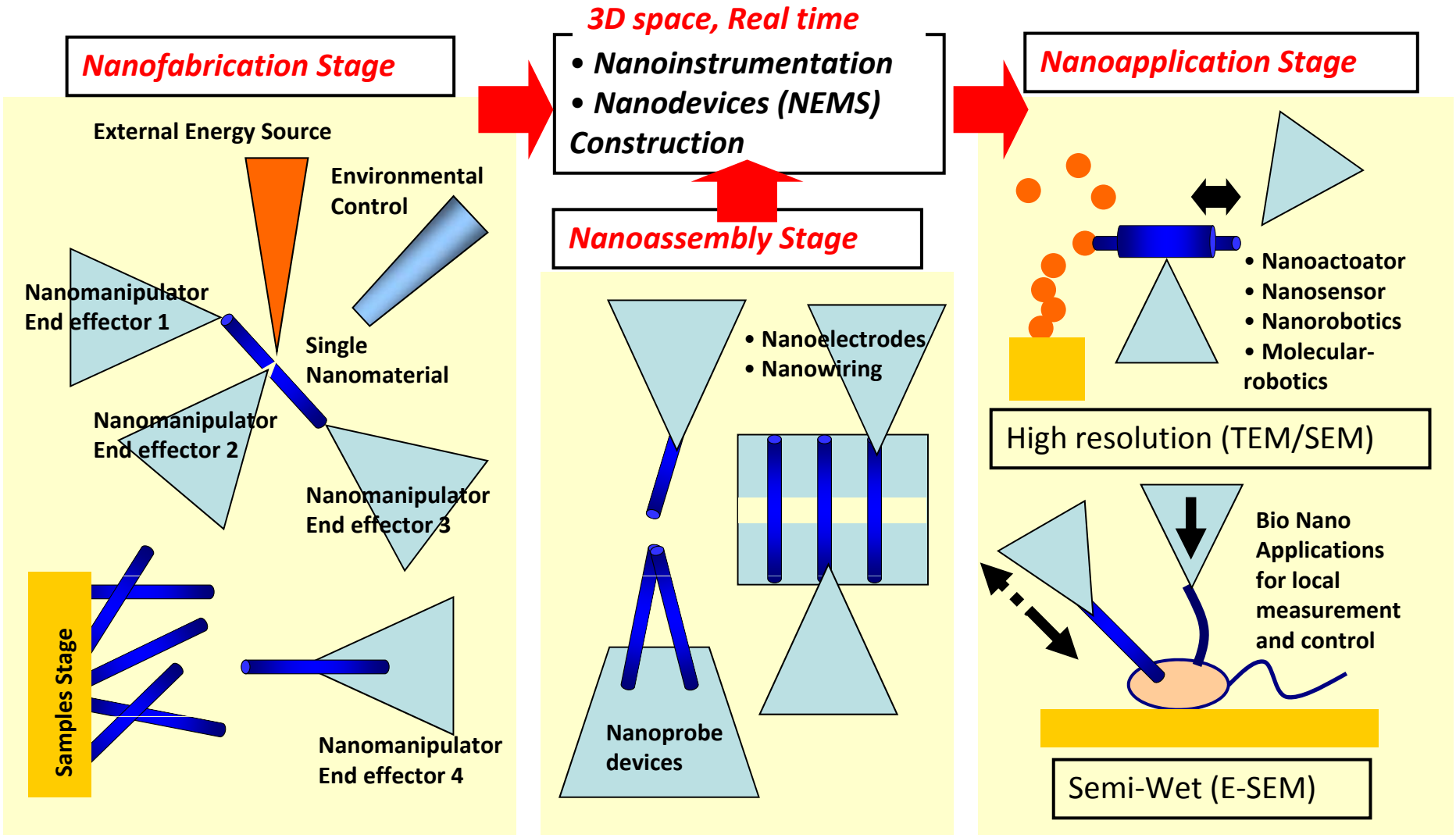
T. Fukuda al. IEEE Nanotechnology Magazine, Vol. 2 Issue 2, pp. 18-31 2008.

Applications of Nanolaboratory



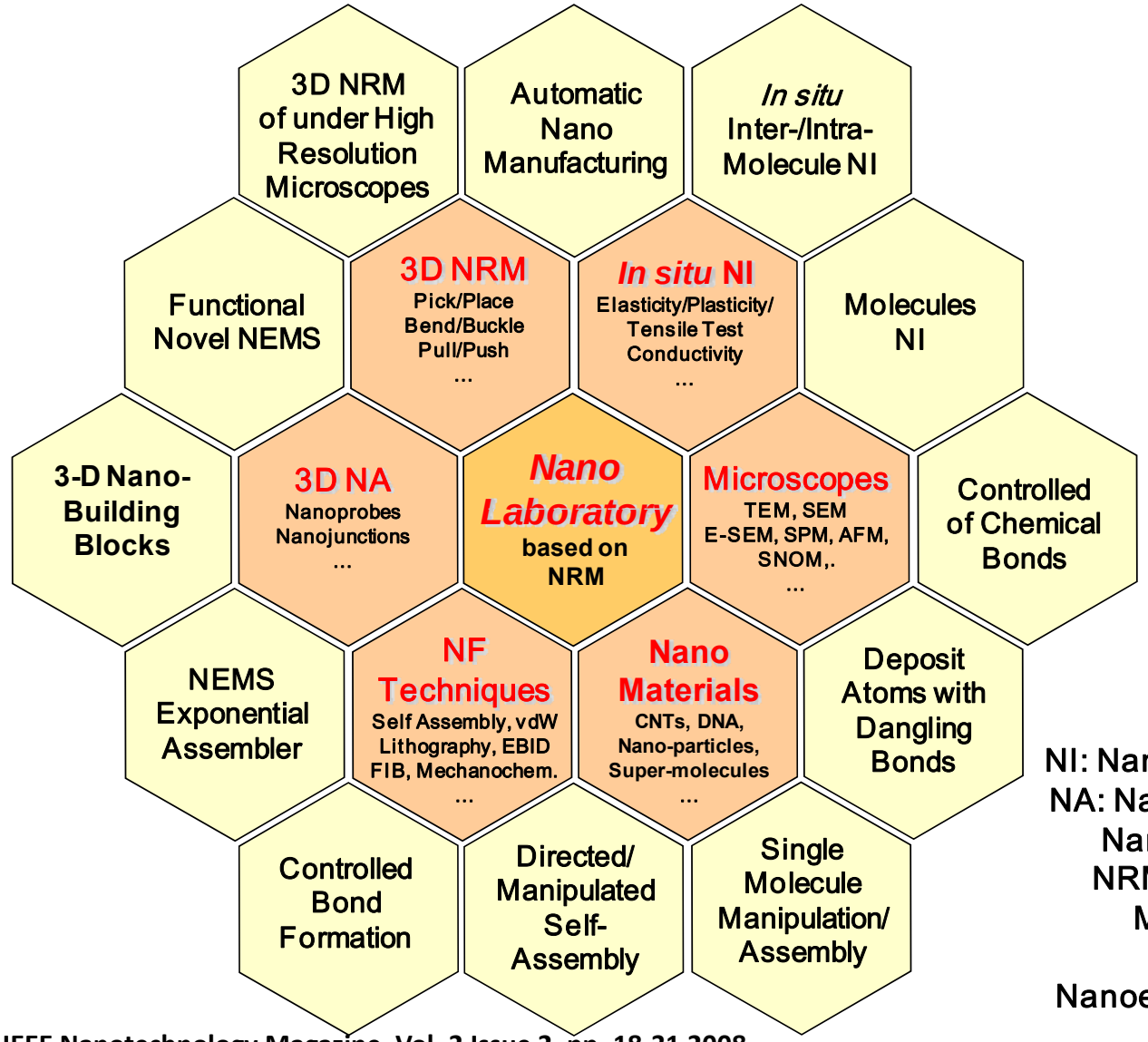
T. Fukuda al. IEEE Nanotechnology Magazine, Vol. 2 Issue 2, pp. 18-31 2008.

Strategies of Nanolaboratory



T. Fukuda al. IEEE Nanotechnology Magazine, Vol. 2 Issue 2, pp. 18-31 2008.

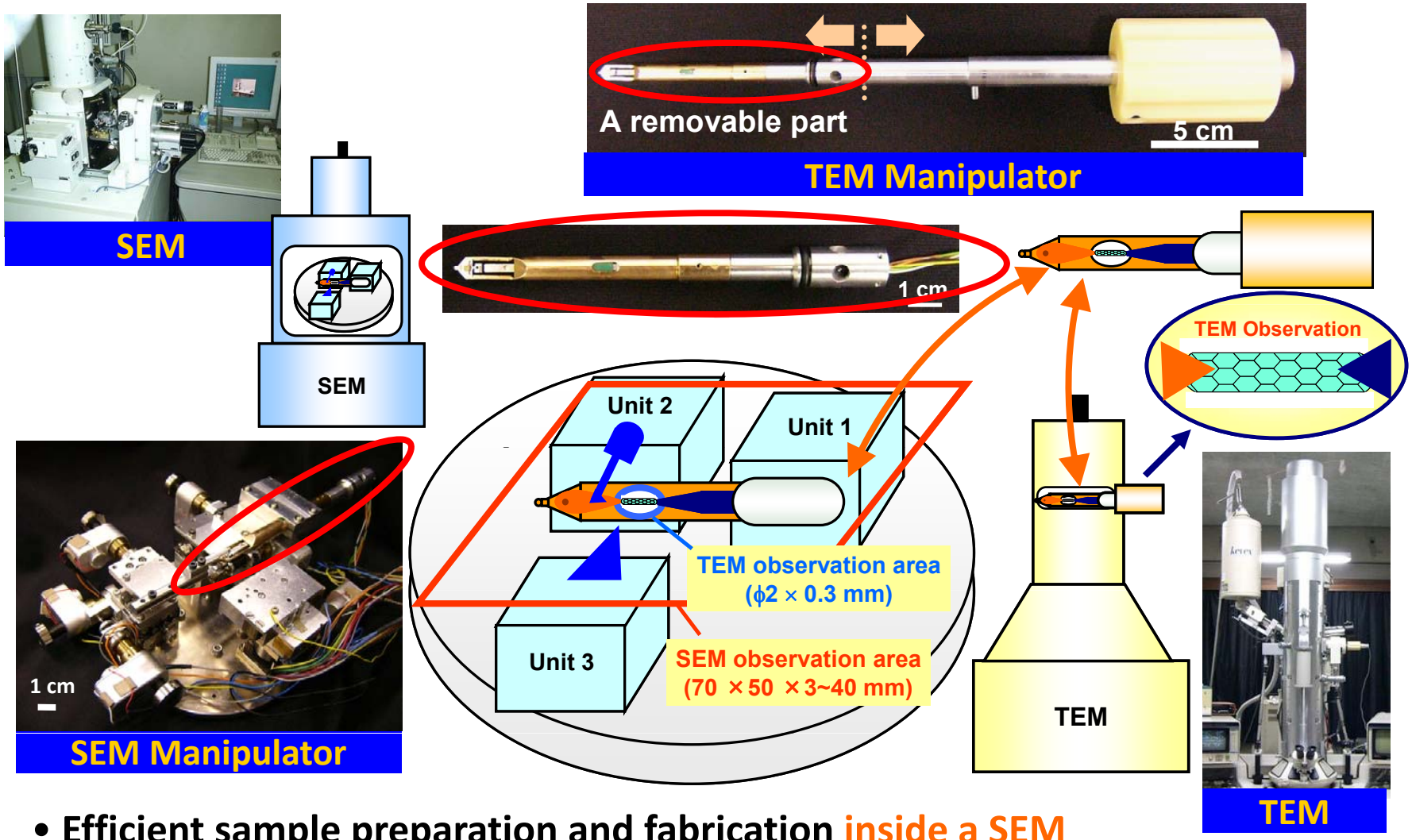
Nanolaboratory



NI: Nano Instrumentation
NA: Nano Assembly NF: Nano Fabrication
NRM: Nanorobotic Manipulation
NEMS: Nanoelectromechanical System

T. Fukuda al. IEEE Nanotechnology Magazine, Vol. 2 Issue 2, pp. 18-31 2008.

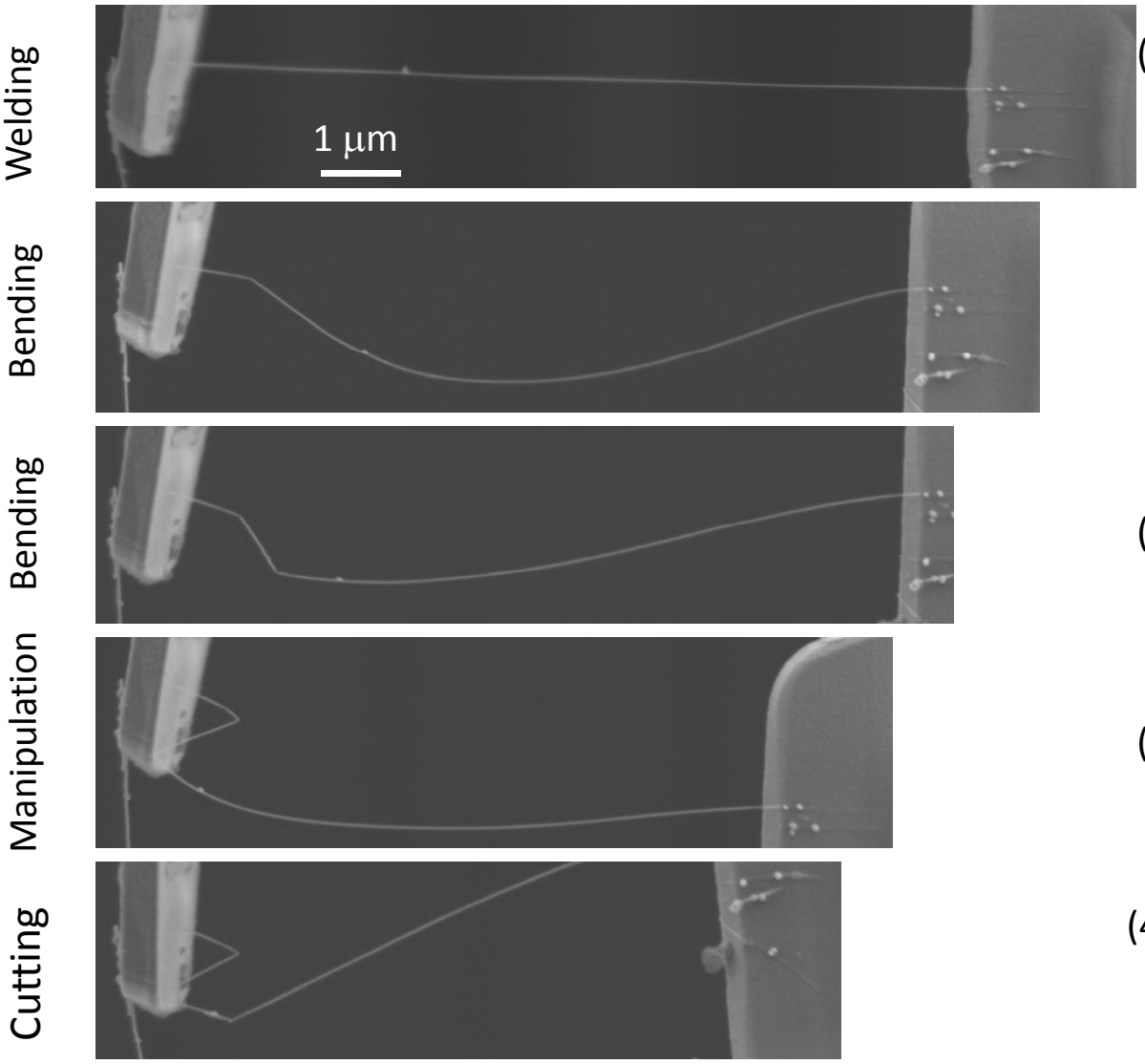
Hybrid Nanorobotic Manipulation System



- Efficient sample preparation and fabrication inside a SEM
- Measurement and evaluation with high resolution inside a TEM

M. Nakajima et al. IEEE Transactions on Nanotechnology, Vol. 5, No. 3, pp. 243-248 2006.

Assembly of 3D Nano-Structure



(1) A CNT was picked up with an AFM cantilever. One end of the CNT was fixed on the surface by deposits. The other was set to touch the surface of another AFM cantilever.

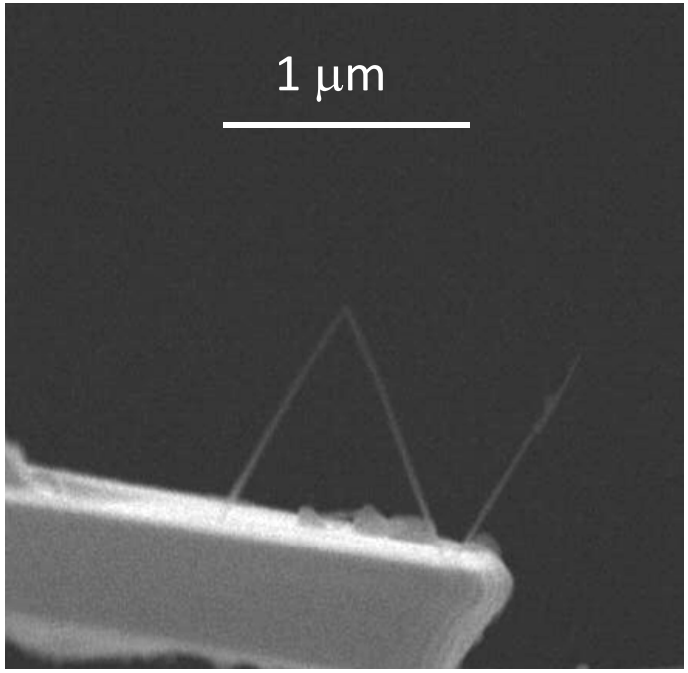
(2) CNT was bent at two points.

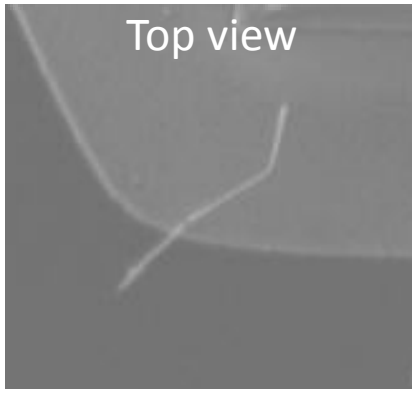
(3) Let the second knick be set to touch the substrate.

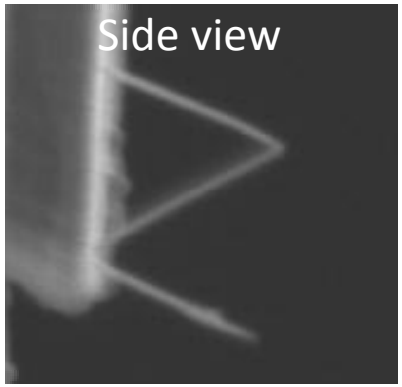
(4) we cut it at the third point to separate the nanostructure apart from the cantilever.

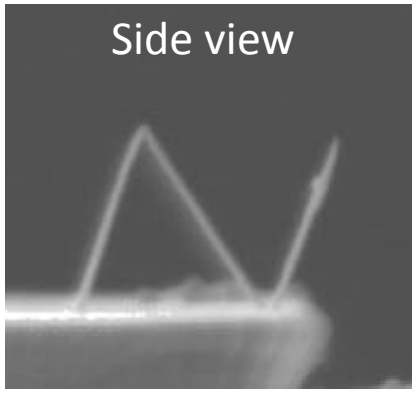
T. Fukuda al. IEEE Nanotechnology Magazine, Vol. 2 Issue 2, pp. 18-31 2008.

A 3D Structure – "N"









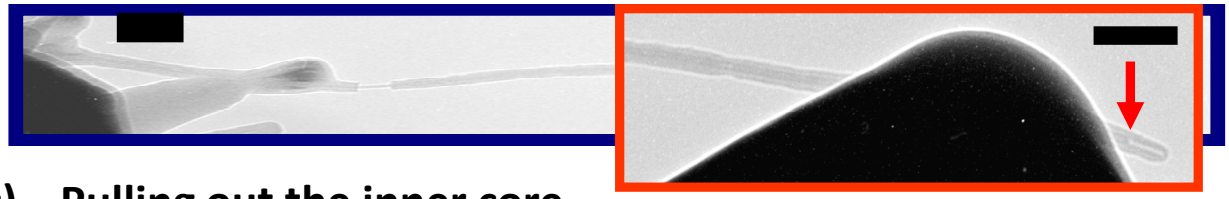
T. Fukuda al. IEEE Nanotechnology Magazine, Vol. 2 Issue 2, pp. 18-31 2008.

Destructive Fabrication Process

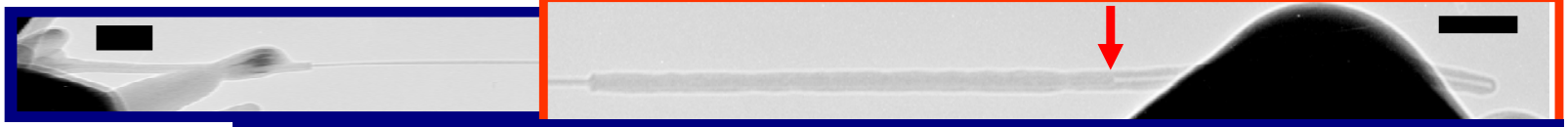
(a) Before destructive fabrication

Outer diameter: ~28 nm,
Inner diameter: ~ 2 nm

(b) At the moment of destructive fabrication

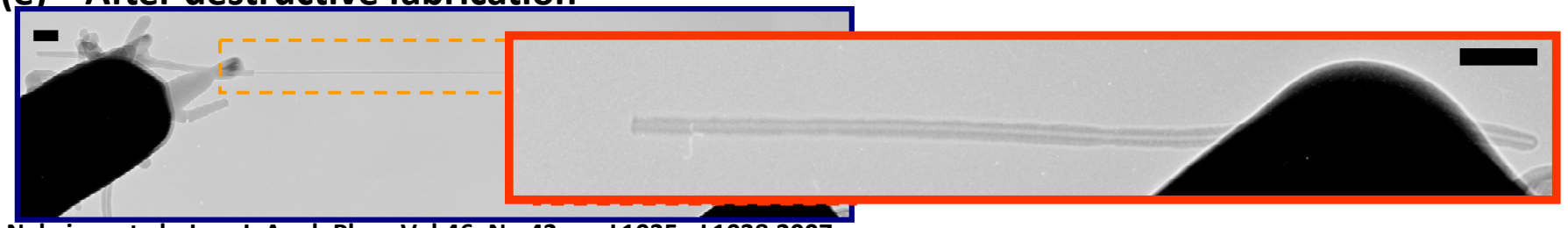


(c) Pulling out the inner core



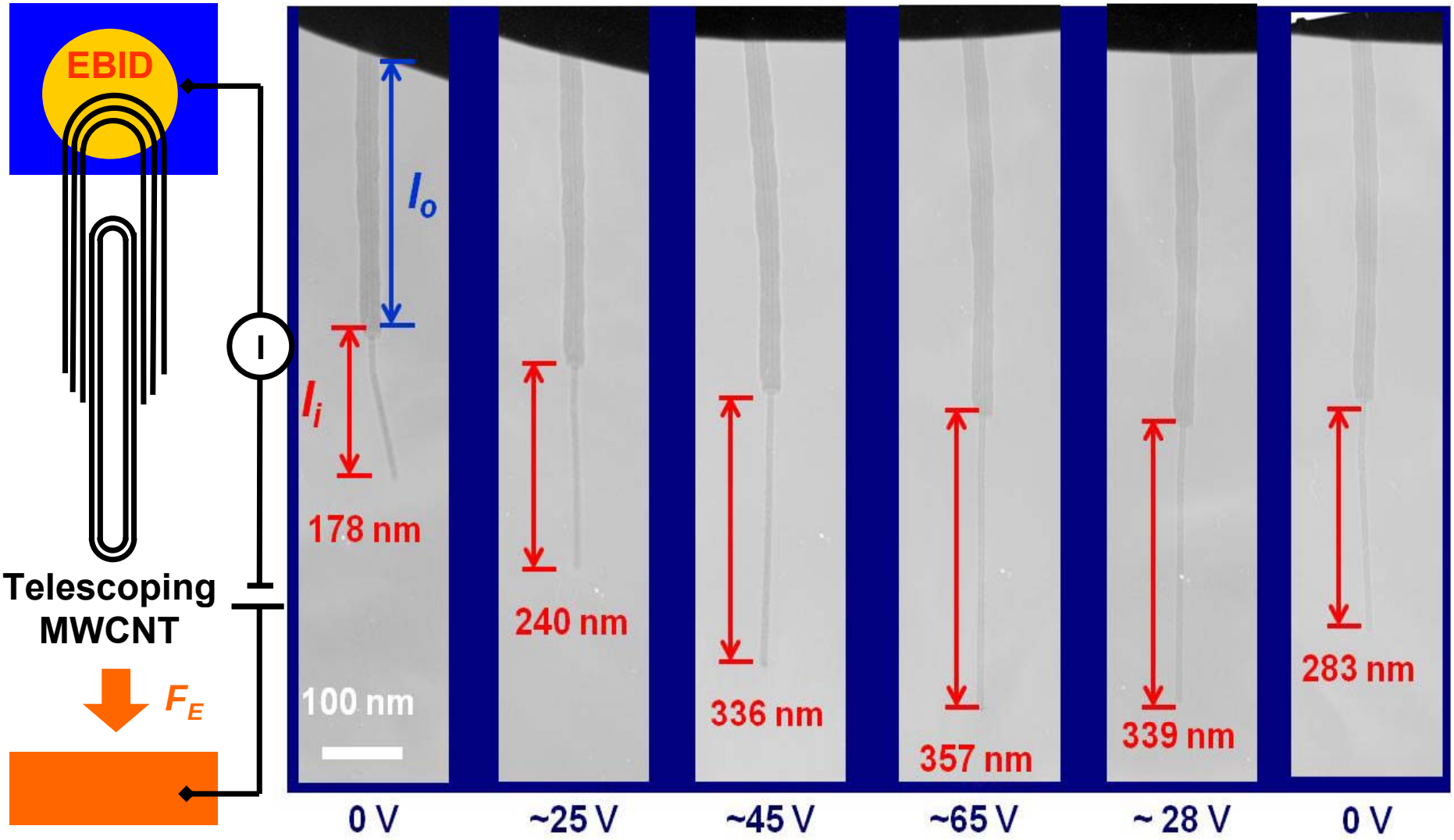
(d) Pulling o

(e) After destructive fabrication



M. Nakajma et al., Jpn. J. Appl. Phys. Vol.46, No.42, pp.L1035 - L1038 2007.

Telescoping Nanotube Nanoactuation



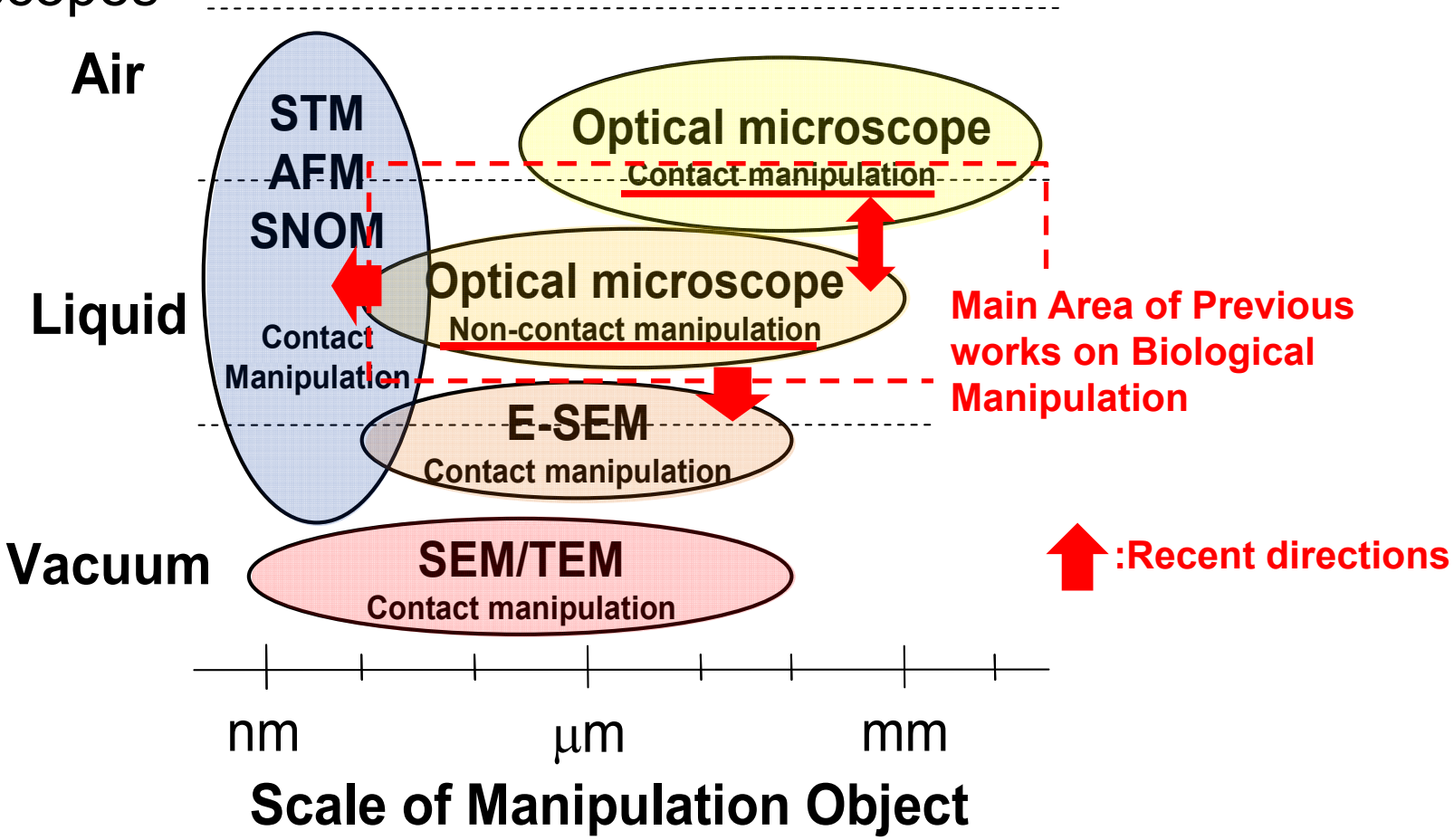
Gap between CNT and Electrode: 1.2 μm

Actuation of Inner-core: ~180 nm

M. Nakajma et al., Jpn. J. Appl. Phys. Vol.46, No.42, pp.L1035 - L1038 2007.

Micro-Nanorobotic Manipulation System

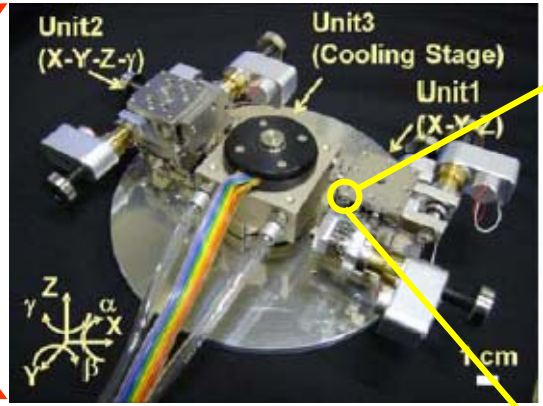
Micro-Nanorobotic Manipulation System under various Microscopes

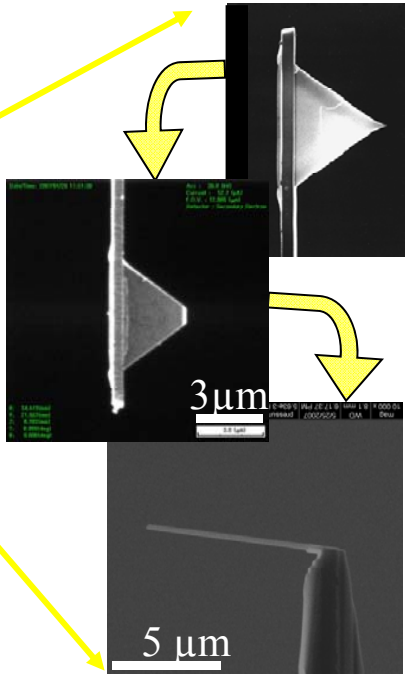


T. Fukuda al. IEEE Nanotechnology Magazine, Vol. 2 Issue 2, pp. 18-31 2008.

ESEM-Nanomanipulation System

Materials & Methods





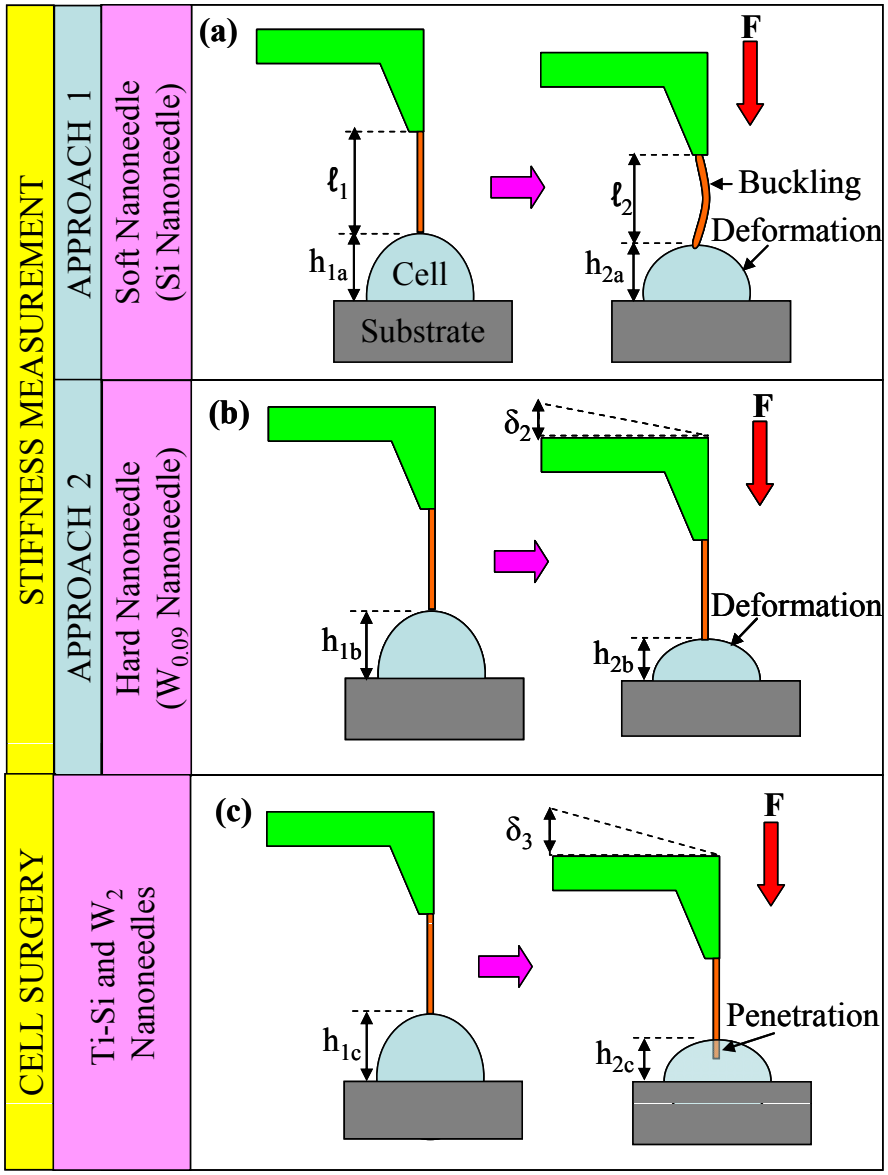
Environmental Scanning Electron Microscope (ESEM)	
Vacuum Mode	E-SEM Mode (10–2600 Pa) Low Vacuum Mode (10–130 Pa) High Vacuum Mode (~10^{-4} Pa)
Acc. Voltage	0.2 ~ 30 kV
Resolution	3.5 nm (E-SEM Mode) 15 nm (Low Vacuum Mode) 3.5 nm (High Vacuum Mode)
Obs. Space	150 mm × 150 mm × 65 mm
Max. Obs. Area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

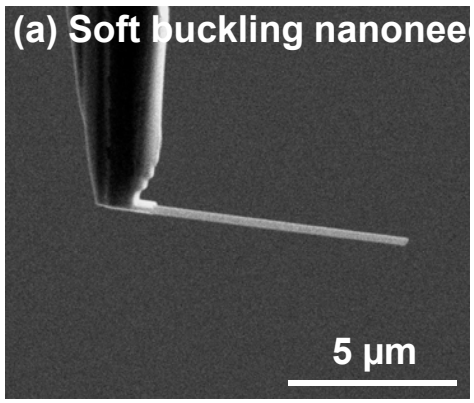
End-effector	
Multi-shapes	•Sharp Tip (0.02-2 N/m spring constants) •Flat Tip (0.2-2 N/m spring constants) •Nanoneedle (0.2, 0.8, 2 N/m spring constants) (< 200 nm diameters)

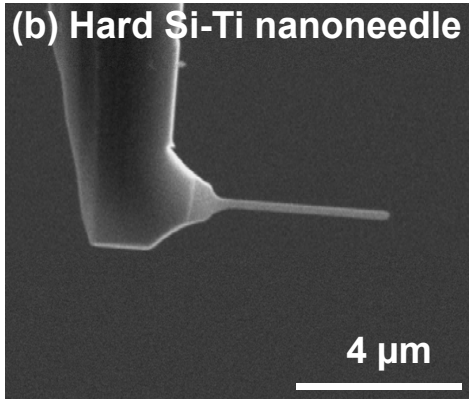
M. R. Ahmad et al., *IEEE Trans. on Nanobioscience*, 7(3), pp. 185-193, 2008.

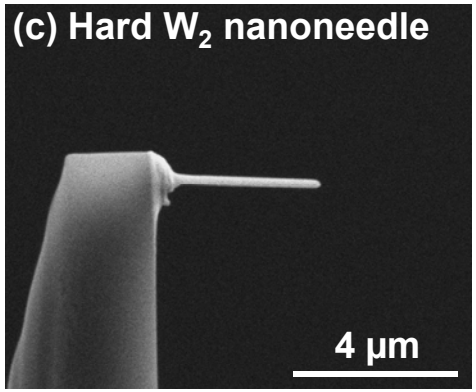
Nano-probes for Single Cells Analysis

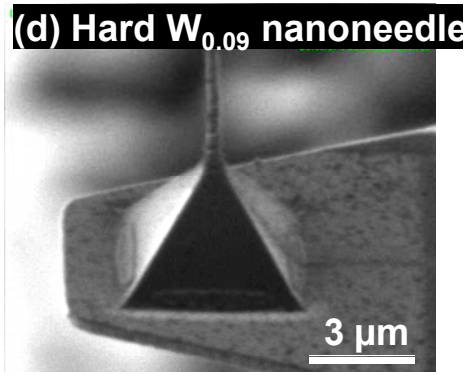


Actual images of the nano-probes







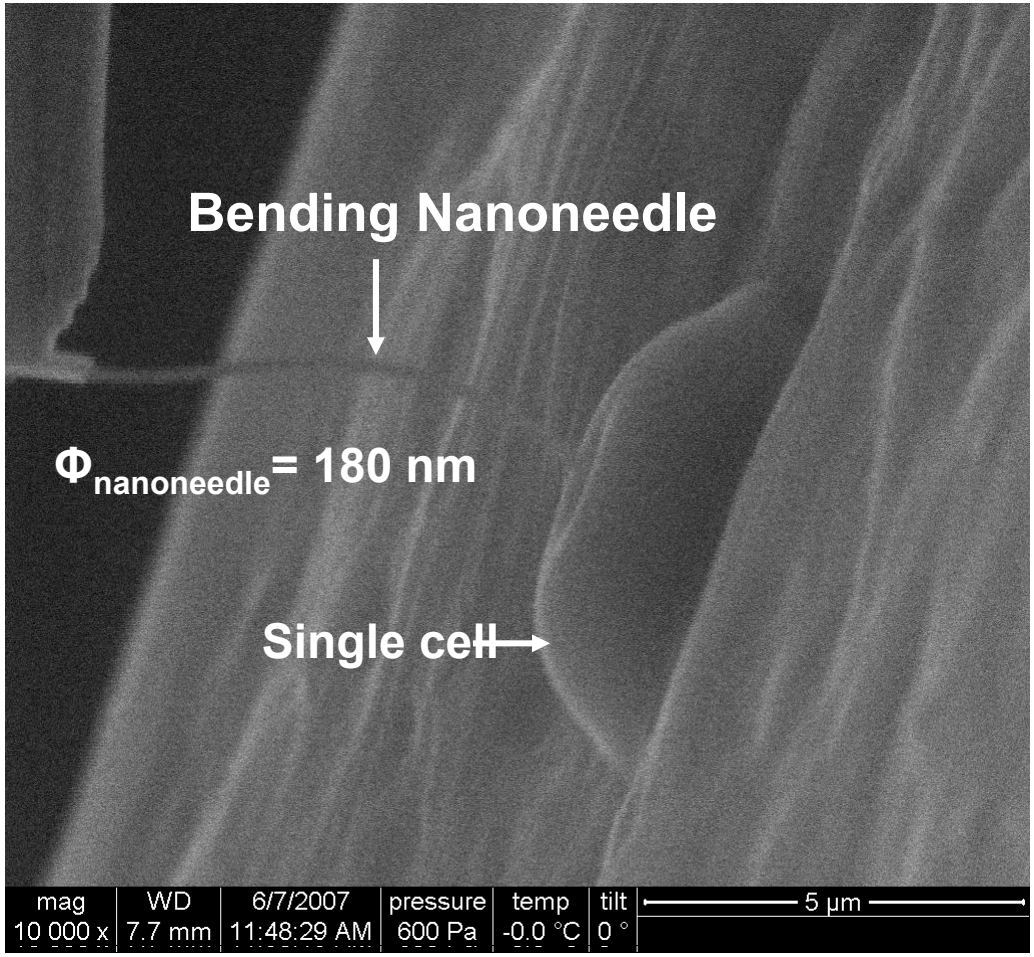


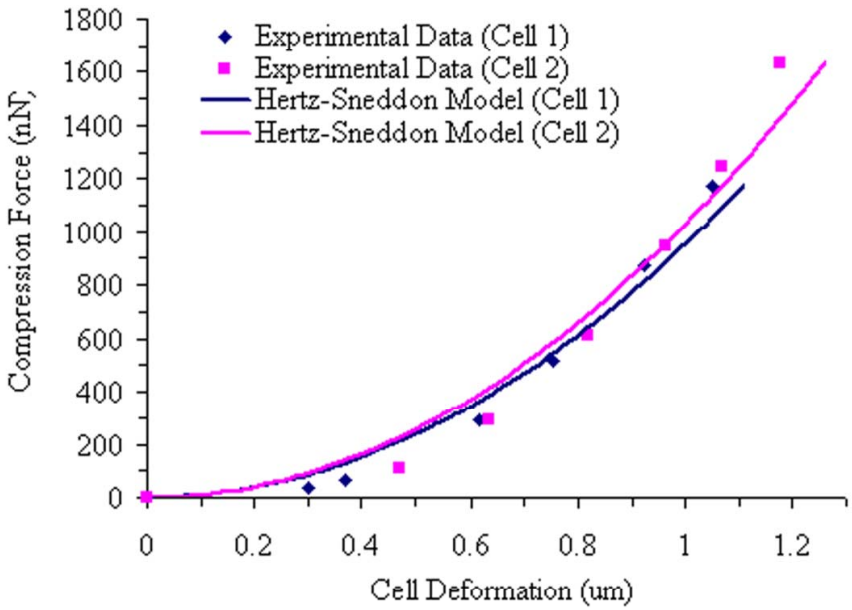
The diameter of the nano-probes is around 170 – 200 nm

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

Results -Nano-probes for single cell analysis-

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.