Advanced 5
Biological Application
～m-TAS～

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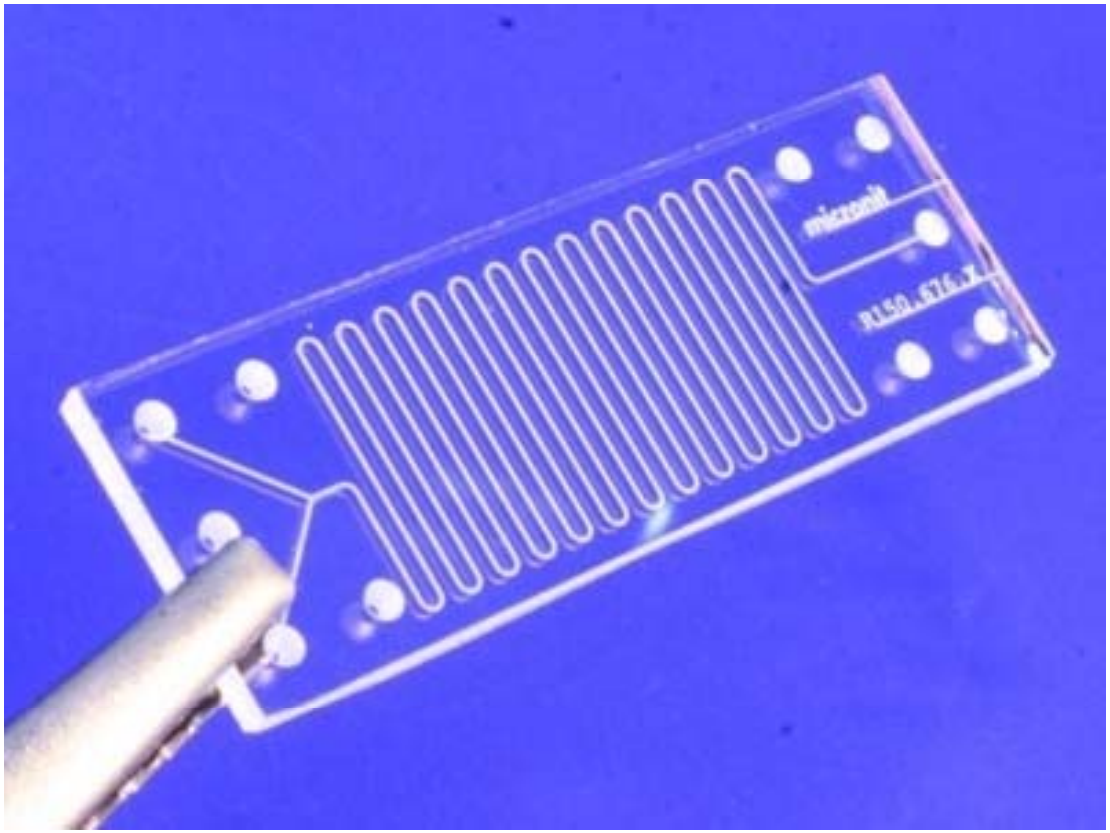
Nagoya University

Lab-on-a-chip



Microreactor-chip (by Micronit Microfluidics)

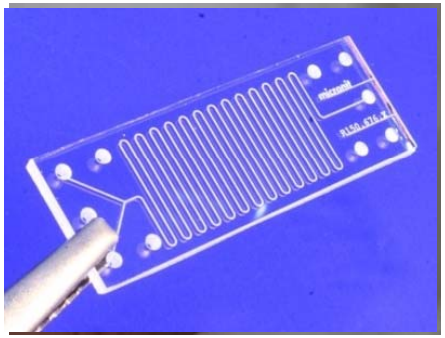
A **lab-on-a-chip** (LOC) is a device that integrates one or several laboratory functions on a single chip of only millimeters to a few square centimeters in size.(Wikipedia)

Microfluidic devices market will exceed $3 billion in 2014.

Why we use Microchip?

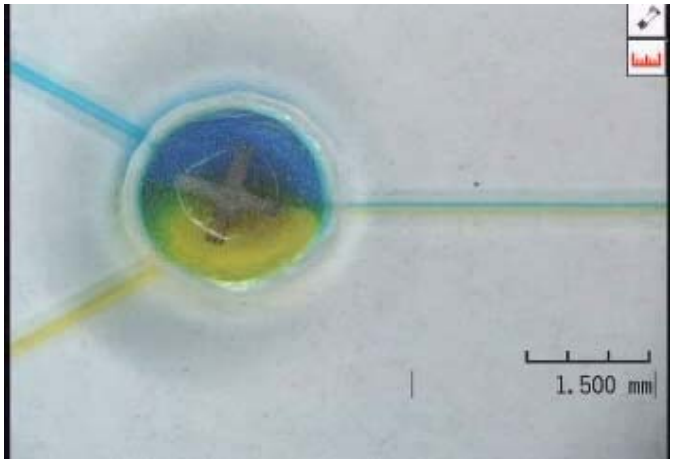
Merits of the biochips.

1. Reduce the consumption of reagent
2. Improve the rate of reaction
3. Parallel processing for different experimental conditions
4. Disposable
5. Low Cost
6. Observation of specific cells



Microchannel

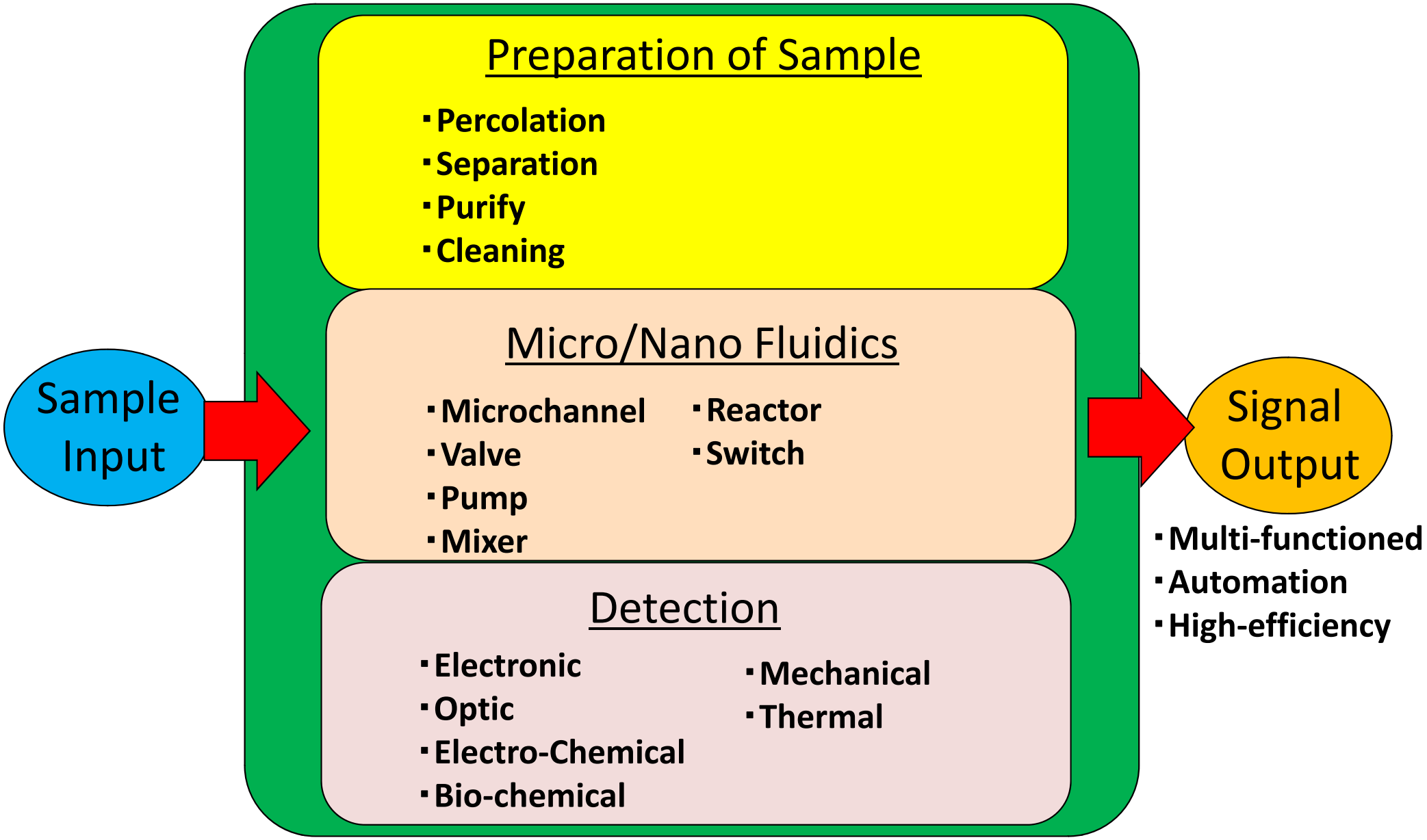
Electrodes



Layered flow: Low Reynolds number

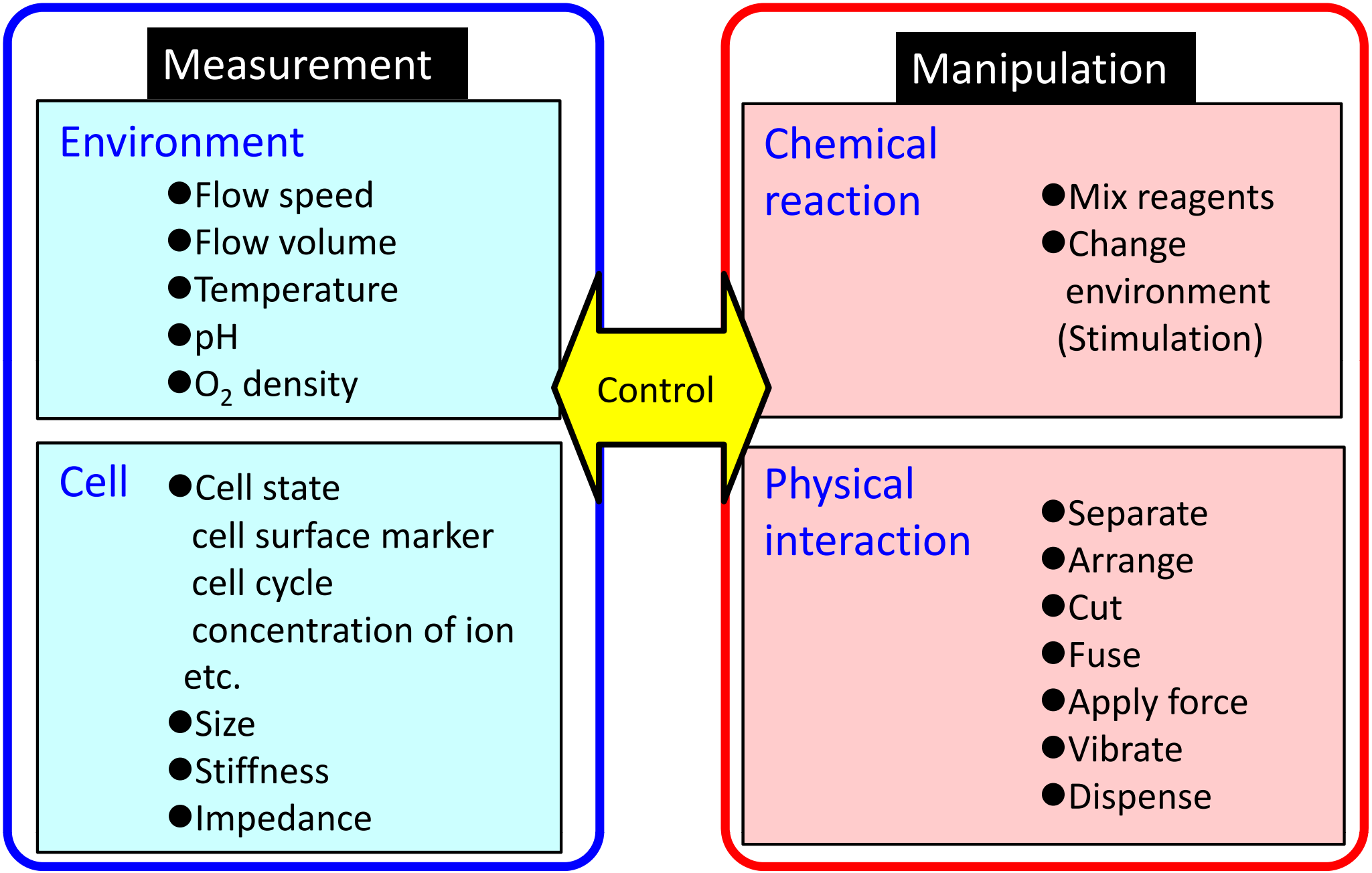
Take advantage of the function.

Concept of μTAS



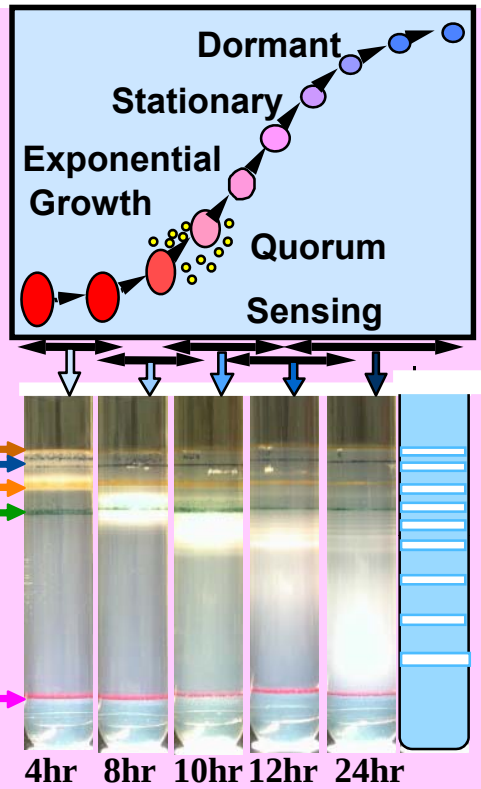
Handbook of Biochip Technology, p.7, NTS Press, (2010).

Works on a biochip



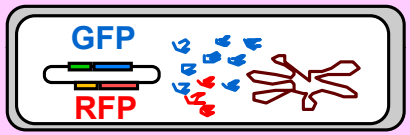
Observation of Immobilized Cell on a Chip

Background (Cell Immobilization on a Chip)



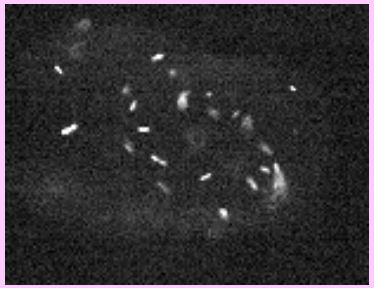
Background

- Heterogeneity of cells
- Analysis of environmental response of the genome transcription is needed.
- Activity of promoters is measured by fluorescence

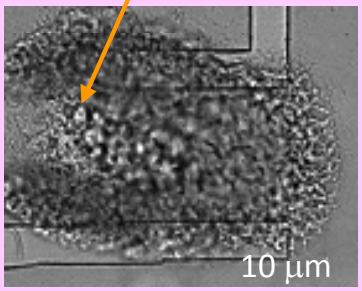


By Prof. Ishihama

E. coli



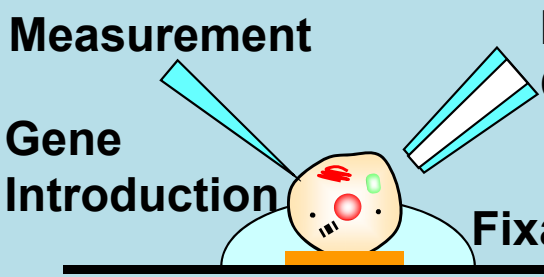
Gene Expression

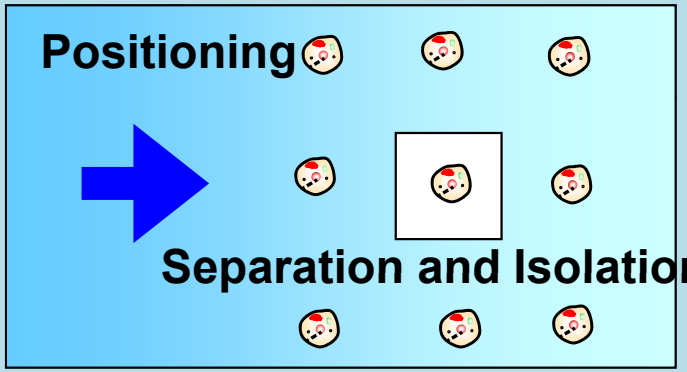


Immobilized E. coli

Measurement and Control of Gene Expression on a chip

Local Control





Immobilization of E. coli

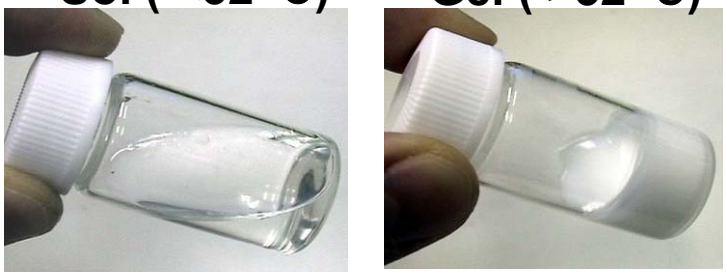
Environmental Response Assay of Genome Transcription

On-chip single cell analysis

1. Repeatability
2. Avoid contamination
3. High success rate

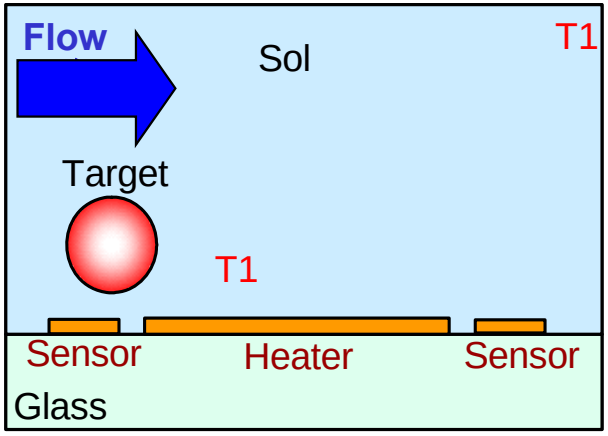
On-chip Separation and Culture with PNIPAAm

Sol (<32 ℃)　　Gel (>32 ℃)

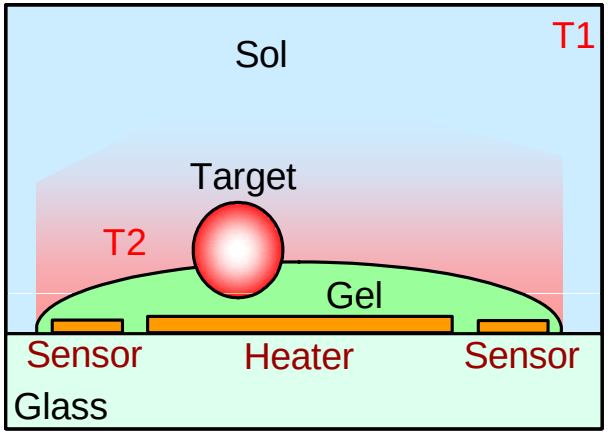


PNIPAAm Sol･Gel Transformation

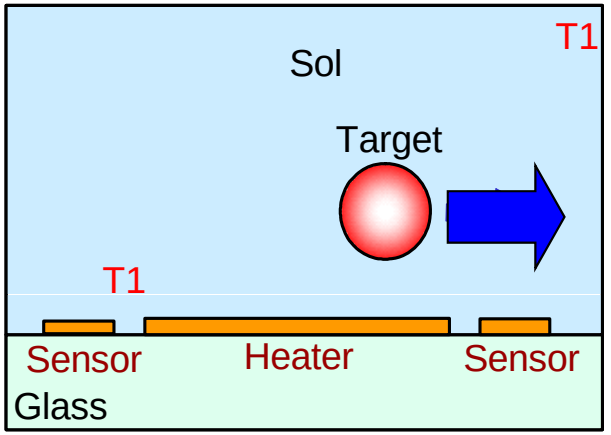
① Before Gelation



② Immobilization

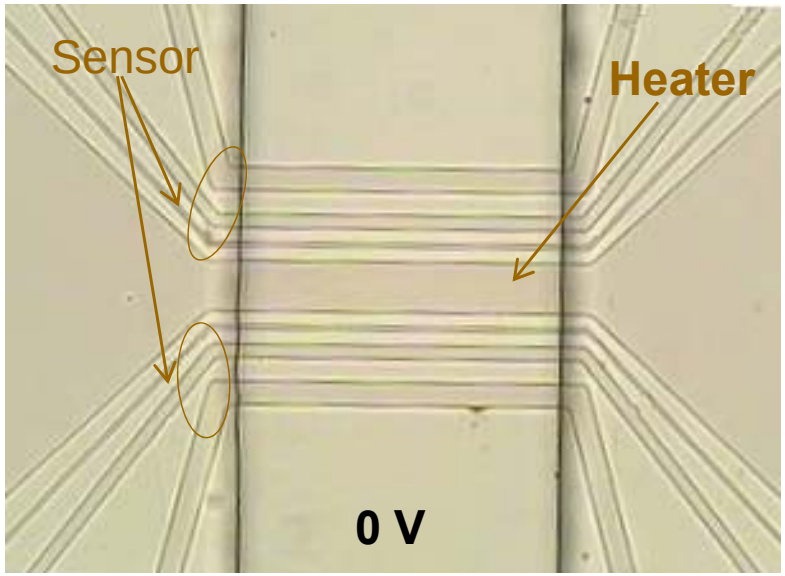


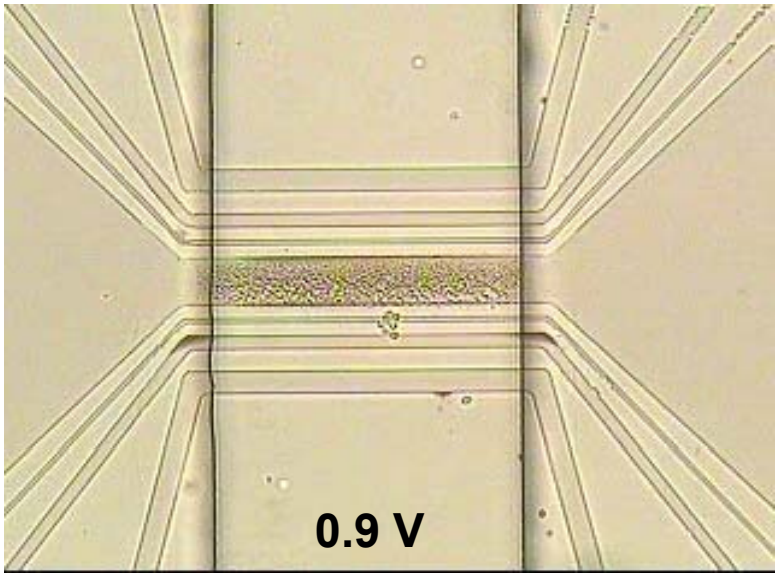
③ Release of Cell



On-chip Separation and Culture with PNIPAAm

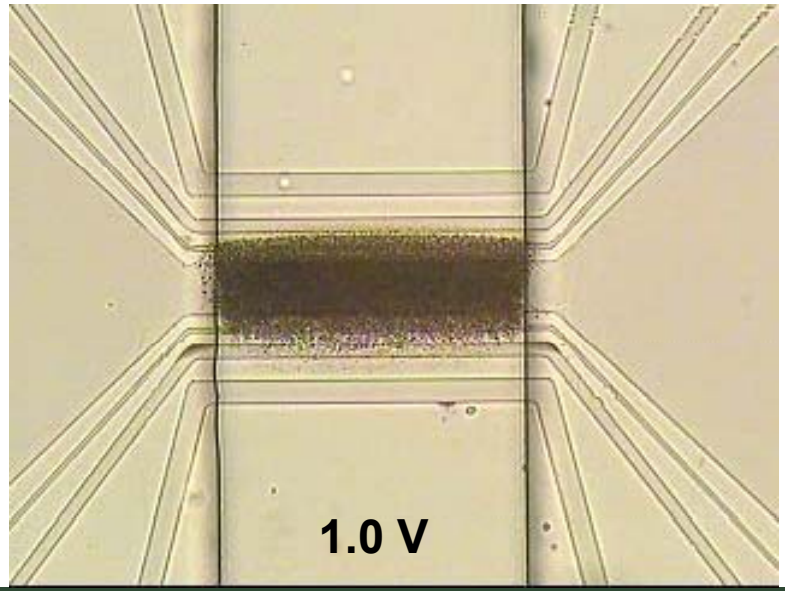
Yeast Cell Immobilization with PNIPAAm by ITO Microheater and Microsensor

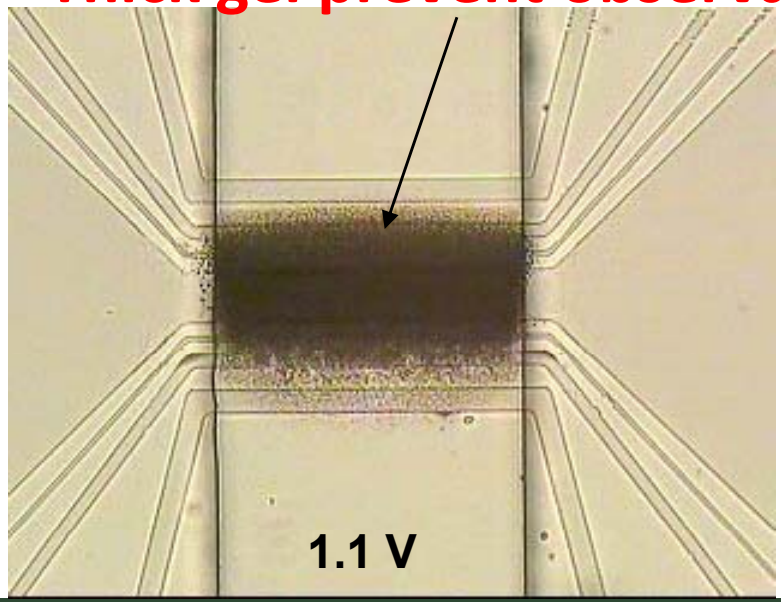




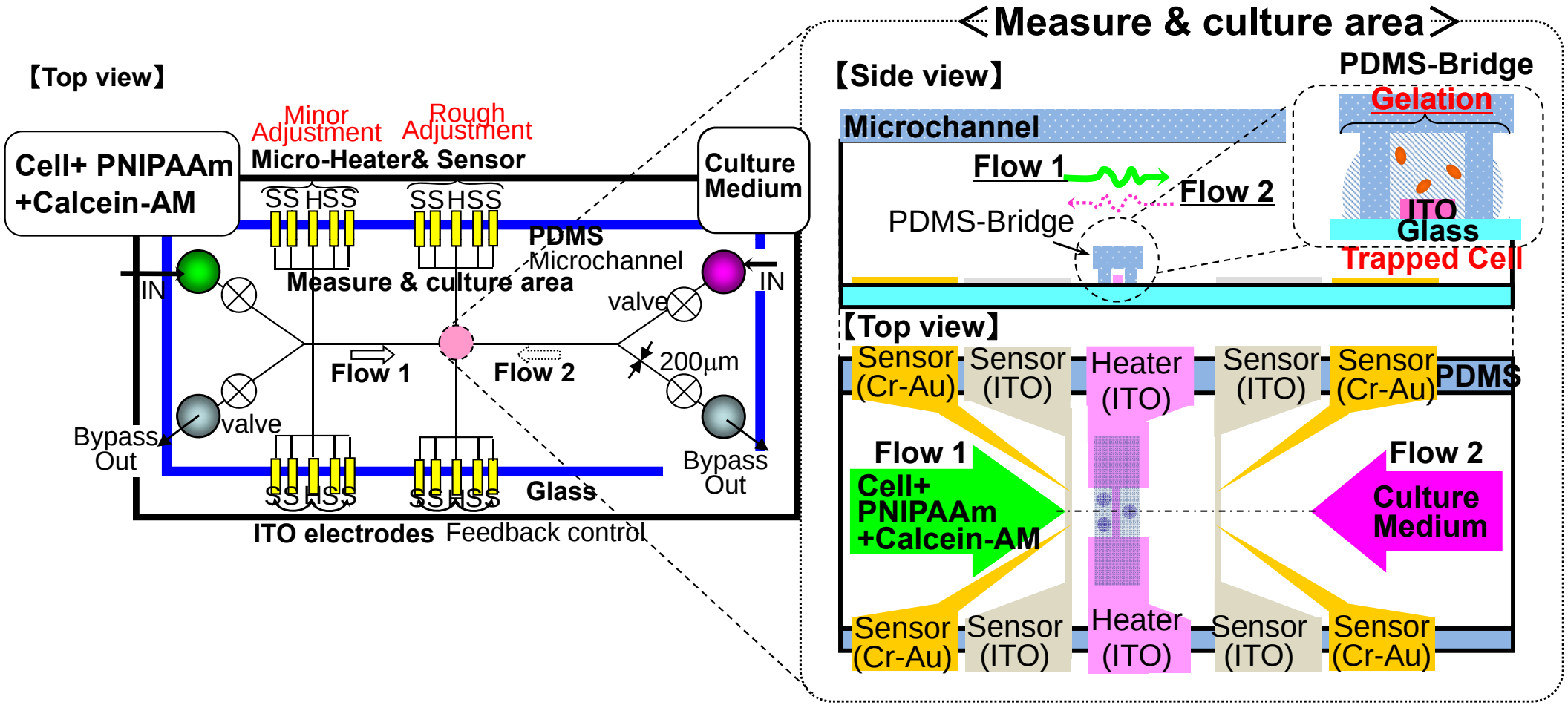
Heater width: 30 μm

Thick gel prevent observations





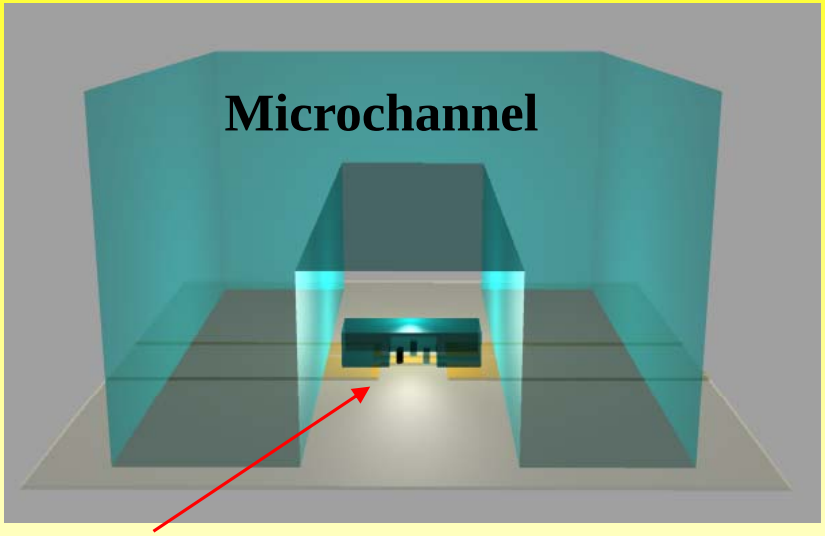
Chip design for a single cell analysis



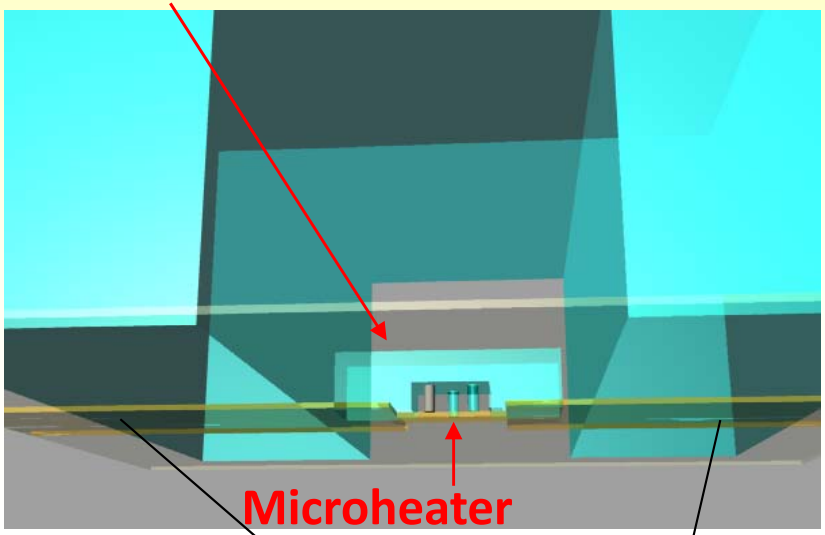
Y. Yamanishi *et al.*, IEEE Transaction on NanoBioscience, Vol.8, No.4, pp.312-317, (2009)

Fabrication of Micro-bridge in Microchannel

Local Temperature Control Micro-bridge

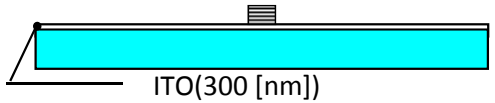


PDMS-Microbridge

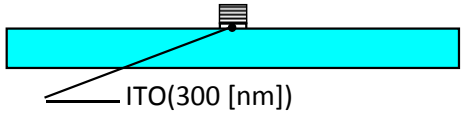


Process Flow

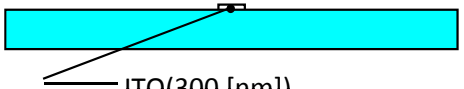
① Photolithography



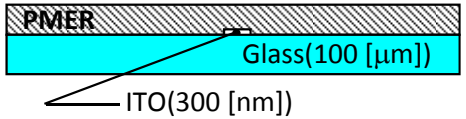
② Wet etching ($FeCl_3$)



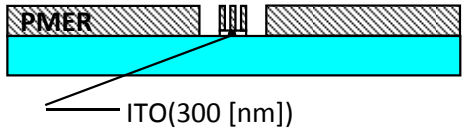
③ Removal of OFPR



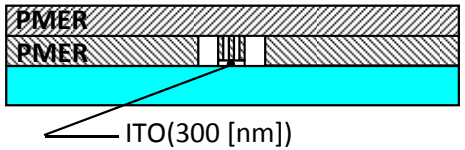
④ PMER Photoresist (1st Step)



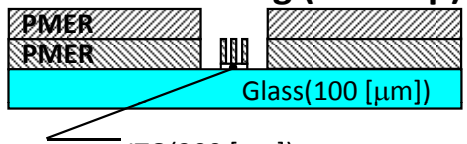
⑤ PMER Patterning (1st Step)



⑥

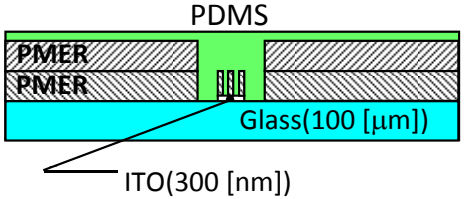


⑦ PMER Patterning (2nd Step)

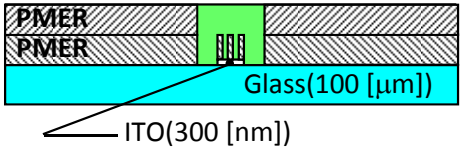


Mold for Micro-bridge

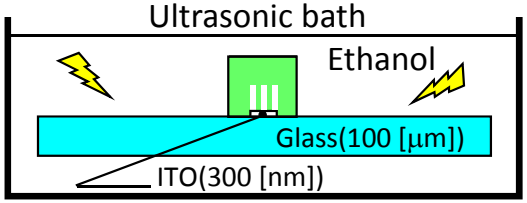
⑧ Spread PDMS



⑨ Removal of PDMS residue

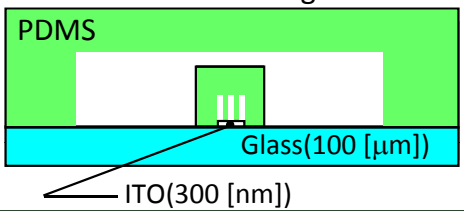


⑩ Removal of PMER

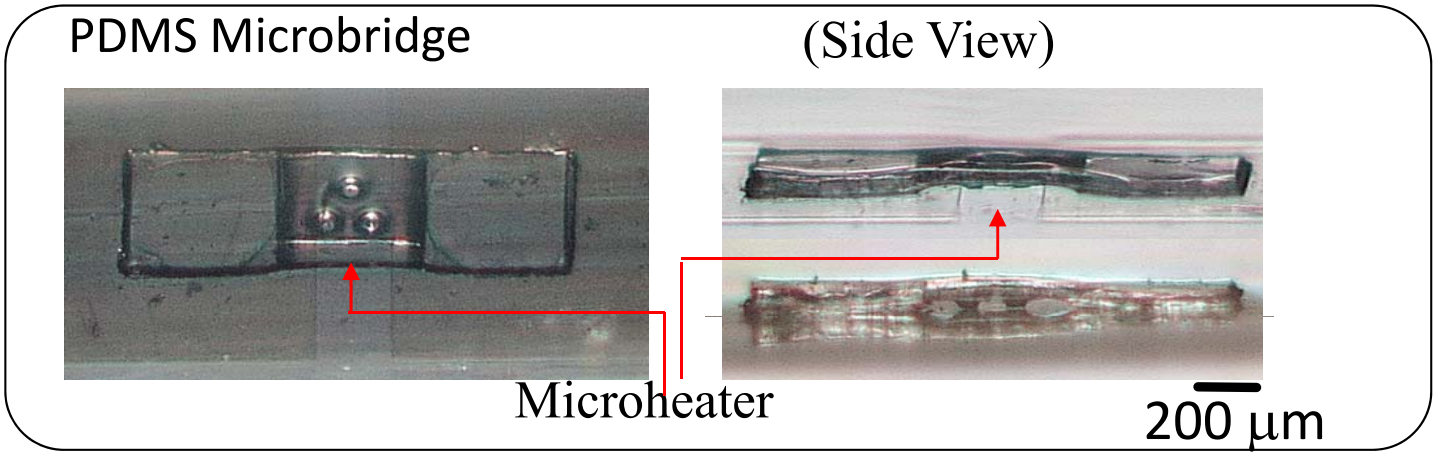


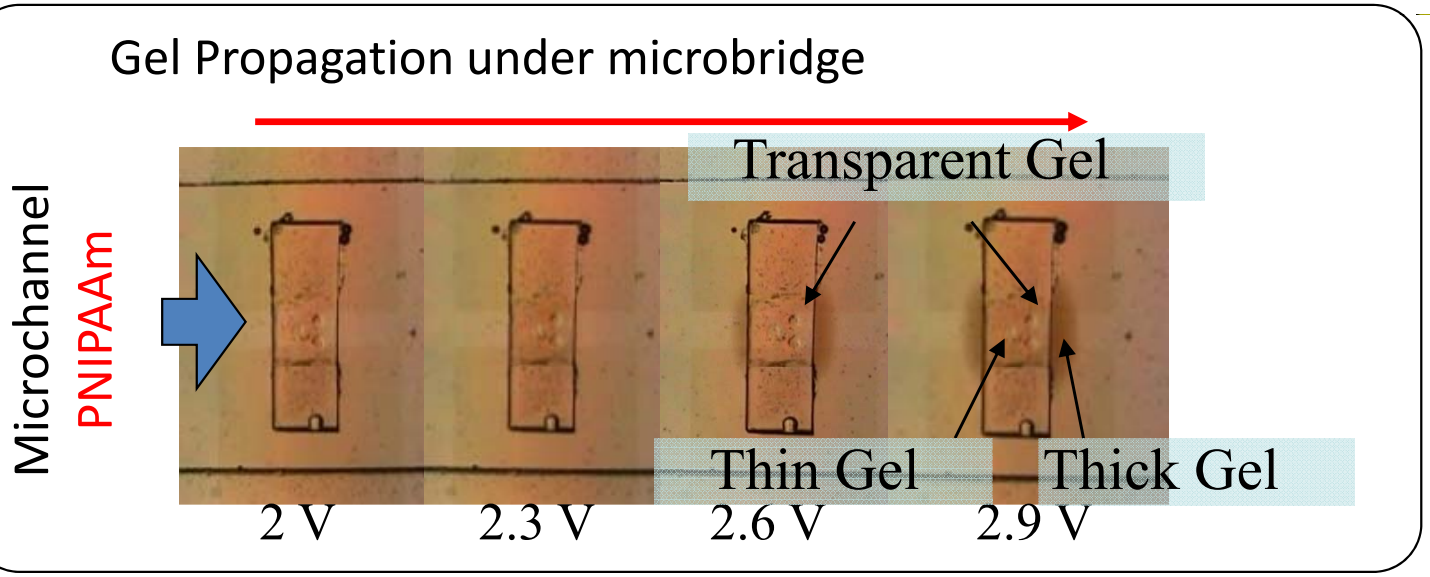
⑪ Assemble of Microchannel

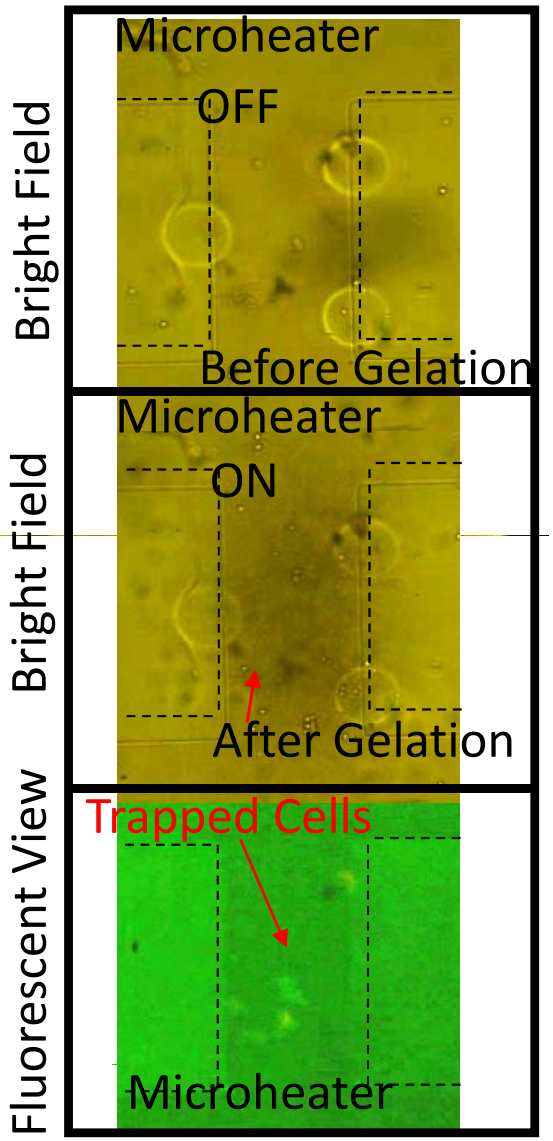
Plasma Bonding



Cell Immobilization under Microbridge



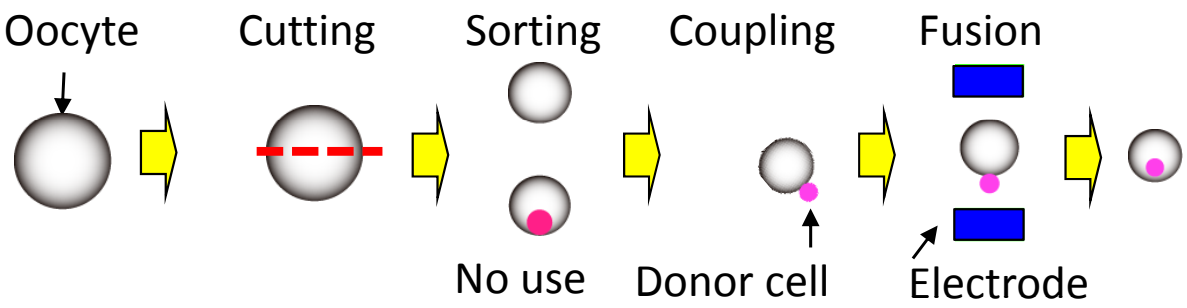




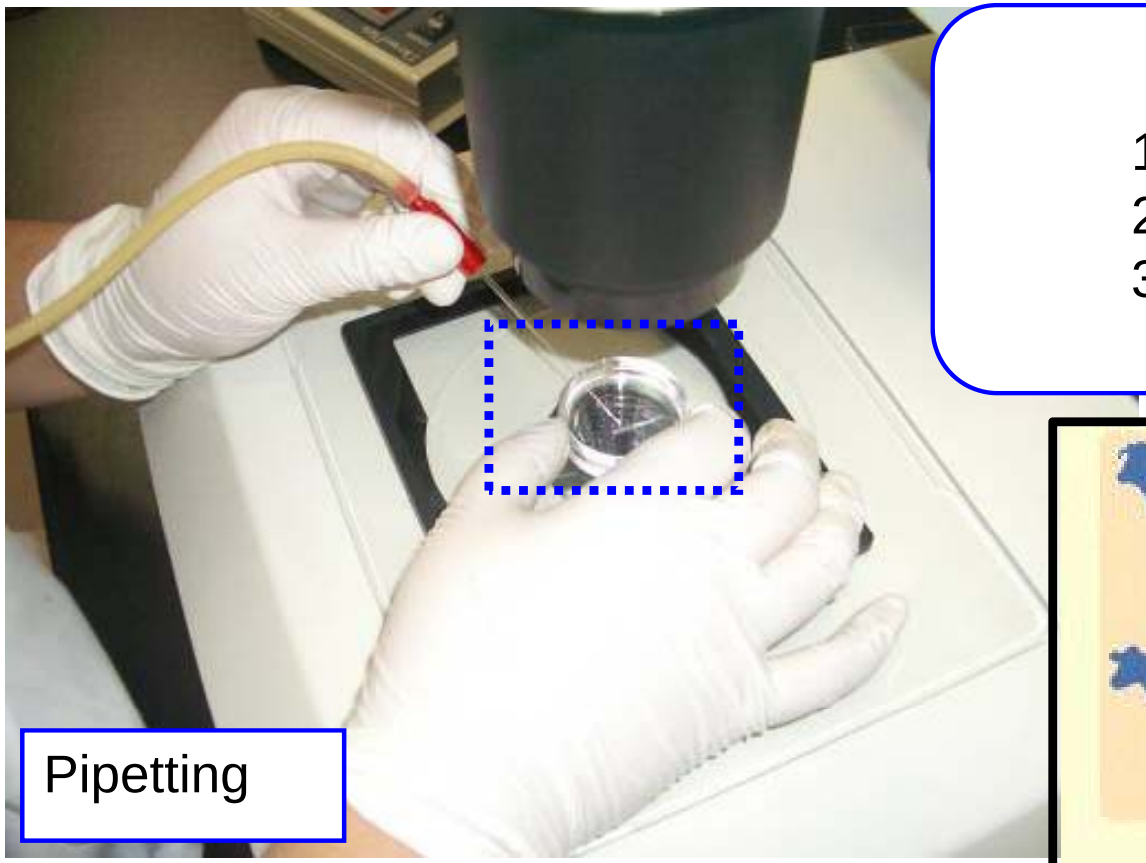
Successfully fabricated micro-bridge in a microchannel and immobilization of cell

Y. Yamanishi *et al.*, IEEE Transaction on NanoBioscience, Vol.8, No.4, pp.312-317, (2009)

Automation of Cloning Chip

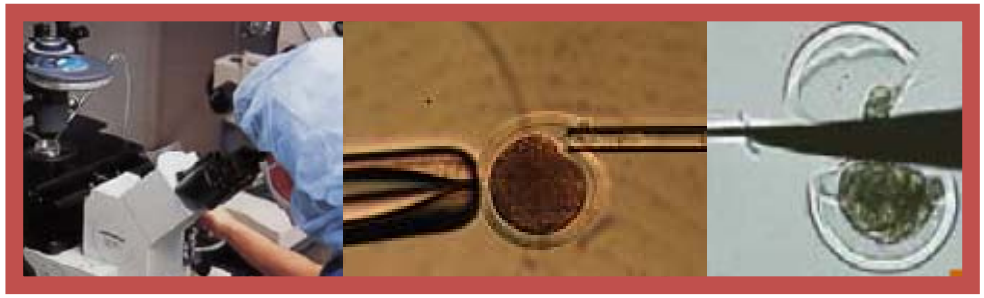


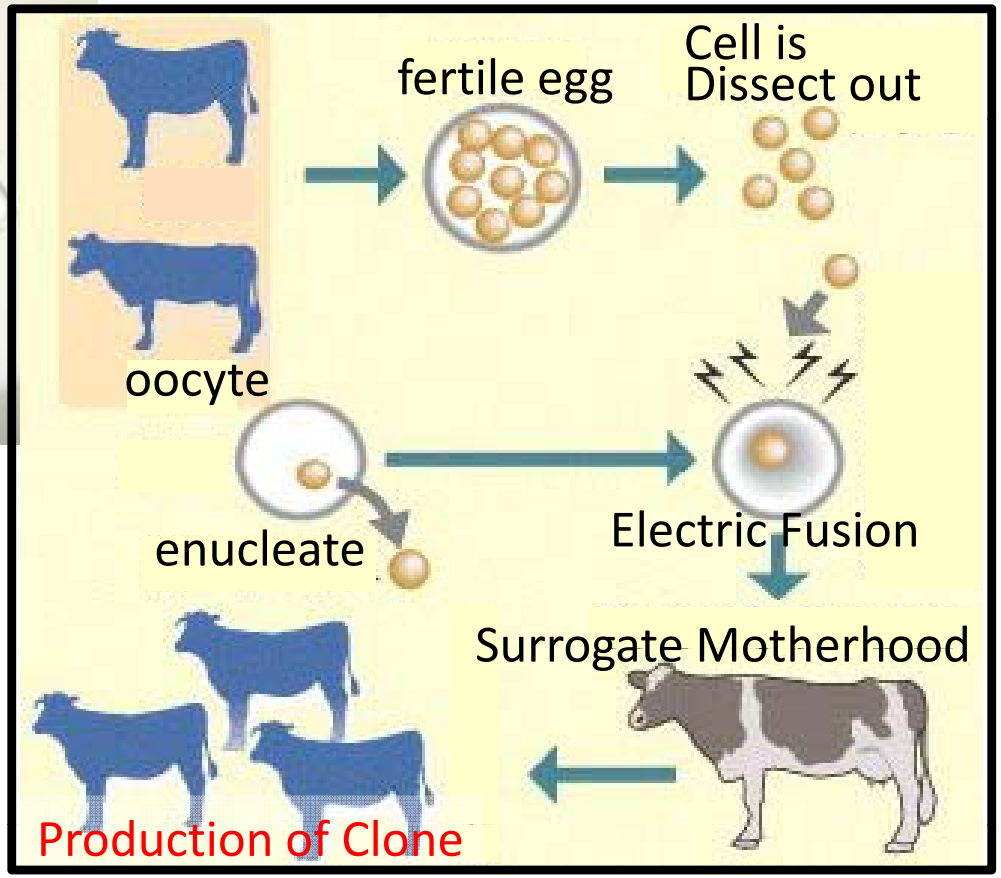
Background



PROBLEMS

1. Repeatability
2. Contamination
3. Success rate





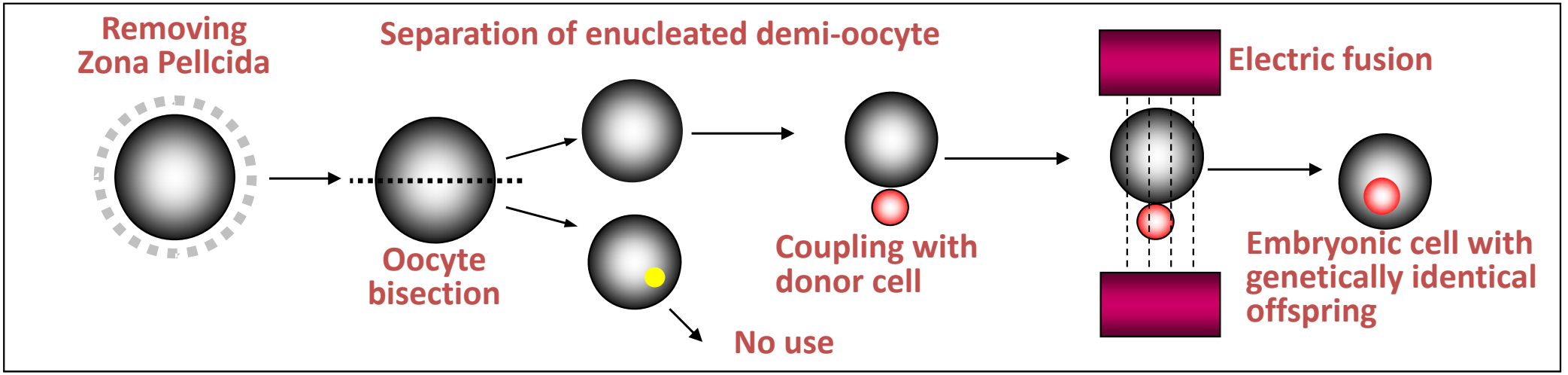
Proposed Protocol for Automation of Cloning

Takahashi et. al.

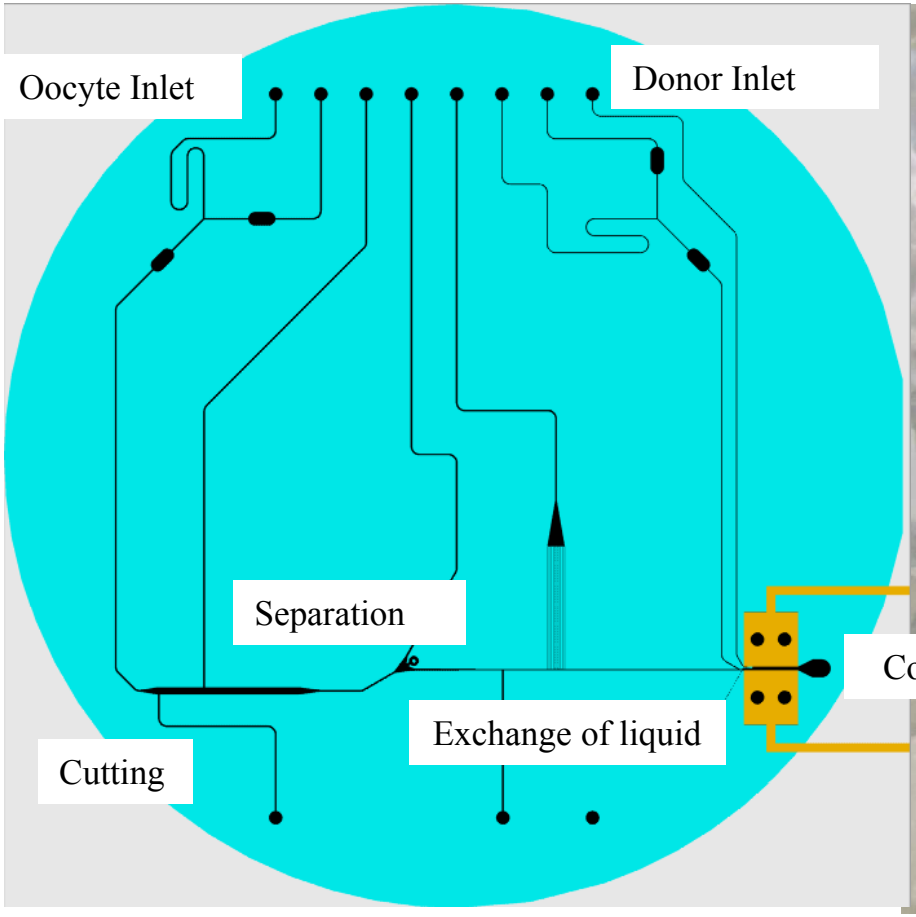
Automation

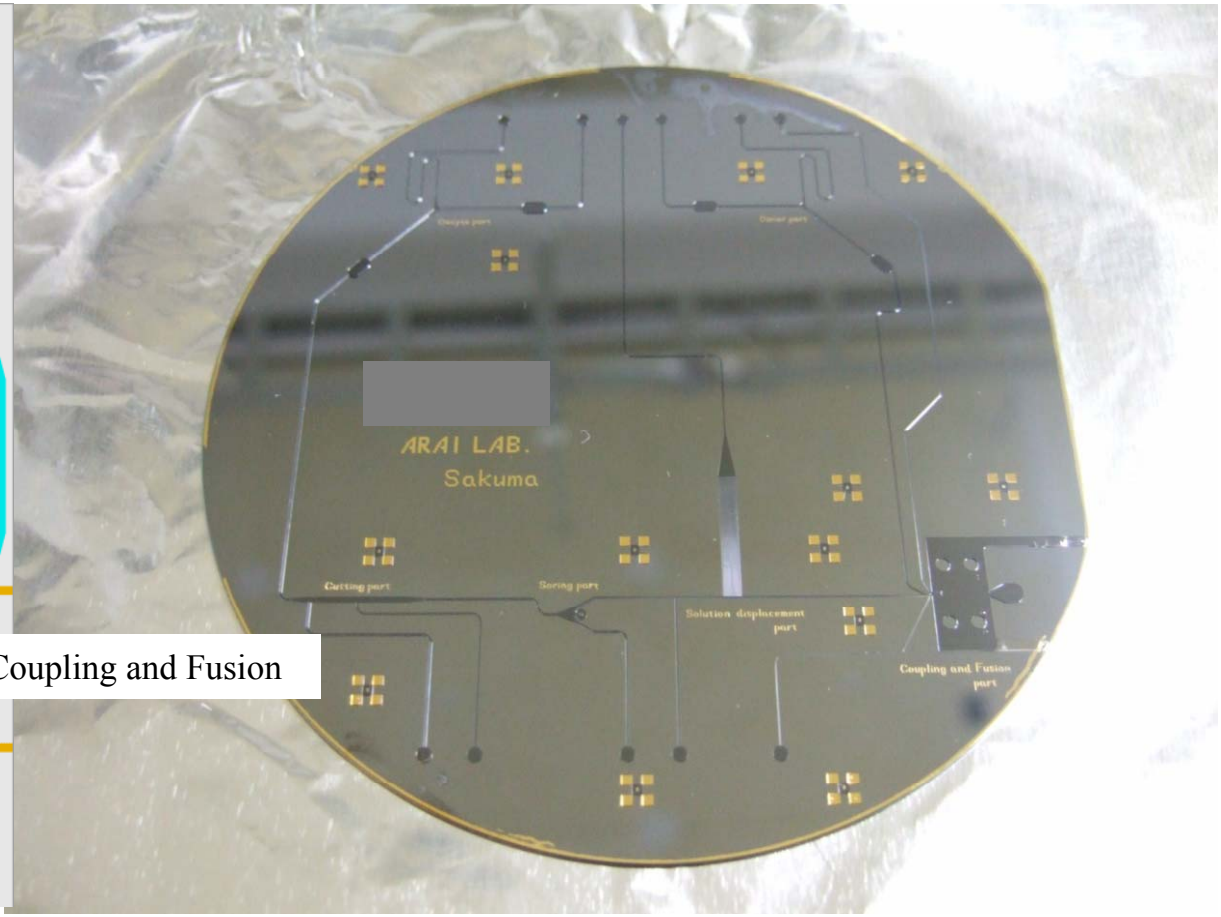
Bio-Chip

1. Automation in closed-space
2. Disposable
3. High efficiency
4. Mass productive and low cost



All-in-one Automation of Cloning Chip



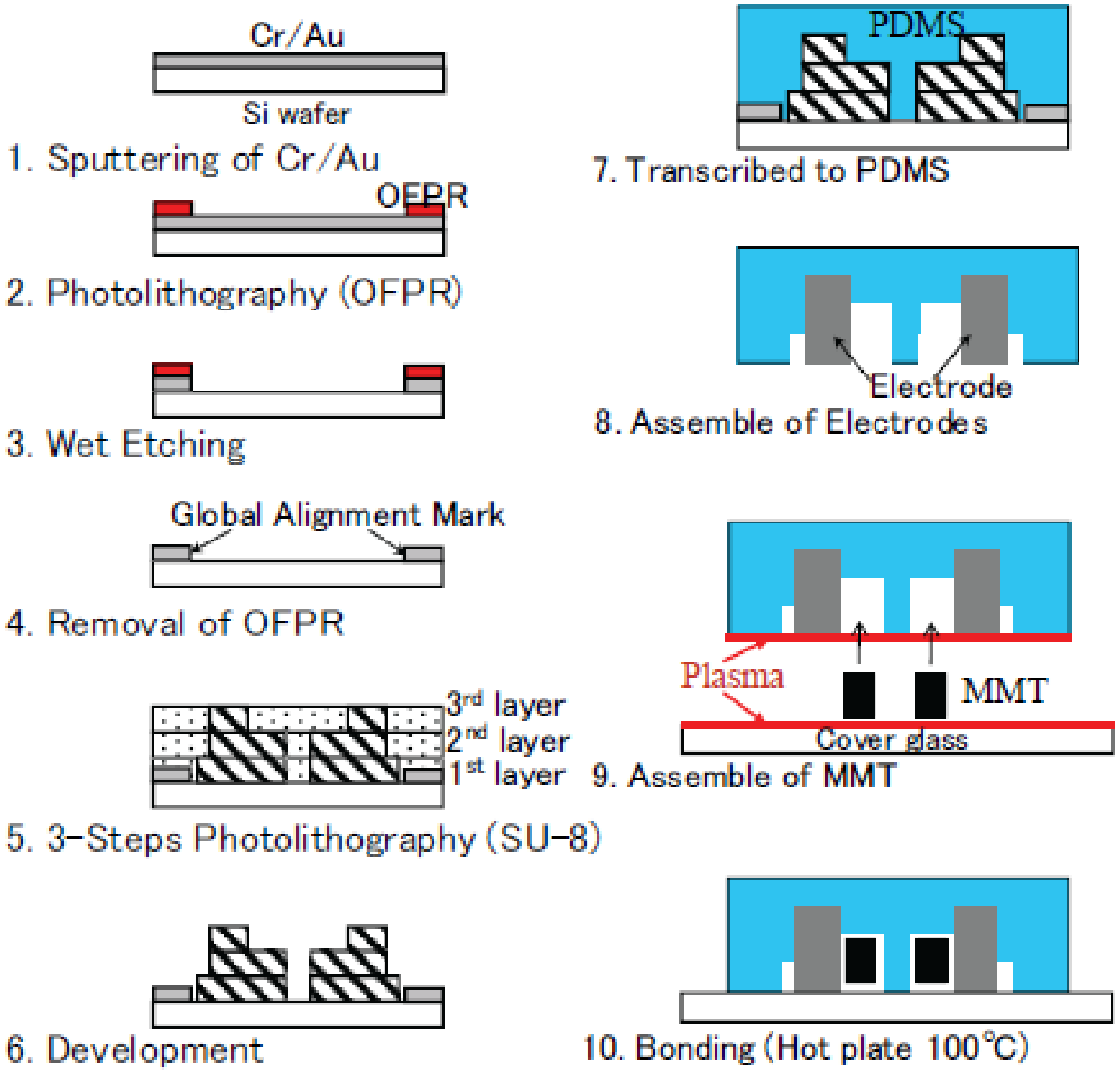


4 inch wafer

Yamanishi et. al. μTAS2009.

Yamanishi et. al. Journal of Robotics and Mechatronics, Vol.22, No.3, p.371-379, (2010).

Process flow to fabricate One-chip

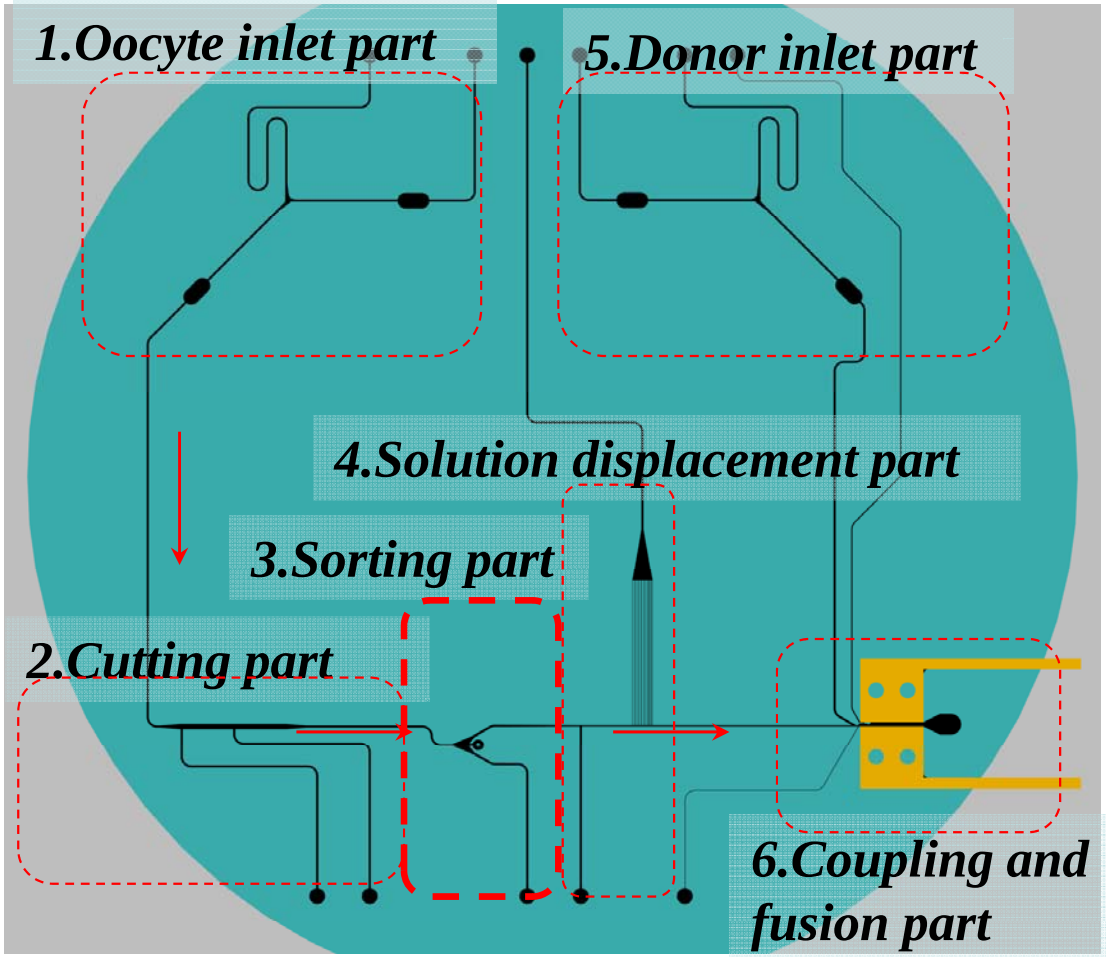


Yamanishi et. al. μTAS2009.

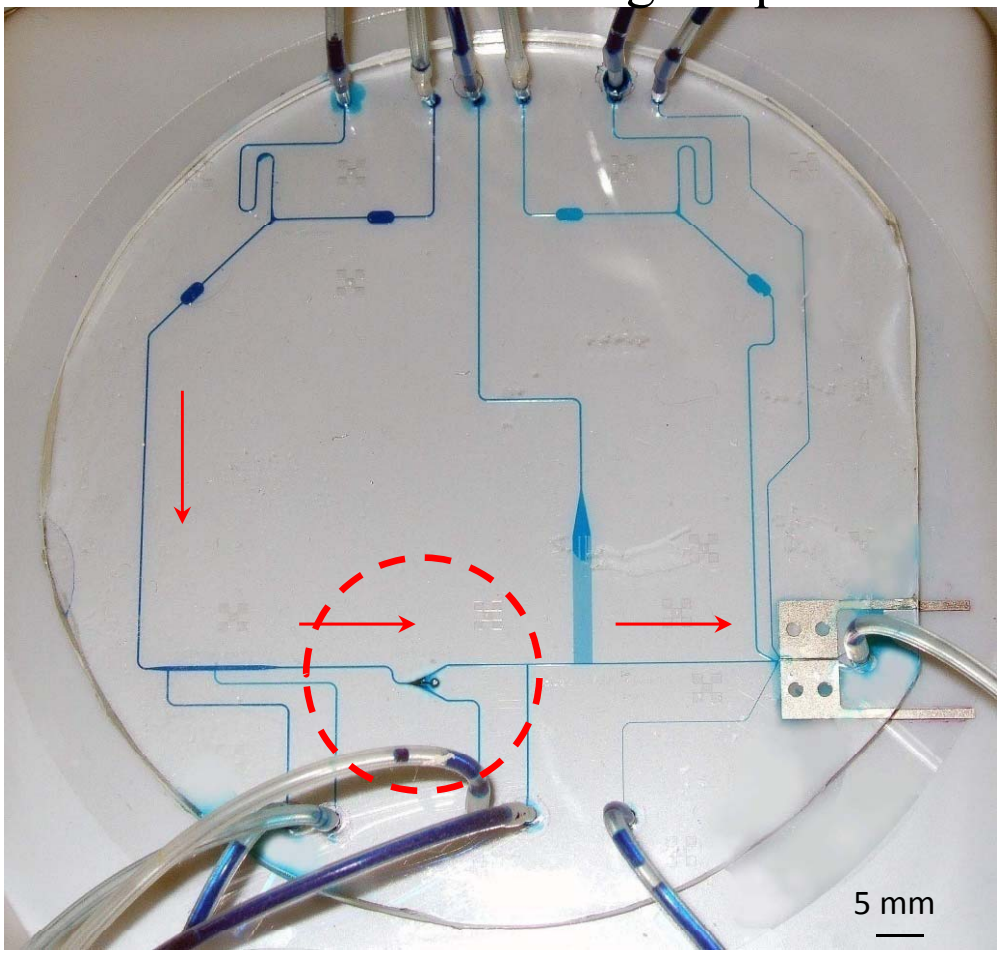
Yamanishi et. al. Journal of Robotics and Mechatronics, Vol.22, No.3, p.371-379, (2010).

All-in-one Unified Microfluidic

Concept View of Cloning Chip



Fabricated Cloning Chip

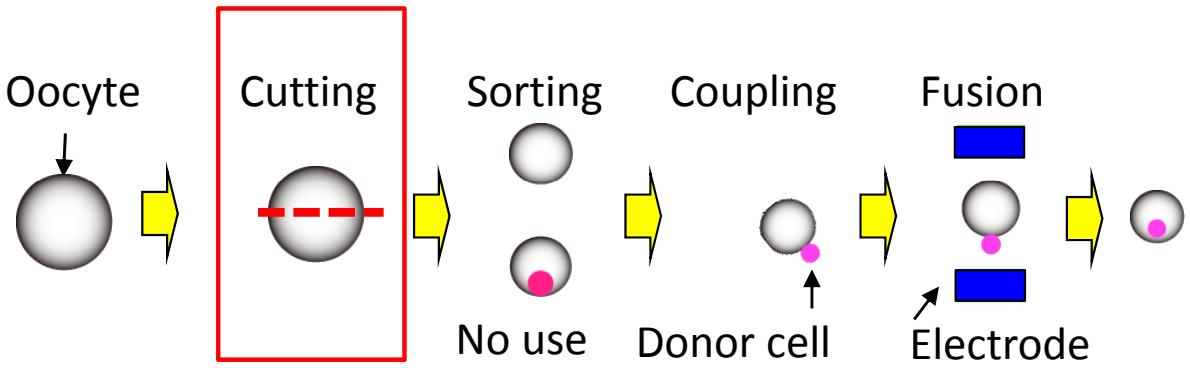


Diameter of cloning chip: φ100 mm

Yamanishi et. al. μTAS2009.

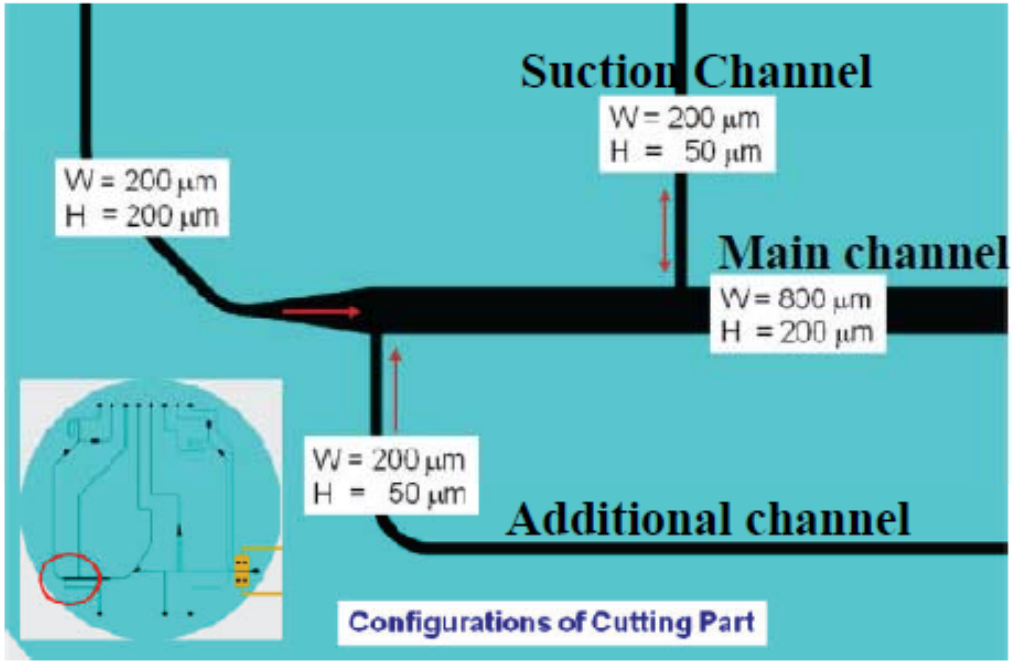
Yamanishi et. al. Journal of Robotics and Mechatronics, Vol.22, No.3, p.371-379, (2010).

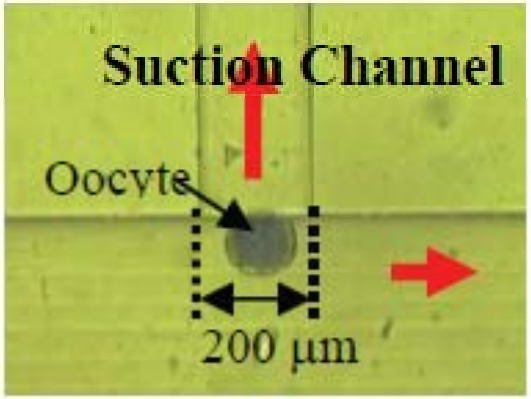
Cutting of Oocyte

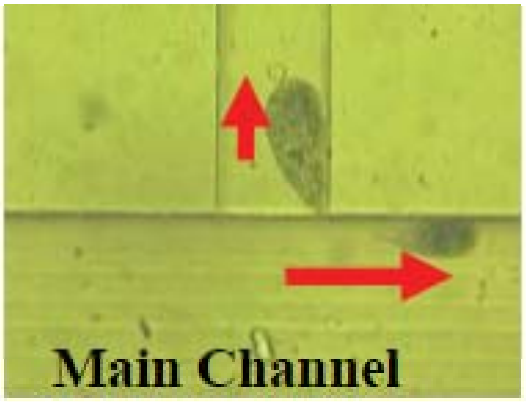


Cutting of Oocyte

Cutting of Oocyte by Microchannel



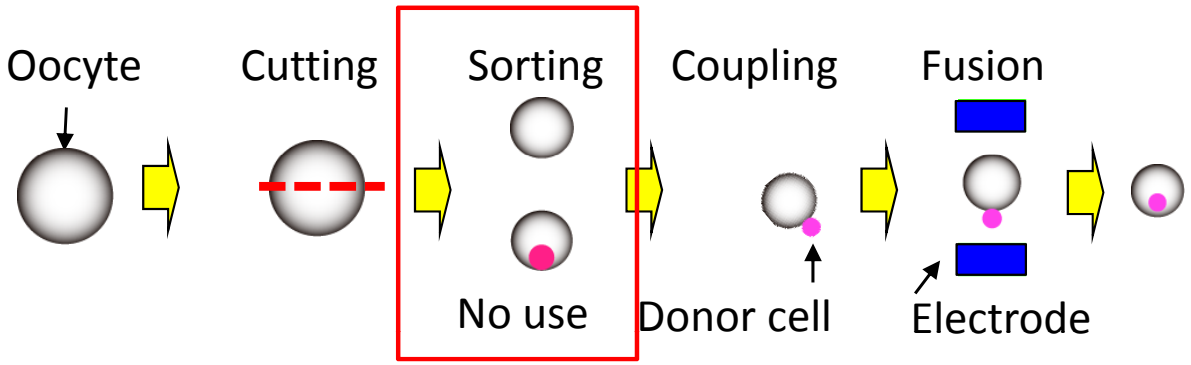




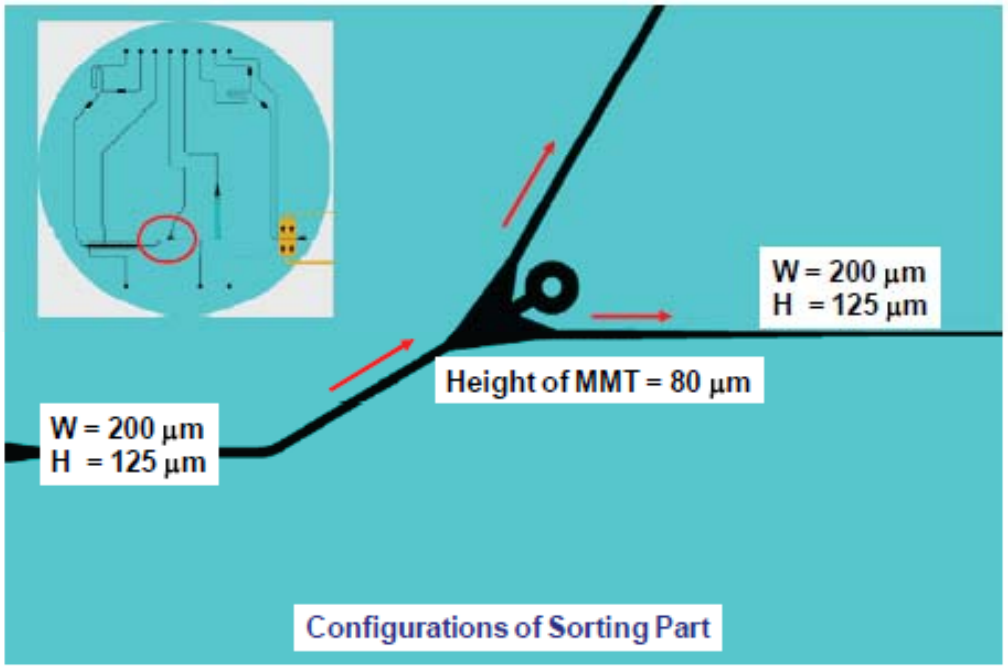
Yamanishi et. al,, Journal of Robotics and Mechatronics, Vol.22, No.3, pp.371-379, (2010).

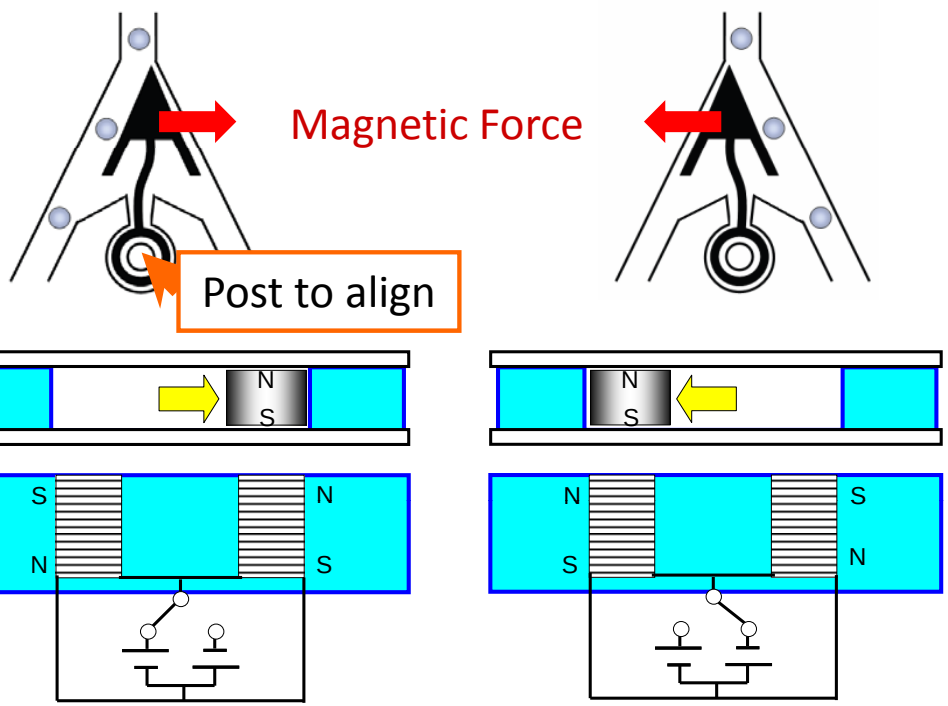
Ichikawa, et. al. (AIST)

Sorting of Oocyte & Exchange of Fluid



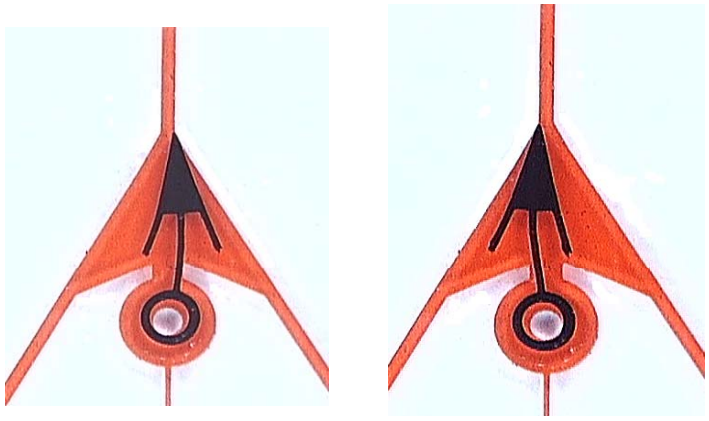
Sorting of Oocyte





Input: 3.85V, 0.15A

Frequency: about 18 Hz

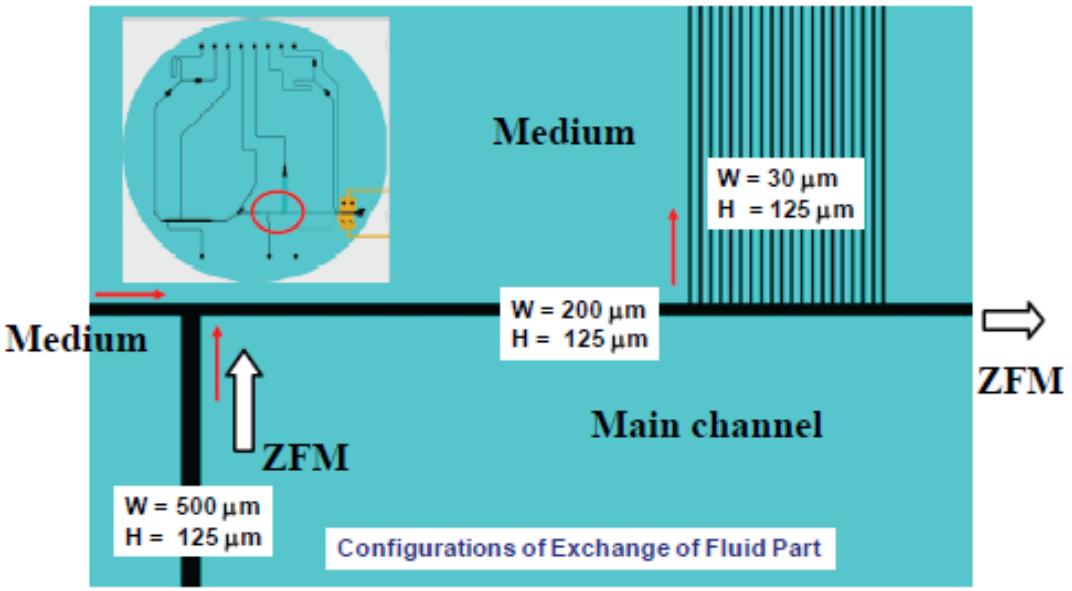


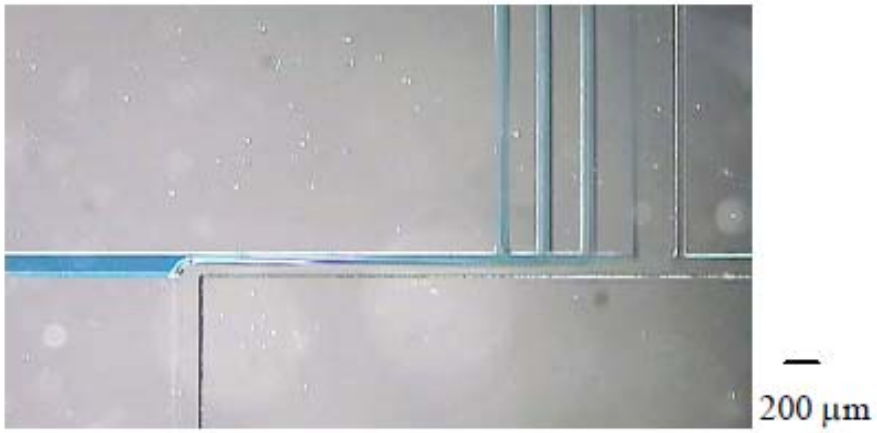
MMT : Magnetically Driven Microtool

Yamanishi et. al,, Journal of Robotics and Mechatronics, Vol.22, No.3, pp.371-379, (2010).

Exchange of Fluid

Require to exchange liquid to apply high voltage in the subsequent Coupling & Fusion Part

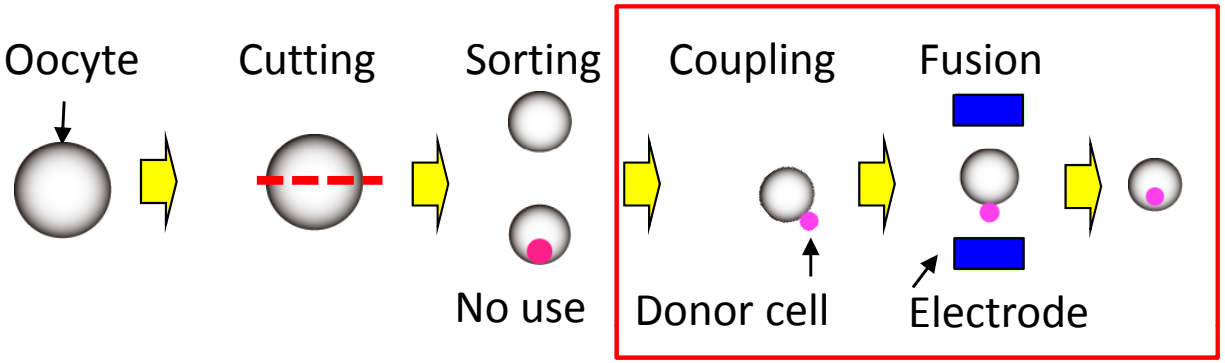




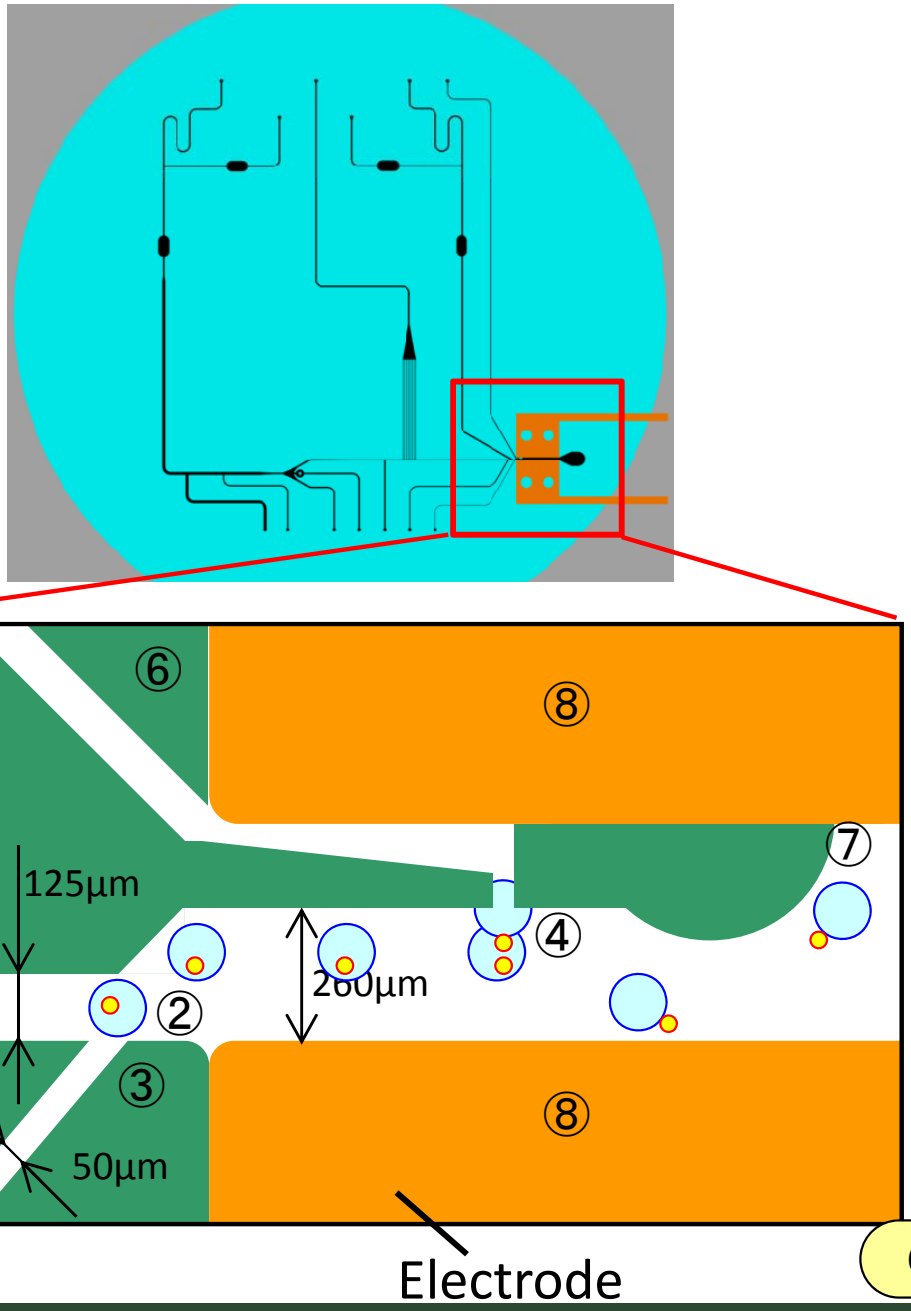
Medium (High Conductivity liquid) → ZFM (Low Conductivity liquid)

Yamanishi et. al,, Journal of Robotics and Mechatronics, Vol.22, No.3, pp.371-379, (2010).

Physical Coupling and Fusion



Coupling by Dielectrophoretic Force



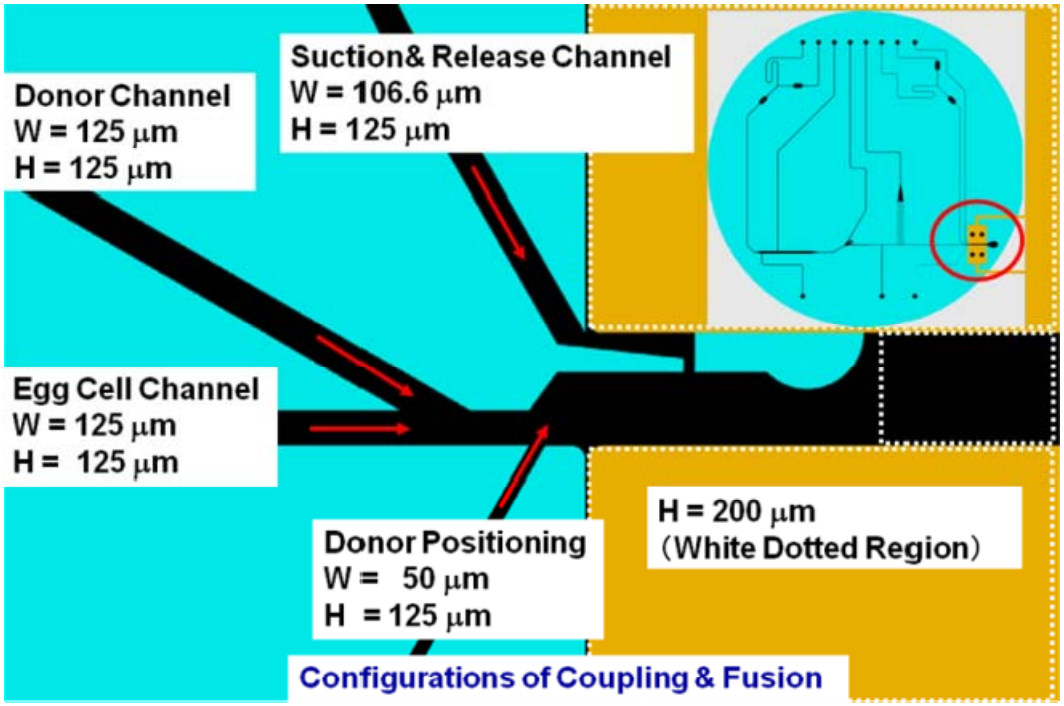
Position control and coupling of cell

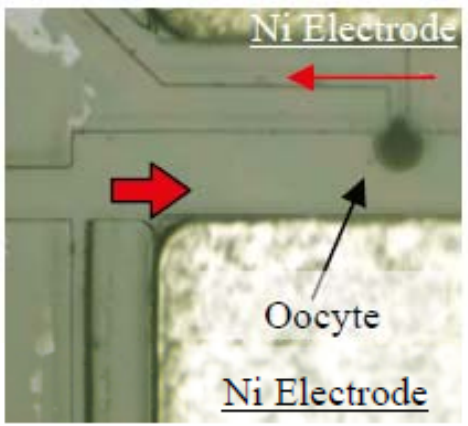
- Fluid control
- Dielectrophoretic Force (DEP)

① Oocyte inlet channel
② Broaden channel
③ Flow control channel
④ Fixing oocyte position
⑤ Donor inlet channel
⑥ Handling channnel
⑦ Cell-to-cell fusion part
⑧Electrode

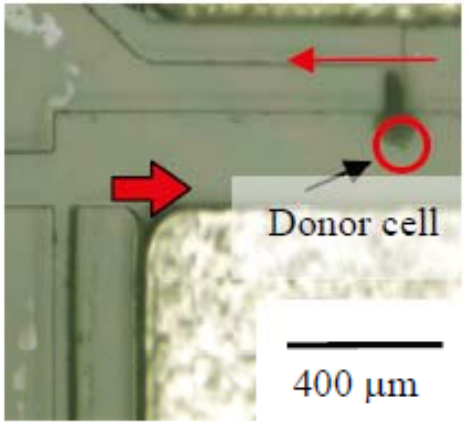
Collaborated work with KHI LTD., JAPAN

Coupling by Fluidic force and DEP

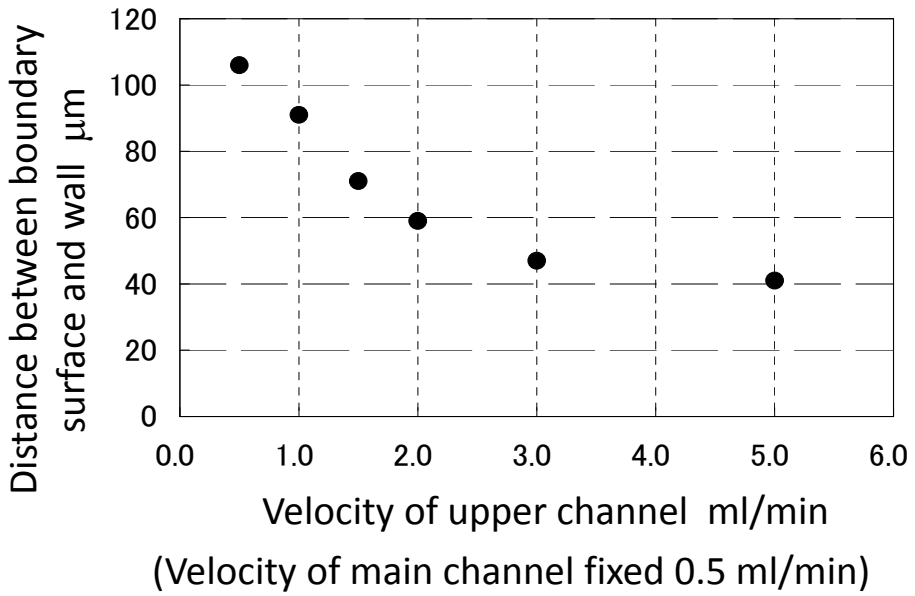




(a) Trapping of Oocyte



(b) Coupling Oocyte and



(Velocity of main channel fixed 0.5 ml/min)

Yamanishi et. al. Journal of Robotics and Mechatronics, Vol.22, No.3, p.371-379, (2010).

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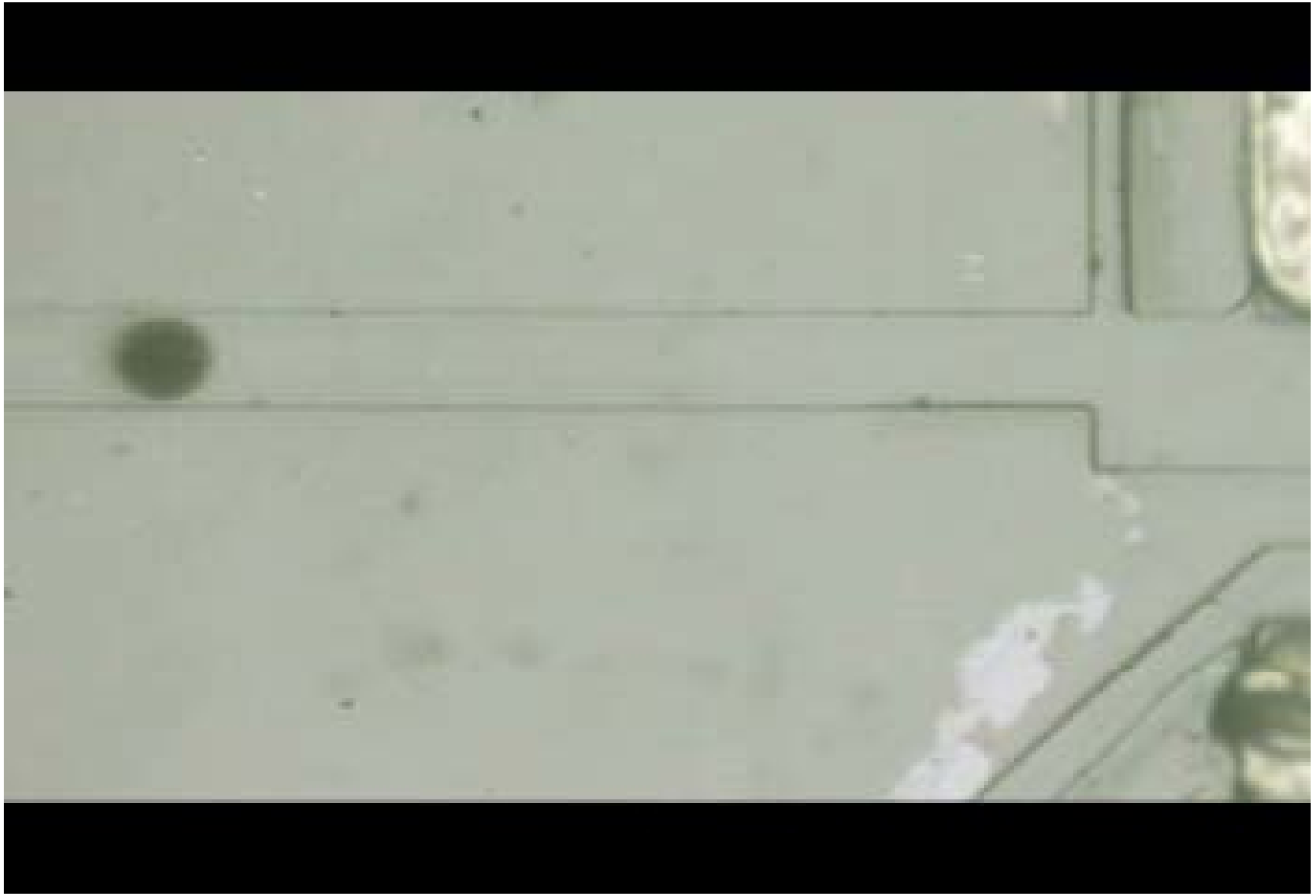
Coupling by Fluidic force and DEP



Yamanishi et. al. μTAS2009.
Yamanishi et. al. Journal of Robotics and Mechatronics, Vol.22, No.3, p.371-379, (2010).

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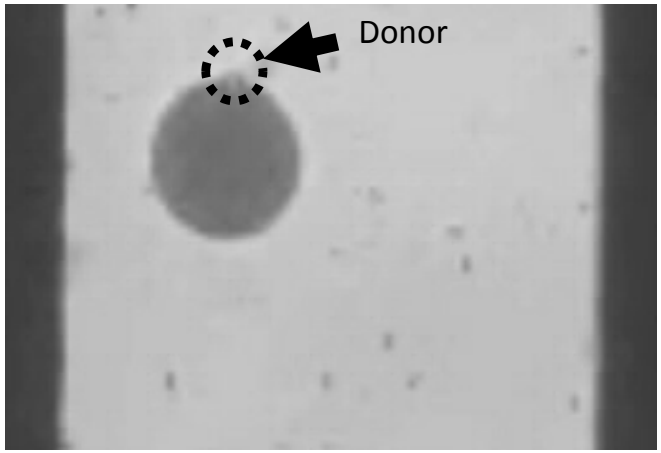
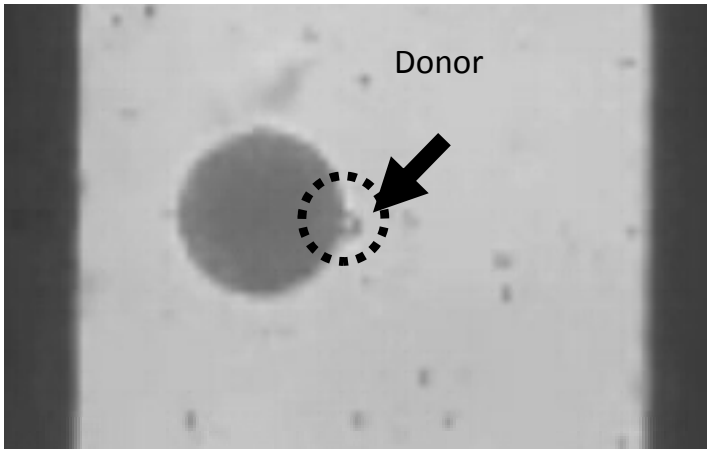
Coupling of Oocyte and Donor Cell

Alignment

AC (8-10 V_{pp}, 1 MHz)



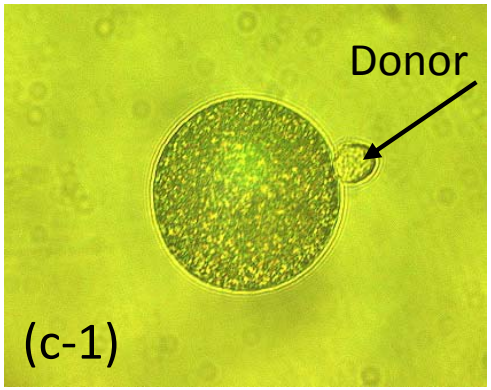
(b-1) 0 sec

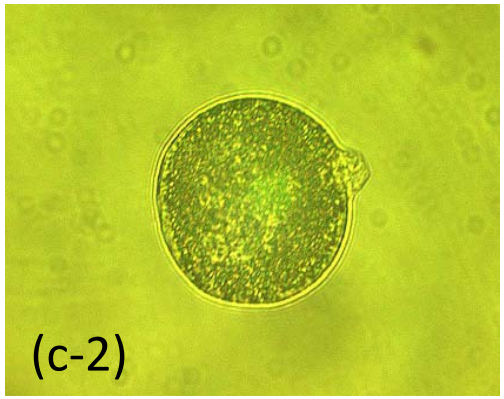


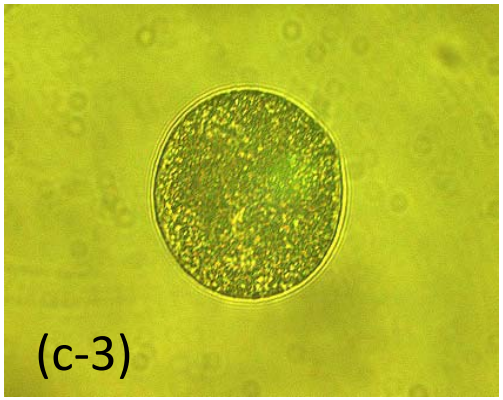
(b-2) 20 sec

Fusion

DC (48 V, 100 μs)





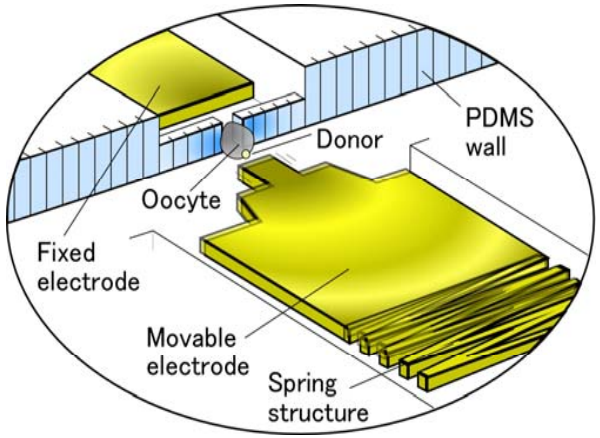


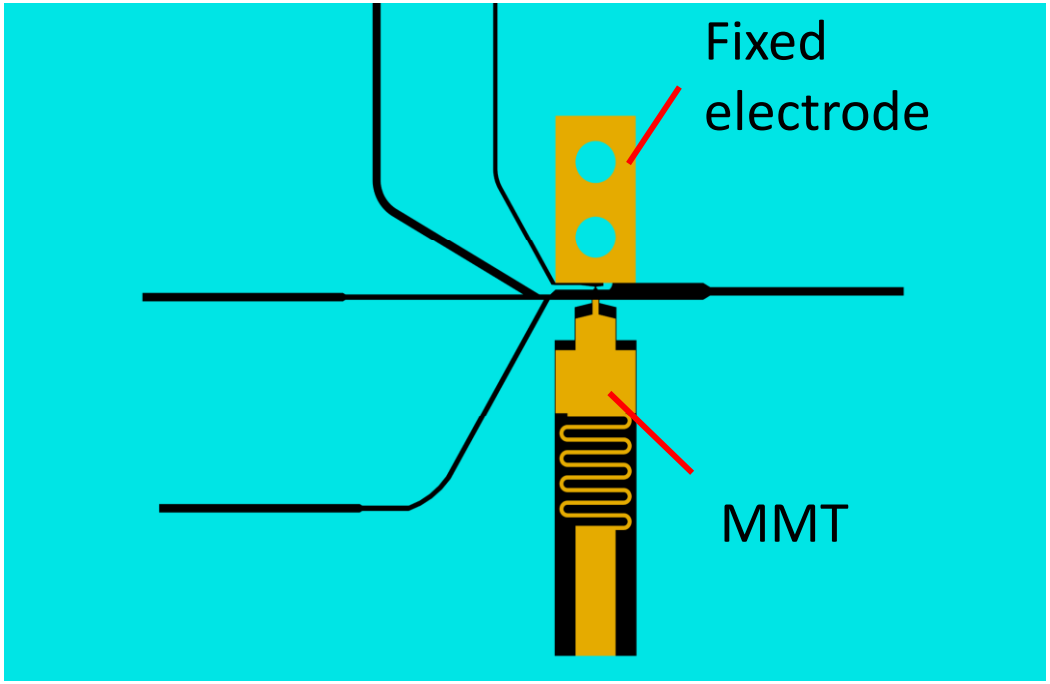
Yamanishi et. al. μTAS2009.

Yamanishi et. al. Journal of Robotics and Mechatronics, Vol.22, No.3, p.371-379, (2010).

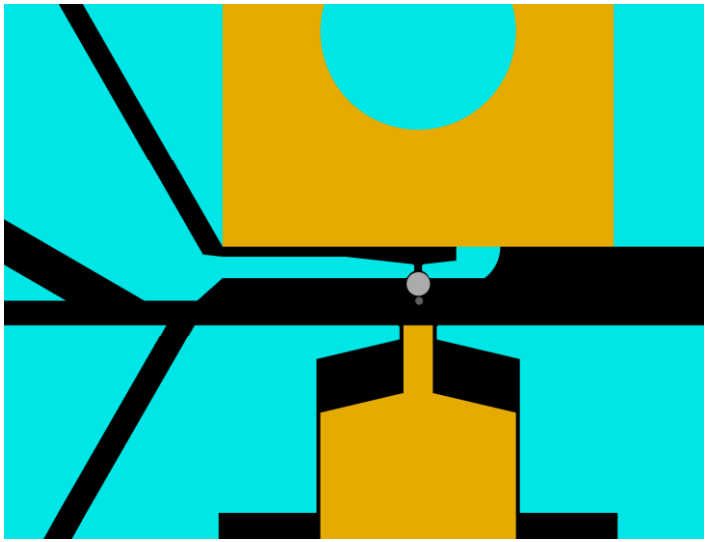
Collaborated work with FHK Co., LTD. JAPAN

Concept of Physical Coupling

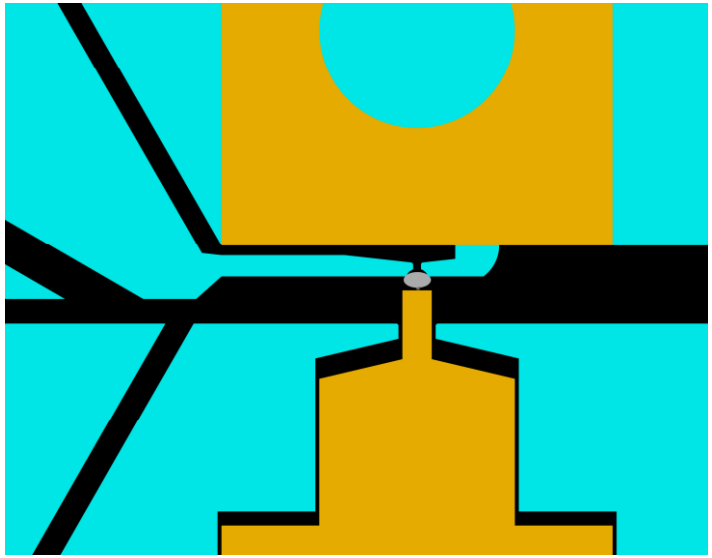




Overall view of physical coupling chip



Before driving MMT



After driving MMT

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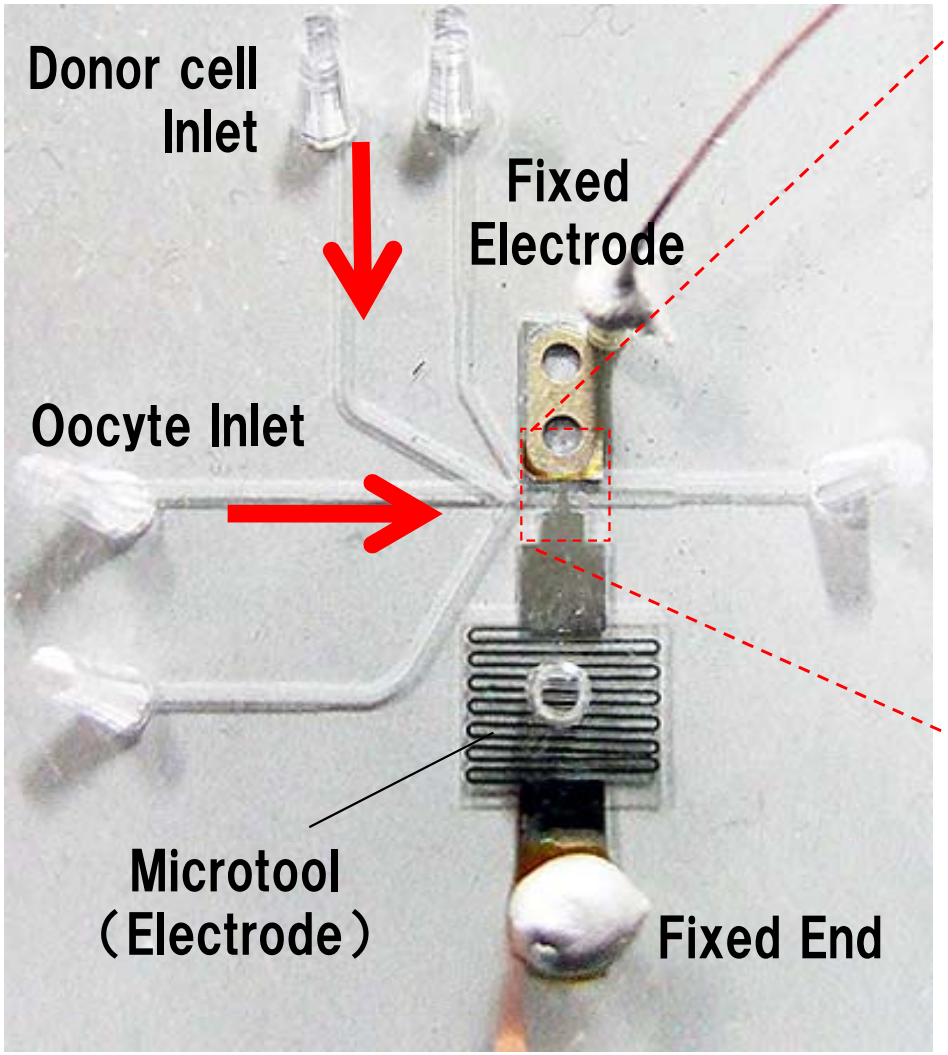
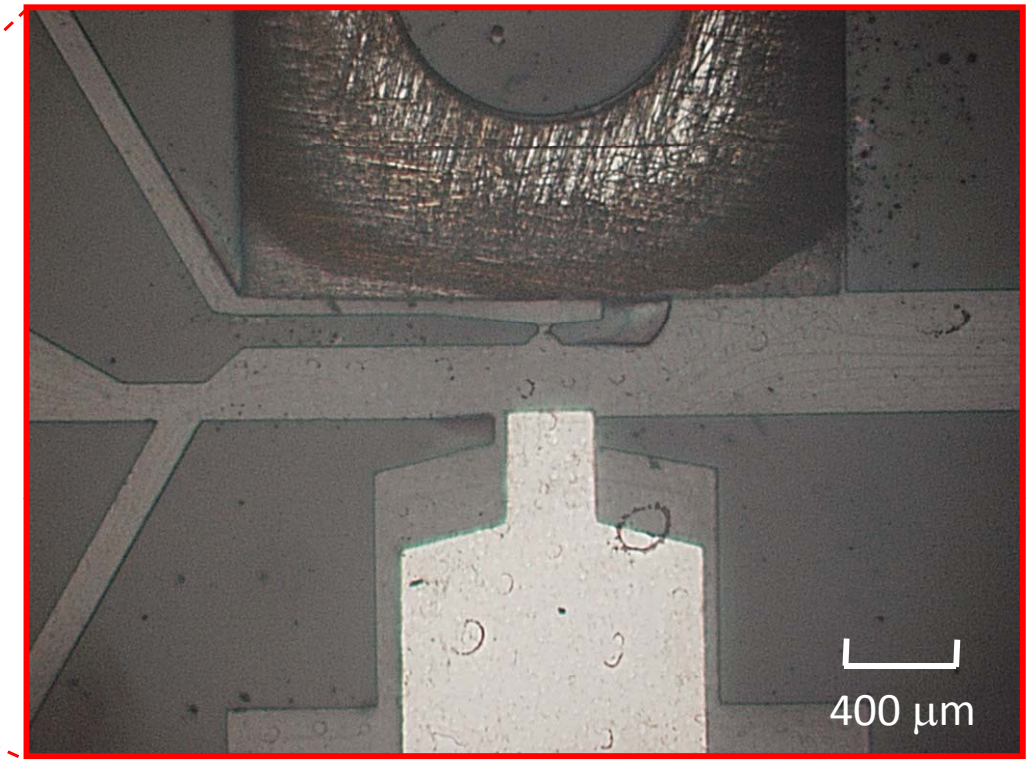
Fabrication of Physical Coupling Chip



Overall view of physical coupling chip



Magnified view of physical coupling chip

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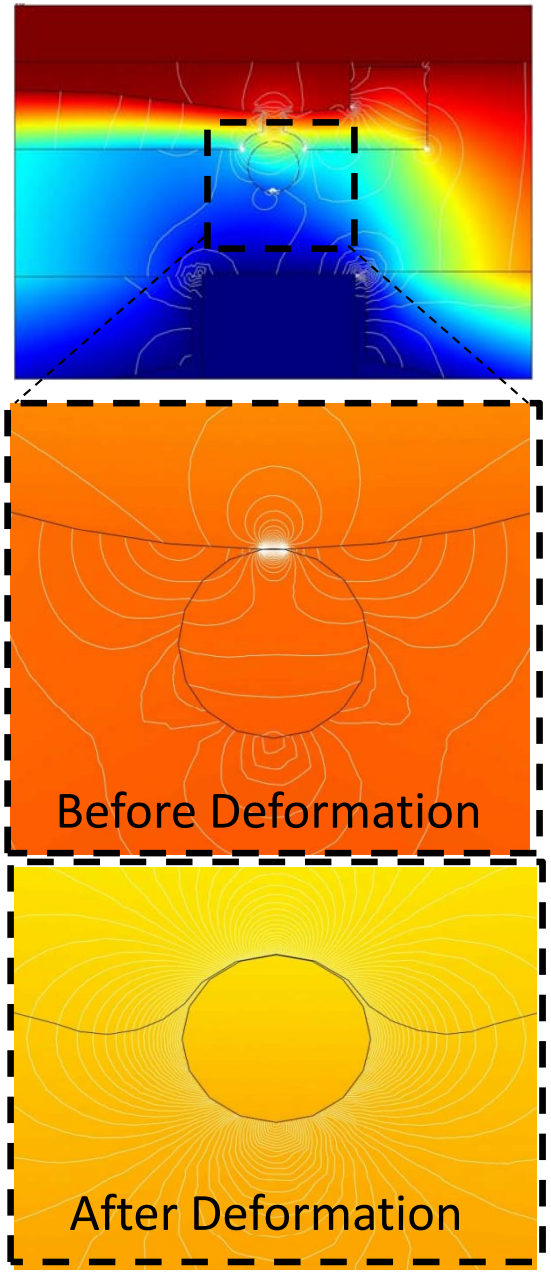
Mechanical and Electrical Stimuli on a Cell



Fixed Electrode side

Spring Movable Electrode to deform the oocyte

Uniformly Distributed of
High Concentration of Electric Field

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