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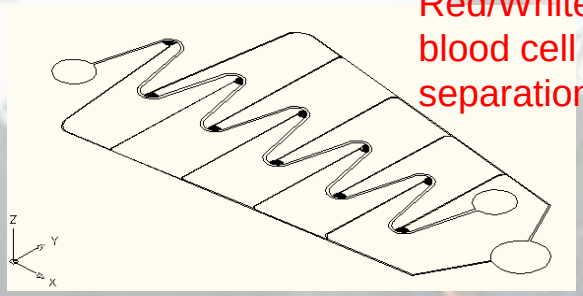
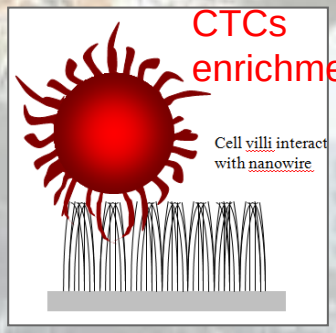
OMICS Group International is a pioneer and leading science event organizer, which publishes around 400 open access journals and conducts over 300 Medical, Clinical, Engineering, Life Sciences, Pharma scientific conferences all over the globe annually with the support of more than 1000 scientific associations and 30,000 editorial board members and 3.5 million followers to its credit.

OMICS Group has organized 500 conferences, workshops and national symposiums across the major cities including San Francisco, Las Vegas, San Antonio, Omaha, Orlando, Raleigh, Santa Clara, Chicago, Philadelphia, Baltimore, United Kingdom, Valencia, Dubai, Beijing, Hyderabad, Bengaluru and Mumbai.

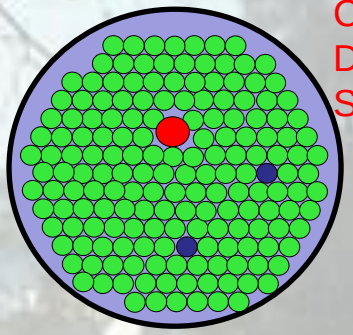
A Nano/Micro Fluidic System for Circulating Tumor Cells (CTCs) Rapid Detection and Diagnosis



Red/White blood cell separation



CTCs enrichment



CTCs Detection by SACA

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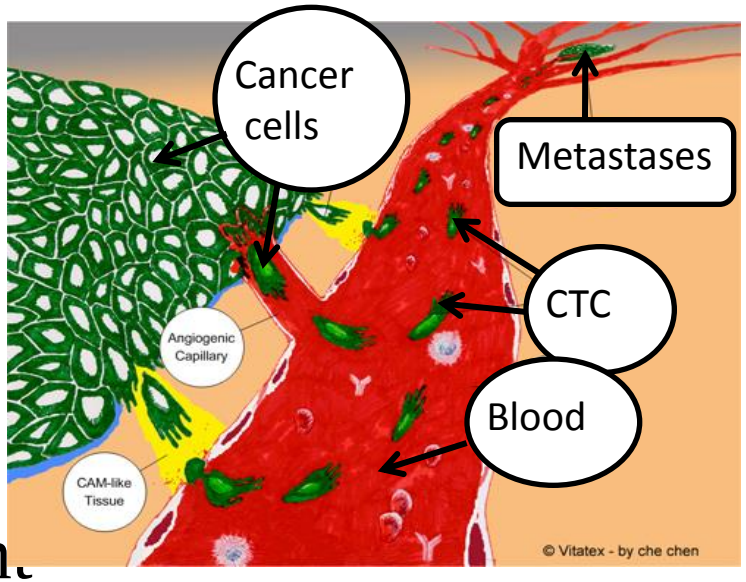
The main technical challenge of CTCs detection



- Main spread way of metastases
  1. nearby tissues spread
  2. blood system spread
  3. lymphatic system spread
- The importance of CTCs amount
- Challenge:

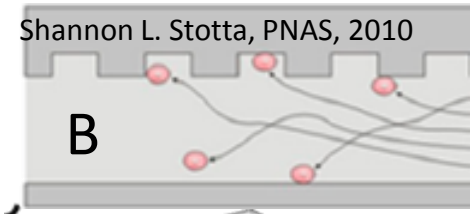
  rare amount of CTC: **1-10 CTCs/1ml**

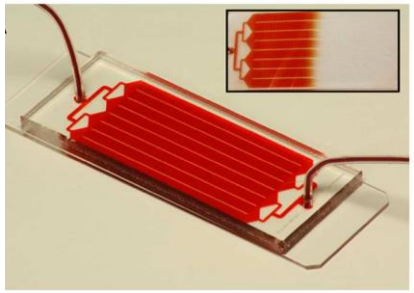
  low separation effcientcy: **1 CTCs/$10^{6\text{-}7}$ WBCs**

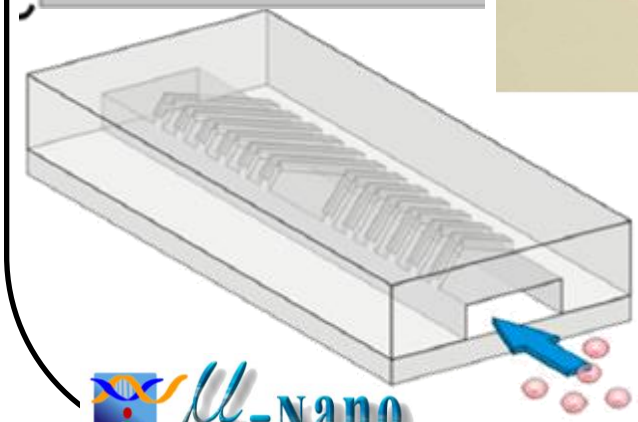
  low sensitivity: **40-50%** (specific antibody)

CTCs separation and capturing in whole blood

- Posts fabricated from Si wafer
  - 100 μm diameter
  - 100 μm tall
- Posts coated with anti-EpCAM
- Whole blood flowed through device by pressure source
- mL-scale volumes



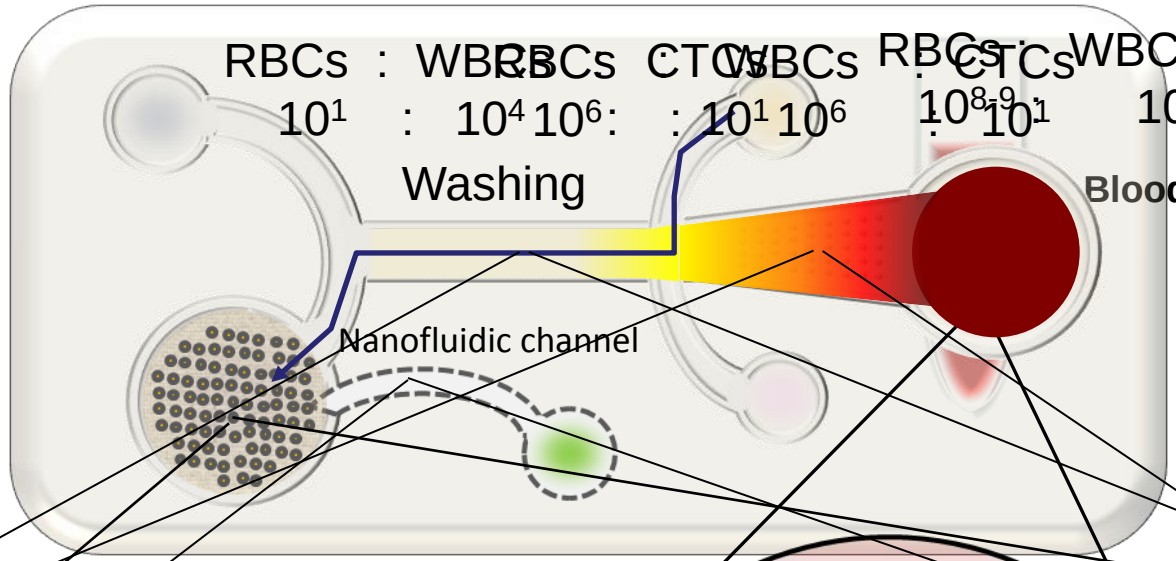


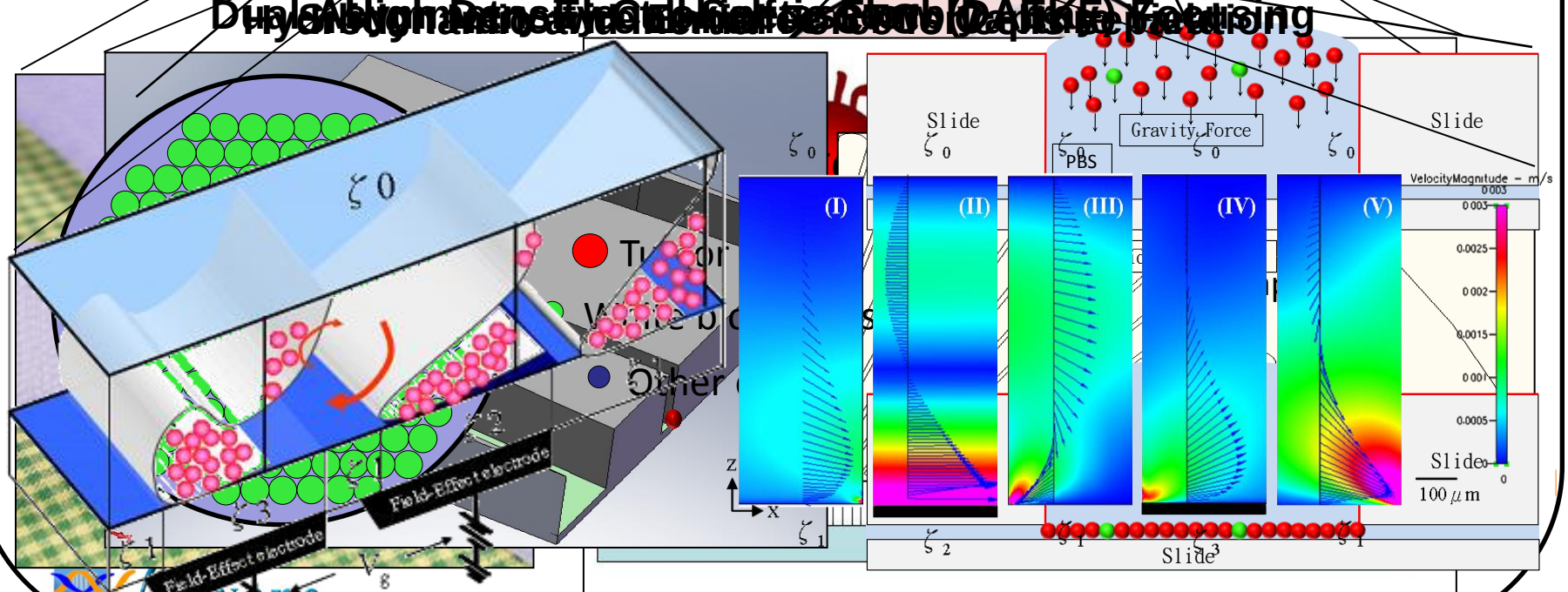


- High sensitivity (1 target cell in 1 billion blood cells)
- Selectivity (47%(A), 63%(B) capture efficiency)
- High yield (99(A)% ,95%(B)).
- Max flow of ~1 mL/hr to 2.5 mL/hr

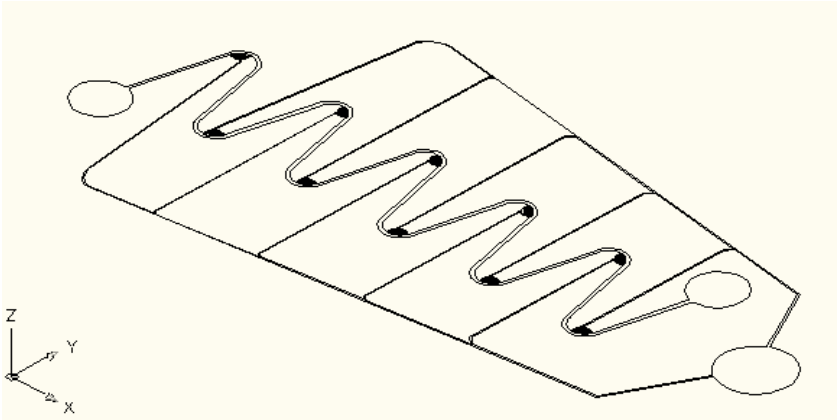
4-12 hours to run sample

- Non-specific binding and clogged
- Without sample preconcentration

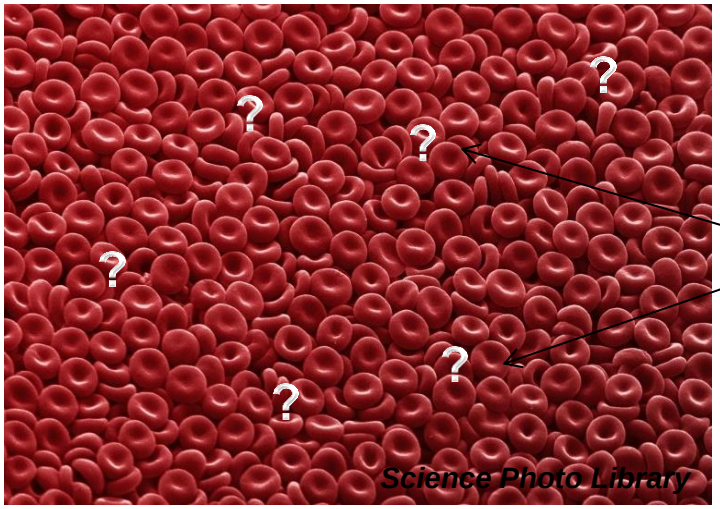

RBCs : WBCs : CTCs
10^1 : 10^4 : 10^1
RBCs : WBCs : CTCs
10^6 : 10^6 : 10^1
RBCs : WBCs : CTCs
10^8-9 : 10^6 : 10^1
Washing
Blood
Nanofluidic channel

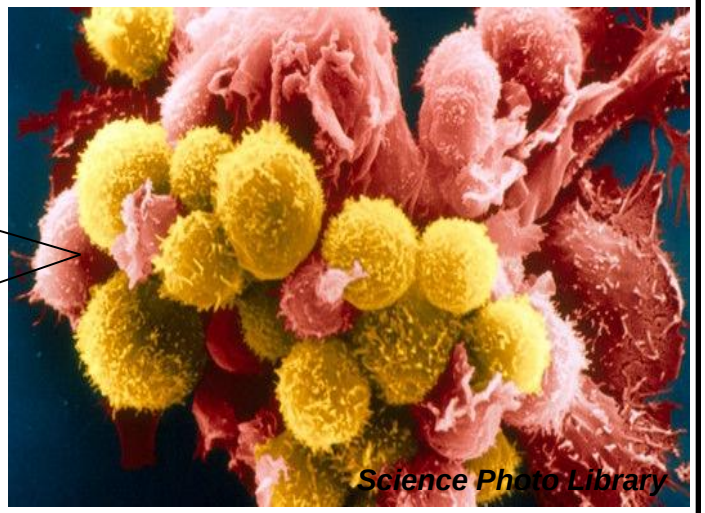

Field-Effect electrode
Field-Effect electrode
Slide
Gravity Force
PBS
Slide
(I)
(II)
(III)
(IV)
(V)
VelocityMagnitude - m/s
Slide
100 μm

Part I:
High-Throughput Blood Cells Separation



The Challenge in early CTCs detection





Red Blood Cell Count (RBCs, 7-9 µm)

4.3-6.2x10^9/mL (Male)

3.8-5.5x10^9/mL (Female)

3.8-5.5x10^9/mL (Infant/Child)

White Blood Cell Count (WBCs, 8-12µm)

4.1-10.9x10^6/mL

Cancer cell (CTCs) count

Stage O/I: 5-20/mL

Stage II : 20-50/ml

Stage III : 50-100/ml

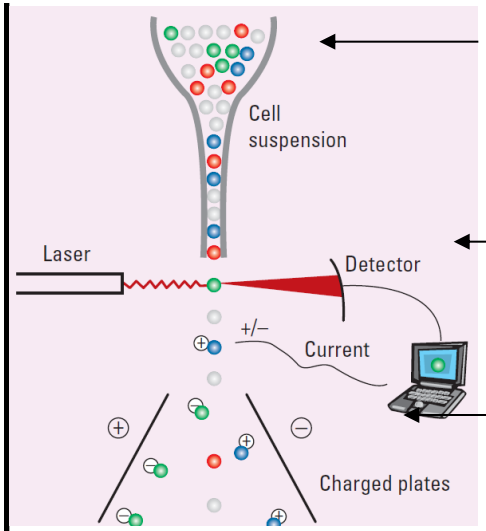
Stage IV: >100/ml

Limits of current detection:

- Low sensitivity and reliability **>20cells/mL (possibility: 20~60%)**
- Take a long processing time and low recovery rate **>1hr & 80~%**

Current technologies for cells separation

1. Flow Cytometry



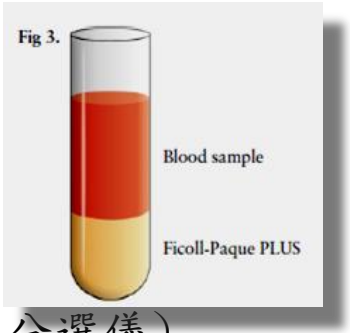
Microfluidic System
Laminar flow
~10^4cells/s

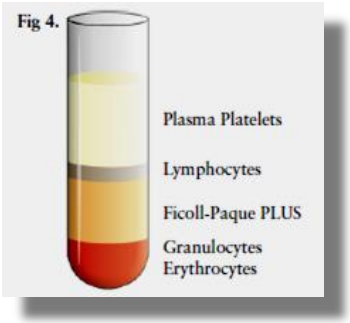
Cells Detection
Optical System
Laser excitation
~28 Hrs for 1cc

Cells Sorting
Electronic System
Histogram FSC & SSC
~ 90~99% purity
Recovery rate~95%

3. Lysis Buffer (細胞裂解溶劑)

- By using chemical buffer to create osmotic pressure difference between the cell membrane and environmental, specifically use to cleavage and removal of mammalian red blood cells or without nuclei cells in whole blood samples ~10^4/s, 1hr for 1cc, 90% purity
  Recover rate~40-50%

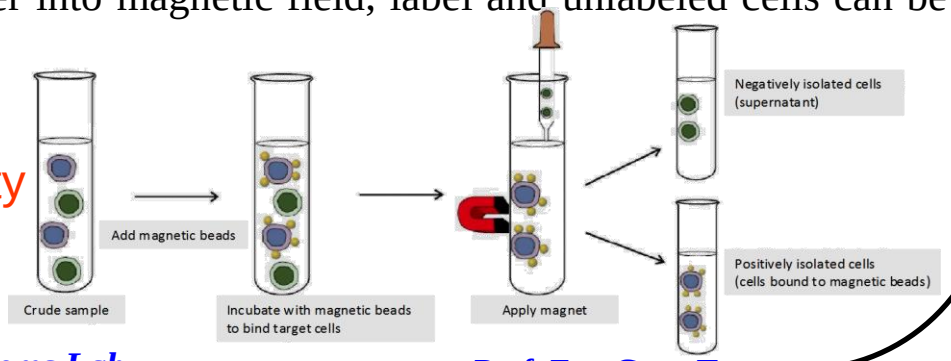




2. Magnetic-Activated Cells Sorting (磁性細胞分選儀)

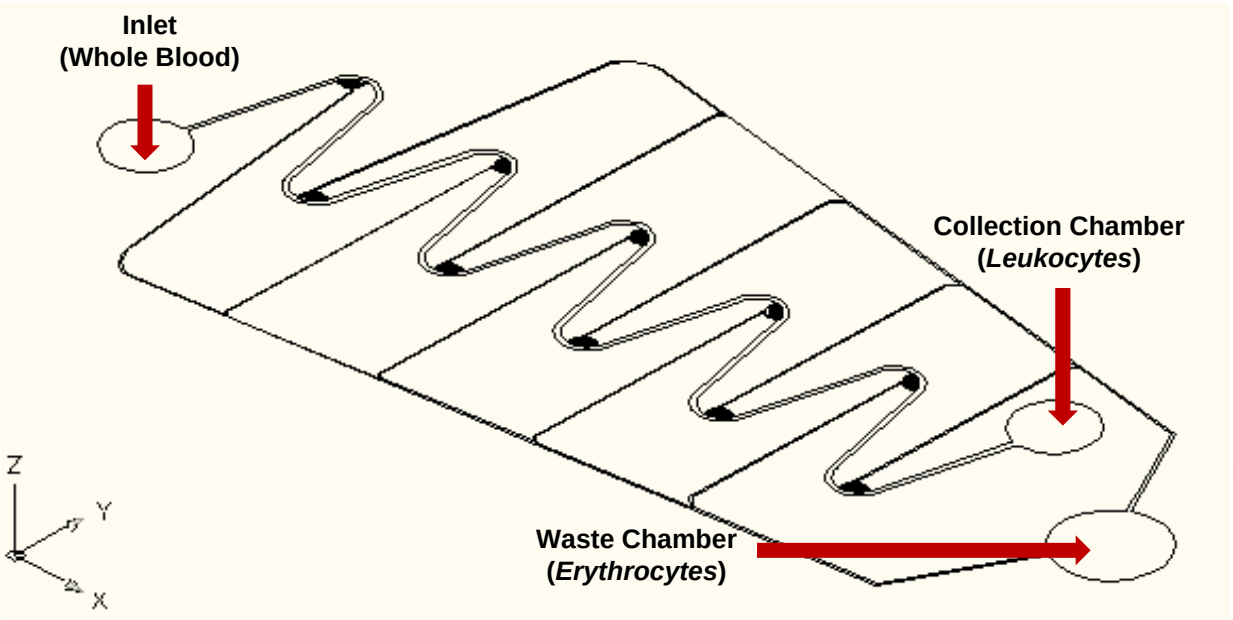
- By using magnetic-beads combine with high specific monoclonal antibody bind onto the surface antigen of target cells。When the cell enter into magnetic field, label and unlabeled cells can be separated 。
- The magnetic-beads diameter is 50nm。

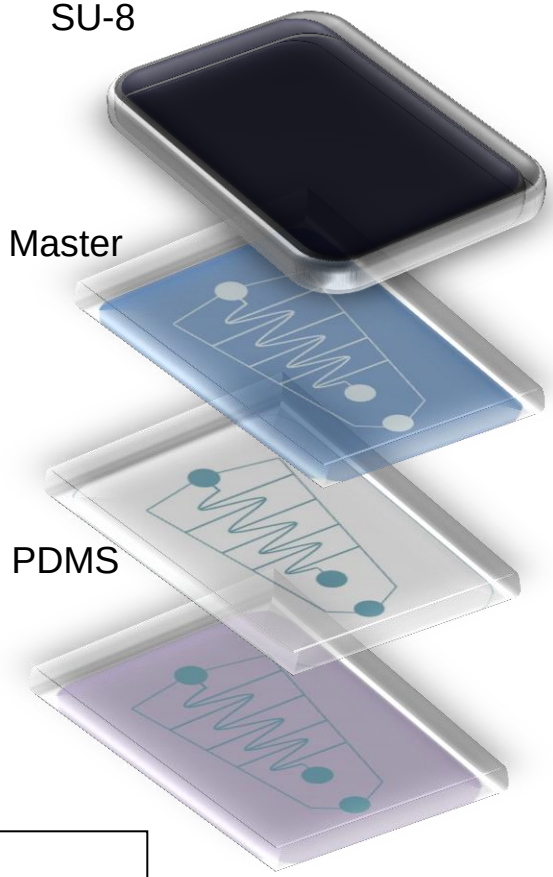
~10^7cells/s, ~10 min for 1cc, 99% purity
Recovery rate~70-90%

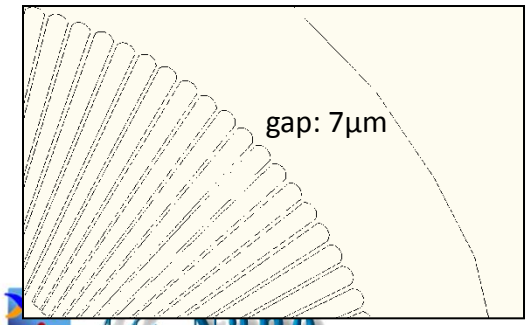


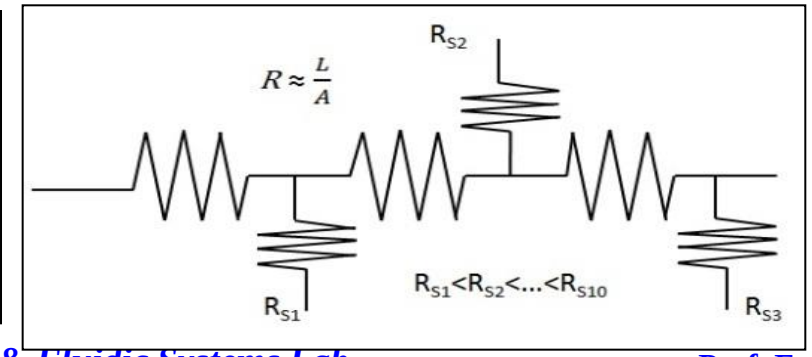
High Efficient Blood Cells Separation

Chip Size	Sample load	Process tIme
Main channel *(L) 28 cm* *(WxH) 200 x 580 μm²*	*10X dilution* *4.3x10⁸ cells/mL*	*(~400μL/min)* *25min.*









Theory analysis of Hydrodynamic and Inertial Force

1. Straight Channel

Lift Force (F_L)

$$F_L = \rho G^2 C_L a_p^4$$

Shear Rate (G)

Stokes Drag (F_D)

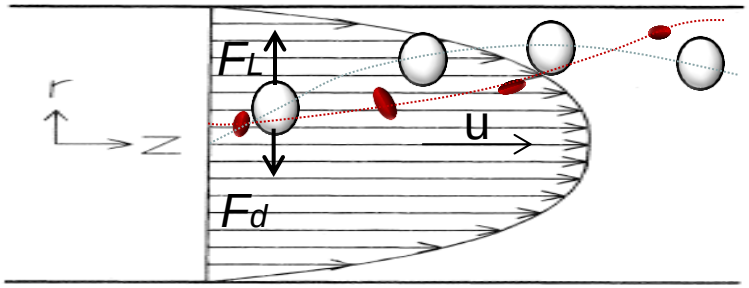
$$F_d = 3\pi\mu a_p U_L$$

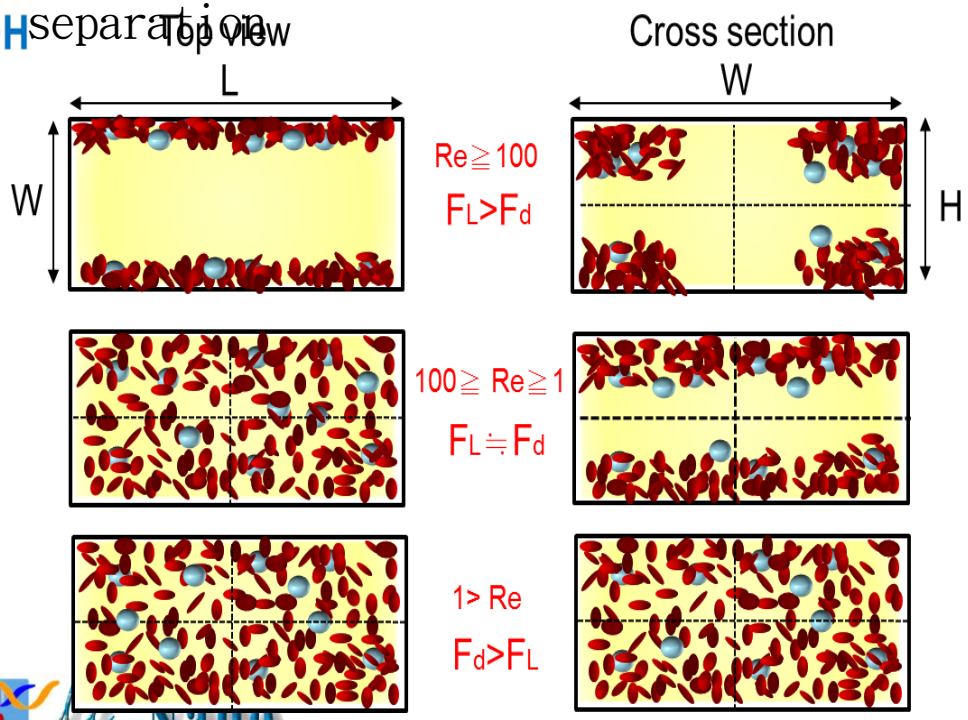
Viscosity (μ)

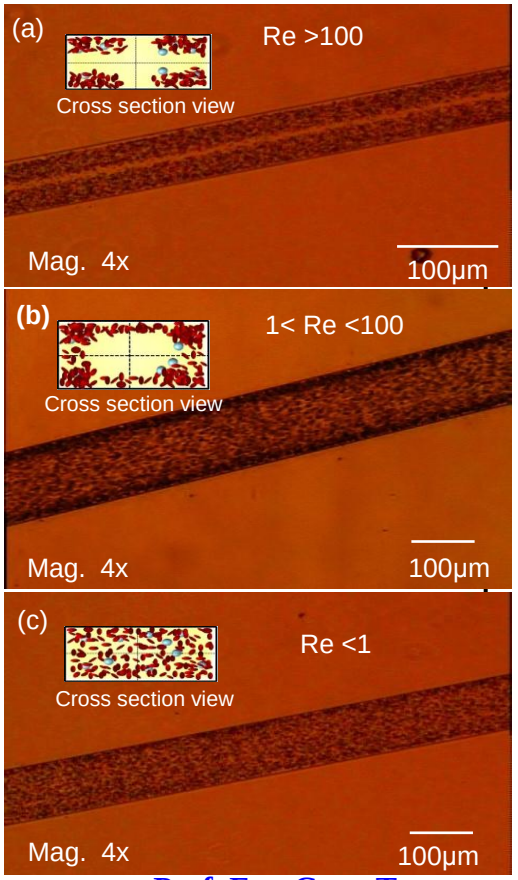
$$L = \frac{2WH}{3(W+H)Re}\left(\frac{W}{a_p}\right)^2$$

L: Length for enough separation

W>>H





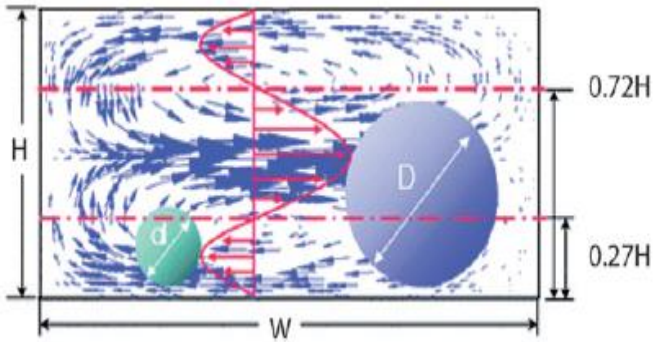


Theory analysis of Hydrodynamic and Inertial Force

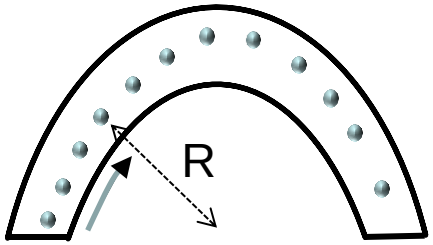
2. Curved Channel

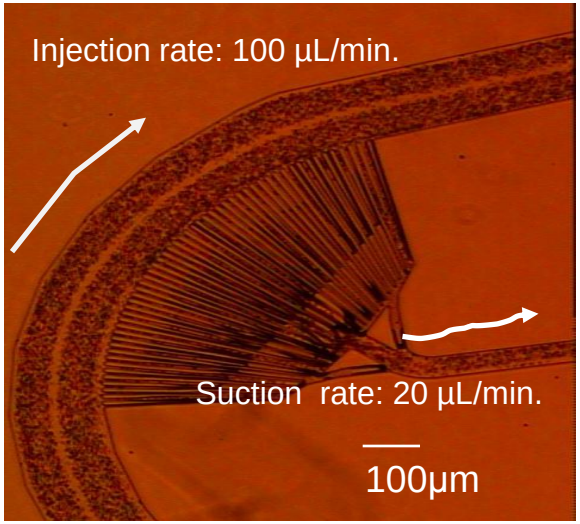
$$\frac{a^2 R}{H^3} > 0.04 \longrightarrow De = \frac{\rho U_f D_h}{\mu}\sqrt{\frac{D_h}{2R}} = Re\sqrt{\frac{D_h}{2R}} > 15$$

Cross section



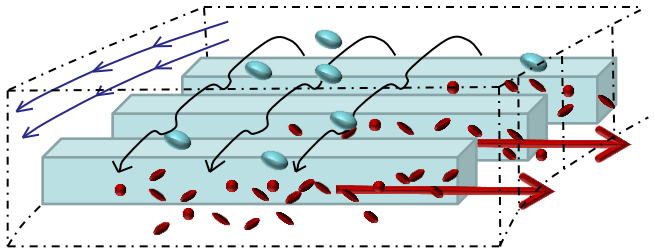
Top view

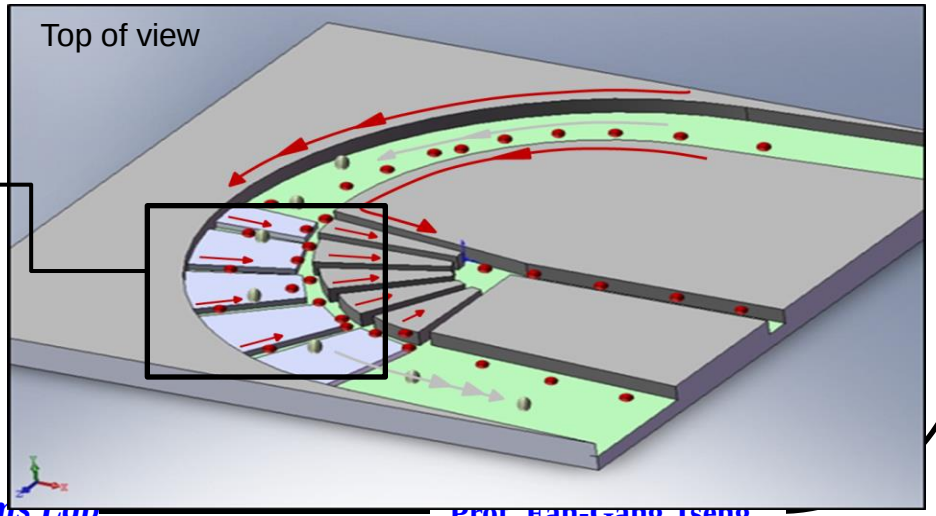




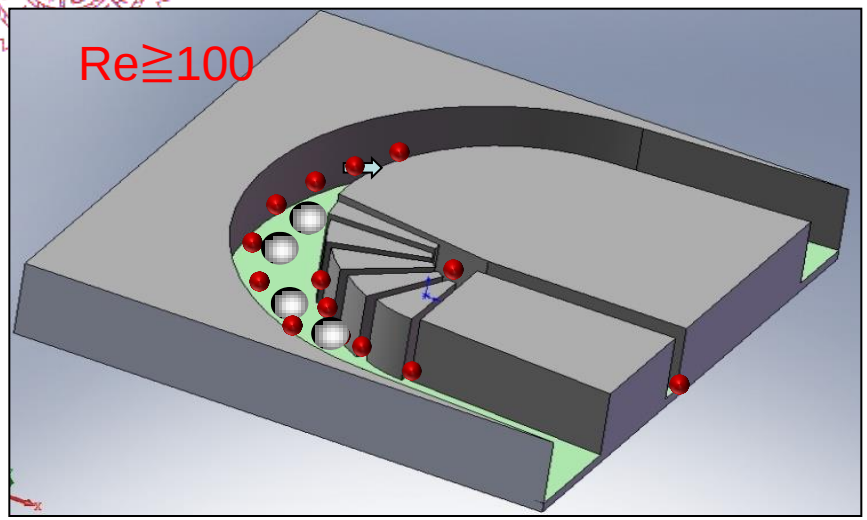
3. Obstacle Channel

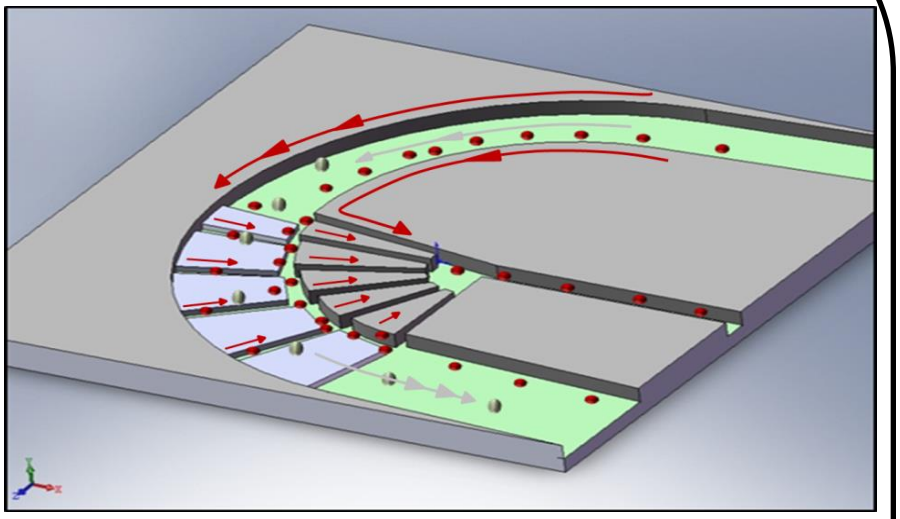
Side of view

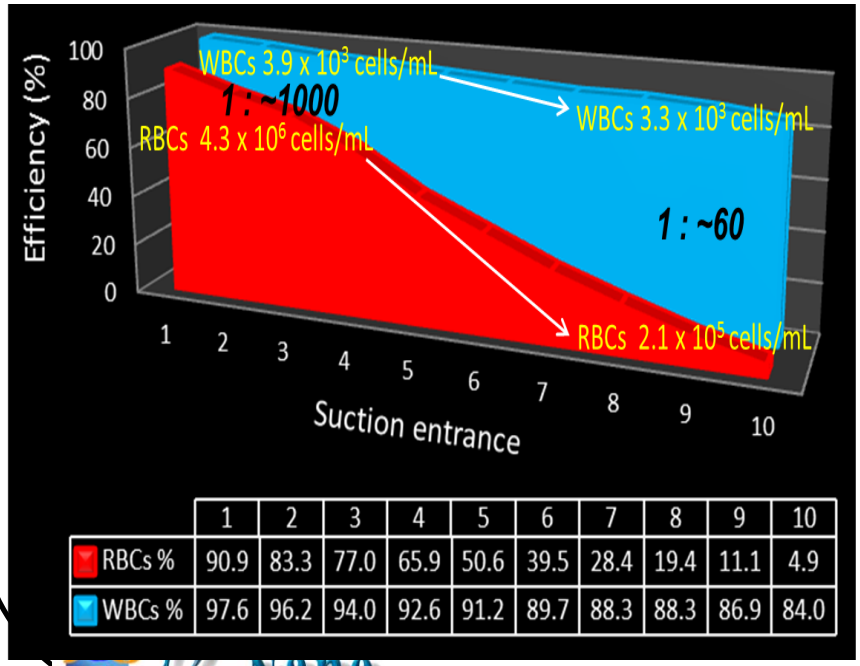




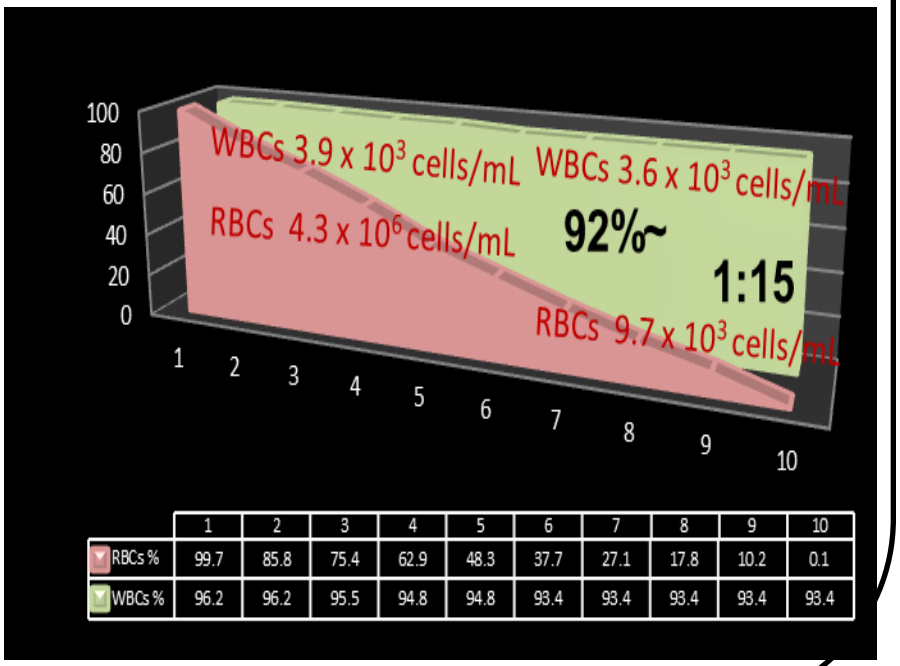
Results







	1	2	3	4	5	6	7	8	9	10
RBCs %	90.9	83.3	77.0	65.9	50.6	39.5	28.4	19.4	11.1	4.9
WBCs %	97.6	96.2	94.0	92.6	91.2	89.7	88.3	88.3	86.9	84.0

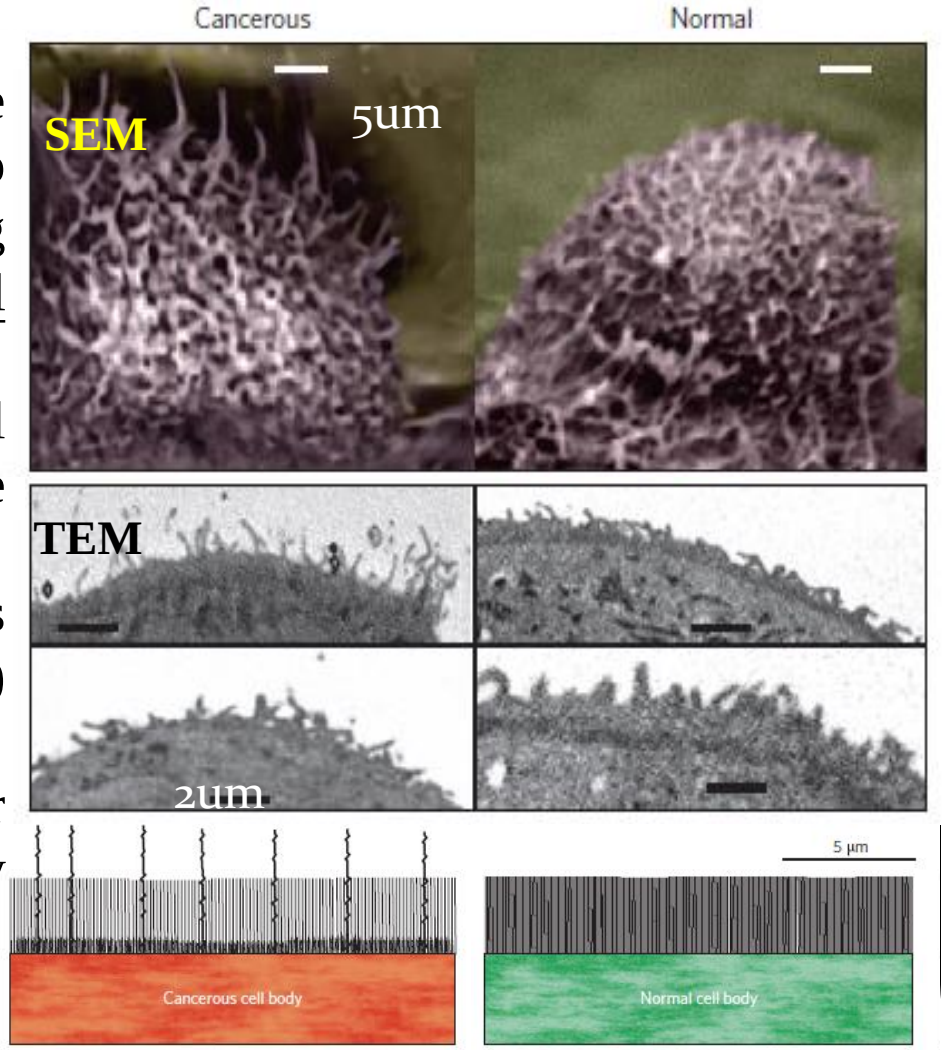


	1	2	3	4	5	6	7	8	9	10
RBCs %	99.7	85.8	75.4	62.9	48.3	37.7	27.1	17.8	10.2	0.1
WBCs %	96.2	96.2	95.5	94.8	94.8	93.4	93.4	93.4	93.4	93.4

Part II: Silicon nanowire biochip applied on circulating tumor cells detection

Nano structure enhance capture yield

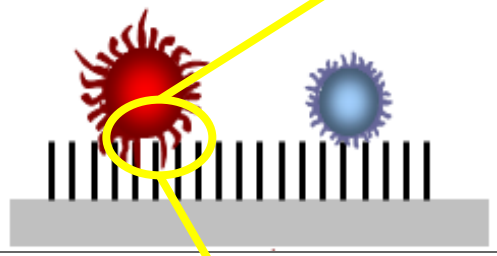
- Microvilli are cellular membrane protrusions, important for of cells to interact with environment, forming brush border, common in epithelial cells
- Microvilli also appears on the cell surface of white blood cells, help the migration of white blood cells.
- The microvilli on the surface appears longer on cancer cell(left, 5&2.6 um) than normal cell(right, 2.4 um).
- our research plan to enhance cancer cell capture rate from WBCs by utilizing the length of microvilli.



Igor Sokolov et al, 2009, Nature nanotechnology

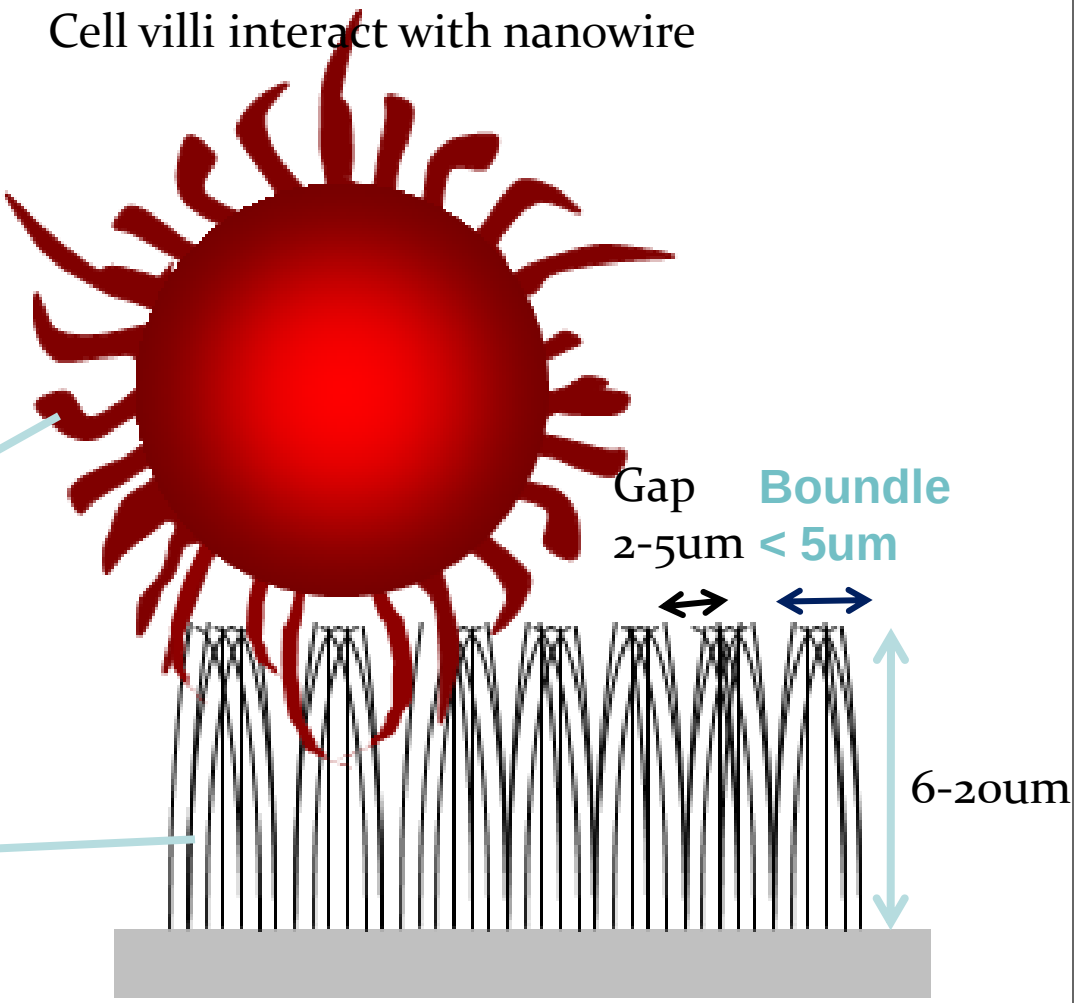
Principle of nanowire-cell villi interaction

Cancer cell capture



Velcro

Cell villi interact with nanowire

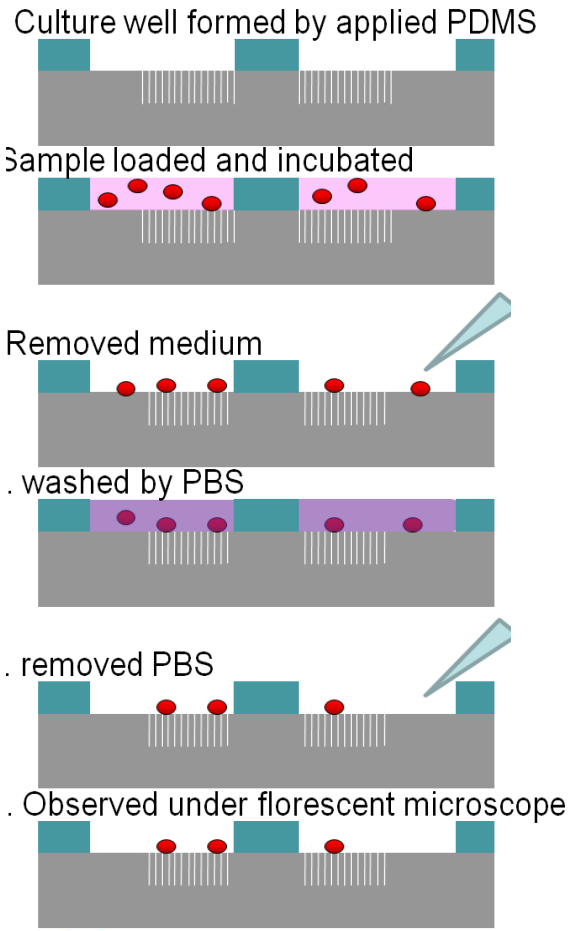


Experimental process and chip fabrication

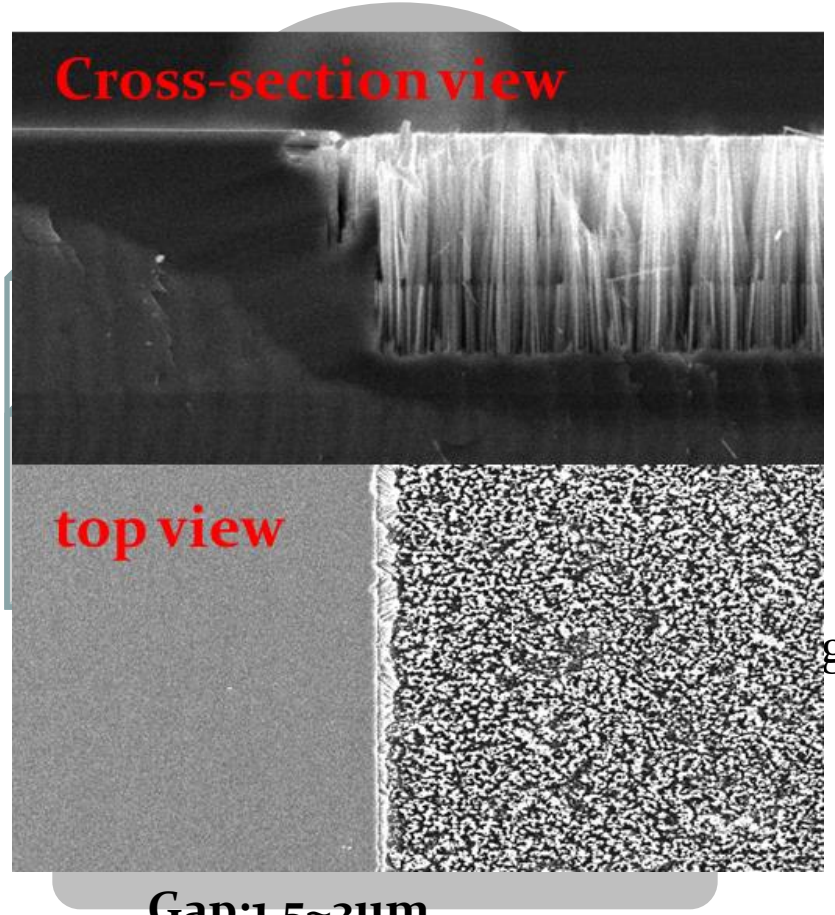
Conditions:

1.Incubated time

2.Nanowire length



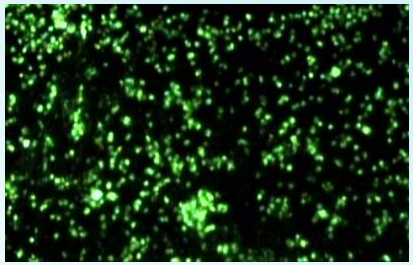
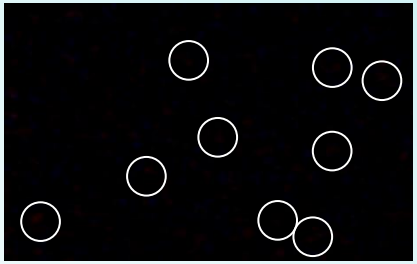
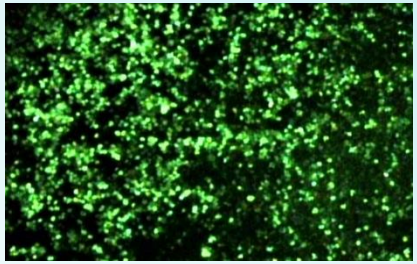
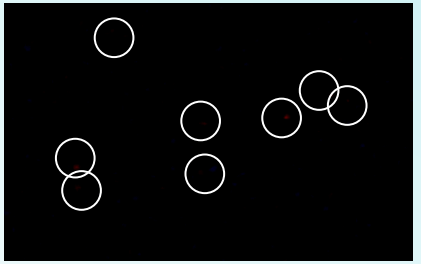
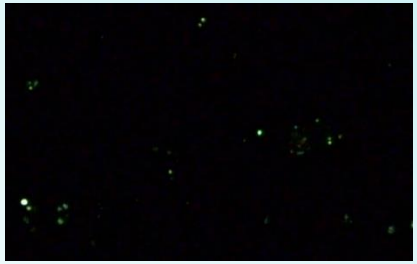
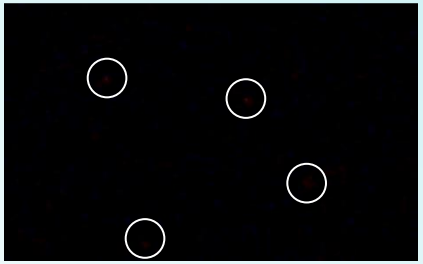
Si nanowire forming



Gap:1.5~2um

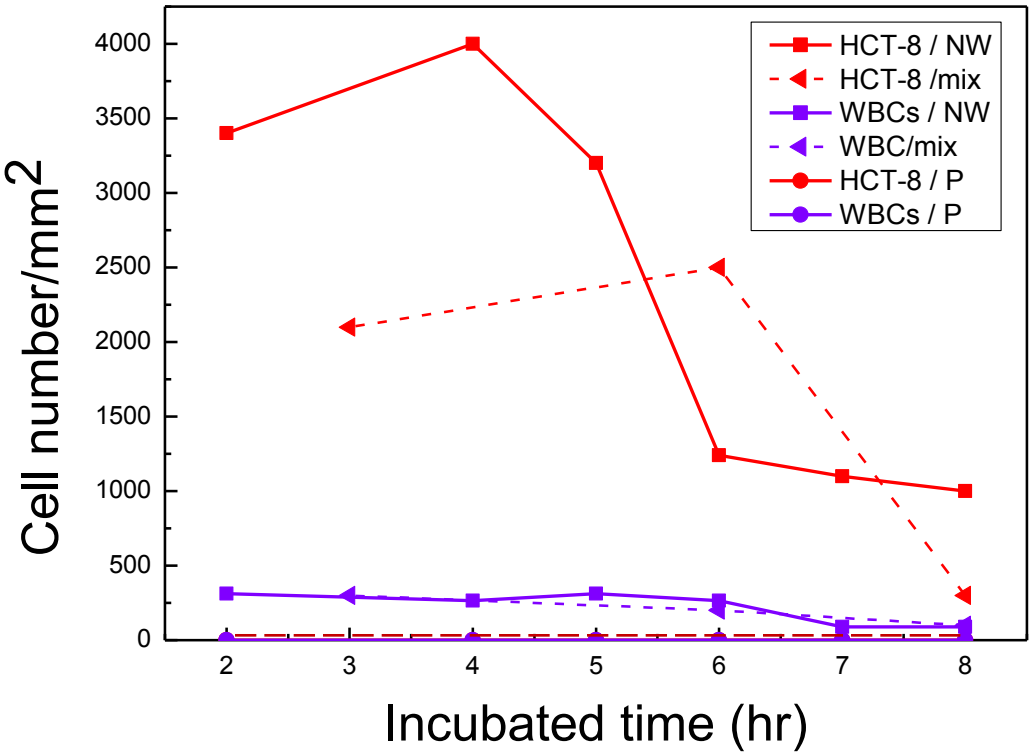
aggregation:1~1.5um

Cancer cell (HCT-8) with WBCs(1:1) capture ratio with variable Incubated time

Incubated time	Green emission	Red emission
3hr		
6hr		
8hr		

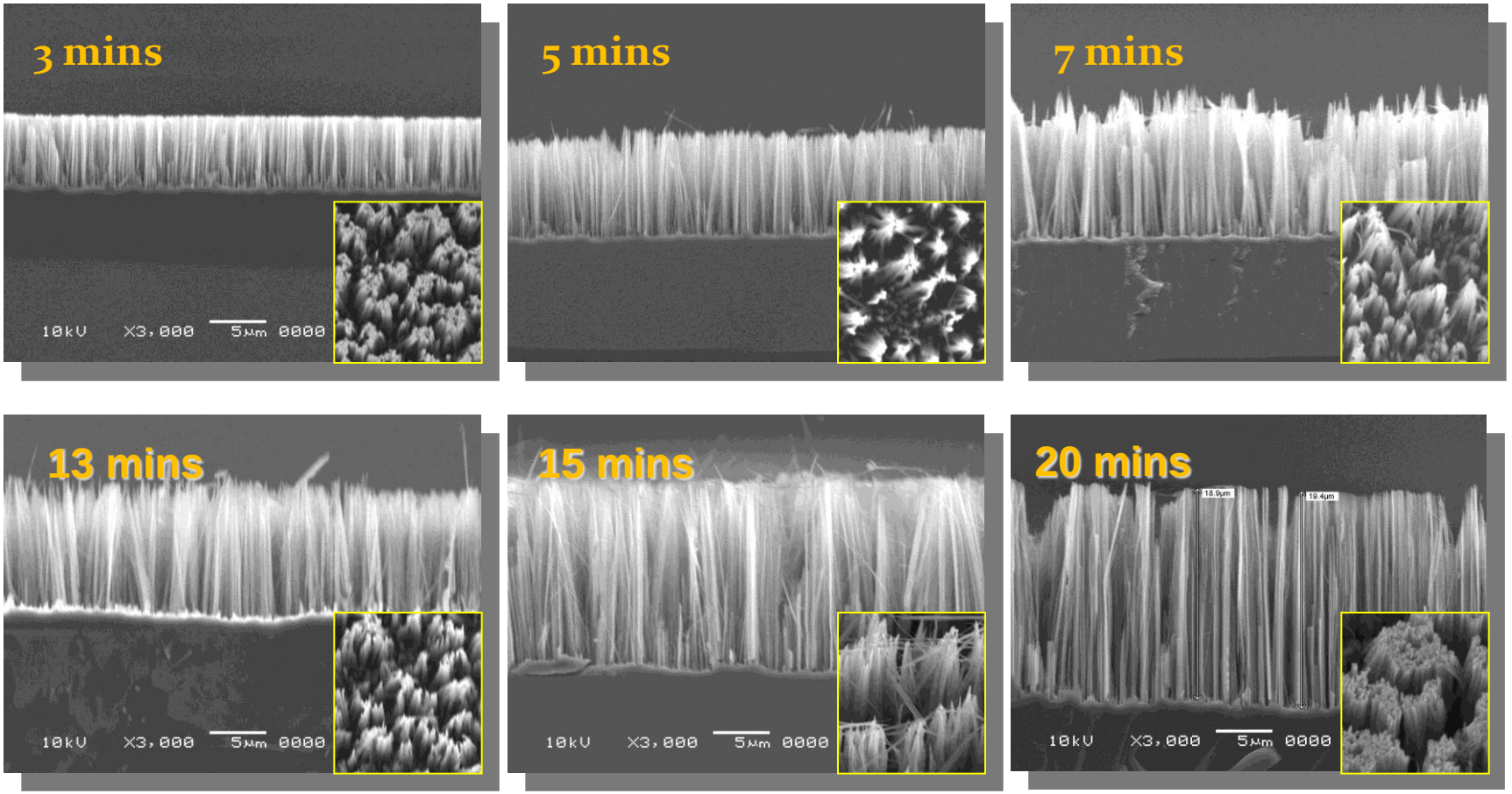
The capture result of different incubated time

Retention rate of two phase chip

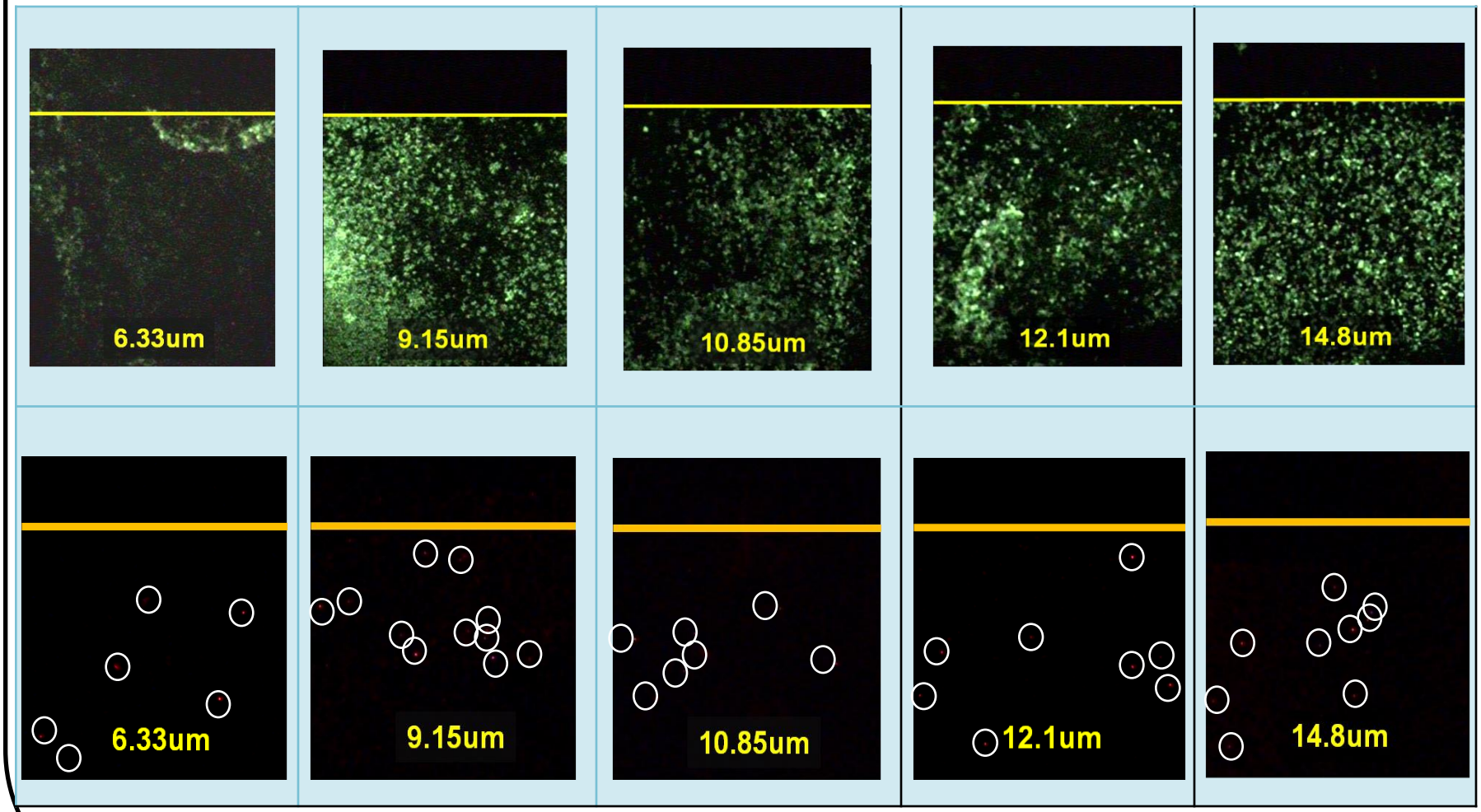


1.Maximun of cancer cell number appears at 4 hours
2.Mixed two cell result in lower capture cell number

Etching time V.S. nanowire length

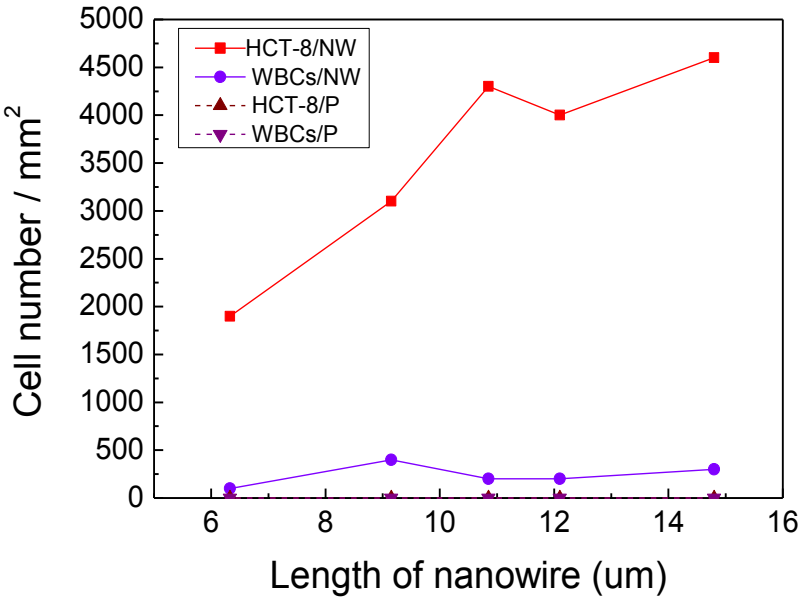


Cancer cell (HCT-8)/WBCs(1:1) capture ratio with variable length



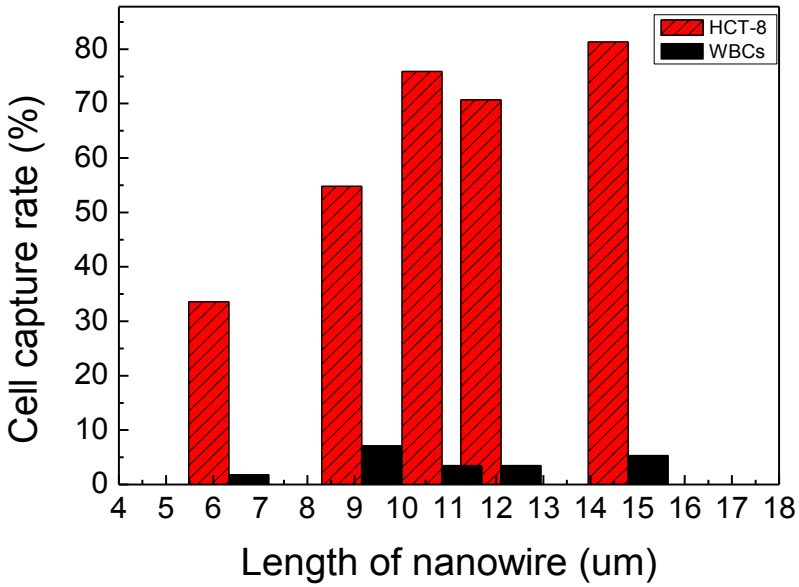
The capture result of different length

Retention rate of two phase chip



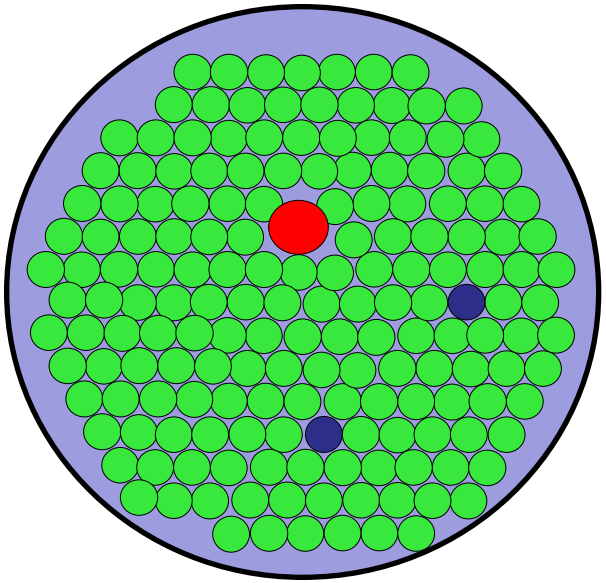
When incubated under the same time, the highest retention rate appeared at 14.8 um length nanowire.

capture rate of two phase chip

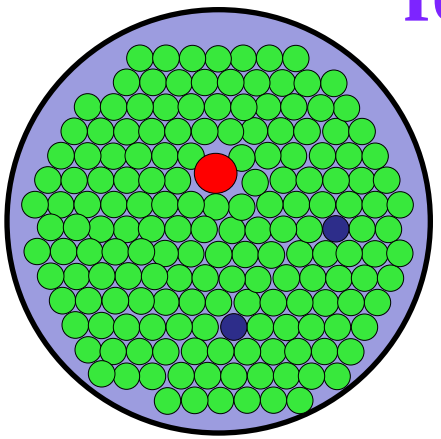


The 14.8 um length nanowire corresponds to 80% capture rate.

Part II: High Density Cell Array Chip for In-Parallel Detection of CTCs



High Density Cell Monolayer Array for in-parallel Detection



Tumor cells

White blood cells

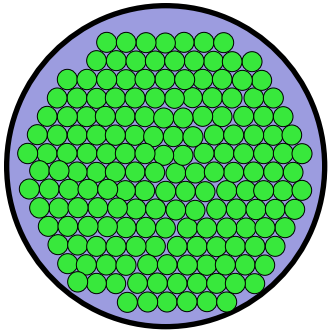
Other cells

Distinguishing ratio>100,000:1

Assembly time <10 min

High throughput: (10^6 cells)/cm^2)

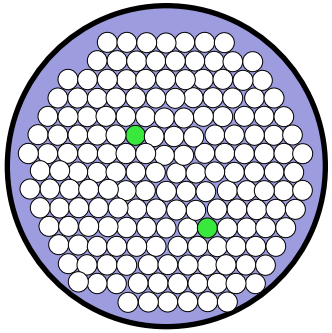
Step 1



Cells stained with dye

- Single type cells

Step 2

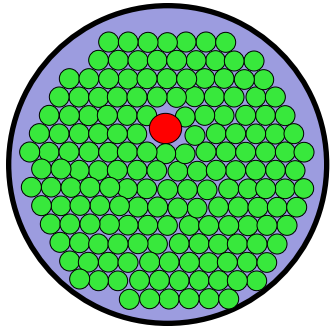


Cells with GTA fixation

Cells stained with dye

- Two type cells with one stained

Step 3



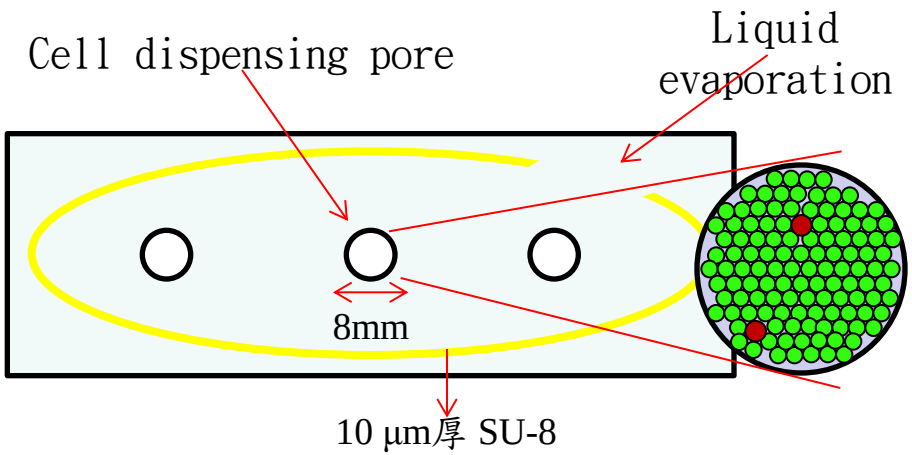
647nm : Background cells

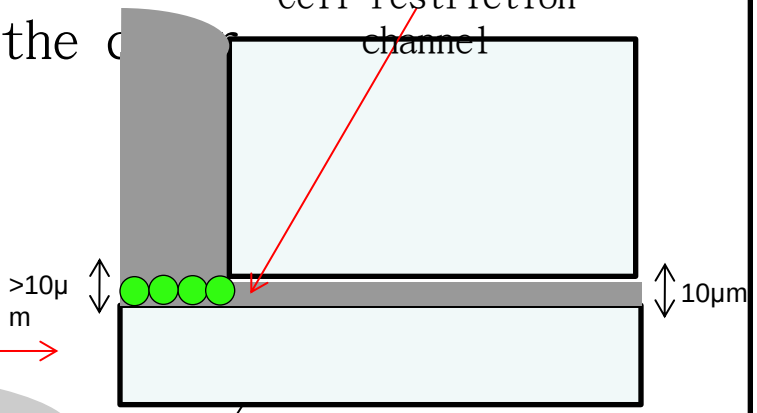
488nm : Target cells

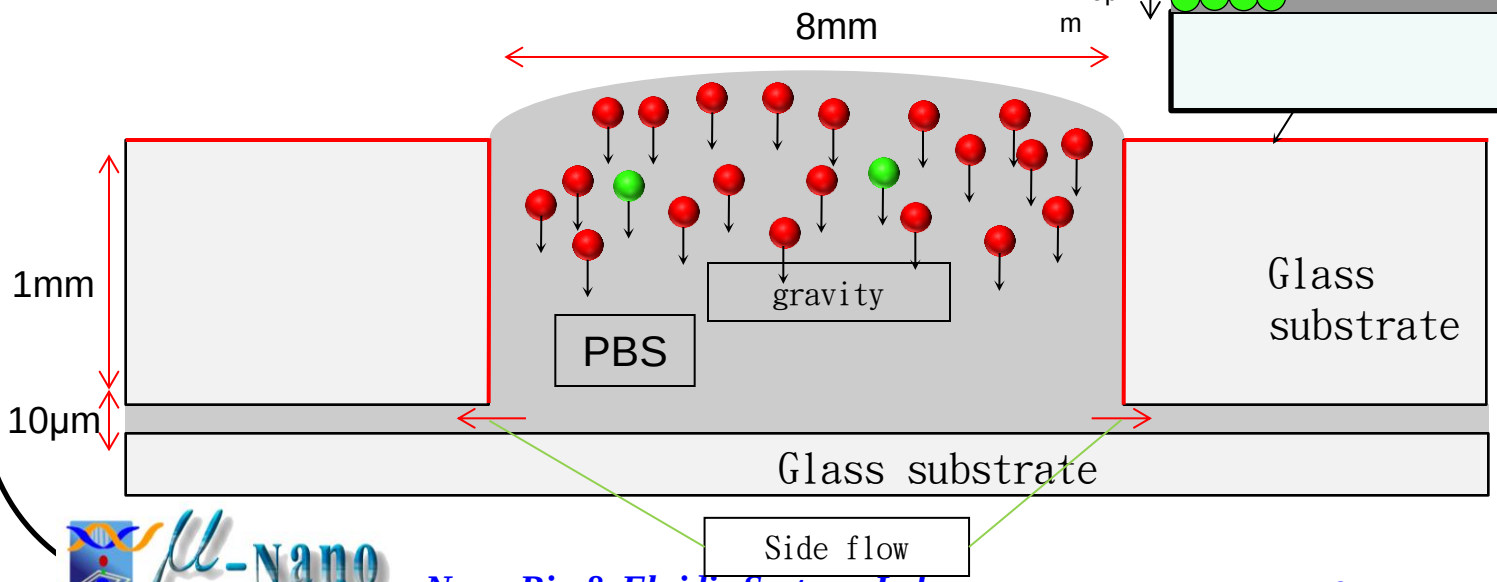
- Two type cells stained differently

Principle of high density Self-Assembly Cell Array (SACA)

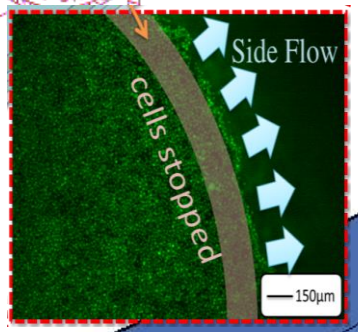
Major mechanisms:
1. Gravitation force
2. lateral flow by evaporation
3. Cell restriction into the c



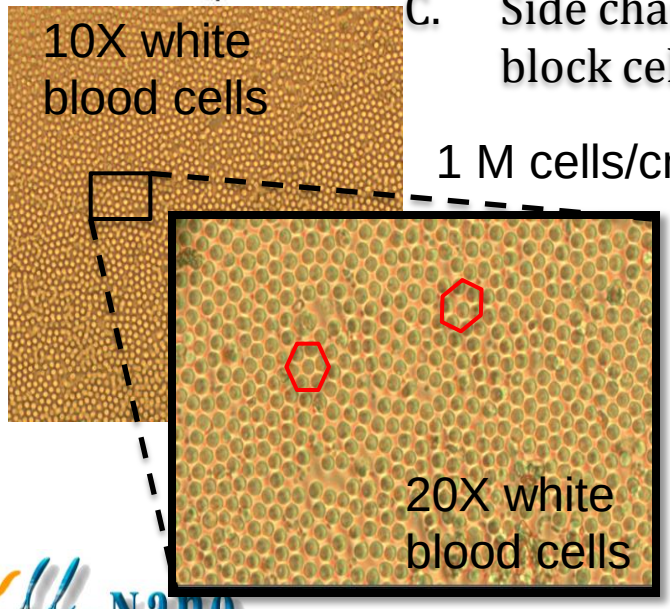




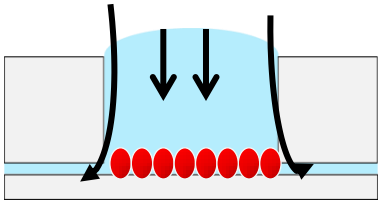
Self Assembled Cell Array (SACA) Chip

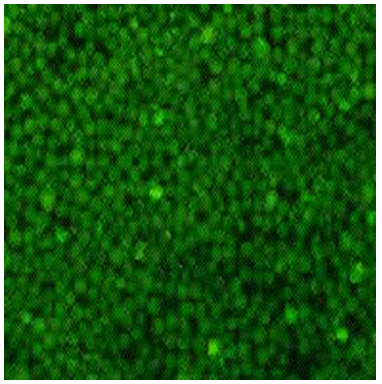


A. Gravity force
B. Side fluid force
C. Side channel block cells

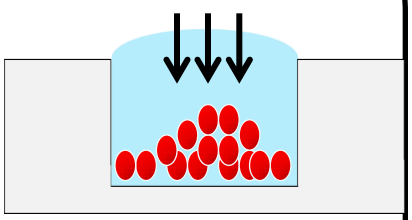


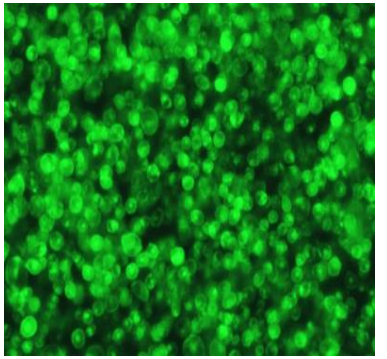
With side channel



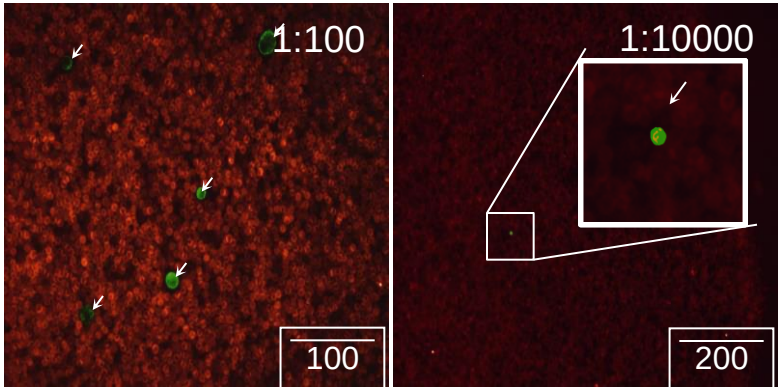


NO side channel



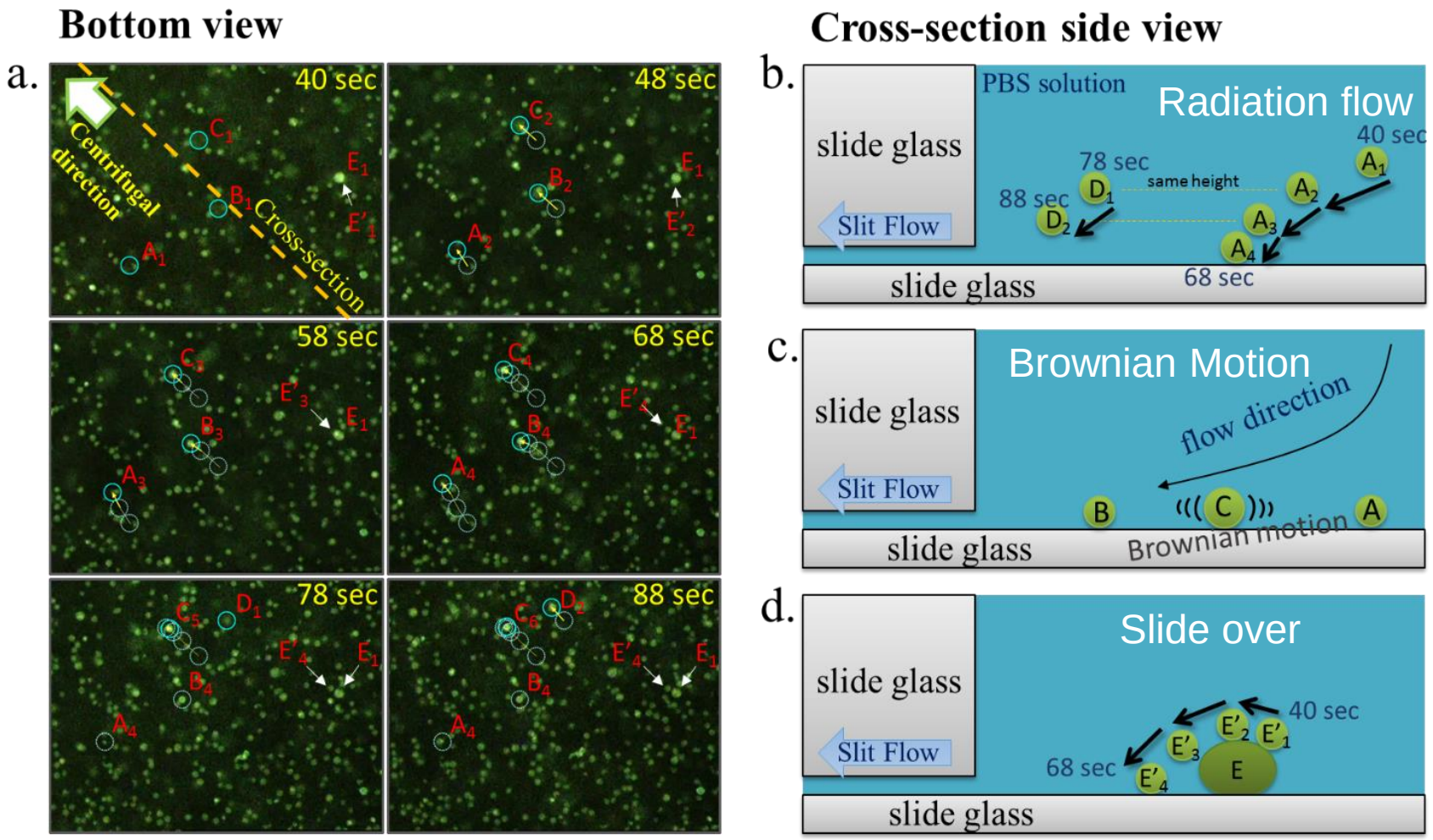


Hela cells in white blood cells



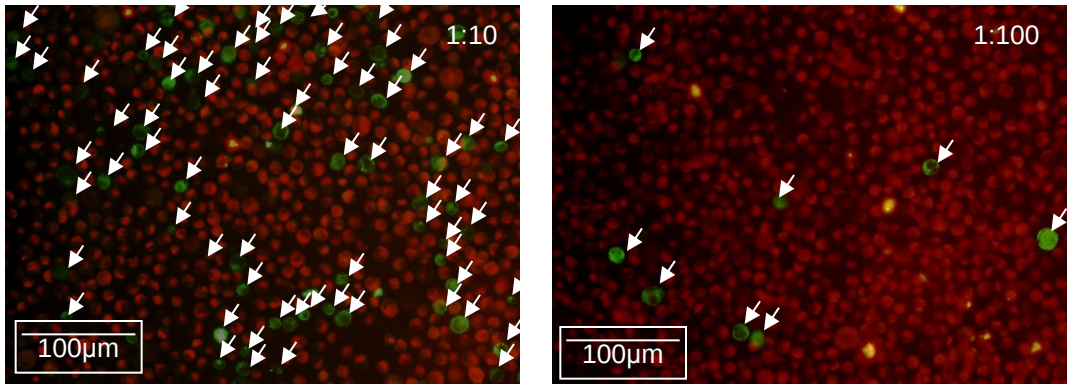
T.J Chang, and FG Tseng, *Biomicrofluidics, 2014*

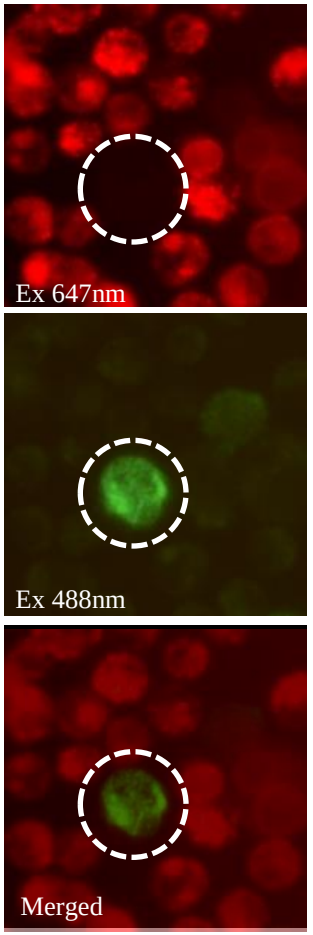
Mechanism for Cell Self-Assembly



Two different cells

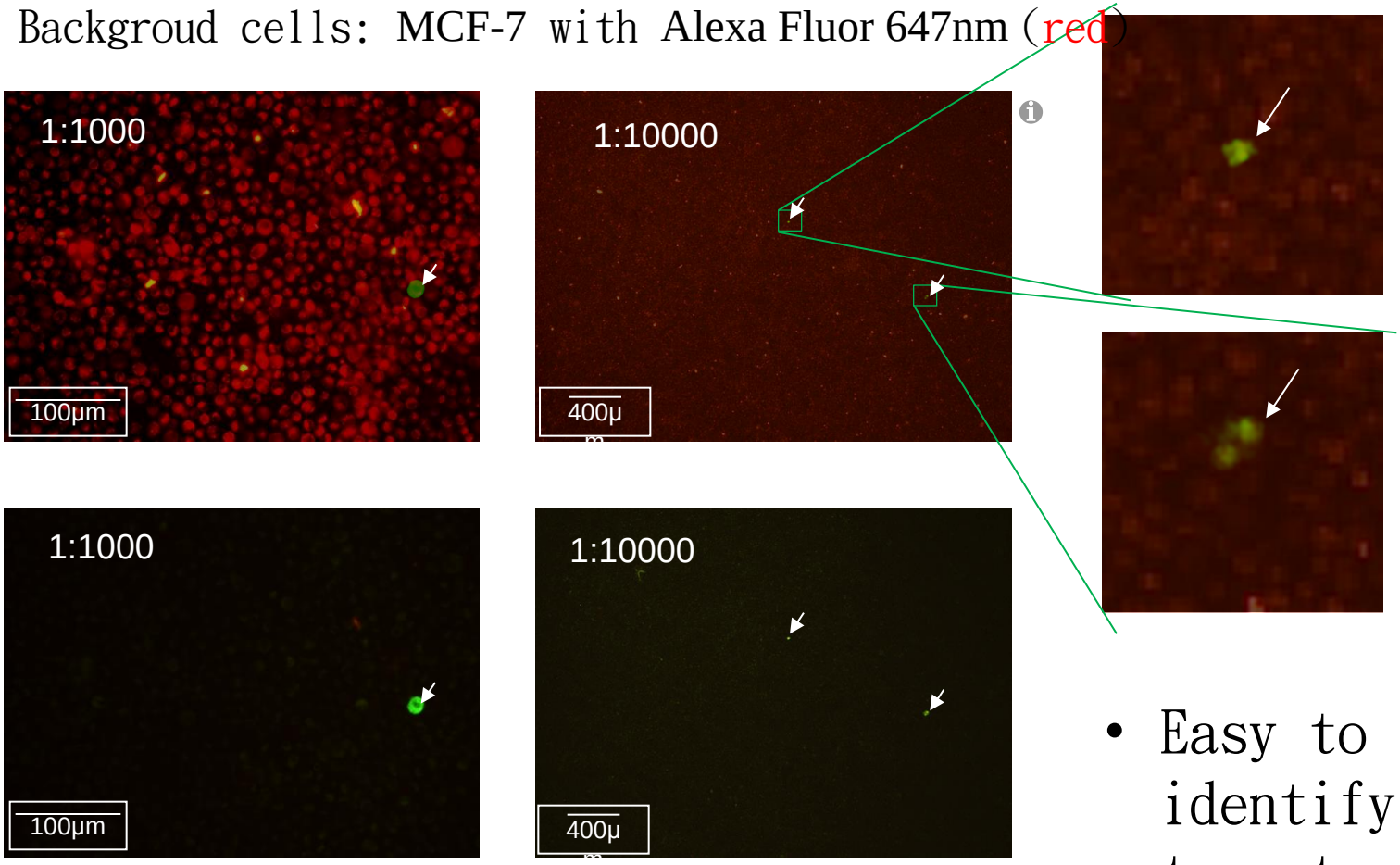
Target cells: HeLa with Alexa Fluor 488nm (green)
Backgroud cells: MCF-7 with Alexa Fluor 647nm (red)





Two different cells (cont.)

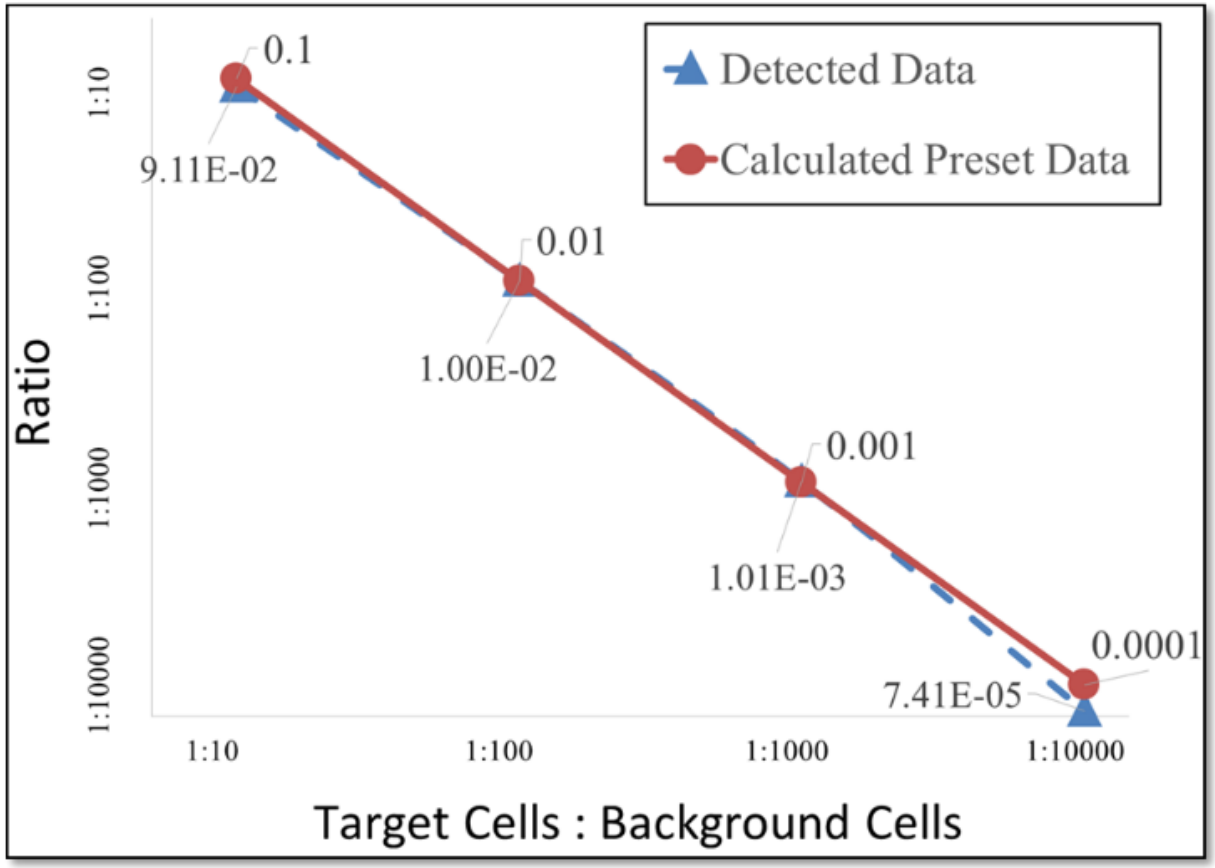
Target cells: HeLa with Alexa Fluor 488nm (green)
Backgroud cells: MCF-7 with Alexa Fluor 647nm (red)



- Easy to identify target cells

Distinguish ratio reaches: 10000:1

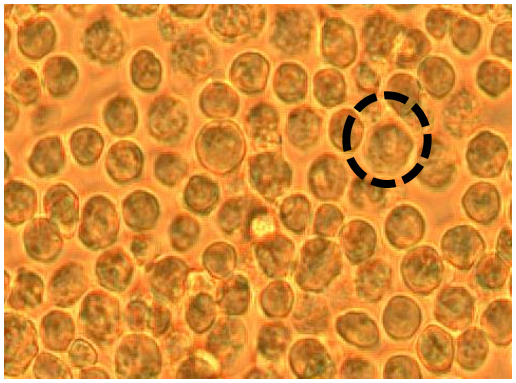
Distinguishing Ratio

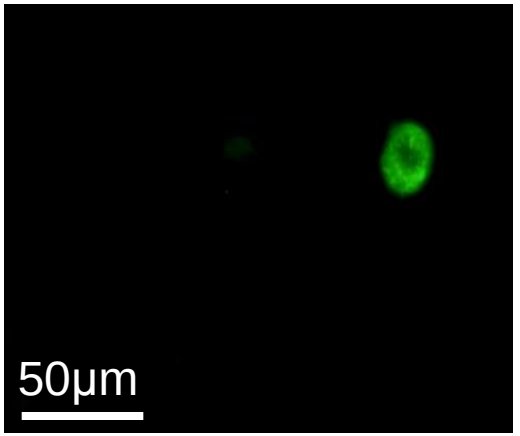


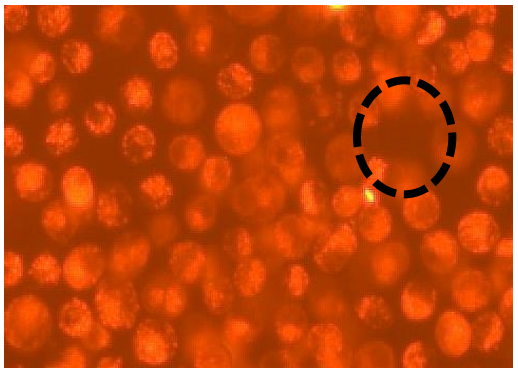
More than 10,000 to 1

Sensitivity Test

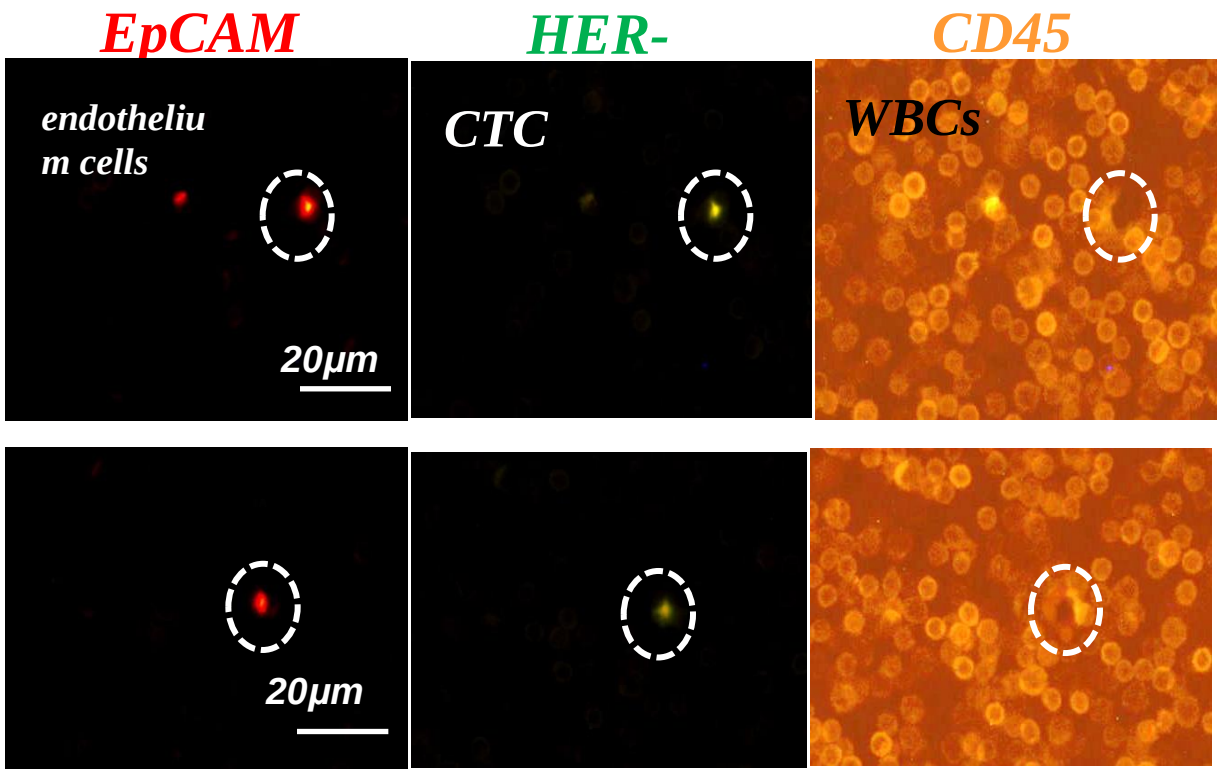
HeLa: WBC	Mixed HeLa cells number	Detected HeLa cells number	Average deviation value
1:10,000	29 34 41	27 30 38	8%
1:100,000	35 39 42	33 34 38	9%
1:1,000,000	34 36 39	30 31 34	12.7%
1:10,000,000	33 37 38	29 30 32	15.2%







CTCs Detection from Real Patient Samples (VGH, Taiwan)



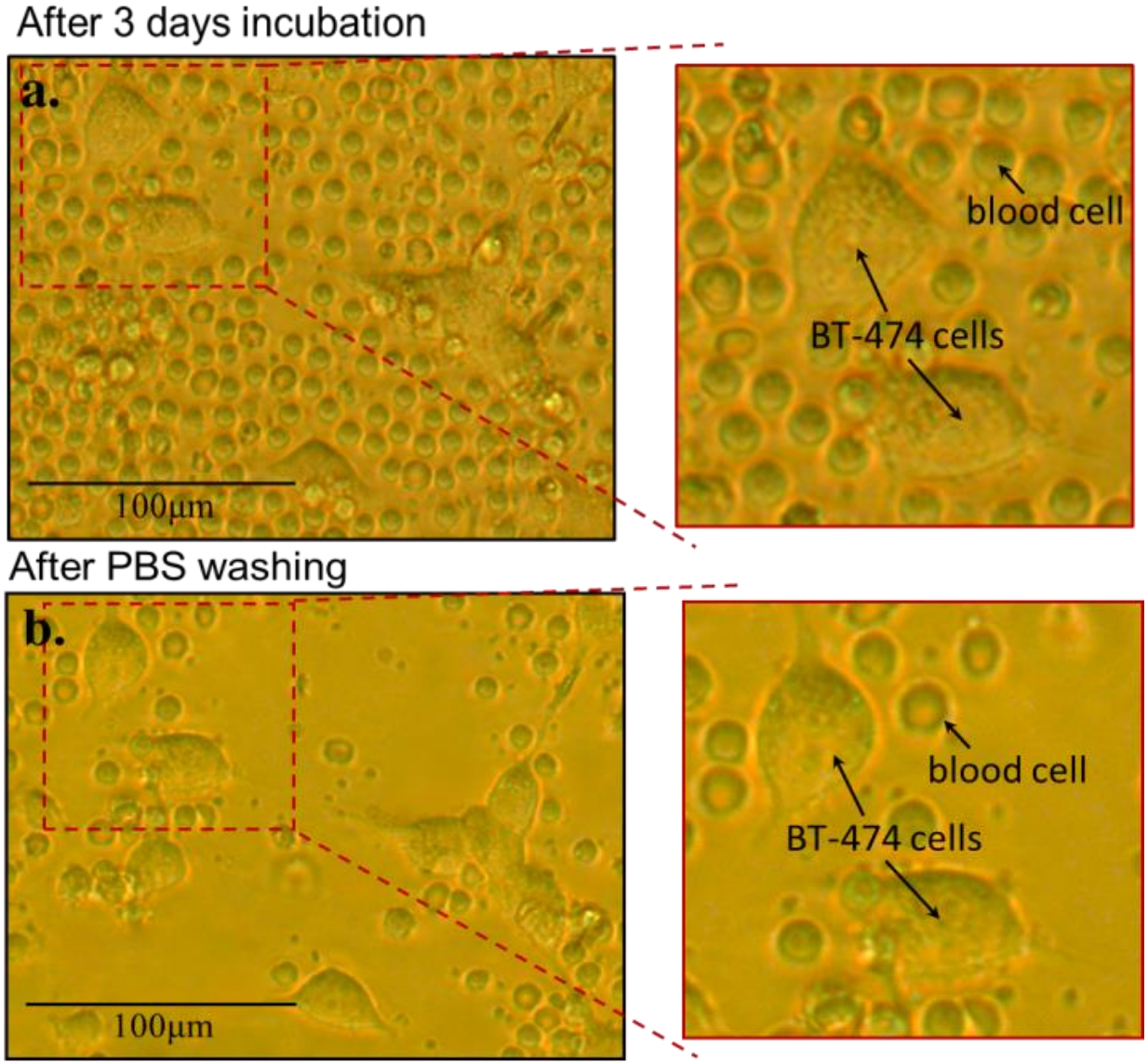
Stage VI breast cancer patient (under treatment)

Spotted average 5 CTCs in wells
A well contain 10^6 WBCs (monolayer)

$6x10^6$ WBCs in 1 ml full blood

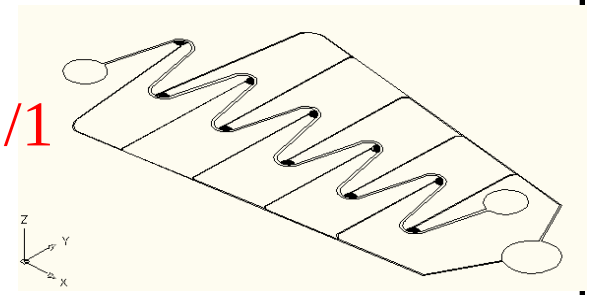
Predicted- 30 CTCs in 8 ml full blood

Tumor cells on-site Incubation

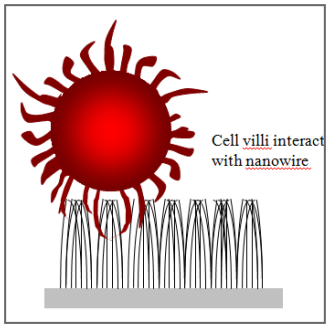


Conclusions

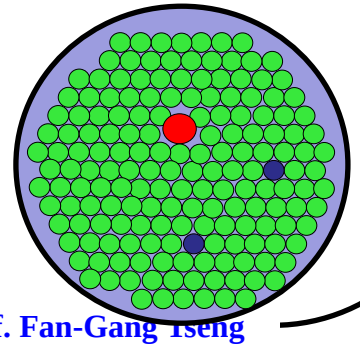
1. Blood cell separation: by using hydrodynamic and centrifugal forces,
   RBC/WBC:1000/1=>60/1=>15/1 ==> <1/1 (goal)



2. CTC enrichment: by using Nanoboundle arrays,
   CTC/WBC: $1/10^7$=>~$1/10^5$ (80%:2% retention)



3. High density Cell Arrays: cell self assembly in descending flow, Density>10^4 cells/mm^2 (>10^6 cells/1cm^2), Distinguish ratio $1/10^6$ => $1/10^7$



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3. NSC Integration Research Program, Taiwan
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Thanks very much for Attention!

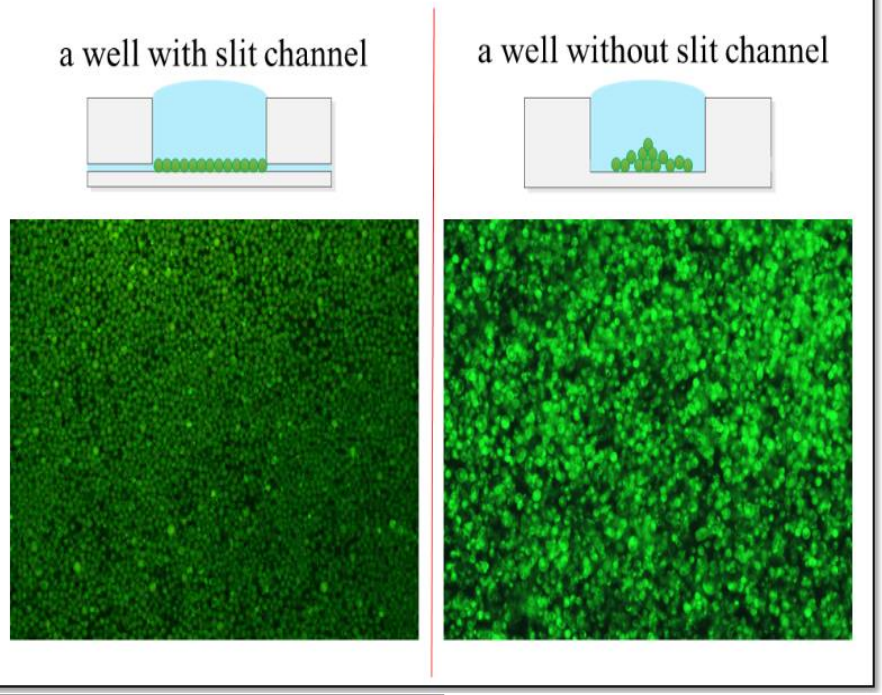
NTHU ESS

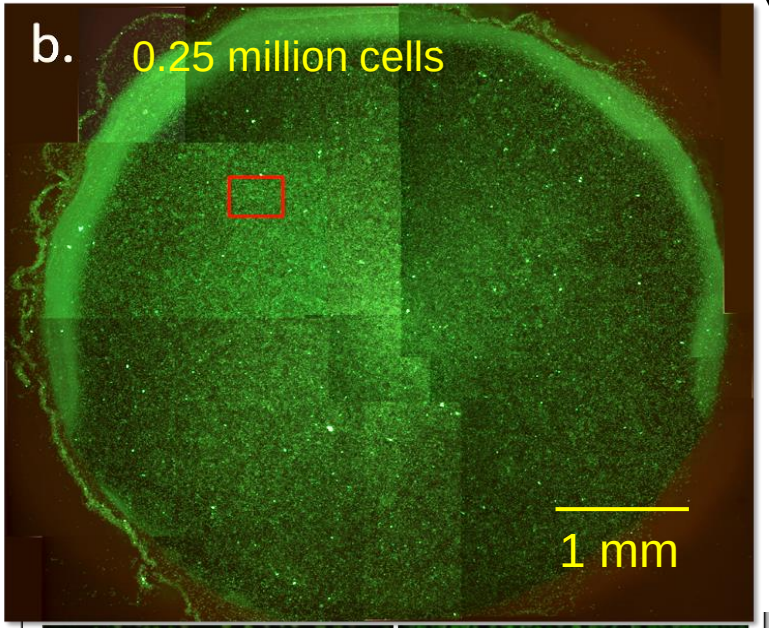
μ-Nano Bio & fluidic Systems Lab

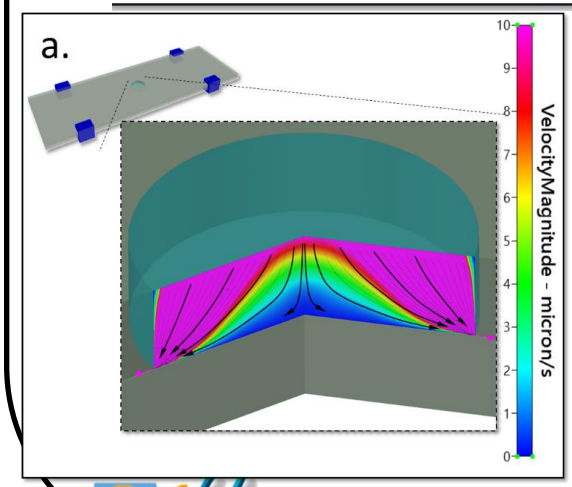
國立清華大學 工科系

奈微米生醫暨流體系統實驗室

The importance of the Micro Slit







High cell arraying density in mono layer:

1,000,000 cells/cm^2!!