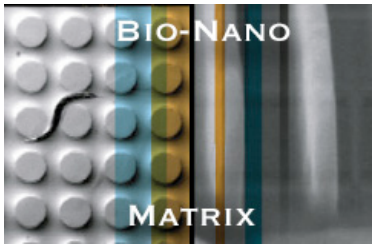
Influence of Microstructures and Nanoparticles on Micron-Sized Cells

Sungsu Park

Division of Nano Sciences and Department of Life Science

Ewha Womans University

Seoul, Korea 120-750



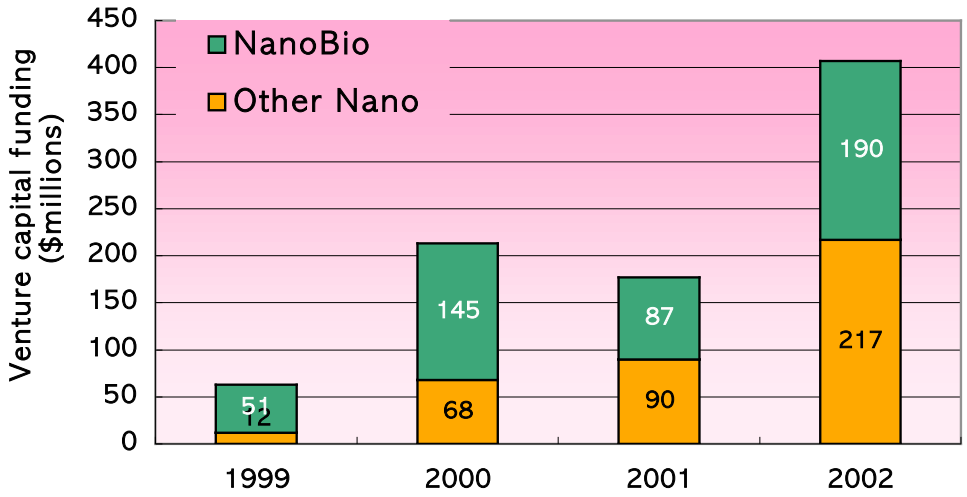
Parts of this talk

- Introduction to Nanobiotechnology
- Influence of microstructures on behaviors of bacteria and nematode
- Influence of nanoparticles on nematode
- Stretching Genomic DNA using Nanochannels (if time is allowed)

Field of Nanobiotechnology

- Nanobio-hybrid materials
- Drug and Gene delivery systems
- Micro- and Nanofluidics
- Biosensors
- Single molecule detection
- Surface chemistry, etc.

U.S. Investment in Nanotechnology



Source: Nature Biotechnology (2003), vol.21,p1144-1147

- **Why so much money goes to NBT?**
  1. **small investment required**
  2. **Fast turnaround!**
  3. **High demands from Pharm. and Biotech. Co.**

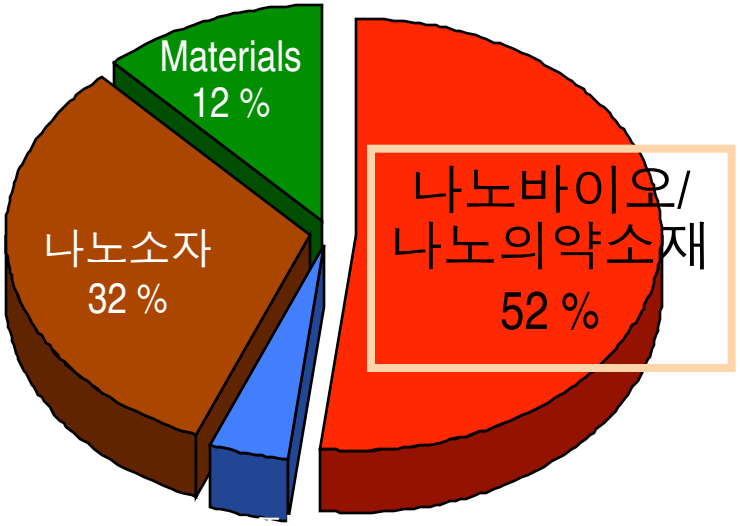
나노관련 미국대학내 연구프로그램

US universities with federally funded nanotechnology programs

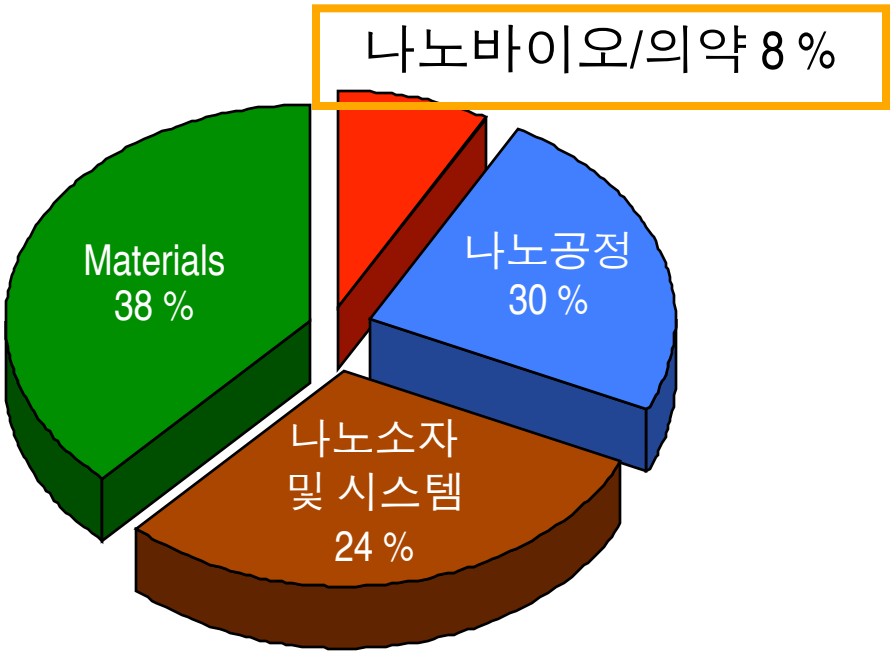
University	Program	Government agency
Rice Univ.	Nanoscience in Biological and Enviromental Engineering	NSF
Northwestern Univ.	Integrated Nanopatterning and Detection	NSF
Rensselaer Polytech. Inst.	Directed Assembly of Nanostructures	NSF
Cornell Univ.	Nanobiotechnology, Science and Technology Center	NSF
Columbia Univ.	Center for Electron Transport in Molecular Nanostructures	NSF
UCLA	Institute for Cell Mimetic Space Exploration	NASA
Texas A&M Univ.	Institute for Intelligent Bio-nanomaterials and Structures	NASA
Princeton Univ.	Bioinspection, Design and Processing of Multifunctional Nanocomposites	NASA
UC, Santa Barbara: MIT; Caltech	Institute for Collaborative Biotechnology	US Army
MIT	Institute for Soldier Nanotechnologies	US Army

전세계 및 국내 나노산업 투자경향

나노바이오 기술 및 소재의 연구개발의 필요성



나노기술관련 미국 내
벤처자금 투자현황 (1998-2004)



한국내 나노기술
관련 특허 동향 (1999-2003)

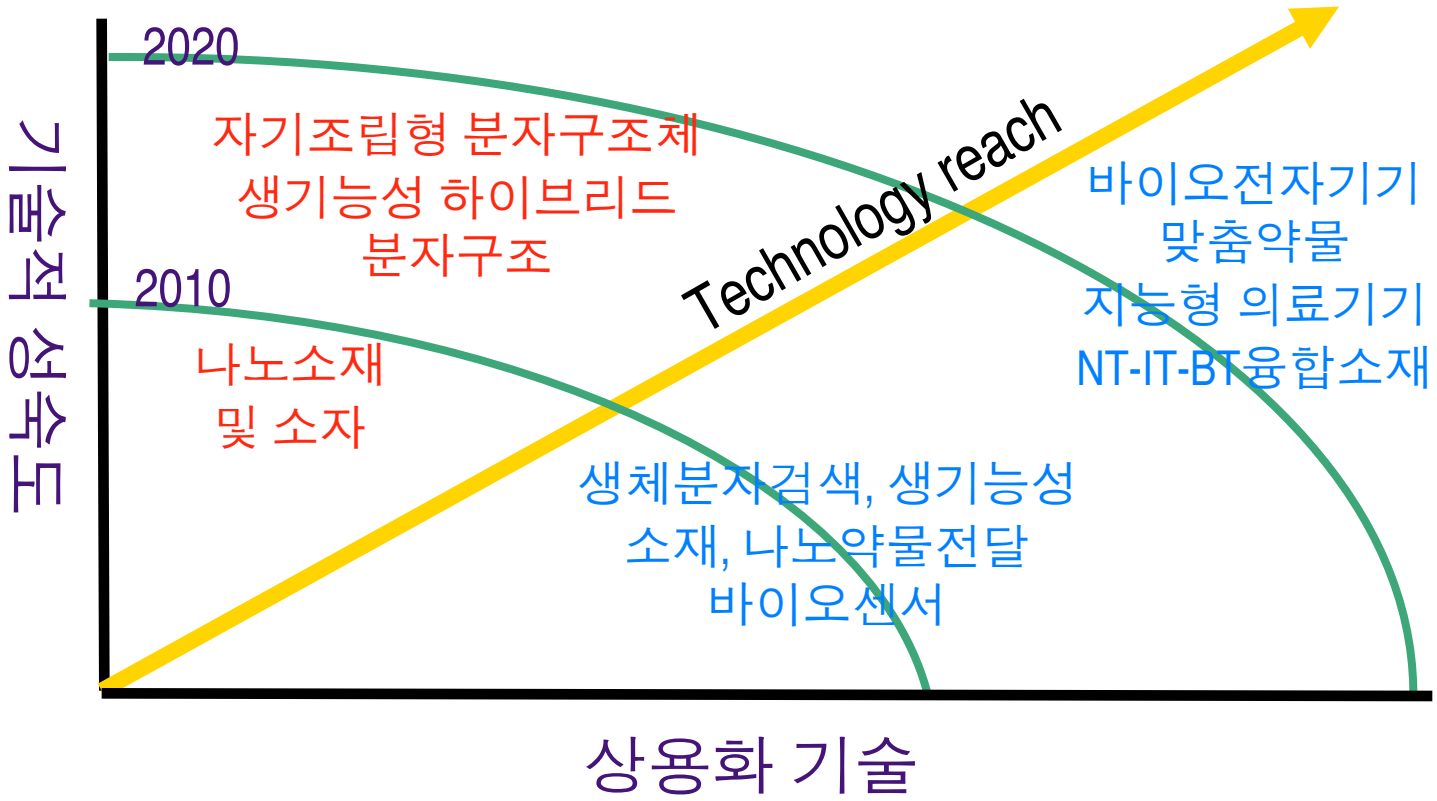
NBT Industry

Table 1: Top 10 nanobiotechnology companies based on amount of venture capital raised.

Company	Funds raised ($Millions)	Sector	Description
Immunicon (Huntingdon Valley, PA)	86.20	Diagnostics	Diagnostic screening using nanoparticles
Quantum Dot (Hayward, CA)	44.50	Biomedical applications	Semiconductor nanocrystals for biological assays
Surface Logix (Brighton, MA)	38.00	Drug discovery	Miniaturized biological assays
Genicon Sciences (San Diego, CA)	34.00	Diagnostics	Nanoscale signal generation and detection
PicoLiter (Sunnyvale, CA)	27.10	Diagnostics	Picofluidics for nanoparticle manufacturing
US Genomics (Woburn, MA)	27.00	Drug discovery	Single molecule analysis assays
Nanosphere (Northbrook, IL)	23.50	Diagnostics	Diagnostic nanoprobes and image analysis systems
Advion Biosciences (Ithaca, NY)	15.00	Drug discovery	Nanoelectrospray bioanalysis using biochips
Ferx (San Diego, CA)	15.00	Drug delivery	Drug delivery using magnetic forces
Nanogram Devices (Fremont, CA)	9.20	Biomedical applications	Nanomaterials for biomedical application components

Source: Lux Capital, Capital IQ

나노바이오 융합분야 기술발전 로드맵

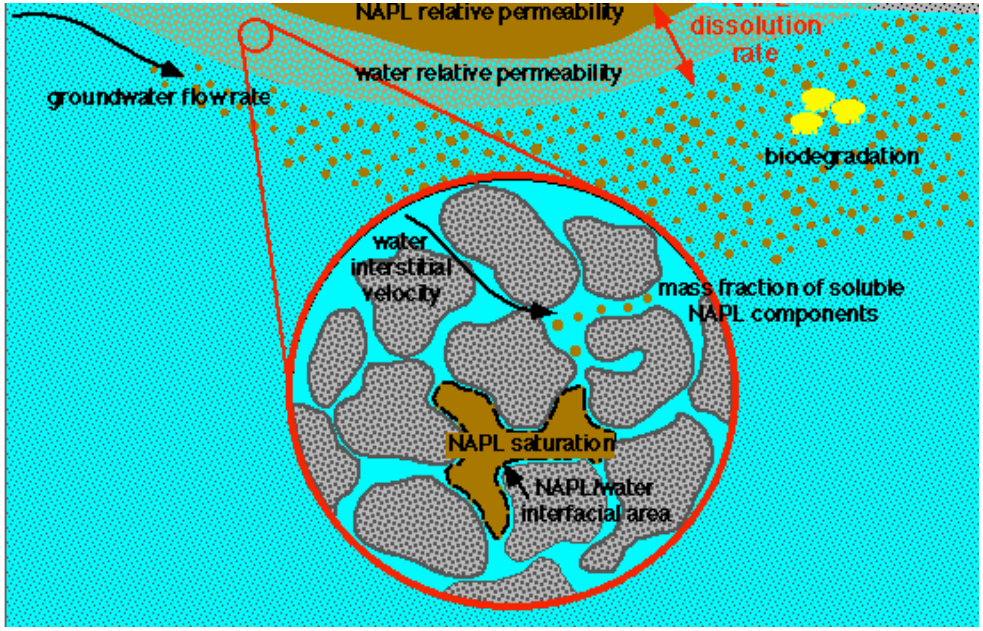


Source: SRI Consulting Business Intelligence (SRIC-BC; Menlo Park, CA, USA) Nature biotechnology 2004 (참고논문)

Influence of micron-scale topology on bacterial social interactions

Collarboration with Robert Austin at Dept. of Physics, Jeffry Stock and Bonnie Bassler at Dept. of Molecular Biology of Princeton University

Microecosystem Vs. agar plate



Microecosystem for bacteria

Typical agar plate

Fabrication of microecosystem chip

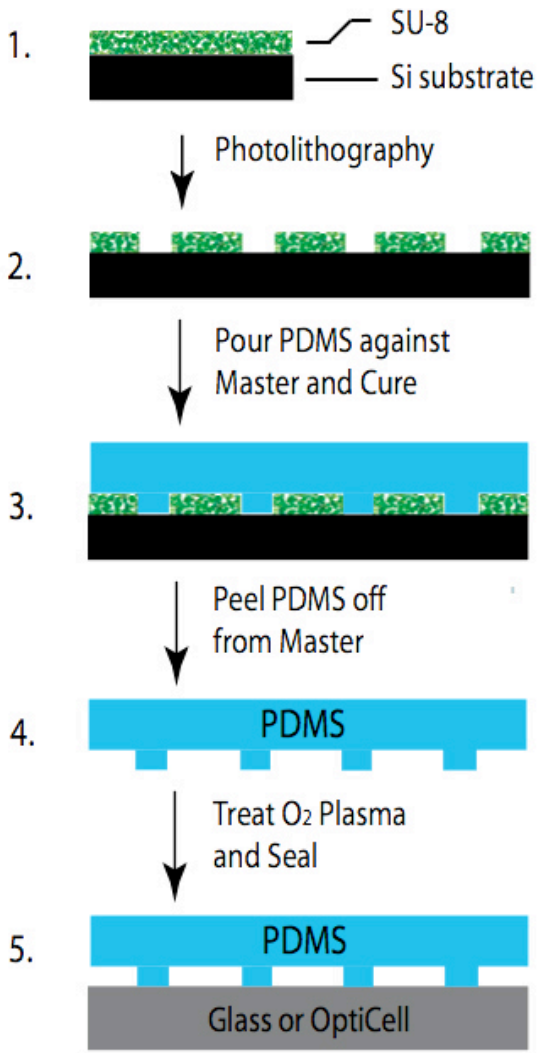


Fig. 1. Fabrication of PDMS structrure.

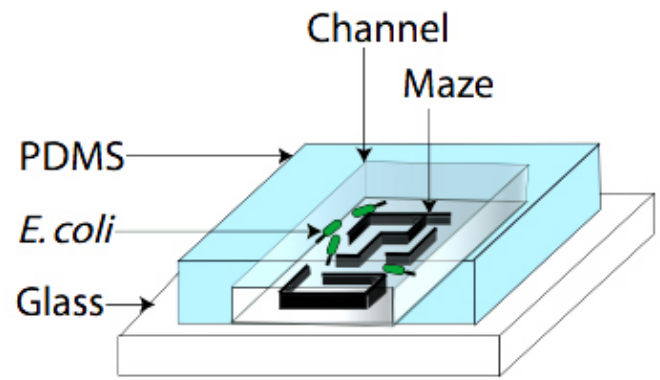


Fig. 2. PDMS maze assembled with glass.

Congregation of *E. coli* carrying GFP to confined structures

Simplified confined geometry: a square with a narrow opening

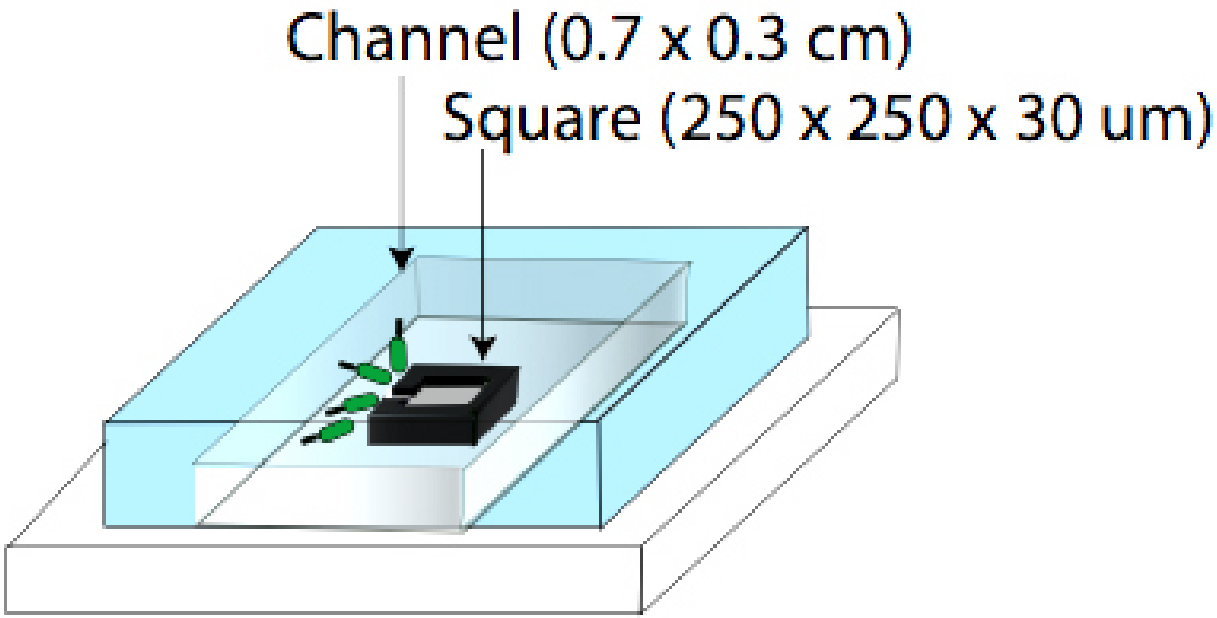
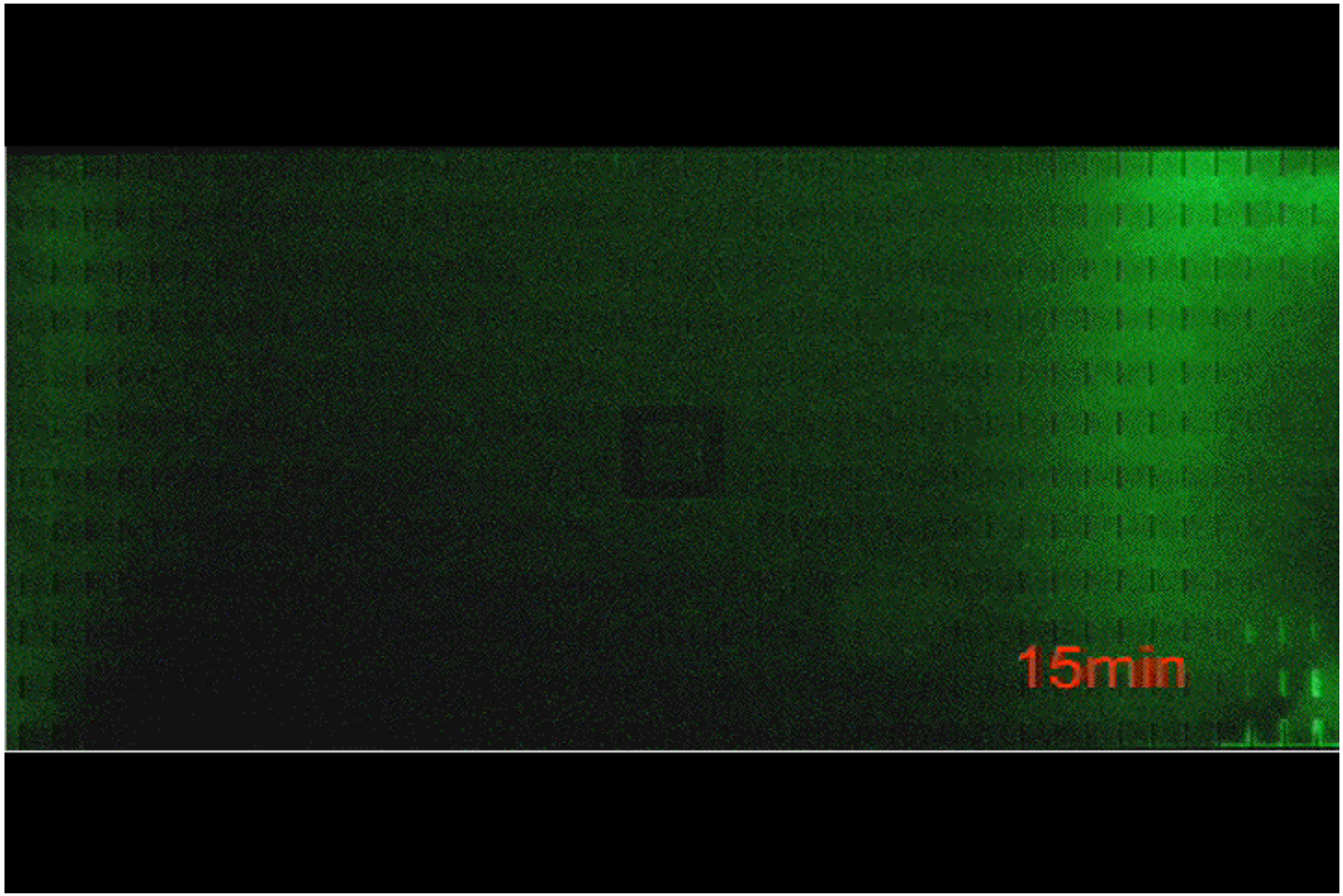


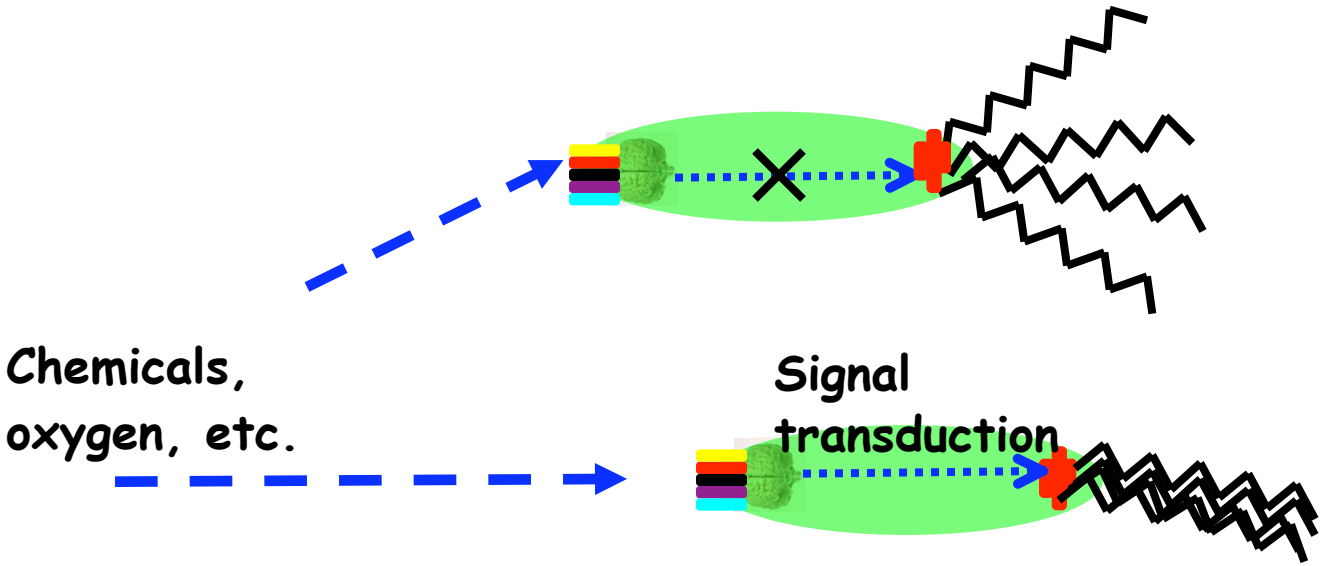
Fig. 3. PDMS square assembled with glass

Congregation of wild-type *E. coli* in M9 glycerol medium



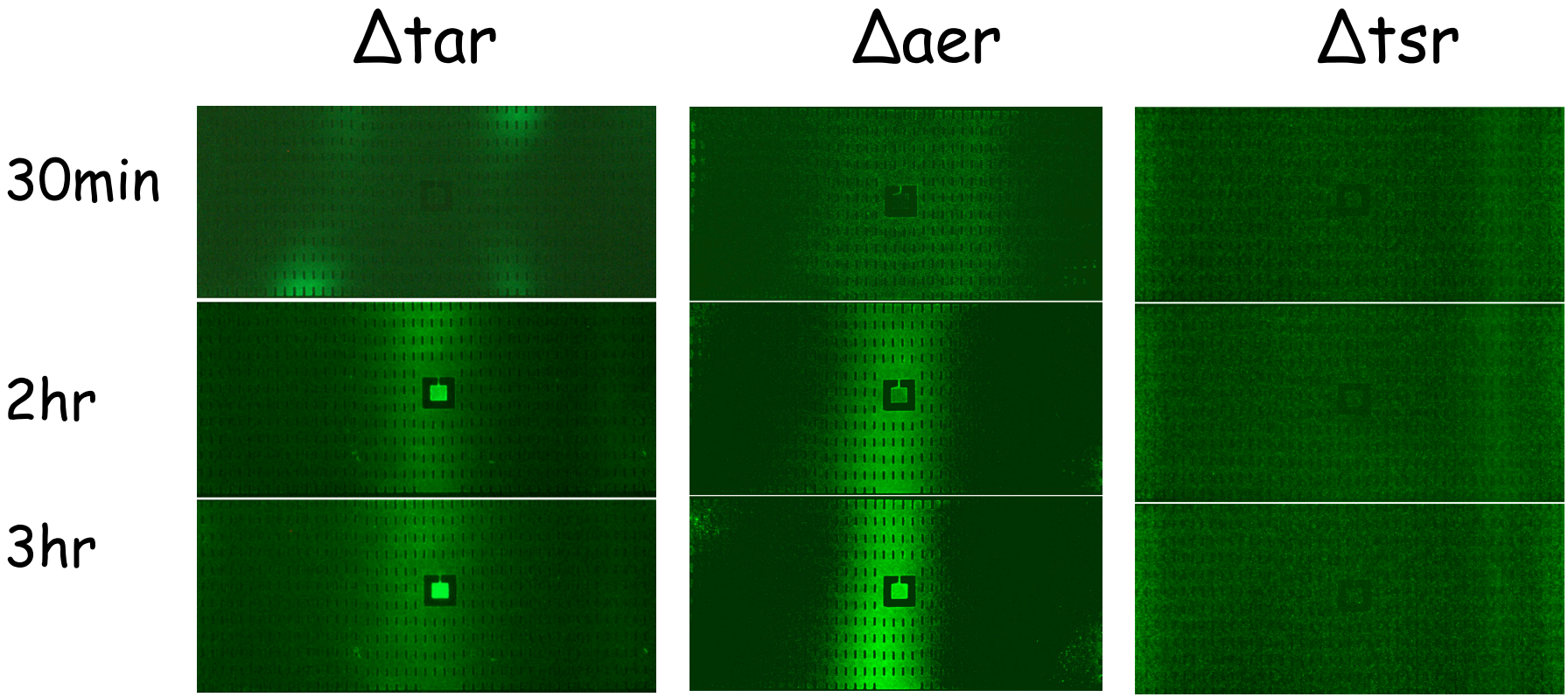
No attractant(s) in the media

Sensing systems affecting bacteria behaviors--Chemotaxis



: Tar (transducer for aspartate)
: Tsr (transducer for serine, glutamic acid, glycine, valine and alanine)
: Aer (aerotaxis receptor)
: Trg (transducer for ribose and galactose)
: Tap (transducer for peptide)

Congregation of chemotactic mutants into a square



M9 glycerol media

Profile of self-attractant concentration in cell-free conditioned M9 media

- **Threshold for the chemotactic response to amino acids**

  - Tar ligands

  L- aspartic acid 30nM

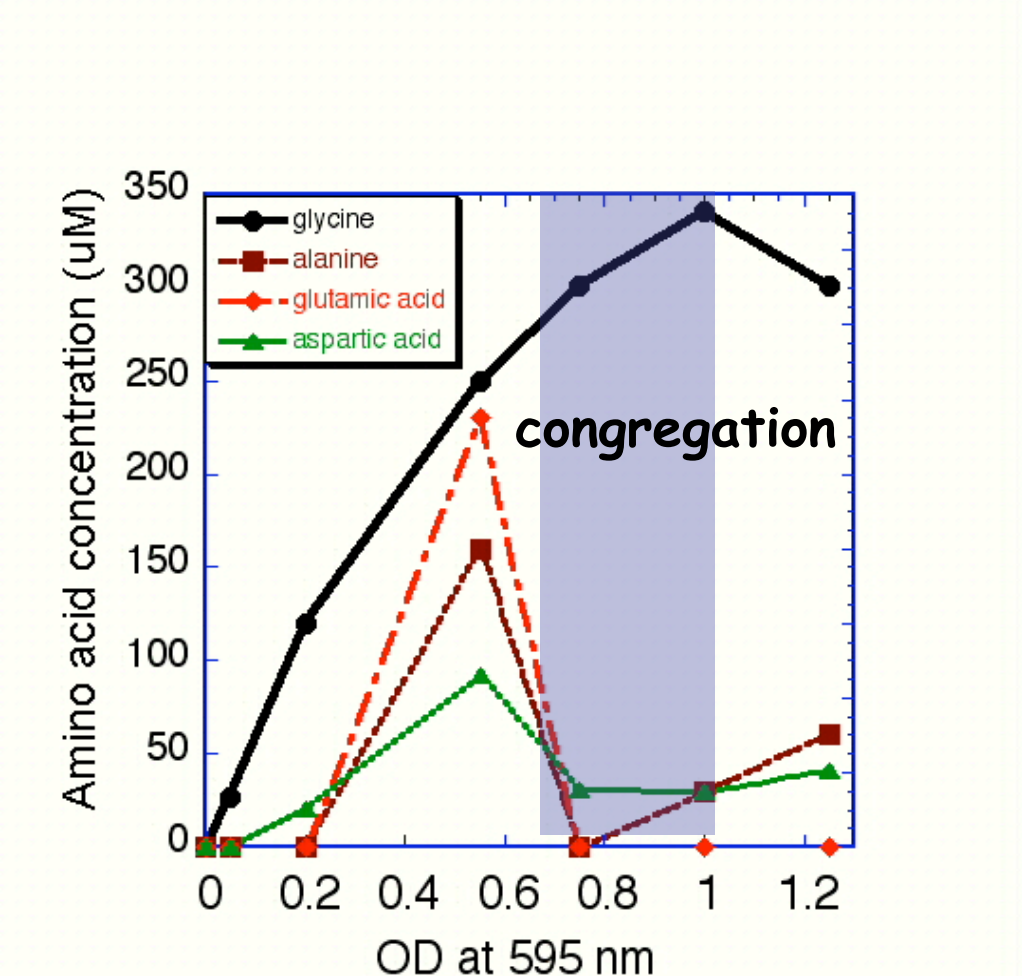
  L-glutamate 30μM

  - Tsr ligands

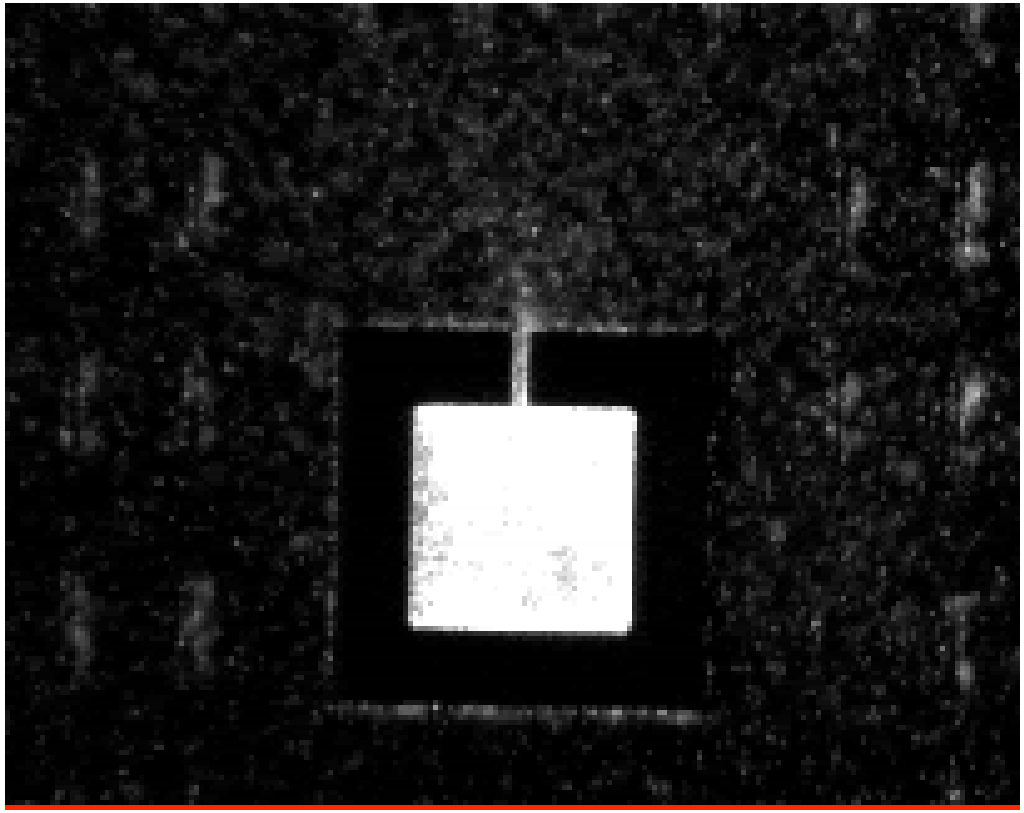
  L-serine100nM

  glycine 60μM

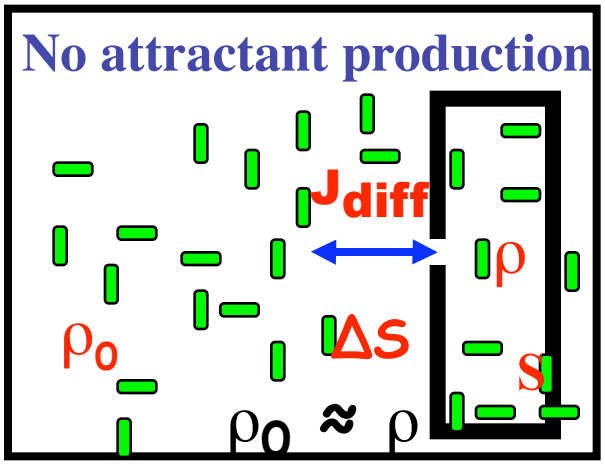
  L-alanine 50μM

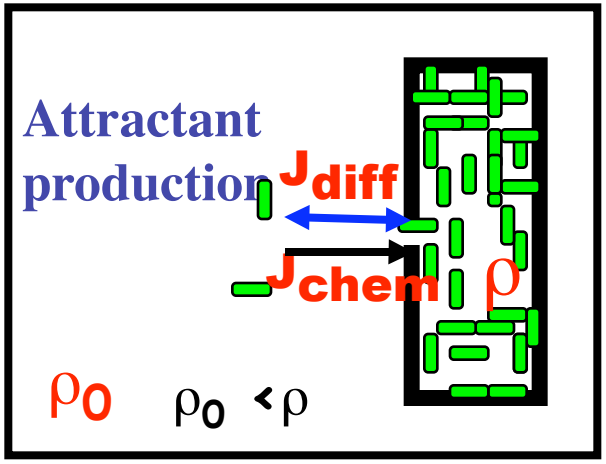


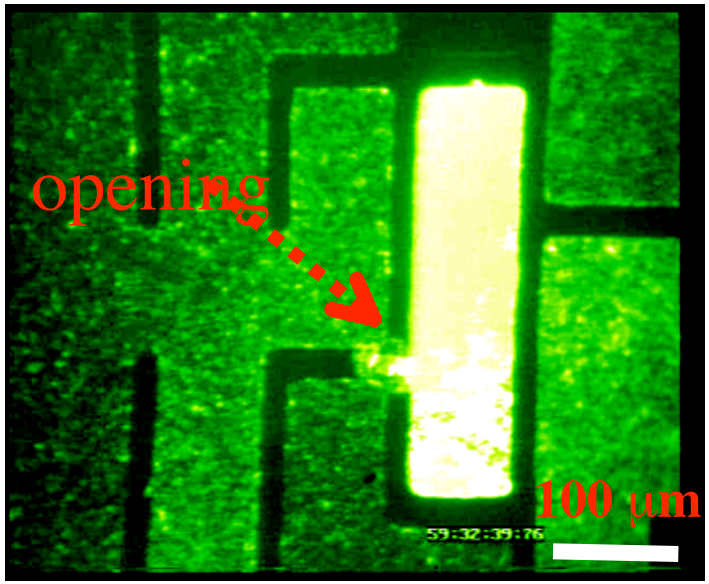
Cells moving out of the square when glycine is not produced!



Furtive congregation theory

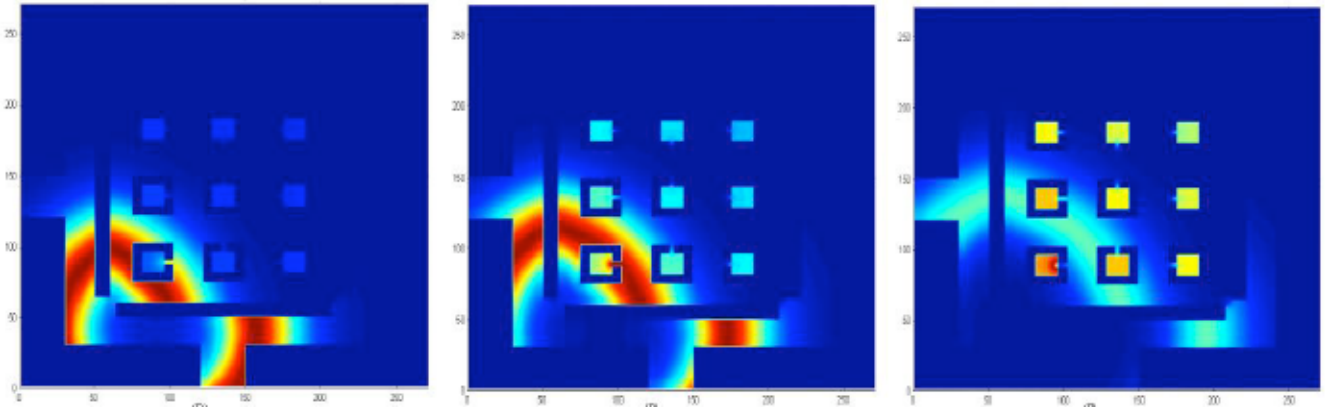


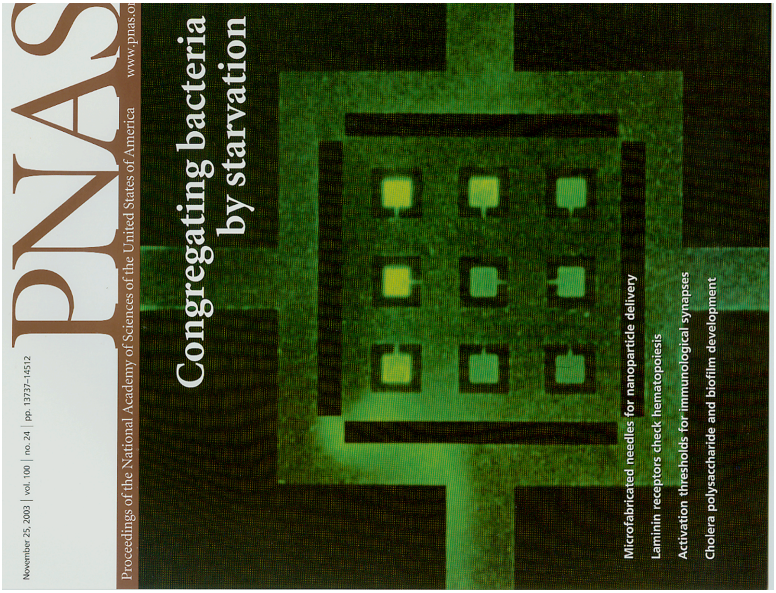




Park, S., et al. “Influence of Topology on Bacterial Social

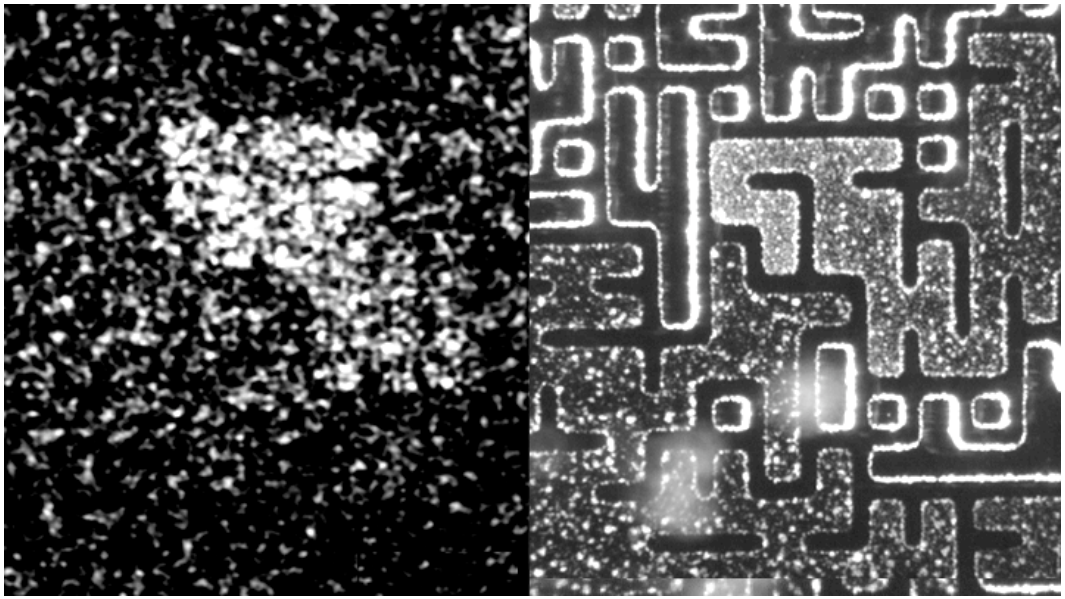
Simulation of congregation wild-type *E. coli* into multiple squares





Congregation-induced quorum-sensing of *Vibrio harveyi*

Photon-counting image **Dark-field image**



Quorum-sensing: cell-density dependent gene expression

Park, S. et al."Motion to form a quoru
Science (2003) 203, 188.

SUMMARY

부산진성 전투, 1592

- **With the appropriate confining geometries, bacteria can chemotactically form clusters under a broad range of metabolism and growth.**

- **This chemotactic behavior is mediated by Tsr (receptor) and glycine (attractant).**

- **Bacteria in congregation can have a higher chance to get access to nutrients released from their buddies and dead cells.**

Microfabricated structures can influence microorganism bigger than bacteria?

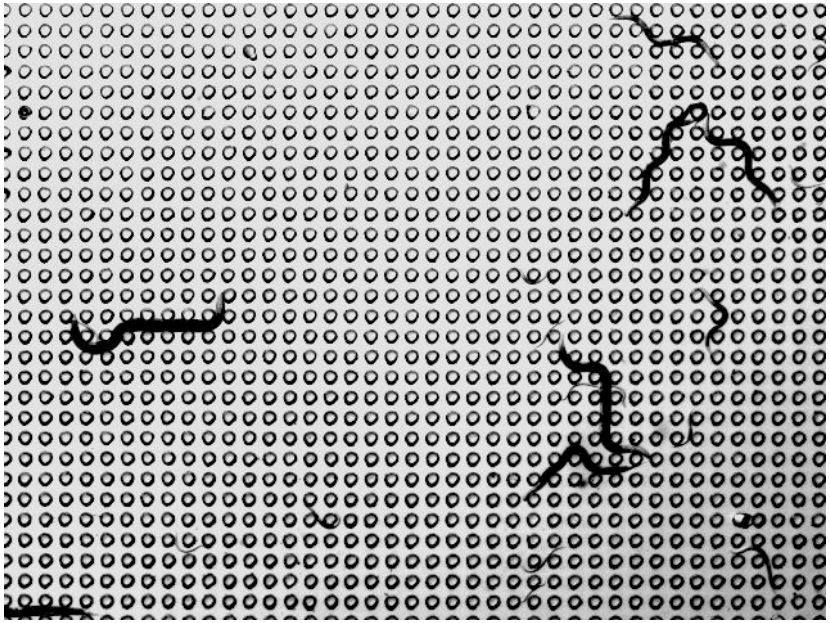
To answer this question, we have selected the Nematode, a predator to bacteria.

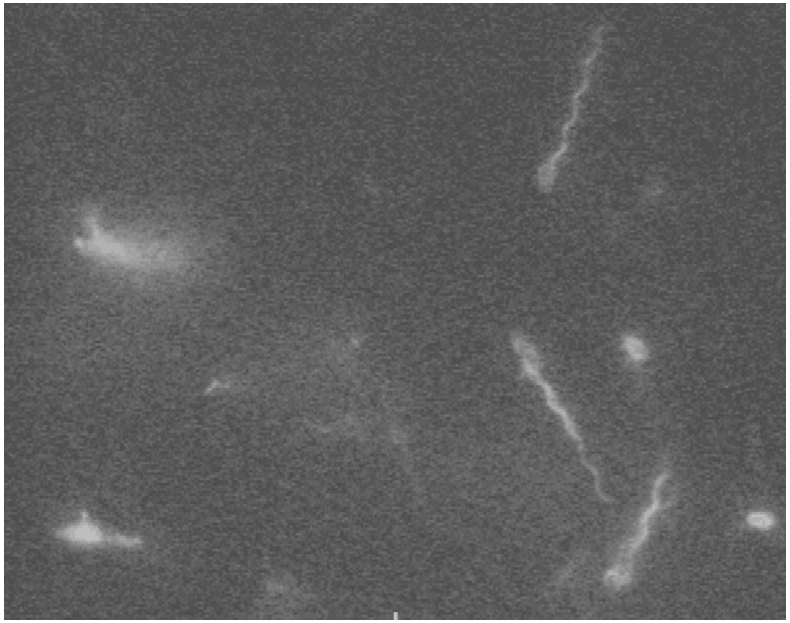
Biology of *Caenorhabditis elegans*

- Grow to about 1 mm in length composed of 959 somatic cells, and lives in the soil, where it survives by feeding on microbes such as bacteria. Capable of executing a variety of behaviors using their nervous system assembled by 302 neurons; thermotaxs, chemotaxis, odortaxis, mechano-sensation, locomotion, and egg laying.

Poor swimmer!

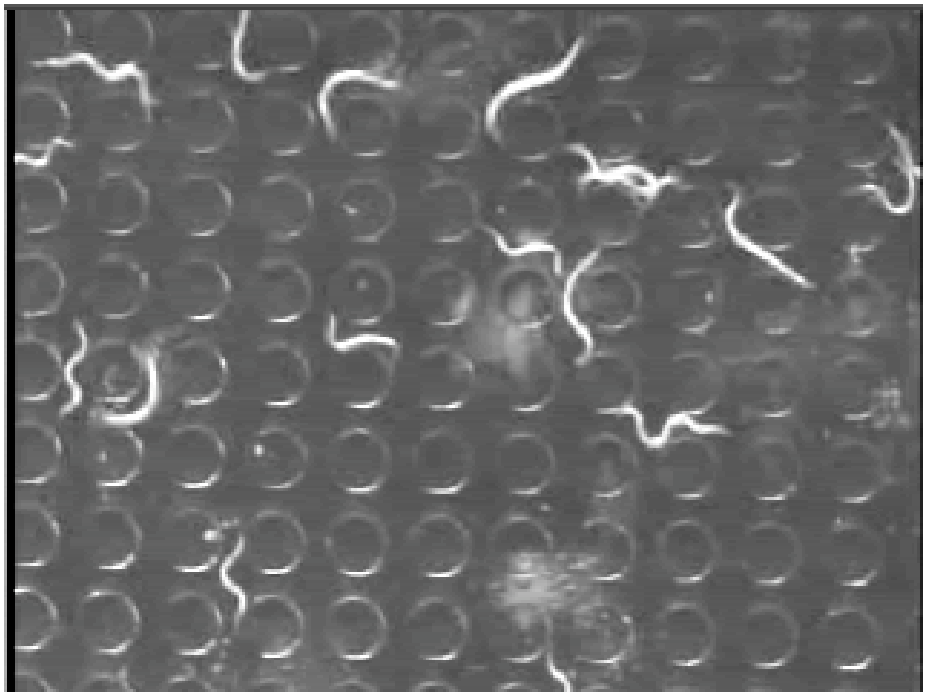
C. elegans in molecular dirt chip



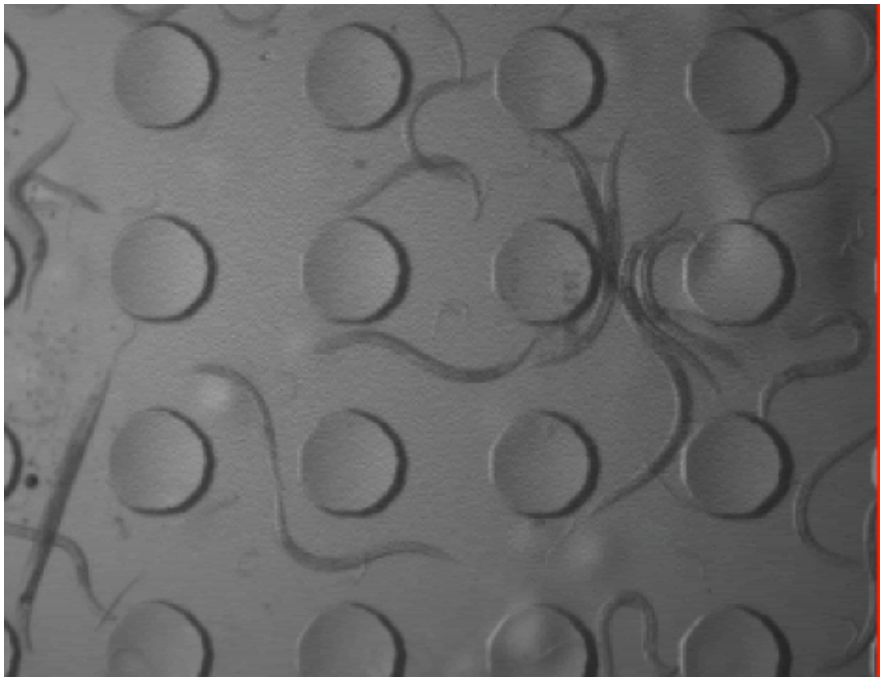


100um post with 100um spacing

선충에게 수영가르치기?

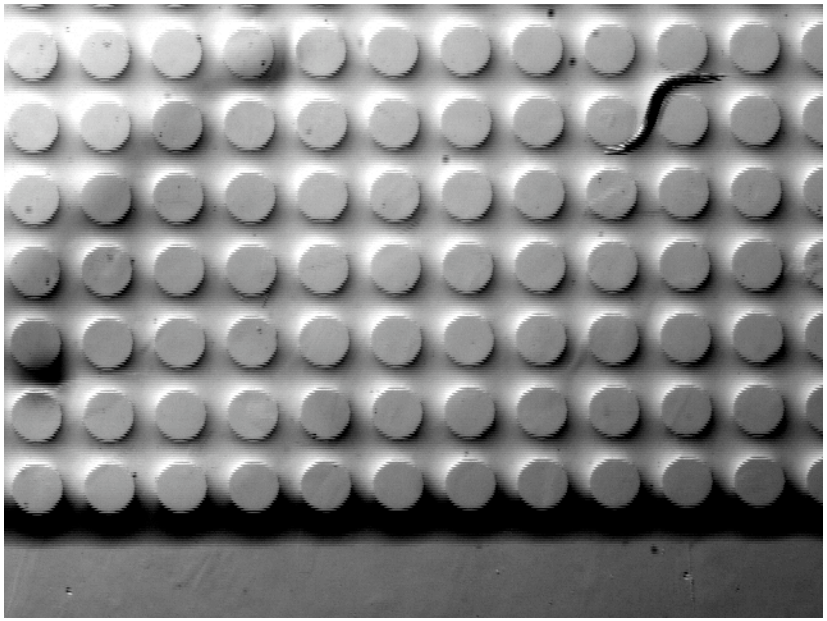


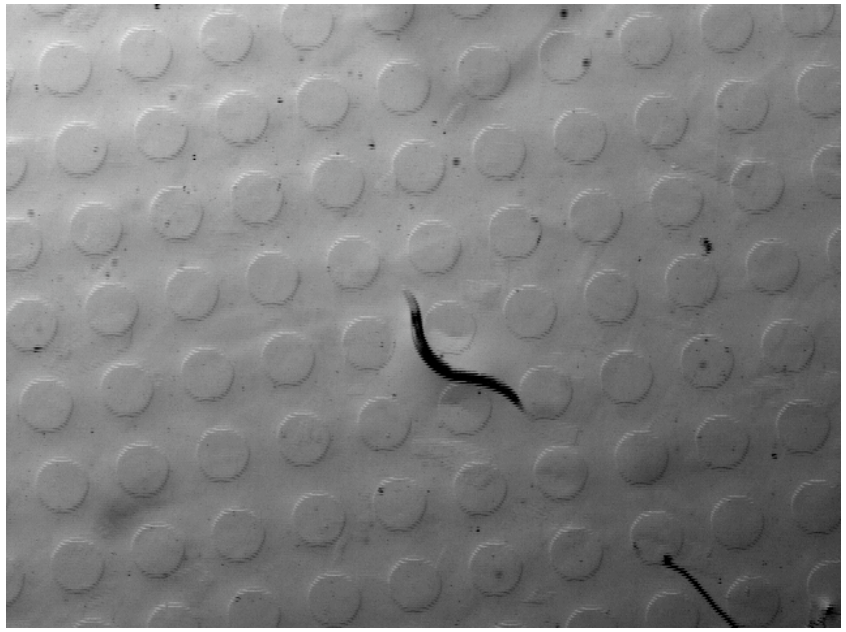
300um직경 기둥과 **150um** 기둥간 간격
선충길이:800um



300um직경 기둥과 **300um** 기둥간 간격
선충길이: not synchronized!!

왜 선충이 45각도로 수영하는 지?





3% Bacto-Agar로 제작된 분자흙알갱이칩
알갱이 직경**:300um,** 알갱이간 공극**: 120-150um**

Conclusions

- C. elegans shows an enhanced motility by pushing themselves against constricted structures.
- When their contour length during swimming matches the size of constricted structures, they swim at 45 degree.
- Worms can be sorted out based on their size using molecular dirt arrays with various space.

Stretched Genomic DNA in Nanochannels

Colloraboration with Stephen Chou group at Nanostructure Laboratory

Princeton

Ultimate goal of this research

Find rare cells

↓

Lyse single cells

↓

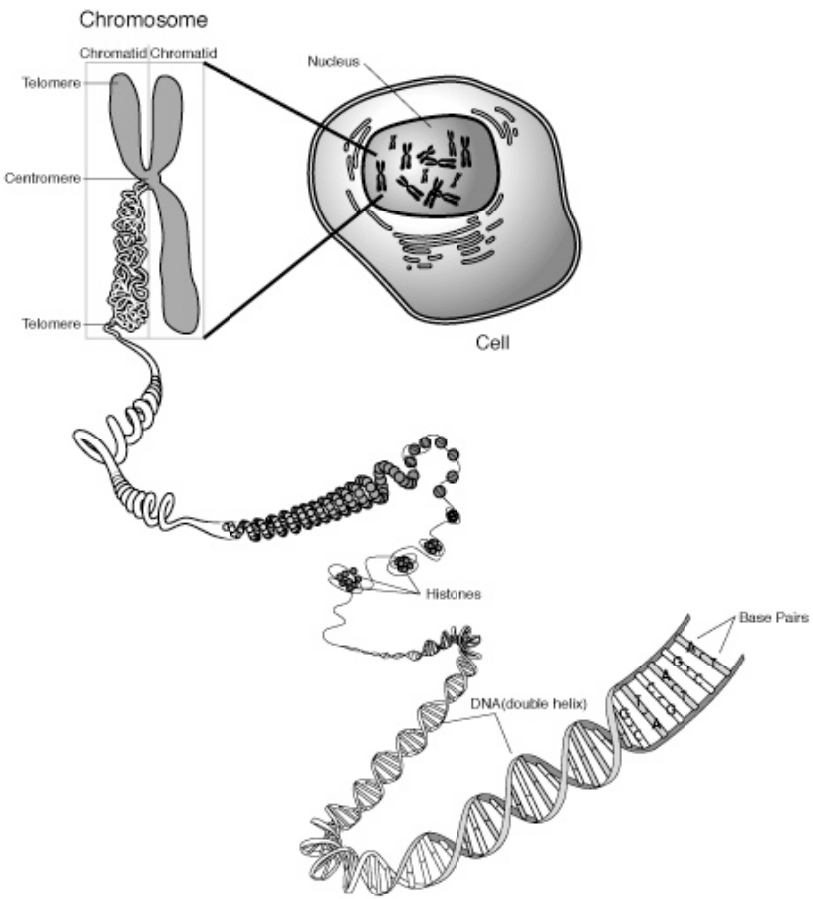
Extract DNA

↓

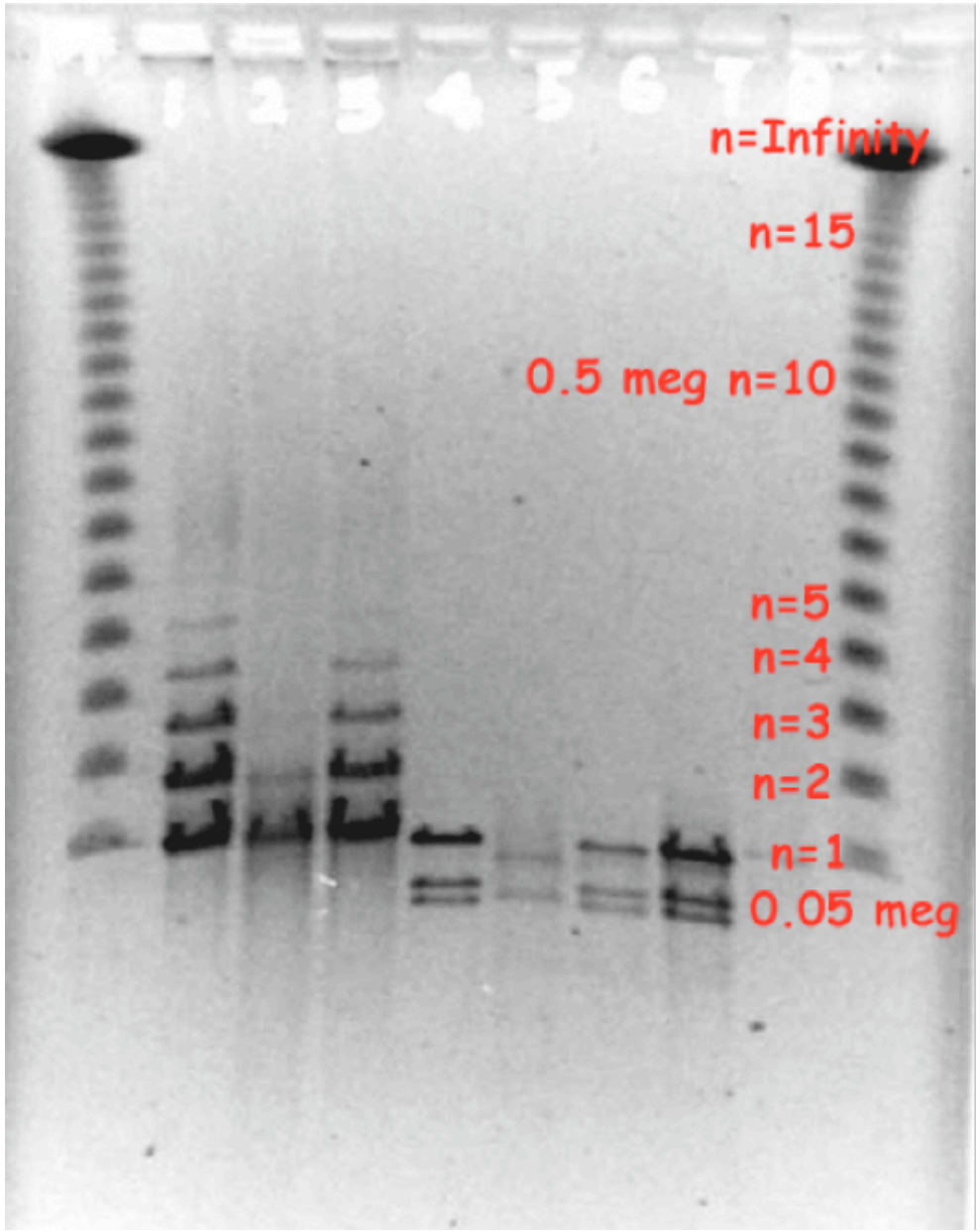
Fractionate and Purify the DNA

↓

Linear analysis of DNA



Lambda DNA on a gel after 12hrs electrophoresis



N=15, 750kbp

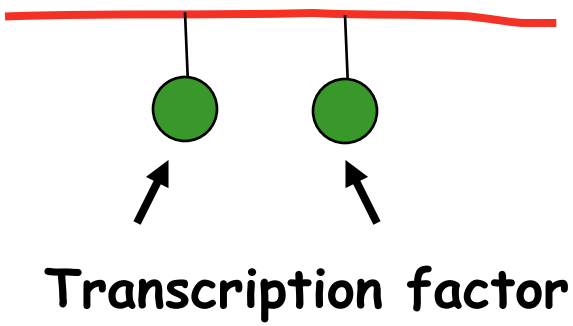
Resolution decreases roughly as 1/L

N=1, 50kbp

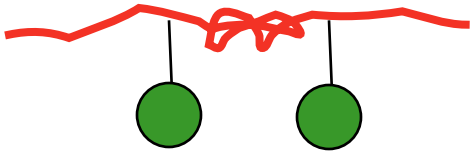
Our approach using Nanochannel array chip

1) Stretch the genomic DNA in the nanochannel
2) Measure the length of the DNA
3) scan the occupied site of the transcription factor.

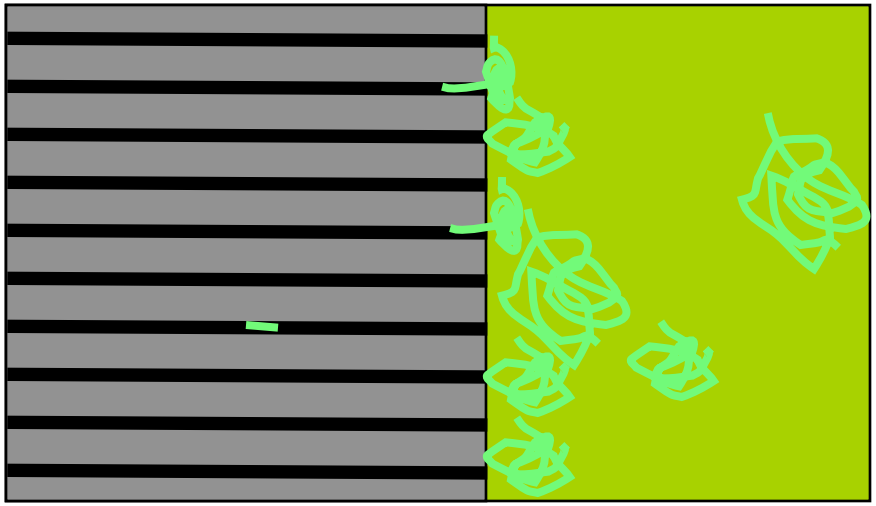
A) Linearized

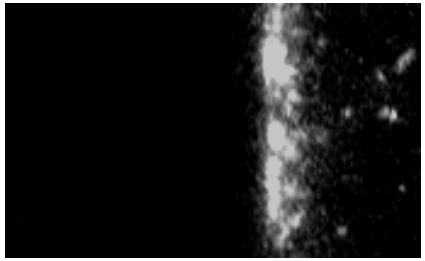


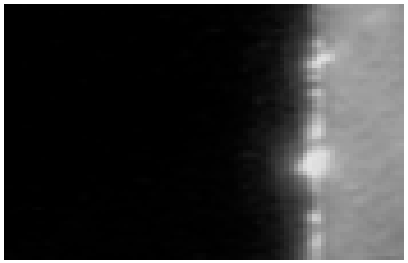
B) Non-linearized

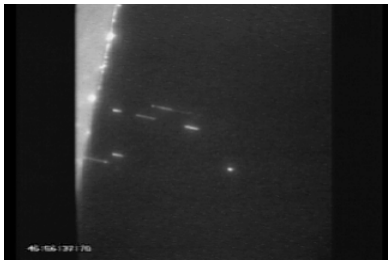


Long DNA molecules stall at edge of the Nanochannel

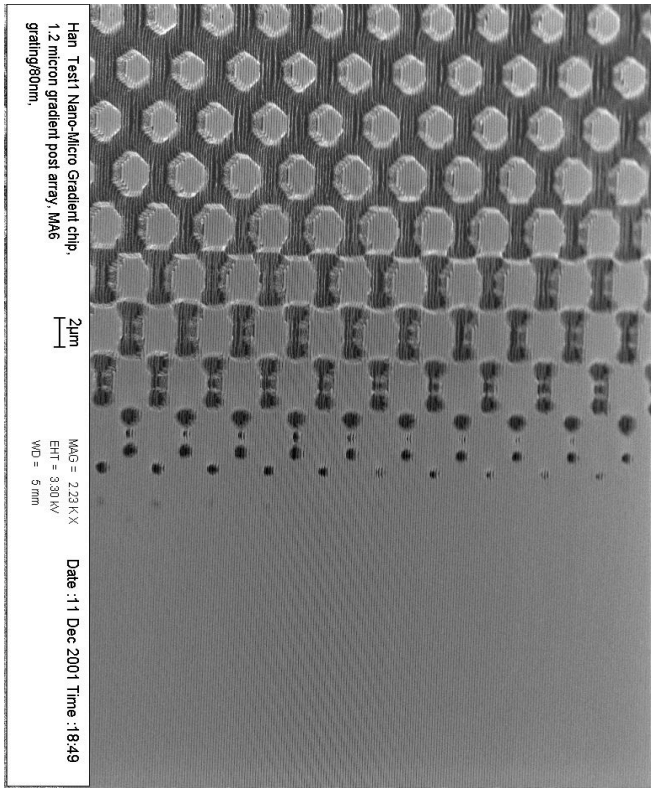


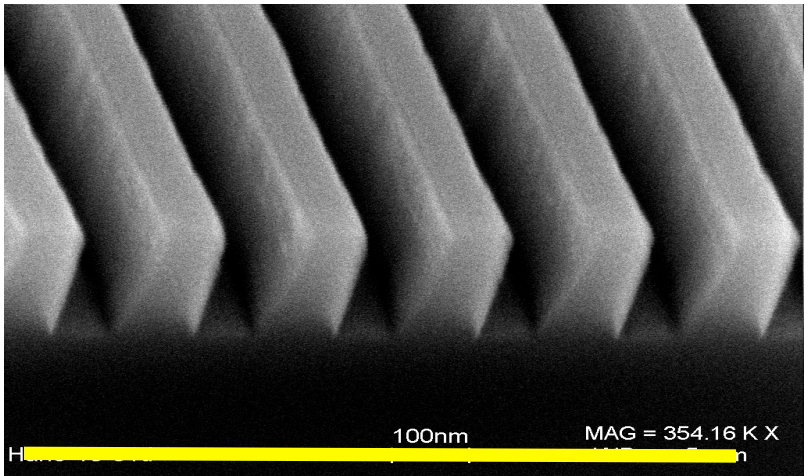




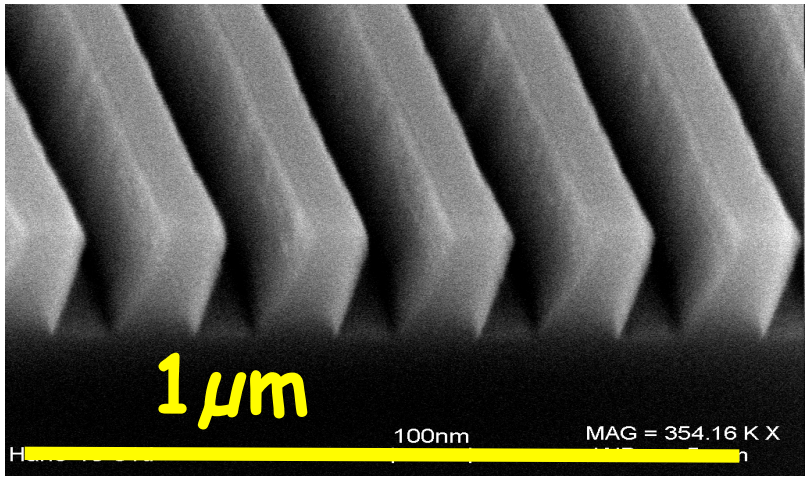


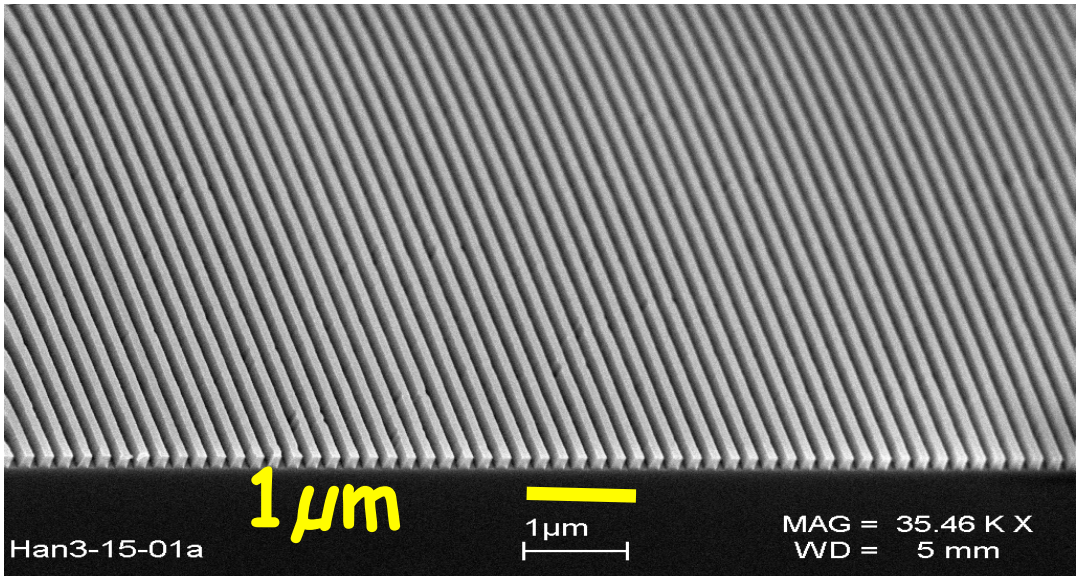
High Throughput Gradient Nanofluidic Chip

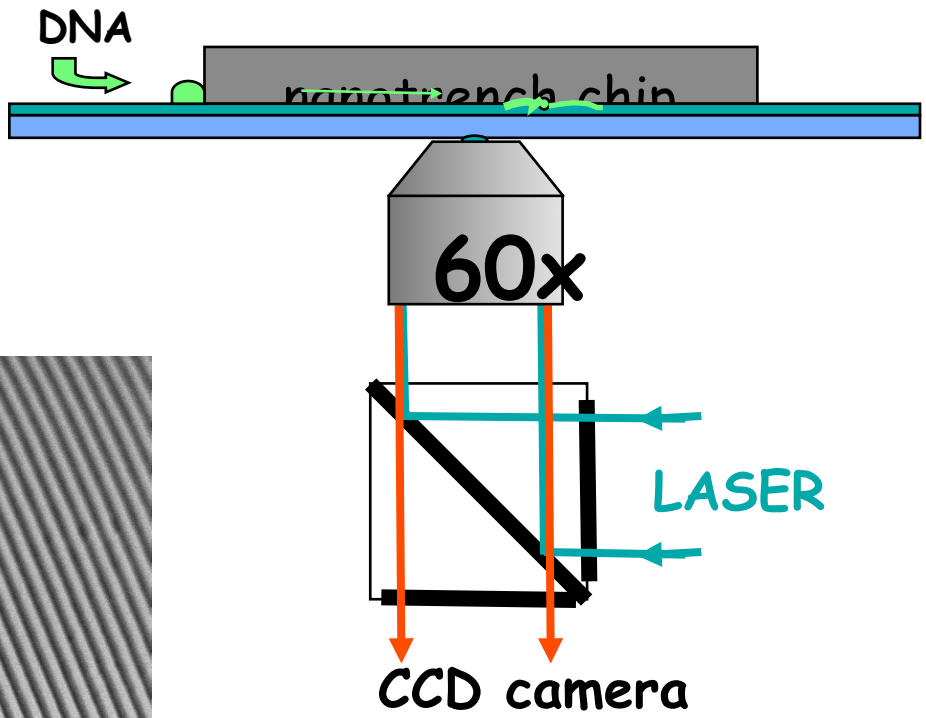




100nm channels are made using nanoimprinting lithography

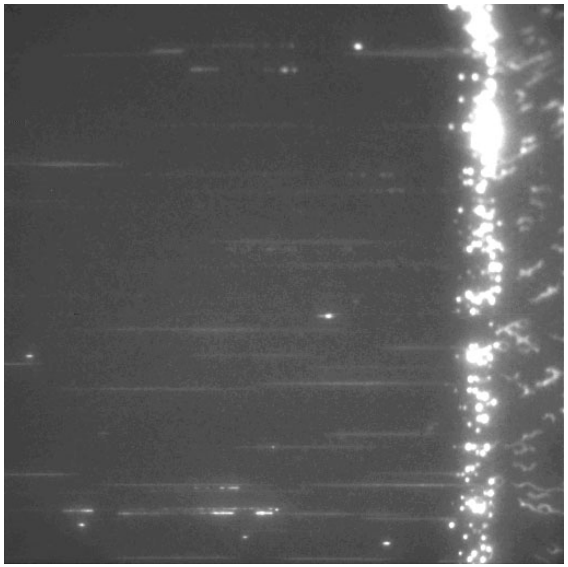




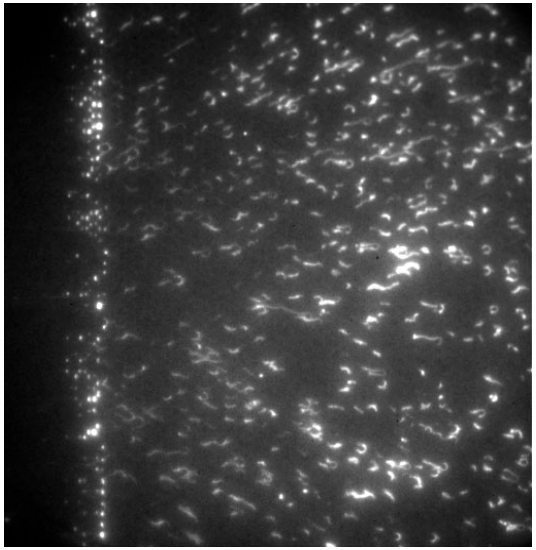


Han, C. et al. (2002) APL

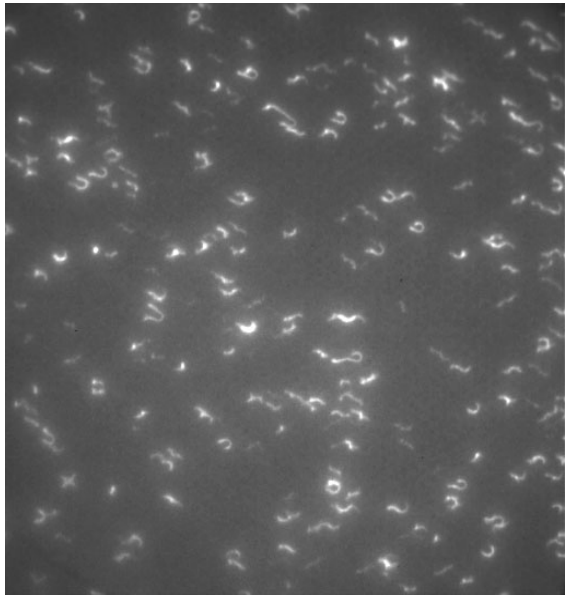
Genomic DNA Analysis On Micro/Nano-Gradient Chip



Entering nanochannels



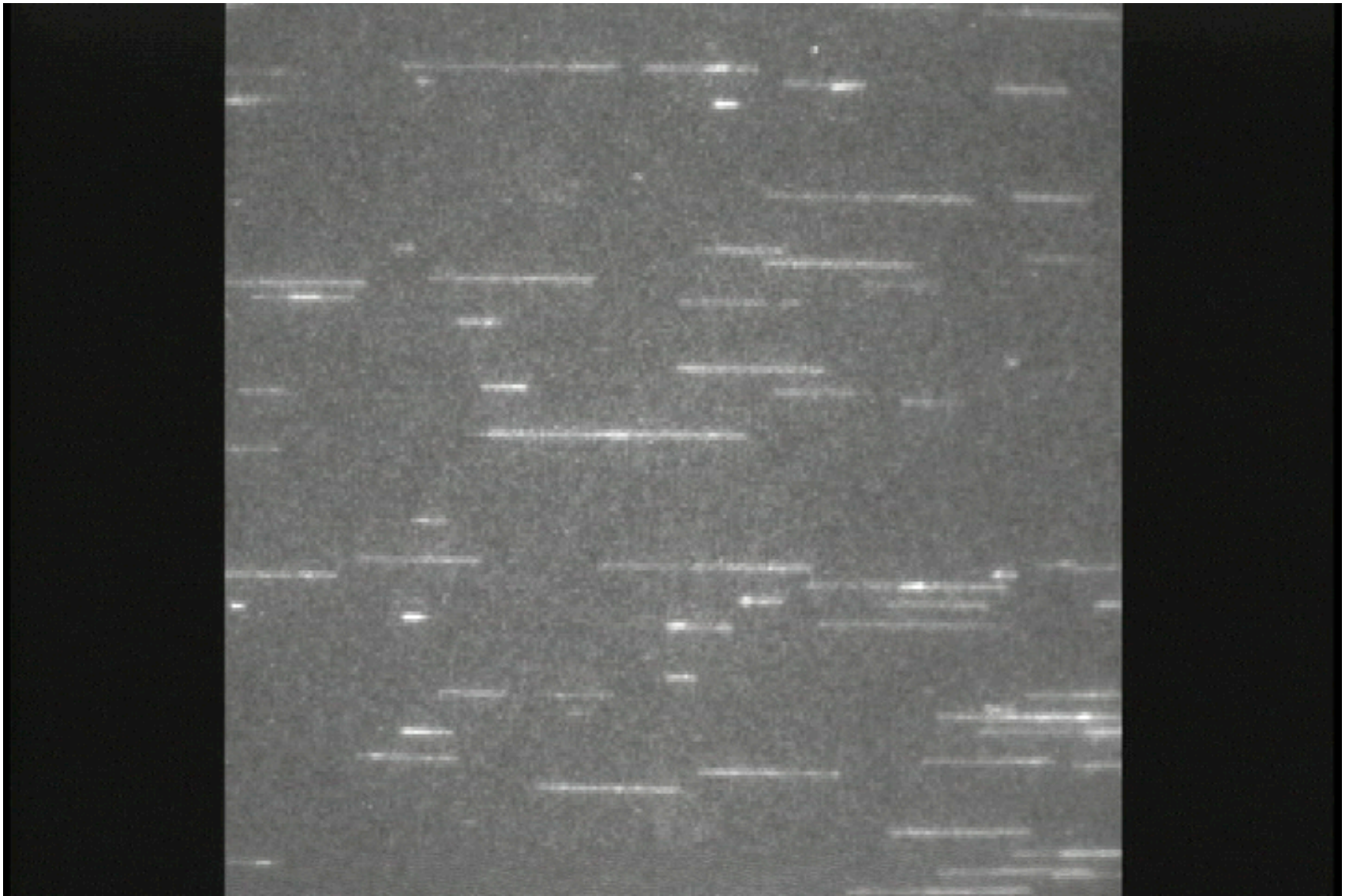
Approaching nanochannels



Prestretched in post array

Lambda phage DNA labeled with TOTO1 in 100 x 200nm channels

ToTo-stained Lamda DNA ladder in nanochannel array chip



Acknowledgement

- Biomatrix 연구실 소개
  - 포스닥: 이내윤(랩온어칩), 김완석(미생물학)
  - 대학원생: 안은영(선충), 김정아(나노입자), 양윤선(랩온어칩)
- 재정지원
  - 학술진흥재단 중점연구소 나노바이오기술연구소,
  - 과학재단 우수연구센터 지능형 나노바이오 소재 연구센터
  - 과학재단 특정연구개발사업 바이오전자프로세서 개발

Thank you for your attention