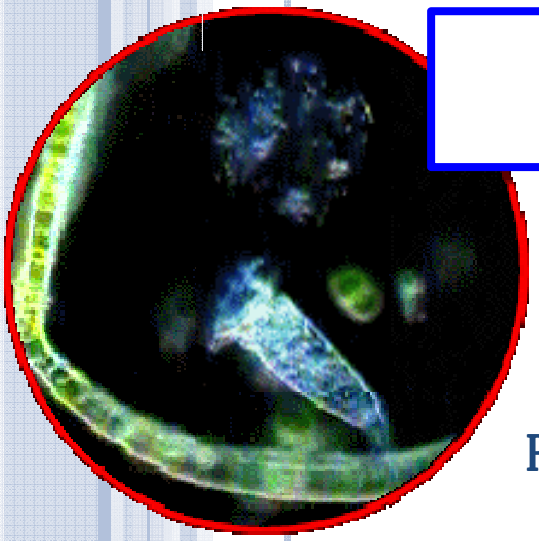


Cells

PART I: The basic ideas about cells

PART II: The applications of nanotechnology about cells

PART III: Basic cell culture

Chia-Fen Hsieh

Dr. Chia-Fu Chou's Lab, NanoBioScience Lab

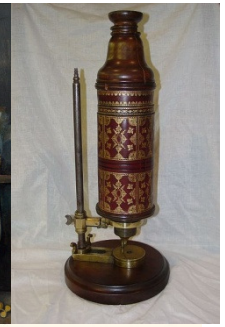
Institute of Physics, Academia Sinica

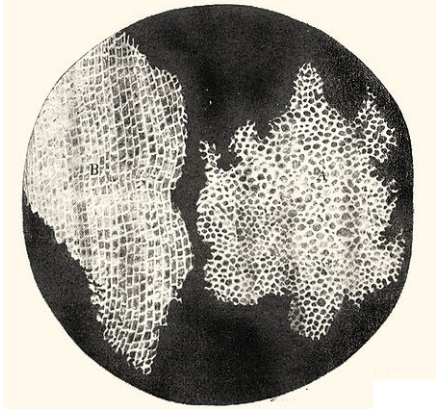
Cell (Biology)

- It comes from the Latin word, *cellula* (a small room)

- It was coined by Robert Hooke

  . . . I could exceedingly plainly perceive it to be all perforated and porous, much like a Honey-comb, but that the pores of it were not regular. . . these pores, or cells, . . . were indeed the first microscopical pores I ever saw
  (Micrographia, 1665)





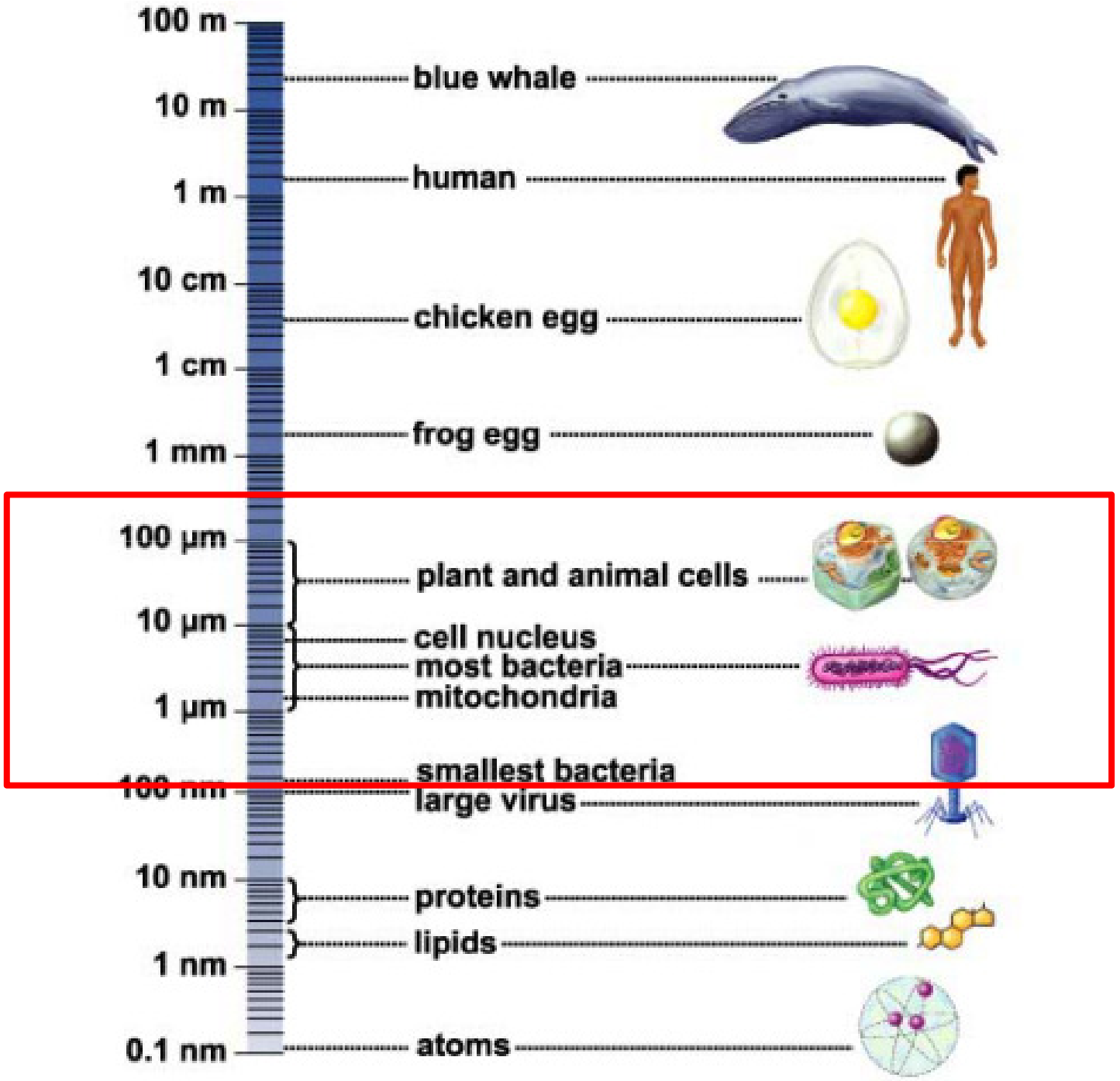
Cork

- It is the fundamental unit of life

What is the size of a cell?

plant and animal cells .....  ? ?

cell nucleus
most bacteria .....  ? ?
mitochondria

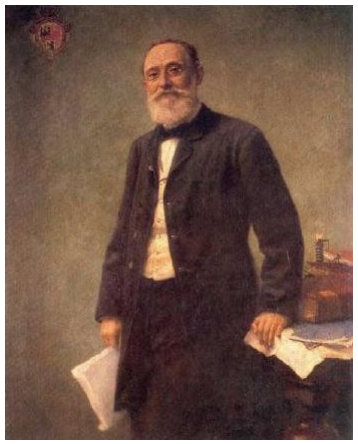

100 m
blue whale
10 m
1 m
human
10 cm
chicken egg
1 cm
1 mm
frog egg
100 µm
plant and animal cells
10 µm
cell nucleus
most bacteria
1 µm
mitochondria
smallest bacteria
large virus
10 nm
proteins
lipids
1 nm
0.1 nm
atoms

Cell theory (1839-1858)

- Theodor Schwann, Matthias Jakob Schleiden, Rudolf Virchow

1810-1882

1804-1881

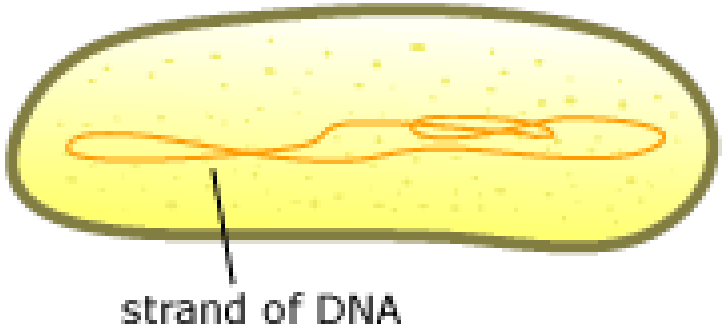


1821-1902

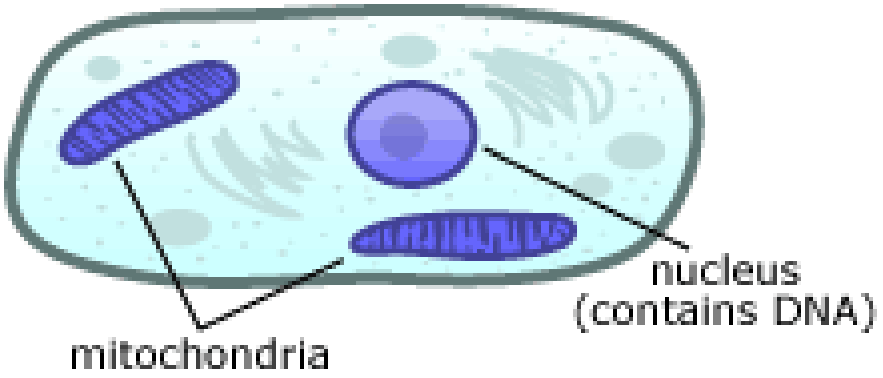
- Modern interpretation of cell theory:
  - All cells come from pre-existing cells by division
  - Energy flow (metabolism and biochemistry) occurs within cells
  - Cells contain hereditary information (DNA) which is passed from cell to cell during cell division

Prokaryotes vs. Eukaryotes

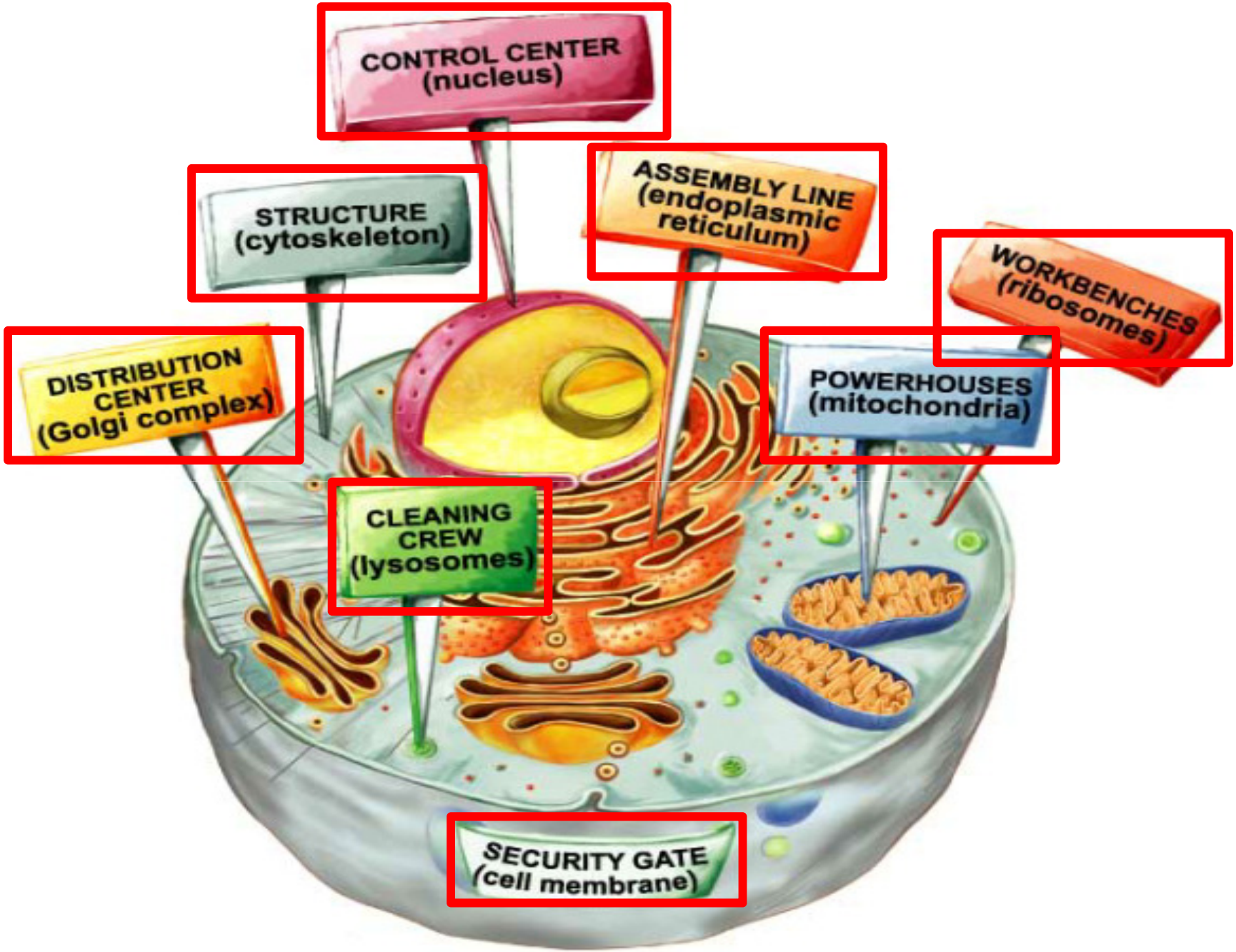
Typical prokaryote cell

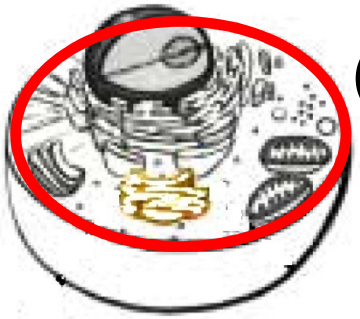


Typical eukaryote cell

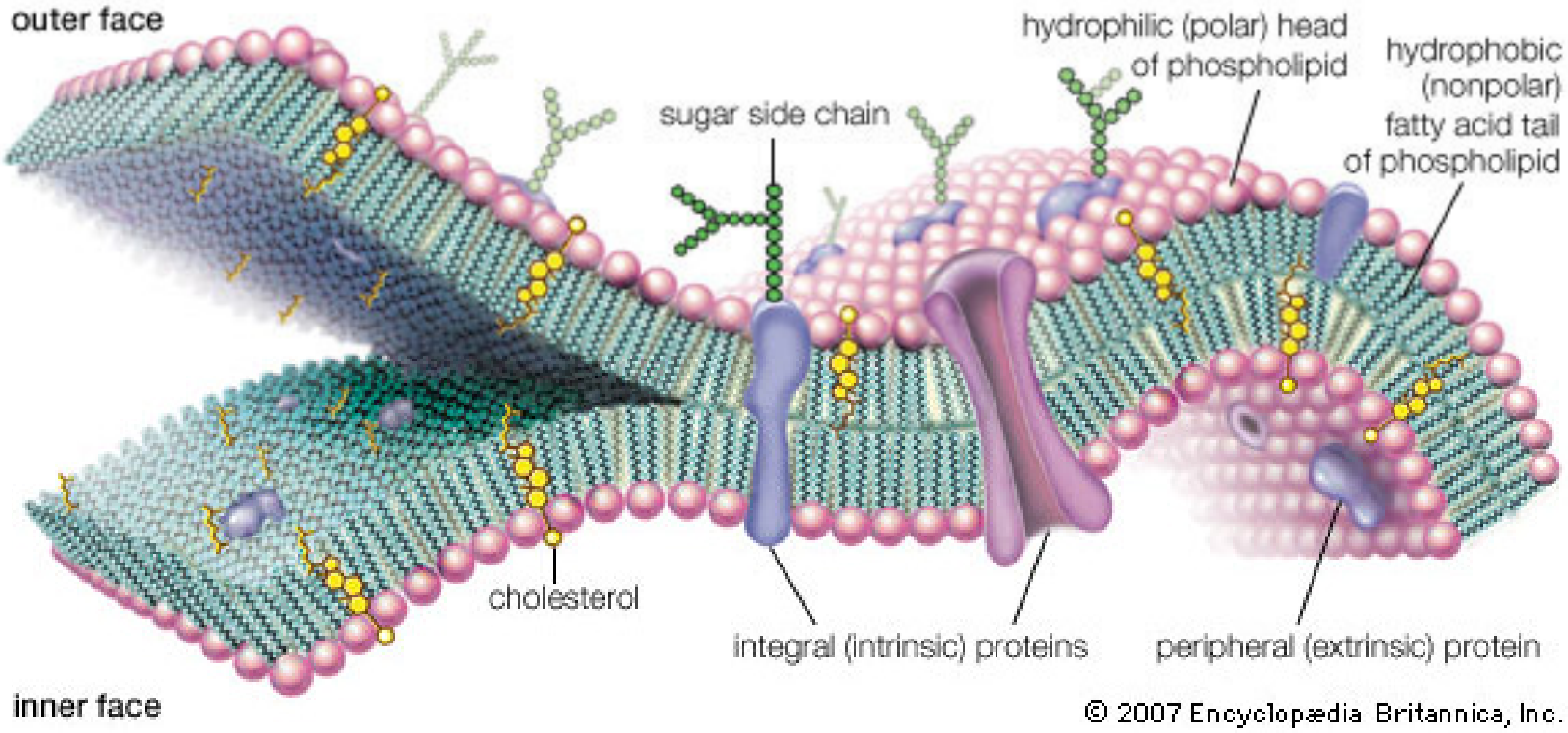


	Prokaryote	Eukaryote
Greek derivation	"before the nucleus"	"true nucleus"
They usually are…	Single-celled organisms	Single-celled organisms or Multi-celled organisms
Including…	Bacteria and Archaea	Yeasts, animals, plants
Size	1-3 μm	10-100 μm

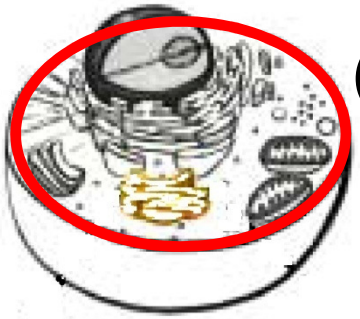

CONTROL CENTER (nucleus)
STRUCTURE (cytoskeleton)
ASSEMBLY LINE (endoplasmic reticulum)
WORKBENCHES (ribosomes)
DISTRIBUTION CENTER (Golgi complex)
POWERHOUSES (mitochondria)
CLEANING CREW (lysosomes)
SECURITY GATE (cell membrane)



Cell membrane (Security Gate)



- Lipid bilayer, protein channel (security guy) , elastic

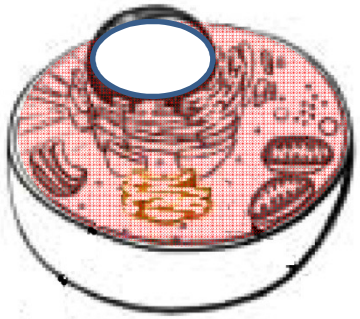


Cell membrane (Security Gate)

TEM observation

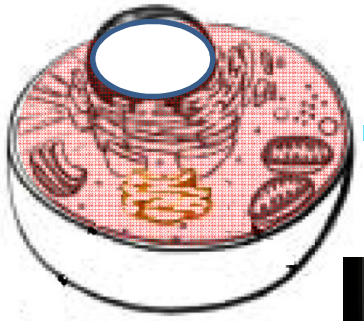
- Protect the cell
- Regulate molecular entry
- Selectively permeable

- Very flexible
- Self-assembly
- Lateral diffusion

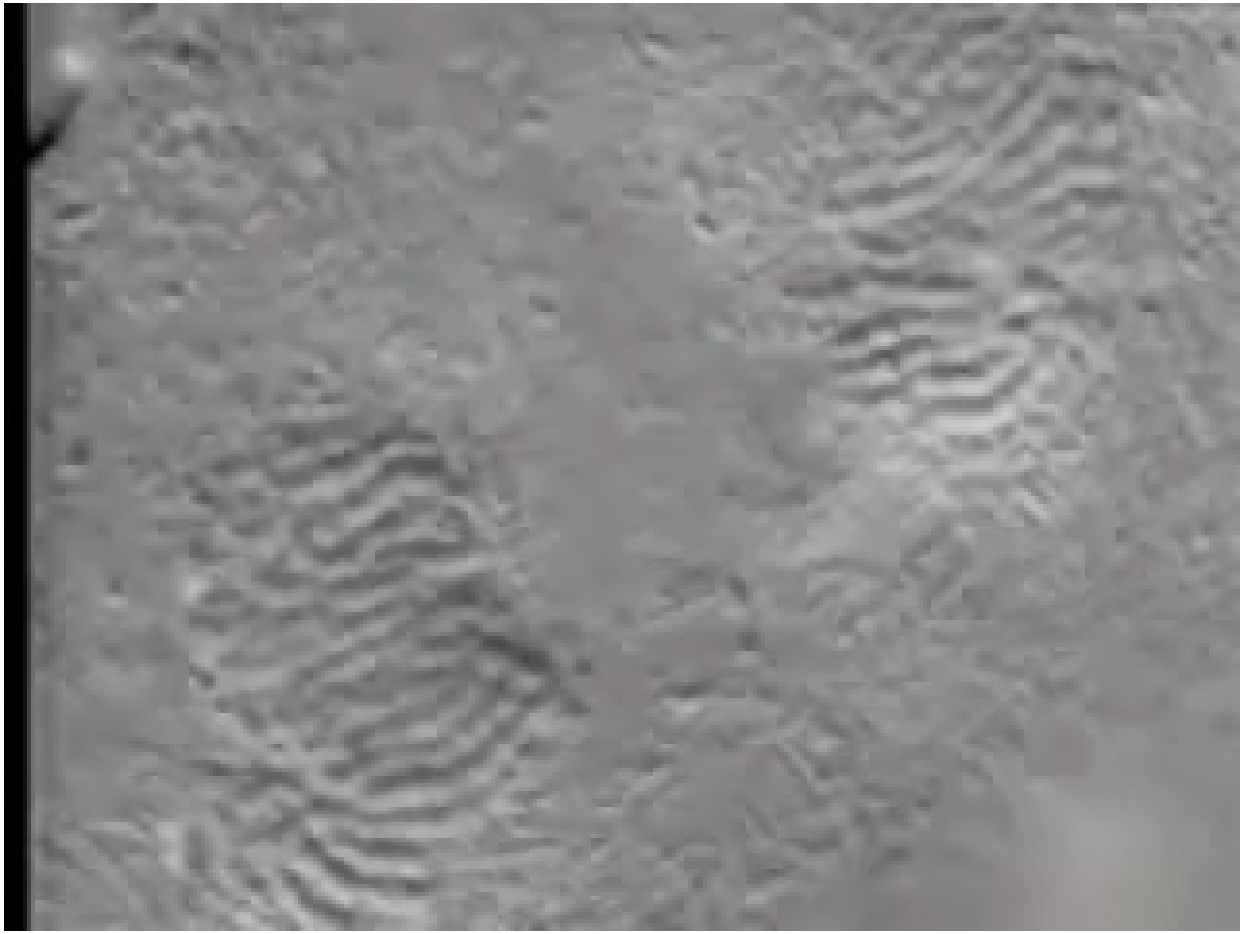


(Cytoplasm vs. Cytosol)

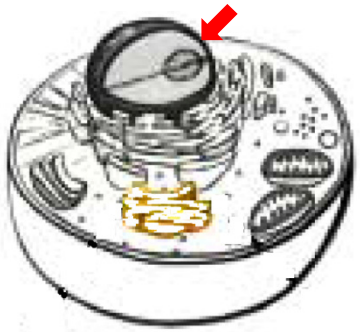
- Cytoplasm is the part of cell that is enclosed within the plasma membrane (except nucleus)
- An organelle is a specialized subunit within a cell that has a specific function, and is usually separately enclosed within its own lipid bilayer
- Cytosol is the part of cytoplasm that is not held within organelles
  - A complex mixture of cytoskeleton filaments, dissolved molecules, and water that fills much of the volume of a cell



Cytoplasm



- Including water, proteins, lipids, and carbohydrates
- Transmit cell signal from one side to another side



Nucleus (Control center)

Double-layered

Nucleolus

rRNA → ribosomes

Nuclear Envelope

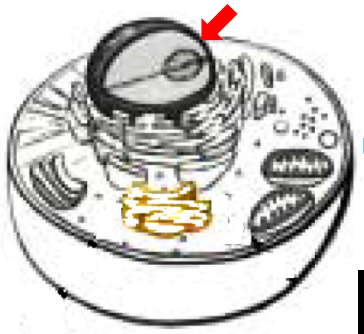
Nuclear Pores

DNA

Chromosomes

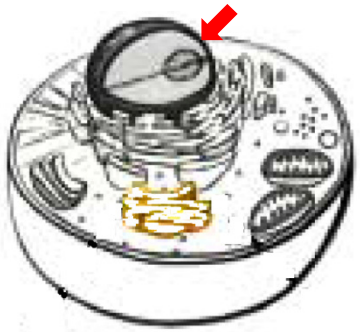
DNA + Proteins

Chromatin



Nucleus (Control center)

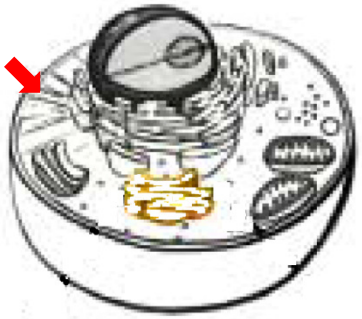
- DNA → RNA → Protein
- Chromosomes are duplicated and separated into two cells



Nucleus (Control center)

- Nucleus is the ultimate control center for cell activities (DNA → RNA → Protein)

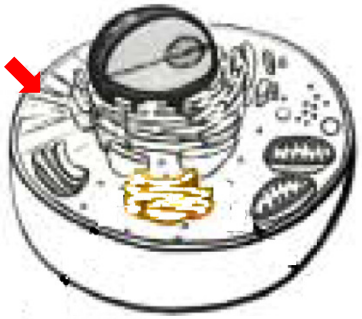
- A second major function of the nucleus involves duplication of the chromatin as a part of cell reproduction
  - When a cell is about to divide, the loosely organized strands of chromatin become tightly coiled, and the resulting chromosomes can be seen under a microscope



Cytoskeleton (Structure)

- A cellular “scaffolding” or “skeleton” contained within the cytoplasm

- It is a dynamic structure that maintains cell shape, protects the cell, enables cellular motion, and plays important roles in both intracellular transport and cellular division

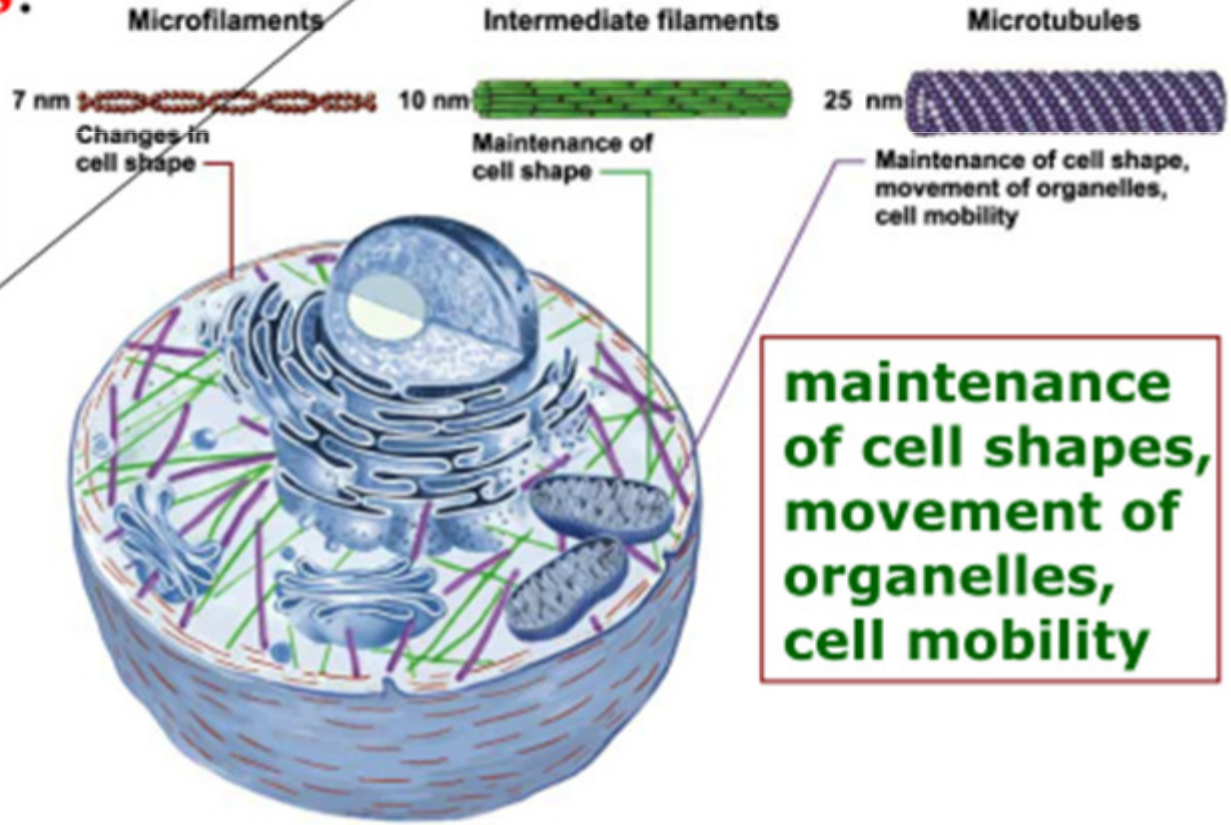


Cytoskeleton (Structure)

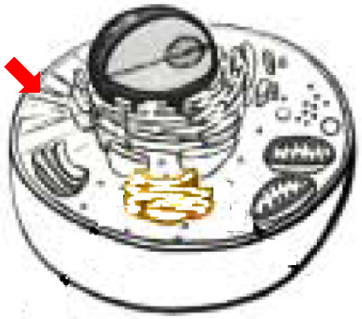
Three main kinds of cytoskeletal filaments: **microfilaments**, **intermediate filaments** and **microtubules**.

changes in cell shapes

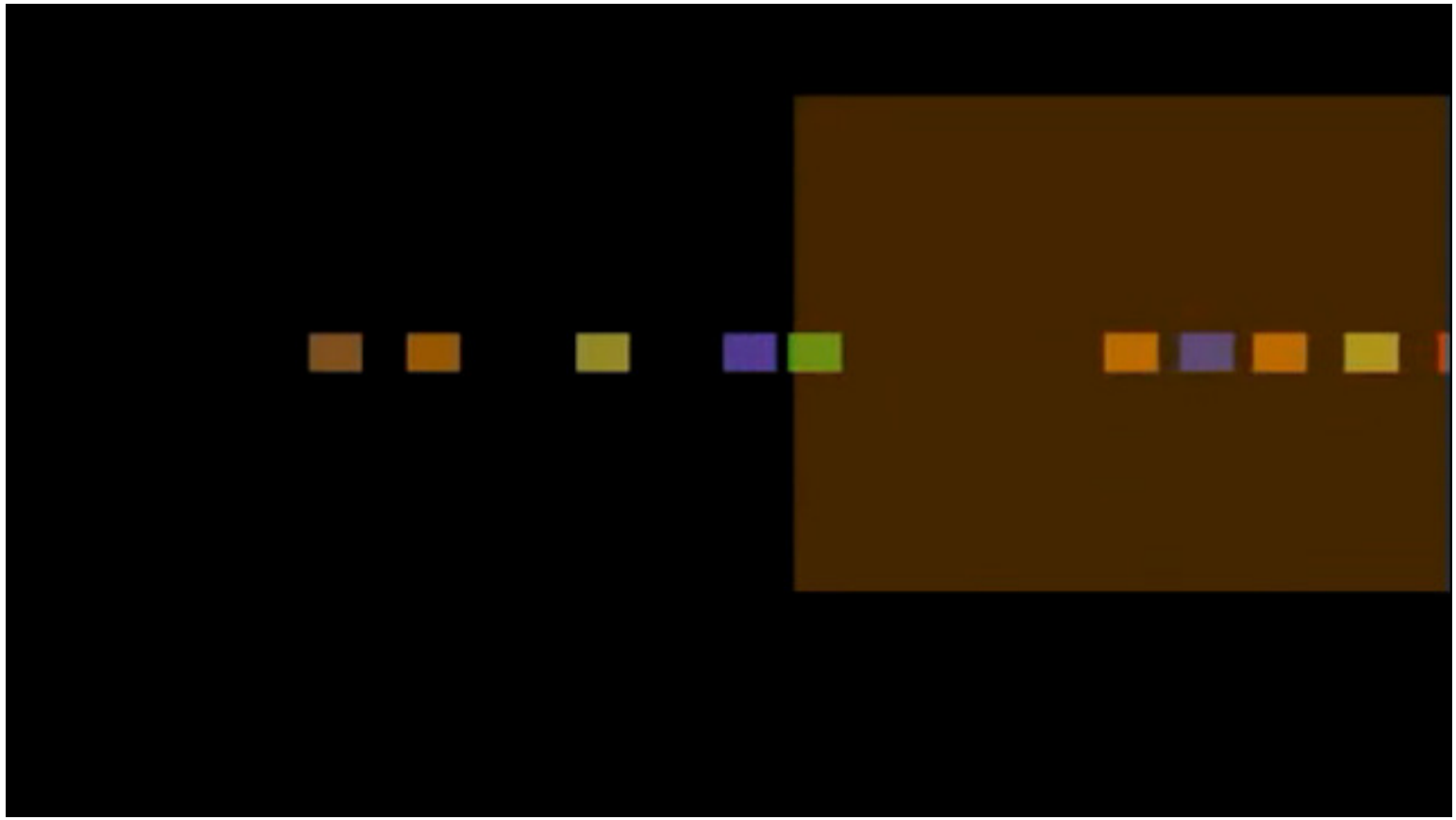
maintenance of cell shapes



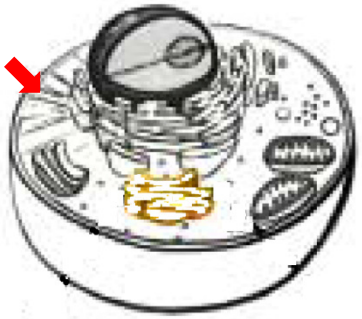
maintenance of cell shapes, movement of organelles, cell mobility



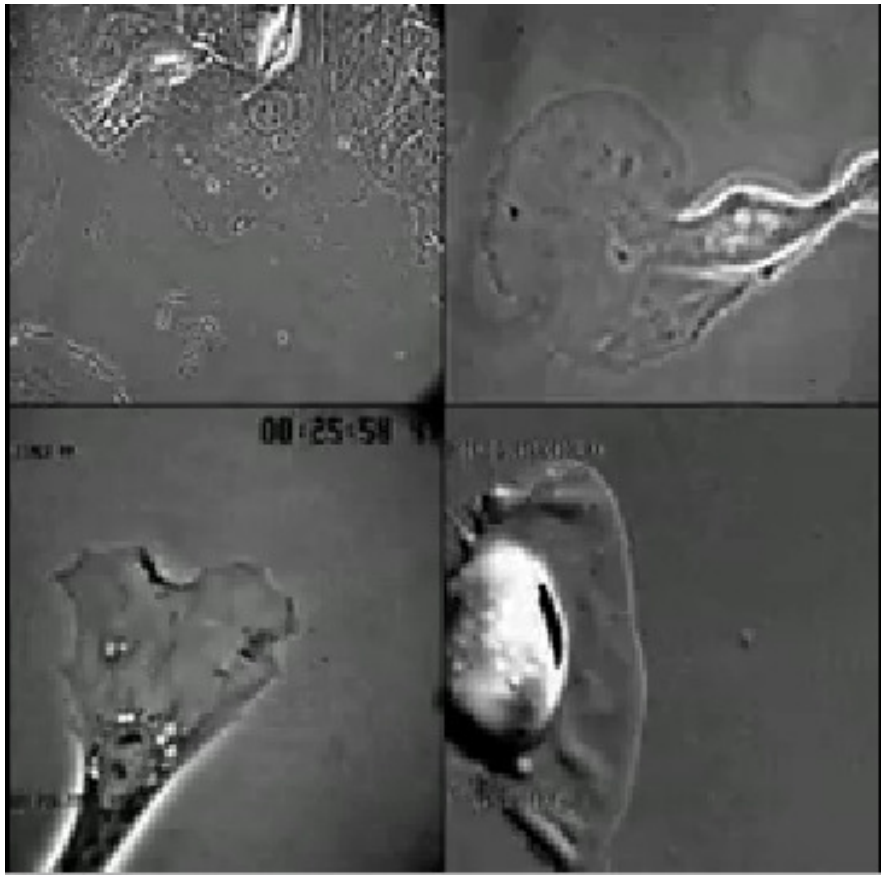
Cytoskeleton – Microtubules

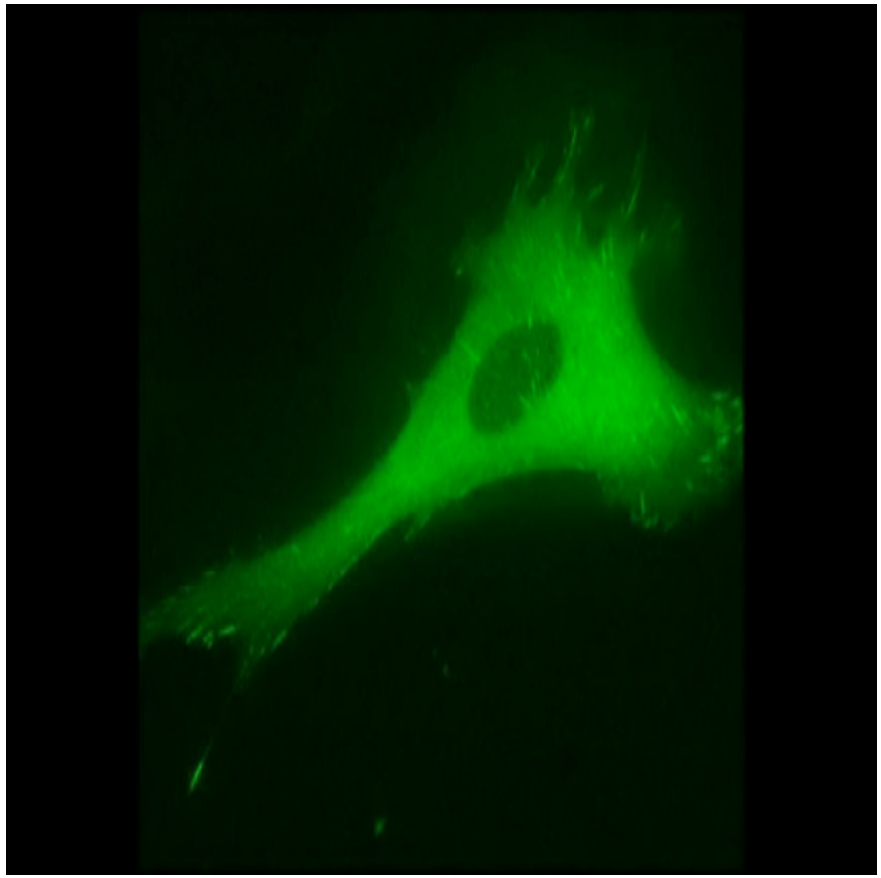


- Start point：MTOC
- Vesicle transport (the rail for the vesicle train)

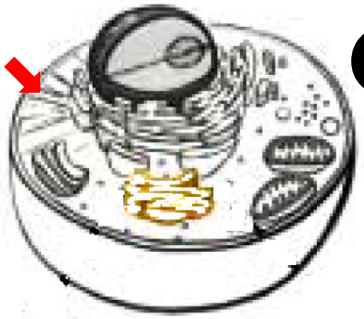


Cytoskeleton – Microfilament

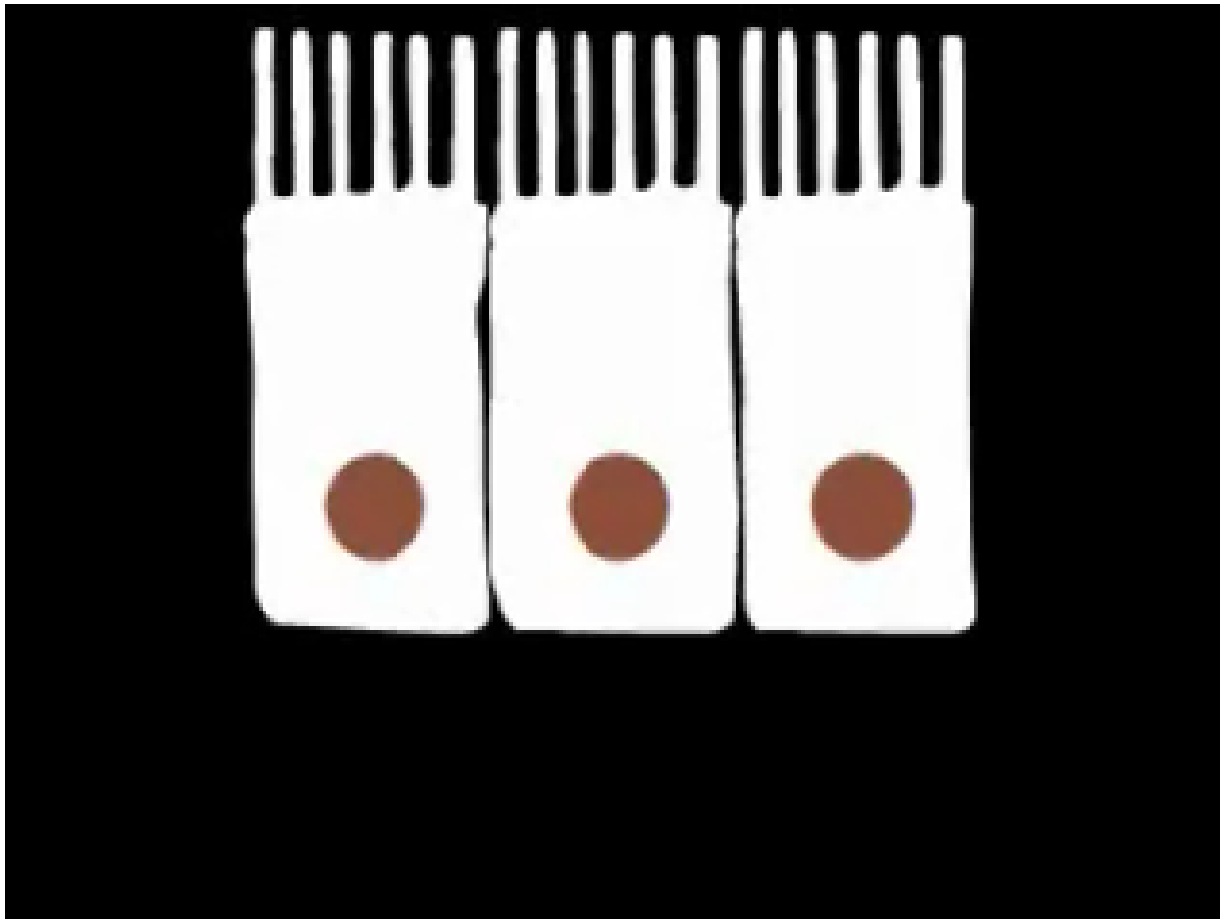




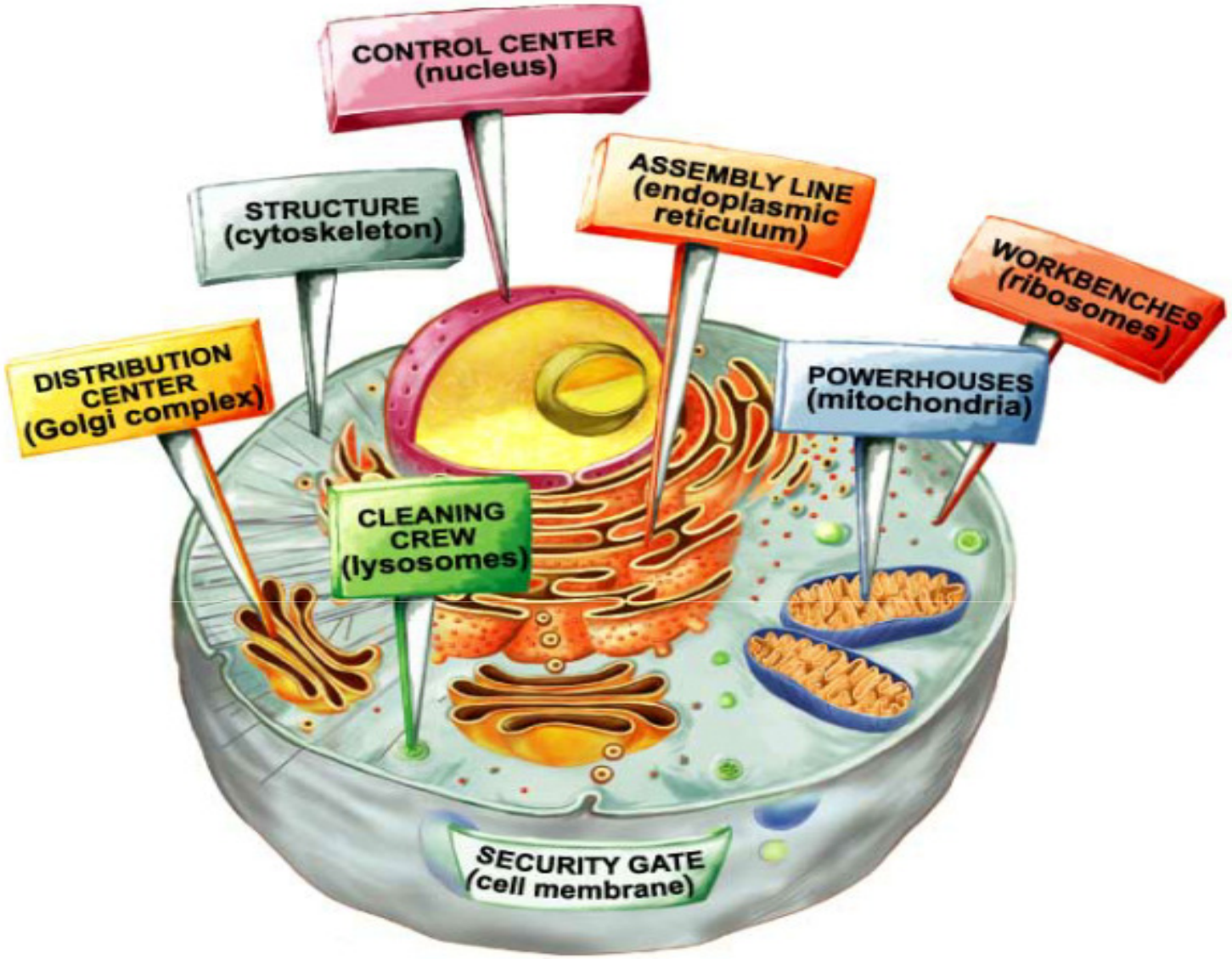
- Changes in cell shapes



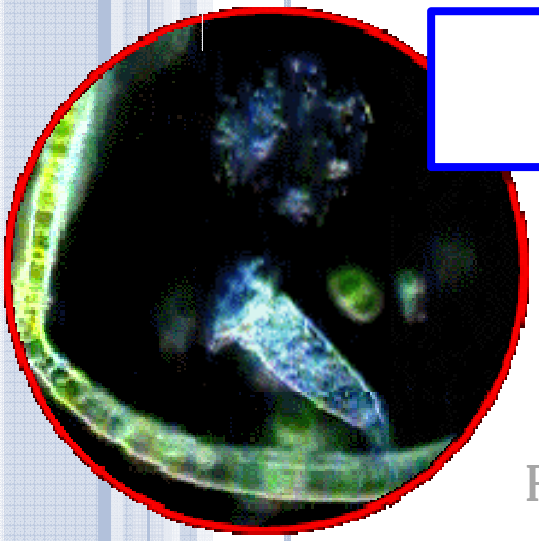
Cytoskeleton – Intermediate filaments



- Only for some animal cells, such as the MDCK cell of kidney



Transcription (DNA → RNA)
Translation (RNA → Protein)



Cells

PART I: The basic ideas of cells

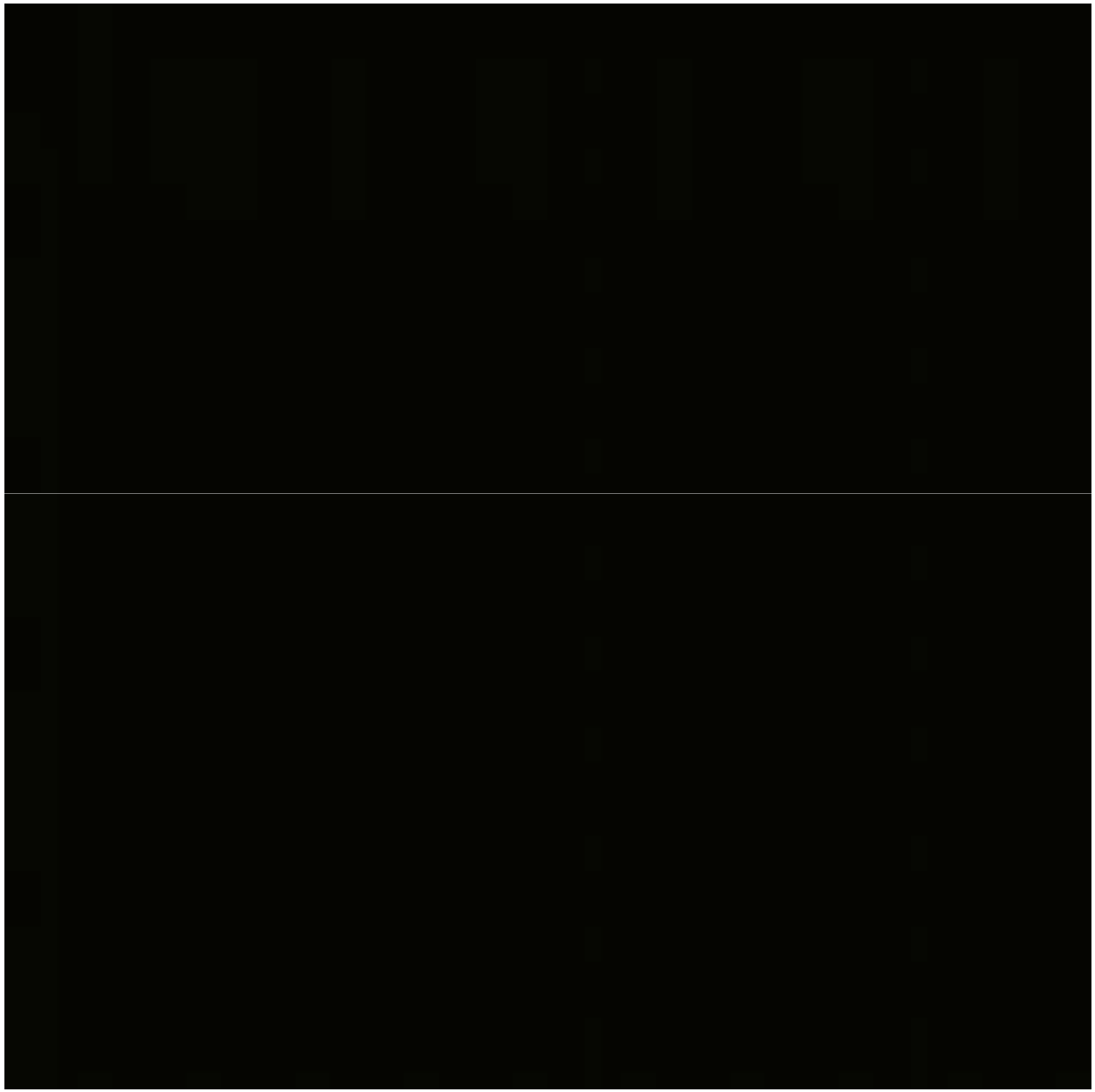
PART II: **The applications of nanotechnology about cells**

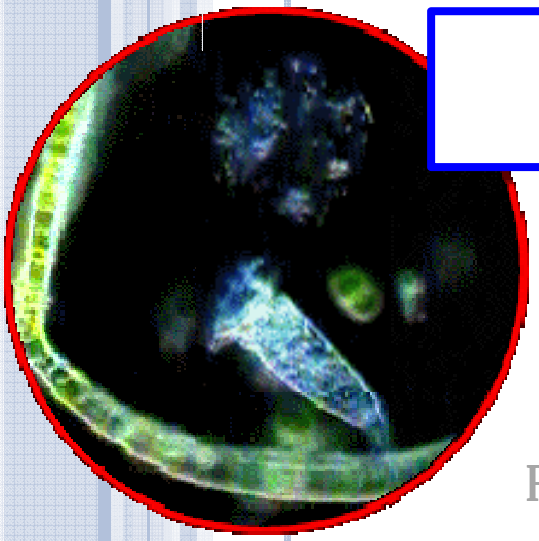
PART III: Basic cell culture

Chia-Fen Hsieh

Dr. Chia-Fu Chou's Lab, NanoBioScience Lab
Institute of Physics, Academia Sinica

“The inner life of a cell” from Harvard university





Cells

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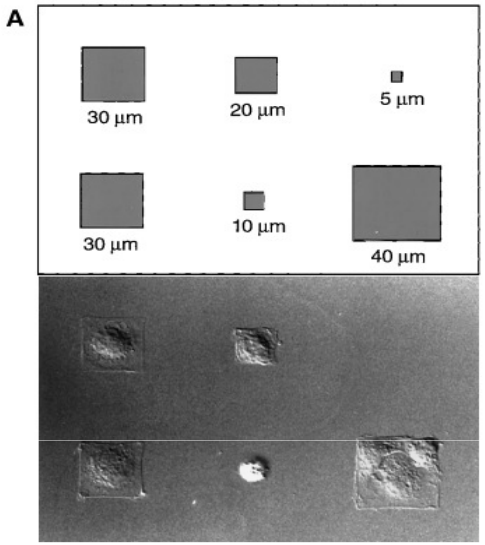
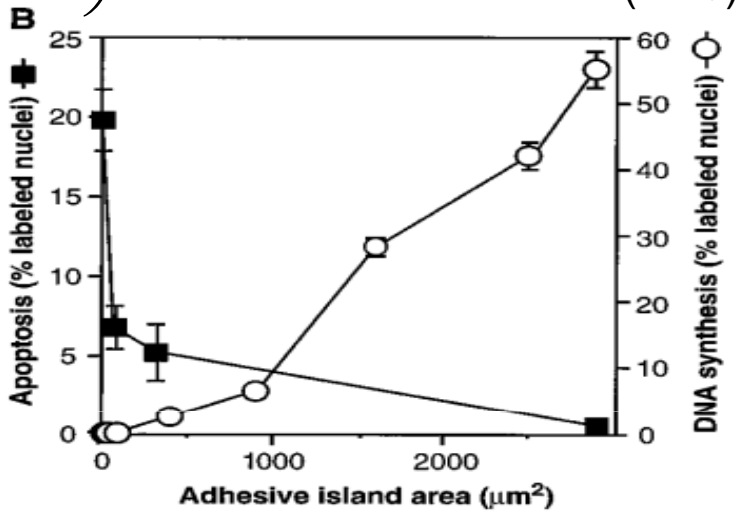
Institute of Physics, Academia Sinica

Geometric Control of Cell Life and Death

SCIENCE • VOL. 276 • 30 MAY 1997

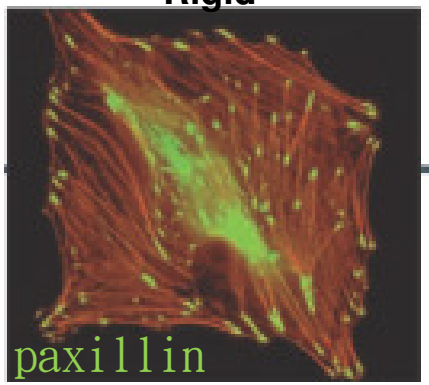


(Death) (Live)



Substrate compliance

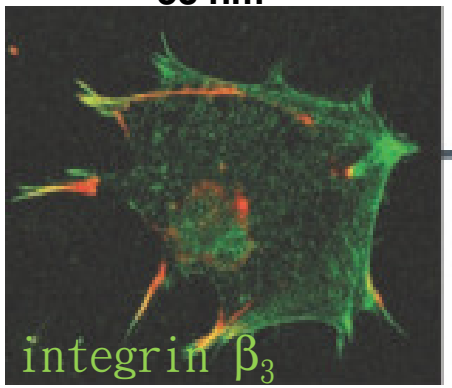
Rigid



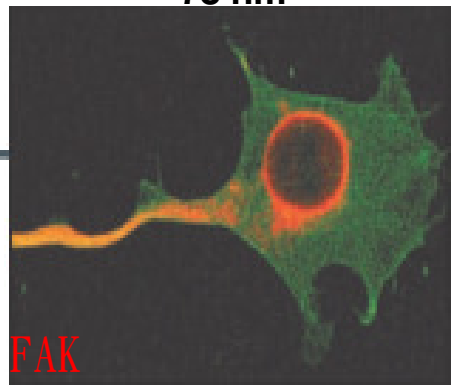
Soft

Lignad spacing

53 nm

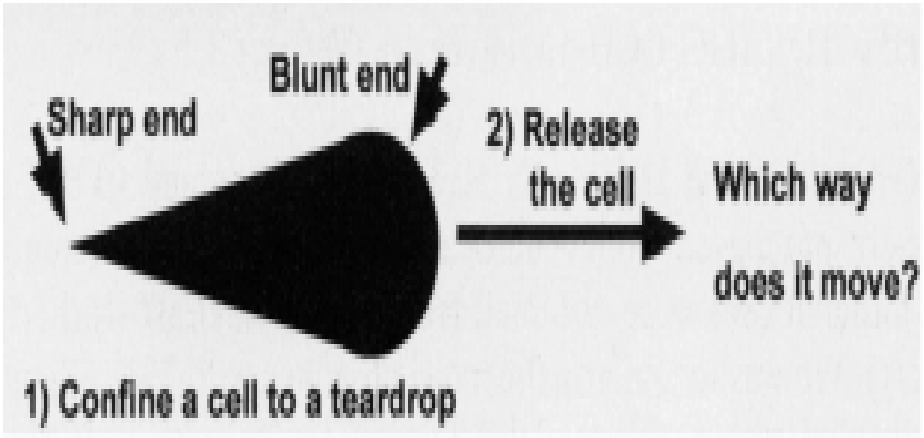


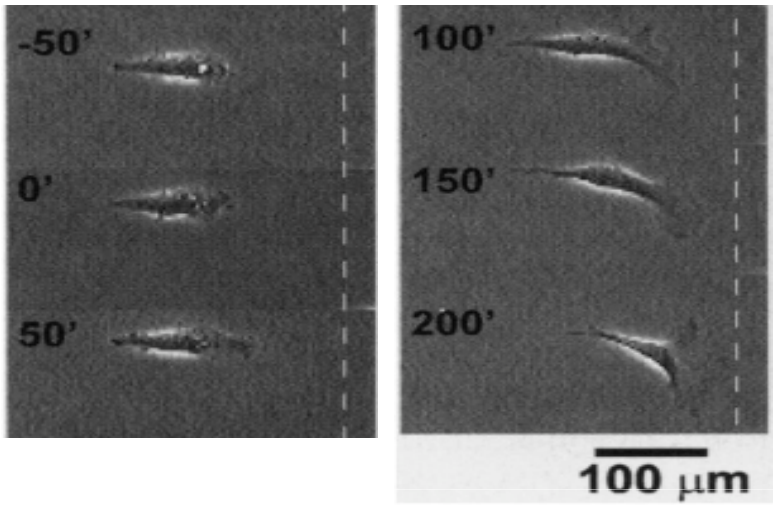
78 nm

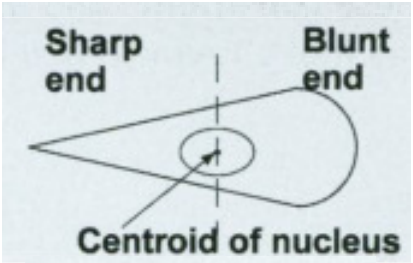


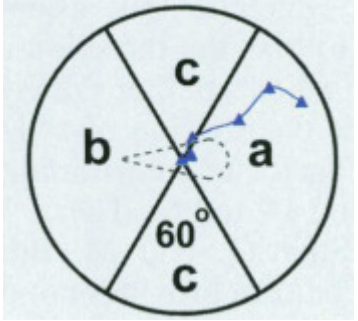
Directing cell migration with asymmetric micropatterns

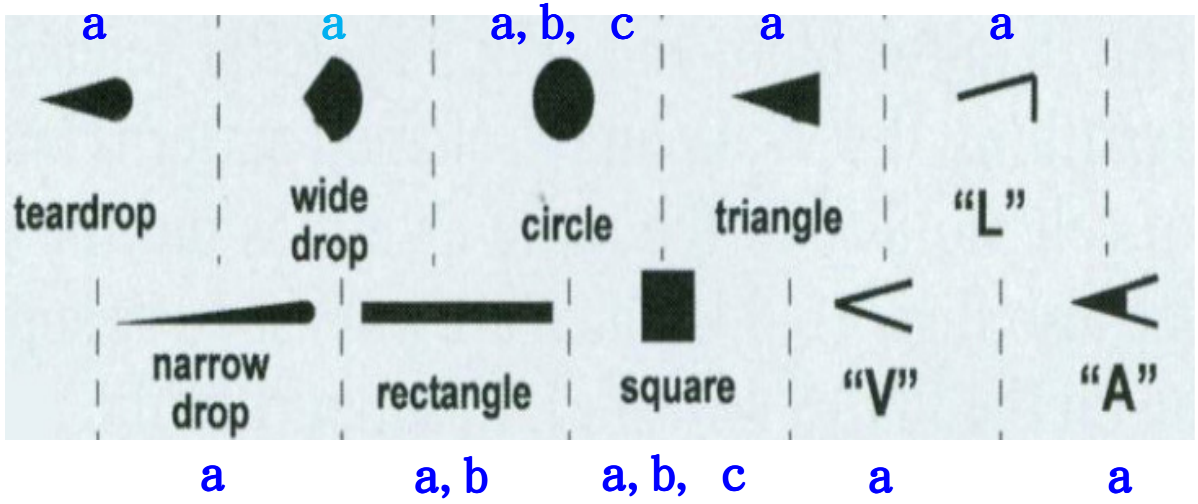
PNAS | January 25, 2005 | vol. 102 | no. 4 | 975–978









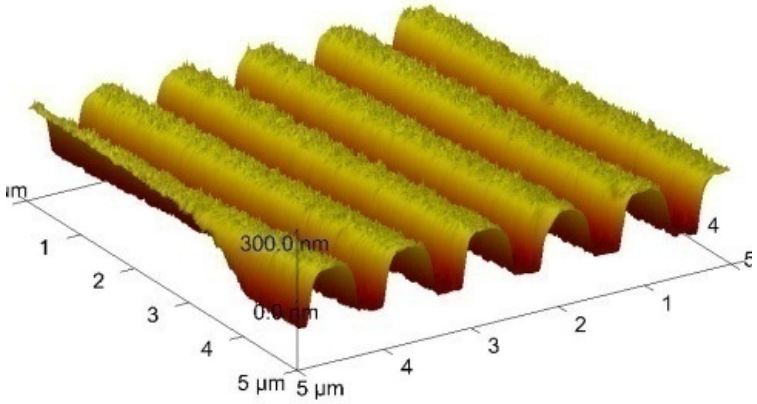


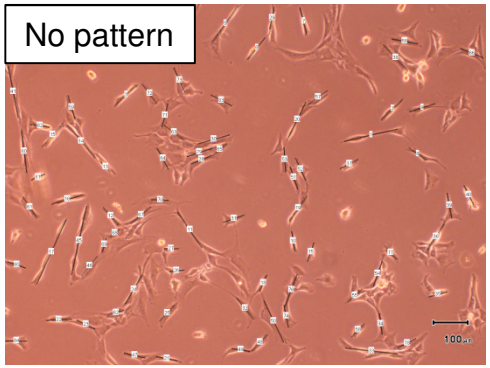
What will cells do on nanopatterns ?

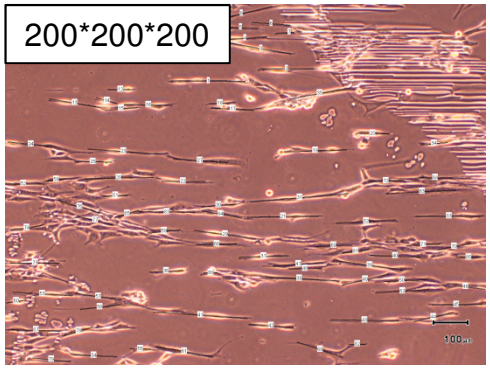
Cells align and migrate along nano-sctructure

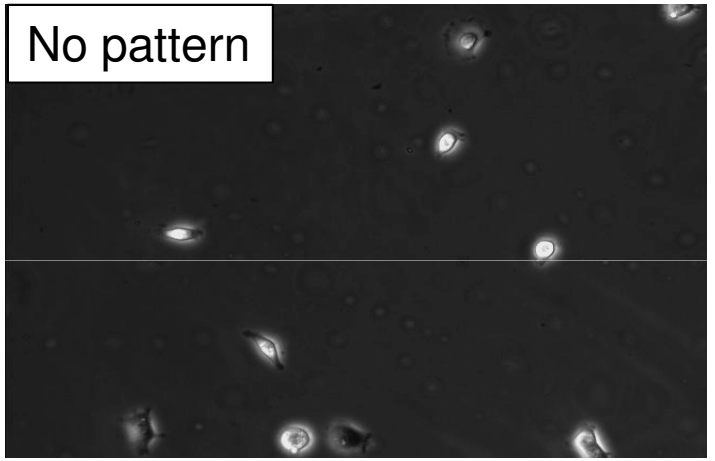
Done by Po-Chieh Chiang  *and Rachel Su* 

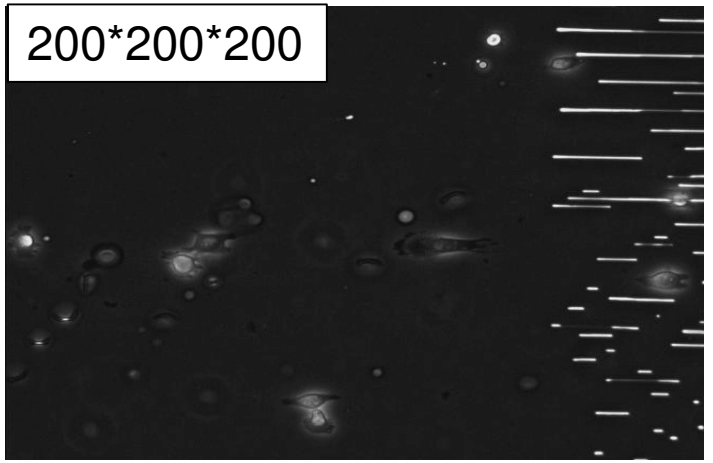
200*200*200 nm



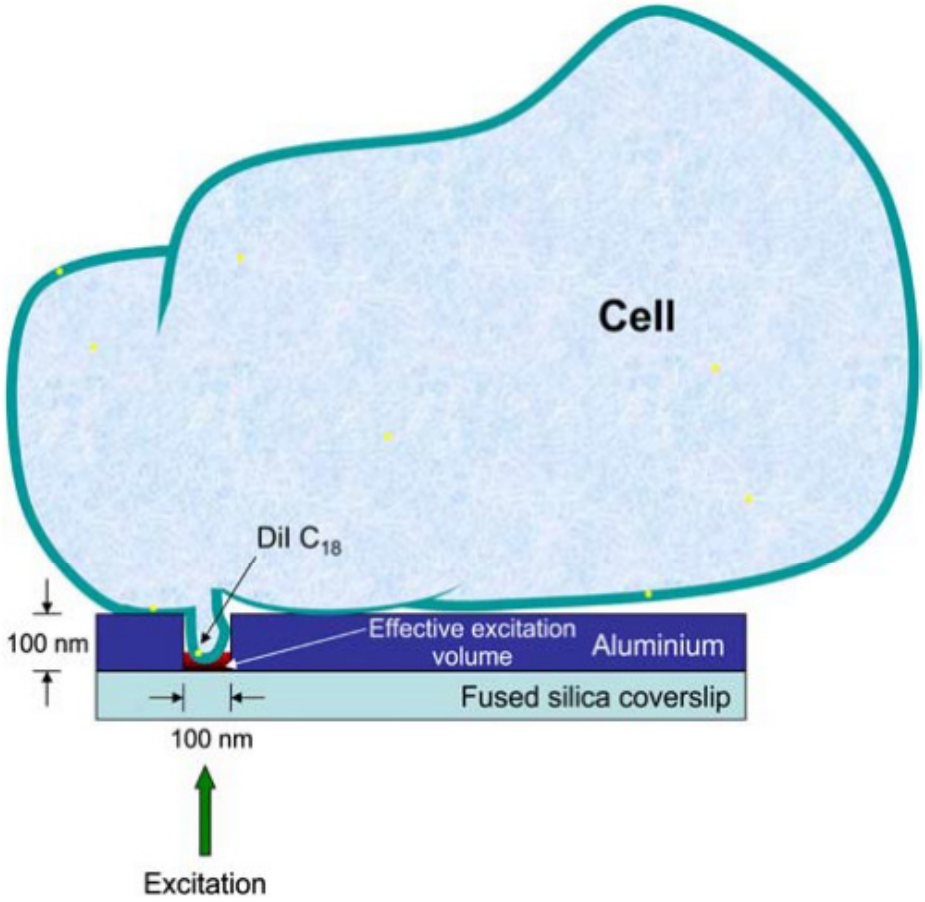




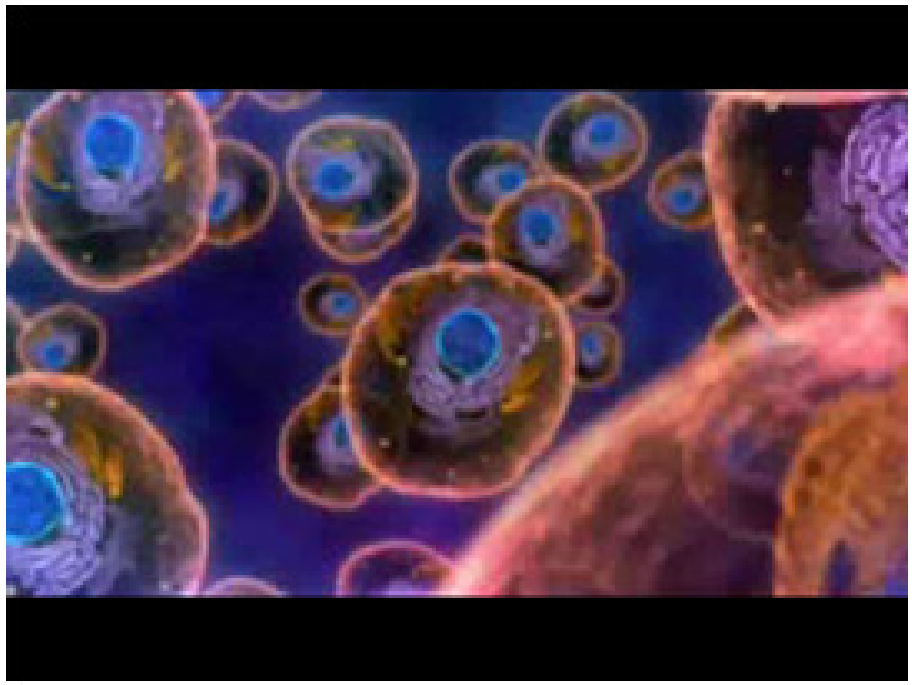




Zero-mode waveguides



Ref: J. B. Edel *et al.*, *Biophys J.*, 88:L43 (2005)



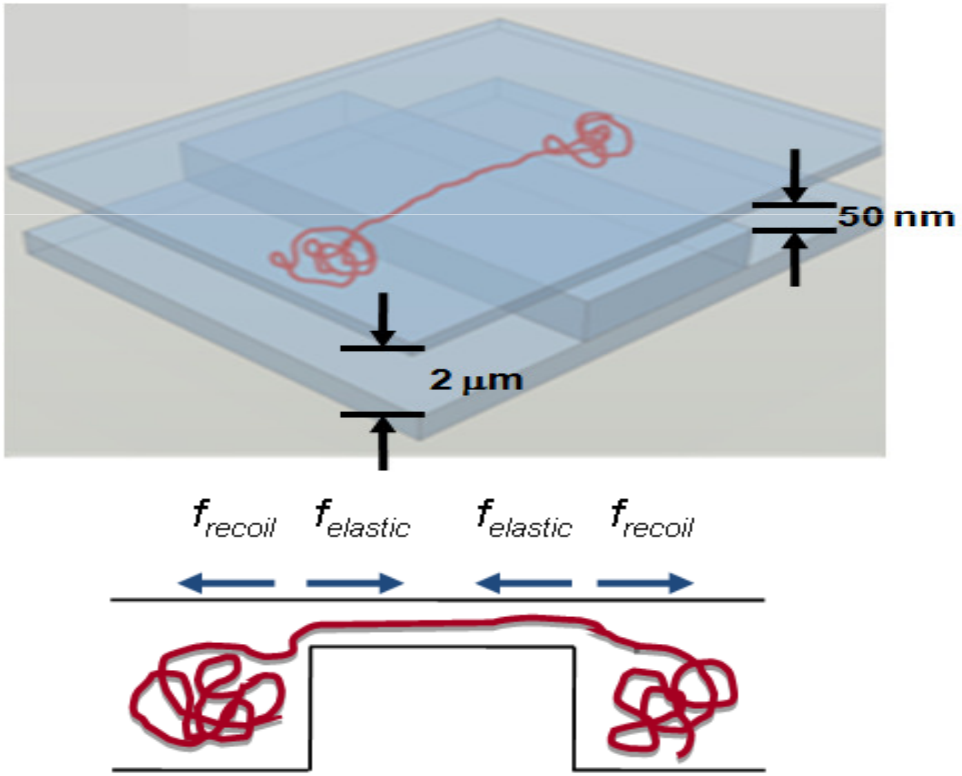
Ref: http://www.pacificbiosciences.com/index.php

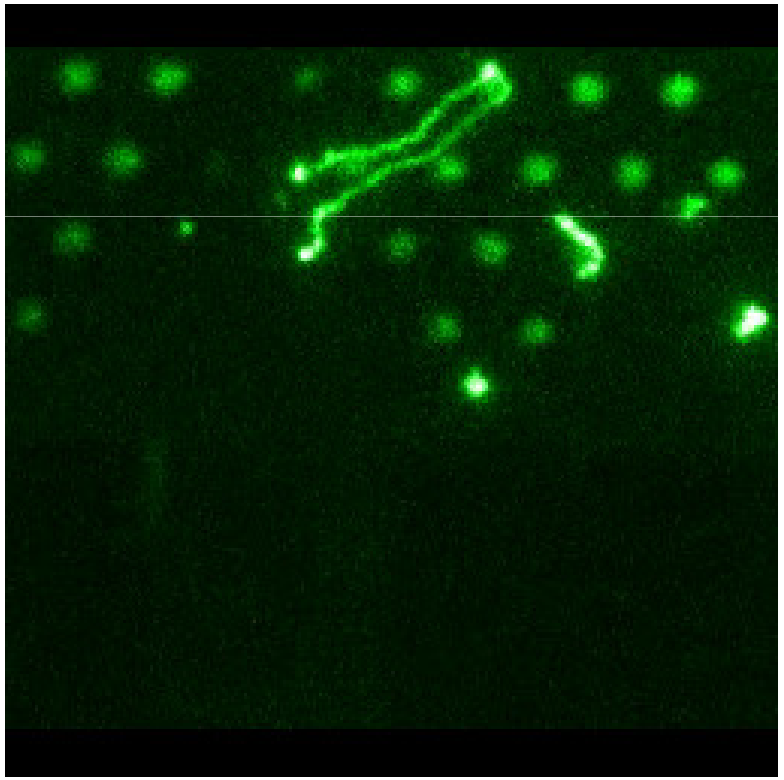
- DNA sequencing

- An optical waveguide that guides light energy into a volume that is small in all dimensions compared to the wavelength of the light
- The focal volume ZMWs – z：10-50 nm, x or y：hole size (50-200 nm)

DNA tug-of-war in nano-channel

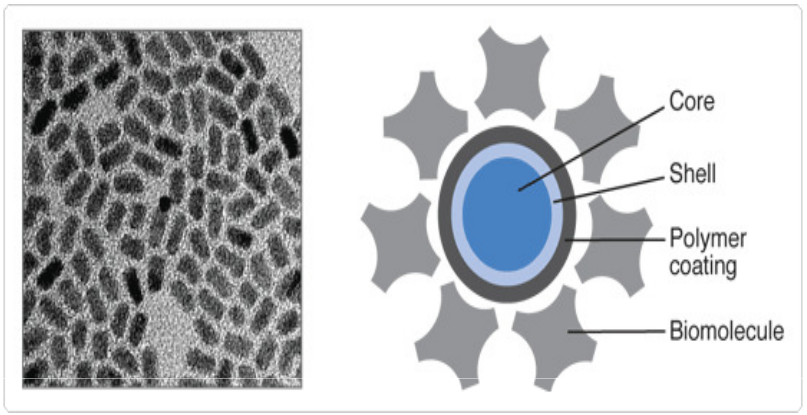
Done by Jia-Wei Yeh

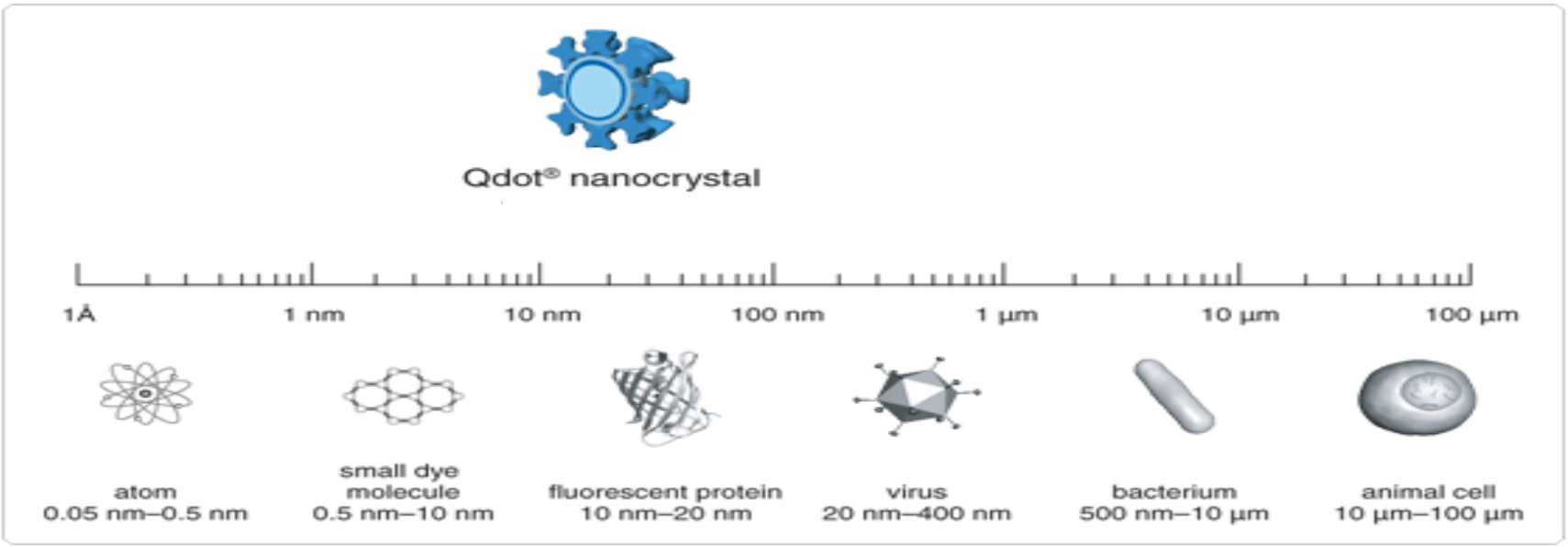




Quantum Dots (QDs)

- Nanometer-scale atom clusters comprising a **core** (cadmium selenide (CdSe)), **shell** (zinc sulfide (ZnS)) and **surface coating**



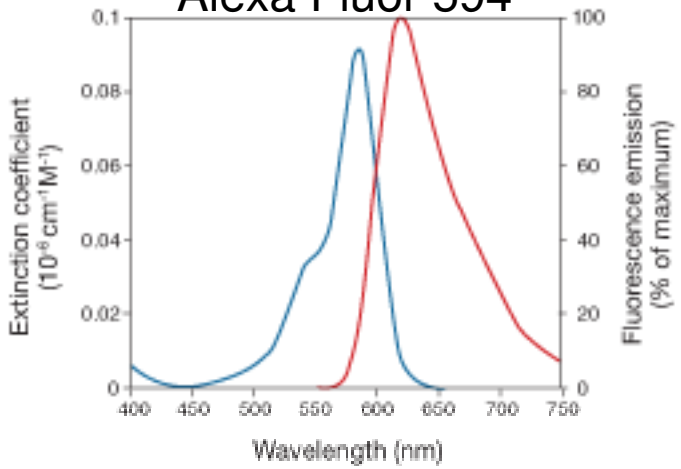


Ref： http://www.invitrogen.com

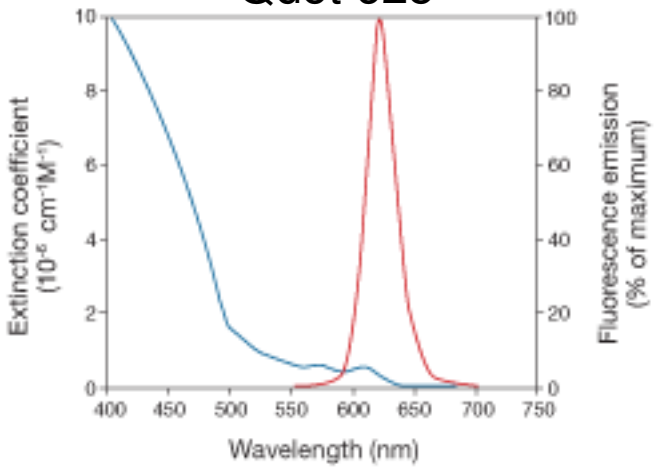
Why QDots ?

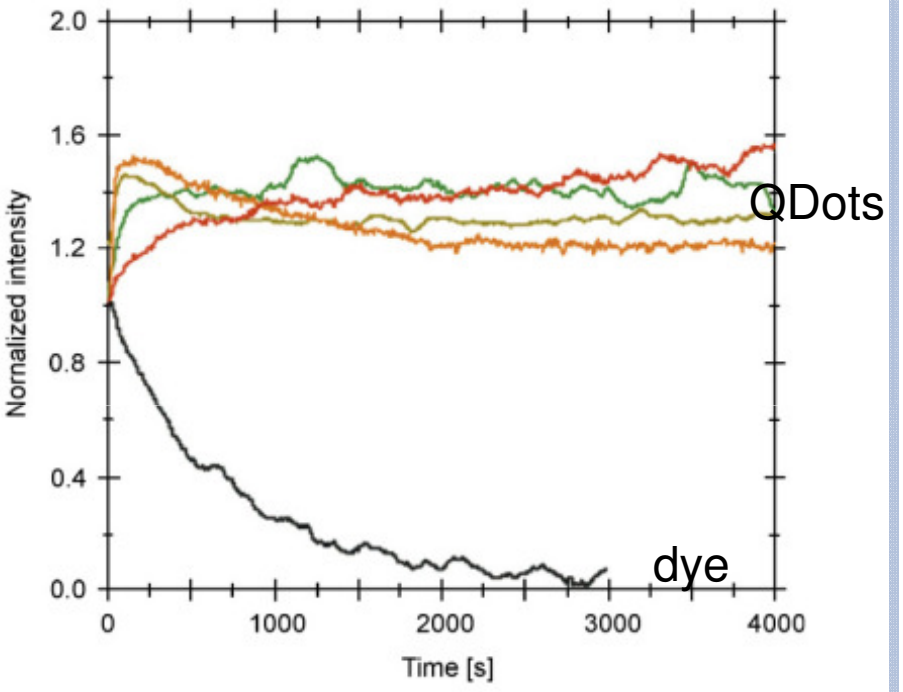
(Broadband Absorption, Sharp Emission, Good Photostability)

Alexa Fluor 594



Qdot 625



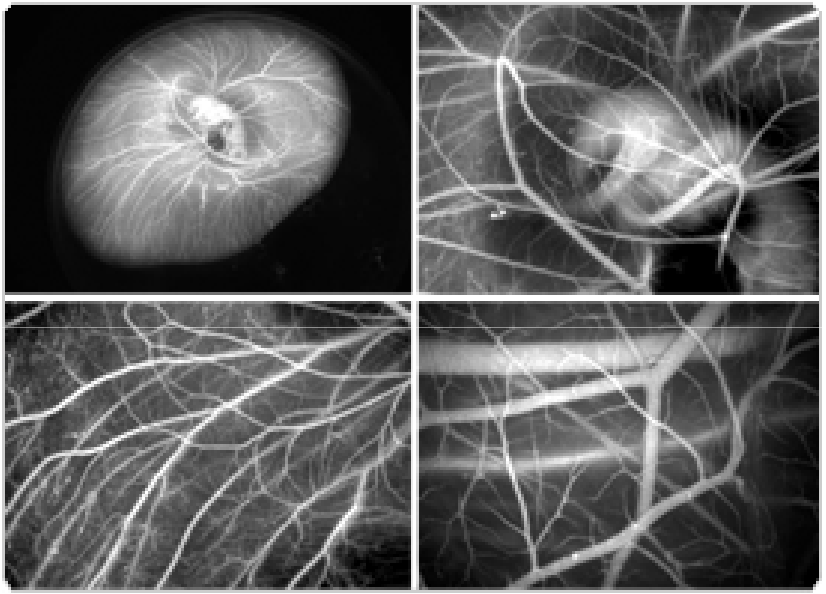


The QDots exhibit a stable emission for at least 4 h, while the dye bleaches after 10 min

Ref： http://www.invitrogen.com

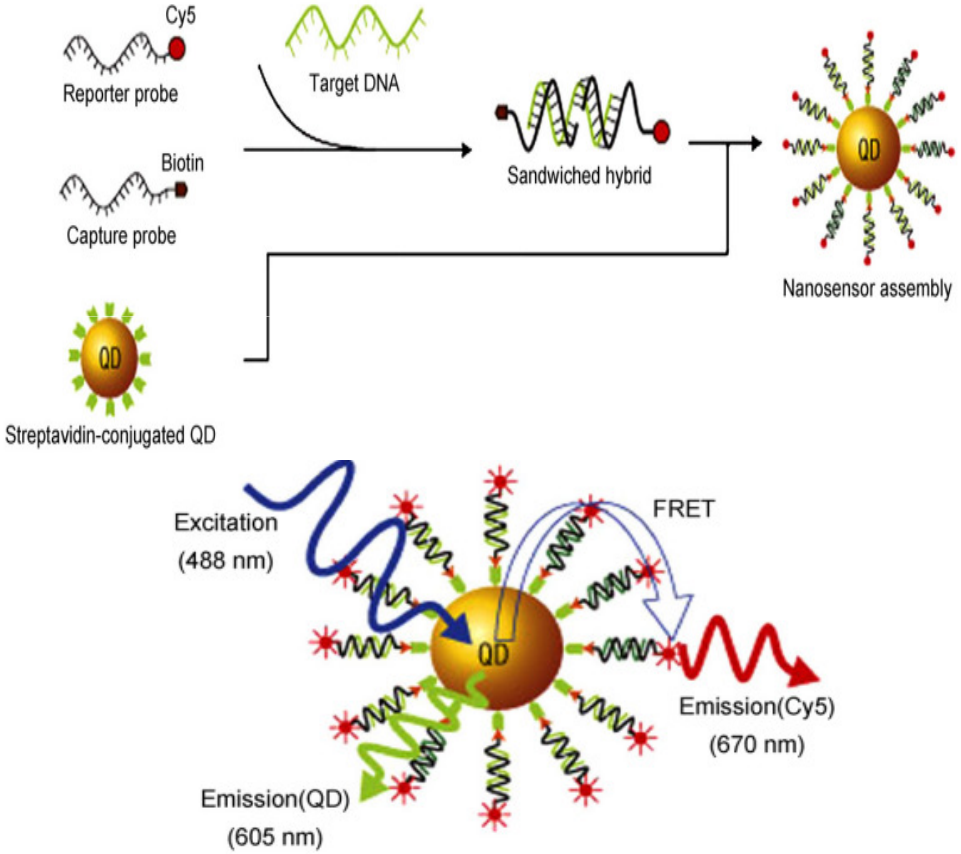
The biological application of QDots

Long-term tissue labeling



Chick embryo injected through the major vitelline vein with non-targeted quantum dots.

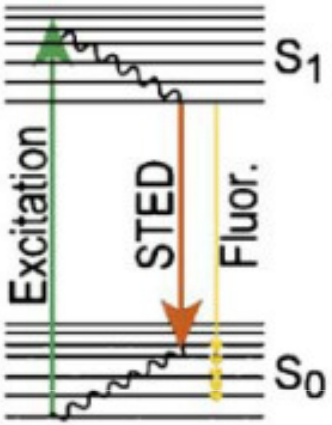
Nano-sensor

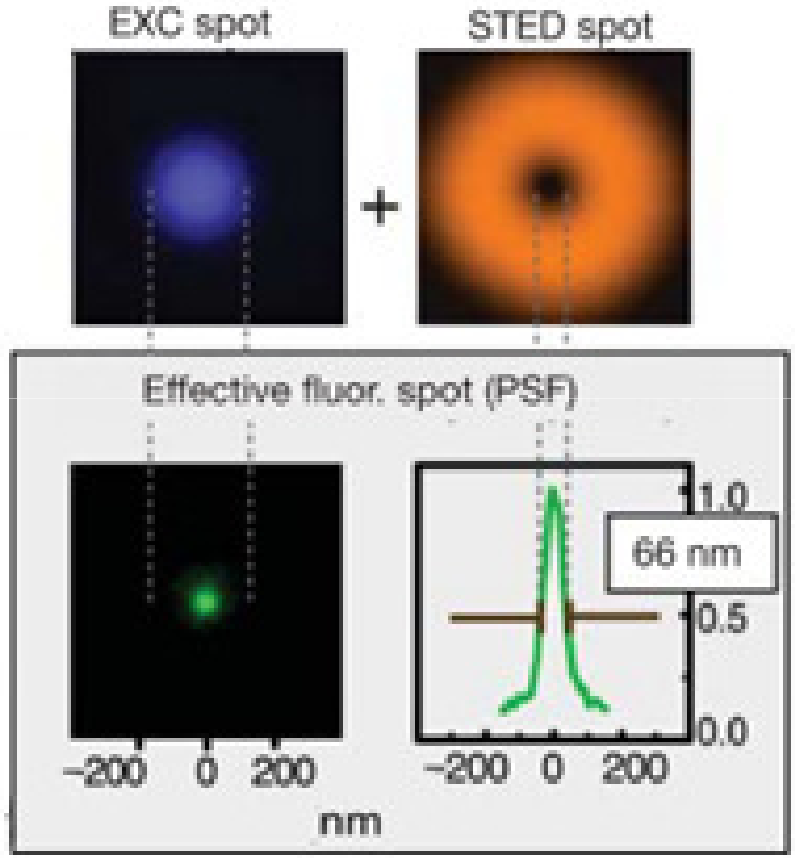


Ref：http://www.invitrogen.com

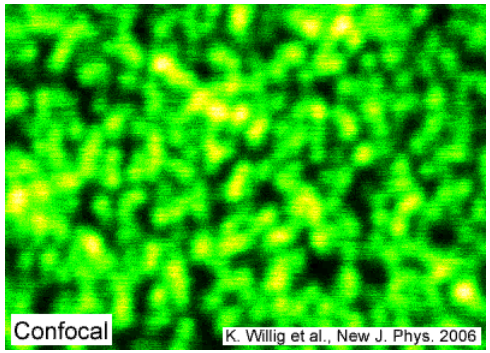
Biomaterials 28 (2007) 4717–4732

Stimulated emission depletion microscopy (STED) provide a way to observe the nano-assemble of lipid raft

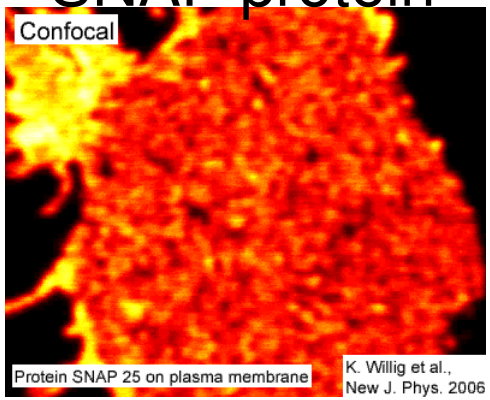




40 nm bead

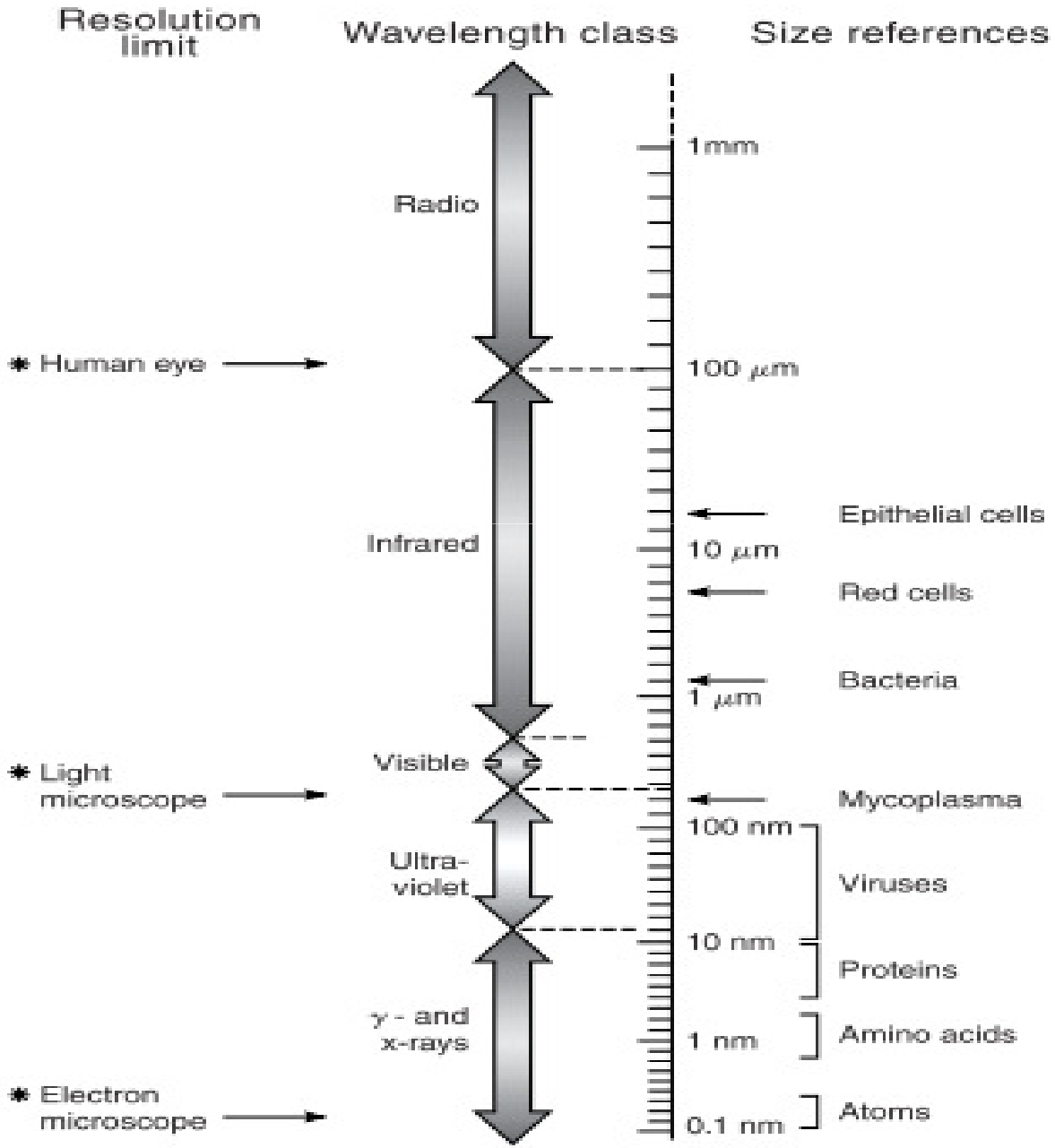


SNAP protein



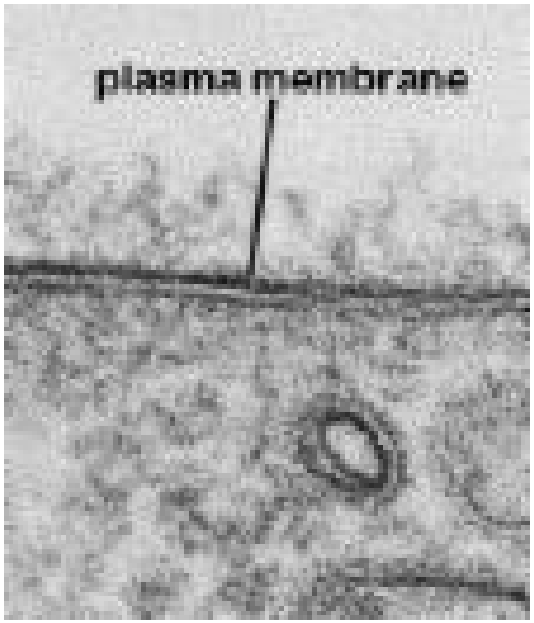
Ref: S. Jakobs, *Biochim Biophys Acta*, 1763:561 (2006)
Ref: K. I. Willig, *et al.*, *Nature*, 440:935 (2006)
Ref: K I Willig ,*et al.*, *New J Phys I.*, 8:106 (2008)
Ref: V. Westphal, *et al.*, *Science*, 320:246 (2008)

Microscopy

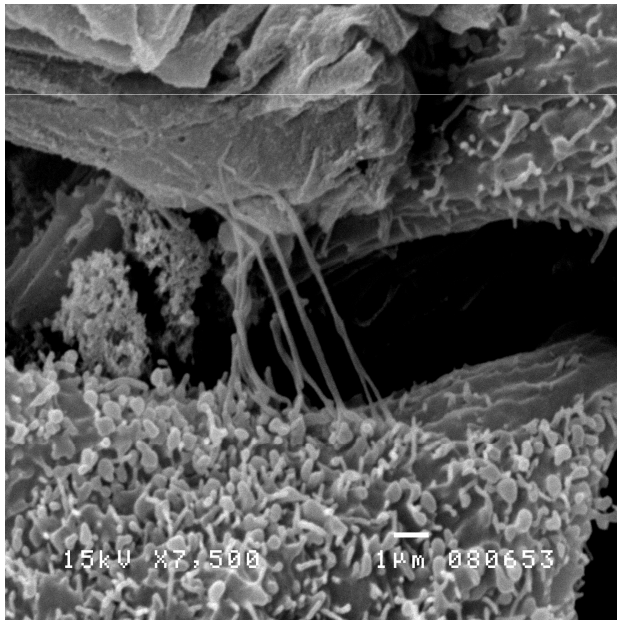

Resolution limit
Wavelength class
Size references
Radio
Infrared
Visible
Ultra-violet
γ - and x-rays
* Human eye
* Light microscope
* Electron microscope
1mm
100 μm
10 μm
1 μm
100 nm
10 nm
1 nm
0.1 nm
Epithelial cells
Red cells
Bacteria
Mycoplasma
Viruses
Proteins
Amino acids
Atoms

Electron Microscopy

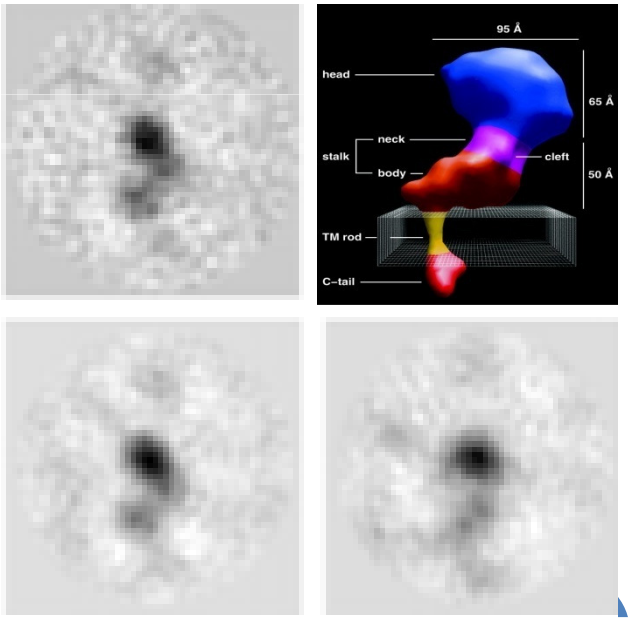
- Transmission Electron Microscope (TEM)
- Scanning Electron Microscope (SEM)
- Cryo Electron Microscope (Cryo-EM)



TEM



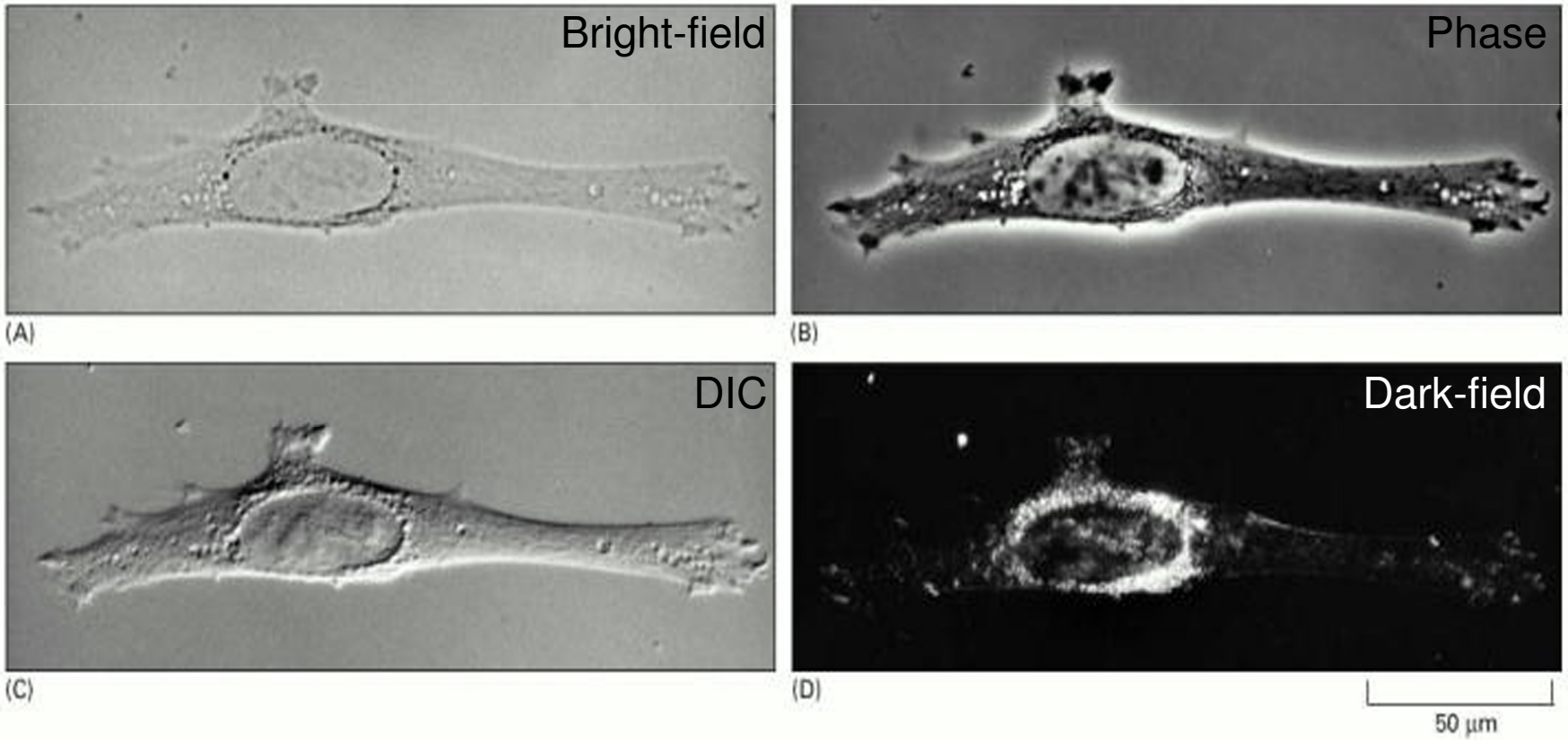
SEM



Cryo-EM

Light Microscopy

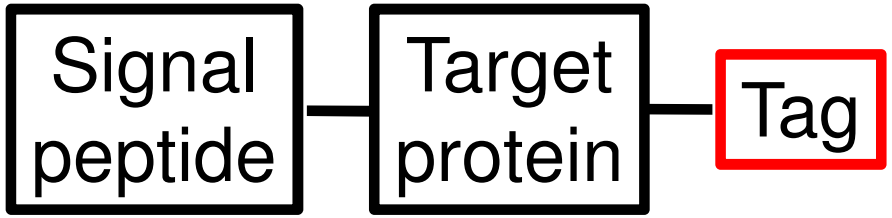
- Bright-field microscopy
- Phase-contrast microscopy
- Differential-interference-contrast (DIC) microscopy
- Dark-field microscopy



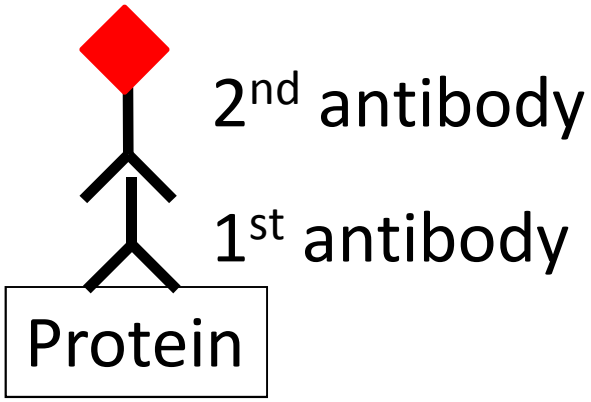
Fluorescence Microscopy

- Fusing protein tag:
  - GFP, EGFP …
  - YFP, EYFP …
  - RFP, DsRed …

One tag, one color

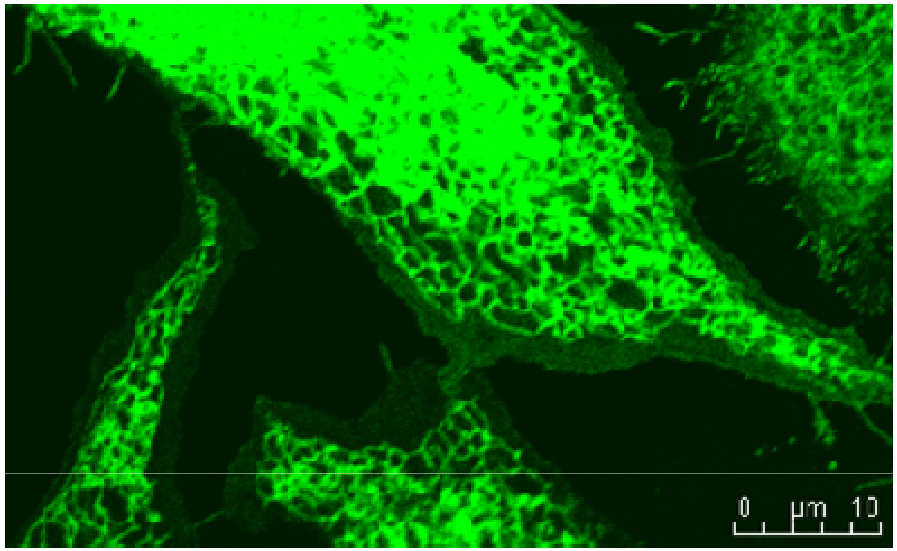


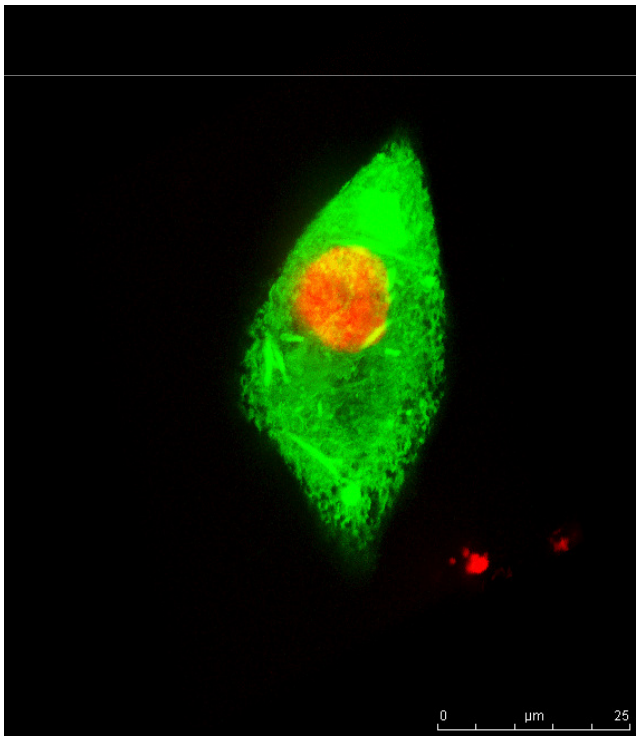
- Fluorescent labeling:
  - FITC TRITC …
  - Analog dye (membrane dye)
  - Oxidizing dye (Mitotracker…)
  - Quantum Dot
  - …
- Immuno-staining:
  - Antibody labeling

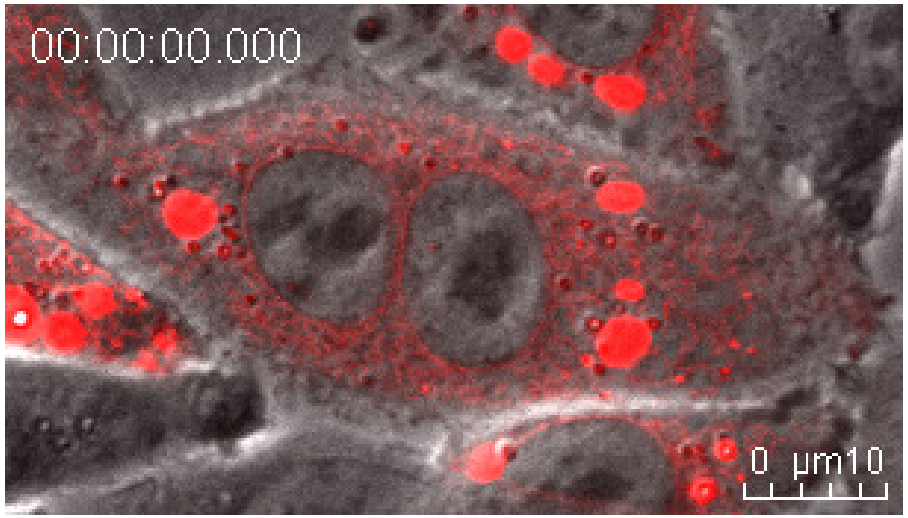


Labeling on biology

- Fusing protein tag:
  - GFP, EGFP …
  - YFP, EYFP …
  - RFP, DsRed …

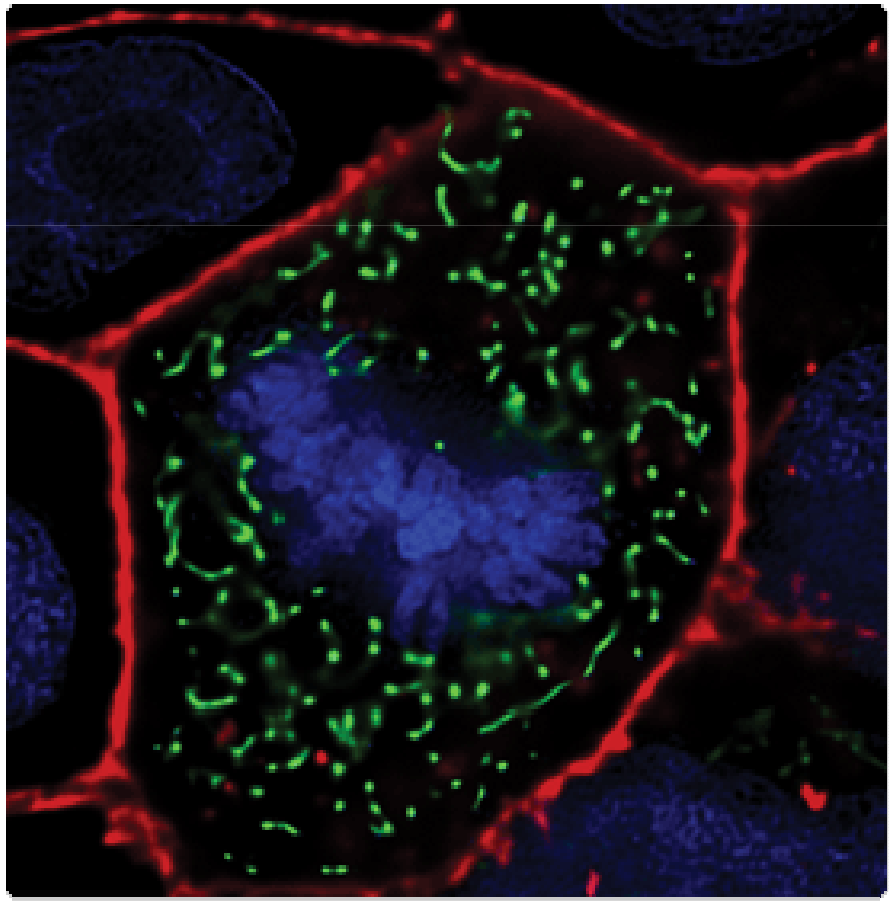






Labeling on biology

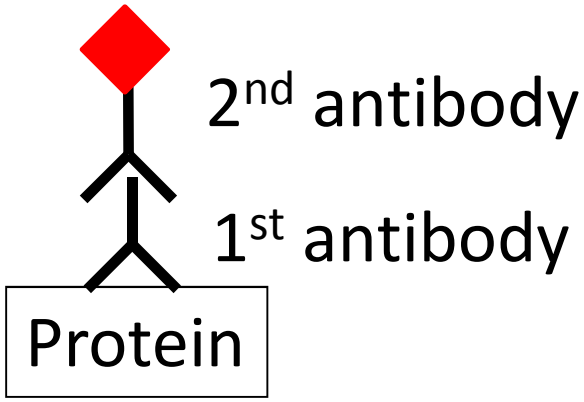
- Fluorescent labeling:
  - FITC TRITC …
  - Analog dye (membrane dye)
  - Oxidizing dye (Mitotracker…)
  - Quantum Dot
  - …

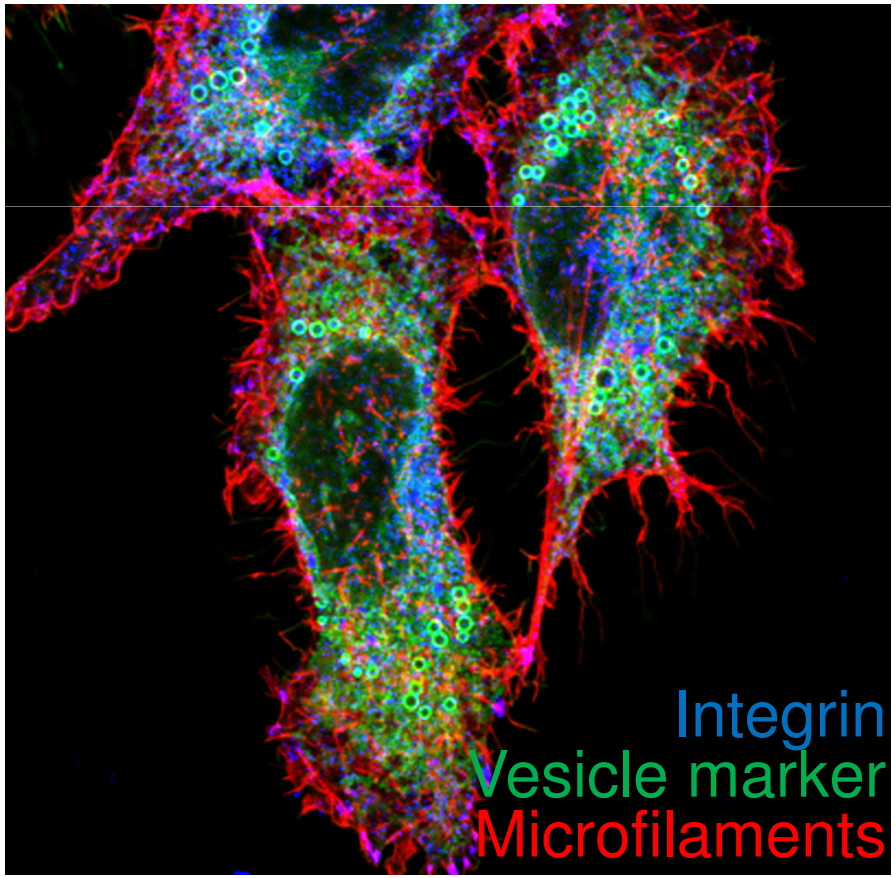


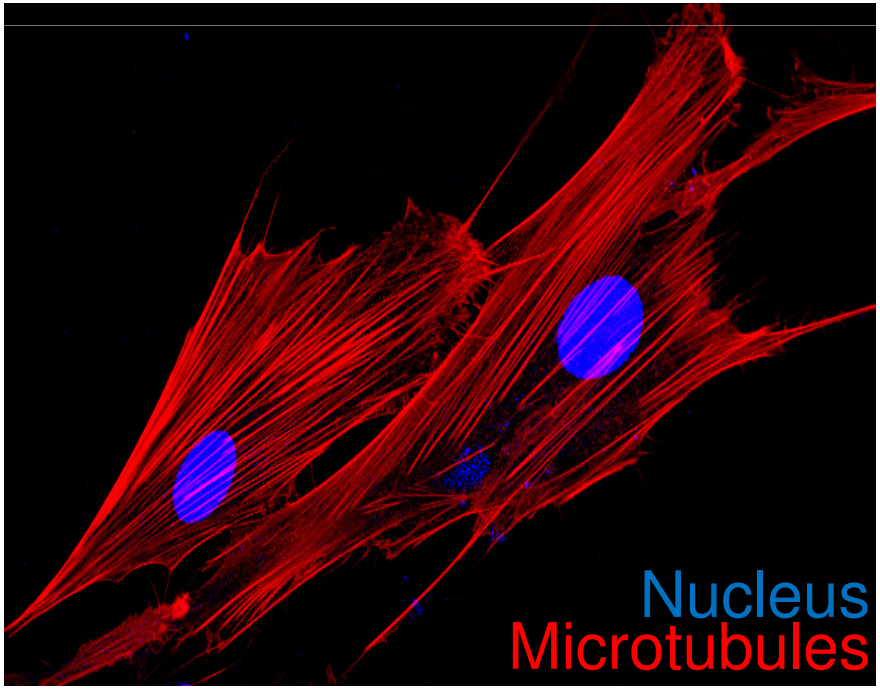
DNA
Mito-tracker
Membrane dye

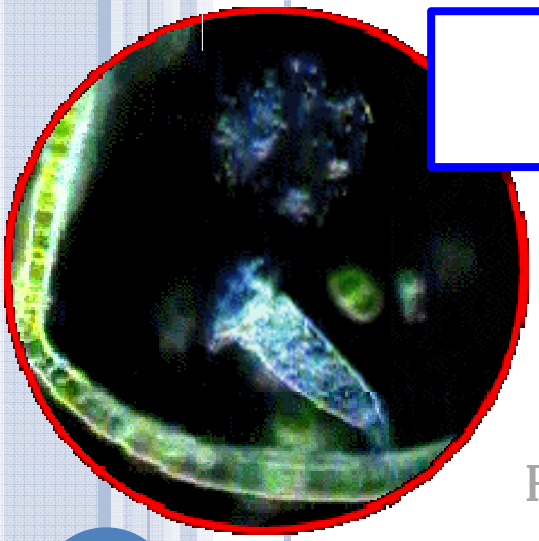
Labeling on biology

- Immuno-staining:
  - Antibody labeling









Cells

PART I: The basic ideas of cells

PART II: The applications of nanotechnology about cells

PART III: **Basic cell culture**

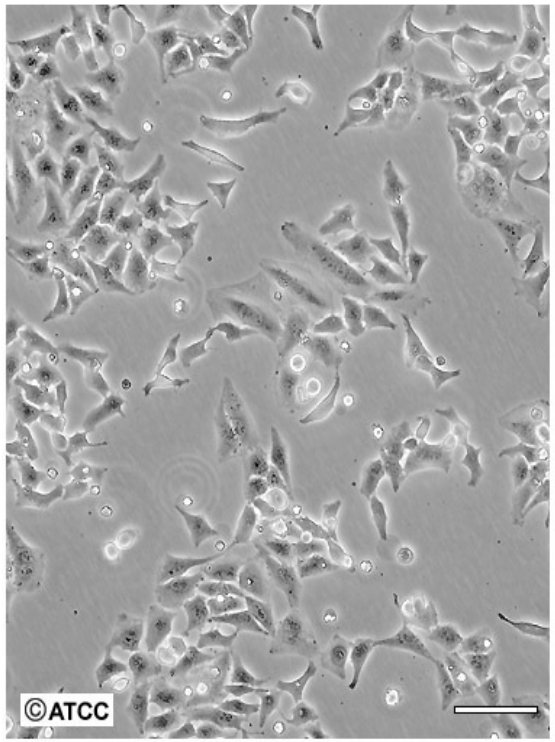
Chia-Fen Hsieh

Dr. Chia-Fu Chou's Lab, NanoBioScience Lab

Institute of Physics, Academia Sinica

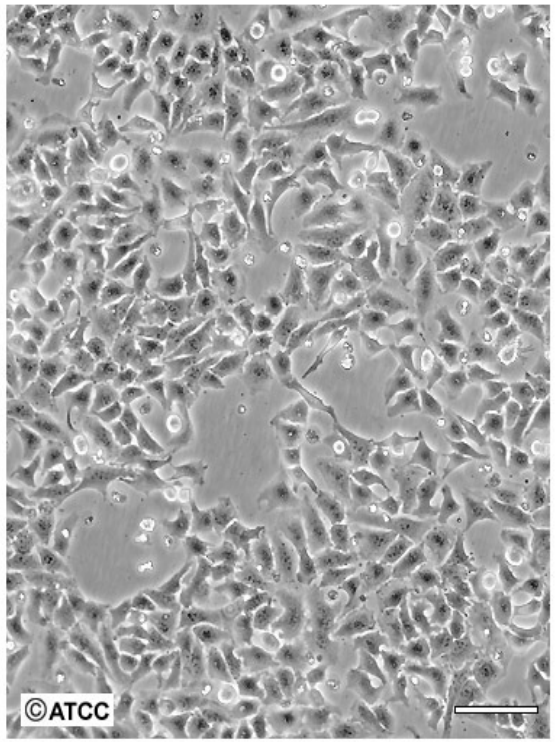
Cell Culture

ATCC Number: **CCL-2**
Designation: **HeLa**



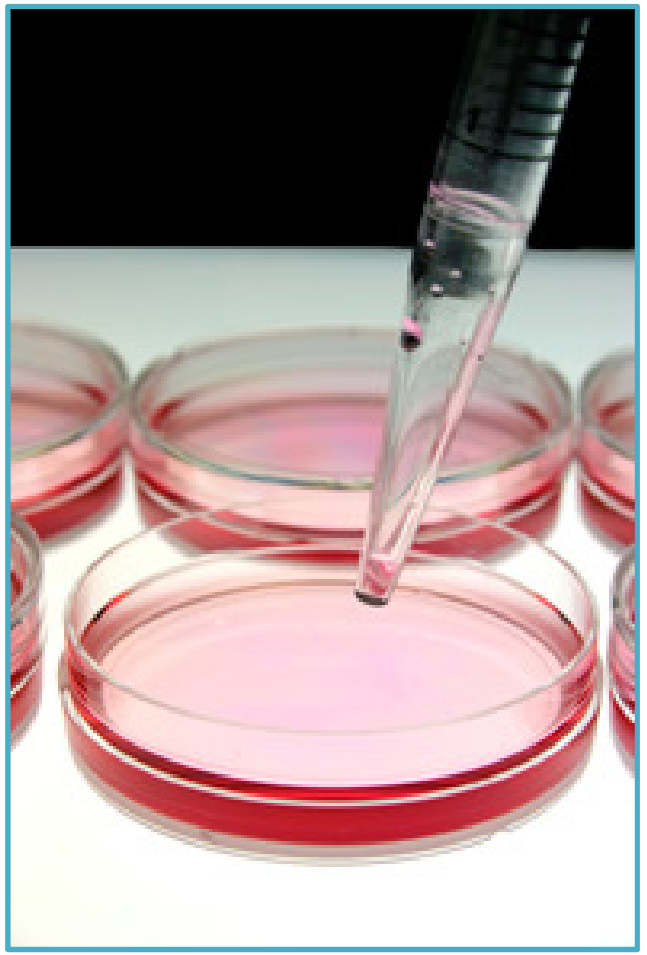
Low Density

Scale Bar = 100µm

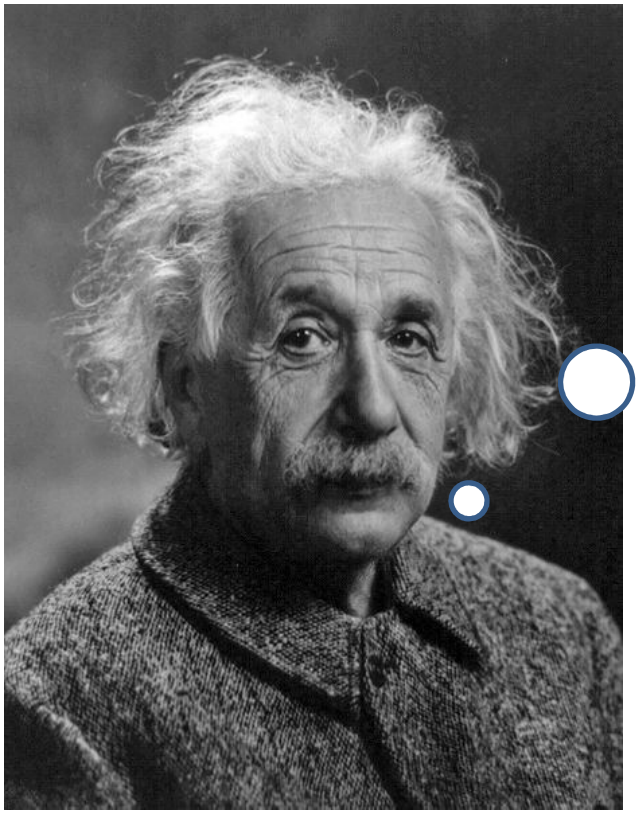


High Density

Scale Bar = 100µm



If we knew what we were doing, it wouldn't be called research, would it?



Albert Einstein

The End