Introduction to Biomedical Engineering
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Design of a neural network using micro-contact printing

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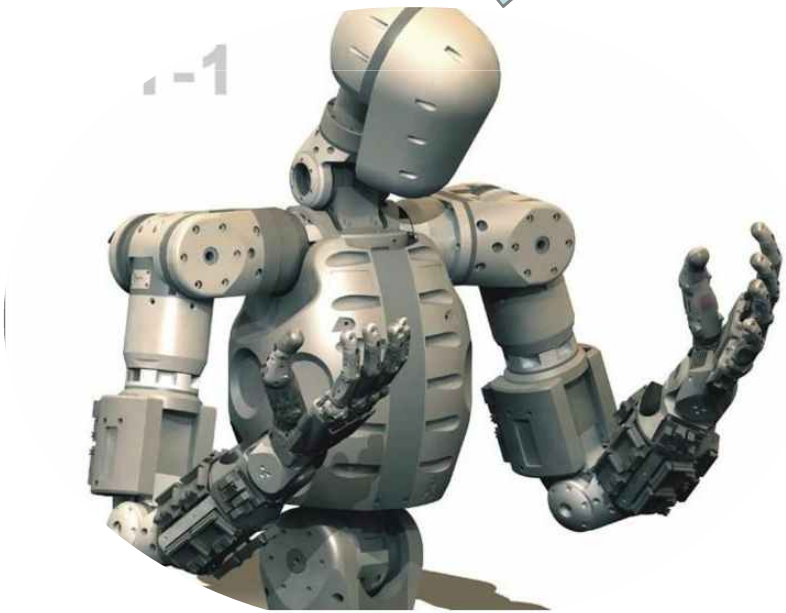
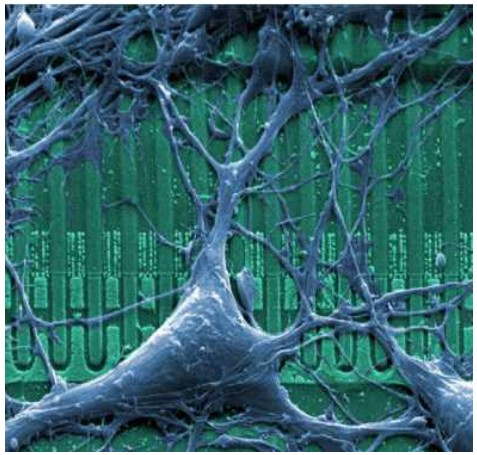
Human + Robot ???

① Culture of neurons on a dish
② Design of neural networks
③ Imaging to verify the networks
④ Training of neural networks





Artificial Brain

How to culture neurons on a dish

Beginning of cell culture

- Harrison (1907) cultivated frog nerve cells and observed the growth of nerve fibers *in vitro* for several weeks. He was considered by some as the father of cell culture.

- **Major development's in cell culture technology ?**
  - First development was the use of antibiotics which inhibits the growth of contaminants.
  - Second was the use of trypsin to remove adherent cells to subculture further from the culture vessel
  - Third was the use of chemically defined culture medium.

Cell Culture Study

- To study "the behavior of animal cells free of systemic variations that might arise in the animal during normal homeostasis and under the stress of an experiment."

- **Advantages over animal studies**
  - Avoid of ethical issues
  - physicochemical environment accurately controlled
  - homogeneity (cell type well defined)
  - many cellular functions can be investigated
  - less costly for screening assays

- **Disadvantages over animal studies**
  - small size (high sensitive techniques to detect changes)
  - scale-up is challenging
  - may not represent *in vivo* phenotype/genotype

Three types of cultured cells

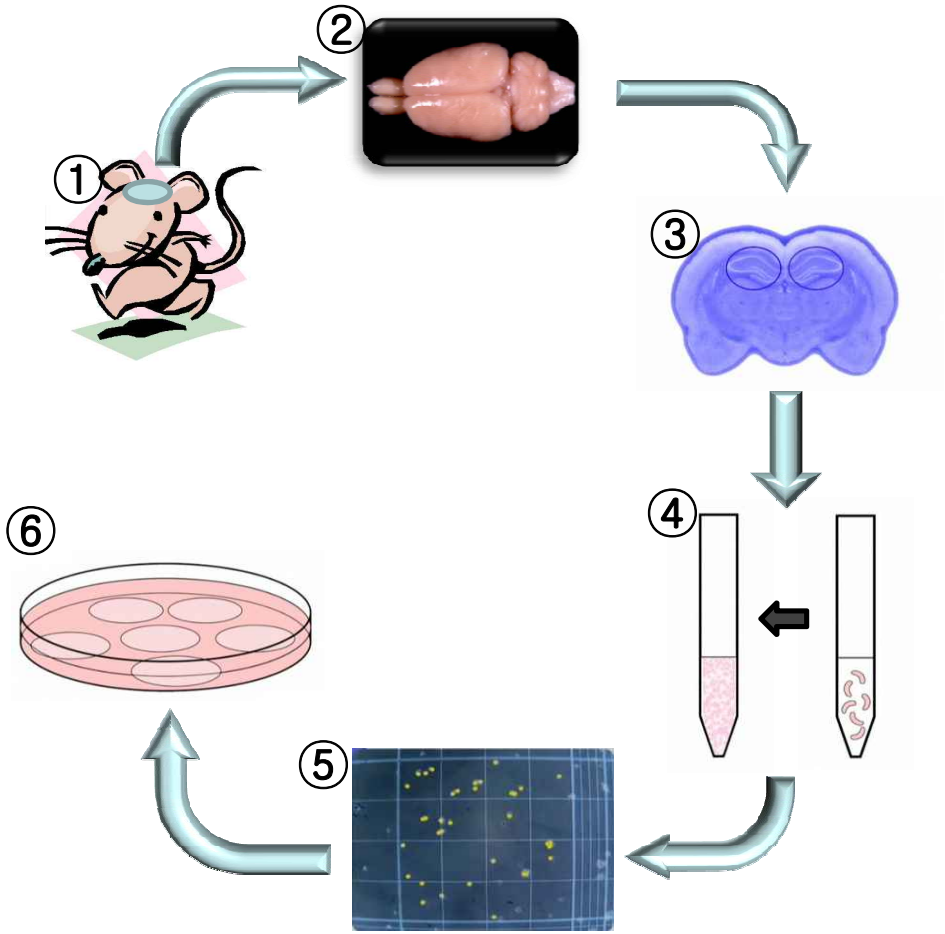
- Primary cultures : Cells derived directly from tissues
- Cell strains (Extended cultures/ multipassage culture) : Cells derived from primary cultures
- Cell lines : Cells derived from several different sources, immortal
  - cell strains : immortalized by rare genetic changes
  - transformed cells : further genetic changes by radiation, chemical carcinogens, tumor viruses
  - tumor cells

Primary culture vs. Cell line culture

- **Primary culture** : Limited growth potential & Limited life span
  - Advantages
    - May represent the best experimental *in vitro* models
    - May retain characteristics of normal cells from that organ
  - Disadvantages
    - Difficult to obtain
    - Susceptible to contamination

- **Cell line culture** : Immortal, Fast Growth, Grow upto higher cell density
  - Advantages
    - Easy to maintain in culture & Easy to obtain large quantities
    - Typically easy to manipulate
  - Disadvantages
    - Cell line may change overtime - genetically unstable
    - Unclear how well they represent function of original cell type

Primary culture procedure of Hippocampal Neurons



① *Rat embryo*

② *Brain 채취*

③ *Hippocampus 채취*

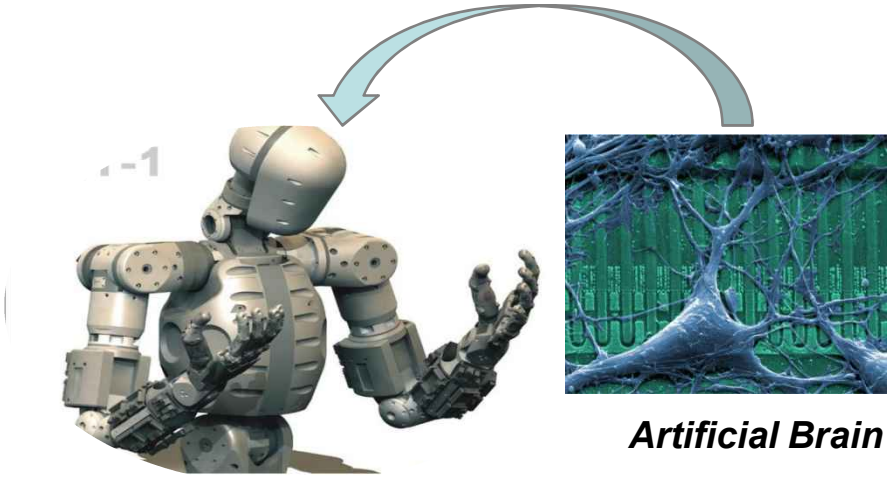
④ *Dissociation*

⑤ *Cell counting*

⑥ *Cell Seeding*

Reference) http://www.jove.com/index/details.stp?ID=895 *(8 min)*

How to design neural networks (micro-contact printing)



Artificial Brain

Logic Circuits made by neurons

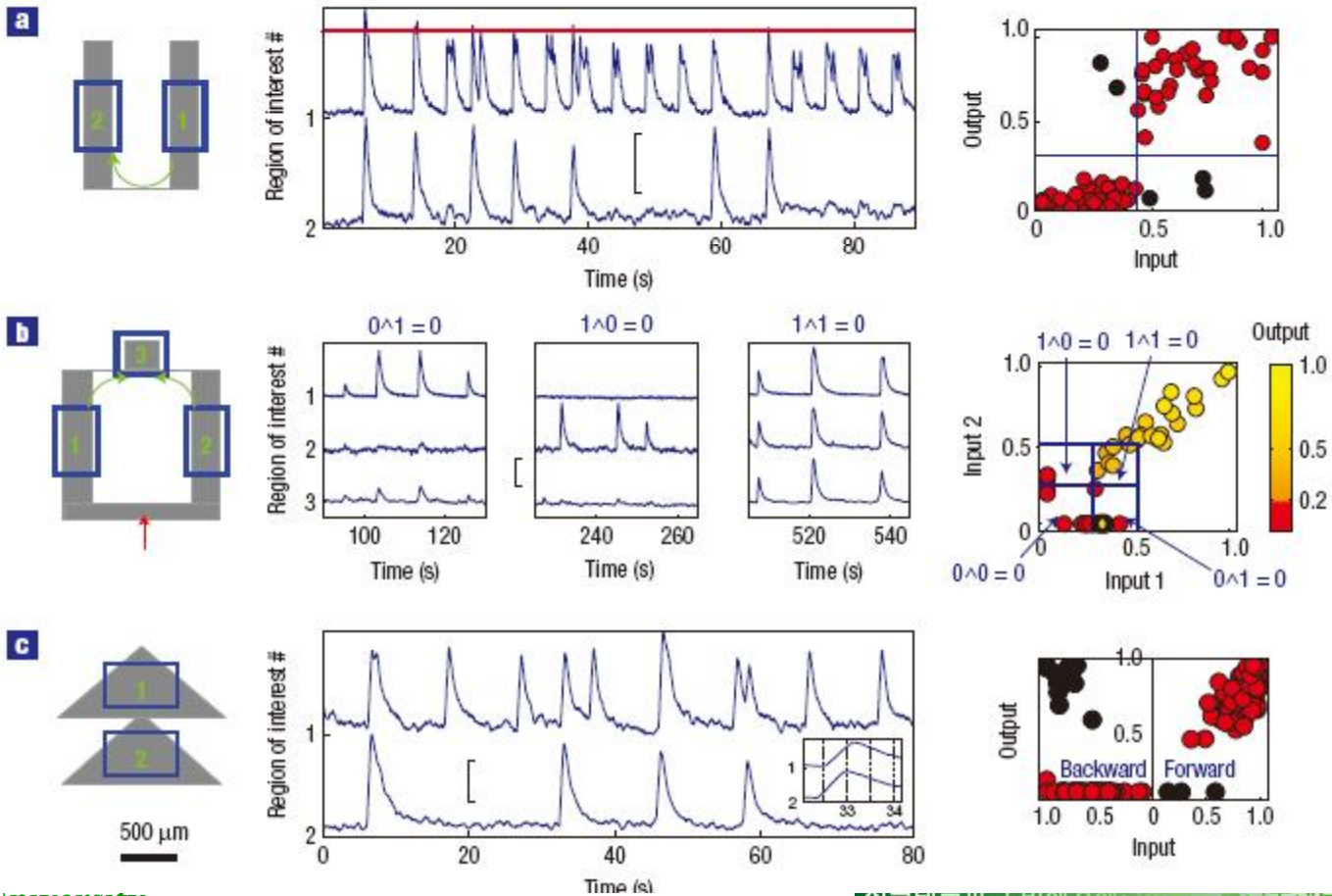
Neuronal Logic Devices from Patterned Hippocampal Cultures

nature physics | VOL 4 | DECEMBER 2008 | www.nature.com/naturephysics

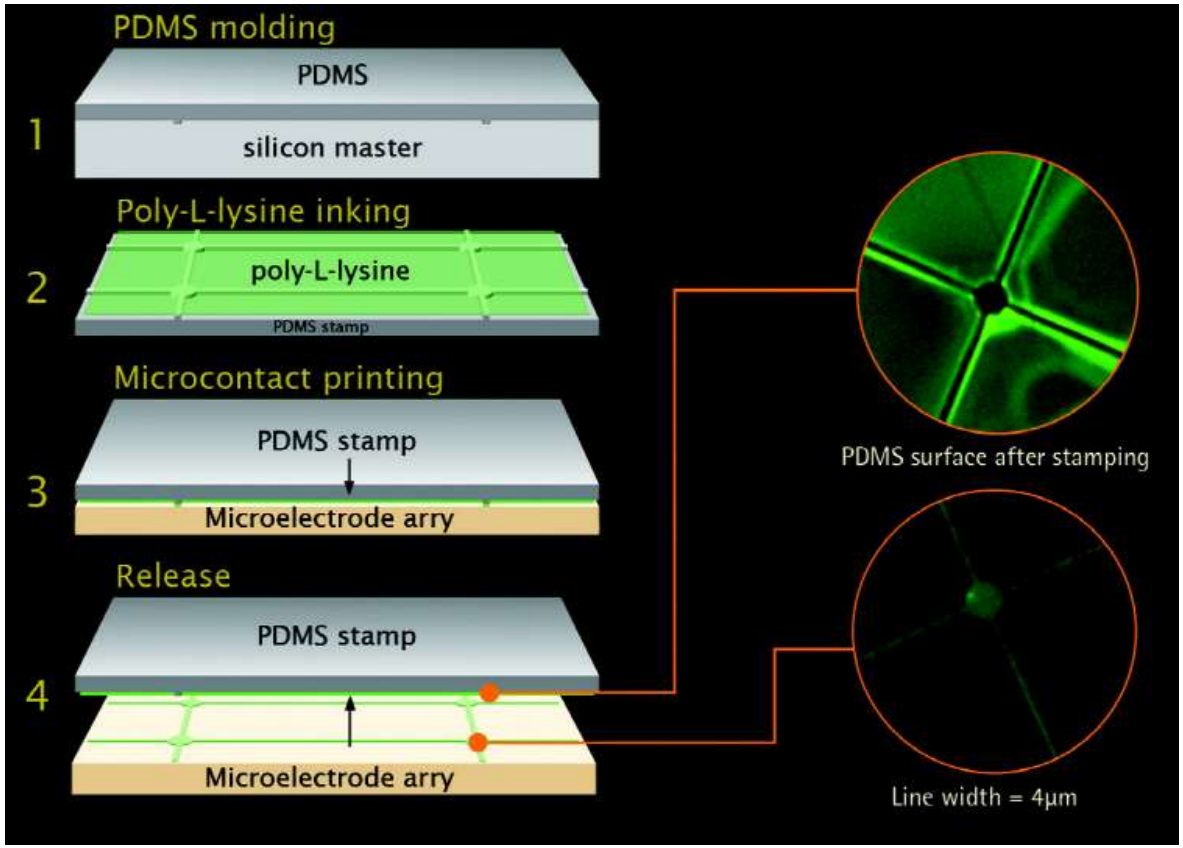
Threshold component

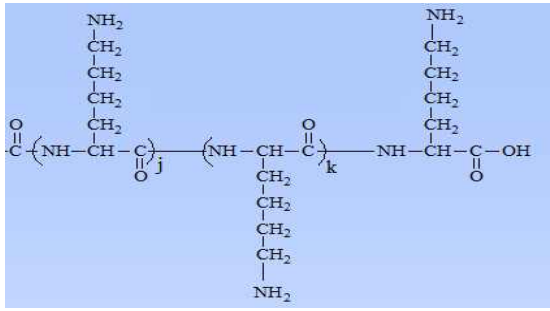
AND Gate

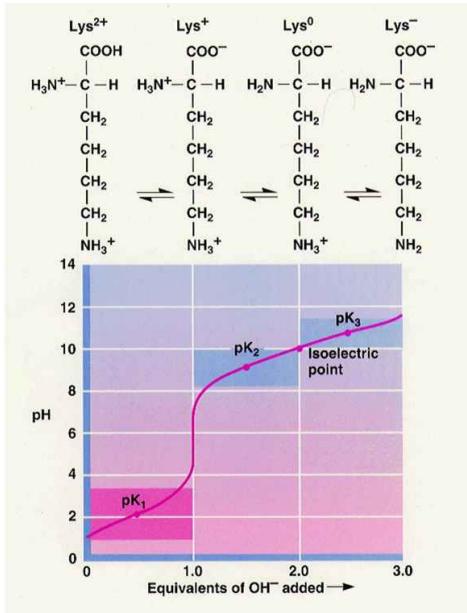
Neural Diode



Procedure of Micro-Contact Printing







Reference) http://www.jove.com/index/details.stp?ID=1065 *(10 min)*

Micro-contact printing

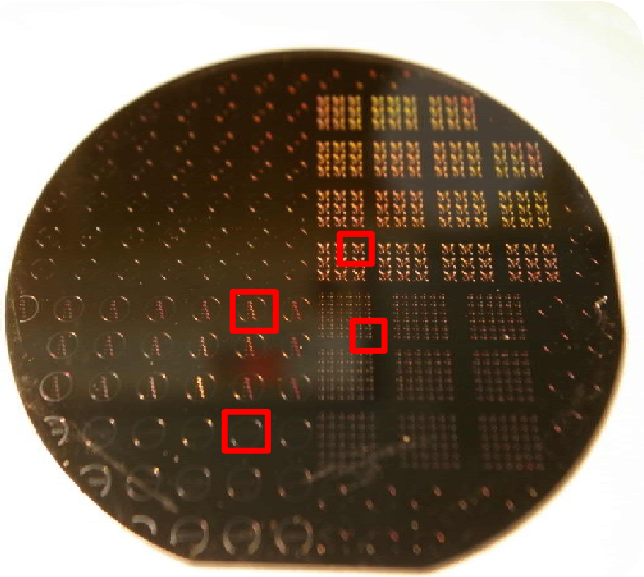
Advantages

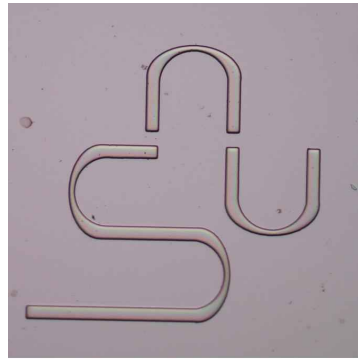
- Fast, simple, and inexpensive
- High resolution
- Not require clean room instrumentation
- Not require absolutely flat surface

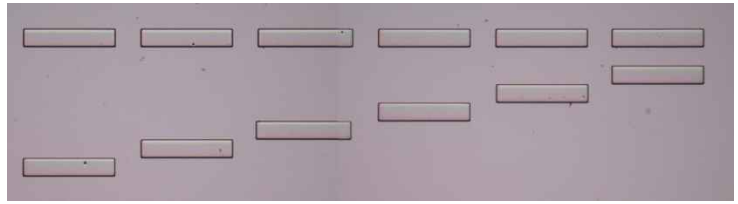
Disadvantages

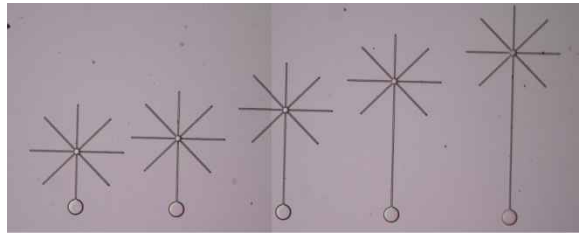
- Dry surface
- Require alignment with MEAs
- Restriction in fine controls (ex. cell positioning,…)

Stamp Master & stamp 제작









<제작과정>

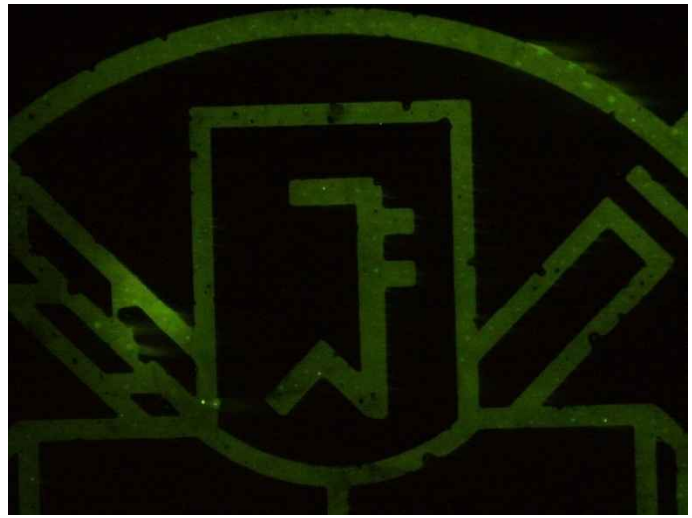
① Cadense 로 Mask Design

② Photolithography & Deep-etching

③ PDMS Molding

Plasma treatment

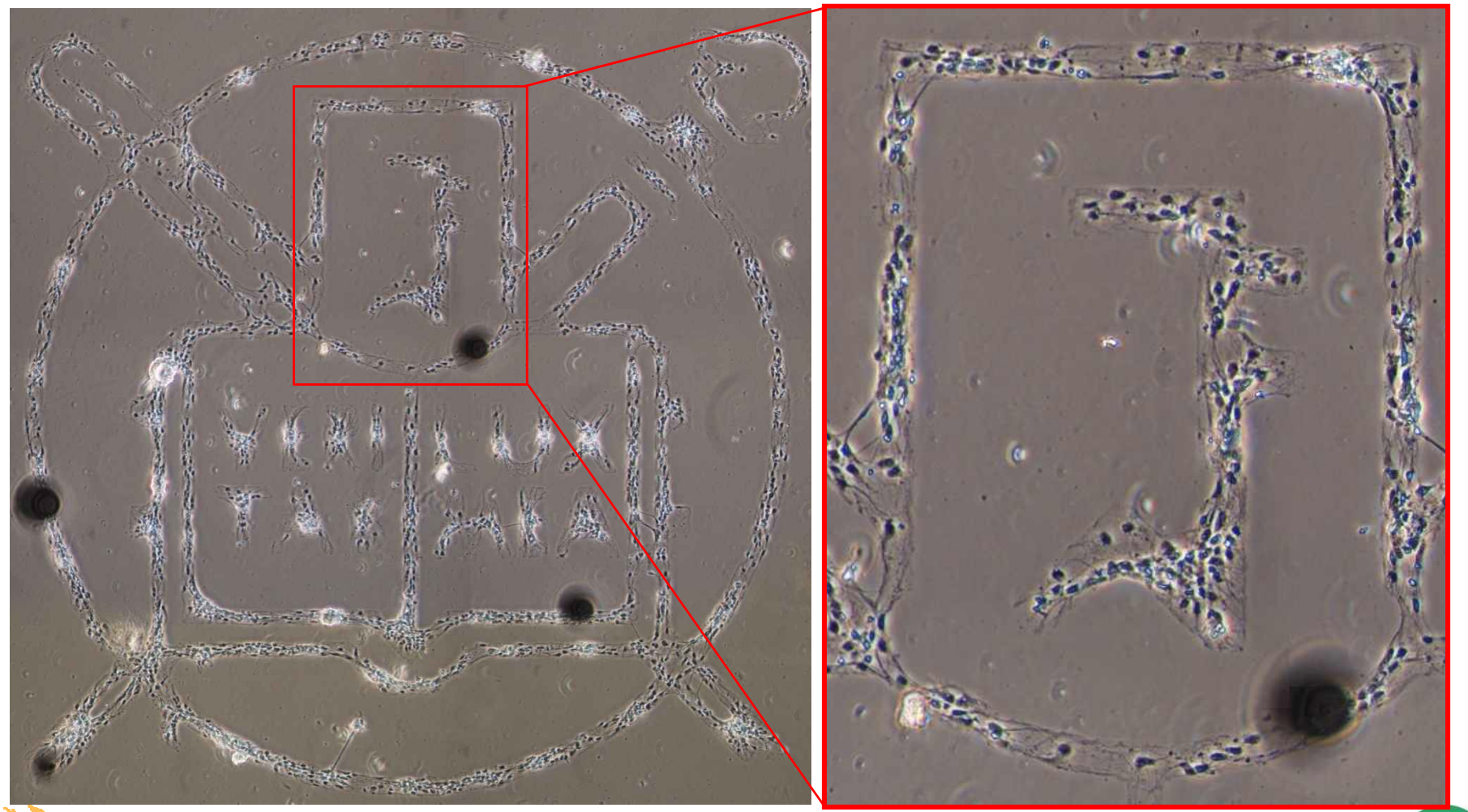
<산소 플라즈마 처리를 하지 않은 경우>



<산소 플라즈마 처리를 한 경우>

- 산소 플라즈마 처리는 유리의 표면을 친수성으로 만들어 주어 상대적으로 소수성인 PDMS 도장으로부터 FITC-PLL 이 유리로 잘 옮겨 오도록 도와준다.

SNU made by Hippocampal Neurons



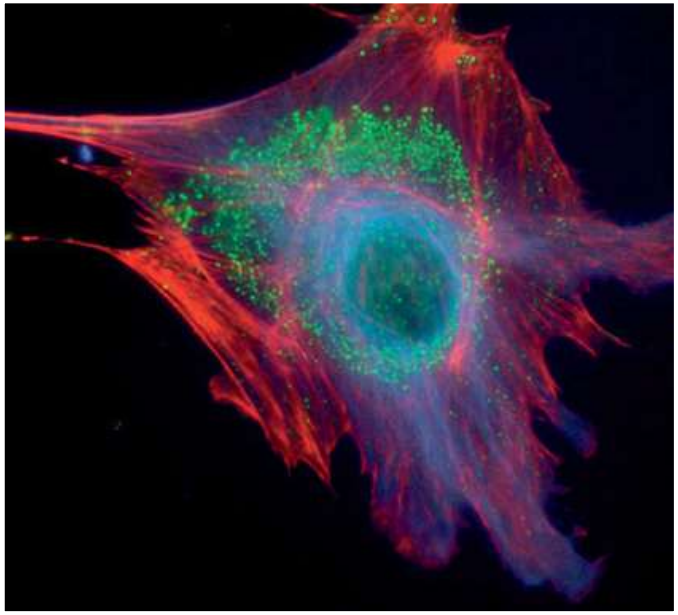
Cell growth on patterned protein

How to Image to verify the networks (immunostaining)

Immunostaining

- **Purpose** : to verify the cell morphology, the conformation of synapses, the location of target protein and so on.

- **Methods** : to use antibodies for the antigen-antibody reactions



Endothelial cell

Blue : tubulin

Red : F-actin

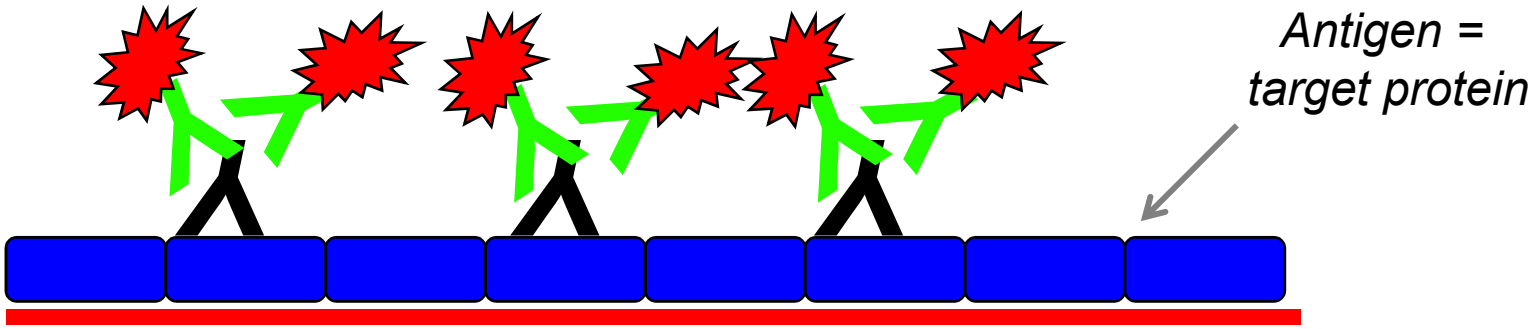
Green : endosomes

Most common method (Indirect Immunofluorescence)

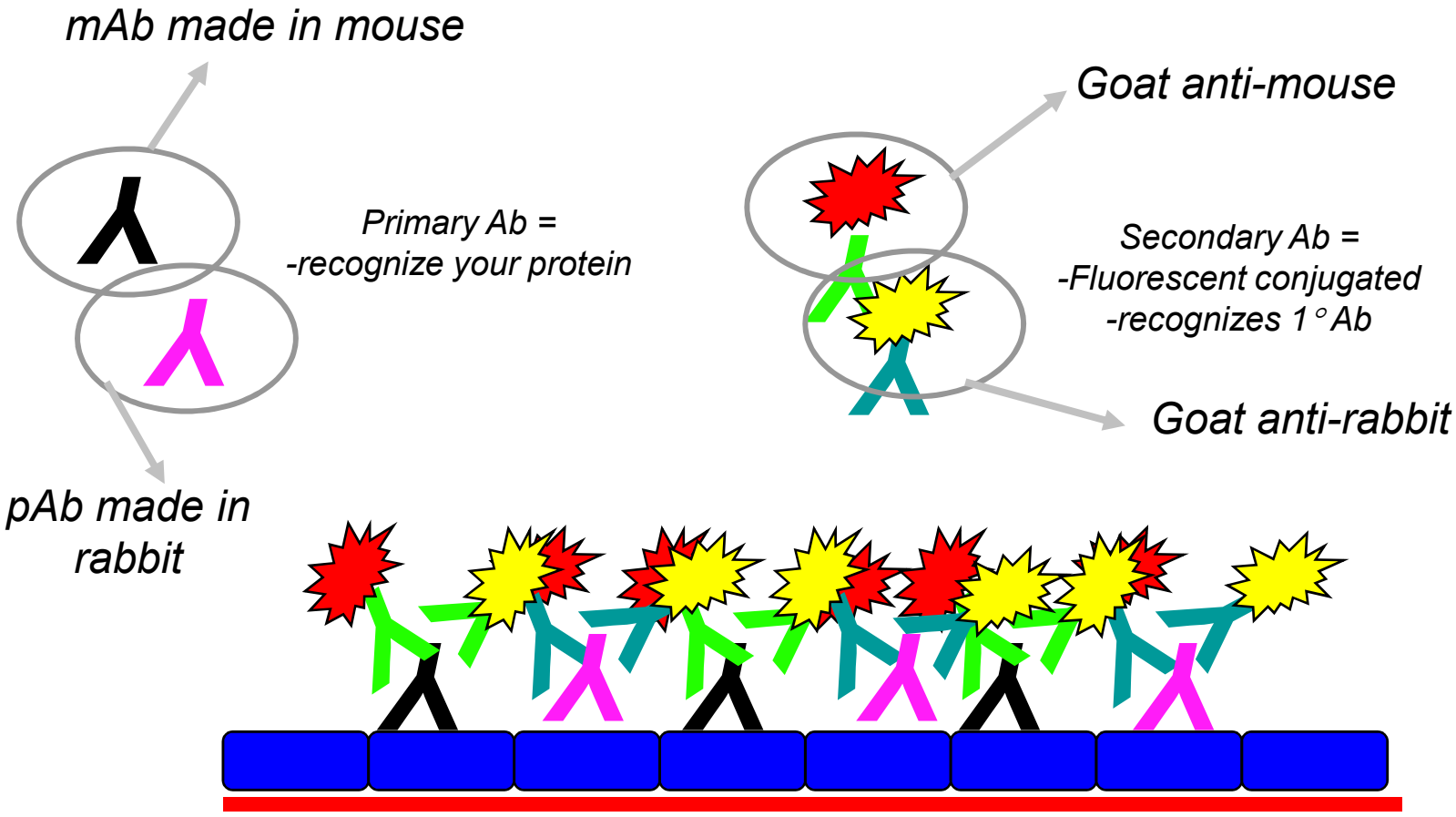
Double antibody technique for signal amplification

Primary Ab =
-recognize your protein

Secondary Ab =
-Fluorescent conjugated
-recognizes 1° Ab



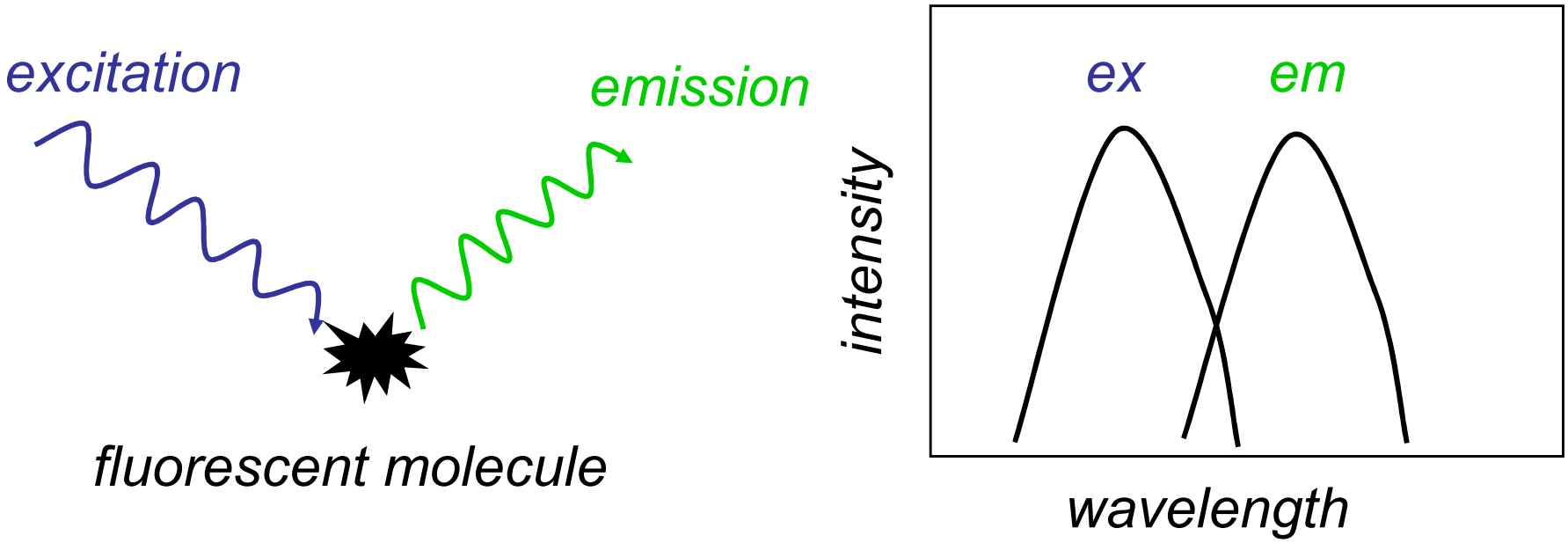
Multiple labeling



Immunostaining Procedure

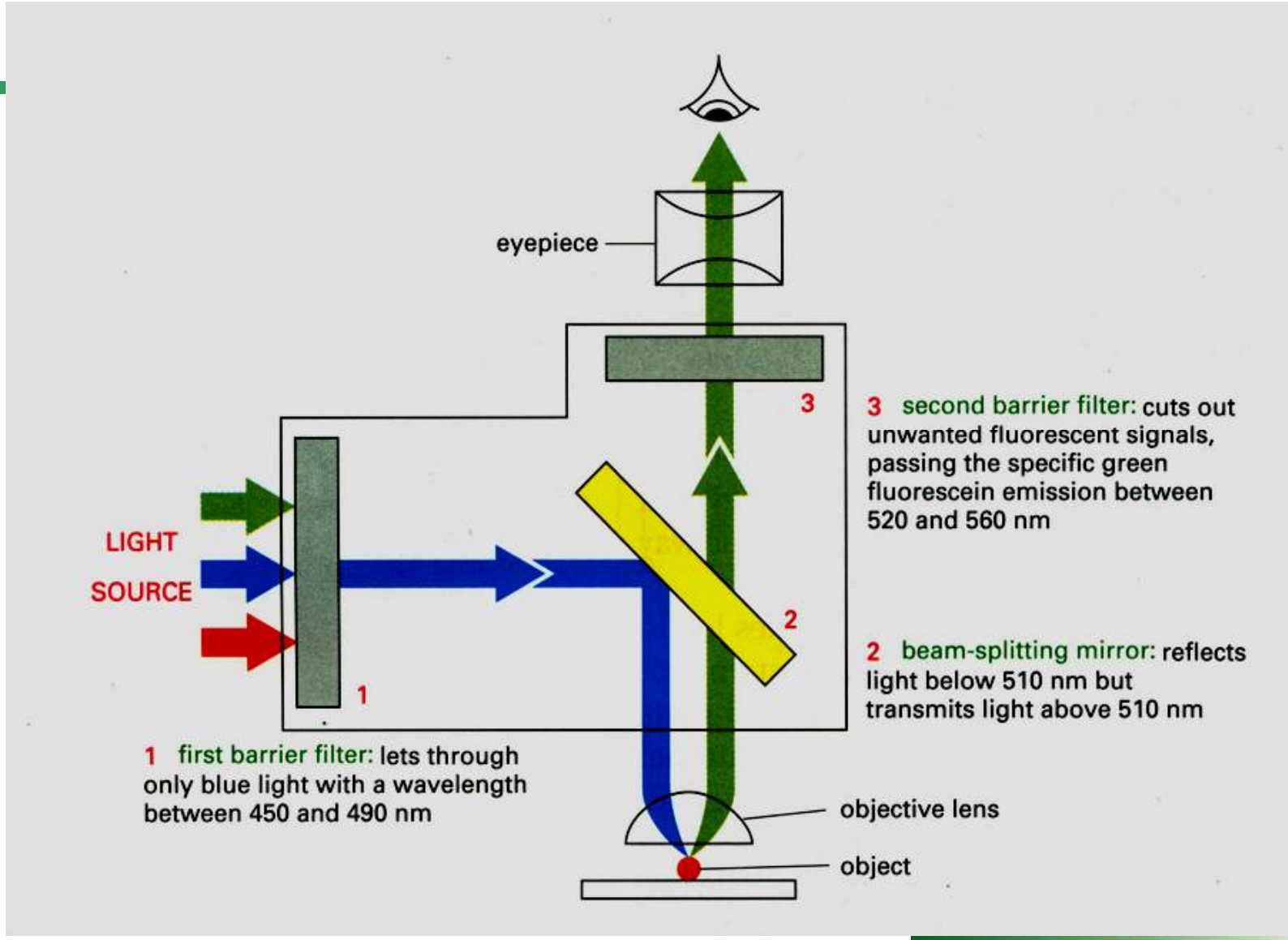
- Prepare sample
- Fixation
  - to preserve the cells/tissue and to immobilize the antigen
- Permeabilization
  - to “punch holes” in the cell membrane so antibodies can diffuse in to bind the target protein
- Blocking
  - to eliminate non-specific binding of antibodies
- Primary antibody incubation
- Secondary antibody incubation
- Prepare for viewing (mounting)

Fluorescence microscopy



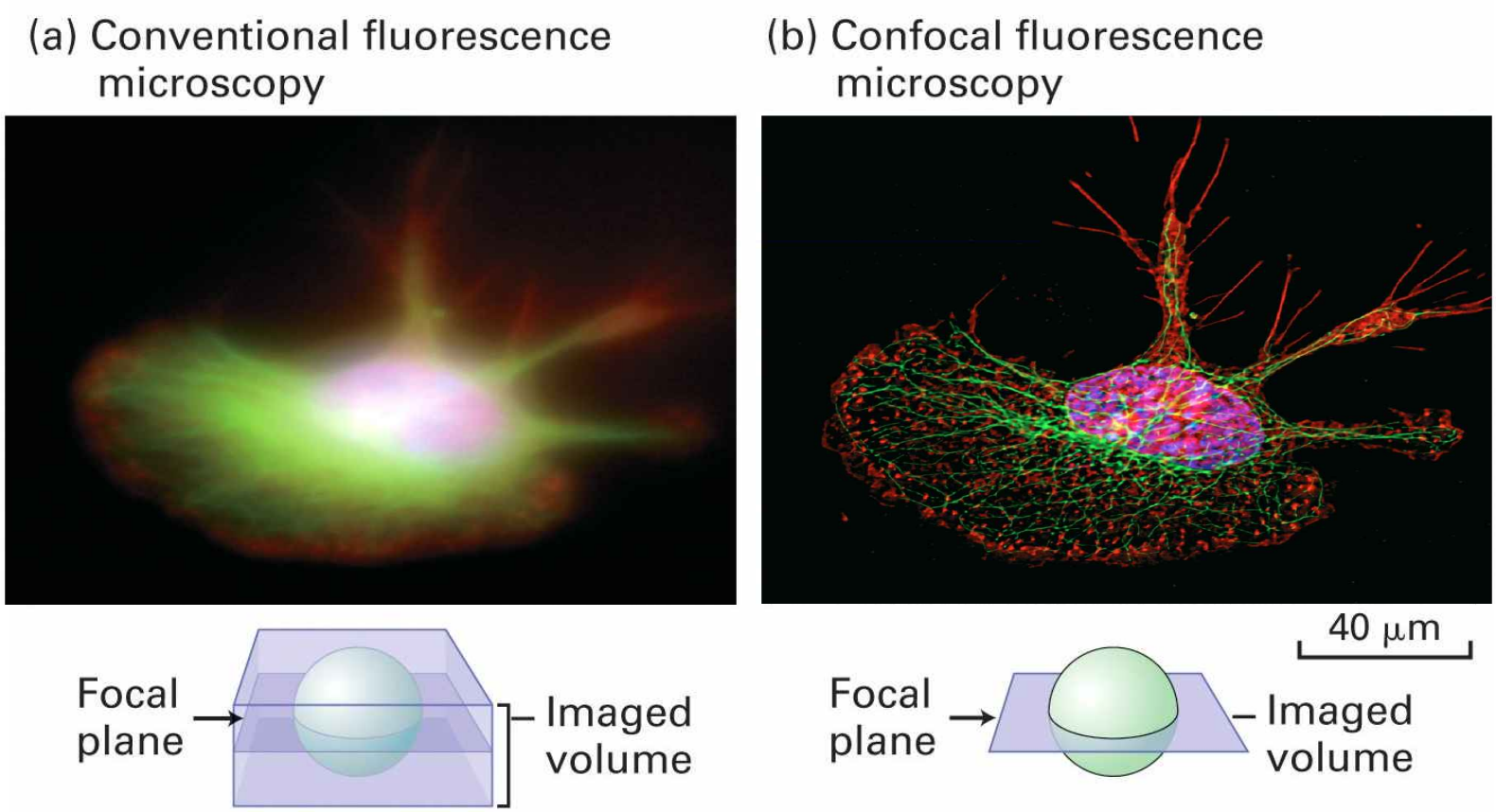
Fluorochrome	Excitation wavelength	Emission wavelength
Fluoroscein	490 - blue	520 - green
Rhodamine	550 – green	580 - red
Hoechst (stains DNA)	345 - UV	455 - blue

Fluorescence microscope

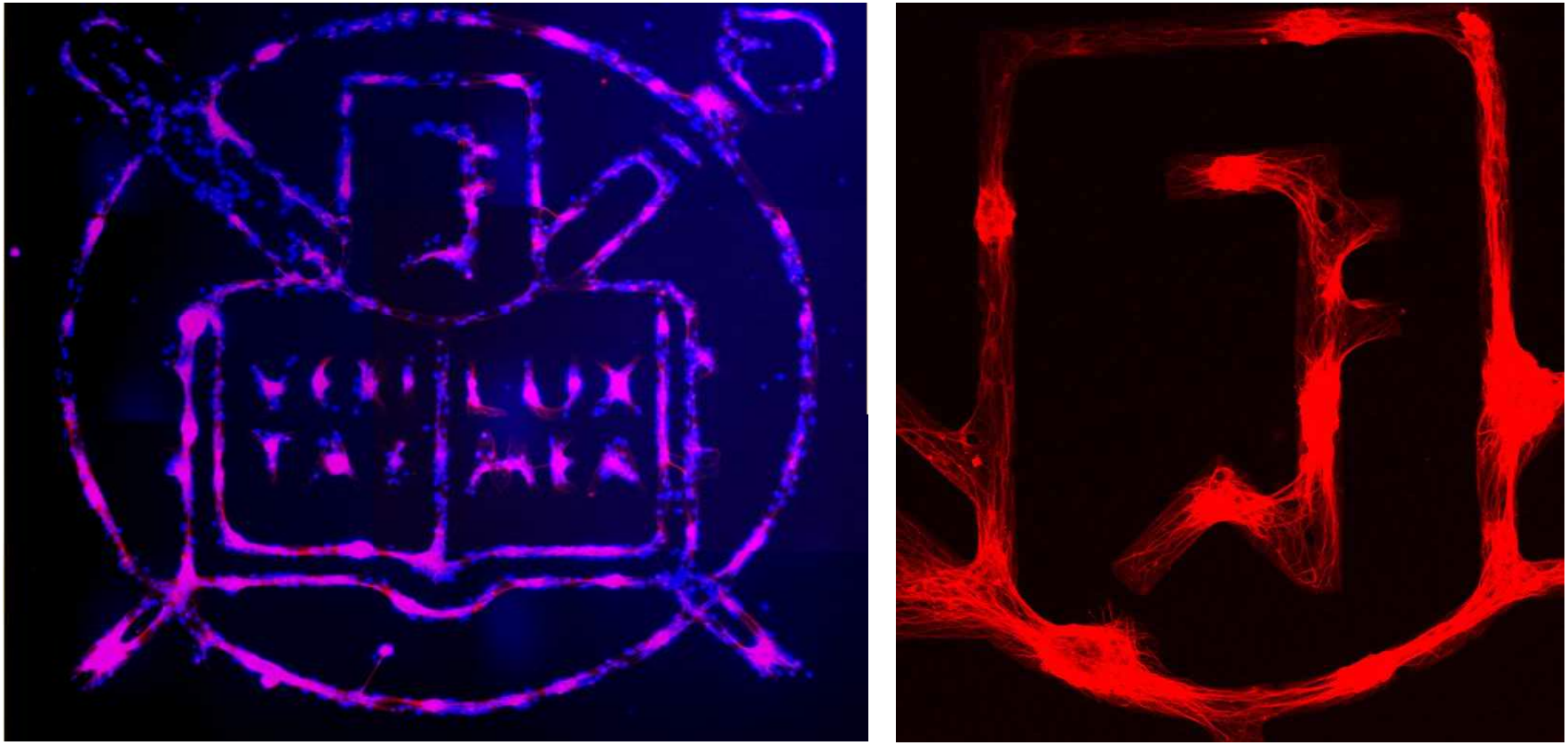


Confocal microscope image

limitations of Fluorescence microscopy : Blurrred images, Thick specimens



immunostaining image of SNU neural network



Reference) http://www.jove.com/index/details.stp?ID=1173 *(15 min)*

Experiment Session Continued...