

Visualizing cell Chapter 9

세포생물학

08.28.25 미생물학교실, 박용환

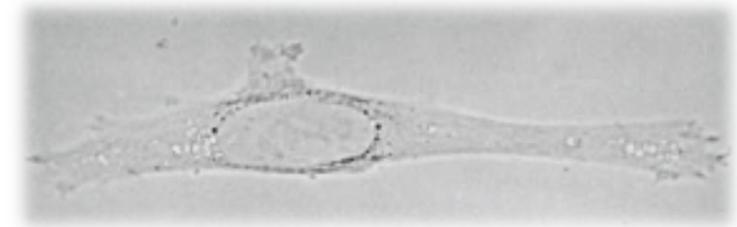
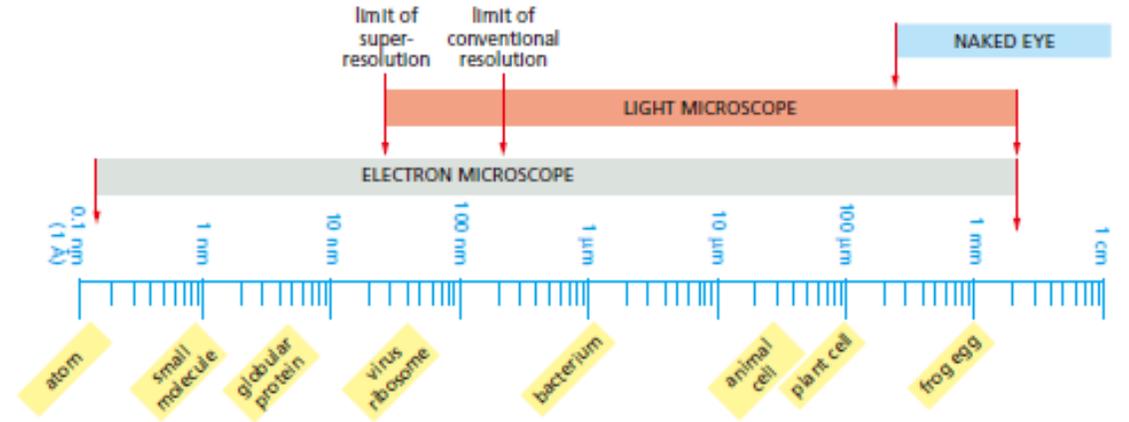
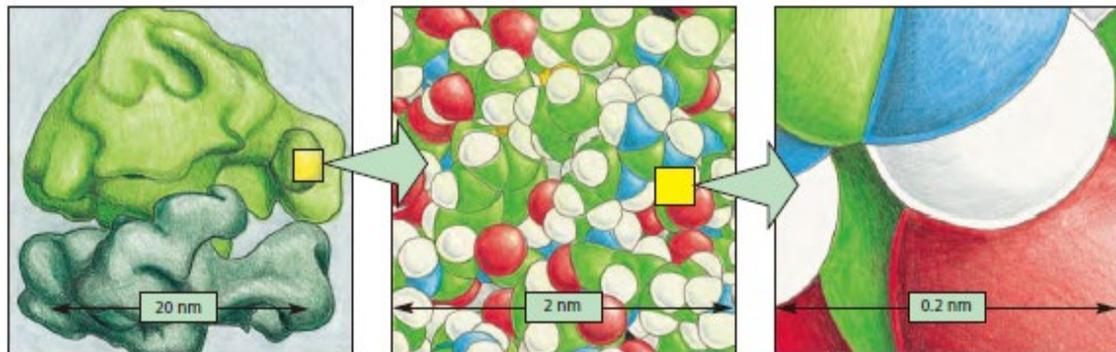
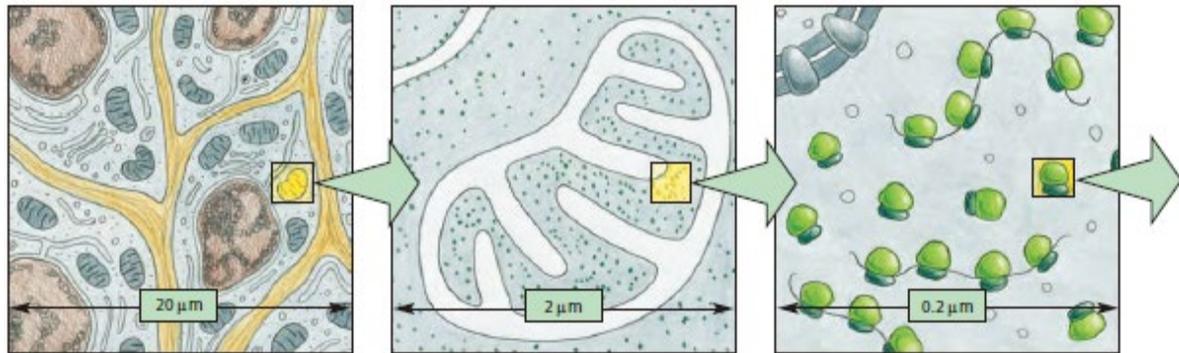
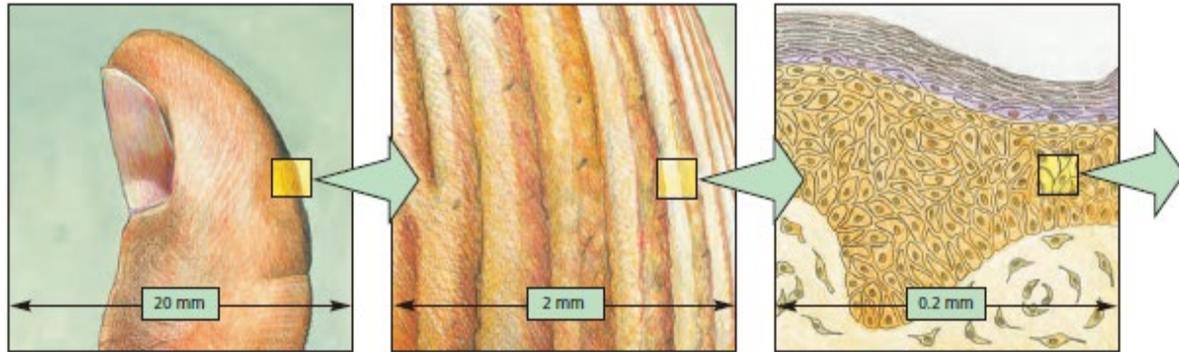
현미경의 개발& 노벨상

물리/화학 분야 ↑ ⇒ 생물 ↑

- ✓ 네덜란드의 물리학자 제르니케(Frits Zernike; 1888-1966): 위상차 현미경을 발명하여 1953년 노벨 **물리학상**
- ✓ 독일의 전기공학자 루스카(Ernst Ruska; 1906-1988): 1986년에 노벨 **물리학상**을 수상
- ✓ 독일의 비니히(Gerd Binnig; 1947-)와 스위스의 물리학자 로러(Heinrich Rohrer; 1933-): 전자현미경보다 훨씬 성능이 좋은 주사형 터널링 현미경(Scanning tunneling microscope; STM)개발로 1986년 공동 노벨 **물리학상** 수상
- ✓ 녹색형광단백질 GFP의 발견과 개발에 관한 업적: 2008년 노벨 **화학상** 수상
- ✓ Eric Betzig, Stefan W. Hell and William E. Moerner: 2014년 노벨 **화학상**, for optical microscopy into a new dimension using fluorescent molecules.
- ✓ Jacques Dubochet, Joachim Frank and Richard Henderson: 2017년 노벨 **화학상**, for the development of cryo-electron microscopy

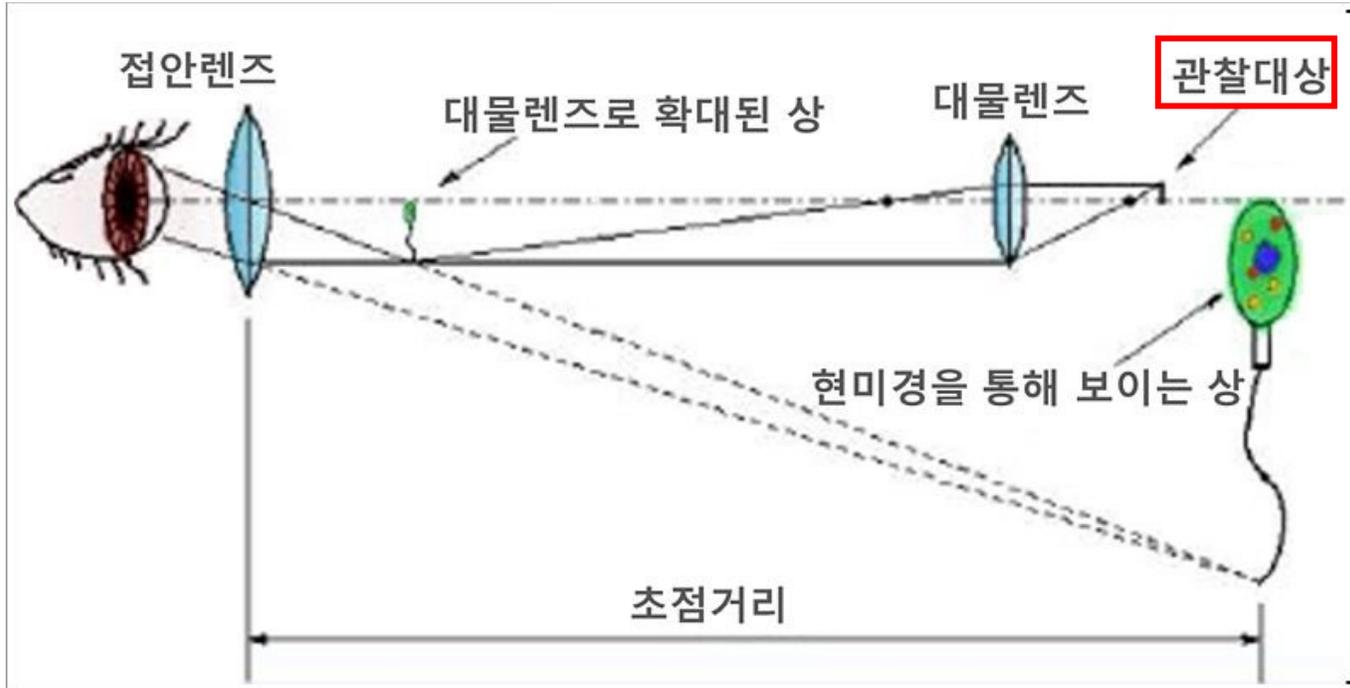
Introduction & Contents

동물세포

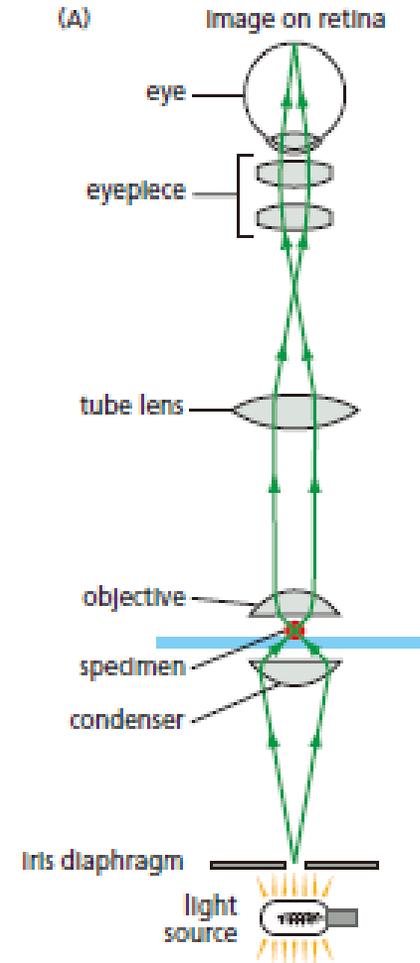


- ✓ 광학 현미경
- ✓ 염색 & GFP
- ✓ 전자 현미경

Optical microscope (Light microscope) 원리



IBS(기초과학연구원)



Optical microscope (Light microscope) 한계; 에돌이 효과 (회절: diffraction)

현미경의 분해능 (resolution): 두개의 점을 구별할 수 있는 최소 거리 - 얼마나 작은 것까지 볼 수 있는가?

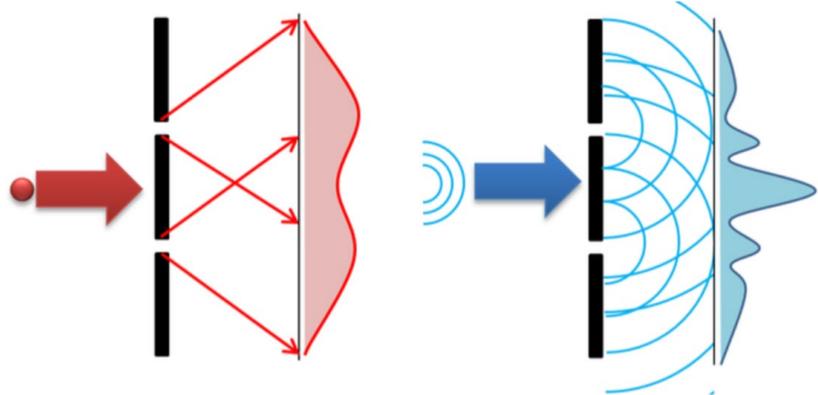
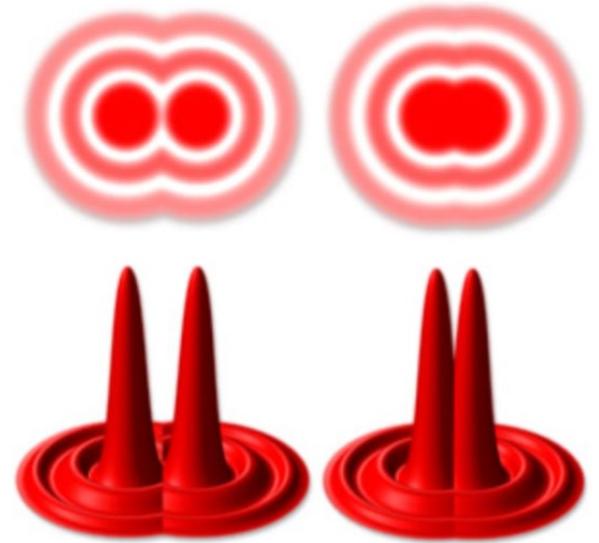
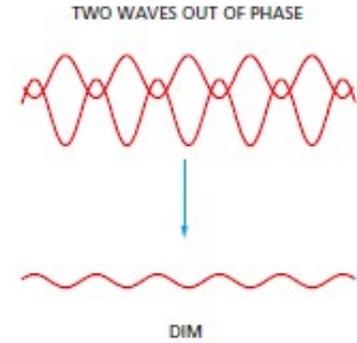
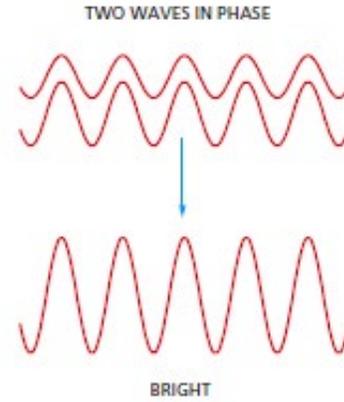
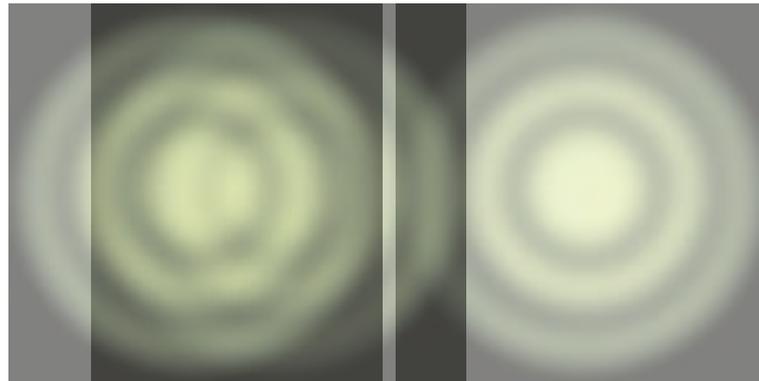
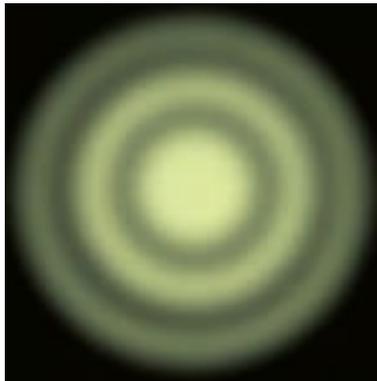


그림 6. 빛이 입자임을 가정했을시 (왼쪽), 빛이 파동임을 가정했을시 (오른쪽)

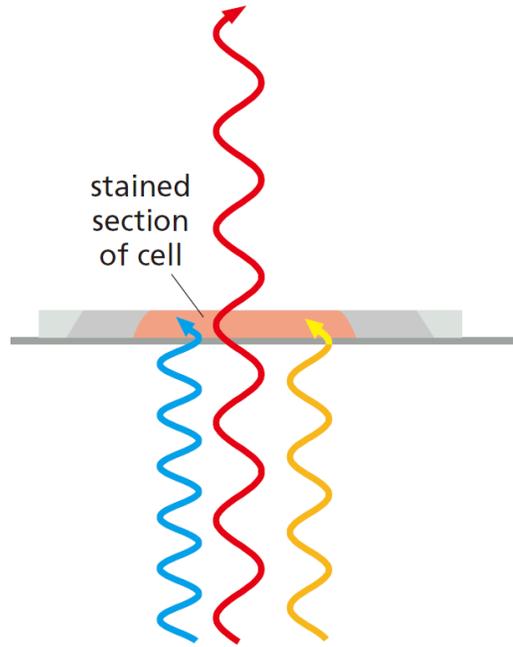
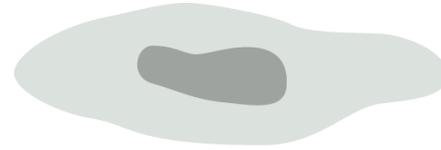
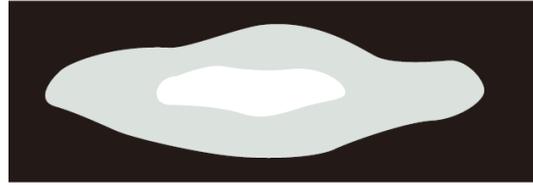
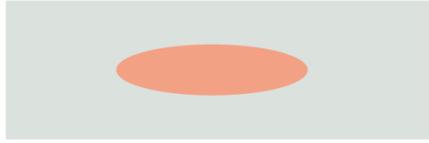
Credit: soma0sd.com



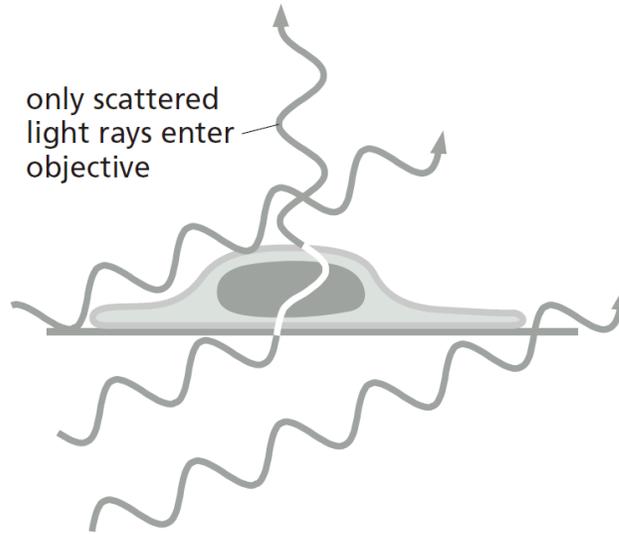
Credit: Zeiss



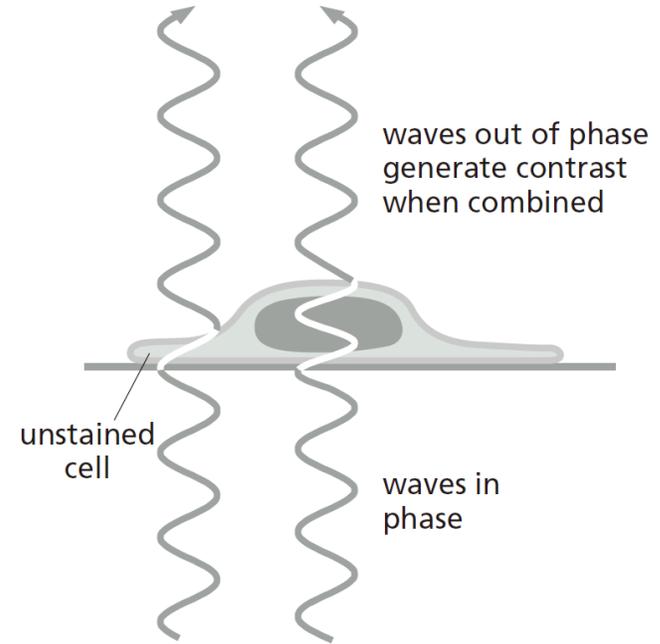
광학 현미경의 대비; contrast



(A) incident light (white)



(B) oblique incident light



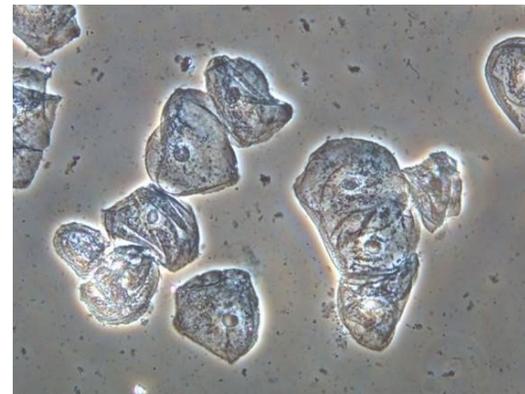
(C) incident light (white)

위상차 현미경 (phase contrast microscopy)

- ✓ 무색 투명한 시료라도 내부의 구조를 뚜렷하게 관찰할 수 있도록 한 특수한 현미경
- ✓ 두께가 다른 혹은 굴절률이 다른 매질: 빛이 통과하는 경로 길이가 달라지거나 서로 다른 굴절률을 가진 매질을 연속해서 통과할 때, 각각의 매질에서의 속도 차이로 인해 위상차가 발생
- ✓ 물질을 통과한 빛이 물질의 굴절률의 차이에 의해 위상차를 갖게 되었을 때 이를 명암으로 바꾸어 관찰
- ✓ Live cell imaging/ No staining
- ✓ 1935년 독일의 F.제르니커에 의해서 개발, 1953년 노벨 물리학상



'위상차용' 대물렌즈 (렌즈에 PH : Phase 라 표기)와 위상차 콘덴서



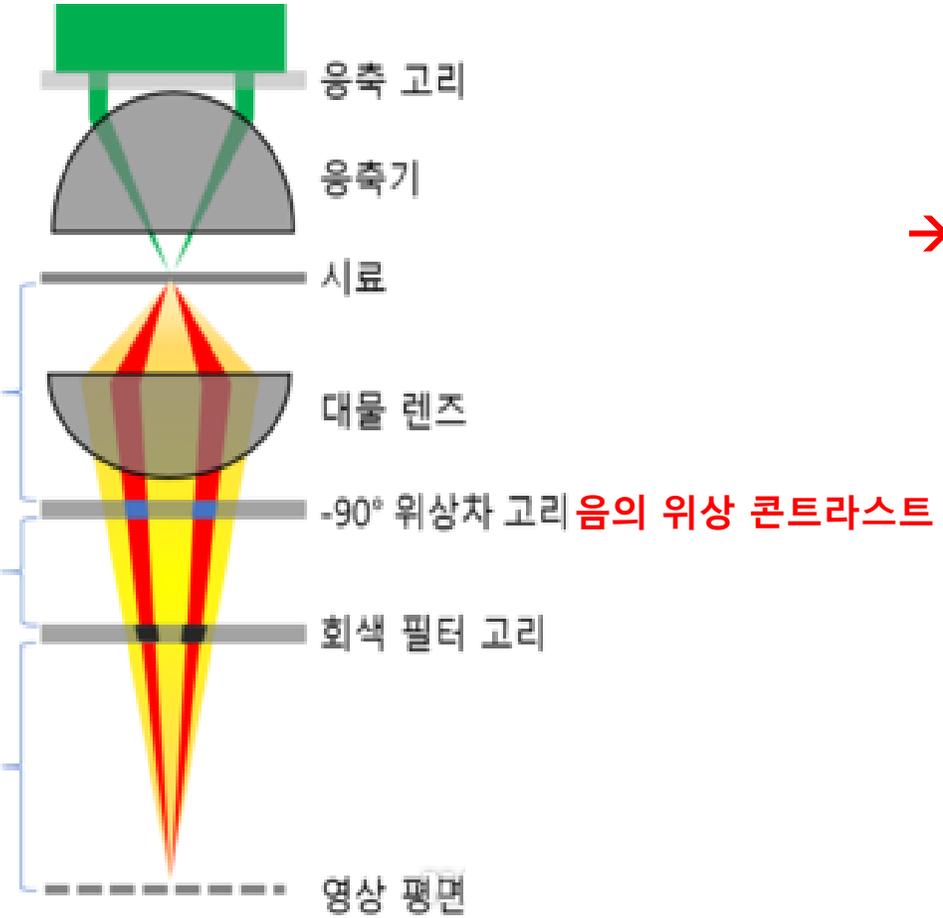
위상차 현미경 원리

- 조명등
- 배경 광선
- 시료에서 산란된 광선
- 전경 = 배경 광선 + 산란 광선

산란 광선 : -90° 위상차
전경 \approx 배경

배경 광선 : -90° 위상차
전경 $>$ 배경

배경 광선 흐릿해짐.
전경 \gg 배경

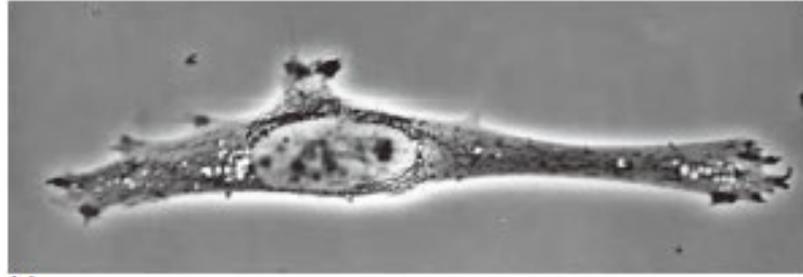


→ 빛의 위상차를 명암 차이로 변환

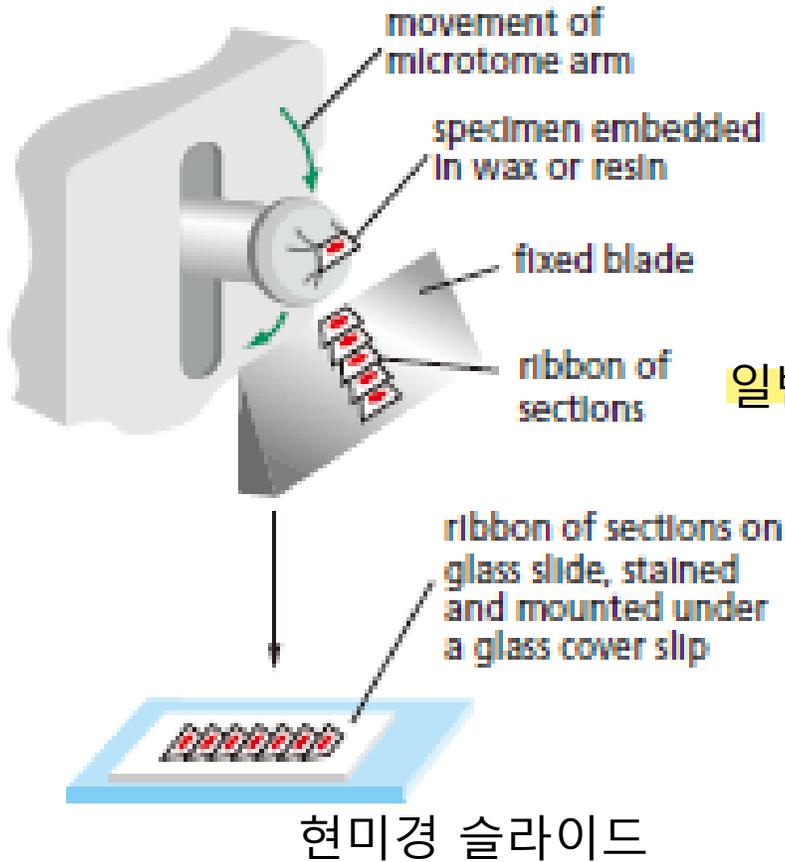
한국물리학회

1. 회절광은 직진광의 위상에 비교하여 대략 $1/4\lambda$ 지연
2. 직진광은 위상판에 의하여 $1/4\lambda$ 또는 $3/4\lambda$ 지연 (강제적)
3. 직진광이 위상판에 의하여 $1/4\lambda$ 지연되어 회절광과 동일한 위상을 갖게 되면 직진광과 회절광의 위상이 서로 보강 간섭 (배경 직진광만 존재) → 샘플이 배경 보다 밝게 보임 (Negative contrast)

시험문제
예시



Intact Tissues Are Usually Fixed and Sectioned Before Microscopy



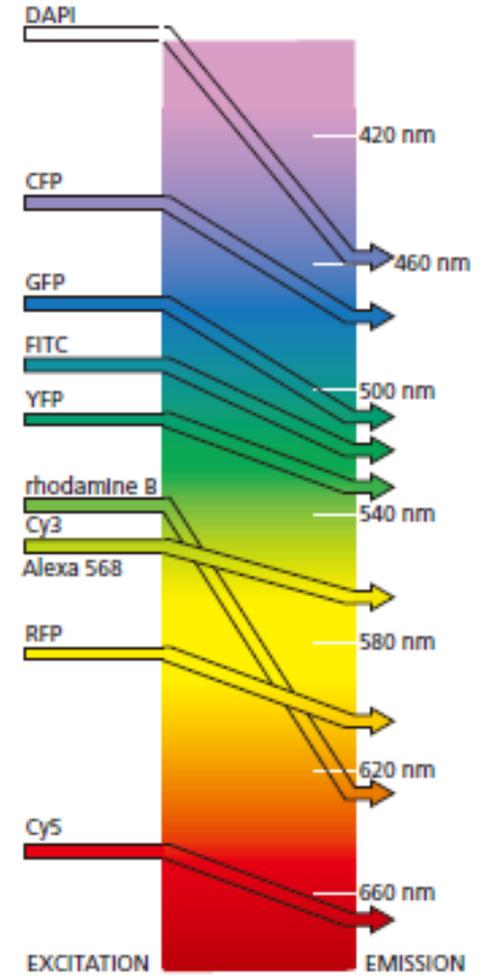
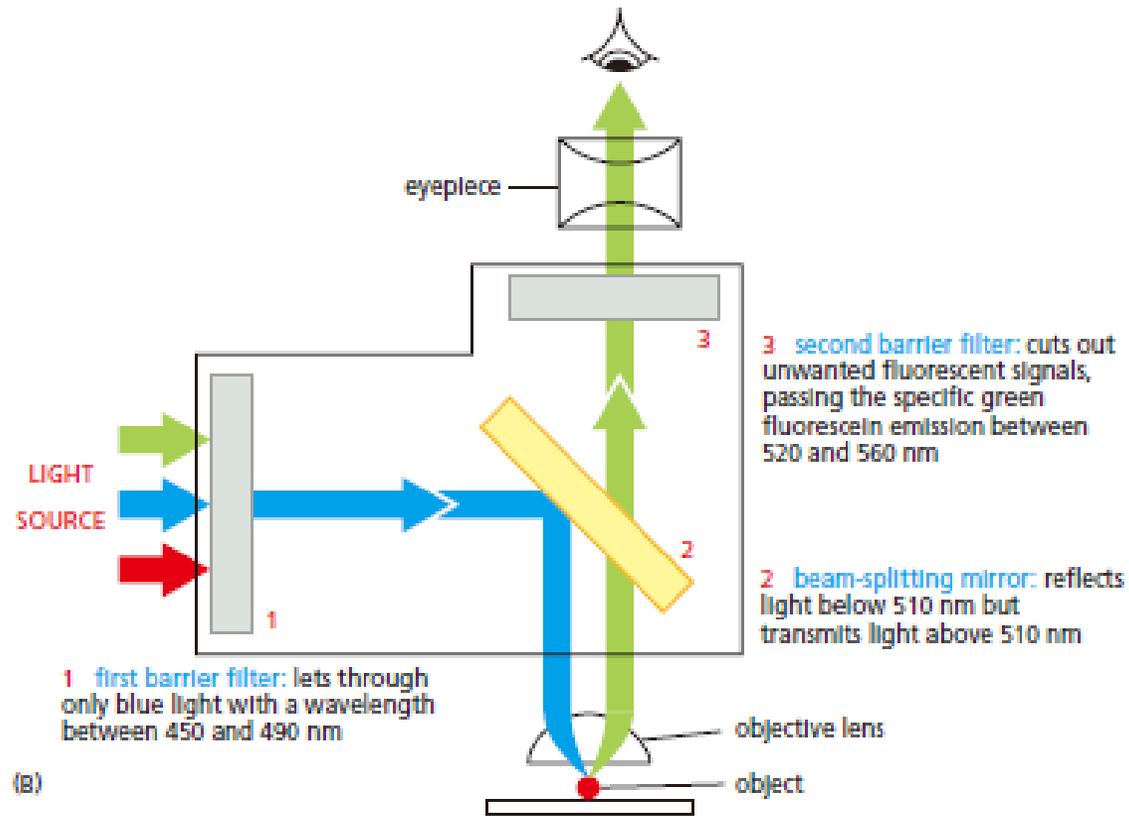
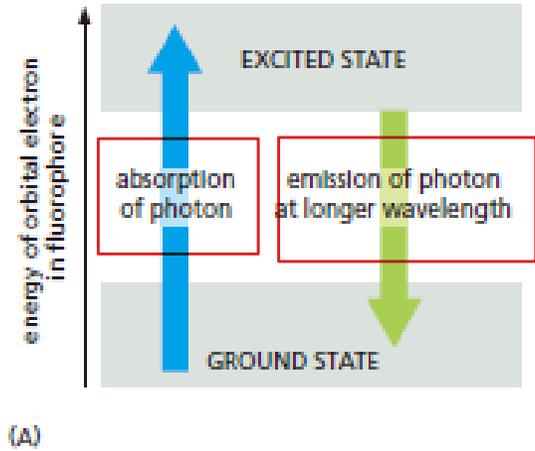
일반적으로 0.5-10 μ m 두께

↪ 온도가 조절 수 있음
↪ 알코올이면 안됨 → 공중 열 → 파라핀 X

고상 보편 안보임

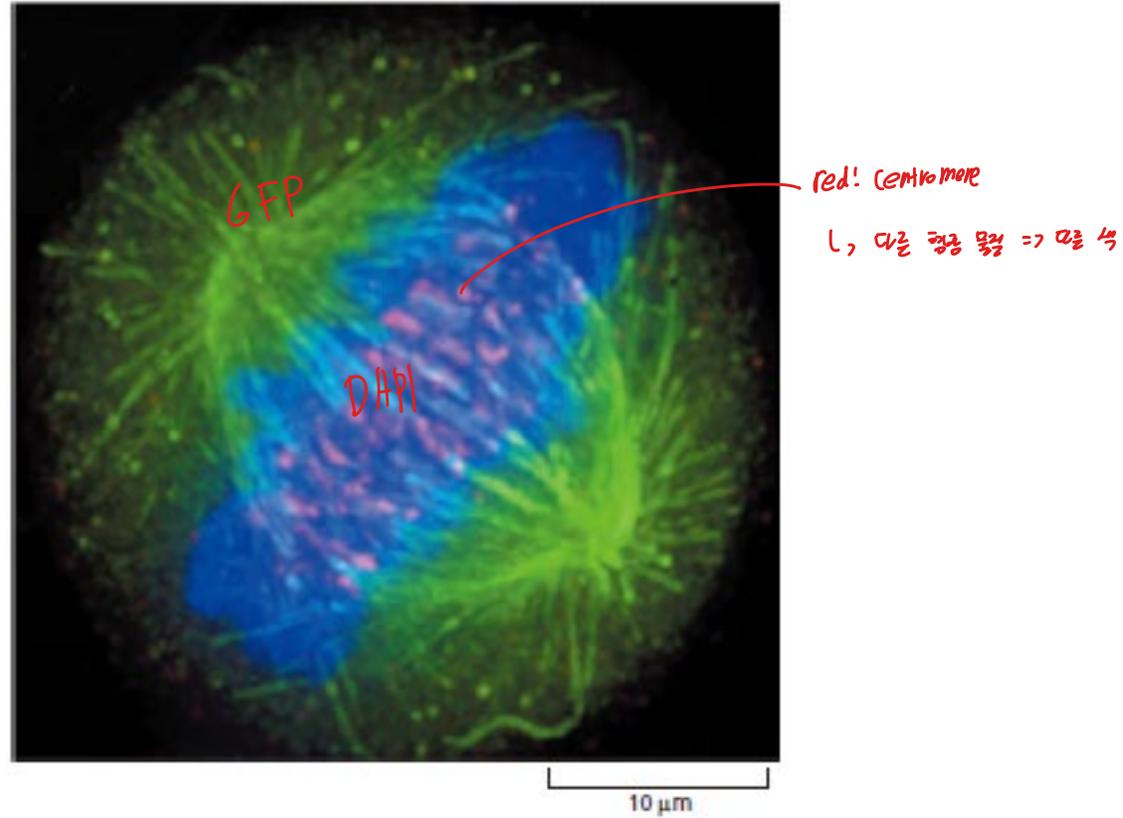
- ❖ DNA/RNA/산성 단백질 염색
- ❖ 안티바디를 활용해서 단백질의 발현 비교
- ❖ 특정 단백질의 위치를 형광 probe와 marker를 이용 확인

형광의 원리, 형광 현미경



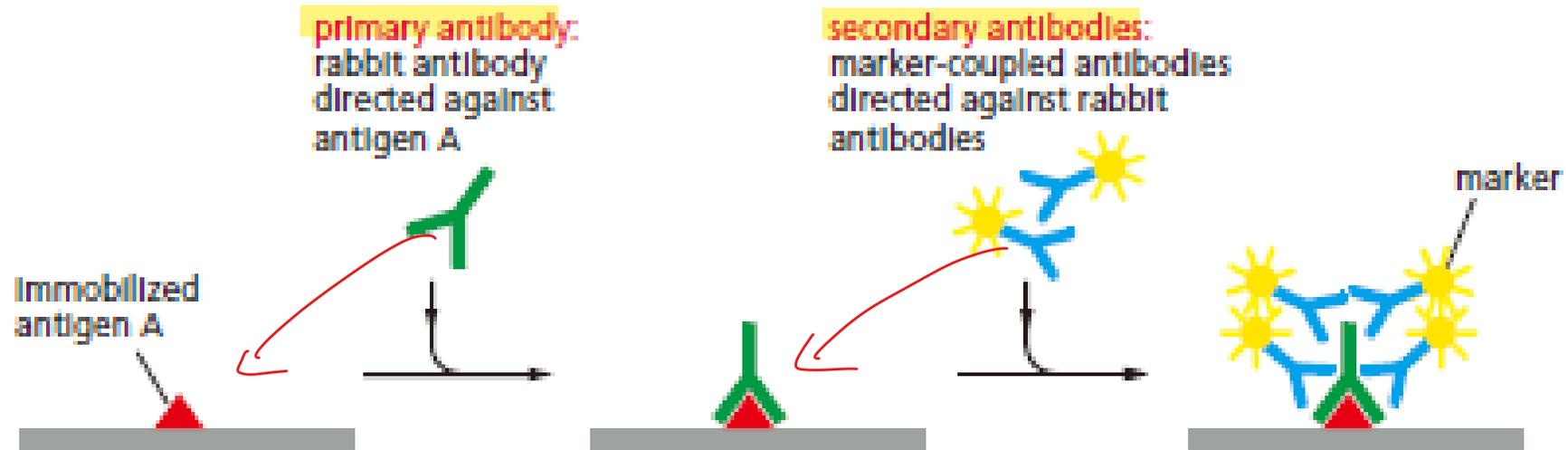
각각 다른 파장이 다 있음

시험 예시: different fluorescent probes can be visualized in the same cell



The spindle microtubules are revealed with a **green** centromeres with a **red** , and the DNA of the condensed chromosomes with the **blue** fluorescent dye **DAPI**

Antibodies Can Be Used to Detect Specific Molecules

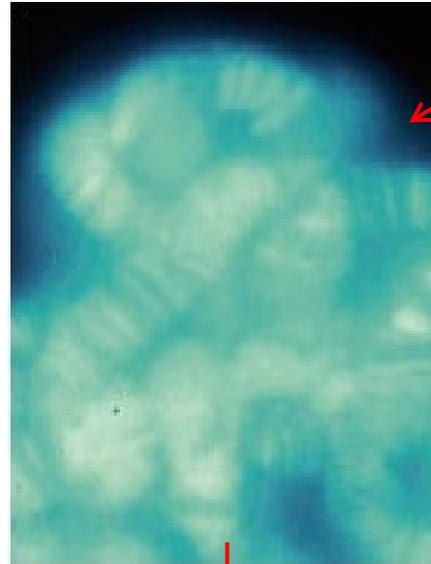


3D images with the Optical Microscope

Image deconvolution (이미지 복원) (computer program)

- ✓ PSF (Point Spread Function):
빛이 주변으로 퍼지는 정도
- ✓ 광학 현미경 분해능 한계, 샘플의 두께, 초점의 부정확성 등으로 인한 이미지 왜곡
→ 복원 알고리즘 도입으로 복원

computed tomography (CT) scanner:
단층 촬영



(A)



(B)

5 μm

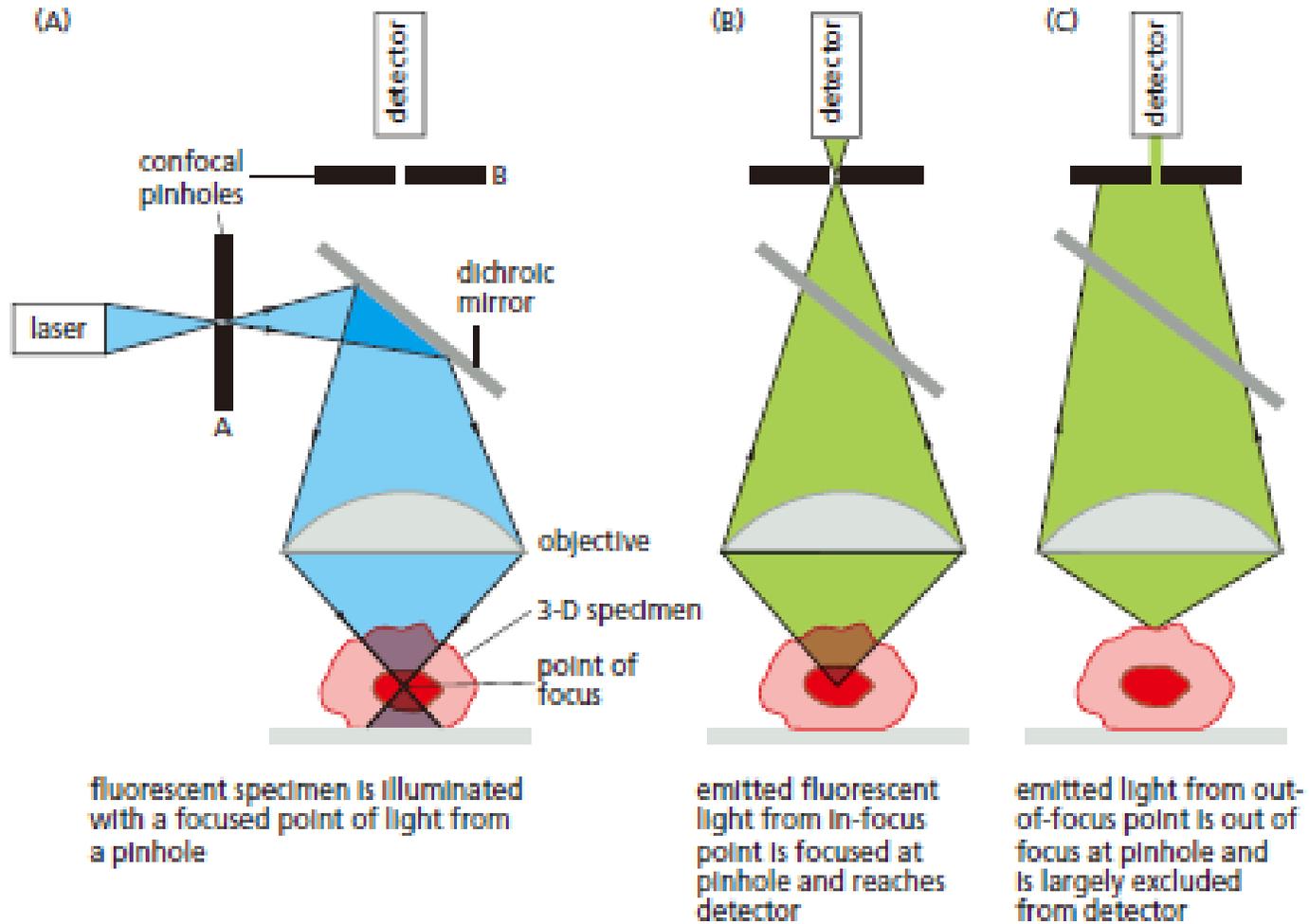
(A) A light micrograph of the large polytene chromosomes from *Drosophila*, stained with a fluorescent **DNA-binding dye**

흐릿/불명확

선명해짐

(B) The same field of view after image deconvolution. Each band is about 0.25 μm thick, approaching the diffraction limit of the light microscope.

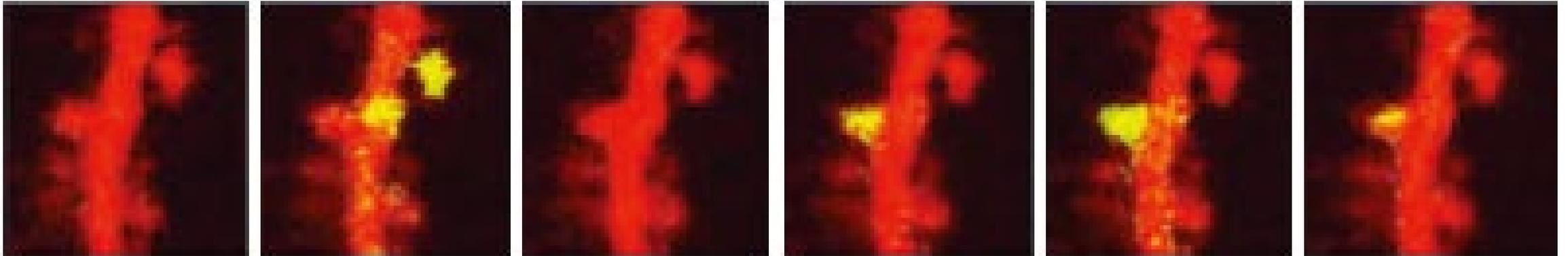
The Confocal Microscope Produces Optical Sections by Excluding Out-of-Focus Light



Deconvolution은 표본에 약 $40\ \mu\text{m}$ 까지/ 공초점 현미경은 약 $150\ \mu\text{m}$ 깊이까지만 이미지를 얻을 수 있음

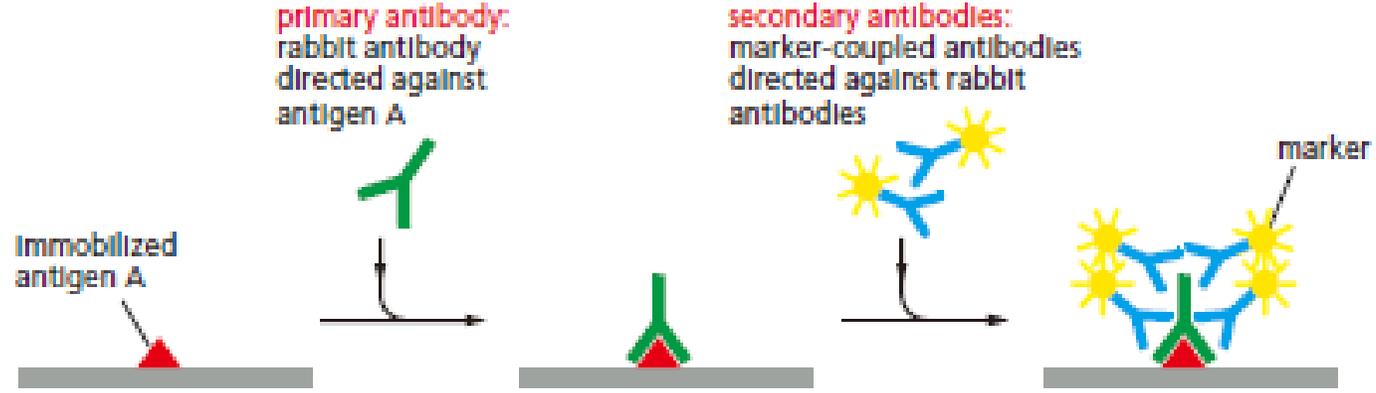
Multiphoton imaging

↳ 2개의 광자를 이용: energy ↓ → 더 깊게 들어가기엔 activation 시키려면 energy ↑
→ 그래서 2개를 사용함 → 평형

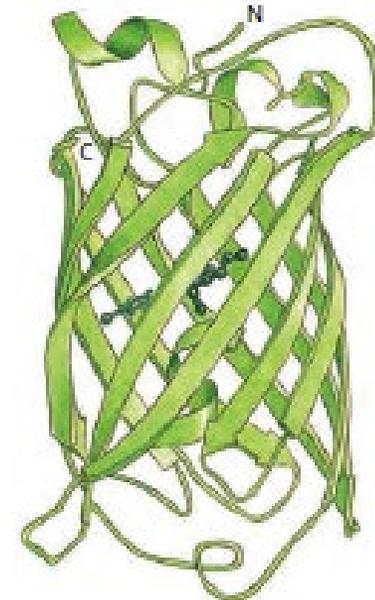
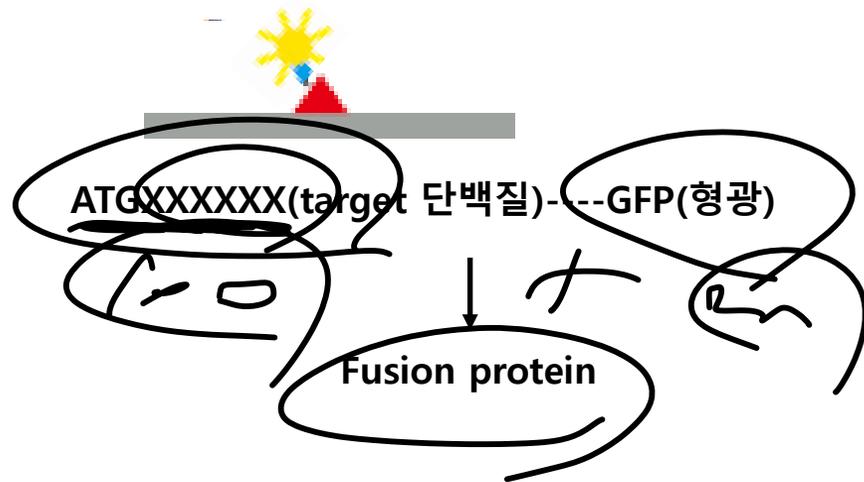


500 μm inside the cortex of a live mouse brain

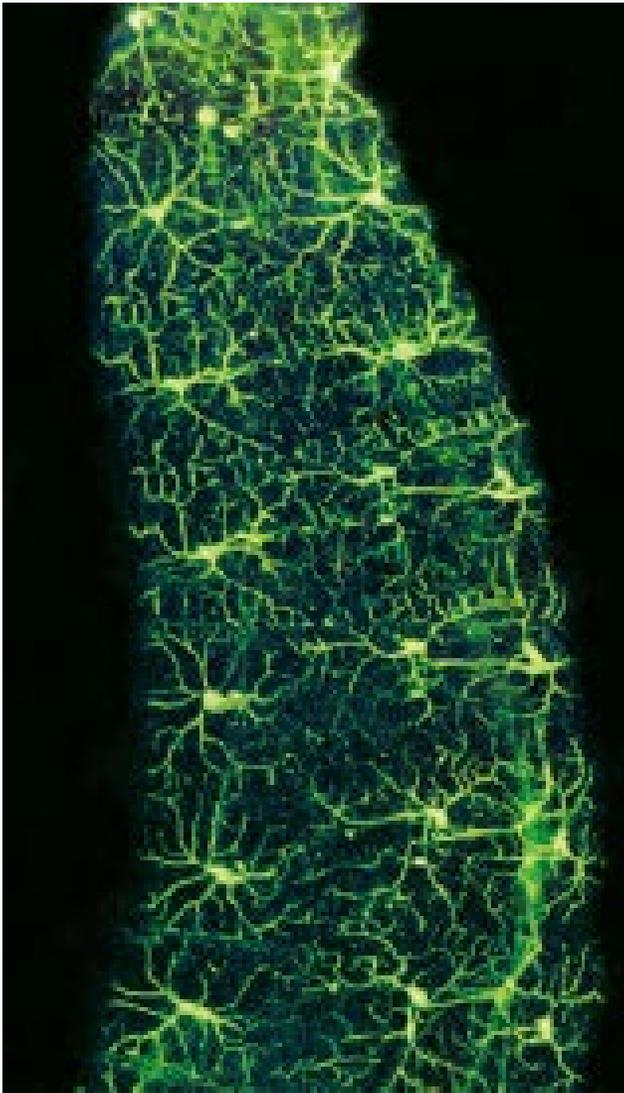
Individual Proteins Can Be Fluorescently Tagged in Living Cells and Organisms



DNA → RNA → protein;
central dogma

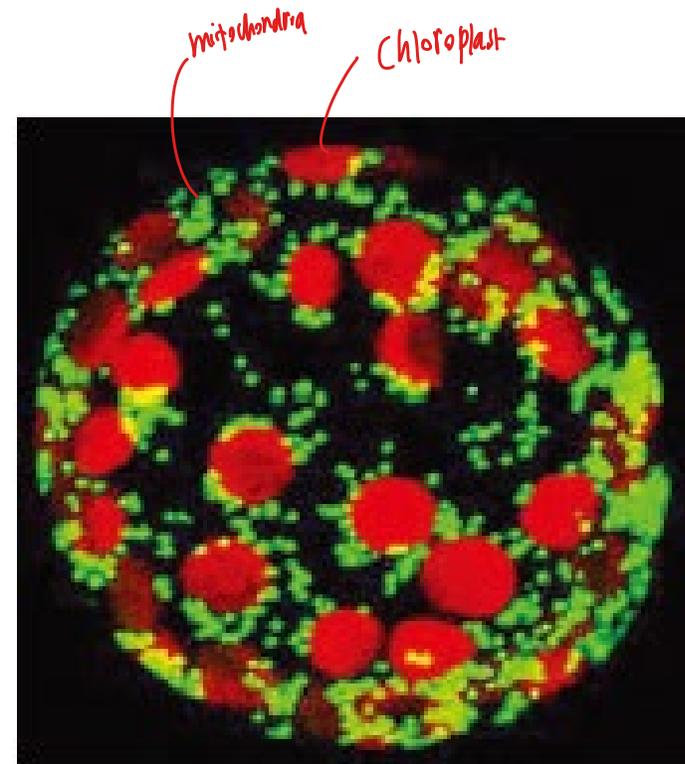
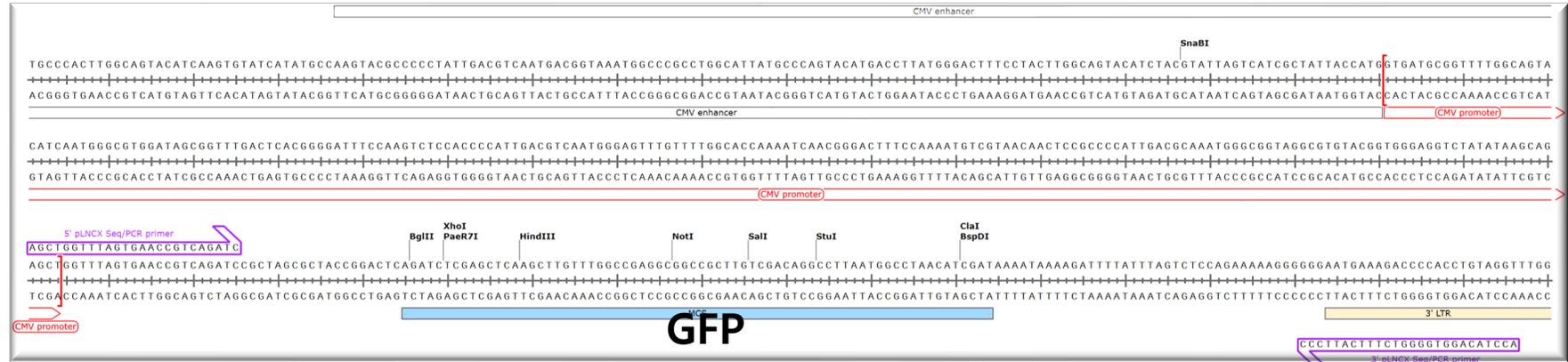


GFP structure



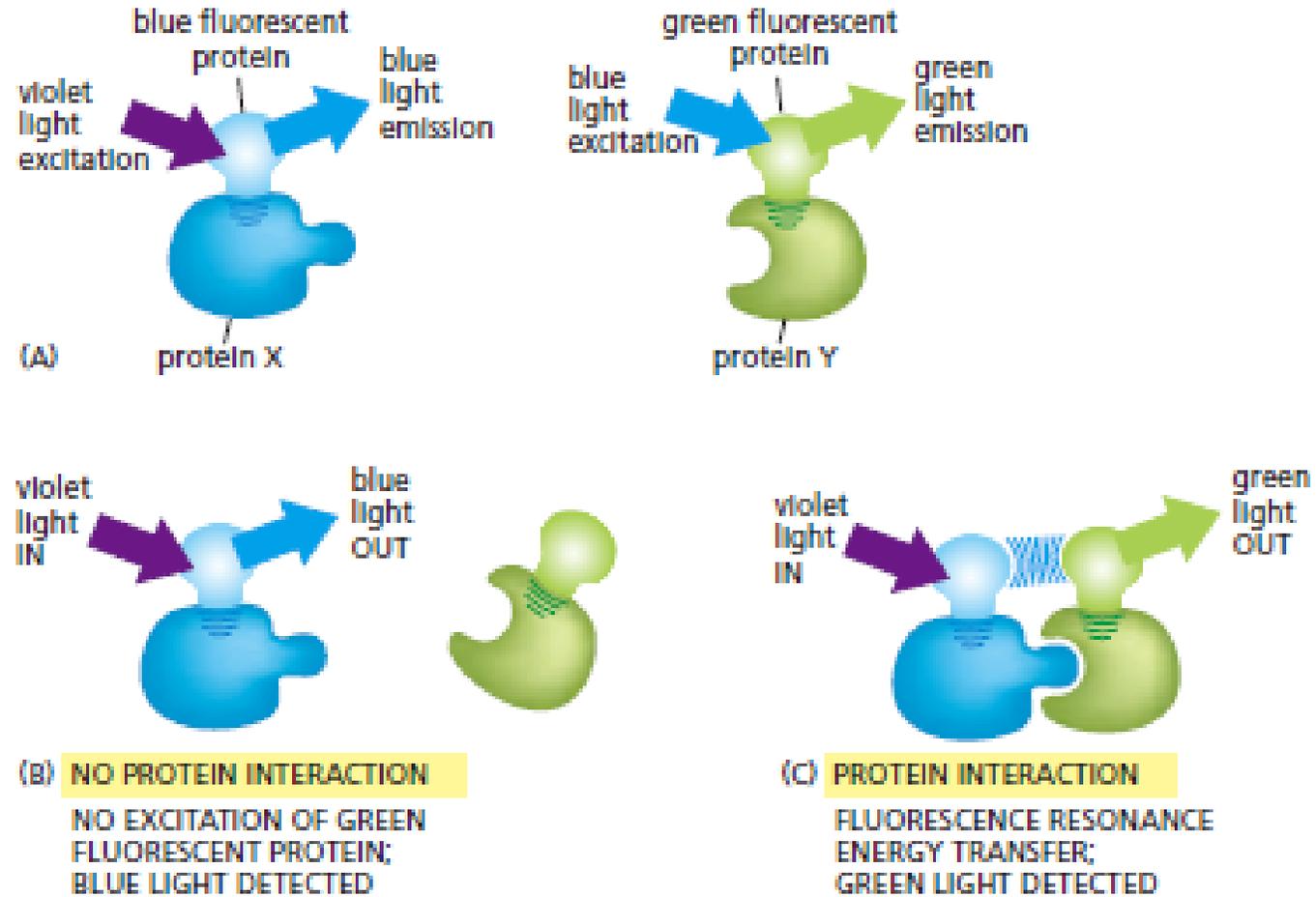
GFP was joined to a fly promoter specific for neuron

Promoter; 전사 (transcription) 조절 인자



Protein Dynamics Can Be Followed in Living Cells- (1st) FRET

Fluorescence resonance energy transfer

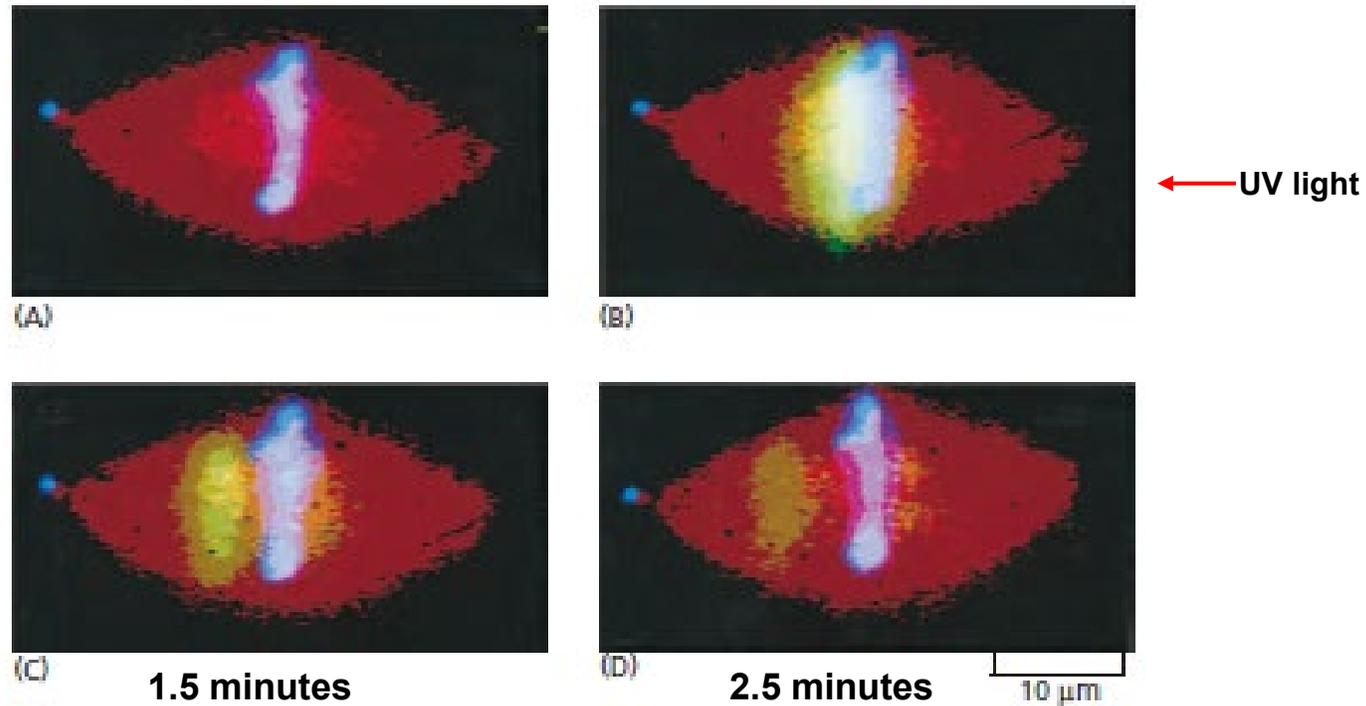


매우 근접하게(약 5nm보다 가깝게)

Protein Dynamics Can Be Followed in Living Cells- (2nd) photoactivation

Reveal the location and movement of proteins

- ❖ 형광: 활성화 과정 없이 특정 파장의 빛을 받으면 형광 나타냄
 - ❖ 광활성화: 빛에 의해 화학 구조가 변해 새로운 형광 특성을 갖음 - 단일 분자 추적
1. 활성화 - 강한 빛 (자외선 or 청색광)
 2. 확인 - 형광을 확인 가능한 빛을 사용

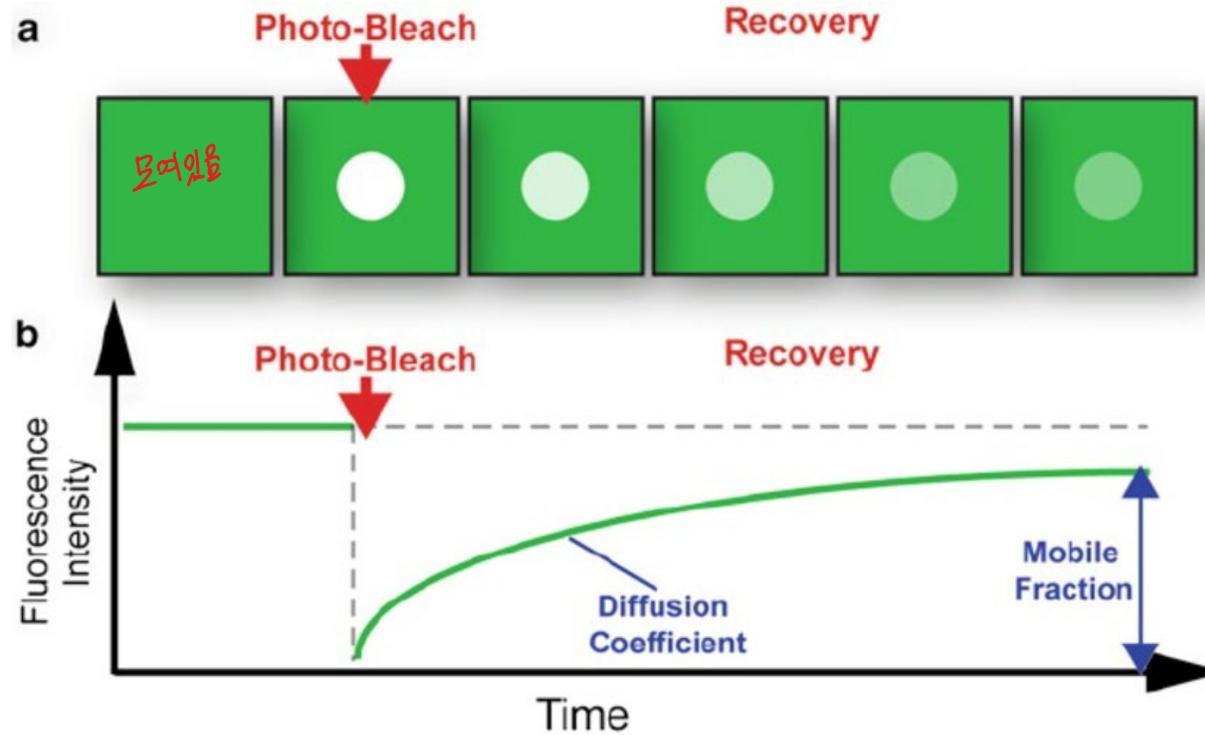


중기의 스피들

Rhodamine-labeled tubulin (*red*) to mark all the microtubules,
Blue DNA-binding dye that labels the chromosomes, and
Caged-fluorescein-labeled tubulin

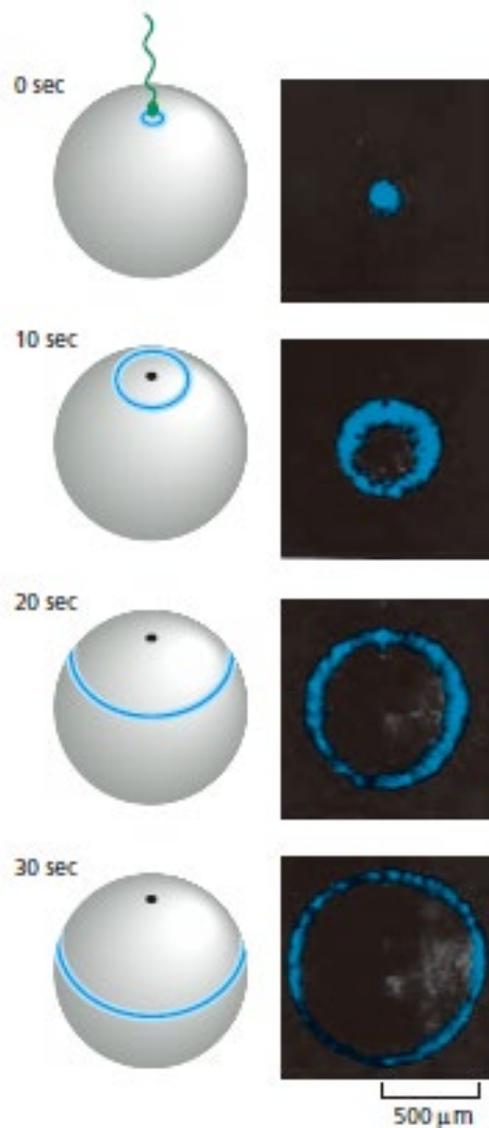
Protein Dynamics Can Be Followed in Living Cells- (3rd) FRAP

Fluorescence recovery after photobleaching (FRAP)

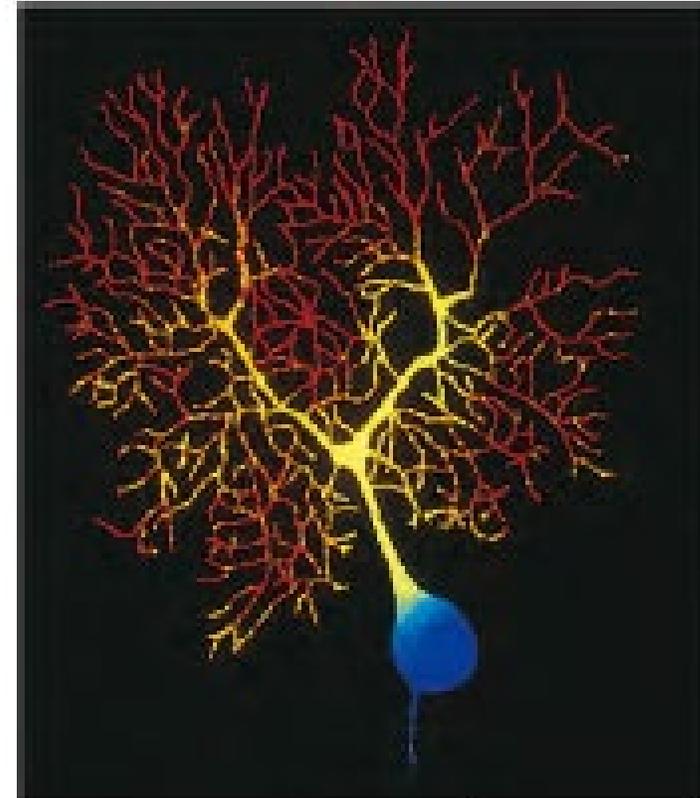


Overview: imaging in the study of integrins. In *Integrin and Cell Adhesion Molecules*, 2011, 159-189.

Light-Emitting Indicators Can Measure Rapidly Changing Intracellular Ion Concentrations



Aequorin은 Ca^{2+} 가 있을 때 청색광을 방출하고 0.5~10μM 범위의 Ca^{2+} 농도 변화에 반응함.

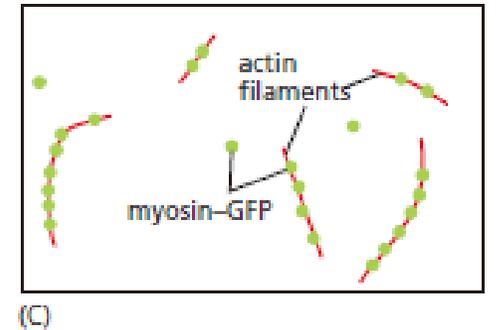
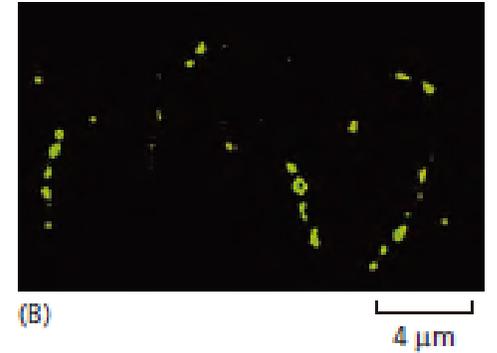
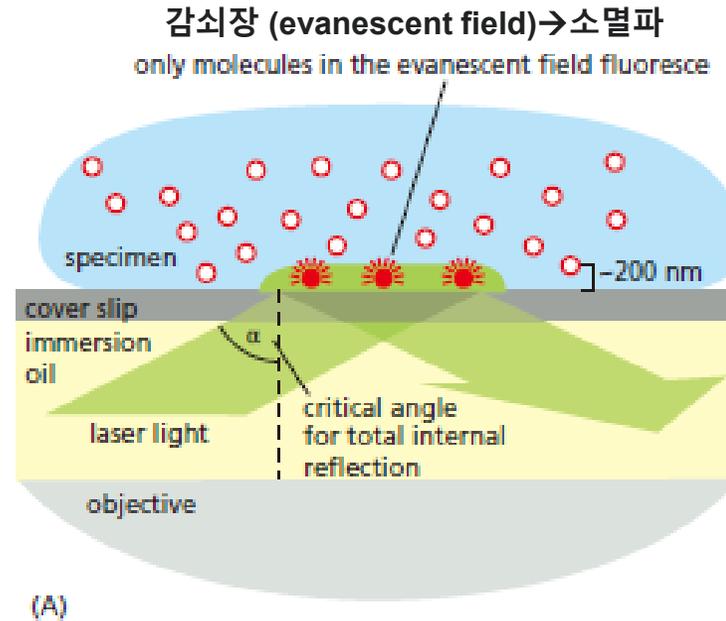
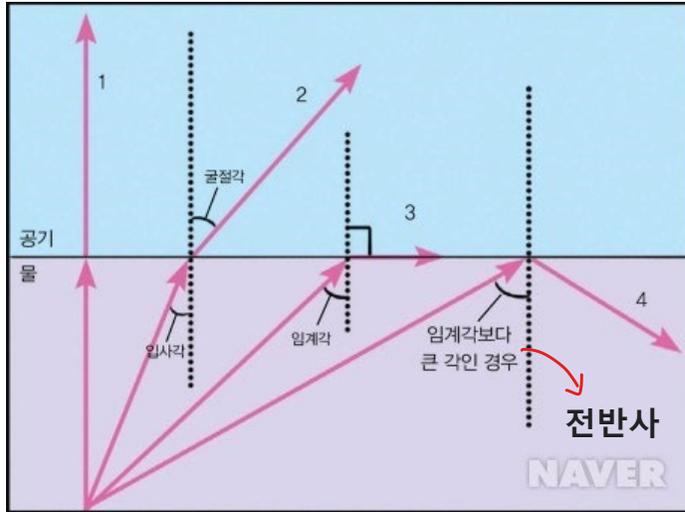


Visualizing intracellular Ca^{2+} concentrations by using a **fluorescent indicator**

Purkinje cells

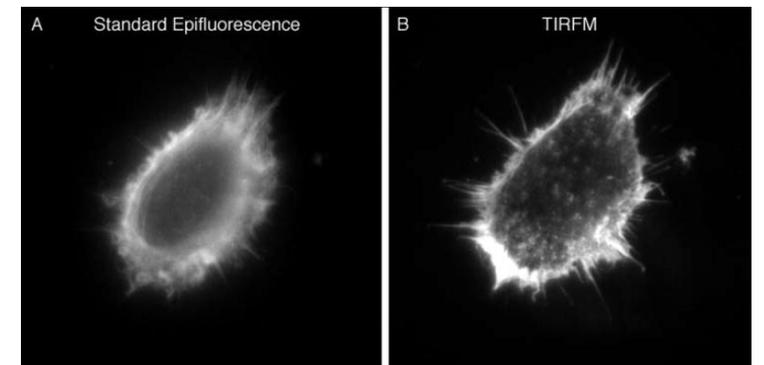
Single Molecules Can Be Visualized by Total Internal Reflection Fluorescence (TIRF) Microscopy

·전반사 형광 현미경



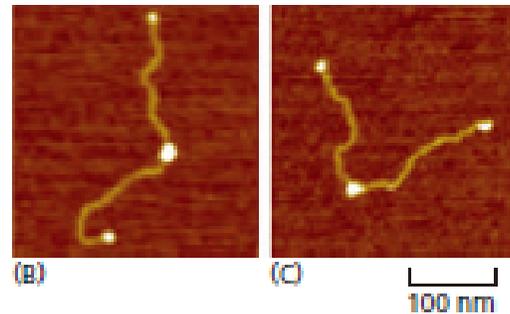
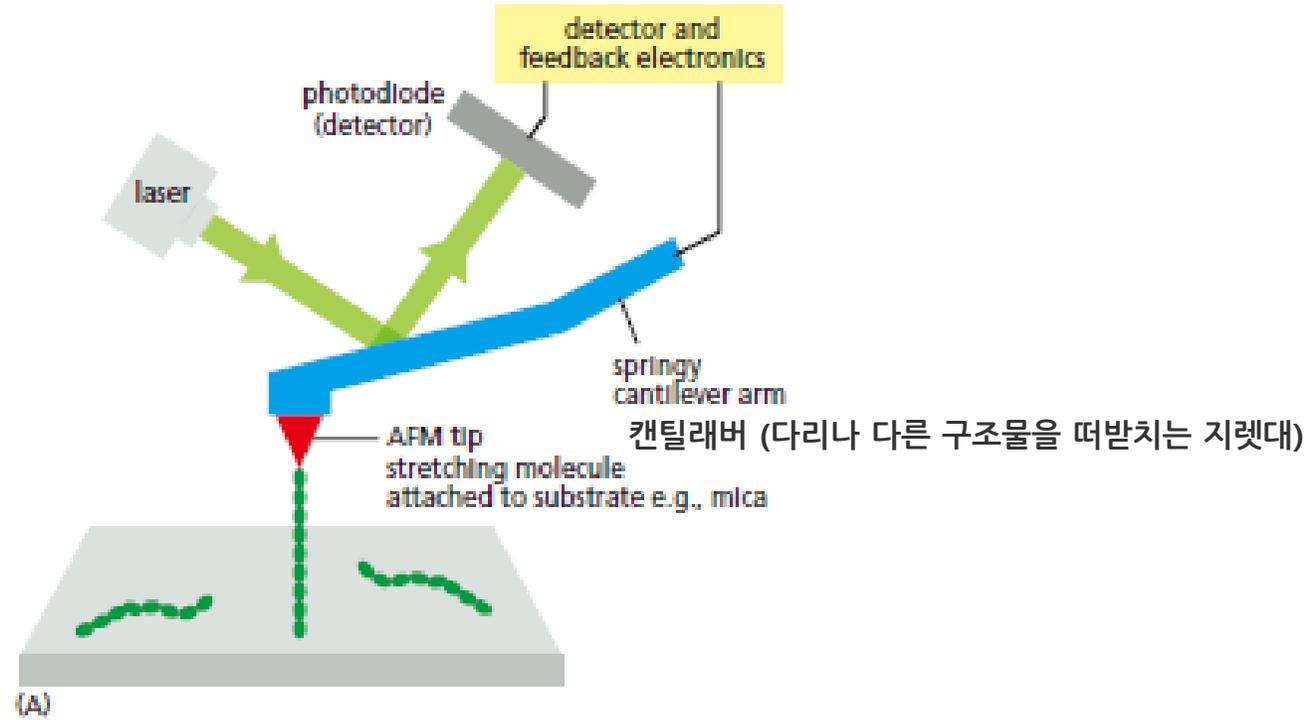
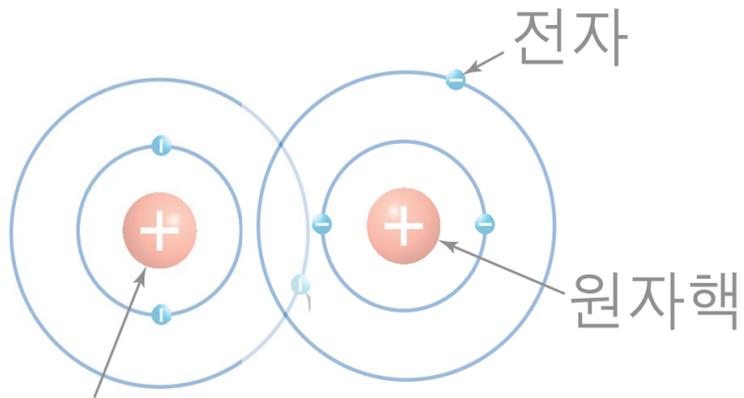
Advantages

- The **background is substantially decreased** so structures → **can be seen clearly**
- There is virtually no out-of-focus fluorescence collected → **decrease blurring effects**
- Cells are exposed to a significantly smaller amount of light → **limits phototoxicity** to cells

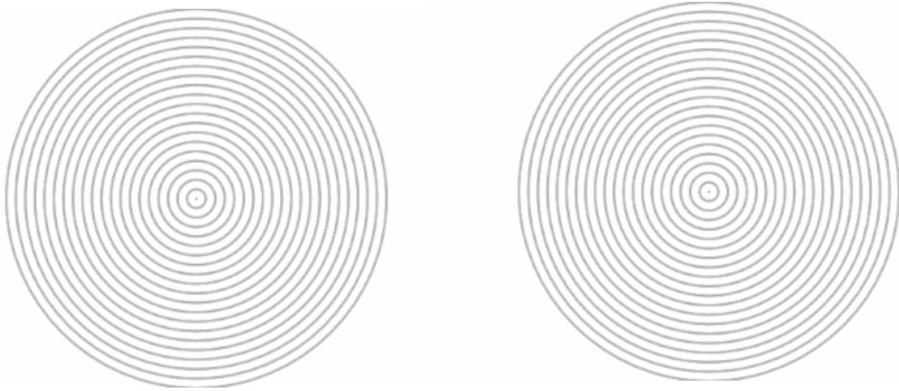


Atomic Force Microscopy (AFM)

- 시료와 탐침 원자간에 작용하는 힘을 검출함으로써 Topology·마찰·정전 상호 작용·도전률·자력 등의 재질 특성을 Image화



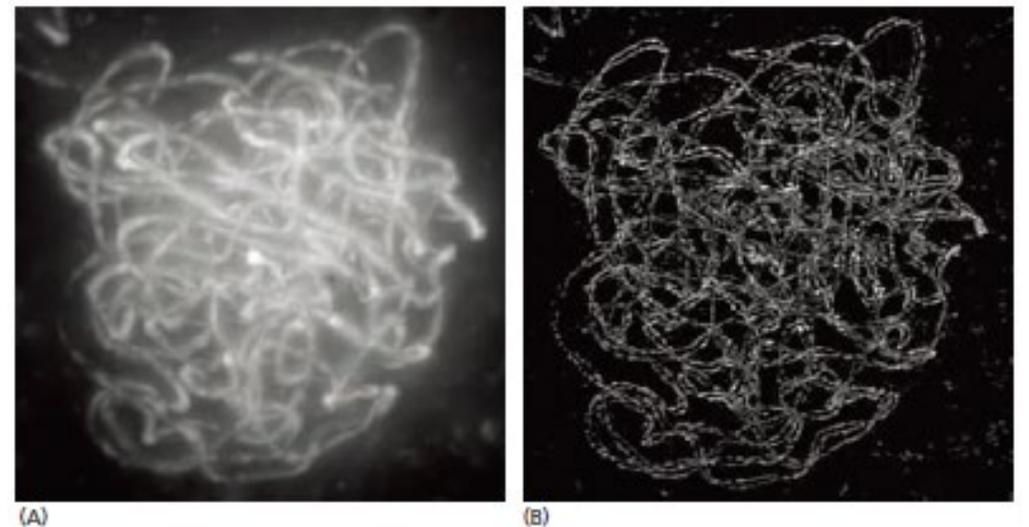
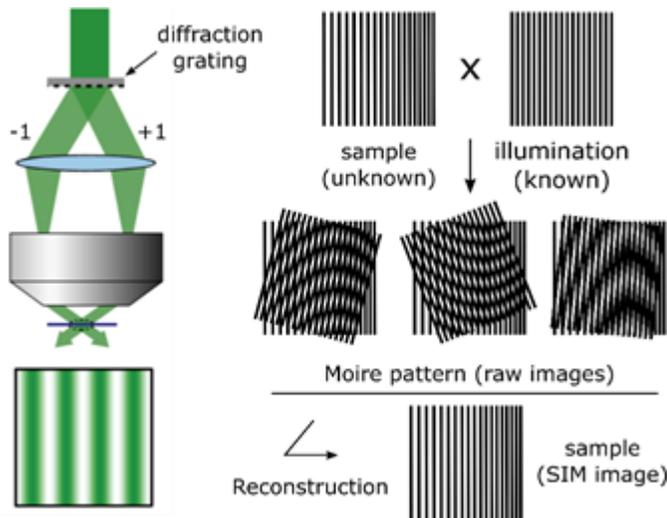
Structured illumination microscopy: SIM 전자현미경보다 나은 광학현미경 나왔다!!!



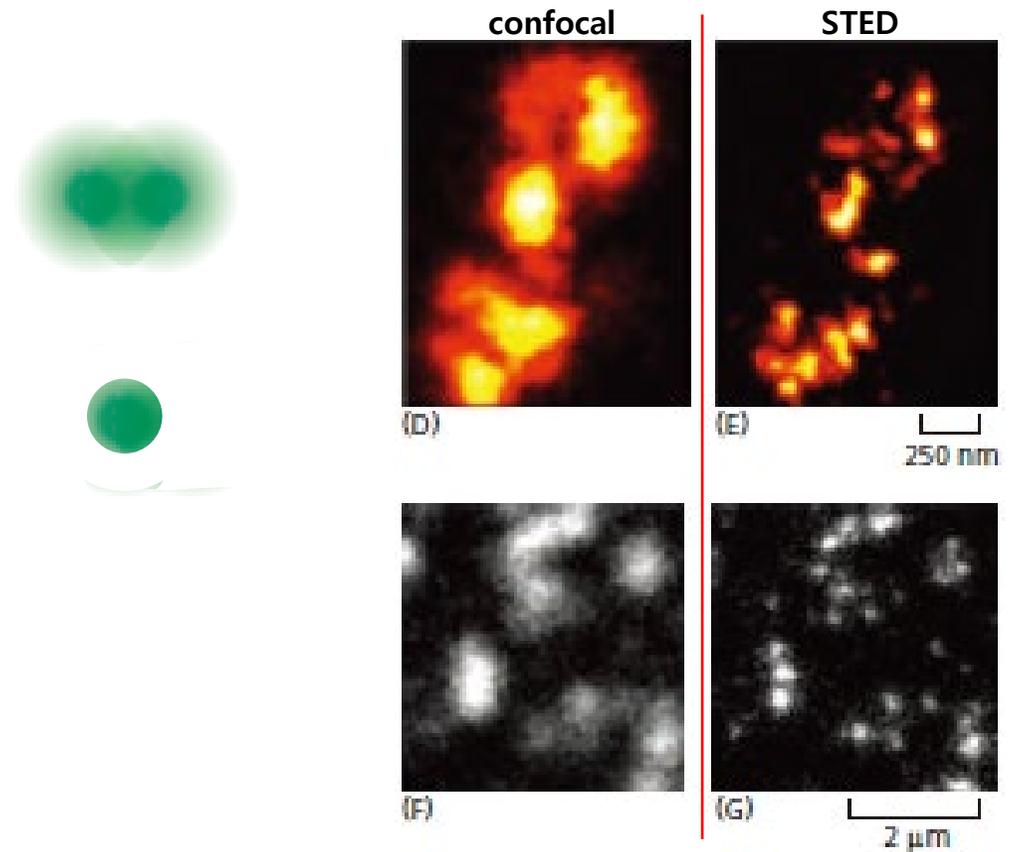
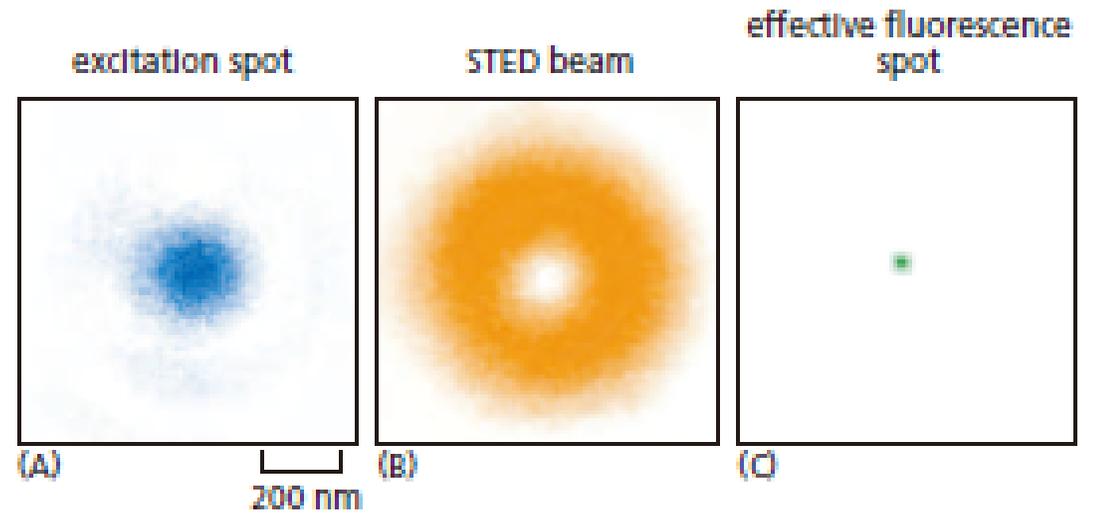
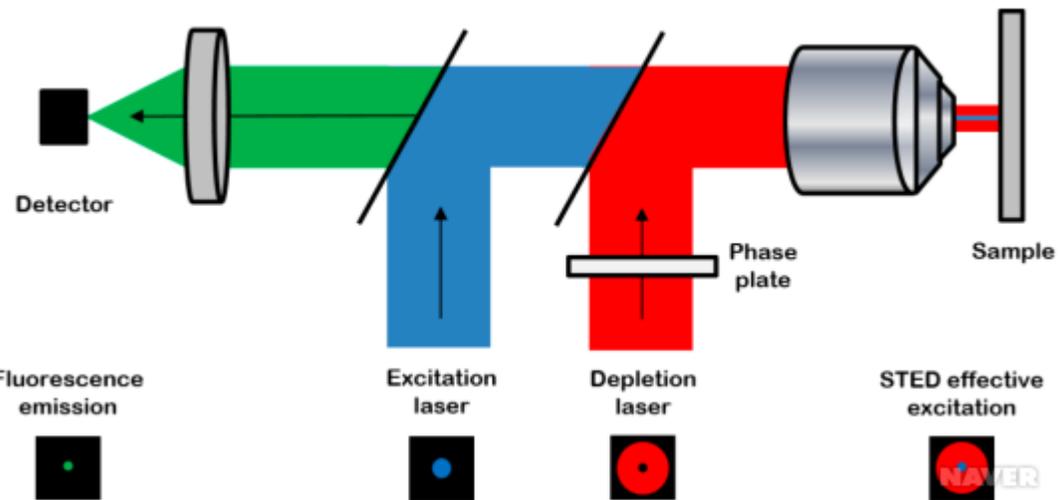
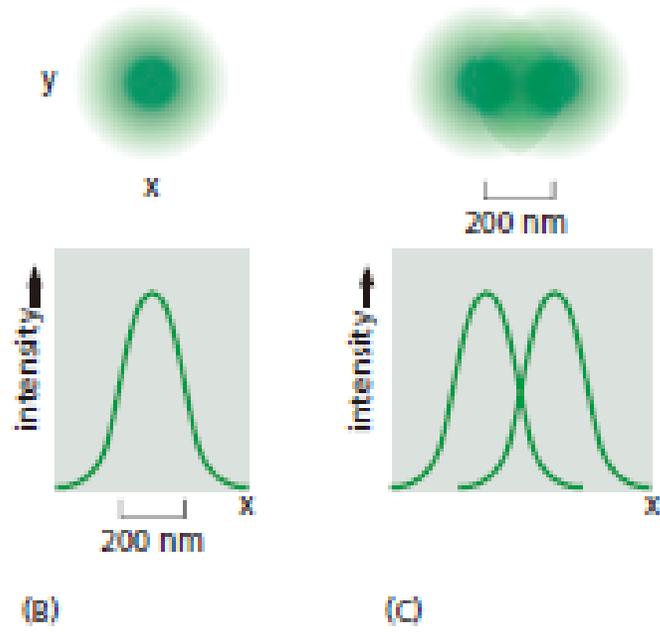
모아레 패턴 (Moiré pattern)

: 두 개의 주기적인 패턴이 겹쳐질 때 생성되는 간섭 무늬

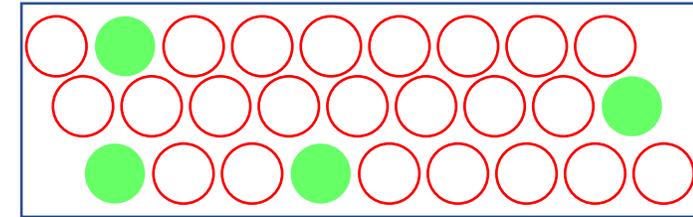
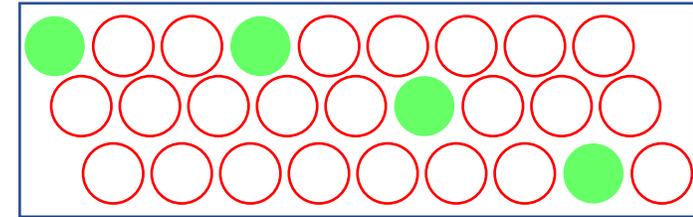
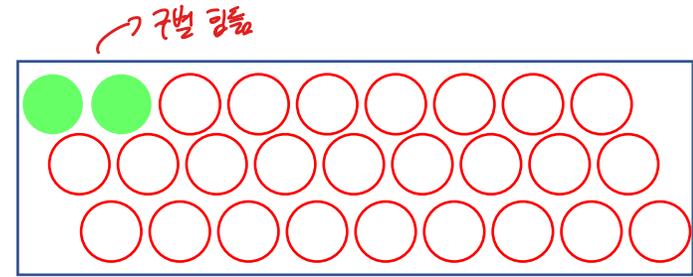
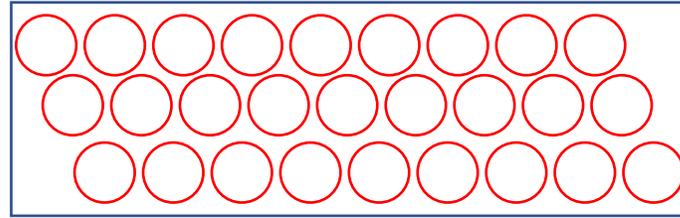
회절한계에 의해 얻을 수 없는 샘플의 고분해능 정보가 관찰 가능한 저분해능 모아레 무늬에 반영



Stimulated emission depletion microscopy: STED

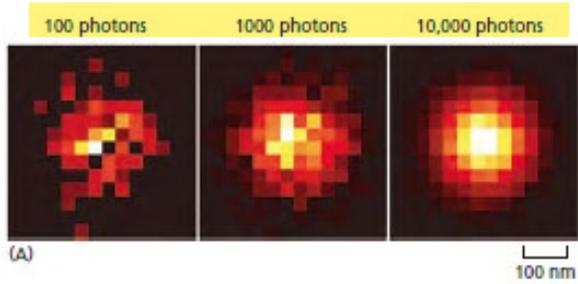


Single Molecule Localization Microscopy: SMLM

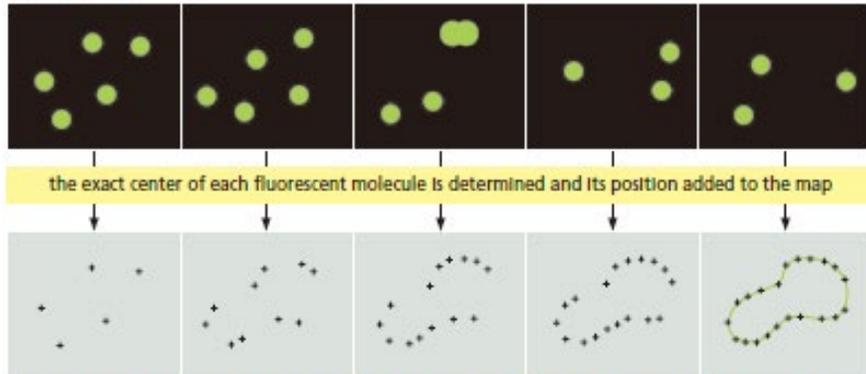


X1000

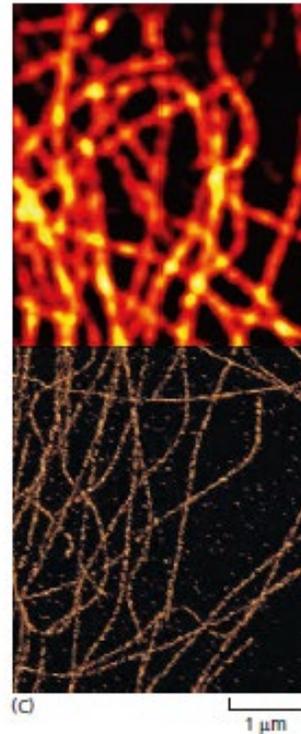
해상도가 높음



successive cycles of activation and bleaching allow well-separated single fluorescent molecules to be detected



a super-resolution image of the fluorescent structure is built up as the positions of successive small groups of molecules are added to the map



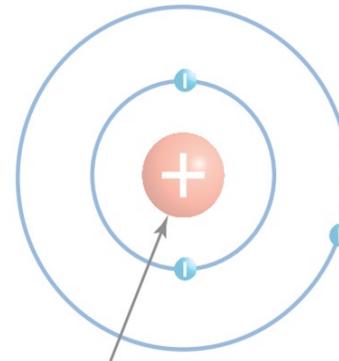
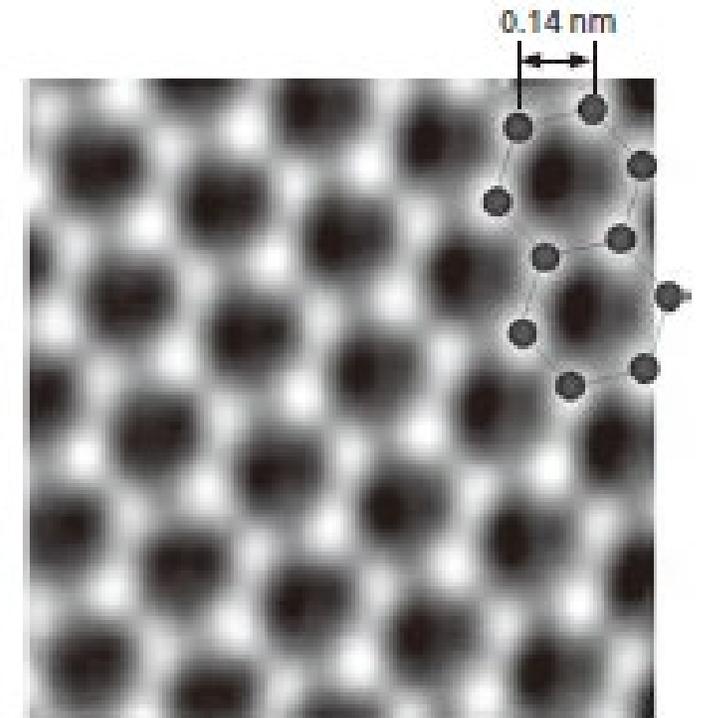
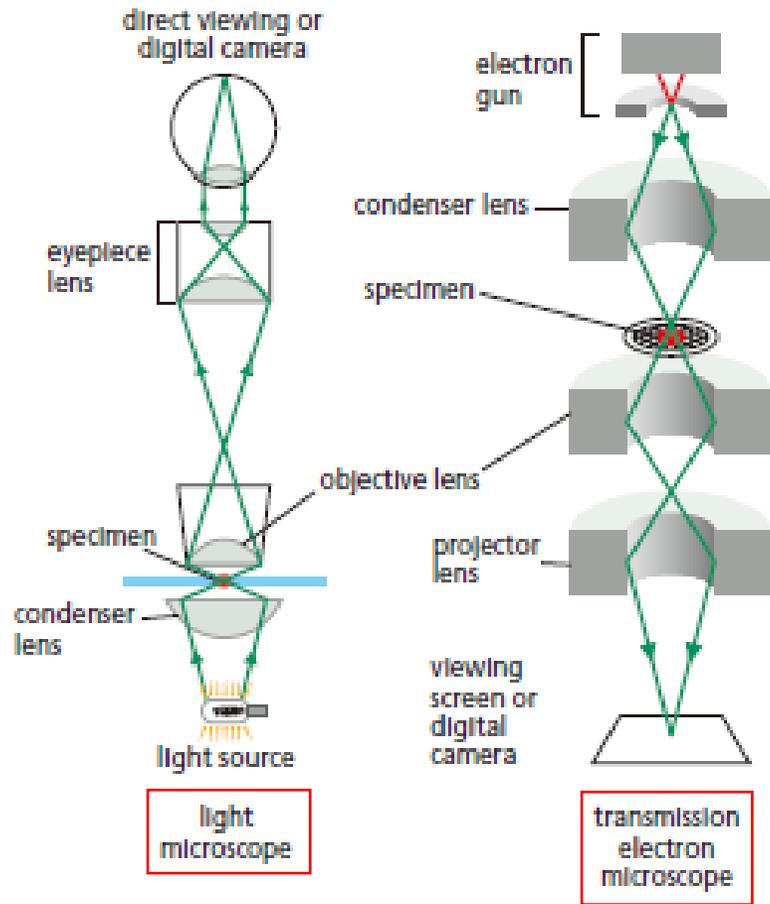
TIRF microscope

(Super-resolution)
PALM microscope

Looking at Cells and Molecules in the Electron Microscope

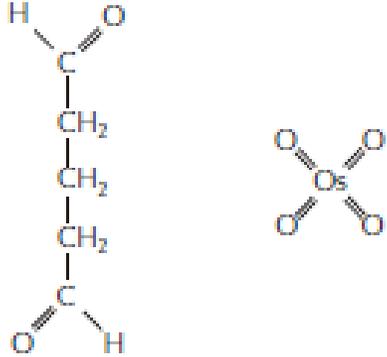
- **투과전자현미경 (Transmission electron microscope, TEM)** : 투과전자현미경은 매우 높은 전압의 전자빔을 시료를 통과시켜 이미지 생성.
- **주사 전자현미경 (Scanning electron microscope, SEM)**: 주사 전자현미경은 전자빔을 시료의 표면을 따라 [탐침](#) 하여 이미지를 생성.
- **초저온 전자현미경 (cryo Electron Microscope, Cryo EM)** : CryoEM은 TEM의 한 종류로, 시료를 초저온 (액체질소 온도)에서 TEM 이미지를 획득하는 현미경. 2017년에는 고해상도 생체 분자구조를 위한 cryoEM 개발로 자크 뒤보셰(Jacques Dubochet), 요킴 프랭크(Joachim Frank), 리차드 헨더슨(Richard Henderson)이 노벨화학상을 수상.

The transmission electron microscope (TEM)

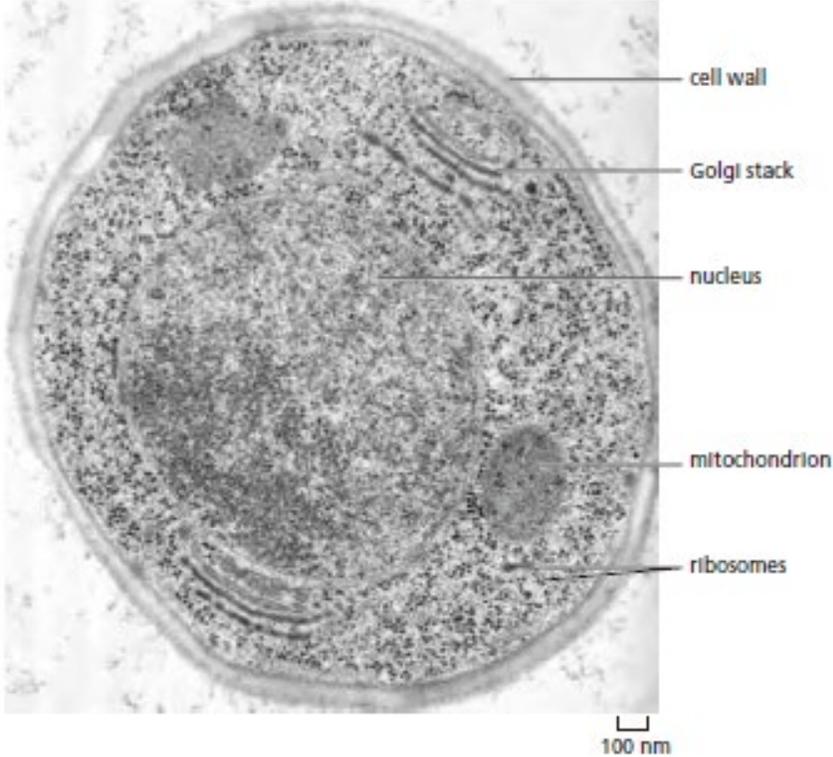
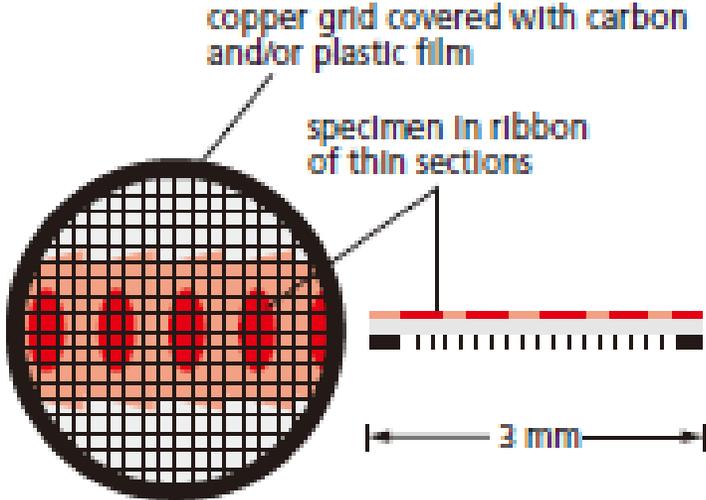


수차 (aberration): 렌즈를 통과한 빛이 한 점에 모일때 색이나 상이 왜곡되는 현상

Biological Specimens Require Special Preparation for Electron Microscopy

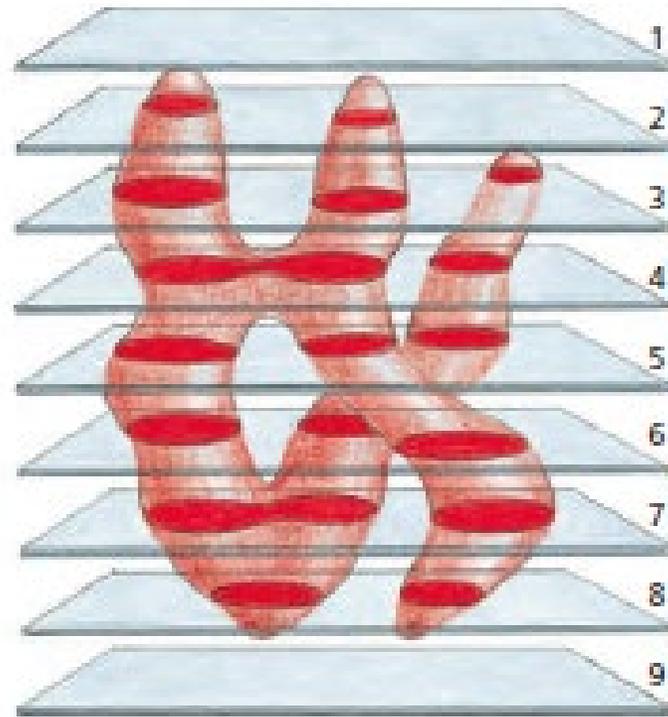


glutaraldehyde osmium tetroxide

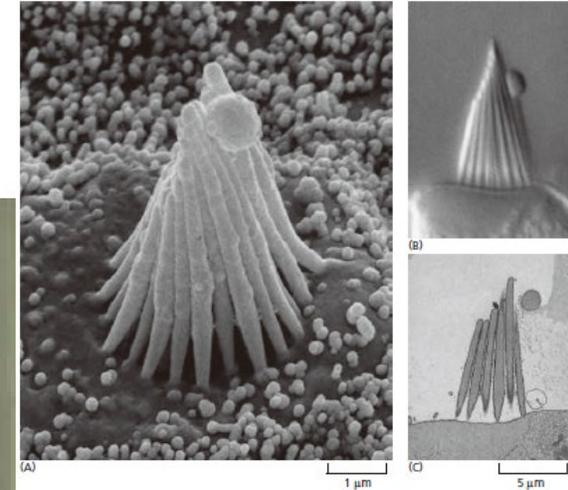
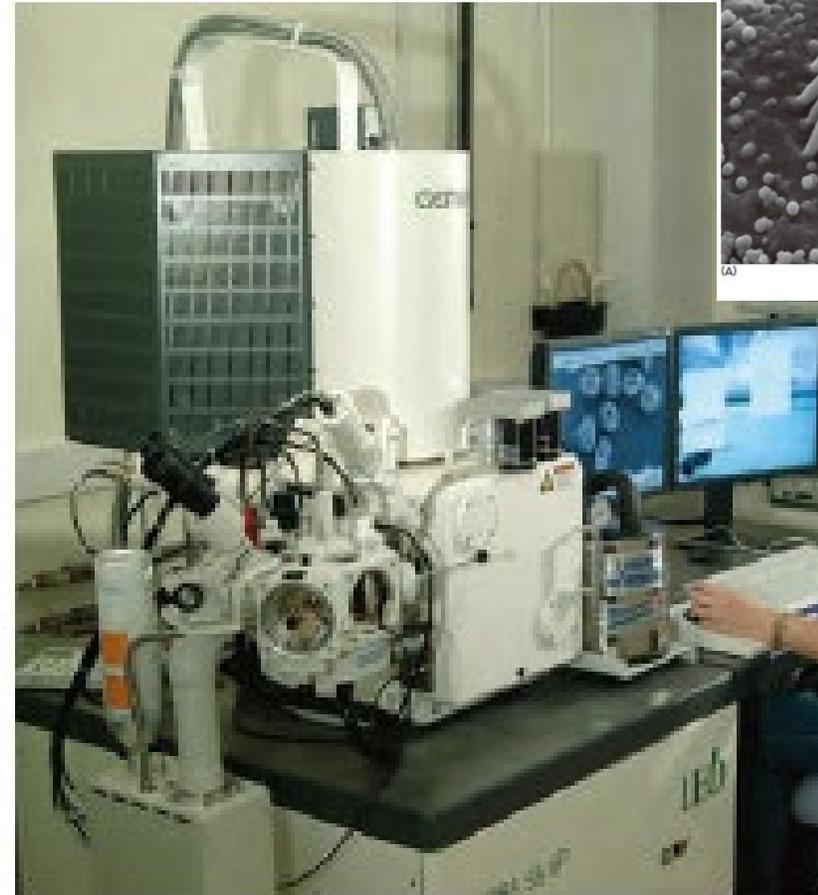
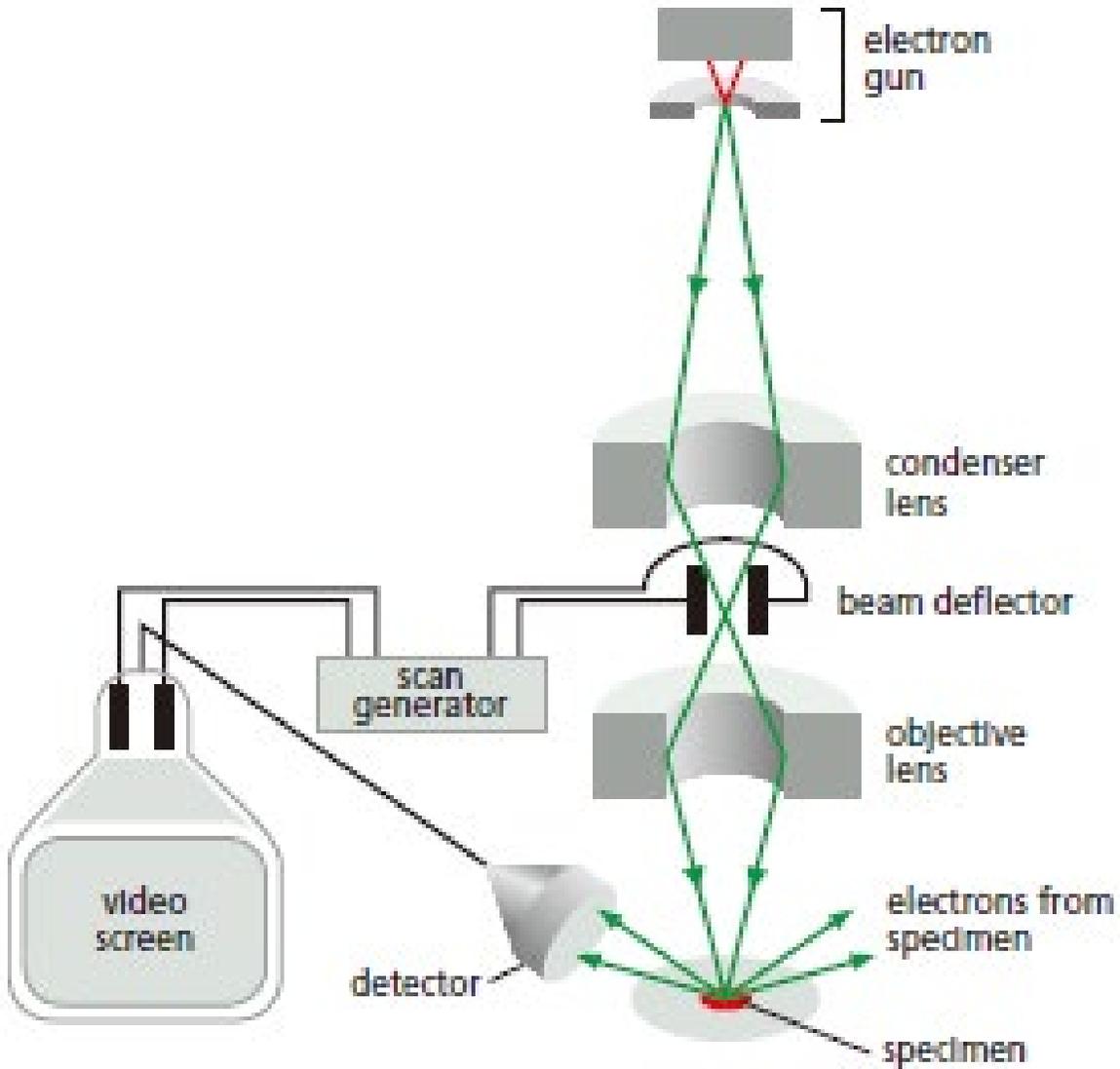


The metal grid that supports the thin sections of a specimen in a transmission electron microscope.

Different Views of a Single Object Can Be Combined to Give a Three-Dimensional Reconstruction



Images of Surfaces Can Be Obtained by Scanning Electron Microscopy (SEM)



Negative Staining and Cryoelectron Microscopy Both Allow Macromolecules to Be Viewed at High Resolution



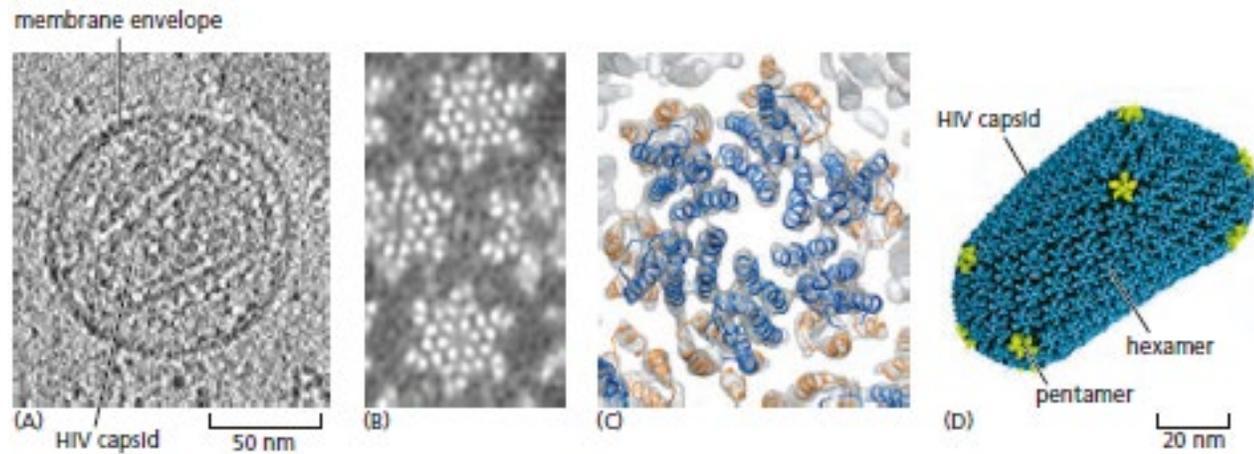
100 nm

Negatively stained actin filaments

Multiple Images Can Be Combined to Increase Resolution

단일 입자 재구성: 많은 동일한 분자의 이미지를 얻고 이를 결합해 평균 이미지를 생성하는 것

인간 면역 결핍 바이러스(HIV) 내부의 단백질 캡시드 구조



(D) (A) 및 (C)에 표시된 상세한 구조를 기반으로 전체 HIV 캡시드의 분자를 재구성해서 216개의 6량체(파란색)와 12개의 5량체(노란색)를 포함하고 있음을 밝힘.

parky5@ajou.ac.kr

수고 하셨습니다.