Advanced 3
Biological Imaging

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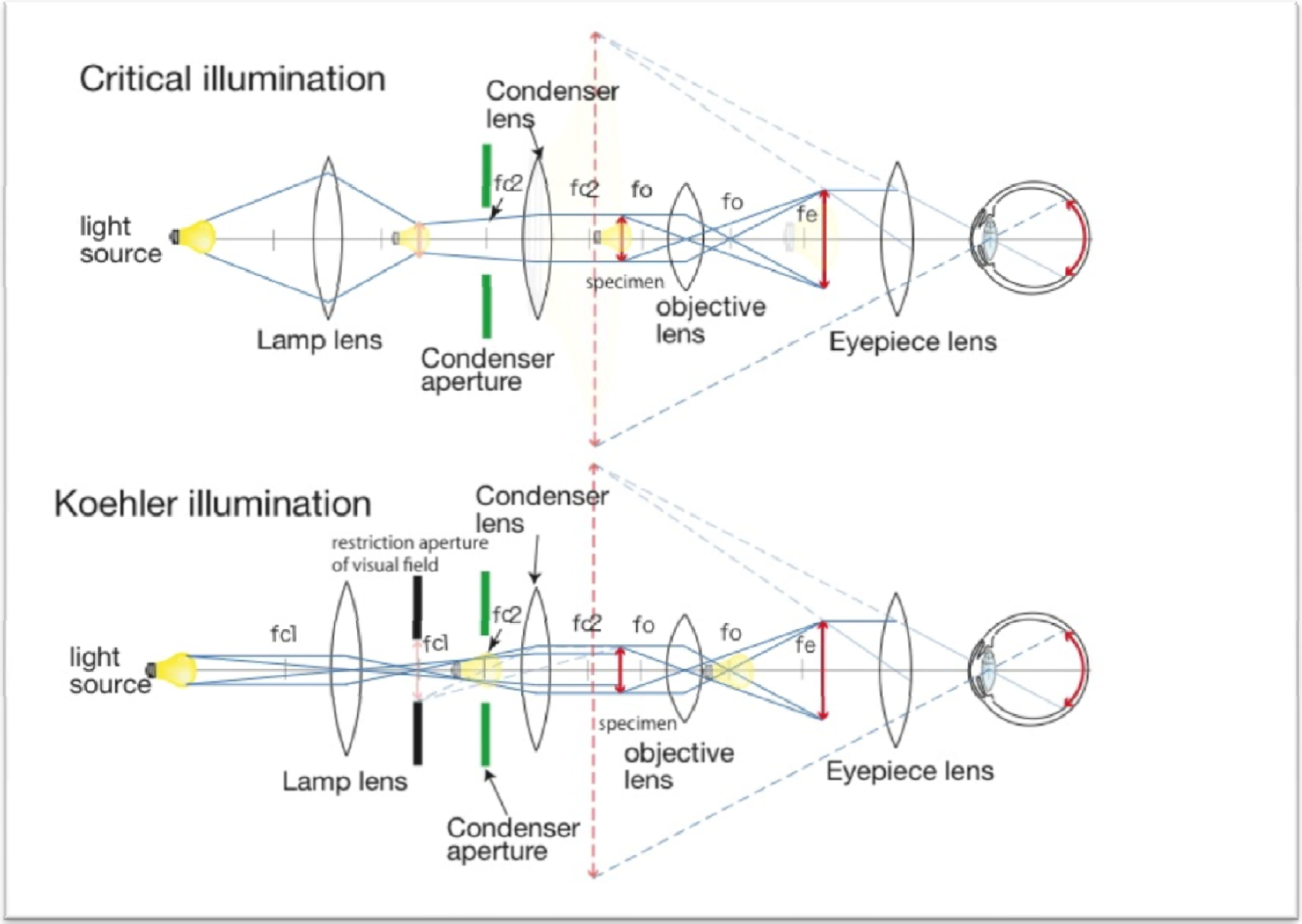

Critical illumination
Condenser lens
fc2
fc2
fo
fo
fe
light source
specimen
objective lens
Lamp lens
Condenser aperture
Eyepiece lens
Koehler illumination
Condenser lens
restriction aperture of visual field
fc2
fc2
fo
fo
fe
fcl
fcl
light source
specimen
objective lens
Lamp lens
Condenser aperture
Eyepiece lens

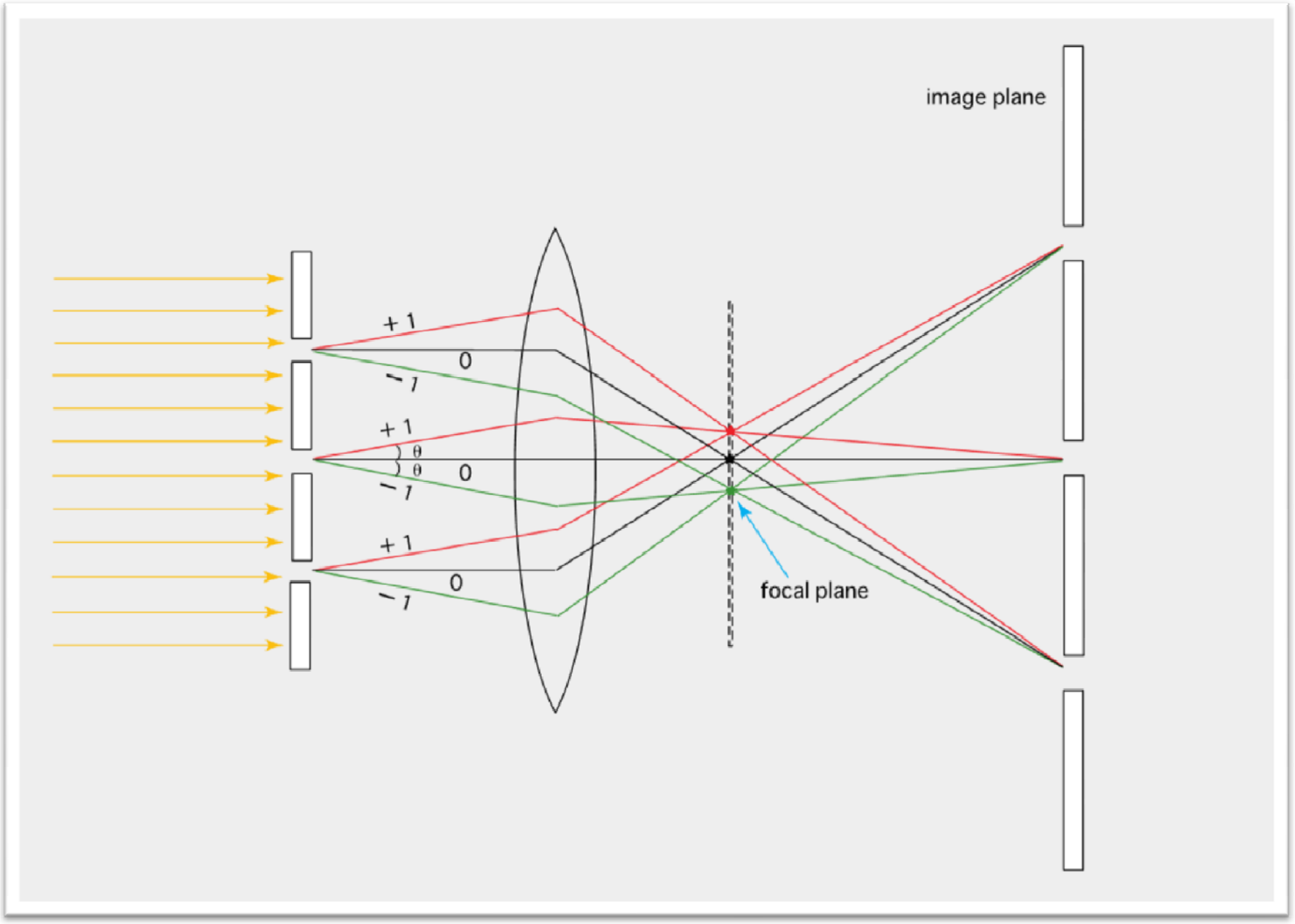
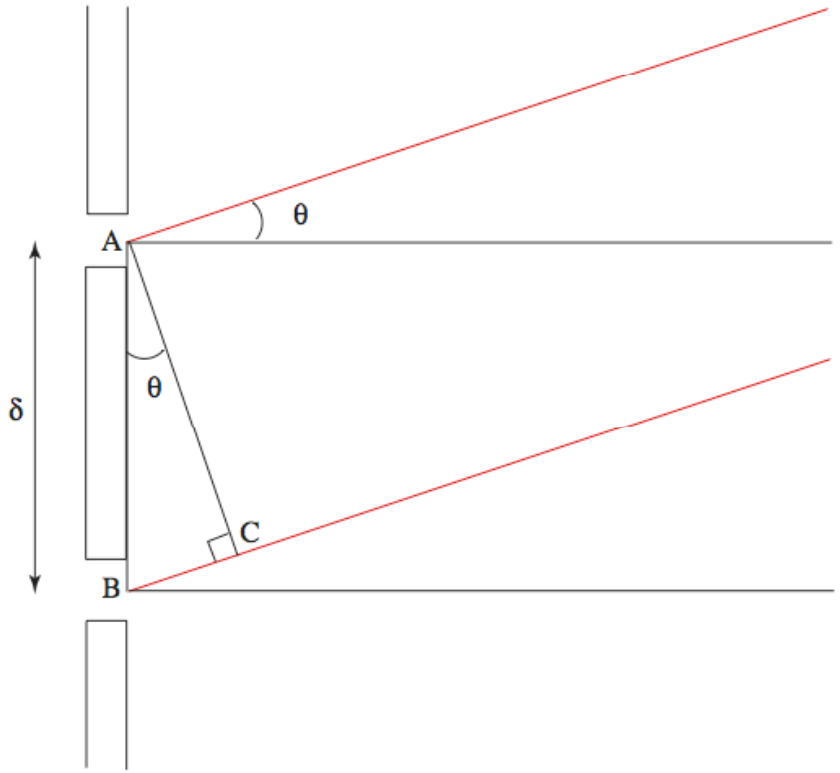


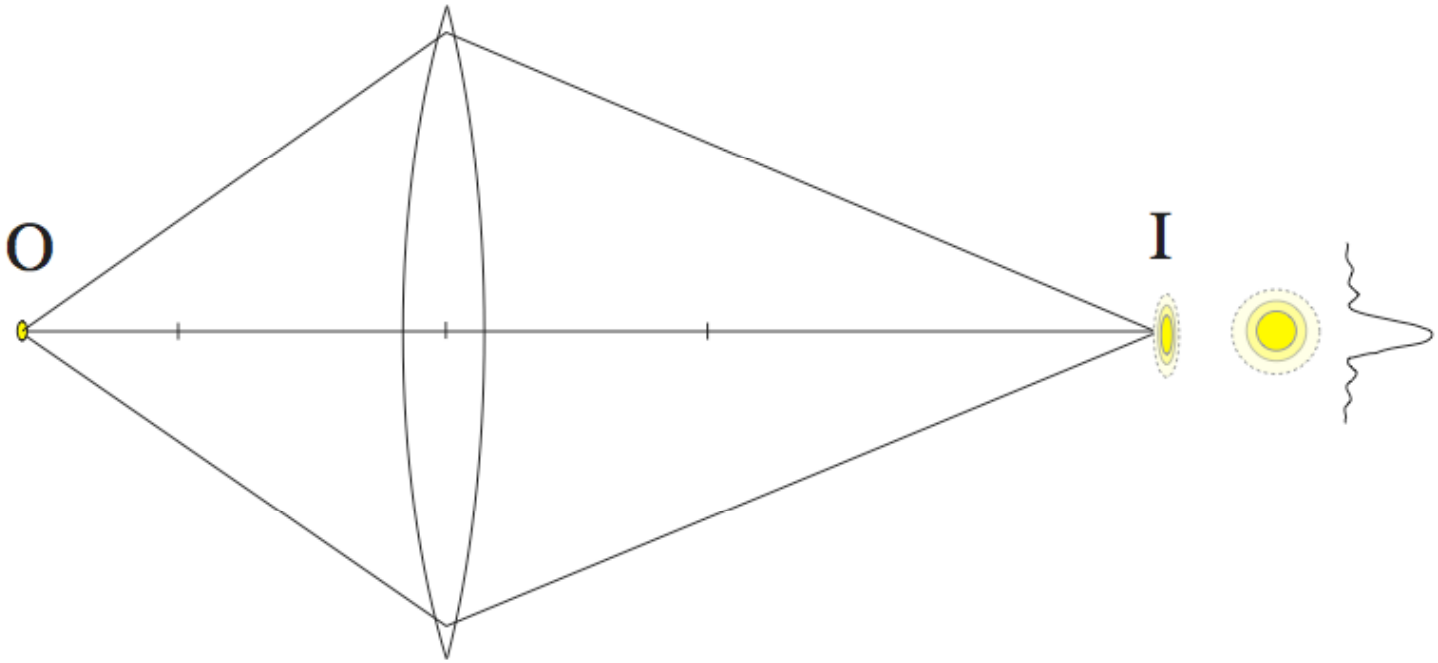
image plane
+1
0
−1
+1
θ
θ
0
−1
+1
0
−1
focal plane



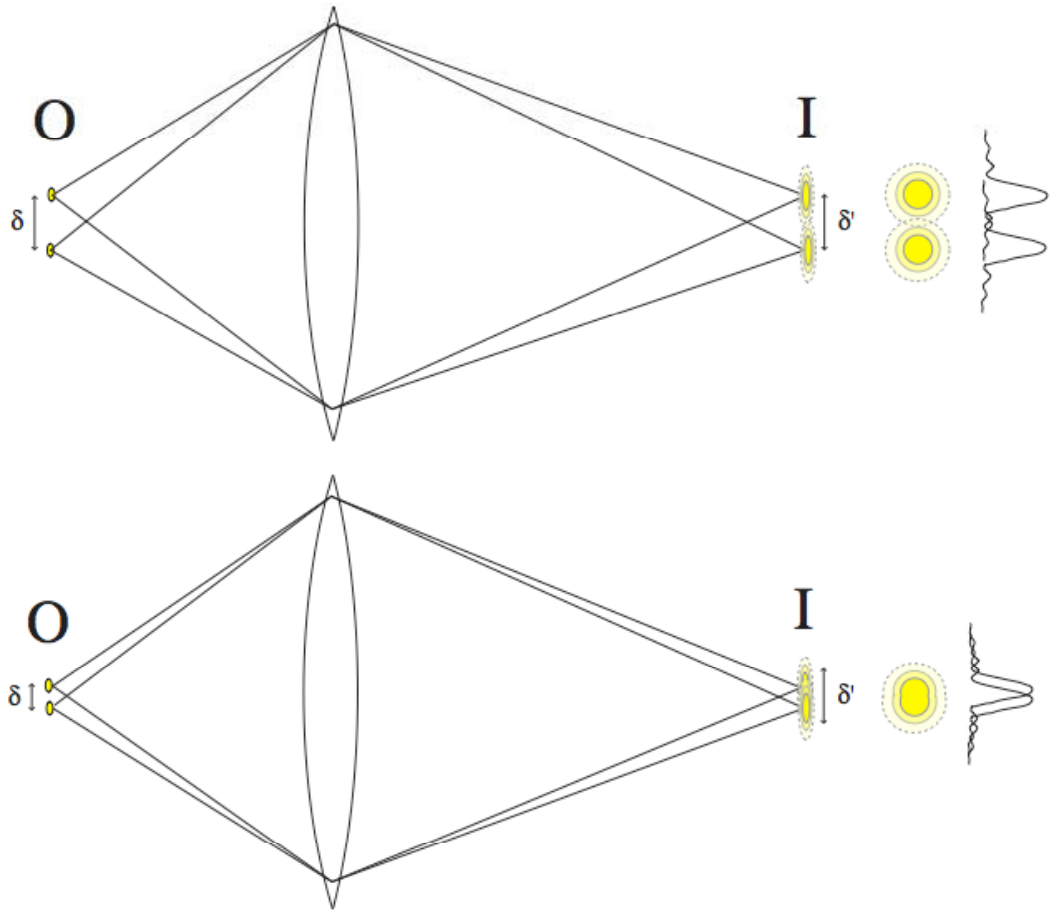
In ⊿ABC、AB Sin∖ = BC ∴ δ Sin∖ = λ. Then δ =λ / Sin∖ (0<θ<□/2) When refractive index of substance between lens and lattice is n, wave length is λ /n. Therefore δ = λ / n Sin∖

In previous slide, relation between lattice constant and wavelength in diffraction was shown. This equation is the same to resolution limit by Ernst Abbe, 1840-1905. That is, it appears that lattice is specimen, and also diffraction angle θ is incident light angle against the lens.

If the largest angle of incident light to the lens is θ_0, $n\mathrm{Sin}\theta_0$ is the numerical aperture of this lens. Then, lattice constant (δ_0) equal to the resolution of the lens. $\delta_0 = \lambda / n\ \mathrm{Sin}\theta_0$ This is a primitive equation on resolution of optical lens by Abbe. He think that resolution is determined by wave length used and numerical aperture of lens.



Imaging of very small bright spot through the lens. Right inset shows focused image (airy disk)

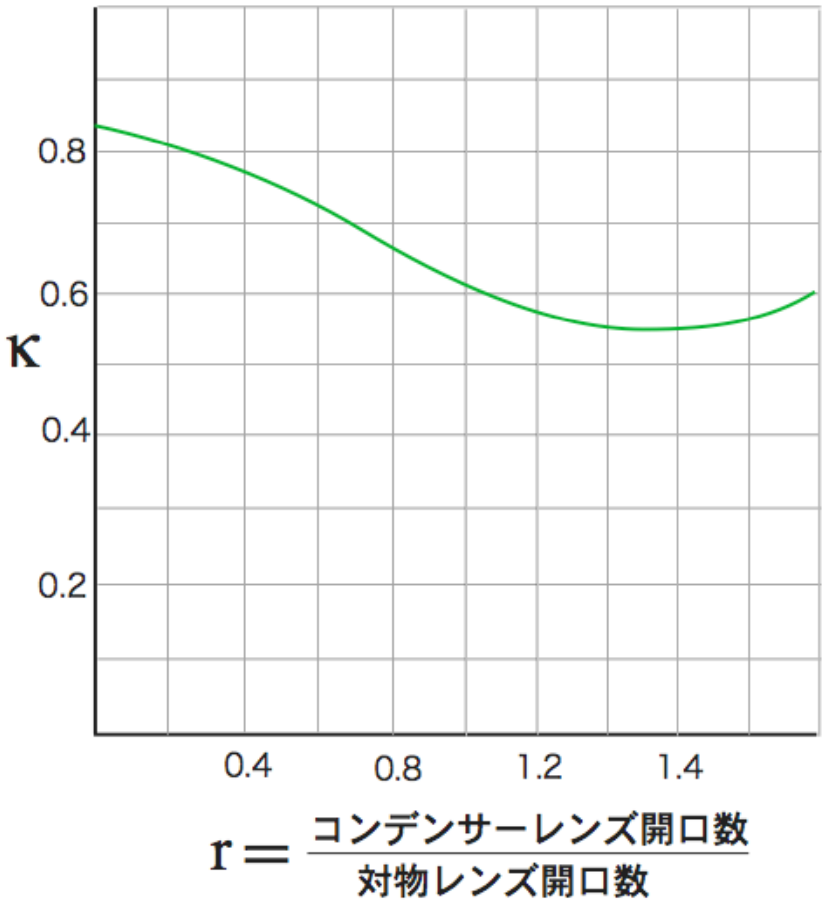


This cartoon shows resolution of two airy disks. In order to discriminate two airy disks distinctly, center of each airy disk must be separated more than half diameter (Lord Rayleight, 1842-1919).

Condenser lens is partially involved in resolution of microscope in a strict sense as well. Limited coherence κ (ratio of numerical aperture of objective and condenser lenses) affects the resolution of microscope as a whole. Therefore, Hopkins defines a following equation in general.

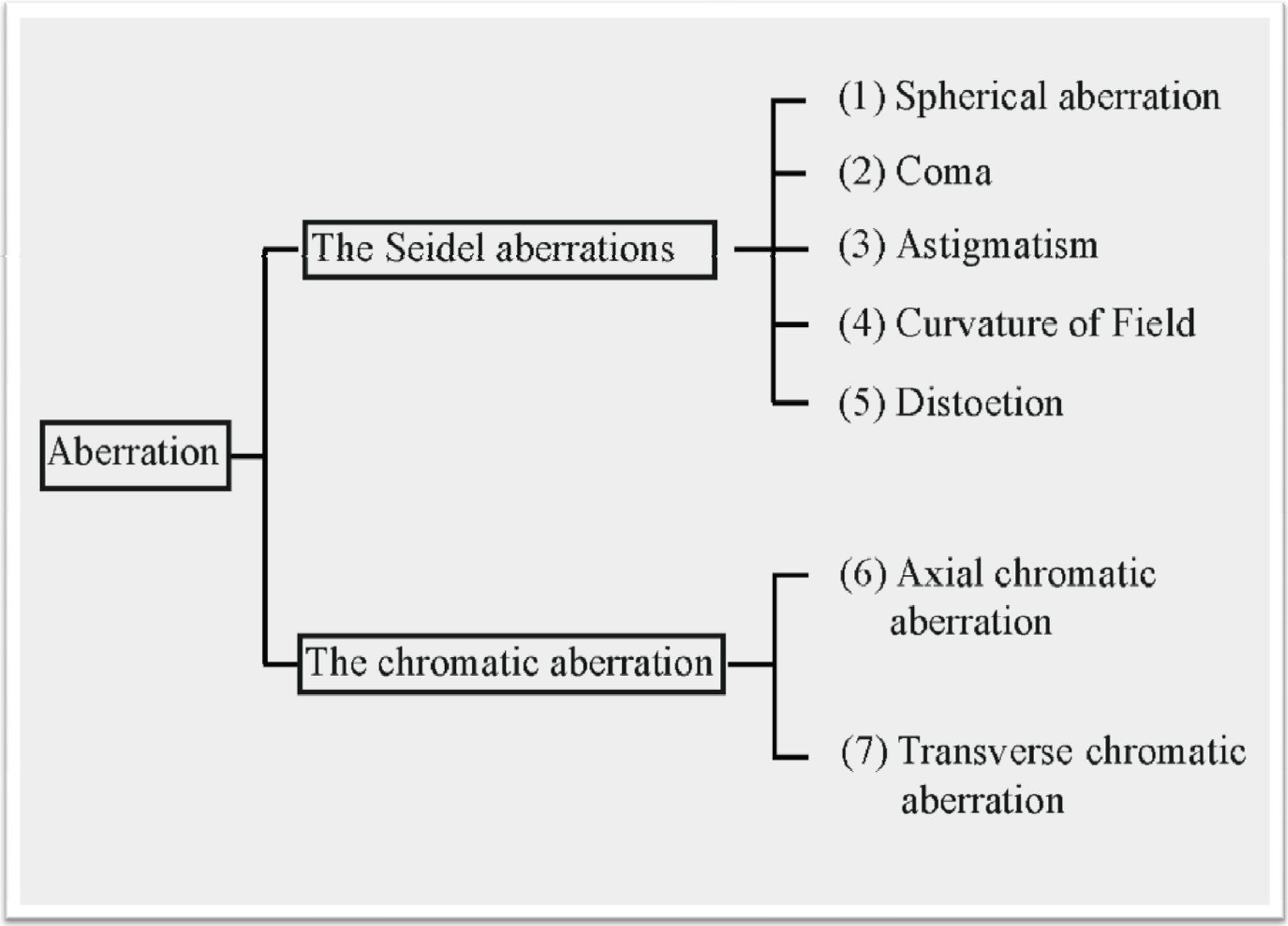
$$\delta_o = \kappa \lambda / n \sin\theta_0$$

κ changes by optical condition.

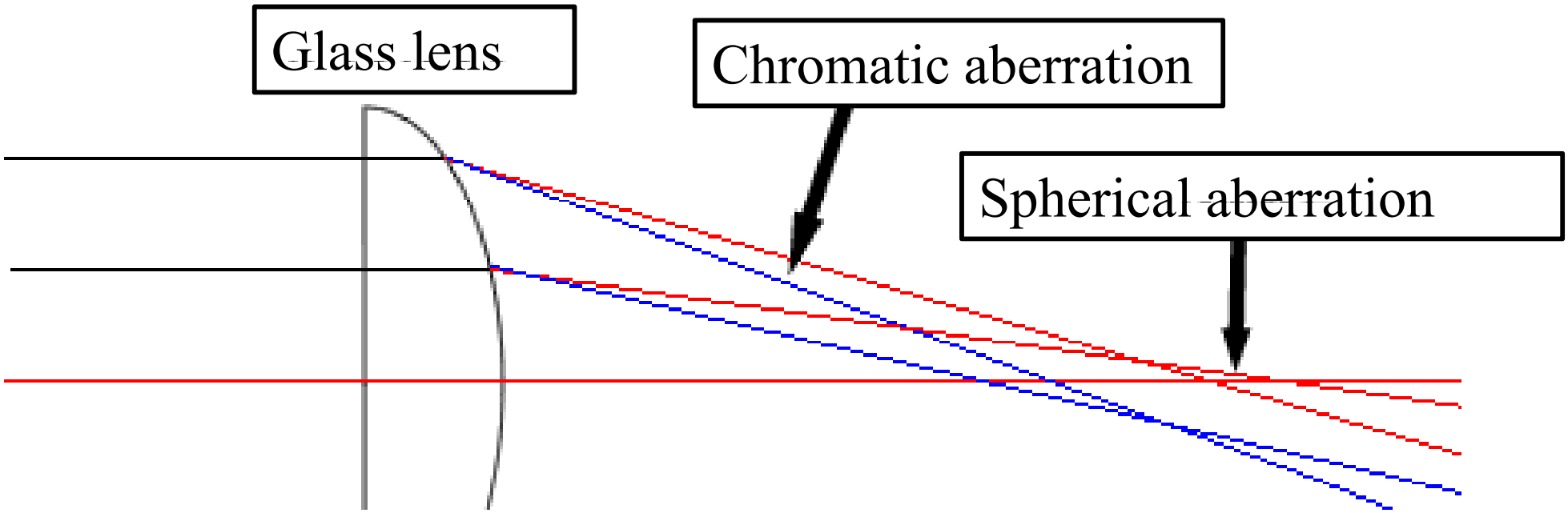


Born and Wolf 1980

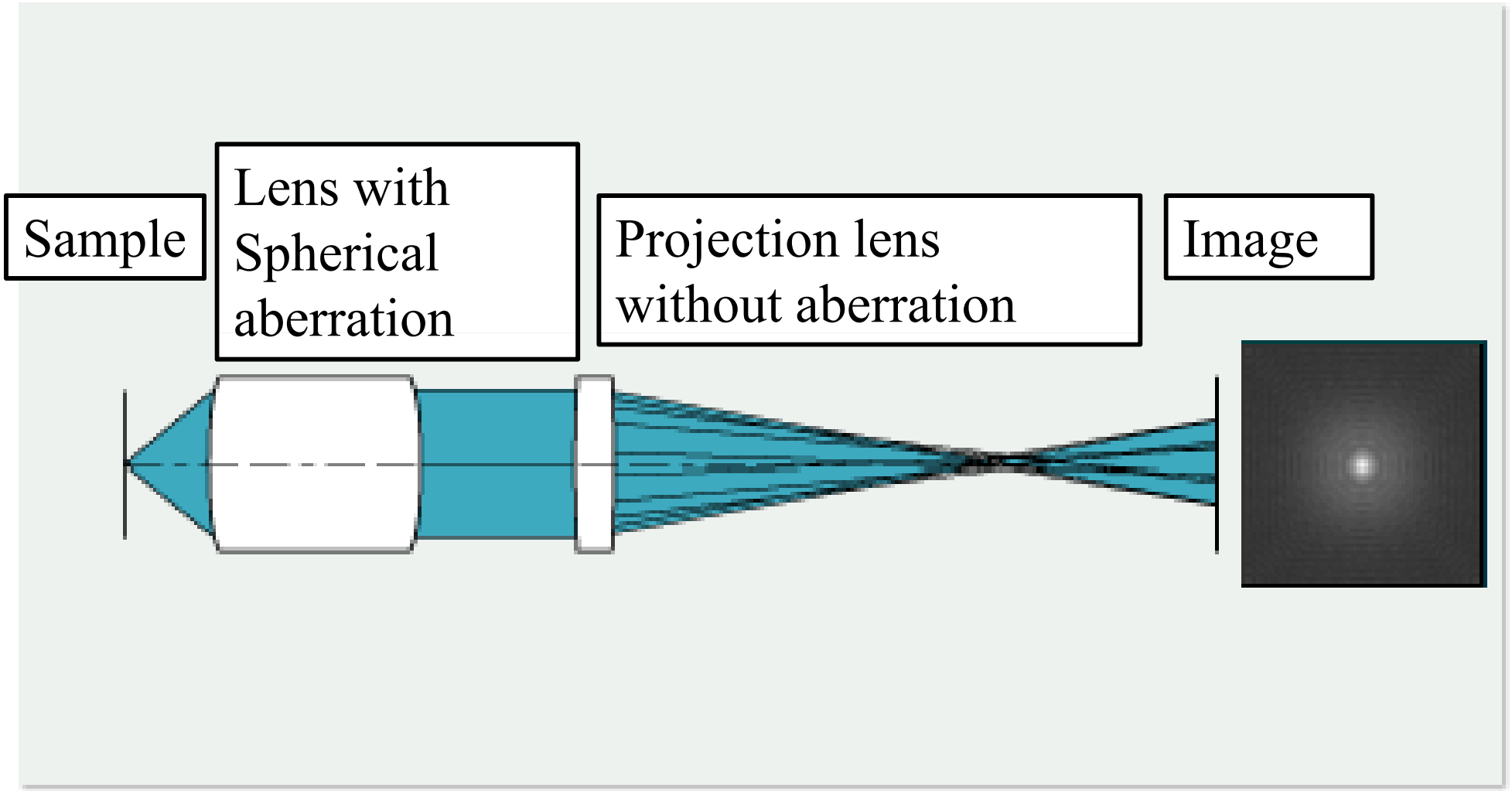
Aberration of lens



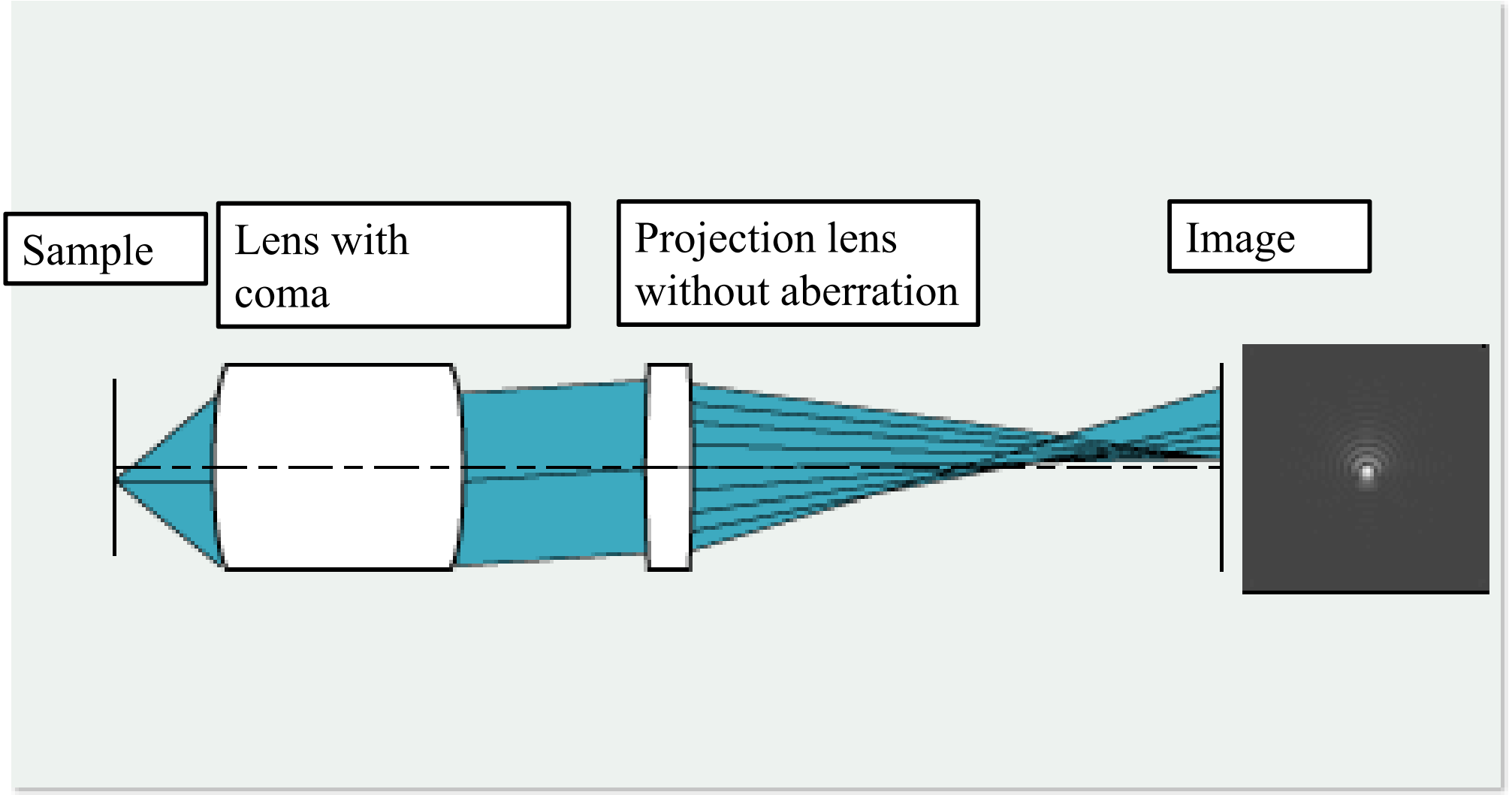
Aberration



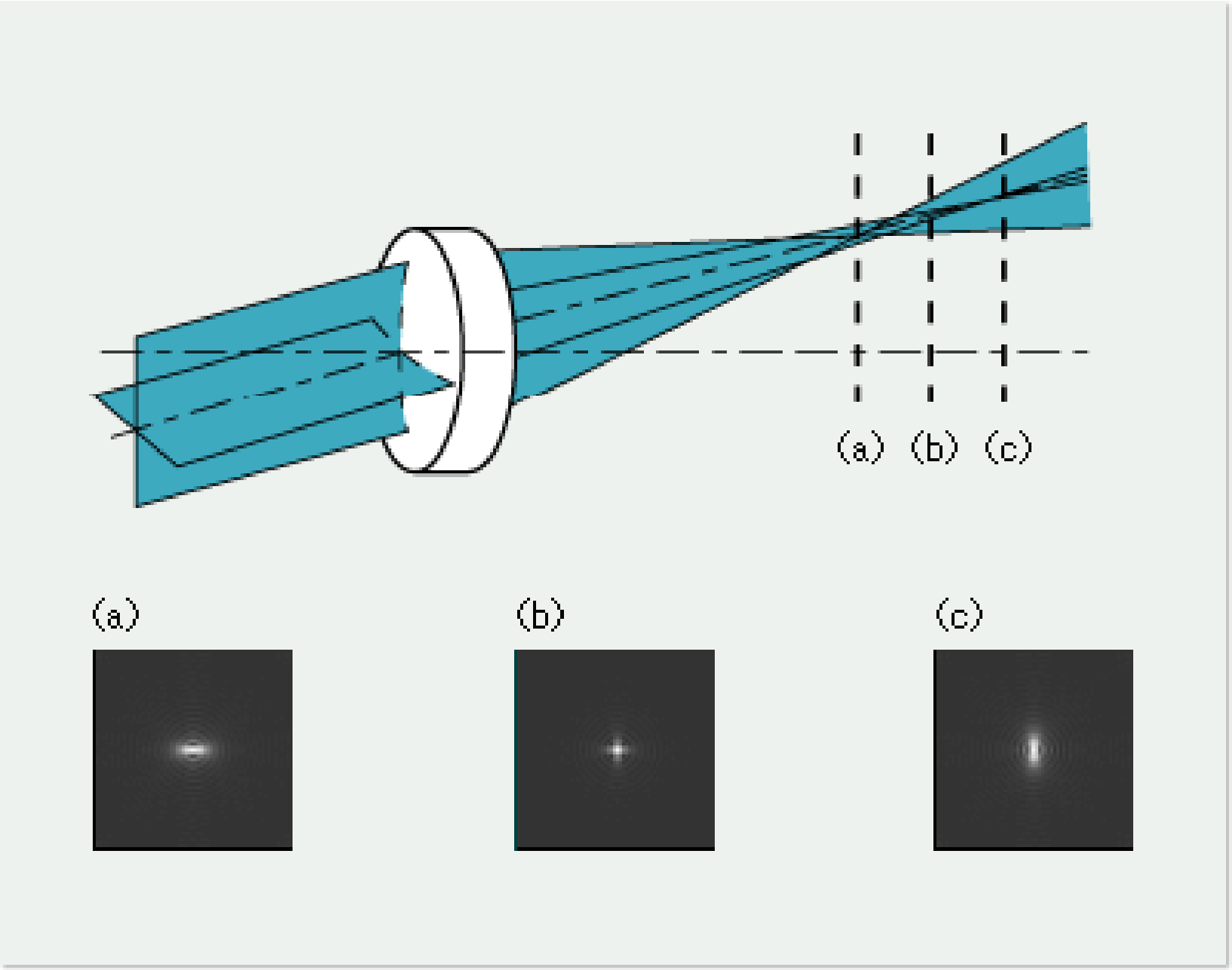
Spherical aberration



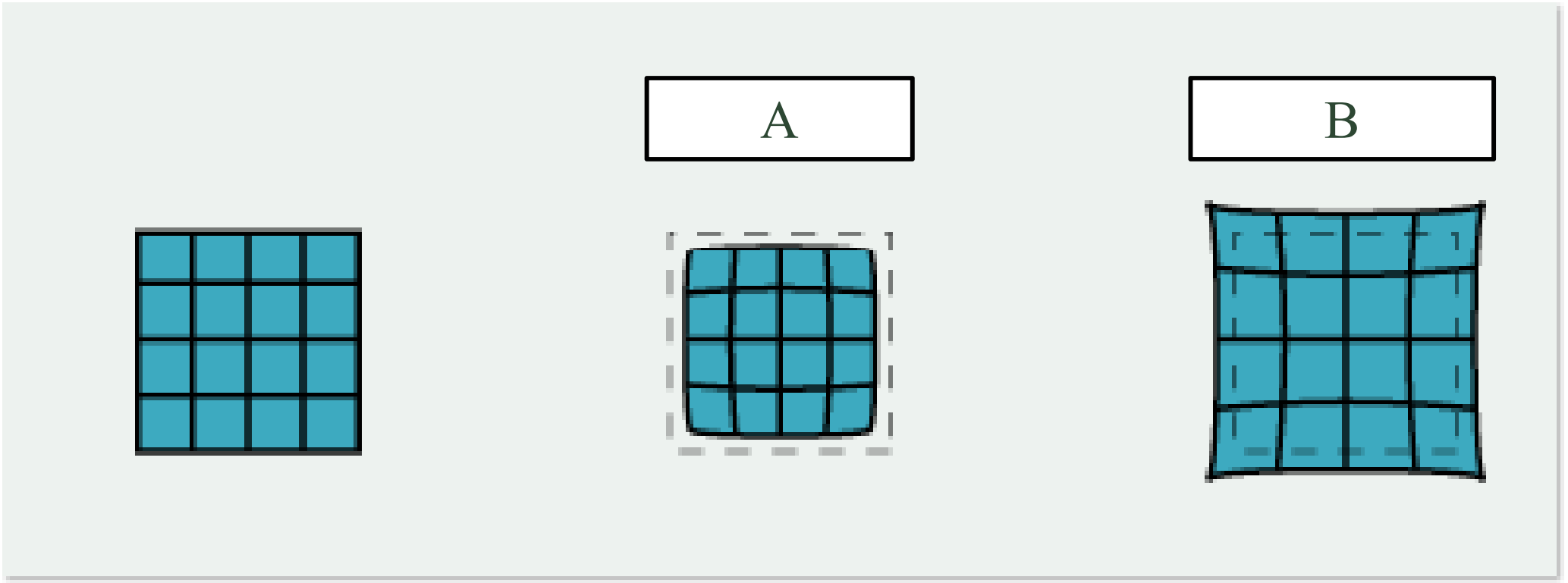
Coma: out of focus in axial periphery

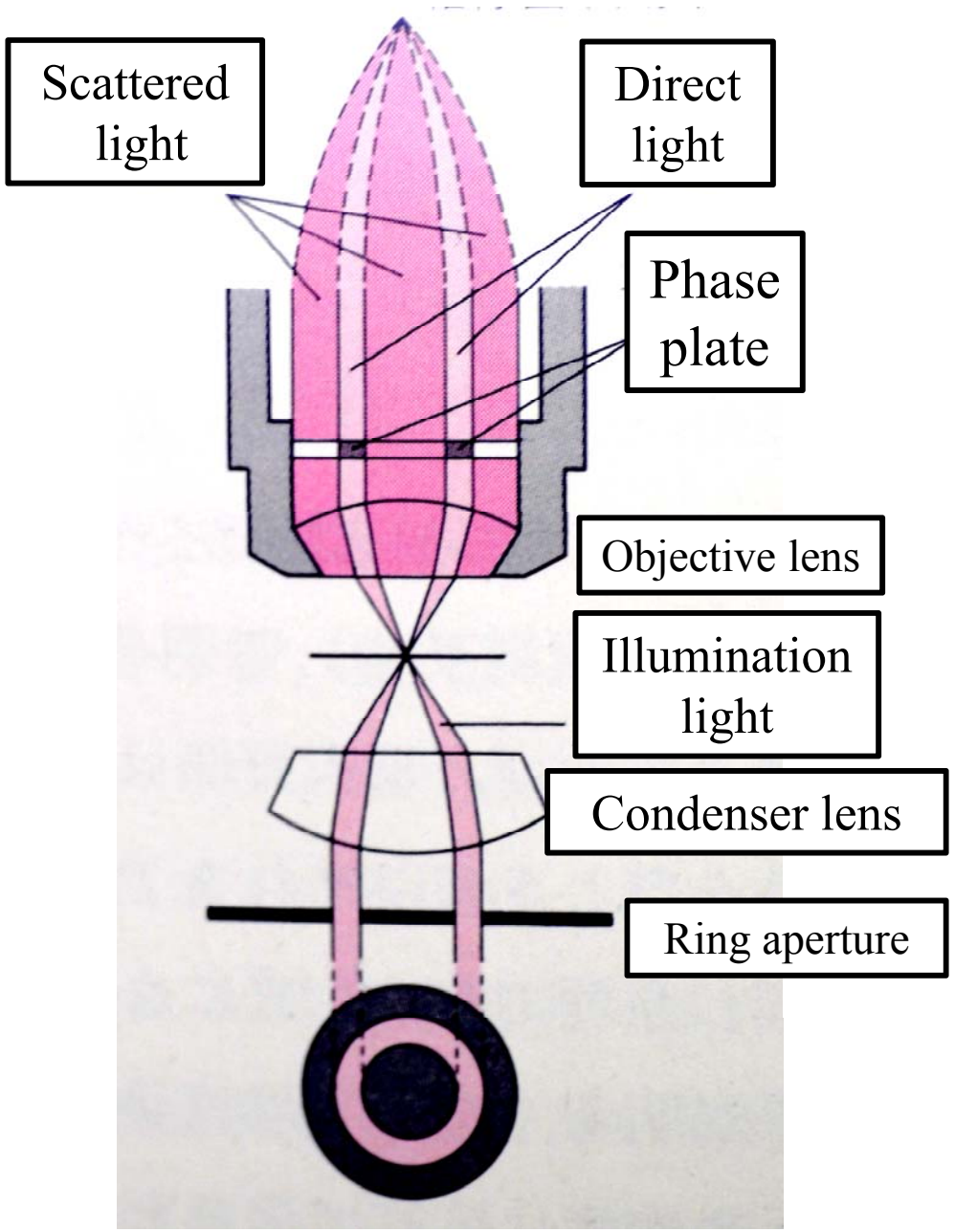


Astigmatism

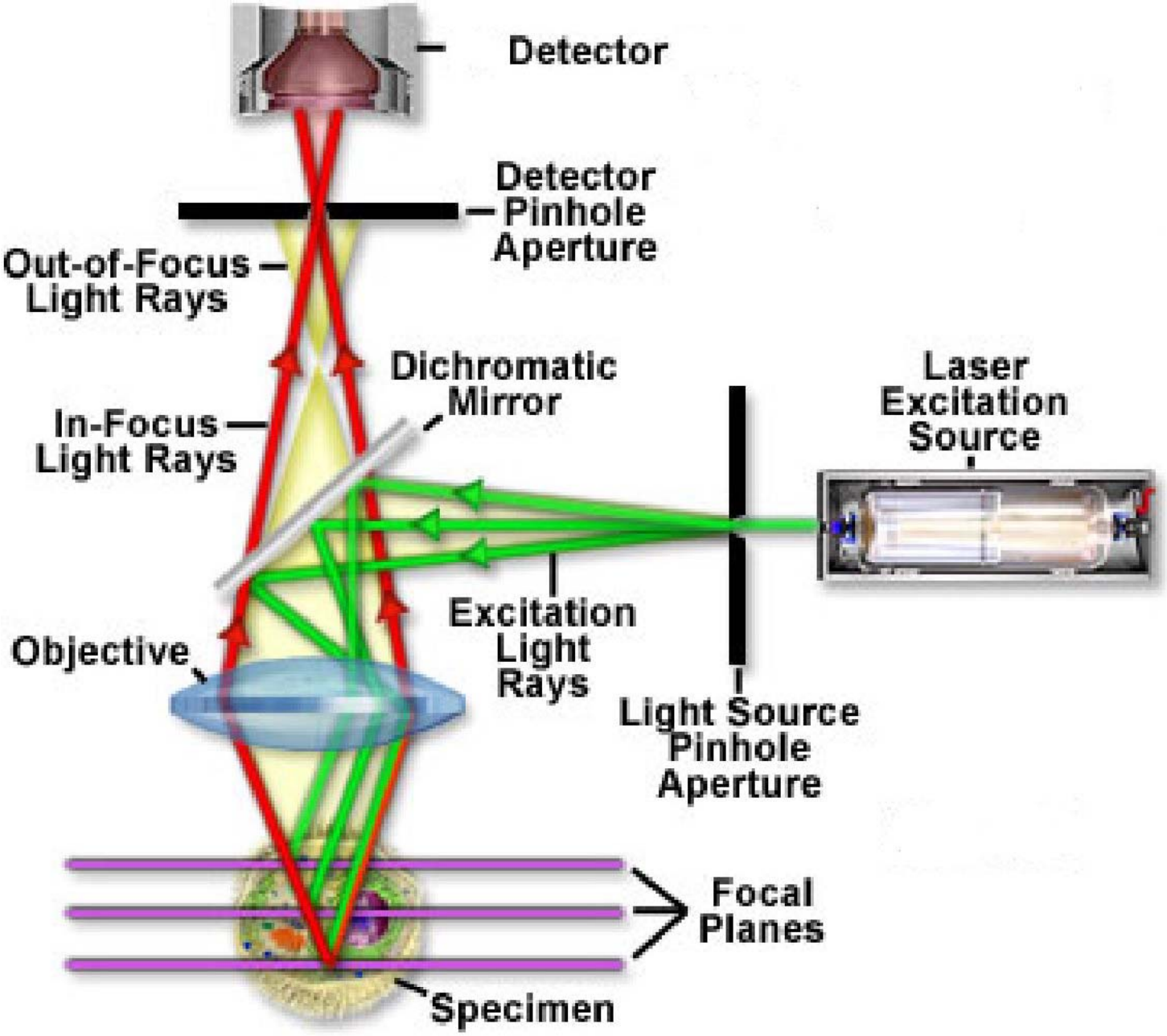


Distortion




Scattered light
Direct light
Phase plate
Objective lens
Illumination light
Condenser lens
Ring aperture

Confocal microscope

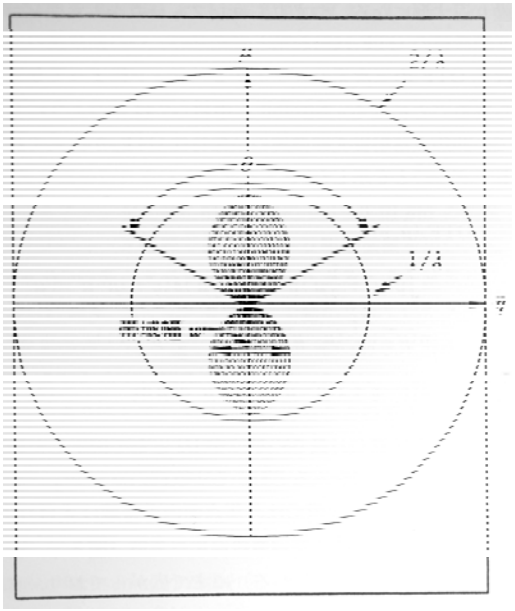


Advantage of confocal optics

1. 3D imaging
2. Tomography
3. Contrast enhancement by reduction of noise

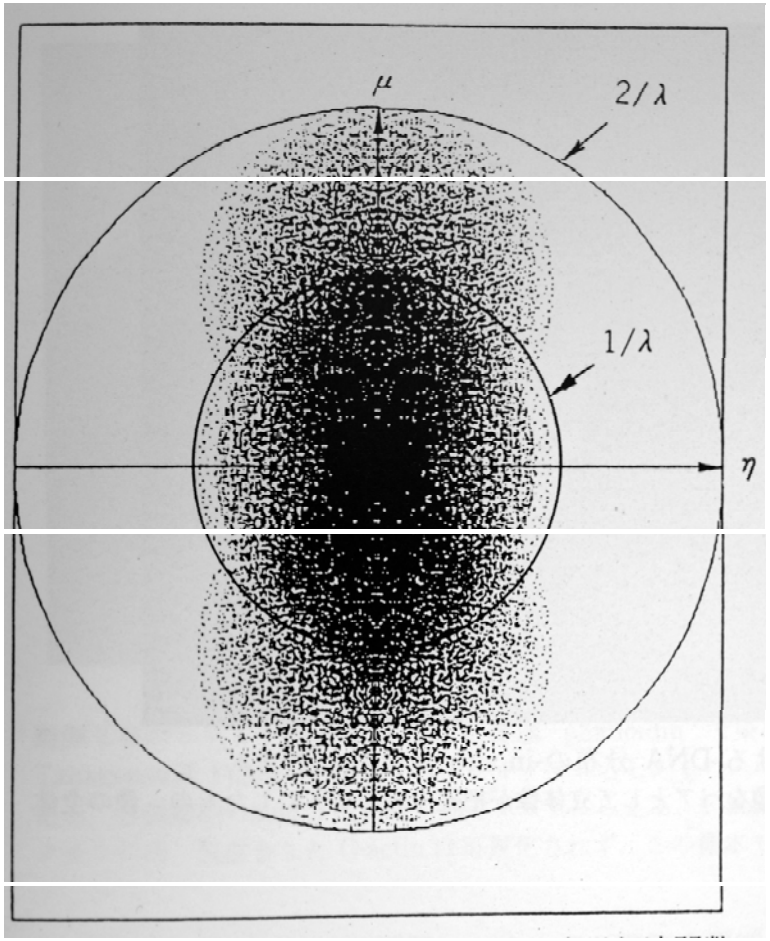
OTF in conventional microscope

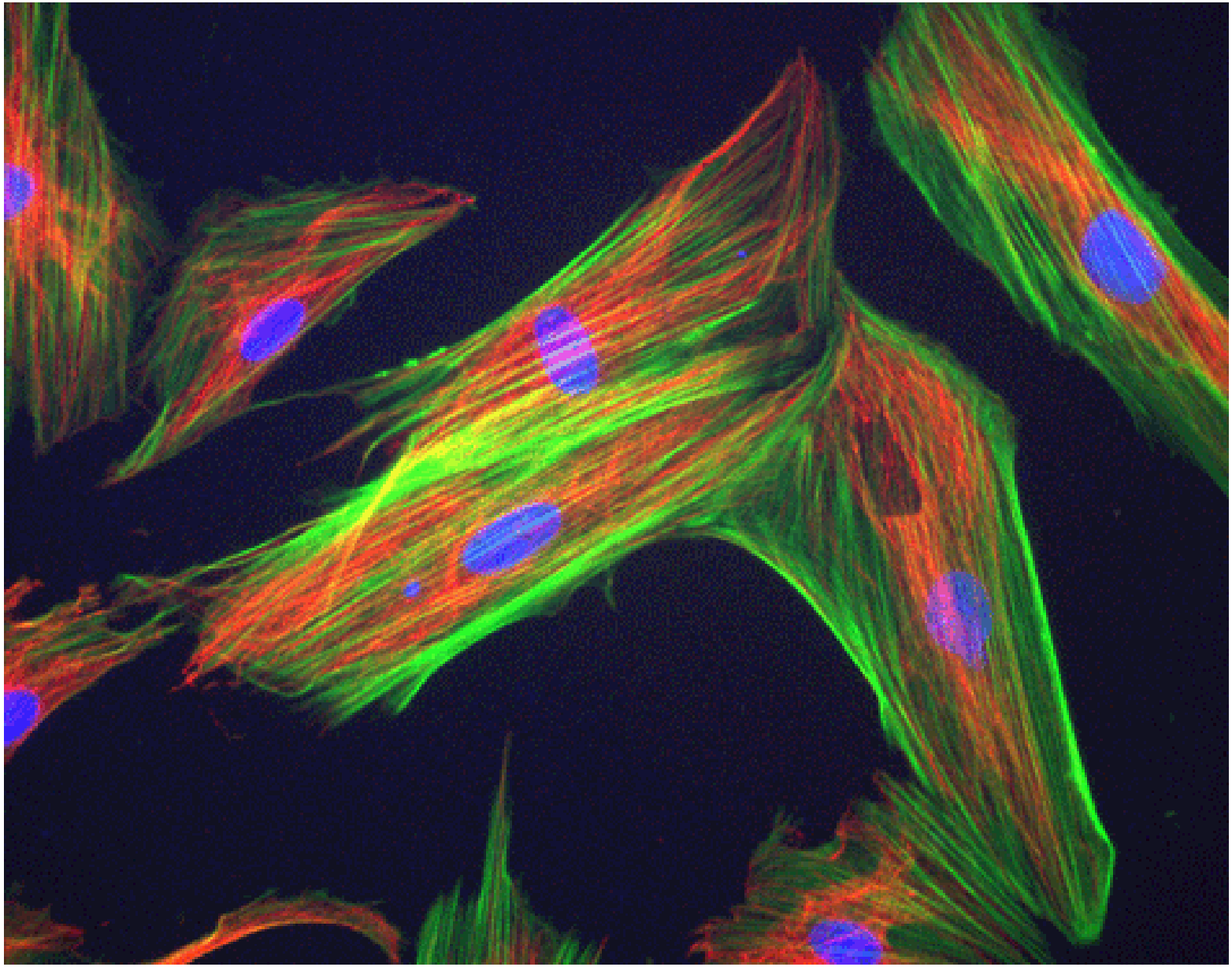
$$\mathrm{OTF}\ n\ (\mu,\eta) = \int p(\mu'+1/2\mu)p^*(\mu'-1/2\mu)\delta(\eta+\lambda\mu \cdot \mu')d^2\mu'$$



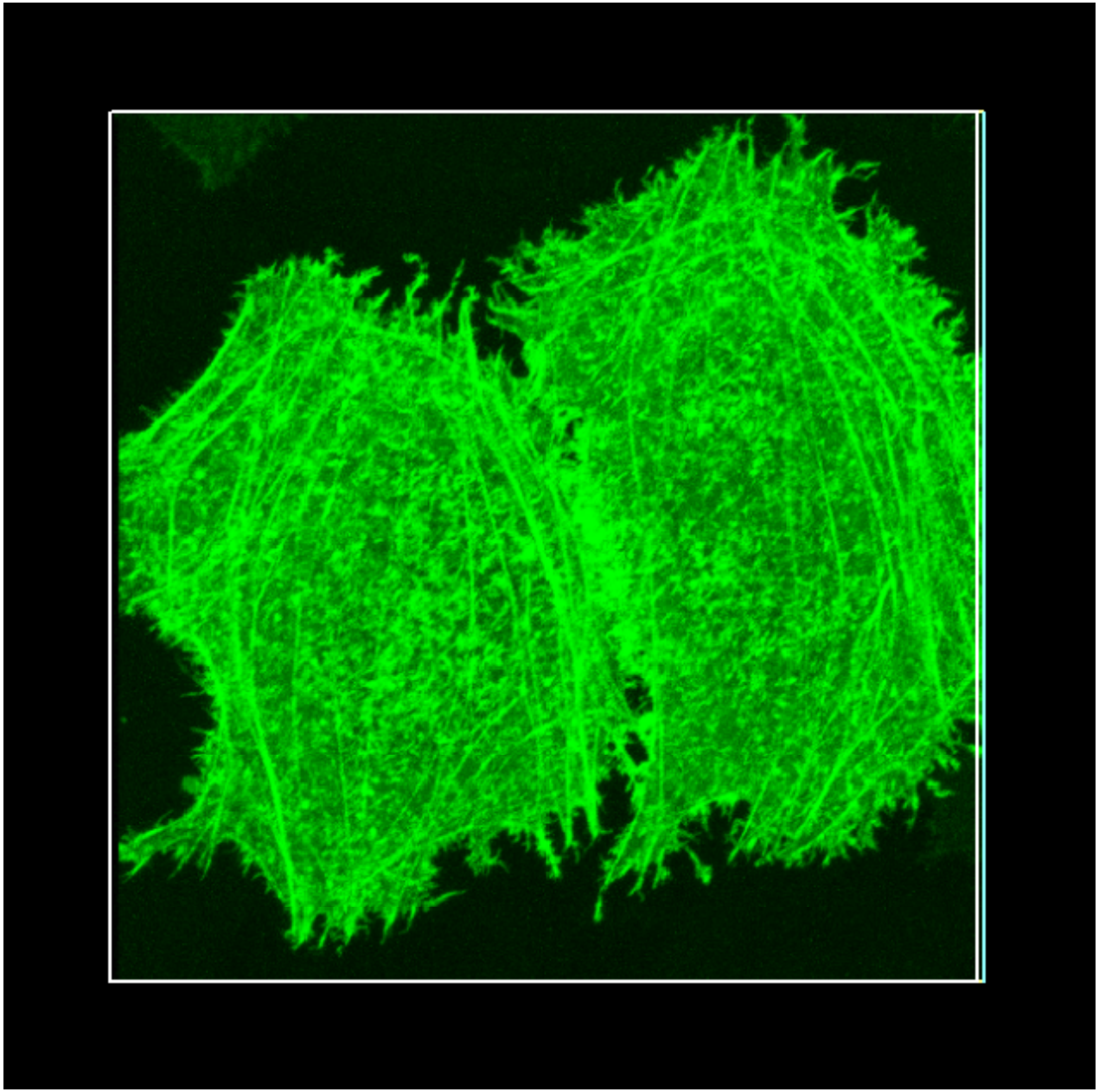
Confocal Optics (OTF)

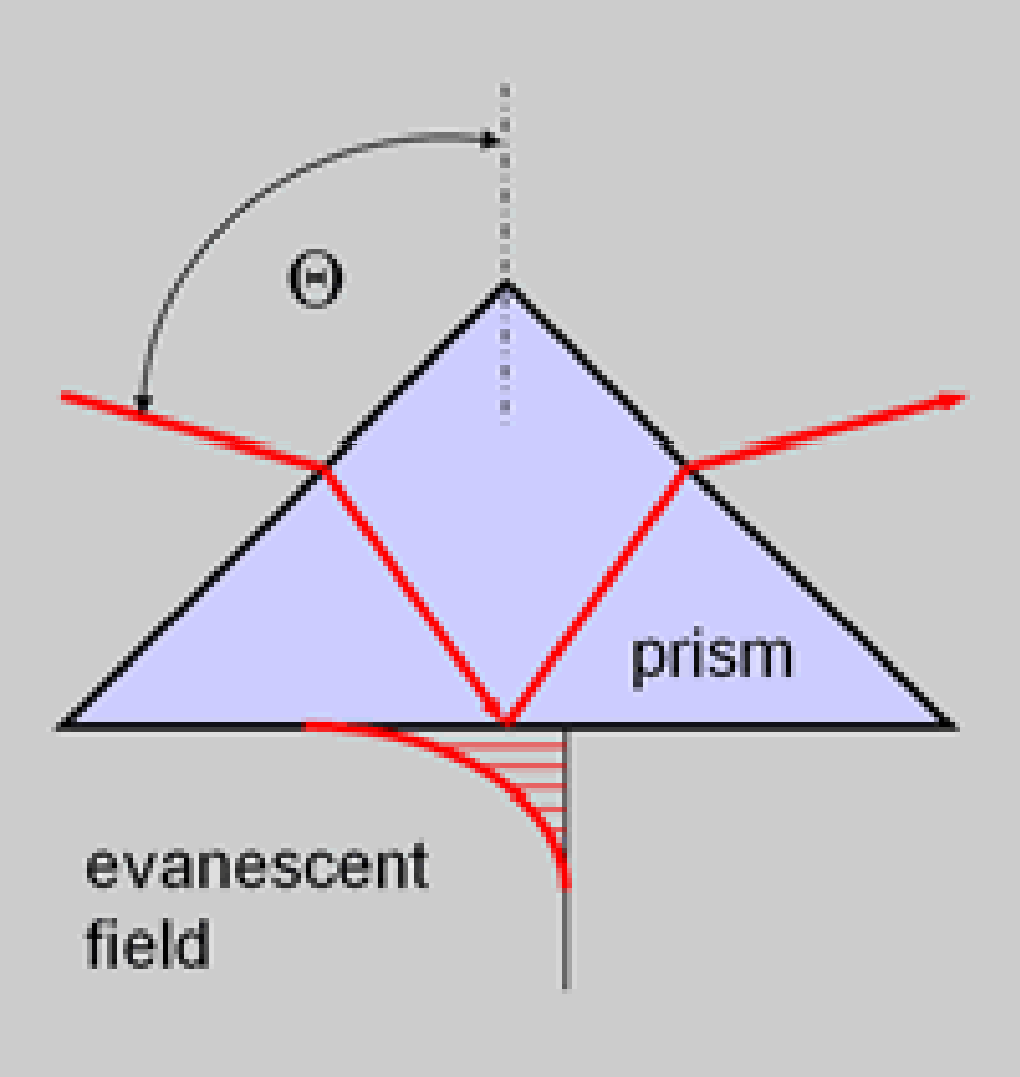
$$\mathrm{OTF}\ c\ (\mu,\eta) = \int p(\mu''+1/2\mu')p^*(\mu''-1/2\mu')\delta(\eta'+\lambda\mu' \cdot \mu'')d^2\mu \cdot p(\mu'''+1/2(\mu-\mu'))\ p^*(\mu'''-1/2(\mu-\mu')) \cdot \delta(\eta-\eta'+\lambda(\mu-\mu')\ \mu''')\ d^2\ \mu'''d^2\eta'$$

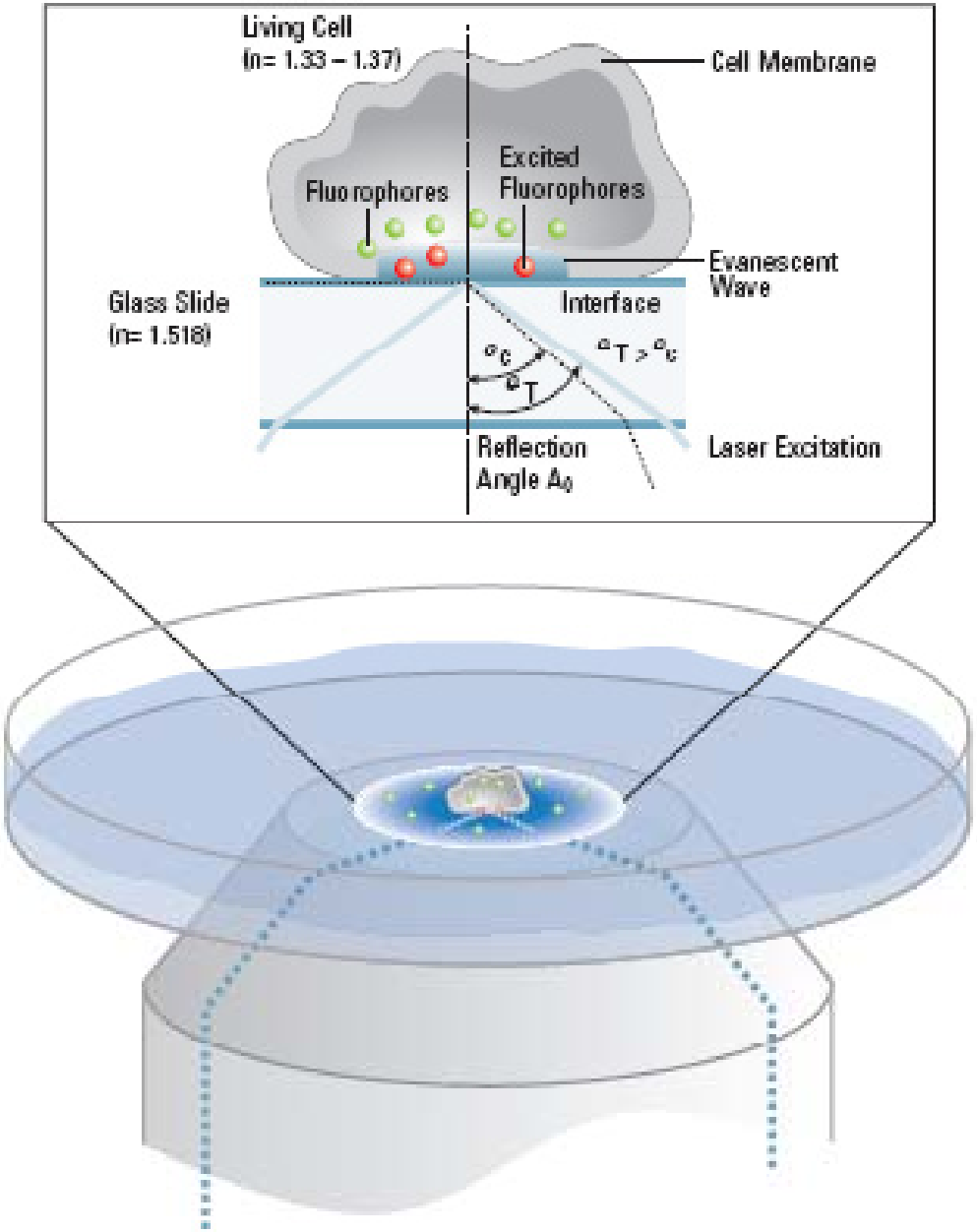




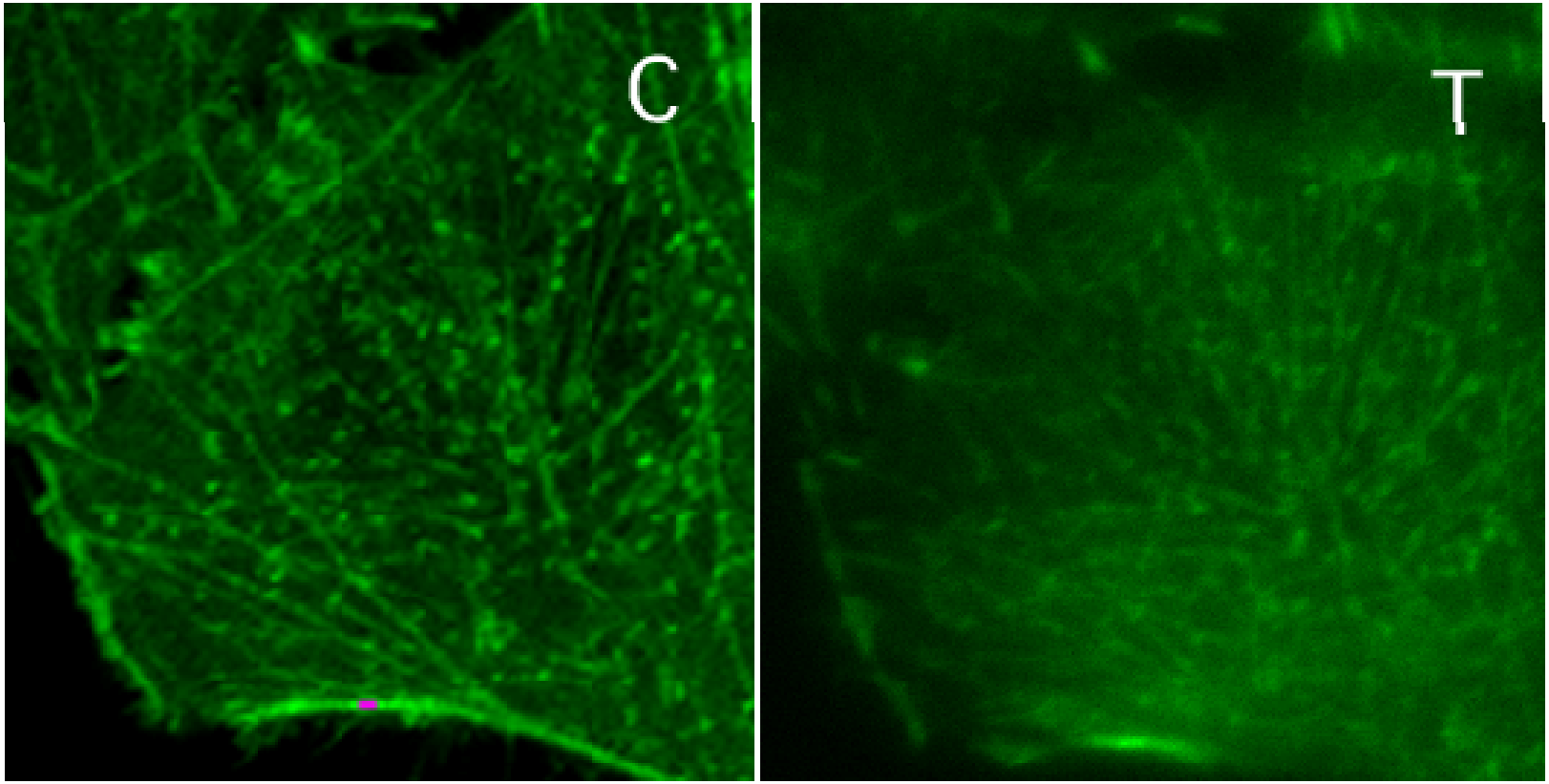
HeLa cells Expressing GFP-actin at stable state



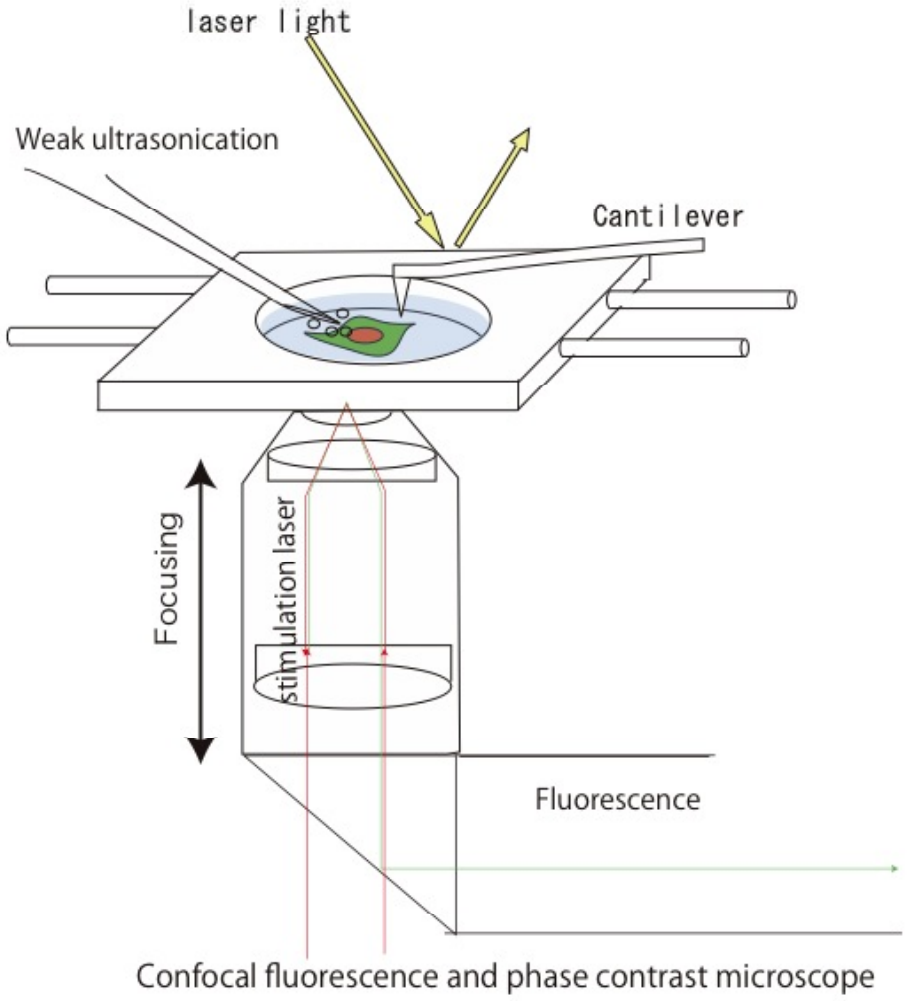

Θ
prism
evanescent
field

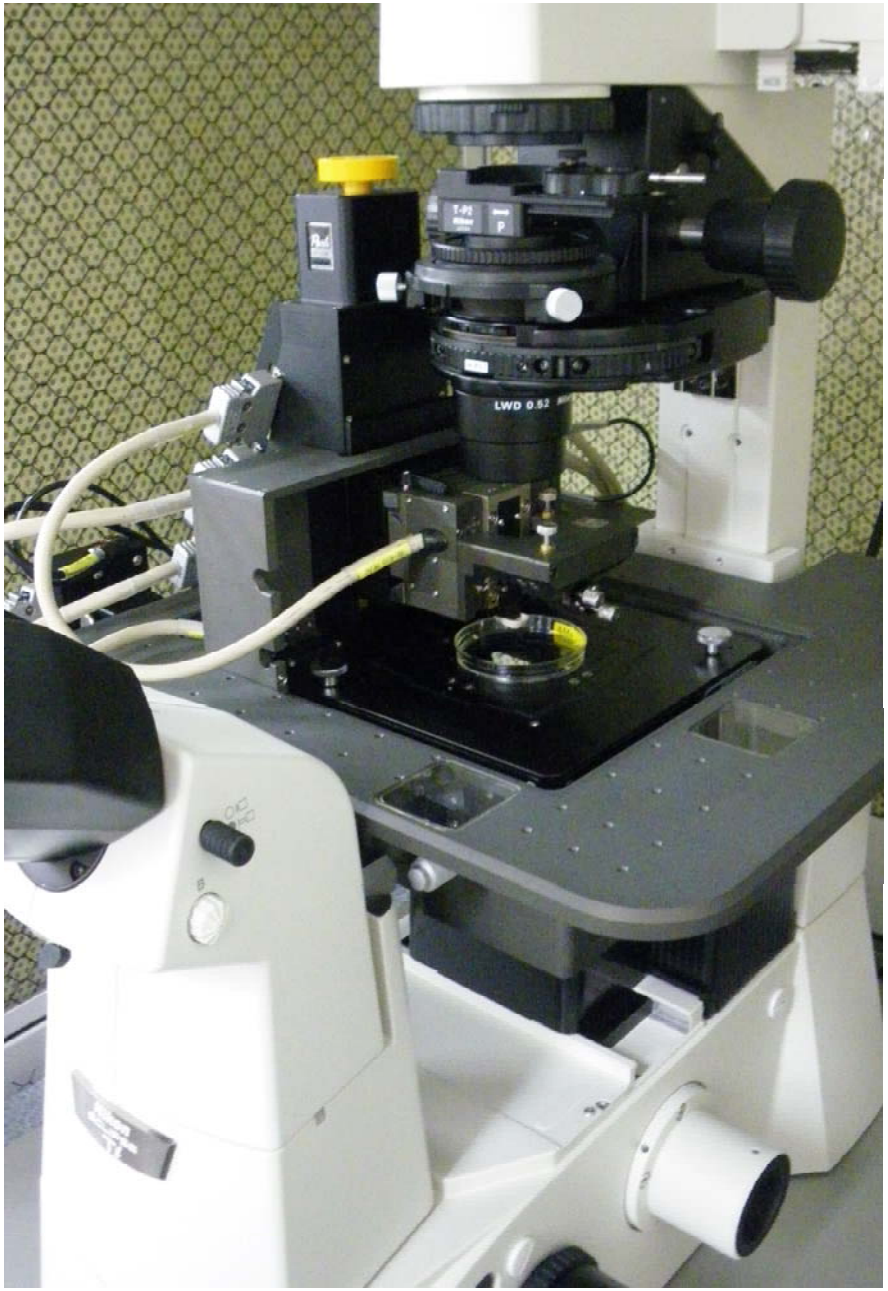

Living Cell
(n= 1.33 – 1.37)
Cell Membrane
Excited
Fluorophores
Fluorophores
Evanescent
Wave
Glass Slide
(n= 1.518)
Interface
θ_C
$\theta_T > \theta_C$
θ_T
Reflection
Angle A_0
Laser Excitation

Comparison of confocal microscope (C) with total reflection microscope (T)



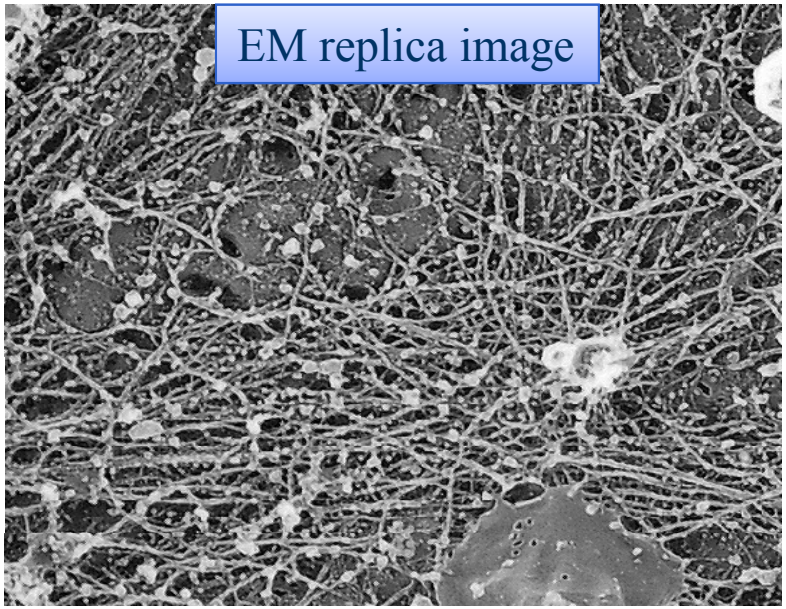
Atomic force microscope we developed

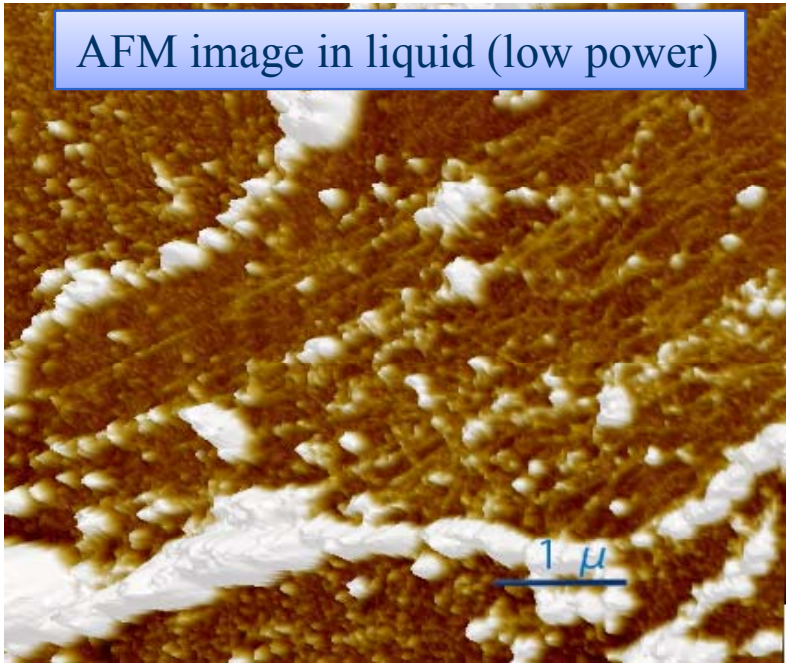


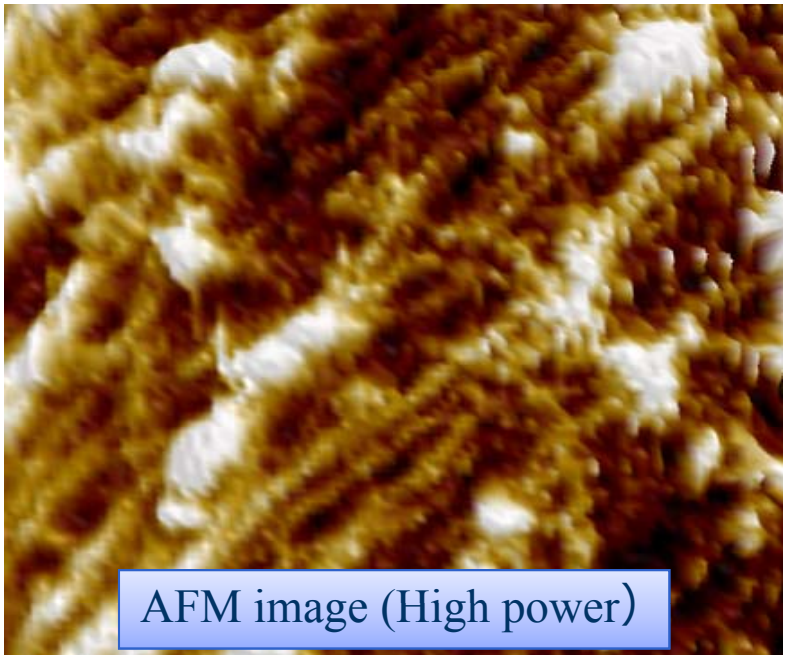


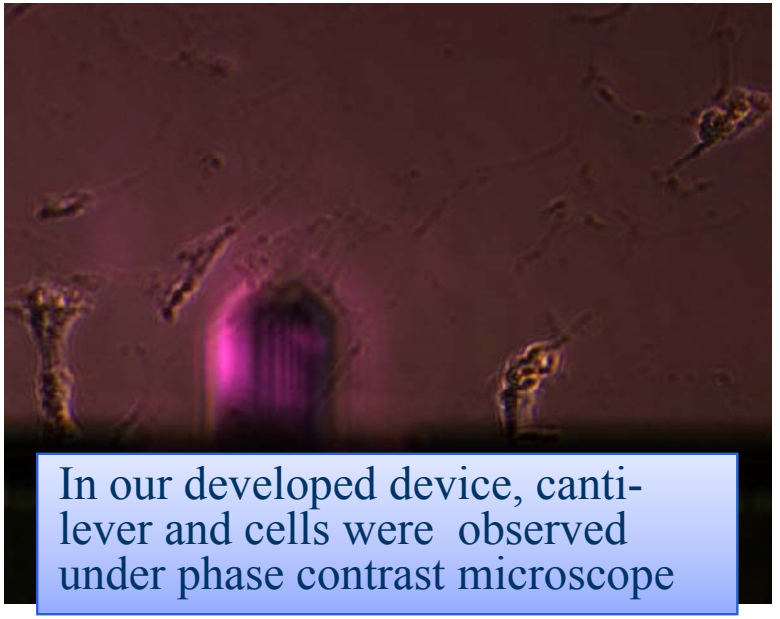
Tip of cantilever

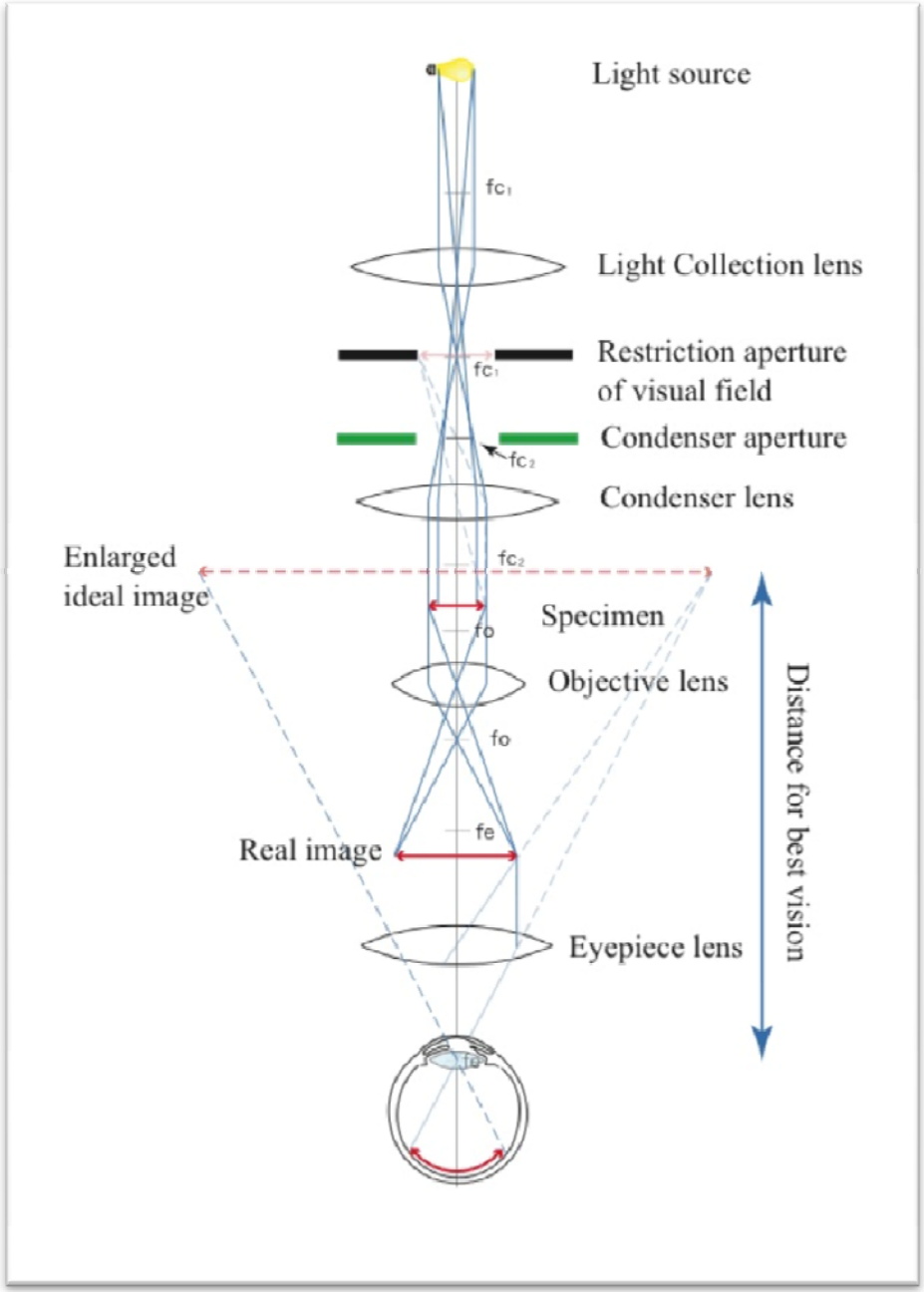
Comparison of AFM view of actin cytoskeleton in liquid with electron microscopic image

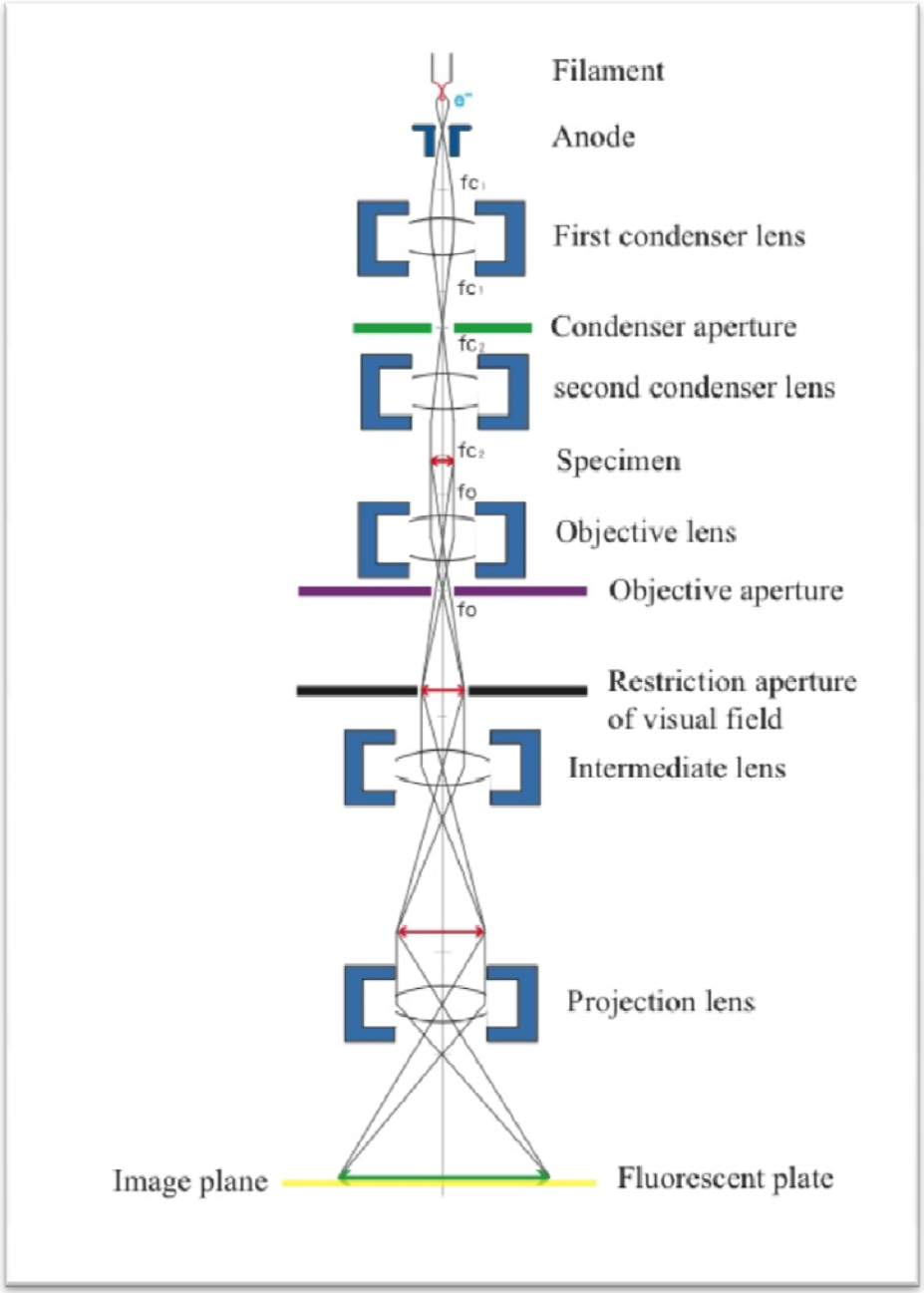









Light source
fc1
Light Collection lens
Restriction aperture
of visual field
fc1
Condenser aperture
fc2
Condenser lens
Enlarged
ideal image
fc2
Specimen
fo
Objective lens
fo
Real image
fe
Eyepiece lens
Distance for best vision


Filament
e⁻
Anode
fc1
First condenser lens
fc1
Condenser aperture
fc2
second condenser lens
fc2
Specimen
fo
Objective lens
Objective aperture
fo
Restriction aperture
of visual field
Intermediate lens
Projection lens
Image plane
Fluorescent plate

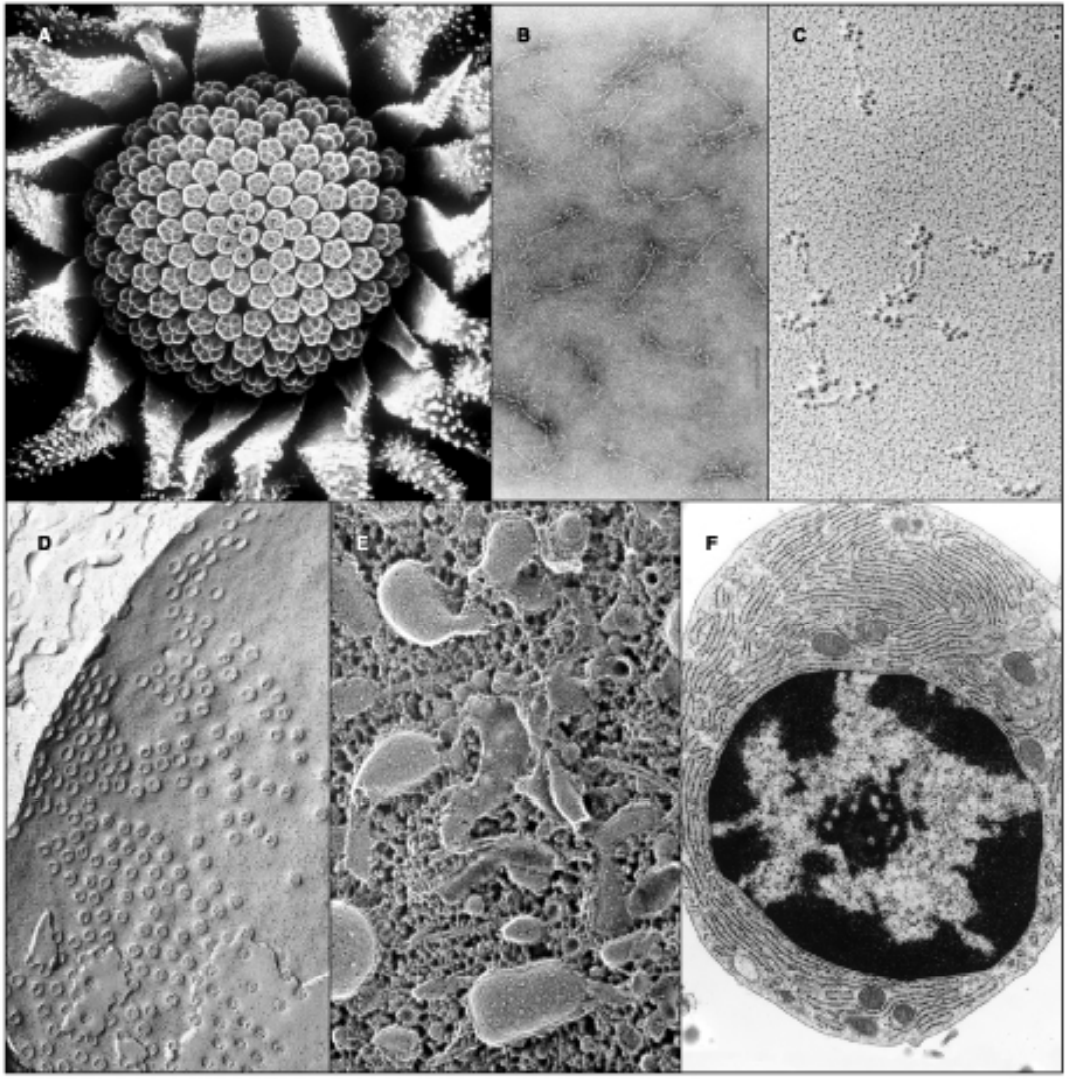
Variety of electron microscope

Scanning electron microscope

Scanning transmission electron microscope

Transmission electron microscope

Structural information is different by methods in electron microscopy



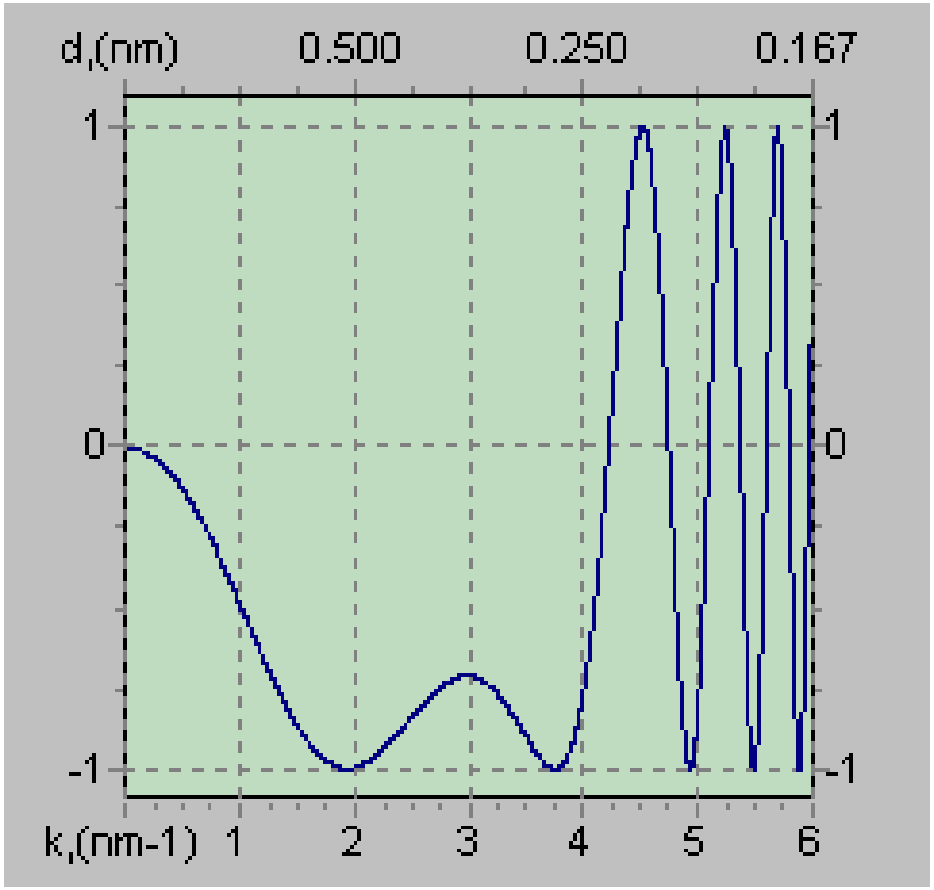
$$T(k) = -\sin\left[\frac{\pi}{2} C_s \lambda^3 k^4 + \pi \Delta f \lambda k^2\right]$$

Minus is positive contrast
Plus is negative contrast

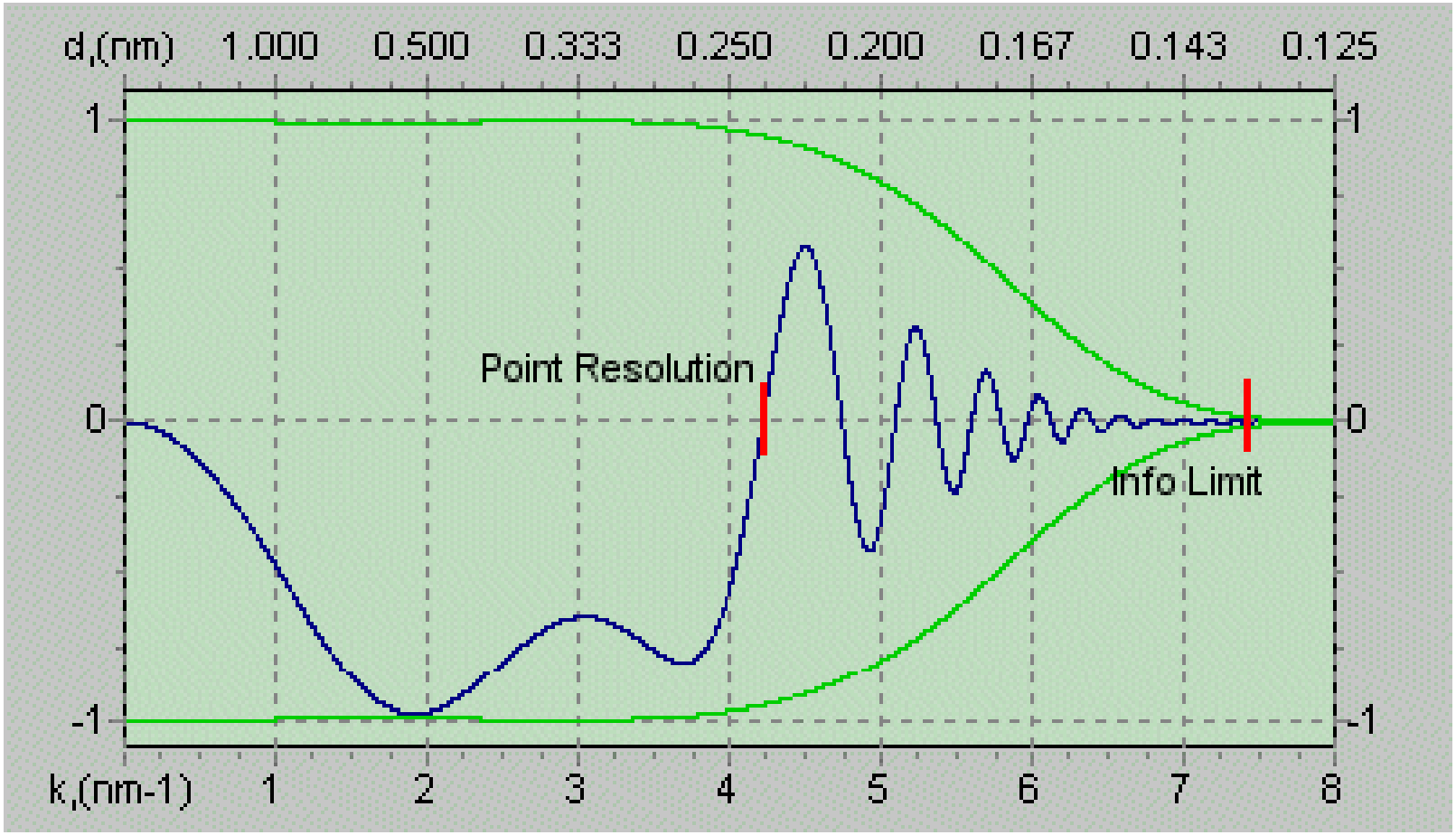
Oscillation is not maintained, and terminate to zero by Envelope Function as follows.

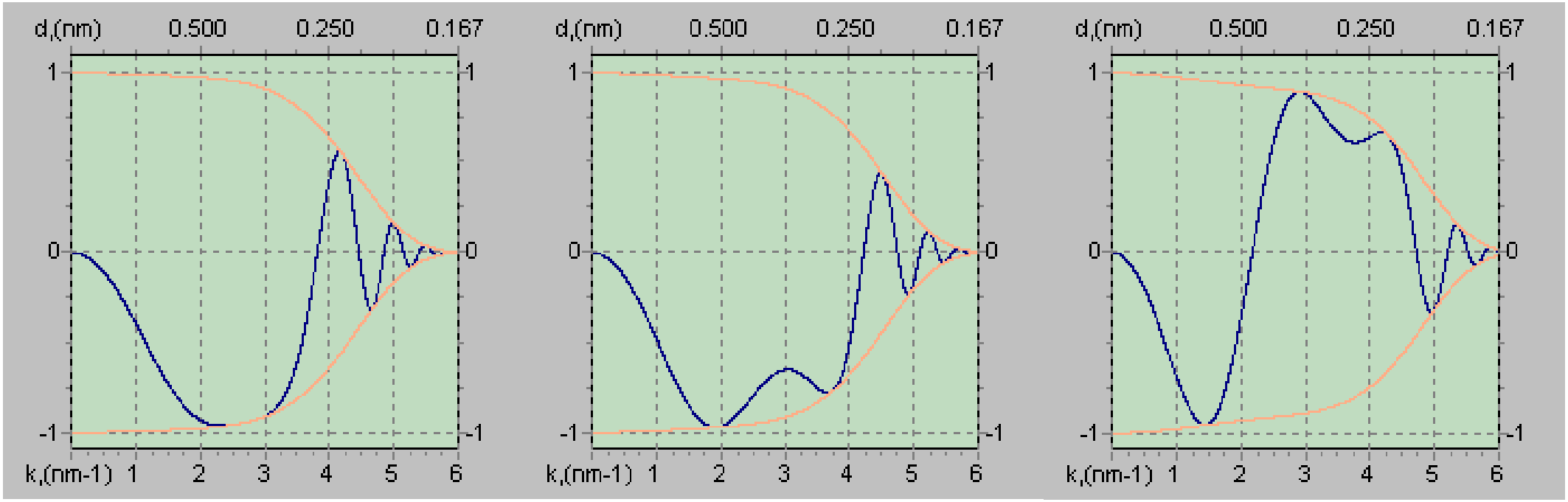
$$T(k)_{eff} = T(k) E_c E_a$$

Temporal coherency envelope
Spatial coherency envelope



$$r_{sch} = 0.66 C_s^{1/4} \lambda^{3/4}$$

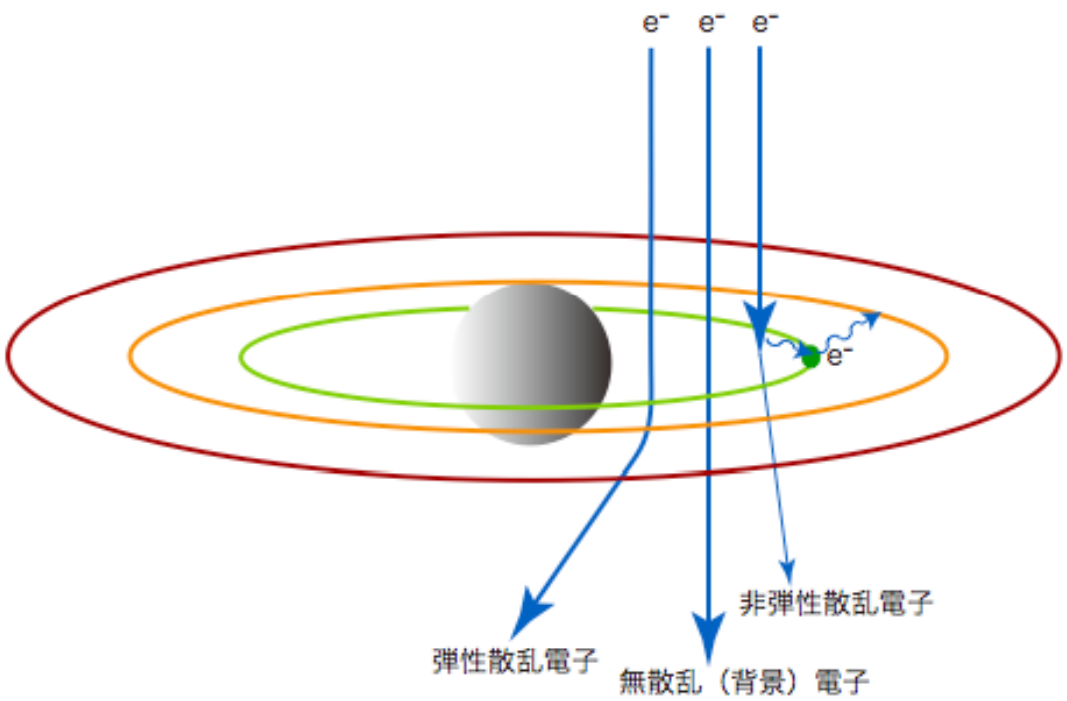




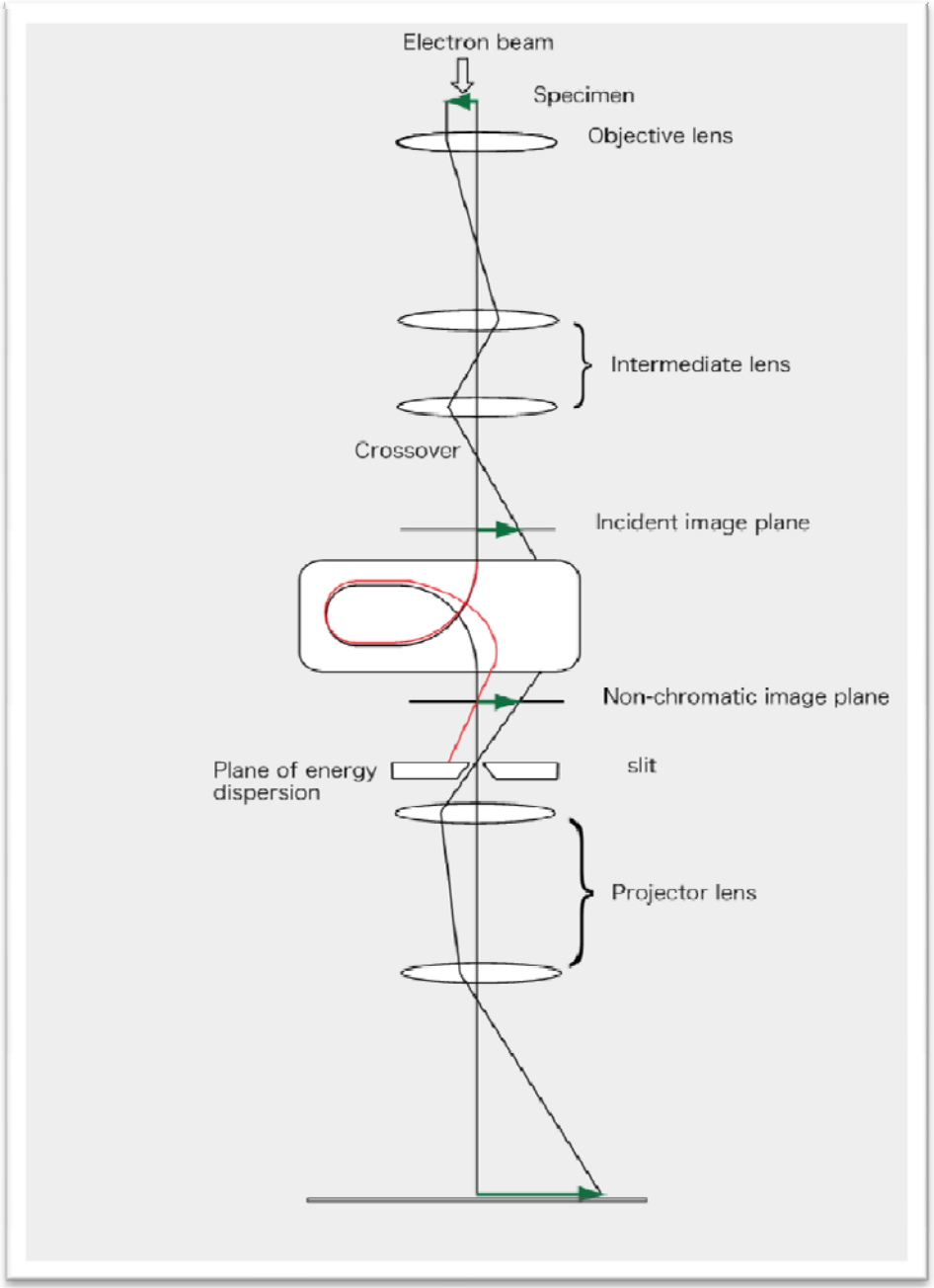
Defocus: 1 scherzer ("True" Scherzer defocus)

1.2 scherzer ("Extended" Scherzer defocus). In general, this is the best defocus to take HR-TEM images.

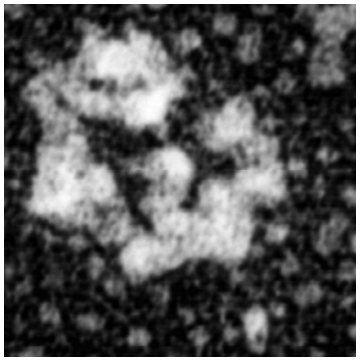
Defocus: 1.9 scherzer
CTF is positive so that it produces a negative phase contrast ("white atoms")


e⁻
e⁻
e⁻
e⁻
非弾性散乱電子
弾性散乱電子
無散乱（背景）電子

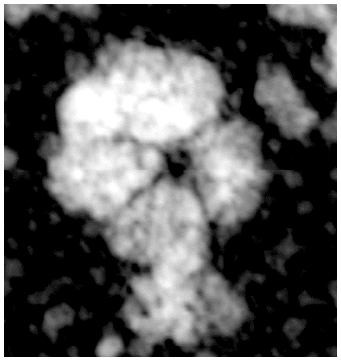
Optical path for Electron Energy Loss Spectral Imaging



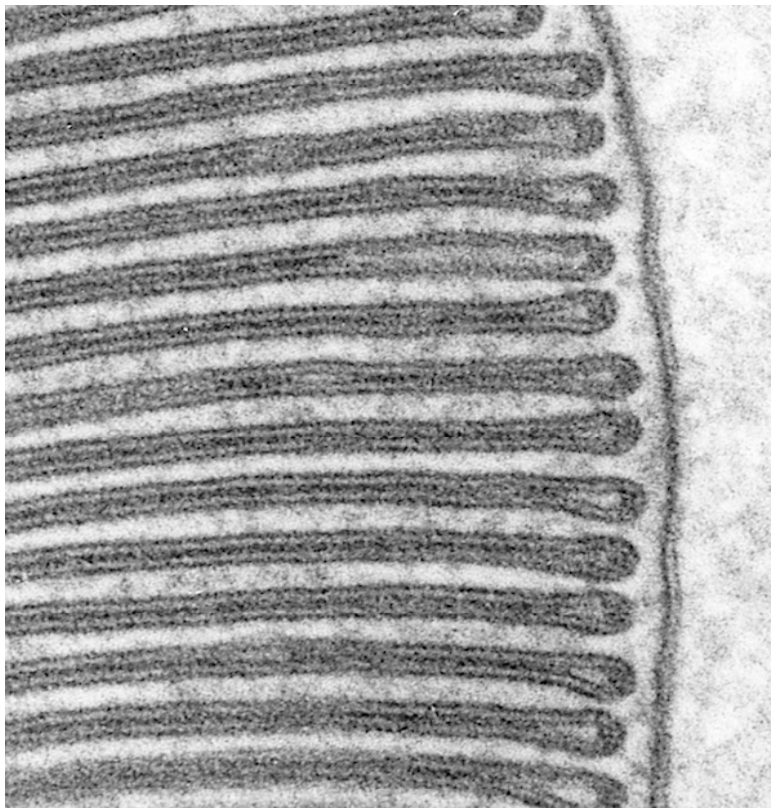
High resolution imaging of outer segment disks in retinal cell by different methods in electron microscopy

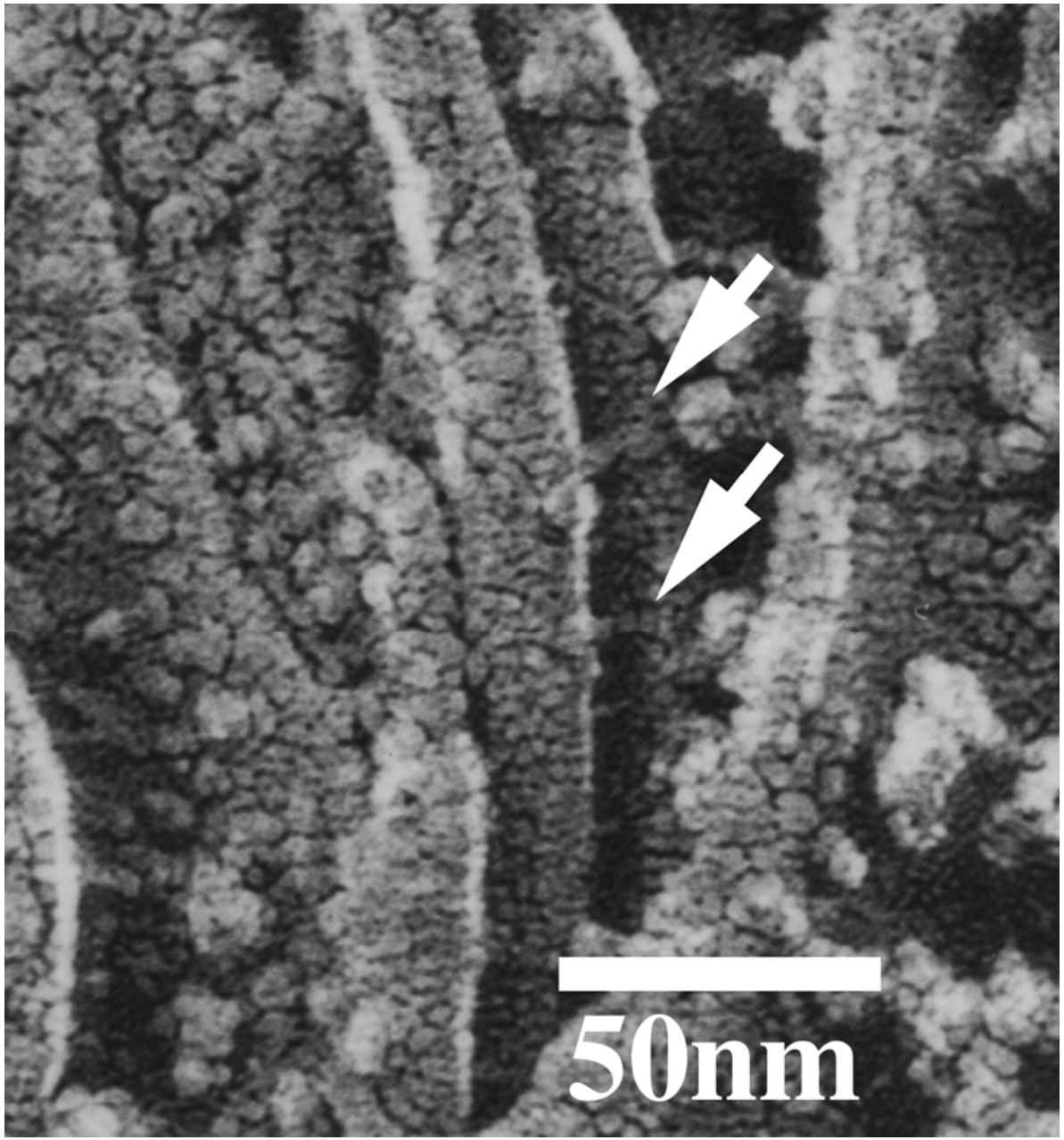


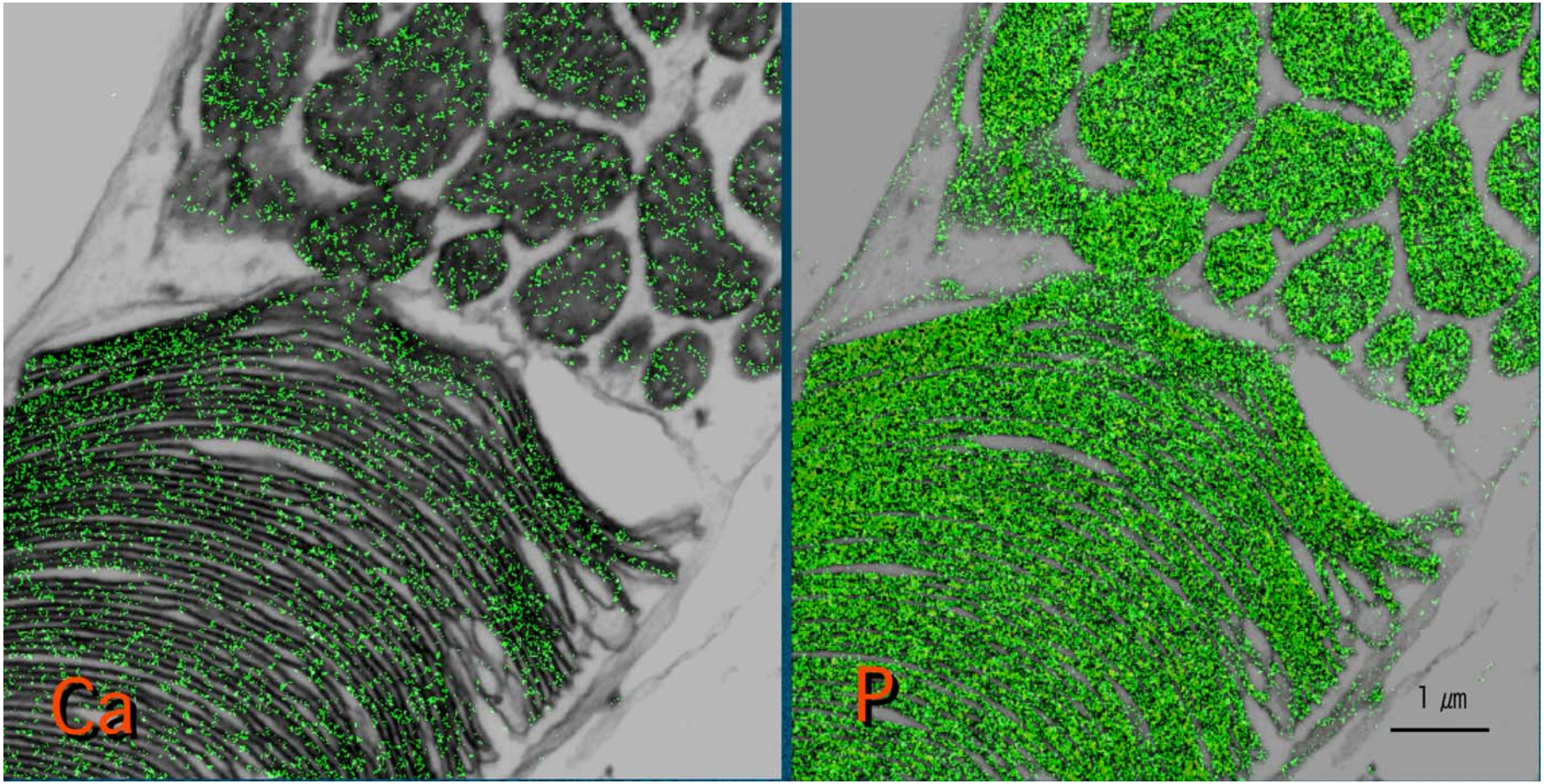
PDE



Tβ

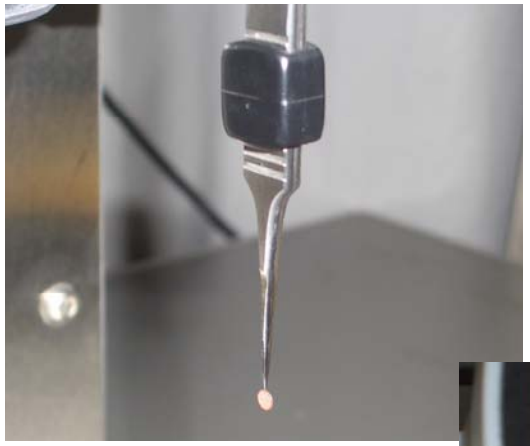




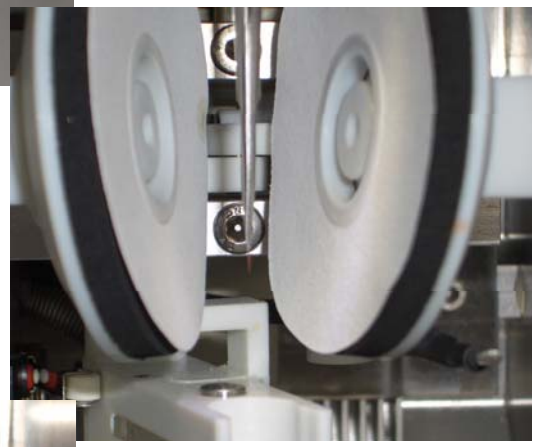

Ca
P
1 μm

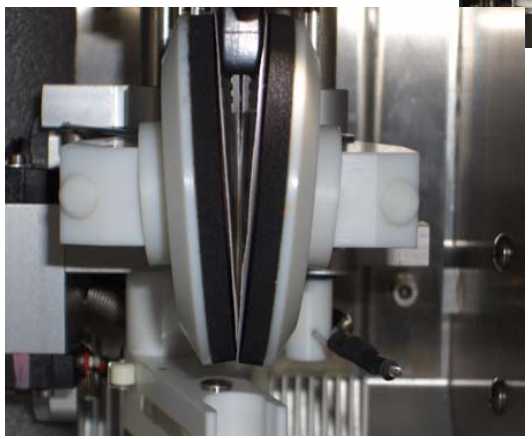
New cryo-electron microscopic techniques for observing the membrane cytoskeleton

Device for rapid freezing (Vitrobot)



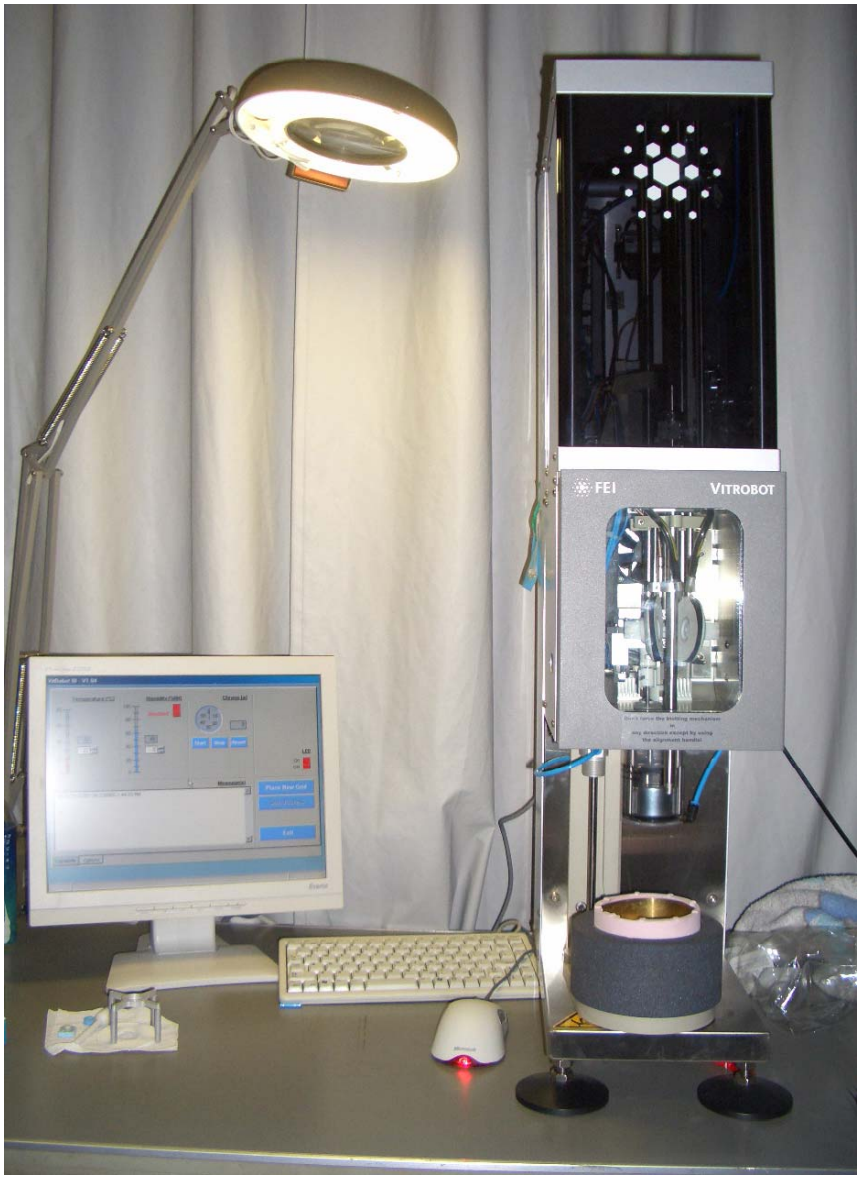
Grid assembled



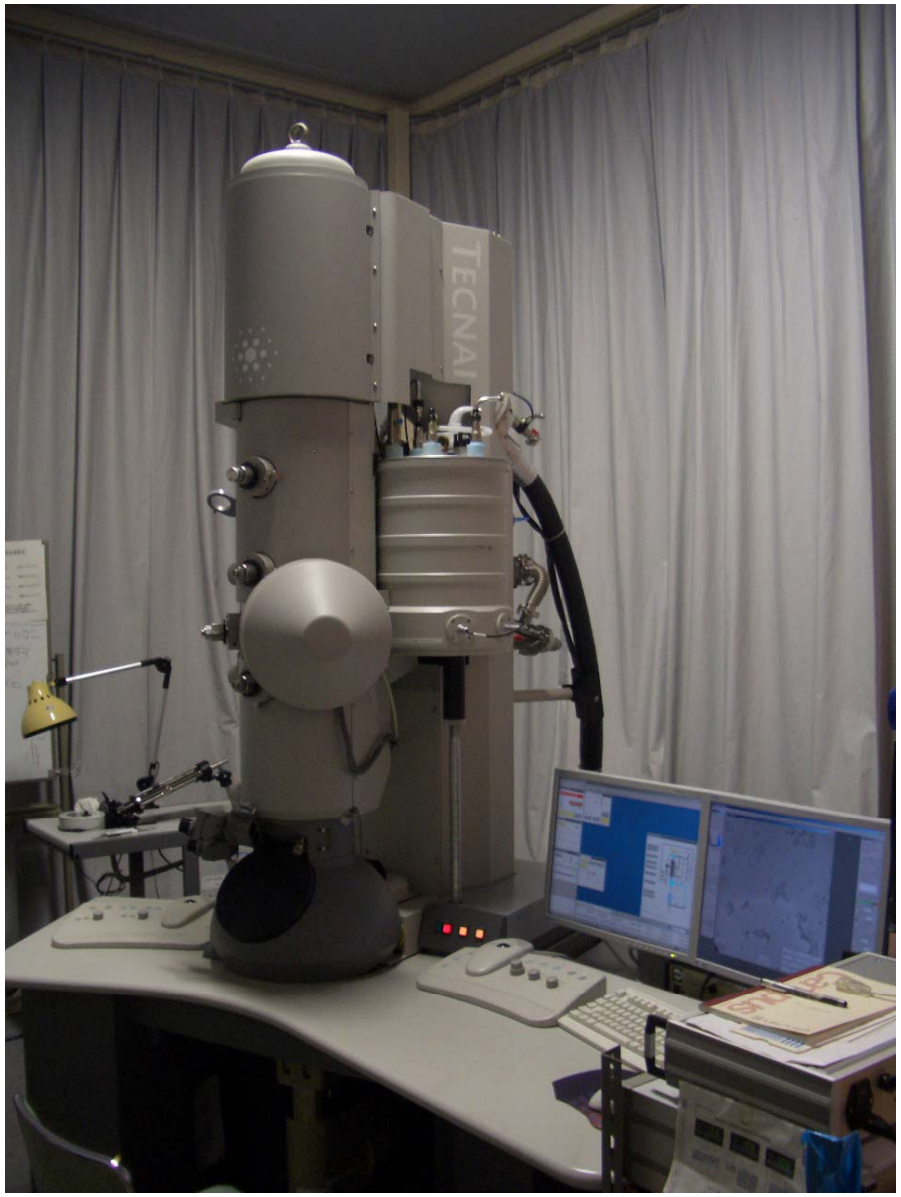


Absorption of excess amount of water

Over view of the device



Cryo-electron microscope (FEI TECNAI G2 Polara)



Cryotransfer

Specimen chamber

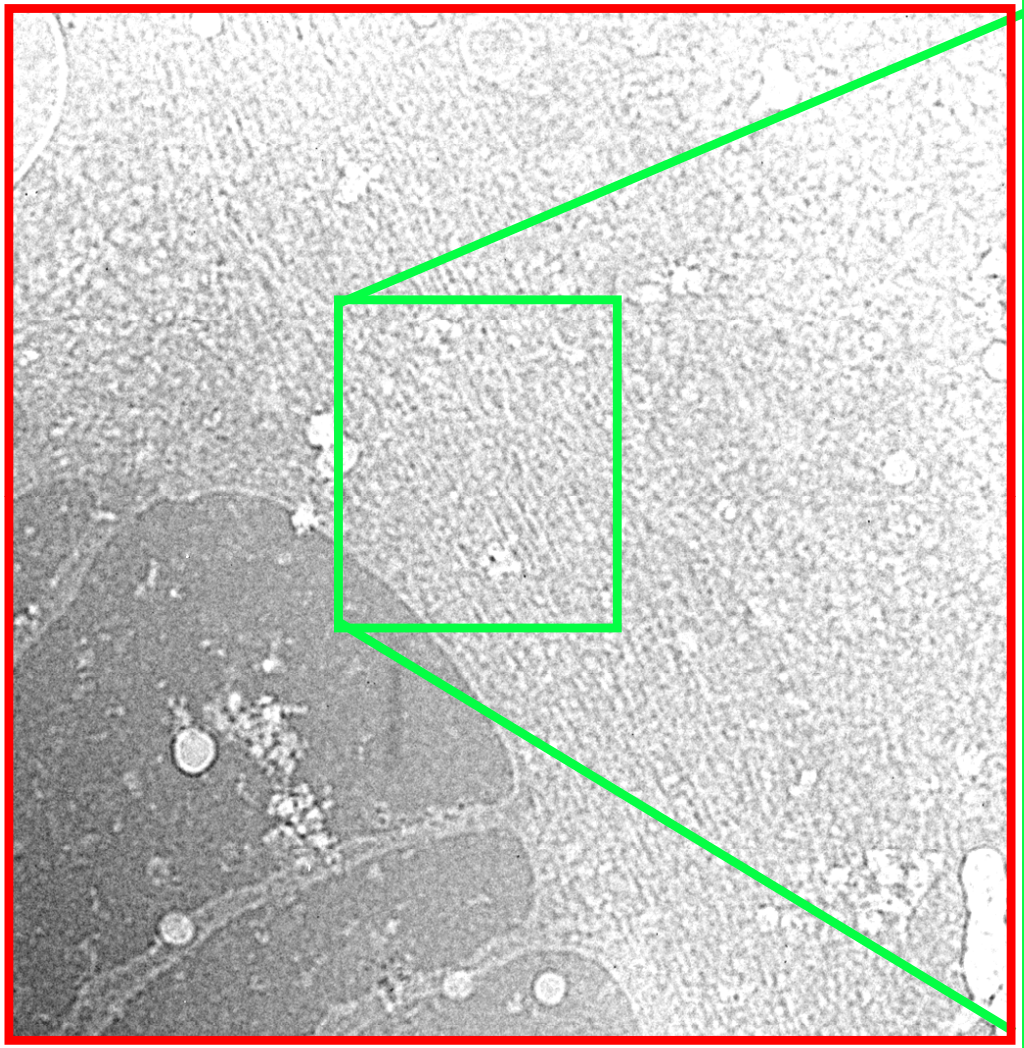
Observation condition

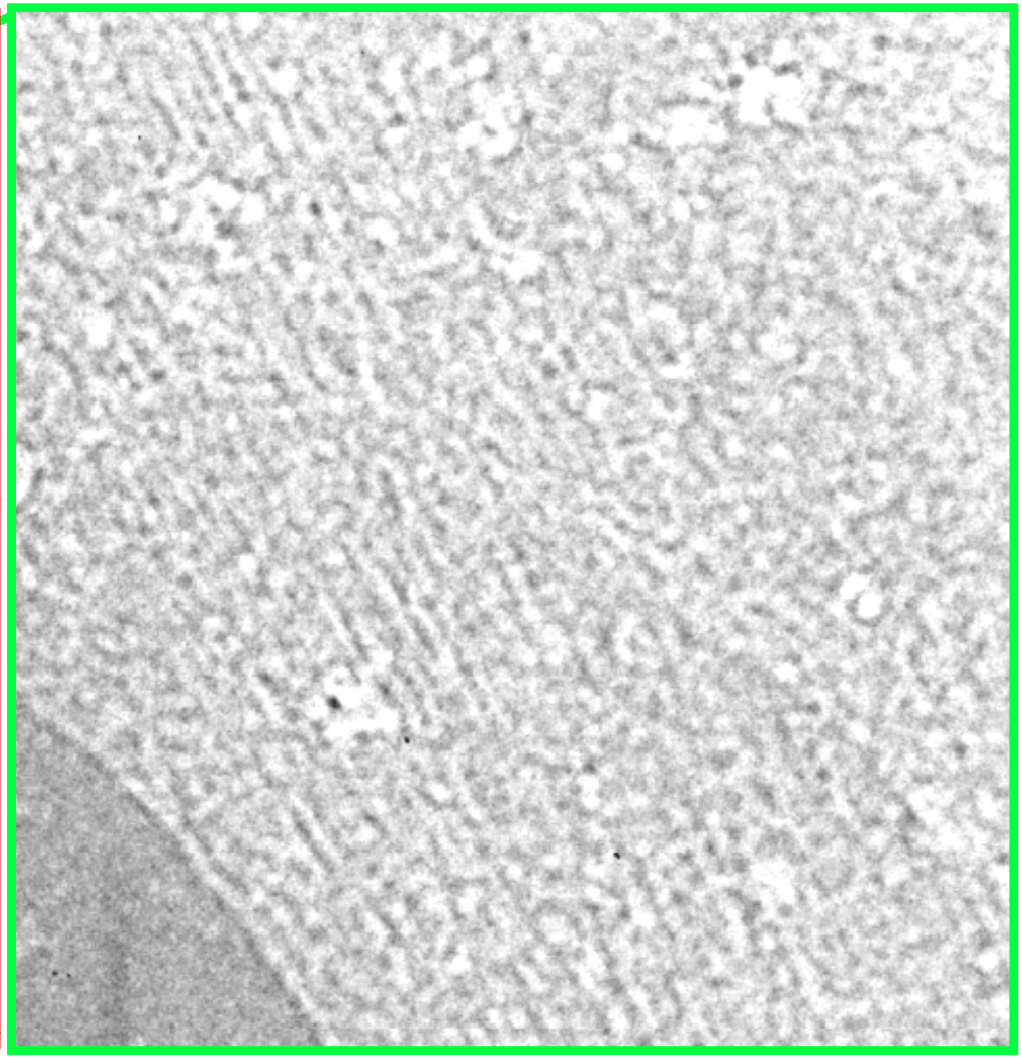
Acceleration voltage: 300 kV

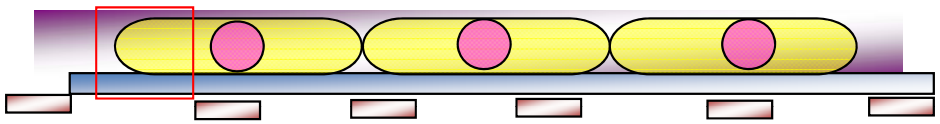
Temperature: 10 K (-263 ºC)

Image recording: CCD(2K×2K)

Whole cell observation

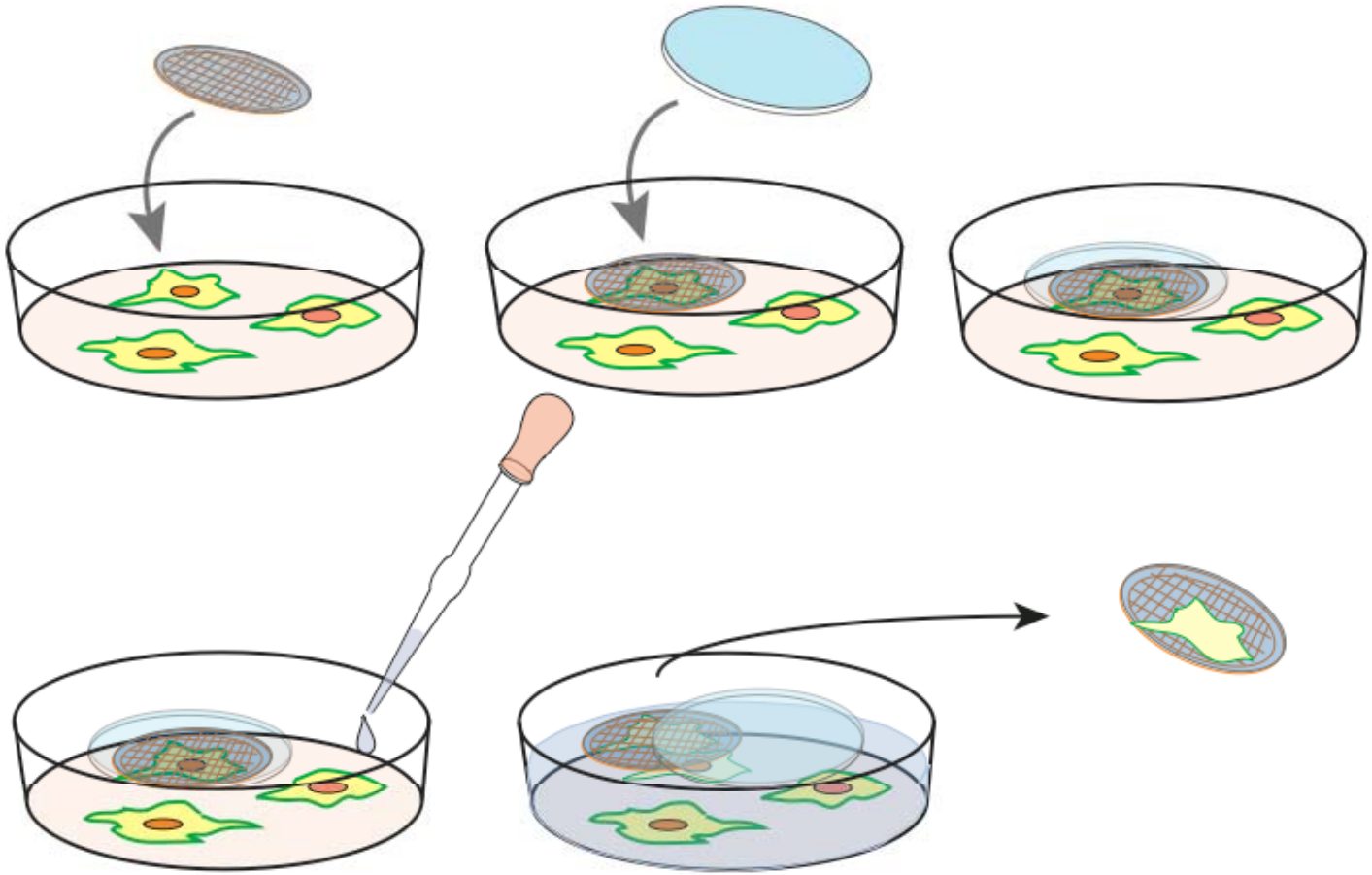






It is impossible to observe fine structure because of much amount of residual cytoplasm.

Our innovation is how apical membrane cytoskeletons of cells are isolated onto a mesh.



1. Culture medium is removed and washed once with Ringer's solution.
2. A sticky mesh is placed on the cells and covered with a small glass cover slip.
3. The mesh is slowly lifted by pouring Ringer's solution.
4. The apical membrane cytoskeleton is adherent to the mesh.

Cryoelectron micrograph of hydrated native membrane cytoskeleton