

超解像蛍光顕微鏡で どこまで見えるか

Principles of Super-resolution Microscopy

平岡 泰

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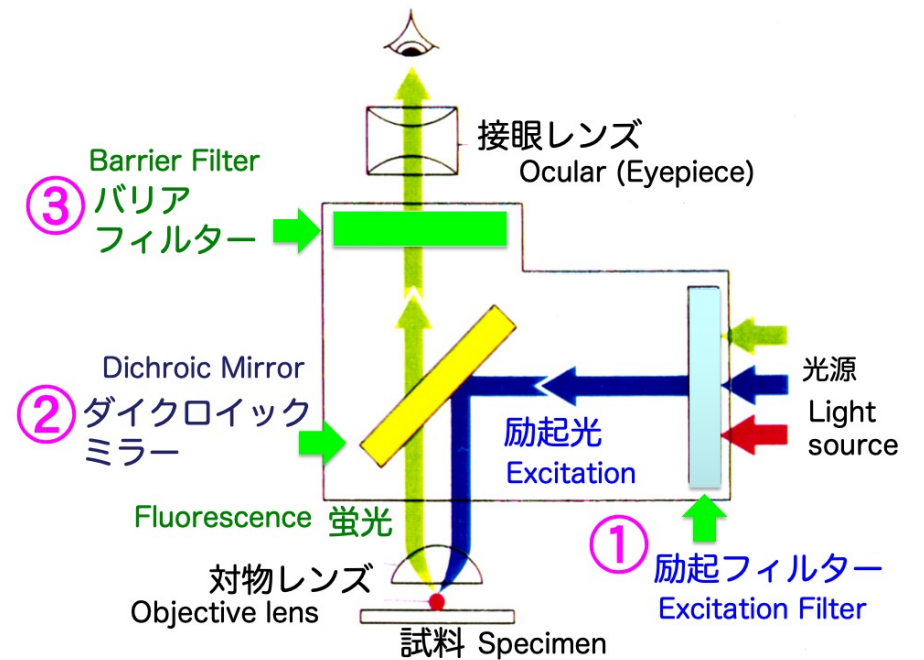
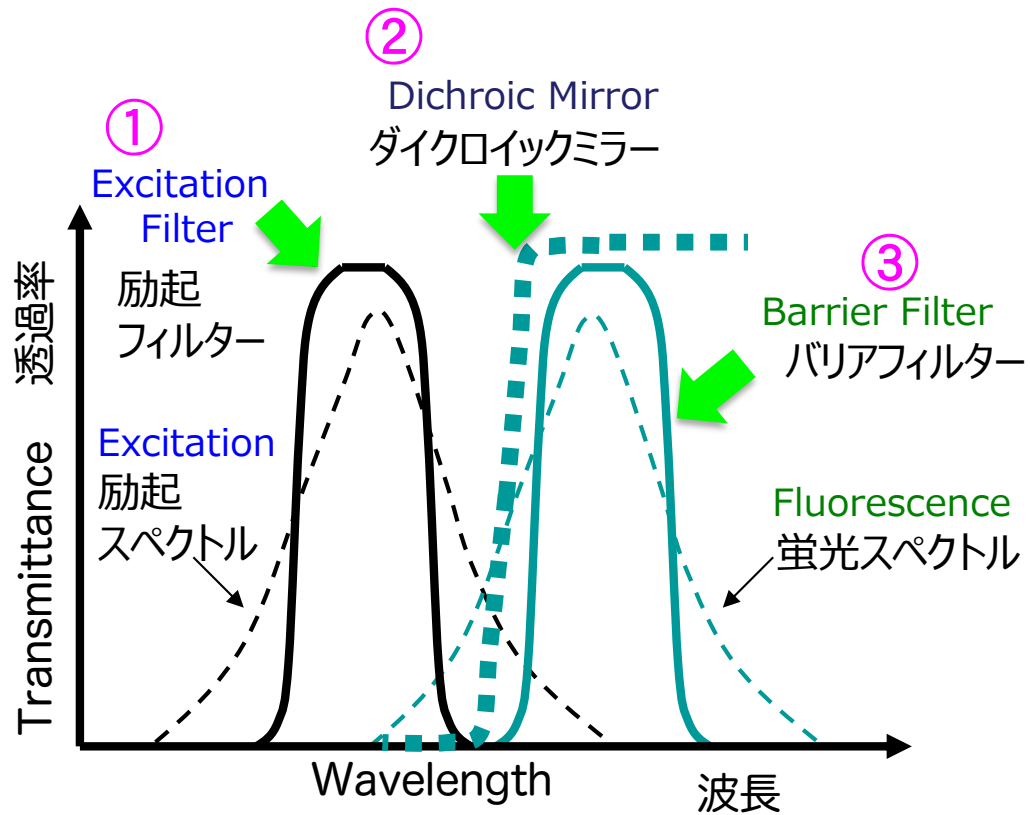
超分解能光学顕微鏡 Super-resolution microscope

- 分解能の限界を超える Beyond the resolution limit

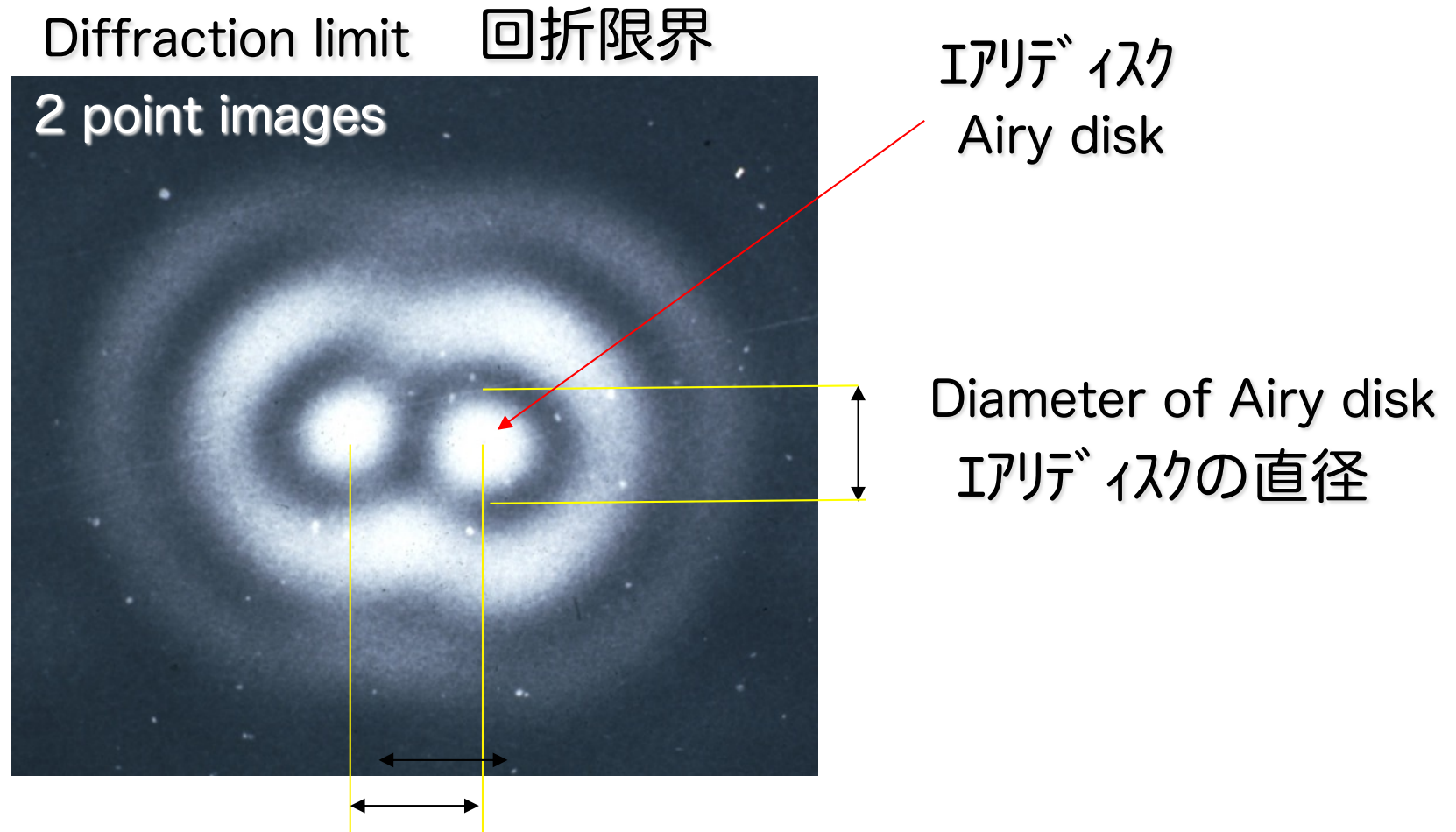
- そもそも、光学顕微鏡の分解能の限界とは
What the resolution limit is
- 顕微鏡像はどのように作られるか
How an image is generated through a microscope
- どのようにして限界を越えるのか
How we can go beyond the resolution limit
- 越えたら、どこまで見えるのか
What we can see beyond the resolution limit
- 分解能を台無しにする方法
How we can destroy the resolution

蛍光顕微鏡の基礎

Principle of fluorescence microscopy



Resolution そもそも、光学顕微鏡の分解能の限界とは

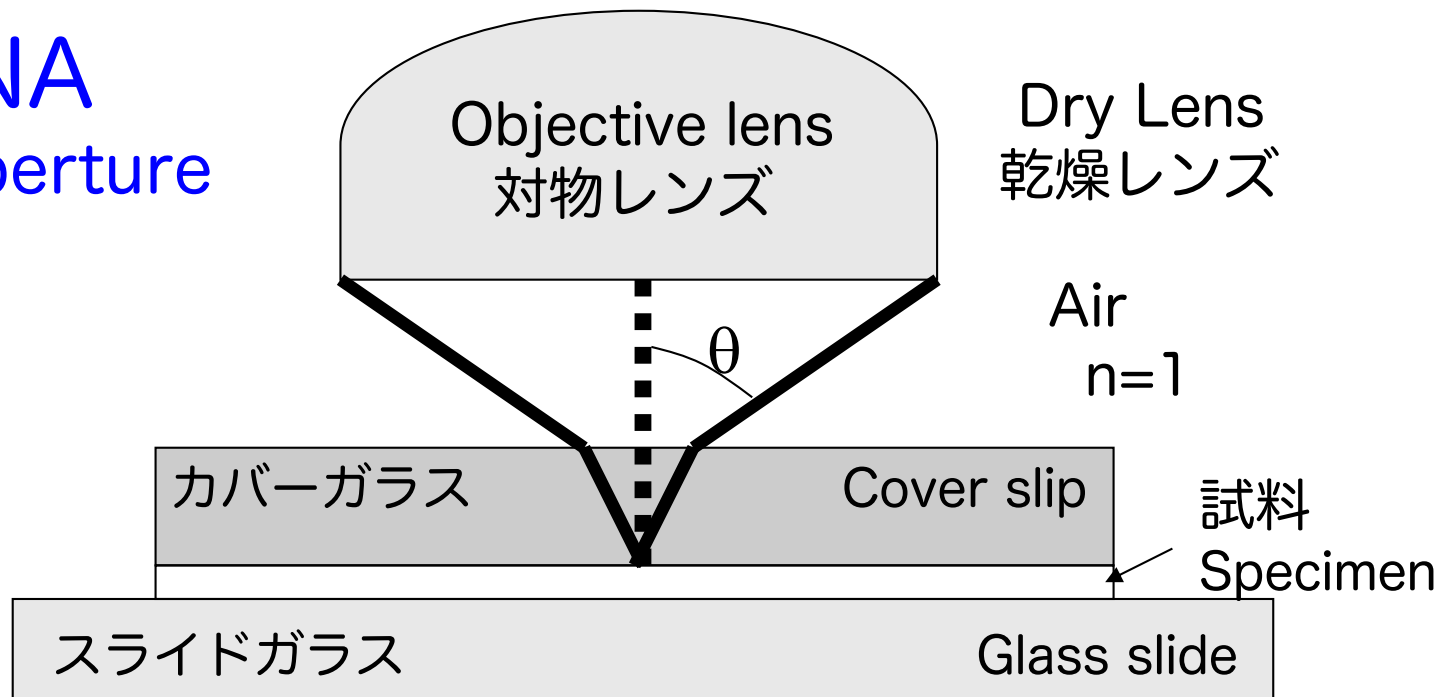


2点に分解して見える最小の間隔 Minimum distance for separation

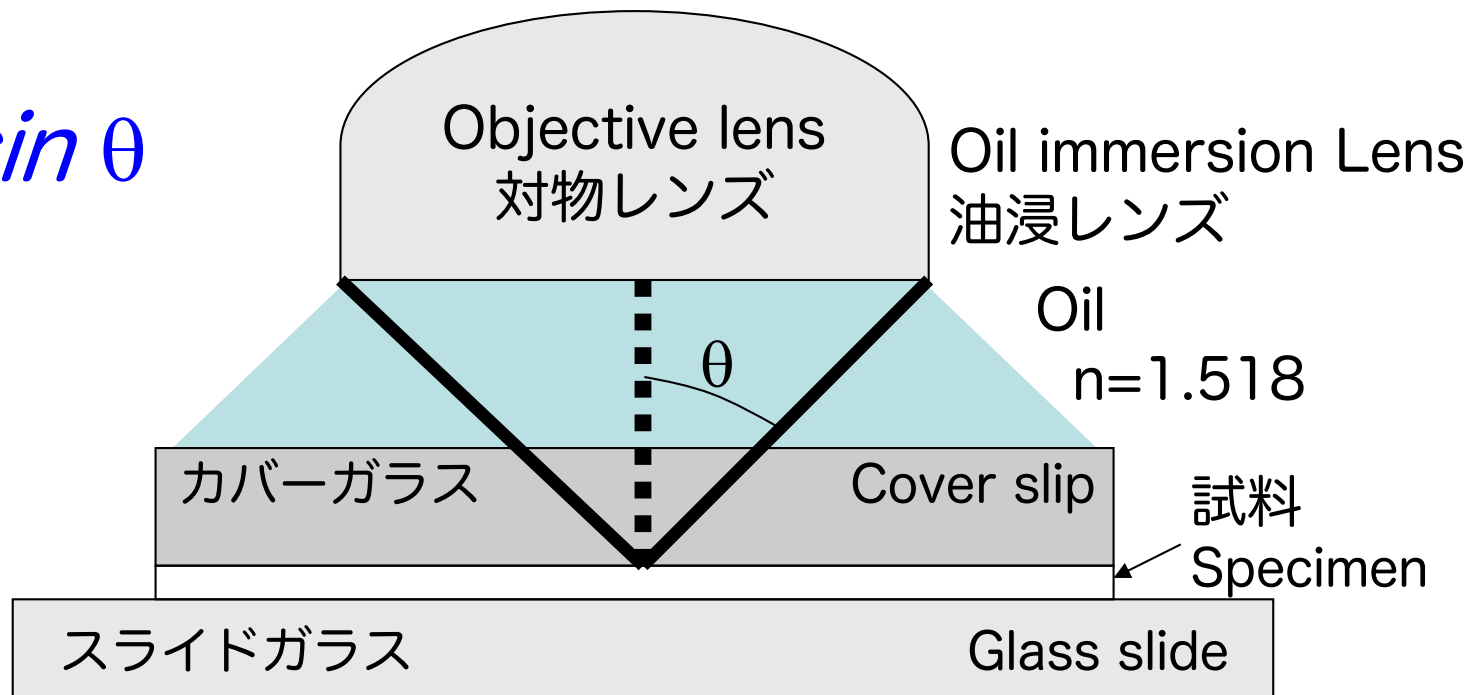
Rayleigh's criterion
レーリーの分解能 $0.61 \lambda / NA$

波長:wavelength λ 開口数:Numerical Aperture (NA)

開口数 NA
Numerical Aperture

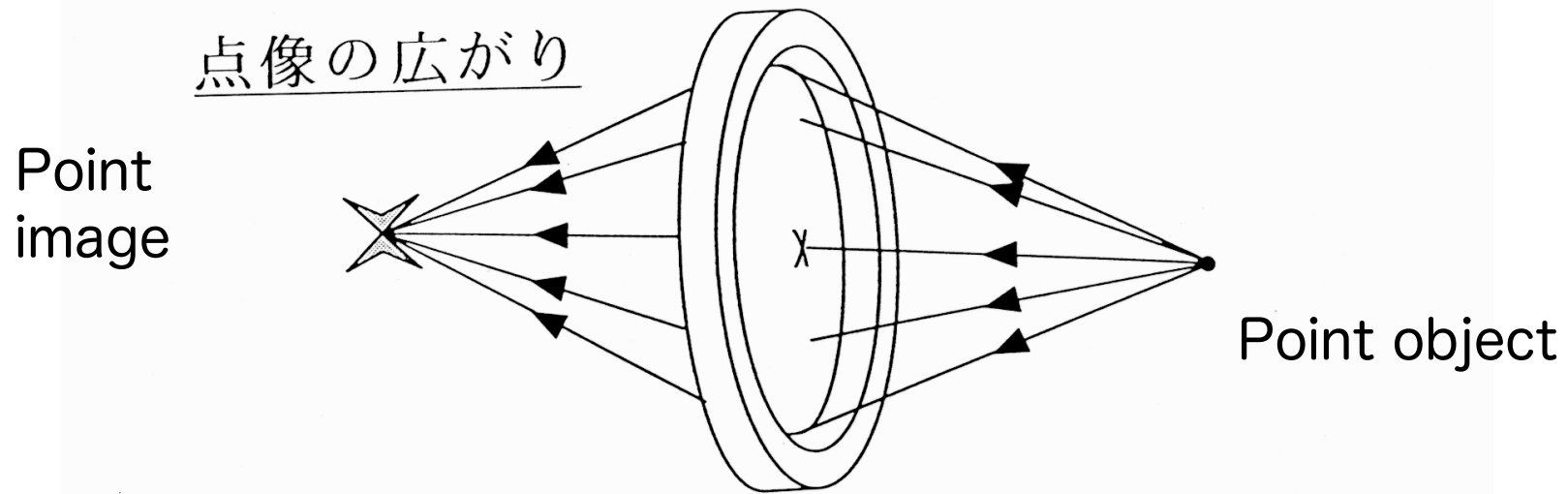


$$NA = n \sin \theta$$

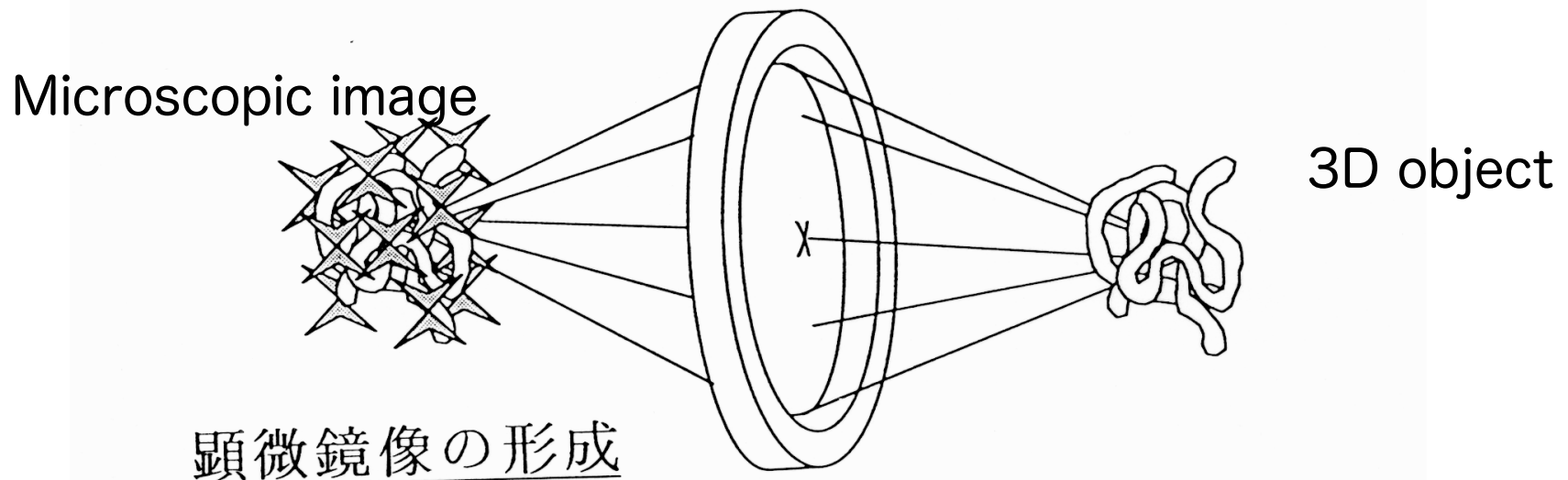


顕微鏡像はどのように作られるか

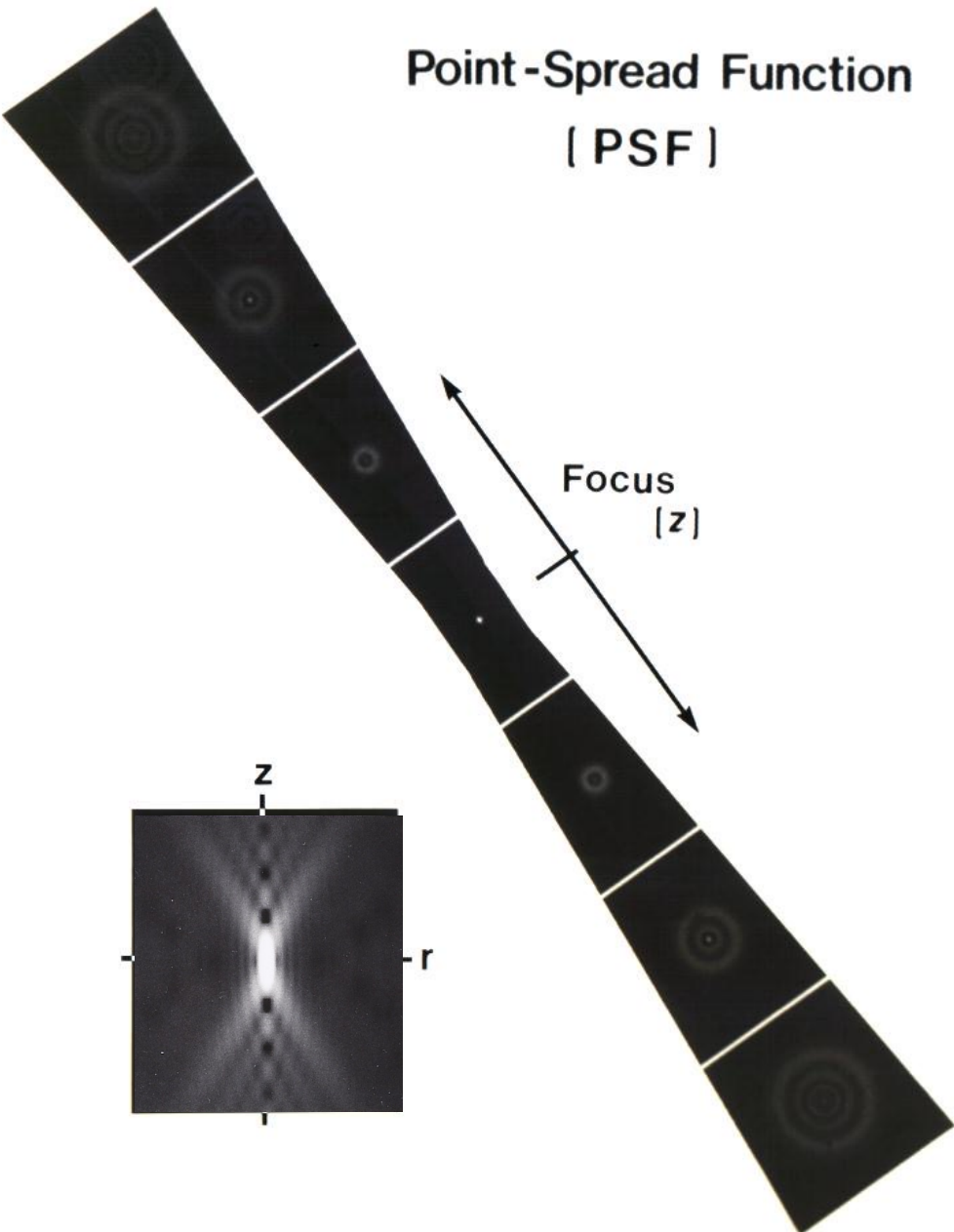
Formation of microscopic images



顕微鏡像 ← [光学顕微鏡] ← 試料物体

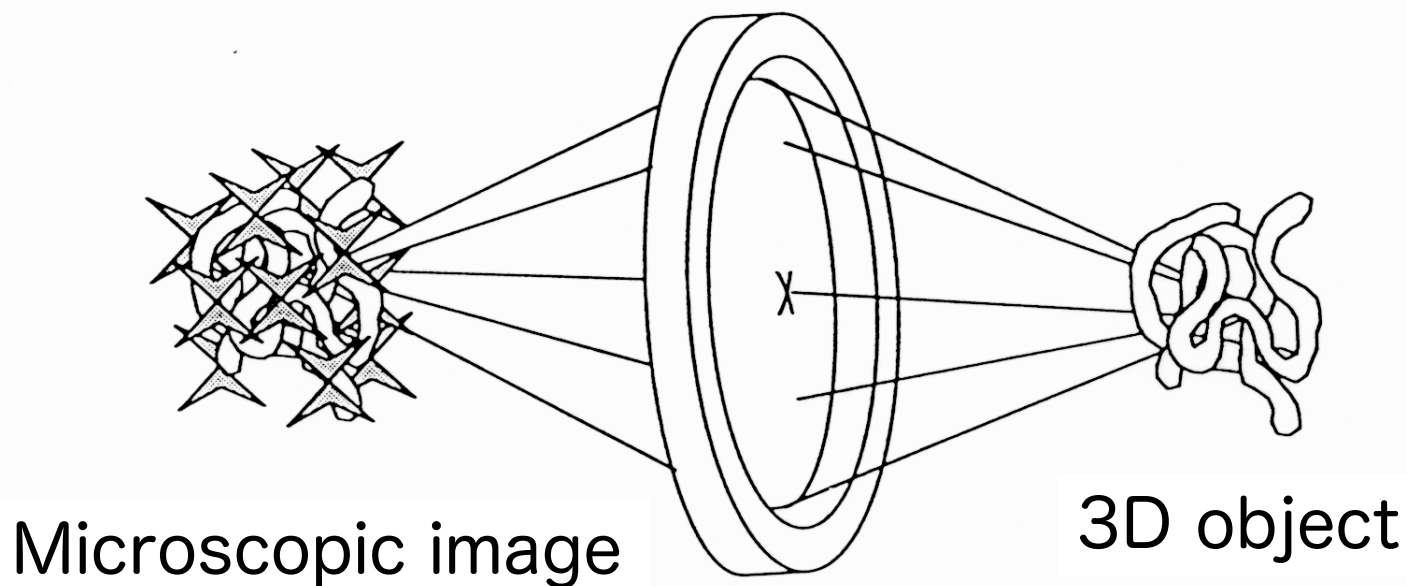


Point-Spread Function (PSF)



Formation of microscopic images

顕微鏡像 ← [光学顕微鏡] ← 試料物体



Convolution 畳み込み積分

$$i(u, v, w) = \iiint o(x, y, z) S(u-x, v-y, w-z) dx dy dz$$

Image

顕微鏡像の
輝度分布

Object

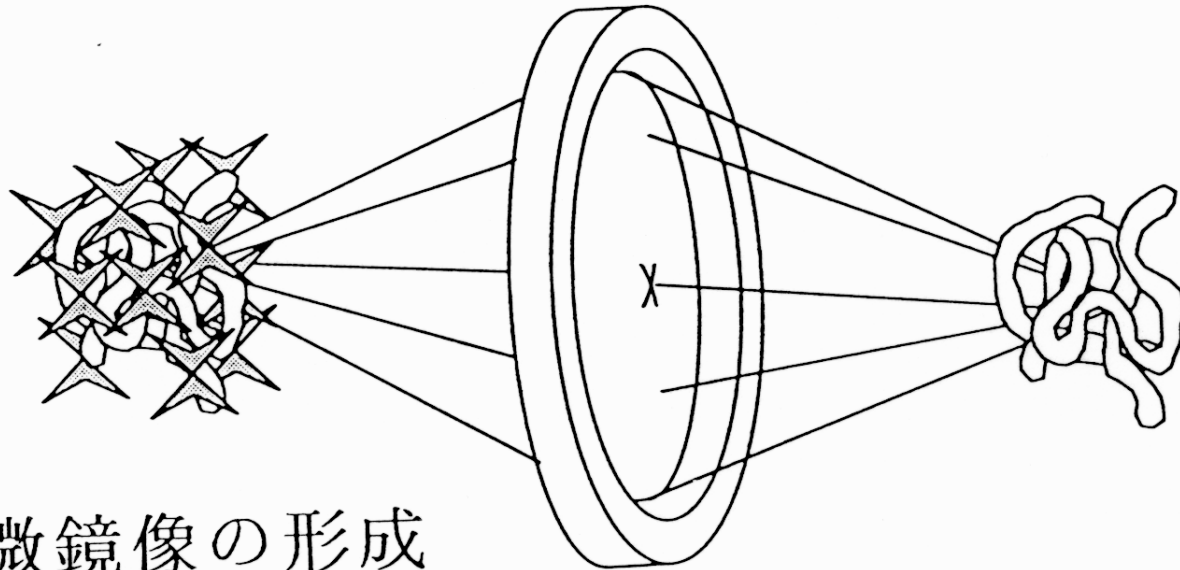
試料物体の
輝度分布

PSF

点像分布関数

Convolution

顕微鏡像 ← [光学顕微鏡] ← 試料物体



顕微鏡像の形成

$$i(u, v, w) = \iiint o(x, y, z) S(u-x, v-y, w-z) dx dy dz$$



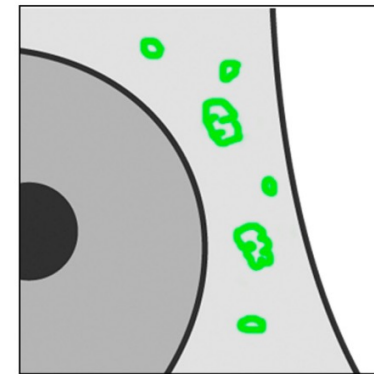
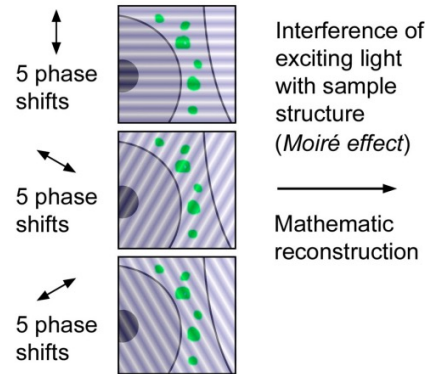
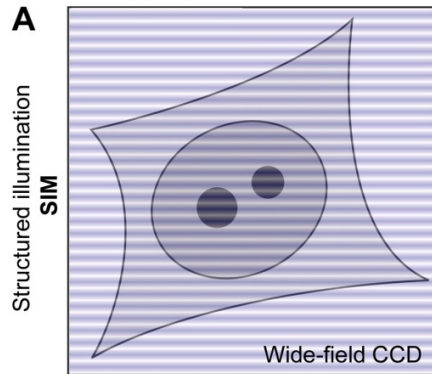
Deconvolution

どのようにして限界を越えるのか

Types of Super-resolution microscopes

SIM

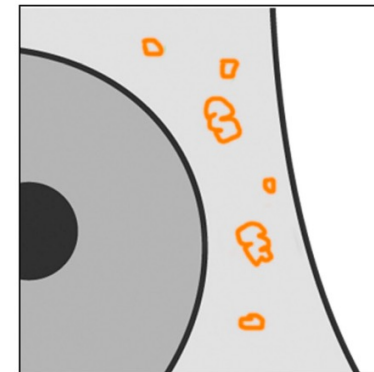
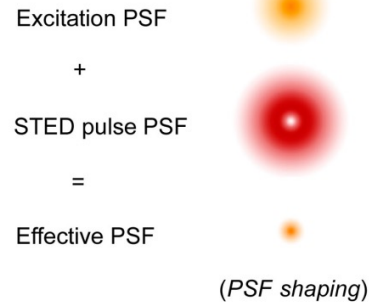
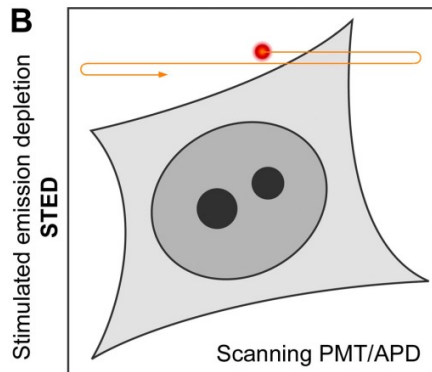
Zeiss
Nikon
API



Wide-field

STED

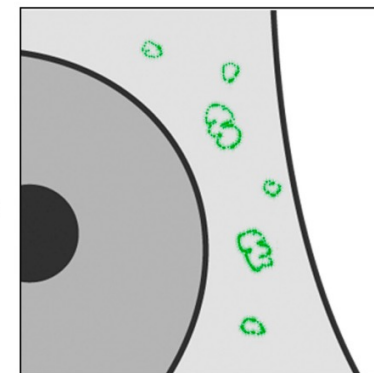
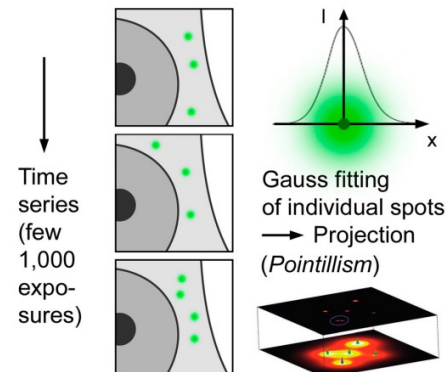
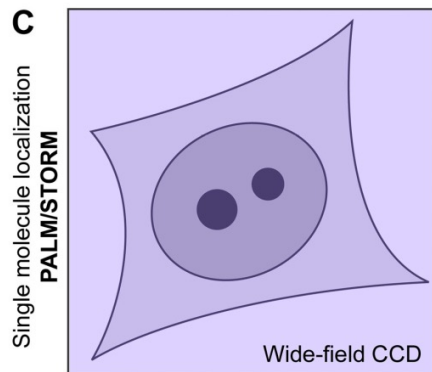
Leica



Confocal

**PALM/
STORM**

Zeiss
Nikon



Wide-field



Eric Betzig
The Nobel prize
2014

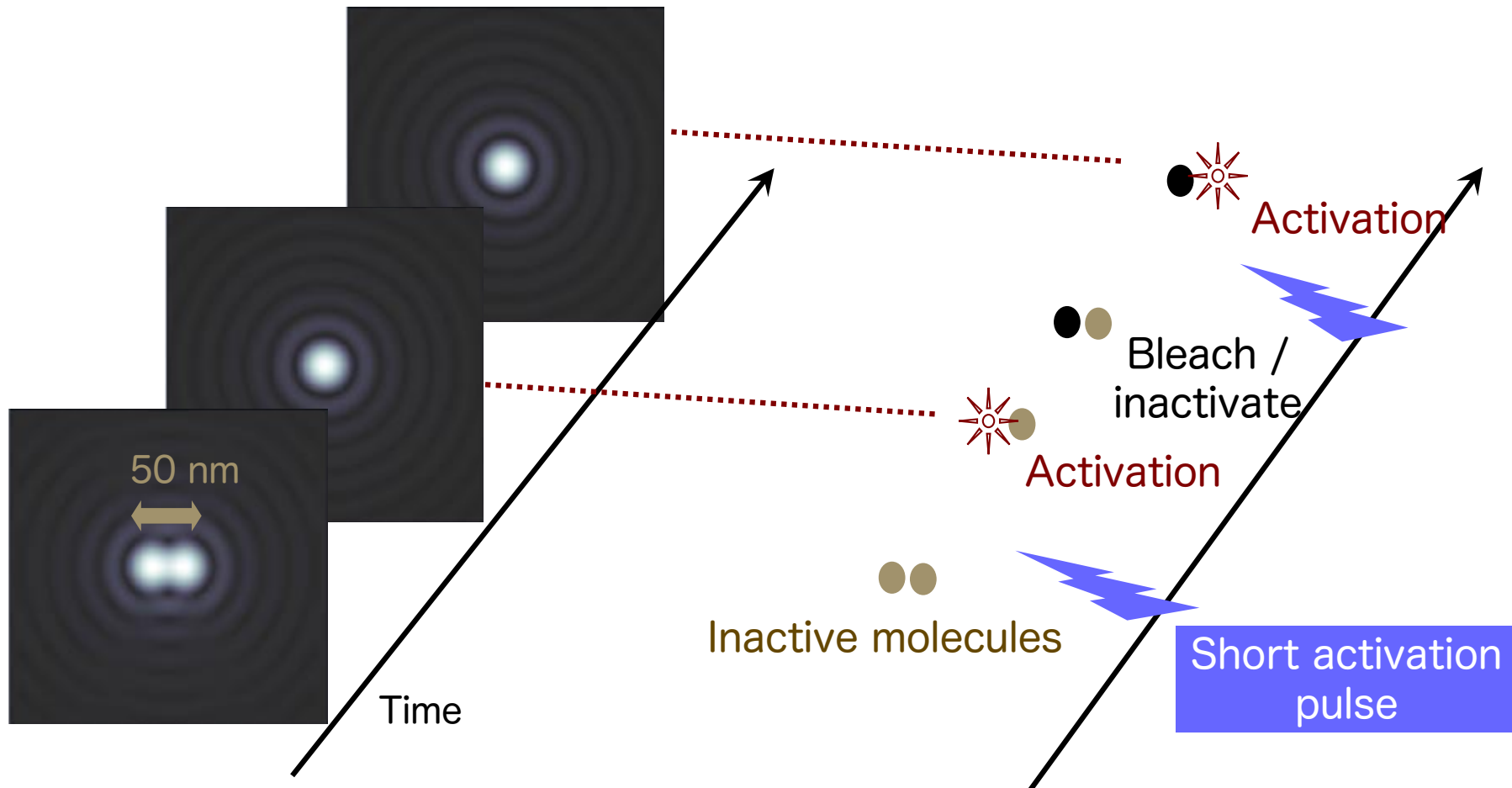
Wide-field microscope

PALM/STORM

Photoactivation Localization Microscopy

Stochastic Optical Reconstruction Microscopy

PALM (PhotoActivated Localization Microscopy)

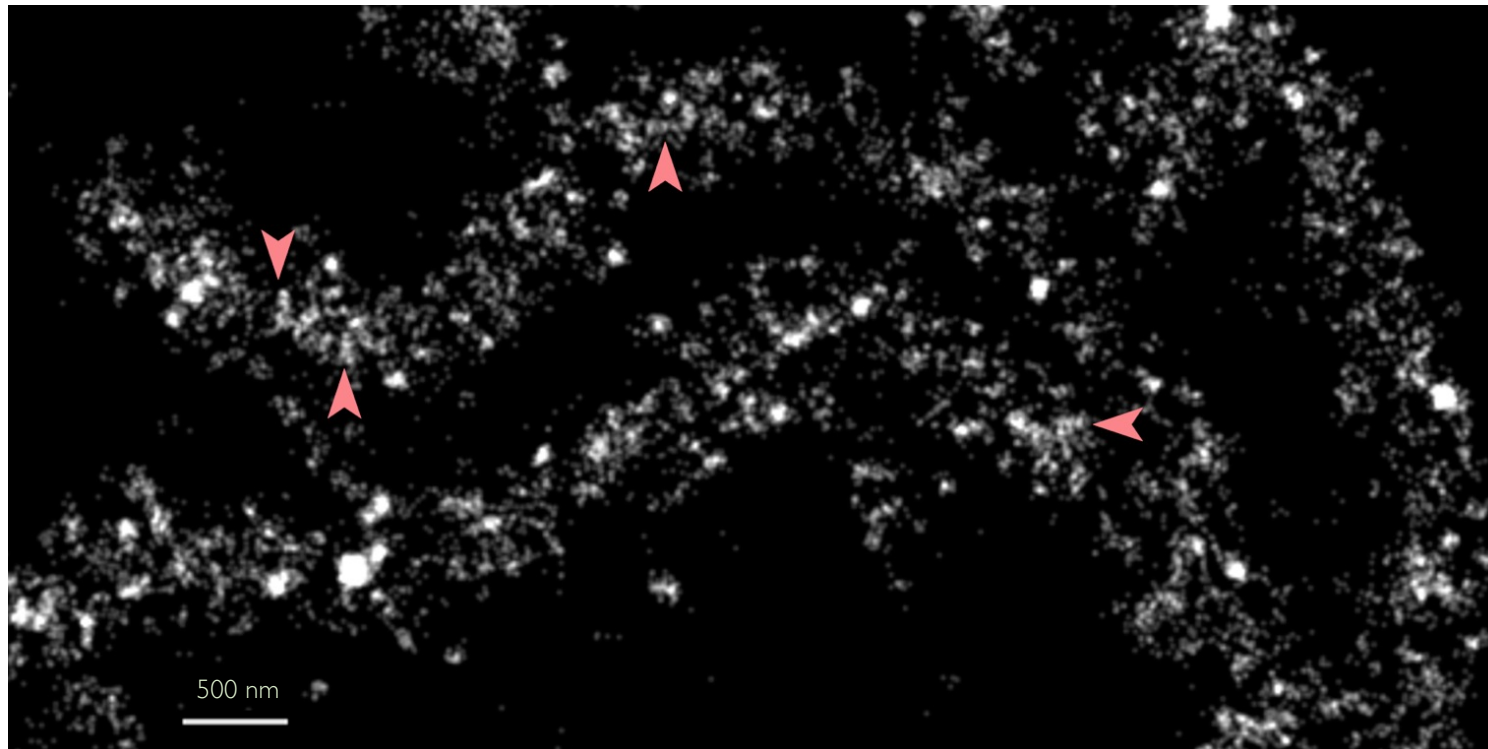


PALM (PhotoActivated Localization Microscopy)



Drosophila Metaphase chromosomes

PALM image



Confocal microscope

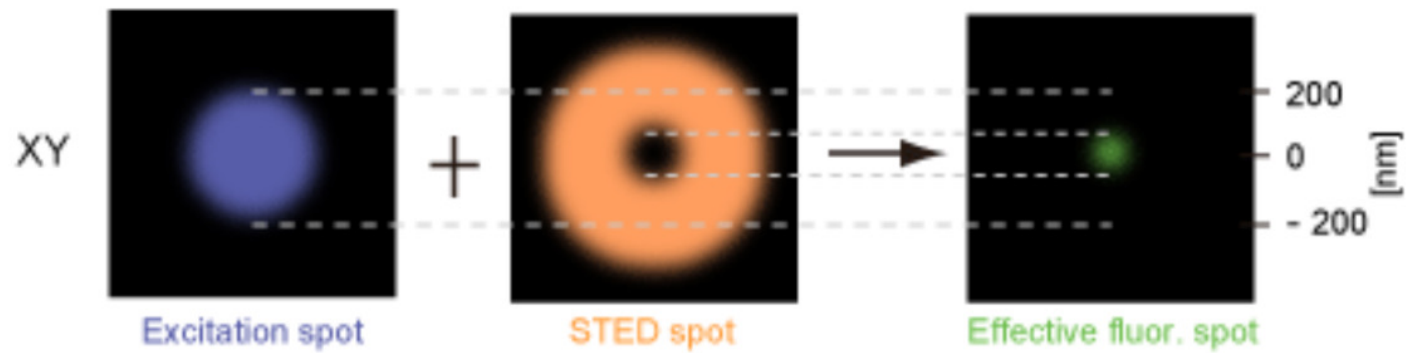
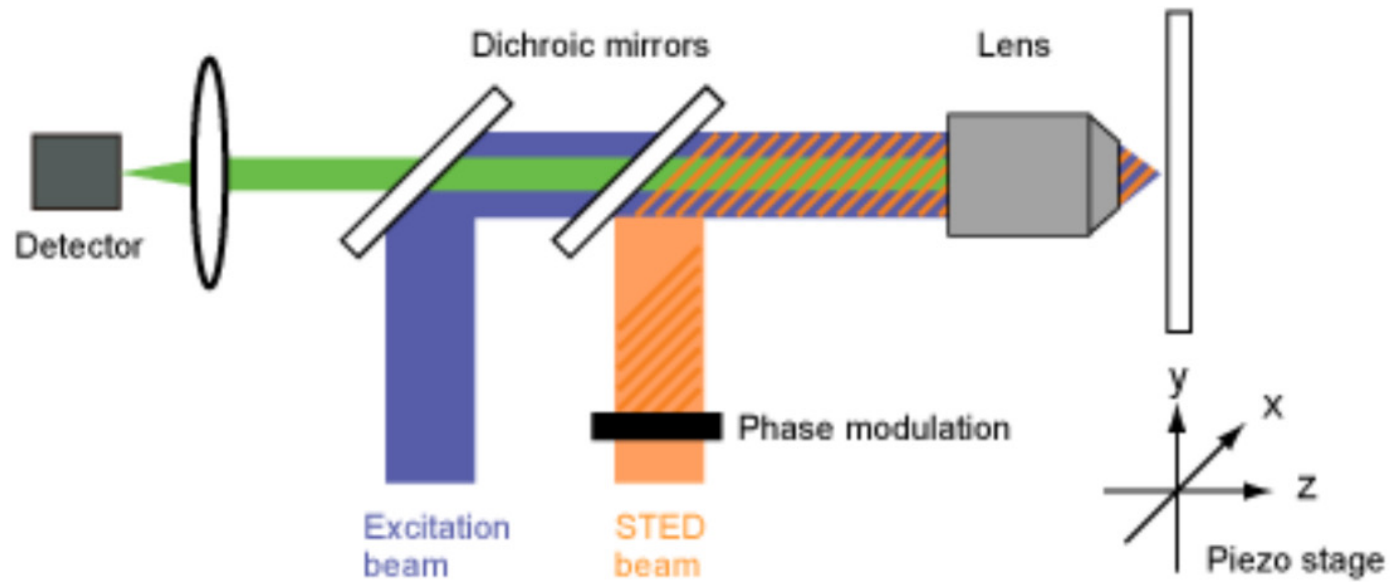
STED

Stimulated Emission Depletion



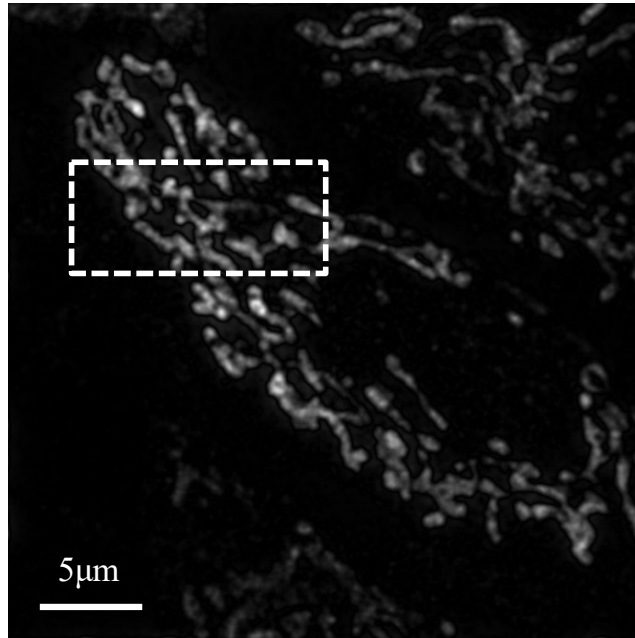
Stephan Hell
The Nobel prize 2014

STED (Stimulated Emission Depletion) microscopy

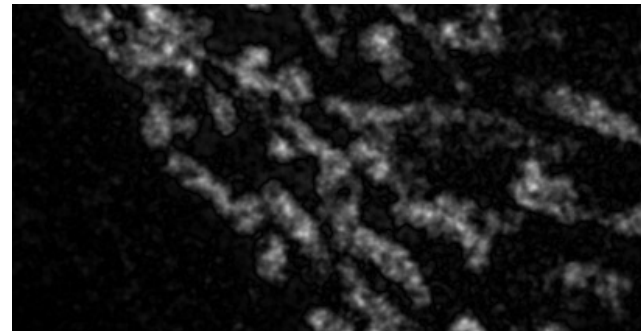
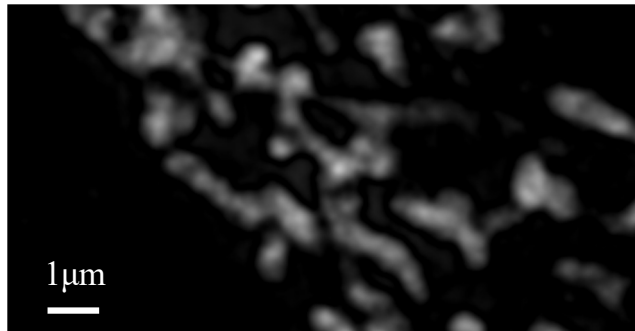
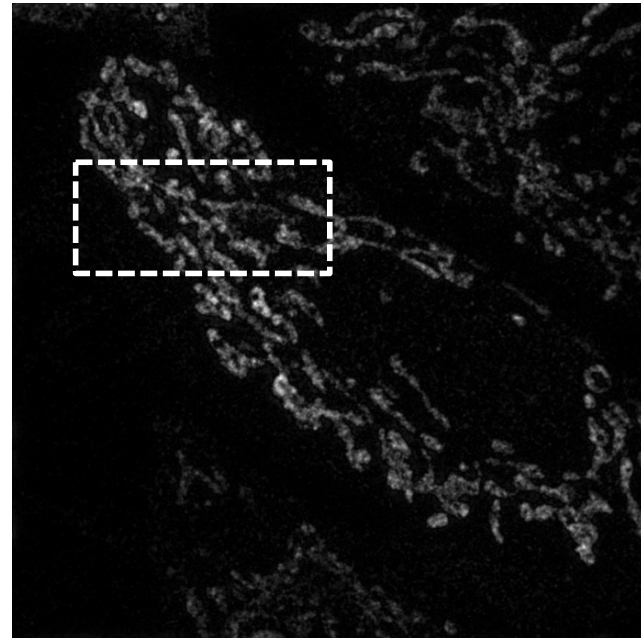


STED (Stimulated Emission Depletion) microscopy

confocal



STED



Mitochondria

Sample: ObgH1-GFP in HeLa cell

Wide-field microscope

SIM

Structured Illumination Microscopy



Mats Gustafsson
(1960-2011)

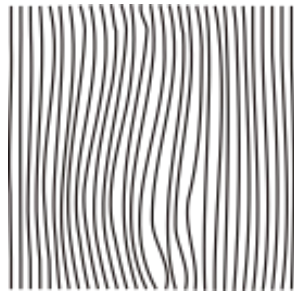
SIMの原理： 縞照明とモアレ模様

Principle of SIM

Structured Illumination and Moiré fringes

未知の微細構造

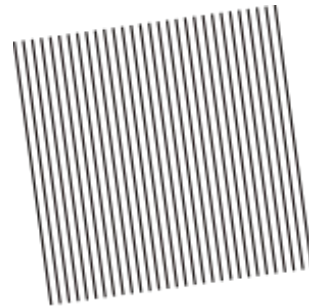
Unknown pattern



×

縞照明

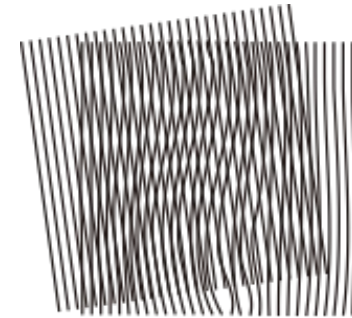
Superposed
known pattern



=

モアレ模様

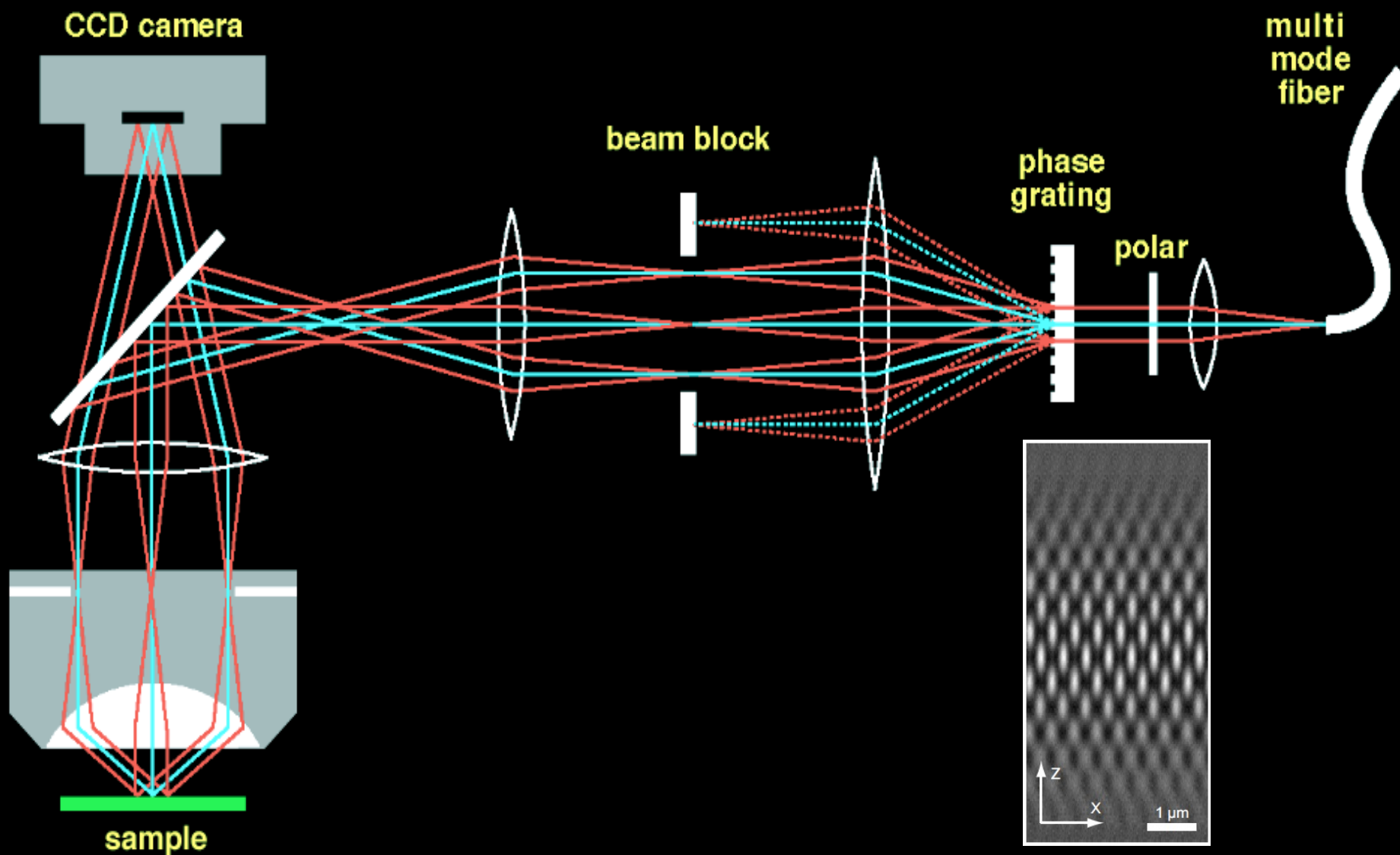
Moiré fringes



Moiré fringes can be resolvable

モアレ模様は分解能より大きくなる

SIM (Structured Illumination Microscopy)



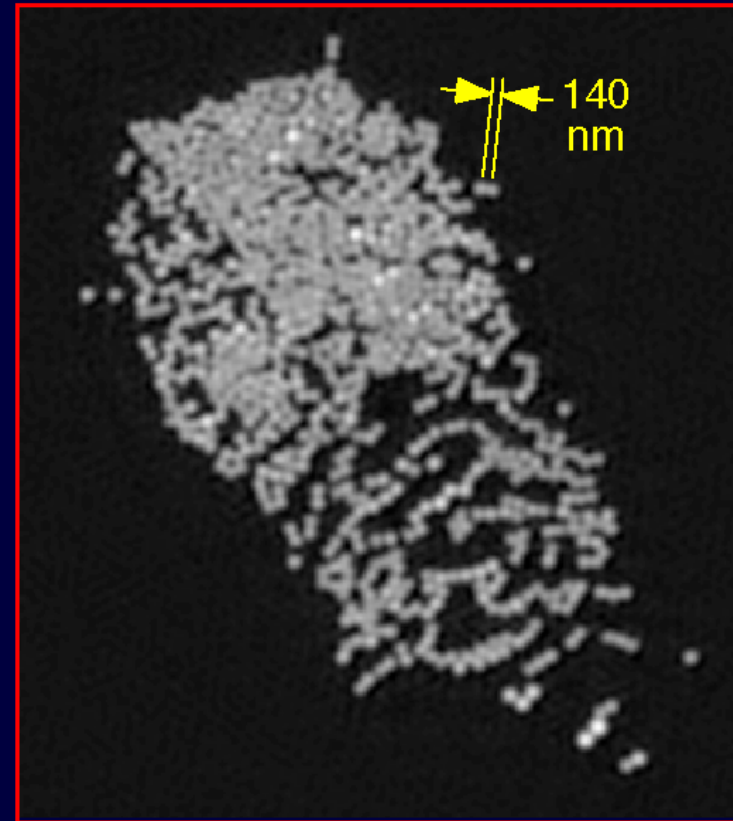
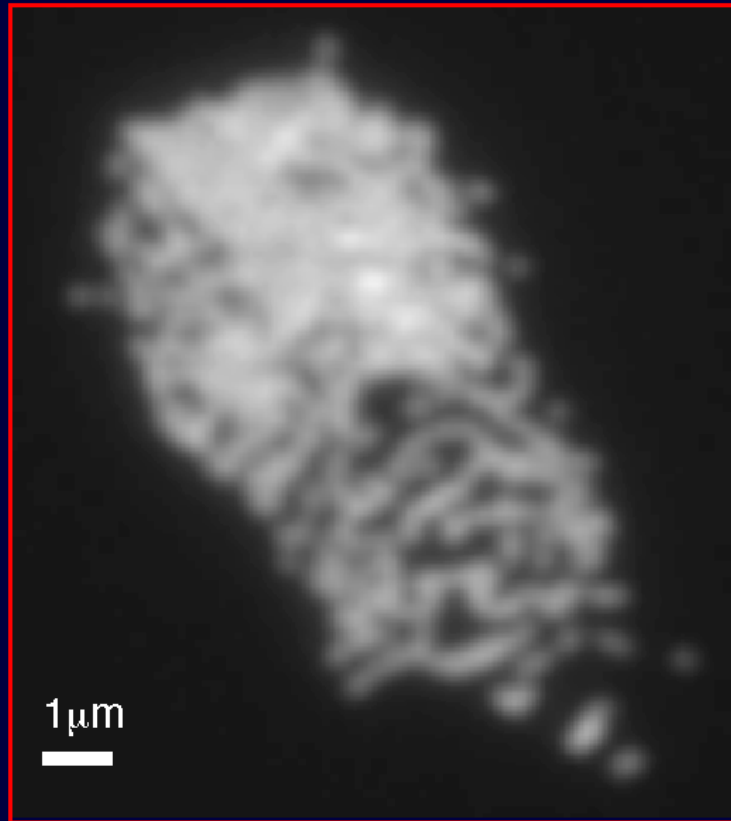
回折限界を越えたら、どこまで見えるのか What we can see beyond the resolution limit

120 nm microspheres

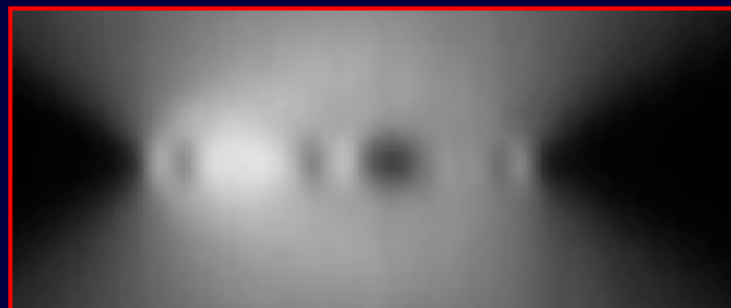
Conventional microscopy

Structured illumination microscopy

XY



XZ



350 nm
axial
FWHM

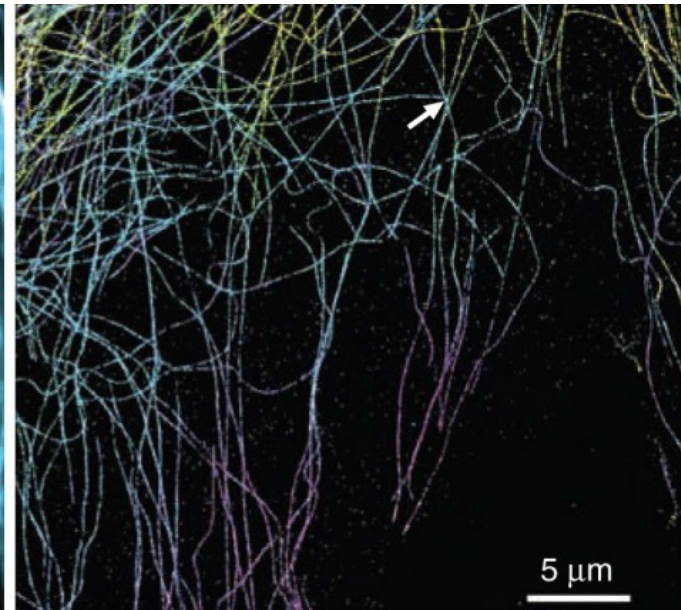
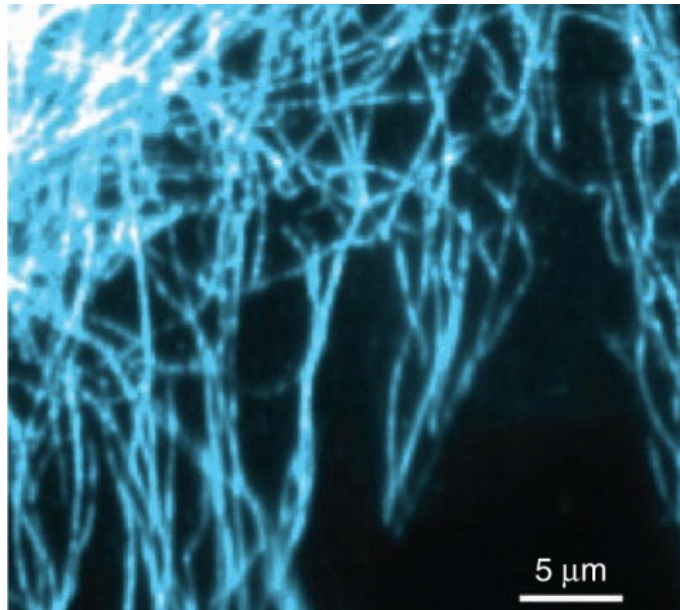
回折限界を越えたら、どこまで見えるのか

What we can see beyond the resolution limit

Microtubules

従来の分解能
Conventional

超解像 (STED)
Super-resolution



回折限界を越えたら、どこまで見えるのか

What we can see beyond the resolution limit

核膜孔複合体
Nuclear pore complex
(NPC)

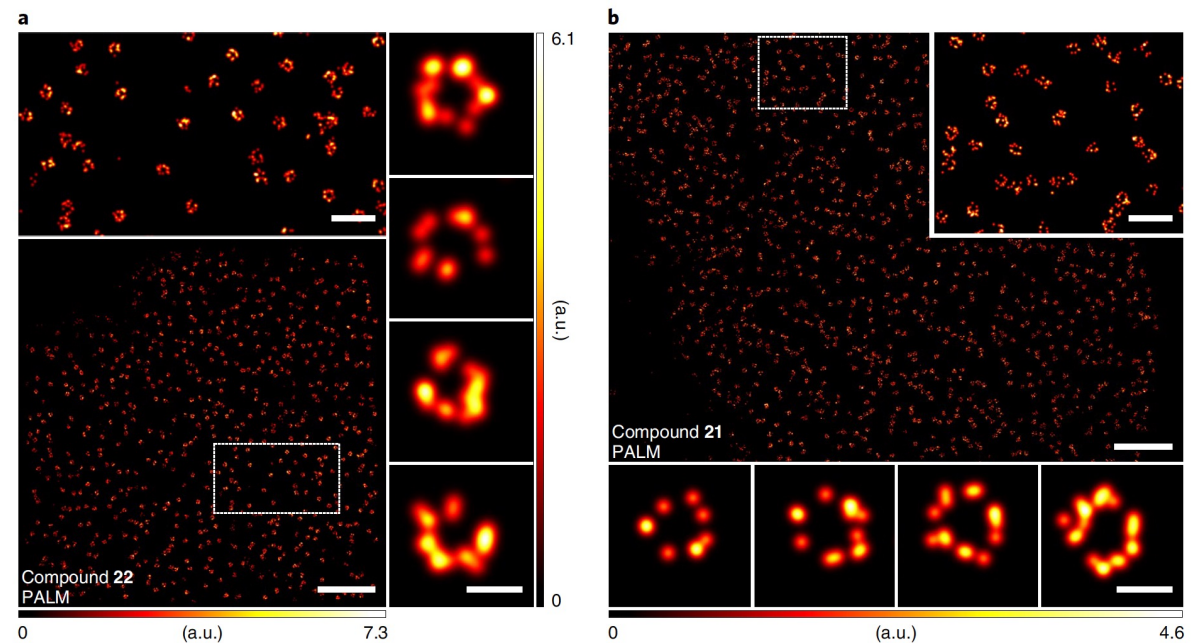
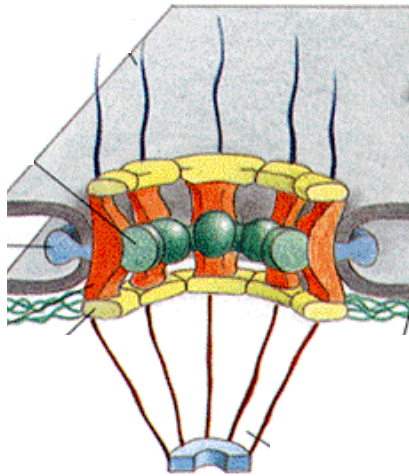


Fig. 5 | PALM imaging of NPCs in living cells using self-labelling PaX₅₆₀ substrates. a, Bottom: PALM image of U2OS stably expressing a NUP107-SNAP-tag construct labelled with **22**. Top: magnified view of the region marked in the overview image. Right column: magnified individual NPCs. **b**, Bottom: PALM image of U2OS cells stably expressing a NUP96-HaloTag construct labelled with **21**. Inset: magnified view of the region marked in the overview image. Bottom row: magnified individual NPCs. Scale bars: 2 μm (**a,b**, main), 500 nm (**a,b**, top insets), 100 nm (**a**, right column; **b**, bottom row).

分解能を台無しにする方法

Aberration of the objective lens can easily destroy the resolution

対物レンズの収差が分解能を破壊する

Important to minimize aberrations especially in super-resolution microscopy

収差を低減することは、超解像イメージングでは特に重要

Chromatic Aberration

色収差

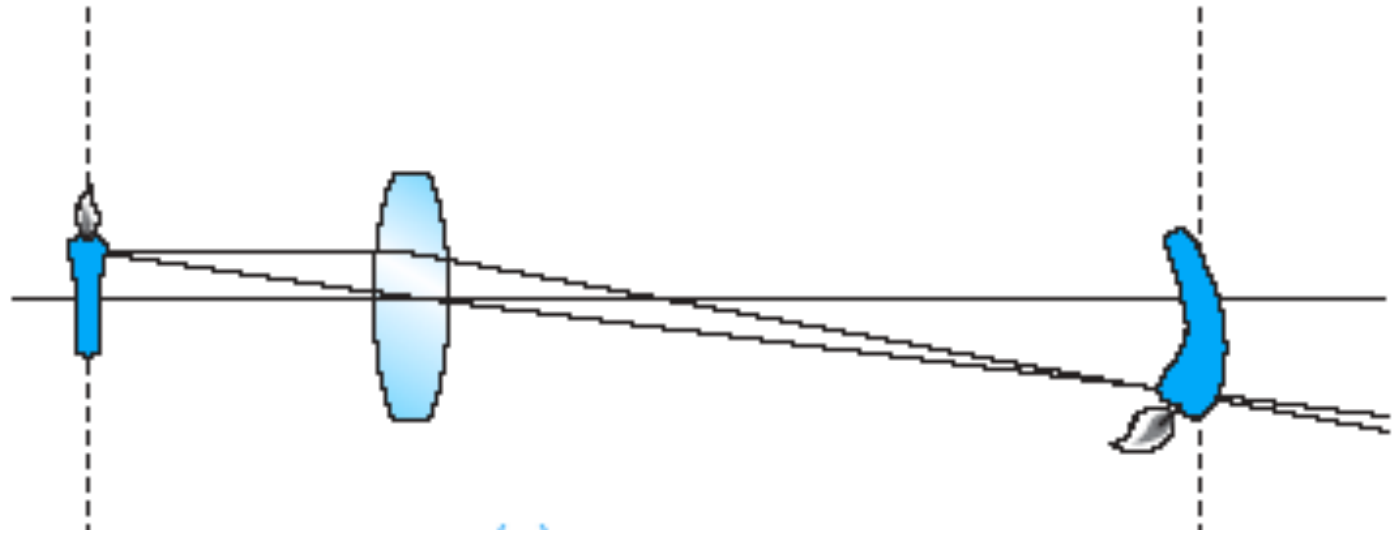
Curvature-of-Field Aberration

像面湾曲収差

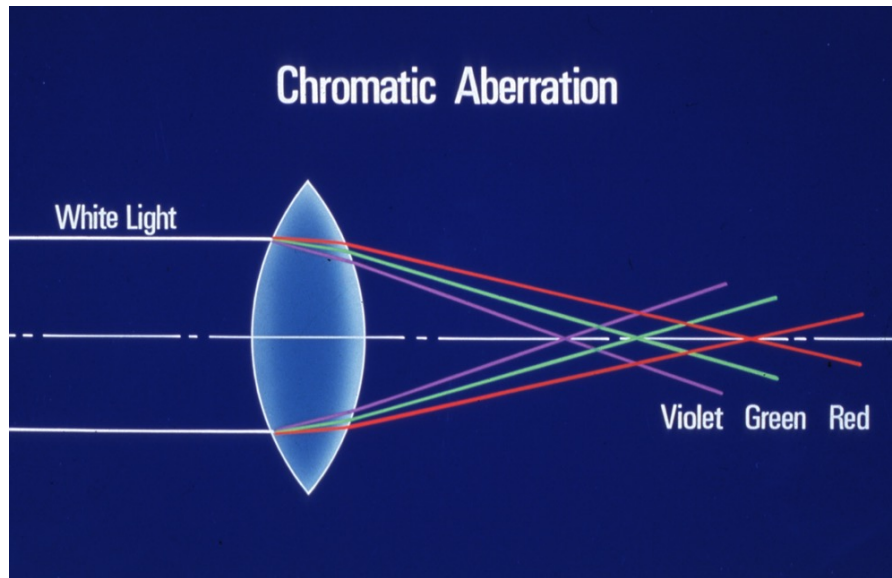
Spherical Aberration

球面収差

像面彎曲収差

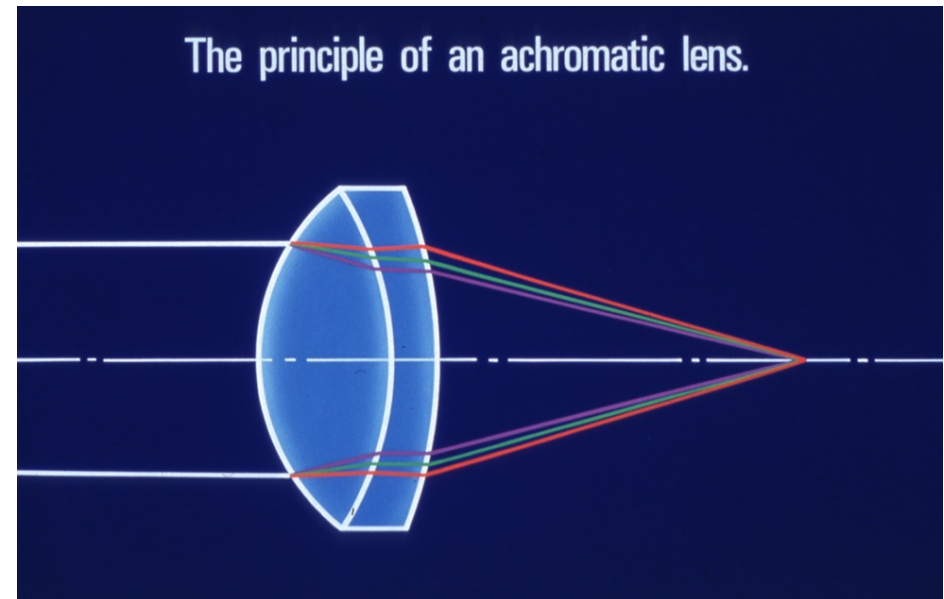


色収差



波長の長短により、集光位置がずれてしまう

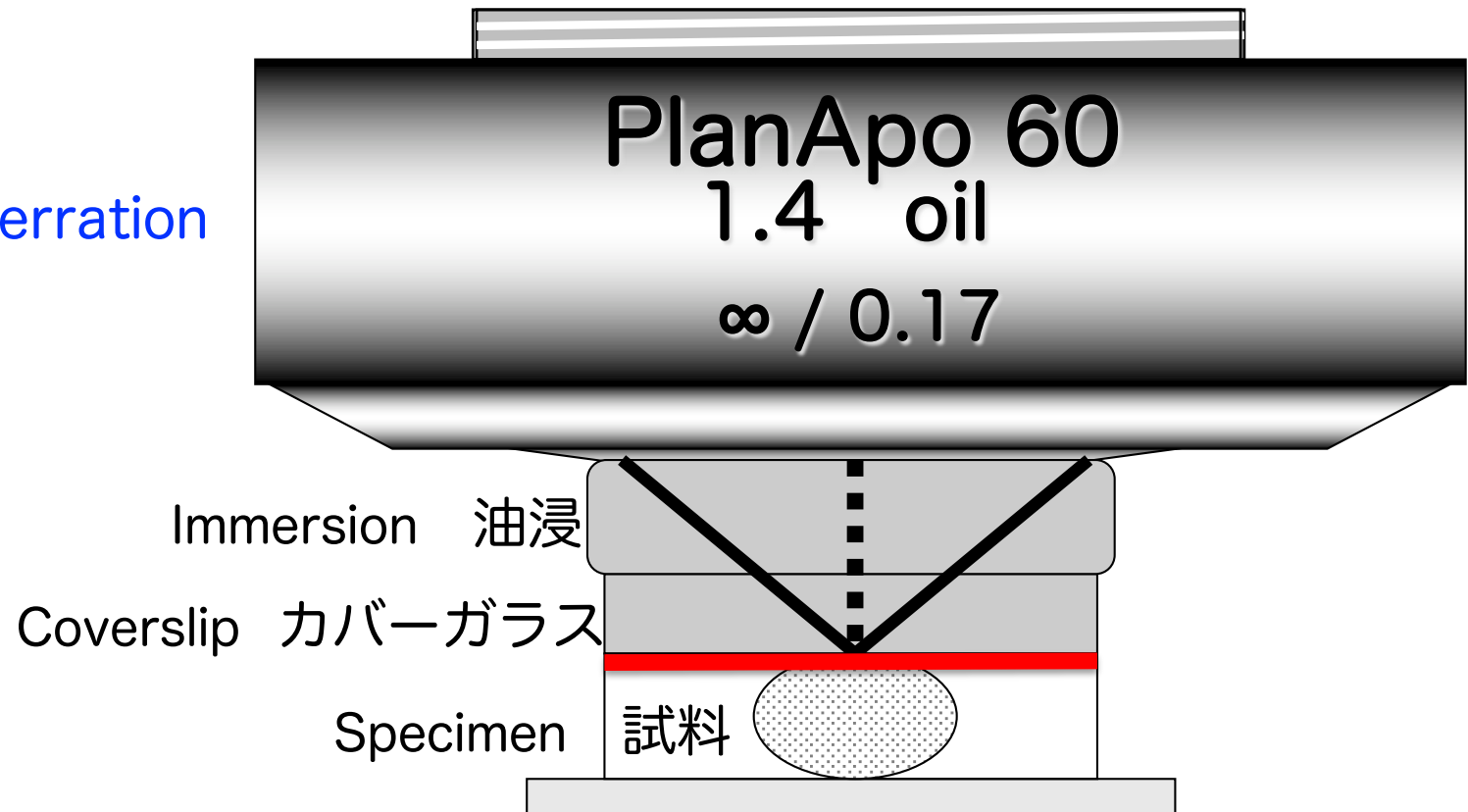
色収差の補正



凸レンズと凹レンズの組合わせで打ち消す

球面収差

Spherical Aberration



対物レンズは、以下の条件を満たす時に球面収差が無いように補正されている

- (1)正しい厚さのカバーズリップを使用し、
- (2)正しい屈折率の液浸（水浸・油浸等）をし、
- (3)試料がカバーズリップの直下（直近）にある

Objective lenses are corrected for spherical aberration when:

- (1) immersion with correct refractive index is used
- (2) a coverslip with correct thickness is used
- (3) the specimen is immediately on the coverslip

球面収差 Spherical aberration

光路長の過不足（屈折率のミスマッチ）

Mismatch of refractive index

光路長の過不足を生じる要因

Causes of spherical aberration

- カバースリップの厚さ
Mismatch of coverslip thickness
- 液浸（水浸、グリセロール浸、油浸）の屈折率
Refractive index of immersion

- 試料溶液の屈折率
Refractive index of sample solution

- **試料自体の屈折率**
Refractive index of specimens

	屈折率
オイル	1.518
グリセロール	1.47
水	1.33
細胞質	~1.4

Textbook (in Japanese)

2007 初版

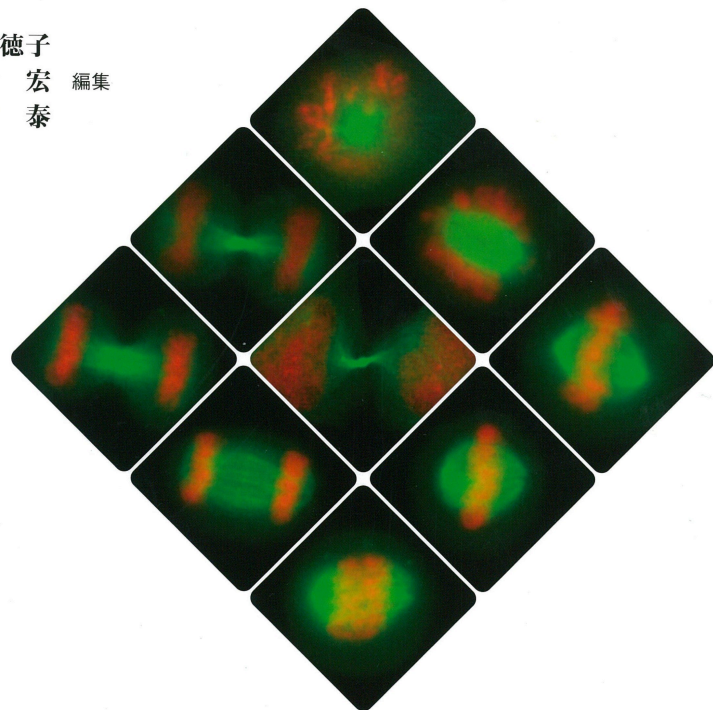
2015 新版

講義と実習

生細胞蛍光イメージング

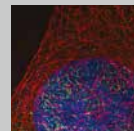
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原口徳子
木村 宏 編集
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