I. Light Microscope.

(*) 0.2 mm ⟶ 0.2 μm. (200 nm).

(*) Noise in a Bright Background (N) : $\sqrt{N}$.

$\frac{\text{Signal}}{\text{Noise}} \geq 1$. 最好. > 2-5.

(*) Four Types of λ_{Light} Microscopy

① Bright field Microscopy { Ordinary one. / Phase-Contrast / Differential interference-Contra

② Dark-field Microscopy : Scattered light is seen.

(*) · Fluorescent Microscopy (Dark field).

Dye: GFP ; DAPI ; Quantum dot (2-20 nm).

DAPI → to mark DNA (blue).

Quantum dot → without photobleaching

Photobleaching : After emitting $10^5 \sim 10^7$ photons.

FRAP : Fluorescence Recovery After Photobleaching
Diffusion of molecule.

FRET : Fluorescence Resonance Energy Transfer
Interactions between molecules. (< 5nm)

"Protein Dynamics" in Living Cells

II. Super-Resolution Imaging

$$d = \frac{k\lambda}{n\sin\theta}.$$

{ $k = \frac{1}{2} = 0.5$. "Abbe"
$k = 0.61$ "Rayleigh"
$k = 0.515$. * FWHM (Full width at half maximum).

Criteria

$d = k\frac{\lambda}{n\sin\theta} \approx \frac{\lambda}{2}$: d → 衍射的程度. $\lambda\downarrow$ $d\downarrow$; $\lambda\uparrow$ $d\uparrow$

↑ "一级明纹的宽度".

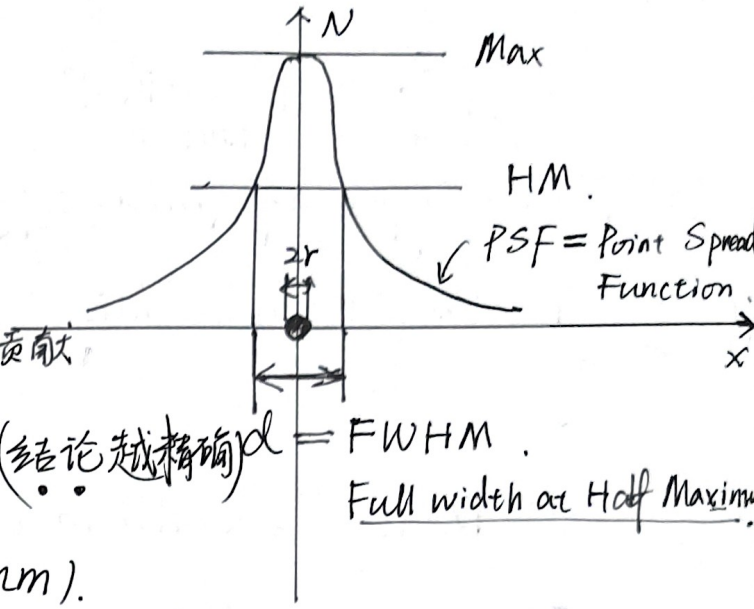
"noise" 实物本径

$d = \sqrt{N}\cdot r \Rightarrow r = \frac{d}{\sqrt{N}}$

number of photons.

$\lambda \xrightarrow{\text{determine}} d = \sqrt{N}\cdot r$

伪张声势的贡献 实实在在的贡献



同样的 d (观测结果) $N\uparrow$ $r\downarrow$ (结论越精确) d = FWHM.

Full width at Half Maximum

1. FIONA ($N\to+\infty$ $r\to 1nm$).

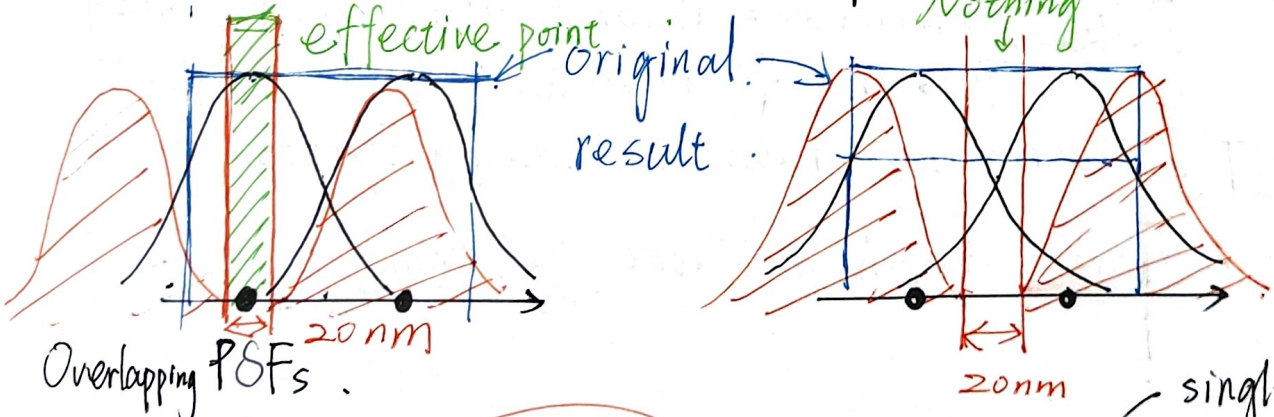
Fluorescence Imaging with One Nanometer Accuracy.

2. SIM. (200nm → 100nm). Resolution × 2.

Structured Illumination Microscopy

3. STED (200nm → 20nm). ×10.

Stimulated Emission Depletion microscopy



Overlapping PSFs.

4. PALM / STORM. (200nm → 1nm) × 200 ← single molecule.

Photoactivated Localization Microscopy

Stochastic Optical Reconstruction Microscopy

Photoswitchable Dye.

Ⅲ. Single molecular Biophysics

TIRF : Total internal Reflection Fluorescence.

DNA curtain.

CRISPR-Cas9.

- Optical Tweezer : F
- ~~Opti~~ Magnetic Tweezer : $F + M(\vec{r} \times \vec{F})$.

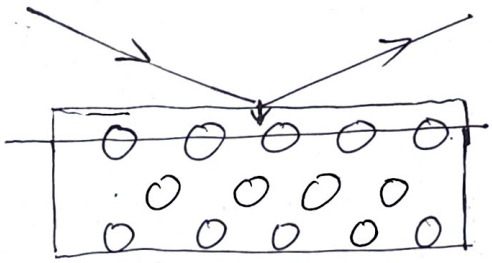
+ AFM.

Patch-Clamp. recording

FRET : Fluorescence Resonance Energy Transfer.

SMLM : (Single Molecule Localization Microscopy)

- STORM : Stochastic Optical Reconstruction Microscopy
  - 取样方式 / 最终目的
- PALM : Photoactivated Localization Microscopy
  - Dye.
  - 初步功能.



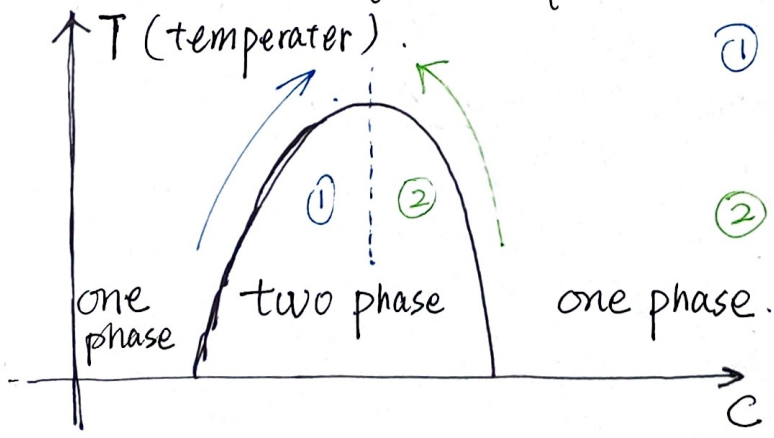
TIRF：使同一时间纵向只有 200nm 接受激光照射.

STED.

SMLM = PALM/STORM } 使横向平面中只有少数分子发荧光.

Biomolecular Condensation.

* LLPS (Liquid-Liquid Phase Seperation).



① 可以理解为横纵轴互换的溶解度曲线 (T-C 与 C-T).

② 可以理解为溶质溶剂对换的溶解度曲线

Both in Liquid Phase