Microscopy { Specimen → ExM

Microscope ↓

Bright Field & Dark Field ~ Fluorescence

to increase contrast ⇓

Phase Contrast

Interference Contrast

To study specific topic:

{ direct
{ indirect

Immunofluorescence

FRAP & FRET

Biosensor

Eyes

To improve resolution :

SIM

STED

SMLM { STORM
PALM

TIRF

Super resolution ↓

Mechanisms

Optics

Light Paths

Digital Processing

To Construct 3D image!

deconvolution ; multiphoton

confocal ; light sheet

Light Source

To Reduce Damage

⑤ multi-photon Imaging (500μm)

deep

* Depth

shallow ← (drawback) (0.2μm) ④ TIRF ←

Feature 1

can be varied ⇒ reconstruct 3D image

optical section ⇒

* eliminate out-of-focus fluorescence

higher contrast — Feature 2

* Less Photon needed

Feature 3 — Lower photo damage

③ image deconvolution (<40μm)

② light sheet microscopy

① confocal microscopy ☆ (150μm)

Variations on the use of Fluorescence

* Detect and assay specific molecules

⇓

Immunofluorescence (coupled with Antibody)

* Amplify the fluorescent Signal

⇓

Indirect Immunocytochemistry { Primary / Secondary }

* Protein Dynamics

⇓

FRET & FRAP

distance & interactions ; kinetic parameters e.g. { diffusion constant / rate }

* Biosensor (for signaling molecules) e.g. Ca^{2+}, cAMP

{ Reporting Component / Sensing Component } via FRET

Sensing Component ↓ conformational Change

* Help by passing Diffraction Limit

⇓

STED ; SMLM { STORM / PALM

relies on

by changing λ — { photoactivable / photoswitchable } dyes

* Dark field to increase contrast

⇓

conventional fluorescent microscope.