

Microscopy { Specimen → ExM  
Microscope

Bright Field &

to increase contrast



Phase Contrast

Interference Contrast

SIM

Superresolution

Mechanisms

Optics

Light Paths

Dark Field ~ Fluorescence

To study specific topic:

Immunofluorescence

FRAP & FRET

Biosensor

{ direct  
indirect

Dyes

To improve resolution:

STED

SMLM

STORM

PALM

TIRF

Digital Processing

To Construct 3D Image!

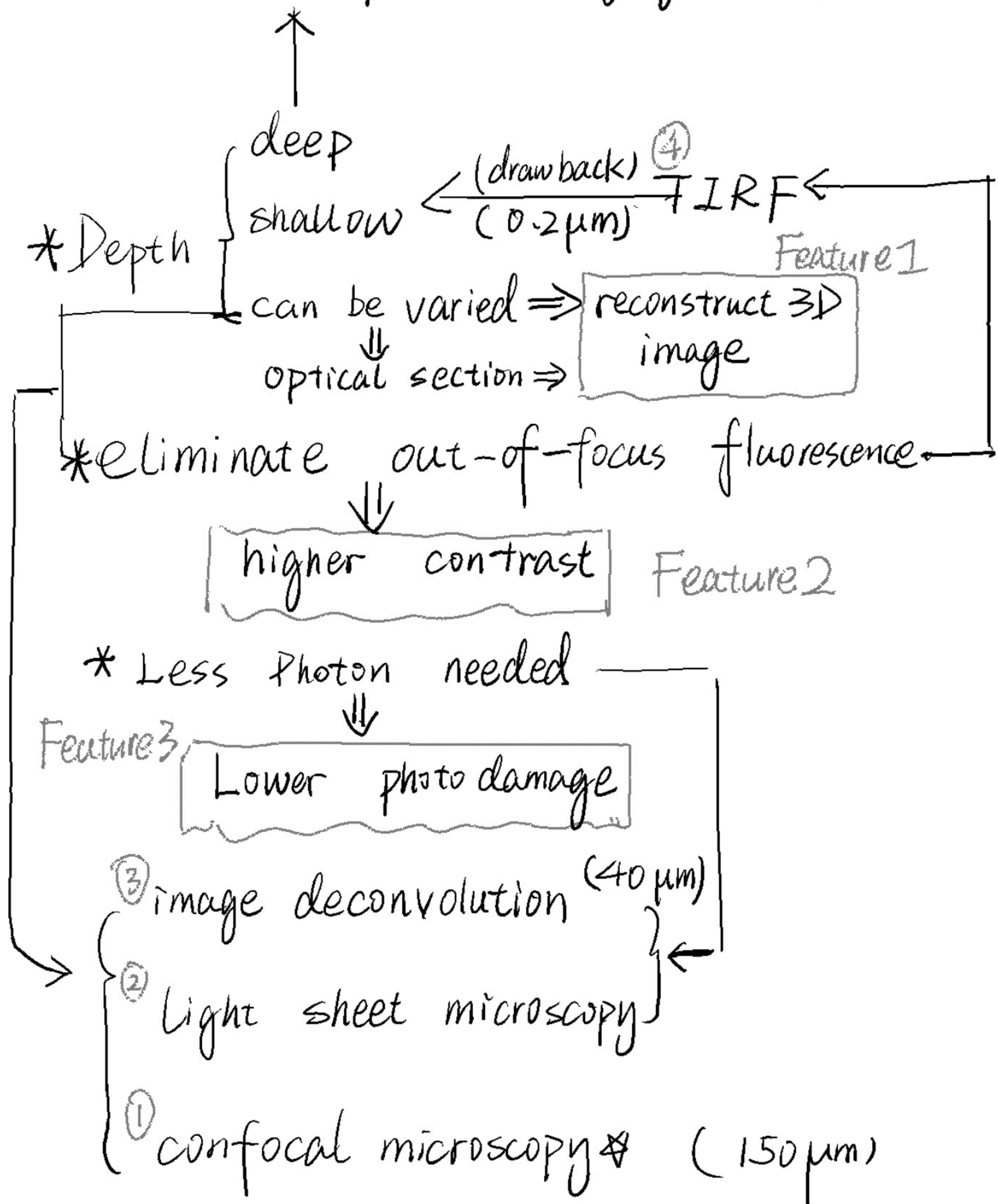
deconvolution; multiphoton  
confocal; light sheet

To Reduce Damage

Light Source



# ⑤ multi-photon Imaging ( $500\mu\text{m}$ )





# 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.  $\text{Ca}^{2+}$ , cAMP

{ Reporting Component via FRET  
Sensing Component

↓  
conformational change



\* Help by passing Diffraction Limit

STED ; SMLM { STORM  
PALM

relies on { photoactivable } dyes  
by changing  $\lambda$  — { photoswitchable }

\* Dark field to increase contrast



conventional fluorescent microscope.