

# Two-Photon Microscopy

## Nonlinear excitation

Two photons absorbed simultaneously

## Deeper penetration

Up to 1mm in tissue

## Reduced photobleaching

Excitation only at focal point

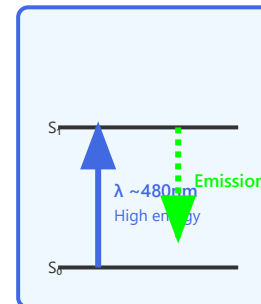
## In vivo imaging

Live animal brain imaging

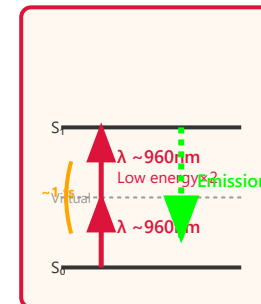
## SHG imaging

Second harmonic generation for collagen

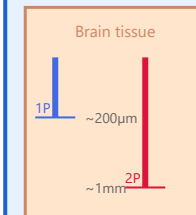
### One-Photon



### Two-Photon

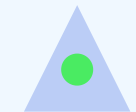


### Penetration Depth



### Excitation Volume

#### One-Photon



Entire cone excited

#### Two-Photon



Only focal point

Pulsed Ti:Sapphire laser  
~100 fs pulses, 80 MHz

### Advantages

- Deep imaging
- Less photobleach
- Lower phototoxicity
- Intrinsic sectioning
- NIR light scatters less

### Clinical & Research Applications

In vivo brain imaging • Deep tissue microscopy • Neuroscience studies  
Intravital microscopy • Tumor microenvironment • Long-term live imaging