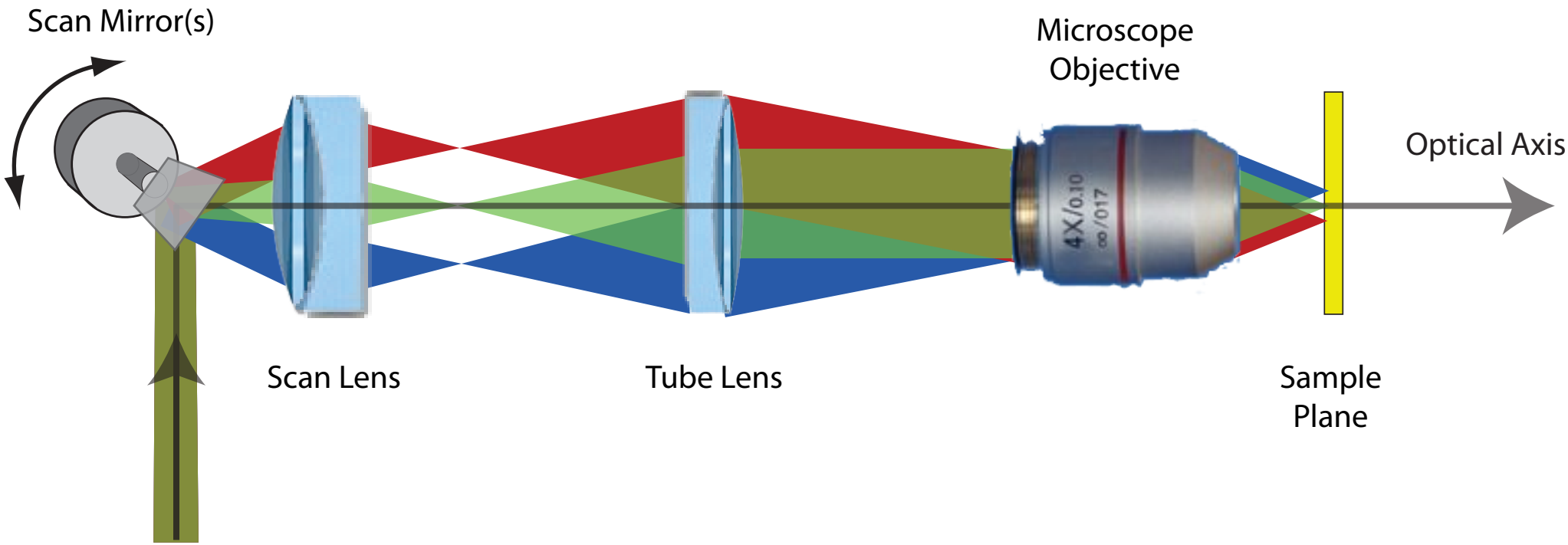
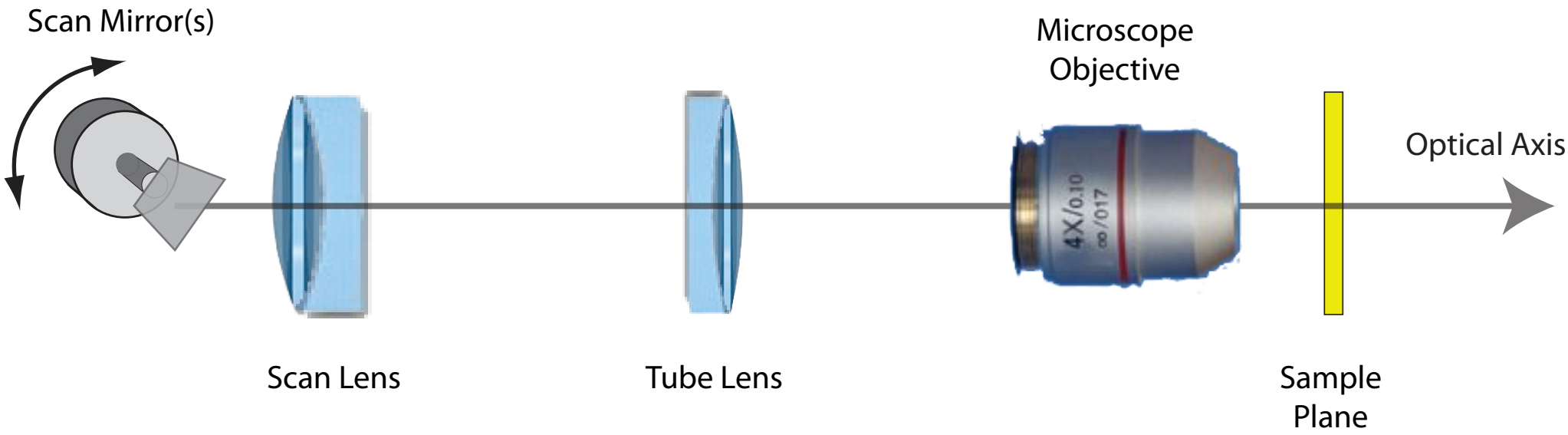
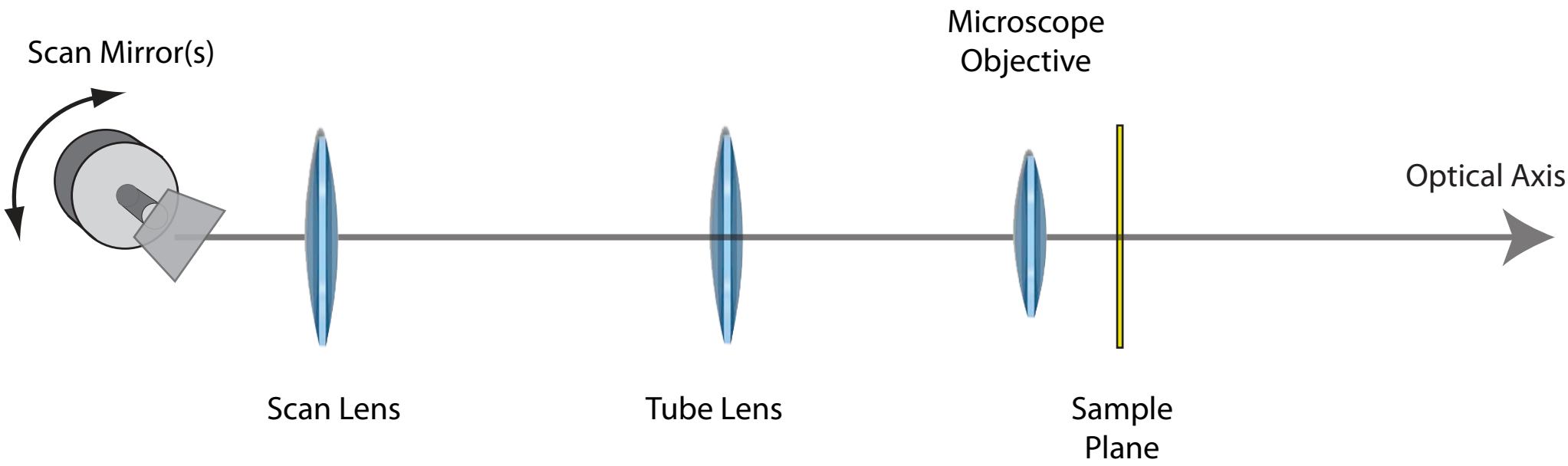
Laser Scanning Microscopy



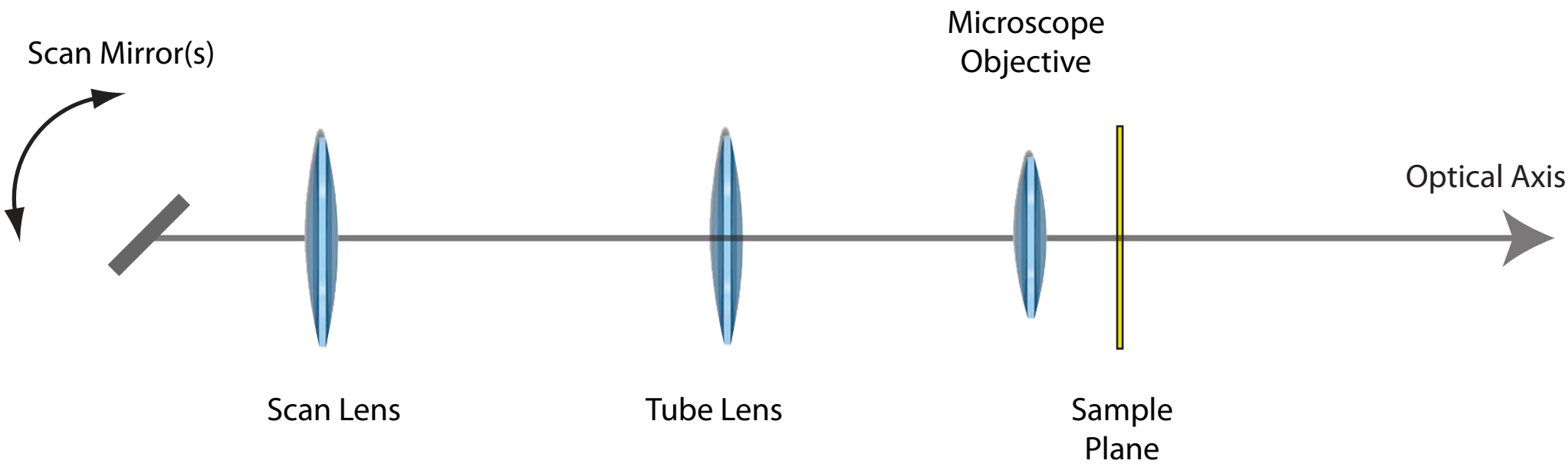
Laser Scanning Microscopy



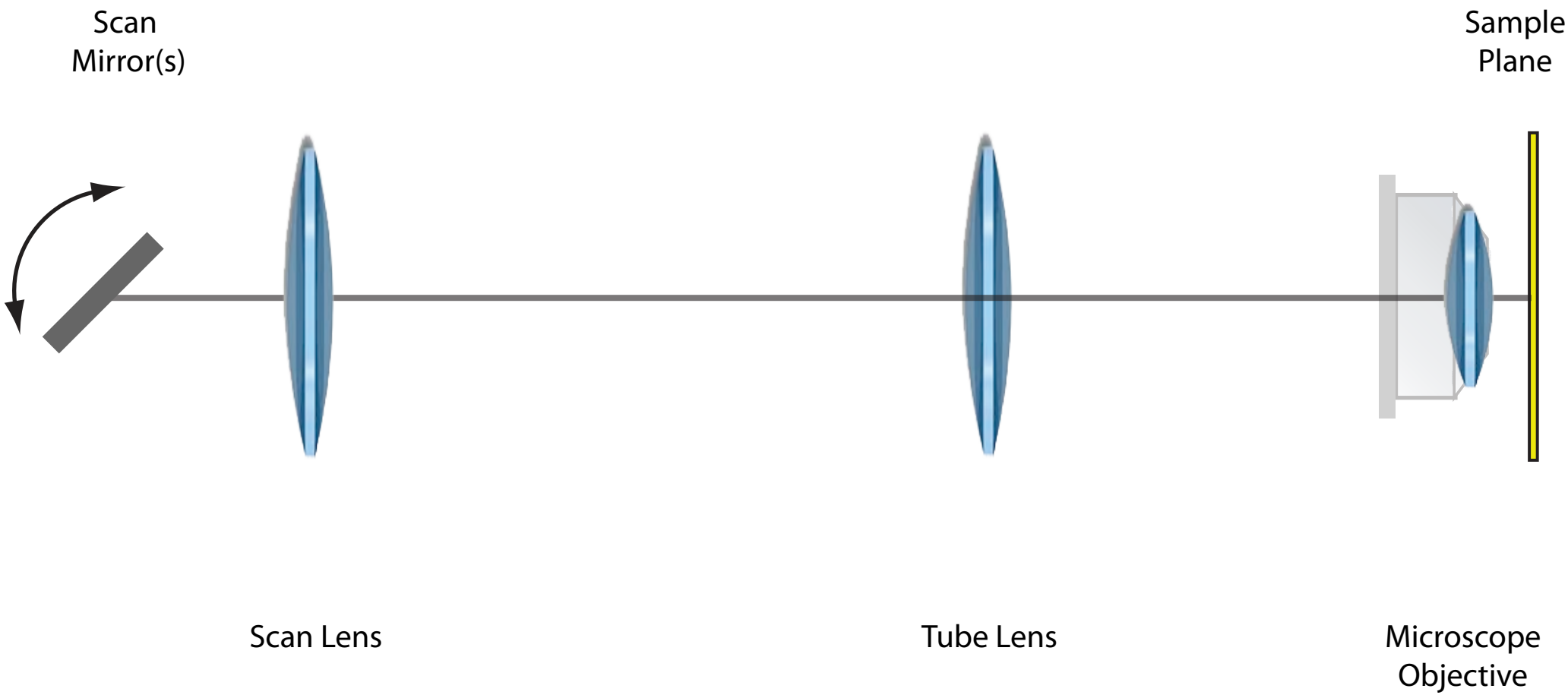
Laser Scanning Microscopy



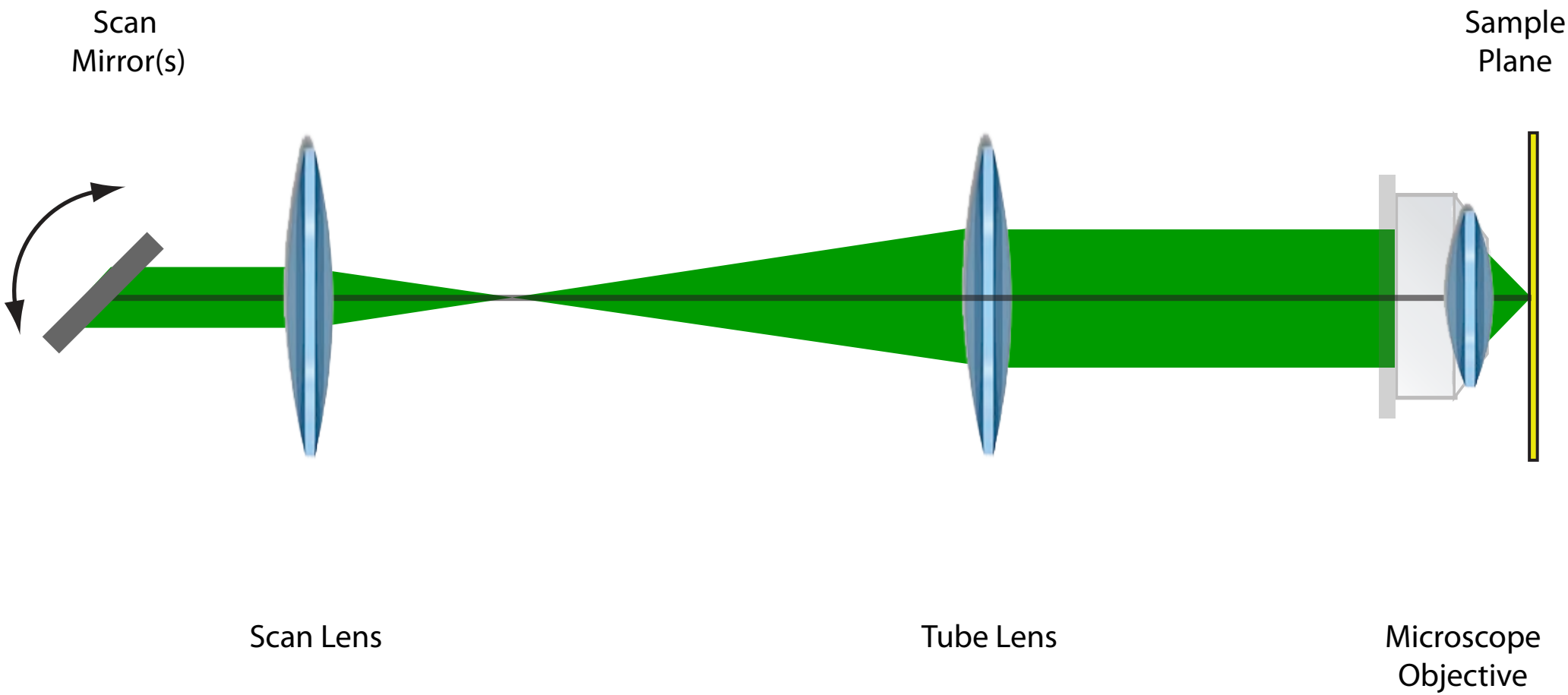
Laser Scanning Microscopy



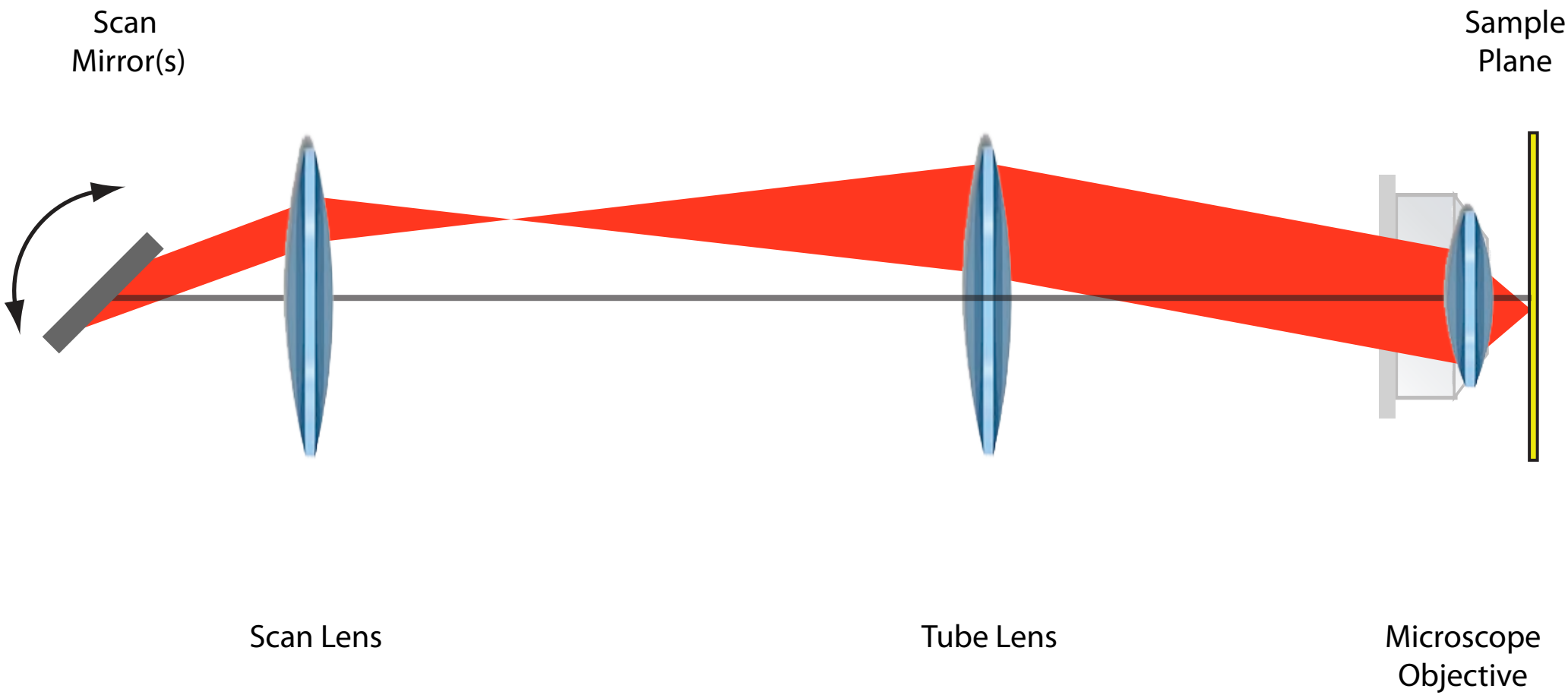
Laser Scanning Microscopy



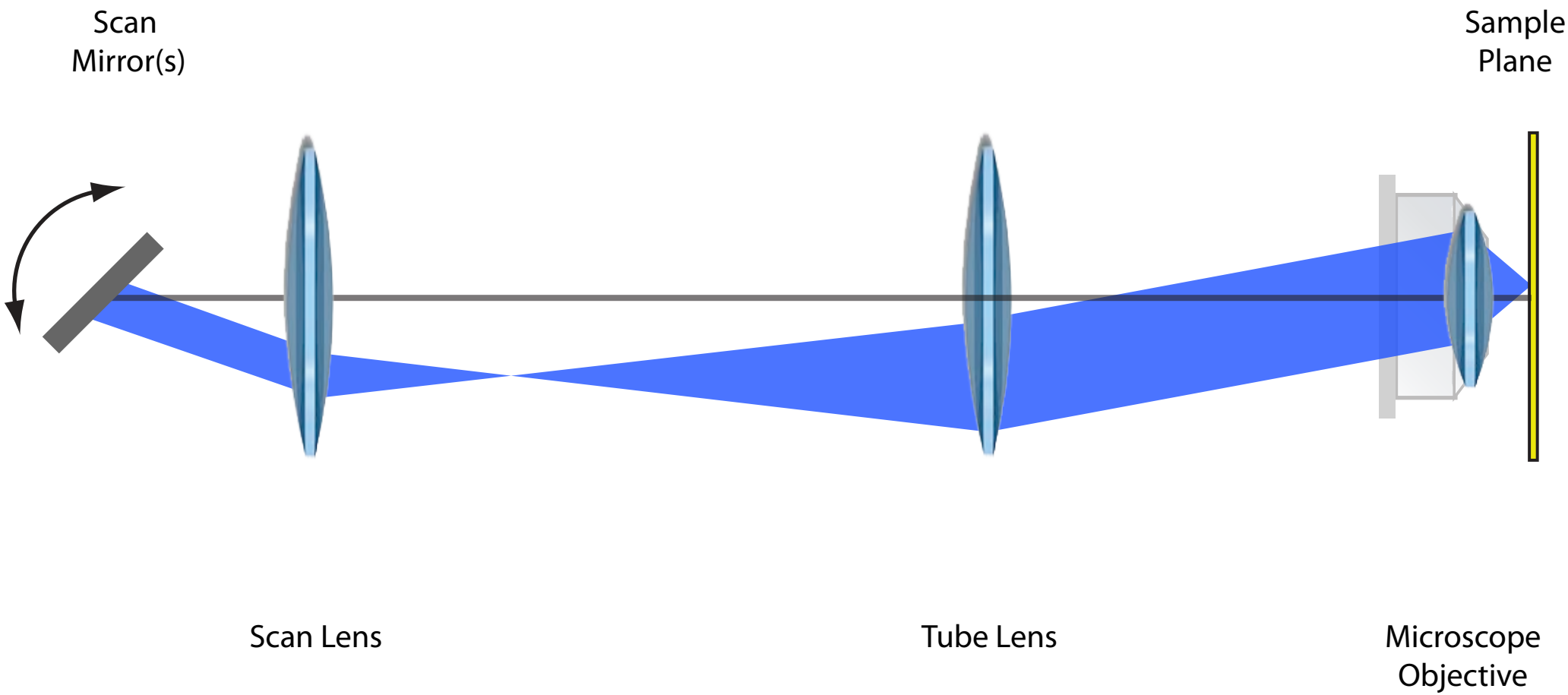
Laser Scanning Microscopy



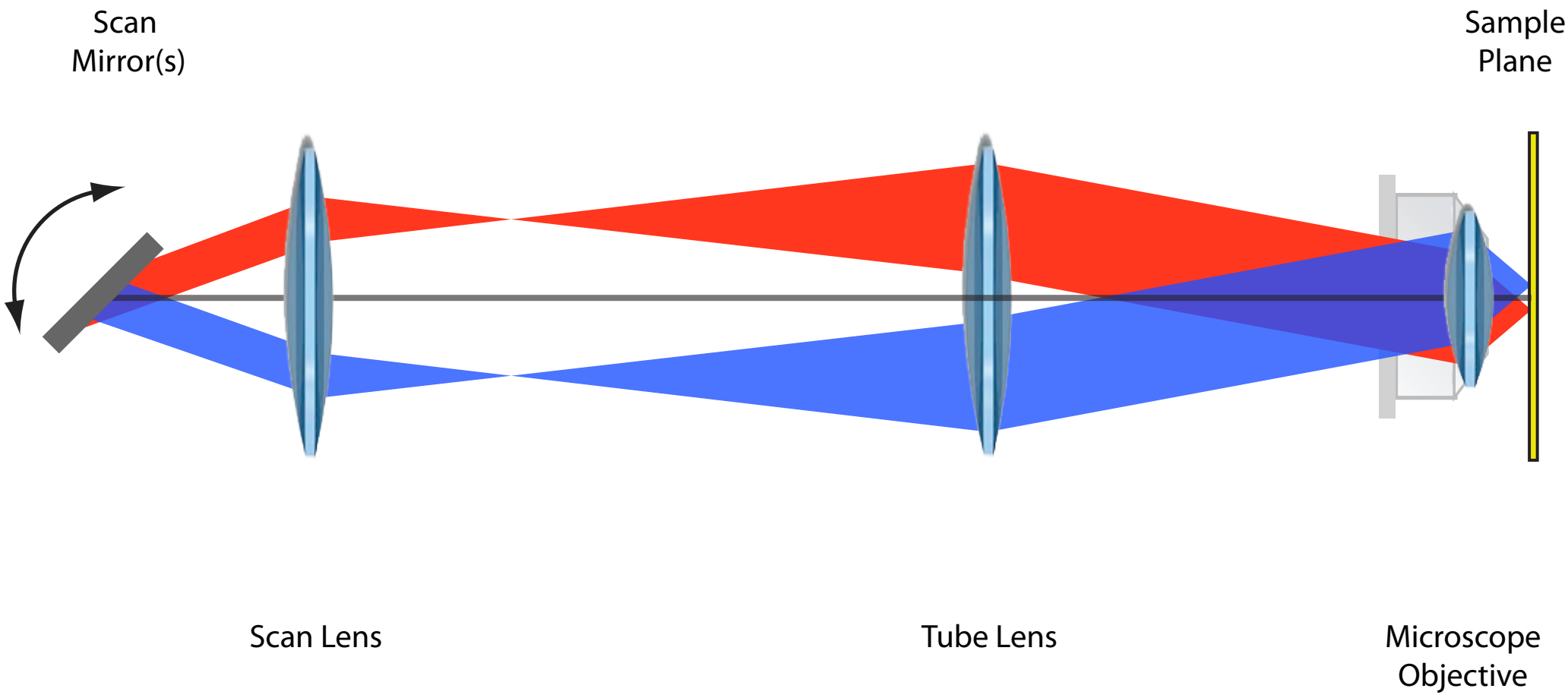
Laser Scanning Microscopy



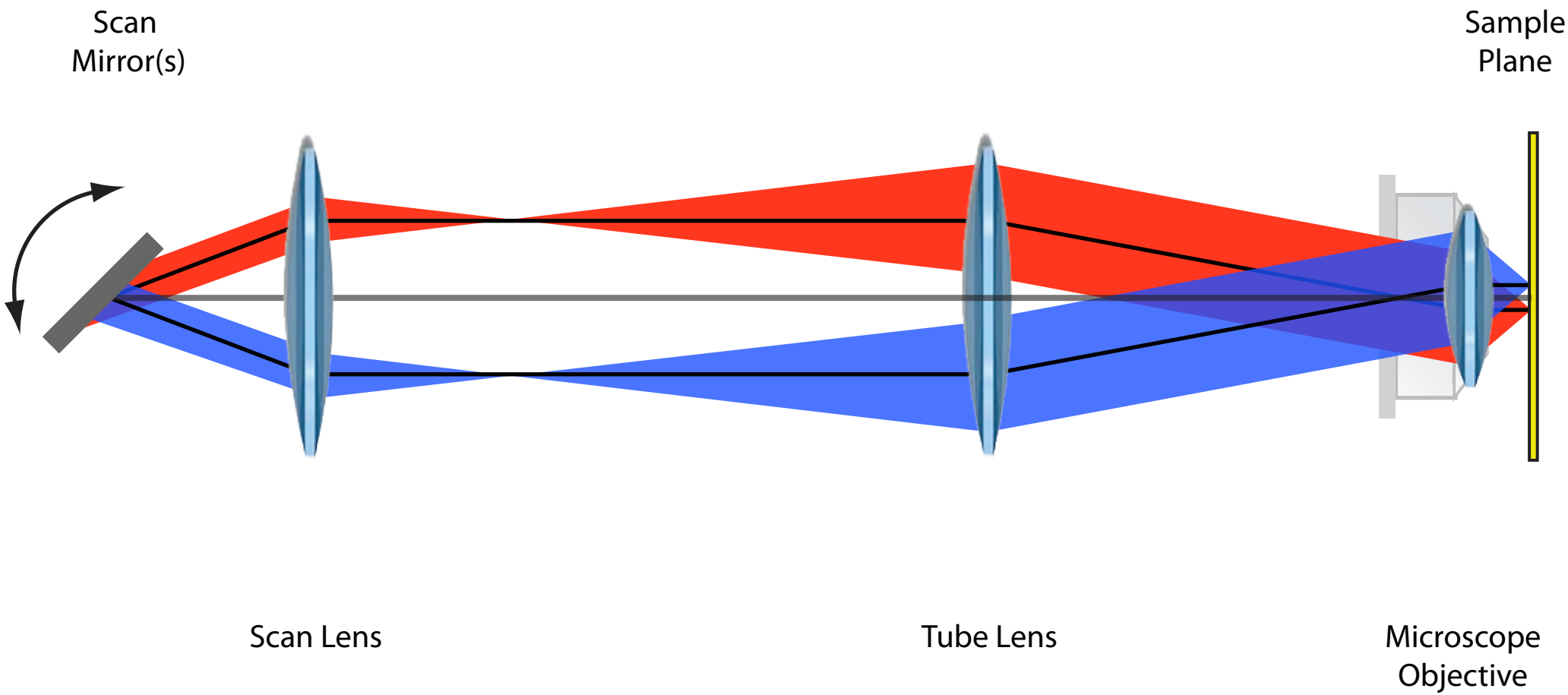
Laser Scanning Microscopy



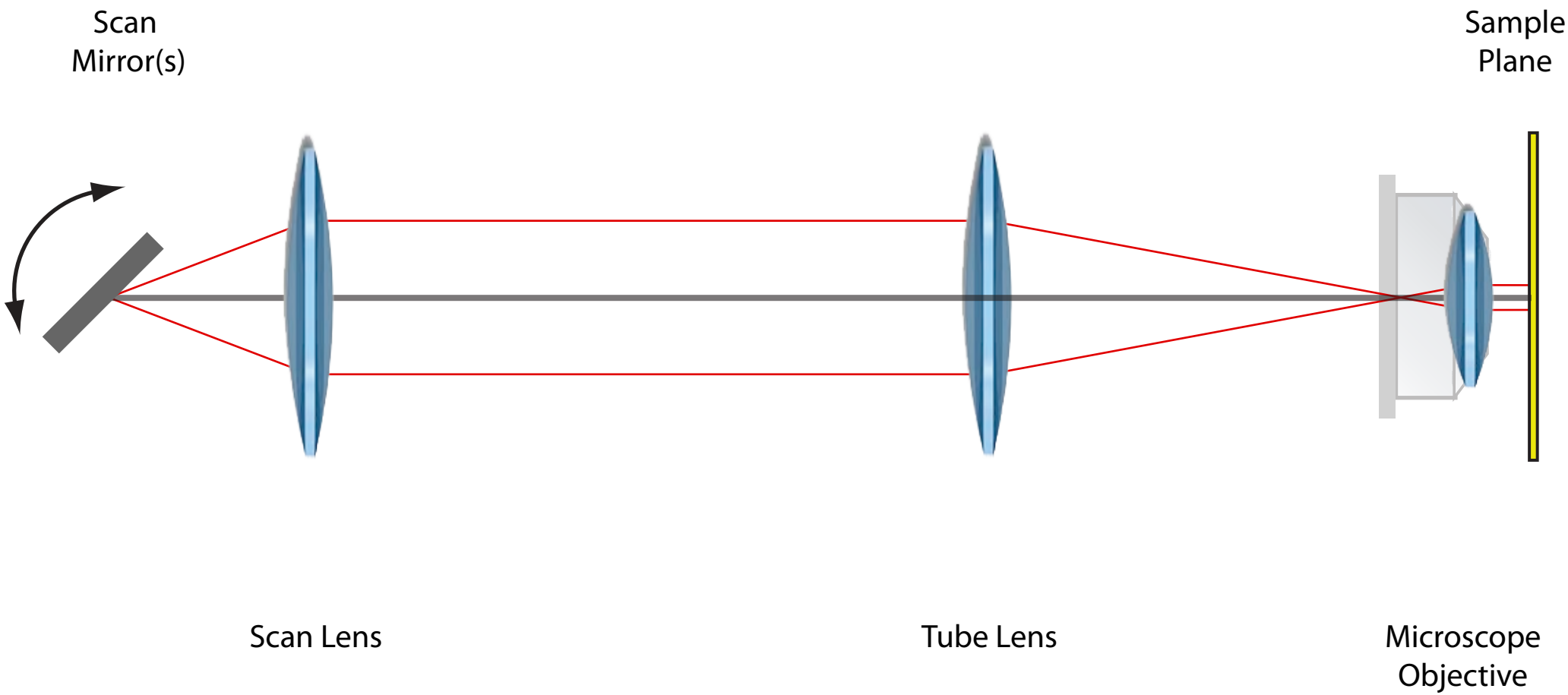
Laser Scanning Microscopy



Laser Scanning Microscopy

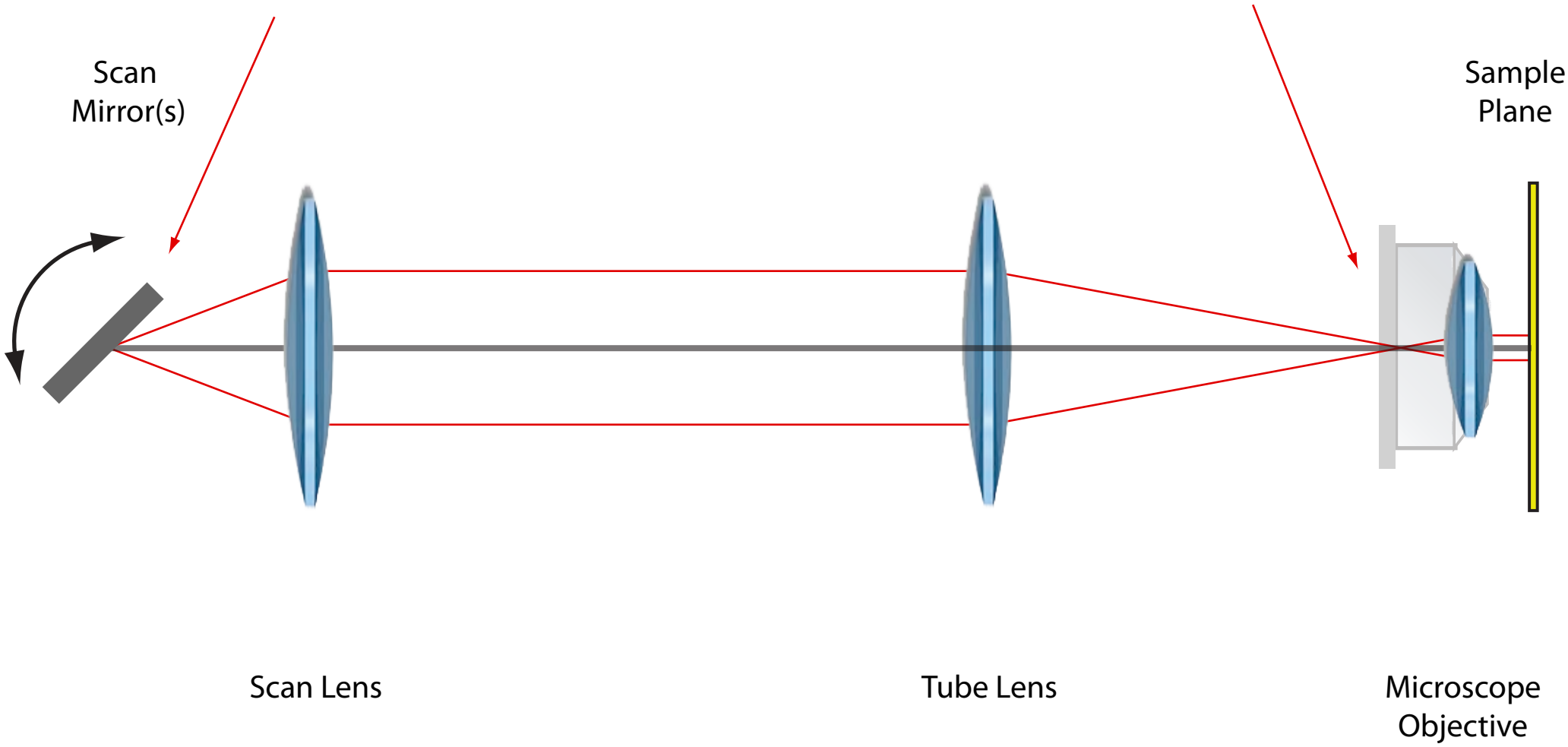


Laser Scanning Microscopy

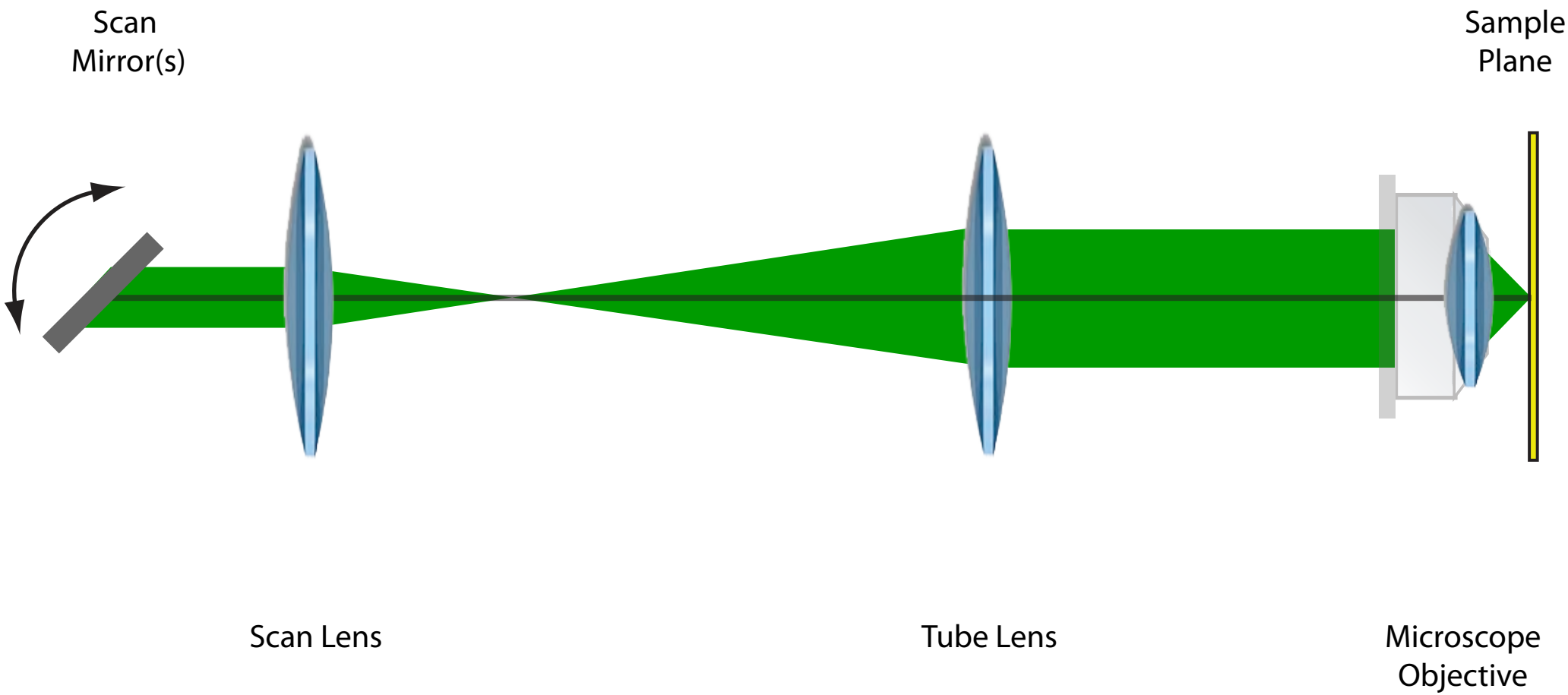


Laser Scanning Microscopy

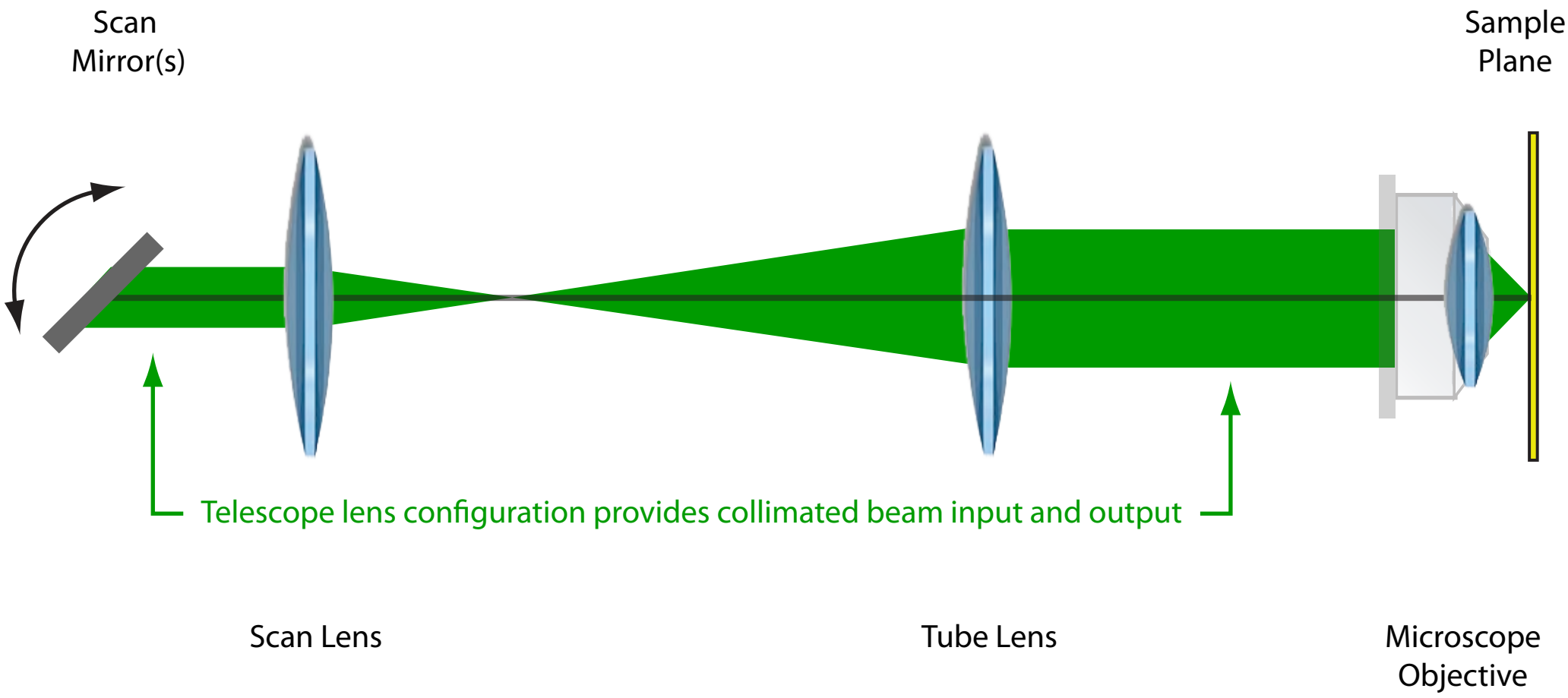
Imaging condition between scan mirror and back aperture of microscope objective



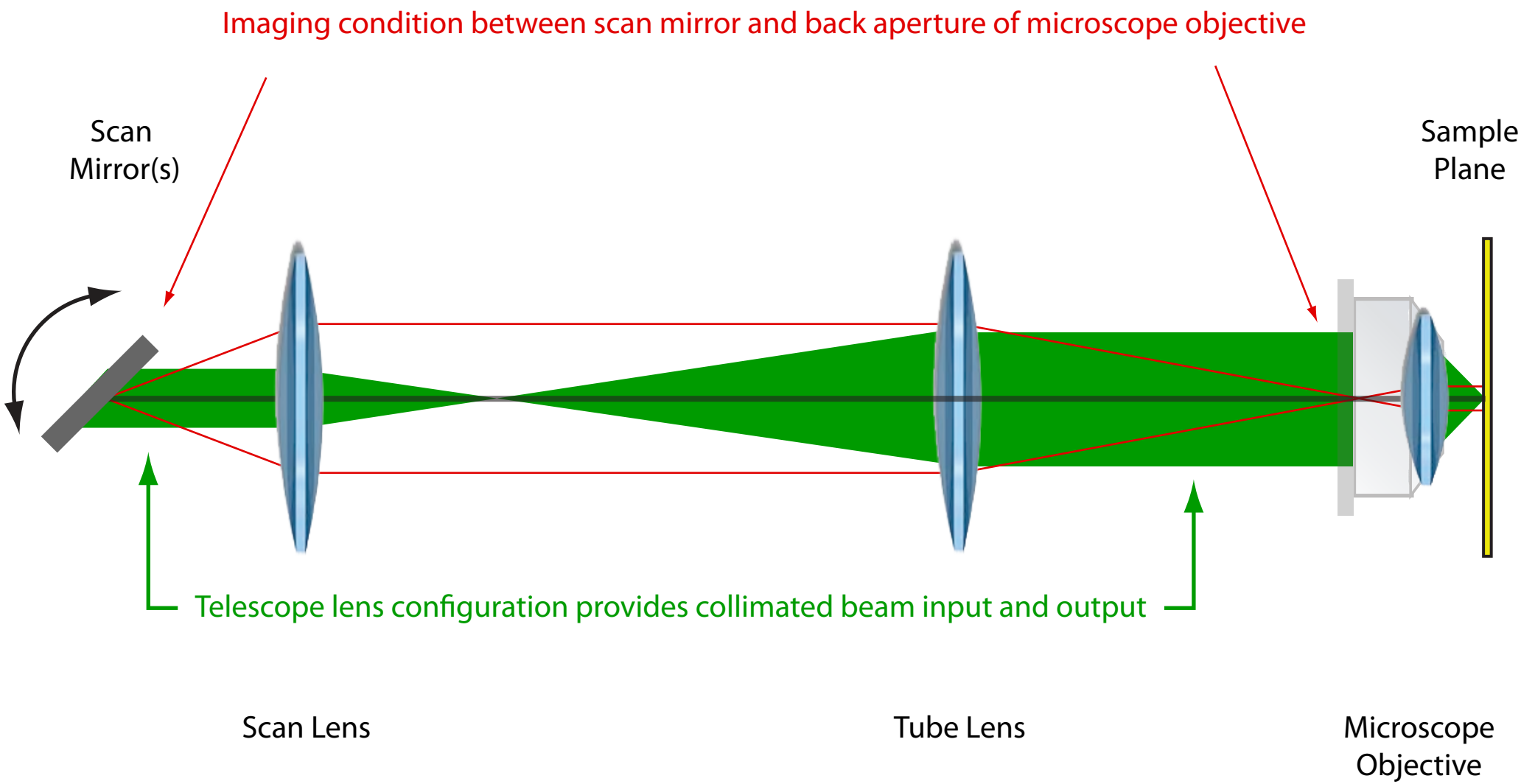
Laser Scanning Microscopy



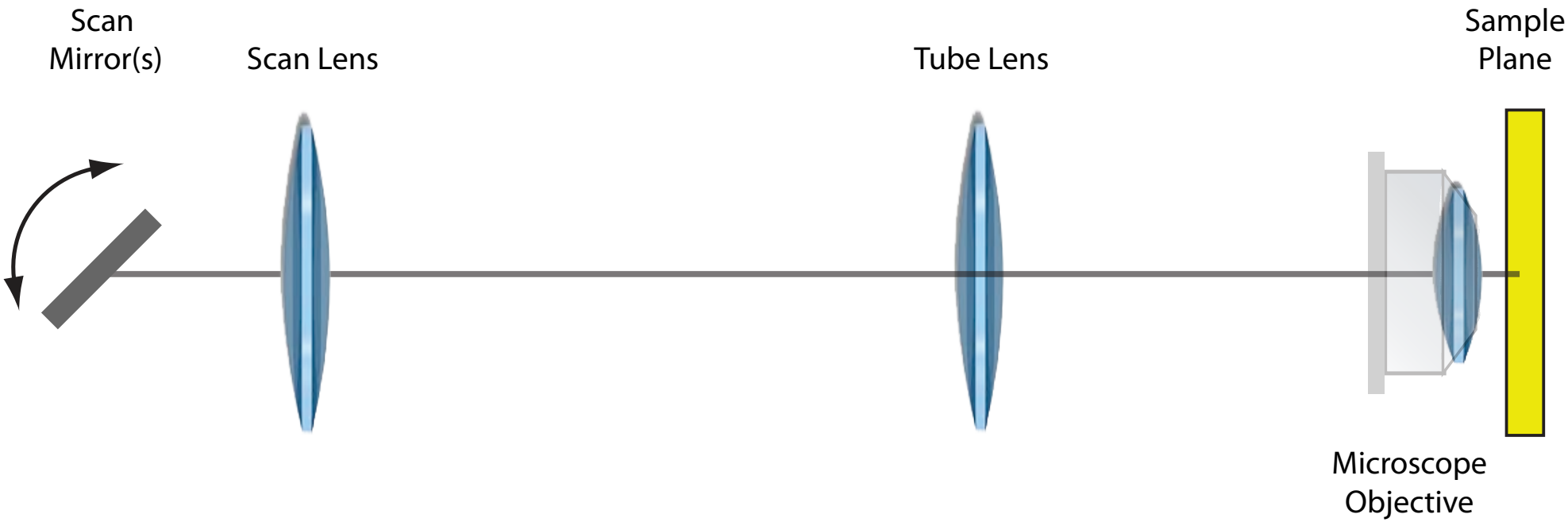
Laser Scanning Microscopy



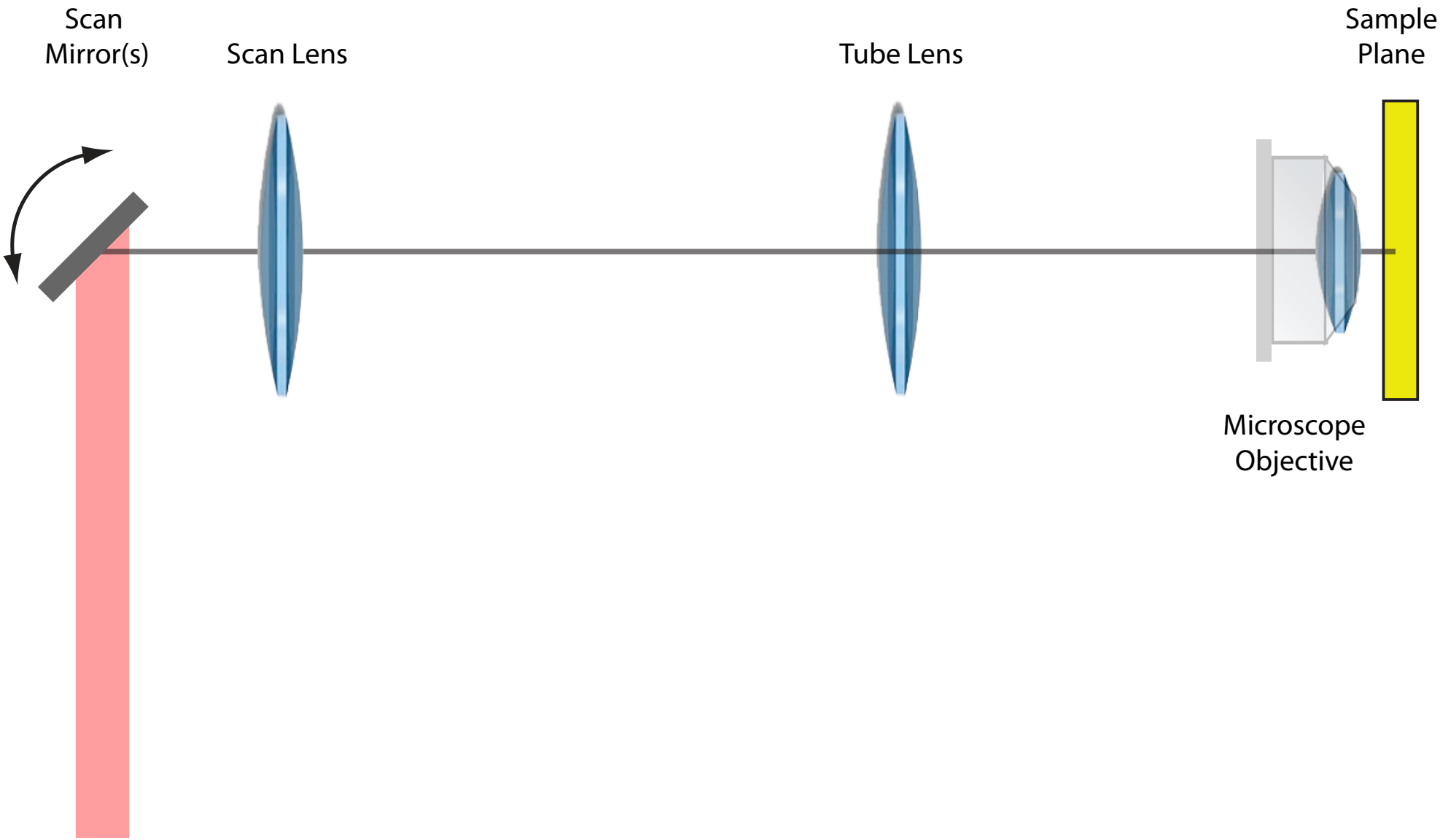
Laser Scanning Microscopy



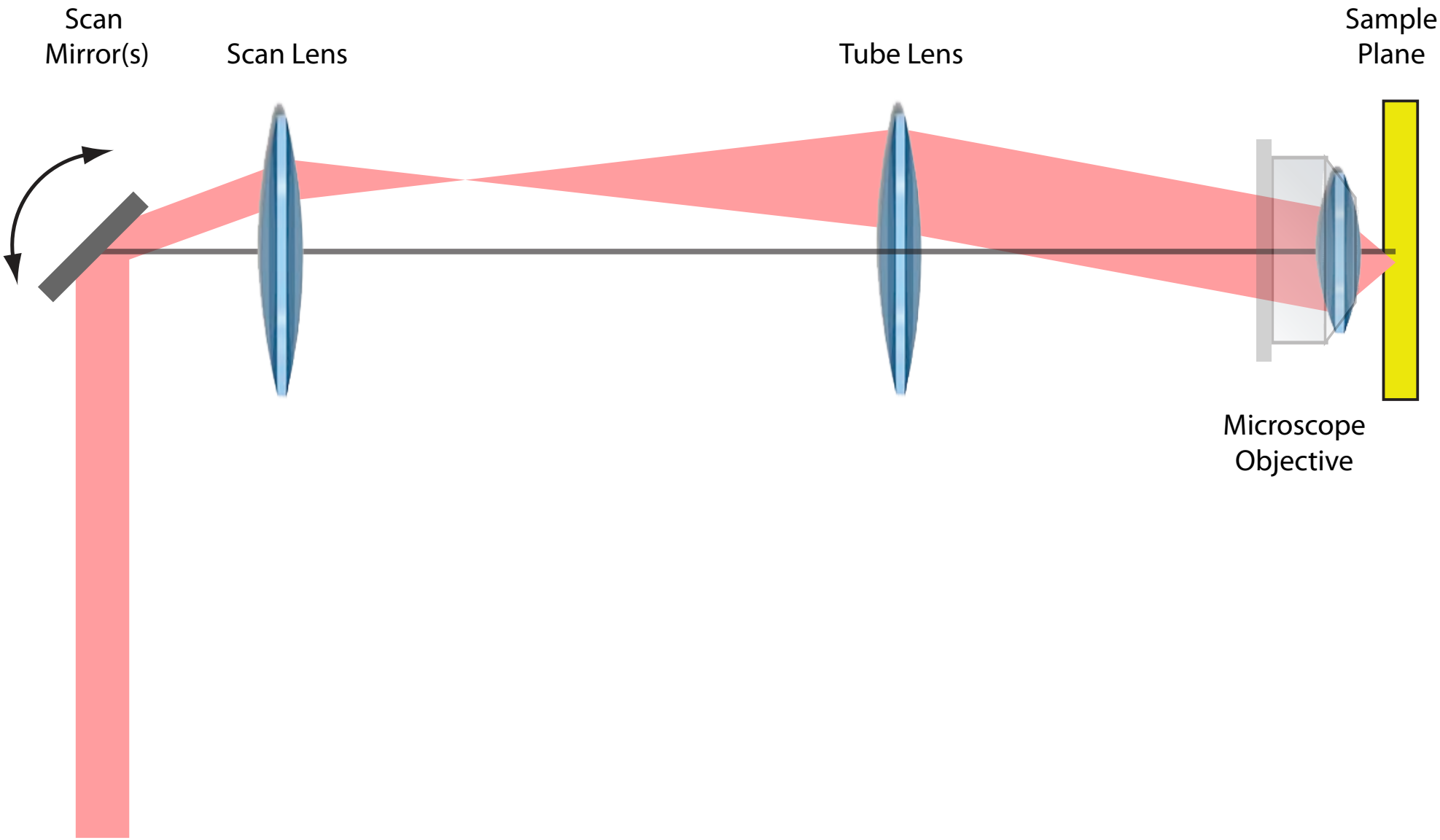
Confocal Laser Scanning Microscopy



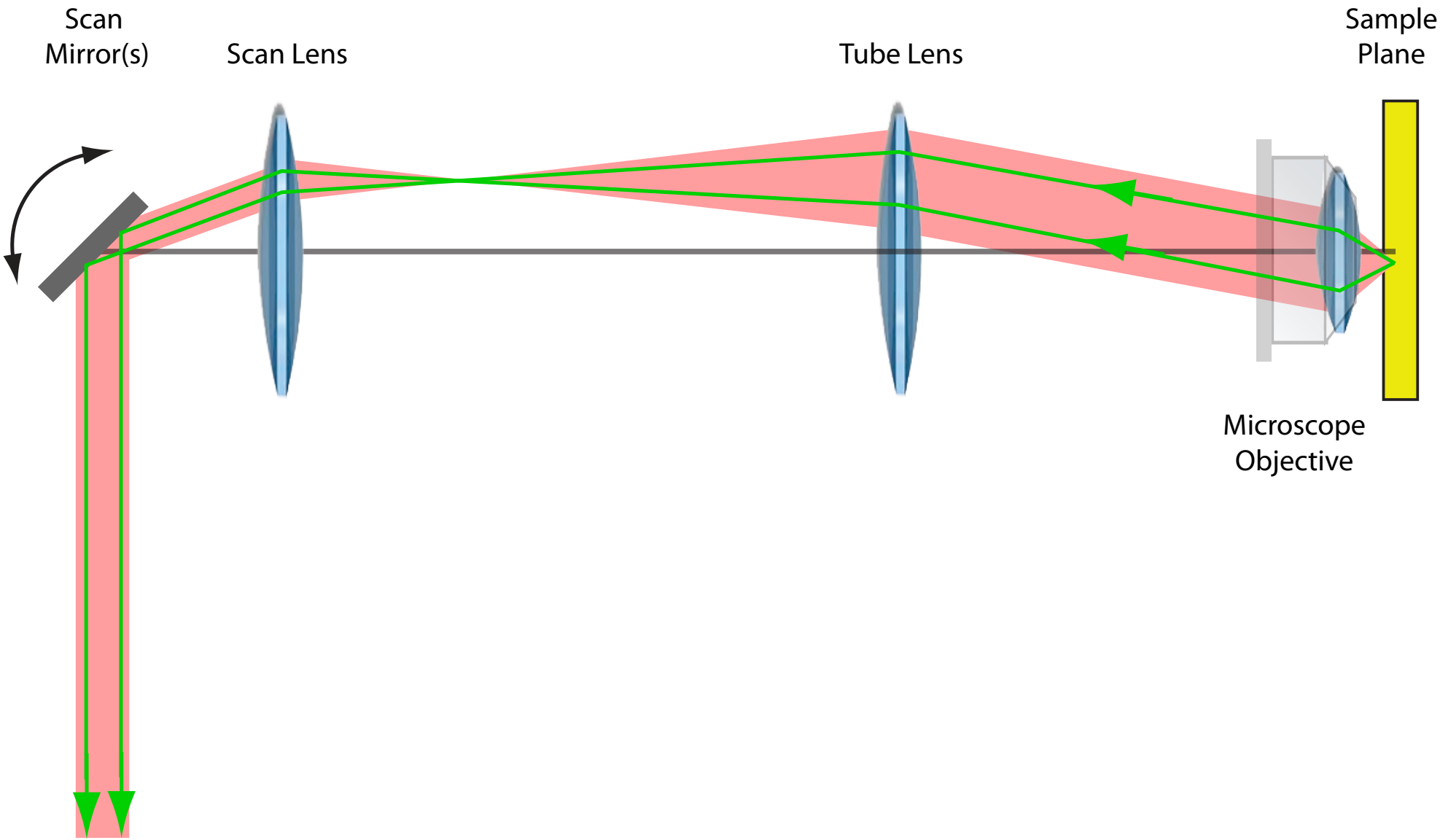
Confocal Laser Scanning Microscopy



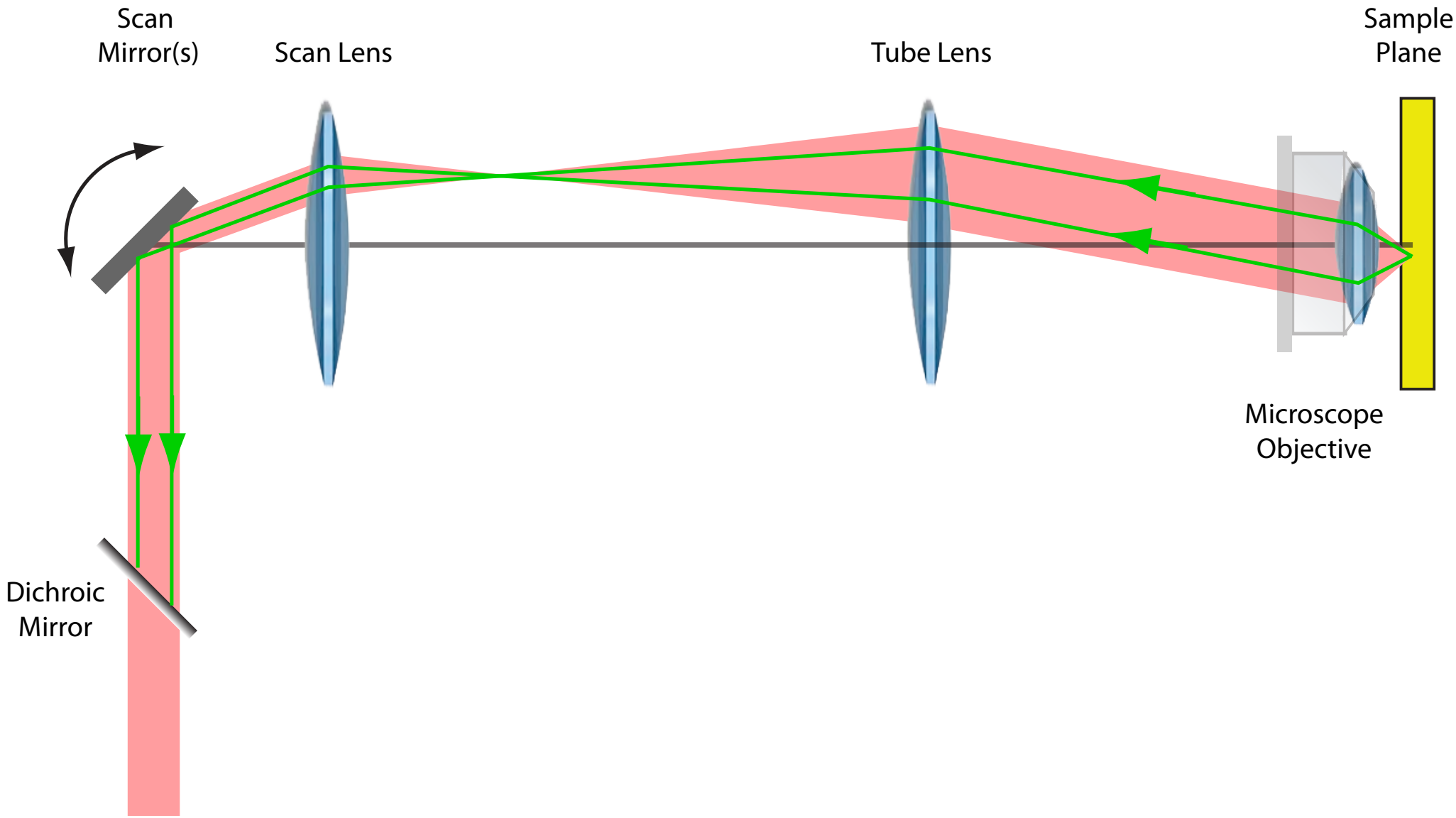
Confocal Laser Scanning Microscopy



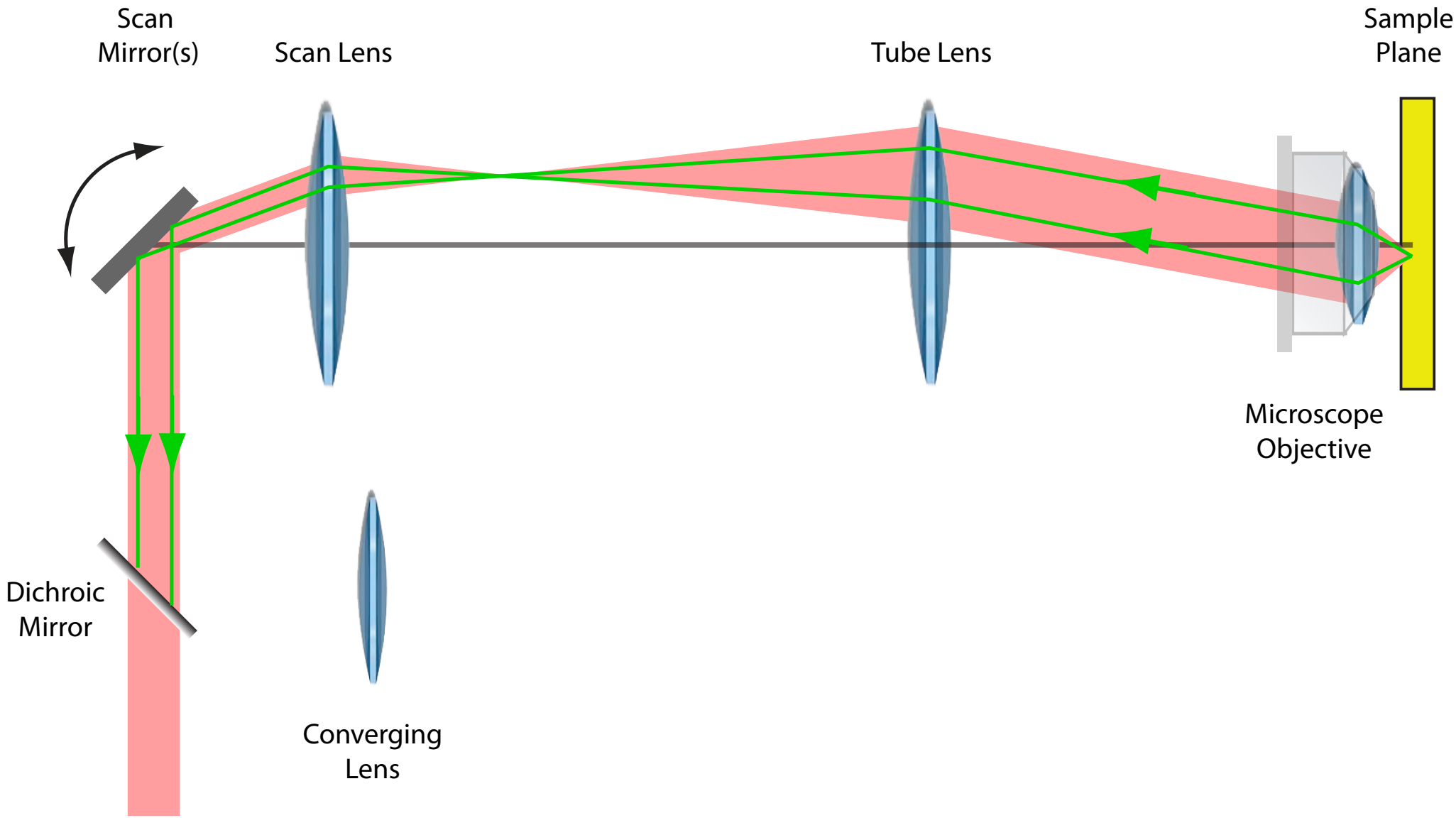
Confocal Laser Scanning Microscopy



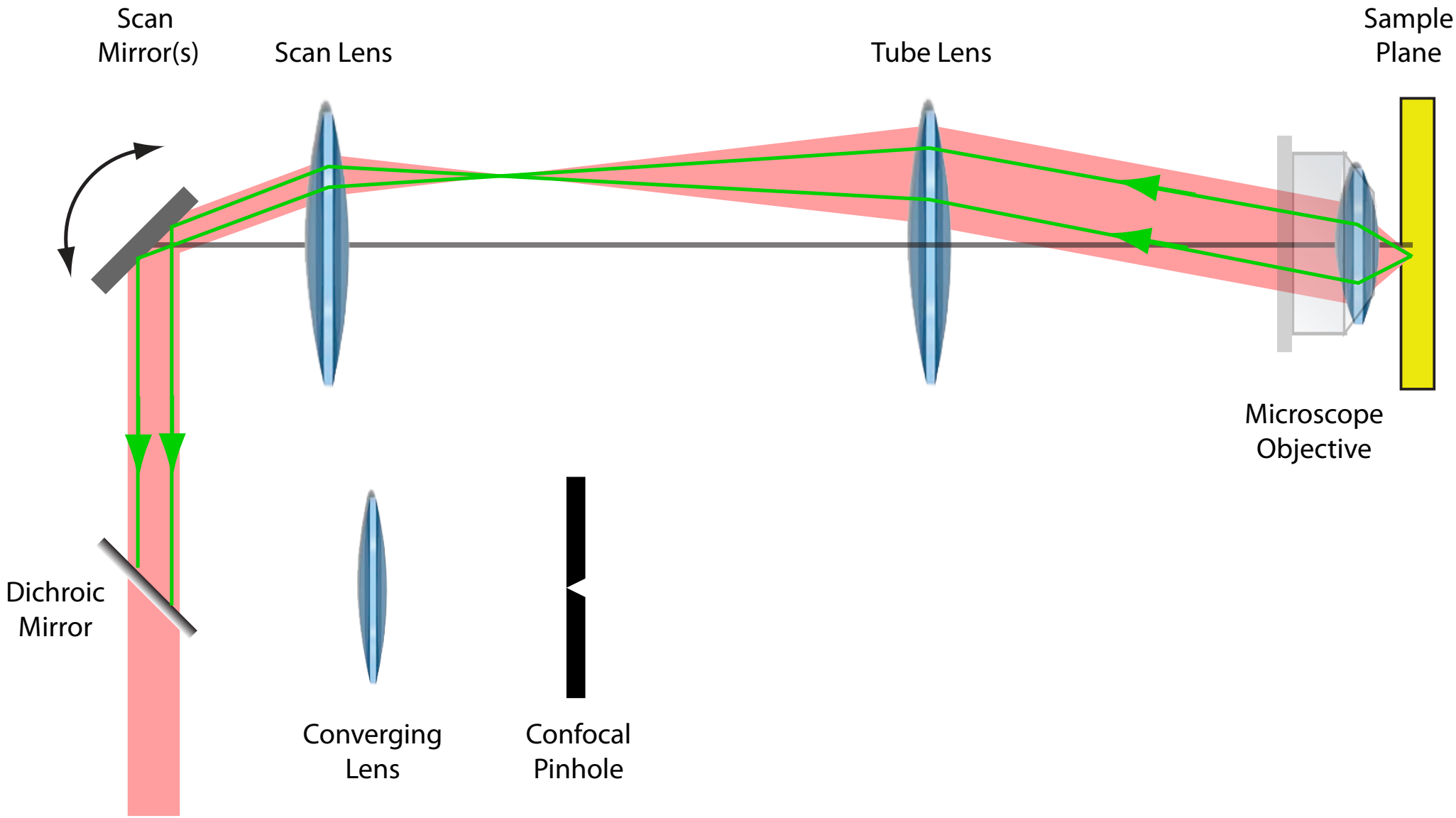
Confocal Laser Scanning Microscopy



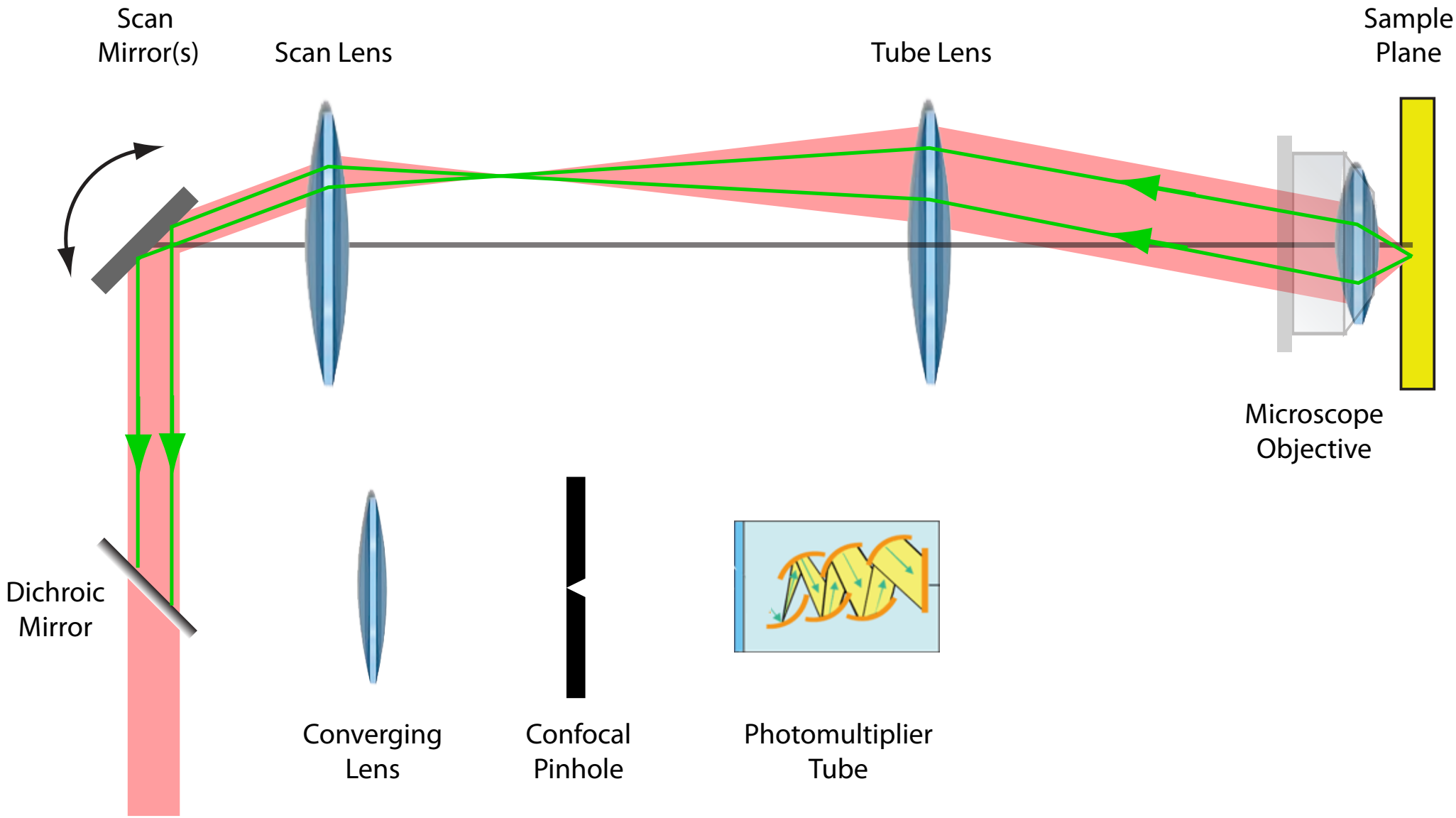
Confocal Laser Scanning Microscopy



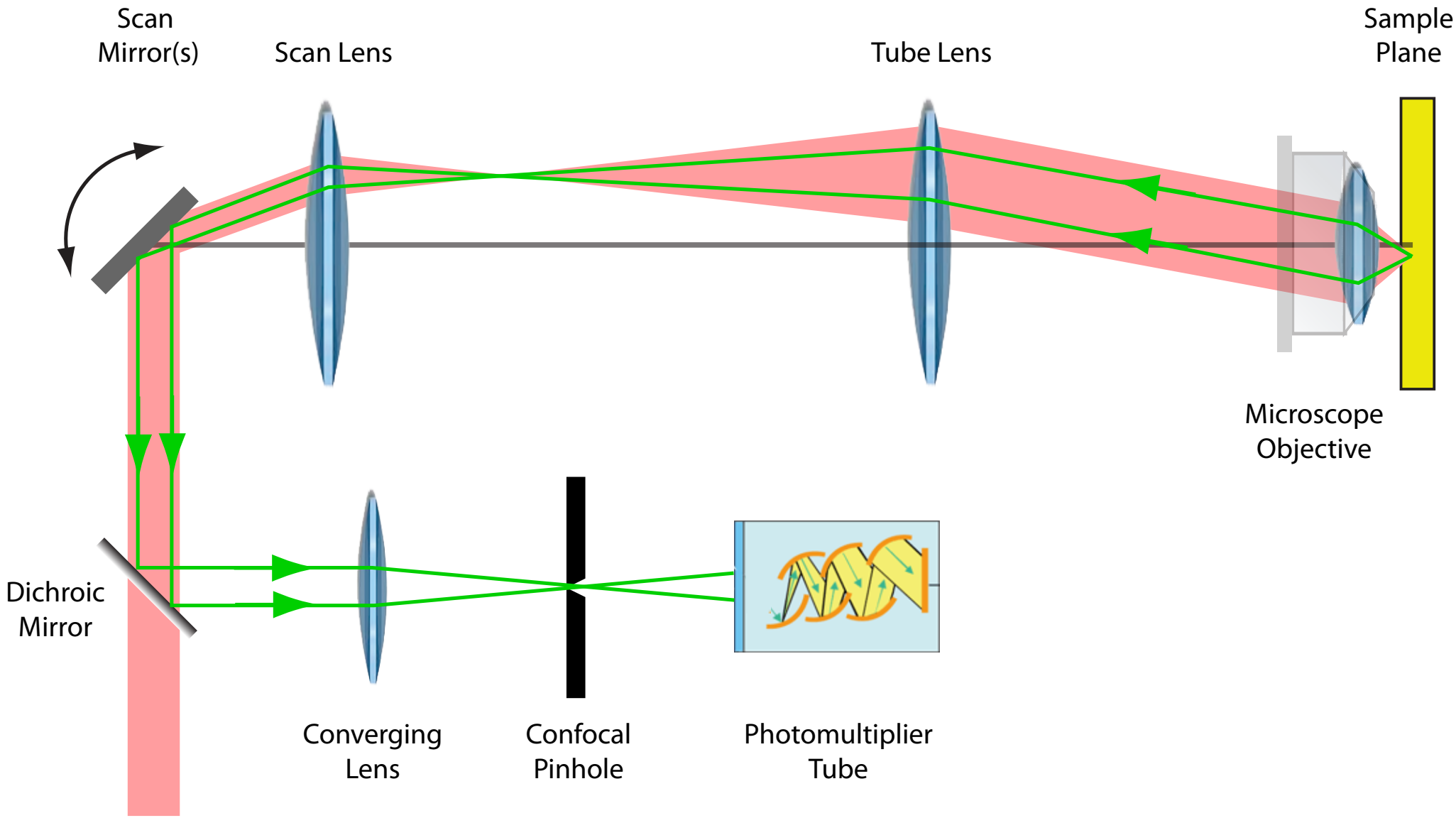
Confocal Laser Scanning Microscopy



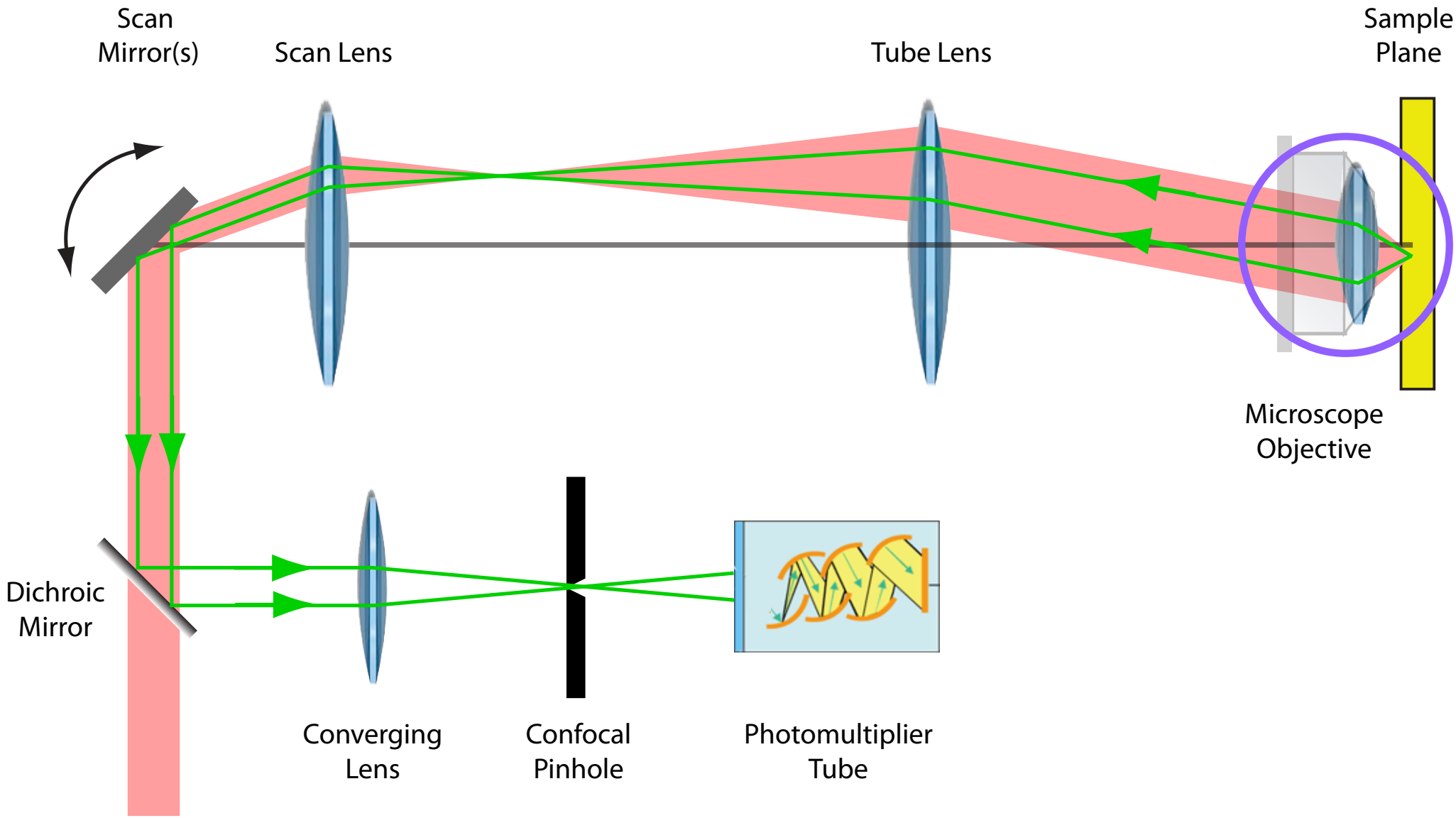
Confocal Laser Scanning Microscopy



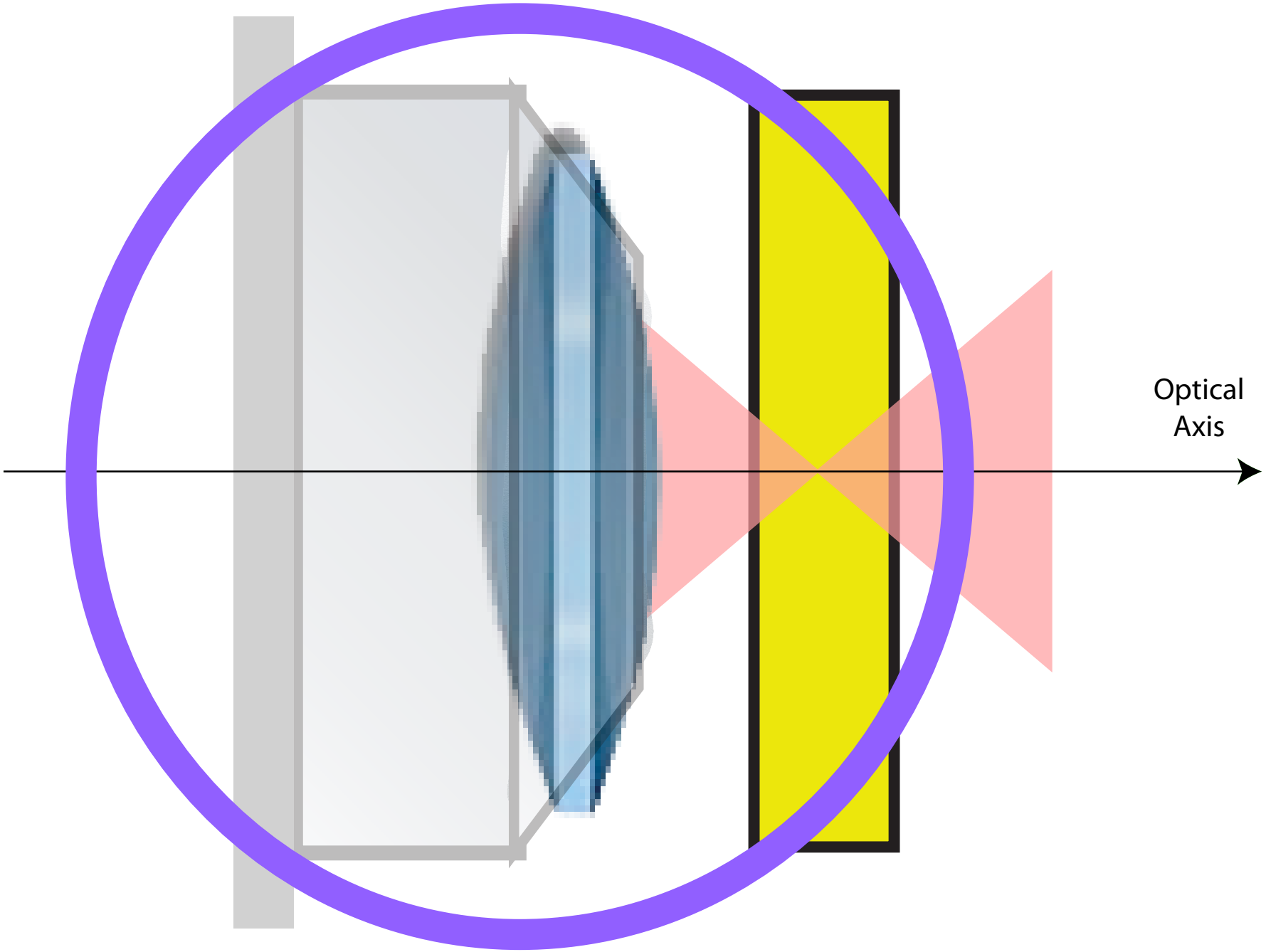
Confocal Laser Scanning Microscopy



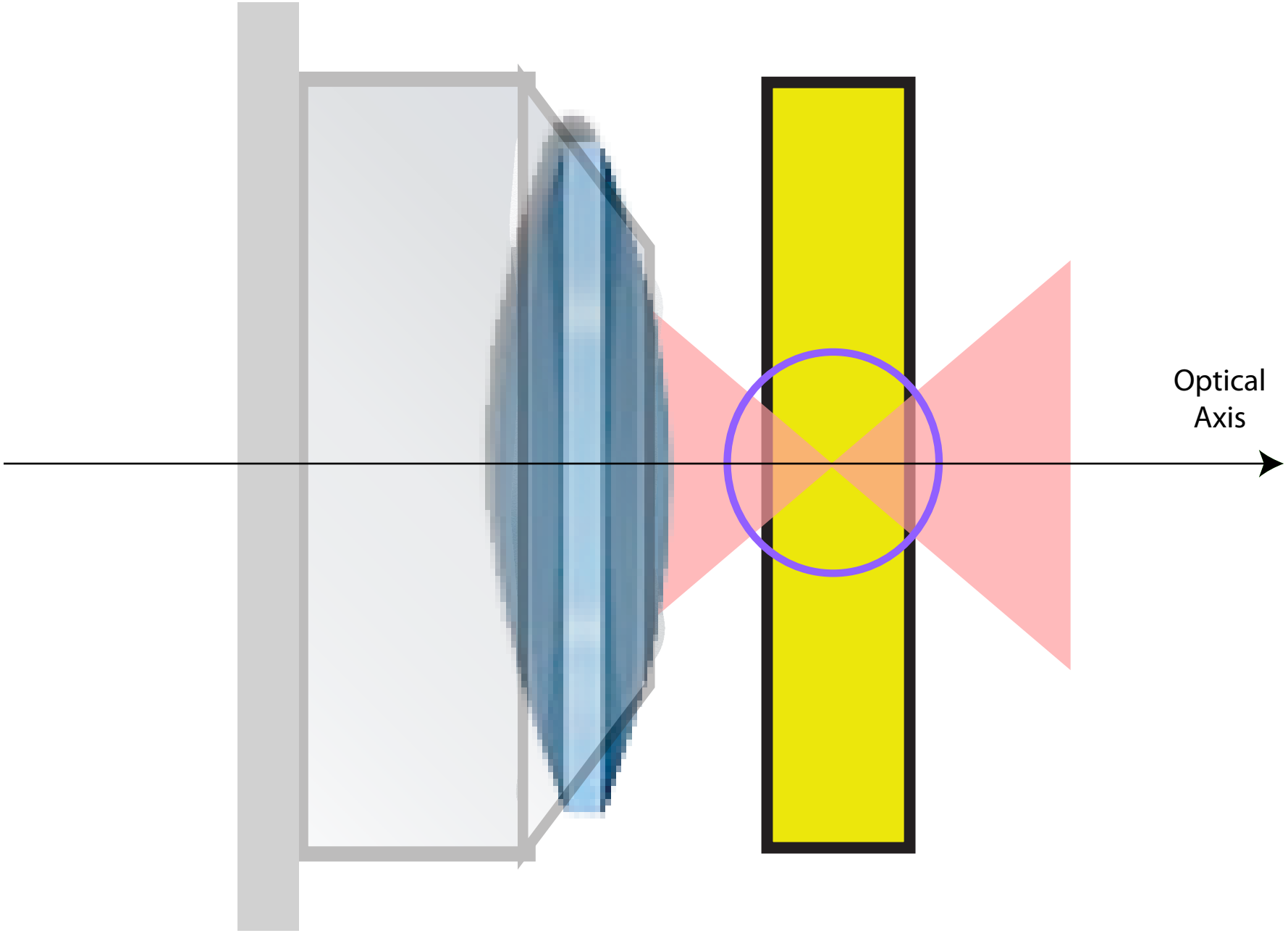
Confocal Laser Scanning Microscopy



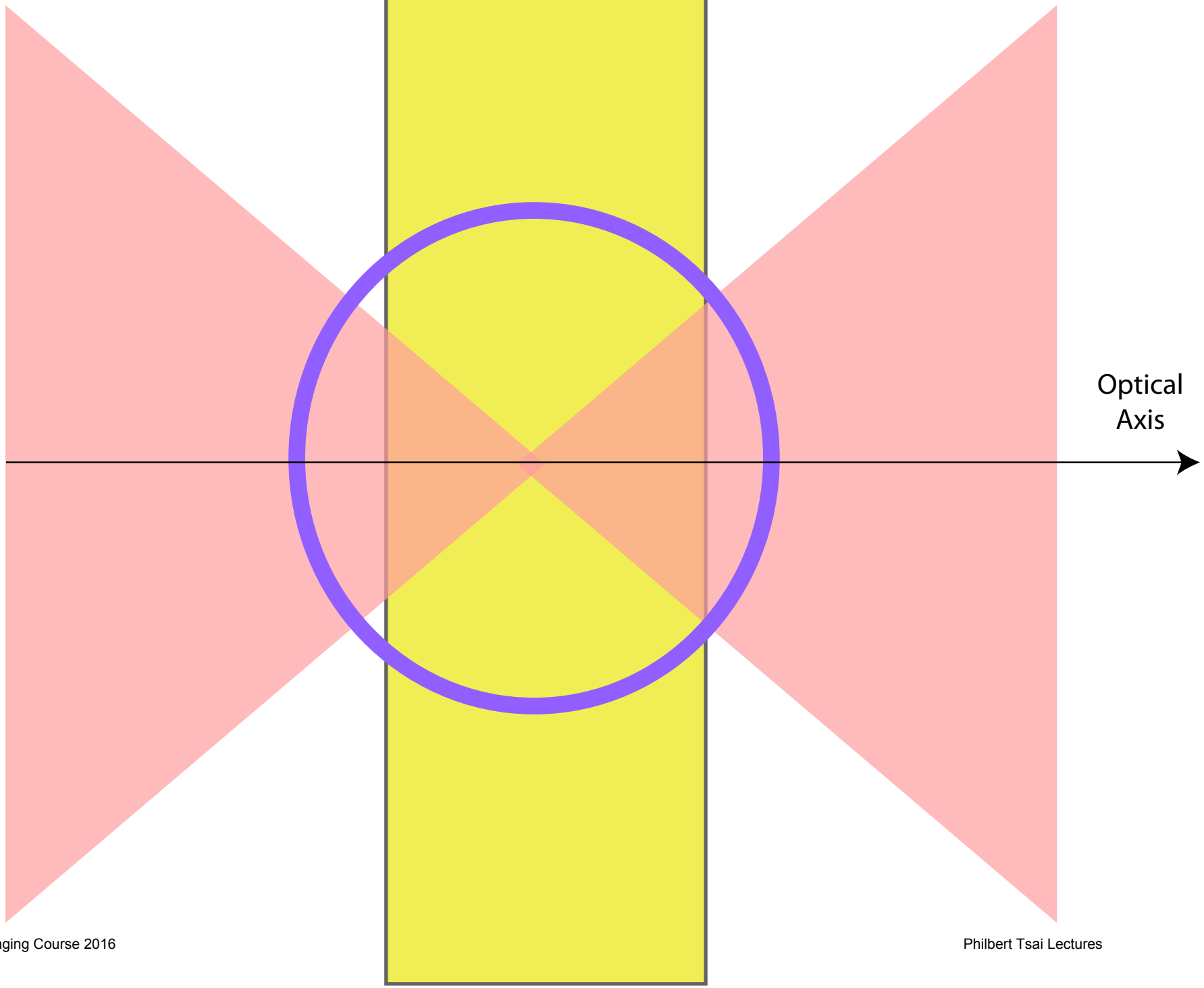
Confocal Laser Scanning Microscopy



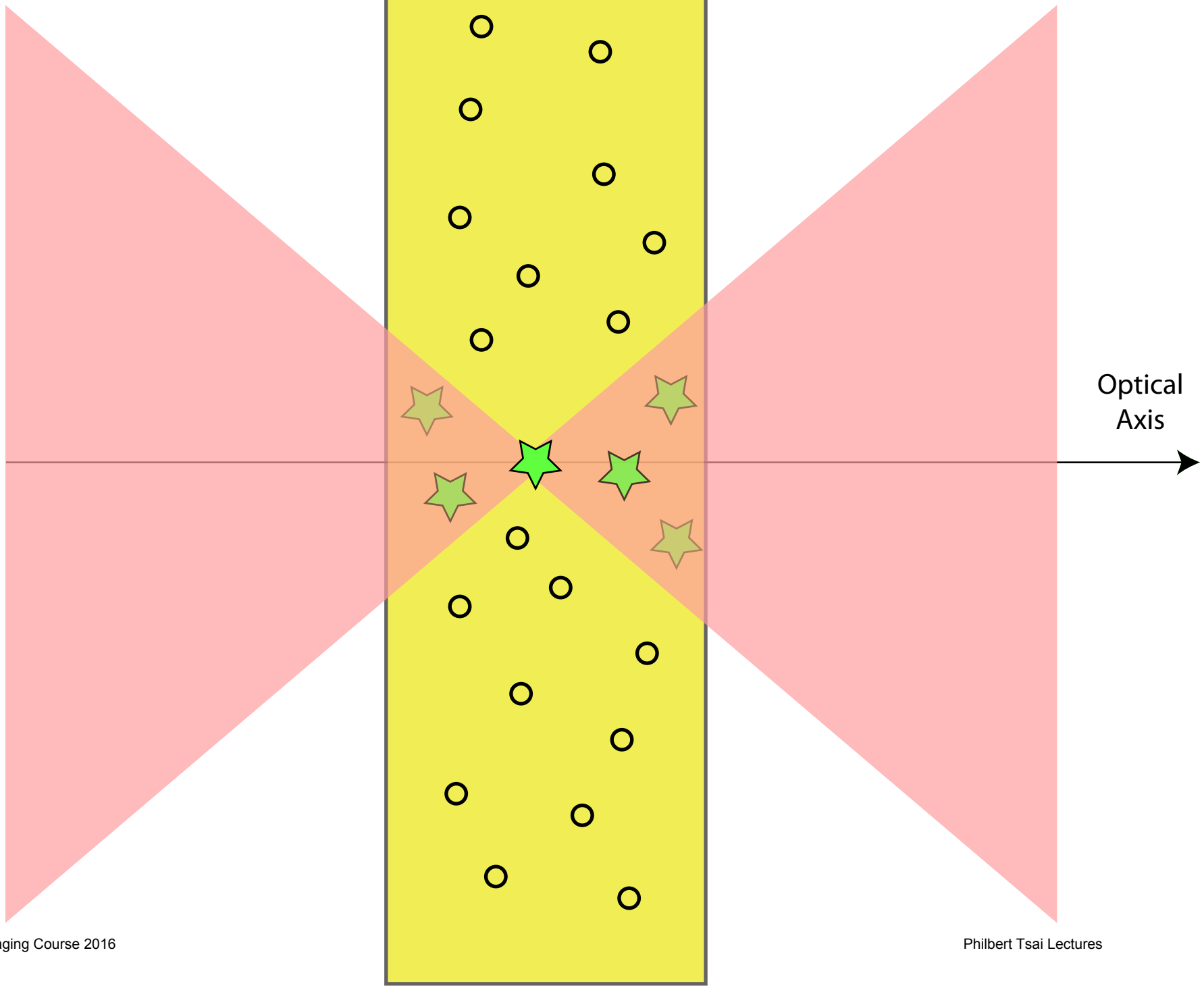
Confocal Laser Scanning Microscopy



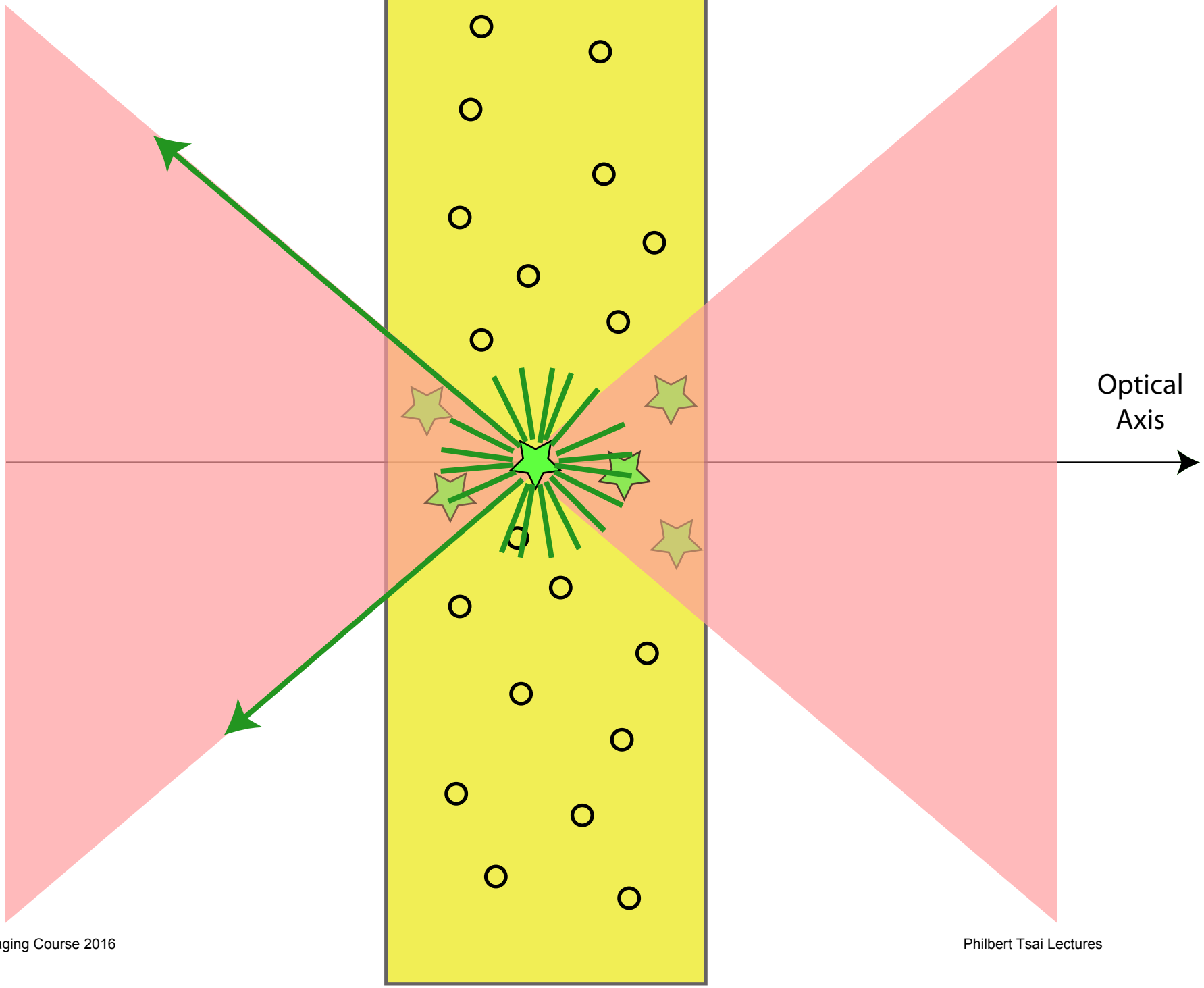
Confocal Laser Scanning Microscopy



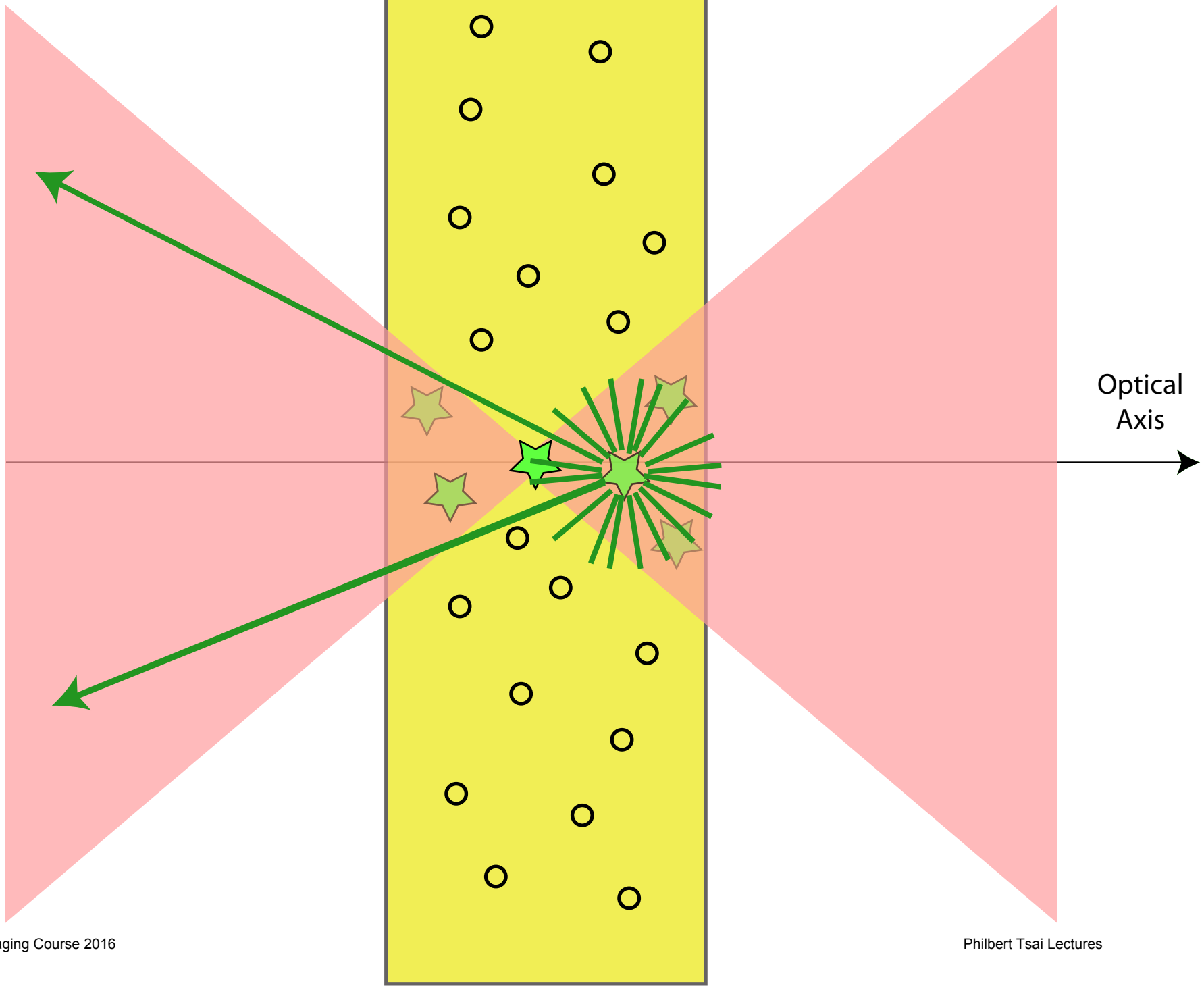
Confocal Laser Scanning Microscopy



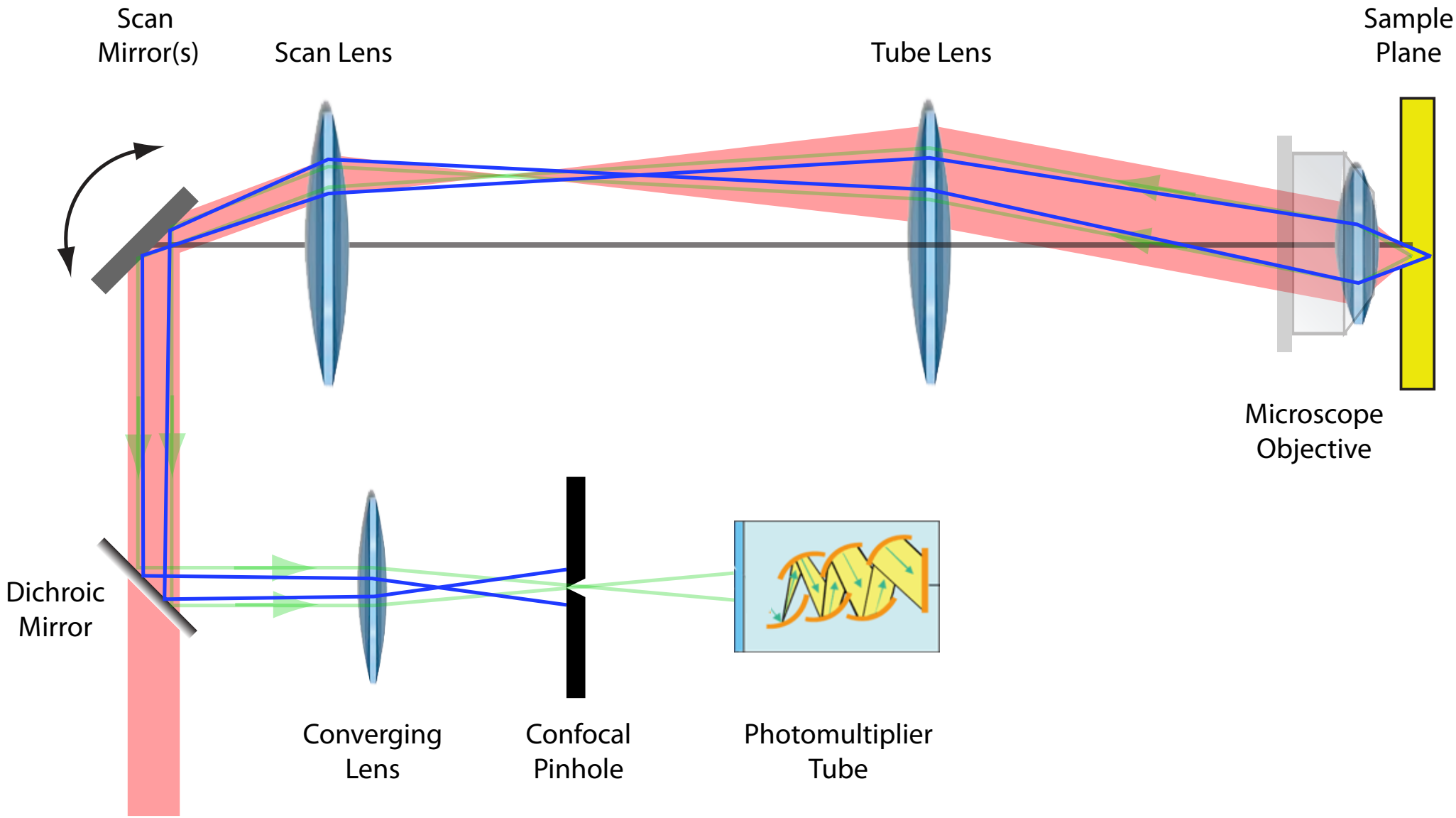
Confocal Laser Scanning Microscopy



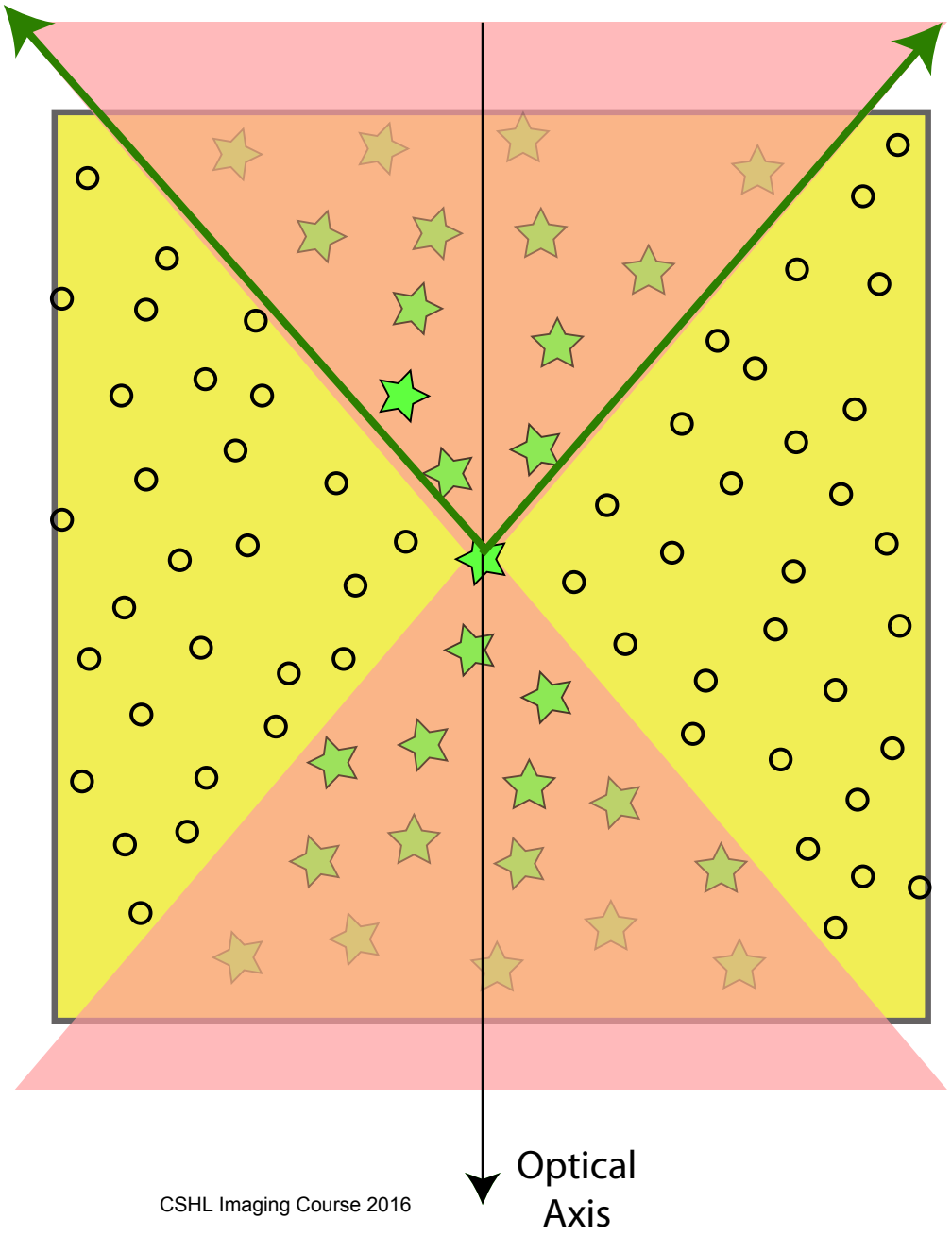
Confocal Laser Scanning Microscopy



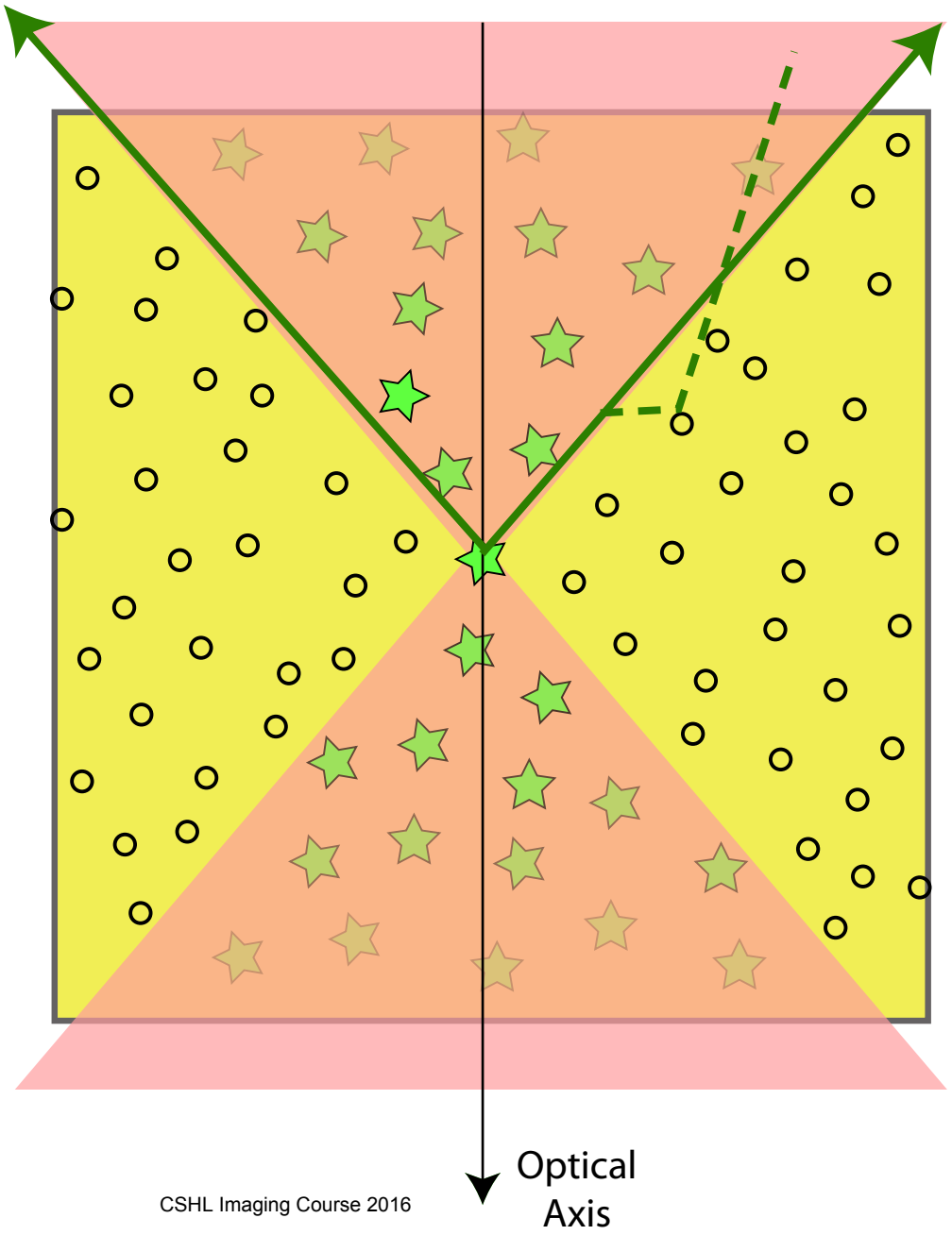
Confocal Laser Scanning Microscopy



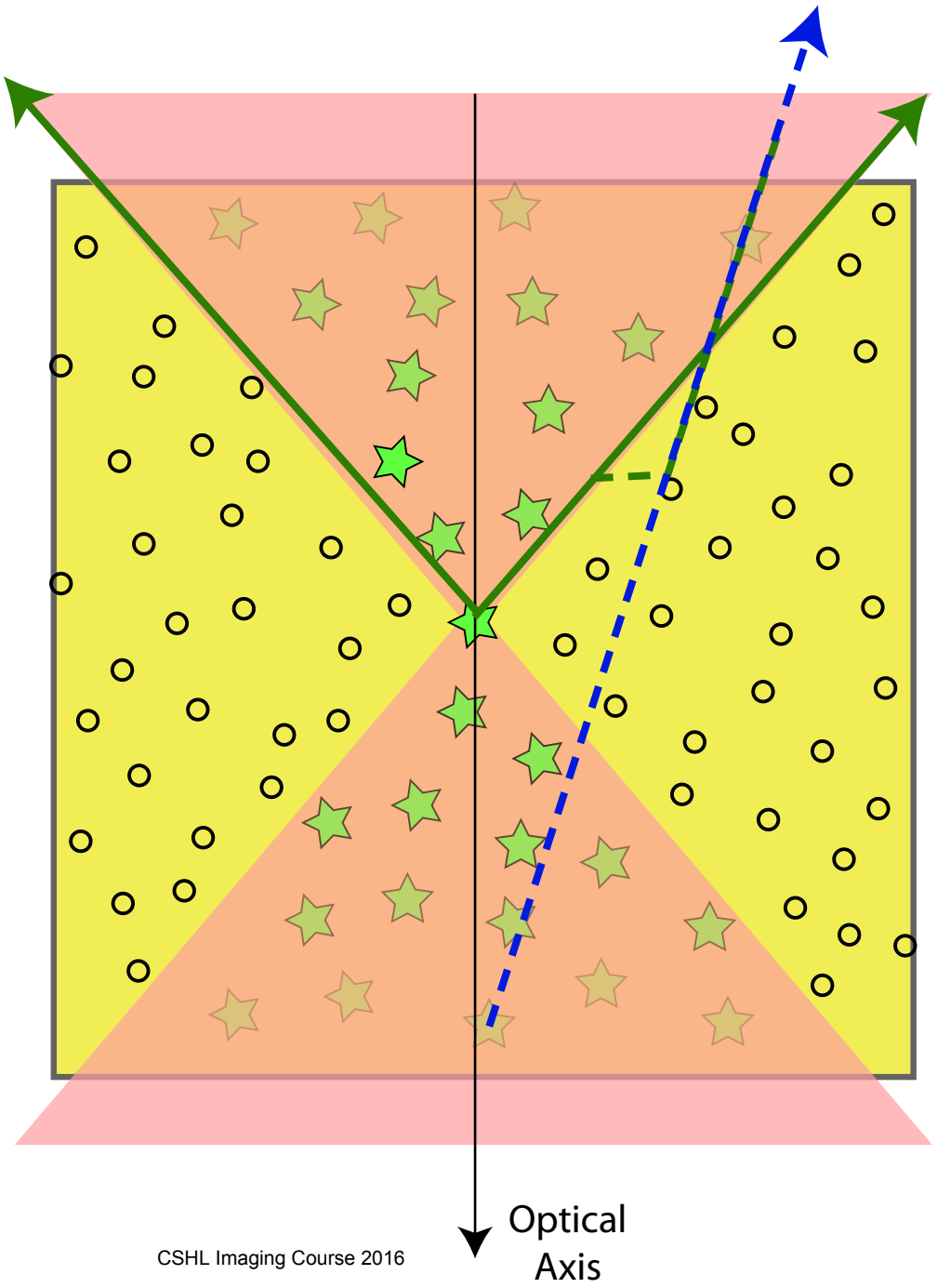
Confocal Laser Scanning Microscopy



Confocal Laser Scanning Microscopy



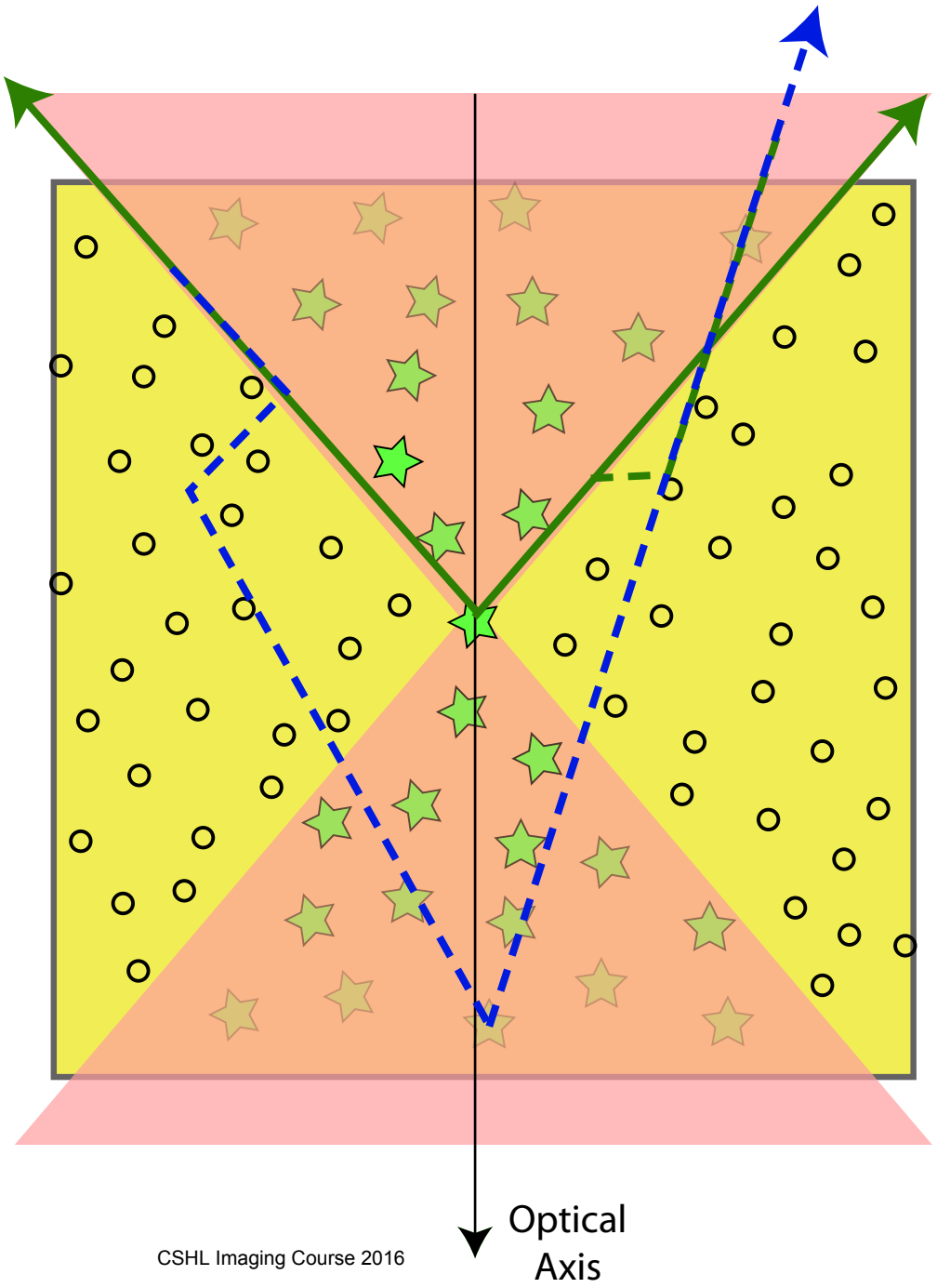
Confocal Laser Scanning Microscopy



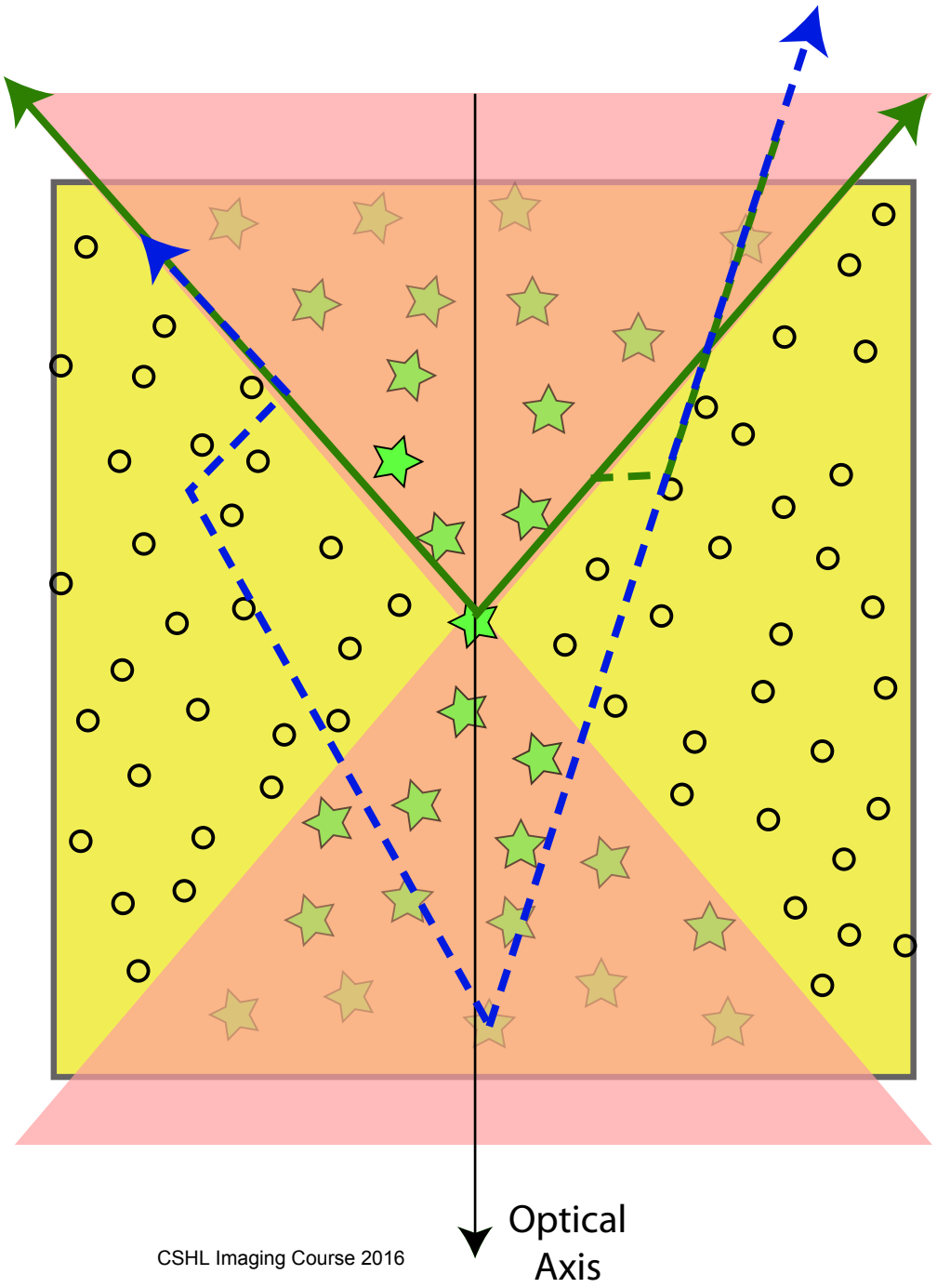
In-focus fluorescence can scatter into the same path as out-of-focus fluorescence, and be blocked by the confocal pinhole.

This decreases the signal reaching the detector.

Confocal Laser Scanning Microscopy



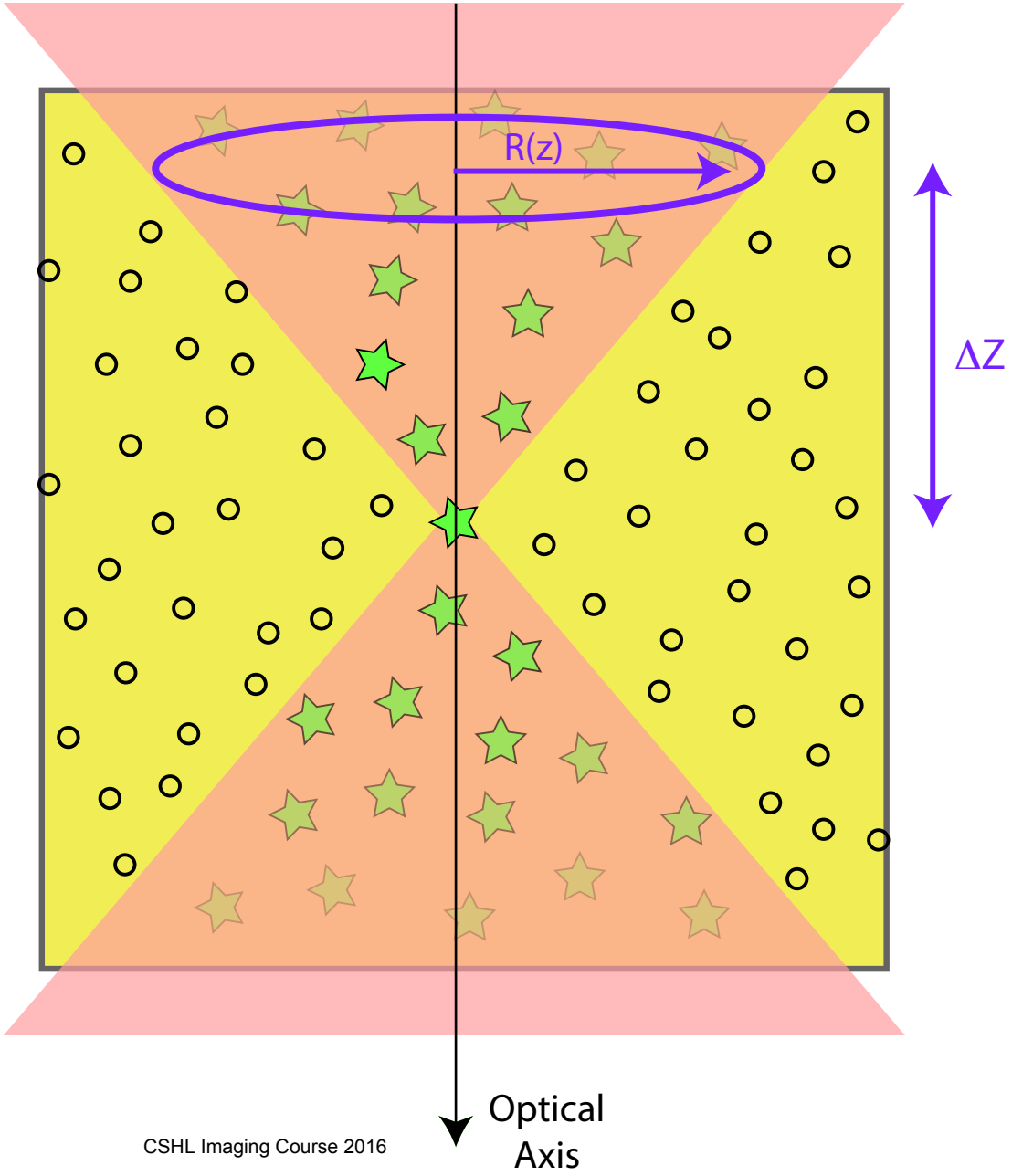
Confocal Laser Scanning Microscopy



Out-of-focus fluorescence can scatter into the same path as in-focus fluorescence, and pass through the confocal pinhole.

This increased background reduces the signal-to-noise ratio.

Two Photon Laser Scanning Microscopy



$R(Z)$ = Radius

A = Area

ΔZ = Distance from Focal Plane

I = Intensity

P = Power

F_m = Fluorescence per Molecule

σ = Dye Cross-section

F_p = Fluorescence per Plane

TWO PHOTON EXCITATION

$R(z) \sim \Delta Z$

$A \sim R^2 \sim (\Delta Z)^2$

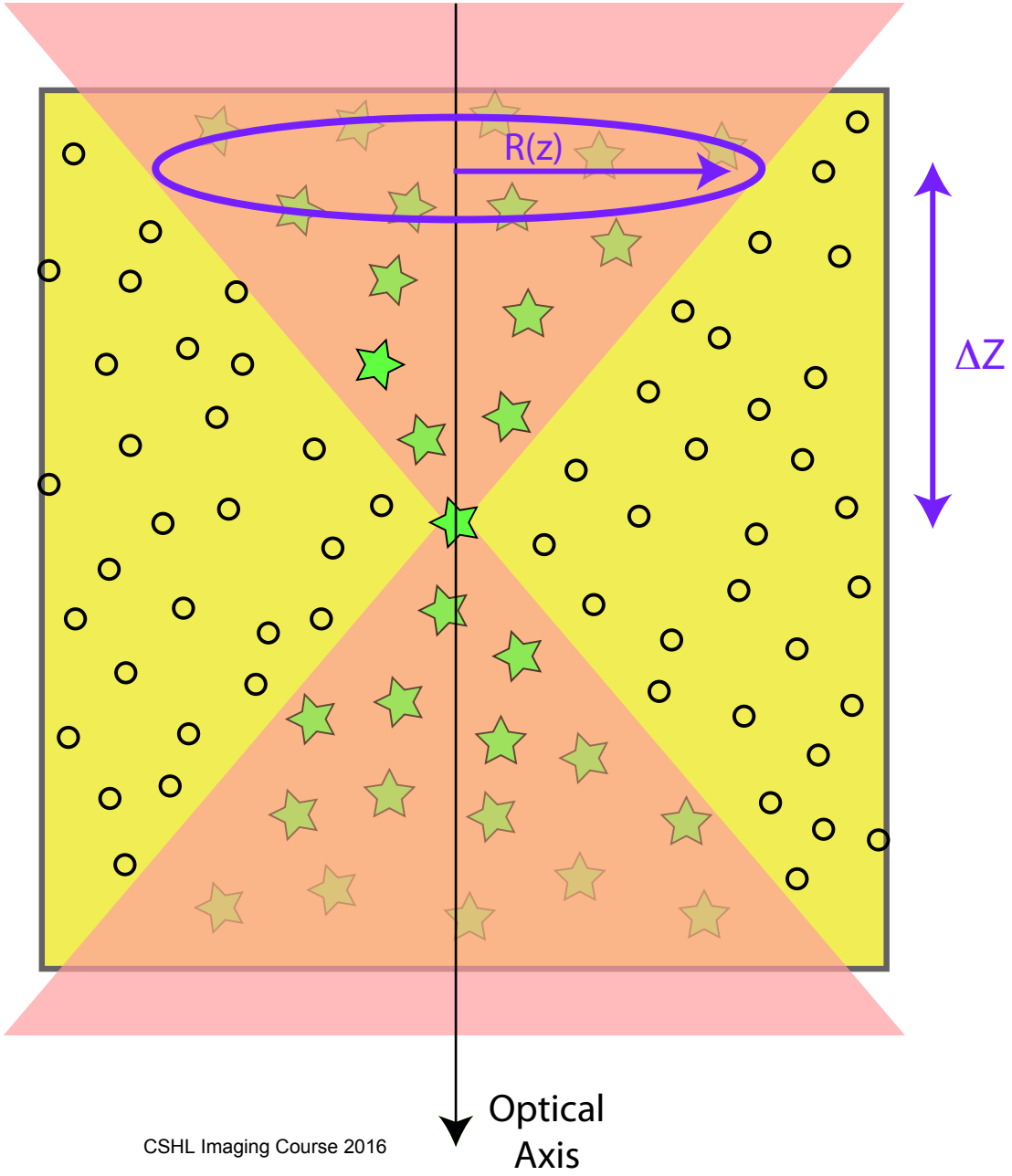
$I = P / A$

$F_m = \sigma * I^2 = \sigma * P^2 / A^2 = \sigma * P^2 / (\Delta Z)^4$

$F_p = F_m * A = \sigma * P^2 / A \sim \sigma * P^2 / (\Delta Z)^2$

For two photon excitation, the fluorescence per plane falls quadratically with distance from the focal plane !

Confocal Laser Scanning Microscopy



R(Z) = Radius

A = Area

ΔZ = Distance from Focal Plane

I = Intensity

P = Power

F_m = Fluorescence per Molecule

σ = Dye Cross-section

F_p = Fluorescence per Plane

SINGLE PHOTON EXCITATION

$R(z) \sim \Delta Z$

$A \sim R^2 \sim (\Delta Z)^2$

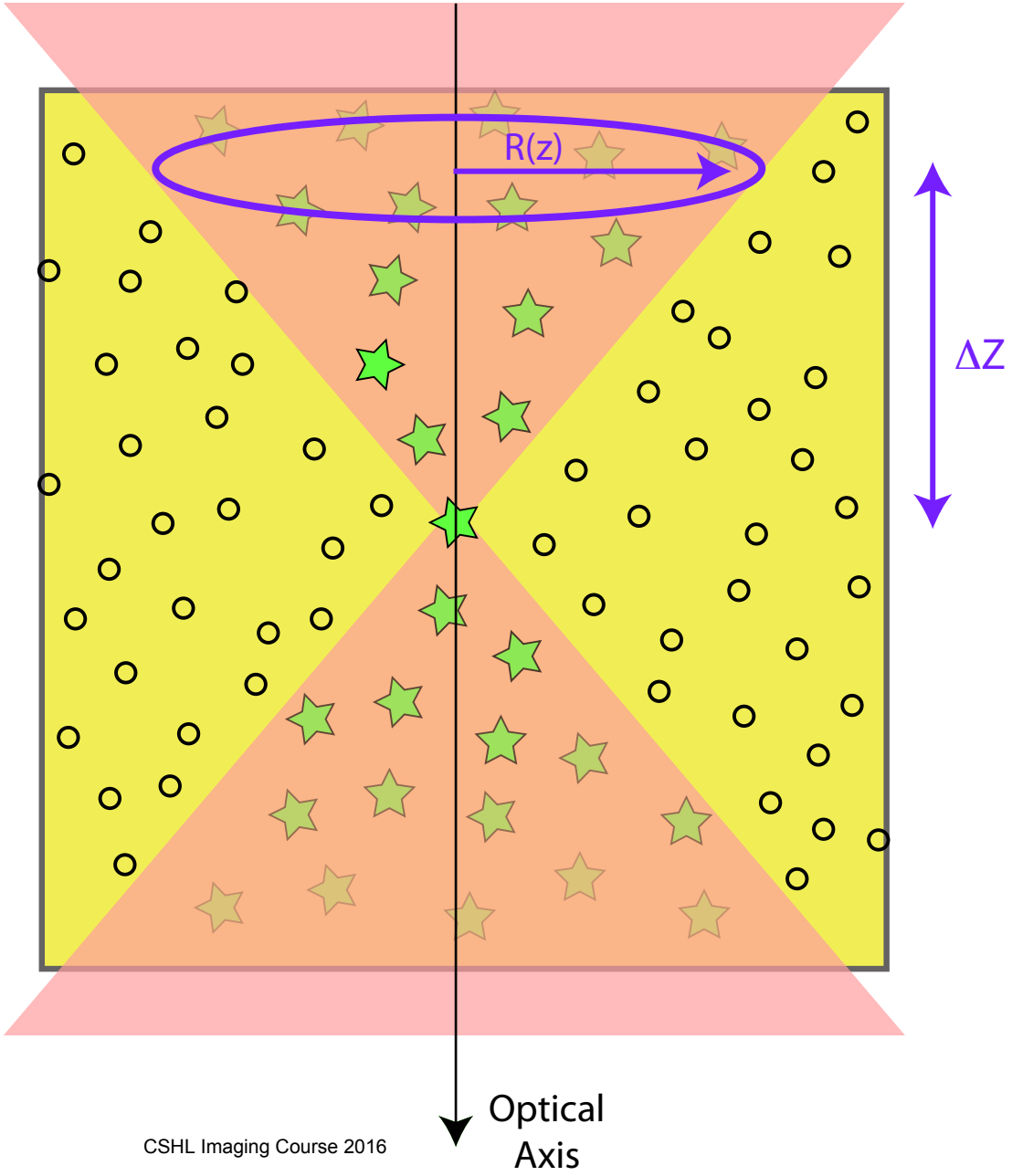
$I = P / A$

$F_m = \sigma * I = \sigma * P/A = \sigma * P/(\Delta Z)^2$

$F_p = F_m * A = \sigma * P$

For single photon excitation, the fluorescence per plane is independent of distance from the focal plane !

Two Photon Laser Scanning Microscopy



$R(Z)$ = Radius

A = Area

ΔZ = Distance from Focal Plane

I = Intensity

P = Power

F_m = Fluorescence per Molecule

σ = Dye Cross-section

F_p = Fluorescence per Plane

TWO PHOTON EXCITATION

$R(z) \sim \Delta Z$

$A \sim R^2 \sim (\Delta Z)^2$

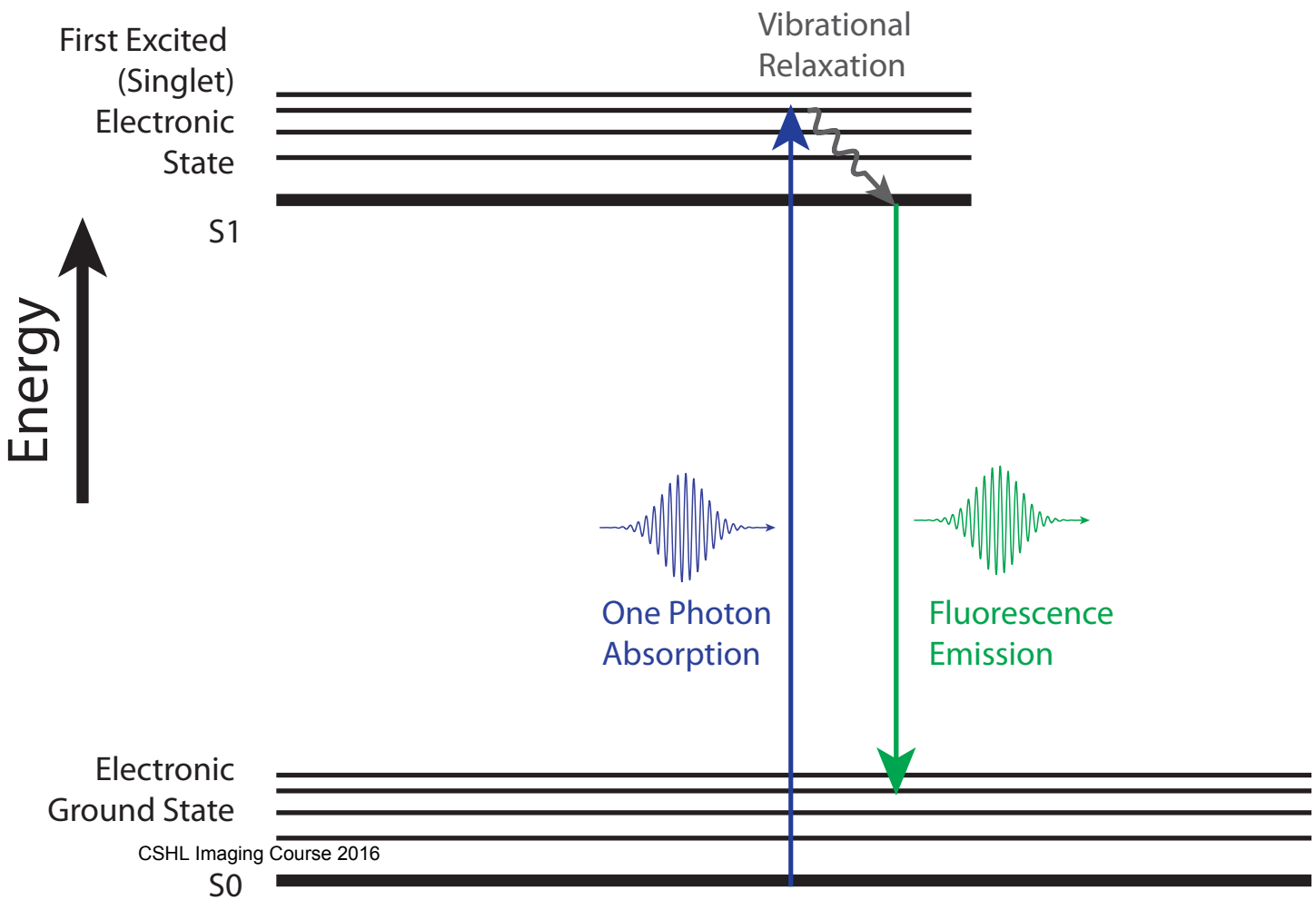
$I = P / A$

$F_m = \sigma * I^2 = \sigma * P^2 / A^2 = \sigma * P^2 / (\Delta Z)^4$

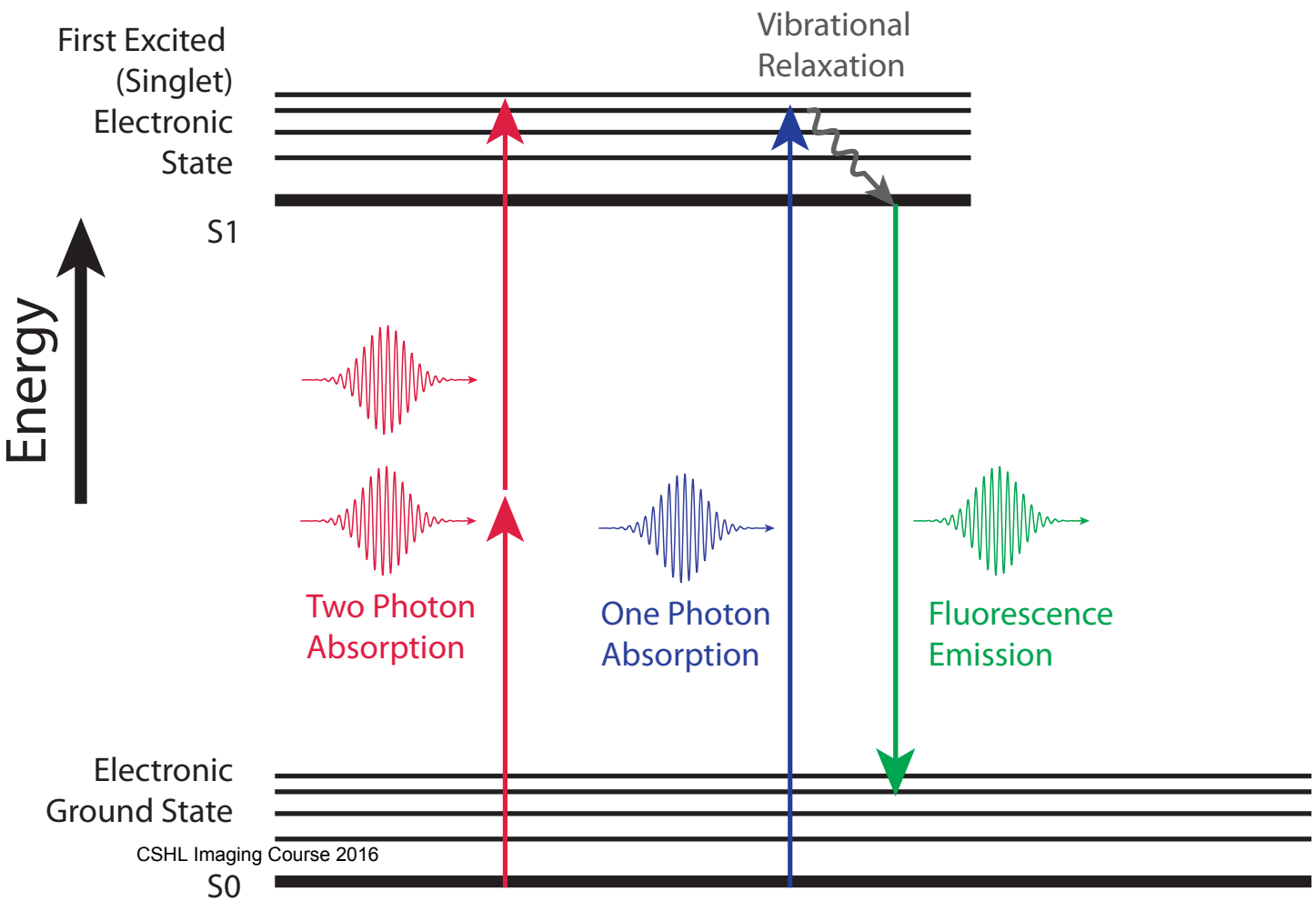
$F_p = F_m * A = \sigma * P^2 / A \sim \sigma * P^2 / (\Delta Z)^2$

For two photon excitation, the fluorescence per plane falls quadratically with distance from the focal plane !

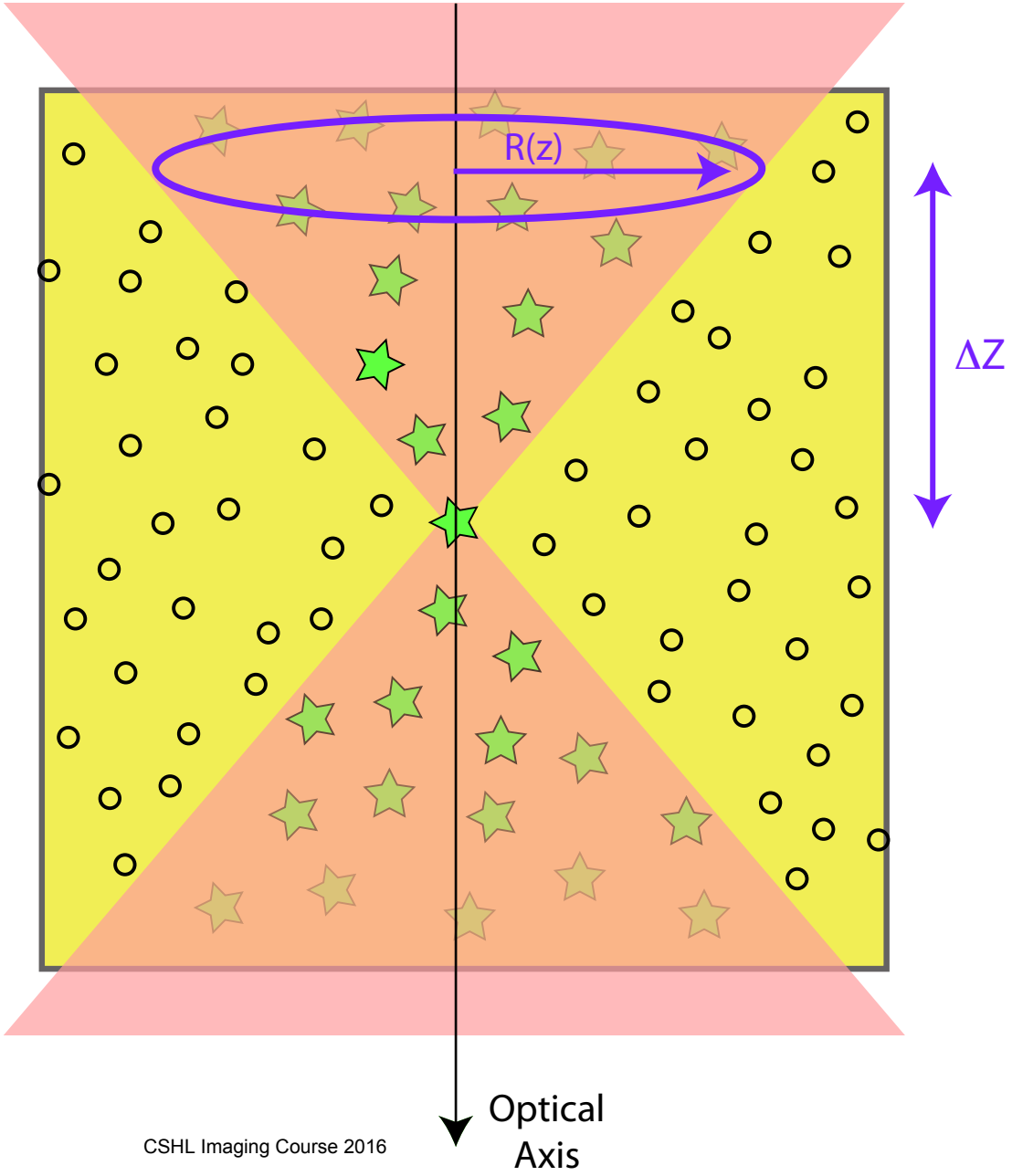
Jablonski Energy Diagram



Jablonski Energy Diagram



Two Photon Laser Scanning Microscopy



$R(Z)$ = Radius

A = Area

ΔZ = Distance from Focal Plane

I = Intensity

P = Power

F_m = Fluorescence per Molecule

σ = Dye Cross-section

F_p = Fluorescence per Plane

TWO PHOTON EXCITATION

$R(z) \sim \Delta Z$

$A \sim R^2 \sim (\Delta Z)^2$

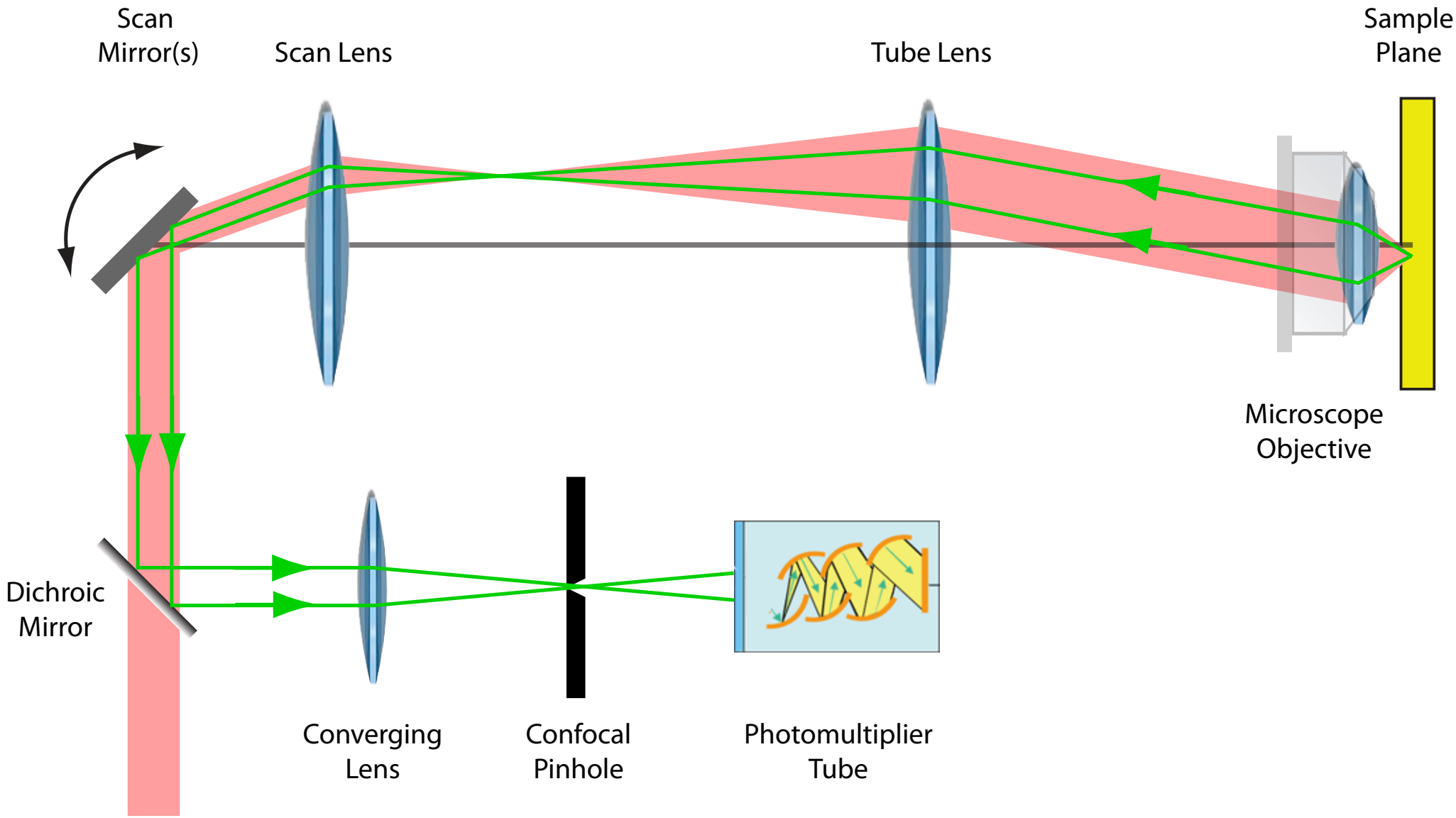
$I = P / A$

$F_m = \sigma * I^2 = \sigma * P^2 / A^2 = \sigma * P^2 / (\Delta Z)^4$

$F_p = F_m * A = \sigma * P^2 / A \sim \sigma * P^2 / (\Delta Z)^2$

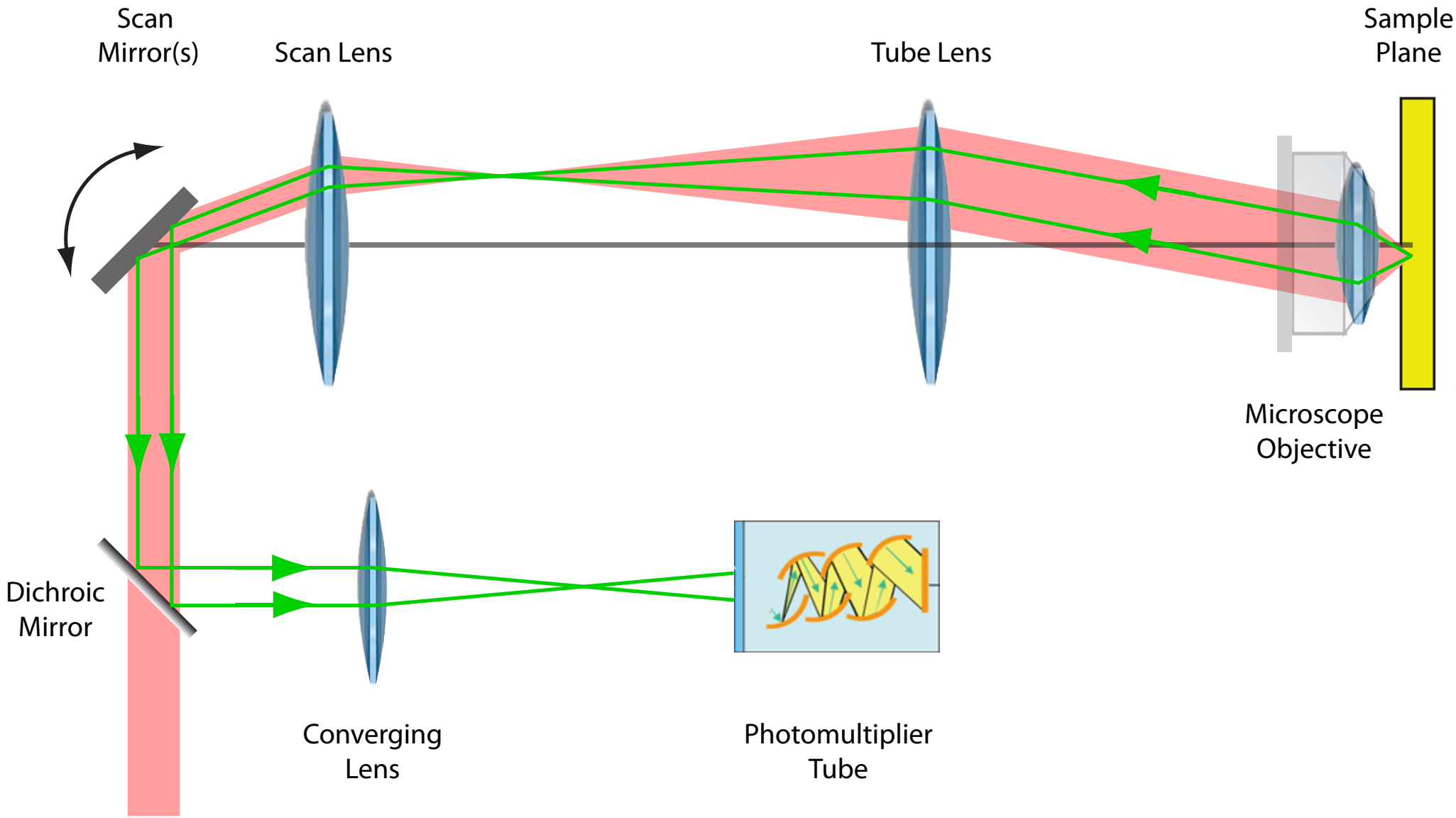
For two photon excitation, the fluorescence per plane falls quadratically with distance from the focal plane !

Confocal Laser Scanning Microscopy



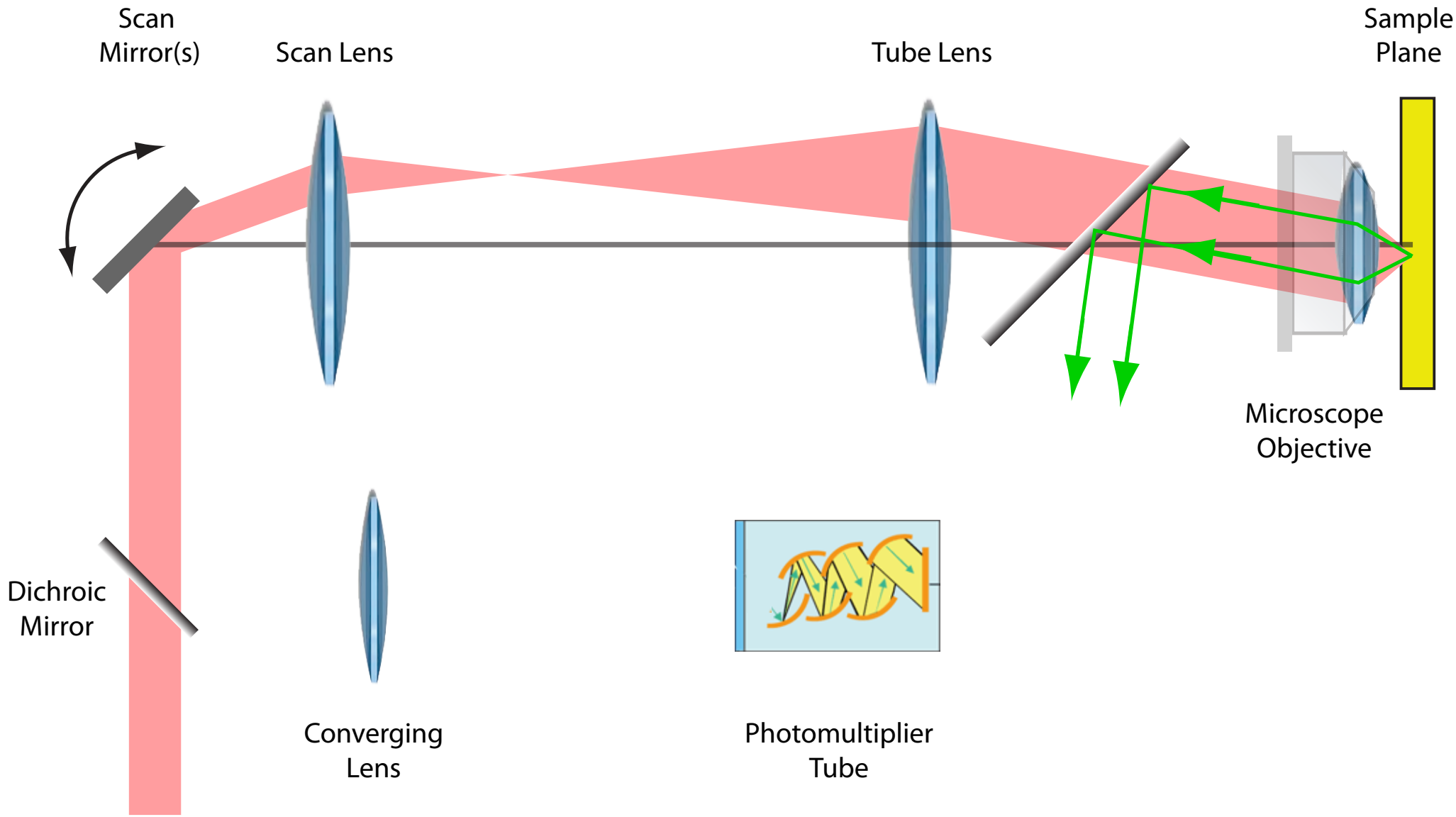
~~Confocal~~ Laser Scanning Microscopy

Two Photon



~~Confocal~~ Laser Scanning Microscopy

Two Photon



~~Confocal~~ Laser Scanning Microscopy

Two Photon