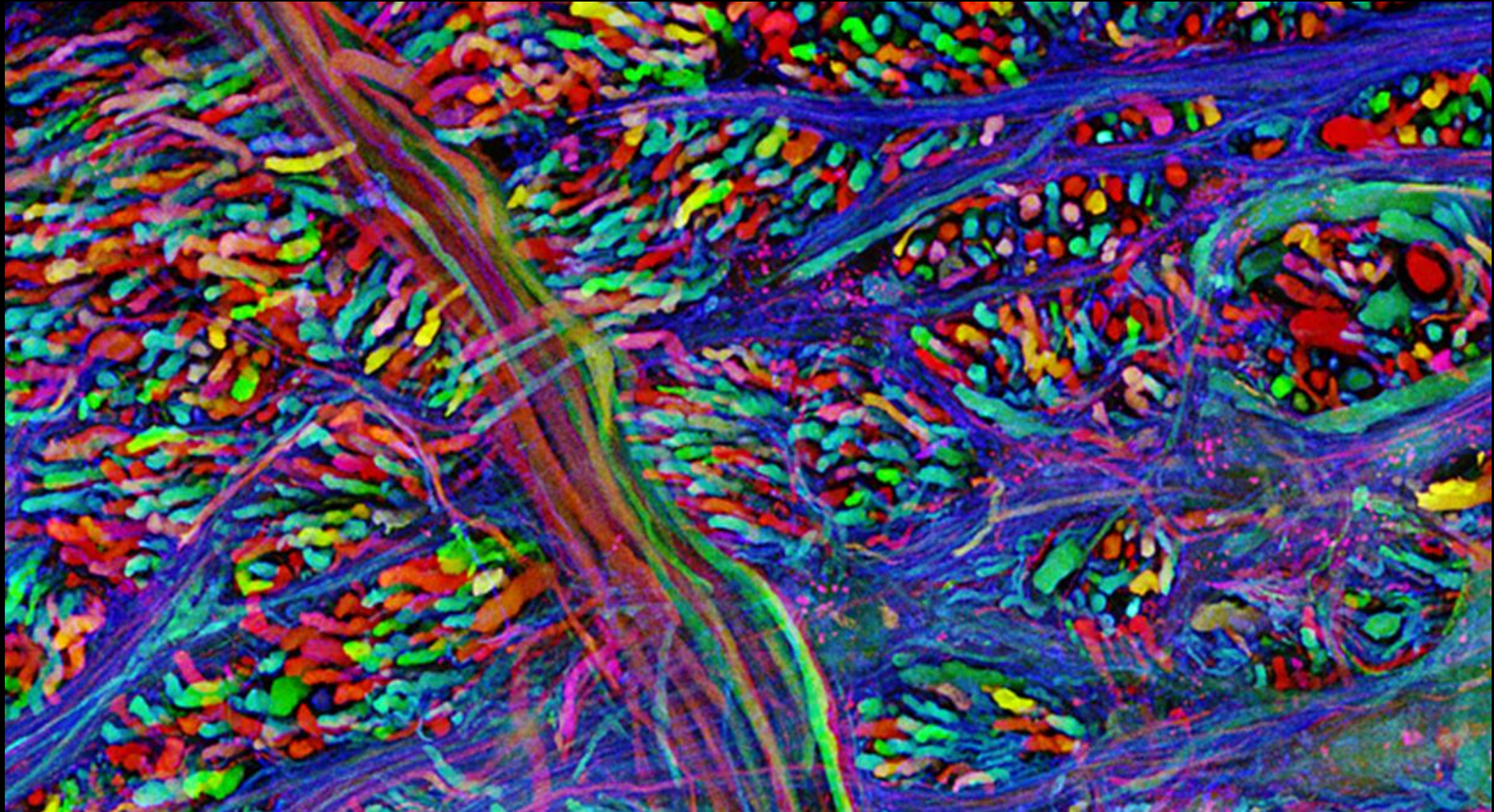


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Your Vision, Our Future



# OLYMPUS

Your Vision, Our Future

## Introduction to Laser Scanning Microscopy NRC CARSLab Course



November 30, 2010  
Olympus Canada  
Andrew Millar

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# BENEFITS OF LASER SCANNING MICROSCOPY

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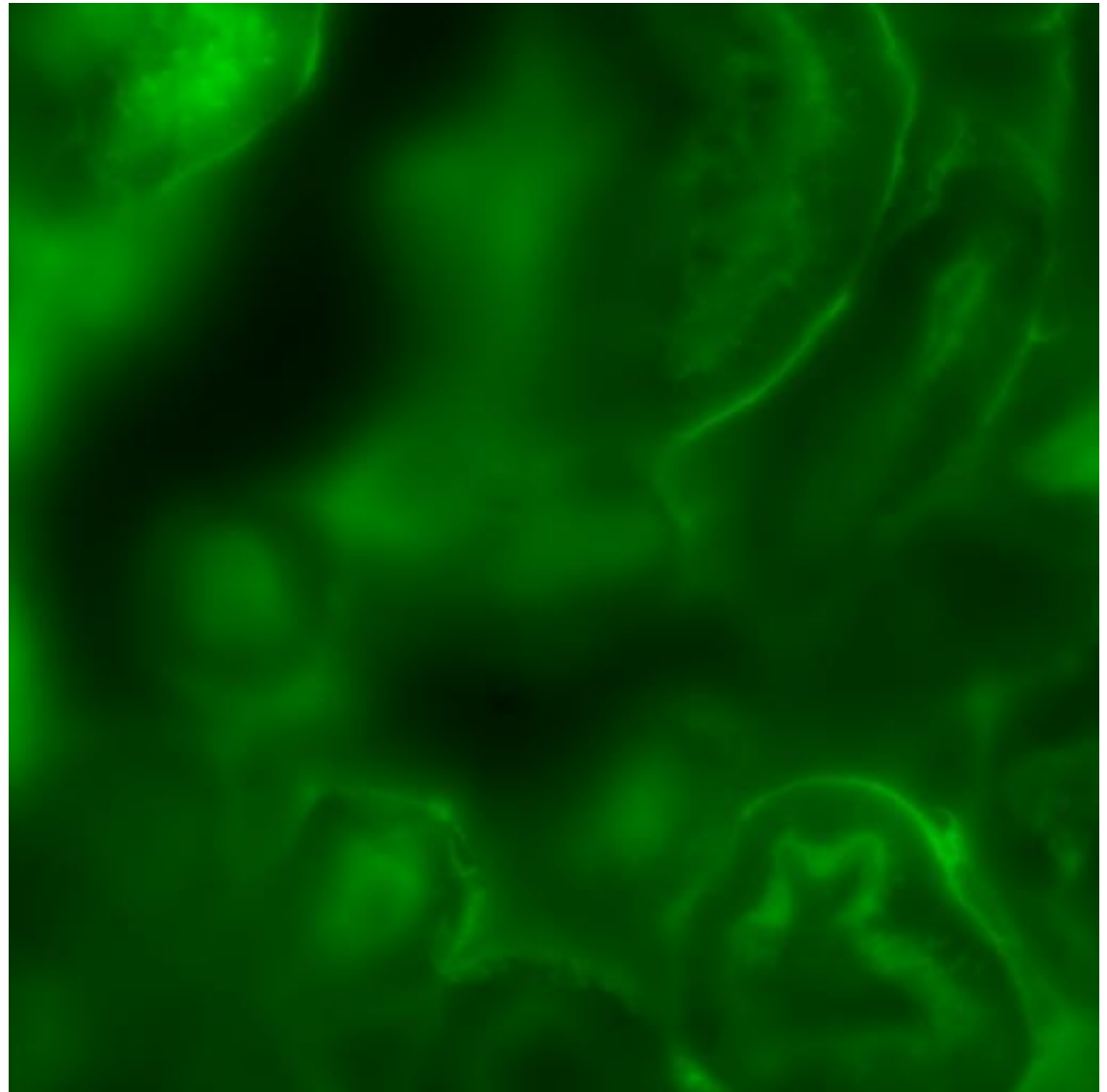
# IMPROVE AXIAL (Z) RESOLUTION

# Improve Z Resolution

## Widefield Fluorescence

- Note blurriness
- Optical section includes 10s of microns of out of focus blur
- Samples thicker than  $\sim 3$   $\mu\text{m}$  will have visible blur – tissue sections, thick cells, in vivo preparations
- This impairs viewing of IN focus data

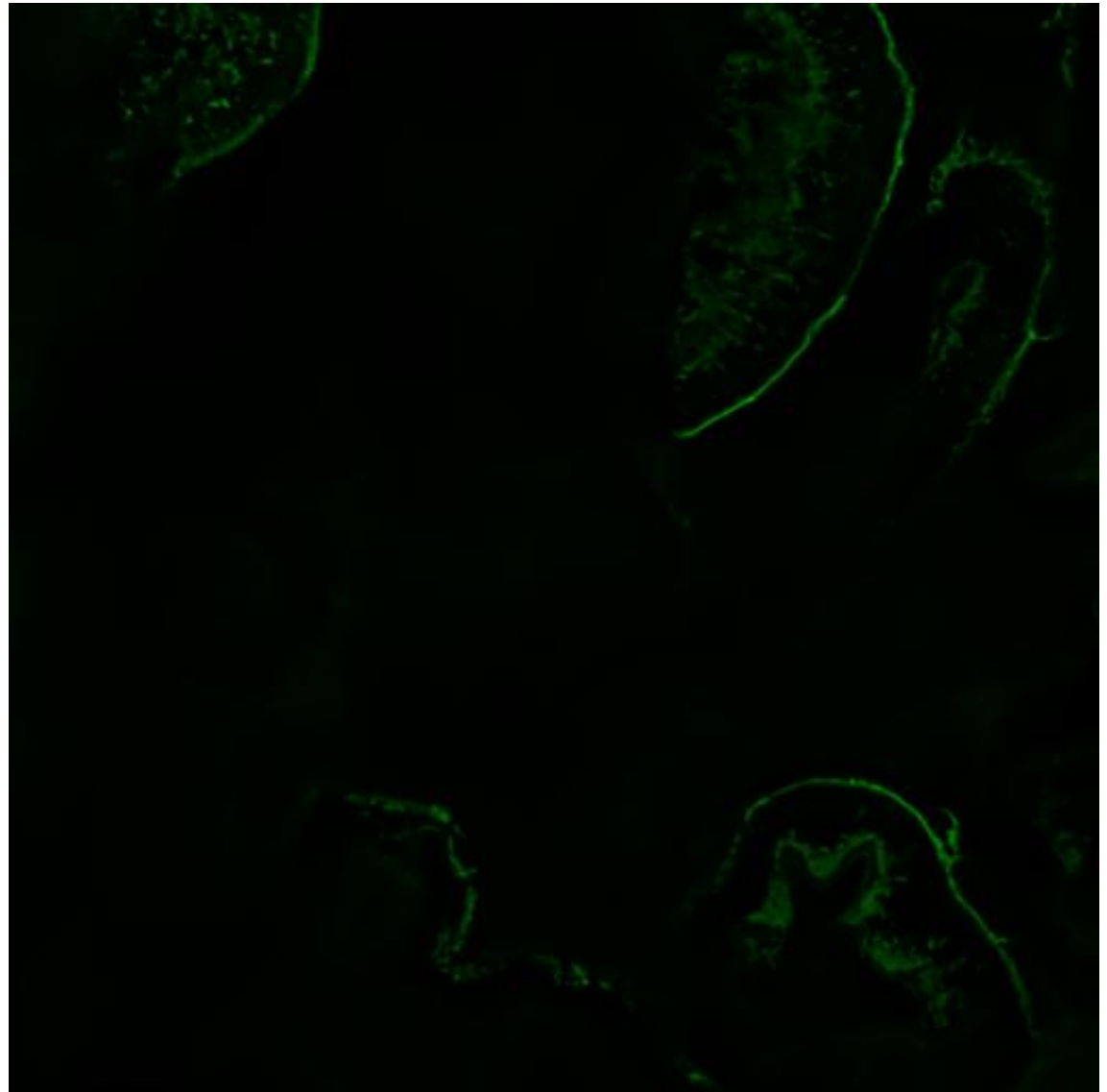
Molecular Probes Kidney Slide  
60x SApo Objective



# Improve Z Resolution

## Confocal Fluorescence

- Note dramatic reduction in out of focus blur
- Optical section is now less than 1  $\mu\text{m}$ , with no out of focus light
- In focus data is clearly visible

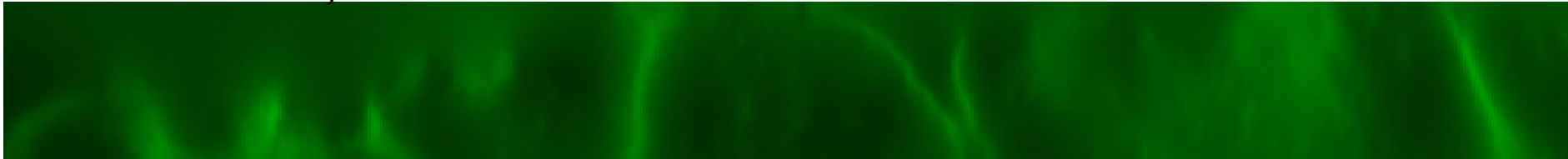


Molecular Probes Kidney Slide  
60x SApo Objective

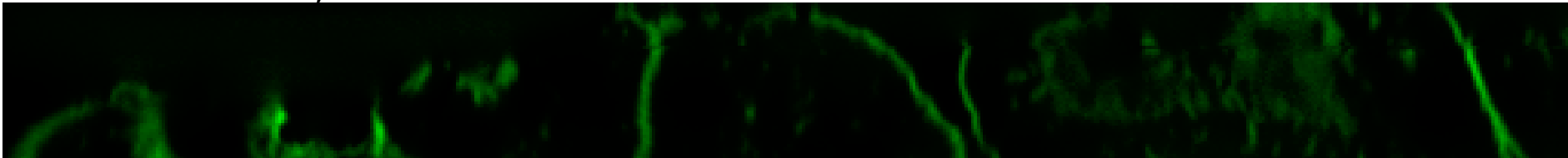
# Improve Z Resolution

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Widefield XZ Projection



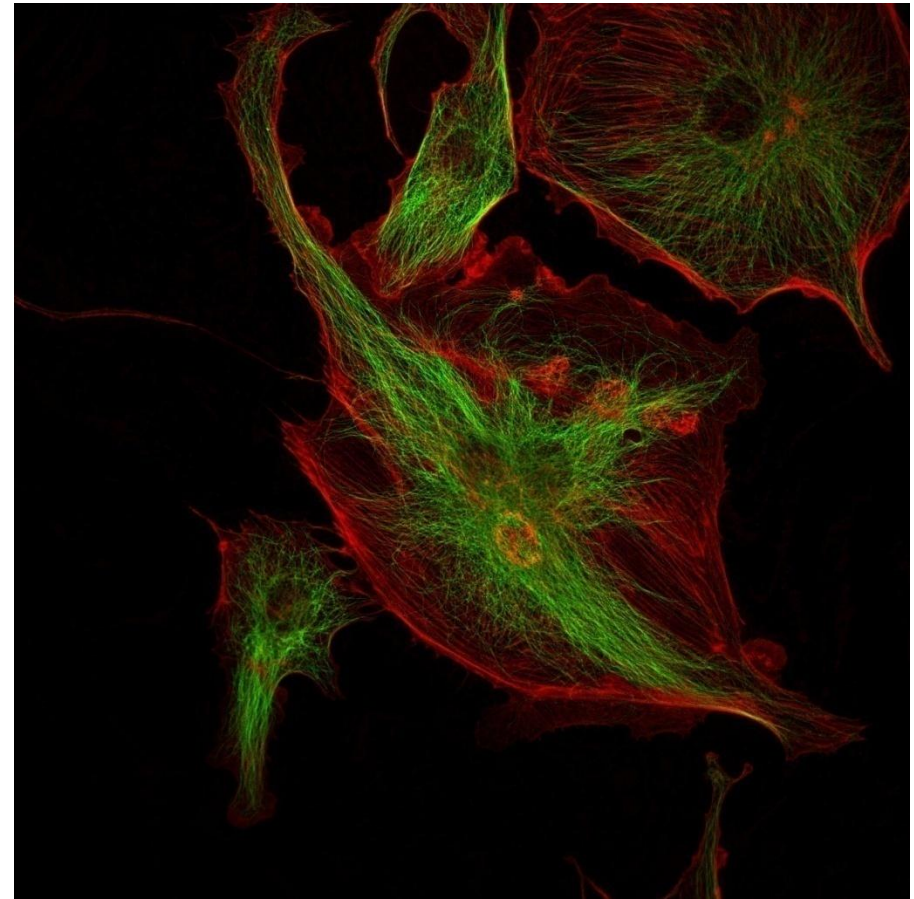
Confocal XZ Projection



# Improve Z Resolution

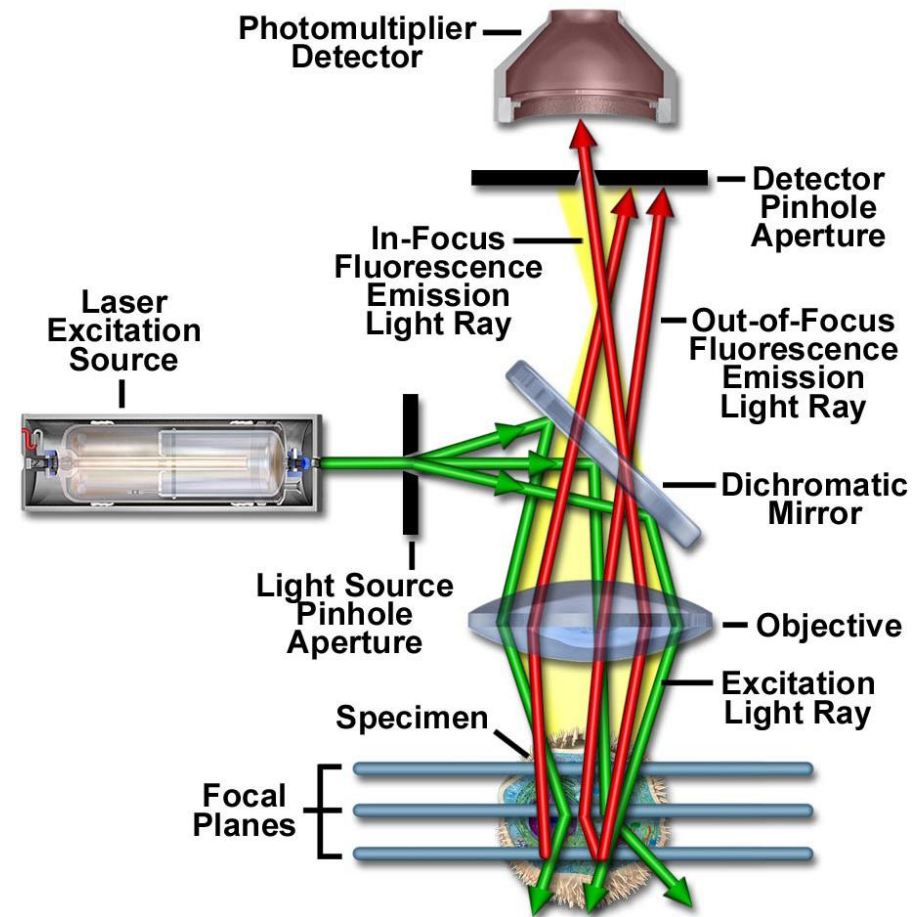
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- This is the primary reason *and* requirement for confocal imaging
- Confocal is still the best technique for imaging thick samples with high resolution in XY and Z
- Z resolution of  $\sim 0.5 \mu\text{m}$ , and can image 50-100  $\mu\text{m}$  into tissue
  - ▶ MPE can image 1 mm into tissue!!



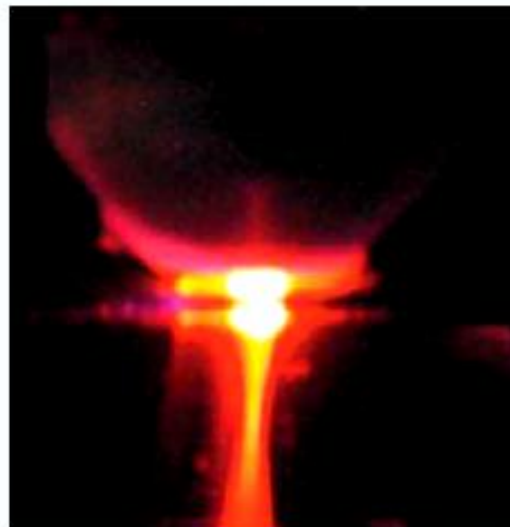
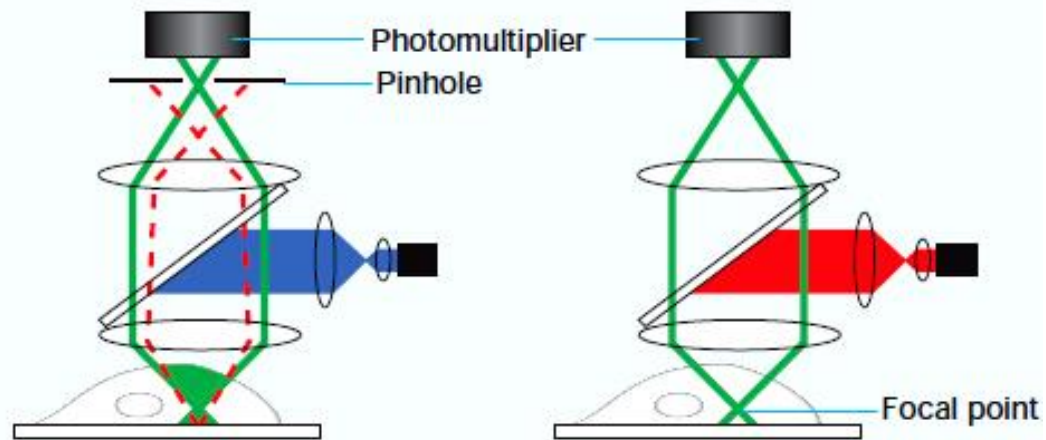
# Improve Z Resolution – Confocal Pinhole

- A laser scanning confocal microscope uses a pinhole to reject out of focus light
- It's size and position is automatically adjusted based on the objective lens, dichroic mirror, and wavelength
- It can be opened or closed to increase or decrease z resolution



# Improve Z Resolution – Multiphoton Excitation

- Multiphoton excitation occurs only at focal point
- No need to use a pinhole to reject out of focus emitted light
- No out of focus light occurs – capture all light with non-descanned detector



Single photon image



Multi photon image

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# SIMULTANEOUS MULTI-LABEL / MULTI-MODAL IMAGING

# Multi-Label / Multi-Modal Imaging

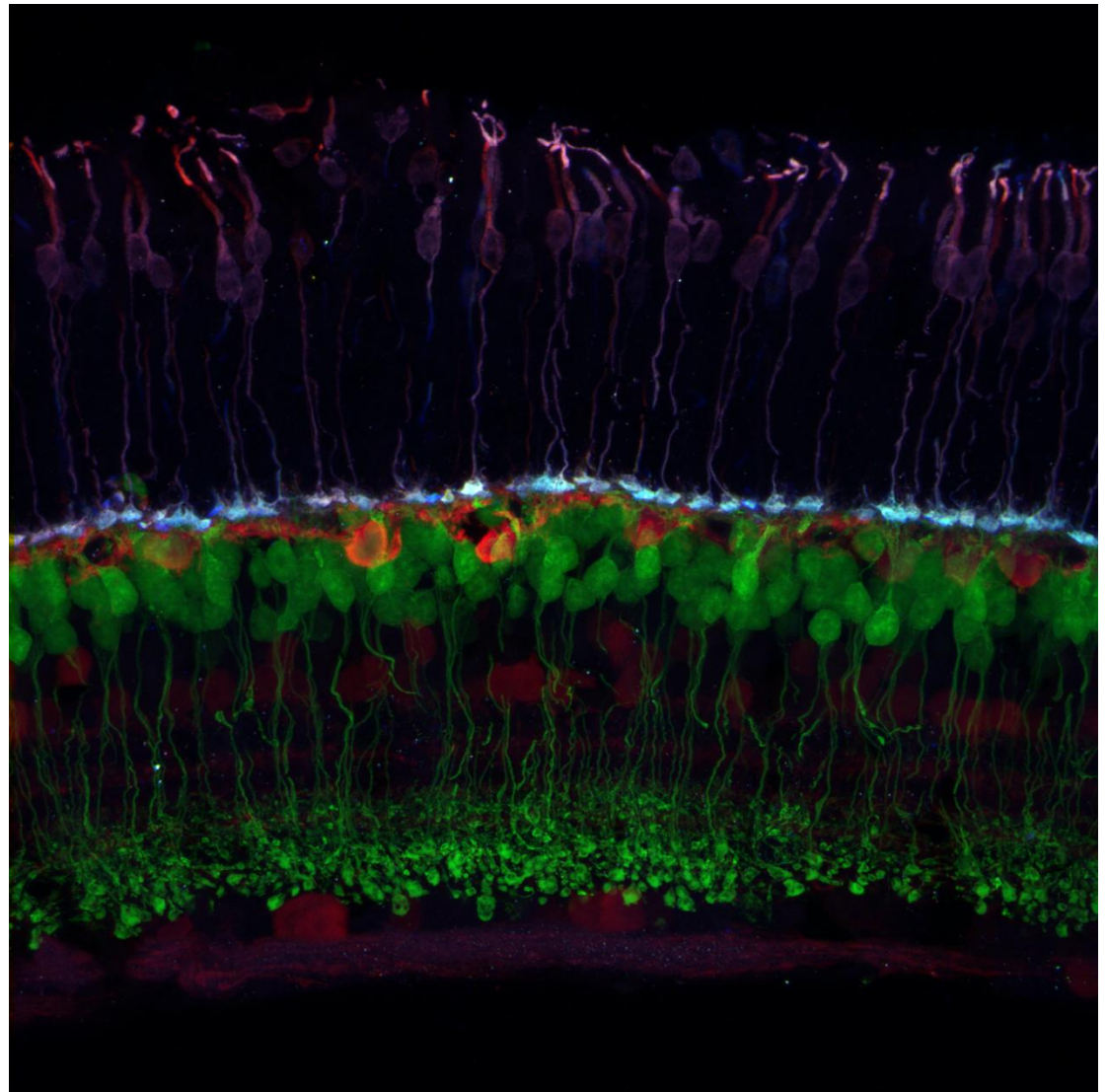
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## Multiple Labels

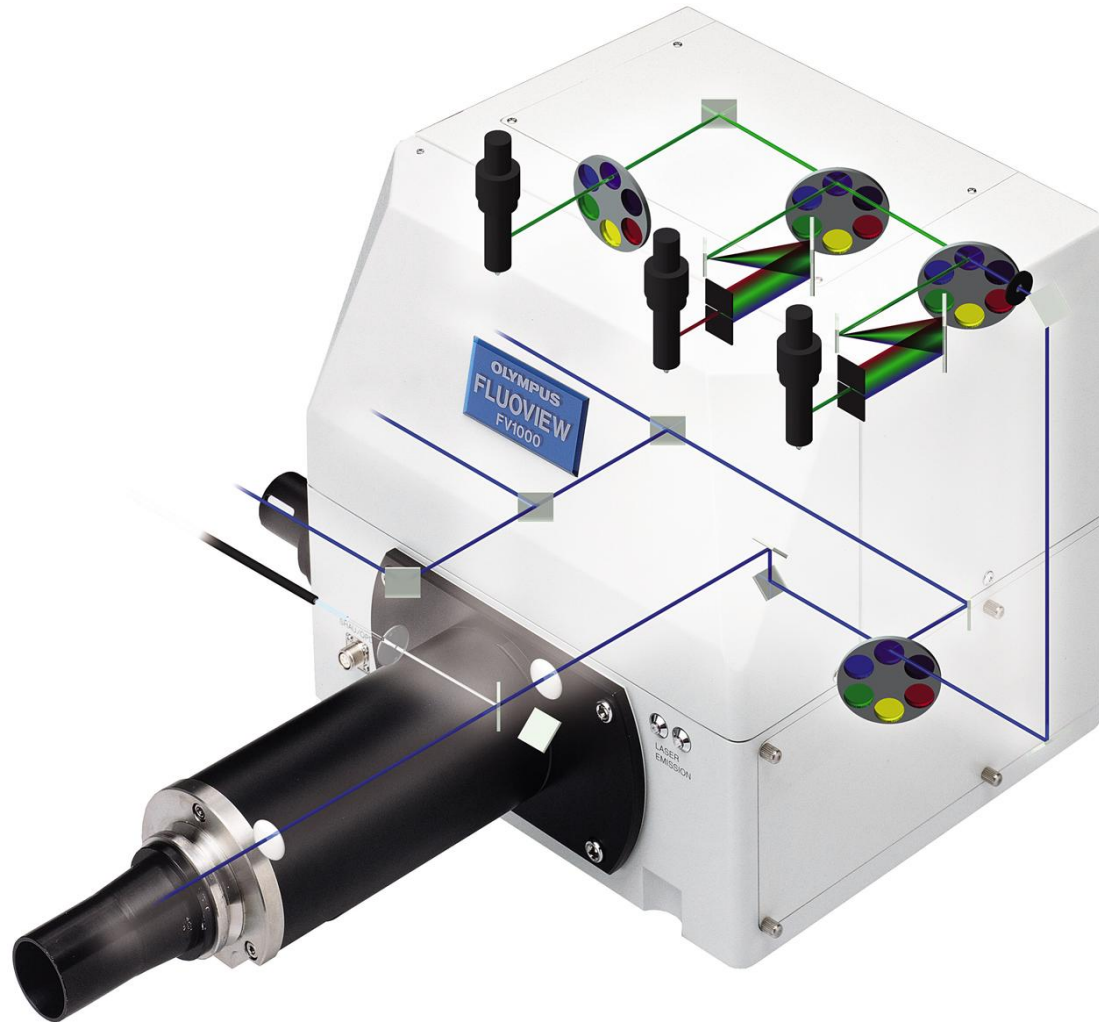
- Used to differentially label specific cellular components or proteins
- 2 or 3 labels (maybe 4)
- DAPI/FITC/TRITC -or similar
- GFP / mCherry
- MPE / SHG/ CARS

## Avoid “Bleedthrough”

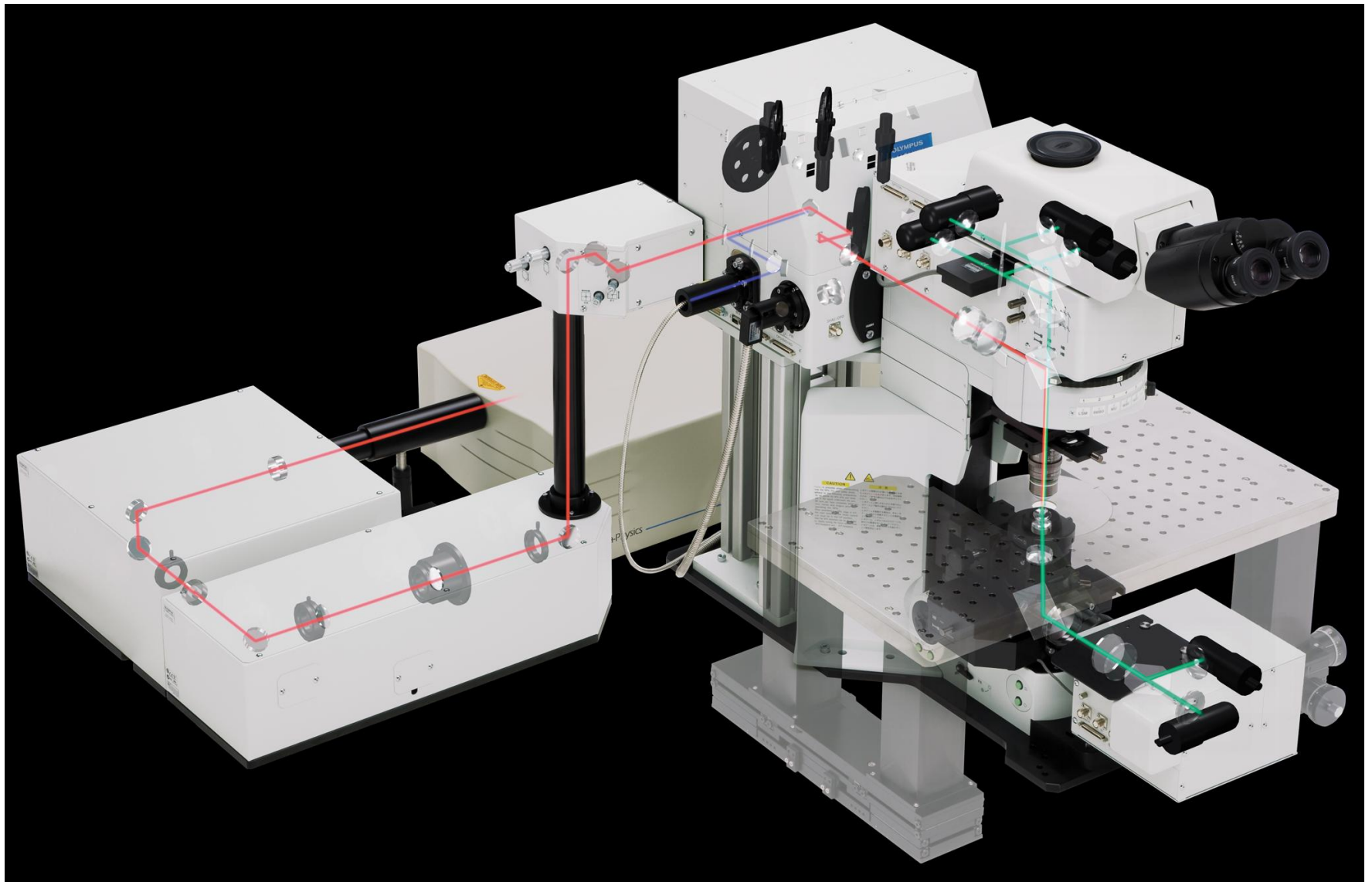
- Spectral Imaging
- Line Sequential



# Detector Arrangement - Confocal



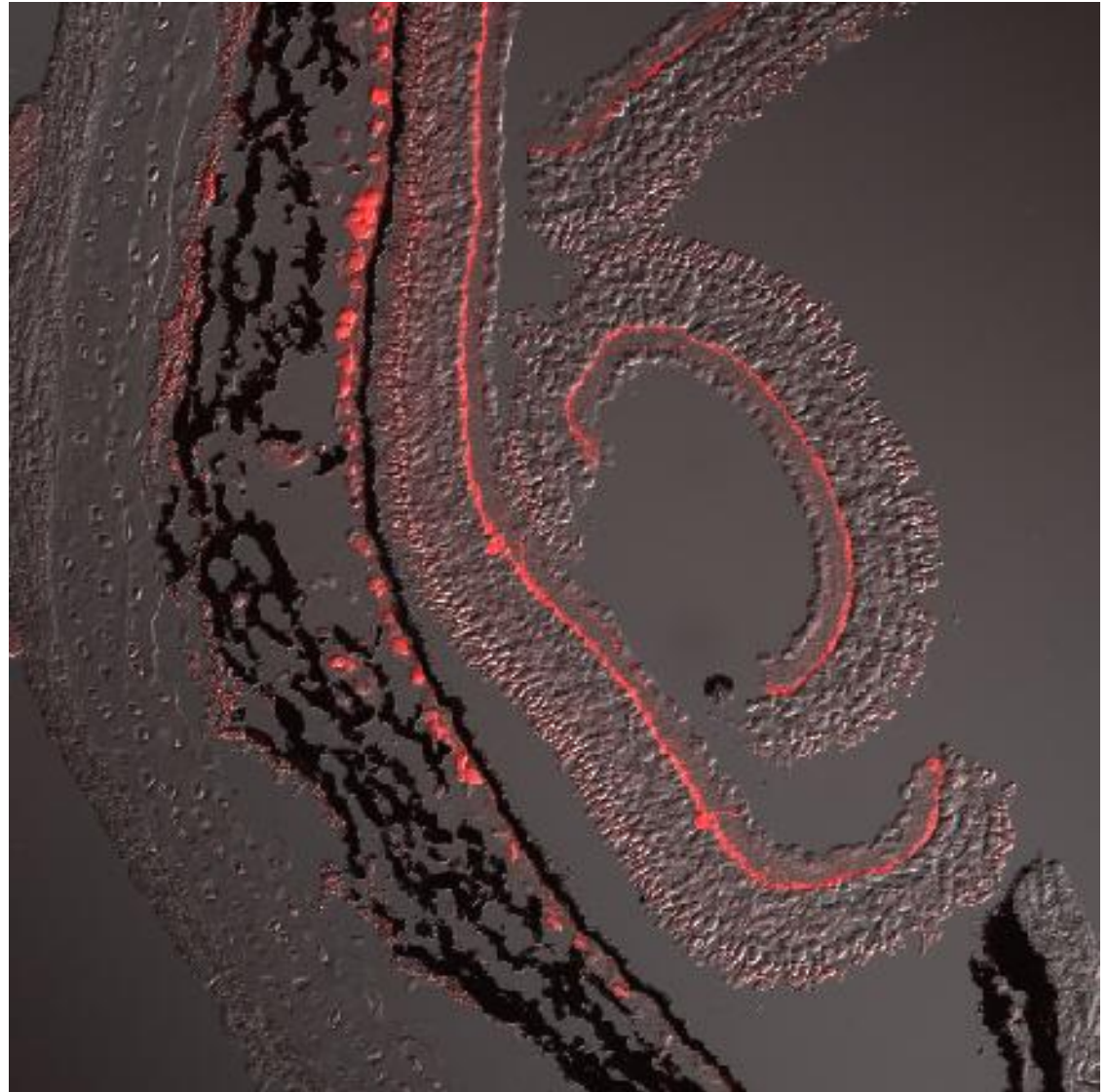
# Detector Arrangement - Multiphoton



# Multi-Label Imaging – Transmitted Light

## Simultaneous detection of DIC / Brightfield

- A transmitted light detector acts in unison with fluorescence detectors
- Captures registered DIC image for context



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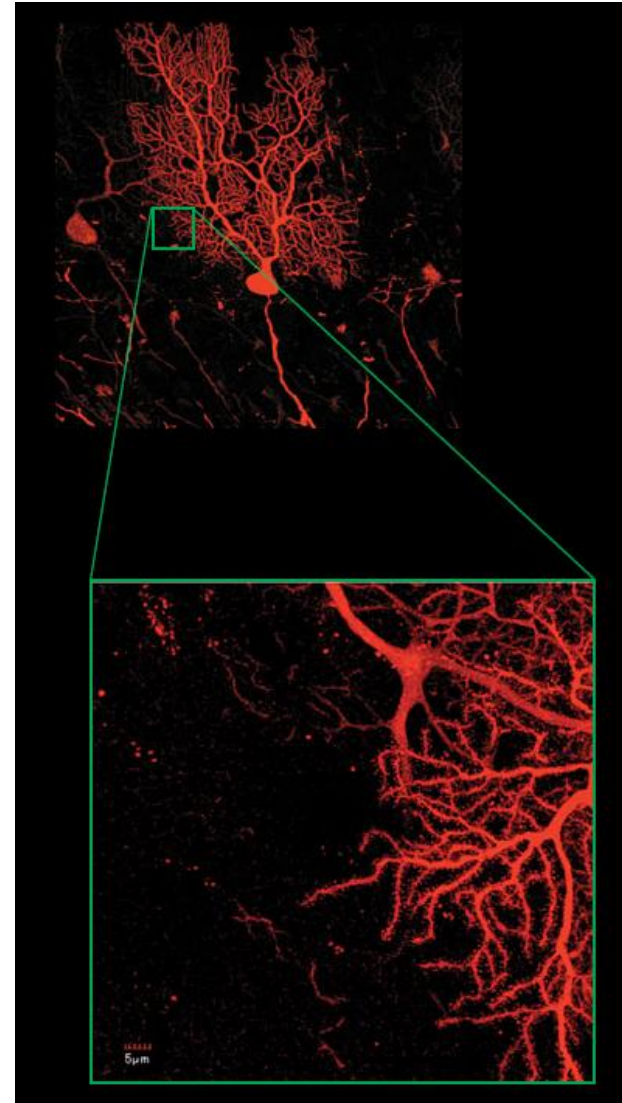
# FLEXIBLE SPATIAL AND TEMPORAL RESOLUTION

# Flexible Resolution

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Most researchers have various magnification and speed requirements

- Need the ability to optimally image their sample while using different objectives (Nyquist sampling)
- Need the flexibility to zoom into areas of interest
- Need to balance speed / resolution / sensitivity



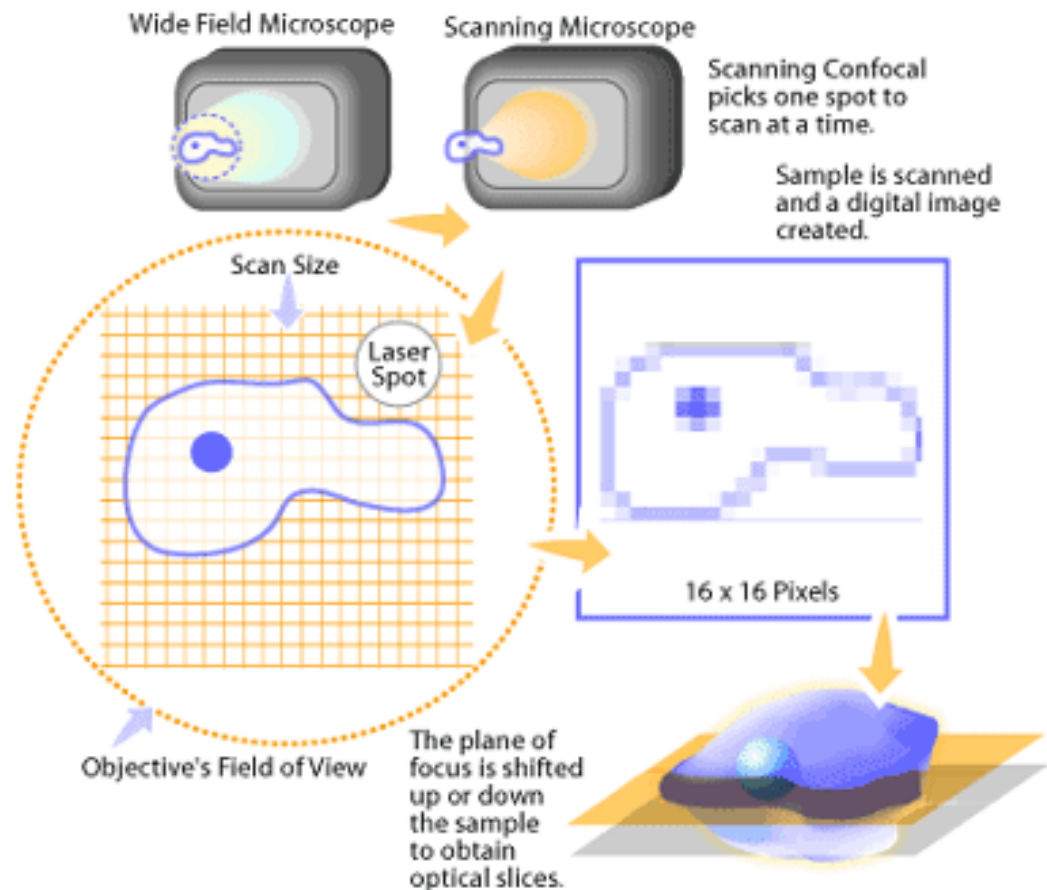
# Flexible XY Resolution

## Widefield Microscope

- Captures the image in one shot with a camera
- Limited to the size and number of pixels on camera
- Fast, but lacks flexibility

## Scanning Microscope

- Laser scans across sample, one pixel at a time
- Image is then generated
- Can adjust number and size of pixels scanned
- This allows optimal sampling (pixel size) at a variety of magnifications and speeds
- Also allows zooming – scanning the same number of pixels across a smaller area



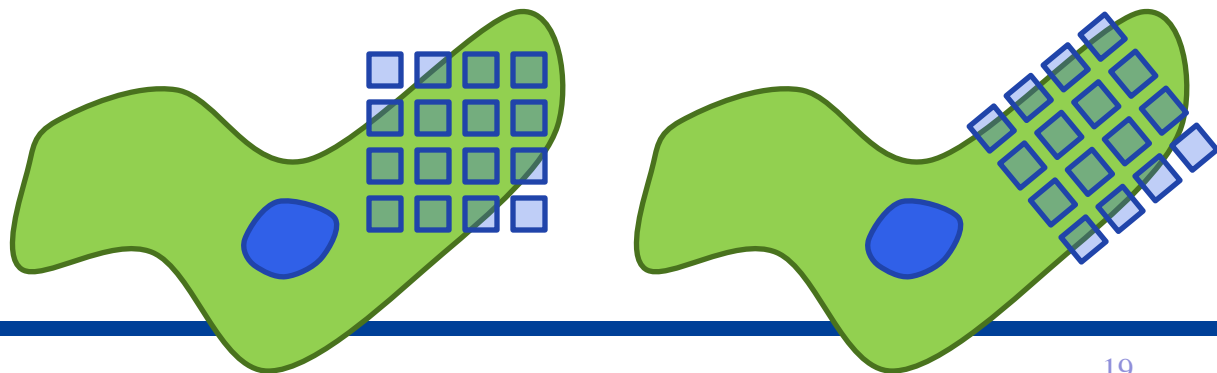
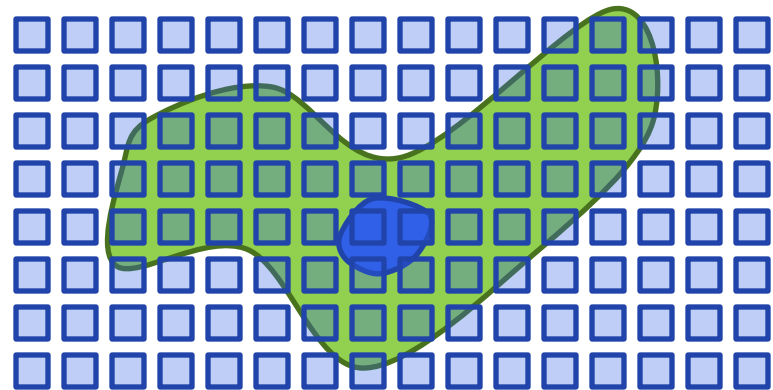
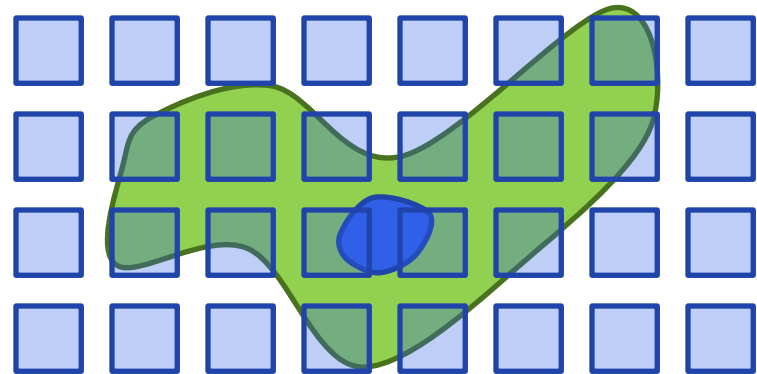
# Resolution Flexibility - Variable Pixel Size

Standard Scan – balance between speed and resolution

High Resolution Scan – increase pixel density but sacrifice speed

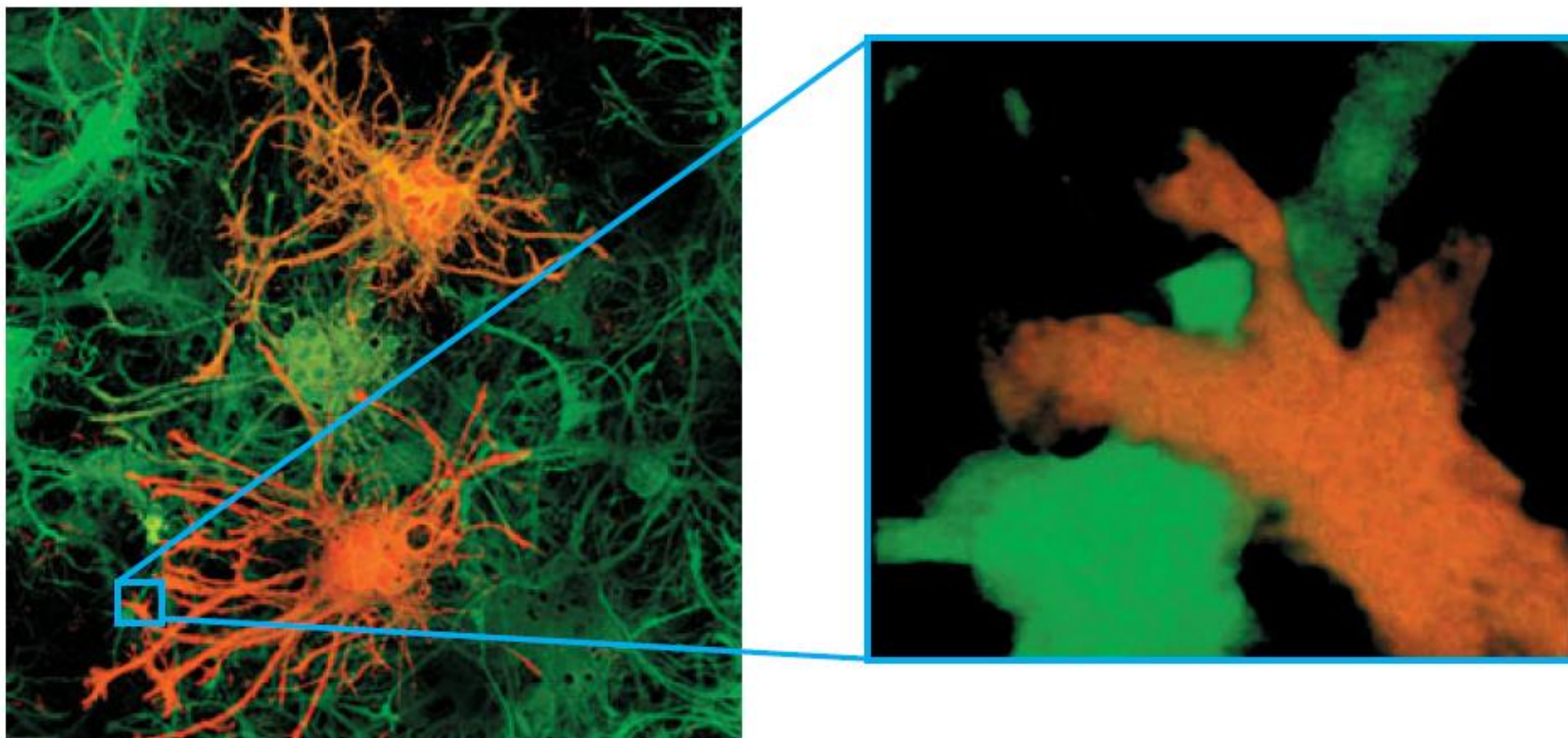
Zoom / Clip Scan – scan at high resolution and speed in smaller area

Rotate Scan – rotate scan angle to efficiently scan irregular areas



# Flexible XY Resolution – Zoom

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# WHY LASER SCANNING MICROSCOPY?

IMPROVE AXIAL RESOLUTION  
MULTI-LABEL/MODAL IMAGING  
FLEXIBLE RESOLUTION



HIGH RESOLUTION IMAGING  
FLEXIBLE PLATFORM

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