

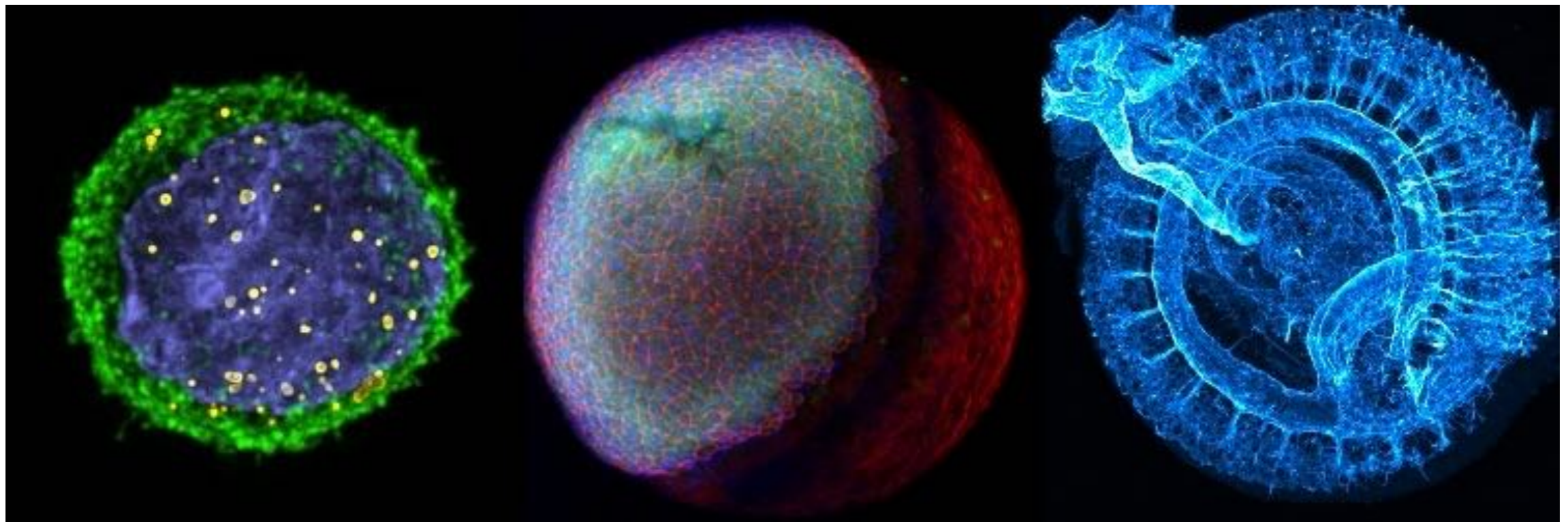
# Overview of light microscopy modalities at the CDB Microscopy Core

PCMD Histology Core

Sept. 11, 2023

Andrea Stout, Ph.D.

Director, CDB Microscopy Core at The Perelman School of  
Medicine University of Pennsylvania



<https://www.med.upenn.edu/cdbmicroscopycore/>

# CDB Microscopy Core Staff

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Office: Room 1108 BRB II/III      Phone: 215-573-7554

# Our Services

**Assisted microscopy:** a core staff member operates the microscope

**Unassisted microscopy:** trained users may access core microscopes 24/7

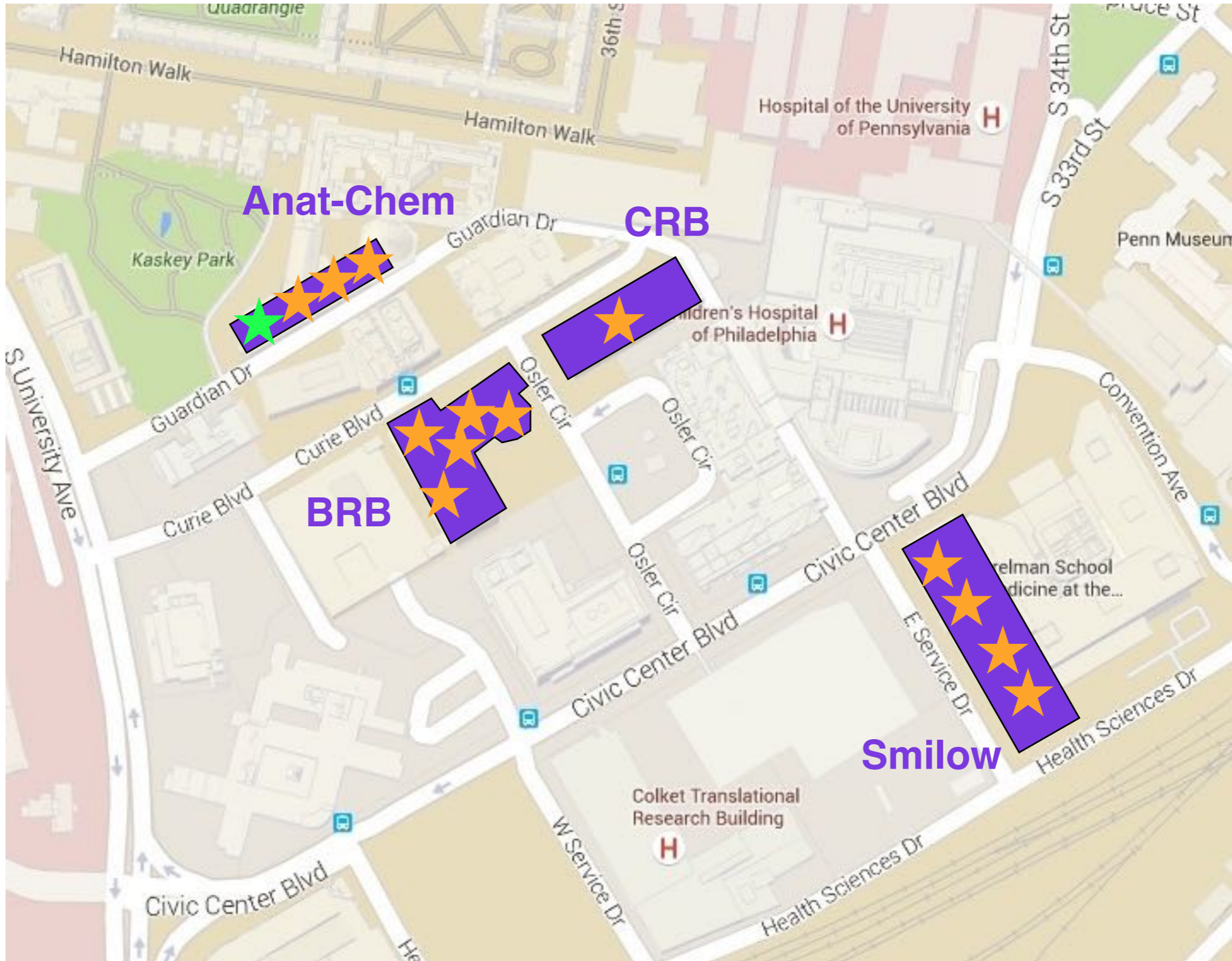
**Scanning EM sample preparation and imaging:** Yuri Veklich provides this service for our core; imaging is done on our FEI Quanta 250 located in the EMRL

**Monthly training lectures:** Covers fundamentals of light microscopy; required for all confocal trainees

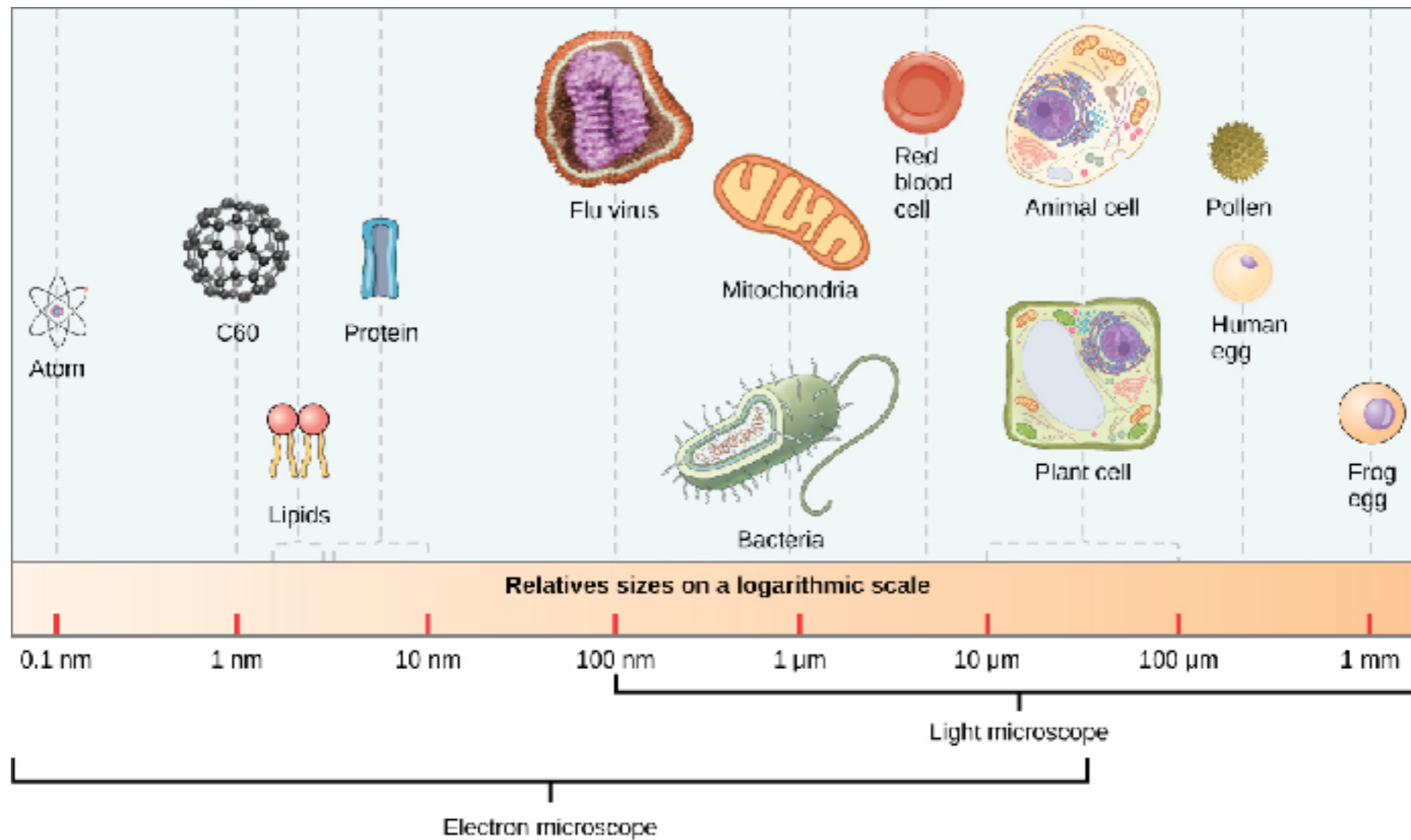
**Consultation on sample preparation & image analysis**

**Access to commercial software:** we have licenses for Imaris, Arivis Vision 4D, and SVI Huygens, along with two powerful Windows 10 workstations in BRB.

# Our locations



# One challenge for any microscopy core is the large range of specimen sizes that people bring



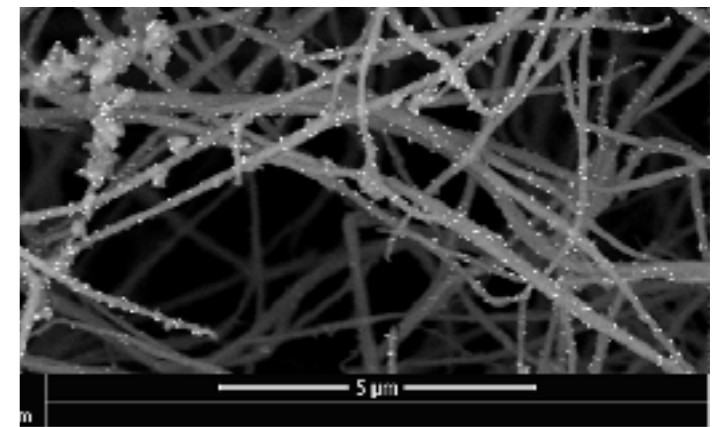
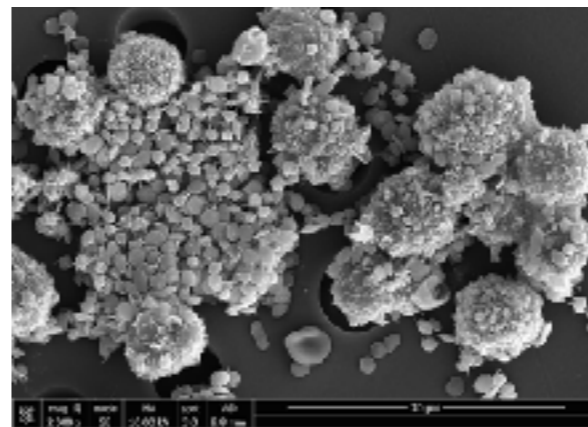
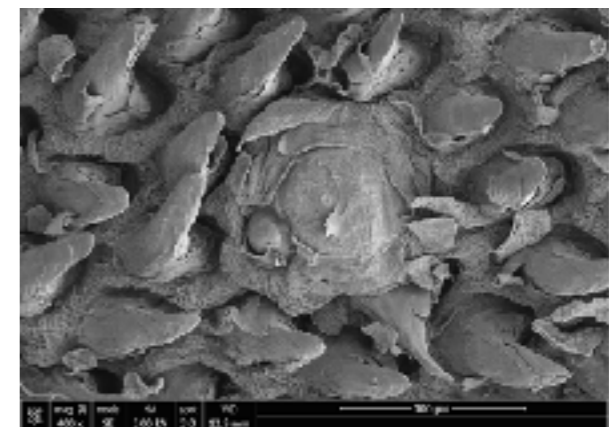
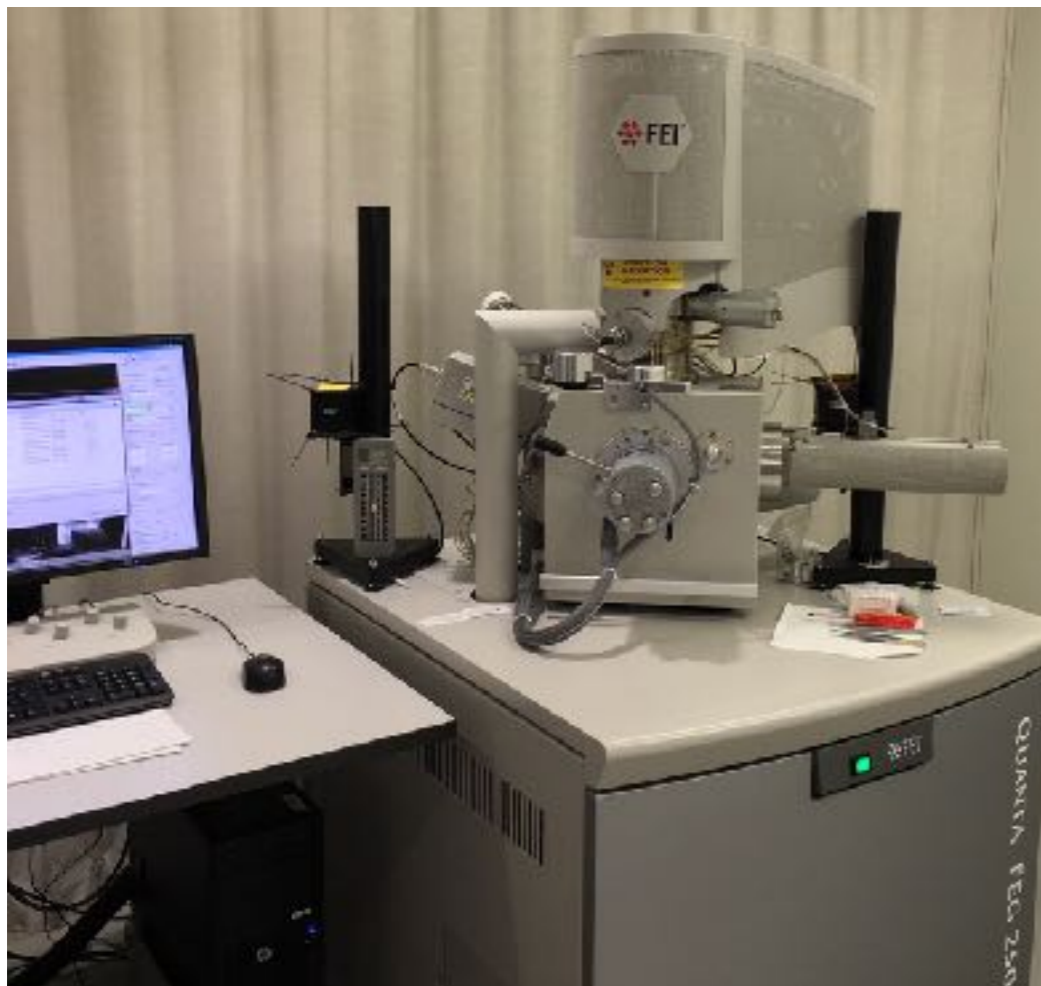
Mouse embryo, ~ 10 mm

# One microscope simply cannot capture images spanning this whole range

**The best microscope for you depends on your sample and what you need from the image data.**

**Always make sure you prepare your sample so that it's compatible with the type of microscope you want to use!**

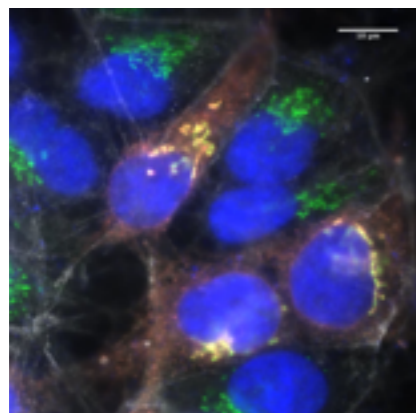
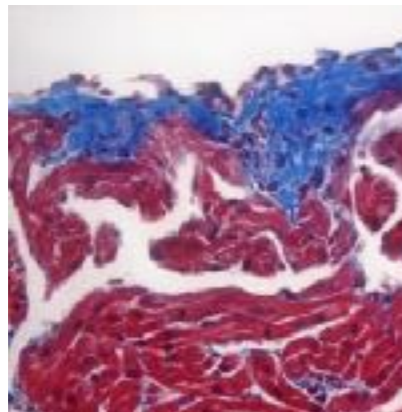
**Scanning electron microscopes can accommodate a fairly large range of sample sizes and are great for characterizing overall morphology and surface structures. Our core offers SEM imaging services on the FEI Quanta FEG 250 SEM in Anat-Chem.**



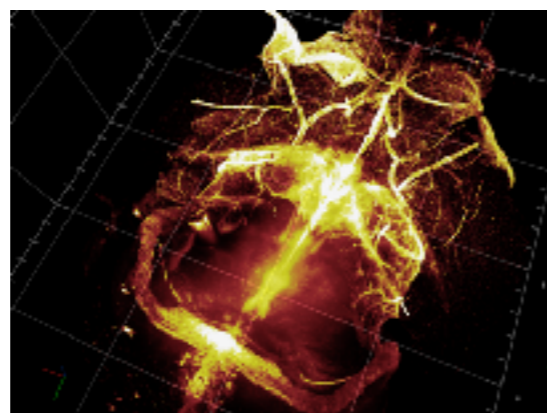
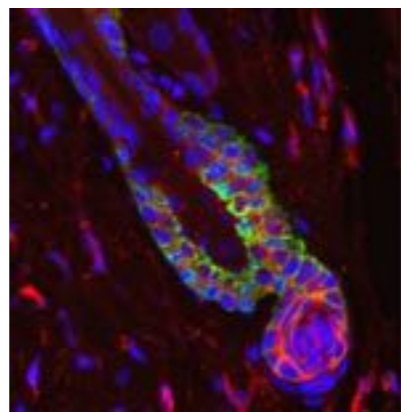
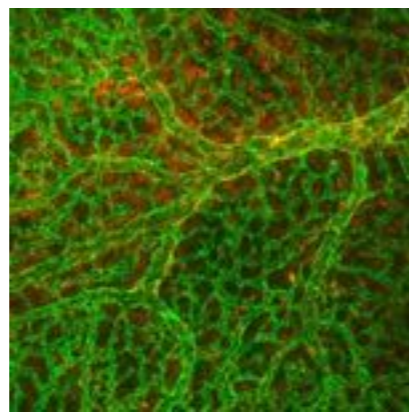
Yuri Veklich provides sample preparation, image acquisition, and, if desired, training on the SEM through our Core.

# Light Modalities Offered In Our Core

## Widefield

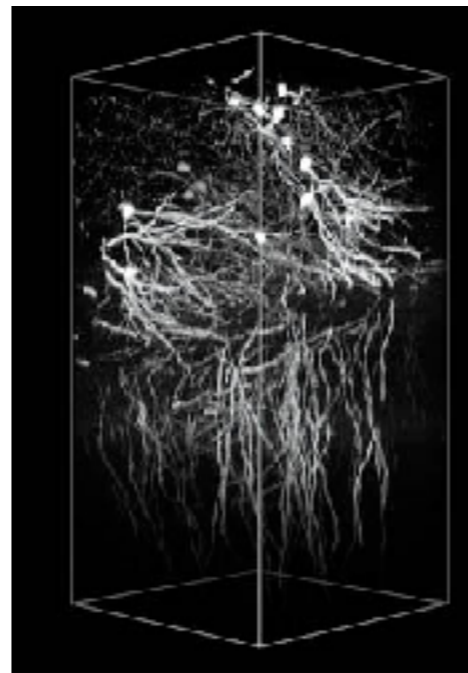


## Confocal & confocal-like

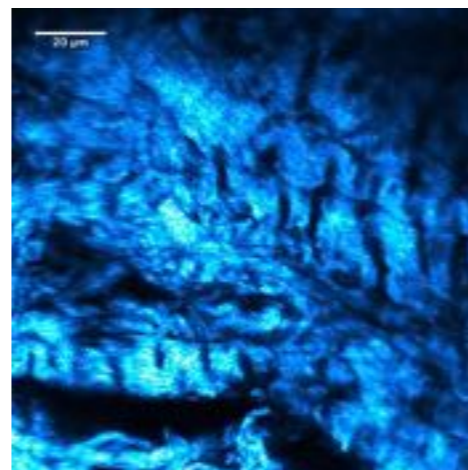


## Non-linear

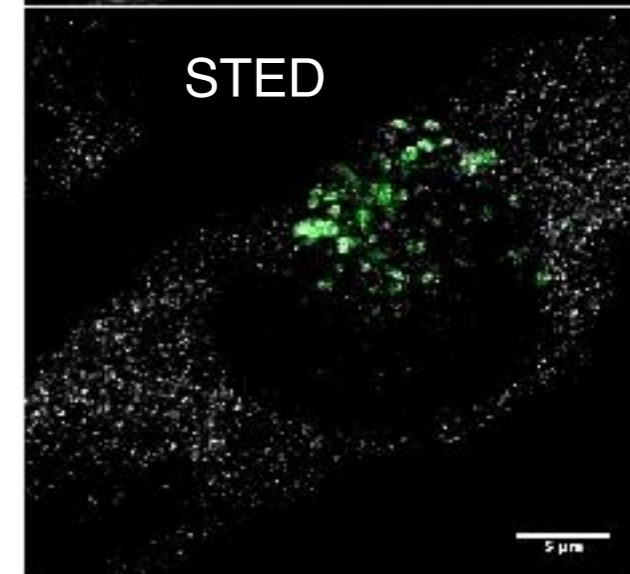
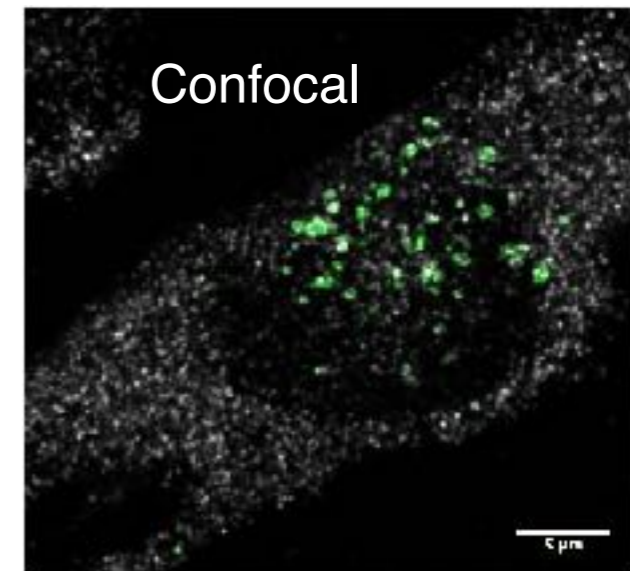
Multi-photon excitation



Second-harmonic generation

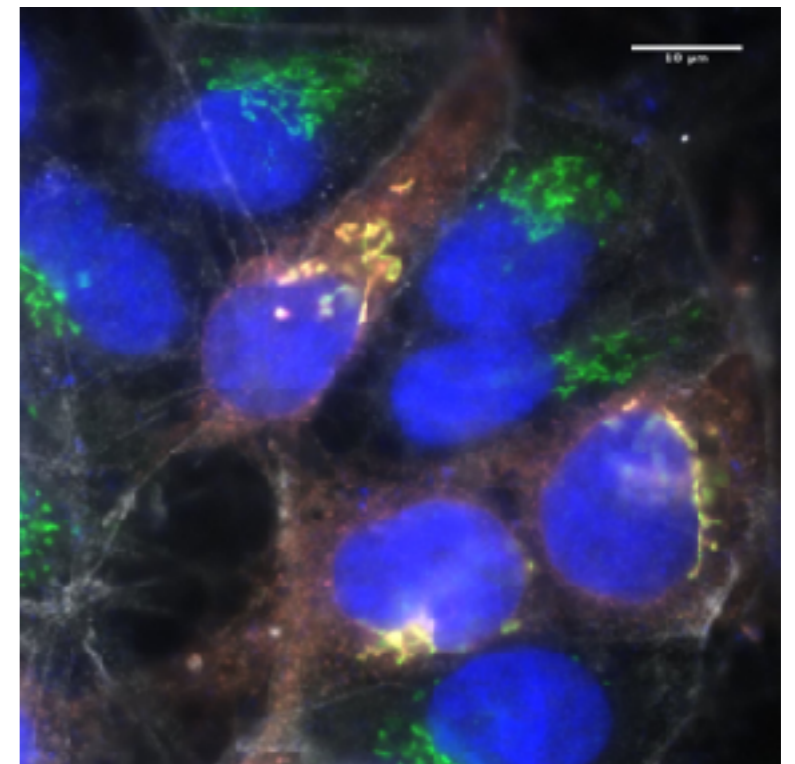
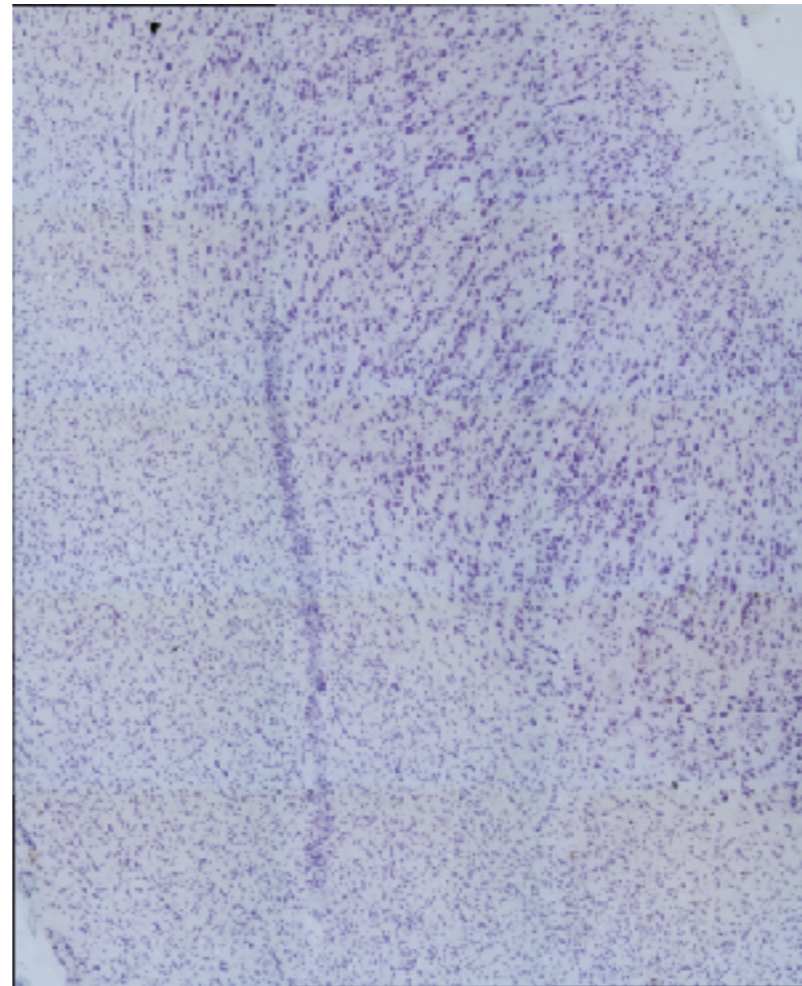
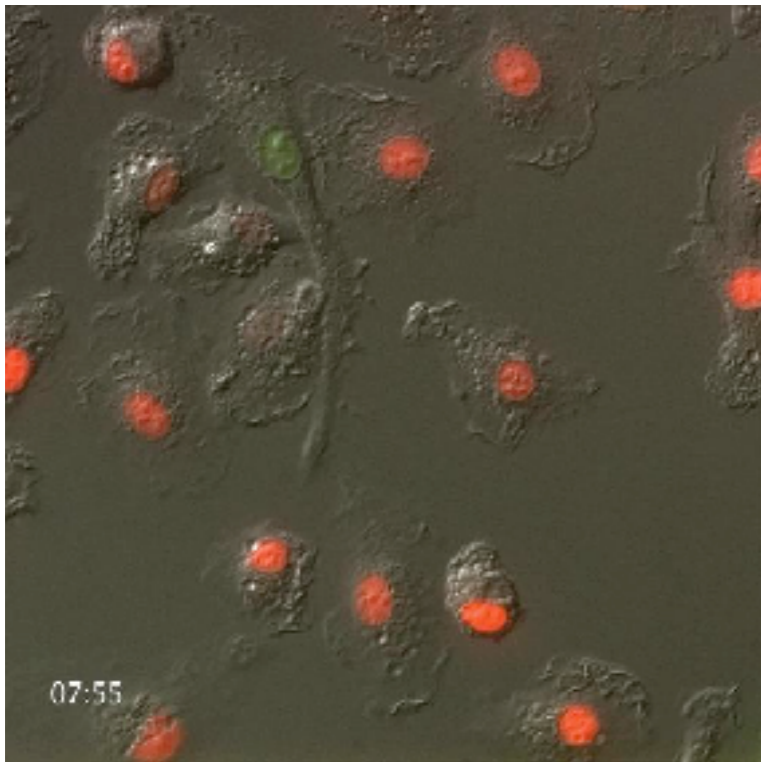


## “Super-resolution”





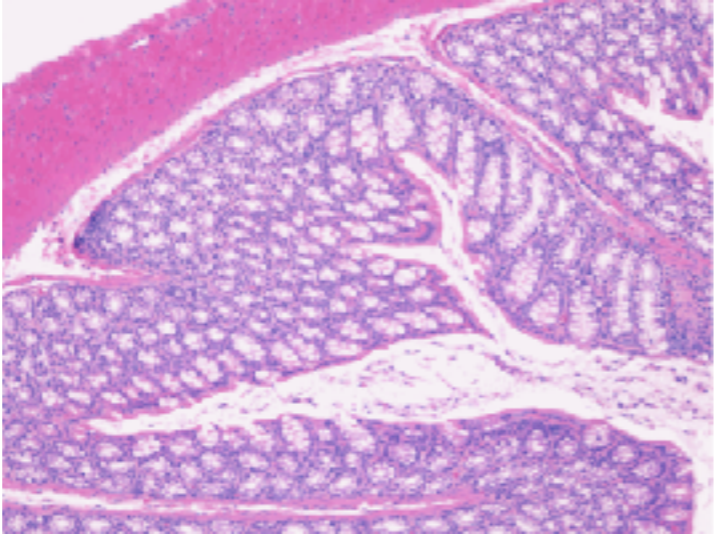
**Widefield microscopes are best for cells and thin tissue sections (less than 20 microns thick). They are also ideal for live cell imaging.**



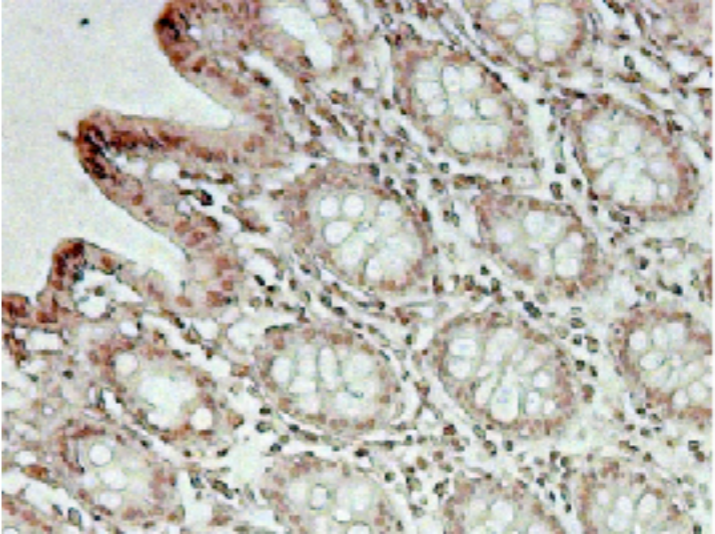
<https://www.med.upenn.edu/cdbmicroscopycore/widefield-microscopes.html>

# Are you staining samples with chromogenic (color) dyes like these?

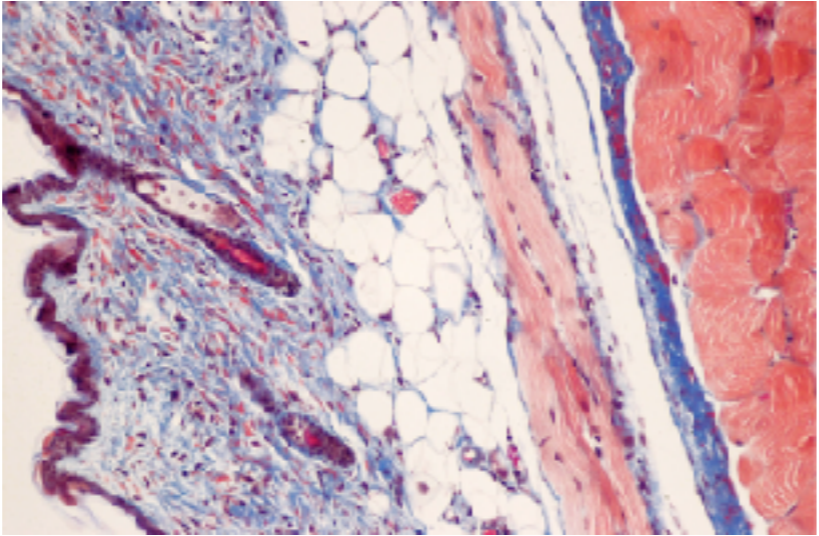
**H & E**  
purple-blue = nuclei  
pink = cytoplasm or ECM



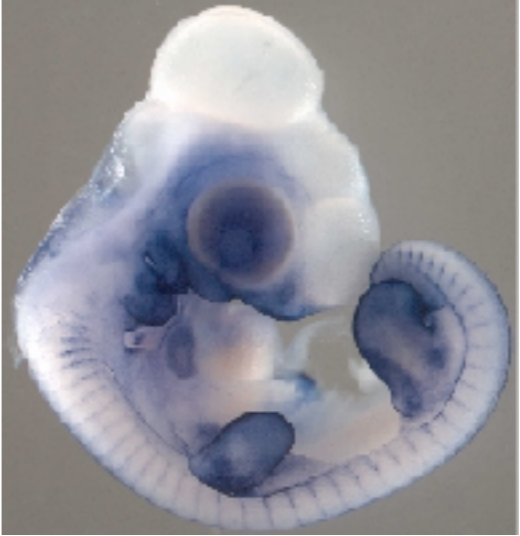
**Horseradish peroxidase**  
(antibody-conjugated)



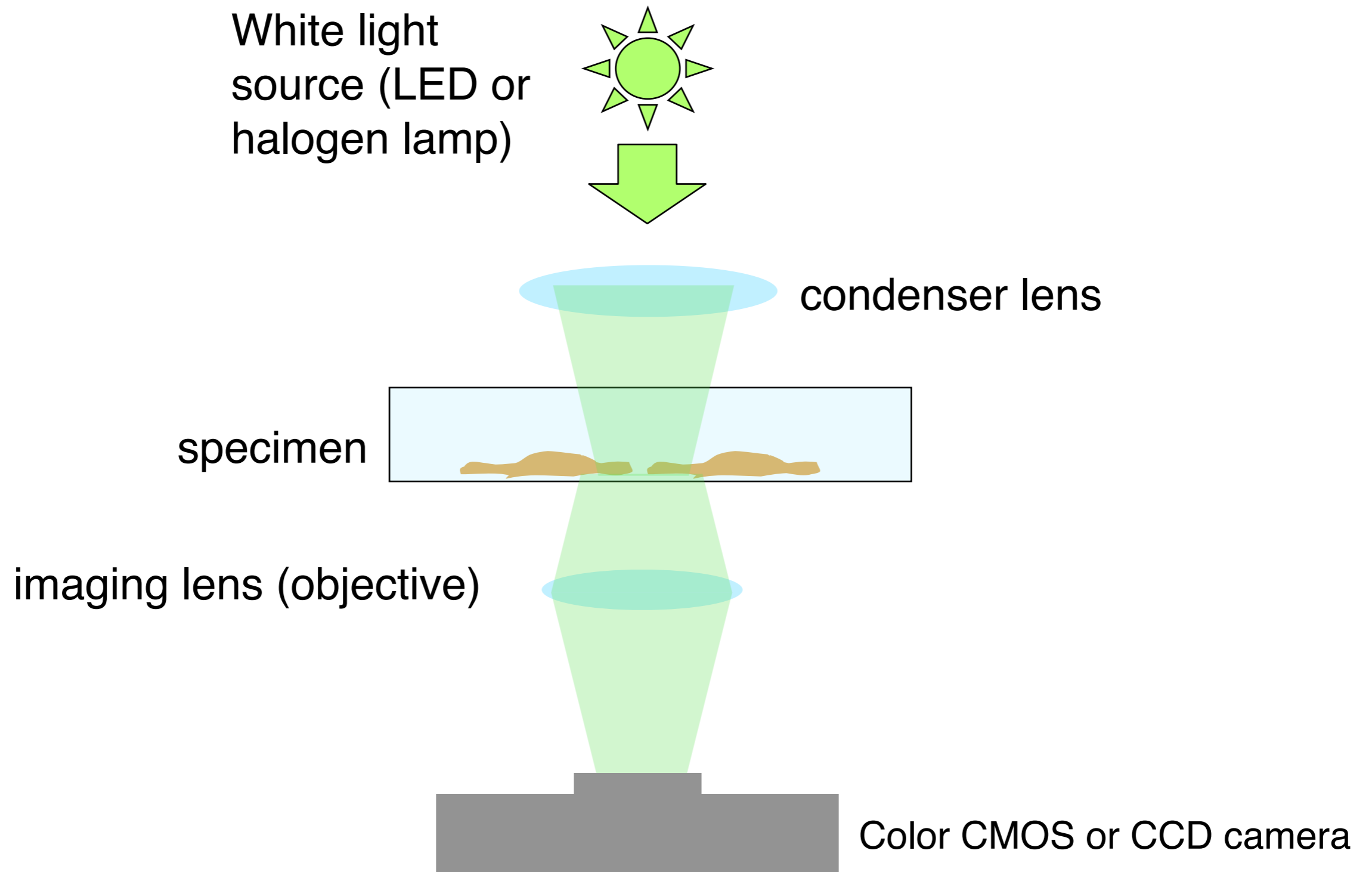
**Masson's Trichrome**  
blue = connective tissue  
purple = nuclei  
red = cytoplasm



**Alkaline phosphatase**  
(specific mRNA sequence)

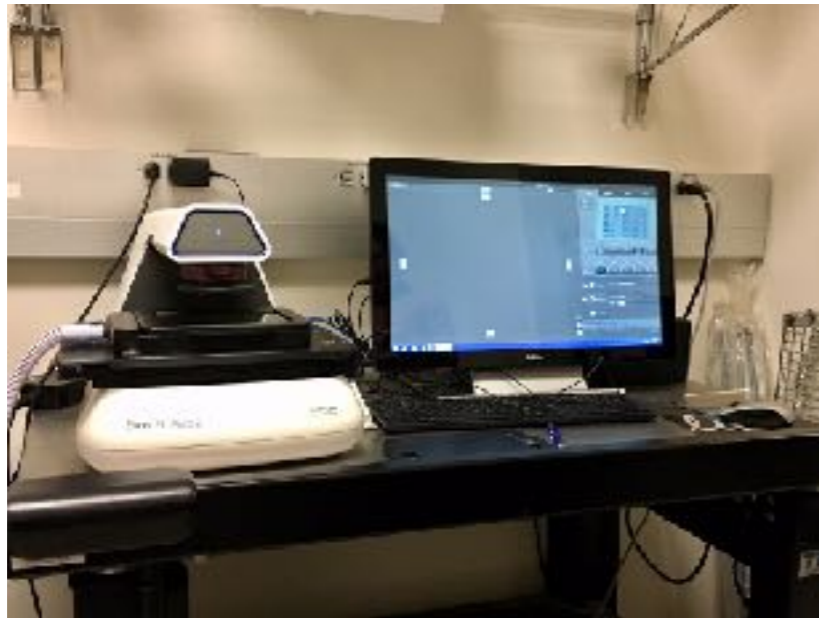


If so, you need to use a widefield microscope equipped with white transmitted light illumination and a **COLOR** camera

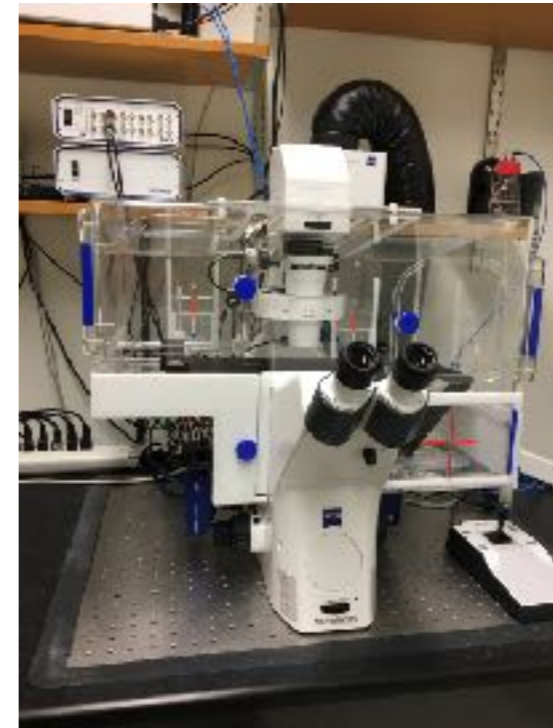


# Two of our three widefield systems have color cameras:

ThermoFisher EVOS FL Auto 2 - Room 1-127 Smilow

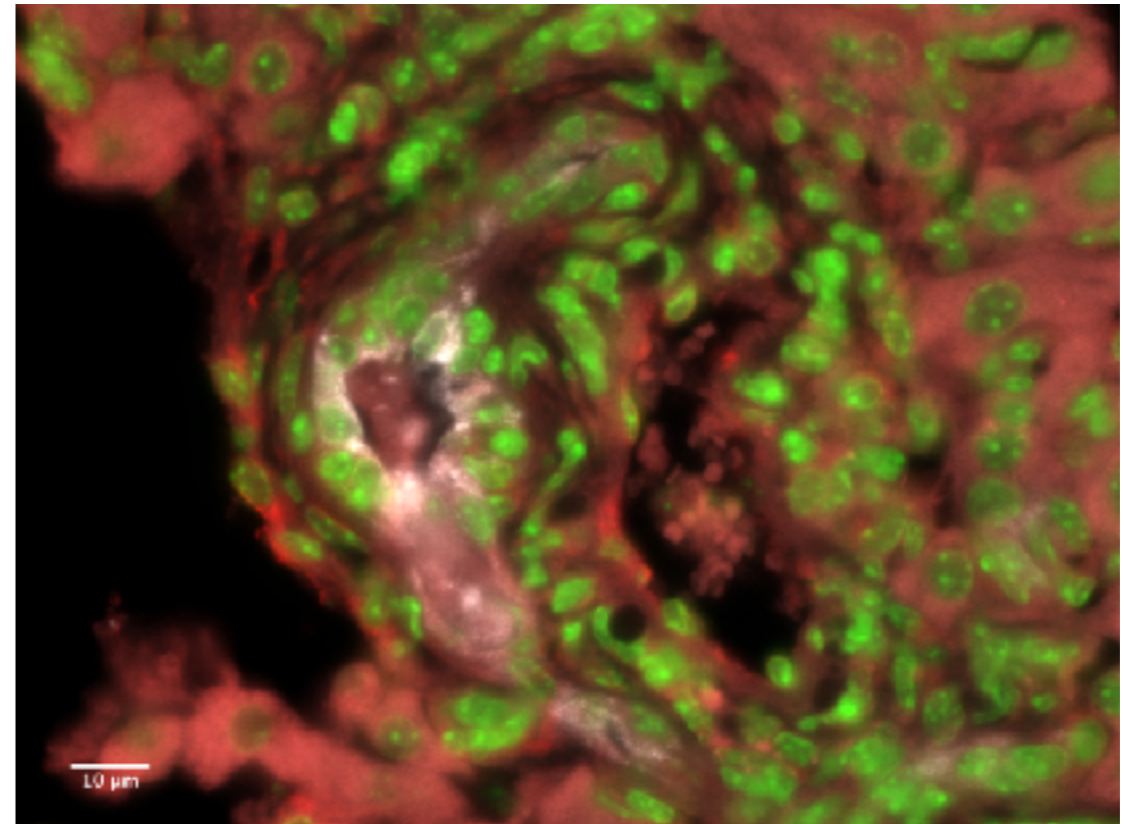
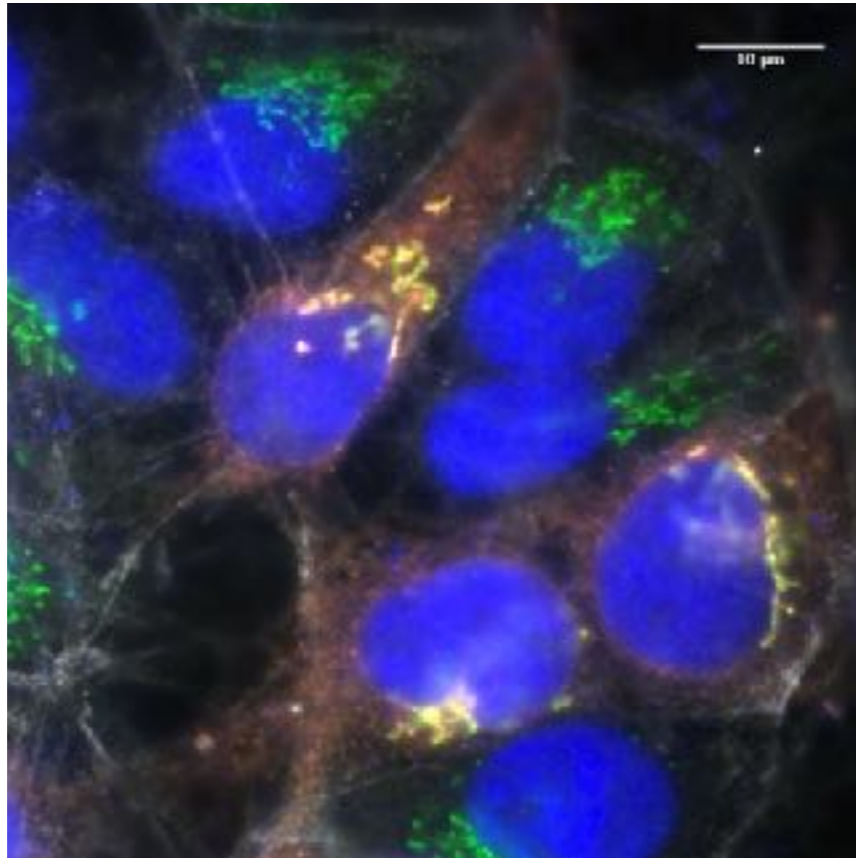


Zeiss AxioObserver 7 - Room 1175B BRB



**Both of these microscopes also have motorized xy stages for scanning whole tissue sections or slides**

**Are you doing immunofluorescence in fixed cells or tissue,  
or imaging fluorescent proteins in live cells?**



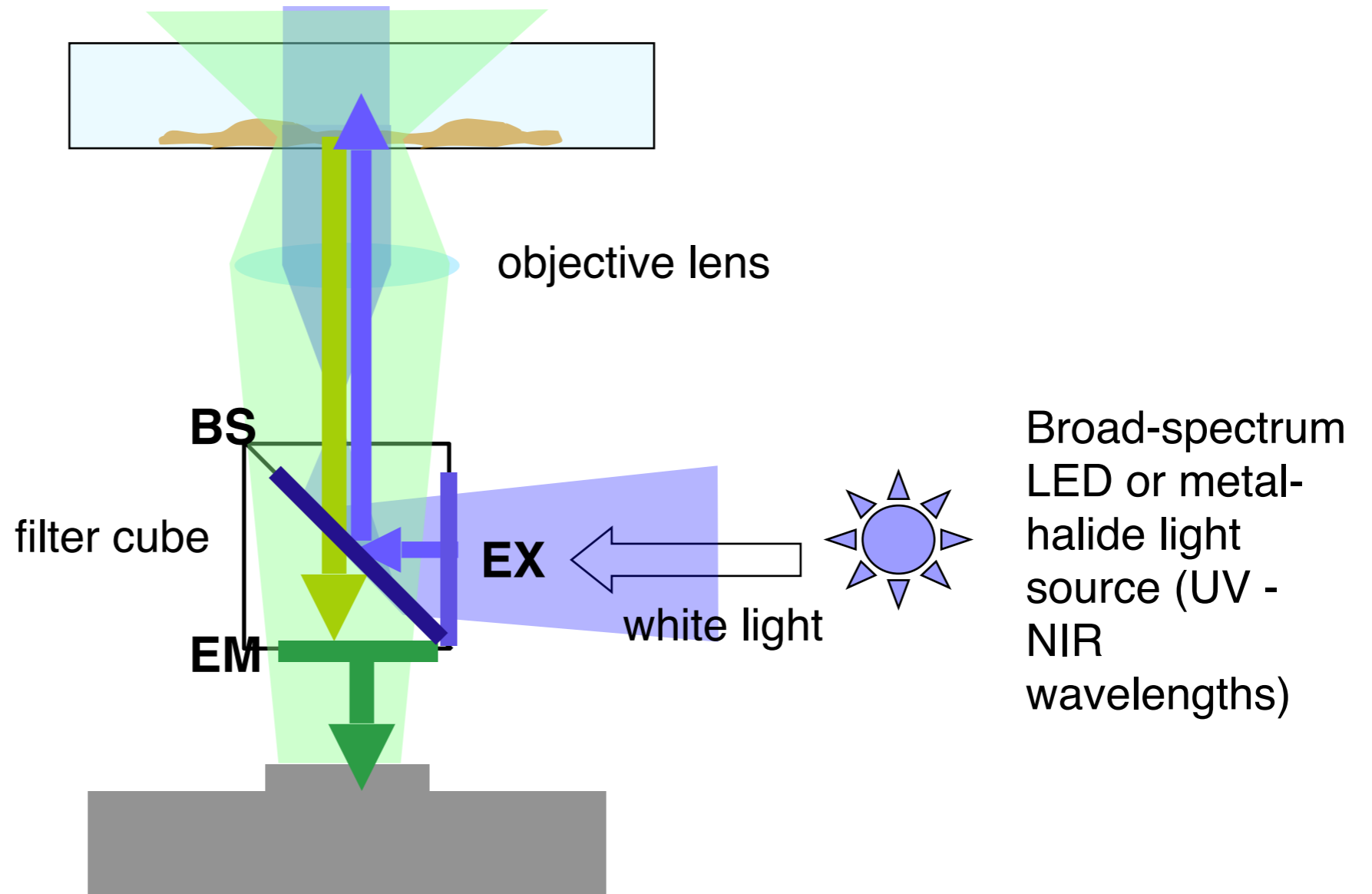
**For thin (< 5 - 10 microns) samples, a widefield  
epifluorescence microscope is the best place to start**

# Widefield epifluorescence microscopy requires different components than transmitted light microscopy

**EX: excitation filter**  
passes wavelengths  
needed to excite specific  
fluorophores

**BS: beamsplitter** reflects  
shorter wavelengths and  
passes longer  
wavelengths

**EM: emission filter** passes  
wavelengths emitted as  
fluorescence by  
fluorophores

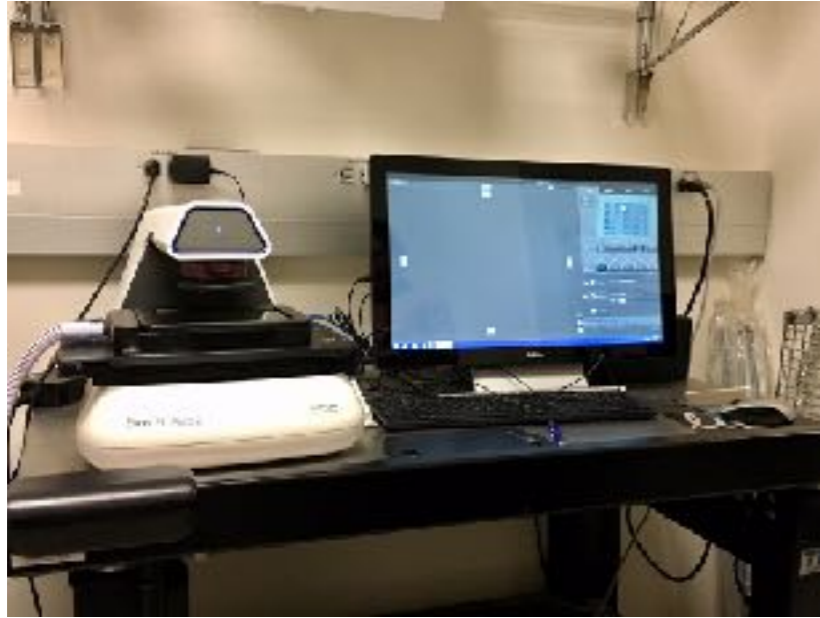


**Monochrome CMOS or CCD camera**

Color cameras should not be used for fluorescence microscopy!

# All of our widefield systems can capture fluorescence

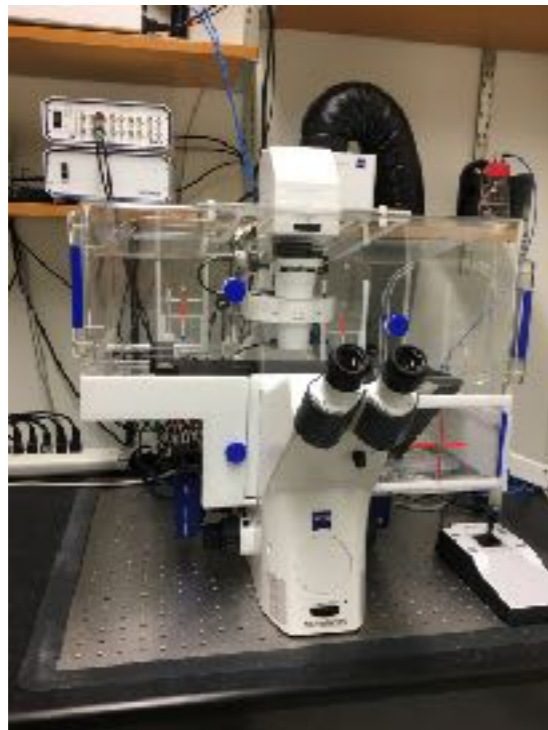
ThermoFisher EVOS FL Auto 2 - Room 1-127 Smilow



Leica DM6000 - Suite 1-12 Smilow



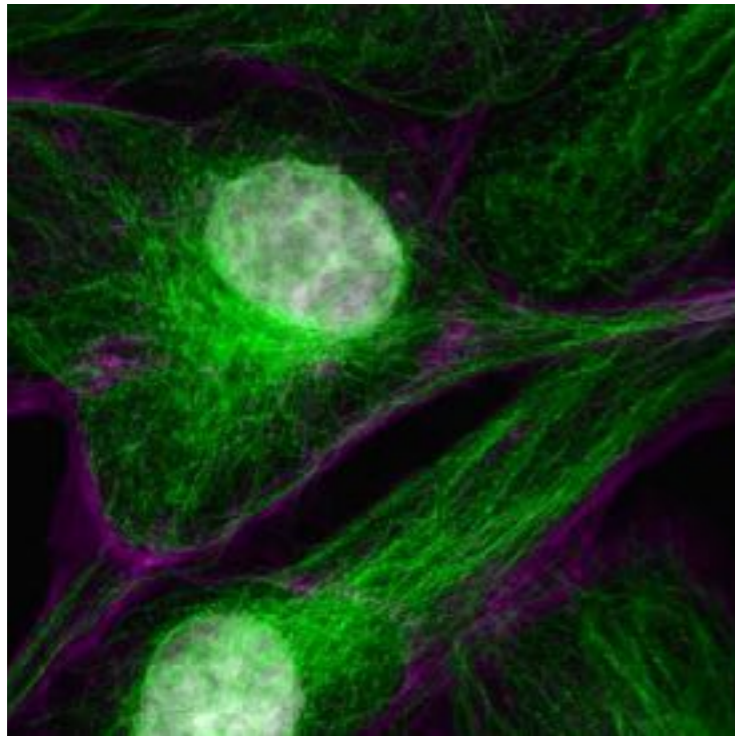
Zeiss AxioObserver 7 - Room 1175B BRB



All have monochrome cameras and a variety of filter sets; the EVOS and the Zeiss have incubation capability for live samples

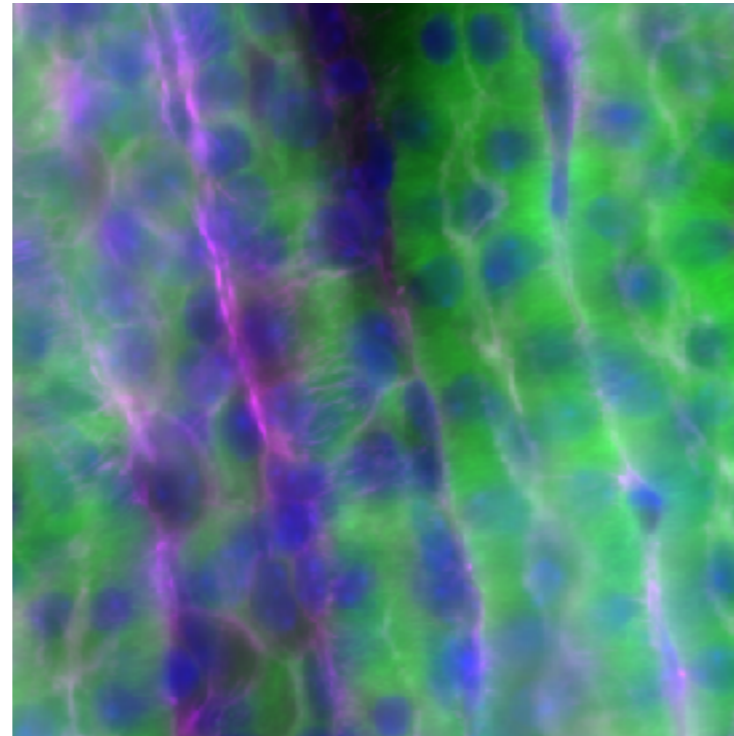
# Confocal microscopes are used for imaging fluorescence in cells & tissues thicker than ~10 microns

**Widefield**



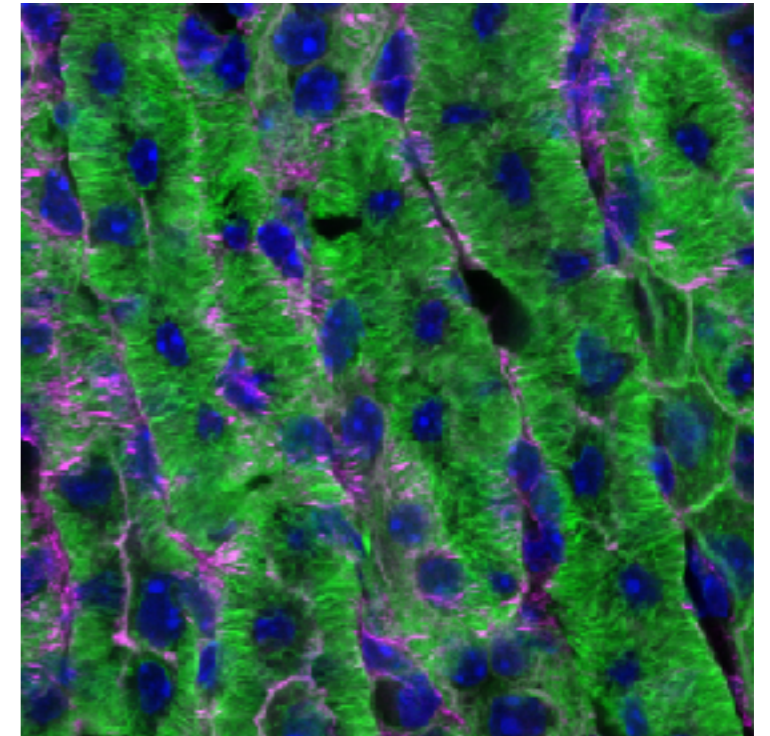
**Fixed BPAE cells,  
~ 3-4 microns thick**

**Widefield**



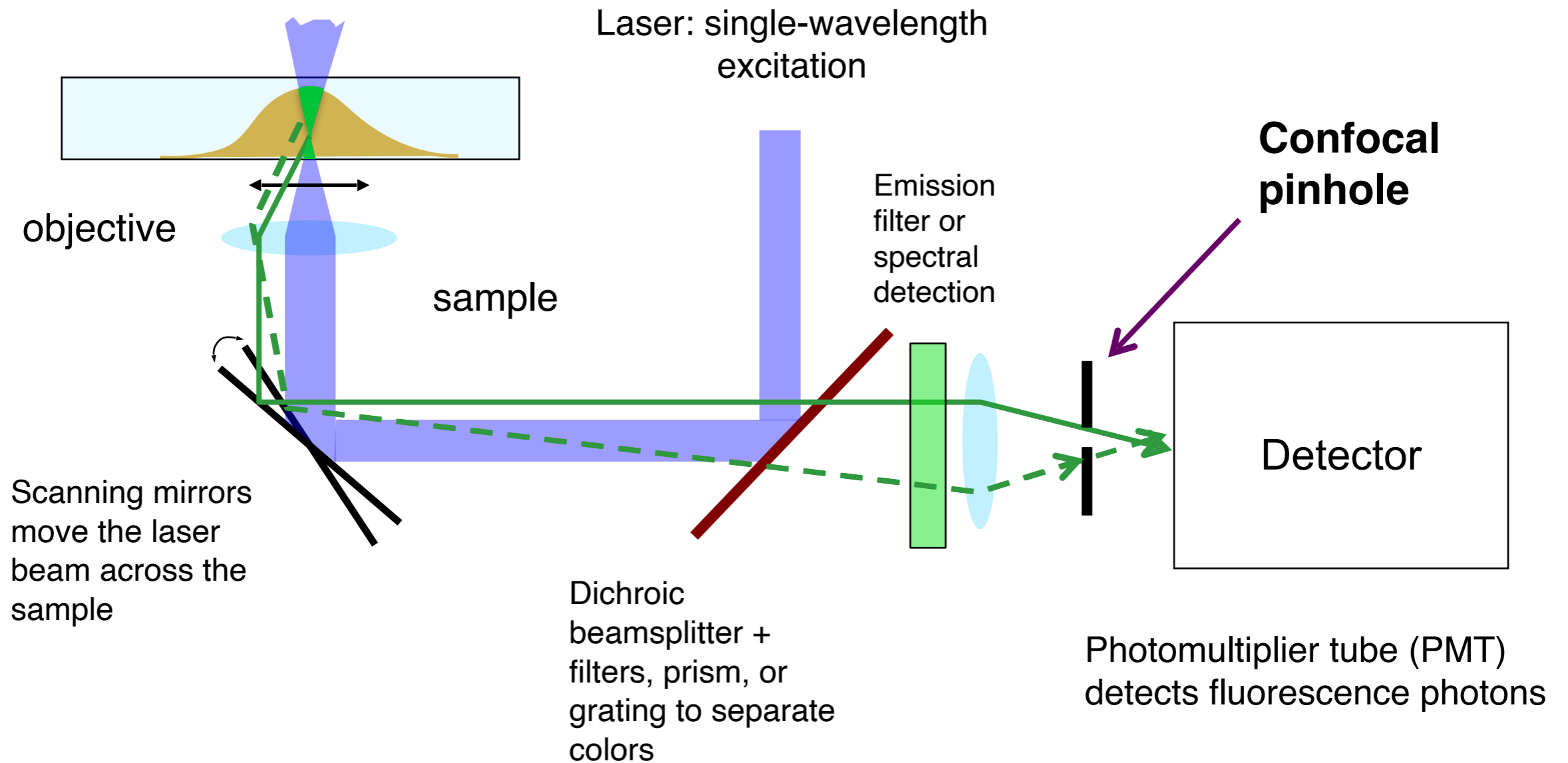
**Cryostat kidney  
section, 16 microns  
thick**

**Confocal**





**In a laser scanning confocal, 2D images are built up one pixel at a time by scanning a focused laser beam across the sample.**

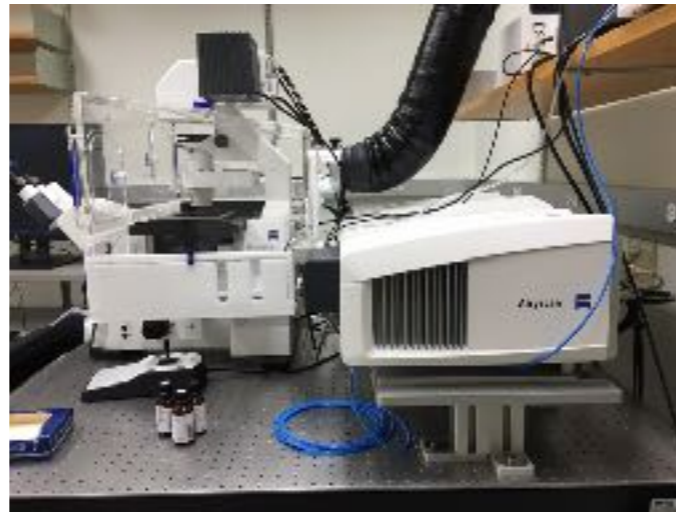


**The confocal pinhole prevents out-of-focus light from entering the detector, giving better contrast when imaging thick samples.**

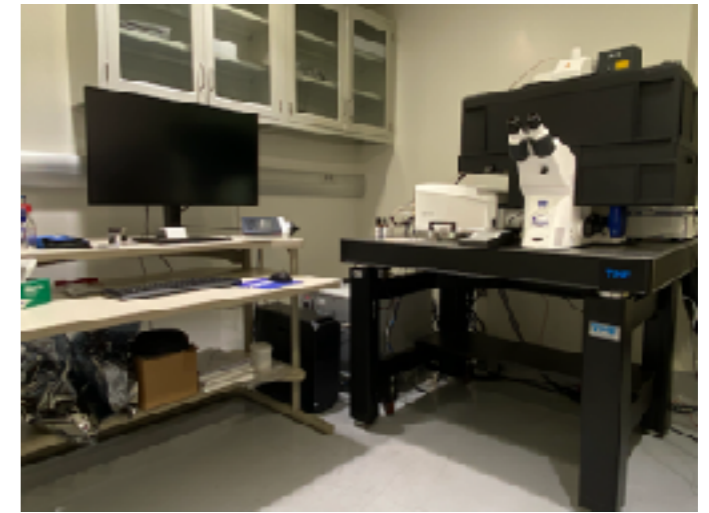
# Our core manages SIX (6) laser-scanning confocals



Zeiss LSM 710  
(Smilow)



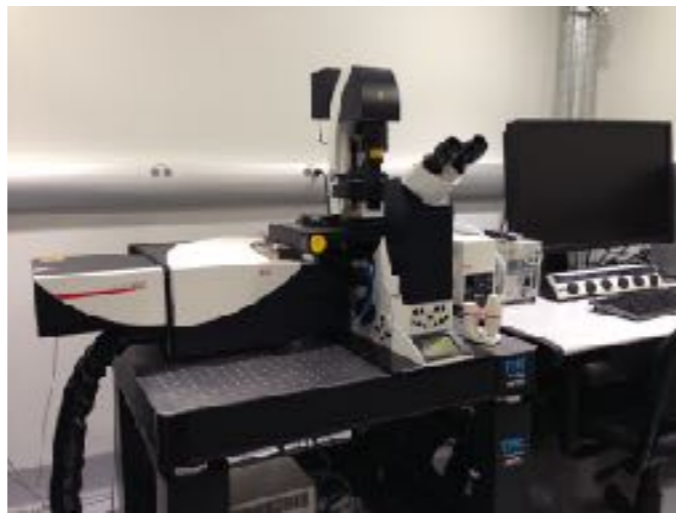
Zeiss LSM 880  
(BRB)



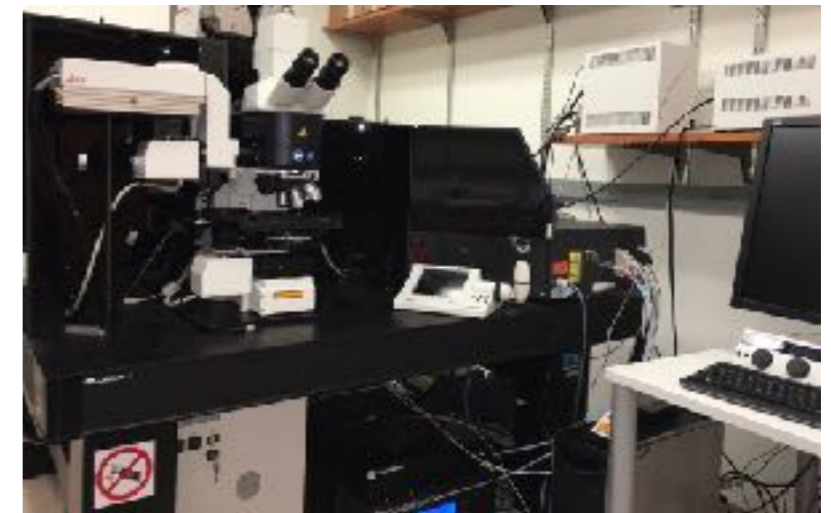
Zeiss LSM 980  
(Anat-Chem)



Leica Stellaris 5  
(Smilow)



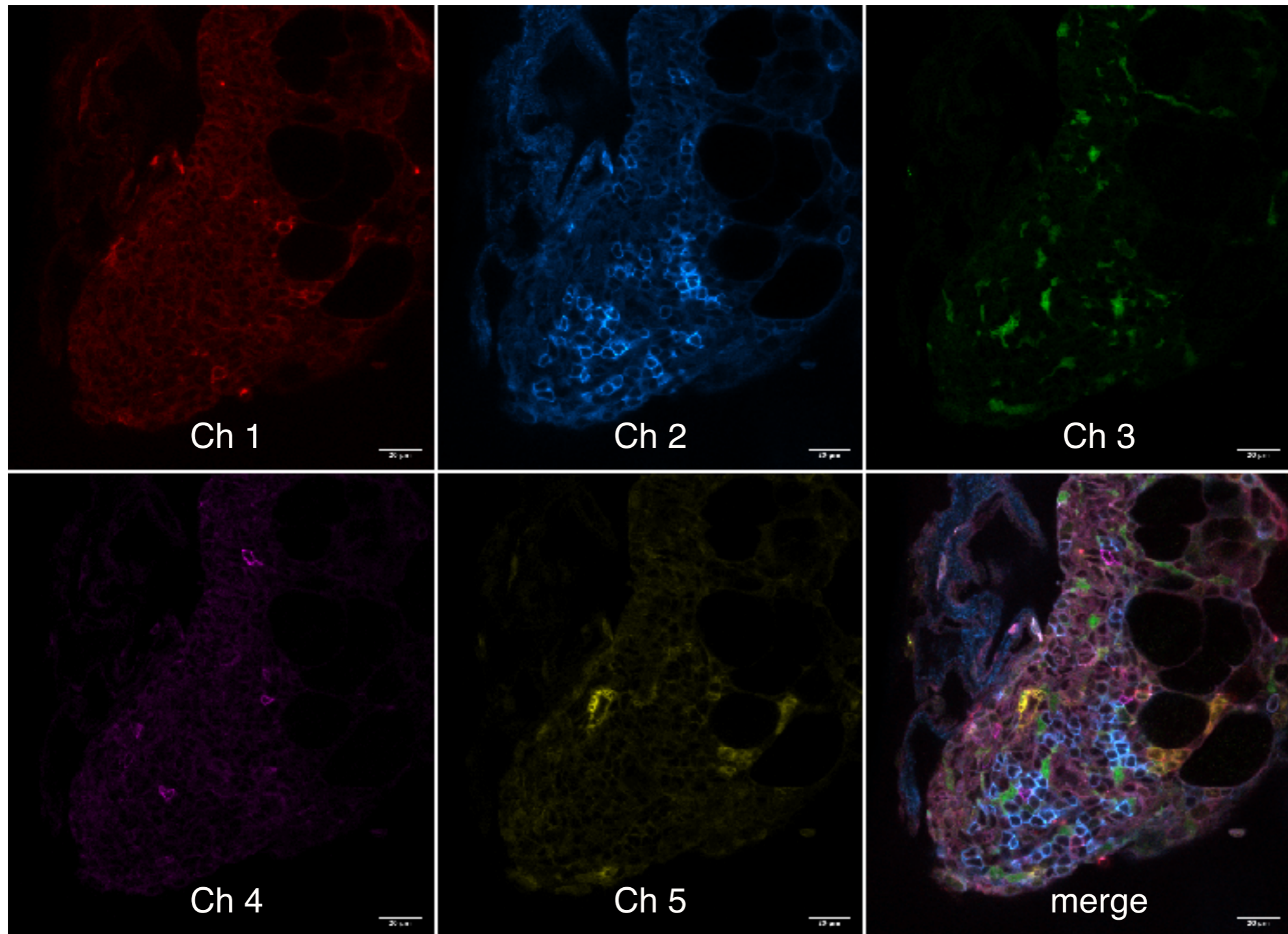
Leica TCS SP8-STED  
(Anat-Chem)



Leica TCS SP8-MP  
(CRB)

**While they are all similar in capability, there are a few differences between them.**

**Two of our Leica confocals have a tunable white light lasers providing many more excitation options than confocals with fixed wavelength lasers**

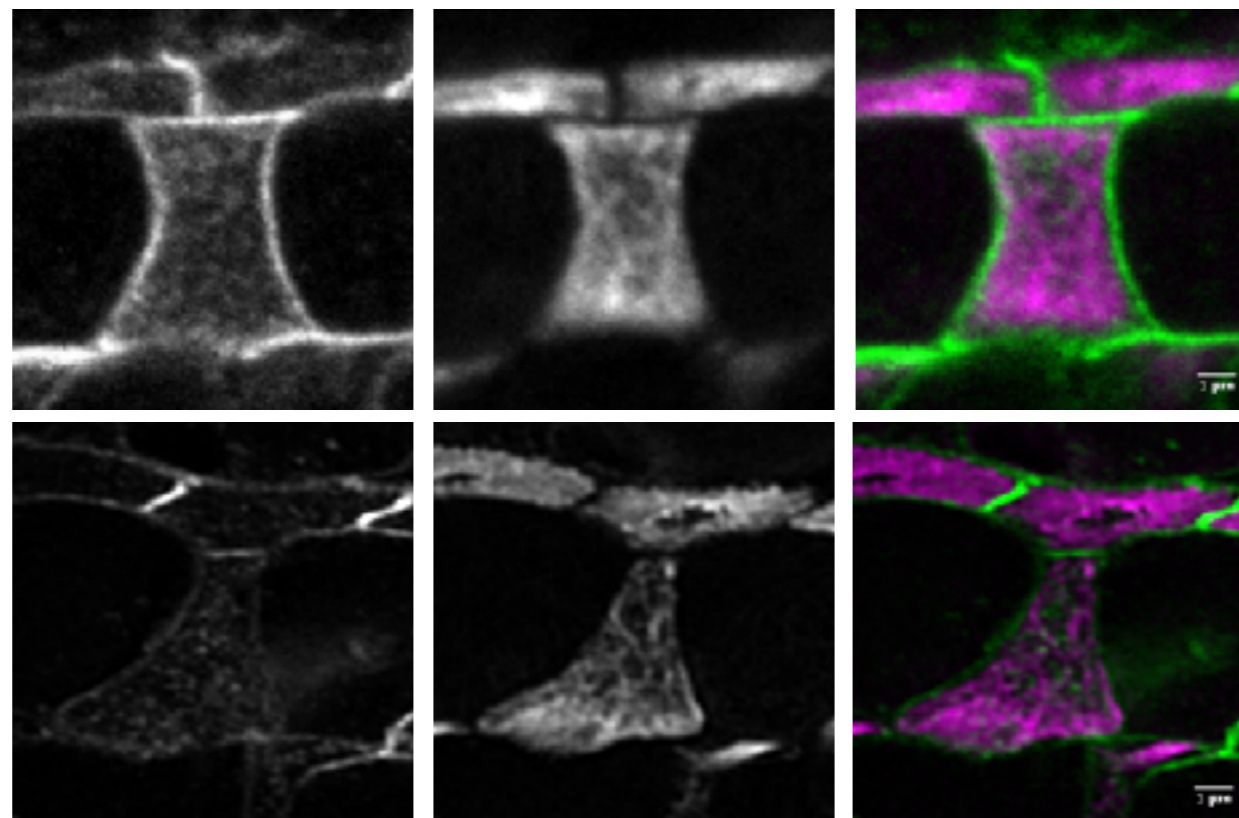


Five-color immunostaining, mouse omentum prepared by David Christian, Hunter lab

**Our Zeiss LSM 880 is equipped with the Airyscan module, which can enhance resolution to  $\sim 120$  nm in  $xy$ ,  $\sim 300$  nm in  $z$**



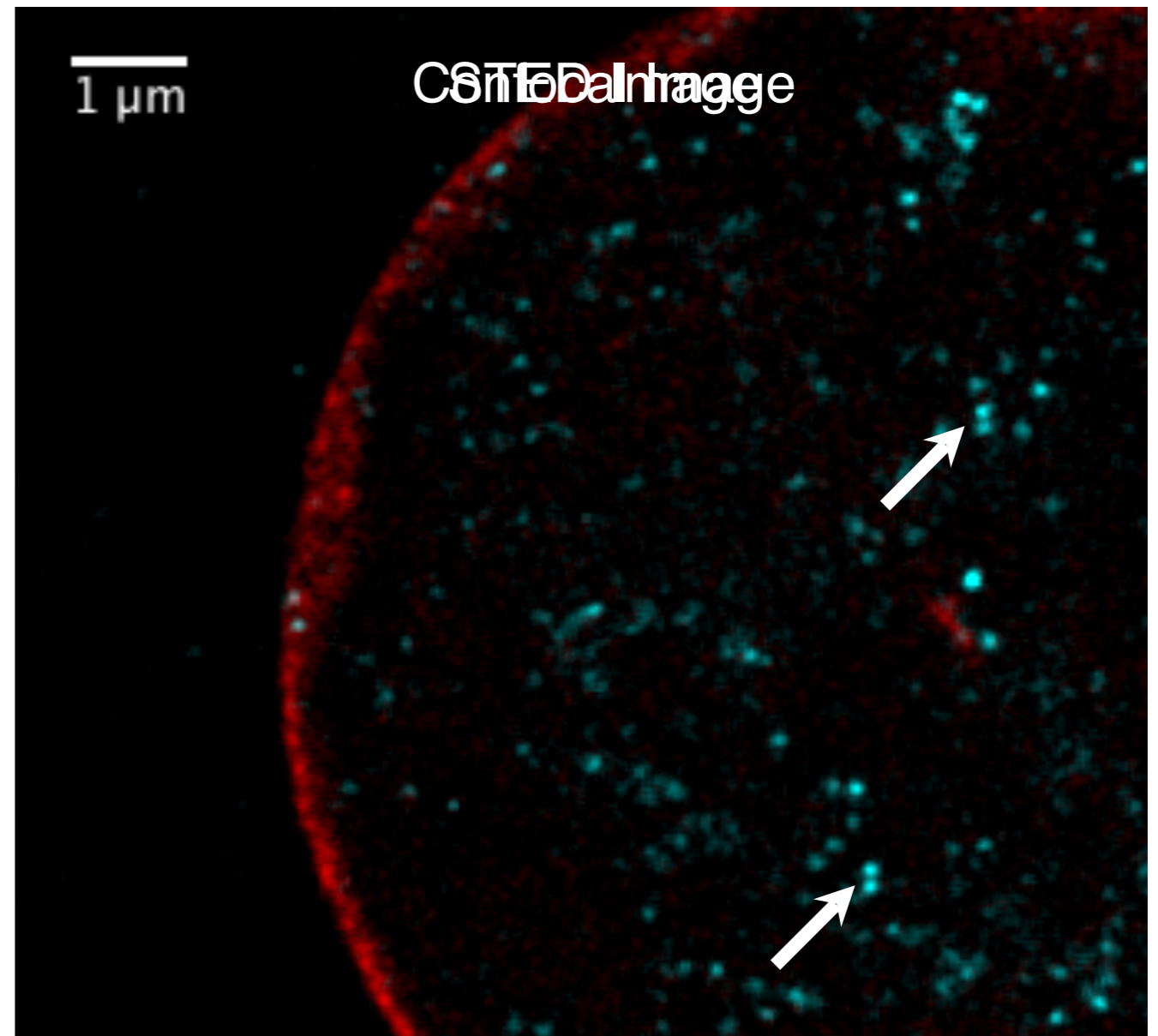
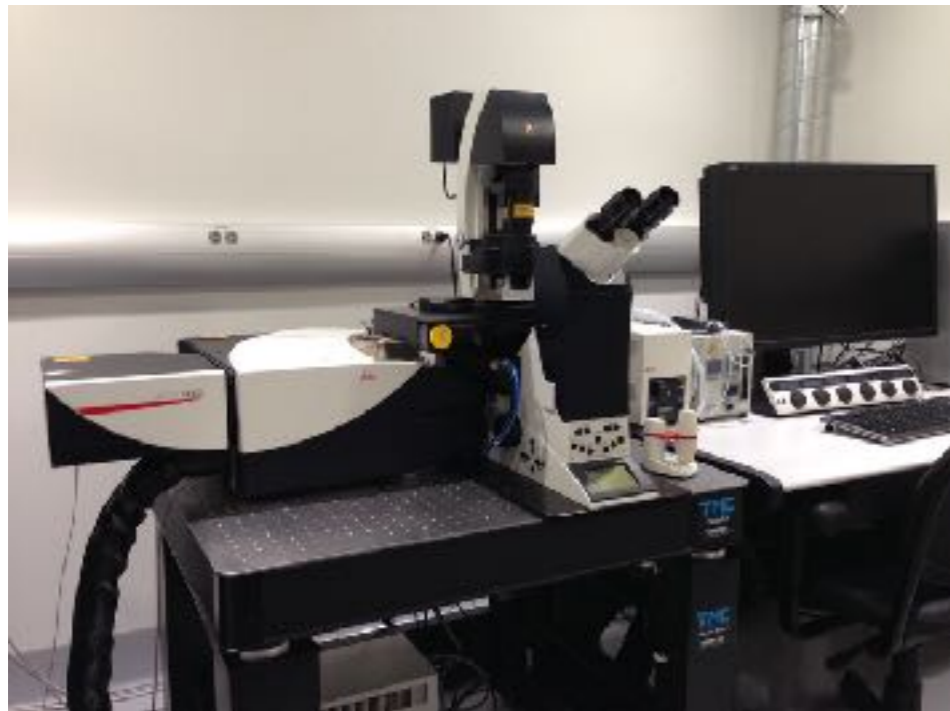
Confocal images  
using optimal  
settings



Airyscan images  
using optimal  
settings

P5 mouse organ of corti from inner ear stained for beta-catenin (green) and detyr-alpha tubulin (magenta), T. Chen from D. Epstein lab

**Our Anat-Chem Leica is also a STED *super-resolution* system, capable of 50-60 nm lateral resolution and ~ 120 nm axial resolution (highest standard confocal resolution is ~250 nm lateral, ~600 nm axial)**



Red: lamin

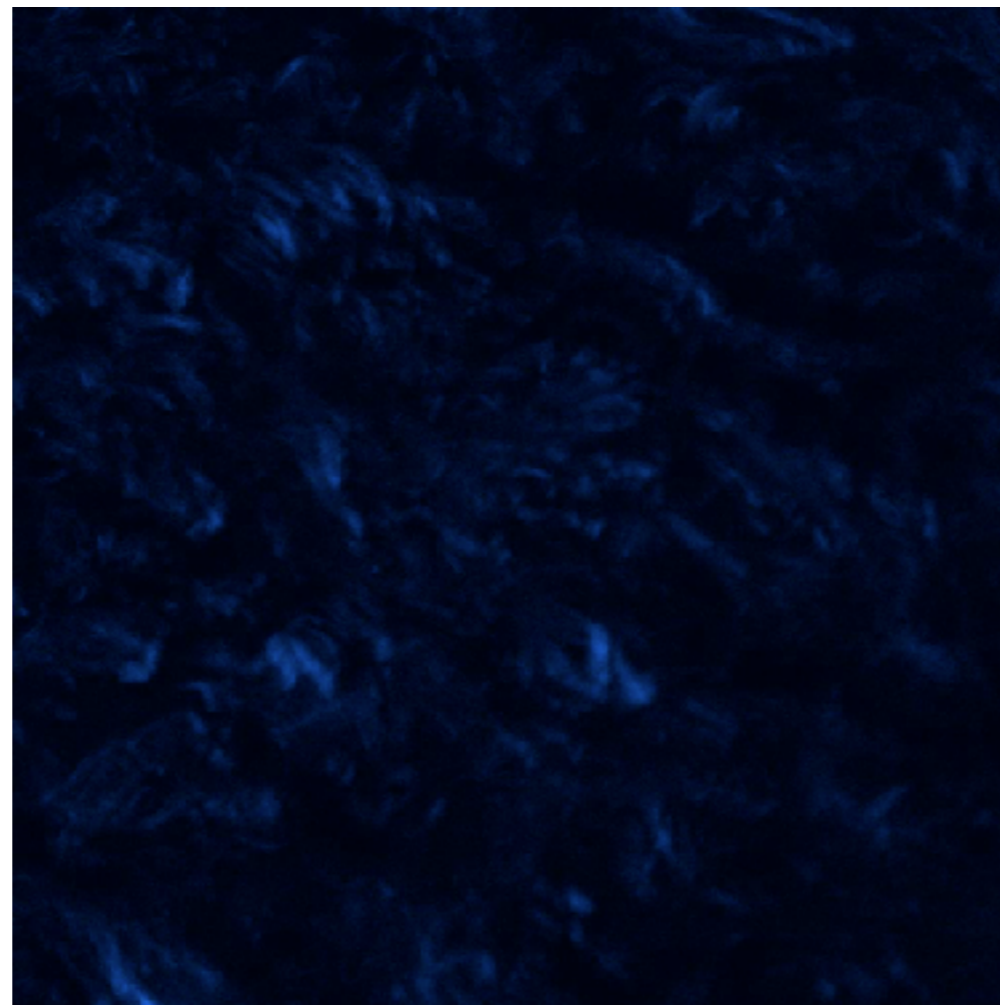
Cyan: histone H3

Sample prepared by A. Poleshko, Epstein lab

# Our CRB Leica confocal has multi-photon and SHG capability (but NO provisions for intravital imaging)



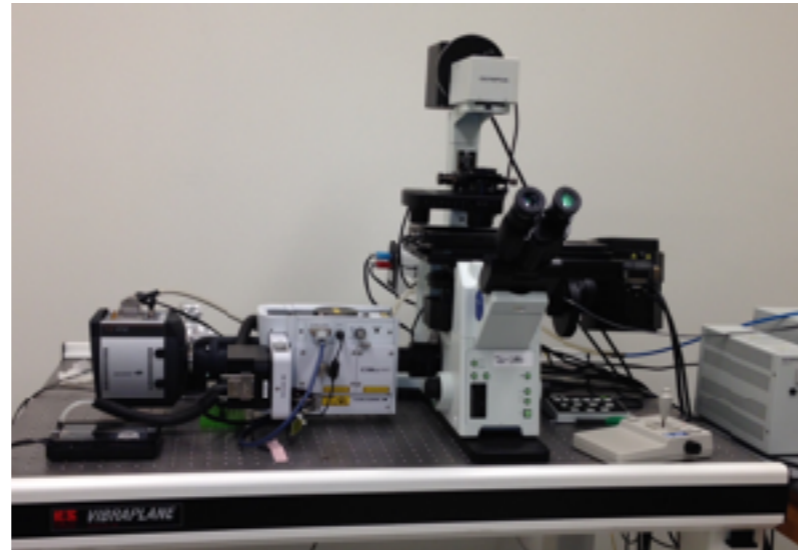
Forward-scattered  
SHG, mouse cervix.  
Sample prepared  
and imaged by  
Carrie Barnum,  
Soslowky Lab.



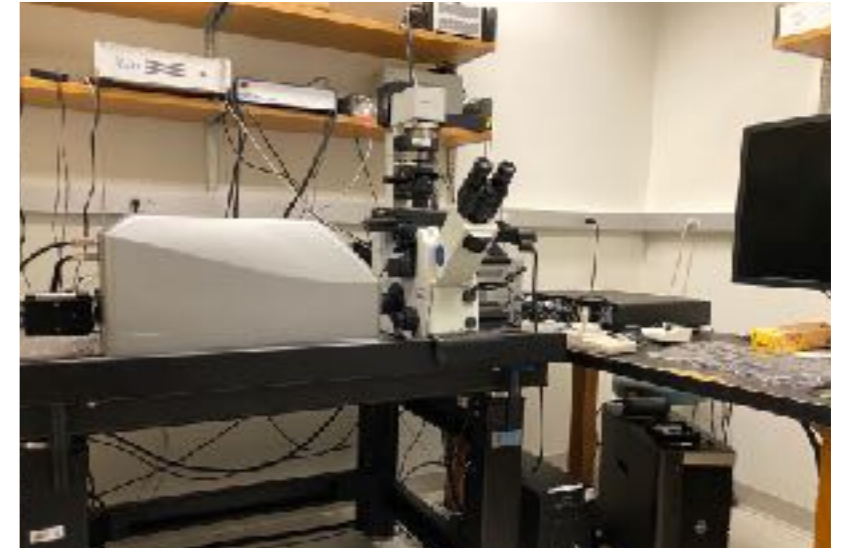
# Camera-based confocals are generally faster than laser-scanning confocals and often less phototoxic.



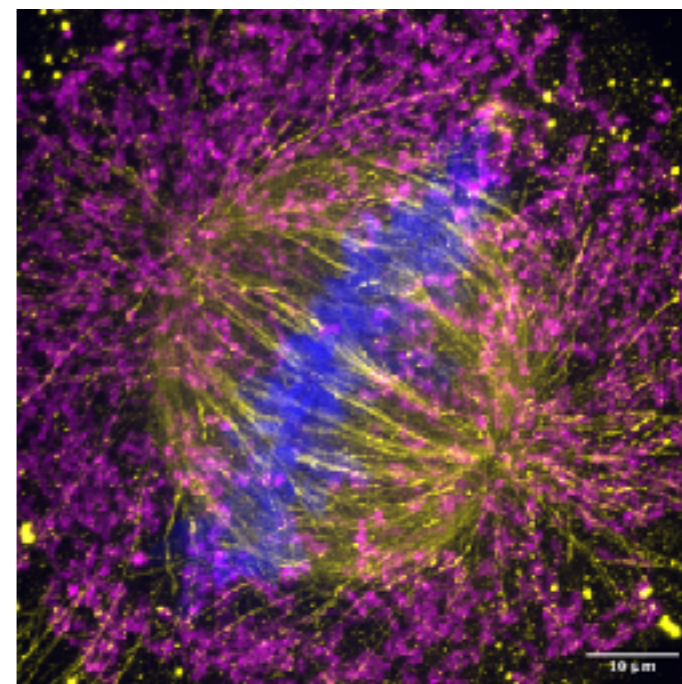
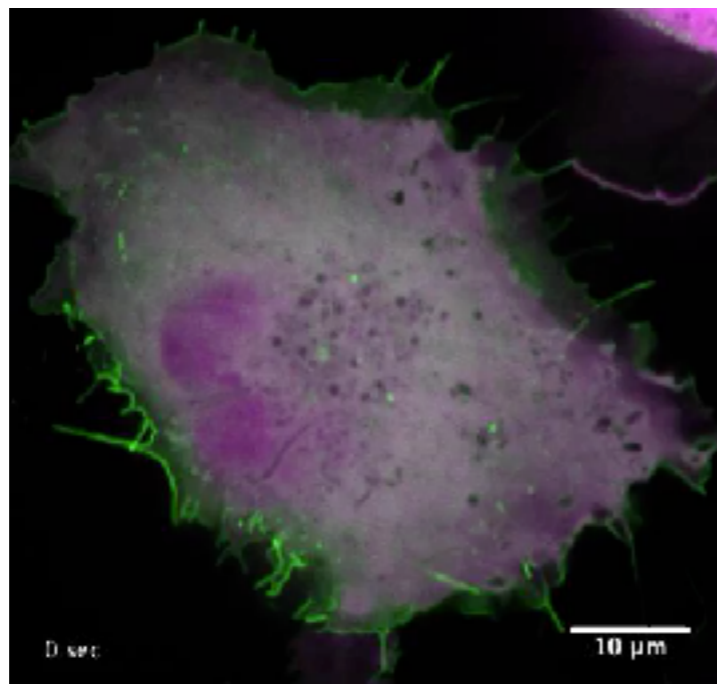
Crest V3 Spinning Disk  
Confocal



Yokogawa CSU-X1 Spinning  
Disk Confocal

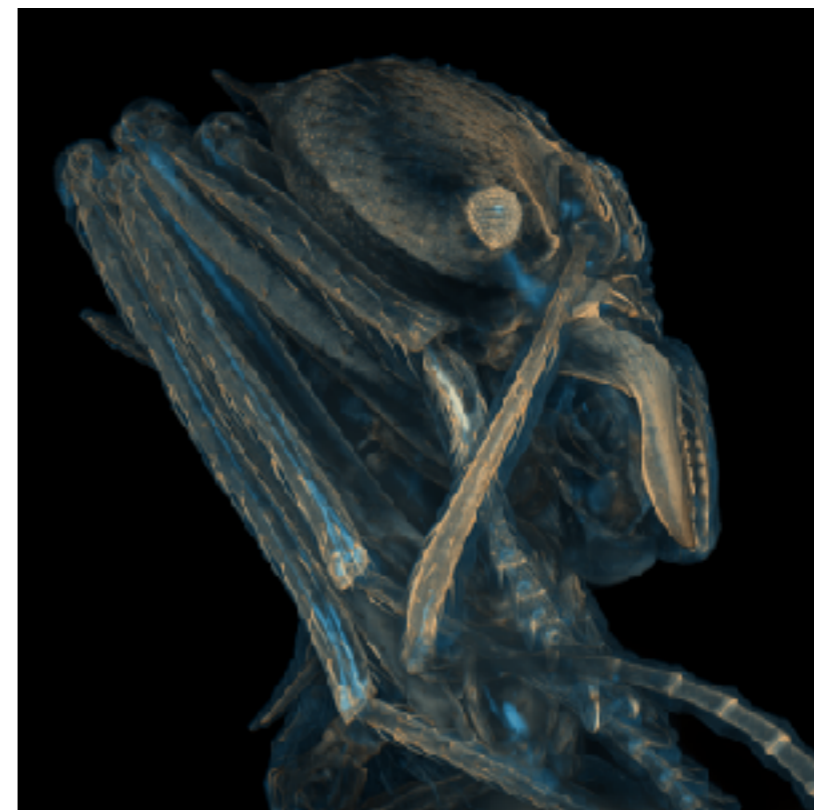
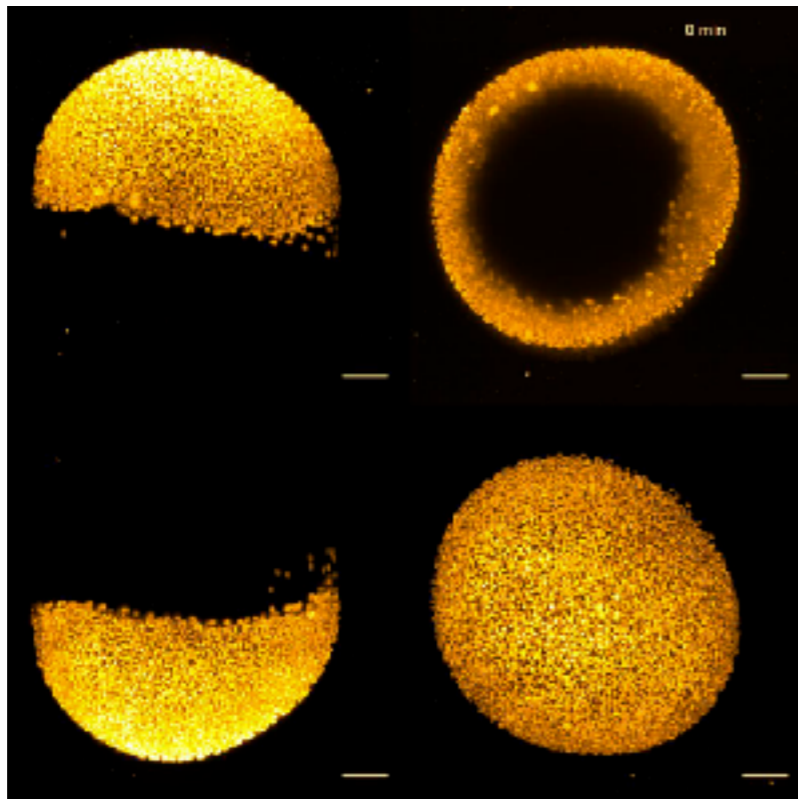
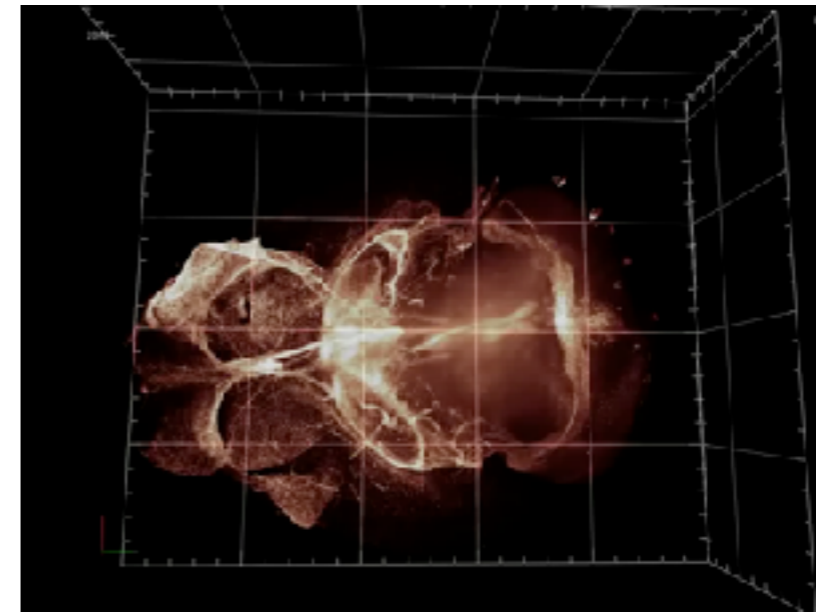


Vistech VT-iSIM



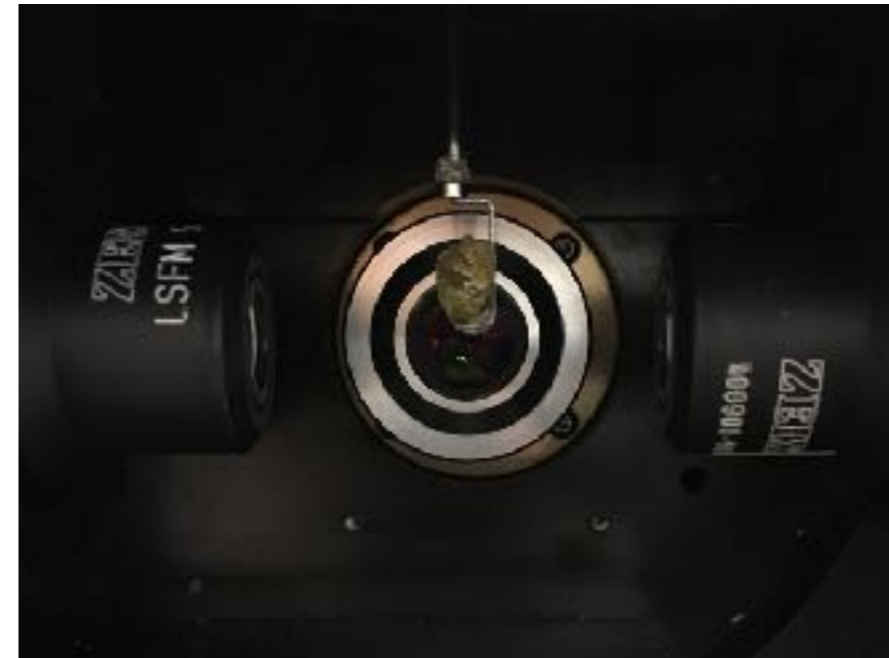
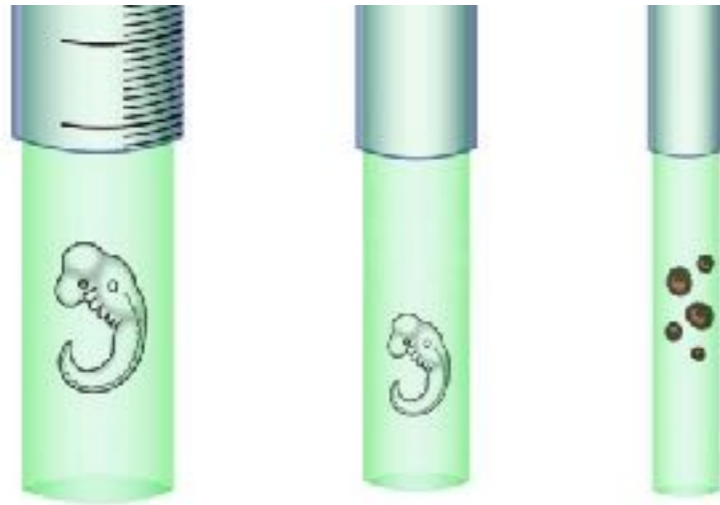


**Our core's Zeiss Lightsheet Z.1 is a specialized instrument designed for rapid confocal-like imaging of large samples (size range from ~0.5 mm - ~7 mm)**



# Sample preparation and mounting for the Zeiss lightsheet is very different than for conventional microscopes

Aqueous samples must be suspended in low-melt agarose



Cleared tissue is usually glued to a support



The sample is then lowered into this fluid-filled chamber for imaging.

**If you are interested in learning more about our microscopes or other services, check out our web site:**

**<https://www.med.upenn.edu/cdbmicroscopycore/>**