Arkansas Bioimaging Facility for Agricultural Research

Confocal Microscopy Workshop 1/9/2023

Fiona L. Goggin

Professor, Department of Entomology and Plant Pathology

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Agenda

- Foundational concepts for beginners (F. Goggin)
- Bio-imaging Facility (F. Goggin)
  - How to use our resources
  - Mini-grant program
- Coffee Break
- Presentation and Live Demo by Leica (~10:00-11:00, Dr. Qing Tang)
- Pizza! (~11:15AM)
- Walk to Plant Science 225

Foundational Concepts for Confocal Microscopy

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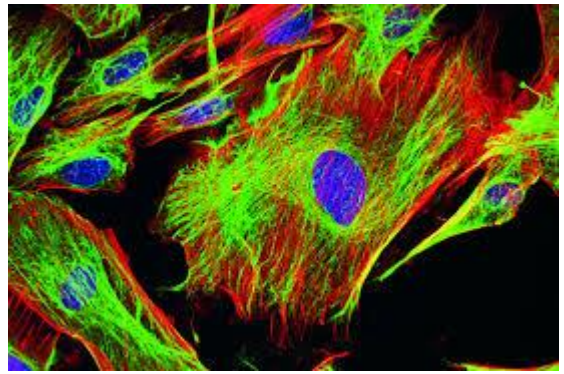
fgoggin@uark.edu
Arkansas Bioimaging Facility for Agricultural Research

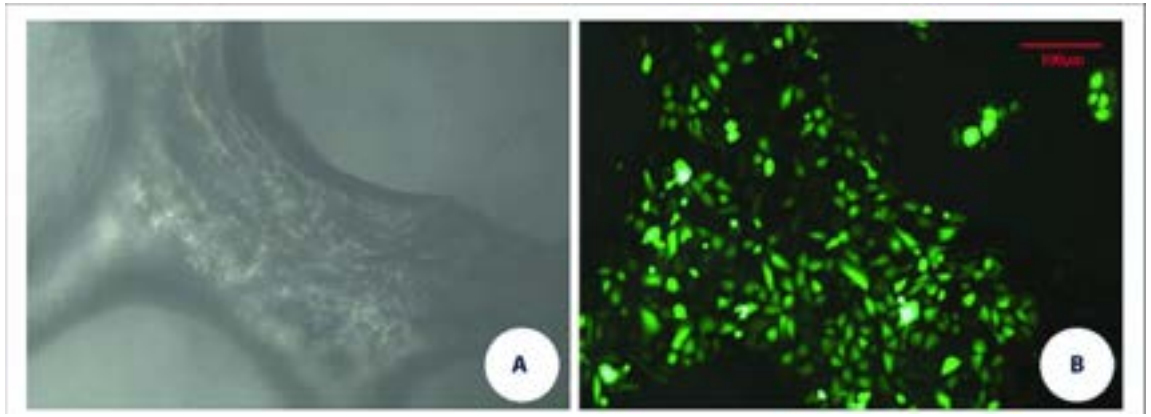
Confocal Microscopy

- **Light microscopy:** visualizes the absorption, reflection, and scattering of visible light by a sample
- **Fluorescence microscopy:** visualizes sample fluorescence
  - **Confocal microscopy:** a refinement on fluorescence microscopy that increases resolution and enables optical sectioning
    - Relies on computational image assembly
    - “False-color” images common



Confocal Microscopy

- Visualizes sample fluorescence
- No fluorescence, no image



Cai et al. 2016. Medical Science Monitor. 22. 4037-4045.

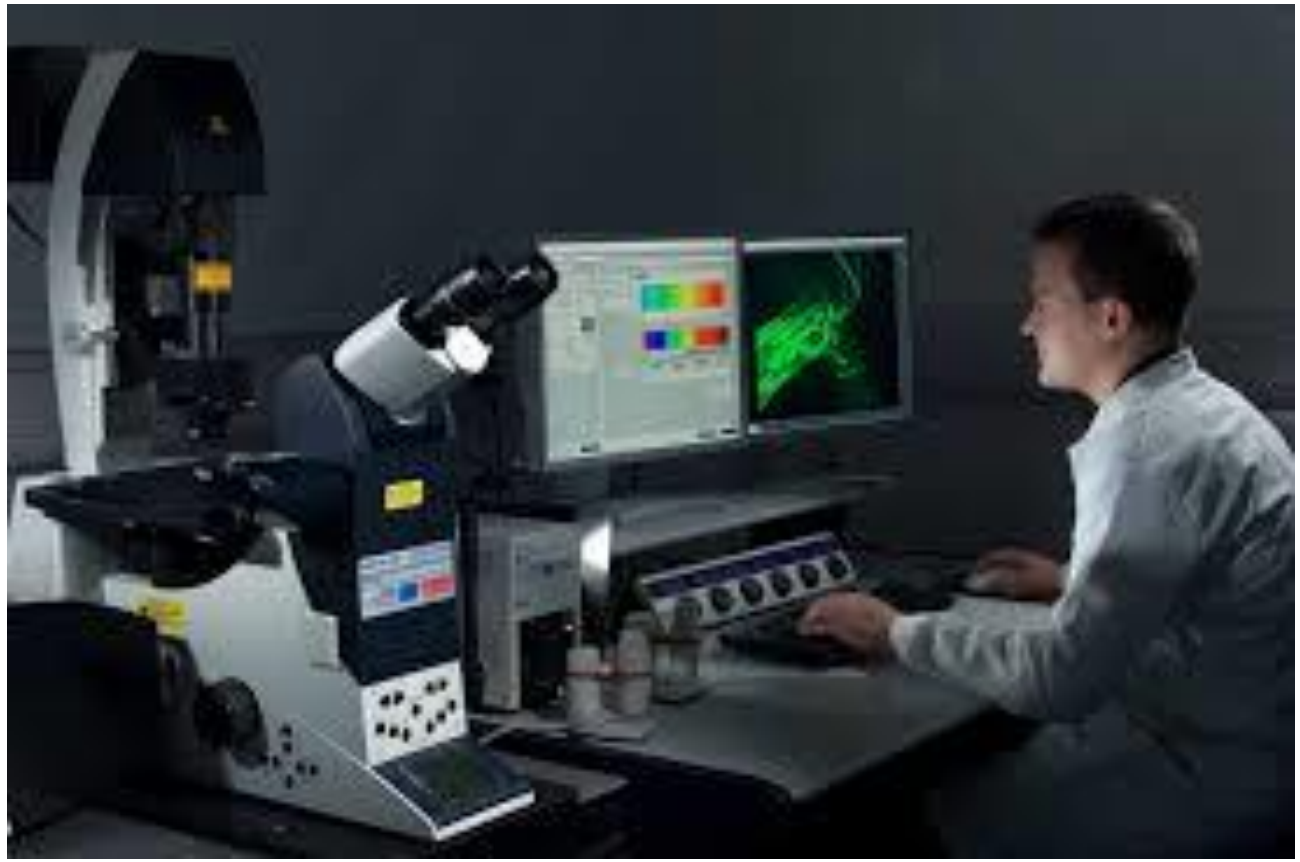
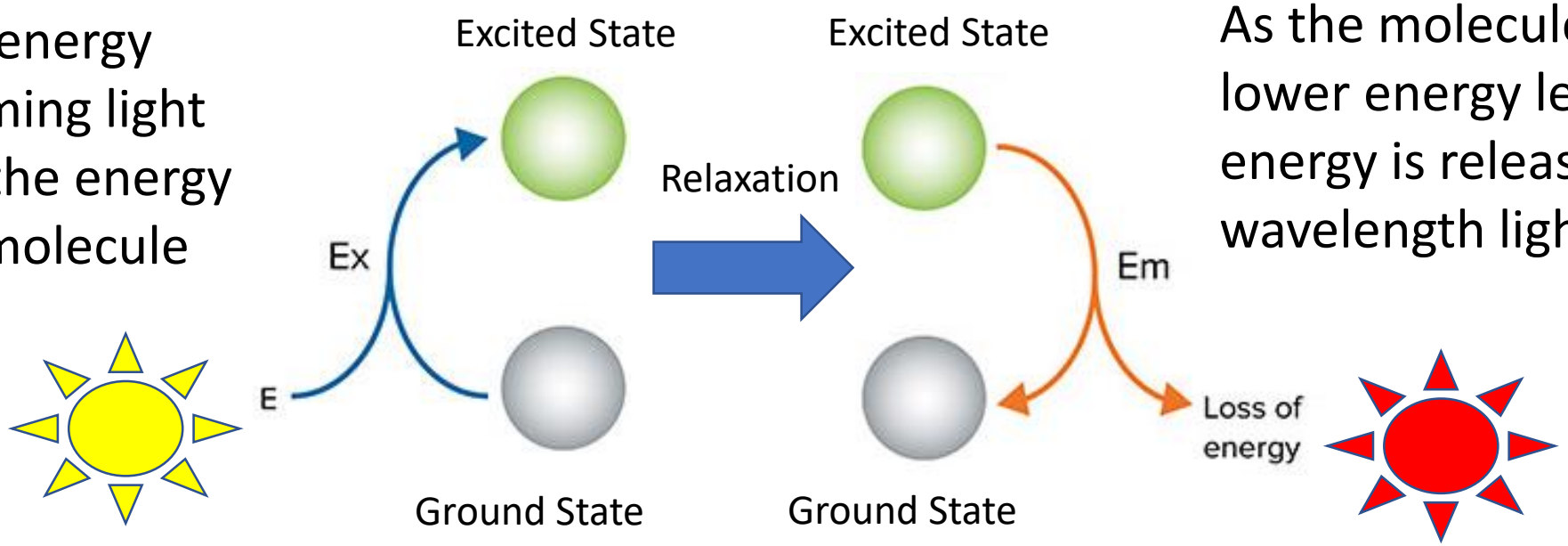


Image from https://warwick.ac.uk/services/ris/impactinnovation/impact/analyticalguide/confocal/

Fluorescence

- The property of absorbing light of a shorter wavelength and emitting light of longer wavelength

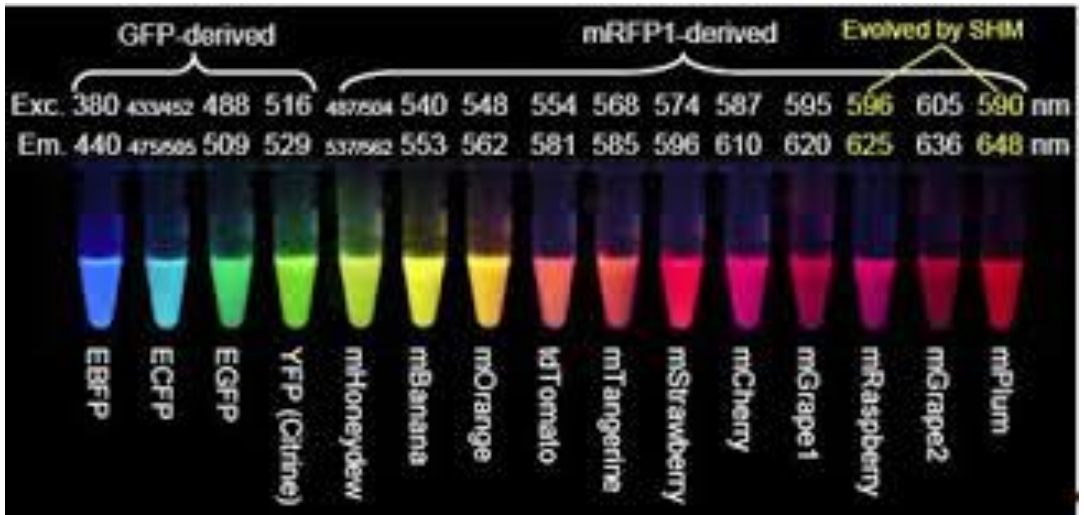
Excitation energy from incoming light increases the energy level of a molecule



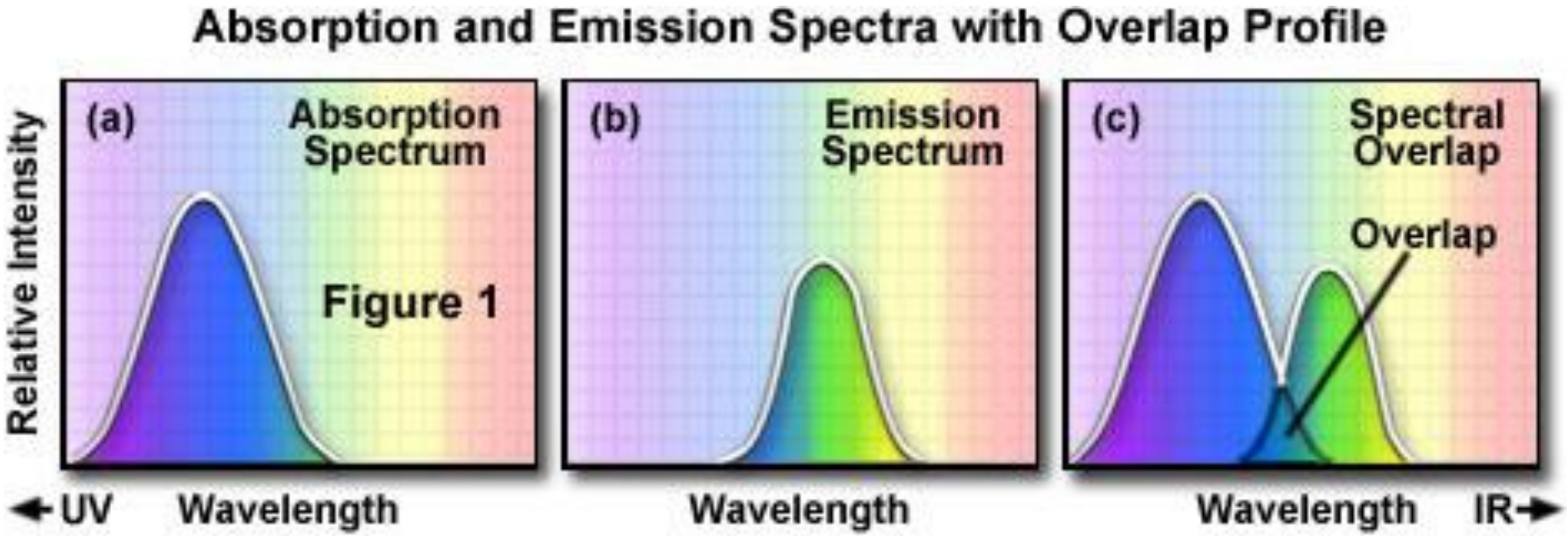
As the molecule returns to a lower energy level, the light energy is released as longer-wavelength light + heat

Fluorescence

- Fluorescent molecules = **fluorophores**
- Different fluorophores are responsive to different excitation wavelengths, so you must have the right light source
- Different fluorophores emit light of different wavelengths



Excitation and Emission Spectra



Many common fluorophores have overlapping spectra

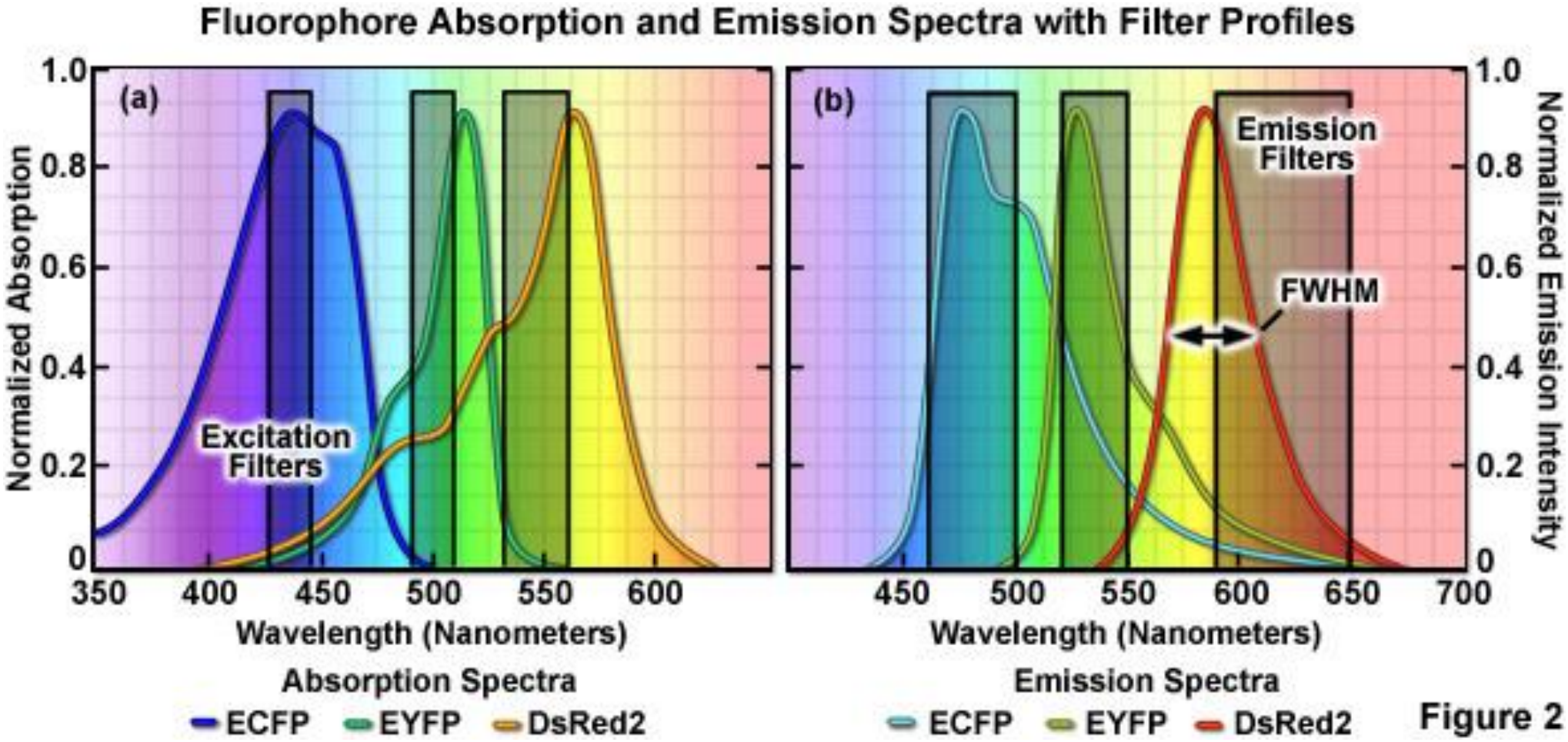


Figure 2

Fluorophores

- Occur in nature
- Can be used for visualization
- Responsible for “autofluorescence”

https://www.saj.usace.army.mil/Media/Images/igphoto/2000758877/

https://www.flickr.com/photos/nickadel/25070765234

Inchworm on rose mallow, white light

Inchworm on rose mallow, fluorescence

Chlorophyll Fluorescence

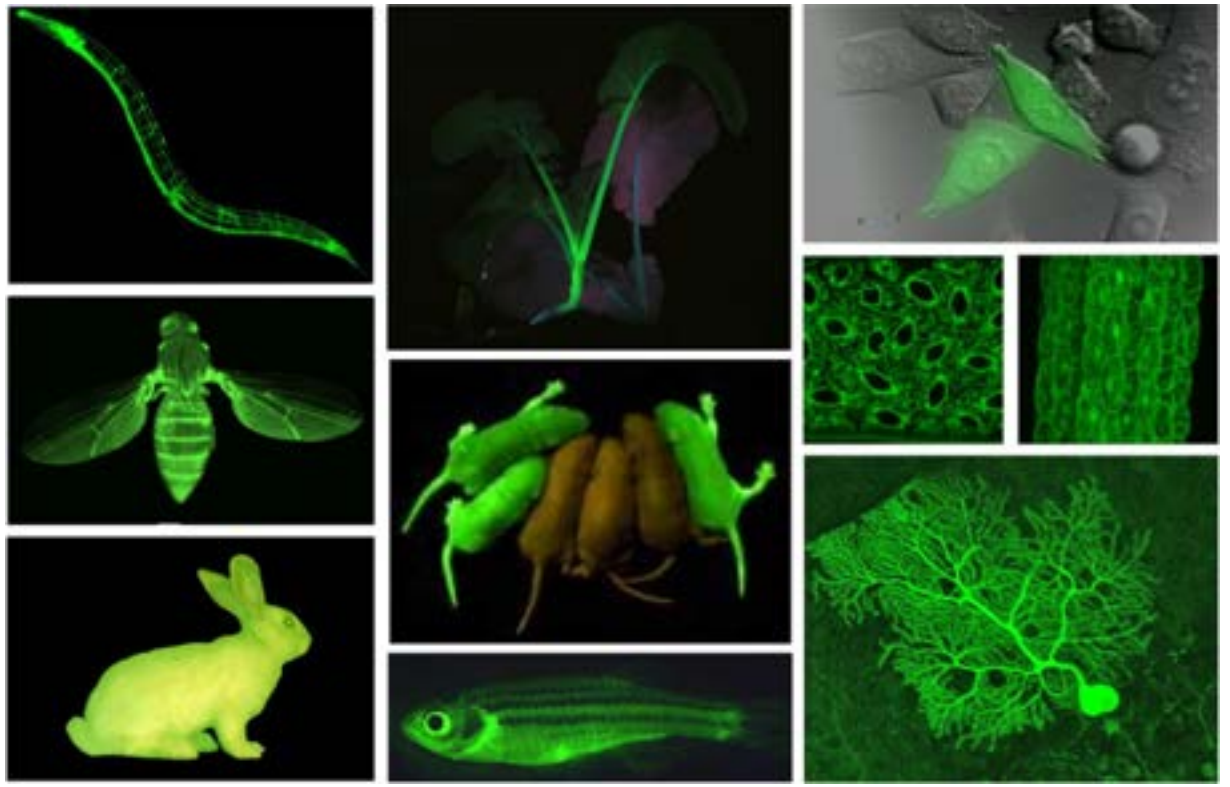
https://www.universityofcalifornia.edu/news/how-basic-research-jellyfish-led-unexpected-scientific-revolution

Green Fluorescent Protein (GFP)

https://nightsea.com/articles/fluorescence-photography-illuminates-chlorophyll/

Fluorophores

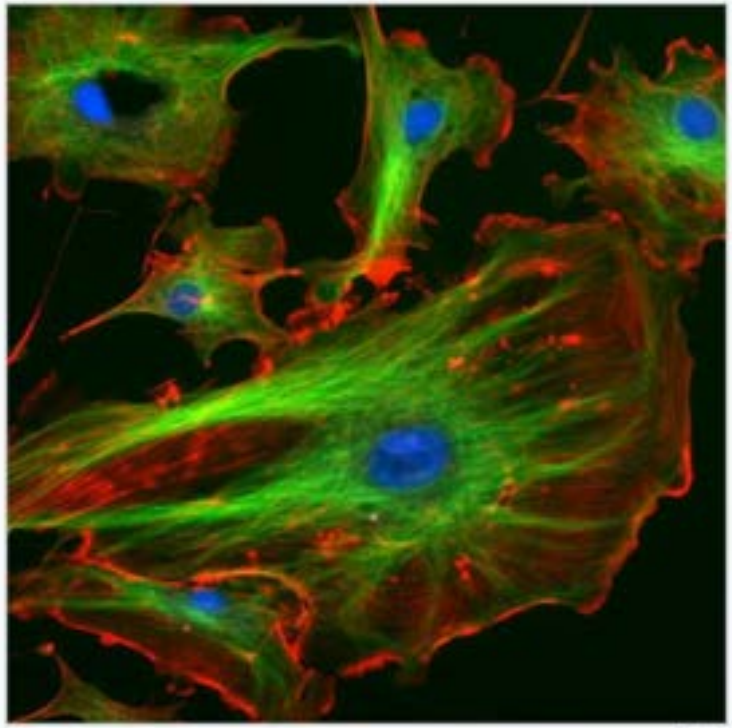
- Can be genetically engineered
  - Transgenic fluorophores can be:
    - Constitutive or inducible
    - Targeted to specific organs, developmental stages, or subcellular compartments



Chalfie, 2009. PNAS. 106 (25) 10073

Fluorophores

- Occur in nature
- Can be genetically engineered
- Or synthesized and added exogenously, for example:
  - DAPI labeling of DNA
  - Phalloidin labeling of actin filaments
  - FITC-labeled antibodies

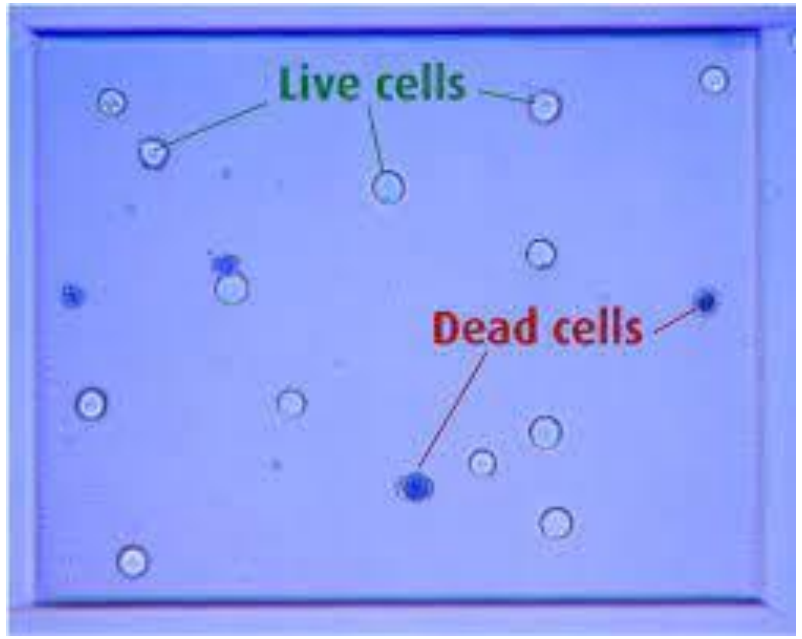


Endothelial cells stained with DAPI (blue), phalloidin (red) and through immunofluorescence via an antibody bound to fluorescein isothiocyanate (FITC) (green).

https://en.wikipedia.org/wiki/DAPI

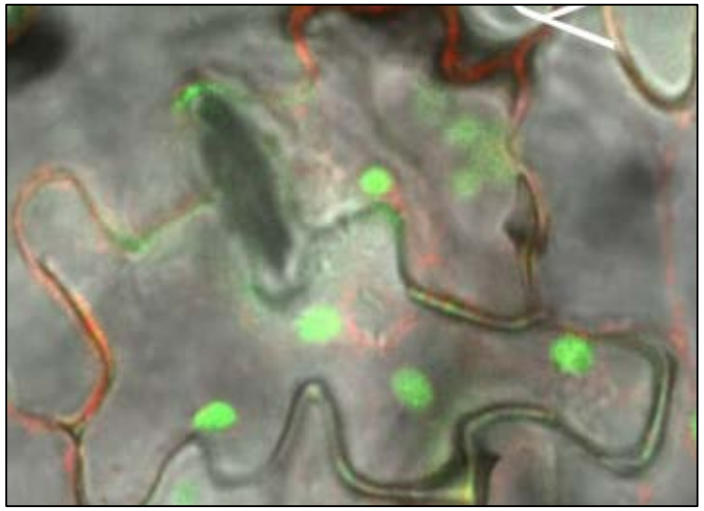
Exogenous Fluorophores

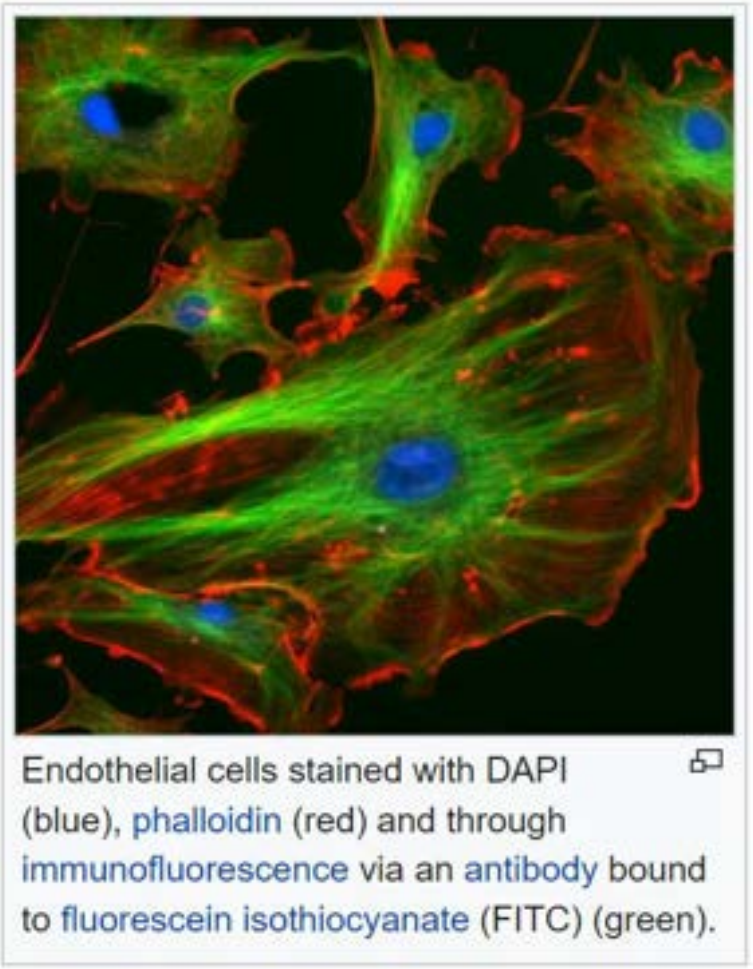
- Uptake by sample can be difficult or heterogenous, and often requires optimization. It is influenced by:
  - Whether label is water-soluble or membrane permeable
  - Sample thickness
- Some assays take capitalize on barriers to uptake
  - Dead cell stains, e.g. Trypan Blue
  - Intracellular vs. extracellular probes



Fluorophores

- May be used in combination
- Help identify cellular “landmarks” and spatial relationships among features of interest
- Can be overlaid onto brightfield images



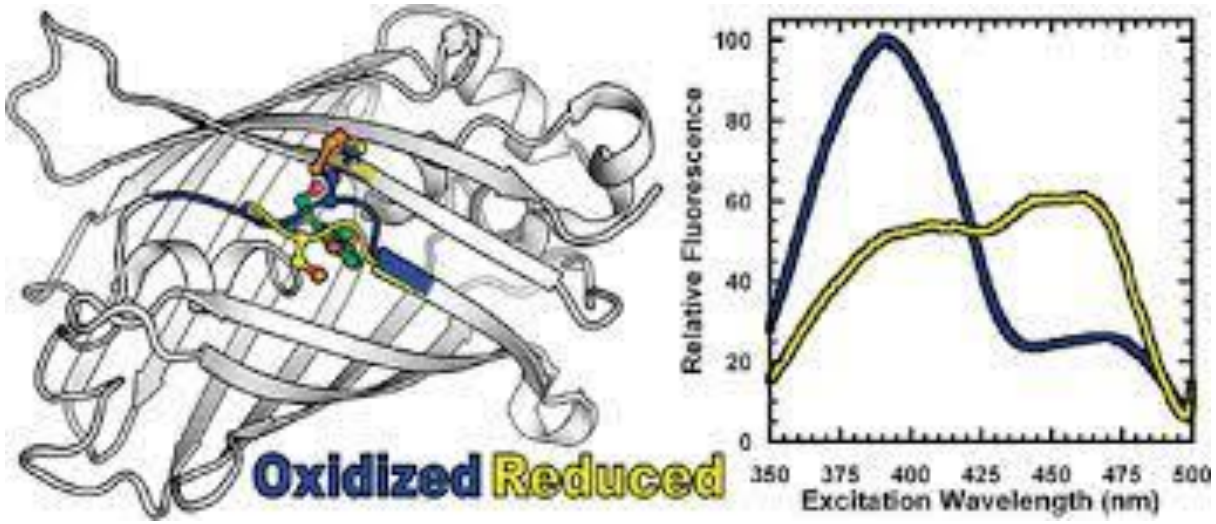


Endothelial cells stained with DAPI (blue), phalloidin (red) and through immunofluorescence via an antibody bound to fluorescein isothiocyanate (FITC) (green).

https://en.wikipedia.org/wiki/DAPI

Fluorophores may be:

- Responsive to their environmental conditions or interactions with other molecules



Redox-sensitive Fluorescent Probe

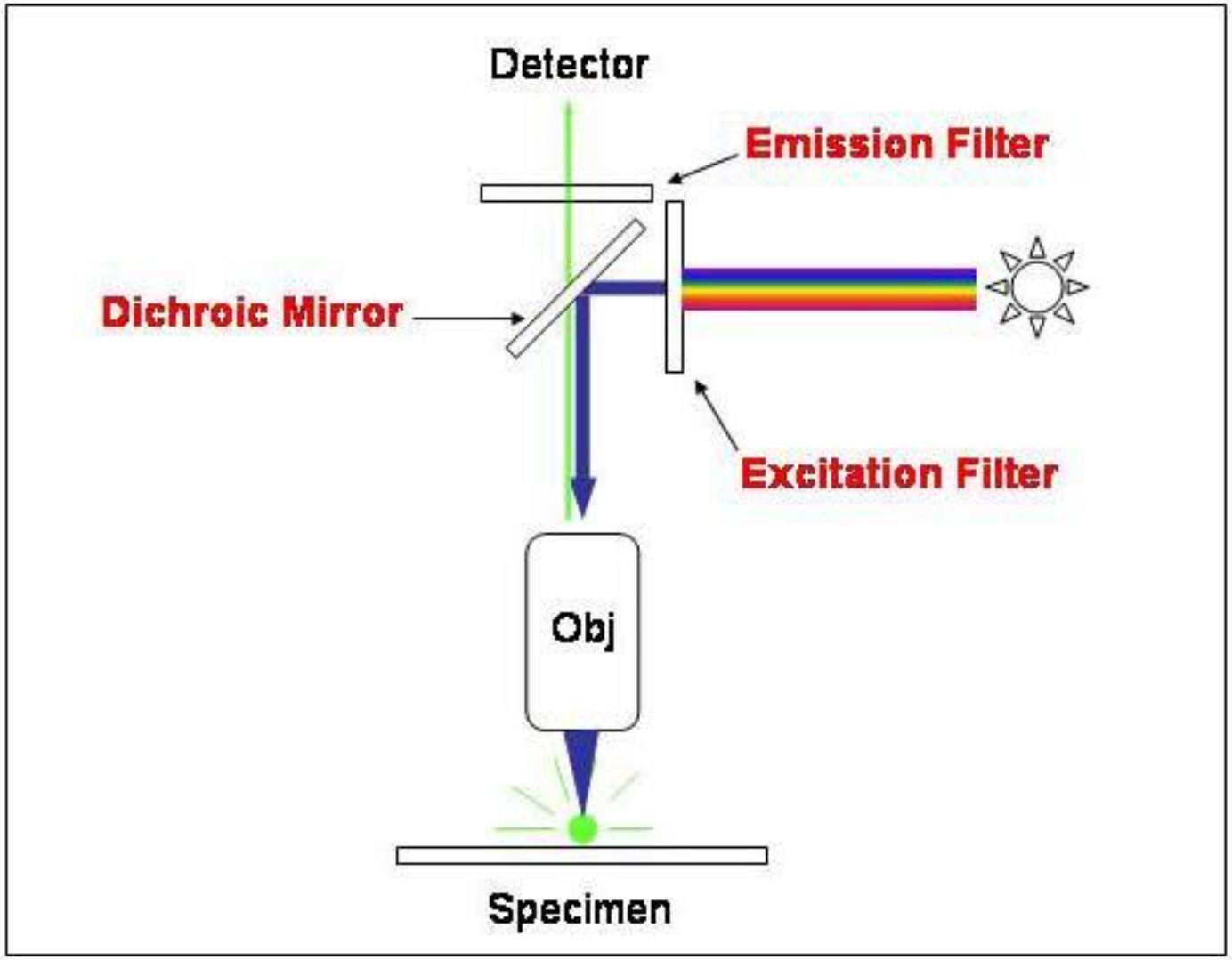
What kinds of questions can be addressed by fluorescence imaging?

- Detection and localization of any molecules for which antibodies or other specific probes are available
- Monitoring of changes in cellular conditions (eg. Redox status, calcium fluxes, pH, etc)
- Observation of the timing and location of gene expression
- Visualization of molecular trafficking, colocalization, and intermolecular interactions
- And many more……

How is Fluorescence Visualized?

Standard Fluorescence Microscopy

- Light source for excitation of fluorophore
- Dichroic mirror to prevent false detection of excitation light source
- Detector for emitted light with emission filter



Selection of Excitation & Emission Filters

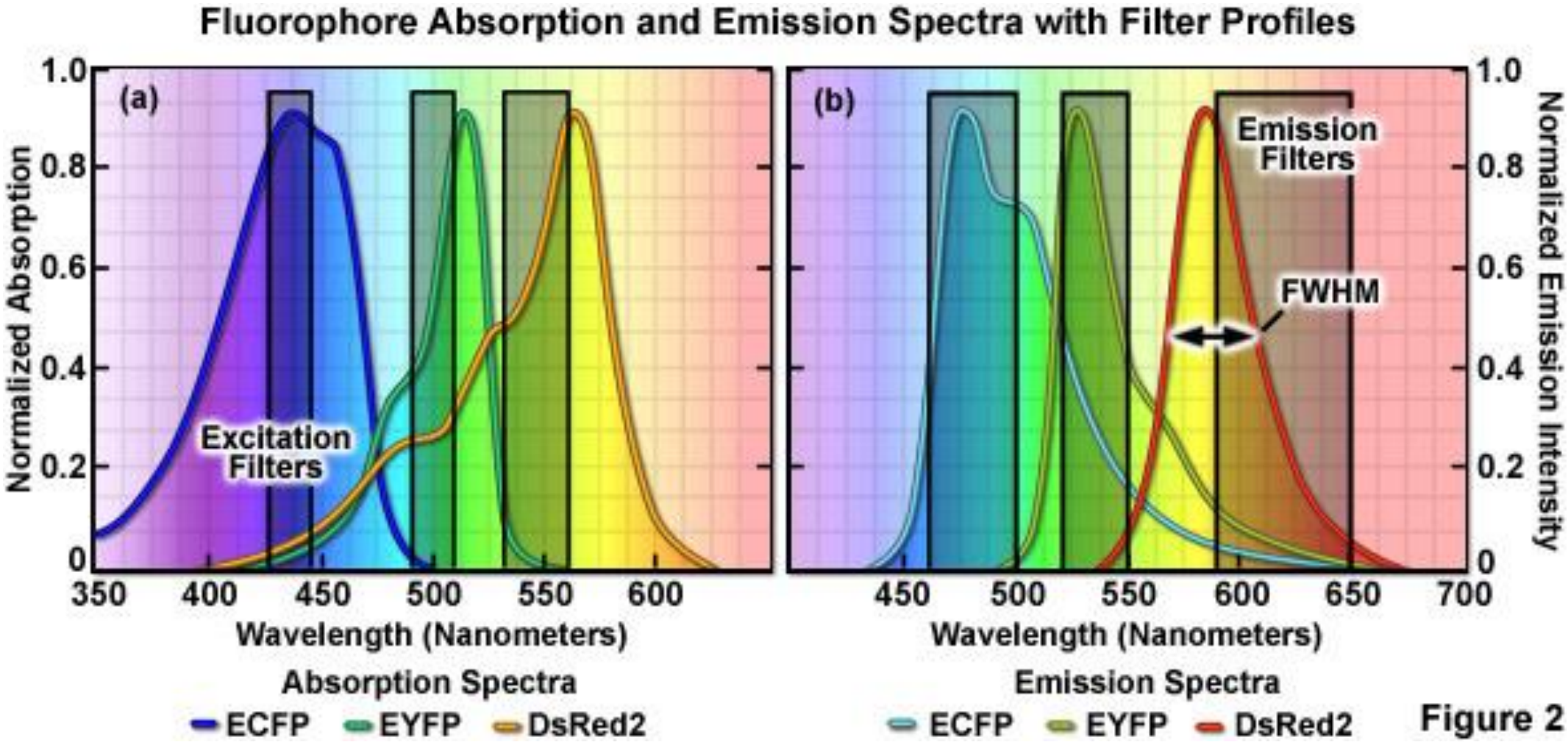
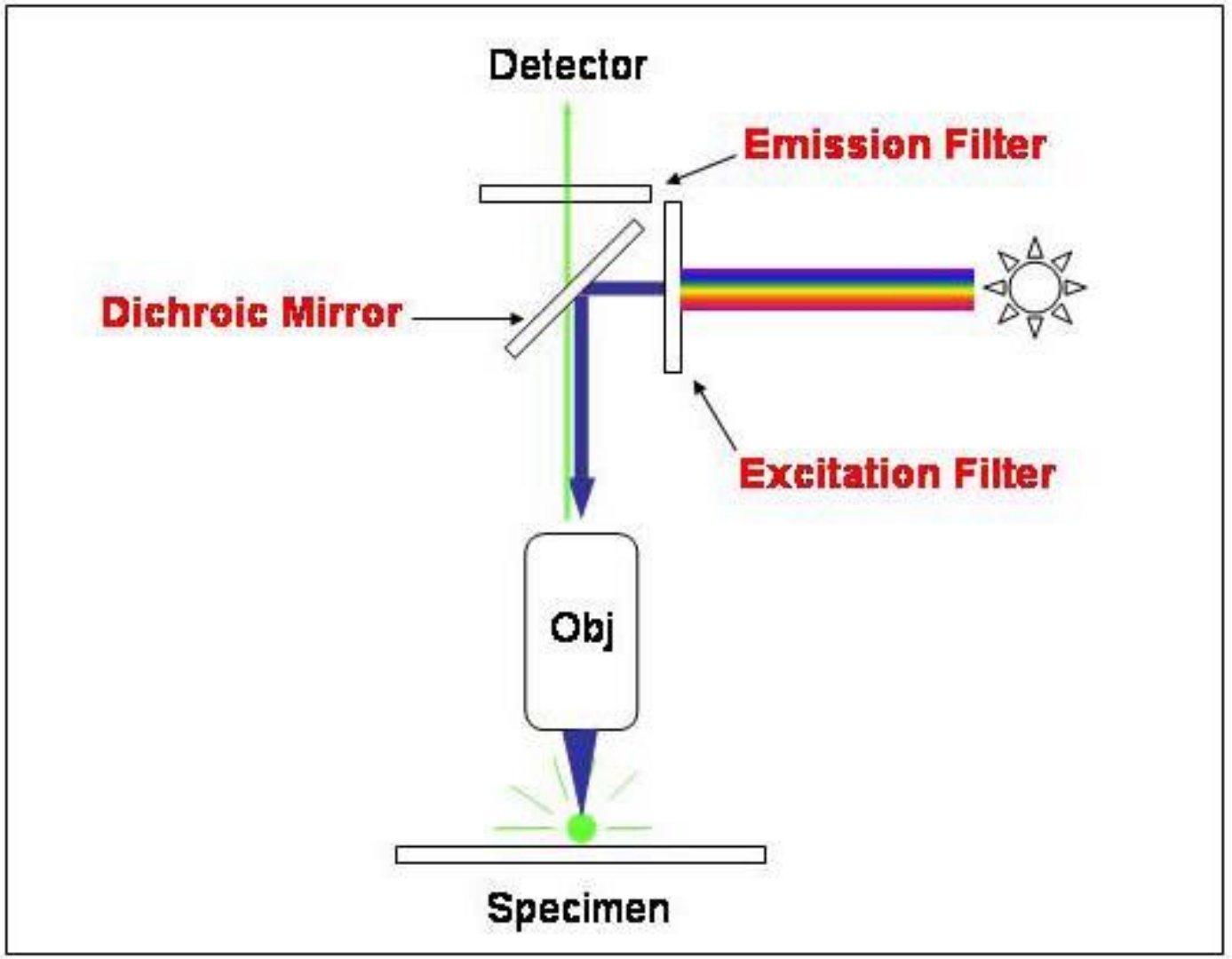
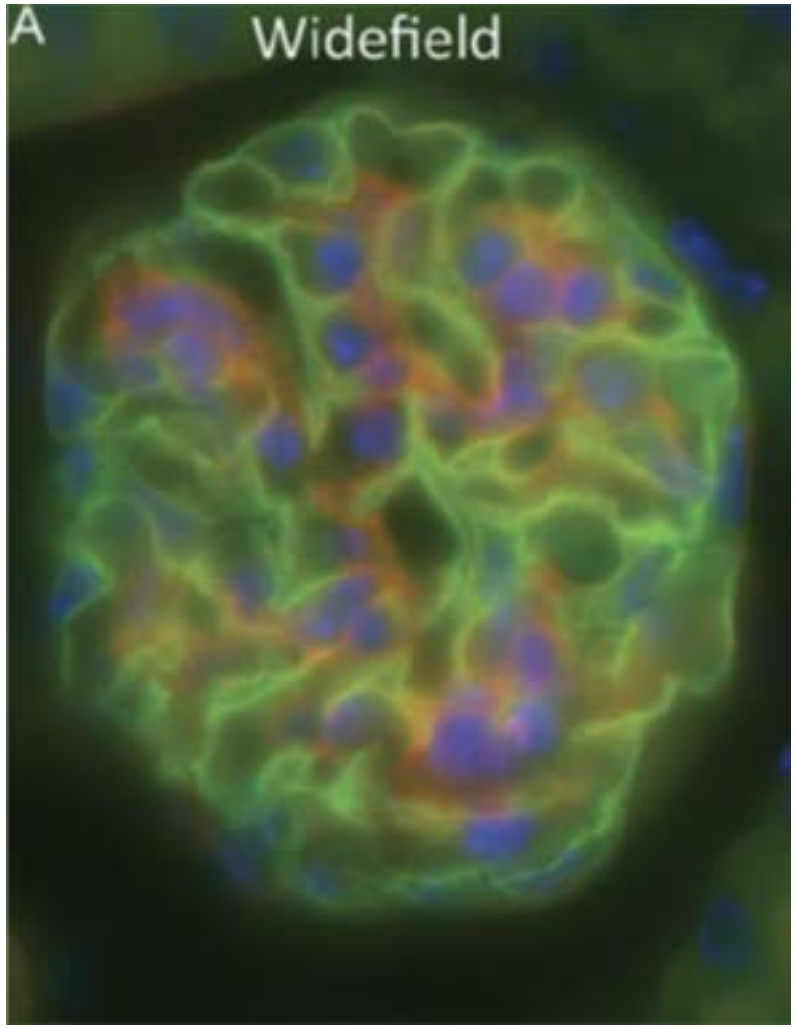


Figure 2

Standard Fluorescence Microscopy

- Issue: detector captures light from multiple planes of focus





Axiom Optics

Confocal Microscopy

- Compared to conventional fluorescence microscopy, it:
  - has increased resolution
  - enables optical sectioning

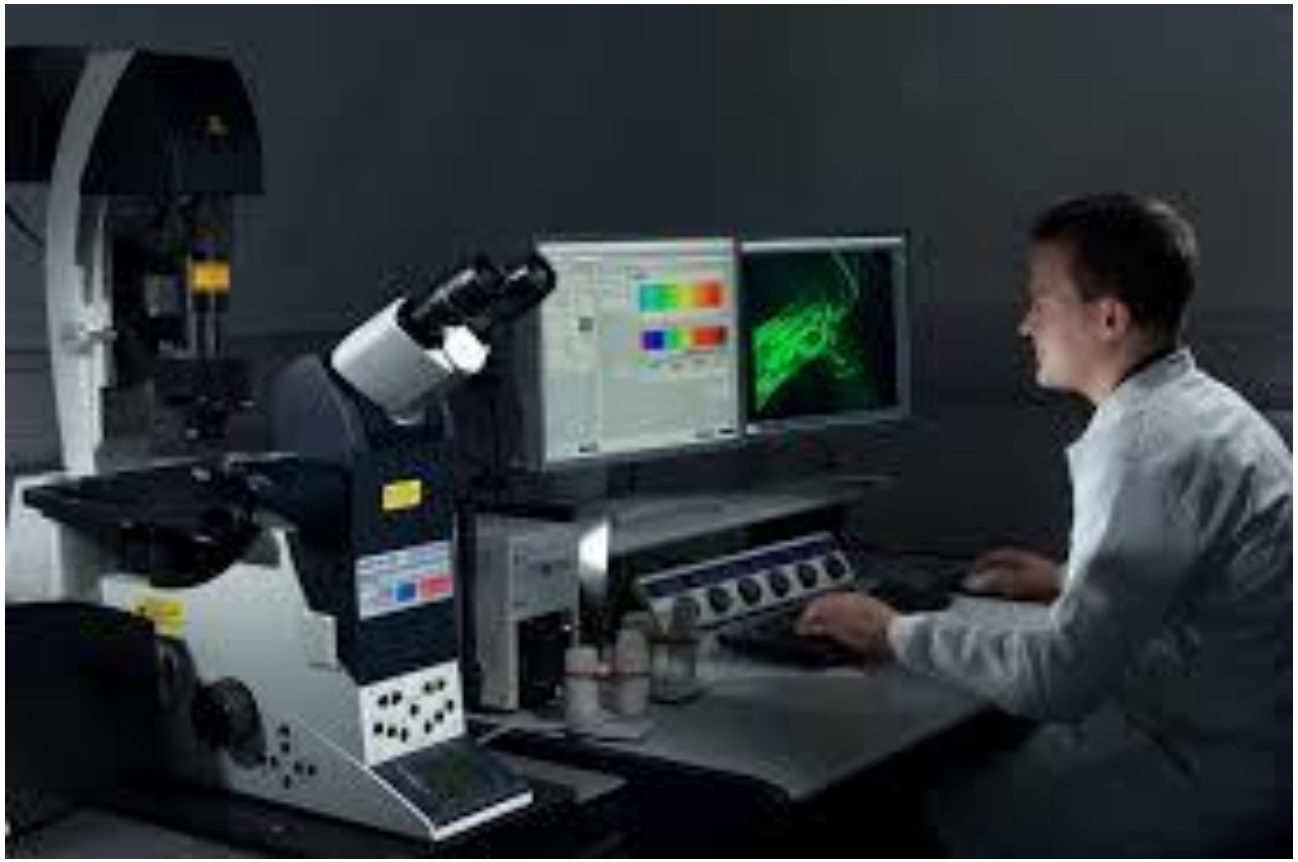
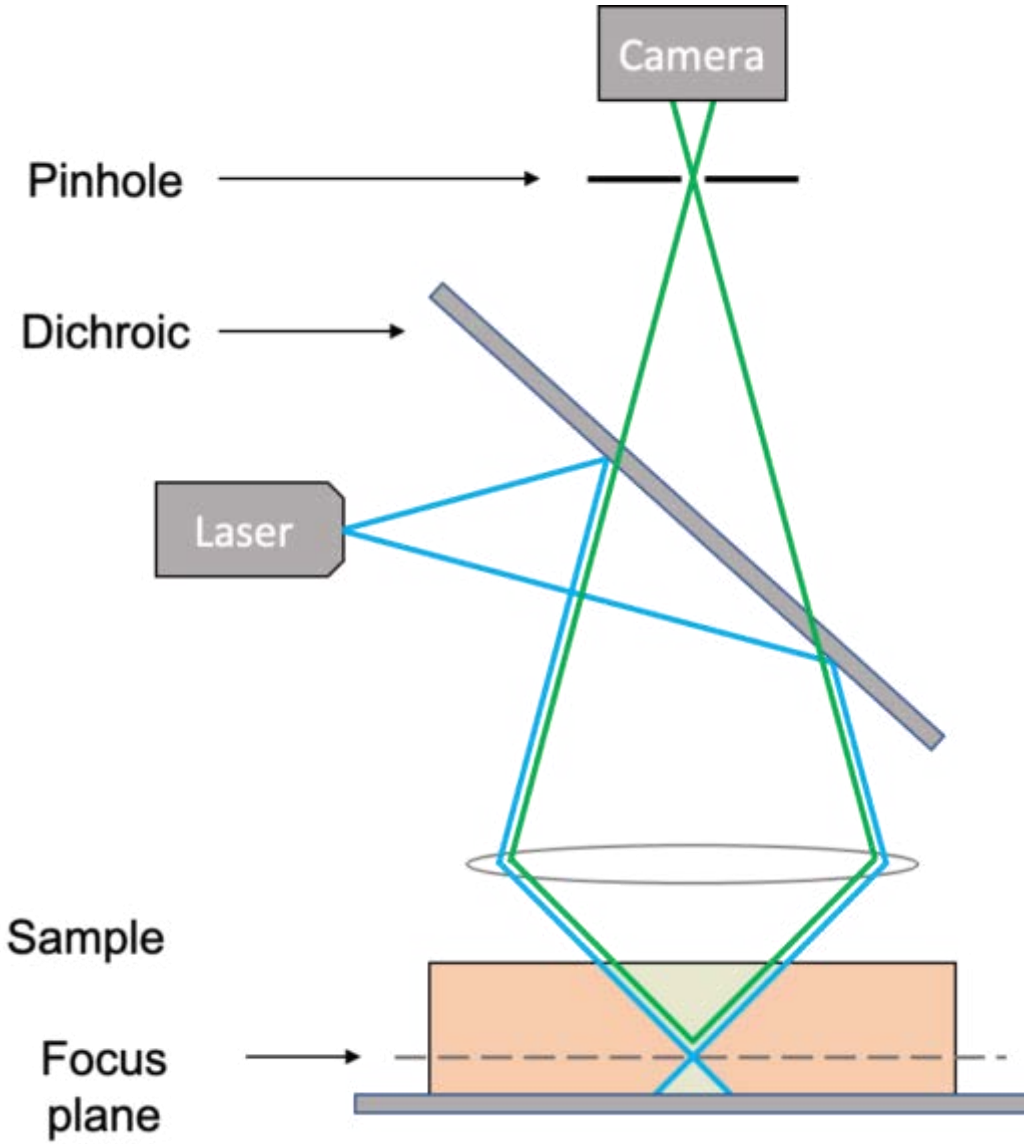


Image from https://warwick.ac.uk/services/ris/impactinnovation/impact/analyticalguide/confocal/

- Video snippet from
- https://www.youtube.com/watch?v=nzQzyuVwils

Confocal Microscopy

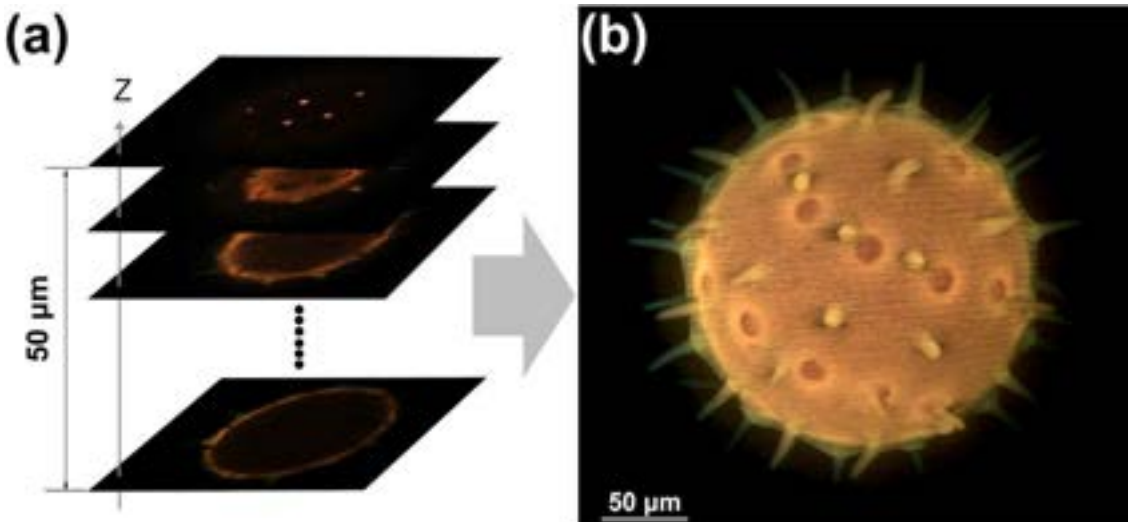
- Excitation wavelengths provided by laser
- Pinhole focuses on a single plane of focus before emitted light reaches detector

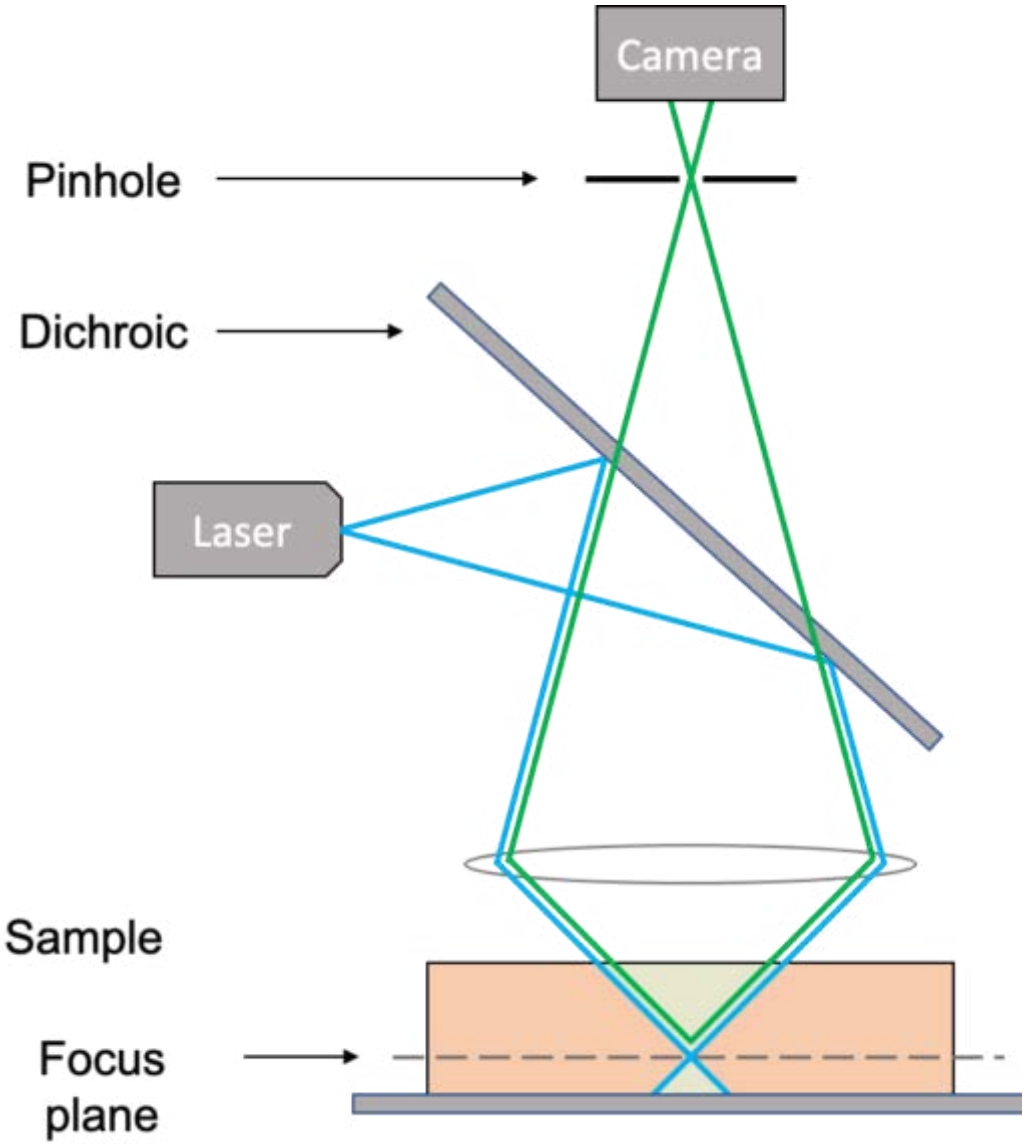


Axiom Optics

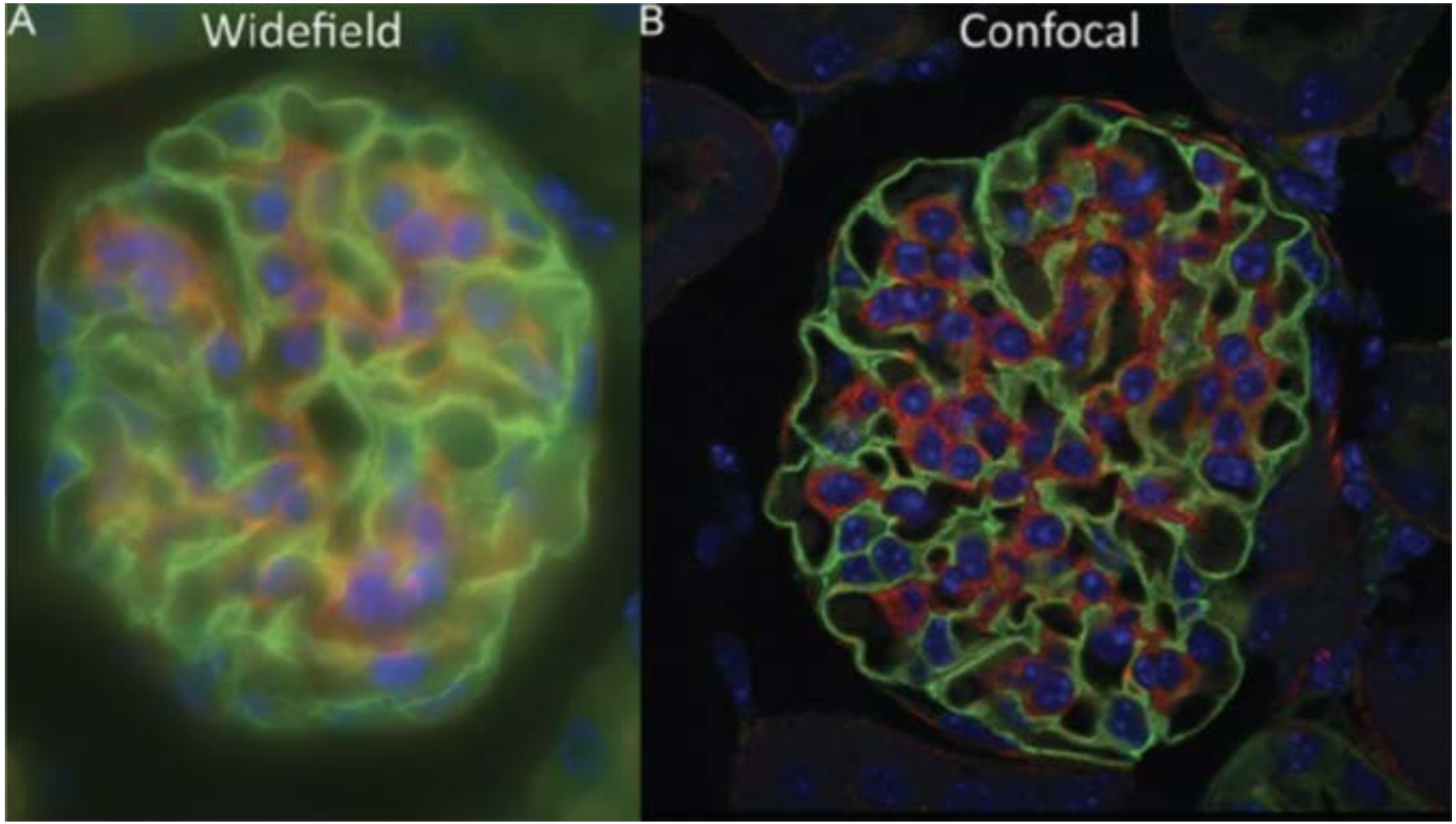
Confocal Microscopy

- Pinhole focuses on a single plane of focus
- Images can be taken at multiple focal planes and computationally stitched together (optical sectioning)





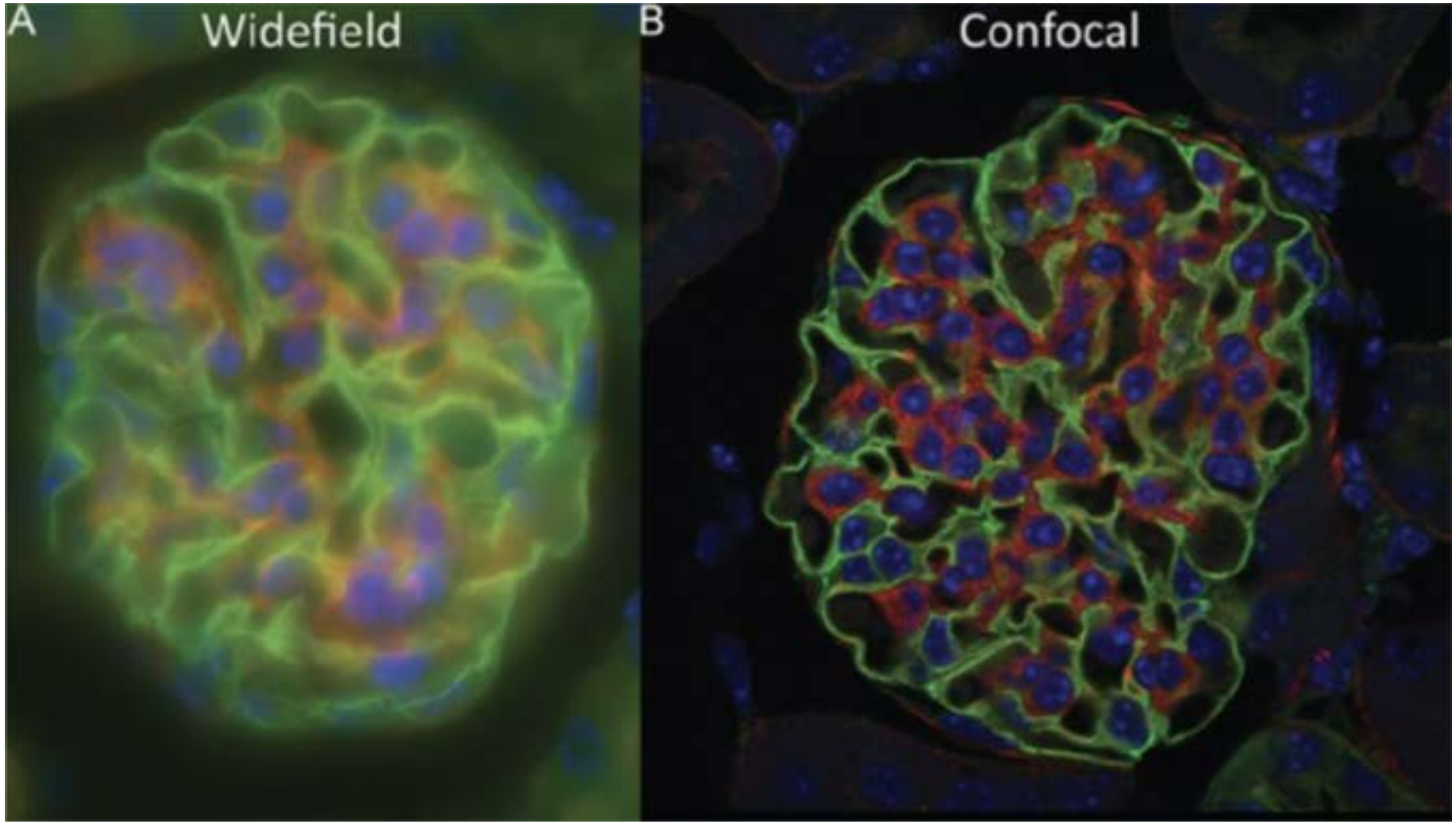
Axiom Optics



Axiom Optics

Confocal Microscopy

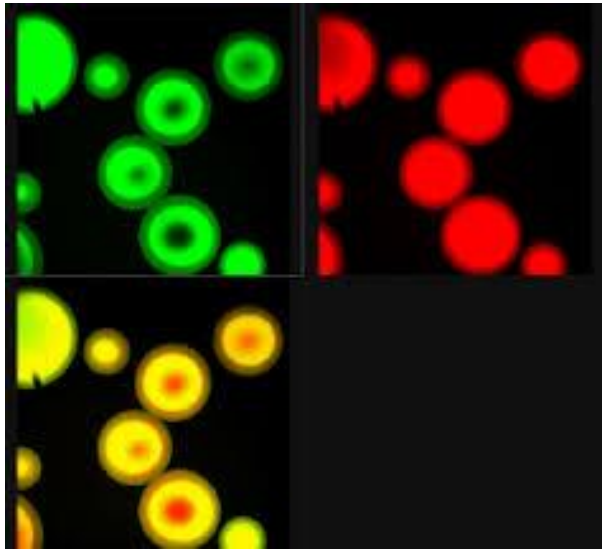
- Reduces need for tissue sectioning
- Facilitates imaging of live tissues

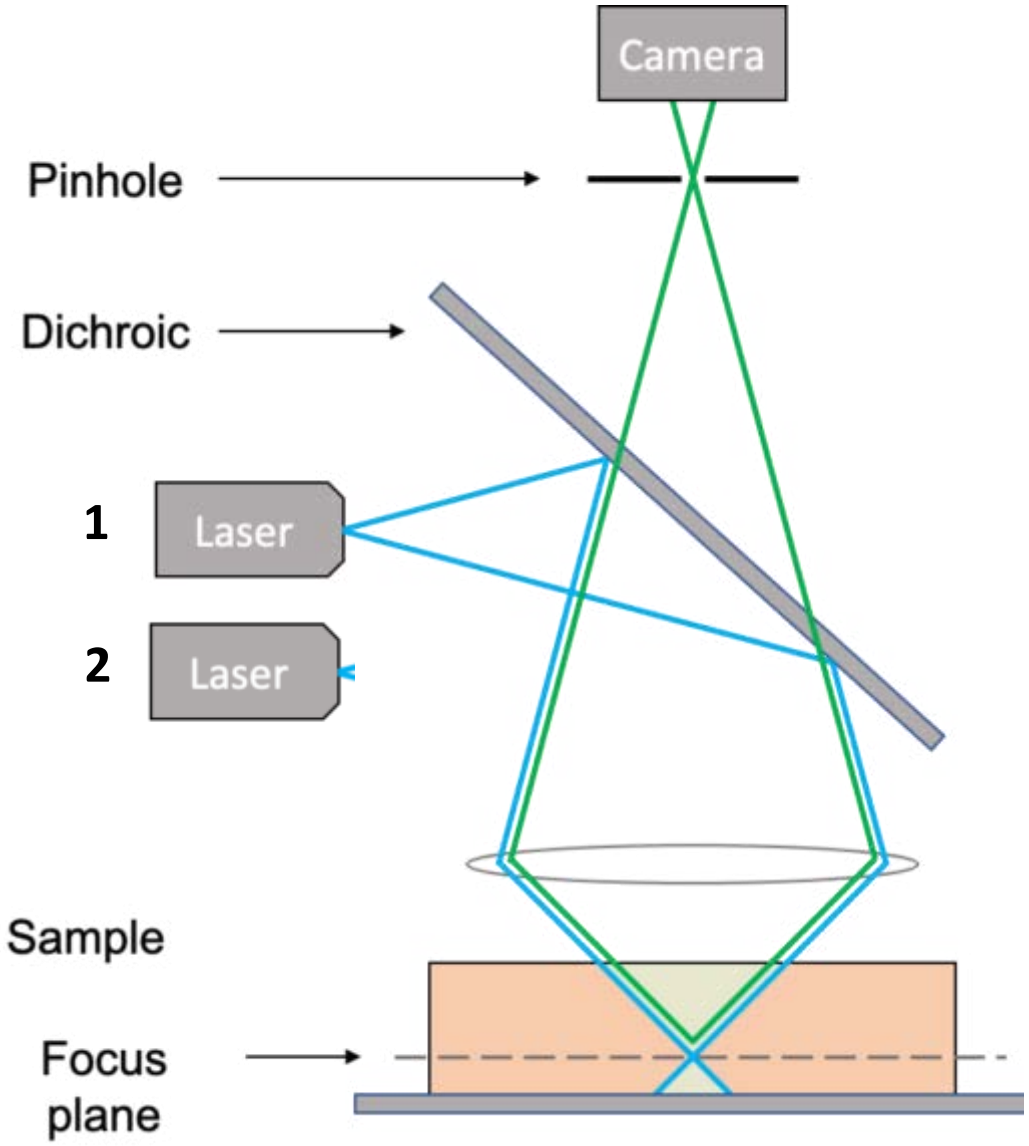


Axiom Optics

Confocal Microscopy

- Multiple fluorophores can be imaged in the same sample through multiple detectors and/or sequential imaging with different excitation lasers





Axiom Optics

Challenges for Confocal Microscopy

- Selecting fluorophores compatible with the excitation capabilities of your system

Challenges for Confocal Microscopy

- Selecting fluorophores compatible with the excitation capabilities of your system
- Distinguishing fluorophores with overlapping spectra
- Discriminating signal from background autofluorescence

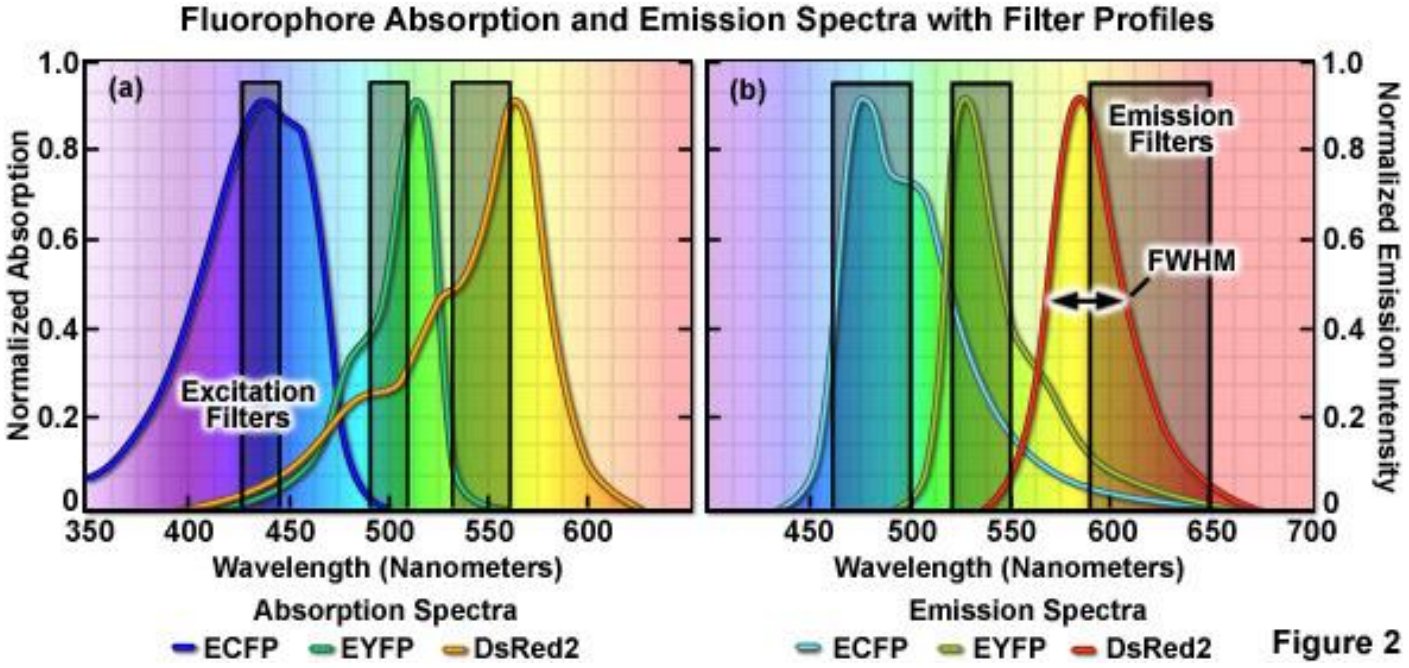


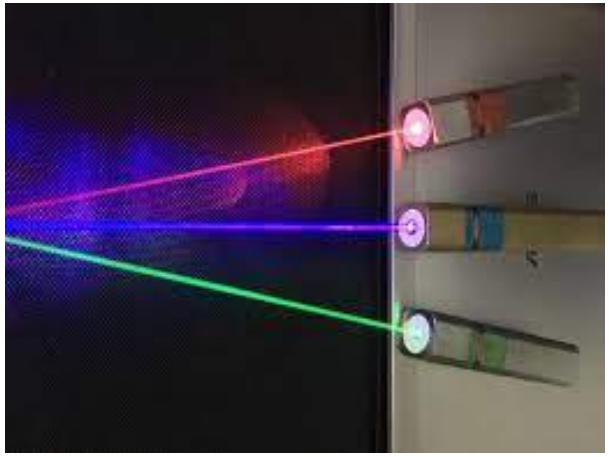
Figure 2

Challenges for Confocal Microscopy

- Selecting fluorophores compatible with the excitation capabilities of your system
- Distinguishing fluorophores with overlapping spectra
- Discriminating signal from background autofluorescence
- Detecting and quantifying low-abundance fluorophores

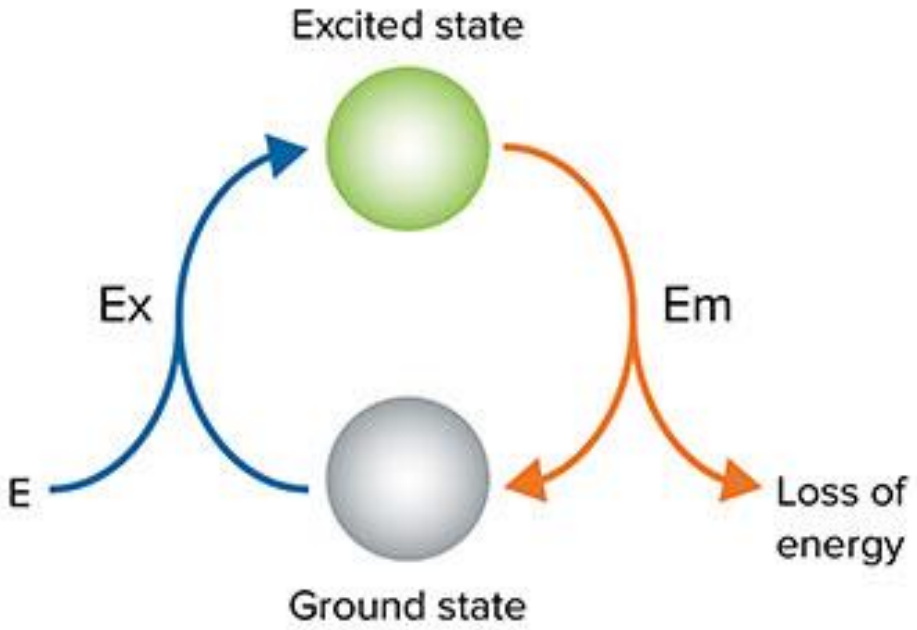
Leica Stellaris 8

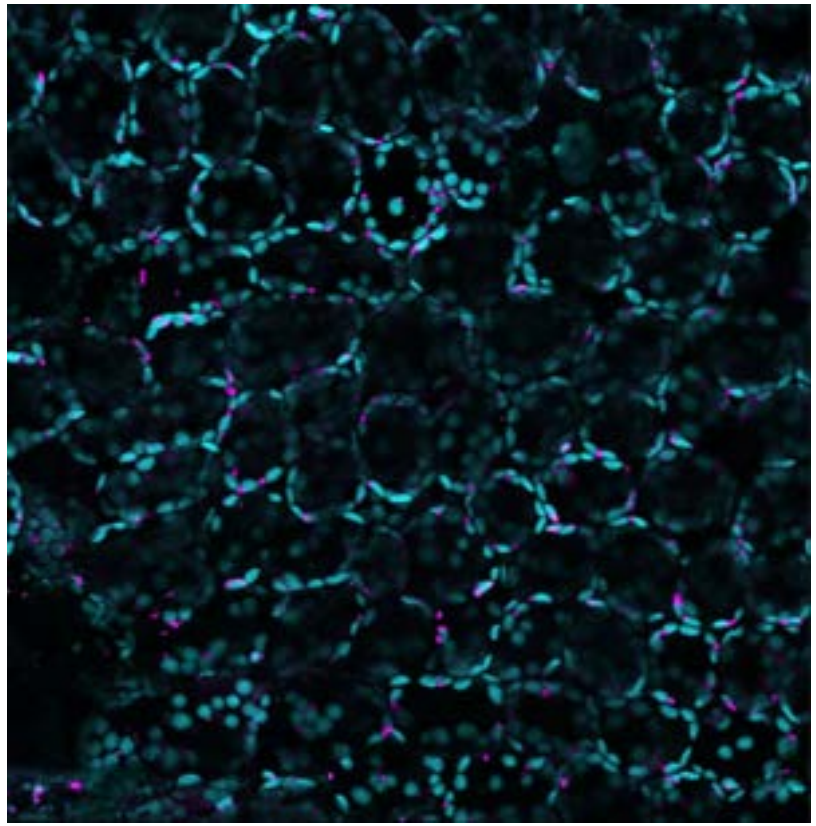
- White Light Laser for customization of excitation wavelengths
  - Allows imaging of a wider range of natural and synthetic fluorophores
  - Facilitates optimization of the imaging of novel fluorophores (lambda scan sequentially tests different excitation wavelengths to ID optimal excitation)

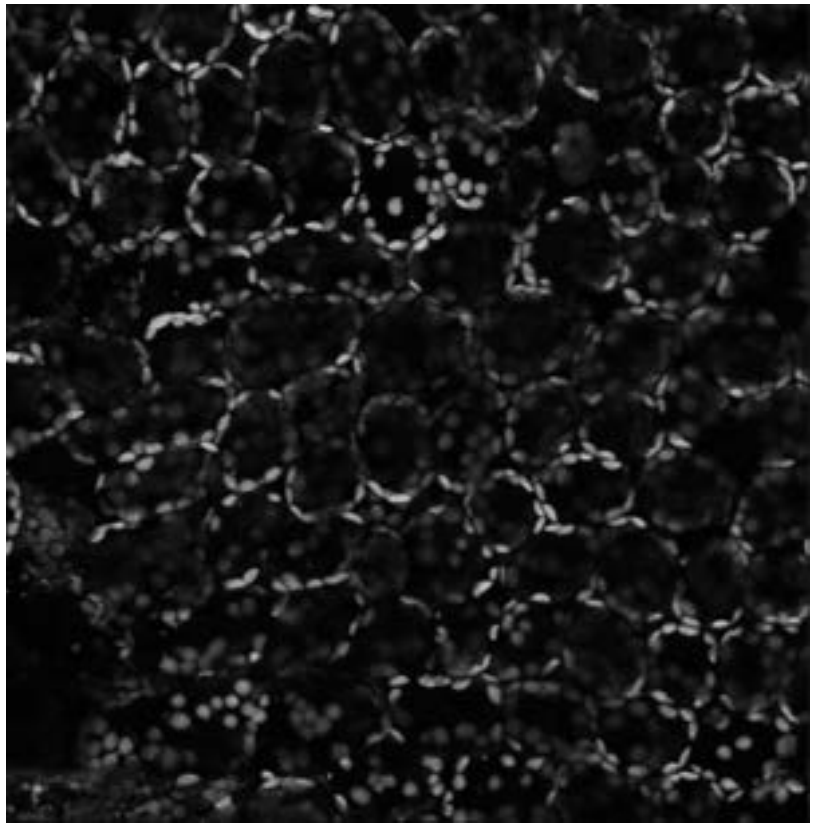


Leica Stellaris 8

- Tausense for discrimination of overlapping emissions on the basis of <u>timing</u> of light emission, not just wavelength of emission





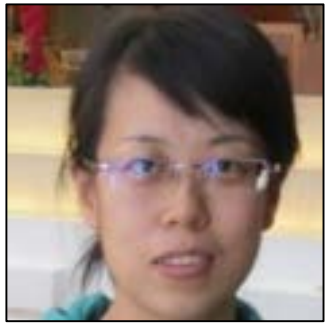


Challenges for Confocal Microscopy

- Selecting fluorophores compatible with the excitation capabilities of your system
- Distinguishing fluorophores with overlapping spectra
- Discriminating signal from background autofluorescence
- Detecting and quantifying low-abundance fluorophores

Arkansas Bioimaging Facility for Agricultural Research

- Located in Plant Science 225
- Funded by USDA NIFA (#2021-05017), UAF, and the Arkansas Bioscience Institute
- Facility Manager: Dr. Jiamei Li
  - jxl080@uark.edu



Jiamei Li

Sami Dridi, POSC | Martin Egan, ENPL | Mary Savin, CEMB





Alejandro Rojas ENPL | Vibha Srivastava CEMB | Jiangchao Zhou ANSC

Arkansas Bioimaging Facility for Agricultural Research

- Leica Stellaris 8 confocal microscope
  - Tunable white light laser: excitation range from 440 to 790 nm
  - 405 nm Diode Laser for UV
  - 3 HyD detectors
  - DMi8 inverted microscope
  - 20X, 40X, and 60X water objectives
  - Okolab humidity chamber
  - LAS X software, including Tausense module for using photon arrival time data

Arkansas Bioimaging Facility for Agricultural Research

- **Other Resources**
  - Olympus SXZ16 Fluorescent Stereoscope equipped with filters to image GFP and m-Cherry fluorescence
  - Olympus BH2 and Nikon Optiphot 2 Epifluorescence Microscopes equipped with filters to visualize DAPI, GFP and RFP fluorescence
  - Free Usage

Arkansas Bioimaging Facility for Agricultural Research

- **Other resources**
  - Biotek Cytation3 Multimode cell imaging system with plate reader and filter cubes for imaging GFP, YFP, and DAPI
    - chrome-extension://efaidnbmnnnibpcajpcglclefindmkaj/https://www.biotek.com/products/literature/Cytation3_Microscopy_Single_Sheet_English_low_res.pdf

Arkansas Bioimaging Facility for Agricultural Research

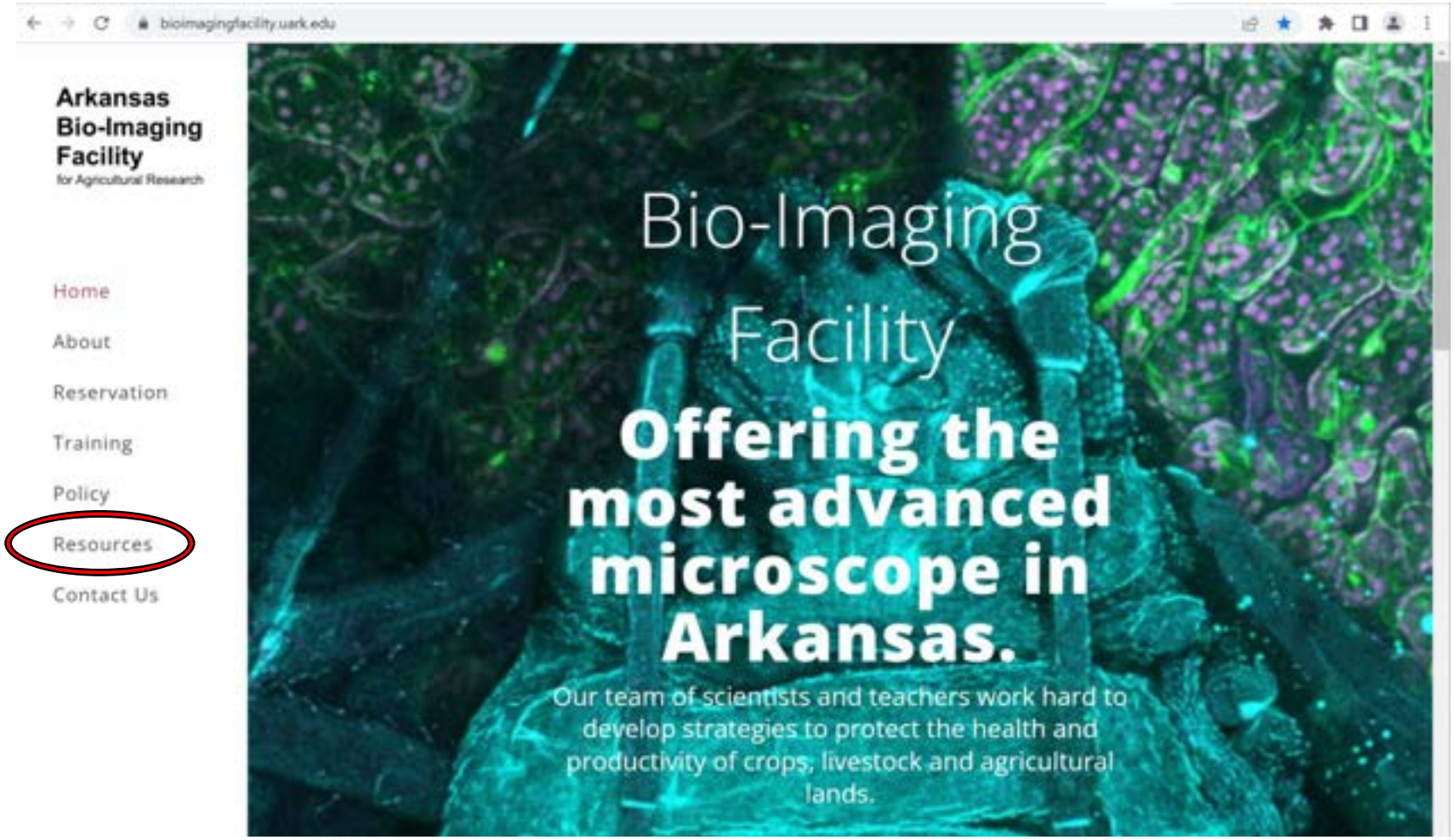
- **Other resources**
  - Bench space and sink for sample preparation
  - Access to Imaris microscopy image analysis software (contact Martin Egan for more information: me021@uark.edu

Martin Egan, ENPL

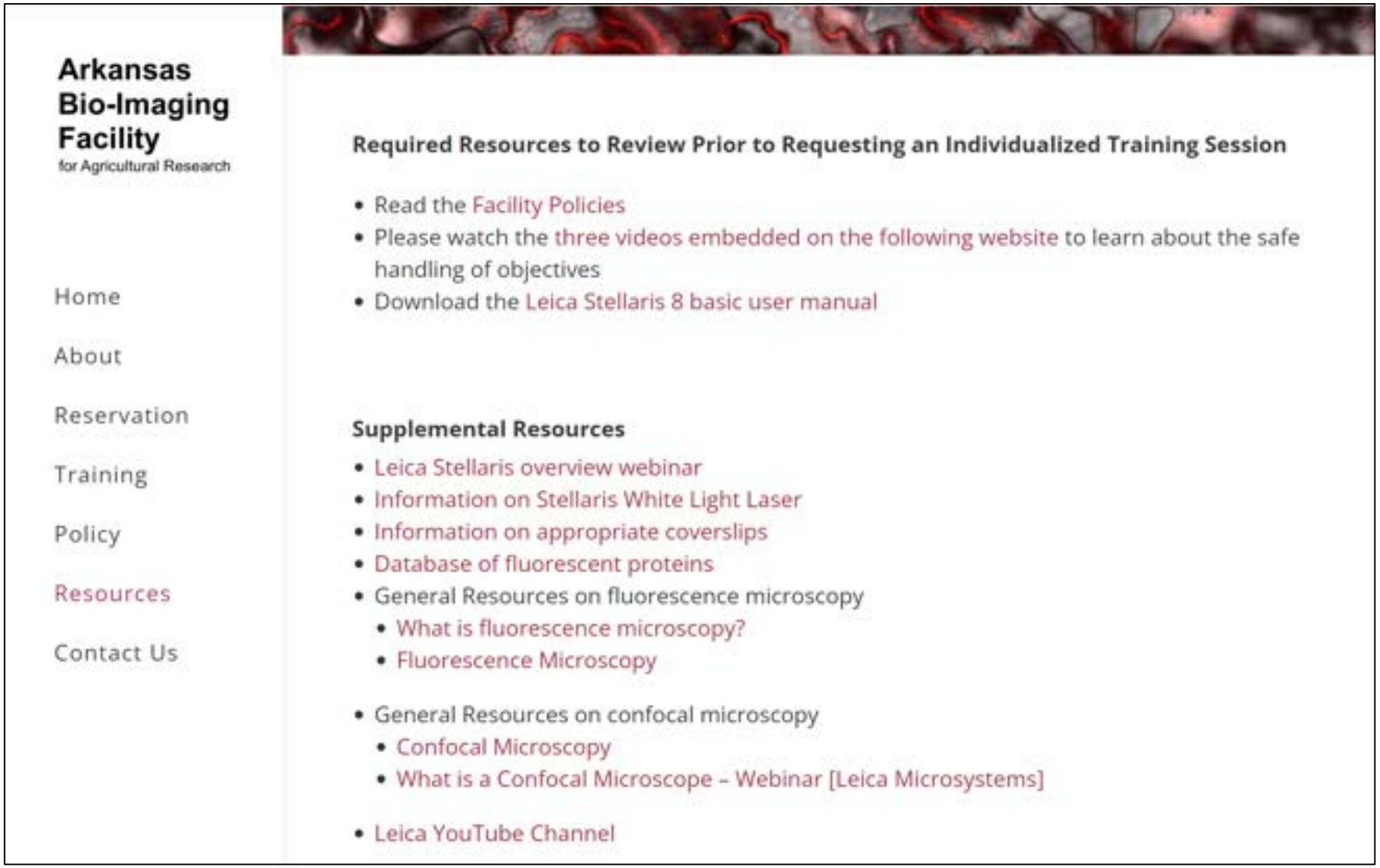
Arkansas Bioimaging Facility for Agricultural Research

- How to use:
  - Review **Resources** pages on website
  - https://bioimagingfacility.uark.edu/

SCAN ME



Resources Page:



Facility Usage Policies

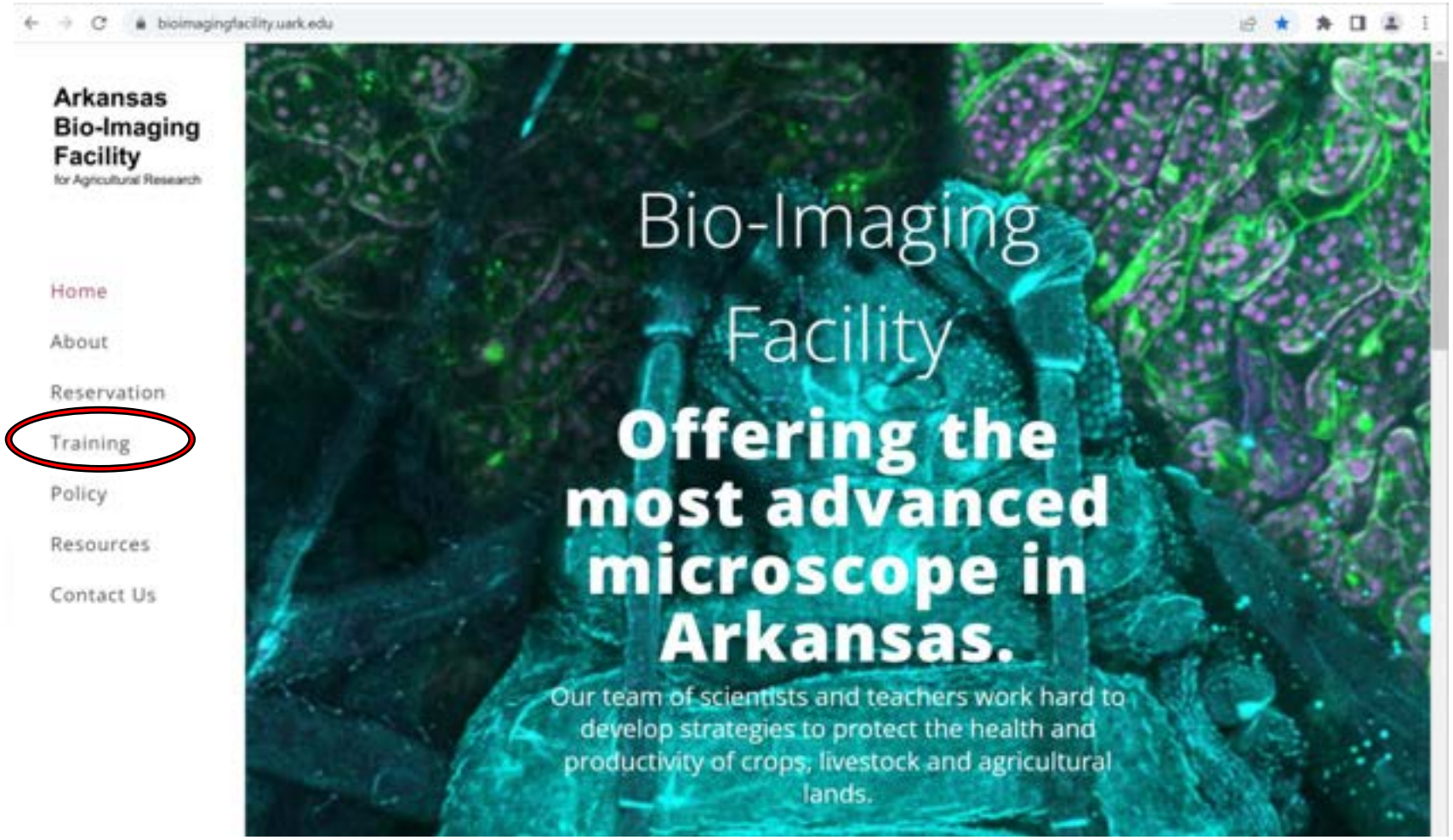
- Confocal usage is $50/hr; 24h cancellation policy
- **Objective care:**
  - Do not use nail polish to attach coverslip to glass slide
  - Immersion objectives must be wiped clean with optical lens paper only
  - **NOTE:** we do not have any oil immersion lenses
- Users' PI responsible for damage
- **Data storage:** back up your data on BOX; external drives not allowed
- Immediately report problems with the instrument to Dr. Li
- **Acknowledgements:** credit NIFA-EGP in publications & presentations: "This work is/was supported by the Equipment Grants Program, award #2021-05017, from the U.S. Department of Agriculture, National Institute of Food and Agriculture."

Arkansas Bioimaging Facility for Agricultural Research

- How to use:
  - Review website
  - Then contact Dr. Jiamei Li for in-person training

Jiamei Li

SCAN ME



Arkansas Bioimaging Facility for Agricultural Research

- How to use:
  - Review website
  - Complete in-person training
  - Then request bookings online or via Jiamei Li
  - jxl080@uark.edu

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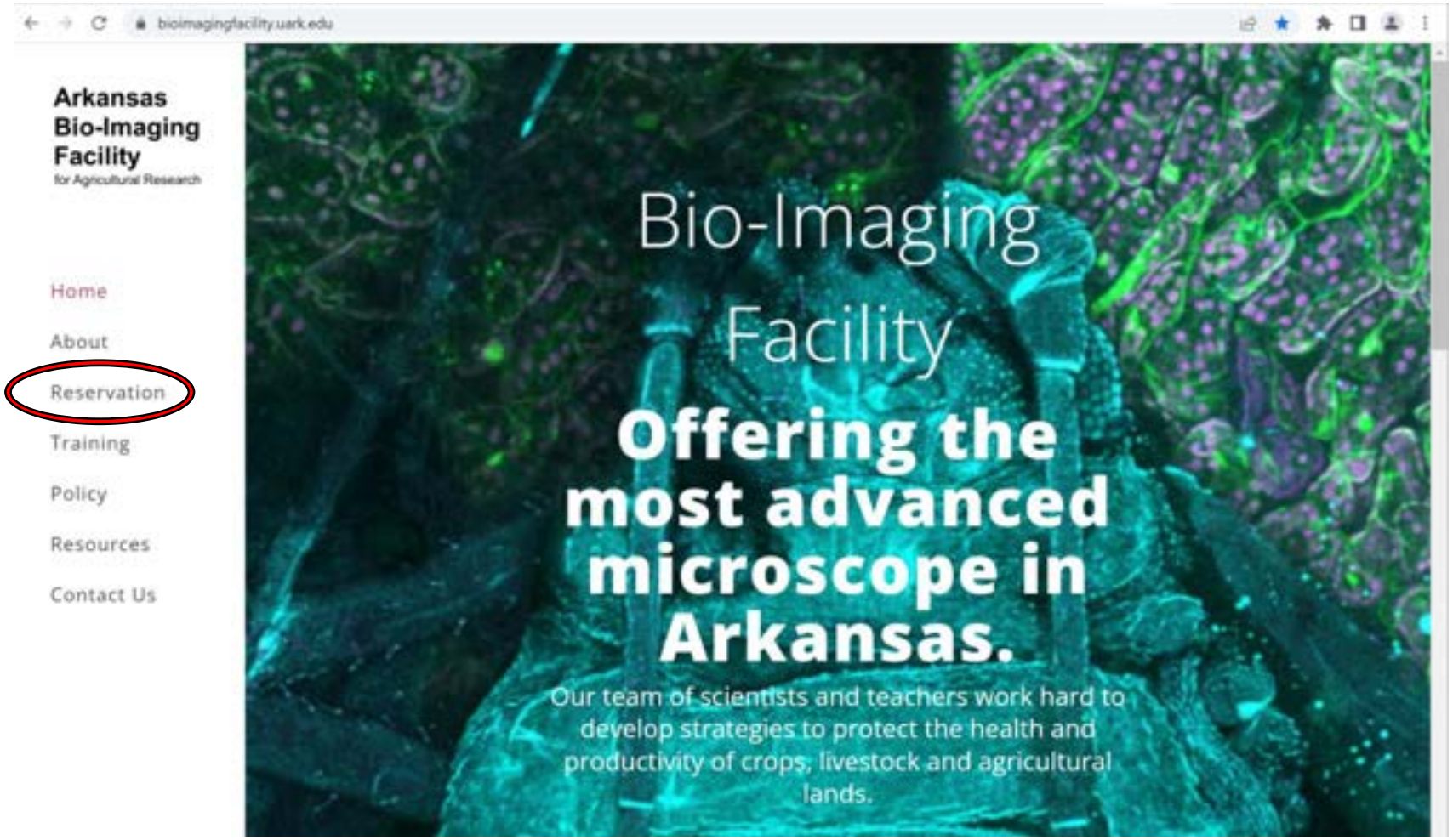


Mini-Grant Program

- **Covers usage fees ($50/Hour) in order to:**
  - Train new users
  - Test the suitability of the Stellaris 8 for your experiments
  - Determine the feasibility of new experimental approaches
  - Collect preliminary data for grant proposals
  - Round out manuscripts for publication
- Funds available for **~200 hours**

Procedure

- Proposals due **February 6th**
- May be submitted by faculty, or by postdoctoral associates or students with faculty co-PI
  - Priority given to UAF/UADA, but opportunity open to all

Proposal Format

- Review Criteria:
  - Clear goals & experimental plan
  - Benefit to current grants and/or future grant submissions
  - Likelihood of publication
  - Number of new users
  - Workforce development---- student & postdoctoral training

Mini-Grant Proposal Template

Your Name:

Your Title (Faculty/ Postdoctoral Associate /Graduate Student/Undergraduate Student):

Your Major Advisor (if you are a student or postdoc):

Your Department:

Your Institution (if other than UAF):

Are you currently using another confocal microscope?:

Proposed users: How many new users of the Stellaris 8 would be involved in this project? Are they faculty, postdocs, graduate or undergraduate students? What is their level of prior microscopy experience?

Time request: Users may request as little as 5h or as much as 25h. Requests for greater than 15h should provide a clear justification for why this amount of time is necessary and can't be charged to other accounts.

Proposed work: Please describe in less than 500 words what you would like to use the Stellaris 8 to do. What are the goals of the experiment? What is the experimental design, including how many samples you plan to observe? How will this benefit your research? For students and postdocs, how will this benefit your training?

Current Funding: Do you (or your advisor) currently have funding that would allow you to continue using the Stellaris 8 once your "free trial" is finished? Please describe briefly.

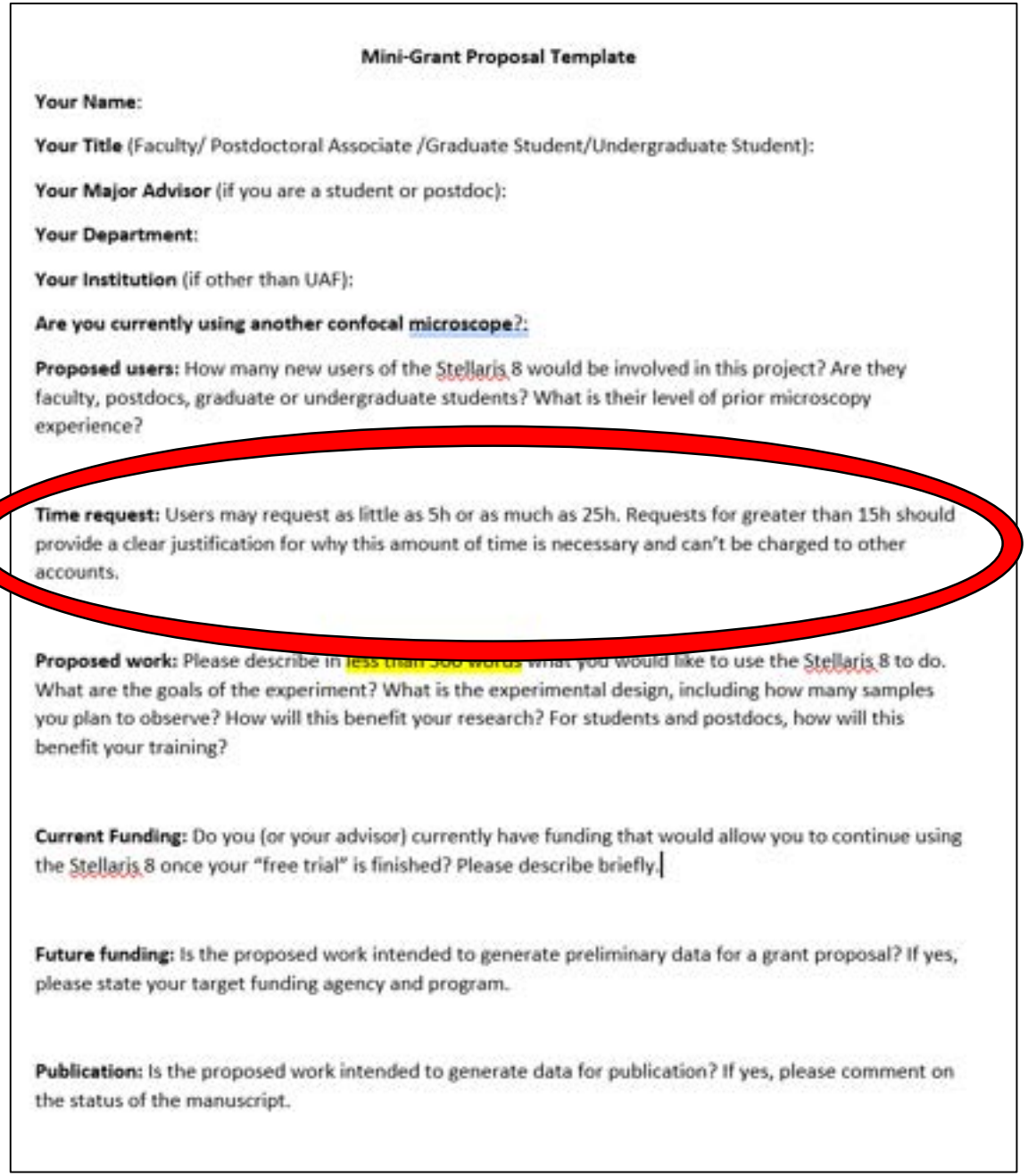
Future funding: Is the proposed work intended to generate preliminary data for a grant proposal? If yes, please state your target funding agency and program.

Publication: Is the proposed work intended to generate data for publication? If yes, please comment on the status of the manuscript.

Review Criteria

- Clear goals & experimental plan
- Benefit to current grants and/or future grant submissions
- Likelihood of publication
- Number of new users
- Workforce development----student & postdoctoral training

- **Note on Time:** What is the minimum time you need to get started? And how much time would you need to complete a useful experimental unit? (You can request a range)



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Your Institution (if other than UAF):

Are you currently using another confocal microscope?:

Proposed users: How many new users of the Stellaris 8 would be involved in this project? Are they faculty, postdocs, graduate or undergraduate students? What is their level of prior microscopy experience?

Time request: Users may request as little as 5h or as much as 25h. Requests for greater than 15h should provide a clear justification for why this amount of time is necessary and can't be charged to other accounts.

Proposed work: Please describe in less than 500 words what you would like to use the Stellaris 8 to do. What are the goals of the experiment? What is the experimental design, including how many samples you plan to observe? How will this benefit your research? For students and postdocs, how will this benefit your training?

Current Funding: Do you (or your advisor) currently have funding that would allow you to continue using the Stellaris 8 once your "free trial" is finished? Please describe briefly.

Future funding: Is the proposed work intended to generate preliminary data for a grant proposal? If yes, please state your target funding agency and program.

Publication: Is the proposed work intended to generate data for publication? If yes, please comment on the status of the manuscript.

Procedure

- Proposals due **February 6th**
  - Email to Jiamei Li at jxl080@uark.edu

- Proposals reviewed by leadership team; decisions announced by March 1
- If selected, PIs will be awarded a usage voucher
  - User training should take place before **July 1**
  - Voucher should be used within **6 months**

Follow-Up

- **UA Imaging Research Symposium?**
  - Invited keynote speakers
  - Presentations selected from mini-grant awardees
  - Poster session for imaging projects by ANY students and postdocs on campus

- **Please email me feedback on:**
  - Your interest in attending/presenting
  - Your suggestions on keynote speakers

  - *fgoggin@uark.edu*