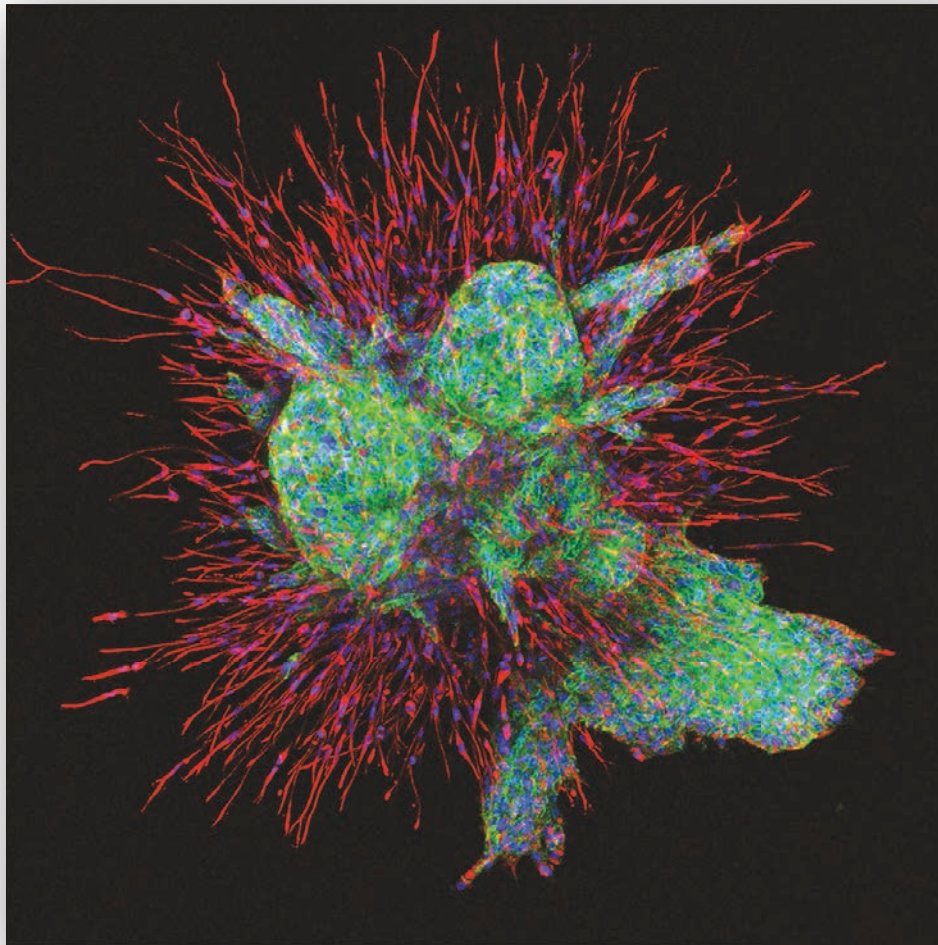


# Fluorescence Photomicrography

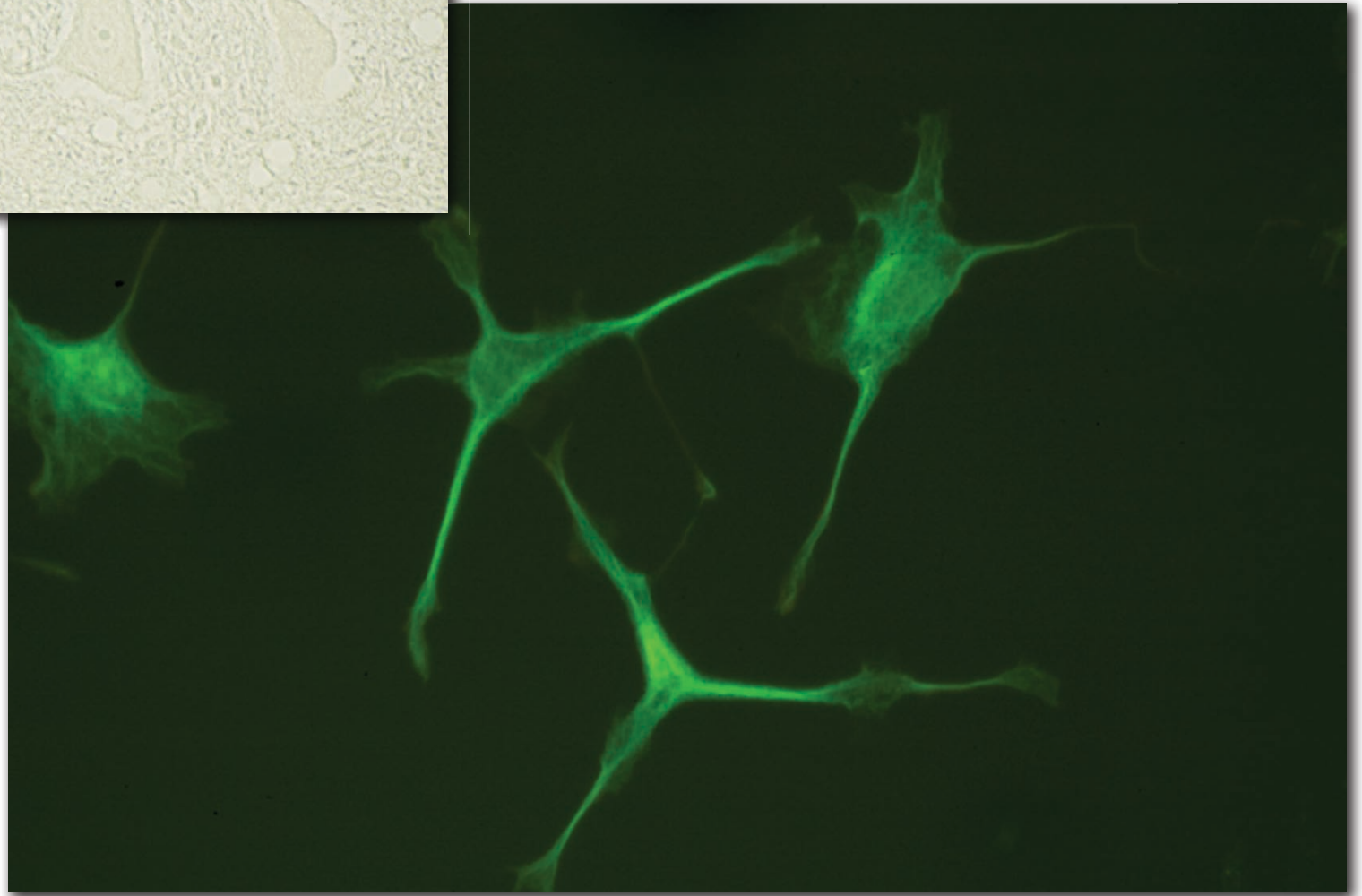
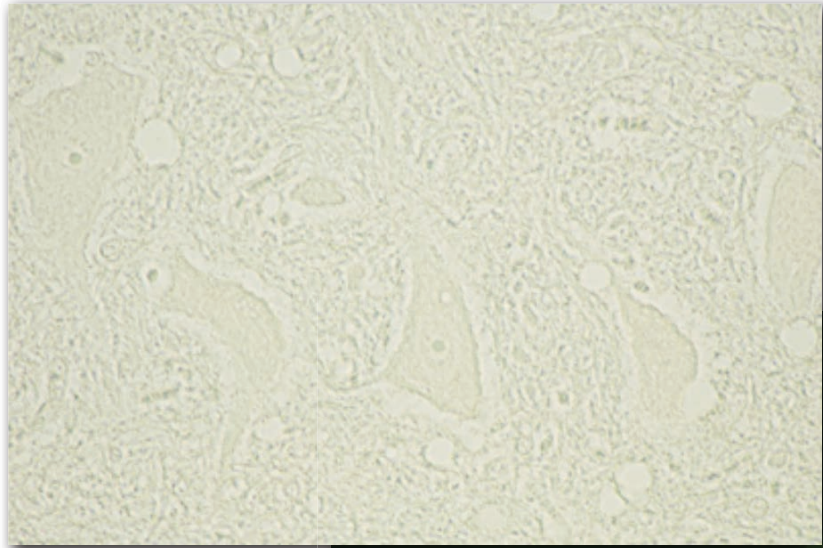
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# Daily House Keeping

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- Assn Four – due Monday & Wednesday
- Assn Five – Due March 2
- Assn Six – Blog & Poster
- Midterm- average grade 79.5%
- Final Exam – March 10



# Fluorescence

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Short  $\lambda$  energy is used to excite a sample that then emits longer  $\lambda$  energy

# Sir Gabriel Stokes -1852

Safari File Edit View History Bookmarks Window Help

George Gabriel Stokes - Wikipedia, the free encyclopedia

http://en.wikipedia.org/wiki/George\_Gabriel\_Stokes

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article discussion edit this page history

## George Gabriel Stokes

From Wikipedia, the free encyclopedia

**Sir George Gabriel Stokes, 1st Baronet FRS** (13 August 1819–1 February 1903), was a mathematician and physicist, who at Cambridge made important contributions to fluid dynamics (including the Navier–Stokes equations), optics, and mathematical physics (including Stokes' theorem). He was secretary, then president, of the Royal Society.

**Contents** [hide]

- Biography
  - Career
- Contributions to science
  - Fluid dynamics
    - Creeping flow
  - Light
  - Fluorescence
  - Polarization
  - Chemical analysis
  - Other work
  - Unpublished research
    - Contributions to Engineering
    - Contributions to Christianity
- Legacy and honours
- Publications
- Notes
- References
- External links

### Biography

George Stokes was the youngest son of the Reverend Gabriel Stokes, rector of Skreen, County Sligo, Ireland, where he was born and brought up in an evangelical Protestant family. After attending schools in Skreen, Dublin and Bristol, he matriculated in 1837 at Pembroke College, Cambridge, where four years later, on graduating as senior wrangler and first Smith's prizeman, he was elected to a fellowship. In accordance with the college statutes, he had to resign the fellowship when he married in 1857, but twelve years later, under new statutes, he was re-elected. He retained his place on the foundation until 1902, when on the day before his 83rd birthday, he was elected to the mastership. He did not enjoy this position for long, for he died at Cambridge on 1 February the following year, and was buried in the Mill Road cemetery.

### Career



Sir George Gabriel Stokes, 1st Baronet (1819–1903)

<b>Born</b>	13 August 1819 Skreen, County Sligo, Ireland
<b>Died</b>	1 February 1903 (aged 83) Cambridge, England
<b>Nationality</b>	United Kingdom of Great Britain and Ireland
<b>Fields</b>	Mathematician and physicist
<b>Institutions</b>	University of Cambridge
<b>Alma mater</b>	University of Cambridge
<b>Academic advisors</b>	William Hopkins
<b>Notable students</b>	Horace Lamb
<b>Known for</b>	Stokes' law Stokes' theorem Stokes line Stokes relations Stokes shift Navier–Stokes equations

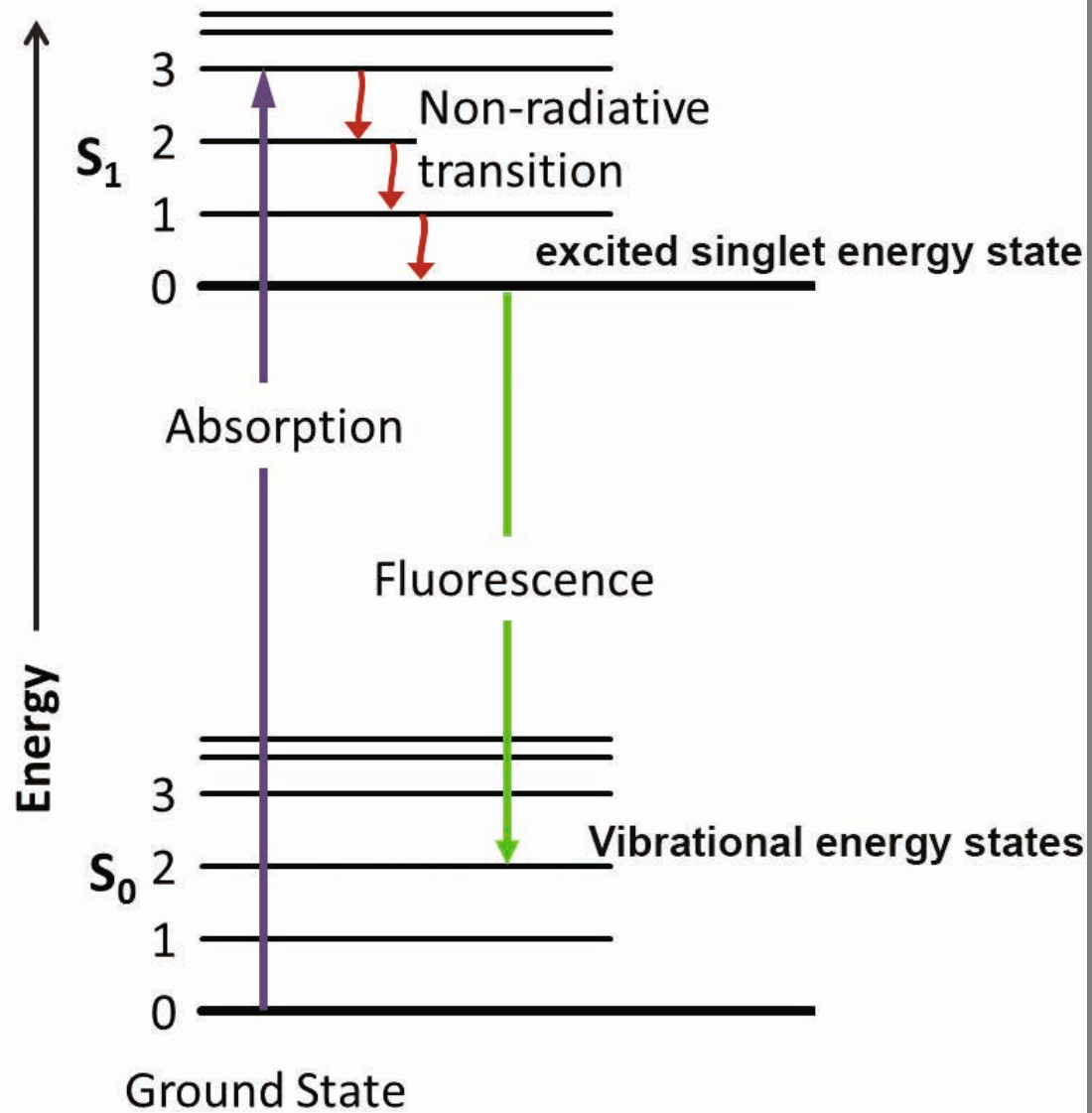
# Stokes' Shift

Vibrational energy is lost as electrons go from an excited state to a ground state.

The excitation is always of a lower  $\lambda$  and higher energy than the emission.

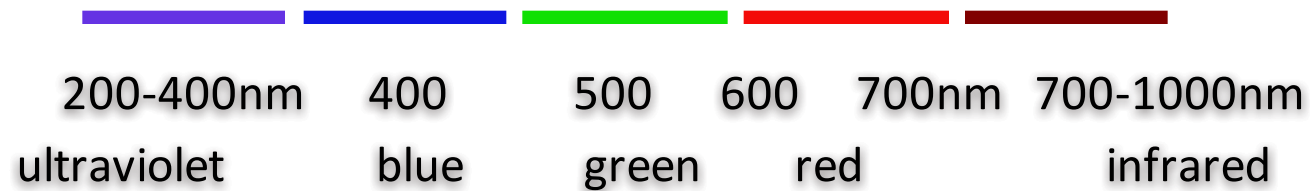
## Jablonski Principle

Professor Alexander Jablonski in 1935 described absorption and emission of light.



# Electromagnetic Spectrum

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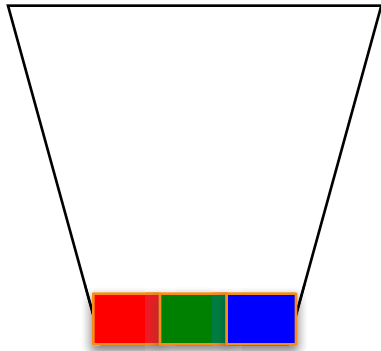
Short  $\lambda$   
High energy

Long  $\lambda$   
Low energy



[http://micro.magnet.fsu.edu/primer/lig  
htandcolor/fluorescencehome.html](http://micro.magnet.fsu.edu/primer/lig<br/>htandcolor/fluorescencehome.html)

Full spectrum RGB light



Exciter Filter



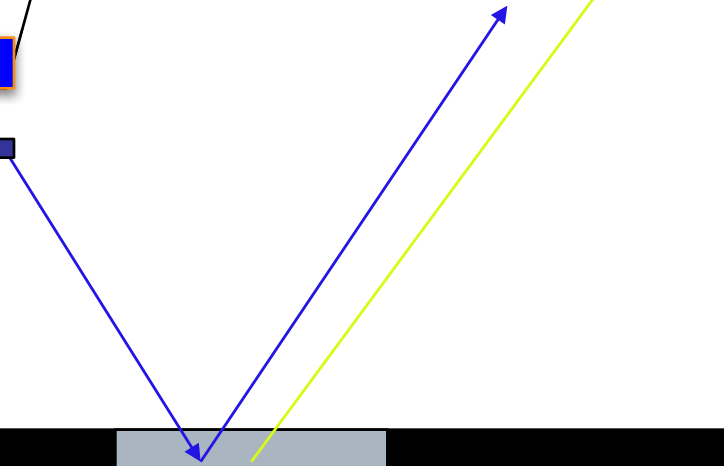
Camera



Barrier Filter



Sample



# What is Required?

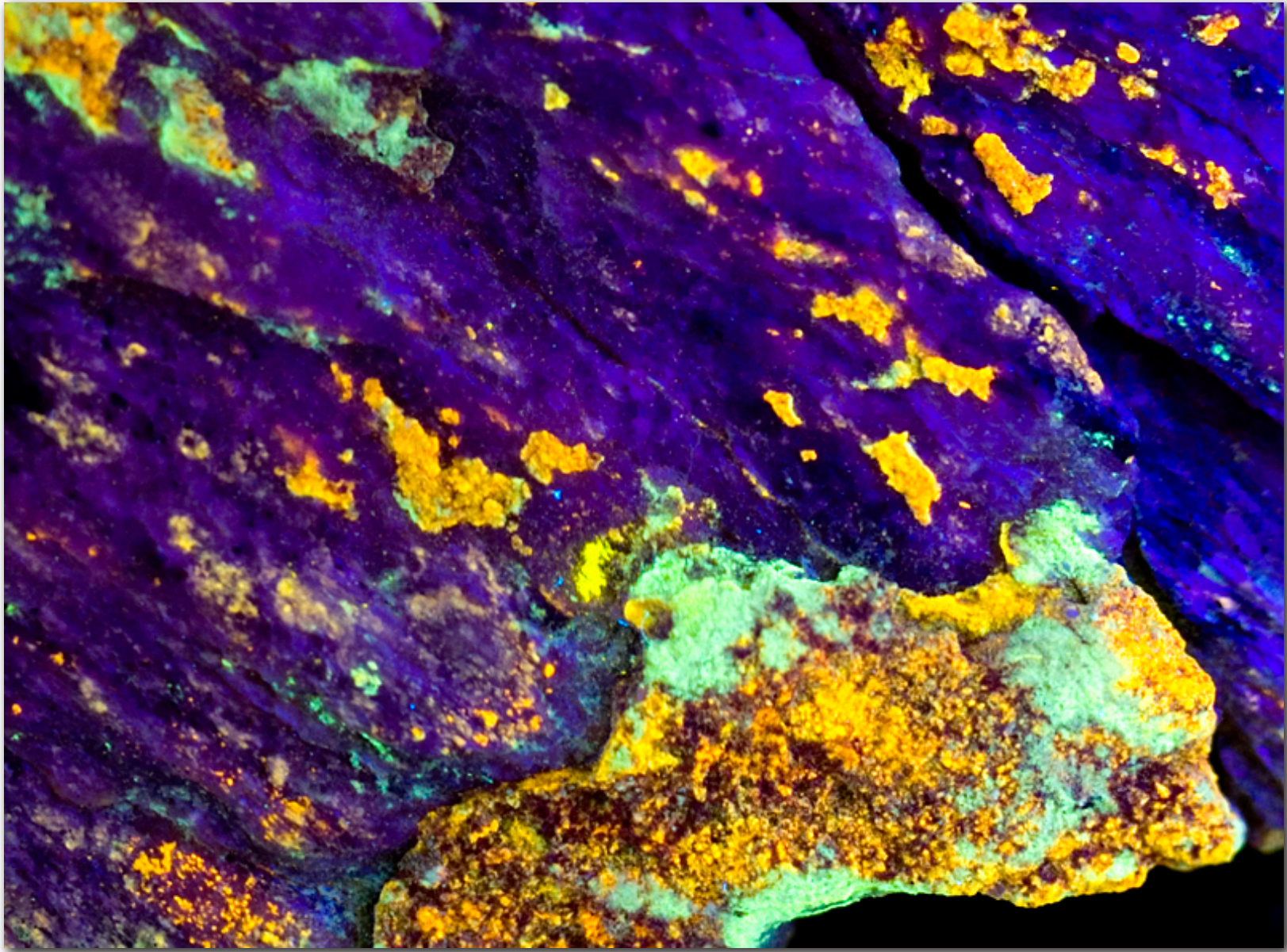
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Excitation Filter

Emission Filter

Light source





# Fluorescence Microscopy

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## Necessary

Light Source  
Reflected Light Path  
Excitation Filter  
Dichroic (beam splitter)  
Emission Filter

## Unnecessary

Special Objectives\*  
Kohler Illumination  
Transmitted light path  
Condenser  
Aperture Diaphragm

# Fluorescence Illumination Path

---

# Light sources for Fluorescence

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Mercury Arc Lamp

Xenon Arc Lamp

Metal Halide Lamp

Light Emitting Diodes (LEDs)

Lasers

Considerations: cost, stability, spectral output, bulb lifetime, ease of use, safety...



# Choosing Filters

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Filtration must be mutually exclusive.

Long pass vs. Band pass  
(wide band vs. narrow band)

# Dichroic Filter

---

(Dichromatic mirror, Beam splitter)

- Is a highly specific color filter
- In one direction filter reflects one color and transmits the other 2 primaries while traveling in the other direction, it transmits 2 primaries

# Objective Selection

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Special objectives are not required for fluorescence, but some are better than others.

- Transmission characteristics of objective
- Aberration correction = more glass, greater light loss
- High NA at lowest magnification possible

# Microscope set up

---

Homogeneous Illumination

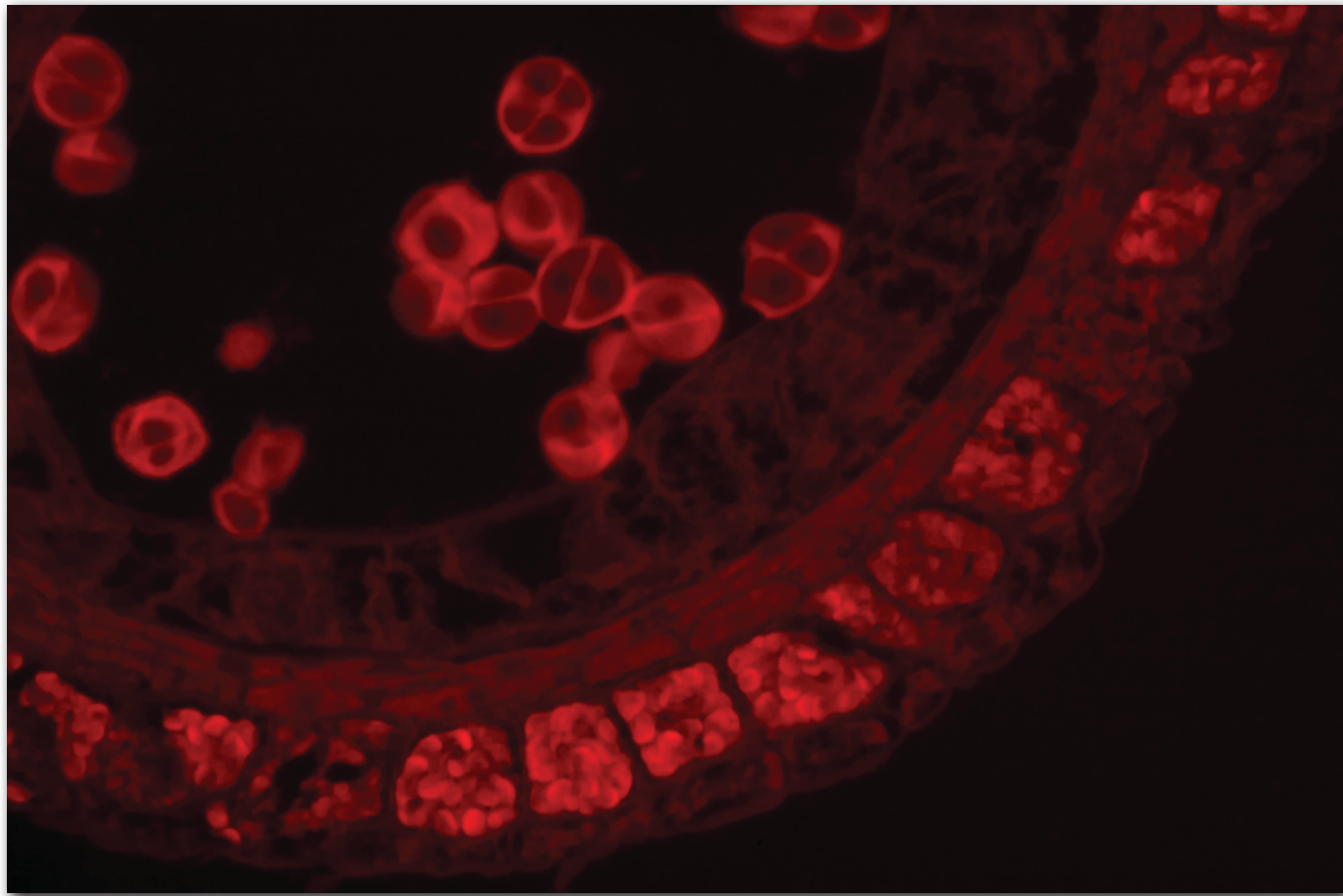
Field stop set

Transmitted light off

FL Filter set in place

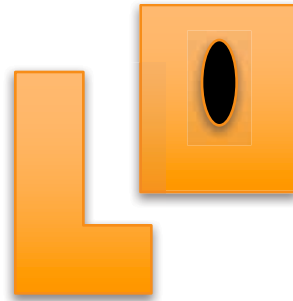
# Autofluorescence

---



# Evaluating the Presence of a Protein

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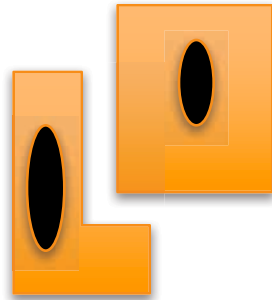
Expression or co-localization

Sometimes called Tagging, Fluorochrome,  
Fluorophore, Probe, or Fluorescence Dye

Antigen – Antibody  
Lock & Key

# Evaluating the Presence of a Protein

---



Sometimes called Tagging, Fluorochrome, Fluorophore, Probe, or Fluorescence Dye

Antigen – Antibody  
Lock & Key

# Common Fluorochromes

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**Dapi**

Fura 2

CFP

**FITC**

**GFP**

YFP

CY3

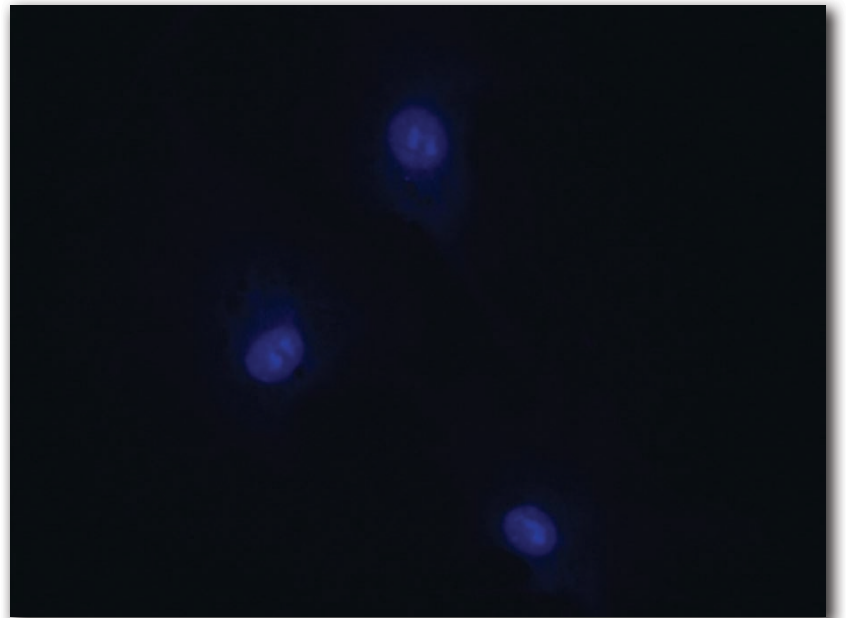
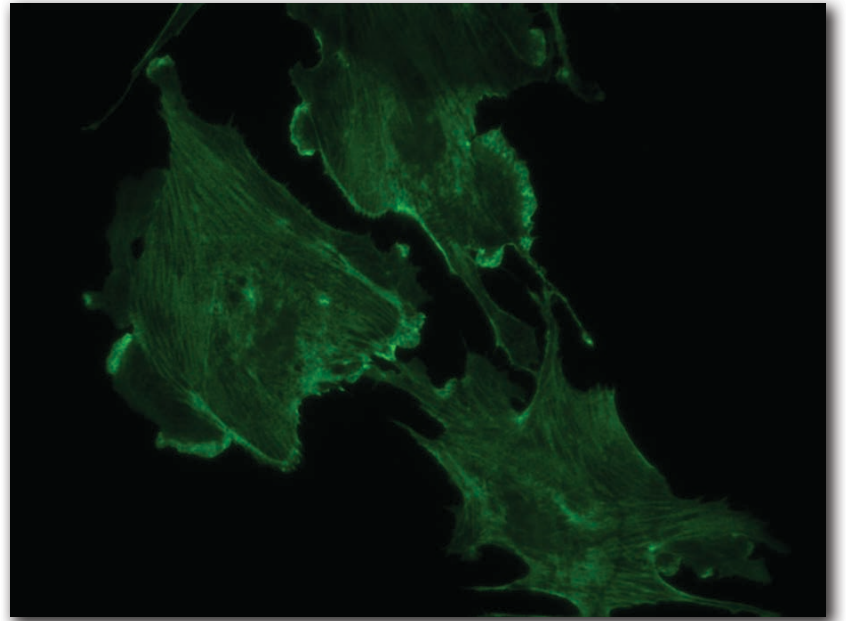
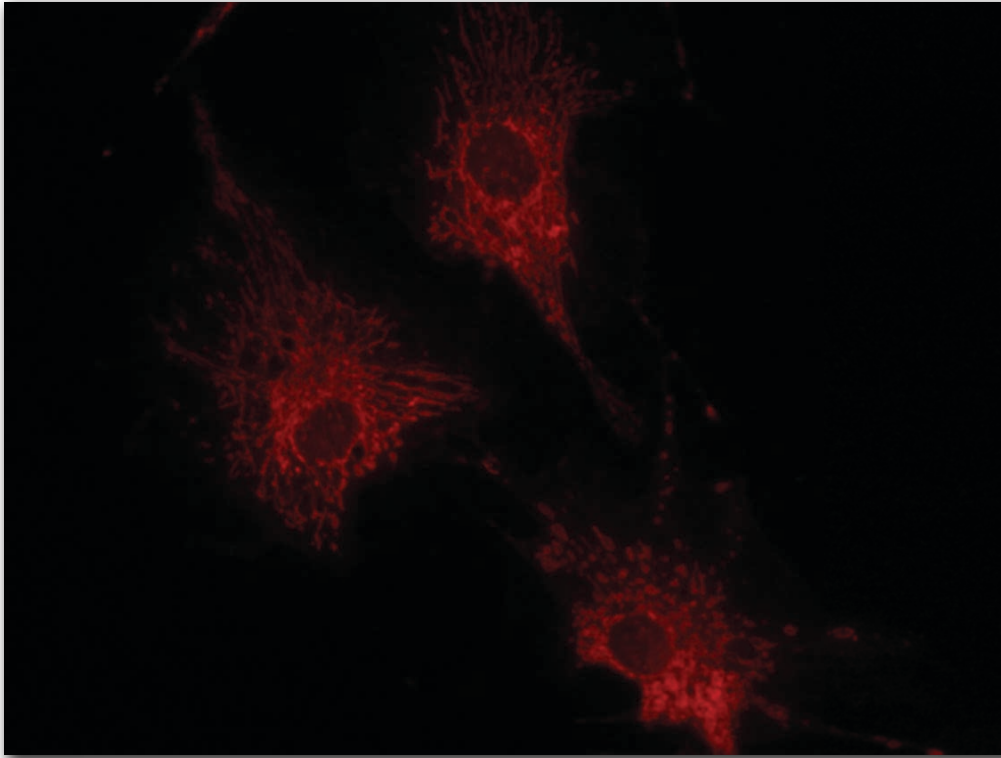
**Texas Red**

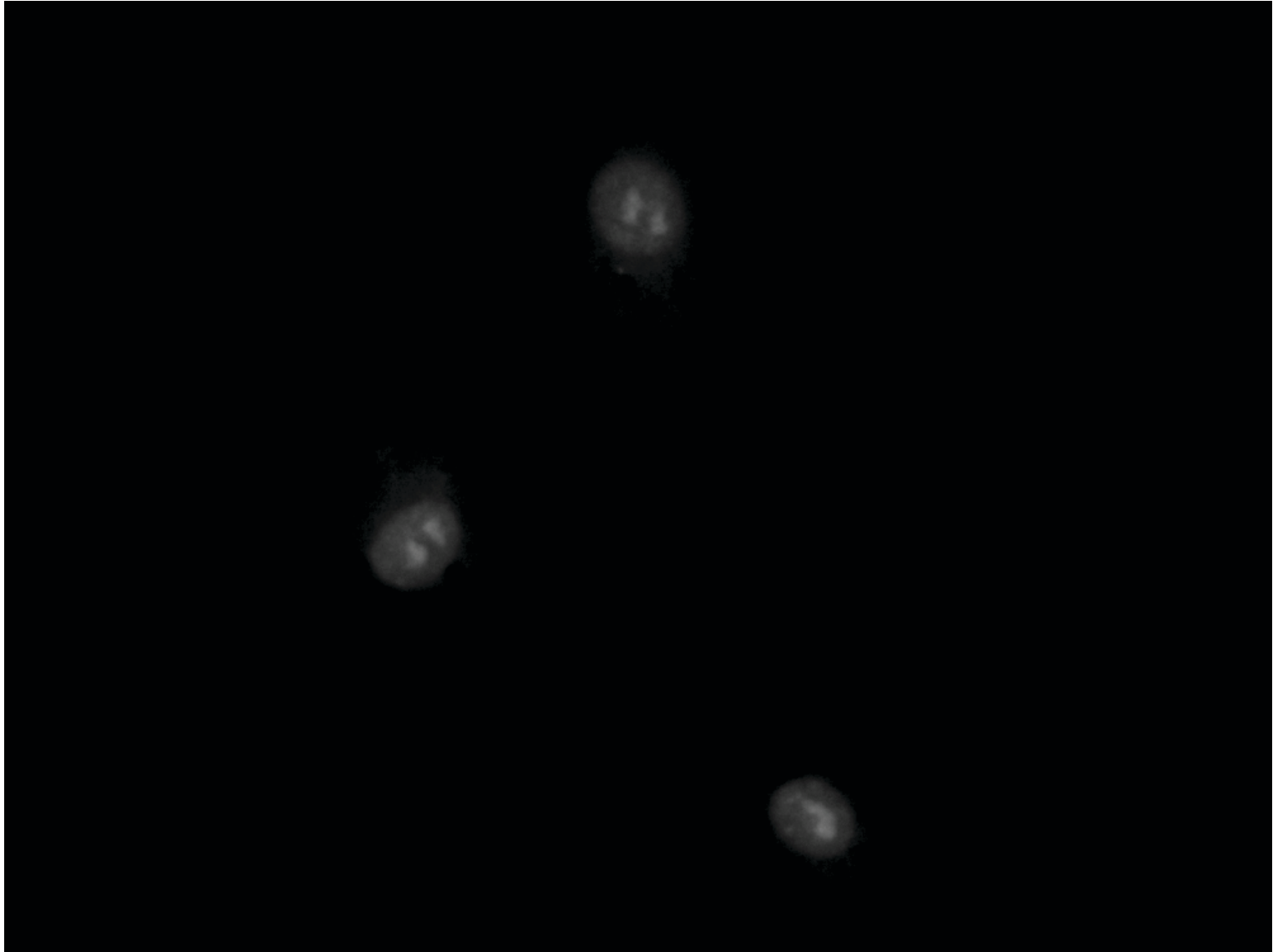
Rhodamine

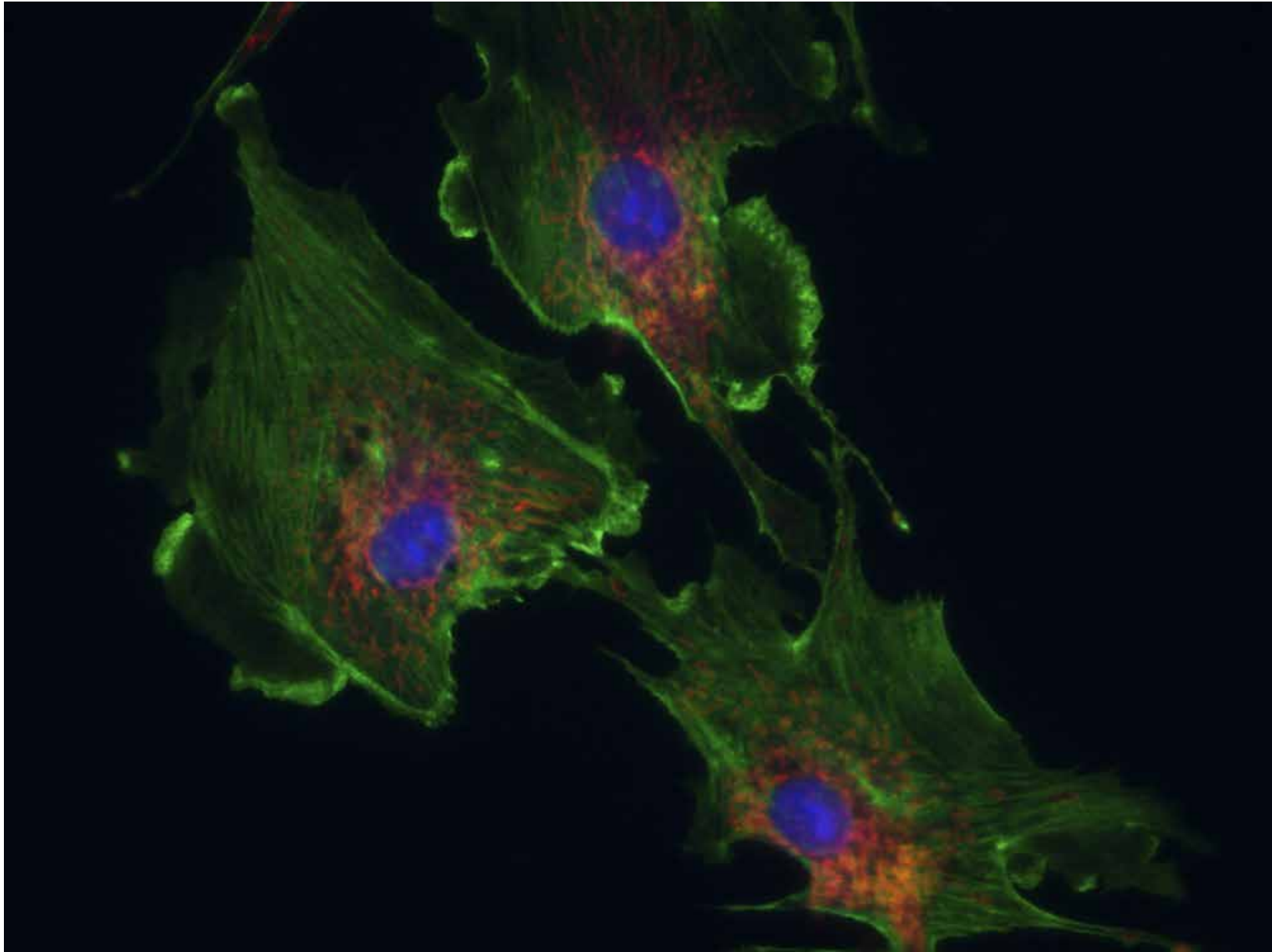
CY5.5



# Immunofluorescence







# Challenges

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Low light

Long exposure

Bleaching / Quenching/Burn Out

High Contrast

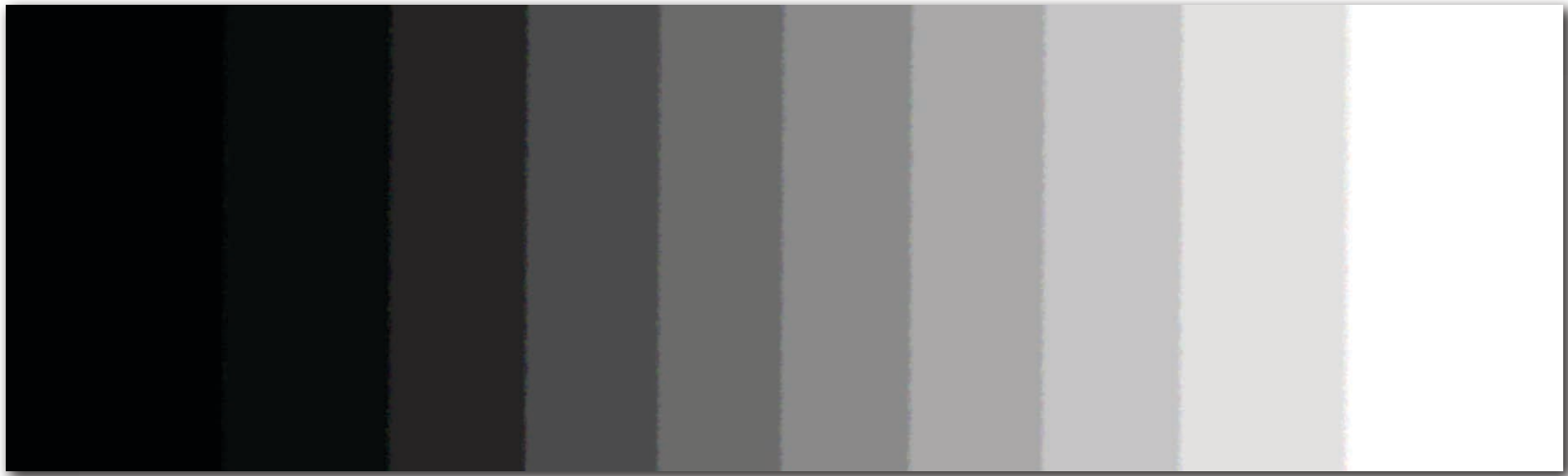
Out of focus Fluorescence areas

Uneven Fluorescence across field

Signal to Noise Ratio

# Exposure

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F1



BF

# Tips, Tricks & Reminders

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Keep the room lights off

Find your sample in brightfield before switching to fluorescence.

Close the reflected light shutter when you are not looking at or taking a picture of sample (if no shutter, cover your sample w/ lens cap.)

It is not necessary to white balance the camera.

# Tips, Tricks & Reminders

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- Binning
- Gain
- Lower Monitor Brightness to help with seeing
- Use a grayscale camera and pseudocolor
- Three shot camera





# Safety Concerns

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Do not touch bulb.

Turn Mercury lamp on before microscope, camera and computer.

Warm up Bulb time

On for the day/off for the day

Log use for lamp lifetime (most power supplies have counter)

Mercury lamps have a 200-300 hour lifespan.

- Flicker, Arching, instability, risk of explosion