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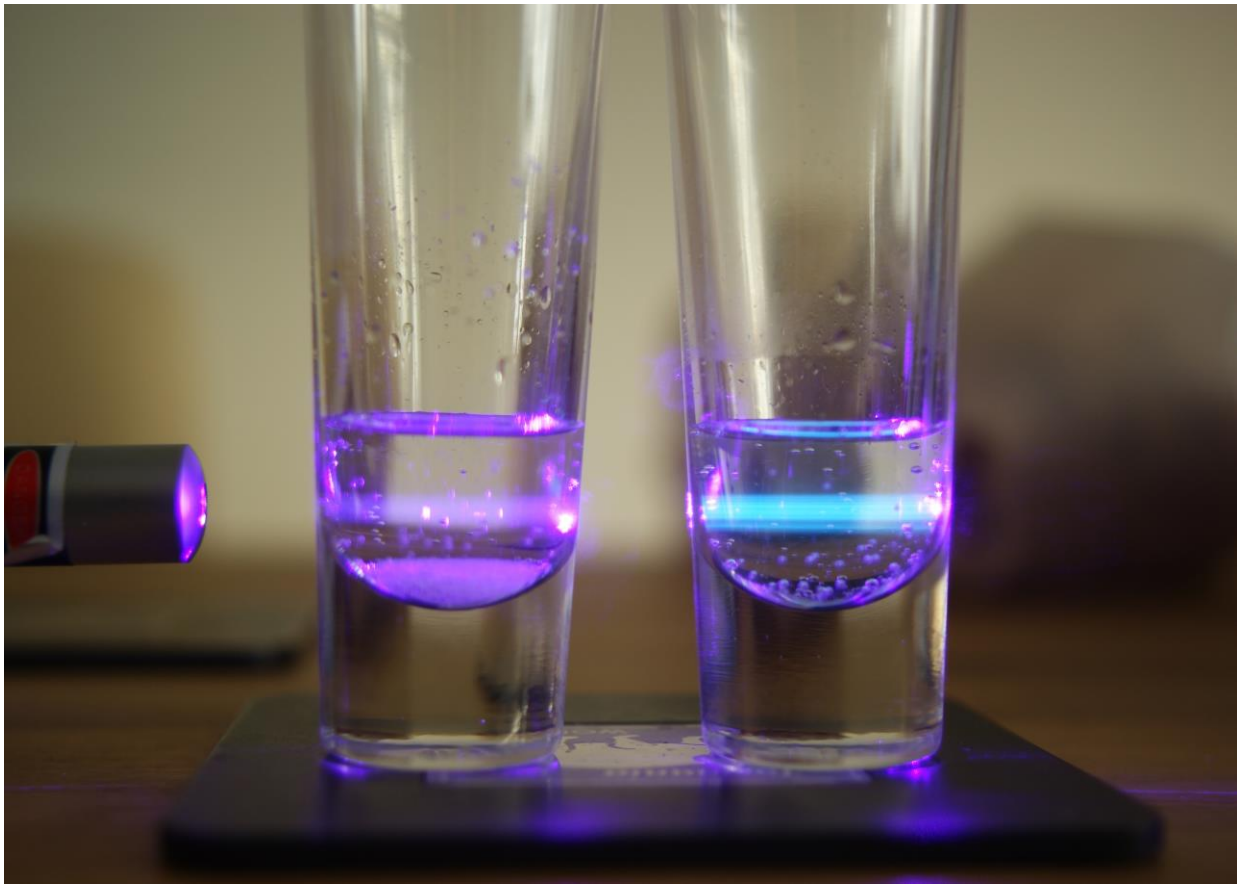
Reliable Instruments for an Unreliable World

# Basics of Fluorescence



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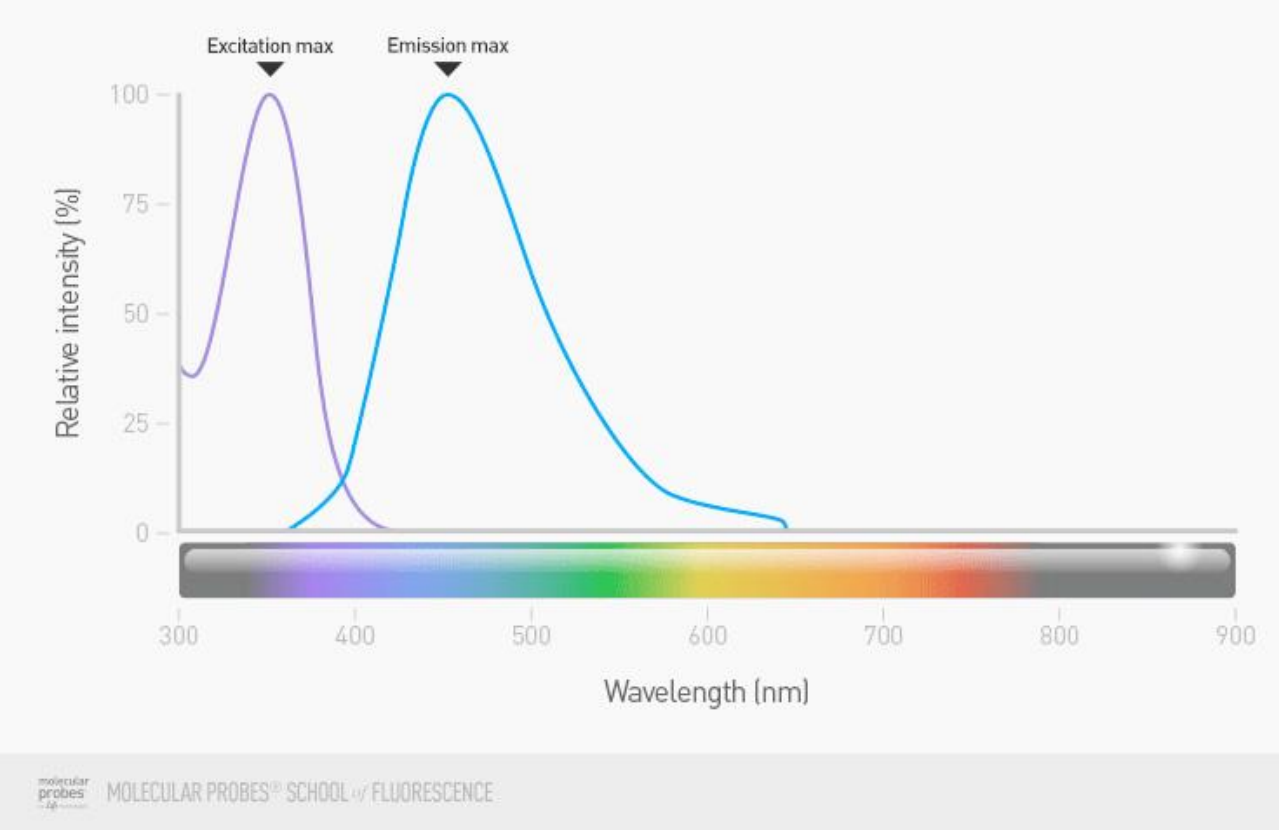
# Fluorescence

- The molecular absorption of light energy at one wavelength and its nearly instantaneous re-emission at another wavelength.
  - Emitted light is always a longer wavelength than the absorbed light due to energy loss by the molecule prior to emission.

# Excitation and Emission

- Fluorescent compounds have two characteristic spectra
  - Excitation spectrum (the wavelength and amount of light absorbed)
  - Emission spectrum (the wavelength and amount of light emitted).



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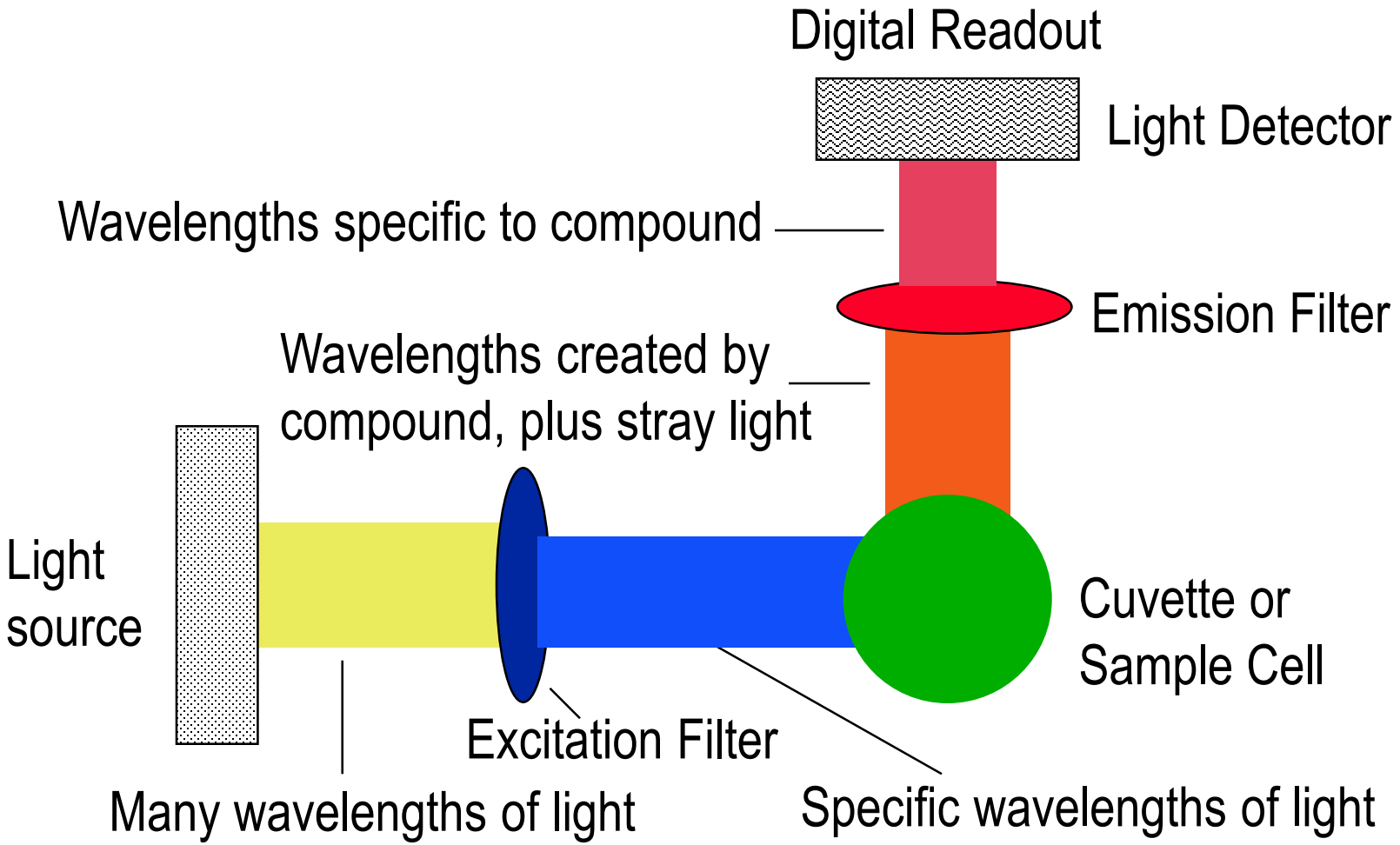
# Filter Fluorometer

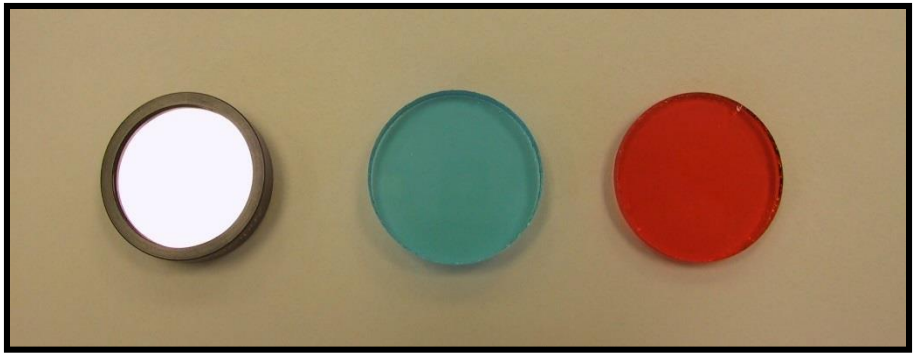


# Light Source

- Provides the energy that excites the compound of interest by emitting light of the desired wavelength
  - Most common are Light Emitting Diodes (LED)

# Optical Filters

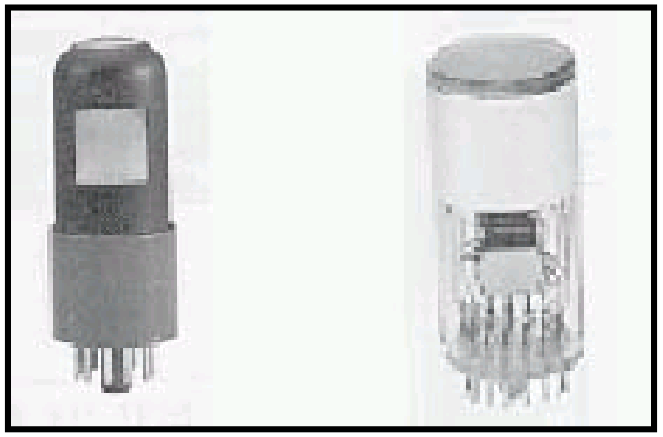
- Colored glass filters have high energy transmittance which enhances sensitivity of fluorometers. However, they are broad bandwidth, typically allowing bandwidths of more than 100 nm to pass and provide marginal wavelength discrimination.



- Interference filters can provide narrow bandwidths, typically 5 to 25 nm wide, which makes them superior for wavelength selection in a fluorometer.

# Light Detectors

- Photomultipliers or photodiodes
  - Emitted light intensity produces a proportional electrical current in the detector. This current is converted to a voltage providing a digital readout.



# Some Fluorescence Applications

- Environmental monitoring
  - Assess health of the ocean
  - Identify harmful algal blooms (HABs)
- Oil spill tracking
- Aquaculture
  - Monitor fish food
  - Determine fish freshness
- Drinking water monitoring
  - Assess health of the intake water
- Wastewater monitoring
  - Identify disposal sites
- Dye tracing
  - Track water flow
- DNA detection

# Some Fluorescence Wavelengths

| | Excitation | Emission |
|---|---|---|
| Chlorophyll | 465nm | 700nm |
| Phycocyanin | 609nm | 643nm |
| Crude Oil | 365nm | 550nm |
| Refined Fuels | 285nm | 340nm |
| Optical Brighteners | 365nm | 445nm |
| DOM | 365nm | 500nm |
| Rhodamine Dye | 530nm | 610nm |
| Fluorescein Dye | 442nm | 530nm |
| Hoechst Dye | 365nm | 510nm |