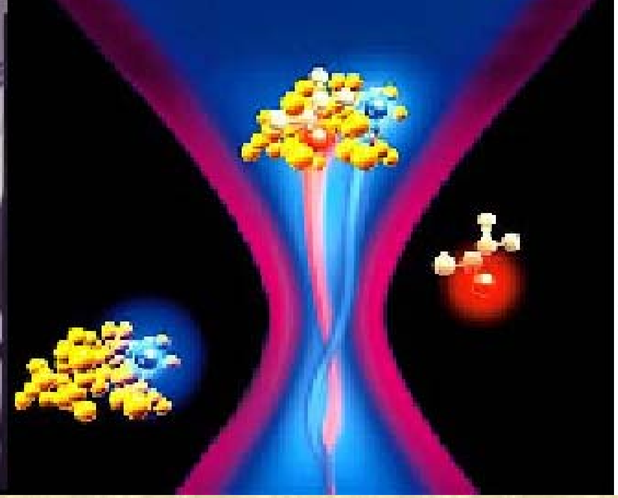
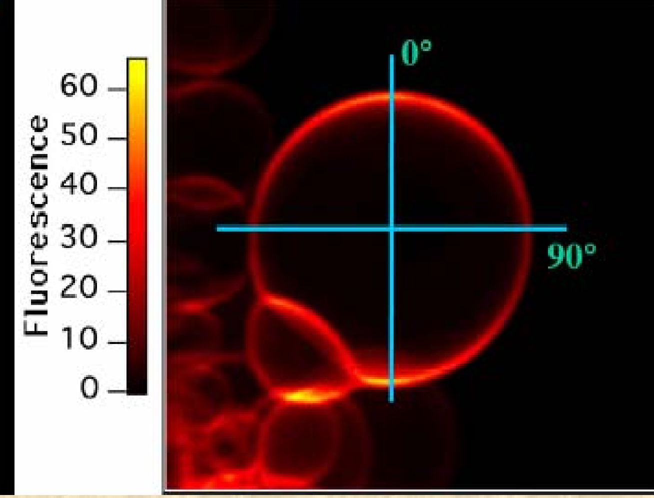




Fluorescence Workshop
UMN Physics
June 8-10, 2006

Steady-state Fluorometer

Joachim Mueller

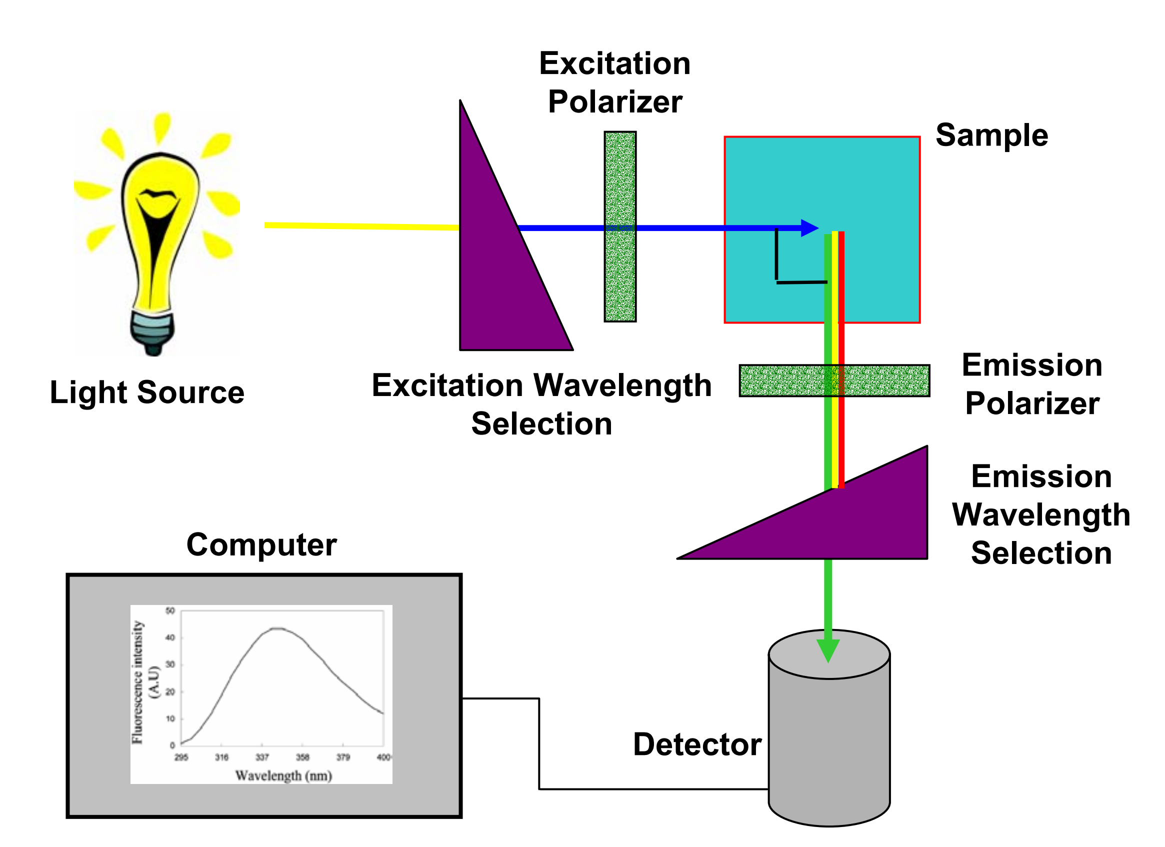
Instrumental Setup, light sources, wavelength selection, detectors, polarizers, corrections, inner filter effect

ISS PC1 (ISS Inc., Champaign, IL, USA)

Fluorolog-3 (Jobin Yvon Inc, Edison, NJ, USA)

QuantaMaster (OBB Sales, London, Ontario N6E 2S8)

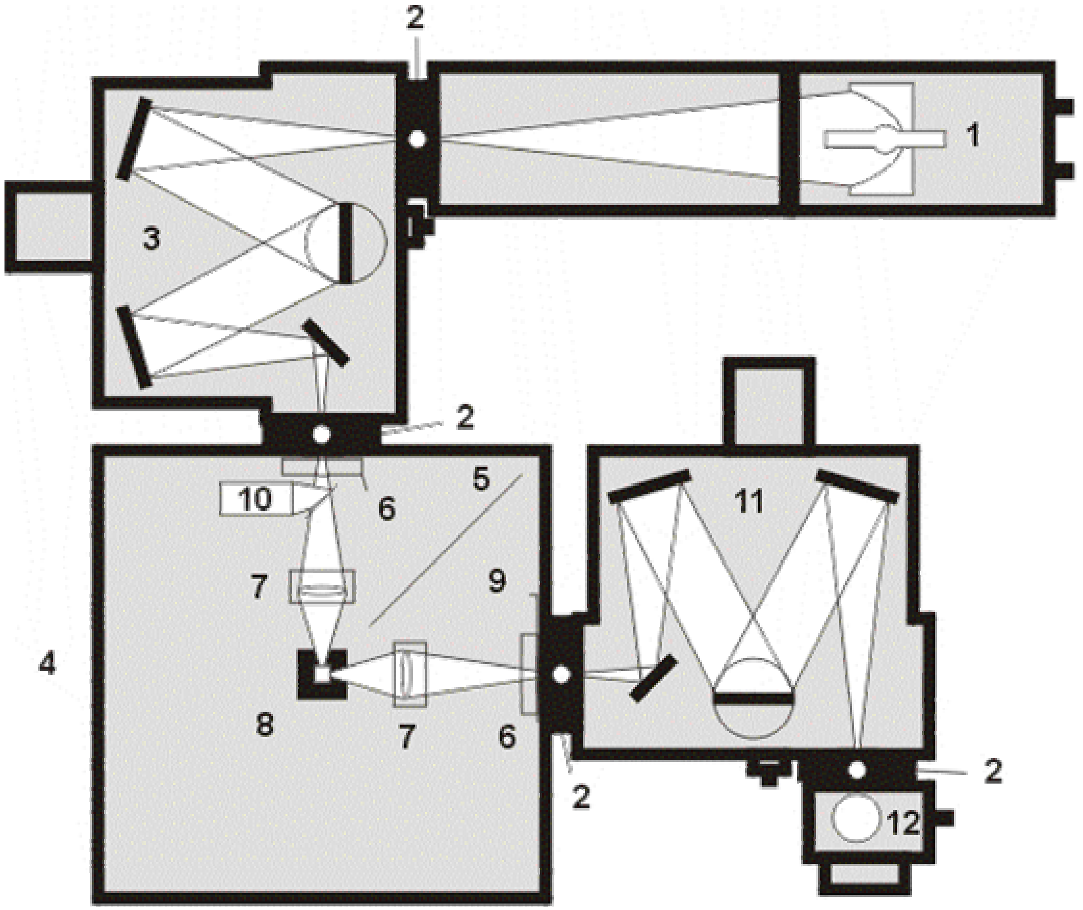
Fluorometer Components



Note: Both polarizers can be removed from the optical beam path

OPTICAL LAYOUT: PTI Model QM-4

1 Lamp housing
2 Adjustable slits
3 Excitation Monochromator
4 Sample compartment
5 Baffle
6 Filter holders
7 Excitation/emission optics
8 Cuvette holder
9 Emission port shutter
10 Excitation Correction
11 Emission Monochromator
12 PMT detector



Light Sources

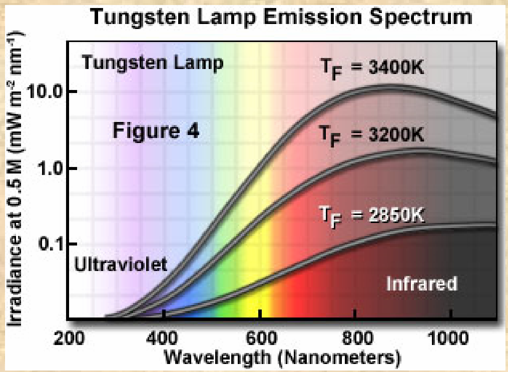
Lamp Light Sources

1. Xenon Arc Lamp (wide range of wavelengths)

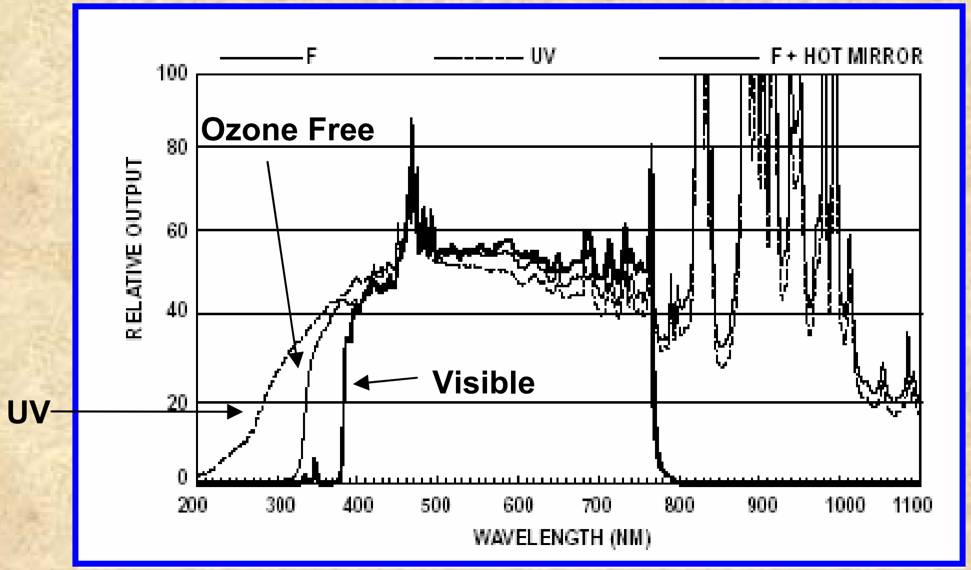
2. High Pressure Mercury Lamps (High Intensities but concentrated in specific lines)

3. Mercury-Xenon Arc Lamp (greater intensities in the UV)

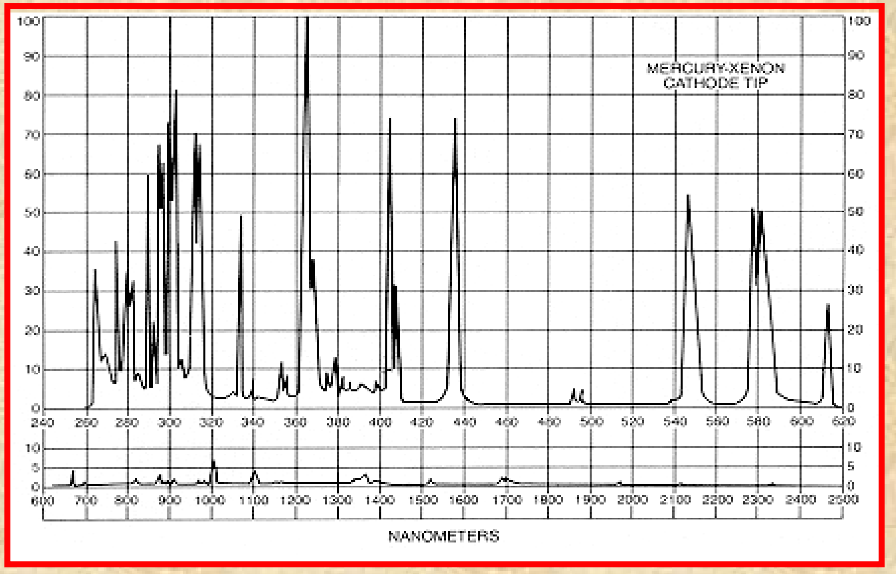
4. Tungsten-Halogen Lamps



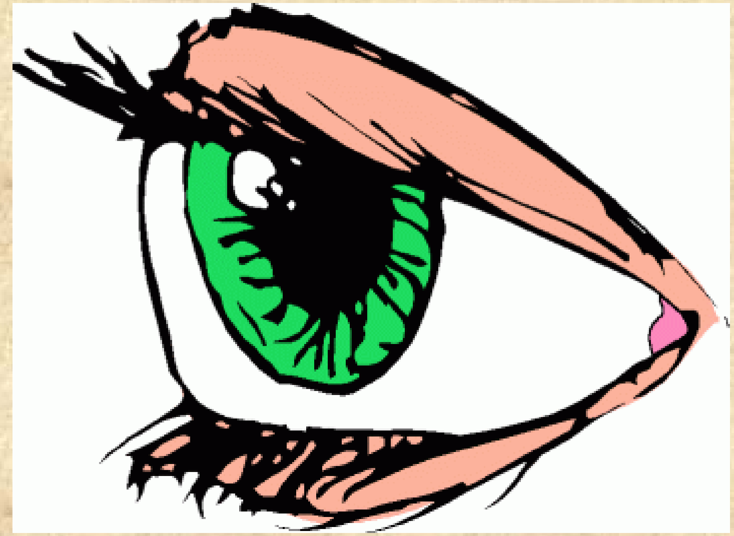
Xenon Arc Lamp Profiles



Mercury-Xenon Arc Lamp Profile



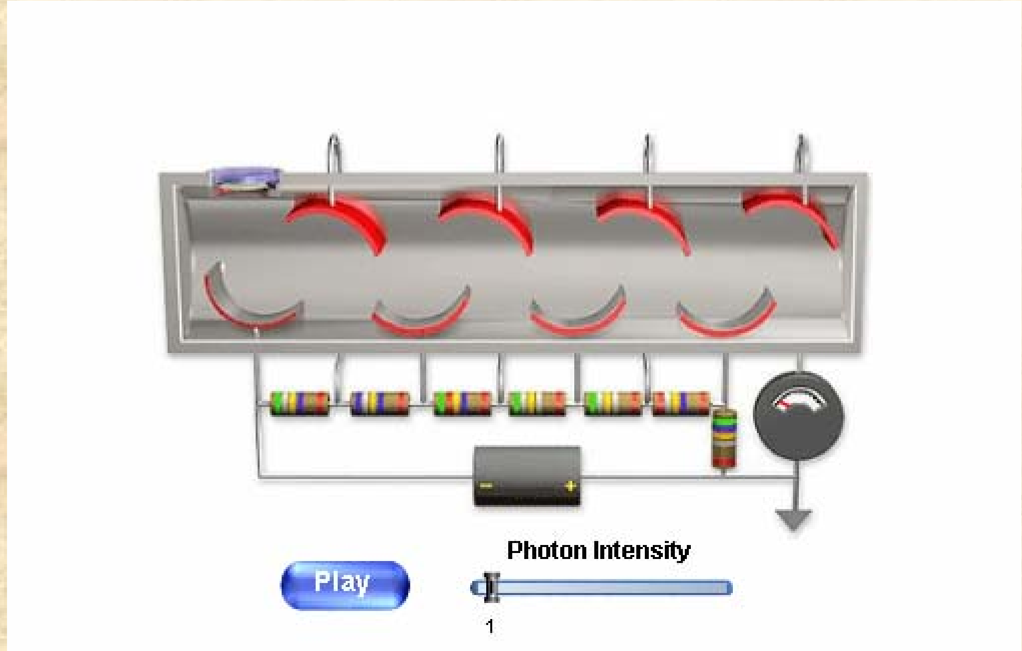
Detectors



Photomultiplier tube (PMT)

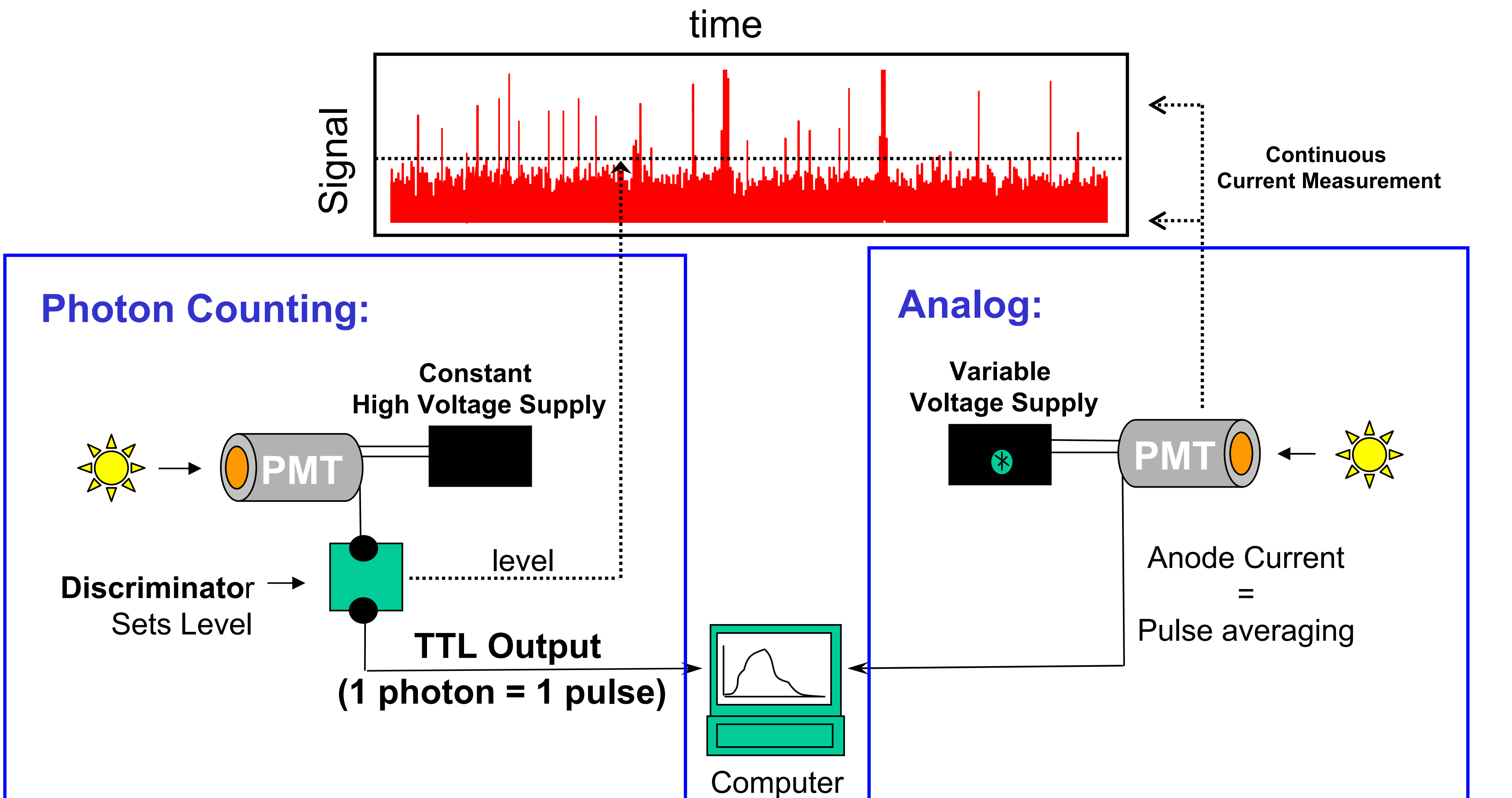
http://micro.magnet.fsu.edu/primer/flash/photomultiplier

Principle of operation:



Pictures:

Photon Counting (Digital) and Analog Detection



Primary Advantages:

1. Sensitivity (high signal/noise)
2. Increased measurement stability

Primary Advantage:

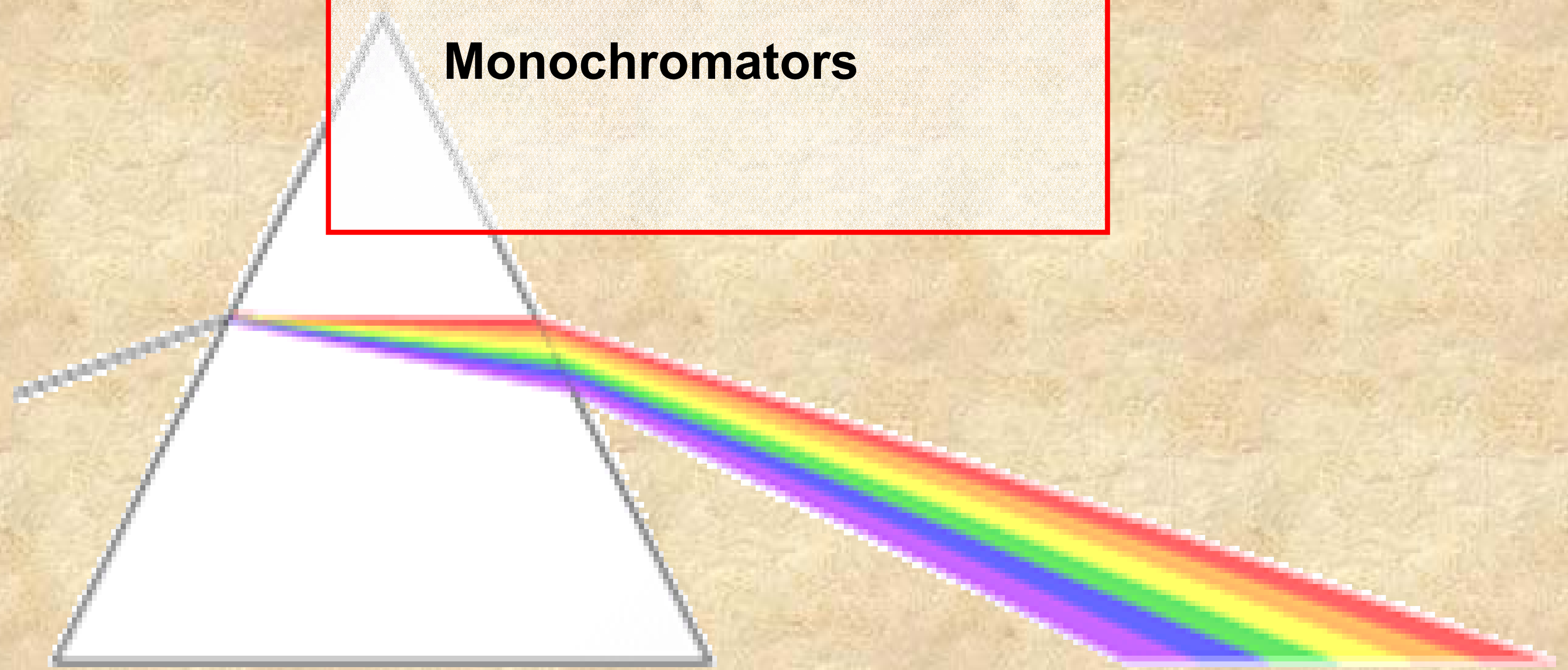
1. Broad dynamic range
2. Adjustable range

Wavelength Selection

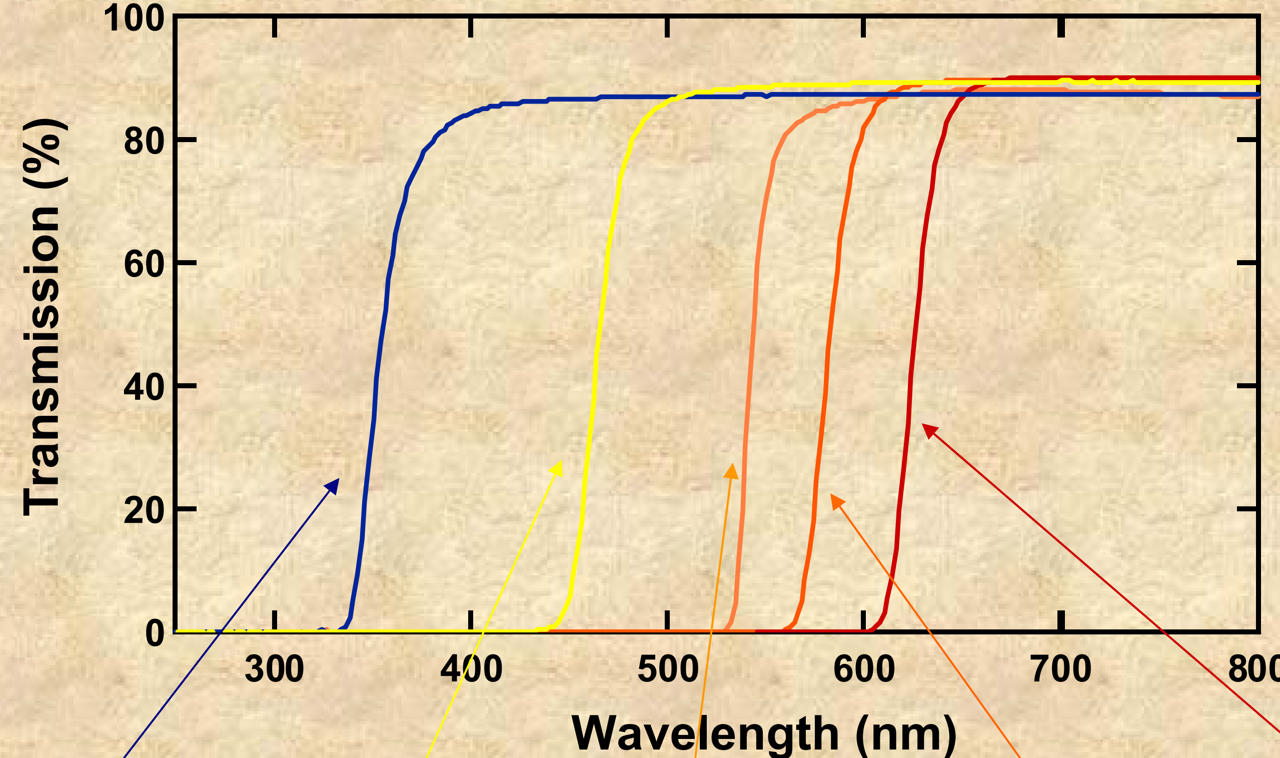
Fixed Optical Filters

Tunable Optical Filters

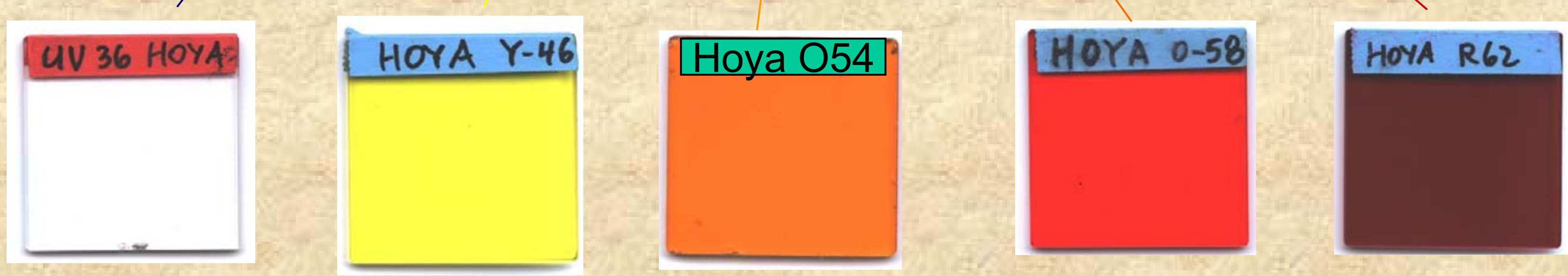
Monochromators



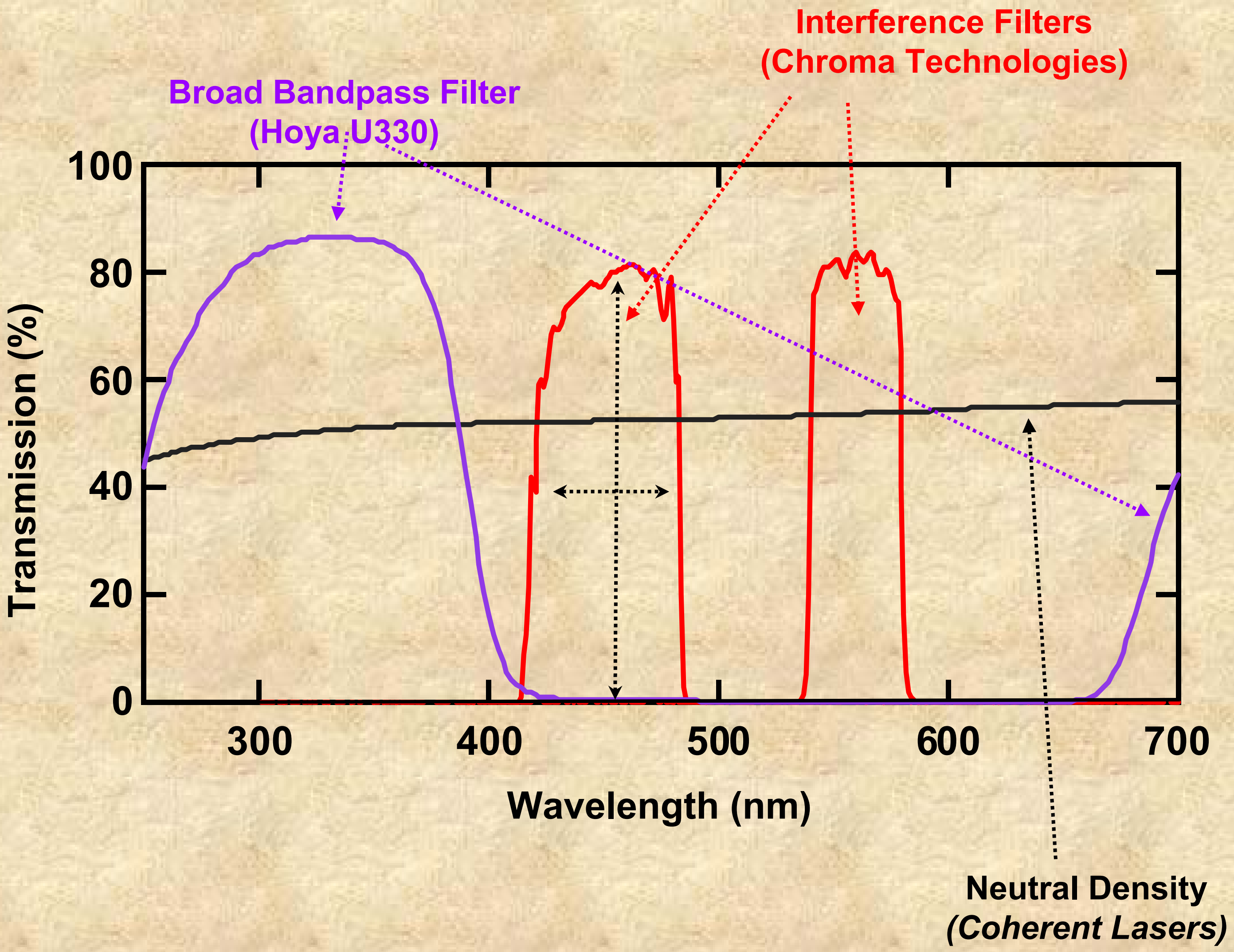
Long Pass Optical Filters



Spectral Shape
Thickness
Size
Fluorescence (!?)



More Optical Filter Types…



Monochromator: Principle

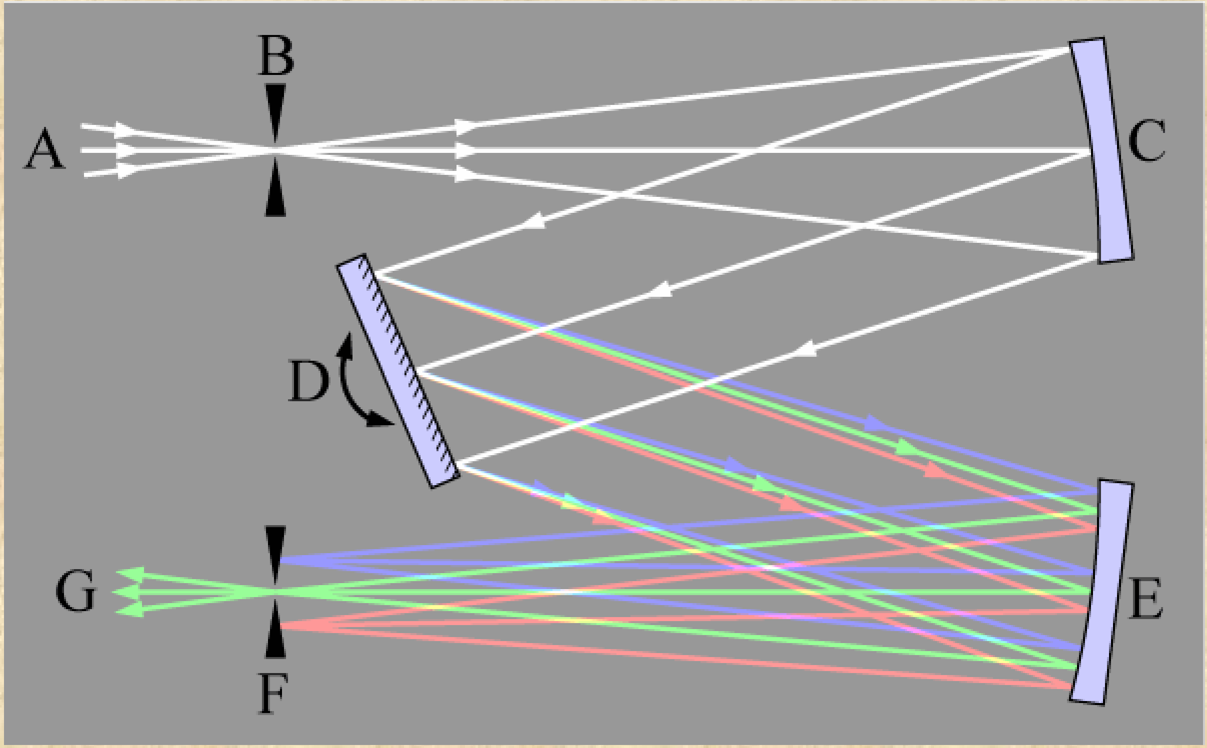
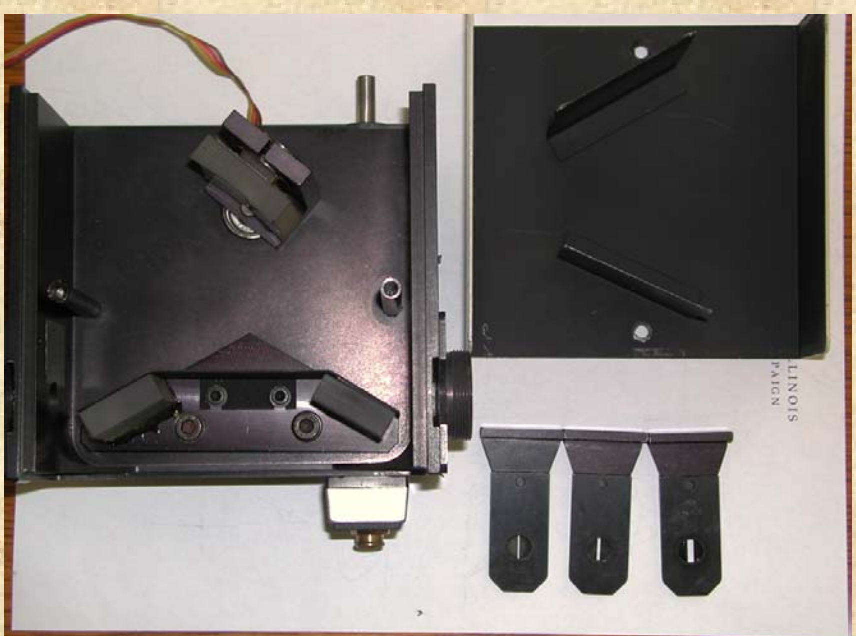
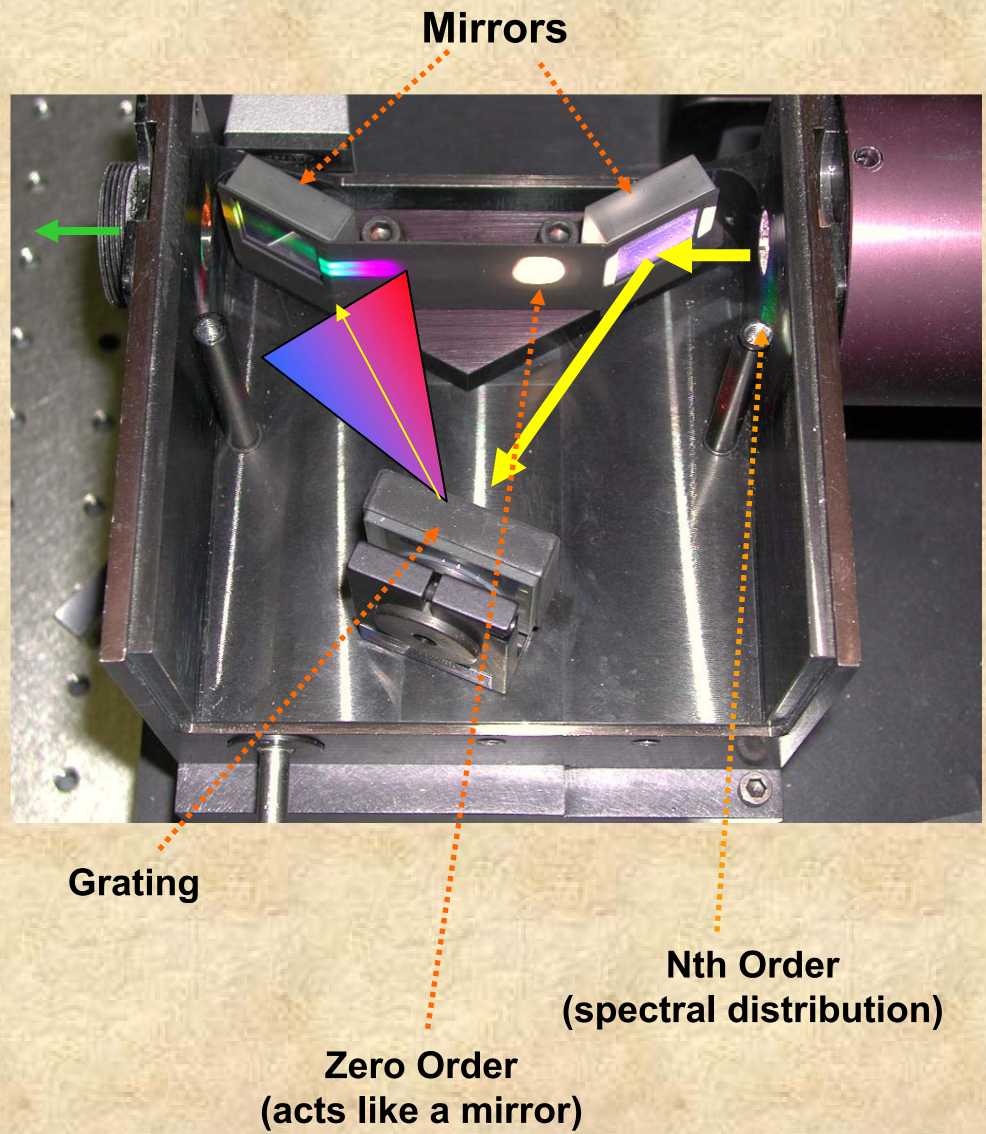


Diagram of a Czerny-Turner monochromator.
Light (**A**) is focused onto an entrance slit (**B**) and is collimated by a curved mirror (**C**). The collimated beam is diffracted from a rotatable grating (**D**) and the dispersed beam re-focused by a second mirror (**E**) at the exit slit (**F**). Each wavelength of light is focused to a different position at the slit, and the wavelength which is transmitted through the slit (**G**) depends on the rotation angle of the grating.

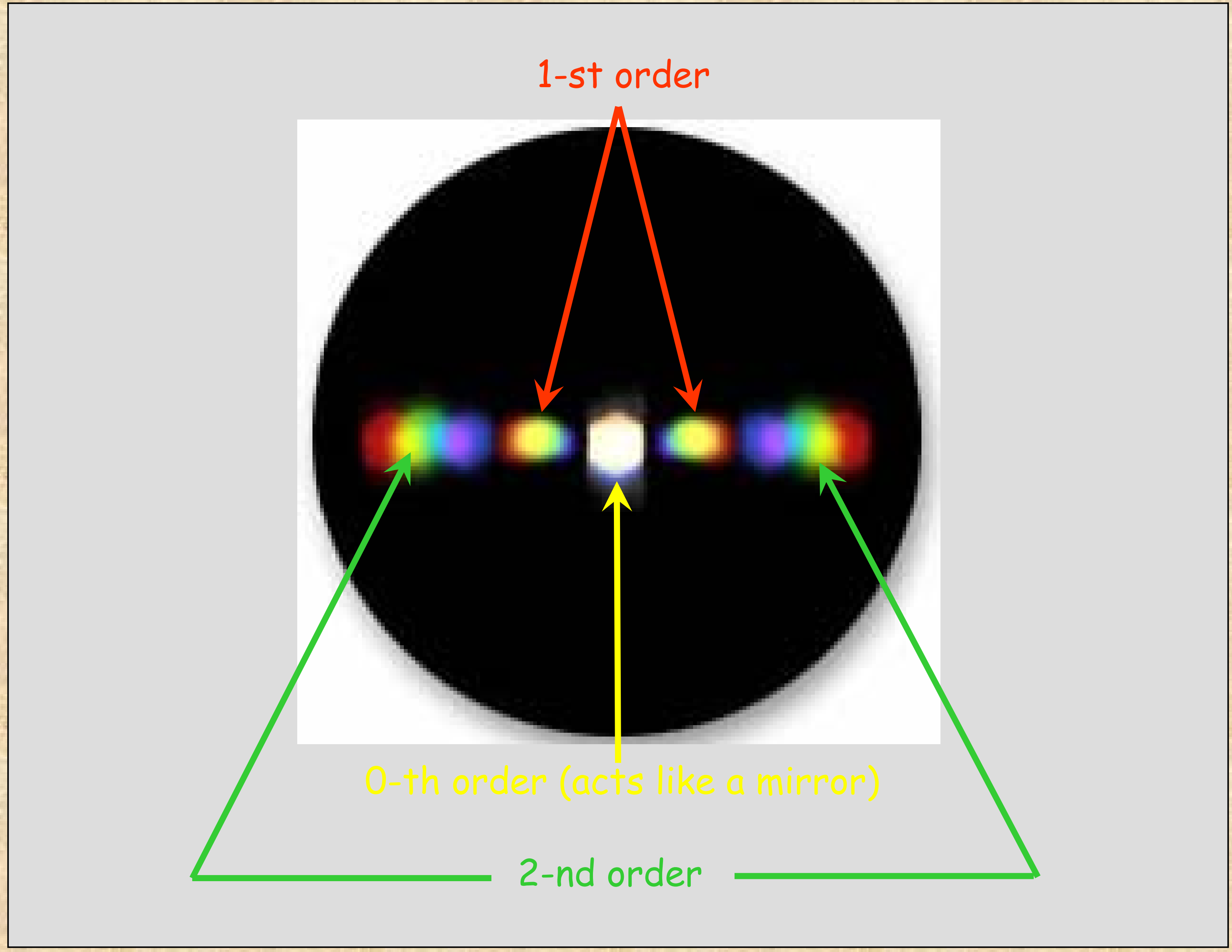
The Inside of a Monochromator

H10 Monochromator



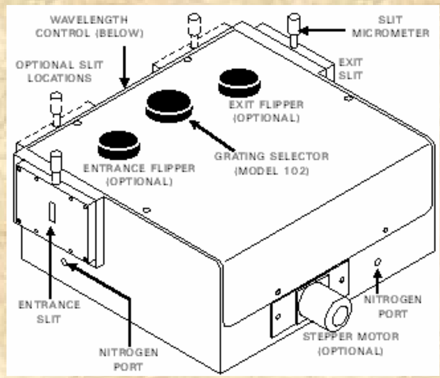


Order of diffraction



Monochromator Slits

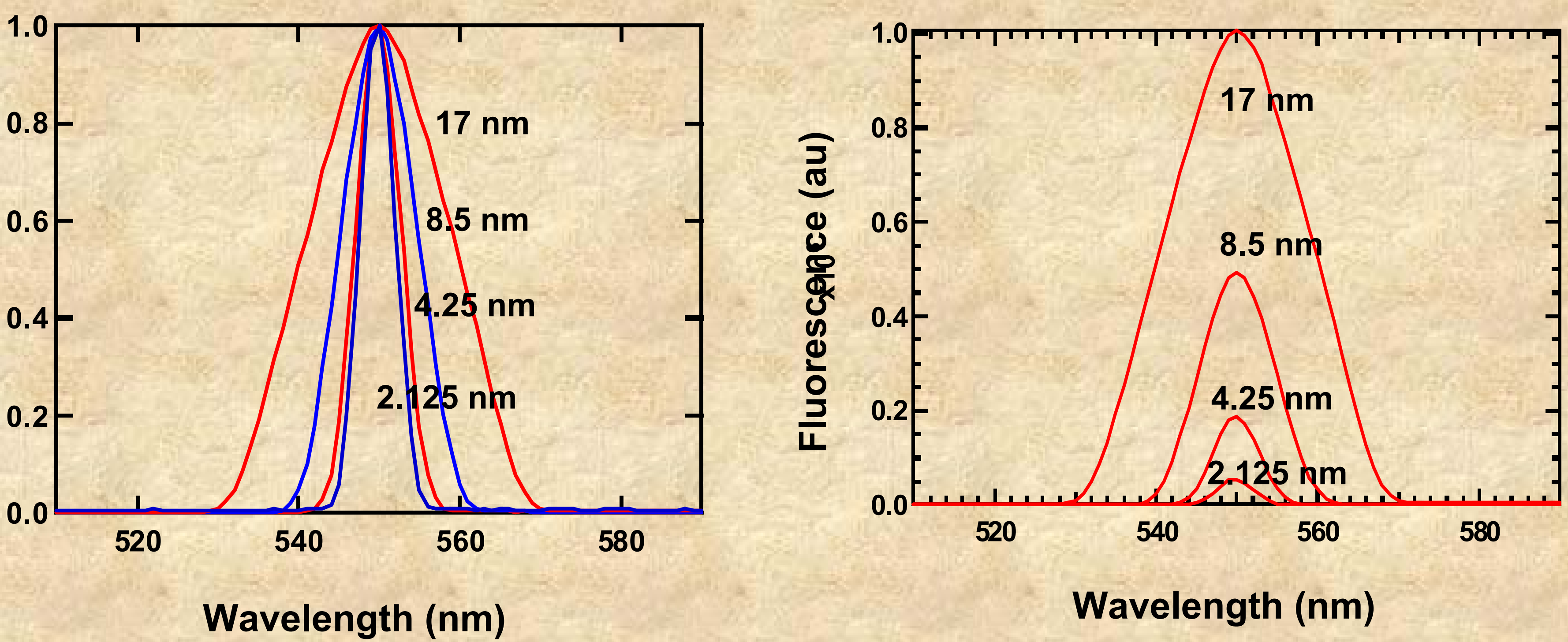
The slit size determines the bandpass and the throughput



Reducing the slit size leads to …

1. Drop in intensity
2. Narrowing of the spectral selection

Changing the Emission Bandpass

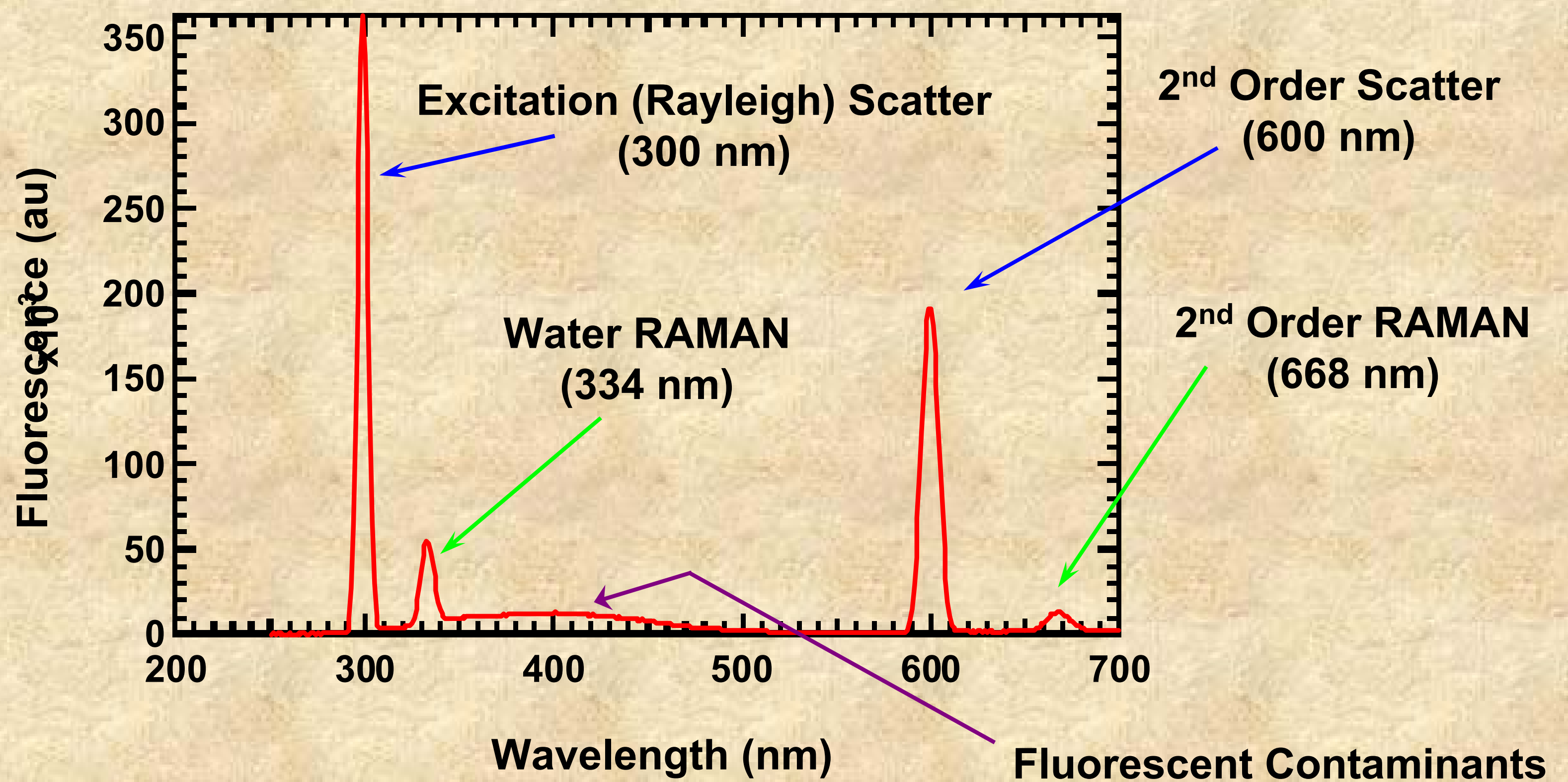


Collected on a SPEX Fluoromax - 2

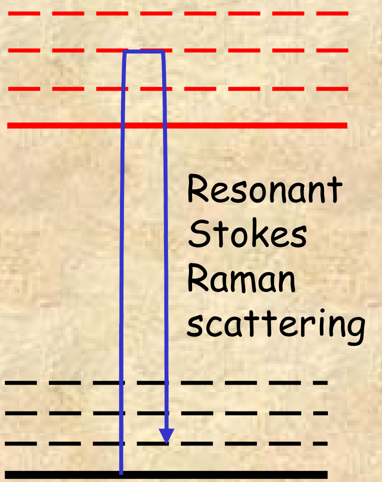
Typical Background Emission Spectrum

Emission Scan:

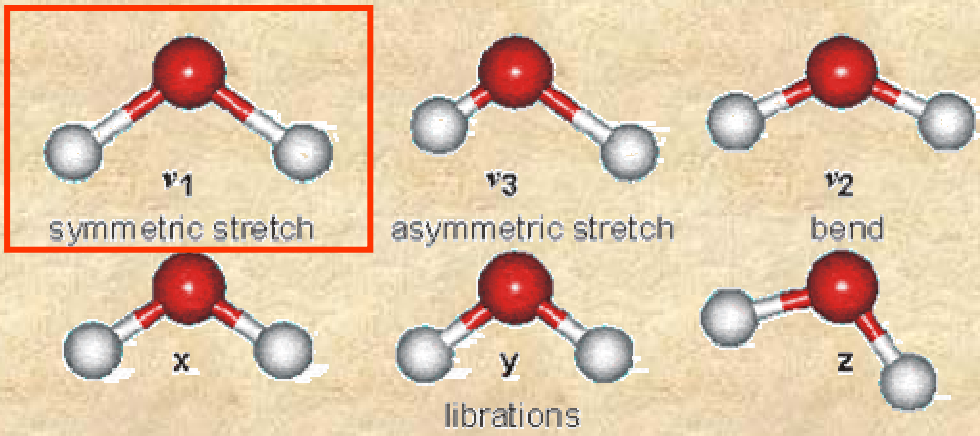
Excitation 300 nm
Glycogen in PBS



Raman scatter of water



Vibrational modes of water



Energy for the OH stretch vibrational mode in water (expressed in inverse wavenumbers): 3400 cm^{-1}

Simple formula to calculate the wavelength of the Raman peak:

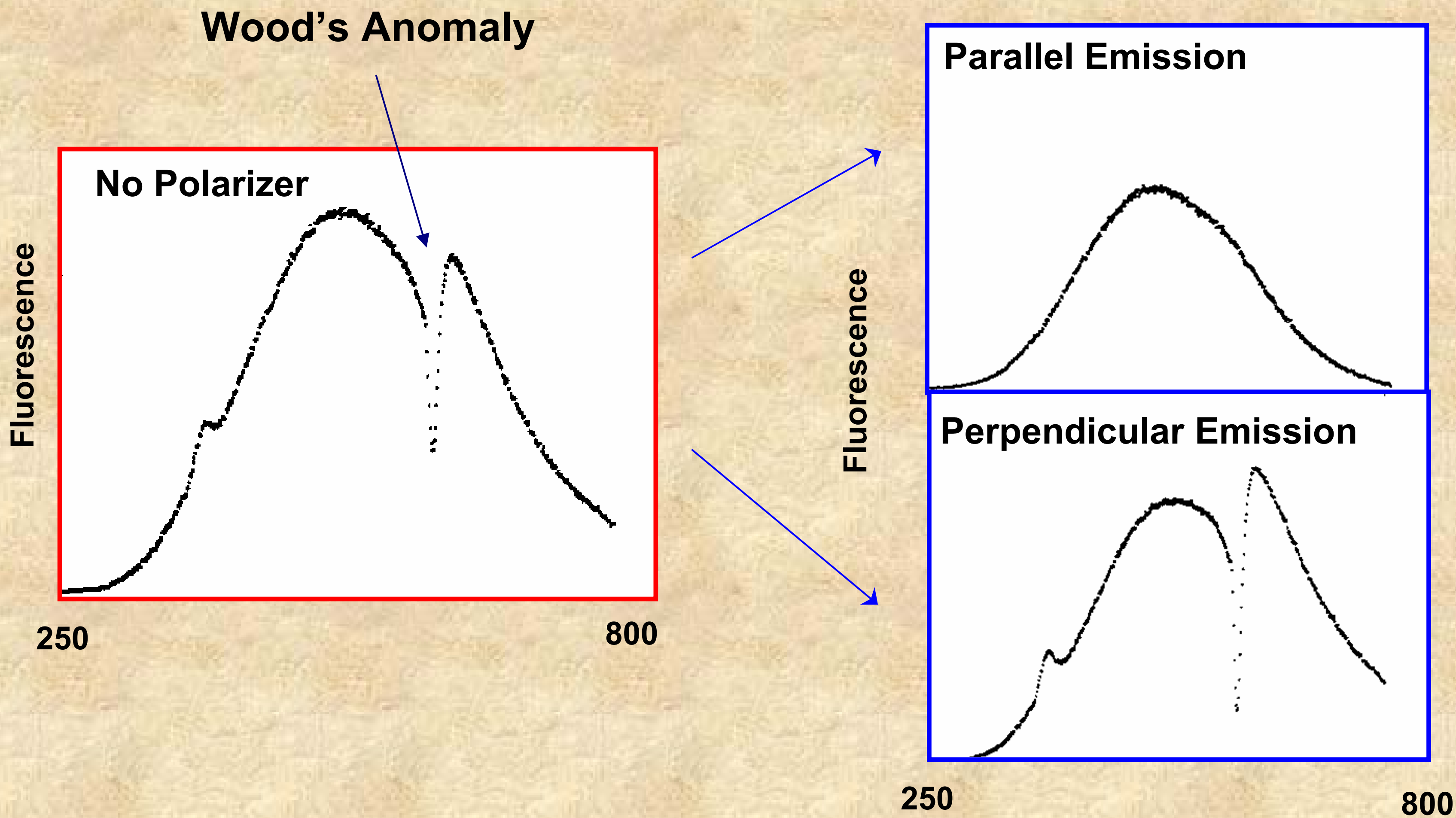
(1) Take the excitation wavelength (say 490 nm) and insert in the following equation:

(2) The result specifies the position of the raman peak in nanometers (i.e. the raman peak is at 587nm for an excitation wavelength of 490nm.

$$\frac{10^7}{\frac{10^7}{490} - 3400} = 587$$

Monochromator Polarization Bias

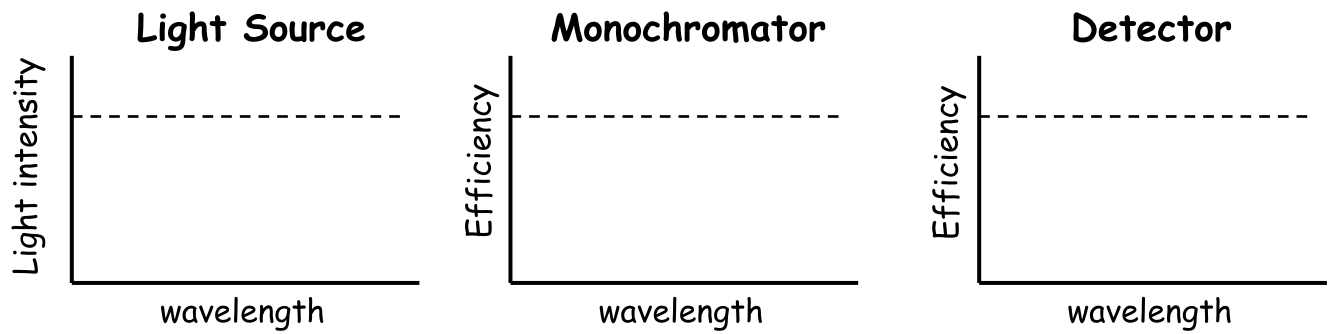
Tungsten Lamp Profile Collected on an SLM Fluorometer



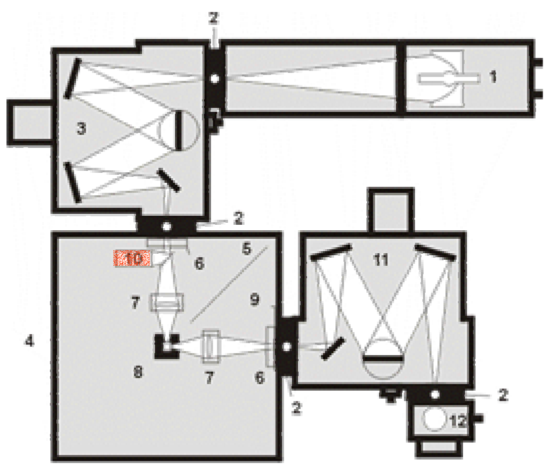
Adapted from Jameson, D.M., Instrumental Refinements in Fluorescence Spectroscopy: Applications to Protein Systems., in Biochemistry, Champaign-Urbana, University of Illinois, 1978.

Distortion of Excitation and Emission Spectra

Ideal Fluorometer:



Excitation Correction:



A beam splitter diverts a few percent of the excitation light impinging on the sample. This light is measured with a calibrated silicon photodiode (10). Thus the instrument monitors at all times the intensity of the excitation light.

Emission Correction:

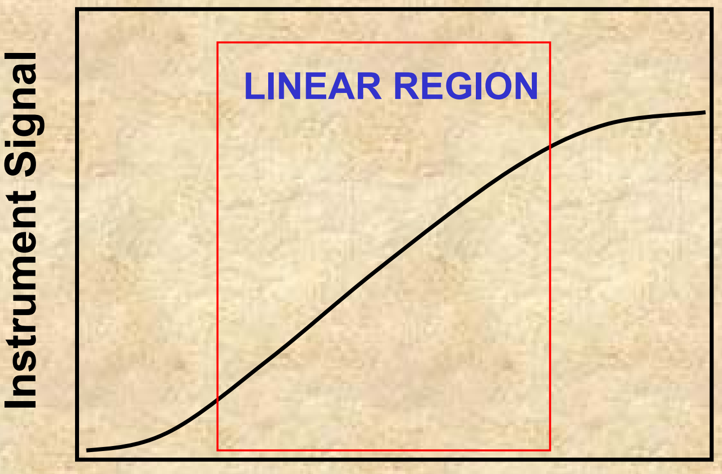
Correcting the emission spectra can be quite complicated and requires specialized techniques.

Luckily, the wavelength and polarization dependent correction factors have been determined and are electronically available (see lab section).

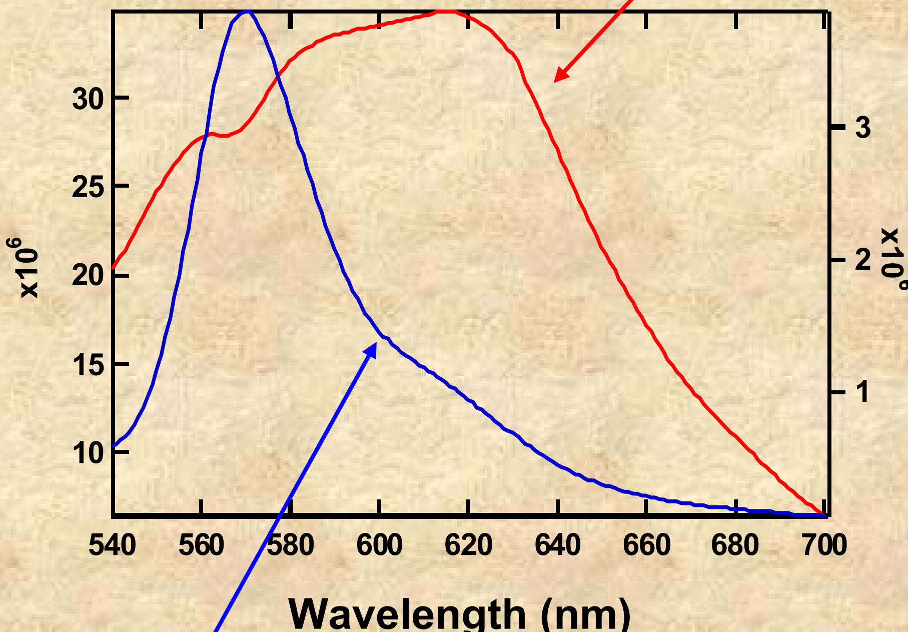
Sample Issues

Signal Attenuation of the Excitation Light
PMT Saturation

Fluorescence vs Signal



Excess Emission



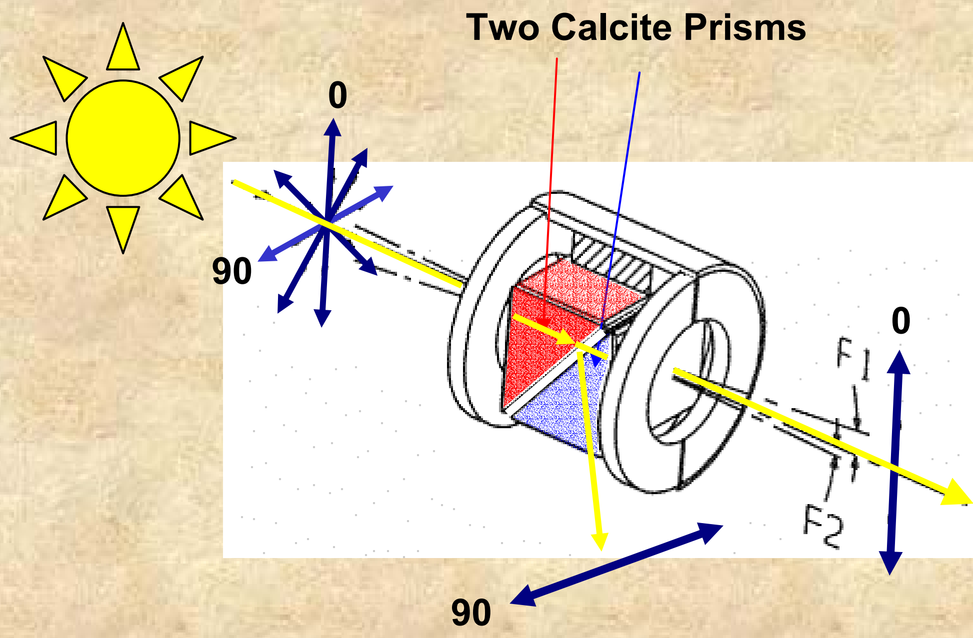
Reduced emission intensity
1. ND Filters
2. Narrow slit widths
3. Move off absorbance peak

Polarizers

Common Types:

- **Glan Taylor (air gap)**
- **Glan Thompson**
- **Sheet Polarizers**

The *Glan Taylor* prism polarizer



Sheet polarizer

Two UV selected calcite prisms are assembled with an intervening air space. The calcite prism is birefringent and cut so that only one polarization component continues straight through the prisms. The spectral range of this polarizer is from 250 to 2300 nm. At 250 nm there is approximately 50% transmittance.