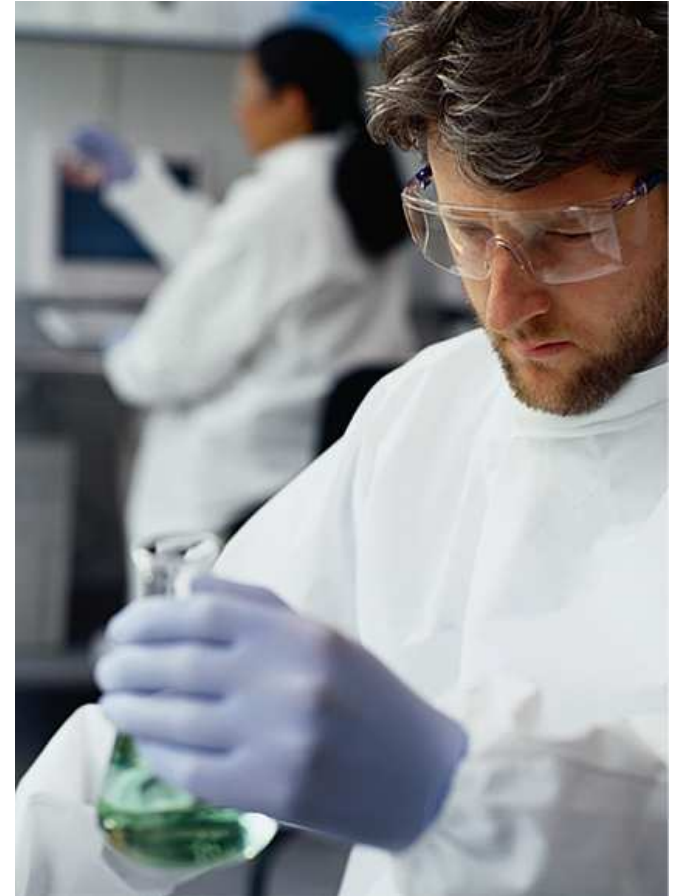


Visible and UV/Vis SPECTROPHOTOMETRY

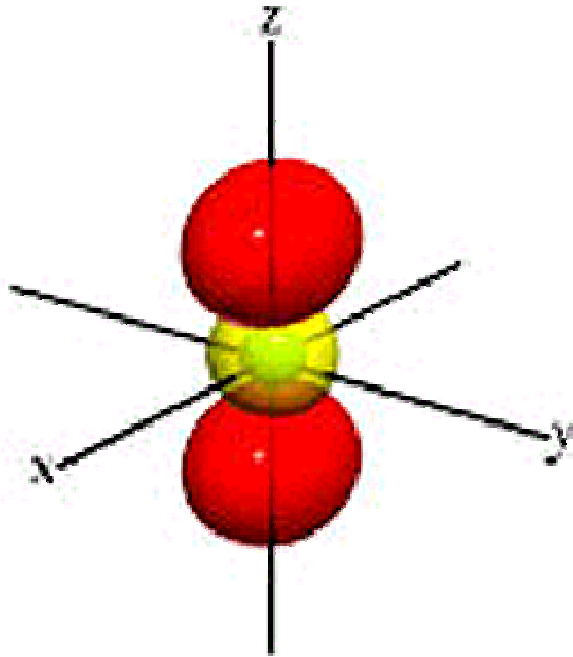
Who uses spectrophotometers?

- Academia & Teaching
- Biological & Biochemical Research
- Bio' & Biochem' Monitoring & Quality Control
- Chemical Research
- Chemical Process Monitoring & QC
- Pharmaceutical Research
- Pharmaceutical Monitoring & QC
- Environmental Laboratories
- Hospital Pharmacy & Clinical Laboratories
- Industrial Laboratories
- etc. etc. etc.
- **Basically just about ANY laboratory!**



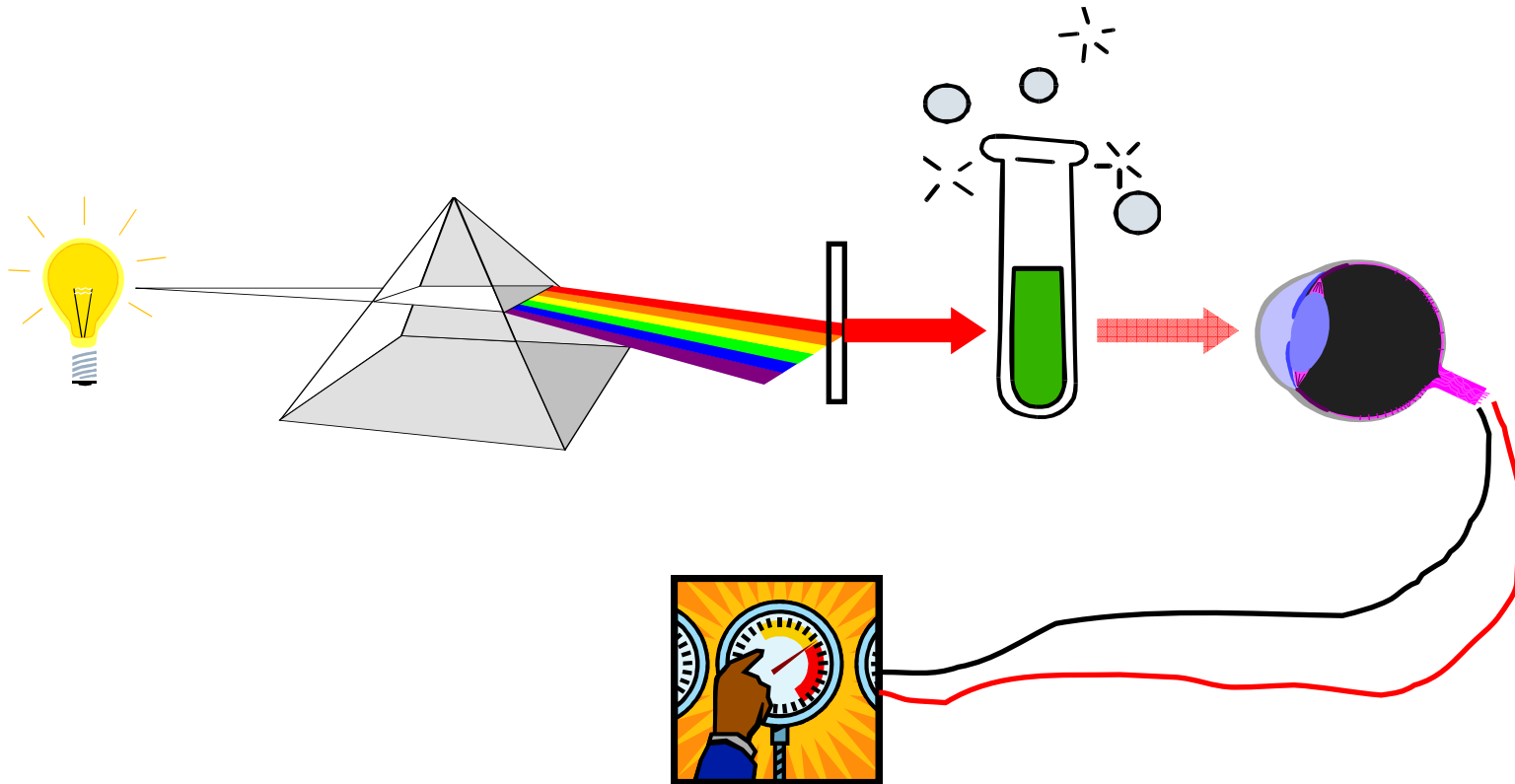
Basic theory

MOLECULAR ORBITALS



- Electrons in molecular orbitals can interact with photons of light.
- Only certain energy levels are allowed.
- Only a photon with the right energy (wavelength) can promote the electron to the “excited state”.
- The orbital returns to the “ground state” by colliding with another molecule and giving up this tiny amount of energy in the form of heat.

What's a spectrophotometer?



Types of Spectrophotometers

- There are different beam systems in Spectroscopy as follows:

-

1- SINGLE BEAM

Types of Single Beam Spectrophotometers:

:

- **A) Visible (VIS) Wavelength (From 330 to 1000nm):**
 - 1. Spectro SC
 - 2. Spectro 20D
 - 3. Spectro 23
 - 4. Spectro 23RS
 - 5. Spectro 2000RS
 - 6. Spectro 2000RSP

Types of Spectrophotometers

- **B) UV-VIS Wavelength (This means it can read Ultra-violet and Visible wavelength, and can read from 190 to 1100 nm.)**

- 7. Spectro UV-VIS RS (UV-2500)
- 8. Spectro UV-Vis AUTO (UV-2602)

-

2- SPLIT BEAM or DUAL BEAM (these are all UV-VIS Spectrophotometers)

Types of Split or Dual Beam Spectrophotometers:

- 9. Spectro 8 Auto Cell (UVS-2700)
- 10. Spectro UV-VIS 8 Auto Cell Variable Bandwidth (UVS-2800)

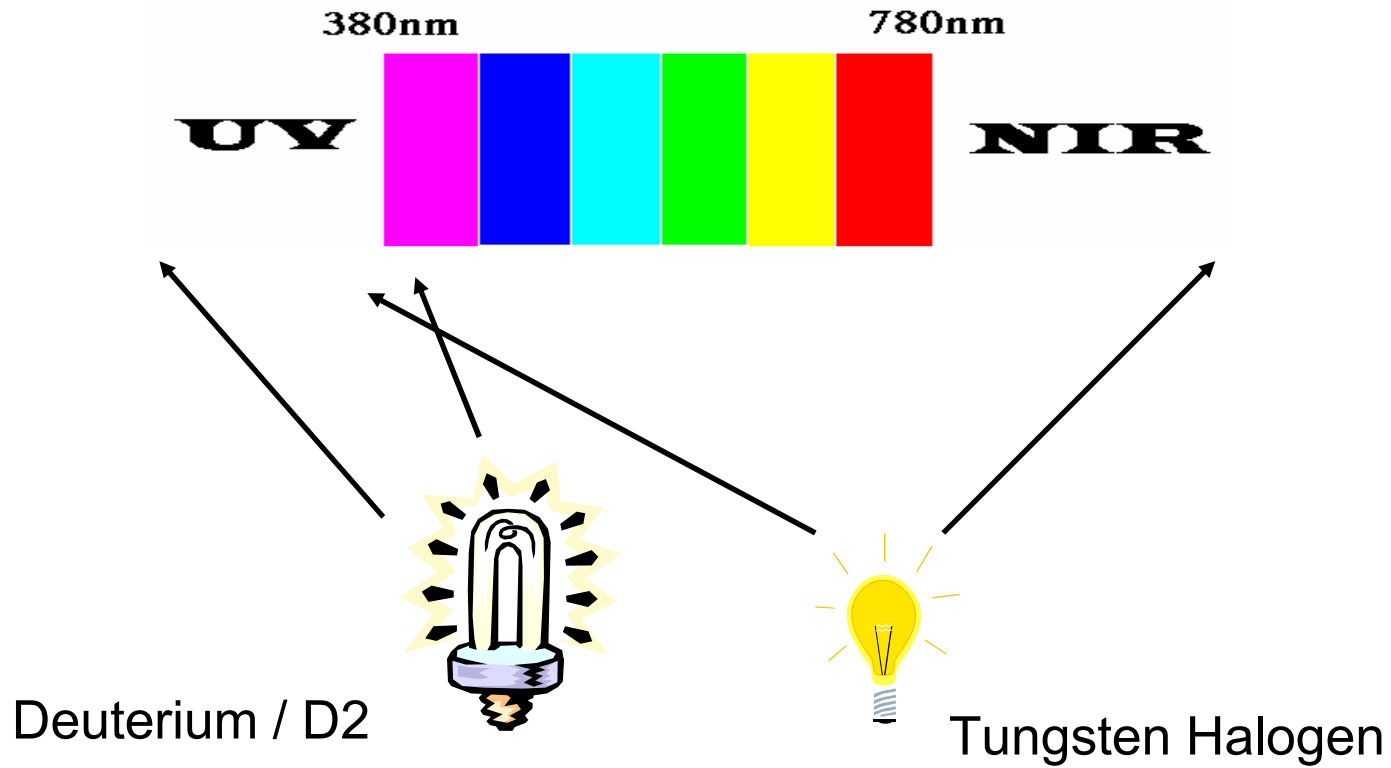
Types of Spectrophotometers

3. DOUBLE BEAM (these are all UV-VIS Spectrophotometers and they can read from 190 to 1100nm.)

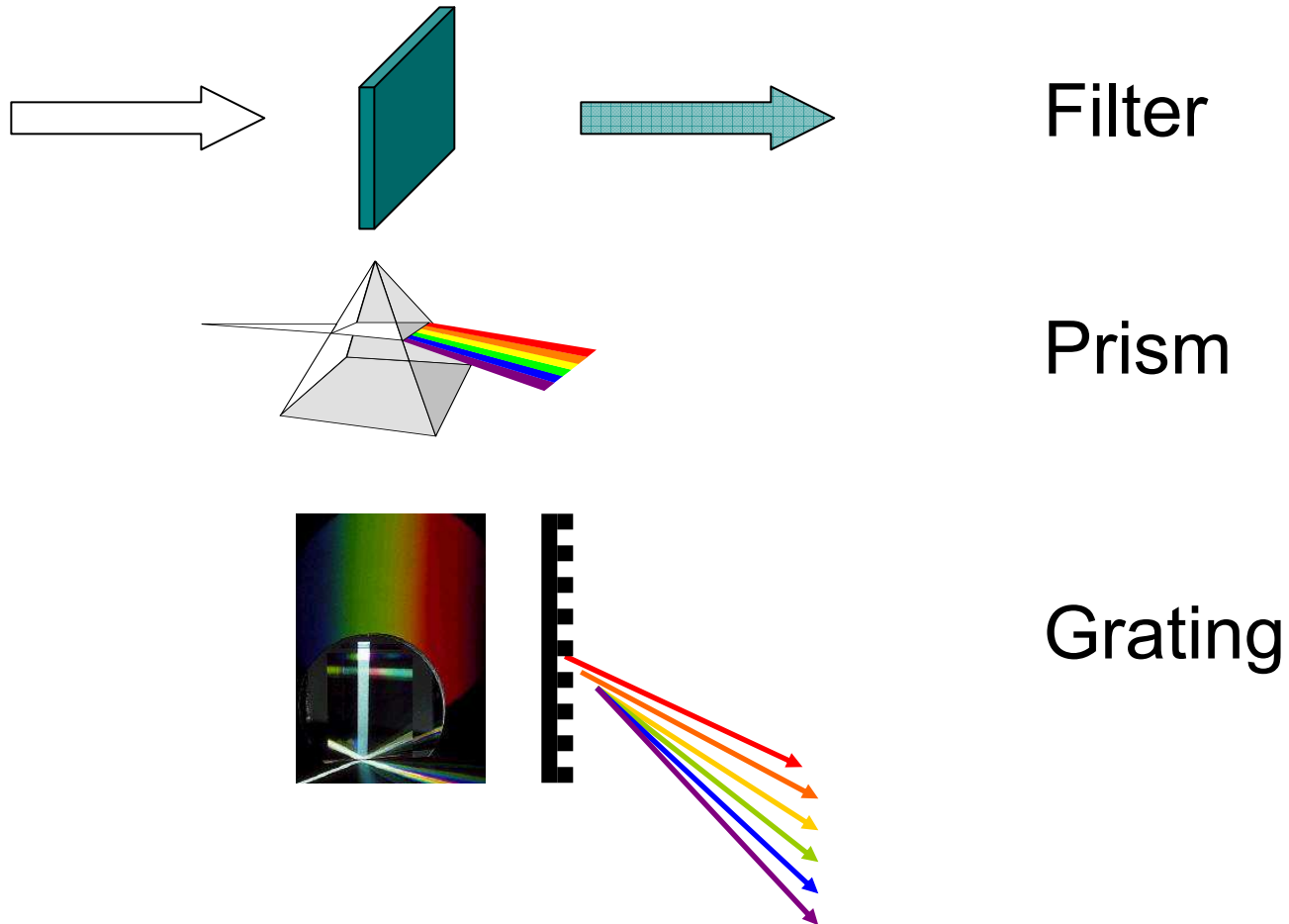
Types of Split or Dual Beam Spectrophotometers:

11. Spectro UV-VIS Double (UVD-2950) 2 cell holder
12. Spectro UV-VIS Double (UVD-3000) – 8 Auto Cell holder and 1 Cell fixed
13. Spectro UV-VIS Double (UVD-3200) – as above with Variable Bandwidth
14. Spectro UV-VIS Research (UVD-3400) – 2 Cell holder
15. Spectro UV-VIS Research (UVD-3500) Variable Bandwidth – 2 Cell holder

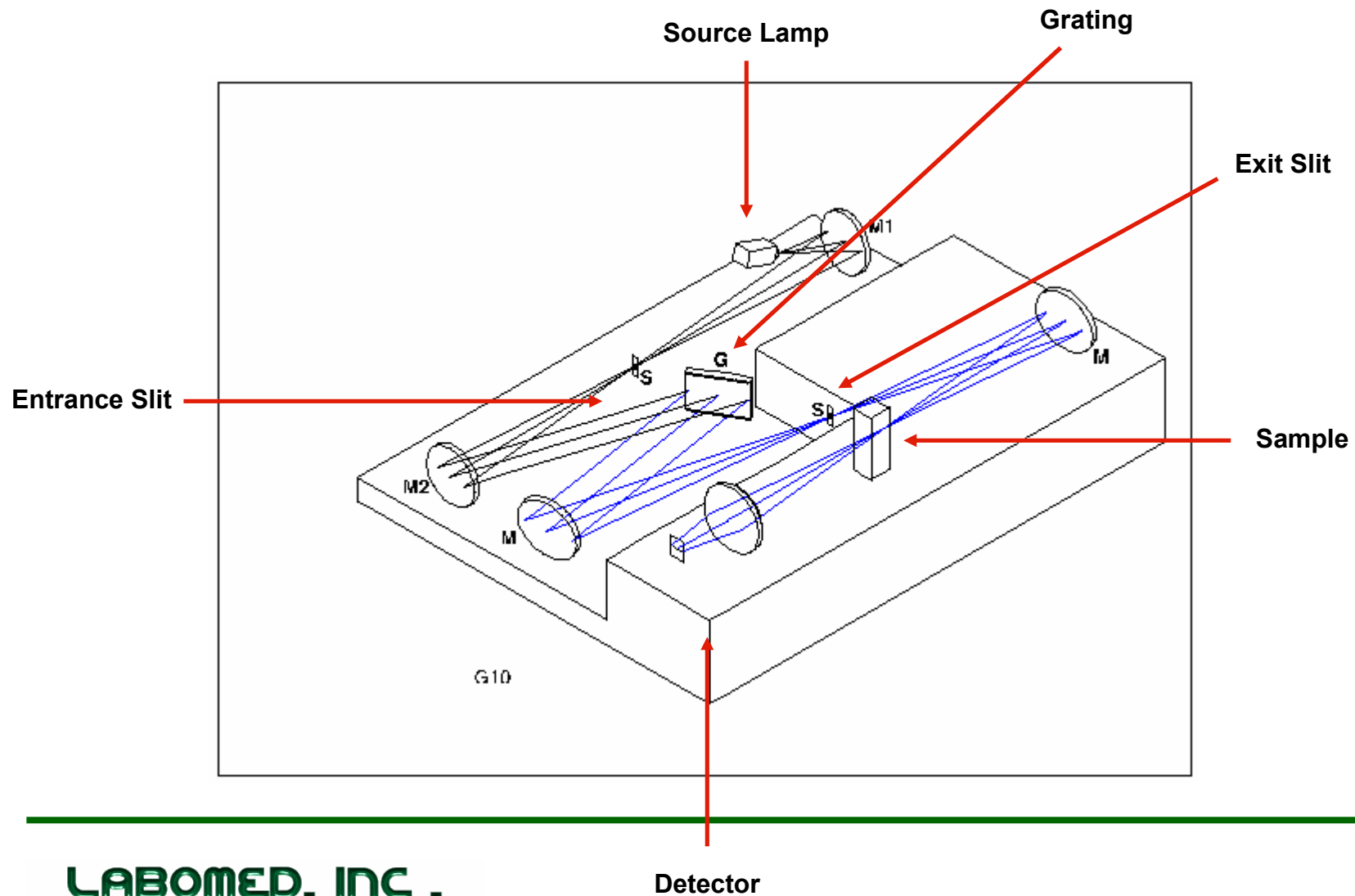
Light sources



Dispersing elements



Overall instrument recap



The Beer-Lambert Law

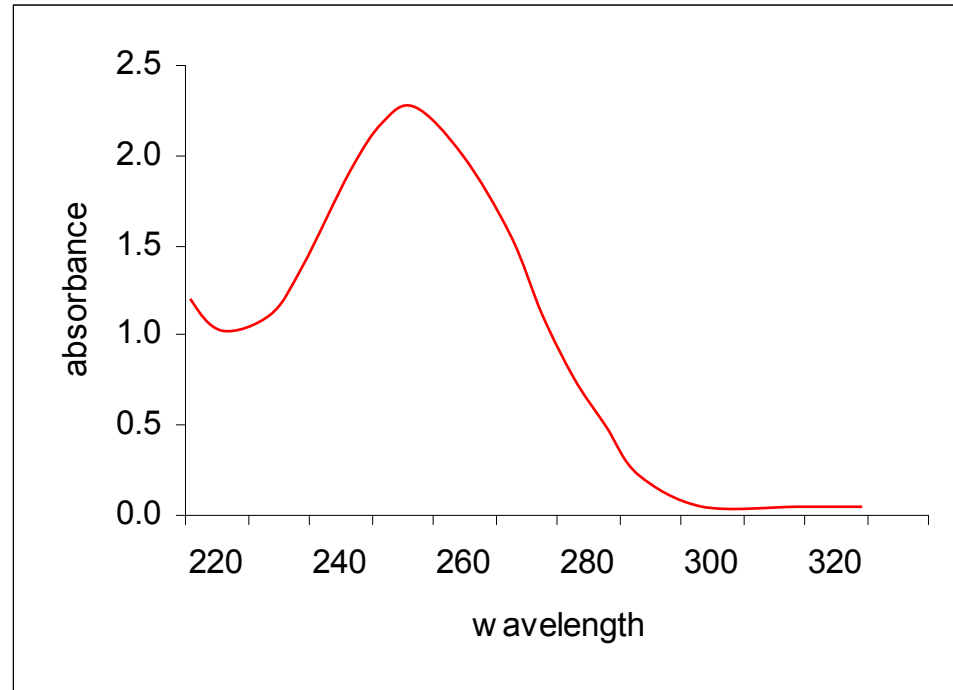
WHEN THE CORRECT WAVELENGTH IS USED:

- Transmittance, $T = I/I_0$
- Absorbance = $\log 1/T$
- The concentration, C of a light absorbing molecule is proportional to the Absorbance:

$$A = (a) \times (b) \times (C)$$

How to find the right wavelength?

Do a spectral scan (i.e., measure the Absorbance at each wavelength) and plot the Absorbances vs the wavelengths

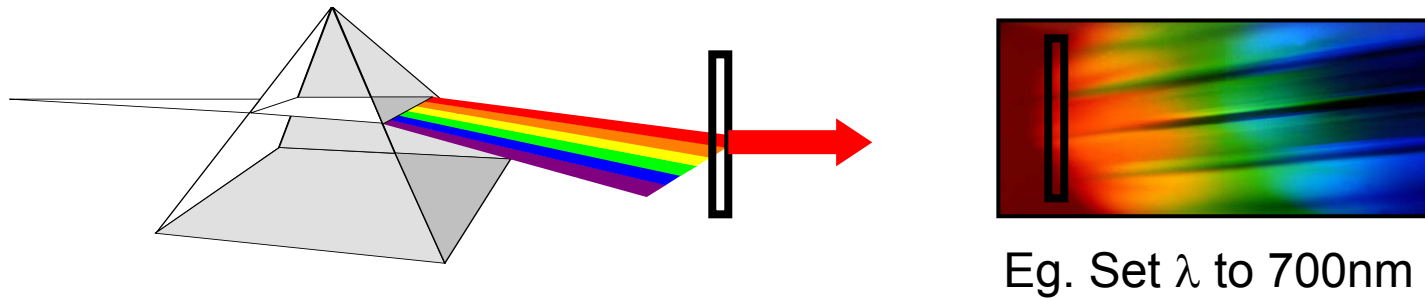


➤ Look for a peak value

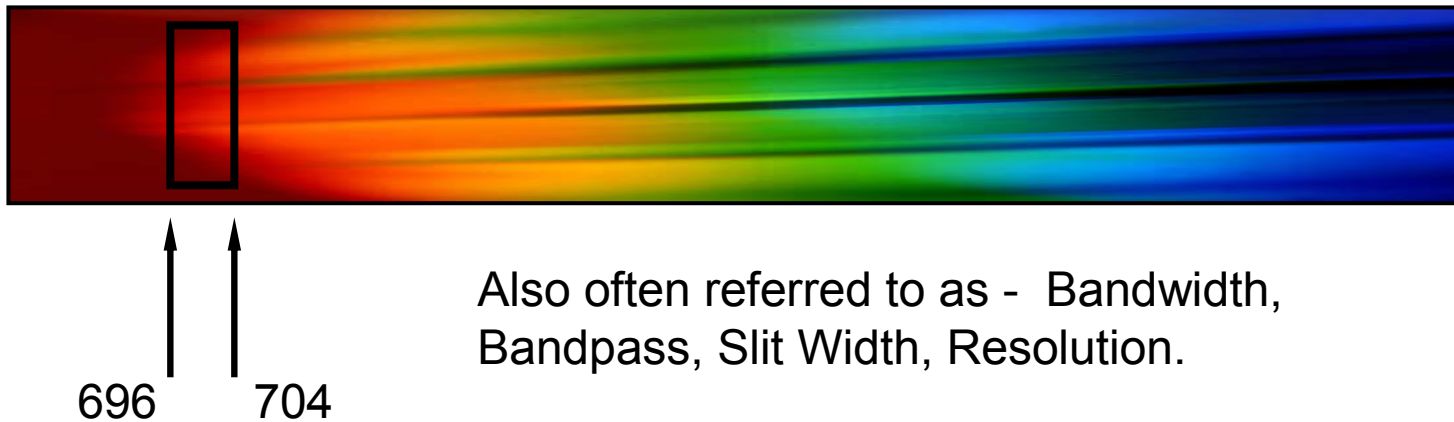
How to find the right wavelength?

- Qualitative analysis: The wavelength(s) at which the peaks occur is a characteristic of only one absorbing molecule
- Quantitative analysis: The height of this Absorbance peak is proportional to the concentration of the light absorbing molecule

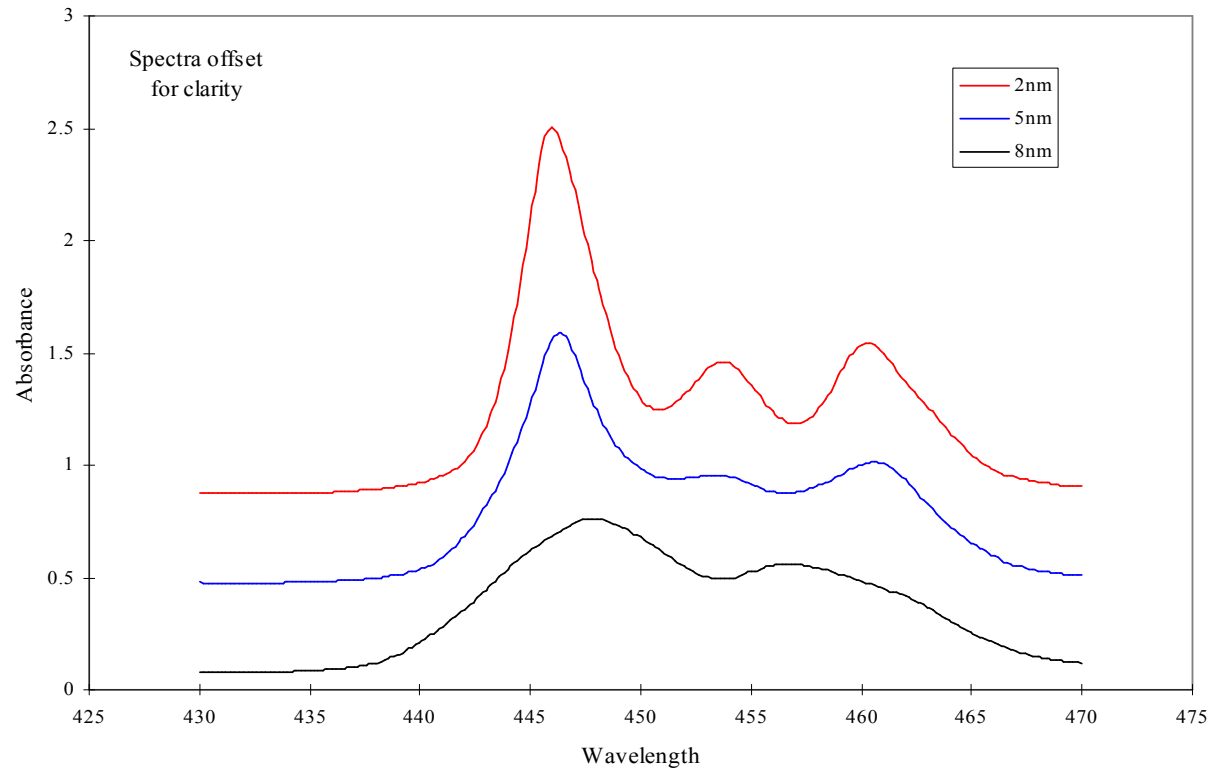
Spectral bandwidth



If SBW of instrument is 8nm



Spectral bandwidth



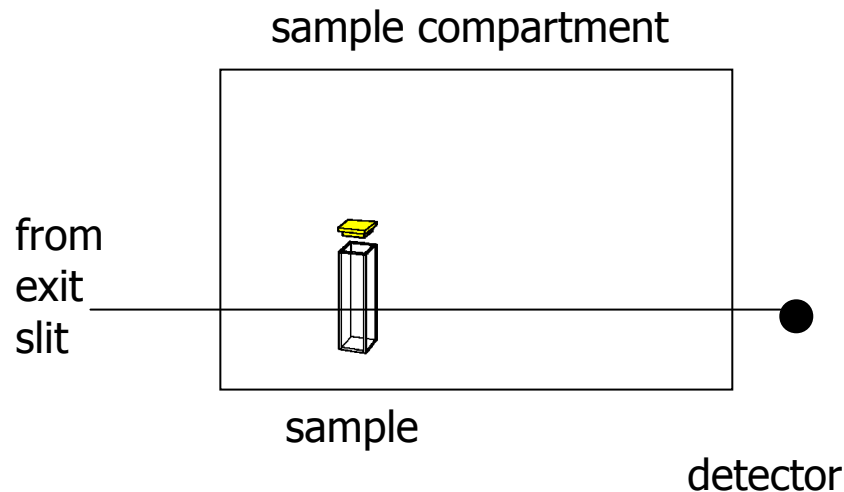
The effect of SBW on the ability to resolve spectral peaks

Range of instrument types

- Simple Fixed λ Filter Instruments
- Grating Instruments (used at one λ at a time)
- λ Scanning Grating Instruments - Fixed SBW
- λ Scanning Grating Instruments - Variable SBW

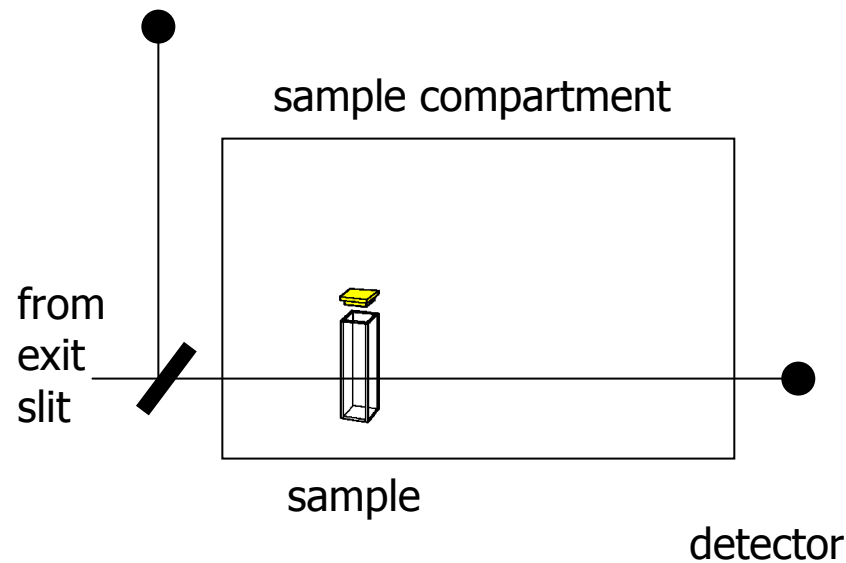
Single beam

FILTER PHOTOMETER AND GRATING SPECTROPHOTOMETER



- Low cost, easy to use and maintain
- but:
- Two separate steps to each measurement
- Cannot easily do spectral scans to find the best wavelength

Single split beam

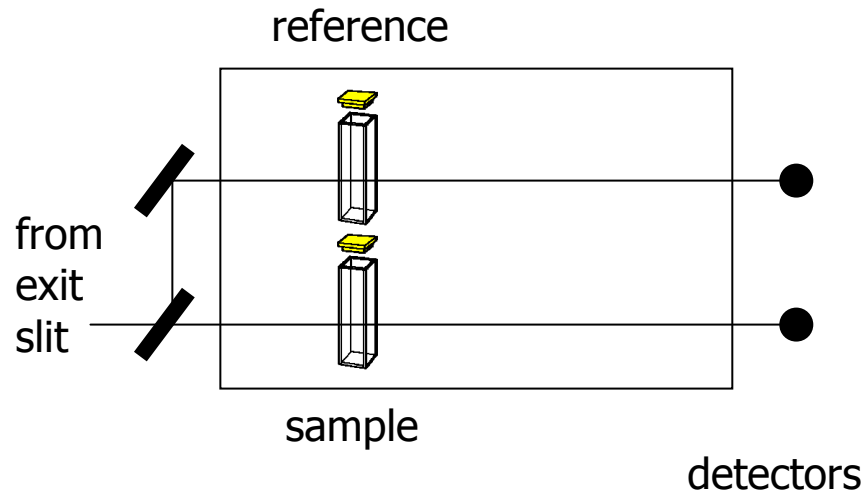


- Low cost, easy to use and maintain, improved stability over the single beam

but:

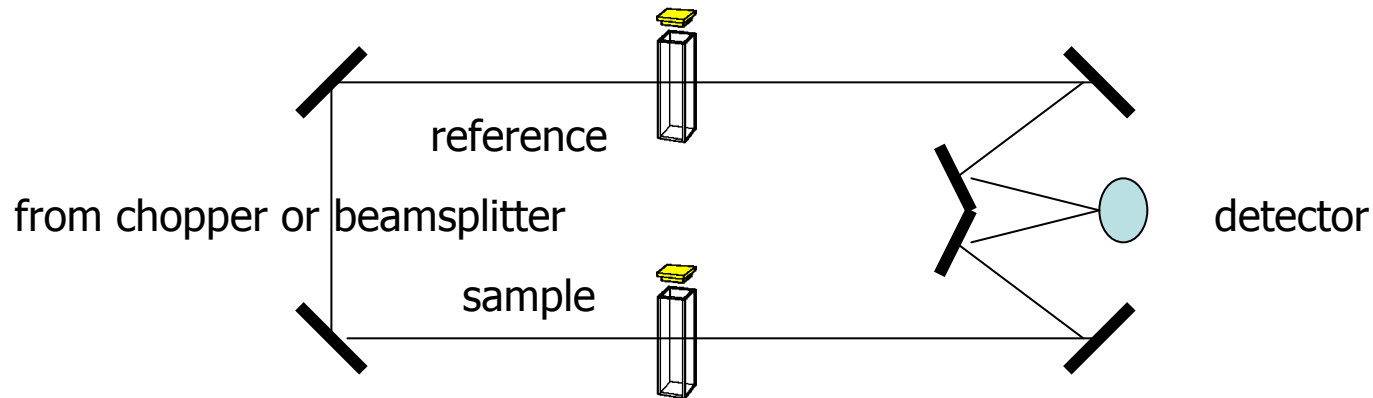
- Still just as much work to make a single wv measurement or a scan measurement

Dual beam



- Improved stability
- Continuously refers to the reference (repeatedly inserting the reference is not necessary)
but:
- Two detectors cannot be perfectly matched – one will detect light differently

True double beam



- Improved stability
- Continuously referencing
- Single light detector measures the sample and reference the same way (If you have two detectors, there is always some optical difference between them but - More expensive

Spectrophotometers from Labomed



LABOMED, INC.

CONCLUSION