Fundamentals and Application of UV-visible/NIR spectroscopy: A guide to best practices and getting good data

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Seminar Overview

I. UV-Visible spectroscopy theory basics
II. Instrument design and components
III. Sampling and experimental guidelines
IV. Sampling accessories and their related applications

What can we probe with UV-Vis Spectroscopy?

Solutions, powders, solids, films

Biomolecules

Semiconductors

Nanostructures

Quantitation

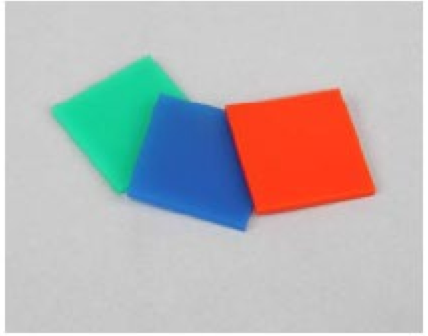
Qualitative

Color Analysis

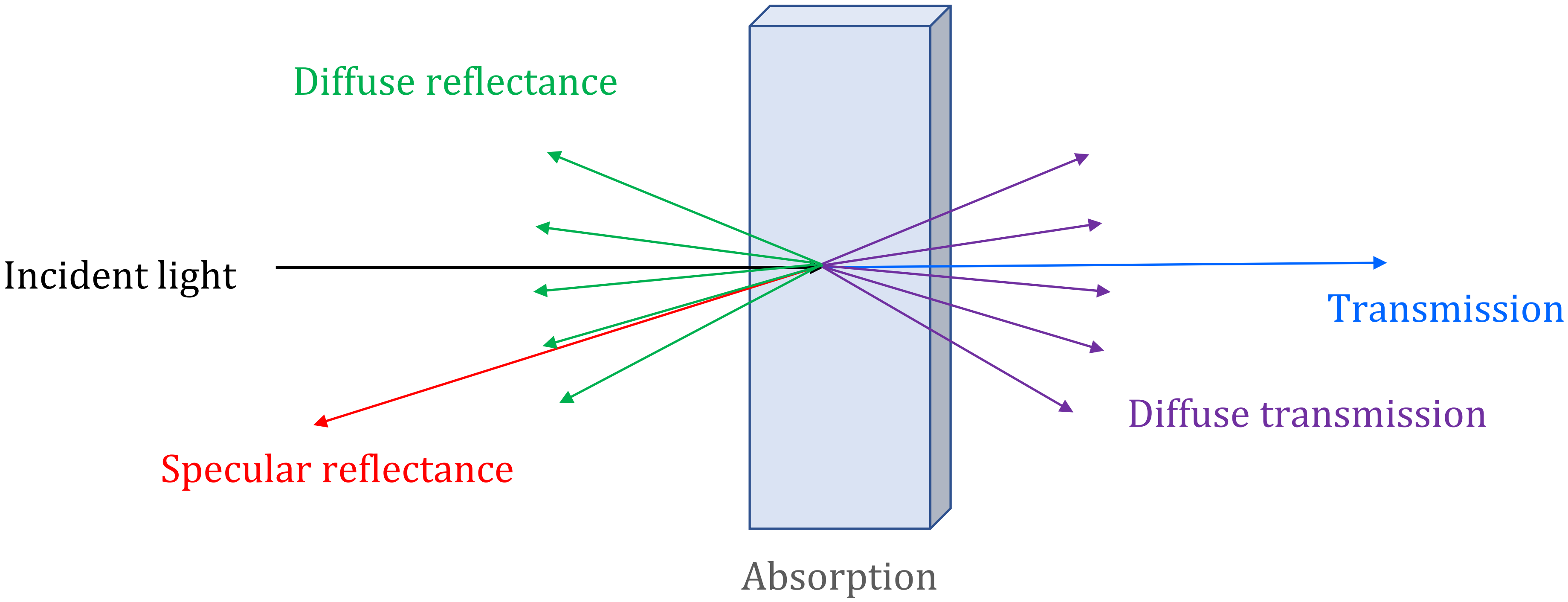
Thermal melts

Species presence

Molecular structure

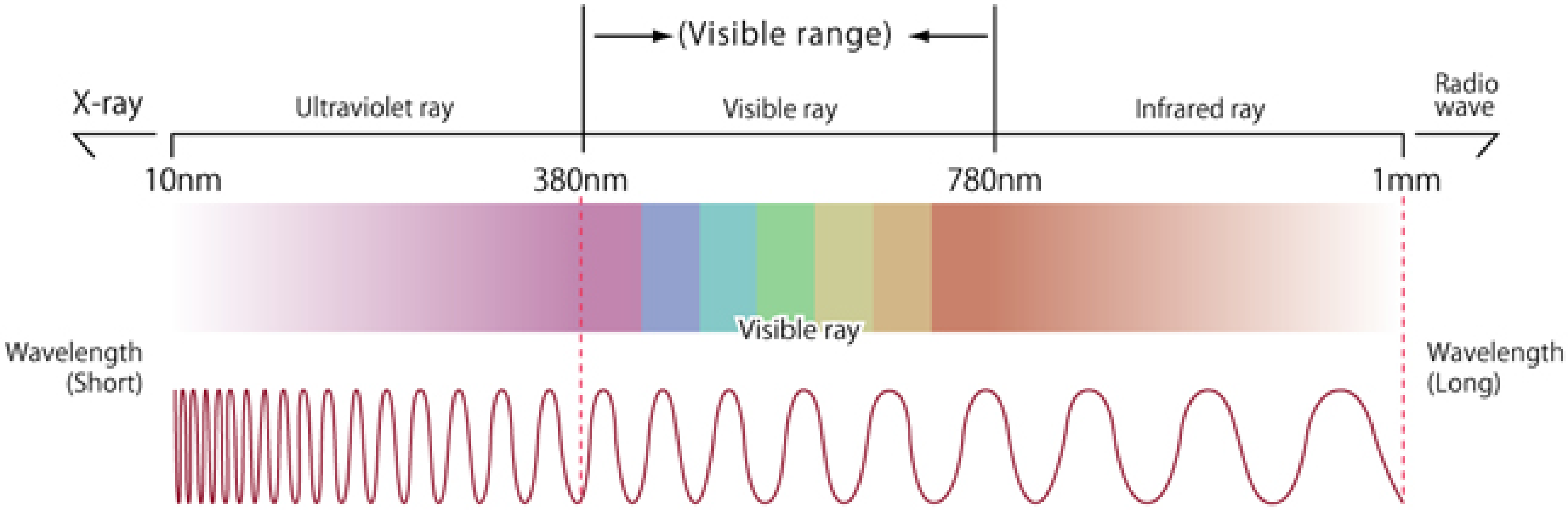


Techniques


Diffuse reflectance
Incident light
Transmission
Diffuse transmission
Specular reflectance
Absorption

Electromagnetic Spectrum

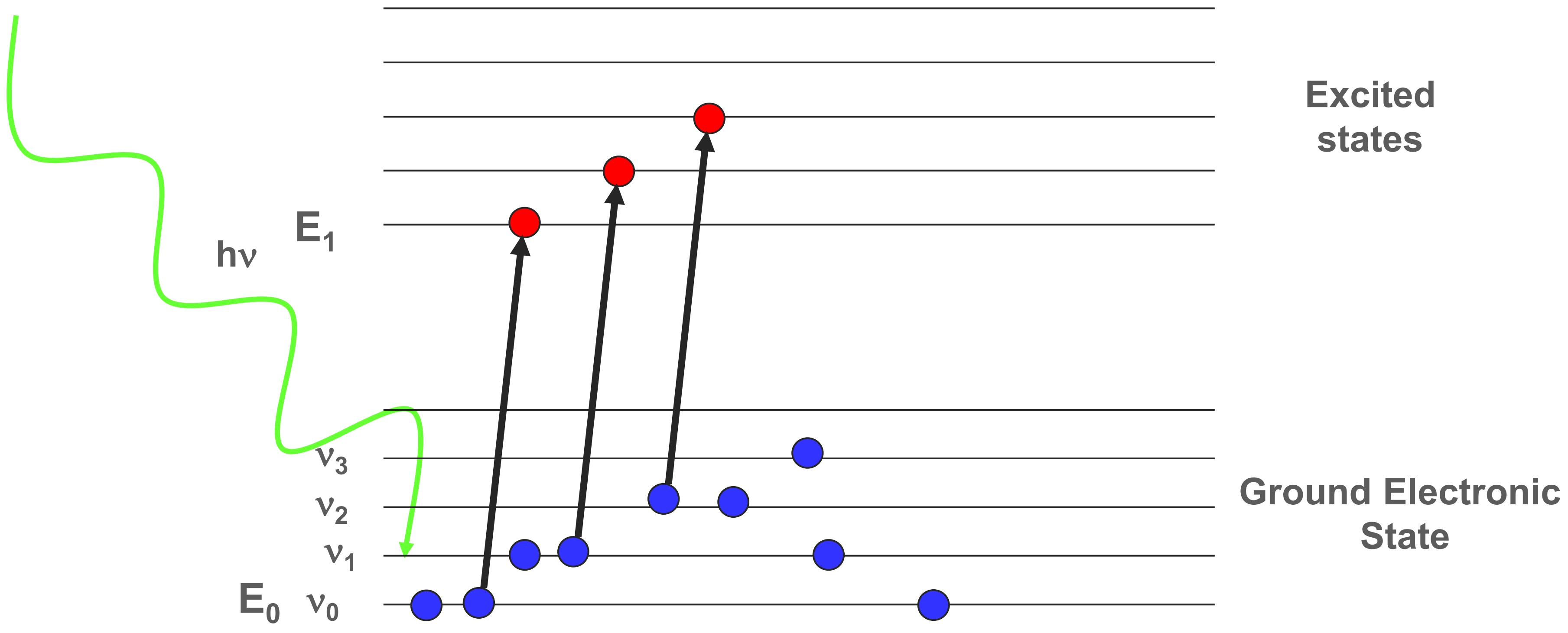
Increasing wavelength



Decreasing energy

https://www.toa.jp/soundoh_wiki/index.php?Soundindex/Secure%20Wireless%20Communication-Infrared%20Wireless%20Microphone%20Systems

Absorption



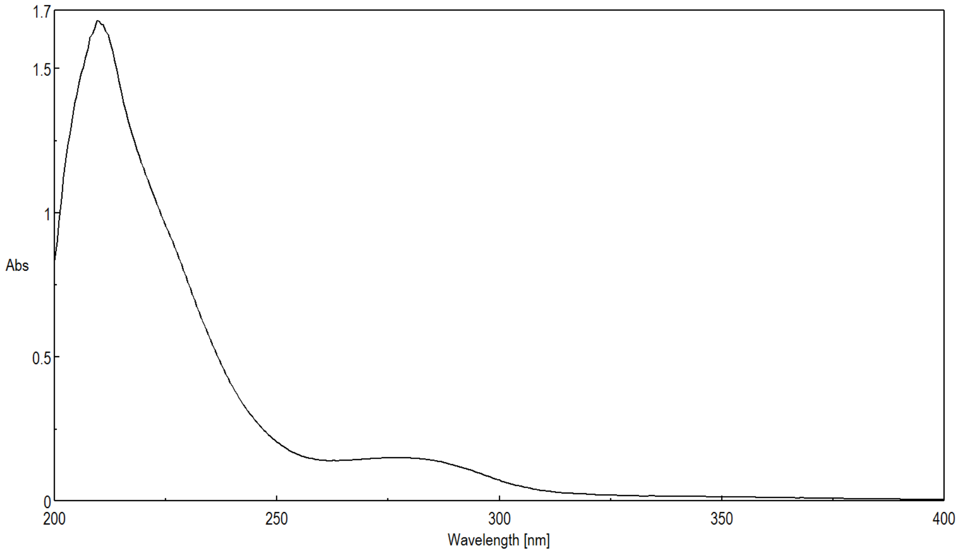
Absorption

- Each ground/excited state pair corresponds to a specific energy (and therefore wavelength) that corresponds to an absorption band.

$$E = h\nu = {}^{hc}/_{\lambda}$$

- The less energy needed to excite the electrons, the longer the wavelength it can absorb.

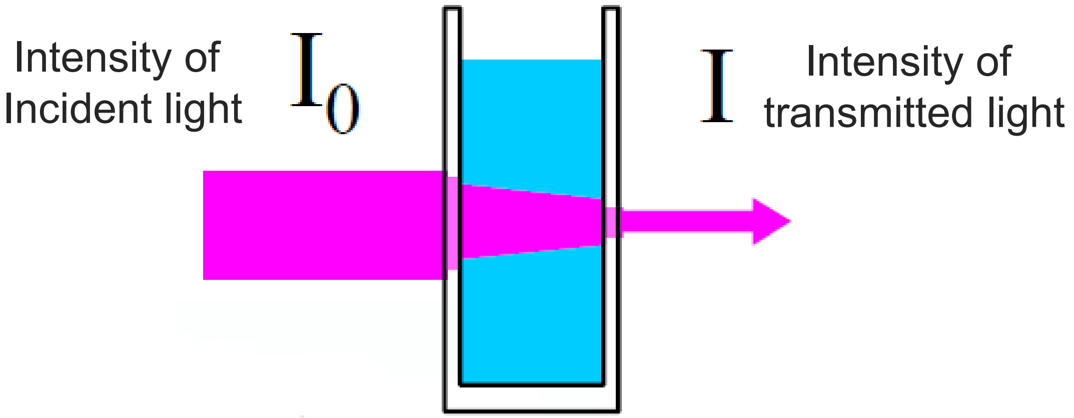
- These absorption bands are indicative of the molecular structure of the sample and will shift in wavelength and intensity depending on the molecular interactions and environmental conditions.



Transmission

UV-Vis Spectrophotometer measures the amount of light absorbed by a sample.

- Measuring the light intensity at each wavelength (ie how much light transmitted through sample)

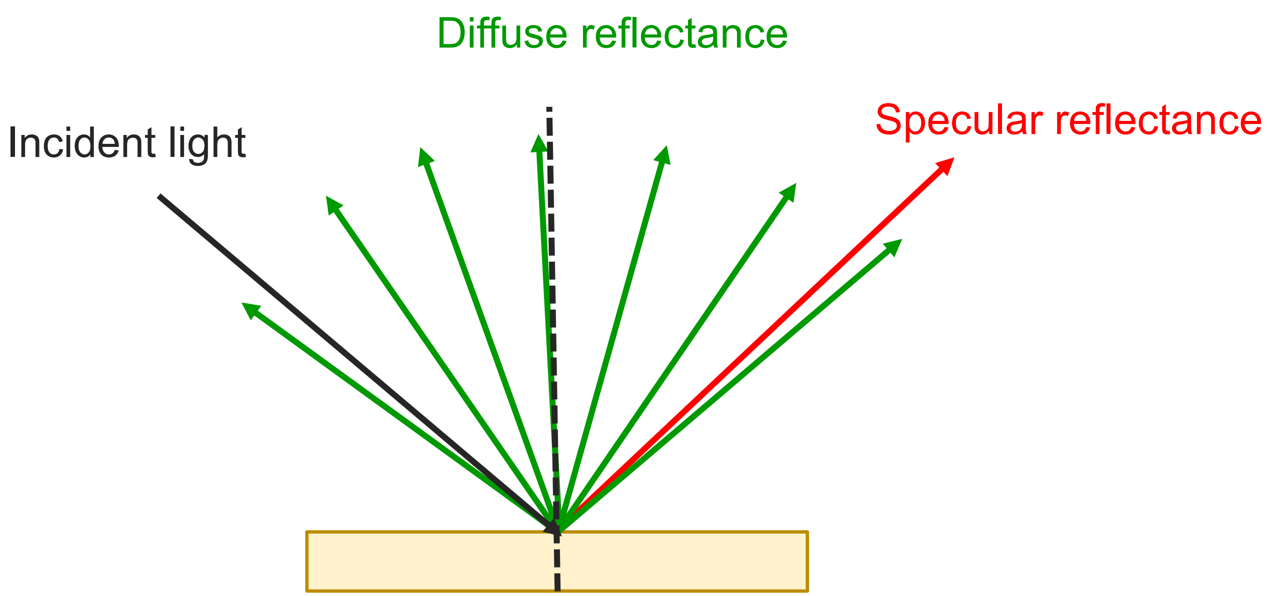


$$T = \frac{I}{I_0}$$

$$Abs = -\log\frac{I}{I_0} = -\log T$$

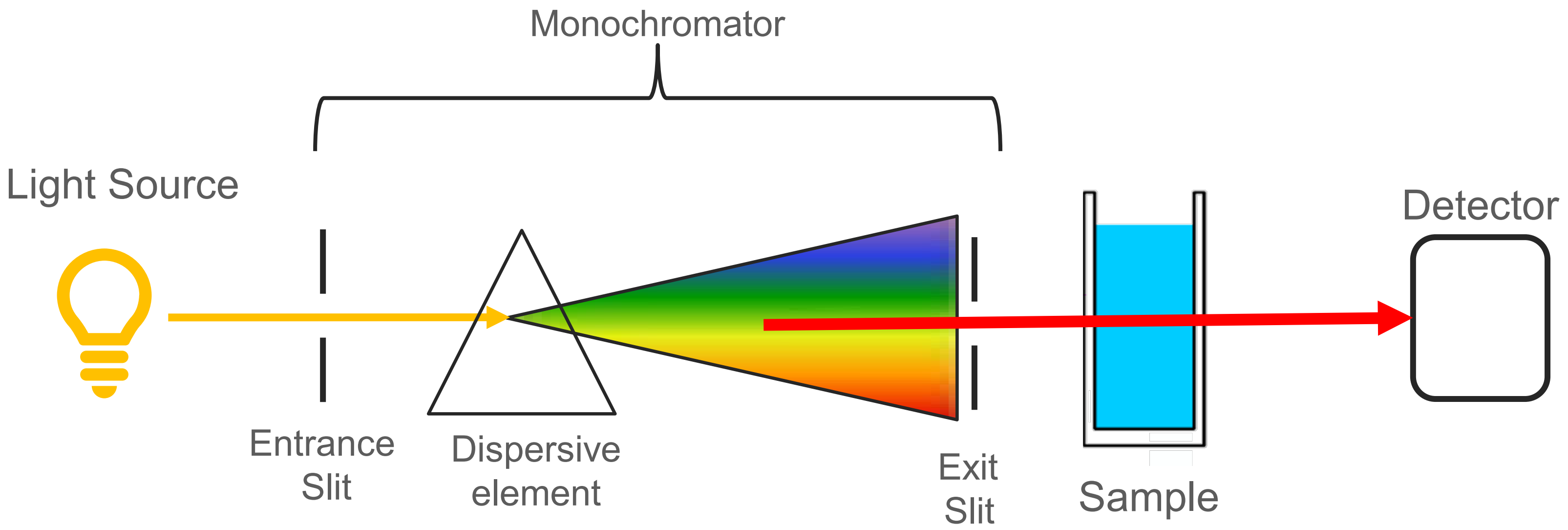
Reflectance

Reflection occurs when light strikes a surface, causing a change in the direction of the light waves

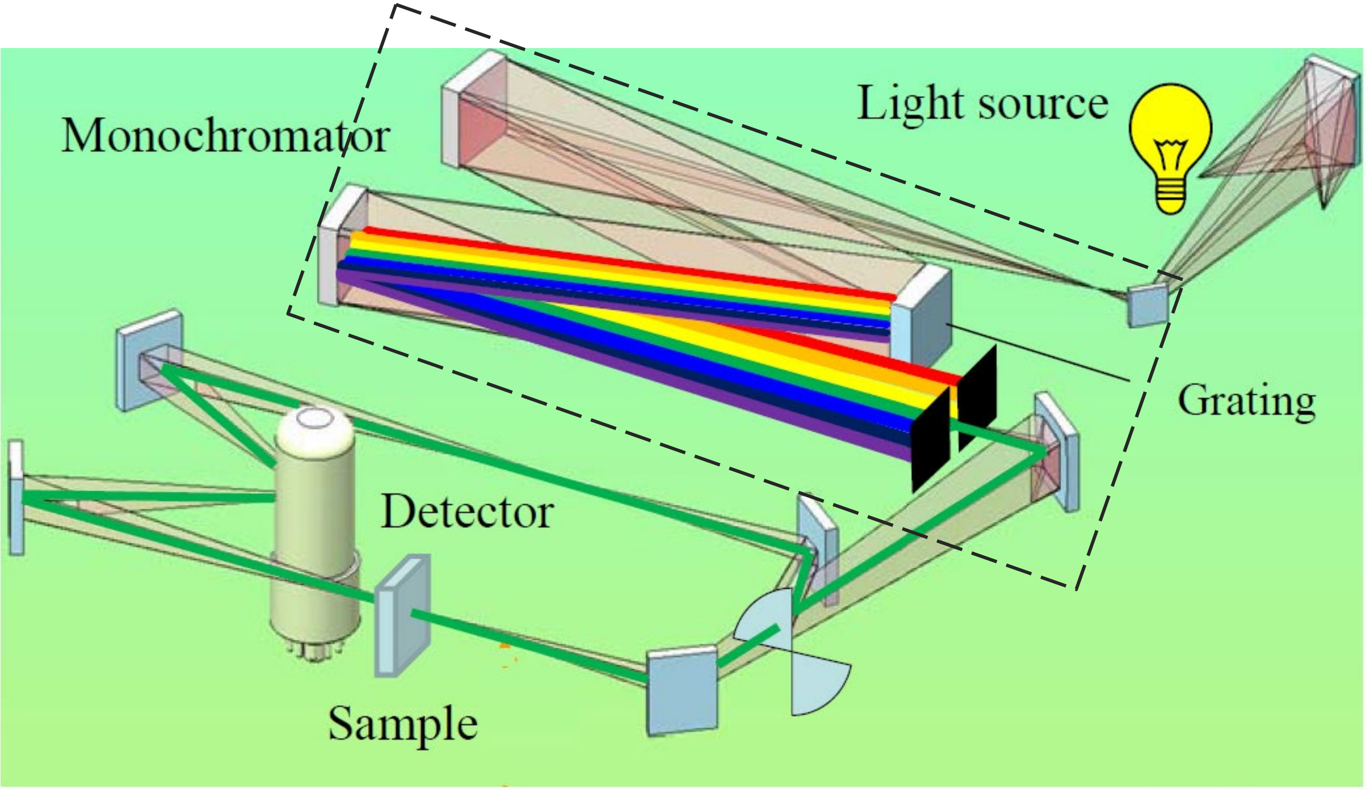


Instrumentation

Principle of Measurement

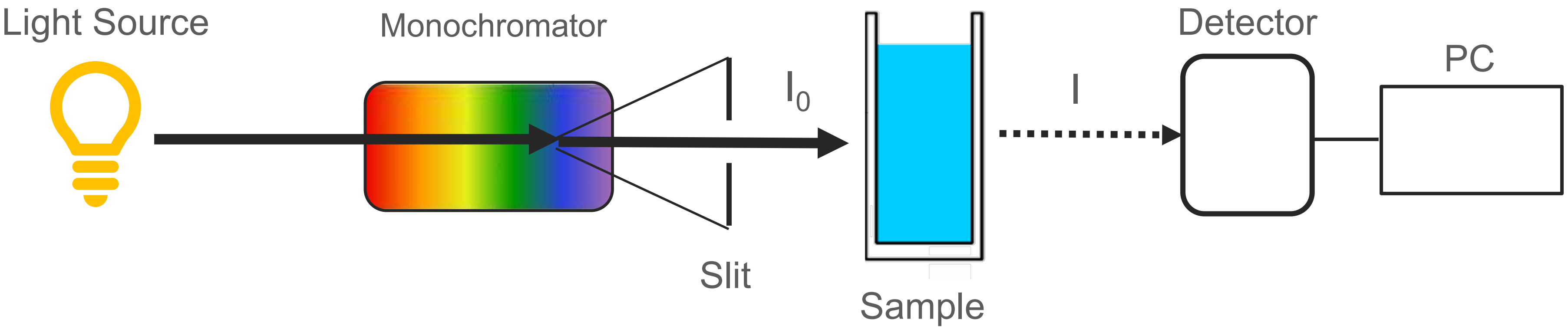


Czerny-Turner Monochromator



*V-760 has two gratings

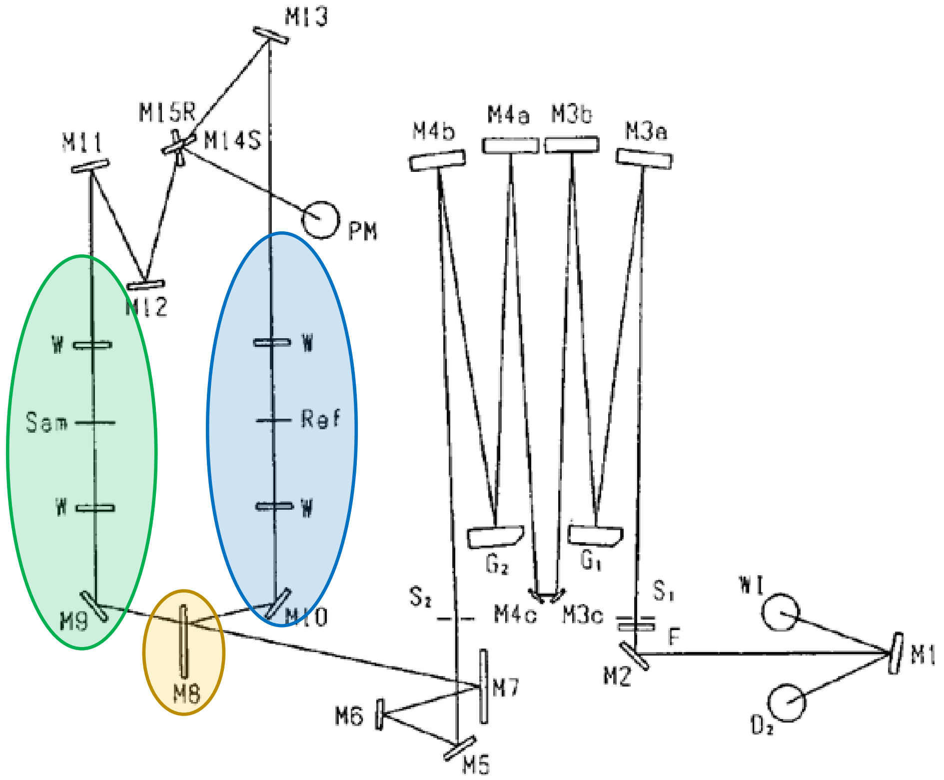
Single Beam Instrument



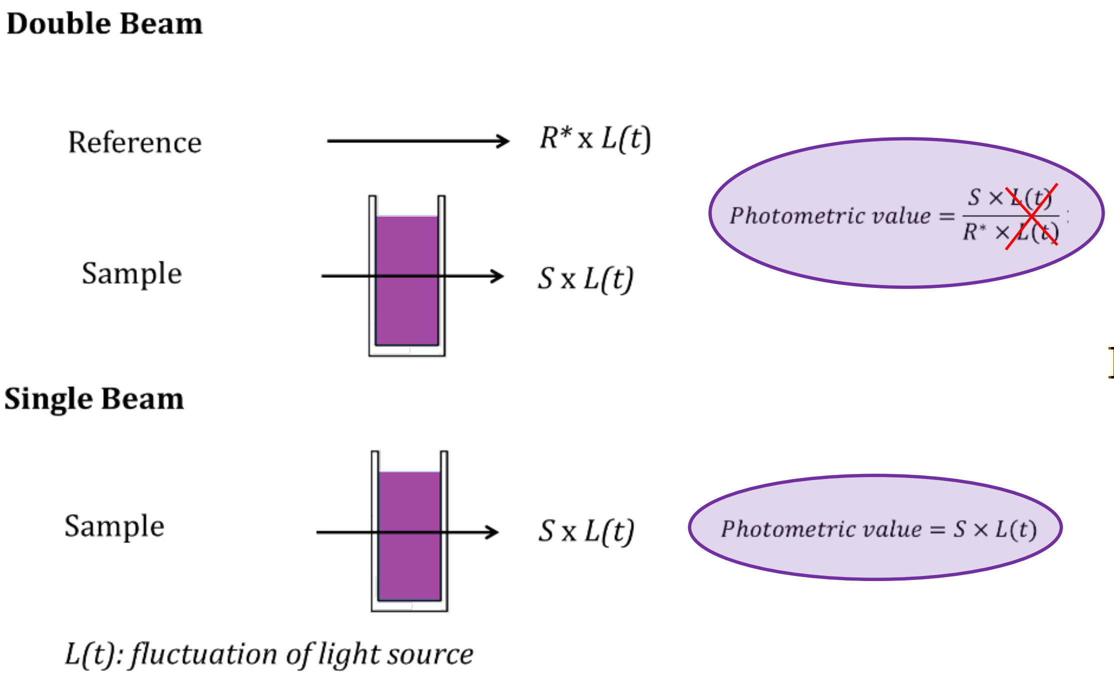
$$T = \frac{I}{I_0}$$

Double Beam Instrument

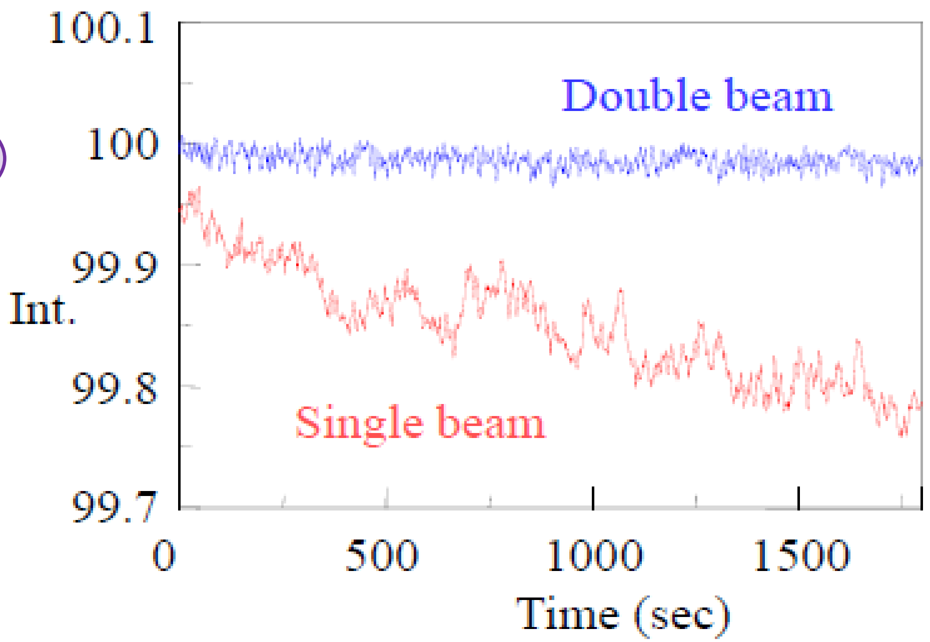
- Light intensity changes with time so reference beam monitors lamp energy and accounts energy differences
- The measured absorbance is the ratio of the sample beam to the reference beam.



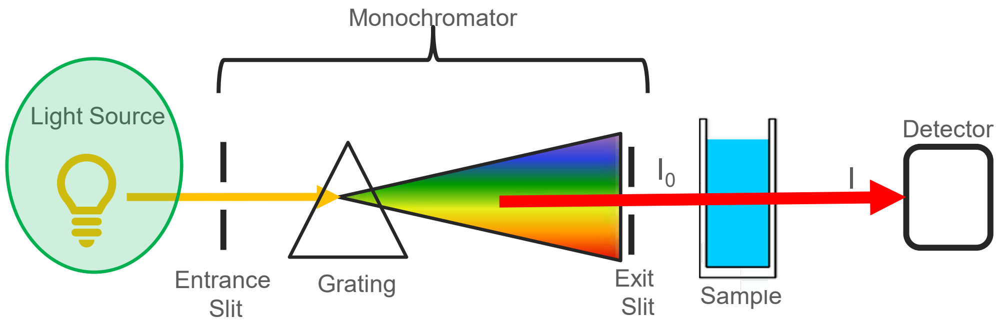
Single vs double beam



Baseline stability



Light Sources

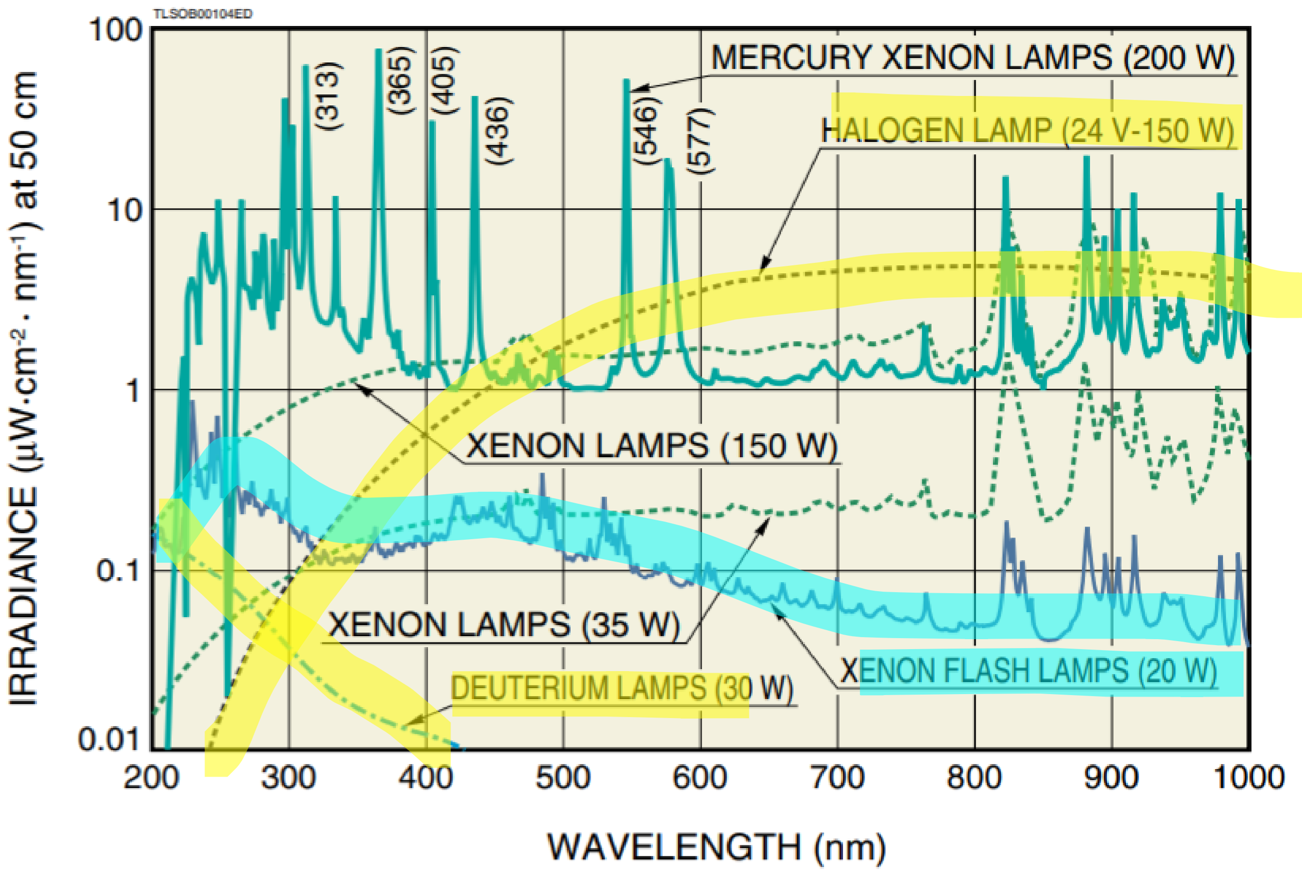


- Wavelength range
- Light throughput
- Stability
- Lifetime
- Cost

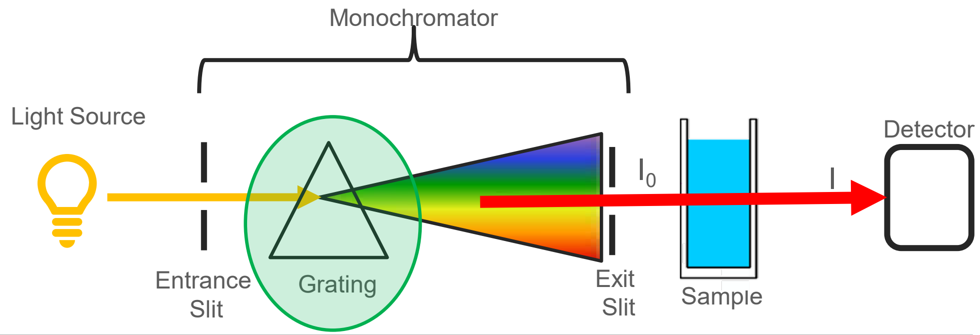
- Deuterium
  - UV: 190-350 nm
  - ~2000 hours
- Halogen (WI)
  - 330-3200 nm
  - ~1000 hours

Continuous and flash sources

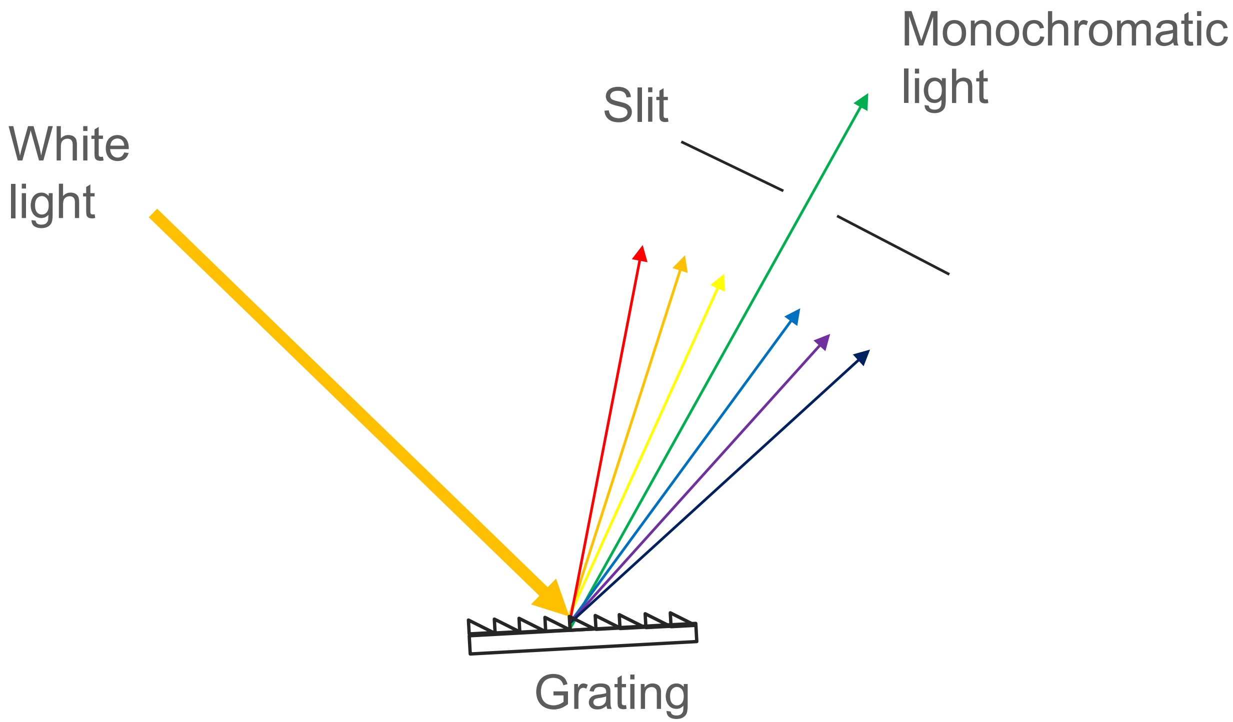
- Continuous lamps provide continuous, *uniform* light output
- Flash lamps release less heat but larger fluctuations in light intensity

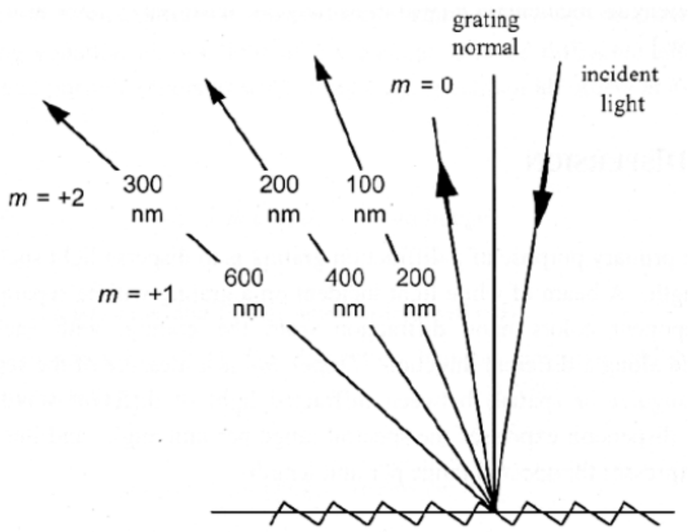


Grating



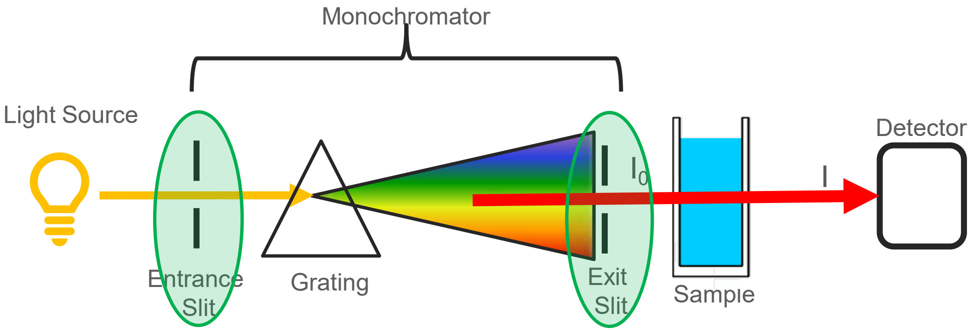
Dispersive element used for wavelength selection

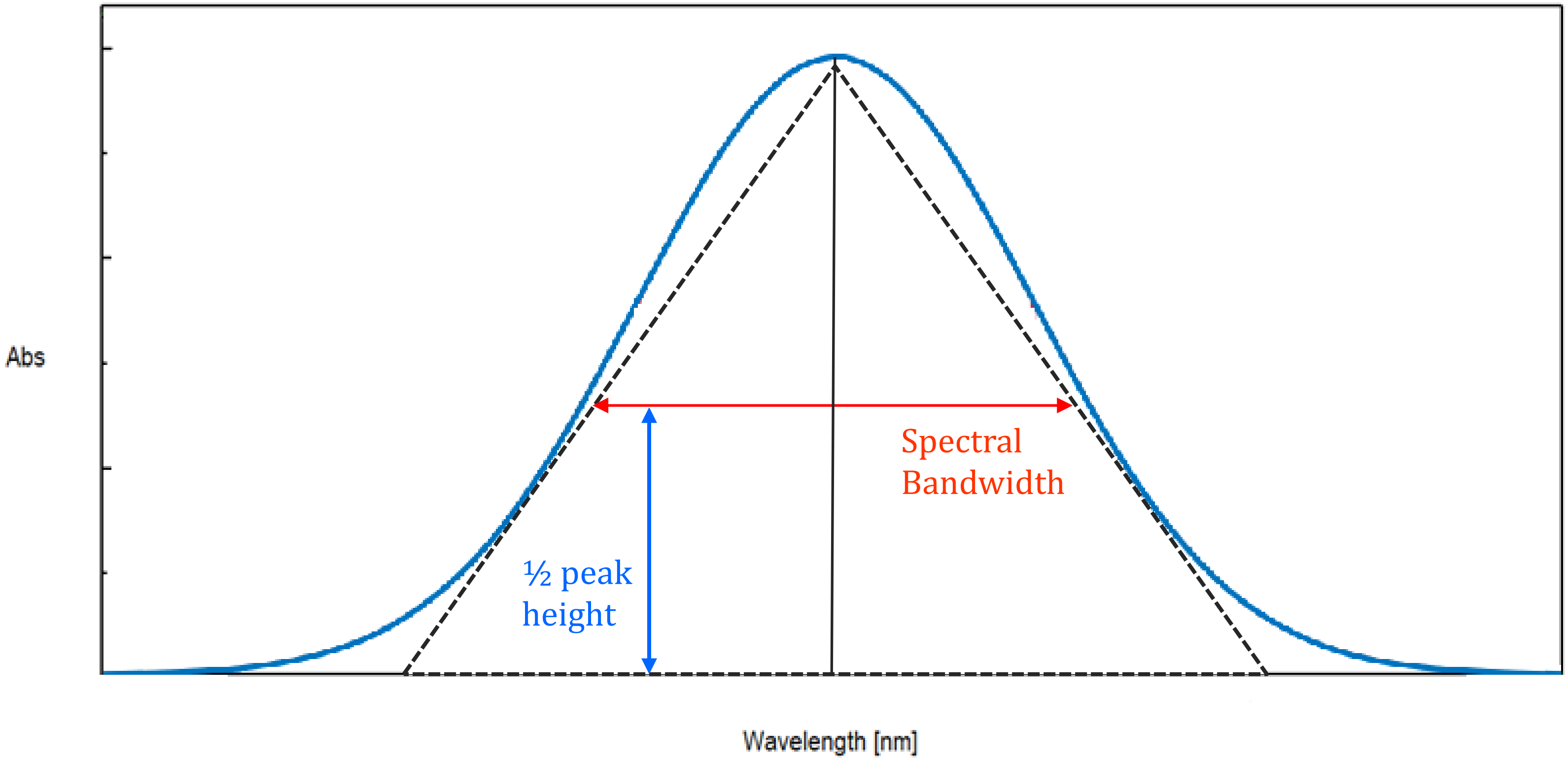




https://www.enlitechnology.com/show/diffraction-orders.htm

Spectral bandwidth



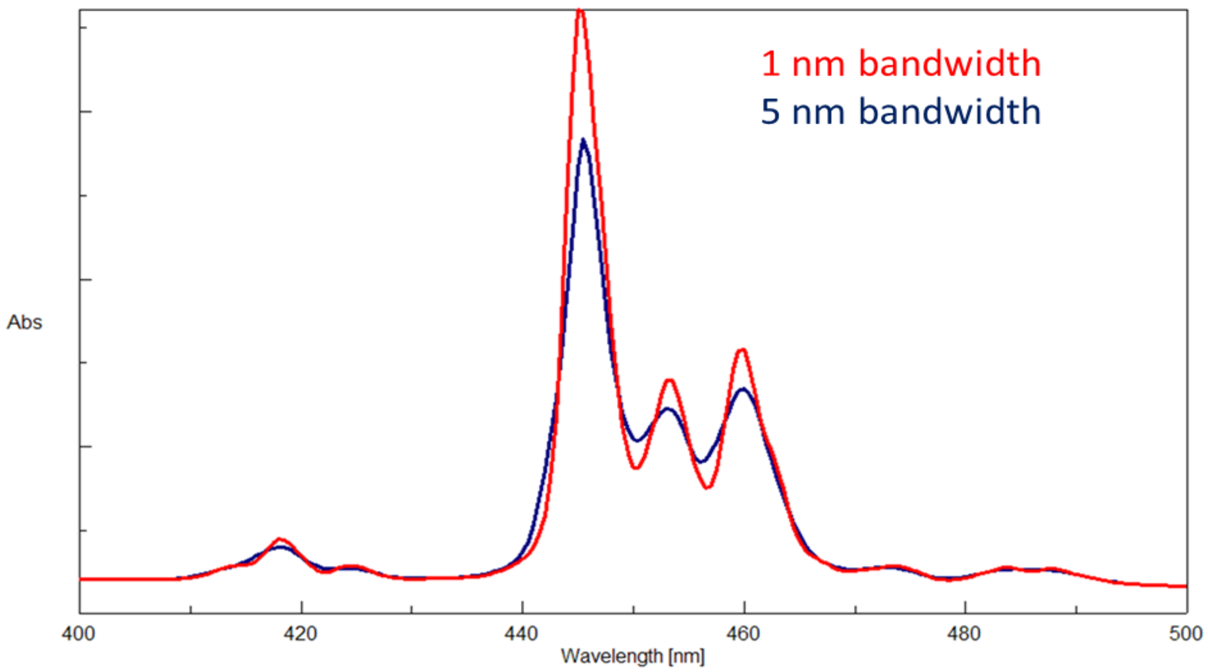


$$\Delta\lambda = \frac{d \cdot \cos\beta}{n \cdot f}\Delta x$$

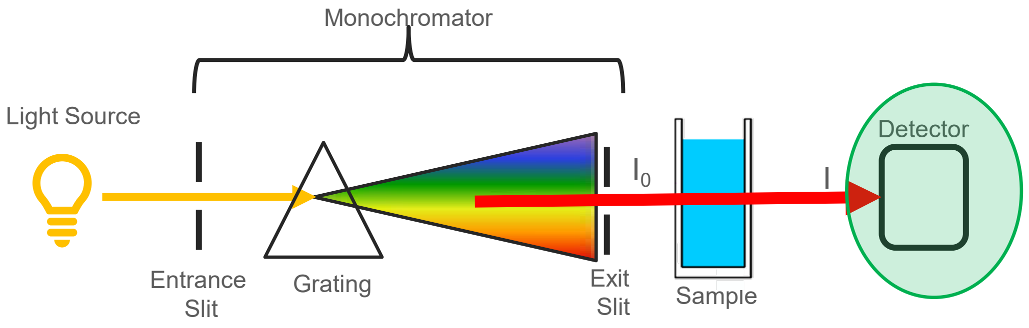
$\Delta\lambda$: bandwidth
d:groove spacing of grating
β: diffraction angle
n: diffraction order
f: focal length
Δx: slit width

Resolution

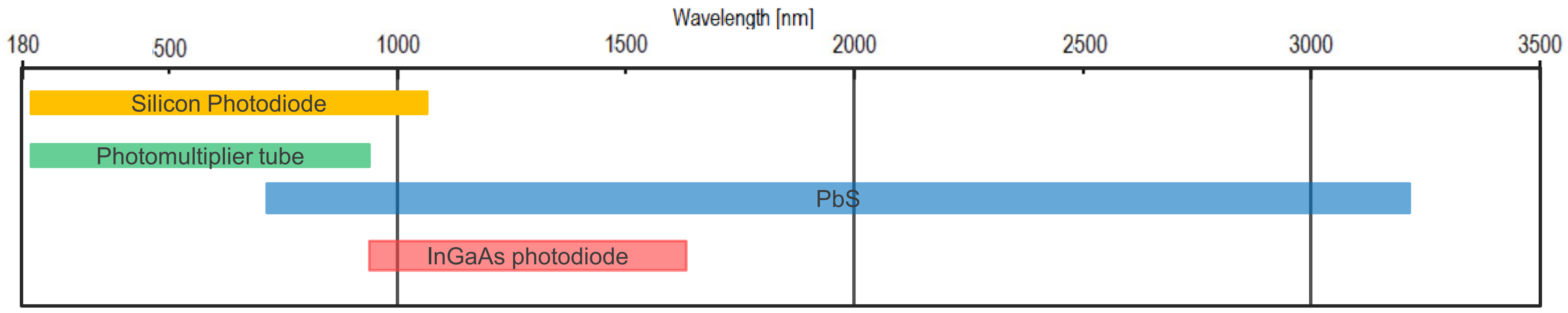
Narrow slit = Narrow bandwidth	→	High noise, better resolution
Wide slit = Wide bandwidth	→	Low noise, poor resolution



Detectors

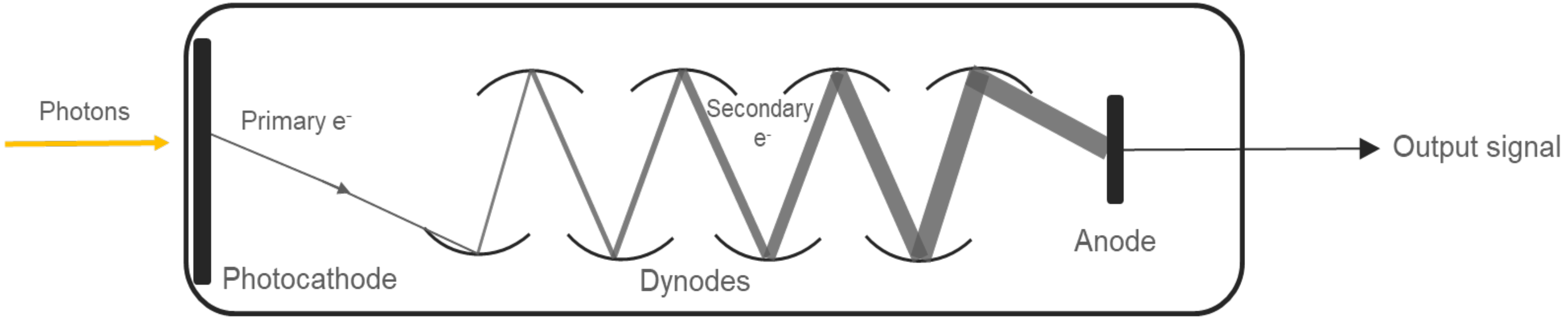


Monochromator
Light Source
Detector
I_0
I
Entrance Slit
Grating
Exit Slit
Sample



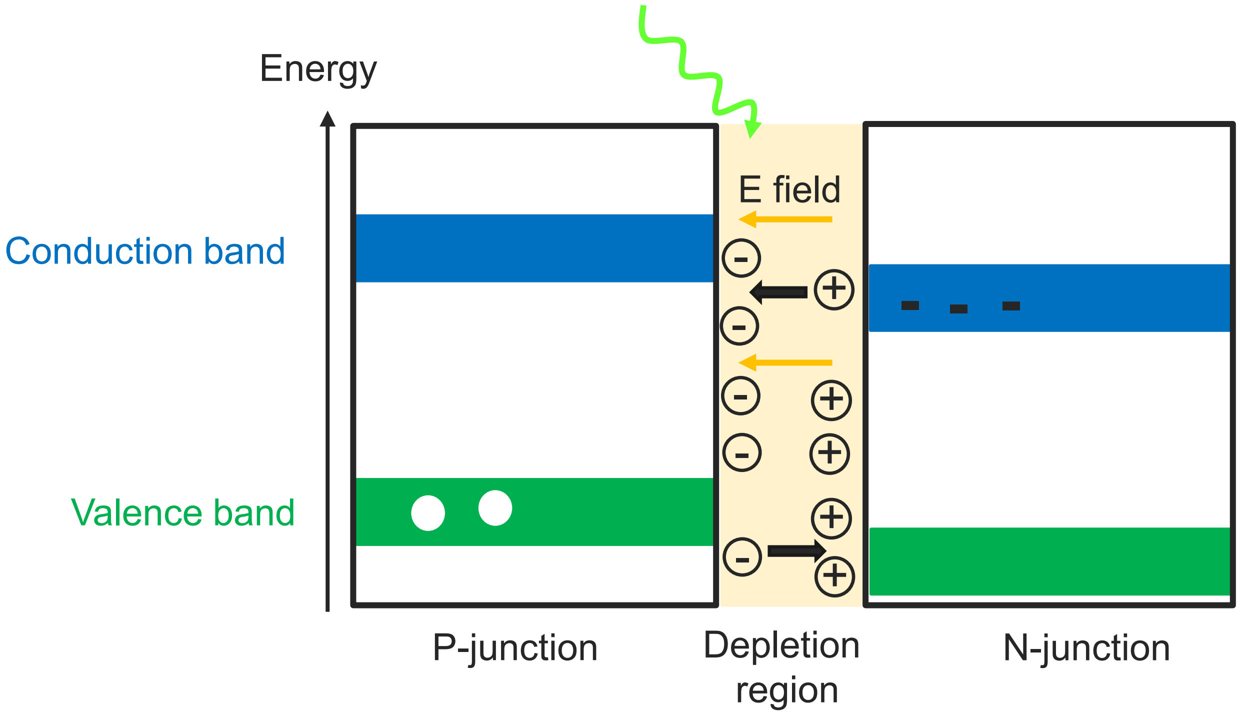
Wavelength [nm]
180
500
1000
1500
2000
2500
3000
3500
Silicon Photodiode
Photomultiplier tube
PbS
InGaAs photodiode

Photomultiplier tube (PMT)



- Wide spectral response
- High S/N output
- High stability

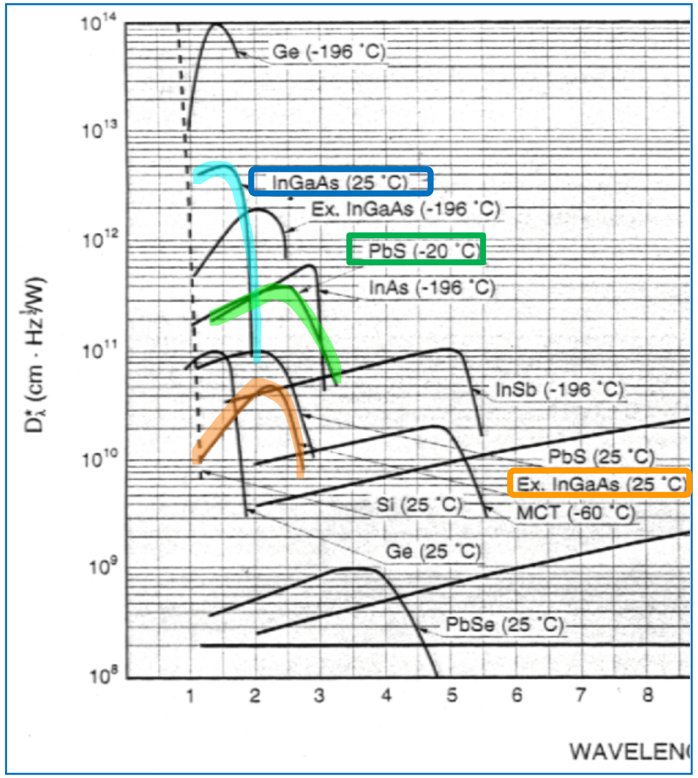
Photodiode

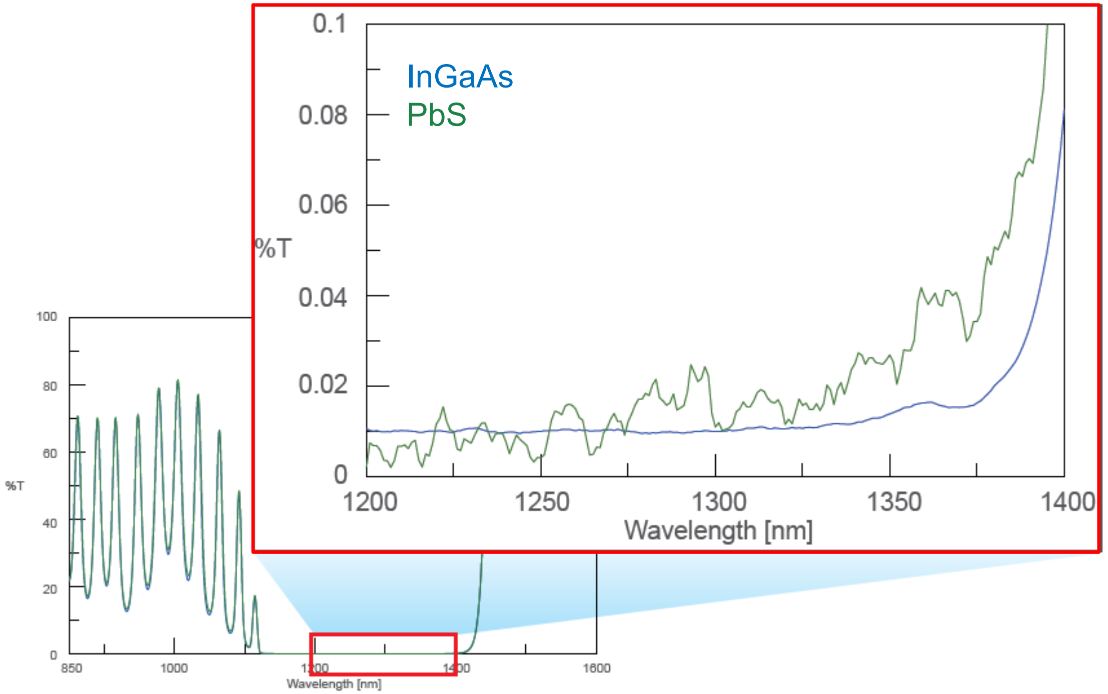


- Quick response
- Slightly broader spectral range (-1100 nm)
- Low noise

NIR Detectors

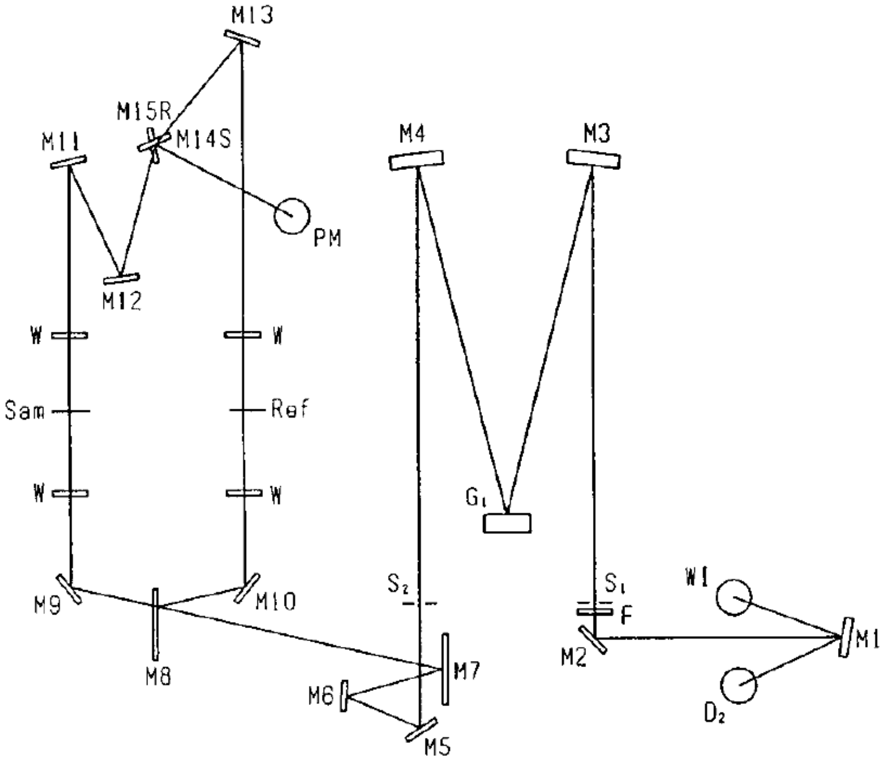
- InGaAs: higher sensitivity, shorter wavelength range
- PbS: wider wavelength range, less sensitive



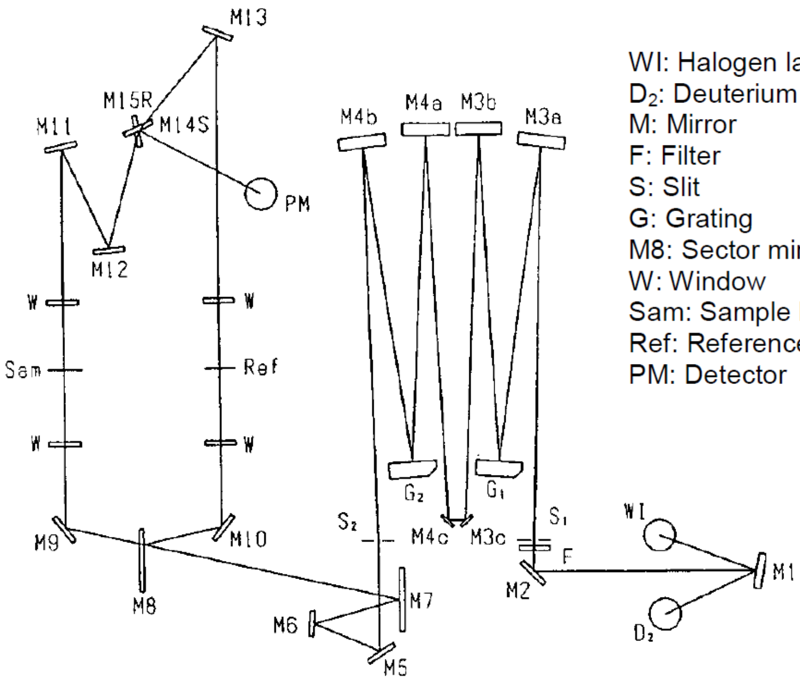


Single vs double monochromator

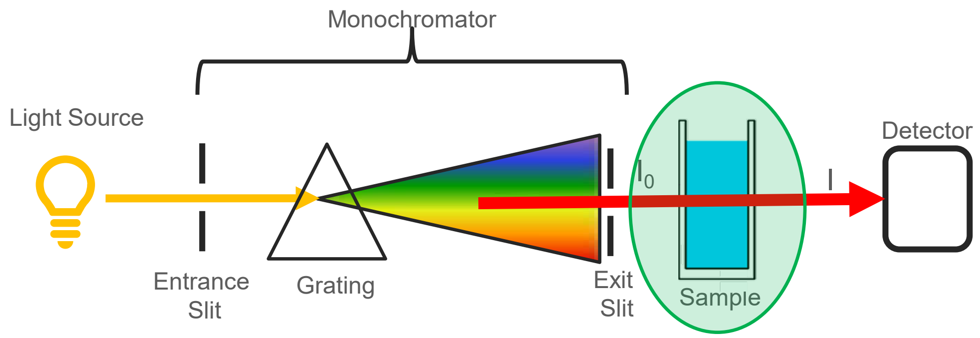
- One grating, one set of slits



- Two gratings, two sets of slits, additional mirrors
- Reduce effects of stray light
- Best for highly absorbing or scattering samples

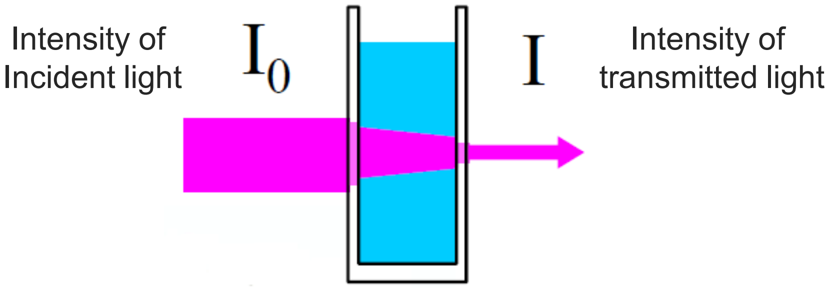


WI: Halogen lamp
D_2: Deuterium lamp
M: Mirror
F: Filter
S: Slit
G: Grating
M8: Sector mirror
W: Window
Sam: Sample beam
Ref: Reference beam
PM: Detector



Beer-Lambert Law

How light is attenuated based on the materials traveled through



$$\boxed{T = {}^{I}/_{I_0}}$$

$$I = I_0 e^{-\varepsilon c l}$$

$$-\log {}^{I}/_{I_0} = \varepsilon c l$$

$$A = \varepsilon \cdot c \cdot l$$

ε: molar absorptivity
c: molar concentration
l: pathlength

Beer-Lambert Law

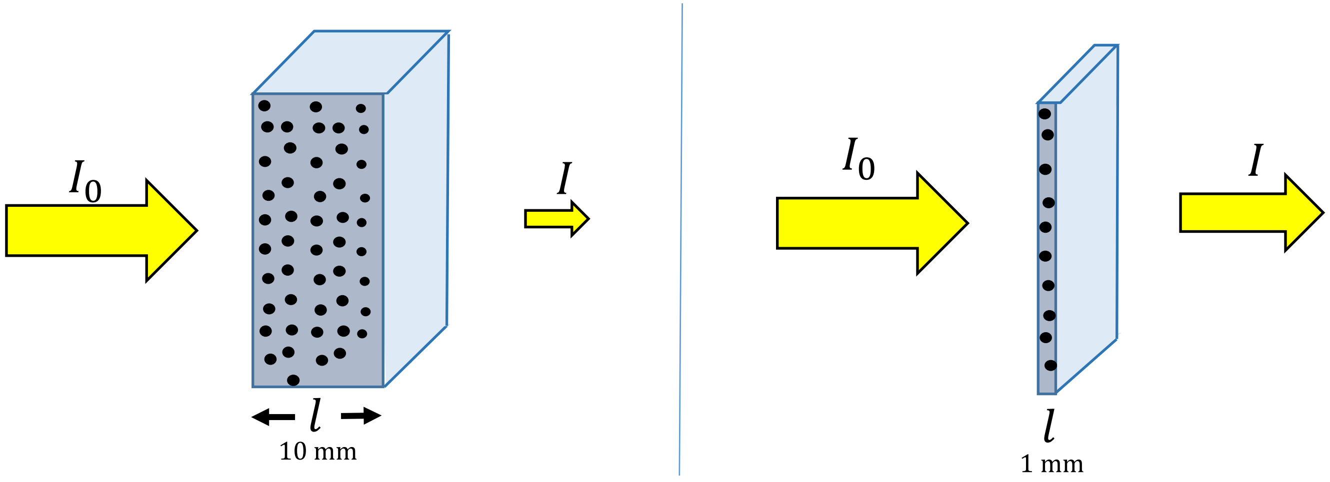
$$A = \varepsilon \cdot c \cdot l$$

ε: molar absorptivity

- Amount of light absorbed per unit concentration
- Higher the *e*, more effective at absorbing light

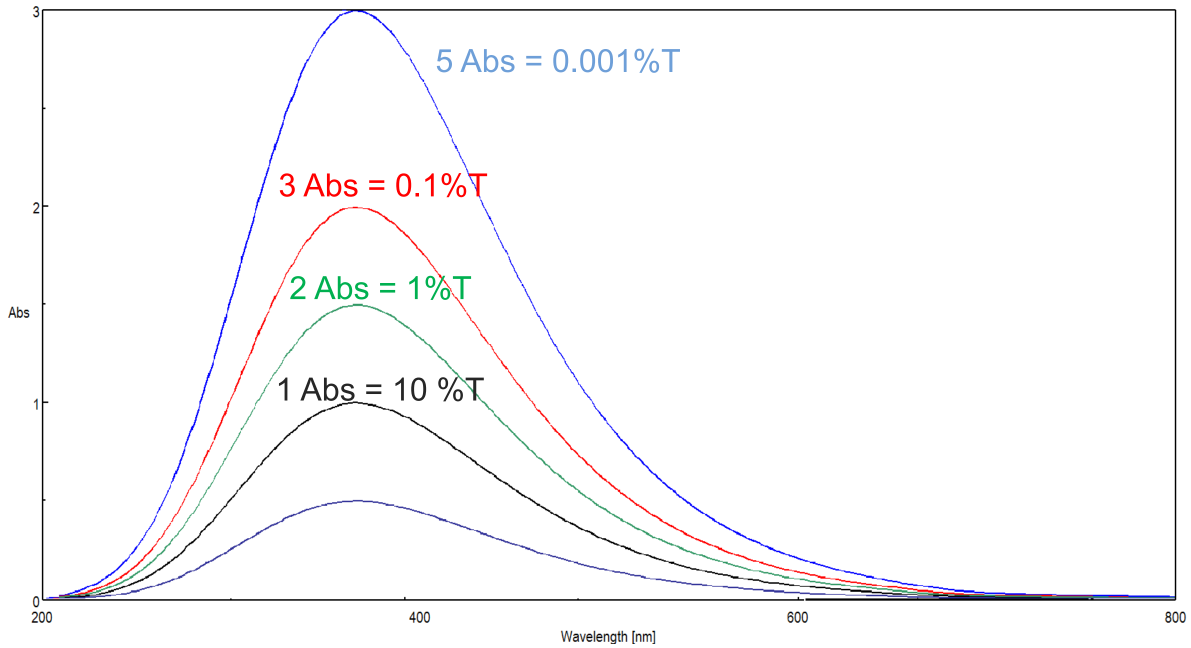
c: molar concentration

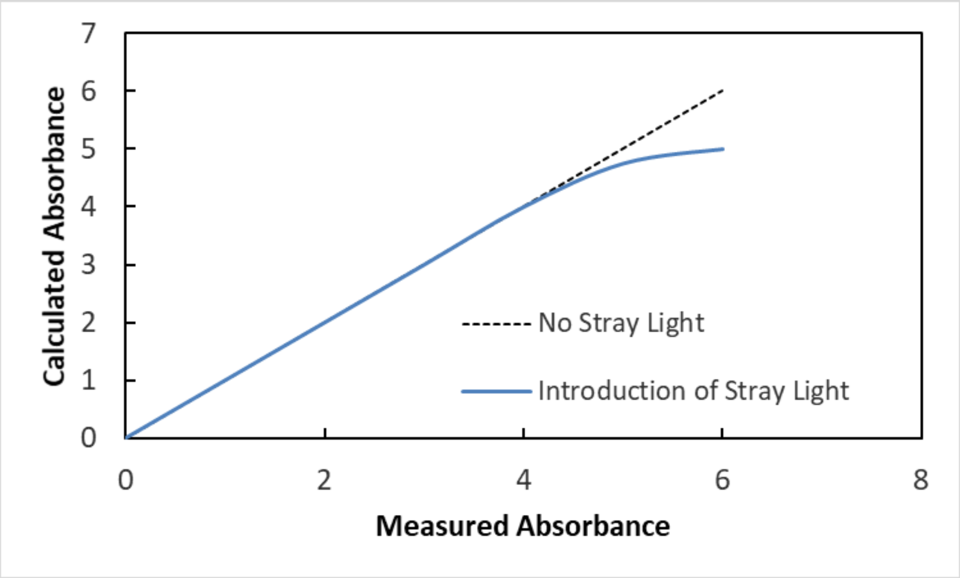
l: pathlength



Stray Light

Any light that does not fall under distribution at a specific wavelength.

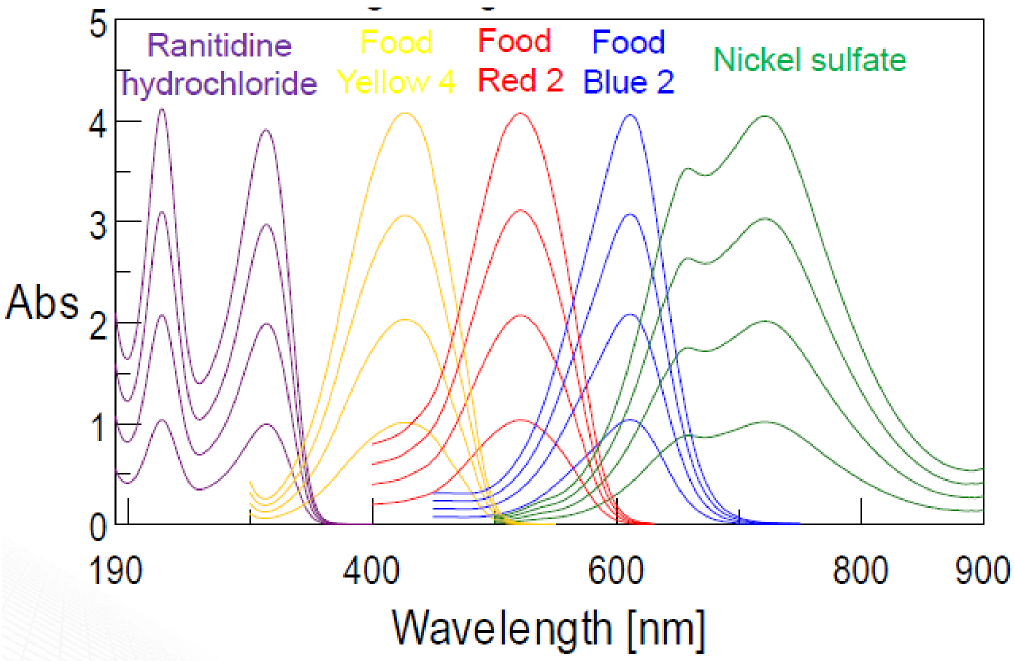




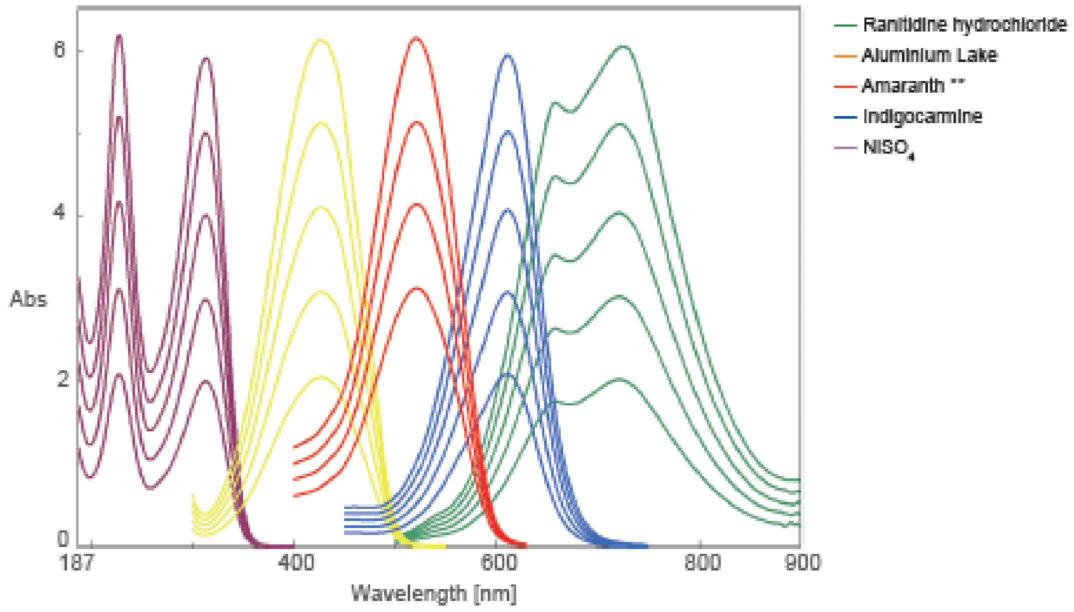
Higher A, lower T

Photometric linearity

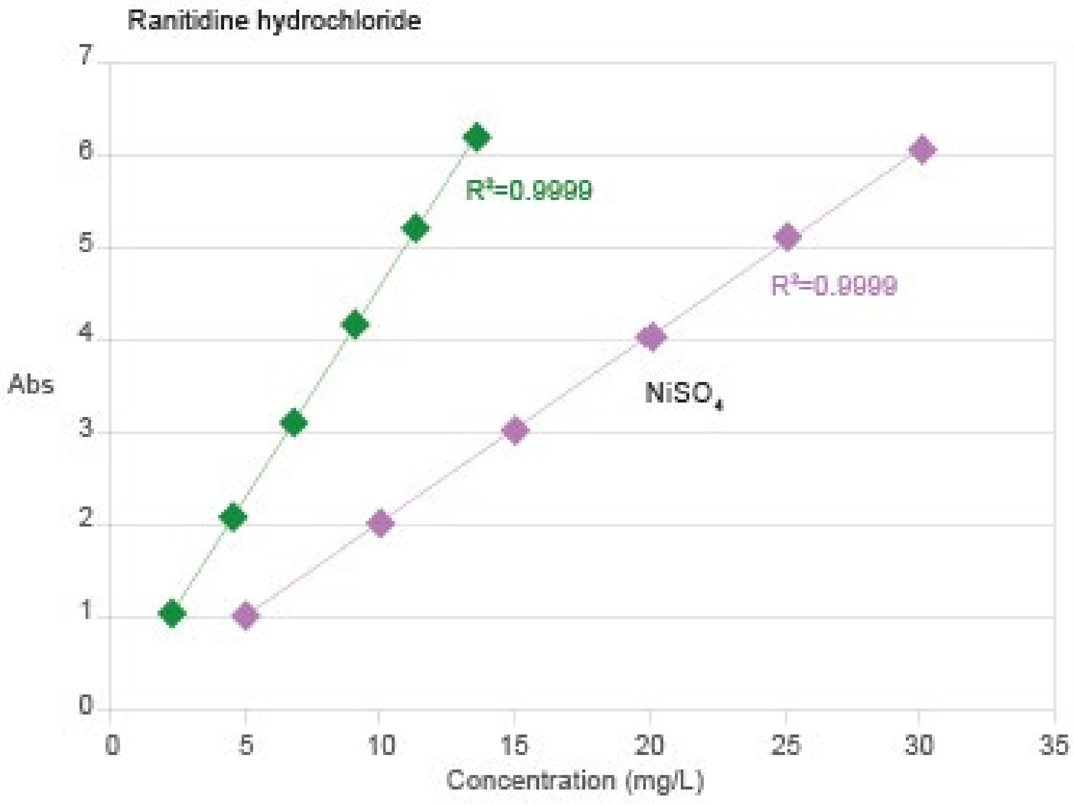
Single mono

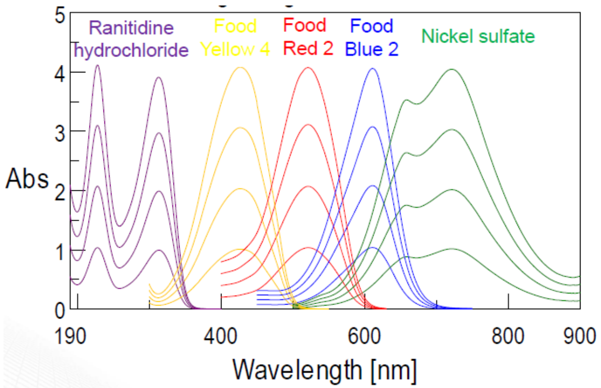


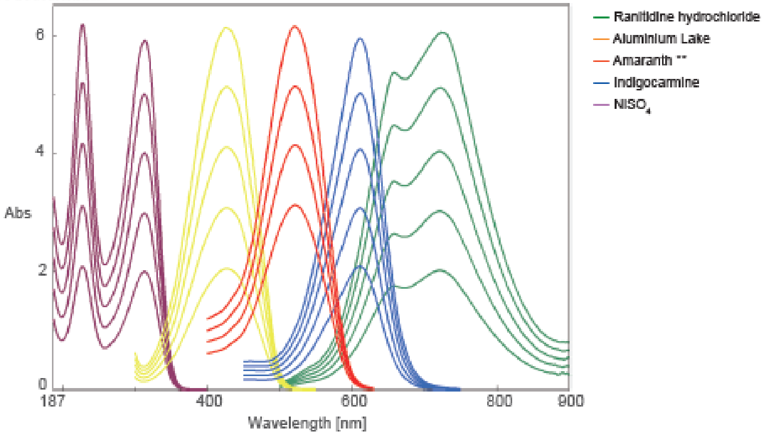
Double mono



Photometric linearity

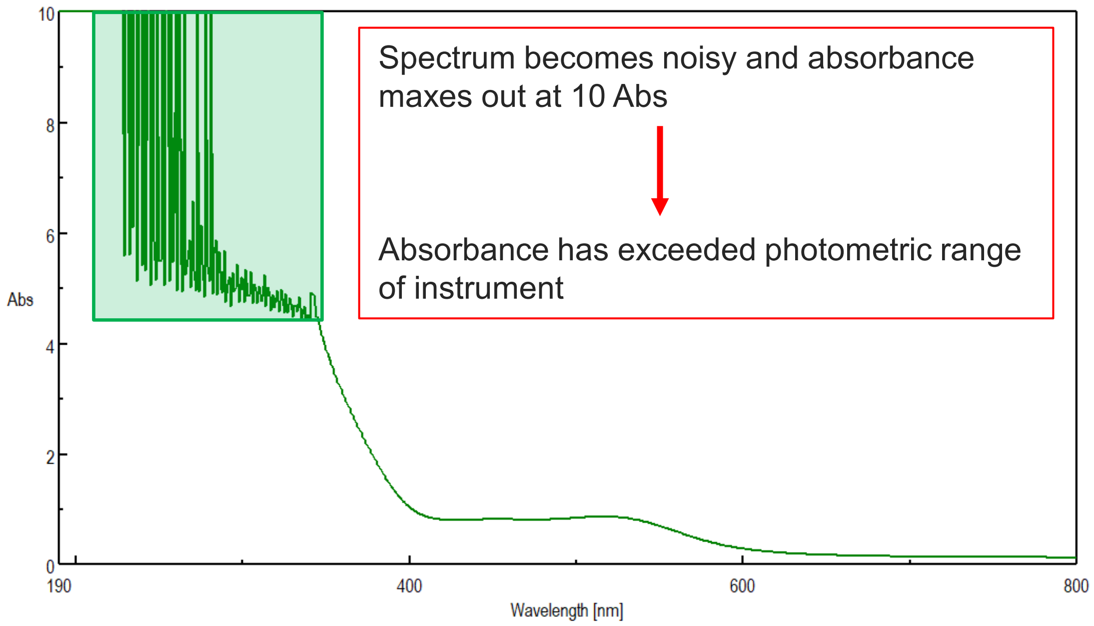


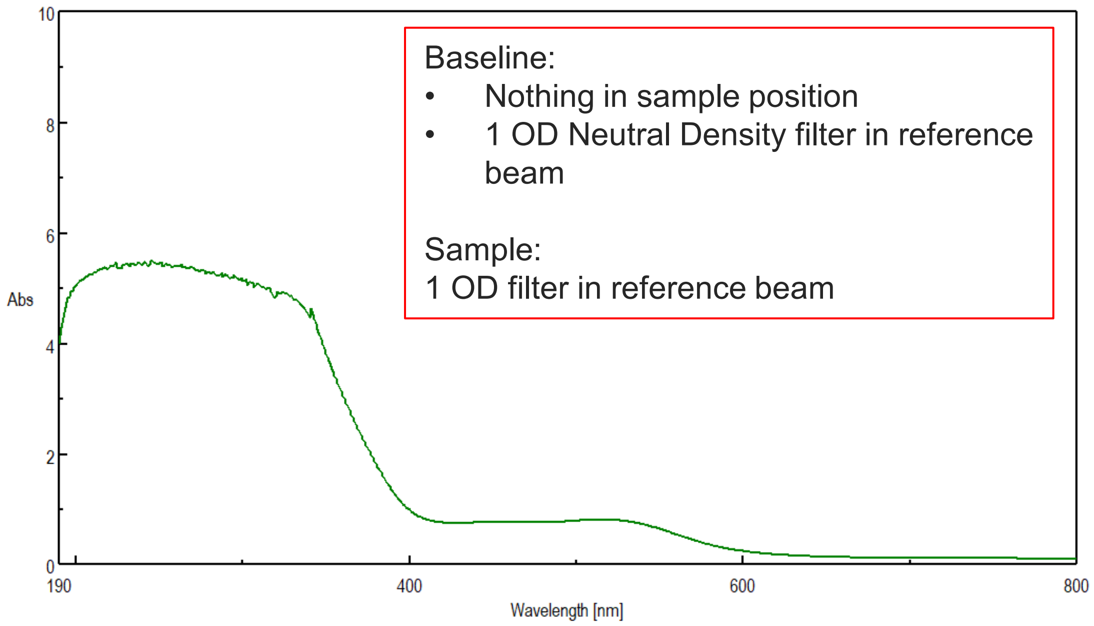




Concentrated samples and rear beam attenuation

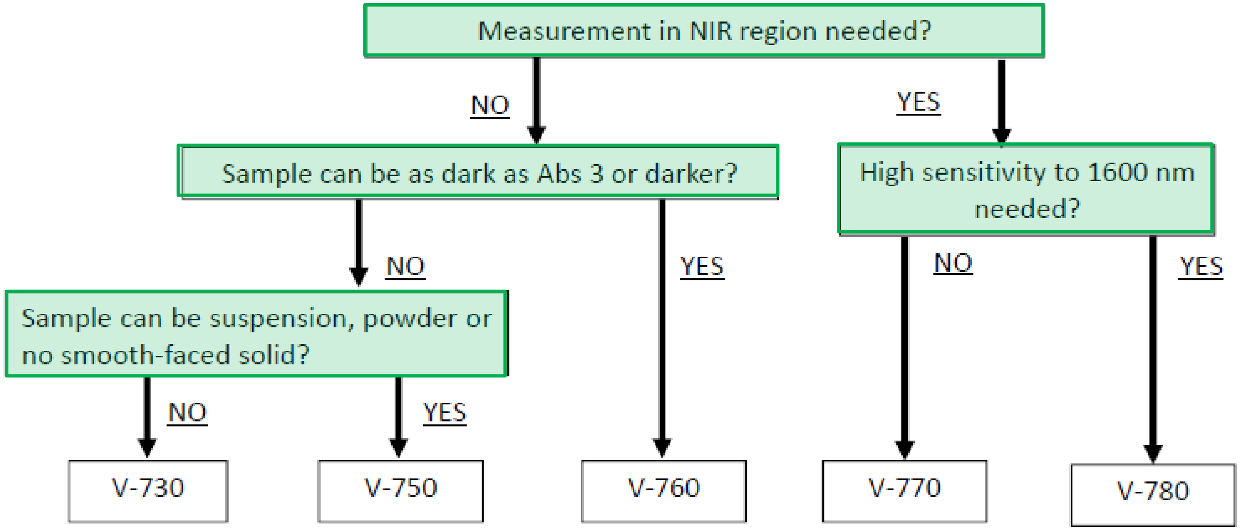
Extends photometric range and sensitivity of instrument for highly absorbing samples





How to select the appropriate model?

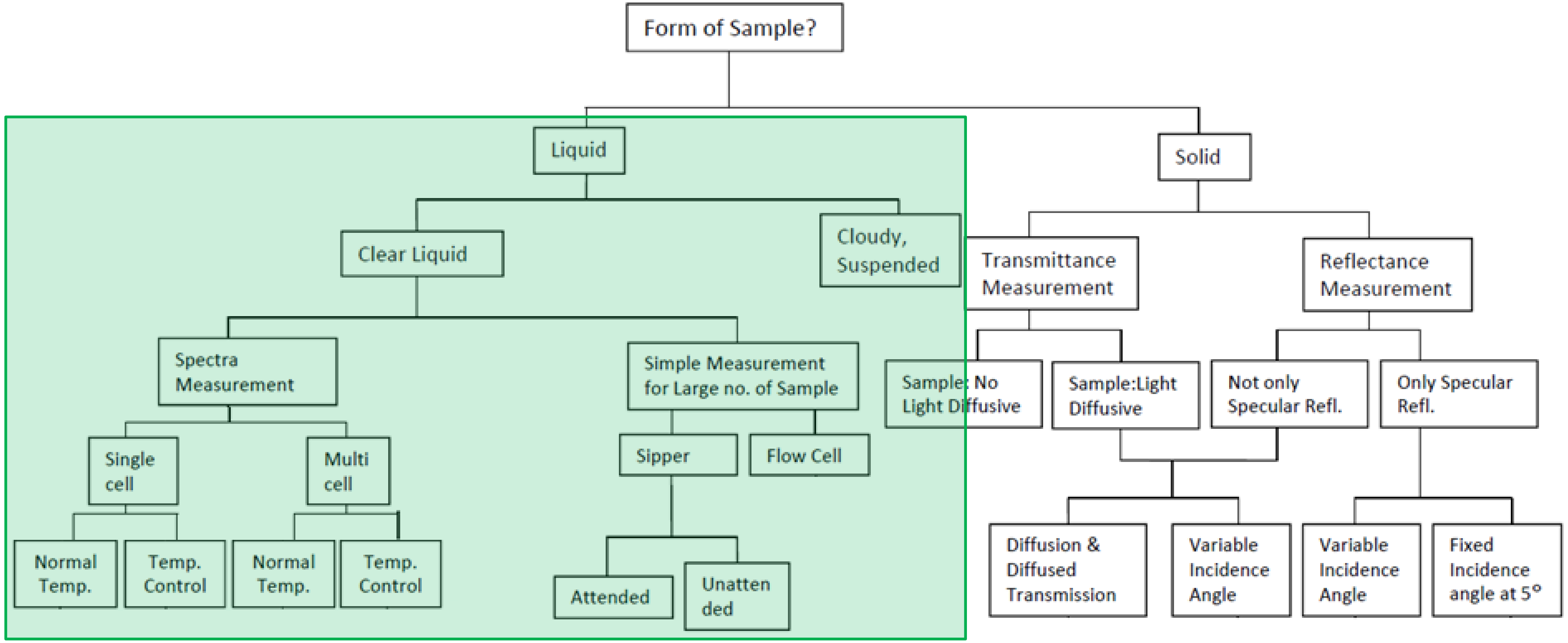
How to select the appropriate model?



Measurement in wide range in NIR	=>	V-770
Double monochromator is preferred	=>	V-760
Less stray light is preferred	=>	V-760
High end instrument	=>	V-770/780
Classical measurements required with minimum budget	=>	V-730

Sampling

Sampling: Liquids



Liquid Samples

1. Wavelength range
2. Concentration and Pathlength
3. Cuvette
4. Solvent

Chromophore	Transition	λ_{max} (nm)	ε_{max}
Alkyne	$\pi \rightarrow \pi^*$	178 196 225	10,000 2, 000 160
Alkene	$\pi \rightarrow \pi^*$	177	13,000
Carbonyl	$n \rightarrow \sigma^*$ $n \rightarrow \pi^*$ $n \rightarrow \sigma^*$ $n \rightarrow \pi^*$	186 280 180 293	1,000 16 12
Carboxyl	$n \rightarrow \pi^*$	204	41
Amido	$n \rightarrow \pi^*$	214	60
Azo	$n \rightarrow \pi^*$	339	5
Nitro	$n \rightarrow \pi^*$	280	22
Nitroso	 $\pi \rightarrow \pi^*$	300 665	100 20
Nitrate	$\pi \rightarrow \pi^*$	270	12

Chromophore		Transition	Wavelength (nm)
-C≡C-		π –> π *	220-190
-C=C-		π –> π *	200-185
-C=C-C=C-		π –> π *	300-250
C=O, -CHO		n –> π *	350-240
-C=C-C=O		n –> π *	400-260
		π –> π *	280-200
-COOH, Lactone, Ester		n –> π *	250-200
S-S		n –> σ *	300-200
Protein, Polypeptide	Aromatic	π –> π *	350-250
	Amide		260-185
			260-200
DNA, RNA		π –> π *	300-200
Co-chelate Complex		D –> d *	700-300
		CT	300-180

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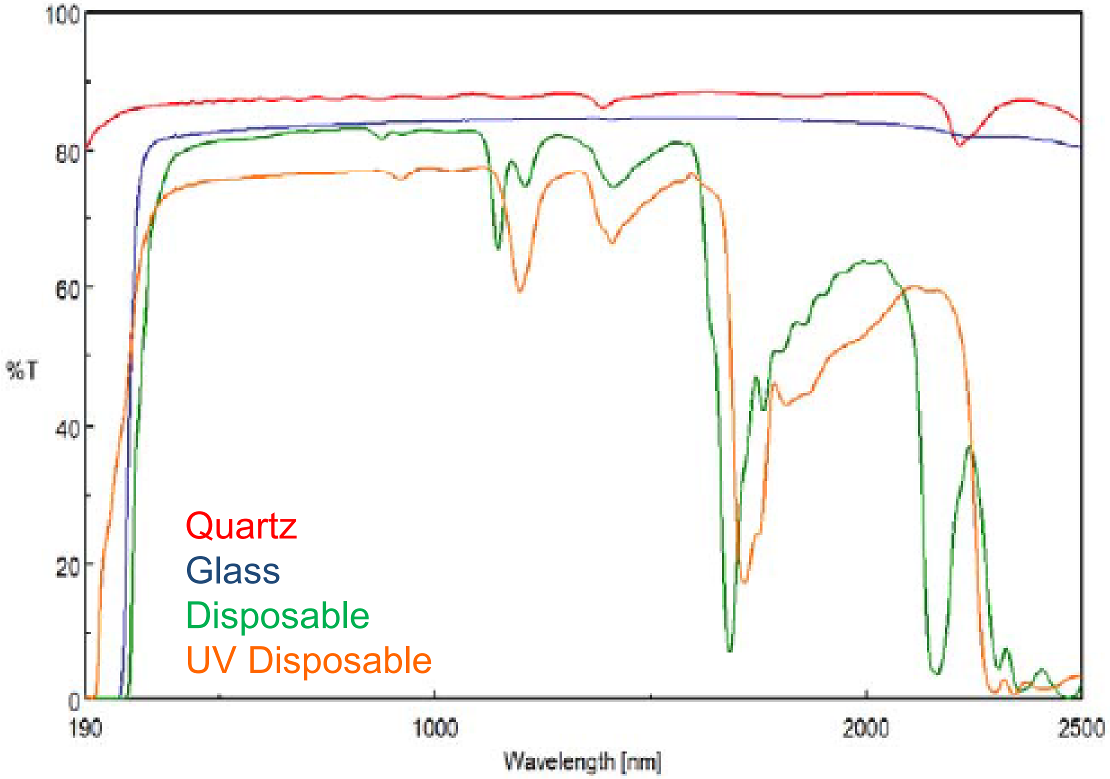
Cuvette Selection



Submicro quartz cell

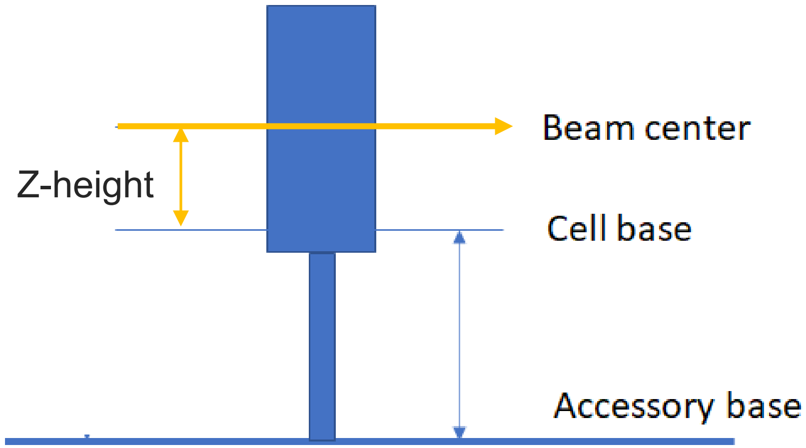
10 mm quartz cell

Micro quartz cell

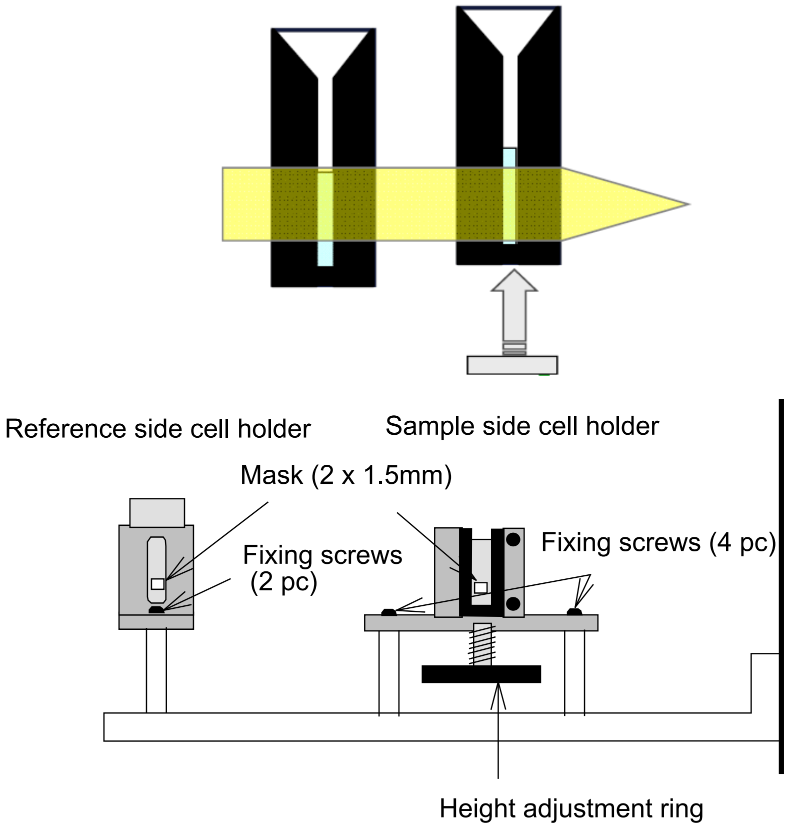
Disposable micro cell

Submicro quartz cell

Z-height



Beam center
Z-height
Cell base
Accessory base



Reference side cell holder
Sample side cell holder
Mask (2 x 1.5mm)
Fixing screws
(2 pc)
Fixing screws (4 pc)
Height adjustment ring

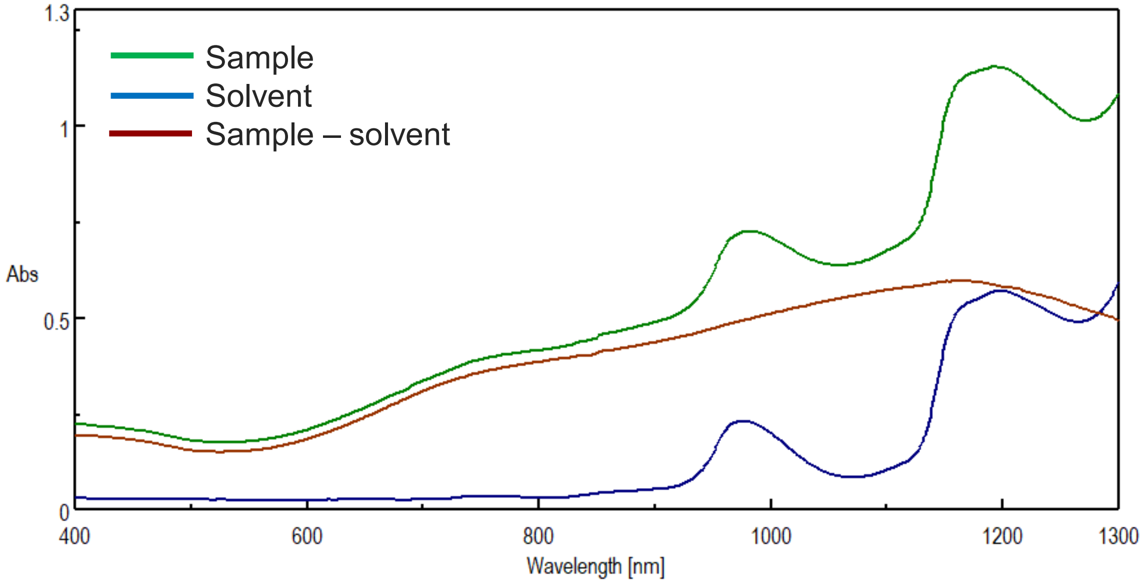
Solvents

- Solubility
- Stability: the sample does not denature and decompose
- Transparency in the measuring wavelength region

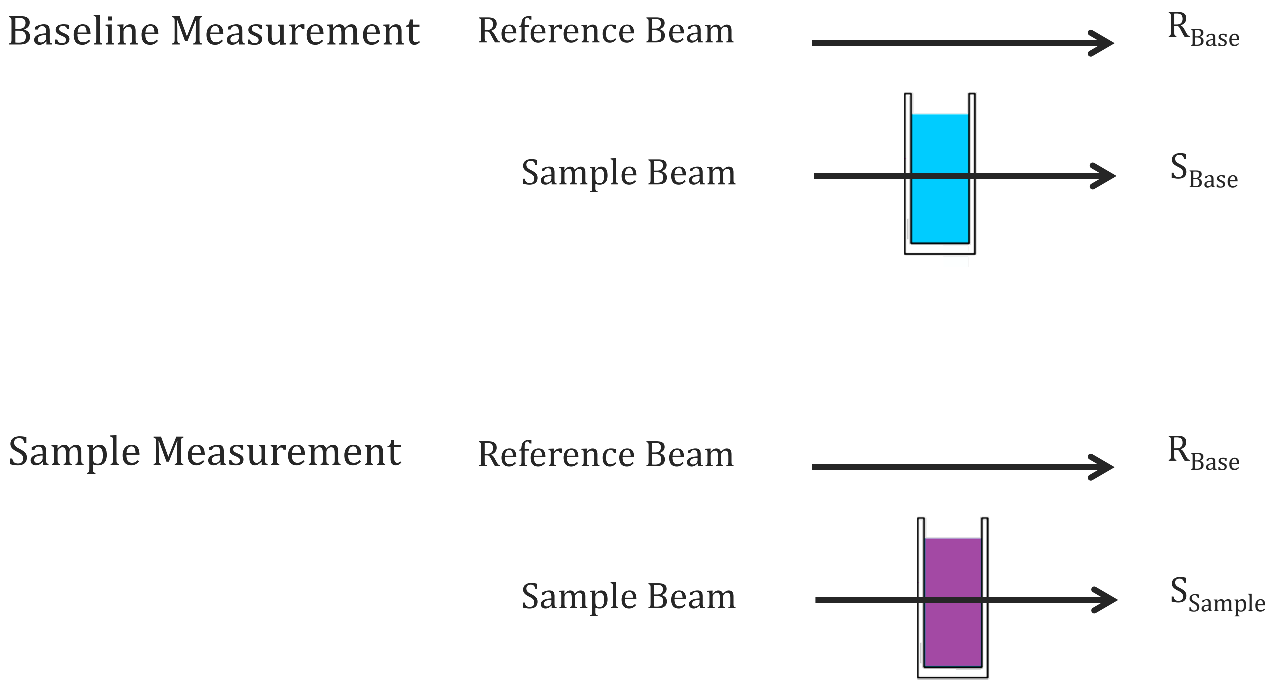
Solvent	
	1 cm
Ethanol/Methanol (4:1)	~220
Distilled Water	~185
10 mM Sodium Phosphate	
0.1 M Sodium Phosphate	
0.1 M Sodium Chloride	
0.1 M Tris-HCl	
0.1 M Ammonium Citrate	
Methanol	~210
Ethanol	~220
Hexanes	~210
Chloroform	~240
Trifluoroacetic Acid	~260
THF	~220

Baseline Measurements

Solvent and cell will absorb or reflect light so the cell with buffer solvent needs to be measured as a reference.



Baseline Measurements

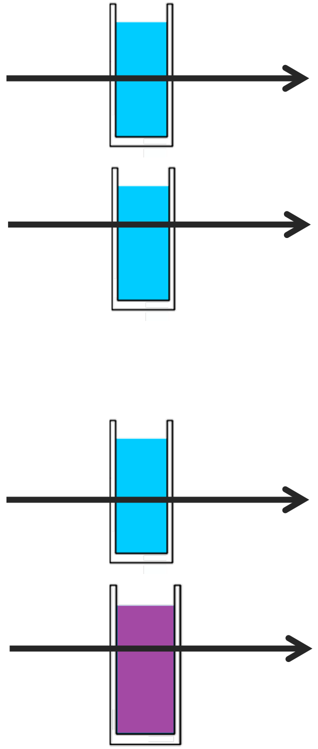


Baseline =
S_{base}/R_{base} x 100

Sample=
S_{sample}/R_{base} x 100

$$T = \frac{\text{Sample}}{\text{Baseline}} \times 100 = \frac{S_{sample}/\cancel{R_{base}} \times 100}{S_{base}/\cancel{R_{base}} \times 100} \times 100 = {}^{S_{sample}}\!/_{S_{base}} \times 100$$

Baseline Measurements



Baseline Measurement

Reference Beam — R_{Base}

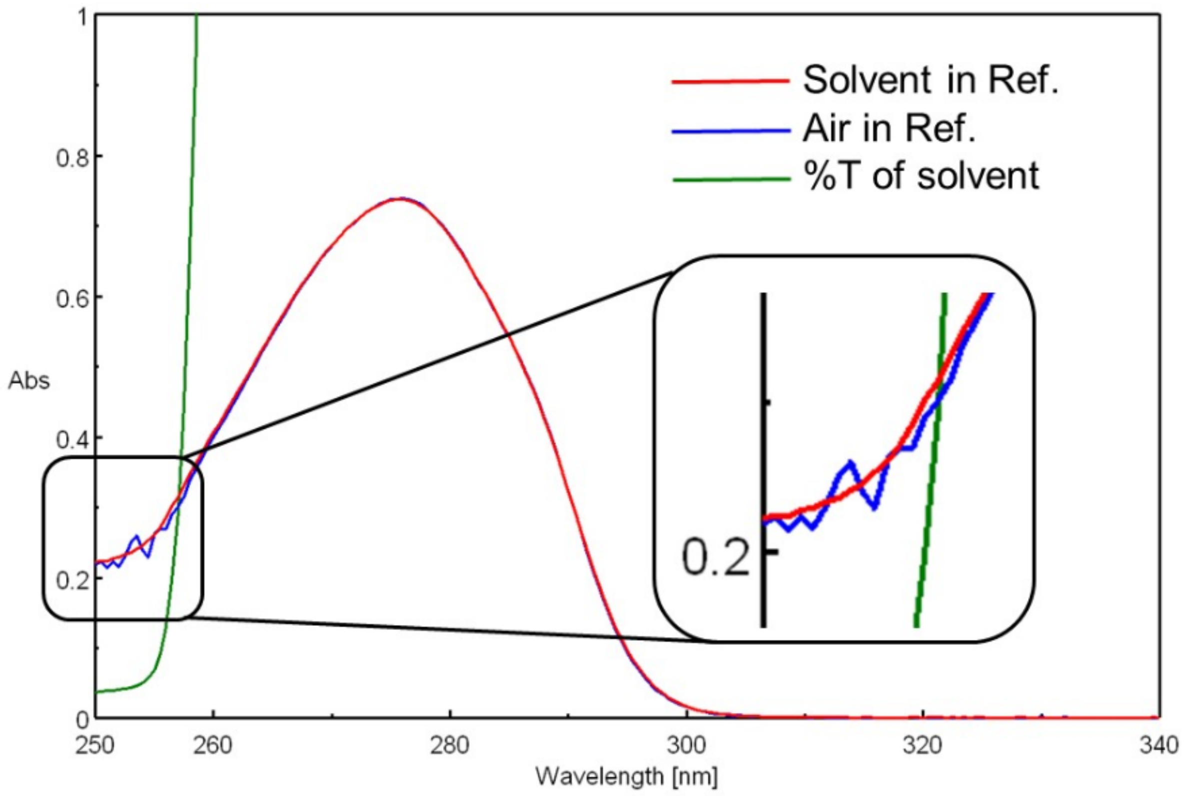
Sample Beam — S_{Base}

Sample Measurement

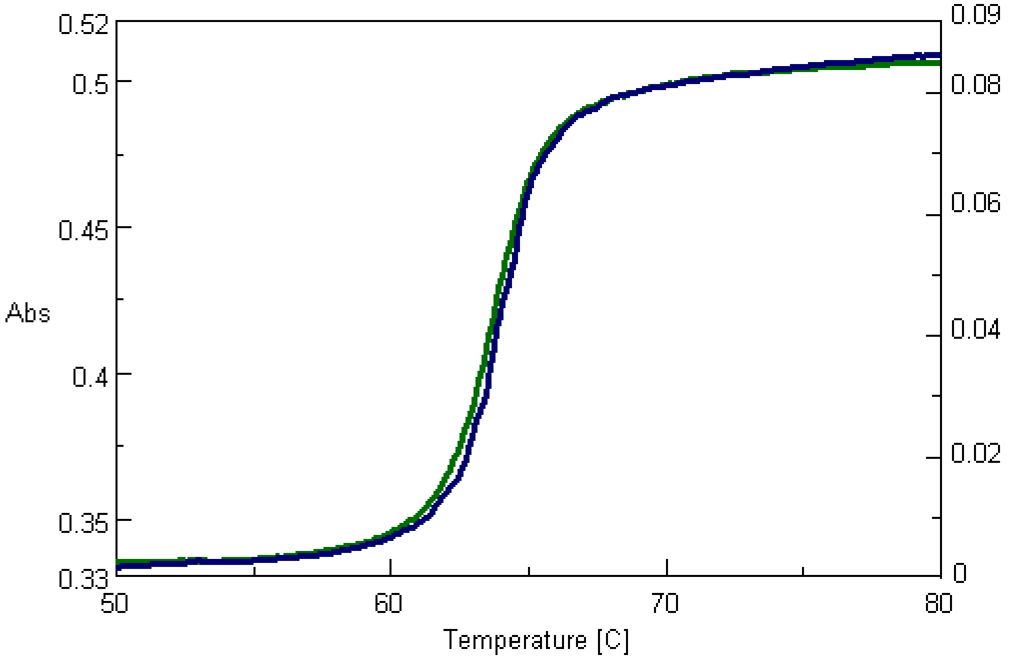
Reference Beam — $R_{Samplee}$

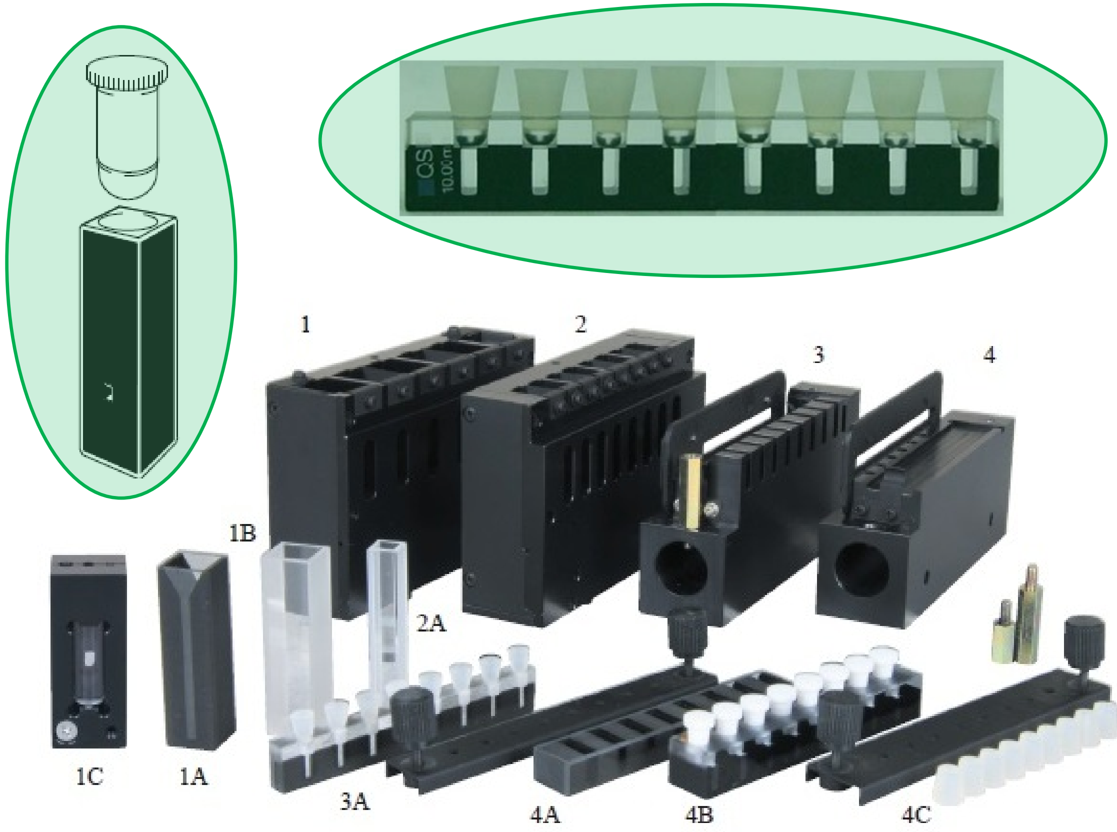
Sample Beam — $S_{Samplee}$

Baseline Measurements



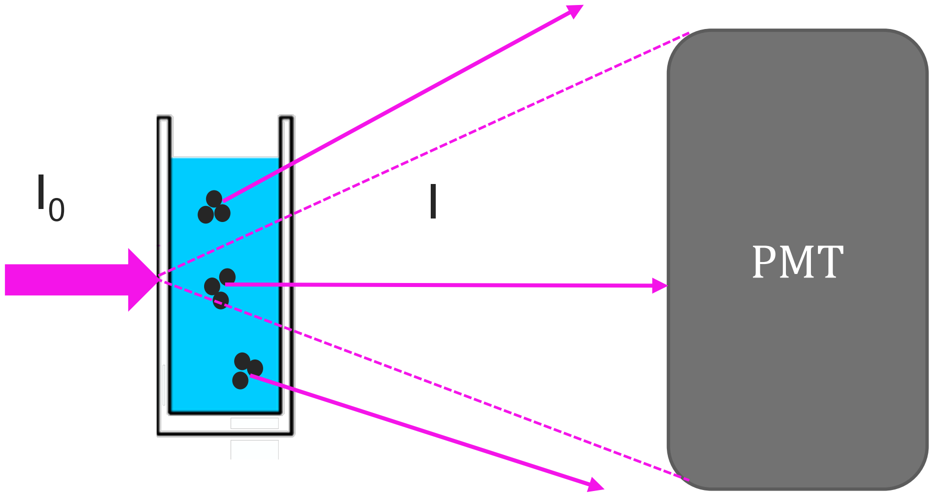
Temperature Studies



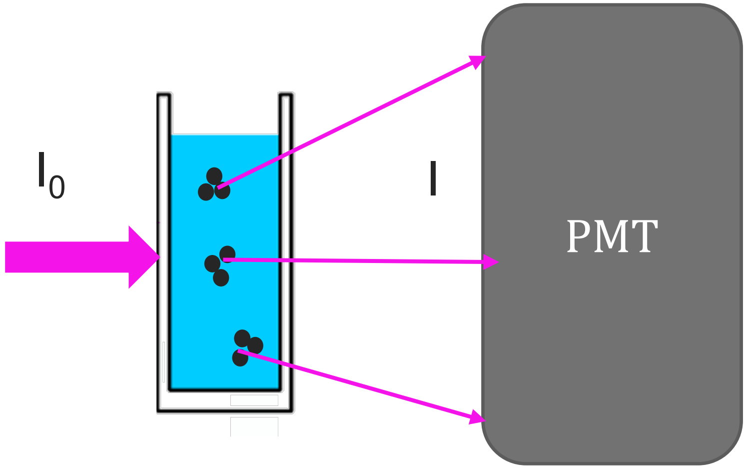


Cloudy/turbid liquid samples

Increase in apparent absorbance due to light loss

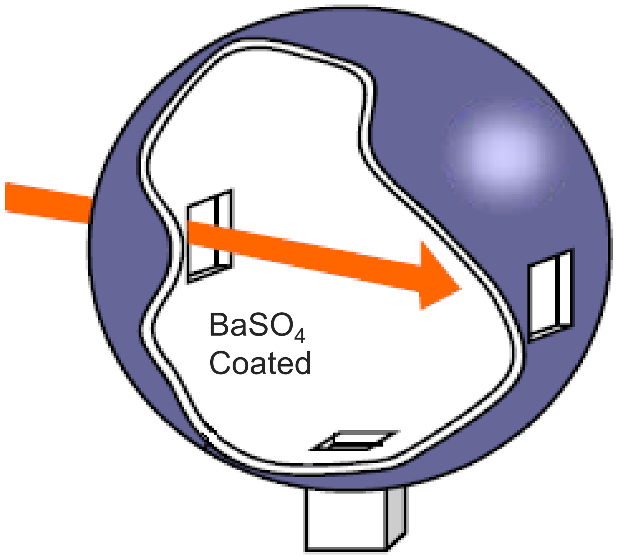


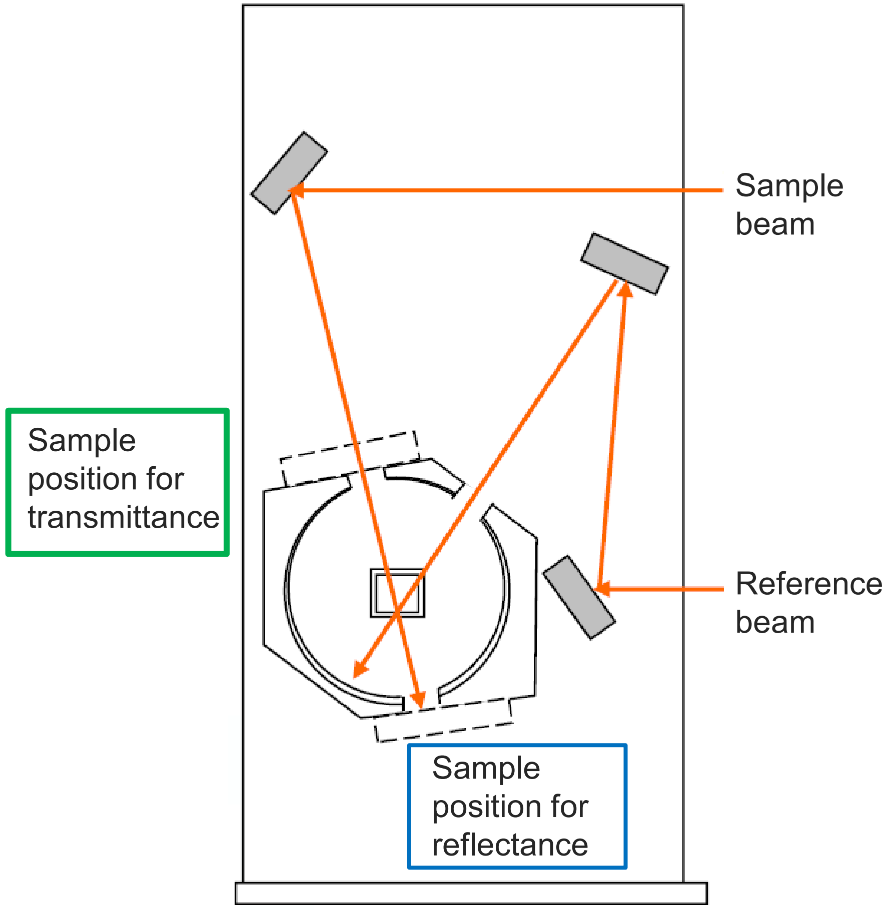
Standard sample position



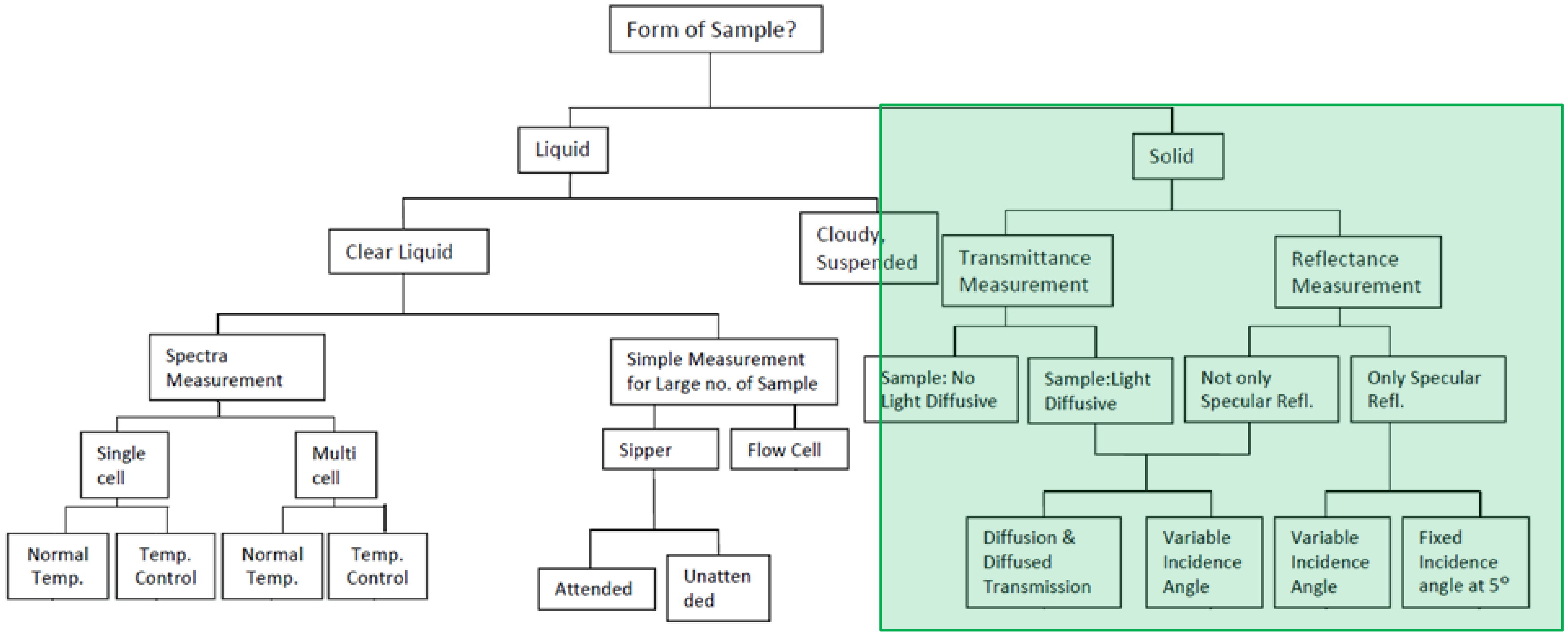
Sample closer to detector

Integrating spheres

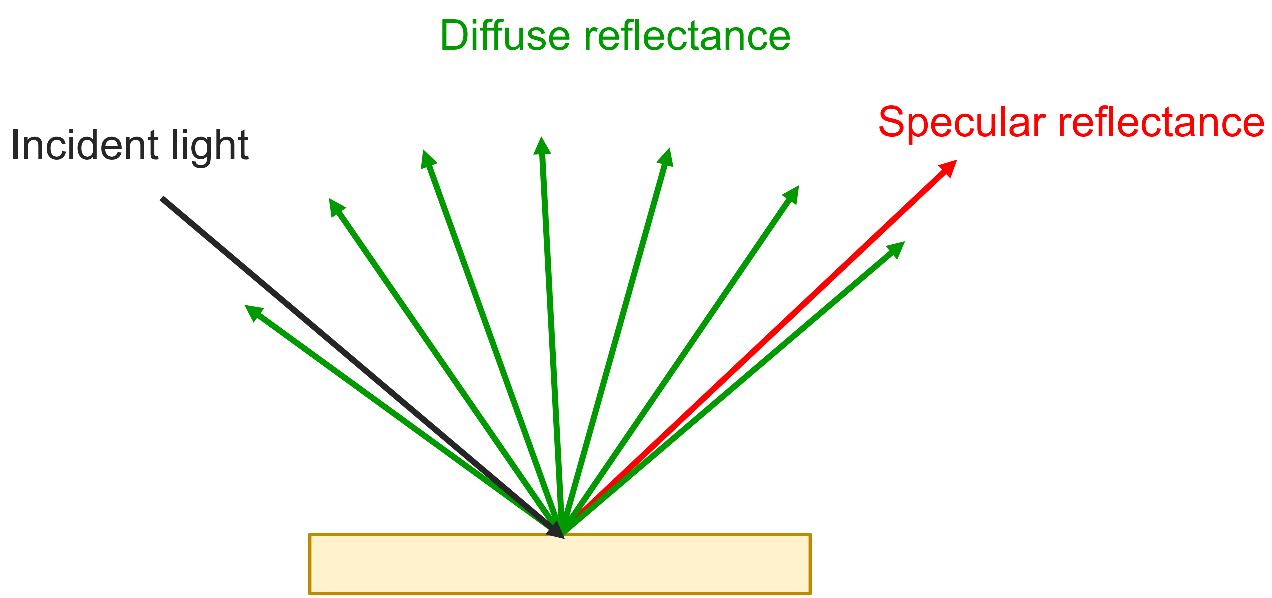




Sampling: Solids

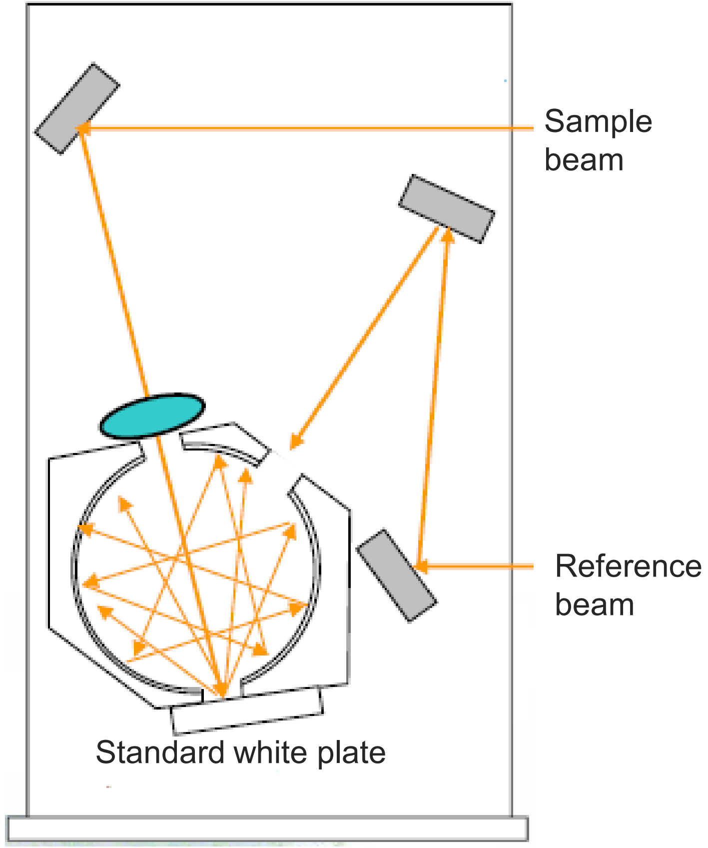


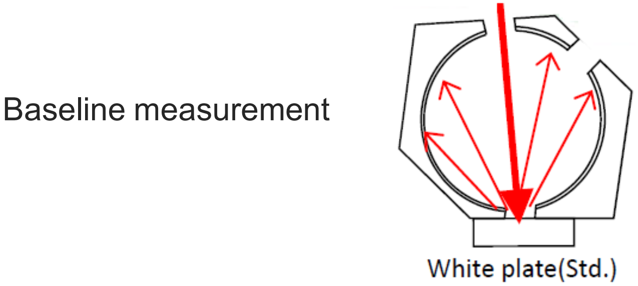
Reflectance

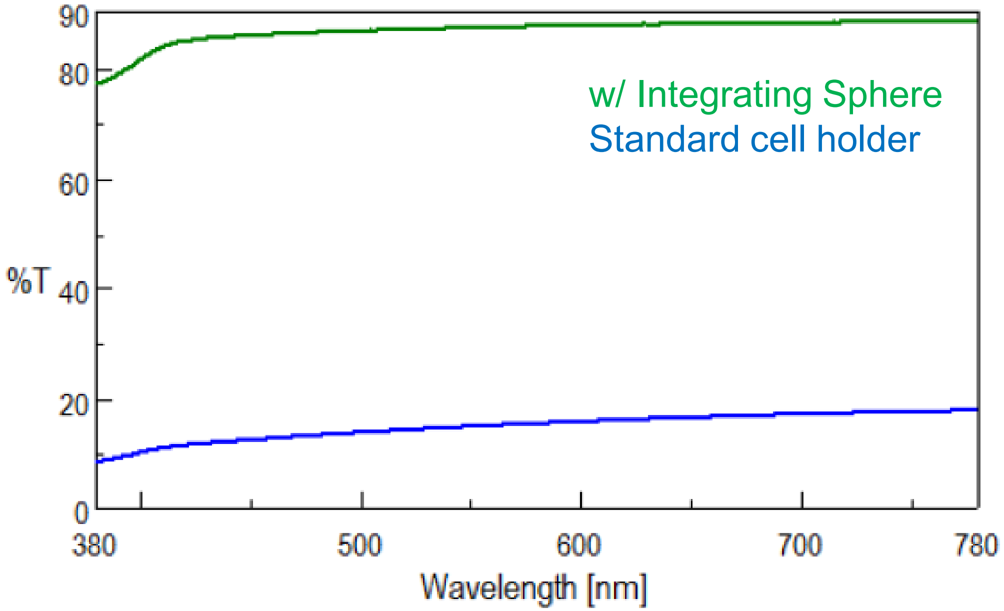


- Relative reflectance: ratios sample reflectance to standard surface reflectance
- Absolute: ratios sample reflectance to reflectance from known output source

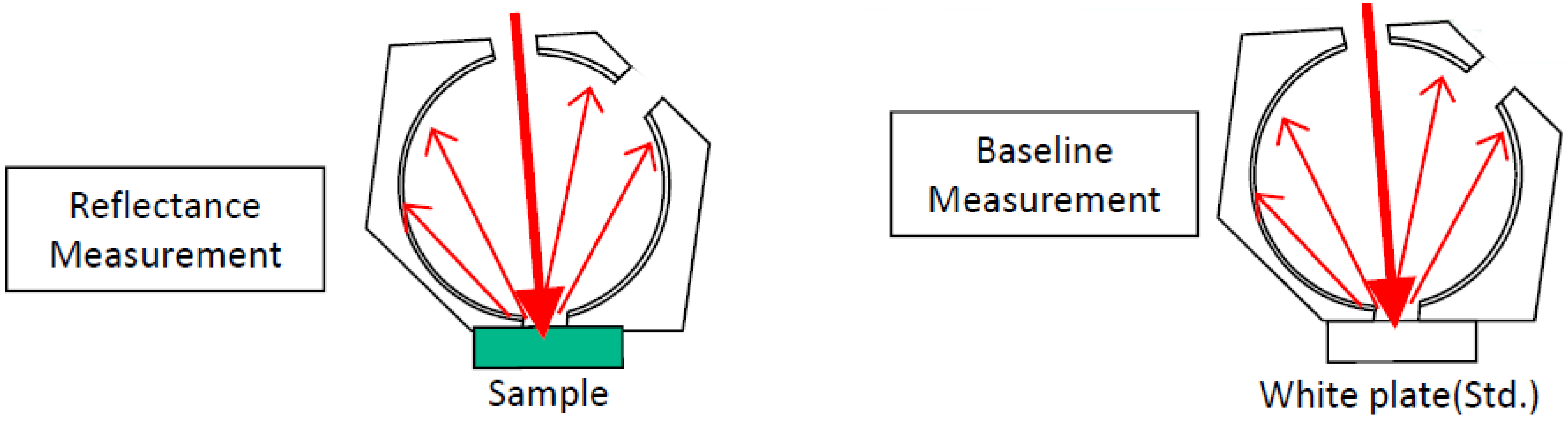
Diffuse Transmittance Measurements



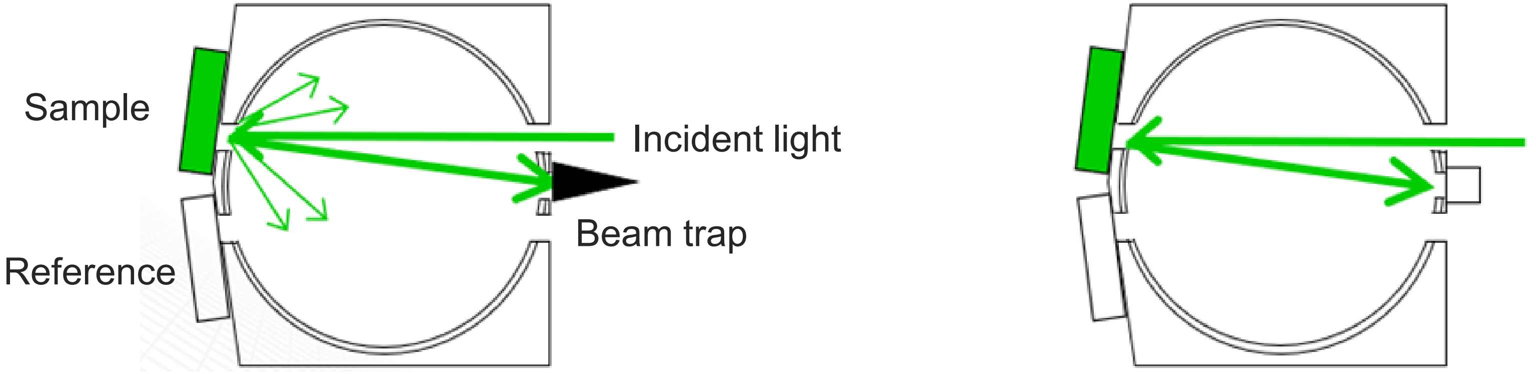




Diffuse and Total Reflectance Measurements



60 mm sphere



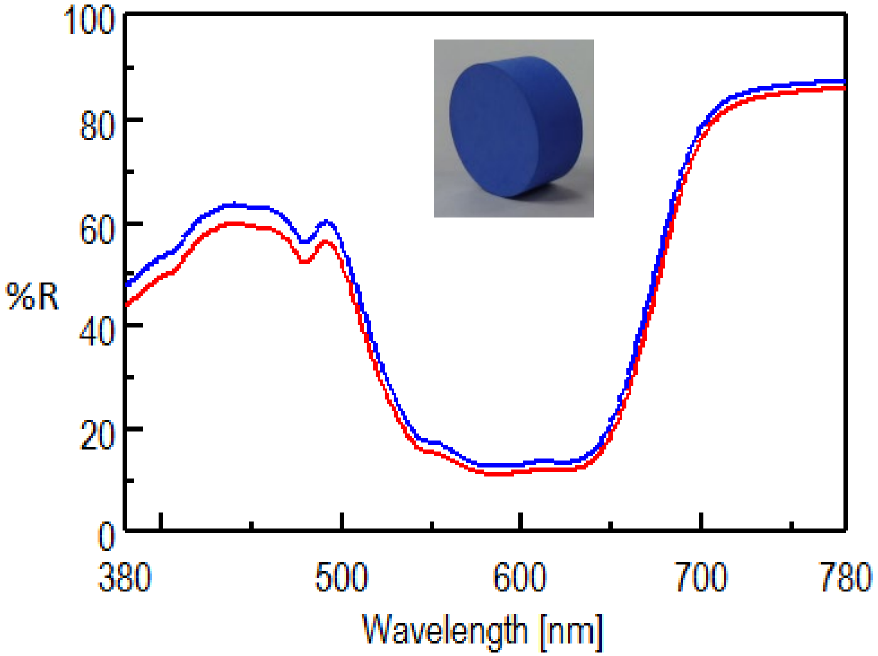
Diffuse and specular reflection (total)

Only diffuse reflection

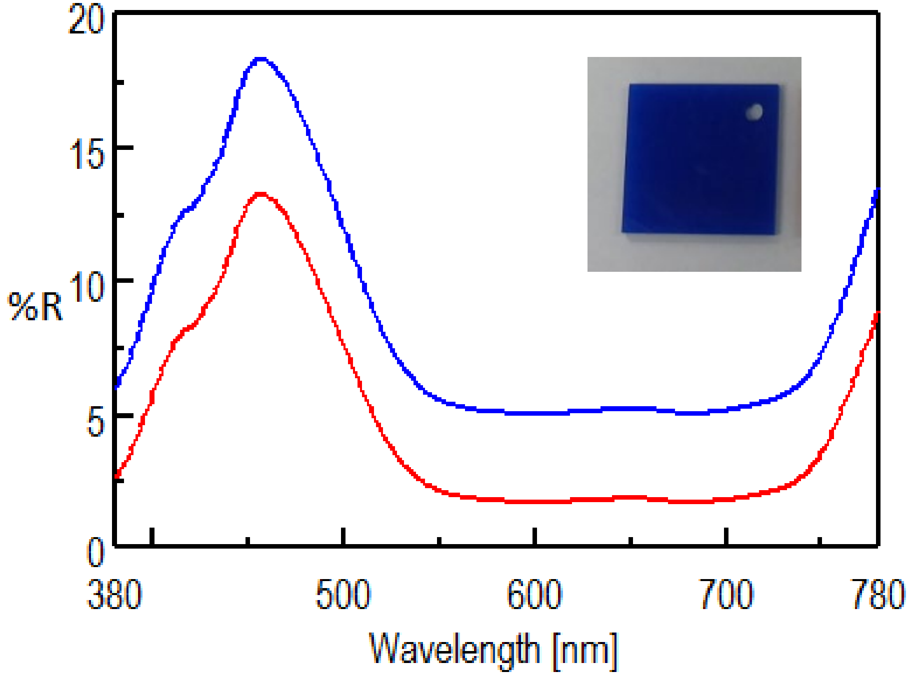
150 mm sphere

Diffuse and Specular Surfaces

Matte



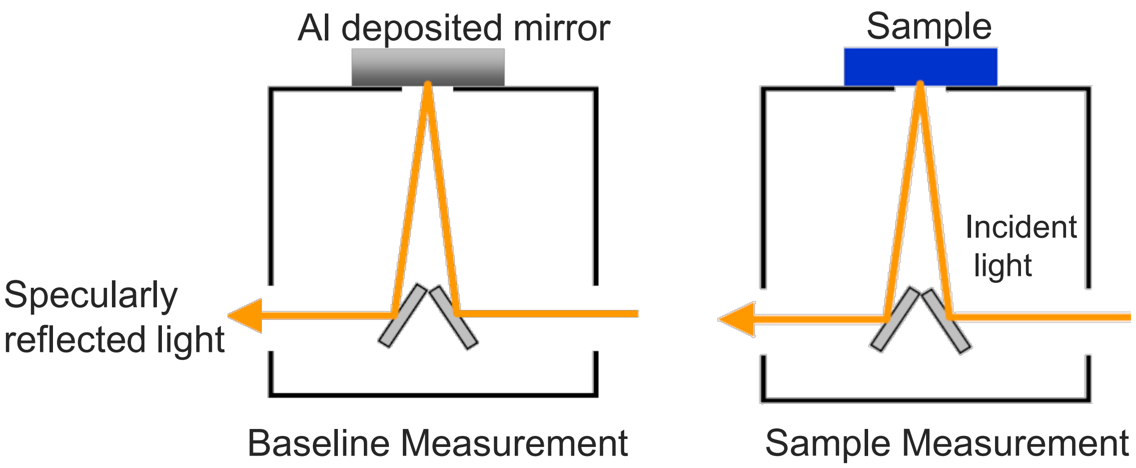
Glossy



— with specular component
— without specular component

Specular reflectance accessory

Ratio of the amount of light reflected from the sample to the amount of light reflected off the reference mirror.

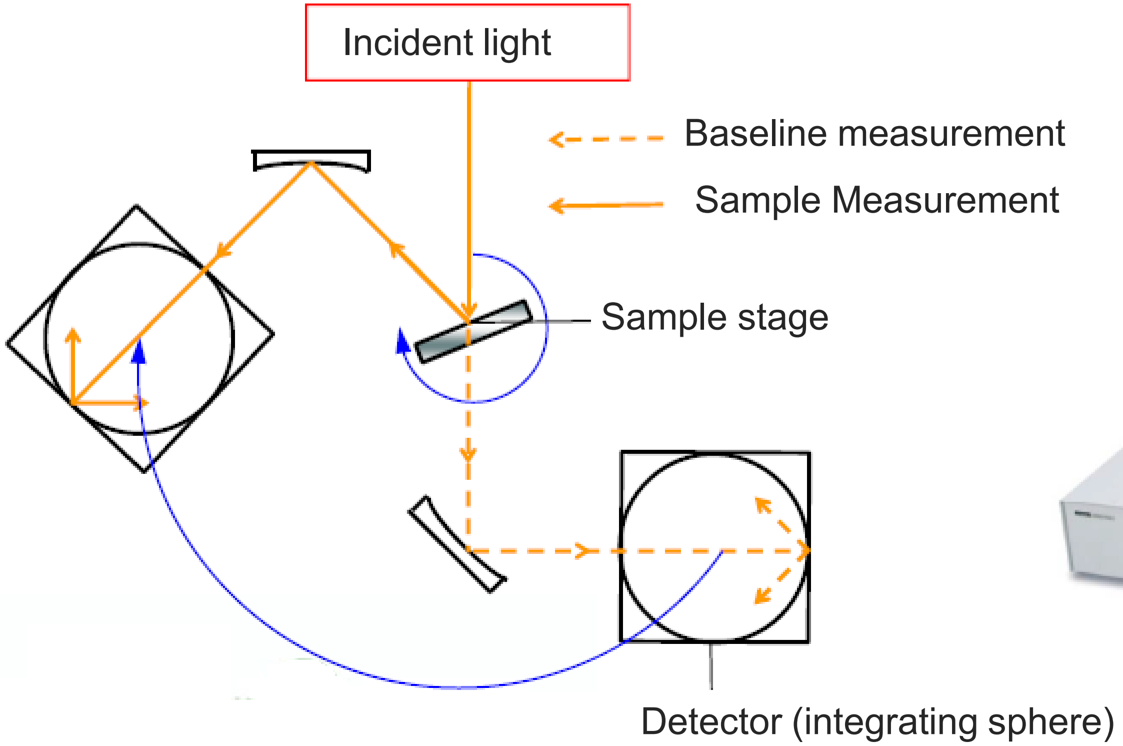


$$\%R = \frac{I_{Sample}}{I_{Al}} \times 100$$

Absolute Reflectance Measurements

$$\boxed{\%R = \frac{I}{I_0} \times 100}$$

%R is the amount of reflected light of the sample against the amount of incident light



Summary

- UV-Vis/NIR spectroscopy probes the absorption, transmission, and reflection of solid, liquids, films, and powders between 190 – 3200 nm.
- Double beam instruments use a reference beam to account for fluctuations in light intensity, I_0, to provide accurate measurements when ratioing the transmitted to incident light.
- The Beer-Lambert law is used to calculate sample concentration and describes how light is attenuated due to sample concentration, pathlength, and the strength of its electronic transition. Stray light introduces deviations to the Beer-Lambert law, leading to inaccurate absorbances and therefore concentrations.
- The cuvette material and sampling volumes need to be considered when selecting an appropriate cuvette for liquid measurements.
- Integrating spheres can be used to collect any scattered light that won't normally reach the detector's surface for cloudy suspensions or solids.

JASCO Educational Resources

Webinars:

- Vibrational Circular Dichroism
- Fluorescence Spectroscopy
- FTIR Theory, Instrumentation, and Techniques
- FTIR Microscopy
- Circular Dichroism Theory and Applications
- Circular Dichroism Measurement Optimization
- Raman Microscopy and Imaging
- SFC Theory and Applications

E-books and Tips and Tricks Posters

- Raman
- Fluorescence
- FTIR
- CD

KnowledgeBase

NEXT WEBINAR WILL BE A LIVE RAMAN MICROSCOPY DEMO

DR. CARLOS MORILLO
TUESDAY JUNE 23RD AT 2:00 PM EDT

Thank you for attending our UV-Vis/NIR Webinar!

ANY QUESTIONS?