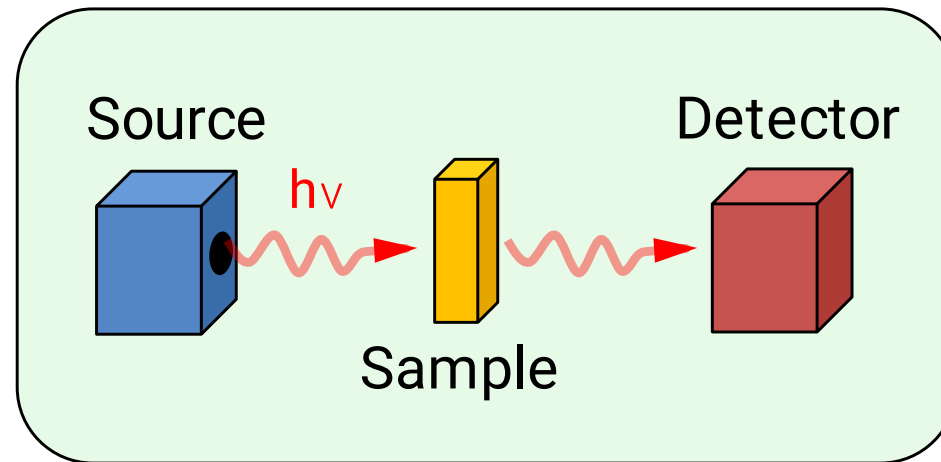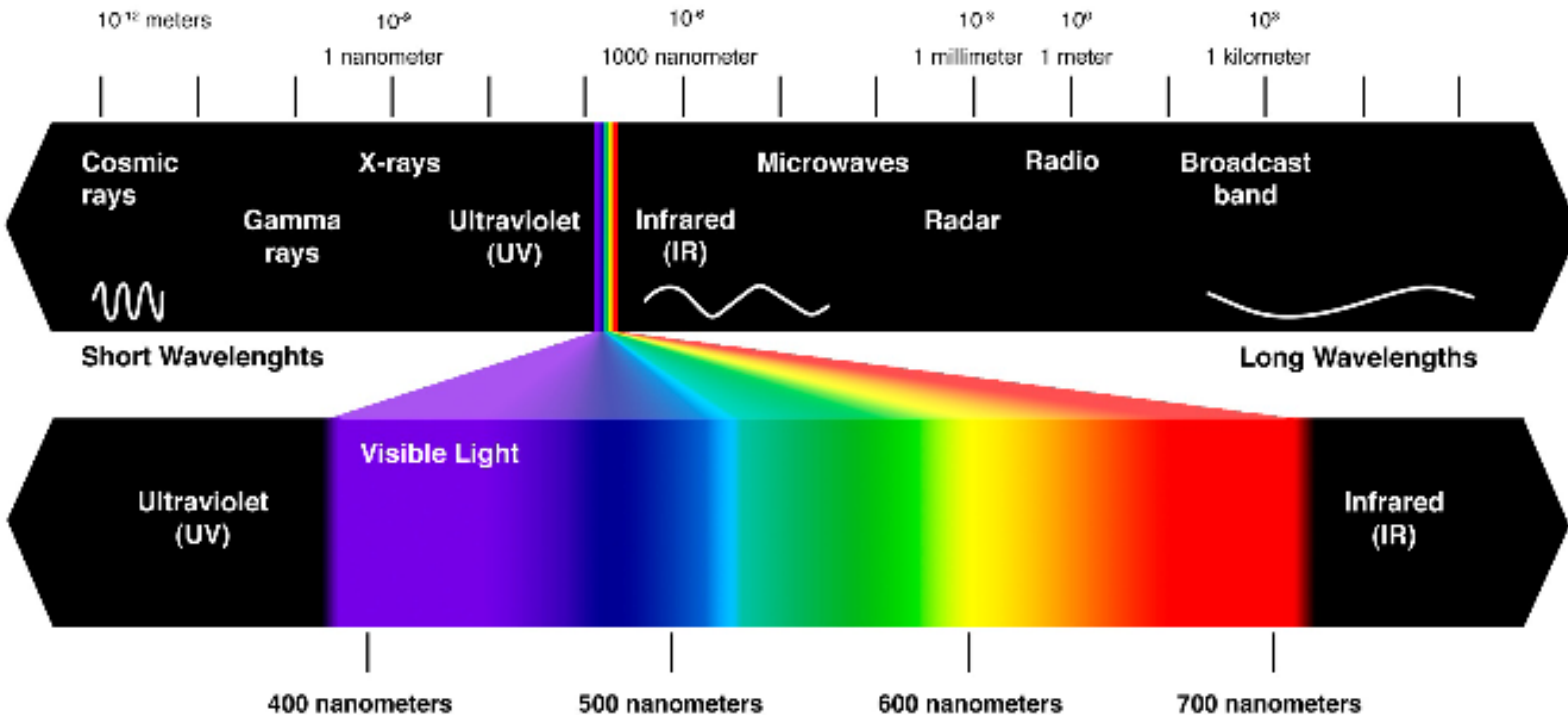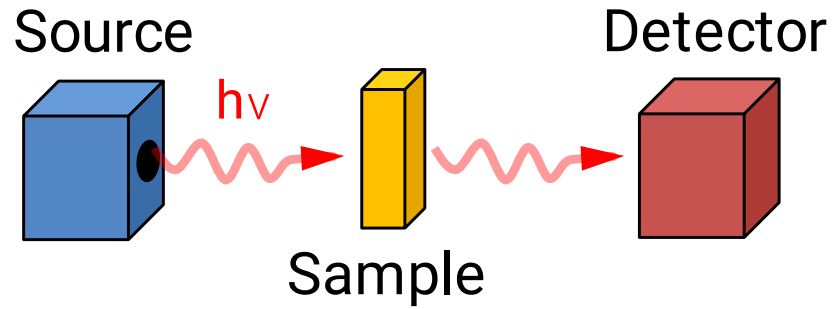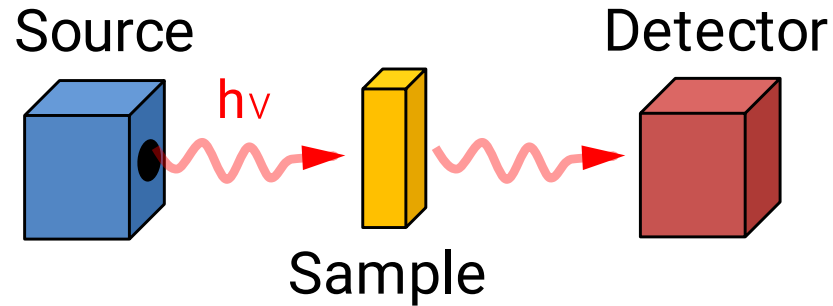# Absorption Spectroscopy

Absorption Spectroscopy

Why Absorption Spectroscopy?

- Color is ubiquitous to humans

- 1000 x more sensitive than NMR

- Qualitative technique (what is in the solution)

- Quantitative technique (concentrations, ratios, etc.)

- Its easy

- It is inexpensive

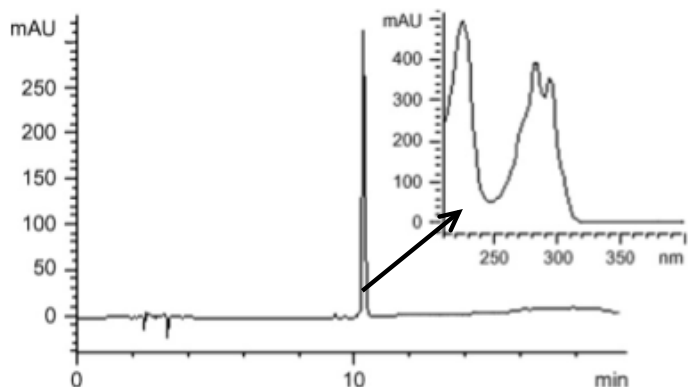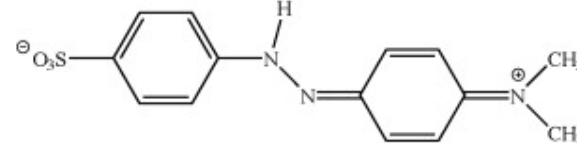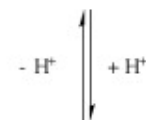- Numerous applications
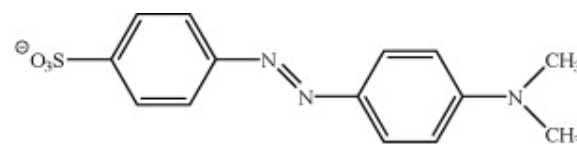
Absorption Spectroscopy in Action

HPLC

Fig. 3. HPLC chromatogram and UV–vis spectra (inset) of Bromo-Dragonfly. The HPLC trace was detected at 210 nm.

Structure Differentiation
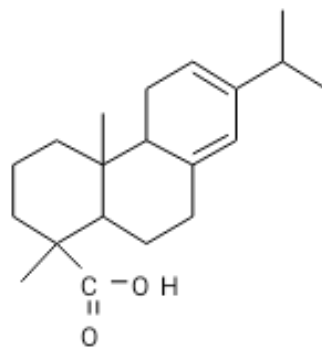
Abietic Acid

214 nm

Levopimaric acid

253 nm

pK_a Determination

Yellow (pH > 4.4)

- H⁺   + H⁺

Red (pH > 3.2)

Examples

Source   Detector

$h\nu$

Sample

3D Glasses

Source

Sample

Detector
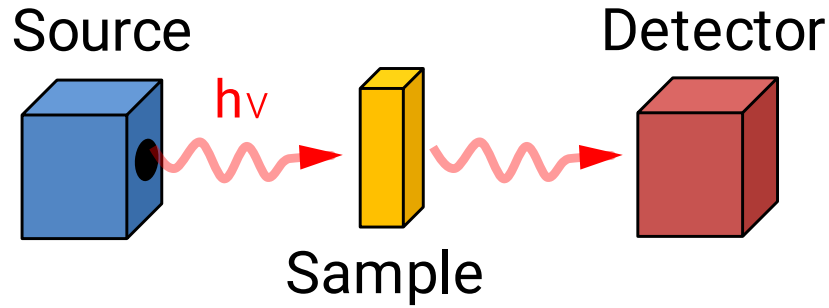
Astrochemistry
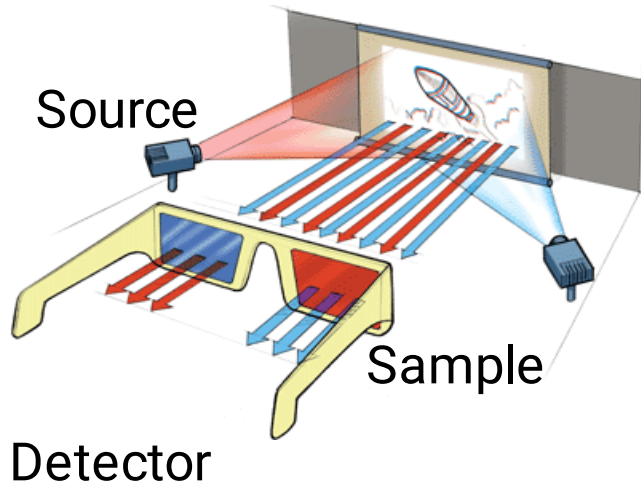
Source

Detector

Sample

First homework (not really): Think of examples of absorption

Outline

1) Absorption

2) Spectrum Beer's Law

3) Instrument Components
   - Light sources
   - Monochrometers
   - Detectors
   - Other components
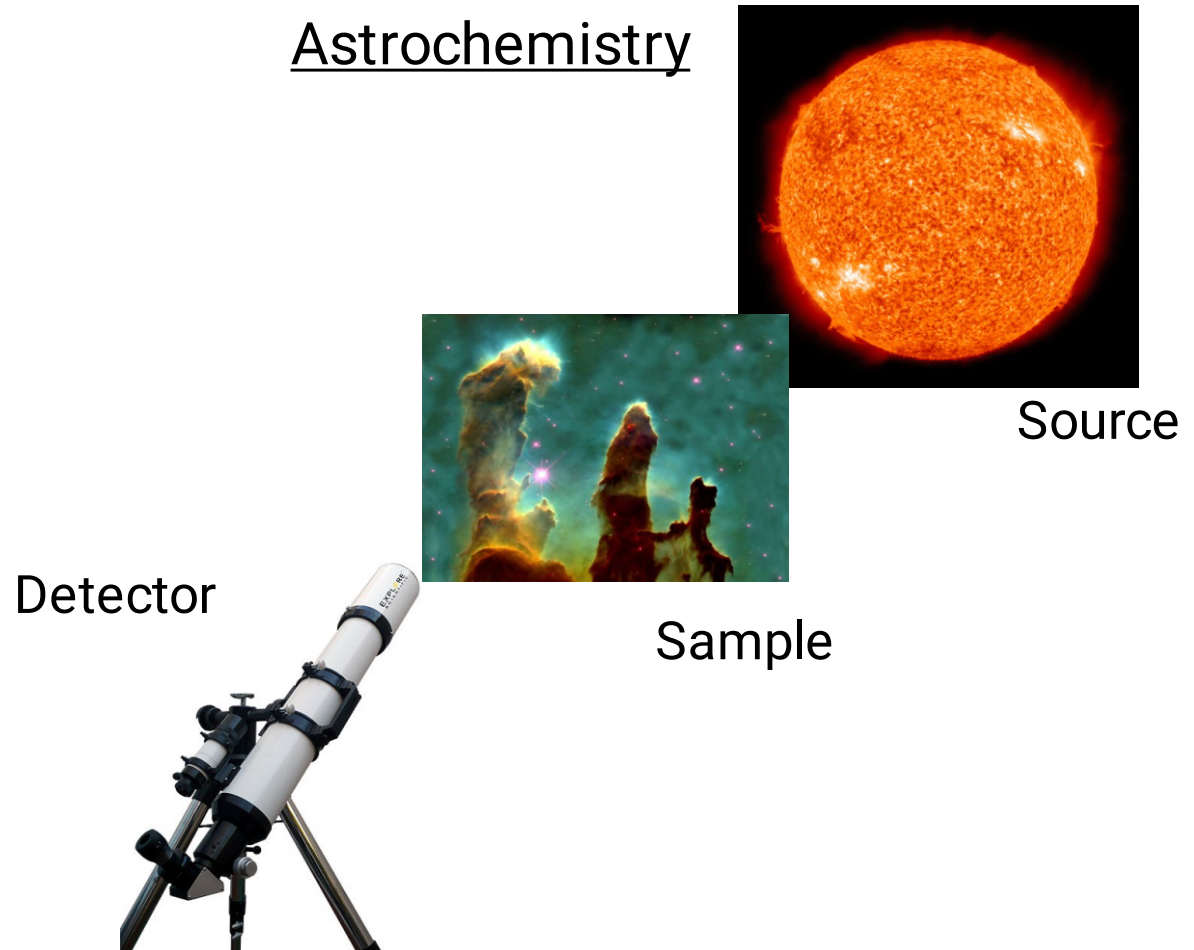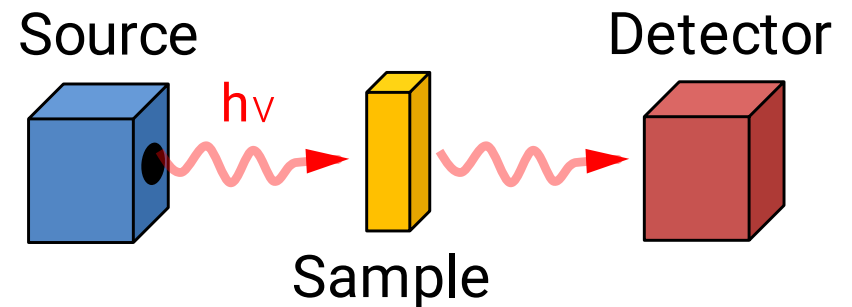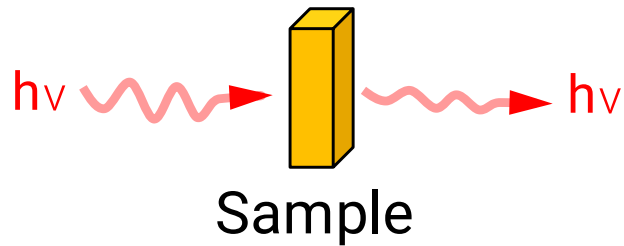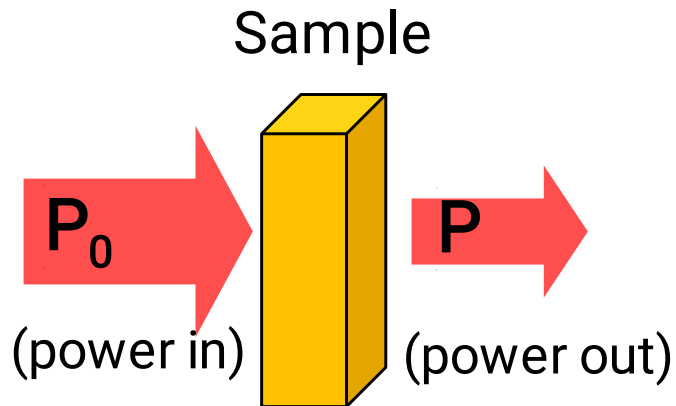   - The sample

4) Instrument Architectures

5) UV-Vis in Action

6) Potential Complications

Source $h\nu$ Detector
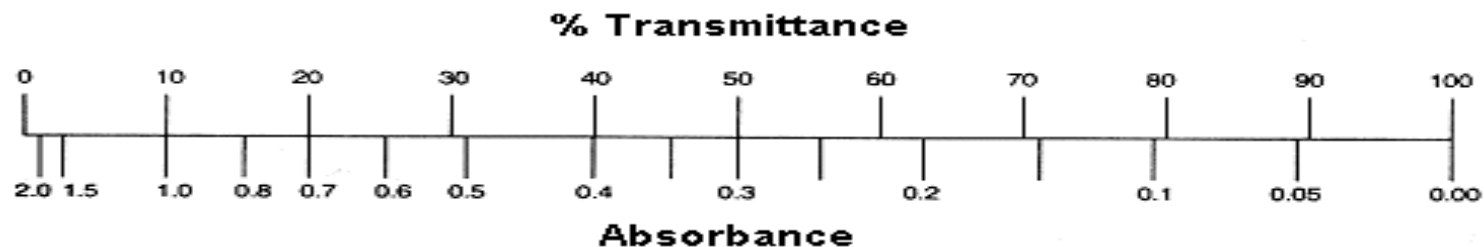
Sample

Absorption by the Numbers

Sample

We don't measure absorbance. We measure transmittance.

Sample

P_0

(power in)

P

(power out)

- Transmittance:

$$T = P/P_0$$

- Absorbance:

$$A = -\log T = \log P_0/P$$

Beer's Law

The Beer-Lambert Law (λ specific):

$$A = \varepsilon\, c\, l$$

A = absorbance (unitless, $A = \log_{10} P_0/P$)

ε = molar absorptivity (L mol^{-1} cm^{-1})

l = path length of the sample (cm)

c = concentration (mol/L or M)

Sample

P_0 (power in)

P (power out)

l in cm

Concentration ⬆ Absorbance ⬆
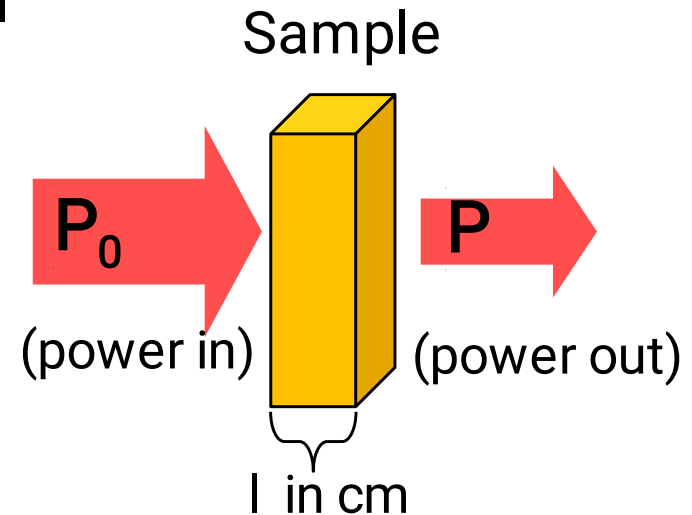
Path length ⬆ Absorbance ⬆
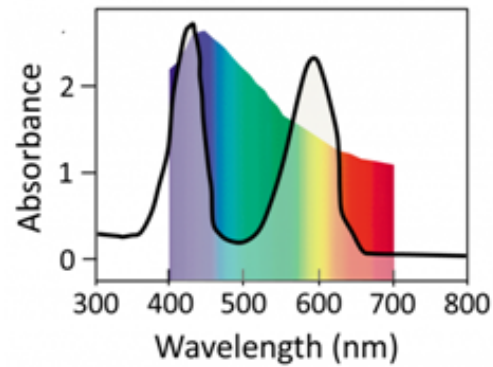
Molar Abs. ⬆ Absorbance ⬆

Absorption Spectrum

Alexandrite Gemstone
$BeAl_2O_4$ (+ Cr^{3+} doping)
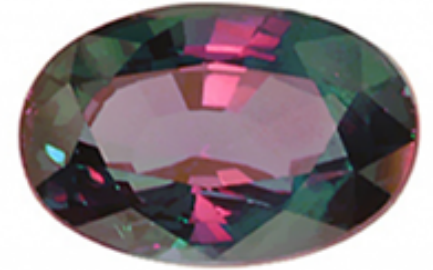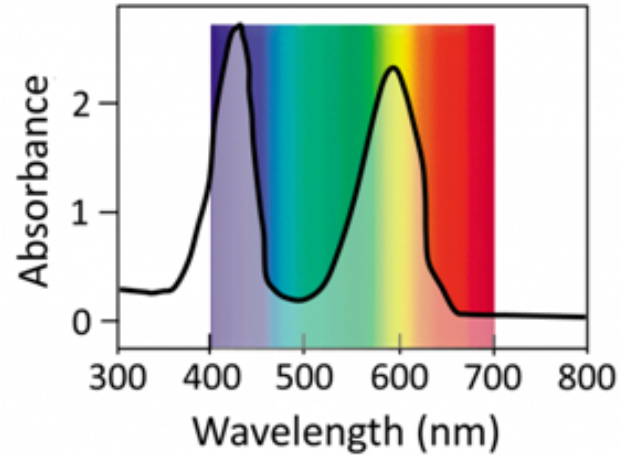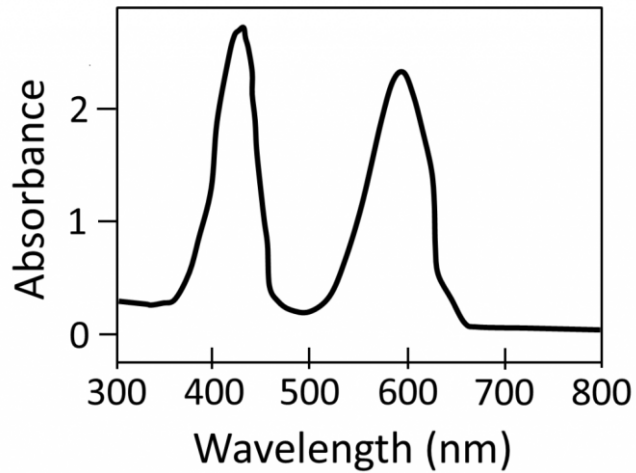
Sunlight

Candle/ Incandescent

Beer's Law

The Beer-Lambert Law:

$A = \varepsilon\, c\, l$

- A = absorbance (unitless, $A = \log_{10} P_0/P$)
- ε = molar absorbtivity (L mol^{-1} cm^{-1})
- l = path length of the sample (cm)
- c = concentration (mol/L or M)

Find ε
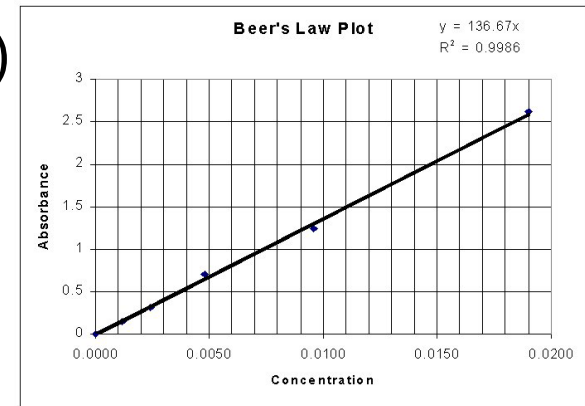1) Make a solution of know concentration (C)
2) Put in a cell of known length (l)
3) Measure A by UV-Vis
4) Calculate ε

$A = \varepsilon\, c\, l$
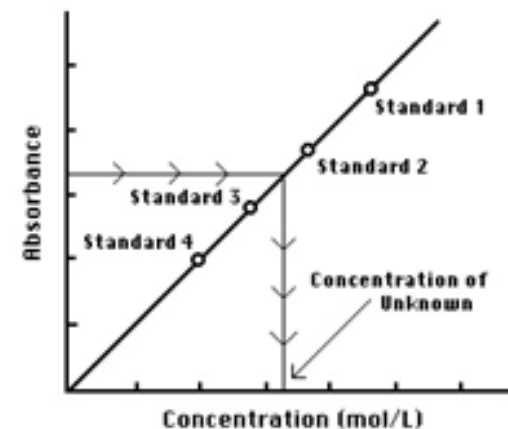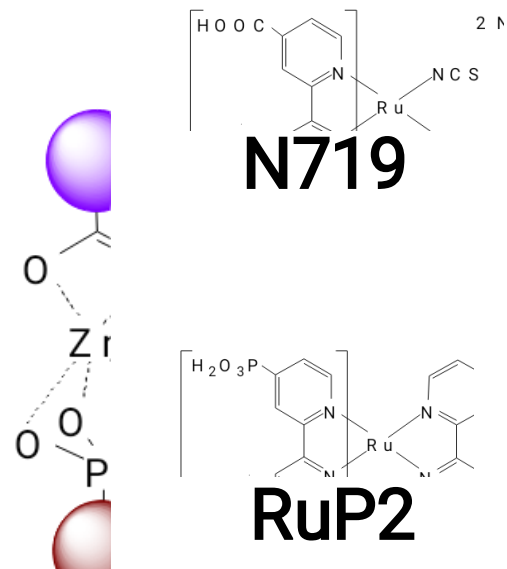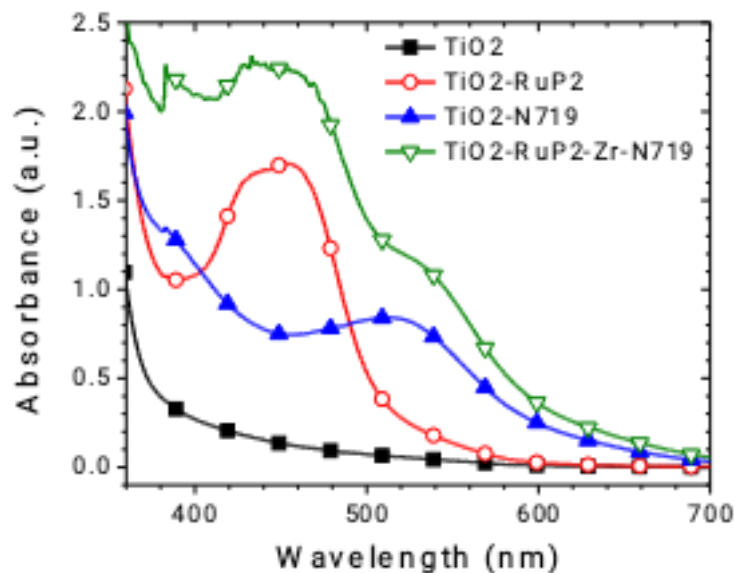
$y = m\, x + b$

Find Concentrations
1) Know ε
2) Put sample in a cell of known length (l)
3) Measure A by UV-Vis
4) Calculate C

$A = \varepsilon\, c\, l$

Beer's Law Applied to Mixtures

N719

RuP2

$$A_1 = \varepsilon_1 \, c_1 \, l$$

$$A_{total} = A_1 + A_2 + A_3 \dots$$

$$A_{total} = \varepsilon_1 \, c_1 \, l + \varepsilon_2 \, c_2 \, l + \varepsilon_3 \, c_3 \, l$$

$$A_{total} = l(\varepsilon_1 \, c_1 + \varepsilon_2 \, c_2 + \varepsilon_3 \, c_3)$$

Limitations to Bear's Law

The Beer-Lambert Law:

$$A = \varepsilon \, c \, l$$

Reflection/Scattering Loss

Figure 13-1 Reflection and scattering losses.

$$A = -\log T = \log P_0/P$$
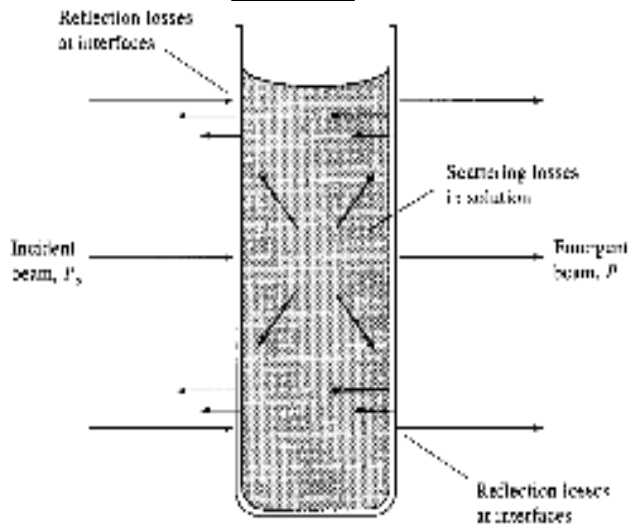
Reflection/Scattering
- Air bubbles
- Aggregates

Lamp effects
- Temperature (line broadening)
- Light source changes
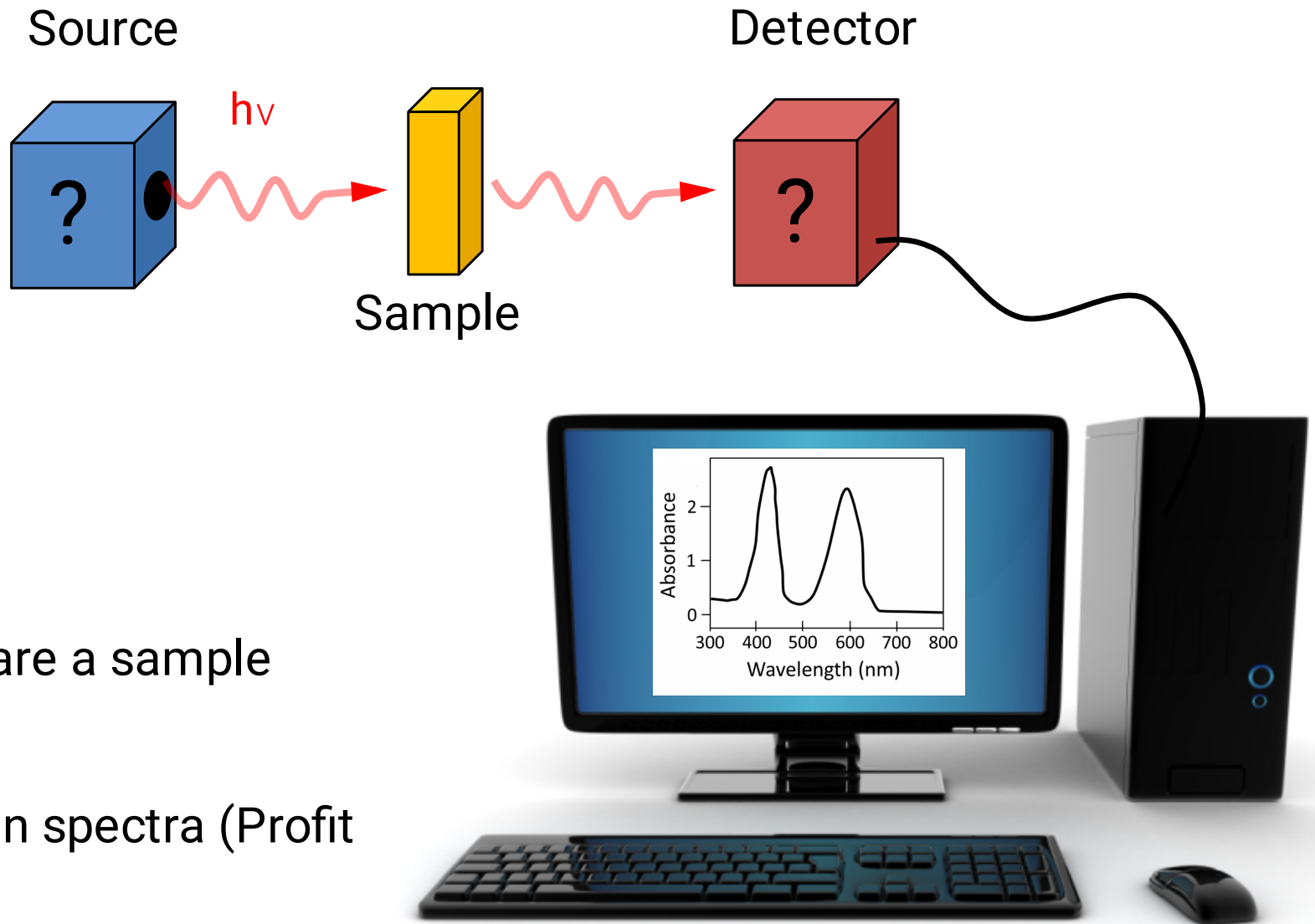- Solvent lensing

Absorbance too high (above 2)
- Local environment effects
- Dimerization
- Refractive index change (ionic strength)

Sample changes
- Photoreaction/decomposition
- Side of the cuvette
- Hydrogen bonding
- Non-uniform through length

Absorption Spectroscopy

Source

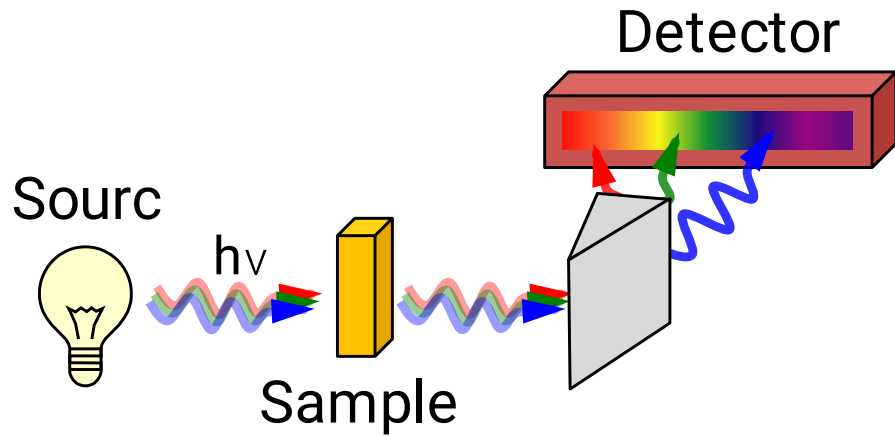Detector

h𝜈

?

Sample

?

Procedure

Step 1: Prepare a sample

Step 2: ???

Step 3: Obtain spectra (Profit !)

Instrumentation

Full spectra detection

Detector

Sourc

hν

Sample

Single λ detection

Detector

Source

hν

Sample

Detector

Sample

Source

hν

Instrumentation

Source

$h\nu$
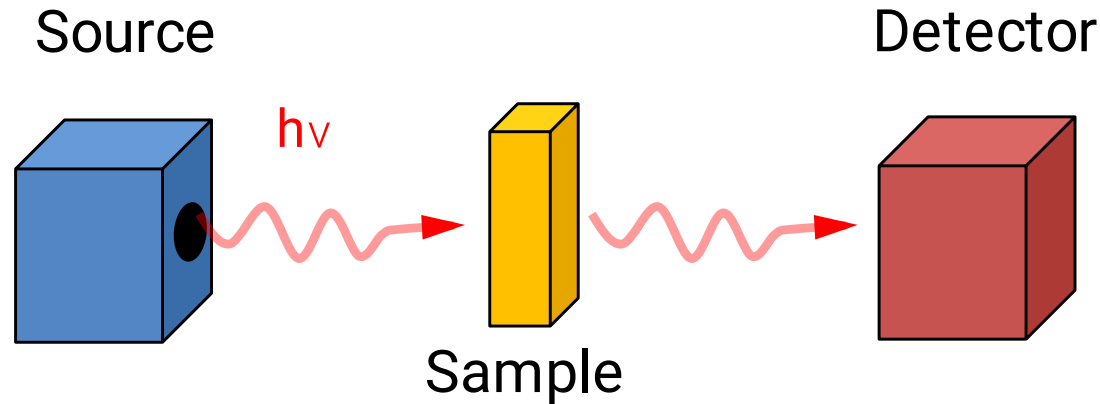
Sample

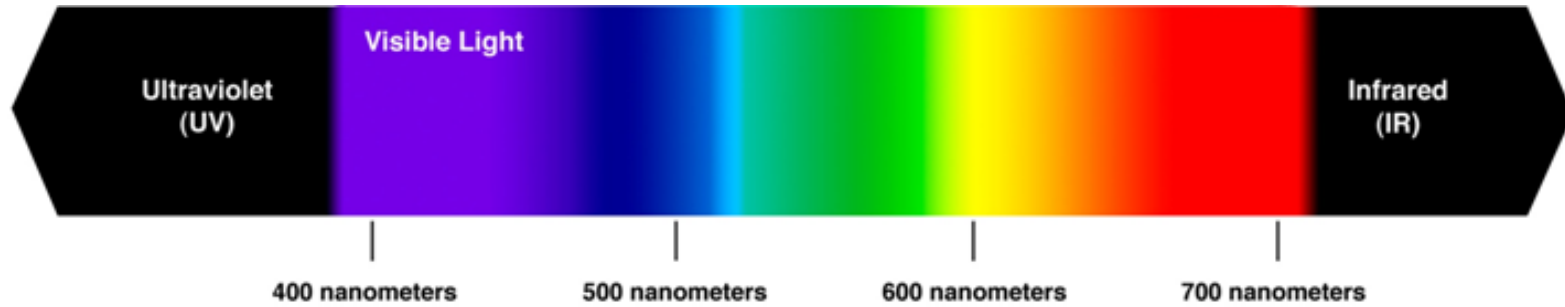Detector

Full spectra detection
- Source
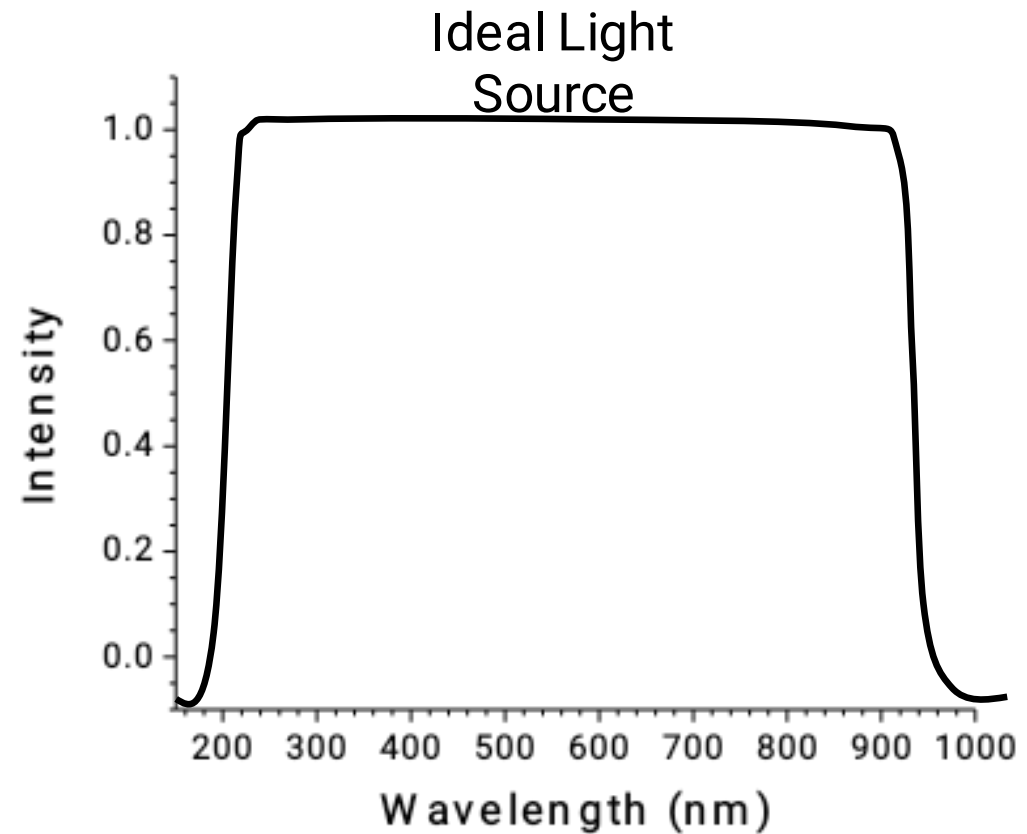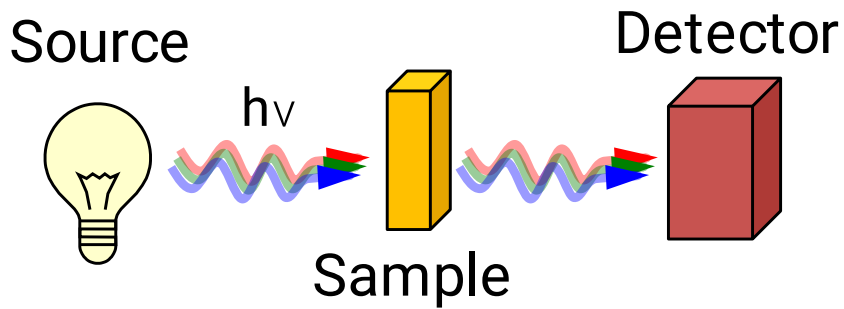- Sample
- Monochrometer
- Area detector

Single λ detection
- Source
- Monochrometer
- Sample
- "Point" detector

1. Light sources
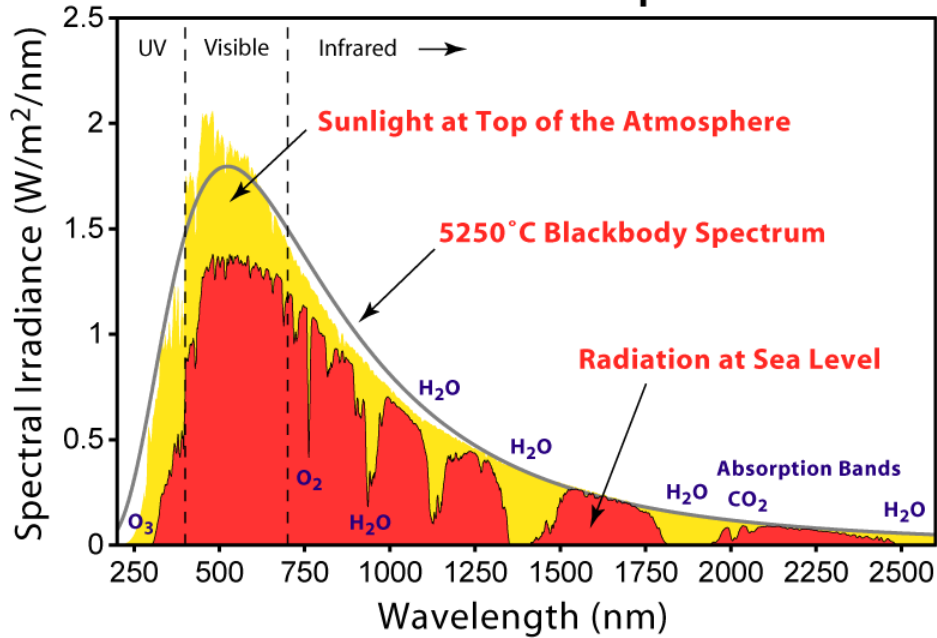2. Monochrometer
3. Detectors
4. Samples

Light Sources, Ideal

Ultraviolet (UV) | Visible Light | Infrared (IR)

400 nanometers   500 nanometers   600 nanometers   700 nanometers

Experimentally we would like ~200 – 900 nm

Source

$h\nu$

Sample

Detector

Ideal Light Source

Light Sources: The Sun

Solar Radiation Spectrum

Pros:

It's free!

Does not die

Relatively uniform from 400-800 nm

Cons:

Inconsistent

Minimal UV-light

Intense absorption lines

$h\nu$

Sample

Detector
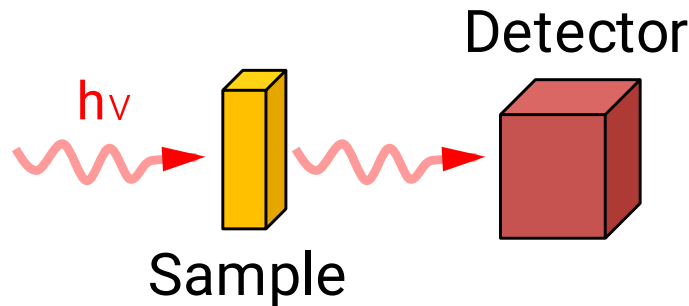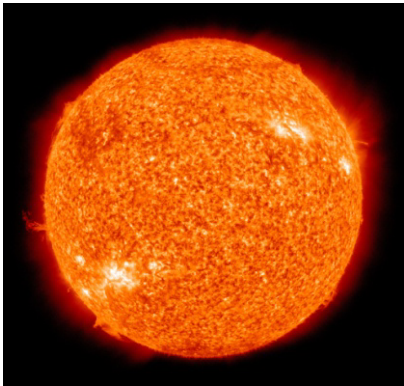
Light Sources: Xe Lamp

Electricity through Xe gas

Pros:
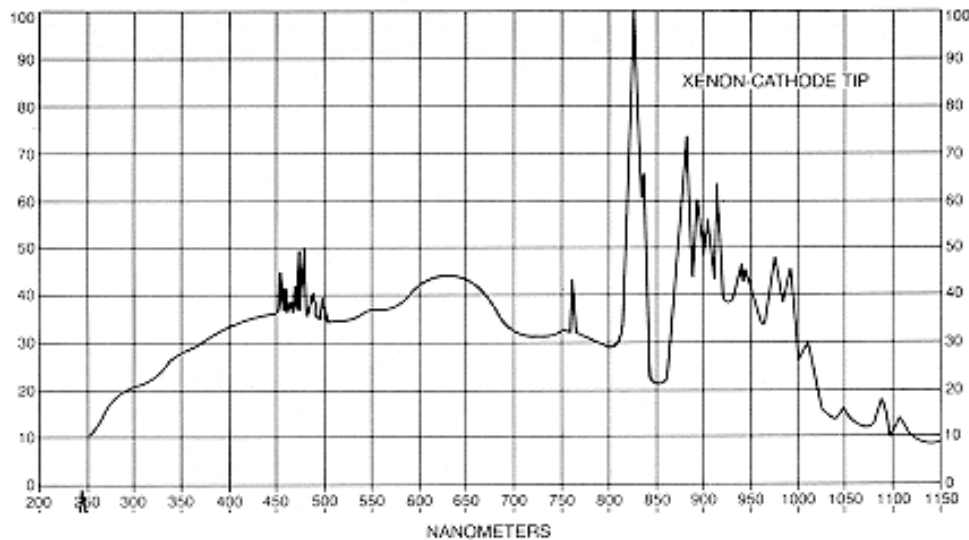
Mimics the sun (solar simulator)

It's simple

Cons:

Relatively Expensive

Minimal UV-light (<300 nm)

Potential Instability

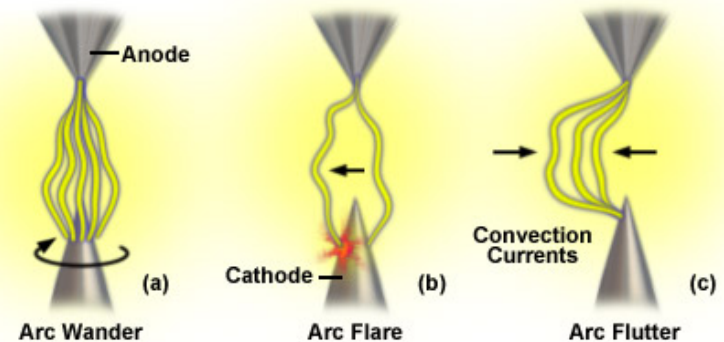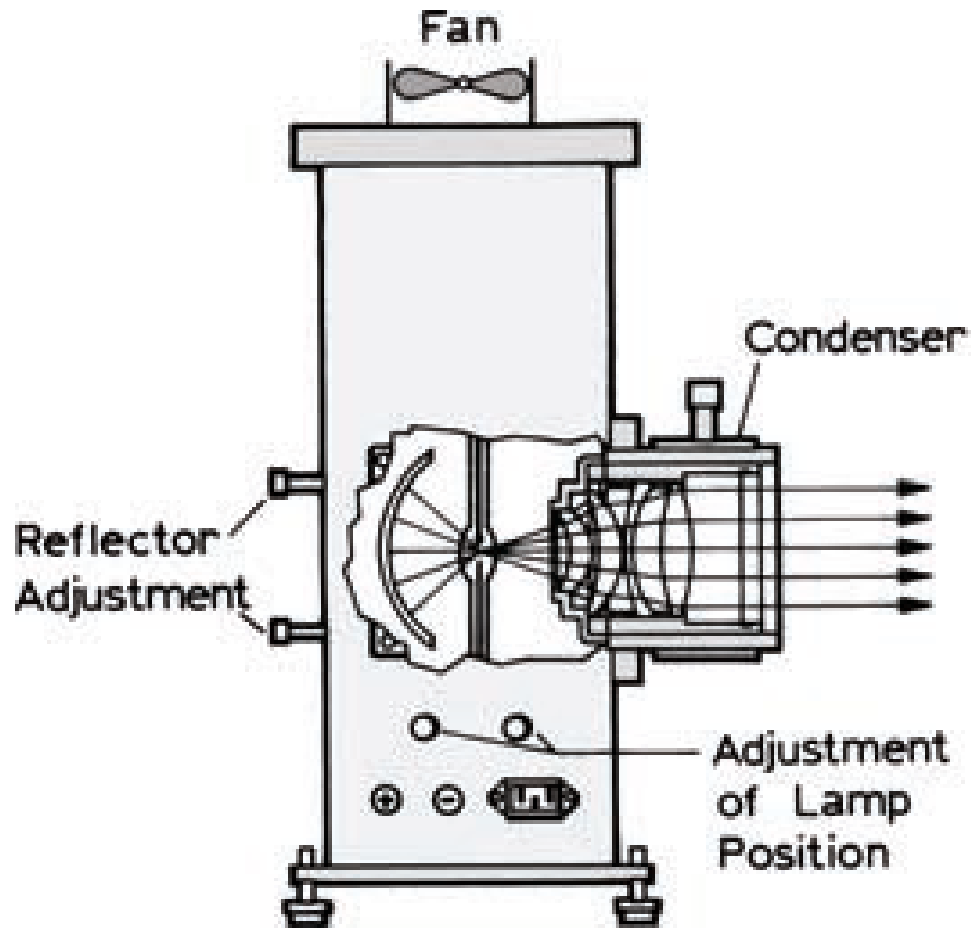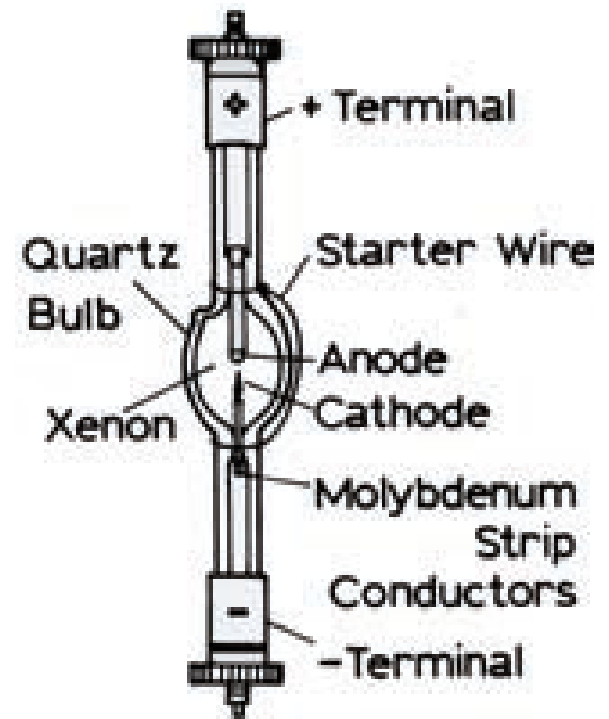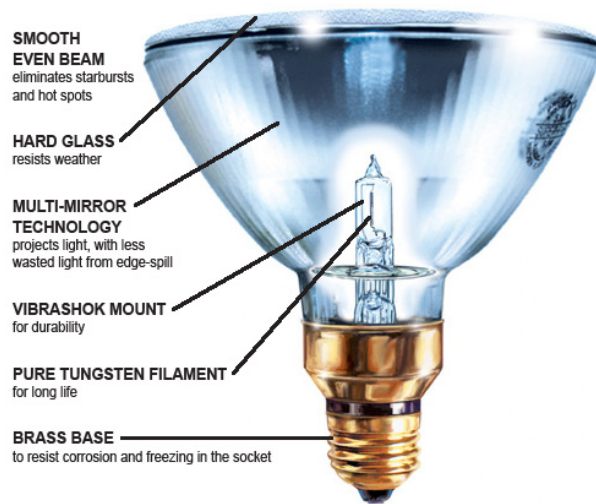Sources of Instability in Xenon Arc Discharge Lamps

(a) Arc Wander — Anode

(b) Arc Flare — Cathode

(c) Arc Flutter — Convection Currents

Figure 3

Light Sources: Xe Lamp

Light Sources: Tungsten Halogen Lamp

SMOOTH EVEN BEAM
eliminates starbursts and hot spots

HARD GLASS
resists weather

MULTI-MIRROR TECHNOLOGY
projects light, with less wasted light from edge-spill

VIBRASHOK MOUNT
for durability

PURE TUNGSTEN FILAMENT
for long life

BRASS BASE
to resist corrosion and freezing in the socket

Halogen gas and the tungsten filament
Higher pressure (7-8 ATM)

Tungsten Lamp Emission Spectrum

Tungsten Lamp T_F = 3400K

Figure 4 T_F = 3200K

T_F = 2850K

Ultraviolet

Infrared

Irradiance at 0.5 M (mW m^{-2} nm^{-1})

Wavelength (Nanometers)

Pros:

Compact size

High intensity

Low cost

Long lifetime

Fast turn on

Stable

Cons:

Very hot

Bulb can explode

Minimal UV-light (<300 nm)
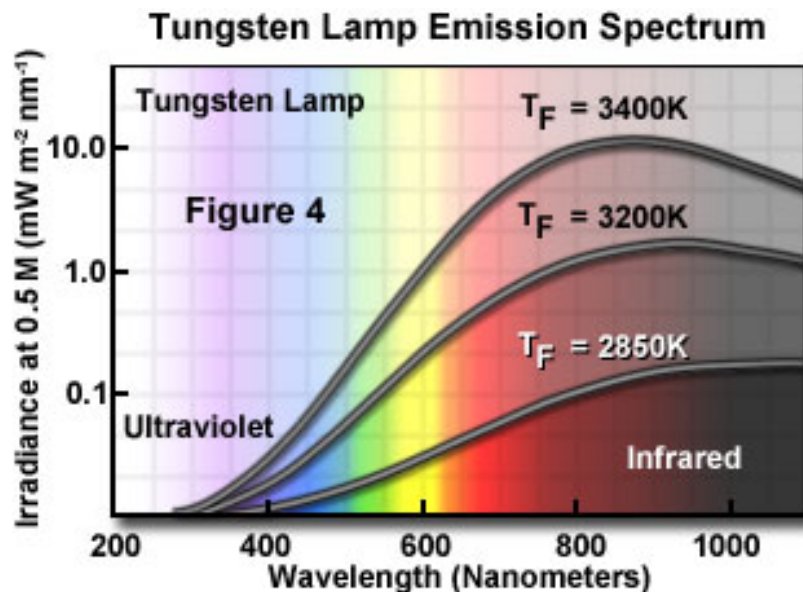
"White" Light

Deuterium lamp
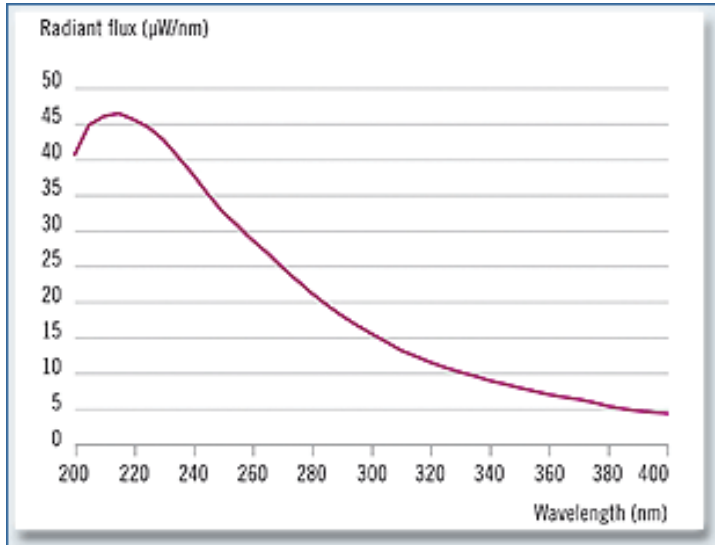
Deuterium lamp −200-330
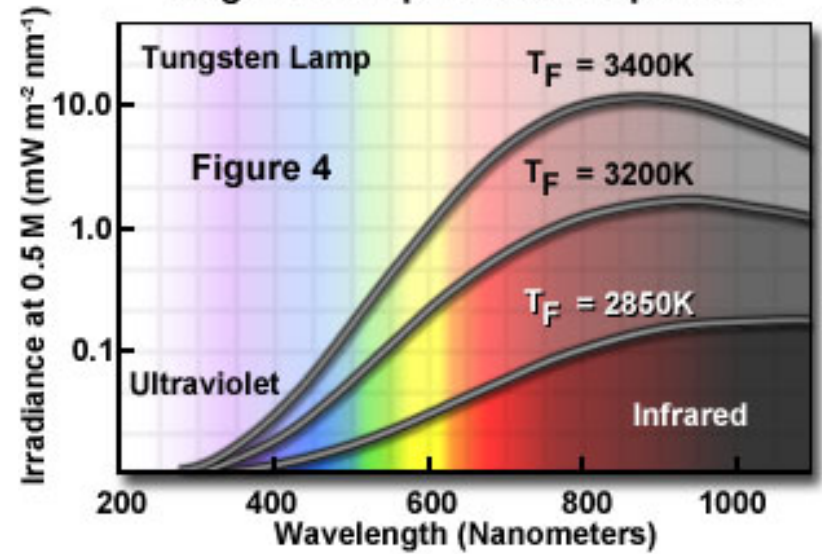
Tungsten lamp − >300 nm

Other Light Sources
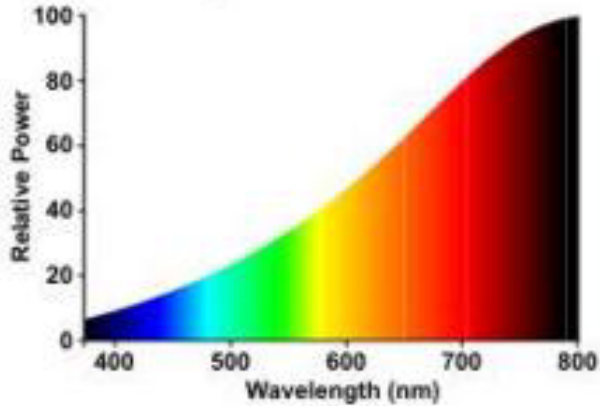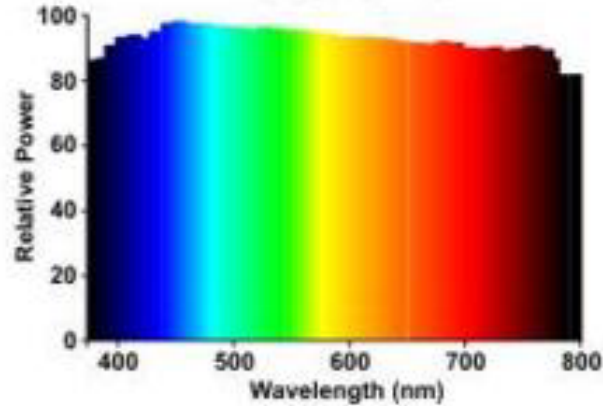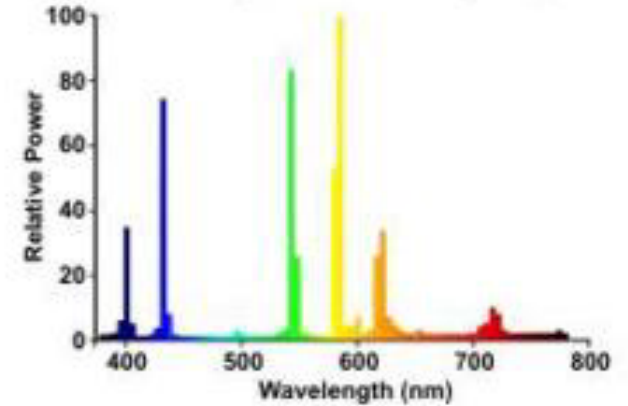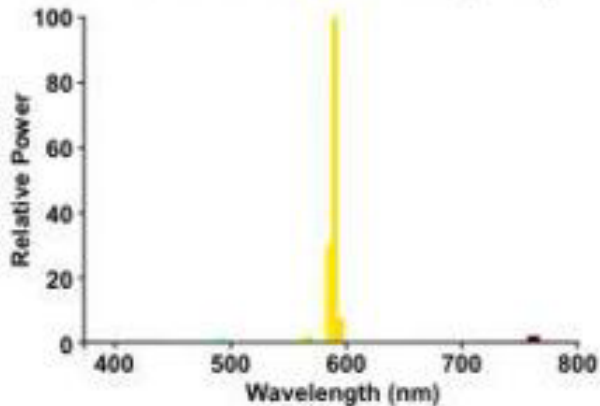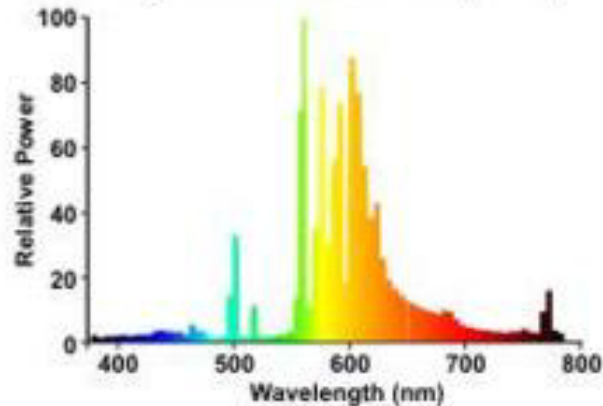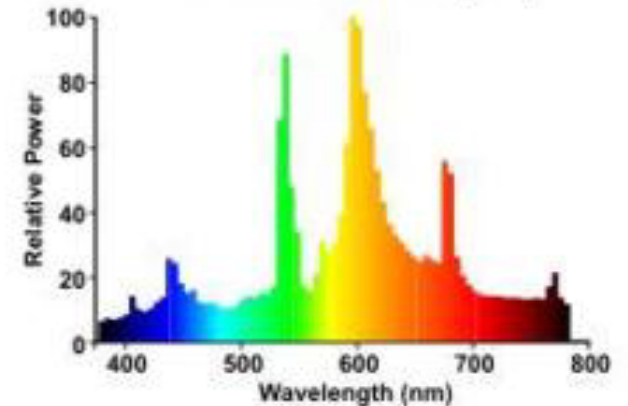
Separating the Light

Sourc

hν

Sample
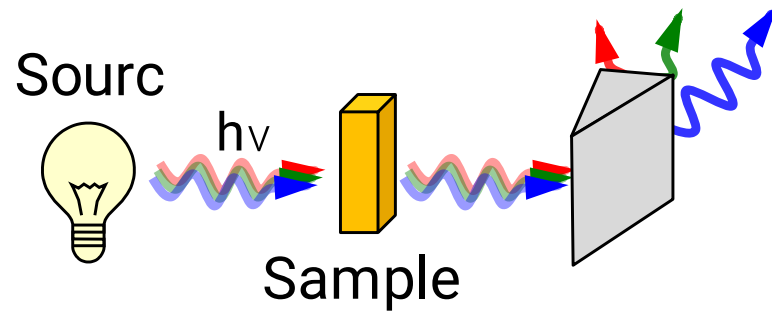
Prism

Grating
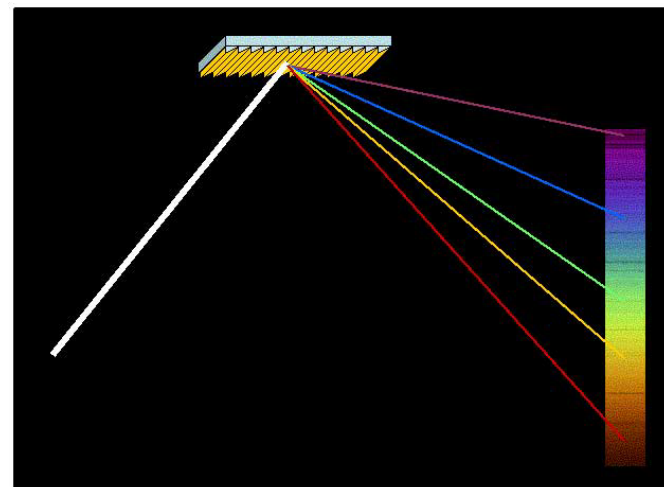
Monochromator: Prism

n_0 is constant
σ is constant
n_{prism} is λ dependent

Wavelength ⬆   Deviation ⬇
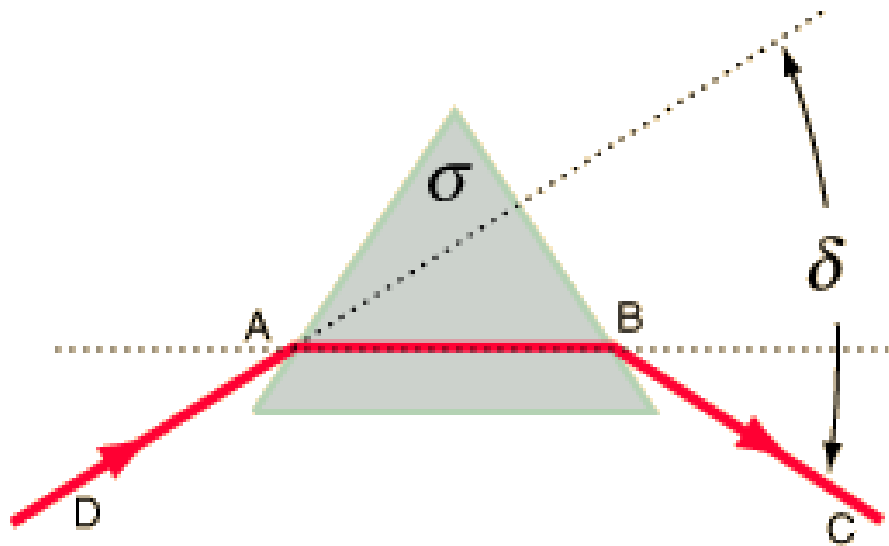
$$\frac{n_{prism}}{n_0} = \frac{\sin \frac{1}{2}(\sigma + \delta)}{\sin \frac{1}{2}\sigma}$$

n_0 = refractive index of air

n_{prism} = refractive index of prism

σ = prism apex angle

δ = deviation angle

Equilateral Dispersing Prism

Figure 1

White Light

$\Delta\theta_d$

θ_d

White Light

Monocromatic Light

Prism

Slit
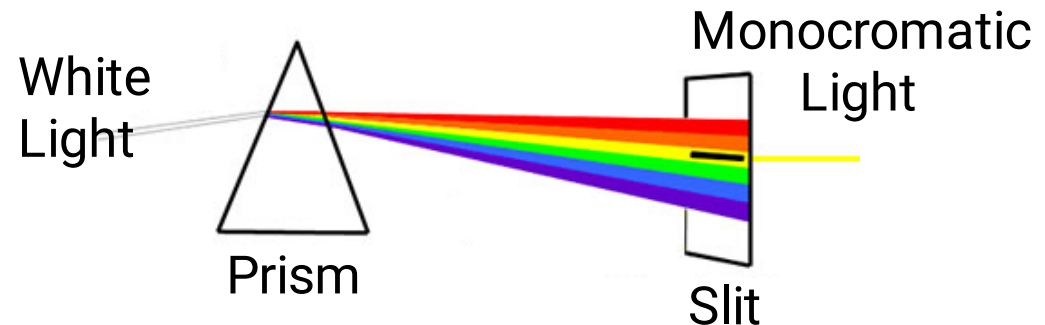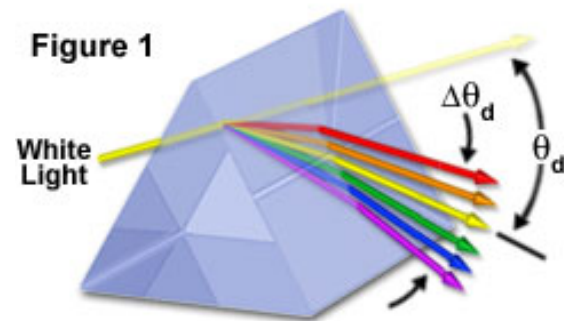
Monochromator: Prism

Monochromator: Grating
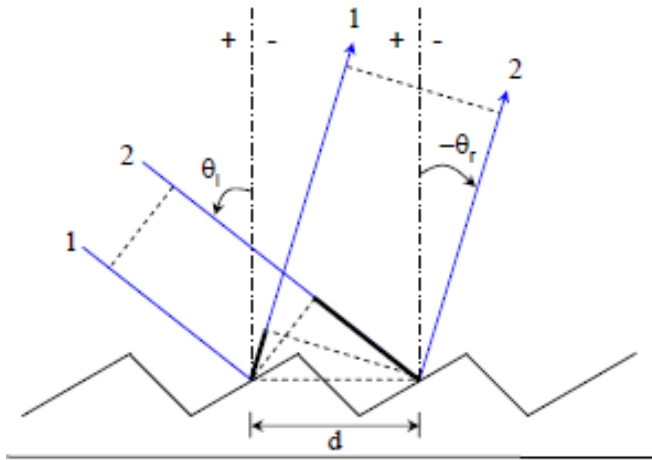
$$\lambda = 2d(\sin \theta_i + \sin \theta_r)$$

λ = wavelength

d = grating spacing

θ_i = incident angle

θ_r = diffracted angle

d is constant

θ_i is constant

θ_r is λ dependent

Wavelength ⬆   Diffraction ⬆

Grating

White Light

Monocromatic Light

Slit
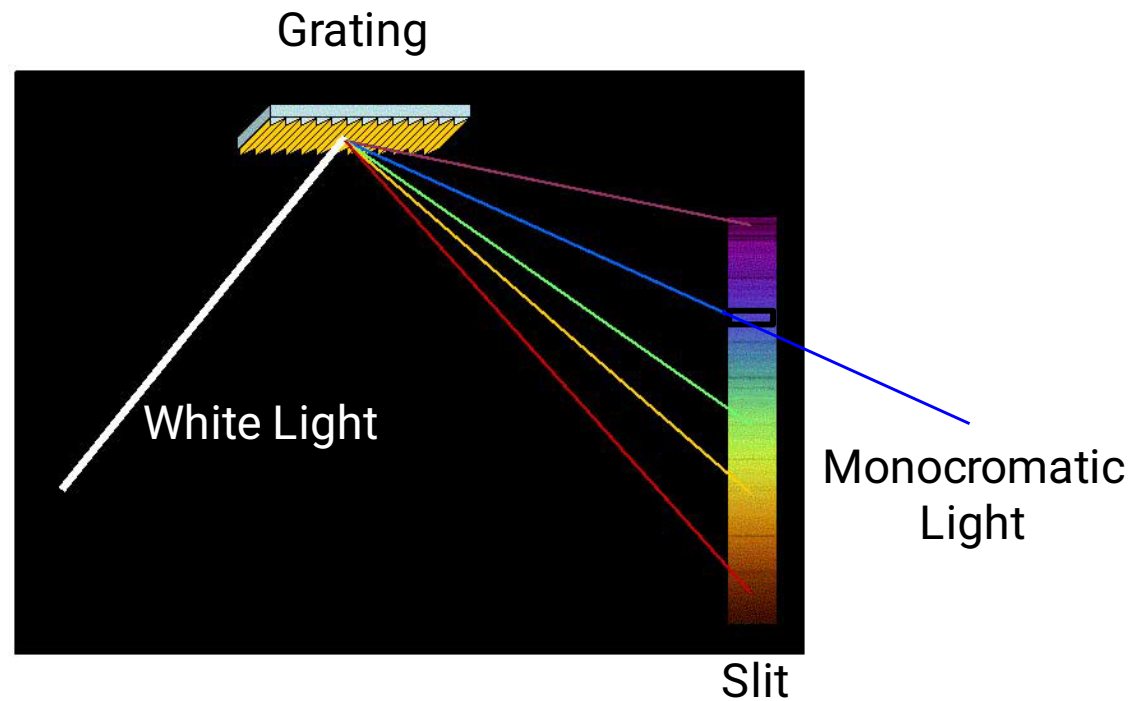
Monochromator: Grating

Detectors

hν

Detector

electrical signal
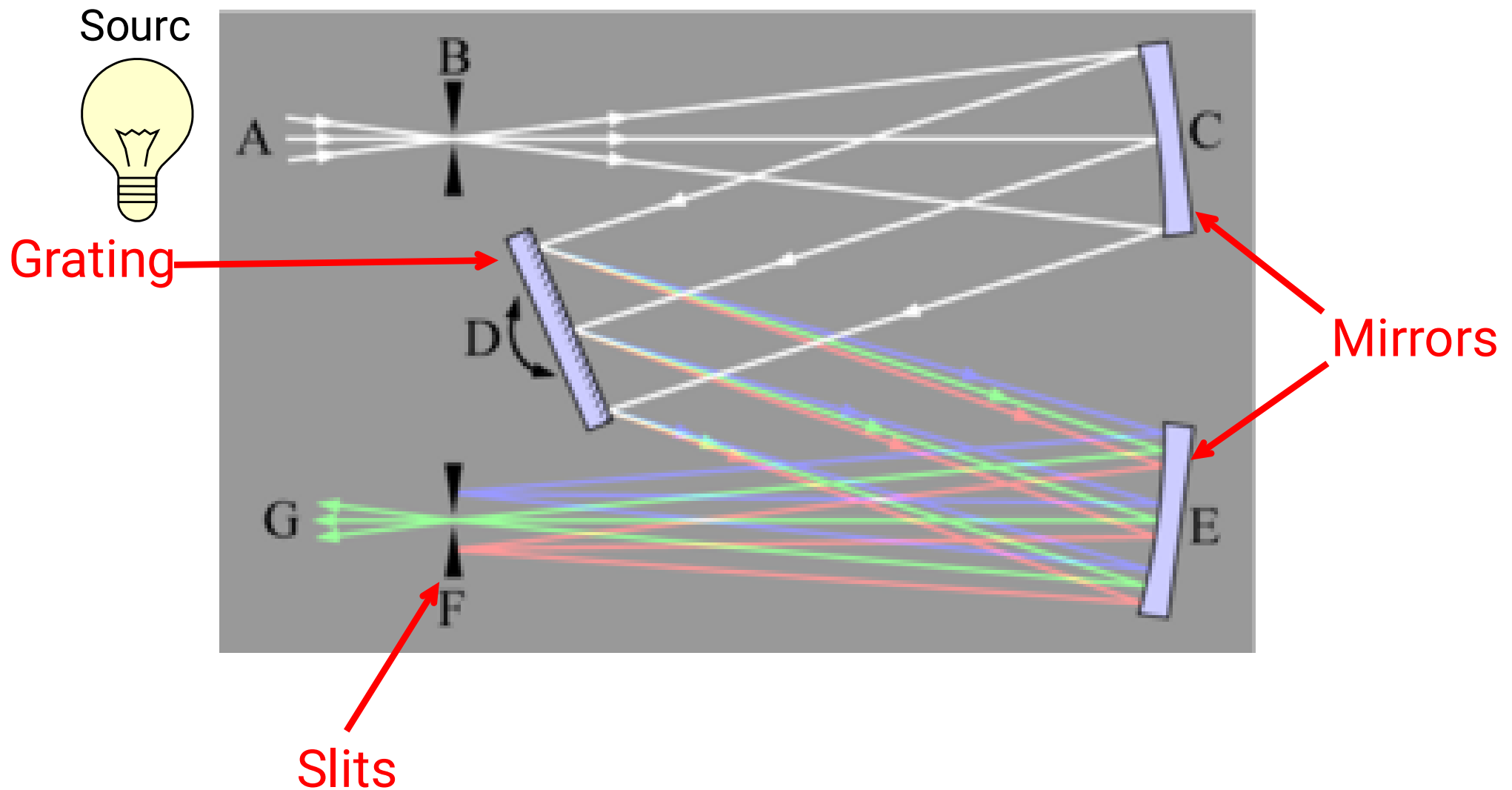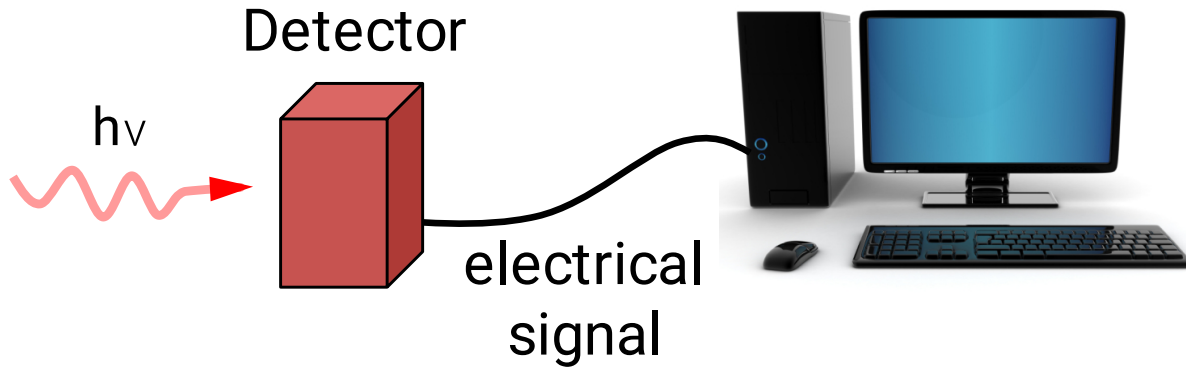
- high sensitivity
- high signal/noise
- constant response for λs
- fast response time

Single λ detection

Diode

PMT

Full spectra detection

CCD
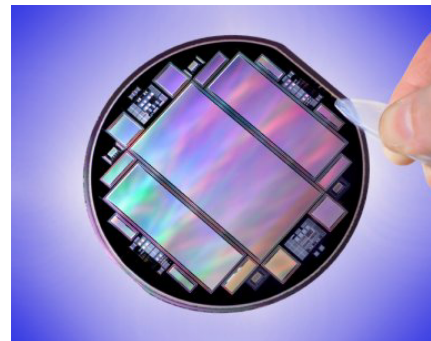
Diode Array

Detectors: Diode

(a) Forward bias (current flows)

(+)   p-Si   n-Si   (−)

(b) Reverse bias (no current flows)

(−)   p-Si   Depletion region (~10^{-6} m)   n-Si   (+)

⊖ Negative electron
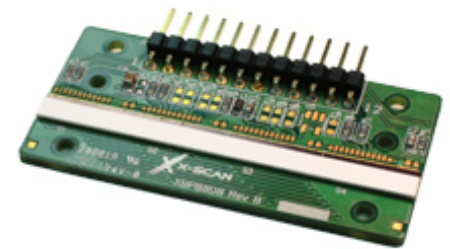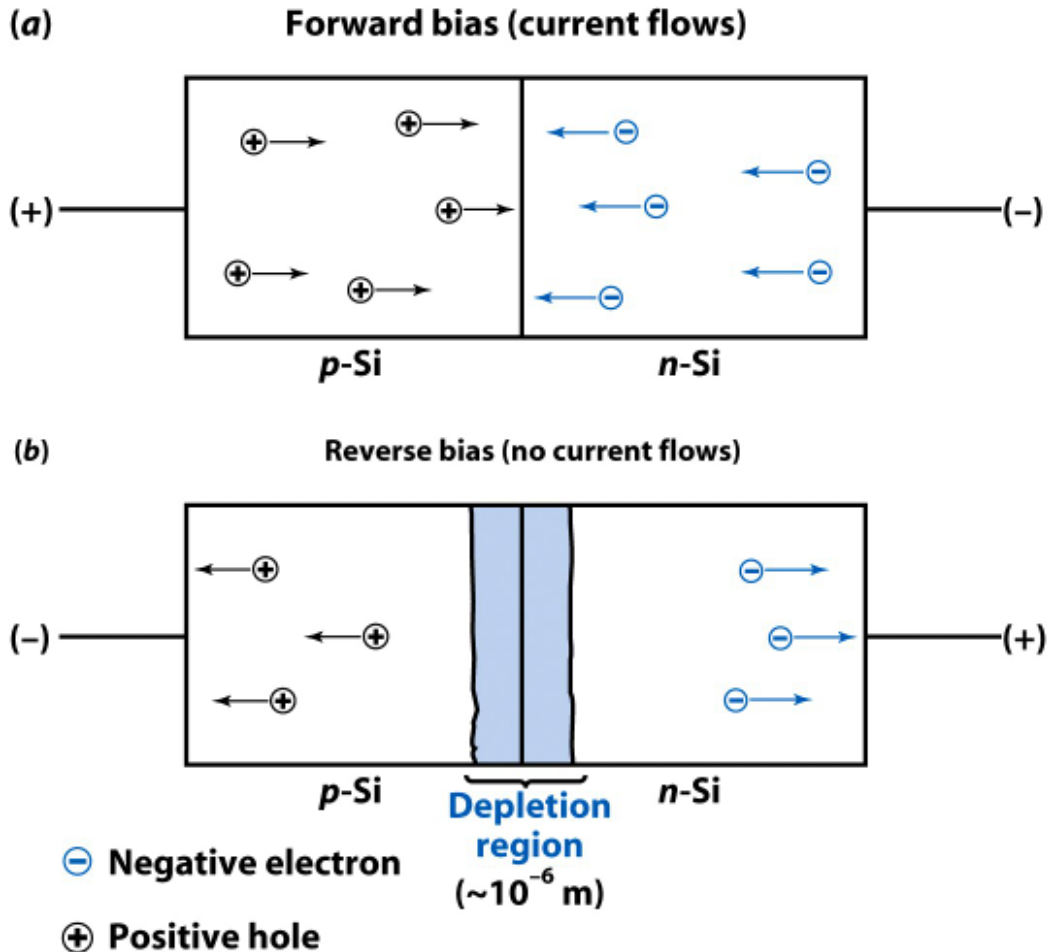
⊕ Positive hole

Figure 15-27
Quantitative Chemical Analysis, Seventh Edition
© 2007 W. H. Freeman and Company

n-type (extra electrons)- P or As doped

p-type (extra holes)- Al or B doped

Forward Bias:
Apply a positive potential
holes + e⁻ = exciton = light
Light Emitting Diode

Zero Bias:
Apply 0 potential
exciton = holes + e⁻ = current
silicon solar cell

Negative Bias:
Apply a negative potential
exciton = holes + e⁻ = more current
photodetector

Detectors: Diode

Pros:

Long Lifetime

Small/Compact

Inexpensive

Linear response

190-1000 nm

Cons:
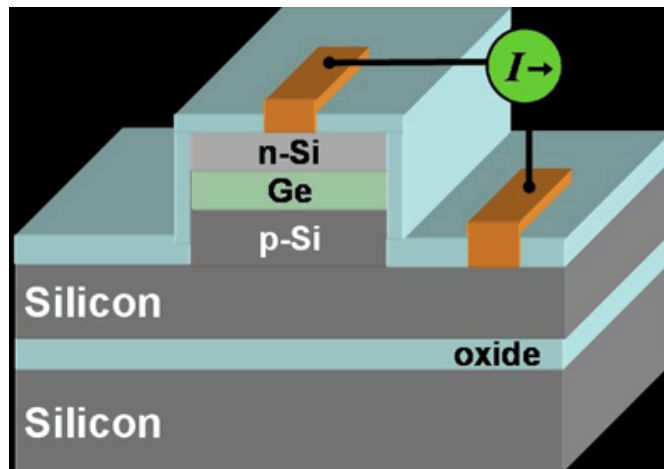
No wavelength discrimination

Minimal internal gain

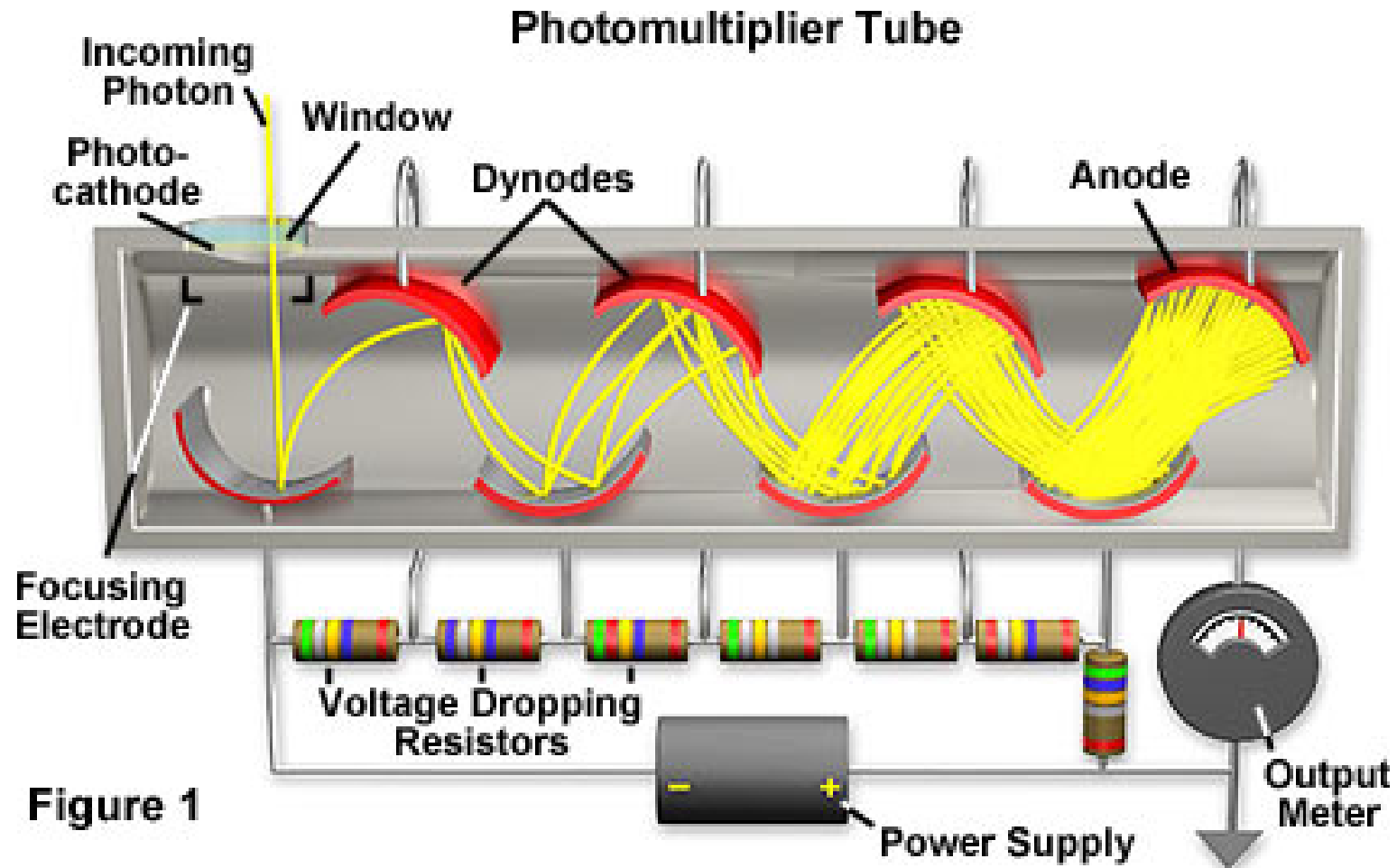Much lower sensitivity

Small active area
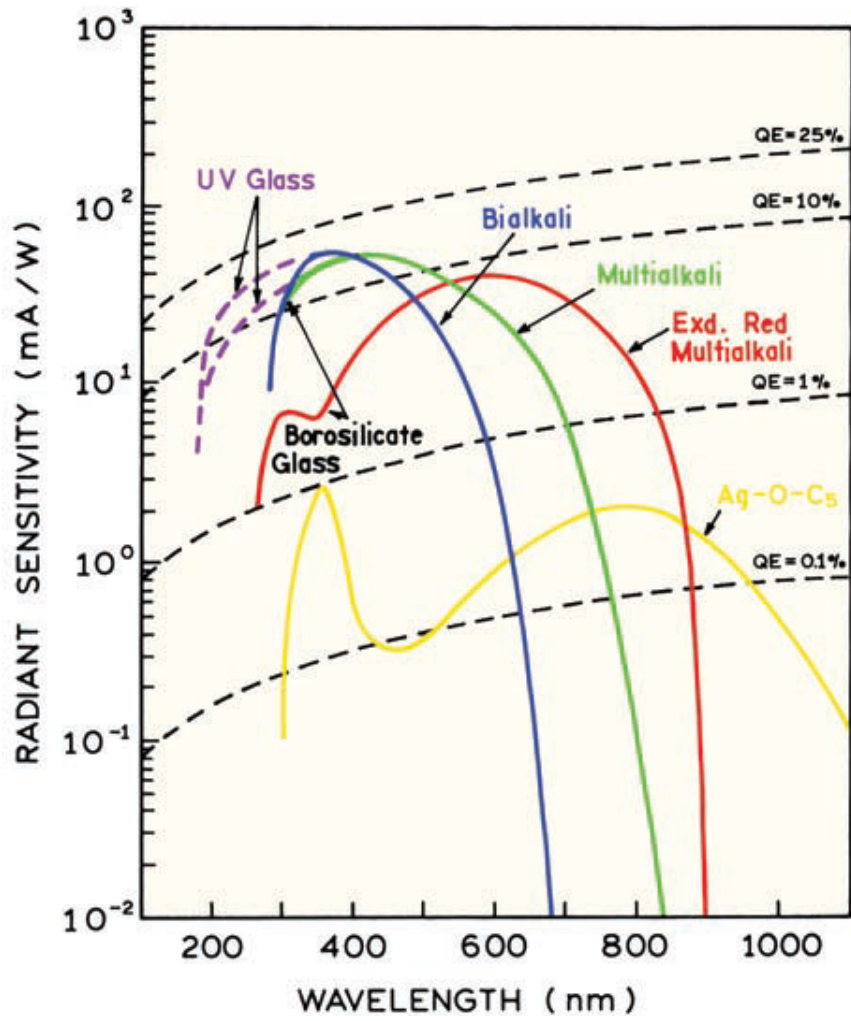
Slow (>50 ns)

Low dynamic range

0.025 mm wide
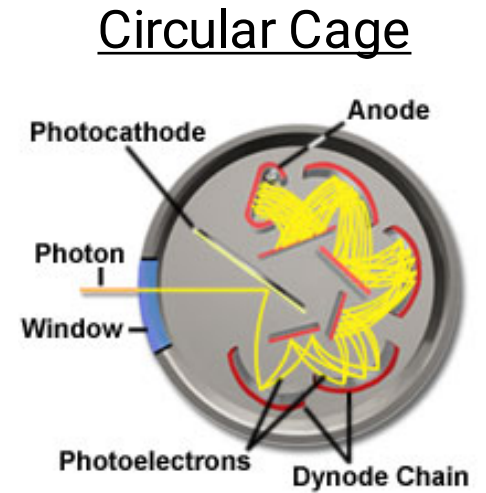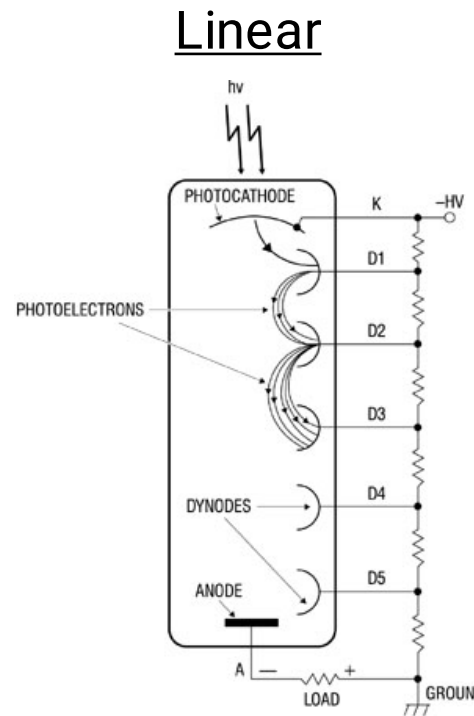
Detectors: PMT

Photomultiplier Tube

Figure 1

- Cathode: 1 photon = 5-20 electrons
- More positive potential with each dynode
- Operated at -1000 to -2000 V

Detectors: PMT

Photocathodes

Architectures

Linear

Circular Cage

Detectors: PMT

Pros:

Extremely sensitive

UV-Vis-nIR

100,000,000x current amplifier

(single photons)

Low Noise

Compact

Inexpensive ($175-500)

Cons:

No wavelength discrimination
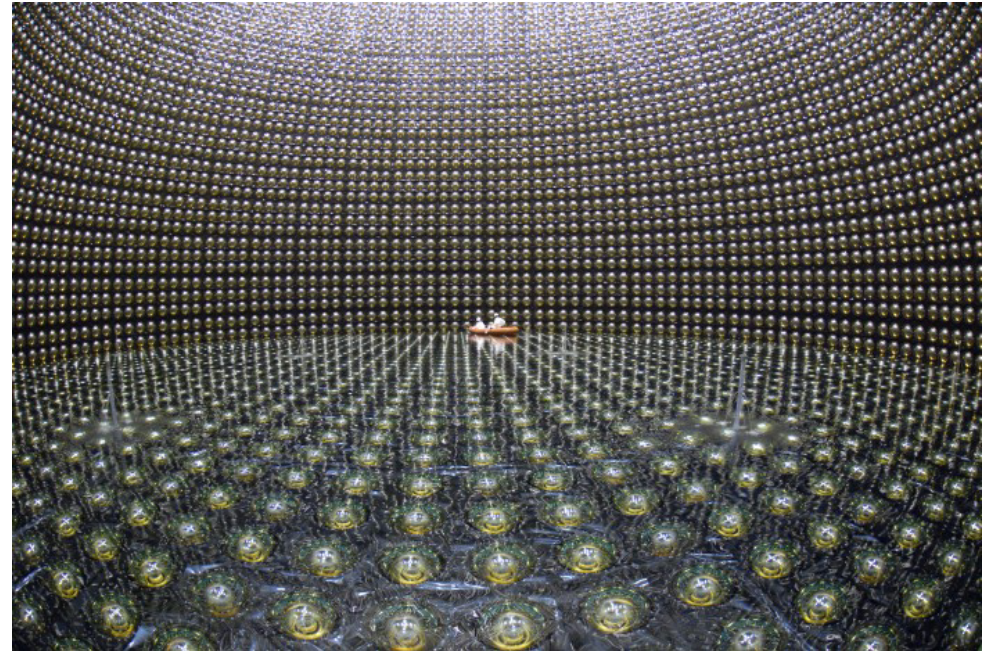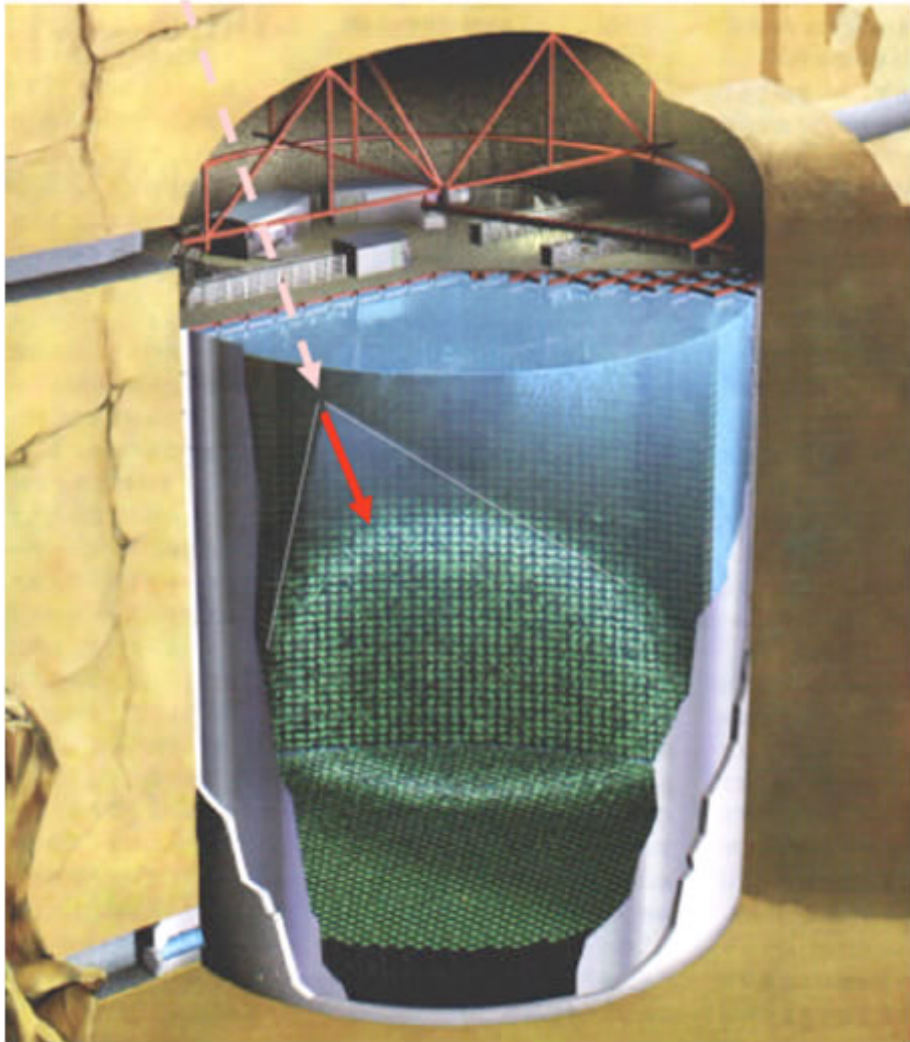
Wavelength dependent τ
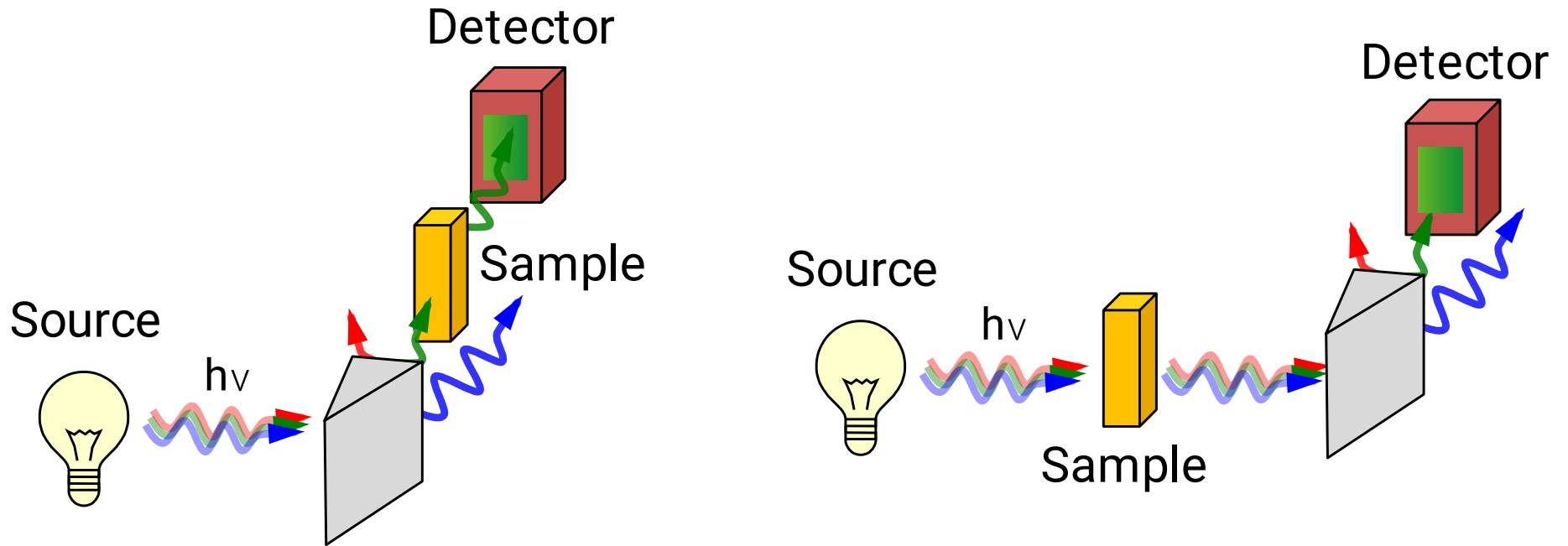
Saturation

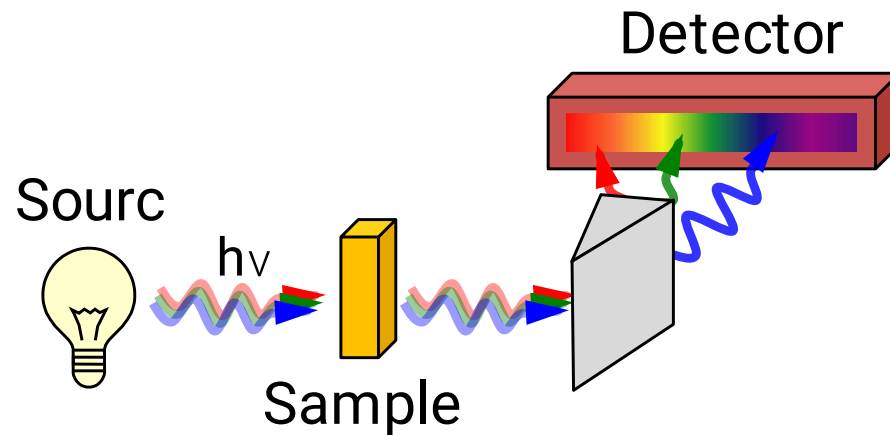Magnetic Field Effects

Detectors: PMT

Super-Kamiokande Experiment

- 1 km underground

- h = 40 m, d = 40 m

- 50,000 tons of water

- 11,000 PMTs

- neutrino + water = Cherenkov Radiation

Instrumentation

Single λ detection
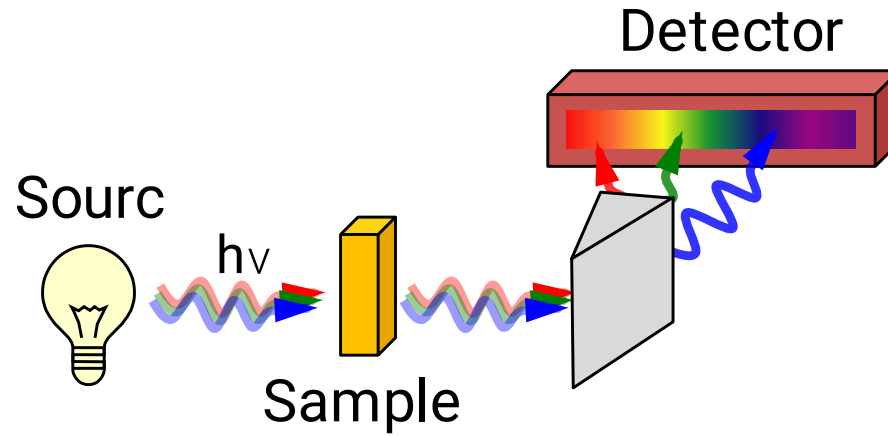
Detector
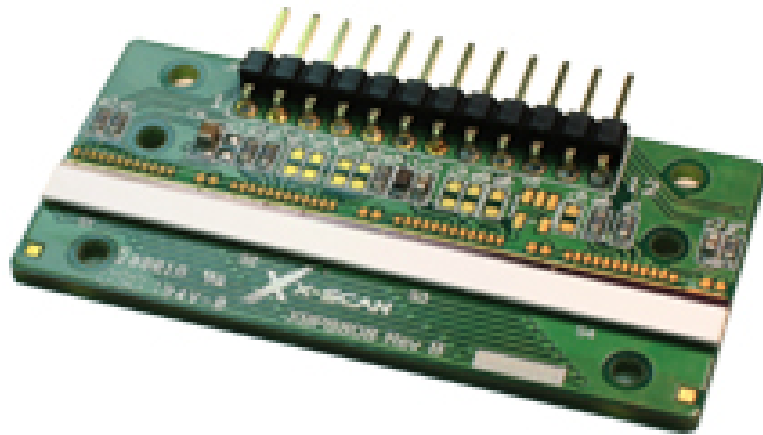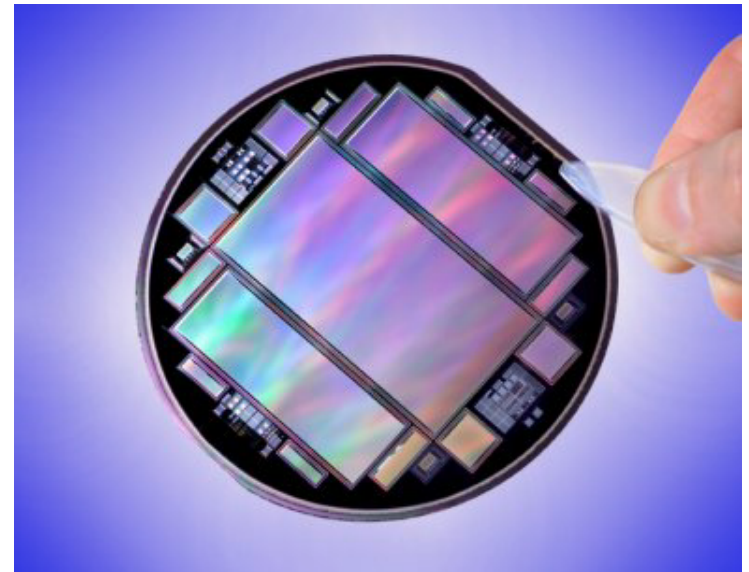
Sample

Source

hν

Detector

Source

hν

Sample

Full spectra detection

Detector

Sourc

hν

Sample

Full Spectrum Detection

Detector

Sourc

hν
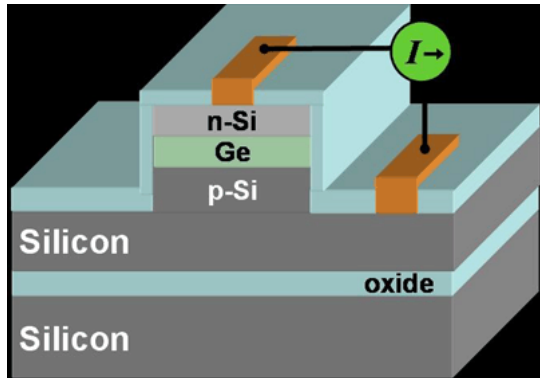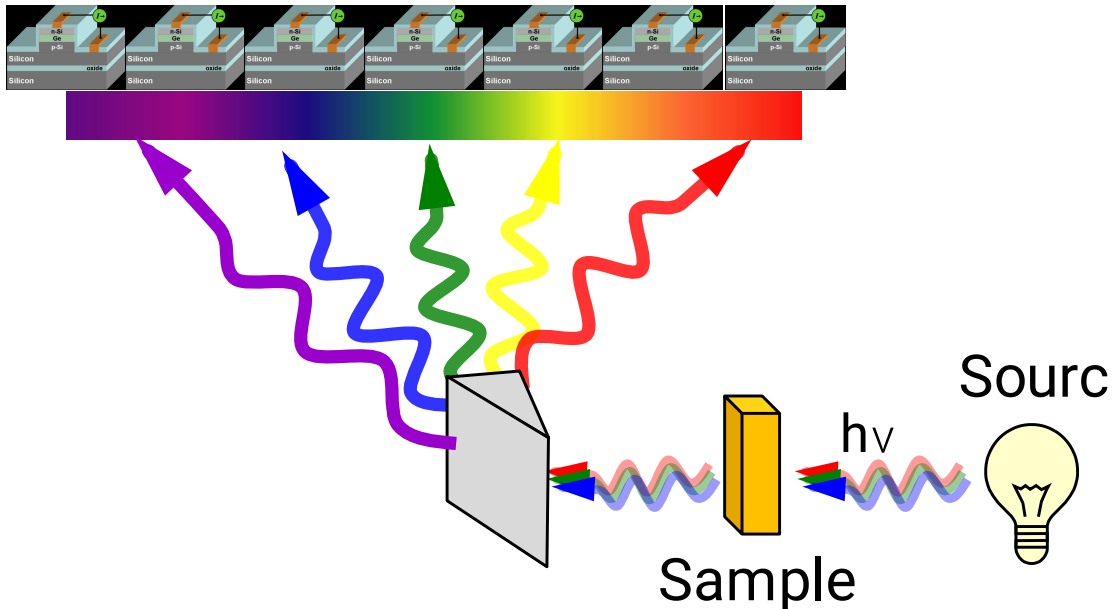
Sample

Diode Array

CCD

Detectors: Diode Array

Diode

Diode Array

Pros:

   Quick measurement

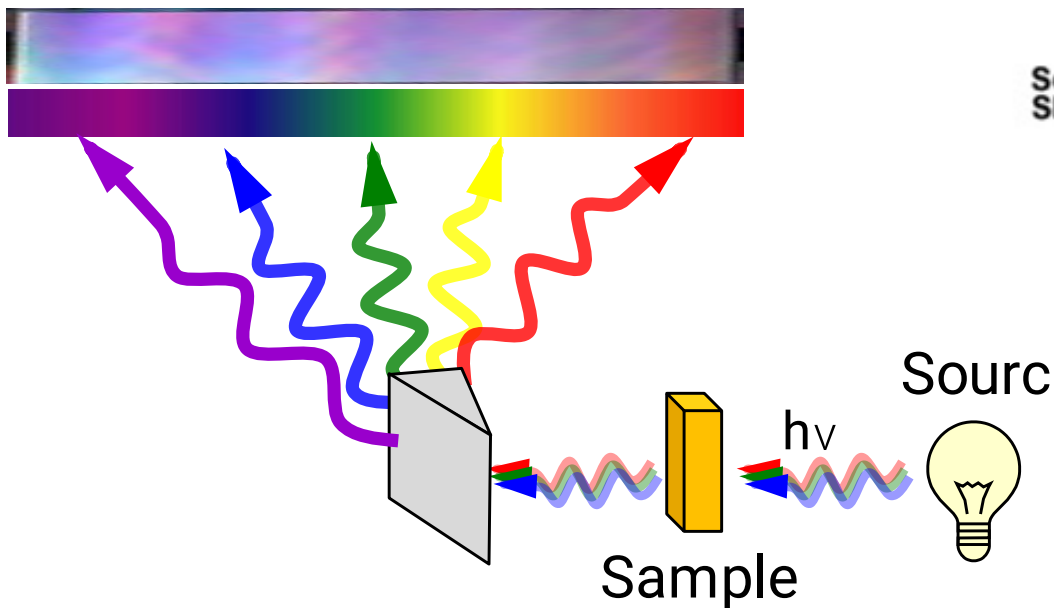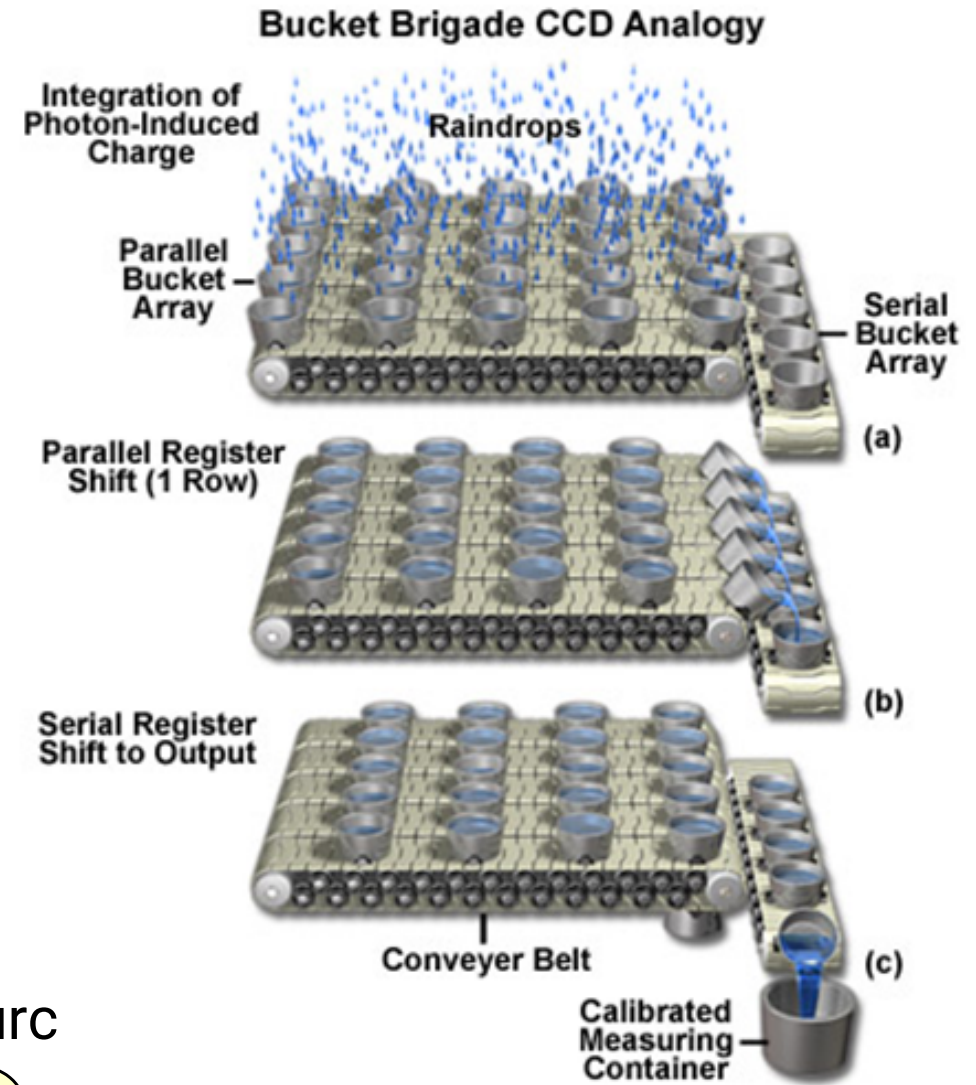   Full spectra in "real time"

   Inexpensive

   Less moving parts
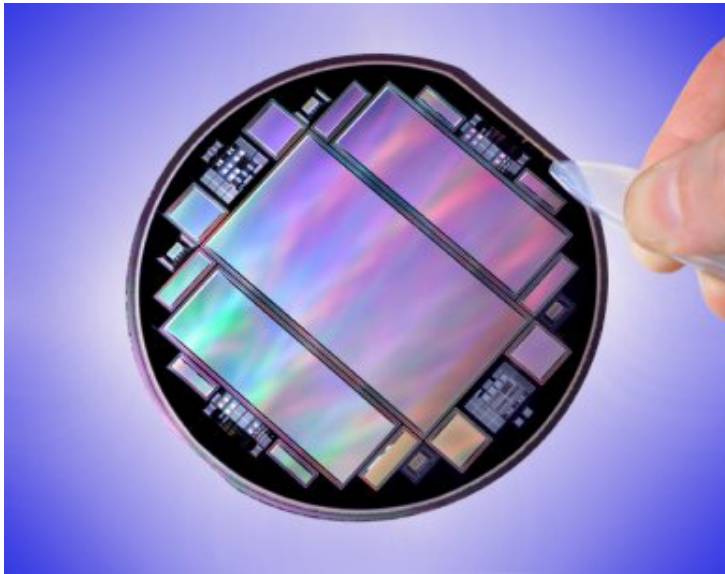
Cons:

   Lower resolution (~1 nm)

   Slow (>50 ns)

   More expensive than a single λ

Detectors: Charge-Coupled Device

Bucket Brigade CCD Analogy

Integration of Photon-Induced Charge — Raindrops

Parallel Bucket Array — Serial Bucket Array (a)

Parallel Register Shift (1 Row) (b)

Serial Register Shift to Output

Conveyer Belt (c)

Calibrated Measuring Container

Sourc

hν

Sample

Detectors: CCD

Anatomy of a Charge Coupled Device (CCD)

Drain Voltage Control

Pixel Reset Gate

Incoming Photons

CCD Gates

Transfer Gate

Buried Channel

n+

n+

Buried Channel

n-

Lateral Overflow Drain

Photodiode Integrated Charge Potential Well

Potential Barrier

p-Silicon

CCD Charge Transfer Potential Well
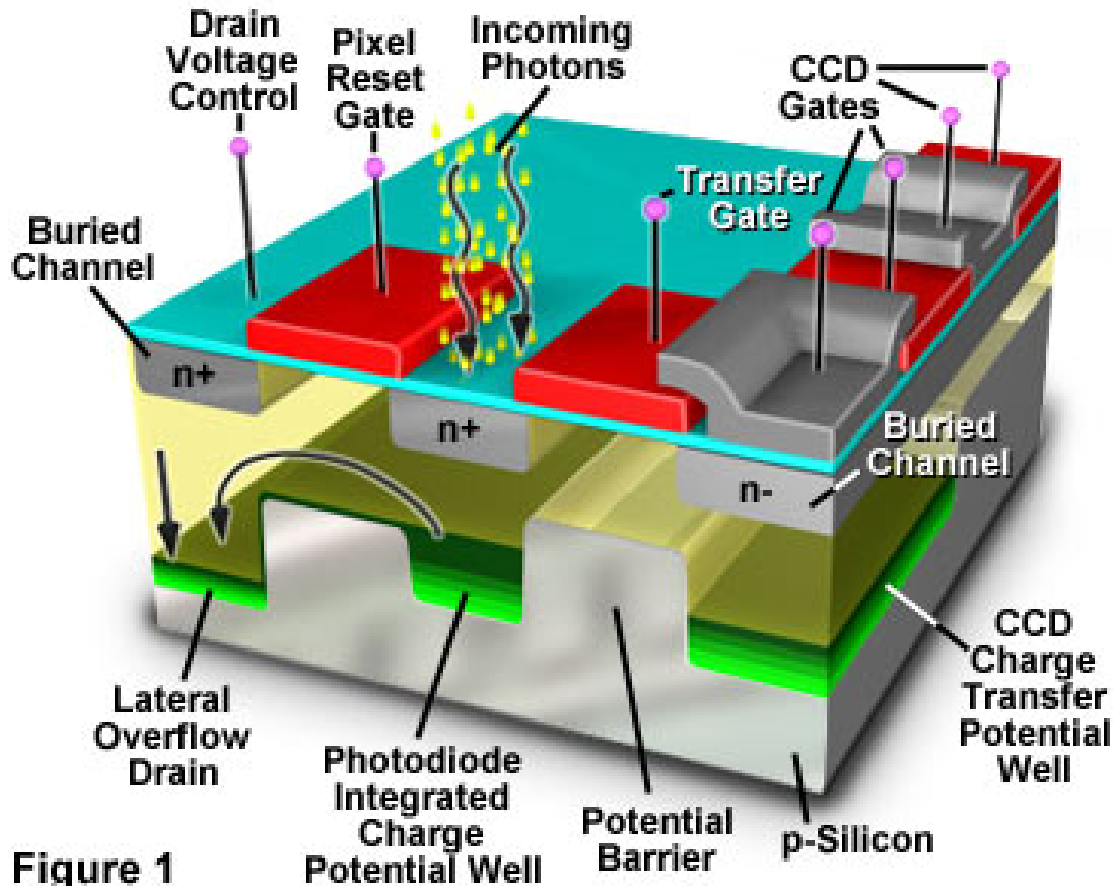
Figure 1

Pros:

Fast

Efficient (~80 % quantum yield)

Full visible spectrum

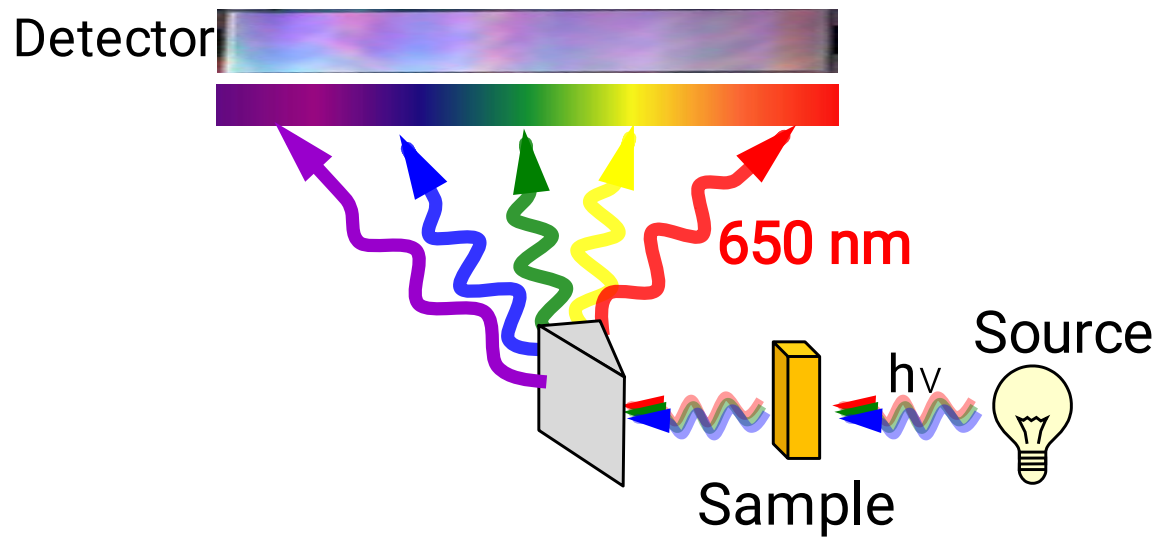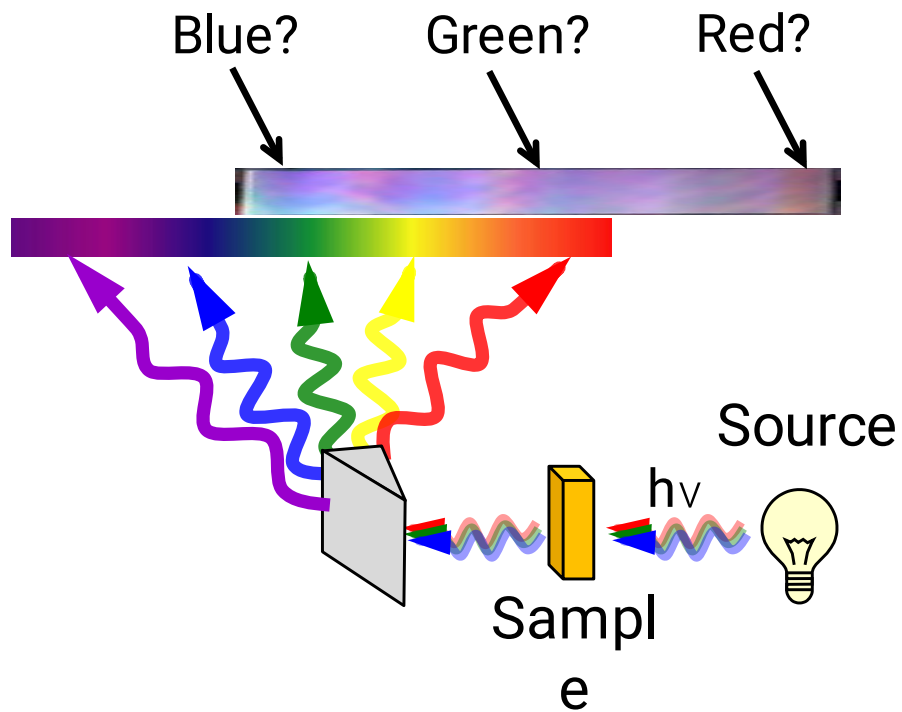Wins you the 2006 Nobel Prize   (Smith and Boyle)

Cons:

Lower dynamic range

Fast (<50 ns)

Gaps between pixels

Expensive (~$10,000-20,000)

Area Detector Calibration

Area Detector Calibration
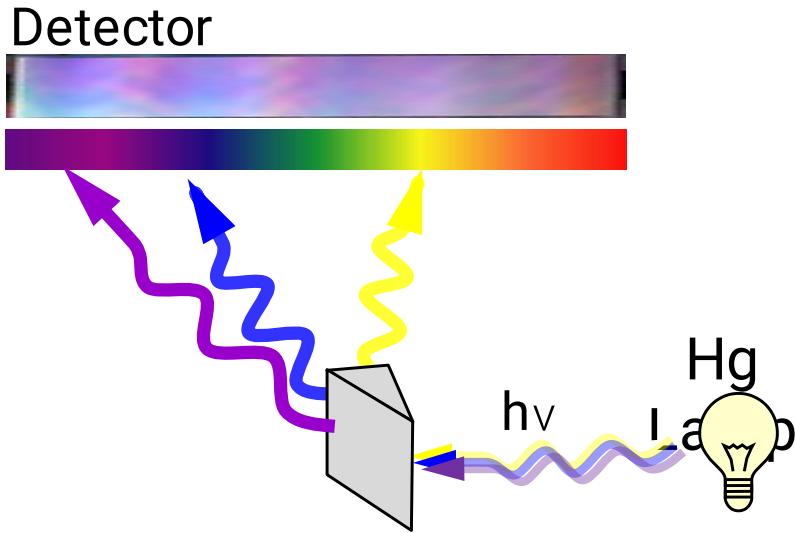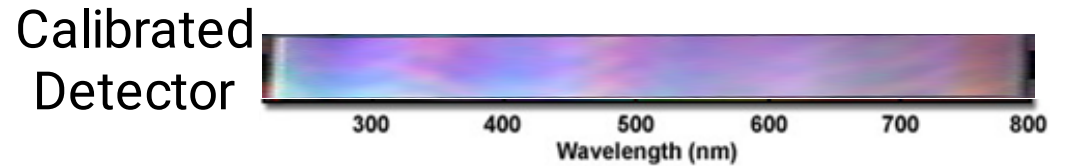
Detector
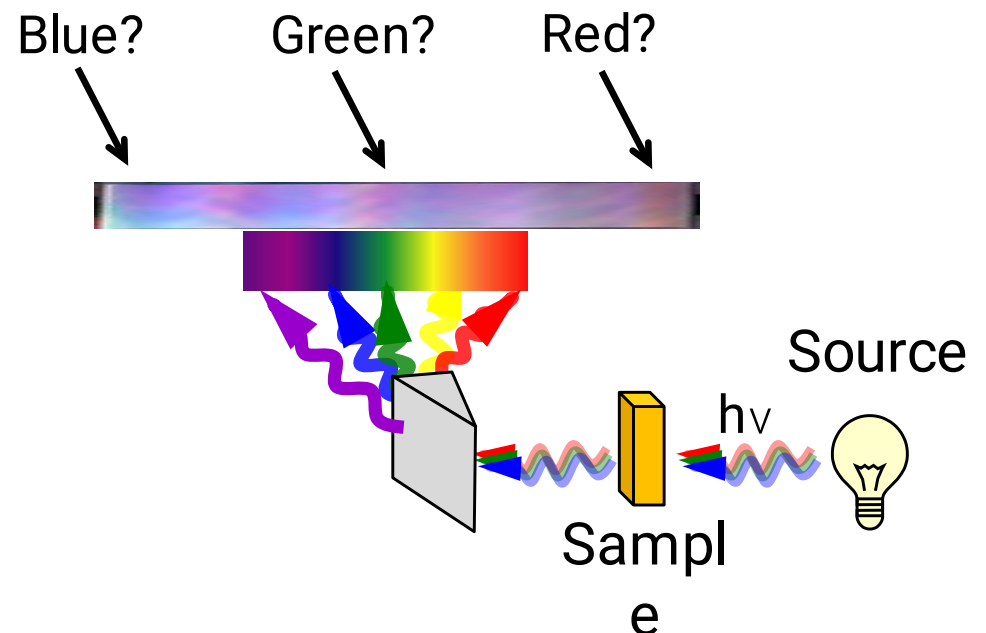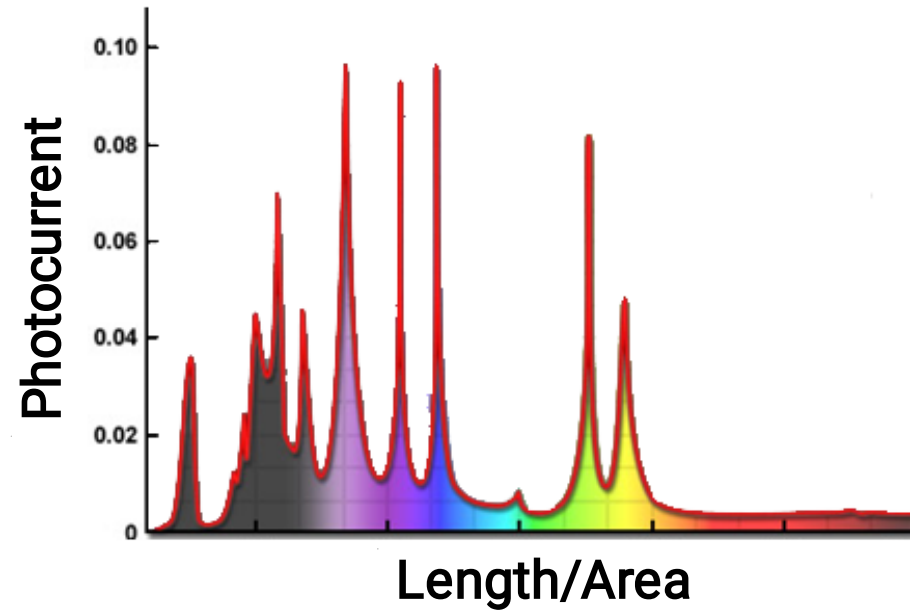
Hg
Lamp

$h\nu$

Hg Lamp Spectrum

Spectral Intensity [W/sr/nm/1000cd]

254
297-302
312-313
334
365
405
436
546
579

HBO 100
Mercury Arc Lamp
Spectral
Distribution

Figure 1

Wavelength (nm)

Photocurrent

Length/Area

Detector

365 nm   436 nm   546 nm

Calibrated Detector

Wavelength (nm)

Instrumentation

Single λ detection

Detector

Sample

Source

hν

Source
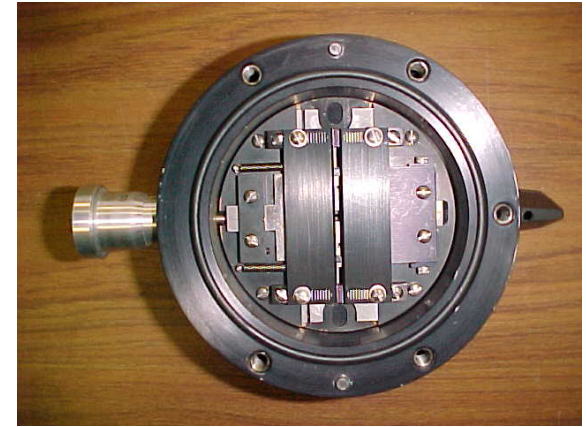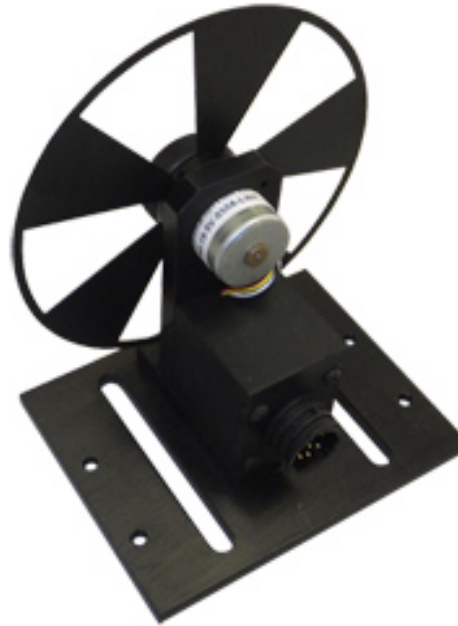
hν

Sample

Detector

Full spectra detection

Detector
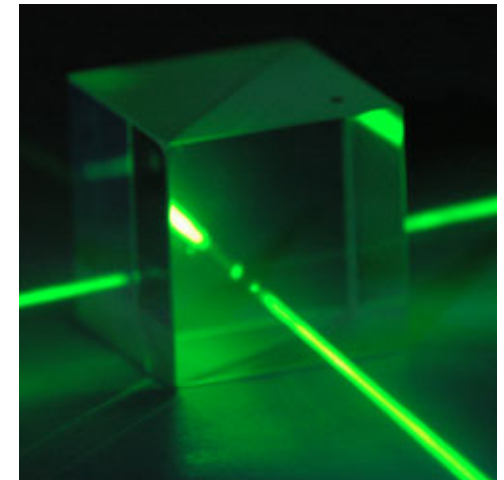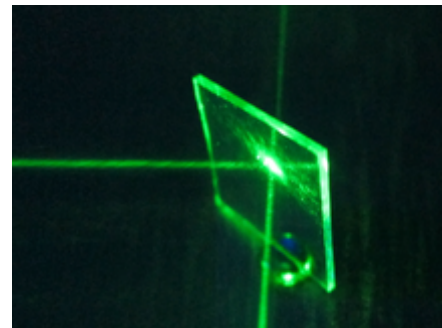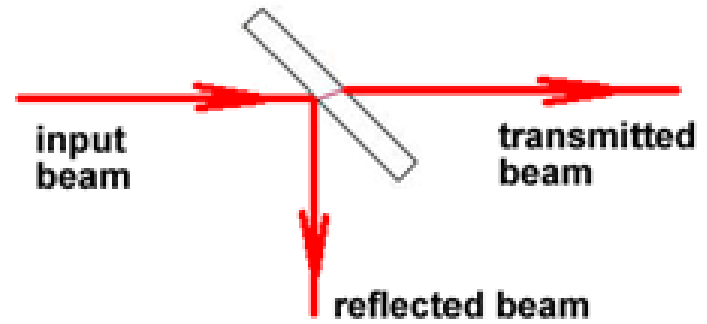
Sourc

hν

Sample
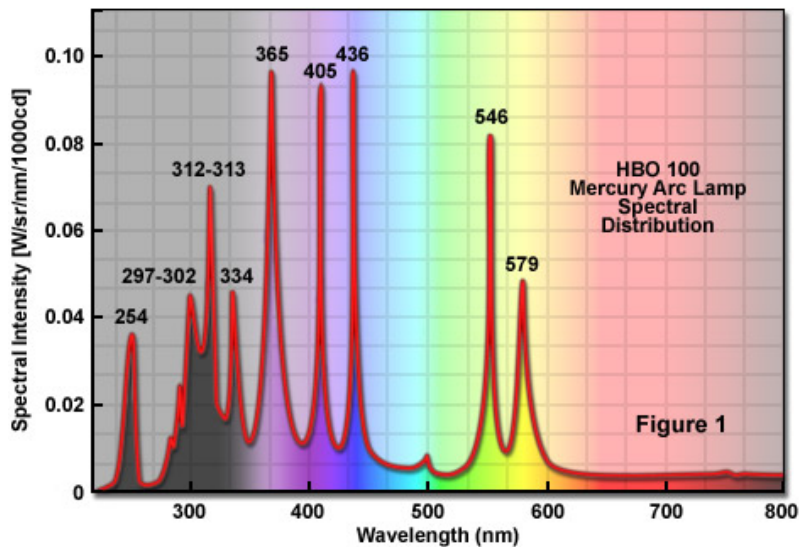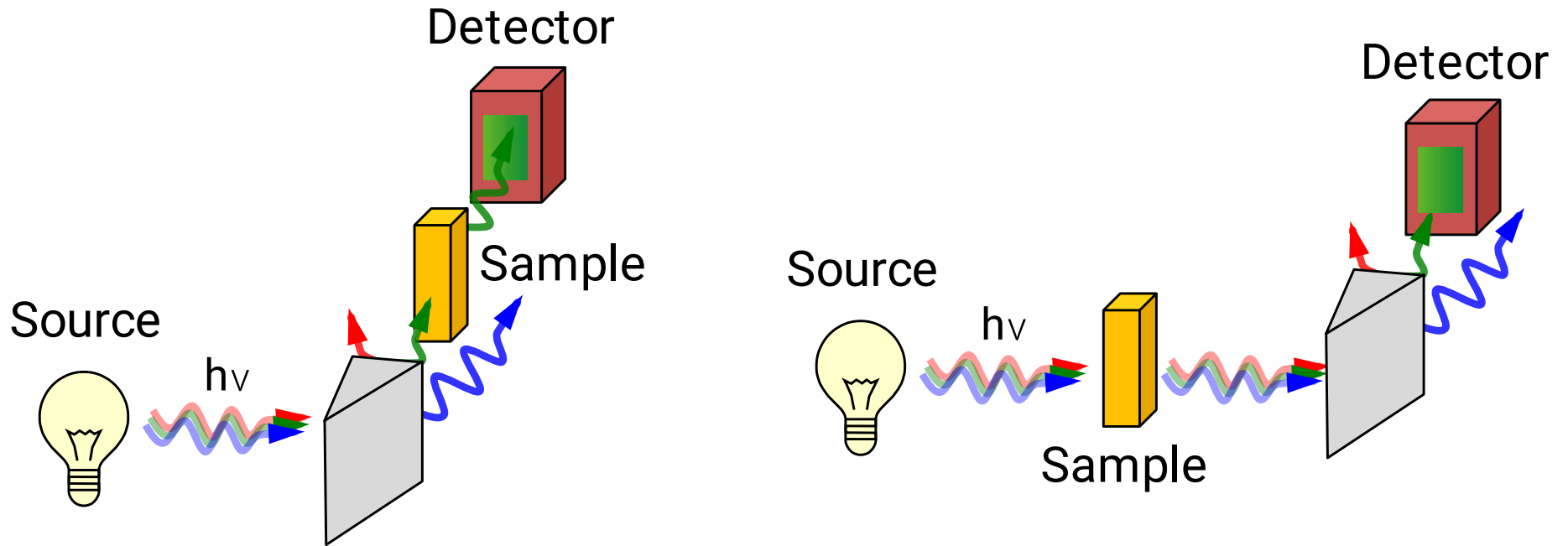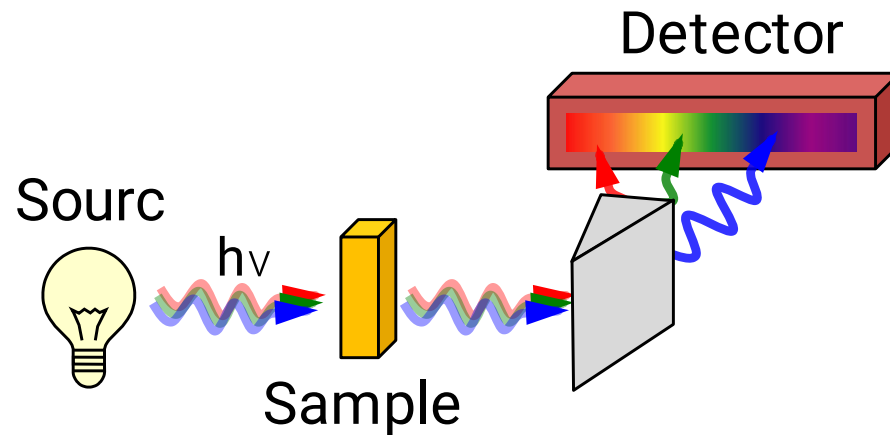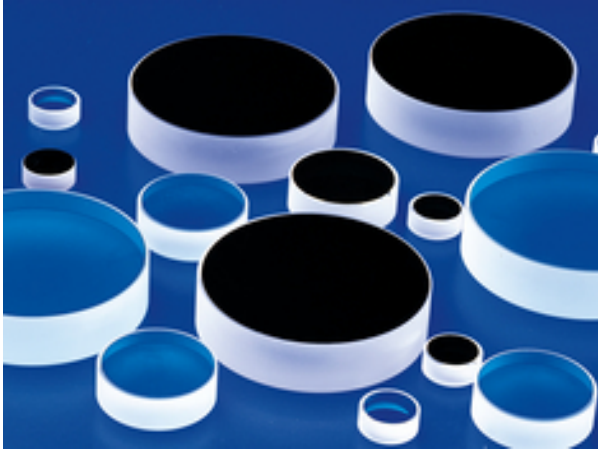
Other Components
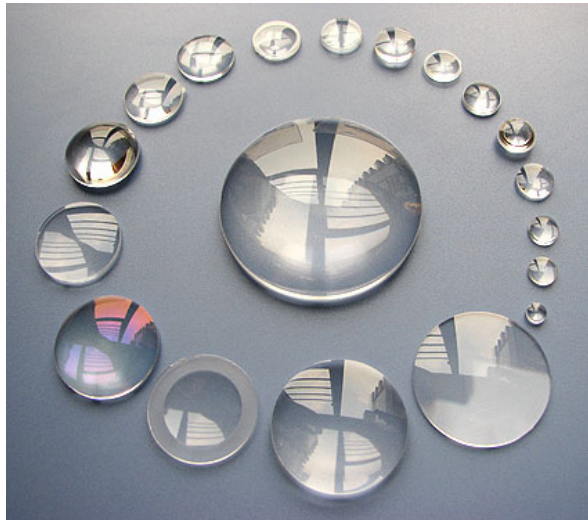
Mirrors

Chopper

Entrance/Exit Slits

Lenses

Shutter

Other Components

Polarizer
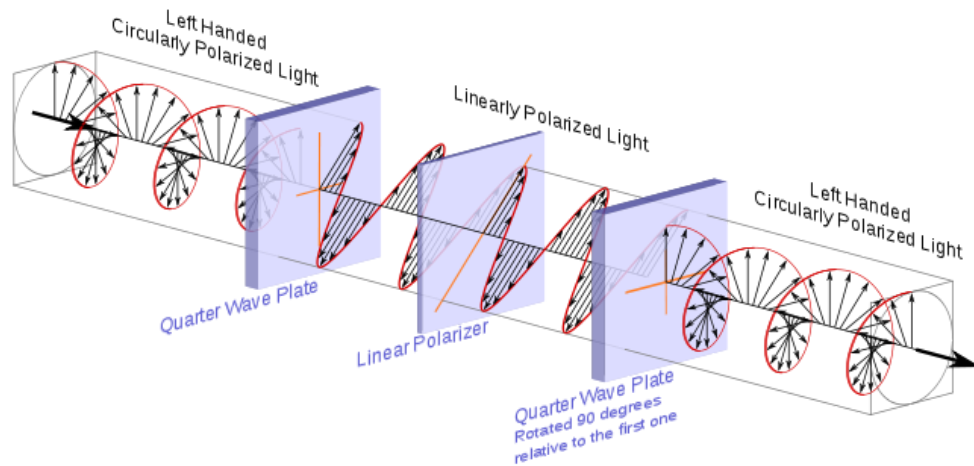
Left Handed Circularly Polarized Light

Linearly Polarized Light

Left Handed Circularly Polarized Light

Quarter Wave Plate

Linear Polarizer

Quarter Wave Plate
Rotated 90 degrees
relative to the first one

Beam Splitter

input beam

transmitted beam

reflected beam

Side Note: Pub Highlight

Side Note: Pub Highlight

(a)

Setting Devices

1: CW 635-nm laser

2: 3mm-thick pork
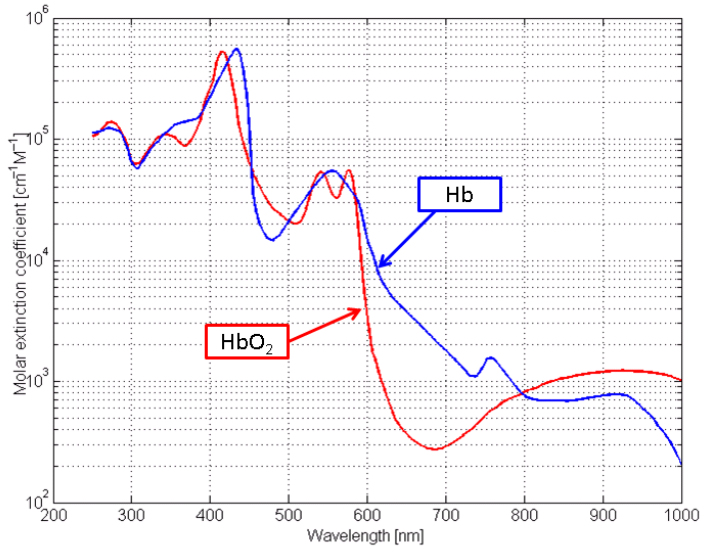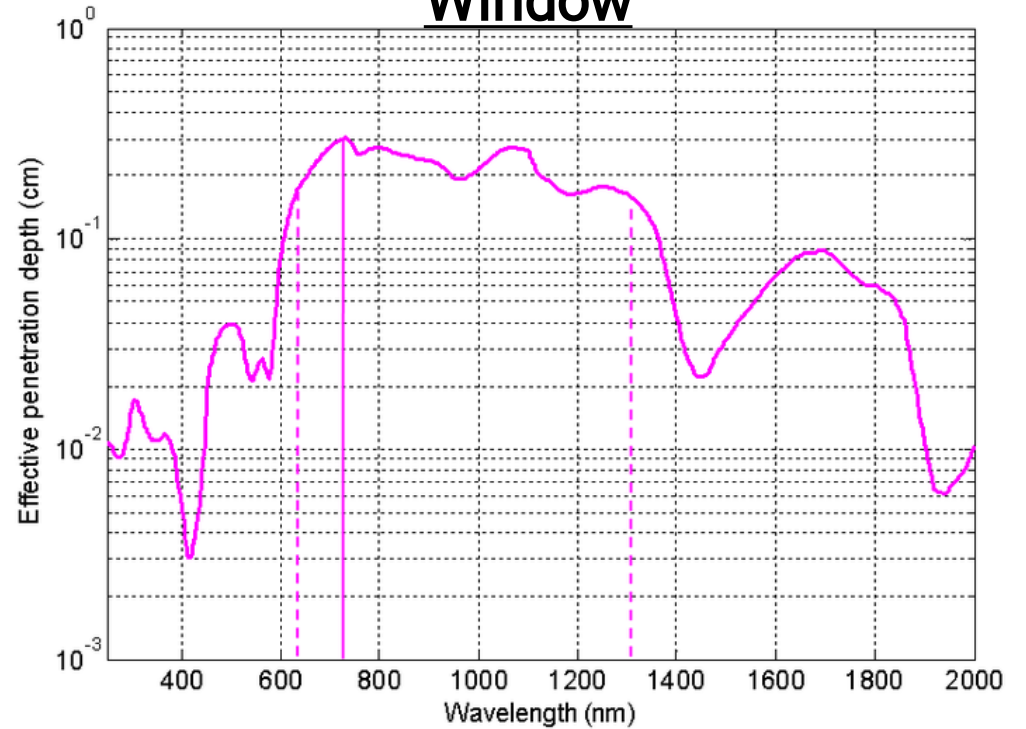
3: Assembly film

(b)

635 nm
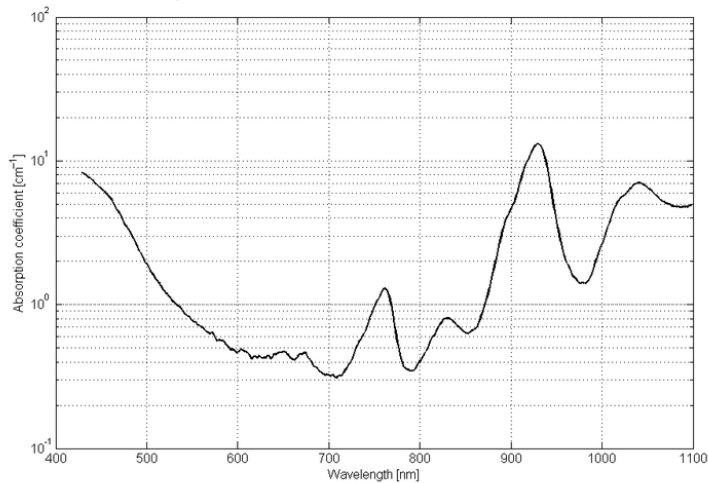1 min
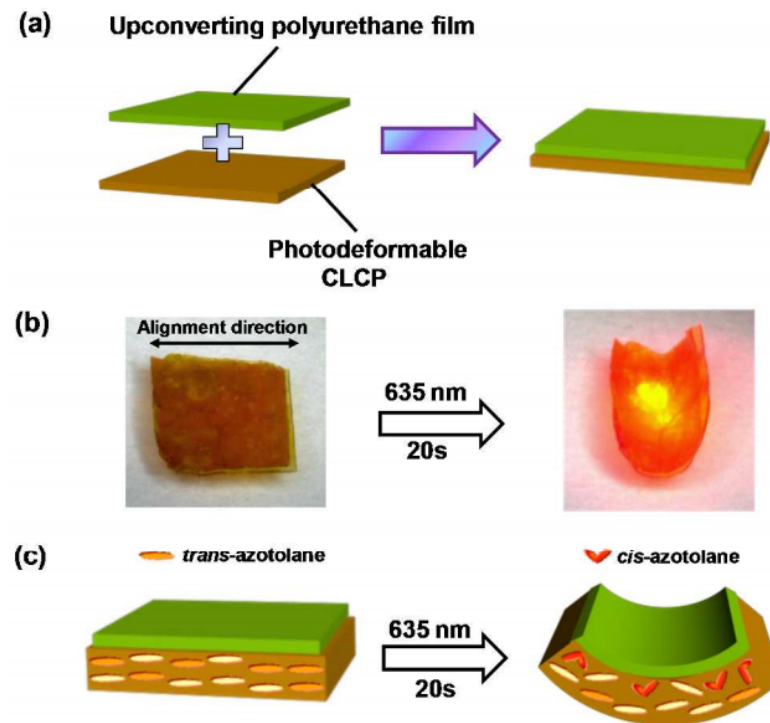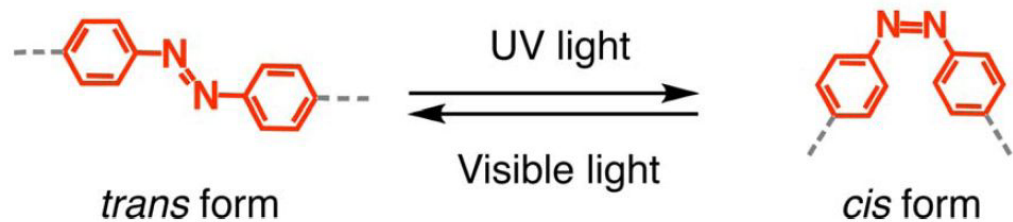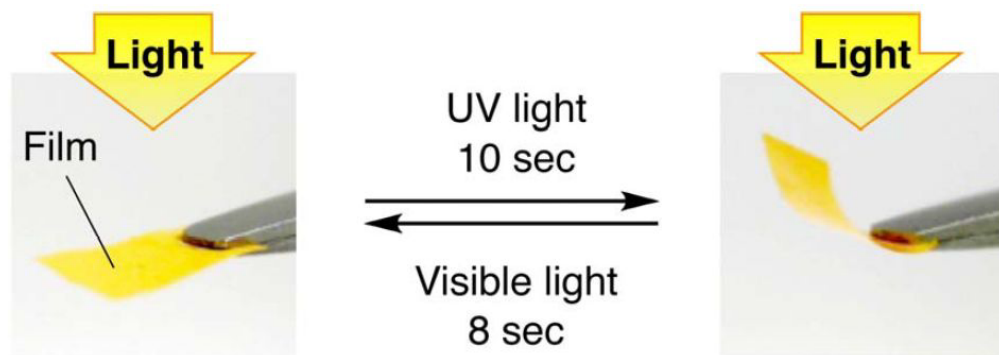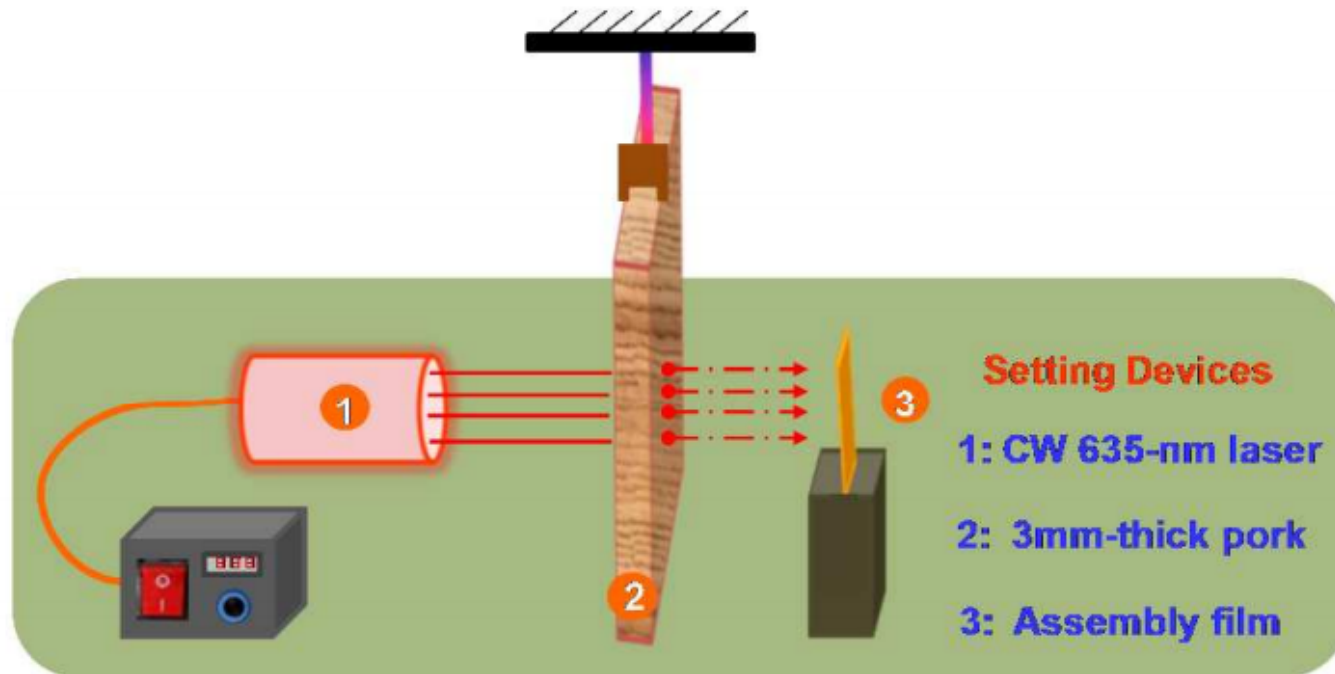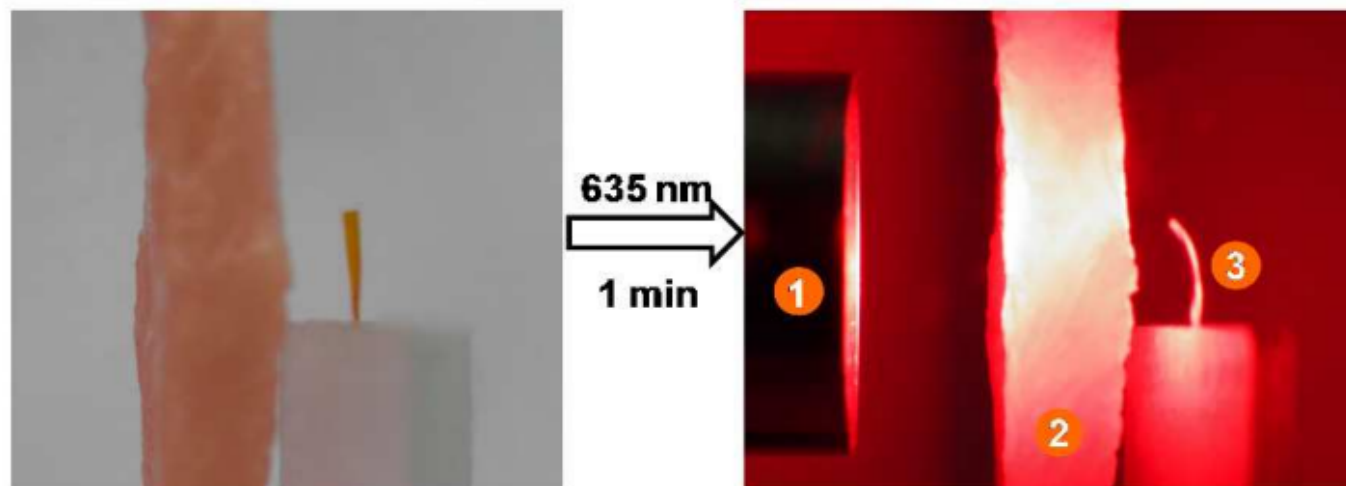
Side Note: Pub Highlight

Hemoglobin Absorption

Biological Tissue Window
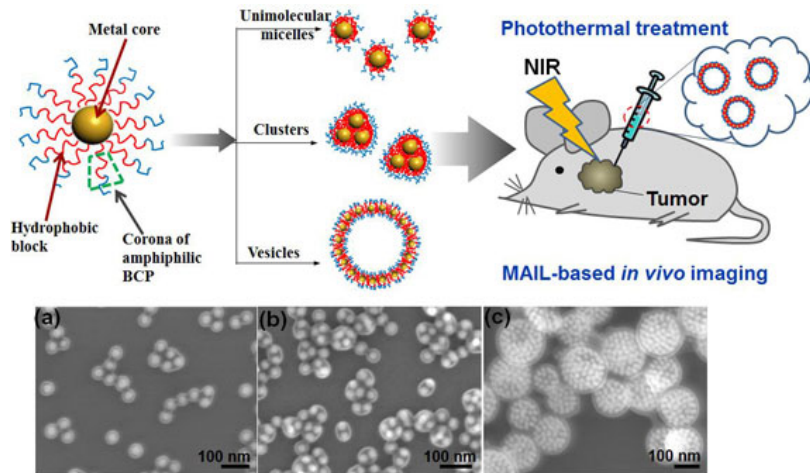

Pig Lard Absorption

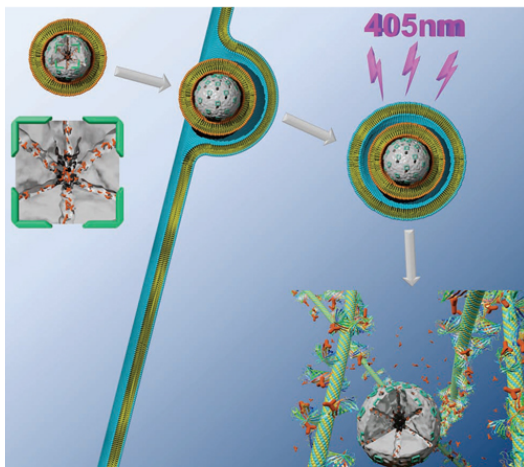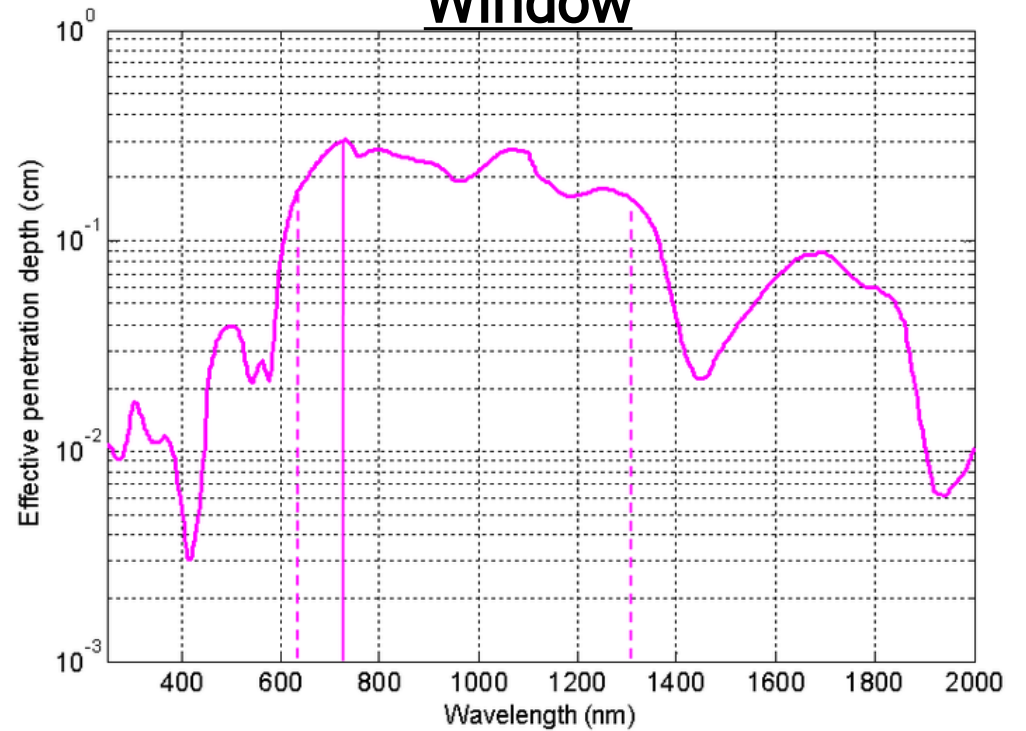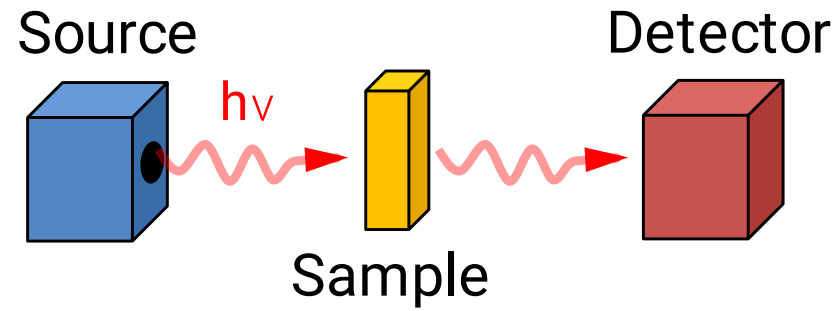# Side Note: Pub Highlight
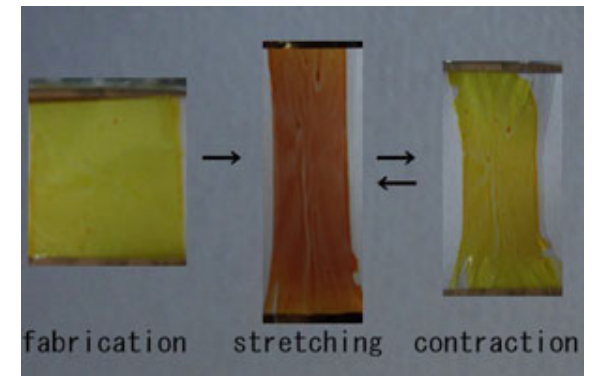
Plasmonic Heating

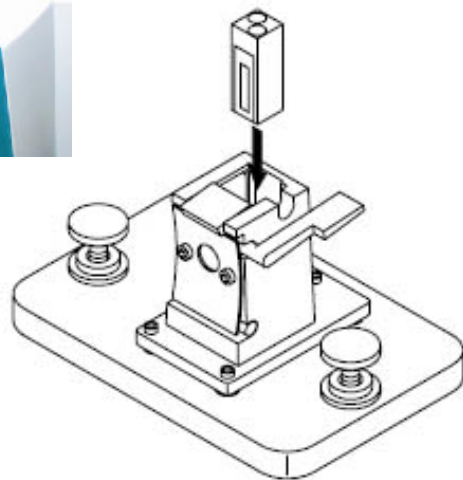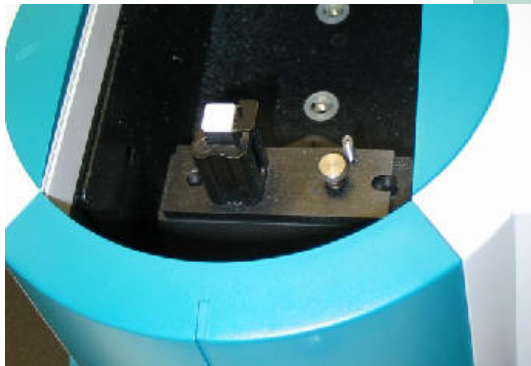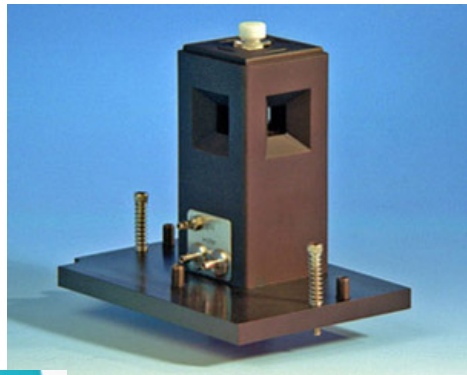## Photo Drug Delivery

Biological Tissue Window

THE SAMPLE

Source   Detector

$h\nu$

Sample

Solutions

Solids

fabrication   stretching   contraction
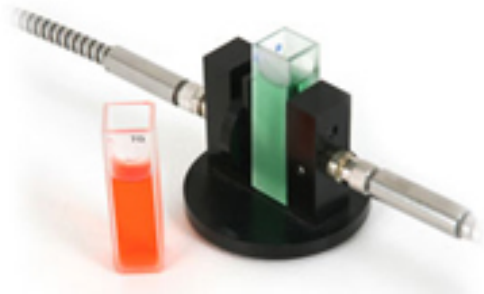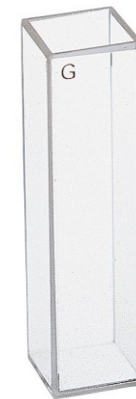
The Sample: Cuvette for Solutions

Plastic   Glass   Quartz
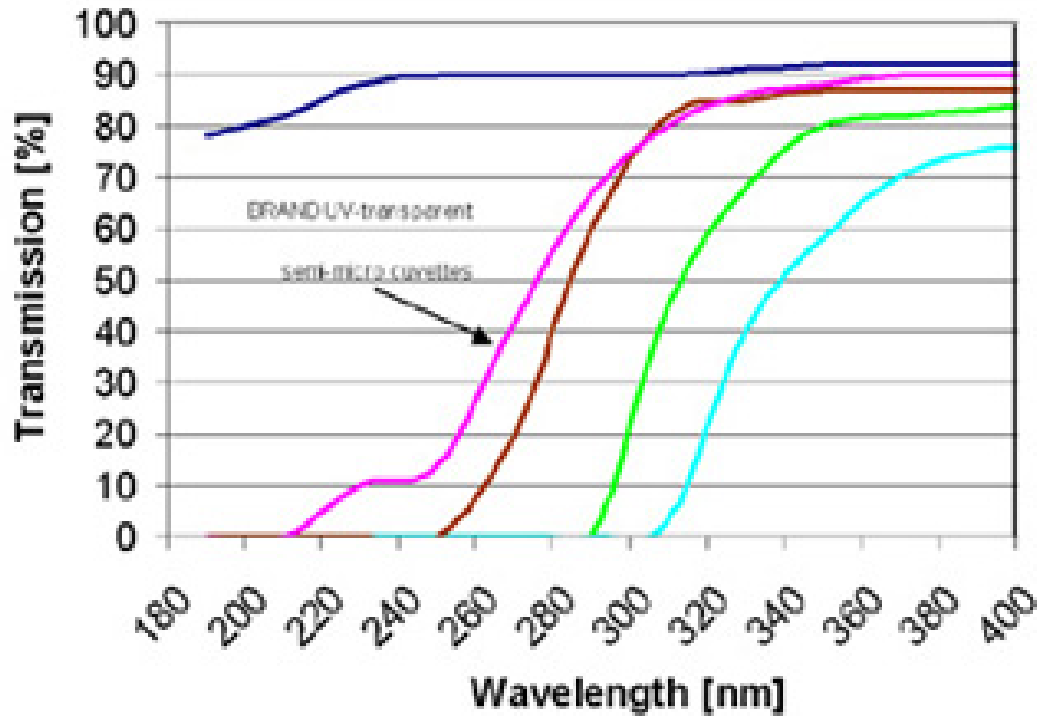
Typically 1 x 1 cm

$$A = \varepsilon\, c\, l$$

The Sample: Cuvette

Transmission Window

(—) PC   > 340 nm

(—) Polystyrene   > 320 nm $0.25

(—) PMMA   > 300 nm $0.29

(—) Glass   > 270 nm $100

(—) Quartz   > 170 nm $200

Polystyrene   Acetone →

The Sample: Specialty Cuvettes

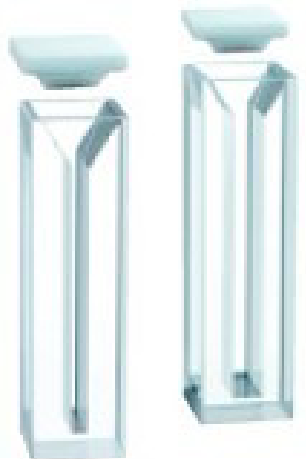## Path length

$$A = \varepsilon\, c\, l$$

Dilute Samples

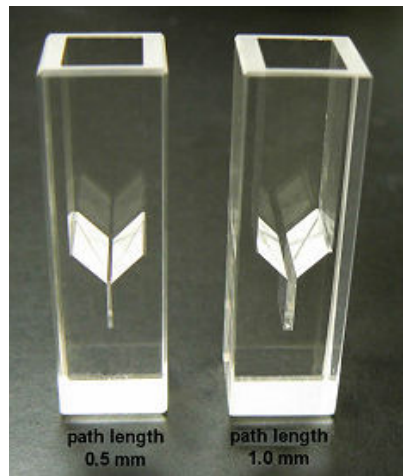10 x 1 cm   1 x 1 cm

Concentrated Samples

0.2 cm   0.5 cm

path length 0.5 mm   path length 1.0 mm
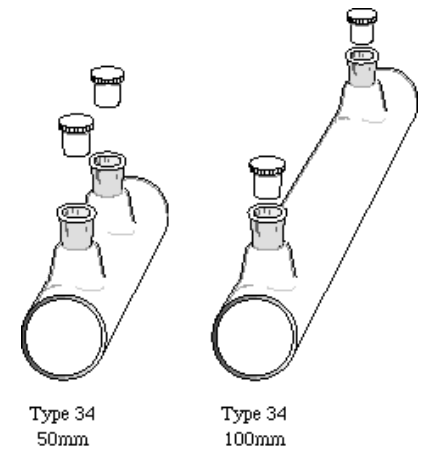
Flow Cell

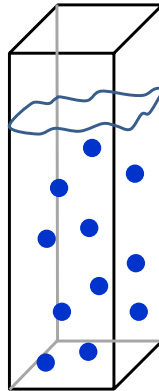## Spec-echem

Air-free

Gas Cell

Type 34 50mm   Type 34 100mm

The Sample: Solvent

- Concentration (typically <50 µM)

- Solubility

- Ionic strength

- Hydrogen bonding

- Aggregation

- π-stacking

- Solvent absorption

Common solvent cutoffs in nm:

water	190
acetonitrile	190
isooctane	195
cyclohexane	200
n-hexane	200
ethanol	205
methanol	210
ether	210
1,4-dioxane	215
THF	220
CH_2Cl_2	235
Chloroform	240
CCl_4	265
benzene	280
toluene	285
acetone	340

The Sample: Solvent

Common solvent cutoffs in nm:

water	190
acetonitrile	190
isooctane	195
cyclohexane	200
n-hexane	200
ethanol	205
methanol	210
ether	210
1,4-dioxane	215
THF	220
CH_2Cl_2	235
Chloroform	240
CCl_4	265
benzene	280
toluene	285
acetone	340

The Sample: Solvent

Vibrational Structure

(a) Vapor

(b) Hexane solution

(c) Aqueous solution
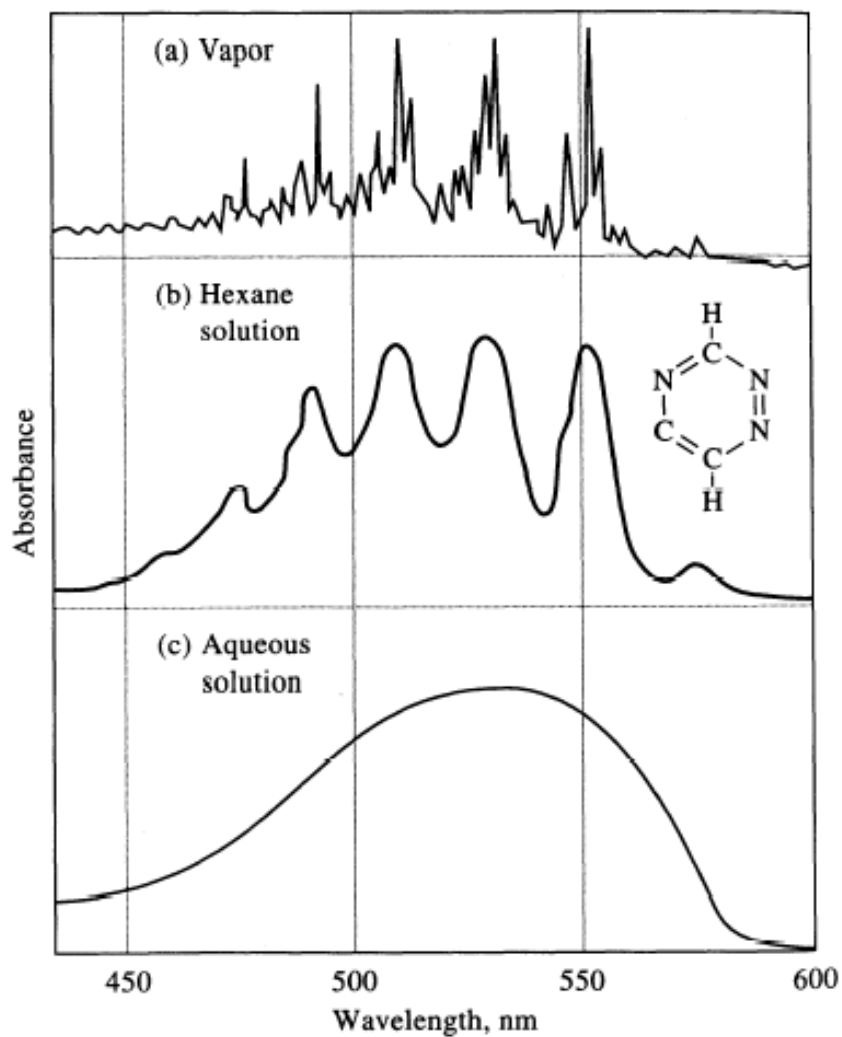
1,2,4,5-Tetrazine

Solvatochromism

Toluene   THF   DCM   Methanol   DMF
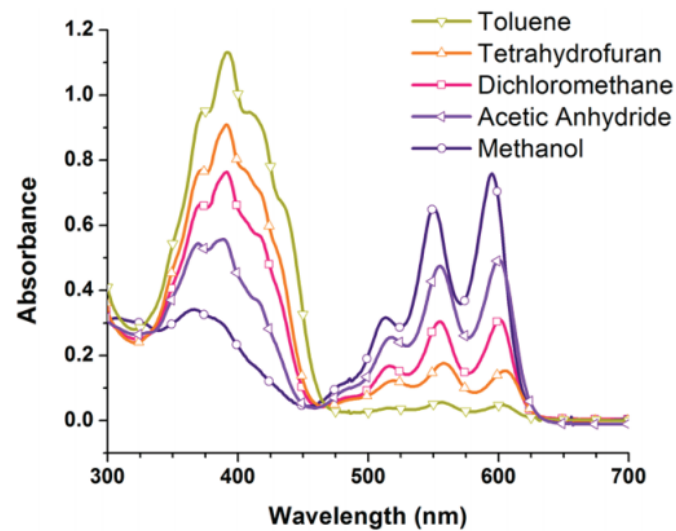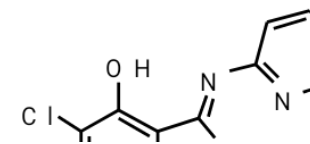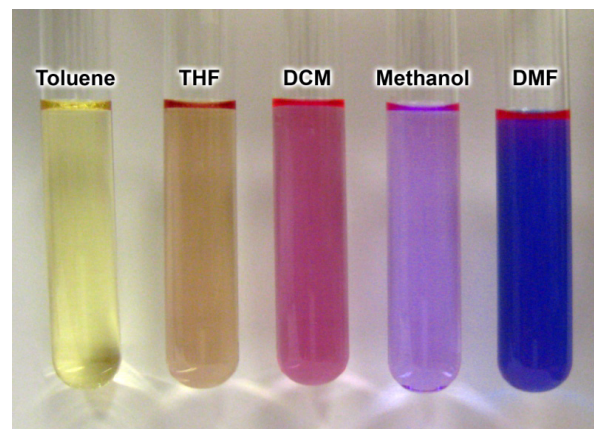
Correcting for background

$$A = -\log T = \log P_0/P$$

Figure 13-1 Reflection and scattering losses.
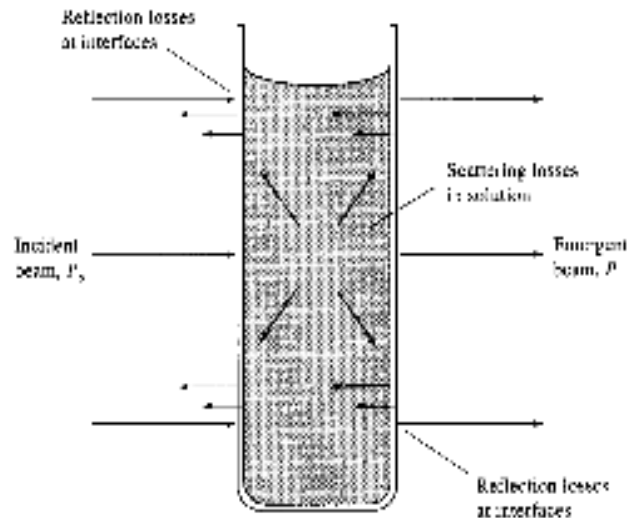
We want to know A (log P_0/P) for only our sample!
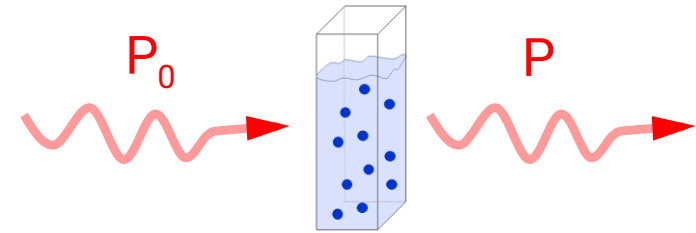
cuvette + solvent + sample

$$A_{all} = A_{cuvette} + A_{solvent} + A_{sample}$$
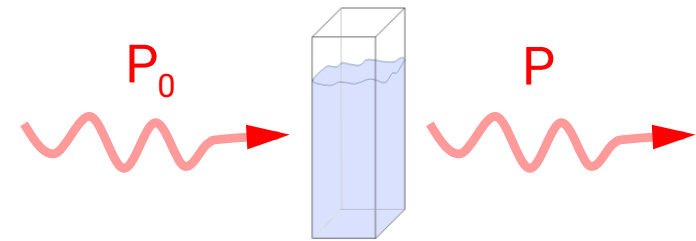
cuvette + solvent

$$A_{background} = A_{cuvette} + A_{solvent}$$

$$A_{all} - A_{background} = A_{sample}$$

Instrument Architectures
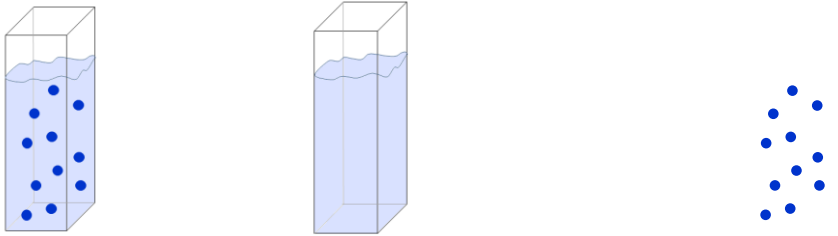
$$A_{all} - A_{background} = A_{sample}$$

How do we measure background
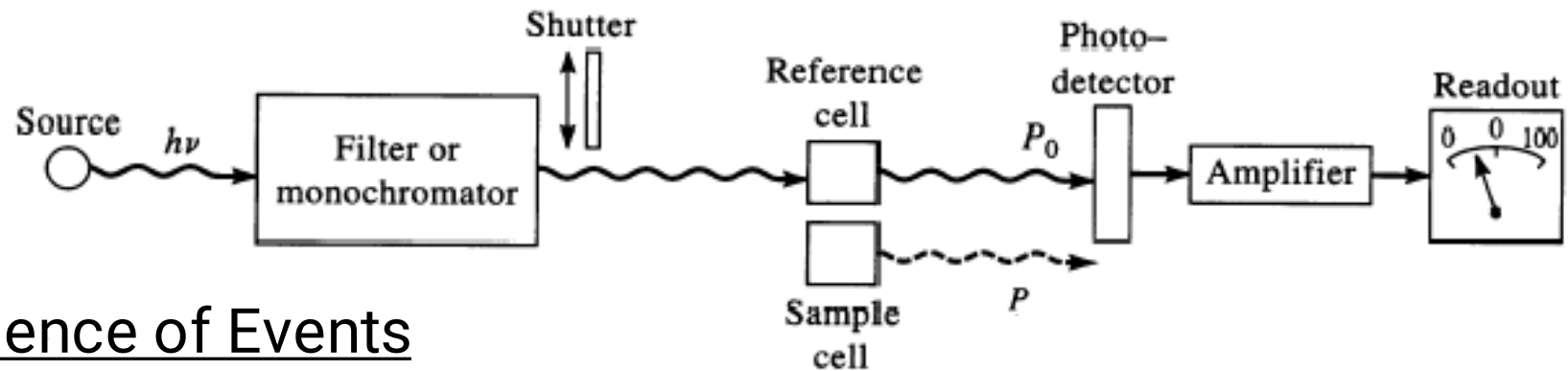(reference) and sample?
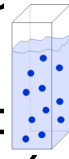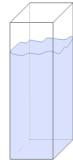
Architectures

1) Single Beam

2) Double Beam

- Spatially Separated

- Temporally Separated

Single Beam Instrument

Sequence of Events

1) Light Source On
2) Reference in holder
3) Open Shutter
4) Measure light (P_0)
5) Raster λ and repeat 4
6) Close Shutter
7) Sample cell in holder
8) Open Shutter
9) Measure intensity (P)
10) Raster λ and repeat 9
11) Close Shutter

Pros:
Simple
Less expensive
Less optics
Less moving parts
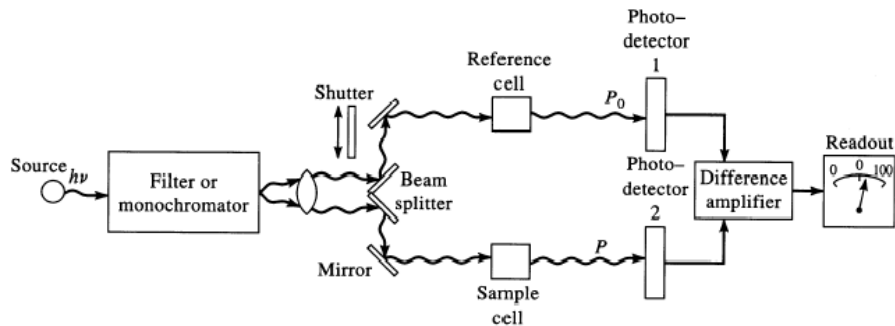Higher light intensity
Can use the same cuvette

Cons:
Changes over time
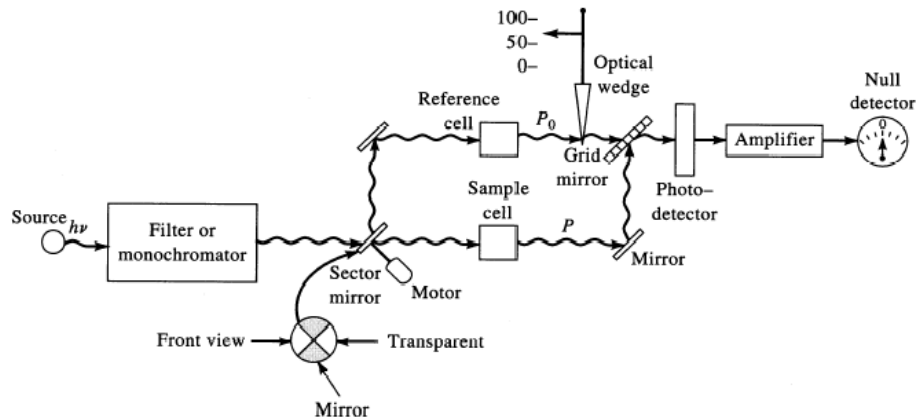Better for short term experiments
Manually move samples

$$A = -\log T = \log P_0/P$$

Double Beam Instrument

Spatially Separated

Compensates for:
1) Lamp Fluctuations

Sources of Instability in Metal Halide Arc Discharge Lamps

—Anode

Convection Currents

(a)   Cathode   (b)   (c)

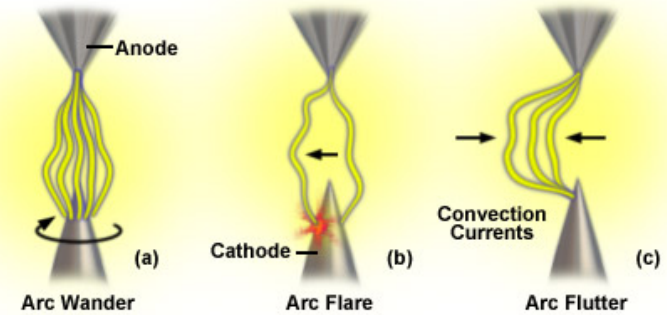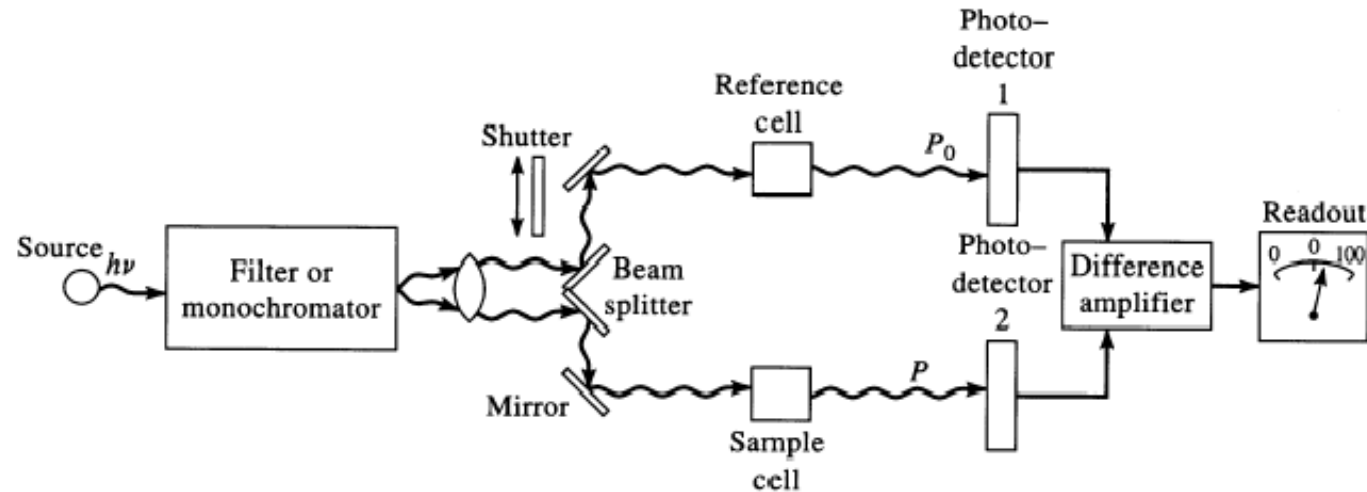Arc Wander   Arc Flare   Arc Flutter

Figure 1

Temporally Separated

2) Temperature changes

3) Amplifier changes

4) Electromagnetic noise

5) Voltage spikes

6) Continuous recording

Double Beam Instrument: Spatial

Sequence of Events
1) Light Source On
2) Reference and sample in holder
3) Open Shutter
4) Measure detector 1 (P_0) and 2 (P)
5) Raster λ and repeat 4
6) Close Shutter

Pros:
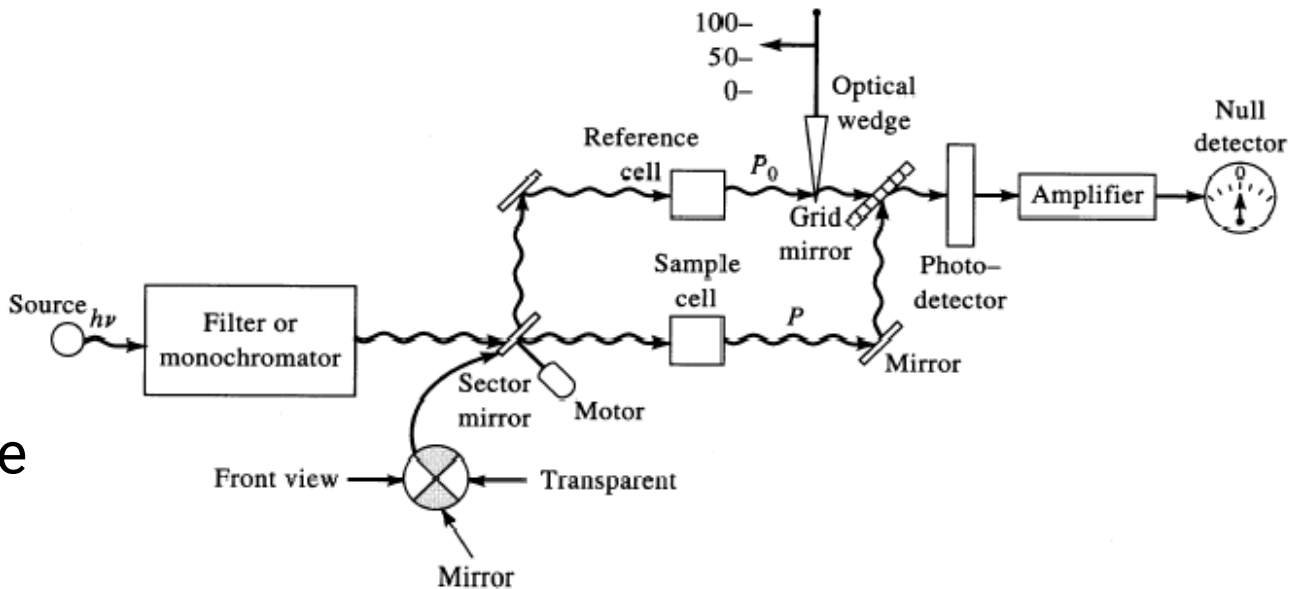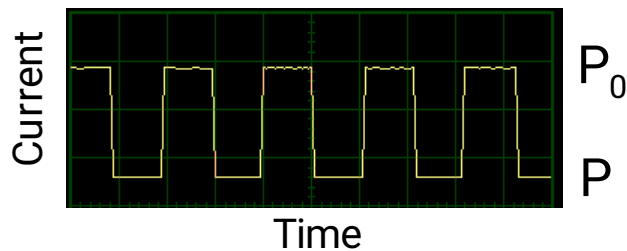Both samples simultaneously
Less moving parts (than temporal)

Cons:
Two different cuvettes
Two different detectors
½ the intensity
More expensive

$$A = -\log T = \log P_0/P$$

Double Beam Instrument: Temporal

Sequence of Events

1) Light Source On
2) Reference and sample
3) Rotate Chopper
4) Open Shutter
5) Monitor detector

6) Raster λ and repeat 4
7) Close Shutter

$$A = -\log T = \log P_0/P$$

Pros:

Both samples "simultaneously"
Same Detector

Cons:

Two different cuvettes
½ the intensity
rotating mirrors
not really simultaneous

Instrument Architectures

Single Beam
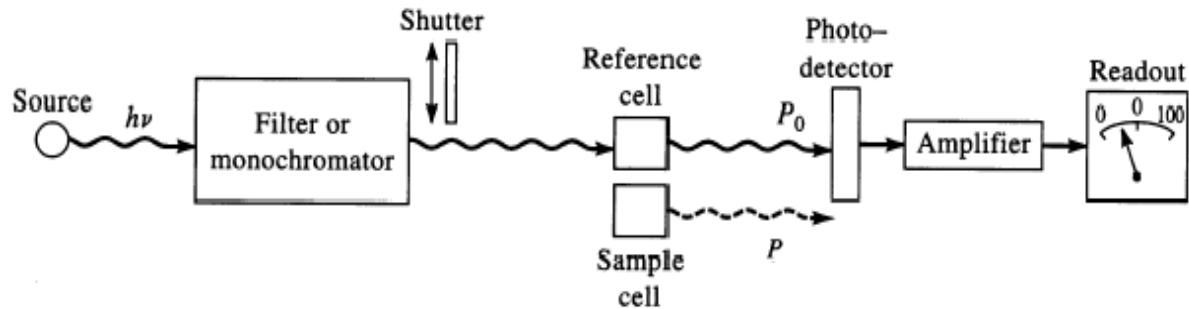
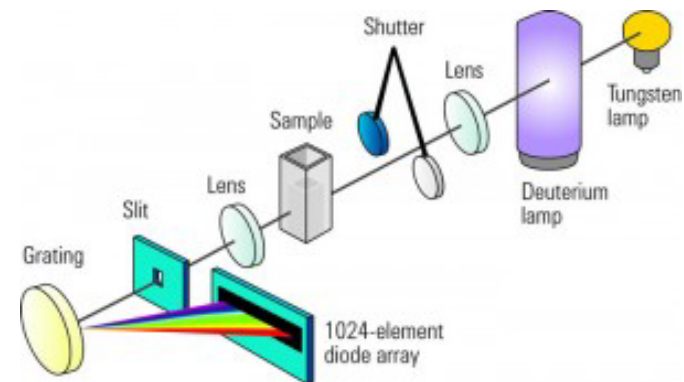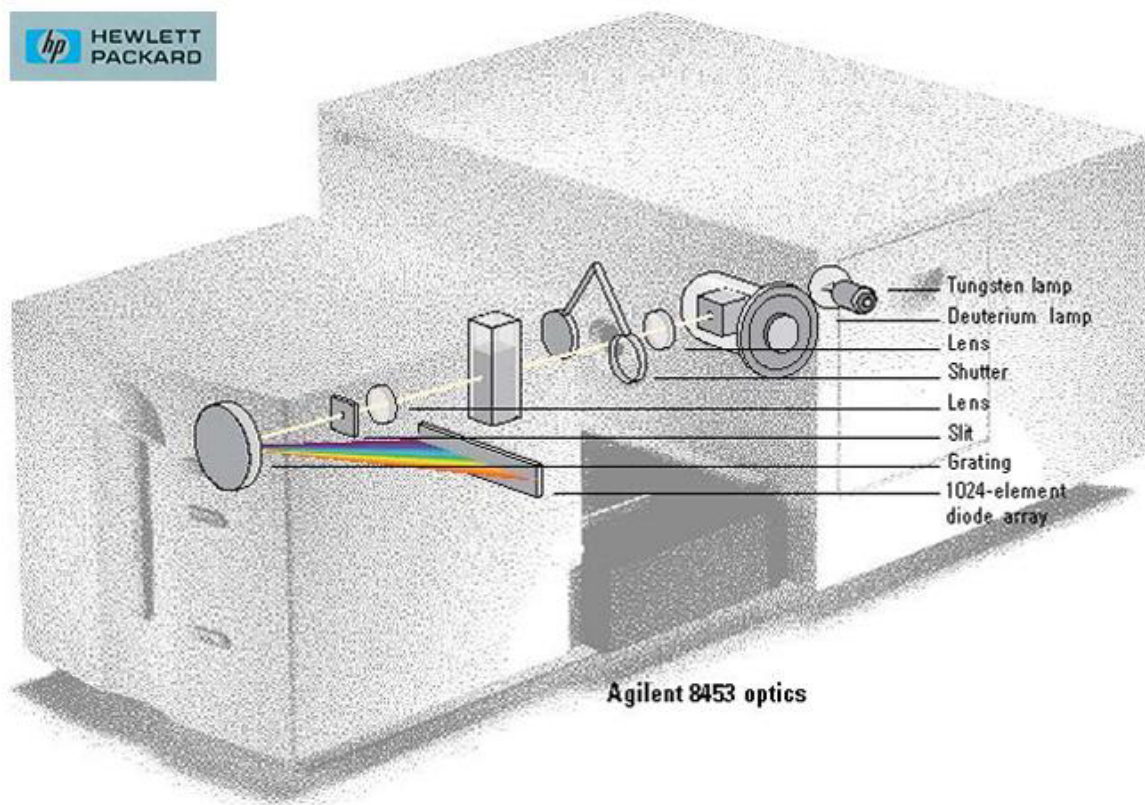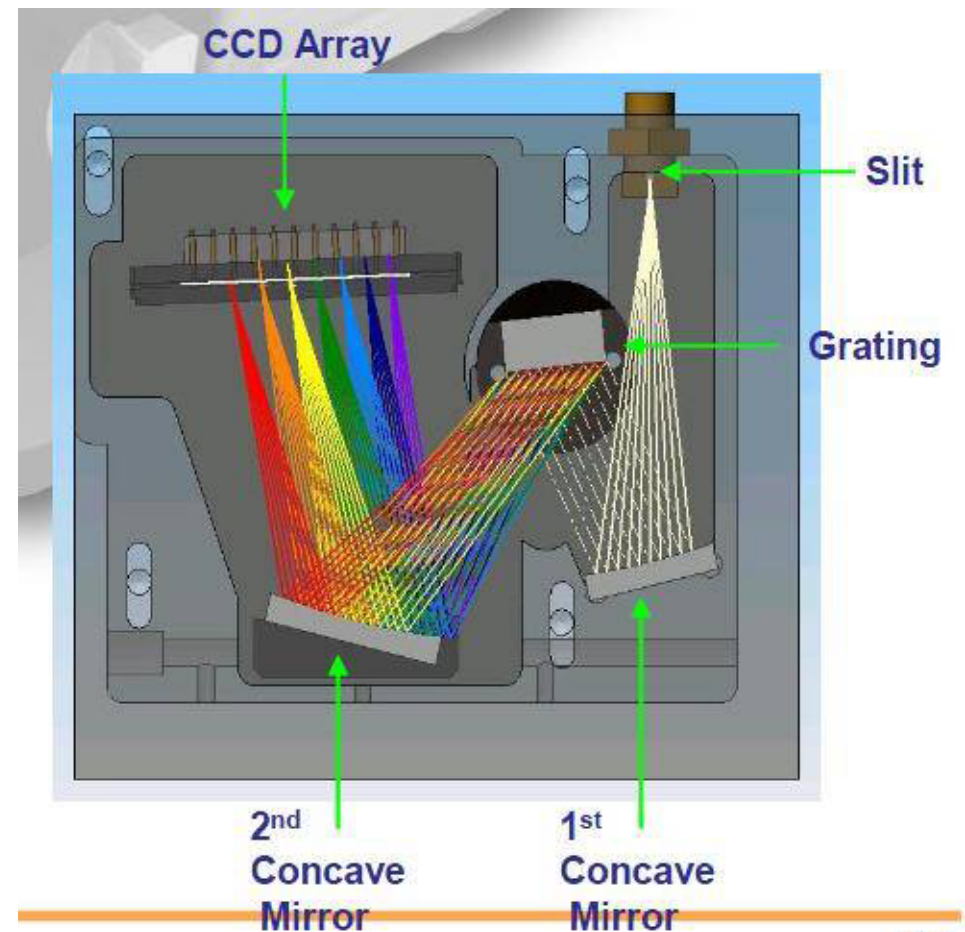## Double Beam
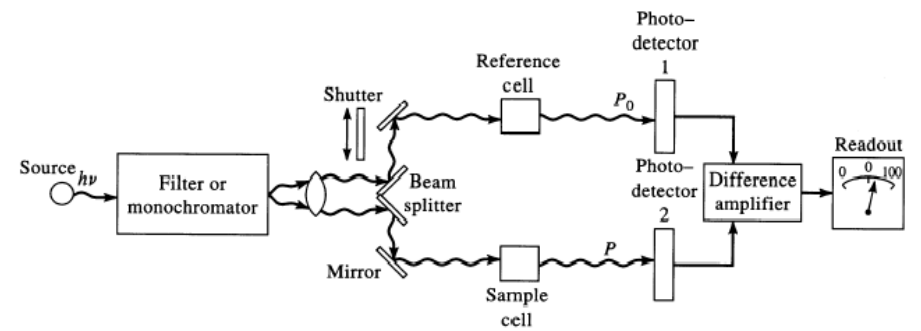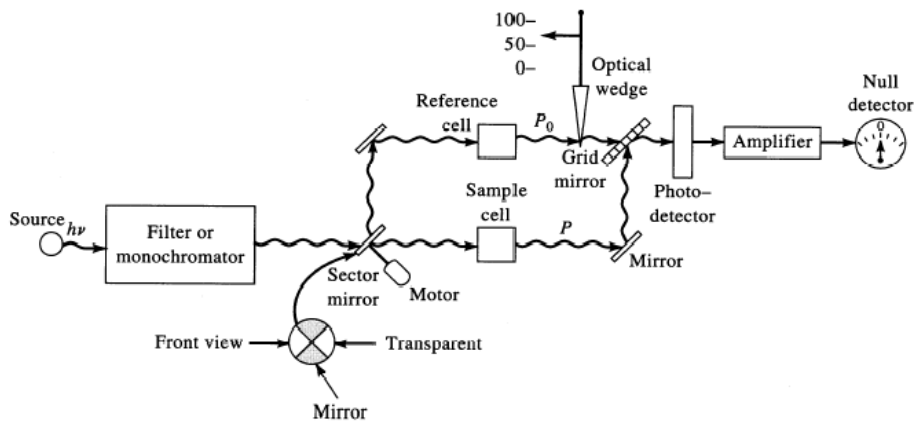
Instrument Architectures

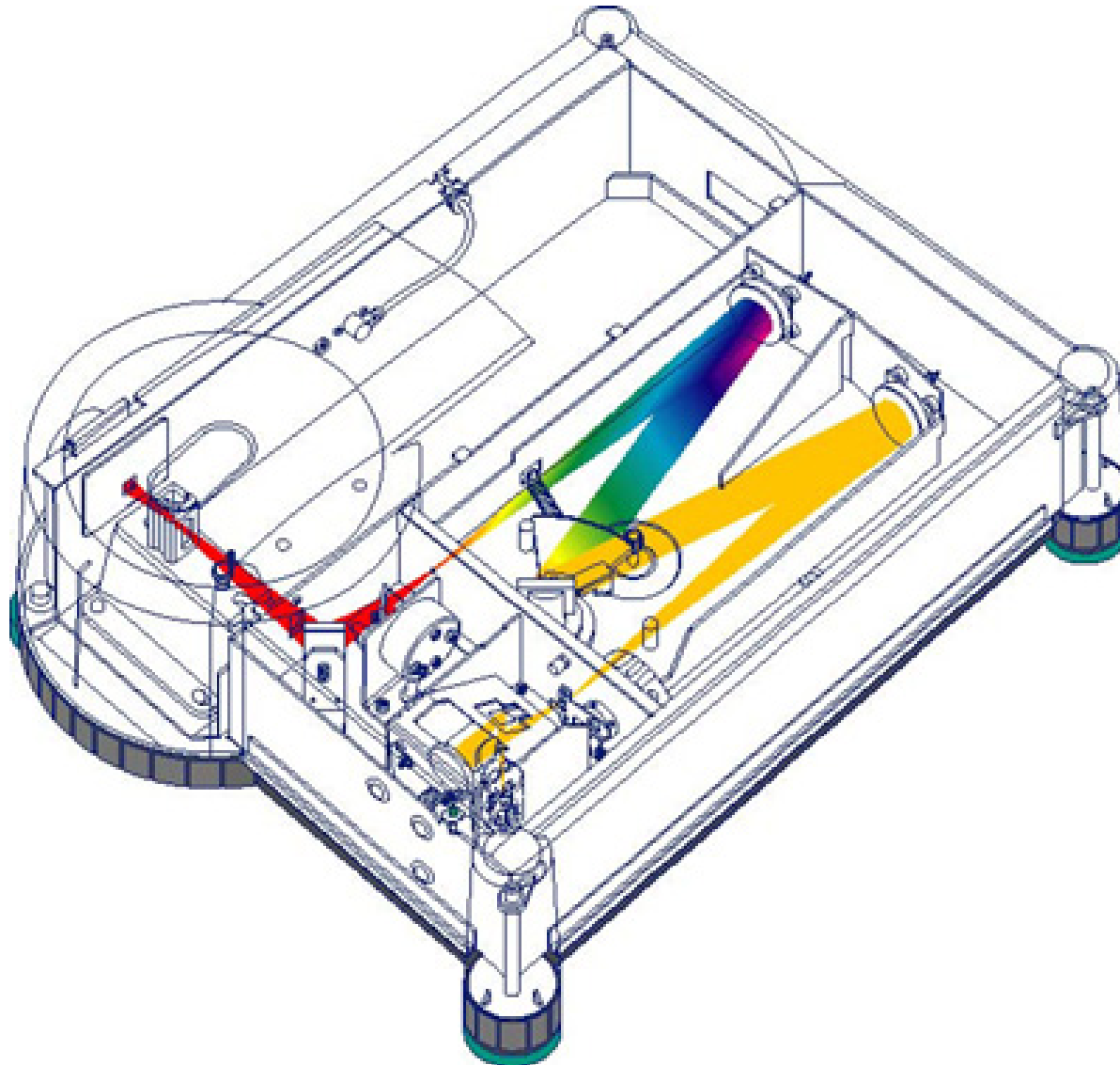Agilent 8453: Single Beam, Diode Array Detector

Agilent 8453 optics

Instrument Architectures
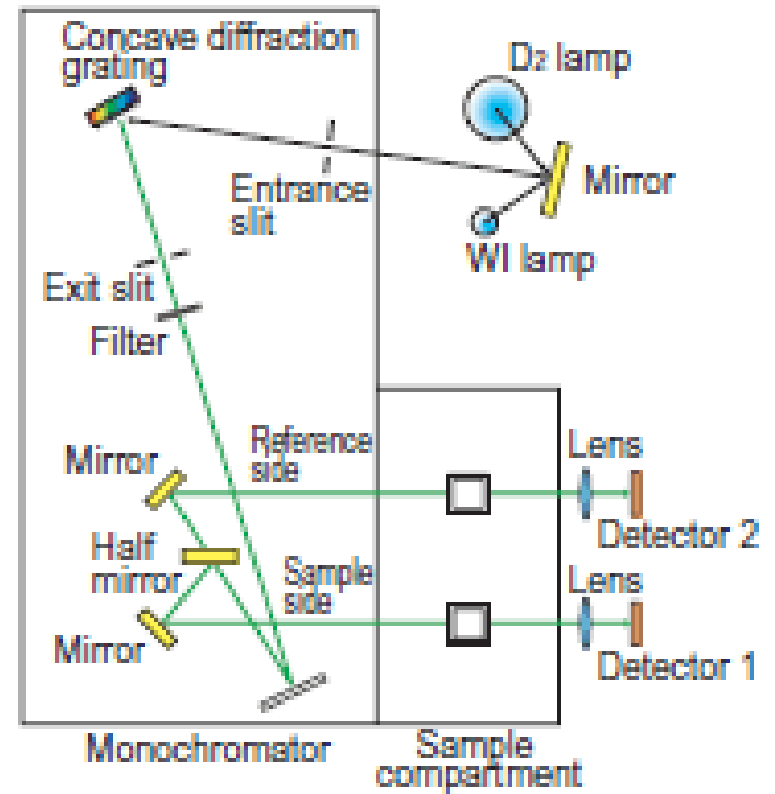
Ocean Optics: Single Beam, CCD Detector

Instrument Architectures
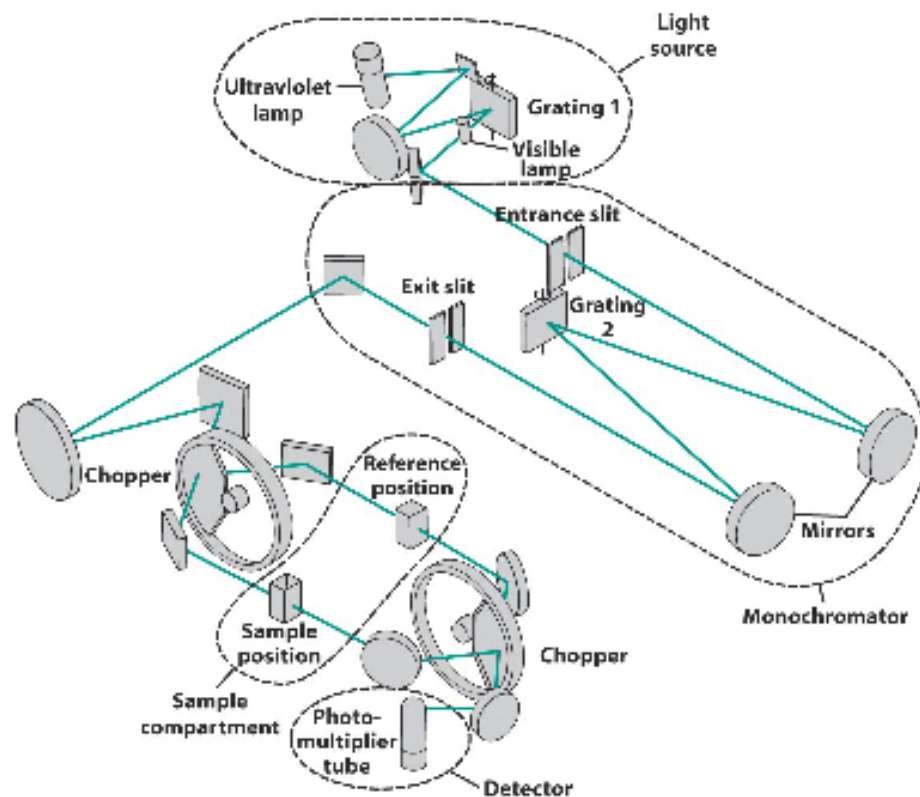
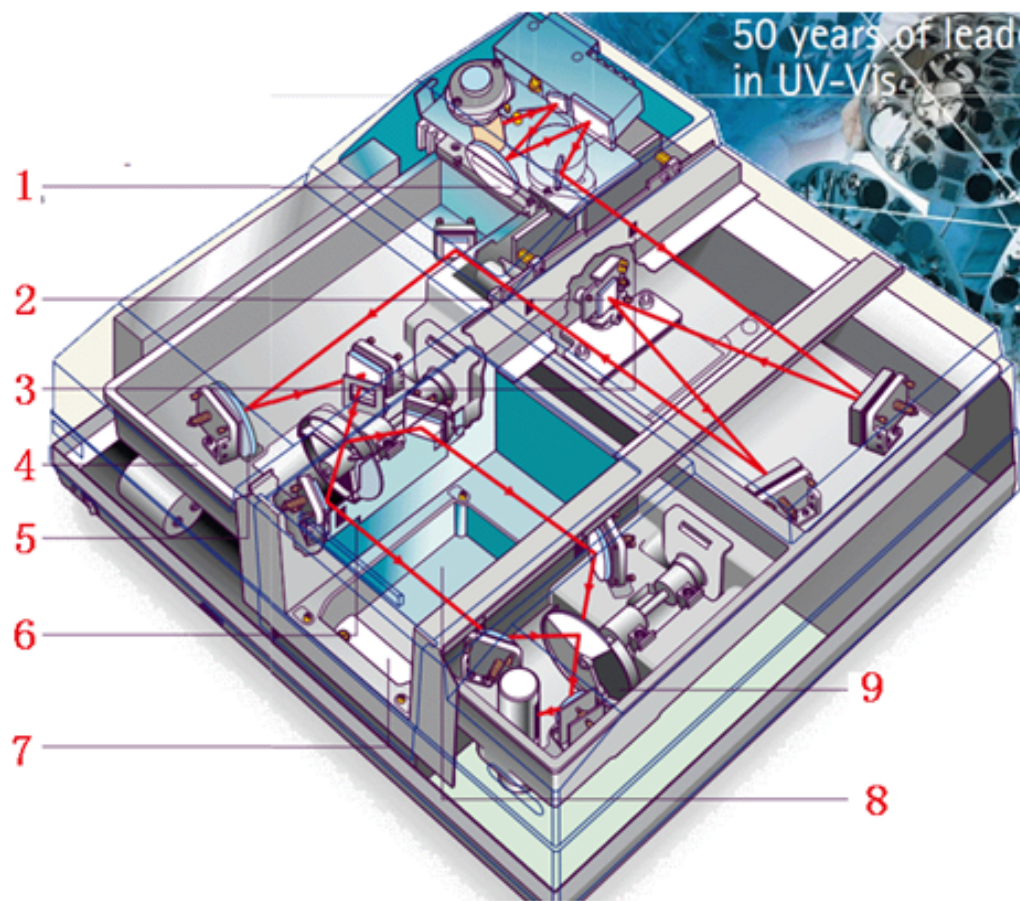Cary 50: Single Beam, PMT detector

Instrument Architectures

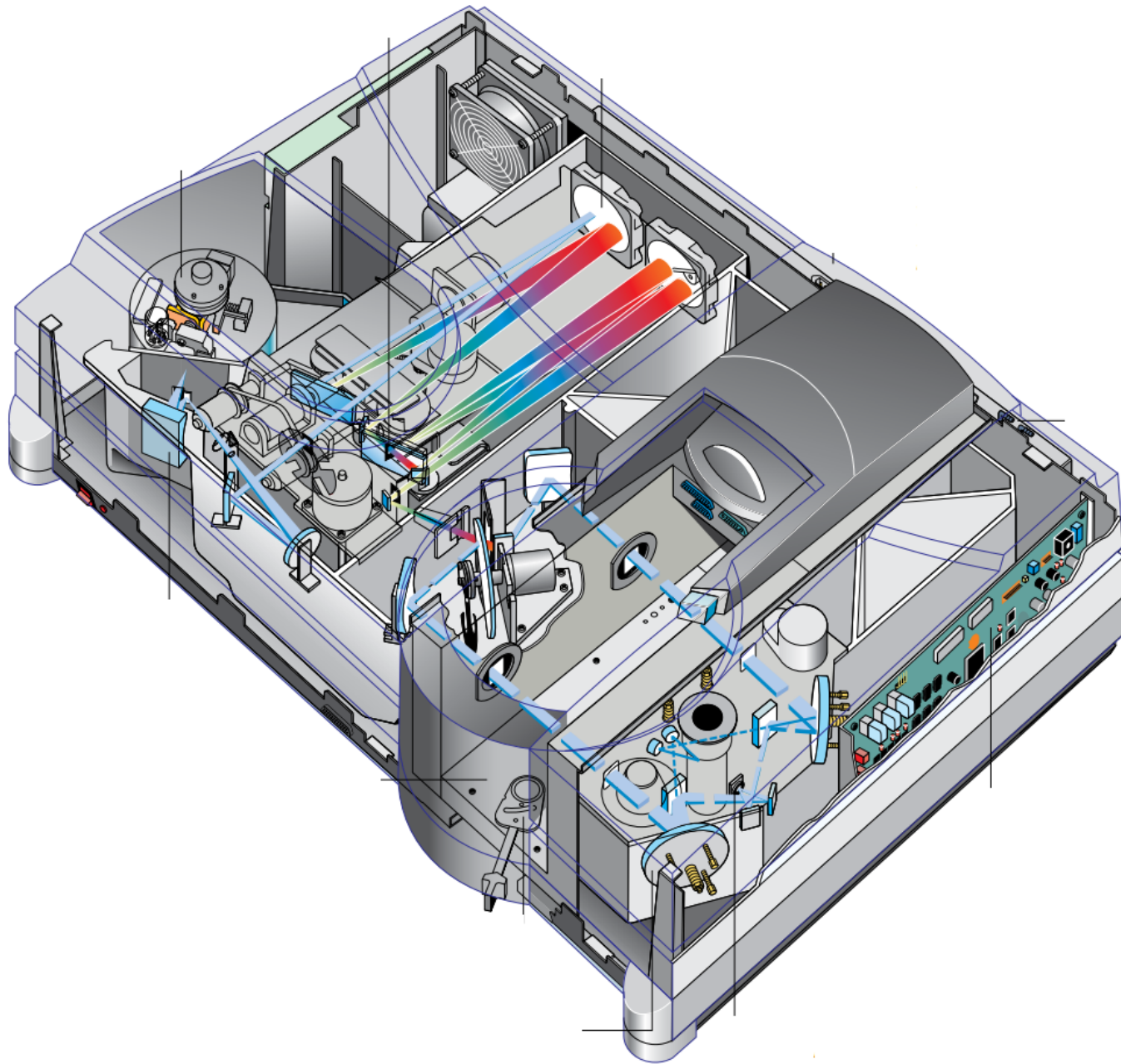Hitachi U-2900: Double Beam, 2 x PMT detector

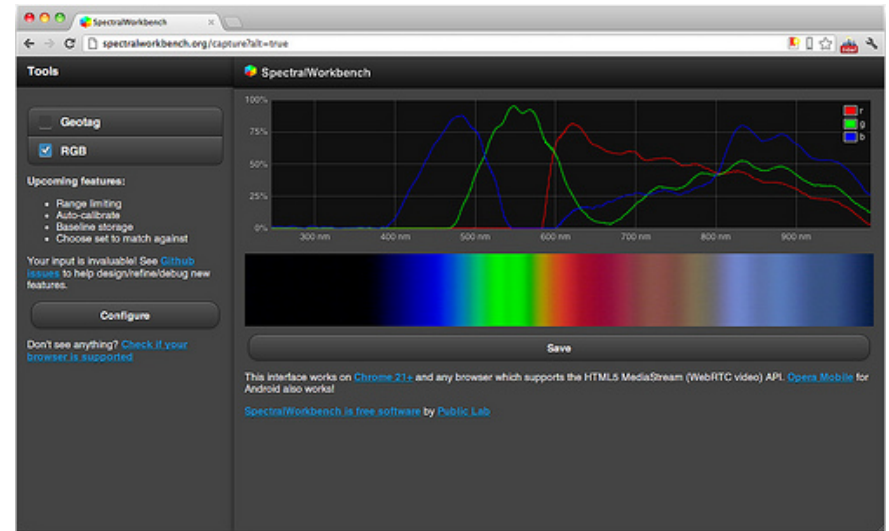# Instrument Architectures

Cary 300: Double Beam, PMT detector

Instrument Architectures

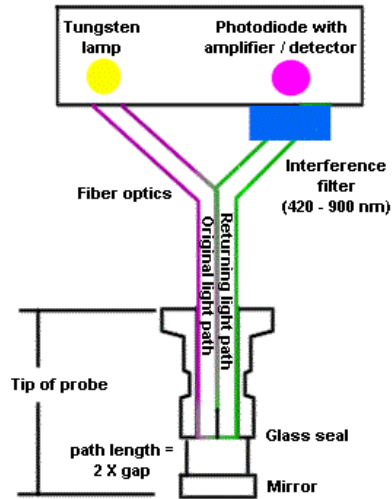Cary 5000: Double Beam, PMT detector

Single Beam Instrument

DIY Spectrometer

$35

http://publiclab.org/wiki/spectrometer

Other Sampling Accessories

Probe-type

Cryostat

Fiber Optics

Microplate Spectrometer

The Sample: Solids

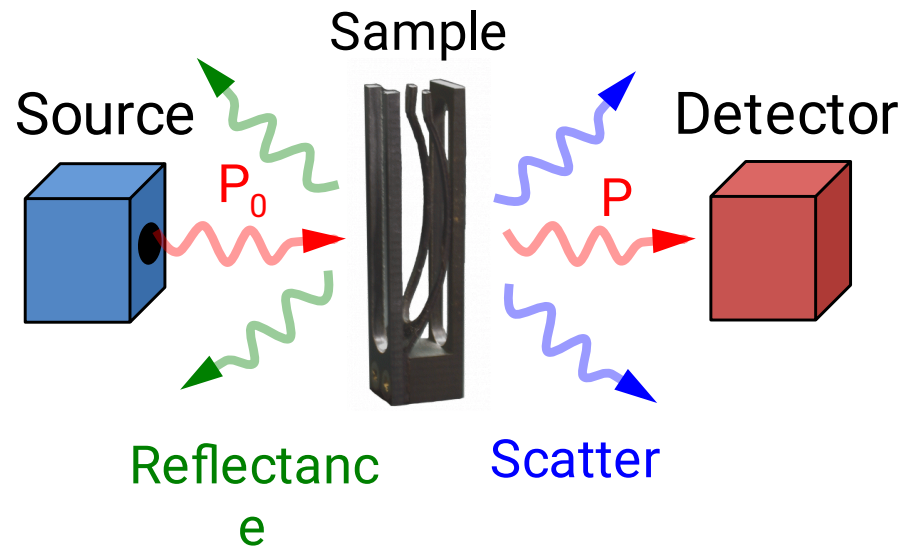Figure 1.8-1   Reflection and scattering losses.

Source   hν

Sample

Detector
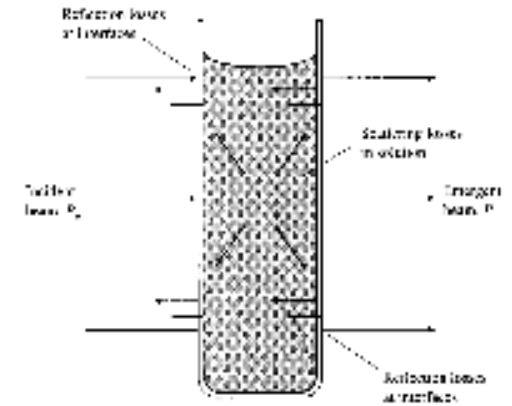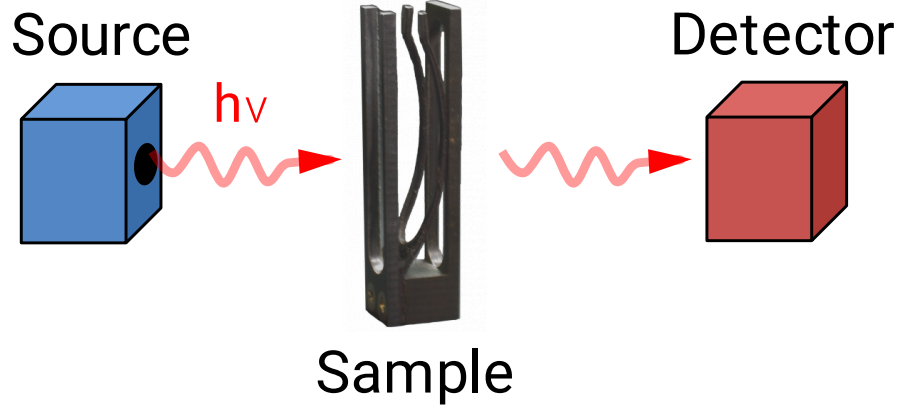
Solids/Films
- More scatter, more reflectance
- No reference

Light rays shining on a surface — Reflected rays — scatter in all directions
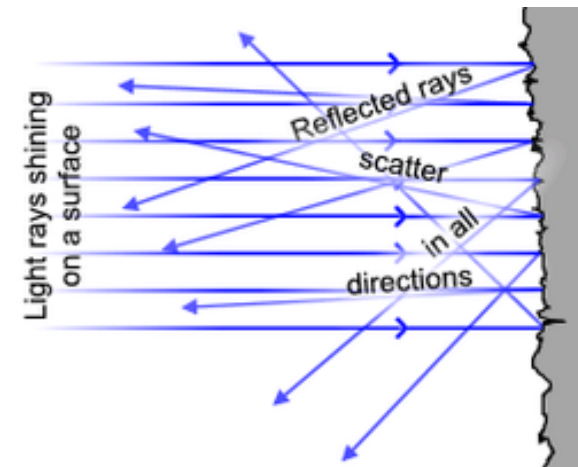
The Sample: Solids

$$A = -\log T = \log P_0/P$$
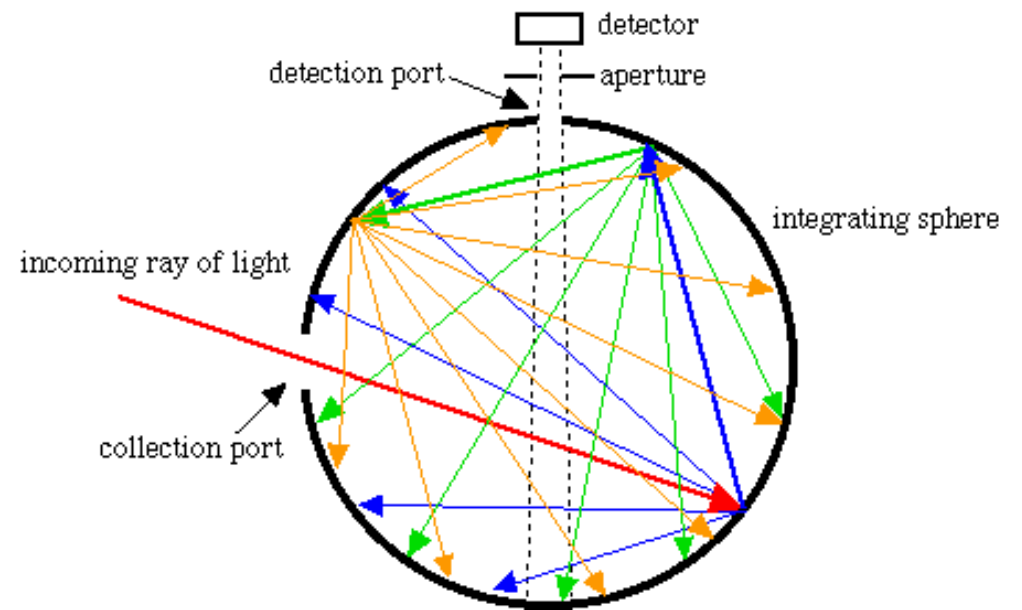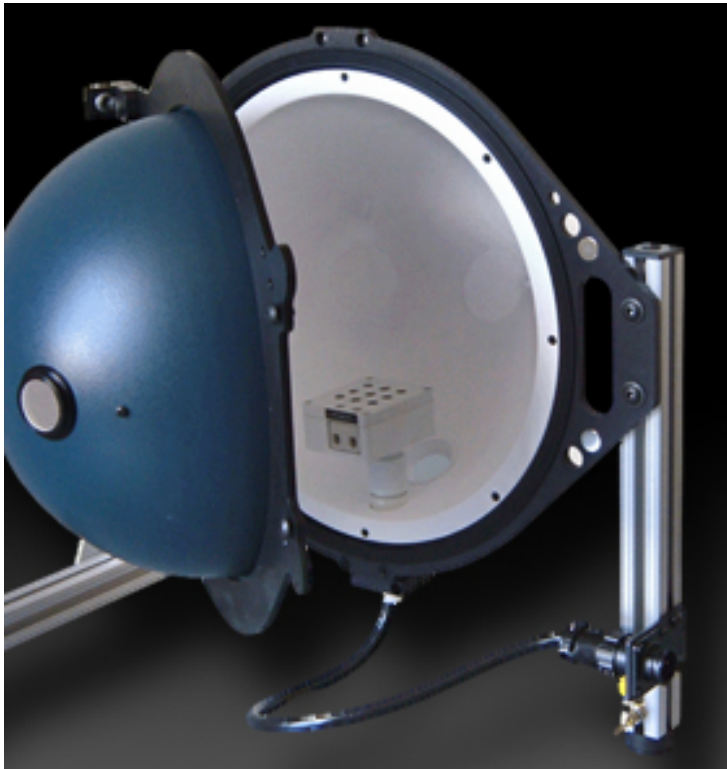

P does not take into account reflectance and scatter!

Measured A > Actual A

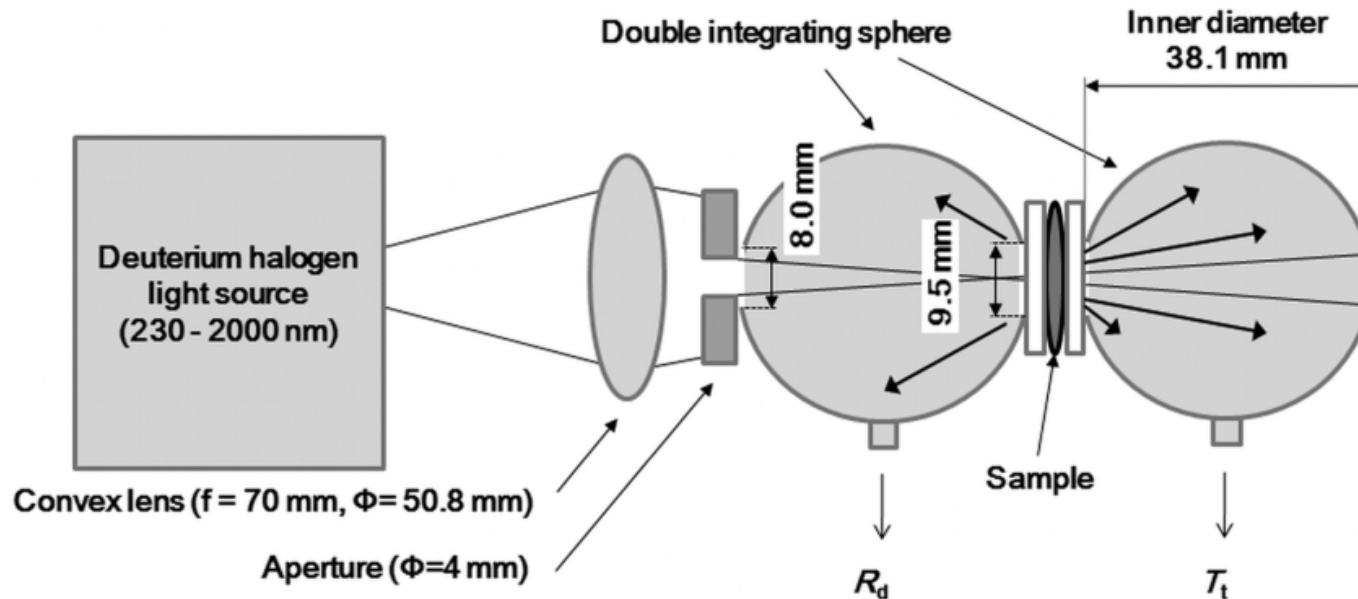More scatter/reflectance = More error

The Sample: Solids

Integrating Sphere

Solid Sample

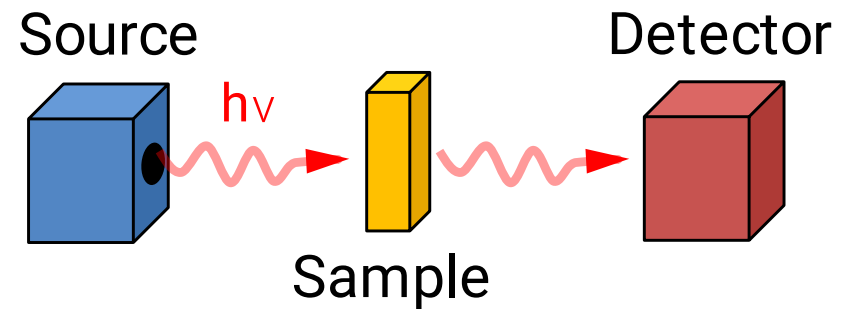$$A = -\log T = \log P_0/P$$

$$P_0 \approx T_{t(\text{without sample})} - R_{d(\text{with sample})}$$

$$P \approx T_{t(\text{with sample})}$$

$$A \approx \log (T_{t(\text{without sample})} - R_{d(\text{with sample})})/T_{t(\text{with sample})}$$

Outline

1) Beer's Law

2) Absorption Spectrum

3) Instrument Components
   - Light sources
   - Monochrometers
   - Detectors
   - Other components
   - The sample

4) Instrument Architectures

5) Applications

6) Limitations
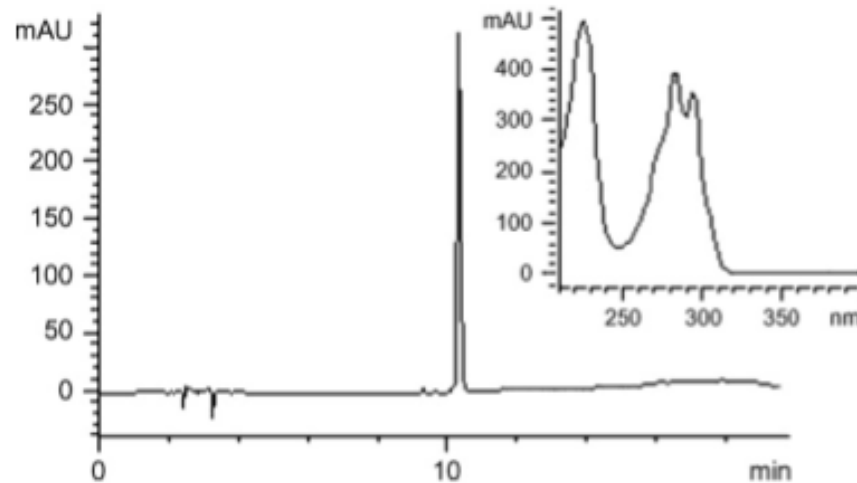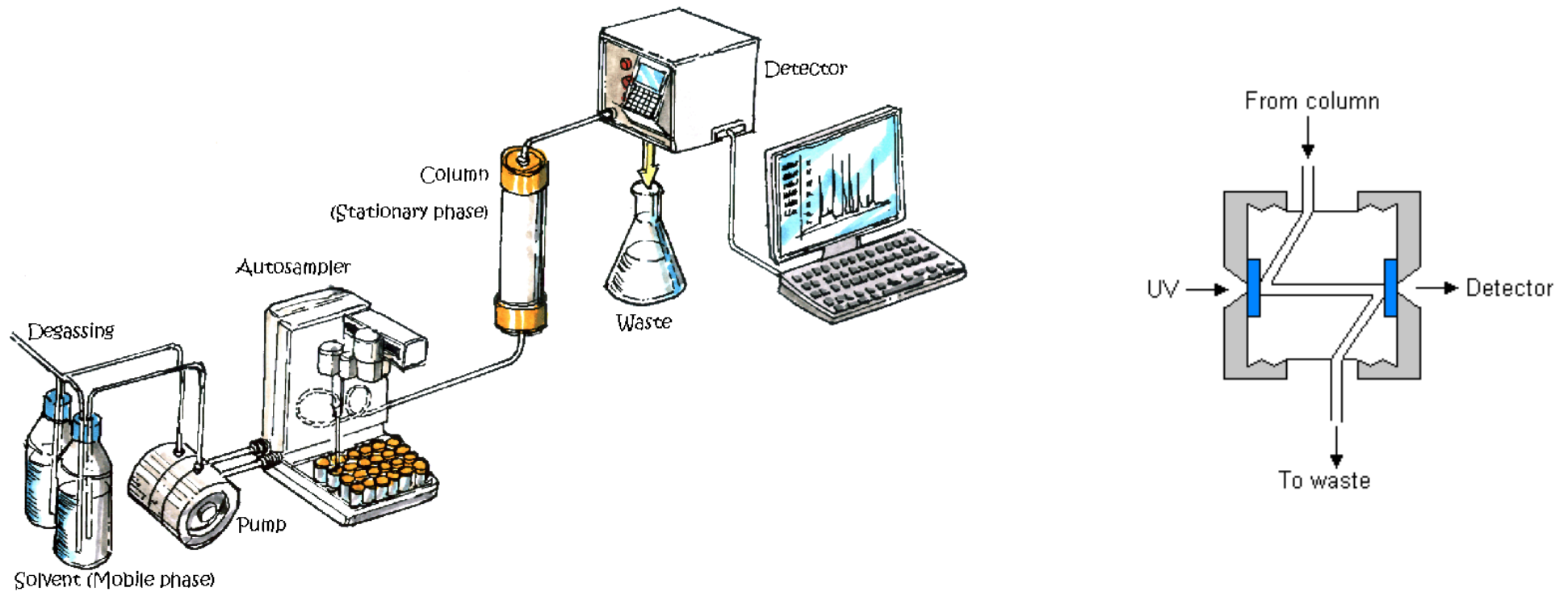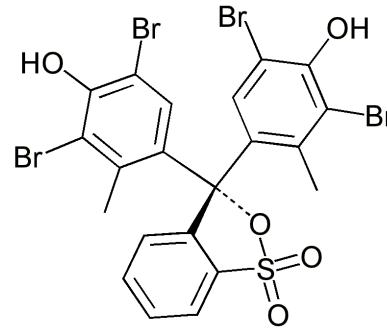
HPLC

Fig. 3. HPLC chromatogram and UV–vis spectra (inset) of Bromo-Dragonfly. The HPLC trace was detected at 210 nm.
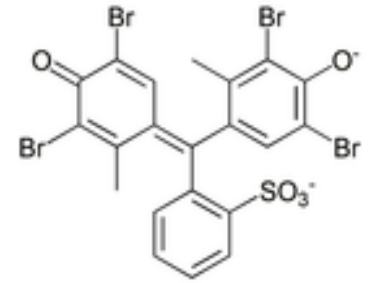
pK_a Determination

Titration of bromocresol green

1) Bromocresol Green in H_2O
2) Titrate with base
3) Monitor pH
4) Monitor Absorption Change
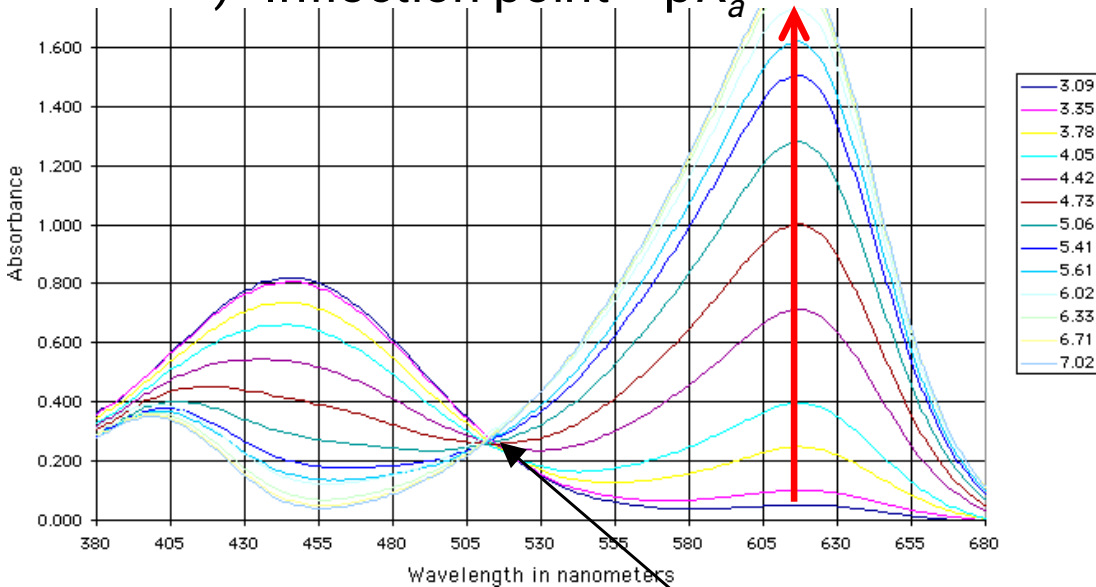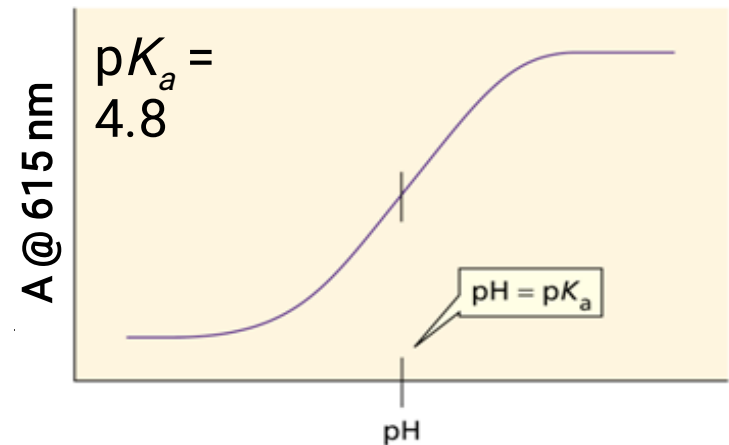5) Graph absorbance vs pH
6) Inflection point = pK_a

yellow   blue

$- H^+$

$+ H^+$
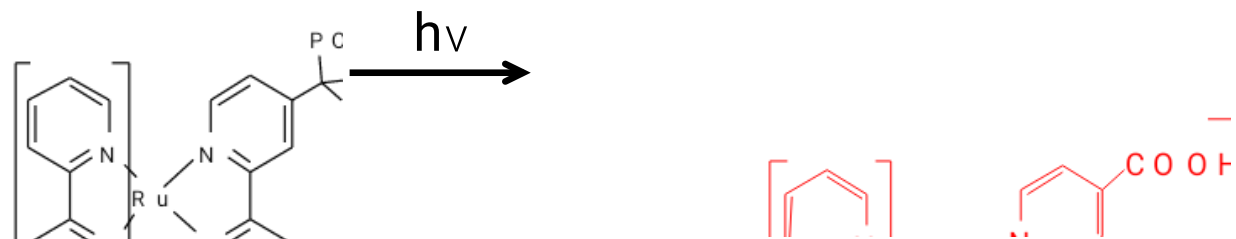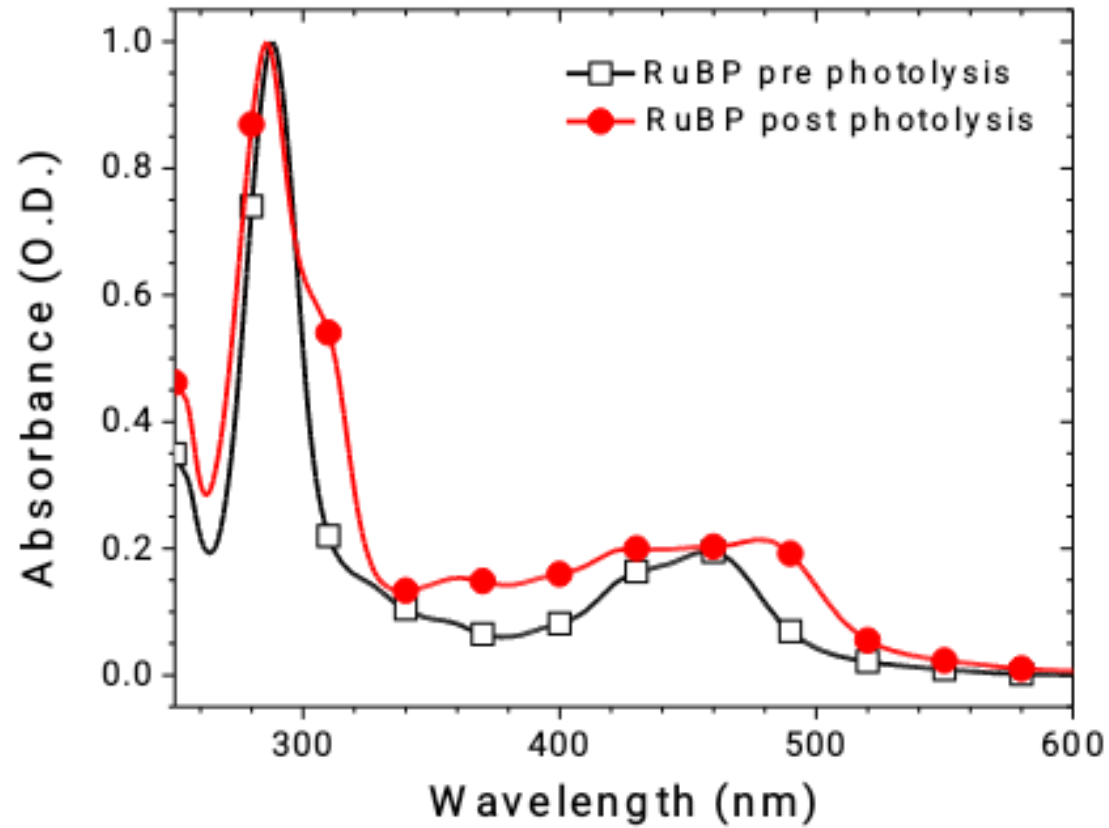

	3.09
	3.35
	3.78
	4.05
	4.42
	4.73
	5.06
	5.41
	5.61
	6.02
	6.33
	6.71
	7.02

Isosbestic point

pK_a = 4.8

A @ 615 nm

pH = pK_a

pH

Reaction Kinetics

Real Time Monitoring

UV-Vis

455 nm source
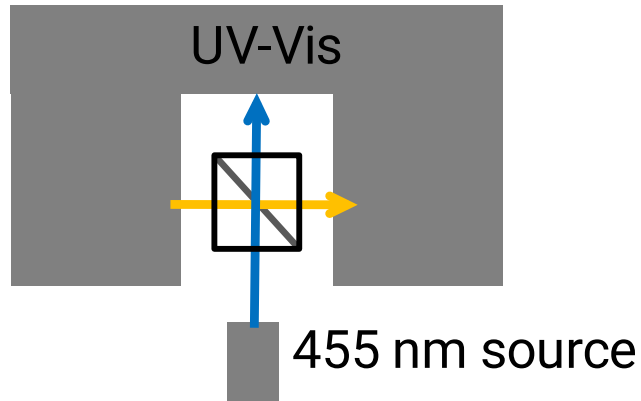
3mL of 40 μM **RuBP** in pH 1, atm

Monitor: Every 5 min for 180 min
Every 30 min for 180 min
Every 60 min for 3420 min

Spectral Fitting

b)

$A \rightarrow B \rightarrow C \rightarrow D \rightarrow E$
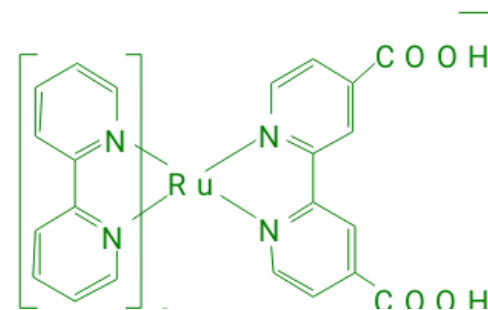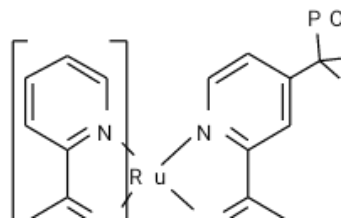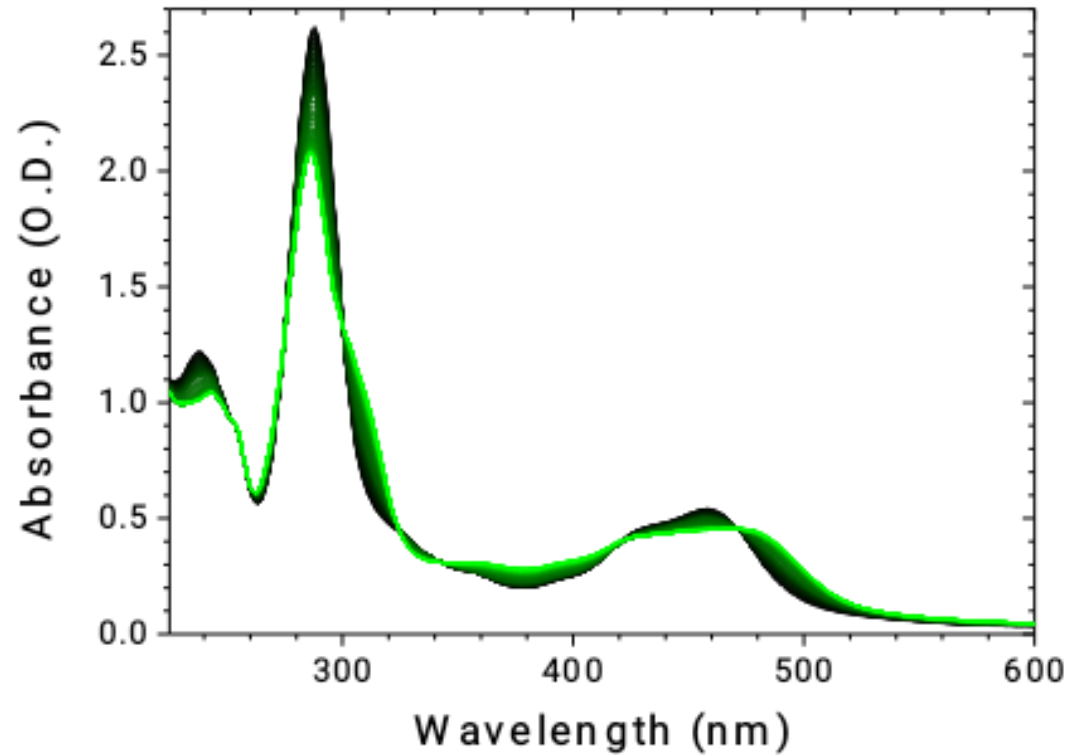
Spectral Fitting

Table 1. Reaction rate constants for the photodecomposition of **RuBP** (error in parentheses).[a]
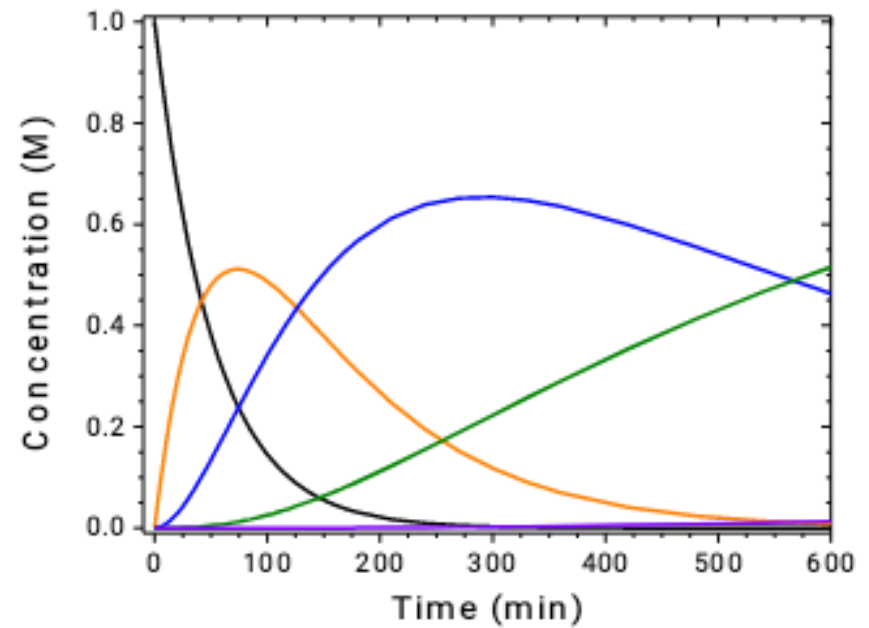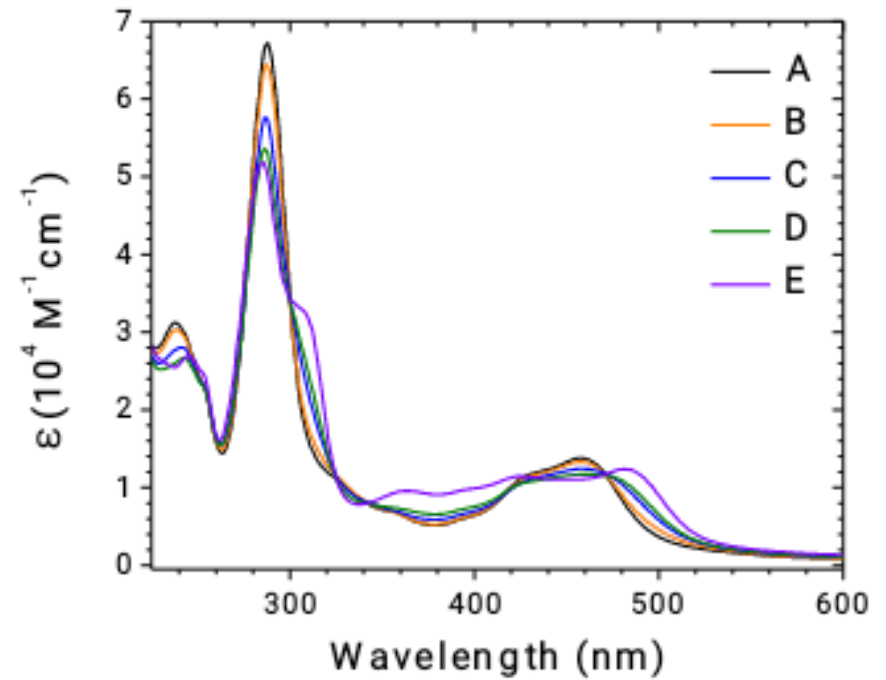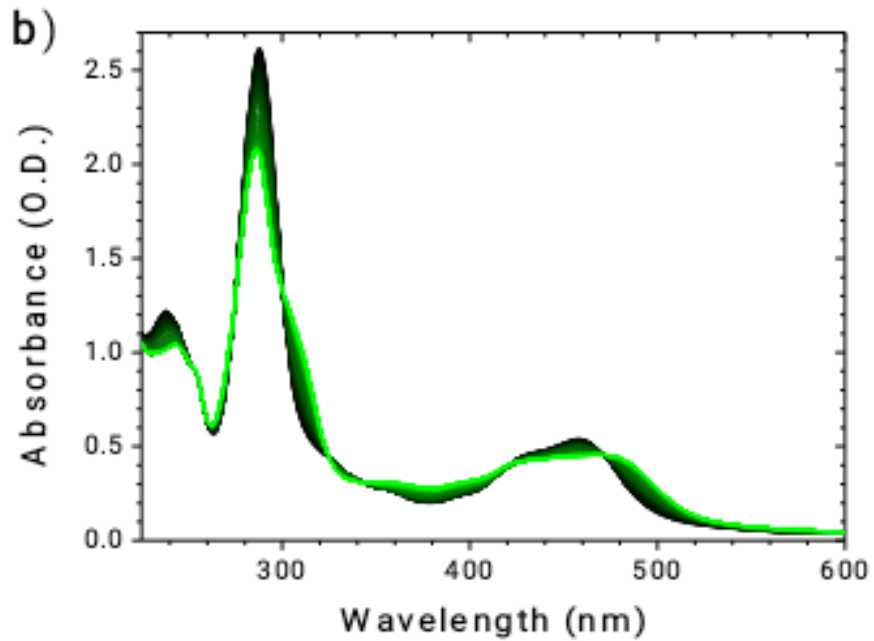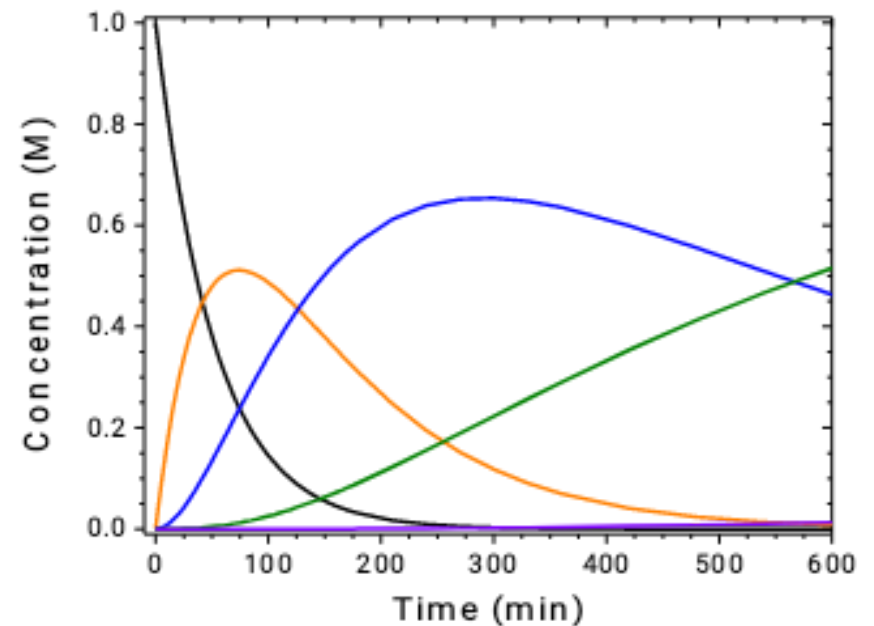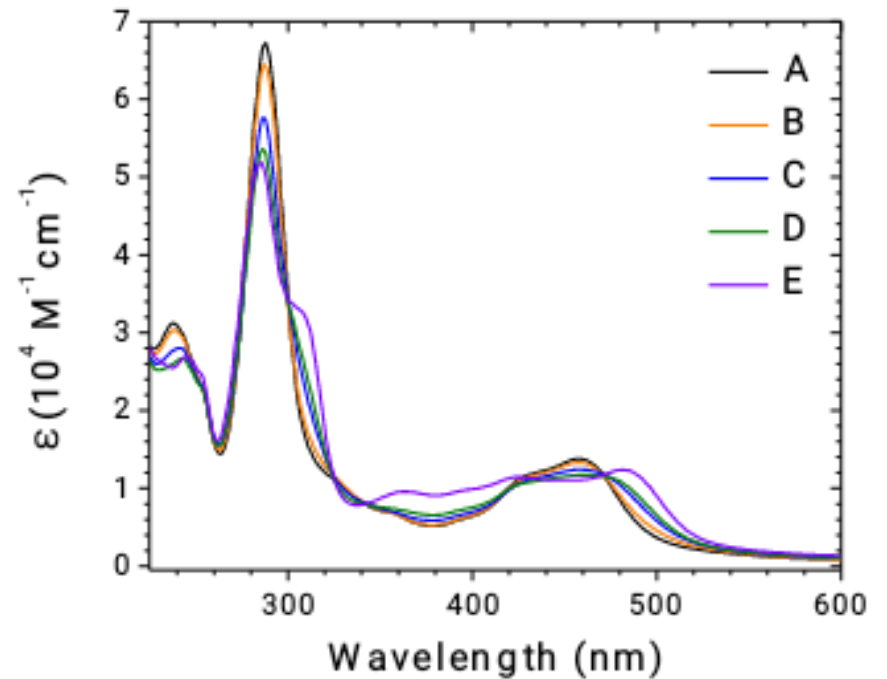
Solvent	$k_{A \rightarrow B}$ $(10^{-4}\ s^{-1})$	$k_{B \rightarrow C}$ $(10^{-4}\ s^{-1})$	$k_{C \rightarrow D}$ $(10^{-5}\ s^{-1})$	$k_{D \rightarrow E}$ $(10^{-6}\ s^{-1})$
H_2O	2.8 (0.06)	1.3 (0.07)	3.4 (0.07)	4.0 (0.6)
D_2O	8.3 (0.08)	1.1 (0.02)	2.9 (0.07)	4.8 (1.1)
$0.1\ M\ HClO_4$	3.2 (0.3)	1.5 (0.06)	2.9 (0.09)	1.6 (0.4)
$0.1\ M\ HClO_4{}^{b}$	16.4 (1.4)	2.9 (0.02)	4.9 (0.04)	2.9 (0.3)

a) In atmosphere with 455 nm (50 mW/cm^2) irradiation unless otherwise noted. b) Bubbled with pure O_2.

Potential Complications
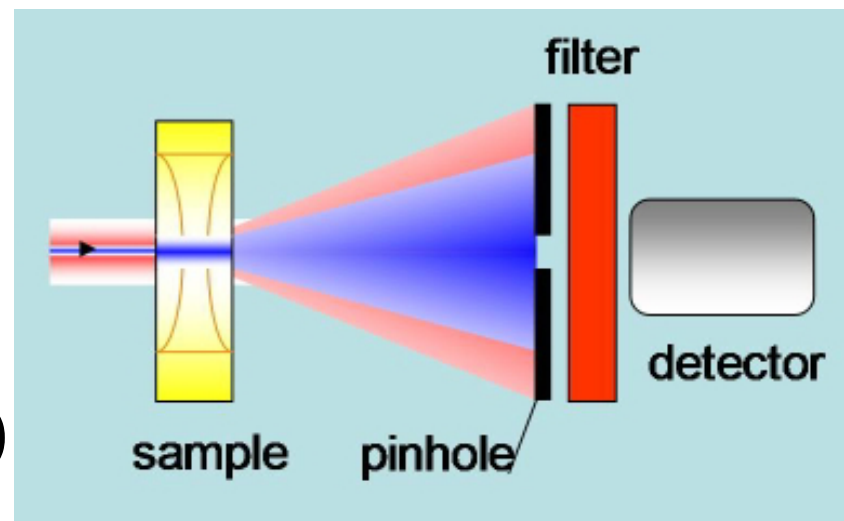
With the Sample
- Photo Reaction/Decomposition
- Concentration to high
    - non-linear (A > 2)
    - Aggregation
    - Refractive index change
- Air bubble generation

With the Cuvette + Solvent
- Cuvette non-uniformity
- Sample holder mobility
- Lensing (abs + heat)
- Temperature (line broadening)

With the Instrument
- Lamp Stability
- Room Lighting
- Noise

Any Questions?