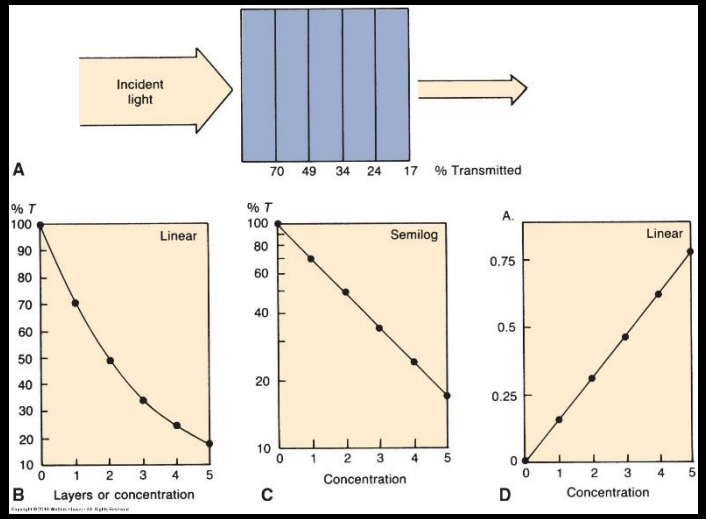
LIGHT BASED METHODS

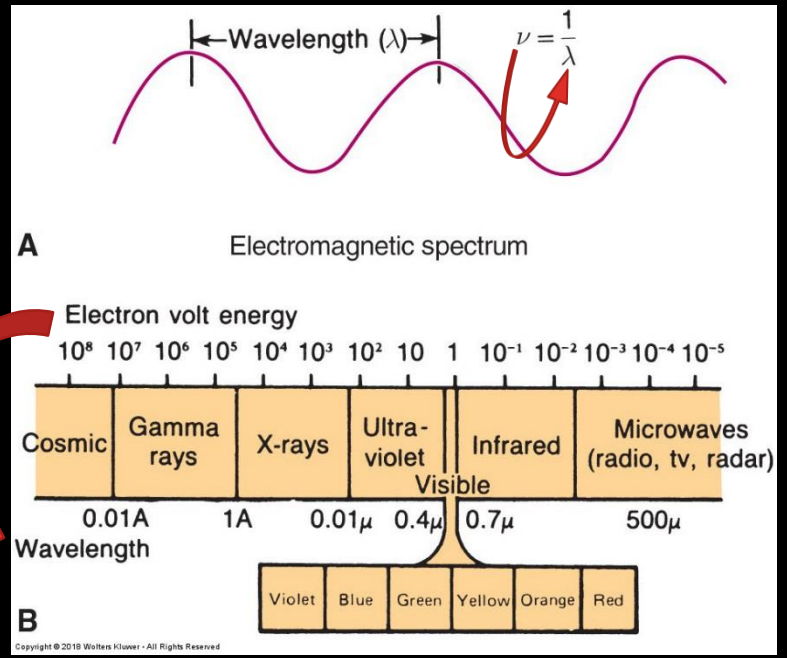
Ryan Collison MLS (ASCP)^{CM}

NATURE OF LIGHT

- Inverse relationships
- UV and visible range most useful
- Substances in solution absorb light
  - Beer's Law A=2-log(%T)



λ ↗ eV↘

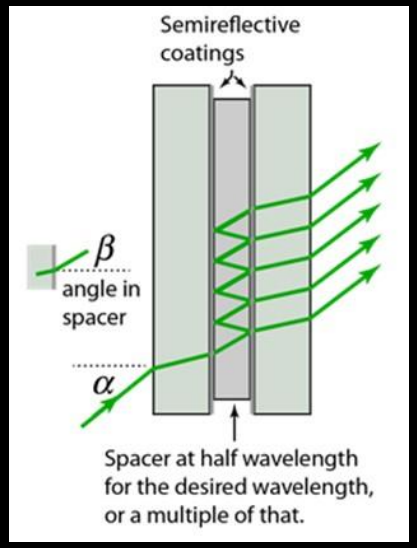


PRODUCTION OF LIGHT

Tungsten/ Tungsten-halogen	Hydrogen/ Deuterium	Mercury (vapor) (low pressure)	Mercury/Xenon (Arc) (high pressure)	Hollow Cathode Lamp
Most common light source, halogen extends life	Continuous spectra of UV light down to 165 nm	Emits a line spectrum in UV and visible	Continuous spectrum UV to mid-visible	Provides emission spectra of element
Near UV-Visible light produced	Deuterium has longer useful life	Unless you're measuring at that wavelength, useless for measurements	Frequently used in UV applications	Used for atomic absorption spectroscopy
Does not work <320nm	Frequently used in UV applications	Useful for calibration		Lamp contains element of interest
Requires heat-cut filter				

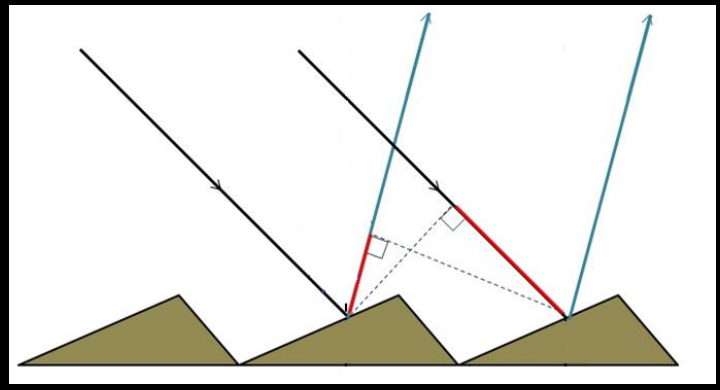
MANIPULATION OF LIGHT

- Monochrometers (wavelength selection)

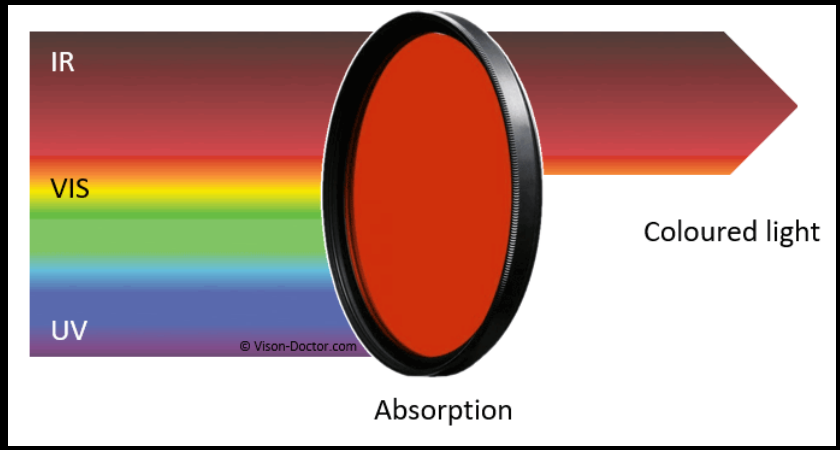


Interference filters produce light with multiples of a given λ. Needs later filters.

Prisms separate light



Diffraction gratings are the most common

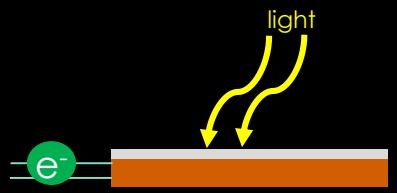


Wide band but at low intensity

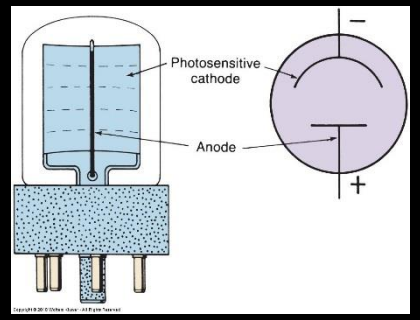
DETECTION OF LIGHT

Increasing Complexity

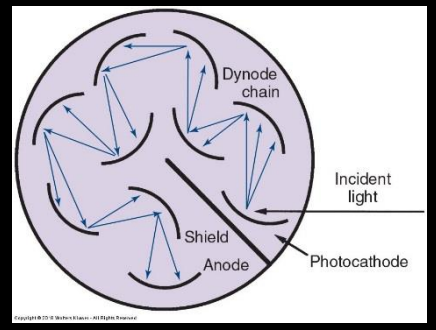
Barrier layer cell: light sensitive material generates electrons



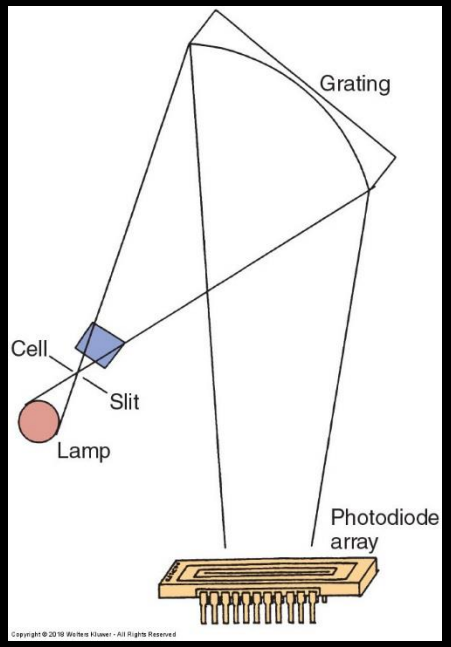
Phototube: similar, but requires voltage input



PM Tube: Series of dynodes amplify signal, extremely sensitive

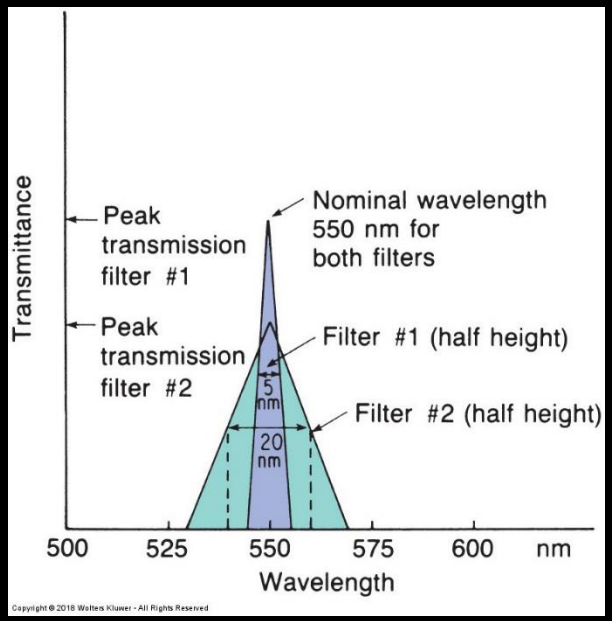


Photodiode array: series of diodes over spectrum can measure whole spectrum but, needs more light



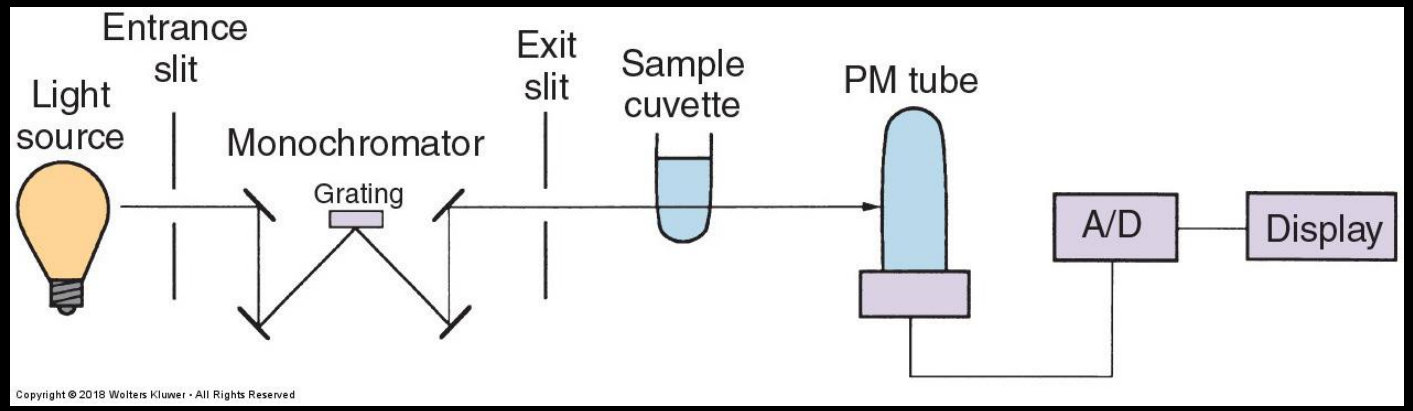
DETECTION OF LIGHT

- Metrics of light detection
  - Bandpass
    - A function of monochrometers
  - Stray light
    - Ideally zero
  - Dark current
    - Always present



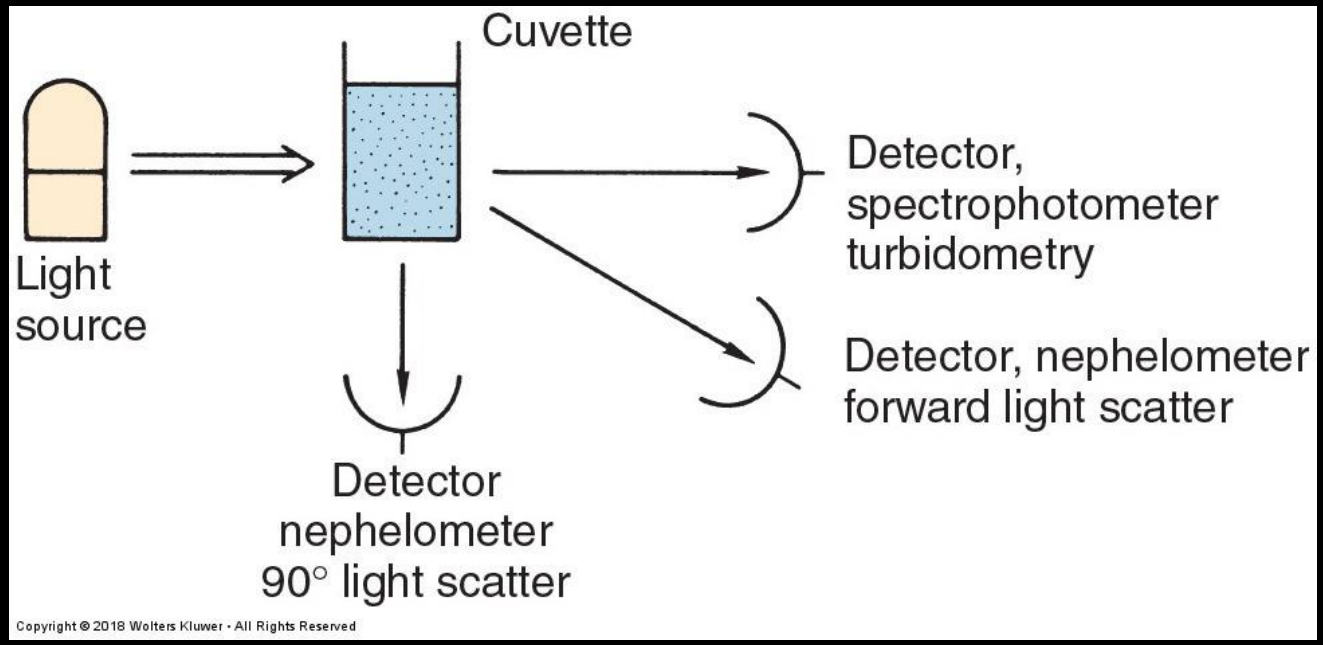
INSTRUMENTS AND APPLICATIONS

- Absorbance Spectrophotometry



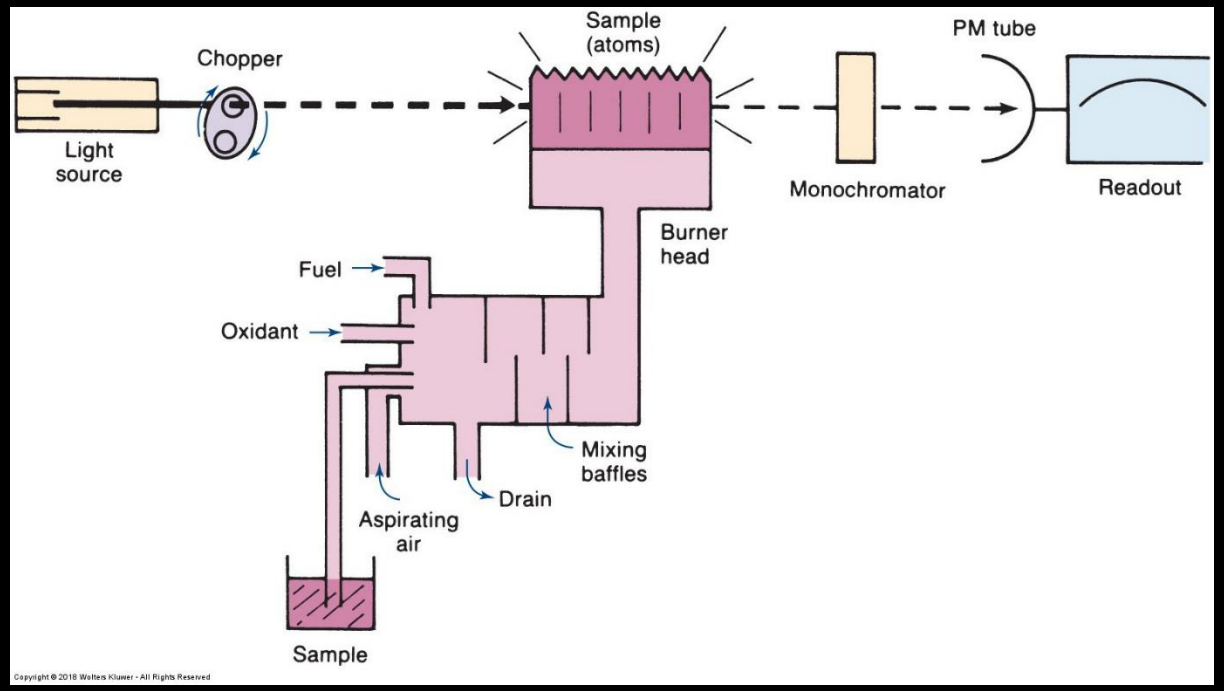
INSTRUMENTS AND APPLICATIONS

- Turbidmetry, nephelometry



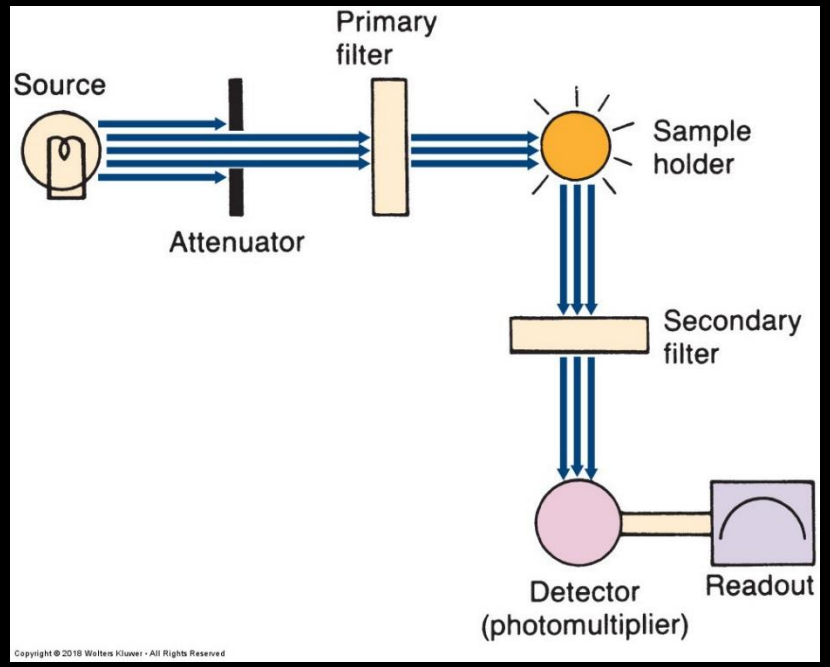
INSTRUMENTS AND APPLICATIONS

- Atomic Absorption Spectroscopy
  - Flameless with graphite furnace



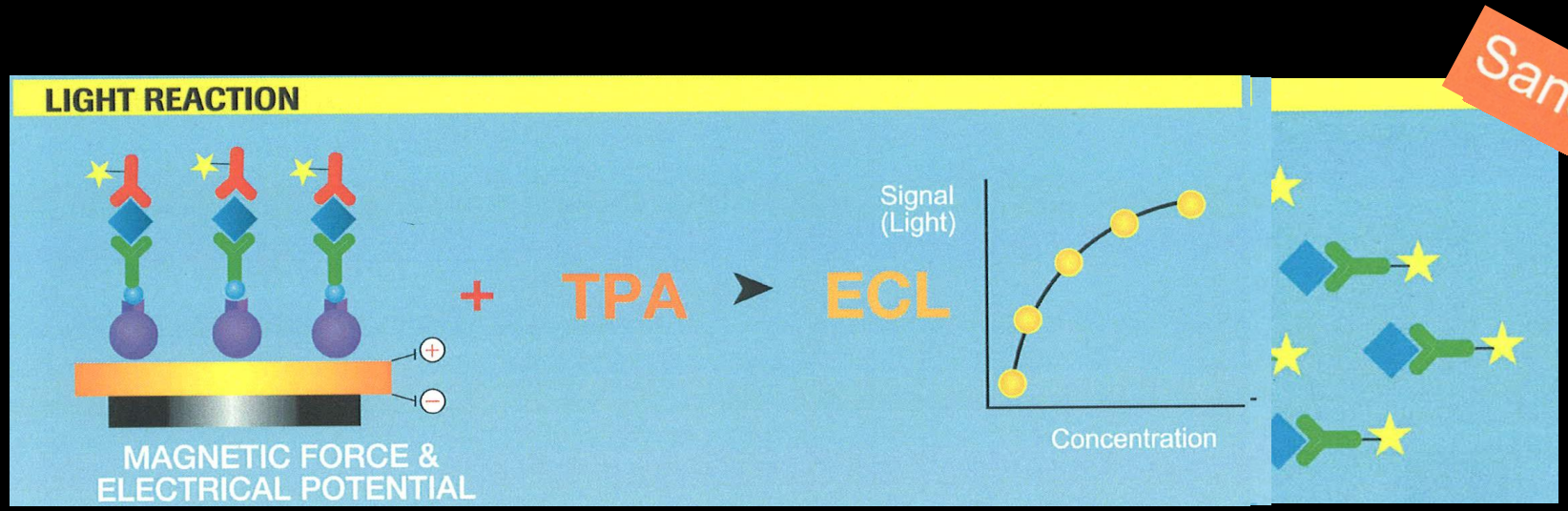
INSTRUMENTS AND APPLICATIONS

- Fluorometry
  - Emitting light of longer wavelengths than those absorbed
  - Sensitivity and specificity



INSTRUMENTS AND APPLICATIONS

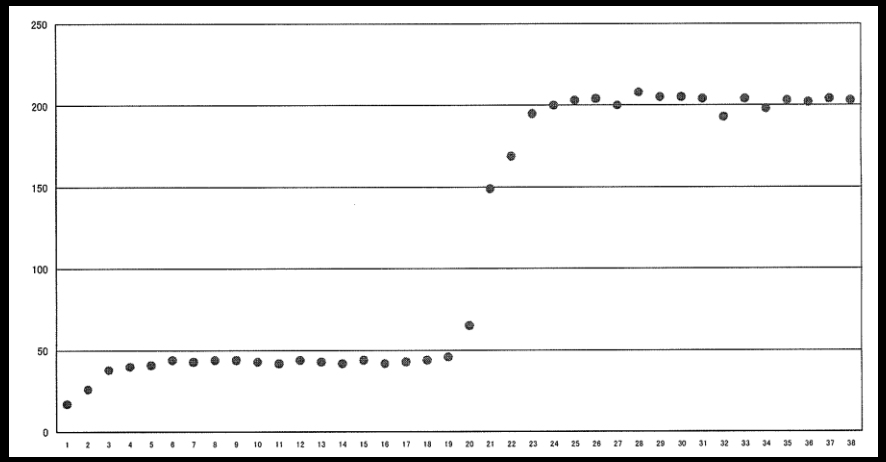
- Chemiluminescence (competitive and non-competitive)



Sandwich Principle

ENSURING QUALITY RESULTS

- Wavelength Accuracy
  - Didymium or holmium oxide
- Stray Light
- Sample Blanks
  - Specific to specimen
- Reagent Blanks
  - Specific to reagent lot
- Bichromatic analysis
  - Measure at secondary λ



Cobas 8000 absorbance readout