12. Biochemistry-2

Physical Measurements of Biochemical Quantities

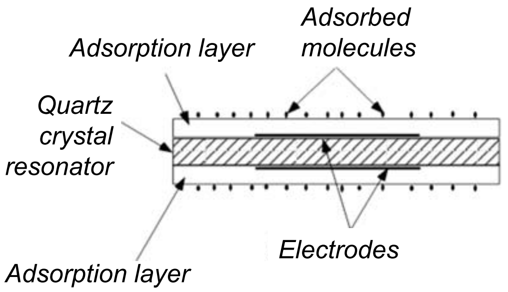
- *Acoustic sensors*
- *Magnetic sensors*
- *Optical sensors*
- *SpO_2*

Mass Sensors

- The amount of a substance can be measured by its mass.
- Weighing a mass is called gravimetry.
- To weigh a very light mass, a microbalance is used.
- If a substance is deposited on a mechanical resonator, the mass will be estimated from the change in its resonance frequency.
- If a substance is uniformly deposited on the surface of a mechanical element through which sound propagates, the propagation pattern will be changed, and thus the mass is estimated from a change in the propagation parameter, such as the sound velocity.
- A mechanical device in which the amount of a substance is measured by its mass is called the mass sensor, or gravimetric sensor.

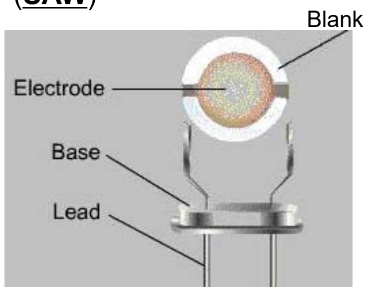
Quartz Crystal Resonator

Bulk Acoustic Wave (**BAW**)
Surface Acoustic Wave (**SAW**)



selectively adsorb specific substance

(a) cross sectional view



(b) actual device

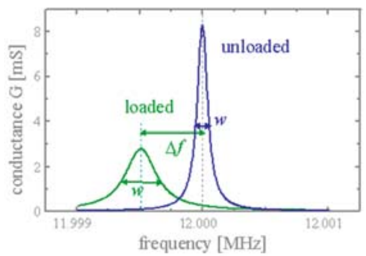
Shear mode or thickness shear mode.
When a substance is adsorbed on it, the mass is loaded to the resonator so that the resonance frequency decreases.

Shift in Resonant Frequency

- Resonance frequency varies according to the mass loaded onto the resonator surface.

Sauerbrey equation

shift in resonant frequency $$\Delta f = -2 f_0^2 \left(\rho_q \nu_q\right)^{-1} \Delta m$$



where
f_0 is resonance frequency
Δm is mass load per unit area
ρ_q and ν_q are density and shear wave velocity

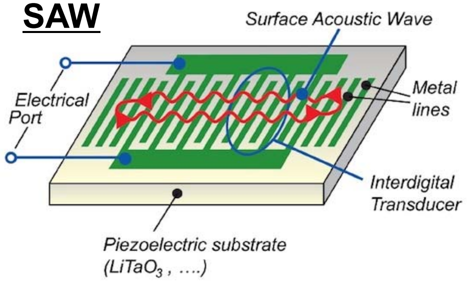
for a quartz crystal

$$\Delta f = -2.26 \times 10^{-6} f_0^2 \Delta m$$

QCM has extremely high sensitivity (500-2500Hz/μg) and has been widely used for detecting gases such as sulfur dioxide, carbon monoxide, hydrogen chloride, and aromatic and aliphatic hydrocarbons.

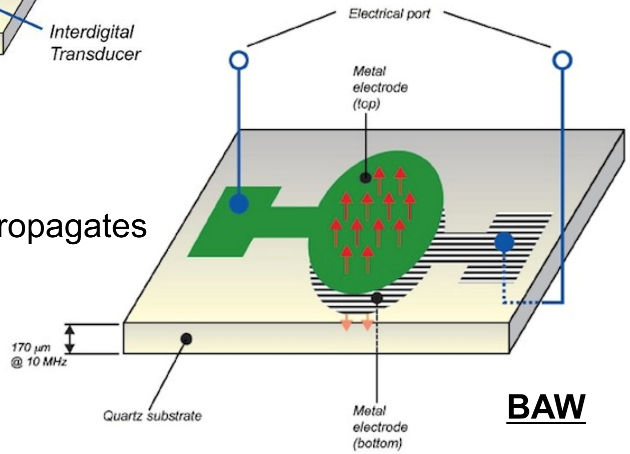
SAW and BAW

SAW



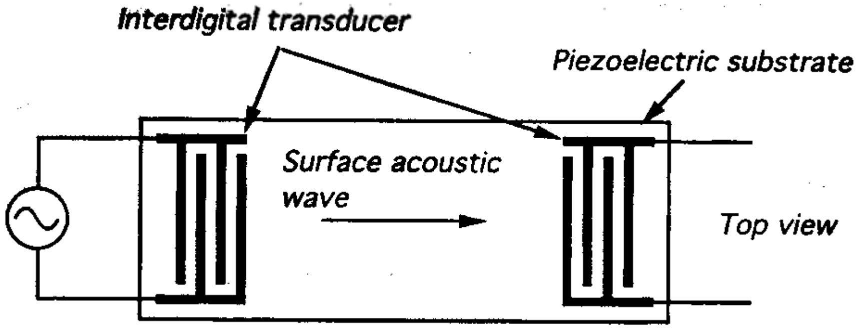
The acoustic wave propagates **horizontally**

The acoustic wave propagates **vertically**

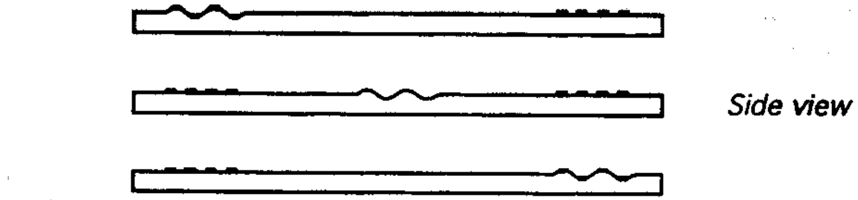


BAW

SAW Sensor

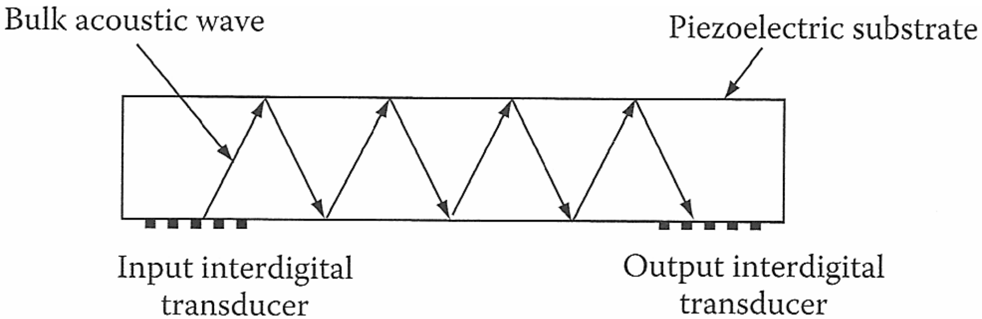


An elastic wave propagating along the surface of a piezoelectric substrate is generated by the electrodes deposited on the same side of the crystal in an interdigital form.



BAW Sensor

Reflection on both surfaces

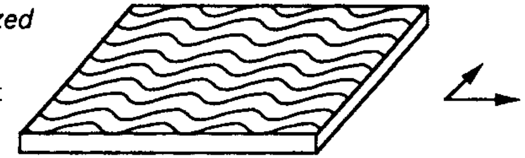


Different types are excited selectively using different **crystalline materials**, **interdigital electrode separation**, and the **operating frequency**.

Elastic Waves

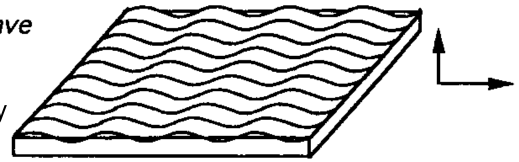
Horizontally polarized shear wave

no surface-normal component and propagate efficiently at a solid-liquid interface.



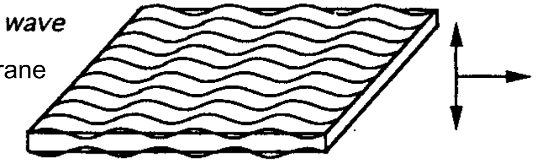
Rayleigh surface wave

a surface-normal component is strongly affected by the viscosity of the adjacent medium
not suited for use in liquid phase



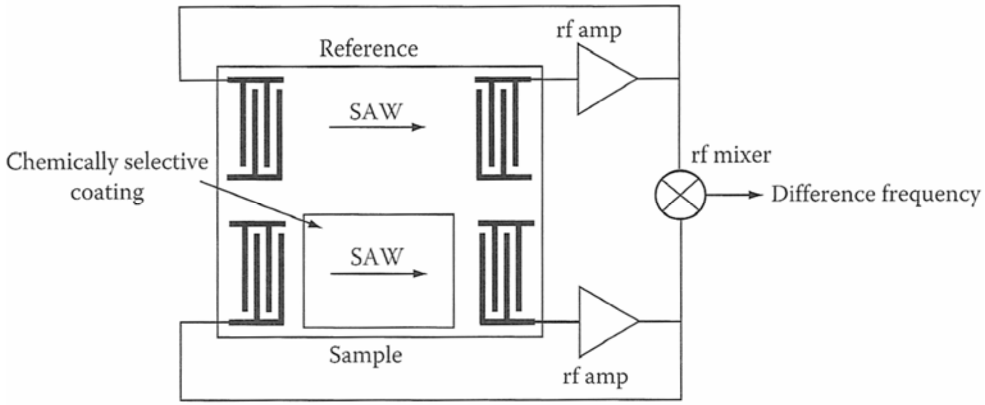
Symmetric Lamb wave

propagate in a thin membrane
higher sensitivity



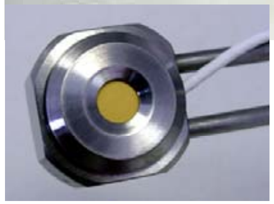
Differential Operation

One delay line has a surface coated by an adsorptive film while the other remains uncoated.



By using the **dual delay line** configuration, the effect of **temperature fluctuation** can be compensated for and the mass loading can easily be detected by the difference in frequency of both delay lines, which are usually on the order of kHz.

Quartz Crystal Microbalance (QCM)

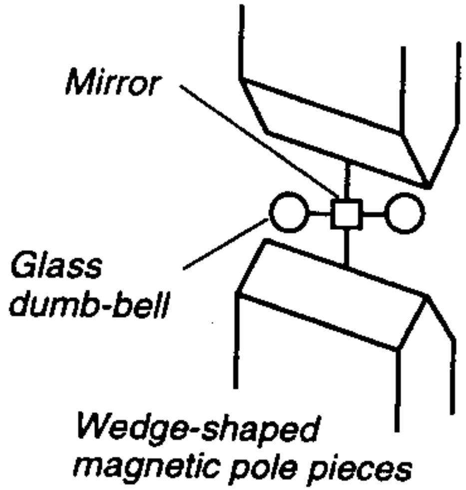


Oscillation Frequency	27 MHz
Sensitivity	30 pg/Hz
Dynamic Range	100 pg～10 μg

Magnetic Susceptibility

- A quantity that determines **magnetization** relative to magnetic field strength.
- When a specific substance in a sample exhibits strong magnetism but others do not, the concentration of that substance can be analyzed by measuring **magnetic susceptibility**.
- **O_2** gas is **paramagnetic** so that its magnetic susceptibility is **positive** and **large**, while **most other gases** in medical use (except N_2O) are **diamagnetic** so that their magnetic susceptibility is **negative** and **small**.
- Two types of paramagnetic pO_2 sensor

Paramagnetic pO_2 Sensor

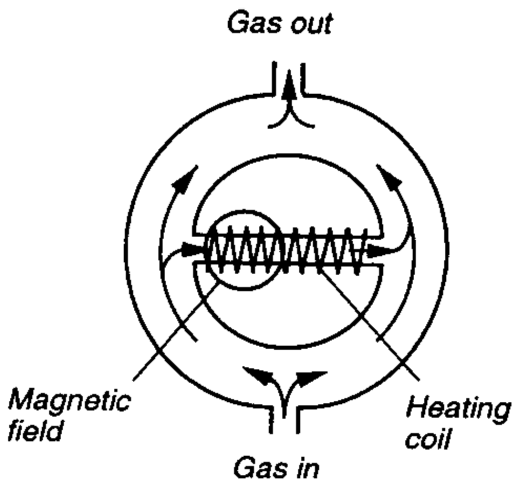


(a) *Dumb-bell type*

- A **glass dumb-bell** is suspended by an elastic wire and stays in equilibrium at the region where the **magnetic field** is strongest in a container with a diamagnetic gas.
- When the dumb-bell is surrounded by a **paramagnetic gas**, the gas tends to move into the region where the magnetic field is stronger, pushing the dumb-bell out from that region.
- **Oxygen content** in surrounding gas can be measured as the **rotation angle** of the dumb-bell that is detected optically

Paramagnetic pO_2 Sensor

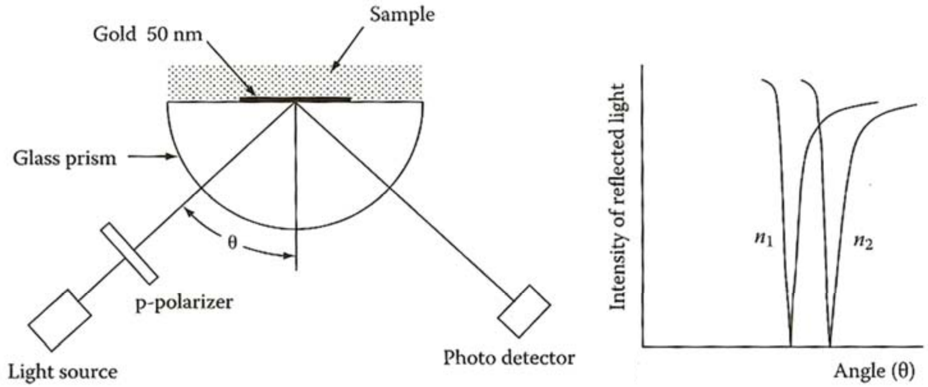
- A central **tube** with a **heating coil**, and a **strong magnetic field** is situated at left side.
- According to **Curie's law**, the **magnetization** of a paramagnetic substance is **inversely proportional** to the **absolute temperature**.
- A **paramagnetic gas** flows from the **cold side** to the **hot side**. The **oxygen content** is then measured by the **gas flow rate** in the tube.



(b) *Gas-flow-detecting type*

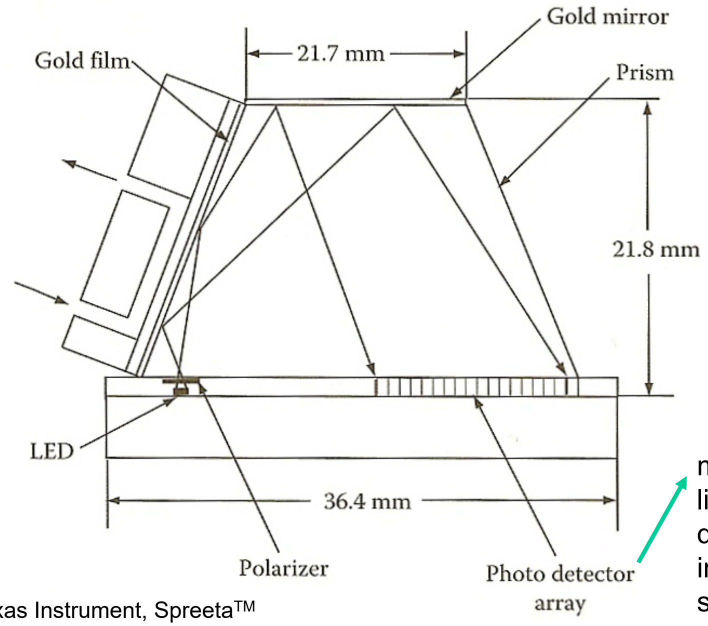
Surface Plasmon Resonance

SPR occurs at a **metal surface** when an **incident light beam** strikes the surface at a **particular angle**. The **condition** depends on the **dielectric property** of the **outside medium** near the metal surface.
The **dielectric property** is not very specific for recognition of molecules. If the metal surface is treated **affinity for specific molecules**, SPR becomes highly selective and can be detected by **reduction of reflected light intensity**.



(a) measurement setup   (b) reflected light intensity vs. incident angle

SPR Sensor – Photo Detector Array



measures reflected light intensities for different angles of incidence simultaneously

Texas Instrument, Spreeta™

Fiber Optic Sensors

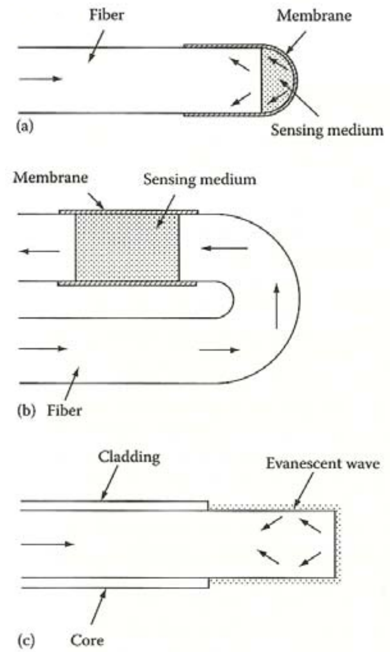
(a) Extrinsic sensor at fiber tip

Light is led by the optical fiber to and from the sensing medium

(b) Extrinsic sensor with U-shaped fiber

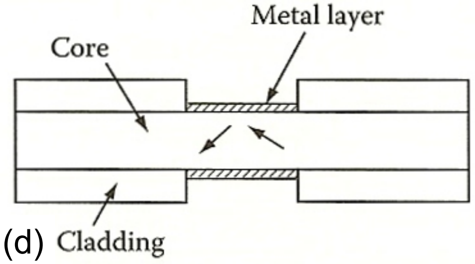
(c) Intrinsic sensor with Evanescent wave sensor

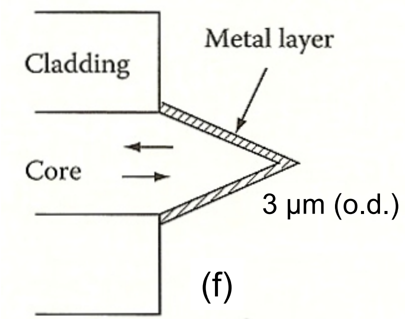
- A part of cladding layer on the optical fiber is removed leaving the core exposed to the measurand.
- The light guided by the probe has an evanescent wave component extending into the medium surrounding the optical fiber.
- Chemical reactions take place in the medium and in a surface layer, such as an immense assay.

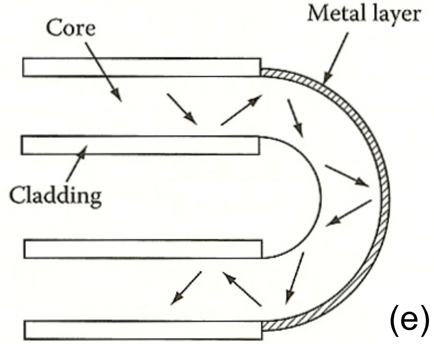


Fiber Optic Sensors

A pair of optical fibers or fiber bundles is used for **excitation** and **detection**. Both the **exciting light** and the **detected signal** can be transmitted along the same optical fiber.





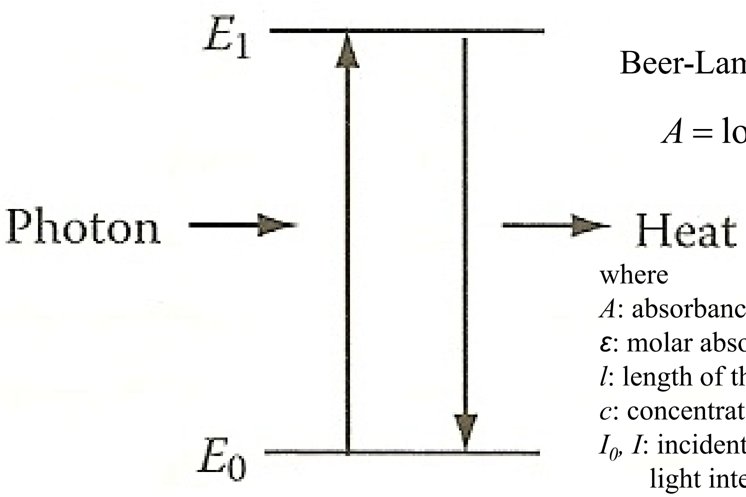


Spectrophotometric Analysis

- Different substances have different colors → possible to identify chemical species optically → spectrophotometric techniques.
- According to quantum theory, every molecule, ion, or atom has **a unique set of discrete energy levels**.
- When a transition from lower to upper energy levels occurs, **energy** equal to the difference between two energy levels has to be **supplied**.
- At a transition from upper to lower level, the same **energy** is **released**.
- The **interaction** between **light** and **molecules**, **ions**, or **atoms** demonstrates different optical phenomena such as **absorption**, **emission**, **fluorescence**, and the **Raman effect** are used in spectrophotometric analysis.

Optical Sensing - Absorption

When a light beam of selected wavelength passes a layer of the object, the power of the light beam is attenuated exponentially in a uniform media according to the relation known as **Beer-Lambert's law**



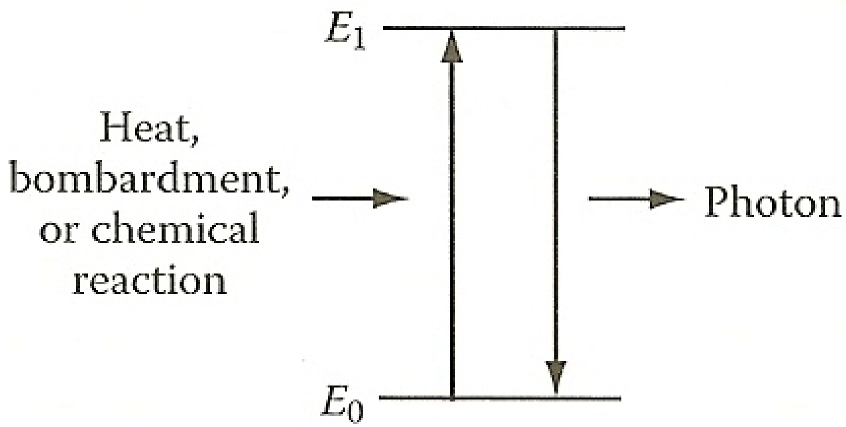
Beer-Lambert's law

$$A = \log \frac{I_0}{I} = \varepsilon l c$$

where
A: absorbance
ε: molar absorptivity
l: length of the optical path
c: concentration
I_0, I: incident and transmitted light intensities

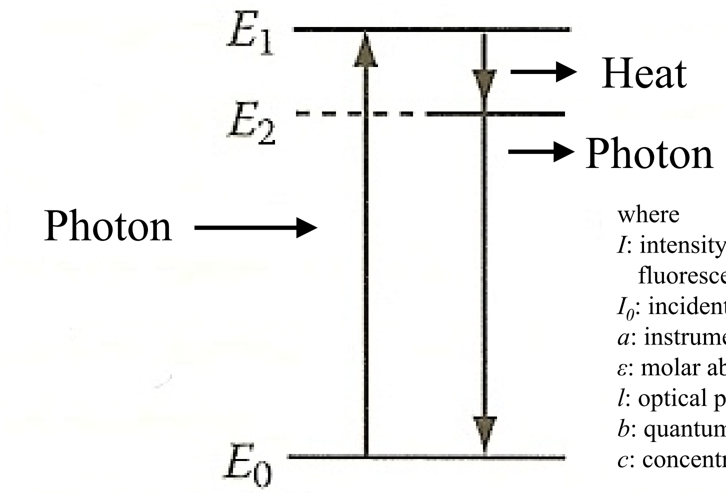
Optical Sensing - Emission

molecules, ions, or atoms are excited to **higher energy levels** by appropriate processes such as heating in a flame or an arc, bombardment of electrons or ions, or exposure to electromagnetic radiation.



Optical Sensing - Fluorescence

The medium is excited optically at a short wavelength and the fluorescence occurs at a longer wavelength.
When the life time of the fluorescence is **longer**, the phenomenon is called the **phosphorescence**.



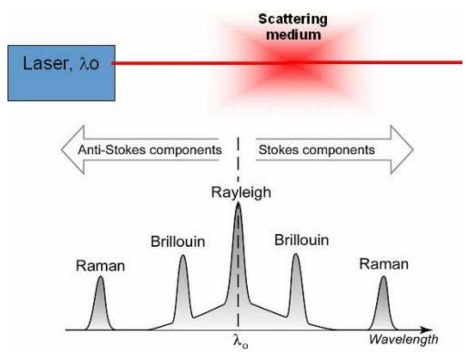
$$I = aI_0\varepsilon blc$$

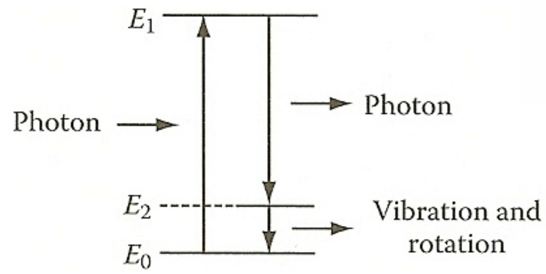
where
I: intensity of the measured fluorescence
I_0: incident light intensity
a: instrument constant
ε: molar absorptivity
l: optical path length in the sample
b: quantum yield of fluorescence
c: concentration of the analyte

direction of light is altered by interacting with small objects

Optical Sensing - Scattering

Rayleigh and Mie scattering → the **wavelength** of the radiation is **not changed**.
Rayleigh scattering is generated at **atomic** and **molecular levels** and dominates in **small particle**.
Mie scattering occurs in **large** particle.
The scattered light component is strongly forward directed and is related to the **concentration** of the scattering particles.





Raman effect generates scattered light with **different wavelength** from that of the incident light.
The shift in the scattered light occurs due to the transition to the **vibrationally or rotationally excited energy level**.

A **set of different wavelength or frequency components** corresponds to **different energy levels of molecular vibration and rotation** ->> the **spectrum of the Raman shift** provides information of the molecular structure.

Optical Sensing - Summary

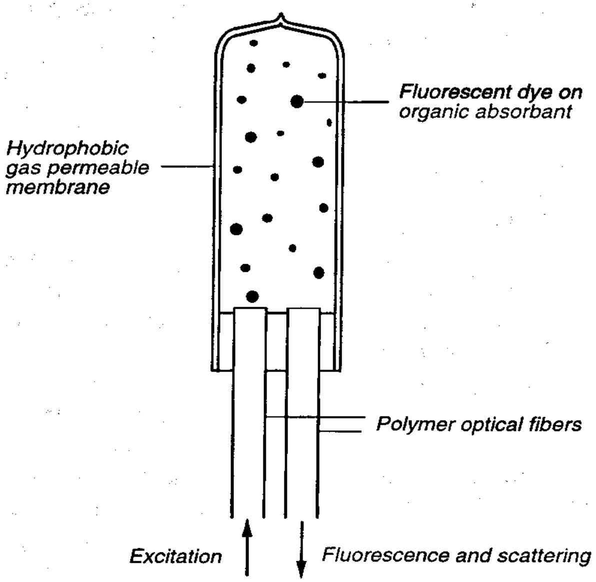
- Absorption, reflection, scattering, and fluorescence → **radiation source**.
- Spectrum of **visible** and **near-infrared** regions ← a tungsten filament lamp or halogen lamp.
- Spectrum of **ultraviolet** (UV) region ← a hydrogen or deuterium lamp.
- Spectrum of **far-infrared** region ← a heated inert solid.
- Monochromatic beam → a prism or a reflection grating.
- Modulating light source + Fourier transform of detected signal → absorption spectrum without using a monochromator → **Fourier-transform spectrometer**.
- Photo detectors = **photomultiplier tube**, **photodiode**.

Quantities and Sensing Principles

Quantities to be Measured	Absorption Transmission	Emission Fluorescence	Fluorescence Quenching
pH	✓	✓	
pO_2		✓	✓
pCO_2	✓	✓	
Glucose		✓	
UO_2^{++}(uranyl)		✓	
Plutonium(III)			✓
Berylium(II)		✓	
Sodium ion		✓	
Metal cations		✓	
Halide ion		✓	✓
Iodine			✓
H_2O_2		✓	
Ammonia	✓		
HCN(vapor)	✓		
Halothane			✓
Immunoassay	✓	✓	
Phosphate esters	✓		
Moisture	✓		

Quenching refers to any process which decreases the fluorescence intensity of a given substance.
A variety of processes can result in quenching, such as excited state reactions, energy transfer, complex-formation and collisional quenching.
Quenching depends on pressure and temperature.
Molecular oxygen, iodide ion, chloride ion and acrylamide are common chemical quenchers.

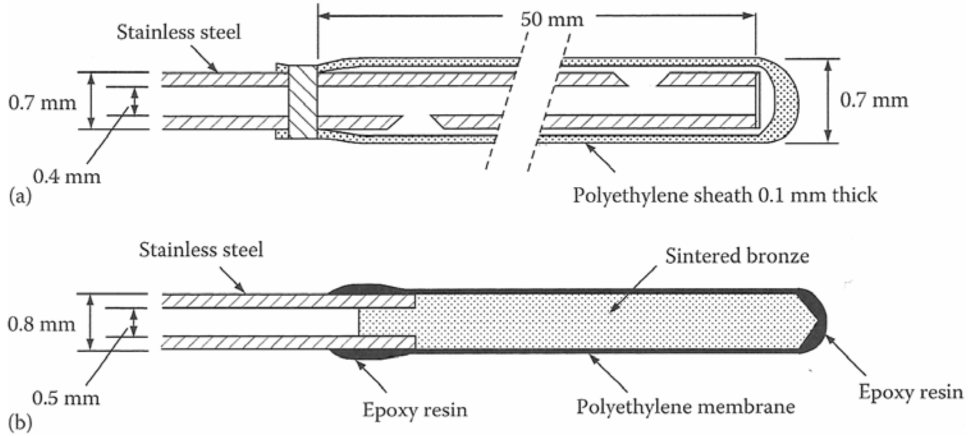
Fiber Optic Intravascular pO_2 Catheter based on Fluorescence



A fluorescent dye, such as perylene dibutyrate, adsorbed in organic beads and contained within a hydrophobic membrane. The dye is excited with blue light at 486nm and it emits fluorescence at 514nm. The fluorescence is quenched by the presence of O_2 and the effect depends on pO_2, so that pO_2 can be determined by the intensity ratio between excitation and the fluorescence.

Catheters-type Mass Spectrometer

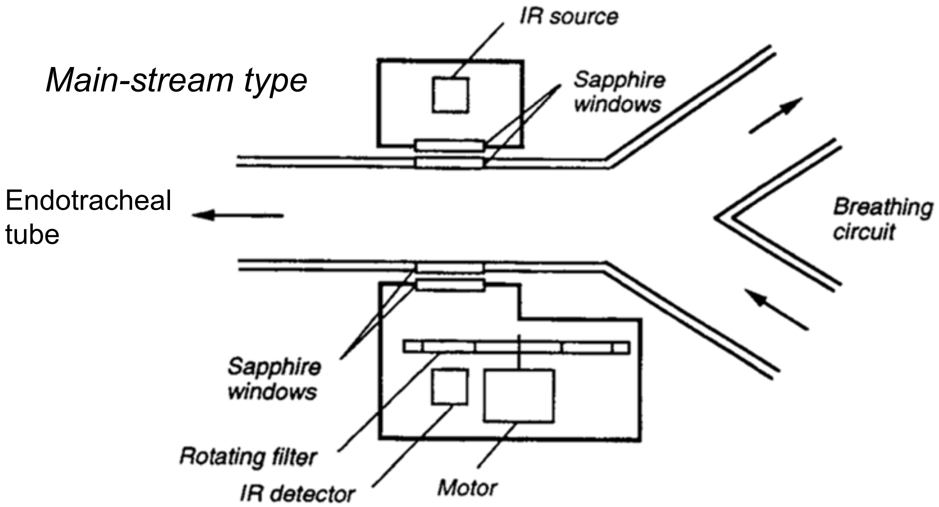
Gas diffusion through a membrane at the tip.
Different gases such as O_2, CO_2, and N_2 can be measured intravascularly and simultaneously



A stainless steel can prevent the diffusion of gases into the catheter, but too inflexible. A sintered bronze plug is used as a membrane support. Polyethylene and silicone rubber are generally used as the membrane.

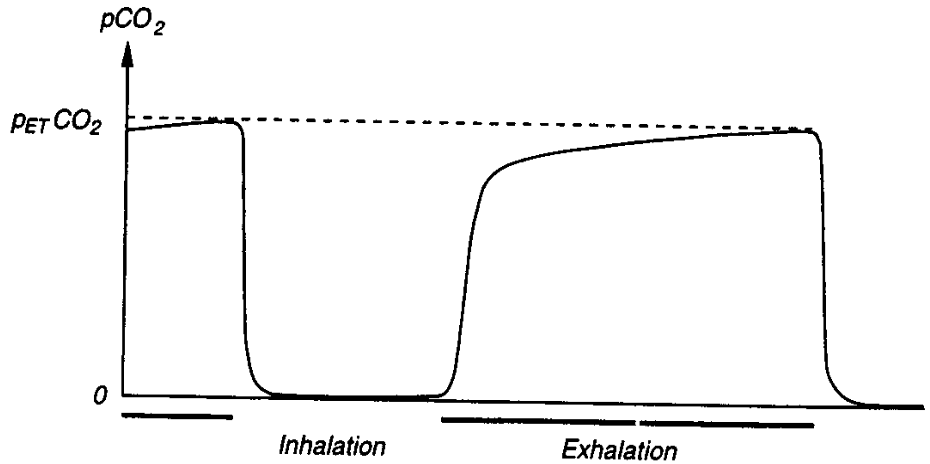
Capnometer (pCO$_2$ Monitor)

pCO$_2$ in expired gas provides information whether the lung ventilates well.
Inspired gas is free of CO_2, thus pCO$_2$ near the mouth varies with the breathing cycle.
Capnometer → instantaneous analysis of pCO$_2$ in expired gas by infrared absorption at a wavelength of 4.26 um or by a mass spectrometer.

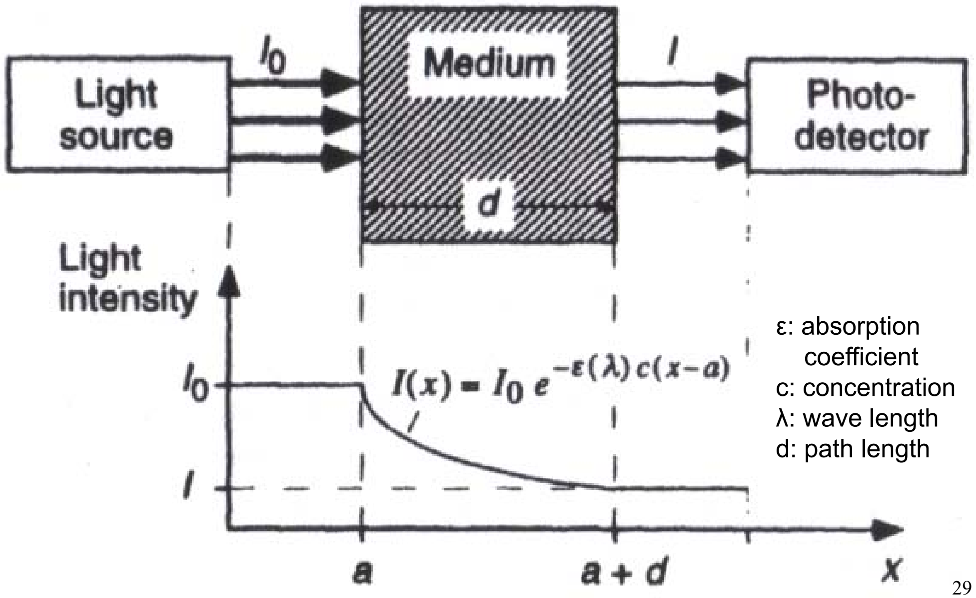


Capnogram

In the exhalation phase, pCO$_2$ increases rapidly due to the mixing of dead space gas with alveolar gas, and then reaches a plateau. At the end of exhalation, pCO$_2$ reaches the maximum which is called the **end-tidal CO$_2$ tension**, or p_{ET}CO$_2$. p_{ET}CO$_2$ indicates the changes in metabolism, pulmonary circulation, and alveolar ventilation.



Beer-Lambert Law



ε: absorption coefficient
c: concentration
λ: wave length
d: path length

Spectrophotometer

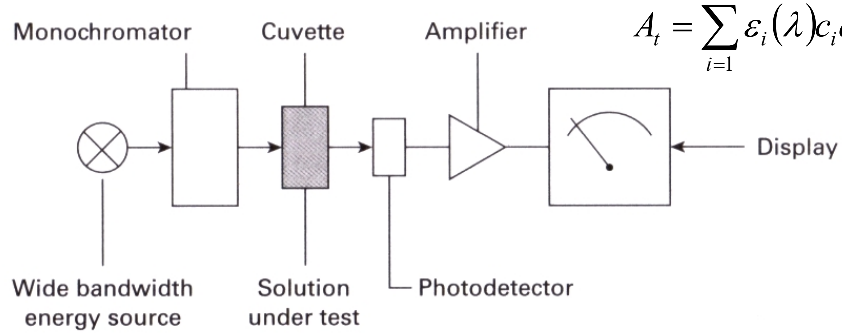
N mixed components can be analyzed using *N* wavelengths of light sources.

- Single or multiple unknown substances
- Clear, non-turbid solution
- Constant path length
- No photochemical reaction
- No reaction between absorbent and solvent

$$T = \frac{I}{I_0} = e^{-\varepsilon(\lambda)cd}$$

$$A = -\ln T = \varepsilon(\lambda)cd$$

$$A_t = \sum_{i=1}^{n} \varepsilon_i(\lambda)c_i d$$



Functional Hemoglobins

- Binding oxygen in the pulmonary capillaries and releasing oxygen in the systemic capillaries
- Oxyhemoglobin(HbO_2)-fully saturated with oxygen
- Reduced hemoglobin(Hb)-partly saturated with oxygen
- Functional oxygen saturation (Functional SO_2)

$$\frac{[HbO_2]}{[HbO_2]+[Hb]} \times 100\% = \frac{c_{HbO_2}}{c_{HbO_2}+c_{Hb}} \times 100\%$$

- Arterial blood ->functional arterial oxygen saturation →functional SaO_2 →SaO_2 →SpO_2

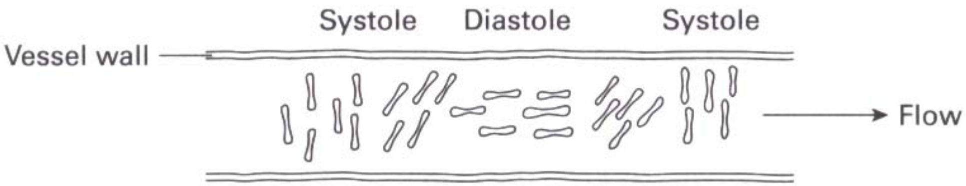
Dysfunctional Hemoglobins

- Dyshemoglobin-no oxygen transportation
- Four most common dyshemoglobins
  - Methemoglobin (MetHb), <0.6%
  - Carboxyhemoglobin (COHb), <2%
  - Sulfhemoglobin and Carboxysulfhemoglobin, not known
- Fractional oxygen saturation (Fractional SO_2)

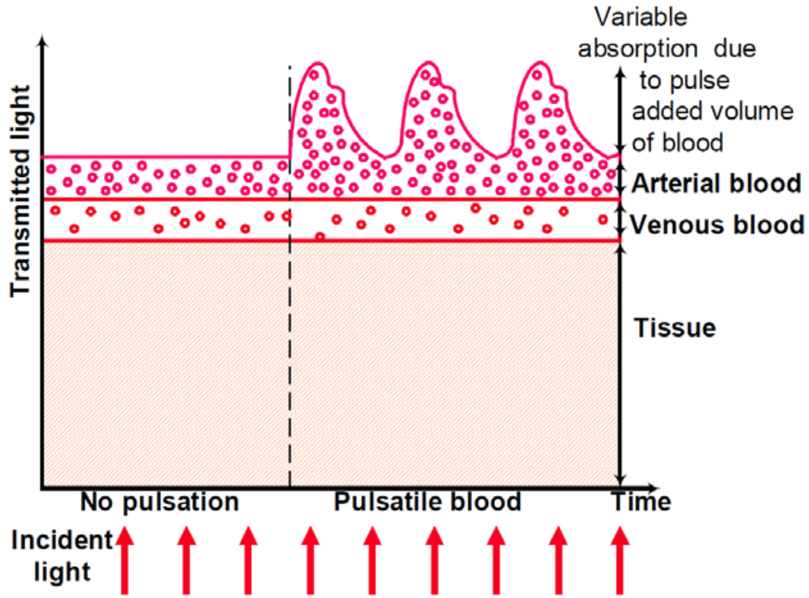
$$\frac{c_{HbO_2}}{c_{TotalHemoglobin}} \times 100\% = \frac{c_{HbO_2}}{c_{HbO_2}+c_{Hb}+c_{MetHb}+c_{COHb}} \times 100\%$$

Erythrocytes Behavior

- The axis of the erythrocytes changes during the cardiac cycle.
- Erythrocytes tend to align their major diameter parallel to the direction of flow during diastole, whereas perpendicular during systole, thereby a greater absorption path length.

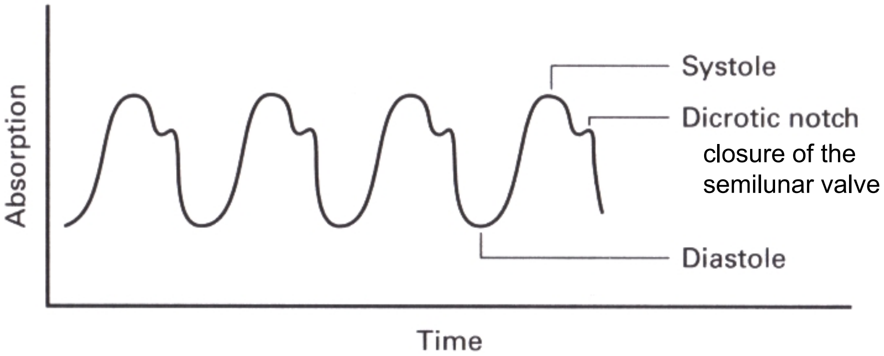


Blood Flow and Transmitted Light

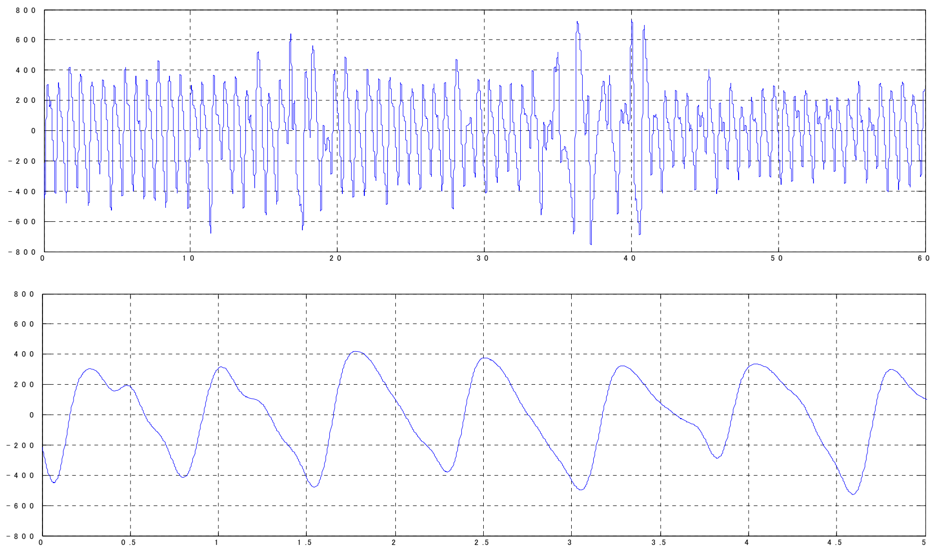


Photoplethysmogram (PPG)

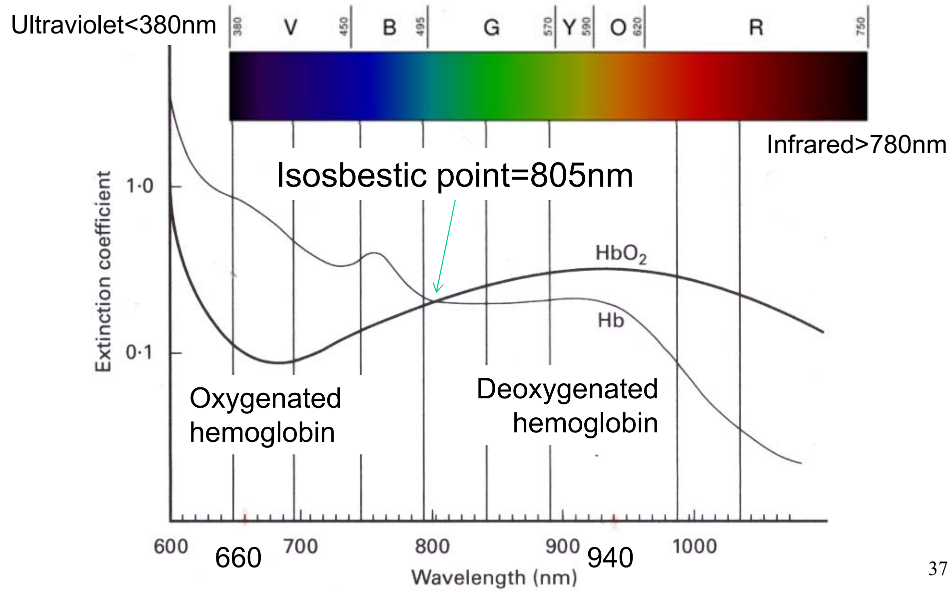
- Owing to the cardiac beating, the absorption of light due to the hemoglobin in the arteries and arterioles increases during systole and decreases during diastole.
- The light absorption through the finger varies by about 1-2% of the total absorption.



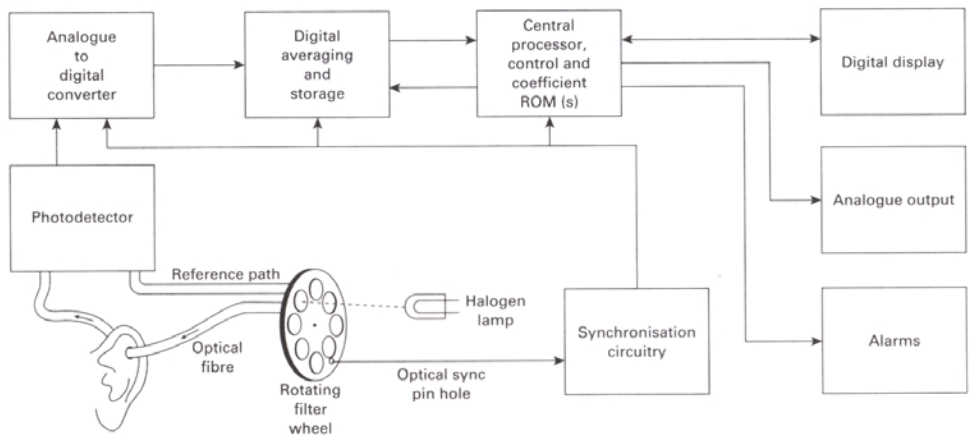
Real PPG Waveforms



Hemoglobin Absorbance at Different Wavelengths

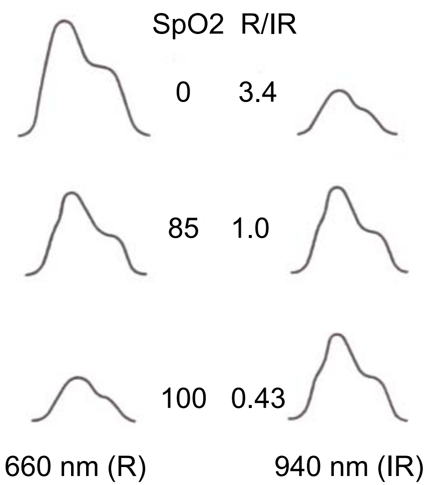


Hewlett-Packard Oximeter



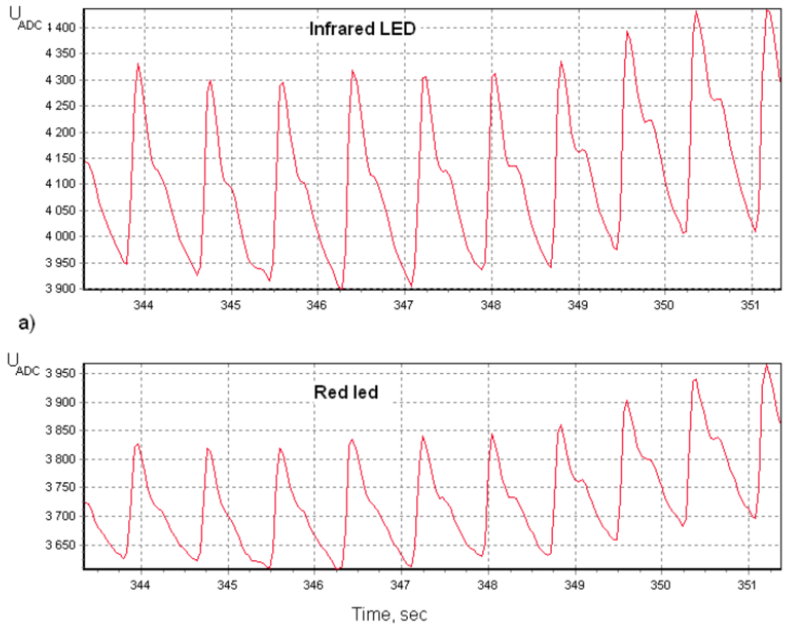
Absorbance at 2 Wavelengths

a comparatively large difference in the absorption between oxygenated and deoxygenated hemoglobin, yielding detectable changes in absorption with small changes in oxygen saturation, i.e. high sensitivity.



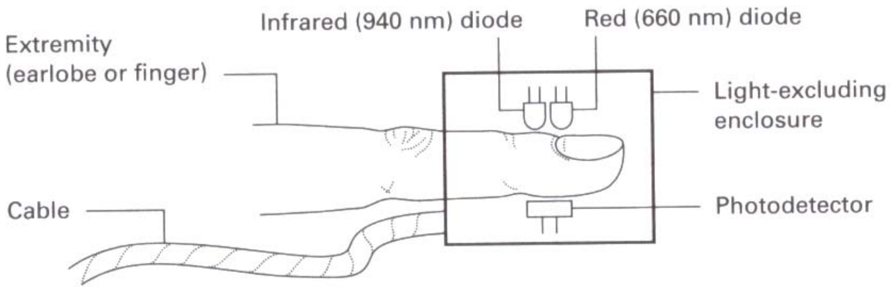
the absorption spectra are reasonably flat at this wavelength so that slight variations in the peak wavelength will make very little difference to calibration.

Waveforms at 2 Wavelengths

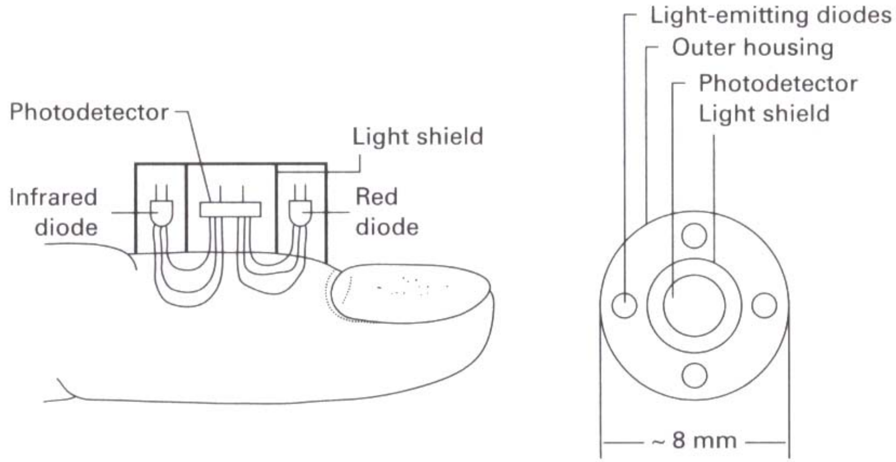


Transmittance Probe

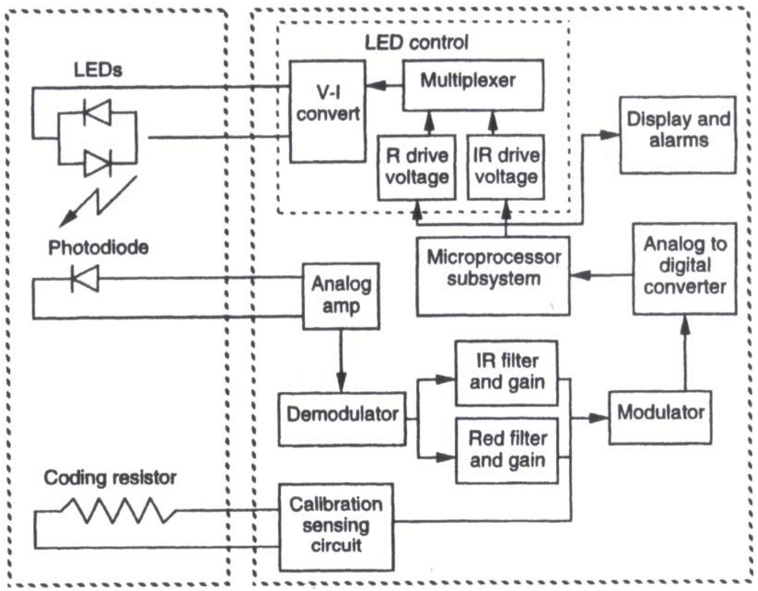
- The whole assembly must be protected from extraneous light over the range of wavelengths to which the detector is sensitive.



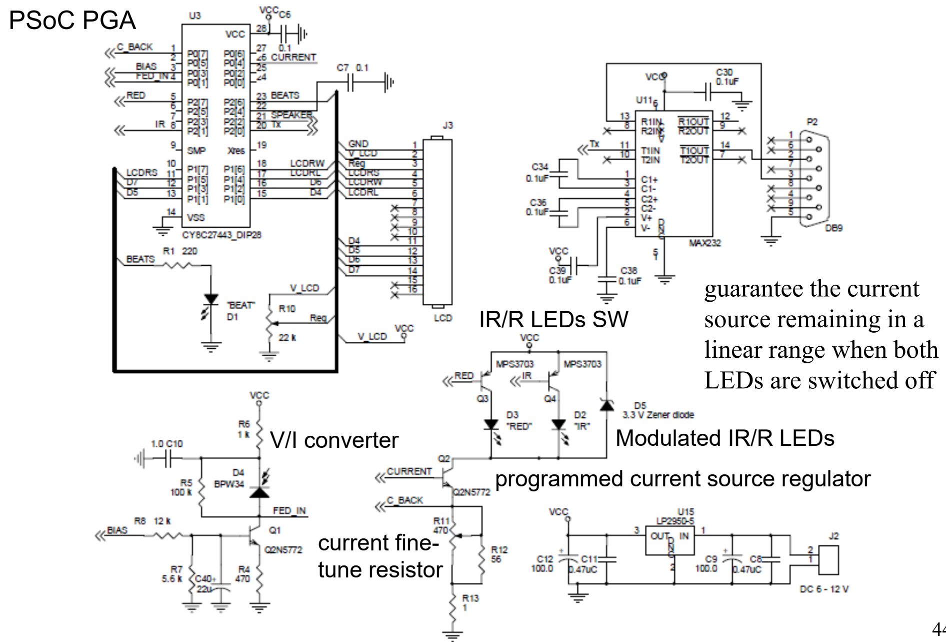
Reflectance Probe



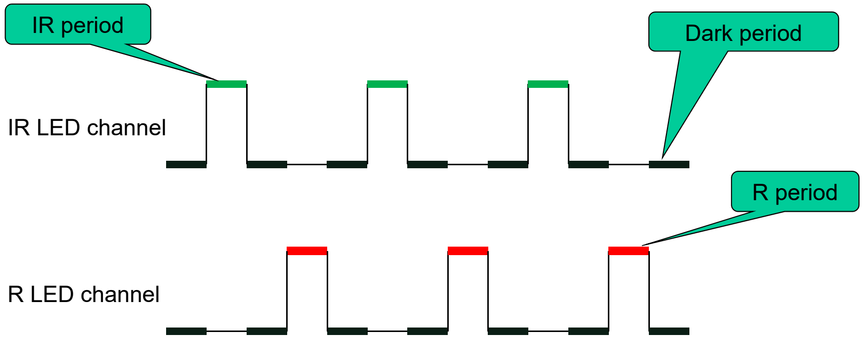
Probe and Circuit



Electronic Circuit



LED Driven Current



Drive signal is a 4kHz square wave generated by "Square Wave Generator". IR/R drive currents are synchronously in amplitudes but keep 180 degree offset in phase.
Two 2kHz square waves are generated a multiplexer to turn on IR/R LEDs sequentially.

SaO_2 Calculation

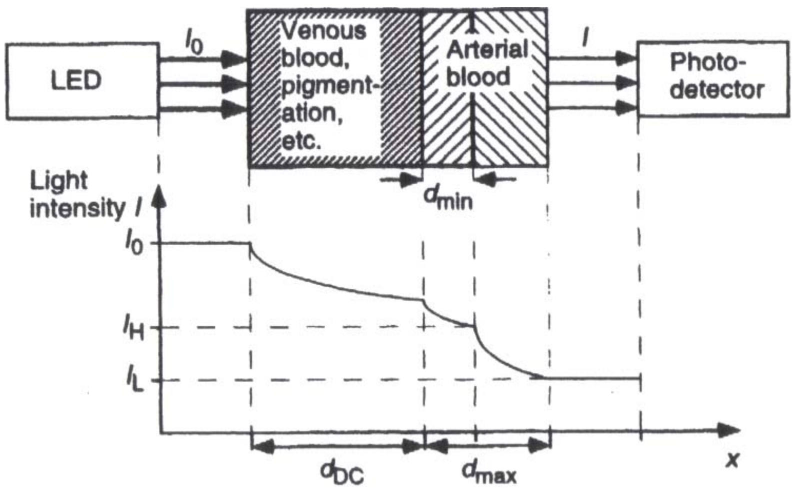
- Absorbencies of oxygenated and deoxygenated hemoglobin differ and vary dependent on incident light wavelength.
- Hemoglobin saturation

$$SaO_2 = \frac{HbO_2}{HbO_2 + Hb} \times 100\%$$

- Two unknown parameters: oxygenated hemoglobin (HbO_2) and deoxygenated hemoglobin (Hb).
- Two equations are required.
- Two wavelengths are used to measure absorption at two wavelength points and calculate outcome as oxygen saturation, i.e. SpO_2.
- SpO2 is usually derived by applying the "R/IR" ratio to a look-up table instead of various empirical equations.

Beer's Law in Blood Vessel

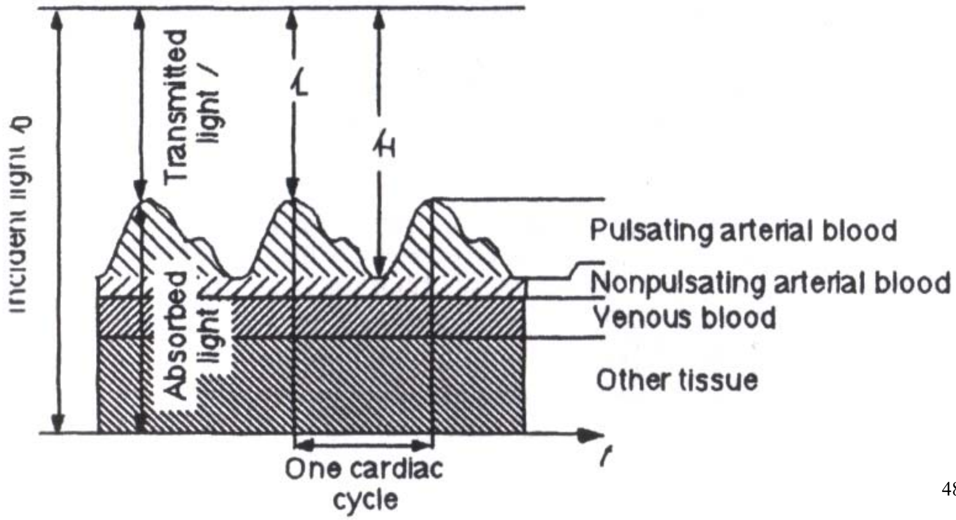
$$I(d) = I_H e^{-\left[\varepsilon_{Hb}(\lambda)c_{Hb} + \varepsilon_{HbO_2}(\lambda)c_{HbO_2}\right]\Delta d} \qquad d = d_{\min} + \Delta d$$



Minimal and Maximal Absorbance

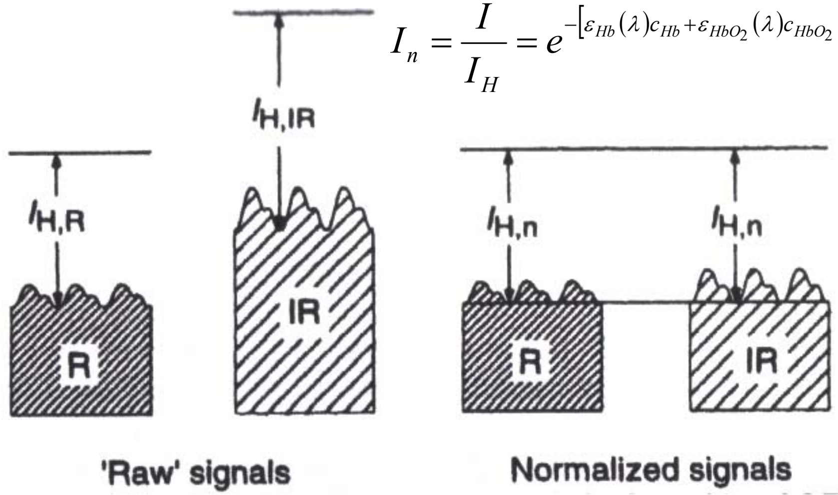
$$I_H = I_0 e^{-\varepsilon_{DC}(\lambda)c_{DC}d_{DC}} e^{-\left[\varepsilon_{Hb}(\lambda)c_{Hb} + \varepsilon_{HbO_2}(\lambda)c_{HbO_2}\right]d_{\min}}$$

$$I_L = I_0 e^{-\varepsilon_{DC}(\lambda)c_{DC}d_{DC}} e^{-\left[\varepsilon_{Hb}(\lambda)c_{Hb} + \varepsilon_{HbO_2}(\lambda)c_{HbO_2}\right]d_{\max}}$$



Signal Normalization

$$I_n = \frac{I}{I_H} = e^{-\left[\varepsilon_{Hb}(\lambda)c_{Hb} + \varepsilon_{HbO_2}(\lambda)c_{HbO_2}\right]\Delta d}$$



Hemoglobin Absorbance

- Four absorbent components
  - Oxy-hemoglobin
  - Deoxy-hemoglobin
  - Other hemoglobins (MetHb, COHb)
  - Unknown sources
- Total absorbance

$$A = \ln\left(\frac{I}{I_0}\right) = \varepsilon_{HbO_2}(\lambda)c_{HbO_2}d_{HbO_2} + \varepsilon_{Hb}(\lambda)c_{Hb}d_{Hb} + \varepsilon_x(\lambda)c_x d_x + A_0$$

Hemoglobin Absorbance

$$c_{HbO_2} = SO_2\left(c_{HbO_2} + c_{Hb}\right) \quad (1)$$

$$c_{Hb} = \left(1 - SO_2\right)\left(c_{HbO_2} + c_{Hb}\right) \quad (2)$$

$$A_t = \varepsilon_{HbO_2}(\lambda)c_{HbO_2}d_{HbO_2} + \varepsilon_{Hb}(\lambda)c_{Hb}d_{Hb} \quad (3)$$

Assuming $d = d_{Hb} = d_{HbO_2}$, and combining (1)-(3) to obtain (4)

$$A_t = \left[\varepsilon_{HbO_2}(\lambda)SO_2 + \varepsilon_{Hb}(\lambda)(1 - SO_2)\right]\left(c_{HbO_2} + c_{Hb}\right)d \quad (4)$$

SpO_2 Calculation

$$R = \frac{A_{t,R}}{A_{t,IR}} = \frac{\ln\left(I_{L,R}/I_{H,R}\right)}{\ln\left(I_{L,IR}/I_{H,IR}\right)} \quad (5)$$

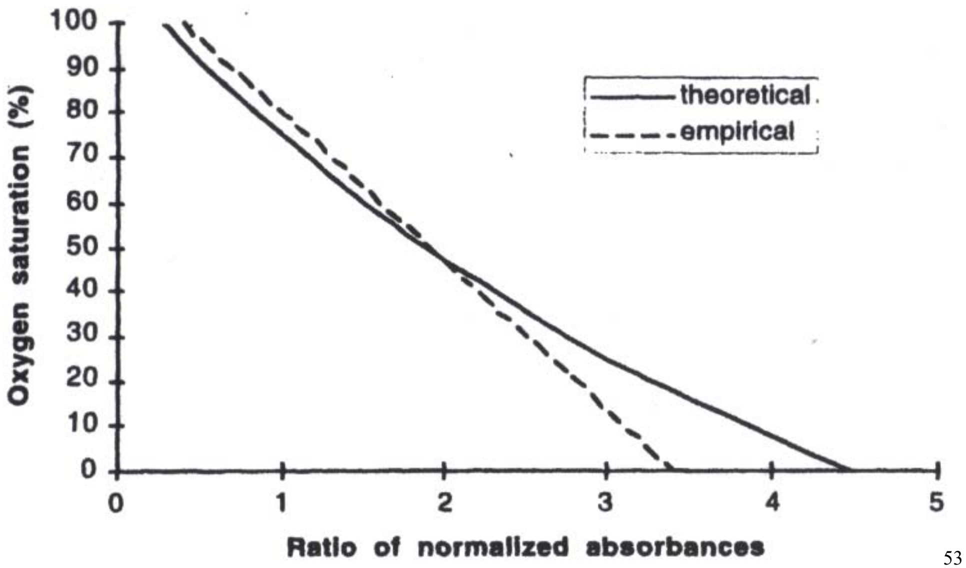
$$R = \frac{\left[\varepsilon_{Hb}(\lambda_R)c_{Hb} + \varepsilon_{HbO_2}(\lambda_R)c_{HbO_2}\right]\Delta d_R}{\left[\varepsilon_{Hb}(\lambda_{IR})c_{Hb} + \varepsilon_{HbO_2}(\lambda_{IR})c_{HbO_2}\right]\Delta d_{IR}} \quad (6)$$

$$R = \frac{\varepsilon_{Hb}(\lambda_R) + \left[\varepsilon_{HbO_2}(\lambda_R) - \varepsilon_{Hb}(\lambda_R)\right]SaO_2}{\varepsilon_{Hb}(\lambda_{IR}) + \left[\varepsilon_{HbO_2}(\lambda_{IR}) - \varepsilon_{Hb}(\lambda_{IR})\right]SaO_2} \quad (7)$$

Assuming $\Delta d_R = \Delta d_{IR}$, and combining (4) and (7) to obtain (8)

$$SpO_2 = \frac{\varepsilon_{Hb}(\lambda_R) - \varepsilon_{Hb}(\lambda_{IR})R}{\varepsilon_{Hb}(\lambda_R) - \varepsilon_{HbO_2}(\lambda_R) + \left[\varepsilon_{HbO_2}(\lambda_{IR}) - \varepsilon_{Hb}(\lambda_{IR})\right]R} \times 100\% \quad (8)$$

Calibration Curves



Empirical Method

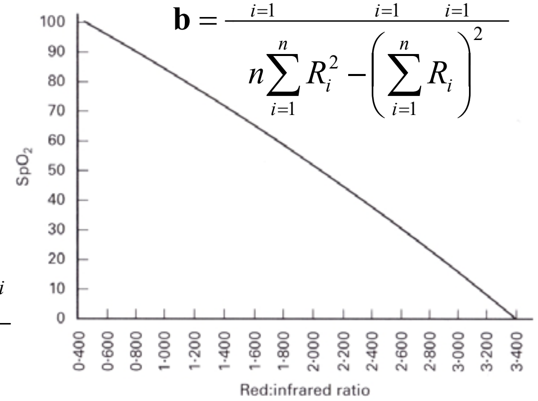
- Ratio of pulse-to-constant

$$R = \frac{AC_{RED}/DC_{RED}}{AC_{IR}/DC_{IR}}$$

- Clinical formula

$$SpO_2 = \mathbf{a} - \mathbf{b}R$$

$$\mathbf{b} = \frac{n\sum_{i=1}^{n} R_i S_i - \sum_{i=1}^{n} R_i \sum_{i=1}^{n} S_i}{n\sum_{i=1}^{n} R_i^2 - \left(\sum_{i=1}^{n} R_i\right)^2}$$



$$\mathbf{a} = \frac{\sum_{i=1}^{n} S_i \sum_{i=1}^{n} R_i^2 - \sum_{i=1}^{n} R_i \sum_{i=1}^{n} R_i S_i}{n\sum_{i=1}^{n} R_i^2 - \left(\sum_{i=1}^{n} R_i\right)^2}$$

- **a** and **b** are coefficients determined through calibration