Tissue Optics

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Light and Bulk Matter

Types of Interactions

- Reflection (Fresnel's law) $R = 1 - T = \frac{(n_1 - n_2)^2}{(n_1 + n_2)^2}$
- Refraction (Snells law) $n_2 \sin(\Theta_2) = n_1 \sin(\Theta_1)$
- Scattering, Diffraction
- Absorption => Variation in Transmission (Beer's law) $I(z) = I_0 e^{-\mu_a(\lambda) z}$
- Phase shifts
- Emission

Light and Turbid Sample

Optical Properties of Turbid Sample

- Refractive index: n
- Absorption: μ_a
- Scattering: μ_s
- Scattering Anisotropy: g
- Reduced Scattering: $\mu_s(1-g)$
- Total Attenuation: $\mu_t=\mu_s+ \mu_a$
- Albedo: μ_s/μ_t
- Transport: $\mu_{tr}=\mu_s(1-g) + \mu_a$
- Diffusion: $1/ (3\ \mu_{tr})$

Refractive Index

$$n = n_r + i n_i$$

$$\mu_a = 2 n_i \frac{\omega}{c}$$

In visible range:

- n_r water: 1.33
- n_r soft tissue: 1.37-1.40
- n_r tooth enamel: 1.62

- Vo-Dinh Chapter 2, Table 2.1

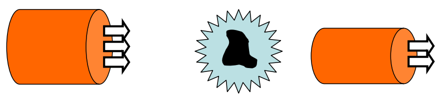
Absorption coefficient

Can describe absorption for a single absorber or a mole of absorbers

Absorption Cross Section [Watt/(Watt/cm²)] $\sigma_a = \frac{P_{Abs}}{I_0}$

Extinction Coefficient cm²/mole $\varepsilon = \frac{P_{Abs}/mole}{I_0}$

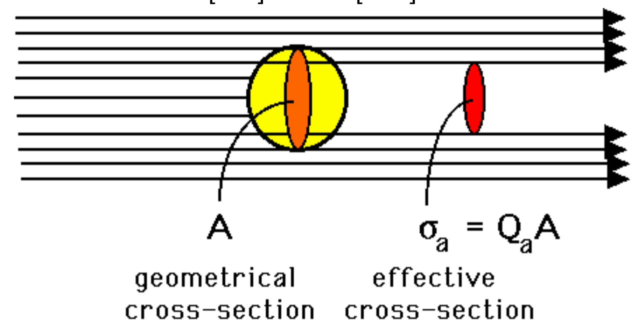
$I_o = P_{in}/A$ $P_{abs} = I_o\sigma_a$ $P_{out} = P_{in} - P_{abs}$



Absorption

$$\sigma_a = Q_a A$$

[cm^2] - [cm2]



Absorption

The Absorption coefficient (μ_a) describes a medium containing many absorbing particles and is defined as:

$\mu_a = \rho_a \sigma_a$

[cm^{-1}] [cm^{-3}] [cm^2]

Where,

σ_a is the effective cross-section [cm^2]

ρ_a is a volume density [cm^{-3}] = absorbers per cm^3

$$T = e^{-\mu_a L}$$

$\mu_a = [c]\varepsilon \ln(10)$

[cm^{-1}] [moles/cm^3] [cm^2/mole]

Where,
c is molar concentration [moles/cm^3]

ε = molar extinction coefficient [cm^2/mole] [(liter/mole)/cm]

M [mole/liter]

$$T = 10^{-\varepsilon c L}$$

Bilirubin Example

- Diameter is about 1 nm
- At 460 nm, ε=53846 [cm^{-1}M^{-1}] (log10 based)
- Typical jaundiced neonates serum bilirubin concentration is 10mg/dL
- Molar weight: 574.65 g/mole
- Concentration: 0.17 10^{-3} M

- What is μ_a ?
- Optical cross section: μ_a / ρ_a = ?
- Ratio optical versus geometrical cross section?

Mean Free Path

$$l_a = \frac{1}{\mu_a}$$

- μ_a ~10 cm^{-1}
- l_a= 1 mm

- Most tissues absorption coefficient is between 0.1-1cm^{-1}

Absorption Spectroscopy

Beer-Lambert

Concentration c, length l, if we pass beam of light with intensity I and wavelength lambda how much light emerges?

Amount of molecules in thin layer: $c \cdot dx$

Change of Intensity due to thin layer: $dI = -\varepsilon \cdot c \cdot dx \cdot I$

ε is the absorption coefficient depending on concentration

Integrating of a path length of l results: $T = \frac{I}{I_0} = 10^{-\varepsilon \cdot c \cdot l} = e^{-\mu_a \cdot l}$

Absorption Spectroscopy

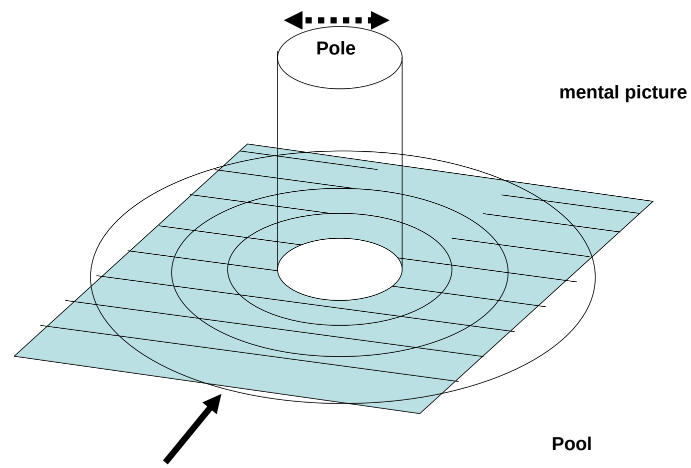
Absorbance is based on log 10 = Optical Density

$$A = \log\frac{I_o}{I} = -\log(T) = \varepsilon cl = \frac{\mu_a}{2.303} l$$

ε is the molar extinction coefficient

%T is Transmission measured

Scattering



Scattering

- Scattering due to spatial distribution of refractive index
- Refractive index spatial distribution due to ultrastructure of object
- Size range of ultrastructure will determine if it is a Mie or Rayleigh scattering
- Inelastic scattering not considered here (Raman scattering)

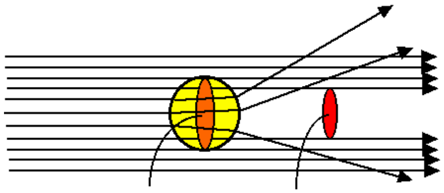
Scattering Cross Section

Can describe scattering for a single scatterer

Scattering Cross Section Watt/(Watt/cm^2)

$$\sigma_s = \frac{P_{scattered}}{I_0}$$

Q_s is the scattering efficiency (can be calculated from Mie theory)
A_s is the area of the scatterer (cm^2)



Scattering Coefficients

- The scattering coefficient (μ_s) describes a medium containing many scattering particles and is defined as:

$$\mu_s = \rho_s \sigma_s$$

[cm^{-1}] [cm^{-3}] [cm^2]

Where,
σ_s is the scattering cross-section (cm^2)
ρ_s is a volume density (cm^{-3}) = # of scatterers per volume

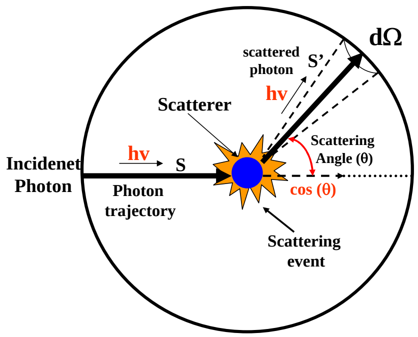
Mean Free Path

$$l_s = \frac{1}{\mu_s}$$

- μ_s ~100 cm^{-1}
- l_s= 0.1 mm
- Most tissues
  μ_s >50cm^{-1} (prostate)
  <1000cm^{-1} (tooth enamel)

Scattering Anisotropy & Phasefunction

- Imagine that a photon is scattered by a particle so that its trajectory is deflected by an angle, θ
- Then, component of a new trajectory aligned forward direction is cos(θ)
- Anisotropy is a measure of forward direction retained after a single scattering event, < cos(θ)>



Scattering Phase Function

Differential scattering cross section: scattering in direction s from input direction s'

$$\frac{d\sigma_s}{d\Omega}(\hat{s},\hat{s}')$$

The angular dependence of scattering is

$$p(\hat{s}\cdot\hat{s}') = \frac{4\pi}{\sigma_s+\sigma_a}\frac{d\sigma_s(\hat{s}\cdot\hat{s}')}{d\Omega}$$

- Often the scattering phase function does not depend on input direction: p(θ)
- p(θ) describes the probability of a photon scattering into a unit solid angle, relative to the original photon trajectory
- p(θ) has historically been called the scattering phase function

Scattering Anisotropy

The proper definition of anisotropy (g) is the expectation value for cos (θ):

Effectiveness of Scattering $g \equiv \frac{\int p(\hat{s}\cdot\hat{s}')\hat{s}\cdot\hat{s}' d\Omega}{\int p(\hat{s}\cdot\hat{s}') d\Omega}$

$$g = \langle \cos\theta \rangle$$

$$= \int_0^{\pi} p(\theta)\cos\theta \, 2\pi \sin\theta \, d\theta$$

$$where, \int_0^{\pi} p(\theta) 2\pi \sin\theta \, d\theta = 1$$

Sometimes also written in terms of cos(θ)

$$= \int_{-1}^{1} p(\cos\theta)\cos\theta \, d(\cos\theta)$$

$$where, \int_{-1}^{1} p(\cos\theta)\, d(\cos\theta) = 1$$

Scattering Anisotropy

- Anisotropy is a measure of forward direction retained after a single scattering event (mean value of cos(θ))

$$g = \begin{cases} -1 \\ 0 \\ 1 \end{cases}$$

Total backward scattering	-1
Backward scattering	-1..0
Forward scattering	0..1
Total forward scattering	1

Biological Tissues, 0.65 < g <0.95

Isotropic Phase Function

Scattering of light at equal efficiency into all possible directions and is defined as:

$$\mathbf{p}(\theta) = \frac{1}{4\pi}$$

Albedo

$$p(\hat{s}\cdot\hat{s}') = \frac{4\pi}{\sigma_s + \sigma_a}\frac{d\sigma_s(\hat{s}\cdot\hat{s}')}{d\Omega}$$

Fraction of light energy incident on a scatterer or absorber from direction s that gets scattered into direction s prime.

$$\int p(\hat{s}\cdot\hat{s}')d\Omega = \frac{\sigma_s}{\sigma_s + \sigma_a} = \frac{\mu_s}{\mu_s + \mu_a}$$

Albedo, in tissue can range from 0.3 to 0.99 depends on wavelength

Ratio of scattering versus total attenuation

Scattering in tissue is dominant!

Heney Greenstein Approximation

Heyney Greenstein scattering phase function is an analytical expression which mimics the angular dependence of light scattering by small particles and is based on the anistropy factor g (used for Monte Carlo Simulations)

$$p(\theta) = \frac{1}{4\pi}\frac{1-g^2}{\left(1+g^2-2g\cos\theta\right)^{3/2}},$$

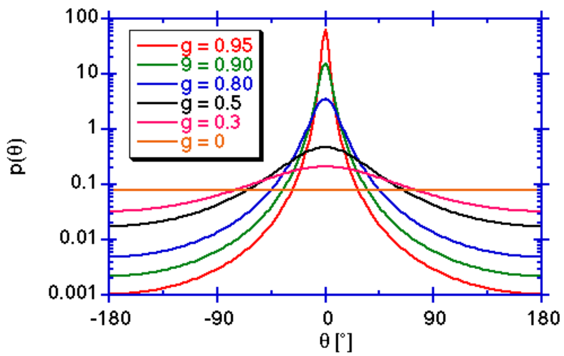
$$where, \int_0^{\pi} p(\theta) 2\pi \sin\theta d\theta = 1$$

$$and, \int_0^{\pi} p(\theta)\cos\theta 2\pi \sin\theta d\theta = g$$

Heney Greenstein

- The function allows the anisotropy factor, g to specify p(θ) such that <cos(θ)> returns the same value of g
- This function is useful in approximating the angular dependence of single scattering events in biological tissue
- The function does not represent true scattering phase functions very well but it is a good average approximation

Example Heyney Greenstein



Reduced Scattering Coefficient

- Lumped property (μ_s') incorporating the scattering (μ_s) coefficient and the anisotropy factor (g):

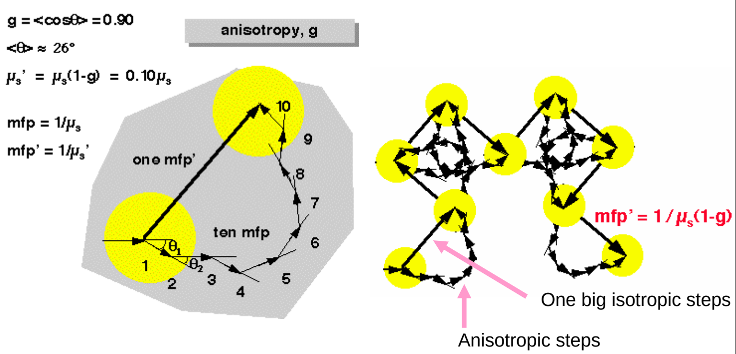
$$\mu_s' = \mu_s(1 - \mathbf{g})$$

Where,

μ_s is the scattering coefficient [cm^{-1}]

g the anisotropy

Reduced Scattering: Isotropic Scattering in average



The following figure shows how many such big steps involving isotropic scattering are equivalent to many small anisotropic steps

Reduced mean free path

- The purpose of the reduced scattering coefficient is to describe the diffusion of photons (isotropic scattering) in a random walk of step size, $1/\mu_s'$, which is the reduced mean free path
- Such a description is equivalent to the description of photon diffusion

Scattering Parameters

- Refractive index fluctuations
- Wavelength
- Particle size and shape

- Small size particles: Rayleigh Theory < 1/10 λ
- Matching size particles: Mie Theory >=λ

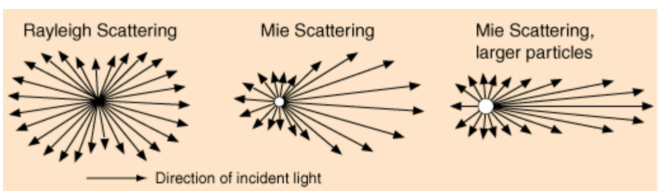
Rayleigh Scattering

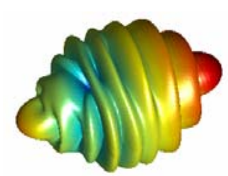
$$I = I_o \frac{8\pi^4 N \alpha^2}{\lambda^4 R^2}(1+\cos^2\theta)$$

λ is the wavelength
I is the intensity of the scattered light
N = # of scatterers
α= polarizability
R=distance from scatterer

- Rayleigh scattering is inversely related to fourth power of the wavelength of the incident light!

Mie Scattering





Mie scattering is not strongly wavelength dependent

Farfield pattern can be calculated by solving Maxwell's equations, e.g. with finite time and finite element methods, grid size is usually 1/10 or less of wavelength

Mie Scattering

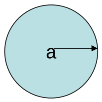
- Gustav Mie in 1908 published a solution to the problem of light scattering by homogeneous spherical particles of any size

- Mie theory describes the way in which spherical, homogeneous particles interact with electromagnetic radiation.

- Particles here means material that constitutes a region with refractive index (n_p) that differs from the refractive index of its surroundings (n_{med})

Mie Calculator

Mie Scattering



$$\left[Q_{s,}\, p(\theta)\right] = Mie(n_r, x)$$

$$\sigma_s = Q_s \pi a^2, \mu_s = \rho_s \sigma_s$$

nr (magnitude of refractive index mismatch)
x (size of the surface of refractive index mismatch)

Normalization used in online Mie calculator

$$g = \frac{\int_0^{\pi} p(\theta) \cos\theta 2\pi \sin\theta d\theta}{\int_0^{\pi} p(\theta) 2\pi \sin\theta d\theta}$$

In depth discussions of Mie theory are presented in van de Hulst (1957), Hansen and Travis (1974), and Bohren and Huffman (1983).

Mie Scattering

The magnitude of refractive index mismatch

- The refractive index mismatch between particle and medium is expressed as the ratio of refractive index for particle and medium:

$$\mathbf{n_r} = \frac{\mathbf{n_p}}{\mathbf{n_{med}}}$$

Where,

n_p is the refractive index of the particle

n_{med} is the refractive index of the medium

Mie Scattering

The surface of refractive index mismatch

- The surface of refractive index mismatch is the ratio of the circumference of the sphere and wavelength of light in medium:

$$\mathbf{x} = \frac{2\pi \mathbf{a}}{\lambda / \mathbf{n_{med}}}$$

Where,

a is the radius of the sphere

λ is the wavelength of light in the medium

Mie Scattering

The values of the parameters “x” and “n”

- The size parameter x has values from 0 – infinity

- The refractive index parameter n_p can have values between 1 – infinity (in a vacuum)

Mie Scattering Approximation

Mie theory can be well approximated to give the expression for the reduced scattering cross-section (σ_s') [Journal of Biomedical Optics, 1(2), 200-11, 1996]:

$$\sigma_s' = 3.28x^{0.37}(\mathbf{n_r} - 1)^{2.09}\mathbf{A_s}$$

Where,

$5 < x < 50$, $1 < n_r < 1.1$, A_s=area of scatterer

These conditions are satisfied in most living tissues

Often tissue scattering is also approximated with:

$$\mu_s' = A \cdot \lambda^{-b}$$

$$\mu_s' \sim \lambda^{-0.5} \ldots \lambda^{-4}$$

Scattering Assumptions

- The optical properties of the particle are completely described by the refractive index
- The medium in which the particles are embedded is considered to be homogeneous
- Only the interactions of a single particle with light of arbitrary wavelength are considered
- The total scattered field is merely the sum of the fields scattered by each particle (i.e. the particles do not affect each other).
- For a collection of particles, the number of particles is large and their separations are random

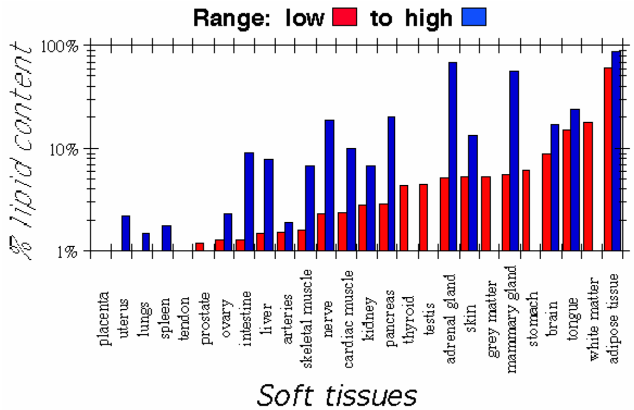
Scattering Example: Soft Tissue

Lipid content of soft tissues

- Soft tissues are dominated by lipid content
- The lipids constitutes the cellular membranes, membrane folds and membranous structures
- The lipid / water interface of membranes presents a strong refractive index mismatch and so plays a major role in scattering

Soft Tissue

Lipid content of example soft tissues



Biological Example Soft: Tissue

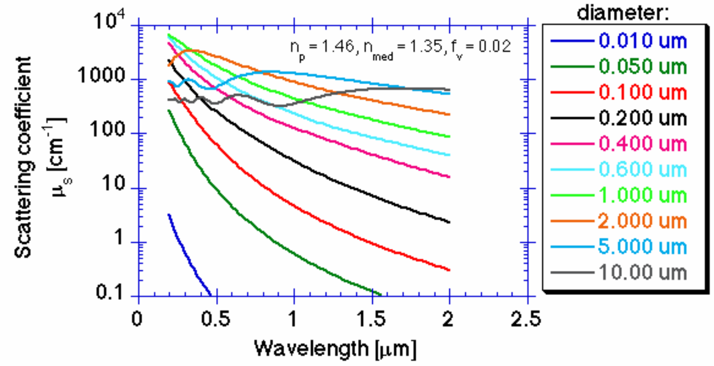
Selection of parameters

- Assume lipid content is 2%
- Assume the lipid is packaged as small spheres of various sizes whose number density maintains a constant volume fraction, f_v
- The volume density, ρ_s is as follows (where a is the radius):

$$\rho_s = f_v / \left((4/3)\pi a^3 \right)$$

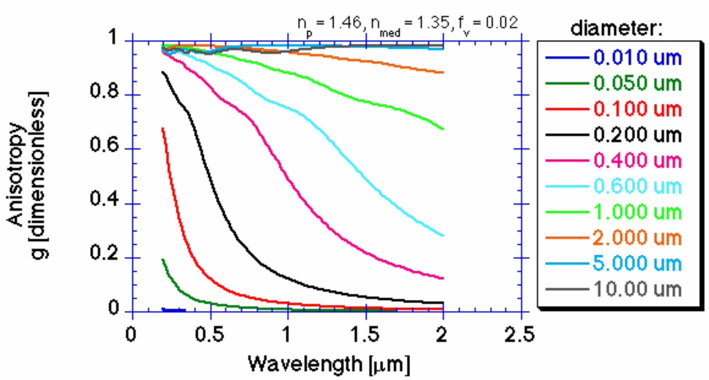
Biological Example: Soft Tissue

Scattering coefficient



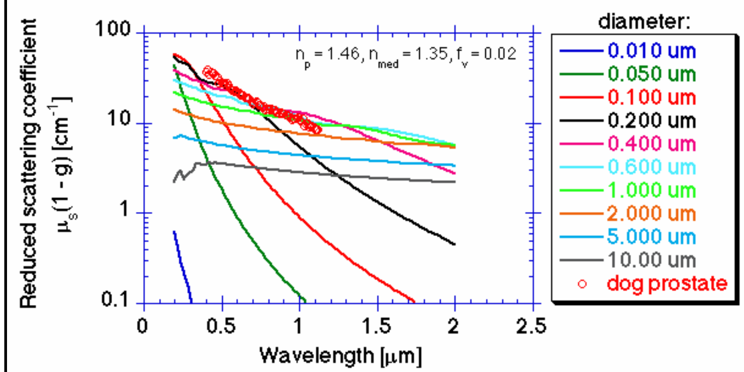
Biological Example: Soft Tissue

Anisotropy



Biological Example: Soft Tissue

Reduced Scattering (combining anisotropy and scattering)



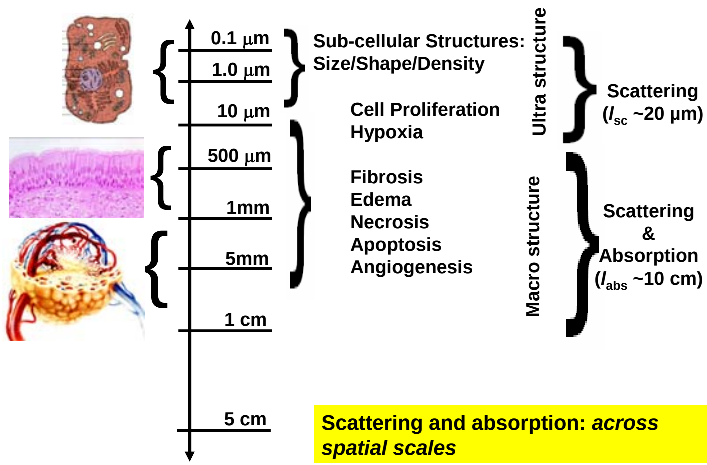
Summary

- Slope: size of particles
- Magnitude: volume fraction, concentration

Tissue

Optical Constituents
Chromophores
Scatterers

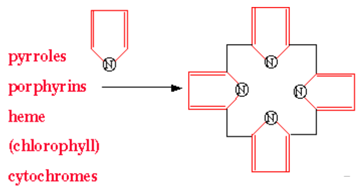
Size Scales



Scattering and absorption: *across spatial scales*

Biological Chromophores

Biologic Chromophores: Evolution



Absorb Sunlight

More efficient absorber

Hemoglobin, Vitamin B12, Cytochrome C

Biologic Chromophores: Nucleic Acids

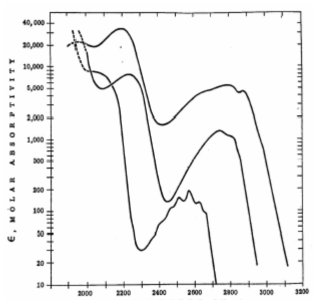
- Purine & Pyrimidine Bases and Derivatives

Molecule	λ (nm)	ε (x10^{-3}) ($cm^2.mol^{-1}$)
Adenine (A)	260.5	13.4
Adenosine	259.5	14.9
Guanine (G)	275	8.1
Guanosine	276	9.0
Cytosine (C)	217	6.1
Thymine (T)	263.75	

ATP: Adenosine Tri Phosphate

Biological Chromophores: Amino Acids

Molecule	λ (nm)	ε (x10^{-3}) ($cm^2.mol^{-1}$)
Tryptophan	280, 219	5.6,47
Tyrosine	274,222, 193	1.4,8,48
Phenylalanine	257,206, 188	0.2,9.3,60
Histidine	211	5.9
Cystine	250	0.3



Biological Chromophores: Vitamins & Cofactors

•Respiratory Enzymes (produce ATP)

Molecule	λ (nm)	ε (x10^{-3}) ($cm^2.mol^{-1}$)
NADH	340,259	6.23, 14.4
NAD+	260	5.9, 18
Flavoprotein (FAD)	530	
Cytochrome a3		

Biological Chromophores: Hemoproteins

- Hemoglobin
- Cytochrome c

contain iron

Each hemoglobin has 4 heme (Fe^{2+}) sites to bind O_2

Responsible for oxygen transport

HbO_2 and Hb oxygen saturation is an indicator of oxygen delivery and utilization as well as metabolic activity

Cytochrome c is part of the electron transfer chain in mitochondria producing ATP

Biological Chromophores: Hemoglobin

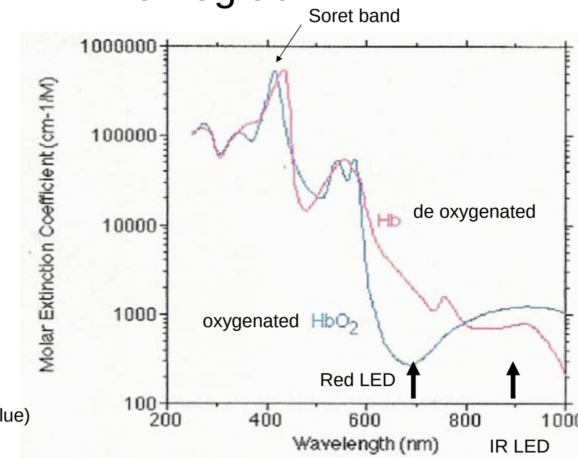
Here units are
1/cm/M
1M=1 mole/liter

Often units are
cm^2/mole

Or

cm^{-1}

red and blue color
should be inverted
(venous blood is blue)



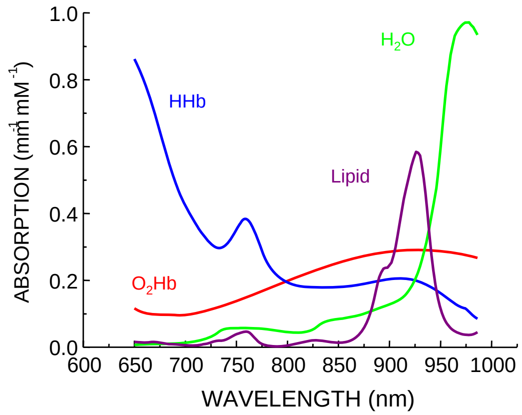
Biological Chromophores: Pigments

- Melanin (skin)
- Carotenoid (plant)
- Chlorophyll (plant)

Biological Chromophores: Structural Proteins

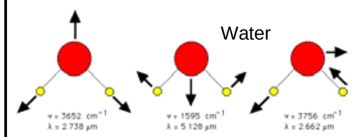
- Keratin (hair, skin)
- Collagen

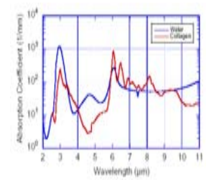
Biological Chromophores: Lipids



Biological Chromophores: Infrared

- Absorption IR





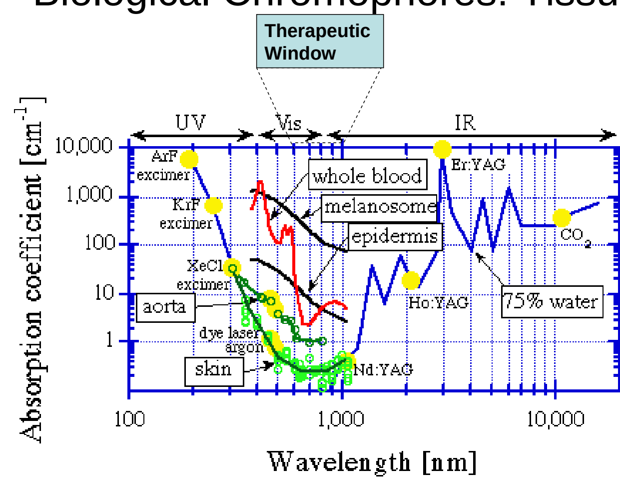
bond	cycles/cm, ν	wavelength, λ = 1/ ν
C-H stretch	2850-2960 [cm^{-1}]	3.378-3.509 [μm]
C-H bend	1340-1465	6.826-7.462
C-C stretch,bend	700-1250	8.000-14.29
C=C stretch	1620-1680	5.952-6.173
C=C stretch	2100-2260	4.425-4.762
CO_3^{2-}	1410-1450	6.897-7.092
NO_3^-	1350-1420	7.042-7.407
NO_2^-	1230-1250	8.000-8.130
SO_4^{2-}	1080-1130	8.850-9.259
O-H stretch	3590-3650	2.740-2.786
C=O stretch	1640-1780	5.618-6.098
N-H	3200-3500	2.857-3.125

ref: PW Atkins, "Physical Chemistry," p. 576, W.H. Freeman and Co., 1978.

Summary: Chromophores

- UV
  - Protein (amino acids) 210-280nm (30% of non water tissue constituents)
  - Water < 180nm
  - DNA < 320nm (resonance at 270nm)
  - Vitamins & Cofactors
- Visible
  - Hemoglobin
  - Lipids
  - Melanin
  - Cytochrome a3, Falvin
- IR
  - Protein (amide excitation) 6.1, 6.45, 8.3 micron
  - Water 0.96, 1.44, 1.95, 2.94, 6.1 micron

Biological Chromophores: Tissue



Wavelength dependence of absorption

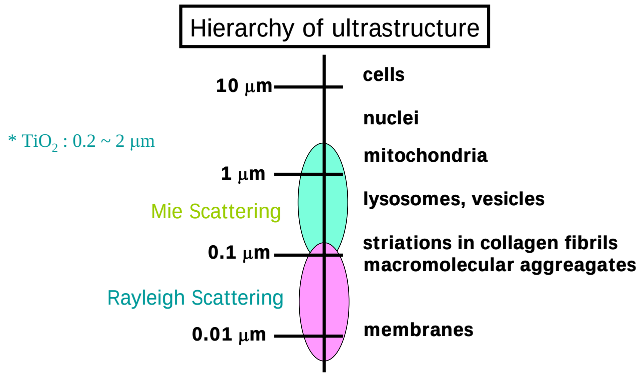
- In the ultraviolet and blue, the absorption increases with shorter wavelengths due to protein, DNA and other molecules
- In the infrared, the absorption increases with longer wavelengths due to tissue water content
- In the red to near-infrared (NIR), absorption is minimal and this region is called the diagnostic and therapeutic window; whole blood is a strong absorber in the red-NIR regime

Biological Scatterers

Tissue scatterers

- The light scattered by a tissue has interacted with the ultrastructure of the tissue
- Tissue ultrastructure extends from membranes to membrane aggregates to collagen fibers to nuclei to cells
- Size range of tissue ultrastructure will determine if it is a Mie or Rayleigh scatterer

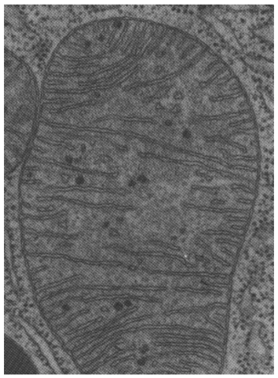
Elastic Scattering: Biological Scatterers

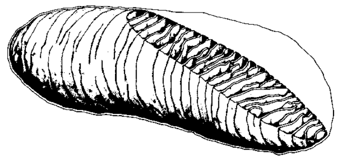


Source of Scattering in Tissue

- Refractive index mismatch between lipid and surrounding aqueous medium
  - Soft tissues are dominated by lipid contents
  - Celluar membranes, membrane folds, and membraneous structure
- Mitochondria, ~ 1micron
  - Intracelluar organelle composed of many folded membrane, cristae
- Collagen fibers, 2 ~ 3micron
  - Collagen fibrils, 0.3 micron
  - Periodic fluctuation in collagen ultrastructure →source of Rayleigh scattering in UV and Visible range (~70nm)
- Cells

Mitochondria



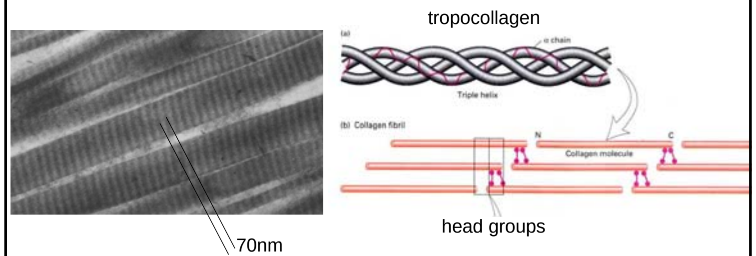


1 micron in size, folded lipid membranes, membranes 9nm thick

Refractive index mismatch between lipid and water causes scattering

Also contains metablic cofactors NAD, FAD used for proton pump over membrane to generate ATP

Collagen fibers, fibrils



Fibers are 2-3 micron in diameter and composed of smaller fibrils 0.3 micron in size (electron micrograph), strong Mie scattering in IR

Fibrils composed of tropocollagen molecules, have banded pattern (70nm period), optical "crystal" 2nd harmonic generators, periodic structure contributes to Raleigh scattering (visible and UV range)

Cross Links, hydroxylysyl pyridinoline and lysyl perydinoline are fluorescent

Skin Example

Collagen Fibers

Length 0.1-8 microns (Mie)

Subunits 50nm (Rayleigh)

Biological Example: Skin/ Collagen

Skin

- Dermis of Skin and sclera of eye are highly scattering and have high collagen content
- **Macroscopic Level**: 0.1 micron fibrils – 8 micron fibers
- Postmortem neonatal skin: 2.8 +/- 0.08 micron fibers
- Fiber Concentration / Volume Density:

ρ_s $=3 \times 10^6 \pm 0.5 \times 10^6$ cm^{-3}.

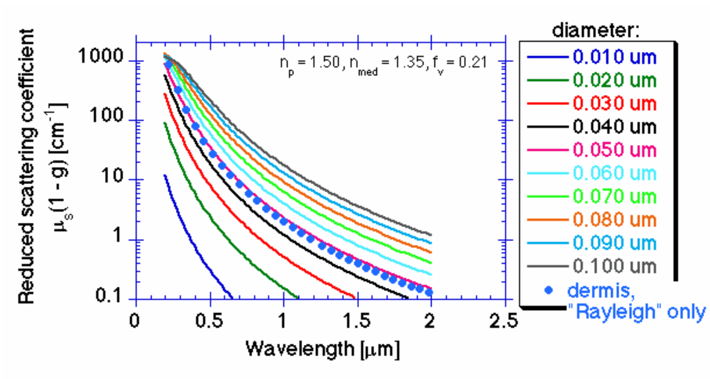
- Volume fraction:

$$f_v = \pi a^2 [1cm] \rho_s$$ =0.21+/-0.1

n_{med}= 1.35 (dermal ground substrate),

n_{fiber}= 1.5 (collagen) – (1.5-1.33)*65.3% (water content)

Rayleigh Component Only



Combined Approach