Tissue Optical Properties
Introduction

• Interaction between Light and Tissue
  • Reflection
  • Refraction
  • Absorption
  • Fluorescence
  • Scattering

• Depends on
  • Constituents of tissue
  • Optical properties of tissue
  • Propagation of light
Absorption

• Extraction of energy from light by a molecular species

• Diagnostic applications: Transitions between two energy levels of a molecule that are well defined at specific wavelengths could serve as spectral fingerprint of the molecule
  • Various types of Chromophores (light absorbers) in Tissue
  • Wavelength-dependent absorption
  • Tumor detection and other physiological assessments (e.g. pulse-oximetry)

• Therapeutic applications: Absorption of energy is the primary mechanism that allows light form a source (laser) to produce physical effects on tissue for treatment purpose
  • Lasik (Laser Assisted in situ Keratomileusis) Eye Surgery, Tatoo Removal, PDT
Absorption

- Absorption occurs when the photon frequency matches the 'frequency' associated with the molecule's energy transition
  - Electrons absorb the energy of the light and transform it into vibrational motion
  - The absorption of a photon results in:
    - quantized change in charge separation
    - quantized excitation of vibrational modes
  - Electrons interact with neighboring atoms → convert vibrational energy into thermal energy
Absorption

- Each electronic energy levels is associated with many vibrational energy levels
- Absorption of UV and visible light promotes transition between electronic energy levels
- Absorption of infrared light promotes transitions between vibrational energy levels
Absorption

- Absorption Cross-section, $\sigma$ [m$^2$]
  - Consider a chromophore idealized as a sphere with a particular geometrical size.
  - Consider that this sphere blocks incident light and casts a shadow, which constitutes absorption.
  - The size of absorption shadow = absorption cross-section
  - $Q_a$: absorption efficiency

\[
\sigma_a = Q_a \cdot A
\]
Absorption

\[ P_{in} = I_o A \]

\[ P_{abs} = I_o \sigma_a \]

\[ P_{out} = I_o (A - \sigma_a) \]

\[ \sigma_a = \frac{P_{abs}}{I_o} \]
Absorption

- **Assumptions**
  - Cross section is independent of relative orientation of the impinging light and absorber
  - Uniform distribution of $N_a$ (molecules/cm$^3$) identical absorbing particles

- **Absorption Coefficient, $\mu_a$ [1/m]**

  $$\mu_a = N_a \cdot \sigma_a$$

  - Absorption cross-sectional area per unit volume of medium

- **Absorption mean free path, $l_a$ [m]**

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

  - Represents the average distance a photon travels before being absorbed
Absorption

- Transmission and Absorbance (macroscopic view)

- Transmission

\[ T = \frac{I}{I_o} \]

- Absorbance (attenuation, or optical density)

\[ A = -\log(T) = \log\left(\frac{I_o}{I}\right) \]
Absorption

• Lambert – Beer Law:
  • The linear relationship between absorbance and concentration of an absorbing species.
  • Relates $\mu_a$, transmission, and absorbance

\[ I = I_o e^{-\mu_a \cdot b} \]

\[ \mu_a = N_a \cdot \sigma_a \]

\[ \sigma_a = \frac{P_{abs}}{I_o} \]

$\sigma$ = absorption cross-sectional area [cm$^2$]
$I_o$ = The intensity entering the sample at $z = 0$ [w/cm$^2$]
$I$ = The intensity of light leaving the sample [w/cm$^2$]
$b$ = pathlength traveled in the sample [cm]
Absorption

Absorbers in Tissue

- **NIR**
  - Hemoglobin
  - Lipids
  - Water

- **UV-VIS**
  - DNA
  - Hemoglobin
  - Lipids
  - Structural protein*
  - Electron carriers*
  - Amino acids*

*Absorbers that fluoresce when excited in the UV-VIS*
Absorption

**UV Absorption**

- Protein, amino acid, fatty acid and DNA absorption dominate UV absorption
- Protein
  - Dominant ‘non-water’ constituent of all soft tissue, ~ 30%
  - Absorption properties determined by peptide bonds and amino acid residues
    - Peptide excitation about $\lambda = 190$ nm
    - Amino acids absorption at $\lambda = 210 - 220$ nm and 260 – 280 nm
- DNA
  - Absorbs radiation for $\lambda \leq 320$ nm
  - Large water absorption $\lambda < 180$ nm
Absorption

Infrared Absorption

- Protein IR absorption peaks at 6.1, 6.45, and 8.3 μm due to amide excitation
- Absorption depth ≤ 10 μm in λ = 6-7 μm region

- Water absorption peak at 0.96, 1.44, 1.95, 2.94 and 6.1 μm
  - Absorption depth
    - ~ 500 mm at λ = 800 nm
    - <1 μm at λ=2.94 μm
    - ≤ 20 μm throughout λ ≥ 6 μm
Absorption

Main Absorbers at visible and NIR
- Hemoglobin
- Lipid

Hemoglobin

- 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
Absorption

Hemoglobin

• **Responsible for oxygen transport**
  • HbO2 and Hb
  • oxygen saturation is an indicator of oxygen delivery and utilization as well as metabolic activity

• **Deoxyhemoglobin has lower absorption than oxyhemoglobin in the blue and green**
  • Bright red arterial blood
  • Bluish venous blood

• **Absorption peaks for HbO₂**
  • 418, 542, 577, and 925 nm

• **Absorption peaks for Hb**
  • 550, 758, 910 nm

• **Isosbestic points**
  • 547, 569, 586, and 798 nm
Absorption

Lipid (Fat)

• Important energy store in the body

• Site-specific measurements of body composition

• Monitoring of physiological changes in female breast tissue

• Tissue layer model

![Graph showing absorption properties of lipid, water, and hemoglobin.](image)
Scattering

• Change of direction of propagation and/or energy of light by a molecular species

• Diagnostic applications: Scattering depends on the size, morphology, and structure of the components in tissues (e.g. lipid membrane, collagen fibers, nuclei).
  
  • Variations in these components due to disease would affect scattering properties, thus providing a means for diagnostic purpose

• Therapeutic applications: Scattering signals can be used to determine optimal light dosimetry and provide useful feedback during therapy
Scattering

Purely absorbing

\[ \text{Photon pathlength} = L \]

With Scattering

\[ \text{Photon pathlength} \gg L \]

Lambert- Beer Law does not apply here!!!

Need to calculate true pathlength of light
Scattering

• Why is the sky blue, clouds white, and sunsets red?
  • Blue skies are produced due to scattering at shorter wavelengths
    • Visible light (violet & blue) are selectively scattered by O2 and N2 – much smaller than wavelengths of the light
    • violet and blue light has been scattered over and over again
  • When light encounters larger particles (cloud, fog), Mie scattering occurs
    • Mie scattering is not wavelength dependent – appears white
    • Cigarette smoke, too
  • At sunset
    • The light must travel over a longer path in the atmosphere
    • Blue/green is scattered away and only red/orange (scattered less) reaches your eyes
Scattering

• **Mechanism for Light Scattering**
  - Light scattering arises from the presence of heterogeneities within a bulk medium
    - Physical inclusions
    - Fluctuations in dielectric constant from random thermal motion
  - Heterogeneity/fluctuations → non-uniform temporal/spatial distribution of refractive index in the medium
    - Passage of an incident EM wave sets electric charges into oscillatory motion and can excite vibrational modes
    - Scattered light is re-radiated by acceleration of these charges and/or relaxation of vibrational transition
Scattering

- **Elastic scattering**: no energy change
  - Frequency of the scattered wave = frequency of incident wave
  - Probes static structure of material
  - Rayleigh and Mie scattering

- **Inelastic scattering**: energy change
  - Frequency of the scattered wave ≠ frequency of incident wave
  - Internal energy levels of atoms and molecules are excited
  - Probes vibrational bonds of the molecule
  - Raman scattering (stokes↓ and anti-stokes ↑)
Scattering

Elastic Scattering

• The light scattered by a system has interacted with the inhomogeneities of the system

• Photons are mostly scattered by the structure whose size matches the wavelength

• Principal parameters that affect scattering
  • Wavelength, $\lambda$
  • Relative refractive index
  • Particle radius
  • Shape and orientation

• Two types of scattering: Rayleigh and Mie
Rayleigh Scattering

- Scattering from very small particles $\rightarrow \leq \lambda/10$
- Rayleigh scattering is inversely related to the fourth power of the wavelength of the incident light

$$I \propto \frac{1}{\lambda^4}$$

$\lambda$ is the wavelength of the incident light
$I$ is the intensity of the scattered light
For scattering of particles comparable or larger than the wavelength, Mie scattering predominates.

Because of the relative particle size, Mie scattering is not strongly wavelength dependent.

Forward directional scattering.
**Scattering**

\[ P_{\text{in}} = I_o A \]
\[ P_{\text{scatt}} = I_o \sigma_s \]
\[ P_{\text{out}} = I_o (A - \sigma_s) \]

- **Scattering Cross Section, \( \sigma_{\text{scatt}} [m^2] \)**
  - ‘area’ of an index-matched, perfectly absorbing disc necessary to produce

- **The measured reduction of light**
  \[ \sigma_{\text{scatt}} = Q_s * A_s \]
  - \( Q_s \): Scattering efficiency (calculated by Mie theory); defined as the ratio of the scattering section to the projected area of the particle on the detector
  - \( A_s \): Area of Scatterer [m2]
Scattering

• Scattering Coefficient, $\mu_s [1/m]$
  - $\mu_s = N_s \sigma_s$,
    - $N_s$ = the number density of scatterers
    - $\sigma_s$ = scattering efficiency
    - Cross-sectional area for scattering per unit volume of medium

• Scattering Mean Free Path, $l_s$
  - Average distance a photon travels between scattering events
  \[ l_s = \frac{1}{\mu_s} \]
Scattering

- **Anisotropy, g**
  - Imagine that a photon is scattered by a particle so that its trajectory is deflected by an angle, $\theta$
  - Then, component of a new trajectory aligned forward direction is $\cos(\theta)$
  - Anisotropy is a measure of forward direction retained after a single scattering event, $\langle \cos(\theta) \rangle$

\[
g = \begin{cases} 
-1 & \text{totally backward scattering} \\
0 & \text{isotropic scattering} \\
1 & \text{totally forward scattering}
\end{cases}
\]

*Biological Tissues: $0.65 < g < 0.95$*
Scattering

- Reduced Scattering Coefficient, $\mu_s'$ [1/m]
  - $\mu_s'$ incorporates the scattering coefficient, $\mu_s$ and the anisotropy factor, $g$
    \[ \mu_s' = (1 - g)\mu_s \]
  - $\mu_s'$ can be regarded as an effective isotropic scattering coefficient that represent the cumulative effect of several forward-scattering events
  - Special significant with respect to photon diffusion theory

\[ \langle \theta \rangle \approx 26^\circ \]
\[ g = \langle \cos \theta \rangle = 0.90 \]
\[ \mu_s' = (1 - g)\mu_s = 0.10\mu_s \]
\[ mpf = 1 / \mu_s \]
\[ mpf' = 1 / \mu_s' = 10mpf = 10 / \mu_s \]
Scattering

- Scattering in Tissue
  - Tissue is composed of a ‘mixture’ of Rayleigh and Mie scattering

![Diagram of a cell with labeled parts and scattering regions]

- Rayleigh Scattering
  - Cells
  - Nuclei
  - Mitochondria
  - Lysosomes, vesicles
  - Striations in collagen fibrils
  - Macromolecular aggregates

- Mie Scattering
  - Membranes

Dimensions:
- 10 μm
- 1 μm
- 0.1 μm
- 0.01 μm
Scattering

- 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, $\sim 1\,\mu m$
    - Intracellular organelle composed of many folded membrane, cristae
  - Collegen fibers, $2 \sim 3\,\mu m$
    - Collegen fibrils, $0.3\,\mu m$
    - Periodic fluctuation in collegen ultrastructure $\rightarrow$ source of Rayleigh scattering in UV and Visible range
  - Cells
Light Transport in Tissue

- Scattering and absorption occur simultaneously and are wavelength dependent
  \[ \mu_t = \mu_s' + \mu_a \]

- Scattering monotonically decreases with wavelength

- Absorption is large in UV, near visible, and IR

- Absorption is low in red and NIR \( \rightarrow \) Therapeutic window \( (600 \leq \lambda \leq 1000 \text{ nm}) \)

  \[ \mu_s' = A \cdot \lambda^{-b} \]
  \[ \mu_s' \sim \lambda^{-0.5} - \lambda^{-4} \]
Light Transport in Tissue

- Modeling of light transport in tissues are often governed by the relative magnitudes of optical absorption and scattering
  - $\mu_a >\!> \mu_s'$: Lambert-Beer Law ($\lambda \leq 300\text{nm}; \lambda \geq 2000\text{nm}$)
  - $\mu_s' >\!> \mu_a$: Diffusion Approximation (600nm ~ 1000nm)
  - $\mu_s' \sim \mu_a$: Equation of Radiative Transfer, Monte Carlo (300nm ~ 600 nm; 1000nm ~ 2000nm)

- Use Monte Carlo, Transport Theory, or Diffusion Theory

\[ \frac{L_o}{L_p} = 4 \text{ or } \uparrow \]
Light Transport in Tissue

- Modeling Photon Propagation

Random Walk depiction of photon propagation in a homogeneous medium. This comprises of combinations of multiple-scatter, absorption and detection.

\( \mu_a, \mu_s, g, \) phase function \( S \)

“Stochastic” Description
Light Transport in Tissue

• Radiative Transport Theory
  • The direct application of EM theory is complicated
  • RTT deals with the transport of light energy
  • RTT ignores wave phenomena (polarization, interference) of EMT

**Steady State Radiative Transport Equation**

\[
\frac{\partial L(\vec{r}, \vec{s}')}{\partial s} = -(\mu_a + \mu_s)L(\vec{r}, \vec{s}')
\]

\[+ \mu_s \int_{4\pi} p(\vec{s}, \vec{s'})L(\vec{r}, \vec{s})d\vec{s}' + S(\vec{r}, \vec{s}) \]

- **Overall Energy balance at position \( r \) and direction \( s \)**
- **Loss due to scatt and abs**
- **gain due to scattering from \( s' \) to \( s \) at \( r \)**
- **Source term**

\( L = \) radiance [W/m^2 sr], propagation of photon power
\( P(s, s') = \) phase (scattering) function
\( s, s' = \) directional vectors of photon propagation
• **Diffusion Approximation**
  • Simplified form of RTT at “diffusion limit”
  • $\mu s' \gg \mu a$
    • the number of photon undergoing the random walk is large
    \[
    \frac{\partial j(\vec{r}, t)}{\partial t} \ll c \left( \mu_a + \mu_s' \right) = c \mu_t'
    \]
  \[
  L(\vec{r}, \hat{s}, t) \approx \frac{1}{4\pi} \phi(\vec{r}, t) + \frac{3}{4\pi} j(\vec{r}, t) \cdot \hat{s}
  \]
  • Isotropic source beyond $1/\mu t'$
    • $\sim 10/\mu t'$ (~ 1mm in biological tissue)
    • far from sources & boundaries
    • assume tissue is “macroscopically homogeneous”

\[
\frac{1}{c} \frac{\partial \phi(\vec{r}, t)}{\partial t} - \nabla \cdot D(\vec{r}) \nabla \phi(\vec{r}) - \mu_a \phi(\vec{r}, t) = S(\vec{r}, t)
\]
where $D(\vec{r}) = 1/3 \left[ \mu_a (\vec{r}) + \mu_s (\vec{r}) \right]$. 
Tissue Optical Properties

- Measurement Strategies

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Optical Source 'input'

“Black Box”

TISSUE H(μa, μs)

Detector 'output'

H: System Function

- Goal: To find out H(μa, μs)
- Requires Non-Static system \(\rightarrow\) Perturbations in either optical source or tissue
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Measurement Schemes

• **CW (Continuous Wave) Measurement**
  • Simplest form of measurement
  • Static, continuous wave input
  • Requires dynamic tissue property changes
  • E.g. pulse oximetry

• **Time-Resolved Measurements**
  • Temporal changes in optical sources
  • Time Domain Photon Migration (TDPM)
  • Frequency Domain Photon Migration (FDPM)

• **Spatially-Resolved Measurement**
  • Spatial changes in optical path
Tissue Optical Properties

- **CW (continuous wave)**
  - pulse oximetry locks into pulse
  - healthy adult calibration accounts for tissue scatter (ms')
  - typically at 2 wavelengths (660, 940 nm)

\[ \mu_t = \mu_a + \mu_s' \]
Tissue Optical Properties

- **Time Domain Photon Migration (TDPM)**
  - Directly measure $m_a$ and $m_s$ from TPSF using Diffusion Equation
  - Complicated and expensive detection system
  - rather low SNR

- **Impulse Function, $\delta$**
  - Scattering and Absorbing Tissue
  - Produce a histogram of the arrival times of many photons

- **Temporal Point Spread Function (TPSF)**
  - $t_0$, $t_u$, $t_s$
Tissue Optical Properties

- Frequency Domain Photon Migration (FDPM)

\[ \phi \sim \text{TIME} \]
\[ M = \frac{AC}{DC} \]