Light and tissue 1

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Outline

Motivation

Absorption

Scattering

Pushing the limits
Cancer biology

- Small tumor
- Sprouting capillary
- Growing tumor

E.g. understanding how tumors form and interact with blood vessels
E.g. monitoring activity in deeper layers of the brain
Immunology

E.g. studying the biology of lymph nodes
Developmental biology

E.g. monitoring early development in real time

Diagnosis

E.g. early detection of tumors in deep tissues
How deep can we see?

With visible light in biological tissues: less than 1 mm, often less than 100 µm

But there are ways to push the limits.
Absorption and Scattering
Absorption in a cuvette
Absorption cross-section

Geometrical cross-section [cm²] \( \sigma_a \): Effective absorption cross-section [cm²] per molecule

N: absorbers per unit volume [1/cm³]

\[ \mu_a = \sigma_a \cdot N \]

The absorption coefficient \( \mu_a \) is the total absorption cross-sectional area per unit volume [cm²/cm³]
Deriving the Beer law

The relative change in intensity equals the total absorption cross-sectional area in the slice divided by the slice area:

\[
\frac{-dI}{I} = \frac{\mu_a \cdot A \cdot dx}{A} = \mu_a \cdot dx \quad \Rightarrow \quad I = I_0 e^{-\mu_ax}
\]
Absorption in a cuvette

\[ I_1 = I_0 e^{-\mu_a x} \]
Absorption spectra
Scattering in a cuvette
Scattering cross-section

\[ \mu_a = \sigma_a \cdot N \]

The scattering coefficient \( \mu_a \) is the total scattering cross-sectional area per unit volume \([\text{cm}^2/\text{cm}^3]\).
Beer law for scattering

\[ I = I_0 e^{-\mu s x} \]

(for ballistic photons)
What happens to the scattered photons?
Scattering Regimes

< $\lambda$  
Rayleigh Regime  
- E.g. particles in the sky  
- Strongly wavelength dependent  
- Mostly isotropic

$\geq \lambda$  
Mie Regime  
- Cells, water droplets (fog)  
- Anisotropic: mostly forward scattering
Anisotropy factor: \( g \)

\[
g = \begin{cases} 
-1 & \text{Backward scattering (anisotropic)} \\
0 & \text{Unidirectional scattering (isotropic)} \\
0 & \text{Forward scattering (anisotropic)} 
\end{cases}
\]

\( g \) for most biological tissues: \( \sim 0.9 \)
(highly forward scattering)
Focusing without scattering

Focusing through isotropic scatterer ($g = 0$)

Focusing through forward scattering medium (high $g$)

Imaging and anisotropy
Mean free path

Mean free path:
\[ \frac{1}{\mu_s} \]

Transport mean free path:
\[ \frac{1}{\mu_s \cdot (1 - g)} \]

- The scattering mean free path is the average distance between scattering events (in biological tissues around 100 µm)
- The transport mean free path can be thought of as the mean distance after which a photon’s direction becomes random (in biological tissues around 1 mm)
Absorbing or scattering?
How do we image deep?

Choose transparent sample, long wavelength

Make sample transparent

Give up on resolution

Push the limits with modern microscopy
Choose transparent samples

*Xenopus laevis* tadpole
Make samples transparent

Make samples transparent

Hama et al (2011)
Optical projection tomography

Sharpe et al (2002)
Optical projection tomography

Sharpe et al (2002)
Sheet illumination

Ntziachristos (2010)
NIR fluorescence imaging

IR Dye emission at ~ 800 nm
Bioluminescence imaging

\[ \text{Luciferin} + O_2 \xrightarrow{\text{Luciferase}} \text{Oxyluciferin} + \text{Light} \ (590\text{nm}) \]
Bioluminescence imaging

Mouse grafted with cells genetically encoding luciferase
Imaging following injection of luciferin

No background autofluorescence.
Bioluminescence imaging
Summary

• In biological tissues, scattering dominates over absorption
• Scattering in most samples is anisotropic (high g)
• Scattering mean free path and transport mean free path are a measure of the penetration depth limit.
• Scattering and absorption are reduced at longer wavelengths

• To image deeper, the simplest solution is to use transparent samples
• Other samples can be cleared optically (but they need to be fixed)
• Optical projection tomography and sheet imaging can be used to image large transparent samples
• NIR fluorescence imaging and bioluminescence have a penetration depth of several mm, but sacrifice resolution.