Measurement of Diffuse and Specular Reflections Through Single Cell Layers

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Aletta E Karsten, J S Dam, A Singh
National Laser Center
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Outline

• **Background**
  Why a Biophotonics group

• **Determination of tissue optical properties**
  Optical properties, Diagnostic window, scattering elements and Integrating Sphere measurements

• **Experimental techniques**
  Phantom and cells

• **Experimental results**
Background

• Biophotonics – new in SA

• Group at NLC aims to:
  • Address health problems through research using lasers and other light sources
  • Stimulate the use of lasers in health related research
  • Focus on Cancer and Diabetes

• Cancer (1993 – 1995)
  • On average 50 000 new cases/year
  • LR at least
    • Male: 1 in 6
    • Female: 1 in 7
Background

- **Diabetes**
  - High prevalence in SA
  - Not a notifiable disease
    - Indian: Av. 17% (11% - 30%)
    - Black: 8%
    - White: 6%
  - Type II diabetes on the increase
  - Limp amputation

- Research aimed at PDT and accelerated wound healing
Experimental work at UJ

WS1 Cell line
Induce wound: sterile pipette

Single cell layers, single scattering events
Laser irradiation
Determination of tissue optical properties

- Definition of optical properties
- Diagnostic window and scattering elements
- Integrating Sphere measurements
Definition of optical properties…

Absorption coefficient

\[ \mu_a = C_a \cdot \text{conc}_{a} \]  
\[ \text{cm}^{-1} \]

Chromophore

Scattering coefficient

\[ \mu_s = C_s \cdot \text{conc}_{s} \]  
\[ \text{cm}^{-1} \]

Scattering element

Anisotropy factor

\[ g = \langle \cos \theta \rangle \]  

Reduced scattering coefficient

\[ \mu'_s = (1 - g) \mu_s \]  
\[ \text{cm}^{-1} \]
Some important tissue chromophores
Scattering spectra of various tissue types

- Breast
- 1% Intralipid
- Forearm
Scattering in Biological Tissue

Human tissue is considered a highly scattering media

Hierarchy of ultrastructure

- 10 μm: cells
- 1 μm: nuclei, mitochondria
- 0.1 μm: lysosomes, vesicles
- 0.01 μm: striations in collagen fibrils, macromolecular aggregates, membranes

ECE532 Biomedical Optics ©1998 Steven L. Jacques, Scott A. Prahl, Oregon Graduate Institute
Integrating Sphere measurements

“Measurements of the total transmittance and reflectance of a thin slab-shaped multiple scattering sample can yield the absorption- and the reduced scattering coefficient of the sample”

\[ R = R_{BS} \left( \frac{I_R}{I_{ref}} \right) \]

\[ T = \frac{I_T}{I_{ref}} \]

Beer-Lambert Law

\[ I = I_0 \exp(\mu_t d) \]
Experimental techniques

- Phantom measurement
- Cell measurements
Phantom measurements

• Used to test the experimental setup
• Itralipid (IL) - Intralipid 20% emulsion from Sigma, Batch # 075K1124
• Vary concentrations
  • Need IL: 1% - 5%/volume
  • Calculate @ $\lambda = 632.8$ nm
    $\mu'_{s} = 1.104 \text{ mm}^{-1} \times \text{conc}$
    $\mu'_{a} = 0.15 \times 10^{-2} \text{ mm}^{-1} \times \text{conc}$
    From K Michielsen et al, Physics Reports,304, (1998) p89-144
• Green food colour
  • Measure $I$ and $I_0$ and calculate $\mu_a$ and $\mu_s$ using:
    $I = I_0 \exp(\mu_{t} \cdot d)$
    Path length: $d = 1.68$ mm
Experimental setup

Phantom

Intralipid: 20%

Green food colourant
Experimental results

- Phantom measurement
- Cell measurements
Data
Results of prediction

(a) "2nd order polynomials"
- Reference
- Prediction, MEP=3.01%

(a) "3rd order polynomials"
- Reference
- Prediction, MEP=7.96%
Thank You

Ann Singh and Thapelo Mabaka