Turbid tissue optics III: Instrumentation and measurements

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Abbe lecture #4

21.01.2014
Roadmap from last time

review of basic concepts from last time

the Virtual Tissue Simulator

reflectance measurements: three types

steady-state

pulsed

sinusoidally-modulated ("frequency domain")

instrument design considerations

various applications
Roadmap for today

- Review of basic concepts from last time
- The Virtual Tissue Simulator
- Reflectance measurements: three types
  - Steady-state
  - Pulsed
  - Sinusoidally-modulated ("frequency domain")
- Instrument design considerations
- Various applications
Time-resolved measurements

\[
R(r,t) = \text{pulse at } t=0 \quad \text{remitted light at } t > 0
\]

\[
\Phi(r, t) = c(4\pi Dct)^{-3/2} \exp\left(-\frac{r^2}{4Dct} - \mu_a ct\right)
\]

Infinite geometry:
Time-resolved measurements

Infinite geometry: \[ \Phi(r, t) = c(4\pi Dct)^{-3/2} \exp \left( -\frac{r^2}{4Dct} - \mu_ac t \right) \]

(similar curves for semi-infinite reflectance)

different source-detector separations

\[ r = 15 \text{ mm} \]
\[ \mu_a = 0.006 \text{ mm}^{-1} \]
\[ \mu_s' = 1 \text{ mm}^{-1} \]
\[ n = 1.4 \]

normalized

\[ r = 15 \text{ mm} \]
\[ 35 \text{ mm} \]
\[ 25 \text{ mm} \]
\[ 15 \text{ mm} \]
Frequency domain diffusion

amplitude modulated source

Decaying photon density distribution

detector

detected signal:

\[ S(t) \]
Frequency domain diffusion

Amplitude modulation
50 MHz - 1000 MHz

Source

\[ \Phi(\omega, r_1) \]

detected intensity

Tissue
Frequency-resolved

The observables:

reference modulation

detected signal

\[ \phi \]

\[ f \]

DC

AC

time

0  5  10  15  20  25  30
Frequency-dependent wave properties: i.e., dispersion!

\[ \mu_a = 0.006 \text{ mm}^{-1} \]
\[ \mu_s' = 1 \text{ mm}^{-1} \]
\[ n = 1.4 \]

- Decay factor
- Propagation factor

Graph showing:
- Constant velocity
- Frequency / MHz
- \( mm^{-1} \)
Validity of photon density wave picture

- Amplitude-modulated source
- Characteristic decay time is $(\mu_a c)^{-1}$
- Typical photon's random walk: time between collisions is $(\mu_s c)^{-1}$

If photon scatters only once during a source modulation cycle, diffusion model does not approximate reality (>10 GHz)

If launched photons "survive" for less than an oscillation cycle, AC effects are no different from DC effects (<10 MHz)
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  pulsed
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instrument design considerations

various applications
Basic instrumentation for all cases

- Near-IR light source(s)
- Detector(s)
- PC
- Calculated optical properties
- Biochemical information
- Clinical decisions

\[ \mu_a(\lambda), \mu'_s(\lambda) \]
Steady state ("CW") + spectrometer

broadband source (lamp)

~250 μW/10nm

delivery fiber
(multimode, e.g. 100 microns)

spectrograph

array detector (e.g. CCD)
Spectrographic CCD display

fiber image: \( \frac{150 \mu m}{\text{fiber}} \cdot \frac{1 \text{ pixel}}{25 \mu m} = 6 \text{ pixels/fiber} \)

integration time: 10’s of seconds

calibration: shine equal light into all channels
Steady state reflectance data

- Optimize fit to steady-state theory and to obtain $\mu_a$ and $\mu'_s$ (e.g., Levenberg-Marquardt)
- Repeat for all wavelengths
Linear probe for *in vivo* diffuse reflectance spectroscopy

Seeing blood oxygenation change

normal air

when rat is breathing 95% O₂

Need to measure both “near” and “far”

\[
\Phi(\rho) = \frac{1}{4\pi D} \left( \frac{\exp(-\mu_{\text{eff}} r_1(\rho))}{r_1(\rho)} - \frac{\exp(-\mu_{\text{eff}} r_2(\rho))}{r_2(\rho)} \right)
\]

dominated by scattering \((D)\)

falls off as \(\exp(-\mu_{\text{eff}} r)\)
Dynamic range requirements: VTS calculations
Non-contact alternative

- absolute reflectance measurable
- fit shape AND height of reflectance curve
Typical power levels: VTS/Matlab calculation
Not so many wavelengths are needed!

Basic instrumentation for all cases

- Near-IR light source(s)
- Detector(s)
- PC
- Calculated optical properties
- Biochemical information
- Clinical decisions

\[ \mu_a(\lambda) \quad \mu'_s(\lambda) \]
Steady state measurements: no spectrometer

diode lasers

5-50 mW

(typically) PMTs or APDs

- much brighter: deeper penetration, or faster acquisition
- fewer wavelengths: less chemical information

no pressing need for a spectrometer, but do need to resolve the wavelengths
Resolving wavelengths: time-sharing

- Time-sharing: tissue sees only 1 wavelength at a time
- Uses full dynamic range of detector(s)

Diode lasers

- Fiber-to-fiber multiplexer (i.e. switch)
Resolving wavelengths: serial modulation

Time-sharing: all lasers on, but only 1 wavelength modulated

- reduced dynamic range
- robust against background light
- higher throughput (multimode fiber switches usually lossy)
Resolving wavelengths: parallel modulation

Frequency-encoding: multiple wavelengths detected simultaneously
- good for higher time-resolution
- greater demand on instrumentation or signal processing
Time-resolved measurements

Infinite geometry:

\[ \Phi(r, t) = c (4\pi D c t)^{-3/2} \exp \left( -\frac{r^2}{4Dc t} - \mu_a c t \right) \]
Time-resolved measurements

Infinite geometry: \[ \Phi(r, t) = c(4\pi Dct)^{-3/2} \exp\left(-\frac{r^2}{4Dct} - \mu_a ct\right) \]

(similar curves for semi-infinite reflectance)

different source-detector separations

\( r = 15 \text{ mm} \)
\( \mu_a = 0.006 \text{ mm}^{-1} \)
\( \mu_s' = 1 \text{ mm}^{-1} \)
\( n = 1.4 \)

35 mm
25 mm
15 mm

normalized

500 1000 1500 2000 2500

time / psec

time resolution needed: psec scale; integration time needed: 100's-1000's of psec
Time-resolved measurements

- ps- or fs-pulse laser (mode-locked dye, Ti:sapph, or fiber; pulsed diode)
- many pulses, builds up a histogram
- typical integration time: ~ 0.1-1.0 sec

**Diagram:**
- Timing pulse
- Ps or fs-pulse laser connected to tissue
- Fast detector:
  - Streak camera
  - Microchannel plate photomultiplier tube
- Time gate
- Signal

**Legend:**
- Timing pulse
- Ps or fs-pulse laser
- Tissue
- Fast detector
- Time gate
Multi-wavelength, time-domain data

Figure 1. Schematic diagram of the system set-up. CD, cavity-dumped; ML, mode-locked; CW, continuous wave; TCSPC, time-correlated single-photon counting; MC, monochromator; MCP-PMT, microchannel plate photomultiplier.

In vivo optical characterization of human tissues from 610 to 1010 nm by time-resolved reflectance spectroscopy
Experimental time-resolved photon diffusion measurements on biological tissue

Fig. 1 Typical best fit of time-resolved reflectance data. The experimental data (♦) are fitted with the convolution (——) of the instrumental transfer function (---) with the theoretical curve (not shown).
Absorption and scattering spectra extracted from fitting time-resolved data to theory.
Important chemical absorbers in tissue
Calculating chemical concentrations

**Table 1** Concentration of absorbers in the female breast

<table>
<thead>
<tr>
<th>Subject</th>
<th>Hb + HbO₂/µM</th>
<th>Y (%)</th>
<th>Water (%)</th>
<th>Lipid (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (44 years)</td>
<td>37</td>
<td>78</td>
<td>13</td>
<td>86</td>
</tr>
<tr>
<td>2 (24 years)</td>
<td>71</td>
<td>75</td>
<td>46</td>
<td>29</td>
</tr>
</tbody>
</table>

**Fig. 3** Absorption spectra of the female breast on 44 year (■) and 24 year (□) old volunteers. The water and lipid content vary appreciably with age.
Reminder of the observables:

- Reference modulation
- Detected signal

Frequency-resolved method
Frequency-resolved instrumentation

AC (MHz)

1
2
3

ref

sig

lockin amplifier

+ +
Frequency-resolved instrumentation: heterodyning

takes RF frequency $\rightarrow$ low frequency (better detection electronics)

http://www-nml.dartmouth.edu/nir/index.html
Frequency domain: optical geometries

Ways to measure:

1) phase and/or amplitude vs. distance
2) phase and/or amplitude vs. frequency

fit to a model to get absorption and scattering coefficients
Multidistance vs. multifrequency

Multidistance

- Single modulation frequency = optimized impedance matching, simpler design

- Multiple detectors = measurements in parallel

- Different distances = different tissue volumes (at least slightly)
Multidistance vs. multifrequency

Multifrequency

- Multiple modulation frequencies = lossier, more complex design

1

2

- Single detector: measurements in series
- Single distance: more consistent tissue volume characterized
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various applications
Application #1: time-domain tissue oximetry

Time-resolved measurements

Resolving wavelengths

Tunable mode-locked laser (many wavelengths):

Simultaneous pulses with delay line (2 wavelengths):

much faster results
2-wavelength tissue oximeter

- Multianode photomultiplier
- 672 & 818 nm diode lasers, 100 ps pulses
- 1 mW average power
- TCSPC board, 80 MHz acquisition
- Integration time $\geq 100$ msec


Fig. 2. Typical time-resolved reflectance curves and reference pulses. Note, in each reference pulse, the presence of the characteristic afterpulse of the PMT. The arrows mark a FWHM of 200 ps.
2-wavelength tissue oximeter

\[ R_f = \frac{1}{2} (4\pi D c)^{-3/2} t^{-5/2} \exp \left( -\mu_a c t \right) \left[ z_0 \exp \left( -\frac{z_0^2}{4Dc} \right) + (z_0 + 2z_b) \exp \left( -\frac{r_i^2}{4Dc} \right) \right] \]

from previous lecture

\[ R(\rho, t) = \frac{1}{2} (4\pi vD)^{-3/2} t^{-5/2} \exp(-\mu_a vt) \exp\left(-\frac{\rho^2}{4Dv t}\right) \]
\[ \times \left\{ z_0 \exp\left(-\frac{z_0^2}{4Dv t}\right) - (z_0 + 2z_e) \right\} \times \exp\left[-\frac{(z_0 + 2z_e)^2}{4Dv t}\right] \]

where \( v \) is the speed of light in the medium, \( z_0 \) \( = (\mu_s')^{-1} \) is the effective mean-free path, \( D \) \( = z_0/3 \) is the diffusion coefficient, \( z_e \) \( = 2D(1 + r_d)/(1 - r_d) \) is the extrapolated distance, and \( r_d \) can be approximated by \( r_d = -1.440 n^{-2} + 0.710 n^{-1} + 0.668 + 0.0636 n \), with indices of refraction of \( n = 1.33 \) for the experiments on phantoms and \( n = 1.4 \) for the in vivo measurements.

Validation: unmixing absorption & scattering

Time domain *in vivo* blood measurements

Application #2: Breast tissue

Breast tissue analysis

Clinical goals:

- Flag abnormal tissue (i.e. tumors)
- Coregister with higher-resolution imaging modalities (e.g. mammographic x-ray)

Instrumentation goals:

- Absorption spectroscopy of breast
- Tomographic reconstructions in breast with tumors
Figure 1. Multiwavelength, multifrequency FDPM instrument.
Simultaneous fit

Data

**DIFFUSION FIT**

absorption and reduced scattering coefficients

concentrations of major chromophores

**Amplitude (a.u.)**

**Frequency (MHz)**

**Phase (deg)**

(courtesy F. Bevilacqua)
Optical scanning of breast tissue

Diffuse optical scanning of tumor vs. normal

Diffuse optical scanning of tumor vs. normal

Tumor vs. normal: contrast in both modalities

Physiological components of fit

- 19 M water
- 32 μM HbO₂
- 11μM Hb
- 0.3 g/cm³ fat

μₐ (mm⁻¹)

wavelength (nm)
Choosing the most diagnostic parameters

Table 1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normal</th>
<th>T\textsubscript{PEAK}</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Median</td>
<td>Mean</td>
</tr>
<tr>
<td>ctHHb</td>
<td>6.73 ± 2.08</td>
<td>6.57</td>
<td>15.3 ± 8.16</td>
</tr>
<tr>
<td>ctO\textsubscript{2}Hb</td>
<td>18.6 ± 6.9</td>
<td>18.9</td>
<td>33.3 ± 12.0</td>
</tr>
<tr>
<td>%Lipid</td>
<td>55.5 ± 8.7</td>
<td>54.9</td>
<td>30.6 ± 13.7</td>
</tr>
<tr>
<td>%H\textsubscript{2}O</td>
<td>27.5 ± 12.1</td>
<td>25.4</td>
<td>49.9 ± 25.4</td>
</tr>
<tr>
<td>Scatter power</td>
<td>0.800 ± 0.362</td>
<td>0.830</td>
<td>1.17 ± 0.503</td>
</tr>
</tbody>
</table>

ctHHb, deoxygenated hemoglobin concentration; ctO\textsubscript{2}Hb, oxygenated hemoglobin concentration; T\textsubscript{PEAK}, peak tumor values.
Pre and post-menopausal breast examples

Figure 2. Measured absorption spectra for a 32-year-old pre-menopausal subject (■, solid line) and a 54-year-old postmenopausal subject (▲, dashed line). Points represent the average of several measurements, and lines represent a least-squares fit (extrapolated to all wavelengths) based on the assumption that breast absorption is due only to Hb-R, Hb-O₂, H₂O, and lipids.

Tumor response to neoadjuvant therapy


Diagram showing a decrease in tissue optical index over days of treatment, indicating a reduction in tumor prior to radiation therapy.
Breast imaging: Computed Tomography

CT-scan (x-ray)

scattering $\ll$ absorption $\Rightarrow$ paths = straight lines

(courtesy F. Bevilacqua)
Breast imaging: *Optical* Computed Tomography

near-infrared light

sources

detectors

numerical reconstruction

scattering $\gg$ absorption $\Rightarrow$ broad probability of paths
$\Rightarrow$ challenges: ill-posed problems (non-unique solution)
poor resolution

(courtesy F. Bevilacqua)

http://www.medphys.ucl.ac.uk/research/borg/index.htm
Tomography in the frequency domain

Fig. 1. Schematic of the automated imaging instrument including hardware and software processing. Source optical fibers are indicated in red and detector optical fibers in green.

Optical mammography: diffuse imaging

http://www.ucl.ac.uk/medphys/research/borl/imaging/monstir/breast
NIR Images of Volunteers with tumors

- **Patient A** -- Fibroadenoma (benign)
- **Patient B** -- Invasive Carcinoma

Sketches by radiologist of tumor location and size

Near infrared images of hemoglobin concentration

2 wavelengths, 3 iterations, $\lambda_c = 10000$
Application #3: brain hemodynamics

light in (690, 830 nm)

light collected
Noninvasive monitoring of hemodynamics

Valsalva Maneuver – 06/03/04

- Optical power measurements
- Increased blood supply
- Heartbeat
- Oxy- and deoxy-hemoglobin concentration changes
Single subject countdown timecourse

Start stimulus
Stop stimulus

Absorptions

Hemoglobin concentrations
Typical headpiece for adults

optical fiber bundles

First Nearest Neighbors  1.3 cm
Second Nearest Neighbors  3 cm
Third Nearest Neighbors  3.9 cm
Visual Stimulation Protocol

- 6 stimulus periods of pattern reversal at 10 Hz

*based upon code by Brian White and Joseph Culver, Washington University (St. Louis)*
Simplified model of reflectance vs. time

\[
\frac{P_{out}}{P_{in}} = e^{-\mu_a L}
\]
Simplified model of reflectance vs. time

\[ \frac{P_{out}}{P_{in}} = e^{-\mu_a L} \]

\[ \frac{P_{\rho}}{P_{in}} = G(\mu_a, \mu_s', \rho) e^{-\mu_a \langle L \rangle} \]

\[ = G(\mu_a, \mu_s', \rho) e^{-\mu_a [DPF \cdot \rho]} \]

“geometry factor”

\[ \langle L \rangle \approx DPF \cdot \rho \]

e.g. \( DPF = 6 \)
Simplified model of reflectance vs. time

Sequential measurements:

\[ \frac{P_\rho(t)}{P_{in}} = G(\mu_a, \mu'_s, \rho) e^{-\mu_a(t)[DPF \cdot \rho]} \]

Changes between measurements:

\[ \frac{P_\rho(t)}{P_\rho(t_0)} = e^{-[\mu_a(t) - \mu_a(t_0)][DPF \cdot \rho]} \]

\[ = e^{-\Delta \mu_a(t)[DPF \cdot \rho]} \]
Simplified model of reflectance vs. time

\[
\frac{P_\rho(t)}{P_\rho(t_0)} = e^{-\Delta\mu_\alpha(t)[DPF \cdot \rho]} \\

\frac{P_{out}}{P_{in}} = e^{-\mu_L} \\

"Modified Beer-Lambert Law (MBLL)"

\[
L_{in} \rightarrow \rho \rightarrow P_{in} \rightarrow P_{out}
\]
Reminder: time domain *in vivo* blood measurements

pressure cuff on

absolute measurements
Reminder: CW *in vivo* measurements

**Valsalva Maneuver – 06/03/04**

- Optical power measurements
- Increased blood supply
- Oxy- and deoxy-hemoglobin concentration changes

![Graph showing Valsalva maneuver with heart rate and hemoglobin concentration changes.](image)
The optical geometry

A real head

A physicist’s head

Scalp
Skull
CSF
Brain

Scalp hemodynamics
Cerebral hemodynamics
Problem: not all blood is in the brain!

Measurement sensitive to both scalp and brain hemodynamics

Want to isolate brain-specific trends

Saager et al., NeuroImage 55(4), 1679--1685 (April 2011)
2 detectors, 2 different depths probed!
Improving signal-to-noise by subtracting “scalp” signal
Good summary case for diffuse spectroscopy
Reviewing the roadmap

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various applications
To learn more

**Tuesdays** in January (7.1, 14.1, 21.1, 28.1), 2:00 pm, IPHT Sitzungssaal

Lecture 2 - **Turbid tissue optics I: Introduction**
Lecture 3 - **Turbid tissue optics II: Instrumentation and measurements**
Lecture 4 - **Turbid tissue optics III: (More) Applications**
Lecture 5 - **A different view of turbidity: elastic scattering analysis**