Introduction to Inverse Solvers in Biophotonics: Inverse Adding-Doubling

Computational Biophotonics Workshop
8/6/14
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Teaching Objectives

• Understand How Adding Doubling…
  – Is an Exact Solution of the Radiative Transport Equation
  – Can be used to describe slabs of tissue (with infinite lateral extent)

• How can we use the solutions of this Adding-Doubling Model (Total Reflectance and Transmittance) to infer the Optical Properties within a homogeneous slab of tissue: Inverse Adding-Doubling

• What does it take to actually measure Reflectance and Transmittance and employ IAD in experimental setting
Outline

• General introduction to inverse solver methods
• Forward Model: What is Adding-Doubling?
  – Principles of Operation
• Inverse Model
  – Solutions from Idealized Measurements
  – Considerations for physical measurements
    • Instrumentation
    • Calibration
    • Sample Geometries/Constraints
  – Strategies for managing experimental conditions
• Summary: “Take Home Messages”
• Examples of IAD used in practice (time permitting)
• Conclusions: When is IAD an appropriate tool to use
Introduction to Inverse Solver Methods

• Refresher of concepts introduced from the first day of this workshop:

• Forward Model
  – Describes “what happens” to light in media as a function of the properties of these media
  – For tissues, these are typically:
    • $\mu_a$, $\mu_s$, g, n, etc...
    – Generating “outputs,” typically in terms of Radiance, L, either within media volumes and/or at boundaries
Refresher of concepts introduced from the first day of this workshop:

Inverse Model

- Infers properties of tissues based on the measurable outputs of an unknown medium
- In general, the measurable outputs are not sufficient to fully describe all the input properties (ill-posed)
- However, through careful selection of
  - a Forward Model
  - assumptions on tissue properties and distribution
  - constraints on the measurement geometry
  - experimental conditions (perturbations)

...inverse solutions are achievable
There are a variety of models and methods that have been developed to date.

– All have their own strengths and weaknesses
  • Computational processing speed vs Accuracy
  • Measurement complexity vs Acquisition time
  • Sensitivity to spatial heterogeneity
  • Instrumentation costs…

– Key to successful research is to understand when are where each method will best address the problem you wish to address
Forward Model: What is Adding-Doubling?

• Calculates the Reflection (Reflectance) and Transmission (Transmittance) of light from a single thin, homogenous layer
  – Doubling
    • Reflectance and Transmittance from an arbitrarily thick slab can then be obtained by repeatedly doubling the thin slab solution until the desired thickness is achieved
Forward Model: What is Adding-Doubling?

- Calculates the Reflection (Reflectance) and Transmission (Transmittance) of light from a single thin, homogenous layer
  - Adding
    - An extension of doubling, permitting layers of differing properties and be added in order to calculate Reflectance and Transmittance in more complex structures including internal reflection at layer boundaries
Radiance (Lecture 2):

- $L(r,\Omega)$: 6-D
- But let's simply this to a 1-D representation:
  - $L_+(x)=c_1 e^{(\lambda x)} + c_2 e^{(-\lambda x)}$
  - $L_-(x)=d_1 e^{(\lambda x)} + d_2 e^{(-\lambda x)}$
  - Where $c$, $d$ are coefficients dependent on input parameters and $\lambda = \sqrt{\mu_a(\mu_a + \mu'_s)} = \sqrt{\mu_a \mu_{tr}}$
- Believe it or not, this is an exact solution of RTE when reduced to a 1-D space
Starting with $L^+$ and $L^-$ from the previous slide, let's see how Adding Doubling calculates to Reflectance and Transmittance for slabs:

- $L^{1+} = T^{01}L^{0+} + R^{10}L^{1-}$
- $L^{0-} = R^{01}L^{0+} + T^{10}L^{1-}$

Here $T$ and $R$ are operators driven by:

- $L_+(x) = c_1 e^{λx} + c_2 e^{-λx}$
- $L_-(x) = d_1 e^{λx} + d_2 e^{-λx}$


How is this a powerful model?
- This is an exact solution for RTE
  - There is no restriction of the ratio of scattering to absorption, so this will work for all optical wavelengths
  - There are no restrictions on scattering anisotropy
  - Internal reflections at boundaries are included (typically described in terms of Fresnel Reflections)

What are the Key Assumptions?
- There is no time dependence (Steady-State)
- Layers must be uniform
  - Finite in thickness
  - Infinite in lateral extent
- Absorption and Scattering must be evenly distributed within the volumes of these layers
- Illumination is uniform, but can collimated or diffuse light
Forward Model: What is Adding-Doubling?

• Summary
  – Adding-Doubling is a Forward model that can describe the Total Reflectance and Transmittance at the boundaries of layers
    • (Note, this method is not optimized to describe within the volumes of these slabs)
  – This model simplifies Radiance into a 1-D function by assuming lateral homogeneity (1 spatial dimension) and integrating over all scattering angles
  – This simplified model reduces the number of input parameters to only 5: $\mu_a$, $\mu_s$, $g$, $n$, and $d$.
    • By assuming values of $n$ and $g$, and measuring the physical thickness of the slab, $d$, it is hence possible to describe Reflectance and Transmittance in terms of only 2 input parameters: mua and mus (addressing the ill-posedness issue)
Inverse Model

• Model Input: \([\mu_a, \mu_s, g, n, d]\)
  – Dimensionless parameters (reduce 3 of the inputs to 2):
    • \(a = \frac{\mu_s}{\mu_a + \mu_s}\)
    • \(\tau = d(\mu_a + \mu_s)\)
  – From Measurements (from exact solution)
    • \(R(\mu_a, \mu_s, g) = L^-(0), T(\mu_a, \mu_s, g) = L^+(d)\)
  – Refractive Index, \(n\), also can manage surface reflections at boundaries (Fresnel Reflections)
  – Assume Henyey-Greenstein scattering, described by \(g\).

• Goal for Inverse Solver:
  – Find \(\mu_a = \mu_a(R, T), \mu_s = \mu_s(R, T)\)

• IAD involves iterative strategy
Inverse Model

• Guess set of optical properties

• Calculate reflectance $R = L^{-}(0)$ and (total) transmittance $T = L^{+}(d)$ from exact formulas (Forward Model)

• Compare calculated and measured values

• Test for goodness of fit

• Repeat until a match is found
• Figure shows the overlay mesh of [albedo, anisotropy] for a fixed thickness slab under index match conditions (no Fresnel reflections)
  – While this mesh demonstrates the uniqueness of the inverse solution, there are regions where the space of the mesh begins to collapse (small changes in $R$ or $T$ would result in large differences in $a$ or $g$)

$$
a \rightarrow a' = \frac{a(1-g)}{1-ag}, \quad \tau \rightarrow \tau' = (1 - ag) \tau$$
Using the “reduced” representation, the inverse solution mesh is now more evenly distributed.

- Over a range of Reflectance and Transmittance values.
- Over a range of typical tissue values.

Fig. 2. Total reflection and total transmission of a slab as a function of the reduced albedo $a'$ and reduced optical thickness $\tau'$. Isotropic scattering is assumed as well as an index of refraction mismatch ($n = 1.4$). Each point on the ($a'$, $\tau'$) grid corresponds to a unique ($R_T$, $T_T$) pair.
How do we measure “Total” Reflectance and Transmittance?

- In the 1-D model we integrated all angular contributions into a single dimension.
- How could we capture and detect over all angles in a physical measurement?
Inverse Model: From Physical Measurements

- Instrumentation
  - Source
  - Integrating Sphere
  - Detector
Inverse Model: From Physical Measurements

• Instrumentation
  – Integrating Spheres: They are what they sound like
    • Interior walls are coated with a highly scattering, highly reflective material (Spectralon, Mg02, etc)
    • Reflect light within the sphere over a 4-pi solid angle (integrates optical signal over all angles that enter sphere)
    • Detector port samples a fraction of this diffusely scattered light
    • For Transmittance, place sample at the entrance port; sphere integrates incoming light
    • For Reflectance, place sample 180 degrees from entrance port; sphere integrates all the diffusely reflected light (specular reflection exits back out the entrance port, if aligned correctly)
Instrumentation

- Integrating Spheres are not perfect
  - Walls are not 100% reflective (more like 99-98%)
  - Not all angles are integrated (angles subtended by sample/entrance/detector ports are lost)
- Thankfully, someone has figured all these details out for you

Inverse Model: From Physical Measurements

![Graph: Relationship between measured reflectance and transmittance.](image)

Figure 1: The relationship between measured values using an integrating sphere and the true values. In this graph, it is assumed that the dark measurement was completely dark and that the reflectance standard was 100%. These effects are typical for a relatively large sample port (50 mm port) in a moderate size integrating sphere (200 mm diameter). In general the measured values using an integrating sphere will tend to underestimate reflectance measurements ($M_R < R$) and overestimate transmittance ($M_T > T$).

### Table 2. Calculation of Detector Power for a Single Sphere with Light Initially Incident upon the Sphere Wall

<table>
<thead>
<tr>
<th>Reflection</th>
<th>Wall Reflection</th>
<th>Sample Reflection</th>
<th>Wall Collection</th>
<th>Sample Collection</th>
<th>Lost through Holes</th>
<th>Detector Collection</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>$n P$</td>
<td>$A P$</td>
<td>$\frac{A}{P} r P$</td>
<td>$\frac{A}{P} m P$</td>
<td>$\frac{A}{P} m P$</td>
<td>$\frac{A}{P} m P$</td>
</tr>
<tr>
<td>2</td>
<td>$n m P$</td>
<td>$R_p \frac{A}{P} m P$</td>
<td>$a (m m P + R_p \frac{A}{P} m P) \frac{A}{P} m P$</td>
<td>$\frac{A}{P} m P + R_p \frac{A}{P} m P$</td>
<td>$\frac{A}{P} m P$</td>
<td>$\frac{A}{P} m P$</td>
</tr>
<tr>
<td>3</td>
<td>$n a (m a + R_p \frac{A}{P} a P)$</td>
<td>$R_p \frac{A}{P} (m a + R_p \frac{A}{P} a P) m P$</td>
<td>$a (m a + R_p \frac{A}{P} a P) m P$</td>
<td>$\frac{A}{P} (m a + R_p \frac{A}{P} a P) m P$</td>
<td>$\frac{A}{P} (m a + R_p \frac{A}{P} a P) m P$</td>
<td>$\frac{A}{P} (m a + R_p \frac{A}{P} a P) m P$</td>
</tr>
<tr>
<td>i</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>a</td>
<td>$n m (m a - R_p \frac{A}{P} a P)^{ \frac{k}{A} } m P$</td>
<td>$R_p \frac{A}{P} (m a - R_p \frac{A}{P} a P)^{ \frac{k}{A} } m P$</td>
<td>$a (m a - R_p \frac{A}{P} a P)^{ \frac{k}{A} } m P$</td>
<td>$\frac{A}{P} (m a - R_p \frac{A}{P} a P)^{ \frac{k}{A} } m P$</td>
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</tr>
</tbody>
</table>
Inverse Model: From Physical Measurements

- **Calibration**
  - Reflectance and Transmittance are *relative* quantities, dependent on the source illumination on the sample
  - To measure R, T, we need to also know the source and account for any other signals we may measure (e.g. stray light, detector dark current)
    - “Empty Sphere”: Measure source illumination, establish what 100% transmission looks like in terms of signal counts
    - “Dark Measurements”: Measures the 0% signal in the sphere configuration in all 3 measurements (Reflectance, Transmittance, 100% signal calibration)
      » Light source is blocked
      » Characterizes the amount of dark current of detector as well as any stray light present in the system
    - Note: Dark Measurements can be both temperature and integration time sensitive
Inverse Model: Strategies for managing experimental conditions

• Inverse Adding-Doubling code is freely available on the web (courtesy of the heroic efforts of Scott Prahl): http://omlc.org/software/index.html
  – Whereas running inverse adding-doubling is a simple process from noiseless, idealized Reflectance and Transmittance data, the bulk of this code has been carefully crafted to address and account for all the imperfections present in experimental settings
    • Integrating Sphere Properties
      – This code has been set up to take in the physical properties of the sphere in use (e.g. sphere size, wall reflectivity, port sizes, etc)
    • Sample geometry
      – Whether this is a bare sample or is a sample sandwiched between slides or cuvette walls
      – Accounting for multiple layers (e.g. index mismatches between sample and glass in addition to the mismatch with air)
    • Side light losses
      – What IAD does not account for is the limited extent of the entrance port and beam size
      – In addition to minimizing error between Forward Model and Measurement Data, this code also runs a forward Monte Carlo simulation to estimate the amount of light that would not enter sphere, given the
        » Relative port and beam sizes
        » Estimated optical properties of the sample and its thickness
        » Physical distance of the sample from the sphere (due to slide thickness)
Sample Considerations

- Inverse Adding-Doubling does not account for:
  - Surface roughness (boundary assumptions, Fresnel reflections)
  - Tissue heterogeneity (Uniformity in infinity lateral extent... what do you consider “infinite” anyway?)
  - Thickness variance (As Adding-Doubling suggests, optical properties should scale with thickness...)
Important Take-Home Messages

• Need to understand the practical constraints/limitations of the model to best exploit the advantages this approach has to offer
Important Take-Home Messages

• Rule #1: Minimize measurement non-idealities
  – Sample Thickness
    • Thinner samples could help minimize side light losses
    • Optical Thickness: span the space of reflectance and transmittance values (keeping this in the range of $[0.75-12]$, is “safe,” beyond this requires a) attention to detail, b) high signal-to-noise)
    • Precision at which you can measure the sample
  – Sample preparation
    • this has to “mimic” a slab of infinite lateral extent
      – no surface roughness
      – even thickness
      – minimal spatial heterogeneities in (and adjacent to) the illumination area
Rule #2: Instrument Stability and Configurability

- These set-ups are very lossy
  - The signal you detect in the end is only a small fraction of the Total Reflection or Transmission.
    - Requires a sensitive and stable detector (or a lot of patience)
  - Single Spheres are easier to calibrate, but collimating light (particularly of a wide spectral range) is difficult and rejects a majority of photons coming from the source
    - Dual Sphere setups are also an option (though not discussed here)
    - In general, Dual Sphere setups do not need collimation, but calibration methods are more challenging.
- Using a variable iris is a convenient means to adjust the amount of light used in these measurements
  - spot size is also limited by
    » Heterogeneity of the sample
    » optical thickness vs side light losses
Important Take-Home Messages

• Rule #3 Have an expectation of what optical properties you hope to measure
  – Validate your results with known references or tissue simulating phantoms
    • Short path-length cuvettes can be used to measure controlled concentration of dyes/Intralipid or microspheres
    • Silicone phantoms can be fabricated in thin sheets and remain stable over large periods of time
    • Check where in parameter space your reflectance and transmittance measurements fall
References Used in this Presentation


- “Determining the optical properties of turbid media by using the adding-doubling method,” Scott A. Prahl, Martin J. C. van Gemert, and Ashley J. Welch, Applied Optics, 32(4), 1993


- Inverse Adding-Doubling Code and (additional references): http://omlc.org/software/index.html

2 Examples where IAD was used at BLI

• Characterization of ex-vivo tissue properties
  – Optical clearing of tissues

• Generation of structured (layered) tissue simulating phantoms
Evaluation of Optical Clearing Agents

- Optical Properties of Mouse Brain Tissue After Optical Clearing with FocusClear

Austin J. Moy, Ph.D., Bernard V. Capulong, B.S., Rolf B. Saager, Ph.D., Matthew P. Wiersma, B.S., Patrick C. Lo, Anthony J. Durkin, Ph.D., Bernard Choi, Ph.D.
Evaluation of Optical Clearing Agents

• Is there a way to quantify:
  – The amount of “clearing” (i.e. the reduction of scattering) of ex-vivo tissue slices?
  – The amount of time needed to optimally clear these tissues?

• Why?
  – Goal is to map tissue microvasculature in brain tissue (mouse model)
  – Reduction in scattering increases the depth these vessels can be traced (less scattering, lower number of tissue slices required)
MIP maps of tissue microvasculature in 1 mm brain slices optically cleared for (a) 1 h (b) 3 h (c) 6 h and (d) 24 h showing the increased density of vasculature that can be visualized with increasing optical clearing time.
Evaluation of Optical Clearing Agents

- \( OCP = \frac{\mu_s'(before\ clearing)}{\mu_s'(after\ clearing)} \)
Validation of Layered Model Spectroscopy

- Biological tissues such as skin are highly structured and layered
- Quantitative optical techniques report chromophore concentrations in terms of a “per volume interrogated”
  - 1) Quantitative measurements represent a fractional contribution from each layer interrogated
  - 2) Volume of tissue is dependent on the optical properties at a given wavelength

Consequences

- Detected concentrations can be confounded by layer thicknesses
- Pure spectral components become distorted as a function of an interrogation volume change
Absorption Model for “Partial Volume Effect”

\[
\mu_{a,\text{meas}}(\lambda)l_{\text{meas}}(\lambda) = c_1 \mu_{a1}(\lambda)l_1 + c_2 \mu_{a2}(\lambda)l_2(\lambda)
\]
where \( l_2(\lambda) = l_{\text{meas}}(\lambda) - l_1 \)

If we can determine a wavelength centered concentration, \( c_{\text{meas}}(\lambda) \), estimate the total depth of interrogation, \( l_{\text{meas}}(\lambda) \), we can estimate the top layer thickness and layer specific concentration.
Simulation - Monte Carlo Results

Simulated SFDI results of Skin Model

\[ l_1 = \frac{(c_{2\text{meas}}(\lambda_1) - c_{2\text{meas}}(\lambda_2))l_{\text{meas}}(\lambda_1)l_{\text{meas}}(\lambda_2)}{(c_{2\text{meas}}(\lambda_1)l_{\text{meas}}(\lambda_1) - c_{2\text{meas}}(\lambda_2)l_{\text{meas}}(\lambda_2))} \]

<table>
<thead>
<tr>
<th>Top Layer Thickness ((\mu m))</th>
<th>Actual</th>
<th>Calc</th>
</tr>
</thead>
<tbody>
<tr>
<td>80</td>
<td>80</td>
<td>81</td>
</tr>
<tr>
<td>135</td>
<td>135</td>
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<td>216</td>
</tr>
<tr>
<td>300</td>
<td>300</td>
<td>243</td>
</tr>
</tbody>
</table>
Experimental Measurements
Skin Phantoms

- **“Epidermal” layers**
  - Range in thickness from 150 – 360 microns
  - TiO$_2$ approximated scattering
  - Naphthol green provides absorption with distinct spectral features

- **“Dermal” layer**
  - 2cm thick (considered semi-infinite)
  - TiO$_2$ approximated scattering
  - Nigrosin provides absorption with distinct spectral features

Silicone phantoms were made to simulate the physical dimensions of layered structures present in skin.

270 µm Naphthol Green

2cm Nigrosin base
High Frequency Ultrasound Image of Skin

Layered Phantom

120 μm

2.01 mm
Validation of phantom properties

- Recipes give general expectations for optical properties, but lacks accurate account of ultimate properties produced in a cured phantom.

- Each cured sheet is measured for optical/physical properties:
  - Thickness is measured by calipers (or ultrasound).
  - Optical properties are determined by Inverse Adding-Doubling.
    - Scott Prahl – code http://omlc.ogi.edu/software/iad/
## Experimental Measurements

### Tabulated results

<table>
<thead>
<tr>
<th>Top Layer Thickness (μm)</th>
<th>Actual</th>
<th>Calc.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Top Basis</td>
<td>150</td>
<td>220</td>
</tr>
<tr>
<td>Bottom</td>
<td>350</td>
<td>355</td>
</tr>
</tbody>
</table>

### Measured Absorption from Layered Phantoms

![Graph showing measured absorption from layered phantoms](image-url)
When Is IAD the appropriate tool?

• When it comes to methods and models to solve for tissue optical properties, IAD is just one of many options available.

• Key: know what you are looking for (or at least what the selected method cannot tell you)
When Is IAD the appropriate tool?

**Strengths**
- Each step has a direct, physical interpretation
- Determines optical properties
  - At any optical wavelength
  - At any anisotropy
- Employs a steady-state method (simplifies instrumentation costs*)
  - *But there is a caveat to this…

**Weaknesses**
- Slow and awkward for calculating internal fluences (outputs are at boundaries)
- Though simplification to 1-D, requires uniform illumination
- Each layer must be homogeneous in depth and spatial extent
Over the next 2 days you will learn more about other (reflectance-based) methods
- Encoding additional information in the light source/detection geometry

Some approaches can be complimentary
- Adds complexity to measurement (instrumentation, acquisition time, calibration, etc)
- Compounds limitations and constraints on tissue
- But can also over come some of the “ill-posedness” that a single technique carries…

General classes of measurements used in inverse solvers
Questions?
This is split in 2 main parts

- Sensitivity analysis of simulated tissue data
  - How do errors (assumptions) of anisotropy, layer thickness or index of refraction affect IAD calculations (and subsequent chromophore calculations)
  - How does noise (and post processing filtering) affect IAD calculations

- Actual physical measurements of a thin “tissue-like” sample
  - “Easy to generate numbers, challenging to trust them”: Experimental Measurements are not trivial.