Optical Tomographic Imaging of Small Animals

Andreas H. Hielscher, Ph.D.
Columbia University, New York City
Dept. of Biomedical Engineering
Dept. of Radiology

Overview

• Introduction
  X-Ray Tomography vs Optical Tomography

• Model-based iterative image reconstruction
  Basic concepts and mathematical background

• Instrumentation
  General optical imaging modalities
  Dynamic optical tomography system

• Applications
  Brain Imaging
  Tumor Imaging
  Fluorescence Imaging
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X-Ray Imaging

Uses X-rays to generate shadowgrams $M(\varphi, \xi)$.

( measurable attenuation )
unknown absorption cross-section
$A(x,y)$

$M(\varphi, \xi)$

X-ray source
electromagnetic wave $\lambda \sim 10^{-10} \text{m}$
energy $\sim 10^4 \text{eV}$

Energy propagates on straight lines through medium
X-Ray Shadowgram

X-Ray Tomography

$M(\phi, \xi)$
X-Ray Tomography

\[ M(\phi, \xi) \]
X-Ray Tomography

unknown absorption cross-section
A(x,y)

M(\phi, \xi)

X-ray source

=>Simple image reconstruction scheme: backprojection of M on lines of transmission. (Inverse Radon Transform)

2D Scan of Head

![2D Scan of Head image]
Optical Imaging

Uses near-infrared light (700 < λ < 900nm)

A(x,y) {unknown absorption & scattering profile}

EM - wave
λ ~ 800 × 10⁻⁹ m
energy ~ 1 eV

Energy does not propagate on straight line between source and detector (light is strongly scattered)

Optical Shadowgram
Optical Imaging

Uses near-infrared light (700< λ<900nm)

A(x,y) {unknown absorption & scattering profile}

EM - wave
λ ~ 800*10^-9m
energy ~ 1 eV

How to reconstruct cross-sectional images A(x,y) from measurement on surface?
(Inverse Problem)

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Model-Based Iterative Image Reconstruction

Theory: Initial guess

Forward Model, \( F \)

depends on \( \times N \) unknowns

predicted detector reading \( I_{P,G} \)

Experiment

sources

detectors

measured detector readings \( I_{M,G} \)

Theory:

\[
D = 1 \text{ cm}^2\text{ns}
\]

Forward Model I

3D-Time-Resolved Diffusion Equation

\[
\frac{\partial U}{\partial t} = \frac{\partial}{\partial x} D \frac{\partial U}{\partial x} + \frac{\partial}{\partial y} D \frac{\partial U}{\partial y} + \frac{\partial}{\partial z} D \frac{\partial U}{\partial z} - c \mu_a U + S
\]

with \( c := \text{speed of light in medium} \), \( S = \text{Source} \),

and diffusion coefficient :

\[
D = c \left( \frac{3}{2} \left[ \mu_a + \mu_s' \right] \right)
\]

with \( \mu_a = \text{absorption coefficient} \) and

\( \mu_s' = \text{reduced scattering coefficient} \).
Diffusion vs Transport Model

**Diffusion equation**
\[
\frac{\partial U}{\partial t} = \nabla c/(3\mu_a + 3\mu_s') \nabla U - c\mu_a U + S
\]

discretize into \(N\) spacial variables
leads to \(N\) finite-difference equations

**Equation of radiative transport**
\[
\frac{\partial \Psi}{\partial t} = S - \Omega \nabla \Psi - (\mu_a + \mu_s)\Psi + \int_0^{4\pi} \frac{\Psi(\Omega') p(\Omega*\Omega') d\Omega'}{4\pi}
\]

with \(U = \int_0^{4\pi} \Psi(\Omega') d\Omega'\) and \(\mu_s' = (1-g) \mu_s\)

discretization into \(N\) spacial and \(A\) angular variables
leads to \(N \times A\) coupled finite-difference equations

slower by factor \(\sim A\)

---

**Limits of Diffusion Model**

laser beam \(\downarrow\)

ring filled
with water

milk

---

Experiments
Transport

Intensity [au]

x [mm]

0 5 10 15 20 25 30 35 40

0 0.5 1 1.5 2 2.5

0 1 1.5 2 2.5

0 0.4 0.6 0.8 1 1.2 1.4 1.6 1.8

Diffusion

Experiments
Transport
Forward Model applied to Mouse Head

\[ \mu_a = 0.1 \text{ cm}^{-1}, \ \mu_s = 10 \text{ cm}^{-1}; \ 14781 \text{ nodes, 24 ordinates} \]

Model-Based Iterative Image Reconstruction

Experiment

\[ \text{sources} \rightarrow \text{detectors} \]

Theory:

\[ \text{initial guess} \rightarrow \text{sources} \rightarrow \text{detectors} \]

Forward Model, \[ F(D) \]

depends on \( N \times N \) unknowns

measured detector readings \( I_{M,i} \)

predicted detector reading \( I_{P,i} \)
Model-Based Iterative Image Reconstruction

Experiment

sources → detectors

measured detector readings $I_{M,i}$

Theory:

sources → detectors

Forward Model, $F(\cdot)$

$D = 1 \text{ cm}^2\text{ns}$

e.g. transport equation

predicted detector reading $I_{P,i}(\cdot)$

Analysis Scheme

$$\Phi = \sum \left( I_{M,i} - I_{P,i}(\cdot) \right)^2$$

Error Value $\Phi(\cdot)$

(This is just one number!)

yes

no

$\Phi < \varepsilon$

Updating Scheme

Model-Based Iterative Image Reconstruction

Experiment

sources → detectors

measured detector readings $I_{M,i}$

Theory:

sources → detectors

Forward Model, $F(\cdot)$

$D = 1 \text{ cm}^2\text{ns}$

e.g. transport equation

predicted detector reading $I_{P,i}(\cdot)$

Analysis Scheme

$$\Phi = \sum \left( I_{M,i} - I_{P,i}(\cdot) \right)^2$$

Error Value $\Phi(\cdot)$

Updating Scheme

$\Phi < \varepsilon$
Model-Based Iterative Image Reconstruction

Experiment

- sources → detectors
- measured detector readings $I_{M,i}$

Theory:

- new guess
- Forward Model, $F(\text{sources})$
e.g. transport equation
- predicted detector reading $I_{P,i}$

Analysis Scheme

$\Phi = \sum \{ I_{M,i} - I_{P,i} \}^2$

Error Value $\Phi$

- $\Phi < \varepsilon$ (yes)
- no

Updating Scheme

- $\Phi < \varepsilon$
Model-Based Iterative Image Reconstruction

Experiment:
- Sources → Detectors
- Measured detector readings $l_{M,i}$

Theory:
- New guess
- Predicted detector reading $l_{P,i}$ (e.g., transport equation)

Forward Model, $F$ (image)

Analysis Scheme:
$$\Phi = \sum \left( l_{M,i} - l_{P,i} \right)^2$$

Error Value $\Phi$

Final new guess

$\Phi < \epsilon$ → Yes

Model-Based Iterative Image Reconstruction

Experiment:
- Sources → Detectors
- Measured detector readings $l_{M,i}$

Theory:
- New guess
- Predicted detector reading $l_{P,i}$ (e.g., transport equation)

Forward Model, $F$ (image)

Analysis Scheme:
$$\Phi = \sum \left( l_{M,i} - l_{P,i} \right)^2$$

Error Value $\Phi$

Final new guess

$\Phi < \epsilon$ → Yes

Updating Scheme
Iteration Example

Initial Guess:
D = 1.0 cm²/ns⁻¹

Homogeneous initial guess
(D = 1 cm²/ns⁻¹)

Iteratively change properties of medium until measurements and predictions agree

Iterative Reconstruction

homogeneous initial guess
(D = 1 cm²/ns⁻¹)
Image Reconstruction as an Optimization Problem

Find image for which error value is smallest!

Contour plot of $\Phi(D, \mu_a)$

Goal: Find minimum of $\Phi(\mu_a, D)$

Employ minimization technique that uses information about gradient $\frac{d\Phi(\mu_a, D)}{d(\mu_a, D)}$.

Data Analysis Scheme

Measurement Data $Y$ Predicted data $U$

$$\Phi(\mu_a, D) = \sum_s \sum_d \sum_t \frac{(Y_{sdt} - U_{sdt}(\mu_a, D))^2}{2\sigma_{sdt}^2}$$

Objective Function $= \chi^2$ Error Function

Goal : Find minimum of $\Phi(\mu_a, D)$

Each image = 40x40 unknowns
Gradient Calculation

**Divided Difference**

1 variable: 2 forward calculations needed to get gradient

\[
\frac{\partial f(\zeta)}{\partial \zeta} = \frac{f(\zeta_2) - f(\zeta_1)}{\zeta_2 - \zeta_1}
\]

Therefore, For problem with N unknowns one needs 2N forward calculations to find gradient.

---

**Adjoint Differentiation**

The evaluation of a gradient requires never more than five times the effort of one forward calculation!


Therefore, adjoint differentiation method is 2N/5 times faster than "traditional" divided difference scheme!
For more details see:


www.bme.columbia.edu/biophotonics

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  - Tumor Imaging
  - Fluorescence Imaging
Optical Imaging Modalities

TIME DOMAIN
FREQUENCY DOMAIN
STEADY-STATE DOMAIN

frequency domain reconstruction ($\omega = 600$ MHz)
steady-state domain reconstruction ($\omega = 0$)

Frequency vs Steady-State Domain

<table>
<thead>
<tr>
<th>target</th>
<th>steady-state domain reconstruction ($\omega = 0$)</th>
<th>frequency domain reconstruction ($\omega = 600$ MHz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>absorption coefficient $\mu_a$</td>
<td><img src="a" alt="Image" /></td>
<td><img src="c" alt="Image" /></td>
</tr>
<tr>
<td>scattering coefficient $\mu_s'$</td>
<td><img src="b" alt="Image" /></td>
<td><img src="f" alt="Image" /></td>
</tr>
</tbody>
</table>
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Instrument Diagram

![Instrument Diagram](image-url)
Dynamic Optical Tomography System (DYNOT)

Up to 10 full tomographic images per second!

Dynamic Optical Tomography System (details)
Detector and Timing Boards

Detector modules (lock-in detection scheme, individual gain settings, 2 amplification stages)

Timing Board

Interfacing Board

From power supply

To DAQ board

Dynamic Optical Tomography System (DYNOT)
Dynamic Range of Measurement

~ $10^{-1} \cdot 0.01$ W

0.1 W

~ $10^{-3} \cdot 0.1$ W

~ $10^{-5} \cdot 0.1$ W

5 cm
Dynamic Range of Measurement

\[ \sim 10^5 \cdot 0.1 \, \text{W} \]

\[ \sim 10^{-3} \cdot 0.1 \, \text{W} \]

\[ 0.1 \, \text{W} \]

\[ 5 \, \text{cm} \]

Dynamic Range of Detectors

3 amplification stages to bring signal within 0.5 - 5 V

Nominal OD value vs. Signal [V]
Timing Scheme

- Move mirror to new fiber, switch gains
- Target illumination (1 source)
- Lock in
- S/H 32 detectors in parallel
- DAQ

Performance Overview

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Modulation frequency</td>
<td>5-10 kHz</td>
</tr>
<tr>
<td>Data acquisition rate</td>
<td>~150 Hz</td>
</tr>
<tr>
<td>Settling time</td>
<td>1-2 ms</td>
</tr>
<tr>
<td>Noise equivalent power</td>
<td>10 pW (rms)</td>
</tr>
<tr>
<td>Dynamic range</td>
<td>$1:10^9$ (180 dB)</td>
</tr>
<tr>
<td>Long term bias drifts</td>
<td>~1% over 30 min</td>
</tr>
<tr>
<td>Background light reject</td>
<td>~100 dB</td>
</tr>
</tbody>
</table>
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Animal Model

- 325 gm Sprague Dawley Rats
- Ventilated at: 40-60 breaths/min, 1-1.5 cc/breath
- Anesthesia: Urethane administered i.p.
- Blood Pressure and derived respiratory rate via Femoral catheter
- Regulate inspired [O₂] and [CO₂]

Probe Geometry

- Forehead shaved
- Animal's head fixed in place using stereotaxic
- Optical probe with fixed geometry positioned in line with lambda (λ) suture line, optodes begin 2 mm anterior to λ.
Carotid Occlusion

Two Wavelengths ($\lambda_1, \lambda_2$)

Reconstruction algorithm provides $\Delta \mu_a$ for each volume element (voxel) of finite element mesh for each wavelength.

For each voxel we get two equations:

$$
\Delta \mu^\lambda_1 = \varepsilon^\lambda_1 Hb \Delta[Hb] + \varepsilon^\lambda_1 HbO_2 \Delta[HbO_2]
$$

$$
\Delta \mu^\lambda_2 = \varepsilon^\lambda_2 Hb \Delta[Hb] + \varepsilon^\lambda_2 HbO_2 \Delta[HbO_2]
$$

$\varepsilon :=$ extinction coefficient (from literature)
Two Wavelengths

Reconstruction algorithm provides $\Delta \mu_a$ for each volume element (voxel) of finite element mesh for each wavelength.

From this we can calculate changes in concentrations of oxy-hemoglobin, $\Delta [Hb]$, and deoxy-hemoglobin, $\Delta [HbO_2]$, for each voxel.

\[
\Delta [Hb] = \frac{\varepsilon_{HbO_2}^{\lambda_2} \Delta \mu_a^{\lambda_1} - \varepsilon_{HbO_2}^{\lambda_1} \Delta \mu_a^{\lambda_2}}{\varepsilon_{Hb}^{\lambda_1} \varepsilon_{HbO_2}^{\lambda_2} - \varepsilon_{Hb}^{\lambda_2} \varepsilon_{HbO_2}^{\lambda_1}}
\]

\[
\Delta [HbO_2] = \frac{\varepsilon_{Hb}^{\lambda_1} \Delta \mu_a^{\lambda_2} - \varepsilon_{Hb}^{\lambda_2} \Delta \mu_a^{\lambda_1}}{\varepsilon_{Hb}^{\lambda_1} \varepsilon_{HbO_2}^{\lambda_2} - \varepsilon_{Hb}^{\lambda_2} \varepsilon_{HbO_2}^{\lambda_1}}
\]
Forepaw Stimulation

Right Forepaw Stimulation

*Oxyhemoglobin*
Reconstruction

Blood Volume

Cut 3
Cut 7
Cut 10

rt.
lt.

-0.003 0 0.004
ΔTHb [mM]

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  - Static Measurements
  - Dynamic Measurements

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Tumors in Mice

- **Tumor is injected into mouse left kidney.**

- **Tumor continues to grow unless treated.**

- **Treatment with VEGF antagonist seeks to stop angiogenesis and reverse tumor growth.**
Tumors in Mice

- Untreated tumors: highly vascularized
- Treated tumors: much less vascularized
- Currently: Many mice are sacrificed to get tumor data
- Only 1 time point per mouse
- We propose to use MRI and OT to study tumor size and vasculature in vivo

More Information:


## fMRI vs Optical Tomography

<table>
<thead>
<tr>
<th></th>
<th>fMRI</th>
<th>Optical Tomography</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Spatial Resolution</strong></td>
<td>0.1mm-1mm</td>
<td>2mm - 10mm</td>
</tr>
<tr>
<td><strong>Sensitive to</strong></td>
<td>Hb (paramag.)</td>
<td>Hb, HbO₂, cytochrome, etc, blood volume, scattering properties</td>
</tr>
<tr>
<td><strong>Speed</strong></td>
<td>0.1 - 1Hz</td>
<td>~50 Hz</td>
</tr>
<tr>
<td><strong>Cost</strong></td>
<td>&gt; $500.000</td>
<td>~ $100.000</td>
</tr>
<tr>
<td><strong>Portability</strong></td>
<td>no</td>
<td>yes</td>
</tr>
<tr>
<td><strong>Continuous Monitoring</strong></td>
<td>no</td>
<td>yes</td>
</tr>
</tbody>
</table>

Combine high spatial resolution of fMRI and high speed and sensitivity of optical tomography!

### 9.4 Tesla MRI (Bruker Avance 400)

- Micro2.5 Imaging set
- 35mm diameter
- Linearly polarized
- Birdcage coil

**Typical imaging time:** 30 - 60 minutes (T1 sequence)
Optical Tomography Set Up

Step 1
Lower mouse into imaging head.

Step 2
Add matching fluid (Intralipid).

Step 3
Illuminate with light (Image!)

Typical imaging time: 10 - 20 minutes

Axial Slice

Optical
Total Hemoglobin

MRI
Kidney
Back Muscle & Spinal Cord
Tumor
### Coronal Slice

<table>
<thead>
<tr>
<th>[HbT] (M)</th>
<th>Optical</th>
<th>MRI</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Total Hemoglobin</td>
<td>Kidney Tumor</td>
</tr>
</tbody>
</table>

### Compare Untreated vs. Treated

<table>
<thead>
<tr>
<th>Untreated [HbT]</th>
<th>Treated [HbT]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated tumor has higher [HbT] than treated tumor because of higher vascularization.</td>
<td>Untreated tumor has higher [Hb] than treated tumor because it is HbO₂ starved.</td>
</tr>
</tbody>
</table>
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  Molecular Fluorescence Imaging
Molecular Imaging

Rheumatoid Arthritis

Light  NIRF

mouse without RA  transgenic mouse with RA

Antigen: glucose-6-phosphate isomerase (GPI)

Mahmood, Weissleder et al MGH-CMIR

Mahmood, Weissleder et al MGH-CMIR
Cancer Detection

Fluorescence Tomography

reconstruction of absorption and scattering profile \( \mu(x,y) \)

reconstruction of fluorescence source profile \( S(x,y) \)

\[ \mu(x,y) \]

\[ S(x,y) \]
Fluorescence Tomography

1) Excitation $\lambda^x$

$\mu_a^{x \rightarrow m}$ absorption of fluorophore

2) Emission $\lambda^m$

$\phi^m$ quantum yield of fluorophore

Inverse Source Problem

$\Omega \cdot \nabla \Psi(r, \Omega) + (\mu_a + \mu_s)\Psi(r, \Omega) = S(r, \Omega) + \mu_s \int_4 \rho(\Omega, \Omega')\Psi(r, \Omega') d\Omega'$

1) Excitation $\lambda^x$

$\Omega \cdot \nabla \Psi^x + (\mu_a^x + \mu_s^{x \rightarrow m} + \mu_s^x)\Psi^x = S^x + \mu_s^x \int_4 \rho(\Omega, \Omega')\Psi^x(\Omega') d\Omega'$

$\phi^x = \int_4 \Psi^x(\Omega') d\Omega'$

2) Emission $\lambda^m$

$\Omega \cdot \nabla \Psi^m + (\mu_a^m + \mu_s^m)\Psi^m = \frac{1}{4\pi} \eta \rho_a^{x \rightarrow m} \phi^x + \mu_s^m \int_4 \rho(\Omega, \Omega')\Psi^m(\Omega') d\Omega'$
Model-Based Image Reconstruction

1) Excitation $\lambda^x$

Forward Model

Prediction P  Experiment M

Inverse Model

$\mu^x$

Model-Based Image Reconstruction

1) Excitation $\lambda^x$  2) Emission $\lambda^m$

Forward Model

Prediction P  Experiment M

Inverse Model

$\mu^x$

$\phi$

Forward Model
Model-Based Image Reconstruction

1) Excitation $\lambda^x$

Forward Model

Prediction $P$

Experiment $M$

Inverse Model

$\mu^x_{\alpha}$

2) Emission $\lambda^m$

Forward Model

Prediction $P$

Experiment $M$

Inverse Model

$\mu^m_{\alpha}$

Image

Mouse Tomography

Surface-weighted Fluorescence

Figure: Example of a mouse tomography reconstruction.
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More Information

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