Fluorescence Molecular Tomography Basics

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Outline

- Scattering
- The basics of FMT
- The Inverse Problem
- Reducing Ill-posedness
WHAT IS SCATTERING?
Each of the rainbow colors bounces away from a raindrop at a slightly different angle relative to the path of the incoming light. Red light bends and reflects so that it leaves a water drop at an angle of 42 degrees or less. Green light bounces away from the drop at an angle of 41 degrees or less. Blue light reflects at 40 degrees or less. Because the water drop is spherical, the reflecting light forms a cone, with the drop at the tip. Each color makes its own cone of light, and all these cones fit inside each other, with the red cone on the outside. The water drop is at the tip of all these cones of colored light.

Water droplets disperse light in a similar way as prism does.
Light Scattering

* Alexander of Aphrodisias (Lived and taught in Athens, 200AD)
Interference effects produce Iridescence
We have seen that each wavelength is scattered at a different angle:
• So what happens if we have a random collection of these?:

We end up with a random distribution of angles for all visible wavelengths.

So, even though each particle may be transparent independently, an ensemble of these will randomize light’s angular distribution mixing all colors in all directions generating diffuse white light.
And clearly, the pattern is present in all:

(a) soap bubbles

(b) adipose tissue

- air
- soap
- adipocyte
Other stuff that multiply scatters light

Sugar
Flour

Mayonnaise

Toothpaste

And the best part: Fat from a nice slice of jamón serrano
In order to image in-vivo...

we need to understand how light interacts with tissue
Statistical Description of optical properties

\[ \mu_{a1} \quad \mu_{s1} \]

\[ \mu_{a2} \quad \mu_{s2} \]
Light Propagation

Governed by:
- Absorption
- Scattering
Scattering Experiment

Yoghourt

Laser Pointer

water
Scattering

1 Drop
4 Drops
8 Drops
12 Drops
Scattering

Ballistic Propagation
(microscopy, OPT, etc.)

Highly Scattering
(FMT, DOT)

Beer’s Law $\sim \exp (-a*z)$

Diffusion $\sim \exp (-a*r)/r$
Imaging in Diffuse Media

ILL-POSED PROBLEM!
FLUORESCENCE MOLECULAR TOMOGRAPHY (FMT)
Highly Scattering Tissues
Image Contrast

- Image Contrast will depend on:
  - Tissue Absorption
  - Autofluorescence
  - Probe Specificity
  - Signal to Noise
Absorption

TurboFP635 excitation/emission spectra

Wavelength (nm)
FMT Setup
FMT Setup

- CCD camera
- Objective
- Filter Wheel
- Mirror
- Galvomirrors
- Flipmount mirrors
- Lasers 1, 2, 3
Non-Contact FMT
Principles of Tomography

FLUORESCENCE

Excitation src.

emission

Tissue

source

Fluorescence

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Bioluminescence vs Fluo.

**BIOLUMINESCENCE**

Bioluminescence

- e.g. luciferin
- emission
Inverse Problem: Using the measurements as a starting point, what distribution of fluorophores or fluorescent proteins caused them?
Forward Problem
Forward Problem
FMT- Inverse Problem
The Weight Matrix Represents the contribution of each voxel to the measurement.
Inverse Problem

\[
[W][F] = \begin{bmatrix}
I_{fl} \\
I_{exc}
\end{bmatrix}
\]

- Weight Matrix
- 3D Fluorescence Map
- Fluorescence Meas.
- Excitation Meas.
Inverse Problem

$$[F] = [W]^{-1} \begin{bmatrix} I_{fl} \\ I_{exc} \end{bmatrix}$$

3D Fluorescence Map

Ill-Posed Problem!

There is not a unique solution to the problem and this solution is not stable.
No anatomical info

Stroke-induced immune depression

J. Ripoll, Munich 2011

FMT: A. Arranz, J. Klohs (ETH)
MRI: J. Grandjean, J. Klohs (ETH)
Recon: A. Zacharopoulos (FORTH)
Cy5.5 capsule implanted

**FMT:** A. Arranz, J. Klohs (ETH)

**MRI:** J. Grandean (ETH)

**Recon:** A. Zacharopoulos (FORTH)
Ill-posedness

- Approaches to reduce ill-posedness:
  - Introducing a spatial dependence on the emission (scanning the source)
  - Introducing spectral measurements
  - Introducing spatial distribution of scattering and absorption
  - Introducing knowledge of the area where signal should originate

PRIORS
• Use of Priors and Fast Inversion Methods:
  – UCL (S. Arridge, T. Correia)
  – FORTH (A. Zacharopoulos)
Multispectral Imaging
**Multispectral Imaging**

**Slab phantom:**
Intralipid + ink + Agar
\( \mu_s' = 16 \text{ cm}^{-1}, \mu_a = 1.5 \text{ cm}^{-1} \)

\[
\begin{bmatrix}
I_1 \\
I_2
\end{bmatrix}
= 
\begin{bmatrix}
S_{G_1} & S_{R_1} \\
S_{G_2} & S_{R_2}
\end{bmatrix}
\ast 
\begin{bmatrix}
C_G \\
C_R
\end{bmatrix}
\]

CFSE (20\(\mu\)M) + ATTO590 (20\(\mu\)M)

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G. Zacharakis
Multispectral Imaging

Geometry

Mixed fluorescence signal

Unmixed CFSE signal

Unmixed ATTO590 signal

Mixed Signal

Green Signal

Red Signal

G. Zacharakis
Multispectral Imaging

ATTO590 (20μM)
CFSE (20μM)

Geometry
mixed fluorescence

unmixed CFSE signal
unmixed Atto590 signal

G. Zacharakis
Acknowledgements

Funding
E.U. Integrated Project “Molecular Imaging”
E.U. EST – MOLEC IMAG
Bill & Melinda Gates Foundation
E.U. Collaborative Project „FMT-XCT“

IN-VIVO IMAGING GROUP
Giannis Zacharakis (Senior Post-doc)
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IDIBAPS
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NIMR - MRC
Dimitris Kioussis

UOC
Alicia Arranz and Christos Tsatsanis

FORTH - IESL:
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GFP-mice:
C. Mamalaki
C. elegans:
Nektarios Tavernarakis
Matthias Rieckher (PhD student)

FLEMING
Stephan Oehler and Babis Savakis

C.A.S. & Xidian University
Dong Di, Shouping Zhou and Jie Tian

ETH
Markus Rudin, Alicia Arranz, J. Grandjean

J. Ripoll, Munich 2011