Fundamentals of fluorescence tomography and its application in biological imaging

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SFB A1: Pre-clinical evaluation of individualized therapy monitoring using Fluorescence Molecular Tomography
SFB Z3: Multimodal Imaging Core
Background

Fluorescence Molecular Tomography
FMT- Fluorescence Molecular tomography

Subject

Active probes

Activatable probes

Tissue absorption

Illumination

Excitation

Emission at lower energy (longer wavelength)

in vivo imaging

Absorption coefficient (cm⁻¹)

Wavelength (nm)

400 500 600 700 800 900

0.01 0.1 1 10 100
FMT- Fluorescence Molecular tomography

Diffusion

\[ -\nabla \left[ D_x (r) \nabla \Phi_x (r) \right] + \mu_{ax} (r) \Phi_x (r) = S_x (r) \]

\[ -\nabla \left[ D_m (r) \nabla \Phi_m (r) \right] + \mu_{am} (r) \Phi_m (r) = -\Phi_x (r) n(r) \]
FMT- Fluorescence Molecular tomography

**Diffusion Equation**

\[-\nabla \left[ D_x(r) \nabla \Phi_x(r) \right] + \mu_{ax}(r) \Phi_x(r) = S_x(r)\]

\[-\nabla \left[ D_m(r) \nabla \Phi_m(r) \right] + \mu_{am}(r) \Phi_m(r) = -\Phi_z(r) n(r)\]
FMT- Fluorescence Molecular tomography

Diffusion Equation

\[-\nabla\left[D_x(r)\nabla\Phi_x(r)\right] + \mu_{ax}(r)\Phi_x(r) = S_s(r)\]

\[-\nabla\left[D_m(r)\nabla\Phi_m(r)\right] + \mu_{am}(r)\Phi_m(r) = -\Phi_x(r)n(r)\]

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<th>Bone</th>
<th>Lung</th>
<th>Heart</th>
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<td>$\mu_a$ (cm(^{-1}))</td>
<td>0.3</td>
<td>0.1</td>
<td>0.25</td>
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<td>$\mu'_s$ (cm(^{-1}))</td>
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<td>20</td>
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FMT- Fluorescence Molecular tomography

Diffusion Equation

\[-\nabla \left[ D_x(r) \nabla \Phi_x(r) \right] + \mu_{ax}(r) \Phi_x(r) = S_x(r)\]

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Experiments

From FMT to Hybrid FMT-XCT
Lung inflammation model

Mouse + LPS from Escheria Coli

E-Coli

LPS molecule

LPS from Escheria Coli

Antigen

Core

Lipid A

Activatable probe

activated by cathepsins
Activatable probe: ProSense (Visen)
FMT- Fluorescence Molecular tomography

Excitation

Excited lifetime

Emission at lower energy (longer wavelength)

Illumination

Excitation / Emission filters

Measurement

NIR laser

CCD camera
Single wavelength experiment

Haller J et al., Visualization of pulmonary inflammation using noninvasive fluorescence molecular imaging, J. Appl. Physiol, 2008
Single wavelength experiment

Emission = Excitation

Haller J et al., Visualization of pulmonary inflammation using noninvasive fluorescence molecular imaging, J. Appl. Physiol, 2008
Single wavelength experiment

A

White light image

E

Normalized

F

x10^{-4}

Fluorescence Tomography

Haller J et al., Visualization of pulmonary inflammation using noninvasive fluorescence molecular imaging, J. Appl. Physiol, 2008
Dual wavelength experiment

Activatable probes
- 750 nm (780-820nm) - ProSense

Active probes
- 680 nm (705-715nm) - AngioSense
Dual wavelength experiment

Reflectance image

Transillumination

Fluorescence Tomography

A

B

C

D

E

F

G

H

I

J

LPS instilled

Control

ProSense

AngioSense

ProSense

Angiosense

Haller J et al., Visualization of pulmonary inflammation using noninvasive fluorescence molecular imaging, J. Appl. Physiol, 2008
Semi-hybrid experiment

Fluorescence Reflectance Images

Fluorescence reflectance

Ntzachristos V et al., Looking and listening to light: evolution of whole-body photonic imaging, Nature Biotechnology, 2005
Semi-hybrid experiment

Fluorescence Transillumination

Fluorescence reflectance  Fluorescence Transillumination

Ntziachristos V et al., Looking and listening to light: evolution of whole-body photonic imaging, Nature Biotechnology, 2005
Semi-hybrid experiment

Fluorescence reflectance

Fluorescence Transillumination

Fluorescence Tomography

Ntziachristos V et al., Looking and listening to light: evolution of whole-body photonic imaging, Nature Biotechnology, 2005
Semi-hybrid experiment

Fluorescence reflectance
Fluorescence Transillumination

Fluorescence Tomography

MRI

Ntziachristos V et al., Looking and listening to light: evolution of whole-body photonic imaging, Nature Biotechnology, 2005
Hybrid FMT-XCT System
Hybrid FMT-XCT system

Schulz et al., Hybrid System for Simultaneous Fluorescence and X-ray computed tomography, IEEE Transactions on Medical Imaging, 2009
Hybrid FMT-XCT system

Schulz et al., Hybrid System for Simultaneous Fluorescence and X-ray computed tomography, IEEE Transactions on Medical Imaging, 2009
Advantages

- seamless coregistration
- assign attenuation coefficients to anatomical regions
- structural prior information
FMT- data

Multiple projections from all sides (360°)

NIR laser → Measurement → CCD camera

Example of resulting data

Fluorescence → Excitation

Normalized data = Fluorescence/Excitation
Example of thorax slice

- Spine
- Lungs
- Heart
- Ribs
Hybrid FMT-XCT

Reconstruction methods
Standard reconstruction method

Simulation - Distributed

Simulation - Localized

Tikhonov regularization

Tikhonov regularization
Reconstruction method

\[ F(x) = \| y - Wx \|^2 + \lambda^2 \| Lx \|^2 \]

- **Residual**
- **Penalty**
- **Regularization parameter**
- **Regularization matrix**
Standard Tikhonov regularization

\[ F(x) = \| y - Wx \|^2 + \lambda^2 \|lx\|^2 \]

residual penalty

\[ L = \text{Identity matrix} \]

Large negative and large positive values are penalized

Smoothing towards zero
Include prior information

Segmentation

$L = \text{identity matrix}$
Include prior information

Segmentation

\[ L = \text{diagonal matrix} \]

Hyde D et al. (2008) New techniques for data fusion in multimodal FMT-CT imaging, 5th IEEE int. symp. on biomedical imaging: From nano to macro
Result with prior information

Simulation - Distributed
Segmentation
Reconstruction

Simulation - Localized
Segmentation
Reconstruction
Hybrid FMT-XCT

Lung inflammation model
Lung inflammation model

Alexa 680 in lungs  Hybrid FMT & XCT  Planar Cryo Imaging
Lung inflammation model

Position of slices

X-ray slice

Reconstruction using priors

RGB (Cryo)

Validation image

Normalized fluorescence (Cryo)
Reconstruction

RGB  Validation image  Reconstruction
Reconstruction
3D Reconstruction
Hybrid FMT-XCT

Biological Applications
Tumor Imaging

Tumor Model

4T1 subcutaneous tumor + Probe
Tumor Imaging with Eva Herzog

Prosense ➔ Indicating proteases

ApoPSS ➔ Indicating Apoptosis
Tumor Imaging

- **Prosense**: Indicating proteases
- **ApoPss**: Indicating Apoptosis
Hybrid FMT-XCT

Osteogenesis Imperfecta
Osteogenesis Imperfecta

With Vladimir Ermolayev in cooperation with Christian Cohrs (Group of Prof. M. De Angelis, Inst. Of Experimental Genetics)

Aga2/+ model

- Bone fractures
- (Lung) bleedings

Lisse et al., 2008

4 x

OsteoSense 680

Bone growth and bone remodelling

FMT - XCT
Bone fractures

Mouse 1
Mouse 2
Mouse 3
Mouse 4
Cryoslices - OsteoSense

Mouse 4 (max 1.3)

Mouse 3 (max 0.5)
Reconstruction: Mouse 4

- Functional prior information
Reconstruction: Mouse 4

Cryoslices

Reconstruction
- Structural and Functional Prior information
Reconstruction: Mouse 4

- Structural and Functional Prior information

Isosurface
Lower threshold

Isosurface
Higher threshold
Reconstruction: Mouse 4

- Structural and Functional Prior information
Reconstruction: Mouse 4

- Structural and Functional Prior information
Thank you

Acknowledgements

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Institute: http://www.helmholtz-muenchen.de/en/ibmi/