Basic principles of quantification using optical techniques

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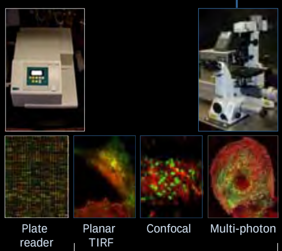
Imaging depth

0 | 0.1mm | 1mm | 1cm | 10cm



?

Resolution

0.1μm | 1μm | 100μm | 1mm

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Imaging with light
aka Optical Imaging

- Contrast
- Multispectral
- Non-ionizing
- Economical

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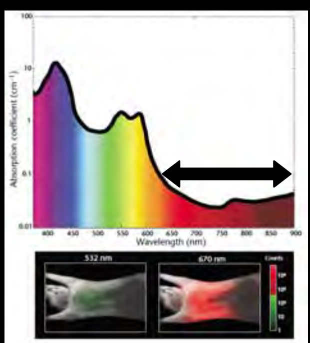
Absorption of light in tissue



Source: Weissleder, Ntziachristos, Nature Medicine, 2003

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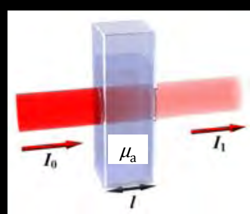
Beer-Lambert Law

$$I = I_0 e^{-\mu_a l}$$

absorption coefficient



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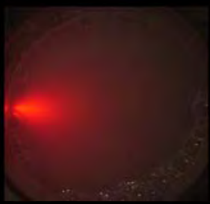
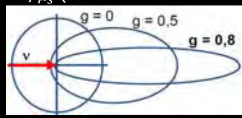
Scattering of light in tissue



Scattering

Scattering coefficient: μ_s [cm^{-1}]
Mean free path: $^1/_{\mu_s}$ (mean distance between scattering events)
Anisotropy: g



Highly scattering media → isotropic (Diffusion) approximation:
Reduced scattering coefficient: $\mu_s' = (1-g)\mu_s$

Tissue	Reduced scattering coefficient (NIR)
Muscle	9 cm^{-1}
Brain	16 cm^{-1}
Breast	12 cm^{-1}
Lung	30 cm^{-1}

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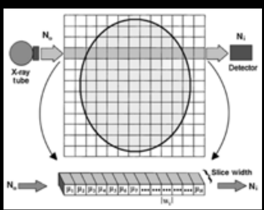
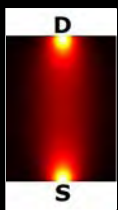
Scattering complicates reconstruction

X-ray CT reconstruction works because X-rays travel in straight lines (insignificant scattering)

This does not apply to optical imaging because scattering in tissue is usually very high





Niedre et al., PNAS 2008

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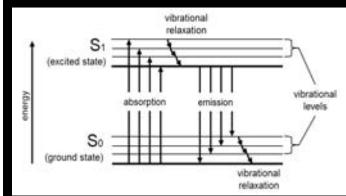
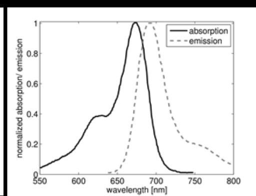
Contrast: Fluorescence





Cy5.5

- Fluorophore-tagged selective targeting
- Activatable fluorescent agents (dequenching)
- Fluorescent proteins

Imaging aim: determine the distribution of fluorophores in tissue

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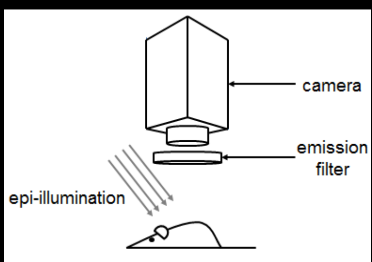
Planar (photographic) Imaging



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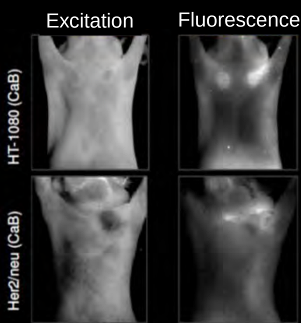
Quantification?

Quantification is hindered by:

- **Absorption** and **scattering** of light below the surface: ***surface weighted***
- Heterogeneous absorption properties



Ntziachristos V. *et al.*, JBO 2005

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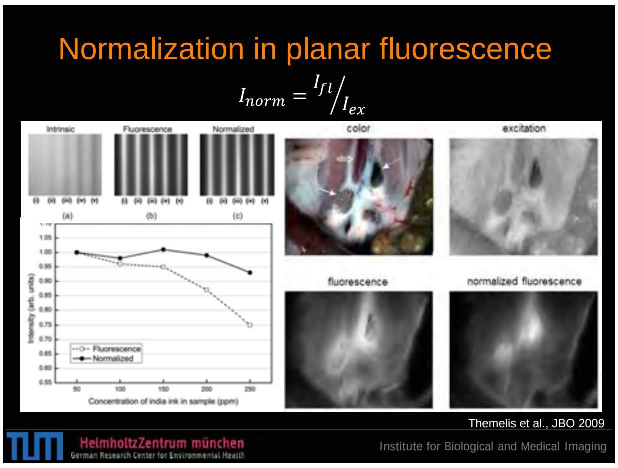
Normalization in planar fluorescence
$I_{norm} = I_{fl} / I_{ex}$
Intrinsic
Fluorescence
Normalized
color
excitation
fluorescence
normalized fluorescence
Fluorescence
Normalized
Concentration of india ink in sample (ppm)
Themelis et al., JBO 2009
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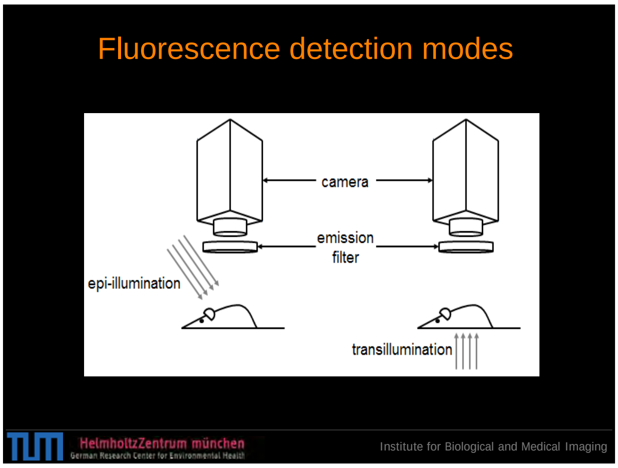
Fluorescence detection modes
camera
emission
filter
epi-illumination
transillumination
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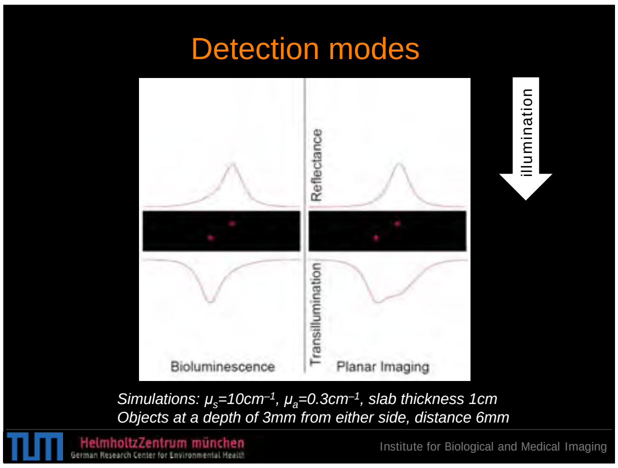
Detection modes
illumination
Reflectance
Transillumination
Bioluminescence
Planar Imaging
Simulations: μ_s=10cm^{-1}, μ_a=0.3cm^{-1}, slab thickness 1cm
Objects at a depth of 3mm from either side, distance 6mm
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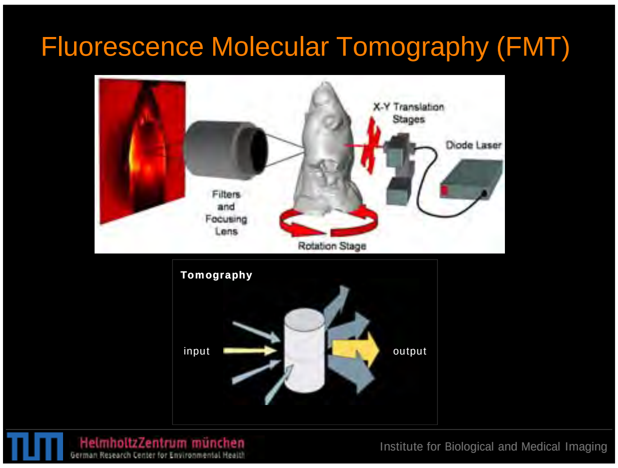
Fluorescence Molecular Tomography (FMT)
X-Y Translation Stages
Diode Laser
Filters and Focusing Lens
Rotation Stage
Tomography
input
output
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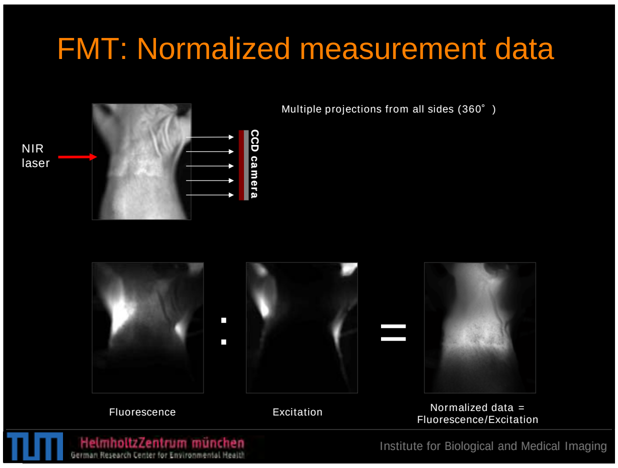
FMT: Normalized measurement data
Multiple projections from all sides (360°)
NIR laser
CCD camera
Fluorescence
Excitation
Normalized data = Fluorescence/Excitation
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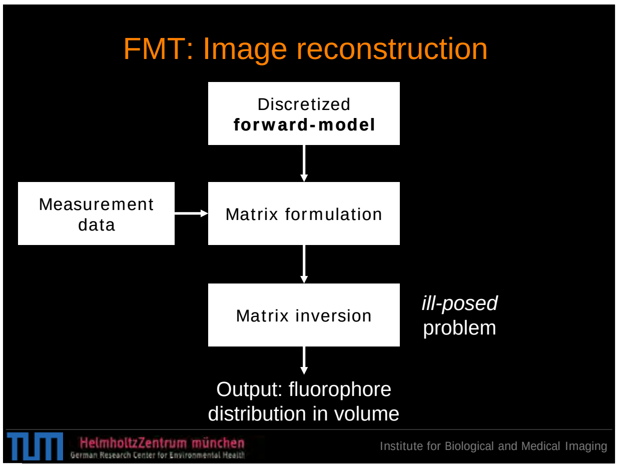
FMT: Image reconstruction
Discretized forward-model
Measurement data
Matrix formulation
Matrix inversion
ill-posed problem
Output: fluorophore distribution in volume
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FMT: Forward model (1)

Diffusion approximation

2 Diffusion Equations

illumination

Excitation light propagation:

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

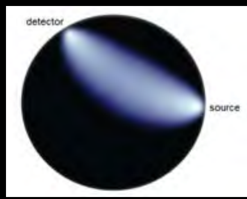
excitation × fluorophore

Emission light propagation:

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

FMT: Forward model (2)

Green's functions
Provides solutions to diffusion equations for **point source illumination**



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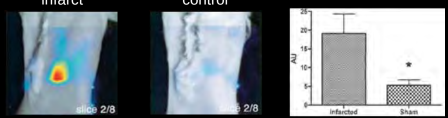
FMT: Deep tissue results

Murine model of myocardial infarction
MNP CLIO-Cy5.5 in **2007**

FMT Coronal slices through heart



Sosnovik et al, Circulation 2007

Quantitative fluorophore distribution in 3D

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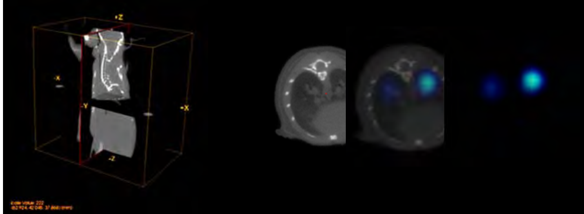
Hybrid FMT-X-ray CT



Improve FMT performance by structural prior information

- **ability to assign different optical properties to CT regions**

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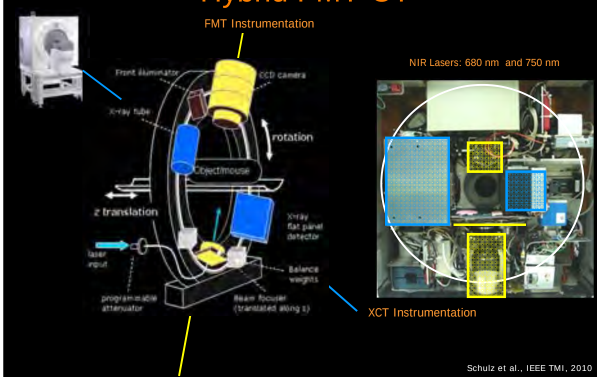
Hybrid FMT-CT



Schulz et al., IEEE TMI, 2010

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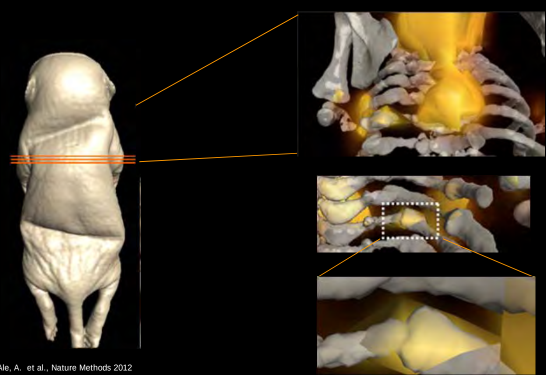
FMT-XCT: Osteogenesis Imperfecta



Ale, A. et al., Nature Methods 2012

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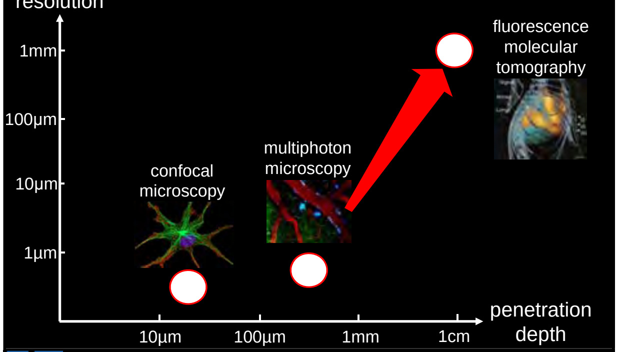
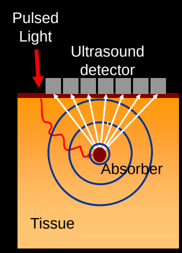
Resolution degrades with depth



Optoacoustic Imaging



$$p_0(x) \propto \phi(x)\mu_a(x)$$

pressure — light fluence — optical absorption coefficient

Imaging based on *photoacoustic effect*
- optical contrast
- ultrasound resolution
- deep inside tissue

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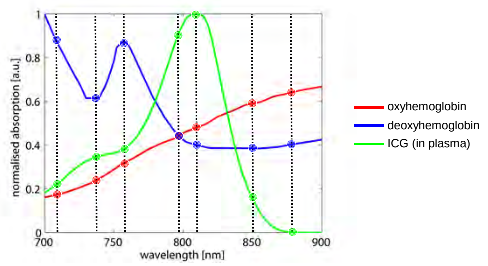
Multispectral Optoacoustic Tomography (MSOT)



Specific **imaging of:**
- intrinsic tissue absorbers: hemoglobin oxygenation (functional)
- optical agents: fluorochromes and nanoparticles

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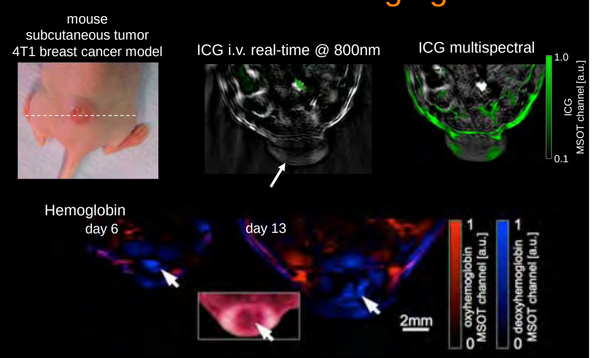
MSOT Tumor Imaging



Herzog, Taruttis, Beziere, Lutich, Razansky, Ntziachristos, Radiology 2012

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Summary

	Contrast	Quantitative accuracy	Spatial resolution	Temporal resolution
Planar Fluorescence	Fluorescence	no	undefined	Real-time
Fluorescence Molecular Tomography	Fluorescence	yes	~1mm	~20 min
Multispectral Optoacoustic Tomography	Absorption	yes	~150µm	Real-time

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Institute for Biological and Medical Imaging **IBMI**

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...

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