Fluorescence and Bioluminescence Tomography for Molecular Imaging

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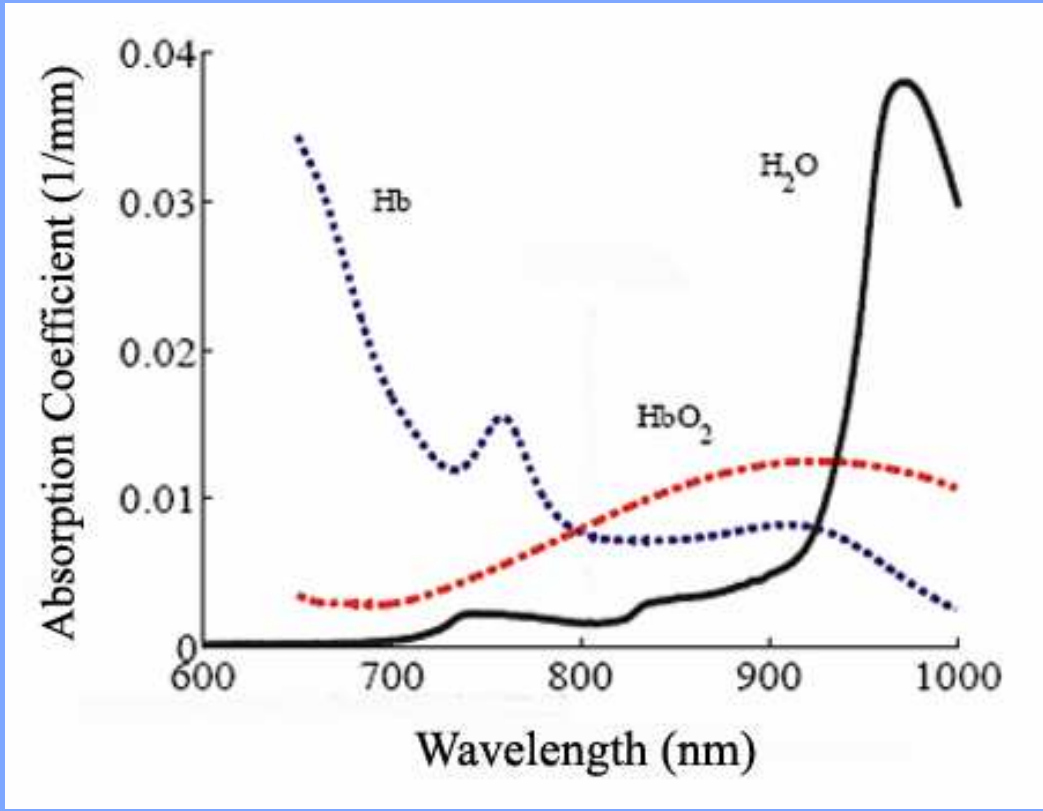
Optical Window Of Biological Tissue



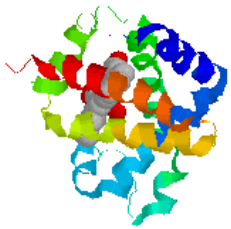
Levels of Optical Imaging

Optical Imaging

imaging of non-specific changes related to morphology and physiology

endogenous chromophores or non-specific contrast agents

developed disease



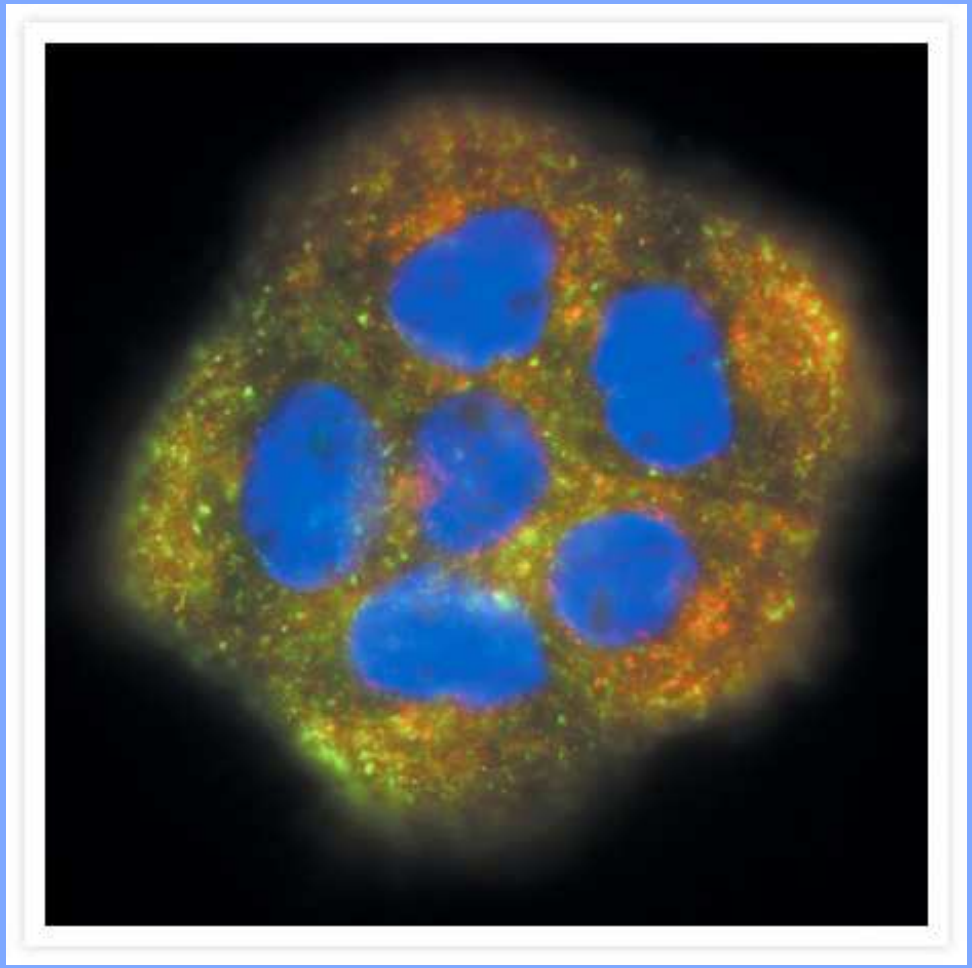
Optical Molecular Imaging

imaging of location and expression levels of specific genes and proteins that are part in the molecular pathways of disease

targeted fluorescent probes

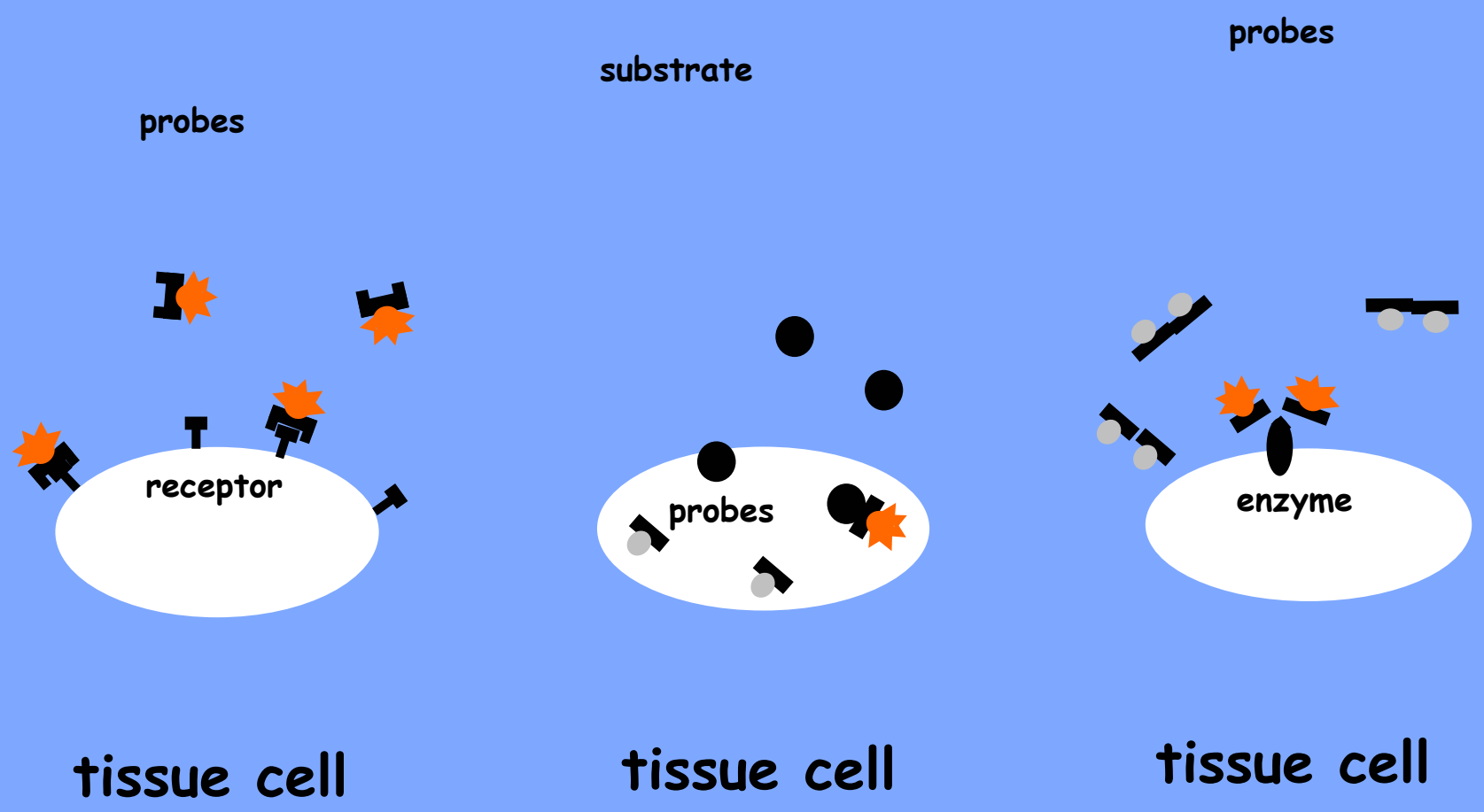
early disease

Optical Molecular Imaging



Cells incubated with green-fluorescent Alexa Fluor 488 transferrin, then fixed and permeabilized. Transferrin receptors were identified with anti–transferrin receptor, mouse IgG1 monoclonal antibody and visualized with red-fluorescent Alexa Fluor 555 goat anti–mouse IgG antibody. Yellow fluorescence indicates regions of co-localization. Nuclei were stained with DAPI. (Source: Invitrogen)

Optical Molecular Imaging

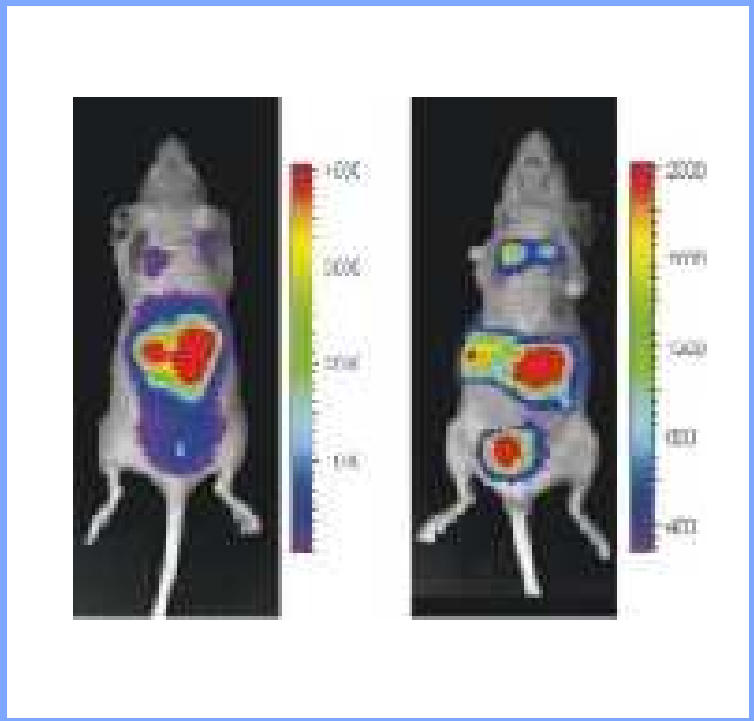


Optical Reporter Probes

Agent	Excitation [nm]	Emission [nm]	Extinction [cm^{-1} M^{-1}]	Quantum Yield
Dye:				
Cy5	649	670	250,000	0.28
Cy5.5	675	694	250,000	0.28
Cy7	743	767	200,000	0.29
Protein:				
GFP	489	508	55,000	0.6
DsRed	558	583	57,000	0.79
Catalyst-Substrate:				
Luciferase/ Luciferin	N.A.	560	N.A.	0.88

Inverse Source Problem

measured light intensity



boundary current J^+
[Watts cm^{-2}]
[photons s^{-1} cm^{-2}]

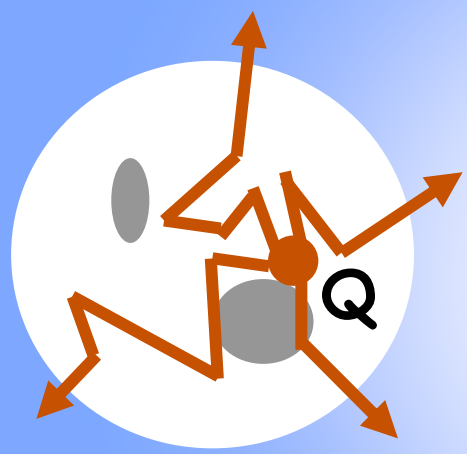
reconstructed source

source power density Q
[Watts cm^{-3}]
[photons s^{-1} cm^{-3}]

Inverse Source Problem

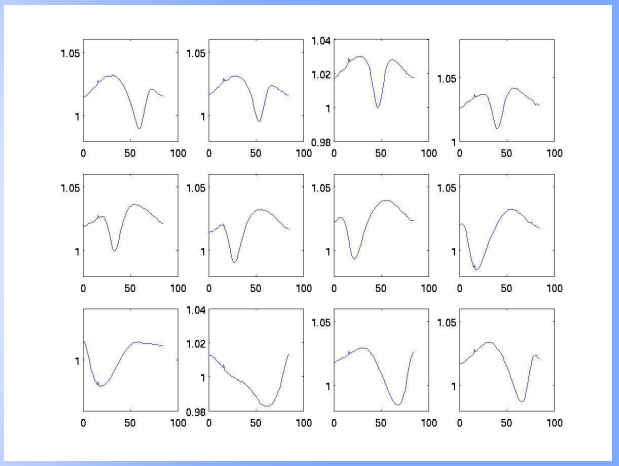
$J^+ = F(Q)$

J^+



10 … 100 mfp

J^+



$Q = F^{-1}(J^+)$

Overview

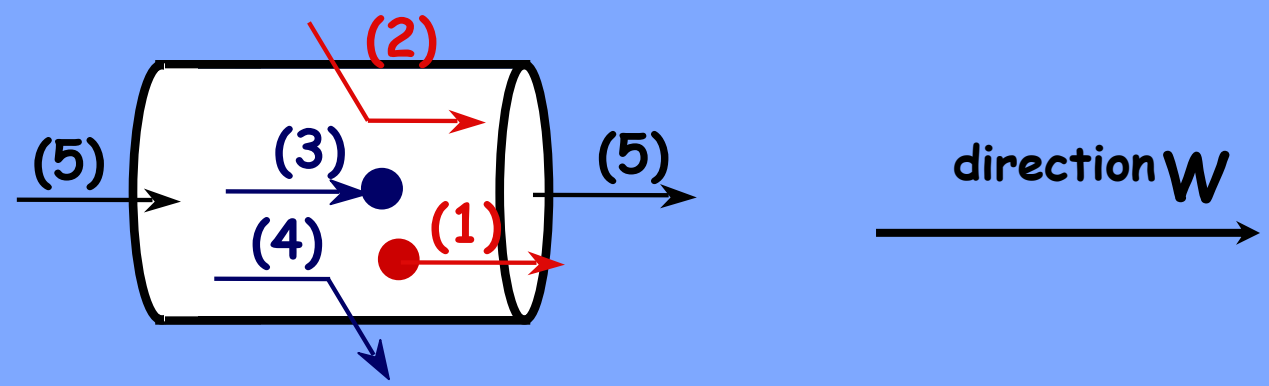
Forward Model $J^+=F(Q)$

Inverse Model $Q=F^{-1}(J^+)$

Fluorescence Tomography

Bioluminescence Tomography

Radiative Transfer Model



(1) gain - source

(2) gain - scattering

(3) loss - absorption

(4) loss - scattering

(5) loss / gain - stream

$$\Omega \cdot \nabla \psi(r,\Omega) + \mu_t \psi(r,\Omega) = Q(r) + \mu_s \int_{4\pi} p(\Omega,\Omega')\psi(r,\Omega')d\Omega'$$

(5) (3+4) (1) (2)

balance equation

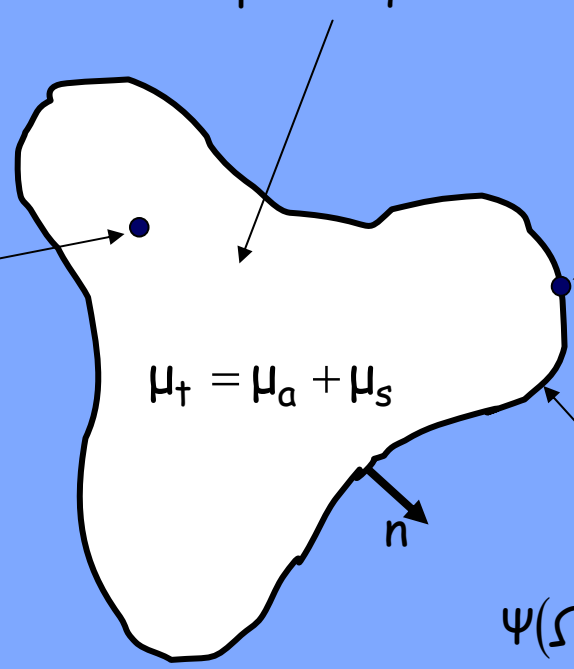
Radiative Transfer Model

$$\Omega \cdot \nabla \psi(r,\Omega) + \mu_t \psi(r,\Omega) = Q(r) + \mu_s \int_{4\pi} p(\Omega,\Omega')\psi(r,\Omega')d\Omega'$$

transport equation

$$\Phi = \int_{4\pi} \Psi(\Omega)d\Omega$$

fluence



$$J = \int_{\Omega \cdot n > 0} (n \cdot \Omega)\Psi d\Omega$$

partial current

$$\Psi(\Omega) = R(\Omega' \cdot n)\Psi(\Omega') + S(\Omega)$$

for $\Omega \cdot n < 0$

partial reflection

Radiative Transfer Model - Approximations

Diffusion Equation:

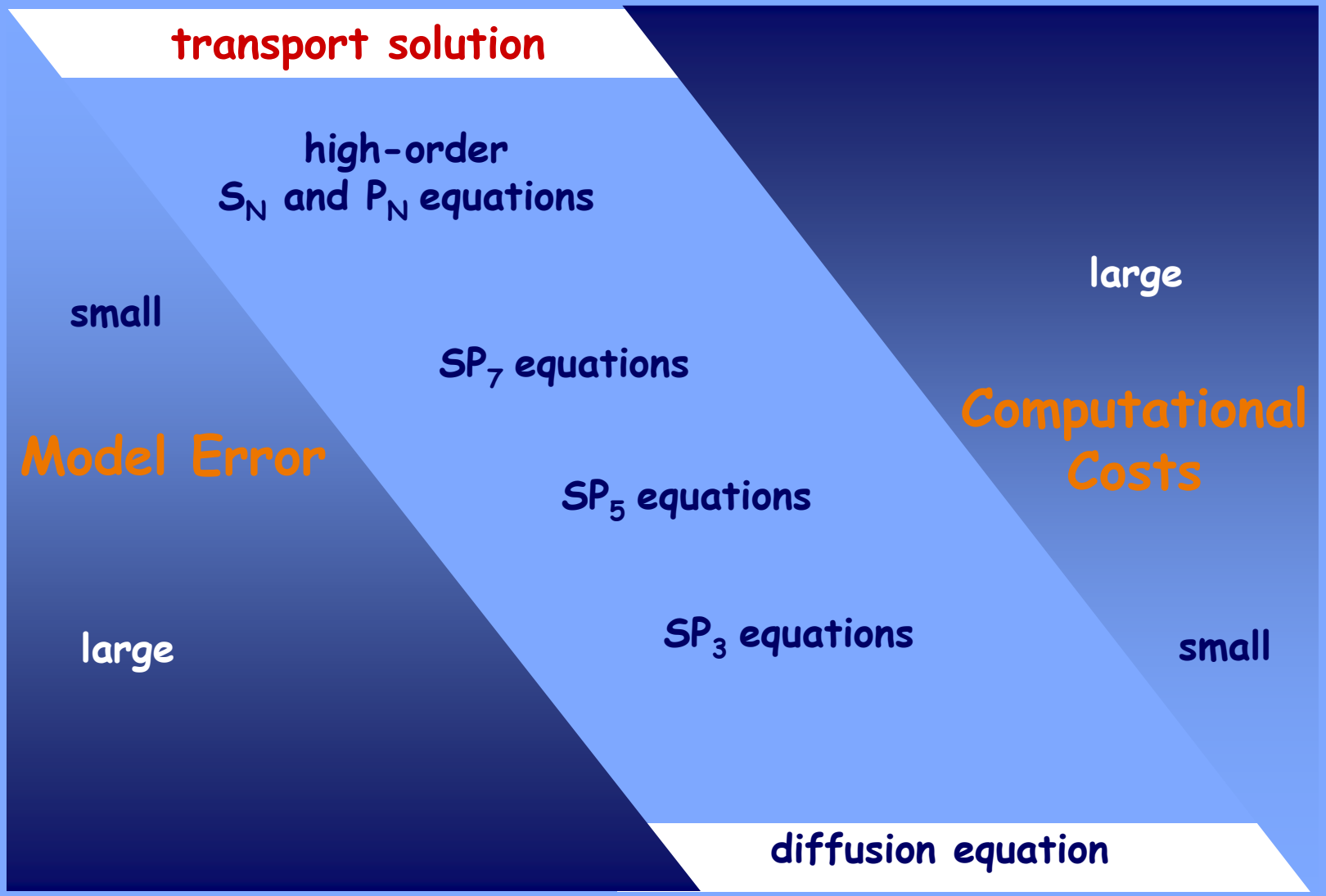
$$-\nabla \cdot \frac{1}{3\mu_{a1}} \nabla \varphi_1 + \mu_a \varphi_1 = Q$$

SP_3 Equations:

$$-\nabla \cdot \frac{1}{3\mu_{a1}} \nabla \varphi_1 + \mu_a \varphi_1 = Q + \left(\frac{3}{2} \mu_a \right) \varphi_2$$

$$-\nabla \cdot \frac{1}{7\mu_{a3}} \nabla \varphi_2 + \left(\frac{4}{9} \mu_a + \frac{5}{9} \mu_{a2} \right) \varphi_2 = -\frac{2}{3} Q + \left(\frac{3}{2} \mu_a \right) \varphi_1$$

Radiative Transfer Models - Benefits vs. Costs



Radiative Transfer Model - Fluorescence

excitation

Φ^x

[W cm^{-2}]

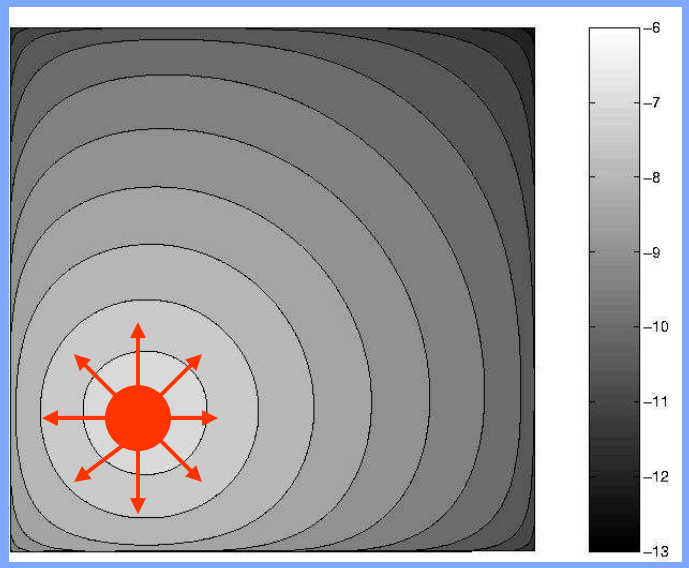
light absorption by fluorophore

$$\mu_a^{x\to m} = c \cdot \varepsilon$$

emission

Φ^m

[W cm^{-2}]



light emission by fluorophore

$$Q^m = \frac{1}{4\pi}\eta\mu_a^{x\to m}\Phi^x$$

Radiative Transfer Model - Fluorescence

excitation

$$\Omega \cdot \nabla \Psi^x + \left(\mu_a + \mu_a^{x\to m} + \mu_s\right)\Psi^x = \mu_s \int_{4\pi} p(\Omega \cdot \Omega')\Psi^x d\Omega'$$

$$\Psi^x = S, \quad n \cdot \Omega < 0$$

excitation field

$$\Phi^x = \int_{4\pi} \Psi^x d\Omega$$

emission

$$\Omega \cdot \nabla \Psi^m + \left(\mu_a + \mu_s\right)\Psi^m = \frac{1}{4\pi} \eta \mu_a^{x\to m} \Phi^x + \mu_s \int_{4\pi} p(\Omega \cdot \Omega')\Psi^m d\Omega'$$

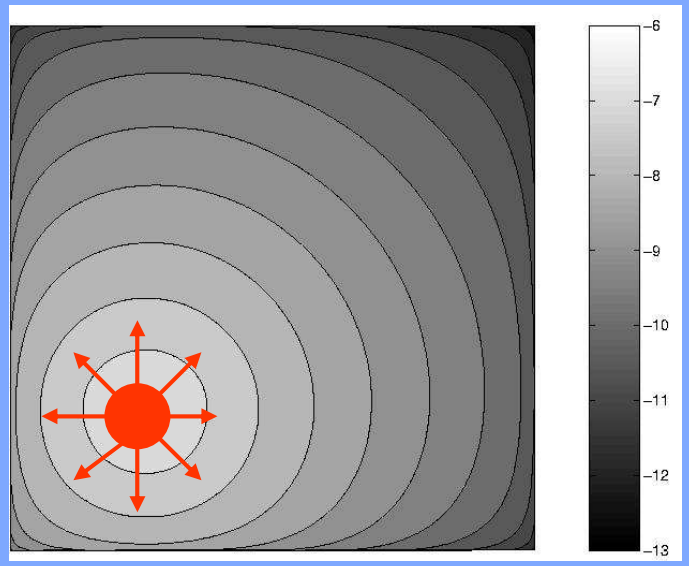
$$\Psi^m = 0, \quad n \cdot \Omega < 0$$

Radiative Transfer Model - Bioluminescence

emission

Φ^m

[W cm^{-2}]



light emission by

bioluminescent source Q

Radiative Transfer Model - Fluorescence

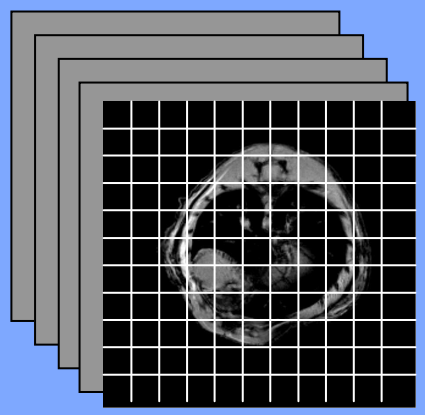
emission

$$\Omega \cdot \nabla \Psi + (\mu_a + \mu_s)\Psi = \frac{q}{4\pi} + \mu_s \int_{4\pi} p(\Omega \cdot \Omega')\Psi d\Omega'$$

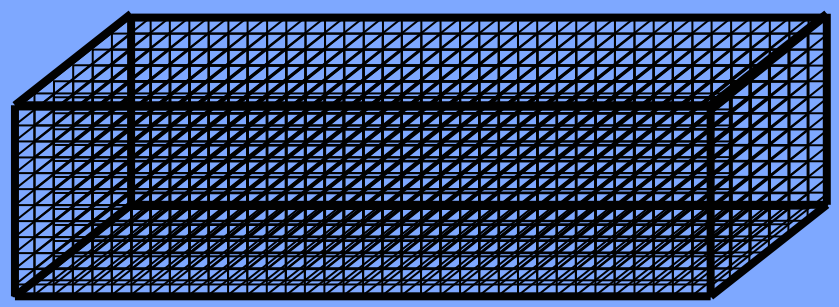
$$\Psi^m = 0, \quad n \cdot \Omega < 0$$

3D Numerical Mouse Model

91 whole-body MRIs of mouse

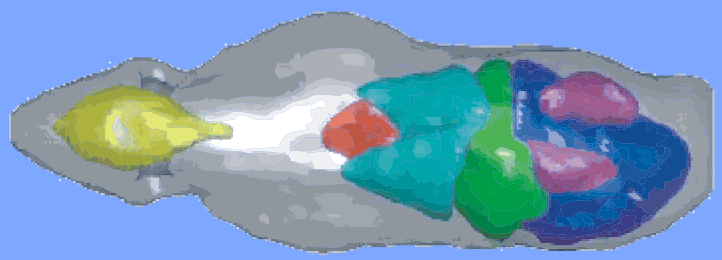


Cartesian grid with 80,000 points



0 mm

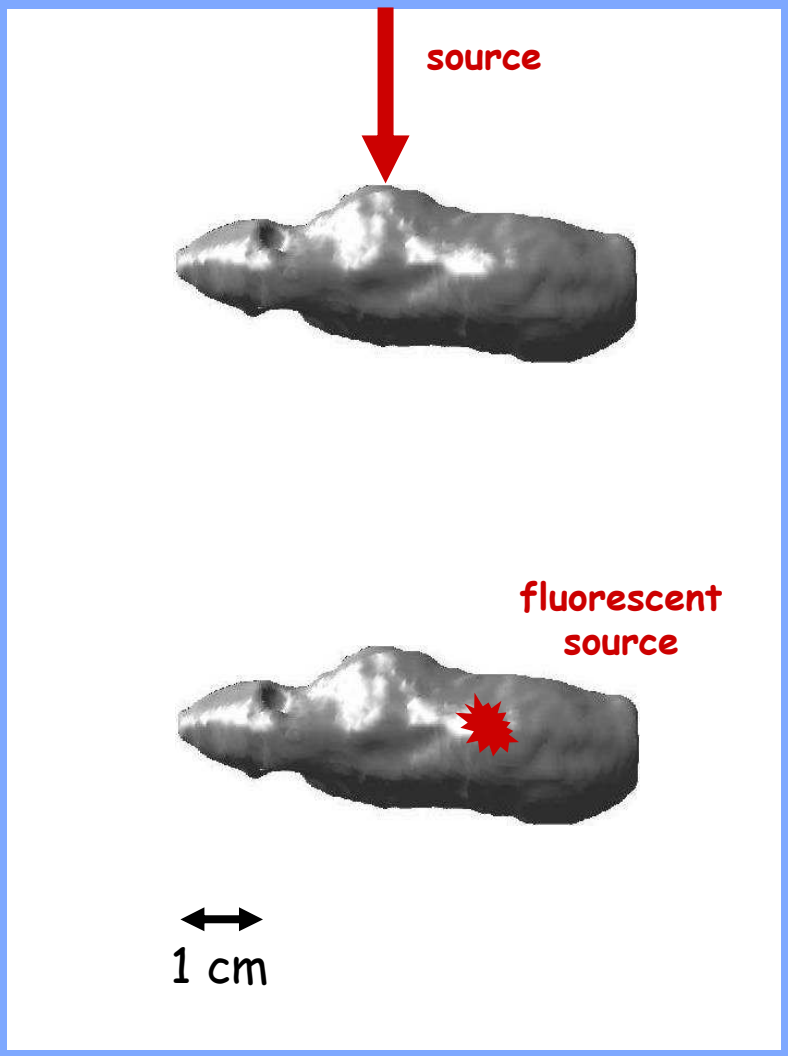
54 mm



Numerical Example

excitation

fluorescence

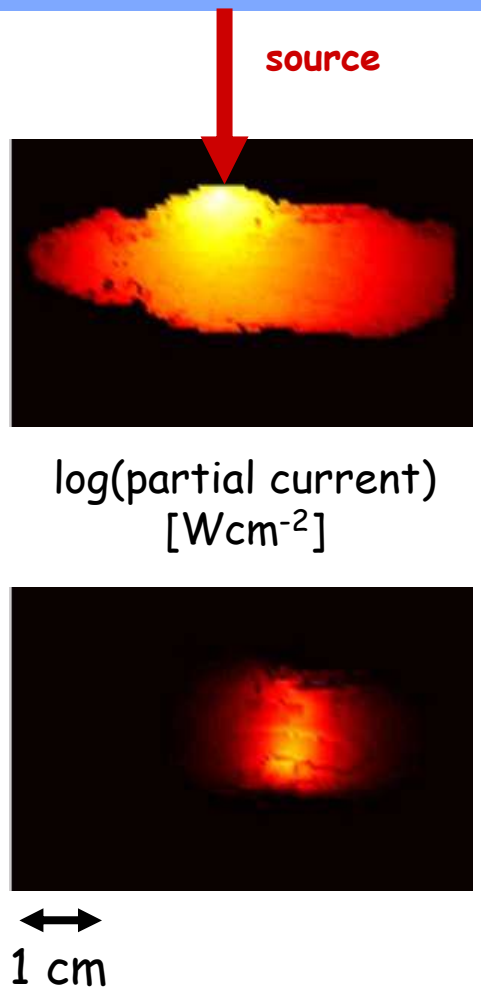


1.8 mm^3, 400nM Cy5.5

Calculated Boundary Flux

excitation

fluorescence



1.8 mm^3, 400nM Cy5.5

Overview

Forward Model $J^{+}=F(Q)$

Inverse Model $Q=F^{-1}(J^{+})$

Fluorescence Tomography

Bioluminescence Tomography

Inverse Model

nonlinear:

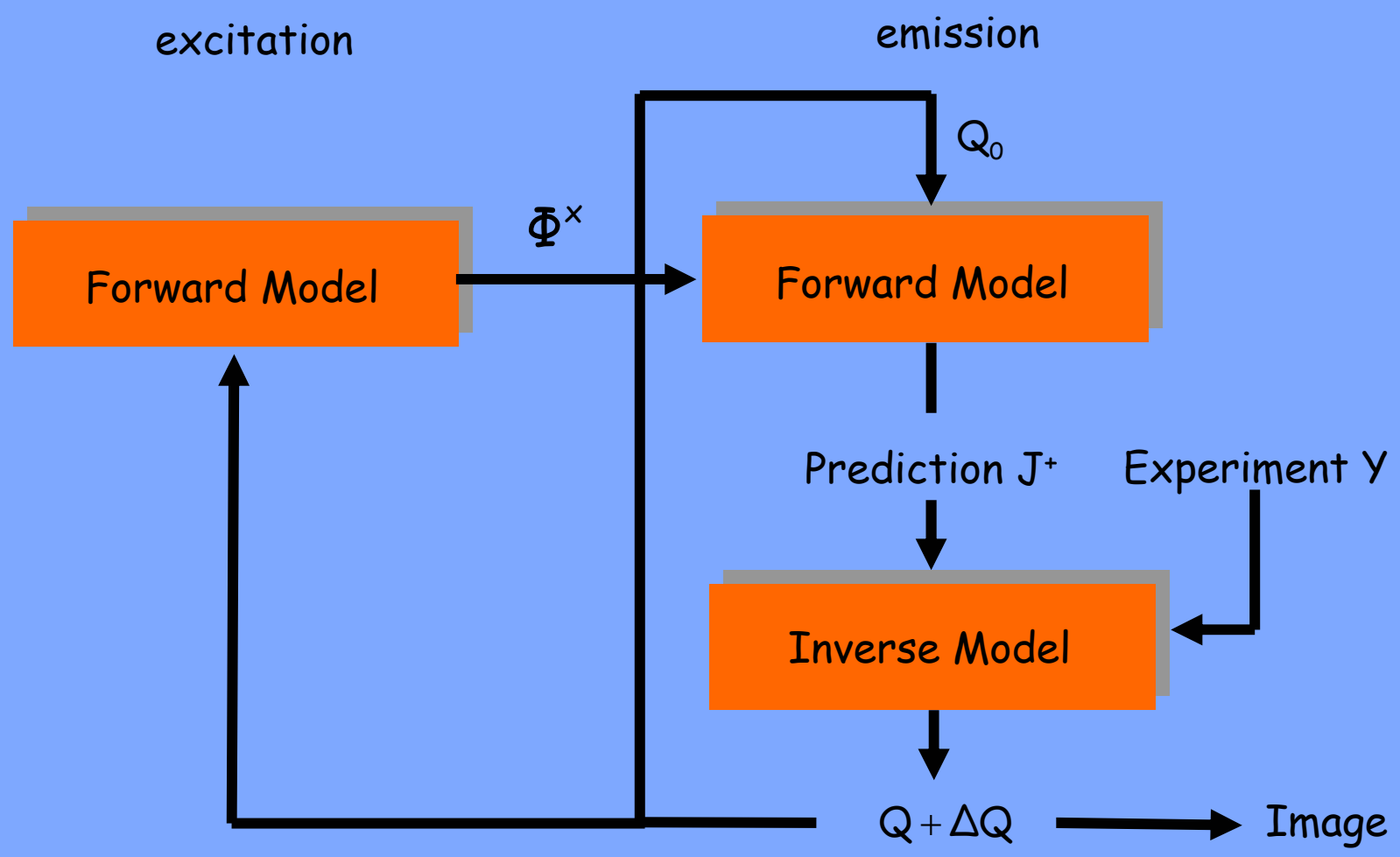
$Q=F^{-1}(J^{+},Q)$ fluorescence

linear:

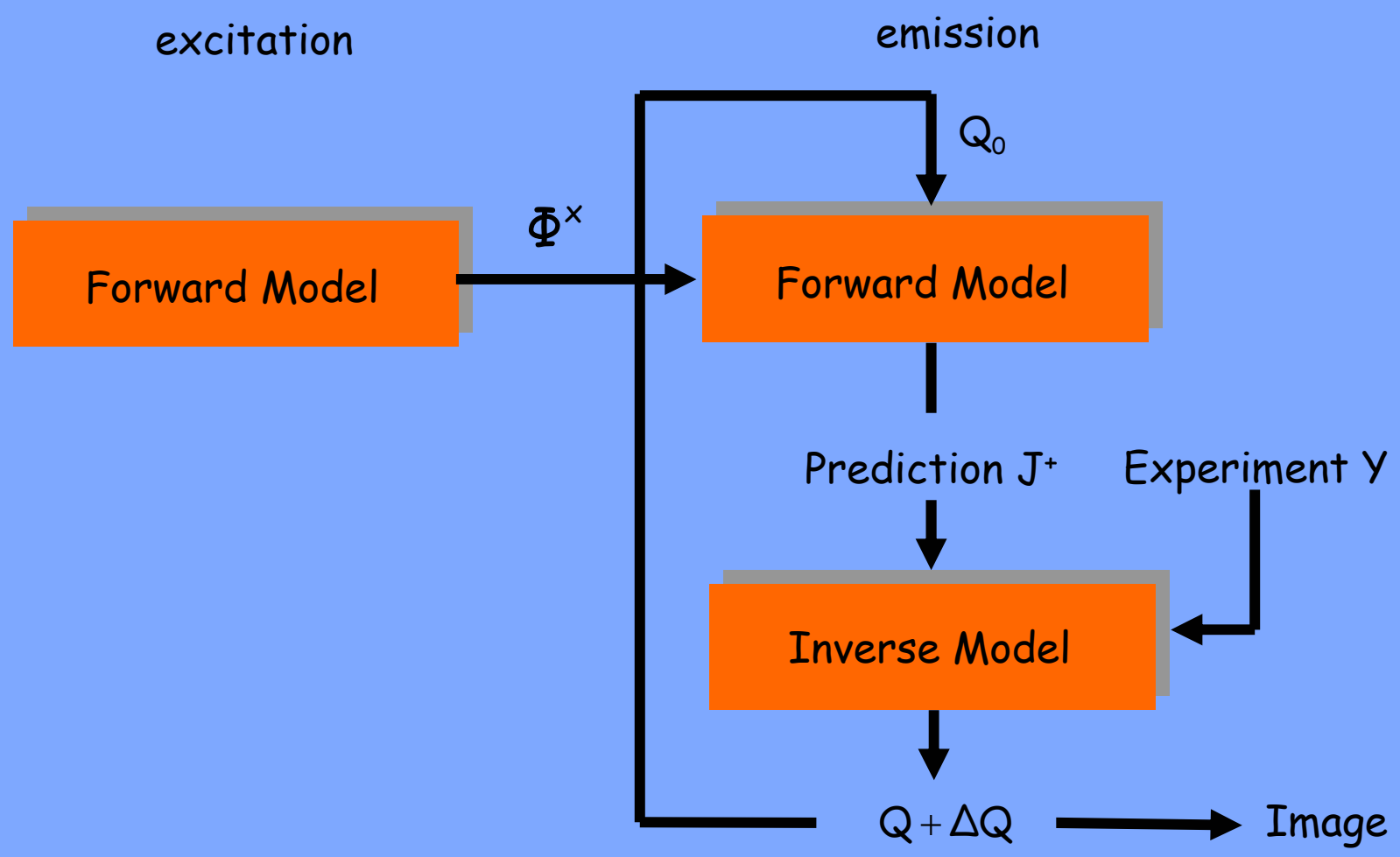
$Q=F^{-1}(J^{+})$ fluorescence approximation

bioluminescence

Nonlinear


excitation
emission
Q_0
Φ^x
Forward Model
Forward Model
Prediction J^+
Experiment Y
Inverse Model
$Q + \Delta Q$
Image

Linear


excitation
emission
Q_0
Φ^x
Forward Model
Forward Model
Prediction J^+
Experiment Y
Inverse Model
$Q + \Delta Q$
Image

Nonlinear: Local Optimization

Forward Model (Q_0)

Measurements → Error Function (Q_0)

$$\Phi(Q) = \frac{1}{N}\sum_{n}^{N}\frac{\left(Y_n - J_n^{+}(Q)\right)^2}{\sigma_n^2}$$

Gradient Calculation

Search Direction

Update Q_n

Forward Model (Q_n)

Error Function (Q_n)

Δ Error Function (Q_n, Q_{n-1}) < ε

no

Search algorithm

Nonlinear: Local Optimization

Forward Model (Q_0)

Measurements → Error Function (Q_0)

$$\Phi(Q) = \frac{1}{N}\sum_{n}^{N}\frac{\left(Y_n - J_n^{+}(Q)\right)^2}{\sigma_n^2}$$

Gradient Calculation

Search Direction

Update Q_n

Forward Model (Q_n)

Error Function (Q_n)

Δ Error Function (Q_n, Q_{n-1}) < ε

yes

New search direction

Computation Of Search Direction

$$\frac{\partial \Phi}{\partial \mu_a} = \left(\frac{\partial \Phi}{\partial \mu_{a1}}, \frac{\partial \Phi}{\partial \mu_{a2}}, \ldots, \frac{\partial \Phi}{\partial \mu_{aN}} \right)$$

amount of image voxels

Adjoint Differentiation

error function Φ is split up into subfunctions Ψ^z given by the radiative transfer model

$$\Phi(\mu) = \left(\tilde{\Phi} \circ \Psi^Z \circ \ldots \circ \Psi^{z+1} \circ \Psi^z \circ \ldots \circ \Psi^2 \circ \Psi^1\right)(\mu)$$

source iteration:
(sub-functions)

$$A\Psi^0 = Q$$

$$A\Psi^1 = B\Psi^0 + Q$$

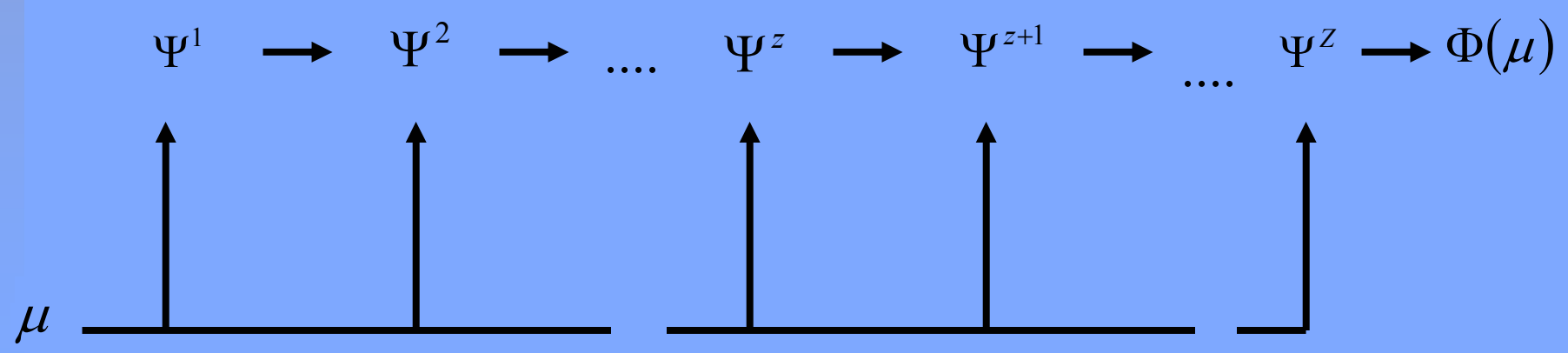
$$A\Psi^2 = B\Psi^1 + Q$$

Adjoint Differentiation

error function Φ is split up into subfunctions Ψ^z given by the radiative transfer model

$$\Phi(\mu) = \left(\tilde{\Phi} \circ \Psi^Z \circ \ldots \circ \Psi^{z+1} \circ \Psi^z \circ \ldots \circ \Psi^2 \circ \Psi^1\right)(\mu)$$

forward direction

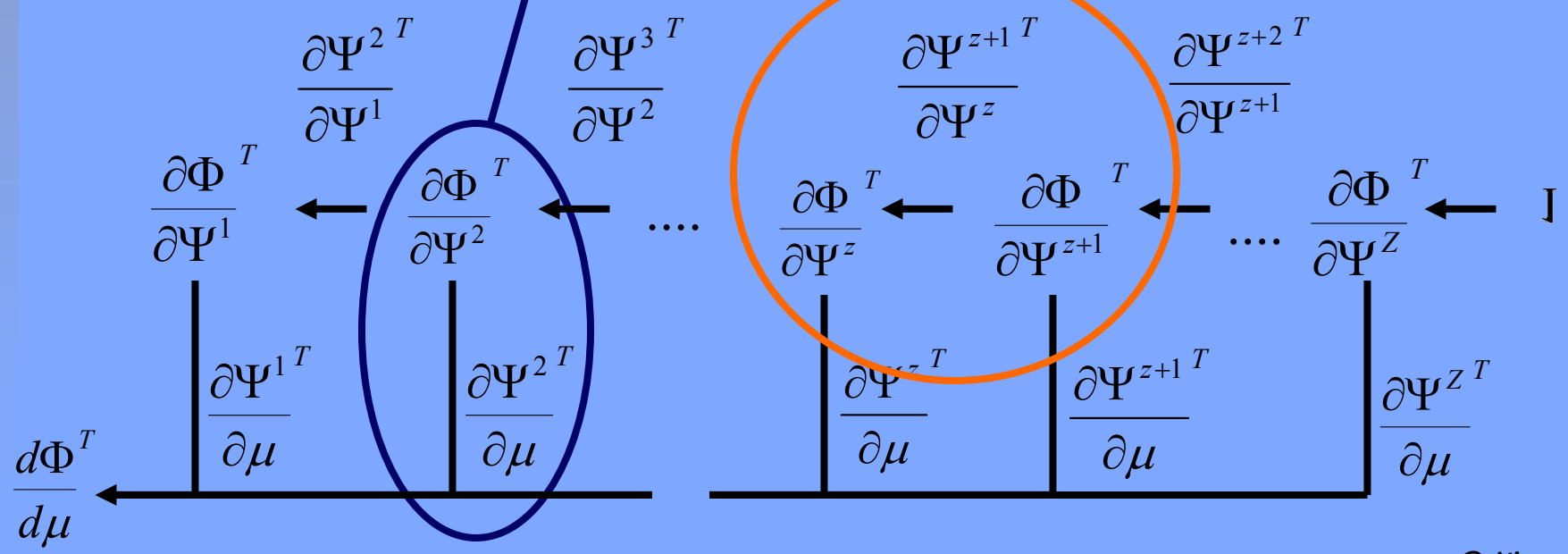


Adjoint Differentiation

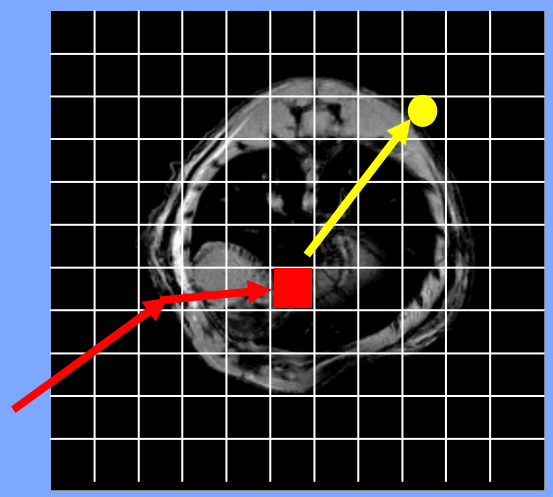
chain rule of differentiation

$$\frac{d\Phi}{d\mu}^T = \sum_z \frac{\partial \Psi^z}{\partial \mu}^T \frac{\partial \Phi}{\partial \Psi^z}^T \qquad \frac{\partial \Phi}{\partial \Psi^z}^T = \frac{\partial \Psi^{z+1}}{\partial \Psi^z}^T \frac{\partial \Phi}{\partial \Psi^{z+1}}^T$$

reverse direction



Linear: Algebraic Reconstruction



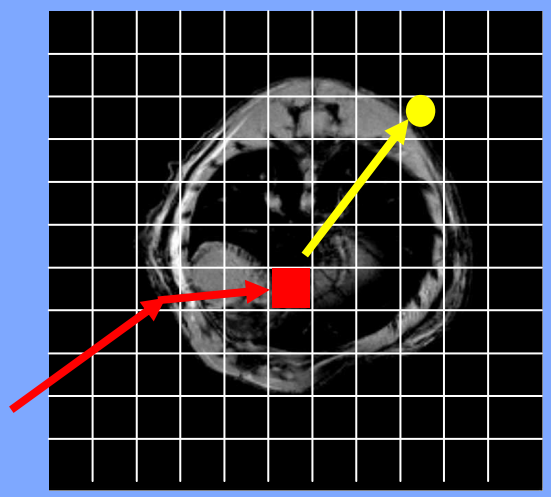
basis function q_n

$$Q(r) = \sum_{n=0}^{N} q_n(r)$$

partial current J^+_m

$$J^+_m = \sum_{n=0}^{N} J^+(q_n)$$

Linear: Algebraic Reconstruction



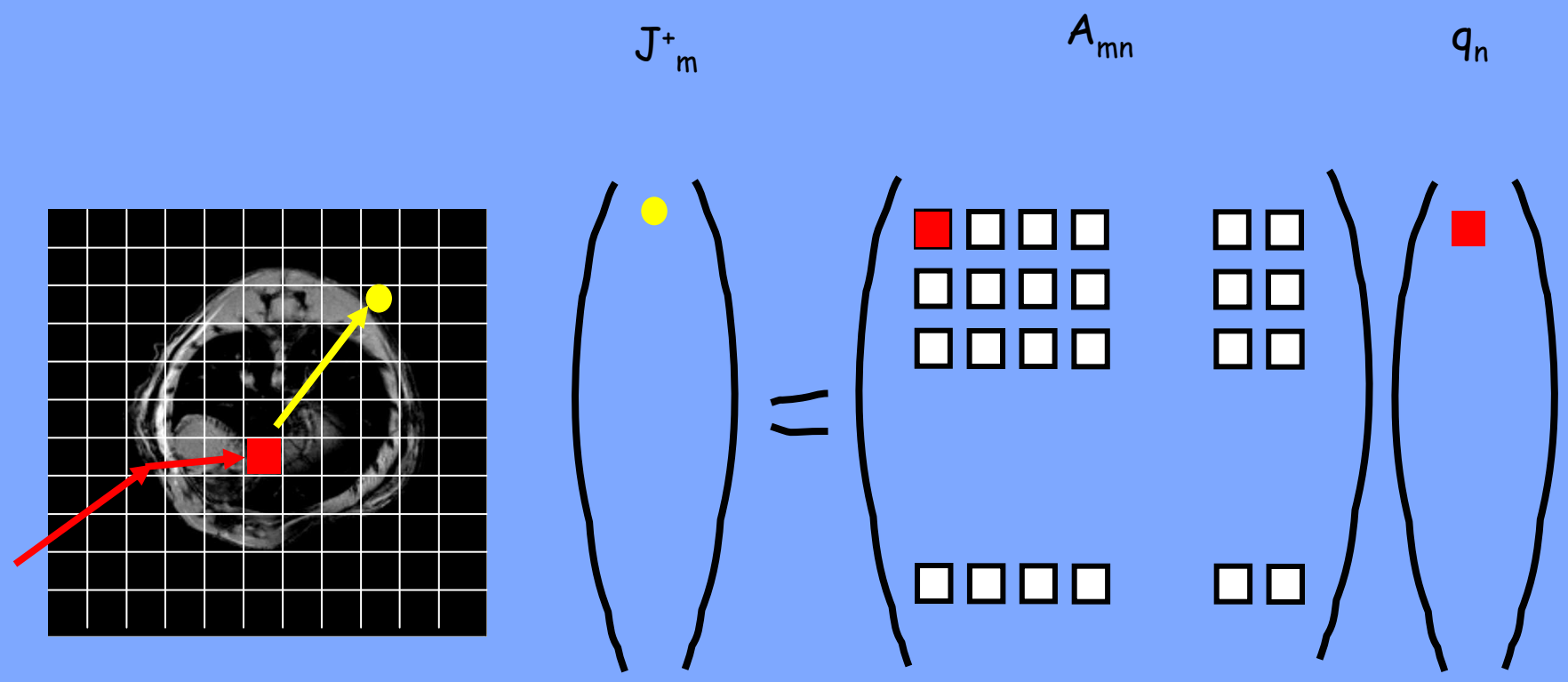
$$\Omega \cdot \nabla \Psi^{x} + \left(\mu_a + \mu_a^{x \to m} + \mu_s\right)\Psi^{x} = \mu_s \int_{4\pi} p(\Omega \cdot \Omega')\Psi^{x} d\Omega'$$

$$\Omega \cdot \nabla \Psi^{m} + \left(\mu_a + \mu_s\right)\Psi^{m} = \frac{1}{4\pi} \eta \mu_a^{x \to m} \Phi^{x} + \mu_s \int_{4\pi} p(\Omega \cdot \Omega')\Psi^{m} d\Omega'$$

10^4 – 10^5 unknowns ■

10^3-10^4 measurement points ●

Linear: Algebraic Reconstruction



10^7 – 10^9 matrix elements

Linear: Algebraic Reconstruction

matrix equation $(J^{+}_{m}) = (A_{mn})(q_n)$

minimization problem $||(A_{mn})(q_n) - (J^{+}_{m})|| = \min$

Kaczmarz algorithm $$q_n^{i+1} = q_n^{i} + \lambda \frac{A_{mn}}{||A^m||^2} (J^{+}_{m} - A^m q^i)$$

robust
good convergence

Overview

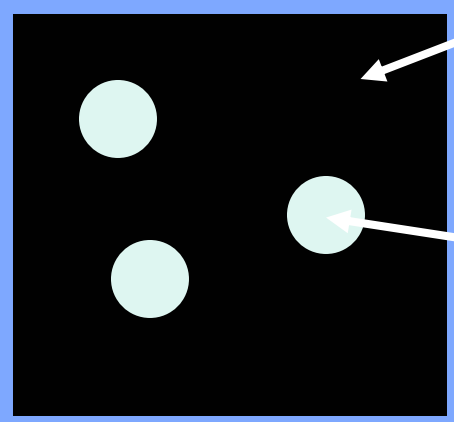
Forward Model $J^{+}=F(Q)$

Inverse Model $Q=F^{-1}(J^{+})$

Fluorescence Tomography

Bioluminescence Tomography

Phantom Experiment



3 cm

solid phantom:

μ_s = 11.6 cm^{-1}

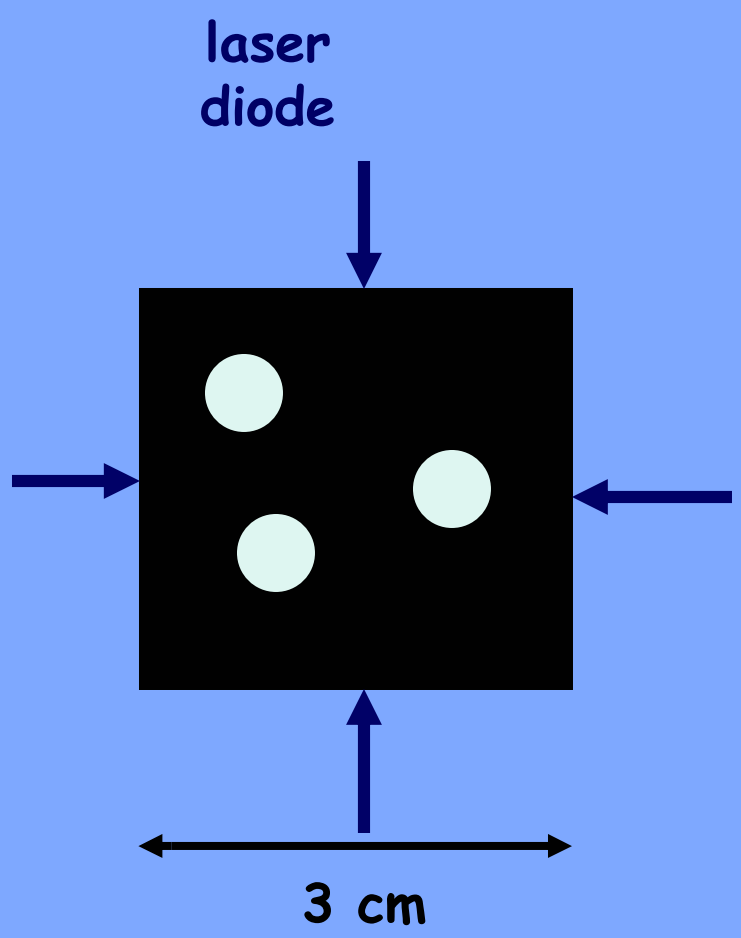
μ_a = 0.01 cm^{-1}

fluorophore:

η= 0.06

$\mu_a^{x \to m}$ = 0.013 cm^{-1}

Phantom Experiment

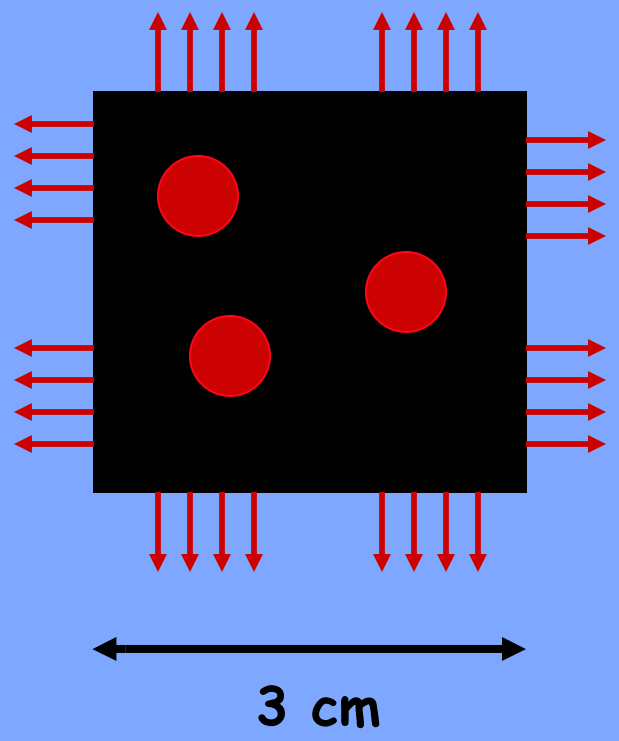


wavelength:

λ^x = 740 nm

1) Excitation

Phantom Experiment



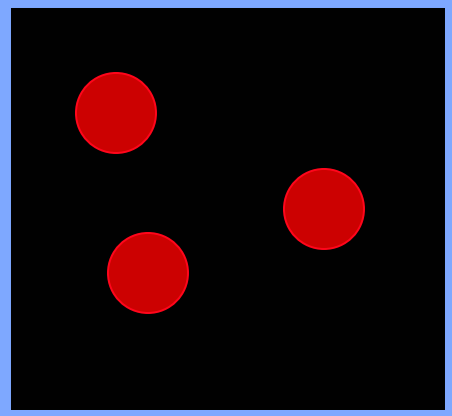
wavelength:

λ^x = 740 nm

λ^m = 802 nm

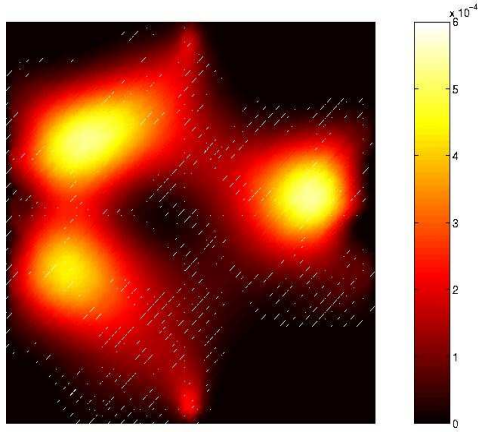
2) Emission

Phantom Experiment

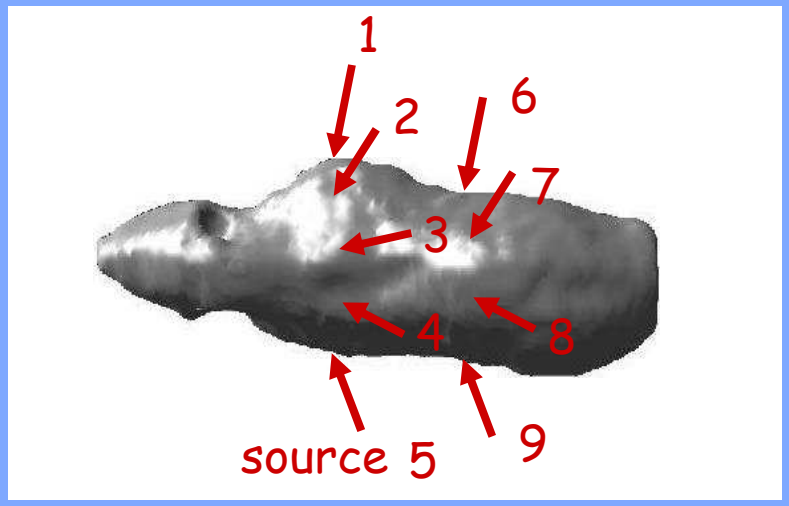


3 cm

$\eta\mu_a$ = 0.0006 cm^{-1}



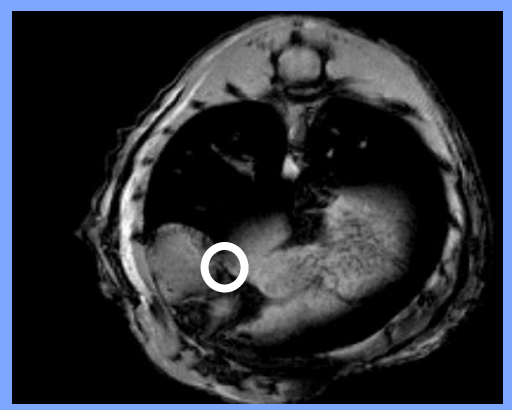
Small Animal Fluorescence Imaging

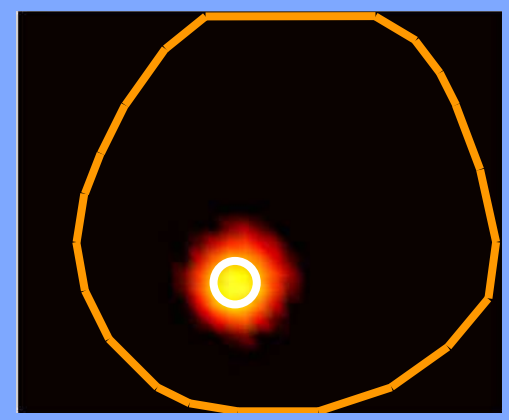


radiatve transfer model is solved
for all source positions on # processors

Small Animal Fluorescence Imaging

1.8 mm^3, 400nM Cy5.5

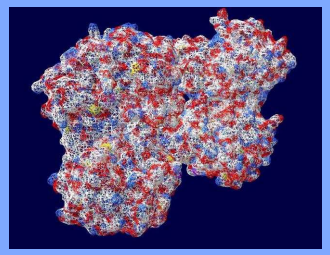


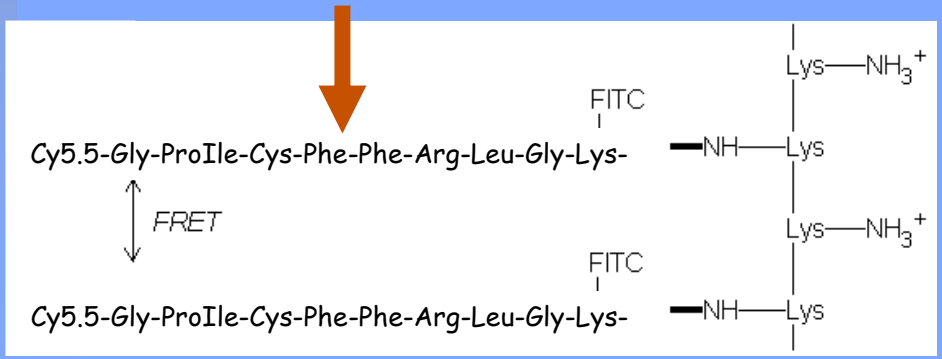


0.5 cm

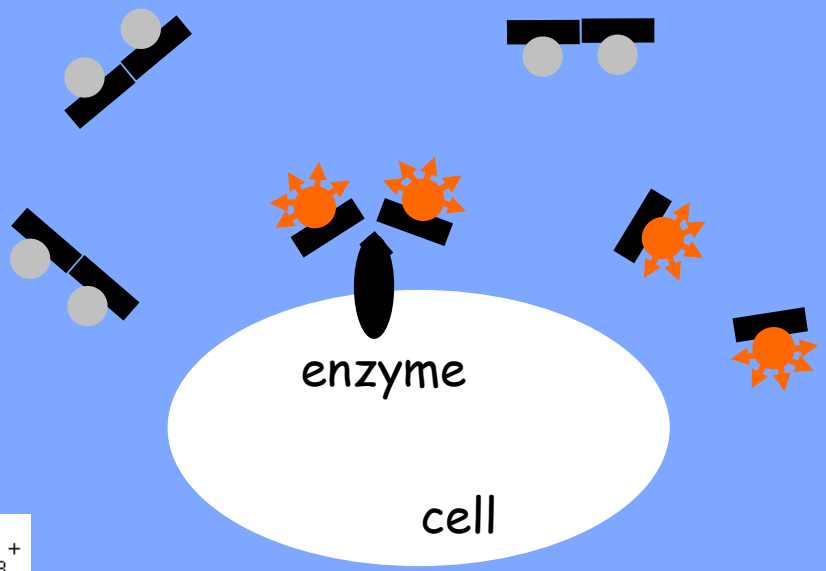
In Vivo Experiments

Fluorescent probe is activated through interaction with a molecular target, e.g. enzyme (Cathepsin D)





fluorescent probe

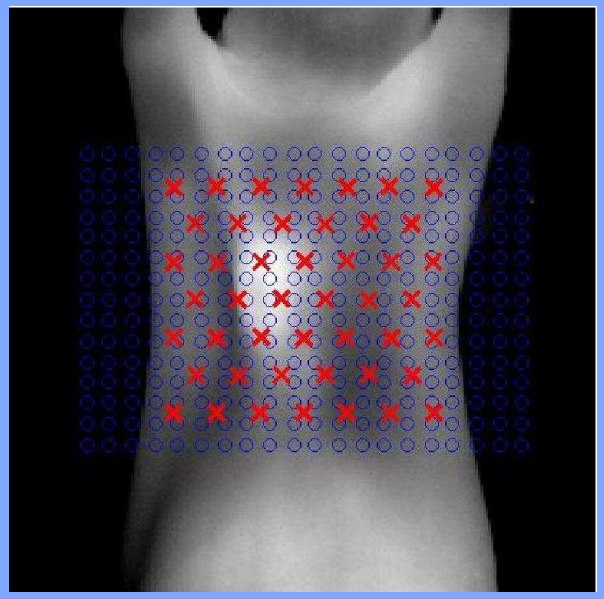


Tung et al, Bioconjugate Chemistry 10, (1999)

Weissleder et al, Nature Biotechnology 17, (1999)

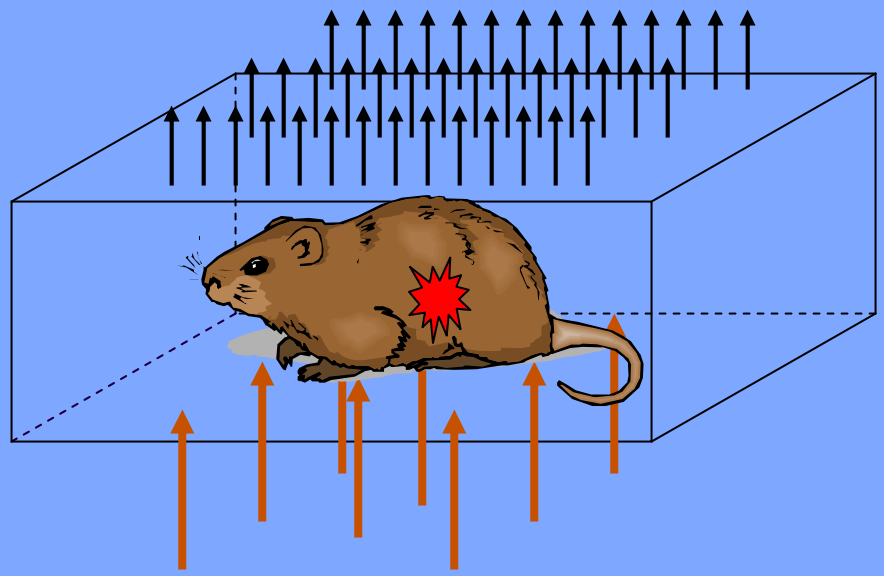
In Vivo Experiments

Mouse with Lewis Lung Carcinoma (LLC)



surface-weighted image

CCD camera



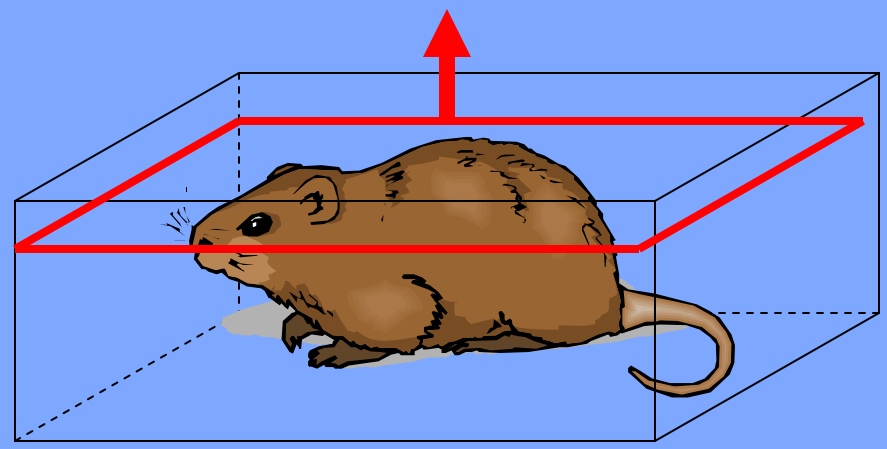
46 source fibers

Experimental data were provided by V. Ntziachristos, MGH

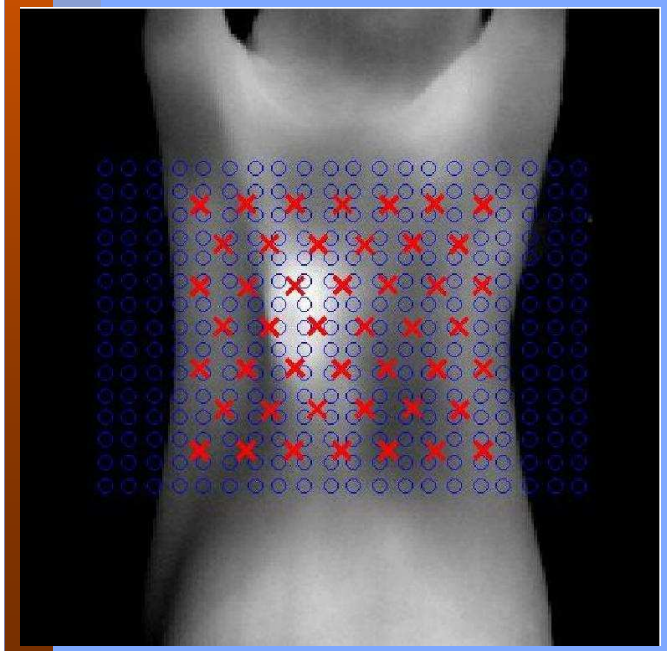
In Vivo Experiments

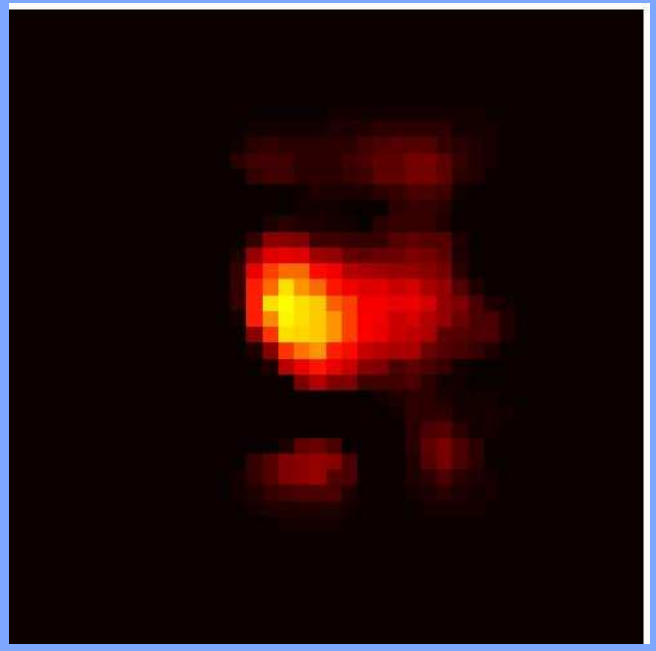
depth

0.2 cm



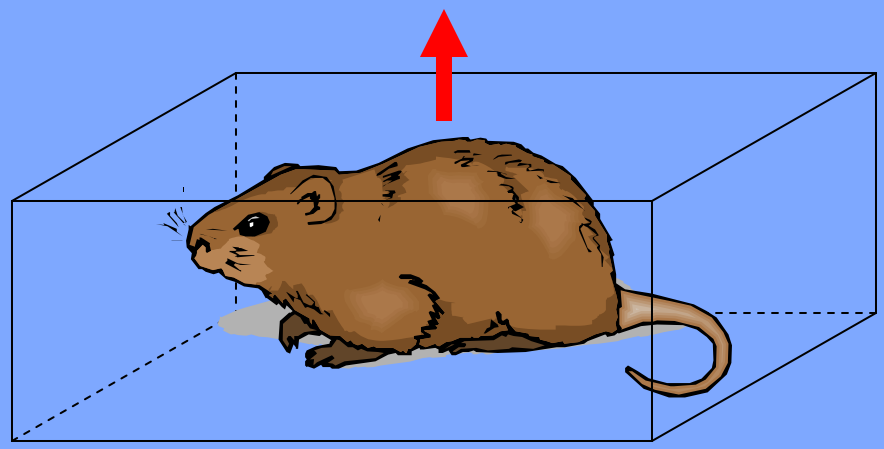
top view



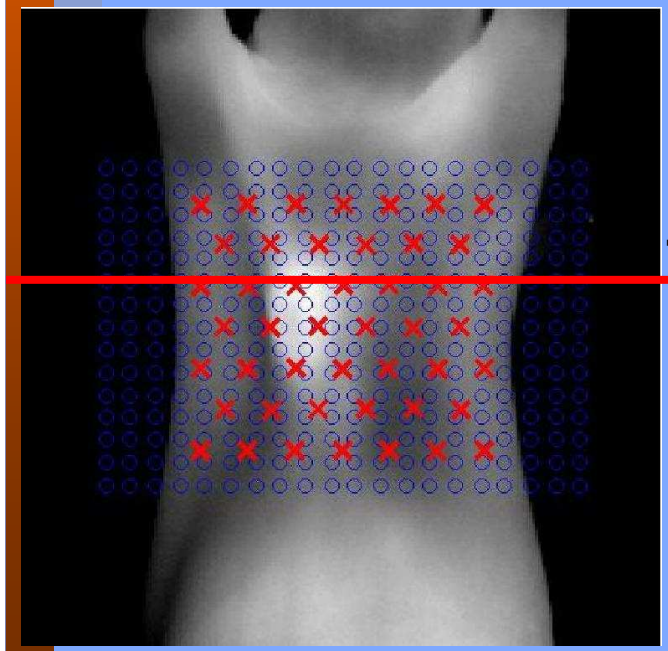


fluorophore
absorption = 0 - 0.032 cm^{-1}

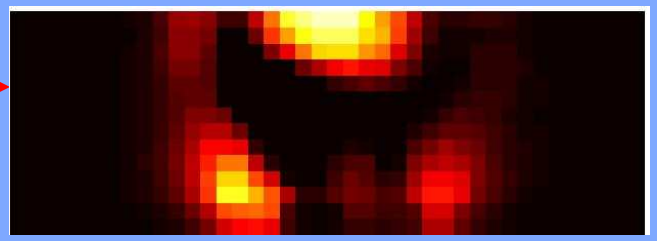
In Vivo Experiments



top view



+0.2 cm



fluorophore
absorption = 0 - 0.032 cm^{-1}

Overview

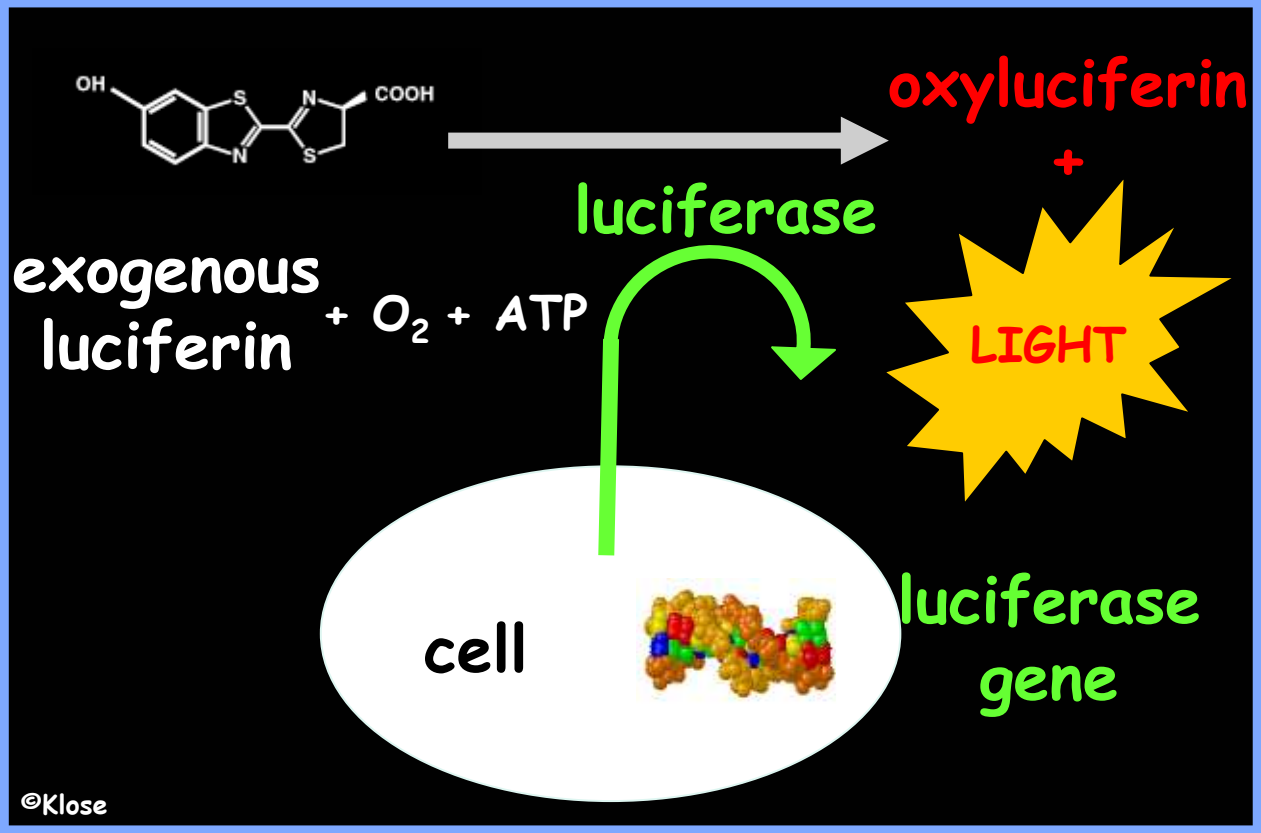
Forward Model $J^{+}=F(Q)$

Inverse Model $Q=F^{-1}(J^{+})$

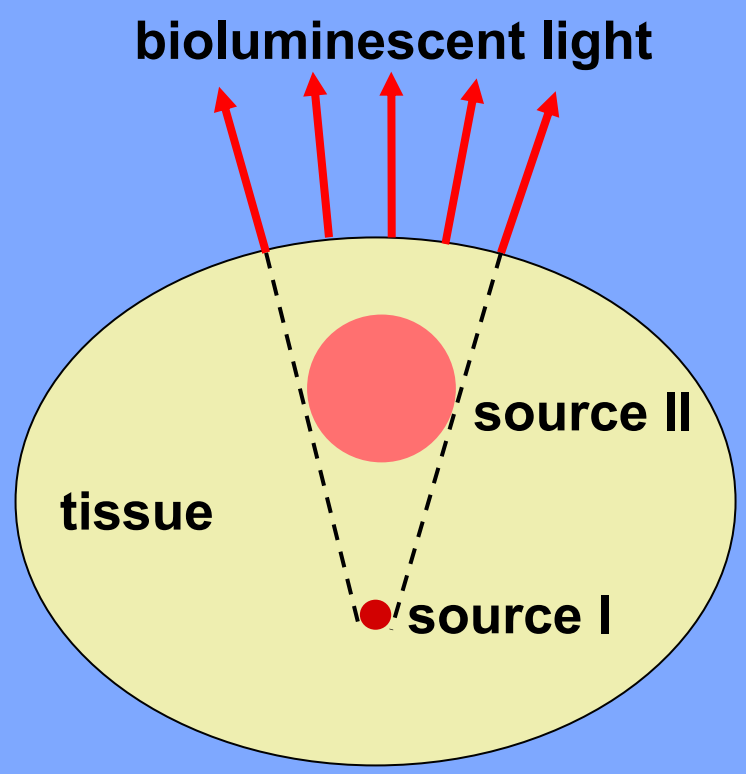
Fluorescence Tomography

Bioluminescence Tomography

Bioluminescence Tomography



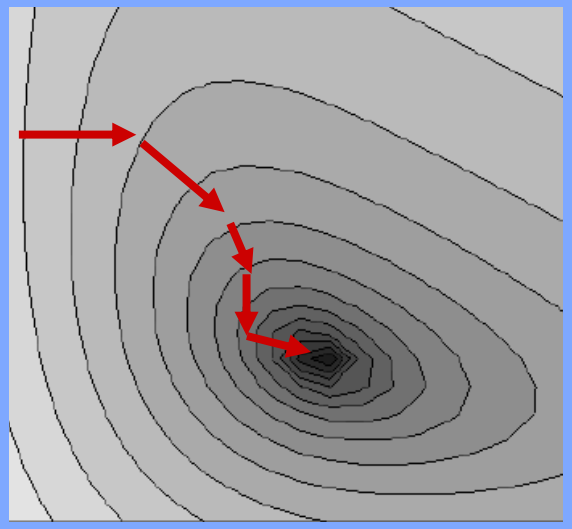
Multiple Solutions



$$\Phi(\theta) = \frac{1}{N}\sum_{n}^{N}\frac{\left(Y_n - J_n^{+}(\theta)\right)^2}{\sigma_n^2}$$

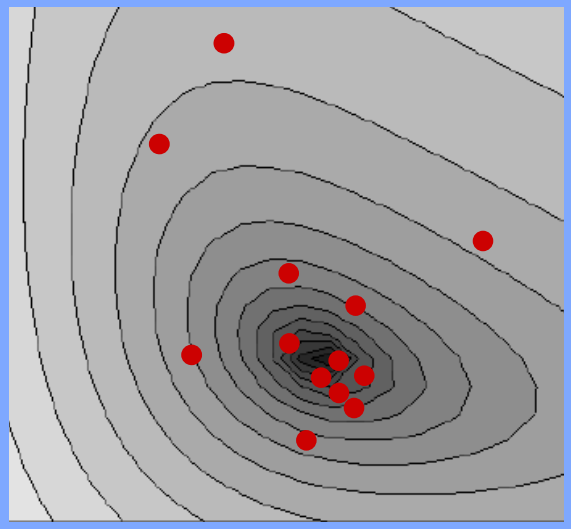
Optimization of $\Phi(\theta) = \frac{1}{N}\sum_{n}^{N}\frac{\left(Y_n - J_n^{+}(\theta)\right)^2}{\sigma_n^2}$

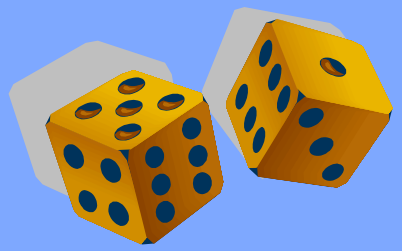
deterministic



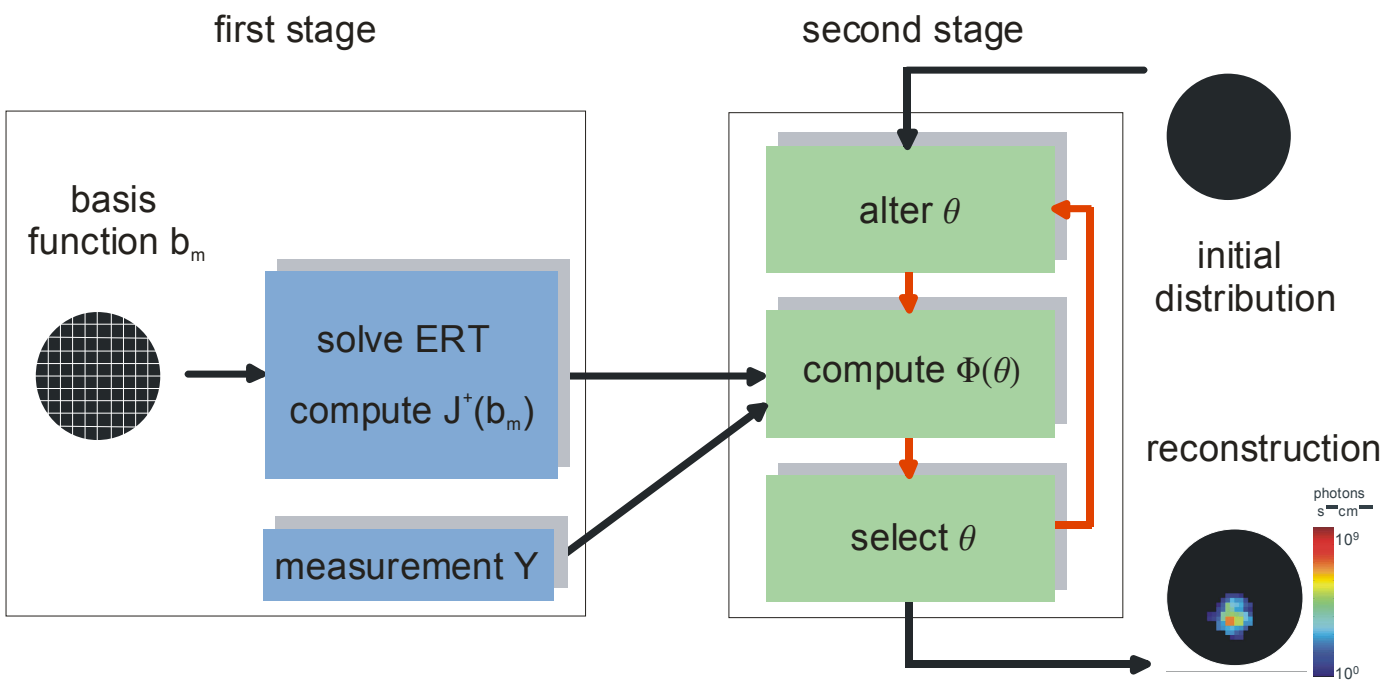
$$\nabla\Phi = \frac{(J^{+}-Y)}{2\sigma^2}\frac{\partial J^{+}}{\partial Q}$$

stochastic





Stochastic Image Reconstruction (SIR)



First Stage - Linear Source problem

source power density is decomposed into source basis functions b_m

$$Q(r) = \sum_{m=0}^{M} \theta_m b_m(r)$$

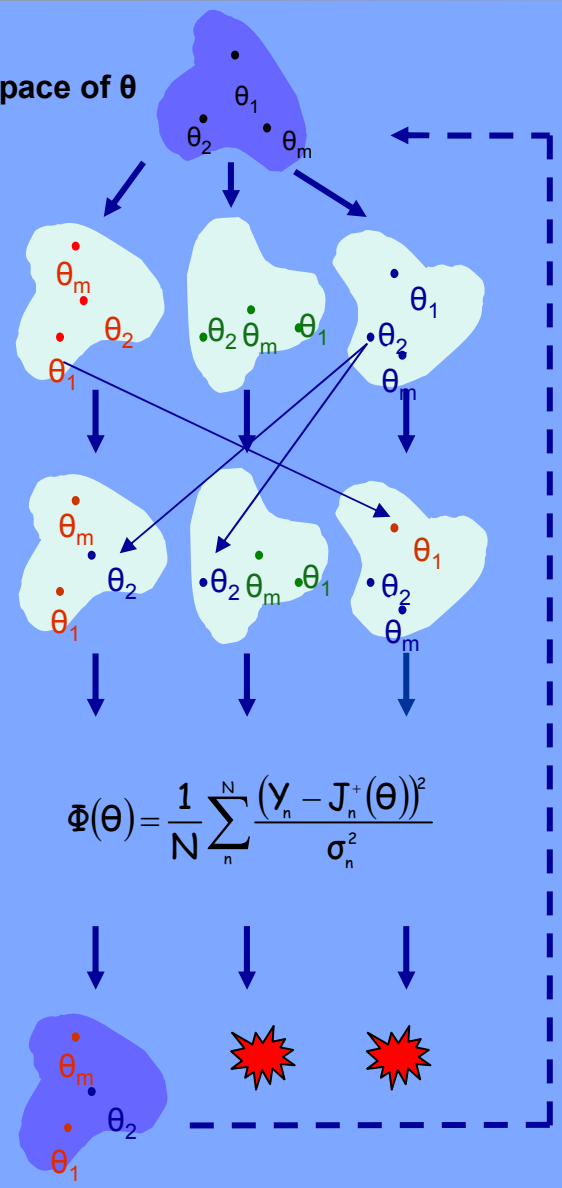
$$\Omega \cdot \nabla \psi(r,\Omega) + \mu_t \psi(r,\Omega) = \frac{b_m(r)}{4\pi} + \mu_s \int_{4\pi} p(\Omega,\Omega')\psi(r,\Omega')d\Omega'$$

boundary flux as function of unknown data variables θ_m

$$J_n(\theta) = \sum_{m=0}^{M} \theta_m J_n(b_m)$$

Second Stage – Evolution Strategy

parameter space of θ



population of
μ parents

population of
λ offspring

1. Alter θ:

recombination
self-adaptation
mutation

$$\theta_m^{new} = \theta_m^{old} + N(0, \sigma_m)$$

2. Compute Φ:

$$\Phi(\theta) = \frac{1}{N}\sum_n^N \frac{\left(Y_n - J_n^+(\theta)\right)^2}{\sigma_n^2}$$

select smallest Φ

"survival of the fittest"

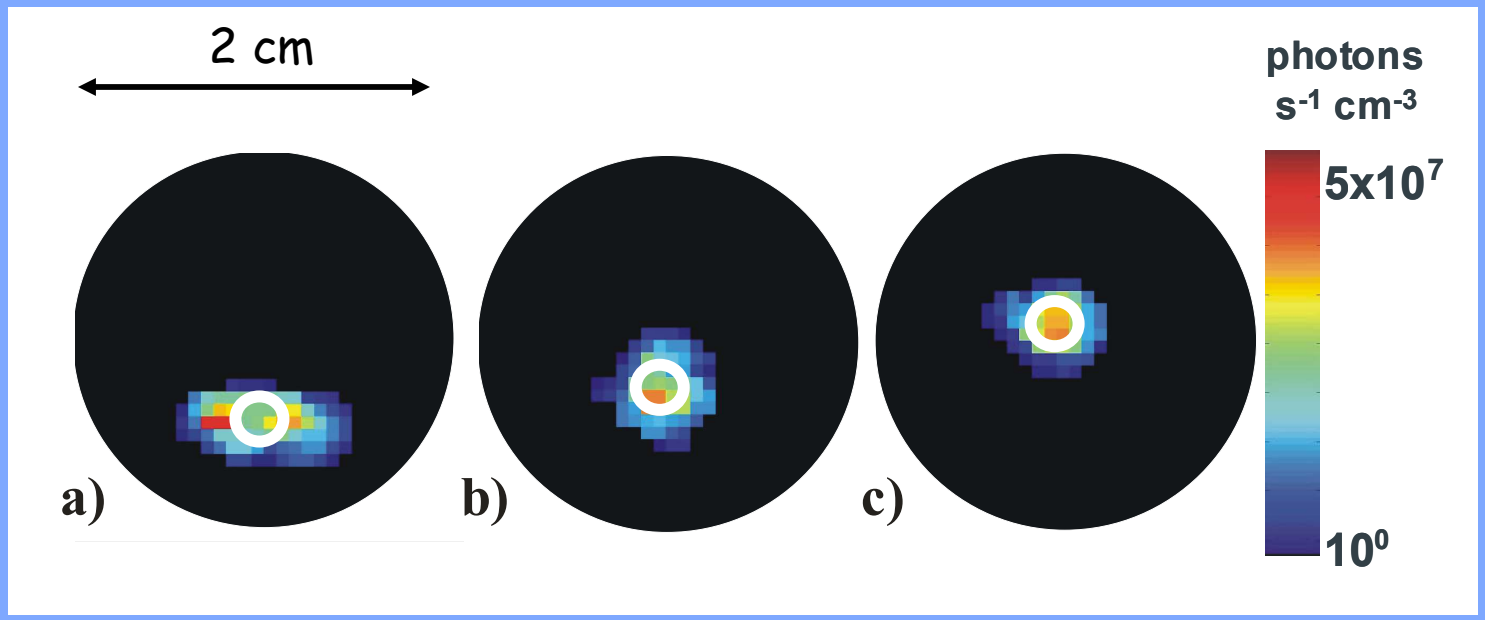
3. Select θ:

NEW
population of
μ parents

Second Stage – Evolution Strategy

- **Recombination:** $$\theta_m^{\lambda} = \frac{\theta_a^{\mu} + \theta_b^{\mu}}{2}$$
- **Self-Adaptation:** $$\hat{\sigma}_m^{\lambda} = \sigma_m^{\lambda} e^{\tau N(0,1)}$$
- **Mutation:** $$\hat{\theta}_m^{\lambda} = \theta_m^{\lambda} + N(0, \hat{\sigma}_m^{\lambda})$$

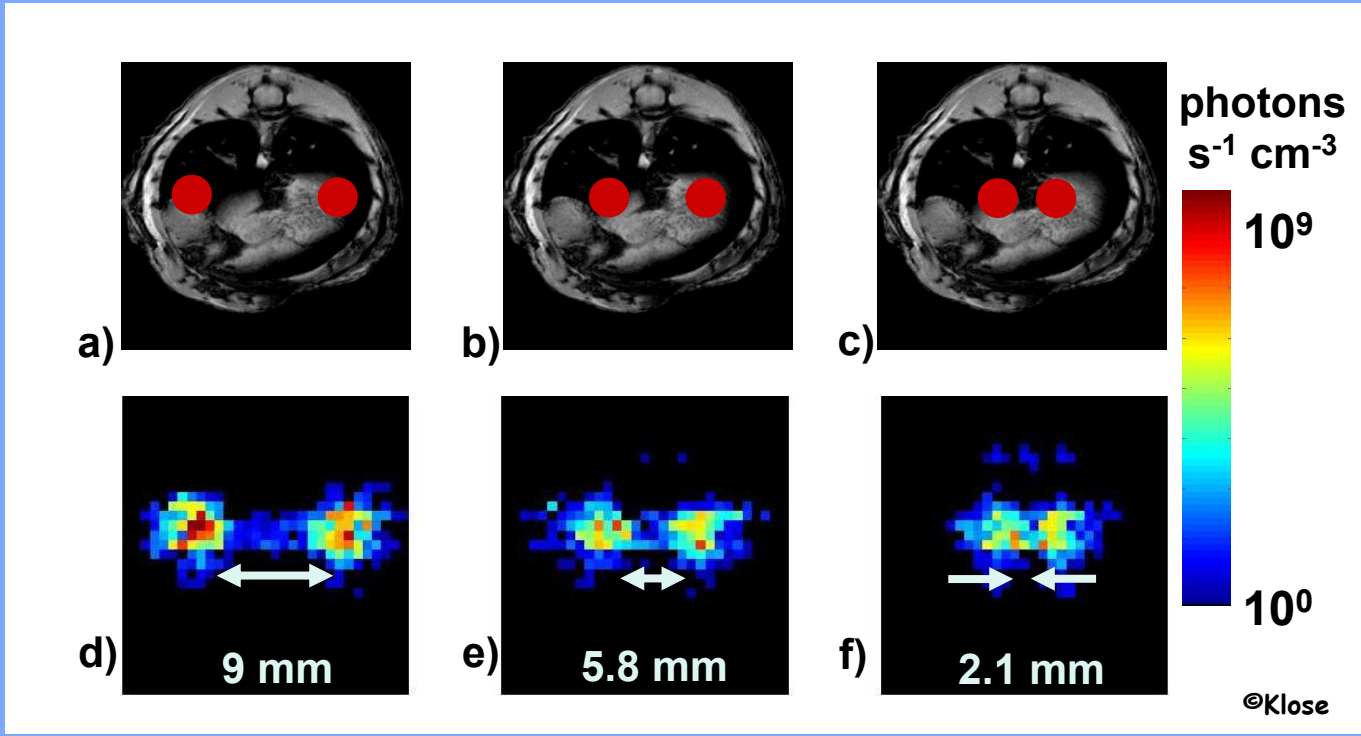
Results - Simulations



675 unknown vector elements θ_m (image)

μ = 3,000 parent members; λ = 18,000 offspring members
900 generations

Results – Simulations



Tomographic reconstruction of two bioluminescent sources.
Optical property maps are based on MRIs.

Summary

Biological tissue is highly scattering

- Equation of Radiative Transfer
- Diffusion/ Simplified Spherical Harmonics

Inverse source problem: linear/nonlinear

- optimization methods
  - gradient techniques (Adjoint Differentiation)
  - Evolution Strategy
- algebraic reconstruction

Computationally very expensive

- cluster computing/efficient processor architecture

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www.columbia.edu/~ak2083/publications.htm