Fluorescence tomography of red fluorescent protein expressed tumors in small animals

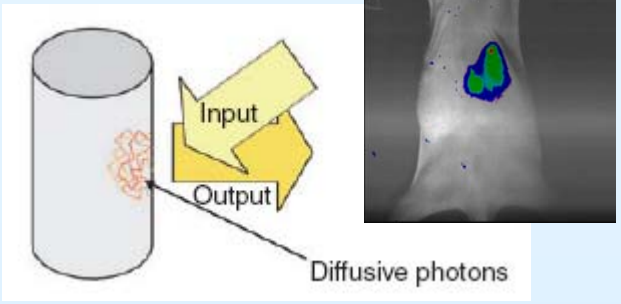
I. Turchin[1], I. Fiks[1], M. Kleshnin[1], A. Orlova[1], I. Balalaeva[2], M. Shirmanova[2,3], A.Rusanov[4], S. Deyev[5], A. Savitsky[4]

[1]Institute of Applied Physics of the RAS, Nizhny Novgorod, Russia
[2]Nizhny Novgorod State University , Russia
[3]Nizhny Novgorod State Medical Academy, Russia
[4]A.N. Bach Institute of Biochemistry of the RAS, Moscow, Russia
[5]Nizhny Novgorod State Medical Academy , Moscow, Russia

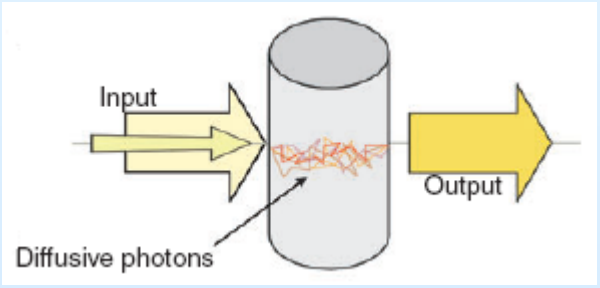
LALS – 2010

Whole-body fluorescence imaging techniques

• **Reflectance (epi-illumination) - 2D images**
Good transverse resolution for subcutaneous tumors
Very fast, no reconstruction

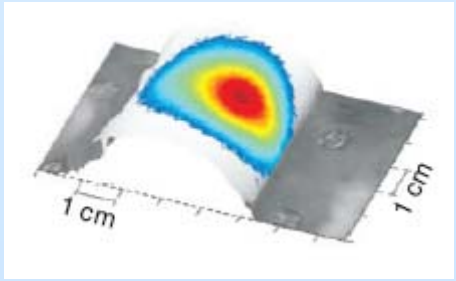


• **Transillumination (or Projection) method**
Visualisation of deep and subcutaneous tumors
Fast, no reconstruction

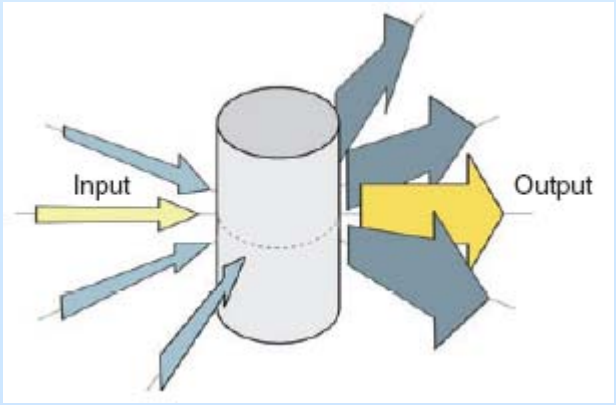


• **Fluorescent diffuse tomography (FDT) allows for 3D reconstruction of the fluorophore concentration**
Complicated,
need for reconstruction.

Inverting matrix is big size,
not sparse (unlike in CT),
ill-conditioned



Diffuse pattern



V. Ntziachristos, et al., Nature Biotechnology **23**, 2005

Whole-body fluorescent imaging applications

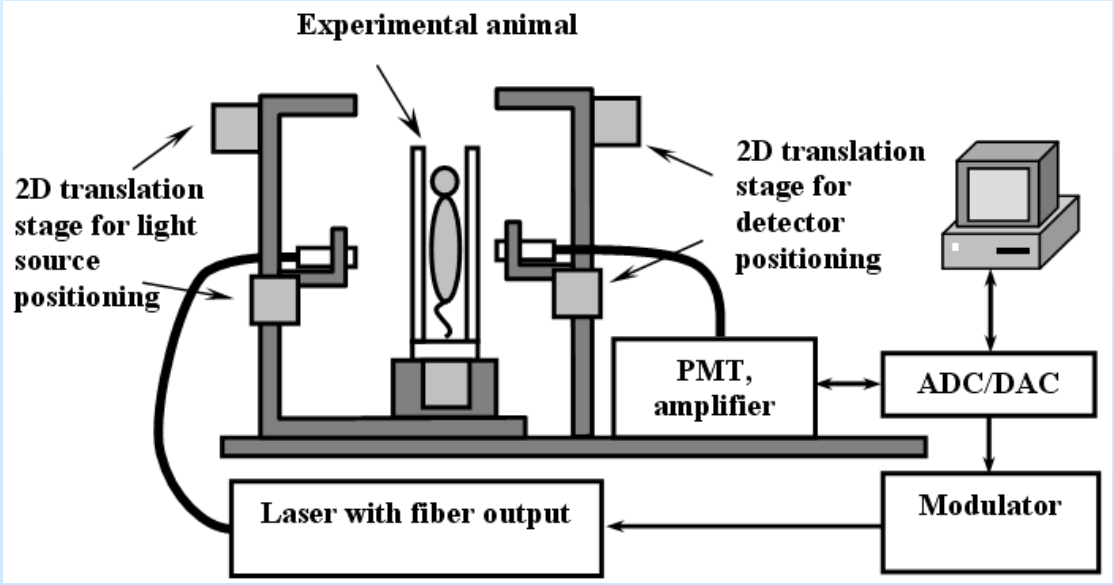
- **Preclinical study of new photosensitizers for fluorescent diagnostics and photodynamic therapy.**
- **Visualization of the drug delivery process using fluorescent labeles (quantum dots or fluorescent proteins).**
- **Investigation of tumor growth, metastasis and retardation under therapy using tumor cells transfected with fluorescent proteins.**
- **Detection of molecular processes, in particular, fluorescence resonance energy transfer (FRET) in experimental animals.**

Hoffman R.M. Lab Animal **31**, 2002

FDT setup with a single source-detector pair

• **Projection method**
2D images, no reconstruction
fluorescent proteins, photosensitizers, quantum dots

• **Fluorescent diffuse tomography**
3D reconstruction if the fluorophore is well localized
fluorescent proteins



IV Turchin, AP Savitsky, et al. // JBO **13**, 041310 (2008)

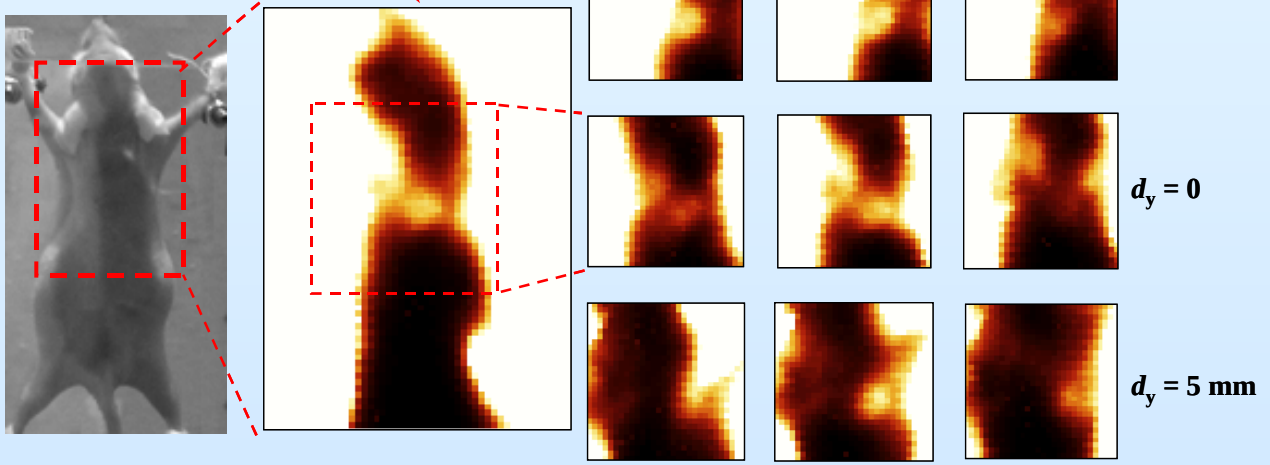
Algorithm of scanning an experimantal animal

projection method

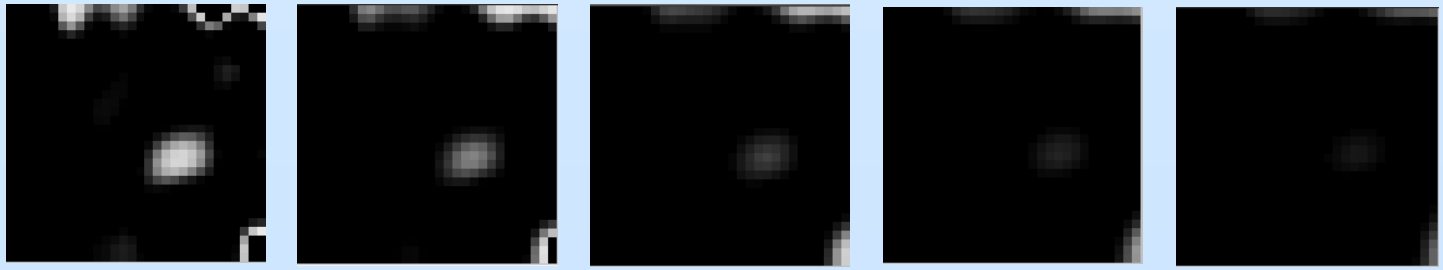
obtaining a general view of the animal (source and detector are moving synchronously)

3D reconstruction

scanning of the selected region with small step sizes and different shifts between source and detector.



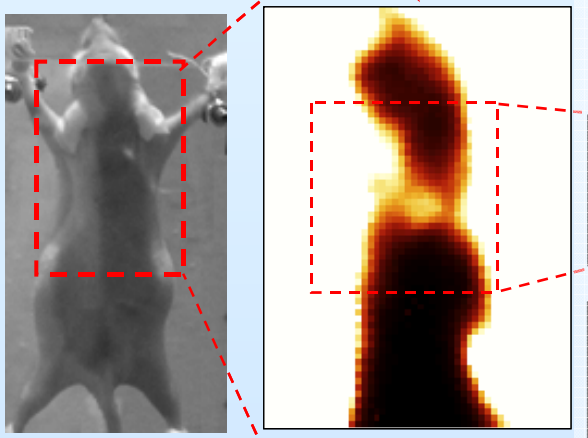
Reconstructed distribution of fluorophore concentration



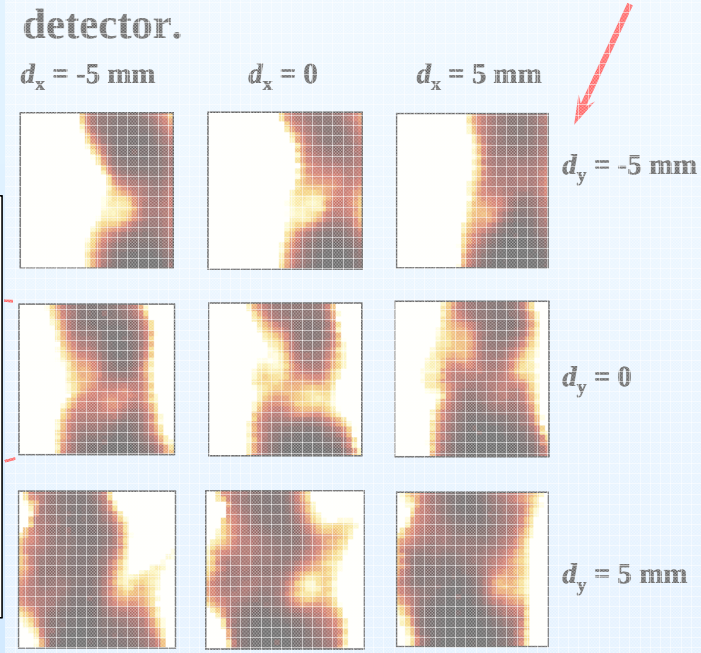
z = 0mm z = 1mm z = 2mm z = 3mm z = 4mm

Algorithm of scanning an experimantal animal

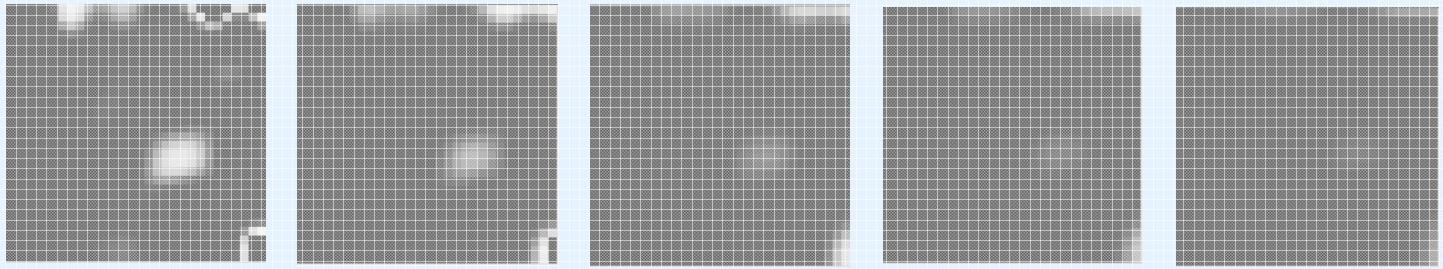
projection method
obtaining a general view of the animal (source and detector are moving synchronously)



3D reconstruction
scanning of the selected region with small step sizes and different shifts between source and detector.

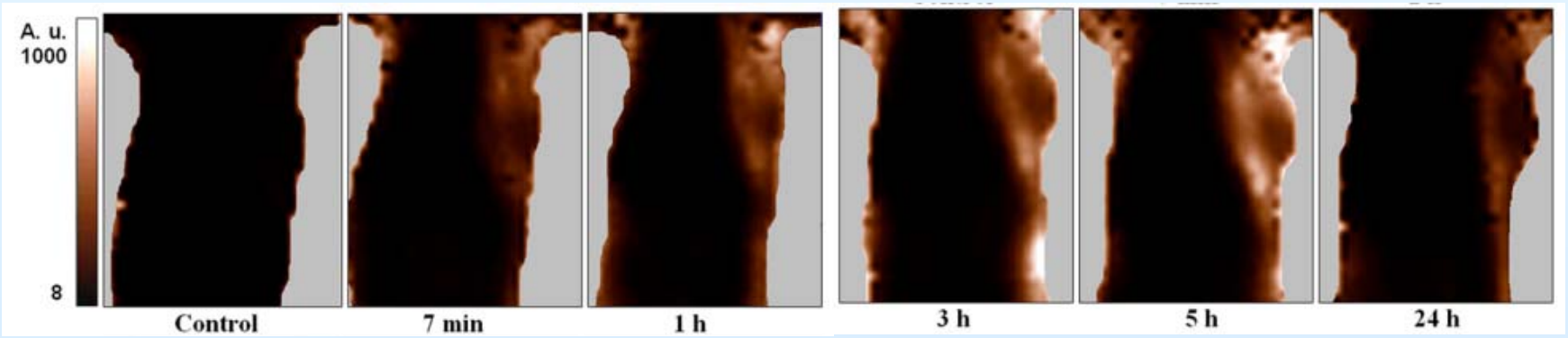


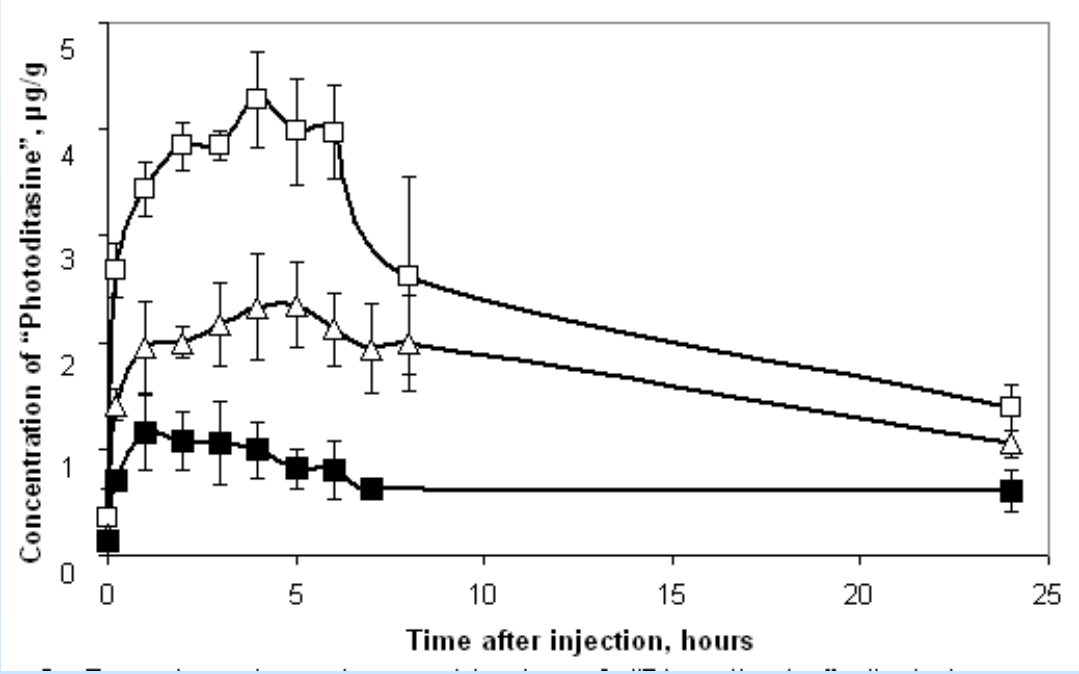
Reconstructed distribution of fluorophore concentration



Preclinical study of new photosensitizers for fluorescent diagnostics and photodynamic therapy (non-tomographic imaging)

Accumulation of “Photosens” (1 mg/kg, i.v.) in mouse cervical carcinoma



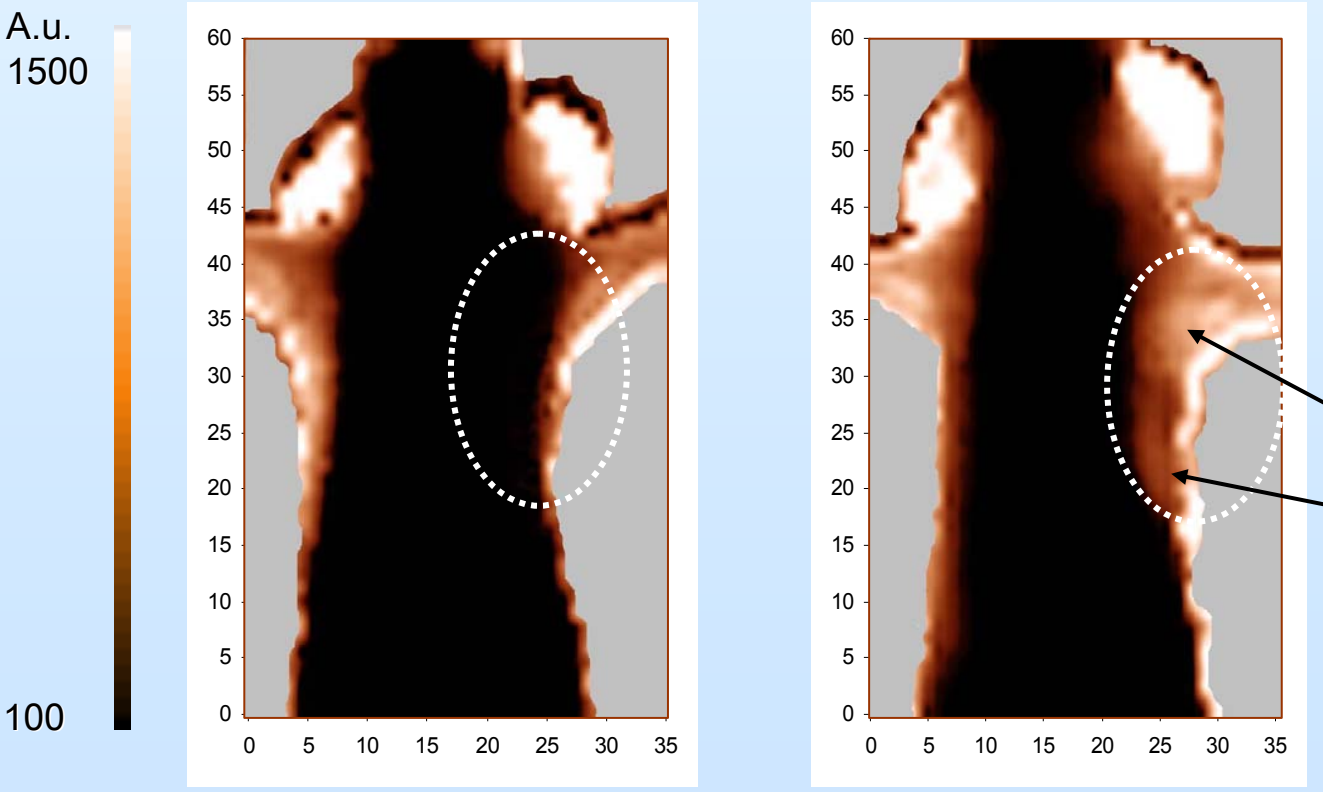


- **Biodistribution**
- **Elimination**
- **Tumor selectivity**
- **Dosage studies**

M.Shirmanova, IV Turchin, et al. // JBO, sent to publication

Whole-body imaging of delivering fluorescent agents to the tumor (non-tomographic imaging)

Human breast carcinoma SKBR-3 in nude mice
Intravenous injection of Qdot 705 ITK (Invitrogen Inc.),
conjugated with anti-HER2/neu 4D5 scFv-antibody
by barnase-barstar protein module
Ex 635 nm, Em_{max} 705 nm

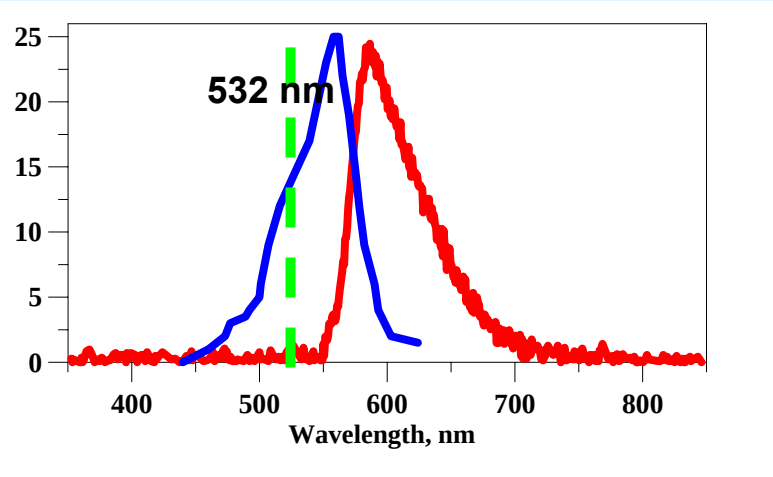


before

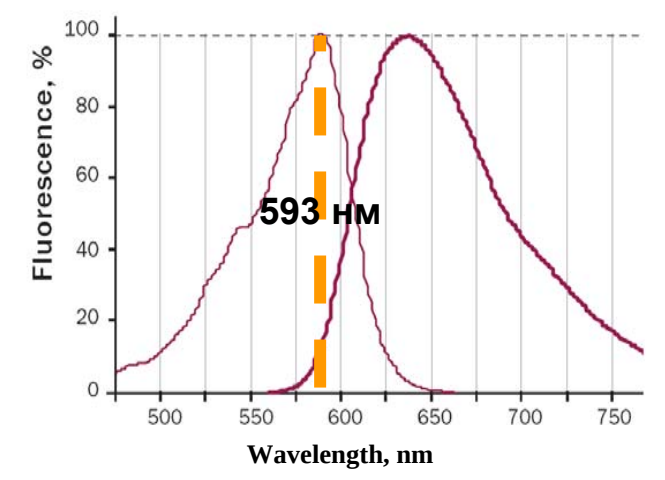
1.5 hours after injection

Investigation of tumor growth, metastasis and retardation under therapy using tumor cells transfected with fluorescent proteins

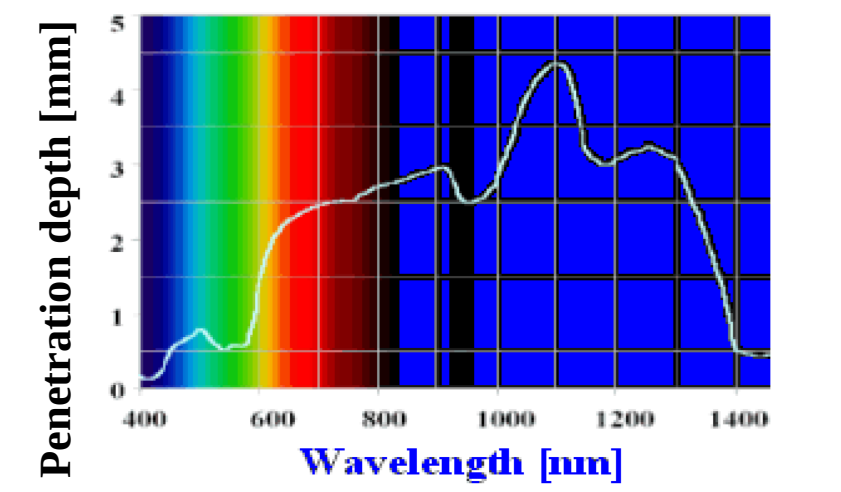
DsRed2



TurboFP635
(scientific name Katushka)

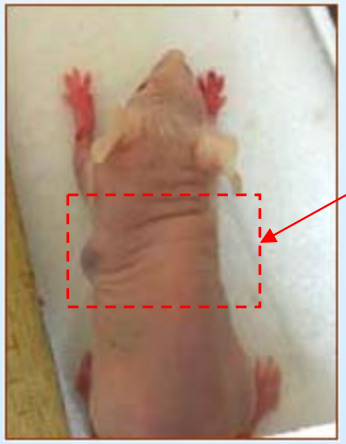


Larger wavelengths gives higher penetration depths.



Intravital monitoring of tumor growth

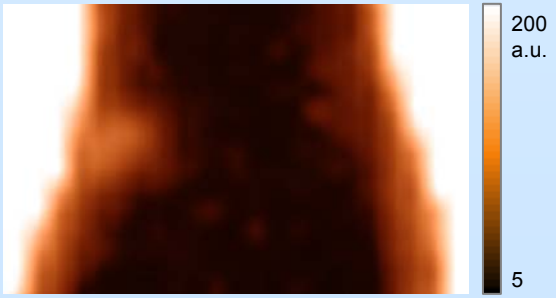
Nude mice carrying human ovarian carcinoma SKOV-3 expressing Katushka



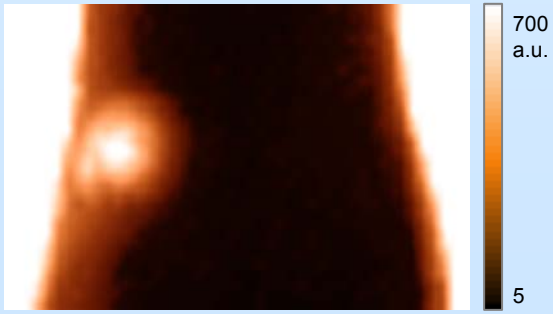
Scanning area

Control before tumor cells injection

15 days



19 days

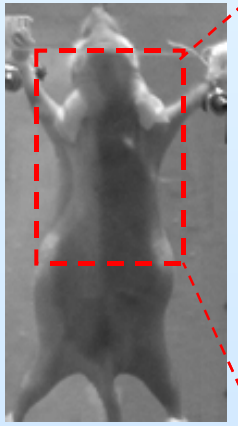


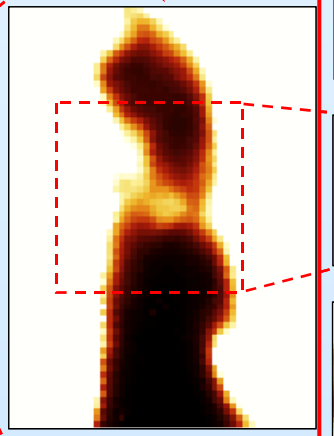
22 days

Algorithm of scanning an experimantal animal

projection method

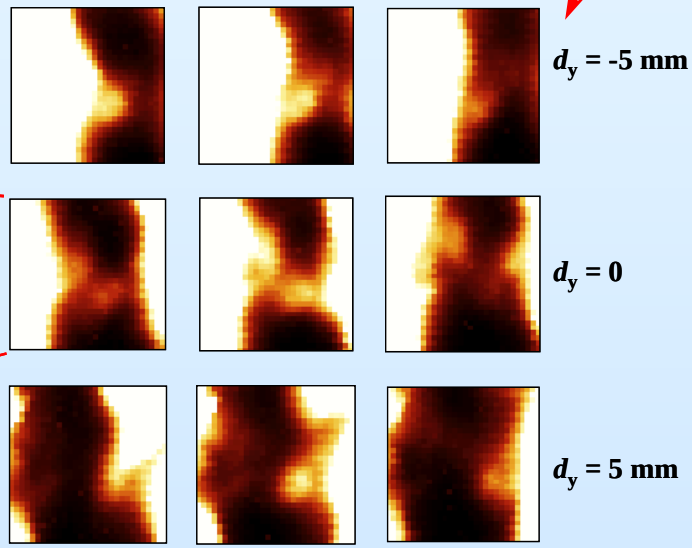
obtaining a general view of the animal (source and detector are moving synchronously)



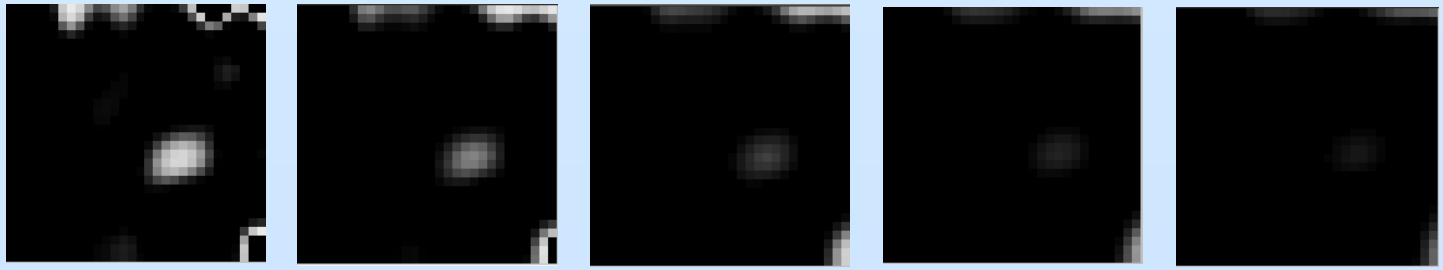


3D reconstruction

scanning of the selected region with small step sizes and different shifts between source and detector.



Reconstructed distribution of fluorophore concentration



z = 0mm | *z* = 1mm | *z* = 2mm | *z* = 3mm | *z* = 4mm

Direct Problem

Radiative transfer equation

$$(\vec{n}\nabla + \mu_a + \mu_s)L(\vec{r},\vec{n}) = \frac{\mu_s}{4\pi}\iiint_{4\pi} L(\vec{r},\vec{n}')\chi(\vec{n},\vec{n}')d\Omega' + Q$$

$$E(\mathbf{r}) = \iiint_{4\pi} L(\vec{r},\vec{n})d\Omega$$

Analytical approximations

Numerical solution
High accuracy,
long time (hours)

Monte Carlo method

Diffusive (at depth > 3-5 мм)

Small-angle (high anisotropic scattering)

Hybrid (at any depth)

$$E^{(diff)}(r) = \frac{3P\varepsilon'}{4\pi}\frac{e^{-\sqrt{3\mu_a\mu_s'}r}}{r}$$

$$E^{(dir)}(\mathbf{r}_\perp, z) = \frac{P}{\pi a^2(z)}\mathrm{e}^{-\mu_t' z - \frac{\mathbf{r}_\perp^2}{a^2(z)}}$$

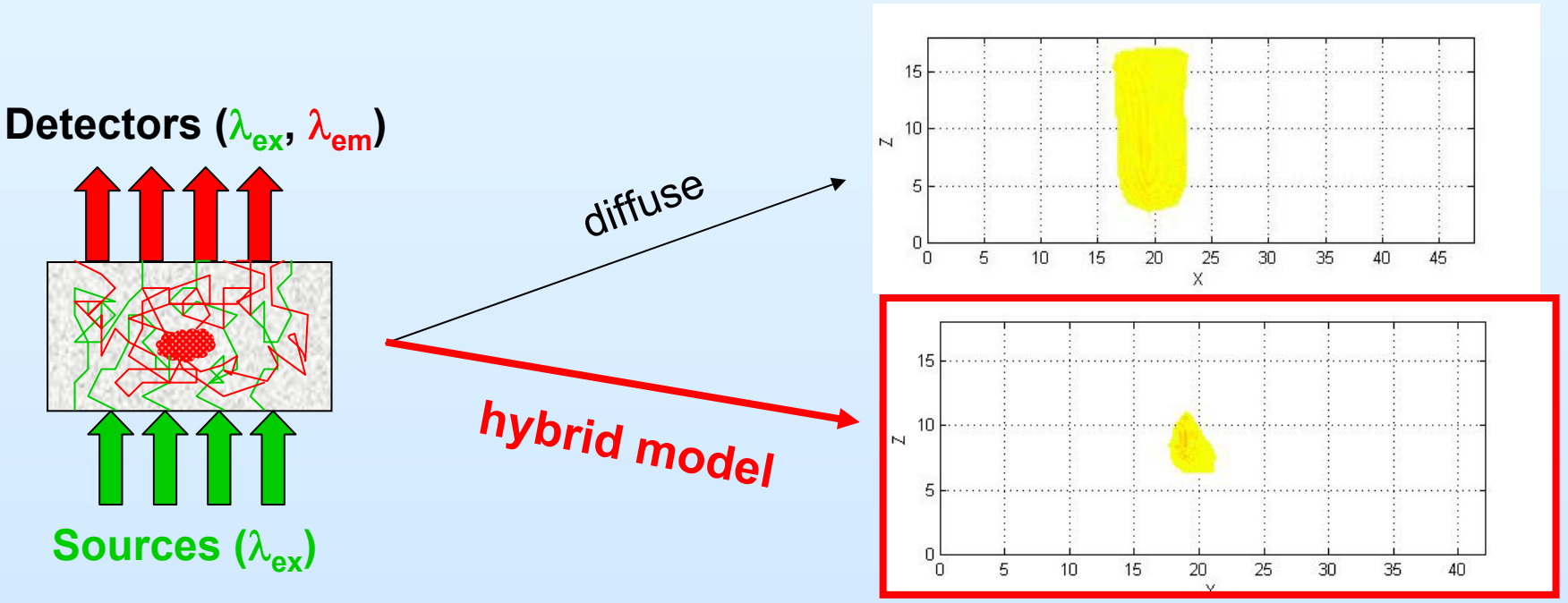
$$E^{(gib)}(\vec{r_1},\vec{r_2},\lambda) = E^{(gdiff)}(\vec{r_1},\vec{r_2},\lambda) + E^{(gdir)}(\vec{r_1},\vec{r_2},\lambda)$$

Point -source field

Directional source field

I. Fiks, M. Kirillin, E. Sergeeva, M. Kleshnin, I. Turchin, Proc. SPIE, 73690B (2009)

3D reconstruction of the fluorophore concentration for plane geometries



3D reconstruction of the fluorophore concentration

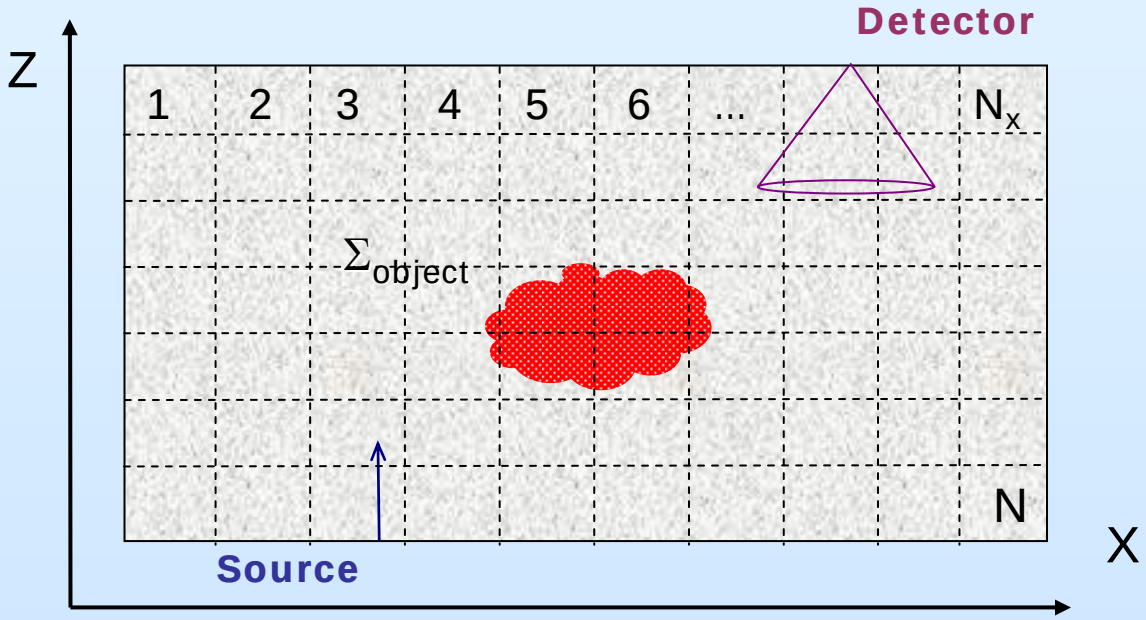
System of linear equations:

$$P(\vec{r}_s,\vec{r}_d)=\iiint \Sigma_{object}(\vec{r}_0)P_{det}(\vec{r}_s,\vec{r}_d,\vec{r}_0)d\vec{r}_0 \quad \Rightarrow \quad Av=p$$

?

solution of direct problem

$$P_{\det}(\vec{r}_s,\vec{r}_d,r_0)=CE(\vec{r}_s,\vec{r}_0,\lambda_{ex})E(\vec{r}_0,\vec{r}_d,\lambda_{em})$$



$$\vec{r}_0 \to j$$

$$(\vec{r}_s,\vec{r}_d) \to i$$

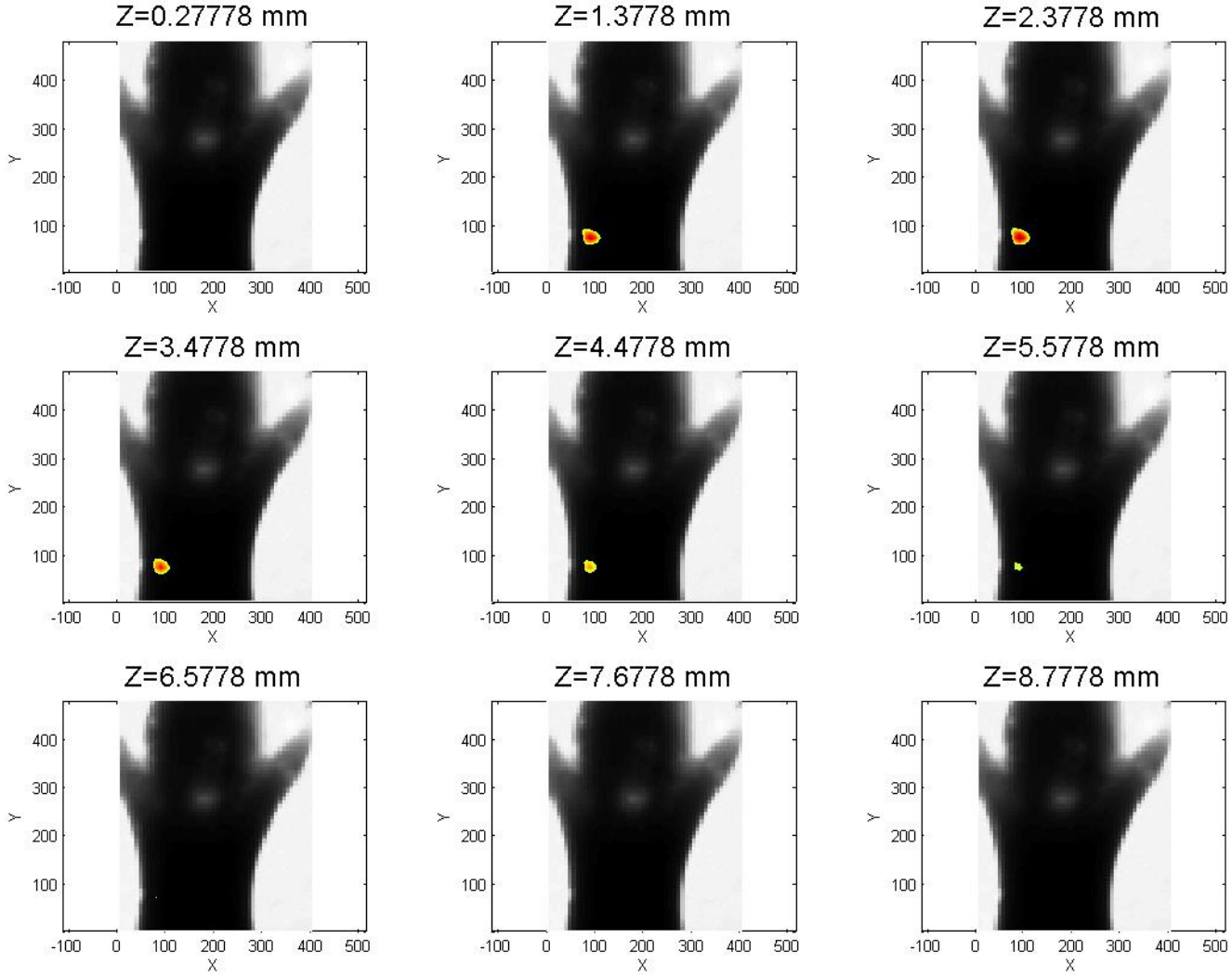
$$\Sigma_{object}(\vec{r}_0) \to v_j$$

$$P(\vec{r}_s,\vec{r}_d) \to p_i$$

$$P_{\det}(\vec{r}_s,\vec{r}_d,\vec{r}_0) \to A_{ij}$$

- **Big size of matrix *A* (about 10^8 elements ~ 1000 Mb Ram)**
- **Matrix *A* is not sparse (in comparison with CT)**
- **Matrix *A* matrix is ill-conditioned ~ 10^{13}**

3D reconstruction of the fluorophore distribution (at depth Z, object width is 1.2 cm)



Improving reconstruction of the fluorophore distribution:

-Many source-detector measurements

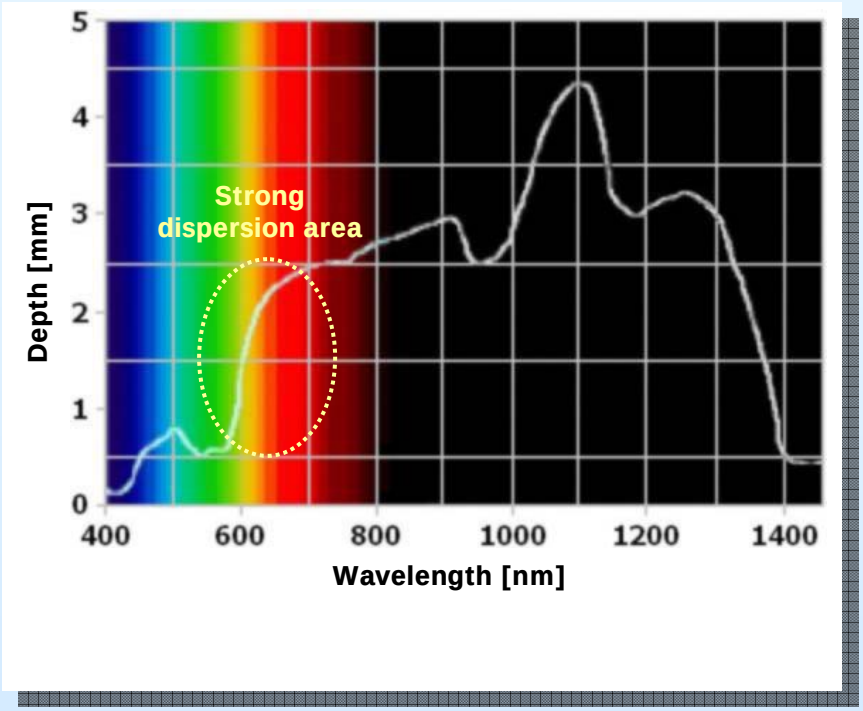
using a sensitive CCD in order to reduce scanning time

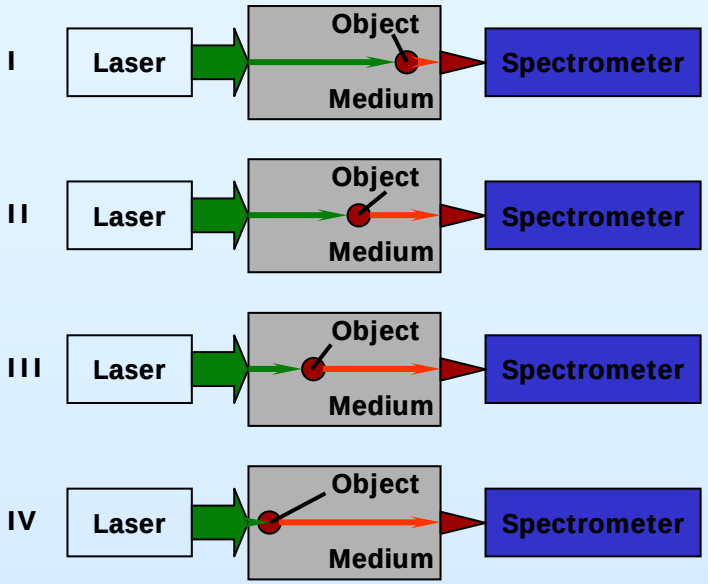
- Additional information

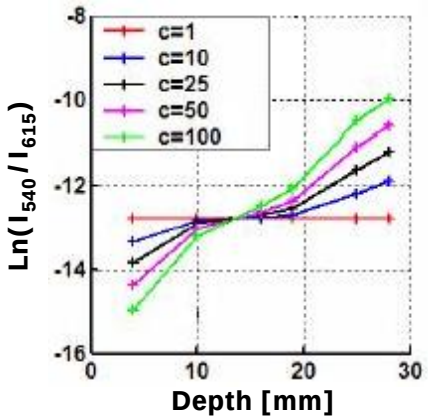
time-domain or frequency-domain FDT

spectroscopic information

FLUORESCENCE SPECTRUM ALLOWS TO LOCALIZE FLUORESCING TUMOR

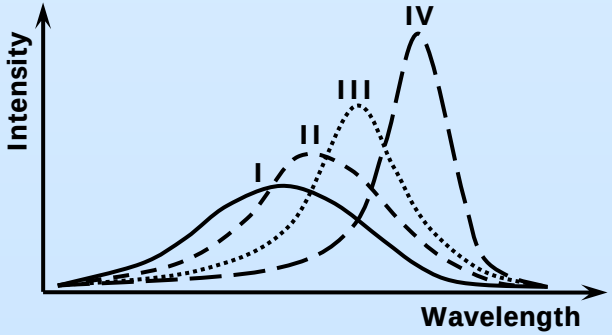






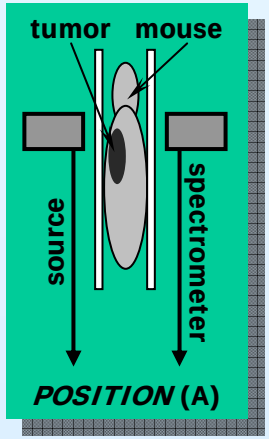
The detected yellow / red intensity ratio as a function of the depth of the fluorescing layer in highly scattering medium (*c* – the contrast between the layer and the rest of the tissue model).

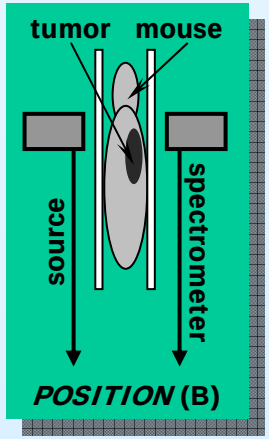
J. Svenssonand, S. Andersson-Engels, Opt. Express, v. 13, p. 4263 (2005)

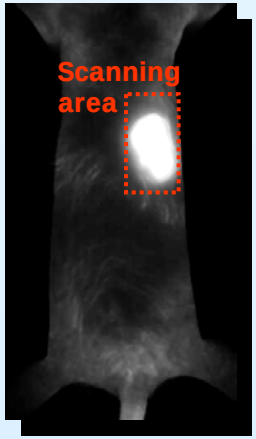


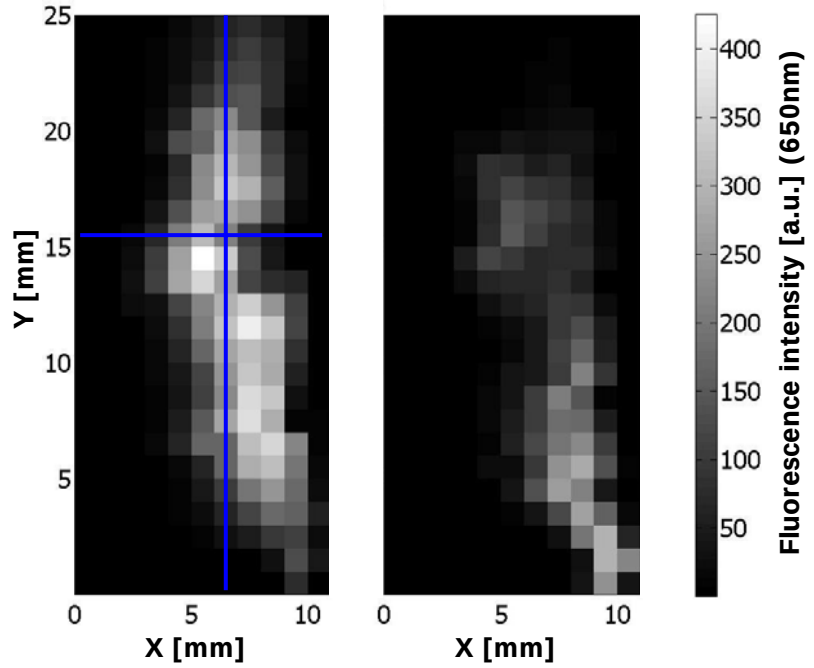
Spectral shape of fluorescence signal as a function of the fluorescence object position

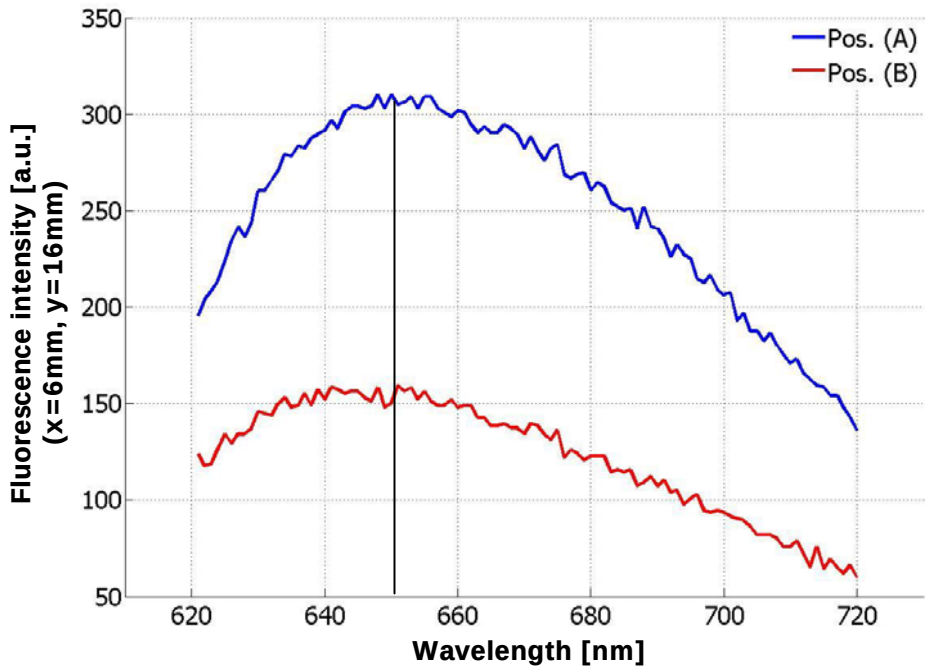
RESULTS OF IN VIVO EXPERIMENTS











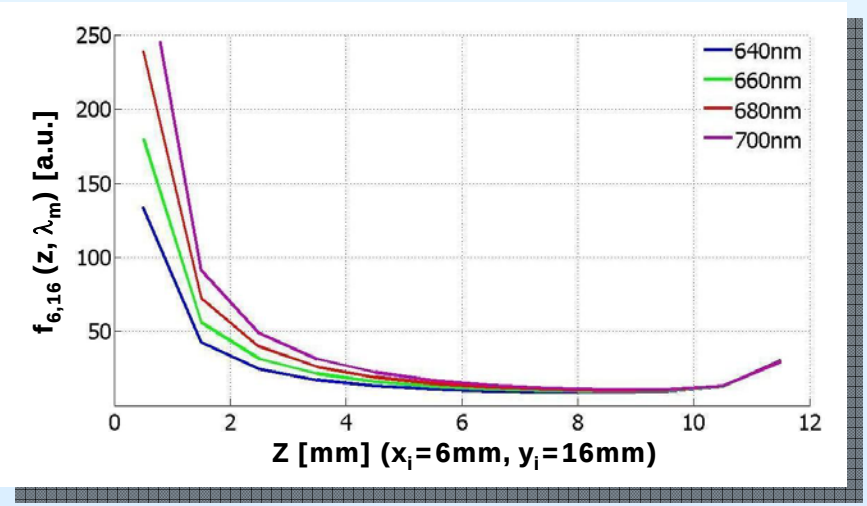
Fluorescence intensity for different scanning positions

Reconstruction of the fluorophore spatial distribution

Matrix coefficients

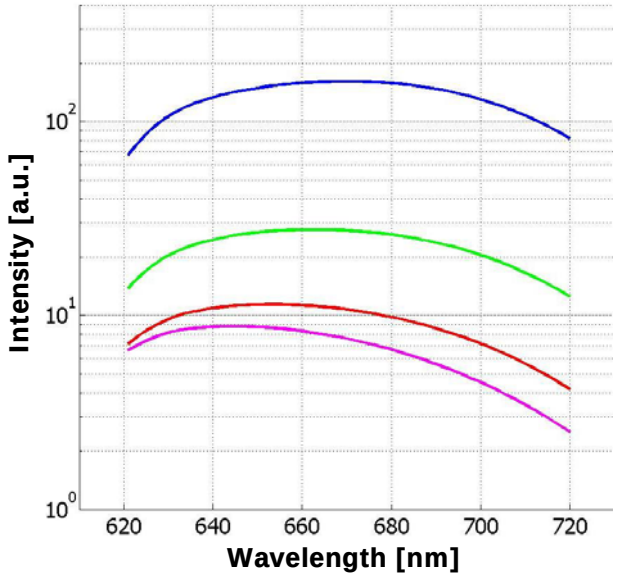
$$f_{i,j}(z_k,\lambda_m)=\frac{\gamma}{P_0}E(x_i,y_j,z_k,\lambda_0)E(x_i,y_j,d-z_k,\lambda_m)$$

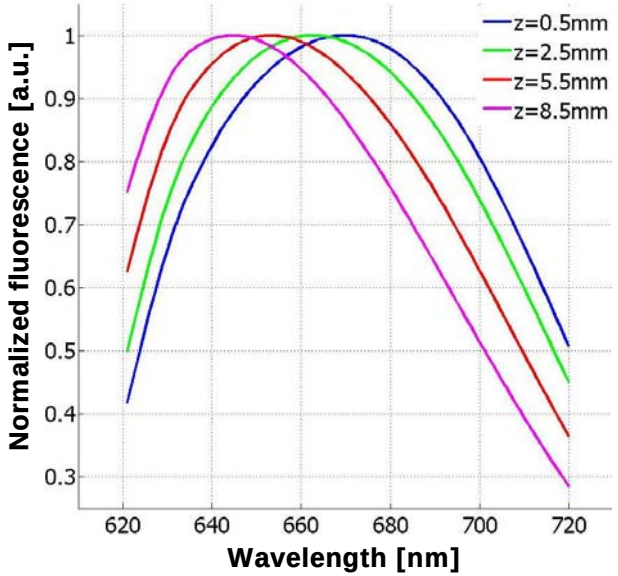
defines the changes of fluorescence spectrum



M.Kleshnin, I. Turchin// Quantum Electronics, excepted for publication

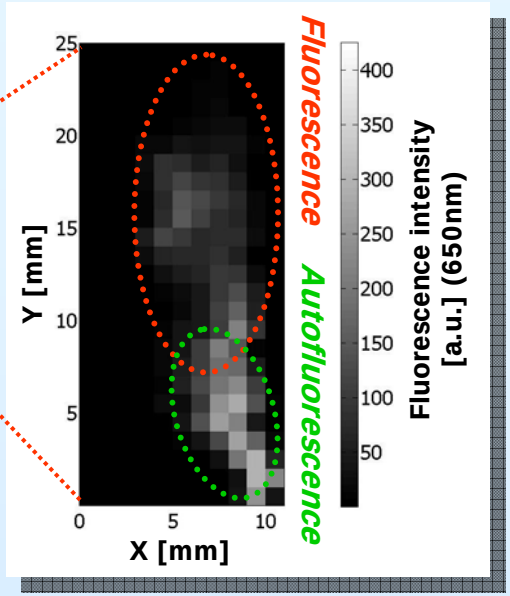
Fluorescence spectra registered from different depths in highly scattering medium (theoretical modeling)

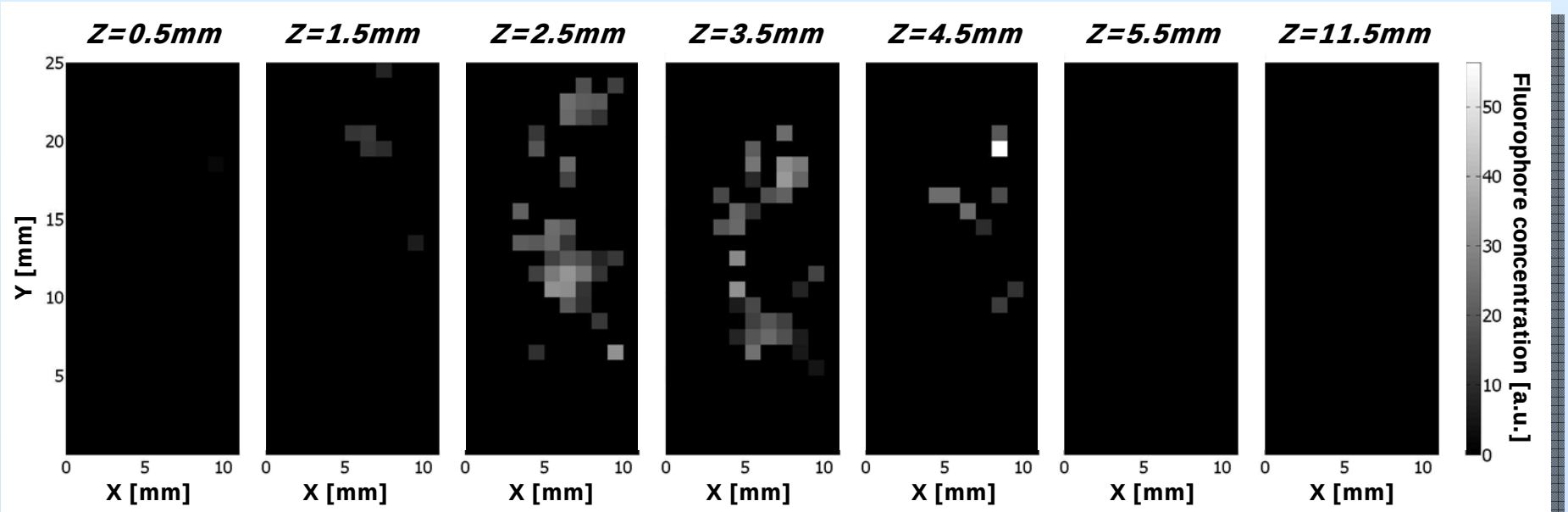




Reconstruction of the fluorophore spatial distribution

Nude mouse with RFP-expressing tumor



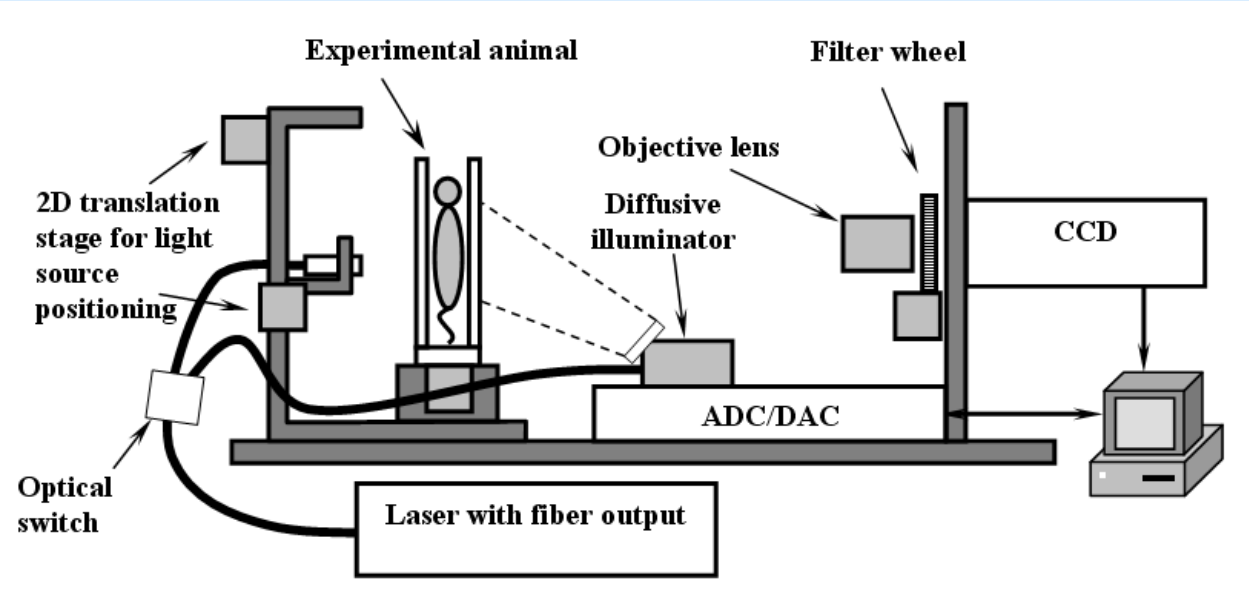


Plane images of fluorescent tumor corresponded to different depths of experimental animal

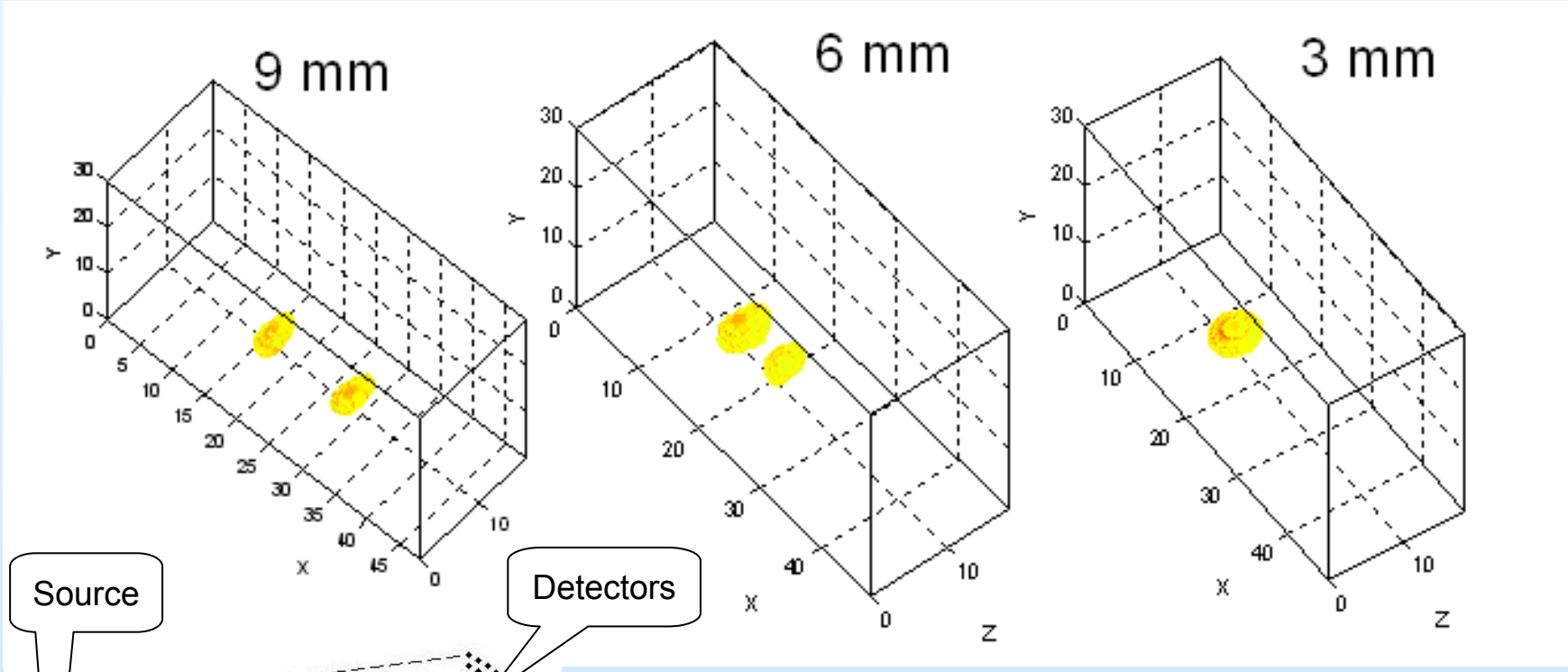
CCD-based FDT experimental setup

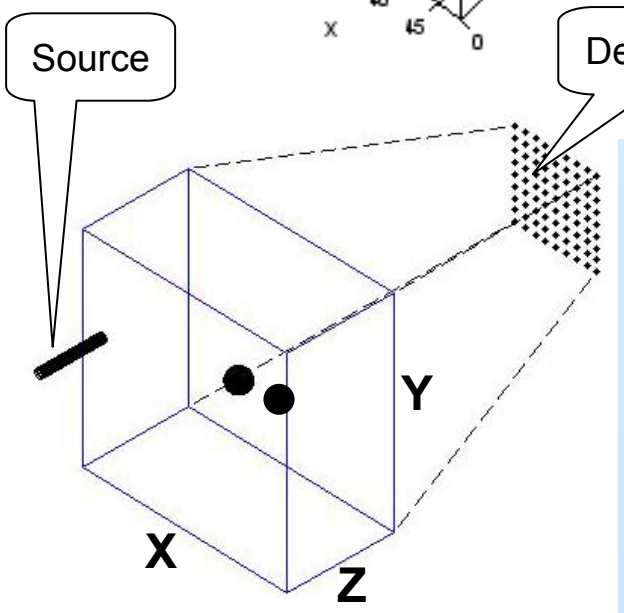
• **Fluorescent diffuse tomography**
3D reconstruction if the fluorophore is well localized *fluorescent proteins*

• **Backreflection for subcutaneous tumors**
2D images, no reconstruction
fluorescent proteins, photosensitizers, quantum dots



Model experiment: reconstruction accuracy





Reconstruction accuracy

- position of the object

$\Delta x, \Delta y = 1$ mm

$\Delta z = 1.5$ mm

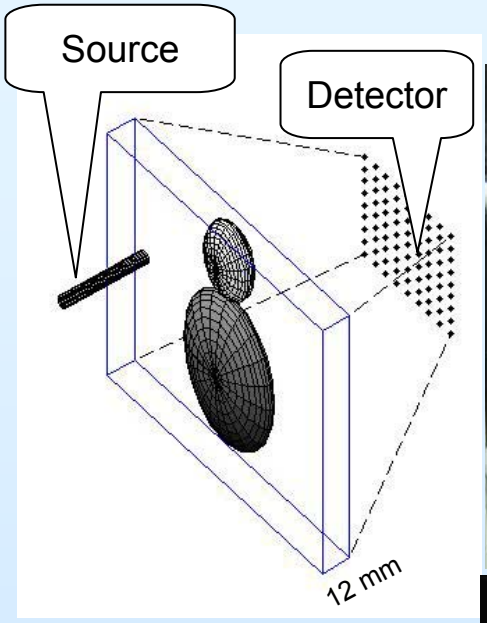
- object shape

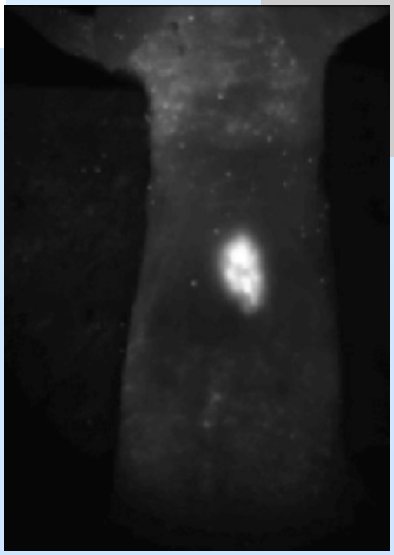
$\Delta R = 0.5$ mm

- separate reconstruction of 2 nearest objects

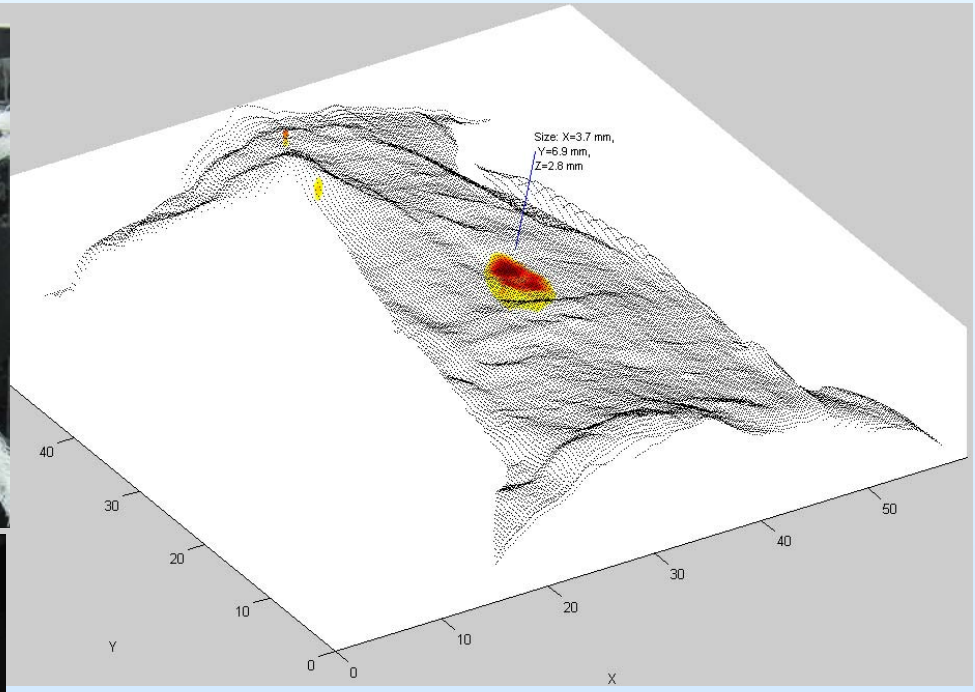
$\Delta x, \Delta y = 3$ mm

In-vivo experiment:
RFP-expressing tumor in NUDE mouse





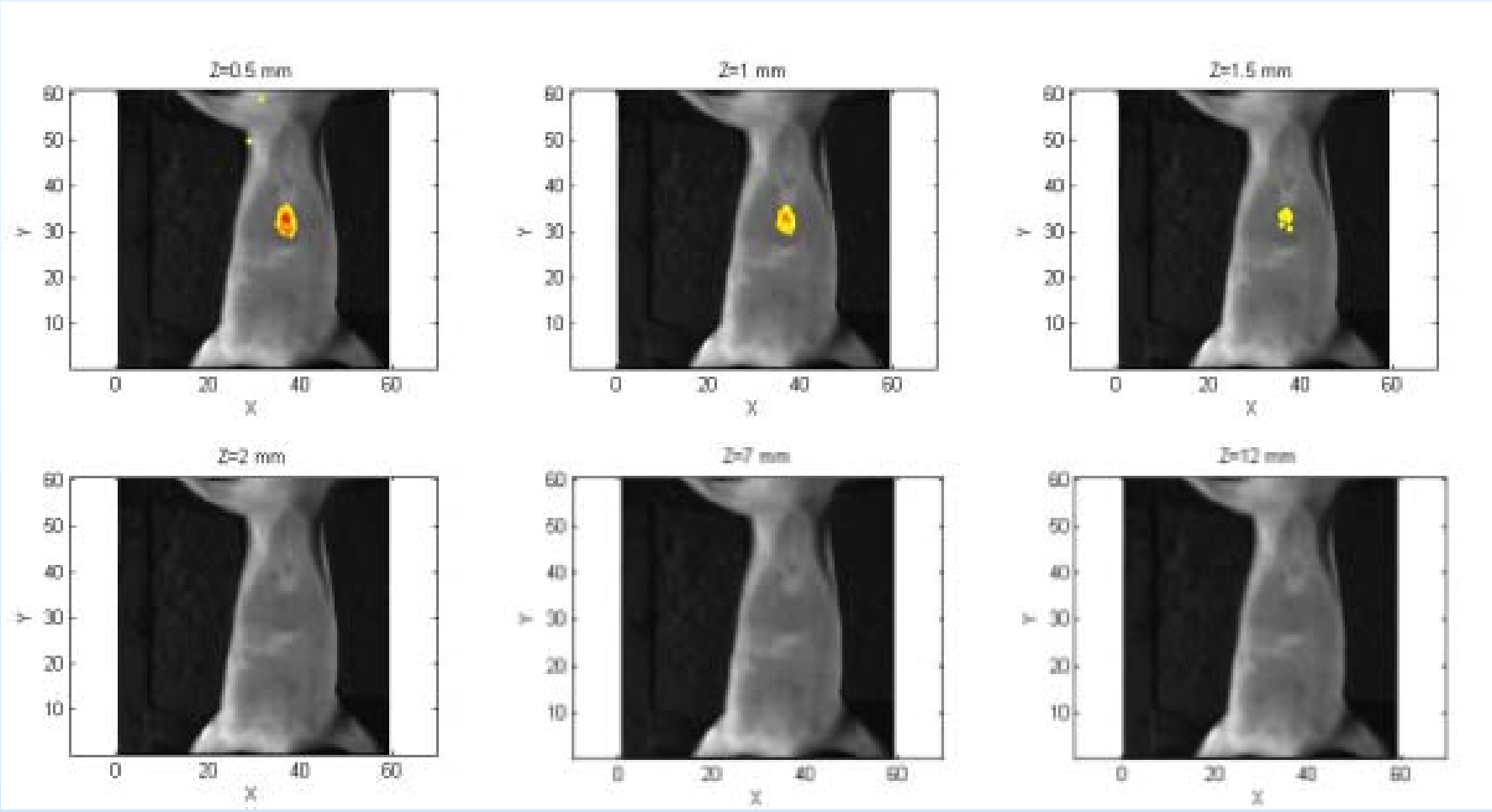
Back Reflection



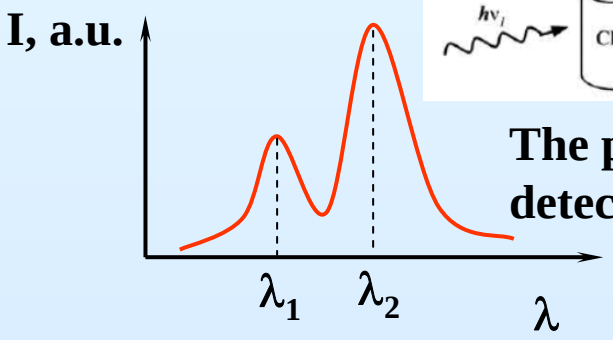
3D reconstruction

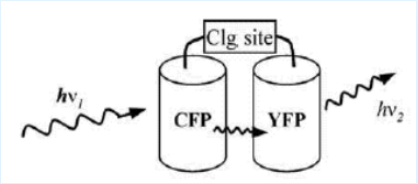
- Inhomogenity medium
- Autofluorescence
- Boundary condition

3D reconstruction of the DsRed2-expressing tumor in-vivo

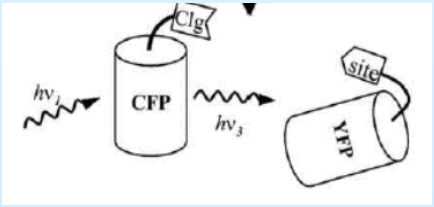


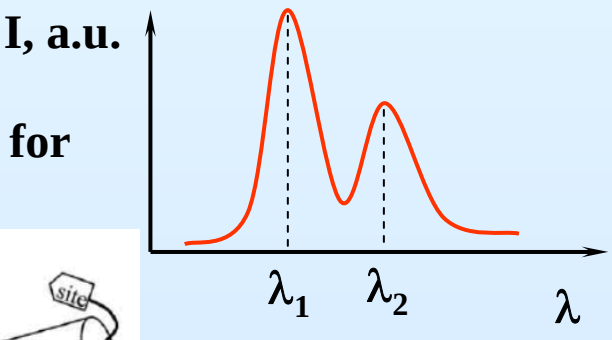
Investigation of molecular processes by whole-body fluorescence imaging
(investigation of mechanism of drug effect)
Based ob spectrum resolution





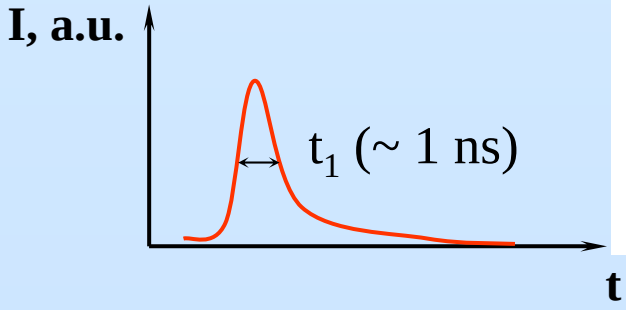
The principal of a FRET sensor for detecting kaspase activation

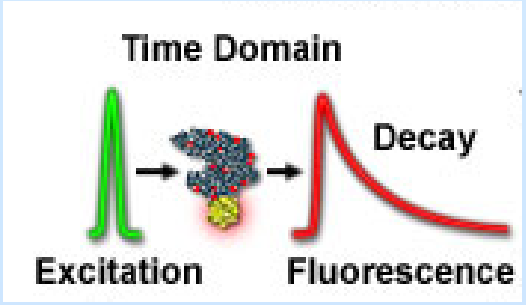


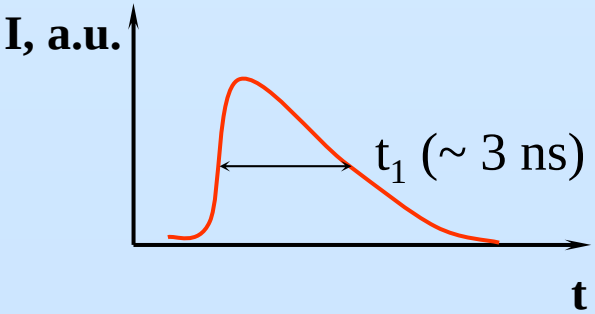


Luo K.Q. *et al*. Biochemical and Biophysical Research Communications 304 (2003) 217–222

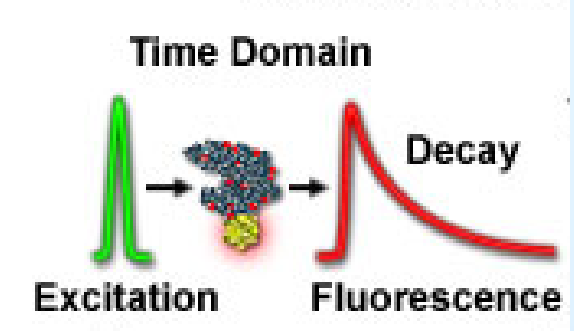
Based on life-time measurement







DFT setup using MCP-based ICCD

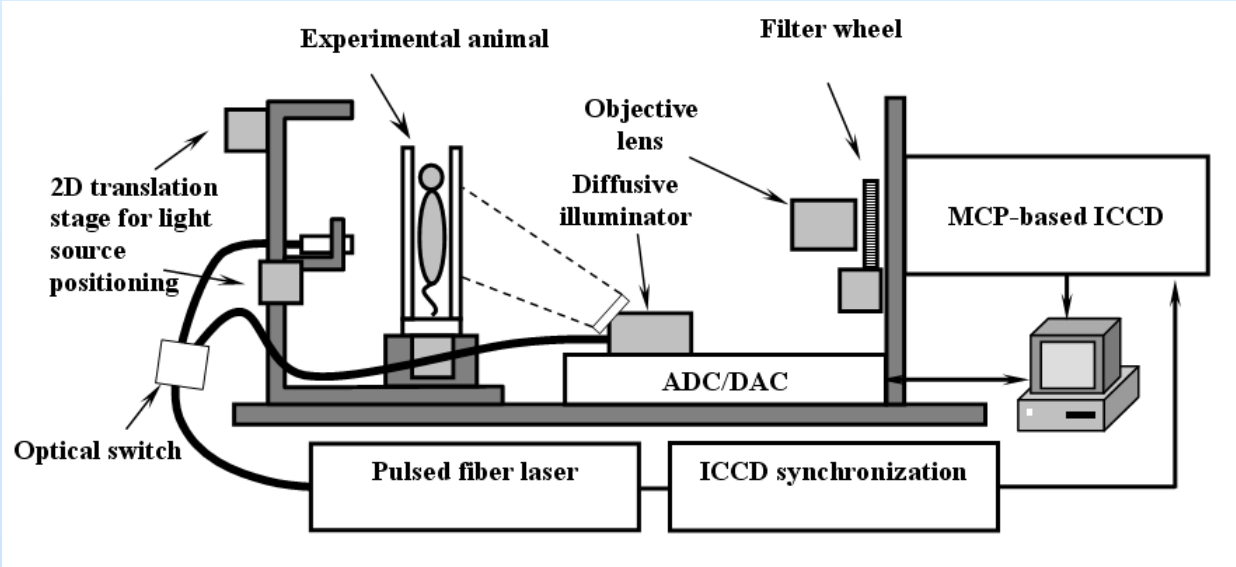


Fiber laser,
wavelength 530 nm
pulse duration <500 ps

Stanfordcomputeroptics ICCD
Minimal gate time 200 ps
Pulse repetion rate 200 kHz

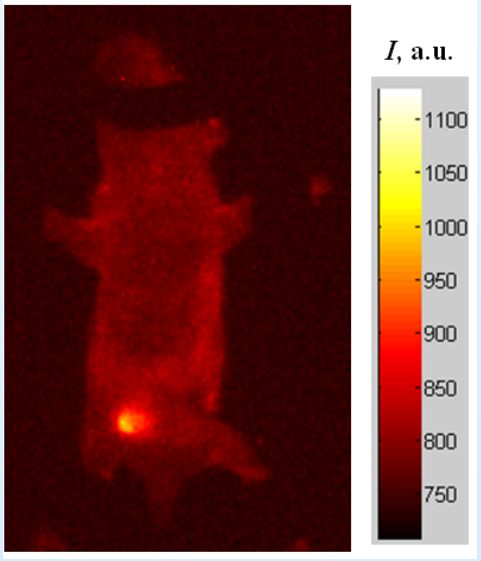
Lifetime measurement provides separation of different fluorophores

Detection of molecular processes, in particular, fluorescence resonance energy transfer (FRET) in experimental animals *in vivo*.

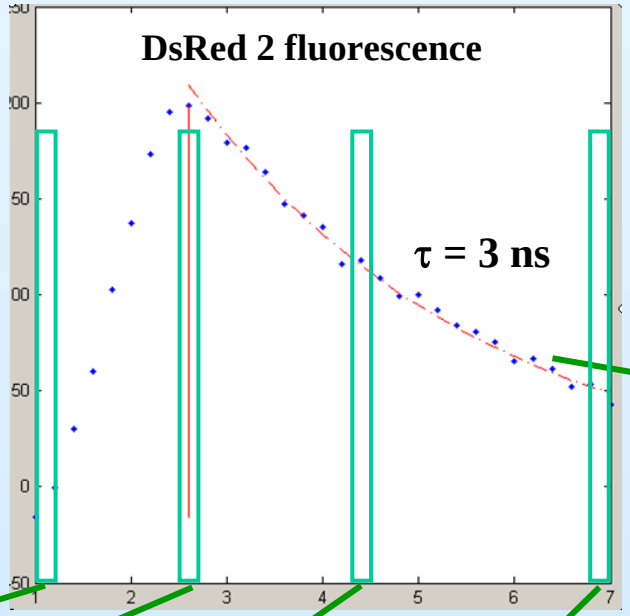


Results of the fluorescence imaging with lifetime resolution
(mouse with the model of subcutaneous tumor expressing DsRed2)

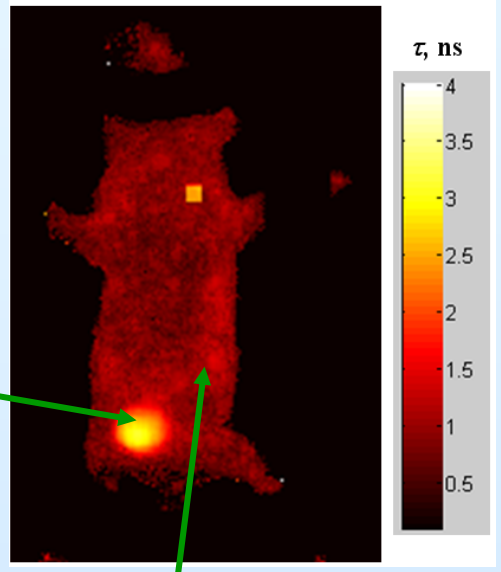
Total fluorescence intensity

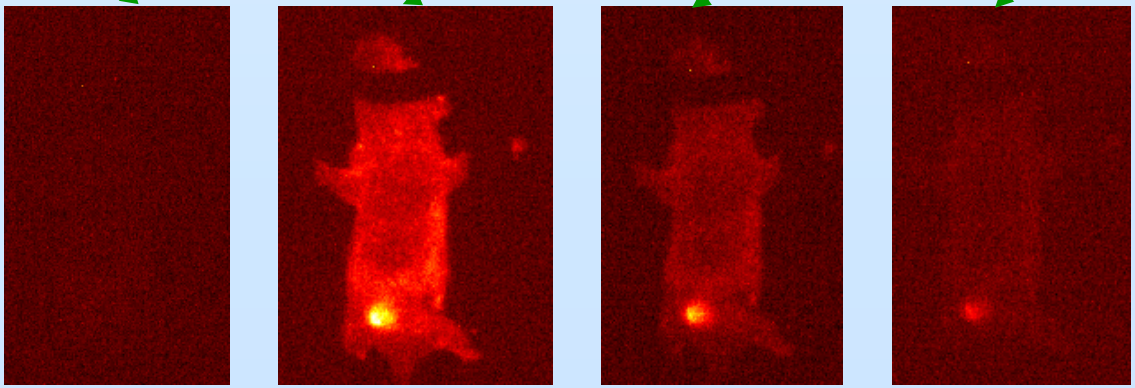


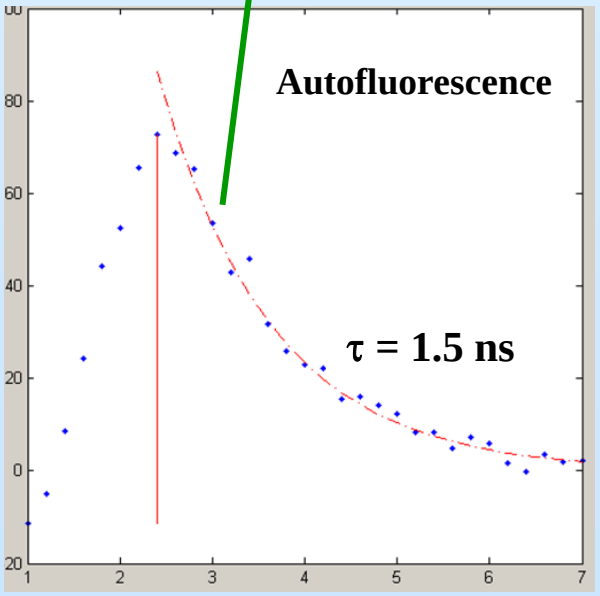
Approximation with exp(-(t-t$_0$)/τ)



Calculated lifetime τ

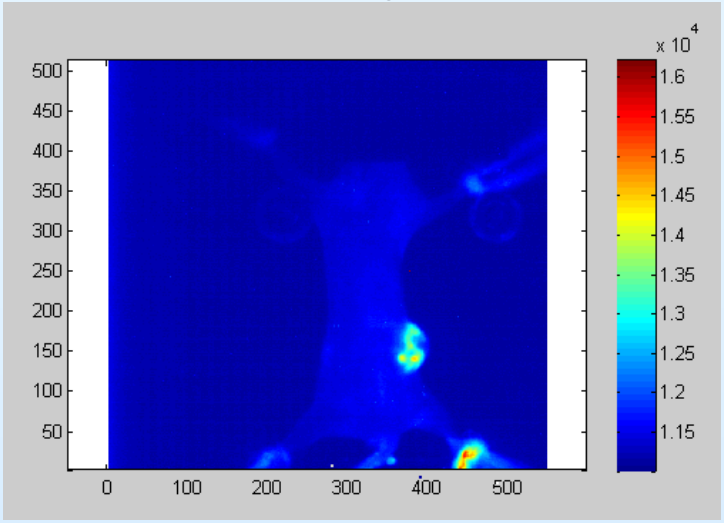


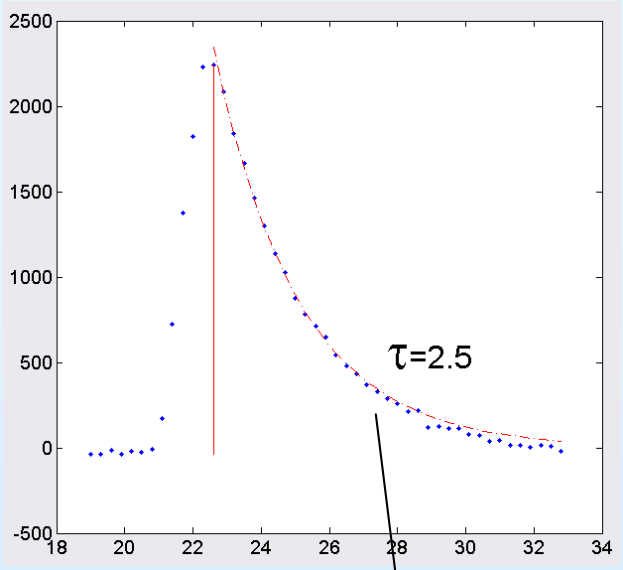




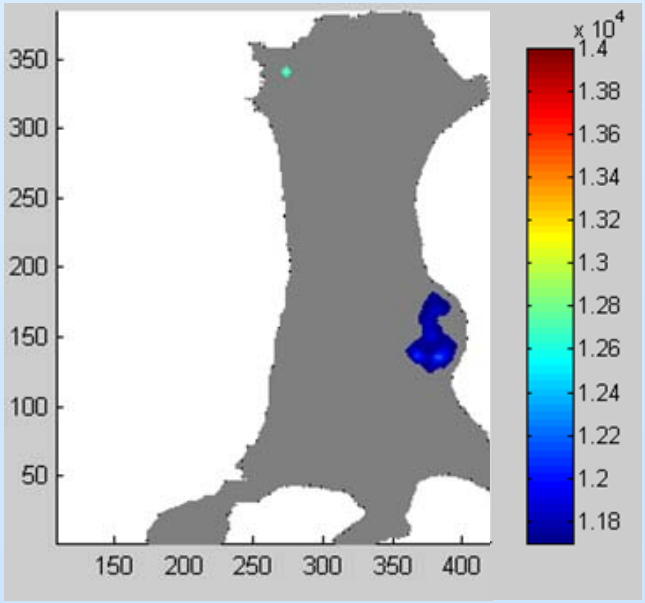
FLIM of the tumor, expressing TurboRFP (in a NUDE mouse)

Fluorescence intensity (in the maximum)

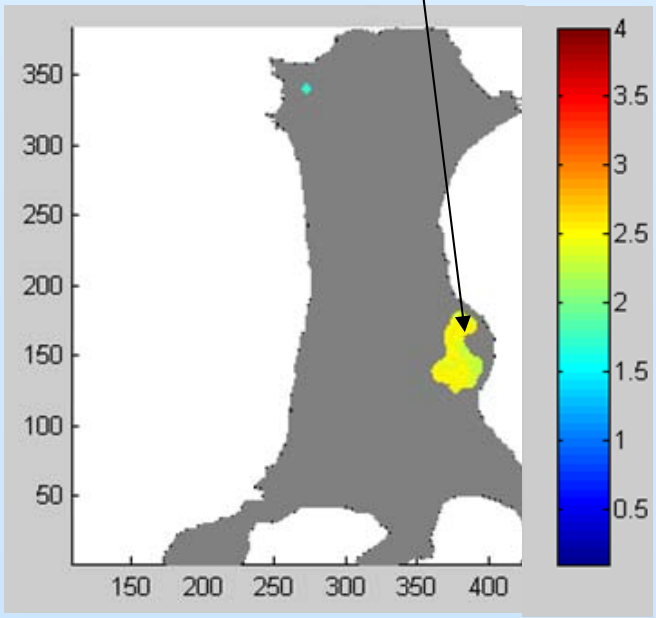




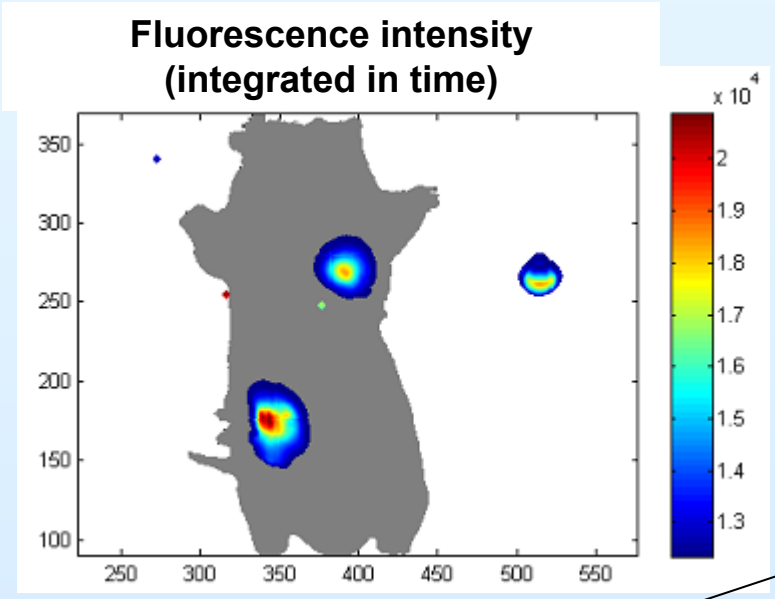
Fluorescence intensity (integrated in time)

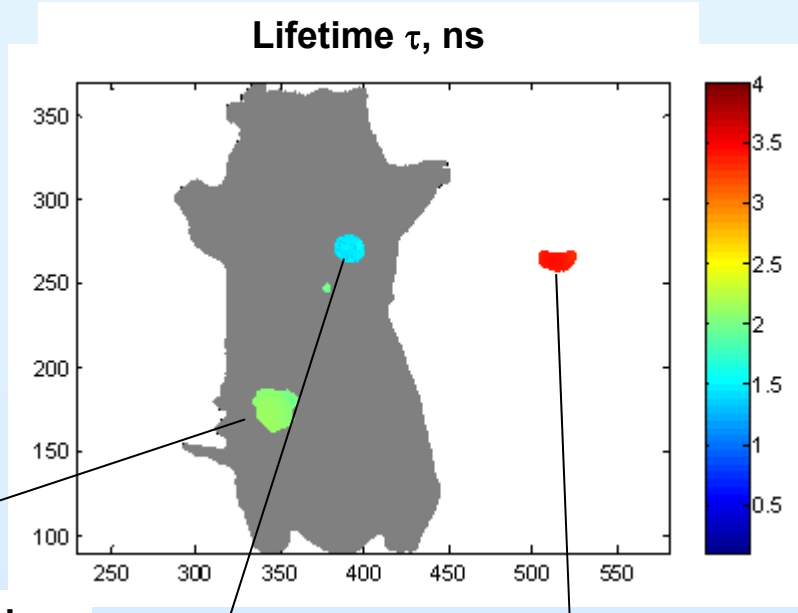


Lifetime τ, ns



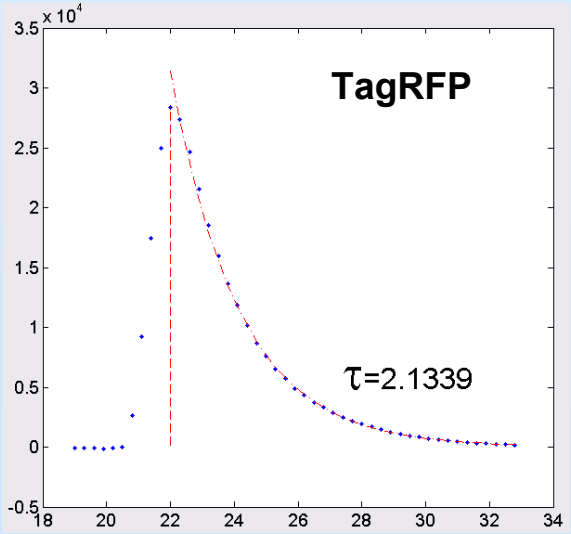
Fluorescence lifetime imaging for FRET (model experiment)

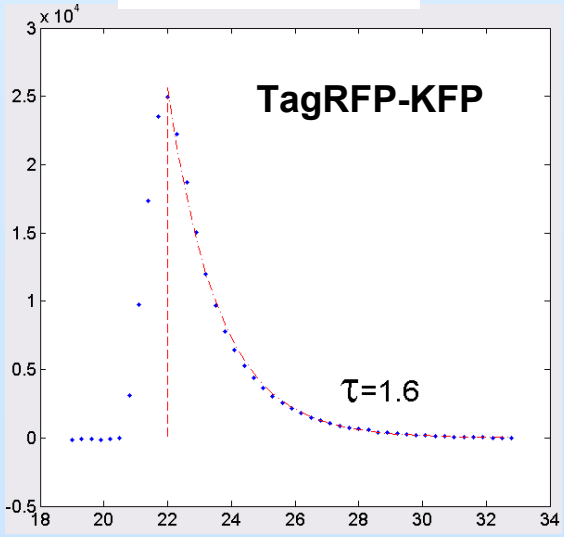


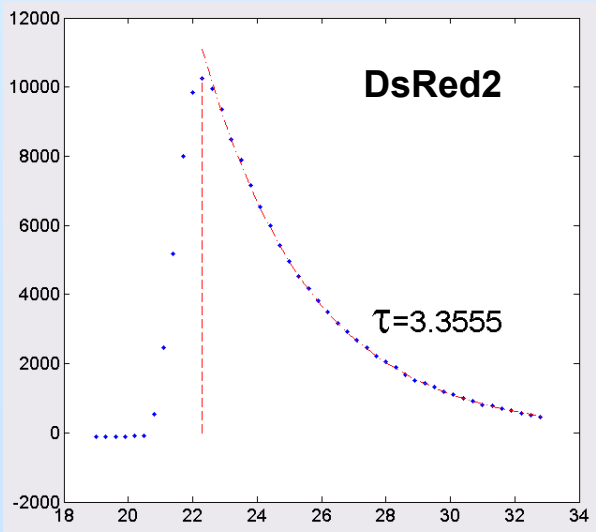


FRET-pair

Testing medium







Conclusion

Fluorescence whole-body imaging allows for:

- **Preclinical study of new photosensitizers for fluorescent diagnostics and photodynamic therapy.**
- **Visualization of the drug delivery process using fluorescent labeles.**
- **Investigation of tumor growth under different conditions using fluorescent proteins (*3D reconstruction is available)*.**
- **(*Potentially*) detection of FRET-processes *in vivo***

IAP, RAS

I.V. Turchin
V.A. Kamensky
A.G. Orlova
N.M. Shakhova
M.Yu. Kirillin
M.S. Kleshnin
I.I. Fiks
V.I. Plehanov
V.A. Vorob'ev
M.B. Prudnikov

Our collaborators

A.P. Savitsky, INBI RAS
I.G. Meerovich, INBI RAS
R.Rusanov, INBI RAS
I.V. Balalaeva, NNSU
A.A. Brilkina, NNSU
E.V. Zagaynova, NNSMA RAMS
M.V. Shirmanova, NNSMA RAMS
S.M. Deyev, IBCH RAS
T.A. Zdobnova , IBCH RAS

Acknowledgements

This work was partly supported by the Russian Foundation for Basic Research (project #10-02-01109,# 07-02-01262) and the Science and Innovations Federal Russian Agency (project # 02.522.11.2002).