Mesoscopic early-photon fluorescence molecular lifetime tomography: first experimental results

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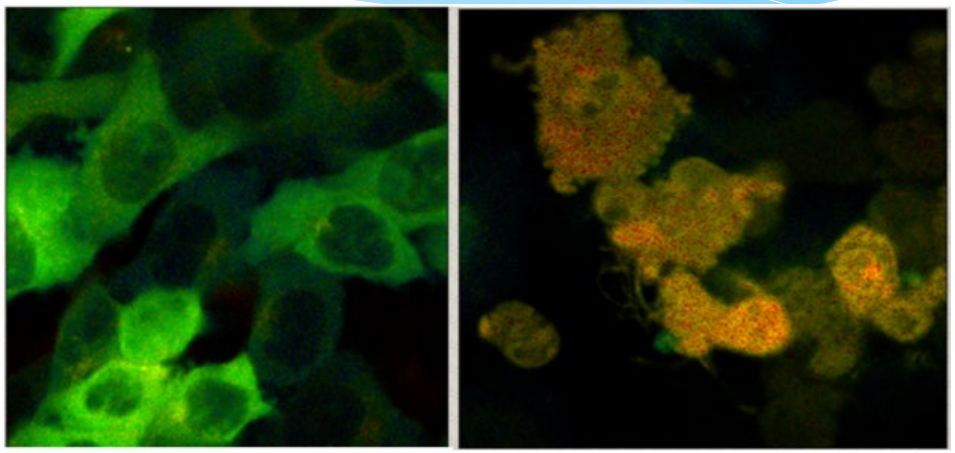
2. Reconstruction model, sensitivity functions, and inverse problem solution
3. Method of fluorescence parameter separation
4. Phantom reconstruction experiment
5. Results, their analysis, and discussion
6. Conclusion and acknowledgement

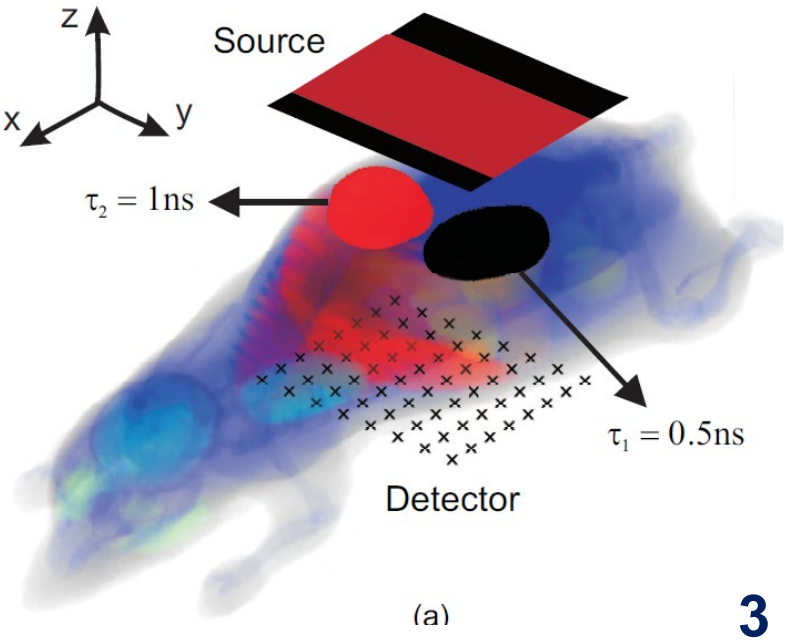
Fluorescence lifetime imaging

- **Fluorescence lifetime microscopy (FLIM): 2D imaging**



Zherdeva V. et al. *J. Biomed. Opt.* 23: 035002 (2018)

- **Fluorescence molecular lifetime tomography (FMLT): 3D imaging**



Chen J. et al. *Biomed. Opt. Express* 2: 871 (2011)

Four ways of reconstructing lifetime

- The first way is to work in the frequency domain. We can identify the function, whose reconstruction for different frequency components will help separate the fluorescence parameters.
  - Godavarty A. *Med. Phys.* 32: 992 (2005)
- The second way is to collect data in time domain and then change to frequency domain or Laplace transform domain to do separation.
  - Gao F. et al. *Opt. Express* 14: 7109 (2006); Nothdurft R.E. et al. *J. Biomed. Opt.* 14: 024004 (2009); Gao F. et al. *Appl. Opt.* 49: 3163 (2010); Gao F. et al. *J. X-Ray Sci. Technol.* 20: 91 (2012)
- The third way is to apply multiplexing that is to reconstruct the fluorophore concentration for different lifetime components and then extract information on the lifetime distribution.
  - Kumar A.T.N. et al. *Opt. Express* 14: 12255 (2006); Raymond S.B. *J. Biomed. Opt.* 15: 046011 (2010); Chen J. et al. *Biomed. Opt. Express* 2: 871 (2011); Hou S.S. et al. *Opt. Lett.* 39: 1165 (2014); Hou S.S. et al. *IEEE Trans. Biomed. Eng.* 66: 2341 (2019)
- The fourth is the "direct" way based on a nonlinear reconstruction model and the solution of nonlinear equations.
  - Cai C. et al. *Opt. Lett.* 40: 4038 (2015); Zhang L. et al. *Chin. Opt. Lett.* 14, 071702 (2016); Cai C. et al. *Biomed. Opt. Express* 7: 1210 (2016); Cai C. et al. *J. Biomed. Opt.* 21: 126012 (2016)

The main idea of our approach

Our approach is based on

- simplification of the expression for the fluorescence source function in the time domain by using its asymptotic approximation [1],
- development of a linear reconstruction model with respect to the simple fluorescence parameter distribution function which includes the fluorophore absorption coefficient and lifetime distributions,
- separation of the fluorescence parameter distributions through derivation and solution of an overdetermined system of linear algebraic equations.

[1] Konovalov A.B. et al. *Int. J. Numer. Meth. Biomed. Eng.* 37: e3408 (2021)

Our reconstruction model

Our reconstruction model is described by the following expression for the time-resolved fluorescence signal

$$\Gamma^f(\mathbf{r}_s,\mathbf{r}_d,t) \propto \int_V f(\mathbf{r},t)\, W_f(\mathbf{r}_s,\mathbf{r}_d,\mathbf{r},t)\, d^3r,$$

$$f(\mathbf{r},t) = \frac{4Dc\gamma\mu_{af}(\mathbf{r})}{\tau(\mathbf{r})v^2(t) + 4Dc}$$ is the fluorescence parameter distribution function (FPDP),

$$W_f(\mathbf{r}_s,\mathbf{r}_d,\mathbf{r},t) = \int_0^t \varphi^e(\mathbf{r}-\mathbf{r}_s,t')\, G^f(\mathbf{r}_d-\mathbf{r},t-t')\, dt'$$ is the sensitivity function,

D, c and γ, μ_{af}, τ are the optical and fluorescence parameters of the object, respectively,

$v(t)$ is the average velocity of photon migration [2],

$\varphi^e(\mathbf{r},t)$ is the density of fluorescence excitation photons,

$G^f(\mathbf{r}-\mathbf{r}',t-t')$ is the fluorescence Green function.

[2] Lyubimov V.V. et al. *Phys. Med. Biol.* 47: 2109 (2002)

Simulation of sensitivity functions [3]

$$W_f(\mathbf{r}_s,\mathbf{r}_d,\mathbf{r}_i,t)=\sum_{n=1}^{N}\sum_{k_n} w_{n,0}\exp\left[-\sum_{i=1}^{p_{k_n}+q_{k_n}}\mu_a(\mathbf{r}_i)l_{n,k_n}(\mathbf{r}_i)\right]\mu_a(\mathbf{r}_i)l_{n,k_n}(\mathbf{r}_i)1(t_{k_n}<t),$$

n and k_n are the indices of the history and the fluorescent photon in history n, respectively,

$w_{n,0}$ is the initial weight of the excitation photon in history n,

$\mu_a(\mathbf{r}_i)=\mu_a^e(\mathbf{r}_i)=\mu_a^f(\mathbf{r}_i)$ is the absorption coefficient in voxel $\mathbf{r}_i$ at excitation and fluorescence wavelengths,

$l_{n,k_n}(\mathbf{r}_i)$ is the length of trajectory section in voxel $\mathbf{r}_i$ for the excitation photon n or fluorescent photon k_n,

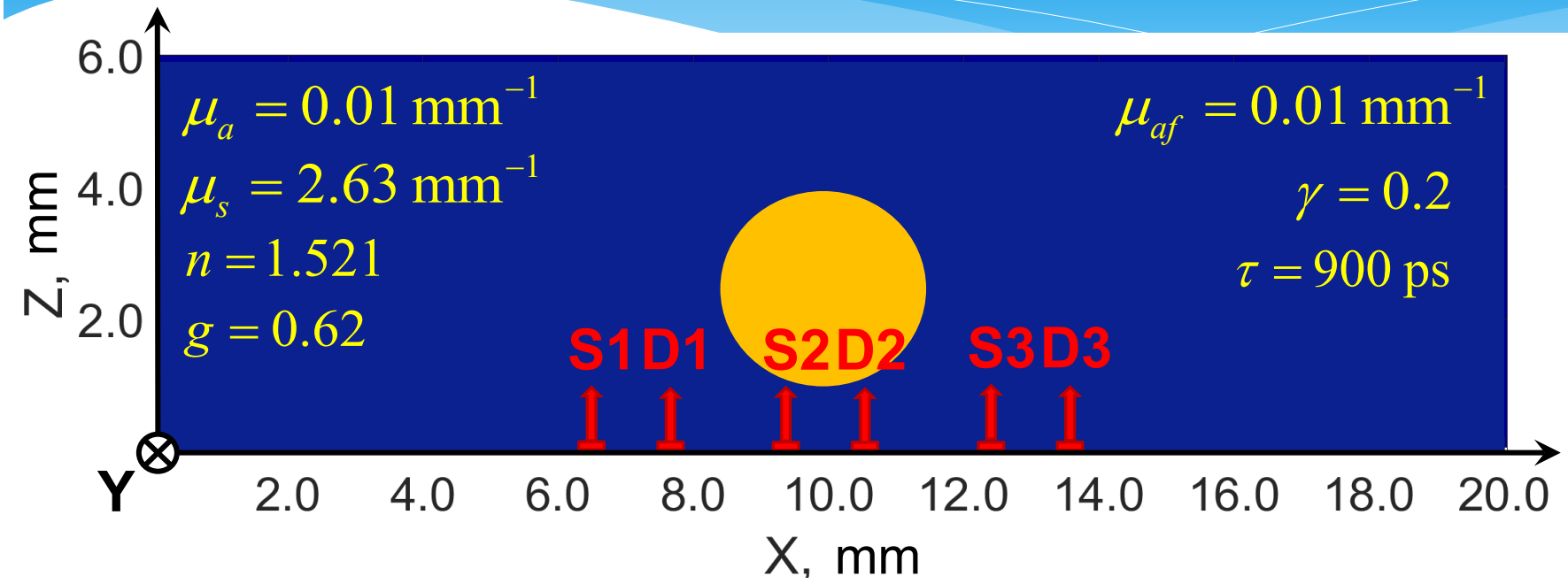
p_{k_n} is the number of voxels the excitation photon of history n crosses when migrates from point $\mathbf{r}_s$ into voxel $\mathbf{r}_i$ and generates fluorescent photon k_n,

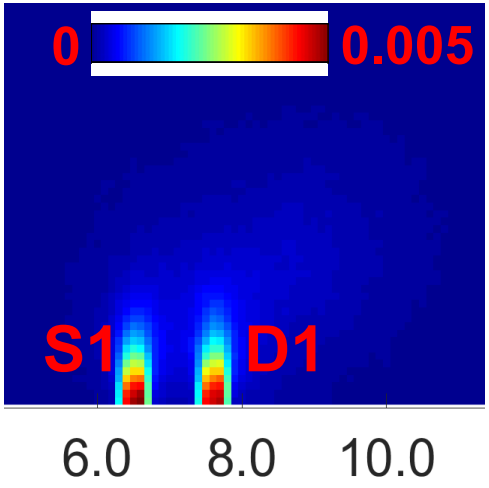
q_{k_n} is the number of voxels fluorescent photon k_n crosses when migrates from voxel $\mathbf{r}_i$ to point $\mathbf{r}_d$,

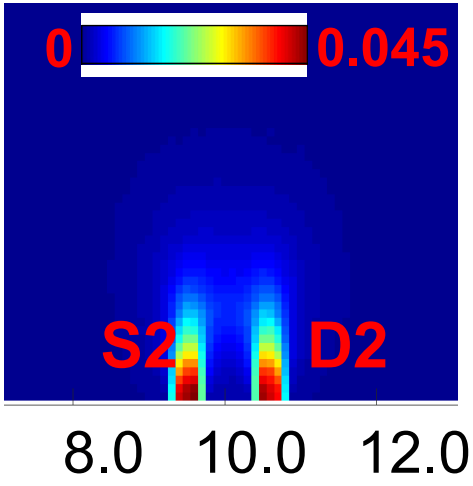
$1(t_{k_n}<t)=1-\Theta(t_{k_n}<t)$, $\Theta(\cdot)$ is the Heaviside function.

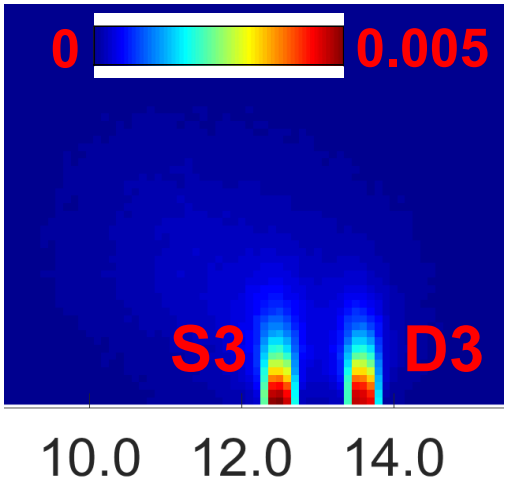
[3] Chen J. et al. *Biomed. Opt. Express* 2: 871 (2011)

Examples of sensitivity functions









Setting up the inverse problem

The inverse problem with respect to the FPDF reduces to the solution of the system of linear algebraic equations (SLAE)

$$\mathbf{W}\mathbf{f} = \mathbf{g},$$

$\mathbf{W}$ is the sensitivity matrix which stores sensitivity functions calculated for all source-receiver pairs involved in reconstruction,

$\mathbf{f}$ is a vector that defines the sought FPDF in the voxels $\{\mathbf{r}_i\}$ of the 3D uniform grid,

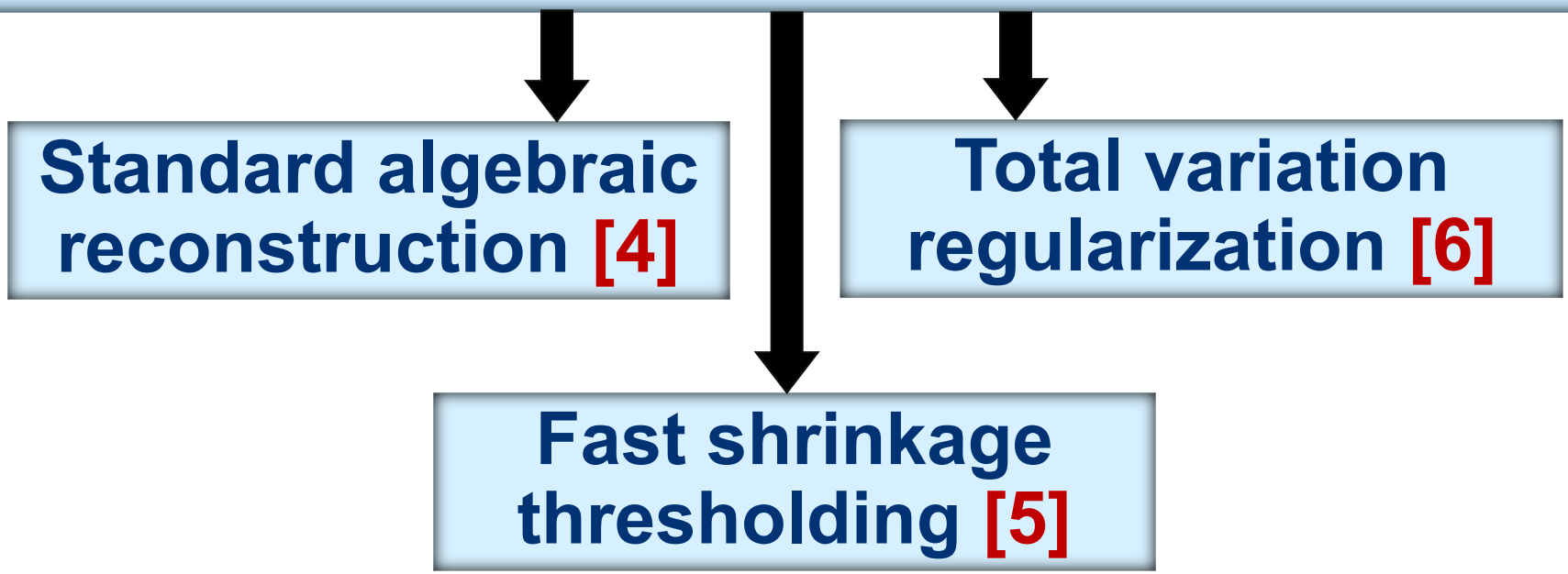
$\mathbf{g}$ is a vector that represents measurement data extracted from the measured fluorescence signals.

As the SLAE is underdetermined, we need regularization to solve it correctly!

Our reconstruction algorithm

The hybrid **compressed-sensing-like** algorithm

ART-FIST-TV



[4] Gordon R. et al. *J. Theor. Biol.* 29: 471 (1970)

[5] Beck A. & TeboulleM. *SIAM J. Imaging Sci.* 2: 183 (2009)

[6] Yu H. & Wang G. *Phys. Med. Biol.* 54: 2791 (2009)

Step-by-step description of the algorithm

- ***Step 1***: Initialize initial approximation $\mathbf{f}^{(0)}$, ART-FIST cycle parameters λ, α, $S_{art-fist}$, and TV cycle parameters β, S_{tv}.
- ***Step 2***: Set $\mathbf{y}^{(1)} = \mathbf{f}^{(0)}$, $t^{(1)} = 1$.
- ***Step 3***: Do $S_{art-fist}$ iterations of the ART-FIST cycle by the formulas:

$$\mathbf{f}^{(s)} = ST_{\alpha,\lambda}\left[ART_{\lambda}(\mathbf{y}^{(s)}) \right], \quad t^{(s+1)} = \frac{1+\sqrt{1+4[t^{(s)}]^2}}{2},$$

$$\mathbf{y}^{(s+1)} = \mathbf{f}^{(s)} + \frac{t^{(s)}-1}{t^{(s+1)}}\left[\mathbf{f}^{(s)} - \mathbf{f}^{(s-1)} \right].$$

- ***Step 4***: Set $\mathbf{f}^{(0)} = \mathbf{y}^{(S_{art-fist})}$.
- ***Step 5***: Do S_{tv} iterations of the TV cycle in by the formula

$$f_i^{(s+1)} = f_i^{(s)} - \beta \frac{\partial \| \mathbf{f}^{(s)} \|_{TV}}{\partial f_i}$$, where $\| \cdot \|_{TV}$ is the TV norm.

- ***Step 6***: Check the stop criterion. If not satisfied, set $\mathbf{f}^{(0)} = \mathbf{f}^{(S_{tv})}$, $\mathbf{y}^{(1)} = \mathbf{f}^{(S_{tv})}$, $t^{(1)} = 1$ and go to ***Step 3***.
- ***Step 7***: End calculations if the stop criterion is satisfied.

Parameters of the algorithm

Parameter	Description
λ	control parameter of ART iterations
α	regularization parameter
$S_{art-fist}$	number of iterations for the ART-FIST cycle
β	step of the gradient descent iterations
S_{tv}	number of iterations for the TV cycle
$ART_{\lambda}(\cdot)$	operator that performs the cycle of standard ART iterations [4]
$ST_{\alpha,\lambda}(\cdot)$	operator that performs image shrinkage in accord with the algorithm from [5]

The separation method

The separation problem is reduced to the solution of the overdetermined system of equations

$$\frac{4Dc\gamma\mu_{af}(\mathbf{r})}{\tau(\mathbf{r})v_m^2 + 4Dc} = f_m(\mathbf{r}), \quad m = 1, 2, 3;$$

that can be written in the form of SLAE

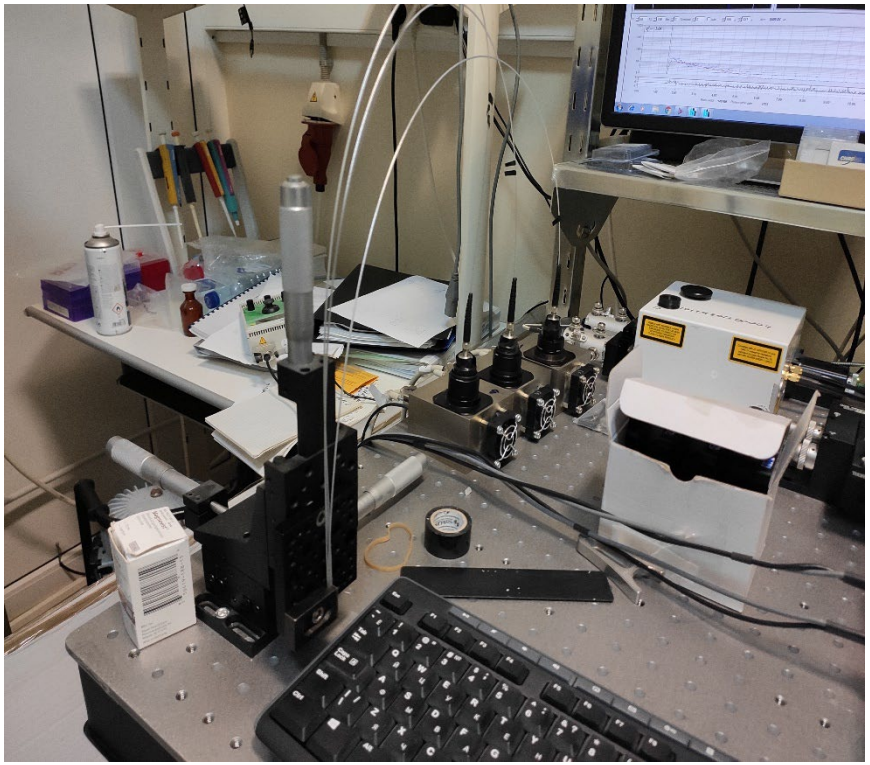
$$\mathbf{Ax} = \mathbf{b}, \quad \mathbf{A} = \begin{pmatrix} 4Dc\gamma & -f_1(\mathbf{r})v_1^2 \\ 4Dc\gamma & -f_2(\mathbf{r})v_2^2 \\ 4Dc\gamma & -f_2(\mathbf{r})v_2^2 \end{pmatrix}, \quad \mathbf{x} = \begin{pmatrix} \mu_{af}(\mathbf{r}) \\ \tau(\mathbf{r}) \end{pmatrix}, \quad \mathbf{b} = \begin{pmatrix} 4Dcf_1(\mathbf{r}) \\ 4Dcf_2(\mathbf{r}) \\ 4Dcf_3(\mathbf{r}) \end{pmatrix}.$$

We seek its solution in terms of least squares with the use of the well-known iterative QR-factorization least square algorithm [7].

[7] Paige C.C. & Sanders M.A. *ACM Trans. Math. Softw.* 8: 43 (1982)

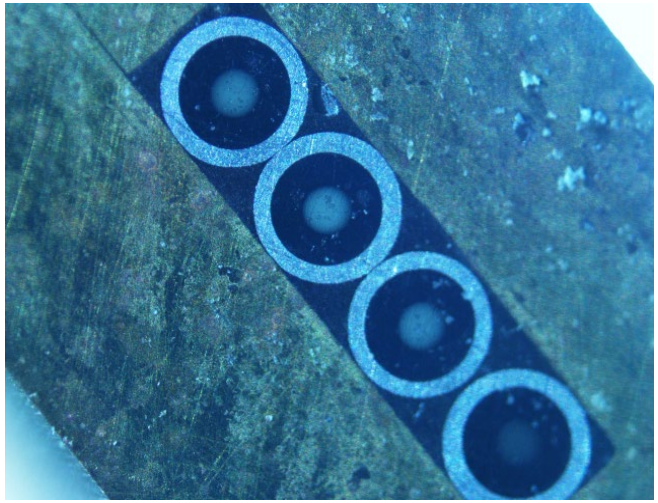
Experimental setup

The experiment for scanning a phantom with fluorophore was done at the Bach Institute of Biochemistry, Research Center of Biotechnology of the Russian Academy of Sciences (Moscow, Russia)

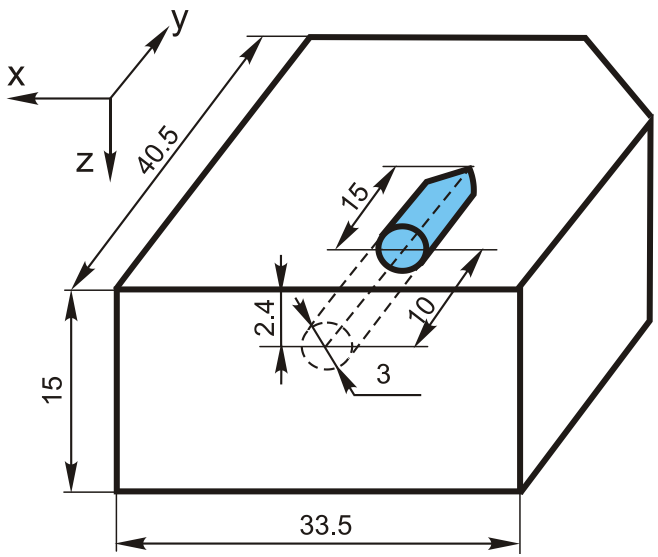


- supercontinuum SC-450-6 laser (Fianium UK Ltd.),
- TCSPC system (Becker & Hickl GmbH): the PMC-100 detector and the SPC-150 module,
- three-channel fiber probe with four fibers,
- tissue-like phantom with fluorophore

Probe and phantom

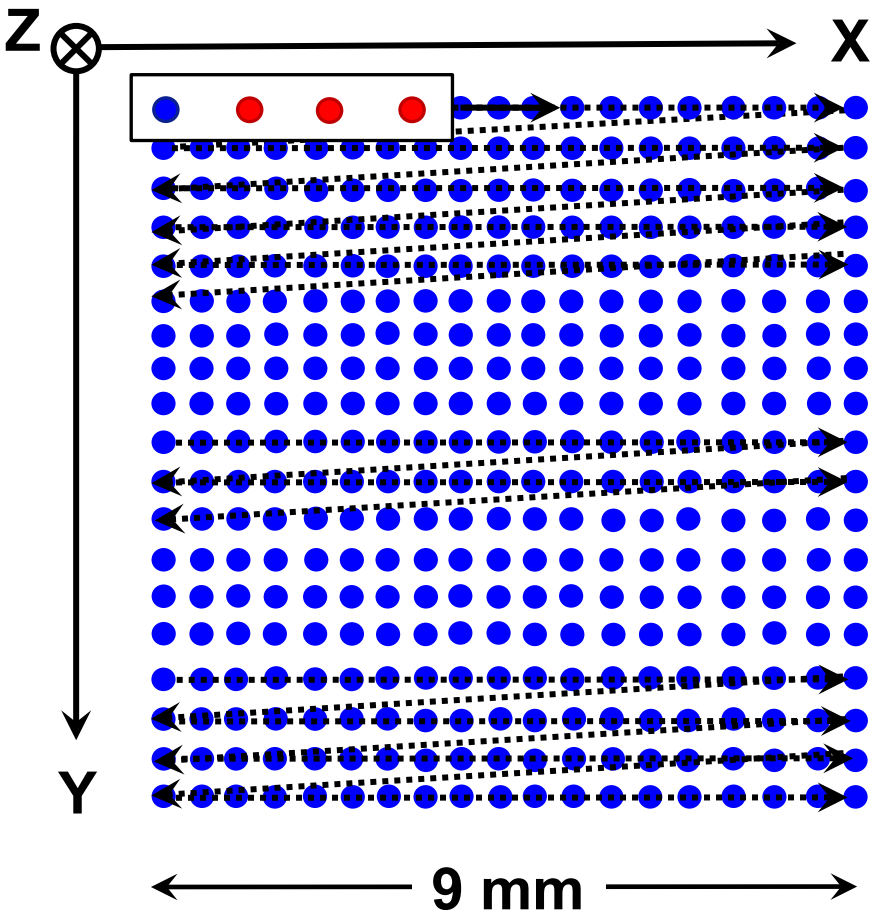


- The three-channel fiber probe has four fibers fixed linearly at an axle spacing of 1.1 mm. Each of them has a 400-μm-diam core and numerical aperture 0.2. The first fiber was used to inject exciting light and the other three for fluorescence registration.



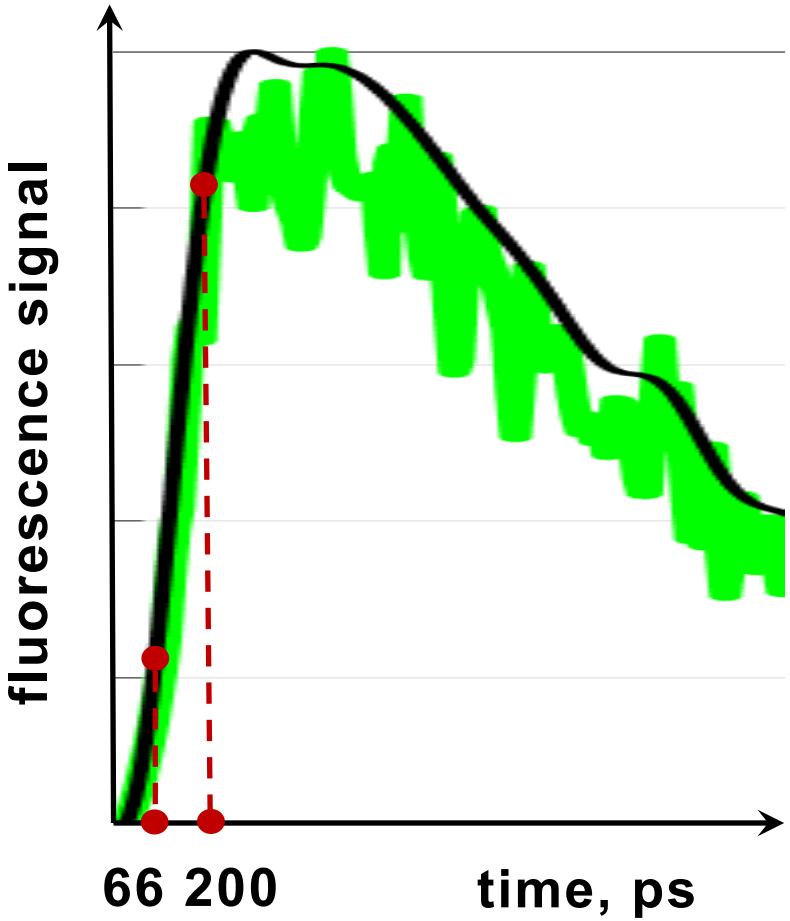
- The phantom is a parallelepiped of the tissue-like material INO Biomimic. Along the parallelepiped there is a cylindrical hole for the fluorescent solution. Fluorophore is Cy5 with concentration $5 \cdot 10^{-7}$ mol/L.

Scanning geometry



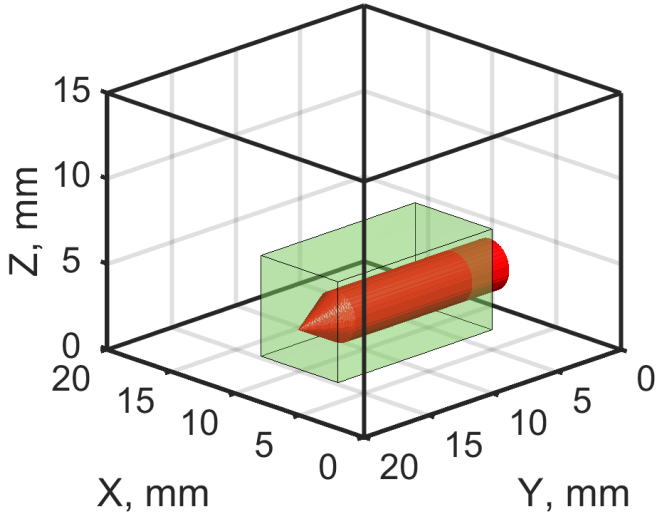
- The fiber probe is moved on the phantom surface at a step of 0.5 mm with a micrometric mover. At the beginning scanning is done in the X direction. Then, after scanning in 19 positions, the probe is moved to the beginning of the next row and runs again along the X direction, and so on in the zigzag pattern.

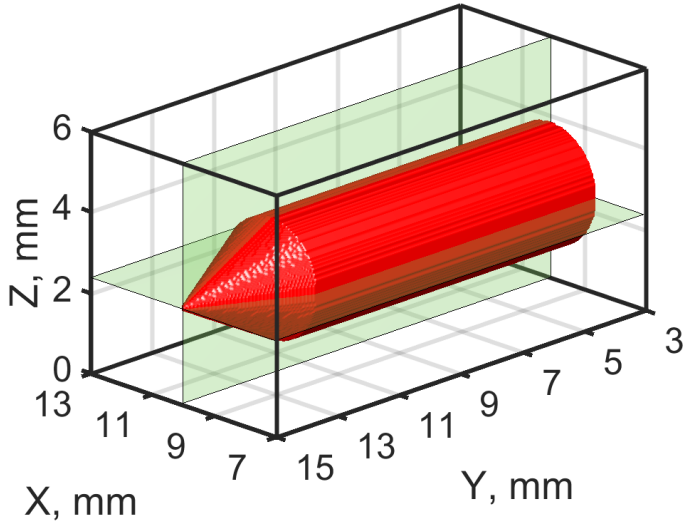
Strategy for generating the array g and matrix W

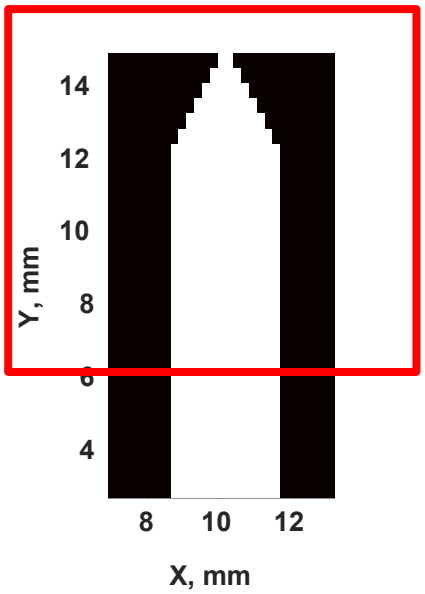


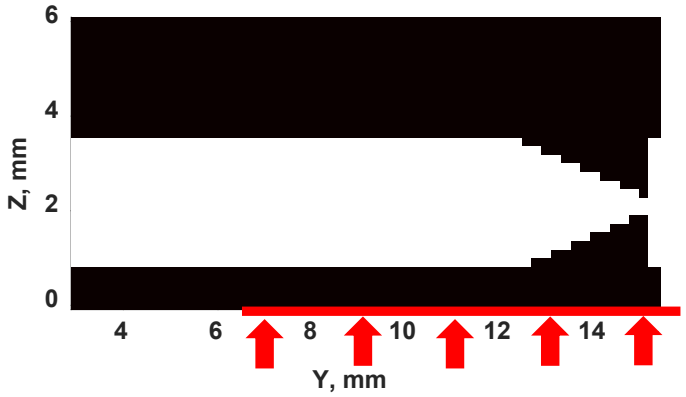
- We use not one but **two or three time gates** for each fluorescence signal in the range **from 66 to 200 ps**.
- This makes it possible to reduce the underdeter-mination of the system. The increased sizes of our sensitivity matrix are **722x6000000** and **1083x6000000**.

Style for reconstructed image presentation



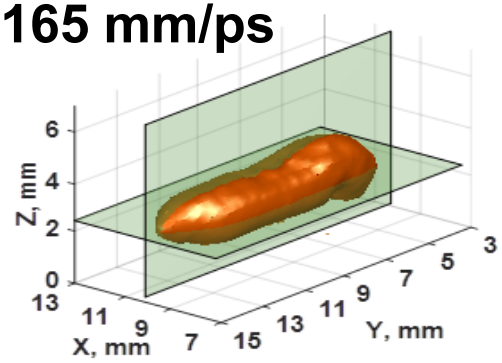


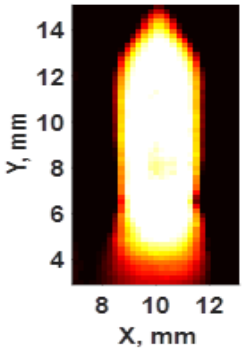


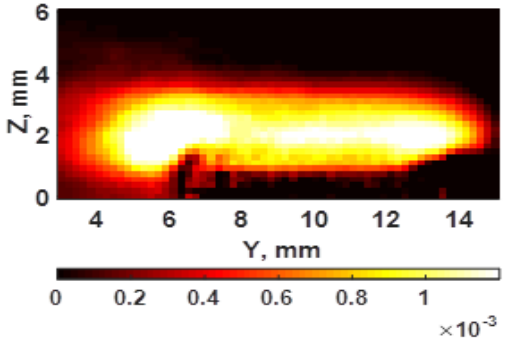


FPDF reconstruction results

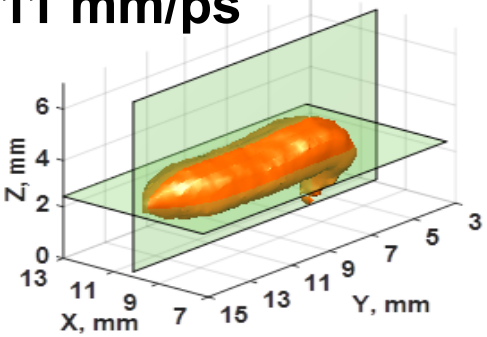
v_1=0.0165 mm/ps

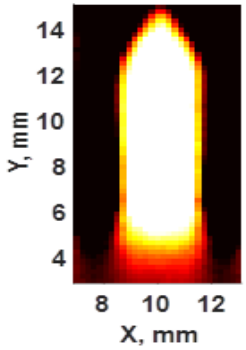


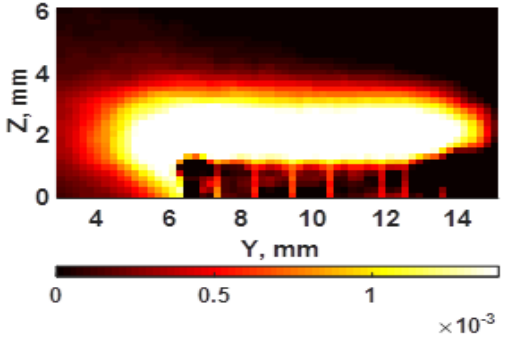




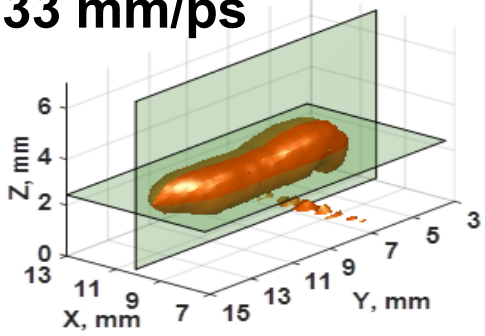
v_2=0.011 mm/ps

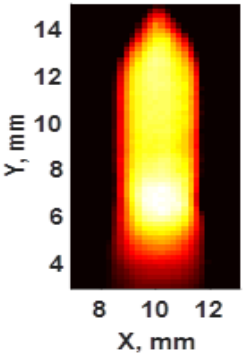


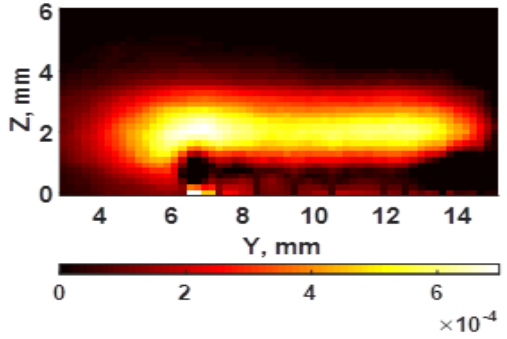




v_3=0.033 mm/ps

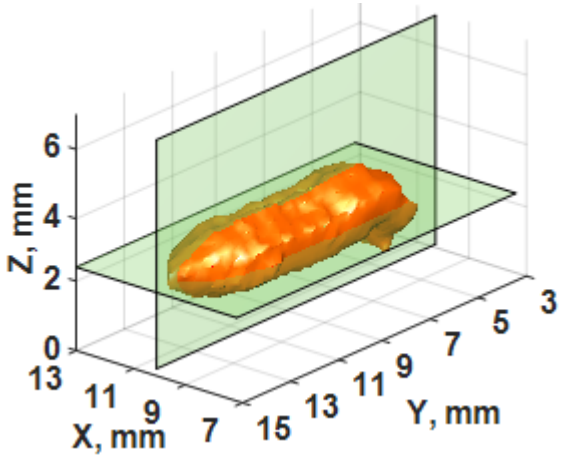


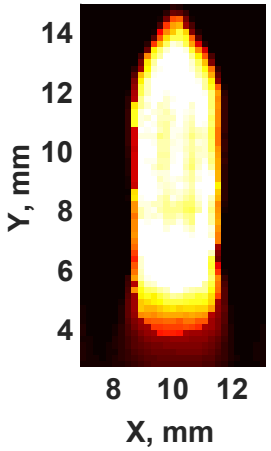


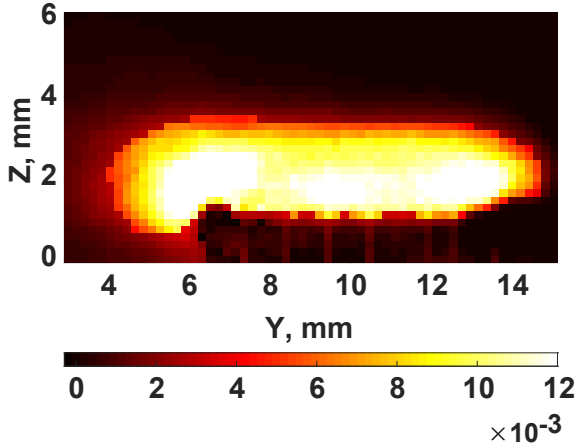


Fluorescence parameter separation result

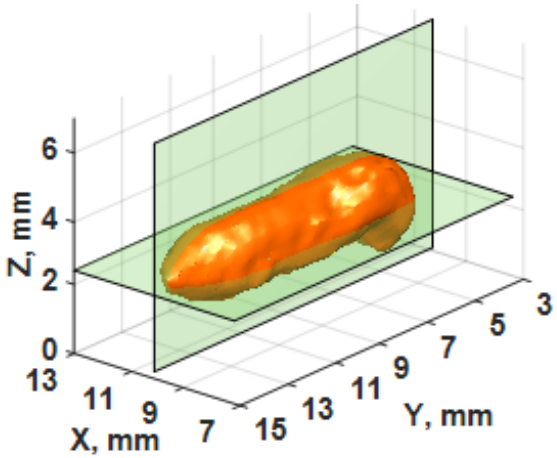
fluorophore absorption coefficient distribution

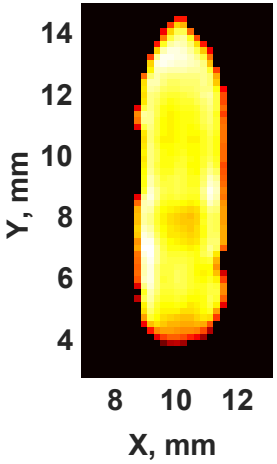


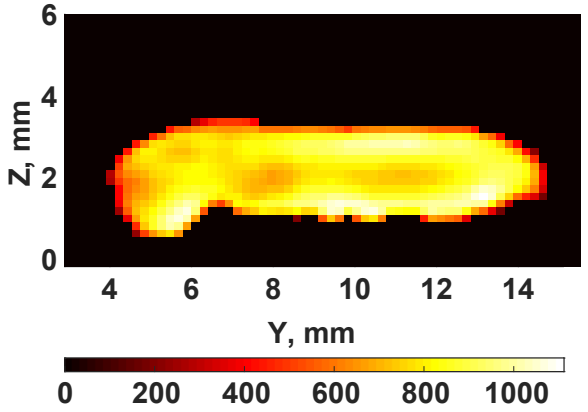




fluorescence lifetime distribution

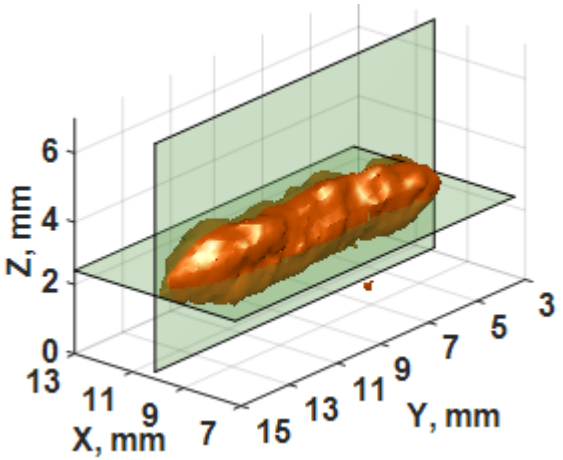


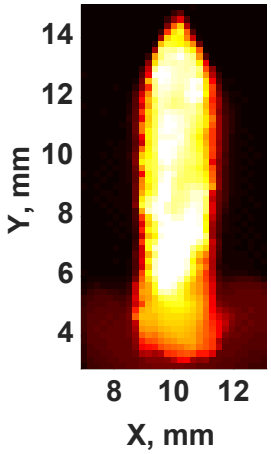


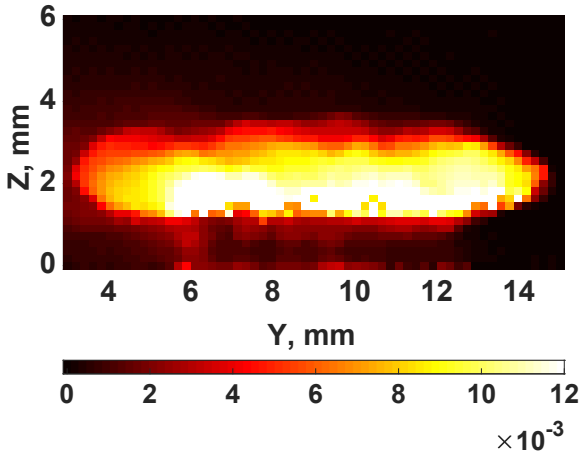


Result for symmetric sensitivity functions

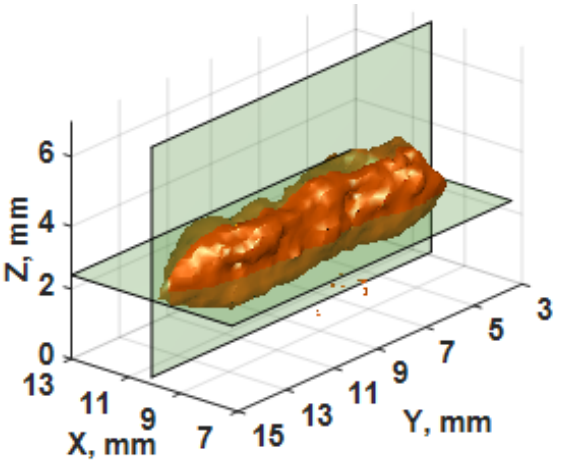
fluorophore absorption coefficient distribution

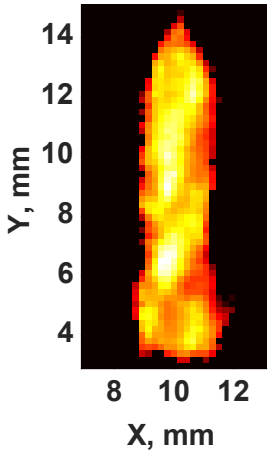


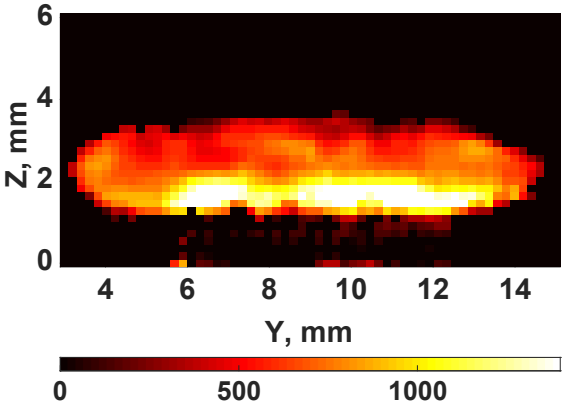




fluorescence lifetime distribution







Some conclusions and question

- Our mesoscopic early-photon FMLT method will be effective if we have the opportunity to use a priori information about the object parameters.
- If we have no information about the object, then we risk getting an inadequate reconstruction of the lifetime distribution.

Can we get the necessary information a posteriori, for example, through multi-step reconstruction? Why not?

Example of two-step reconstruction

- **Step 1**: Reconstruct the fluorophore absorption coefficient using some initial approximation of the object parameters. Update some of these parameters and simulate the spatially dependent sensitivity functions.
- **Step 2**: Apply the proposed method of fluorescence parameter separation to the updated data.

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Thank you for your attention!