Russian Federal Nuclear Center –
Zababakhin Institute of Applied Physics

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“ROSATOM” STATE CORPORATION

Separate reconstruction of fluorophore absorption and fluorescence lifetime using early arriving photons

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Why lifetime FMT?

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Among all fluorescence parameters, it is neither the fluorophore absorption coefficient, nor the quantum yield that is most sensitive to the molecular surrounding of fluorescent sensors. The most sensitive is fluorescence lifetime. It is this parameter that allows us to get information on the space and time characteristics of processes that occur in the cells and molecules of animal tissues.

Time-domain FMT problem formulation

$$\begin{cases} \dfrac{1}{c^{e,f}}\dfrac{\partial \varphi^{e,f}(\mathbf{r},t)}{\partial t} - \nabla\cdot\left[D^{e,f}(\mathbf{r})\nabla\varphi^{e,f}(\mathbf{r},t)\right] + \left[\mu_a^{e,f}(\mathbf{r}) + \mu_{af}(\mathbf{r})\right]\varphi^{e,f}(\mathbf{r},t) \\ \quad = S^{e,f}(\mathbf{r},t) \\ S^e(\mathbf{r},t) = I_0\delta(\mathbf{r}-\mathbf{r}_s)\delta(t-t_s) \\ S^f(\mathbf{r},t) = \dfrac{\gamma(\mathbf{r})\mu_{af}(\mathbf{r})}{\tau(\mathbf{r})}\displaystyle\int_{t_s}^{t}\varphi^e(\mathbf{r},t')\exp\dfrac{t'-t}{\tau(\mathbf{r})}dt' \\ \varphi^{e,f}(\mathbf{r}_d,t) + 2AD^{e,f}(\mathbf{r}_d)\dfrac{\partial\varphi^{e,f}(\mathbf{r}_d,t)}{\partial q} = 0 \end{cases}$$

C. Darne et al. *Phys. Med. Biol.* 88: R1 (2014)

Ways to visualize the fluorescence lifetime

- The **first way** is to work in 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 to 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 fluorescence 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)

Our simplifying assumptions

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- We use the asymptotic approximation for the fluorescence source function
- The contribution of fluorophore absorption to the solution is negligible
- Fluorescence quantum yield is constant
- The scattering medium and fluorescent inclusions have identical optical parameters

$$c^e \cong c^f \cong c \qquad \mu_a^e \cong \mu_a^f \cong \mu_a \qquad D^e \cong D^f \cong D$$

- We change the Robin boundary condition by the Dirichlet one

A.B. Konovalov et al. *7th Int. Symp. "Topical Problems of Biophotonics"* (2019)

The simplified system

$$\begin{cases} \frac{1}{c}\frac{\partial \varphi^{e,f}(\mathbf{r},t)}{\partial t} - D\Delta\varphi^{e,f}(\mathbf{r},t) + \mu_a \varphi^{e,f}(\mathbf{r},t) = S^{e,f}(\mathbf{r},t) \\ S^e(\mathbf{r},t) = I_0 \delta(\mathbf{r}-\mathbf{r}_s)\delta(t-t_s) \\ S^f(\mathbf{r},t) = \frac{\gamma\mu_{af}(\mathbf{r})\cdot 4Dct^2}{\tau(\mathbf{r})|\mathbf{r}|^2 + 4Dct^2}\varphi^e(\mathbf{r},t) \\ \varphi^{e,f}(\mathbf{r}_d,t) = 0 \end{cases}$$

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Solution of the simplified system

$$g(\mathbf{r}_s, t_s, \mathbf{r}_d, t_d) = \frac{\Gamma^f(\mathbf{r}_s, t_s, \mathbf{r}_d, t)\big|_{t=t_d}}{\Gamma^e(\mathbf{r}_s, t_s, \mathbf{r}_d, t)\big|_{t=t_d}}$$

is the time-resolved normalized datum

$$\Gamma^{e,f}(\mathbf{r}_d, t) = -c^{e,f} D^{e,f}(\mathbf{r}_d) \frac{\partial \varphi^{e,f}(\mathbf{r}_d, t)}{\partial q}$$

are the temporal diffusion responses

$$g(\mathbf{r}_s, t_s, \mathbf{r}_d, t_d)$$

$$= \int_V \frac{4\gamma D c^2 t_d^2 \mu_{af}(\mathbf{r})}{\tau(\mathbf{r}) |\mathbf{r}_d - \mathbf{r}_s|^2 + 4Dct_d^2} \int_{t_s}^{t_d} \frac{G^e(\mathbf{r} - \mathbf{r}_s, t - t_s) \partial G^e(\mathbf{r}_d - \mathbf{r}, t_d - t) / \partial q}{\partial G^e(\mathbf{r}_d - \mathbf{r}_s, t_d - t_s) / \partial q} dt\, d^3 r$$

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Fundamental equations and sensitivity functions

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1. τ does not depend on $\mathbf{r}$

$$g(\mathbf{r}_s,t_s,\mathbf{r}_d,t_d)=\int_V W_{\mu_{af}}(\mathbf{r}_s,t_s,\mathbf{r}_d,t_d,\mathbf{r})\,\mu_{af}(\mathbf{r})d^3r$$

$$W_{\mu_{af}}(\mathbf{r}_s,t_s,\mathbf{r}_d,t_d,\mathbf{r})=\frac{4\gamma Dc^2t_d^2}{\tau|\mathbf{r}_d-\mathbf{r}_s|^2+4Dct_d^2}\int_{t_s}^{t_d}\frac{G^e(\mathbf{r}-\mathbf{r}_s,t-t_s)\partial G^e(\mathbf{r}_d-\mathbf{r},t_d-t)/\partial q}{\partial G^e(\mathbf{r}_d-\mathbf{r}_s,t_d-t_s)/\partial q}dt$$

A.B. Konovalov et al. *7th Int. Symp. “Topical Problems of Biophotonics”* (2019)

2. $\tau(\mathbf{r})$ depends on $\mathbf{r}$

$$g(\mathbf{r}_s,t_s,\mathbf{r}_d,t_d)=\int_V W_f(\mathbf{r}_s,t_s,\mathbf{r}_d,t_d,\mathbf{r})\,f(\mathbf{r})d^3r$$

$$W_f(\mathbf{r}_s,t_s,\mathbf{r}_d,t_d,\mathbf{r})=c\int_{t_s}^{t_d}\frac{G^e(\mathbf{r}-\mathbf{r}_s,t-t_s)\partial G^e(\mathbf{r}_d-\mathbf{r},t_d-t)/\partial q}{\partial G^e(\mathbf{r}_d-\mathbf{r}_s,t_d-t_s)/\partial q}dt$$

$$f(\mathbf{r})=\frac{4\gamma Dct_d^2\mu_{af}(\mathbf{r})}{\tau(\mathbf{r})|\mathbf{r}_d-\mathbf{r}_s|^2+4Dct_d^2}=\frac{4\gamma Dc\mu_{af}(\mathbf{r})}{\tau(\mathbf{r})v^2(t_d)+4Dc}$$

is the fluorescence parameter distribution function

The main idea

$$\left.\begin{aligned}\frac{4\gamma Dc\mu_{af}(\mathbf{r})}{\tau(\mathbf{r})v^2(t_{d1})+4Dc} &= f_1(\mathbf{r})\\ \frac{4\gamma Dc\mu_{af}(\mathbf{r})}{\tau(\mathbf{r})v^2(t_{d2})+4Dc} &= f_2(\mathbf{r})\end{aligned}\right\} \qquad \begin{aligned}\mu_{af}(\mathbf{r}) &= \frac{1}{\gamma}\cdot\frac{f_1(\mathbf{r})f_2(\mathbf{r})\left[v^2(t_{d1})-v^2(t_{d2})\right]}{f_1(\mathbf{r})v^2(t_{d1})-f_2(\mathbf{r})v^2(t_{d2})}\\ \tau(\mathbf{r}) &= \frac{4Dc\left[f_2(\mathbf{r})-f_1(\mathbf{r})\right]}{f_1(\mathbf{r})v^2(t_{d1})-f_2(\mathbf{r})v^2(t_{d2})}\end{aligned}$$

$$\left.\begin{aligned}\frac{4\gamma Dc\mu_{af}(\mathbf{r})}{\tau(\mathbf{r})v^2(t_{d1})+4Dc} &= f_1(\mathbf{r})\\ &\vdots \quad n=1\ldots N\\ \frac{4\gamma Dc\mu_{af}(\mathbf{r})}{\tau(\mathbf{r})v^2(t_{dN})+4Dc} &= f_N(\mathbf{r})\end{aligned}\right\} \qquad \sum_{n=1}^{N}\left(f_n(\mathbf{r})-\frac{4\gamma Dc\mu_{af}(\mathbf{r})}{\tau(\mathbf{r})v^2(t_{dn})+4Dc}\right)^2 \to \min$$

Semi-infinite space

$$G(\mathbf{r}-\mathbf{r}',t-t')=\left[4\pi Dc(t-t')\right]^{-3/2}\exp\left[-\mu_a c(t-t')\right]$$

$$\times\left\{\exp\left[-\frac{(x-x')^2+(y-y')^2+(z-z')^2}{4Dc(t-t')}\right]-\exp\left[-\frac{(x-x')^2+(y-y')^2+(z+z')^2}{4Dc(t-t')}\right]\right\}$$

$$W_f(\mathbf{r}_s,t_s,\mathbf{r}_d,t_d,\mathbf{r})=\left(W_f\right)_- -\left(W_f\right)_+$$

$$\left(W_f\right)_\pm=\frac{z}{\pi(4D)^{3/2}(ct_d)^{1/2}z_s}\exp\left[\frac{x_d^2+y_d^2+z_s^2}{4Dct_d}-\left(\sqrt{p}+\sqrt{q_\pm}\right)^2\right]$$

$$\times\left(q_\pm^{-1/2}+2p^{-1/2}+\frac{1}{2}p^{-3/2}+p^{-1}q_\pm^{1/2}\right)$$

$$p=\frac{(x-x_d)^2+(y-y_d)^2+z^2}{4Dct_d},\quad q_\pm=\frac{x^2+y^2+(z\pm z_s)^2}{4Dct_d}$$

A.B. Konovalov, V.V. Vlasov *Quantum Electron.* 44: 719 (2014)

A.B. Konovalov, V.V. Vlasov *Proc. 38th PIERS*: 3487 (2017)

Discretization of the fundamental equation

$$g_{i,j} = \sum_{m=1}^{M}\sum_{n=1}^{N}\sum_{l=1}^{L} W_{m,n,l}^{i,j} f_{m,n,l} \qquad (\mathbf{g} = \mathbf{Wf})$$

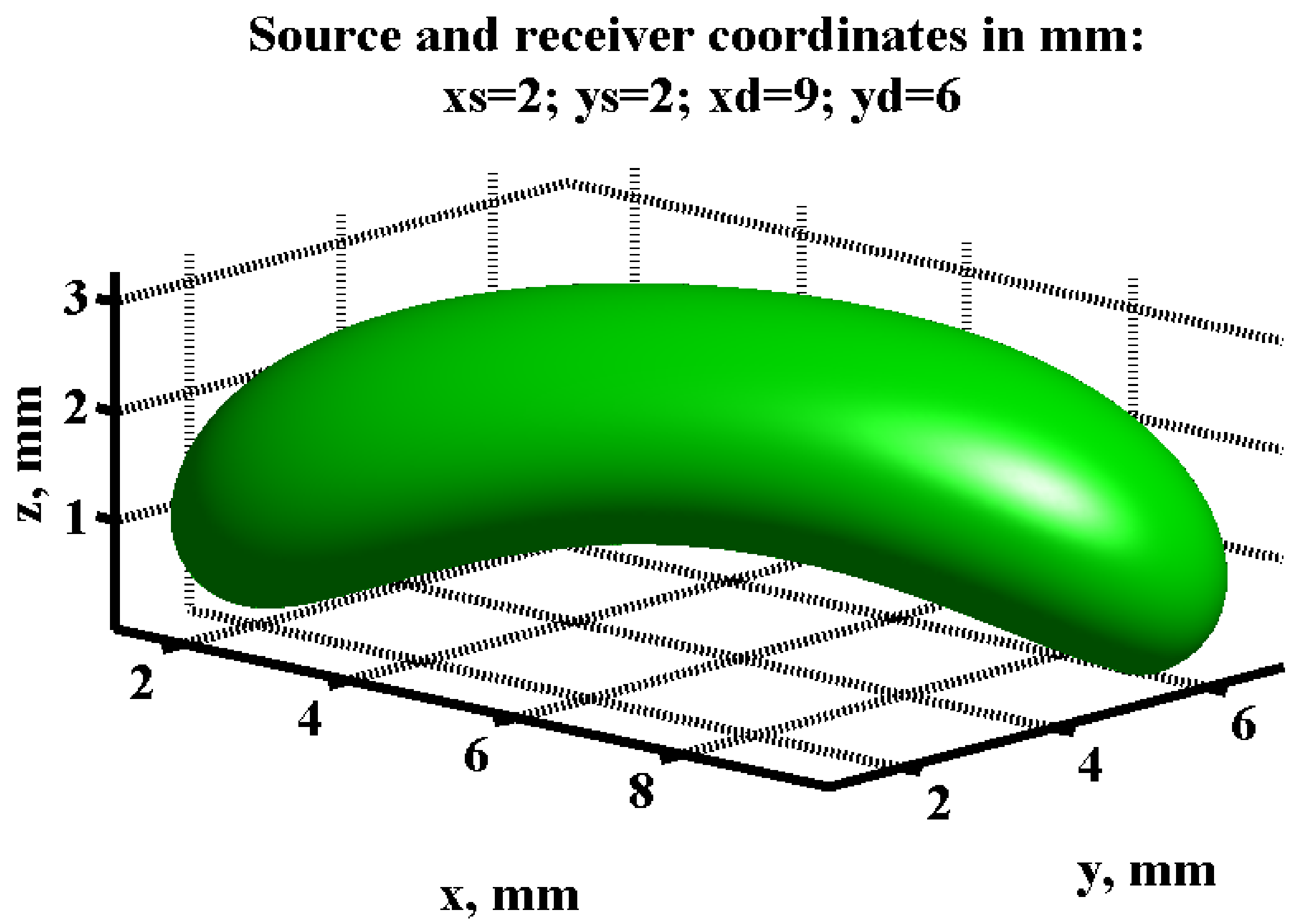
$$\left(W_{m,n,l}^{i,j}\right)_{\pm} = \frac{\Delta^3 z_l}{24\pi D^{5/2}(ct_d)^{1/2}} \cdot \exp\left[\frac{x_i^2 + y_i^2 + (3D)^2}{4Dct_d} - \left(\sqrt{\frac{(x_n - x_i)^2 + (y_m - y_i)^2 + z_l^2}{4Dct_d}} + \sqrt{\frac{(x_n - x_j)^2 + (y_m - y_j)^2 + (z_l \pm 3D)^2}{4Dct_d}}\right)^2\right]$$

$$\times\left\{\left[\frac{(x_n - x_j)^2 + (y_m - y_j)^2 + (z_l \pm 3D)^2}{4Dct_d}\right]^{-1/2} + 2\left[\frac{(x_n - x_i)^2 + (y_m - y_i)^2 + z_l^2}{4Dct_d}\right]^{-1/2}\right.$$

$$+\frac{1}{2}\left[\frac{(x_n - x_i)^2 + (y_m - y_i)^2 + z_l^2}{4Dct_d}\right]^{-3/2} + \left[\frac{(x_n - x_i)^2 + (y_m - y_i)^2 + z_l^2}{4Dct_d}\right]^{-1}$$

$$\left.\times\left[\frac{(x_n - x_j)^2 + (y_m - y_j)^2 + (z_l \pm 3D)^2}{4Dct_d}\right]^{1/2}\right\}$$

Example of sensitivity function



$$c = 0.214 \text{ mm / ps} \qquad D = 0.194 \text{ mm}$$

Cancelled scanning fiber probe

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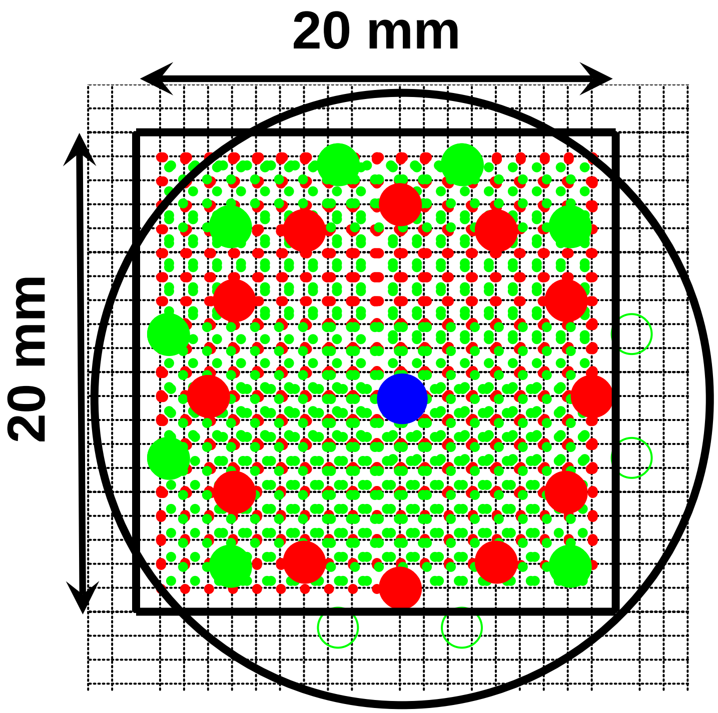

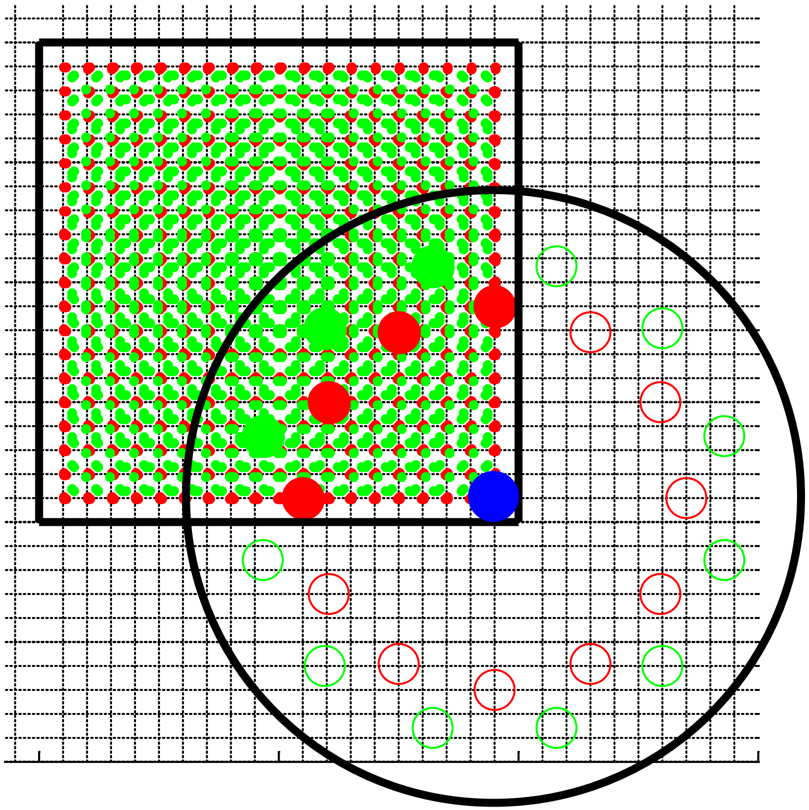

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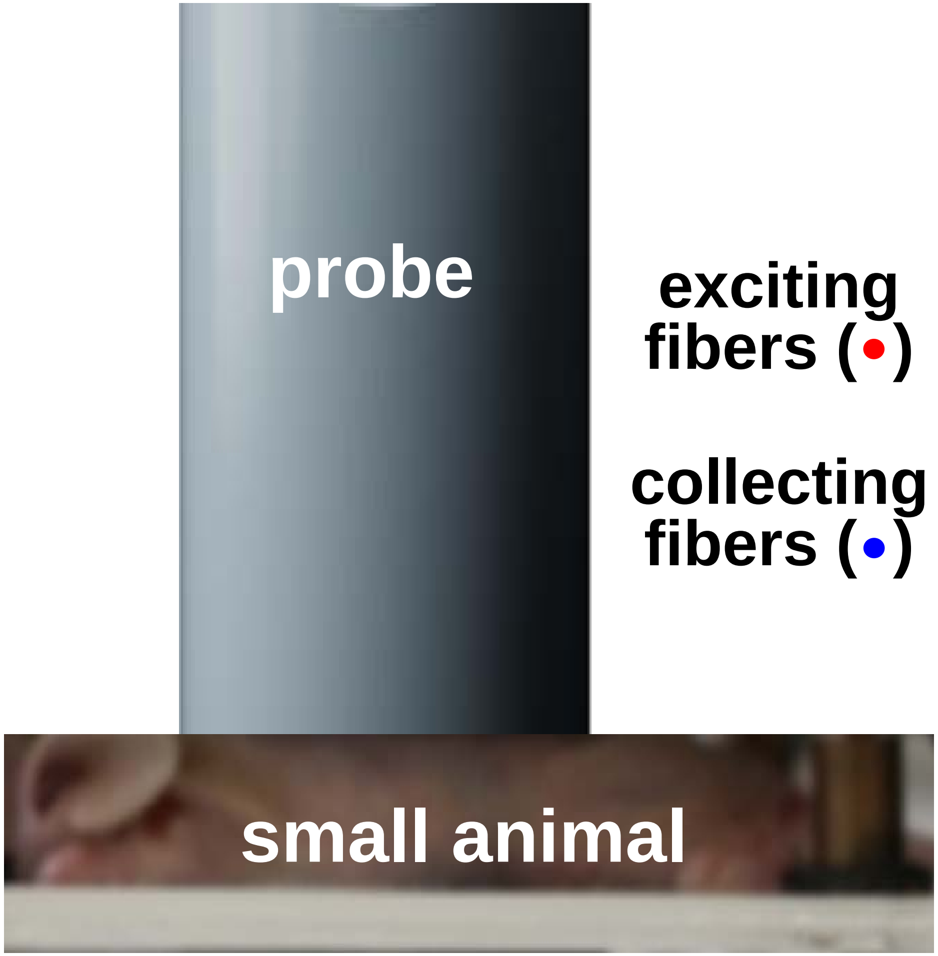
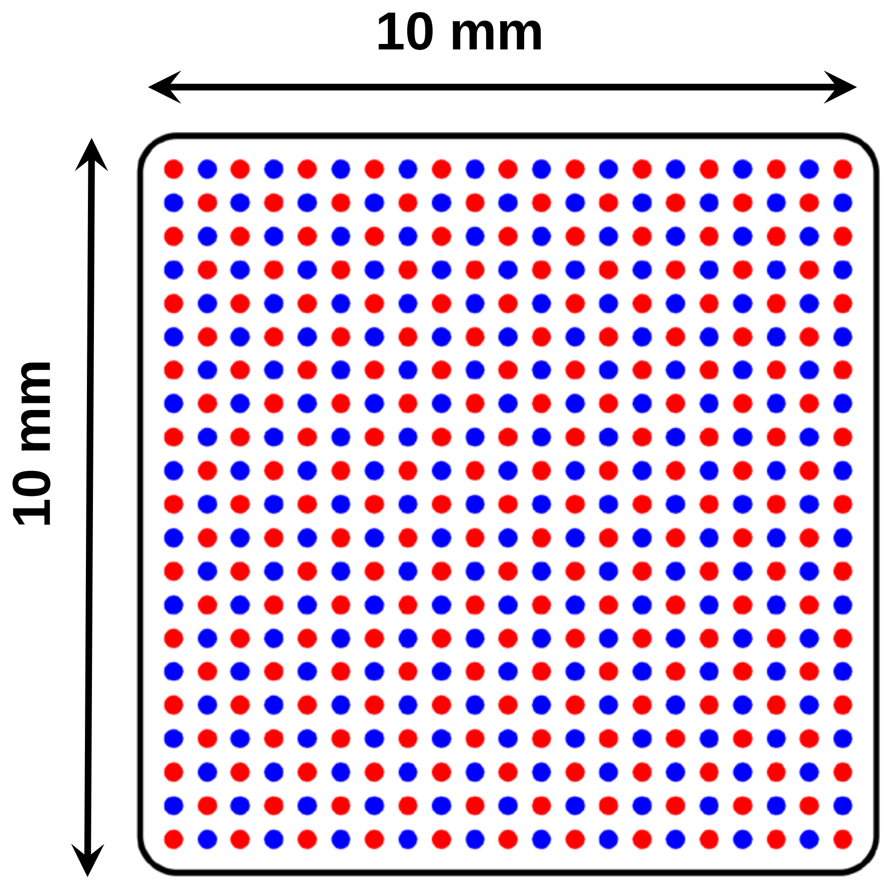
New virtual fiber probe

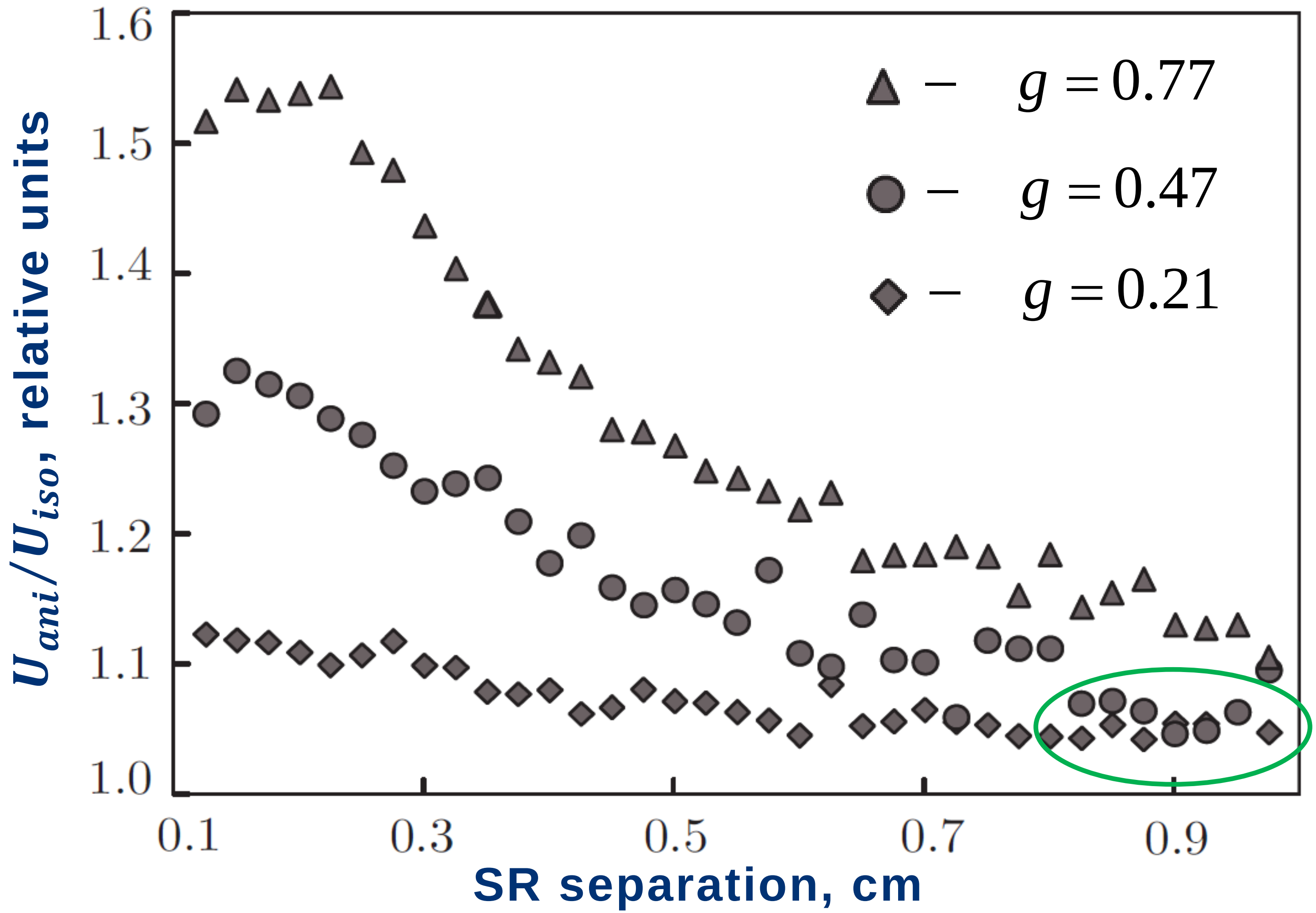


exciting fibers (●)

collecting fibers (●)

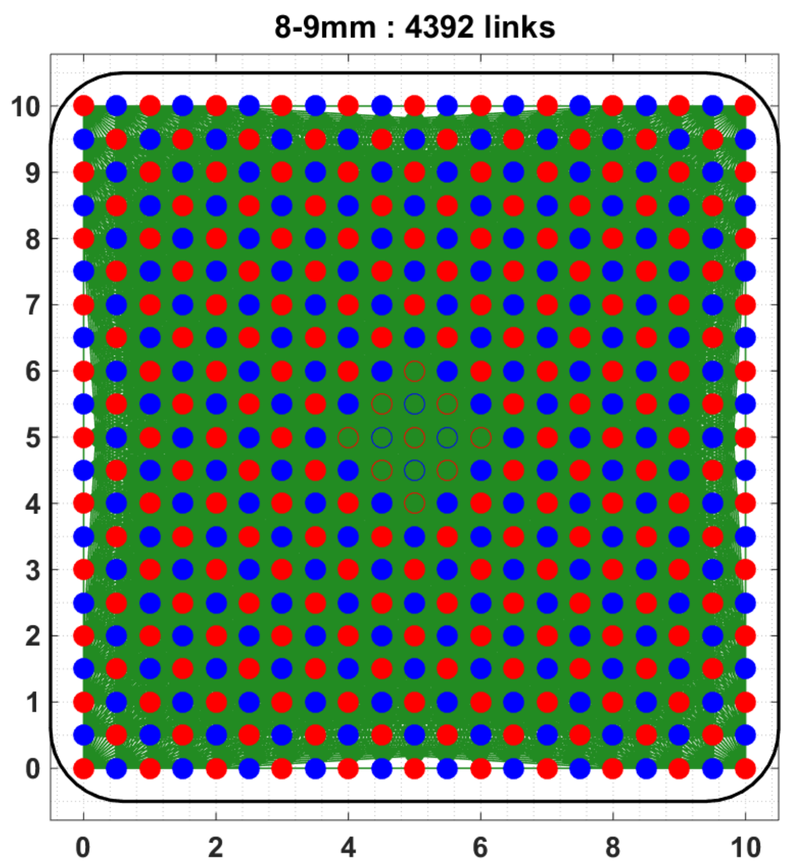


Choice of source-receiver (SR) separations

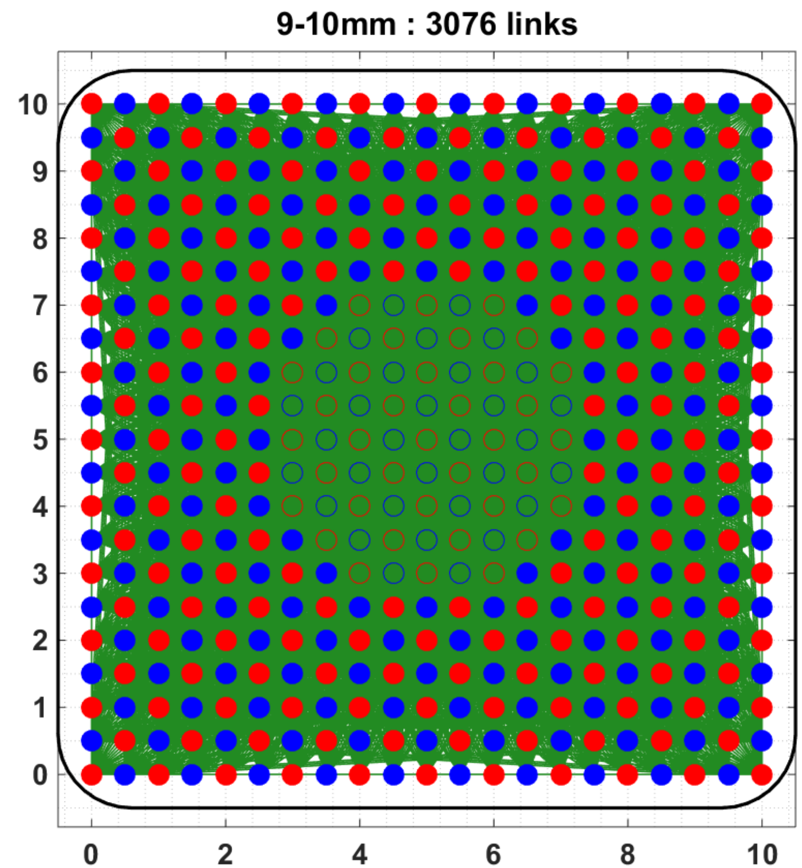


V.L. Kuzmin et al. *Zh. Eksp. Teor. Fiz.* 155: 460 (2019)

Choice of time gates

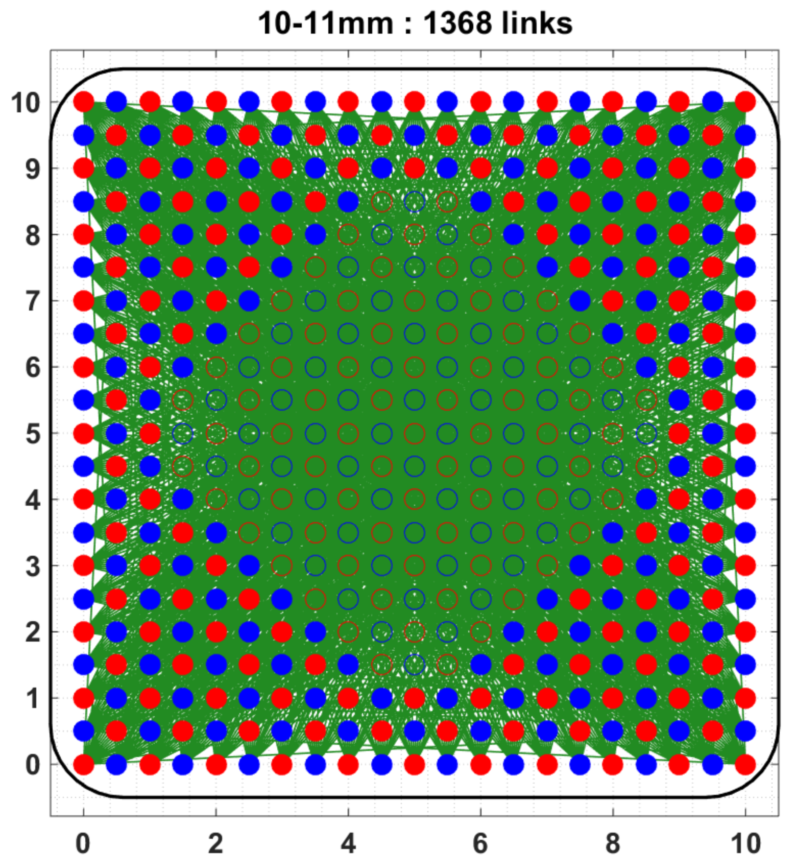


$$t_d = 110 \text{ ps}$$

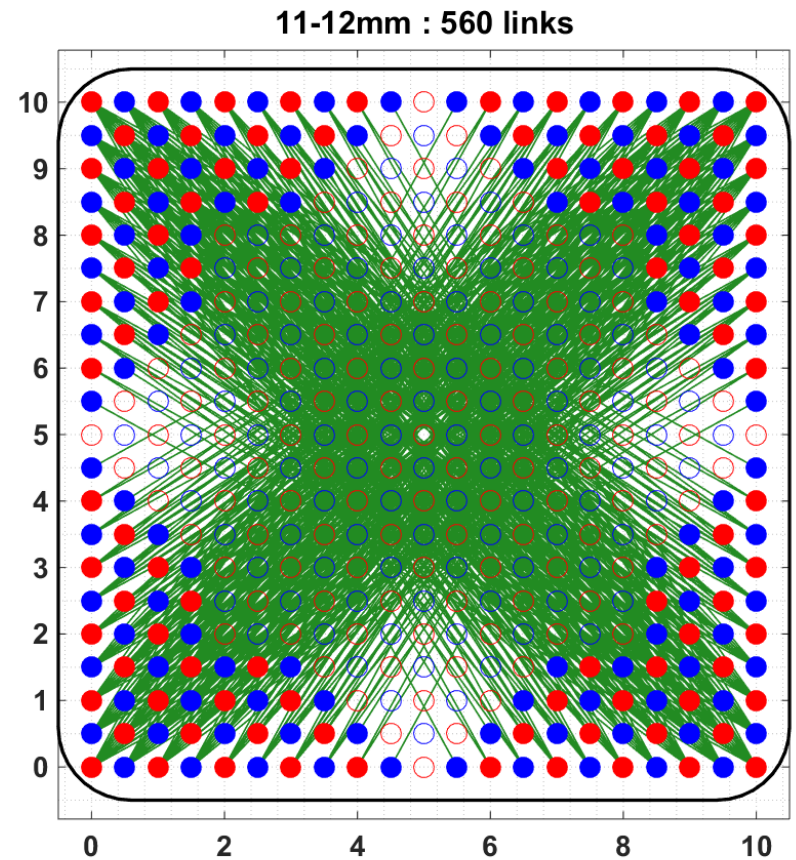


$$t_d = 135 \text{ ps}$$

Choice of time gates



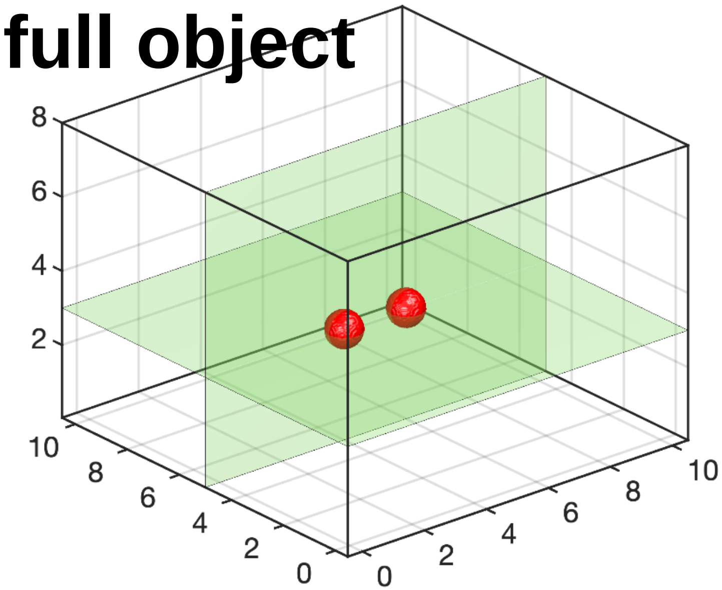
$$t_d = 160 \text{ ps}$$



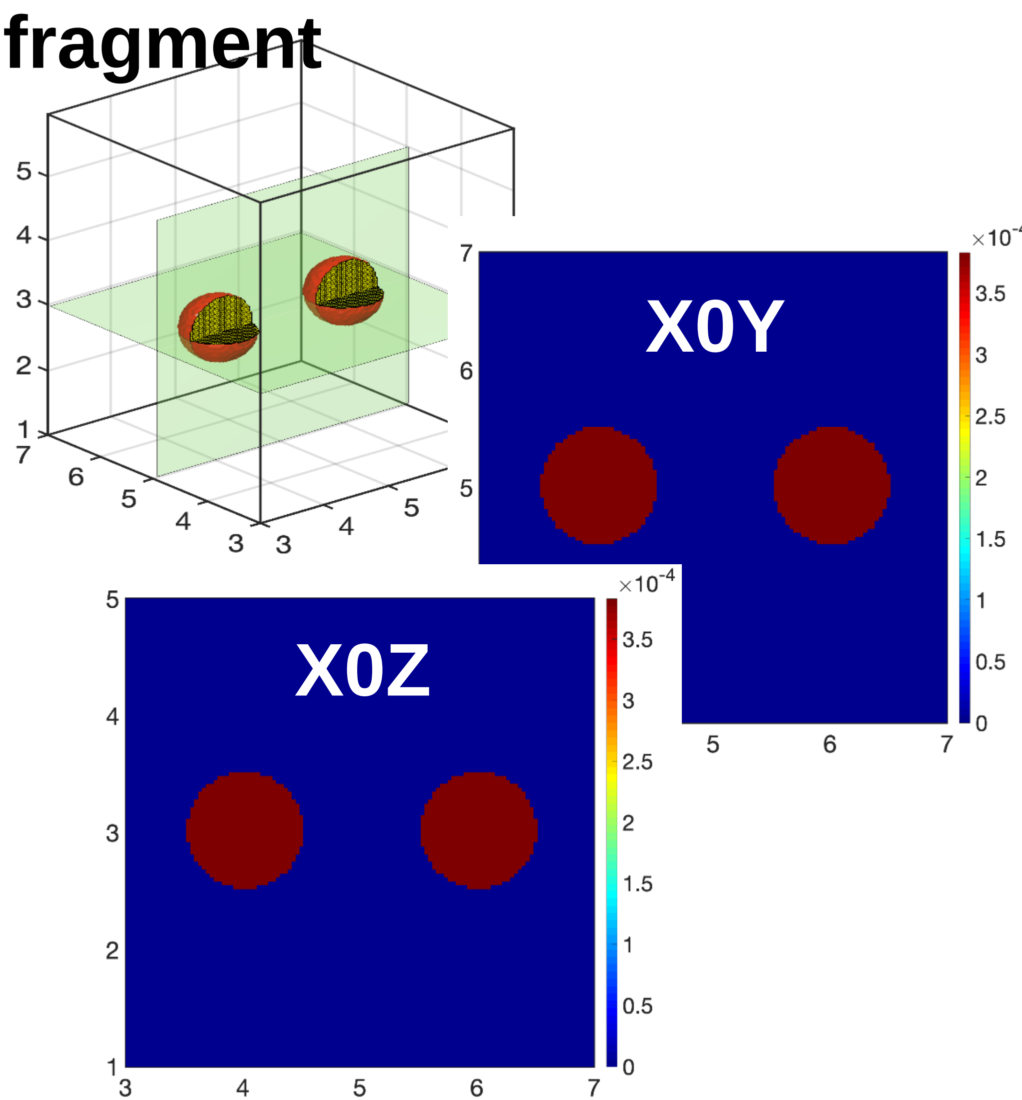
$$t_d = 185 \text{ ps}$$

The object to be reconstructed

full object



fragment



$$c = 0.214 \text{ mm / ps}$$

$$D = 0.194 \text{ mm} \qquad \gamma = 1$$

$$\tau = 1000 \text{ ps}$$

$$\mu_{af} = 0.01 \text{ mm}^{-1}$$

The ART-TV algorithm

$$\|\nabla \mathbf{f}\|_1 \to \min \quad \text{s.t.} \quad \mathbf{Wf} = \mathbf{g}$$

Lagrangian: $$\|\mathbf{Wf} - \mathbf{g}\|_2^2 + \lambda \|\nabla \mathbf{f}\|_1 \to \min$$

1. ART-iterations

$$f_j^{(k+1)} = f_j^{(k)} + \lambda \frac{g_i - \sum_j W_{ij} f_j^{(k)}}{\sum_j W_{ij}^2} W_{ij}$$

2. TV-iterations

$$f_j^{(n+1)} = f_j^{(n)} - \tau \frac{\partial \| \mathbf{f}^{(n)} \|_{TV}}{\partial f_j}$$

A.B. Konovalov & V.V. Vlasov *Proc. SPIE* 9917: 99170S (2016)

V.V. Vlasov et al. *J. Electron. Imaging* 27: 043006 (2018)

The results of 3D reconstruction: $f(\mathbf{r})$

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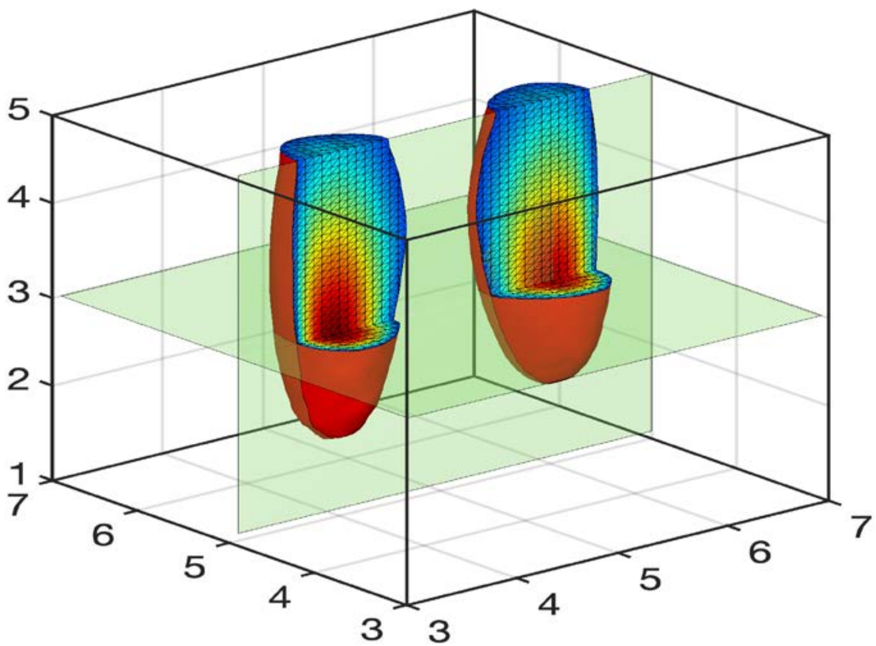
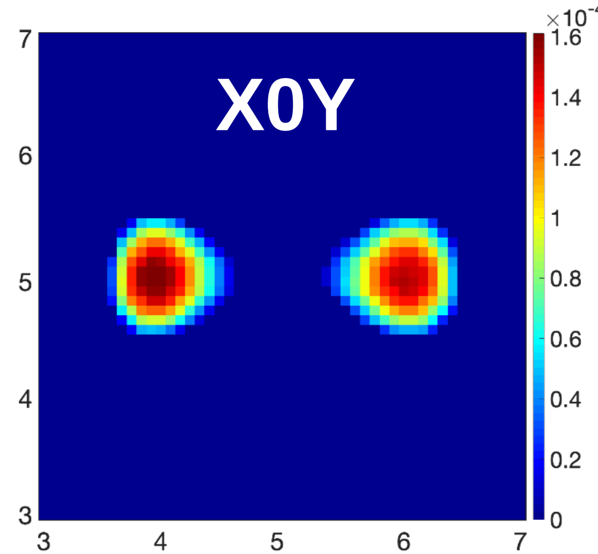
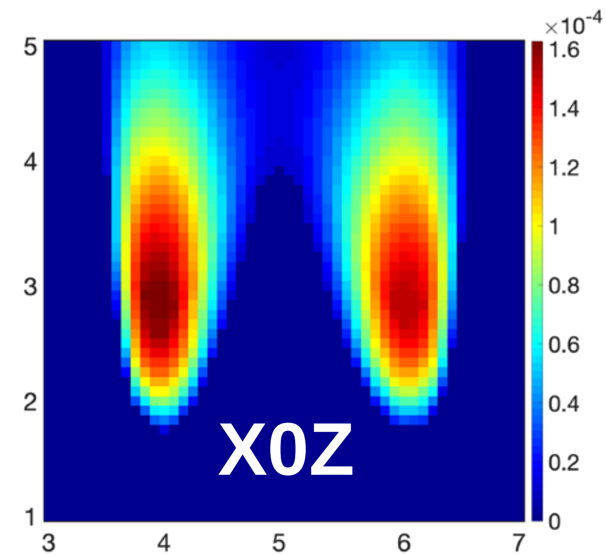
SR separations of 8-9 mm

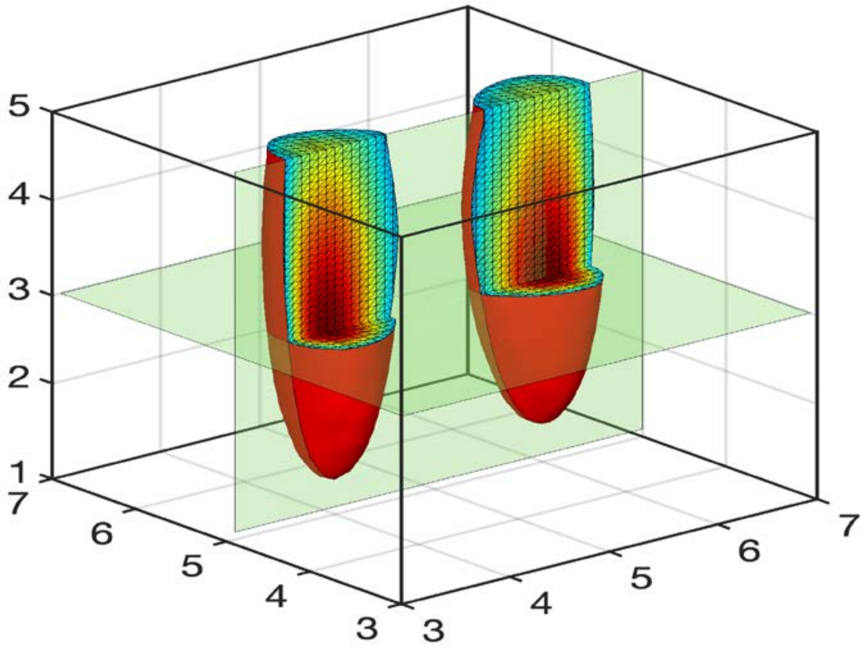


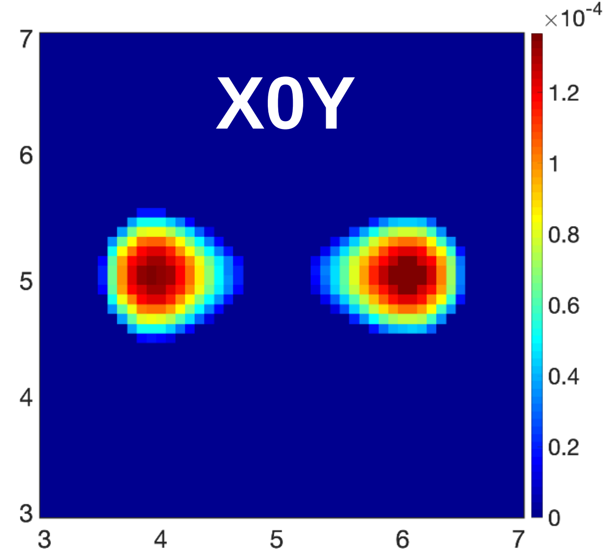


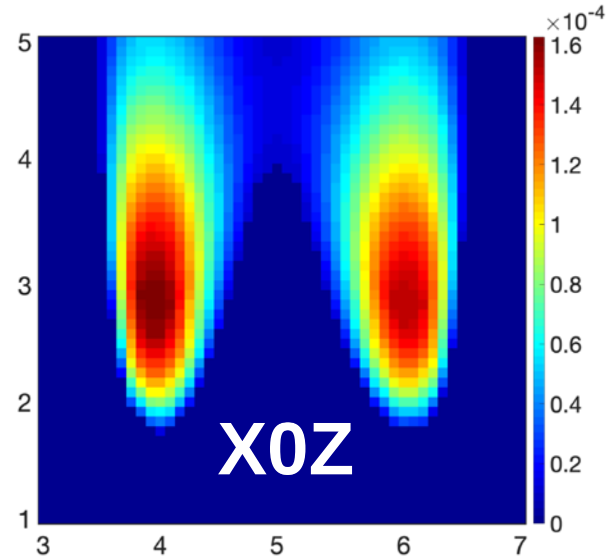


The results of 3D reconstruction: $f(\mathbf{r})$

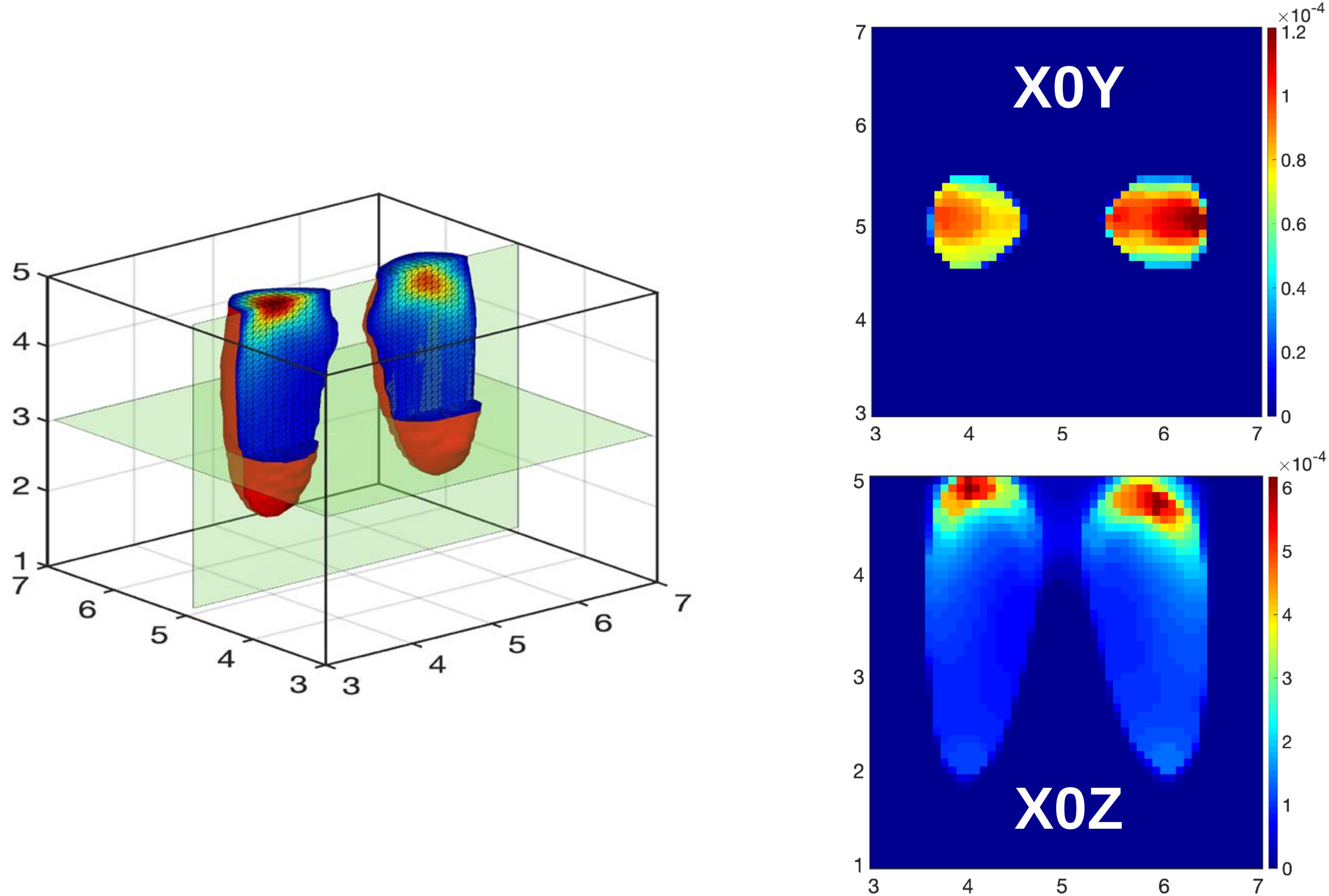
SR separations of 10-11 mm



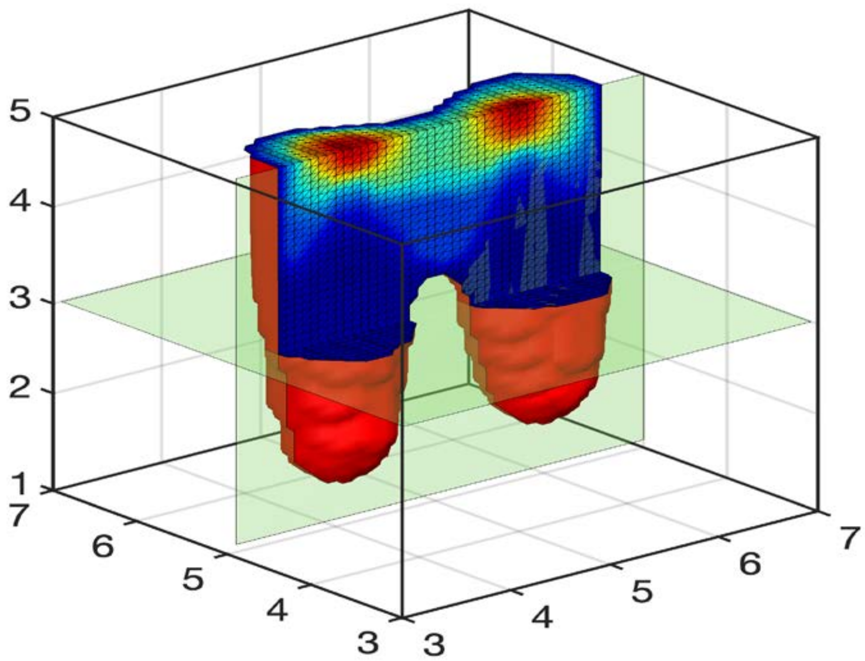


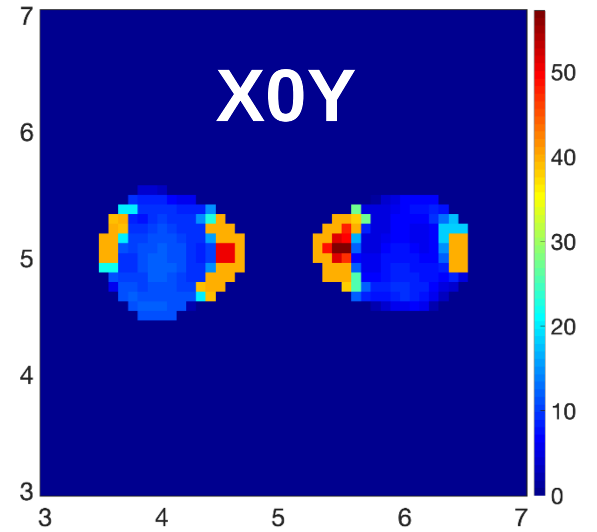


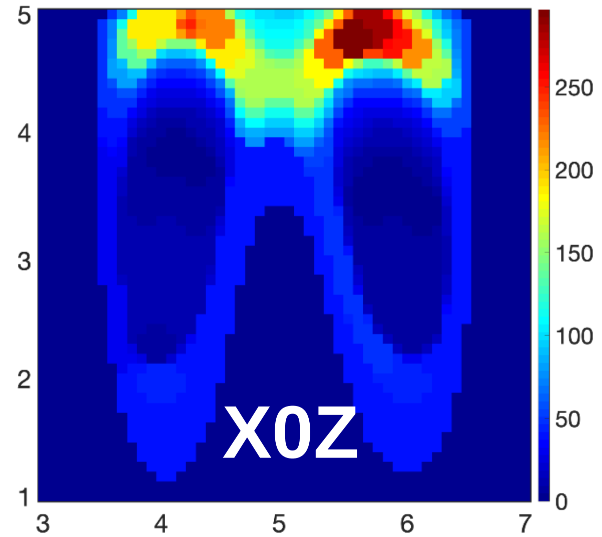
The results of separation: $\mu_{af}(\mathbf{r})$



The results of separation: $\tau(\mathbf{r})$







Ways to improve the results obtained

- Possibly, we should try separate with regularization as we do for the reconstruction of the function f(r).
- We must continue to study registration geometry. Possibly, the function f(r) can be reconstructed more accurately.
- We must exploit the full potential of the probe and implement mesoscopic fluorescence tomography. The latter will be done in any case.

Thank you for your time!

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We are very thankful to professor Savitsky and professor Tuchin for useful discussions of the new data registration geometry.

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