Locating and classifying fluorescent tags behind turbid layers using time-resolved inversion

Supplementary information

Supplementary Note 1 - Forward model expression derivation

For a single fluorescent particle (ignoring spatial dependence, which results in a time shift),

$$R(t) = \rho e^{-t/\tau} u(t). \qquad (1)$$

For an incident pulse train, the convolution in Eq. 1 above is

$$\begin{aligned} R(t) \underset{t}{*} \sum_{m=-\infty}^{\infty} \delta(t - mT) &= \sum_{m=-\infty}^{\infty} e^{-\left(\frac{t-mT}{\tau}\right)} u(t - mT) \qquad (2) \\ &= e^{-\frac{t}{\tau}} \sum_{m=-\infty}^{M} \left(e^{T/\tau}\right)^m \\ &= \frac{1}{1 - e^{-T/\tau}} e^{-\frac{T}{\tau}\left(\frac{t}{T} - \left\lfloor \frac{t}{T} \right\rfloor\right)}, \end{aligned}$$

where $M = \lfloor t/T \rfloor$. We can calculate the contrast about the instant $t = 0$. For $t_\varepsilon > 0$,

$$R^{(+)} \equiv \lim_{t_\varepsilon \to 0} R(t_\varepsilon) = \frac{1}{\tau\left(1 - e^{-T/\tau}\right)} \qquad (3)$$

$$R^{(-)} \equiv \lim_{t_\varepsilon \to 0} R(-t_\varepsilon) = \frac{e^{-T/\tau}}{\tau\left(1 - e^{-T/\tau}\right)} \qquad (4)$$

The contrast V is defined as

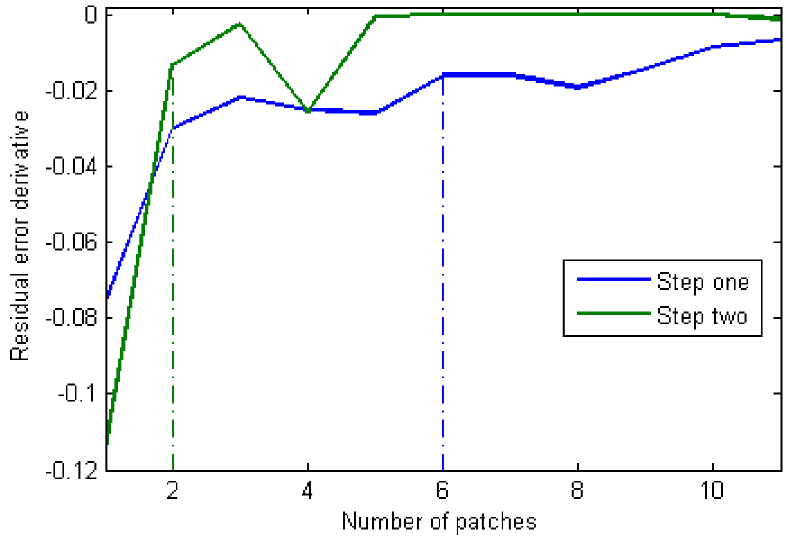
$$V \equiv \frac{R^{(+)} - R^{(-)}}{R^{(+)} + R^{(-)}} = \tanh(T/2\tau) \qquad . \qquad (5)$$

Supplementary Note 2 - Stopping criterion

The relaxed stopping criterion was chosen in order to make sure all the correct atoms are included in the first step even if incorrect atoms are temporarily selected during the process. This is important since the second OMP step is based on the selection from the first step and cannot add new atoms. The first step in our method can be thought of as an online dictionary generation step which generates all plausible locations. This stage is robust as long as the number of constructed atoms is significantly larger (about 3-5x) than the actual number of patches, and that is why we have chosen this relaxed criterion.

The second stage requires a more gentle care, as the number of atoms is not known *a priori*. This stopping criterion is a known problem with no closed-form solution once noise appears. Our algorithm handles this limitation by monitoring the residual energy decrease rate, which provides information on the correct number of patches. Supplementary fig. 1 demonstrates this; we note that the residual error of the first step decreases slowly, while in the second step the error decreases very fast and reaches almost zero at the correct number of patches. Needless to say that if we know the number of patches, any search procedure can be used.

Supplementary figure 1



Residual error decrease rate versus number of patches considered in the OMP solution. The dashed lines represents the location in which the stopping criterion has reached (we empirically select a threshold of -0.02 as a stopping criterion).

Supplementary Note 3 - Comparison to previous methods

Our method is compared to other existing methods in supplementary table 1. To the best of our knowledge only fluorescence diffuse optical tomography (FDOT)[1] is capable of recovering fluorescence lifetime and localizing patches. However, it requires more acquisition steps (full rastering of the sample), direct positioning of fluorescent probes in line of sight of the detector since it is based on transmission mode and depends on ballistic photons. It is noteworthy that a simple optimization[2] and back-projection technique[3] for localizing fluorescent tags would fail because of the significant lifetime of the fluorescent tags; hence a streak-based method with use of a dictionary-based algorithm is needed to simultaneously reconstruct both.

In order to compare the number of acquisition steps we counted the number of required images for each method. In case of FDOT[1], there are 84×46=3856 required measurements with an ICCD. In the case of [4] the required number of images is equal to the number of optimization steps, which is a multiplication of the spatial light modulator resolution (in the order of 600×600) times the number of phase steps.

As seen in supplementary table 1 our method is the only one that offers simultaneous 3D location and lifetime estimation in reflective mode after three consequent scattering events. This is crucial for recovering the patches that are not directly in front of the camera but their fluorescent emission can reach the camera lens in the time window of the acquisition. The noninvasive reflective mode also makes our method more appealing for applications that require deeper localization of the fluorophores.

Supplementary Table 1. Comparison between different methods for looking through turbid media and around the corner.

	Reconstruction model	Fluorescence lifetime recovery	Recovery scale	Acquisition raster steps	Mode - Number of scatterings
Our approach - (streak-based localizing fluorescent tags through diffuser)	**Sparsity-based dictionary**	**Yes**	**Macro**	**12**	**Reflective-3**
ICCD-based fluorescence diffuse optical tomography [1]	Ballistic photons- Standard convex optimization	Yes	Macro	3856	Transmissive-1
Streak-based albedo recovery through diffuser [2]	Standard convex optimization	No	Macro	16	Reflective-3
Streak-based looking around the corner [3]	Back projection	No	Macro	60	Reflective-3
Spatial light modulator - based method [4]	Adaptive wavefront correction	No	Micro	<600×600× phase steps	Transmissive-1
Speckle-based approach [5]	Using memory effect and speckle statistics	No	Micro	10	Reflective-1

Supplementary References:

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3. Velten, A. et al. Recovering three-dimensional shape around a corner using ultrafast time-of-flight imaging. *Nat. Commun.* **3**, 745 (2012).
4. Katz, O., Small, E. & Silberberg, Y. Looking around corners and through thin turbid layers in real time with scattered incoherent light. *Nat. Photonics* **6**, 549–553 (2012).
5. Katz, O., Heidmann, P., Fink, M. & Gigan, S. Non-invasive single-shot imaging through scattering layers and around corners via speckle correlations. *Nat. Photonics* **8**, 784–790 (2014).