Simple imaging protocol for autofluorescence elimination and optical sectioning in fluorescence endomicroscopy: supplementary material

RUlKANG ZHANG1,a, RAJA CHOuKeT1,a, ALISSOn G. TEBO1, MARIE-AUDE PLAMONT1, ZSOlT KElMEn2, LIOnEL GISSOt2, JEA-N-DENISS FAURE2, ARNAUD GAuTIER1, VinCenT CroQUETTE3,4,* , LuDoVIc JuLIEN1,* , And THOMAS Le SAUX1,*

1PASTEUR, Département de Chimie, École normale supérieure, PSL University, Sorbonne Université, CNRS, 75005 Paris, France
2Laboratoire de Physique Statistique, École normale supérieure, PSL Research University, 75005 Paris, France
3Laboratoire d’Optique Quantique, École normale supérieure, PSL Research University, 75005 Paris, France
4Institut de biologie de l’École normale supérieure (IBENS), École normale supérieure, CNRS, INSERM, PSL Research University, 75005 Paris, France

aThese authors contributed equally to this manuscript.
*Corresponding authors: vincent.croquette@ens.fr, ludovic.jullien@ens.fr, thomas.lesaux@ens.fr

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This document provides supplementary information to “Simple imaging protocol for autofluorescence elimination and optical sectioning in fluorescence endomicroscopy,” https://doi.org/10.1364/OPTICA.6.000972. It provides (i) details related to the endoscopic setup for fluorescence imaging, simulations of the Pre-OPiOm and Speed OPiOM signals in fluorescence endomicroscopy, protocols of calibration of light intensities, evidence of autofluorescence of the fiber bundle and materials, and (ii) supplementary figures.

1. ENDOscopIC SETUP FOR FluoresceNce IMAGINg

A. Setup configuration

The optical layout of the device is illustrated in Fig. 2. The setup uses two high power color LED chips (LXZ1-PB01, LHUV-0400, LXZ1-PX01; Lumileds, Amsterdam, Netherlands) as excitation lights. Their lights are collimated by optical condensors L1 (ACL2520U-A, f = 20 mm, Thorlabs, Newton, NJ) and filtered by band pass filters (ET470/40x, ET405/20x; Chroma Technology, Bellows Falls, VT). The UV and blue beams are combined by a dichroic mirror (T425LPXR, Chroma Technology) and sent to an afocal system, which consists of two convergent lenses (L2: AC254-100-A, f = 100 mm, L3: AC254-040-A, f = 40 mm; Thorlabs). An iris (SM1D12C, Φmax = 12 mm, Thorlabs) is positioned at the exit pupil of the afocal system through which lights with different field angles pass. The iris is also at the focal plan of the lens L4 (AC254-100-A, f = 100 mm, Thorlabs), which is used to conjugate the iris stop to the focal plan of the objective (10×, NA=0.5, Zeiss, Jena, Germany). The effective NA of the objective matches the NA of the fiber to secure a maximal light delivery. The imaging fiber (FIGH-30-850N, Fujikura, Tokyo, Japan) is a fused silica-based 30 cm long coherent fiber bundle, which consists of 30000 cores of about 5 μm in diameter with a core-to-core distance of around 4.3 μm. A GRIN lens (NEM-100-06-08-520-S, GrinTech, Jena, Germany) with a magnification of 1:1 is cemented onto the distal tip of the fiber. The distal tip of the fiber and the GRIN lens are embedded in a 2.5 cm long tube of stainless steel and glued together to ensure mechanical stability of the ensemble during experiments. The fluorescence image transferred from the fiber is magnified by the objective, filtered by an emission filter (ET525/36m, Chroma Tech.) and focused onto the camera (3080cp, iDS, Obersulm, Germany) by a tube lens of f = 50 mm (AC254-050-A, Thorlabs). The effective magnification goes to 3X.
B. Video acquisition
In the imaging experiments, we record films for \( m \) periods of light modulation (\( m \) is an integer). The acquisition frequency of the camera is set so as to obtain \( 2N \) (\( N \) is an integer) frames per period of modulation. Thus the acquisition frequency is \( f_{acq} = 2N f_m \), where \( f_m \) is the modulation frequency of the excitation light. The fluorescence emission acquired at pixel\((x,y)\) of the \( k \)th frame is equal to
\[
I_p(x,y,k) = T_s \left( \gamma_p^0(x,y) + \sum_{n=1}^{N} \left( \gamma_p^{n \sin}(x,y) \sin \left( \frac{n \pi f_s}{N} \left( \frac{k}{f_s} + \phi_{acq} \right) \right) \right) + \gamma_p^{n \cos}(x,y) \cos \left( \frac{n \pi f_s}{N} \left( \frac{k}{f_s} + \phi_{acq} \right) \right) \right) \tag{S1}
\]
where \( T_s = \frac{1}{f_s} \) refers to the exposure time of one frame, \( \gamma_p^{n \sin}(x,y) \) and \( \gamma_p^{n \cos}(x,y) \) are respectively the sinus and cosinus components of the fluorescence signal at harmonic \( n \) around the average value \( \overline{I_p}(x,y) \), and \( \phi_{acq} \) is a time lag, which may originate from distinct starting times for the light modulation and the acquisition of the camera. \( \phi_{acq} \) can be easily calibrated by using the fluorescence emission from instantaneously responding fluorophores (such as EGFP or Fluorescein).

Pre-processing has been performed over the video to compensate for possible photobleaching of the fluorophores. By assuming the photobleaching to exhibit a linear decay, the compensation factor has been calculated from the average of two successive periods:
\[
K(x,y) = \frac{\langle I_p(x,y,k) \rangle_{k=2N} - \langle I_p(x,y,k) \rangle_{k=0}}{2N} \tag{S2}
\]
Then all the frames over the two periods are corrected as in Eq.(S3)
\[
I_p^{corr}(x,y,k) = I_p(x,y,k) - K(x,y) \times k \tag{S3}
\]
All the frames over the whole video have been corrected for photobleaching by applying the same algorithm for all the successive pairs of two periods.

The Pre-OPIOM image \( \gamma_p^0(x,y) \) is calculated by averaging the frames over the whole film and normalizing to unit time
\[
\gamma_p^0(x,y) = \frac{f_s}{2mN} \sum_{k=0}^{2mN-1} I_p^{corr}(x,y,k) \tag{S4}
\]
The demodulation is done by multiplying the \( k \)th frame \( I_p^{corr}(x,y,k) \) with \( \cos \left( \frac{n \pi f_s}{N} \left( \frac{k}{f_s} + \phi_{acq} \right) \right) \) and by averaging over the whole film to get the first order cosine component, namely the Speed OPIOM image:
\[
\gamma_p^{n \cos} = \frac{f_s}{mN} \sum_{k=0}^{2mN-1} I_p^{corr}(x,y,k) \times \cos \left( \frac{n \pi f_s}{N} \left( \frac{k}{f_s} + \phi_{acq} \right) \right) \tag{S5}
\]
C. Reconstruction of the Pre-OPIOM and Speed OPIOM images by removal of the comb pattern
The images shown in the Main Text have been corrected from the artifact originating from the comb structure of the fiber bundle by applying an algorithm based on spatial interpolation on the crude images (see Fig.S1)[1]. The algorithm first localizes the centers of the individual fiber core through the image, which defines a set of local maximum points \( P_i \) identified by their coordinates and absolute fluorescence points. The image is then segmented with a Voronoi diagram based on the set of points \( P_i \) so that the natural neighbours of any point in the rest of the image \( Q \notin P_i \) can be easily defined. The fluorescence level at the point \( Q \) is eventually calculated as the weighted average of its neighbours, where the weight depends on its geometric relation to each neighbouring points.

D. Matlab Code to generate Speed OPIOM images with interpolation algorithm

```matlab
%% 'im' is the raw image.
%pre-processing helps better find the regional maxima
ref=imaaussfilt(im,0.4);
%find regional maxima of the image
BW=imregionalmax(ref,4);
%the maximal points representing the fiber cores are selected
im_core=im.*BW;
%find the coordinates and the absolute values of the cores
[Xs,Ys,Vs]=find(im_core);
%natural interpolation
F = scatteredInterpolant(Xs,Ys,Vs,'natural');
[Xq,Yq]=find(~BW);
Wq=F(Xq,Yq);
%%
%replace the other points
%with the calculated interpolation values
im_inter=zeros(size(im));
ind=sub2ind(size(im),Xq,Yq);
im_inter(ind)=Vq;
% artifact-removed image
im_final=im_inter+im_core;
figure; h=imagesc(im_final);
axis equal tight; axis off;
```

\[ \text{Fig. S1. Removal of the comb pattern in a Pre-OPIOM images acquired in endoscopy. a: Crude image acquired from the endoscope. b: Reconstructed image. Note that the algorithm retains the original fluorescence level imaged from the fiber and smooths the whole image without losing the lateral resolution defined by one bundle core. See Text for details.} \]
E. Acquisition parameters used for Speed OPIOM imaging

| Figure | Periods | $f_s$ | $f_m$ | $\lambda_{ex}$ | $L^0_1$ | $\lambda_{em}$ | $L^0_2$ |
|--------|---------|-------|-------|---------------|---------|---------------|---------|
| 3g,j,l | 8       | 60    | 3.0   | 480           | $4.8 \times 10^{-2}$ | 405  | 2.3 $\times 10^{-2}$ |
| 5a-d   | 8       | 60    | 3.0   | 480           | $4.8 \times 10^{-2}$ | 405  | 2.3 $\times 10^{-2}$ |
| 6a-d-f | 8       | 60    | 3.0   | 480           | $4.8 \times 10^{-2}$ | 405  | 2.3 $\times 10^{-3}$ |
| 6b-c-g | 2       | 10    | 1/8   | 480           | 5 $\times 10^{-2}$    | 405  | 1.6 $\times 10^{-2}$ |

Table S1. Acquisition parameters used for Speed OPIOM imaging in Figures 3, 4, 5, and 6 of the Main Text. $f_s$ and $f_m$ respectively refer to the sampling rate and to the modulation frequency of the excitation lights.

2. SIMULATION OF THE PRE-OPIOM AND SPEED OPIOM SIGNALS IN FLUORESCENCE ENDOMICROSCOPY

A. Calculation of the spatial profiles of exiting light intensity at the distal end of the fibers

A.1. Assumptions

The emergent light from a 1X magnification GRIN lens\(^1\) attached to the distal end of the fiber bundle plunging into a medium of refractive index $n$ was computed by making several approximations:

- The intensity is considered to be constant across the distal surface of the fiber. This approximation results from uniformly illuminating the fiber thanks to the Köhler system (see Fig. 2 d,e);

- The radiance $L$ of the fiber distal end is angularly constant (Lambertian source) within the acceptance angle. Indeed, the radiance from each point of the LED source is homogeneous. After being imaged to infinity after the optics, the spatial homogeneity of the LED chip is transferred to the angular homogeneity of the radiance at the distal end;

- Absorption or scattering of light in the propagation medium can be characterized as: $I(\lambda) = I_0 10^{-\alpha \lambda}$, where $I$ designates light intensity. $\lambda$ is the light path through the medium and $\alpha$ is the characteristic length of penetration, after passing through which the intensity falls to $\frac{1}{10}$ of its value at the origin;

- The simulation starts from the focal plan, which can be considered close to the GRIN lens surface ($< 50 \mu$m).

A.2. Calculation of the spatial intensity profile

We first analyzed the spatial profile of the exiting light intensity at the distal end of a fiber of radius $R_0$. Since the light distribution is rotationally symmetric to the optical axis, we only computed the light irradiance $I$ at medium points $(r, 0, z)$ identified by their cartesian coordinates with origin set at the center of the exit surface of the GRIN lens (see Fig. S2).

\(^1\)The magnification of the GRIN lens being 1, Abbe sine condition $y' \cdot n \sin(\alpha) = y' \cdot n' \sin(\alpha')$ (where $n, n'$ is the refractive index of object space and image space, $\alpha, \alpha'$ is the angle of the ray to the optical axis when it reaches object and image plane; $y, y'$ is object and image heights) implies that $NA$ at the exit of the GRIN lens equals to that of the fiber ($NA = NA' = n \sin(\alpha_n) = n' \sin(\alpha'_n)$).

\[ I(r, 0, z) = \frac{d\Phi_F(r, 0, z)}{dS} \]  

where $r$ is the distance to the optical axis of the fiber, $z$ is the depth from the end of the GRIN lens and $\Phi_F(r, 0, z)$ is the photon flux passing through an element of area $dS$ at $(r, 0, z)$.

When analyzing the points $(r, 0, z)$ close to the fiber ($z \sim 2R_0$), the exit end of the fiber is considered as a surface source $\Sigma$. Each element of surface $d\Sigma$ at $(x_s, y_s, 0)$ is considered lambertian within an acceptance angle $\theta_m$. The radiance can be expressed as:

\[ L(\theta) = \begin{cases} 
L_\theta & \theta \leq \theta_m \\
0 & \theta > \theta_m 
\end{cases} \]

where $\theta$ is the angle between the light ray and the normal of the surface $\Sigma$. The acceptance angle is defined by the numerical aperture of the fiber in use, $NA = n \sin(\theta_m) = 0.39$, where $n$ is the refractive index of the medium around the fiber.

The flux received by $d\Sigma$ at $(r, 0, z)$ from the source element $d\Sigma$ at $(x_s, y_s, 0)$ is:

\[ d^2\Phi_F = L(\theta) 10^{-\frac{\alpha}{\lambda}} \frac{d^2G}{dS} = L(\theta) 10^{-\frac{\alpha}{\lambda}} \frac{dSd\Sigma \cos^2 \theta}{d^2} \]

In Eq.(S7), $G$ is the etendue defined as

\[ d^2G = d\Sigma \cos \theta d\Omega = \frac{dSd\Sigma \cos^2 \theta}{d^2} \]

where $d$ is the distance between $d\Sigma$ and $dS$, and $\cos \theta = \frac{z}{\sqrt{(x-x')^2 + (y-y')^2 + z^2}}$ (see Fig. S2).

Finally we get the irradiance at $(r, 0, z)$ from the entire surface for $d\Sigma = \int d\Sigma$:

\[ I(r, 0, z) = \int_0^{2\pi} \int_{\theta_m}^{\theta} L(\theta) 10^{-\frac{\alpha}{\lambda}} \frac{dSd\Sigma \cos^2 \theta}{d^2} d\theta d\phi \]
where the ratio \( \rho \) depends on the photoswitching properties of the fluorescent label and \( \tau_{12}^{I_{12}}(r,0,z) \) designates the relaxation time associated to photoswitching under illumination with \( I_1(r,0,z) \) and \( I_2(r,0,z) \) (see Eqs.(101,102) of the Supporting Information of the reference [2]). Neglecting any chromatic aberration of the fluorescent endomicroscope and assuming that the medium exhibits similar absorption and scattering towards lights at the two wavelengths 480 and 405 nm, we then conclude that the ratio \( \rho_{I} \) is constant within the irradiance profile.

Equipped with the preceding information, one can easily calculate the spatial evolution of the Pre-OPIOM and Speed OPIOM response from the computed spatial profiles of light intensity. Indeed they exhibit a well-defined dependence on the light intensities \( I_1 \) and \( I_2 \) which are given in Eqs.(98,100) of the Supporting Information of the reference [2].

- **Pre-OPIOM response.** At constant ratio \( \rho_{I} \), the profile of Pre-OPIOM fluorescence intensity \( \gamma_F^{0} \) can be written

\[
\gamma_F^{0} = \phi^0 \times I_1(r,0,z)
\]

where \( \phi^0 \) designates a constant, which depends on the brightness of the fluorophore states, \( \rho_{I} \), the cross sections for forward and backward photoswitching at both wavelengths, and the fluorophore concentration;

- **Speed OPIOM response.** At constant ratio \( \rho_{I} \), Eqs.(97,100) in reference [2] can be used to write the profile of Speed OPIOM fluorescence intensity \( \gamma_F^{\text{1,cos}} \) as

\[
\gamma_F^{\text{1,cos}} = \phi^{\text{1,cos}} \times I_1(0,0,0) \frac{[I_1(0,0,z)]^2}{[I_1(0,0,0)]^2 + [I_1(r,0,z)]^2}
\]

where \( \phi^{\text{1,cos}} \) designates a constant, which depends on the brightness of the fluorophore states, \( \rho_{I} \), the cross sections for forward and backward photoswitching at both wavelengths, and the fluorophore concentration. The first Speed OPIOM resonance condition (S10) is verified at each position of the illuminated zone since \( \phi_{I} \) remains there constant. In contrast, the second Speed OPIOM resonance condition (S11) directly involves the light intensities (see Eqs.(13–16,18) of the Supporting Information of the reference [2]) and not anymore their ratio. As a consequence, it is only verified in the focal plane, which correspondingly generates a faster spatial decay of the response and an associated better axial selectivity in Speed OPIOM than in Pre-OPIOM.

### C. Simulation of the collected Pre-OPIOM and Speed OPIOM signals from variable thickness of solution

In the following, we still assume that the Speed OPIOM resonance conditions are satisfied at the focal plane at \( z = 0 \). The fluorescence collected by the fiber arises from the solution under illumination of the fiber. We consider a \( dz \)-thick layer of fluorescent solution at depth \( z \). The intensities of the Pre-OPIOM and Speed OPIOM signals generated from the layer are respectively given in Eqs.(S14,S15)

\[
\begin{align*}
\frac{dI_{\text{Pre-OPIOM}}(z)}{dz} &= \int \phi^0 \cdot I_1(r,0,z) \cdot 2\pi r dr \\
\frac{dI_{\text{OPIOM}}(z)}{dz} &= \int \phi^{\text{1,cos}} \\
I_1(0,0,0) \frac{[I_1(0,0,z)]^2}{[I_1(0,0,0)]^2 + [I_1(r,0,z)]^2} \cdot 2\pi r dr
\end{align*}
\]

The fluorescence signals collected from the solution of depth \( z \) by the fiber with \( S_{\text{fiber}} \), detection surface integrate the contribution of all the layers within the depth of \( z \). As the \( S_{\text{fiber}} \) and the illuminated layer at depth \( z \), \( S_{\text{layer}}(z) \), are small with respect to the distance \( z \) between them, we write

\[
\begin{align*}
I_{\text{Pre-OPIOM}}(z) &= \int_0^z S_{\text{fiber}} dI_f(z) \\
I_{\text{OPIOM}}(z) &= \int_0^z S_{\text{fiber}} dI_{\text{OPIOM}}(z)
\end{align*}
\]

The calculation was performed using Matlab, the \( \phi^0 \) and \( \phi^{\text{1,cos}} \) were deduced according to the experimental measurements of Pre-OPIOM and Speed OPIOM respectively.

### D. Matlab Code for the calculation of Pre-OPIOM and Speed OPIOM responses at the exit of the fiber

% 'R0' is the radius of the fiber
% 'NA' is the numerical aperture of the fiber
% 'n_r' is the refractive index of the medium
% 'lambda' is the characteristic penetration length
% of the light in scattering medium
% acceptance angle of the fiber
theta=asin(NA/n_r);
% size of the differential element on the distal end
delta=0.0005;
xs=-R0:delta:R0;
ys=-R0:delta:R0;
[X,Y]=meshgrid(xs,ys);
% the fiber is round shape
fiber=sqrt((X.^2+Y.^2)<R0);
%discretization of the coordinates (r,z)in the space d_r=0.01;d_z=0.5;
%for i=1:m
%distance between the two elementary surfaces
D = sqrt((x(i)-x(j))^2+(y(i)-y(j))^2);
%acceptance cone
cone = sqrt(((x(i)-x(j))^2+y(i)^2)<(z(j)*tan(theta)));
%effective area of the fiber facet that contributes to the illumination of this point
surface = fiber*cone;
%solid angle and scattering coefficient
G = delta^2*(z(j)./D).^4./(z(j)^2).*10.^(-D/lambda);
I(i,j) = sum(G(surface)); irradiance on point (r,0,z)
end

B=I(:,2:m);
B=fliplr(B);
%symmetrical extension
I=[B I]

%normalization
IN=I/max(I(i,:));
%normalization
IN_omiop=I_omiop/max(IN_omiop);

3. CALIBRATION OF LIGHT INTENSITY

Speed OPIOM implementation requires to determine the intensity of the two excitation lights at the sample (typically at 480 and 405 nm) so as to fulfill the resonant illumination conditions for the desired reversibly photoswitchable fluorescent proteins. Instead of using a powermeter, we directly exploited the dynamical photochemical properties of the RSFPs for calibrating the light intensities.

The principle of the calibration experiments is to measure the relaxation time associated to the conversion between the on and off RSFPs states by applying light jumps on a RSFP-containing sample. We typically used fixed HeLa or U2OS cells expressing Dronpa-2 or Padron as the calibrating sample put at the focal plan of the endoscope. In the first step, the sample was submitted to a light jump at 405 nm at constant light intensity \( I_0 \). A reversed switch took place. The temporal evolution of the fluorescence emission was recorded (see Fig. S3b,S4b) and fitted with Eq.(S20):

\[
I_F(t) = I_F(0,\lambda_1,\lambda_2) + \lambda_1\lambda_2 \left[ 1 - \exp\left( -\frac{t}{\tau_{\lambda_1\lambda_2}} \right) \right] \quad (S20)
\]

where \( \lambda_{1,2} \lambda_{1,2} \) is a pre-exponential term, which accounts for the molecular brightnesses of the ON and OFF states as well as their relative proportions (see [2]). \( \tau_{\lambda_1} \) was extracted from the fit and \( I_0 \) could be then retrieved from Eq.(S19):

\[
\frac{1}{\tau_{\lambda_1}} = k_{21}^\Delta + (\sigma_{12,\lambda_1} + \sigma_{21,\lambda_1}) I_0^1 \quad (S19)
\]

where \( \sigma_{12,\lambda_1} \) and \( \sigma_{21,\lambda_1} \) are respectively the molecular action cross-sections for photoisomerization, which converts 1 to 2 and 2 to 1 (see [2]).

In the second step, while \( I_0 \) was still maintained at its original value, the sample was submitted to a light jump at 405 nm at constant light intensity \( I_0^2 \). A reversed switch took place. The temporal evolution of the fluorescence emission was recorded (see Fig. S3a,S4a) and fitted with Eq.(S18):

\[
I_F(t) = I_F(0,\lambda_1,\lambda_2) + \lambda_1\lambda_2 \left[ 1 - \exp\left( -\frac{t}{\tau_{\lambda_1\lambda_2}} \right) \right] \quad (S18)
\]

where \( \lambda_{1,2} \lambda_{1,2} \) is a pre-exponential term, which accounts for the molecular brightnesses of the ON and OFF states as well as their relative proportions (see [2]). \( \tau_{\lambda_1} \) was extracted from the fit and \( I_0^2 \) could be then retrieved from Eq.(S21):

\[
\frac{1}{\tau_{\lambda_1\lambda_2}} = k_{21}^\Delta + (\sigma_{12,\lambda_1} + \sigma_{21,\lambda_1}) I_0^1 + (\sigma_{12,\lambda_2} + \sigma_{21,\lambda_2}) I_0^2 \quad (S21)
\]

Fig. S3. Photoisomerization kinetics of Dronpa-2. a: Evolution of the fluorescence emission of Dronpa-2 upon illumination at 480 nm (\( I_0^1 = 4.8 \times 10^{-2} \text{Ein.m}^{-2}\text{s}^{-1} \)); b: Evolution of the fluorescence emission of Dronpa-2 upon illumination at both 480 and 405 nm (\( I_0^1 = 4.8 \times 10^{-2} \text{Ein.m}^{-2}\text{s}^{-1} \) and \( I_0^2 = 2.3 \times 10^{-2} \text{Ein.m}^{-2}\text{s}^{-1} \)). Markers: Experimental points; line: Fit with Eqs. (S18) (in a) and (S20) (in b).
4. AUTOFLUORESCENCE OF THE FIBER BUNDLE

The fiber bundle exhibits a significant autofluorescence under illumination in the UV range. For illustration, we imaged at 525 nm in the air the distal end of the fiber bundle upon reducing the illumination zone by the iris so that only the central area of the fiber bundle was illuminated at 480 (F0 = 4.8 × 10^{-2} Ein. m^{-2}.s^{-1}) and 405 (F0 = 1.6 × 10^{-2} Ein. m^{-2}.s^{-1}) nm. After averaging 20 images, we obtained the image displayed in Fig. 2f.

5. MATERIALS

A. Reversibly photoswitchable fluorescent proteins

The RSFPs used in this study are Dronpa-2[3, 4] and Padron[5], which belong to the Dronpa[6] family. Dronpa-2 contains only one mutation M159T and Padron contains eight mutations: T59M, V60A, N94I, P141L, G155S, V157G, M159Y and F190S.

B. Plasmids

The plasmids for bacterial and cellular expression of Dronpa-2, Padron and EGFP, and the plants (Camelina) expression vector expressing p35S::Dronpa-2; pCVMV::DsRED construct have been previously described[2].

C. Protein production and purification

The plasmid expressing Dronpa-2 carrying an N-terminal hexahistidine tag was transformed in E. coli DH10B strain. Cells were grown in Terrific Broth (TB). Expression was induced by addition of isopropyl β-D-1-thiogalactopyranoside (IPTG) to a final concentration of 1 mM at OD(600 nm)=0.6. Cells were harvested after 16 h of expression and lysed by sonication in Lysis buffer (30/40 mM imidazole, 50 mM Tris/HCl at pH 7.5, 400 µM 1-((2-Aminoethyl) benzylsulfonyl) fluoride hydrochloride, 5 mg/mL DNAse, 5 mM MgCl2 and 1 mM dithiothreitol). Insoluble materials were removed by centrifugation and the soluble protein extract was batch absorbed onto Ni-NTA agarose resin (Thermofisher). The protein loaded Ni-NTA column was washed with 20 column volumes of 50 mM TRIS/HCl pH 7.5, 20 mM imidazole, 150 mM NaCl. Bound protein was eluted in 50 mM TRIS/HCl pH 7.5, 500 mM imidazole, 150 mM NaCl. Protein fractions were dialyzed on cassette Slide-A-Lyzer Dialysis Cassettes (Thermofisher) against 50 mM TRIS/H2SO4 pH 8.0.

D. Cultures of Escherichia coli

Dronpa-2 was expressed in E. coli BL21 strain. Cells were grown into 2 mL of lysogeny broth (LB) at 37°C, 220 RPM for 1 h. Cells were plated at low density on LB agar plates, and plates were incubated overnight at 37°C. Dronpa-2 single colonies were then separately transferred to LB-ampicillin media and incubated at 37°C, 220 RPM. Cells were then diluted and grown in LB at 37°C, 220 RPM. Expression was induced at 30°C by addition of isopropyl β-D-1-thiogalactopyranoside (IPTG) to final concentration of 1 mM at OD(600)= 0.6.

E. Plant transformation and growth

The plants expressing 35S::DRONPA2 were produced as described previously[2, 7] Camelina sativa(cv Celine) seeds expressing or not 35::DRONPA2 construct were sown on soil compost with a total of 5 plants in 15 cm wide pot and grown for 12 days in glasshouse.

F. Mammalian cell culture

U2OS HTB-96 (ATCC lot 64048673) cells were cultured in McCoy’s 5A medium supplemented with phenol red and 10% (vol/vol) fetal calf serum (FCS), at 37°C in a 5% CO2 atmosphere. For imaging, cells were seeded in αDish IBIDI (Biovalley) coated with poly-L-lysine. Cells were then transiently transfected with 2 µg total DNA using Lipofectamine 2000 (Invitrogen) according to the manufacturer’s protocol for 24 hours prior to fixing. Samples were washed with 1X DPBS and then fixed for 15 minutes in 4% paraformaldehyde. Imaging was performed in 1X DPBS.
6. SUPPLEMENTARY FIGURES

**Fig. S5.** Quantitative analysis of the normalized excitation light intensities at 480 and 405 nm across the distal end of the fiber. Distribution of the normalized light intensity at the pixels of the image recorded in Fig. 2d (a) and 2e (b).

**Fig. S6.** Dependence of the intensity (a,c) and phase (b,d) of the normalized excitation light at 480 (left) and 405 (right) nm as a function of the bending of the fiber bundle (a,b: 0°; c,d: 90°). No noticeable modification of the homogeneity of the intensity or of the phase can be observed over the surface of the fiber bundle when its proximal end is illuminated at 405 or 480 nm. The normalized light intensity and phase are displayed in linear scale at the right of the images.
Supplementary Material

Fig. S7. Normalized Speed OPIOM signals collected from three Dronpa-2 containing samples exhibiting different opacities. ●: Quasi-transparent Dronpa-2 solution; ■: Suspension of Dronpa-2-expressing bacteria; ▲: Dense suspension of Dronpa-2-expressing bacteria obtained by concentration of the preceding suspension by centrifugation. The Pre-OPIOM and Speed OPIOM signals have been obtained after spatial averaging over a disk of 50 pixels (equivalent to a disk 60 µm in the sample) of the Pre-OPIOM and Speed OPIOM images.

Fig. S8. Normalized Pre-OPIOM (a,c) and Speed OPIOM (b,d) responses to the spatial profiles of exiting light intensity at the distal end of the fiber bundle (a,b) or of a single microfiber (c,d) based on simulated illumination pattern through a scattering medium with a penetration length of $\lambda_c=100$ µm, which is representative of attenuation distances in the mice brain[8]. Intensities are displayed in common decimal logarithmic scale. Axis unit: µm.

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