SUPPORTING INFORMATION

Photon avalanche in lanthanide doped nanoparticles for biomedical applications: superresolution imaging

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Emission branching ratio

\begin{figure}
\centering
\includegraphics[width=\textwidth]{emission_branching_ratio.png}
\caption{The theoretical emission branching ratio as a function of spectroscopic Nd parameter $X_{Nd} = \Omega_4/\Omega_6$.}
\end{figure}

Following W. Macfarlane et al.\textsuperscript{1} one may estimate the emission branching ratio $\beta_{JJ'} = I(^4\text{F}_{3/2} \rightarrow ^4\text{I}_{J'}) / \beta_J I(^4\text{F}_{3/2} \rightarrow ^4\text{I}_{J'})$, where $J'=15/2, 13/2, 11/2, 9/2$ based on Judd-Ofelt theory as

$$\beta_{JJ'}(X_{Nd}) = \frac{(a_J \cdot X_{Nd} + b_J) \cdot \lambda_{JJ'}^{-3}}{\sum_J (a_J \cdot X_{Nd} + b_J) \cdot \lambda_{JJ'}^{-3}}$$
Where \( a_{J'} \) and \( b_{J'} \) are constants equal to the squared matrix elements of the irreducible tensor operators of rank 4 and 6

\[
a_{J'} = \left| \langle \left. F_{3/2} \right| U^{(4)} \left| I_{J'} \right. \rangle \right|^2
\]

\[
b_{J'} = \left| \langle \left. F_{3/2} \right| U^{(6)} \left| I_{J'} \right. \rangle \right|^2
\]

And the spectroscopic parameter of \( \text{Nd}^{3+} - X_{\text{Nd}} \) is defined as the ratio between phenomenological \( \Omega_4 \) to \( \Omega_6 \) Judd-Ofelt parameter. Figure S1 presents the emission branching ratio versus \( X_{\text{Nd}} \), where vertical lines indicate \( J_{JJ'} \) values for different hosts. The \( \beta_{JJ'} \) is important in our discussion, because it determines the effectiveness, the intermediate \( 4I_{11/2} \) level is populated by radiative transitions from \( 4F_{3/2} \) metastable level. This in turn determines the avalanche threshold and temperature dependent \( 4F_{3/2} \to 4I_{9/2} \) emission transients.

**Sub-diffraction imaging vs non-linearity of emission center**
Figure S2. The role on in-out non-linearity in improving the sub-diffraction imaging (a) the in-out non-linear relationships $I_{OUT} = (I_{EXC})^N$, for $N = 1..80$; the original diffraction limit is defined as $\delta_0 = \lambda / (2\ NA)$; (b) the point-spread function cross-sections obtained for non-linear in-out characteristics of materials for $N=1..80$; (c) the full-width at half maximum obtained from data in panel (b) and fitted with $\delta/\delta_0^N$ equation; (d) resolution enhancement $\Delta = \delta/\delta_0 = N^{0.5}$ versus the non-linear order $N$.

The rate-equation model

Based on the energy levels in Nd$^{3+}$, we have developed a set of rate-equations, which describe the averaged behavior of the system

$$\frac{\partial n_1}{\partial t} = W_{NR} \cdot n_2 + \frac{\beta_9 n_5}{\tau_{Nd}} - W_{CR} \cdot n_1 \cdot n_5 - W_{AT} \cdot n_1 \cdot \exp\left(\frac{-\Delta E}{kT}\right)$$  Eq.S1

$$\frac{\partial n_2}{\partial t} = W_{NR} \cdot (n_3 - n_2) - \frac{\sigma_{1064 \ \text{i}EXC}^{\text{ESA}}}{h\nu} \cdot n_2 + \frac{\beta_{11} n_5}{\tau_{Nd}} + W_{AT} \cdot n_1 \cdot \exp\left(\frac{-\Delta E}{kT}\right)$$  Eq.S2

$$\frac{\partial n_3}{\partial t} = W_{NR} \cdot (n_4 - n_3) + \frac{\beta_{13/2} n_5}{\tau_{Nd}}$$  Eq.S3

$$\frac{\partial n_4}{\partial t} = 2 \cdot W_{CR} \cdot n_1 n_5 - W_{NR} \cdot n_4 + \frac{\beta_{15/2} n_5}{\tau_{Nd}}$$  Eq.S4

$$\frac{\partial n_5}{\partial t} = \sigma_{1064 \ \text{i}p}^{\text{ESA}} \cdot n_2 - W_{CR} \cdot n_1 n_5 - \frac{n_5}{\tau_{Nd}}$$  Eq.S5

$$\sum_{J=9,11,13,15} \beta_J = 1; \quad \sum_{l=1,5} n_l = 1;$$

The equations marked by gray background, indicate thermal population of $^4I_{11/2}$ in respect to the ground $^4I_{9/2}$ level. Because these rate equations can be solved analytically only for low excitation regime, we have decided to solve them numerically. The following phenomenological parameters have been set:

1. rate of non-radiative transitions ($W_{NR}$=1/10$^5$ s$^{-1}$) – this parameter is characteristic for a given host material
2. rate of cross-relaxation ($W_{CR}$=1/10$^5$ s$^{-1}$ ) – this parameter is proportional to the concentration of Nd$^{3+}$ ions
3. variable intensity of photoexcitation (I_{\text{exc}} \text{ from } \ldots \text{ to } \ldots), \text{ where the pumping rate is defined by the absorption cross section } \sigma_{1064}^{\text{ESA}} \text{ and pumping intensity } I_p \text{ at } h\nu=1064 \text{ nm and.}

4. photoexcitation pulse width (s) – setting reasonably long photoexcitation pulse, enables to find the steady-state intensity

5. energy gap (\Delta E = 1900 \text{ cm}^{-1}) – this energy gap is energy difference between highest Stark level of the ground \(_4^I_{9/2}\) state and lowest stark level of the \(_4^I_{11/2}\) level.

6. All in silico experiments were performed at 42 °C. Grey part of the equations correspond to thermal initial population of the first \(_4^I_{11/2}\) excited state, which shall contribute to photon avalanche threshold

All other parameters have their typical meaning: h=Planck constant, k=Boltzmann factor, \(\beta_J\) (\(J'=9/2,11/2,13/2,15/2\)) are branching ratios (see below). First, the transients were calculated for \(n_1\) to \(n_5\) levels. To evaluate steady-state emission intensity, the photoexcitation pulse was fixed long enough to get saturation (Fig.S3, I_{\text{SS}}) in transient of the population \(n_5\), i.e. \(I(t)\sim n_5=f_{\text{exc}}(W_{\text{NR}}, W_{\text{CR}}, \Delta E, T, t_{\text{PULSE}}, t)\). In a similar way the half-rise times were calculated \(I(t_{1/2}) = \frac{1}{2} \cdot I_{\text{SS}}\). The variability of these factors were subsequently presented as a function of excitation intensity (I_p), non-radiative (1/W_{\text{NR}}) and cross-relaxation (1/W_{\text{CR}}) rates.

**Table S1.** Energies of \(Z_5\) (\(_4^I_{9/2}\)) and \(Y_1\) (\(_4^I_{11/2}\)) Stark levels of Nd\(^{3+}\) ions and energy differences between \(Z_1\) and \(Y_1\) (\(\Delta E\)) and between \(Z_5\) and \(Y_1\) (\(\Delta E_{\text{min}}\)) Stark levels of different host materials

<table>
<thead>
<tr>
<th>Host material</th>
<th>(Z_5)</th>
<th>(Y_1)</th>
<th>(\Delta E_{\text{min}})</th>
<th>(\Delta E)</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Y_3\text{Al}<em>5\text{O}</em>{12})</td>
<td>848</td>
<td>2001</td>
<td>1153</td>
<td>2001</td>
<td>9</td>
</tr>
<tr>
<td>(Y_2\text{O}_3)</td>
<td>640</td>
<td>1899</td>
<td>1259</td>
<td>1899</td>
<td>2</td>
</tr>
<tr>
<td>(\text{Gd}_2\text{O}_3)</td>
<td>610</td>
<td>1900</td>
<td>1290</td>
<td>1900</td>
<td></td>
</tr>
<tr>
<td>(\text{Y}_{\text{Gd}}\text{O}_3)</td>
<td>580</td>
<td>1910</td>
<td>1330</td>
<td>1910</td>
<td></td>
</tr>
<tr>
<td>(\text{YAlO}_3) (YAP)</td>
<td>671</td>
<td>2023</td>
<td>1352</td>
<td>2023</td>
<td></td>
</tr>
<tr>
<td>(\text{LiLaP}<em>3\text{O}</em>{12})</td>
<td>326</td>
<td>1939</td>
<td>1613</td>
<td>1939</td>
<td>3</td>
</tr>
</tbody>
</table>

Based on Judd-Ofelt theory the Einstein coefficient (radiative rates) is proportional to line strength of electric dipole transition, which is expressed as:

\[
A_{ij} \sim S_{j'i'}^{\text{ad}} \sum_{\lambda=2,4,6} \Omega_\lambda \cdot |\langle 4f^N\alpha[S\lambda]J||U_j^{(\lambda)}||4f^N\alpha[S'\lambda']J' \rangle|^2
\]
The $\langle|U^{(0)}|\rangle$ is reduced matrix elements of the irreducible tensor operators, which indicates theoretical probability of transition between SLJ and S’L’J manifolds and the $[\Omega_2 \Omega_4 \Omega_6]$ are intensity parameters characteristic for given ione in given matrix. Taking into account the $[\Omega_2 \Omega_4 \Omega_6] = [6.07 3.05 10.52]$ Judd-Ofelt parameters for e.g. fluorides as well as reduced tensor $[U_2 U_4 U_6] = [0.001 0.2371 0.3972]$ for $^4I_{9/2} \rightarrow ^4F_{5/2}$ at ~800 nm (which is typically used to pump Nd based lasers), and $[U_2 U_4 U_6] = [0.0000 0.1136 0.4104]$ for $^4I_{11/2} \rightarrow ^4F_{3/2}$ at ~1064 nm (which is typically used as laser emission), one may calculate $s^\text{ed}_{4I_{9/2} \rightarrow 4F_{5/2}}$ (800nm) = 4.907769·10^{-20} \text{cm}^2, while $s^\text{ed}_{4I_{11/2} \rightarrow 4F_{3/2}}$ (1064nm) = 4.663888·10^{-20} \text{cm}^2, which means, the integrated absorption cross section of ESA should be around 95% of GSA, if only the $^4I_{11/2}$ level is fully populated. The latter $\sigma$ was actually measured to be 10.75·10^{-20} \text{cm}^2 in Nd$^{3+}$ doped NaYF$_4$ single crystals. Based on E.S. Levy et al. estimations and the modeling performed there for Nd$^{3+}$ ions, the $\sigma_{4I_{9/2} \rightarrow 4F_{3/2}}$ (1064nm) ~1·10^{-25} \text{cm}^2. Therefore the $A_{\text{GSA}}/A_{\text{ESA}}$ ratio equals to $\sigma_{4I_{9/2} \rightarrow 4F_{3/2}}$ (1064nm) / $\sigma_{4I_{11/2} \rightarrow 4F_{3/2}}$ (1064nm) ~ 1·10^{-25} \text{cm}^2 / 95%-1.75·10^{-20} \text{cm}^2 = 0.098·10^{-5} = 0.98·10^{-4}, which satisfy the condition $R<10^{-4}$ and thus should classify the process as photon avalanche$^5,6$. It is important to mention, the Nd doped NaYF$_4$ single crystals exhibited 26%, 100% and 389% higher absorption cross section and 54%, 147% and 231 % higher emission cross section than Nd$^{3+}$ doped YAG, YLF, BYF. $^4$ This is also in agreement with calculations made by Joubert et al$^7$. The only studies on PA in Nd$^{3+}$ ions demonstrated 4$g_{13}$ nm PA emission under 603.6 nm in LiYF$_4$ single crystal, at temperatures <40K with lasing slope factor efficiency up to 11%. The critical parameter $R$ has reached 1.7·10^{-4} for avalanche at T 40 K$^{1,7}$.

Not too much information can be found on non-radiative rates, which in fact determine the balance between non-radiative losses and looping, which is a fundamental requirement for PA to occur. In Nd$^{3+}$, the energy gap between $^4I_{15/2}$, $^4I_{13/2}$ and $^4I_{11/2}$ and the ground $^4I_{9/2}$ state are equal to around 1540, 1700 and 1480 cm$^{-1}$ respectively, with $W_{NR} \sim 10^3$-10$^{-4}$ s$^{-1}$ (based on LaF$_3$ host$^6$). Such $W_{NR}$ corresponds to $A_{NR} \sim 10^{-3}$-10$^{-4}$ s, which, according to Fig.S3 makes the presented system closer to looping rather than PA. The $W_{NR}$ has a tremendous impact on the PA behaviour in Nd$^{3+}$ (as shown in Fig.3e,f), because this mechanism is present and necessary both to get looping, and simultaneously deplete the $^4I_{11/2}$ level. However, based on data available in the literature and the energy gap law, it is impossible to get univocal and reliable values for other hosts such as NaYF$_4$ (see discussion in chapter 4.2 of Kaminski). We have therefore presented the performance of the studied PA system for variable $W_{NR}$ values.
The impact of host material parameters on the PA behavior

It is necessary to find a material, which through a proper balance between CR and NR processes enhances PA and enables to get very high orders of PA reaching > 50. As one may see from Fig.3, lower CR rates (lower Nd$^{3+}$ concentrations) enhances PA effect, while increasing A$_{CR}$ leads to more looping like behavior, and in consequence to lower N and higher PA threshold. Higher NR rates significantly reduces PA threshold, change PA into looping.

Since the power dependent in-out process is described by allometric relationship

$$P_{OUT}(P_{IN}) = a \cdot (P_{IN})^N$$

To simplify and speed up the analysis, both sides of the equation can be recalculated with a logarithmic function, which gives

$$\ln(P_{OUT}) = \ln[a \cdot (P_{IN})^N]$$
$$\ln(P_{OUT}) = \ln(a) + N\ln(P_{IN})$$

thus substituting

$$P'_{OUT} = \ln(P_{OUT}) ; a' = \ln(a) ; P'_{IN} = \ln(P_{IN})$$

one gets a linear function $P'_{OUT} = a' + N \cdot P'_{IN}$, which can be easily fitted with 1-order polynominal. This procedure was implemented in a matlab code presented below, which enabled, based on W$_{CR}$ and W$_{NR}$ dependent in-out curves, to calculate power dependent nonlinearities $N(P_{IN})$, as presented in Fig.3d and f, respectively. Such ‘adaptive’ approach is required because of variable and steep changes of the photon avalanche in-out relationships make the evaluation of N susceptible to the manual selection of the range on the in-out curve.

```matlab
function TableN = CalculateTableN( PowerDepTable)

TableX = log(PowerDepTable(:, 1)); % Excitation intensity
TableY = log(PowerDepTable(:, 2)); % Emission intensity

SizeT = max(size(TableX));
SD = 0; % standard deviation
SDErr = 0.02; % acceptable SD
MinimalCheck = 20; % start fitting with at least 10 data points

PowerNTable = zeros(SizeT-10,2); % define table for power dependent N

for idxT=1:SizeT-MinimalCheck % go through all (but last MinimalCheck) data points
    \n```
Didx = MinimalCheck;
SD = 0;
% as long as dataset is nicely fitted with p1 * X^N + p2
% enlarqre the dataset beyond MinimalCheck datapoints
while (SD < SDErr) & (idxT + Didx < SizeT)
% extract Didx datapoint for fitting
TableX2 = TableX( idxT: idxT + Didx) - TableX( idxT);
TableY2 = TableY( idxT: idxT + Didx);
% fit the dataset with p1 * X^N + p2

[p,ErrorEst] = polyfit(TableX2,TableY2,1);
% generate fitted curve
[f, delta] = polyval(p,TableX2, ErrorEst);
% calculate SD based on residuals
SD = sum((delta.^2)./(f.^2));
% build the result table using original X and power factor N
PowerNTable(idxT,1) = PowerDepTable(idxT, 1);
PowerNTable(idxT,2) = p(1);
Didx = Didx + 1;
end;
end;

% plot(TableX2, TableY2,'o', TableX2, f, '-') hold on;

TableN = PowerNTable;
clc;
sprintf(’Progress of analysis %3.1f %% ’, [100*idxT / (SizeT-MinimalCheck)]);
end;
The impact of excitation and materials properties of spatial resolution of PASSI

Figure S3. Resolution versus cross-relaxation rate and excitation intensity. Top row presents the excitation intensity (y axis) dependent profile measured in silico for single 10 nm PA NP at looping strength ($A_{CR}$=1,2,5,10,20 e-5 s$^{-1}$) at subsequent columns (these data served to obtain the Fig.2e data). Bottom row presents (similar to Fig.2b,g) the cross section of 3 PA NPs (10nm in diameter) displaced (by 5,10,20,30,40,50,75,100 and 125 nm) for different looping strength ($A_{CR}$=1,2,5,10,20 e-5 s$^{-1}$) at subsequent columns and at the optimum $I_0$=1.07e5, 1.15e5, 1.3e5, 1.8e5, 3.0e5 excitation intensities, which were adjusted to respective $A_{CR}$. 
Figure S4. The impact of excitation intensity on the spatial resolution. Cross sections of the 3 NP phantom (i) versus distance between NPs (along y axis) and for different $I_0$ excitation intensity (a-h).

Lateral optical resolution of PASSI

Figure S5. Spatial resolution enhancement using photon avalanche labels. (a) Phantoms composed of 3 NPs ($\phi=20$ nm PA NPs) placed at $L = 60$ nm distances are reconstructed using optimal PA conditions, and (b) respective cross sections of graph for distance $L = 60$ nm along a light blue line. Phantom composed of 20 nm PA NPs (distance $L = 60$ nm) reconstructed with (c) steady state avalanche and (d) time-gated avalanche models. (e) Emission intensity cross section obtained along the line showed in graph (a) was reconstructed with (e) steady state avalanche and (f) time-gated avalanche models, respectively for $L = 30, 40, 60$ and $100$ nm.

Figure S6. Spatial resolution enhancement using photon avalanche labels. (a) Phantoms composed of 5 NPs ($\phi=20$ nm PA NPs) placed at $L = 60$ nm distances are reconstructed using optimal PA conditions, and (b) respective cross sections of graph for distance $L = 60$ nm along a light blue line. Phantom composed of 20 nm PA NPs (distance $L = 60$ nm) reconstructed with (c) steady state avalanche and (d) time-gated avalanche models. (e)
Emission intensity cross section obtained along line showed in graph (a) and reconstructed with (e) steady state avalanche and (f) time-gated avalanche models, respectively for L = 30, 40, 60 and 100 nm.

**Axial optical resolution of PASSI**

For a Gaussian-profile beam, the 1/e beam radius is given by \(^9\):

\[
w(z) = w_0 \cdot [1 + (z/z_R)^2]^{1/2}
\]

Eq.1.

Where \(w_0 = \lambda/NA \cdot \pi\) (Eq.2) is the spot size at the focus at \(z=0\), and \(z_R = w_0/NA\) is Raleigh length. The pump intensity is expressed as \(^9\):

\[
I_p(x, y, z) = I_0 \cdot [w_0/w(z)]^2 \cdot \exp\left(-\frac{x^2+y^2}{w^2(z)}\right) \cdot \exp(-\varepsilon \cdot z)
\]

Eq.3

where \(\varepsilon\) is an attenuation coefficient of the pump beam in the sample. Let’s assume small attenuation coefficient of the sample for pump beam \((\varepsilon=0.001)\), \(\lambda=1064\), NA=1.4. This enables to visualize the beam cross section.

According to the ref.\(^{10}\), diffraction for super-resolution localization of single molecule is defined as \(\delta_0/(N^{0.5})\), where \(\delta_0\) is width of the diffraction-limited point spread function and \(N\) number of detected photons.

The intensity of conventional (liner, Stokes) fluorescence excited by Gaussian beam (ZX plane) is presented in Figure S7a and b based on the above equations. The luminescence of non-linear fluorophore is presented in Fig.S7 at panels c and d (for \(N=2\)), e and f (for \(N=10\)), g and h (for \(N=30\)) and i and j (for \(N=60\)).

**Figure S7:** Linear axial (along z axis) intensity for Gauss profile of laser beam (a), (b). Intensities of emission from \(N^{th}\) order non-linear processes, respectively for \(N=2\) (c,d), 10 (e,f), 30 (i,j) and 60 (g,h).
Supporting information references


