Visualization of upconverting nanoparticles in strongly scattering media

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Abstract: Optical visualization systems are needed in medical applications for determining the localization of deep-seated luminescent markers in biotissues. The spatial resolution of such systems is limited by the scattering of the tissues. We present a novel epi-luminescent technique, which allows a 1.8-fold increase in the lateral spatial resolution in determining the localization of markers lying deep in a scattering medium compared to the traditional visualization techniques. This goal is attained by using NaYF4:Yb3+Tm3+@NaYF4 core/shell nanoparticles and special optical fiber probe with combined channels for the excitation and detection of anti-Stokes luminescence signals.

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1. Introduction

Nanosized luminescent particles are becoming important universal markers for optical visualization applications in biomedicine. Interest in the in vivo visualization of biomarkers located deep in biotissues by the fluorescence diffuse optical tomography (FDOT) technique is constantly increasing [1–4]. FDOT is a highly sensitive, rapid, and compact technique for the in situ three-dimensional reconstruction of the distribution of fluorescent markers situated deep in strongly scattering biological tissues and is used to indicate various pathologies, such as cancerous growths [5], Alzheimer’s disease [6], or the results of drug therapy [7]. The markers usually used for optical tomography purposes are either dye molecules or quantum dots emitting light in the Stokes region of the spectrum. Unfortunately the self-luminescence of biological tissues in that case contributes substantially to the signal being detected and thus reduces the spatial resolution of the technique [8]. To exclude the wideband autoluminescence of biotissues to be excluded when detecting luminescence signals. The use of multispectral techniques [10], a priori information [11, 12], and time separation of marker luminescence and tissue self-luminescence signals, based on the application of pulsed excitation sources [13], only insignificantly improve spatial resolution.

Actively studied in the past decade have been nanoparticles comprising rare earth metals [14]. These particles are characterized by anti-Stokes luminescence, which allows the effect of self-luminescence of biotissues to be excluded when detecting luminescence signals. The use of upconverting nanoparticles (UCNPs) for FDOT purposes was limited because of their low quantum yield. However, particles have recently been synthesized whose conversion efficiency comes to a few percent, which is sufficient for their visualization in the bulk of diffuse scattering biotissues at excitation intensities below their optical damage threshold [15–18]. Xu and associates [19–21] have demonstrated that the use of the NaYF4:Yb3+Tm3+ UCNPs that feature a nonlinear dependence of their conversion efficiency on the excitation intensity allows the spatial resolution of the trans-luminescent FDOT technique to be
increased by a factor of 1.41 by comparison with the dye molecules widely used nowadays, whose Stokes luminescence intensity depends linearly on the intensity of the exciting radiation.

We suggest a novel epiluminescent technique with which the photoluminescence (PL) excitation source and the PL signal detector are found on the same side of the object under study. The technique makes use of raster scanning fiber imaging (RSFI) for the visualization of UCNPs. A special optical fiber probe with combined PL signal excitation and detection channels makes it possible to raise the resolution of the FDOT technique to an unprecedented level. The lateral resolution of the RSFI technique using UCNPs of quadratic nonlinearity exceeds that of the wide-field imaging (WFI) technique by a factor of 1.8.

2. Materials and methods

In this work, we used optically bright NaYF₄:Yb³⁺Tm³⁺@NaYF₄ core/shell nanoparticles synthesized by us by the method described in [16]. Figure 1 presents the size distribution histogram of the nanoparticles. The average size of the nanoparticles is 16 nm. As shown by X-ray diffraction, the nanoparticles are in the β-phase. Figure 2 shows the PL spectrum of the UCNPs obtained with a Model Fluorolog-3 HJY spectrofluorimeter using excitation from a 975-nm laser. Present in the PL spectrum is a strong line at a wavelength of 800 nm and two weak lines in the region of 470 and 650 nm. The radiation emitted in the short-wave region of the spectrum was filtered out, and it was only the 800 nm radiation that was used in experiment. Radiation at 975 and 800 nm suit well the purposes of deep optical probing, for they fall within the window region of biological tissues [22]. The inset in Fig. 2 shows the intensity of the 800-nm emission of the nanoparticles as a function of the exciting radiation intensity. It can be seen from the slope of the curve on a log-log scale that the emission of the UCNPs is quadratic in intensity. The PL conversion efficiency defined as a ratio of the emitted PL power and absorbed excitation power (measured in terms of W/W) of the as-synthesized upconversion nanoparticles was measured by an integrating sphere as 4% at the excitation light with light intensity of 70 W/cm² in intensity.

To study the spatial resolution of nanoparticle visualization techniques, we used a biotissue phantom made of polyvinylchloride plastisol (M-F Manufacturing Co., USA) by the method described in [23], with addition of TiO₂ nanoparticles (Sigma-Aldrich, Germany) 510 nm in average size and 3 mg/dl in concentration. The scattering coefficient μs = 50 cm⁻¹, absorption coefficient μa = 0.2 cm⁻¹, and anisotropy factor g = 0.57 of the phantom depended but weakly on the radiation wavelength in the near IR region.
In this work, we studied two epiluminescent visualization techniques: raster scanning fiber imaging and wide-field imaging (Fig. 3). Polymer strips with homogeneously embedded UCNPs 10 volume percent in concentration were used as markers. The marker strips (1.5 mm wide and 8 mm long) were immersed in the phantom to a depth of 4 mm. Where two marker strips were used, the distance between their centers was varied from 3 to 12 mm.

With the WFI technique, the collimated radiation of a CW semiconductor laser ($\lambda = 975$ nm) passed through a dichroic mirror and illuminated the biotissue phantom. The diameter of the light spot on the phantom surface exceeded the distance between the marker strips. The PL signal ($\lambda = 800$ nm) was recorded by a Model Falcon EMCCD camera (Raptor Photonics). The position of the camera was adjusted so as to make its matrix and the top surface of the phantom lie in optically conjugate planes. Rejection filters were used to suppress the excitation signal in the detection system. All the components of the experimental setup were fixed.
With the RSFI technique, the laser radiation was coupled into an optical fiber probe. The probe with spatially combined PL excitation and detection channels comprised an assembly of optical fibers 0.6 mm in diameter and 0.22 in numerical aperture. The central, metalized fiber was used for excitation, while the fibers along the periphery served to detect the PL signal. The distribution of the intensity of scattered radiation on the top surface of the phantom was determined by moving the probe over the surface. The 975 nm radiation was filtered out and the PL signal was recorded with the EMCCD camera used as a linear detector, the matrix of the camera and the end face of the probe being located in optically conjugate planes. The gap between the probe and the surface of the phantom was 0.5 mm. Increasing the distance from the probe to the surface of the phantom up to 3 mm had no effect on the spatial resolution of the technique.

To quantitatively elucidate the question as to the lateral spatial resolution of the WFI and RSFI techniques, we conducted experiments with two marker strips beneath the scattering medium of the phantom.

In what follows, we will present the theory of the methods being considered, based on the definition of radiance for a diffuse scattering medium, as well as a comparative quantitative evaluation of their lateral spatial resolution and an estimate of the sensitivity of the RSFI technique as regards the determination of the occurrence depth of UCNPs under a scattering medium.

3. Results and discussion

Let the scattering medium be a plane-parallel plate of thickness \( d \) formed by the planes \( z = 0 \) and \( z = d \), as shown in Fig. 4. Light beam of wavelength \( \lambda = 975 \) nm and intensity \( I_0 \) is normally incident on the medium. \( r_p = (x_p, y_p, z = 0) \), \( r_0 = \{x_0, y_0, z = 0\} \) are the coordinates of the light beam center and sampling point, respectively. We assume a negligibly small marker site \( \Delta S(r_0) = \Delta x \Delta y \) located in the vicinity of the point \( r_e = \{x_e, y_e, z = d\} \). In the case where the PL signal is excited and detected with an optical fiber probe, \( r_p = r_0 \). The probe cross-section \( \Delta \sigma(r_0) \) is small on the scale of the light intensity variation.

In our theoretical analysis, we proceeded from the following definition of the radiance \( B^{-0}(r_s, r_0) \) of the surface \( z = 0 \) [23]:

\[
B^{-0}(r_s, r_0) = \frac{\Delta \Phi(r_0)}{\Delta \sigma(r_0) \cos \theta(r_s, r_0) \Delta \Omega},
\]

\[
\Delta \Omega(r_s, r_0) = \sin \theta(r_s, r_0) \Delta \theta(r_s, r_0) \Delta \phi = \frac{\Delta S(r_0) \cos \theta(r_s, r_0)}{R_s},
\]

\[
R_s = \sqrt{(x_s - x_e)^2 + (y_s - y_e)^2 + d^2}, \quad \cos \theta(r_s, r_0) = d / R_s.
\]

Here \( \theta, \phi \) are spherical coordinates, \( \Delta \Phi(r_0) \) is the power emitted by the site \( \Delta \sigma(r_0) \) within the solid angle \( \Delta \Omega(r_s, r_0) \), and \( B^{-0}(r_s, r_0) \) is the radiance in the direction of the vector \( R_0 = \{(x_s - x_0), (y_s - y_0), d\} \). In the case of isotropic scattering medium considered below, \( B^{-0}(r_s, r_0) = B^{-0}(| R_0 |) \).

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Fig. 4. A schematic diagram of the scattering of the light beam incident on a spatially inhomogeneous medium. The extended and narrow light beam geometries are considered. \( \mathbf{N} \) is a normal to the surface \( z = 0 \), \( r_p \) is the coordinate of the center of the incident beam, \( r_0 \) is the coordinate of the sampling point situated at the plane \( z = 0 \), \( r_s \) is the coordinate of the photoluminescent marker, \( (\mathbf{R})_0 = r_0 - r_s \). \( I_{i}(r_0 - r_p) \) denotes the intensity of the light beam, \( \Delta \sigma(r_0) \) – the fiber probe cross-section, \( \Delta S(r_s) \) – the photoluminescent marker surface site of the size small compared with the light beam intensity variation, \( \theta \) is the angle between \( \mathbf{N} \) and \((\mathbf{R})_0 s\).

Taking into account the fact that the power incident on a unit area of the surface \( z = 0 \) is equal to the power scattered from a unit surface area in all directions, we find from expressions (1)

\[
I_{i}(r_0 - r_p) = \int \int B^{z=0}(\theta, \phi) \cos \theta d\Omega(\theta, \phi) = \int \int B^{z=0}(|\mathbf{R}_0|) \frac{d^2}{R^2_0} dx dy.
\]

(2)

Since the absolute value of radiance is determined by the illuminance \( I_{i}(r_0 - r_p) \) of the site \( \Delta \sigma(r_0) \), it is appropriate to introduce into consideration the normalized radiance \( F(R_0) \):

\[
B^{z=0}(|\mathbf{R}_0|) = I_{i}(r_0 - r_p) F(R_0),
\]

\[
B^{z=0}(|\mathbf{R}_0|) = I_{i}(r_0 - r_p) F(R_0) \exp(-\mu_\sigma R_0),
\]

(3)

where \( \mu_\sigma \) is the absorption coefficient. The normalized radiance \( F(R_0) \) is a characteristic of the spatially inhomogeneous medium, which is determined by its scattering properties. Substitution of the first expression of (3) into the second equality of (2) leads to the normalization limitation on the form of the scattering function \( F(R_0) \):

\[
\int \int F(R_0) \frac{d^2}{R^2_0} dx dy = 1.
\]

Such media as uniformly scatter light in all directions are called ideally scattering or Lambertian media. It follows from the first expression of (2), relation (1) for the solid angle \( d\Omega(\theta, \phi) \), and the first equality of (3) that for Lambertian media

\[
B^{z=0}(|\mathbf{R}_0|) = \frac{I_{i}(r_0 - r_p)}{\pi}, \quad F(R_0) = \frac{1}{\pi}.
\]

(4)
The intensity emitted as a result of some nonlinear optical process (in the given case, it is the intensity emitted by UCNP at a wavelength of $\lambda = 800$ nm) depends on the intensity of the incident radiation ($\lambda = 975$ nm), the intensity of the beam emitted from the point $r_p$ of the surface $z = 0$ within a unit solid angle being equal to $\Delta\Phi(r_0) / [\Delta\sigma(r_0) \cos \theta]$. In that case, we find from relations (1) and (3) that the total radiation intensity $I_{\lambda=975}(x, y, z = d)$ at the wavelength $\lambda = 975$ nm in the vicinity of the point $(x, y)$ of the surface $z = d$, which is determined by the contributions from the entire surface $z = 0$, may be represented in the form

$$I_{\lambda=975}(x, y, z = d) = \int \int F \left( R_{i0} \right) I_i \left( r_0 - r_p \right) \exp \left( -\mu_p R_{i0} \right) \frac{d}{R_{i0}} \, dx_0 \, dy_0. \tag{5}$$

Considering the quadratic relationship between the photoluminescence intensity at the wavelength $\lambda = 800$ nm and the incident radiation intensity at the wavelength $\lambda = 975$ nm, $I_{\lambda=800} = \eta^2 I_{\lambda=975}$, the intensity of photoluminescence produced by the marker strip in the vicinity of the point $(x, y, d)$ of the site $\Delta S(r_i)$ may be represented, according to expression (5), in the form

$$I_{\lambda=800}(x, y, z = d) = \eta^2 \left[ I_{\lambda=975}(x, y, z = d) \right]^2 = \eta \left[ \int \int F \left( R_{i0} \right) I_i \left( r_0 - r_p \right) \exp \left( -\mu_p R_{i0} \right) \left( \frac{d}{R_{i0}} \right)^3 \, dx_0 \, dy_0 \right] \tag{6}$$

where $\eta$ is the proportionality coefficient associated with the upconversion efficiency.

The radiance of the marker strip at the wavelength $\lambda = 800$ nm in the direction of the vector $r_1$ (from the point $(x, y)$ of the plane $z = 0$ toward the point $(x, y)$ of the plane $z = d$) is given by the formulas

$$B_{\lambda=800}^d(x, y) = F(R_{i1}) I_{\lambda=800}(x, y, z = d), \quad R_{i1} = \sqrt{(x_i - x)^2 + (y_i - y)^2 + d^2}. \tag{7}$$

To calculate the radiation intensity at the point $(x, y)$ of the plane $z = 0$, we use formula (5) subject to the substitution

$$F \left( R_{i0} \right) I_i \left( r_0 - r_p \right) \rightarrow B_{\lambda=800}^d(x, y), \quad R_{i0} \rightarrow R_{i1}, \quad dx_0 \, dy_0 \rightarrow dx \, dy.$$

We will take it that the surface area of the marker site $\Delta S(x, y)$ is small, so that the functions $F(R_{i1})$ and $(d / R_{i1})^3 \exp(-\mu_p R_{i1})$ are changed but little upon variation of the coordinates $x_i, y_i$ within the limits of this site. In that case, considering relations (6) and (7), we get the following expression for the light intensity at the wavelength $\lambda = 800$ nm at the point $(x, y)$ of the plane $z = 0$:

$$I_{\lambda=800}^{\text{beam}}(x, y, z = 0) = A_{\text{beam}} \left( r_i, r_p \right) F \left( R_{i0} \right) \left( d / R_{i1} \right)^3 \exp \left( -\mu_p R_{i1} \right), \quad A_{\text{beam}} \left( r_i, r_p \right) = \eta \frac{\Delta S(r_i)}{dx \, dy} \int \int F \left( R_{i0} \right) I_i \left( r_0 - r_p \right) \exp \left( -\mu_p R_{i0} \right) \left( d / R_{i0} \right)^3 \, dx_0 \, dy_0 \frac{d}{d^3}. \tag{8}$$

where the function $A_{\text{beam}} \left( r_i, r_p \right)$ is independent of $r_i$ and can be considered constant at fixed $r_p$ and $r_i$. The distance $R_{i1}$ is defined by formula (7).

If the plane $z = 0$ of the phantom and the image plane of the CCD camera are optically conjugate, the illuminance $E$ measured by the camera in its image plane is proportional to the
product of the luminance of the source into the relative aperture \((D/f)^2\) of the objective lens, where \(D\) is the diameter of the lens and \(f\), its focal length [24]. For this reason, when the surface \(z = 0\) is illuminated by a wide light beam, the illuminance measured by the CCD camera is proportional to the total light intensity \(I_{A,800}(x, y, z = 0)\) given by expressions (8).

The RSFI technique is a particular case described by formula (8) with due regard for the following specificities. First, the incident beam with an intensity of \(I_1(r_0 - r_p)\) is narrow, secondly, the incidence spot \(r_0 = r_p\) of the light beam with \(\lambda = 975\) nm is matched with the spot \(r_1\) at which the scattered light with \(\lambda = 800\) nm is detected in the plane \(z = 0\). Let the surface area of the probe be \(S_{probe} = \pi r_{probe}^2\), where \(r_{probe}\) is the radius of the optical fiber. We will assume that in the plane \(z = 0\)

\[
I_1(r_0 - r_p) = \begin{cases} 
I_{0} = \text{const}, & \text{if } |r_0 - r_p| \leq r_{probe} \\
0, & \text{if } |r_0 - r_p| > r_{probe}
\end{cases}
\]

In addition, we will assume that the probe surface area is so small that the functions \(F(R_{\lambda,0})\) and \((d / R_{\lambda,0})^3 \exp(-\mu R_{\lambda,0})\) in the localization region of the incident light intensity \(I_1(r_0 - r_p)\) remain practically unchanged. In that case, we have from relation (8)

\[
I_{probe}^{probe}(x, y, z = 0) = A_{probe}(R_{\lambda,0})[F(R_{\lambda,0})] \left( (d / R_{\lambda,0})^3 \exp(-3\mu R_{\lambda,0}) \right),
\]

where the distance \(R_{\lambda,0}\) is defined by formula (1).

Thus, the shape of the scattered radiation signal is determined by the product of the normalized radiance \(F(R_{\lambda,0})\) and the function \(\Omega_0(R_{\lambda,0}) = (d / R_{\lambda,0})^3 \exp(-\mu R_{\lambda,0})\) conditioned by the radiation decay and the dependence of the solid scattering angle on the coordinates of the marker and the observation point. In the case of strongly scattering media wherein the dependence \(F(R_{\lambda,0})\) is much weaker than \(\Omega_0(R_{\lambda,0})\) (Lambertian media (4) with \(F(R_{\lambda,0}) = 1/\pi\) can serve as an example), the shape of the scattered radiation signal is determined by the function \(\Omega_0(R_{\lambda,0})\). In the opposite case, it will be governed by the normalized radiance \(F(R_{\lambda,0})\) of the medium. In comparing between theoretical and experimental results, we will assume that the medium of the phantom is strongly scattering (\(F(R_{\lambda,0}) = \text{const}\)). Note that the decay coefficient \(\mu_a\) found from comparison between experimental and theoretical dependences (8), (9) is somewhat overrated, for the actual ray trajectories are curved [24] and their length is greater than the distance \(R_{\lambda,0}\) assumed in the theory (the second formula of (3)) to allow for radiation decay.

In expressions (8), (9) in the case of strongly scattering medium, \(F(R_{\lambda,0}) = \text{const}\) and \(d / R_{\lambda,0} \leq 1\). Therefore, normalized distribution (9) is narrower than normalized distribution (8), the relation between them being as follows:

\[
\frac{I_{\lambda,800}^{probe}(x, y, z = 0)}{A_{probe}[F(R_{\lambda,0})]} = \left( \frac{I_{\lambda,800}^{beam}(x, y, z = 0)}{A_{beam}[F(R_{\lambda,0})]} \right)^3.
\]
Figure 5 presents the experimental dependences of PL at $\lambda = 800$ nm obtained when irradiating the marker strip with the wide light beam (the WFI technique, full circles) and with the fiber probe (the RSFI technique, open circles). Solid curves 1 and 2 are constructed by formulas (8) and (9), respectively, at $\mu_s = 0.2$ cm$^{-1}$, $z = d = 4$ mm, $x_0 = x_s = 36.65$ mm, $y = y_p = \text{const}$, $A_{\text{beam}} = \text{const}$, $F(R_{s0}) = \text{const}$.

It can be seen that the width of the PL signal recorded by the RSFI technique is narrower by a factor of 1.77 than that of the signal recorded by the WFI technique (width ratio at half maximum). There is good agreement between the theoretical and experimental results.

Consider the case where two marker strips are localized in the plane $z = d$ at the points with the coordinates $r_a = \{x_a, y_a\}$ and $r_b = \{x_b, y_b\}$. Theoretical relations (8) and (9) in that case are reduced to the form

$$I_{\lambda=800}^{\text{probe}}(x_0, y_0, z=0) = A_{\text{probe}} \left\{ \left[ F(R_{a0}) \right]^3 \left( d / R_{a0} \right)^9 \exp(-3\mu_a R_{a0}) \right\} + \left[ F(R_{b0}) \right]^3 \left( d / R_{b0} \right)^9 \exp(-3\mu_a R_{b0}) \right\},$$

$$I_{\lambda=800}^{\text{beam}}(x_0, y_0, z=0) = A_{\text{beam}} \left\{ F(R_{a0}) \left( d / R_{a0} \right)^3 \exp(-\mu_a R_{a0}) \right\} + F(R_{b0}) \left( d / R_{b0} \right)^3 \exp(-\mu_a R_{b0}) \right\},$$

where $A_{\text{probe}}$ and $A_{\text{beam}}$ are constants.

In our experiments, the PL signal ($\lambda = 800$ nm) was detected in the plane $z = 0$ with one and the same optical fiber probe, or with a wide light beam with one and the same transverse intensity distribution. The distance between the marker strips $(x_b - x_a)$ was taken at 3.5, 4, 5.1, 6, 7.1, 7.8, and 9.2 mm.

Figure 6 presents PL intensity distributions recorded by the WFI and RSFI techniques in the case of two marker strips spaced a distance of $(x_b - x_a) = 5.1$ mm apart. Solid curves 1 ($I_{\lambda=800}^{\text{probe}}(x_0, y_0 = y_a = y_b, z=0)$) and 2 ($I_{\lambda=800}^{\text{beam}}(x_0, y_0 = y_a = y_b, z=0)$) are constructed using
expressions (11) and (10), respectively, where it is assumed that \( \mu_a = 0.2 \text{ cm}^{-1}, x_a = 33.4 \text{ mm}, x_b = 38.5 \text{ mm}, d = 4 \text{ mm}, \) \( F(R_{ab}) = F(R_{00}) = \text{const}, \) \( A_{\text{beam}} = \text{const}, \) \( A_{\text{probe}} = \text{const}. \)

One can see from this figure that, first, there is good agreement between the experimental and theoretical data and secondly, the probe-assisted excitation and detection of the PL signal (RSFI technique) provides for higher spatial resolution of the localizations of the marker strips found beneath the strongly scattering phantom, as compared to the WFI technique.

Quantitatively to evaluate the lateral spatial resolution of the techniques under study here, we will use the Rayleigh criterion [25]. According to this criterion, for two radiation peaks formed by two luminescent markers located at points \( \{x_a, y_a = y_0\} \) and \( \{x_b, y_b = y_0\} \) in the plane \( z = d \) to be spatially resolved, it is necessary that the following condition be satisfied:

\[
R(x_a, x_b) = \frac{I_{\text{max}}(x_a, y_b) - I_{\text{min}}(x_a, y_b)}{I_{\text{max}}(x_a, y_b)} \times 100 \geq 20\%.
\]  

(12)

Considering good agreement between the experimental and theoretical results, we use expressions (10) and (11) to construct the functions \( R(x_a, x_b) \) by substituting either \( (I_{\text{probe}}^{\text{max,min}})_{x_a,y_b} \) or \( (I_{\text{beam}}^{\text{max,min}})_{x_a,y_b} \) into expression (12). The dependences of the parameters \( R(x_a, x_b) \) corresponding to the RSFI technique (curve 1) and the WFI technique (curve 2) on the distance between the marker strips are presented in Fig. 7. One can see from this figure that in the case of the RSFI technique the Rayleigh criterion is satisfied at \( (x_b - x_a) \geq 3.6 \text{ mm} \), whereas with the WFI technique it is satisfied at \( (x_b - x_a) \geq 6.5 \text{ mm} \). This means that the lateral spatial resolution of the RSFI technique is higher by a factor of \( (6.5/3.6) \) than that of the WFI technique.
It follows from the above analysis that in the case where a dye with a linear dependence of the PL intensity on the intensity of the incident light beam is used instead of UCNPs, formulas (8) and (9) are respectively transformed into

\[ I_{\text{beam}}^{\lambda=975}(x_0, y_0, z=0) = D_{\text{beam}} F(R_{\text{p}})(d/R_{\text{p}})^3 \exp(-\mu_d R_{\text{p}}), \]  

\[ I_{\text{probe}}^{\lambda=975}(x_0, y_0, z=0) = D_{\text{probe}} \left[ F(R_{\text{p}}) \right]^2 (d/R_{\text{p}})^6 \exp(-2\mu_d R_{\text{p}}), \]  

where \( D_{\text{beam}} \) and \( D_{\text{probe}} \) can be considered to be constant at fixed \( r_p \) and \( r_s \). It follows from comparison between formulas (13) and (8) that the replacement of the marker strip with a dye by that with UCNPs does not improve the lateral spatial resolution of the WFI technique (the dependence of the PL intensity on \( R_{\text{p}} \) remains unchanged). It can be seen from expressions (13), (8), and (14) that the RSFI technique using a dye marker has the advantage of higher lateral spatial resolution over the WFI technique using either dye of UCNPs markers. The dependence \( I_{\text{probe}}^{\lambda=975} \sim (d/R_{\text{p}})^6 \) is stronger than \( I_{\text{beam}}^{\lambda=975}, I_{\lambda=800}^{\text{beam}} \sim (d/R_{\text{p}})^3 \). And finally, it follows from comparison between distributions (14) and (9) that the change-over from dye to UCNPs markers in the RSFI technique is accompanied by an additional improvement of its lateral spatial resolution. The dependence \( I_{\text{probe}}^{\lambda=975} \sim (d/R_{\text{p}})^6 \) is replaced by \( I_{\text{probe}}^{\lambda=975} \sim (d/R_{\text{p}})^9 \).

Now let us offer an estimate of the capability of the RSFI technique to determine the occurrence depth \( d \) of UCNPs. Figure 8 presents an experimental dependence of \( I_{\text{RSFI}}^{\lambda=975} \) on \( x_0 \) (cf. Fig. 6) and theoretical dependences (10) constructed at various \( d \) values for the case where two marker strips are located beneath the phantom. It follows from this figure that changing the thickness of the phantom from its true value of 4 mm by a mere \( \pm 0.5 \) mm leads to a perceptible difference between the experimentally measured depth of the valley between the peaks and the value predicted by the theory at \( d = 3.5 \) and 4.5 mm. This means that by comparing between experimental and theoretical results one can determine the occurrence depth \( d \) of the marker correct to \( \pm 0.5 \) mm.
Fig. 8. Experimental dependence of the intensity of the PL signal ($\lambda = 800$ nm) obtained by moving the probe along the intersection line of the planes $z = 0$ and $y = y_p = \text{const}$ (open circles, see Fig. 6). Solid curves 1 through 3 are constructed by formula (10) at $\mu_a = 0.2$ cm$^{-1}$, $x = 33.4$ mm, $x_0 = 38.5$ mm, $x (s_{op}) = x (s_{op}) = \text{const}$, and $A_{\text{probe}} = \text{const}$. The distance between the marker strips is $(x_0 - x_a) = 5.1$ mm. The thickness of the phantom: $d = 3.5$ mm (1), $d = 3.5$ mm (2), $d = 4.5$ mm (3).

Now consider the case where only one strip with nanophosphor markers is located in the plane $z = d$ in the vicinity of the point $\{x_s, y_s\}$. In this case, the depth of occurrence of the markers is impossible to determine within sufficiently high accuracy, because the shape of the scattered PL signal changes but little as a function of $d$. For this reason, we suggest using two probes. Let $\{x, y\}$ and $\{x + h, y\}$ be the coordinates of the first and the second probe in the plane $z = 0$, $h$ being the distance between the probes. Both probes are being concurrently moved in the $x$-axis direction along the intersection line of the planes $z = 0$ and $y = y_s$, with the distance $h$ between them remaining constant. We will assume that the first and the second probe are sources of radiation with a wavelength of $\lambda = 975$ nm and that the PL signal with a wavelength of $\lambda = 800$ nm is detected by the second probe. The expressions describing the distribution of the PL radiance along the scanning line $(y = y_s, z = 0)$ have the form

$$F(R_{1,2}) = \text{const}$$

$$I_{KSF}' = I_{\text{probe}}(x, y = y_s, z = 0) =$$

$$D_{\text{probe}} \left[ (d/R_1)^3 \exp(-\mu_a R_1) + (d/R_2)^3 \exp(-\mu_a R_2) \right],$$

$$R_1 = \sqrt{(x - x_s)^2 + d^2}, \quad R_2 = \sqrt{(x + h - x_s)^2 + d^2}, \quad D_{\text{probe}} = \text{const.}$$

(15)
Note that the fixed distance $h$ between the probes should be slightly greater than the half-width of the incident light beam at the base in the case of single-probe PL signal detection (see curve 2 in Fig. 5). In that case, distribution (15) becomes asymmetric and sensitive to the variation of the thickness $d$ of the phantom. This is illustrated by Fig. 9 wherefrom one can see that a ± 0.5 mm change in $d$ is accompanied by a noticeable deformation of the shape of the distribution. This means that by fitting the theoretical distribution to its experimental counterpart one can determine the depth of occurrence of the luminescent marker beneath the scattering medium to an accuracy of at least no worse than ± 0.5 mm.

4. Conclusion

A comparison is made between the WFI and RSFI techniques for diffuse optical visualization of luminescent markers in strongly scattering media. The shape of the PL signal is theoretically demonstrated to be determined by the mutual arrangement of the radiation source and detector. An agreement is obtained between the theoretical and experimental results. It is proved that the lateral spatial resolution of the probe-assisted excitation and detection of PL signals (RSFI technique) is 1.8 times that of the WFI technique. The RSFI technique is demonstrated to be capable of determining the depth of occurrence of luminescent markers beneath a scattering medium accurate to within ± 0.5 mm. The RSFI technique suggested may prove useful in exact localization of cancerous formations in biological tissues.

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