Would near-infrared fluorescence signals propagate through large human organs for clinical studies?

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Received November 26, 2001

We predict the capacity of near-infrared fluorescent signals to propagate through human tissue for non-invasive medical imaging. This analysis employs experimental measurements of a biologically relevant local fluorochrome embedded in tissue-like media and predicts the equivalent photon counts expected from breast, lung, brain, and muscle as a function of diameter by use of an analytical solution of the diffusion equation that can take into account large arbitrary geometries. The findings address feasibility issues for clinical studies and are relevant to recent development of near-infrared fluorescent probes and molecular beacons for in vivo applications. © 2002 Optical Society of America

OCIS codes: 170.3880, 170.3860.

The recent interest in novel Indocyanine derivatives, targeted dyes, and molecular beacons for in vivo imaging opens exciting pathways for new modalities of disease detection based on functional and molecular contrast. The clinical utility of such probes, however, depends on imaging of fluorochromes localized deep in tissues. Currently, there exists only limited information on the detection capacity of fluorochromes distributed in the human body. In this Letter we predict the fluorescence strength expected from a small fluorescent lesion, such as a tumor, embedded in human tissue. To achieve realistic calculations, we employed a recently developed tomographic system to measure experimentally the fluorescence detected from a known fluorochrome implanted in a diffuse medium. These experimental measurements were subsequently used to calibrate an analytical solution of the diffusion equation for cylindrical geometries. Using the calibrated solution, we computed the fluorescence signals expected from human breast, lung, and brain tissue as a function of diameter.

To obtain straightforward insight into fluorescence-detection capacity in tissues, we opted for a prediction model that yields a linear relationship among fluorescence counts, tumor characteristics, and instrument gain. We chose an analytical solution of the diffusion equation that is accurate when one is simulating small objects (smaller than 5-mm diameter), which is based on the Born approximation:

\[ U_{\Omega}(r_s, r_d) = S_0 \gamma \epsilon \cdot [F(r)] \cdot G(r, r_s, r_d) \cdot V, \quad (1) \]

where \( U_{\Omega}(r_s, r_d) \) is the fluorescence signal detected at position \( r_d \) that is due to a source at position \( r_s \) and a fluorochrome distribution of concentration \([F(r)]\) and volume \( V \) at position \( r_s \). \( S_0 \) is a gain factor that contains the laser strength and overall system gain, \( \gamma \) is the fluorochrome quantum yield, \( \epsilon \) is the fluorochrome extinction coefficient, and \( G(r, r_s, r_d) \) is the Green function solution of the diffusion equation, which combines intrinsic and fluorescence signal propagation and is calculated for cylindrical geometry by use of the Kirchhoff approximation adapted for diffusive waves. The Kirchhoff approximation is a very time-efficient computation method and has been used extensively in acoustics and physical optics (see Ref. 9 and references therein). All the simulations were done for a diffusive cylinder of refractive index 1.33 surrounded by air. The error introduced as a result of the Kirchhoff approximation with respect to the exact solution was, for all cases shown here, <1%.5

Equation (1) is very useful for this analysis, since it efficiently separates the problem that is being investigated into an optical-property-dependent nonlinear component (Green function) and linearly related experimental factors, such as the laser power or detection gain (via the term \( S_0 \)), and the tumor characteristics, such as volume or fluorochrome concentration. This separation means that, for a given medium, calculations of the fluorescence counts that are expected when the laser power is improved or the tumor volume is increased, for example, can be obtained by linear scaling of an existing simulation.

A generic geometry was employed for the simulations performed. Figure 1 depicts the middle slice of a three-dimensional setup consisting of a 12-cm-long cylinder with variable diameter and a smaller cylinder simulating a tumor, immersed in the middle of the larger cylinder at 0.5 cm off center. The small cylinder attains an equivalent volume of \( \sim 100 \) \( \mu \)L (4-mm diameter, 8-mm length), which represents a typical small cancer detected by current radiological techniques. The geometry was purposely asymmetric because symmetric artifacts or errors that are due...
to symmetric structures, if they are present, are not easily separated from real signals. We chose to simulate four basic human tissues, i.e., breast (of a young and an old adult), lung, brain, and muscle. The optical properties chosen for these tissues were generic for the near-infrared region and are summarized in Table 1. We further assumed a typical uptake of macromolecules into malignant tumors, as previously determined by biodistribution studies,10 which yields concentrations of ~100 nM of agent (i.e., 10 pmol of dye in the 100-μL tumor that was selected) 24 h after administration.

Fluorescence measurements were obtained experimentally and were subsequently used to calibrate Eq. (1). The experimental setup was identical to the geometry and dimensions shown in Fig. 1, with the large-cylinder diameter set to 2.5 cm. The small cylinder was made from fused quartz (Wilmad Glass, Buena, N.J.) and contained 100 nM of the infrared fluorochrome Cy5.5 (Amersham Pharmacia Biotech, Piscataway, N.J.) in 0.5% Intralipid. The Cy5.5 dye has quantum yield $\gamma = 0.28$, extinction coefficient $\varepsilon = 250,000 \text{ M}^{-1} \text{ cm}^{-1}$, absorption peaks at 675 nm, and fluorescence emission peaks at 694 nm. The large cylinder was constructed from black Delrin and contained a water solution of 0.5% Intralipid. An array of 12 140-μm/100-μm-diameter clad/core source fibers (Fiber Instrument Sales, Oriskany, N.Y.) was placed equidistantly around the periphery of the cylinder at the middle plane and excited the fluorochrome from multiple angles. A similar ring of 12 detection fiber bundles (Dolan-Jenner Industries, Lawrence, Mass.), each 3 mm in diameter, was placed 0.5 cm above the source ring, which was rotated by an angle of 15°. Both the source and the detector fiber optics came face to face with the inner wall of the cylinder. The light source was a 670-nm laser diode (B&W TEK, Newark, Del.) set to 20 mW of output power at the fiber tip. Sequential light coupling into the different source fibers was accomplished with an optical switch (DiCon FiberOptics, Richmond, Calif.). For light detection, the fiber bundles were arranged on a 6 cm × 6 cm flat black surface (the fibers were placed 1 cm apart) and imaged on a 1.2 cm × 1.2 cm, −40° C-cooled CCD chip by a VersArray 512B CCD camera (Roper Scientific, Acton, Mass.) and a Micro-Nikkor f/2.8D lens (Nikon Tokyo JP). A reference fiber was used to correct for laser light amplitude variations throughout the measurement.

Fitting the experimental measurements to Eq. (1) yielded the gain factor, $S_0$, of the setup employed. By use of this experimentally determined $S_0$, Eq. (1) becomes a realistic prediction model that relates fluorescence photon counts to tissue optical properties, dimensions, volume, concentration, and quantum yield. Figure 2(a) depicts the average fluorescence strength, $\bar{S}$, on a logarithmic scale, that is expected at the peripheries of the different tissues simulated as a function of organ diameter. $\bar{S}$ is measured in counts per second per square millimeter of CCD area, and it is calculated as the average signal measured with the fluorochrome present minus the signal measured without the fluorochrome (the background measurement that is due to CCD offset and noise and bleed-through signals allowed by the cutoff filter). $\bar{S}$ is a measure of the physical quantity of interest in this study, i.e., the fluorescence strength, and allows a direct observation of bulk fluorescent signals at the boundary.

Figure 2(a) indicates that the attenuation rate of breast tissue and the adult lung in the near infrared is approximately 1 order of magnitude every 4 cm, the attenuation rate in denser breast approaches 1 order of magnitude every 3 cm, muscle yields 1 order of magnitude attenuation every 2 cm, and brain tissue attenuates near-infrared light by 1 order of magnitude every 1.5 cm. In absolute terms, Fig. 2(a) denotes that fluorescent photons from small cancerlike volumes can be detected in most tissues examined, even after the photons propagate for several centimeters. This estimate is conservative, since technological advances could lead to significant detection improvements relative to the experimental setup used in this study. More-efficient photon detection can be achieved by direct fiber coupling on the CCD chip or a 1:1 lens system, yielding more than a 40-fold detection improvement. The use of higher light power could significantly increase the expected fluorescence photon strength, especially since we have found that no saturation or photobleaching effects occur for laser powers up to 100 mW and

![Diagram](image)

**Table 1. Optical Properties Used in the Simulations**

<table>
<thead>
<tr>
<th>Tissue Type</th>
<th>Absorption Coefficient $\mu_a$ (cm$^{-1}$)</th>
<th>Reduced Scattering Coefficient $\mu_s'$ (cm$^{-1}$)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast (ages 40–70)</td>
<td>0.03</td>
<td>9</td>
<td>6</td>
</tr>
<tr>
<td>Breast (ages 20–40)</td>
<td>0.05</td>
<td>11</td>
<td>6</td>
</tr>
<tr>
<td>Lung</td>
<td>0.01</td>
<td>20</td>
<td>7</td>
</tr>
<tr>
<td>Muscle</td>
<td>0.15</td>
<td>9</td>
<td>8</td>
</tr>
<tr>
<td>Brain (adult)</td>
<td>0.15</td>
<td>12</td>
<td>9</td>
</tr>
</tbody>
</table>
Fig. 2. (a) Average fluorescent photon counts expected at the peripheries of different organs as a result of the fluorochrome shown in Fig. 1, as a function of diameter. (b) Average fluorescent photon counts predicted for 2-orders-of-magnitude improvement in detection technology. Three signal-to-noise ratio levels for shot-noise-limited detection are also plotted (dashed lines).

fluorochrome concentrations that exceed 800 nM (results not shown). For imaging purposes, increasing the exposure time and using cooled CCD chips and lower read-noise circuitry than used in this study can further improve the detection capacity.

Figure 2(b) illustrates the photon counts expected for a moderate, 2-orders-of-magnitude increase in detection capacity. There are also three horizontal lines plotted, marking the 10-, 20-, and 30-dB signal-to-noise levels achieved, assuming shot-noise-limited detection. In reality, background fluorescence (which is due primarily to nonspecific dye distribution) limits the signal-to-noise ratio achieved, since background signals can be seen as biologically induced noise after appropriate subtraction schemes.\textsuperscript{11} Data set optimization\textsuperscript{12} and appropriate algorithms\textsuperscript{13} can be used to image at a low signal-to-noise ratio, and such considerations should be addressed on a case-to-case basis; this study, however, demonstrates that fluorescence imaging of human tissues becomes an issue of uptake-to-background contrast rather than of propagation feasibility. It is important to the latter argument that fluorescence offers mechanisms for multifold background suppression by use of quenching and activation (dequenching) of fluorescent probes or fluorescence resonance energy transfer. These technologies may significantly minimize background signals \textit{in vivo} and, combined with advances in drug delivery and hardware improvements, surpass the predictions of this study.

We acknowledge discussions with Alexei Bogdanov, Jr., Lee Josephson, Umar Mahmood, and Margaret Eppstein. V. Ntziachristos is grateful to the Cancer Research Fund of the Runyon-Winchell Foundation and to the U.S. Army Congressionally Directed Medical Research Programs for concept award BC995360, J. Ripoll received European Union Training and Mobility of Researchers grant FMRX-CT96, and R. Weissleder received National Institutes of Health grant P50 CA86355. V. Ntziachristos’s e-mail address is vasilis@helix.mgh.harvard.edu.

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