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Recovery of intrinsic fluorescence from single-point interstitial measurements for quantification of doxorubicin concentration

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Abstract

Background and Objective—We developed a method for the recovery of intrinsic fluorescence from single-point measurements in highly scattering and absorbing samples without *a priori* knowledge of the sample optical properties. The goal of the study was to demonstrate accurate recovery of fluorophore concentration in samples with widely varying background optical properties, while simultaneously recovering the optical properties.

Materials and Methods—Tissue-simulating phantoms containing doxorubicin, MnTPPS, and Intralipid-20% were created, and fluorescence measurements were performed using a single isotropic probe. The resulting spectra were analyzed using a forward-adjoint fluorescence model in order to recover the fluorophore concentration and background optical properties.

Results—We demonstrated recovery of doxorubicin concentration with a mean error of 11.8%. The concentration of the background absorber was recovered with an average error of 23.2% and the scattering spectrum was recovered with a mean error of 19.8%.

Conclusion—This method will allow for the determination of local concentrations of fluorescent drugs, such as doxorubicin, from minimally invasive fluorescence measurements. This is particularly interesting in the context of transarterial chemoembolization (TACE) treatment of liver cancer.

Keywords

Fluorescence spectroscopy; P_3 approximation; Radiative transport equation; TACE

INTRODUCTION

Fluorescence spectroscopy can be a valuable tool in the analysis of tissues. There exist a number of biologically relevant compounds that are naturally fluorescent, such as nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide (FAD)[1]. The fluorescence of these and other naturally occurring fluorophores has been used to differentiate between healthy and malignant tissue in the oral cavity[2], the larynx[3], and the colon[4], among others. A number of drugs are fluorescent, and can be tracked and analyzed using fluorescence spectroscopy. One notable example of this is doxorubicin, which is a chemotherapeutic agent used to treat a wide variety of malignancies[5]. There have been a number of studies done to track doxorubicin concentration after injection using fluorescence *in vivo* in mouse models[6, 7] and in excised human breast cancer tissue after treatment[8].

In thin or dilute samples, collected fluorescence spectra can be analyzed directly in order to yield the concentration of the fluorophore. However, in samples with high scattering or absorption, the detected fluorescence spectrum can be heavily distorted. This is due to emitted fluorescence being absorbed and scattered within the sample. Furthermore, the propagation of the excitation light is affected by the background absorption and scattering, resulting in detected fluorescence that may not be proportional to fluorophore concentration. Both the shape and magnitude of detected fluorescence spectra can be altered by background optical properties.

Therefore, if we wish to deduce information about a fluorophore based on a distorted, detected fluorescence spectrum, we need to correct for the effects of propagation through the turbid medium. This has been studied by a number of research groups. Solutions can roughly be divided into two categories: 1) techniques that rely upon knowledge of the sample's optical properties and 2) techniques that retrieve intrinsic fluorescence without pre-existing knowledge of the sample's optical properties. In the first class of techniques, the optical properties of the tissue sample are often determined using diffuse reflectance spectroscopy. These optical properties are then used with a model of light propagation in order to correct for their effects on the fluorescence spectrum. The model often takes the form of Monte Carlo simulation, with the optical properties determined by diffuse reflectance used as inputs to the simulation. Liu *et al* demonstrated accurate recovery of intrinsic fluorescence using an inverse Monte Carlo model informed by diffuse reflectance measurements[9]. Wang *et al* also showed recovery of intrinsic fluorescence, but in a more complex layered model of the skin[10].

Other groups have used approximations to the radiative transport equation in order to model the propagation of fluorescence through a sample. Zhadin and Alfano used a simplified diffusion model in order to correct for distortions to intrinsic fluorescence[11]. Diamond *et al* demonstrated recovery of intrinsic fluorescence using a full diffusion model and spatially resolved reflectance and fluorescence measurements[12]. Reflectance measurements were fit using Monte Carlo simulations in order to extract optical properties, which were then used with a diffusion model of fluorescence in order to recover fluorophore concentration.

Photon migration theory can also be used to correct fluorescence spectra using measured diffuse reflectance, as was demonstrated by Wu, Feld, and Rava[13]. This can result in very simple corrections that consist of dividing the measured fluorescence by the diffuse reflectance spectrum. Finlay *et al* expanded this method by introducing an empirical correction to the amplitude of the detected fluorescence based on a measurement of the diffuse reflectance at the excitation wavelength[14].

Techniques that do not require the optical properties to be known *a priori* have not been investigated as thoroughly. Finlay and Foster used a forward-adjoint fluorescence model in order to recover intrinsic fluorescence by assuming that the absorbers and scatterers in a sample were known, but not their concentrations[15]. This allowed for the simultaneous recovery of fluorophore concentration and background optical properties. Chang *et al* used an analytical expression based on diffusion theory in order to extract intrinsic fluorescence from a 2-layered skin model[16]. This was then used to provide possible diagnostic information on cervical dysplasia.

In this study, we demonstrate a forward-adjoint method for the recovery of intrinsic fluorescence and optical properties from turbid samples using a single interstitial, isotropic source and detector. This recovery does not require a separate diffuse reflectance measurement. Verification of the method is performed in tissue simulating phantoms containing doxorubicin.

MATERIALS AND METHODS

Forward-adjoint fluorescence model

In order to correct for sample-induced distortions to the fluorescence, a model of fluorescence propagation and detection is required. Here, we use the forward-adjoint method, first proposed by Crilly *et al* [17].

In this method, the generation and detection of fluorescence is represented by two functions, the fluence distribution generated by the source and the positional importance. The fluence distribution can be calculated or simulated by forward-modeling techniques, and the adjoint portion of the method is accounted for in the positional importance. Rather than represent the propagation of fluorescence as a combination of photon sources at the locations of fluorescence generation, the adjoint approach determines the locations that contribute to the detected signal as a function of the optical properties. The positional importance is therefore the probability that a given detected photon originated from a specific point in the sample.

The forward-adjoint fluorescence model is expressed mathematically as

$$F_{\text{det}} = (\lambda_x / \lambda_m) \mu_{a,f} \varphi_f \int \Phi_x(\vec{r}) \tilde{\Phi}_m(\vec{r}) dV \quad (1)$$

where λ_x and λ_m are the excitation and emission wavelengths, respectively, and $\mu_{a,f}$ and φ_f are the absorption coefficient of the fluorophore at the excitation wavelength and the fluorescence quantum yield, respectively [15]. These terms combined represent the intrinsic fluorescence, F_0 . $\Phi_x(\vec{r})$ is the fluence generated by the source, and $\tilde{\Phi}_m(\vec{r})$ is the positional importance, both of which depend on the optical properties of the sample. The subscripts x and m correspond to excitation and emission, respectively. This integral represents the propagation of excitation and emission light through the sample, and therefore accounts for the distortion of the intrinsic fluorescence spectrum.

Here we represent the fluence and positional importance using the P_3 approximation to the radiative transport equation. This approximation is more accurate than the diffusion approximation close to the source, and is described in detail by Hull and Foster [18]. We are interested in the use of an isotropic source and detector, so we consider the case of an isotropic point source in an infinite medium. In this case, the fluence at a distance r is given by

$$\Psi_0(r) = B^- \frac{e^{-v^- r}}{v^- r} + B^+ \frac{e^{-v^+ r}}{v^+ r}, \quad (2)$$

where

$$B^\pm = \frac{(v^\pm)^5 [3\mu_a \mu_t^{(1)} - (v^\mp)^2]}{12\pi \mu_a^2 \mu_t^{(1)} [(v^\pm)^2 - (v^\mp)^2]}, \quad (3)$$

$$v^\pm = \left(\frac{\beta \pm \sqrt{\beta^2 - \Gamma}}{18} \right)^{1/2}, \quad (4)$$

$$\beta = 27\mu_a \mu_t^{(1)} + 28\mu_a \mu_t^{(2)} + 35\mu_t^{(2)} \mu_t^{(3)}, \quad (5)$$

$$\Gamma = 3780 \mu_a \mu_t^{(1)} \mu_t^{(2)} \mu_t^{(3)}, \quad (6)$$

and $\mu_t^{(n)} = \mu_a + (1 - g^n) \mu_s$. This simplification of $\mu_t^{(n)}$ is due to the assumption of the Henyey-Greenstein phase function. The absorption and scattering coefficients are represented by μ_a and μ_s , respectively, and g is the scattering anisotropy.

The form of the positional importance is identical, but with optical properties corresponding to the emission wavelength[17]. In order to obtain an expression for detected fluorescence we evaluate equation (1), inserting equation (2) for $\Phi_x(r)$ and $\Phi_m(r)$. This results in an integral of the form

$$F_{\text{det}} = F_0 \int_{r_x=0}^{\infty} \int_{\theta=0}^{\pi} \int_{\phi=0}^{2\pi} \Phi_x(r_x) \tilde{\Phi}_m(r_m) r_x^2 \sin(\theta) dr_x d\theta d\phi. \quad (7)$$

Since we are using the same isotropic probe as a source and detector, $r_x = r_m = r$, and the problem is symmetrical in θ and ϕ . This simplifies the integral to

$$F_{\text{det}} = 4\pi F_0 \int_{r=0}^{\infty} \Phi_x(r) \tilde{\Phi}_m(r) r^2 dr. \quad (8)$$

With the expressions for fluence and positional importance given by equation (2), this results in the expression for detected fluorescence given in equation (9),

$$F_{\text{det}} = 4\pi F_0 \sum_{i=(+,-)} \sum_{j=(+,-)} \frac{B_x^i B_m^j}{v_x^i v_m^j (v_x^i + v_m^j)}, \quad (9)$$

with the coefficients again found in equations (3–6). This expression is identical to equation (5) from Finlay and Foster[15], in the limit of the source-detector separation going to zero.

In techniques that have access to diffuse reflectance measurements of the sample, equation (9) would be evaluated directly using the measured optical properties in order to determine the intrinsic fluorescence, F_0 . Here, we want to determine the intrinsic fluorescence directly without *a priori* access to the sample's optical properties. In order to do this, we invoke knowledge of the absorbers present in the sample and a scattering spectrum shape given by

$$\mu_s(\lambda) = a \left(\lambda / \lambda_0 \right)^{-b}, \quad (10)$$

where λ is the wavelength, in nm, λ_0 is a fixed normalization wavelength, and a and b are constant coefficients. An iterative fitting procedure can then be utilized in order to determine the intrinsic fluorescence.

In order to determine the value of the distortion to the intrinsic fluorescence, the scalar error metric

$$\chi^2 = \sum_{\lambda} [F_{\text{det}, \text{norm}}(\lambda) - F_{\text{calc}, \text{norm}}(\lambda)]^2 \quad (11)$$

is minimized for the entire spectrum using a constrained non-linear optimization (fmincon, MATLAB, Mathworks, Natick, MA), with the absorber and scatterer concentrations constrained to be positive. Here $F_{\text{det}, \text{norm}}$ is the measured, detected fluorescence spectrum

divided by its value at some wavelength λ_0 . $F_{calc, norm}$ is a calculated detected fluorescence spectrum, computed using equation (9), the known shape of the intrinsic fluorescence spectrum and a trial set of absorber concentrations and scatterer parameters, divided by its value at λ_0 . The absorption spectra consist of the absorption due to the background absorbers, as well as the absorption due to the fluorophore. In the computation of F_{calc} , F_0 is assumed to consist of only one fluorophore. However, the fitting procedure could be expanded to include multiple fluorophores, as described in Finlay and Foster[15].

After the optimum set of optical properties is determined, these are used to generate the spectral distortion term

$$D = 4\pi \sum_{i=(+,-)} \sum_{j=(+,-)} \frac{B_x^i B_m^j}{v_x^i v_m^j (v_x^i + v_m^j)}, \quad (12)$$

from equation (9), where the coefficients correspond to the minimized set of optical properties. This term represents an attenuation spectrum that accounts for the effects of background optical properties on the propagation of the excitation and emission. The intrinsic fluorescence is then found by

$$F_0 = \frac{F_{det}}{D}. \quad (13)$$

In principle, the optical properties found should correspond to the sample optical properties. However, we found that there was some cross-talk between the absorption due to the fluorophore and that due to the background absorbers. Therefore, a second fitting step is performed in order to separate these effects. In order to do this, F_0 is fixed at the value found in equation (13). As described above, this F_0 consists of the absorption of the fluorophore and the quantum yield. The quantum yield is assumed to be a constant value and is derived by calibration with known concentrations of the fluorophore in question. The value of F_0 can therefore be used to calculate $\mu_{a,f}$ and the concentration of the fluorophore. This concentration of fluorophore is then used to set the contributions of the fluorophore to the absorption at the excitation and emission wavelengths. Minimization is then performed over the error metric

$$\chi^2 = 1000 \sum [F_{det} - F_{calc}]^2, \quad (14)$$

where F_{det} is the detected fluorescence spectrum and F_{calc} is the detected fluorescence spectrum calculated using equation (9), the known F_0 , and a trial set of background absorber concentrations and scattering parameters. The minimization is performed with a constrained global minimizer (MultiStart, MATLAB), in order to ensure that local minima are not found. MultiStart is used as a global optimizer in order to ensure deterministic results. The absorber concentrations and scattering parameters, a and b , are constrained to be non-negative. The factor of 1000 is included to ensure that the optimization runs to completion.

Phantom preparation

Tissue-simulating phantoms consisted of deionized water, Intralipid-20% (Baxter Healthcare Corporation, Deerfield, IL) as a background scatterer, and manganese meso-tetra (4-sulfanatophenyl) porphine (MnTPPS, Frontier Scientific, Inc., Logan, UT) as a background absorber. MnTPPS was chosen due to the similarity of its absorption spectrum to hemoglobin, and its negligible fluorescence in the wavelength range of interest. Intralipid was chosen due to its tissue-like scattering properties. The fluorophore used was

doxorubicin hydrochloride (Doxil®, Ortho Biotech, Bridgewater, NJ). Phantoms were prepared with 80 mL of deionized water and 4 mL of Intralipid-20%, to which MnTPPS was added at concentrations ranging from 2–12 μM and doxorubicin was added at concentrations ranging from 1.5–50 μM . MnTPPS was prepared at a stock solution of 10 mg/mL by dissolving powdered MnTPPS in deionized water.

Absorption basis spectra for MnTPPS and doxorubicin were measured using a commercial spectrophotometer (Varian 50 Bio, Palo Alto, CA). The MnTPPS spectrum was found by measuring MnTPPS in deionized water at a concentration of 25 μM , and averaging the results of multiple measurements in order to improve the signal-to-noise ratio. The doxorubicin basis spectrum was found by measuring doxorubicin HCl in deionized water at a concentration of 85 μM . Normalized versions of these spectra are shown in Figure 1a.

The fluorescence basis function for doxorubicin was found by making measurements, as described in the next section, of doxorubicin fluorescence in a phantom consisting of 80 mL of deionized water, 0–4 mL of Intralipid-20%, and doxorubicin at concentrations of 1.5–15 μM . These spectra were then divided by D , calculated using equation (12) and the known scatterer and doxorubicin concentration, in order to retrieve the intrinsic doxorubicin fluorescence. These spectra were normalized and averaged over all measured data in order to create the basis spectrum shown in Figure 1b.

Data collection and correction

Spectra were collected using the system shown in Figure 2. Fluorescence was excited by a 488 nm laser diode (iBeam Smart 488-S, Toptica Photonics, Victor, NY), filtered by a bandpass filter (model 52650, Oriel Instruments, Stratford, CT). This excitation light was passed through a dichroic mirror (488trans-Pc-xr, Chroma Technology Corporation, Bellows Falls, VT), and focused into a fiber-coupled isotropic probe (model IP85, Medlight SA, Switzerland) with an outside diameter of 0.8 mm. The emitted fluorescence was collected by the same isotropic probe, collimated, reflected off of the dichroic mirror, and filtered with a long-pass filter (HQ500LP, Chroma Technology Corporation). This was then focused into an optical fiber and detected by a TE-cooled, 16-bit spectrometer (B&W Tek, Newark, DE). Integration times varied by experiment, but were generally in the range of 50–500 ms. Data collection and instrument control were performed with a laptop computer through a custom LabVIEW interface (National Instruments, Austin, TX).

After data collection, raw fluorescence spectra were corrected for dark background, system response, integration time, and excitation power. Dark spectra collected without excitation light were subtracted from each measured fluorescence spectrum. After dark subtraction, each spectrum was divided by a wavelength-dependent system response. This was found by placing the isotropic probe into a 6" integrating sphere, with a baffle between the detector port and the probe (Labsphere, North Sutton, NH). A NIST-traceable lamp (model LS-1-CAL, Ocean Optics, Dunedin, FL) was then used to illuminate the sphere through the baffled detector port, and the spectrum detected. This detected spectrum was divided by the known lamp spectrum to get the system response. All spectra were also divided by the integration time and excitation power, in order to get all measured spectra on the same scale. Excitation power was measured using the integrating sphere mentioned previously with a silicon photodiode (SDA-050-U, Labsphere) and calibrated radiometer (SC-6000, Labsphere). Excitation power was typically on the order of 5 mW.

Spectra were also corrected for fluorescence created within the spectroscopy system, as shown in Figure 3. The spectrum shown by the solid line was measured in a phantom containing doxorubicin, with the large features at wavelengths longer than 660 nm coming from the spectroscopy system. In order to correct for this, spectra were measured in either 80

mL of water or 80 mL of water with 1–4 mL of Intralipid-20%. The spectra taken in phantoms containing Intralipid were then corrected for scattering-induced distortion using equations (12) and (13). The resulting spectra were then normalized and averaged to produce the spectrum shown by the open circles in Figure 3. System fluorescence was found to come largely from the isotropic probe. This system fluorescence spectrum had peaks at 678 and 693 nm, which were outside the range used for fluorescence measurement and in a region of low absorption and scattering. Therefore, this region could be fit using a non-linear optimization from 665–700 nm, as shown in Figure 3, to determine the contribution of system fluorescence to the detected signal. For each fluorescence spectrum collected, two measurements were made. One was at low integration time (40–50 ms) in order to capture the system fluorescence peaks without saturating the detector. This spectrum was used for the fitting procedure shown in Figure 3. Another spectrum was then taken at a longer integration time (typically 100–500 ms), in order to use the full dynamic range of the spectrometer in the 500–650 nm spectral window. The system fluorescence fit from the first spectrum was then scaled to the integration time of the second spectrum and subtracted off. The fitting could not be performed directly on the spectrum measured at higher integration time because the integration times used caused the system fluorescence to saturate the detector.

RESULTS

Experimental verification of the technique was performed in tissue-simulating phantoms as described above, at doxorubicin concentrations ranging from 1.5–50 μM . At each doxorubicin concentration, spectra were collected for five MnTPPS concentrations from 2–12 μM . For each phantom, the isotropic probe was submerged in the phantom to a depth of 2.5 cm, fluorescence measurements were made, as described previously, and corrected for dark background, system response, and system fluorescence.

After correction, fluorescence spectra were analyzed using the fitting algorithm described previously. The results for a representative experiment are shown in Figure 4. The open circles correspond to the recovered intrinsic fluorescence spectrum, while the solid line represents the known intrinsic fluorescence spectrum, based on the doxorubicin concentration in the phantom. For this experiment, the doxorubicin concentration was 12 μM and the MnTPPS concentration was 4.75 μM .

A summary of doxorubicin concentration recovery over all measured phantoms is shown in Figure 5a. In Figure 5a, data points represent the average recovered doxorubicin concentration for an experiment consisting of measurements at five MnTPPS concentrations, with error bars representing standard deviation. As can be seen the recovery is good up to a doxorubicin concentration of approximately 25 μM . Beyond this point, self-quenching of the doxorubicin fluorescence occurs. In this process, there is direct energy transfer between adjacent fluorophores[19]. Unlike inner filtering, which is re-absorption of fluorescence by the fluorophore, self-quenching does not have an appreciable effect on the shape of the detected fluorescence spectrum. The only effect is a reduction in overall fluorescence magnitude. Therefore, this method cannot correct for the effects of self-quenching, meaning that a doxorubicin concentration of 25 μM represents an apparent fundamental upper limit for recovery. This value is comparable to the results of fitting doxorubicin fluorescence spectra measured in a commercial fluorometer, as shown in Figure 5b. Here doxorubicin fluorescence was measured in a 1 cm quartz cuvette with increasing fluorophore concentration. The detected fluorescence spectrum at each concentration was then fit using a non-linear least squares method and the doxorubicin fluorescence emission basis function in order to determine the magnitude of the fluorescence. In clinical studies, the maximum doxorubicin concentration in tumor tissue was found to be 819 ng per g of tumor tissue[20].

Assuming a tumor density of 0.95 g/mL[21], this translates to a doxorubicin concentration of 1.3 μM , which is well below the 25 μM threshold. If only the range of doxorubicin concentrations below 25 μM is considered, mean error in the recovery of doxorubicin concentration is 11.8%, with a maximum error of 30%.

After determination of doxorubicin concentration, the second step of the fitting algorithm is run in order to determine the background optical properties. While the primary goal of this method is to recover intrinsic fluorescence, recovery of optical properties can be considered as a secondary benefit of the technique. The results of this for an individual experiment are shown in Figure 6. Here the open circles represent recovered values, while solid lines represent the known optical properties of the phantom. These are derived from the same measurements that were used to determine the intrinsic fluorescence shown in Figure 4.

The recovered absorption of MnTPPS can then be used to calculate concentration, based on the known molar extinction of MnTPPS. A summary of this is shown in Figure 7. The data points represent the average recovered MnTPPS concentration over four experiments at doxorubicin concentrations of 1.5–25 μM , with error bars corresponding to standard deviation. The recovery of background absorption tracks linearly with increasing MnTPPS concentration, but is not as accurate as the recovery of fluorophore concentration. The mean error in recovery of MnTPPS concentration was 23.2%, with a maximum error of 33.9%. As previously stated, recovery of doxorubicin concentration is of primary importance, so the seemingly large errors in MnTPPS concentration recovery are not of deep concern.

The recovery of the scattering parameters shown in equation (10) is summarized in Figure 8. Here, the value of λ_0 was set to 488 nm so that the value of a was equal to the scattering coefficient at the excitation wavelength. The solid lines represent the actual value computed for the phantom based on the Intralipid concentration, while data points represent averages over measurements made in phantoms with the same MnTPPS concentration, but varying doxorubicin concentration. Error bars again represent standard deviation. The values of scattering parameters were systematically over-predicted, with mean errors of 24.4% and 21.2% in a and b , respectively. The mean error in the recovery of the overall μ_s spectrum was found to be 19.8%.

DISCUSSION

To the best of our knowledge, this is the first time that intrinsic fluorescence has been recovered from point fluorescence measurements made in a turbid medium without *a priori* knowledge of the optical properties. As mentioned in the Introduction, there has been a great deal of work done on the recovery of intrinsic fluorescence informed by diffuse reflectance measurements of optical properties made at the surface of the sample, with comparatively little done without *a priori* knowledge of the optical properties. In the interstitial regime, there has also been some work done on the recovery of intrinsic fluorescence. Finlay *et al* demonstrated recovery of motexafin lutetium fluorescence in the human prostate using a single side-firing optical fiber to excite and detect fluorescence[22]. This recovery utilized the forward-adjoint model, but required a separate measurement of the tissue optical properties.

Compared to schemes that employ a separate determination of optical properties, the accuracy of fluorophore concentration that we demonstrate here is similar. Weersink *et al* demonstrated recovery of fluorophore concentration with a root mean square error of 14.6%, using a ratio of detected fluorescence to reflectance as a metric of fluorophore concentration[23]. Diamond *et al* demonstrated recovery with error of 8%, using a diffusion model of fluorescence informed by reflectance measurements to determine optical

properties[12], and Müller demonstrated accuracy of approximately 90%, using a method based on photon migration theory[24]. Given that these techniques employ external knowledge of optical properties, the 11.8% error for the technique described here compares favorably with these estimates.

The method presented here is also capable of extracting optical properties. As mentioned previously, this has been previously performed in surface-contact geometry. Chang *et al* reported that their method was capable of providing good recovery of optical properties, but did not provide any data[16, 25]. However, their recovery of fluorescence is quite robust, so it can be surmised that the recovery of optical properties is of good quality as well. Finlay and Foster demonstrated recovery of absorption from hemoglobin that was approximately 20% lower than the value determined by diffuse reflectance[15]. They were able to recover oxygen saturation and the Hill parameters accurately, though, which is often the goal of tissue spectroscopy. The 23.2% mean error in the recovery of absorption reported here is therefore comparable to previously reported results.

The technique described in this paper does not require a separate measurement in order to determine the tissue optical properties before recovery of intrinsic fluorescence. This represents a significant reduction in clinical complexity, as illustrated in the simple system diagram depicted in Figure 2. Simplified instrumentation should ease the transition of the technique in the clinic, where it can be used to examine a number of medically relevant fluorophores.

This method also has some limitations. Since the determination of fluorescence distortion requires the background absorbers to induce a shape change in the detected spectrum, the technique will only work in the presence of absorbers with distinct absorption features in the emission window. This is why the technique works well in the 500–620 nm region, where both MnTPPS and hemoglobin have strong absorption features with distinct shapes. In the NIR region, the absorption of hemoglobin is much lower and lacks strong features. This was evident in a study done by Cottrell *et al* [26]. The authors employed a divide-by-reflectance method for correction based on Wu *et al* [13], and found that the measured reflectance spectrum was featureless and stable. This division was therefore omitted. The method presented here will also not be as effective for fluorophores with a large Stokes shift. Currently, the absorption at the excitation wavelength can be determined because the effects of this absorption are also apparent in the emission spectrum. For fluorophores with large Stokes shifts, the absorbers in the excitation and emission windows may be different, making it impossible to determine the effects of excitation attenuation from detected fluorescence spectra. This would require a separate measurement at the excitation wavelength, as was employed by Finlay *et al* [14]. Fortunately, doxorubicin has emission around 550–600 nm and does not possess an overly large Stokes shift.

As mentioned previously, doxorubicin is a potent chemotherapeutic agent used to treat a number of malignancies. We are particularly interested in the determination of doxorubicin concentration in the liver after transcatheter arterial chemoembolization (TACE). For this therapy a chemotherapeutic agent, in this case doxorubicin, is selectively delivered to the arteries feeding a tumor. Embolization beads are delivered either simultaneously with the drug or directly afterwards in order to occlude the blood vessels feeding the tumor. This leads to higher drug concentration in the tumor, lower drug concentration in systemic circulation, and reduced blood-flow to the tumor. TACE has been used successfully as a palliative therapy for a number of liver cancers, with significantly improved survival times[27, 28].

The concentration of doxorubicin delivered to a tumor can have a significant impact on the tumor outcome[20]. However, in TACE the doxorubicin concentration delivered to the tumor is not routinely measured. In a typical TACE procedure, the physician injects doxorubicin embedded in embolization beads until there is stasis of blood flow into the tumor. This can result in individual patients receiving differing concentrations of doxorubicin, which could have implications for the outcome of the treatment. Measurements of doxorubicin concentration in systemic circulation after TACE have been made[29], but there have not been measurements made of the local doxorubicin concentration in the tumor after TACE. Fluorescence-based recovery of intrinsic doxorubicin fluorescence has previously been demonstrated in mouse models[7], but this again requires a separate measure of tissue optical properties and is performed at the tissue surface.

We anticipate a clinical trial in order to assess the feasibility of measuring the concentration of doxorubicin present in liver tumors, specifically hepatocellular carcinoma, after TACE using the forward-adjoint method presented here. In order to do this, a 19-gauge biopsy needle will be inserted percutaneously into the tumor mass under image guidance, at some point after the TACE procedure. Upon insertion, the core of the needle will be removed and the sterile, single-use isotropic optical probe will be inserted into the tumor and fluorescence measurements made. In the future, such measurements may be used to investigate a correlation between doxorubicin concentration in the tumor after TACE treatment and tumor response.

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Figure 1.

(a) Normalized absorption spectra of MnTPPS (solid line) and doxorubicin (dashed line) in deionized water. Spectra were measured using a commercial spectrophotometer. (b) Normalized fluorescence spectrum of doxorubicin.

Figure 2. System used for collection of fluorescence spectra. BPF and LPF refer to band-pass and long-pass filters, respectively.

Figure 3.

Detected fluorescence spectrum showing contributions from the desired doxorubicin fluorescence and the system fluorescence. The solid line represents a detected fluorescence spectrum measured in a phantom containing doxorubicin with evidence of system fluorescence. The open circles represent a fit of the system fluorescence in the range of 665–700 nm to the measured data. This fit is used to remove the effects of system fluorescence.

Figure 4. Recovered intrinsic fluorescence spectrum (○) using the forward-adjoint model, compared to the known intrinsic fluorescence spectrum (solid line) for a doxorubicin concentration of 12 μ M.

Figure 5.

(a) Recovery of doxorubicin concentration using the forward-adjoint fluorescence model in tissue-simulating phantoms containing Intralipid-20% and MnTPPS. The accuracy of concentration recovery breaks down beyond a doxorubicin concentration of approximately 25 μM , due to self-quenching. The solid line represents perfect agreement. (b) Fitted magnitude of doxorubicin fluorescence with increasing doxorubicin concentration, measured using a commercial fluorometer.

Figure 6.

Recovery of (a) μ_a and (b) μ_s using the forward-adjoint fluorescence model. In both cases, open circles are recovered optical properties and solid lines are known optical properties. The absorption spectrum contains contributions from 12 μ M doxorubicin and 4.75 μ M MnTPPS.

Figure 7.

Recovery of MnTPPS concentration from fluorescence measurements analyzed using the forward-adjoint model. The data points represent the average recovered MnTPPS concentration over four experiments at doxorubicin concentrations of 1.5–25 μM , with error bars corresponding to standard deviation. The solid line represents perfect agreement.

Figure 8.

Recovery of scattering parameters (a) a and (b) b , according to equation (10), using the forward-adjoint fluorescence method. The solid lines represent the known values, while data points represent averages over measurements made in phantoms with the same MnTPPS concentration, but varying doxorubicin concentration. Error bars represent standard deviation.