Do fluorescence decays remitted from tissues accurately reflect intrinsic fluorophore lifetimes?

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Fluorescence spectroscopy and imaging methods, including fluorescence lifetime sensing, are being developed for noninvasive tissue diagnostics. The purpose of this study was to identify and quantify those factors affecting the accurate recovery of fluorophore lifetimes from inhomogeneous tissues *in vivo*. A Monte Carlo code was developed to numerically simulate time-resolved fluorescence measurements on layered epithelial tissues. Simulations were run with experimental parameters matching previously reported clinical studies in the gastrointestinal tract. The results demonstrate that variations in fluorescence decay time as large as those detected clinically between normal and premalignant tissues (~2 ns) could be simulated by variations in tissue morphology or biochemistry, even when intrinsic fluorophore lifetimes were held constant.

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Fluorescence spectroscopy and imaging methods are being developed for a variety of applications in clinical tissue diagnostics, including early cancer detection in epithelial tissues such as the cervix, gastrointestinal (GI) tract, and lungs.[1] Methods that sense fluorophore excited-state lifetimes are of interest because intrinsic biomolecular lifetimes reflect local tissue biochemistry while remaining unaffected by excitation laser intensity or sources of optical loss,[2] both of which can be influenced by tissue optical scattering and absorption. The first time-resolved fluorescence studies conducted endoscopically on lower GI-tract tissues[3,4] demonstrated this fact and indicated that measured fluorescence decays varied with tissue pathology, with cancerous and precancerous lesions exhibiting faster decay times than normal tissues and benign lesions. Figure 1 shows representative fluorescence decay curves obtained *in vivo* from normal and premalignant (adenoma) colon tissues and indicates the large difference in recovered average decay time $\langle\tau\rangle$ between tissue pathologies: 4.6 and 2.6 ns, respectively.[5] Although the observed differences were diagnostically significant, their physical origins, including whether they must necessarily be attributed to changes in the intrinsic fluorophore lifetime with varying disease state, remained unknown. Answering this question for complex tissues (such as epithelial tissues, wherein 85% of all cancers occur[6]) is important for the continued development and improvement of clinical optical diagnostic methods based on fluorescence lifetime sensing.

In this Letter we address whether physical factors other than intrinsic fluorophore lifetime can have a significant effect on the fluorescence decay time recovered from inhomogeneous tissues by numerically modeling time-resolved fluorescence measurements in epithelial tissues by use of simulation parameters that match the clinical studies described above.[3,4] We employ Monte Carlo methods to model photon transport in turbid media, as they are both accurate and versatile for complex tissue and probe geometries.[7] Here, for what is to our knowledge the first time, we develop a time-resolved Monte Carlo code to model fluorescence in layered turbid media with multiple fluorophores and apply it to quantitatively simulate tissue fluorescence decays measured *in vivo* during GI endoscopy. The results are generally applicable to diagnostic fluorescence lifetime sensing applications in layered tissues, using endogenous or exogenous fluorescent biomarkers.

Numerical studies employed a previously validated time-resolved Monte Carlo code[8] that was modified to incorporate a two-layer, turbid, model epithelial medium[9] and a single optical fiber for fluorescence excitation and detection.[5] The inset of Fig. 1 shows a schematic of the simulation, which approximated lower GI-tract epithelial tissue as a bilayered stack,[10] with a mucosal layer [Layer (1)] of finite thickness

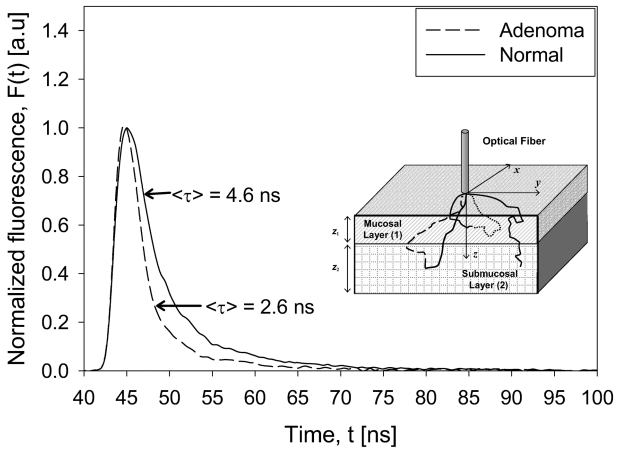

Fig. 1. Representative time-resolved fluorescence decays measured *in vivo* (excitation at 337.1 nm, emission at 550 ± 20 nm) for normal colon tissue and for a premalignant adenomatous polyp.[5] Inset, tissue model used for numerical simulation of fluorescence decay.

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(z_1) atop a semi-infinite submucosal layer [Layer (2)]. Each layer was characterized by its optical transport coefficients (absorption μ_a, scattering μ_s, and anisotropy g) at the fluorescence excitation (λ_x) and emission (λ_m) wavelengths and contained a uniformly distributed fluorophore characterized by its fluorescence absorption coefficient (μ_{af}), intrinsic excited-state lifetime (τ), and quantum yield (Φ_{QY}).

Appropriate model inputs for both optical transport coefficients (μ_a, μ_s, g) and fluorophore properties (μ_{afx}, τ, Φ_{QY}) were based on previous studies of endogenous fluorescence spectra from epithelial tissues in the lower GI-tract and the cervix.[10–12] Previous work in the colon[11] found that the dominant mucosal fluorophore was intracellular nicotinamide adenine dinucleotide (NADH), whereas the dominant submucosal fluorophore was extracellular collagen. Together these studies provided the framework for the simplified computational model of a complex biological tissue that we employ here. In the simulations each fluorophore was characterized by its lifetime τ and quantum yield Φ_{QY} that were both held fixed, while other physical parameters were varied. Table 1 lists the known ranges of the optical transport coefficients for lower GI-tract tissues as well as fluorophore properties published in previous reports.[2,5,10,12–19] For all pathologies, colonic submucosa was considered to have the optical transport coefficients of normal mucosa.[10]

All simulations of model tissues presented here held each fluorophore lifetime and quantum yield fixed, with NADH lifetime $\tau_1 = 1.5$ ns, quantum yield ${}^1\Phi_{QY} = 0.05$, collagen lifetime $\tau_2 = 5.2$ ns, and quantum yield ${}^2\Phi_{QY} = 0.3$, consistent with the ranges listed in Table 1. Simulations used a single optical fiber (diameter 600 μm) to deliver and collect light from the mucosal surface of the tissue to match previous clinical experiments.[3,4] Simulated time-resolved decay curves were fitted via an iterative least-squares method to biexponential fluorescence decays to calculate the resulting average fluorescence decay time $\langle\tau\rangle$.[5] This permitted direct, quantitative comparisons between the simulated fluorescence decays and the clinically measured fluorescence traces.

Figure 2 shows simulated time-resolved fluorescence decays for a set of five colon tissue models (Tissues 1–5), as detailed in Table 2. The baseline normal tissue, Tissue 1, was created by taking both the mucosal and submucosal layer to be normal colon tissue, according to the values reported in Table 1. The simulation for Tissue 1 yielded an average decay time of $\langle\tau\rangle = 4.5$ ns, which is consistent with average fluorescence decay times measured clinically for normal colon tissues,[4] as indicated in Fig. 1. To simulate premalignant (adenomatous) colonic epithelium and determine which tissue properties other than intrinsic fluorophore lifetimes might affect the recovered fluorescence decay remitted from tissue, simulation parameters were varied in a controlled manner, as shown, e.g., for Tissues 2–5. For Tissue 2 the optical transport coefficients of Layer (1) were taken to be those of adenomatous tissue, which resulted in an average decay time of $\langle\tau\rangle = 4.5$ ns, unchanged from that of Tissue 1. Indeed, our study found that very large and physically unreasonable changes (approximately 100–300%) in optical transport coefficients were required before tissue fluorescence decay times were appreciably affected, as would be expected for the single-fiber geometry.[8] For example, the fluorescence decay simulated from Tissue 3 resulted in an average decay time of $\langle\tau\rangle = 3.2$ ns but required unphysical optical transport coefficients with values greatly exceeding the ranges reported in Table 1. These results indicate that variations in optical transport coefficients alone between normal and precancerous colon tissues are not likely to account for the large variations in fluorescence decay times (~2 ns) measured clinically between tissue pathologies.[3,4]

In Fig. 2, other simulations of adenomatous colon tissue (Tissues 4 and 5) indicated that physically reasonable variations (i.e., values within ranges specified in Table 1) in mucosal layer thickness (z_1) and collagen absorption or concentration (${}^2\mu_{afx}$) could account for

Table 1. Model Inputs Required for Simulations[a]

Optical Transport Coefficient Symbol ($\lambda_{ex} \pm$ SD) (nm)	Range	Units	Tissue Type (Pathology)	Ref.
$\mu_{ax}(337 \pm 10)$	20 ± 2	cm^{-1}	Colonic mucosa (adenoma)	10
$\mu_{am}(550 \pm 20)$	$\sim 10 \pm 2$			
$\mu_{sx}(337 \pm 10)$	$\sim 120 \pm 5$	cm^{-1}	Colonic mucosa (adenoma)	10
$\mu_{sm}(550 \pm 20)$	$\sim 70 \pm 5$			
$\mu_{ax}(337 \pm 10)$	$\sim 12 \pm 2$	cm^{-1}	Colonic mucosa (normal)	10,18
$\mu_{am}(550 \pm 20)$	$\sim 3 \pm 2$			
$\mu_{sx}(337 \pm 10)$	$\sim 200 \pm 5$	cm^{-1}	Colonic mucosa (normal)	10,18
$\mu_{sm}(550 \pm 20)$	$\sim 90 \pm 5$			

Fluorophore Optical Coefficient Symbol; λ_{ex} (nm)	Range	Units	Fluorophore	Ref.
${}^1\mu_{afx}$, ~340	0.2–6.1	cm^{-1}	NADH	13,14
${}^2\mu_{afx}$, ~325	~0.3–1.5	cm^{-1}	Collagen	15
τ_1, ~337	0.4–2.5	ns	NADH	2,16
τ_2, ~337	4.5–6	ns	Collagen	16,17
${}^1\Phi_{QY}$, ~355	0.02–0.1	–	NADH	19
${}^2\Phi_{QY}$, ~337	0.1–0.4	–	Collagen	16

[a]Superscripts $i = 1, 2$ denote layer i. Subscript x (m) refers to coefficient value at the excitation (emission) wavelength. For all models, ${}^x g = {}^m g = 0.9$ for each layer.[10,12,18] For normal colon tissues, $z_1 \sim 200$–450 μm; for adenomatous lesions, $z_1 \sim 450$–900 μm.[10] SD, standard deviation.

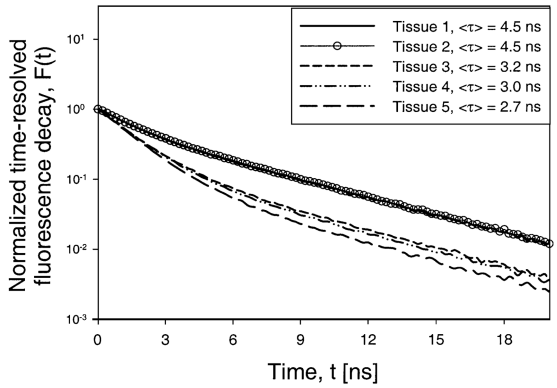

Fig. 2. Normalized time-resolved fluorescence simulations for five tissue models (see Table 2).

Table 2. Parameters for Simulated Tissues[1–5] in Fig. 2[a]

Parameter	1	2	3	4	5
Optical transport coefficients (cm^{-1})					
${}^{1}\mu_{ax}$, ${}^{1}\mu_{am}$	12, 3	20, 10[b]	30, 15[b]	20, 10[b]	20, 10[b]
${}^{1}\mu_{sx}$, ${}^{1}\mu_{sm}$	200, 90	120, 70[b]	240, 140[b]	120, 70[b]	120, 70[b]
${}^{2}\mu_{ax}$, ${}^{2}\mu_{am}$	12, 3	12, 3	24, 10[b]	12, 3	12, 3
${}^{2}\mu_{sx}$, ${}^{2}\mu_{sm}$	200, 90	200, 90	300, 150[b]	200, 90	200, 90
z_1 (μm)	400	400	400	700[b]	700[b]
Fluorophore absorption (cm^{-1})					
${}^{1}\mu_{af}$	0.5	0.5	0.5	0.5	0.5
${}^{2}\mu_{af}$	1.5	1.5	1.5	1.5	1.0[b]
Decay time, $\langle\tau\rangle$ (ns)	4.5	4.5	3.2	3.0	2.7

[a]Superscripts and subscripts are as in Table 1;
[b]Values changed relative to Tissue 1.

variations in remitted fluorescence decay times as large as those measured clinically between normal and premalignant colonic epithelium. For example, relative to Tissues 1 and 2, Tissue 4 exhibited a significantly shortened average decay time ($\langle\tau\rangle = 3.0$ ns) when z_1 increased to 700 μm (while all parameters were kept within ranges reported in Table 1) because of the increased sampling of the short-lived fluorophore in that layer. Likewise, Tissue 5 displayed a further decrease of average decay time ($\langle\tau\rangle = 2.7$ ns), when the increase in z_1 was combined with a decrease in ${}^{2}\mu_{afx}$. The average decay times recovered from Tissues 4 and 5 are consistent with clinical measurements of premalignant colonic epithelium,[4] as shown in Fig. 1. In epithelial tissues both a thickened mucosal layer and a decrease in submucosal collagen content are associated with premalignant change,[10,12] indicating that these simulations are both physically reasonable and biologically relevant. Recalling that for all simulations presented here the intrinsic fluorophore lifetimes were held constant, these data indicate that even large variations in remitted fluorescence decay time measured clinically between tissue pathologies do not necessarily imply changes in intrinsic fluorophore excited-state lifetime.

In turbid homogeneous media, fluorescence lifetime measurements are generally considered to be independent of fluorophore concentration, but in turbid inhomogeneous (layered) media, such as epithelial tissues, such measurements can be affected significantly by fluorophore properties other than the intrinsic excited-state lifetime, including fluorophore spatial distribution and concentration. Photon-migration models like that employed here may be useful for developing complex fiber-optic probe geometries for clinical studies on layered tissues, for example, to enhance (or diminish) the effects of the aforementioned properties on the detected fluorescence signal. Future computational models could incorporate multiple fluorophores per tissue layer, permitting more realistic simulations. These approaches should be useful for further optimization and validation of clinical diagnostic methods based on fluorescence lifetime sensing, potentially leading to better noninvasive optical discrimination between disease states of tissue *in vivo*.

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