The technique of modulated luminescence of bones was developed experimentally and theoretically and was subsequently used to interpret measurements performed on the cortical layer of human skull bones. The photophysical theory is based on the optical excitation and decay rate equations of the fluorescent endogenous chromophore and on the molecular interaction parameter with the photon field density in the matrix of the bone. An effective mean relaxation lifetime, $\tau_M$, of skull cortical bone was derived theoretically and was found to depend on the endogenous chromophore decay lifetime, $\tau_2$, in the upper energy state, on the generated luminescence field density through its dependence on the incident photon field density and on the thickness of the bone. A linear dependence of $\tau_M$ on laser beam intensity, $I_0$, was found and sensitivity of the value of $\tau_M$ to bone thickness, $L$, was observed for $L \approx 6.2$ mm. Both experimental dependencies of $\tau_M$ on $I_0$ and $L$ were in excellent agreement with the theoretical model. The unusually long relaxation luminescence lifetime was accounted for theoretically by means of an excited-state manifold invoking intersystem crossing to a forbidden state followed by decay to the ground state of the chromophore. Best fits to the data were able to yield measurements of the following chromophore and photon field parameters: $\tau_2=19.7$ ms, optical scattering coefficient $\mu_s(659\text{ nm})=4.4\times10^4$ m$^{-1}$, optical absorption coefficient $\mu_a(659\text{ nm})=13$ m$^{-1}$, and coupling coefficient $B_{21}=1.6\times10^8$ m$^3$/J/s, the decay coupling coefficient of the endogenous chromophore participating in the optical interaction in the form of stimulated luminescence emission mediated by the luminescence photon field between the long-lived excited state $E_2$ and the lower (ground) state $E_1$. The method of modulated luminescence can be used to measure photophysical properties of the chromophore in cortical skull bones, being a sensitive marker of bone diseases, namely, osteoporosis and cancer.

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I. INTRODUCTION

Bone is a dynamic living porous mineralized structure consisting of organic components (collagen and long chains of noncollagenous proteins that intertwine in elastic fibers) and inorganic mineral matter in the form of hydroxyapatite, $\text{Ca}_{10}(\text{PO}_4)_{6}(\text{OH})_2$, of poor crystalline quality. Mechanically, the ratio of organic matrix to mineral concentration is very important. Newly deposited bone is low in mineral density. As mineral density increases and bone crystals increase in size, the stiffness of bone increases [1]. Bone crystallinity is also a measure of turnover rate, where the average crystal size is smaller in young bone because newly formed crystals are smaller than mature crystals. Bone mineral crystals are colloidal in size, so there is a large amount of surface area on which normal lattice ions can be substituted by other ions in solution. The calcium site can be substituted by [2] $\text{Na}^+$, $\text{K}^+$, $\text{Mg}^{2+}$, $\text{Sr}^{2+}$, and $\text{Pb}^{2+}$ and the anionic sites can be substituted with $\text{CO}_3^{2-}$, $\text{HPO}_4^{2-}$, $\text{Cl}^-$, and $\text{F}^-$. These substitutions into the hydroxyapatite lattice are very important to bone strength, flexibility, and the process of remodeling. This structure is constantly undergoing renewal processes as old material is removed and new added.

Investigations of the optical properties of human bones have been growing rapidly in recent years as a result of the perceived need to develop new diagnostic tools to combat the increased incidence of osteoporosis (OP), a major public health problem, especially in aging populations: in the U.S. alone 10 million persons (mostly postmenopausal Caucasian women) have osteoporosis and 18 million more have low bone mass, a condition known as osteopenia [3]. Miller et al. [4] have used far infrared fluorescence spectroscopy of tibia bones from ovariectomized female monkeys (potentially during the onset of postovariectomy OP) and reported sensitivity of several substitutional mineral absorption bands to mineral content, crystallinity, and the content/nature of the organic matrix. Takeuchi et al. [5] have performed preliminary measurements of elastic optical-range light scattering from the hydroxyapatite of bone and they found a correlation between optical attenuation and bone mineral density (BMD). This sets the stage for a noninvasive BMD measurement technique. Recently Ugryumova et al. [6] used white light, an integrating-sphere and optical coherence tomography (OCT) to perform diffuse reflectance and transmittance measurements of intentionally demineralized cortical horseleg bone over the spectral range of 520–960 nm. An approximately linear relationship between scattering coefficient, $\mu_s$, ($\sim 25–35$ mm$^{-1}$) and BMD was found and attributed to the presence of hydroxyapatite crystals. Values of the optical absorption coefficient, $\mu_a$, spectra were $\sim 0.12$ mm$^{-1}$ and also exhibited strong decreases with increasing demineralization, as well as a directional dependence of the optical absorption coefficient on bone anisotropy controlled by collagen fiber orientation. This is an important result as it shows the feasibility of optical absorption- and scattering-based bone loss diagnostic probes. Similar effects were reported during mid-infrared measurements of human trabecular bone using a Fourier-transform infrared (FTIR) spectrometer [7].
By way of OP quantification, the World Health Organization (WHO) proposed diagnostic guidelines for OP [8,9] based on the measurement of BMD by dual energy x-ray absorptiometry (DXA or DEXA), the major diagnostic technique to date [10–12]. Besides DEXA, the following bone densitometry techniques are currently used for density measurements of bone: quantitative computed tomography, radiographic absorptiometry, peripheral quantitative computed tomography, quantitative ultrasound (Q-US), and quantitative measurements of trabecular bone through magnetic resonance images [13].

Purely ultrasonic techniques such as Q-US use the attenuation of nonionizing ultrasound waves and changes in the speed of sound to diagnose variations in bone density and osteoporosis. However, a wide acoustic frequency range is required [14], i.e., large transducer arrays, which affect spatial resolution. Intimate contact with the bone is required which may be inconvenient for nonflat body areas and compromises signal reproducibility. Besides the aforementioned experimental limitations, a potential practical drawback is the complexity of the interaction mechanisms between the incident ultrasonic field and the bone [15]. This issue adds nonspecificity to purely ultrasonic measurements which are not yet fully understood.

The foregoing overview of wave-based bone diagnostic techniques shows or implies that there are measurable differences in bone optical and acoustic properties, with optical excitation providing superior specificity to that of the low-contrast ultrasound. Therefore, the investigation of the response of bone to optical stimuli may be important toward the development of sensitive diagnostic techniques of the onset of OP at the very earliest stage and for detailed characterization of bone matter without the ionizing effects of x rays. Modulated luminescence (LUM) of hard tissues is a method which is based on direct near-infrared radiative photon emissions from chromophores responding to optical excitation. Our preliminary measurements on human cranial vault (skull) bones [16] have revealed strong luminescence emission with modulation frequency dependence very similar to that exhibited by dental enamel luminescence characteristic of hydroxyapatite [17]. Mineral loss was simulated using 37% phosphoric acid gel (3M ESPE AG, Scotchbond™) and was validated using transverse microradiography. An etching treatment series was done sequentially with LUM signals measured before and after each treatment time. The luminescence amplitude and phase showed very small differences which, however, were measurable due to the very high signal stability and signal-to-noise ratio (SNR; several hundred). Bone luminescence emission was observed in the 700–850 nm range, generally the same spectral range as hydroxyapatite [18] which has approx 1 cm optical absorption length and is thus very sensitive to the value of the optical absorption coefficient, $\mu_{\rho}$. The observed high SNR implies that synchronous lock-in detection of laser-source-filtered LUM can be used to easily detect minute changes in bone texture by means of sensitive photodetectors, e.g., Si or InGaAs. LUM signals can subsequently be linked to intrinsic bone photophysical properties, a major goal of this paper. A fundamental understanding of bone photophysics can thus be obtained and used to introduce and quantify two-dimensional LUM imaging using laser scanning or near-infrared cameras.

The dependence of the modulated LUM signal on the optical properties of bones (absorption and scattering coefficients) renders this technique sensitive to minute geometrical changes in bone, e.g., the void fraction, due to density changes (Fig. 1). In addition, the optical-to-radiative energy conversion associated with modulated luminescence is shown to be sensitive to intrinsic photophysical properties of endogenous LUM generating bone chromophore(s). The combination of these factors may potentially act additively to render LUM an optimal noninvasive diagnostic technique capable of detecting the onset of bone density changes and osteoporosis at the earliest stages. In this paper we construct a photophysical theoretical model of LUM to explain its dependence on the optical properties of the bone and of the experimentally observed endogenous chromophore excited-state relaxation lifetime in the bulk (matrix) of the skull. We use the model to measure the dependence of modulated luminescence of human cortical skull bone on the excitation laser beam modulation frequency. In the next section we put forth a photophysical theoretical model based on the diffuse luminescence field present in skull cortical bone considered as a turbid medium.

II. MODULATED LUMINESCENCE PHOTOPHYSICS OF BONE

A. Exact rate theory

It is well known that endogenous fluorophores such as collagen in bone exhibit typical fluorescence lifetimes on the order of nanoseconds or shorter. However, the luminescence emission observed in the reported measurements has a very long lifetime (ms). Therefore one is led to conclude that a simple two-state model of the excited-state energy manifold of the luminescent endogenous chromophore in cortical human skull bones [16] is not an accurate depiction of the photophysical processes occurring upon optical excitation, but instead a forbidden transition associated with intersystem crossing of is a more likely process, as shown in Fig. 2. Given that little is known about the photophysics of this newly observed luminescence emission from skull bone, the formalist will be kept as general as possible and the theoretical predictions will be applied to the data. $W_p(t)$ is the optical pump rate represented by the laser beam which excites the chromophore in the skull bone to the upper inter-
mediate (virtual) state \( E_v \). The chromophore on this level crosses into the virtual level \( E_i \) and eventually decays into the upper state \( E_2 \) of the observed transition either as heat (nonradiatively) or radiatively with effective lifetime \( \tau_{2} \). Once in state \( E_2 \) the chromophore decays as the observed luminescence photon emission. The characteristic decay time from energy level 2 to 1 is denoted as \( \tau_2 \). The fact that the direct pathway \( E_v \rightarrow E_1 \) is also possible and very likely determines the relative populations in states 2 and 1 proportionally to the decay times of \( E_v \) and \( E_2 \) into the ground state \( E_1 \). The lifetime \( \tau_{1} \) associated with the direct deexcitation pathway is typically assumed to be on the order of \( \sim \)ns. Upon incidence of laser light on the surface of the hard turbid tissue of the cortical bone, a diffuse photon field is established in the bulk of the bone, Fig. 3, past the ballistic photon range. The bone matrix is assumed to have optical absorption coefficient \( \mu_a(\lambda) \) and scattering coefficient \( \mu_s(\lambda) \), Fig. 3. Here \( \lambda \) is the wavelength of the excitation laser beam. The presence of the laser field of wavelength \( \lambda \) in the matrix results in the optical density \( \rho(z,\lambda,\omega)[1\text{ m}^{-3}] \):

\[
\rho(z,\lambda;\omega) = \frac{1}{2} \rho_0(\lambda)(1 + e^{i\omega t}),
\]

where \( \omega=2\pi f \) is the angular modulation frequency of the excitation laser intensity \( I_0 \). The three-dimensional nature of the optical density field \( \rho_0(z,\theta;\lambda,\omega) \) has been suppressed and simplified into a one-dimensional (1D) field \( \rho \):

\[
\begin{array}{c|c|c|c}
I_0 & \mu_a & \mu_s & g \\
\hline
0 & L & z & \end{array}
\]

FIG. 3. Bone chromophore excitation manifold involving intersystem crossing into a state \( E_i \) from a virtual excited state \( E_v \). A forbidden transition from the vertical-decay cross state \( E_2 \) to the ground state takes place stimulated by the presence of the luminescence field in the luminescence-reabsorption dynamic model.

- \( = \rho_0(z,\lambda;\omega) \) because (a) the skull bones dealt with in this research exhibited essentially isotropic optical behavior (\( \theta \) independent) and (b) the diffuse optical scattering in the skull matrix broadened the extent of the field considerably beyond the radius of the excitation laser beam spot size, thereby rendering the luminescence frequency response essentially independent of the radial coordinate within the approx (50 \( \mu \)m)\(^2 \) viewing area of the near-infrared detector of the emitted luminescence. In any case, in the theoretical model it will be seen that the spatial integral of the optical field does not affect the details of the frequency response of the modulated luminescence. The relation between the optical density and total photon field \( \Psi_t \) in the turbid medium is

\[
\rho(z,\lambda;\omega) = \frac{1}{c} \Psi_f(z,\lambda;\omega),
\]

\[
\Psi_f(z,\lambda;\omega) = \Psi_d(z,\lambda;\omega) + \Psi_s(z,\lambda;\omega),
\]

where \( \Psi_f \) and \( \Psi_s \) are the diffusive and coherent components of the total optical field in the bone matrix, respectively [19]. The latter component largely accounts for optical propagation within the ballistic photon regime (ca. 50 \( \mu \)m from the surface) [20]. The photophysical picture evoked in this model to interpret the LUM data is one in which the photon density field induces luminescence through deexcitations in the chromophore and luminescence photons, in turn, stimulate further decay of the chromophore in the excited state \( E_2 \) cross-linked to the ground state \( E_1 \) through a forbidden transition with long lifetime \( \tau_2 \). The number density of stimulated deexciting chromophores depends on the optical (and generated luminescence) intensity in their neighborhood and is a function of depth, following optical absorption, scattering, and attenuation in the turbid medium. Therefore, the dynamic stimulation parameters of the decay process to the ground state are functions of depth in the bone. This process occurs in parallel with the spontaneous decay of the excited molecular system which is characterized by the lifetime \( \tau_2 \). At the same time the direct deexcitation process \( E_v \rightarrow E_1 \) is also occurring very efficiently, thus returning the majority of the excited chromophores to the ground state via this route with relaxation time \( \tau_{1} \), the competing process being the crossover one with decay lifetime \( \tau_{2} \). In keeping with the 1D approximation, the excited-state concentration of luminous molecules \( N_f(z,\omega) \) is the source of the LUM signal at mean wavelength \( \lambda_{\text{LUM}} = c/f_{21} \), where \( f_{21} \) is the mean optical frequency of the emitted luminescence and \( c = \mu_0 / n \) is the speed of light in skull cortical bone; \( \mu_0 \) is the speed of light in the vacuum and \( n \) is the refractive index of bone:

\[
Q(z,\lambda_{\text{LUM}};\omega) = \eta_\text{R} h f_{21} N_f(z,\omega), \quad [1 \text{ m}^{-3}],
\]

where \( \eta\text{R} \) is the radiative energy conversion efficiency and \( h \) is Planck’s constant.

The rate equations for the populations in the excited-state manifold, including the intermediate state, are \( N_s(z,\lambda,\omega) \), \( N_f(z,\lambda,\omega) \), and \( N_2(z,\lambda,\omega) \) respectively. They can be written as
\[ \frac{\partial N_1(z; \omega)}{\partial t} = -(B_{1c}/c) \Psi_1(z; \omega) N_1(z; \omega) - \left( \frac{1}{\tau_{t1}} + \frac{1}{\tau_{t2}} \right) N_1(z; \omega) \\
+ (B_{1c}/c) \Psi_1(z; \omega) N_1(z; \omega), \quad (4a) \]

\[ \frac{\partial N_2(z; \omega)}{\partial t} = \frac{N_j(z; \omega)}{\tau_{t2}} = -(B_{2j}/c) \Psi_2(z; \omega) N_2(z; \omega) - \frac{1}{\tau_2} N_2(z; \omega), \quad (4b) \]

\[ \frac{\partial N_i(z; \omega)}{\partial t} = (B_{i+1}/c) \Psi_i(z; \omega) N_i(z; \omega) \\
+ (B_{2j}/c) \Psi_j(z; \omega) N_2(z; \omega) + \frac{N_j(z; \omega)}{\tau_{v1}} + \frac{N_j(z; \omega)}{\tau_{v2}} \\
- (B_{1c}/c) \Psi_{i-1}(z; \omega) N_{i-1}(z; \omega). \quad (4c) \]

Here the excitation wavelength, \( \lambda \) dependence of the total optical field, \( \Psi_1 \), was suppressed. The product \((B_{i+1}/c)\Psi_i\) is the probability per unit frequency that optical transitions are induced by the diffuse luminescence field from level 1 to level \( j \). Figure 2 depicts the intersystem crossing and decay to the state \( E_2 \) of the arbitrary excited state \( E_0 \) populated by the optical pump field \( W_{p0}(\lambda, \omega) \). Luminescence photons of energy \( \Delta E = E_2 - E_1 = h\omega_{21} \) are emitted. \( E_1 \) is the chromophore ground state. Alternatively, the ground state may be a different energy level labeled \( E_0 \) not shown in Fig. 2. \( B_{i+1} \) [m \(^3\) J \(^{-1}\) s \(^{-1}\)] are the optical coupling coefficient between levels \( i \) and \( j \). \( c \) is the speed of light in the medium. Adding Eqs. (4a)–(4c) one can readily show that \( \frac{\partial}{\partial \lambda} [\sum_i N_i(z; \omega) + N_2(z; \omega) + N_1(z; \omega)] = 0 \), i.e., the overall chromophore density is constant: \( N_0(z; \omega) + N_2(z; \omega) + N_1(z; \omega) = \text{const} = N(z; \omega) \). The total number of chromophores in a bone sample is fixed and this leads to the molecular concentration conservation condition,

\[ \int_0^L N_1(z; \omega) dz + \int_0^L N_2(z; \omega) dz + \int_0^L N_1(z; \omega) dz = N_L = \text{const}, \quad (5) \]

where \( N_i \) is the (uniform) total concentration of all chromophores (all states) at any depth \( z \). Since there is no spatial transport of chromophores, instead of Eq. (5) a local conservation principle will apply at all coordinate points \( z \) within the turbid medium:

\[ N_1(z; \omega) + N_2(z; \omega) + N_1(z; \omega) = N(z; \omega) = N_i. \quad (6) \]

The coupling of the two assumed states (1) and (2) in the bone matrix by means of rate equations (4) mixes the modulated total optical field,

\[ \Psi_j(z; \omega) = \frac{1}{2} \Psi_{0j}(z)(1 + e^{i\omega t}), \quad (7) \]

with the full Fourier spectrum of the chromophore molecules at all states,

\[ N_j(z; \omega) = \sum_{k=0}^{\infty} N_{jk}(z)e^{ik\omega t}, \quad j = 1, 2, u, \quad (8) \]

where \( k=0 \) denotes the dc component of the \( N_j \) molecular distribution. Given that the experimental system only monitors the fundamental component \((k=1)\) of each molecular excited state because the lock-in amplifier filters out the dc baseline and all other harmonics, Eqs. (7) and (8) can be inserted in Eq. (4) and the various harmonic orders \( O(e^{ik\omega t}) \) can be calculated. Each harmonic order, \( k_0 \), and the one higher, \((k+1)\), are coupled through an infinite system of algebraic equations, the result of equating terms of the same harmonic order on the left- and right-hand sides of Eq. (4). The set of coupled rate equations (4) actually reduces to two, Eqs. (4b) and (4c), as \( N_1(z; \omega) = N_j(z; \omega) - N_2(z; \omega) \) from the conservation conditions. The contribution of \( N_2 \) in the form of the Fourier component \( N_{2k} \) in each order is used as an input to the next higher harmonic order. Thus, the dc and components of the excited-state concentration \( N_2(z, \omega) \) and \( N_1(z, \omega) \) are calculated from the dc terms in Eqs. (4b) and (4c):

\[ \left[ \left( \frac{B_{21}}{2c} \right) \Psi_{01}(z) + \frac{1}{T_2} \right] N_{20}(z) = \frac{1}{\tau_{t2}} [N_j - N_{10}(z)], \quad (9a) \]

\[ \left[ \left( \frac{B_{2z}}{c} \right) \Psi_{0}(z) + \frac{1}{T_v} \right] N_{20}(z) = \left[ \left( \frac{B_{1v}}{2c} \right) \Psi_{0}(z) + \frac{1}{\tau_{v1}} \right] N_j \\
- \left[ \left( \frac{B_{1v}}{c} \right) \Psi_{0}(z) + \frac{1}{\tau_{v1}} \right] N_{10}(z). \quad (9b) \]

Here the following definitions were made:

\[ \overline{B_{1v}} = \frac{1}{2}(B_{1v} + B_{21}), \]

\[ \overline{B_{2v}} = \frac{1}{2}(B_{1v} - B_{21}), \]

\[ T_v = \left( \frac{1}{\tau_{v1}} - \frac{1}{\tau_{v2}} \right)^{-1}, \]

\[ T_2 = \left( \frac{1}{\tau_{t2}} + \frac{1}{\tau_{t2}} \right)^{-1}. \quad (10) \]

In addition, we may define the following transition rates (see Fig. 2):

\[ R_1(z) = \left( \frac{B_{21}}{2c} \right) \Psi_0(z) + \frac{1}{T_2}, \]

\[ R_2(z) = \left( \frac{B_{2z}}{c} \right) \Psi_0(z) + \frac{1}{T_v}, \]

\[ R_3(z) = \left( \frac{B_{1v}}{c} \right) \Psi_0(z) + \frac{1}{\tau_{v1}}. \]
\[ R_3(z) = \left(\frac{B_{12}}{2c}\right) \Psi_{\omega}(z) + \frac{1}{\tau_{12}} \]  

(11)

The solution of the algebraic system [Eqs. (9a) and (9b)] leads to the following expressions for the dc populations:

\[ N_{10}(z) = N_t \left[ \frac{\tau_{c2} R_1(z) R_4(z) - R_3(z)}{\tau_{c2} R_1(z) R_3(z) - R_2(z)} \right] \]  

(12a)

and

\[ N_{20}(z) = N_t \left[ \frac{(B_{12}/2c) \Psi_{\omega}(z)}{\tau_{c2} R_1(z) R_3(z) - R_2(z)} \right]. \]  

(12b)

It is important to note that setting \( \Psi_{\omega}(z) = 0 \) in the foregoing expressions results in \( N_{10}(z) = N_t \) and \( N_{20}(z) = 0 \), as expected. Also, when \( \Psi_{\omega}(z) > 0 \), but, \( \tau_{c2} \gg \tau_{12} \), then, again, \( N_{10}(z) = N_t \) as the vast majority of the excited chromophore molecules returns to the ground state through the direct (vertical) deexcitation pathway.

Now, equating the \( O(\epsilon^{\omega}) \) terms on both sides of Eqs. (4b) and (4c) yields algebraic equations which involve the dependence of the Fourier components \( N_{11}(z; \omega) \) and \( N_{21}(z; \omega) \) on \( N_{10}(z) \) and \( N_{20}(z) \):

\[ [i\omega + R_1(z)] N_{21}(z; \omega) = -\frac{N_{11}(z; \omega)}{\tau_{c2}} - \left(\frac{B_{12}}{2c}\right) \Psi_{\omega}(z) N_{20}(z), \]  

(13a)

\[ [i\omega + R_3(z)] N_{11}(z; \omega) = -R_2(z) N_{21}(z; \omega) + \left(\frac{B_{12}}{2c}\right) \Psi_{\omega}(z) N_t, \]  

\[ -\left(\frac{B_{12}}{c}\right) \Psi_{\omega}(z) N_{10}(z) \]  

\[ -\left(\frac{B_{12}}{c}\right) \Psi_{\omega}(z) N_{20}(z). \]  

(13b)

After considerable manipulation, the solution for the experimentally significant population \( N_{21}(z; \omega) \) can be written in the form

\[ N_{21}(z; \omega) = N_t \Psi_{\omega}(z) \left\{ \left( \frac{B_{12}}{c} \right) \left( \frac{\tau_{c2}}{\tau_{12}} \right) \left( \frac{R_4(z)}{R_1(z)} \right) - \left( \frac{R_2(z)}{R_1(z)} \right) \right\} + \left( \frac{B_{12}}{2c} \right) \left( \frac{\tau_{c2}}{\tau_{12}} \right) \left( \frac{1}{i\omega + R_3(z)} \right) \left( \frac{R_3(z)}{R_1(z)} \right) \]  

\[ -\frac{1}{4} \left( \frac{B_{12}}{2c} \right) \Psi_{\omega}(z) \left( \frac{\tau_{c2}}{\tau_{12}} \right) \left( \frac{R_3(z)}{R_1(z)} \right) \]  

\[ \times \left( i\omega + R_1(z) - \frac{R_3(z)}{\tau_{c2}(i\omega + R_3(z))} \right)^{-1}. \]  

(14)

This general formula can be significantly simplified using the fact that vertical fluorescence transitions take place at times very short compared to our experimental luminescence frequency probe, i.e., one may set \( \tau_{c2} \ll \tau_2 \). In this limit \( B_{12} \sim \frac{\tau_{c2}}{\tau_{12}} \) and \( \frac{(B_{12}/c) \Psi_{\omega}(z)}{\tau_{c2} R_1(z) R_3(z) - R_2(z)} \approx \frac{1}{\tau_{12}} \) for any state coupling constant \( B_{lm} \). This implies

\[ \tau_{c2} R_1(z) R_4(z) - R_2(z) \approx \tau_{c2} R_2(z) R_3(z) - R_2(z) \]  

\[ \approx \left( \frac{\tau_{c2}}{\tau_{12}} \right) \left( \frac{B_{12}}{2c} \right) \Psi_{\omega}(z) + \frac{1}{\tau_2}. \]  

(15)

Now Eq. (12b) for the dc population of state \( E_2 \) can be simplified:

\[ N_{20}(z) = N_t \left[ \frac{\tau_{c2}/\tau_{12}}{\tau_{c2}} \right] \left[ \frac{(B_{12}/2c) \Psi_{\omega}(z)}{(B_{12}/2c) \Psi_{\omega}(z) + \tau_{12}} \right]. \]  

(16)

Similarly, Eq. (14) for the population of state \( E_2 \) modulated at the fundamental frequency \( f = \omega/2\pi \) can be simplified considerably using the foregoing approximations. In addition, the approximation \( i\omega + R_1(z) \approx 1/\tau_1 \) is used for modulation frequencies in the range of our experiments (\( \leq 1 \text{ kHz} \)) and the rate

\[ R_3(z) = \left(\frac{B_{21}}{2c}\right) \Psi_{\omega}(z) + \frac{1}{\tau_2} \]  

(17)

is defined. Then

\[ N_{21}(z; \omega) = N_t \left( \frac{\tau_{12}}{\tau_{c2}} \right) \left( \frac{\tau_2}{\tau_2} \right) \left( \frac{B_{12}}{2c} \right) \left( \frac{R_3(z)}{R_1(z)} \right) + \left( \frac{\tau_{12}}{\tau_{c2}} \right) \left( \frac{B_{21}}{2c} \right) \Psi_{\omega}(z) \]  

\[ \times \left( \frac{B_{21}}{2c} \right) \Psi_{\omega}(z). \]  

(18)

Taking into account that the theoretical best fits to our experimental data yield values \( (B_{12}/c) \sim 10^{-3} \text{ m}^3 \text{ J}^{-1} \text{ s}^{-1} \), we may ignore the products \( (B_{12}/2c)(B_{12}/c) \) and \( (B_{12}/c)(B_{12}/2c) \). This yields the simplified equation

\[ N_{21}(z; \omega) = N_t \left( \frac{\tau_{12}}{\tau_{c2}} \right) \left( \frac{B_{12}}{2c} \right) \frac{\tau_2}{1 + i\omega \tau(z)} \]  

\[ \times \left( \frac{B_{21}}{2c} \right) \Psi_{\omega}(z). \]  

(19)

Here, a depth-dependent effective relaxation time constant \( \tau(z) = 1/R_3(z) \) was defined as follows:

\[ \frac{1}{\tau(z)} = \frac{1}{\tau_2} + (B_{21}/2c) \Psi_{\omega}(z). \]  

(20)

Physically, \( \tau(z) \) represents the combined effect of spontaneous and luminescence-field-induced chromophore forbidden transition from the intersystem crossed upper excited state \( E_2 \).
to the lower state \( E_1 \). The modulated population of the exited state \( N_{21}(z; \omega) \) depends on the branching ratio \( \beta_1 \) of deexcitation lifetimes between the direct vertical transition \( E_0 \rightarrow E_1 \) and the indirect (and much slower) transition \( E_0 \rightarrow E_2 \rightarrow E_1 \). The decay time also depends on the coupling coefficient \( B_{21} \) as expected. The luminescence photon density of energy \( \Delta E = \hbar f_{21} \), in turn, is the result of the presence of the laser excitation field \( \Psi_{e0}(z) \) which populates (one or more) states \( E_n \) contributing to the population of \( E_2 \). Therefore, as the optical (and luminescence) field density varies across the thickness of the optical field is switched off, \( \tau \) reduces to the intrinsic decay time constant \( \tau_v \) characteristic of the molecular excited state of the chromophore. When the external optical field is present, the total decay time constant decreases proportionately to the field intensity as expected from stimulated emission.

From Eqs. (3) and (19) the measured LUM signal at the fundamental frequency \( f = \omega / 2 \pi \) is given by

\[
S(\omega) = \text{const} \int_0^L N_{21}(z; \omega) dz = \text{const} \int_0^L \frac{\tau(z) \Psi_{e0}(z) dz}{1 + i \omega \tau(z)}. \tag{21}
\]

It should be noted that if the three-dimensional character of the laser excitation photon field were fully taken into account in the model, i.e., \( \Psi_{e0} = \Psi_{e0}(r, z) \), the Hankel integral of the form \( \Psi_{e0}(r, z) = \int_0^L \tilde{\Phi}_{e0}(k, z) J_0(kr) dk \) would have to replace \( \Psi_{e0}(z) \) in Eq. (21). Here \( \tilde{\Phi}_{e0}(k, z) \) and \( J_0(kr) \) are the Hankel transform of \( \Psi_{e0}(r, z) \) and the Bessel function of the first kind of order zero, respectively [19]. This is a more complicated expression, however, it does not alter the modulation frequency dependence of the emitted luminescence which only appears in the denominator of the integral in Eq. (21). This mathematical argument shows why the dimensionality of the modulated luminescence response in Eq. (21) is not an issue in studies of the frequency response of the LUM field emitted from skull bones.

The result of Eq. (21) with or without the radial dependence of \( \Psi_{e0} \) is exact provided \( \tau_v \ll \tau_v \), however, the integral does not have an analytical solution and is difficult to use for fitting purposes of the theory to the data due to the complexity of the optical field expression for \( \Psi_{e0}(z) \). Therefore, an approximation is introduced to the general theory which allows for further comparison with the experimental results.

**B. Approximate mean-value rate theory**

The dc terms in Eqs. (4b) and (4c) lead to the expression for \( N_{10}(z) \) and \( N_{20}(z) \), Eqs. (9a) and (9b). Integrating over the thickness of the bone matrix yields

\[
\frac{1}{T_2} \int_0^L N_{20}(z) dz + \left( \frac{B_{21}}{2c} \right) \int_0^L \Psi_{e0}(z) N_{20}(z) dz = \left( \frac{L}{\tau_v} \right) \frac{N_1}{\tau_v} - \frac{1}{\tau_v^2} \int_0^L N_{10}(z) dz \tag{22a}
\]

and

\[
\left( \frac{B_{1e}}{c} \right) \int_0^L \Psi_{e0}(z) N_{10}(z) dz + \frac{1}{T_2} \int_0^L N_{10}(z) dz \tag{22b}
\]

The approximation involves equating two definitions of the mean value of the dc terms of the excited endogenous chromophore

\[
\langle N_{20} \rangle = \frac{\int_0^L \Psi_{e0}(z) N_{20}(z) dz}{\int_0^L \Psi_{e0}(z) dz} = \frac{1}{L} \int_0^L N_{20}(z) dz, \quad j = 1, 2, \tag{23}
\]

which is valid for turbid media where slowly spatially attenuated photon fields can be established. Using these definitions in Eqs. (22a) and (22b), after some manipulation one obtains the mean-value counterpart of Eq. (12b):

\[
\langle N_{20} \rangle = N_1 \left( \frac{R_{3M} - R_{4M}}{\tau_c R_{1M} (R_{3M} - R_{2M})} \right) \tag{24}
\]

and a similar expression for Eq. (12a). Here the mean-value counterparts of Eq. (11) were defined as follows:

\[
R_{1M} = \left( \frac{B_{21}}{2c} \right) \langle \Psi_{e0} \rangle + \frac{1}{T_2}, \tag{25a}
\]

\[
R_{2M} = \left( \frac{B_{1e}}{c} \right) \langle \Psi_{e0} \rangle + \frac{1}{T_2}, \tag{25b}
\]

\[
R_{3M} = \left( \frac{B_{1e}}{c} \right) \langle \Psi_{e0} \rangle + \frac{1}{\tau_v}, \tag{25c}
\]

\[
R_{4m} = \left( \frac{B_{21}}{2c} \right) \langle \Psi_{e0} \rangle + \frac{1}{\tau_v}. \tag{25d}
\]

Using the approximations \( \tau_1 \ll \tau_2 \) and \( (B_{1m}/c) \langle \Psi_{e0} \rangle + 1/\tau_v \approx 1/\tau_v \) yields

\[
\langle N_{20} \rangle = N_1 \langle \Psi_{e0} \rangle (B_{21}/2c) \tau_M \langle \Psi_{e0} \rangle, \tag{26}
\]

where

\[
\frac{1}{\tau_M} = \frac{1}{\tau_2} + \left( \frac{B_{21}}{2c} \right) \langle \Psi_{e0} \rangle. \tag{27}
\]

Similarly, the contribution of the excited-state chromophore molecules to the fundamental Fourier component, Eqs. (13a) and (13b), can be integrated in the mean-value sense. The results are in a compact form:

\[
(i \omega + R_{1M}) N_{21} = -\frac{\langle N_{11} \rangle}{\tau_c} - \frac{B_{21}}{2c} \langle N_{20} \rangle \langle \Psi_{e0} \rangle, \tag{28a}
\]

\[
021920-6
\[(i\omega + R_3M)\langle N_{1}\rangle = N_0 \left( \frac{B_{12}}{2c} - \frac{B_{12}}{c} \right) \langle \Psi_R \rangle + \left[ \frac{B_{12}}{c} \right] \langle R_{2M} \rangle \langle \Psi_R \rangle - R_{2M} \langle N_{21} \rangle \]  

(28b)

From these two equations and the mean-value approximations above, a simplified mean-value expression for \(\langle N_{21}(\omega) \rangle\) may be obtained:

\[\langle N_{21}(\omega) \rangle = N_0 \left( \frac{\tau_{12}}{\tau_{22}} \right) \left( \frac{B_{12}/2c}{1 + i\omega \tau_M} \right) \]  

(29)

This expression bears resemblance to the local Eq. (19), however, it involves the mean optical field value \(\langle \Psi_R \rangle\) and definition of the effective excited-state lifetime \(\tau_M\), Eq. (27).

The derived expression (29) is relatively simple and physically transparent. It involves the expected state-coupling constants \(B_{12}\) which allows for chromophore molecule excitation by the optical pump and \(B_{21}\), which allows coupling to the ground state from level \(E_2\) as the means of producing delayed luminescence photons as a fraction of the originally excited chromophore controlled by the branching ratio \(\tau_{12}/\tau_{22}\). However, there is an important limitation in the definition of effective lifetime \(\tau_M\) due to its mean-value nature stemming from the equality of the two definitions of mean value embedded in the assumed equivalence (23). The usual definition of an “average” value \(\langle \Psi_R(L) \rangle\) = \(\int_0^L \Psi_R(z)dz\) assigns decreasing values of the generated optical and luminescence photon density fields as thickness increases. For thin nonscattering slabs optical confinement in the slab determined by mismatch of optical properties and reflections at the boundaries (especially the back boundary) increases optical (and expected luminescence density) with decreasing thickness. This is not the case with strongly scattering turbid media, such as bone slabs, for two reasons: (1) the diffuse component of the optical excitation and luminescence fields does not allow efficient optical confinement at the back interface, with the result that thinner slabs undergo a much more significant loss of optical flux through the back interface which steepens the density gradient inward from the interface and decreases the overall photon density. This will result in weakening of the luminescence density in the body of the skull bone with thinner slabs or increasing the photon density with thicker slabs. (2) Related to the scattering nature of the medium, if two slabs of thickness \(L_1 < L_2\) are irradiated identically, and one considers a virtual slice of thickness \(0 < z < L_1\) in both slabs, the photon density within the thickness \(z < z_0\) will be higher in the case of the thicker slab \(L_2\) because \(z > z_0\) regions contribute more backscattered photons into the \(z \leq z_0\) region, thus increasing the photon density with increasing slab thickness; the thicker the slab the larger the contribution and the higher the photon density. Given the attenuating character of the absorbing and scattering field, the backscattered photon density is expected to saturate at large thicknesses \(L\) compared to the optical extinction length in the turbid medium. Both effects (1) and (2) are synergistic and have been observed in our measurements with bone thickness as a parameter. These physical effects including depth saturation are captured by the diffuse photon density theory developed in Sec. II B, but the usual definition of mean value which includes the \(1/L\) factor is inconsistent with the turbid character of the medium, so a different normalization factor other than thickness was sought for this expression. It will be noted that if the exact definition, Eq. (20), of the effective deexcitation lifetime is allowed, no thickness scale \(1/L\) enters the integral of the optical field \(\Psi_R(z)\) and the mathematical form of this function (see below) allows for a correct description of the phenomena.

To remain consistent with the mean-value approximation and calculate a physically acceptable mean value \(\langle \Psi_R \rangle\), the following definition of “mean” is used:

\[\langle \Psi_R(L) \rangle = \frac{1}{D} \int_0^L \Psi_R(z)dz,\]  

(30)

where \(D\) is the characteristic optical extinction depth where the photon density field drops to the \(1/e\) of its surface value. The value of \(\langle \Psi_R(\omega) \rangle\) in the limit of a semi-infinite turbid medium (or of a medium thickness large enough so that back-propagated photon contribution saturation to the total number density of local photons will occur) can be used to normalize the value for the finitely thick medium:

\[\langle \Psi_R(L) \rangle = \langle \Psi_R(\omega) \rangle \int_0^L \Psi_R(z)dz = C_1 \int_0^L \Psi_R(z)dz,\]  

(31)

where \(C_1\) is a thickness-independent constant. This expression can now be substituted into Eq. (27) for the effective relaxation time constant:

\[\frac{1}{\tau_M} = \frac{1}{\tau_2} + (B_{21}/2c)C_1 \int_0^L \Psi_R(z)dz.\]  

(32)

Finally, the expression for the measured LUM signal becomes

\[S(\omega) = \text{const} \langle N_{21}(\omega) \rangle\]  

(33)

and Eq. (29) must be used.

C. Photon density field

According to Eq. (2), \(\Psi(z) = \Psi_R(z) + \Psi_{\lambda}(z)\). The coherent component of the optical field in the bone matrix is represented by an exponentially decaying function in the direction of the incident laser energy [19],

\[\Psi_{\lambda}(z) = i_0(1 - R)\exp(-\mu_z),\]  

(34)

where \(R\) is the Fresnel reflection coefficient of the surface and \(\mu_s = \mu_a + \mu_s\) is the optical attenuation coefficient combining the effects of both the absorption coefficient, \(\mu_a\), and the scattering coefficient, \(\mu_s\). Although more accurate models of the ballistic photon field exist which describe propagation at early diffuse times [20] (corresponding to high modulation frequencies), we have found that at the relatively low frequencies involved in this work the simple exponential decay, Eq. (34), is adequate to model the frequency response of the...
system. Besides, the high scattering coefficients measured in the experiments render the ballistic photon contributions negligible. The diffuse component of the photon field is described [21] by the boundary value problem of the differential equation,

\[
\frac{d^2}{dz^2} \Psi_d(z) - 3 \mu_s' \mu_t' \Psi_d(z) = -3(1 - R)I_0 \mu_s(\mu_t + \mu_s g) \exp(-\mu_t z),
\]

and the boundary conditions of optical flux continuity at surfaces at \( z = 0, L \):

\[
\Psi_d(0) - A \frac{d}{dz} \Psi_d(z) \big|_{z=0} = 0,
\]

(36a)

\[
\Psi_d(L) + A \frac{d}{dz} \Psi_d(z) \big|_{z=L} = 0.
\]

(36b)

In Eq. (35), \( \mu_t' = \mu_s + \mu_t (1 - g) \) and \( g \) is the mean cosine of the scattering angle. The particular solution is

\[
\Psi_{d,p}(z) = (1 - R)I_0 \frac{\mu_s(\mu_t + \mu_s g)}{\mu_s'(\mu_a - \mu_t'^3/3 \mu_s')} \exp(-\mu_t z).
\]

(37a)

The homogeneous solution is of the form

\[
\Psi_{d,h}(z) = a \exp(Qz) + b \exp(-Qz),
\]

(37b)

where \( Q = \sqrt{3 \mu_s' \mu_t'} \). The integration constants \( a \) and \( b \) are determined by boundary conditions (36). Here the constant \( A \) is given as \( A = \frac{1 + AQ}{1 - AQ} \), where \( r \) is the internal reflectance, defined as the ratio of the upward-to-downward hemispherical diffuse optical fluxes at the boundary [22, 23].

It can be shown that

\[
b = \frac{e^{Qz} X e^{Qz} (dY) - e^{-Qz} (dY)}{1 - X^2 e^{Qz}},
\]

(38a)

\[
a = -bX - dY,
\]

(38b)

where

\[
X = \frac{1 + AQ}{1 - AQ}, \quad Y = \frac{1 + AQ}{1 - AQ}, \quad Z = \frac{1 + AQ}{1 - AQ},
\]

\[
d = (1 - R)I_0 \frac{\mu_s(\mu_t + \mu_s g)}{\mu_s'(\mu_a - \mu_t'^3/3 \mu_s')}. \]

(38c)

Combining both the particular and homogeneous solutions, the total photon density field expression describing the optical field in the bone matrix in Eqs. (32) and (33) becomes

\[
\Psi(z) = ae^{Qz} + be^{-Qz} + [d + (1 - R)I_0]e^{-\mu_t z}.
\]

(39)

III. MATERIALS AND METHODS

The cortical layer of human skull bones was stripped off its trabecular underlayer and then chosen for this study because of its mechanical strength which allowed filing its thickness down to 1.2 mm without crumbling. The skull samples used for this study were harvested from a cadaver from the Toronto General Hospital. They were stored in a container filled with saline solution when they were not in use. The saline solution was used to prevent the bone samples from developing cracks due to excessive dryness. Before the start of an experiment, a sample was removed from the storage container and was dried using a piece of paper towel. It was then left out to dry and thermalyze at the ambient temperature for approximately 20 min before commencing measurements. This procedure ensures that the presence of water inside the bone does not affect the optical properties of the sample and also minimizes the influence of temporal variations of the dc temperature on the measured LUM signal. A photograph of a typical skull sample is shown in Fig. 4. Skull bones were attached to LEGO™ blocks using multipurpose epoxy (5 min Epoxy, Lepage). The block was then snapped on a LEGO™ block holder fastened to the circular rotational stage that allows rotation around the \( z \) axis. The holder was mounted on a micrometer stage. This configuration allowed the small skull samples to translate in three orthogonal directions and rotate around the vertical axis (\( z \) direction). These four degrees of freedom enable the accurate control and the precise positioning and orientation of the skull samples. As a consequence, reproducibility of experimental data was excellent as verified by studies conducted to monitor signal variation at different regions on the sample surface and the effects of changing laser radiation incidence angle.

A schematic representation of the experimental system is displayed in Fig. 5. The main components of the system include a low-powered semiconductor laser diode, a lock-in amplifier, a laser diode controller, an infrared detector, a photodetector, and a desktop computer. The semiconductor laser diode emitting at 659 nm (Mitsubishi ML101U27, maximum power: 120 mW) with a beam diameter of 0.5 mm was used as the sample excitation source. During an experiment, the
The purpose of the first series of experiments conducted with human skull bones was to evaluate the penetration depth of the diffuse luminescence field generated by the total (coherent and diffuse) photon density field in the bone created by the incident laser radiation. The extent of LUM signal penetration depth was tested by measuring the signal, painting the back surface with black paint, remeasuring and comparing the LUM signals with and without the paint layer. This procedure was repeated several times following the filing off of approx 1 mm thickness after each set of measurements.

The second type of experiments carried with a skull bone was measurements testing the effects of changing laser intensity on the frequency dependence of the LUM signal. For those experiments, a variable neutral density filter (Newport, 960-RC) was added in front of the laser diode to control the laser intensity impinging on the bone. The laser power transmitted through four filter positions was measured by a power meter (Melles Griot, power/energy meter 13PEM001). The entire scan procedure was automated and the modulation frequency and lock-in amplifier sensitivities were adjusted automatically by our MATLAB program.

FIG. 5. Experimental setup for modulated luminescence of bones.

Tests were conducted to investigate the influence of hydration level on the LUM signal. It was found that LUM is relatively insensitive to changes in hydration level. There was a slight increase in phase lag with decreasing hydration level but overall the influence was minimal. Figure 6 shows the frequency scan results with and without the back-surface paint layer. It was found that LUM amplitude decreases with the black layer present, as expected from the enhanced absorption of the luminescence and/or excitation photons by the paint at the back interface. The LUM phase lag increased with the black paint thus indicating that the observed effects were not a simple change in the LUM field density in the body of the skull bone, but rather the interaction of the LUM field with the chromophore(s) responsible for the generation of luminescence. In Fig. 6 results for thicknesses above 3.2 mm are not shown since those scans were insensitive to the application of black paint. Therefore, it can be concluded that cortical skull bone of thickness higher than 3–4 mm is effectively optically semi-infinite but higher depth resolution may be obtained with higher fluence lasers. Nevertheless, following the photophysical analysis of the frequency scans, it will be seen that the LUM decay (relaxation) lifetime is a substantially more sensitive thickness probe than the presence of the back-surface paint layer.

IV. RESULTS

Figure 7 shows the dependence of the LUM frequency scans on laser power (intensity). It is seen that an increase in laser power increases the LUM amplitude, as expected, but not equally at all modulation frequencies. High frequencies increase proportionately more than low frequencies. Correspondingly, the LUM phase lags decrease with increasing power and the minimum shifts to higher frequencies. At the highest frequencies the phase lags converge to essentially the same value. Experiments conducted as a function of modulation frequency with thickness as a parameter yielded results very similar to those of Fig. 6. The salient features were that LUM amplitude decreases and phase lag increases with decreasing sample thickness. The LUM amplitude decrease for thinner bone can be understood within the framework of the two mechanisms discussed above: by a larger portion of the laser energy being transmitted directly through the bone rather than becoming absorbed and internally converted to luminescence radiation and by the smaller total number of backscattered photons in the turbid medium.
The theory developed in Sec. II has been applied to the interpretation of the behavior of laser-irradiated cortical skull bone observed in Fig. 6 without back paint and Fig. 7 and has been used to extract optical properties of the bone as well as physical quantities associated with the coupling of the optical field to the molecular excitation process of the luminescent chromophore. The theoretical model has also been found to be consistent with the sample LUM behavior in the presence of back-surface paint shown in Fig. 6.

### V. DISCUSSION

The theory developed in Sec. II has been applied to the interpretation of the behavior of laser-irradiated cortical skull bone observed in Fig. 6 (without back paint) and Fig. 7 and has been used to extract optical properties of the bone as well as physical quantities associated with the coupling of the optical field to the molecular excitation process of the luminescent chromophore. The theoretical model has also been found to be consistent with the sample LUM behavior in the presence of back-surface paint shown in Fig. 6.

#### A. Lifetime dependence on laser intensity

The effective relaxation time constant $\tau = \tau(I_0, L)$ as shown in Eq. (32) involves an integral which can be calculated from Eq. (39) as follows:

$$\int_0^L \Psi(z) dz = \frac{a}{Q} (e^{QL} - 1) + \frac{b}{Q} (1 - e^{-QL}) + \frac{[d + I_0(1 - R)]}{\mu_t} (1 - e^{-\mu_t L}).$$

The frequency dependence of the amplitude and phase of the LUM signal in Figs. 6 and 7 can be fitted to the theoretical expression (33) using Eqs. (28) and (40). Since the photon field $\Psi(z)$ linearly depends on the incident laser intensity $I_0$, the dependence of $1/\tau(I_0, L)$ on this quantity is also expected to be linear. The experimental LUM amplitude and

![Figure 6](attachment:image6.png)

**FIG. 6.** LUM amplitude (a) and phase (b) frequency scans for various skull bone thicknesses with and without the presence of a back-surface black paint layer. The continuous lines are theoretical best fits to the experimental curves. Extracted effective lifetimes, $\tau_{\text{eff}} = 9.167$ ms ($L = 3.2$ mm, no paint); $9.381$ ms ($L = 3.2$ mm, with paint); $10.33$ ms ($L = 2.2$ mm, no paint); $11.09$ ms ($L = 2.2$ mm, with paint); $13.01$ ms ($L = 1.2$ mm, no paint); $14.18$ ms ($L = 1.2$ mm, with paint).

![Figure 7](attachment:image7.png)

**FIG. 7.** LUM amplitude (a) and phase (b) frequency scans for various incident laser beam powers: 54, 41.6, 13.7, and 5.9 mW. The continuous lines are theoretical best fits to the experimental curves.
DYNAMIC PHOTOPHYSICAL PROCESSES IN LASER-

PHYSICAL REVIEW E 80, 021920 (2009)

affected the optical scattering and absorption coefficients of bones [4,6]. This close similarity may possibly point to a connection of the endogenous chromophore responsible for the observed strong luminescence emission in cortical skull bone to HAp crystals known to be contained in the bone along with collagen [6]. A difficulty for this assignment is the effective order of magnitude difference in the extracted lifetimes from skull bone (6–16 ms, Table I) and that for dental enamel HAp (2.1 ms) [17]. It is possible that the different chemical structure of skull bone HAp and/or the presence of other constituents such as collagen may have a strong effect on the LUM relaxation response of cortical bone HAp, affecting the relaxation lifetime. Similar relaxation perturbation phenomena are widely known to occur in inorganic solid-state luminescent materials under the name “crystal-field effects” when substitutional atomic or ionic impurities alter the internal potential fields responsible for emission and shift values of radiative lifetimes. Additional similarities between HAp and skull bone optical properties have been found and are discussed below (Sec. V B).

The effective lifetime decrease with optical field intensity increase in the bone matrix reveals the type of photophysical interaction mechanism between luminescence photons and chromophore excited state. In this picture the presence of a LUM photon in the vicinity of a luminescent molecule in the upper (excited) state increases the probability for inducing the molecular decay into the lower state. This probability increases with photon density. This mechanism is also consistent with the back-surface paint results in Fig. 6. The LUM density (and amplitude) decreases due to enhanced absorption of incident photons by the paint layer, photons which otherwise would have been backscattered into the bone matrix. The decreased photon density eliminates some induced decays of the photoexcited fluorophore, resulting in longer effective lifetimes, as observed from the theoretical best fitting of Eq. (33) to the various bone-thickness data of Fig. 6 with and without the black paint layer. The LUM signal dependence on laser intensity and the calibration of bone LUM signals with respect to it by means of effective lifetime measurements is an important mechanism to consider when using this technique to study detailed optical interactions of laser fields with chromophores in the bone structure and extract intrinsic and effective lifetimes.

B. Lifetime dependence on skull bone thickness

Using the same procedure, the LUM amplitude and phase experimental curves from skull thickness studies, Fig. 6 and data from additional thicknesses (4.2, 5.2, and 6.2 mm; not shown), were fitted to Eq. (33) to extract the effective lifetime, $\tau$ as a function of thickness $L$. The best fits for the thinner slices are shown in Fig. 6 and extracted effective lifetimes for all thicknesses are listed in Table II. It is clearly seen that LUM effective lifetime measurements are sensitive to bone thickness as large as 6.2 mm and beyond, while the application of black paint on the back surface does not affect the depth-integrated LUM signal for thicknesses more than 3–4 mm, Fig. 6. To understand the effective lifetime dependence on the thickness of the sample, the integral $\langle \Psi_{0f}(L) \rangle$...
\(= C_1 \int_0^L \Psi_0(z) dz\) must be computed. In the previous case, Sec. V A, there was no need for this detailed calculation as the \(I_0\) dependence can be separated out of the integrand and is always linear, while the rest of the integral is carried along as a constant. Several simulations were performed with \(C_1 = 1\) for simplicity. Selected results are shown in Fig. 10. The family of curves shown in that figure has fixed value of \(\mu_a\) and three different values of \(\mu_s\). It is seen that increasing \(\mu_s\) decreases the value of the integral for large thicknesses, as expected, since less photon flux can reach deeper inside the turbid bone matrix under enhanced scattering. Nevertheless, for very thin layers (\(L < 1.3\) mm), the trends are nonmonotonic because the value of the integral depends on trade-offs between photon losses through the front and back surfaces (low \(\mu_s\) and photon density enhancement through multiple interreflections and/or backscattering at those surfaces. Figure 9 also shows that the integral tends to saturate with increasing thickness: the higher the value of \(\mu_s\), the smaller the saturation thickness value. This is due to the strong attenuation of the photon field at large depths which then contribute little or nothing to the integral. For smaller values of \(\mu_s\), the photon field density is larger at deeper locations in the bone matrix, thus contributing larger values to the integral. Other simulations showed that the value of the integral decreases substantially with increasing \(\mu_s\), as expected from the greater photon loss to the bone matrix as a result of enhanced absorption. Physical consequence of these simulations is that a thicker medium captures a higher number of incident photons, thus contributing to higher cumulative LUM signal amplitude, as shown in Fig. 6(a). Another expectation is that more induced excited-state decay events will occur in a thicker matrix, thus producing a larger density of luminescence photons contributing to an enhanced mean decay rate of the chromophores and a shorter effective lifetime, \(\tau\) as per the theoretical mechanism of Sec. II. This expectation was borne out using the extracted values of \(\tau\) in Table II in a plot of the inverse effective lifetime vs thickness shown in Fig. 10. Equation (32) was used to fit the data to the theory. As expected, \(\tau\) decreases with increasing \(L\). The simplex down-hill multiparameter fitting algorithm was used to determine the best-fitted optical absorption and scattering coefficients and \((B_{21}/2c)C_1\) values [27].

In many multiparameter fitting problems, the solution set may contain parameter values that give the best agreement between the theoretical and experimental curves mathematically but are physically invalid. To solve this difficulty, the down-hill simplex algorithm was modified so that the fitted parameters only varied within a set range of values. Based on literature values, \(\mu_s\) was allowed to vary between 25 000 and 45 000 m\(^{-1}\) while \(\mu_a\) varied from 2 to 80 m\(^{-1}\). Using this enhanced algorithm, the optimal values of \((B_{21}/2c)C_1, \mu_s,\) and \(\mu_a\) were found to be 0.0079 mJ, 44 340 m\(^{-1}\), and 13 m\(^{-1}\), respectively. The best-fitted curve is plotted on Fig. 10. The aforementioned \(\mu_s\) and \(\mu_a\) values for laser excitation at 659 nm are comparable to \(\mu_s \sim 35 000\) m\(^{-1}\) and \(\mu_a \sim 60\) m\(^{-1}\) reported for horse-leg bones by Uglyumova et al. [6] who attributed these values to the presence of HA\(p\) crystals in the bone, and \(\mu_s \sim 9–13\) m\(^{-1}\) reported for human calcaneus by Cubbedu et al. [28].

The foregoing interpretation of the experimental results in terms of chromophore photophysics can be used to estimate the value of the mean optical coupling coefficient \(B_{21}\) assuming the extinction depth to be defined as the depth beyond which diffuse luminescence attenuates adequately so

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**TABLE II.** Extracted effective lifetimes \(\tau_M\) for various skull bone thicknesses.

<table>
<thead>
<tr>
<th>Thickness (mm)</th>
<th>Extracted lifetime (ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.2</td>
<td>7.132</td>
</tr>
<tr>
<td>5.2</td>
<td>7.141</td>
</tr>
<tr>
<td>4.2</td>
<td>7.439</td>
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<tr>
<td>3.2</td>
<td>9.167</td>
</tr>
<tr>
<td>2.2</td>
<td>10.330</td>
</tr>
<tr>
<td>1.2</td>
<td>13.006</td>
</tr>
</tbody>
</table>

**FIG. 9.** Photon density integral \(I_0\) with \(\mu_a\) and \(\mu_s\) values as parameters.

**FIG. 10.** Inverse effective lifetime \(\tau_M(I_0, L)\) as a function of bone thickness \(L\).
that the sample is essentially semi-infinite for all larger thicknesses. This defines the luminescence saturation length $L_{sat}$. Our frequency scans with cortical bone thicknesses larger than those shown in Fig. 6, both amplitude and phase, showed that $L_{sat} \approx 5.2$ mm. Using Eqs. (30) and (31), $D \approx L_{sat} = 1/C_1$ or $C_1 = 192$ m. Therefore, from the best-fit values in Fig. 10 we find $B_{21}/2c = 6.4 \times 10^{-4}$ m$^2$/J. Assuming the refractive index of bone to be approx 1.55 at 700 nm, attenuation tomography with multipliers $/H_20849$ can be easily detected using synchronous lock-in detection and correlation length. This means that laser-source-filtered LUM can be generated in the 700–850 nm range, although strongly scattered by bones. Similarly, the bone luminescence thus shown that the sample is essentially semi-infinite for all larger thicknesses. This defines the luminescence saturation length $L_{sat}$.

A final comment can be made regarding the potential of modulated luminescence lifetime markers for clinical in vivo detection of BMD with the presence of an overlying skin layer. LUM is a method which is based on direct near-infrared radiative photon emissions from hard tissue chromophores. Optical absorption depth in soft tissue in the near-infrared (NIR; 650–1000 nm) spectral range is largely determined by that of water [30] and can be large (ca. 10 mm at 700 nm). However, scattering controls the overall optical attenuation [19]. These facts imply that a relatively small number of incident NIR photons have the ability to penetrate deeply into human tissue overlying bone structures and to interact with bones. Similarly, the bone luminescence thus generated in the 700–850 nm range, although strongly scattered by overlying tissue, has approx 1.0 cm optical absorption length. This means that laser-source-filtered LUM can be easily detected using synchronous lock-in detection and sensitive state-of-the-art photodetectors, e.g., Si or InGaAs, as in the experimental configuration of Fig. 5, or even photomultipliers (e.g., Hamamatsu NIR photomultiplier model H9170–75) for cases of deeper seated bones, like at the base of the spine or the wrist.

VI. CONCLUSIONS

A photophysical theory of modulated diffuse luminescence (LUM) of bones was developed and used to interpret associated measurements performed on the cortical layer of human skull bones. The purpose of the study was to assess the feasibility of applying LUM as a marker for BMD measurements for monitoring the onset of osteoporosis. The theory was based on a general excited-state manifold of the chromophore molecules constructed on the basis of experimental behavior and leading to a set of optical excitation and decay rate equations of the luminescent chromophores in the bone and on the photophysical molecular interactions with the photon field in the matrix of the bone. An effective depth-dependent relaxation lifetime $\tau(z)$ and its mean value $\tau_M$ of skull cortical bone was derived theoretically and was found to depend on the photon field density, as well as on the thickness of the bone. A linear dependence of $\tau_M$ on laser beam intensity, $I_0$, was found and sensitivity of the value of $\tau_M$ to bone thickness, $L$, was observed for $L \leq 6.2$ mm. Both $I_0$ and $L$ experimental dependencies were in excellent agreement with the theoretical model. The values of optical absorption and scattering coefficients of the endogenous chromophore in the bone matrix at the excitation wavelength were calculated from plots of $\tau(I_0, L)$ vs laser intensity and bone thickness. The values of the coupling coefficient $B_{21}$ and the intrinsic lifetime of the chromophore were also measured. Using similarities between skull bone LUM frequency responses, both amplitude and phase, and the frequency responses of dental LUM generated by enamel HA/0, as well as the reported optical properties of human and horse-leg bones, it was shown that the luminescent chromophore in cortical human skull bone may be related to the form of HA/0 encountered in it. However, more work remains to be done to clarify the origin of skull bone modulated luminescence, mainly at the spectroscopic level, which can be aided by the application of the theory developed in this paper.

In summary, quantitative LUM can be used as a sensitive method to measure optical properties of the active chromophore in cortical skull bones and the optical-field-induced molecular interaction parameter, potentially as markers of bone health or disease, such as osteoporosis or cancer. When calibrated vs laser intensity, the LUM technique can also be used to measure human skull thickness. More LUM studies involving tissue-covered bones are underway to assess the technique’s clinical relevance and sensitivity to BMD in the presence of a skin overlayer.

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