Čerenkov radiation emission and excited luminescence (CREL) sensitivity during external beam radiation therapy: Monte Carlo and tissue oxygenation phantom studies

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Abstract: Radiotherapy generates Čerenkov radiation emission in tissue, and spectral absorption features appearing in the emission spectrum can be used to quantify blood oxygen saturation (S\textsubscript{tO2}) from the known absorptions of hemoglobin. Additionally, the Čerenkov light can be used to excite oxygen-sensitive phosphorescence of probe PtG4, whose emission lifetime directly reports on tissue oxygen partial pressure (pO\textsubscript{2}). Thus, it is feasible to probe both hemoglobin S\textsubscript{tO2} and pO\textsubscript{2} using external radiation therapy beam to create as an internal light source in tumor tissue. In this study, the sensitivity and spatial origins of these two signals were examined. Emission was detected using a fiber-optic coupled intensifier-gated CCD camera interfaced to a spectrometer. The phosphorescence lifetimes were quantified and compared with S\textsubscript{tO2} changes previously measured. Monte Carlo simulations of the linear accelerator beam were used together with tracking of the optical signals, to predict the spatial distribution and zone sensitivity within the phantom. As the fiber-to-beam distance (FBD) varied from 0 to 30 mm, i.e. the distance from the fiber tip to the nearest side of the radiotherapy beam, the effective sampling depth for CR emission changed from 4 to 29 mm for the wavelengths in the range of 600-1000 nm. For the secondary emission (phosphorescence) the effective sampling depth was determined to be in the range of 9 to 19 mm. These results indicate that sampling of S\textsubscript{tO2} and pO\textsubscript{2} in tissue should be feasible during radiation therapy, and that the radiation beam and fiber sampling geometry can be set up to acquire signals that originate as deep as a few centimeters in the tissue.

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1. Introduction

Recent work demonstrated the occurrence of Čerenkov emission light during external beam radiotherapy [1], using linear accelerator (LINAC) irradiation from electrons and photons. The emitted radiation has broad spectrum, and both absorption and emission properties of the medium can be quantified using this radiation [2]. The generation of the broadband light from within irradiated tissue opens up potential possibility to sample the tissue microenvironment in vivo during therapy. In this study, the particular focus is on determining the potential for sensing oxygenation in vivo and determining the spatial origins of the signal. While a previous study demonstrated that spectroscopy of tissue hemoglobin oxygen saturation (SO2) is feasible using Čerenkov emission as an internal light source, the spatial origins of the signal and the detection sensitivity distribution were not examined. The present study considers this, as well as the potential to induce phosphorescence of exogenous oxygen-sensitive probes [3] in tissue, thereby measuring both tissue SO2 and oxygen partial pressure (PO2) [4]. This combined approach may be useful in tumor radiotherapy, as it may allow comprehensive quantification of tissue hypoxia as well as changes in hypoxia during the course of fractionated radiation therapy. The spatial origins of both absorption-based SO2 and phosphorescent-based PO2 signals have been quantified in our study through experiments and Monte Carlo modeling.

Čerenkov emission occurs when charged particles move through a dielectric medium at a phase velocity greater than the speed of light in that medium [5], inelastically losing energy through electrical field interactions with the transiently polarized medium. This emission can happen in biological media such as human tissue [6]. Unlike atomic or molecular luminescence spectra that have characteristic spectral peaks corresponding to quantized energy transitions, Čerenkov emission has continuous spectrum across the entire UV, visible and NIR spectrum with intensity varying as the inverse-square of the wavelength [7]. Thus, the emission at higher frequencies (shorter wavelengths) is more intense, giving rise to its characteristic blue glow appearance, and it tends to be directionally emitted at an acute angle relative to the path of travel [7]. The Čerenkov emission originating from several biomedical radiotracers (18F, 64Cu, 68Ga, etc.) has recently been characterized in both phantoms and small animals [8–12]. The discovery of optical molecular Čerenkov luminescence imaging (CLI) has been recently reported [10], and later this approach has been extended on 3D tomography [6,13–16]. Nonetheless, the exploitation of Čerenkov emission in radiation
therapy is quite recent, and it is still unexplored as a method of sampling tumor tissue microenvironment.

It is well known that tissue oxygenation is a major factor influencing the outcome of radiation therapy [17,18], since oxygenated cells are more effectively killed by radiation than hypoxic cells. Additionally, chronically hypoxic tumor tissues are insensitive to vascularly delivered drugs, e.g. in chemotherapy, due to poor delivery. Thus techniques for measuring tumor $pO_2$ during fractionated therapy may be extremely useful for tuning the treatment conditions and monitoring therapeutic outcome. However, current measurements of $pO_2$ are usually invasive [19], and the obtained signals are heterogeneous on a microscopic scale, requiring sampling of large volumes to gain information about individual tumor’s oxygenation status [20,21]. Optical spectroscopy is a noninvasive technique that has been used to measure oxygen hemoglobin saturation, which is related to tissue $pO_2$, during the course of fractionated radiation therapy in experimental tumors [22]. Since Čerenkov light has the spectrum allowing quantification of hemoglobin $S_tO_2$ [2], measuring this spectrum during treatment has the potential for real-time monitoring of blood oxygen saturation during treatments. In the previous work, alterations in the Čerenkov emission spectroscopy (CES) measured during radiation treatment of living tissue have been combined with diffuse optical tomography (DOT) to estimate microvascular $S_tO_2$ [2]. Further exploration of the utility of CES for determination of tumor blood oxygen saturation may prove to be valuable for potential clinical applications. Using simulations, this study takes an in-depth look at the spatial origins of the CR signal induced by an electron beam and sampled by an optical fiber, with varying fiber-to-beam distances.

Čerenkov emission has been shown to be able to serve as an excitation source for secondary luminescence, most recently with quantum dots (Qtracker705) [23]. The UV-blue-green components of this emission are higher in intensity, but the attenuation of this light is orders of magnitude higher than of the red-near-infrared components due to the hemoglobin absorption. Thus Čerenkov Radiation Excited Luminescence (CREL) can be used to induce emission at wavelengths in the near-infrared (NIR) range, where there is less absorption and light originating from deep tissues reaches the surface. One of the advantages of Čerenkov emission is that it is generated only within the targeted tissue of interest, such as within tumor during targeted radiation therapy. This provides a broadband source within the irradiated tissue with a well-known spatial origin, whereby CREL detection can be optimized by arranging the detection system with respect to the incident electron or photon beam.

Here we explore the possibility of combining Čerenkov emission with the phosphorescence quenching method [4] for tissue oxygen imaging. CES of hemoglobin and CREL in combination with phosphorescence has been shown to have the potential to reveal spatial distributions of two important tissue parameters: $S_tO_2$ and $pO_2$. Spatial origins of the two signals have been evaluated in order to evaluate the technique's potential for pre-clinical or clinical radiation therapy. This study was based on tissue phantoms and Monte Carlo simulations to characterize spatial signal distributions for CES and CREL. The experimental CREL lifetime measurements were carried out using a well-characterized oxygen sensitive probe, PtG4 [3]. Since the lifetime of CREL of PtG4 is sensitive to changes in $pO_2$, phosphorescence lifetime measurements could be used to obtain information on oxygenation of the sampled region. Simulations were carried out to investigate the influence of spatial arrangements of the radiotherapy beam relative to the sampling optical fiber.

2. Methods

2.1 CR-induced phosphorescence lifetime measurements

To couple Čerenkov Radiation (CR) with Phosphorescence Lifetime Imaging (PLI) we used dendritic phosphorescent probe PtG4. PtG4 is the direct analog of recently reported probe G4 [3], with the difference being that PtG4 contains platinum(II) instead of palladium(II)
tetraaryltetrabenzo[porphyrin as its core phosphorescent sensor. A number of closely related Pt tetrabenzo[porphyrin-dendrimers have been recently disclosed [24]. PtG4 phosphoresces at room temperature in deoxygenated aqueous solutions with quantum yield of 0.07 (measured against fluorescence of Rhodamine 6G in ethanol, 0.94). The phosphorescence lifetime of PtG4 in the absence of oxygen at 22°C is $\Theta_0 = 47 \mu s$. The phosphorescence lifetime imaging system used in the present study resembled that used recently [3]. PtG4 has been introduced in the phantom. Phosphorescence of PtG4 was excited by Čerenkov radiation originating from the radiotherapy beam. A lifetime imaging system, based on an ICCD camera, operated in time domain, as described below.

2.1.1 CREL tissue phantom

This study utilized liquid optical phantoms composed of phosphate buffered saline solution (D-PBS, Mediatech Inc., Manassas, VA), with 1% v/v Intralipid© (Fresenius Kabi, Uppsala, Sweden) used to provide scattering, and 1% v/v porcine whole blood to provide absorption. For the pO$_2$ luminescence studies, PtG4 was added to the phantom to reach the final concentration of 5 µM. The phantom was deoxygenated using D-glucose, glucose oxidase and catalase [25]. Fully oxygenated and fully deoxygenated states were investigated. The phantom was sealed during the deoxygenation process to avoid re-oxygenation.

2.1.2 CREL lifetime measurement system

The experiments were performed with a linear accelerator (Varian Clinic 2100C, Varian Medical Systems, Palo Alto, USA) at the Norris Cotton Cancer Center. As shown in Fig. 1(A), the initial beam shape was 60 mm by 60 mm square, which irradiated the entire top of the phantom. An electron beam with energy of 18 MeV was used for these measurements. The measurement system consisted of a single fiber bundle (CeramOptec, Germany) 15 meters in length, which housed nineteen 200 micron diameter silica fibers. This fiber bundle collected the light at the boundary of the phantom at a point location and guided the signal into a vertical line of fibers at the entrance slit of the spectrometer. The fiber bundle tip was positioned in contact with the flat surface and 25 mm from the top surface of the liquid. The spectrometer (Acton Insight, Princeton Instruments, Acton, USA) was connected to a fast gated ICCD (PI-MAX3, Princeton Instruments, USA). Since the spectrometer and ICCD were placed outside the radiotherapy room, no shielding from the radiation generated by the LINAC was necessary. The ICCD was cooled to −25°C and the grating used in all experiments was 300 lines/mm. Continuous wavelength spectra were acquired with an integration time of 20 seconds. For all the measurements, background files were measured for the same setting up, same time-line and same radiotherapy beam with empty container. Each spectrum was processed by normalizing the total collected counts by the integration time (yielding counts/sec), background subtraction and smoothing by averaging ten neighboring spectral data points into bins 0.49 nm wavelength width. The background subtraction technique accounted for Čerenkov emission induced in the fiber and containers during phantom measurements. The lifetime and oxygen partial pressure for both fully oxygenated and deoxygenated states were measured independently using a frequency-domain phosphorometer [24,26] to validate the CREL lifetime measurements.

2.1.3 CREL lifetime measurement sequence

Since the linear accelerator (LINAC) pulses electrons in 5 µs bursts at a repetition rate near 180 Hz, a fast time gated system was designed to measure the lifetime of CREL gating to these pulses. As shown in Fig. 1(B), the external radiotherapy beam triggers when the beam is on, to sample the CREL signal and reject sampling when the beam is off. By choosing the gate delays with respect to the trigger signal, the intensity of CREL at different time points was measured and the lifetime was calculated by fitting the intensity decay with a single exponential model (Eq. (1)):
\[ I_t = I_0 \exp\left(\frac{-t}{\tau}\right), \] 

(1)

where \( I_0 \) is the initial intensity, \( I_t \) is the intensity measured for different time points and \( \tau \) is the lifetime. Once the lifetime been calculated from the exponential fitting, \( pO_2 \) values could be calculated from Stern-Volmer model (Eq. (2)):

\[ \frac{1}{\tau} = \frac{1}{\tau_0} + k_q \times pO_2, \]

(2)

where \( \tau \) is the phosphorescence lifetime at a specified oxygen pressure \( pO_2 \), \( \tau_0 \) is the phosphorescence lifetime in the absence of oxygen \( (pO_2 = 0) \), and \( k_q \) is the quenching constant [3].

In this work, delays were set with respect to the falling edge of the trigger signal. About 35 time points were measured by choosing the delays varied from 8 \( \mu \)s to 200 \( \mu \)s. The gate width was chosen to be 3 ms to make sure that all the CREL emitted after the delay was measured. The intensifier of the ICCD was turned on to be a gain of \( \times 100 \). The measurement for every time point was an on-CCD accumulation for 500 trigger pluses. Each time point takes 2.5 sec to measure and thus 35 time points measurement for the lifetime exponential fitting takes 87.5 sec approximately. The dose rate of the LINAC has been kept to be 4 Gy/min which leads to an entrance dose of approximately 5.83 Gy delivered to the phantom for the CREL lifetime assessment of one oxygenation state.

To prove that this gating technique could also be applied to measure CR instead of CREL for fully oxygenated and deoxygenated states, gated measurement of CR has also been done by setting the delay to 3 \( \mu \)s and gated width to 5 \( \mu \)s to only detect the CR signal for a phantom without adding PtG4. \( S_O2 \) values have been measured by an ischemia monitoring system (Spectros T-stat, Spectros, Portola Valley, CA).

Fig. 1. The geometry of the measurement system and temporal acquisition process are shown with (A) the fast time gated spectrometer system, (B) the time line of how the linear accelerator works in pulsed mode and the way to measure decays of CREL. The data were acquired while choosing gate width (D to E) of 3 ms and a gate delay varying (A to D) starting with 10 \( \mu \)s and ending up with 200 \( \mu \)s.

2.2 CR and CREL detection sensitivity simulation

This study uses the Geant4 toolkit for Monte Carlo modeling to stochastically simulate radiation transport associated with CR and CREL measurements. This C++ code was developed by an international consortium to model particle (such as photons, electrons or
protons) transport through matter using subroutines that describe the physics of particle-matter interactions; these subroutines have been validated against National Institute of Standards and Technologies reference data [27]. The experimental geometry was modeled in the GEANT4 code to study the detection sensitivity for both CR and CREL. Optical properties of tissue phantom were pre-defined. Adjoint method was adopted to simulate the detection sensitivity distribution.

2.2.1 Geometry and tissue phantom

The GAMOS toolkit [28], was used to mimic the geometry utilized in experiments in the simulations performed by Geant4. The present study simulates fiber optic measurement of an irradiated phantom that is defined here as slab geometry with specified dimensions of 60 x 60 x 100 mm, which was voxelised into cubes with 0.5 mm dimension. Figure 2(A) shows a schematic of this simulated geometry. A fiber with a diameter of 1.2 mm and numerical aperture of 0.22 was placed in contact with the surface of the container at several positions (30 mm, 0 mm, and 75 mm) along the +y direction. Parallel external beam were used to irradiate the phantom from the top surface. To illustrate the configuration, Fig. 2(B) shows the simulated detection sensitivity of CR within a homogeneous, tissue-mimicking volume with absorption and scattering spectral properties given in Fig. 2(C). To investigate how the detected sensitivity distribution and effective sampling depth are influenced by changes to the arrangement of the radiation beam and the fiber bundle, the electron beam has been shifted further and further away from the fiber tip with fiber-to-beam distance (FBD) of 0 mm, 10 mm, 20 mm and 30 mm.

To match the experimental conditions, both the components and optical properties of phantom for simulation have been defined to match the experimental phantom described in 2.1.1. For the CR detection sensitivity simulation, the phantom was composed of water with 1% v/v intralipid as a scattering standard and 1% v/v porcine whole blood as an absorber. The oxygen saturation \( S_{O_2} \) in these phantoms was defined as 90%. For CREL detection sensitivity simulation, the oxygen-sensitive luminescent agent PtG4 was defined as an additional component of 5 \( \mu \)M concentration. Phantom optical properties were defined for all the materials for the simulations. In details, reflective index and absorption coefficient was defined for water from previous work [29,30]. The absorption coefficients of whole blood were calculated by weighting the absorption of hemoglobin and absorption of deoxy-hemoglobin according to predefined \( S_{O_2} \) value [31,32] at all relevant wavelengths needed. The absorption coefficient, emission spectrum and quantum yield were defined for PtG4 as measured in previous studies [3], and are displayed in Fig. 3(A) and Fig. 3(B). The total absorption coefficient of the phantom for simulation was calculated by (Eq. (3)):

\[
\mu_a = c Hb_o \times [S_{O_2} e_{HbO} + (1 - S_{O_2}) e_{Hb}] + C_w \mu_{H2O} + C_{G4} \mu_{G4},
\]  

(3)

where \( c Hb_o \) is the total hemoglobin concentration, and \( e_{HbO} \) and \( e_{Hb} \) are the extinction coefficient for oxy- and deoxy-hemoglobin, respectively. \( C_w \) is the water fraction, and \( \mu_{H2O} \) and \( \mu_{G4} \) is the absorption coefficient of pure water and PtG4. The exact scattering coefficient and g factor values for intralipid [33,34] were calculated for the chosen concentration and wavelength range by Eq. (4) and Eq. (5):

\[
\mu_s = C_{intralipid} \times 2.54 \times 10^7 \times \lambda^{-2/4},
\]  

(4)

\[
g = 1.1 - 0.58 \times 10^{-3} \times \lambda.
\]  

(5)

All the optical properties have been defined in data arrays corresponding to wavelength from 300 nm to 1000 nm with increment of 2 nm. An 18 MeV electron parallel beam was defined for all simulations.
Fig. 2. The geometry of simulations and optical properties of phantom are shown with (A) a 60x60x100 mm cuboid container defined and voxelised into 0.5 mm cubes. The coordinate system was as indicated here, and a fiber with diameter 1.2 mm and numerical aperture 0.22 was posited right on the surface of the container at position (x = 30 mm, y = 0 mm, z = 75 mm). The external electron beam irradiated the phantom from the top surface. In (B) the intersections of a typical simulation to show how the sensitivity distribution appeared in 3D, and in (C) the optical properties are shown of the tissue mimicking phantom made of water, 1% Intralipid, 1% whole blood and PtG4 with a concentration of 5 μM.

2.2.2 Monte Carlo simulations

CR detection sensitivity simulations included subroutines to perform the following processes: 1) propagation of primary particles through the medium, 2) generation and propagation of secondary particles through the medium, 3) the generation of Čerenkov emission from charged particles. For CREL detection sensitivity simulations, additional subroutines 4) the propagation of Čerenkov photons through the medium subject to scatter and absorption, 5) absorption and emission of photons by phosphor [35] were included. This study utilized an adjoint method for Monte Carlo modeling, which independently determines 1) the emission fluence field for Čerenkov and phosphor emission and 2) the fiber capture field. The details of these calculations are given below.

During simulation of Čerenkov emission, primary electron transport is initialized by selecting a random location within the illumination cross-section x-y area and assigned z-direction trajectory normal to the interface. Geant4 accounts for complex processes of electron transport [35] such as Columbic interactions, and soft and hard collisions. Secondary particles generated during the transportation of primary particles have been taken into consideration when calculate Čerenkov emission. For every step of a charged particle track, Čerenkov radiation is stochastically sampled with the probability of emission given by application of the Frank-Tamm formula (Eq. (6)):

\[
\frac{dN}{ds} \approx \frac{2\pi d\lambda}{137\lambda^2} \left( 1 - \frac{1}{\beta^2 n^2} \right). \tag{6}
\]

where \( n \) is refractive index, \( \beta \) is the phase speed of the particle over the speed of light in vacuum, \( \lambda \) is the wavelength of Čerenkov photons and thus \( dN \) is the number of generated Čerenkov photons for the movement of step size \( ds \) in the wavelength range \( d\lambda \).

To avoid overflowing of Čerenkov photons generated per step, a maximum number of two Čerenkov photons per step were instituted. The two photons limitation ensures that for each movement, the step size of charged particles will be calculated small enough to make sure no more than two Čerenkov photons will be generated. Upon Čerenkov emission, the location, wavelength, intensity, and trajectory of the Čerenkov photons were logged and returned as the CR field. For simulation of phosphorescence emission, Čerenkov photons are propagated following emission, with the probability of absorption of Čerenkov photons by PtG4 determined by the absorption coefficient of PtG4 at the Čerenkov wavelength. Following absorption, the phosphorescence quantum yield of PtG4 was taken into consideration to calculate the number of CREL photons emitted by the corresponding absorption event.
Finally, the known emission spectrum of PtG4 was sampled to determine the wavelength of the CREL photons. The wavelength shifting process built in Geant4 was adopted to simulate the luminescence process. Upon phosphorescence emission the location, wavelength, and intensity were sampled and returned within the CREL field. For each radiotherapy beam, 100 millions of electrons has been launched as primary particles.

This study utilized the adjoint method to determine detection sensitivity profiles for an optical collection fiber. This is accomplished by assuming the detection fiber is actually a light source and simulating the optical photons at chosen wavelengths. Initial photon trajectories were sampled within the fiber cone of acceptance, which is governed by the numerical aperture. Photon mean free paths were determined using Mie scattering, Fresnel’s equations and Snell’s law were used to determine reflection and refraction, and the Beer-Lambert law modeled attenuation due to absorption. The propagation histories of photons throughout the voxelized volume was tracked, and used to return the fiber capture field. Using the reciprocity principle, the detected sensitivity distribution for Čerenkov emission was calculated as the product of the normalized fiber field and the normalized CR field. Similarly, the detected sensitivity distribution for CREL was calculated by multiplying the normalized fiber field and the normalized CREL field. The effective sampling depth was calculated by summing the corresponding coordinate of each voxel, weighted by the sensitivity value of that voxel (Eq. (7)):

\[
\text{Depth}_{\text{eff}} = \frac{\sum W_i d_i}{\sum W_i},
\]

where \(W_i\) is the sensitivity value for each voxel and \(d_i\) is the corresponding depth. This calculation returns a statistical depth value where the signal will be detected averagely. Not only the detected spatial sensitivity distribution, but also the effective sampling depths for different wavelengths will yield the wavelength dependent intensity spectra recorded during measurements.

3. Results

The continuous wavelength measurement of CREL and gated measurement of CREL and CR will be shown. By applying the time-gating technique, intensity of CREL for different time points has been measured for fully oxygenated and deoxygenated states. Phosphorescence lifetime and \(pO_2\) information have been recovered by fitting the decay of CREL with an exponential model and applying Stern-Volmer equation. Finally, simulation results about the detection sensitivity distribution and effective sampling depth for both CR and CREL will be shown and discussed.

3.1 Experimental measurements of CR and CREL

The absorption and emission spectra of PtG4 are shown in Fig. 3(A) and Fig. 3(B). Spectra measured using continuous wave (CW) and gated CREL techniques for different \(pO_2\) levels (\(pO_2 = 141.95\) Torr and \(pO_2 = 2.31\) Torr) are shown in Fig. 3(D). These results clearly show the phosphorescence peak at 772 nm, suggesting that Čerenkov radiation emission can be used to excite PtG4. As expected, the intensity of CREL from PtG4 increases upon removal of oxygen. Comparing the CW and gated spectral measurements demonstrates that time gating can be used to effectively isolate the phosphorescence signal from the Čerenkov emission. The lifetime measurements and exponential fitting are shown in Fig. 3(E). The recovered lifetime values from the fitting were listed in Table 1 with 95% confidence range for each \(pO_2\) level and were compared with the values measured independently by a frequency domain phosphorometer [36]. A gated spectrum measurement of CR from tissue mimicking phantoms with different \(S_tO2\) (\(S_tO2 = 92\%\) and \(S_tO2 = 5\%\)) is shown in Fig. 3(C). The spectrum is similar to the previous results of continuous wavelength measurements [2], which indicate...
that besides continuous wavelength measurements, the time gating technique could also be adopted to measure CR spectrum and fitting the $S_tO_2$ value similarly. One of the major advantages of the time gating technique is the ability to reduce ambient light and thus there is no need to black out light in the radiotherapy room [37]. Another advantage is that time-resolved measurement is independent on the probe concentration, reporting on the amount of oxygen in the probe environment.

Fig. 3. Experimental measurements of CREL are shown for PtG4 in different $pO_2$ levels. In (A) the Molar extinction coefficient of PtG4 is shown, with (B) the emission spectrum and (C) a gated spectrum measurement of CR shown from tissue mimicking phantoms with different $S_tO_2$ ($S_tO_2 = 92\%$ and $S_tO_2 = 5\%$). (D) A continuous wavelength and gated spectrum measurement of CREL shown from tissue mimicking phantoms with different $pO_2$ levels ($pO_2 = 141.95$ Torr and $pO_2 = 2.31$ Torr). In (E) the lifetime fitting of the fast time gated CREL intensity data is shown.

| $pO_2$ = 141.95 Torr | $pO_2$ = 2.31 Torr |
|-----------------------|---------------------|
| Lifetime values from fitting | 20.79 μs (20.08 μs, 21.56 μs) | 45.64 μs (45.13 μs, 46.19 μs) |
| Reference lifetime values | 21.29 μs | 45.62 μs |

### 3.2 Sensitivity simulations of CR emission

The calculated sensitivity distributions in the y-z plane at $x = 30$ mm are shown in Fig. 4(A-D) and sensitivity vs. depth profiles in Fig. 4 (E-H) for wavelengths from 300 nm to 1000 nm and FBDs of 0 mm, 10 mm, 20 mm and 30 mm. As shown in the figure, the detection sensitivity distribution changes with wavelength for CR which is basically due to wavelength dependent optical properties of the phantom. For FBD = 0 mm, i.e. the fiber is in touch with the side of radiotherapy beam, regions near the fiber tip are more sensitive than other regions. When the beam is shifted further away with FBD = 10 mm, 20 mm or 30 mm, regions near the beam where most of the CR have been generated showed increasing sensitivity with respect to regions near the fiber tip, especially for wavelengths greater than 500 nm which generally have better penetration through tissue. Thus, the detection sensitivity distribution of CR depends on wavelengths and the relative arrangement of the fiber and the radiotherapy beam.

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Fig. 4. Detective sensitivity distribution of Čerenkov radiation emission and sensitivity vs. depth profiles. In (A)-(D) the Detective sensitivity distribution of Čerenkov radiation emission is shown in y-z plane while x = 30 mm for broad electron beam with 18 MeV energy and fiber-beam distances = 0 mm, 10 mm, 20 mm and 30 mm. The coordinate system was the same shown in Fig. 2(A). The external radiotherapy beam propagated in –z direction initially and the fiber was put at (y = 0, z = 75), pointing +y direction. For each fiber-beam distance, wavelength from 300 nm to 1000 nm with 25 nm increment have been investigated and shown here (left to right, top to bottom). In (E)-(H) the sensitivity vs. depth profiles for broad 18 MeV electron beams are shown with fiber-beam distances of 0 mm, 10 mm, 20 mm and 30 mm and wavelength from 300 nm to 1000 nm with 100 nm increment.

Effective sampling depth and intensity were calculated and shown in Fig. 5 for wavelengths from 300 nm to 1000 nm in 25 nm increments and for all the FBDs investigated here. As anticipated, the effective sampling depth changes with wavelength because of different penetration ability in tissue. Also, as the beam moves away from the fiber tip, the effective sampling depth increase because of most of CR has been generated and detected from deeper in tissue phantom. What’s worth to note here is that the simulated spectrum for broad beam with FBD = 0 mm and for S_{O2} = 0.9 is quite similar to both the gated spectrum of CR with S_{O2} = 0.92 in Fig. 3(C) and the continuous wave measurement under similar conditions we have done in the previous paper [2], which validates the simulation method.
3.3 Simulations of CREL

Since the phosphorescence of PtG4 peaks at 772 nm, for the detected sensitivity distribution and effective sampling depth of CREL, only the wavelength of 772 nm has been considered as the rest of the spectrum is assumed to behave similarly in tissue. For this reason, the detection sensitivity distribution for CREL should be less sensitive than that of CR. As in the Čerenkov radiation emission simulations, detected sensitivity distributions are plotted in the y-z plane at x = 30 mm in the sensitivity vs. depth profiles shown in Fig. 6. To compare the effective sampling depth of Čerenkov radiation and CREL, the effective sampling depths for 772 nm wavelength in both cases are listed in Table 2. From Table 2, it is seen that although the effective sampling depth for CREL increases with increasing FBDs, it does not increase as much as that of CR. In other words, compared with CR, the detection sensitivity distribution and effective sampling depth for CREL is less sensitive to the relative arrangement of fiber and radiotherapy beam.

| FBD  | Čerenkov emission | CREL |
|------|------------------|------|
| 0 mm | 7.6 mm           | 9.3 mm |
| 10 mm| 14.8 mm          | 12.1 mm |
| 20 mm| 22.8 mm          | 15.8 mm |
| 30 mm| 29.2 mm          | 18.9 mm |

4. Discussion

One of the advantages of Čerenkov emission is that it is emitted within a targeted tissue of interest such as a tumor, where the LINAC beam delivery is well known and in fact extensively planned for each subject. The position and intensity of emission depends highly on where the charged primary particles deposit most of their energies, and thus the detection
can be optimized by the relative arrangement of the optical detector system and external beam incidence. It is well known the optical elements themselves can generate CR, and so care must be taken experimentally to ensure that the signal measured is from the tissue and not from the instrumentation. However given the high degree of knowledge about the 3D treatment plan, it is feasible to plan light pick up instrumentation around the planned delivery of the radiation. The purpose of this work was to provide a comprehensive interpretation of the locations where detected CR signals originate from within tissue, and develop some estimates on the limits of sensing if applied in external beam radiation therapy to monitor oxygen in tumors.

The detected sensitivity distributions shown in Fig. 4 vary with wavelength and fiber to beam distance. For broad beams which irradiate the entire top surface of the phantom, tissue regions nearest the fiber tip are more readily detected and the extension of detection sensitivity increases non-linearly with wavelength due to optical properties affecting the attenuation traveling through tissue. For FBD not equal to 0 mm, the sensitivity vs. depth profiles has two peaks in most of the cases (Fig. 4 bottom right). Besides regions near the fiber tip, regions inside or near the beam are also very sensitive because CR photons are generated in those regions. For longer wavelengths (~600 nm), regions inside the beam are even more sensitive than the near the fiber due to better penetration ability in tissue. As shown in Fig. 5, while fiber-beam distance changes from 0 to 30 mm, the effective sampling depth varies roughly from 4 to 29 mm for 600-1000 nm wavelength range.

Taking detected sensitivity distributions and effective sampling depth into consideration, some limitations exist for the SO₂ measurements based on Čerenkov radiation emission spectrum fitting [2]. At first, this method requires the pre-existence of hemoglobin and deoxy-hemoglobin in the region of interest (ROI). Secondly, since the sensitivity distributions and effective sampling depths depend on wavelength, the recovered value of SO₂ should be an effective value for multiple SO₂ sampled at different regions and depths. Lastly, even though the system could be optimize to detect the Čerenkov radiation emission generated in the ROI, in most cases the signal needs to propagate through regions surrounding the ROI before detection which leads to a mixed SO₂ value of the ROI’s and other unwanted regions. Thus while SO₂ signals can be derived from measurements that propagate through several centimeters of tissue, the actual value derived will be from a mixture of attenuations of the source tissues as well as those tissues that the signal has propagated through. This is one inherent limitation of SO₂ sensing with this approach to CR detection. In comparison CREL signal measurement has a slightly different spatial sensitivity which may lead to better localization of the resulting pO₂ estimate.

The CREL signal from PtG4 has a lifetime which is sensitive to tissue background pO₂ level at the point where the luminescence emission is generated. The emission is peaked in the near-infrared which provides maximal tissue penetration, and is only weakly attenuated by absorption. Similar to CR, the detected sensitivity distribution and effective sampling depth for CREL provides information about regions and relative intensities that the signal has been detected from and through. However, since CR photons stimulate the CREL where the phosphor is located, the CREL field and sensitivity distribution is more spread out than that of Čerenkov radiation emission (Fig. 5) and is less affected by absorptive attenuation. This explains why CREL is less dependent on fiber to beam distance than is CR (see Table 2). For this reason, the effective sampling depth of CREL for broad beam while fiber to beam distance of 0 mm is about 2 mm larger than that of CR. While the distance changes from 0 to 30 mm, the effective sampling depth has been shown to vary from 9 mm to 19 mm. Since both the intensity and lifetime of CREL from PtG4 depend on the pO₂ value of the environment, either intensity or lifetime measurement could provide potential ways to measure pO₂ in the tissue. However lifetime has the advantage that it is not affected by either spectral changes or the strong spatial variation of intensity which may occur in tissues.

The concentration of PtG4 used in this study was 5 μM while some previous preclinical studies [38–40] have used much higher concentrations of similar probes. In this study, we
take 500 pulses on-CCD accumulations for each time point data and measured about 35 time points for the exponential fitting which leads to the measurement time to be 87.5 sec approximately. However, the measurement could be further improved by taking less on-CCD accumulation and less time points for the exponential fitting. The intensity of phosphorescence showed dependence on pO₂ in the phantom. As shown in Fig. 3(D), the intensity of CREL from PtG4 increases with deoxygenation. To isolate the CREL signal from Čerenkov emission, the time gating system was designed to detect CREL only by setting the time line properly. Comparing the continuous wavelength measurements and gated measurements in Fig. 3(D), CREL has been isolated from Čerenkov radiation emission. As shown in Fig. 3(E), combined with the fast time gated measurement system, the intensity of phosphorescence could be measured at different time points and lifetime could be calculated correctly by fitting the decay of phosphorescence (Table 1).

Compared with the SₐO₂ recovery method based on CR spectrum fitting, several advantages exist in the pO₂ recovery method based on CREL lifetime measurement. Lifetime measurement avoids difficulties associated with measurements and fitting of the whole detected spectrum. Unlike CR spectroscopy which uses a wide wavelength range for fitting, CREL from PtG4 emitted with a peak centered at 772 nm, therefore, the sensitivity distribution and sampling depth are less wavelength-dependent. Furthermore, the lifetime of CREL is not affected by the propagation in tissue and yields the oxygenation information exactly where the CREL has been stimulated.

5. Conclusions

Čerenkov emission spectroscopy during external beam radiotherapy was used to sample blood and tissue oxygenation with computation and phantom experiments. The lifetime of PtG4, an oxygen sensitive phosphor, recovered using CREL was calculated by CREL correctly by fitting the decay and thus could be potentially adopted to reveal the tissue pO₂ value in the sampling region. In order to assess the depth from which signals can be measured, extensive Monte Carlo simulations were carried out, and results show that both CR and CREL could be detected at depths of a few centimeters into tissue. The detected sensitivity profiles and effective sampling depth are highly dependent on the wavelength and the relative arrangement of external radiotherapy beams. The near-infrared wavelengths penetrate up to 20-30 mm while sensitivity to the blue, green and red wavelengths was substantially shallower. Information about the detected sensitivity distributions and effective sampling depths could be linked to treatment planning in order to spatially recover the regions where the signals have been detected. The simulation for different SₐO₂ values could be similarly done and small animal models could be potentially adopted as the geometry. Further testing of this approach in phantoms and animals with realistic blood SₐO₂ tissue pO₂ changes in tumors should follow to validate this methodology.

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