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erenkov Excited Fluorescence Tomography using External Beam Radiation

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Abstract

Radiation treatment of cancer induces an optical erenkov emission throughout the treated volume, which could be used to excite molecular reporters in vivo, allowing molecular sensing of tissue response during fractionated therapy. In this letter, the idea that spatial mapping of this signal can be achieved with tomographic recovery of the fluorophore distribution is tested for the first time, using 6 MeV photons from a linear accelerator in a heterogeneous tissue phantom.

erenkov light excited fluorophore throughout the tissue phantom, and diffuse tomography was used to recover images. Measurements from 13 locations were used, with spectrometer detection and spectral fitting, to separate the fluorophore emission from the erenkov continuum. Fluorescent diffuse tomographic images showed a linear response between the concentration and the reconstructed values. The potential to apply this molecular imaging in treatment with molecular reporters appears promising.

External beam radiation therapy (EBRT) is used in the treatment of many cancers, but the methods for monitoring changes in the tumor volume during the course of treatment are dependent on image guidance by CT or MRI [1], and the ability to guide therapy based upon molecular signals has been heavily examined yet remains unsuccessful to date. A course of treatment lasts 2 to 10 weeks with daily radiation treatment fractions; ideally image guidance or molecular response would be measured during treatment, maximizing the targeting of radiation and individualizing therapy based upon tumor volumes and molecular signals.

In this study, a method is proposed that combines EBRT with optical measurements taken during treatment to measure the signal of a targeted fluorophore with excitation by the phenomenon known as erenkov radiation [2]. When charged particles travel with a velocity greater than the speed of light in dielectric medium they generate erenkov photons. Charged particles with these properties are produced with linear accelerators (LINAC). erenkov emission generated from a LINAC treatment beam has previously been

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used for both absorption and fluorescence emission imaging using single fiber measurements [3, 4].

Photons emitted through bremsstrahlung radiation, exhibit a spectral dependence that is inversely proportional to the wavelength squared. A majority of the photons generated are in the ultraviolet and blue regions of the spectrum; however, these wavelengths are largely absorbed in tissue. Therefore the ability to measure bremsstrahlung light in patients is limited to shallow depths of light generation and longer wavelengths. By targeting a large Stokes shift fluorophore to the tumor region, it is possible to shift some of the bremsstrahlung photons to longer wavelengths increasing the intensity of surface measurements [5,6]. This principle has been shown previously by using radioactive probes to excite fluorescence on quantum nanoparticles through bremsstrahlung radiation energy transfer (CRET) [6].

An ideal fluorophore would have a large Stokes shift with large absorption in the shorter wavelengths and emission within the near infrared (NIR) window, where light can propagate further through tissue. In this study, Cyto500LSS was used (absorption max 500nm and emission max 630nm), which can be readily conjugated to targeting agents and has a large Stokes shift.

Experiments were conducted using a cylindrical tissue equivalent phantom containing 1% Intralipid with a height of 164 mm and diameter of 86 mm. An anomaly with height 114 mm and 33 mm diameter was placed 13 mm from the outer boundary of the phantom wall (Fig. 1c). The anomaly was filled with 1% Intralipid inclusions with varying concentrations of fluorophore ranging from 0.1 mg/mL to 0.8 mg/mL to represent different binding rates.

Thirteen fiber bundles were placed around the exterior of the phantom to collect surface spectra. Fiber bundles contained seven 400-micron detection fibers with NA of 0.37 and 13 m long. Each bundle has an individual spectrometer and cooled CCD on an imaging cart kept outside of the lead treatment door in order to decrease noise in the measurements due to scattered radiation. Individual detection channels allow for varying collection times and large flexibility in fiber placement. The fiber arrangement for these measurements can be seen in Fig. 2c. Fibers were placed 88 mm above the treatment couch and data was collected for 30 seconds during LINAC treatment to the phantom with a 6 MV photon beam from 500nm to 800 nm.

The LINAC beam size and shape can be altered to match a treatment plan for individual patients using a multi-leaf and collimator system. Beam size is measured at the isocenter, located 1 m from the source, but in this experiment measurements were acquired at 1.36 m. The beam size was expanded to include the angular variation leading to a square beam with sides of length 58 mm (40 mm at isocenter). Beam size and shape were restricted only by the requirement of not passing directly through a collection fiber as large amounts of bremsstrahlung radiation would be generated within the fiber and detected by the CCD.

Measured spectra were subject to dark signal subtraction prior to correction for the spectral response of the optical components. This correction factor was determined by dividing a measured white light source by its known spectra for each fiber bundle. Butterworth filters were used to decrease the spectral noise and pixel-to-pixel variations. A least squares (LS)

fitting algorithm was used to separate the signal from the fluorophore from the signal generated by the γ -ray radiation. A basis spectrum of the background γ -ray radiation level was generated by measuring surface data when the anomaly was filled with a 1% Intralipid vessel. Determining the background spectra for a more complex imaging domain could either be measured before the administration of fluorescence or modeled by accounting for scattering and absorption parameters of tissue (i.e. water, HbO, deoxyHb).

Figure 2a and b are examples of LS fitting of the measured spectra and its two basis functions, the background γ -ray and the fluorophore emission curve. The emission curve of the fluorophore was allowed to shift up to 5 nm in order to get the best fit. Previous work has shown that a distortion of the emission spectra is expected when generated at depth in tissue [7]. Figure 2c is a 2D representation of the slice containing the detection fibers. The blue square represents the beam size and γ -ray light field region and the fiber positions are numbered. Figure 2d shows the result of integrating a 20 nm region centered on the emission peak maximum for each of the 13 detectors for the four concentrations of fluorophore. A poor LS fit for detector 1 in the 0.1 mg/mL concentration caused by poor fiber contact with the phantom surface led to the removal of that data point prior to reconstruction.

Images of the fluorophore distribution were generated using the NIRFAST software package [8, 9]. Reconstructions were completed with and without the addition of spatial priors on a finite element mesh, created using simple shape geometries and a defined mesh resolution. Nodes within the anomaly region were marked. Optical properties were applied to each node of the mesh as the average values over the 20 nm region described previously. For patient experiments mesh generation and region assignment could be determined through DICOM files provided from previous imaging studies of the patient.

The reconstructions used an iterative process to match the measured surface data to a diffusion model of light propagation. Unlike traditional fluorescence tomography measurements, where excitation is done with a laser entering the medium at the surface, our fluorophore was excited by γ -ray radiation, which was generated throughout the interior of the phantom. To approximate the excitation field, distribution and photon intensity, Monte Carlo modeling was performed using GAMOS [10, 11]. The resultant field calculations were interpolated onto our mesh and used in the diffusion forward model, see Figure 3a.

The fluorescence yield recovered for the various concentrations had high spatial correlation without the inclusion of spatial information, although the values were surface weighted as is sometimes encountered in tomographic reconstructions. A linear relationship exists between the concentration of fluorophore and the difference between the mean reconstructed value in the anomaly region and the mean reconstructed value in the background for both the no priors and hard priors reconstruction results. The bias in this relationship could arise from a variety of factors; in this case it is likely caused by data-model mismatch or incorrect optical properties during reconstruction. γ -ray light was generated throughout most of the phantom due to the beam location and size, but through spectrally resolving the two light

components, Cherenkov and fluorescence; reconstructions of the signal were calculated with relatively high contrast to background values (4.5-6 for no priors and 20-28 for priors).

Using a single light source, in this case the Cherenkov light field, creates a different reconstruction problem than typical fluorescence molecular tomography, which uses multiple source projections to build up a data set [12, 13]. A single source generated beneath the surface of the imaging domain is similar to the bioluminescence problem that is known to be more difficult to reconstruct [14-17]. Additional difficulties in reconstruction of the fluorescence signal and location lie in the amount of signal generated per treatment, complexity of LS fitting, and accounting for any degradation of the fluorophore over time due to photobleaching from prolonged Cherenkov light generation due to treatment by the LINAC beam.

Future work will require further optimization of the LS fitting to include absorption properties and other optical parameters of the system allowing for the addition of biologically relevant material to the phantom (i.e. blood). It is expected that measurements regarding the presence of the fluorophore would still be effective due to the Stokes shift to longer wavelengths. Additional experiments will look at varying the beam size and shape as well as changing the size, position, or number of anomalies present within the tissue-mimicking phantom.

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Figure 1.

a) A schematic of the experimental setup shows the location of the phantom and measurement devices. b) The phantom schematic shows the region of the phantom where Čerenkov would be generated in a 3D volume. c) The top view of the phantom has a white light image of the experimental set up with the phantom exterior highlighted with red.

Figure 2.

Least squares fitting of the background Raman radiation and the fluorophore emission spectrum were done for each detector location shown in (c). a) Example of 0.1 mg/mL concentration fitting. b) Example of 0.8 mg/mL concentration fitting. c) The integrated intensity of the signal measured in each detector for increasing concentrations of fluorophore in the anomaly.

Figure 3.

a) The calculated field of Cerenkov radiation is shown in the plane of the detection fibers. b) The reconstructed images are shown when using no spatial information for each of the concentrations marked. c) The linear relationship between the concentration of the anomaly and the reconstructed values are shown in the graph.