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Imaging luminescent tattoo inks for direct visualization of linac and cobalt irradiation

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Abstract

Purpose: Tattoo fiducials are commonly used in radiotherapy patient alignment, and recent studies have examined the use of UV-excited luminescent tattoo ink as a cosmetic substitute to make these visible under UV illumination. The goal of this study was to show how luminescent tattoo inks could be excited with MV radiation and imaged during beam delivery for direct visualization of field position.

Methods: A survey of 9 UV-sensitive tattoo inks with various emission spectra were investigated using both UV and MV excitation. Images of liquid solutions were collected under MV excitation using an intensified-CMOS imager. Solid skin-simulating phantoms were imaged with both surface-painted ink and in situ tattooing during dose delivery by both a clinical linear accelerator and cobalt-60 source.

Results: The UV inks have peak fluorescence emission ranging from approximately 440nm to 600nm with lifetimes near 11–16μs. The luminescence intensity is approximately 6x higher during the X-ray pulse than after the pulse, however the signal-to-noise is only approximately twice as large. Spatial resolution for imaging was achieved at 1.6mm accuracy in a skin test phantom. Optical filtering allows for continuous imaging using a cobalt source and provides a mechanism to discriminate ink colors using a monochromatic image sensor.

Conclusions: The present study demonstrates how low-cost inks can be used as fiducial markers and imaged both using time-gated and continuous modes during MV dose delivery. Phantom studies demonstrate the potential application of real-time field verification. Further studies are required to understand if this technique could be used as a tool for radiation dosimetry.

Introduction

The use of permanent tattoos with black ink has been a common practice in the field of fractionated radiotherapy patient alignment as a way of providing permanent fiducial

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Conflicts

Brian Pogue is founder and president of DoseOptics LLC a company developing camera systems and software for radiotherapy imaging of Cherenkov light for dosimetry. The camera system used in this study was donated by DoseOptics. The other authors declare no competing interests.

markers.¹⁻³ The use of UV-sensitive fluorescent ink has been studied in human trials to assess the value for preserving positional alignment accuracy while allowing for near invisible appearance to the patient.^{4,5} These inks are less noticeable under normal room or day light conditions, while still being visible by eye under UV “black light” (~395nm) illumination. While these markings are used as an initial coarse alignment, and modern systems use kV imaging for final positioning, this is not always the case, especially in low and middle income countries. The current work examines the potential to expand the application of these UV-sensitive inks to include field verification through luminescence imaging with direct excitation by MV radiation. A clinical linear accelerator using both time-gated and continuous image acquisition, as well as a cobalt-60 source were used for irradiation of skin-simulating phantoms and topically applied UV-sensitive tattoo inks.

Tattoo ink is composed of a non-specific mixture, with the chromatically active components being pigmented polymorphous granules. These are inserted into the skin, with static preservation of them at the junction of the papillary and reticular dermis.⁶ Tattoo pigments have been shown to be approximately 0.2–5µm in diameter.^{5,6} The set of UV-sensitive inks investigated in the present study does not have an exact composition that is well documented, but they are manufactured and sold for human use, however in the United States the inks are not regulated by the FDA. The inks under investigation all exhibit luminescent properties under X-ray excitation. This luminescence phenomenon could be direct scintillation where X-ray energy is converted into luminescent emission, or Cherenkov-excited luminescence, where the predominantly UV Cherenkov emissions in tissue excite luminescence in the ink. In either case, in the presence of high-energy X-rays these inks emit light with a microsecond lifetime. This light can be imaged during treatment to provide real-time feedback on dose delivery. While tattoo inks are traditionally injected into the skin, surface application provides a semi-permanent delivery mechanism. Both methods are demonstrated in the current work as potential fiducial markers or methods of entrance/exit field visualization.

The central questions addressed in this study was an assessment of which combination of spectral properties and light yields provide the most detectable signal from skin-equivalent phantoms, and what image acquisition parameters could be utilized for detection during the pulsed X-rays of a linear accelerator or the continuous dose delivered by a cobalt-60 source. The ultimate goal of this work is not to replace modern patient-alignment and field verification mechanisms, but instead to demonstrate the potential of additional tools for field verification, especially in low-resource settings.

Materials and Methods

Optical properties were measured for a set of 9 colors of UV-sensitive tattoo inks (Mom's Nuclear Millennium Colors Inc., West Babylon, NY). The inks consist of a proprietary pigment in a solution of distilled water, glycerin, propylene glycol and Hamamelis Virginiana Extract (witch hazel). The inks were further diluted with ethanol and their emission spectra and lifetime were measured on a spectrofluorometer (Fluoromax4, Horiba, Kyoto, Japan). 500µL of ink was diluted with 1500µl of 70% ethanol, then 30µL was immediately aliquoted into a quartz cuvette and measured on the spectrofluorometer.

Excitation was maintained at 380nm with a 5nm slit for all samples and emission was measured from 400nm to 700nm with a 2nm slit. The 70% ethanol was measured as a control and subtracted from the emissions measurements. The phosphorescence lifetime was measured by delay with excitation again at 380nm with 5nm slit, and peak emission wavelengths for each sample were monitored with a 5nm slit. A total of 100 flashes per measurement were collected with 80ms between flashes. A 100 μ s window was used for all measurements, with an initial delay of 5 μ s incrementing by 5 μ s up to a max delay of 500 μ s.

The diluted samples with longer emission wavelengths (Red Dawn, Radiant Pink, Smoldering Orange) as well as the white ink (Invisible Fallout) were chosen to be measured using 6MV X-ray radiation delivered by a clinical linear accelerator (Clinac 2100CD, Varian Medical Systems, Palo Alto, CA) delivering approximately 2Gy dose. 150 μ L of each diluted sample, were placed in a black-wall 96-well plate. The well plate was placed on top of a 1.4cm water-equivalent phantom and a 15cm $\times$ 15cm beam was delivered from beneath the samples, similar to geometries reported previously.^{7,8} A time-gated intensified CMOS camera (C-DoseTM, DoseOptics, Lebanon, NH) was used to image the delivery of the X-rays and the luminescence with the room lights off. The linac delivers irradiation in short 4 μ s pulses at a 360Hz repetition rate. Images of Cherenkov and fast scintillation emissions were collected during the 4 μ s X-ray pulses over a 56ms exposure time, equating to 20 pulses per frame. Delayed phosphorescence was imaged with a 5 μ s delay after the start of each pulse for a duration of 100 μ s over the same 56ms CMOS exposure time. This latter acquisition approach eliminated the Cherenkov light from the tissue, which is only detectable during the x-ray pulse, thereby cutting the background signal down significantly.

Three of the more optimal ink colors (Red Dawn, Smoldering Orange, Afterglow Yellow) were chosen to be painted on the surface of a 2mm thick PDMS-based skin-simulating phantom. The optical properties of the phantom (μ_s' : 3.7–2.4 mm⁻¹, μ_a : 0.1–0.03 mm⁻¹ for λ $\approx$ 471–851nm) were measured using spatial frequency-domain imaging (Reflect RS, Modulim Imaging, Irvine, CA). A second 3mm thick silicone-based skin-simulating phantom (Practice Skin Silicone Pad, Segbeauty, Alhambra, California) with similar optical properties (μ_s' : 2.8–1.1 mm⁻¹, μ_a : 0.2–0.01 mm⁻¹ for λ $\approx$ 471–851nm) was given to a tattoo artist to make a resolution chart using the red ink. Both skin phantoms were placed on a 1.4cm water-equivalent phantom and a 15cm $\times$ 15cm beam was delivered from beneath the samples. Three imaging techniques were compared: (1) during the x-ray pulses using time-gating, which captures both Cherenkov and fast scintillation signals, (2) after the x-ray pulses, using time-gating to capture just the phosphorescence images, and (3) continuous measurement with the camera always on throughout the irradiation, not time-gated to the pulse. To capture emissions during the X-ray pulse the CMOS exposure was again 56ms with no pulse delay and 4 μ s intensifier on-time. Phosphorescence mode also has a CMOS exposure of 56ms with 5 μ s delay and 100 μ s duration. The continuous mode was not triggered by the linac X-ray and repeatedly enables the intensifier for 4ms every 40ms. These three modes were used to image the skin phantom without any filtering, and again using a 600nm long pass filter placed before the photocathode.

The PDMS-based skin phantom was also imaged during irradiation with a cobalt-60 source (Equinox 100, Theratronics, Ottawa, Ontario). The phantom was placed on a 0.5cm silicone

base with the bottom surface at 100cm SSD and irradiated from below with the gantry head at 180°. The camera was placed on a tripod next to the couch at an angle of approximately 45° and imaging distance of 0.86m. The room lights were off, however, numerous security cameras equipped with LED rings emitting at 850nm remained on. The camera system used a bandpass filters centered at 550nm (FB550–40, Thorlabs, Newton, NJ) or 635nm (RPB610–660, Omega Optical, Brattleboro, VT). Images were collected in a continuous mode for a 5ms duration at a rate of 30 frames per second. A background image was collected before each irradiation to collect and subtract any stray ambient light in the room. The phantom was irradiated for 1.46 minutes, or approximate dose of 2Gy.

Results

The normalized emission spectra of each of the 9 UV-sensitive inks is provided in Figure 1A. The white ink, “Invisible Fallout” most closely matches the spectral properties of inks used in previous studies, meaning it is barely visible under room lights, whereas the other 8 colors are visible under room light, and fluoresce brighter under UV-excitation. While the white appears to have the highest intensity (Figure 1A), in our phantom imaging the orange, red and yellow reported the highest intensity. This is likely due to the red-sensitive photocathode used in the intensified-CMOS system which has peak sensitivity between approximately 570nm and 800nm.⁹ The lifetimes of each color range from approximately 11–16µs (Figure 1B). The proprietary compounds used in these inks which would correspond to the observed emission spectra and lifetimes are still unknown.

Integrating the emission spectra above 550nm collected during UV-excitation (380nm) provides an estimate of the relative sensitivity of our imaging system (Figure 2A). A subset of these samples show intensities higher than the control both during the X-ray pulse (Figure 2B), and after the pulse (Figure 2C). The intensity observed during the X-ray pulse was approximately six times greater than that of the phosphorescence observed after the pulse. It was unclear whether the light generated during the X-ray pulse was due to direct X-ray scintillation, or due to the heavily UV-weighted Cherenkov excitation. The low white intensity was likely due to red-sensitive photocathode used in the imaging system.

Skin phantoms with ink painted on the surface (Figure 3) or tattooed into the phantom (Figure 4) were imaged in 3 modes: During the X-ray pulse, after the pulse (phosphorescence), and continuous, all with and without a 600nm long pass filter. The optical properties of both skin phantom fall within expected biological values for skin.¹⁰ A room-light color photo of the painted phantom is shown as an inset in Figure 3E, and each unfiltered mode is shown with different intensity scales in Figure 3A–C. The image collected during the X-ray delivery (Figure 3A) shows the highest intensity for each ink, however it also has the highest background values. The phosphorescence image shown in Figure 3B has the lowest overall intensity but also very low background, whereas the continuous-mode image (Figure 3C) has intensities between both. The signal-to-noise ratio (1) is used as a metric to compare the quality of the respective imaging methods:

$$SNR = \frac{\mu_{ink}}{\sigma_{bg}} \quad (1)$$

where μ_{ink} is the mean intensity of each ink region and σ_{bg} is the standard deviation of a similar-size region on the skin phantom without ink. From this metric it can be observed the image collected during the pulse has the highest SNR without a filter (Figure 3E) and with a 600 long-pass filter. The SNR is increased during the X-ray pulse and continuous mode with the addition of a 600nm longpass filter, whereas it is decreased for phosphorescence (Figure 3F). This can be attributed to the noise characteristics observed during each imaging mode, where phosphorescence imaging has inherently lower background due to the time gating of the intensifier after the X-ray pulse.⁷ The increase in SNR is seen more predominantly by the red ink because the filter better matches the emission spectrum of this ink.

Filtering during continuous mode helped improve the SNR by approximately 10%. This mode most closely resembles a cobalt-60 source, which delivers dose continuously. An image of the same phantom collected during cobalt-60 irradiation and using a 635nm bandpass filter is shown in Figure 3D, where an affine image transformation has been applied to account for the differing imaging geometry. Figure 3G compares the SNR observed for each color of ink using the two bandpass filters. When the cobalt-60 gantry was moved from 180° to 315°, the observed SNR was reduced by approximately 20%, likely due to the smaller surface dose of the entrance beam, but the background was also observed to be higher which may be caused by the camera's closer proximity to the source.

A resolution test phantom was made by tattooing the red ink into a silicone skin phantom. A monochrome room-light image of the phantom is shown in Figure 4 (top row) where the 3 lines are distinguishable to the 3rd largest size, or 1.4mm. The images shown in Figure 4 are all background subtracted based on the mean background intensity of a region on the skin phantom, this resulted in the filtered image collected during the X-ray pulse having slightly higher intensity due to the decreased background signal. A line profile was generated by taking the mean along the vertical axis defined by the white box overlaid on each image and the maximum resolution was defined as the smallest region where the 3 peaks are still distinguishable. Figure 4 shows how this resolution target was nearly indistinguishable without a filter, whereas the first and possibly second line pairs are detectable using a 600 long-pass filter for an estimated resolution of 1.6mm.

Discussion

The use of tattoo and semi-permanent inks is a widely used a method for treatment alignment in radiation therapy.^{1,2} Utilizing UV-excited ink provides patients with a cosmetically appealing option of a tattoo that is less visible, resulting in improved self-image.⁵ In these instances the patient is aligned under the aid of a UV light source by the therapist, who uses UV-A and visible light in the range of 385–405nm to visualize the ink. At these wavelengths there is minimal carcinogenic potential to the therapist and safety glasses can be worn to minimize any adverse effects of prolonged ocular exposure. While these methods provide a mechanism to align patients before daily dose fractions, many

modern radiotherapy systems use kV imaging often delivered via cone-beam CT. Patient alignment systems such as Vision RT and ExacTrac can also be used to monitor patient alignment, but are not directly monitoring beam delivery. However, in low and middle income countries these modern resources for alignment are often not available. The visualization of these inks during treatment is one method to provide feedback on both field delivery and alignment, which would otherwise not be available.

The active compounds that exist within these inks are commonly undocumented due to the nature of the tattoo industry. Previous reports of similar UV inks indicate the active compound was coumarin-based,⁵ but the spectral properties coumarins generally absorb UV and emit wavelengths under 530nm,^{11,12} which does not explain the red emission observed in some of these inks or the microsecond lifetime. Nevertheless, these inks provide a potentially low-cost method for patient alignment, and also direct verification of radiation delivery, either by scintillation or Cherenkov-excited luminescence. The methods demonstrated in the current work are similar to those reported by Jenkins *et al* where thin (0.8mm) scintillating silicone films were used for beam visualization.¹³ However, we have demonstrated commercially available inks (Available for \$15/30mL) can be directly painted or transferred onto skin-phantoms covering a large area. This application demonstrates how currently used tattoo-based fiducial markings could utilize the same inks over a larger area, and applied in a semi-permanent topical manner, to visualize entrance and exit beams for field delivery verification. If surface delivery of the ink (Figure 3) was calibrated, this could provide a low-cost and low-resource mechanism for dosimetry similar to how scintillating disks have been used to correlate with dose measured by TLDs.¹⁴ In this geometry, tissue optical properties are not a concern, so the brightest inks could be matched with peak photocathode quantum efficiency. As such, the white or yellow inks appear as ideal candidates for surface imaging as long as the corresponding similar photocathode quantum efficiency profile.

While the resolution test sample created by delivering the ink below the phantom surface has a lower overall intensity, it provides evidence that these inks may still be visible at low concentration even in the presence of tissue optical properties. The absolute pixel resolution attained by the imaging system under normal room illumination is 1.4 mm, whereas the resolution observed during the X-ray pulse is approximately 1.6 mm, which is slightly larger than the average deviation reported by Jenkins (mean: 0.5mm, max: 2.0mm) using a similar experimental setup.¹³ The observed resolution is likely limited by camera detection optics, as the resolution degrades only slightly between room illumination and X-ray excitation. In situations where portal imaging is used for alignment, which is common in low to middle income countries, average treatment variations of 3mm have been shown.¹⁵ While the modern trend aims for sub-mm accurate patient alignment, luminescent inks show promise as a potential metric for visualizing radiation dose delivery with high spatial accuracy.

The long microsecond lifetime of the measured inks was unexpected, but can prove to be a beneficial contrast mechanism when high background environments are imaged. The X-rays delivered by the linear accelerator will scatter high energy electrons, which generate Cherenkov emission in dielectric media such as tissue. The observed intensity of these Cherenkov emissions will fluctuate depending on parameters such as dose build-up and

tissue optical properties. By imaging after the X-ray pulse, the variable Cherenkov emission can be suppressed.

Another method to suppress background is to use optical filters. While Cherenkov emissions are largely blue, the spectral properties follow a $1/\lambda^2$ distribution.¹⁶ Additionally, since much of the blue light is absorbed within the tissue, before reaching the surface, the Cherenkov surface emissions are largely red. In the linac study, a 600nm longpass filter was used to understand the possibility of suppressing excess light generated during the X-ray pulse. As shown in Figure 3F, this filter boosts the SNR of the red ink by approximately 8–15% during the X-ray and continuous imaging modes. Filtering was used during cobalt-60 irradiation to suppress excess light mainly due to IR LEDs on security cameras in the bunker. Filters centered at 635nm were able to discriminate the red and orange inks with similar SNR, whereas the filter centered at 550 suppressed red and orange and allowed for detection of the yellow ink. On the linear accelerator, filtering after the pulse lowers the SNR because much of the light is generated by the ink and there is very low background, so in this low-photon environment filtering is not beneficial.

The continuous mode imaging demonstrated on the linear accelerator was translated for use with a cobalt-60 source currently used in Honduras. While cobalt-60 systems are becoming less common and currently account for 22–33% of all radiotherapy systems worldwide, their prevalence is estimated to be much higher in low and middle income countries.^{17,18} These systems generally lack many modern features such as cone beam CT alignment. In these situations, UV- and MV-excited fiducial markers which have the dual purpose of real-time field verification, provide an additional tool to help reduce potential delivery errors.

Conclusions

The UV-sensitive fluorescent tattoo inks investigated in the present study exhibit luminescent properties which were imaged during radiation therapy. The practice of tattooing or placing semi-permanent markings during medical procedures has been reported previously and is a common clinical procedure. The current work demonstrates the ability to image UV-sensitive fluorescent inks during radiation therapy using both a linear accelerator or cobalt-60 source. Using a linear accelerator, imaging can be time-gated with the X-ray delivery as well as after the pulse due to the phosphorescent properties of the inks. Phosphorescence imaging provides the ability to greatly reduce the background intensity due to the reduced Cherenkov emissions during this period. In addition to imaging in solution, the inks are visible as ink painted on the surface or tattooed into skin-simulating phantoms with a spatial resolution near 1.6mm. The spectral variation of these inks could be used to provide multiplexed imaging during dose delivery which was demonstrated using bandpass filters with cobalt-60 irradiation. Further work is needed to study the clinical efficacy of this workflow.

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Figure 1: Normalized fluorescence emission of 9 UV-sensitive inks (A) and their corresponding normalized lifetime (B) based on 380nm excitation.

Figure 2:

Mean intensity of select UV-sensitive tattoo inks in ethanol solution, measured with 380nm UV excitation (A,) during the MV X-ray pulse (B) and after the pulse (C).

Figure 3:

Select UV-sensitive ink was painted on the surface of a PDMS-based skin-simulation phantom (E inset) and imaged during the X-ray pulse (A), after the pulse (B), and without time gating (C). The same phantom was imaged using a cobalt-60 source and 635 bandpass filter (D). The signal-to-noise (SNR) was measured for each ink using each imaging method (E), and was compared to the same configuration with a 600 longpass filter. The ratio of the SNR with (SNR_F) and without (SNR_{NF}) the filter provide insight on the noise (F) characteristics of each method. The SNR for each ink was measured using both a 550nm bandpass filter (left) and 635 bandpass filter (right, where the inset show the filter pass band (gray shaded) in relation to the ink emission (G)).

Figure 4:

Red UV ink was tattooed into a skin-simulating phantom (top inset) to test system resolution under room-lights (top), time-gate to X-ray delivery (middle), and continuous acquisition during dose delivery (bottom).