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Development and characterization of a scintillating cell imaging dish for radioluminescence microscopy

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Abstract

Radioluminescence microscopy is an emerging modality that can be used to image radionuclide probes with micron-scale resolution. This technique is particularly useful as a way to probe the metabolic behavior of single cells and to screen and characterize radiopharmaceuticals, but the quality of the images is critically dependent on the scintillator material used to image the cells. In this paper, we detail the development of a microscopy dish made of a thin-film scintillating material, $\text{Lu}_2\text{O}_3:\text{Eu}$, that could be used as the blueprint for a future consumable product. After developing a simple quality control method based on long-lived alpha and beta sources, we characterize the radioluminescence properties of various thin-film scintillator samples. We find consistent performance for most samples, but also identify a few samples that do not meet the specifications, thus stressing the need for routine quality control prior to biological experiments. In addition, we test and quantify the transparency of the material, and demonstrate that transparency correlates with thickness. Finally, we evaluate the biocompatibility of the material and show that the microscopy dish can produce radioluminescent images of live single cells.

Graphical abstract

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Conflicts of interest:

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Introduction

This paper describes the development of a scintillating cell imaging dish for radioluminescence microscopy that is optimized for imaging performance, experimental reproducibility and potential for large-scale manufacturing. In this study, we demonstrate methods to rigorously study the radioluminescence, transparency, and biocompatibility of the dish.

Radioluminescence microscopy is an emerging modality that allows radiotracer imaging with high resolution. Among other applications, this technique has been used to image glucose uptake in single cancer cells¹. The technique can also be multiplexed with other measurements such as fluorescence and bioluminescence, making it a powerful, dynamic method to probe multiple metabolic and biological processes² at the same time on a single-cell level³. The technique has many applications in cancer research and diagnosis given the metabolic heterogeneity inherent in cancer cells⁴. The need for single-cell imaging tools is particularly important given that even single melanoma cells can result in efficient tumor formation⁵. Single-cell analysis can also provide valuable information about the dynamics of tumor evolution⁶. Therefore, single-cell methods that can detect cell abnormalities are extremely important in understanding cancer initiation and progression. Our research has already demonstrated that single-cell imaging using radiotracers can provide important information on live cells that is not readily available on a bulk level³. Further, it is possible to perform radioluminescence microscopy using a commercially available bioluminescence microscope or a custom-built low-light microscope⁷.

While other techniques exist to image radionuclides, radioluminescence microscopy offers unique advantages due to its high spatial resolution. To the best of our knowledge, this technique is the only method able to reliably measure the radioactive uptake of single living cells with high resolution and sensitivity. Microautoradiography is another sensitive technique that can image tissue sections and cells, but it can only image fixed and sectioned samples, as opposed to live single cells. In addition, other microplate-based products such as Cytostar and Scintiplate use organic scintillators that do not produce enough light to be imaged through a microscope, making microplates more appropriate for bulk measurements as opposed to live single cell imaging.

The key aspects of a successful radioluminescence microscope system include a moderate magnification (typically 5–10×), high numerical aperture, low acquisition noise, and high frame rate. Most importantly, one must use an imaging dish containing a scintillator component, so that the output from radioactive emissions may be converted into light that the microscope can detect.

Previously, radioluminescence microscopy has been used in conjunction with a commercially available CdWO₄ scintillating plate¹. These plates were placed on top of live radioactive cells, creating multiple layers between the microscope objective and the scintillator surface. Further, the CdWO₄ material has limited light yield (12–15 photons/keV⁸) and a non-ideal emission wavelength peak (475 nm) that does not match the detector's peak sensitivity⁹. We assume that published light yield values, which are usually

measured using an X-ray source, accurately represent the efficiency of beta-particle luminescence.

In subsequent work, our lab has shown that radioluminescence images of higher quality could be produced using a $\text{Lu}_2\text{O}_3\text{:Eu}$ thin-film scintillator¹⁰ in place of the thicker CdWO_4 scintillator¹¹. This material is known to have a high light yield (37–48 photons/keV), with an emission wavelength peak (610 nm) corresponding to a higher quantum efficiency of the EMCCD camera^{12, 13}. In addition, because of the greater light yield of the material, a thin coating of $\text{Lu}_2\text{O}_3\text{:Eu}$ provides enough light to localize individual radioactive decay events. By physically truncating the ionization track in such a way, it is possible to localize each event closer to the origin of the particulate emission to improve spatial resolution. An additional advantage of thin-film scintillators is that the material cost can be dramatically reduced relative to bulk materials, which is important for large-scale production of this type of consumable device.

Another advantage of the approach being considered here is that the thin-film scintillator can be placed at the bottom of the dish and yet still allow imaging using a short-working distance objective. Our previous setup involved seeding cells as a monolayer on a glass-bottom dish and placing a 500 μm -thick scintillator on top of the cells, creating multiple layers between the scintillator and the objective. Here, we are able to seed the cells directly on top of the scintillator, enabling easier access to the cells and minimizing any possible stresses that result from the scintillator being placed on top of the cells. Additionally, the light emitted in the scintillator has a more direct path to the objective, compared to the case where the scintillator is on top of the cells and the scintillation light has to traverse the sample. This represents a significant improvement over our previous radioluminescence experimental set-up¹¹.

However, there are some remaining challenges with using a thin-film scintillator. First, ceramic materials may cause the light to scatter, degrading the quality of brightfield and fluorescence images that are acquired in conjunction with radioluminescence imaging. The ideal microscopy dish should be optically transparent, with minimal absorption and scattering of the light. Second, the material should be biocompatible, allowing for longer-term cell viability. Finally, a practical method is needed to ensure that manufactured thin-film scintillators meet rigorous quality standards to ensure reproducible imaging performance. In contrast to the reproducible fabrication of CdWO_4 scintillators, $\text{Lu}_2\text{O}_3\text{:Eu}$ thin films are made using an electron-beam physical vapor deposition method that is subject to greater variability, hence the need for quality assurance testing.

In this study, we evaluate and compare thin-film $\text{Lu}_2\text{O}_3\text{:Eu}$ scintillators of different sizes with respect to their scintillation, optical, and biocompatibility properties. We first test the variability in the manufacturing process through a robust yet rapid quality assurance protocol that tests the physical scintillation properties of the dish. We then measure the optical transparency of the different samples. Finally, we study the biocompatibility of these materials.

Experimental methods

Scintillator fabrication

High-density europium-doped Lu_2O_3 was deposited onto c-plane oriented, single-crystal sapphire wafers using the electron-beam physical vapor deposition (EBPVD) method¹⁴. The target material was synthesized by means of co-precipitation using Lu_2O_3 and 5% (mol) Eu_2O_3 powders (Stanford Advanced Materials, Irvine, CA). The powder was calcined in 5 sccm high purity oxygen flow at 1000°C for 2 hours. Then it was isostatically cold pressed into the form of 1" diameter pellets with an average thickness of 5 mm. The targets were evaporated by electron beam. The substrate was placed above the target and mounted on a heater heated up during deposition to improve crystallinity. The scintillators were glued into bottom-free microscopy dishes (Cellvis D35-14) using a fast-curing epoxy glue (Loctite). The scintillator was allowed to adhere to the microscopy dish for 24 hours and then sterilized by filling with ethanol for 24 hours. CdWO_4 scintillators (1 cm × 1 cm × 0.5 mm; two-sides polished) were ordered from MTI Corporation and used as a reference standard.

Radioluminescence microscopy setup

All imaging was performed using a low-light microscope outfitted with a 20X/0.75 NA oil objective (CFI Plan Apochromat λ , Nikon), and a deep-cooled electron-multiplying charge-coupled device (EM-CCD; ImageEM C9100-13, Hamamatsu). The C9100-13 CCD is a back-thinned frame transfer device, with a 512×512 array of 16 μm ×16 μm pixels. Samples were imaged using 4×4 binning and an electron-multiplication gain of 1200. Details of the system have been provided elsewhere⁷.

Scintillator physical characterization

For quality assurance purpose, the physical properties of the different scintillators was characterized using an alpha radiation source (Am-241, 108.2 nCi, Isotope Products Laboratories, 107.3 nCi at the time of experiment) and a beta radiation source (Tl-204, Spectrum Techniques, 0.60 μCi at the time of experiment). A total of 1000 frames were acquired. Transparency was measured with a Stover scatterometer that uses a red laser to quantify the light transmission and scatter through the sample. Further, a microgrid (R1L3S3P, Thorlabs) was imaged through the scintillator using a ThermoFisher EVOS microscope (brightfield mode).

Image reconstruction

Radioluminescence image correction and analysis were performed using MATLAB R2016 (Mathworks, Natick, MA). Radioluminescence micrographs were corrected by subtraction of a dark image, taken with the same exposure time but with a non-radioactive sample in the microscope. These images were further corrected for field flatness using a flat-field calibration map acquired using a uniform distribution of FDG. Gaussian filtering was applied where appropriate to reduce noise. Hot spots produced by gamma rays or annihilation photons were removed by applying a custom algorithm that can detect sharp features well above neighboring pixels. A Matlab algorithm was used to segment individual

ionization tracks and to compute the total intensity of each track. A violin plot was then constructed in order to display the intensity from each individual ionization track.

Cell imaging using RLM

MDA-MB-231 human breast cancer cells were purchased from the American Type Culture Collection (ATCC, Manassas, VA) and cultured in DMEM (Gibco) medium supplemented with 10% fetal bovine serum. For radioluminescence experiments, the scintillator dish was adsorbed with fibronectin (10 $\mu\text{g}/\text{ml}$) for 1h. The scintillator was then washed thrice with PBS, and 10^5 cells were seeded directly onto the scintillator plate. Imaging was performed the following day. The cells were incubated with FDG (500 $\mu\text{Ci}/\text{mL}$ for up to 30 minutes), washed thrice in PBS, and then imaged using radioluminescence microscopy for 10,000 frames (100 ms/frame).

WST-1 assay

A WST-1 assay (Roche) was performed on cells seeded onto $\text{Lu}_2\text{O}_3:\text{Eu}$ and CdWO_4 scintillators. 10^5 cells were seeded onto fibronectin-adsorbed scintillators in a 12 well plate, and were cultured for 48 hours. Prior to the assay, the scintillators were transferred to a cell-free 12 well plate so that only the cells on the scintillators were assayed. The results were standardized to absorbance/ μm^2 and normalized to the CdWO_4 scintillator.

Scanning electron microscopy (SEM)

Cells were fixed for 30 minutes in 4% paraformaldehyde. For variable-pressure scanning-electron microscopy (VP-SEM), samples were post-fixed in 1% Osmium tetroxide (OsO_4) for 30 mins, followed by rinsing in water (3 $\times$ 5 min). Samples were imaged with a Hitachi 3400N-VP-SEM operated at 15 kV and using Backscattered Electron (BSE) Detection. Hydration was simultaneously controlled from 4°C to -25°C with a cool stage and Deben Peltier pump. For FESEM imaging, samples were fixed in 4% paraformaldehyde and post-fixed in 1% OsO_4 were freeze-dried and sputter-coated with a 50 Angstrom layer of gold-palladium before imaging with a Zeiss Sigma FESEM operated at 2–3 kV and using InLens Secondary Electron (SE) and Everhardt Thornley (SE2) detection.

Inductively coupled plasma mass spectrometry (ICP-MS)

10^5 cells were seeded onto fibronectin-adsorbed scintillators in a 12 well plate, and were cultured for 48 hours. Cells were then trypsinized and lysed using RIPA buffer. The lysate was filtered with a 0.2 μm filter. In addition, samples were also submerged in distilled water for 48 hours. ICP standards for Eu and Lu (Ricca Chemical) were used to create concentration standards for both metals. Samples were analyzed using a Thermo Scientific XSERIES 2 ICP-MS.

Results and Discussion

Scintillator Characterization

Due to the use of the electron beam vapor deposition method, we have control over the thickness of the material. In this study, we test a range of thicknesses from 2.5 μm to 13 μm .

In addition, to create a head-to-head comparison, we also test a 500 μm -thick scintillator that is comparable in thickness to the CdWO_4 samples previously used.

Figure 1A is a schematic that demonstrates our setup. While we previously used thin sections of frozen FDG to measure the absolute sensitivity of the scintillator¹¹, this set-up was cumbersome and not suitable for rapid quality testing of the manufactured scintillators films. Here, we demonstrate a faster and more reproducible set-up for this purpose, which uses a long-lived sealed radioactive source instead of a short-lived radionuclide. We test the luminescent properties of the scintillator using both an alpha source (Am-241) and a beta source (Tl-204). Alpha decay is the emission of a positively charged helium ion (two protons and two neutrons). Beta decay is the emission of an electron or its antiparticle, the positron. Alpha particles typically have a very short mean path, which means that the particles are expected to deposit most of their energy in the sample along a straight line. In contrast, beta particles are smaller and lighter, and they are expected to travel longer distances through relatively more convoluted paths. In addition, alpha and beta particles have different average energies; the Am-241 source generates alpha particles with an energy of 5486 keV, while the Tl-204 beta source generates beta particles with a continuous energy spectrum and an endpoint energy of 764 keV, resulting in a calculated range approximately 584 μm for the beta particle (continuous slowing-down approximation⁹) and 10.4 μm for the alpha particle¹⁵. The alpha source is used to test the properties of the scintillator material independently its thickness, since the particle range is comparable to the thickness of the scintillator film. Alpha particles are expected to deposit almost all of their energy in all but the thinnest scintillators. On the other hand, beta particles penetrate deep into the sample and are impacted by both the quality of the scintillator material and the thickness of the film. The results using the beta source are more predictive of actual performance given the fact that the microscope is primarily used for beta imaging.

It is important to note that we do not capture every alpha or beta particle. Our event processing algorithm deliberately excludes events that are not bright enough or not focused enough to be included in the final image. Therefore, the analysis of the tracks includes only a subset of the events that are detected.

In Figure 1B (left panel), we compare the distribution of track intensities for individual alpha particles interacting with different scintillator materials. Here, we use a 500 μm -thick CdWO_4 scintillator and a similarly thick $\text{Lu}_2\text{O}_3\text{:Eu}$ scintillator as our reference samples. Track intensity is calculated as the sum of the intensities of the pixels within individual ionization tracks, as observed by the microscope. We specifically study this parameter because differences in pixel intensity are expected to directly correlate with system sensitivity. The detection of single ionization events is critically dependent on a bright scintillator, especially for low-energy beta particles that may not deposit enough energy in the narrow region of the scintillator that is in focus to yield a detectable event. Using this method, thousands of tracks are analyzed and summarized using a violin plot. As expected, alpha particles produce much less intense tracks in CdWO_4 than in $\text{Lu}_2\text{O}_3\text{:Eu}$. Notably, the 4–5 fold difference in measured intensity is greater than the 2–4 fold difference in light yield for the two materials. This may be due to other properties of the scintillators, such as

spectral emission properties, alpha particle stopping power, surface roughness, and crystallinity of the material.

As shown in Figure 1B (right panel), the difference is less pronounced for the beta source, possibly due to greater reabsorption of the light in $\text{Lu}_2\text{O}_3:\text{Eu}$. Nevertheless, these results confirm our previous finding that $\text{Lu}_2\text{O}_3:\text{Eu}$ is a better scintillator material for radioluminescence microscopy. These data also demonstrate the suitability of alpha and beta sealed radiation sources for rapid quality control of thin-film radioluminescence microscopy dishes. Our results are qualitatively similar to what we have previously found¹¹.

Next, we test 5 different $\text{Lu}_2\text{O}_3:\text{Eu}$ films, with thicknesses ranging from 2.5 to 500 μm , using both alpha and beta sources. Figure 1C (left panel) shows the response of different-thickness scintillators to alpha particles. These particles create optical emissions that are bright and punctate, because the particle itself is heavy and does not significantly scatter within the scintillator. Thickness appears to make a small but noticeable impact on the resulting track intensity. Because alpha particles are densely ionizing, we are able to observe a signal from even the thinnest scintillator sample, although at a significantly lower intensity ($5.7 \pm 4.9 \times 10^4$ A.U.) than that of the other thin films (mean intensity ranging from $1.8 \pm 0.9 \times 10^5$ A.U. for the 13 μm scintillator to $2.0 \pm 1.0 \times 10^5$ A.U. for the 7 μm scintillator).

We note that two peaks are visible for the 7 and 8.3 μm scintillator coatings and not for the other coatings. This finding, which was described by another group¹⁵, could be explained by the fact that some alpha particles do not deposit their energy entirely in the scintillator, since their range in Lu_2O_3 is greater than the thickness of the scintillator. The higher peak likely corresponds to alpha particles that impinge onto the scintillator at an oblique angle, and therefore deposit all their energy in the scintillator film. The lower peak corresponds to particles that, due to their angle of incidence, travel through the scintillator into the adjoining substrate. In light of this, for the purpose of quality assurance, the proposed Am-241 source is suitable for scintillators thicker than 10 μm . For thinner scintillators, it may be necessary to use an alpha source with a lower energy. This could also be achieved using a thin layer of attenuating material to uniformly reduce the energy of the alpha particle.

Our experiment also finds that the thick scintillator yields 28% higher mean intensity ($2.6 \pm 0.1 \times 10^5$ A.U.). This could be explained by the fact that this scintillator is made using a different manufacturing process, suggesting that the process of thin-film deposition results in lower light yield or worse light extraction than the optimal bulk material.

In contrast to the alpha source, the beta source more realistically simulates the physics of radioluminescence imaging, which is most commonly used for imaging short-lived positron-emitting isotopes. Figure 1C (right panel) demonstrates that scintillator thickness has a more noticeable impact on signal intensity when a beta source is used. Contrary to alpha particles, beta particles are sparsely ionizing and therefore propagate hundreds of microns deep inside the scintillator. Because of this, we are unable to register a discernible signal for the thinnest 2.5 μm scintillator. It is likely that any signal produced by beta particles in the thin coating is too weak to be distinguished from background noise. We do observe signal from the samples in the 7–13 μm range when they are tested with the beta source. The mean intensity of these

samples ranges from $2.6 \pm 1.6 \times 10^4$ A.U. for the 7 μm scintillator to $2.9 \pm 2.0 \times 10^4$ A.U. for the 13 μm scintillator. We also test the 500 μm -thick sample using the beta source and observe a nearly five-fold increase in signal compared to the thin films ($1.7 \pm 1.0 \times 10^5$ A.U.). This effect illustrates the fact that the response of the scintillator to long-range beta particles is affected both by the properties of the material and by its thickness. From these data, we conclude that while signal intensity depends on thickness, the effect is relatively small for the range of scintillators being considered (i.e. 7 to 13 μm). This may be because gains in light emission are offset by greater self-absorption and scattering of the light in the scintillator, as will be shown in the next section. Therefore, we conclude that scintillator thickness does not need to be accurately controlled within this range for the purposes of radioluminescence microscopy.

Scintillator transparency

In addition to the radioluminescence signal produced by the thin films, it is also important that the samples be transparent. Optical clarity of the scintillator is required not just for radioluminescence imaging but also to enable the collection of optical images of the same cells using brightfield and fluorescence microscopy. Therefore, we have developed methods to quantify optical transparency so that manufactured scintillator films that fail to meet our requirements may be discarded. In Figure 2A, we image a microscopic grid through various scintillator coatings using a brightfield microscope to test the transparency of these samples. The transparency of the coatings appears to be inversely correlated with their thickness. As the samples get thicker, transparency becomes poorer. This visual result is corroborated by scatterometry in Figure 2B. The central peak represents light that has passed through the sample unscattered. As the sample thickness increases, the amount of light that passes through the sample without any angular deviation decreases. From these data, we conclude that a sample with a thickness of less than 13 μm would appropriately balance scintillation performance with desired transparency.

These findings therefore demonstrate that transparency measurements combined with radioluminescence screening using long-lived radioactive sources can provide important and rapid quality control testing for scintillator thin films. As an example, we performed the same analysis with two samples with similar thicknesses that were prepared during the same EBPVD run. We found significant differences between the scintillation intensities of these two samples when tested using an alpha source and the methods described above (Figure S1), and using scatterometry (Figure S2). Our quality control methods are thus able to accurately identify outliers. The quality control process is demonstrably necessary to identify scintillators that do not meet performance requirements.

Biocompatibility

We then test the biocompatibility of the $\text{Lu}_2\text{O}_3\text{:Eu}$ thin films. As previously reported, cells grown for 2 days on a $\text{Lu}_2\text{O}_3\text{:Eu}$ film show worse viability (measured by a standard WST-1 assay) than those grown on a standard CdWO_4 scintillators (Figure 3A). To improve cell viability, we coated $\text{Lu}_2\text{O}_3\text{:Eu}$ scintillators with a variety of materials; unfortunately, none of the coatings tested (SiO_2 , Al_2O_3 and PDMS) produce the desired effect (Figure 3B). The data here are normalized to the sample with no coating. A thin 100 nm coating with PDMS even appears to decrease biocompatibility slightly.

A possible explanation for the lower viability of cells grown in contact with the $\text{Lu}_2\text{O}_3\text{:Eu}$ thin-film scintillator is that rare earth metals leach from the scintillator into the culture medium. This would also explain why scintillator coatings failed to improve viability. To test this hypothesis, we use ICP-MS to analyze water samples that have been in contact with the $\text{Lu}_2\text{O}_3\text{:Eu}$ scintillator surface for 48 hours (Table 1). We also analyze the lysate and media of cells, which have been cultured on these scintillators for 48 h to assess the presence of Lu or Eu. In all of the samples analyzed, we find trace amounts of both Lu and Eu. Though Eu is not considered very toxic below millimolar concentrations (about 75 ppm¹⁶), Lu_2O_3 is considered toxic¹⁷, so it is possible that the reduced viability of the cells is due to the presence of Lu_2O_3 . We note significant variability in both Lu and Eu concentrations found in water as well as in cell lysate. However, it is possible that due to the filtering, dilution and processing steps required to prepare samples for ICP-MS, the amount of Lu and Eu present inside cells may be underestimated. For example, protein solids, which may bind heavy metals, are filtered out prior to ICP-MS. In conclusion, Eu and Lu uptake may impact longer-term cell viability when using these microscopy dishes. However, this lowered biocompatibility might not be a limitation if cells are only in contact with the scintillator for a short period of time. For longer-term applications, material toxicity may be a concern and other materials such as CdWO_4 may be more appropriate even though spatial resolution and intensity will be compromised.

We further analyze the surface properties of the scintillator using scanning electron microscopy (SEM). Figure 4A is a VP-SEM image of MDA-MB-231 cells on the surface of the thin-film scintillator. The darker objects on the image are individual cells, and the white layer is the $\text{Lu}_2\text{O}_3\text{:Eu}$ scintillator coating deposited on a sapphire substrate. Figure 4B is a conventional SEM image, where the $\text{Lu}_2\text{O}_3\text{:Eu}$ surface roughness can be seen in more detail. Individual cells (as shown) are able to spread out onto the rough scintillator surface. It has previously been demonstrated that cell surface roughness can lead to enhanced cell growth and biocompatibility in some cell types¹⁸. It is possible that the roughness of the $\text{Lu}_2\text{O}_3\text{:Eu}$ scintillator contributes to its interaction with live cells.

Live cell imaging

Finally, the scintillator dish (Figure 5A; *right*) is able to perform well when tested using a standard radioluminescence microscopy set-up. We are able to image the uptake of FDF by MDA-MB-231 cells plated onto a manufactured $\text{Lu}_2\text{O}_3\text{:Eu}$ -coated imaging dish (Figure 5B–5D). Notably, the cells are imaged clearly using the brightfield settings on the microscope and individual cells are easily distinguished. The optical transparency of these scintillator films is much better than shown in previous work¹¹. Radioluminescence signal can also be matched to individual cells, as shown in Figure 5C. These dishes are therefore capable of imaging radioluminescence signals and may also be multiplexed with other types of imaging modalities such as brightfield and fluorescence.

Conclusions

In conclusion, we have developed a method to fabricate and characterize thin-film scintillator dishes for radioluminescence microscopy. Radioluminescence microscopy is an

important technique that has already been used for microscopy applications beyond FDG imaging; radioluminescence has shed light on the localization characteristics of hexadecyl-4- ^{18}F fluorobenzoate (^{18}F HFB)¹⁹ as well as the distribution pattern of 3'-deoxy-3'- ^{18}F fluorothymidine (^{18}F -FLT) in proliferative vs. non-proliferative cells³.

Further, given that we are able to distinguish between the tracks of alpha and beta particles, it is possible in the future that this system may be used for the multiplexed detection of different radionuclides. A variety of factors including scintillator film composition and thickness as well as pixel binning, microscope magnification, and the electron-multiplication gain would impact further advances in spatial resolution. We have discussed the optimization of these factors using computer simulation in a separate manuscript²⁰. While radioluminescence microscopy has thus far been used for single cell visualization, additional applications are possible as the technique is refined. In particular, improvements in spatial resolution can allow for intracellular visualization as well as the visualization of radiotracer signals from cells in clusters.

We have also developed methods to reproducibly test the quality of thin-film scintillators, and have determined a range of thicknesses in which optical transparency and radioluminescence properties of the scintillator are optimized. We further demonstrate the biocompatibility of these scintillator materials, determine that cells can grow on and cover the surface of the scintillator, and perform radioluminescence microscopy with a prototype scintillating dish. This study is the first step towards the development of a large-scale manufacturing and testing scintillator-coated imaging dishes for radioluminescence microscopy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Bibliographic References and Notes

1. Pratz G, Chen K, Sun C, Martin L, Carpenter CM, Olcott PD, Xing L. PLoS One. 2012; 7:e46285. [PubMed: 23056276]
2. Gallina ME, Kim TJ, Shelor M, Vasquez J, Mongersun A, Kim M, Tang SK, Abbyad P, Pratz G. Anal. Chem. 2017; 89:6472. [PubMed: 28562033]
3. Sengupta D, Pratz G. J Nucl. Med. 2016 jnumed. 115.167734.
4. Sengupta D, Pratz G. Mol. Cancer. 2016; 15:1. [PubMed: 26728659]
5. Quintana E, Shackleton M, Sabel MS, Fullen DR, Johnson TM, Morrison SJ. Nature. 2008; 456:593. [PubMed: 19052619]
6. Navin N, Kendall J, Troge J, Andrews P, Rodgers L, McIndoo J, Cook K, Stepansky A, Levy D, Esposito D. Nature. 2011; 472:90. [PubMed: 21399628]
7. Kim TJ, Türkcan S, Pratz G. Nat. Protoc. 2017; 12:1055–1076. [PubMed: 28426025]

8. Knoll, GF. Radiation detection and measurement. 3. Zobrist, B., editor. Vol. ch. 8. John Wiley & Sons, Inc.; 2000. p. 235
9. [Accessed 8 Jan 2018] National Institute of Standards and Technology: Stopping Power and Range Tables for Electrons. 2018. <http://physics.nist.gov/PhysRefData/Star/Text/ESTAR.html>
10. Huan J, Hu L, Fang X. ACS Appl. Mater. Interfaces. 2014; 6:1462–1469. [PubMed: 24387245]
11. Sengupta D, Miller S, Marton Z, Chin F, Nagarkar V, Pratz G. Adv. Healthc. Mater. 2015; 4:2064–2070. [PubMed: 26183115]
12. Marton Z, Bhandari HB, Brecher C, Miller SR, Singh B, Nagarkar VV. High performance microstructured Lu₂O₃: Eu thin film scintillator for X-ray computed tomography. 2013
13. Dujardin C, Garcia-Murillo A, Pedrini C, Madej C, Goutaudier C, Koch A, Petrosyan A, Ovanesyan K, Shirinyan G, Weber M. Synthesis and scintillation properties of some dense X-ray phosphors. 1999
14. Marton Z, Bhandari HB, Brecher C, Miller SR, Singh B, Nagarkar VV. IEEE T. Nucl. Sci. 2014; 60
15. Prusa P, Nikl M, Mares JA, Kucera M, Nitsch K, Beitlerova A. Phys. Status Solidi (a). 2009; 206:1494–1500.
16. Feyerabend F, Fischer J, Holtz J, Witte F, Willumeit R, Drücker H, Vogt C, Hort N. Acta Biomater. 2010; 6:1834–1842. [PubMed: 19800429]
17. Rim KT, Koo KH, Park JS. Saf. Health Work. 2013; 4:12–26. [PubMed: 23516020]
18. Deligianni DD, Katsala ND, Koutsoukos PG, Missirlis YF. Biomaterials. 2000; 22:87–96.
19. Kiru L, Kim TJ, Shen B, Chin FT, Pratz G. Mol. Imaging Biol. 2017:1–10.
20. Wang Q, Sengupta D, Kim TJ, Pratz G. J Biophotonics. 2017

Figure 1.

A: Scintillator optical characterization. (A) Schematic showing thin-film scintillators characterized by imaging the ionization tracks produced using a sealed alpha or beta source. (B) A comparison of the track intensities of 500 μm-thick CdWO₄ and Lu₂O₃:Eu scintillators when exposed to alpha and beta radiation sources. (C) A comparison of the track intensities of Lu₂O₃:Eu scintillators with different thicknesses when exposed to alpha and beta radiation sources.

Figure 2. Scintillator transparency measurements. (A) Images of a microscopic grid demonstrate that as the scintillator thickness increases, the transparency of the sample decreases. (B) Scatterometer data confirms that as the samples become thicker, the amount of light that passes through without angular deviation decreases.

Figure 3. Scintillator biocompatibility. (A) WST-1 cell viability assay for a CdWO_4 scintillator (set to 100% as the reference) and for a representative $\text{Lu}_2\text{O}_3:\text{Eu}$ thin-film scintillator. (B) WST-1 assay for $\text{Lu}_2\text{O}_3:\text{Eu}$ thin-film scintillators coated with a variety of materials, where the unmodified scintillator is set to 100% as the reference.

Figure 4.
(A) VP-SEM of cells plated on an Lu₂O₃:Eu scintillator. (B) SEM of the same sample.

Figure 5.

(A) Photograph of a $\text{Lu}_2\text{O}_3\text{:Eu}$ -coated imaging dish. (B) Bright-field image of cells plated on the microscopy dish. (C) Corresponding radioluminescence image. (D) Overlay of brightfield and radioluminescence images from 5B and 5C.

Table 1

ICP-MS data of samples exposed to $\text{Lu}_2\text{O}_3\text{:Eu}$ scintillators showing the presence of europium and lutetium in the leachate.

Material Analyzed	Concentration of Lutetium (PPm)	Concentration of Europium (PPm)
Water from 12.5 μm $\text{Lu}_2\text{O}_3\text{:Eu}$	2.86	0.483
Cell lysate (plated on 10 μm $\text{Lu}_2\text{O}_3\text{:Eu}$)	0.021	0.045
Filtered media (cells plated on 10 μm $\text{Lu}_2\text{O}_3\text{:Eu}$)	0.084	0.057