Published in final edited form as:

J Biophotonics. 2018 March ; 11(3): . doi:10.1002/jbio.201700138.

***In silico* optimization of radioluminescence microscopy**

Qian Wang, Debanti Sengupta, Tae Jin Kim, and Guillem Pratx^a

Department of Radiation Oncology, Stanford University, California 94305, United States

Abstract

Radioluminescence microscopy (RLM) is a high-resolution method for imaging radionuclide uptake in live cells within a fluorescence microscopy environment. Although RLM currently provides sufficient spatial resolution and sensitivity for cell imaging, it has not been systematically optimized. This study seeks to optimize the parameters of the system by computational simulation using a combination of numerical models for the system's various components: Monte Carlo simulation for radiation transport, 3D optical point-spread function for the microscope, and stochastic photosensor model for the EMCCD camera. The relationship between key parameters and performance metrics relevant to image quality is examined. Results show that $\text{Lu}_2\text{O}_3:\text{Eu}$ yields the best performance among 5 different scintillator materials, and a thickness: 8 μm can best balance spatial resolution and sensitivity. For this configuration, a spatial resolution of $\sim 20 \mu\text{m}$ and sensitivity of 40% can be achieved for all three magnifications investigated, provided that the user adjusts pixel binning and EM gain accordingly. Hence the primary consideration for selecting the magnification should be the desired field of view and magnification for concurrent optical microscopy studies. In conclusion, this study estimates the optimal imaging performance achievable with RLM and promotes further development for more robust imaging of cellular processes using radiotracers.

Graphical abstract

Radioluminescence microscopy (RLM) is a new method for imaging radionuclide probes in a microscopy environment. This study uses a combination of numerical models for RLM to optimize its performance. How RLM performance metrics are affected by parameters of different system components is explored. With these relationships, we found efficiently the optimized parameters enhancing RLM performance (cover figure: a comparison of radioluminescence cell images simulated using experimental and optimized parameters).

^aCorresponding author, pratx@stanford.edu.

Keywords

radioluminescence microscopy; imaging system optimization; computational simulation; Lu_2O_3 thin film scintillator

1. Introduction

Radioluminescence microscopy (RLM), an emerging technique for high-resolution radionuclide imaging, has the capability to visualize positron emission tomography (PET) radiotracers at the single cell level [1-6]. Unlike other optical imaging modalities (*e.g.*, bioluminescence and fluorescence imaging), radioluminescence imaging detects the dim light resulting from the interaction of ionizing particles with a scintillator substrate. In RLM, ionizing particles are alpha or beta particles produced during the decay of radionuclides used to label biological molecules. By detecting the spatiotemporal distribution of radiotracers in tissues or live cells, RLM can probe specific biochemical processes to gather information about the diversity of cellular behaviors. Through this process, it is also possible to investigate the targeting of PET tracers at the cellular level, which can be used in the discovery and validation of new tracers.

A number of technologies have been developed for high-resolution imaging of beta particles. Direct detection can be achieved using semiconductor detectors such as complementary metal–oxide–semiconductor (CMOS) chips [7-9] and position-sensitive avalanche photodiodes (PSAPD) [10]. Other devices use scintillators in combination with light sensors such as charge coupled device (CCD) or CMOS camera for indirect beta sensing [11-13]. RLM combines a transparent scintillator with a low-light microscope to enable microscopic examination of radionuclide accumulation in live cells, while also providing conventional microscopy capabilities [1, 2].

Given the challenging nature of imaging radionuclides at the single-cell level with high sensitivity, the performance of RLM in terms of spatial resolution and sensitivity must be as high as possible. Currently, the design of the microscope (*e.g.*, the parameters of the microscopy objective, scintillator and camera) has been optimized empirically, through trial and error [4, 14]. For instance, a 10 μm -thin Lu_2O_3 scintillator has been used in some experiments to enhance spatial resolution and sensitivity. A non-standard tube lens has also been investigated to increase image brightness by demagnification [14]. Finally, samples are prepared carefully to optimize spatial resolution by seeding cells directly onto the scintillator to minimize the source-scintillator distance.

However, while this empirical performance optimization can yield useful information, the optimal combination of system parameters is still unknown. This study aims at solving this problem using a computational simulation model of the RLM we previously established and validated [15]. Here we aim to find the optimal parameters for (1) the material and thickness of the scintillator; (2) the magnification of the microscope; and (3) the settings of the EMCCD camera (EM gain and binning). The optimal design is chosen by considering its impact on the spatial resolution and sensitivity of the simulated system.

2. Models and methods

Figure 1 presents the experimental RLM setup for single-cell imaging (*left side*) and the corresponding simulation models (*right side*). The process of imaging cells with this method involves a sequence of physical events. First, positrons emitted from radiotracers localized in single cells propagate through matter until they annihilate, exciting a large number of optical photons along their trajectories in the scintillator. Photons produced by scintillation are then focused by a low-light microscope onto the detection plane of an EMCCD camera, where it is read out. As shown in Figure 1, RLM can use either thin-film scintillators ($<20\ \mu\text{m}$ thick) or thick ($>100\ \mu\text{m}$ thick) scintillators. The focal plane of the microscope can be manually adjusted to focus on the region of the scintillator closest to the cells being imaged, to ensure that the scintillation light is in focus.

2.1. Numerical model of radioluminescence microscopy

Numerical models describing radiation transport and scintillation, light propagation through the microscope and photon sensing by the EMCCD camera were established and validated in our previous study [15]. One advantage of computational modeling is that the space of the system's parameters can be efficiently explored to derive optimal device parameters for imaging.

The physical processes related to the interactions of ionizing particles with the scintillator and surrounding structures (*e.g.*, particle transport, elastic and inelastic collisions) are simulated by Monte-Carlo simulation using the Geant4 package (version: Geant4 10.1) [16, 17]. The location and energy deposition of positron interactions, and the resulting number of scintillation photons produced are saved by the simulation package for further analysis. The maximum step size between two adjacent interactions is set to be $1\ \mu\text{m}$, consistent with the resolution of RLM. An example of parameters used for the Monte-Carlo simulation of RLM using a Lu_2O_3 thin-film scintillator is listed in table 1 (please refer to Supplement S1 for the complete set of parameters used in the simulation). Other scintillators are also investigated in this study, and the parameters are adjusted accordingly.

Scintillation photons produced by ionizing positrons are focused by a low-light microscope objective, modeled after the Olympus LV200, onto the camera detection plane. This process is simulated by convolving the 3D distribution of the emitted scintillation light in object space with a depth-dependent point-spread function (PSF). The variable refractive index Gibson-Lanni model is used to generate the PSF of the microscope and accounts for the refractive index (RI) mismatch between the scintillator layer, the cell medium layer ($RI=1.33$), and the immersion oil layer ($RI=1.5$) [18-20]. In addition to the distance from the source to the front focal plane in object space, the PSF is also determined by the scintillation light wavelength, the working distance and the numerical aperture. To enhance the computational efficiency, photons are assumed to have a fixed wavelength corresponding to the scintillator emission peak. We have verified in additional experiments (not shown) that this assumption has only a minor influence on the imaging performance. To increase image brightness, the microscope combines a high-numerical-aperture microscopy objective with a 36 mm-focal-length tube lens [14], resulting in 5 times lower magnification than specified by the objective. In the PSF calculation, the effective magnification is used.

For computational purpose, the scintillator space is divided evenly into discrete 3D voxels. The lateral size of the voxels is defined to match the pixel size of the EMCCD camera before magnification by the microscope. For instance, for the 8× magnification, the scintillator lateral size of the voxels is set to 1.625 μm to match the physical size of the camera pixels (13 μm). The voxel size in the depth direction is determined by the scintillator thickness. For scintillators thinner than 20 μm, the voxel thickness is set to 1 μm, otherwise, for thicker scintillators, it is set to 5 μm. Photons generated within the same scintillator depth layer are convolved with the corresponding 2D PSF, which is a matrix of 100×100 pixels with pixel size consistent with that of the scintillator layer (e.g., 1.625 μm for the 8× magnification).

The process of light capture by the EMCCD camera is modeled by a photo-sensor model. Photon shot noise (σ_{shot}), dark current noise (σ_{dark}), excess noise (σ_{excess}), clock induced charge noise (σ_{CIC}) and readout noise (σ_{read}) are all included in this model. We model photon shot noise, dark current and readout noise using a Poisson random process. The use of an electron multiplier is a unique feature of EMCCD cameras. This component helps suppress readout noise but it introduces excess noise [21, 22]. A method to model this noise has been presented in our previous paper [15]. The standard deviation of the total output noise N can be estimated as

$$N = \sqrt{\sigma_{\text{read}}^2 + F^2 M^2 (\sigma_{\text{shot}}^2 + \sigma_{\text{dark}}^2 + \sigma_{\text{CIC}}^2)} \quad (1)$$

where M is the total gain of cascaded electron multiplication elements and F is the excess noise factor, which approaches 2 when the number of incident electrons and multiplying stages are large. The digital camera output is obtained from the number of detected photoelectrons using a multiplicative conversion factor of 5.8 counts/electron and an offset of 3040 counts, which is used to avoid clipping negative camera values.

At the end of the pipeline, the simulated camera frames are processed by a reconstruction method to produce an image of the radiotracer distribution in the microscope field of view. We use a simplified version of the optical reconstruction of beta-ionization track (ORBIT) method, which is described in previous papers [2, 15]. Briefly, the method segments individual positron ionization tracks from raw camera frames, then applies a center-of-mass calculation to a selected subset of ionization tracks to estimate the emission location of the positron. These locations are then aggregated to form the reconstructed image. To improve spatial resolution, tracks extending more than 13 pixels (assuming 8× magnification and 4×4 pixel binning) in the x or y direction (denoted by figure 1) are not included in the reconstruction.

2.2. System parameters being optimized

The main goal of this study is to optimize RLM for better system performance, especially higher spatial resolution and system sensitivity, which are both critical for visualizing individual cells. First, we investigate scintillators made of five materials: Lu₂O₃:Eu, CdWO₄, LSO, CaF₂ and YAG, in thin film (10 μm thick) and thick (100 μm) form. These scintillators are chosen for their physical properties compatible with radioluminescence

microscopy, listed in table 2. The different wavelengths of the emitted photons affect the shape of the PSF and the quantum efficiency of the photon detection (see Table 2), which are modeled in the computational framework through the Gibson-Lanni model of the PSF and the photosensor model, respectively.

Next, the influences of parameters related to the microscope and camera, such as the magnification, electron multiplication (EM) gain and pixel binning on the system performance are investigated. We consider three microscope objectives, chosen for their high numerical aperture: a 20X/0.75NA air objective (UPLSAPO20X, Olympus), a 40X/1.3NA oil objective (UPLFLN40XO, Olympus), and a 100X/1.35NA oil objective (UPLAPO00XOI3, Olympus) [1, 23]. Due to the non-standard tube lens in the LV200, the effective magnification of these objectives is 4×, 8× and 20×, respectively. The overall numerical aperture of the system using the non-standard tube lens is not affected for the microscope with 8× and 20× effective magnifications but it slightly decreases from 0.75 to 0.63 for the microscope with 4× effective magnification [14], which is accounted in our simulation. The lateral voxel size used in the simulation is adjusted based on the effective magnification to match the camera pixel size (13 μm), resulting in lateral voxel sizes of 3.25, 1.625 and 0.65 μm, respectively. We also vary the EM gain from 200 to its maximum value of 1200 and the pixel binning (1×1, 2×2 and 4×4) to examine how these parameters affect image quality. For all simulations, the default scintillator, magnification, EM gain and pixel binning are Lu₂O₃ (10 μm thick), 8×, 600 and 4×4, unless specified otherwise.

Finally, the optimization scheme is evaluated for different radioisotope sources. In addition to ¹⁸F, we investigate four positron emitters (¹¹C, ¹³N, ¹⁵O and ⁶⁸Ga) and one electron emitter (⁹⁰Y), whose key properties are given in table 3.

2.3. Performance metrics

To find the optimal set of system parameters, we measure various performance metrics. Two of the metrics, the positron energy deposition and the positron penetration depth, are computed directly from Monte Carlo data for the different scintillators under investigation. These metrics indicate how much energy individual positrons effectively deposit in the scintillator, and how deep in the scintillator they travel.

Next, to quantify how different system parameters affect the characteristics of the raw scintillation tracks seen by the camera, we compute two other metrics. The *track intensity* is obtained by summing the intensity of pixels contained within a segmentation mask computed for each individual track captured by EMCCD camera. The *track dispersion* estimates the spatial dispersion of the light around the track centroid. This metric is defined as

$$\sum_{i=1}^N [\sqrt{(x_i - \bar{x})^2 + (y_i - \bar{y})^2} \cdot I_i] / \sum_{i=1}^N I_i \quad (2)$$

where (x_i, y_i) and I_i are the coordinates and the intensity of the i th ($i=1, 2, \dots, N$) pixel within a track, respectively, and $(\bar{x}, \bar{y})$ denote the intensity-weighted centroid of the track.

The segmentation of the tracks is implemented by applying an H-maxima transform to the image and setting a binary threshold above the noise. The threshold is chosen to be 100 camera counts above the simulated mean dark noise (averaged over 1000 frames), which is sufficient to reject 95% of the noise.

Finally, to evaluate the system's performance as a whole, an ^{18}F point source producing 10^5 decays is placed 4 μm from the scintillator (the effective source-scintillator distance in cell imaging experiments) to test different scintillators, objectives and imaging parameters. The sensitivity of the system is defined as the fraction of the positrons that are detected by the system. The spatial resolution of the system is defined as the full-width half-maximum of the point-source image, computed by fitting a 2D Gaussian function.

3. Results

3.1. Scintillator material selection

First, to select the optimal scintillator material, we simulate RLM imaging using scintillators made of different materials (table 2). Monte Carlo simulations show that Lu_2O_3 has the lowest mean penetration depth for ^{18}F positrons among the five scintillators investigated: 15.8 μm , compared to 20.1 μm , 20.8 μm , 48.5 μm and 36.4 μm for CdWO_4 , LSO, CaF_2 and YAG scintillators, respectively (assuming a 100- μm -thick scintillator). Furthermore, a greater fraction of the positron energy is deposited in a 10- μm -thick coating of Lu_2O_3 (45.3 keV) than any other scintillator: the energy deposition is only 34.6 keV, 38.5 keV, 26.2 keV and 32.5 keV for CdWO_4 , LSO, CaF_2 and YAG scintillators, respectively.

We now examine the properties of the scintillation tracks as seen by the EMCCD camera. Figure 2 shows the mean track dispersion (a), mean track intensity (b), spatial resolution (c) and sensitivity (d) for either thin (*light shading*) or thick (*dark shading*) scintillators. Compared with other scintillators, the Lu_2O_3 scintillator yields much brighter scintillation tracks: 2.2×10^4 and 5.2×10^4 counts for 10- μm - and 100- μm -thick scintillator, respectively, which is around 2 times brighter than tracks from other scintillators. In addition, scintillation tracks produced by the Lu_2O_3 thin-film scintillator (10 μm thick) display the smallest amount of spatial dispersion (8.1 μm). As a result of the combined effect of track intensity and dispersion, the highest spatial resolution (20.9 μm) and sensitivity (41.6%) are achieved for the Lu_2O_3 thin-film scintillator. Although the Lu_2O_3 thin-film scintillator is dimmer than its thicker counterpart, it achieved higher sensitivity, presumably because fewer extended tracks are discarded during the reconstruction. The opposite trend is seen for dimmer scintillators, for which tracks are too dim in the thin-film geometry to be reliably detected. As a result, the physical properties of the Lu_2O_3 scintillator allow the highest spatial resolution and sensitivity found in this study.

3.2. Scintillator thickness selection

Having selected Lu_2O_3 as the optimal scintillator material, we examine the characteristics of raw ionization tracks as a function of scintillator thickness (2-200 μm). Mean track dispersion initially increases quasi-linearly as scintillator thickness increases, up to a thickness of 25 μm , and it continues to increase beyond this point, albeit at a much more

gradual rate (figure 3a). Here, the increase in dispersion is due to the fact that the ionization track extends deeper in the scintillator for thicker scintillators, thus leading to greater dispersion of the light. Track intensity follows a similar pattern (figure 3b). The increase in track intensity with scintillator thickness is due to the increased amount of energy deposited in a thicker scintillator. It is noted that increasing the scintillator thickness beyond 25 μm does not substantially affect the characteristics of the track. This effect is thought to be due to the limited depth of field of the microscope.

At the system level, increasing scintillator thickness from 2 to 25 μm leads to a loss of spatial resolution of 0.3 μm for every 1 μm thickness increase. For thicknesses above 25 μm , spatial resolution reaches a plateau and additional increases in scintillator thickness do not cause significant loss of spatial resolution (figure 3c).

The sensitivity of the scintillator initially increases in concert with the thickness of the scintillator, as more energy is deposited in the material. However, as the scintillator becomes thicker, more tracks are rejected by the ORBIT reconstruction algorithm because their elongated and unfocused shape does not permit accurate reconstruction. The combination of these two effects leads to the trend seen in figure 3d for the sensitivity, consisting of a rapid increase, followed by a plateau between 8 and 25 μm , and finally a slow decrease.

As shown in figure 3, the scintillator thickness optimal for spatial resolution is different than the thickness optimal for sensitivity. To balance out these two objectives, we built a cost function (ϕ) accounting for the concurrent contribution of spatial resolution (ε) and signal-to-noise ratio, proportional to the square root of the sensitivity (s):

$$\phi(\tau) = \sqrt{\frac{s(\tau)}{s_{\max}(\tau)}} - \frac{\varepsilon(\tau)}{\varepsilon_{\max}(\tau)} \quad (3)$$

where $\tau \in [2 \mu\text{m}, 200 \mu\text{m}]$ is the scintillator thickness, $s_{\max}$ and $\varepsilon_{\max}$ are the maximum achievable spatial resolution and sensitivity. This function assumes signal-to-noise ratio and spatial resolution are equally important; different weights can be used for applications where one metric is more important than the other. The cost function reaches its maximum value for an 8- μm -thick scintillator (figure 4). Therefore, considering both spatial resolution and sensitivity, the optimal thickness of the Lu_2O_3 scintillator is determined to be 8 μm .

3.3. Microscope optimization

The brightness of the image captured by the camera is substantially affected by the parameters of the microscope. Image brightness, defined as photon intensity per surface area, is proportional to the square of the numerical aperture of the objective lens and inversely proportional to the square of the magnification. The imaging performance of RLM is investigated for objectives with different magnifications and numerical apertures. As shown in table 4, spatial resolution and track dispersion improve with higher effective magnification. Track intensity per pixel, which reflects image brightness, decreases with higher effective magnification but increases with higher numerical aperture. The sensitivity

of the system is not significantly different for the three objectives being considered, despite of differences in track intensity per pixel, suggesting that all three objectives collect a sufficient amount of light to achieve near-optimal sensitivity.

It is important to note that magnification also affects the size of the field of view (FOV). For many applications, the choice of the magnification will be driven more by the requirement for a FOV of a specific size rather than by performance considerations. Moreover, the selected magnification also affects the quality of the images taken using the fluorescence and brightfield capabilities of the RLM. As will be shown in Section 3.5, optimal resolution and sensitivity can be achieved for RLM irrespective of the magnification used in the instrument.

3.4. Camera optimization

After evaluating the influence of parameters pertaining to the scintillator and the microscope, we examined the influence of EMCCD camera parameters. Track dispersion increases with higher EM gain (figure 5a), presumably because track intensity increases with EM gain and, therefore, dimmer areas of the track become visible (figure 5b). The combination of these two effects has a minor effect on spatial resolution, with only a slow degradation noticeable as gain increases. The sensitivity of the RLM system also increases with higher EM gain due to the brighter track intensity (figure 5d). Owing to these trends in spatial resolution and sensitivity, an EM gain between 600 and 800 is recommended for RLM imaging.

Next, the influence of pixel binning on image quality is estimated. For some cameras, pixel binning is advantageous because the pixels are combined before the gain register and readout circuitry, thus resulting in an improvement in signal to noise ratio. Our simulation assumes that pixel binning occurs at the electronic level, before the EM gain. The performance characteristics of RLM imaging with 8 $\times$ magnification and 1 $\times$ 1, 2 $\times$ 2 or 4 $\times$ 4 binning are presented in figure 5. Track intensity is not affected by binning since the total number of counts is preserved by this operation. In contrast, track dispersion increases with pixel binning due to the reduced spatial sampling. At low EM gain (*i.e.*, <400), 4 $\times$ 4 binning is required to maintain high sensitivity, at the cost of a decrease in spatial resolution. For higher EM gain (*i.e.*, >600), 1 $\times$ 1 pixel binning can be chosen to enhance the spatial resolution.

3.5. Global optimization

The sequential optimization of the scintillator (material and thickness), microscope objective and camera settings (EM gain and pixel binning) may yield a local rather than a global maximum. Therefore a global optimization approach, *i.e.*, simultaneous search over the set of all the system parameters, was performed. The selection of the scintillator material and thickness was not included in the global optimization because the Lu₂O₃ thin-film scintillator was clearly the best candidate, independently of the parameters of the imaging system. Having selected this scintillator material, we searched simultaneously and globally for the best combination of effective magnification, EM gain and pixel binning. As shown in figure 6, different combinations of effective magnification and pixel binning, namely 4 $\times$ and 1 $\times$ 1, 8 $\times$ and 2 $\times$ 2, and 20 $\times$ and 4 $\times$ 4, result in similar spatial resolution and sensitivity,

provided that the EM gain is adjusted accordingly. This indicates that a user can select a magnification based on considerations not related to RLM imaging performance, such as field of view or fluorescence image quality, and yet still achieve optimal spatial resolution and sensitivity for RLM imaging, independent of magnification.

Finally, the performance metrics simulated with the optimized parameters are compared with those simulated with the parameters intensively used in the radioluminescence cell imaging experiment. The two sets of parameters and corresponding performance metrics (using an ^{18}F point source, 4- μm from the scintillator) are given in table 5. Further, we compared the simulated radioluminescence cell images (illustrated in our previous paper [15]) obtained using experimental and optimized parameters, as shown in figure 7a and 7b, respectively, given the same planar cell source which is 4 μm from the scintillator and same total radioactive decays: 10^6 over all cells. Positrons are assumed to be emitted evenly from the cells extracted from the brightfield cell image. The radioluminescence cell image is fused with brightfield cell image in figure 7 to give an intuitive impression of the cell morphology. Using the optimized parameters (8- μm -thick Lu_2O_3 scintillator, 8 $\times$ magnification, 2 $\times$ 2 pixel binning and EM gain = 800), the radioluminescence cell image embraces obviously greater performances (*i.e.*, higher spatial resolution and sensitivity) than those obtained using the experimental parameters (500- μm -thick CdWO_4 scintillator, 8 $\times$ magnification, 4 $\times$ 4 pixel binning and EM gain = 800).

3.6. Optimization for different radioisotopes

The aforementioned RLM optimization scheme is further evaluated for different radioisotopes to ensure that the optimal parameter combination is optimal for a wide variety of radioisotopes (^{11}C , ^{13}N , ^{15}O , ^{18}F , ^{68}Ga and ^{90}Y). First of all, we compute the dispersion (figure 8a) and intensity (figure 8b) of single tracks, and the image spatial resolution (figure 8c) and sensitivity (figure 8d), using either a 10- μm -thick (light blue shading) or a 100- μm -thick (dark blue shading) Lu_2O_3 scintillator exposed different radioisotopes. Track dispersion and intensity are found to be proportional to the particle mean penetration depth and mean energy deposited in scintillators, respectively, given by table 3, and they in turn determine the spatial resolution and sensitivity, respectively.

For the thin scintillator for which the decay energy is only partially absorbed, beta particles with higher energies (*e.g.*, ^{68}Ga positrons and ^{90}Y electrons) deposit less energies and therefore have lower track intensity and sensitivity. In contrast, track dispersion is similar for different radioisotopes, resulting in similar spatial resolution. For the thick scintillator, high-energy beta particles tend to deposit more energies at the end of their trajectories, too deep within the scintillator for the scintillation photons to be focused properly by the microscope. These long, unfocused tracks are more likely to be discarded during the reconstruction process. Therefore, isotopes with higher-energy emissions have lower spatial resolution and sensitivity, as shown by figures 8c & 8d.

Overall, RLM has higher performance for imaging isotopes with low-energy emissions. Additionally, a 10- μm thin scintillator performs better than a 100- μm thick scintillator for all isotopes investigated. Finally, the global optimization results obtained using ^{18}F also apply

to the other isotopes considered in this study (see Supplement S2), indicating that the choice of camera and objective parameters is independent of the isotope being considered.

4. Discussion

Based on the simulation methodology and RLM system model that was previously established, we optimized our radioluminescence imaging system by systematically finding the optimal combination of imaging parameters. We considered various parameters involving every element of importance within the system, and we examined the influence of these parameters on the imaging performance of the system, represented by the spatial resolution, sensitivity, track intensity and track dispersion. Moreover, the optimal combination of parameters found for ^{18}F also applies to other radioisotopes. This approach demonstrates that computational simulation is an efficient and effective approach for improving the performance of RLM imaging and extending its ability to image cellular processes in dense populations of cells.

To illustrate the advantages of the Lu_2O_3 thin-film scintillator, we present an example of simulated and measured ^{18}F positron tracks acquired with either a 10- μm -thick Lu_2O_3 or a 500- μm -thick CdWO_4 scintillator, as shown in figures 9a-d. The track from the Lu_2O_3 scintillator is noticeably shorter and brighter. Also, radioluminescence cell images acquired using the system with a Lu_2O_3 thin-film scintillator (figures 9e-f) present higher brightness and spatial resolution in comparison with those from the system with a thick CdWO_4 scintillator (figures 9g-h).

The conventional Gibson-Lanni model used to generate the depth-dependent PSF of the microscope considers the sample (here, the scintillator), cover glass and immersion oil layer, and accounts for the refractive index mismatch between these three layers. However, it is not adequate for simulating the PSFs for the “sandwich” geometry, where the cells are arranged between the scintillator and the cover glass (as in figure 1). By using a variable refractive index Gibson-Lanni model, we account for the refractive index mismatch between the scintillator, cell sample, cover glass and immersion oil layer [18-20]. The introduction of an additional layer causes a shift of the back focal plane in the image space, which is corrected by adjusting the stage of the microscope. The PSF obtained after refocusing the microscope is very similar to that generated with conventional Gibson-Lanni model.

This study is based on a numerical model of the RLM system that we have previously developed and validated. To achieve optimal image quality, the model should be accurate enough to predict the imaging performance for different system parameter. There may exist some discrepancies between the model and the realistic system due to uncertainty in the design of some of the system components. For instance, we modeled the light passing through the microscope lens by convolving with a depth-dependent PSF. This approximation may not be sufficient to include all physical effects introduced by the microscope. A Monte-Carlo simulation of photon transmission through the lens would be a more accurate approach. However, since the details of the commercial lens design are not available, we have to make a compromise between the accuracy of the model and feasibility.

5. Conclusion

Using a previously developed model of the RLM, we systematically explored the influence of key parameters on imaging performance and identified the optimal combination of these parameters. This *in silico* approach is a more effective than empirical adjustments as many more parameters can be considered. The simulation results indicate that optimal RLM imaging performance is achieved for an 8- μm -thick Lu_2O_3 film scintillator. On the instrument side, a key finding was that similarly high resolution and sensitivity could be achieved for different magnifications, provided that the camera binning and gain were adjusted accordingly. This feature gives the user the flexibility to select the magnification of the system based on experimental requirements rather than performance considerations. This optimized configuration was confirmed for a range of different radioisotopes. It is hoped that the improved RLM will play a more important role in the single-cell characterization of small molecules such as PET tracers and radiolabeled pharmaceuticals.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This study was supported by National Institutes of Health (NIH) grants R01CA186275 and R44GM110888.

References

1. Pratz G, Chen K, Sun C, Martin L, Carpenter CM, Olcott PD, Xing L. PLoS ONE. 2012; 7:e46285. [PubMed: 23056276]
2. Pratz G, Chen K, Sun C, Axente M, Sasportas L, Carpenter C, Xing L. J Nucl Med. 2013; 54:1841–1846. [PubMed: 24003077]
3. Sengupta D, Pratz G. J Nucl Med. 2016; 57:1136–1140. [PubMed: 27081170]
4. Sengupta D, Miller S, Marton Z, Chin F, Nagarkar V, Pratz G. Adv Healthc Mater. 2015; 4:2064–2070.
5. Türkcan S, Naczynski DJ, Nolley R, Sasportas LS, Peehl DM, Pratz G. Sci Rep. 2016; 6:1–11. [PubMed: 28442746]
6. Kim TJ, Türkcan S, Pratz G. Nat Protocols. 2017; 12:1055–1076. [PubMed: 28426025]
7. Liu Z, Jian Z, Wang Q, Cheng T, Feurecker B, Schwaiger M, Huang SC, Ziegler SI, Shi K. J Nucl Med. 2016; 57:1548–1555. [PubMed: 27363838]
8. Wang Q, Tous J, Liu Z, Ziegler S, Shi K. J Instrum. 2014; 9:C05067.
9. Wang Q, Liu Z, Ziegler SI, Shi K. Phys Med Biol. 60:5261–5278.
10. Vu NT, Yu ZTF, Comin-Anduix B, Søndergaard JN, Silverman RW, Chang CYN, Ribas A, Tseng HR, Chatziioannou AF. J Nucl Med. 2011; 52:815–821. [PubMed: 21536929]
11. Barthe N, Chatti K, Coulon P, Maitrejean S, Basse-Cathalinat B. Nuclear Inst and Methods in Physics Research, A. 2004; 527:41–45.
12. Ding, Y., Caucci, L., Barrett, HH. 2014 IEEE Nuclear Science Symposium and Medical Imaging Conference (NSS/MIC 2014). Seattle, Washington: United States; Nov 8-15. 2014 p. 1-4.
13. Chen L, Gobar LS, Knowles NG, Liu Z, Gmitro AF, Barrett HH. J Nucl Med. 2008; 49:1141–1145. [PubMed: 18552136]
14. Kim TJ, Türkcan S, Ceballos A, Pratz G. Biomed Opt Express. 2015; 6:4585–4598. [PubMed: 26601020]
15. Wang Q, Sengupta D, Kim TJ, Pratz G. Med Phys. 2017; 44:1782–1795. [PubMed: 28273348]

16. Agostinelli S, Allison J, Amako K, Apostolakis J, Araujo H, Arce P, Asai M, Axen D, Banerjee S, Barrand G, Behner F, Bellagamba L, Boudreau J, Broglia L, Brunengo A, Burkhardt H, Chauvie S, Chuma J, Chytrcek R, Cooperman G, Cosmo G, Degtyarenko P, Dell'Acqua A, Depaola G, Dietrich D, Enami R, Feliciello A, Ferguson C, Fesefeldt H, Folger G, Foppiano F, Forti A, Garelli S, Giani S, Giannitrapani R, Gibin D, Gómez Cadenas JJ, González I, Gracia Abril G, Greeniaus G, Greiner W, Grichine V, Grossheim A, Guatelli S, Gumplinger P, Hamatsu R, Hashimoto K, Hasui H, Heikkinen A, Howard A, Ivanchenko V, Johnson A, Jones FW, Kallenbach J, Kanaya N, Kawabata M, Kawabata Y, Kawaguti M, Kelner S, Kent P, Kimura A, Kodama T, Kokoulin R, Kossov M, Kurashige H, Lamanna E, Lampén T, Lara V, Lefebvre V, Lei F, Liendl M, Lockman W, Longo F, Magni S, Maire M, Medernach E, Minamimoto K, Mora de Freitas P, Morita Y, Murakami K, Nagamatsu M, Nartallo R, Nieminen P, Nishimura T, Ohtsubo K, Okamura M, O'Neale S, Oohata Y, Paech K, Perl J, Pfeiffer A, Pia MG, Ranjard F, Rybin A, Sadilov S, Di Salvo E, Santin G, Sasaki T, Savvas N, Sawada Y, Scherer S, Sei S, Sirotenko V, Smith D, Starkov N, Stoecker H, Sulkimo J, Takahata M, Tanaka S, Tcherniaev E, Safai Tehrani E, Tropeano M, Truscott P, Uno H, Urban L, Urban P, Verderi M, Walkden A, Wander W, Weber H, Wellisch JP, Wenaus T, Williams DC, Wright D, Yamada T, Yoshida H, Zschesche D. Nuclear Inst and Methods in Physics Research, A. 2003; 506:250–303.
17. Allison J, Amako K, Apostolakis J, Araujo H, Dubois PA, Asai M, Barrand G, Capra R, Chauvie S, Chytrcek R. IEEE Trans Nucl Sci. 2006; 53:270–278.
18. Kirshner H, Aguet F, Sage D, Unser M. J Microsc. 2013; 249:13–25. [PubMed: 23126323]
19. Gibson SF, Lanni F. J Opt Soc Am A. 1992; 9:154–166. [PubMed: 1738047]
20. Gibson SF, Lanni F. J Opt Soc Am A. 1989; 6:1357–1367. [PubMed: 2795290]
21. Hirsch M, Wareham RJ, Martin-Fernandez ML, Hobson MP, Rolfe DJ. PLoS ONE. 2013; 8:e53671. [PubMed: 23382848]
22. Chao J, Ram S, Ward ES, Ober RJ. Proceedings of SPIE. 2013; 8589:858905. [PubMed: 25075263]
23. Natarajan A, Türkcan S, Gambhir SS, Pratz G. Mol Pharm. 2015; 12:4554–4560. [PubMed: 26460685]

Figure 1.

Diagram of positron radioluminescence imaging of single tumor cells using a thin film ($\approx 10 \mu\text{m}$ thick, coated onto a substrate; left hand side) scintillator or a thick scintillator ($\approx 100 \mu\text{m}$ thick; right hand side); and computational models used for the simulation of radioluminescence imaging (right side).

Figure 2.

Imaging performance comparison for different scintillators (Lu_2O_3 , CdWO_4 , LSO, CaF_2 and YAG): mean track dispersion (a), mean track intensity (b), spatial resolution (c), sensitivity (d) for thin (light shading) or thick (dark shading) scintillators.

Figure 3. Effect of scintillator thickness on imaging performance. Track dispersion (a), track intensity (b), spatial resolution (c), and sensitivity (d) as a function of Lu_2O_3 scintillator thickness.

Figure 4. Cost function used for jointly optimizing spatial resolution and sensitivity as a function of scintillator thickness

Figure 5. Effect of camera parameters on imaging performance. Track dispersion (a), track intensity (b), spatial resolution (c), sensitivity (d) as a function of EM gain and pixel binning

Figure 6. Global optimization of magnification, pixel binning and EM gain on imaging performance. Spatial resolution (a) and sensitivity (b) as a function of EM gain, pixel binning and effective magnification (mag)

Figure 7.

(a) RLM cell images simulated using experimental parameters (500- μm -thick CdWO_4 , 8 $\times$ magnification and 4 $\times$ 4 binning, EM gain = 800) compared with (b) RLM cell images simulated using optimized parameters (8- μm -thick Lu_2O_3 , 8 $\times$ magnification and 2 $\times$ 2 binning, EM gain = 800). The RLM images are displayed using quantitative units of Bq/s/mm^2 and therefore are normalized for sensitivity, total acquisition time and pixel area.

Figure 8.

Track dispersion (a), track intensity (b), spatial resolution (c) and sensitivity (d) for different radioisotope sources (^{11}C , ^{13}N , ^{15}O , ^{18}F , ^{68}Ga and ^{90}Y). 10-μm-thick (light blue) or 100-μm-thick (dark blue) Lu_2O_3 scintillator is used.

Figure 9.

(a)-(d): representative example of simulated and measured ^{18}F positron track imaged with RLM for a thin Lu_2O_3 and a thick CdWO_4 scintillator. (e)-(f): simulated and measured ^{18}F radioluminescence cell map for Lu_2O_3 -scintillator-integrated RLM compared with those from CdWO_4 -scintillator-integrated RLM.

Table 1
Parameters used in Monte-Carlo simulation of Lu₂O₃ RLM system

Parameters	Values	Parameters	Values
Scintillator density	9.5 g/cm ³	Scintillator thickness *	10 μm
Scintillation light yield	48 photons/keV	Substrate thickness	200 μm (Sapphire)
Scintillator emission peak	610 nm	Substrate density	4.1 g/cm ³
Scintillator refractive index	1.9	Substrate refractive index	1.8
Scintillator reflectivity	0.1	Substrate reflectivity	0.08

* The scintillator thickness is adjusted in the investigation of its influence on imaging performance.

Table 2
Simulation parameters for different scintillators

Scintillator	Lu ₂ O ₃	CdWO ₄	LSO	CaF ₂	YAG
Material composition	Lu ₂ O ₃ : Eu	CdWO ₄	Lu ₂ SiO ₅ : Ce	CaF ₂ : Eu	Y ₃ Al ₅ O ₁₂ : Ce
Density (g/cm ³)	9.5	7.9	7.4	3.2	4.6
Light yield (photons/keV)	48	12	29	19	8
Emission peak (nm)	610	475	420	435	550
Refractive index	1.93	2.30	1.82	1.47	1.82
QE	0.9	0.8	0.7	0.7	0.85
Decay time (ns)	2.5×10 ⁴	10 ⁴	40	940	100

Table 3**Physical properties of different isotopes**

Isotopes	^{11}C	^{13}N	^{15}O	^{18}F	^{68}Ga	^{90}Y
Emission	β^+	β^+	β^+	β^+	β^+	β^-
Half-life	20.4 min	9.96 min	2.03 min	109.8 min	68.3 min	64.1 h
Branching (β) in %	99.8	99.8	99.9	96.9	87.7	99.99
Max. emission energy (keV)	959	1199	1732	634	2912	2280
Mean emission energy (keV)	386	492	735	250	836	937
Mean depth in 10- μm -thick Lu_2O_3 scint. (μm) *	7.2	7.6	8.2	6.2	8.3	8.2
Mean depth in 100- μm -thick Lu_2O_3 scint. (μm) *	27.2	34.7	45.3	15.7	50.2	48.6
Mean energy deposited in 10- μm -thick Lu_2O_3 scint. (keV) *	40.9	39.6	35.4	45.5	35.6	36.1
Mean energy deposited in 100- μm - Lu_2O_3 thick scint. (keV) *	178.5	199.1	216.2	127.2	225.0	216.5

* The mean depth and mean energy deposited in the Lu_2O_3 scintillator (scint.) are obtained from Monte-Carlo simulations.

Table 4
Ionization track characteristics for different objectives (8- μm -thick Lu_2O_3 scintillator)

Effective magnification	4	8	20
Immersion medium	air	oil	oil
Numerical aperture	0.63 [*]	1.3	1.35
Image brightness	0.025	0.026	0.005
Objective light collection efficiency (%)	11	25	28
Spatial resolution (μm) [*]	23.4	20.5	17.6
Sensitivity (%) [*]	36.3	40.8	40.0
Mean track dispersion (μm) [*]	9.6	7.5	7.4
Mean track intensity (counts $\times 10^4$) [*]	0.93	1.84	1.99
Mean track intensity per pixel (counts $\times 10^3$ /pixel) [*]	2.26	2.53	1.10

^{*} Effective numerical aperture. Specified NA is 0.75

Table 5
RLM performance comparison: experimental vs optimized parameters

	Simulation with experimental settings	Simulation with optimized settings
Scintillator	500- μm -thick CdWO_4	8- μm -thick Lu_2O_3
Microscope and EMCCD settings	8 $\times$ mag. [*] and 4 $\times$ 4 binning, EM gain=600-800	8 $\times$ mag. and 2 $\times$ 2 binning or 20 $\times$ mag. and 4 $\times$ 4 binning, EM gain = 600-1200
Spatial resolution (μm)	30	18
Sensitivity (%)	29	45

^{*} mag. = effective magnification