Performance evaluation of ^{18}F radioluminescence microscopy using computational simulation

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Purpose: Radioluminescence microscopy can visualize the distribution of beta-emitting radiotracers in live single cells with high resolution. Here, we perform a computational simulation of ^{18}F positron imaging using this modality to better understand how radioluminescence signals are formed and to assist in optimizing the experimental setup and image processing.

Methods: First, the transport of charged particles through the cell and scintillator and the resulting scintillation is modeled using the GEANT4 Monte-Carlo simulation. Then, the propagation of the scintillation light through the microscope is modeled by a convolution with a depth-dependent point-spread function, which models the microscope response. Finally, the physical measurement of the scintillation light using an electron-multiplying charge-coupled device (EMCCD) camera is modeled using a stochastic numerical photosensor model, which accounts for various sources of noise. The simulated output of the EMCCD camera is further processed using our ORBIT image reconstruction methodology to evaluate the endpoint images.

Results: The EMCCD camera model was validated against experimentally acquired images and the simulated noise, as measured by the standard deviation of a blank image, was found to be accurate within 2% of the actual detection. Furthermore, point source simulations found that a reconstructed spatial resolution of 18.5 μm can be achieved near the scintillator. As the source is moved away from the scintillator, spatial resolution degrades at a rate of 3.5 μm per μm distance. These results agree well with the experimentally measured spatial resolution of 30–40 μm (live cells). The simulation also shows that the system sensitivity is 26.5%, which is also consistent with our previous experiments. Finally, an image of a simulated sparse set of single cells is visually similar to the measured cell image.

Conclusions: Our simulation methodology agrees with experimental measurements taken with radioluminescence microscopy. This *in silico* approach can be used to guide further instrumentation developments and to provide a framework for improving image reconstruction. © 2017 American Association of Physicists in Medicine [https://doi.org/10.1002/mp.12198]

Key words: computational simulation, imager performance evaluation, positron imaging, radioluminescence microscopy

1. INTRODUCTION

In the past few years, a series of high-resolution beta imaging devices have been developed for biological and physiological applications. These devices use a variety of technologies to directly or indirectly localize beta emissions in samples, such as charged coupled detectors (CCD), complementary metal-oxide semiconductors (CMOS), and position-sensitive avalanche photodiodes (PSAPD).^{1–6}

Direct beta imaging approaches can sensitively detect the emitted particle without the need for a scintillator. Medipix/Timepix, a hybrid CMOS image sensor fabricated by CERN, is a widely used detector for direct beta particle measurement. The device has been applied in biomedical imaging applications to image radiotracer distribution both *in vivo* and *in vitro*, using radioisotopes such as ^{18}F , ^{14}C , and $^{90}\text{Y}/^{90}\text{Sr}$.^{7–12} For example, the Timepix detector has been used in microfluidic experiments to monitor tumor cell metabolism in real time. FDG uptake in tumor cells was visualized dynamically by adjusting the available concentration of fluorodeoxyglucose

(^{18}F]FDG) through an external pump connected to a microfluidic chip inlet.¹³ Position-sensitive avalanche photodiodes are also used for direct beta imaging—for example, PSAPDs have been used to image glucose metabolism of cells incubated on a microfluidic chip.^{6,14}

Several indirect beta imagers have also been developed, including gaseous avalanche chambers (e.g., β -imager $^{\text{T}}\text{Ra}$ -cer), scintillator sheets (e.g., β -imager $^{\text{D}}\text{Fine}$), and phosphor films coupled with a lens and a semiconductor sensor array. These systems have used tracers that contain ^3H , ^{14}C , ^{35}S , $^{90}\text{Y}/^{90}\text{Sr}$, and $^{99\text{m}}\text{Tc}$ to produce images with a higher resolution.^{4,15}

Radioluminescence microscopy is another indirect beta imaging technique for functional biological imaging on the single-cell level.^{16–19} This technique enables the visualization of cellular processes by capturing the light generated during the scintillation of beta particles emitted from radioactive compounds accumulated in individual tumor cells. A scintillator converts the kinetic energy from emitted beta particles into optical photons that can be imaged using a microscope

with a low magnification and high numerical aperture. This imaging modality can provide us with valuable information on the diversity found in individual tumor cells, which cannot be fully captured by conventional radionuclide imaging techniques.

Compared with other previous beta imaging systems, radioluminescence microscopy is capable of sensing dimmer light from single living cells in a microscopic environment.¹⁷ The excellent performance of the radioluminescence microscope is because of the properties of the cadmium tungstate (CdWO_4) scintillator used, which include high density and stopping power, and good light yield. In addition, image quality is further improved because of the high numerical aperture (NA) of the microscope and high photon sensitivity of the EMCCD camera. This system has been successfully utilized to image metabolism,¹⁶ proliferation,²⁰ and drug binding²¹ in single tumor cells.

While this technique has already demonstrated biological utility, we aim to improve the design of the microscope by developing a computational model of this system that precisely describes the different physical processes involved. Such a model would be useful to numerically explore the performance of the system as the design variables are varied (e.g., scintillator characteristics, objective properties, image reconstruction parameters, etc.). In this study, we establish a multiphysical computational model that accurately simulates the operation of the radioluminescence microscope, including

production, transport, conversion, and detection of ionizing radiation and secondary particles. Spatial resolution, sensitivity, and background noise of ^{18}F positron imaging obtained from numerical simulations are all compared with experimental measurements. The imaging characteristics of other beta emitters (^{11}C , ^{32}P , ^{68}Ga , and ^{90}Y) are explored and compared with those of ^{18}F . In addition, the entire process of imaging ^{18}F radiolabeled cells is tested from end to end and compared with experimentally acquired cell images.

2. METHODS

2.A. Physical implementation of radioluminescence microscopy

The operation of the microscope has been described elsewhere.^{16,17,22} Briefly, sparsely distributed cells are labeled with a radioactive tracer such as $[^{18}\text{F}]\text{FDG}$. Positrons emitted from the cell-localized radiotracer travel into the scintillator and produce scintillation light (Fig. 1). Two imaging configurations can be used: one with cells seeded on the glass dish and scintillator placed on top, as shown in Fig. 1(a); and the other with cells seeded directly on top of the scintillator, as shown in Fig. 1(b). The latter configuration can only be used with scintillators thinner than 100 μm to accommodate the short working distance of the 40X objective (200 μm), but it has the advantage of enabling easy access to the cells (for

FIG. 1. Diagrams of positron imaging using a radioluminescence microscope. Two imaging setups are presented here: (a) Cells are seeded onto a glass dish, immersed in cell medium, and placed under a 500- μm -thick CdWO_4 scintillator; (b) Cells are seeded directly onto a 100- μm -thick CdWO_4 scintillator. A thinner scintillator is required for the second setup due to the short working distance of the objective (200 μm). The volume ± 5 μm around the focal plane in the z direction, labeled with darker shading, indicates the depth of field of the microscope, that is, the region of the scintillator in focus. [Color figure can be viewed at wileyonlinelibrary.com]

staining and other manipulations). The dim scintillation light is captured by the microscopy objective, refocused by the tube lens, and finally recorded by the EMCCD camera. An image reconstruction method called ORBIT (optical reconstruction of the beta-ionization track) is then applied to estimate the position of each individual decay event and to produce an image of the distribution of the radiotracer.¹⁷

The radioluminescence microscope can be thought of as three major subsystems, namely the scintillator, the microscope, and the image sensor. Each subsystem involves physical processes that are complex to model, therefore, requiring dedicated simulation procedures. As a result, we have developed a multiphysical workflow to simulate radioluminescence microscopy acquisitions from end to end, as shown in Fig. 2.

2.B. Monte-Carlo simulation of radiation transport and scintillation

The physics of ^{18}F positron generation, transport, and absorption in the scintillator are simulated using Geant4, a Monte Carlo toolkit to simulate the passage of particles through matter.²³ Positrons interact with the scintillator through elastic scattering and inelastic collisions with nuclei and atomic electrons. Inelastic collisions with atomic electrons are the primary mechanism of kinetic energy loss and photon excitation.

The geometric model used in the simulation is consistent with experimental conditions. The electromagnetic, optical, decay, and transportation processes of positrons and their secondary particles (e.g., photons) are modeled by registering the corresponding physics model classes owned by Geant4 in the physics list. Production of scintillation light follows a Poisson distribution and accounts for the energy deposition and the material properties of the modeled scintillator (Table I). The positron decay of ^{18}F is simulated by the radioactive decay package in Geant4. The number of incident positrons that are generated due to radioactive decay is determined by statistical considerations. The reflection and refraction of optical photons at the interface of two media

(such as air-scintillator interface) are also considered in the simulation.

During Monte-Carlo simulation, we record the discrete interactions of the positrons with the scintillator (position, deposited energy, and number of scintillation photons). The simulated data are further processed to match the input format of the next stages of the simulation. During data processing, the scintillator is discretized into $1024 \times 1024 \times T/5$ voxels (T : scintillator thickness) with a voxel size of $1.625 \mu\text{m} \times 1.625 \mu\text{m} \times 5 \mu\text{m}$ (length $\times$ width $\times$ depth), and the data collected are voxelized. The tracking step limit and range cut values (below which particles do not interact nor produce secondary particles) are adjustable. The max step size is chosen to be consistent with the pixel size of system's eventual output image, and the default cut value of 1 mm is used in Geant4 simulation. Other optional step limits and cut values have been tested and proven to have little influence on the simulation results. The geometrical, material, and optical parameters of the CdWO_4 scintillator and other pertinent structures (culture medium and glass-bottom dish) are specified in Tables I and II, respectively.

2.C. Optical modeling of the microscope

In our set-up, scintillation light produced in the scintillator is captured by a special bioluminescence microscope (Olympus LV200 system), which consists of a high numerical aperture microscope objective (Olympus UPLFLN 40XO) coupled with a special 36-mm-focal-length tube lens.²² The image brightness in microscopy is directly proportional to the square of the numerical aperture (NA) of the objective lens and inversely proportional to the square of magnification (M) of the image.²² The magnification is the ratio between focal lengths of tube lens and objective lens. Therefore, the purpose of the short focal length is to increase the image brightness by reducing its magnification thus focusing the light onto the camera while preserving the high numerical aperture. As a linear system, the microscope can be accurately modeled by its depth-dependent point-spread function (PSF). For sources near the focal plane of the microscope, the spatial

Fig. 2. Flowchart of the simulation of positron imaging using radioluminescence microscopy. [Color figure can be viewed at wileyonlinelibrary.com]

TABLE I. Parameters of scintillator (CdWO_4) used in the simulation.

Parameters	Values
Thickness	500 μm^a
Length and width	1.664 mm
Density	7.9 g/cm^3
Light yield	12 photons/ $\text{keV}\gamma$
Wavelength of emission max.	475 nm
Lower wavelength cutoff	330 nm
Refractive index at emission max. ^b	2.3
Reflectivity at emission max. ^b	0.25

^aTypical scintillator thickness; thickness can be varied based on experimental requirements.

^bThe refractive index and reflectivity in a medium are dependent on the photon wavelength. Their values are given at the wavelength where the scintillation intensity is the highest.

TABLE II. Parameters of the medium (except the scintillator) used in the simulation.

Parameters ^a	Values
Culture medium density	1.0 g/cm^3
Culture medium thickness	10 μm
Culture medium refractive index	1.35
Culture medium reflectivity	0.1
Glass dish density	2.4 g/cm^3
Glass dish thickness	170 μm
Glass dish diameter	1.664 mm
Glass dish refractive index	1.49
Glass dish reflectivity	0.05

^aExcept culture medium and glass dish, other related structures such as immersion oil and objective lens are not included in the Monte Carlo simulation.

response of the microscope is limited by optical diffraction. However, in the case of radioluminescence imaging, scintillation light is not constrained to the focal plane but is emitted from the entire volume of the scintillator. Due to the high numerical aperture, the microscope has a relatively shallow depth of field (on the order of 10 μm) and light emitted away from the focal plane will be subject to spatial blurring.

This effect is modeled in the simulation by convoluting the 3D scintillation distribution with a depth-dependent PSF. The PSF represents the 2D image recorded by the microscope when a point source is placed at different locations along the main optical axis (Fig. 3). In this work, we chose the Gibson and Lanni model, which accounts for the refractive index mismatch among the scintillator, the glass cover-slip, and the oil immersion layer. The modeled geometry corresponds to the upright geometry (Fig. 1(b)). The upside down geometry (Fig. 1(a)) cannot be easily modeled using this approach. We used a Java implementation of this model written by Hagai Kirshner and Daniel Sage (Biomedical Imaging group at EPFL)²⁴ to generate realistic 3D microscope PSFs. As predicted by the depth of field, significant blurring occurs for photons generated more than 5 μm away from the focal

plane. This effect is especially prominent for photons produced along positron tracks that extends deep within the scintillator. Figs 3(a)–3(c) display y-z cross-sections of different PSFs with the point source located 0, 5, and 10 μm from the front focal plane in object space. For a point source placed at the front focal plane, the PSF is symmetrical about the back focal plane in image space. This symmetry is lost when moving the source away from the front focal plane due to the shift-varying nature of the PSF in the z direction. Figs 3(d)–3(f) show cross-sections of the PSFs at the camera detection plane for different positions of the source (0, 5, and 10 μm) and demonstrate depth-dependent blurring of the light.

In the optical simulation, the scintillator is discretized into 5- μm -thick layers in the depth direction. The PSF is further assumed to be independent of the source location in the x-y plane due to shift-invariance of the PSF in the x-y direction. The 3D scintillation light is projected onto the image plane of the camera, layer by layer, by convolving the planar scintillation intensity with the corresponding depth-dependent PSF. Each layer of the PSF is represented using 100×100 pixels and is normalized to sum to 1. The parameters used in PSF generation are listed in Table III.

The number of photons is reduced when passing through the microscope due to the finite numerical aperture of the microscope and loss during transmission. It is estimated that approximately 16% of photons near the focal plane reach the EMCCD camera, considering the light collection efficiency (25% for $\text{NA} = 1.3$), transmission (80%), and throughput (80%) of the optical train (tube lens and objective).¹⁷ This attenuation is modeled by multiplying the mean photon intensity at EMCCD photodiodes with a uniform factor of 0.16. The magnification of the microscope ($8\times$) is also accounted for in the simulation.

2.D. EMCCD camera model

The EMCCD cameras were developed to reduce read noise found in conventional CCD cameras. In this type of camera, an electron multiplication register multiplies electronic signals before readout noise is added. The signals can be amplified up to 1200 times with the EMCCD camera used in this study (Hamamatsu, ImagEM C9100-14). To accurately model the operation of the EMCCD camera, many different types of noise must be simulated, including photon shot noise, dark current noise, clock induced charge (CIC) noise, excess noise, and readout noise. Modeling the various sources of noise requires performing a sequence of operations, as shown in Fig. 2 (right panel). Photon shot noise is due to the random fluctuation in photon number. For each camera pixel, the number of photons follows a Poisson distribution:²⁵

$$I_{\text{ph},s} \sim \text{Poisson}(I_{\text{ph}}) \quad (1)$$

where I_{ph} is the mean incident photon intensity and $I_{\text{ph},s}$ represents the number of discrete photons arriving at each camera pixel. It follows from this definition that the variance of the photon shot noise is given as $\sigma_s^2 = I_{\text{ph}}$.

FIG. 3. Microscope depth-dependent point-spread function (PSF). (a)–(c) Cross-sections of the PSF in a plane perpendicular to the microscope focal plane (x-y plane) using point sources placed on 5 μm and 10 μm from the focal plane in object space, respectively; (d)–(f) cross-sections of the PSF at the camera detection plane. [Color figure can be viewed at wileyonlinelibrary.com]

TABLE III. Parameters used in the generation of PSF.

Parameters	Values
Voxel size in x and y direction ^a	1.625 μm
Layer thickness (z direction) ^a	5 μm
Voxel number in x and y direction	100
Number of layers ^b	100
Refractive index immersion	1.5
Refractive index sample	2.3
Numerical aperture	1.3
Working distance	200 μm

^ax-y plane is assumed to be parallel to the scintillator; Z direction is perpendicular to x-y plane.

^bThe layer number is variable with the scintillator thickness.

The conversion of the incoming photons into electrons is defined by the quantum efficiency (QE , unit: electron/incident photon) of the photodiodes, which is assumed to be identical for all pixels. The number $I_{e^-,s}$ of electrons created at each pixel can be expressed by

$$I_{e^-,s} = \text{Poisson}(I_{ph} \cdot QE) \quad (2)$$

Dark current refers to electrons thermally generated in the pixel in the absence of light. According to vendor specification, the average dark current D is 0.001 electron/pixel/s (at

working temperature of -70°C). For a frame with the integration length of t_i , the mean dark signal $I_{e^-,D,\text{mean}}$ can be expressed as

$$I_{e^-,D,\text{mean}} = D * t_i \quad (3)$$

Like the photon shot noise, the dark current noise $I_{e^-,D}$ due to the random arrival of the generated electrons is modeled by a Poisson process with mean $I_{e^-,D,\text{mean}}$ and variance $\sigma_d^2 = I_{e^-,D,\text{mean}}$ ^{26,27}

$$I_{e^-,D} \sim \text{Poisson}(I_{e^-,D,\text{mean}}). \quad (4)$$

The CIC noise induced by the charge transfer process is integrated in the EMCCD camera model by a Poisson process. These noise properties are fixed for fixed readout clock and clock duty cycle. The average CIC noise over frames $I_{e^-,CIC,\text{mean}}$ is 0.01 electron/pixel/frame for our camera. Within each camera frame, the CIC noise, $I_{e^-,CIC}$ at each pixel is described by:

$$I_{e^-,CIC} \sim \text{Poisson}(I_{e^-,CIC,\text{mean}}) \quad (5)$$

Compared with conventional CCD cameras, EMCCD cameras incorporate an extra multiplier register, which multiplies electrons through a sequence of gain stages, leading to high overall gain. The mean total electron multiplication (EM) gain G can be expressed by the following formula:^{24,28}

$$G = (1 + g)^N \quad (6)$$

where g indicates the probability of generating an extra electron at each stage, and N is the total number of stages. For each pixel and each multiplication stage, the number of new electrons $I_{e^-, \text{extra}}$ generated at a pixel satisfies a nomial distribution

$$I_{e^-, \text{extra}} \sim \text{Binominal}(I_{e^-}, g) \quad (7)$$

where I_{e^-} is the initial number of electrons entering the multiplication stage. For multiple stages, the total charges $I_{e^-}^{i+1}$ obtained at the $(i+1)^{\text{th}}$ stage can be computed recursively as

$$I_{e^-}^{i+1} = I_{e^-}^i + I_{e^-, \text{extra}}^{i+1}, \quad i = 1, 2, \dots, N - 1 \quad (8)$$

where $I_{e^-}^i$ represents the number of electrons at the output of the i th stage and $I_{e^-, \text{extra}}^{i+1}$ the additional electrons created at the $(i+1)$ th stage. The initial number of electrons entering the multiplier register is given by the sum of the signal and the noise (dark and CIC), that is, $I_{e^-}^0 = I_{e^-, S} + I_{e^-, \text{DC}} + I_{e^-, \text{CIC}}$.

To enhance the signal-to-noise ratio (especially for weak ionization tracks), pixels are binned using 4×4 binning, such that the readout from the camera is a matrix of 256×256 pixels with a pixel size of $6.5 \mu\text{m}$.

Readout noise produced during the electronic charge-voltage conversion affects the signal after electron multiplication and binning. Compared with other noises, which are amplified by the EM gain, readout noise is relatively small. The average relative readout noise of our camera $I_{e^-, r, \text{mean}}/G$ is reported to decrease with EM gain and pixel clock speed, and becomes less than 1 electron/pixel/frame at a gain of $50\times$. At maximum gain, the average relative readout noise can achieve a value that is less than 0.01 electrons/pixel/frame. The readout noise $I_{e^-, r}$ is emulated by a Poisson process with variance $\sigma_r^2 = I_{e^-, r, \text{mean}}$:

$$I_{e^-, r} \sim \text{Poisson}(I_{e^-, r, \text{mean}}) \quad (9)$$

where $I_{e^-, r, \text{mean}}$ is the average readout noise. Electronic charges are subsequently converted into voltages and then digitized, which is modeled by a conversion factor (cf). This factor represents the ratio between pixel electron number and output digital number. For our camera, this ratio is specified as $cf = 5.8$ electrons/count and assumed uniform for all pixels. Based on the photo-sensor model established above, the final digital readout for a pixel can be expressed as:

$$\text{Readout} = \{B[M(I_{e^-, S} + I_{e^-, \text{DC}} + I_{e^-, \text{CIC}})] + I_{e^-, r}\}/cf \quad (10)$$

where $M(\cdot)$ and $B[\cdot]$ indicate operators for the implementation of electron multiplication and binning, respectively.

In this simulation, the photo-response nonuniformity noise (systematic spatial variation in pixel efficiency) and the dark current fixed pattern noise (systematic spatial variation in dark current) are not considered as they are small compared with the noises involved. These effects are caused by pixel manufacturing defects, such as surface defects at the SiO_2/Si interface and randomly distributed discrete charge generation centers.^{29–31} The parameters used in the photon sensor model are given in Table IV.

TABLE IV. Parameters used in the simulation of EMCCD camera.

Parameters	Values
Quantum efficiency for photon conversion into electrons QE	0.8 electron/incident photon
Mean dark current ($I_{e^-, \text{D}, \text{mean}}$)	0.001 electron/pixel/s
Mean CIC noise $I_{e^-, \text{CIC}, \text{mean}}$	0.01 electron/pixel/frame
^a Mean relative readout noise $I_{e^-, r, \text{mean}}$	0.0075 electron/pixel/frame for $G=1200$; 0.025 electron/pixel/frame for $G=600$
Number of multiplication stages N	~ 450
Probability that an electron generates a new electron at each stage g	0.0143
Conversion factor cf	5.8 electrons/count

^aThese values are evaluated using 11 MHz pixel clock speed. The relative readout noises at other gains can be obtained by checking the relative readout noise-gain curve provided by the camera supplier.

2.E. Image reconstruction

The raw output frames from the EMCCD are further processed and reconstructed into an image. For consistency, simulated and experimentally measured tracks are processed using the same reconstruction method, a simplified version of optical reconstruction of beta-ionization track (ORBIT) described in our previous report.¹⁷ A mean dark image, taken by averaging 2000 blank camera frames, is subtracted from each radioluminescence frame prior to reconstruction. Individually ionization tracks are segmented from the radioluminescence images. In order to realize the segmentation, each frame of radioluminescence image is first smoothed by applying a Gaussian low-pass filter to bridge the gaps between fired pixels belonging to the same positron track. Then, a binary threshold is applied to segment individual tracks. All track areas within a frame are segmented. Dim tracks with intensity close to the noise floor or with area smaller than four connected pixels are automatically filtered out by masking during the segmentation. Long tracks or defocused tracks with more than 10^5 total camera counts or 15 fired pixels over the segmented track area are rejected as they degrade spatial resolution. Finally, medium tracks and bright short tracks are retained, and their centroids are taken as the position of the incident positron. Different from the original ORBIT method, the bright-field cell image is not used as prior information for finding the primary position of incident positrons. The simplified version ORBIT requires far less computation time and it is less prone to bias, particularly when the bright-field image is imperfect.

2.F. Computational model validation

To validate the accuracy of the simulation model, we compare the characteristics of raw positron tracks, as measured by radioluminescence microscopy. The area and total intensity of individual positron tracks are measured in simulated and experimental images. Track area is defined as the area of

the segmented ionization track. Total track intensity is defined as the sum of all pixels within the segmentation mask. The statistical distribution of these two metrics over a large set of measured and simulated events is compared. Furthermore, to better characterize positron tracks, the positron penetration depth is plotted versus the emission energy exploiting Monte-Carlo simulation. In each trial, 10^5 positrons are emitted with a single energy (within the ^{18}F positron energy range) from a point source. The event distribution with positron penetration depth and total deposited energy in the scintillator using an ^{18}F positron point source with 10^6 decays is also explored.

We also compare simulated dark noise with measured dark noise (obtained without any radioactive or light source) to validate the noise model of the EMCCD. The statistical properties of the noise are compared over 2000 frames.

2.G. Live cell imaging

A radioluminescence cell image is acquired for comparison with our simulation framework. Human breast cancer cells (MDA-MB-231) are used and cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum. For imaging using our system, the cells are seeded directly onto a fibronectin-coated, glass-bottom, imaging dish and cultured for 48 h. On the day of imaging, the cells are incubated in glucose-free medium for 1 h, then in FDG-containing medium (FDG concentration: 750 $\mu\text{Ci/ml}$) for 1 h 45 min before washing the cells three times to remove unbound FDG. Before imaging, a CdWO_4 scintillator (1 cm $\times$ 1 cm $\times$ 0.5 mm) is gently placed directly on top of the cells to convert the energy released by the positron into light. Bright-field imaging is used to select a suitable field of view and focus the microscope. Radioluminescence images (8000 frames) are then acquired, each with an exposure time of 150 ms, maximum EM gain, and 4×4 pixel binning (consistent with simulation settings). With the selected frame exposure setting, around 20 positron tracks per frame can be captured on average. While this amount is suitable for image reconstruction, generally it is preferable to acquire no more than 10 events per frame to minimize the amount of spatial overlap between individual ionization tracks.¹⁷

The same experiment was simulated using the computational method described previously. In the simulation, the cells were assumed to be flat objects located 5 μm from the scintillator. Their outline was segmented from the acquired bright-field image. The cells were assumed to be uniformly filled with ^{18}F activity. The parameters of the simulation and reconstruction were chosen to be consistent with the experiment.

2.H. System performance evaluation

In radioluminescence microscopy, spatial resolution is determined by the physics of positron propagation, the optical response of the microscope, the noise and resolution of the camera, and the reconstruction procedure. In simulations, we

define the spatial resolution as the full width at half maximum (FWHM) of the image of an ideal ^{18}F point source. The FWHM is obtained by fitting the 2D image to a 2D Gaussian function. Spatial resolution is assessed for images acquired at four different stages of the simulation. (a) A scintillation image is obtained by summing scintillation photons along the z direction to form a 2D intensity image for each frame, and summing these images again. (b) An image of the scintillation light as seen by the microscope is computed by applying the depth-dependent PSF (convolution) to each z -layer prior to summing along the z direction and over all the frames. (c) The EMCCD camera readout image is obtained by applying the EMCCD model to the image obtained in the previous step. (d) A reconstructed image is obtained by running the ORBIT algorithm on the raw camera frames. The ability to measure spatial resolution computationally is unique to a simulation framework, since it is not easily possible to fabricate and position an ideal positron point source. In real experiments, spatial resolution is assessed by the ability to distinguish two cells in close proximity in the image.¹⁸ Spatial resolution was computed for varying source-scintillator distance of 0, 2, 4, 6, 8, and 10 μm .

Similarly, the sensitivity of the system is estimated for different source-scintillator distances and incident positron energies. Sensitivity is defined as the ratio of the number of counts in the reconstructed image to the initial number of radioactive decays from the point source (here, 10^5 decays). A major advantage of computer simulations is that the energy detection limit for positrons can be accurately analyzed as a function of incident positron energy.

3. RESULTS

3.A. Track characteristics

Examples of representative ^{18}F positron tracks of different lengths are shown in Fig. 4. For simulated tracks, the simulation workflow is demonstrated by displaying the scintillation light produced in the scintillator (1), the same light after passing through the microscope (2), and finally the EMCCD camera output (3). Three representative measured tracks are included for qualitative comparison (Fig. 3, 4th row).

In order to evaluate the similarity between measured and simulated track images, the area and total intensity of individual positron tracks are assessed and compared for both images (Fig. 5). The statistical distribution of the track area is summarized for a large set of simulated and measured events in Figs. 5(a) and 5(b) using a violin plot (the width of the "violin" is proportional to the relative frequency of occurrence of a particular y value). In a similar fashion, the distribution of total track intensity per event is shown in Figs. 5(c) and 5(d). The mean area and intensity for all simulated tracks are 77.2 $\mu\text{m}^2/\text{track}$ (8.7% higher than experiment), 13,300 camera counts/track (9.3% lower than experiment), respectively. Differences between the simulated and experimental system are expected, given that many parameters are not precisely known. In this context, the differences found here are

FIG. 4. Three representative simulated tracks produced by incident ^{18}F positrons. Short (a), medium (b), and long tracks (c), are shown at three stages of the simulation: (1) positron transport and scintillation, (2) image formation by the objective and (3) EMCCD signal output. Three comparable tracks, which were experimentally measured, serve as references (bottom row). The positron energy for the three simulated tracks shown in this figure is approximately 100, 250, and 400 keV, respectively.

considered reasonable. Possible sources of discrepancy are detailed in the Discussion section.

To better understand the visible features of ionization tracks, Monte-Carlo simulation data was processed to estimate the penetration depth of positrons in the CdWO_4

scintillator as a function of incident positron energy (Fig. 6). As expected, the penetration depth of positrons in the scintillator increases with incident energy. Figure 6(a) presents the variation in average positron penetration depth as a function of incident positron energy. The average is taken over all

FIG. 5. Comparisons of the frequency distribution of track area and intensity for a large set of simulated and measured positron tracks. The distribution is normalized by the total number of detected events. [Color figure can be viewed at [wileyonlinelibrary.com](#)]

FIG. 6. (a) Average positron penetration depth as a function of positron energy; (b) Frequency distribution showing deposited energy and penetration depth for a set of positrons emitted from an ^{18}F source. [Color figure can be viewed at [wileyonlinelibrary.com](#)]

positrons reaching the scintillator following 10^5 decays. Figure 6(b) shows the distribution of penetration depth and total deposited energy for positrons emitted from an ideal ^{18}F point source located $5\text{ }\mu\text{m}$ from the scintillator. In this configuration, the mean penetration depth and total energy deposition are $24.5\text{ }\mu\text{m}$ and 140.6 keV . The source distance ($0\text{--}10\text{ }\mu\text{m}$ from the scintillator) has minimal influence on the penetration depth and deposited energy through our test.

3.B. Spatial resolution

To better understand the contribution of the various physical processes to the spatial resolution, the response of the

system to an ^{18}F point source was measured at different stages of the simulation (Fig. 7). Together, these results show that spatial resolution as a function of source-scintillator distance is primarily determined by the physics of positron transport in the specimen and scintillator. The microscope objective contributes to further degrade the spatial resolution by $5\text{ }\mu\text{m}$ on average. Noise in the EMCCD camera does not have a significant effect on spatial resolution. Finally, the ORBIT reconstruction algorithm can improve the spatial resolution by an average of $15\text{ }\mu\text{m}$, primarily through the rejection of unsuitable tracks and through centroiding of the ionizing tracks. The highest spatial resolution achievable is $18.5\text{ }\mu\text{m}$, with the source directly in contact with the scintillator. The

FIG. 7. Spatial resolution as a function of the source-scintillator distance. Spatial resolution is shown at various stages through the simulation, namely after scintillation, microscopy, camera recording, and ORBIT reconstruction. Experimentally measured spatial resolution is shown by gray shading. [Color figure can be viewed at [wileyonlinelibrary.com](#)]

spatial resolution of the system is analyzed using linear regression as a function of source-scintillator distances, as shown in Fig. 7, resulting in the following linear relationship between spatial resolution (SR) and source-scintillator distance (D): $SR = 3.5D + (19.3 \mu\text{m})$. This relationship shows that spatial resolution decreases by approximately $3.5 \mu\text{m}$ for each incremental $1 \mu\text{m}$ in source-scintillator distance.

These results are in good agreement with our experimental cell studies. Previously, we have demonstrated $30\text{--}40 \mu\text{m}$ resolution using a 0.5 mm thick CdWO_4 scintillator,²¹ shown in Fig. 7 in gray shading. This level of spatial resolution would correspond to a source distance of $3\text{--}6 \mu\text{m}$. This estimated source-scintillator distance is verified experimentally by measuring the thickness of MDA-MB-231 cells using a holographic 3D microscope (3D Cell Explorer, Nanolive, Switzerland; data not shown). The cell thickness is measured to be $10 \mu\text{m}$ on average and the average emission depth for a whole cell is estimated to be $4.6 \mu\text{m}$. The average emission depth accounts for the fact that positrons emitted closer from the scintillator are more likely to be detected than those

emitted further away from it. Overall, the estimate of the spatial resolution derived from simulations agrees well with our measurements of spatial resolution. This thus confirms that the proposed simulation framework is an appropriate model of the system.

3.C. Sensitivity

The system sensitivity is plotted as a function of the source-scintillator distance [Fig. 8(a)] and incident positron energy (Fig. 8(b)). The average sensitivity is assessed to be $\approx 26.5\%$ under realistic simulation parameters. The sensitivity (S) varies linearly with the source-scintillator distance (D) as shown in Fig. 8(a), and the linear relationship can be expressed by $S = -(0.8\%/\mu\text{m}) \times D + (30.8\%)$. The sensitivity decreases 0.8% for every $1 \mu\text{m}$ increase in source-scintillator distance on average and it is strongly influenced by the energy of the incident positrons. The maximum sensitivity is achieved around 200 keV . Below this value, fewer events deposit enough energy in the scintillator to be detected reliably. Above this value, sensitivity is lost due to the rejection of long tracks in the ORBIT reconstruction procedure. Based on the simulation, it is estimated that the lowest detectable positron energy is in the order of 15 keV .

3.D. Dark noise

To validate the stochastic camera model, simulated and measured dark noise are compared by calculating the mean value and standard deviation of the noise over 2000 frames, with an EM gain of 600. The mean dark signal over the entire frames is found to be 3051.4 and 3051.8 counts/pixel for the simulation and measurement, respectively. This mean signal includes a uniform baseline value set to 3038 counts/pixels to match the experimentally measured dark images. This baseline value is consistent with camera specifications. Furthermore, the mean standard deviation of the dark noise is found to be 39.9 and 39.4 counts/pixel for the simulation and measurement, respectively. These values indicate that the stochastic camera model is a good approximation of the EMCCD, as far as dark noise is concerned.

FIG. 8. (a) System sensitivity as the function of source-scintillator distance; (b) System sensitivity as a function of incident positron energy. [Color figure can be viewed at [wileyonlinelibrary.com](#)]

3.E. Characterization of imaging performance using different beta emitters

While most of this work was conducted using ^{18}F positron sources, our system also enables the visualization of other beta-emitting radionuclides. To evaluate the performance of radioluminescence microscopy for other radionuclides, we simulated ^{11}C , ^{32}P , ^{68}Ga (positron-emitting) and ^{90}Y (electron-emitting) sources. These sources are widely used in the radiation therapy and medical imaging. The emission energy spectra are compared in Fig. 9(a).

By Monte-Carlo simulation of scintillation, we estimated mean penetration depth and mean deposited energy for single positrons over 10^5 radioactive decays, as presented in Figs. 9(b) and 9(c). The particle penetration depth and deposited energy in the scintillator are not obviously influenced by the source-scintillator distance (2 μm shown in light shading; 5 μm in dark shading) but they are significantly different from isotope to isotope. In particular, these two metrics are positively correlated with mean positron emission energy. For instance, ^{90}Y electrons have the highest mean penetration depth and deposited energy, reaching $\approx 120\text{ }\mu\text{m}$ and $\approx 480\text{ keV}$, respectively. This property is due to the fact that electrons emitted from ^{90}Y have on average more energy than the other isotopes considered in this study. The opposite is true for ^{18}F , which has the lowest mean beta energy.

The spatial resolution and sensitivity of the system is also evaluated for different emitters (Figs. 9(d) and 9(e)). ^{18}F positrons provide the highest spatial resolution and sensitivity. Results indicate that spatial resolution and sensitivity is

negatively correlated with the emission energy. This trend is expected given that more energetic particles give rise to longer ionization tracks, which are more difficult to reconstruct accurately. In addition, regardless of the isotope, placing the source closer to the scintillator enhances the spatial resolution and sensitivity.

3.F. Simulation of [^{18}F]FDG imaging in single cells

As a final validation, the acquisition of live cancer cell image using radioluminescence microscopy is simulated. To generate a realistic distribution of [^{18}F]FDG, we created a digital phantom that replicates an experimentally acquired dataset. The simulated and measured reconstructed cell images are both shown in Fig. 10, where the radioluminescence image is merged with the bright-field image to better visualize cells. The amplitudes of both images share the same unit: counts/s/mm² to facilitate a visual comparison.

The two images can be visually compared to each other to provide a qualitative, end-to-end validation of the computational framework. Visually, the simulated and measured radioluminescence images are comparable with respect to spatial resolution, contrast, and noise. In particular, the cluster of cells designated by the blue arrow appears very similar in both images. Generally, isolated single cells present the same level of spatial blur but different intensity level. This discrepancy is of course expected, because the simulation assumes that all the cells have the same activity concentration, whereas in reality each cell contains a different amount of activity. Other cell clusters (yellow and green arrow) had

FIG. 9. (a) Simulated energy spectra for beta particles emitted from ^{11}C , ^{18}F , ^{32}P , ^{68}Ga (positrons) and ^{90}Y (electrons); (b) & (c) Mean penetration depth and mean deposited energy in the scintillator, per event, for different beta isotopes, using a point source placed 2 μm (light shading) or 5 μm (dark shading) from the scintillator; (d) & (e) Spatial resolution and sensitivity obtained by using the same isotopes, after reconstruction. [Color figure can be viewed at wileyonlinelibrary.com]

FIG. 10. Comparison between simulated (a) and measured (b) reconstructed radioluminescence cell images. The radioluminescence information is added to the grayscale bright-field image as a color overlay. Three cell clusters are highlighted by blue, yellow, and green arrows, respectively.

substantial differences when simulated. It is probable that this was due to intrinsic limitations in the definition of the activity distribution: we assumed that [^{18}F]FDG was distributed within the flat outline of the cells, as segmented on bright-field images, but in fact the cell is a 3-dimensional object, and the boundaries of the cell may be misrepresented by the bright-field image.

The signal-to-background ratio, defined as the ratio of the background-corrected average signal to the standard deviation of the background, is evaluated for both simulated and measured radioluminescence cell images. The signal regions are segmented by setting a lower threshold equal to 20% of the maximum pixel value. Similarly, the background region comprises pixels with value less than 10% of the maximum pixel value. Using this definition, the global signal-to-background ratio for the simulated and the measured cell images are 19.3 and 18.5, respectively.

4. DISCUSSION

In this study, we perform a computational simulation of the process of imaging positrons with radioluminescence microscopy to understand the mechanisms behind the process of visualizing FDG uptake in single cells. The physical processes of energy deposition, scintillation, photon propagation through the microscope, and signal capture and readout by the EMCCD are all incorporated into our simulation using different numerical models. This simulation allows us to theoretically estimate the performance and limitations of the system, such as spatial resolution, sensitivity, and noise. Furthermore, we can use this computational framework to optimize the experimental setup and, for instance, determine the optimal source-scintillator distance or the optimal camera parameters. Furthermore, this study provides a framework for improving image reconstruction and opens new possibilities to estimate the origin of measured positrons more accurately.

Overall, our simulation results are in good agreement with the experimental measurements. For instance, the intensity and the area of the measured and simulated tracks are within

10% of each other. The simulated spatial resolution of the microscope is consistent with our experimental findings. Finally, a positron sensitivity of $\sim 27\%$ was found both in simulations and experimental acquisitions. Together, these results indicate that the simulation framework we developed can model the intricate physical processes that occur during radioluminescence microscopy data acquisition.

The simulation methodology is a useful tool to optimize the experimental configuration of the radioluminescence microscopy. For instance, the scintillator thickness was found to affect both spatial resolution and sensitivity (Fig. 11(a), an ^{18}F point source $4\text{ }\mu\text{m}$ from the scintillator is used). Using this model, we found that a thin CdWO_4 scintillator should perform better than a thick one. This could be verified experimentally, provided that such a thin scintillator is robust enough to be used in experiments. Our methodology could also be used to better understand how image quality is impacted by microscope parameters such as numerical aperture, depth of field, and magnification. Based on that, we are hopeful to optimize the microscope to maximize spatial resolution and sensitivity. Additionally, optimal EMCCD camera parameters for binning (2×2 or 4×4 binning) and EM gain can be determined by the methodology. In Fig. 11(b), the spatial resolution and the sensitivity as the function of EM gain are both presented. These data show a trade-off between the spatial resolution and the sensitivity for EM gain larger than 1000. EM gain values of 600–800 are shown to better balance spatial resolution and sensitivity.

There exist some discrepancies between the simulation and the measurement. The image distortion caused by photon propagation through the objective is simulated by a classic analytical model (Gibson–Lanni model), which recapitulates the individual response of the objective and tube lenses in the microscope.^{32,33} Because it is an approximation, the model may contain limitations so that it cannot fully agree with the experimental conditions. Monte-Carlo simulation is a more accurate method for simulating photon transmission through the objective but it is challenging to implement because the exact geometry of the microscope objective is not precisely

FIG. 11. Spatial resolution (circle solid curve) and sensitivity (star dash curve) as the function of scintillator thickness (a) and EM gain (b). [Color figure can be viewed at wileyonlinelibrary.com]

known. Furthermore, the computational requirements would be prohibitive considering the immense number of generated photons. For these reasons, the Gibson–Lanni PSF model was chosen in this study.

The discrepancies between simulated and measured cell images come from the nonuniform FDG uptake of tumor cells in the experiment. Variations in FDG uptake result from biological heterogeneity, such as differential expression of enzymes (e.g., hexokinase II) and/or transporters (e.g., glucose transporter 1).^{34–36} In addition, as a simplification, the simulation models cells as flat objects 5 μm away from the scintillator. In reality, the radiotracer is distributed within a 3D volume and positrons originate from a variety of locations with varying distances from the scintillator, which decreases image resolution.

The iterative electron multiplication model we used in the simulation of EMCCD sensor (section 2.D) is time-consuming, especially when the initial number of electrons and stages is high. A technique is proposed to accelerate this procedure. It has previously been shown that the distribution of charges after N stages of multiplication follows a normal distribution if the number of input electrons is large enough (typically, $N > 30$).^{24,28} The parameters of the normal distribution can be computed as

$$\mu_{EM} = G \cdot I_{e-}^0 \quad (11)$$

$$\sigma_{EM}^2 = F^2 G^2 I_{e-}^0 \quad (12)$$

where I_{e-}^0 is the mean number of charges converted entering the multiplier, μ_{EM} and σ_{EM} are the mean and standard deviation of the normally distributed signal at the output of the multiplier, G is the EM gain, and F denotes the excess noise factor, which is equal to 1.4 for high gain. In the case where the number of input charges is less than 30, a different modeling approach can be applied, which utilizes the Gamma distribution.²⁴ Both techniques are able to enhance the computational efficiency greatly.

The ORBIT reconstruction method has been described in details previously.¹⁷ The original method is capable of retracing long ionization tracks to localize the origin of positron events. The optical bright-field image is further used to identify the correct end of long tracks. However, in view of its complexity and greatly increased time-consumption, we use a simplified version of ORBIT. Here, the bright-field prior image is not used. Long or defocused ionization tracks are also rejected because they contain relatively less spatial information compared to shorter tracks. The removal of dim and long defocused tracks will not bias the final reconstructed image as these types of tracks occur randomly, without any correlation with the parameters of interest. The remaining tracks are processed using a simple centroiding of the scintillation intensity distribution. This simplified version of ORBIT achieves lower sensitivity (due to the loss of the long tracks) but better spatial resolution and 10× faster processing. The simplified ORBIT reconstruction is similar to other processing methods used for digital autoradiography.⁷ The conventional method (all the events are used in the reconstruction and the photon intensity-weighted track centroid is taken as the event position) higher sensitivity since all the events are included but worse spatial resolution due to the inclusion of low-quality events. ORBIT effectively trades-off sensitivity for spatial resolution, which is required for imaging of delicate biological structures. Sufficient events can be captured by extending the number of exposure frames in the experiment, which can compensate for the loss of lower quality events.

In radioluminescence imaging of cancer cells, an exposure time of 20 min is usually chosen. The loss of radioactivity over the duration of the long exposure may cause a “loss” in the number of detected counts (compared to the ideal case where the radioactivity is constant). For ^{18}F and ^{11}C , this loss amounts to a decrease in the number of detected counts of 6.1% and 28.8%, respectively, and represents a theoretical decrease in SNR of 3% and 15%, respectively. Therefore, radionuclides with half-life as short as 20 min can be imaged

with this system with no significant loss of SNR. For shorter lived isotopes, higher labeling radioactivity could be used, but the signal may be difficult to be reconstructed due to the accumulation of too many events in the raw camera frames.

5. CONCLUSION

A computational simulation of positron imaging using radioluminescence microscopy is implemented and validated in this study. Monte Carlo, analytical, and statistical models are established to describe the sequence of physical processes leading to image formation. Results obtained using our simulation methodology agree with experimental measurements taken using radioluminescence microscopy. This *in silico* approach can be used to guide further instrumentation development, and also provides a framework to improve image reconstruction. This will help improve the quality of single-cell imaging and facilitate a deeper understanding of tumor cell biology.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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