Quantitative single-particle digital autoradiography with α -particle emitters for targeted radionuclide therapy using the iQID camera

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(Received 29 December 2014; revised 15 May 2015; accepted for publication 18 May 2015; published 11 June 2015)

Purpose: Alpha-emitting radionuclides exhibit a potential advantage for cancer treatments because they release large amounts of ionizing energy over a few cell diameters (50–80 μm), causing localized, irreparable double-strand DNA breaks that lead to cell death. Radioimmunotherapy (RIT) approaches using monoclonal antibodies labeled with α emitters may thus inactivate targeted cells with minimal radiation damage to surrounding tissues. Tools are needed to visualize and quantify the radioactivity distribution and absorbed doses to targeted and nontargeted cells for accurate dosimetry of all treatment regimens utilizing α particles, including RIT and others (e.g., Ra-223), especially for organs and tumors with heterogeneous radionuclide distributions. The aim of this study was to evaluate and characterize a novel single-particle digital autoradiography imager, the ionizing-radiation quantum imaging detector (iQID) camera, for use in α -RIT experiments.

Methods: The iQID camera is a scintillator-based radiation detection system that images and identifies charged-particle and gamma-ray/x-ray emissions spatially and temporally on an event-by-event basis. It employs CCD-CMOS cameras and high-performance computing hardware for real-time imaging and activity quantification of tissue sections, approaching cellular resolutions. In this work, the authors evaluated its characteristics for α -particle imaging, including measurements of intrinsic detector spatial resolutions and background count rates at various detector configurations and quantification of activity distributions. The technique was assessed for quantitative imaging of astatine-211 (^{211}At) activity distributions in cryosections of murine and canine tissue samples.

Results: The highest spatial resolution was measured at $\sim 20 \mu\text{m}$ full width at half maximum and the α -particle background was measured at a rate as low as $(2.6 \pm 0.5) \times 10^{-4} \text{ cpm/cm}^2$ (40 mm diameter detector area). Simultaneous imaging of multiple tissue sections was performed using a large-area iQID configuration ($\phi 11.5 \text{ cm}$). Estimation of the ^{211}At activity distribution was demonstrated at $\text{mBq}/\mu\text{g}$ -levels.

Conclusions: Single-particle digital autoradiography of α emitters has advantages over traditional film-based autoradiographic techniques that use phosphor screens, in terms of spatial resolution, sensitivity, and activity quantification capability. The system features and characterization results presented in this study show that the iQID is a promising technology for microdosimetry, because it provides necessary information for interpreting alpha-RIT outcomes and for predicting the therapeutic efficacy of cell-targeted approaches using α emitters. © 2015 American Association of Physicists in Medicine. [<http://dx.doi.org/10.1118/1.4921997>]

Key words: alpha imaging, autoradiography, iQID, targeted radionuclide therapy, alpha particles, ^{211}At -radioimmunotherapy

1. INTRODUCTION

Cancer treatments fail when metastatic lesions are not adequately eradicated. Radioimmunoconjugates incorporating α -particle emitting isotopes should theoretically provide a highly effective approach to cell-directed treatment of metastatic lesions in cancer therapy due to their short range in tissue (50–80 μm) and high linear energy transfer (LET).^{1–5} In comparison, the LET of β particles emitted by radionuclides such as iodine-131 (^{131}I) and yttrium-90 (^{90}Y) is two or three orders of magnitude lower than that of α particles.⁶ A key strategy in radioimmunotherapy (RIT) is to selectively irradiate cancer cells while minimizing the radiation dose to healthy cells and organs using radiolabeled tumor-specific antibodies. This approach has been successfully studied in a variety of preclinical models of both solid and hematologic malignancies^{7–10} and translated into promising clinical trials of hematologic and solid tumor malignancies.^{2,11–14} Given the potential of these initial studies, methods are needed to evaluate and assess the effectiveness of targeted therapy for both cancer lesions and metastatic disease. Digital autoradiography and microdosimetry provide tools for evaluating the specific and nonspecific uptake of radioimmunoconjugates in cancer cells and normal tissue¹⁵—information that can be critical for ascertaining the therapeutic index/window and for adjusting experimental treatment regimens.

Autoradiography serves as an important *ex vivo* imaging technique in many applications for quantifying and visualizing the distribution of small amounts of radiotracers within thin tissue sections. The most common biological use of autoradiography is with β particles and low-energy γ rays.¹⁶ In recent years, digital autoradiography of α emitters has become an integral *ex vivo* imaging tool in targeted alpha therapy research, as it provides the uptake and retention data needed for accurate microdosimetry of target and nontarget tissues.¹⁵

We present a novel single-particle digital autoradiography imaging method that leverages the latest advances in CCD and CMOS cameras and computing hardware for real-time imaging of tissue sections approaching cellular resolutions. This digital autoradiography technique is based on the ionizing-radiation quantum imaging detector (iQID), a scintillation camera originally developed for γ -ray imaging applications, such as scintigraphy and single-photon emission computed tomography (SPECT).^{17–23} The detector's response to a broad range of ionizing radiation was recently investigated, including neutrons, spontaneous fission, conversion electrons, and α and β particles with individual quanta imaged and localized, both spatially and temporally, on an event-by-event basis.¹⁸

1.A. Description of imaging system

1.A.1. iQID

The iQID comprises a scintillator in direct contact with a microchannel plate (MCP) image intensifier and a lens for imaging the intensifier screen onto a CCD or CMOS camera. Charged particles or photons (x-ray/ γ ray) absorbed in the

scintillator crystal or a phosphor screen produce a flash of light that is amplified via the image intensifier by a factor of 10^4 – 10^6 and then imaged onto the camera sensor. The scintillation flash is finely sampled with an array of pixels and referred to as an “event cluster.” A cross-sectional view of an iQID camera is shown in Fig. 1(A). In real time, each event is identified and pixel data are used to estimate the location of the α emitter to subpixel precision using a centroid or maximum-likelihood calculation as illustrated in Fig. 2.

The iQID camera used in these studies is shown in Fig. 3(A), incorporating a 4-cm diameter ProxiVision MCP-PROXIFIER™ image intensifier with two microchannel plates in a V-stack assembly. It has fiber-optic input/output windows with a bialkali photocathode and a P43 phosphor screen. The imaging area is readily increased from 4 to 11.5-cm diameter to accommodate imaging studies with large numbers of tissue samples by coupling a fiber-optic taper (Incom, Inc.; magnification ratio 2.875:1) to the intensifier input window [Fig. 1(B)]. Configurations with imaging areas up to 20 cm in diameter are possible. Real-time image acquisition software supports a variety of cameras from Point Grey Research, Inc., that can be tailored to different imaging experiments, in terms of spatial resolution and counting-rate capability. Currently, the highest resolution camera being tested in imaging experiments is a four-megapixel (2048×2048 pixels, pixel size 5.5 μm) Point Grey Research Grasshopper® 3 with a CMOSIS CMV4000 CMOS sensor. A F1.4, 12.5-mm focal length lens (Fujinon CF12.5HA-1, 1 in. sensor format) is used to image the intensifier 40-mm output window on to this camera sensor. The camera connects to a laptop computer via a USB 3.0 interface and acquires full-resolution images at rates up to 90 frames/s. The effective pixel size of the detector is 19.5 μm when imaging with a 4-cm detector area or 61 μm in the 11.5-cm area configuration. Both detector area and effective pixel size can be modified with appropriate selection of camera lens focal length and spacing distance between the lens and intensifier output window.

1.A.2. Acquisition software

The iQID acquisition software uses a LabVIEW™ interface for setting calibration and acquisition parameters such as camera gain, frame rate, regions of interest (ROIs), and energy thresholds. An image viewer is included to visualize activity distributions as they are constructed in real time. Each camera frame is processed using the high-performance computing capability of the laptop computer's NVIDIA graphics processing unit (GPU) via the CUDA (Ref. 31) programming platform. For each event cluster, the estimated position (centroid), time, and pixels associated with the scintillation flash are stored as list-mode data that can be used for further postprocessing, if needed.^{18,32}

1.A.3. Scintillators

Various scintillators are available for single-particle α imaging. One of the brightest scintillators is silver-doped zinc sulfide (ZnS:Ag), which generates $\sim 95\,000$ photons/MeV.²⁴

FIG. 1. Cross-sectional view of the iQID camera (not to scale). (A) iQID imaging configuration for detecting α particles using a ZnS:Ag phosphor screen; (B) example large-area iQID configuration incorporating a fiber-optic taper.

Zinc sulfide is a historically important material that played a significant role in nuclear physics experiments during the early 20th century, including the discovery of the atomic nucleus by Rutherford.³³ The ZnS:Ag used in these experiments is commercially available as a 25 μm thick phosphor layer deposited on a 250 μm thick transparent polyester plastic sheet (Eljen Technology, EJ-440). The EJ-440 phosphor has an area density of $3.25 \pm 0.25 \text{ mg/cm}^2$ (volumetric density of $\sim 1.3 \text{ g/cm}^3$). The projected range for a 5.5 MeV α particle in this material is $\sim 67 \mu\text{m}$; therefore, a thin phosphor layer

($\leq 25 \mu\text{m}$) is used for high-resolution imaging. α particles emitted with a trajectory normal to the phosphor layer will deposit approximately 1.64 MeV in the EJ-440 material, calculated using GEANT4.³⁴ The high light output of ZnS:Ag enables the iQID to operate essentially blind to other forms of background radiation when imaging α particles.¹⁸

In addition to phosphor powder screens, thin single-crystal scintillators and scintillating fiber-optic faceplates³⁵ can be used for α -particle imaging. The properties of several high-density, high-atomic number single-crystal scintillators that

FIG. 2. Example single-particle event detection algorithm for α particles. (A) Raw CCD camera frame where the magnified region shows three α particles; (B) median-filtered image with noisy pixels removed; (C) filtered image with individual particles identified using a connected components labeling algorithm; and (D) the estimated 2D interaction position. The position estimate, time stamp, summed pixel values, and all associated event pixels are concatenated and written to disk as a list-mode event entry. [Reprinted with permission from Miller *et al.*, Nucl. Instrum. Methods Phys. Res., Sect. A 767, 146–152 (2014). Copyright 2014 Elsevier.]

FIG. 3. (A) PNNL iQID camera with a 40-mm diameter image intensifier; the detector area diameter is increased to 115 mm using a fiber-optic taper. [Reprinted with permission from Miller *et al.*, Nucl. Instrum. Methods Phys. Res., Sect. A **767**, 146–152 (2014). Copyright 2014 Elsevier.] This portable iQID system operates using a laptop computer and the configuration can be easily modified for testing various detector components and to accommodate a variety of imaging experiments. (B) Example imaging setup for a canine lymph node and bone marrow biopsy experiment, with the corresponding superimposed α -particle (^{211}At) autoradiograph in (C).

are nonhygroscopic and have a relatively high light yield are listed in Table I. Although these alternatives exhibit a significantly lower light yield compared to ZnS:Ag, they have a much higher stopping power for α particles, with ranges of less than 20 μm , and can be made thin ($\sim 100\ \mu\text{m}$) without a supporting substrate.

1.A.4. Sample preparation for quantitative single-particle digital autoradiography

Candidate α -particle emitting isotopes for cancer treatment include ^{211}At ($t_{1/2} = 7.2\ \text{h}$), ^{225}Ac ($t_{1/2} = 10\ \text{days}$), ^{212}Bi ($t_{1/2} = 60\ \text{min}$), ^{213}Bi ($t_{1/2} = 46\ \text{min}$), ^{233}Ra ($t_{1/2} = 11\ \text{days}$), ^{149}Tb ($t_{1/2} = 4\ \text{h}$), and ^{255}Fm ($t_{1/2} = 20\ \text{h}$).^{6,36} Astatine-211 is of particular interest because of its high LET (5.869 and 7.450 MeV α particles), biologically relevant half-life, and absence of α -emitting daughters. The sample preparation involves cryosectioning of the tissues of interest into slices of approximately 10 μm thickness, to minimize α -particle absorption. Sections are then placed in contact with the scintillation screen, which is laid on the light-sensitive region of the image intensifier or fiber-optic taper, as shown in Fig. 3(B), with superimposed α -particle digital autoradiographs in Fig. 3(C). For α particles, the scintillator provides approximately 50% (2π) detection efficiency, which, in theory, can be increased to nearly 100% if the tissue sections are sandwiched between two pieces of scintillation screen. A light-tight cover shields the intensifier during imaging experiments. Side-by-side comparisons between the radioactivity distribution and histological features can be made after hematoxylin and eosin (H&E) staining of imaged tissue sections or neighboring slices.

In this study, we report initial system characteristic tests of the iQID camera for quantitative digital α -particle autoradiography. We present initial imaging results of ^{211}At -labeled radioimmunoconjugates distributed within tissue cryosections in radioimmunotherapy studies, demonstrating the potential of the iQID camera as an important tool for α -emitter microdosimetry studies.

2. MATERIALS AND METHODS

2.A. Evaluation of imaging system

2.A.1. Spatial resolution—Line spread function (LSF) and modulation transfer function (MTF)

To quantify the intrinsic detector spatial resolution at various imaging configurations, LSFs and corresponding MTFs were obtained using a 5 $\mu\text{m} \times 5\ \text{mm}$ laser-drilled slit (Lenox Laser) in 50- μm thick tungsten foil. A microscope image of the slit is shown in Fig. 4(A). The slit collimator was placed on the scintillator and illuminated with a 4.25-kBq (115-nCi) electroplated ^{239}Pu disc source (5.15 MeV α particles; active area diameter $\sim 5\ \text{mm}$) to create a pseudo-line source. The interaction location of each event was estimated to subpixel precision by fitting a 2D Gaussian function to event clusters using nonlinear least squares methods. Next, finely sampled LSFs were generated at subpixel levels, using a method similar to that described by Fujita *et al.*³⁷ but on an event-by-event basis, by histogramming event locations perpendicular to the slit. A least-squares Gaussian fit to the LSF was then used to estimate the detector spatial resolution in terms of a full width at half maximum (FWHM) and to generate the MTF.

TABLE I. Properties of various scintillators.

Scintillator	Density (g/cm ³)	Projected range ^a (μm)	Luminosity (photons/MeV)
ZnS:Ag (phosphor)	1.3	65.05	95 000 (Ref. 24)
CdWO ₄	7.9	14.48	27 000 ^b (Ref. 25)
GSO (Gd ₂ SiO ₅ :Ce)	6.71	15.31	7 400 ^b (Ref. 26)
LSO (Lu ₂ SiO ₅ :Ce)	7.4	15.44	26 000 ^b (Ref. 26)
LYSO	7.1	15.65	34 000 ^b (Ref. 27)
(Lu _{1.8} Y _{0.2} SiO ₅ :Ce)			
YAG	4.55	16.05	16 700 ^b (Ref. 28)
(Y ₃ Al ₅ O ₁₂ :Ce)			
BGO (Bi ₄ Ge ₃ O ₁₂)	7.13	16.79	8 000 ^b (Ref. 29)

^a5.5 MeV α particle; calculated using SRIM (Ref. 30).
^bGamma-ray luminosity values.

Fig. 4. iQID slit resolution tests. (A) Microscope image of a 5- μm , laser-drilled slit in 50- μm tungsten. (B) iQID slit image acquired at the highest resolution configuration tested ($28.85 \times 28.85 \text{ mm}^2$, 14.08- μm effective pixel size) using a 100 μm thick CdWO_4 and ^{239}Pu α particles (20.8 FWHM). (C) Data from the CdWO_4 slit experiment processed to generate an image analogous to traditional autoradiography where the signal is summed for all cluster events (89.86 FWHM). (D) The same imaging configuration using a ZnS:Ag phosphor screen (EJ-440) (45.5 FWHM). (E) Line spread functions of (B)–(D). (F) Modulation transfer functions of (B)–(D).

Slit images were acquired at multiple detector configurations (with and without the fiber-optic taper) where the effective

pixel size, i.e., the physical area of the scintillator that is imaged by a single pixel, was varied. A smaller effective pixel size corresponds to a reduced detector area and field of view (FOV) for a given camera sensor, with the potential benefit of higher-resolution imaging compared to a large-area FOV configuration. The effective pixel size for spatial resolution tests was varied from approximately 14 to 62 μm (detector areas $28.85 \times 28.85 \text{ mm}^2$ to $\phi 115 \text{ mm}$), and slit images were obtained using both a 100 μm thick CdWO_4 crystal ($5 \times 5 \text{ mm}^2$) and an EJ-440 ZnS:Ag phosphor.

2.A.2. Spatial resolution—Double slit

A double-slit collimator was used to evaluate the image resolution with 25 $\mu\text{m} \times 3 \text{ mm}$ slits, laser drilled in a 100- μm thick tungsten foil with a 50- μm center-to-center spacing between slits. iQID double-slit images were then generated as described for the single-slit experiment. A microscope image of the double slit is shown in Fig. 5(A).

2.A.3. Spatial resolution—Microcapillary array resolution phantom

Another spatial resolution assessment of the system was performed using a 1-mm thick microcapillary array plate (Incom, Inc.), consisting of fused bundles of hollow glass-capillary tubes (100- μm diameter holes) with a rounded hexagonal morphology at a pitch of 125 μm (wall thickness 25 μm). An imaging phantom was constructed by resting the microcapillary array plate on the surface of the CdWO_4 crystal with the electroplated ^{239}Pu α source placed on top of the microcapillary array. A microscope image of the microcapillary array is shown in Fig. 6(A). With an aspect ratio of 10:1 (length-to-hole diameter), the microcapillary array served as a collimator, limiting the maximum lateral range traveled by the α particles incident at the edge of capillary hole/scintillator interface to approximately 1.32 μm for CdWO_4 . The imaging test was performed with an effective detector pixel size of 14.2 μm , and the source was imaged for 140 h due to the low count rate after collimation.

2.A.4. Detection efficiency

To verify the theoretical 100% detection efficiency for α particles (2π geometry), a 14-mm diameter, NIST traceable electroplated disc source (Eckert & Ziegler Analytics) with α particles ranging from 3.70 to 7.95 MeV was imaged using a ZnS:Ag (EJ-440) screen in the large-area iQID configuration for 48 h. The estimated radioactivity was then compared to the reported certified total α -particle radioactivity of $4.32 \pm 0.06 \text{ Bq}$.

2.A.5. Background rate

To determine a lower bound on the minimum detectable activity when imaging α particles, the background rate was estimated for both detector area configurations with ZnS:Ag (EJ-440). The iQID was calibrated for α -particle detection

FIG. 5. iQID double-slit resolution test. (A) Microscope image of a double slit with $\sim 25 \mu\text{m}$ wide slits and $50 \mu\text{m}$ pitch laser drilled in $100 \mu\text{m}$ thick tungsten. [(B) and (C)] Double-slit images using the high-resolution configuration with the (B) CdWO_4 crystal and (C) ZnS:Ag scintillator. (D) Profiles of the double-slit images.

using the ^{239}Pu disc source. The 40-mm configuration was run for 160 h (10-h time windows) and the 115-mm configuration for 60 h (5-h time windows), with shielding covers placed on the phosphor layers to reduce any potential α -particle

background (e.g., radon and decay products). Background α -particle events for the 40-mm configuration were filtered from other background events—e.g., cosmic-ray events interacting in the physical CMOS pixel, intensifier MCP, photocathode, or ZnS scintillator—using a combination of pixel intensity and spatial extent of the light distribution. No background event filtering was performed for the 115-mm configuration, as real events and background events are similar both in spatial extent and pixel amplitude, due to minification and light loss using the fiber-optic taper.

2.A.6. Uniformity

To assess detector uniformity for the 40 and 115-mm configurations, a uniform α source was generated by placing the 5-mm diameter ^{239}Pu disc source on a cylinder at a distance approximately 1.6 cm above the detector and collimated to a 4.6-mm diameter circle. The collimated source was placed at the center and edge locations of the detector in a cross pattern and imaged for 60 min at each location, to obtain approximately 50 000 events, such that the total uncertainty in the number of counts was less than 1%. The uniformity across the detector was then estimated through comparison of the total measured counts for the different positions.

2.B. Imaging applications

2.B.1. Anti-CD20 targeting B-cell lymphoma, large-area iQID imaging

The ability to simultaneously image multiple tissue sections is an important feature of an autoradiography imager, especially for biodistribution studies using short-lived isotopes, like ^{211}At . This was demonstrated using the large-area iQID in a tissue biodistribution study with female athymic nude mice (The Jackson Laboratory; aged 6–7 weeks) bearing subcutaneous human B-cell lymphoma xenografts (10^7 Ramos cells injected in the right flank). B-lymphocytes were targeted using the anti-CD20 monoclonal antibody (mAb) 1F5 labeled with ^{211}At using a *closo*-decaborate(2-) (B10) reagent (^{211}At -B10-1F5) and compared with an ^{211}At -labeled isotype-matched control mAb (anti HSV-1, $\mu\text{IgG}2\text{a}$; ^{211}At -B10-HB8181).³⁸ Twelve animals with tumors of approximately 5-mm diameter

FIG. 6. Resolution imaging experiment using a microcapillary array and a $5 \times 5 \times 0.1 \text{ mm}$ CdWO_4 crystal. (A) Microscope image of a 1 mm thick microcapillary array with $100\text{-}\mu\text{m}$ diameter pores at a pitch of $125 \mu\text{m}$ ($25\text{-}\mu\text{m}$ wall thickness). [(B) and (C)] iQID α image using a 4.25-kBq ^{239}Pu source with an active area of 5-mm diameter that was imaged for 144 h at an effective pixel size of $14.2 \mu\text{m}$.

were assigned to groups of $n = 3$, receiving 1.85 MBq (50 μ Ci) of either $^{211}\text{At-B10-1F5}$ or $^{211}\text{At-B10-HB8181}$. Tumor, liver, and kidney samples were harvested 4 and 20 h after injection (p.i.), and 10 μ m thick sections of each tissue type, one per group, were imaged for approximately 9.4 h (4 h p.i.) and 36 h (20 h p.i.).

The ^{211}At radionuclide distribution in suborgan regions was estimated at the necropsy time point using the spatiotemporal information of each event. Activity estimation was performed by selecting a ROI within the autoradiograph and plotting the event frequency as a function of time. The activity concentration (mBq/ μ g) at the necropsy time point was estimated by least-squares fitting an exponential function to these data.

2.B.2. Anti-CD45 targeting non-Hodgkin lymphoma (NHL)

The iQID imaging approach was assessed in preclinical studies investigating anti-CD45 mAb (CA12.10C12) RIT conditioning to substitute for total body external beam irradiation in hematopoietic cell transplantation (HCT) preparative regimens for NHL.^{39,40} Lymph nodes from a beagle dog were biopsied 2 and 19 h after ^{211}At -anti-CD45 mAb infusion. The subject weighed 8.7 kg and received 0.75 mg/kg of $^{211}\text{At-B10-CA12.10C12}$ (13.9 MBq/kg). The lymph node tissue sections (12 μ m thick) were sandwiched between two sheets of EJ-440 ZnS and imaged for 15 h (2 h p.i.) and 22 h (19 h p.i.). The activity for the entire section was estimated at the corresponding biopsy time point using the technique previously described, and visually “hot” and “cold” regions were selected and their estimated activity concentrations compared to the total mean activity concentration. The radionuclide distribution images were then compared with histological features and morphology of neighboring H&E stained sections.

3. RESULTS

3.A. Evaluation of imaging system

3.A.1. Spatial resolution—LSF and MTF

Spatial resolution results from the 5- μ m slit are shown in Table II. For the highest resolution configuration, slit images, LSF, and MTF plots are shown in Figs. 4(B)–4(F). The highest

spatial resolution measured with this technique was 20.8 μ m FWHM acquired with the 100- μ m thick CdWO₄ crystal and 45.5 μ m FWHM for ZnS:Ag in a 28.85 $\times$ 28.85 mm² FOV (effective pixel size $\sim$ 14 μ m).

3.A.2. Spatial resolution—Double slit

Image results from the double-slit experiment (25- μ m width, 50- μ m pitch) at the highest resolution configuration are shown in Figs. 5(B) and 5(C). A finely sampled, cross-sectional view of the double slit is shown in Fig. 3(D). A double Gaussian fit to the slit profile gave an estimated center-to-center spacing distance (peak-to-peak) between slits of 49.59 μ m for CdWO₄ (FWHM 32.91 slit 1 and FWHM 33.57 slit 2) and 50.96 μ m (47.07 μ m slit 1 and 46.02 μ m slit 2). A combination of a finite slit thickness (increased solid angle) and lateral range of α particles within the scintillator resulted in geometric blur that was estimated to 3.59 μ m for CdWO₄ (effective slit width 32.18 μ m for a 25- μ m slit) and 6.25 μ m for ZnS (effective slit width 37.5 μ m for 25- μ m slit). The maximum peak-valley ratios were 2.34:1 and 1.17:1 for ZnS and CdWO₄, respectively.

3.A.3. Spatial resolution—Microcapillary array resolution phantom

Image results from the microcapillary resolution phantom experiment are shown in Figs. 6(B) and 6(C). The edges of the 5 $\times$ 5 mm CdWO₄ crystal are visualized along with regions of uniform/nonuniform activity in the 5-mm diameter ^{239}Pu electroplated disc source. Note that the 25- μ m thick walls of the microcapillary array are resolved.

3.A.4. Spatial resolution—Event size and substrate thickness

The impact of crystal or substrate thickness on the event cluster size is demonstrated in Fig. 7, with single α events from the 100- μ m thick CdWO₄ crystal (event cluster width of 76 μ m FWHM), 25 μ m thick ZnS:Ag phosphor layer on 250- μ m thick polyester plastic (EJ-440, event cluster width 291 μ m FWHM), and the same phosphor layer but in approximate direct contact with the image intensifier window (77 μ m FWHM).

3.A.5. Detection efficiency

A total of 3.6727×10^5 counts were acquired from the electroplated α source during the 48-h image acquisition. This translates to an estimated total α radioactivity of 4.25 ± 0.01 Bq compared to 4.32 ± 0.06 Bq reported in the certified α source certificate.

3.A.6. Background rate

The mean background rate (α -particles only) using ZnS:Ag for a total imaging time of 160 h with the 40-mm diameter iQID configuration was found to be $(2.6 \pm 0.5) \times 10^{-4}$ cpm/cm²,

TABLE II. iQID spatial resolution tests.

Scintillator ^a	Detector area	Effective pixel size (μ m)	LSF FWHM (μ m) ^b
CdWO ₄	28.85 $\times$ 28.85 mm ²	14.08	20.8
ZnS:Ag	28.85 $\times$ 28.85 mm ²	14.08	45.5
CdWO ₄	$\varnothing$ 40 mm	24.36	32.3
ZnS:Ag	$\varnothing$ 40 mm	24.36	46.6
CdWO ₄	$\varnothing$ 115 mm ^c	61.95	68.9
ZnS:Ag	$\varnothing$ 115 mm ^c	61.95	64.1

^aCdWO₄—100 μ m thick crystal; ZnS:Ag—25 μ m thick phosphor on 250 μ m thick polyester substrate (EJ-440).

^b α -particle interaction locations estimated to the nearest subpixel quadrant.

^c40:115 fiber-optic taper.

Fig. 7. Impact of substrate thickness on event cluster size. (A) ^{239}Pu α -particle event cluster in a $100\text{ }\mu\text{m}$ thick CdWO_4 crystal. (B) ^{211}At α -particle interaction in ZnS:Ag (Eljen Technology, EJ-440) on a $250\text{ }\mu\text{m}$ thick polyester film. (C) An ^{211}At α -particle event from the same ZnS:Ag scintillator, but with the phosphor layer in direct contact with the image intensifier fiber-optic input window. Pixel signals are normalized to the highest value in each event cluster.

corresponding to an α -particle source with an activity of approximately $4\text{ }\mu\text{Bq}/\text{cm}^2$. For the 115-mm diameter iQID configuration (60 h acquisition time), the background rate was estimated to $1.4 \times 10^{-1} \pm 8.5 \times 10^{-4}\text{ cpm}/\text{cm}^2$ (approximately $2\text{ mBq}/\text{cm}^2$).

3.A.7. Uniformity

The mean number of events acquired at the center and edge locations for the 40-mm diameter configuration (effective pixel size $21.3\text{ }\mu\text{m}$) was 4.9725×10^4 counts with a maximum difference of 1.33% between locations and 4.8866×10^4 counts for the 115-mm diameter configuration (effective pixel size $61.1\text{ }\mu\text{m}$) with 1.07% maximum difference between locations.

3.B. Imaging applications

3.B.1. Anti-CD20 targeting B-cell lymphoma, large-area iQID imaging

Figure 8(A) shows images from the anti-CD20 mouse biodistribution study using ^{211}At -B10-1F5 and ^{211}At -B10-HB8181, with an array of tissue sections simultaneously acquired using the large-area iQID configuration. Sections of tumor, kidney, and liver are shown from three treatment groups (4 h p.i.), imaged for approximately 9.4 h. The iQID images reveal higher activity concentration in tumors and kidneys with lower activity concentration in liver samples for ^{211}At -B10-1F5 groups compared to the nonspecific control mAb.

3.B.2. ^{211}At activity estimation

Figure 8(C) shows a magnified image of the α -particle distribution in a kidney section 20 h after ^{211}At -B10-1F5 injection, using a 36-h imaging time. Figures 7(D) and 7(E) show the event frequency and exponential fit to data acquired in selected low- and high-activity ROIs. The activity concentration for the selected high-activity region was estimated to 19.3 mBq ($24.2\text{ mBq}/\mu\text{g}$) 20 h p.i.; the corresponding number was 4.4 mBq ($5.5\text{ mBq}/\mu\text{g}$) for the low-activity region. The half-life for the exponential fit was left as a free parameter whose estimate deviated only by 8.02 min [Fig. 8(D)] and 11.4 min [Fig. 8(E)] from the 7.214-h half-life of ^{211}At .

3.B.3. Anti-CD45 targeting non-Hodgkin lymphoma

Figure 9 shows the activity distribution and the corresponding morphology in a canine lymph node 2 h after ^{211}At -anti-CD45 RIT, displaying a clearly heterogeneous ^{211}At uptake pattern. The estimated total activity in the section at the 2-h biopsy was 8.82 Bq, with a mean activity concentration of $27.71\text{ mBq}/\mu\text{g}$. The activity concentration in selected high-activity ROIs ranged from approximately 300 to $540\text{ mBq}/\mu\text{g}$; the corresponding range in low-activity ROIs was $10\text{--}20\text{ mBq}/\mu\text{g}$. For the 19-h lymph node section (image not shown), the mean activity concentration at the time of biopsy was $11.58\text{ mBq}/\mu\text{g}$ with activity concentrations in high-activity ROIs ranging from approximately 40 to $74\text{ mBq}/\mu\text{g}$ and $3\text{--}5\text{ mBq}/\mu\text{g}$ in low-activity ROIs. The iQID image and its neighboring H&E-stained section jointly demonstrated efficient targeting of ^{211}At -anti-CD45 RIT to lymphocyte-rich regions in the lymph node. Ongoing studies will reveal if this uptake suffices for curative therapy of relapsed NHL in dogs, without incurring dose-limiting toxicities in nontarget tissues.

4. DISCUSSION

A pinnacle feature of the iQID imaging methodology is the ability to localize charged particles, spatially and temporally, on an event-by-event basis. Applied to RIT, iQID digital autoradiography builds upon the pioneering work of Bäck and Jacobsson and the development of the α -camera for high-resolution bioimaging of α particles.¹⁵ Compared to time-integrated digital autoradiography imaging methods, where individual interactions are not discerned, iQID imaging enables improvements both in terms of spatial resolution and activity quantification capability. Single-particle imaging improves the spatial resolution by removing the inherent blur from the scintillation event and estimating the location of each event to subpixel precision. Additional noise sources that otherwise limit or impede accurate activity quantification, especially at low levels, are completely removed by single-particle imaging. These include read noise from the CCD-CMOS camera, variation in the scintillation light production that is inherent to the scintillator material, light scattering within the scintillator, and a variable energy deposition in thin

FIG. 8. ^{211}At anti-CD20 RIT mouse biodistribution study. (A) Tissue sections simultaneously imaged using the large-area iQID camera. The estimated activity for the magnified kidney section (B) was 31.24 Bq (844 pCi) 2 h p.i. The pixel colorbar scales correspond to the number of α -particle events detected during the image acquisition. (C) The ^{211}At distribution in a mouse kidney 20 h p.i. using a 36-h imaging time, with an estimated total activity of 4.81 Bq (130 pCi). [(D) and (E)] Decay plots of high- (D) and low-activity (E) ROIs ($282 \times 282 \mu\text{m}^2$) with estimated activities of 19.3 mBq (24.23 mBq/ μg) and 4.4 mBq (5.52 mBq/ μg).

phosphors that is dependent on the trajectory of the emitted α particle.

4.A. Spatial resolution—Event size and substrate thickness

A notable finding from our resolution tests is the higher spatial resolution obtained with CdWO_4 compared to the EJ-

440 ZnS:Ag phosphor screen. The spatial resolution difference is clearly visible from the maximum peak-to-valley ratios in the double-slit experiment (2.34:1 for CdWO_4 and 1.17:1 for ZnS) with substrate thickness, although relatively thin at $250 \mu\text{m}$, hypothetically causing the observed resolution loss with increased uncertainty in the estimated interaction location.

FIG. 9. Images of a cryosectioned canine lymph node, biopsied 2 h after ^{211}At -anti-CD45 RIT. [(A) and (B)] iQID α images with pixel values normalized to the mean activity concentration at the time of biopsy. The heterogeneous uptake includes activity concentrations up to 19.6 times higher than the mean activity for the whole section. (C) Neighboring H&E-stained section. Comparison of the ^{211}At distribution with the corresponding morphology demonstrated targeting of areas within the T lymphocyte-rich paracortex, with little or no accumulation in lymphoid follicles and medulla.

Accordingly, the spatial resolution could be improved when using the EJ-440 material by placing it in an upside down configuration with the tissue sample in direct contact with the image intensifier fiber-optic entrance window or preferably separated using a thin Mylar film (e.g., $3\text{ }\mu\text{m}$) to avoid surface contamination. As demonstrated in Fig. 7(C), ZnS:Ag EJ-440 in this configuration would reduce the event cluster size, possibly improving the resolution as a result. However, this approach is not ideal since scintillation light must pass through the tissue and could potentially be attenuated or scattered below detection threshold settings. In addition, it precludes the option of increased detection efficiency by sandwiching tissue sections between scintillation screens or using the same tissue section, mounted on a microscope slide, for both α imaging and histological processing. Alternatively, the spatial resolution could be improved while maintaining the benefits of the imaging geometry described in this paper, by depositing ZnS:Ag on a thinner substrate, e.g., $50\text{--}100\text{ }\mu\text{m}$ thick polyester.

5. FUTURE WORK

5.A. Scintillators

The slit imaging experiments provide the intrinsic detector resolution for α particles with a trajectory approximately at normal incidence using collimation. However, the lateral range of α particles in the scintillator becomes an important resolution factor to consider and will be investigated in future work. Measuring the effect of the lateral α -particle range is challenging, given that a noncollimated line source must be constructed that is much smaller than the effective pixel size. To minimize the effect of the lateral range of α particles (see Table I), high-density crystals ($<15\text{ }\mu\text{m}$) are of interest as well as very thin layers ($<10\text{ }\mu\text{m}$) of the low-density ZnS:Ag phosphors. Future detector development will investigate trade-offs between substrate thickness, phosphor layer thickness, and high-density crystals and incorporate results to optimize

the spatial resolution in quantitative digital autoradiography imaging experiments.

5.B. Energy resolution

A current limitation of the iQID detector is its poor energy resolution and inability to distinguish between α particles of different energies. This is likely a combination of gain noise from the image intensifier, read noise from the CCD or CMOS camera, and a lack of full energy deposition for α -particles with trajectories that are approximately at normal incidence to the thin ZnS:Ag scintillators. The impact of these effects and potential improvements in energy resolution will be the subject of future studies.

5.C. Background rate

Filtering α -particle background from other background is challenging when imaging with fiber-optic tapers. In future work, we propose to investigate the use of advanced image analysis techniques to distinguish events for improved background rates in large-area configurations.¹⁷

5.D. Microdosimetry

A primary goal of RIT is to improve the cure rates of malignancies such as myeloid leukemia and lymphoma by eradicating micrometastases and minimal residual disease using targeted α -particle therapy. This requires defining the absorbed doses of α -particle-emitting radioimmunoconjugates and assessing their targeting efficacy and toxicity. Our initial results demonstrate that the iQID camera significantly aids these efforts by providing a means whereby the spatial distribution of radionuclides can be observed in tissue sections, along with estimated activity concentrations quantified at $\text{mBq}/\mu\text{g}$ -levels. For the next phase of development, we will investigate and implement microdosimetric methods for estimating the 3D dose distribution of tissue sections near cellular resolutions.^{41,42} Additionally, we propose to develop image analysis

methods for optimally combining and extracting relevant histological dosimetric data.

6. CONCLUSION

The detector characterization and single-particle digital α autoradiography results presented in this study demonstrate that the iQID camera is a valuable tool for targeted alpha therapy research. The iQID is a portable, laptop-operated system that requires no cooling and leverages the ever-increasing advances in CCD-CMOS technology. Single-particle imaging with subpixel position estimation enables imaging studies to be performed with intrinsic detector resolutions as high as $\sim 20\ \mu\text{m}$, and even higher resolution imaging may be possible if a smaller FOV ($< 28.85 \times 28.85\ \text{mm}^2$) is acceptable, or using higher-resolution cameras, e.g., $> 4\ \text{MP}$. Large-area iQID configurations accommodate studies requiring simultaneous imaging of an array of tissue sections. The high detection efficiency, low background rate, and event-by-event spatiotemporal information allow activity distributions to be quantified using the iQID at mBq-levels in small volumes (e.g., $10 \times 10 \times 10\ \mu\text{m}^3$) even with short-lived α emitters. These features and initial ^{211}At imaging results demonstrate the potential for the iQID camera as an integral tool for assessing and optimizing the use of targeted α -particle therapy regimens for treatment of cancer.

ACKNOWLEDGMENTS

Research reported in this publication was supported by the Laboratory Directed Research and Development Program at Pacific Northwest National Laboratory, a multiprogram national laboratory operated by Battelle for the U.S. Department of Energy; by the National Cancer Institute under Award Nos. R01 CA076287, R01 CA109663, R01 CA136639, R01 CA154897, and R01CA172582; and by a Walker Immunotherapy Research Fellowship from Doug and Maggie Walker (SHLF). B. W. Miller is grateful for the support of a Linus Pauling Distinguished Postdoctoral Fellowship at PNNL. The authors thank the United States Transuranium and Uranium Registries (USTUR) for use of their certified α source. The iQID camera development is in collaboration with the Center for Gamma-Ray Imaging, NIH Grant No. P41EB002035. The authors declare that they have no conflict of interest.

Research involving human participants and/or animals: All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All animal study protocols were approved by the FHCRC Institutional Animal Care and Use Committee (IACUC), and the experiments executed according to principles outlined in the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources).

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