Beta-radioluminescence imaging: A comparative evaluation with Cerenkov luminescence imaging

Martin T. King1, MD, PhD; Colin M. Carpenter1, PhD; Conroy Sun2, PhD; Xiaowei Ma3,4, MD; Quynh-Thu Le1, MD; John Sunwoo5, MD; Zhen Cheng3, PhD; Guillem Pratx1, PhD; Lei Xing1, PhD

1Department of Radiation Oncology, Stanford University, Stanford, CA
2College of Pharmacy, Oregon State University, Portland, OR
3Department of Radiology, Stanford University, Stanford, CA
4Department of Nuclear Medicine, Xijing Hospital, Fourth Military Medical University, Xi’an, Shaanxi China
5Department of Otolaryngology, Stanford University, Stanford, CA

First/corresponding Author (Resident):
Martin King, M.D., Ph.D.
Department of Radiation Oncology
Stanford University School of Medicine
875 Blake Wilbur Drive
Stanford, CA 94305
Phone: 650-725-4782
Fax: 650-725-8231
E-mail: mking1@stanford.edu

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Abstract

Rationale: Cerenkov luminescence imaging (CLI) can provide high resolution images of 18F-fluorodeoxyglucose (FDG)-avid tumors, but requires prolonged acquisition times due to low photon sensitivity. In this study, we propose a new modality, termed beta-radioluminescence imaging (beta-RLI), which incorporates a scintillator with a gamma rejection strategy for imaging beta particles. We perform a comparative evaluation of beta-RLI with CLI in both in vitro and in vivo systems.

Methods: Using in vitro phantoms, we characterized the photon sensitivity and resolution of CLI and beta-RLI. We also conducted a series of in vivo experiments with xenograft mouse models using both amelanotic (A375, UMSCC1-Luc) and melanotic (B16F10-Luc) cell lines. The B16F10 and UMSCC1 cell lines were transfected with the luciferase gene (Luc). CLI images were acquired over 300 seconds, and beta-RLI images were acquired using two 10 second acquisitions. We correlated 18F-FDG activities, as assessed by positron emission tomography, with tumor radiances for both beta-RLI and CLI. We also compared tumor signal-background ratios (SBR) between these modalities for amelanotic and melanotic tumors.

Results: For in vitro experiments, the photon sensitivity for beta-RLI was 560-fold greater than that for CLI. However, the spatial resolution for beta-RLI (4.4 mm) was inferior to that of CLI (1.0 mm). For in vivo experiments, correlations between 18F-FDG activity and tumor radiance were 0.52 (p < 0.01) for beta-RLI, 0.81 (p = 0.01) for amelanotic lesions with CLI, and -0.08 (negative contrast; p = 0.80) for melanotic lesions with CLI. Nine of 13 melanotic lesions had an SBR < 1 for CLI, despite an SBR > 1 among all lesions for beta-RLI.

Conclusion: Beta-RLI can produce functional images of both amelanotic and melanotic tumors in a shorter timeframe than CLI. Further engineering developments are needed to realize the full clinical potential of this modality.

Keywords: Beta, Cerenkov, radioluminescence, melanoma, surgery
Introduction

Cerenkov luminescence imaging (CLI) is an optical radionuclide imaging technique that can produce high resolution (1-2 mm) functional images of $^{18}$F (1–3). Since CLI signals demonstrate a strong linear relationship with positron emission tomography (PET) activity (3), this technology can be used for measuring tumor burden after chemotherapy administration (4,5) and surgical resection (6,7). Many radiotracers including $^{18}$F-fluorodeoxyglucose (FDG) are already Federal Drug Administration-approved. As a result, CLI has immediate translational potential, and studies have already been reported in human patients (8-10).

However, CLI is limited by its relatively low photon sensitivity. Investigators have estimated that a 0.635 MeV positron from $^{18}$F can produce only 20 photons with wavelengths between 250-600 nm (1). As a result, prolonged acquisition times on the order of 3 to 5 minutes in a darkened environment are required (3,7,9). These stringent acquisition conditions may be difficult to satisfy in certain clinical settings, including the operating room.

One potential strategy for improving photon sensitivity is to utilize a scintillator, which can convert beta particles and gamma rays from $^{18}$F into optical light. Scintillators can produce between 1,000 to more than 100,000
photons per MeV depending on the material used (11). Scintillators have been incorporated into handheld radioactive probes and cameras for radio-guided surgical applications (12-16).

In recent years, non-Cerenkov optical radionuclide imaging techniques, termed radioluminescence imaging (RLI), have been evaluated (17). In particular, investigators have performed in vitro and in vivo imaging of the pure gamma emitter, $^{99m}$Tc, by placing bismuth germanate oxide scintillator crystals between the radioactive source and the camera within a commercial small animal imaging system. The system resolution was 1.3 mm, and a collimator was present (18). Non-scintillator-based RLI experiments of $^{99m}$Tc (19) and an alpha emitter (20) have also been reported. In our group, one investigator performed RLI of $^{18}$F-FDG-uptake in single cells, by imaging a radioactive cell monolayer in direct contact with a scintillator using microscopy (21). Another investigator designed a fiber-optic system for imaging $^{18}$F-FDG from ex vivo atherosclerotic plaques that were covered by a scintillator (22).

In this study, we propose a new method for imaging beta particles from $^{18}$F using a scintillator. This method, termed beta-radioluminescence imaging (beta-RLI), incorporates RLI with a gamma rejection strategy adapted from beta probes (12). We hypothesize that beta-RLI may provide
enhanced photon sensitivity compared with CLI. Furthermore, beta-RLI may produce images of intact $^{18}$F-FDG-avid tumors in a shorter timeframe than that required with CLI. The purpose of this study is to perform a comparative evaluation of beta-RLI with CLI in both *in vitro* and *in vivo* settings.

**Methods**

**Materials**

$^{18}$F-FDG was produced from the radiochemistry facility (Stanford, CA). Two melanoma cell lines, the melanotic B16F10 and amelanotic A375 cell lines, were obtained from the America Type Culture Collection. The head and neck UMSCC1 cell line was obtained from the University of Michigan. The B16F10 and UMSCC1 cell lines were transfected with the pGL4.10[luc2] vector from Promega (Madison, WI) using the jetPRIME transfection reagent (Polyplus, New York, NY), in order to create B16F10-Luc and UMSCC1-Luc cell lines.

**Optimal imaging modalities**

All images were acquired using an IVIS system (200 or Spectrum) with medium binning and a 10-13 cm imaging field-of-view. Essential acquisition
parameters for all imaging modalities are listed in Table 1. A schematic for beta-RLI is shown in Fig. 1.

Optical imaging modalities are described as follows: 1) CLI: No material was placed between the camera and the object of interest. 2) RLI: A scintillator was positioned ~1 mm above the object, but below the camera. Optical signals from both beta particles ($\beta$) and gamma rays ($\gamma$) were collected.

Eqn 1: $\text{RLI} = \beta + \gamma$.

3) Block-RLI: A 1 mm-thick stainless steel slab (Evansville Sheet Metal Works, Evansville, IN) was placed between the scintillator and the object. Stainless steel was utilized because of its ability to block almost all beta particles with energies < 1.5 MeV (12). The optical signal captured by the camera can be expressed as:

Eqn 2: $\text{Block-RLI} = \alpha_1\beta + \alpha_2\gamma$,

where $\alpha_1$ and $\alpha_2$ are the percent beta and gamma transmissions through stainless steel. For this study, estimates of $\alpha_1$ and $\alpha_2$ were 0.03 and 0.91, respectively (Supplemental S1). 4) Beta-RLI (with gamma rejection): This image was calculated as the difference between the RLI and block-RLI, which was multiplied by $1/\alpha_2$ in order to eliminate the gamma component.

Eqn 3: $\text{Beta-RLI} = \text{RLI} - 1/\alpha_2*\text{block-RLI} = \beta*(1-\alpha_1/\alpha_2)$. 
All RLI and block-RLI were acquired using a radioisotopic screen (Bruker Biosciences Corporation, Billerica, MA), although other scintillators were also evaluated (Supplemental S2).

**Optical image processing**

Median filtration (3 pixel width) was applied to CLI, RLI, and block-RLI. RLI and block-RLI also underwent additional Gaussian smoothing (sigma = 2 mm) in order to reduce noise. Bias correction, flattening field correction, and cosmic ray correction (median filtration of pixel values more than 10 standard deviations from the image mean) were also applied. All processing was performed using software written in Python 3.2.3 (Python Software Foundation, Beaverton, OR).

**In vitro: Signal uniformity**

The outer shell of the Micro Deluxe Phantom (Data Spectrum Corp. Hillsborough, NC) was filled with 20 mL of 1% agarose (Sigma-Aldrich Chemical Co., St. Louis, MO) in order to create a uniform base. A 30 mL solution of 5.1 MBq (137.5 μCi) $^{18}$F-FDG (254 kBq/mL or 6.9 μCi/mL) was prepared. RLI, block-RLI, beta-RLI, and CLI were obtained. Profiles across images were extracted. An additional experiment evaluating signal
uniformity over a non-uniform background was also conducted (Supplemental S3).

In vitro: Resolution

A 0.5 mm internal diameter capillary tube (VitroCom, Mountain Lakes, New Jersey) was filled with $^{18}$F-FDG (370 kBq/mL or 10 $\mu$Ci/mL). Beta-RLI and CLI were acquired with the scintillator placed directly against the capillary tube. Small binning was used. Profiles were drawn perpendicular to the capillary tube, and full-width half maximum (FWHM) values were extracted.

In vitro: Object-scintillator distance dependencies for beta-RLI

A 0.5 mm diameter capillary tube was filled with 259 kBq/mL (7 $\mu$Ci/mL) $^{18}$F-FDG, and positioned on a stack of ten 1 mm thick acrylic slabs. Serial beta-RLI images were acquired at distances between the capillary tube and scintillator of 1-10 mm. FWHM values and maximum signal ratios (maximal signal at specified distance/maximal signal at 1 mm distance for air) from profiles drawn perpendicular to the capillary tube were acquired for these air distances. In order to evaluate depth dependency, maximum signal ratios were also acquired for a capillary tube placed under 1-3 mm of acrylic.

In vitro: Photon sensitivity analysis
Serial 10-fold dilutions of $^{18}$F-FDG droplets (370 kBq (10 μCi) to 0.037 kBq (0.001 μCi)) dissolved in 50 μL matrigel (BD Biosciences, Franklin Lakes, NJ) were prepared on microscope cover glasses (Fisher Scientific, Pittsburgh, PA) in triplicate. Final concentrations ranged from 7.4 MBq/mL (200 μCi/mL) to 0.74 kBq/mL (0.02 μCi/mL). CLI and beta-RLI were acquired. The average intensities of regions of interests (ROIs) over the droplets were plotted against $^{18}$F-FDG activities. The slopes of the linear regression curves represented the photon sensitivities for each modality. A similar analysis involving serial dilutions of $^{18}$F-FDG-avid B16F10 cells is described in Supplemental S4.

In vivo: Tumor model

All animal studies were conducted in accordance with the institutional Administrative Panel on Laboratory Animal Care. B16F10-Luc, A375, and UMSCC1-Luc cells were cultured in Dulbecco’s Modified Eagle Medium supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin (Invitrogen Life Technologies, Carlsbad, CA) at 37°C. Approximately 1.0E6 cells suspended in phosphate buffered saline were inoculated into the right flank of female athymic nude mice (Charles River Laboratories, Cambridge,
MA) at 6-8 weeks of age. Mice were imaged after 9-10 days for B1F10-Luc and A375 cells, and after 5-6 weeks for UMSCC1-Luc cells.

In vivo: PET imaging

PET images were acquired using a microPET/CT scanner (Inveon, Siemens Medical Solutions USA, Inc, Knoxville, TN). Tumor-bearing animals were injected with 11.1-14.8 MBq (300-400 μCi) $^{18}$F-FDG dissolved in 150 μL PBS via the tail vein. After 30-60 minutes, mice were anesthetized with 2% isoflurane (Aerrane’ Baxter, Deerfield, IL), positioned in the prone position, and imaged. PET images were reconstructed using the ordered subsets expectation maximum algorithm with attenuation correction if CT data were available. Tumor activities were quantified using commercial software.

In vivo: Optical imaging

Acquisition times were 300 seconds for CLI, and 10 seconds for both RLI and block-RLI. For CLI, 1-3 mice were imaged at the same time. For beta-RLI, one mouse was imaged each time. BLI was acquired over 60 seconds at 10 minutes after intraperitoneal injection of D-Luciferin (150 mg/kg).

- Melanotic B16F10-Luc (n = 14): After tumor exposure (skin excision), beta-RLI and CLI were acquired. Given negative tumor contrast with
CLI, BLI was performed and mice were sacrificed. After confirming that photons from BLI did not pass through the scintillator (data not shown), beta-RLI was performed after partial and full resections for 5 and 10 mice, respectively. After full resection, BLI was acquired for evaluating the presence of residual disease. CLI was not performed after partial and full resections due to the presence of D-Luciferin.

- *Amelanotic A375 (n = 5):* Beta-RLI and CLI were acquired after tumor exposure, partial resection, and full resection. Mice were not repositioned between beta-RLI and CLI. Mice were sacrificed after initial imaging of the exposed tumors.

- *Amelanotic UMSCC1-Luc (n = 13):* After tumor exposure, beta and CLI were acquired for 13 and 4 mice, respectively. Mice were then sacrificed.

*In vivo: Tumor signal-to-background ratio*

Tumor ROIs were delineated using white-light and functional (CLI, RLI, beta-RLI, and BLI) images using ITK-SNAP 3.0 software (23). Background ROIs were drawn around the tumor ROI using a circular tool with a diameter of 4-6 mm. However, regions covering intact skin were excluded. Tumor signal-background ratios (SBRs) were then computed.
In vivo: Statistical analysis

The following analyses were conducted:

1. Pearson’s correlation coefficients between $^{18}$F-FDG PET activity (scaled to the time of optical imaging) and tumor radiance after tumor exposure were calculated for beta-RLI, CLI for amelanotic tumors, and CLI for melanotic tumors.

2. Pearson’s correlation coefficients for SBRs from CLI versus beta-RLI were calculated for amelanotic and melanotic tumors. A comparative analysis for SBR values from beta-RLI versus RLI was also conducted (Supplemental S5).

3. One-sided t-tests were conducted in order to determine if tumor SBR declines between successive stages of serial resection were statistically significant. Tests were employed for CLI and beta-RLI of A375 tumors (n = 5), and for beta-RLI of B16F10-Luc tumors (n = 5).

4. Among the grossly resected B16F10-Luc tumors with residual disease, as visualized with BLI, the median SBR and the percentage of cases with an SBR > 1.2 were tabulated.
Results

In vitro experiments

Fig. 2 shows RLI, block-RLI, beta-RLI, and CLI of the cylinder with $^{18}$F-FDG. As exhibited in the profiles, the elevated background in the RLI was suppressed in the beta-RLI. However, the resulting beta-RLI was not nearly as uniform as CLI. In another experiment, beta-RLI was effective in suppressing gamma rays from a non-uniform background (Supplemental S3).

As shown in Fig. 3a, maximum signal ratios degraded as the air distance between the scintillator and the capillary tube increased. Furthermore, marked declines in maximum signal ratios were observed at 1-3 mm depths below the acrylic slabs. With respect to resolution, the FWHM values of profiles perpendicular to the 0.5 mm capillary source were 1.0 mm for CLI and 4.4 mm for beta-RLI when the scintillator was placed against the capillary tube. Fig. 3b shows that the beta-RLI resolution decreased, as the distance between the scintillator and the capillary tube increased. Fig. 3c depicts intensity values for serial FDG dilutions. Photon sensitivity values for beta-RLI and CLI were 391.8 p/s/cm$^2$/sr/Bq (37.3 counts per second (cps)/kBq) and 0.7 p/s/cm$^2$/sr/Bq (0.07 cps/kBq) respectively. Beta-RLI was 560 times more sensitive than CLI based on this experiment. Beta-RLI
also exhibited greater photon sensitivity in imaging B16F10 cells
(Supplemental S4).

In-vivo studies: Tumor exposure

Fig. 4a shows the relationship between pre-resection tumor radiance and $^{18}$F-FDG activity (kBq/mL) for beta-RLI, CLI for amelanotic lesions, and CLI for melanotic lesions. The correlation coefficients between radiance and $^{18}$F-FDG activity were 0.52 ($p < 0.01$) for beta-RLI, 0.81 ($p = 0.01$) for amelanotic lesions with CLI, and -0.08 ($p = 0.80$) for melanotic lesions with CLI. Fig. 4b shows the relationships between SBR values between CLI and beta-RLI. Correlation coefficients were 0.57 ($p = 0.11$) for amelanotic lesions, and 0.55 ($p = 0.55$) for melanotic lesions. For CLI, 9 of 13 melanotic lesions had an SBR < 1, despite an SBR > 1 among all lesions for beta-RLI. SBR values for RLI (without gamma rejection) were significantly lower than those for beta-RLI (Supplemental S5).

Fig. 5 shows optical images of a mouse with a 71.0 kBq (1.92 μCi) B16F10-Luc tumor after tumor exposure. Although, the tumor was clearly visible on beta-RLI, there was negative contrast over the pigmented lesion on CLI. BLI confirms the presence of the tumor. On the other hand, the
amelanotic A375 tumor (31.1 kBq; 0.84 μCi) was clearly visible on the CLI, as shown in the left panel of Fig. 6.

In-vivo studies: Serial resections

Fig. 6 shows CLI and beta-RLI of the A375 tumor after partial and full resections. The tumor signals were less apparent after partial resection and almost indiscernible after full resection for both modalities. On the beta-RLI, the high background activity anterior to the tumor bed corresponds to the right knee. This structure may have been closer to the scintillator than the tumor bed, especially after serial restrictions of the initially exophytic tumor.

Table 2 shows SBRs of B16F10-Luc and A375 tumors after stages of serial resections for CLI and beta-RLI. For CLI, partial and full resections of the A375 tumors demonstrated statistically significant decreases in SBR. However, for beta-RLI, only full resections of the A375 and B16F10-Luc tumors demonstrated statistically significant SBR declines (p < 0.05 based on one-sided t-tests).

In-vivo studies: Residual disease after full resection

All 10 mice that underwent full resections of B16F10-Luc tumors had residual disease based on BLI. The median SBR was 1.0 [minimum 0.8,
maximum 1.6]. Four of 10 tumors (40%) had an SBR > 1.2, and appeared discernible from background in Fig. 7. The other six tumors were not easily discernible.

**Discussion:**

In this study, we evaluated a method for the functional imaging of $^{18}$F called beta-RLI. This method incorporates scintillator-based RLI with a gamma rejection strategy in order to preferentially image beta particles. We then performed a comparative evaluation of beta-RLI with CLI. Using *in vitro* experiments, we showed that beta-RLI has 560-fold greater photon sensitivity, which may allow for shorter acquisition times and less stringent requirements regarding ambient lighting intra-operatively. However, beta-RLI also exhibited poorer spatial resolution (FWHM 4.4 mm versus 1 mm for CLI), limited depth penetration secondary to the positron range of $^{18}$F (2.4 mm in water), as well as declines in both signal intensity and spatial resolution with increasing distance between the object and scintillator.

Using *in vivo* mouse models, we demonstrated statistically significant correlations between $^{18}$F-FDG activity and radiance for amelanotic lesions using beta-RLI and CLI, but not for melanotic lesions using CLI. Furthermore, 9 of 13 melanotic lesions had an SBR < 1 for CLI, despite an SBR > 1 among all
lesions for beta-RLI. These results suggest that Cerenkov photons were absorbed by melanin. However, beta-RLI images often exhibited high background signal after resection, especially for surrounding structures closer to the scintillator (see Fig. 6). Furthermore, CLI may provide a more quantitative assessment of tumor burden, as demonstrated by the statistically significant declines in SBR after both partial and full resections of the A375 tumors. Lastly, beta-RLI is not suitable for imaging microscopic disease, as only 4 of 10 B16F10-Luc tumors with microscopic residual disease had an SBR > 1.2. Although CLI was not evaluated in this manner, its photon sensitivity is likely insufficient for evaluating microscopic disease (Supplemental S4).

The imaging properties for beta-RLI and quite similar to those for beta probes/cameras. With respect to photon sensitivity, a beta prototype utilizing a phoswich detector for electronic gamma rejection exhibited lower photon sensitivity (2.5-14.0 cps/kBq depending on collimation) (14) than this beta-RLI system (37.3 cps/kBq). However, beta prototypes utilizing the gamma subtraction technique that was adapted for beta-RLI demonstrated a greater photon sensitivity (>100 cps/kBq) (12, 13, 16). Beta prototypes have a similar spatial resolution (1.6-5 mm) (13, 15, 16), as well as limited depth penetration (16). Furthermore, beta probes also exhibit decreasing signal
intensity and spatial resolution with increasing object-scintillator distance 
(14, 15).

However, over the past two decades, beta probes have undergone intensive experimental and clinical evaluations for 18F-FDG guided surgery. Experimental studies suggest that beta probes offer superior real-time localization of tumor deposits compared with gamma probes (24,25). In addition, clinical studies for the intraoperative tumor localization of melanomas (13) and other cancers (15,26) have yielded promising results.

Given the parallels between beta-RLI and beta probes/cameras, we would expect 18F-FDG-guided surgery to be the main clinical application for beta-RLI. However, beta-RLI has potential advantages over beta probes/cameras. First, beta-RLI has optical image overlay, which can allow the surgeon to correlate areas of increased activity with anatomic features. Second beta-RLI can provide a large imaging field-of-view for efficient functional mapping of the operative bed.

There are other methods for improving the sensitivity of CLI without scintillators. Down-conversion involves utilizing nanoparticles to shift the wavelength of Cerenkov light for better tissue penetration (27). However, most nanoparticles are exogenous agents that have not been Federal Drug Administration-approved for human use. High energy beta-emitting
radionuclides, such as $^{90}$Y exhibit superior sensitivity compared with $^{18}$F. However, $^{90}$Y requires conjugation to peptides, such as arginine-glycine-aspartate (RGD), for tumor targeting (28).

Future research developments may revolve around scintillator design for improving the acquisition and image quality of RLI. First, a single acquisition beta-RLI technique could be realized by attaching a stainless steel grid onto a scintillating sheet. Since each image contains information from both RLI and block-RLI, interpolation algorithms could be used to recover the missing portions of these images prior to image subtraction for gamma rejection. This may allow for decreased acquisition time and reduced image noise. Furthermore, gamma rejection may be improved, since the scintillator and object often shift between RLI and block-RLI acquisitions. Second, spatial resolution may be improved through the incorporation of collimation (14), but at the expense of photon sensitivity. Third, flexible scintillators (29) may improve sensitivity by maximizing the contact between the tumor and scintillator, and minimizing the effect of distance variations between the tumor and scintillator on signal intensities.
Conclusion

Beta-RLI can produce high contrast functional images of both amelanotic and melanotic tumors in a shorter timeframe than CLI. Beta-RLI limitations include poor spatial resolution, limited depth penetration, high background, as well as the need for acquiring two images. Further engineering developments are needed to realize the full clinical potential of this modality.

Disclosure

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References


**Fig. 1**: Beta-RLI acquisition schematic. A beta-RLI is obtained by obtaining the difference between a RLI (containing both beta particles ($\beta$) and gamma rays ($\gamma$)) and a block-RLI (containing mostly gamma rays). The schematic is not drawn to scale. The scintillator thickness and stainless steel thickness were <1 mm and 1 mm, respectively.
**Fig. 2:** Signal uniformity across a cylindrical phantom filled with 5.1MBq (137.5 μCi) $^{18}$F-FDG. A) Optical images (RLI, block-RLI, beta-RLI, CLI). Intensity values for profile analysis were extracted from the purple lines across the phantom. B) Intensity profiles.
Fig 3: Resolution and photon sensitivity analyses. A) Effect of distance (air and acrylic) between a 0.5 mm diameter capillary tube and scintillator on maximal signal ratio (maximal signal at specified distance/maximal signal at 1 mm distance for air). B) Effect of air distance on full-width half maximum (FWHM) resolution. C) Radiance values of beta-RLI and CLI for serial $^{18}$F-FDG dilutions.
Fig. 4. A) Radiance versus $^{18}$F-FDG activity (kBq/mL) for beta-RLI among all tumors, CLI for amelanotic (A375 and UMSCC1-Luc) tumors, and CLI for melanotic (B16F10-Luc) tumors. Radiance has units (p/s/cm$^2$/sr). B) Signal-background ratios between CLI and RLI for amelanotic and melanotic tumors.
**Fig 5.** Beta-RLI, CLI, and BLI of a melanotic B16F10-Luc tumor with 71.0 kBq (1.92 μCi) $^{18}$F-FDG by PET after tumor exposure. For beta-RLI and CLI, red contours encircle the tumor. White contours encircle the background. SBRs are 3.6 for beta-RLI and 0.7 for the CLI.
Fig. 6. Beta-RLI and CLI of an amelanotic A375 tumor with 31.1 kBq (0.84 μCi) $^{18}$F-FDG by PET after serial resections. Serial resections included tumor exposure, partial resection, and full resection. For beta-RLI and CLI, red contours encircle the tumor. White contours encircle the background. White arrows point to high signal from the mouse knee, which may have been closer to the scintillator than the tumor bed.
**Fig 7.** BLI and beta-RLI after full tumor resections for 5 mice with B16F10-Luc tumors. For beta-RLI, red contours encircle the tumor. White contours encircle the background. Each beta image is scaled between the minimum and maximum values within the red contour in order to accentuate signal contrast within the tumor bed. As such, background may appear saturated. High signal over the tail region may be secondary to FDG accumulation from tail vein injection and/or urine. The image on the very right corresponds to the mouse in Fig. 5.
Table 1: Components required for acquiring Cerenkov luminescence images (CLI), radioluminescence images (RLI), and block-RLI. Scintillator and steel plates were placed directly above the object. Beta-RLIs were computed by subtracting block-RLI from RLI.
### Table 2:

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* denotes statistically significant declines in tumor SBR after partial and full resections, based on a one-sided t-test (p < 0.05).
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