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Practical Guidelines for Cerenkov Luminescence Imaging with Clinically Relevant Isotopes

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Abstract

Cerenkov luminescence imaging (CLI) is a relatively new imaging modality that utilizes conventional optical imaging instrumentation to detect Cerenkov radiation derived from standard and often clinically approved radiotracers. Its research versatility, low cost, and ease of use have increased its popularity within the molecular imaging community and at institutions that are interested in conducting radiotracer-based molecular imaging research, but that lack the necessary resources and infrastructure. Here, we provide a description of the materials and procedures necessary to conduct a Cerenkov luminescence imaging experiment using a variety of imaging instrumentation, radionuclides, and animal models.

Keywords

Cerenkov luminescence imaging; Isotope; Radiation; Radiotherapy; Antibody; Nanoparticle; Peptide; Reporter gene expression; Optical imaging

1 Introduction

Molecular imaging is defined as the noninvasive, visualization, characterization, and measurement of biological processes at the molecular and cellular levels in living organisms using 2-or 3-dimensional imaging techniques such as positron emission tomography, single photon computed tomography, magnetic resonance imaging, optical imaging, and ultrasound [1]. This dynamic scientific discipline has had a significant impact on health care since it enhances precision medicine strategies through improving patient diagnosis, risk stratification, and monitoring therapy response [2]. Preclinically, molecular imaging has enabled scientists to expand their knowledge of cellular function in health and disease; rapidly identify novel biomarkers for diagnosis and prognosis, and accelerate drug discovery and therapeutic development while reducing research costs in academia and industry [3].

Cerenkov luminescence imaging (CLI) is a new imaging modality that continues to gain popularity within the molecular imaging community since it utilizes conventional optical imaging instrumentation to detect Cerenkov radiation [4–9]. Cerenkov radiation, first described in the early twentieth century [10, 11], is electromagnetic radiation in the ultra-violet and blue portion of the visible spectrum that is emitted when charged particles, such as positrons (β^+) or beta minus (β^-) particles, travel beyond the speed of light in a dielectric

medium [7, 12]. For example, as a β^- particle travels with superluminal velocity through water, it creates a non-homogeneous electromagnetic field among the water molecules within the medium. As homogeneity to the electromagnetic field is restored, Cerenkov radiation is emitted and can be detected with charge-coupled devices designed to detect bioluminescent and fluorescent light. Figure 1 illustrates this principle and its application to CLI. Since the discovery of Cerenkov radiation, additional publications have described the physics behind this phenomenon and its potential applications to molecular imaging research [12–16]. Moreover, since CLI was first reported by Robertson and coworkers [17], scientists have expanded its applications to include clinical CLI [18–24]; CLI-monitored gene expression [25–28]; and the CLI-based, high-throughput screening of imaging agents [29–33], nanoparticles [34–40] and radiotherapeutics [41–44],

The adaptability of CLI to a variety of research questions will only serve to increase its popularity among molecular imaging scientists. However, considering this imaging modality's relatively low cost and ease of use, it has the potential to dramatically impact the investigations of those who are not molecular imaging scientists, but wish to utilize molecular imaging techniques to answer fundamental questions related to their scientific research. This document has been prepared with these scientists as the primary audience, and provides a description of the materials and procedures necessary to conduct a Cerenkov luminescence imaging experiment.

2 Materials

Prepare all solutions using ultrapure water (18 M Ω -cm resistivity (25 °C)) and analytical grade reagents. Prepare and store all reagents at room temperature (unless indicated otherwise).

1. An appropriate radiotracer to probe the biological phenomenon of interest (*see* Note 1). Use appropriate shielding for storage.
2. An appropriate mouse model (*see* Note 2).
3. An optical imaging system with applicable software and computer interface (*see* Note 3).
4. A rodent anesthesia system (*see* Note 4).
5. Isoflurane (USP) or equivalent inhalant anesthetic and non-reflective black paper (Edmunds Optics; 54–571).
6. Non-reflective black tape (ThorLabs; T743–2.0).
7. Geiger Counter (Ludlum Measurements, Inc.; Model 3 with 44–9 GM pancake detector).
8. Dissection tools and pipettes.
9. Pipette tips.
10. Microiuge tubes.

11. Absorbent blue pads and spare mouse cage(s) with adsorbent bedding, food and water (*see* Note 5).
12. 0.5 mL insulin syringe with fixed 29-gauge needle for animal injection.
13. 70% alcohol in a spray bottle and alcohol wipes.
14. Gamma counter such as the Wizard® gamma counter (Perkin Elmer; 2470–0020) or scintillation counter such as the TRI-CARB 4810TR (Perkin Elmer; A481000) (*see* Note 6).
15. Radioactive dose calibrator (*see* Note 7).
16. Saline or phosphate buffered saline (PBS).
17. Radioactive decontaminant.

3 Methods

Use Caution! Radioactive materials (RAM) emit ionizing radiation and are extremely dangerous. They are colorless, odorless, tasteless, and are detected only with an appropriate survey meter. Before beginning any experiments, seek approval from your institution's Radiation Safety committee, Environmental Health and Safety committee, Animal Resources Program and Institutional Animal Care and Use committee. Observe safe work practices and ALARA (**A**s **L**ow **A**s **R**easonably **A**chievable) rules associated with RAM. Use appropriate personal protective equipment such as lab coats; gloves; safety goggles; Teflon® sleeve and shoe covers; dosimetry badges and environmental controls such as lead and Plexiglas® materials to minimize radiation exposure. It is advisable to never work alone with RAM, and to regularly use a survey meter to determine hand, foot, or body contamination. Always use the survey meter to assess work areas before and after experiments. Follow your institution's guidelines for handling RAM contamination. Dispose of RAM and contaminated materials using protocols established by your institution's Radiation Safety committee.

3.1 Evaluating a CLI Phantom

1. Pipette 200 (7400), 100 (3700), 50 (1850), 25 (925), 12 (444), 6 (222) and 0 (0) μCi (kBq) of radioactivity into seven separate clear Eppendorf tubes. Adjust the volume in each tube to 200 μL with saline and seal.
2. Confirm the total radioactivity in each microfuge tube using a dose calibrator that has been configured to detect the radionuclide of interest.
3. Place the tubes on non-reflective black paper in the optical imaging system chamber. They may be fixed to the paper with non-reflective black tape if necessary (*see* Note 8).
4. Image the tubes (Initial settings: f-stop: 2; binning: 1, FOV: B; background correction applied; 1 min acquisition time; bioluminescent mode). These settings are listed as a reference.

5. Using the optical imaging system software, draw regions-of-interest (ROI) around the areas of detected luminescence and calculate the radiance within the ROI.
6. Using productivity software, plot the average radiance on the ordinate (Y) axis and the activity in each tube on the abscissa (X) axis. Perform linear regression analysis to determine the correlation coefficient between the observed radiance and dispensed activity (*see* Note 9).

3.2 Cerenkov Luminescence Imaging In Vivo

1. Prepare the radiotracer in a suitable injection buffer such as medical saline or PBS (*see* Note 10).
2. Anesthetize the animal to be imaged. Supply heat to the animal during the injection process. This can be accomplished using a heating pad underneath an absorbent blue pad.
3. Inject each animal with the formulated radiotracer (*see* Note 11).
4. After injection, return the animal to its cage and allow the animal to recover from anesthesia. The animal should be able to ambulate efficiently and not be in distress.
5. After the prescribed biodistribution period, anesthetize the animal as in Subheading 3.2, **step 2** (*see* Note 12).
6. Place the anesthetized animal on non-reflective black paper in the imaging chamber and the animal's nose in the anesthesia nose cone of the imaging system.
7. Take a photograph of the animal in the imaging chamber to verify the animal is positioned within the instrument's field of view (FOV).
8. Image the animal in bioluminescence or fluorescence mode to detect the Cerenkov radiation emanating from the animal (*see* Notes 13, 14, and 15). If using fluorescent mode, the excitation lamp must be off and short wavelength filter sets removed.
9. After in vivo imaging is completed, the animal may be returned to its cage for further imaging in the future (*see* Note 16).
10. Using the optical imaging system software, draw ROIs around the areas of detected luminescence and calculate the radiance associated with the ROIs (*see* Notes 17 and 18).

4. Notes

1. Consult your Radiology and Nuclear Medicine Departments for the availability of CLI-applicable radiotracers. Vendors such as PETNET Solutions (<https://usa.healthcare.siemens.com/molecular-imaging/petnet-solution>), Cardinal Health (<http://www.cardinalhealth.com/en/product-solutions/pharmaceutical-products/>

nuclear-medicine/radiopharmaceuticals.html), PerkinElmer (<http://www.perkinelmer.com/category/radiochemicals>), or Nordion (<http://www.nordion.com/>) sell radiotracers that can be used in CLI experiments.

2. In general, due to the absorbance of Cerenkov radiation by deep tissue, subcutaneous tumors are visualized more easily than orthotopic tumors within the body cavity. Also, the presence of fur will cause optical scattering and reduce the observed Cerenkov luminescence signal detected by the instrument. If necessary, depilate the animal before radiotracer injection. Use clippers to remove bulk fur followed by a depilatory cream to remove any fine fur from the animal. Consult your institution's veterinary staff for an appropriate protocol.
3. Consult the optical imaging instrument manufacturer to determine if your particular instrument is configured to detect Cerenkov radiation.
4. An inhalant anesthesia system is preferred for anesthetizing the rodent during radiotracer injection. Typically, the optical imaging system will be configured with an anesthesia machine that delivers inhalant anesthesia in a stream of medical oxygen to the rodent while in the imaging chamber. Inhalant anesthesia is preferred over the use of injected anesthetics since delivery of the former is more consistent. It reduces the possibility of animal movement during the imaging session.
5. These should be available from the institution's animal resources program. Transferring the animals to new cages at any point after radiotracer injection reduces the animal's exposure to radioactive materials in the bedding. This also minimizes body surface contamination of the animal, which may increase the background luminescence signal during the imaging session.
6. Gamma counters and liquid scintillation counters detect radiation in different ways. Gamma counters measure the gamma ray photons emitted by a decaying radionuclide. This is a "dry" technique. If using a gamma counter, ensure that the energy window is configured to detect the gamma photon(s) of interest. Liquid scintillation counting is a "wet" technique used primarily to detect alpha or beta particles emitted from a decaying radionuclide. Liquid scintillation counters measure the radioactivity in a sample by detecting luminescence, which is generated when charged particles interact with a liquid scintillant that has been mixed with the radioactive material. If using a liquid scintillation counter, scintillation fluid, and scintillation vials with cap will be required.
7. Be sure your instrument is configured to detect the radionuclide of interest.
8. The tubes should be arranged in the center of the instrument's FOV. This can be accomplished by laying the sealed tubes on their sides in a circular pattern on non-reflective black paper, and taking a photograph of the tubes in the imaging chamber to verify their position.
9. The better the agreement between the calculated value and the theoretical value of 1.00, the more meaningful the correlation between the dispensed activity and the Cerenkov radiation detected by the instrument.

10. The injection volume should not exceed 250 μ L, or as defined by your institution's IACUC.
11. Tail vein injection is preferred since it delivers the radiotracer directly into the circulatory system.
12. The imaging of animals injected with small molecule radiotracers can begin immediately. The imaging of animals injected with large molecular weight radiotracers (i.e., a radiolabeled antibody or nanoparticle) should be delayed for 12–24 h to allow for the accumulation of the radiotracer at the tissue of interest and systemic clearance.
13. For radiotracers that emit prompt Cerenkov radiation, imaging can occur immediately. When Cerenkov radiation is produced by the daughter decay products of alpha particle emitting radionuclides, secular equilibrium must be achieved before imaging. For ^{225}Ac this period is 10 h, and reference 13 lists equilibrium periods for additional alpha particle emitting radionuclides.
14. During imaging, keep the animal under anesthesia. While the number of animals imaged at any one time will be dependent upon the injected radiotracer and instrument settings, it is recommended that not more than three animals be imaged at the same time.
15. Table 1 lists several CLI applications. While the instrumentation settings are provided, they should be utilized as an initial reference only. Final imaging parameters should be determined empirically and optimized beforehand by imaging one mouse that has been injected with the radiotracer. Once the imaging parameters have been optimized, all other mice can be injected with the radiotracer and imaged using the finalized imaging parameters. This practice reduces radiotracer waste, and utilizes time more efficiently when short-lived radiotracers are being used in CLI applications.
16. After the final imaging time point, additional data can be obtained through ex vivo imaging of the animal's organs and tissues. This is accomplished by euthanizing the animal, removing organs and tissues of interest, and imaging them on non-reflective black paper in the imaging chamber with the parameters established for the in vivo imaging experiments.
17. Background correction can be performed through (a) use of dark images acquired at the equivalent instrument settings immediately before experimental image collection, or (b) ROI analysis of a region in the same experimental image but remote from the area of interest.
18. Radiotracer in vivo specificity, retention, and clearance can be determined from comparing the average radiance observed in each tissue or by generating target tissue-to-non-target tissue ratios ($T/NT = \text{average radiance of target tissue} / \text{average radiance of non-target tissue}$).

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Fig. 1.

Illustration depicting the generation of Cerenkov radiation. **(A)** A tumor (**T**) bearing mouse injected with a radiotracer that emits charged (β^-) particles, which travel with subluminal velocities ($v < c/n$). These particles are unable to distort the electromagnetic field among the surrounding water molecules and Cerenkov radiation is not generated at the site of radiotracer localization. **(B)** A tumor (**T**) bearing mouse injected with a radiotracer that emits charged (β^-) particles, which travel with superluminal velocities ($v > c/n$). These particles distort the electromagnetic field among the surrounding water molecules. As this distortion in the electromagnetic field is removed, Cerenkov radiation is produced and is detected by the optical imaging instrumentation. The red color in B is a software-generated pseudo-color, which denotes radiotracer accumulation and detection of Cerenkov radiation.

Light gray circles represent bulk water molecules. Dark gray ellipsoids represent water molecules interacting with the charged particles. Arrows represent the paths of the particles

Table 1
Summarized imaging parameters of selected applications involving Cerenkov luminescence imaging

Entry	Radiotracer	Activity injected (MBq/ μ Ci)	Application	Data collection parameters and notes	Biological model	Reference
1	[¹⁸ F]F-FDG	7.3/270	In vivo tumor visualization	IVIS 100/200 bioluminescence mode, 1 min data acquisition, 1 h post-injection (p.i.)	CWR22-RV-1 human prostate xenograft	[17]
2	[¹⁸ F]F-FHBG	8.1/300	Reporter gene expression/tumor visualization	IVIS 200 bioluminescence mode, 1 h and 2 h p.i. data acquisition	C6 rat glioma xenograft transfected with HSV1-tk reporter gene system	[26]
3	[¹²⁴ I]NaI	11/297	Reporter gene expression in human mesenchymal stem cells (MSCs)	IVIS 100 bioluminescence mode, 1 min data acquisition, 0–8 days p.i.	Athymic nude mice injected with Fluc-hNIS transfected MSCs	[27]
4	[⁹⁰ Y]Y-DOTA-30F11	11/300	In vivo visualization of systemic radiotherapy delivery	IVIS Spectrum fluorescence mode (lamp off), 1.5 min acquisition time (0–3 d p.i.), f-stop: 1; binning: 4; FOV: B; cosmic ray normalization, flat field correction and background correction applied	Athymic nude mice bearing murine myeloid SJL leukemia cells, SJLB6F1/J mice, SJL/J mice	[41]
5	[¹⁷⁷ Lu]Lu-DOTA-30F11	11/300	In vivo visualization of systemic radiotherapy delivery	IVIS Spectrum fluorescence mode (lamp off), 1.5 min acquisition time (0–3 d p.i.), f-stop: 1; binning: 4; FOV: B; cosmic ray normalization, flat field correction and background correction applied	Athymic nude mice bearing murine myeloid SJL leukemia cells, SJLB6F1/J mice, SJL/J mice	[41]
6	[²²⁵ Ac]Ac-DOTA-c(RGDyK)	1.9/50	In vivo visualization of systemic radiotherapy delivery	IVIS 100, fluorescence mode (lamp off), 5 min acquisition time (24 h p.i.), f-stop: 2; binning: 1; FOV: B; background correction applied	Athymic nude mice bearing human U87 mg xenografts	[43]
7	[⁸⁹ Zr]Zr-DFO-J591 mAb	11/300	In vivo tumor visualization	IVIS 200, bioluminescence mode 1 min acquisition time (24–96 h p.i.), f-stop: 1; binning: 1; FOV: B; background correction applied	Athymic nude mice bearing human LNCAP xenografts	[9]
8	[⁶⁴ Cu]Cu-OTA-anti-GD2 mAb	11/300	In vivo tumor visualization	I VIS Spectrum 1 min acquisition (0–48 h p.i.), f-stop: 1; binning 16; FOV: 12.5 cm; cosmic ray, flat field and background corrections applied.	SHO mice bearing GD2 ⁺ neuroblastoma xenografts	[45]
9	[¹⁸ F]F-FDG	15/400	Nanoparticle mediated biomarker imaging	IVIS200 0.5 min acquisition	Athymic nude mice bearing SCC7 or BT20 xenografts	[16]