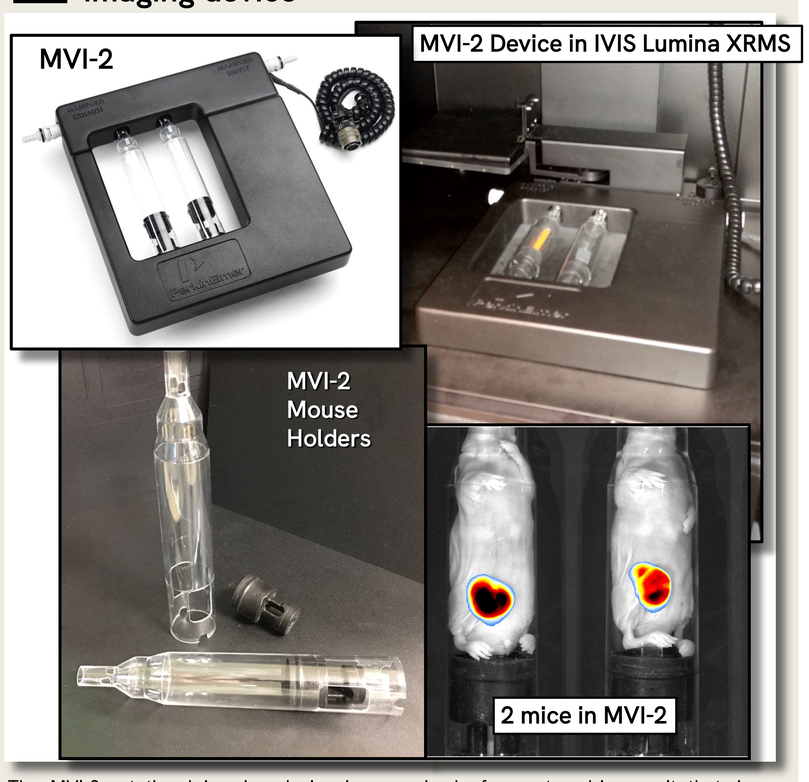
Novel optical 2D image acquisition and analysis of tumor metastasis using a two-mouse, 360-degree positioning device

Rich MacKendrick, Craig McMannus, Kristine Vasquez, Lee Tambeau, Josh Kempner, Pete Harvey, and Jeffrey D. Peterson. Life Sciences and Technology, **Revvity, Inc.**

Abstract

Preclinical 2D optical imaging by bioluminescence (BLI) offers a quick and robust approach for assessing biological changes associated with a variety of disease states, including cancer, inflammation, infection, and toxicology. The procedure is simple when focused on a single disease site; however more complex disease models may display biological changes at multiple regions of interest, requiring imaging of all sides of the experimental animals. We have developed a computer-controlled, two-mouse, 360-degree rotational system (MVI-2) that allows complete 2D surface imaging of two mice for the identification, localization, and quantification of whole body BLI. The device utilizes two mouse restraining tubes that lock into two parallel rotational anesthetic-delivery slots driven by a motor that rotates the tubes through user-defined position/time intervals for acquisition of optical and X-ray data on the IVIS™ Lumina XRMS. To assess this novel imaging device female nu/nu mice were implanted intra-splenically with $5x10^5$ HCT-116 Red-FLuc colorectal cancer cells, generating a metastasis model in which Luciferase expressing cells migrate from the spleen throughout the peritoneum starting at day 10 post-surgical implant. Mice were screened for BLI Total Flux (Photons/sec) and sorted into 2 cohorts of n= 6 mice per group. Mice were dosed IP with 5-fluorouracil (5-FU; 50 mg/kg/day) treatment on days 9 and 10, and a second cohort received only vehicle (5mL/kg 10% DMSO). All animals were imaged for BLI signal on days 0, 5, 7, 11, and 14 on the 2-mouse rotational system for 360-degree surface BLI signal (16 rotations every 22.5 degrees). All analyses were performed using Region of Interests (ROIs) placed centrally in each image position, generating quantitative line profiles for which AUC (area under the curve) values were calculated. The results showed that as little as two doses of 5-FU dramatically affected primary tumor burden by 80%, with regional AUC analysis revealing 95% inhibition of metastasis. In summary this system is an advance over previous rotational systems, both in the ability to image 2 mice simultaneously and in the novel analysis approaches and full-surface image representations. Analysis is not just used to identify optimal positions for conventional analysis, rather positional AUC analysis is used to provide quantification that offers additional sophistication and graphical representation. These results suggest that this novel approach is useful for assessment of tumor metastasis models of cancer as well as enhanced disease state quantification when paired with a 2D optical bioluminescence imaging system.

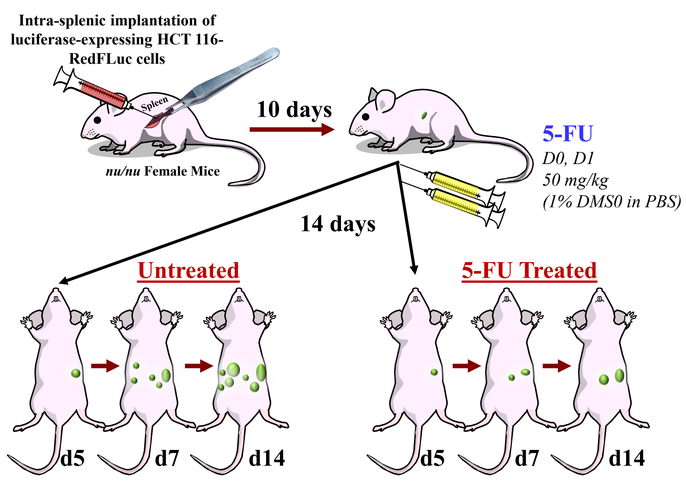
1 MVI-2: a two-mouse, 360-degree surface FLI/BLI imaging device



The MVI-2 rotational imaging device is comprised of a motor driven unit that, in a computer-controlled fashion, can simultaneously rotate two removable, transparent mouse holding tubes. Mice receive anesthesia within the holders as they rotate through a series of stepped positions, from as few as 4 positions to as many as 36 positions for image acquisition. This device is designed for fluorescent or bioluminescent 2D imaging (with or without X-ray) on the IVIS Lumina series of instruments and is controlled within the Living Image™ software.

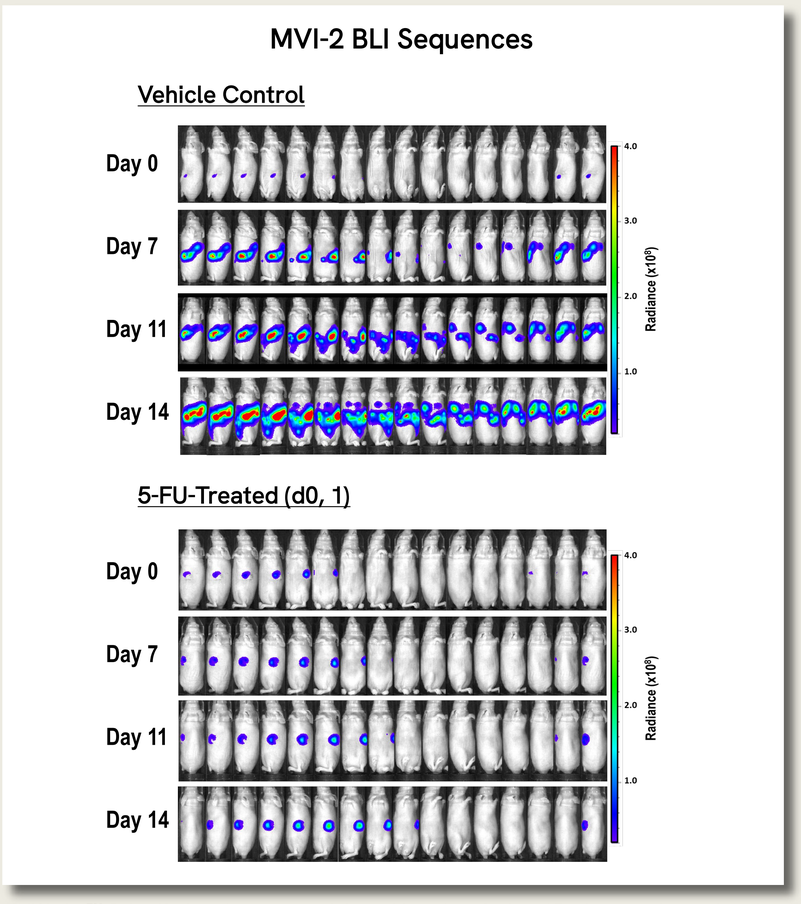
2 Experimental Protocol

Intrasplenic HCT-116 Tumor Metastasis Model



All animal studies were performed under protocols approved by Revvity's Institutional Animal Care and Use Committee (IACUC). Athymic nude mice (6-8 weeks old) were purchased from the Charles River (Wilmington, MA). IVISbrite™ HCT116 Red F-luc colon adenocarcinoma cells (Revvity Inc.), 5 $x10^5$ per injection, were implanted intra-splenically in nude mice. After 10 days (Day 0), animals were randomized into vehicle or treatment (5-fluorouracil; 5-FU) groups based on bioluminescence signal as indicated (high and low outlier animals were removed). Treatment of vehicle were given for 2 days (Days 0 and 1) by IP injection at the indicated dose, and mice were imaged on the MVI-2 on days 0, 5, 7, 11, and 14.

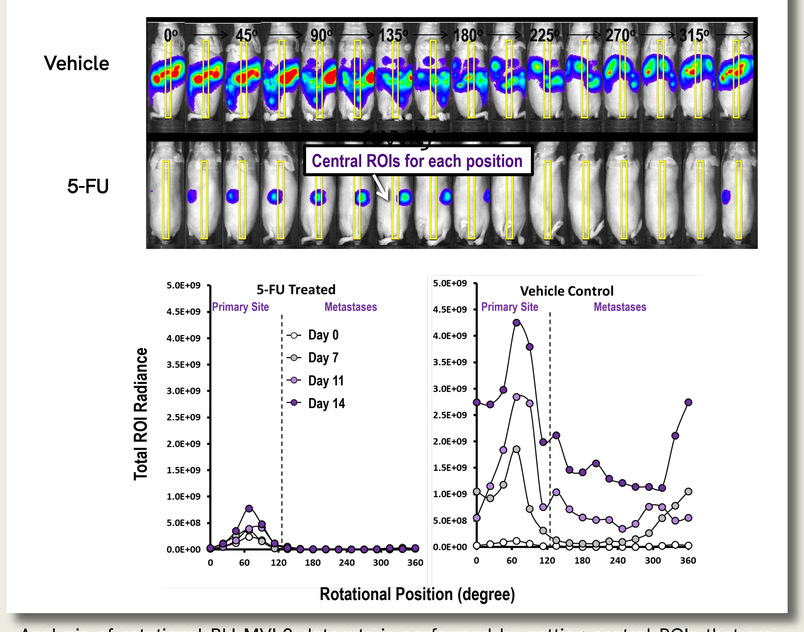
3 Surface BLI sequence acquisition on MVI-2 over time



IVISbrite™ D-Luciferin substrate was injected in pairs of mice, 15 minutes prior to imaging on the MVI-2. A total of 16 rotational positions (every 22.5°) were acquired, allowing acquisition of the image series in 10 minutes or less within the plateu of bioluminescence signal output. The same animals were re-imaged on the MVI-2 on repeated days, as indicated. Shown are rotational and temporal series of two representative mice imaged using the MVI-2 on the IVIS Lumina XRMS.

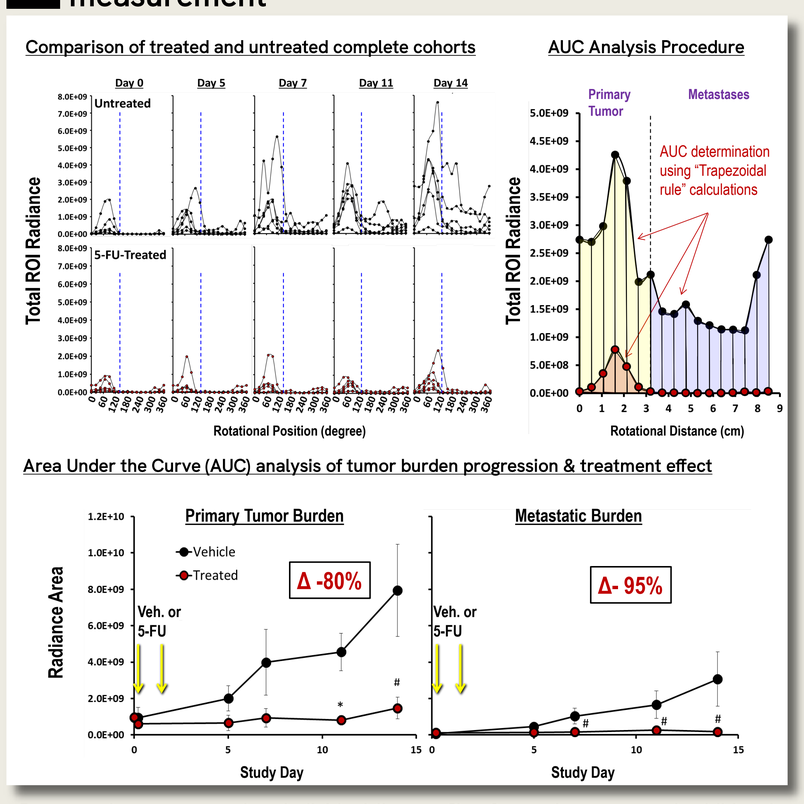
4 Quantification of MVI-2 surface BLI scans

Analysis Approach: 2 representative individual mice



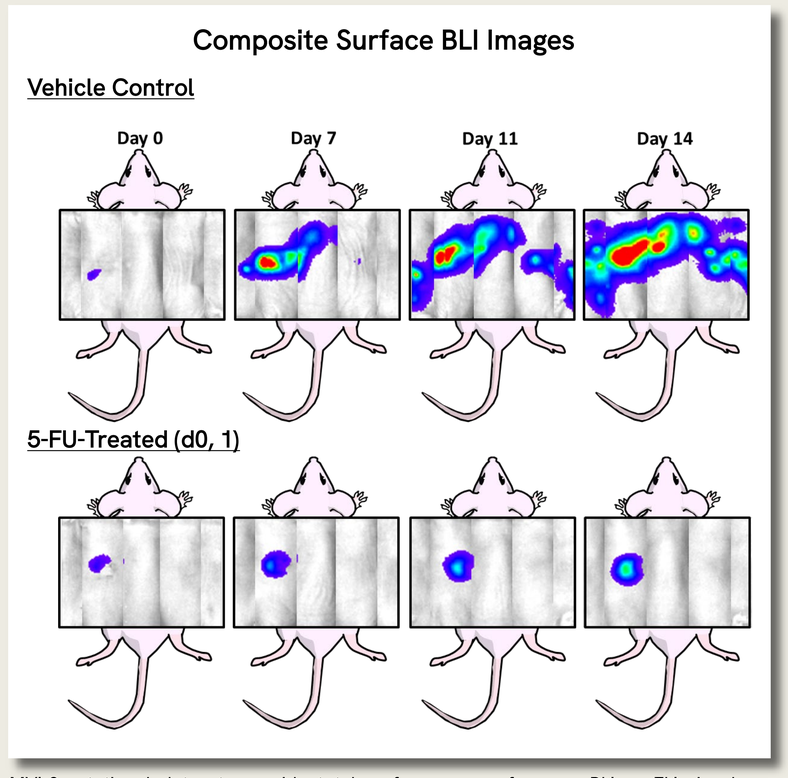
Analysis of rotational BLI MVI-2 datasets is performed by setting central ROIs that are sized based on the surface distance traveled for each rotation (width) and the length of the torso (height). This analysis assures the capture of all data and prevents overlap of ROIs. The rotational location can be used to identify the primary tumor site, with outside regions of signal attributed to metastasis.

5 Detailed group results and advanced AUC measurement



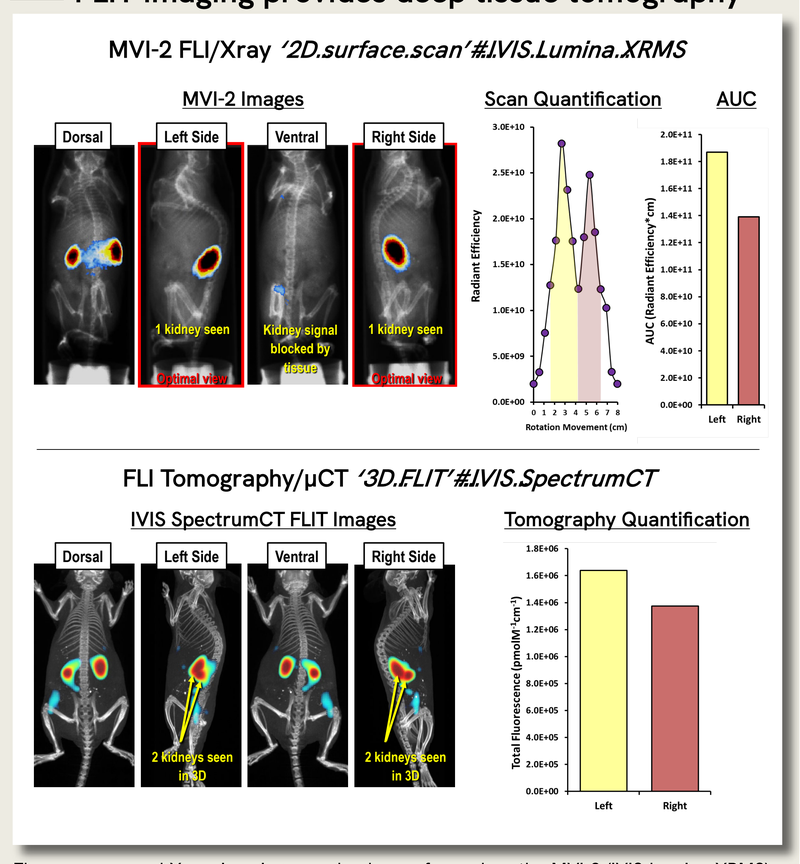
Data can be represented as individual line profiles for each mouse, and additional quantitative information can be derived from an "area under the curve" (AUC) analysis, which can provide regional tumor burden information. AUCs are measured using the "Trapezoid rule" for breaking curves into quantifiable geometric shapes that can be combined to generate the total signal AUC for a given region. This allows the separate quantification of primary tumor masses as well as the total metastatic burden as well as treatment effects.

6 Surface representation of BLI by merging four cardinal positions at each 90° rotation



MVI-2 rotational datasets provide total surface scans of mouse BLI or FLI signal, so appropriate subregions of signal can be combined as a "surface map" of the signal. Shown are composite surface maps for 2 representative mice using 0°, 90°, 270°, and 360° images, appropriately cropped to remove overlap and to show only torso signal. Such images can also be generated with smaller slices of all 16-positions. This representation provides a simple way to represent views of the scope of tumor metastasis and the impact of therapeutic intervention.

7 MVI-2 FLI imaging provides surface scans; 3D FLIT imaging provides deep tissue tomography



Fluorescence and X-ray imaging can also be performed on the MVI-2 (IVIS Lumina XRMS), and as an example we imaged kidney contrast using IVISense™ Folate Receptor 680 fluorescent probe (Revvity Inc.) at 24h and compared the results to tomographic imaging on the IVIS SpectrumCT. MVI-2 datasets provide a surface map of the kidney fluorescence emitted (overlayed on the X-ray images) but clearly signal from the kidneys cannot be seen when on the opposite side of the animal (ventral imaging). In contrast, tomographic images show the kidneys independent of depth and animal position. Despite these significant differences, the MVI-2 approach is superior to conventional 2D, single-position imaging and generated a kidney AUC quantitative profile in a manner similar to the quantitative results by tomography for this particular application.

Summary

Tumor metastasis models in mice present a number of challenges for monitoring and quantification. Typical imaging by bioluminescence is often performed using mice in either a single position or by imaging the front and back of the experimental mice. As the quality and quantification of signal can be greatly affected by the position of the animal, these standard approaches may lead to significant variability. We developed a rotational imaging system (MVI-2) that allows imaging of two mice simultaneously at a number of rotational positions precisely defined by a computer-controlled motor system. Custom-designed transparent mouse holders were designed for optimal mouse stabilization and the open section at the nose further allowed for the delivery of gas anesthesia delivered via the MVI-2. This rotational imaging approach allows researchers to image multiple sides of experimental mice (BLI or FLI), either employing a minimal front/sides/back strategy or a full 16-20 position acquisition (i.e. every 10-90 degrees). Analysis focuses on ROIs in the center of each image, the ideal region for data quality and consistency.

Our results highlight some of the benefits of full 2D surface optical imaging, including the improvement in the scope of data collected, the ability to represent composite surface images, and the ability to apply novel AUC analyses to the data. Line graphs readily convey both quantitative and locational changes in optical signal, and this is ideal for measuring progression and treatment in tumor metastasis models. Although the MVI-2 cannot provide tomographic-quality optical data, it can be a reasonable approach for assessing whole body surface signal heterogeneity that is superior to conventional 2D imaging strategies.