Localization of Subcutaneous, Intraperitoneal, and Satellite Tumors Utilizing an Animal Rotation Device in a Multimodal Imaging System

Introduction

Non-invasive in vivo optical imaging has been increasingly used in the pre-clinical arena. Tumorigenesis studies, in particular, have lent themselves to fluorescent tracking as a means of tumor detection, growth monitoring, and determining the efficacy of treatment(s). Monitoring the growth and early detection of tumors at both the primary and secondary sites is of principal interest to the researcher. Light-scattering, narrow visualization windows in deep tissue, and inability of fluorescent signal to penetrate completely through animals further compounds the difficulties of visualization and analysis. As a result, detection of signal often requires the animal to be positioned at an optimal orientation - one in which the origin of the fluorophore is positioned so that the light path through diffusive tissue is minimized. Further, in many studies tumors originate at unanticipated locations, making it difficult to properly position the mouse for optimal detection of tumors. To overcome these issues, we have developed a murine rotation device for use in the commercially available Carestream In Vivo Imaging Systems that enables visualization of the mouse at multiple calibrated angles. Application of this novel animal rotation device demonstrated its utility for the detection and longitudinal monitoring of subcutaneous, intraperitoneal, and secondary tumor sites non-invasively in live mice.

Methods

Cell Labeling
Kodak X-SIGHT 761 Nanospheres (Carestream Health, Woodbridge, CT, USA) were diluted to a concentration of 1 µM in 3 mL of RPMI containing 2% FBS. Ovarian cancer stem-like cells (OCSCs) were trypsinized and washed 3X with 2% RPMI. After the final centrifugation, the media was removed, replaced with the diluted nanospheres and incubated at 37° C, 5% CO2 with gentle shaking for 6 hr. The cells were spun, resuspended in 10% RPMI and returned to tissue culture plates overnight.

In vivo Imaging
OCSCs labeled with Kodak X-SIGHT 761 Nanospheres were injected subcutaneously at the flank (3x106 cells) and intra-peritoneally (IP) (3x106 cells) into athymic nude mice. Animals were anesthetized with 2% isoflurane and imaged at 7, 14, and 21 d post injection of labeled OCSCs utilizing a Multimodal Animal Rotation System (MARS) (Bruker BioSpin, Woodbridge, CT, USA) (Fig. 1) and the In-Vivo MS FX PRO Imaging System (MS FX PRO) (Bruker BioSpin, Woodbridge, CT, USA). Near-infrared fluorescence (NIRF) images with camera and filter settings optimal for the X-SIGHT 761 label (capture settings: 10 sec exposure, 2x2 binning, f-stop 2.8, Field of View (FOV) 120, excitation: 760nm, emission: 830nm) were taken for detection of tumors and X-ray images (capture settings: 10 sec exp, 2x2...
binning, f-stop 2.8, FOV 120, 35 KVP, 0.4mm Al) were taken for precise anatomic co-registration of optical signal. On Day 21 the animals were sacrificed and tumor fluorescence was analyzed ex vivo for further verification. Analysis was completed in MI Software v5.03.65 and image overlays were compiled in ImageJ (http://rsbweb.nih.gov/ij/, v1.43). Region of Interest (ROI) analysis in Fig. 2 was completed by obtaining the mean pixel intensity after automatically thresholding the area to 50% of the maximum pixel intensity for each tumor region.

**Results/Discussion**

To enhance detection of optical signals originating from mouse tumors, data was collected at every 10° of a 360° rotation under multiple modalities (NIRF, X-ray, reflectance). Acquiring images at these precise angles enhanced quantitation and detection of tumors. The primary subcutaneous tumor and two major IP tumors were visualized and NIRF intensity was measured at each incremental image (Figure 2). The optimal position to obtain the best data, as indicated by the greatest fluorescent signal capture, can be determined through the initial 360° imaging of the animal. For future imaging time points, the mouse can then be precisely driven to that position using the MARS system, or subsequent 360° rotations can be completed to observe any changes in fluorescent reporters over time as done in this study.

Low-level tumor detection was also optimized by this procedure. Small size tumors with low-level fluorescent emissions were localized at only a 30° range (Fig. 3). Without this comprehensive scan, these tumors would have gone undetected at the standard prone, supine and laterally recumbent imaging positions (0, 180, 90, 270).

![Figure 1. Commercially available MARS. System is fully automated and compatible with all In Vivo Imaging Systems](image-url)
Figure 2. Localization and quantitation of subcutaneous and major intraperitoneal tumors

Figure 3. Intraperitoneal satellite tumor visualization. White arrows indicate NIRF masses (tumors). Rotation permits detection of small tumors with low-level fluorescent emissions
Conclusion

In fluorescent imaging, the amount of optical signal captured is highly dependent on the path between emitted light and the detector. The inherent limitation of single-view in vivo optical imaging is mitigated by rotating the subject to positions that minimize the depth of optically scattering tissues between the fluorescent source and the detector - thus optimizing for the "sweet spot" of in vivo signal capture. In this study, animal rotation demonstrated its utility in improving visualization and quantitation of fluorescently labeled tumors. Furthermore, by completing 360° imaging of the animal, primary and satellite tumors that may have gone undetected were imaged and localized within the peritoneum. In conjunction with optical imaging, the described system may prove as a useful tool for the localization of tumors and other fluorescently labeled structures from unanticipated positions inside a mouse, as well as longitudinal tracking of processes associated with these structures.