Early In Vivo Detection of Tumors Using Multimodal Imaging Systems

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Molecular imaging, or the non-invasive spatial and temporal detection of signatures within the intact living organism that relate to cellular and intracellular events, has become essential for assessing targeted therapeutics as well as individualized progression of disease. For the pre-clinical research field, imaging techniques that measure both functional as well as anatomical changes in the whole body are vital for treatment follow up and co-registration of molecular signals.

Groups actively participating in molecular imaging studies have sought to superimpose functional images onto anatomic images taken from a different imaging system at a different time, or in the extreme case, superposition onto computer-generated models. These practices are obviously unreliable and dangerously misleading simply because anatomy changes dramatically across individuals and even across a single individual who is imaged at different times and under different treatment conditions.

Therefore, a simple, non-invasive, and easy-to-use molecular imaging system that provides both functional and anatomic features is critically needed for researchers who do not require tomographic images but, rather, simpler imaging tools to validate chemistry or other biological and molecular questions pertinent in molecular imaging. A combined functional and structural imaging tool may lead to the understanding of several questions involving physiological changes, contrast agent distribution, early disease detection, and therapeutic response.

In a recent study described below, the fluorescence imaging capabilities of the Bruker system were validated by detecting the selective binding of a near infrared (NIR) fluorescent contrast agent to tumor cell receptors after intravenous injection. Subsequent to fluorescent imaging, the In-Vivo MS FX PRO multimodal imaging system was then used to capture high-resolution X-ray images to co-register the emitted fluorescent signals with the anatomical location where tumor cell inoculation occurred. Subsequent to optical and X-ray image acquisition, histological examination confirmed the presence of a tumor less than 1 mm in size. Our data demonstrate the ability by the MS FX PRO to accurately combine functional and anatomic images on the same living subject.

Materials and Methods
The experimental procedures for the aforementioned study follow. The fluorescent dyes, cyanine 5.5 (Cy5.5) (excitation/emission, 675/694 nm) and indocyanine green (ICG) (excitation/emission, 720/775 nm), were used in a conjugated form. That is, both dyes were covalently bonded to a peptide. The Cy5.5 was conjugated to a peptide that targets matrix metalloproteinases (MMPs), and ICG was conjugated to the RGD peptide, which has a high binding affinity to membrane αvβ3 integrin receptors. Human prostate cancer cells PC3 (1 million) naturally expressing MMPs were inoculated intra-tibia into a nude mouse.

Additionally, human melanoma tumor cells M21 (1 million) that positively express αvβ3 integrin were inoculated intracranially. Imaging was performed on the fourth day post-prostate cancer inoculation or on the eighth day post-melanoma inoculation. All images were acquired 24 hr following tail vein injection of either the Cy5.5 or ICG contrast agent at equivalent doses of 15 nmol. The optical imaging exposure times ranged between 10 sec to approximately 1 min.
Results
The results from our imaging study are described below. Whole-body images of a nude mouse bearing a human prostate tumor are displayed in Figure 1. The fluorescent optical image clearly depicts the distribution of the Cy5.5 conjugate throughout the body, including Cy5.5 uptake into the prostate tumor and wash-out into the bladder. X-ray images are also displayed to provide a whole-body view of the skeletal anatomy. Merged optical and X-ray images were accomplished for co-registration of the anatomy and location of Cy5.5. Similarly, images were acquired of the same nude mouse; however, the field of view was reduced to capture only the prostate tumor inoculation site (Figure 2). In other words, the imaging system “zoomed in” on the diseased site of interest. Again, optical images, X-ray scans, and merged images are presented to further emphasize the specific uptake of Cy5.5 and its precise location at the animal’s knee. Also, unexpectedly, the Cy5.5 conjugate was found to be accumulated at the wrist of the nude mouse. Therefore, a high-resolution image of the mouse’s wrist is displayed in Figure 3. Accordingly, a pathological H&E stain of the wrist region is presented in Figure 4, confirming the positive binding of Cy5.5 to cancer cells that were metastasized into the wrist bone.

Whole-body images of a second nude mouse bearing the human melanoma tumor are provided in Figure 5. The fluorescent image clearly shows a high-intensity region in the brain representing the presence of ICG fluorescence due to binding of the contrast agent to the melanoma tumor cell. The fluorescence signal delineates the inoculation site in the brain, as well as ICG uptake in the kidney. The tumor mass could not be detected but visual, therefore, a fluorescence based imaging approach using the In-Vivo multimodal imaging system was vital for identifying the presence of this cancer in the brain. Again, pathology studies demonstrated tumor cells in the brain and in the skull (Figure 6).
Conclusion

Our data demonstrate that the In-Vivo MS FX PRO multimodal imaging system facilitates combining functional and anatomical imagery of a subject. Such an imaging technique may be used for pre-clinical research in early disease detection as well as for identifying treatment and therapy responses. The performance of the MS FX PRO, however, is not limited to optical and X-ray. The system is also capable of planar nuclear imaging, thus it is the only imaging system on the market that provides optical, nuclear, as well as X-ray detection for small-animal research. The instrumentation specifications include a light-tight box with an automatic switch to prevent leakage of X-ray for research safety. The box also houses up to several animals with multichannel connections that can be used for anesthesia gas, humidified air at a constant temperature, and intravenous injection catheter.

The optical imaging component of the In-Vivo system broadly includes an excitation source, detector, and optical filters. A 40 mW power-adjustable xenon lamp first provides a broad band source of light. This range is suitable for fluorescent dye excitation including the fluorophores families of Alexa and Cy dyes, as well as nearIR alternatives such as ICG and IRDye800.

A powerful 10X zoom lens focuses the fluorescent signal onto the CCD camera chip at a variable field of view from 20 x 20 cm2 to 2 x 2 cm2. The CCD chip has a 16-bit dynamic range for imaging both bright and dim signals in the same image.

The excitation light source is filtered with a band pass filter; the system can hold up to four optical excitation filters to change the source output (at the request of customer). Additionally, a wide-angle filter is used at the detector end to pass only the fluorescent emission signal. Again, the system can hold, via a filter wheel, up to four optical emission filters. The central wavelength, bandwidth, and optical density of all filters are available upon request. Lastly, “white light” images can be taken with no optical filtering.

In addition to the system itself, the cost includes a central processing unit (CPU), as well as three copies of a lifetime software license. The multiple software licenses enable the user to analyze and process data on different computer workstations as well as reduce the cost for repeated annual license fees. Image acquisition and processing is accomplished with the versatile and flexible MI software, which can control the exposure times as well as the bin size of the image for increasing signal-to-noise ratios and integration times. Other processing techniques, such as image overlay, normalization, contrast control, and image subtraction, are options to aid the researcher in correcting uneven light/energy sources and high background signals.

With this tri-modality imaging system, a vast combination of studies can be performed to study cell proliferation, growth, and the response of disease to drugs or other agents. For example, the combination of fluorescence imaging with X-ray imaging on several animals at once may enable identification of specific cells, the exact anatomical locations, and the expression of a protein owing to drug or other gene therapies. Another example includes the combination of optical with nuclear imaging to determine contrast agent distribution and interaction of a fluorophore or a radiopharmaceutical on the body and cell.

Overall, the benefits of the In-Vivo system include (i) being able to image multimodalities on the same day for hourly time-course data, (ii) the ability to detect nanomole quantities of fluorescent agent non-invasively for the reduction of toxicity and morbidity, (iii) a versatile software package and support for accurate molecular imaging studies, and lastly (iv) a flexible hardware package that allows the user to start the optical imaging option or “add” multimodal options, such as X-ray, depending on budget and research needs. Ultimately, the system is designed and engineered for pre-clinical animal research in cell biology, tumor biology, early disease detection, drug screening, drugs/agents interactions, treatment regimen testing, and therapy responses evaluations.

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