Kodak *in vivo* imaging system: precise coregistration of molecular imaging with anatomical X-ray imaging in animals

Kodak Molecular Imaging Systems introduces a line of small-animal *in vivo* imaging instruments that provide a new level of molecular signal localization in live animals. The Image Station In-Vivo FX allows precise multi-modal coregistration of optical or radioisotopic molecular images with high-resolution anatomical X-ray images in animals

Traditional research on disease mechanisms using animal models has relied mainly on the detection of morphological changes of the diseased tissues, with physical measurements and anatomical imaging or on the excision and pathological study of the tissues of interest. These methods often require long time periods for measurable changes to occur and require a large number of animal cohorts as multiple animals are often sacrificed at each time point for histological testing.

Over the last few years, exciting new molecular imaging agents have emerged from research laboratories that allow highly specific fluorescence-, luminescence- and radioisotope-based imaging of disease processes at the molecular level within living animals. These *in vivo* molecular imaging agents provide the potential for rapid detection of specific molecular and metabolic changes within target tissues in animals (or humans) long before morphologic changes can be detected. In addition, these molecular changes can be monitored *in vivo* without sacrificing the animal, resulting in lower cost, time savings and improved data by using the same live animal for continued studies.

The need for multimodal imaging

One major advantage of optical molecular imaging over anatomical imaging is the use of 'dark-field' imaging methods that allow high levels of target signal over the surrounding background signal. Dark-field contrast, however, does not typically provide the appropriate contextual anatomic information for useful localization of the molecular imaging signals within the animal. Limited anatomic context of dark-field agents has been provided using digital imaging overlay techniques in which the dark-field contrast is superimposed on a reflection image of an experimental animal.

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Although the overlay methods are beneficial, repeated imaging of the same animal in different imaging sessions often results in misinterpretation of the signal localization as animal repositioning is difficult. The white-light reference image may be suitable for localization of large tumor masses, but lacks the anatomical context required for repeatedly localizing smaller signals of interest and/or mapping the molecular signals to bones or other anatomical structures within the animal.

Hoping to realize the full potential of the dark-field molecular imaging agents, researchers are beginning to apply multimodal instrumentation that combines dark-field contrast with penetrating radiographic anatomical imaging in one system.

Kodak Image Station In-Vivo F/FX

Kodak Molecular Imaging Systems has recently introduced a line of *in vivo* small-animal imaging systems, including a model that allows the capture of X-ray images. These X-ray images provide the detailed penetrating anatomical guideposts that greatly enhance the localization of the *in vivo* optical or radioisotopic molecular imaging agents.

The product line consists of the Kodak Image Station In-Vivo F and the Kodak Image Station In-Vivo FX. The In-Vivo F allows for very high resolution, multi-wavelength fluorescence, luminescence and radioisotopic imaging in small animals. The In-Vivo FX includes all of the capabilities of the In-Vivo F and the high-resolution X-ray Imaging Module using a Radiographic (X-ray) Imaging Screen.

For both radiographic and radioisotopic imaging, patented Kodak phosphor screens coupled to speed-enhancing interference optics efficiently convert the ionizing radiation into light. The light is emitted by the screens and captured by the charge-coupled device (CCD) camera to form the image. Two different screen assemblies are available. One is optimized for the high-energy radioisotopes such as ^{111}In, ^{99}Tc and ^{18}F, and the other is optimized for the low-energy, high-resolution requirements of X-ray imaging.

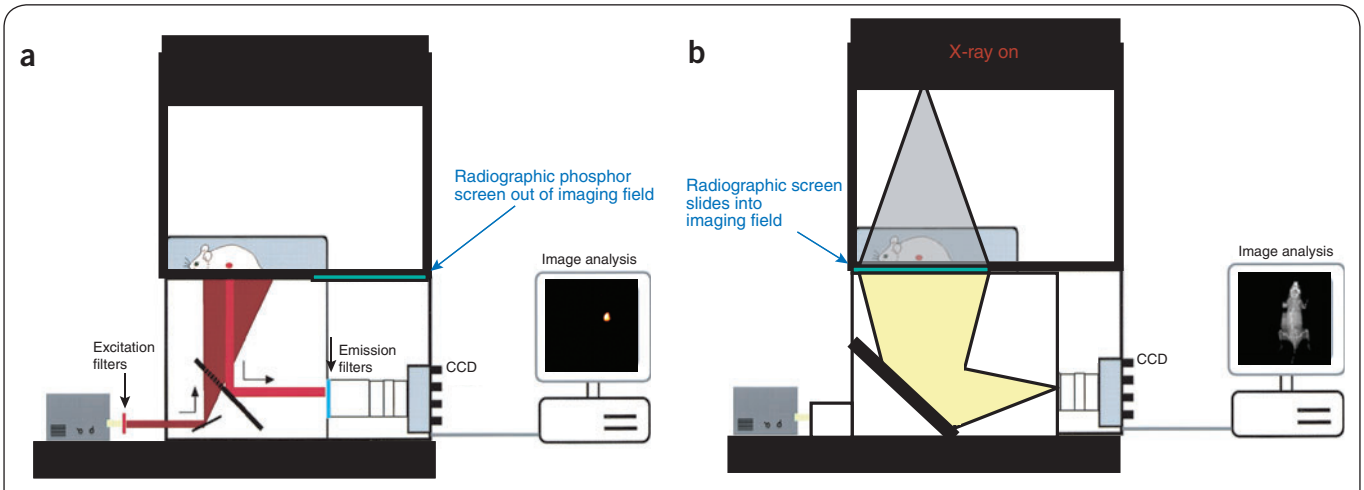

Figure 1 | Image Station In–Vivo FX—multimodal operation. (**a**) A near IR example of the optical imaging modality. With the radiographic screen out of the imaging field the white light illuminator is powered, the appropriate excitation and emission filters are selected for the fluorochrome of interest and the image is captured. (**b**) This can then be followed by the radiographic (X-ray) mode by simply sliding the radiographic screen under the animal chamber without moving the animal. The excitation is set to black (no excitation light) and the emission filter is set to open (no emission filter). The X-ray mode is selected in software and the X-ray generator is activated, producing an X-ray field that is transduced to light by the phosphor screen and captured by the CCD camera at very high resolution. As both the optical and radiographic images are captured at the same focal plane, they can be easily and precisely overlayed into one in Kodak MI software to provide the coregistered multimodal images. (**a,** adapted from ref. 1.)

Multimodal imaging operation

The Kodak Image Station In-Vivo F and FX systems use the same operation and hardware for optical imaging of an animal (or multiple animals) immobilized and positioned in the animal chamber directly above the imaging chamber window (**Fig. 1a**). For fluorescence, excitation light from a high-intensity lamp is directed through the selected excitation filter to the animal. Fluorescence from the imaging agent inside the animal is then emitted and separated from the excitation light as it passes through the patented Kodak Wide Angle emission filter. The fluorescence enters the 10× zoom lens and is focused onto a 4 million pixel, cooled CCD. The digitized read–out is efficiently interfaced to a personal computer (Windows or Mac).

Multiple optical images of different molecular entities with different fluorescent tags can be captured in the same animal by simply selecting different filters and capturing additional images. In Time-Lapse mode, multiple images can be captured in the same session to track the bio-distribution of the imaging agent.

Once the desired optical images are captured, the radiographic (X-ray) phosphor screen can be moved into the imaging field by simply sliding the screen under the animal chamber (**Fig. 1b**). The phosphor screen comes into close contact with the thin plastic sheet that supports the animal in the animal chamber, placing the screen essentially at the same focal plane setting used with the optical images. The image capture setting in software is switched to X-ray and the microfocus X-ray generator emits a maximum energy of 35 Kvp for the desired imaging time (typically <30 s). The X-rays are differentially absorbed by bone and soft tissue, creating a projection of the animal's anatomical structure on the phosphor screen. The bright-field image of the phosphor screen is captured and digitized in the camera and read into the computer.

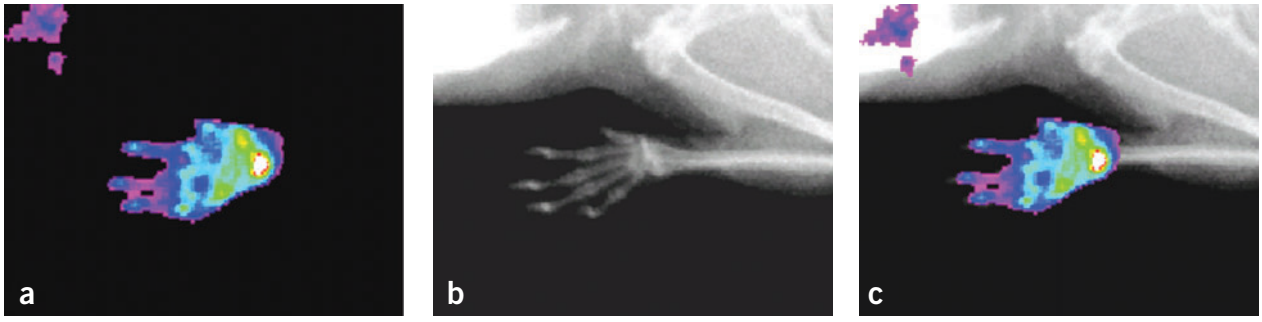

Figure 2 | Precise multimodal coregistration is demonstrated with images of a mouse injected with Osteosense probe (VisEn Medical), which contains a near IR fluorochrome and binds to bone. (**a**) Near IR fluorescence using ex720 and em790WA filters for 30 s. (**b**) X-ray image (30 s) with the same field of view and focal plane as the fluorescence image. (**c**) Overlay of **a** and **b** in Kodak MI 4.0 software, showing precise coregistration of the probe's near IR fluorescence and the X-ray absorbance in the right forepaw 'finger' bones of the mouse. Images courtesy of B. Bednar, Imaging Research, Merck Research Laboratories, Merck Co.

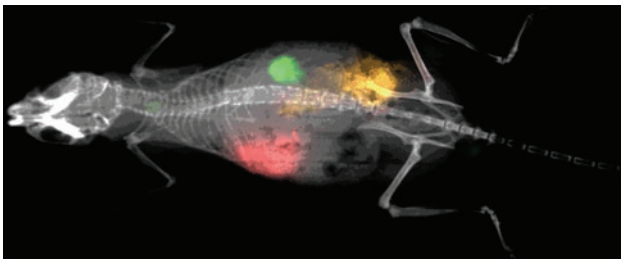

Figure 3 | A combination of multiwavelength imaging with X-ray imaging in a mouse with three different fluorochrome-labeled probes injected into different regions of a mouse abdomen. Successive 10-s images were taken with ex465 and em535WA for FITC (green), ex 535 and em600WA for phycoerythrin (yellow), and ex625 and em700WA for Cy5 (red). A 30-s X-ray image was then taken with the radiographic screen engaged. The fluorescent images were pseudocolored and the four images were merged via 'Add Image' function in Adobe Photoshop. Image courtesy Jingmei Biotech Co. Ltd., Beijing, China.

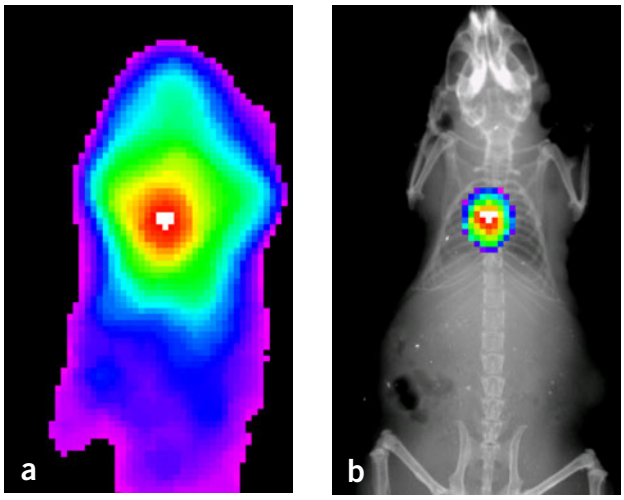

Figure 4 | The combination of Radioisotopic and X-ray modalities of imaging in a mouse tail vein injected with 287 µCi of [^{18}F]FDG PET. (**a**) Approximately 1 h after injection, with the radioisotopic screen engaged and the camera set to highest binning state (16 x 16), the [^{18}F]FDG PET image was captured for 8 min and pseudocolored. (**b**) A 30-s X-ray image was then taken with camera in highest resolution binning state (1 x 1). The radioisotopic image was contrasted to show only the top 5% of the image intensity and overlayed on the X-ray image showing the highest activity coregistered with the anatomical location of the mouse heart.

As the images of each modality are captured without movement of the animal and with no change in optical focus or zoom, the images can easily be merged or overlayed in the Kodak MI software for precise coregistration.

Multimodal imaging examples

Demonstration of the coregistration of fluorescence and X-ray imaging is shown in **Figure 2**. The mouse was injected with Osteosense™ 750, a near-infrared fluorescent diphosphonate probe that binds to bone. This high-resolution image of the animal's paw shows the fluorescent signals coming from the probe attached to the digits in the paw (**Fig. 2a**). The X-ray image details the bones in the digits of the animal paw (**Fig. 2b**), and the overlay image demonstrates the expected colocalization and the precise coregistration of these two modalities in the Kodak instrument (**Fig. 2c**).

Combined multiwavelength fluorescence and X-ray imaging is shown in **Figure 3.** We injected three different fluorescently tagged imaging agents subcutaneously into different regions of the mouse abdomen. Fluorescence imaging with different filter sets appropriate for each fluorochrome was followed by X-ray imaging. The four images, representing the three different fluorescent channels and the X-ray image, were easily contrasted and pseudocolored in Kodak MI 4.0 software and merged in Adobe Photoshop™.

The image in **Figure 4** demonstrates the combination of radioisotope ^{18}F imaging (typically used in positron emission tomography (PET) imaging) with X-ray imaging. The mouse was injected with [^{18}F] fluordeoxyglucose (FDG) PET and imaged with the Kodak Image Station In-Vivo FX using the radioisotopic imaging screen for 8 min with the camera in the highest-binning state. The image was contrasted and overlayed on the subsequent X-ray image to show the localization of the isotope in the heart of the animal.

Conclusion

Kodak has developed and commercialized powerful multimodal *in vivo* imaging systems that greatly enhance the localization of molecular signals in live animals. These systems are now used by top academic, biotechnology and pharmaceutical research institutes worldwide. The flexibility of the system allows the combination and coregistration of multiple wavelengths and multiple modalities of imaging including optical, radioisotopic and radiographic imaging. Several studies are now in progress that will further detail the utility of combining, coregistering and performing the appropriate analysis of the multiple imaging modalities provided by the Kodak Image Station In-Vivo F/FX systems.

1. Mahmood, U. & Weissleder, R. Near-infrared optical imaging of proteases in cancer. *Mol. Cancer Ther.* **2**, 489–496 (2003).