Fast Imaging System with Maximized Flexibility for Fluorescent Protein Imaging in Vivo

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Abstract

Through use of fully automated large format optics, the UVP iBox is capable of fluorescent protein imaging with a range of cameras that use front and back illuminated CCDs with sizes up to a 43 mm diagonal, greatly expanding the applications for high resolution, large field of view and increased throughput imaging. The iBox imaging system can be configured with both monochrome and color CCDs, with CCD resolution currently up to 8.3 megapixels. The range of fast lenses includes several interchangeable, fully automated optics: a 50 mm f1.2, 28 mm f 1.8, and a 24 -70 mm f2.8 zoom lens. These lenses give maximum imaging flexibility, with the field of view ranging from one to several animals, plants, culture dishes etc. At f1.2, the typical exposures are less than 50 msec, minimizing the effect of animal movement. The camera, optics, sample platform position, and excitation and emission filters are under full software control, permitting reproducible and rapid imaging with software presets and macros. Analysis of mouse models using CFP, GFP, and RFP for cancer metastasis and treatment will be used to illustrate the imaging advantages of the iBox.

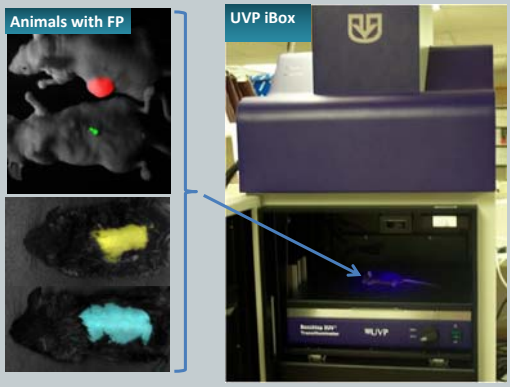
Background

Fluorescence can be used to report the location of bio-molecules or tissue of interest in vivo with high contrast to background. Through a series of fluorescent images taken at different time points, the development of diseases can be tracked in living organisms. Fluorescent proteins (FPs), because of their endogenous expression, allow the observation with minimal disturbance to the subject (Hoffman and Yang, 2006 a, b). For example, cancer cells can be engineered to carry FPs stably and implanted into the subject to allow monitoring of metastasis and the effectives of cancer treatment.

A variety of FPs had been engineered to suit the need of different applications. A specific FP can be chosen for its color which separates the signal from background and/or for its ease of use (well-control molecular biology, compatibility with the physiology of the subject, environmental stability). In multi-labeling experiments, pairs of FPs that are distinguished spectrally can be use to create contrast and to observed interactions between several targets. Popular pairing included CFP-YFP and GFP-RFP. The UVP iBox system, equipped with a wide spectral range light source, distinguished filter sets and the high QE, cooled camera, accommodates the full range of FP applications.

Methods

Images of the mice were taken with UVP iBox in vivo imaging system configured with the GFP, RFP, YFP and CFP excitation and emission filter sets, fast f 1.2 50 mm large area Biolens, 4.2 mpx high resolution BioChemi 500 camera, motorized sample platen, UVP heater (set to 37C), automated BioLite™ excitation light source, and VisionWorks®LS analysis software.

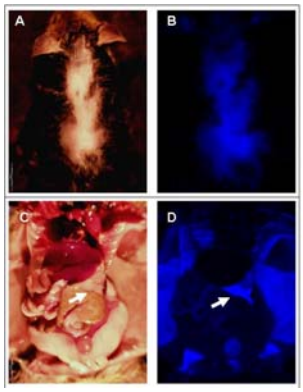


Both white light and fluorescent images were taken and combined using the blend control, with the fluorescent image colorized according the color of fluorescence-in the case red for RFP. Typical exposures were: 0.010 sec for white light and 1 sec for the RFP.

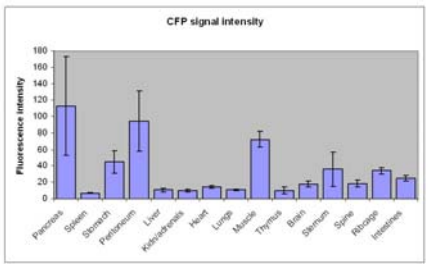
Results

Identifying the non-uniform distribution of CFP on nude CFP mice

From Cao, 2008, CK6/ECFPMouse showing CFP expression. A, C are brightfield images while B and D are CFP fluorescent images. A, mouse skin showing CFP fluorescence, B, visualizing the abdominal and thoracic areas for CFP. Pancreas is highlighted by arrow.

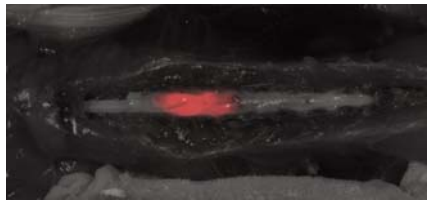


CFP signal intensity verified with complete necropsy.

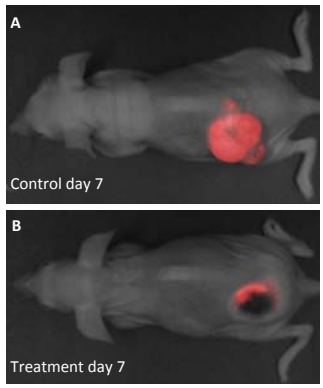


Localizing Tumor Expressing RFP

RFP expressing spinal cord tumor imaged on UVP iBox



Evaluating Cancer Treatment



A time controlled sequence of fluorescent images were taken on two groups of mice. Both groups received injection of RFP-U87 brain cancer cells. Comparing the untreated control (A) to the treatment (B) at day 7 indicates that the tumor was targeted by Salmonella A1-R given intravenously via weekly injections, and this resulted in a significant reduction in the tumor growth. The use of single color imaging of tumors, illustrated here, is critical to time course studies and illustrates the importance of fluorescent protein imaging in cancer research.

Conclusion

Rapid quantitative acquisition and analysis of CYP, GFP, YFP, and RFP has been demonstrated with the UVP iBox small animal imaging system. Advantages of the system include:

- ✓ Combined with UVP f1.2 optics, very fast (e.g., 50 to 200 msec) exposures are routine yielding sharp images unaffected by breathing of the animal.
- ✓ Tumor margins are easily defined with the bright fluorescence, and using VisionWorks software and Area Density tools, the quantitating the tumor area (mm^2) is straightforward.
- ✓ Epi illumination is built into the darkroom and include 365nm for quick inspection of the animals.
- ✓ The BioLite supplies a direct source using fiber optic bundles and a closed optical path to tightly control the output spectrum for consistent, repeatable measurements
- ✓ Standard matched excitation/emission filters for typical fluorescent proteins are available in addition to the fully customizable filter combinations.
- ✓ Access port for connecting to an external anesthesia unit

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