



Focal Points

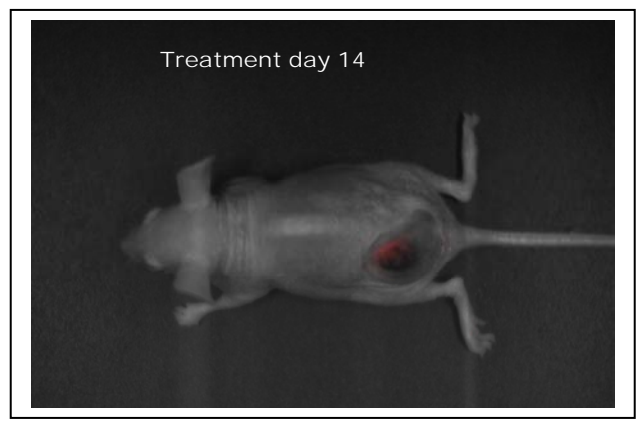
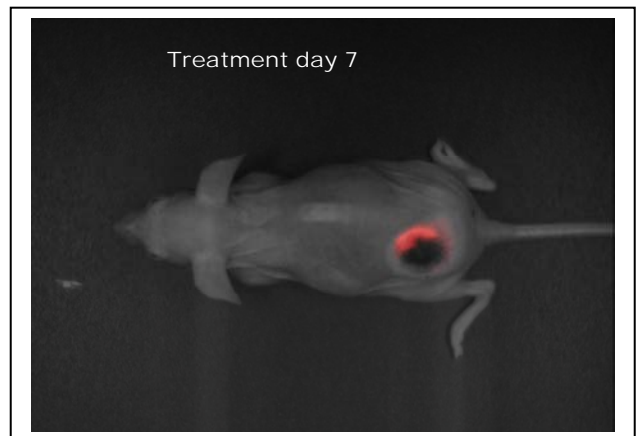
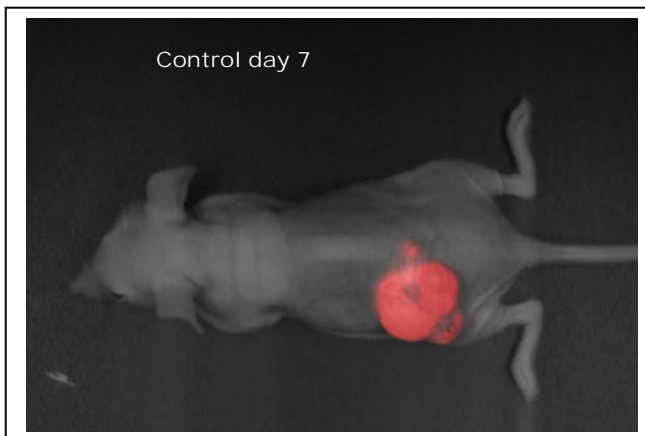
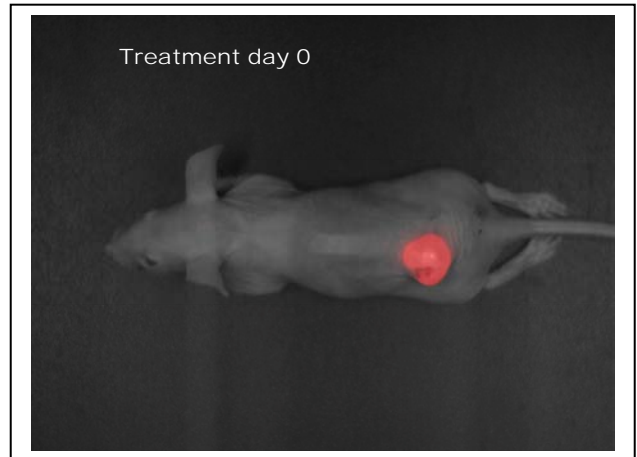
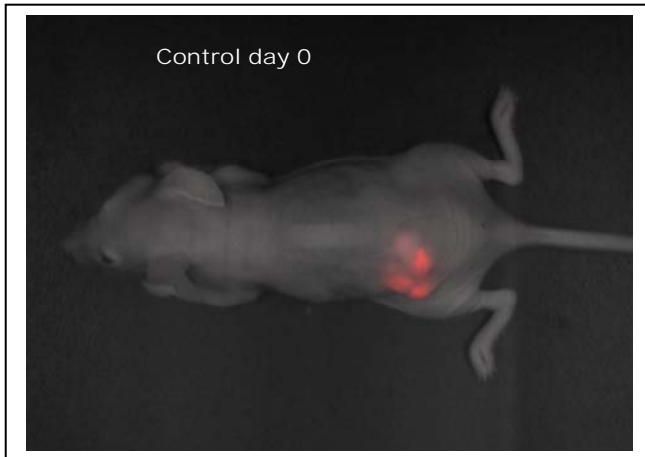


Application Note FP-130

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Salmonella Typhimurium Treatment of RFP Tagged U87 Tumors in Nude Mice

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Introduction

Fluorescent protein imaging with the UVP iBox® Small Animal Imaging System has a number of distinct advantages for whole animal imaging (Ref: FP-129, 1, 2).

Fluorescent proteins with a wide range of emission wavelengths are available, simplifying multiple label studies. The proteins are naturally fluorescent and require no substrates or cofactors.

In addition, the fluorescent properties of the reporter proteins are constantly being expanded, and now include a range of extremely bright red emitting proteins. Genes for these reporters are readily available from commercial and academic sources.

Expressed fluorescent proteins are bright. 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.

Quantitation. Tumor margins are easily defined with the bright fluorescence, and using VisionWorks software and Area Density tools, the quantitating the tumor area (mm²) is straightforward.

The application images illustrate imaging RFP orthotopic tumors in nude mice over a period of 14 days with the UVP iBox® Small Animal Imaging System. The treatment, described in detail by Zhao et al. (2007), consists of daily injections of a mutant of *Salmonella typhimurium* that concentrates in tumors and, minimizes or eliminates metastasis with PC-3 human prostate tumor cells. In the illustration below, U87 Red fluorescent protein expressing human glioma cell line was used.



Materials and Methods

See Zhao et al. (2006, 2007). UVP iBox in vivo imaging system configured with the GFP, RFP, YFP and CFP excitation and emission filter sets, fast f1.2 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.

The procedure for imaging is straightforward. A small number of U87 cells were inserted subcutaneously into the nude mice. Once the tumor had reached 1 mm² in volume, observations began and were designated Day 0. The treatment group received weekly injections of 5×10^7 /100 μ l *Salmonella* A1-R via a tail injection. Using the RFP excitation and emission filter set, images were taken with the UVP iBox system. Both white light and fluorescent images were taken and combined using the blend control (paste special with source at 75% and destination at 25%), with the fluorescent image colorized according to the color of fluorescence-in this case red for RFP. Typical exposures were: 0.010 sec for white light and 1 sec for the RFP.

Results

Comparing the untreated control to the treatment at day 7 and 14 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 fluorescent imaging of tumors, illustrated here, is critical to time course studies and illustrates the importance of fluorescent protein imaging in cancer research.

References

1. Hoffman, R.M. and Yang, M. Whole-body imaging with fluorescent proteins. *Nat. Protoc.* **2006**, *1*, 1429-1438.
2. Hoffman, R.M. and Yang, M. Color-coded fluorescence imaging of tumor-host interactions. *Nat. Protoc.* **2006**, *1*, 928-935.
3. Zhao, M.; Geller, J.; Ma, H.; Yang, M.; Penman, S.; Hoffman, R.M. Monotherapy with a tumor-targeting mutant of *Salmonella typhimurium* cures orthotopic metastatic mouse models of human prostate cancer. *Proc. Natl. Acad. Sci. U. S. A.* **2007**, *104*, 10170-10174.
4. Zhao, M.; Yang, M.; Ma, H.; Li, X.; Tan, X.; Li, S.; Yang, Z.; Hoffman, R.M. Targeted therapy with a *Salmonella typhimurium* leucine-arginine auxotroph cures orthotopic human breast tumors in nude mice. *Cancer Res.* **2006**, *66*, 7647-7652.

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