Dual-color, whole-body imaging in mice

To the editor:

In the March issue, Ntziachristos *et al.* (*Nat. Biotechnol.* **23**, 313–320, 2005) reviewed new optical techniques for whole-body imaging of small animals and compared them with current methods. We wish to refute some of the statements pertaining to dual-color tumor mouse models that appear in their Perspective.

Ntziachristos *et al.* claim that spectral separation has advantages in distinguishing red fluorescent protein (RFP)-expressing tumors growing in a green fluorescent protein (GFP)-expressing transgenic nude mouse; however, they use an animal with weak expression of RFP in the tumor, which is highly uncharacteristic for this model, according to previous results obtained in our laboratories[1,2] (see **Fig. 1a**). In the dual-color RFP-tumor GFP mouse model, our experience has been that it is not necessary to separate the spectra to obtain a bright dual-color image because there is a strong signal from both GFP and RFP[1,2].

Ntziachristos *et al.* also state that one of our papers[3] uses whole-body fluorescence imaging to detect GFP-expressing "tumors implanted superficially." However, the fluorescent tumor cells in this paper were not implanted superficially; they were implanted either on the colon or injected into the tail vein, where they metastasized to the brain, liver and bone, and were brightly visualized by whole-body imaging. An example of simultaneous dual-color, whole-body imaging of GFP and RFP tumors in the brain is presented in **Figure 1b**. These data and those from another of our publications[4] thus contrast with the statement of Ntziachristos *et al.* that what they term "planar" imaging is limited to superficial observations.

In contrast to the statements of Ntziachristos *et al.* that nonlinear effects can lead to erroneous interpretation in planar imaging and that current fluorescent imaging techniques are "a crude qualitative tool," a study coauthored by one of us (R.M.H., ref. 5) has shown that whole-body fluorescence imaging of RFP pancreatic tumors and metastasis, quantified as pixel area, correlates well with actual tumor volume.

We eagerly await further advances in the spectral separation and tomographic imaging described by Ntziachristos *et al.* for whole-body imaging of small animals.

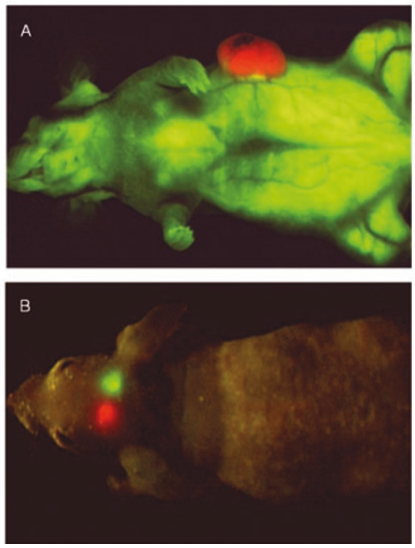

Figure 1 Whole-body, multicolor fluorescent imaging. (**a**)Whole-body image of an RFP-expressing tumor implanted in the mammary fat pad of a GFP transgenic nude mouse. (*Cancer Research* **64**, 8651–8656, 2004. Reprinted by permission of the American Association for Cancer Research). (**b**) RFP- and GFP-expressing tumors implanted in the brain of a nude mouse. (*Biotechniques* **39**, **in press**, 2005. Reprinted by permission.) Imaging was carried out at 470 nm using either fiber optic illumination **a** or illumination with a blue light emitting diode (LED) flashlight **b**. Image capture was with a Hamamatsu CCD camera with appropriate emission filters (Chroma Technology; see refs. 1 and 2 for details).

However, we also hold that whole-body imaging of dual-color mouse models of tumors, such as those produced by our company AntiCancer, can be broadly applied to *in vivo* research work using existing imaging techniques and widely available inexpensive instruments.

Robert M. Hoffman[1,2] & Meng Yang[1]

[1]AntiCancer, Inc., 7917 Ostrow Street, San Diego, California 92111, USA. and [2]Department of Surgery, University of California, San Diego, 200 West Arbor Drive, San Diego, CA 92103-8220, USA. e-mail: all@anticancer.com

1. Yang, M., *et al. Proc. Natl. Acad. Sci. USA* **100**, 14259–14262 (2003).
2. Yang, M., Reynoso, J., Jiang, P., Li, L., Moossa, A.R. & Hoffman, R.M. *Cancer Res.* **64**, 8651–8656 (2004).
3. Yang, M. *et al. Proc. Natl. Acad. Sci. USA* **97**, 1206–1211 (2000).
4. Hoffman, R.M. *Lancet Oncol.* **3**, 546–556 (2002).
5. Katz, M.H. *et al. J. Surg. Res.* **113**, 151–160 (2003).