Facile whole-body imaging of internal fluorescent tumors in mice with an LED flashlight

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The use of green fluorescent protein (GFP) for detection and visualization of cells in vivo has been established by our laboratory. With this fluorescent tool, tumor cells were detected and visualized for the first time at the microscopic level in fresh viable tissue in their normal host organ (1).

The use of GFP in whole-body imaging was also established by our laboratory (2). Fluorescent tumors growing and metastasizing in live mice were imaged in real time. Whole-body optical imaging, based on GFP, is external and noninvasive. It affords unprecedented continuous visual monitoring of malignant growth and spread within intact animals without the need of substrate injection or anesthesia. Quantitative measurement of tumor growth on internal organs was determined using digitized whole-body images. Imaging was with either a transilluminated epifluorescence microscope or a fluorescence light box and thermoelectrically cooled color charge-coupled device (CCD) camera (2).

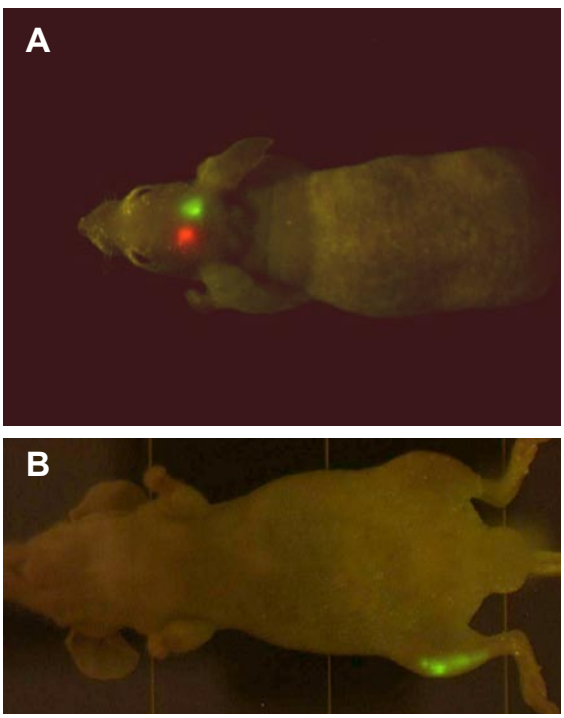

Figure 1. Whole-body imaging of green fluorescent protein (GFP) and red fluorescent protein (RFP) tumors in the brain in a nude mouse. (A) GFP- and RFP-expressing tumors implanted in the brain in a single nude mouse. The excitation light was produced with a simple blue-LED flashlight equipped with an excitation filter with a central peak of 470 nm. The image was acquired with a Hamamatsu charge-coupled device (CCD) camera. (B) GFP-expressing tumor implanted in the tibia of the right hind leg of a nude mouse imaged with the blue-LED flash light as in panel A.

Recently, Tyas et al. (3) used a blue LED flashlight to genotype GFP transgenic mice. GFP was expressed at high levels in most tissues in these mice (3). Tyas and coworkers used a blue LED flashlight to excite the GFP fluorescence in living animals with appropriate excitation and emission filters to identify GFP-positive transgenic mouse pups (3).

It is reported here that a blue LED flashlight (LDP LLC, Woodcliff Lake, NJ, USA; www.maxmax.com/OpticalProducts.htm) with an excitation filter (midpoint wavelength peak of 470 nm) and an emission D470/40 filter (Chroma Technology, Brattleboro, VT, USA) for viewing could be used for whole-body imaging of mice with GFP and red fluorescent protein (RFP)-expressing tumors growing in or on internal organs (2).

Figure 1A shows whole-body imaging of two tumors, one expressing GFP and the other expressing RFP implanted in the brain. The image shows that the GFP and RFP tumors are simultaneously excited with the blue LED flashlight and readily imaged. Figure 1B shows a GFP-expressing tumor implanted in the bone and whole-body imaged with the blue LED flashlight.

Figure 2A shows a GFP-expressing tumor implanted on the colon and whole-body imaged with the blue LED flashlight. The animal was also opened and imaged in the same way with the blue LED flashlight (Figure 2B). Comparison of Figure 2A and 2B shows the high accuracy of the whole-body image in Figure 2A compared to the image of the opened animal in Figure 2B.

Figure 2C shows a whole-body image of an RFP-expressing tumor implanted on the liver and a GFP-expressing tumor on the pancreas of the same mouse. As with the RFP and GFP tumors implanted in the brain in Figure 1, the RFP tumor implanted on the liver and GFP tumor implanted on the pancreas are simultaneously excited with the blue LED flashlight. Figure 2D shows an image, excited with the blue LED flashlight, of an RFP-expressing tumor implanted on the pancreas that has metastasized.

The images were captured with a Hamamatsu C5810 three-chip cooled color CCD camera (Hamamatsu Photonics, Hamamatsu City, Japan). However, a much simpler digital camera could be used with acceptable results. The images shown in Figures 1 and 2 were readily seen by the naked eye with no anesthesia, substrate, or restraint of the animal needed.

A software program was used to calculate the number and intensity of pixels in each image and then convert the numbers to mm^2 for each tumor (Table 1). The fluorescence intensity is an average value of all detectable GFP signals. The fluorescence intensity is measured in relative units defined by the gray scale of the green channel of the CCD camera. A threshold value was used that was detectably above background as visualized by eye. Table 2 compares the size and intensity of images of the tumor implanted on the colon visualized by both whole-

body and open imaging (Figure 2). It can be seen from Table 2 that the size of the imaged tumor is comparable for both the whole-body and open images. Even more striking is that the intensity of the whole-body image is 70% of the open image. Although some information is lost with whole-body imaging due to light scattering, a remarkable amount of information is obtained, even with such simple instrumentation. It has been previously shown that whole-body imaging correlates with actual tumor volume (4). Low-fluorescence tumors, however, may require more sophisticated equipment for whole-body images.

Table 1. Quantitation of Tumor Images

Tumor Location	Pixels	mm^2
GFP brain	912	6.00
RFP brain	811	5.34
GFP bone	3561	23.42
GFP colon (whole-body image)	2465	16.22
GFP colon (intra-vital image)	2731	17.97
GFP pancreas	2813	18.51
RFP liver	2254	14.83
RFP pancreas	25103	165.15

GFP, green fluorescent protein; RFP, red fluorescent protein.

Table 2. Comparison of Whole-Body and Intravital Images of GFP Tumor on Colon

Image Type	Tumor Size in Pixels	Fluorescence Intensity
Whole-body image	2465	137
Open image	2731	196

Images were acquired with excitation by blue-LED flashlight. The fluorescence intensity is measured in relative units defined by the gray scale of the green channel of the charge-coupled device (CCD) camera. GFP, green fluorescent protein.

The data in this report result in the following conclusions: (*i*) very strong signals emit from GFP- and RFP-expressing tumors inside the animal; (*ii*) the images are readily quantifiable; (*iii*) there is negligible interference from autofluorescence; and (*iv*) very simple and low-cost instruments can be used for GFP and RFP whole-body macro-imaging.

These data show the great potential of fluorescent protein-based whole-body imaging for high-throughput in vivo screening of drug efficacy and other applications. The data also correct serious misconceptions in the literature stating "limits" of in vivo fluorescence protein imaging (5,6).

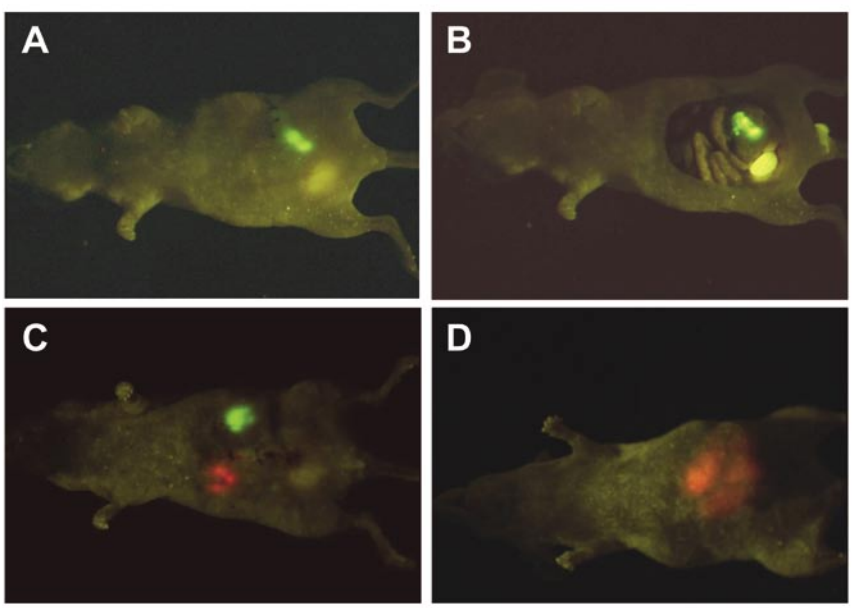

Figure 2. Whole-body and open imaging of green fluorescent protein (GFP) and red fluorescent protein (RFP) tumors in nude mice. (A) Whole-body image shows the GFP-expressing tumor on the colon imaged with the blue-LED flashlight. (B) Same as panel A, with animal opened. (C) Image of RFP-expressing tumor on the liver and GFP-expressing tumor on the pancreas in a nude mouse. The tumors were imaged with the blue-LED flashlight. (D) Whole-body image of a metastasizing RFP-tumor on the pancreas in a nude mouse. (A–D) The blue-LED flashlight was used as in Figure 1.

COMPETING INTERESTS STATEMENT

The authors declare no competing interests.

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