Task

Practice in vivo imaging technique for researchers who have limited access to in vivo samples

Solution

Simple user interface and one touch automation enables researchers and students to practice in vivo imaging technique with silicone animal sample without any need for training

The Utility of GFP and RFP for In Vivo Fluorescence Imaging

Introduction

In vivo imaging using fluorescent proteins permits rapid and simple tracking of primary and metastatic cancer development research in live animals. Green fluorescent protein (GFP) is a commonly used fluorescent dye for in vivo imaging, being discovered decades ago in the jellyfish Aequorea victoria as a primary feature of bioluminescence[1]. Upon establishing its novel utility for fluorescence labelling in other living organisms, the development of new molecules has expanded the range of available fluorescent proteins to cover almost the entire visible spectrum from 350nm (UV) to 750nm (Infrared). The unique characteristics and enhanced properties of fluorescent proteins have since enabled the non-invasive visualization of structural organization and dynamic processes in living cells and organisms by in vivo imaging[2]. This technology allows subjects to be monitored longitudinally over the course of a study to provide real-time information on internal changes.

Application of in vivo imaging can be seen across a variety of disciplines from cell biology and physiology to medicine and drug discovery. However, preparing in vivo samples with fluorescent-labeled proteins usually involves extensive training and specific experience working with live animals. Besides complicated sample preparation procedures, imaging the fluorescent proteins within small animals requires specialized instrumentation comprising of a high-performance camera, an excitation light source, emission filters and associated software. These requirements for preparing samples and acquiring images largely limits opportunities for students or entry level researchers to participate in research involving in vivo applications. The UVP GelSolo, an easy-to-use entry level imager,

is an ideal option for school laboratories, multi-user laboratories and practical trainings where users may not have experience with in vivo imaging or access to relevant samples. Researchers are quickly able to work with the UVP GelSolo and a silicone mouse sample to practice in vivo imaging techniques and get a taste of how in vivo imaging works in a laboratory setting. This application note will demonstrate how in vivo fluorescent imaging of a typical mouse model used in research labs is performed, substituting a fluorescent silicone mouse. The UVP GelSolo, equipped with a 5.0-megapixel camera, 8-48mm f/1.2 manual zoom lens, UV transilluminator, white and blue overhead LED light sources will be used to image the silicone mouse. The Blue LED light source, with a peak wavelength at 450nm, is a commonly used excitation source for green and red fluorescent protein labels used in in vivo imaging. Students at non-research institutions who might not have access to animal models but are interested in learning in vivo imaging can use the UVP GelSolo to practice the in vivo imaging technique. School laboratories can use these instruments for teaching purposes and can use this application note to supplement STEM training, especially for students pursuing research careers and advanced degrees.

Materials and Methods

The UVP GelSolo was used to image a fluorescent silicone mouse embedded with both red and green round plastic fluorescent rods. The fluorescent rods were chosen because green fluorescent protein (GFP) and red fluorescent protein (RFP) are commonly used in in vivo imaging. Their spectral profiles are shown in Figure 1. Blue LED Light can excite these fluorescent proteins, having a wavelength of 445nm to 470nm and a peak wavelength near 456nm, which lies in the GFP excitation spectrum. The emission filter for GFP has been selected to be 510nm to 523nm with a peak wavelength of 513nm. The emission filter designed for RFP has a wavelength range of 575nm to 640nm and a peak wavelength of 605nm.

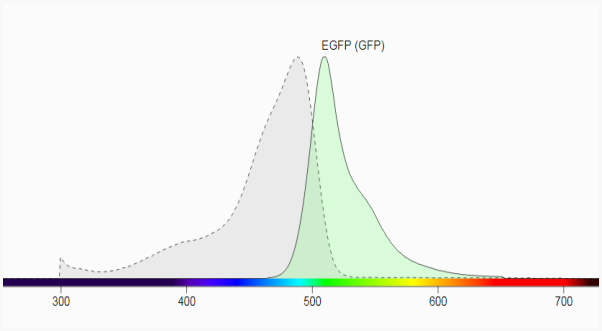

Figure 1. a) Excitation and emission spectrum of GFP (Source: Biotium)

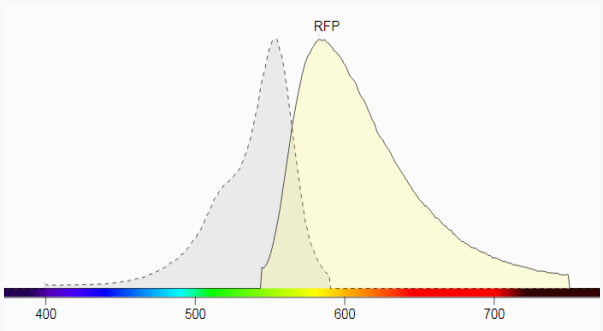

Figure 1. b) Excitation and emission spectrum of RFP (Source: Biotium)

Silicone Mouse Creation

The mouse model used for this experiment is made of colored silicone rubber. Red and white acrylic paints were applied to mimic the look of the true nude mouse model commonly used in fluorescent in vivo imaging. Before the silicone model was completely solidified, two pieces of 1cm red round plastic fluorescent rods were inserted into the head of the silicone mouse and two pieces of 1cm green-yellow round plastic fluorescent rods were inserted into the back of the mouse, to visually mimic tumors. The silicone rubber mouse then set for four hours and was subsequently imaged.

Silicone Mouse Imaging

The silicone mouse was imaged using the UVP GelSolo imager. Selections for exposure time, light sources and emission filter are detailed in Table 1.

Table 1: Capture Parameters for GFP and RFP Signal

Capture Parameters	White	GFP	RFP
Exposure Time	150ms	3 seconds	2 seconds
Excitation Light	White LED	Blue LED	Blue LED
Emission Filter	Clear	510nm-523nm, Peak 513nm	575nm-640nm, Peak 605nm

Image Acquisition Step-by-Step Procedure

1. Turn on the Epi White light on the system.
2. Place the silicone mouse in the darkroom.
3. Open the filter slot located on top of the system and place the 513nm and 605nm emission filters into the filter slots. Leave one filter slot empty for capturing white light images.
4. Open the VisionWorks software on the desktop and start Live View.
5. Adjust the manual lens until the previewed image is clear and sharp.
6. Click Capture to acquire a white light image.
7. Turn off Epi White light and turn on Epi Blue light.
8. Slide the filter tray until the 513nm filter is in place.
9. Start Live view and capture the green fluorescent image.
10. Switch the filter to 605nm and capture the red fluorescent image.
11. The Capture parameters for both GFP and RFP signals are recorded in Table 1 and captured images are shown in Figure 2.

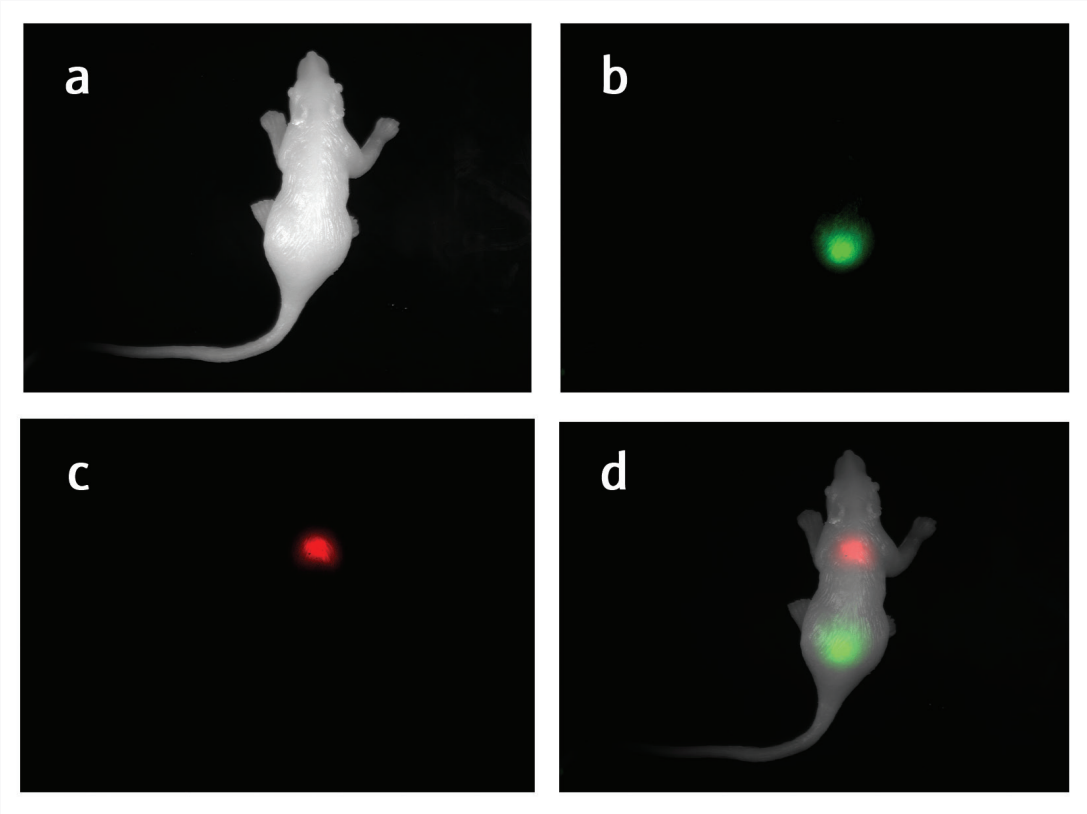

Figure 2: a) White image of the silicone mouse. b) GFP signal in the silicone mouse. c) RFP signal in the silicone mouse. d) Composited white channel, GFP channel and RFP channel to help visualize the GFP and RFP signal

Results

As displayed in Figure 2a-c, the silicone mouse and the individual GFP and RFP signals are clearly detected with the UVP GelSolo imaging system. Figure 2d reveals the location of each signal in a composite image. The results demonstrate distinct imaging of fluorescent signals within an organism in a non-invasive and easily reproducible manner.
In addition to the in vivo image acquisition technique, students and researchers will also be able to learn post-processing for captured in vivo images. As displayed in Figure 2b and c, the emission filter blocks unwanted wavelengths and allows only the selected wavelengths to pass through. The GFP emission filter allows fluorescent signal with wavelengths of 510nm to 523nm to pass through; and the RFP emission filter allows signals with 575nm to 640nm to pass through. These images confirm that the emission filter blocks unwanted signal efficiently and delivers a clean image. However, it is hard to tell where the signal is located. In this case, the pseudocolor and compositing functions in the software become helpful. The fluorescent signal will usually be pseudocolored depending on the spectrum of the fluorescent protein as shown in Figure 2b and c. Compositing the colored fluorescent images with a white light image helps to locate the fluorescent signals on the animal. For more in-depth analysis, intensity of the signal can be analyzed using the Area Density feature in the software.
As an easy-to-use system, the UVP GelSolo is designed for imaging and analysis without the need for any additional training. The user-friendly automation and straightforward features make the UVP GelSolo an ideal entry level imager for school laboratories, multi-user laboratories and practical trainings.

References

1. Day, Richard N and Michael W Davidson. “The fluorescent protein palette: tools for cellular imaging” Chemical Society reviews vol. 38,10 (2009): 2887-921.

2. Dmitriy M. Chudakov, Mikhail V. Matz, Sergey Lukyanov, and Konstantin A. Lukyanov. “Fluorescent Proteins and Their Applications in Imaging Living Cells and Tissues” Physiological Reviews 2010 90:3, 1103-1163

Analytik Jena US LLC
2066 W. 11th Street
Upland, CA 91786 · USA

Phone +1 909 946 3197
Fax +1 909 946 3597

info@us.analytik-jena.com
www.us.analytik-jena.com

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