In vivo imaging and monitoring of RFP+ tumors in mice using the Sapphire FL

Background/Introduction

In vivo fluorescent imaging of model organisms such as mice is a powerful approach allowing visualization of structures labeled with markers or dyes. Such imaging is particularly relevant to cancer research. The ability to image tumors in vivo makes it possible to assess tumor size and monitor changes in tumor size over time without sacrificing the animal. Measuring changes in tumor size over time allows study of tumor progression and of the effectiveness of anti-tumor treatments.

Options for in vivo imaging include X-rays, magnetic resonance imaging (MRI), computer tomography (CT), and positron emission tomography (PET), all of which require access to expensive, highly specialized equipment. The ability to image model organisms using a bench-top imaging system such as the Sapphire FL Biomolecular Imager from Azure Biosystems presents an accessible option to researchers which can be easily incorporated into their workflow.

This application note describes the in vivo imaging of tumors in living mice using the Sapphire FL including tumor measurement and tracking tumor growth over time.

Materials and Methods

Cancer model

Tumors were introduced in five 6-week-old female BALB/c mice (Jackson Laboratory) by injecting 4T1 tumor cells. The 4T1 cell line is derived from a mouse mammary gland tumor. 4T1 tumors resemble human breast cancer and mice with 4T1 tumors serve as an animal model of stage IV human breast cancer.

Mice were anesthetized with isoflurane and injected subcutaneously with $3x10^5$ 4T1 cells. Two control mice (RFP-) were injected with wildtype 4T1 cells (ATCC, catalog #4T1-CRL-2539) and three experimental mice (RFP+) were injected with 4T1/RFP stable cells expressing a fluorescent reporter, TurboRFP, driven by the CMV promoter (Cellomics, catalog #SC-1217). Mice were then monitored for any abnormal behavior.

Mice were injected with 4T1 cells on experimental day 1. Tumors at the injection site were imaged in vivo on days 4 and 11 after injection. After in vivo imaging, four mice (two RFP- and two RFP+) were sacrificed and their tumors excised and imaged ex vivo. The fifth mouse was

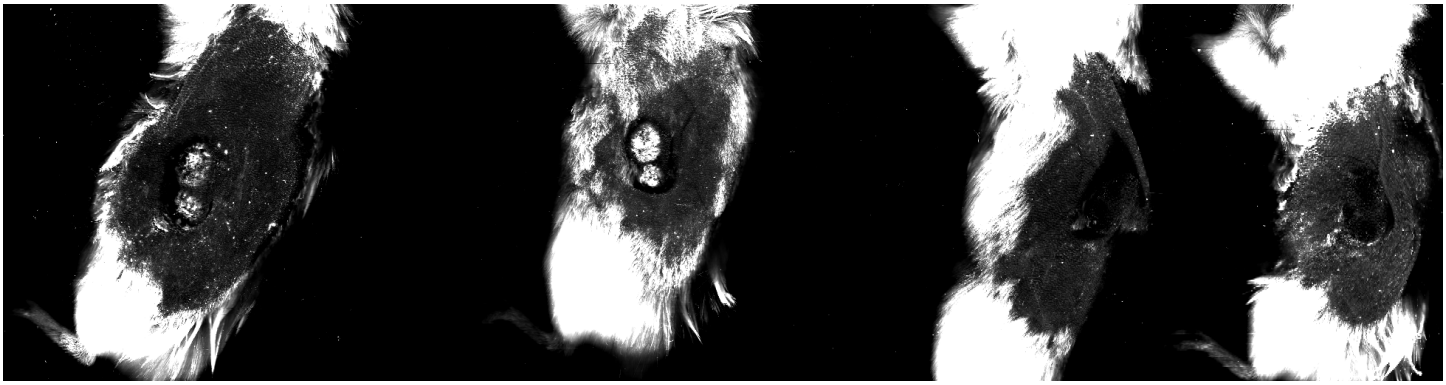


Figure 1. In vivo fluorescent images of 4T1 tumors in four mice on post-injection day 11.

sacrificed on day 14 and its tumor imaged post-mortem. This tumor was then excised and imaged again ex vivo.

Tumor length and width were measured on days 4 and 11 using an external caliper. Tumor volume was calculated using a modified ellipsoidal formula: Volume = ½(Length x Width^2).

In vivo tumor imaging

For in vivo imaging, mice were shaved and then prepped with Nair hair removal lotion (Church & Dwight Co., Inc.) (lotion was applied and wiped away after three minutes).

While imaging was performed within the Sapphire FL, mice were anesthetized with 2% isoflurane delivered through an Anesthesia Nose Cone (Azure, catalog #IS4050) and Anesthesia Tubing (Azure, catalog #IS4051). The oxygen/isoflurane flow rate was set to 2L/min.

The following conditions are recommended for imaging mice in vivo:

- Imaging time should not exceed one hour and should be limited to 30 minutes when possible. Heating should be provided for long-term imaging.
- Proper nose cones should be used to provide isoflurane

In vivo images of mouse tumors were captured on the Sapphire FL at 100 µm resolution using the 532 Standard Optical Module with intensity setting 6. The focus height was set to 0.00 mm.

Ex vivo imaging of mouse tumors

After excision, tumors were imaged on the Sapphire FL at 50 µm resolution using the 532 Standard Optical Module with intensity setting 8. The focus height was set to 0.00 mm.

Results

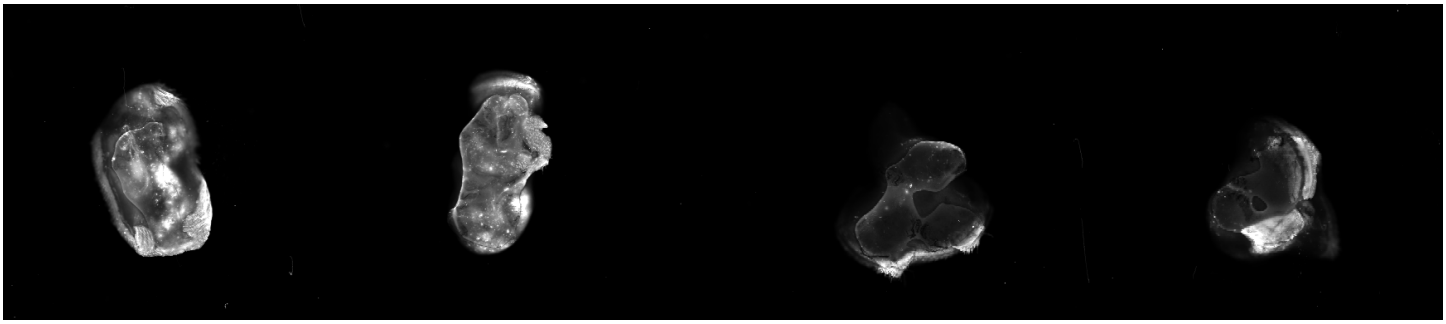
Both fluorescently labeled (RFP+) and non-fluorescent, negative control (RFP-) tumors are visible in in vivo images of whole mice captured on the Sapphire FL (Figure 1). Fluorescently labeled tumors are easier to visualize due to the expressed reporter gene. Average tumor volume was 66.59 mm^3 on post-injection day 4 and 443.88 mm^3 on post-injection day 11 (Figure 1, Table 1). Measurements were in vivo taken with calipers.

After in vivo imaging on post-injection day 11, four mice were sacrificed, and their tumors were excised and imaged on the Sapphire FL (Figure 2). The fluorescence of the tumors expressing fluorescent RFP is apparent compared to the control (RFP-) tumors (Figure 2).

The fifth mouse (Mouse ID #1), which had been injected with fluorescently labeled T41 cells (RFP+), was sacrificed on post-injection Day 14. Its tumor was imaged post-mortem, then excised and imaged ex vivo. The changes in this tumor over time are shown in Figure 3.

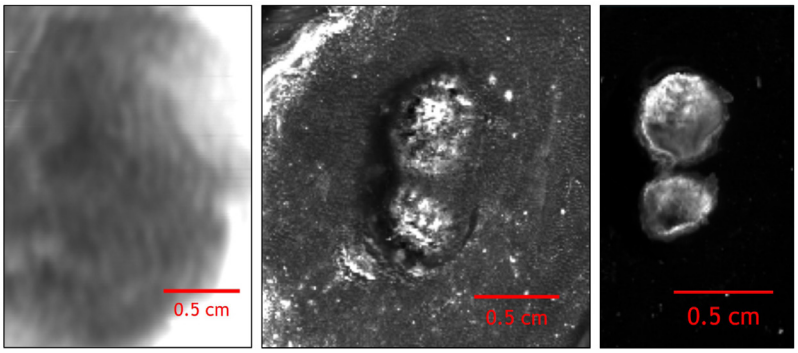
Groups	Mouse ID	Post-Injection Day 4			Post-Injection Day 11		
		Length	Width	Vol mm^3	Length	Width	Vol mm^3
4T1 RFP+	1	6.62	4.97	81.76	12.09	10.15	622.77
	2	6.65	4.21	58.93	10.94	7.22	285.14
	3	4.67	3.08	22.15	12.27	7.93	385.80
4T1 RFP-	4	7.1	4.9	85.24	10.32	10.09	525.33
	5	5.51	5.55	84.86	10.34	8.8	400.36
			Average	**66.59**		Average	**443.88**

Table 1. Tumor measurements on day 4 and day 11 after injection.



RFP+ RFP+ RFP- RFP-

Figure 2. Ex vivo tumors imaged on day 11 after injection (Mouse ID #s 2-5, in order from left to right).



Day 4 Day 11 Day 14

Figure 3. Tumor in Mouse #1 (RFP+) imaged on days 4, 11, and 14 after injection of 4T1/RFP cells.

Discussion/Conclusions

This experiment demonstrates the in vivo imaging of mouse tumors using the Sapphire FL Biomolecular Imager. Tumors expressing a fluorescent reporter are easily visualized using fluorescent detection. Unlabeled tumors can also be detected well enough to allow measurement and calculation of tumor volume.

The ability to image and measure tumors in live mice allows tumor size to be measured over time. This facilitates studies of disease progression and of the effectiveness of anti-cancer treatments.

Several attributes of the Sapphire FL imager make imaging of live animals possible. The Sapphire FL has integrated anesthesia ports; combined with appropriate tubing and nose cones, living mice can be imaged under anesthesia. In addition, the imaging platform of the Sapphire FL accommodates specimens with depth (up to 4 cm) allowing mice, plants, petri dishes, and other large biological samples to be imaged. In addition to fluorescent imaging as demonstrated here, the Sapphire FL can also image near-infrared fluorescence and chemiluminescence.

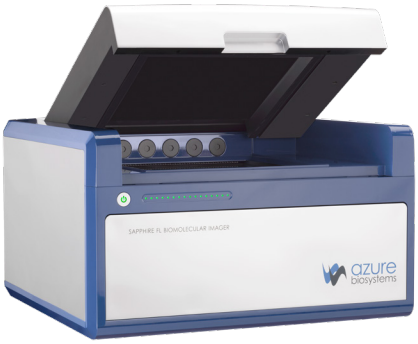


Figure 4. Sapphire FL is a laser scanner designed for multicolor fluorescent imaging, NIR fluorescent imaging, chemiluminescence, phosphor imaging, and more.

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