Focal Points

Application Note FP-169

UVP, LLC Upland, CA | (800) 452-6788 | (909) 946-3197 | info@uvp.com
Ultra-Violet Products Ltd. Cambridge UK | +44(0)1223-420022 | uvp@uvp.co.uk
Web Site: uvp.com

Detecting a Mouse Pancreatic Cancer Cell Line Using the iBox® Explorer™ Imaging Microscope

Introduction

iBox Explorer Imaging Microscope

In vivo imaging of small animals for pre-clinical research using fluorescent markers is a novel approach to visualizing cellular processes occurring at the micron level. There is growing interest in understanding what occurs at the level of tumor metastasis through direct visualization. For example, studies have shown that it is possible to use green fluorescent protein (GFP)- and red fluorescent protein (RFP)-labeled tumor cells to observe their behavior in the vasculature in vivo, such as tumor shedding and extravasation[i]. The iBox Explorer Imaging Microscope (UVP, LLC), a system that can visualize the tumor microenvironment both accurately and reliably, will expand the understanding of the mechanism of metastasis. In addition, the ability to view fluorescence ranging from the organ level down to the cellular level can add valuable information of in vivo processes.

In a pilot study, a pancreatic tumor tissue sample was imaged at two magnifications: 2.5x and 8.8x. A red fluorescent protein (RFP) filter was used to highlight the RFP-tagged cytoplasm and observe the distinct morphologic characteristics of individual cells as well as the tissue microenvironment ex vivo. Thus, use of the iBox Explorer can aid in the visualization of many cellular processes through fluorescence technology. Visualization can occur across a wide range of magnifications and spanning the visible to near infrared spectrum.

Materials and Methods

Visualization of a fluorescent pancreatic tumor cell line ex vivo

An XPA1 dual-color mouse pancreatic cancer cell line (AntiCancer, Inc.) was grown orthotopically, surgically resected, sectioned with a microtome at a 20-micron thickness, and transferred to a slide for imaging. Two tissue slices were selected from the center of the bulk tumor and placed side by side. The sample was then stored in a -20°C freezer until it was ready for imaging.

The BioLite™ Xe MultiSpectral Light Source (UVP, LLC), part of the iBox Explorer system, provided luminous excitation with a high level of intensity. For excitation of the RFP flurophore, a filter with a peak wavelength of 525nm and a bandpass of 502-547nm was placed in the filter wheel. A darkroom emission filter with a peak wavelength of 605nm and a bandpass of 580-630nm was used to highlight

the RFP-labeled cytoplasm. Images were captured using the iBox Explorer with VisionWorks®LS Software (UVP, LLC) controls. Illumination was directed coaxially and via side-lighting through the iBox Explorer's optics.

Post-editing

Captured images were edited using the VisionWorksLS software. Background fluorescence was removed using histogram adjustment and monochrome images were pseudo-colored according to the filtration specifications (RFP vs. GFP).

Results

Mouse pancreatic cancer cell line

Figure 1 shows 2.5x magnification of an illuminated specimen. The cellular orientation, tissue architecture and cytoplasmic morphology can be clearly delineated using an RFP emission filter. Further magnification (8.8x) of the same sample (Figure 2) reveals more details of the cytoplasmic structure, specifically areas of high concentration of RFP in addition to cytoplasmic extensions and cellular orientation.

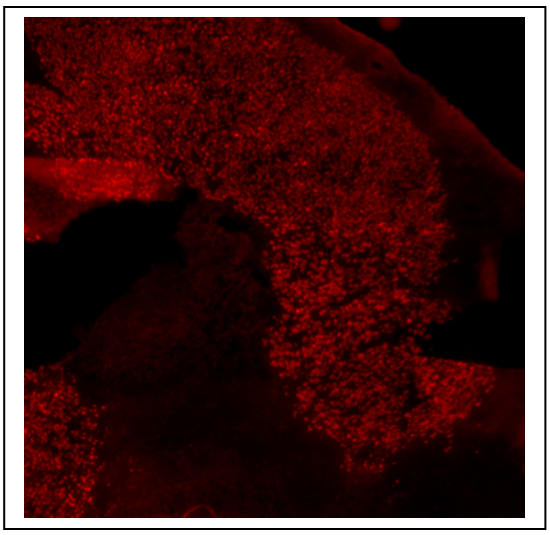

Figure 1. Mouse pancreatic cancer cell line XPA1. This histological specimen was viewed at 2.5x magnification, corresponding to a field of view (FOV) of 5.8 x 5.8mm. Coaxial lighting was used to illuminate the specimen with an exposure time of 11 seconds.

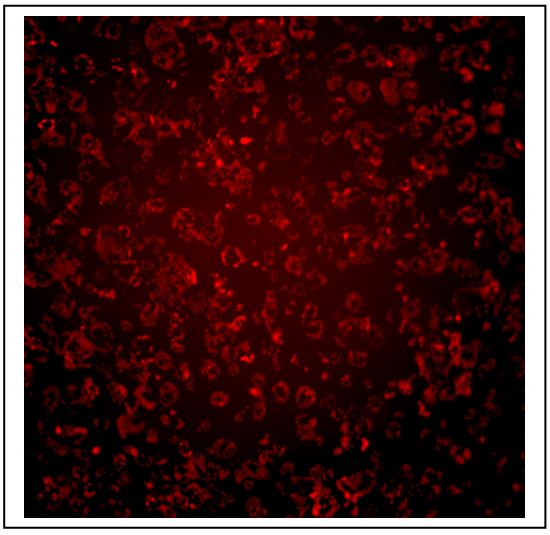

Figure 2. Mouse pancreatic cancer cell line XPA1. The corresponding FOV is 1.7 x 1.7mm. Further magnification to 8.8x reveals more detail of the tissue, cell orientation, areas of high RFP concentration, and cytoplasmic morphology. Side lighting was used to illuminate the sample with an exposure time of 4 seconds.

Conclusion

This application illustrates the functionality of the iBox Explorer in imaging fluorescently-labeled histology samples of tumor tissue. Using the iBox Explorer's high-specification, cooled camera and RFP filters, very crisp images of tumor cells at two different magnifications were obtained. These images clearly delineate cell and tissue borders as well as cellular morphology. The applications of the iBox Explorer Imaging Microscope can expand to include other aspects of cancer study, such as elucidating the nature of the tumor microenvironment, hematogenous trafficking of metastases, tumor angiogenesis and tumor shedding. In fact, with a resolution of 2.28 pixels per micron, the use of the iBox Explorer has the capability to image a wide variety of in vivo applications on the microscopic level.

[i] Jiang P, Yamauchi K, Yang M, Xu M, Maitra A, Bouvet M, Hoffman RM. Tumor cells genetically labeled with GFP in the nucleus and RFP in the cytoplasm for imaging cellular dynamics. Cell Cycle. 2006 Jun; 5(11): 1198-201.