



# Focal Points

Application Note FP-171



**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

## Intravital Imaging of a Mouse Ventral Skin-Flap Using the iBox<sup>®</sup> Explorer<sup>™</sup> Microscope

Tony Sanchez, MD, Life Scientist | UVP, LLC, Upland, CA

### Introduction

The field of cancer biology has been aided by intravital imaging systems which elucidate the microenvironment of tissues and the migration of cells. These imaging systems allow for visualization and quantification of cell movement, host and tumor interactions, intravasation and extravasation. The information gathered from quantitative and qualitative analyses of such studies sheds light on the largely unknown processes associated with migration of a tumor cell into the stroma and ultimately into distant sites. Understanding the mechanisms of invasion, intravasation, transendothelial migration and extravasation sheds light on the metastatic process and impacts the field of cancer therapeutics, especially since the vast majority of deaths associated with cancer are linked to metastasis from a primary lesion<sup>1</sup>.



*iBox Explorer Imaging Microscope*

Critical to the investigation of intravital migration imaging is the use of fluorescent proteins. In particular, tumor cells transfected with viral vectors encoding for genes of green fluorescent protein (GFP) and red fluorescent protein (RFP) have been used extensively in preclinical research<sup>2</sup>. These tagged cells faithfully express the fluorescent phenotype which can be detected by a fluorescence microscope. In a study of nude athymic mice injected with GFP/RFP-labeled human fibrosarcoma cells, the iBox Explorer Imaging Microscope (UVP, LLC) investigated the migration of cells within the venous circulation. Using an optical train that allows for imaging from the whole organ to cellular level (2.24 pixels/micron resolution), cancer cells were visualized migrating within the systemic circulation.

### Materials and Methods

#### *Cancer Cell Line and Animal Model*

The development of dual-colored human fibrosarcoma cancer cells (HT-1080, AntiCancer, Inc.) results in GFP expression in the nucleus and RFP expression in the cytoplasm<sup>3</sup>. To visualize intravascular tumor cell trafficking, a medium containing  $1 \times 10^5$  dual-colored HT-1080 cells was injected into the proximal region of the epigastrica cranialis vein of a nude mouse 12 to 18 hours prior to imaging. When the animal was ready for imaging, a skin flap was prepared<sup>4</sup>. A ketamine mixture was used to

anesthetize the animal and an arc-shaped incision was made in the abdominal skin to expose the epigastrical cranialis artery and vein. The inside surface of the skin flap was directly imaged.

### *Intravital Fluorescence Optical Imaging*

Two color channels were selected to image the transit of cancer cells within the venous circulation. High intensity excitation is supplied by the BioLite™ Xe MultiSpectral Source (UVP, LLC), a xenon excitation light with a blue excitation filter producing a peak wavelength of 475 nm and a bandpass of 20 nm (475/20). Illumination of the field was achieved through either side (epi) or coaxial (direct) lighting. Side lighting directs light at the stage through two fiber optic cables positioned 180 degrees relative to each other. Coaxial lighting transmits excitation light coaxially through the iBox Explorer's microscope optics to the sample stage. For images captured using coaxial lighting, a 515 long pass (515lp) emission filter was employed. An emission filter with a peak wavelength of 535 nm and a bandpass of 22 nm (535/22) was used to image green fluorescence. Finally, the red channel (the filter set that selects for red fluorescence) employed the 615lp emission filter.

### *Imaging Software Analysis*

Images captured using the iBox Explorer's high specification, scientific grade cooled CCD camera were edited using VisionWorks®LS Acquisition and Analysis software (UVP, LLC). Background fluorescence was removed using histogram adjustment and monochrome images were pseudocolored according to the filtration specifications (RFP and GFP). Images were then multiplexed to overlay multiple image channels.

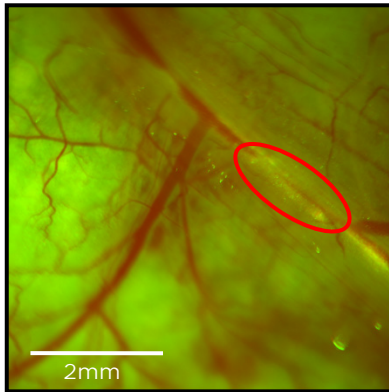
## Results

Real-time in vivo imaging of HT-1080 cancer cells was accomplished using the iBox Explorer at several levels of magnification. Figure 1 highlights the visual field at 2.5x, which corresponds to a field of view of 5.8 x 5.8 mm. The vasculature can be seen packed with HT-1080 cells (bright yellow) distal to the site of injection, especially at a point of bifurcation. Higher magnification, at 8.8x, highlights the behavior of small clusters of cells. These clusters are seen in the perivascular space in Figure 2. At the highest magnification, individual cell behavior is observed. In Figure 3, two cells are visualized in transit within a venule. Both cells' nuclei are at the leading edge of the cell facing in the direction of blood flow. Both cells are also adherent to the vessel wall.

## Conclusion

The iBox Explorer provides in vivo visualization from the macro to micro level, from whole organs to individual cells, and is an important tool for in vivo imaging of cancer cell biology. Using the skin-flap technique, dual-colored HT-1080 cells were visualized in real-time moving through the vasculature of a murine host. As a result, detailed information of intravascular transit of individual cells as well as large clusters of cells was able to be captured. Thus, visual information regarding cellular trafficking within the vasculature<sup>5</sup> as well as the movement of cells within the microenvironment of a tumor<sup>6,7</sup> has provided insight into the metastatic process. These results show that the iBox Explorer is both a powerful and easy-to-use tool that supports researchers in further elucidating cellular behavior within tumors.

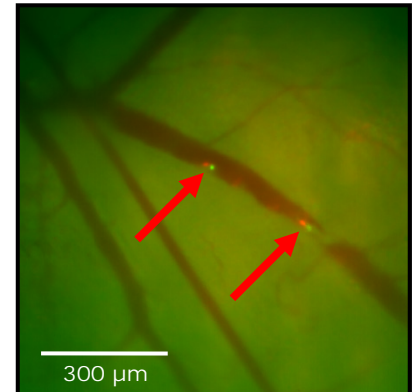
## Figures



**Figure 1:** A multiplexed image of a skin flap, 2.5x. The major vessel in the field corresponds to the epigastrica cranialis vein of a nude mouse. Clusters of dual-colored HT-1080 cells can be visualized moving through the vasculature in the right-most aspect of the field. The field of view is 5.8 x 5.8mm.



**Figure 2:** 8.8x magnification of a skin-flap. Migrated dual-colored HT-1080 cells (bright yellow) within a distal vessel have begun to extravasate. The field of view corresponds to a 1.7 x 1.7mm window.



**Figure 3:** High magnification (16.5x) of two HT-1080 cells migrating within the vasculature, each with a GFP-labeled nucleus and an RFP-labeled cytoplasm. The field of view is 900 x 900 microns.

## References

1. Weigelt, B., Peterse, J. L. & van 't Veer, L. J. Breast cancer metastasis: markers and models. *Nature Rev. Cancer* 5, 591–602 (2005).
2. 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.
3. Yamamoto N, Tsuchiya H, Hoffman RM. Tumor imaging with multicolor fluorescent protein expression. *Int J Clin Oncol*. 2011 Apr;16(2):84-91.
4. Yamauchi K, Yang M, Jiang P, Xu M, Yamamoto N, Tsuchiya H, Tomita K, Moossa AR, Bouvet M, Hoffman RM. Development of real-time subcellular dynamic multicolor imaging of cancer-cell trafficking in live mice with a variable-magnification whole-mouse imaging system. *Cancer Res*. 2006 Apr 15;66(8):4208-14.
5. Yamauchi K, Yang M, Jiang P, Yamamoto N, Xu M, Amoh Y, Tsuji K, Bouvet M, Tsuchiya H, Tomita K, Moossa AR, Hoffman RM. Real-time in vivo dual-color imaging of intracapillary cancer cell and nucleus deformation and migration. *Cancer Res*. 2005 May 15;65(10):4246-52.
6. Wyckoff JB, Wang Y, Lin EY, Li JF, Goswami S, Stanley ER, Segall JE, Pollard JW, Condeelis J. Direct visualization of macrophage-assisted tumor cell intravasation in mammary tumors. *Cancer Res*. 2007 Mar 15;67(6):2649-56.
7. Kedrin D, Gligorijevic B, Wyckoff J, Verkhusha VV, Condeelis J, Segall JE, van Rheenen J. Intravital imaging of metastatic behavior through a mammary imaging window. *Nat Methods*. 2008 Dec;5(12):1019-21. Epub 2008 Nov 9.