



# Focal Points



## Application Note FP-172

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## Monitoring Tumor Progression Using the iBox<sup>®</sup> Scientia<sup>™</sup> Imaging System

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### Introduction

Molecular imaging offers benefits over *ex vivo*, cross-sectional techniques like immunohistochemistry or H&E staining due to the non-destructive nature of fluorescence imaging. By preserving the native environment of the target, repeated experimentation of a particular animal or tissue sample can be conducted. With the appropriate reporter, such as green fluorescent protein (GFP) or red fluorescent protein (RFP), molecular imaging can localize a biological phenomenon to a particular location and track that process over time. Thus, by targeting a gene with a genetic reporter such as fluorescent proteins or downstream expression with a fluorophore conjugate, *in vivo* fluorescence imaging can yield anatomical, functional and molecular data.



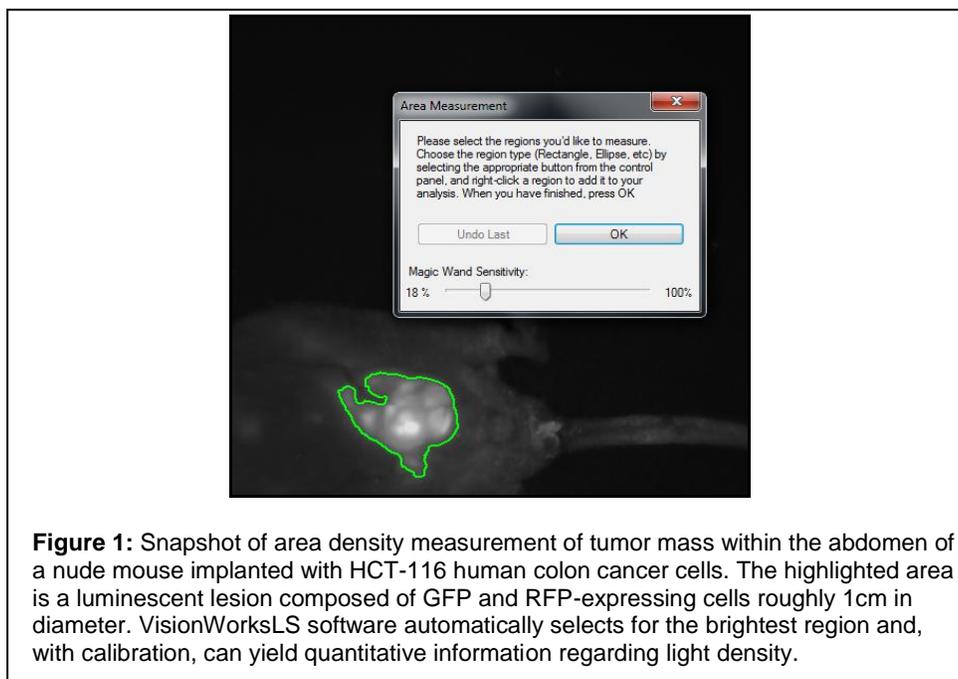
Molecular imaging has many applications that span multiple therapeutic areas. One application with great utility in cancer biology is the tracking of tumor lesions implanted within mice. Monitoring of tumor growth over time is a useful tool for observing the efficacy of chemotherapeutic agents in either shrinking or stabilizing tumor lesions. In a recent experiment, mice were implanted with a cancer cell line expressing fluorescent proteins and imaged on a weekly basis. Through the use of the iBox Scientia Imaging System (UVP, LLC), tumor lesions can be visually tracked and quantified with a high quantum efficiency camera, powerful light source, quality lenses and interchangeable filter sets to image fluorescence across a wide spectral range from 450 nm to near infrared (NIR being 700nm and greater).

### Materials and Methods

Four adult nude female mice (AntiCancer, Inc., San Diego, CA) were injected with  $3 \times 10^6$  human colon HCT-116 dual-color cancer cells directly into the peritoneal cavity. Once the implanted tumor lesions reached 1mm in diameter, all mice were monitored and GFP/RFP fluorescence images were captured weekly for a total of six weeks to monitor the growth of the implanted cancer cells.

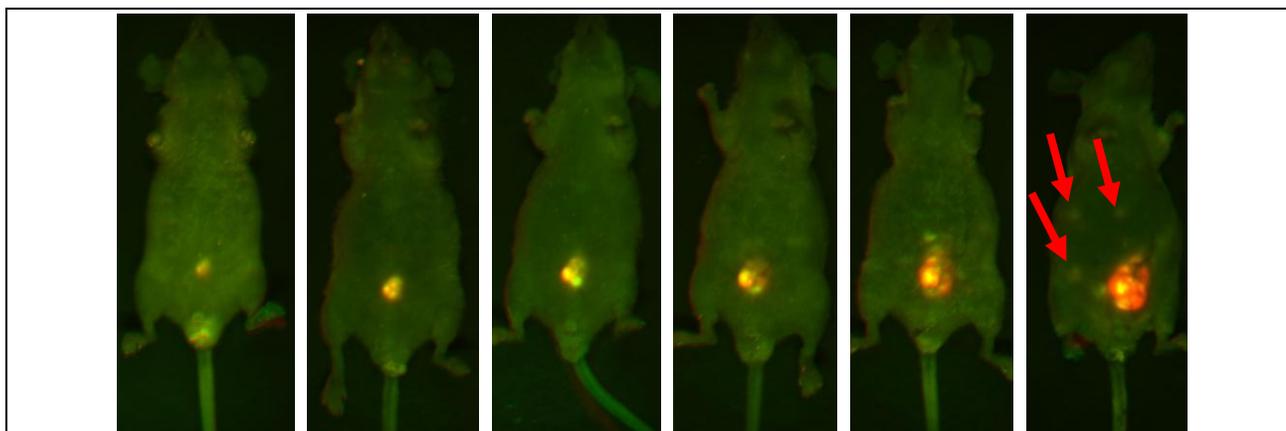
Two color channels were selected to image the growth of tumors within each mouse. All samples were excited using the 150 watt halogen BioLite<sup>™</sup> MultiSpectral Light Source (UVP, LLC) and color-specific excitation filters. A blue excitation filter with a peak wavelength of 475nm and a bandpass of 40nm (475/40) was used to excite GFP. A green excitation filter (525/45) was used to excite RFP. To select for green fluorescence, an emission filter with a peak wavelength of 535nm and a bandpass of 44nm (535/44) was used. The red channel employed the 605/50 filter. Images were captured using the BioChemi 500, a 4.2 MP cooled monochrome CCD camera (UVP, LLC).

Images captured using the BioChemi 500 camera were acquired and multiplexed using VisionWorks®LS software (UVP, LLC). Background fluorescence was removed using histogram adjustment and monochrome images were pseudocolored according to the emission specifications (RFP and GFP). Images were multiplexed in order to overlay multiple images. Area density measurements were acquired using multiplexed monochrome images. Finally, tumor size was determined by taking the square of the pixel density for each lesion at a given time interval (Figure 1).



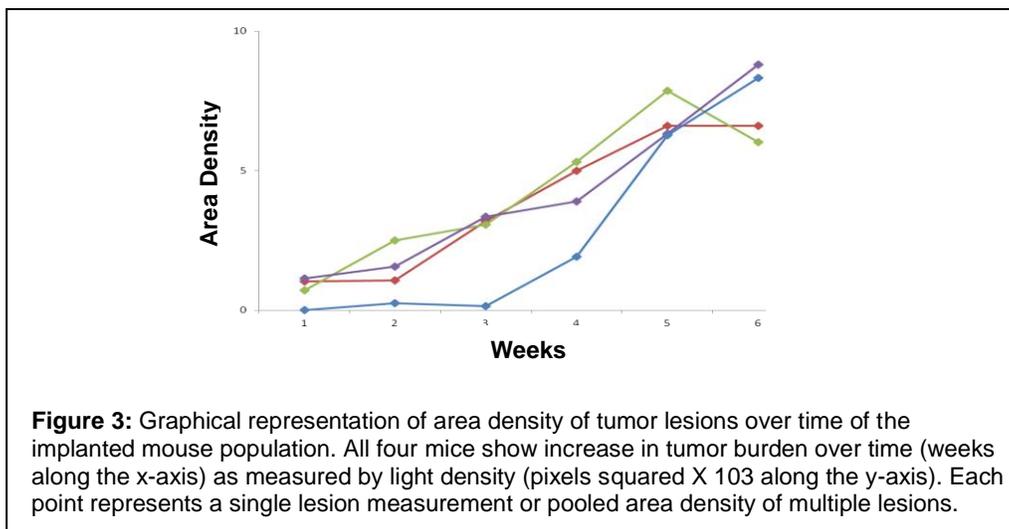
## Results

Figure 2 shows the progression of one tumor lesion over the course of several weeks. In the last image, metastatic lesions are observed emerging within the peritoneum (smaller areas of fluorescence).



**Figure 2:** Representative mouse highlighting the progressive increasing luminosity of an implanted lesion in the peritoneum. Each image was captured using both GFP and RFP filters and then multiplexed. The intense fluorescent signal shown in the implanted tumor is significantly brighter than the surrounding tissue. Note the formation of metastatic lesions beginning to develop distal to the site of implantation and becoming visible within the abdomen in the final slide (red arrows).

Pooled data for tumor measurements can be seen in Figure 3. Tracking the lesions over time reveals a relatively linear increase in tumor burden with an average maximum area density of  $7.4 \times 10^3$  pixels squared.



## Conclusion

In this experiment, implanted colon cancer cells, HCT-116, were tracked over time within the abdominal cavity of mice. The growth of tumors showed a steady increase in area density within all four mice, reaching a peak at six weeks. This steady increase in growth was measured using the area density function of VisionWorksLS software, and correlates strongly with volume.<sup>1</sup>

The ability to non-invasively monitor functional or molecular changes offers advantages over immunohistochemical and other cross-sectional techniques due to the non-destructive nature of in vivo imaging. A disease process can be tracked by simply transfecting a cell or organism with a fluorescent protein or tagging a downstream molecule with a fluorophore conjugate and monitoring the changes in real time.

The iBox Scientia is developed to image fluorescence-labeled cells by maximizing signal-to-noise through the use of a high resolution cooled CCD camera, highly sensitive optics, a directed illumination source and a large selection of excitation and emission filters. Given the breadth of fluorescent proteins and fluorophores available to researchers, applications using the iBox in pre-clinical studies include, but are not limited to, cancer biology, cardiovascular disease and immunology.

## References

1. J. of Surgical Research, Vol. 113, No. 1, July 2003