



Focal Points

Application Note FP-175



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Imaging of Fluorescent Microspheres Using the iBox[®] Explorer[™] Imaging Microscope

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Introduction

Fluorescent microspheres are round spherical micro particles that typically range from 1 to 1000 μ m in diameter. They emit bright colors when illuminated by excitation sources spanning from the UV to IR regions of the spectrum.

Fluorescent microspheres are loaded with a variety of proprietary dyes, making them the brightest fluorescent microspheres available.

Typical applications of fluorescent microspheres include medical imaging, biomedical technology research, qualification and validation of medical devices, and biomedical diagnostics. Fluorescent microspheres have been used extensively in

fluorescence microscopy as markers for cellular antigens, as well as biological tracers to investigate phagocytic processes and to determine blood flow in tissues. They have also been utilized as standardization reagents for flow cytometry.



iBox Explorer Imaging Microscope

Materials and Methods

The sample was prepared by loading 20 μ L of Invitrogen's carboxylate-modified microspheres, 15 μ m, Red fluorescent (580/605) on a glass slide.

Fluorescence Microscopy

The sample slide was excited with the BioLite[™] Xe MultiSpectral Source (UVP, LLC), a xenon excitation light with a green excitation filter producing a peak wavelength of 560nm and a bandpass of 54nm (560/54). Illumination of the field was achieved through coaxial (direct) lighting. Coaxial lighting transmits excitation light coaxially through the iBox Explorer's microscope optics to the sample stage (UVP, LLC). For images captured using coaxial lighting, a 515 long pass dichroic mirror was employed for optimum illumination of the sample. An emission filter with a peak wavelength of 605nm and a bandpass of 50nm (605/50) was used to selectively image red fluorescence.

Imaging Software

Images were captured using the iBox Explorer's high specification, scientific grade cooled CCD camera and 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 color specifications.

Results and Conclusions

Imaging of fluorescent microspheres was achieved with iBox Explorer Imaging Microscope at different levels of magnifications. Figures 1 and 2 illustrate higher resolution images of fluorescent microspheres (15 μ m) at 8.8x and 16.5x magnifications which correspond to a field of view of 1.7 x 1.7mm and 0.9 x 0.9mm respectively. As the average size of tumor cells range from 13-20 μ m, these results successfully validate the imaging proficiency of iBox Explorer down to the single cell level.

The iBox Explorer is a powerful resource in both in vivo and ex vivo research. This study further complements the multiple application capabilities of iBox Explorer from in vivo and ex vivo research to medical diagnostics and flow cytometry applications.

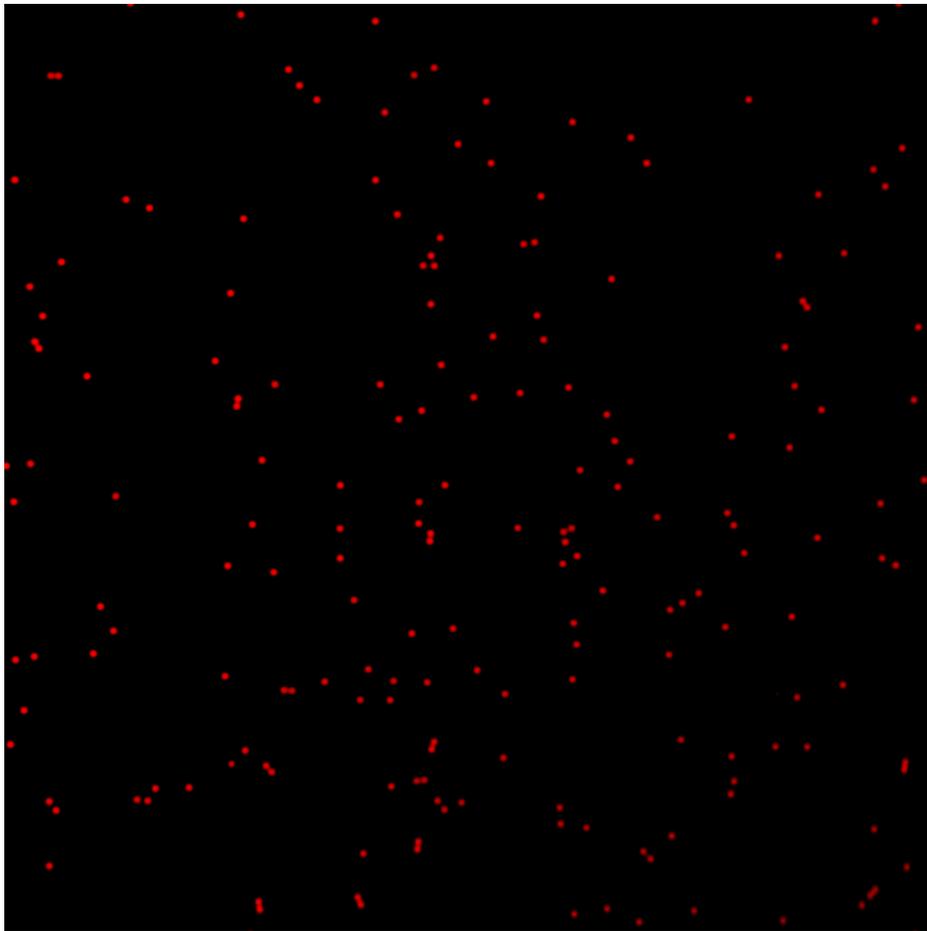


Figure 1: Fluorescent microspheres imaged with iBox Explorer at 8.8x magnification corresponds to a field of view of 1.7 x 1.7mm window.

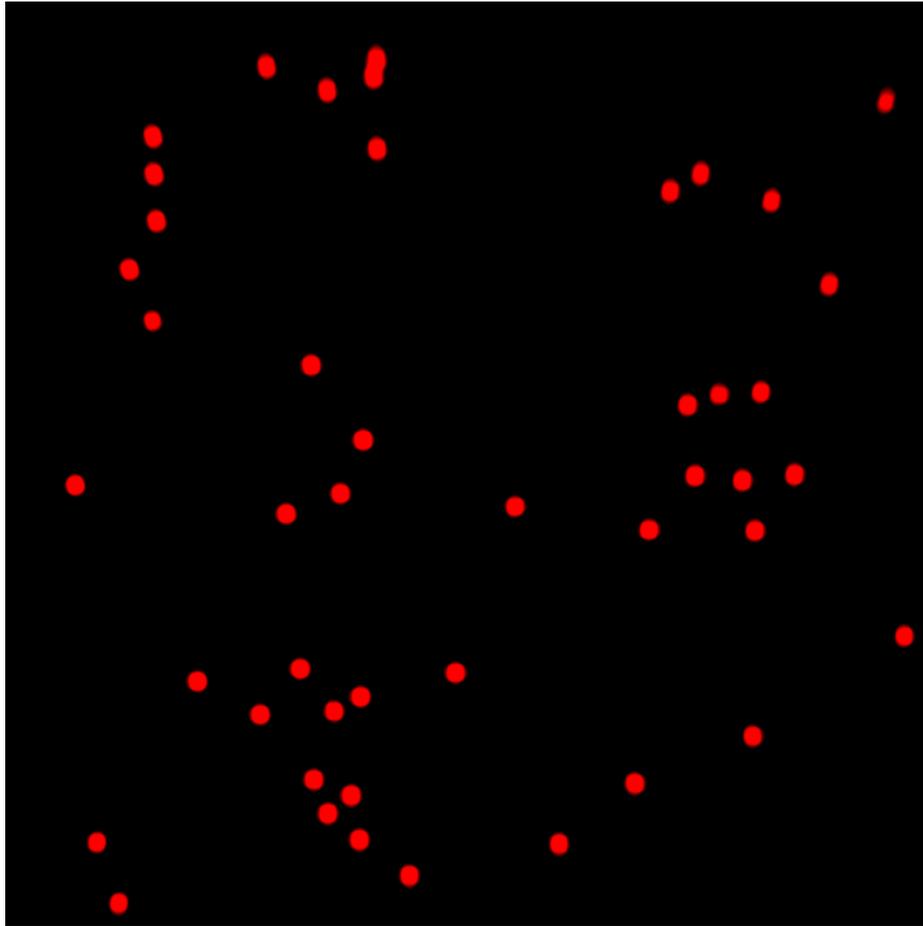


Figure 2: Fluorescent microspheres imaged with iBox Explorer at 16.5x magnification corresponds to a field of view of 0.9 x 0.9mm window.