Focal Points

Application Note FP-173

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Imaging of Near Infrared Dyes In Vivo

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Near Infrared (NIR) fluorophores have been developed to overcome the limitations of light propagating through biological material and have been successfully used for in vivo applications. Expansion of NIR dye development will continue to produce more robust fluorophores, thereby addressing the limitations of current dyes. Limitations include photostability, low quantum yield and stability within aqueous media[i].

iBox Explorer Imaging Microscope

Both the iBox® Scientia™ Imaging System and iBox® Explorer™ Imaging Microscope (UVP, LLC) are developed to detect low fluorescent signals across a wide spectral range within living animals. Through the use of high quantum efficiency cameras, detectable emission spectra extend into higher wavelengths, including NIR. In addition, the BioLite™ Xe MultiSpectral Light Source, an external xenon arc lamp, generates intense light across the UV to NIR range. Thus, the iBox line of imagers provides an ideal tool for the study of NIR fluorophores within small animals.


iBox Scientia Imaging System

Figure 1 shows a multiplexed image of a mouse excited with the BioLite Xe and captured using the iBox Scientia. Both mice in the image were injected with an antibody-conjugated NIR dye, Qdot®800 (Life Technologies, Grand Island, NY). Distribution of the dye was monitored 24 hours later.

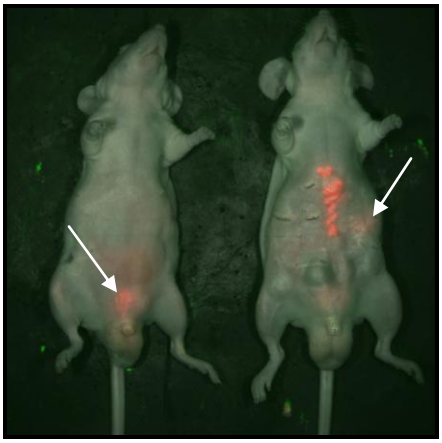

Figure 1: Qdot800 fluorophore-antibody conjugate distribution within two mice using the iBox Scientia. Both animals were implanted with a cancer cell line four weeks prior to experimentation. Areas of red show aggregation of the fluorophore-antibody conjugate. Bright field, GFP (503-523nm) and NIR (800 long pass) color channels were multiplexed to generate the image shown. Note areas of intense fluorescence of the suture material (right) as well as the conjugate in the abdominal cavity (arrows).

Using the iBox Explorer Imaging Microscope, Figure 2 illustrates a higher magnification of an exposed mouse abdominal cavity into which a green fluorescent protein-tagged cancer cell line was implanted.

NIR dyes overcome three properties of photon transmission within tissue that limit observation of emitted light: absorption, autofluorescence and scatter. Light in the visible spectrum, particularly at shorter wavelengths (400-600 nm), is heavily absorbed by tissue. This absorption limits the penetration depth to which excitation can be used to image a magnified target to a few millimeters. The two greatest absorbers of visible light include both oxy- and deoxy- hemoglobin (Figure 3). Absorption of NIR emitted light, however, is 1-2 orders of

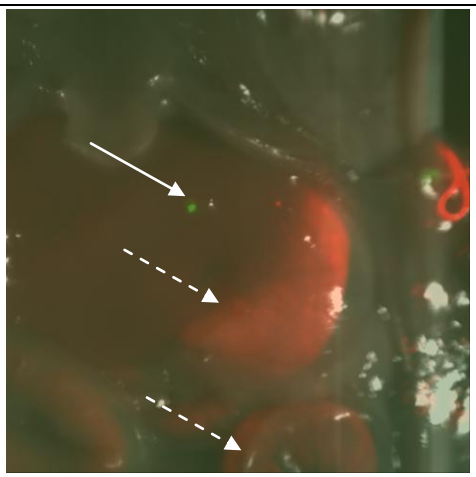

Figure 2: View of fluorophore-antibody conjugate distribution within an exposed abdomen captured with the iBox Explorer Imaging Microscope. Note the presence of a GFP-expressing tumor lesion within the liver (solid white arrow) and collection of conjugate (red) within the abdominal organs (spleen and loops of bowel, dashed white arrows). The image represents three color channels which were multiplexed using VisionWorks®LS software (UVP, LLC).

magnitude less when compared to visible light. In addition, imaging with NIR dyes eliminates the background noise typically present due to autofluorescence caused by endogenous biomolecules distributed within cells and tissues (Figure 3). Finally, light scatter within tissue is minimized at higher wavelengths. Therefore, a larger percentage of both excitation and emitted light reach the target and camera, respectively, using NIR dyes.

UVP's iBox line of imagers is well equipped to detect fluorescent signals above background levels and across a wide spectral range. Both systems, the iBox Scientia and iBox Explorer, are designed to complement the current in vivo fluorophore technology and are useful for imaging NIR dyes in addition to widely used fluorescent proteins.

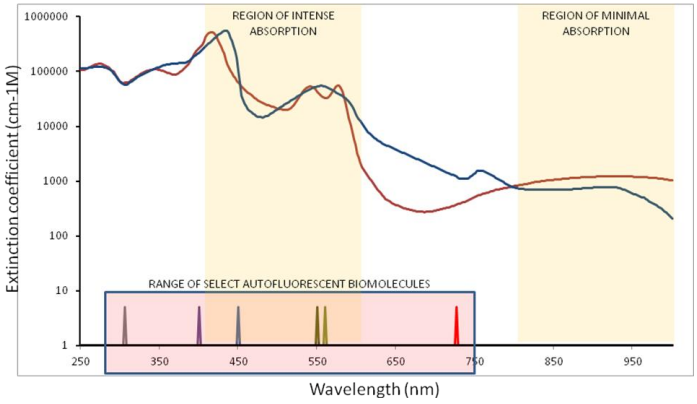

Figure 3: Absorption spectra of oxy- and deoxy-hemoglobin, adopted from Optical Clearing of Tissues and Blood, SPIE 2005. Absorption of light by hemoglobin is greatest at lower wavelengths. The oxyhemoglobin curve is in red and the deoxyhemoglobin is in blue. The box in the lower left represents the most common biomolecules present in plants and animals, and illustrates the wavelength at which autofluorescence occurs. From left to right: NAD(P)H, collagen, riboflavin, tyrosine, melanin and chlorophyll.

[i] Ghoroghchian PP, et al. In vivo fluorescence imaging: a personal perspective. Wiley Interdiscip Rev Nanomed Nanobiotechnol. 2009; 1(2): 156-67.