

Near Infrared Technology and Optical Agents For Molecular Imaging

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ABSTRACT

Optical imaging is a rapidly developing biomedical technology that enables the examination of cellular processes in the context of a living animal. While several optical imaging modalities employing fluorescent proteins or bioluminescent reporter systems have shown utility in life science research, targeted ligands labeled with near infrared emitting fluorochromes have the additional potential to translate to human clinical use. Described here is a brief overview of the optical imaging technologies currently in use with a particular focus on the use of ligand-targeted near infrared fluorochromes as imaging agents.

INTRODUCTION

Optical Imaging consists of several technologies which can be used to non-invasively interrogate an animal model for the progression of a disease, determine the effects of drug candidates on the target pathology, assess the pharmacokinetic behavior of a drug candidate, compare candidate drugs for target binding affinity, and develop biomarkers indicative of disease and treatment outcomes. Optical Imaging is comprised of three approaches which offer the potential for high sensitivity and good spatial resolution.

Bioluminescent imaging is an indirect technique based on the expression of firefly luciferase from recombinant plasmids inserted into hybrid cell lines that can be transplanted into animals. In some cases the gene is constitutively expressed and the introduction of luciferin, either injected or inhaled, allows the

production of light in the target cells. In a second case, the luciferase gene can be activated by chemical induction of the promoter controlling the gene's expression with the subsequent generation of light upon contact with luciferin. This approach requires the creation of modified cells, and analysis is limited to cells expressing the luciferase gene. Thus it is not translatable to clinical practice.

In a second approach, cells engineered to express fluorescent proteins can be used to mark tumors. The recombinant tumor cells can be implanted into animals and, following excitation with an appropriate light source, the fluorescence from the expressed fluorescent proteins can be detected by means of a CCD camera. The excitation and emission wavelengths of commercially available fluorescent proteins are generally in the visible region of the spectrum. As discussed in this publication, this region can be

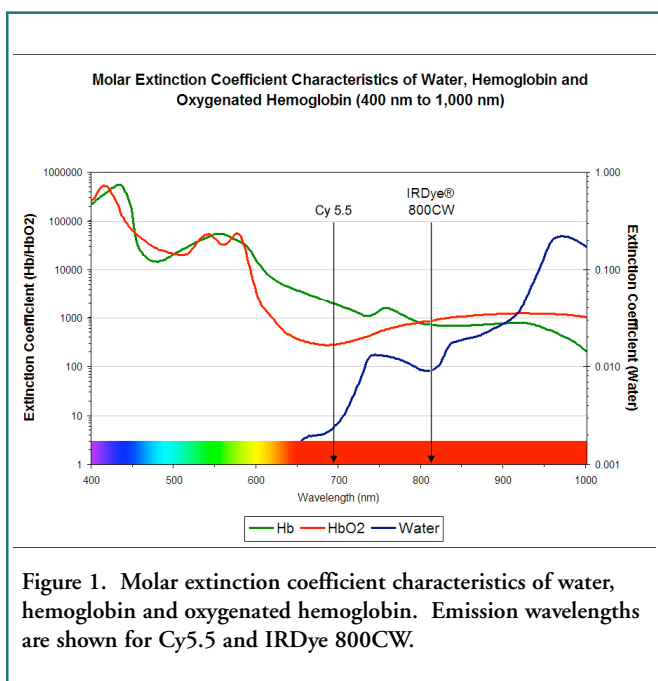
compromised by tissue autofluorescence and non-specific background. Also, this method requires transgenic cell lines and is thus limited in its ability to detect a variety of potential targets. Similar to bioluminescent imaging, this method cannot translate to the clinic.

A more flexible and direct approach employs targeted imaging agents consisting of antibodies, receptor binding ligands, small molecules, or peptides labeled with fluorochromes. The fluorescent labels can be visualized by excitation with an appropriate light source and the emitted photons captured via a CCD camera or other optical detector.

For fluorescent imaging, there are generally three parameters which are used to characterize the interaction of photons with tissues. The three processes are light absorption, light scattering, and fluorescent emission. A fundamental consideration in optical imaging is maximizing the depth of tissue penetration. Absorption and scattering of light is largely a function of the wavelength of the excitation source¹. In general, light absorption and scattering decreases with increasing wavelength². Below 700 nm, tissue absorption results in small penetration depths of only a few millimeters¹. Thus in the visible region of the spectrum, only superficial assessment of tissue features is possible.

The light absorption is due to oxy- and deoxyhemoglobin, melanin, lipid, and other compounds found in living tissue^{2,3,4}. These compounds cause tissue autofluorescence throughout the visible spectral range up to approximately 700 nm^{5,6}. Because the absorption coefficient of tissue is considerably smaller in the near infrared region (700 nm-900 nm), light can penetrate more deeply into the tissues to depths of several centimeters^{3,7,8}.

A key to enabling optical imaging has been the development of suitable NIR fluorochromes with high molar extinction coefficients, good quantum yields and low non-specific tissue binding. There are several commercially available candidate fluorochromes



which can be used for optical imaging, including IRDye[®] 800CW, IRDye[®] 680, IRDye[®] 700DX, Cy5.5, and Alexa[®] Fluor 750. Quantum dots have been used; however, their size often precludes efficient clearance from the circulatory and renal systems and there are questions about their long-term toxicity⁴.

A number of studies have been published using NIR dyes. Two of the dyes commonly used for optical imaging are Cy5.5 and IRDye[®] 800CW. Cy5.5 has been used in the past primarily due to the lack of other candidate dyes suitable for imaging. Cy5.5 has excitation/emission maxima at 675 nm/694 nm, making it a borderline candidate labeling agent^{1,3}. In contrast, a recently developed fluorochrome, IRDye 800CW, has its excitation/emission maxima at 785 nm/810 nm precisely centered in the region known to give optimal signal to background in optical imaging (see **Figure 1**)^{1,8}.

In a cell based assay system using IRDye 800CW labeled secondary antibodies to assess cell signaling pathways, IRDye 800CW was shown to yield superior signal-to-background ratios and enabled quantification of low levels of protein phosphorylation⁹.

In contrast, the signal to background using Cy5.5-labeled secondary antibodies was too low to allow its use in the assay (data not shown). Further experiments showed that Cy5.5 exhibited a high level of non-specific binding to cells (data not shown). This observation, coupled to the fact that Cy5.5 is outside the optimal NIR region, makes it less suitable for imaging studies requiring high signal to background.

The performance of IRDye 800CW has been compared to radiochemical detection in animal studies. Using gamma scintigraphy and NIR imaging, Houston *et al.* compared the ability of a cyclopentapeptide dual-labeled with 111 indium and IRDye 800CW to image $\alpha v\beta 3$ -integrin positive melanoma xenografts¹⁰. The tumor regions were clearly delineated by optical imaging of the IRDye 800CW signal. In contrast, the tumor boundaries could not be identified by scintigraphy due to high noise levels.

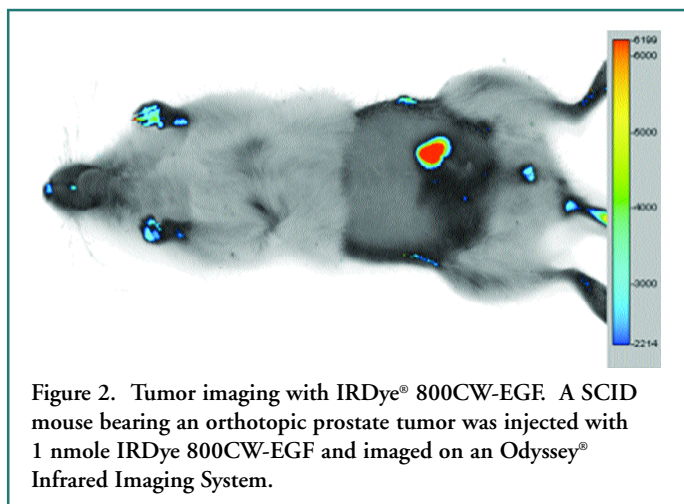


Figure 2. Tumor imaging with IRDye[®] 800CW-EGF. A SCID mouse bearing an orthotopic prostate tumor was injected with 1 nmole IRDye 800CW-EGF and imaged on an Odyssey[®] Infrared Imaging System.

We have successfully used IRDye 800CW conjugated to epidermal growth factor (IRDye 800CW-EGF) as an optical agent for imaging tumor progression¹¹. Figure 2 shows an example of a prostate tumor-bearing SCID mouse injected with IRDye 800CW-labeled EGF. The IRDye 800CW-EGF showed good sensitivity with very low background from autofluorescence or non-specific binding.

For animal imaging, targeted NIR-labeled ligands can be used to visualize virtually any pathology without the need for engineered cell lines as required for

imaging by bioluminescence or fluorescent proteins. In addition, NIR optical imaging has a further advantage in that it has the potential to translate into the clinic. Several NIR imaging instruments for use on humans are currently under development. De Grand and Frangioni have described a prototype NIR optical imaging system for use with NIR fluorochrome-labeled optical agents in non-invasive intraoperative imaging procedures¹². The authors envision the system being eventually used for image-guided cancer resection with real-time assessment of surgical margins, sentinel lymph node mapping, and intraoperative mapping of normal and tumor vasculature. Furthermore, Gurfinkel *et al.*, Hawrysz and Sevick-Muraca, and Chen *et al.*, have described NIR-based imaging instruments directed at the early detection of breast cancer^{3, 13, 14}. These instruments potentially could be used for guiding fine needle biopsies and sentinel lymph node monitoring during surgery.

There are several biological barriers that should be taken into consideration when using NIR dye-labeled optical probes. The probe must be able to reach its target in sufficient concentration and with sufficient binding affinity that it can be imaged. In this respect, optical probes are similar to a pharmaceutical agent in that considerations of absorption, distribution, metabolism, excretion, and toxicity need to be evaluated. In addition to non-specific binding, trapping, rapid excretion, and metabolic effects, there are delivery barriers to be overcome. For example, the size and characteristics of the dye labeled ligand may prevent it from crossing the blood-brain barrier. However, the combination of an NIR labeling agent such as IRDye 800CW and NIR-based imaging instruments used for both small animal and clinical imaging has the potential to provide both good spatial resolution and sensitive detection of targeted molecules. In the future, NIR imaging technology should augment current imaging technologies and provide a means of characterizing disease processes and monitoring therapeutic efficacy, enabling earlier detection through the identification of molecular biomarkers in both the research laboratory and the clinic.

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