

# Optical Molecular Imaging: Time Domain advantages with eXplore Optix™

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## Executive Summary

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Molecular imaging is defined as the *in vivo* characterization of cellular molecular events involved in normal and pathologic processes. With the advent of optical molecular imaging, specific molecules, proteins and genes may be tagged with a luminescent reporter and visualized in small animals. This powerful new tool has pushed *in vivo* optical imaging to the forefront as it allows for direct determination of drug bio-distribution and uptake kinetics as well as an indicator of biochemical activity and drug efficacy. In fact, the development of molecular imaging technologies has been tagged by the National Cancer Institute as one of six extraordinary opportunities for development.<sup>(1)</sup>

Up to now, two optical modalities have been used for *in vivo* molecular imaging: intensity based bioluminescence systems and intensity based fluorescence systems. Although these systems can provide analysts with pertinent data, they are not sufficient for many types of *in vivo* applications. However, the application of ART's proprietary Time Domain (TD) optical technology to molecular imaging has led to a unique system that can meet these demands. This innovative small animal molecular imaging system, eXplore Optix™, not only provides enhanced information but leads to superior 3D imaging and fluorophore quantification. New heights in molecular imaging are attainable with optical TD technology, including:

- depth and concentration recovery of fluorescently tagged cells and proteins from planar imaging,
- fluorescence lifetime information for fluorescent species confirmation and quantification,
- recovery of tissue scattering and absorption characteristics for robust quantification, and
- 3D tomographic imaging

Fluorescence lifetime data may be used to gain insight into tissue pH, oxygenation and other information pertaining to the local biochemical environment of the fluorophore, and precision 3D imaging with accurate quantification of inclusion position, size and concentration. It can be concluded that this type of imaging system yields much more informative data and permits an enhanced understanding of biochemical reactions and drug efficacy *in vivo*.

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# 1

## INTRODUCTION

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The tracking of molecular and cellular events in small animals is a means of quantifying drug efficacy, side effects, toxicity and disease progression. The ability to monitor these events is, however, limited by traditional methods that require a time-consuming analysis of multiple animals and the extrapolation of *in vitro* results to the *in vivo* situation.

In order to improve the reliability of these animal models and decrease the time and costs associated with such studies, ART Advanced Research Technologies Inc (ART) has developed a small animal molecular imaging system called eXplore Optix™. This system permits the detection, localization and tracking of biomolecular events, non-invasively and in real-time, using proprietary Time Domain (TD) optical technology. To date, optical molecular imaging systems on the market have been based on Continuous Wave (CW) imaging technologies. In the following sections, the advantages of TD molecular imaging are compared to CW imaging in terms of theory (in Sections 2 and 3) and in terms of system performance (in Section 4). Since eXplore Optix is the sole TD optical molecular imager available, whereas there are a number of CW instruments on the market, technical comparisons will be based on the capabilities of eXplore Optix versus two main classes of CW imaging technologies: bioluminescence and CW fluorescence intensity.

# 2

## TD OPTICAL IMAGING

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In its simplest form, optical medical imaging involves illuminating the part of the body of interest with non-ionizing radiation and analyzing the emergent pattern for signs of pathology. Current CW methods are the direct evolutionary result of early trans-illumination techniques. In these methods the total light attenuation, that is the total loss of photons through tissue, is measured and analyzed.

The key, however, to successful imaging is to overcome the challenges posed by scattering and to separate the absorption and scattering processes effectively. In TD optical imaging, photons are classified based on the time at which they emerge from the tissue. The generated time-of-flight distribution (generally referred to as a TPSF or temporal-point-spread-function) can be thought of as a statistical distribution of all possible photon paths between the point of illumination and the point where the light exits the tissue. This distribution can be used to recover the optical characteristics of the specimen and thus discriminate scattering from absorption.

By investigating both the scattering and absorption properties of tissue, the manner in which light propagates through tissue can be elucidated. Thus, when normal tissue differs from abnormal in its absorption or scattering of light for some physiological or morphological reason, it then becomes possible to differentiate optically between normal and abnormal conditions.

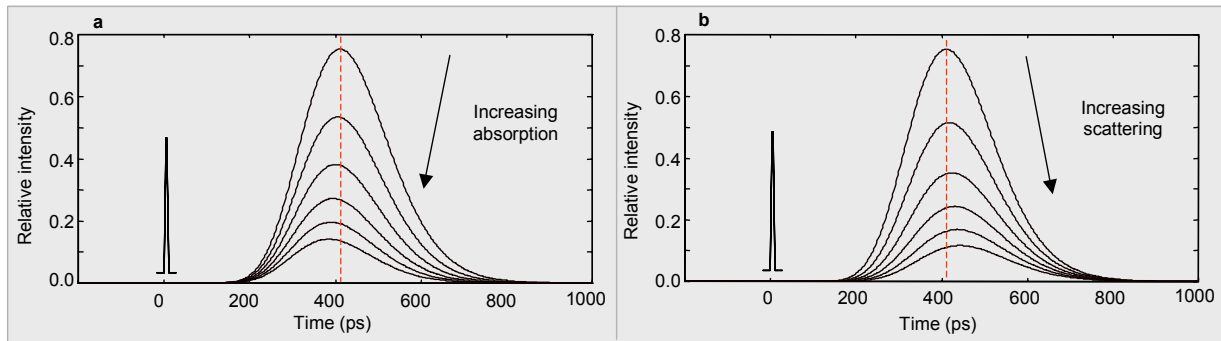


Figure 1: Effect of absorption (a) and scattering (b) changes on a theoretical photon time-of-flight distribution. Changes in absorption and scattering are made in +2 % increments from their nominal values. Arrows point in the direction of the shifting peak maximum as the sample absorption or scattering is varied.

In a time-resolved reflectance measurement, the scattering and absorption coefficients may be determined independently by fitting to the time-varying diffusion equation:

$$I(r,t) = (4\pi Dc/n)^{-3/2} z_0 t^{-5/2} \exp\left(\frac{r^2 + z_0^2}{4Dct/n}\right) \exp(-\mu_a ct/n) \quad (1)$$

where  $I(r,t)$  is the positional time-dependent intensity,  $r$  is the illumination / collection separation distance,  $z_0 = 1/\mu_s'$ ,  $D = 1/(3\mu_s' + 3\mu_a)$ ,  $\mu_s'$  is the reduced scattering coefficient,  $\mu_a$  is the absorption coefficient and  $n$  is the refractive index of the medium.

With a CW measurement, it is more difficult to account for variations in intensity due to imperfections in animal illumination and light collection. This potential problem is alleviated in the TD when Equation 1 is used since the scattering and absorption properties are recovered directly from the shape and position of the TPSF. Additionally, with single illumination / detection point CW intensity measurements, only the product of the scattering and absorption coefficients is recovered. Furthermore, it has been reported that TD measurements provide more consistent recovery of the absorption coefficient than a CW system.<sup>(2)</sup>

## 3 OPTICAL MOLECULAR IMAGING

The advent of fluorescent reporters for a number of disease-related biomolecular processes has pushed optical molecular imaging to the forefront. Both CW and TD based molecular imaging systems collect optical signals, which can be used to determine the intensity and location of fluorescent emissions from an animal. In CW measurements, light emanating from the surface predominates the signal and masks sub-surface constituents that may be present. In addition, although CW intensity measurements provide relative measures of *in vivo* concentrations of fluorescent contrast agents, absolute quantification is difficult. This is because the intensity of both the excitation light reaching the targeted tissue region and the luminescent emissions detected are a function of the surrounding tissue's optical properties. Quantification therefore requires knowledge of the optical properties of the tissues under interrogation, which is by definition an unknown.

However, when tissue is irradiated with a picosecond light pulse, the temporal characteristics of the pulse change as it propagates through tissue. With a time-resolved detection system, the shape and intensity of the elongated pulse (the TPSF) is recorded (see Figure 3). By analyzing the shape characteristics of the TPSF, the optical properties of the tissue can be determined. Specifically, tissue absorption is encoded in the falling curve of the TPSF whereas the peak maximum and the breadth of the TPSF encodes tissue scattering. Additionally, when fluorescently-labeled tissue is irradiated with a (continued on page 7)

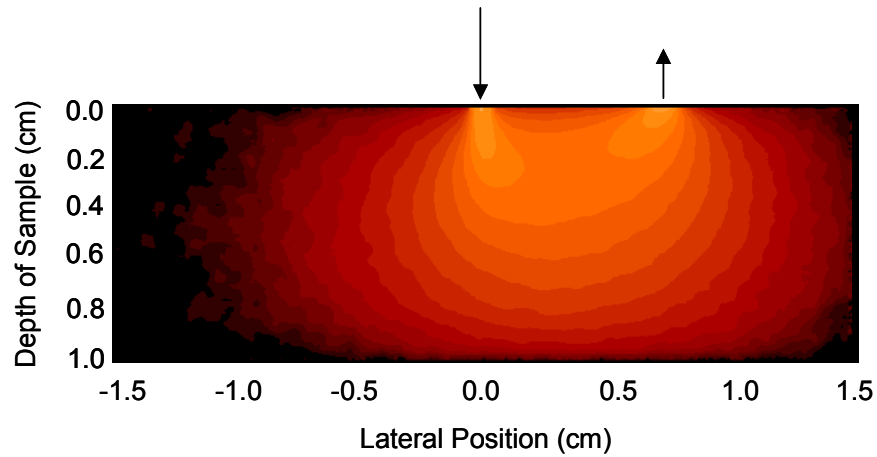


Figure 2: An illustration describing the propagation of CW light from a point source toward an exit point 0.7 cm away in a turbid medium mimicking the optical properties of tissue. Note that light does not travel in a straight line between the point of illumination and the point of collection.

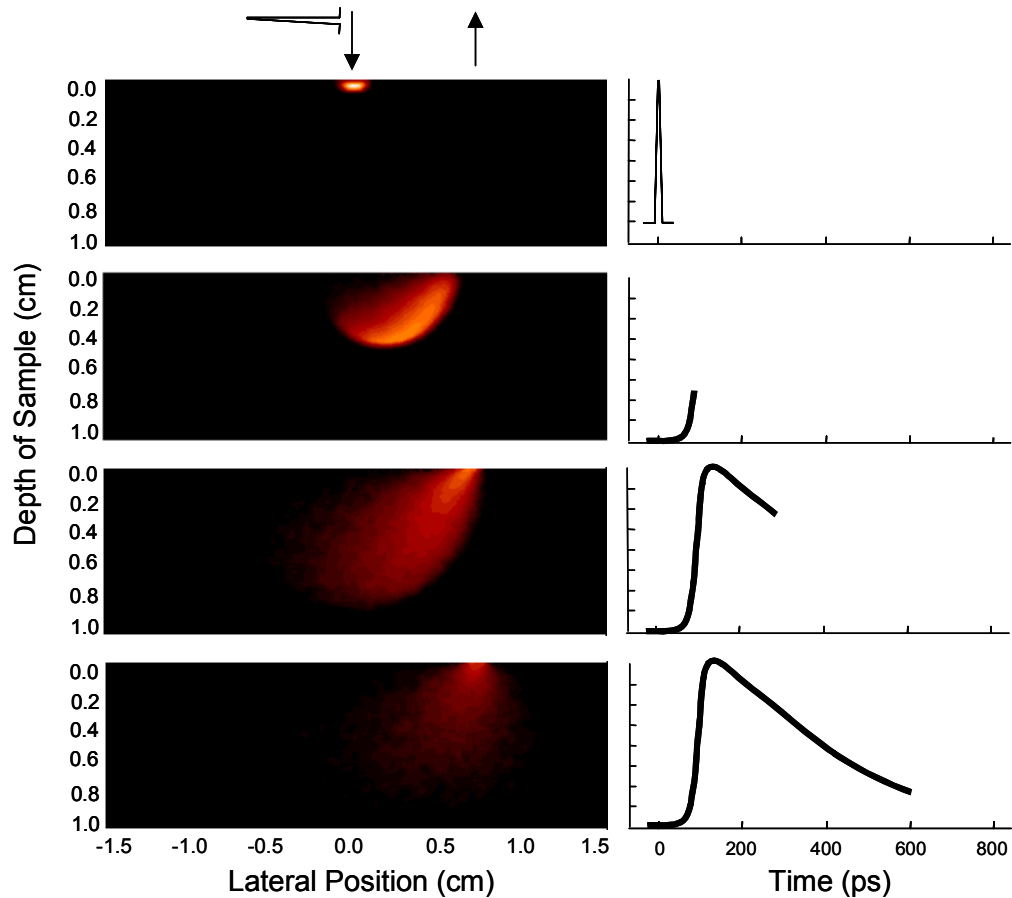


Figure 3: Time lapsed photon distributions of a picosecond light pulse traveling between an irradiation point at 0.0 cm and an exit point at 0.7 cm. Tissue parameters:  $\mu_a = 0.006 \text{ mm}^{-1}$   $\mu'_s = 1.0 \text{ mm}^{-1}$  No fluorescent inclusion is present.

short light pulse, the reporter does not emit instantaneously, but over a characteristic lifetime. By resolving the optical signal into its temporally varying components, rich information about the state of the tissue absorption and scattering, as well as fluorescence lifetime, is directly ascertained.

These arguments can be validated by considering the relative fluorescence intensity  $I_F / I_X$  from a non-scattering solution using a first order relaxation process.<sup>(3)</sup> In the case of CW excitation, the relative fluorescent intensity is:

$$-\log(I_F / I_X) = k(\mu_{a,F} / \ln 10) k_F \int_0^{\infty} \exp(-t / \tau) dt = k \varepsilon [F] (k_F / \tau) = k \varepsilon [F] \phi_F \quad (2)$$

and for time-resolved measurements, the equivalent expression is:

$$-\log\{I_F(t) / I_X(t=0)\} = k \varepsilon [F] k_F \int_0^t \exp(-t' / \tau) dt' \quad (3)$$

where  $I_F$  is the detected emission intensity,  $I_X$  is the incident excitation intensity,  $\varepsilon$  is the molar extinction coefficient of the fluorophore,  $k$  is a constant which contains the optical interaction length,  $[F]$  is the concentration of the fluorophore,  $\mu_{a,F}$  is the absorption cross section of the fluorophore ( $\mu_{a,F} = \ln 10 \varepsilon [F]$ ),  $k_F$  is the radiative decay rate via fluorescence,  $\tau$  is the fluorescence lifetime and  $\phi_F$  is the quantum yield of fluorescence ( $\phi_F = k_F / \tau$ ).

As demonstrated in *Equation 2*, a CW measurement of fluorescence cannot distinguish between a change in fluorophore concentration from a change in quantum yield or lifetime. It is demonstrated in *Equation 3* that time dependent decay kinetics may be obtained independently from the fluorophore concentration with a time-resolved measurement. This, in combination with an analysis of the TPSF shape, makes it possible to decouple the fluorophore concentration-quantum yield product from lifetime and tissue optical properties using TD data.<sup>(3)</sup>

It should be noted that in addition to fluorescence intensity based CW imaging technology, another CW technology, bioluminescence, is also used in molecular imaging. In this modality, the excitation energy is supplied by a chemical reaction rather than from a source of light. Typically two agents are required for bioluminescence — the light producer, which is generically referred to as a luciferin, and a driving or catalyzing agent, the luciferase. The luciferase catalyzes the oxidation of luciferin, resulting in light.<sup>(4)</sup> In most cases, fresh luciferin must be brought into the reaction system, either through diet or by internal synthesis. The luciferase is tagged to proteins or genes incorporated into the organism.

The capabilities of CW fluorescence, bioluminescence and TD fluorescence technologies are illustrated in *Table 1*, below. Fluorescence intensity may be measured with both CW and TD systems, but not with bioluminescence imagers, as they do not contain a light source that can excite the fluorophore. Likewise, both CW and TD fluorescence imaging systems may be used to determine bioluminescence intensity when the illumination source is off. Nanomolar detection limits are possible with all three types of systems.<sup>(5,6)</sup> Depth recovery is possible to a degree with a bioluminescence imaging system, however, the optical transport properties of the tissue must be assumed.<sup>(7)</sup> With a TD imaging system, the depth is directly recoverable by using the mean transit time of fluorescent emissions. Concentration recovery is also possible from a planar scan with TD information, if the depth and surface emission shape and size is known.<sup>(6)</sup> Such concentration recoveries are generally not possible with bioluminescence imaging systems unless one has *a priori* knowledge about the luminescent species or has access to calibration data.<sup>(8,9)</sup> Optical transport parameters (scattering and endogenous and exogenous absorption coefficients) may be directly recovered from TPSF data. Although these parameters may be estimated using a CW

fluorescence imaging system, they are more difficult to obtain since one must use spatially dependent diffuse reflectance information. This is particularly difficult and inaccurate in mice due to their limited size. Given that TD imaging systems measure fluorescence intensity, lifetime and tissue transport parameters readily, tomographic reconstruction is generally superior. Two other advantages of TD systems include their ability to distinguish and quantify two different fluorophores simultaneously based on their overlapping decay kinetics and to distinguish when certain classes of fluorophores are in a given chemical environment based on variations in fluorescence lifetime.

Table 1: A global theoretical comparison of optical molecular imaging instruments.

	Continuous Wave		Time Domain
	Bioluminescence	Fluorescence	Fluorescence
<b>Direct measures of fluorescence intensity</b>		✓	✓
<b>Direct measures of bioluminescence intensity</b>	✓	✓	✓
<b>Nanomolar detection limits</b>	✓	✓	✓
<b>Tomographic imaging</b>	✓	✓	✓
<b>Direct recovery of fluorophore depth</b>	✓		✓
<b>Accurate concentration recovery from planar scan</b>			✓
<b>Fluorescence lifetime</b> <ul style="list-style-type: none"> <li>• <b>Distinction between endogenous and exogenous fluorescence from a single scan</b></li> <li>• <b>Distinction between variations in chemical environment and concentration</b></li> </ul>			✓
<b>Direct recovery of fluorochrome and tissue absorption</b>			✓
<b>Direct recovery of tissue scattering</b>			✓

## 4 TECHNOLOGY COMPARISON

eXplore Optix is unique in that it is the only TD small animal optical imaging device on the market. The following subsections will benchmark the performance of eXplore Optix by comparing it with commercially available CW imaging methods, focusing on the following areas:

- a. depth and concentration recovery
- b. tomography
- c. fluorescence lifetime
- d. detection sensitivity / limits
- e. illumination geometry and system ergonomics

### **a. Depth and Concentration recovery**

An important issue in luminescence imaging is the localization of luminescent material in tissue. Without accurate depth information, lumophore quantification is not possible unless many assumptions are made. A CW fluorescence system cannot distinguish between an intensely fluorescent, deep inclusion and a weakly fluorescent inclusion at shallow depths without using tomography. With CW bioluminescence systems, estimates of depth are possible if it is assumed that luminescent signals originate from a point-like location within the animal and that the spot size on the animal surface is a function of depth. Additionally, knowledge of the tissue properties, which are not directly obtainable, is required. The user therefore must perform calibrations. The TD eXplore Optix system, however, is the only system that can recover depth and concentration directly using planar scans. This powerful ability has important consequences when assessing tumor growth and metastasis.

The way in which a TD system resolves depth of a fluorescently labeled probe relies on the unique properties of the TPSF. The technique, which is similar to acoustic reflection or depth echo, uses shifts in the time corresponding to the maximum in peak fluorescence intensity as a measure of inclusion depth. The method should provide accurate recovery over a wide range of fluorophore concentrations and lifetimes.<sup>(10,11)</sup>

As an example, the eXplore Optix system localized Cy5.5<sup>TM</sup> fluorescent inclusions buried in liquid phantoms.<sup>(6)</sup> In this experiment, the phantom's absorption and scattering coefficients were  $0.006 \text{ mm}^{-1}$  and  $1.0 \text{ mm}^{-1}$  respectively, and the inclusion had a fluorophore concentration of 50 nM. Depth recoveries were made by fitting the measured TPSF to a theoretical model for varying concentrations. The results demonstrated that when the inclusion was positioned 3 mm below the surface, the recovered depth was found to be 3.1 mm. Likewise, at 5 mm depth, a value of 5.95 mm was computed. Even at depths beyond 10 mm, the technique allows for depth recovery to 25% of its true value. Additionally, this method has been found to provide accurate depth recovery independent of fluorophore concentration<sup>(6)</sup>.

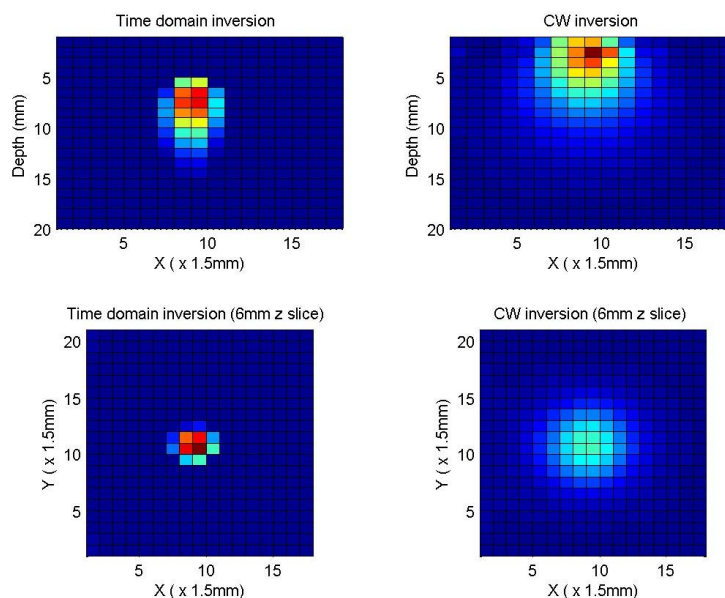
Once the depth of the fluorescent inclusion and its size are ascertained, concentration recovery becomes possible. Initial results with eXplore Optix have indicated that the relative concentration of the fluorophore may be measured to an accuracy of 15% for high fluorophore concentrations and to 30% for low concentrations.<sup>(6)</sup>

### **b. Tomography**

One of the ultimate goals of bio-optical imaging is the generation of 3D full body images via tomographic scanning and reconstruction. Research activity has been particularly intense in this area during the last decade. Although published research has demonstrated the abilities of a particular apparatus and configuration, be it CW or TD technologies, only the unique time resolved properties of fluorescent emissions, as discussed above, allow for accurate tomographic imaging with a single planar scan. This result is exciting since a typical reconstruction traditionally uses multi-view optical information to generate 2D slice or 3D volume images of a specimen. The reconstruction process itself requires the solution to an inverse model, which satisfies certain physical constraints as well as the physics of photon migration and fluorescence excitation and emission in scattering media. In many cases a modified Born approximation model is used for tomographic reconstruction.<sup>(12)</sup> This tomographic reconstruction recovers the shape, location, concentration and fluorescence lifetime of fluorescent inclusions resulting in a 3D volumetric representation of the animal's interior.

As previously discussed, it is difficult to distinguish between a deeply embedded concentrated fluorophore and a weak fluorophore near the tissue surface using CW intensity information. In order to distinguish between these two cases, tomographic reconstruction with multi-angle data recorded from many source and detector positions is usually required. The apparent location and intensity of fluorescent emissions, as seen from different perspectives, changes depending on the illumination position. These parallax and intensity shifts provide the necessary information to the reconstruction algorithm. However, unlike X-ray CAT scan images, the scattering properties of light lead to image ambiguities and blurring. Current research in the field of tomographic medical imaging is addressing these problems. However, directly providing the reconstruction algorithm with an initial image, physical constraints, or non-ambiguous data can dramatically improve information output.<sup>(13,14)</sup>

As mentioned previously, eXplore Optix has demonstrated remarkable abilities in providing accurate tomographic images at low concentrations with a single view planar scan. In order to compare the reconstruction performances obtained using integrated CW information to those with incorporating time-resolved data, a 4 mm solid scattering cube containing a low concentration of Cy5.5<sup>TM</sup> dye was placed between 5 and 9 mm below the surface of a scattering solution and scanned at a resolution of 1.5 mm in both the X and Y directions. Only one view was used. As may be seen in the top left and right panels of *Figure 4*, the tomographic reconstruction that incorporates temporal information performs well in locating the correct depth of the inclusion, whereas the reconstruction based on CW data performs rather poorly when imaged using the same planar geometry. In the bottom panels, the viewpoint is different. Instead of a depth resolved axial slice, a lateral XY view at a depth of 6 mm is shown. The image reconstructed from time-resolved information clearly demonstrates that the block is indeed of the correct size, whereas the CW image suggests that a larger circular object is present. The blurring in the time-resolved image is also markedly diminished when compared to the CW result. When the blurring is reduced, greater confidence may be placed on subsequent volume and concentration recovery of localized tissue uptakes.



*Figure 4: Tomographic reconstructions of a 4 mm<sup>3</sup> fluorescent inclusion placed between 5 and 9 mm below the surface of a scattering solution. Both TD and CW results are shown.*

In order to improve these results even further, the unique galvanomirror based illumination and collection system of eXplore Optix may be used to obtain multi-view information. In this scanning mode, multi-view reflection data is obtained. Additionally, profilometry data is used to define the contour of the animal. Since scattering and absorption of the excitation and emission light also change throughout the animal, providing accurate information about tissue optical properties ( $\mu_s'$  and  $\mu_a$ ) to the reconstruction algorithm is also critical to the overall image quality. As mentioned above, incorporating information about the shape of the animal and its tissue optical properties can reduce image ambiguities and provides a solid basis for robust tomographic reconstruction. A discussion of how tissue optical properties are obtained with the galvanomirror scanning system and TD reflectance data is discussed in the following sections.

### **c. Fluorescence Lifetime**

Although the field of intensity-based CW fluorescence methods is mature and they continue to be extensively applied in biotechnology, the use of fluorescence lifetime measurements is becoming more prevalent. This is because fluorescence lifetime, unlike intensity-based CW methods, is essentially independent of fluorophore concentration and is less affected by photo-bleaching processes. The measurement of fluorescence lifetime however, requires the use of time-resolved optical techniques, which only eXplore Optix provides.

Another advantage of lifetime measurements is that it is possible to discriminate between two fluorophores whose emission spectra overlap. Additionally, it may be possible to employ fluorescent dyes having different decay kinetics in diseased and normal tissues. Such characteristics are known to occur with varying tissue pH and oxygenation, which provide rich biochemical information of the local tissue environment.<sup>(15)</sup> For these reasons time-resolved fluorescence spectroscopy is considered to provide a more realistic description of tissue structure and environment than intensity-based measurements.

The resolving power of fluorescent lifetime imaging is demonstrated below in *Figure 5*, which shows a fluorescent intensity and lifetime map of a mouse that was fed standard rodent chow immediately prior to scanning. From the two images, a total of four hot spots are observed of which two has an abnormally long fluorescence lifetime. Since the top two regions correspond to tumours, while bottom two are centered in the GI tract, it was determined that the animal feed was producing the endogenous fluorescent signal. The variations in fluorescent lifetime are also clearly seen in *Figure 6*, which contains a histogram of fluorescence lifetimes recorded over the entire animal. The exogenous fluorescence clearly has a fluorescence lifetime of 2 ns while the endogenous fluorescence from the feed has a lifetime of 2.5 ns. This important result shows that different fluorophores that are excited with the same source and have similar emission spectra may be separated based on their fluorescent lifetimes. In a companion article, applications of fluorescence lifetime imaging are discussed further.<sup>(15)</sup>

### **d. Detection sensitivity and limits**

The detectors employed in CW intensity measurements and in TD imaging systems are based on two different technologies. A cooled CCD camera system is used almost exclusively for CW fluorescence and bioluminescence systems<sup>(16-19)</sup> whereas for a TD instrument, time-correlated single photon counting or TCSPC is preferred. Contemporary CCD and TCSPC systems are extremely sensitive and equally applicable to intensity-based optical molecular imaging. However, current gated ICCD technology simply does not have the capability to resolve short optical pulses to sufficient resolution. Due to this limitation, CCD based systems do not allow for depth recovery, accurate tomographic imaging or

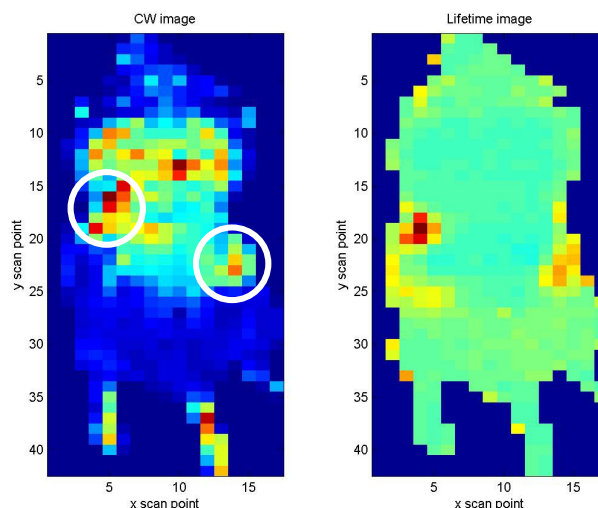


Figure 5: Comparison between a fluorescence intensity and lifetime imaging of a tumor mouse. Wavelength of excitation: 668 nm. Wavelength of emission 700 nm. White circles indicate endogenous fluorescence.

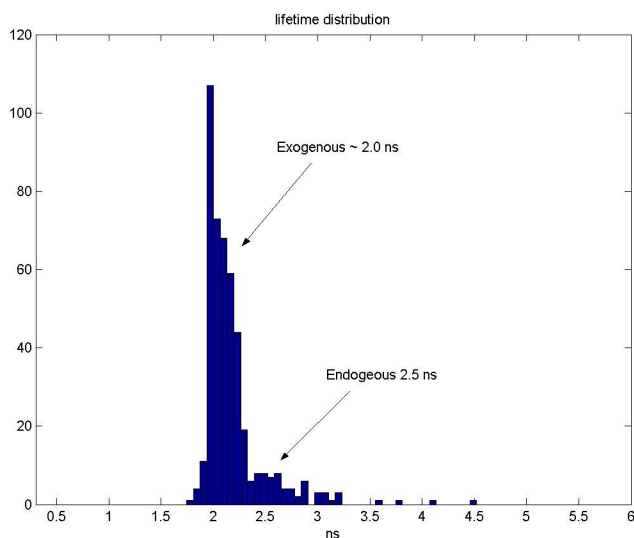


Figure 6: Histogram of fluorescence lifetimes from Figure 5 recorded over the entire mouse.

fluorescence lifetime determination. Additionally, CCD cameras are subject to read-out noise, which limits the accuracy of low-light level measurements. This problem does not exist with a TCSPC system because it generates a large pulsed signal whenever a photon is detected.<sup>(20)</sup> One problem that plagues both CCD and TCSPC systems however, is dark noise. In order to reduce the dark count rate, the detection element is thermoelectrically cooled. Such technology is standard in CW and TD systems.

At low fluorophore concentrations, a TCSPC system is linear over 6 orders of magnitude and has been proven to be sufficiently sensitive for use in single molecule detection equipment and to be advantageous for weakly fluorescent samples.<sup>(20,21)</sup> In order to obtain acceptable TPSF photon statistics, the integration time over which light is collected must be carefully chosen in such a way that the overall scan time is kept to a minimum. The balance is made with eXplore Optix and scan times are of the order of minutes.

The limits of detection for CCD and TCSPC based technologies are also very comparable. With the eXplore Optix TCSPC detector, the detection limit is approximately 100 photons per TPSF. This signal level corresponds to a sub-nanomolar fluorophore concentration at depths of 5 mm below the tissue surface, on the order of 1 nM at depths between 5 mm and 9 mm and approximately 50 nM at a depth of 12 mm. Although the detection sensitivities between TCSPC and CCD are similar, a TCSPC system only requires low power lasers. This is particularly advantageous when using unstable fluorophores that are easily photo-bleached.

A CW imaging system employing a cooled CCD camera is, however, more sensitive toward weak signals because the integration time may be much longer and all points on the animal are inspected simultaneously. Although the individual pixel integration time may be longer with a CCD camera than what a TCSPC system can achieve, for certain applications such as tomography, the advantages are not nearly as pronounced. This is due in part to the choice of illumination and collection geometry, which will be expanded upon in the following sections.

#### e. Illumination geometry / system ergonomics

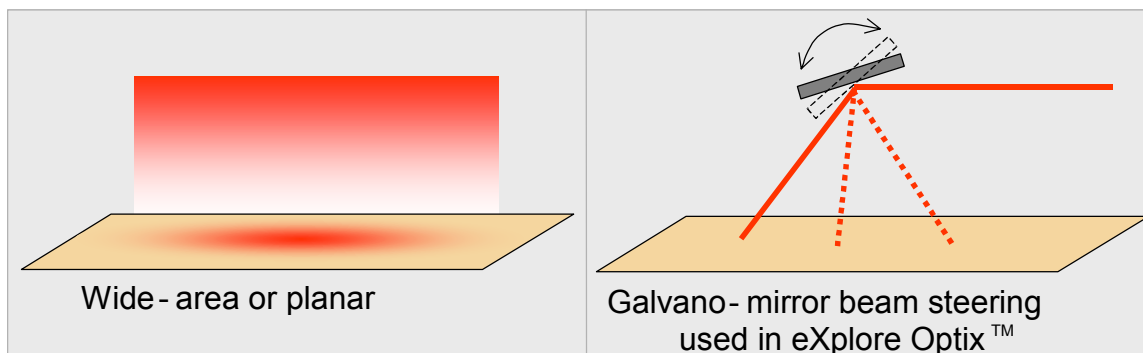


Figure 7: Illumination modalities.

There are essentially two methods of illuminating exogenous fluorophores—wide-area and point-wise. These illumination configurations, as shown in Figure 7, are fundamentally different and as a consequence, a detection system recording diffusely reflected light emanating from the animal would record dissimilar information. As previously mentioned, a CCD camera may be used to image light from all points on the animal simultaneously. In order to make use of this advantage however, the animal needs to be illuminated with a wide area beam. Wide-area illumination unfortunately necessitates the use of a laser that is many times more powerful and bulky. Worse however, the recorded optical signals contain spatially integrated information. For planar intensity-based fluorescence imaging this is not necessarily problematic, however, for tomographic applications, this illumination configuration is not ideal.

A natural choice for tomographic imaging is point-wise illumination and collection. From the previous discussion about tomography, recovery of the optical properties of tissue is an important aspect to consider for accurate quantification of the concentration of a fluorescently-labeled probe.<sup>(8)</sup> With a CW intensity based system, a single illumination / detection point measurement gives only the effective total optical coefficient which is a product of the scattering and absorption coefficients. However, a TD-based system in the same configuration gives absolute measures of the scattering and absorption coefficients (see Equation 1).

Ease of use, mechanical simplicity, and minimal animal preparation are paramount considerations when assessing any molecular imaging system. eXplore Optix's unique free-beam non-contact scanning design, permits independent illumination and light collection over a wide range of points over the surface of the animal. Such a system based on galvano-mirrors and beam steering optics is extremely advantageous both for planar imaging and tomographic applications.

In tomography, an important factor that affects image quality is the number of illumination and collection points (or views) used.<sup>(22)</sup> Since the beam steering optics of eXplore Optix are adjustable, optimal beam and collection positioning may be made without the need to manually adjust animal position, as is required of fiber-optic based systems. Additionally, galvanomirror illumination and light collection allow the collection of multi-angle information leading to enhanced 3D tomographic images.

For planar imaging, a point illumination system vastly reduces variations in intensity between longitudinal scans. CW fluorescence intensity based systems employ one or two fiber-optic illuminator heads for wide-area illumination, which is typically not uniform. Problems also arise when the animal is repeatedly repositioned in the light field. If it is not placed in the same location, the measured fluorescence intensity will differ due to changes in illumination intensity. With galvanomirror optics, this problem is greatly reduced since the laser spot illuminates the tissue uniformly at every point.

# 5

## SUMMARY AND CONCLUSION

This article presented the salient features of CW bioluminescent, continuous-wave fluorescent and TD fluorescent technologies for optical molecular imaging applications. A comparison between these three imaging modalities has demonstrated that *in vivo* bioluminescent markers may be imaged in animals with all three modalities, while *in vivo* fluorescence imaging may be made only with fluorescence CW and TD imaging systems. In addition, detection sensitivities are similar for all three types of systems if the best detection equipment for each modality is used. However, for applications listed in the table below, a TD imaging system not only gives enhanced information, but leads to superior 3D imaging and fluorophore quantification. It may be concluded that this type of system provides much more informative data to an analyst and permits an enhanced understanding of biochemical reactions and drug efficacy *in vivo*.

Advantages of TD fluorescence imaging
<b>Depth recovery from planar imaging</b>
<b>Concentration recovery from planar imaging</b>
<b>Fluorescence lifetime information for fluorophore species confirmation</b>
<b>Recovery of tissue scattering, tissue absorption and fluorochrome absorption</b>
<b>Simultaneous quantification of two fluorophores</b>
<b>Fluorescence lifetime data provides tissue pH, oxygenation and other information pertaining to a local biochemical environment of the fluorophore</b>
<b>Much more precise 3D imaging with accurately quantification of inclusion position, size and concentration.</b>

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