Fluorescence lifetime and quantitative volumetric measurements using time-domain small animal optical imaging

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Originally presented during the Annual Meeting of the American Association for Cancer Research on March 28, 2004.

ABSTRACT

The interest in optical modalities for molecular imaging has been increasing steadily in recent years. Optical imaging makes use of fluorescent contrast agents that can be chemically targeted to specific biomolecules and/or activated by specific physiological processes. Fluorescence based optical imaging has prospective applications in many biomedical areas ranging from the detection of pathologies to measuring specific cellular and molecular pathways. Here, we present results of experiments that highlight the capacity of ART Advanced Research Technologies' optical imaging system, eXplore Optix, to quantitatively recover depth, volume, concentration and fluorescent lifetime information *in vivo*, using both photon time-of-flight and intensity data. Depth and concentration sensitivity are shown to be at least 11 mm and 10 nM of Cy5.5 fluorophore, respectively. Image reconstruction using time-domain data demonstrates inclusion recovery of the correct size, shape and position. Results on animals show the capacity of the device to recover depth and concentration and provide volumetric data using a simple planar geometry. Different fluorophores with spectrally overlapped emission profiles can also be distinguished by their lifetime and *in vivo* fluorescence lifetime results of mice injected with various dyes show separation of endogenous and exogenous fluorescence. This type of system provides informative data to an analyst permitting a thorough understanding of biochemical reactions and drug efficacy *in vivo*.

MATERIALS AND METHODS

In vitro and *in vivo* time-domain [1] fluorescence measurements (Figure 1) were performed on an eXplore Optix molecular imager (ART Advanced Research Technologies, Saint-Laurent, Quebec) with a pulsed laser diode emitting at 670 nm, 80 MHz repetition rate, pulse length < 100 ps (Figure 2).

In vitro studies

The eXplore Optix system was tested to assess its performance capabilities, with particular regard to concentration recovery and depth sensitivity. A group of experiments were performed using varying concentrations, volumes and cross-sectional areas of solid and liquid Cy5.5 (Amersham Biosciences) fluorescence inclusions buried in phantoms. The liquid optical absorption and scatter coefficients were $\mu = 0.006 mm^{-1}$ and $\mu = 1.0 mm^{-1}$. The temporal point spread function (TPSF) was analyzed in a number of different ways (e.g. intensity, gated-intensity with varied gate width, fitted lifetime and absorption coefficient). In addition, raw time-domain data was processed in order to view volumetric images of the scanned regions. By using the Born approximation in the time-domain, light propagation can be modeled and used as a forward modeler for the tomographic inversion problem[2].

FIGURE 1. Principle of time-domain near-infrared (NIR) fluorescence imaging.

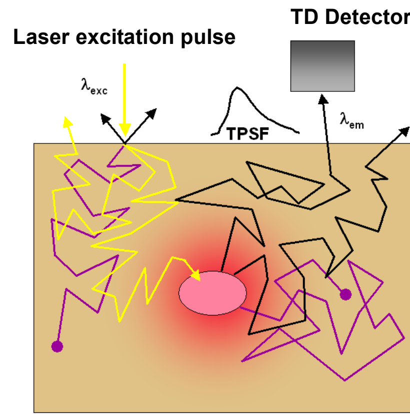
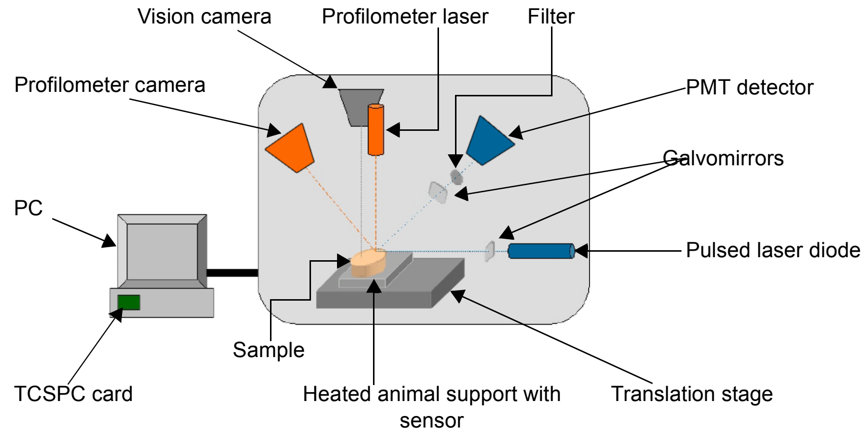


FIGURE 2. Diagram depicting the eXplore Optix acquisition system. The animal is positioned on the animal table and a region of interest is selected via a top and side live camera. The animal is then translated into the imaging section of the system where the optics are housed. The entire system is light tight. (TCSPC = time correlated single photon counting, PMT = photon multiplier tube).



In vivo studies

In the first experiment, one normal BALB-c mouse wa simplanted with a solid cubic inclusion(10nM) under the skin and a series of experiments were performed. In the second experiment, mice (n=8) were given an I.V. injection of either a liver targeting (Cy5.5-DTPA-galactosyl-dextran) or control (Cy5.5-DTPA-dextran) agent. Uptake of both agents was observed over 30 minutes. Data sets were reconstructed. The experiments were conducted at LAB Pre-Clinical Research International Inc. (Laval, Quebec). The experimental protocol was approved by the LAB Pre-Clinical Animal Care Committee. Animals were anesthetized (ketamine 87 mg/kg, xylazine 13mg/kgi.p.) for the imaging experiments.

RESULTS

In vitro studies

An important issue of fluorescence imaging is localizing the depth of fluorescent material in tissue. Without accurate depth information, any attempt at evaluating fluorophore concentration quantitatively is not possible without large assumptions leading to potential errors. This ability has important consequences in assessing tumor growth and metastasis.

The results of the system's depth sensitivity evaluation (Figure 3, Table 1, Figure 4) demonstrate that the temporal information contained in the signal enables the quantification of inclusiondepth with a high degree of precision. Fluorescence signal of 1 nM at 5-9 mm below the phantom surface was detected (40 µW power). With higher laser poser (750 µW), 1 nM could be resolved at 12 mm with 5% contrast and 50 nM at 13 mm with >10% contrast. Concentration estimates are proportional to the actual values at each depth. The time-domain data type allows accurate reconstruction with good spatial resolution (Figure 5). The measurements presented were all acquired with planar acquisition geometry (1 source, 1 detector raster scanned).

FIGURE 3. Peak position of TPSF moves with inclusion depth, but does not change with concentration.

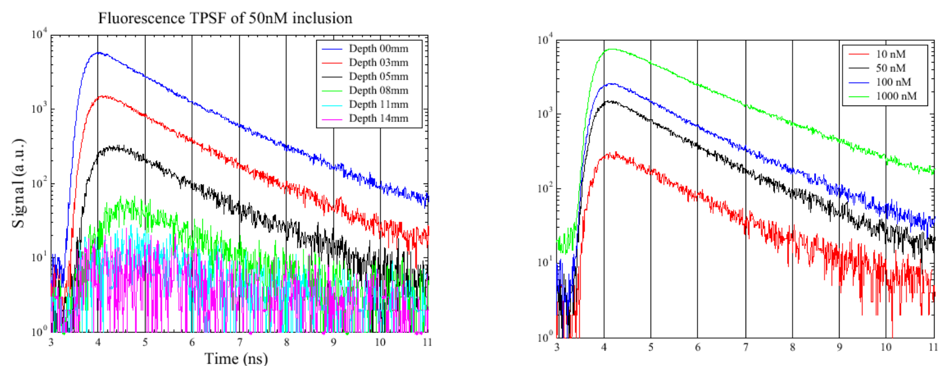


TABLE 1. Results of depth recovery algorithm from planar time-domain measurements on liquid.

	Recovered depth (mm) for different C (nM)			
Real depth (mm)	**C=1**	**C=10**	**C=50**	**C=100**
0-4	0.15	0.15	0.15	Not done
3-7	3.7	3.4	3.1	3.4
5-9	6.15	5.65	5.95	6.15
8-12	Noise	Noise	9.95	10.2
11-15	Noise	Noise	13.65	13.8

FIGURE 4. Depth and concentration estimation results.

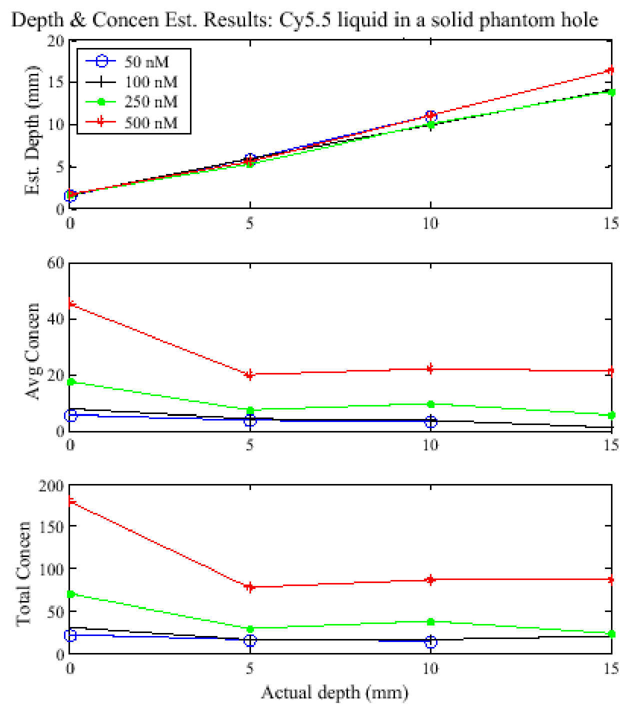
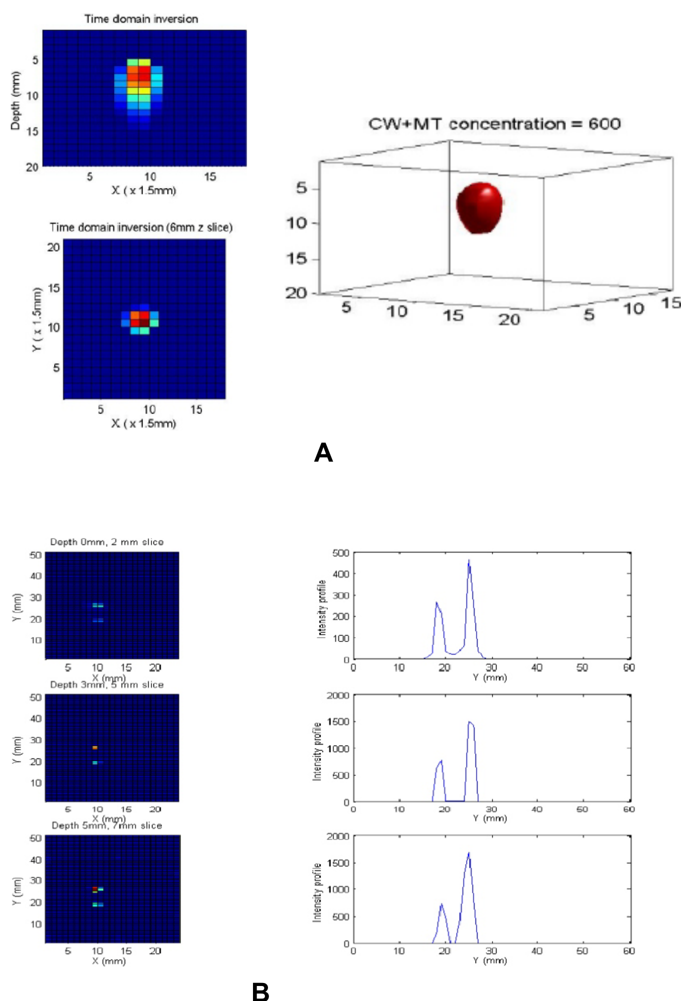


FIGURE 5. (A) A fluorescent cube of dimensions 4 x 4 x 4 mm was inserted in liquid. The top of the cube is positioned at z = 5 mm and the bottom is at z = 9 mm. Time-domain reconstruction shows the cube in the correct position and the correct size. (B) A 50 nM and 100 nM cube was inserted in liquid 2 mm apart and placed at different depths. Time-domain reconstruction shows distinction of the two inclusions at all depths.



In vivo studies

To assess the ability to distinguish fluorophores with spectrally overlapped emission profiles, but different fluorescence lifetimes, a solid cube inclusion was implanted under the skin in such a way that both fluorescent signals from the inclusion and the autofluorescence in the stomach could be selected in a region of interest. The 10 nM inclusion was easily detected with a signal to noise ratio of >10 (Figure 6). Time-resolved measurement of Cy5.5 fluorescence decay exhibited a signal decay time of 2.17 ns whereas autofluorescence from the stomach was 4.7 ns. In order to confirm that the 4.7 ns signal

originated from the stomach, the animal was euthanized and the internal organs of the abdominal cavity were imaged before and after resection of the stomach.

An *in vivo* application is presented, which demonstrates that fluorescence results are similar to published radionuclide results with [TC-99m]-labeling [3]. With the liver targeted agent there is a rapid uptake phase and subsequent accumulation. In contrast, the control (non-targeted) agent resembles a blood pool agent without any tissue specificity (Figure 7). Volumetric reconstruction (Figure 7B) of time-domain data greatly enhances image detail when compared to intensity maps (Figure 7A).

FIGURE 6. Fluorescent lifetime data. (A) Endogenous fluorescence from mouse chow cannot be distinguished from that of Cy 5.5 on the intensity image. (B) Endogenous fluorescence is well differentiated on the fluorescence lifetime scale. (C) Histogram showing the lifetime differentiation. (D) Reconstruction result.

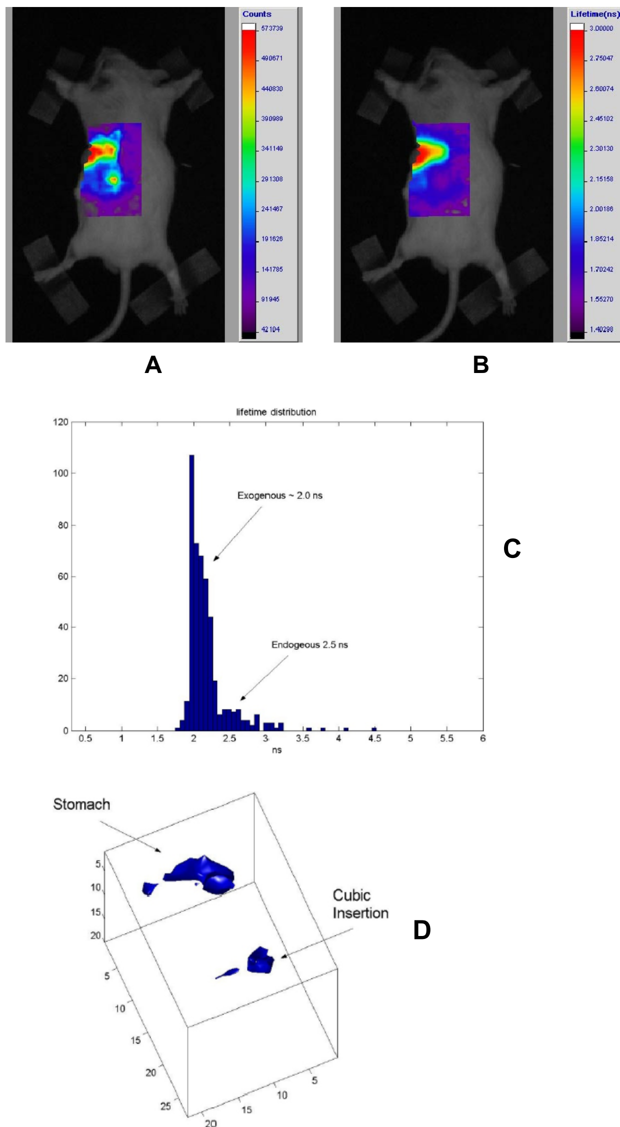
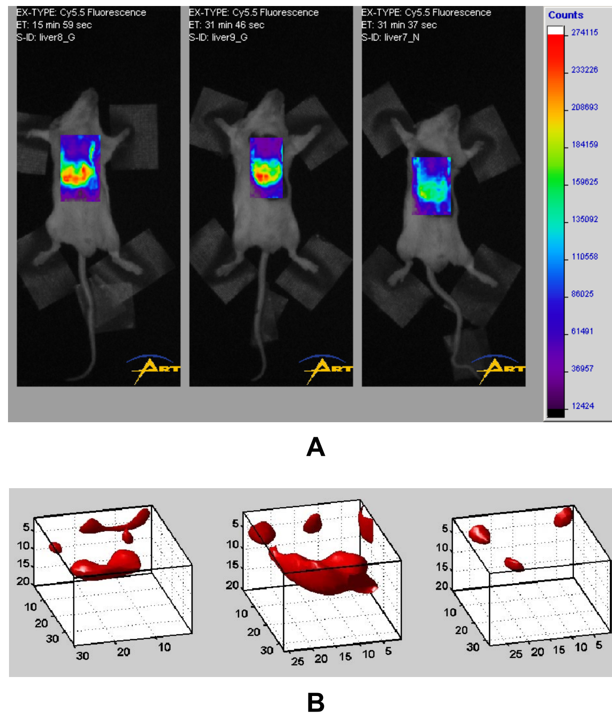


FIGURE 7. *In vivo* fluorescence results showing the accumulation of a liver targeted agent. Left, 15 minutes post-injection, targeted agent. Middle, 30 minutes, targeted. Right, 15 minutes, non-targeted. (A) Topographic images. (B) Tomographic images of the same animals. Reconstruction was performed with the same data set as shown in A from planar reflection geometry.



CONCLUSION

- Time-domain optical imaging permits high sensitivity depth probing and tomography from a planar imaging geometry.
- Nanomolar concentration sensitivity at depths between 0-13 mm were achieved.
- Accurate depth recovery (±2 mm error) was achieved.
- Fluorescence lifetime information allows discrimination of endogenous and exogenous fluorescence or of differentiating multiple exogenous fluorophores based on fluorescent lifetime information.

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