A novel system for in vivo fluorescence lifetime imaging of small animals using supercontinuum wavelength-tunable picosecond laser

SoftScan HealthCare Group

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Introduction

- New fluorescent proteins, dyes and nanoparticles are developed and intensively used for revealing biological processes at the cellular and sub-cellular levels [1].
- While fluorescence imaging is intensively used for in-vitro assays the transfer towards in-vivo whole body visualization did not follow the same pace and the preclinical in-vivo validation is perceived as the main bottleneck in the translational medicine.
- We present a new time-domain instrument for in-vivo fluorescence lifetime imaging of small animals, with a wider and more flexible spectral coverage.
- The benefits of its flexibility, very high dynamic range, ability to convert the time-domain information into fluorescence lifetime and quantitative 3D volumetric views are explored in different experimental conditions.
- A simple method using the fluorescence lifetime sensitivity to pH for probing those changes noninvasively in vivo is also illustrated.

Instrument description

- Sensitive and flexible time-domain platform for in vivo molecular imaging in small animals (figure 1).
- Configured in reflection geometry, performs a 3D raster scanning with adjustable spatial resolution and pixel wise excitation optimization.
- Sensitive time correlated single photon detection.
- Extended spectral coverage of a pulsed supercontinuum laser (Figure 2).
- From time-domain measurements a model based software package generates:
  - Fluorescence lifetime images;
  - Depth profiling and 3D volumetric evaluations [2] of fluorophore concentration for the whole Vis-NIR spectral range (450nm to 850nm).

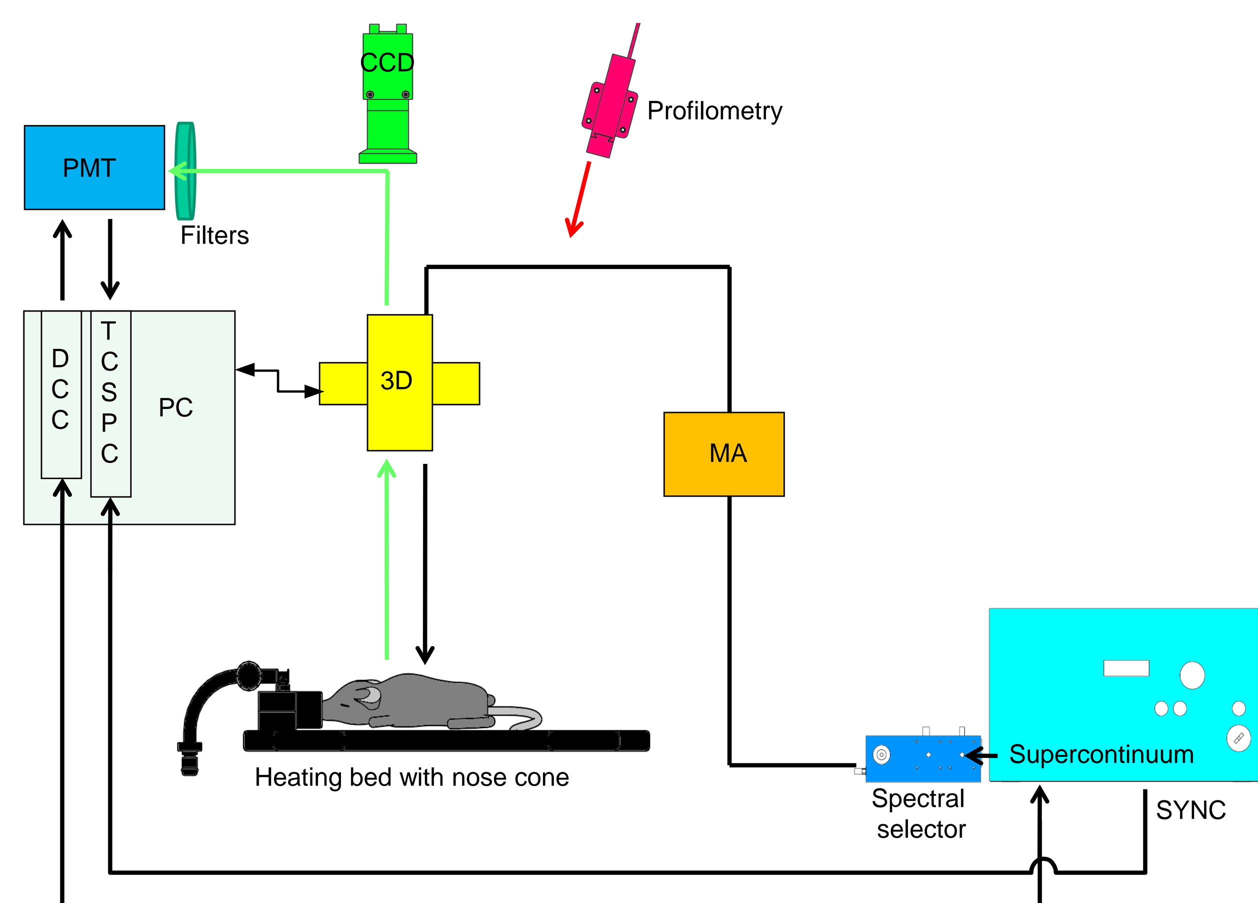

Figure 1. Diagram of the system: all the subassemblies are fully automated and software controlled from simple and intuitive user interface. The 3D raster scan uses the profile of the animal acquired before data acquisition. The system includes accessories for gas anesthetic delivery and specimen temperature control.

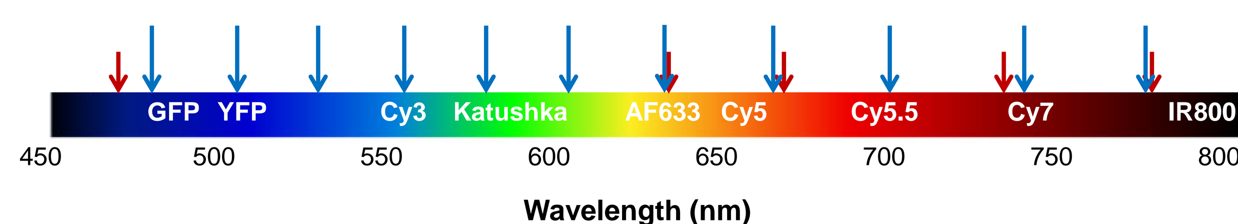

Figure 2. Spectral distribution of the most popular fluorophores. Single wavelength pulsed lasers traditionally used for fluorescence lifetime imaging (red arrows) limit the selection of the fluorophore probe that can be used for in vivo imaging. Tunable laser adds potential selections (as some examples are shown with the blue arrows) that could optimally cover the whole Vis-NIR spectrum.

In vitro experimental results

1. Sensitivity and repeatability of the lifetime value of ICG at different concentrations

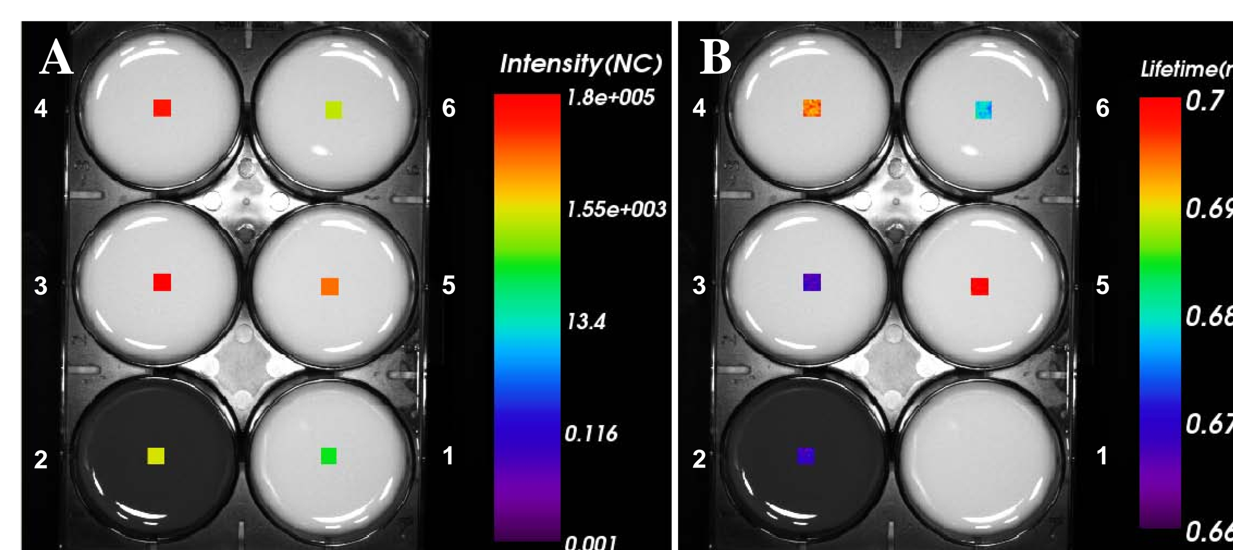

Figure 3. Intensity (**A**) and lifetime (**B**) maps of ICG samples having a large dynamic range of concentration (>10^4) and medium absorption coefficient. All IGC samples maintained similar SNR generating very stable lifetime evaluation.

Sample	ICG concentration (nM)	Scattering coefficient (1/mm)	Absorption coefficient (1/mm)	Lifetime (ns)	Lifetime fluctuation (ns)
1	0	1.0	0.004	NA	NA
2	17.70	1.0	0.020	0.671	0.0020
3	1770.00	1.0	0.004	0.669	0.0023
4	177.00	1.0	0.004	0.695	0.0022
5	17.70	1.0	0.004	0.704	0.0022
6	0.18	1.0	0.004	0.682	0.0022

Table 1. Larger optical properties and ICG concentration variations have minimal impact on lifetime results. Negligible lifetime fluctuations were observed between all samples.

2. Lifetime variation of pH sensitive SNARF-1

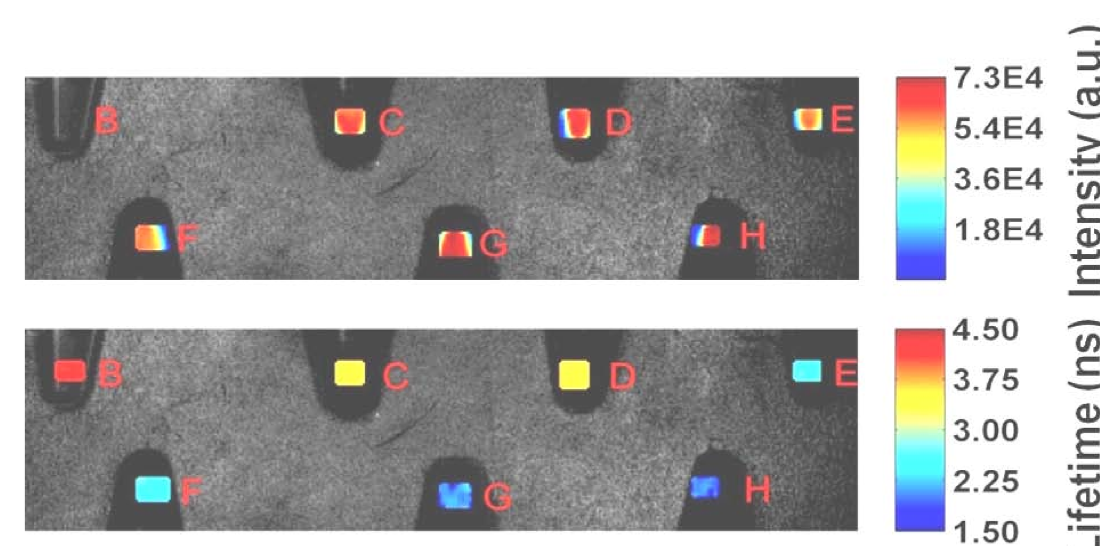

Figure 4. Intensity and lifetime images of samples with different pH. Each vial contained 100µL SNARF-1 [3] at concentration of 100 nM but mixed in solution with different pH values. B: solvent, C&D: pH=6, E&F: pH=7, G&H: pH=8.

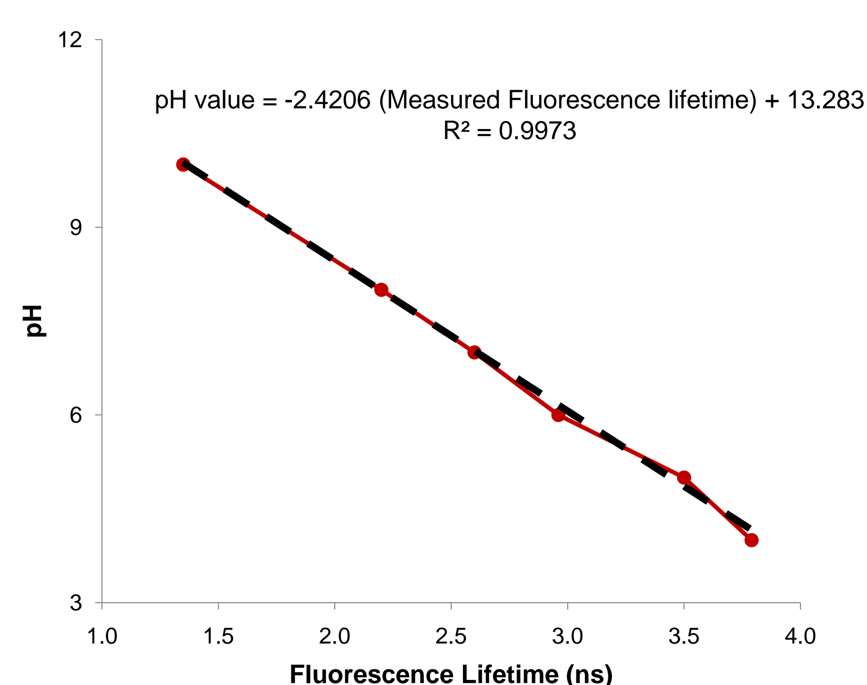

Figure 5. The measured lifetime values of SNARF-1 in different pH solutions (similar to figure 4) were used to generate a calibration curve that can be used to compute the pH value of a SNARF-1 solution.

In vivo fluorescence imaging

3. Lifetime gating

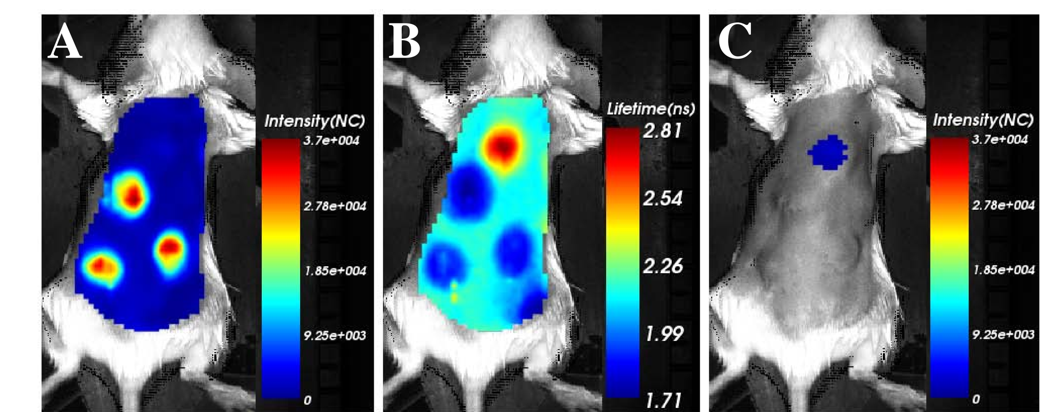

Figure 6. Comparison of fluorescence intensity (**A**) and lifetime (**B**) images demonstrates that the lifetime analysis (**C**) can discriminate fluorophores that have very similar yields. Also could reveal small contaminations with fluorophores that have a negligible contribution to the intensity image but significant difference in lifetime. All procedures were in accordance with the Canadian Council on Animal Care.

4. Fluorescent lifetime based pH indicator

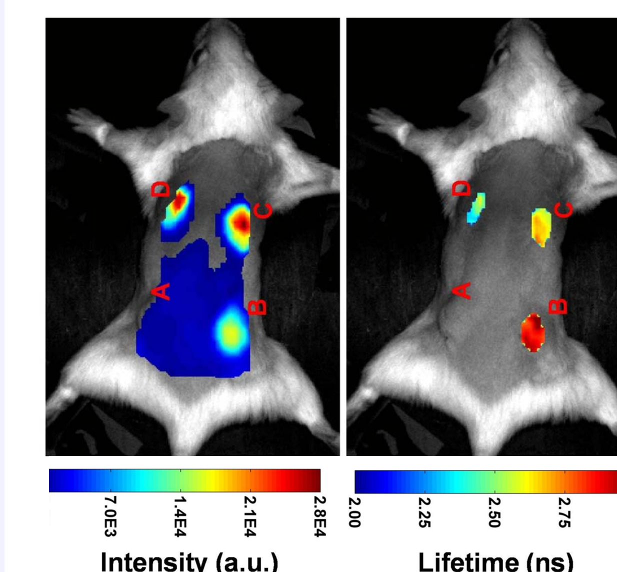

Figure 7. The mouse received sub-cutaneous injection of either the solvent (i.e. matrigel, A) or SNARF-1 based cocktails having initially a pH of 6 (B), 7 (C) or 8 (D). Fluorescence intensity (left panel) shows comparable intensity level for all injection sites. However, lifetime analysis reveals that the lifetime value of each injection site is different (right panel).

Sample	pH of the solution	Measured Lifetime (ns)		pH computed from the calibration curve	ΔpH
		In vitro	In vivo		
A	Matrigel	4.10			
B	6	2.96	2.86	6.36	0.36
C	7	2.59	2.62	6.94	-0.06
D	8	2.17	2.39	7.50	-0.50

Table 2. In-vivo pH monitoring using the calibration curve of pH versus lifetime for SNARF-1 indicator. The in vivo pH computed from the calibration curve is diverging from the initial pH value. This may be explained by the tendency of the body to shift the pH towards the normal physiological range.

Summary and Conclusions

- A versatile Time-Domain instrument with flexible wavelength selection covering the whole Vis-NIR spectral range was presented.
- It offers the possibility to explore and exploit the specificity of fluorescence lifetime imaging in-vivo and to translate and validate in-vitro results.
- Fluorescence lifetime analysis and lifetime gating help for clearly discriminating fluorophores with similar spectra and yields.
- A faster and easier method is proposed for using pH sensitive probes (as SNARF-1) for the diagnosis of microenvironment pH changes in-vivo.

References

1. Han, *et al.*, *Chem Rev.* **110,** 2709 (2010)
2. Mincu *et al.*, *WMIC poster presentation* (2011)
3. The Molecular Probes Handbook, *www.invitrogen.com*