

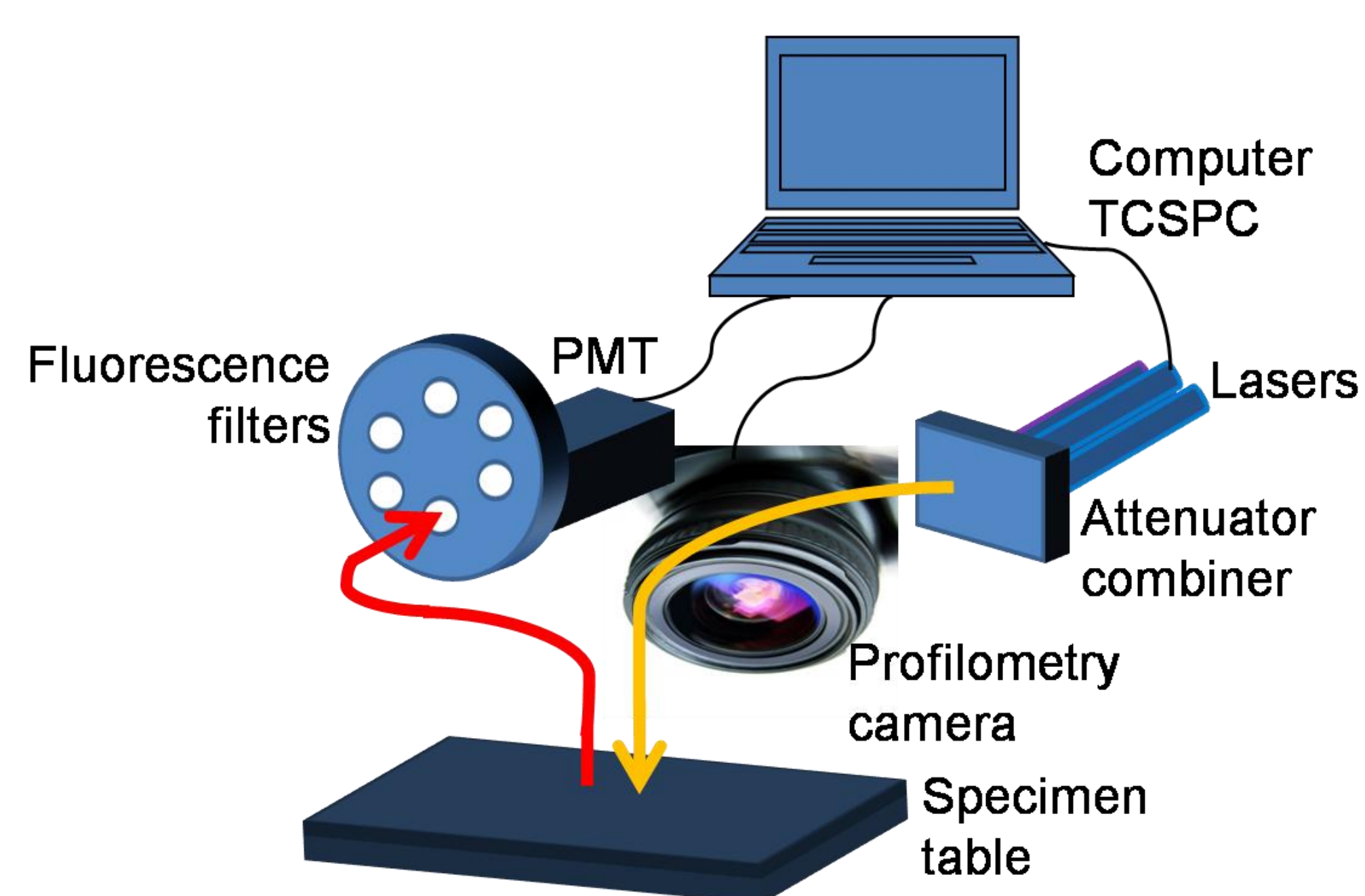


Guobin Ma, Dao Chao Huang, Niculae Mincu, Muriel Jean-Jacques, and Mario Khayat
ART Advanced Research Technologies Inc., Montréal, Québec, Canada
gma@art.ca

Introduction

- Fluorescent lifetime imaging¹ (FLIM) is a technique based upon the intrinsic characteristics of fluorophores
- FLIM is independent of signal intensity and fluorophore concentration, thus providing an efficient and robust means of extracting functional information
- Detection of possible alterations of physiological parameters (e.g. pH and temperature) resulting from malignant transformation of tissue can be a powerful diagnostic tool for earlier cancer detection and prognosis^{2,3}
- FLIM of specifically targeted fluorescent labeled antibodies can be sensitive to such variations and provide functional images of the regions of interest⁴
- FLIM has been widely used in microscopy
- We present a method of *in vivo* animal imaging to sense the local pH microenvironment by FLIM using a time-resolved fluorescence imaging platform⁵

In Vivo FLIM Imaging System



- Pico-second pulsed laser diode excitation
- High sensitivity photomultiplier tube (PMT) to collect photons
- Time-correlated-single-photon-counting (TCSPS) board to generate time point spread function
- Optimized fluorescence filters
- Specially designed illumination/collection configuration to minimize tissue autofluorescence in measured signal
- Computer controlled turn-key system
- Commercially available

Materials and Method

Fluorescent label and pH control

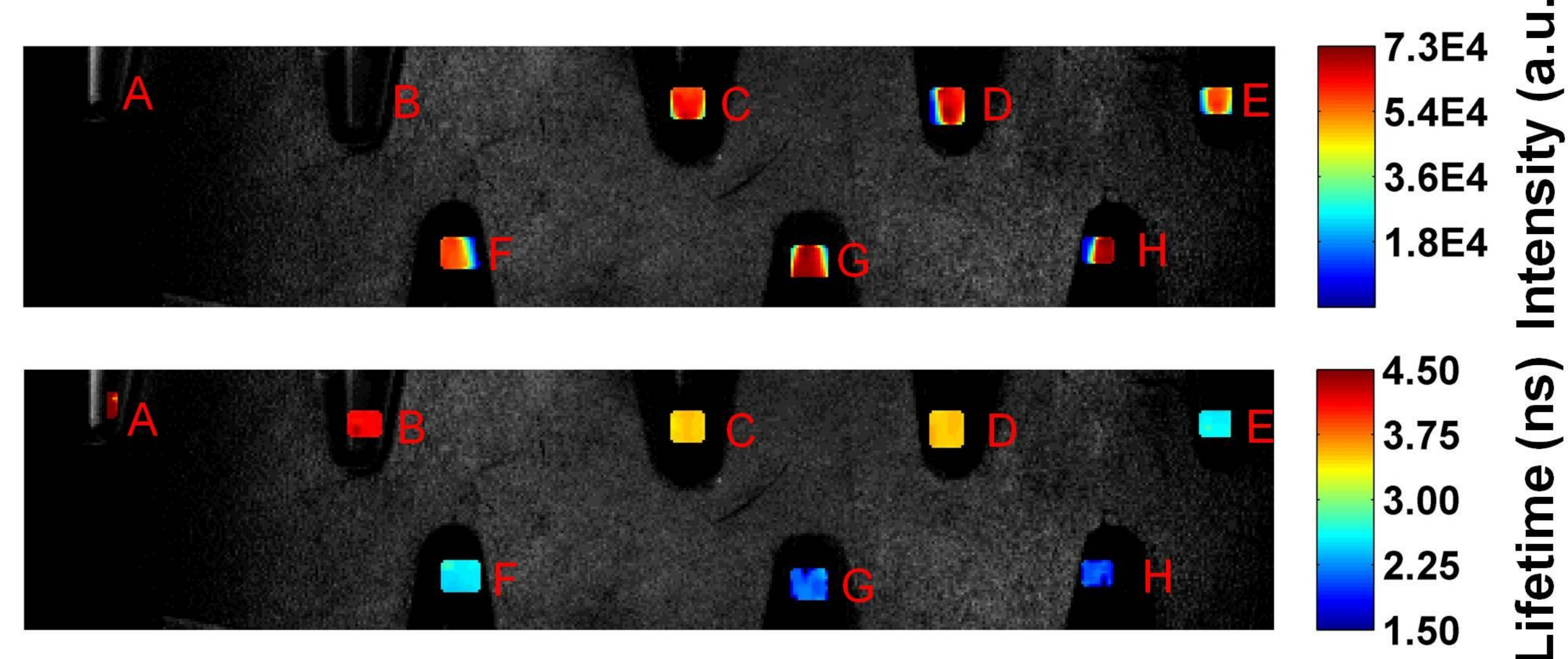
- pH sensitive fluorescent dye, SNARF-1⁶
- Absorption peak: ~550 nm;
- Emission peak: ~640 nm
- Mix with different pH buffers
- pH value verified by pH meter

Mouse model and *in vivo* imaging

- CD-1 mice shaved with Nair cream
- Subcutaneous injection of Matrigel + SNARF-1 mixed with different pH buffer
- Imaged immediately after injection of the sample
- All procedures were in accordance with the Canadian Council on Animal Care
- Intensity and lifetime analysis by OptiView⁷

Results

1. *In vitro* imaging of the pH sample SNARF-1



Sample	pH	Lifetime (ns)
A	empty	
B	Matrigel	4.10
C,D	6	2.96
E,F	7	2.59
G,H	8	2.17

Figure 1. Typical fluorescence intensity and lifetime images of *in vitro* measurement of SNARF-1 mixed with Matrigel at different pH environments. The samples are confined in Eppendorf tubes. In the images, A is an empty tube. B contains Matrigel only. C and D are samples with pH 6; E and F are pH 7; and G and H are pH 8. The concentrations of all SNARF-1 samples are controlled as the same. The intensities of the samples are similar, but the lifetimes are quite different. This difference enable us to probe the pH environment.

2. Lifetime change of SNARF-1 over pH value

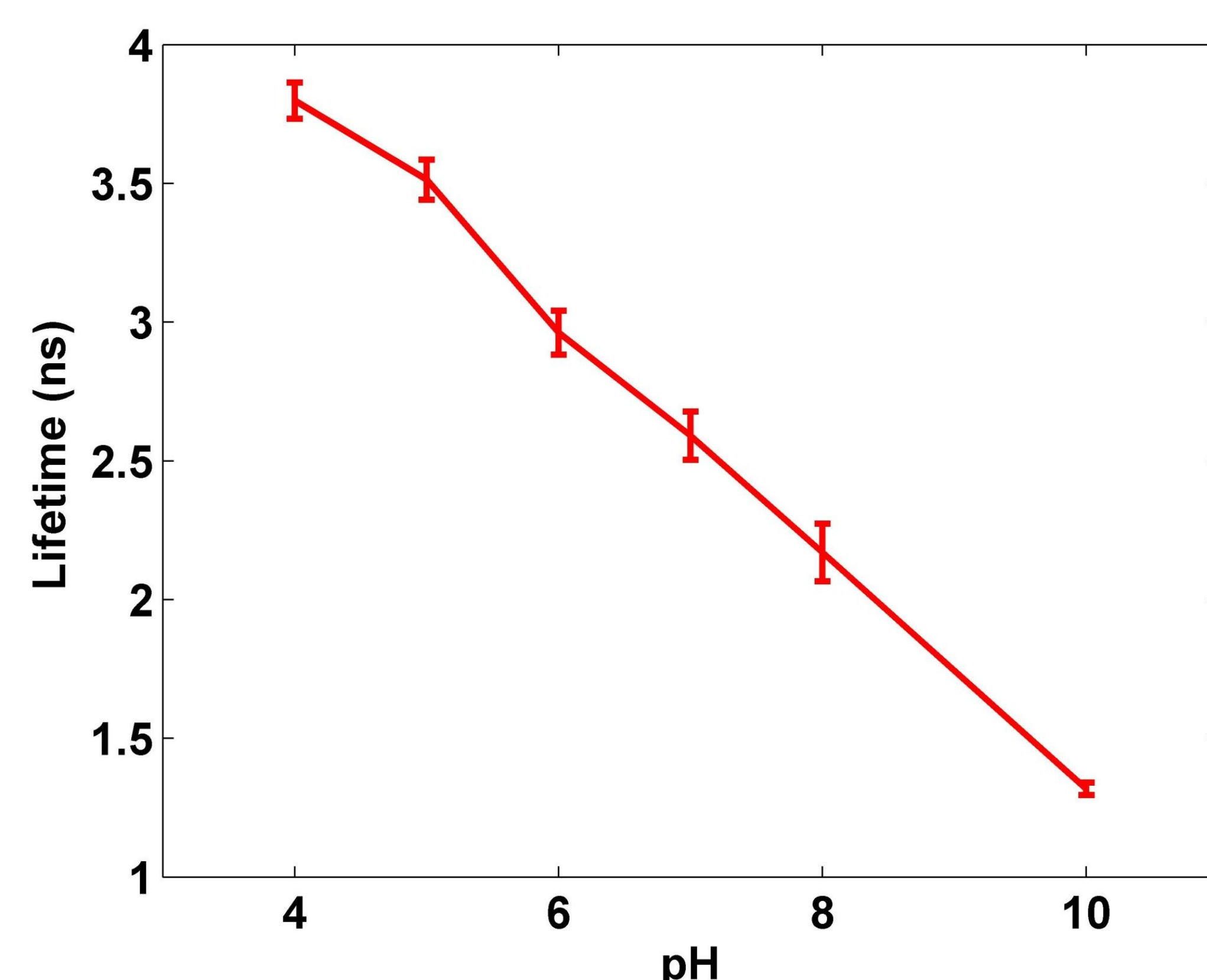
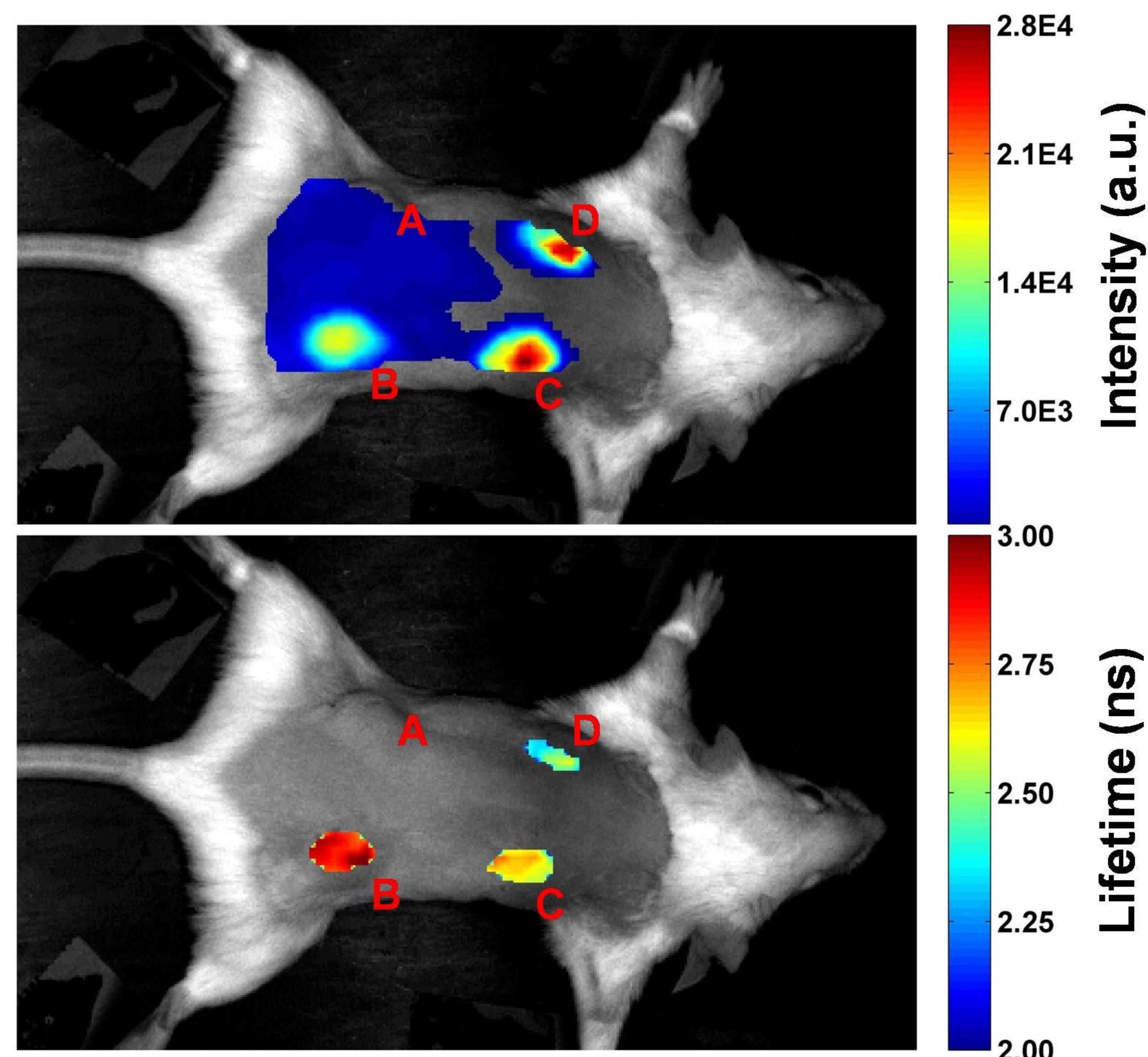


Figure 2. Estimated fluorescence lifetime of SNARF-1 at different pH environments *in vitro*. Data extracted from many images similar to those shown in Figure 1. The error bar represents the standard deviation of 6 or more measurements of samples with the same pH. Results indicate that the fluorescence lifetime of SNARF-1 decreases nearly linearly over pH value.

3. Image pH sensitive SNARF-1 *in vivo*



Sample	pH	Lifetime (ns)
A	Matrigel	
B	6	2.86
C	7	2.62
D	8	2.39

Figure 3. Fluorescence intensity and lifetime images of *in vivo* measurement of SNARF-1 mixed with Matrigel at different pH environments. The samples are injected in CD-1 mice subcutaneously. Location A is Matrigel only. B is the location injected with sample of pH 6; C is pH 7; and D is pH 8. The concentrations of all SNARF-1 samples are controlled as the same. It is not convincing to use the intensity image of these samples (top panel) to distinguish the pH. However, the lifetimes (bottom) of these samples are quite different (range from 2.39 ns for pH 8 to 2.86 ns for pH 6) and closely related to the pH values which enable us to probe the pH environment.

Summary and Conclusions

- We demonstrated proof of concept for using fluorescence lifetime imaging to probe micro pH environment *in vivo*
- The fluorescence lifetime of pH sensitive dye SNARF-1 decreases with pH value
- The less pronounced change of lifetime over pH *in vivo* versus *in vitro* may be attributed to the biological process in mice that has a tendency to neutralize the pH
- Further studies using pathological models with varied pH microenvironments caused by physiological effect are under way

References

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