



SoftScan
HealthCare Group



In vivo fluorescence lifetime imaging of ICG pharmacokinetics in rodent model



Marilyse Piché, Dao Chao Huang, Muriel Jean-Jacques, Salim Djeziri, Zhiqiang Xu, Niculae Mincu, and Guobin Ma

ART Advanced Research Technologies Inc., Montréal, Québec, Canada

gma@art.ca



Introduction

- Indocyanine green (ICG) is a near infrared fluorescent dye providing higher sensitivity and specificity than visible labels for in vivo imaging.
- ICG is clinically approved in several fields in medicine. Because of its selective uptake by hepatocytes, ICG is used to directly reflect the physiology and pathology status of the hepatobiliary system of small animal, e.g. hepatic dysfunction⁽¹⁾.
- Fluorescent lifetime improves the specificity of imaging results. With lifetime, the liver, gallbladder and intestines can be distinguished for further distinct investigation.
- The aim of this study was to provide a method using Fluorescent Lifetime Technology to in vivo image ICG biodistribution in mouse and assess its pharmacokinetics in the liver, gallbladder and intestines in order to study the physiology and pathology of the hepatobiliary system.

Materials and Method

- CD-1 nude female adult mice aged 6-8 weeks received tail vein injection of 1µg, 5µg or 10µg of ICG in 100µl saline solution.
- Mice were maintained with isoflurane anesthesia and were repetitively imaged during one hour, and at 6 and 24 hours post-injection.
- In vivo imaging was performed with the fluorescence lifetime imager Optix MX2⁽²⁾ with a laser that has an excitation of 785nm. One mouse was also scanned in the Skyscan Micro-CT.
- All data were processed using the OptiView™ analysis software. In vivo ICG fluorescence signal was analyzed in terms of fluorescence intensity, lifetime, depth and concentration. Co-registration of optical-CT images was performed in Amira.
- All procedures were in accordance with the Canadian Council on Animal Care.

Results

1. ICG in vivo imaging: Fluorescence intensity

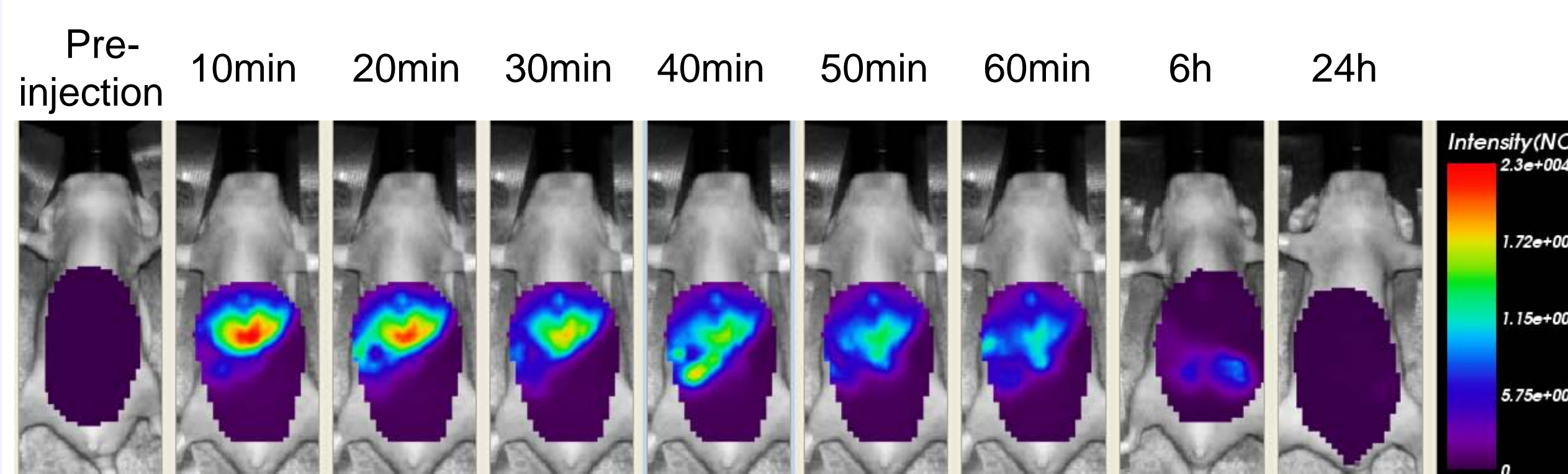


Figure 1. Once ICG was injected, the same mice was imaged at different time point. Maximal fluorescent signal was detected in the liver at 10 minutes. Thereafter, the intensity decreases over time. Low level ICG fluorescent signal was detected in the gallbladder and intestines up to 24 hours post-injection.

2. ICG in vivo imaging: Lifetime map

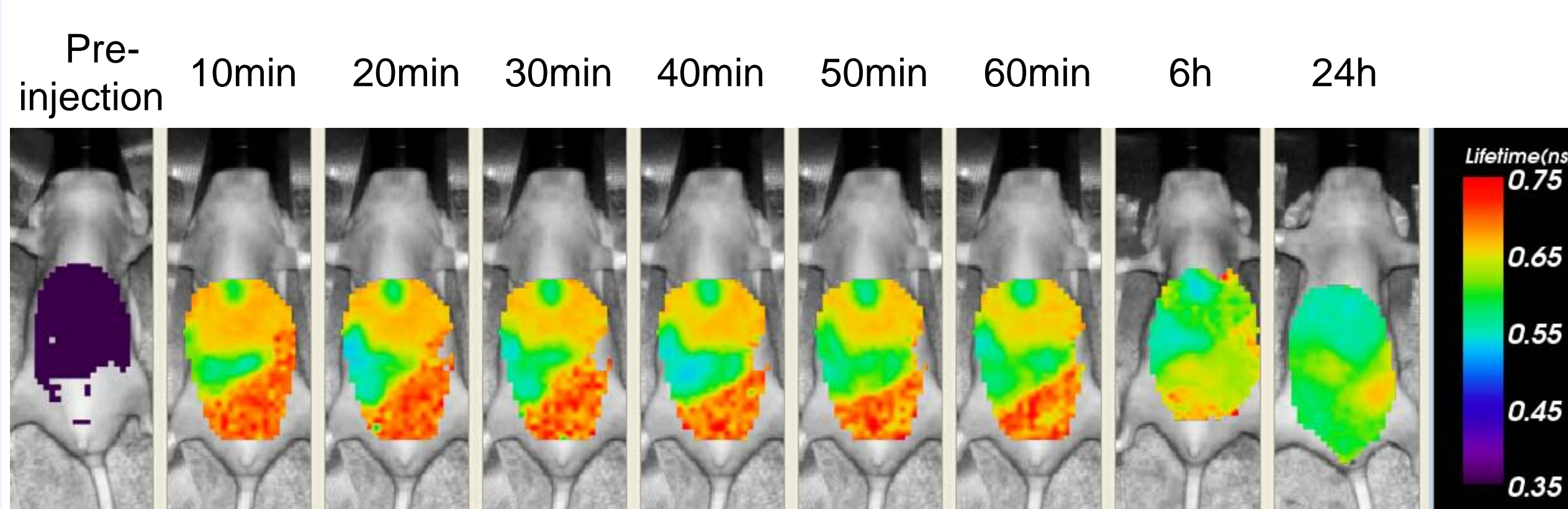


Figure 2. Lifetime analysis revealed that at 10 minutes post-injection, ICG was not restricted in the liver but was also found in gallbladder, intestines and probably in circulation. At 24 hours, there was still some ICG all over the body.

3. Fluorescent lifetime imaging and gating

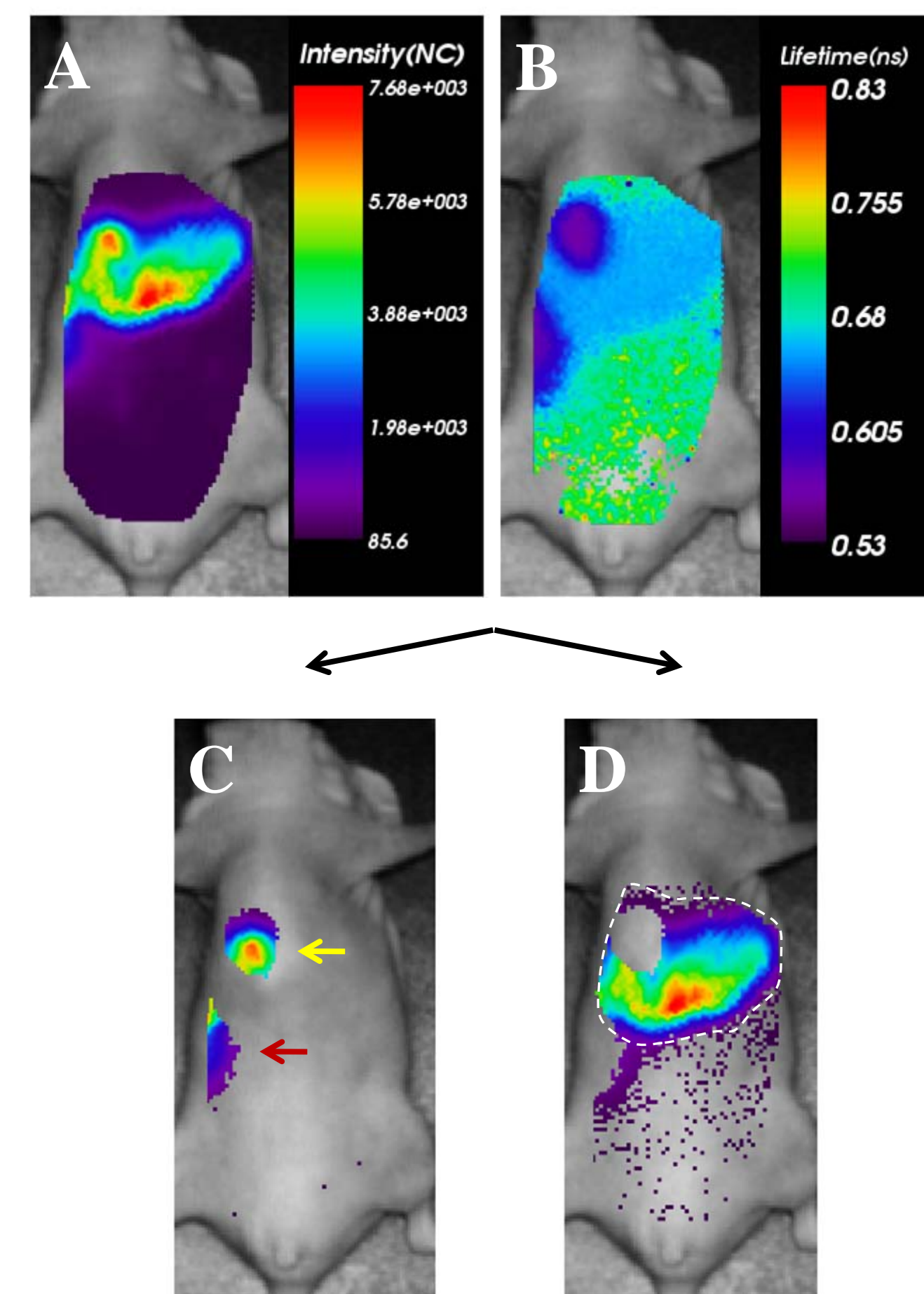


Figure 3. Fluorescent intensity map (A) shows that ICG is mainly detected in liver and gallbladder. However, lifetime map (B) reveals that ICG is found in other parts of the body. Lifetime value of ICG can be used to differentiate organs. Thus, gallbladder (C, yellow arrow) and intestines (C, red arrow) can be visualized by a lifetime gating below 0.62ns, and the liver can be discriminated with a lifetime gating of 0.62-0.675ns (D). Precise quantification of the fluorescent signal in the liver is achieved in OptiView by drawing an ROI (dash white line) specifically around this organ.

4. Pharmacokinetics of ICG

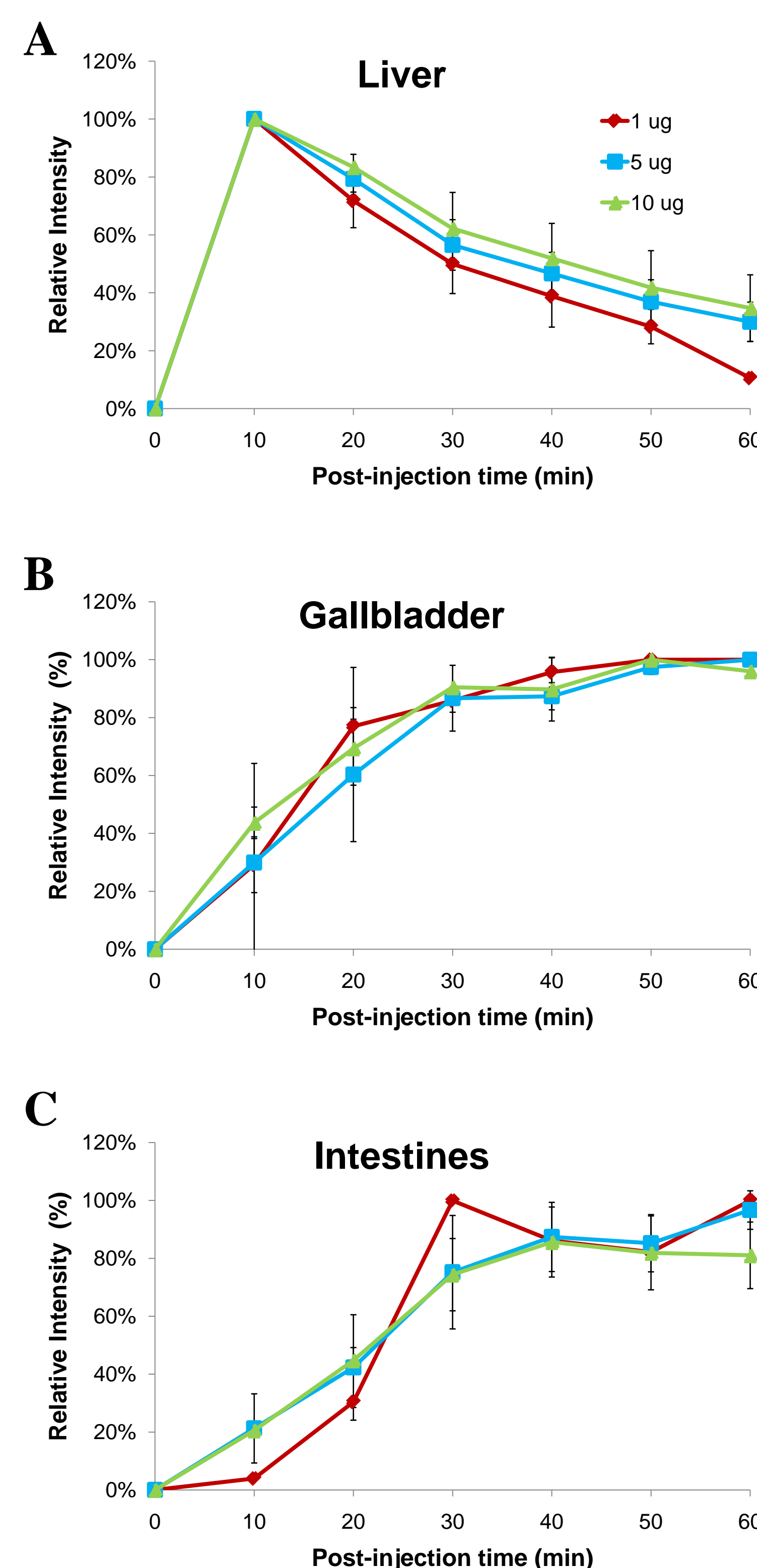


Figure 4. Using lifetime gating, ICG fluorescent signal from the liver (A), gallbladder (B) or intestines (C) was quantified over time and was reported as relative intensity.

In the liver (A), the maximal fluorescent intensity was observed at 10min and decreases rapidly, whereas for the gallbladder (B) and the intestines (C), the maximal fluorescent signal was obtained after 50 min post-injection.

The pharmacodynamic of three different dosage of ICG (1, 5, 10µg) was similar for the gallbladder and the intestines (B&C), but in the liver (A), the elimination rate was slower for higher dosages. n=3 for each dosage.

5. Ex vivo imaging

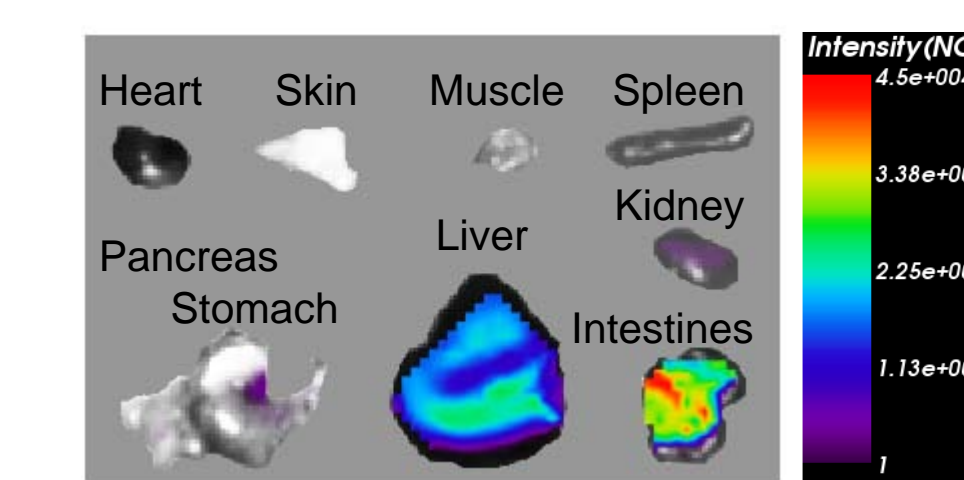


Figure 5. Ex vivo imaging was performed at 70min post-injection of 10µg of ICG. High fluorescent intensity was detected in intestines, and in liver. Very low intensity was also detected in the kidney, spleen and stomach.

6. Depth and concentration

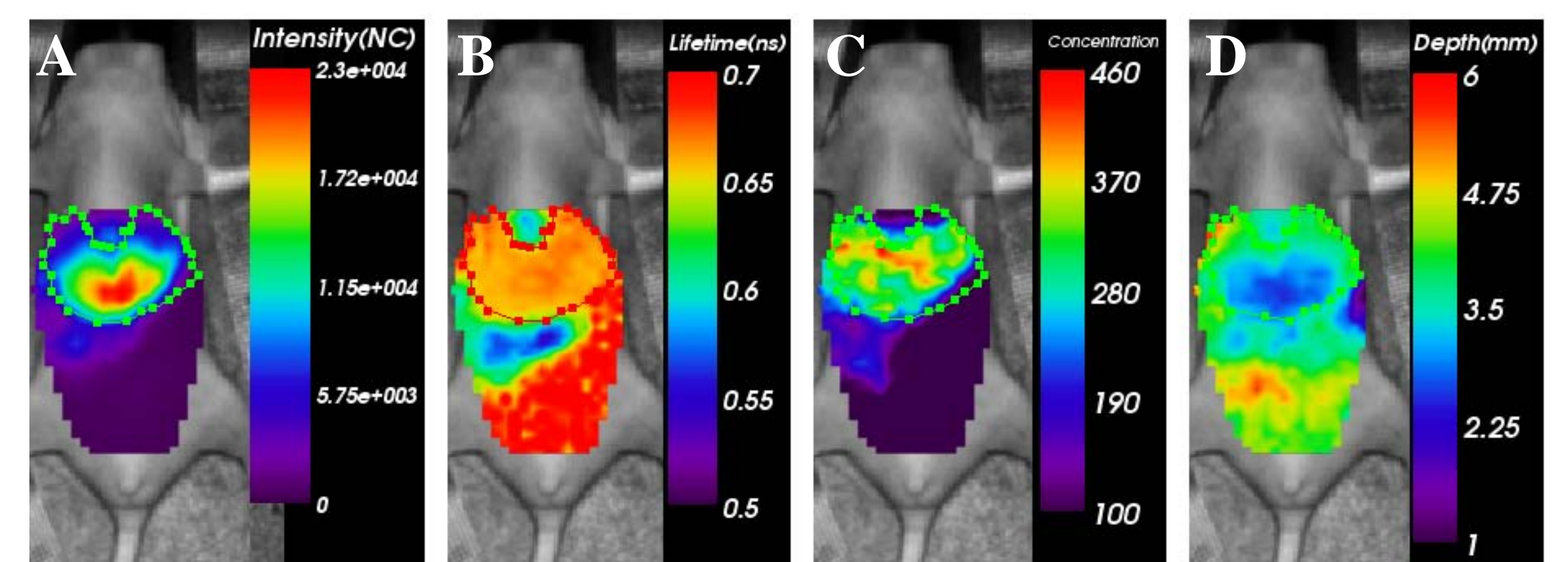


Figure 6. Maximal fluorescent signal in the liver (A) was always seen in the lower part of the ROI delimited by the lifetime map (B, red dotted line). True concentration map (C) revealed that ICG is concentrated mostly in areas in the upper and middle part of the liver. Depth map (D) confirmed that the liver is not at a constant depth relative to the skin; the lower part of the liver is closer to the skin (depth ≈2.5mm) compared to the upper part that is covered by the thoracic cage (depth ≈ 4mm).

7. Multi-modality in vivo imaging

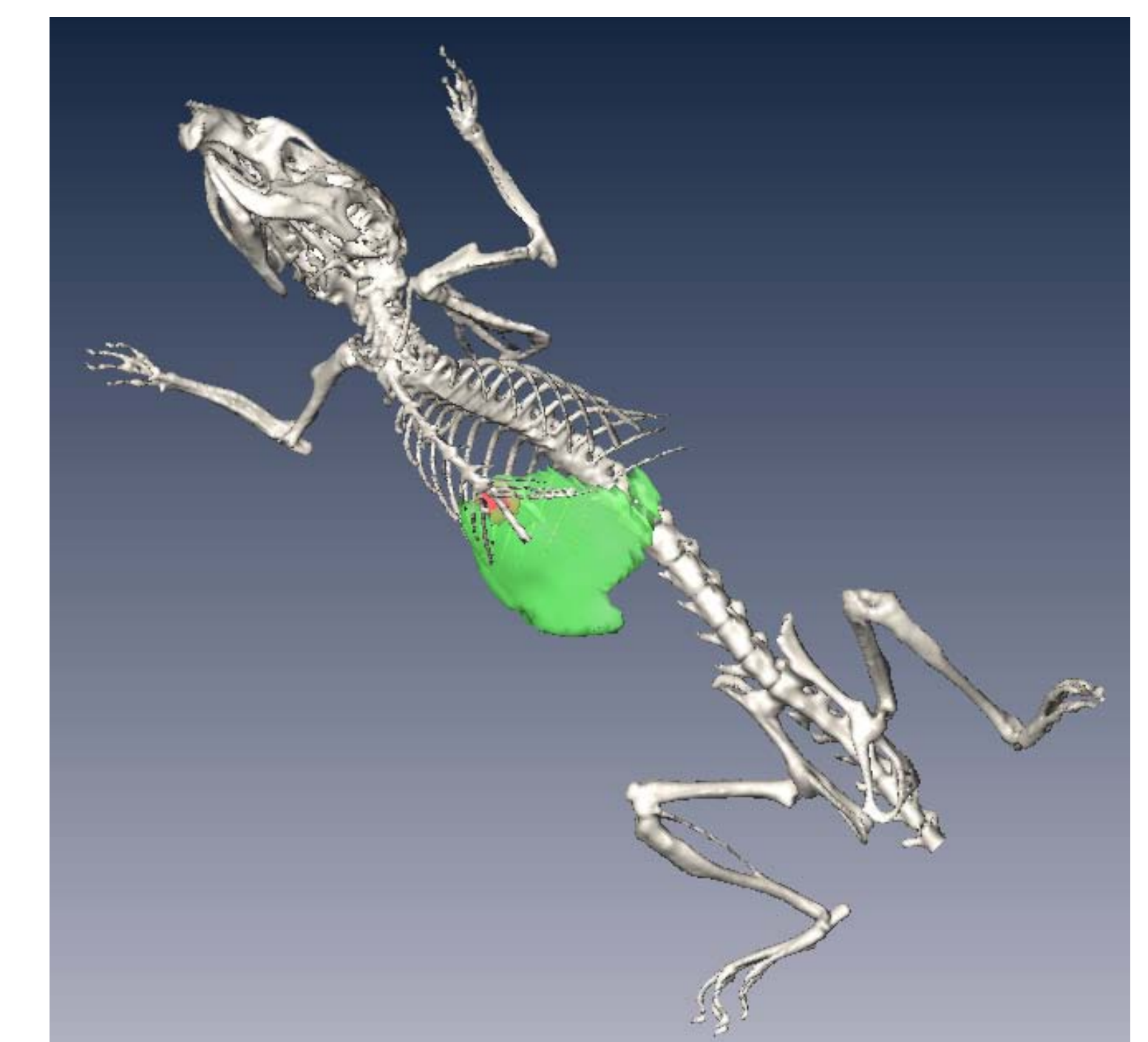


Figure 7. The same mouse was scanned in Optix and Micro-CT using the micro-ct bed⁽³⁾. In OptiView, the liver (green) and gallbladder (red) were separately analyzed and reconstructed in 3D. Those organs were superposed with the skeleton of the mouse.

Summary and Conclusions

- We demonstrated a new method to separate and quantify ICG signals originated from the liver, gallbladder and intestines using fluorescence lifetime imaging for physiology and pathology studies.
- Characterization of hepatic ICG clearance by optical fluorescence lifetime imaging provide a direct and noninvasive measure of functional state of the liver and the gallbladder.
- The technology could be applied as a non-invasive, quantitative and direct tool to monitor liver-gallbladder function status and treatment effect, as well as to monitor drug delivery in pharmacokinetic research.

References

- El-Desoky, et al., *Br J Surg.*, **86**, 1005 (1999).
- Ma, et al., *Appl. Opt.* **46**, 1650 (2007).
- Mincu et al., *WMIC poster presentation* (2011)