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Case Study

Cryo-Fluorescence Tomography Outperforms 2D *In Vivo* Fluorescence Imaging

Introduction

In vivo fluorescence imaging (FLI) is a common imaging technique used by researchers to study disease models and the biodistribution of molecular probes and drug therapies. However, 2D *in vivo* FLI has significant limitations, including a lack of anatomical information, no depth information, low resolution, and low sensitivity. While this method is easy to deploy, in the case of novel research, these limitations impart the risk of significant misinterpretations. This case study highlights research from Massachusetts General Hospital, Harvard Medical School utilized Cryo-Fluorescence Tomography (CFT) alongside 2D *in vivo* FLI in a murine thrombosis model. The correct interpretation of the *in vivo* image was only possible with the addition of the CFT imaging, highlighting the superior performance of CFT to accurately study both anatomical and molecular endpoints.

In vivo FLI Provided Misleading Data in Fibrin-Targeting Drug Study:

Study Overview:
A study using a ferric chloride-induced ($FeCl_3$) murine thrombosis model aimed to track the localization of a Cy-5.5-labeled fibrin-targeting peptide (EP-2105) after tail vein injection. Mice were imaged 10 minutes post-injection of 50 µg EP-2105 by 2D *in vivo* FLI on the IVIS SpectrumCT platform, and the data suggested that the fibrin-targeting peptide was localized to the thrombus (Figure 1A). Due to the known limitations, specifically the lack of anatomical and depth information, the researchers sought additional information to confirm the initial interpretation.

CFT Intervention and Findings:
To identify the peptide's true localization, 3D, high resolution CFT was performed to provide overlaid anatomical and fluorescence images for more accurate information. CFT revealed that the fibrin-targeting peptide was not localized to the thrombus as initially interpreted. Instead, the peptide distributed to the submandibular gland, which given the proximity to the thrombus and limitations of the 2D imaging, required CFT to correctly determine (Figure 1B,C,D).

Conclusion:
This case study underscores the importance of 3D CFT in accurately determining the anatomical localization of compounds, avoiding the misinterpretations that can arise from 2D *in vivo* optical imaging techniques.

Figure 1.

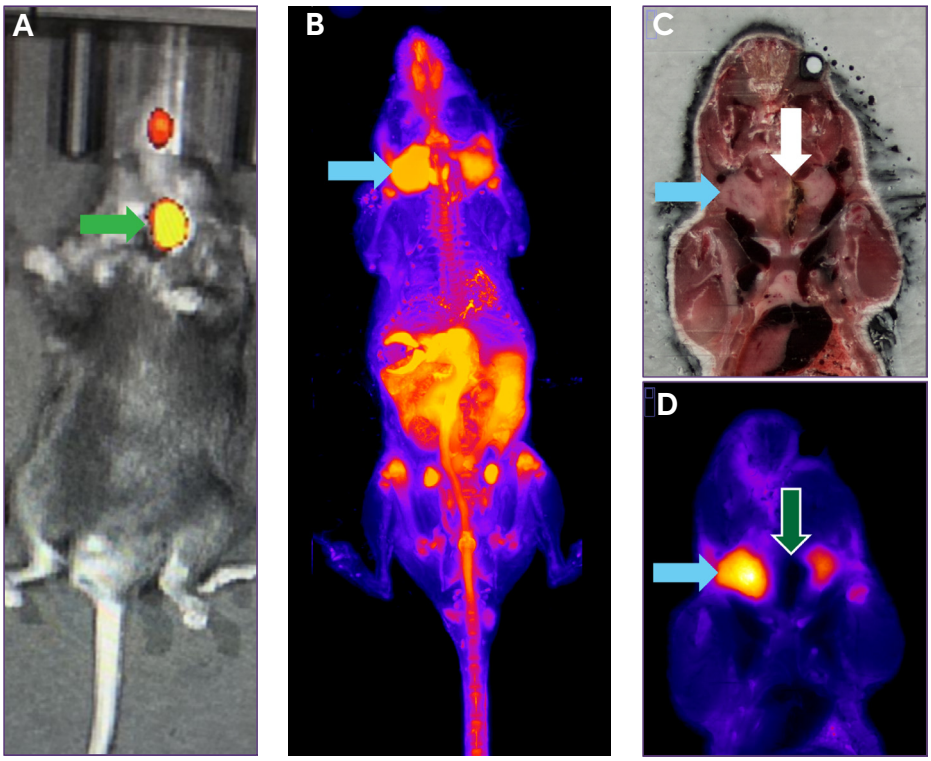

Figure 1: A) Signal interpreted as localization to thrombus from *in vivo* fluorescence imaging on the IVIS platform; B) Signal shown from submandibular gland in 3D CFT Fluorescence image; C) Anatomical identification of thrombus and submandibular gland from CFT; D) No accumulation of fluorescence labeled peptide in the thrombus.

High Sensitivity CFT Identified Signal in Thrombus Where *In Vivo* FLI Failed:

Study Overview:
An additional study used a vascular crush injury thrombus model to evaluate the localization of the same Cy-5.5 labeled fibrin-targeting peptide (EP-2105) after tail vein injection. Mice were imaged 10 minutes post-injection of 50 µg EP-2105 using 2D *in vivo* FLI on the IVIS SpectrumCT platform. The *in vivo* imaging failed to detect the fibrin-targeting peptide due to low sensitivity (Figure 2A).

CFT Intervention and Findings:
Previous studies confirmed that EP-2105 targets fibrin and localized in thrombus, so the researchers again turned to CFT to increase spatial resolution and sensitivity. CFT successfully identified the presence of Cy-5.5-EP-2105 within the thrombus (Figure 2B,C).

Conclusion:
This case highlights the superiority of CFT in detecting low-abundance signals in complex biological models. The use of CFT ensured that the expected localization of the peptide was accurately identified, providing reliable data for the study.

Figure 2.

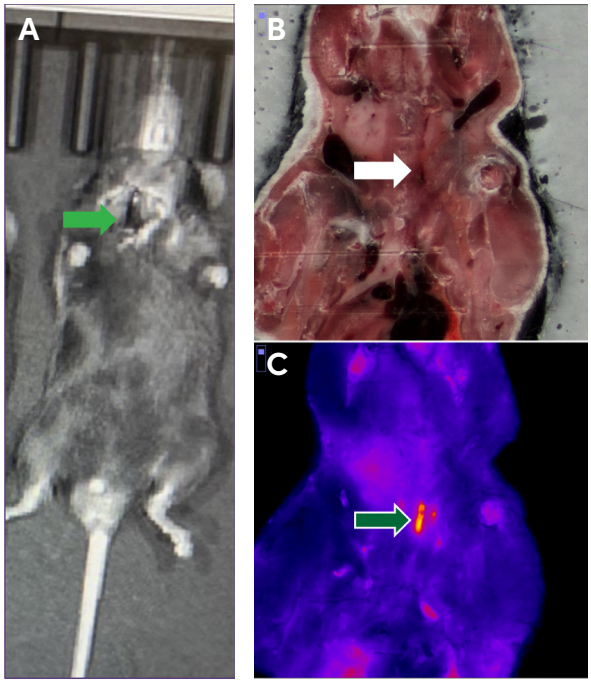

Figure 2: A) No signal or localization was observed in the thrombus with 2D in vivo imaging on the IVIS Platform; B) Presence of thrombus observed from high resolution anatomical CFT data; C) Visualization of fibrin-targeting peptide to the thrombus observed via CFT.

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Conclusions

This case study demonstrates the limitations of 2D *in vivo* FLI in accurately localizing compounds within complex biological models. Whether due to insufficient anatomical information or low resolution and sensitivity, these limitations can lead to significant misinterpretations, affecting the reliability of study outcomes. CFT is superior to *in vivo* FLI in detecting low-abundance signals and was utilized in this study to identify a missrepresentation of existing *in vivo* data and provided accurate information on the peptide localization. CFT's ability to offer high sensitivity, high resolution, and detailed anatomical context ensures that researchers can trust the localization data, leading to more reliable conclusions and better-informed decisions in their preclinical research.