Visualizing Tumor Metastasis Throughout a Whole Mouse Using 3D Ex Vivo Cryo-Fluorescence Tomography (CFT)

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Abstract

Preclinical tumor metastasis models are common, but are difficult to visualize and subsequently quantify accurately. MRI lacks sensitivity and specificity at early stage metastasis, while PET, SPECT and optical techniques lack resolution. Cryo-Fluorescence Tomography (CFT) is a new ex-vivo imaging modality combining cryoslicing with serial fluorescence and anatomical white light imaging. This technique provides a whole-body molecular 3D data set which shows the extent of metastasis.

A 4T1 mouse mammary tumor cell line having a high incidence of metastasis was used in a preliminary study. This line expresses both luciferase and DsRed which allows for a comparison of bioluminescence and fluorescence imaging techniques. Half a million cells were injected into the left ventricle of the heart to ensure thorough distribution throughout immunodeficient NSG mice. Tumor progress was monitored regularly via an in-vivo bioluminescence imaging system. After 10 days the animals were sacrificed and frozen whole. The animals were embedded in optimum cutting temperature material after which the subject was serially sectioned at 50 microns, with white light anatomical and red fluorescence images acquired of the block face after each slice. Data from the fluorescent and white light images were reconstructed into a 3D data including corrections for alignment and other biological and optical aberrations.

In vivo bioluminescence data shows diffuse signal, indicating metastatic tumor lesions throughout the animal. While this imaging technique provides marked specificity, it is also highly surface weighted and unable to resolve small metastases below approximately 1mm in depth. Tumor localization using BLI normally requires post mortem imaging of the dissected subject. However, the CFT data provides clear localization of tumor metastasis using the anatomical reference provided by the white light images.

One-view BLI missed an order of magnitude of the tumors, the majority of which were under 1 mm in size. The bioluminescence signal suffers from dynamic range artifacts, as large tumors near the surface will mask the signal of smaller tumors, even if they are adjacent. Using CFT, tumor lesions measuring less than 400 um were easily detected in this model.

CFT as an endpoint of bioluminescence imaging has provided a complete account of metastatic disease in our subject animals. The high resolution molecular 3D data is invaluable to measure tumor burden, and is supported with whole body white light imaging for anatomical landmarking. CFT also provides 3D optical molecular data without suffering from depth artifacts due to limited penetration and high scatter of diffuse optical light in tissue. The ability to see much smaller tumor sites means that CFT can be employed at an early stage in tumor metastasis studies, studying whole-body metastatic proliferation at an earlier time point than is typically available with other modalities.

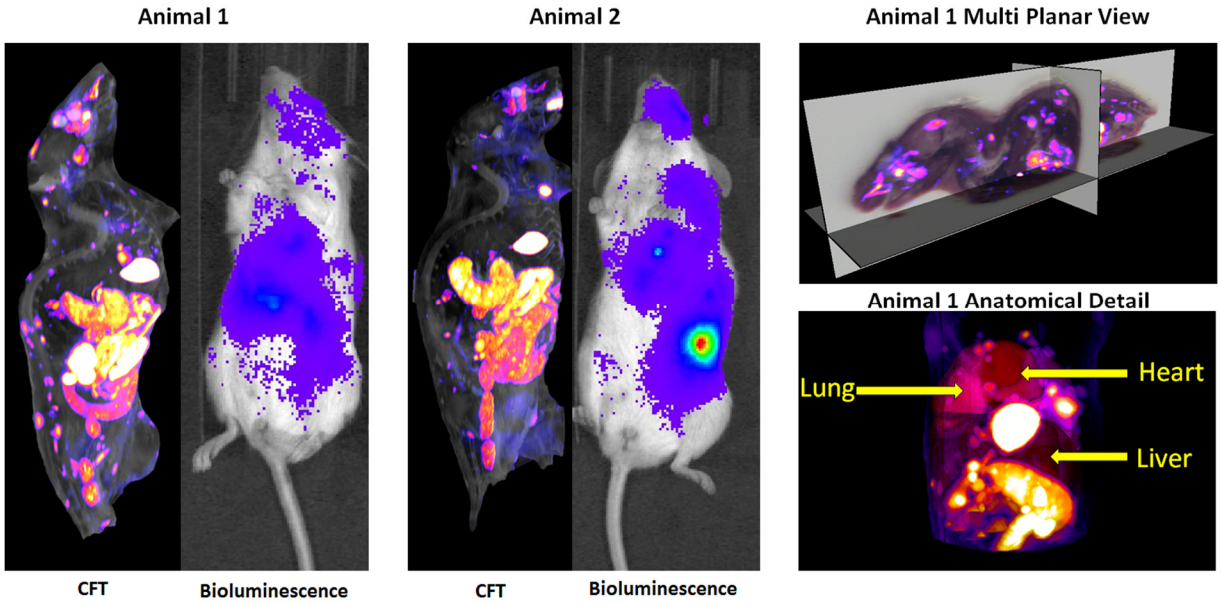

Figure 1. Bioluminescence imaging has excellent specificity but lacks sufficient resolution to quantify tumor metastases, especially at depth. Cryo-Fluorescence Tomography can be used as an ex-vivo imaging compliment to bioluminescence imaging to identify tumors as small as 300um in this study.