Cryo-fluorescence Tomography as a new tool in 3D visualization of tumor heterogeneity, metastatic proliferation and immuno-oncology.

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Introduction

Cryo-Fluorescence tomography (CFT) is serial slicing with off-the-block anatomical and fluorescence imaging and is capable of imaging a whole animal into a 3D dataset. This technique can also image isolated tumors either from rodent models, or human patients, at higher resolution. CFT has a unique ability to provide both high resolution anatomical images along with very high sensitivity and specificity. Sections can also be transferred to slide for further histology and co registration back to the whole sample data set. In this study, we utilize CFT in several tumor models to track tumor microenvironment and heterogeneity, metastatic spread, and expression of specific markers in excised human tumors.

Methods

In the first study, a 4T1 mouse mammary tumor model expressing both luciferase and DsRed was used to study metastasis. Bioluminescence imaging was conducted serially on animals until tumor expression was detected. Animals were then sacrificed and frozen whole before being embedded in Optimum Cutting Temperature material. The specimen is then imaged using CFT with a section thickness of 50um. Three images are collected for each section: a white light image and two fluorescence images.

For the next study, immunocompromised mice were intracranially inoculated with GL26-luc2 cells in the right hemisphere of the brain. After two weeks, the subject was imaged with a T1-weighted MR sequence with Gadolinium contrast enhancement. A bioluminescence image was also acquired. Finally, the subject is intravenously administered 100uL of 0.1mM indocyanine green (ICG) and 100uL of 0.2mM of Angiosense680Ex then sacrificed 24-hours later. The brain is then removed and embedded in Optimum Cutting Temperature material. The specimen is then imaged using CFT with a section thickness of 25um.

To validate clinical translatability, enrolled patients were administered with 2.5mg/m2, 25mg/m2 or 62.5mg/m2 Cetuximab-IR800Dye during tumor resection. Tumor samples were embedded in paraffin and sectioned in the imaging system. Fluorescent and bright field images were captured off the block face.

For all studies, the resulting image stacks are aligned, corrected for biological optical effects, and reconstructed to recover a 3D distribution of fluorophore as well as 3D white light information. 3D visualization and co-registration was done with VivoQuant (Invicro)

Results

For metastatic tumor progression, CFT showed a near complete account of metastatic disease in our subject animals when compared to bioluminescence imaging. The high resolution molecular 3D data is invaluable to measure tumor burden, even with very small tumors, and is supported with whole body white light imaging for anatomical landmarking.

In the GBM models, the CFT data had very high specificity to the tumor and a very high correlation to the MRI meaning the 2 techniques are equally good at assessing tumor angiogenesis. Further fluorescent markers can also be employed in the CFT study to provide additional information on gene/protein expression or presence of immunologic factors as well.

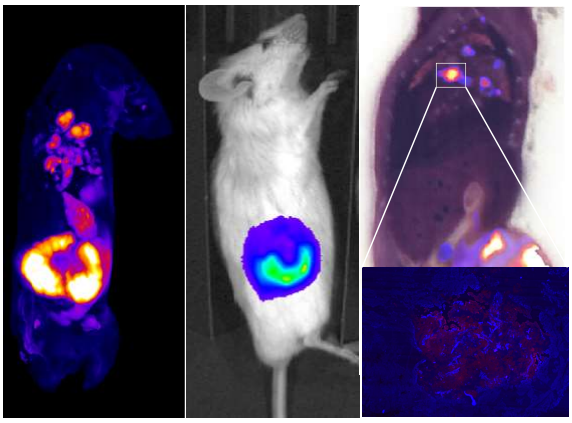

Figure 1: Molecular fluorescence data shown as a 3D maximum intensity projection (left) while Bioluminescence (BI) data as a 2D overlay (middle), in the same animal. CFT provides more comprehensive information about the primary tumor compared to BI. Additionally, BI completely misses the secondary metastatic tumors that are prolific in the lungs. CFT allowed for the guided collection of a section of a major metastatic site by allowing us to transfer the section immediately below a region of strong signal (upper right). This section was mounted and stained before high resolution digital fluorescence microscopy (lower right).

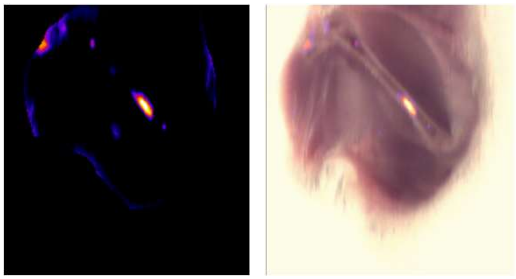

Figure 2: CFT shows additional sites of tumor metastasis in the femur. Fluorescence signal alone (left) has very high specificity with low background. Whitelight images with fluorescence overview (right) show very specific signal localization within the bone marrow space.

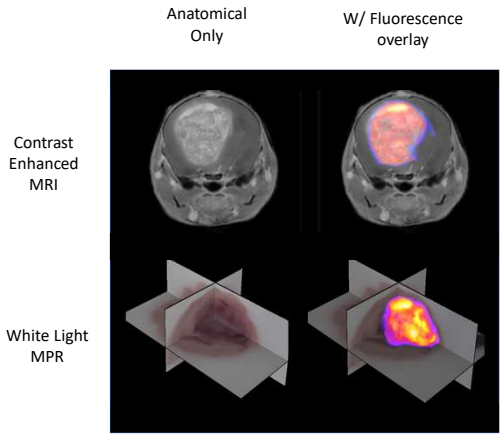

Figure 3: A zoomed in view of the Glioma. The MRI contrast enhances image shows Structural heterogeneity. Low background signal of the Fluorescence shows specificity to the cancer region in the brain. Overlay of the images show good correlation of the signal in the region of interest

Conclusion

Using CFT as a complementary imaging platform adds additional information that bridges the gap between in vivo imaging and traditional histopathology. CFT shows good correlation with bioluminescence imaging and contrast enhanced MRI, while providing an additional level of molecular specificity.

The fluorescence provides an enormous amount of specificity in this study, as it is only the tumor site that is appreciably fluorescent in the imaging domain. However, the imaging technique does not sacrifice structural information for specificity, as there is also a collection of white light images for anatomical landmarking. Thus, imaging with CFT provides valuable but complimentary information in preclinical oncology studies.

The CFT process also allows for the collection for tissue samples for traditional histology analysis.

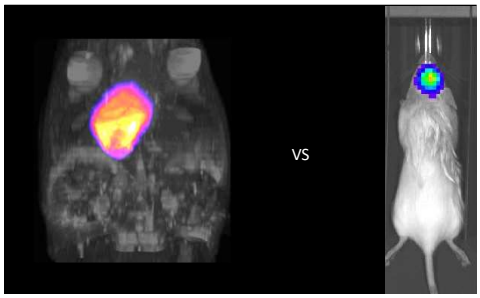

Figure 4: Traditional BLI (right) offers a highly sensitive imaging technique used in a variety of oncology models. However the modality is limited in its ability to accurately give information on 3D structure. It is common practice to incorporate MRI into Murine GBM studies. Using CFT (left) as an additional data point the researcher can also evaluate how the fluorescent reporter or the white light visualization correlate structural information from the MRI.

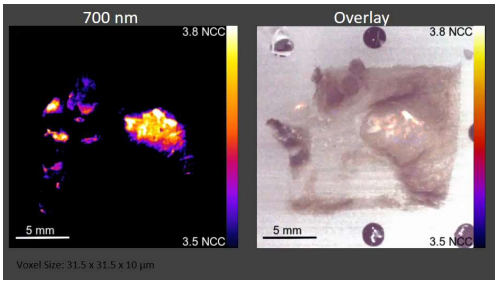

Figure 5: Fluorescence and white light planar images were sequentially acquired off the block-face throughout the entire sample as it was sectioned. Collected 2D white light and fluorescent images (left) were co-registered (right) to provide anatomical reference to Cetuximab-IR800Dye. Collected voxel size: 31.5µm x 31.5µm x 10µm

Cryo-Fluorescence Tomography

CFT has proven to be a powerful technique for oncology investigations. This technique can characterize the 3D bio-distribution and localization of antibody drug conjugates, or diagnostic antibodies, across an entire sample. More complete and precise data sets fluorescent labeled CAR T cells or tumor associated macrophages can aid the development of immuno-oncologic therapies.

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Work was done in collaboration with:
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