3D High Resolution Fluorescence Imaging as a Complimentary Assay to *In Vivo* Gadolinium-Enhanced T1-Weighted MRI and Bioluminescence

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Introduction

Murine models are a valuable tool in medical research, commonly imaged using MR or bioluminescent techniques. Although MR has excellent soft tissue contrast and resolution, the cost of imaging can be high. Bioluminescence imaging has excellent specificity, but suffers poor resolution and sensitivity due to the high absorption and scatter of light in biological tissue. Cryo-Fluorescence Tomography (CFT), an imaging modality based on serial slicing and off-the-block fluorescence imaging, is examined for its utility as a complimentary assay in preclinical oncology studies. CFT is incorporates into existing workflows and allows for registration and evaluation with in vivo imaging techniques (Figure 1).

Methods

An immunocompromised mouse is intracranially inoculated with GL26-luc2 cells in the right hemisphere of the brain. After a waiting period of two weeks, the subject is imaged with a T1-weighted MR sequence with Gadolinium contrast enhancement. A bioluminescence image is also acquired. Finally, the subject is intravenously administered 100uL of 0.1mM indocyanine green (ICG) and 100uL of 0.2mM of Angiosense680Ex. At the 24-hour time point, the subject is sacrificed. 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. Three images are collected for each section: a white light image and two fluorescence images. One fluorescence image is taken for Angiosense detection, and one is taken for ICG detection. Using VivoQuant, the resulting image stacks are aligned, corrected for biological optical effects, and reconstructed to recover a 3D distribution of Angiosense and ICG, as well as 3D white light information.

WorkFlow

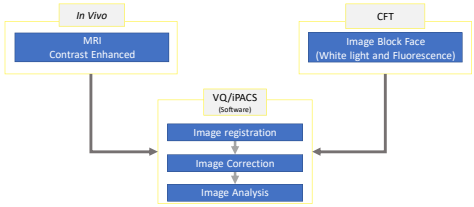

Figure 1: This diagram represents the workflow used to integrate CFT in this workflow as a complementary technology. Using VivoQuant as the analysis and reconstruction engine in vivo and ex vivo samples are registered, oriented and analyzed.

Results

A comparison of the Angiosense and the ICG distributions yields a linear correlation of greater than 0.90, demonstrating the consistency of the imaging method among fluorophores. This further indicates that the imaging technique is poised for clinical ICG distribution analysis in samples such as resected tumor margins. The bioluminescence image provides a strong indication of the presence and the activity of the cell line, but lacks resolution at depth since it is highly surface weighted. The MR image is co-registered to the fluorescence data (Figure 2, 3, 4). Multi-planar images were generated in VivoQuant using the CFT information. Comparing the fluorescence and MR intensities within the tumor site shows a linear correlation of greater than 0.7, suggesting comparable image information content between the MR and the Fluorescence

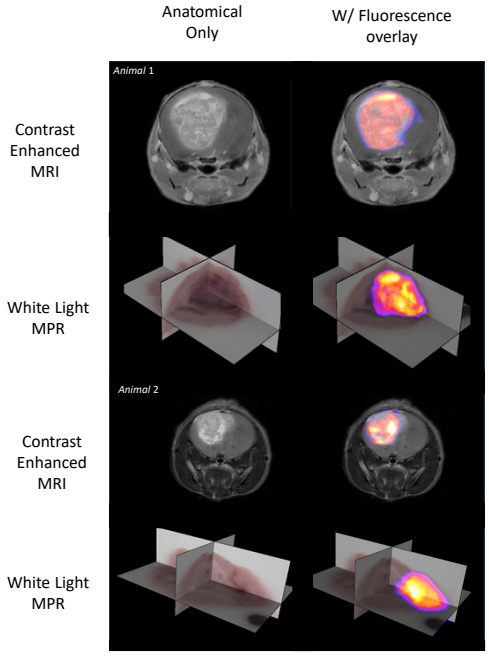

Figure 2: Molecular fluorescence data is shown as a 3D maximum intensity projection with a multi-planar slice view of corresponding white light data. In this way, molecular fluorescence can be analyzed in the accessible context of white light images.

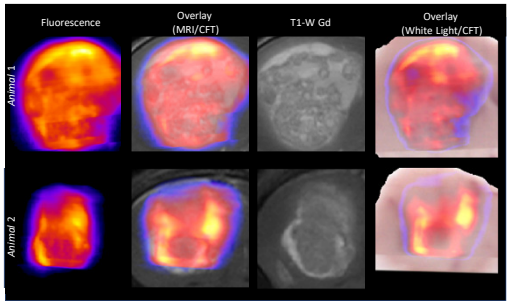

Figure 3: A zoomed in view of the Glioma. The MRI contrast enhances image shows Structural heterogeneity. Low background signal of the Fluorescense shows specificity to the cancer region in the brain. Overlay of the images show good correlation of the signal in the region of interest

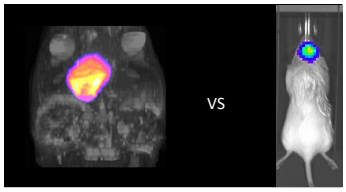

Figure 4: Traditional BLI offers a highly sensitive imaging technique used in a variety of oncology models. However the modality is limited in its ability to accurately give information on 3D structure. It is common practice to incorporate MRI into Murine GBM studies. Using CFT as an additional data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

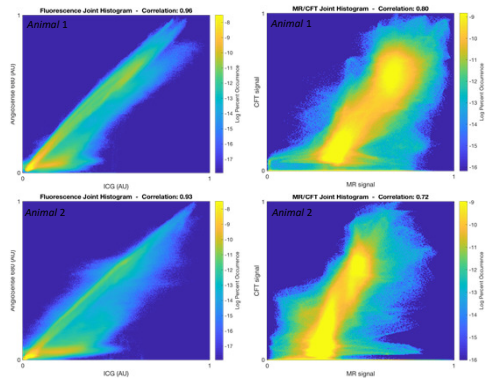

Figure 5: Comparison graphs between AngioSense and ICG were plotted. Correlation between AngioSense and ICG were greater than .90. Comparison between the contrast enhanced MRI and the Angiosence was performed to show a 0.80 and 0.72 Correlation between animal 1 and 2, respectively.

Conclusion

Using CFT as a complementary imaging platform adds additional information that bridges the gap between in vivo imaging and traditional histopathology. Both Angiosense and ICG are non specific tracers that have shown good correlation with contrast enhanced MRI in this model.

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. Future research will incorporate models that use molecular targeted probes as well as the use of transgenic models that express fluorophores.

Cryo-Fluoroscence Tomography

Cry-Fluorescence Tomography (CFT) is an emerging molecular imaging technique where a specimen is prepared with exogenous or endogenous fluorescence, fresh frozen or paraffin embedded, and mounted into a slicing instrument. The specimen is serially sectioned, and for each slide a white light and at least one fluorescence image is taken (although it is possible to simultaneously capture fluorescence at multiple wavelengths for multi-tracer imaging or spectral unmiximg). The images are then aligned based on anatomical or embedded fiducial markers, corrected for off-the-block subsurface fluorescence, and rendered as a three-dimensional volume. This produces 3D molecular fluorescence distributions that are perfectly co-registered to white light images, which can then be used for tissue identification, segmentation, or 3D rendering.

The technique allows for co-registration between a wide variety of imaging technologies that vary in resolution and sensitivity. The reconstruction and 3D rendering into isotropic voxels allows easy registration and visualization with 3D in vivo imaging modalities. CFT offers a range of resolutions. The flexible platform design allows for the researcher to interrogate anything from tissue samples to entire animals at a variety or resolutions.

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Work was done in collaboration with: