Precise Anatomical Mapping and Quantitative Analysis of Mouse Brains Using Cryo-Fluorescence **Tomography on Xerra™**

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BACKGROUND

Cryo-Fluorescence Tomography (CFT) on Xerra™ (EMIT Imaging, Inc.) provides proven efficacy for studying whole animal distribution of both gene expression and drug delivery[1,2,3]. Within the fields of drug delivery and distribution, there is particular interest in evaluating expression and distribution within brain tissue of animal models.

The alignment of the Allen Mouse Brain Atlas (CCFv3)[4] with the Xerra data serves as a crucial step tool in brain research. It not only facilitates accurate and repeatable measurements of specific brain areas within the fluorescent data, but also enables the masking of designated regions for visual comparison in both white light and fluorescent volumes. This dual functionality significantly enhances the ability to correlate structural and functional data, providing deeper insights into brain distribution and protein expression.

The described methodology ensures controlled and reproducible measurement of CFT image data, advancing the capabilities of this novel imaging tool in brain research. The combination of the Xerra's precise color imaging and the robust alignment with the Allen Mouse Brain Atlas presents a powerful tool for neuroanatomical studies and the investigation of fluorescently-labeled biological probes and processes.

OBJECTIVES

- Provide deeper insights into brain distribution and protein expression by correlating structural and functional data generated by the volumetric white light and fluorescent data generated by Xerra
- Demonstrate the importance of white light color imaging when analyzing detailed structures
- Leverage CFT's white light and fluorescent images to improve the data analysis pipeline
- Standardize analysis of neurological studies on CFT datasets.
- Increase the accessibility of CFT through publicly available toolsets, including ImageJ/Fiji and the Allen brain atlas

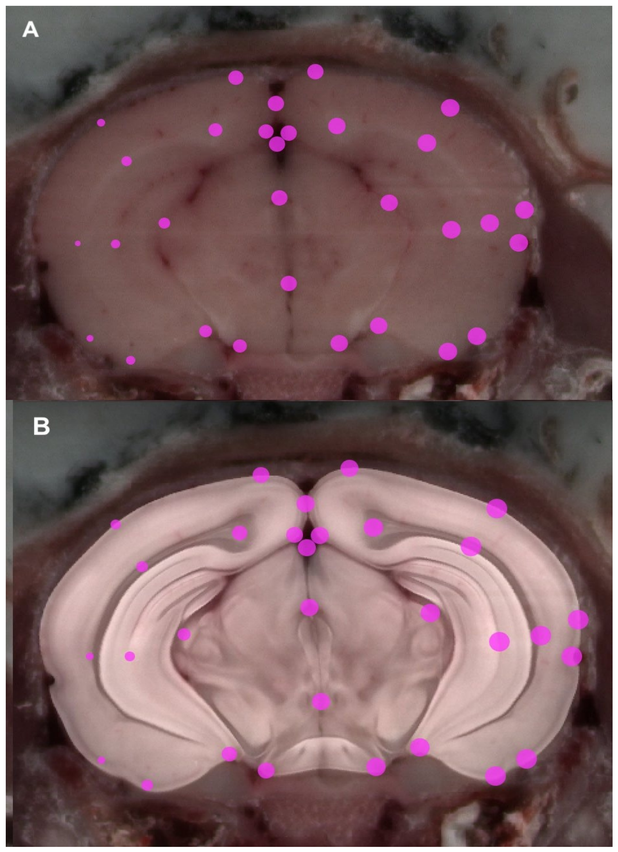

FIGURE 1: Key point selection on CFT dataset (A) and overlay of atlas warped to key points (B)

METHODS

- White light and fluorescent CFT datasets were captured and reconstructed using Xerra
- Using the Allan CCF 2017 V3[4] atlas, the CFT dataset is set as the reference dataset and the brain atlas is the target
- The BigWarp[5] tool for Fiji was used to select key points on the reference and target image volumes. After key point selection the target dataset is transformed via Thin Plate Spline (Figure 1).
- For many analysis tasks the full 461 region list is not required, so we reduced the Reference atlas to 16 component regions (Figure 2)
- On an example dataset, analysis of signal intensity in selected 3D regions was performed using Fiji's ROI manager

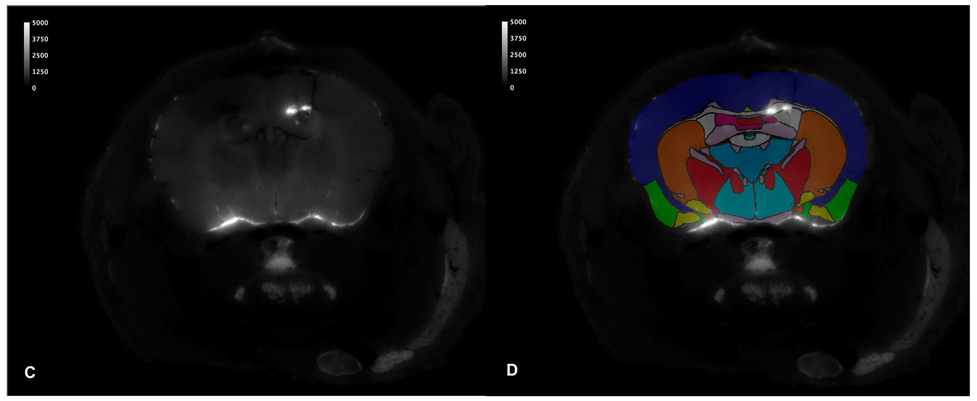

FIGURE 2: CFT fluorescence image (C) and fluorescence image with reduced region color-coded atlas overlaid

RESULTS

- Fiji's Big Warp tool is sufficient for working with CFT data for atlas alignment using key point selection (Figure 1A). The overlay of the warped atlas with the white light CFT image (Figure 1B) demonstrates the accuracy of the alignment and the structural information available in the white light CFT volume.
- Using the example dataset, the fluorescent data (Figure 2C) shows elevated signal in the striatum compared to the adjacent cerebral cortex, orange and blue respectively
- Further, the brightest brain subregion by average voxel intensity was measured to be the olfactory area (Figure 3)
- The olfactory area is difficult to visualize in the sagittal and axial planes using the full fluorescent volume (Figure 4 E, G).
- Using the atlas to mask out the brain (Figure 4 G,H) makes anatomical visualization much clearer. The olfactory area is now clearly distinguishable from other anatomy (Figure 4 G).

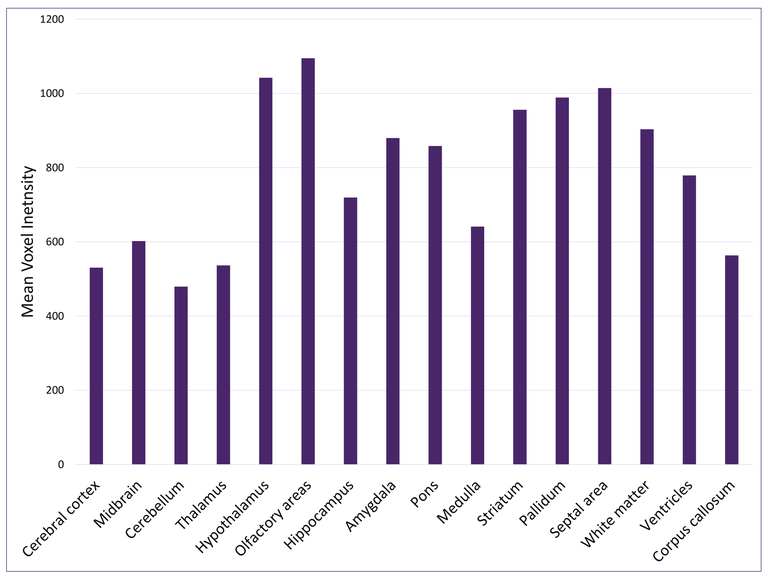

FIGURE 3: Average signal value of the 16 measured atlas regions.

RESULTS (Cont.)

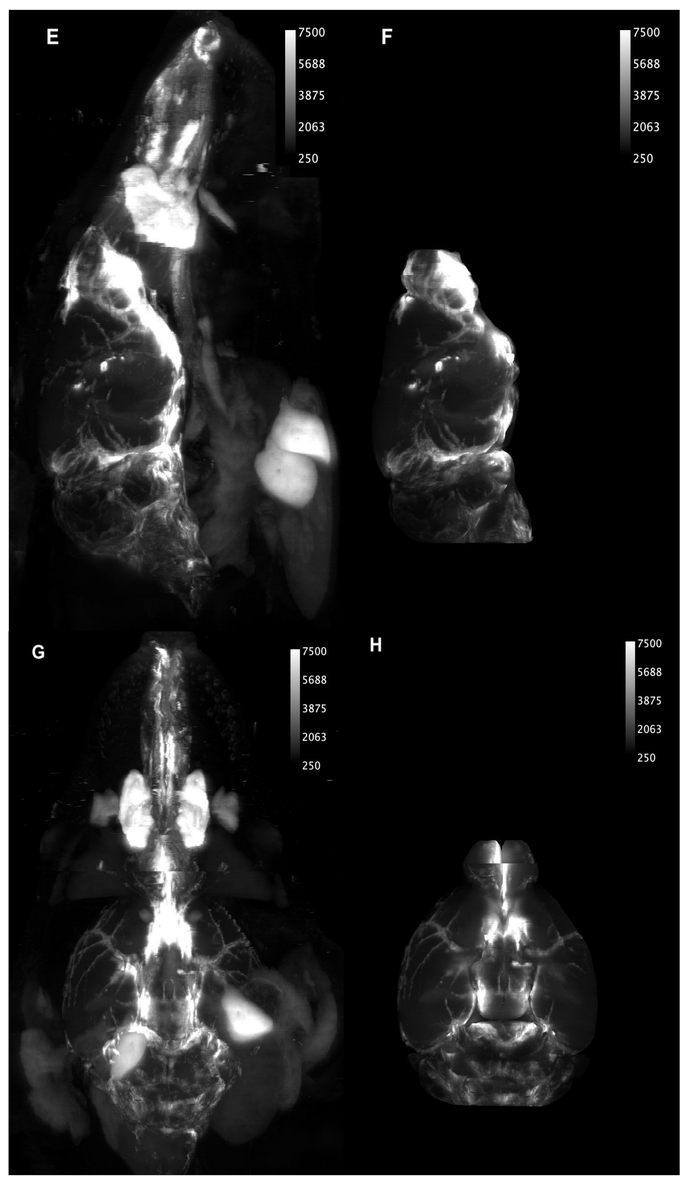

FIGURE 4: Sagittal maximum intensity projections of whole CFT dataset (E) and brain only dataset (F). Axial datasets (G-H)

CONCLUSIONS

- A CFT dataset of a sectioned mouse skull compatible with atlas alignment and downstream analysis of fluorescent signal provides valuable regional information on drug delivery/diffusion and gene/protein expression
- White-light, color CFT data alone contains enough information for precise registration to a standard brain atlas, enabling unbiased regional analysis
- Registration can be performed to either map the atlas to the CFT image or to map all CFT datasets to a common space
- Future work includes automation of the atlas alignment and further study of auto-fluorescence signal observed at some imaging wavelengths

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