Development and Optimization of Standalone Multi-Wavelength Fluorescence Add-on for a Cryoslicer

Robert W. Holt[1], Mohammed Q. Qutaish[1], Jacob Hesterman[1], Ildiko Polyak[1], Phuong Diep[1], Jack Hoppin[1], John V. Frangioni[3], Ajay Verma[2]

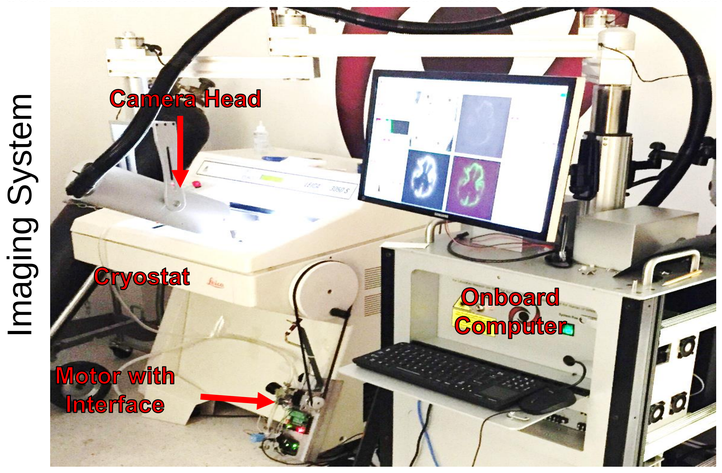
[1]InviCRO, LLC, Boston, MA [2]Biogen, Cambridge, MA [3]Curadel, LLC, Worcester, MA

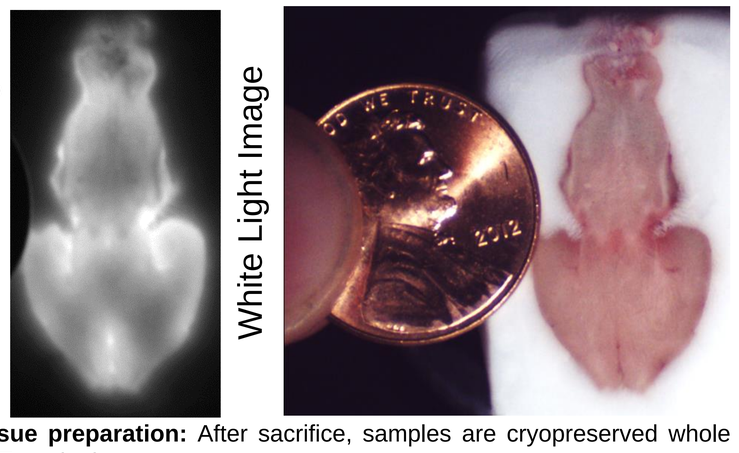
Objective

Multispectral fluorescence cryoslice imaging has been previously used to measure drug distribution ex-vivo in standalone or retrofitted cryoslice imagers [1,2]. Such specificity in a single imaging system can result in high cost per scan. For high throughput and low cost, it would be valuable to construct a fluorescence imager with a corresponding software package that can work in tandem with common cryoslicing instruments which are already in place. The methods outlined here demonstrate a workflow of cryofluorescence imaging techniques for a versatile, transportable add-on to existing cryoslicing instruments.

Methods

Instrument: A FLARE® Model R1 Open Space Imaging System (Curadel ResVet Imaging, Marlborough, MA) is manually aimed at a Leica 3050S cryostat (Leica Camera AG, Wetzlar, Germany) for simultaneous imaging of color video and two channels of NIR fluorescence from the tissue block.





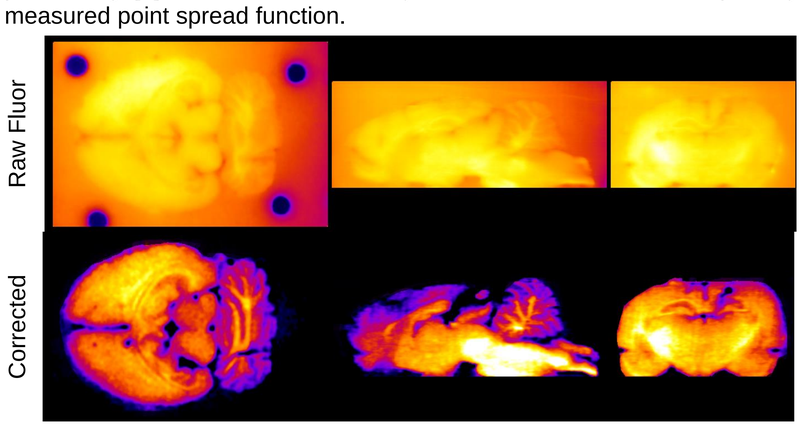
Tissue preparation: After sacrifice, samples are cryopreserved whole in OCT on dry ice.

Fluorescent and White Light Image Acquisition: Once fully frozen, blocks are loaded into a cryostat or macrotome and sectioned in 25 µm thick increments. Fluorescent and white light images are acquired for every section. Images are taken of the block face to eliminate artifacts from tape transfer.

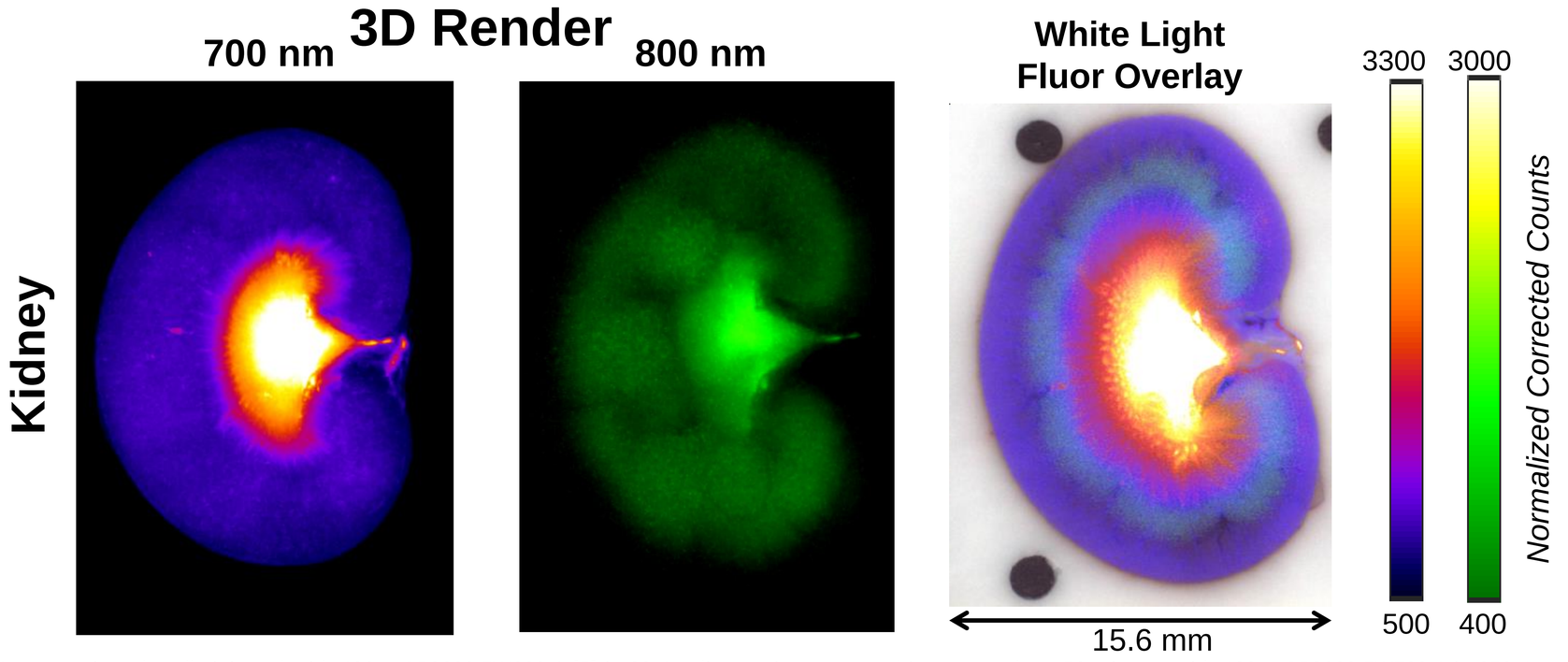
Image Processing and 3D Model Generation: Fiducial markers are visible in white light and are used to align images.

Subsurface Fluorescence Correction

Subsurface fluorescence correction is performed using either next-image processing [3] or a Richardson-Lucy deconvolution with an empirically measured point spread function.



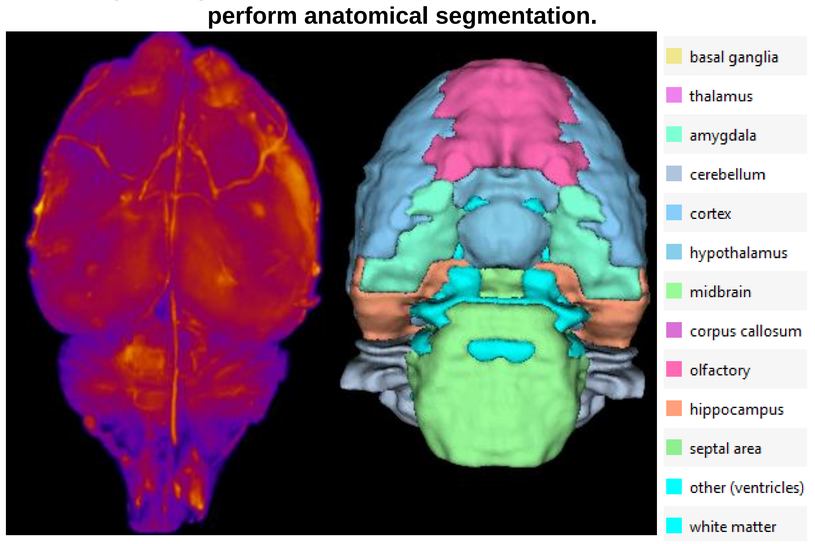
Resected Rat Kidney Imaging



A rat was intrathecally injected with 400µg PEG909-ZW800-1 [5] in 30µL saline at time zero, and intravenously at 50 minutes with 400µg PEG909-ZW700-1 in 250µL saline. The subject was sacrificed at one hour and the left kidney and brain were resected. The samples were frozen and imaged using a cryoslice thickness of 25 microns.

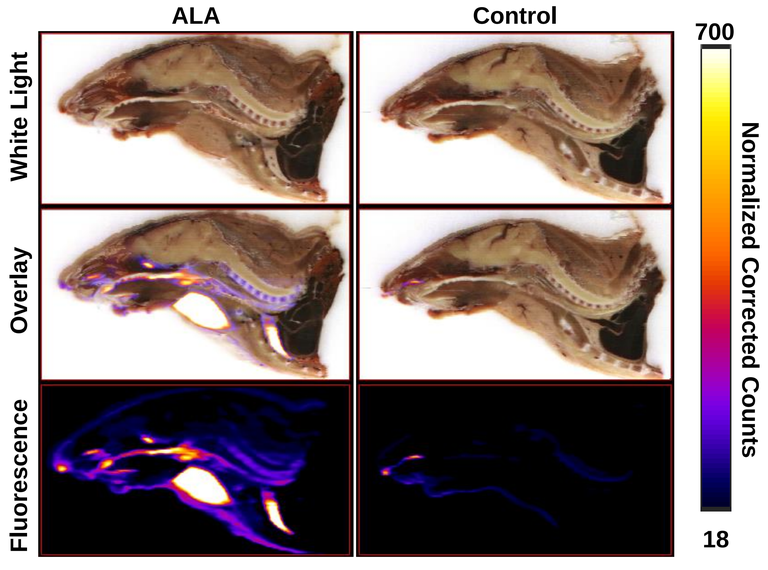
Anatomical Segmentation

White light images can be used with established reference tools to perform anatomical segmentation.



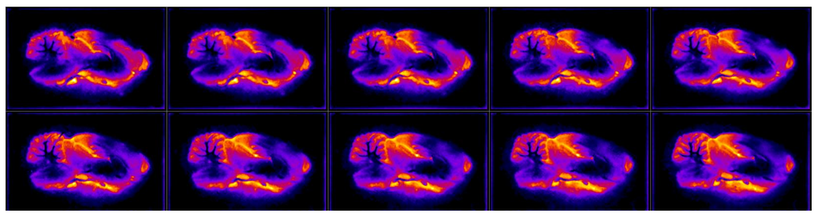
PPIX Imaging

A male immunocompetent hairless mouse was injected IV with 200mg/kg 5-Aminolevulinic acid hydrochloride (ALA, Sigma-Aldrich, A3785). A control subject was injected IV with 200uL saline. Subjects were sacrificed at one hour, frozen, sectioned, and imaged.

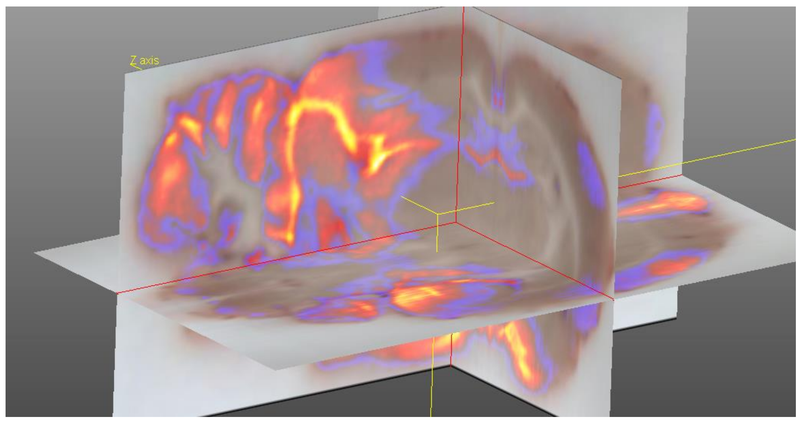


2DG Imaging

A male Sprague-Dawley rat was dosed with XenoLight 750 RediJect 2DG probe (PerkinElmer, Waltham, MA) to show glucose uptake [4].



Fluorescent images of sequential sections displayed after subsurface fluorescence removal. 2DG uptake in brain parenchyma is evident.



Fluorescent and white light images are registered and overlaid showing 2DG distribution.

Summary

The system supports hybridization of the white light and fluorescence information to perform a more accurate fluorescence distribution recovery. The current workflow is constructed to interface with existing tools, including the assignment of metainformation so that the resulting images can be accessed through the cloud and viewed anywhere in the world. The cryofluorescence tomography pipeline provides meaningful, accessible molecular information.

References

[1] Roy et al., Anat Rec (2009).
[2] Sarantopoulos et al., Mol Imaging Biol (2011).
[3] Steyer et al., Annals of Biomed. Eng. (2009).
[4] Seaman et al., WMIC abstract (2015).
[5] Choi et al., Angew Chem Int Ed Engl (2011).