



High-Throughput Three-Dimensional Tissue Imaging

VIB Training/Imaging @ VIB IX: The Mesoscopy Challenge

Disclosure

- Co-Founder at Emit Imaging
- Adjunct Instructor at the University of Wisconsin – Madison



Xerra™

Bridging The Gap Between
In Vivo and Histopathology



What is Emit Imaging

Integrated hardware and software to enable bright-field, fluorescent and nuclear tomography



What Emit Imaging does:

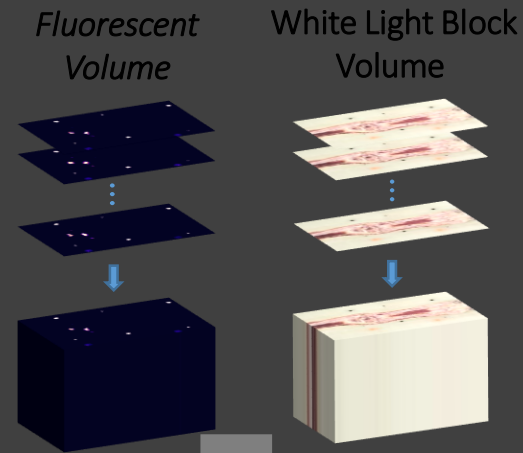
- Automated multimodal imaging of ex-vivo tissue in slices
- Overlay molecular images with anatomical reference

Applications in:

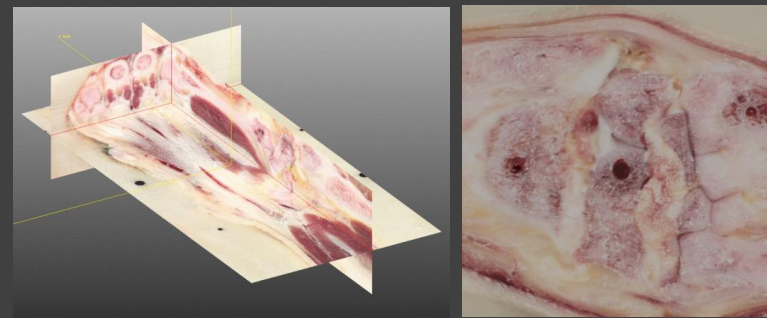
- Pharmacokinetics/Pharmacodynamics
- Phenotyping and model development
- Biodistribution/Bioavailability
- Immuno-Oncology
- Gene Expression/Editing (CRISPR)
- Cell tracking/Regenerative medicine
- Clinical molecular pathology

Surpasses Current Technologies:

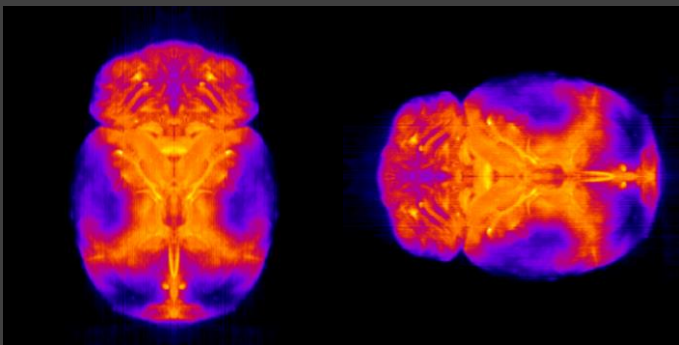
- Fluorescent Histology
- Autoradiography
- Tissue Scintillation (Grind & Bind)
- Immunohistochemistry (IHC)



Emit Imaging Drug Delivery Study



What is Cryo-Fluorescence Tomography CFT?



CFT is a Molecular Tissue Imaging (MTI) modality where ex vivo fluorescence from individual 2D sections are captured and compiled into a 3D volume merged with anatomical white light reference.

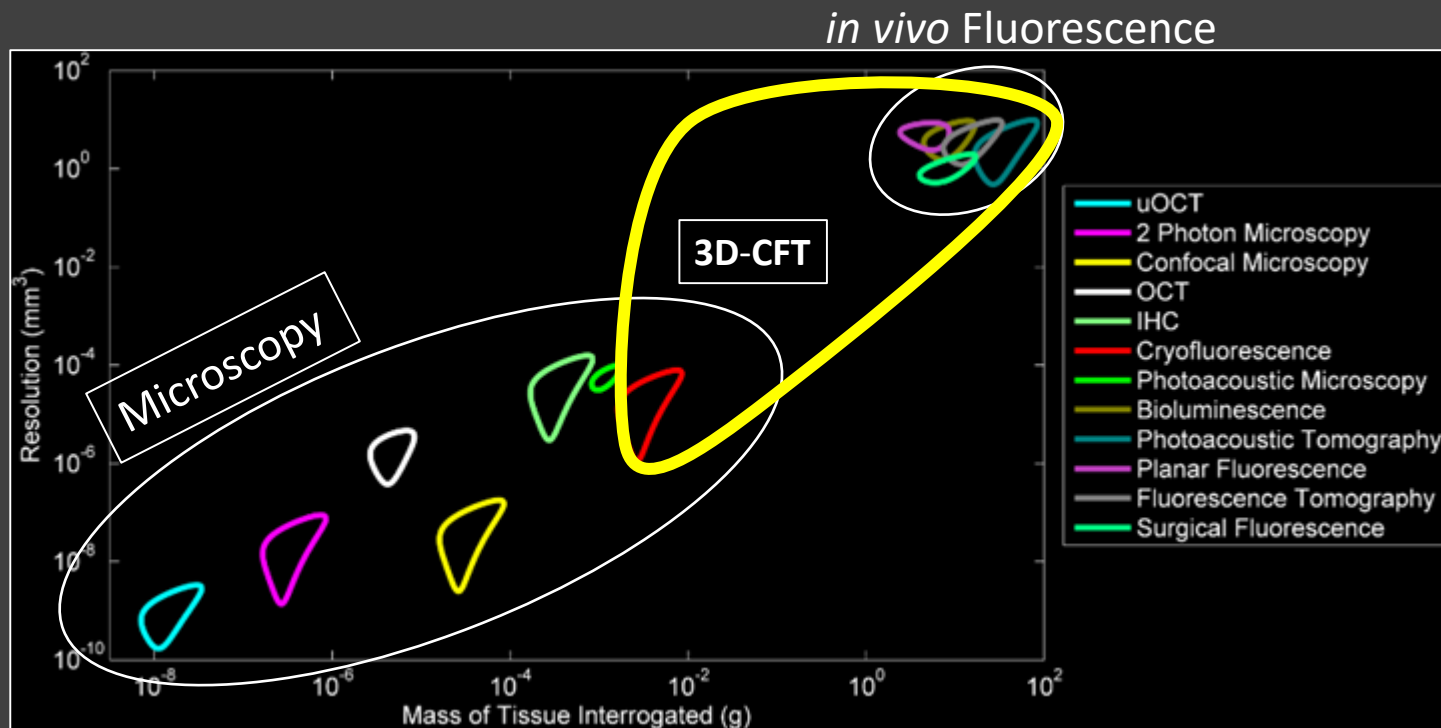
Benefits

- Image size scales from cell clusters
- Can image samples from rodent small organs to NHP brains.
- Fits into any research workflow
 - Implemented after *invivo* imaging
 - Tissues can be collected for histology (auto-rad, IHC, etc)

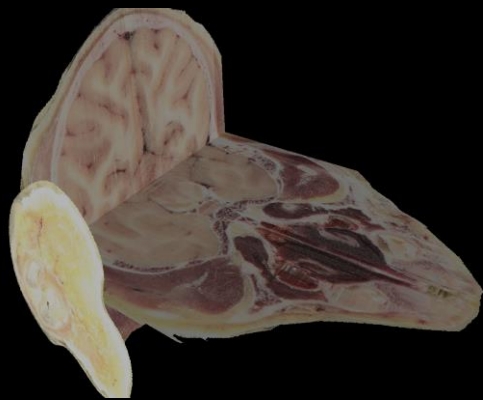
3D-CFT Fills the Gap in Tissue Imaging



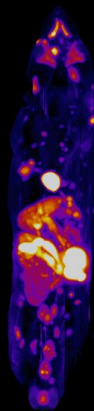
Enables a “big picture” view of the impact of a therapeutic with the ability to investigate in high resolution, off of the same sample



3D-MTI images across a wide range of volumes



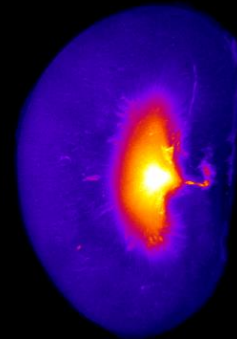
100mm



50mm



25mm

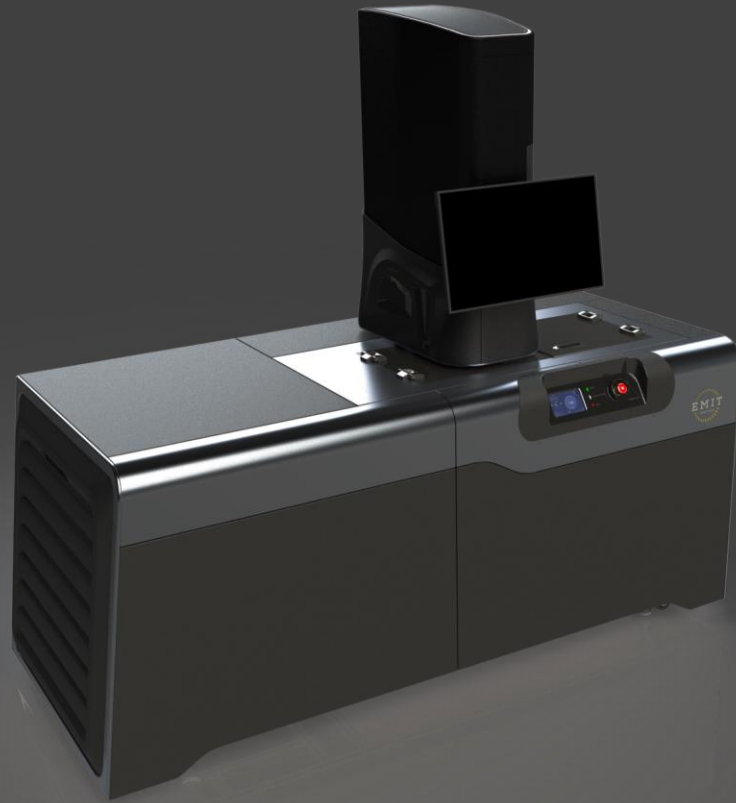


10mm

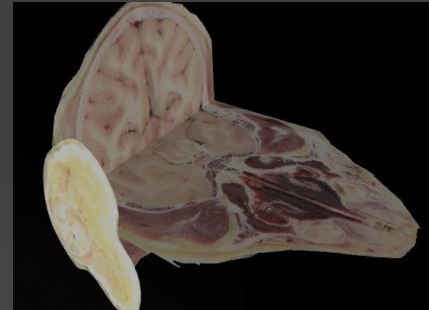
Volume

Resolution

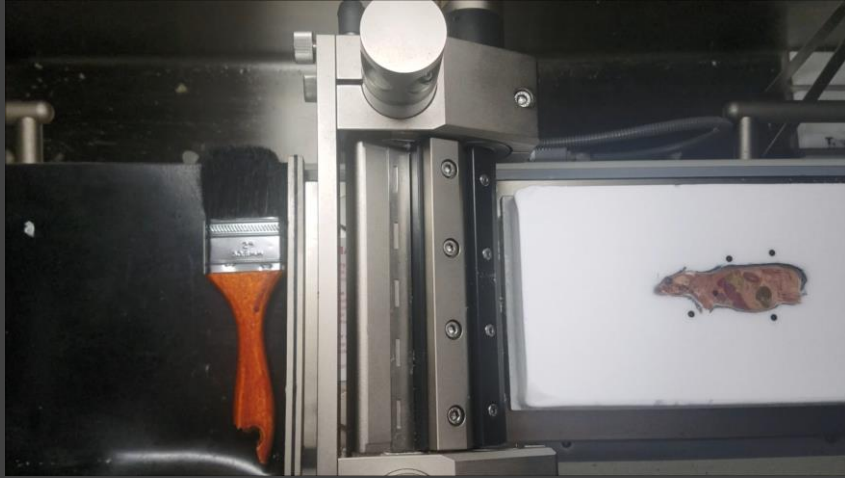
Cryo-Fluorescence Tomography (CFT) Technology



- Block of whole tissue is mounted in a slicer
- Fluorescent camera images the labeled tissue at each and every slice
- Image stack is error corrected and reconstructed into 3D model and analyzed



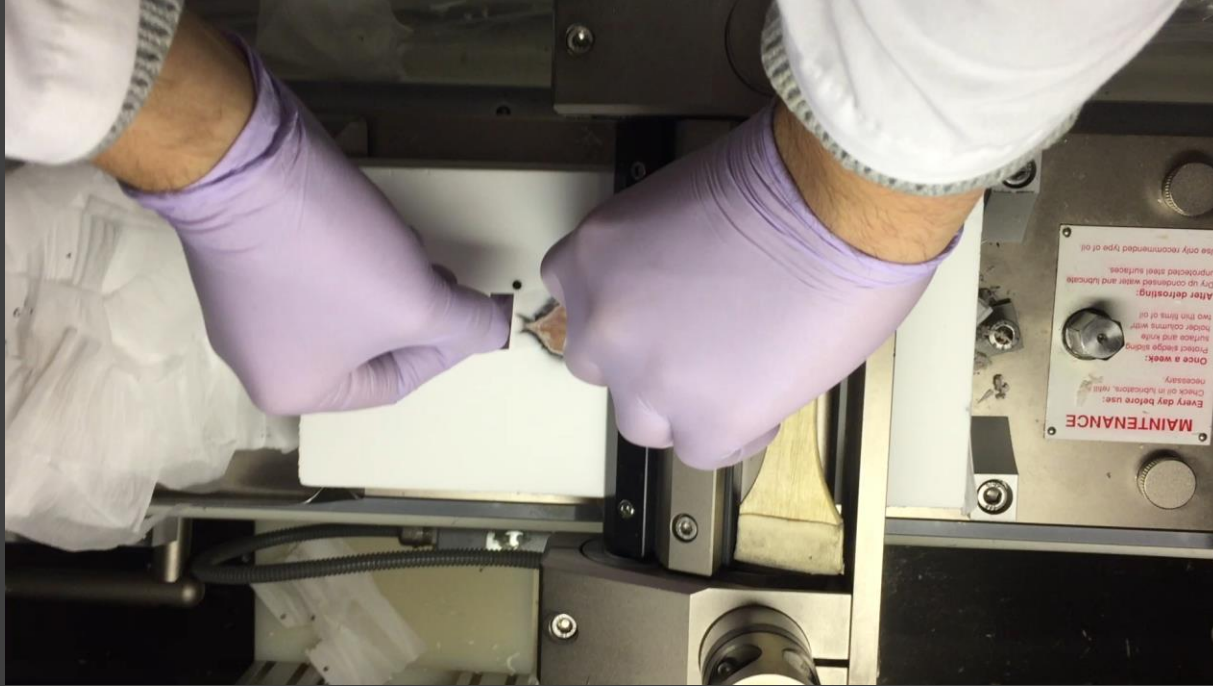
Cryo-Fluorescence Tomography (CFT) Technology



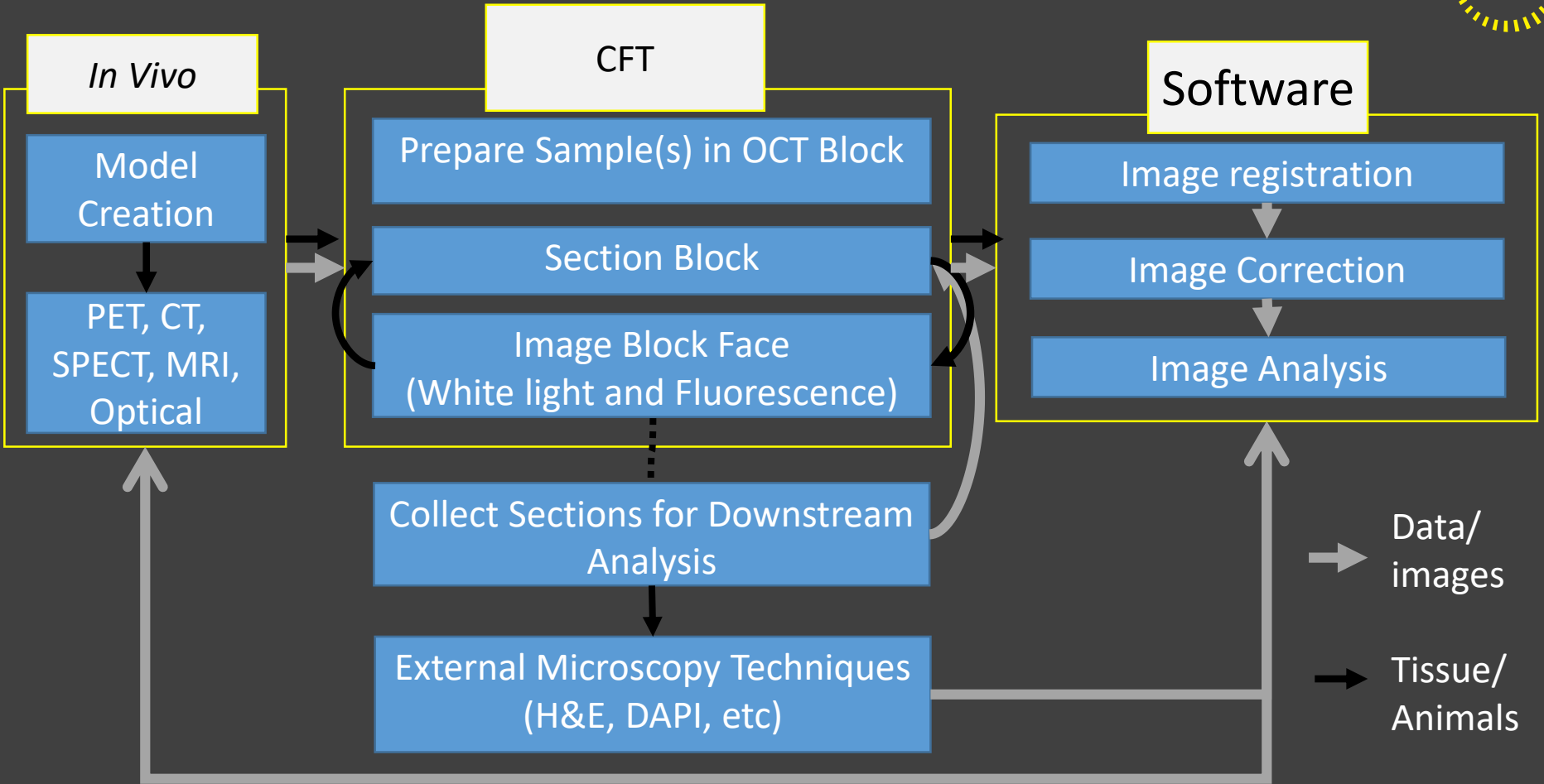
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Tissue Collection

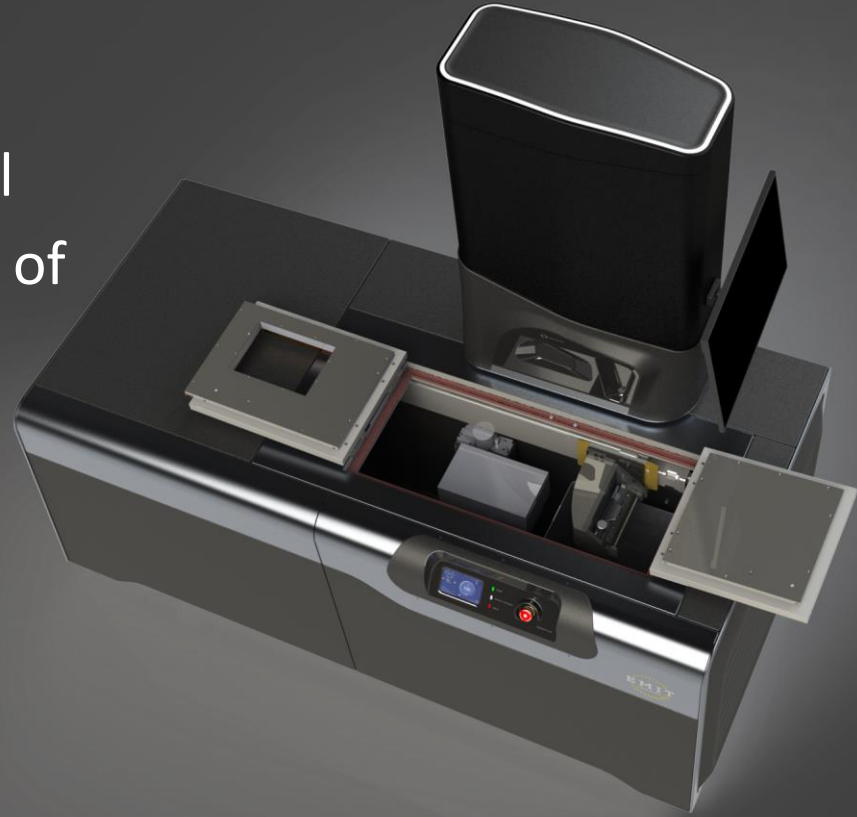


CFT Workflow



Software

- Dedicated acquisition interface with role based access control
- Automated reconstruction kernel
- VivID™ Multi resolution viewer of native data
- Supports output to VQ or other analysis package



GFP Imaging

Monitoring Gene Expression with CFT

Study Design



1. Animals

- Animal 1: AAV9-CAG-GFP, IV
- Animal 2: Control (PBS)

2. Mice embedded in an OCT block in a prone orientation, fiducials installed

3. MTI with the with a White Light and Fluorescent camera with below parameters:

- 25um sections in the cryo-macrotome
- Image acquisition in the white light and GFP

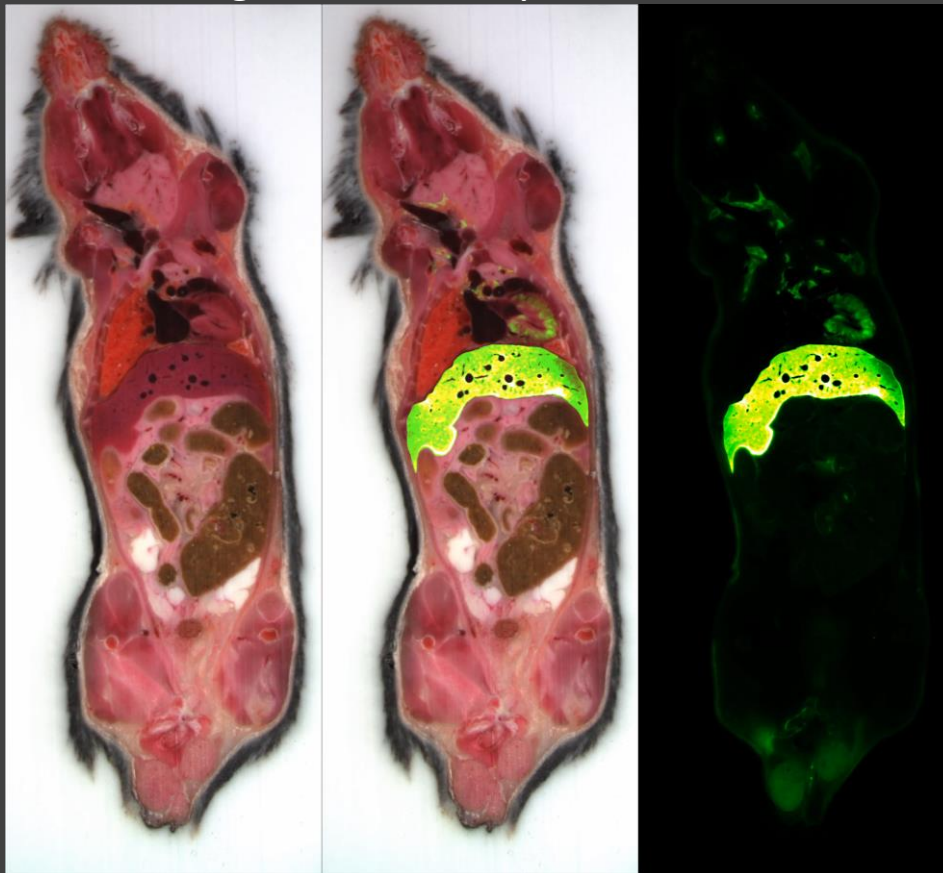
Flythroughs

AAV9-GFP; IV; 25um section; 470nm EX/510nm EM and white light

White Light

Overlay

Fluorescence



760

Normalized Corrected Counts

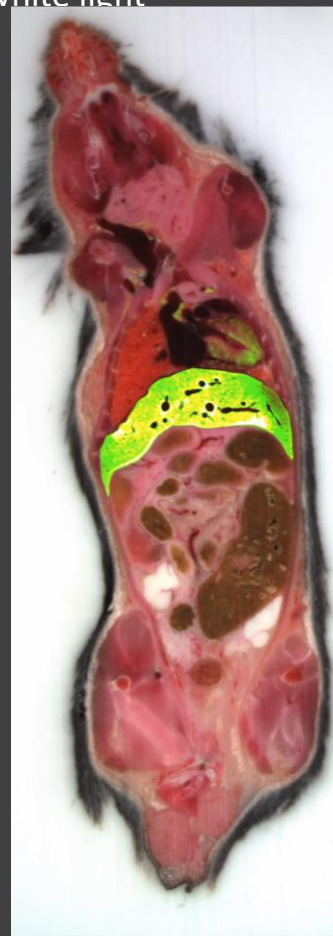
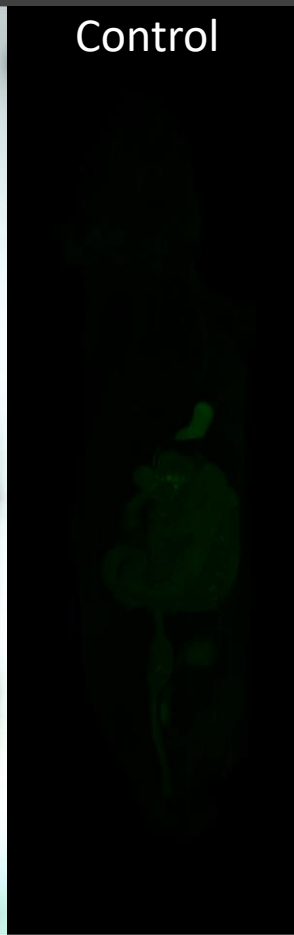
0.3

Flythroughs

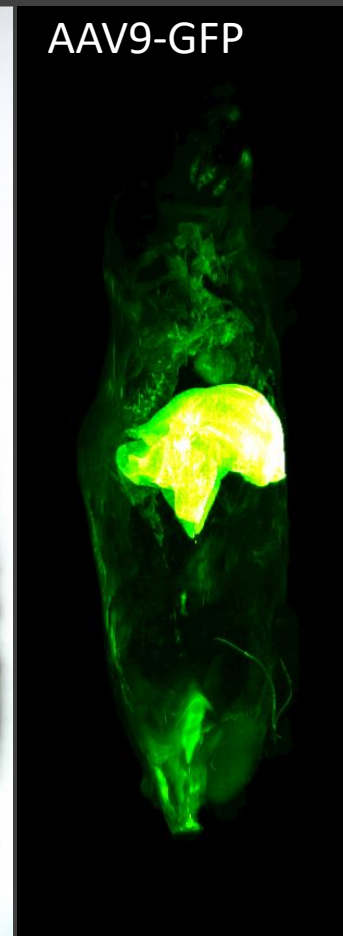
Control vs AAV9-GFP; IV; 25um section; 470nm EX/510nm EM and white light



Control



AAV9-GFP



1000

0.3

Normalized Corrected Counts

Dual Expression Tumor Models

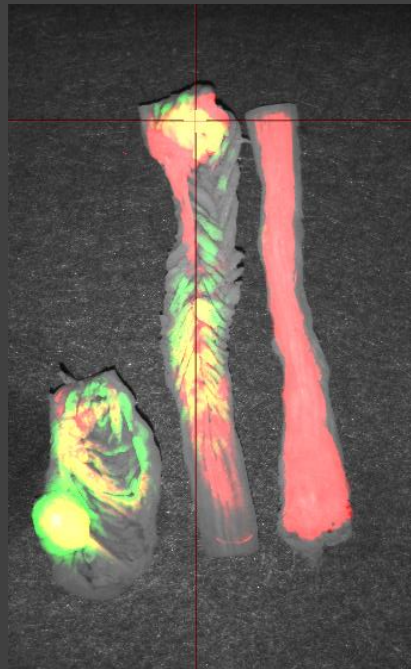
“Rainbow Colon” progresses from RFP to GFP as tumors establish and grow

Colorectal Cancer imaging

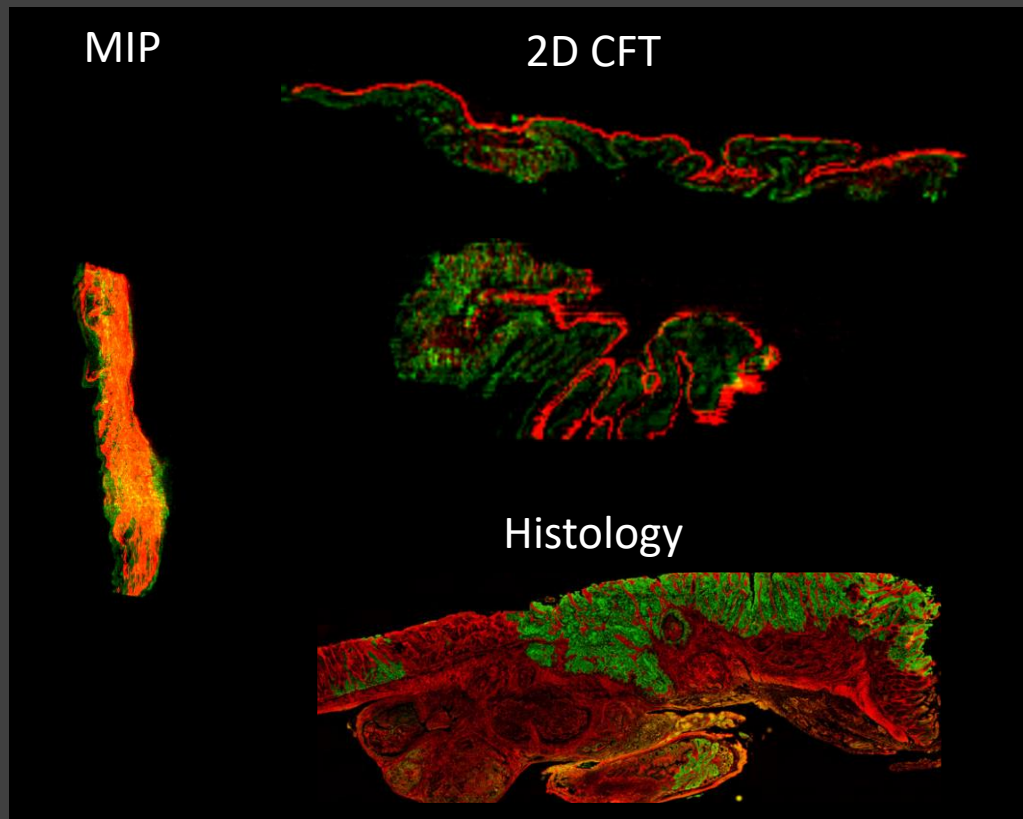
- Objective
 - Characterization and visualization of the tdTomato and GFP expression of the Colorectal tumors at multiple resolutions
- Animal Model
 - **Fabp1-Cre⁺ mT/mG⁺ Pik3ca^{*+} Apc^{Min/+}**
 - **EGFP expression represents high expression of PI3K and a transition from Adenoma to Carcinoma**
- **Excised tissue was imaged at 10um resolution**
- **Ongoing studies to image entire animal**

Rainbow Colon Heterogeneity

Ex Vivo Fluorescence



Ex Vivo CFT and Histology



Rainbow Colon Whole Mouse



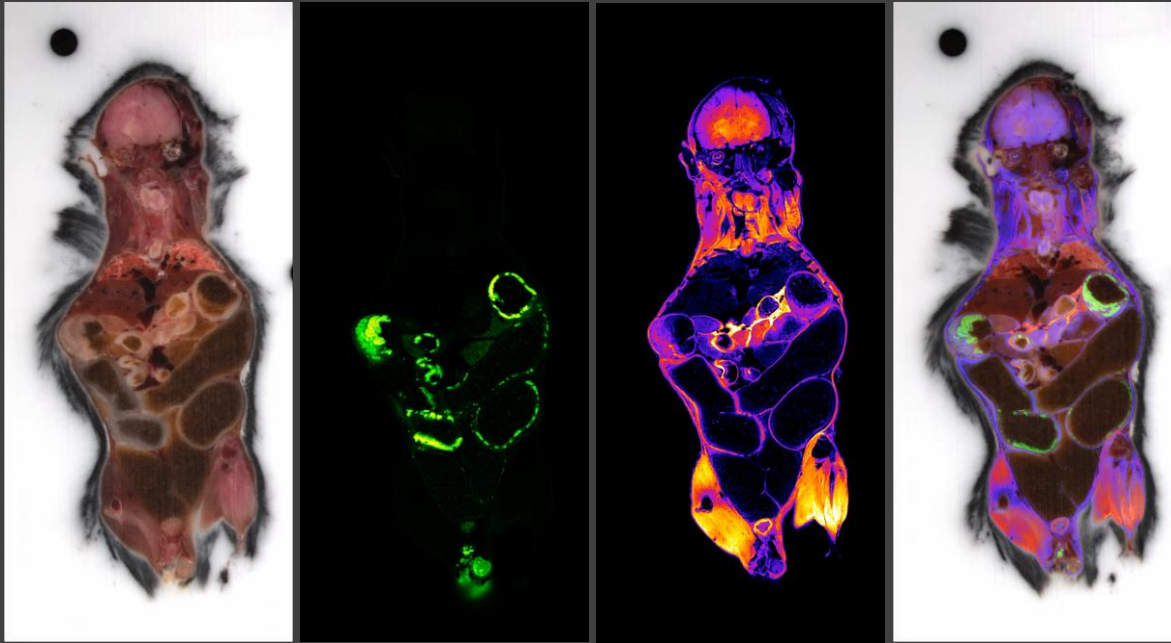
Coronal Fly-throughs

White Light

470 nm

528 nm

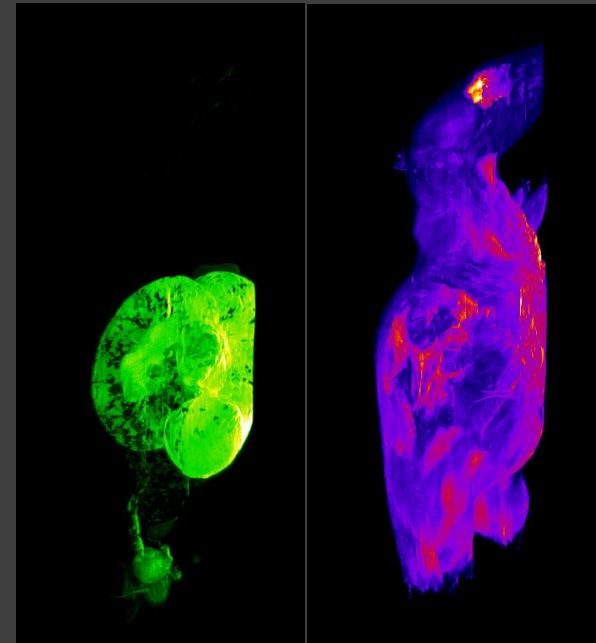
Overlay



MIPS

470 nm

528 nm



Macrophage Imaging

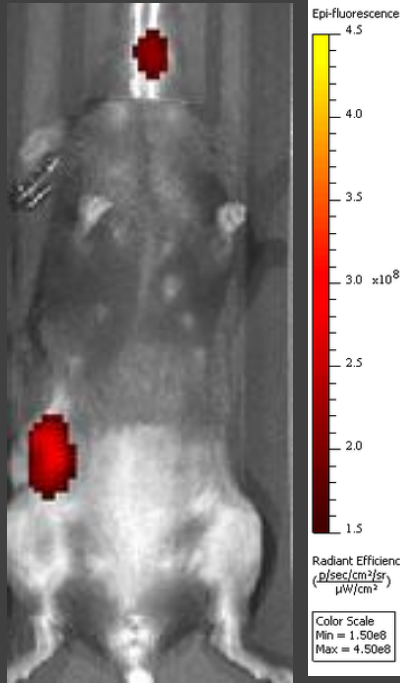
Dual labeled V-Sense was administered to the animal for imaging using ^{19}F -MRI, in vivo Fluorescence, and CFT.

Multimodality Macrophage Imaging in Xenograft Mice

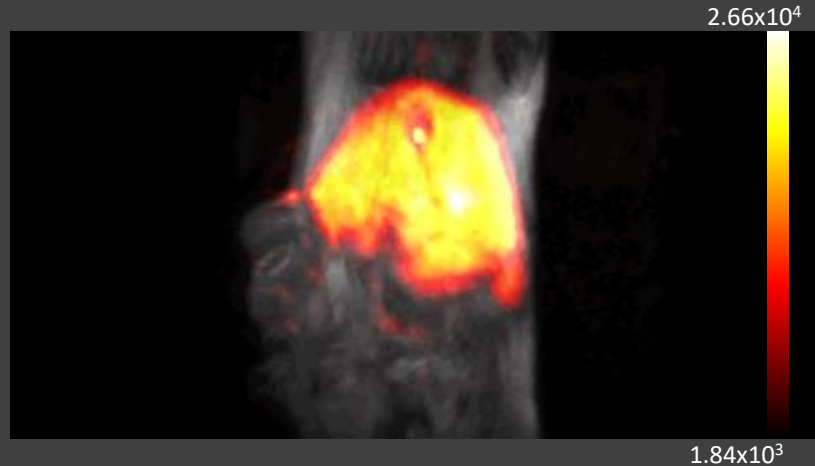


MC38 syngeneic, V-sense-NIR

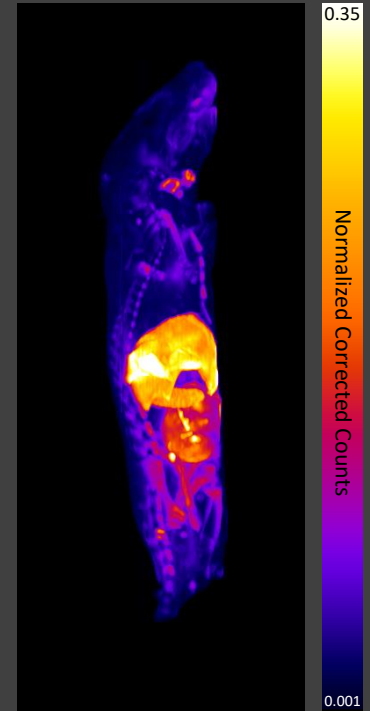
IVIS



19F MRI



CFT



Multi-Spectral Macrophage Imaging in Xenograft Mice



MC38 syngeneic (green), V-sense – (red)

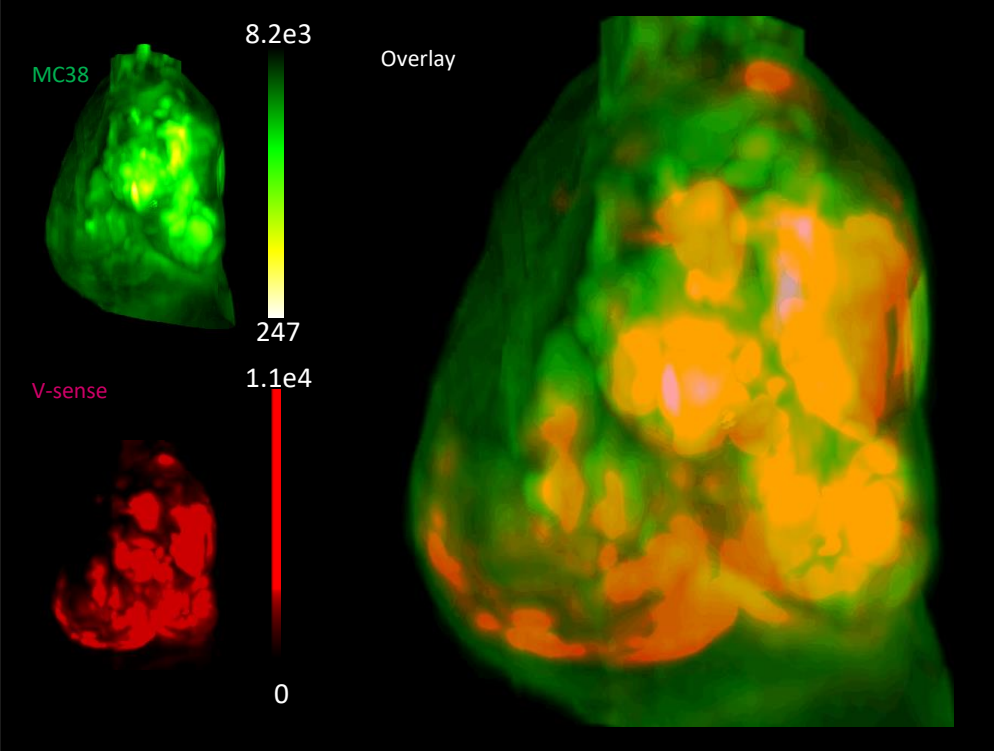
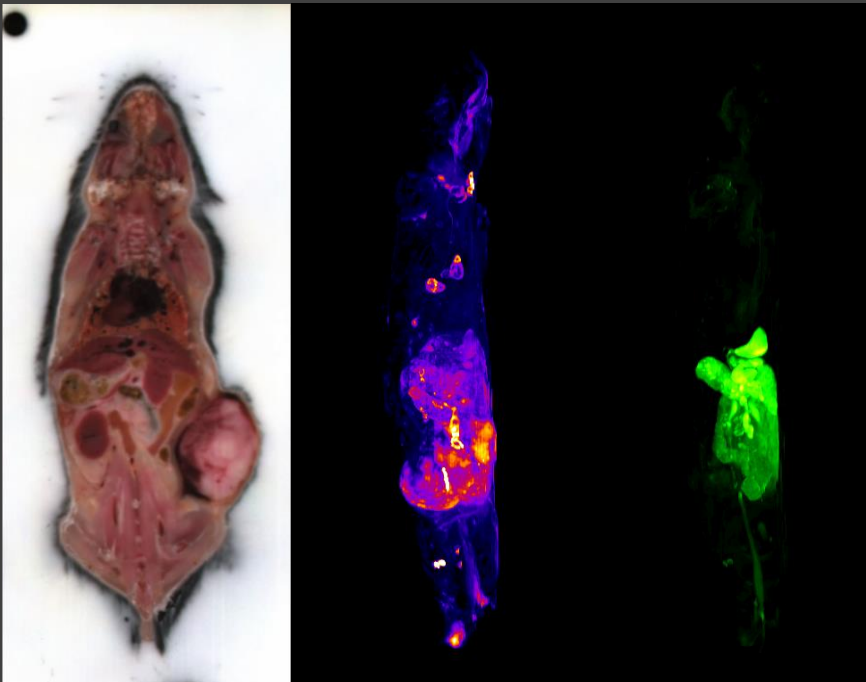
Whole Animal Evaluation

Tumor Heterogeneity Evaluation

2D Fly-through

3D MIP (RED)

3D MIP (GREEN)



Ovarian Cancer

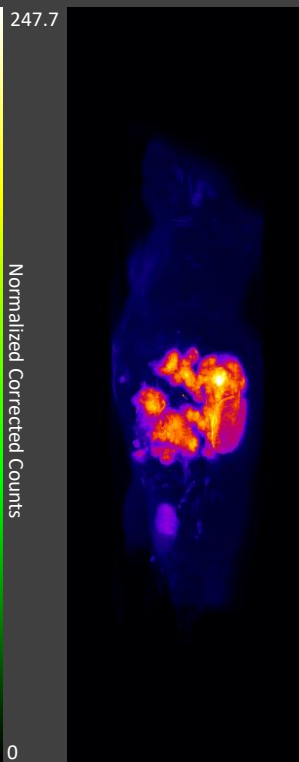
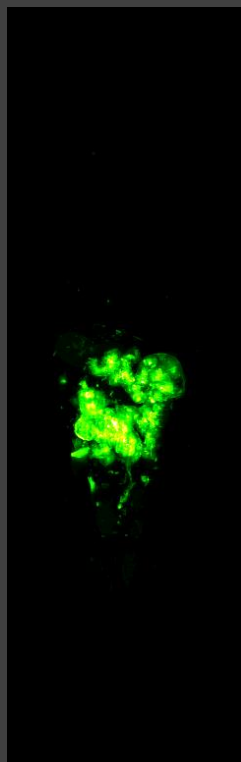
SKOV3ip cell line (Her2-positive) expressing Luc2 and tdTomato

CFT Imaging vs in vivo Fluorescence Imaging



Ovarian Cancer cells
tdTomato

Her2-Specific Protein
AF647 label

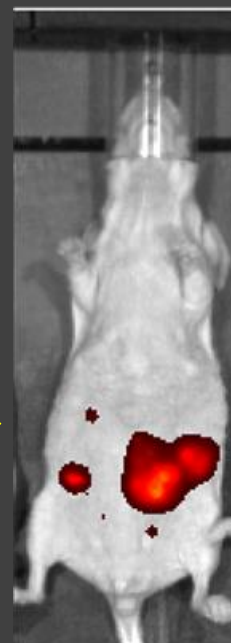


← CFT Imaging

→ In vivo Fluorescence

Ovarian Cancer cells
tdTomato

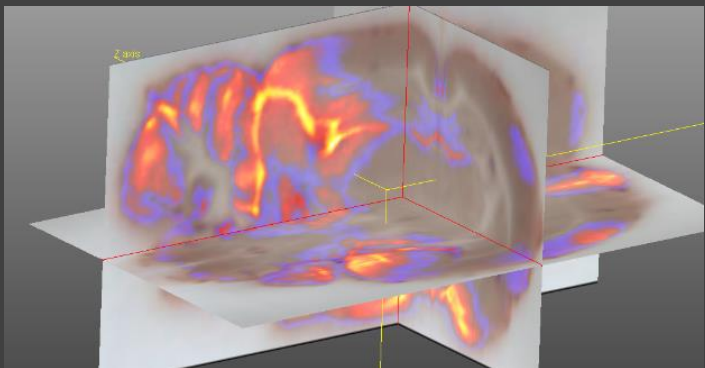
Her2-Specific Protein
AF647 label



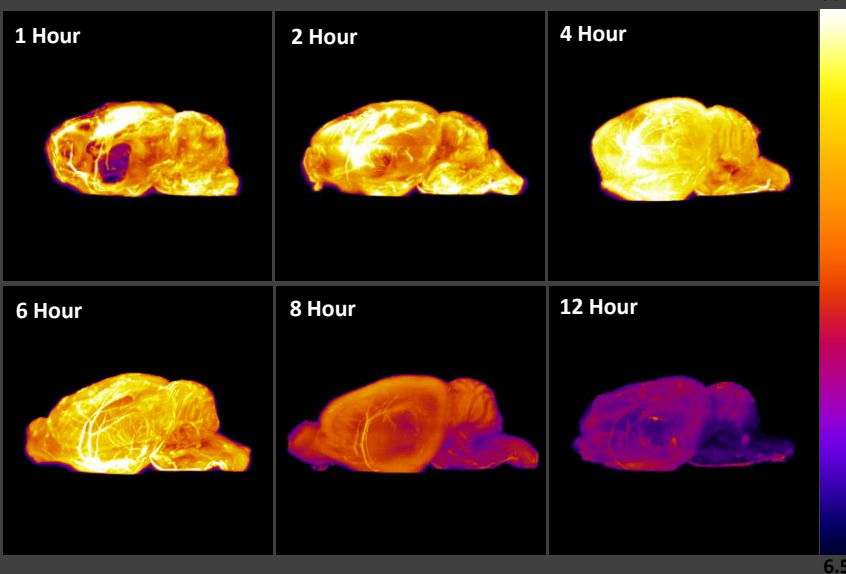
Neuro PK/PD

Simultaneously Collect Structural and Functional Information
from multiple samples in the same scan

Temporal Capabilities with 3D-CFT



3D-CFT PK Analysis of Rodent Brain

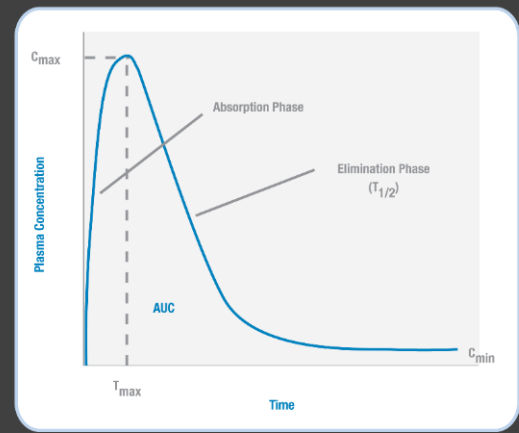


Biodistribution over time (Rodent Brain)

VS

Traditional PK Analysis

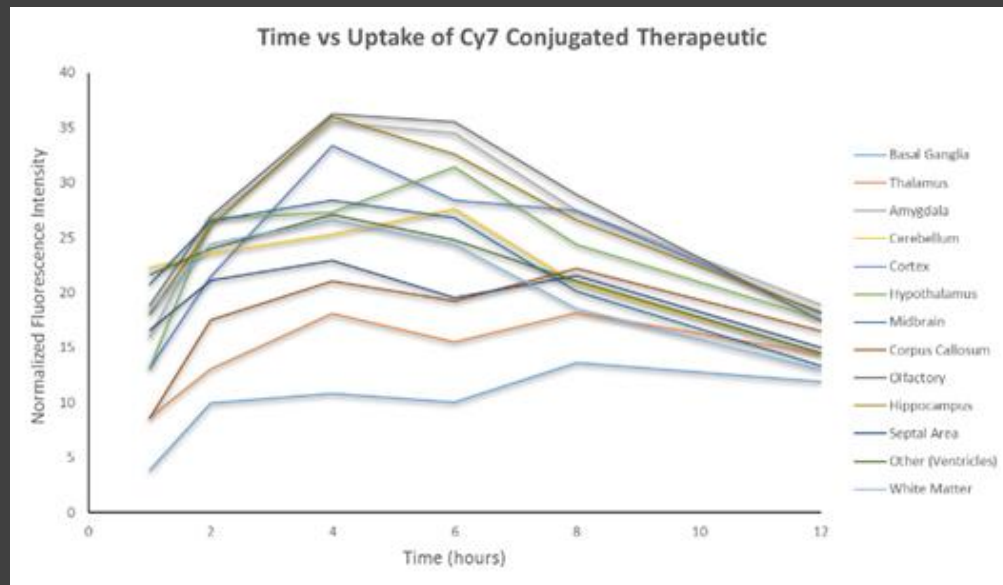
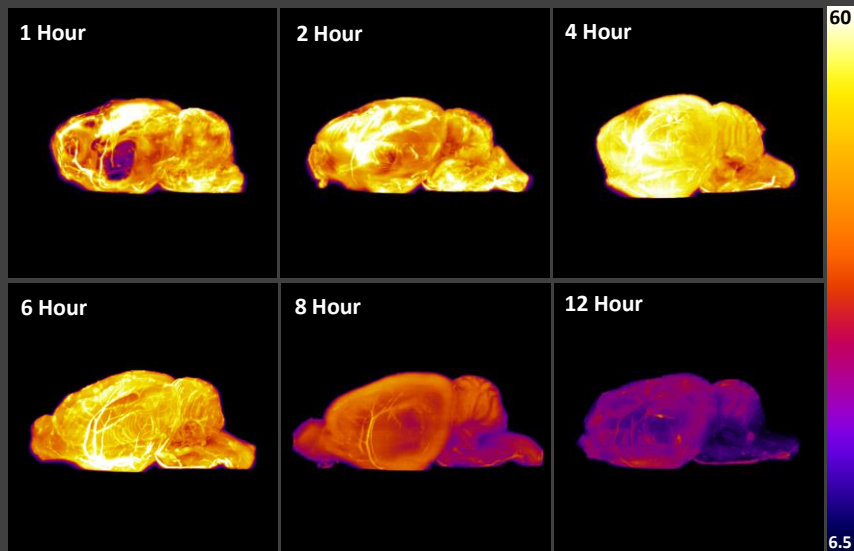
(no indication of where the compound is taken up or how it is clearing)



Traditional analysis could be used to complement 3D-CFT analysis

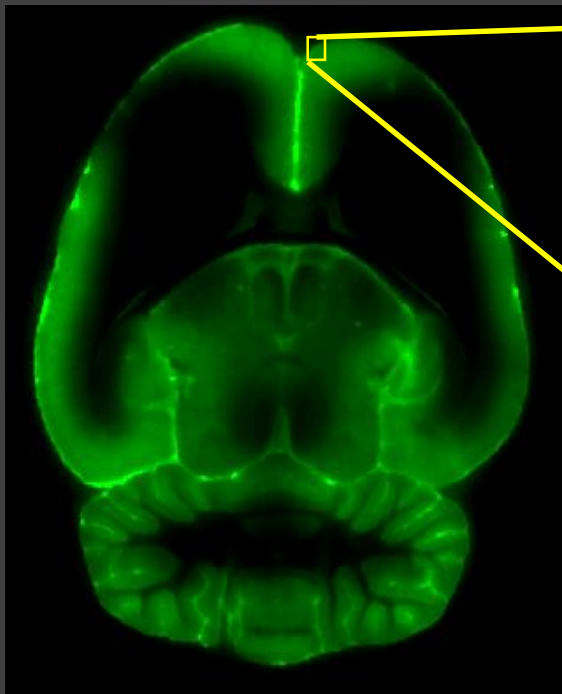
Time Course of a Labeled Therapeutic

Atlas driven data analysis



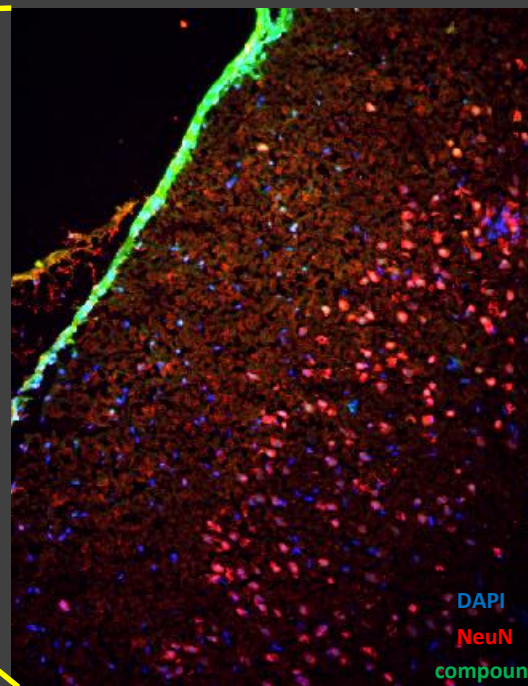
3D-CFT is Compatible with Standard Histology

Gives multi-resolution capability: Structural to Cellular resolution on one Sample



3D-CFT
Fluorescent image of
compound in mouse brain

Global PK with Histology
proof in same sample
Example: Therapeutic
compound localizes in
neuronal nuclei



**Standard histology of 3D-CFT
tissue section of mouse brain**

Glioblastoma Multiforme Imaging

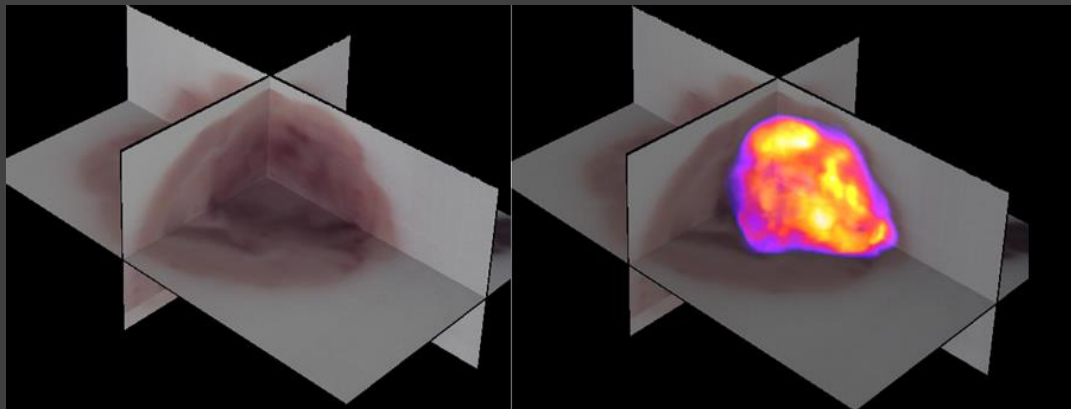
Evaluating multimodal / multi-resolution registration of a mouse GBM model with MRI and CFT

Study design



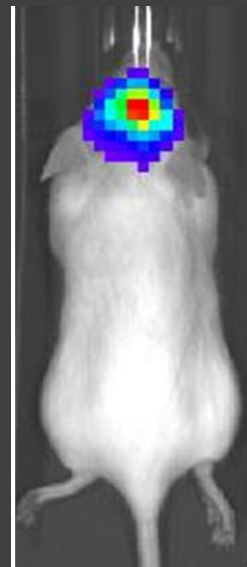
- GL26-luc2 cells were implanted intracranial into a murine model.
- Tumor growth was monitored using BLI and MRI.
 - T1-weighted MR sequence with Gadolinium contrast enhancement
 - Standard BLI measurement
- Administration of ICG and AngioSense680
- 25 um CFT section were collected

MPR CFT vs. Traditional BLI



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.

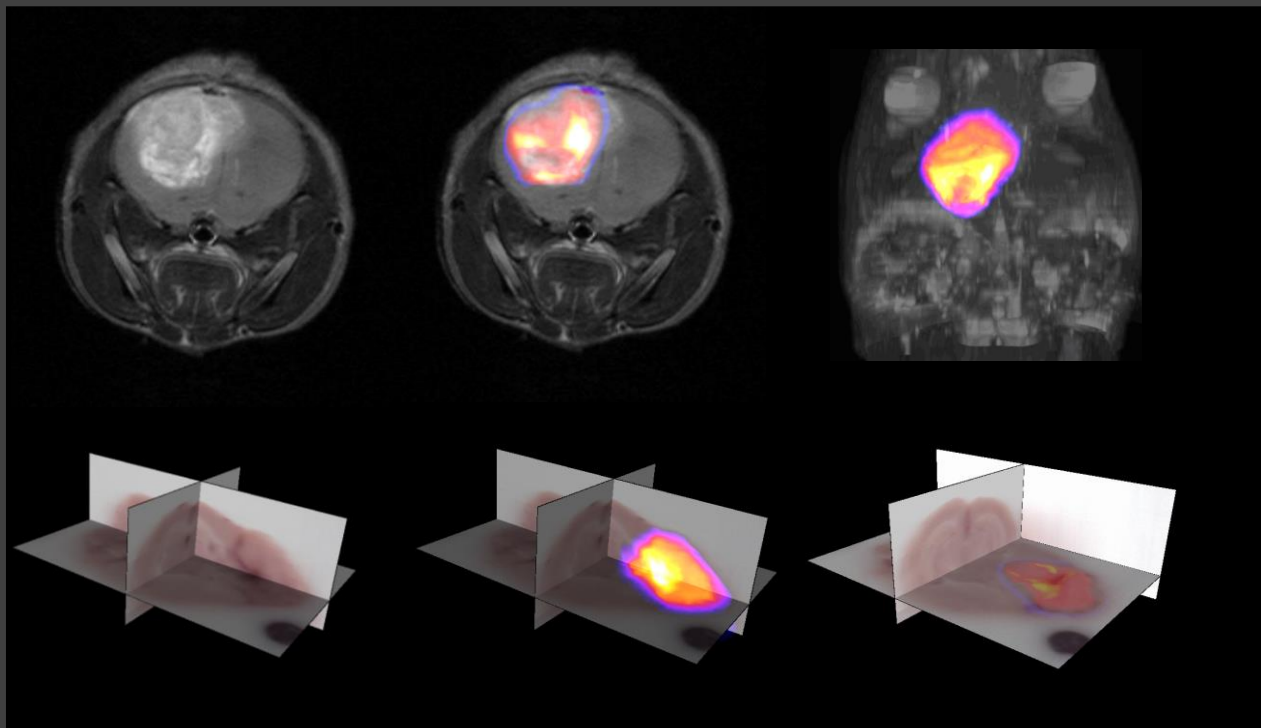
VS



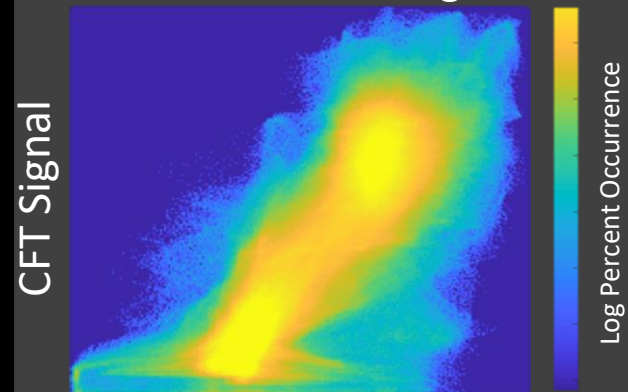
Traditional BLI offers a highly sensitive imaging technique used in a variety of oncology models. However the modality is limited in its ability to accurately give information on 3D structure.

MRI and CFT Imaging of GBM

Evaluating multimodal / multi-resolution registration of a mouse GBM model



MR/CFT Joint Histogram



MR Signal

MRI signal shows a 0.80
Correlation with the CFT
Signal

Metastatic lesion detection and visualization

Murine breast cancer (4T1) expressing DsRed and Luciferase.

Left ventricle administration to promote metastatic lesions

Orthotopic model with metastatic lung cancer

CFT of Metastatic Tumor Spread

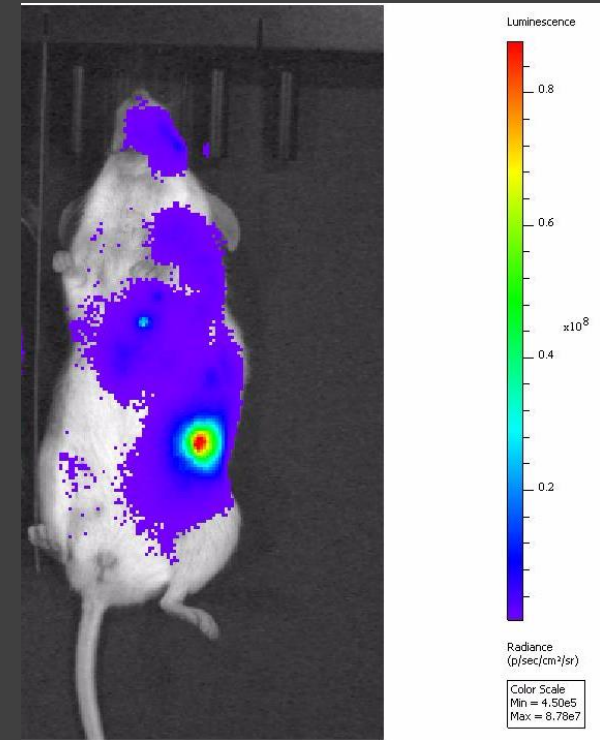
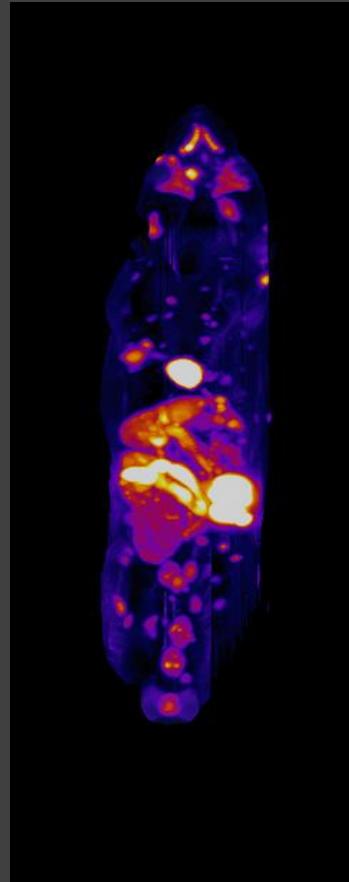


- 4T1 mouse mammary tumor cell line
- expresses both luciferase and DsRed
- immunodeficient NSG mice
- 2 animals injected via left ventricle
- 2 animals injected subcutaneous
- BLI and CFT imaging conducted for all animals

Whole Body MIP Shows Position of Metastatic Tumors

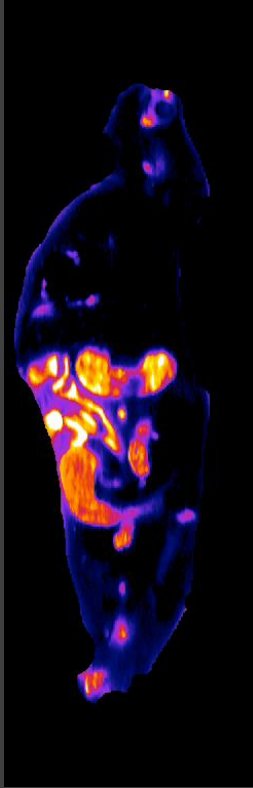


- Shows many lesion sites that are not detected with BLI
 - Abdominal
 - Lung
 - Bone
- Even post mortem BLI would not see all lesions

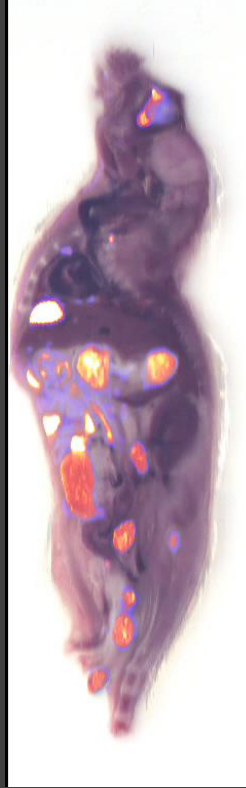


Fly-through in 3 views.

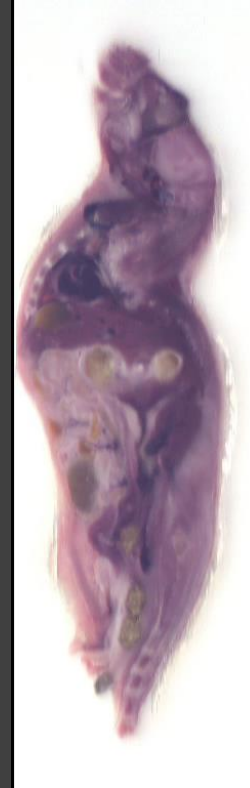
Fluorescence



Overlay



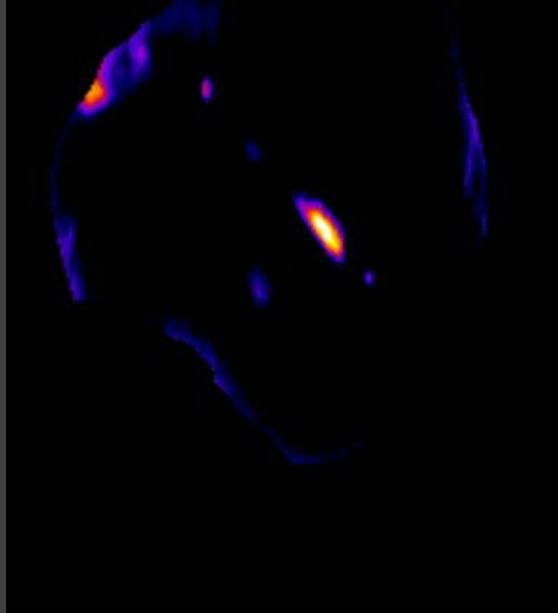
White Light



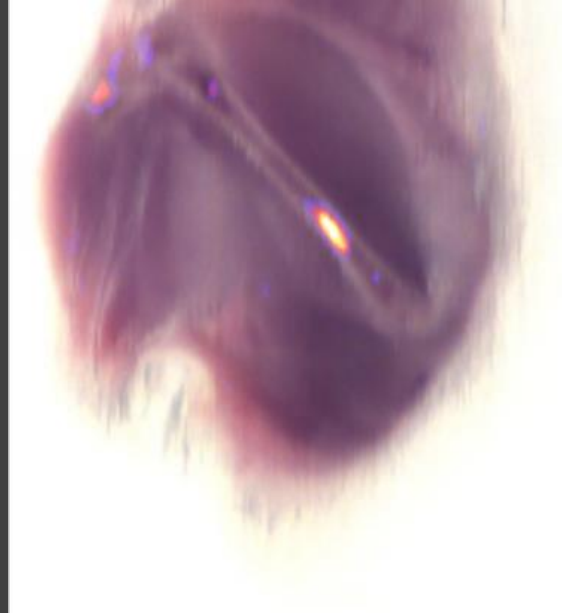
Femur Metastasis Zoomed In



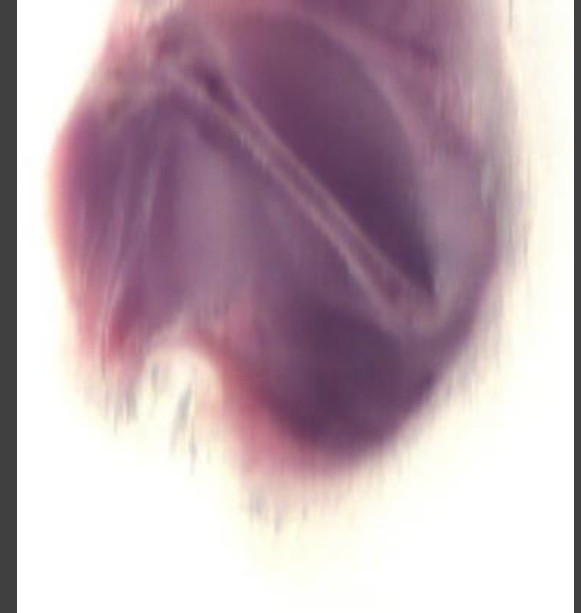
Fluorescence



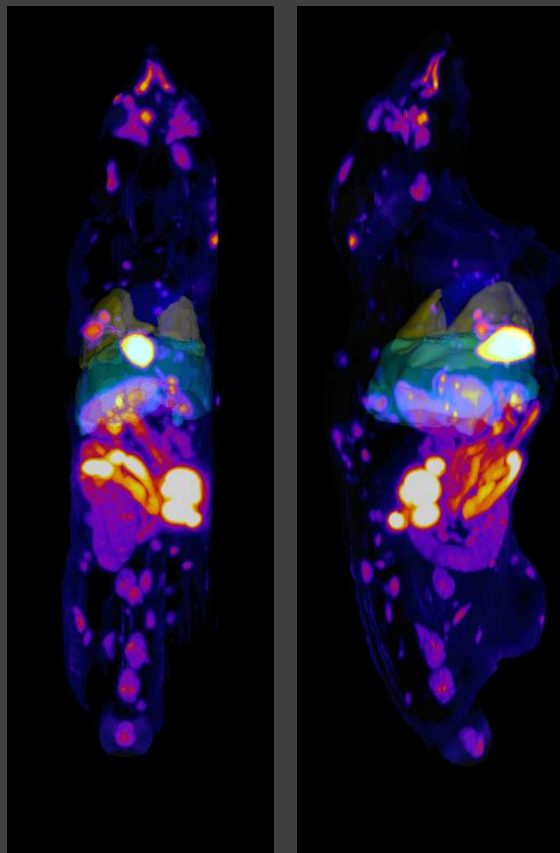
Overlay



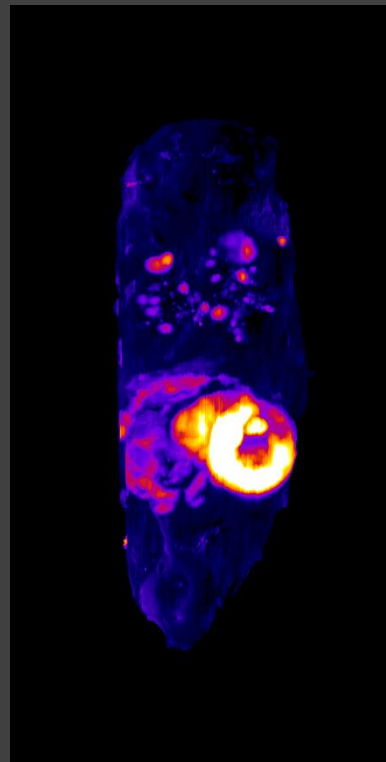
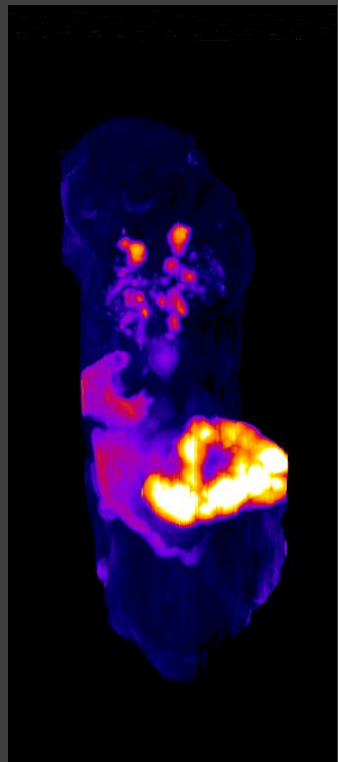
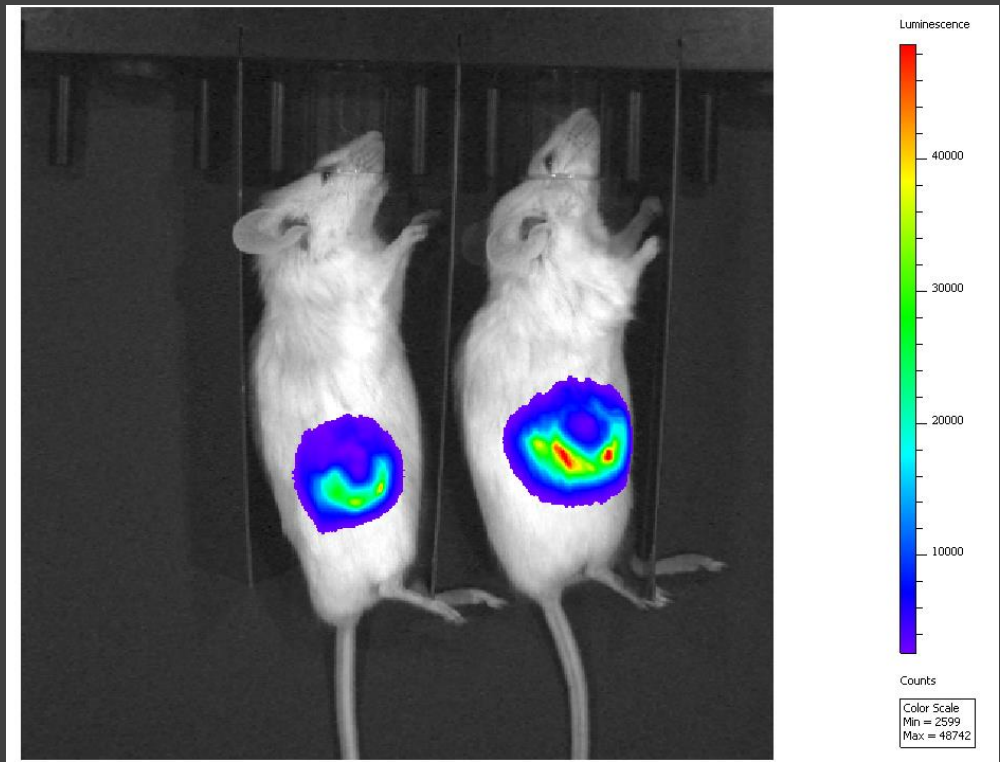
White Light



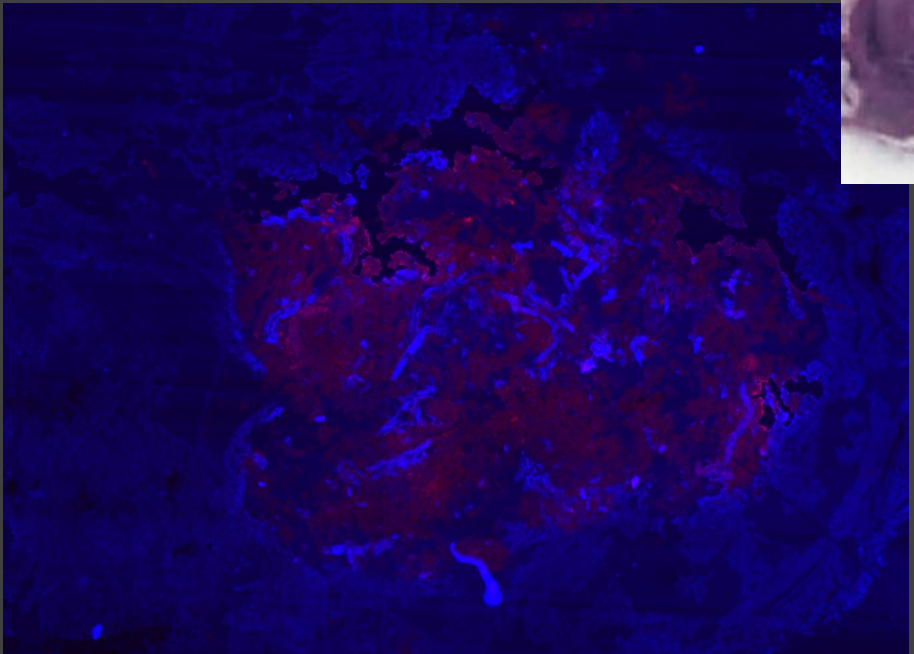
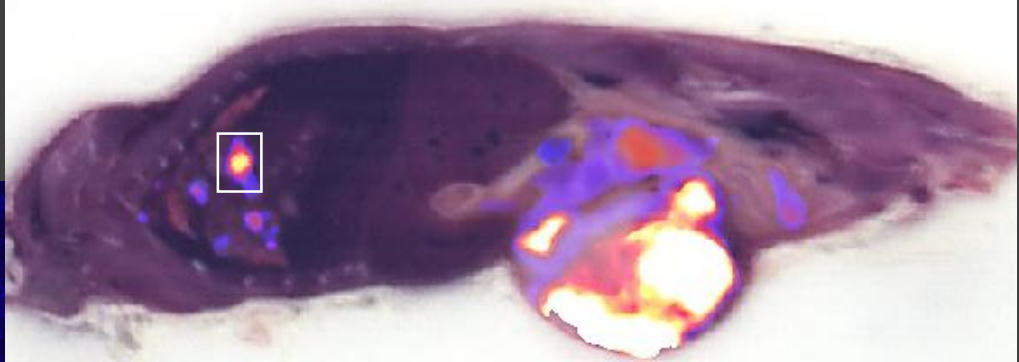
Whole Body MIP with segmentation of the lung and liver



Primary->Metastatic mice BLI Vs. CFT



Tissue Collection for Histology of Secondary Lung Tumor



ADME Imaging of Multiple Administration Routes

Evaluating compound clearance with CFT

High Resolution Multichannel ADME Imaging

Dual probe imaging in the 700nm and 800nm

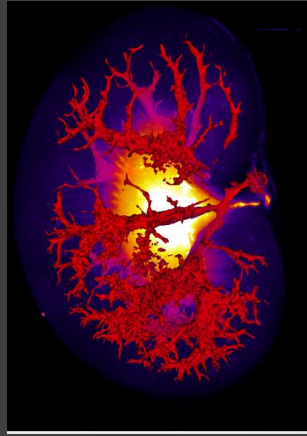
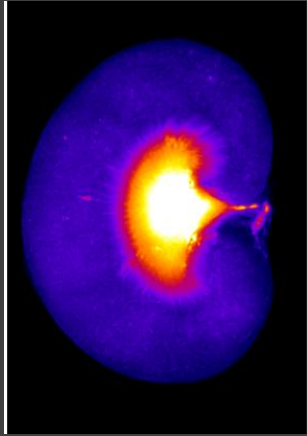


- Simultaneous imaging with white light and the NIR probes.
 - Two routes of administration
 - Two Fluorescent signals
 - White light vascular segmentation
- *Intrathecal administration 400μg PEG909-ZW800-1 in 30μL saline at time zero,*
- *Intravenously administration at 50 minutes with 400μg PEG909-ZW700-1 in 250μL saline*
- *Vascular structure shown in red was determined white light segmentation and overlaid in the figures for assessment.*

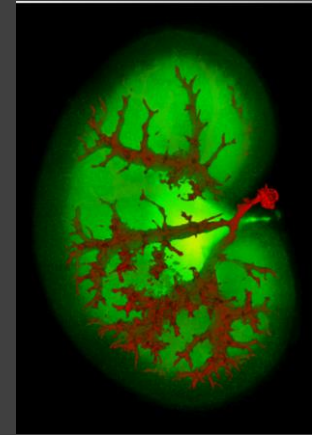
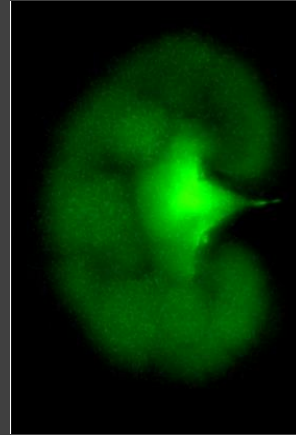
Dual-Tracer Kidney Imaging w/ White Light



PEG909-ZW700-1



PEG909-ZW800-1



Biodistribution of two probes in one organ including segmented vascular structure from the white light information

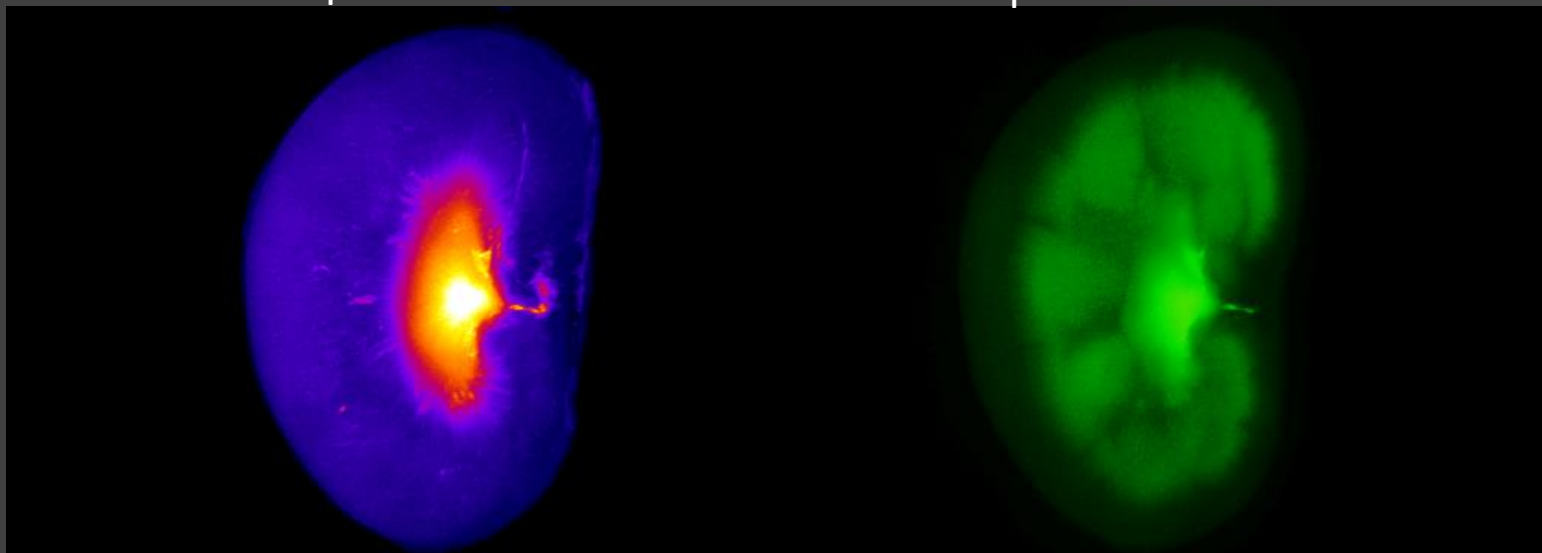
Dual-Tracer Kidney Imaging

One Hour Post Injection



Compound A – 700nm

Compound B – 800nm



Two different fluorophores imaged
simultaneously

Voxel size (μm): 21.7 X 21.7 X 25

Imaging Markers Used in Fluorescence Guided Surgery

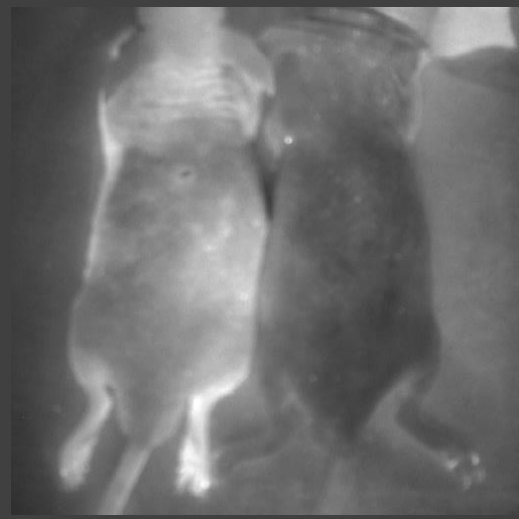
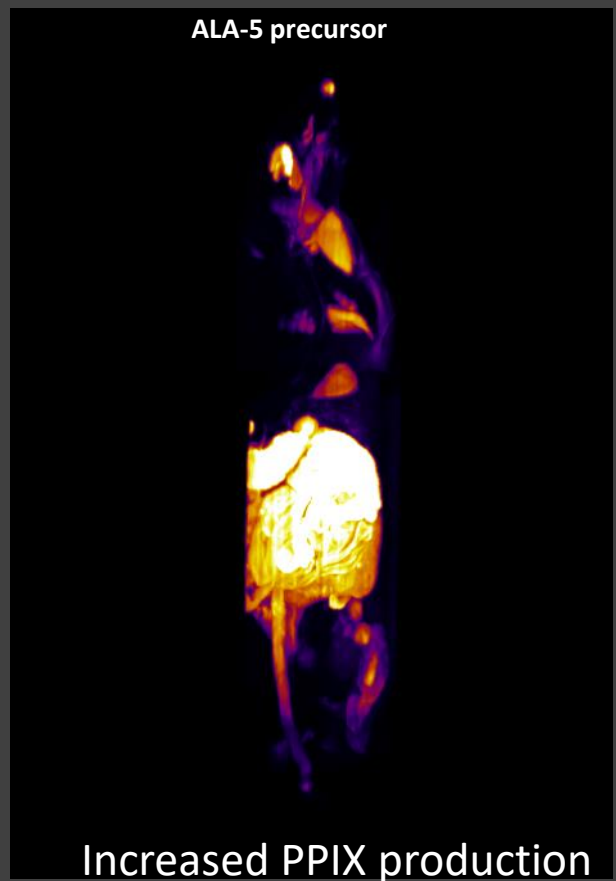
Using an endogenous marker for tumors

- 5-Aminolevulinic Acid (5-ALA) is used in the synthesis of the porphyrin pathway.
 - When injected in vivo, promotes the production of fluorescent porphyrins.
 - Commonly used in fluorescent guided surgery
 - Lymph node and glioblastoma detection during surgery

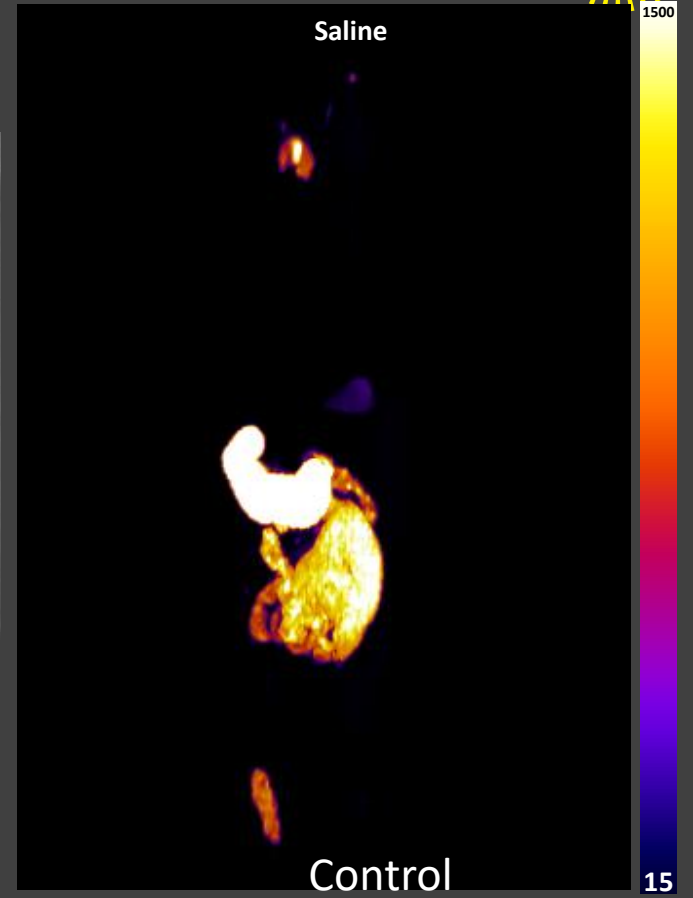
Surface Fluorescence Vs. Whole Body CFT



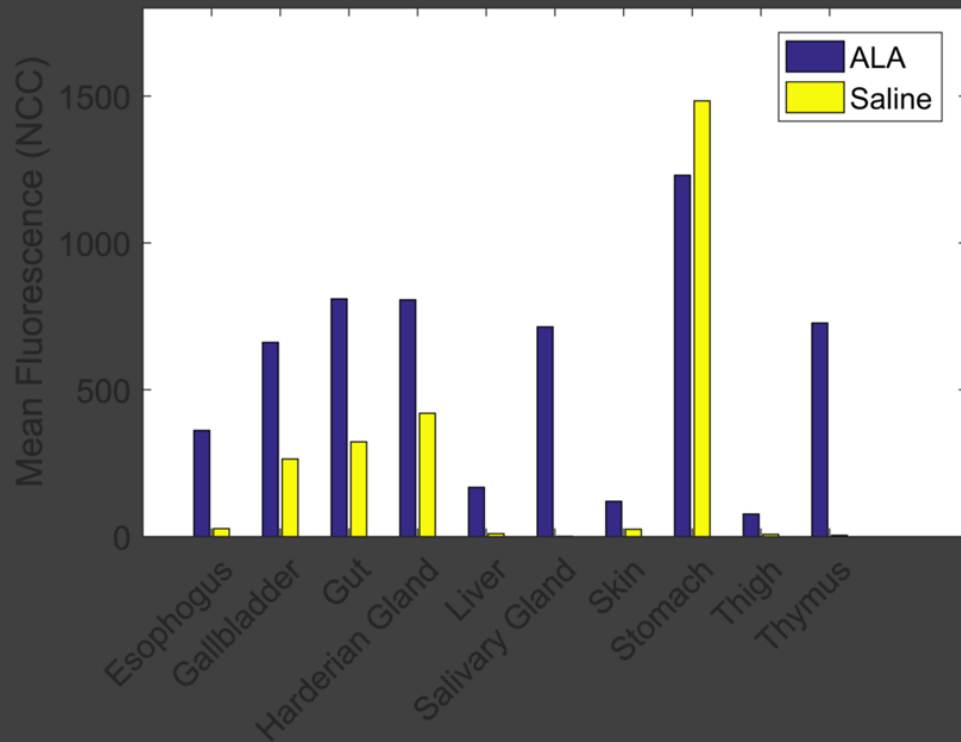
ALA-5 precursor, IV at ~1 hour post-injection, PPIX Fluorescence, Mouse Whole Body



Voxel size: (40µm x 40µm x 40µm)



Quantitative Evaluation of ALA vs Control



Segmented Regions	Percent Increase
Esophogus	1230
Gallbladder	151
Gut	150
Harderian Gland	92.2
Liver	1600
Salivary Gland	99400
Skin	392
Stomach	-17.1
Thigh	908
Thymus	17300

Discussion

