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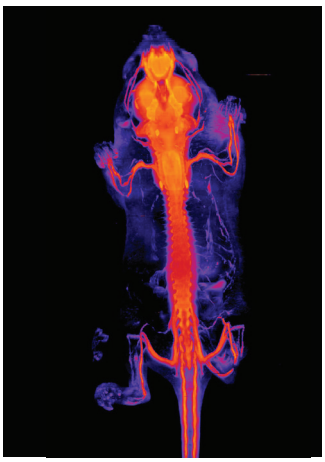
CRYO-FLUORESCENCE TOMOGRAPHY

Transformative 3D Imaging to Monitor Drug PK/PD

Cryo-Fluorescence Tomography (CFT) is a transformative 3D approach to imaging drug distribution and protein expression in whole-animals. EMIT Imaging offers both instrumentation and services via our platform, Xerra™, a high resolution and high sensitivity preclinical imaging tool designed to advance discoveries in biological and drug research. See what you're missing with standard 2D *in vivo* fluorescence imaging techniques and utilize CFT to better visualize:

- Whole-body drug **distribution and delivery**
- **Screening** candidate drugs and delivery systems
- Whole-body therapeutic **protein expression**
- **Multiplexed** co-localization of drug with targets
- On and off target **tissue identification**



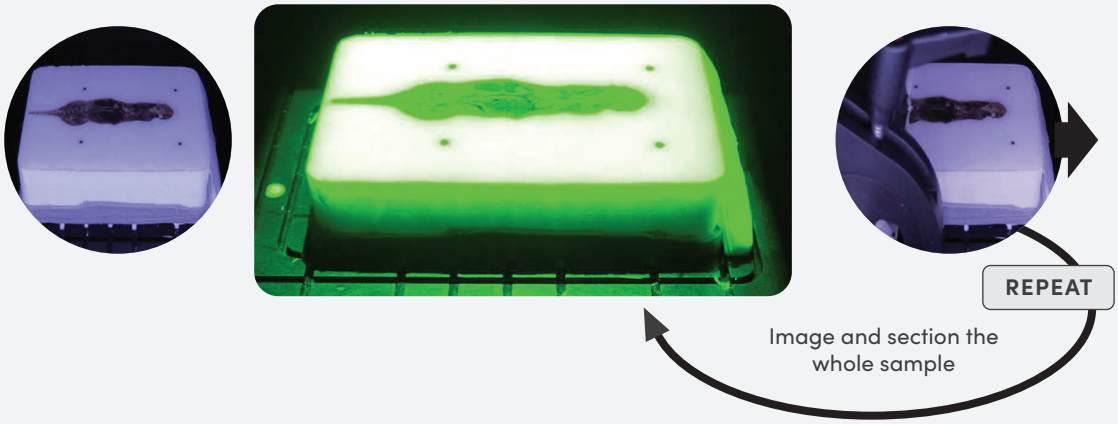
HOW CFT WORKS

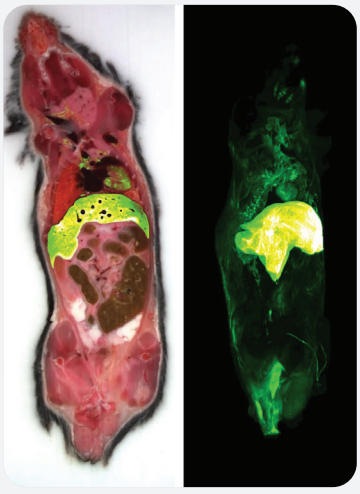
1.PREP
Cryo-preserve & embed the sample

2. IMAGE
High resolution, high sensitivity white light & flourescence imaging

3. SECTION
Remove 20-55 µm from the block surface

OUTPUT
3D image stacks





HIGH RESOLUTION
Resolution down to 20 µm

HIGH SENSITIVITY
nM sensitivity, comparable to nuclear medicine

MULTIPLEXING
6 lasers and 7 filters for multiplexed applications

AUTOMATION
Xerra is a fully automated

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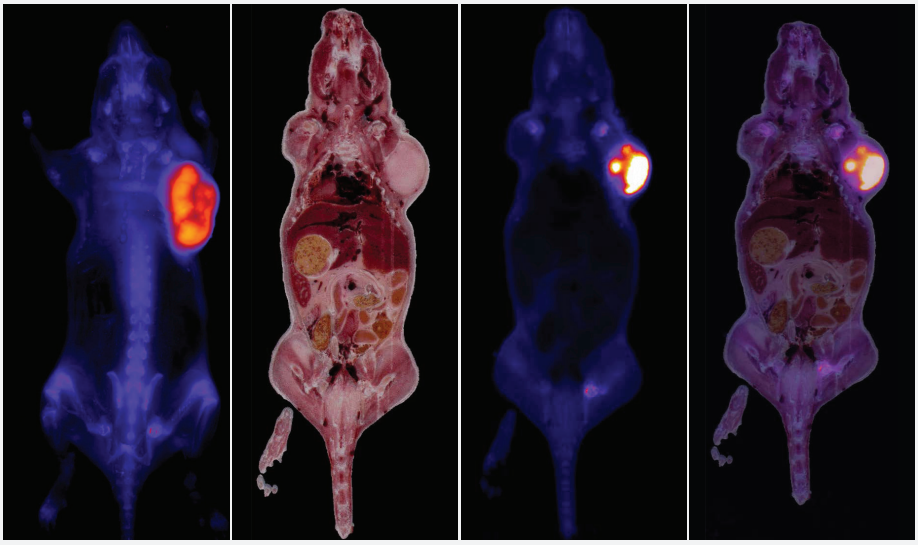
APPLICATIONS

Fibroblast-activation protein (FAP): Whole-Body Drug Biodistribution



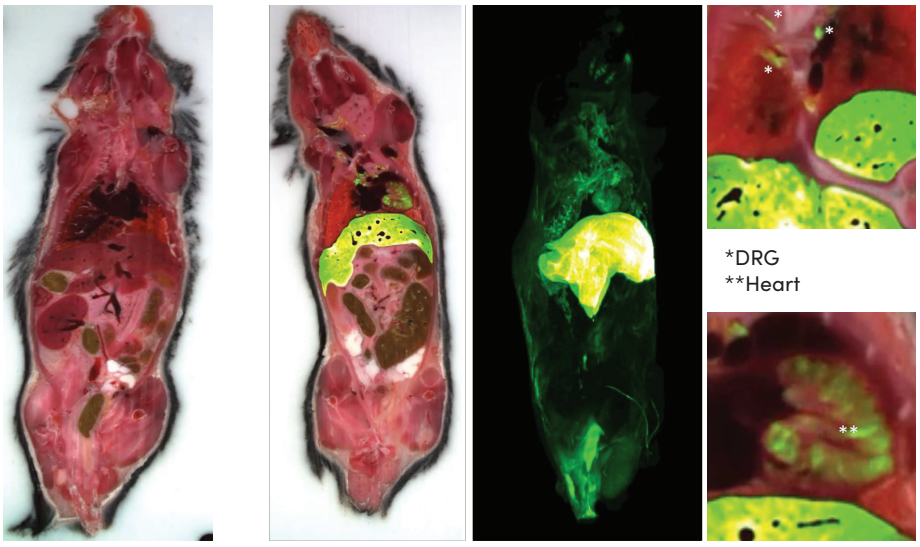
3D Fluorescence Image | Anatomical White-light | Fluorescence Image | Anatomical + Fluorescence

FINDINGS: Data confirms drug preferential uptake in the tumor with minimal uptake in other organs/tissues

DETAILS:

- 1µg dose ofZ W800-1-labeled FAP-targeting small molecule
- U87 xenograft
- 4hrs post iv administration
- Imaged at 35 µm

AAV-mediated Protein Expression Following IV Administration



AAV9 Control | AAV9-GFP

FINDINGS: CFT uniquely achieves the required combination of resolution and sensitivity in a whole animal

DETAILS:

- GFP expression at day 15 following IV injection
- No expression in the control
- Liver is major organ where GFP expression occurs
- Note signal in dorsal root ganglion (DRG) and heart

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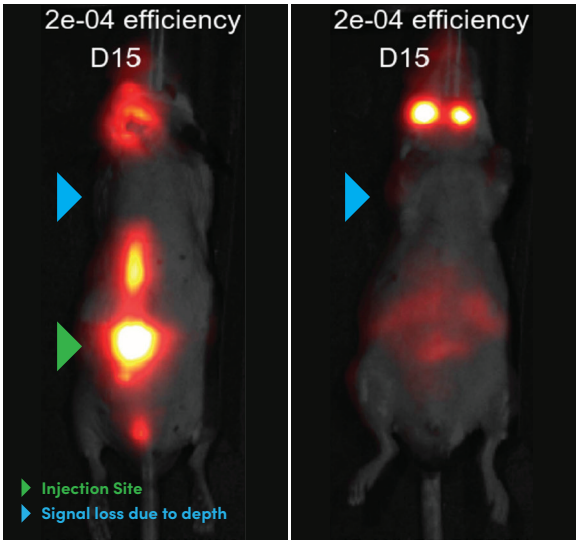
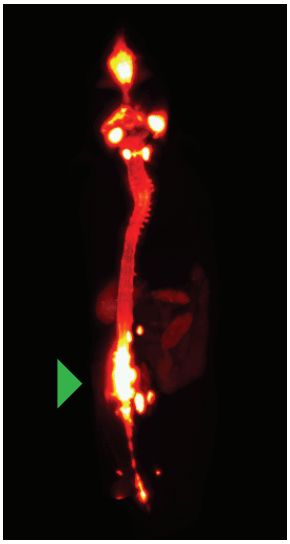
APPLICATIONS

Whole-Body Distribution of a Labeled ASO Following IT Injection



2D *In Vivo* Fluorescence



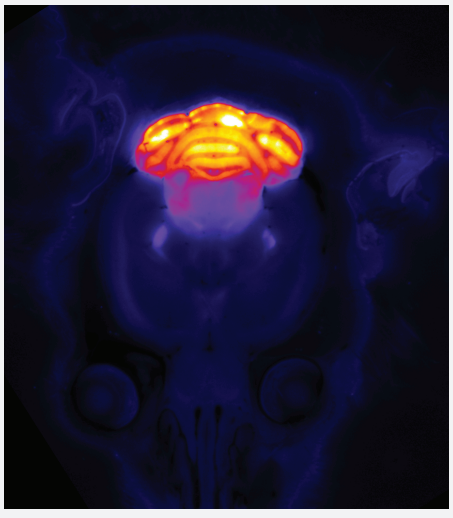
3D CFT

FINDINGS: CFT is superior to surface fluorescence due to its depth limitations (signal loss) and poor resolution

DETAILS:

- Cy5-labeled ASO at day 15 following IT injection
- Full visualization of spinal column into the cisterna magna
- CFT detects bioD and clearance: GI, lympathic, nasal turbinate, etc.
- CFT is 3D and high resolution: <50 µm versus >1mm in optical and nuclear medicine

Cell-specific Fluorescence Reporter Imaging



DETAILS:

GAD67-GFP (line G42) mice selectively express enhanced green fluorescent protein (EGFP) in the parvalbumin (Pv)-expressing subclass of basket interneurons (soma, dendrites, and axons) and also in putative presynaptic boutons.

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XERRA™ CFT IMAGING SYSTEM

INSTALL XERRA IN YOUR LAB

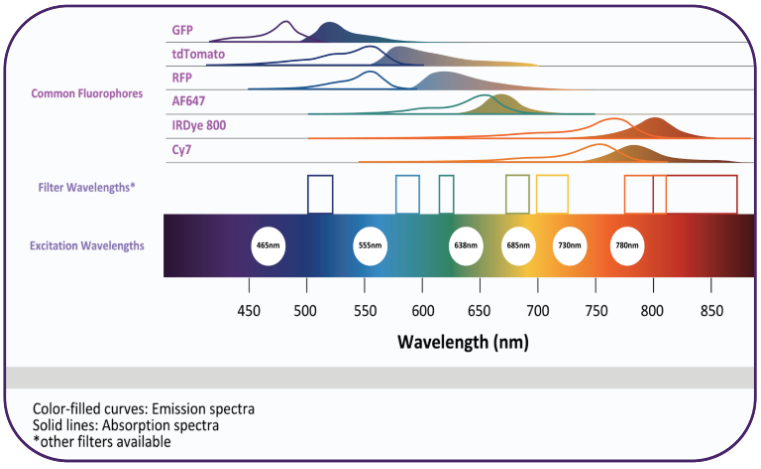
- Xerra automates the CFT workflow
- Anatomical white-light images co-registered with fluorescence images, capable of multiplexing
- 5 magnifications: 20-55 µm pixel resolution
- 6 excitation lasers: 470 to 780 nm
- 7 emission filters: 500 to 850 nm
- Xerra is CE marked

RUN CFT AS A SERVICE

- EMIT offers services, including imaging and analysis
- Animals/tissues are prepared at the sponsor or CRO
- Shipped frozen to EMIT for blocking and imaging
- Images are reviewed with the sponsor and additional analysis support available

FLUOROPHORE CHARACTERIZATION

Xerra is equipped with laser and filter combinations that match most applications in biological research



The **Limit of Detection (LOD)** and **Limit of Quantification (LOQ)** have been evaluated for the following fluorophores:

Fluorophore	LOD (nM)	LOQ (nM)
Cy3	1.7	5.1
Cy5	2.6	7.7
Cy5.5	4.1	12.3
AF488	4.3	12.9
AF546	6.8	20.5
AF647	0.9	2.9
AF700	3.8	11.7
AF750	3.7	11.3
IRDye 800CW	9.5	28.9
ZW800-1	11.7	35.3

Fluorophore Queue:

- GFP
- mCherry
- tdTomato
- mKate
- FITC
- Texas Red
- AF594

Additional can be added upon request