Practical Applications of MRI in Toxicologic Pathology

Part of the Imaging Workshop of the International Academy of Toxicologic Pathology (IATP)

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In vivo Imaging- The Future is Now

Invited Review

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Toxicologic Pathology in the 21st Century

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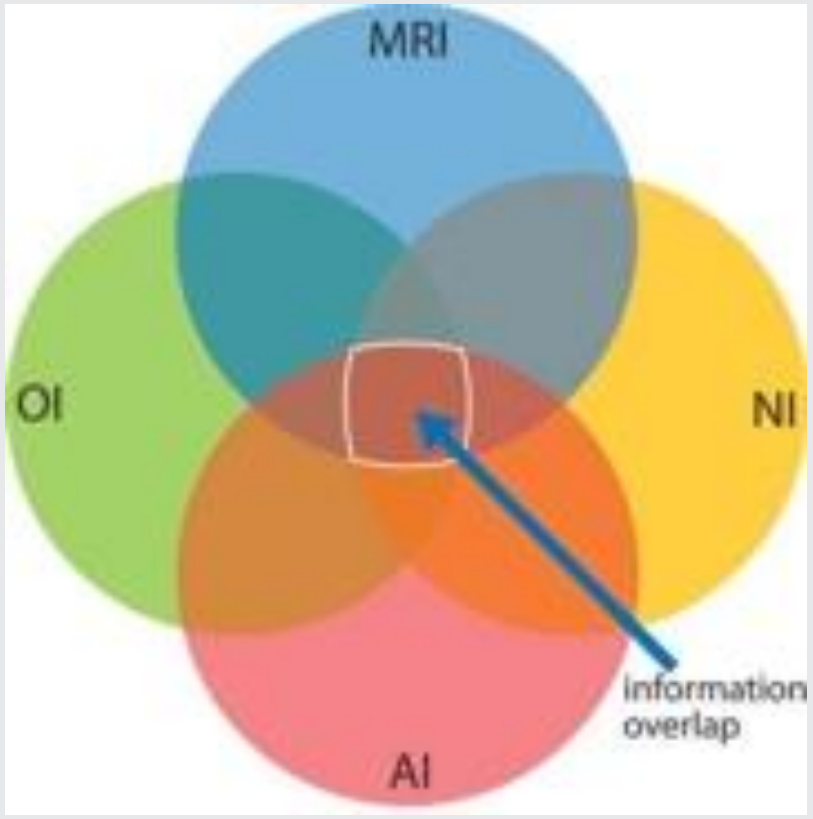
ABSTRACT

Toxicology is and will be heavily influenced by advances in many scientific disciplines. For toxicologic pathology, particularly relevant are the increasing array of molecular methods providing deeper insights into toxicity pathways, *in vivo* imaging techniques visualizing toxicodynamics and more powerful computers anticipated to allow (partly) automated morphological diagnoses. It appears unlikely that, in a foreseeable future, animal studies can be replaced by *in silico* and *in vitro* studies or longer term *in vivo* studies by investigations of biomarkers including toxicogenomics of shorter term studies, though the importance of such approaches will continue to increase. In addition to changes based on scientific progress, the work of toxicopathologists is and will be affected by social and financial factors, among them stagnating budgets, globalization, and outsourcing. The number of toxicopathologists in North America, Europe, and the Far East is not expected to grow. Many toxicopathologists will likely spend less time at the microscope but will be more heavily involved in early research activities, imaging, and as generalists with a broad biological understanding in evaluation and management of toxicity. Toxicologic pathology will remain important and is indispensable for validation of new methods, quality assurance of established methods, and for areas without good alternative methods.

In Vivo Imaging- Don't Choose, Fuse!



Advantages of MRI in Toxicology

- **Non invasive** - Permits longitudinal *in vivo* imaging to follow disease in the same animal (better statistics, less animals required)
- Can acquire numerous **digital slices** from whole fixed organs in **any plane** without destroying the specimen
- High **soft tissue contrast** as well as good bone visualization
- Provides a means to obtain **quantitative data**

Provides complimentary information to conventional pathology- Better practice, safer drugs/products

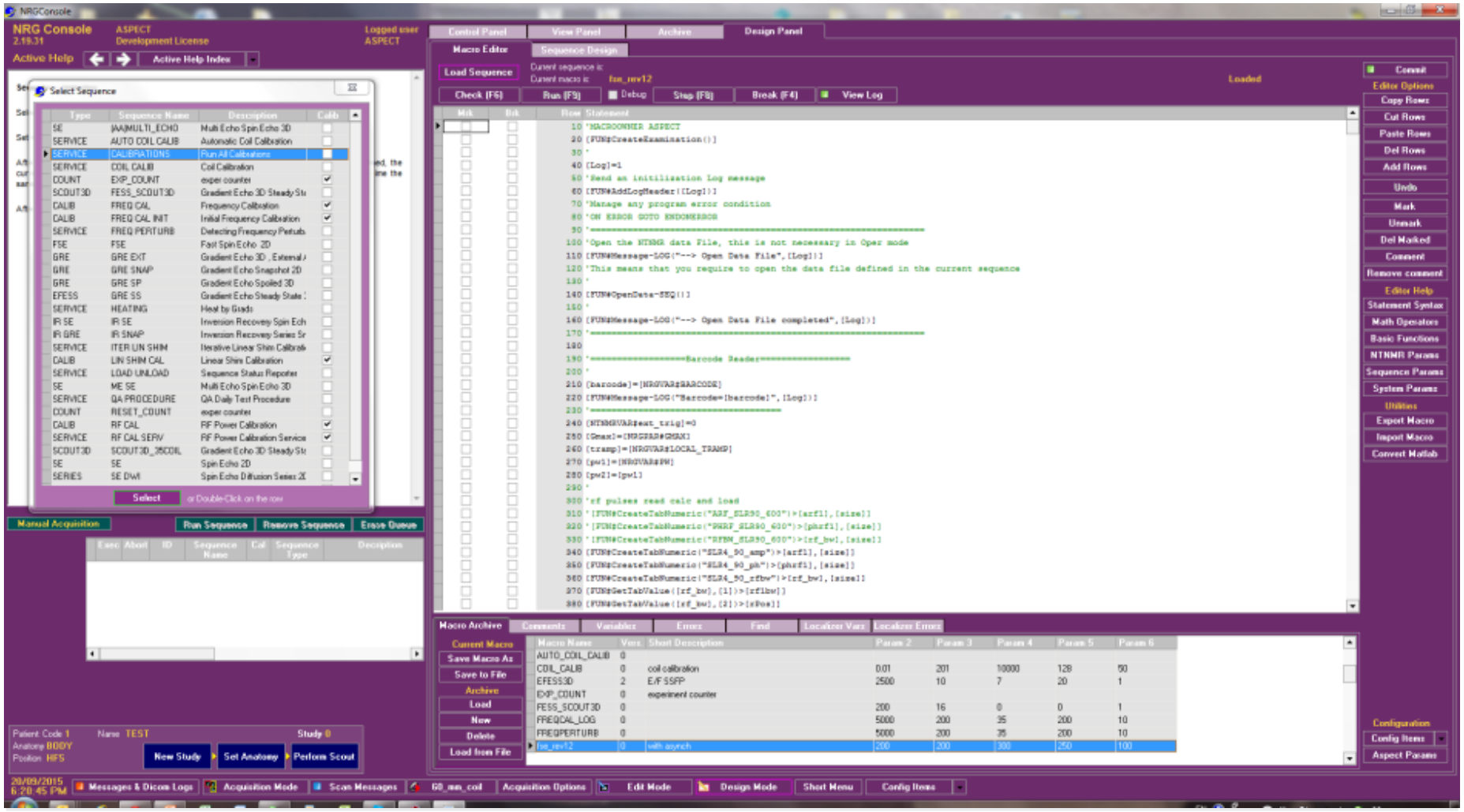
MRI- A Psychological Barrier

- Expensive equipment
- Expensive maintenance
- Special facility required (shielded room)
- Safety issues (no metals around)
- Hard to operate
- For "MR gurus" only – High level of expertise required

Shift Happens – Compact MRI for **Everyone**

- Compact
- Quiet
- Affordable
- Easy to use
- Safe
- No special facility
- Maintenance-free

Complicated Software - No more



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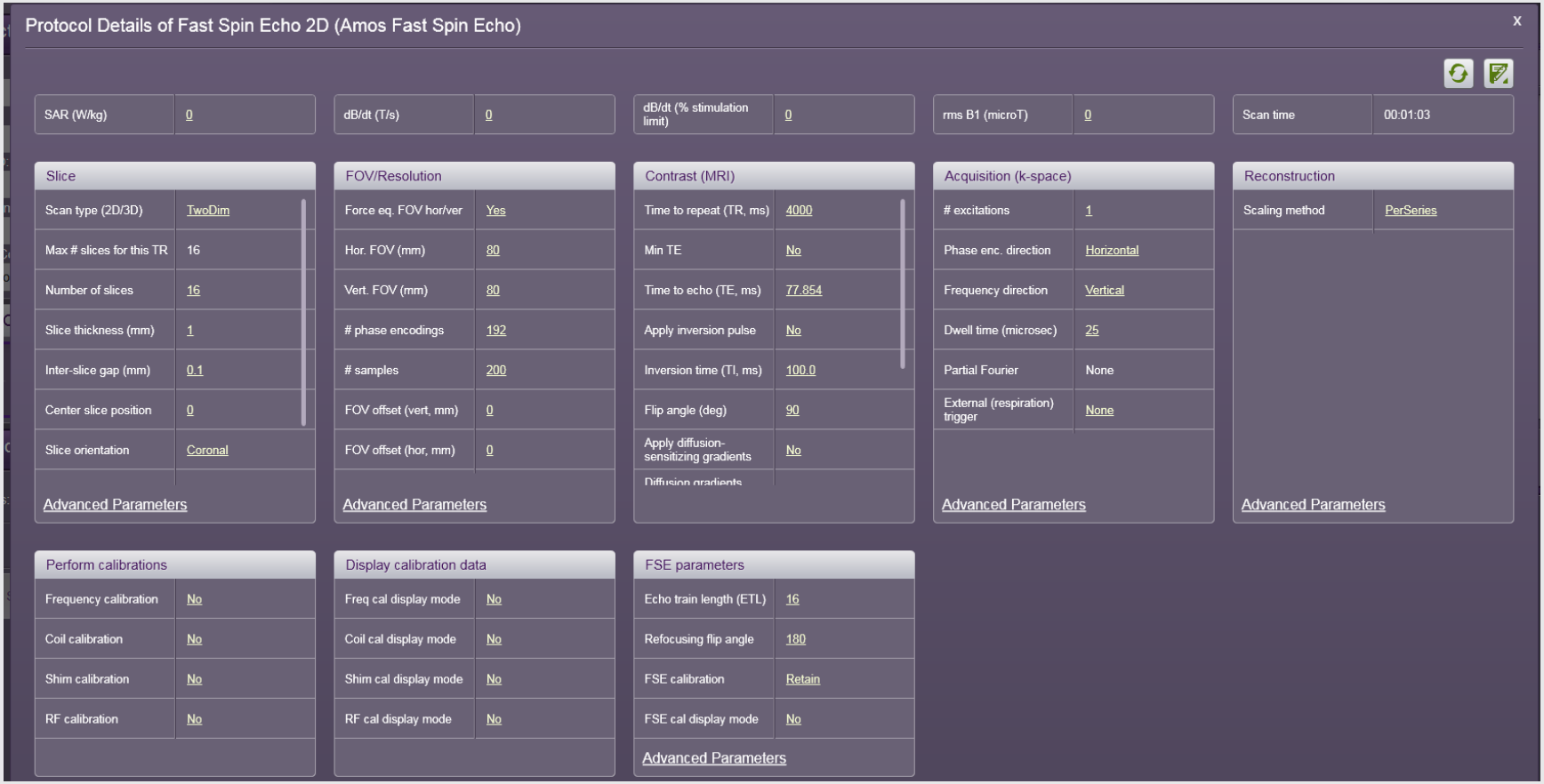
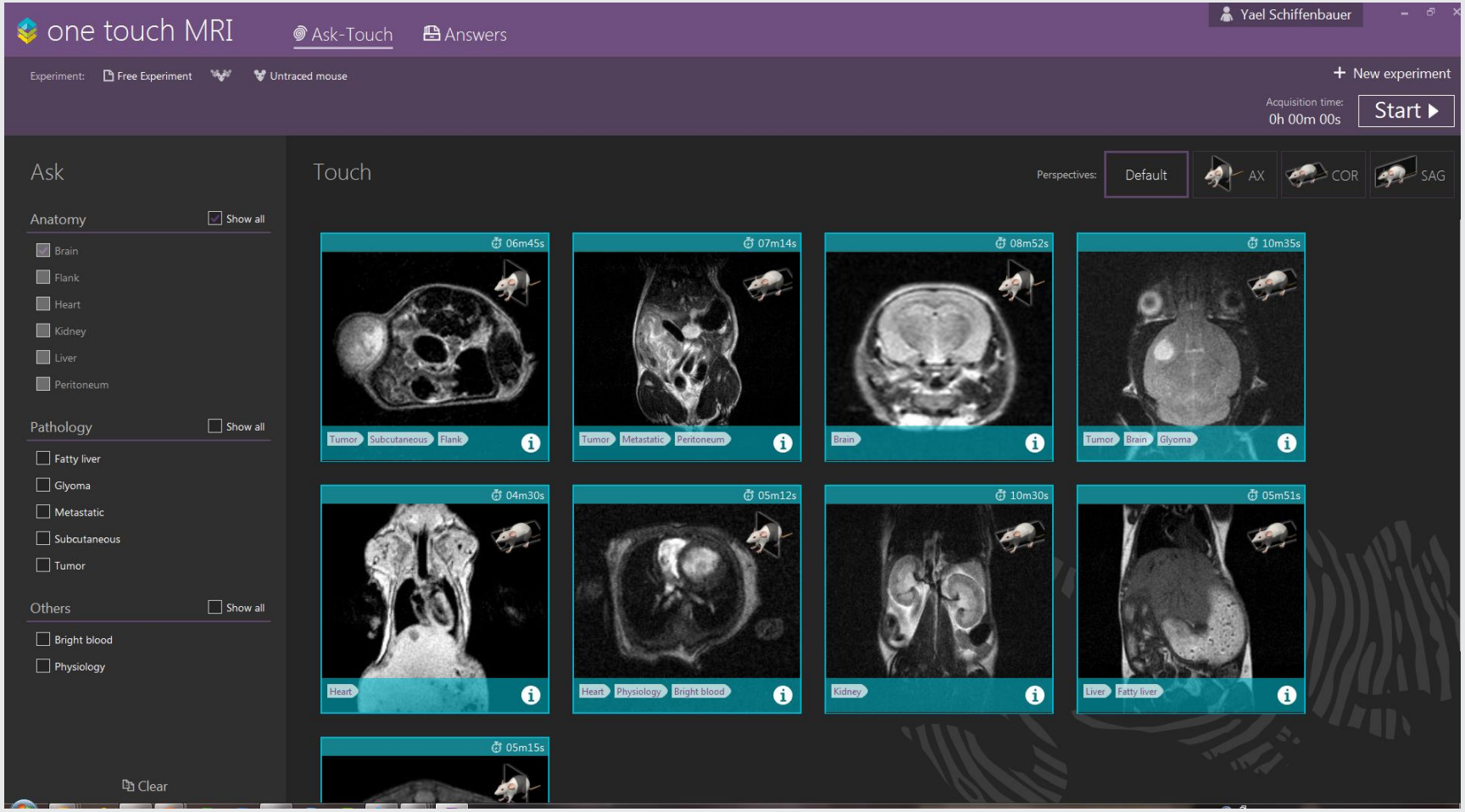
Tons of Parameters to Optimize… No more



One Touch MRI - Ask. Touch. Answer.

Just Choose What You Need to Scan



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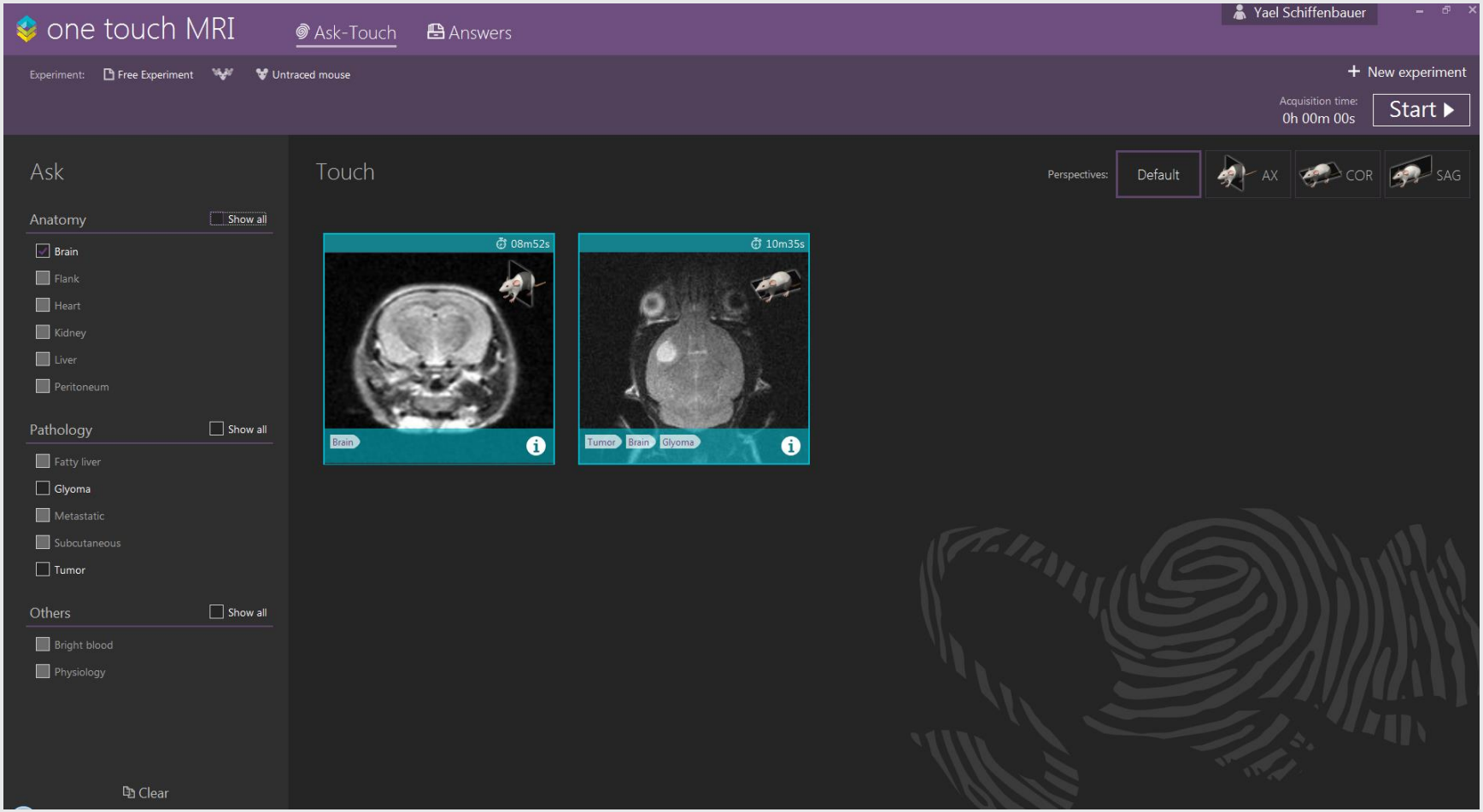
Brain Protocols?



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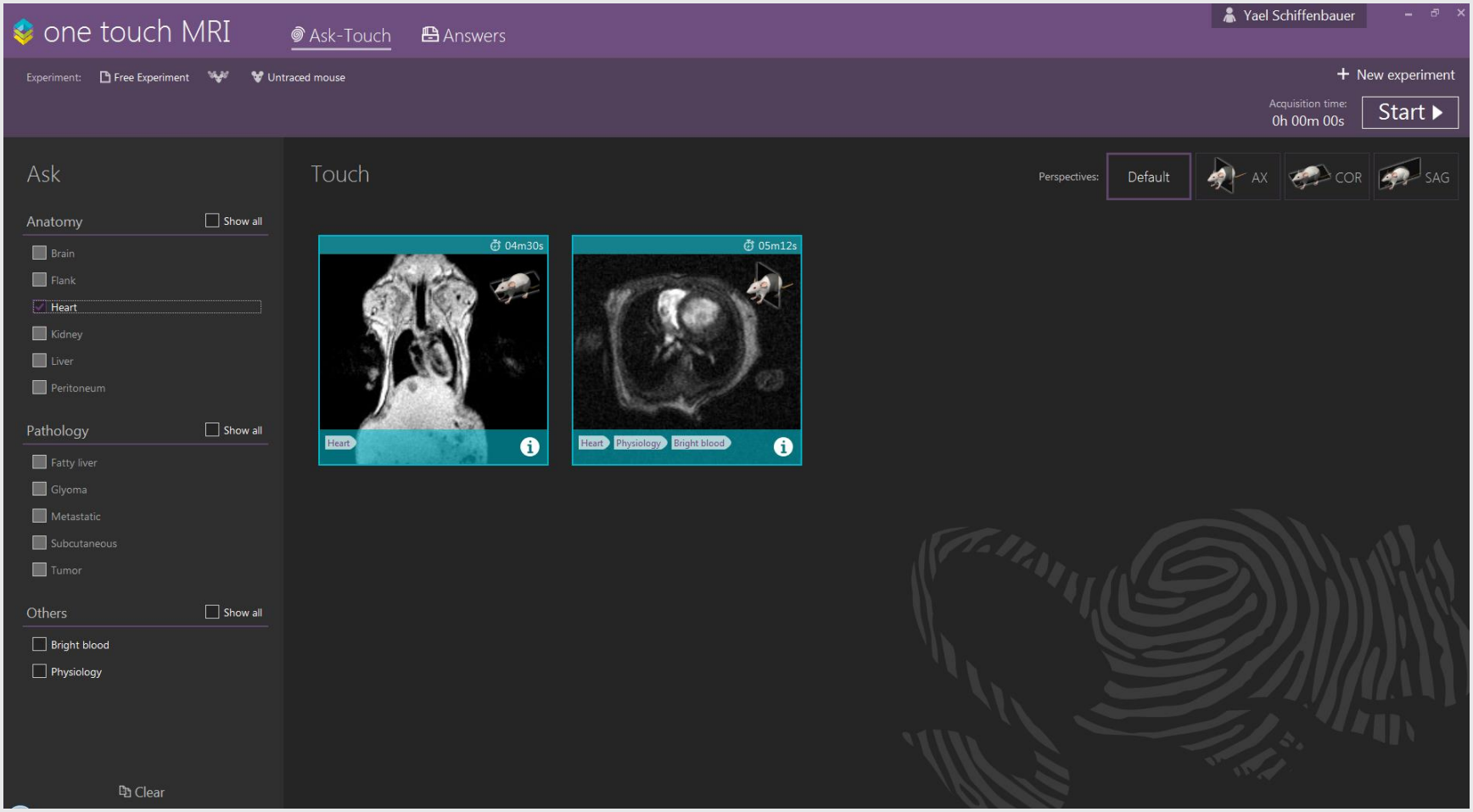
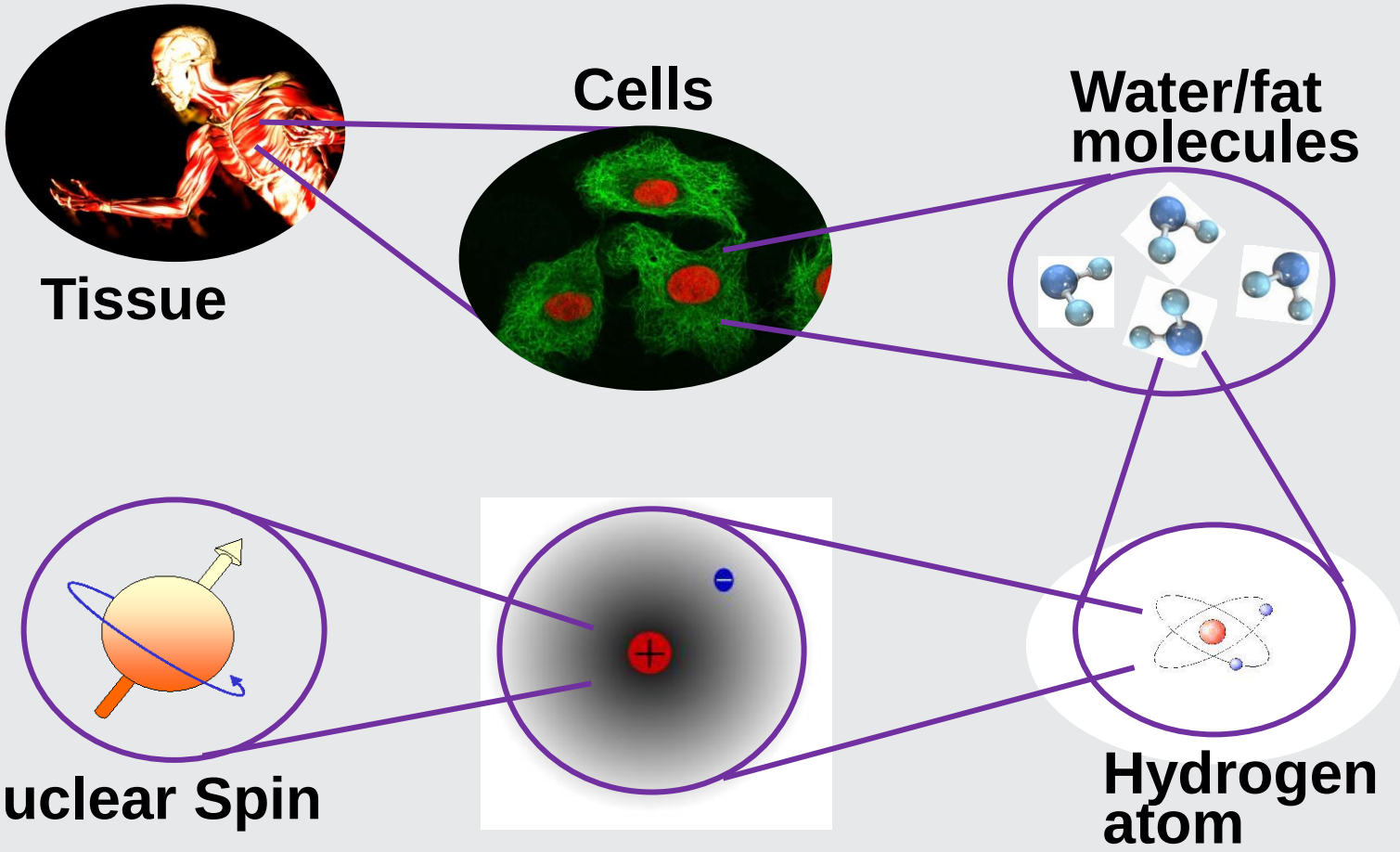
Heart protocols?



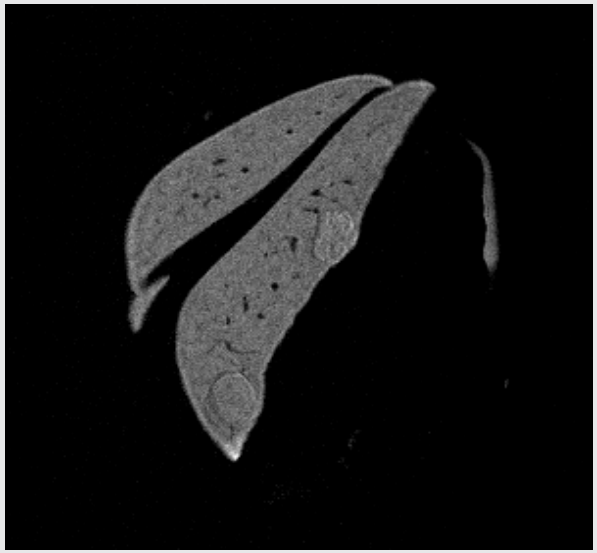
Source of Signal in MRI

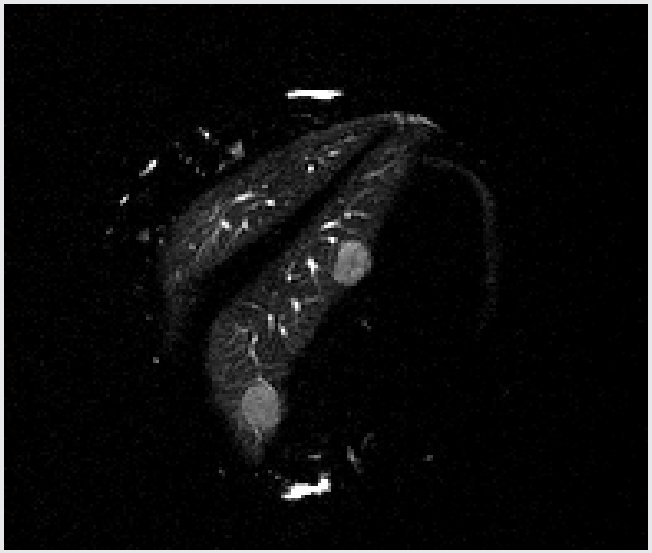


MRI Terminology

- By changing the frequency, duration and timing of applied magnetic fields and radio frequency (rf) pulses, MRI can provide what are basically "MRI stains"
- Most common "MRI stains" (Types of contrast) – T1 and T2

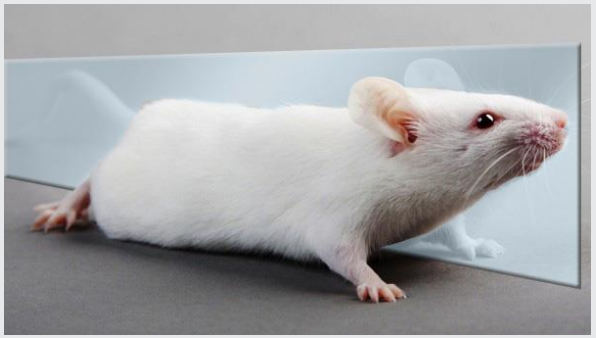
T1



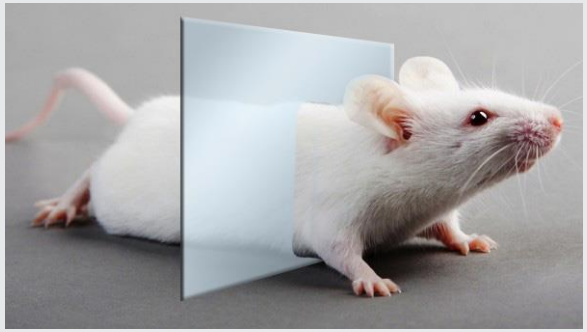


T2

Any plane can be imaged

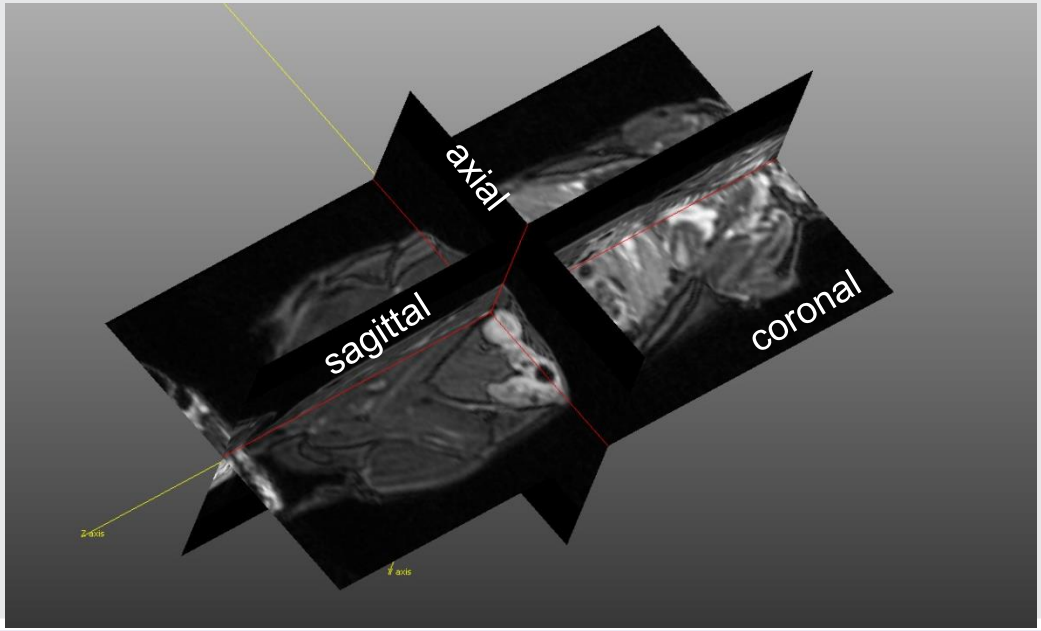


sagittal

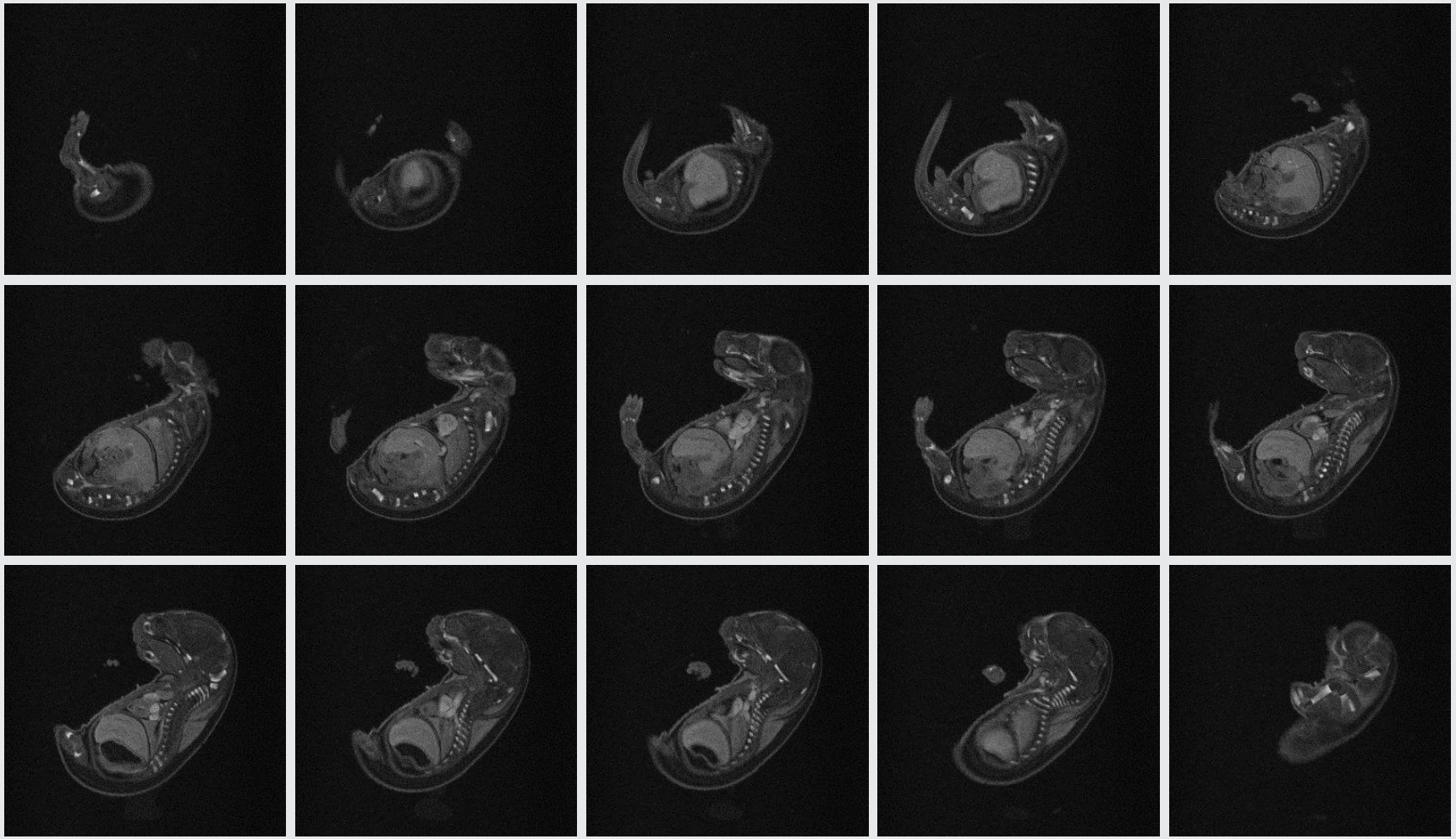


axial

coronal

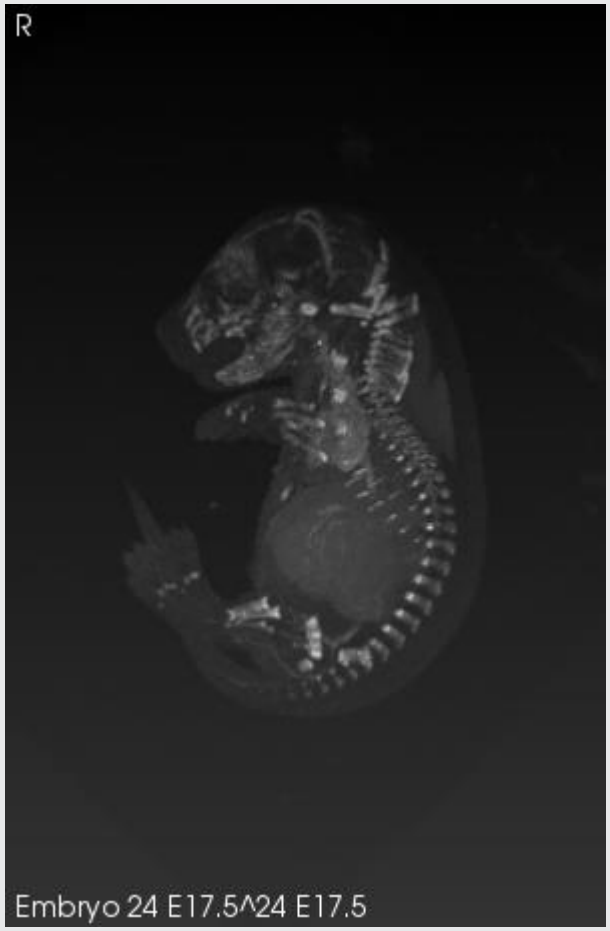


MRI Data Presentation- Individual Slices



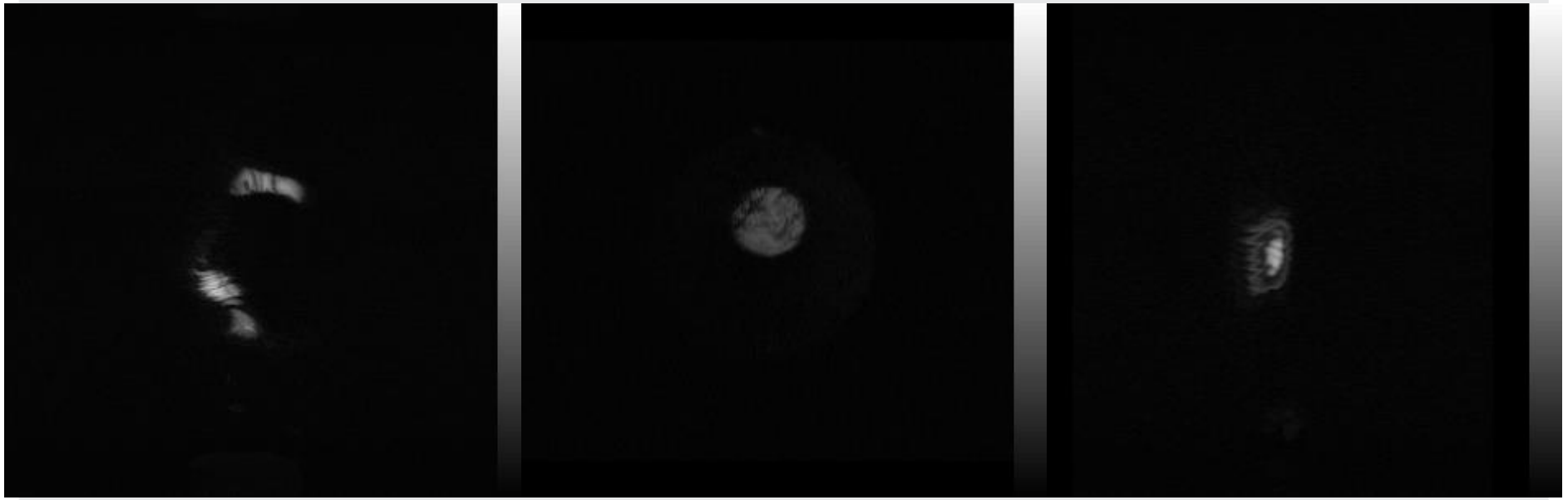
MRI Data Presentation –Slice through Animation

MRI Data Presentation – See through 3D Rendering (MIP)

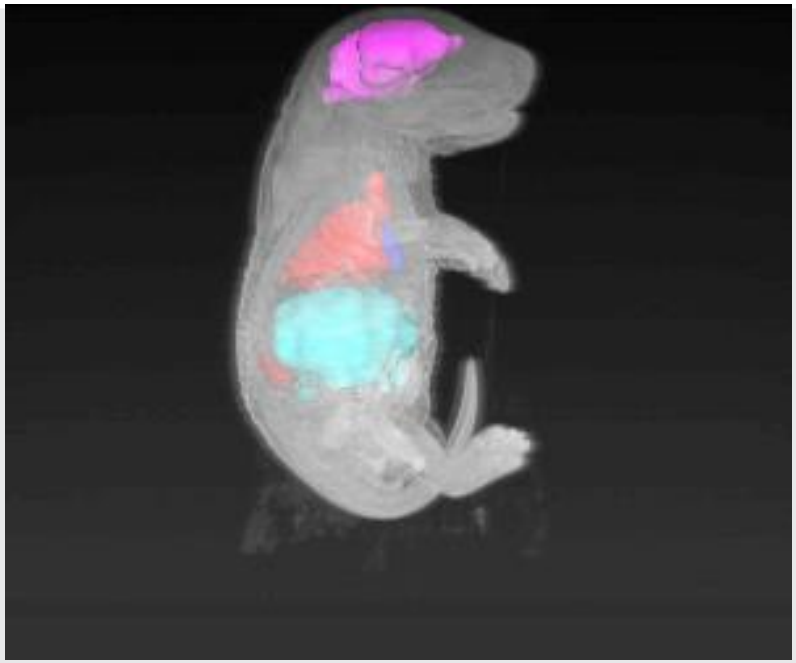


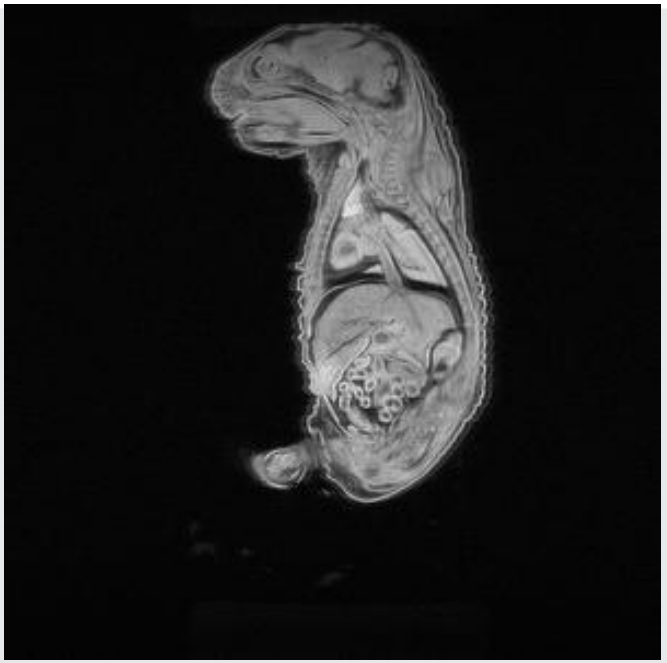
Segmentation – Volume Calculation

Sagittal **axial** **coronal**



Segmentation – Volume Calculation





ROI	Rat	Color	Voxels	Volume mm³
Lungs	rat embryo control E20	red	28910	97.5713
Heart	rat embryo control E20	blue	8218	27.7358
Liver	rat embryo control E20	cyan	84566	285.41
Brain	rat embryo control E20	magenta	38770	130.849
Left Kidney	rat embryo control E20	dark red	3044	10.2735
Right Kidney	rat embryo control E20	dark cyan	2745	9.26438

Scientific Collaboration

NIH National Institute of Environmental Health Sciences

National Toxicology Program
U.S. Department of Health and Human Services

harlan®
Harlan Biotech Israel Ltd.

האוניברסיטה העברית בירושלים
The Hebrew University of Jerusalem

NeuroDerm

aspectimaging

Eisai

RTC
RESEARCH TOXICOLOGY CENTRE - ROMA

ACTELION

Methods

All scans performed on a M-series compact MRI by Aspect Imaging.

Animals:

- -Anesthetized with isoflurane
- -Heated
- -Physiological monitoring

Fixed samples:

- - In any fixative solution
- - Fluorinert

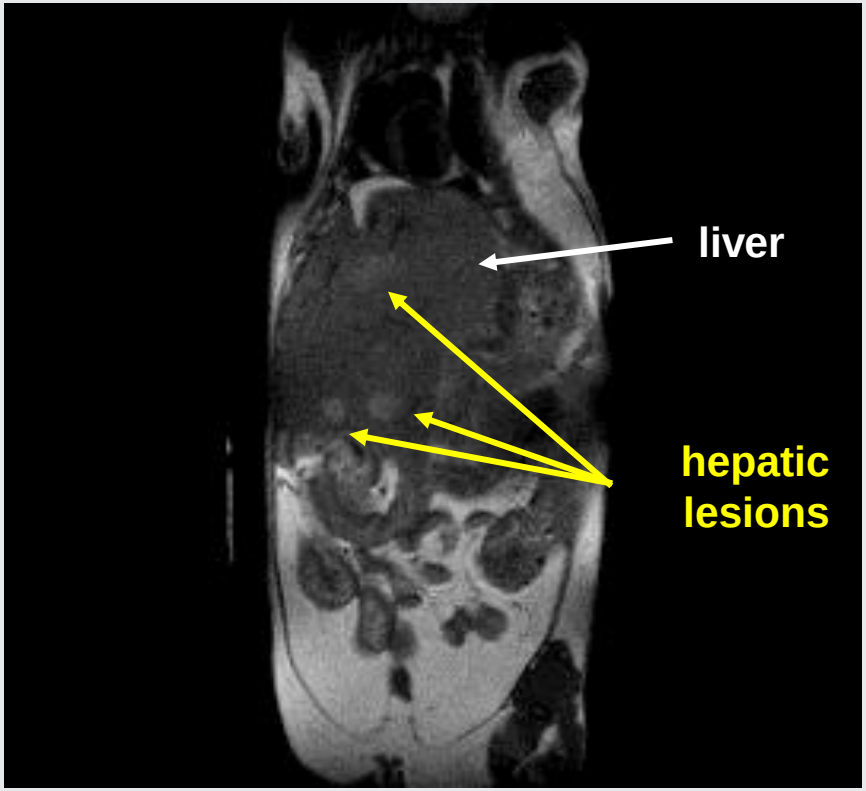
Models That Will be Presented

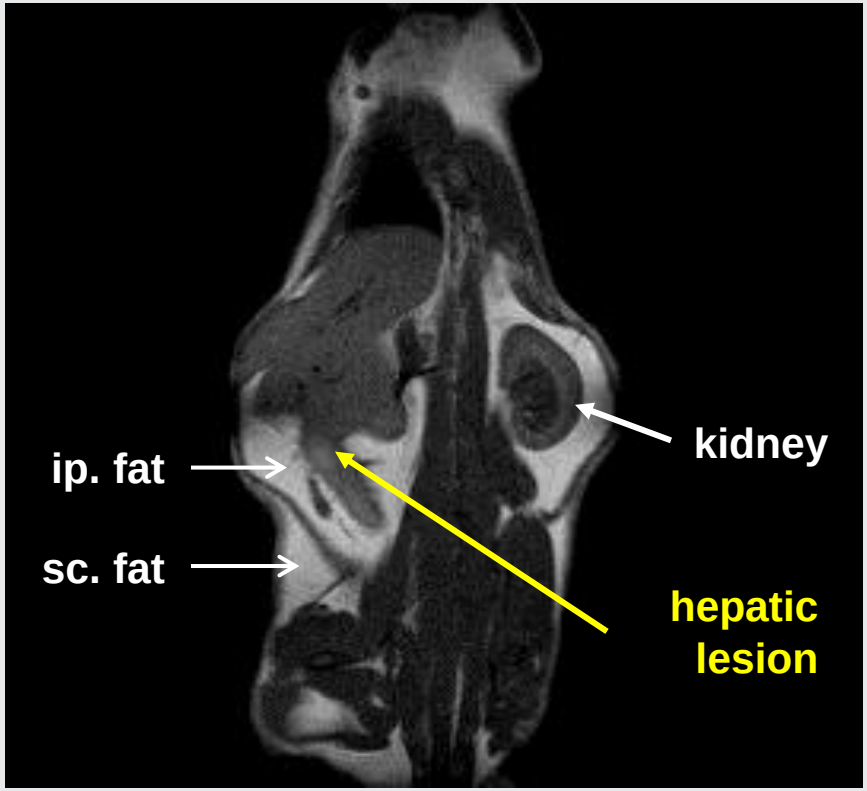
- Focal liver lesions
- Acute kidney injury
- Local Safety of SC formulations
- Biodegradable implant
- Brain tumor growth
- Rat lung fibrosis
- Neurotoxicity

Focal Hepatic Lesions

- **Model:** Mdr-/- mouse develops multiple focal hepatic lesions.
- **Objective:** Detect and measure volume of multiple focal lesions

Detection of Multiple Focal Lesions in Mouse Liver – *In Vivo* MRI

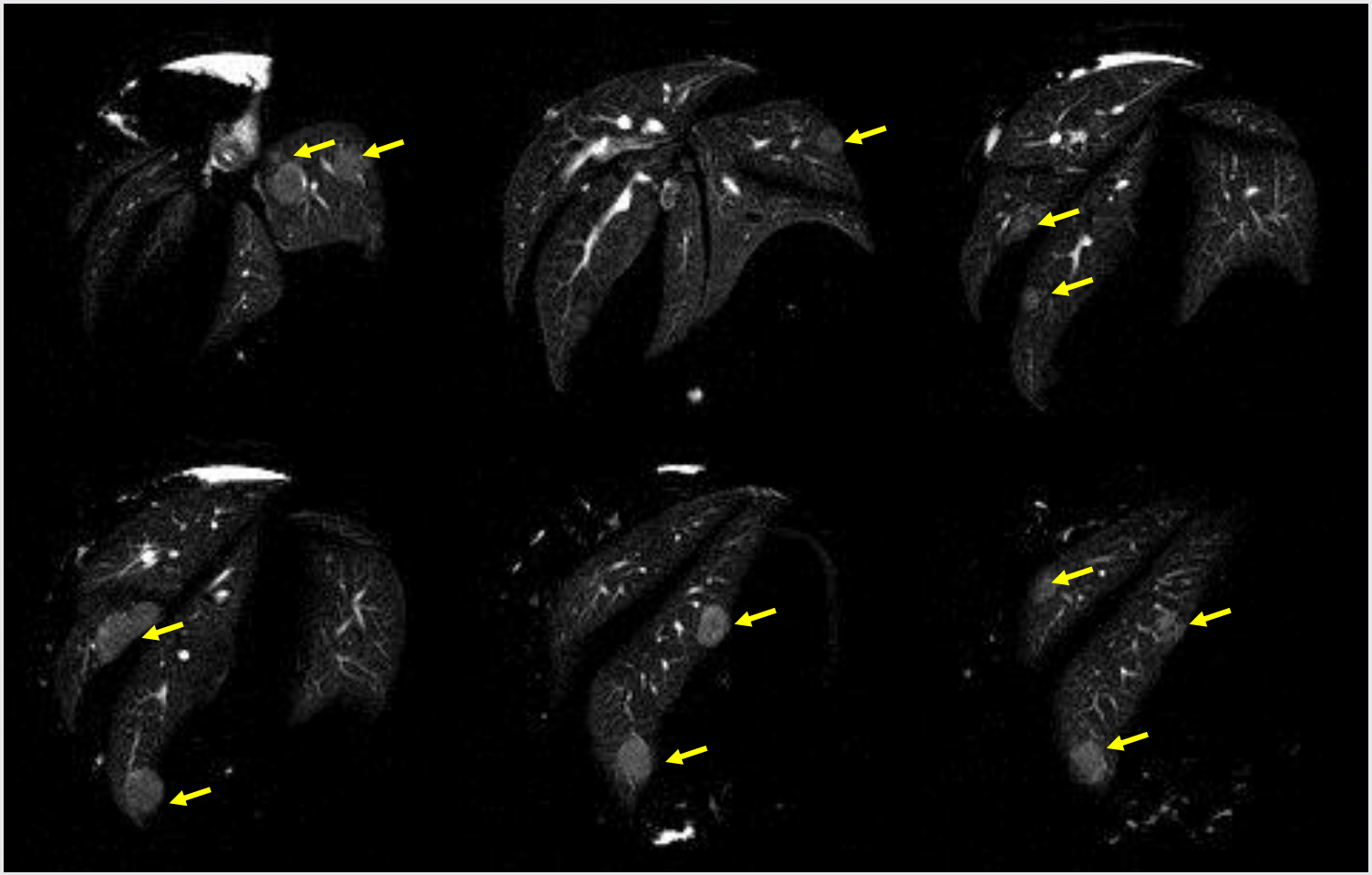




resolution 270 μm; slice thickness 1mm; acquisition time 3.5 min

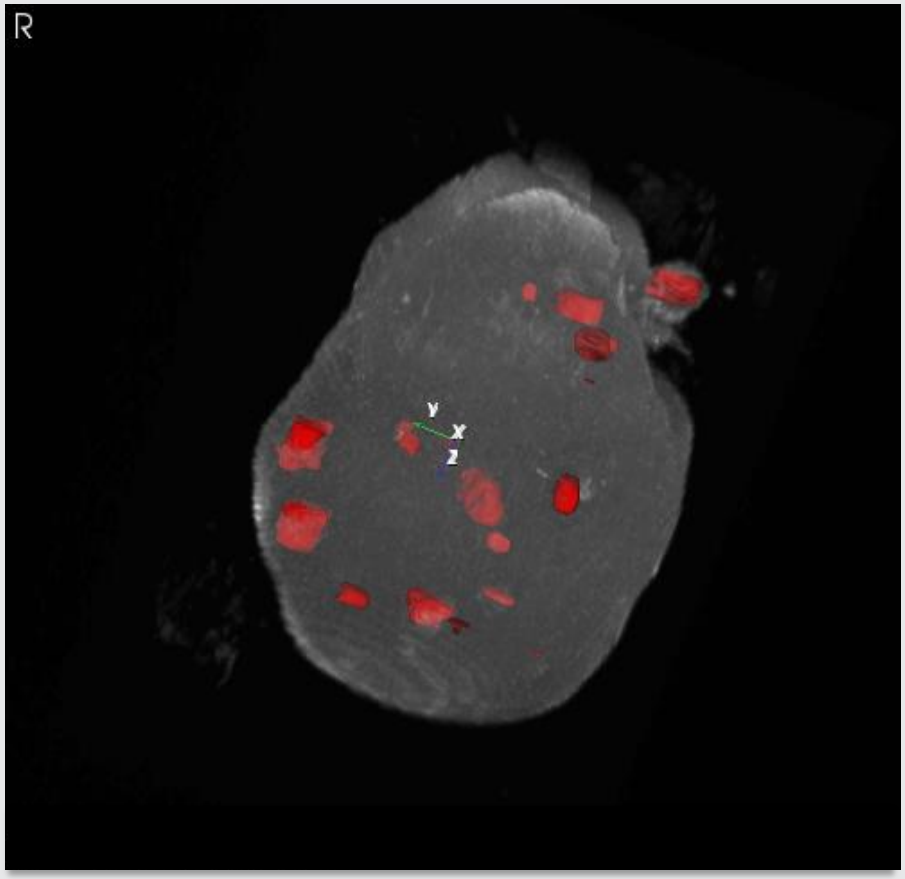
Multiple Focal Lesions in Mouse Liver

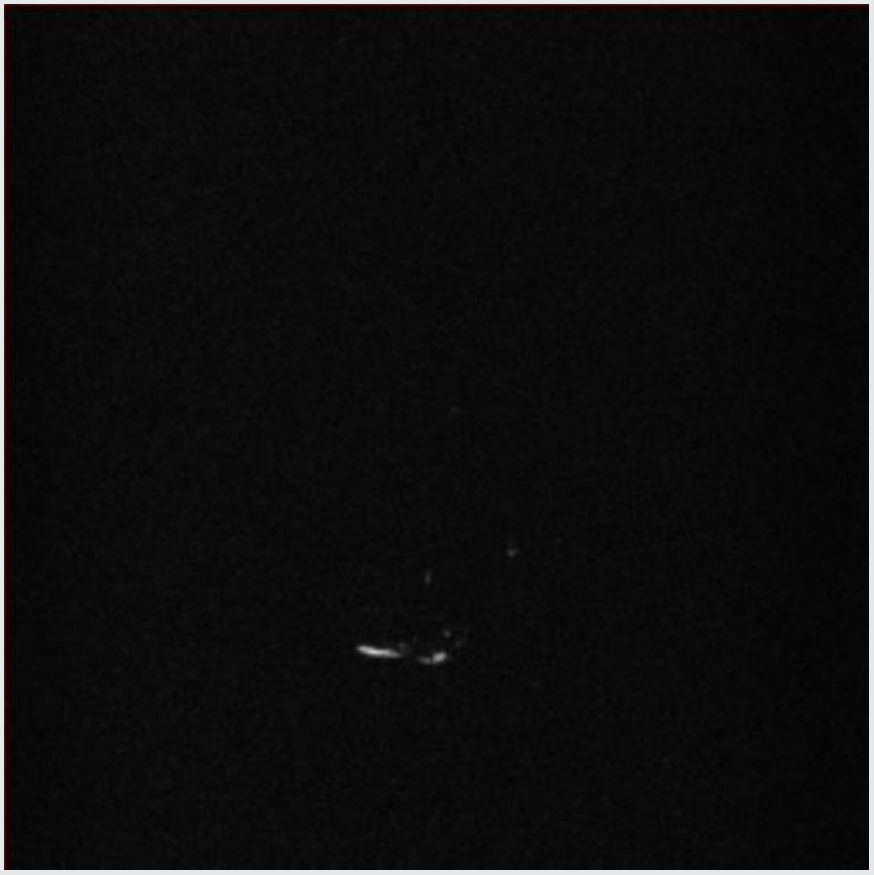
Ex vivo MRI



resolution 156 μm; slice thickness 0.7 mm; acquisition time 35 min

Segmentation of Lesions Based on *Ex Vivo* MRI

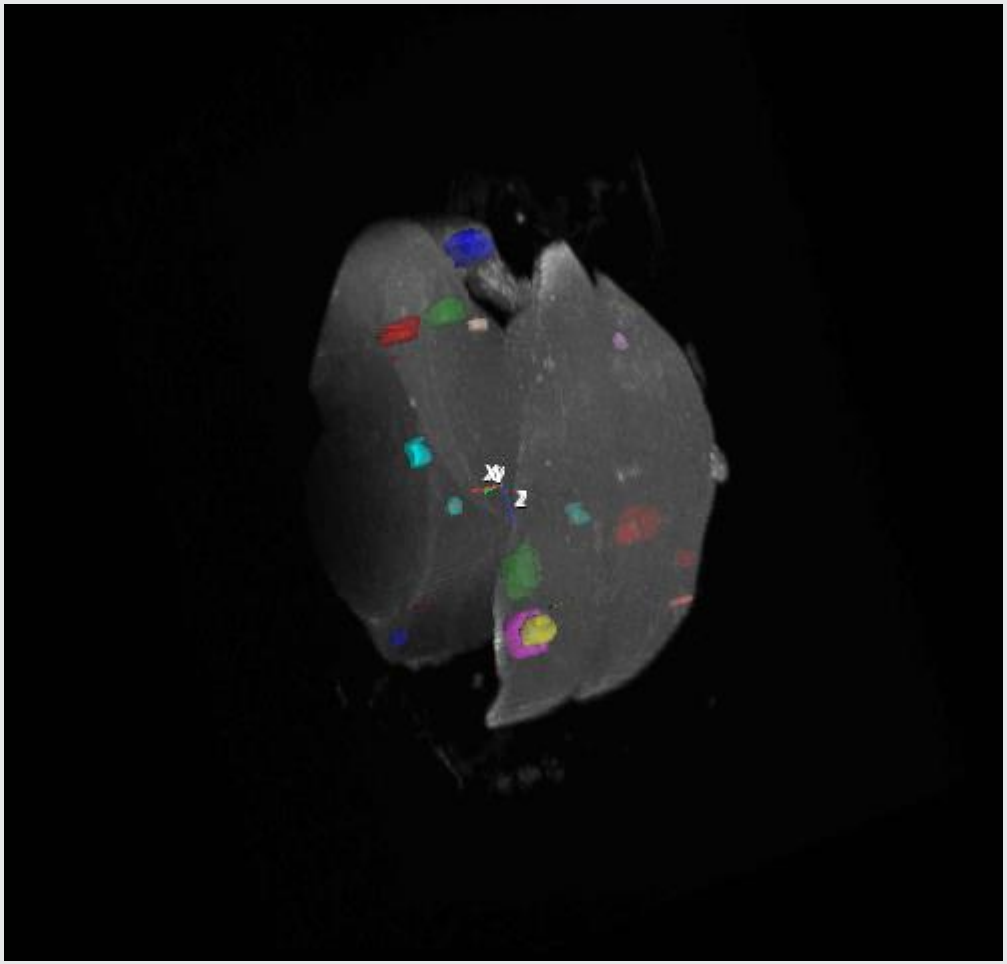




Quantification of Lesions Based on *Ex Vivo* MRI

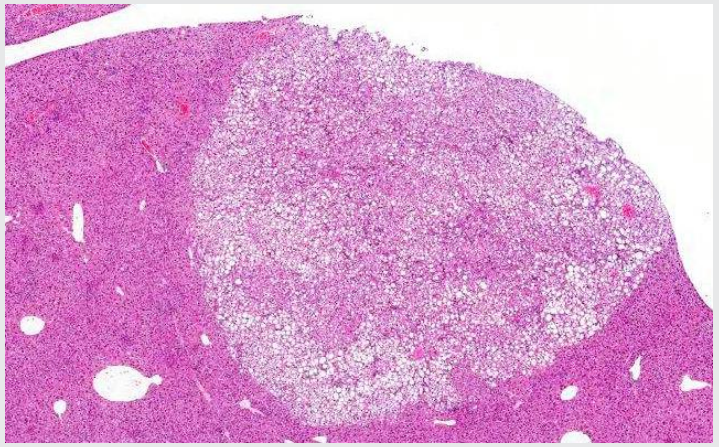
- **15** distinctive lesions were detected
- The smallest lesion detected had a diameter of **0.6 mm**
- The largest lesion had a diameter of **4.8 mm**
- Total liver mass **2593 mm^3**
- Total lesion mass **60.3 mm^3 (2.3%)**

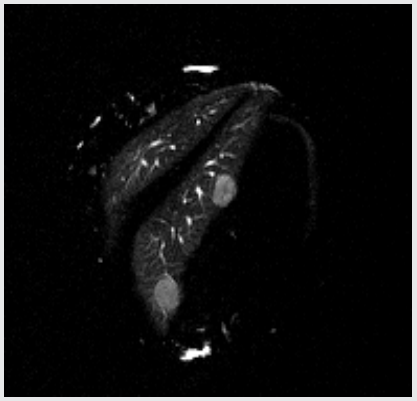
Segmentation of Lesions Based on *Ex Vivo* MRI

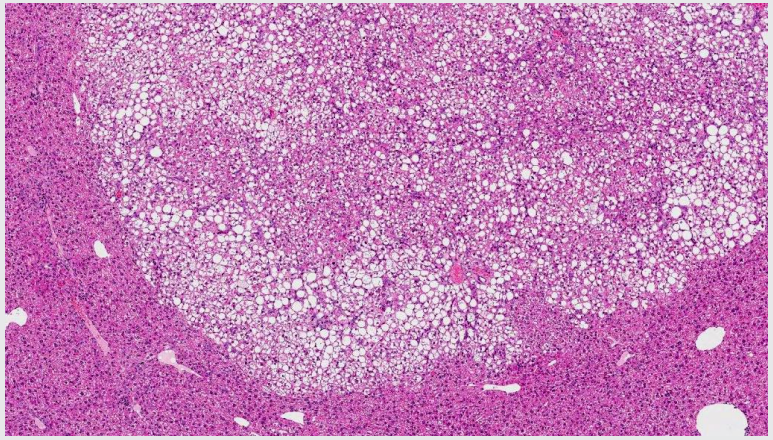


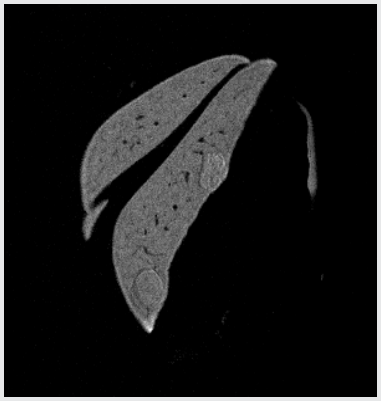
ROI	Color	Voxels	Volume mm³
1	red	701	2.6741
2	green	950	3.62396
3	blue	1123	4.28391
4	cyan	342	1.30463
5	magenta	2047	7.80869
6	yellow	831	3.17001
7	dark red	2271	8.66318
8	dark green	1961	7.48062
9	dark blue	204	0.778198
10	dark cyan	245	0.934601
11	tomato	201	0.766754
12	maroon	197	0.751495
13	orchid	99	0.377655
14	peach puff	143	0.545502
15	light sea green	152	0.579834

Classification of Liver Lesions as Focal Fatty Changes by Histopathology









Summary & Comment

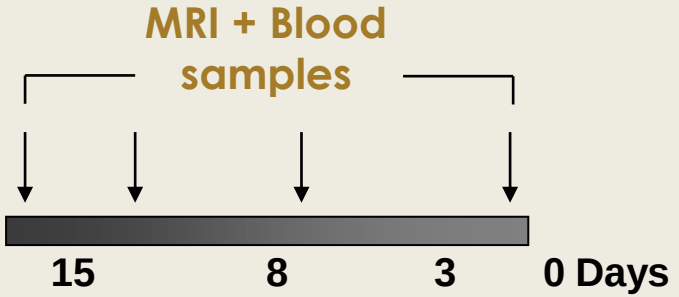
- I*n vivo* and *ex vivo* MRI evaluation were effective in identifying the location and measuring the volume of focal changes in the liver.
- This approach using *in vivo* MRI would allow for following lesion development over time
- In this study the MRI was done after lesions were fully developed, however, longitudinal studies using *in vivo* MRI would easily be feasible in this model

Rhabdomyolsis-Induced Acute Kidney Injury (AKI) in Mouse

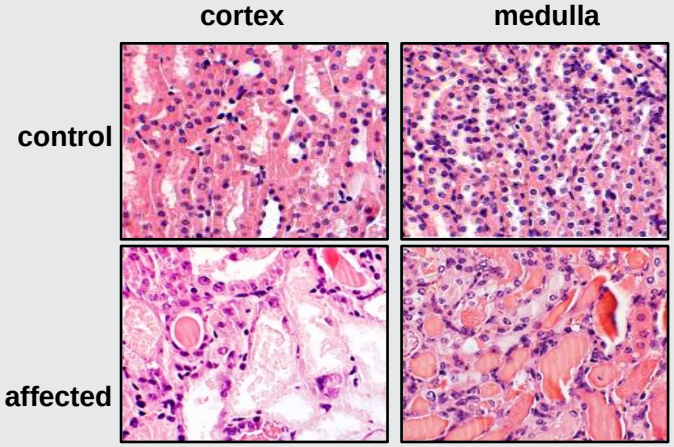
Mice Model of Glycerol-Induced AKI

CB6F1 Mice

IM 50% Glycerol



Histopathology – Day 3



Control vs Affected Kidney

In Vivo MRI

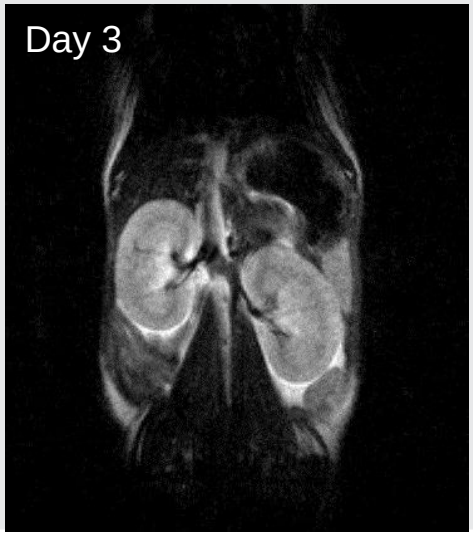
resolution 234 μm; slice thickness 1mm; acquisition time 10 min

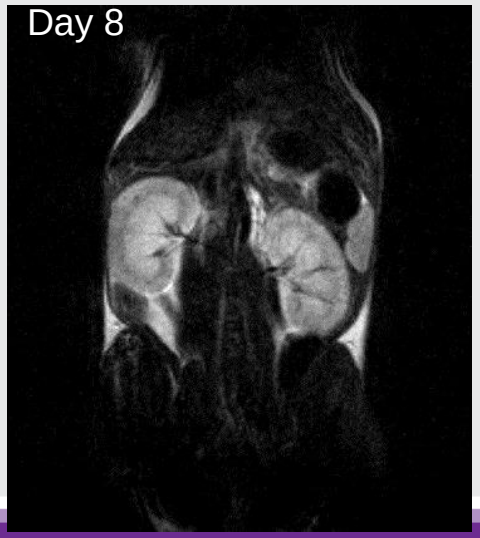
- **Loss of contrast**
- **Enlarged kidneys**

Following Disease Progression

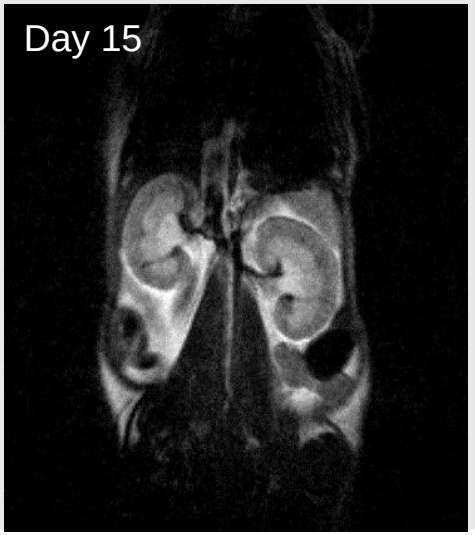
In Vivo MRI

Contrast lost and kidney enlargement





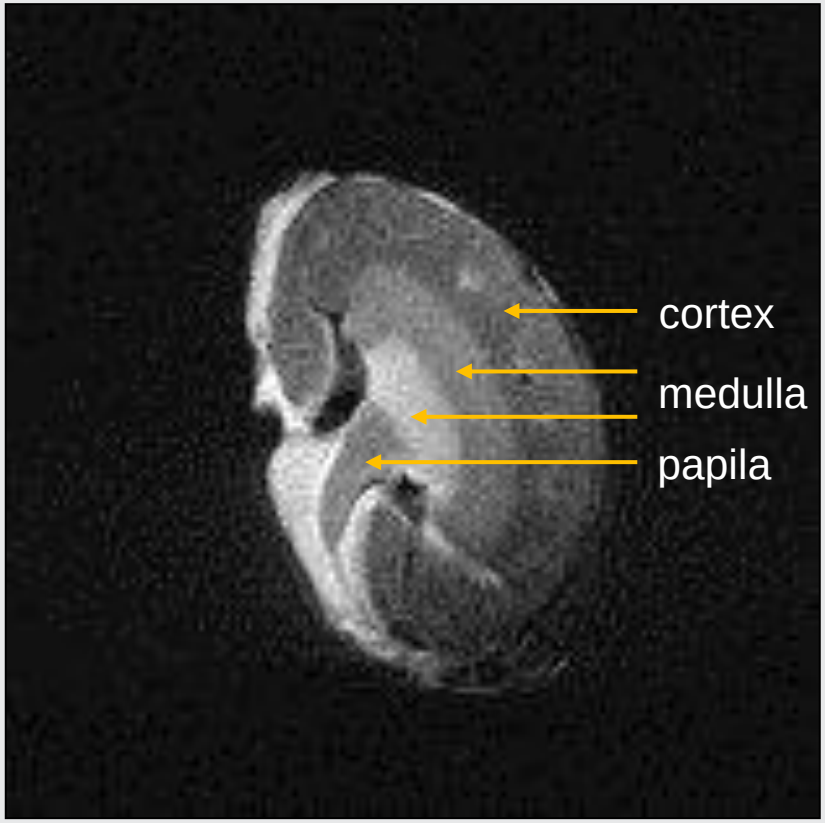
Contrast and size recovered



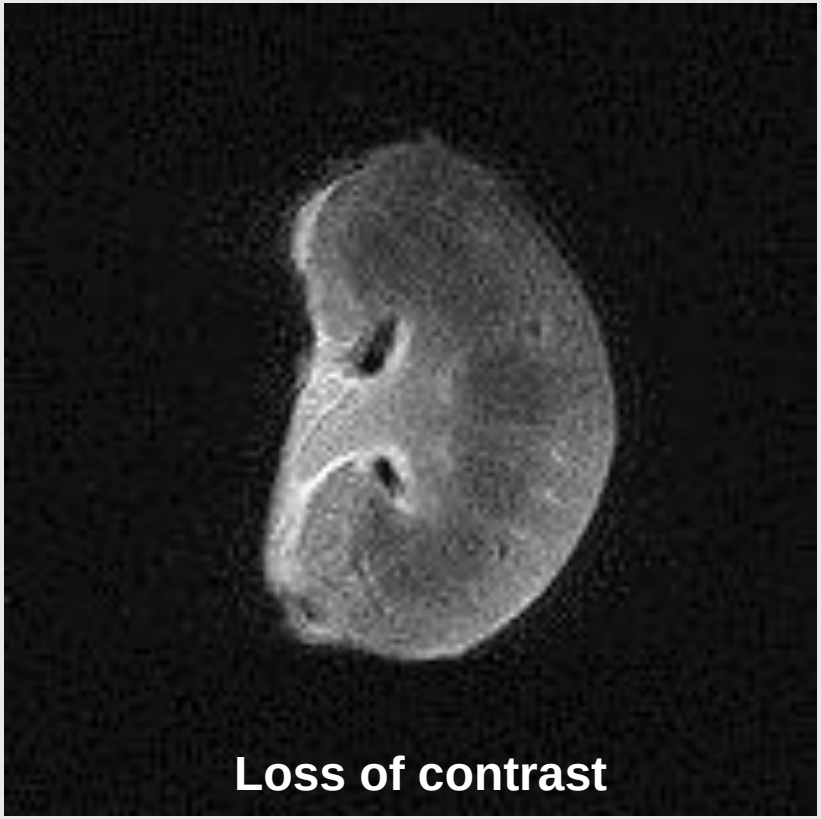
Control vs Affected Kidney

Ex Vivo MRI

Control



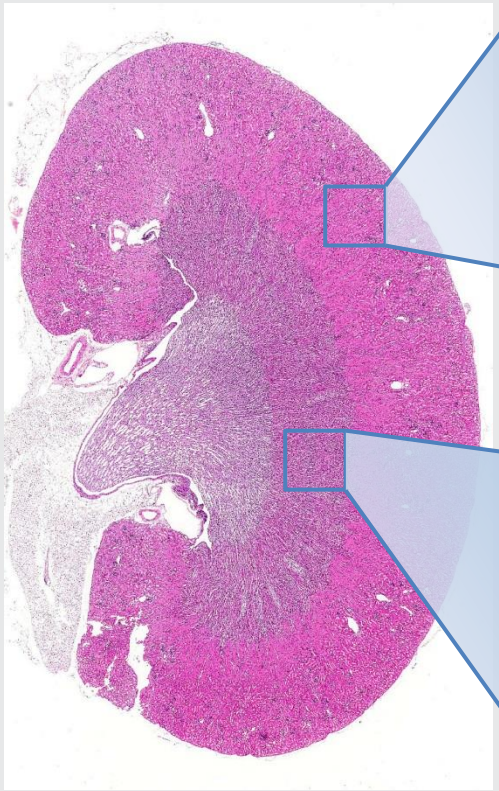
Affected



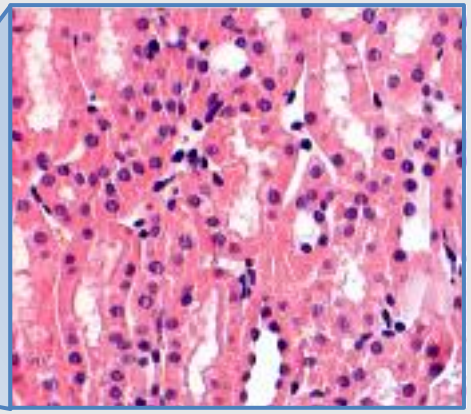
resolution 117 μm; slice thickness 0.5 mm; acquisition time 56 min

MRI & Histology – Control Kidney

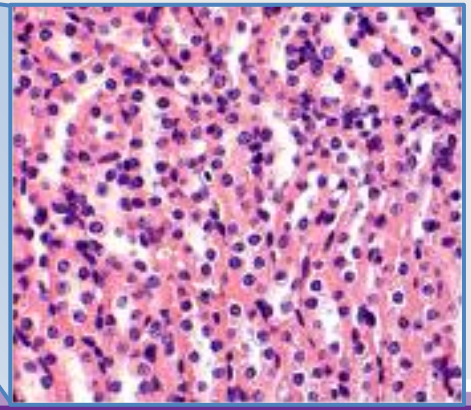
Ex Vivo



cortex X 200

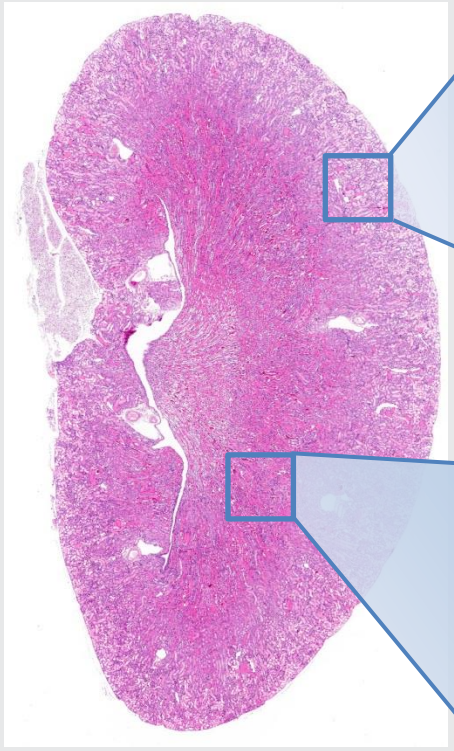


medulla X 200

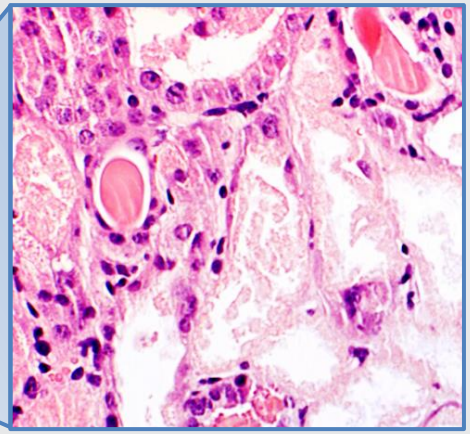


MRI & Histology – Affected Kidney

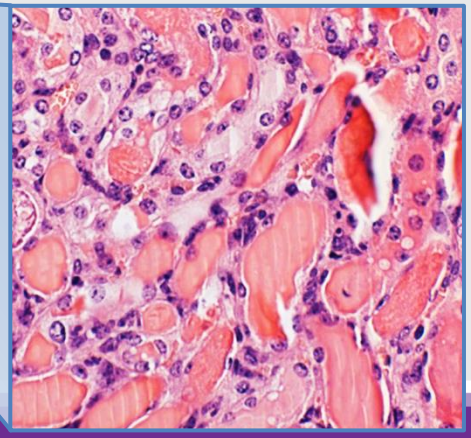
Ex Vivo



cortex X 200



medulla X 200



Summary of Findings & Comment

- *In-vivo* and *ex-vivo* MRI were effective in identifying alterations in the cortex and medulla
  Histopathology: Maximal extent of cortical necrosis and medullary hyaline cast formation.

- *In-vivo* and *ex-vivo* MRI confirmed organ recovery.
  Histopathology: The previously necrotic tubules were replaced by regeneration.

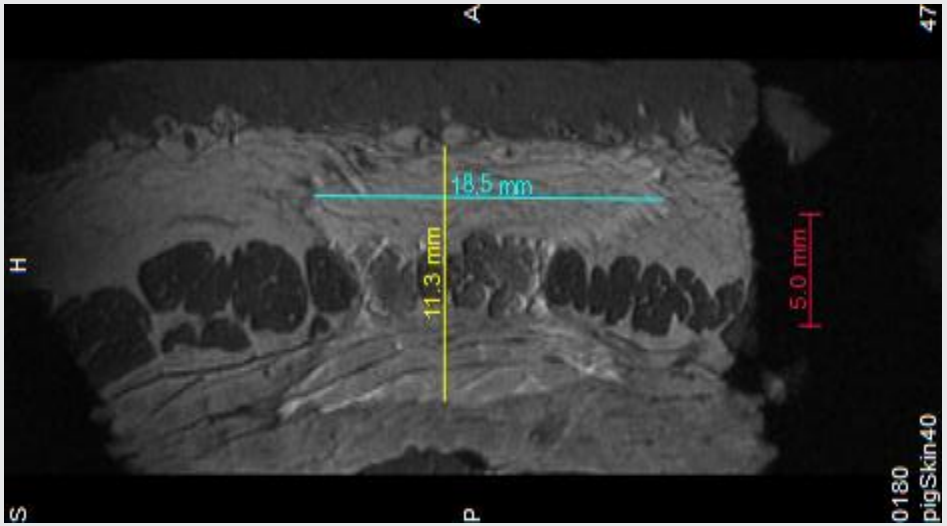
Local Safety of Subcutaneous Formulations

- **Model:** In this study, subcutaneous lesions were analyzed by MRI 2 weeks after a 24-hour continuous infusion of different formulations.

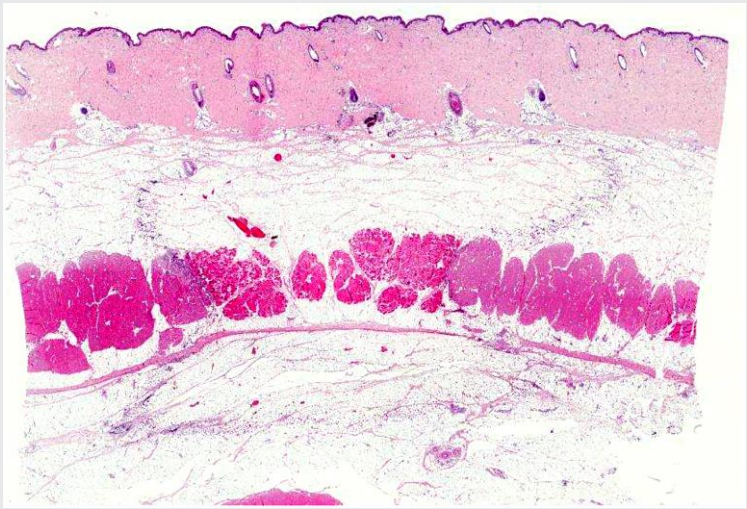
- **Objective of the experiment:** This was a feasibility study for application of the *Ex-Vivo* MRI in order to evaluate the subcutaneous toxic effects induced at the injection site of test compounds.

Subcutaneous Drug Injection Into Pig Skin MRI vs. Histology

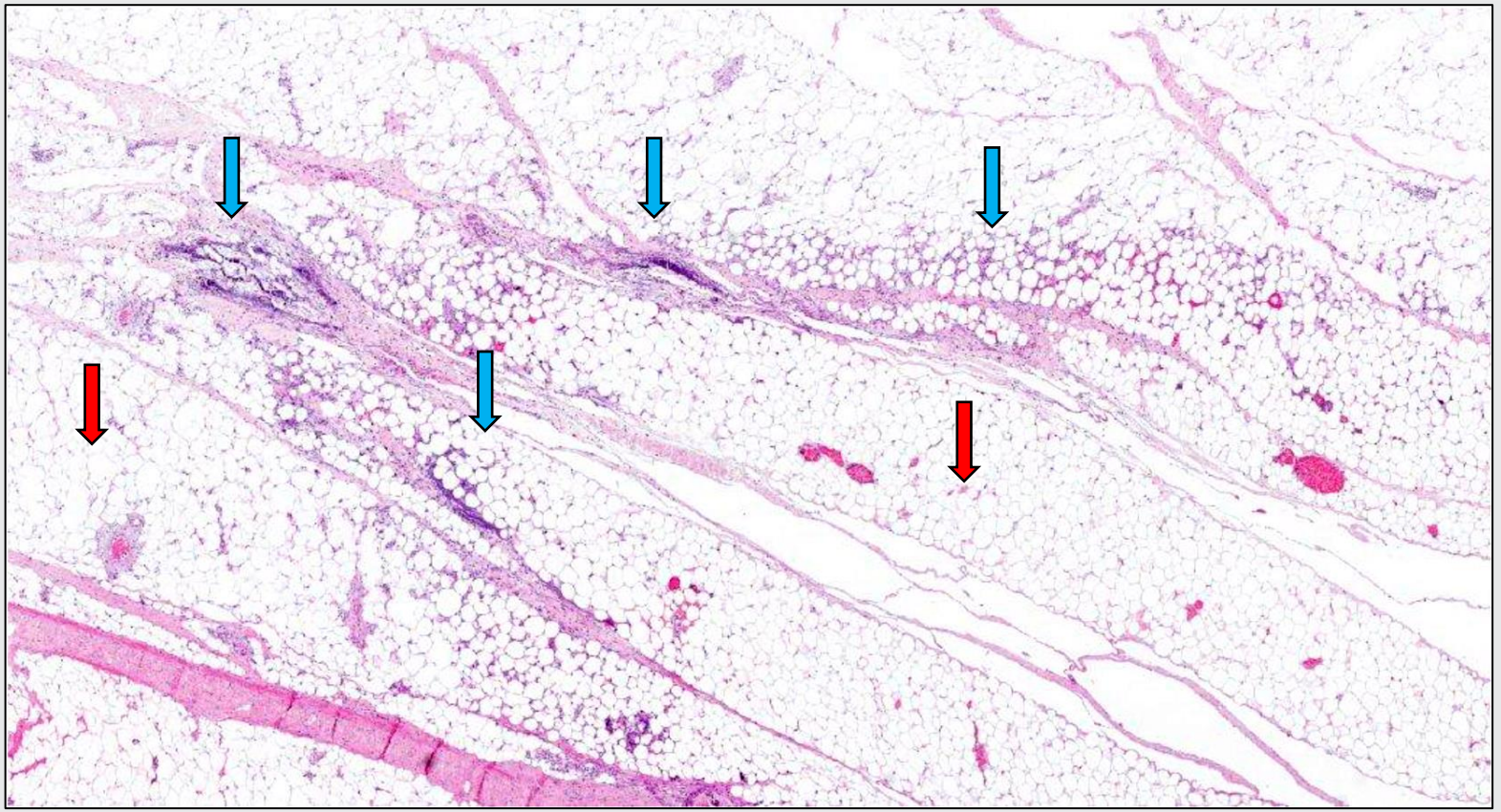
MRI (T1)



Histology

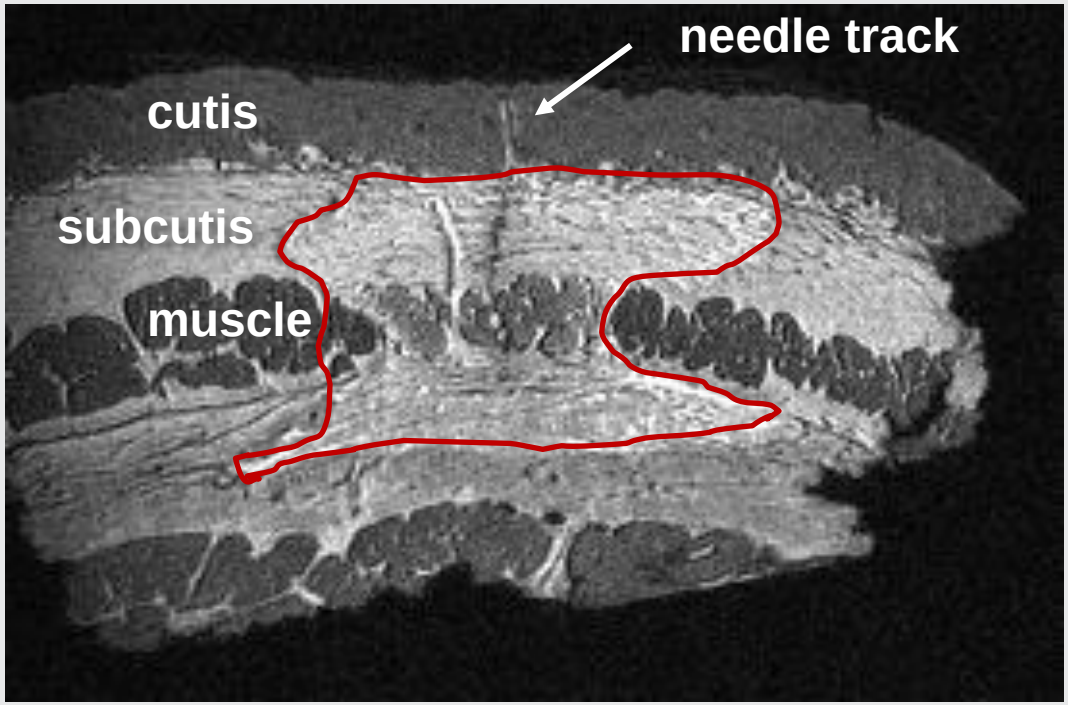


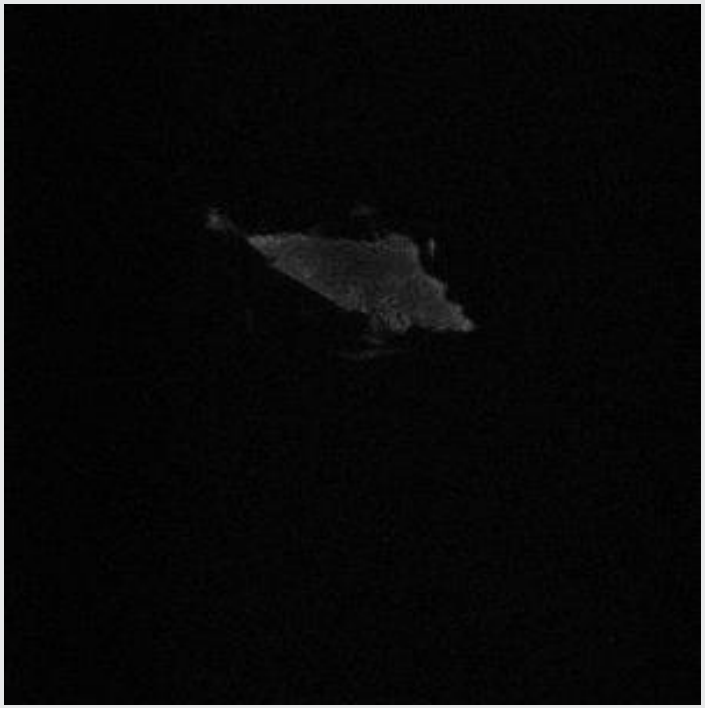
H&E Histopathology



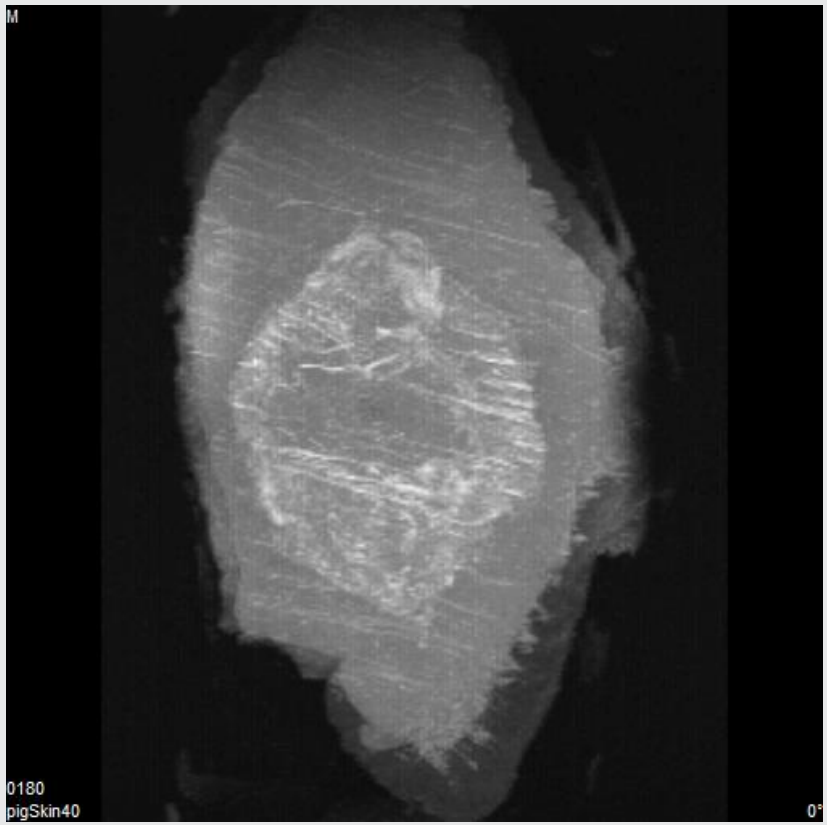
- Blue = Multifocal areas of fat necrosis & associated inflammation
- Red = Normal adipose tissue

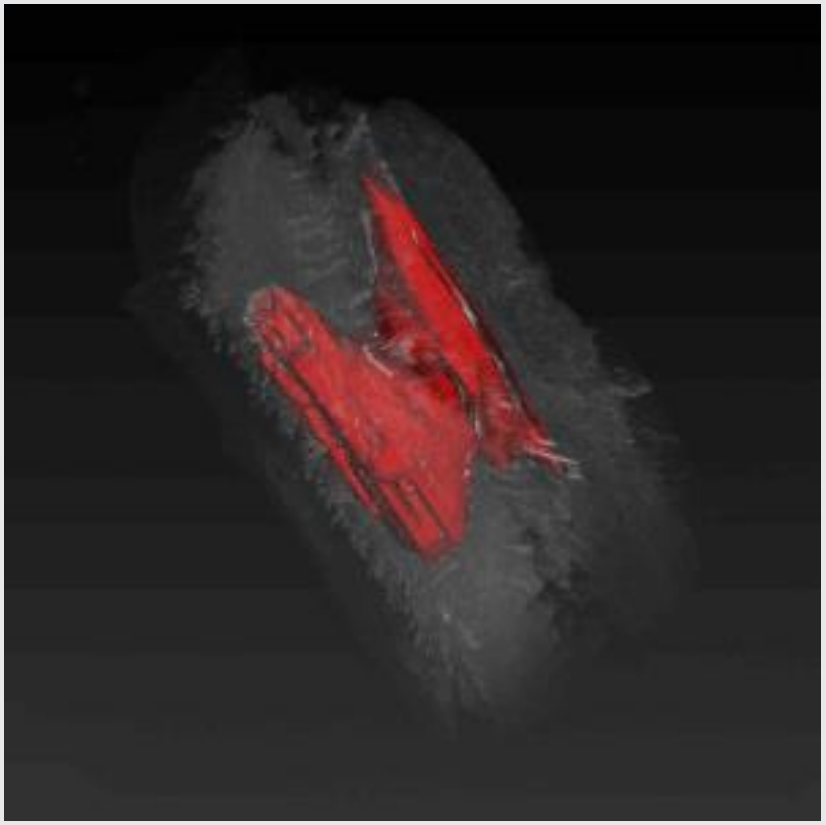
Subcutaneous Drug Injection Into Pig Skin - *Ex Vivo* MRI





Segmentation and Quantification of Affected Volume - *Ex Vivo* MRI





Affected Volume **2200 mm^3**

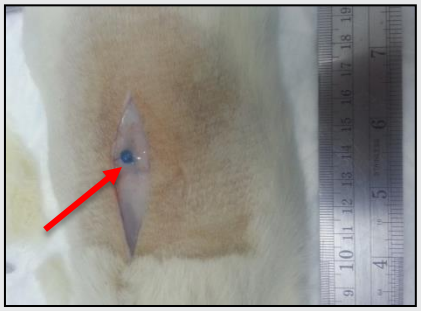
Summary & Comment

- *Ex vivo* MRI was effective in identifying the location and quantifying the extent of subcutaneous necrosis and inflammation caused by different formulations.

- Applying this method on fixed tissues samples derived from different dose formulations provides a quantitative determination of relative irritancy of different injected formulations.

Biodegradable Implanted Device

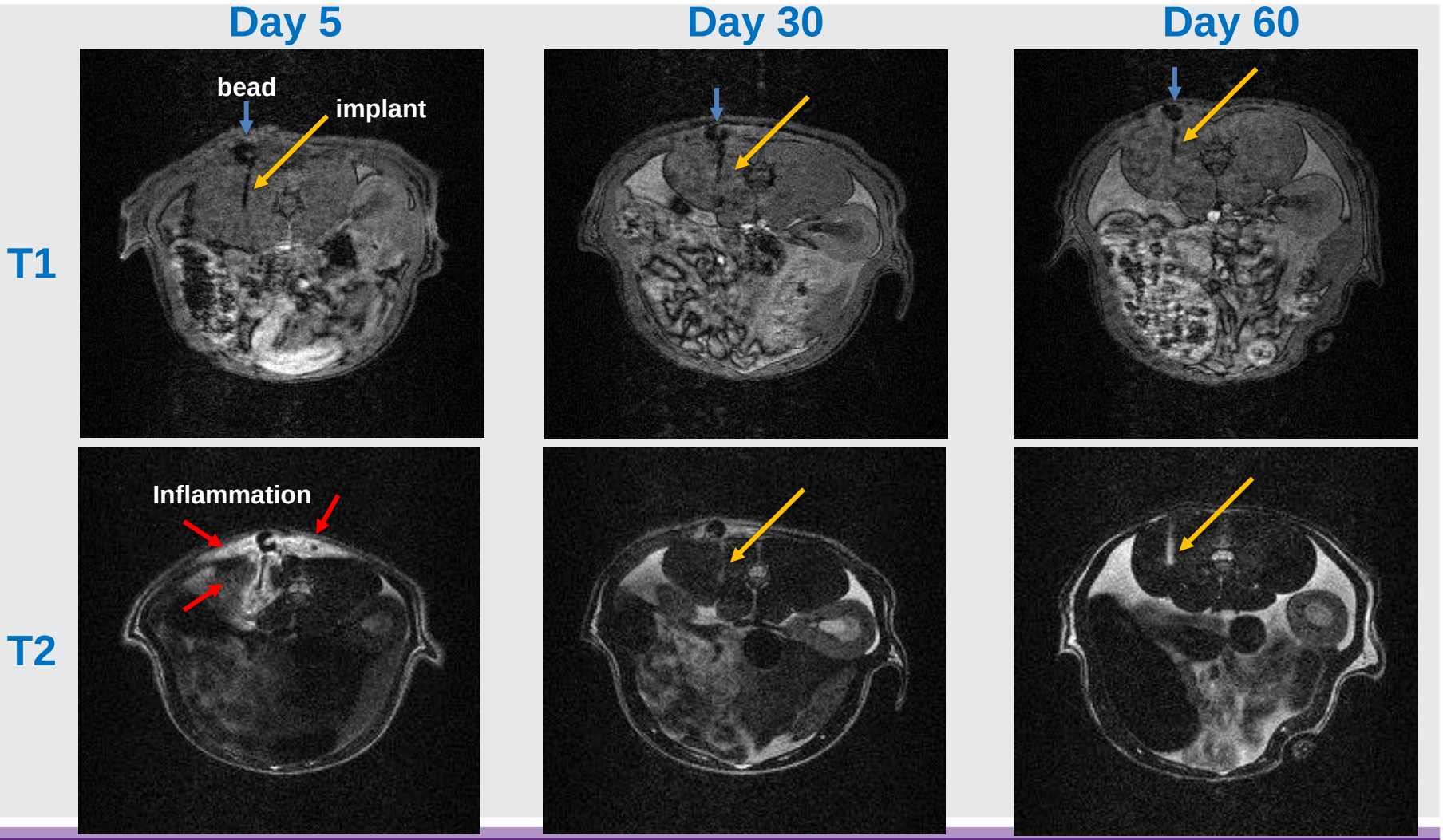
- **Model:** A double layer of a 5x5 mm^2 device was implanted in the right paralumbar muscle of Sprague Dawley rats.



- A plastic bead was implanted subcutaneously just over the device to enable accurate localization and follow-up of the implantation site.

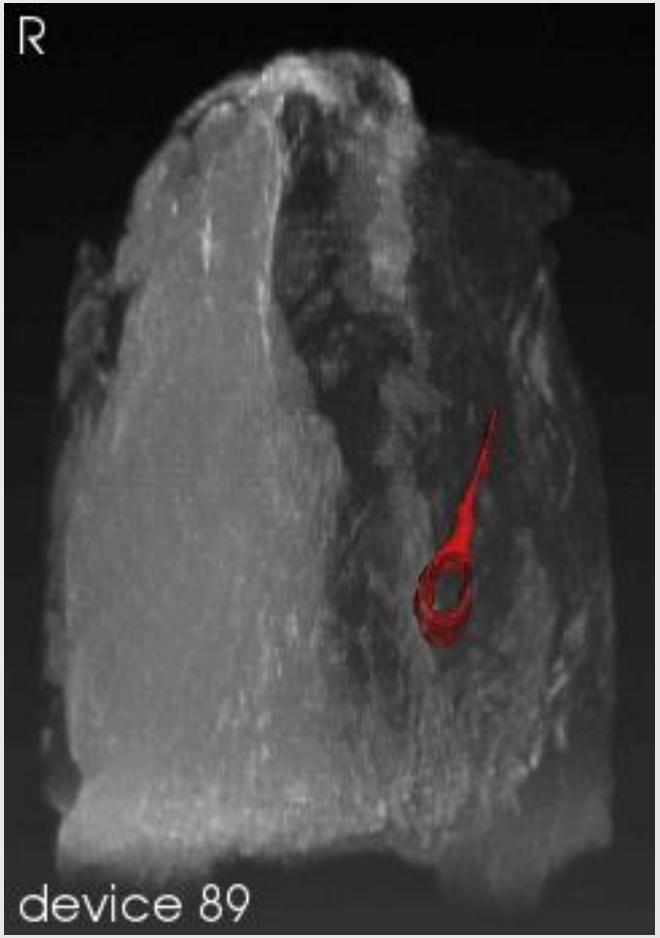
- **Objective:** Evaluation of *in vivo* MRI as a tool for assessment of degradation of a bio-degradable device.

In Vivo MRI of Implanted Device



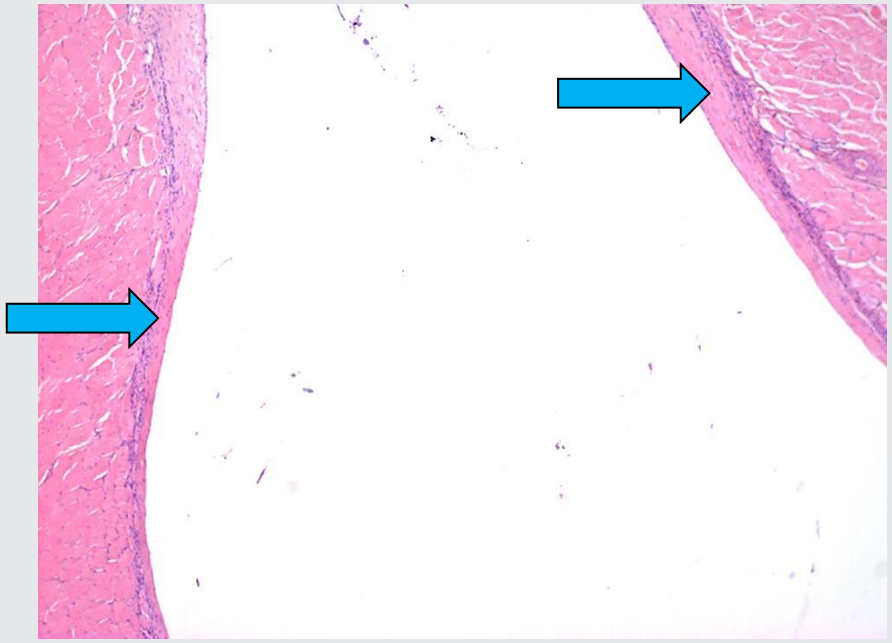
aspectimaging

Ex Vivo MRI of Implanted Device Segmentation and Quantification



Volume of device: **32.2 mm^3** at day 60

Histopathology of Implantation Site After 60 Days



mature connective tissue capsule

cavity of device

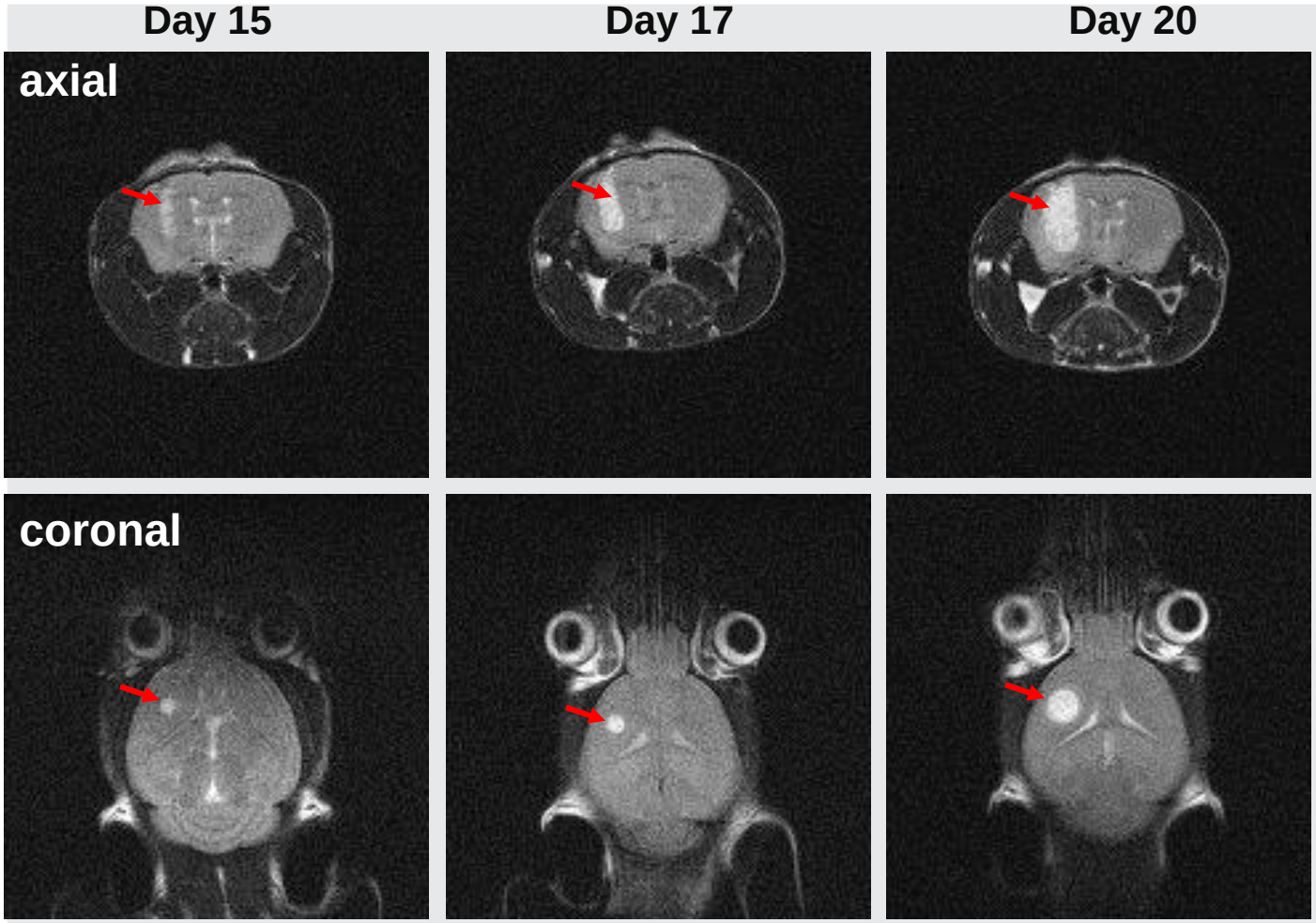
Longitudinal Growth of a Brain Tumor

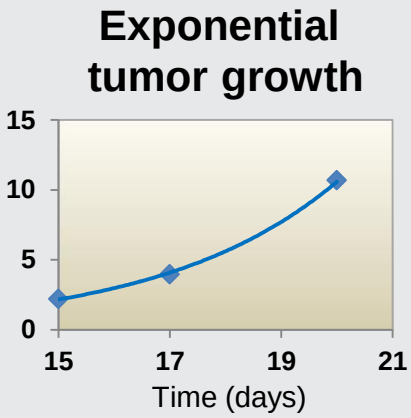
- **Model:** GI-261 glioma cells stereotactically injected into the right brain hemisphere of CB6F1 mice

- **Objective:** Longitudinal evaluation of tumor growth

Longitudinal Evaluation of Tumor Growth

In Vivo MRI

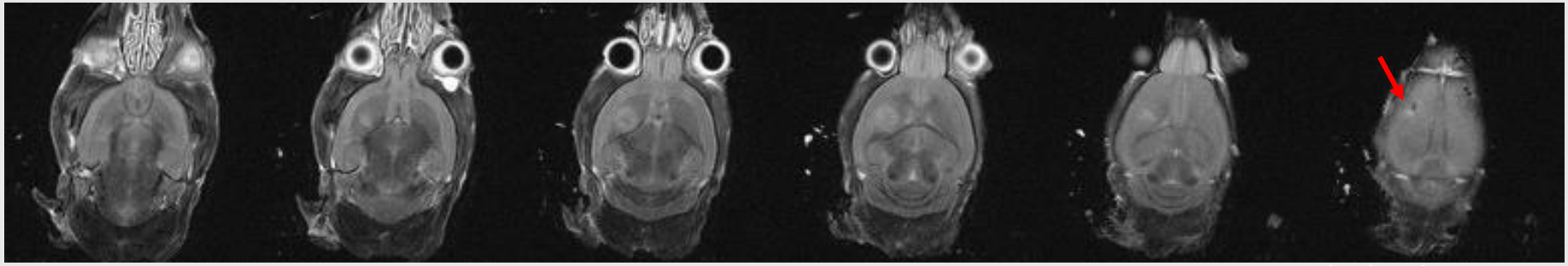




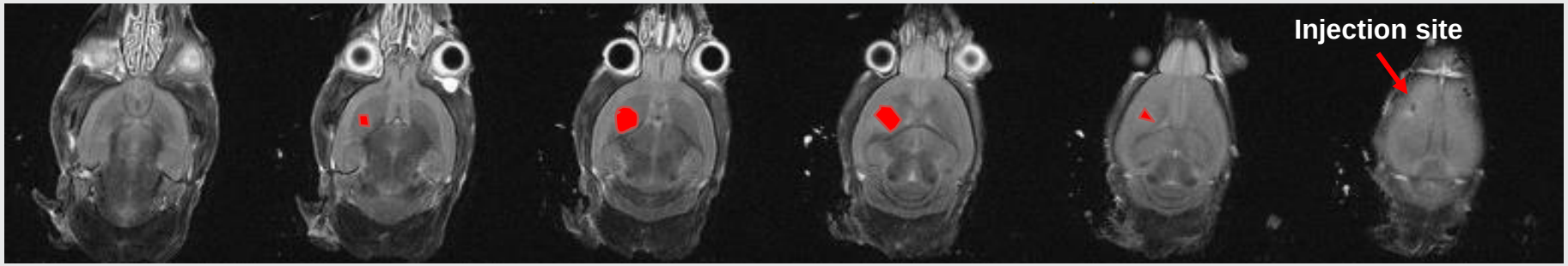
resolution 156 μm; slice thickness 1 mm; acquisition time 13 min

Tumor Segmentation – *Ex Vivo* MRI

Day 17 – coronal



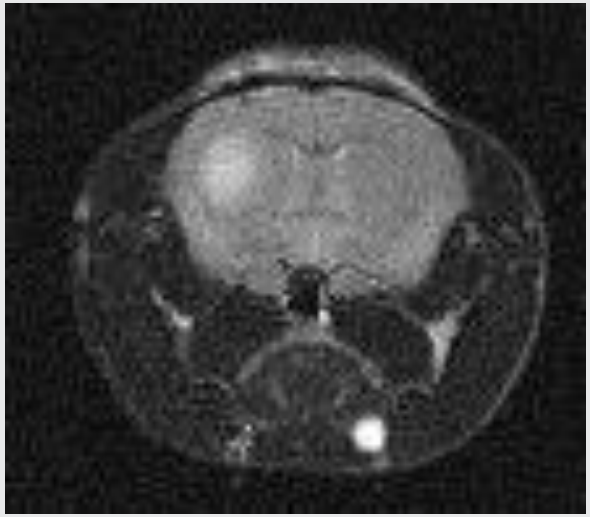
Injection site

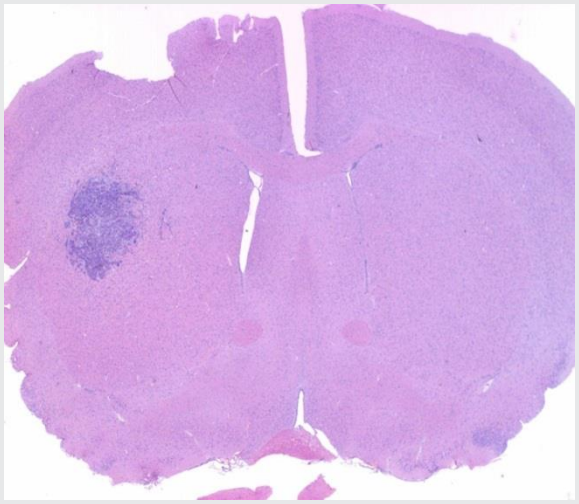


Tumor volume 6.6 mm^3

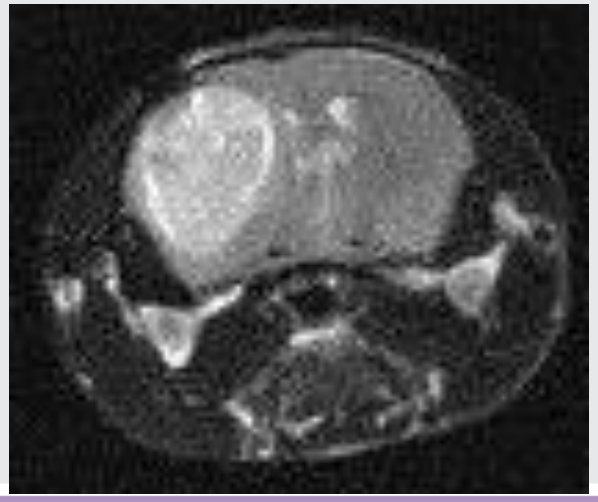
Ex Vivo MRI vs Histology

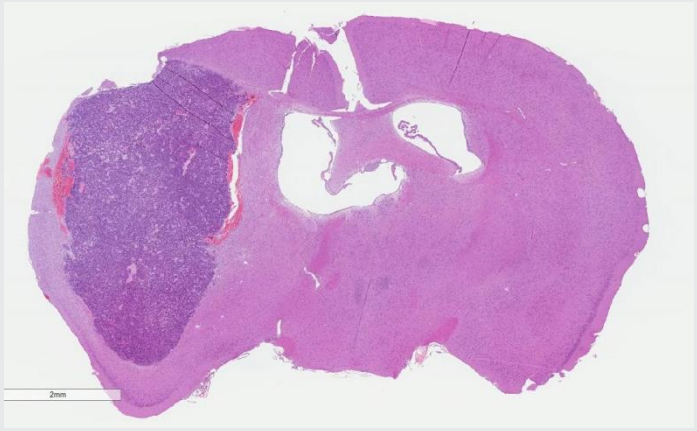
Day 15





Day 20





Summary & Comment

- *In-vivo* and *ex-vivo* MRI evaluation provided a way to follow the time-related growth of an induced tumor in the brain and to determine the volume of the tumor.

- This model demonstrates the utility of using MRI for longitudinal studies and would be useful for testing the efficacy of anti-cancer drugs.

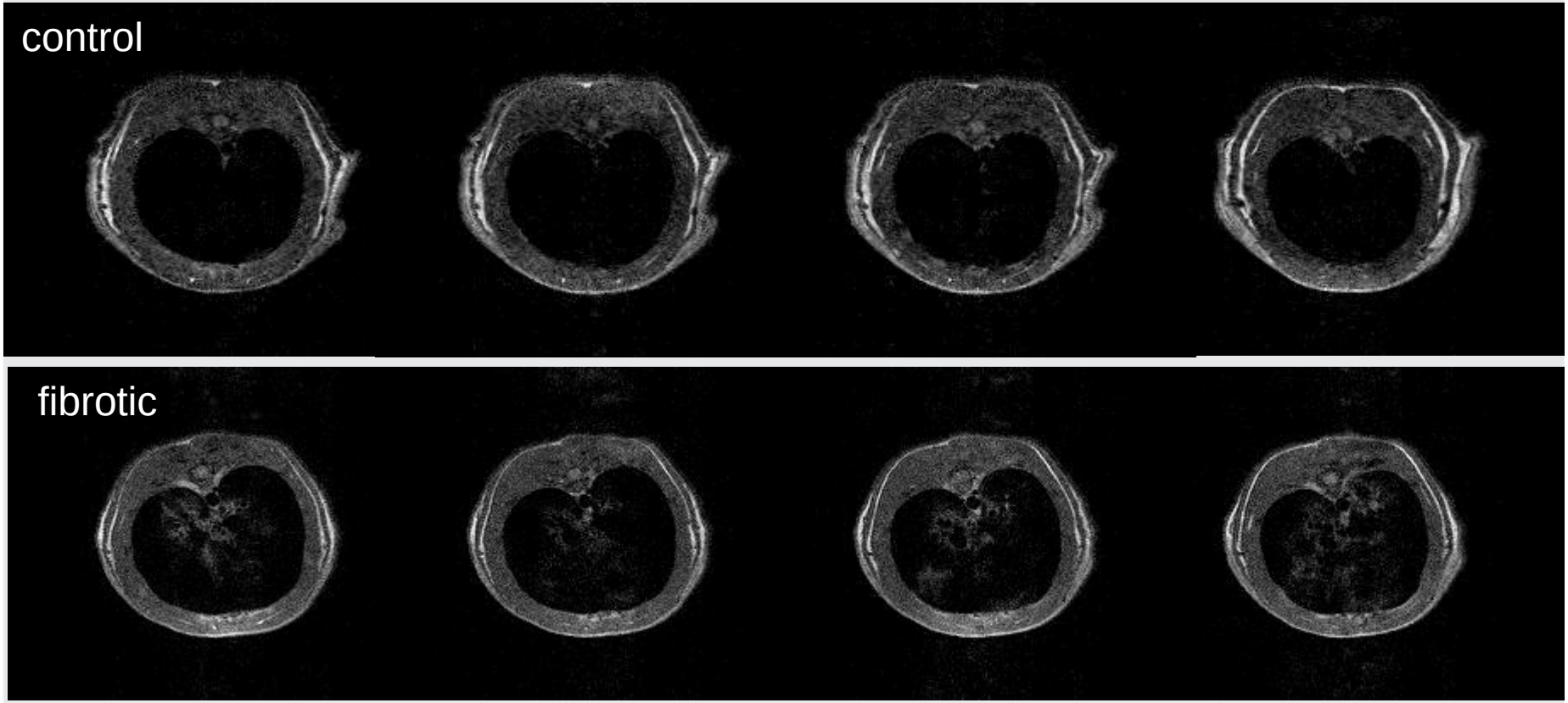
Rat Lung Fibrosis

- **Model:** Single intratracheal instillation of bleomycin into 6 week-old Sprague Dawley rats

- **Objective:** Monitor lung fibrosis in rats using *in vivo* and *ex vivo* MRI as a tool for following temporal progression of the pathological process

Rat Lung Control vs. Fibrosis
In Vivo MRI

Day 11 Post Instillation

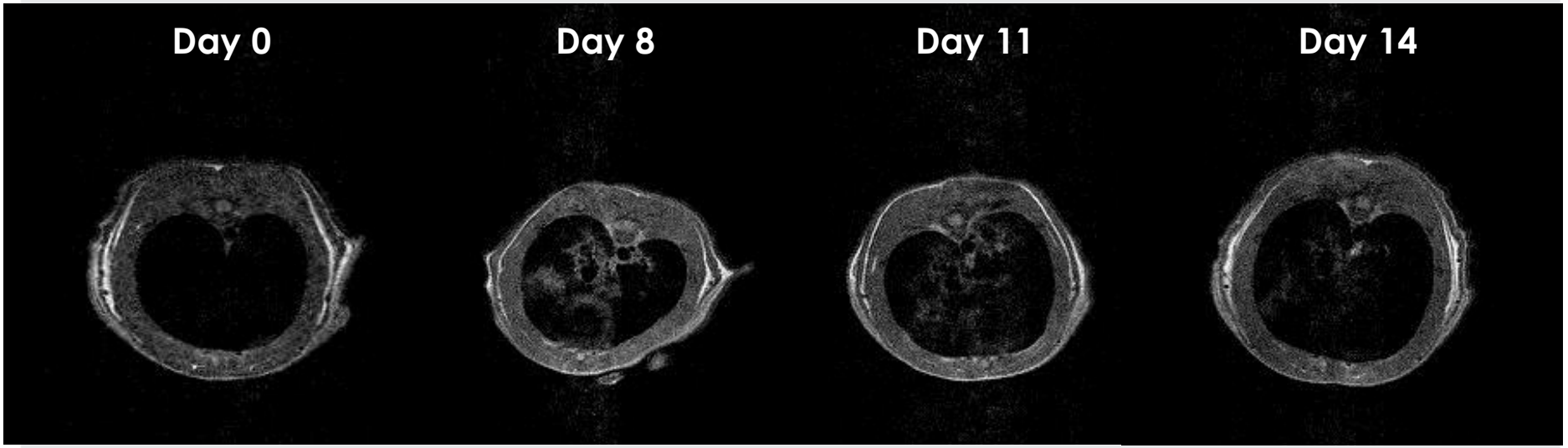


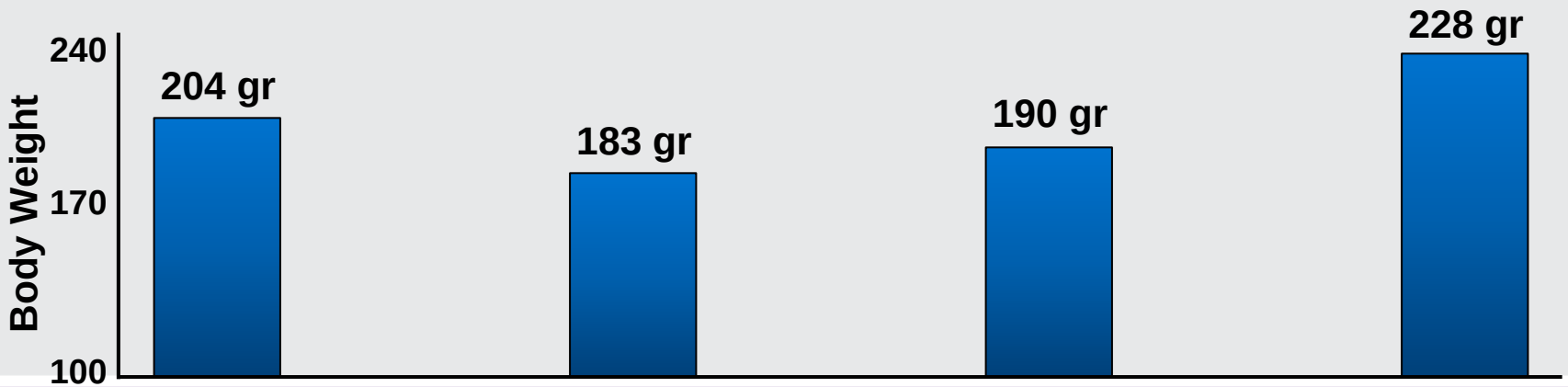
resolution 274 μm; slice thickness 1.2 mm; acquisition time 4.5 min

aspectimaging

Time Course of Disease

In Vivo MRI

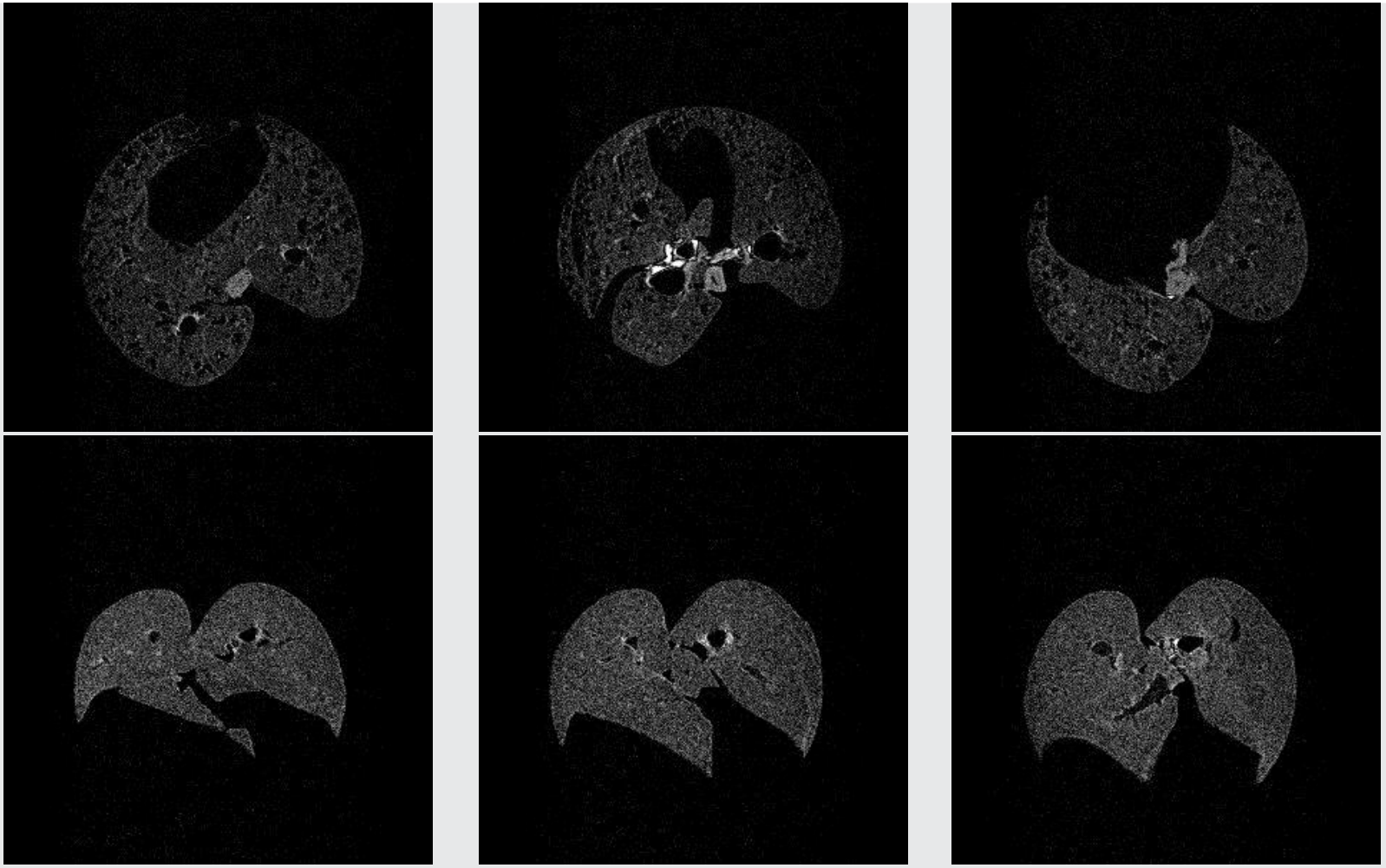




Control rat lungs inflated with air vs instilled in formalin (the ones instilled with formalin are a bit brighter)

control air

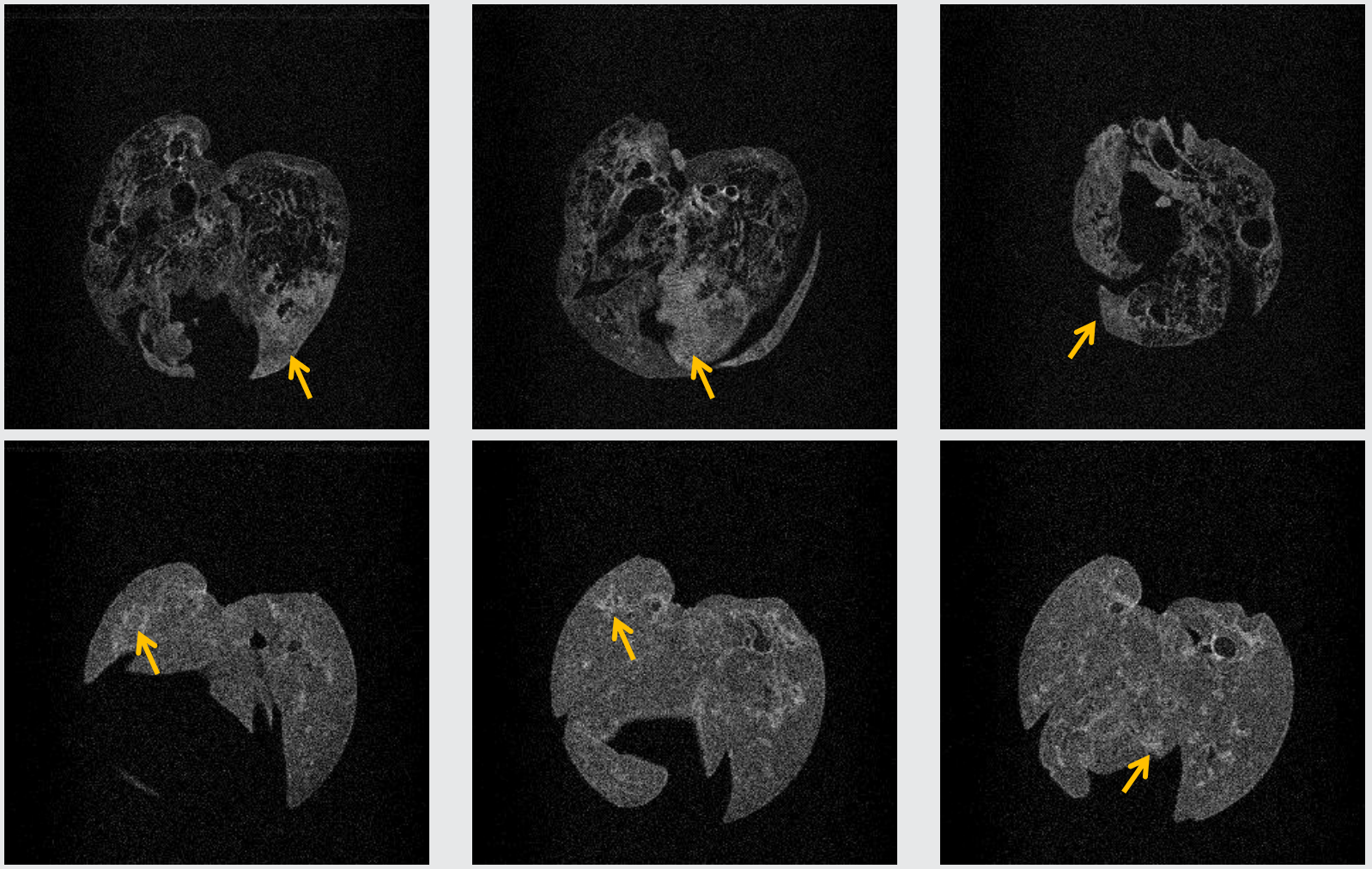
control formalin



BLM rat lungs inflated with air vs instilled in formalin (fibrosis in "air" lungs is more visible on the darker background)

BLM air

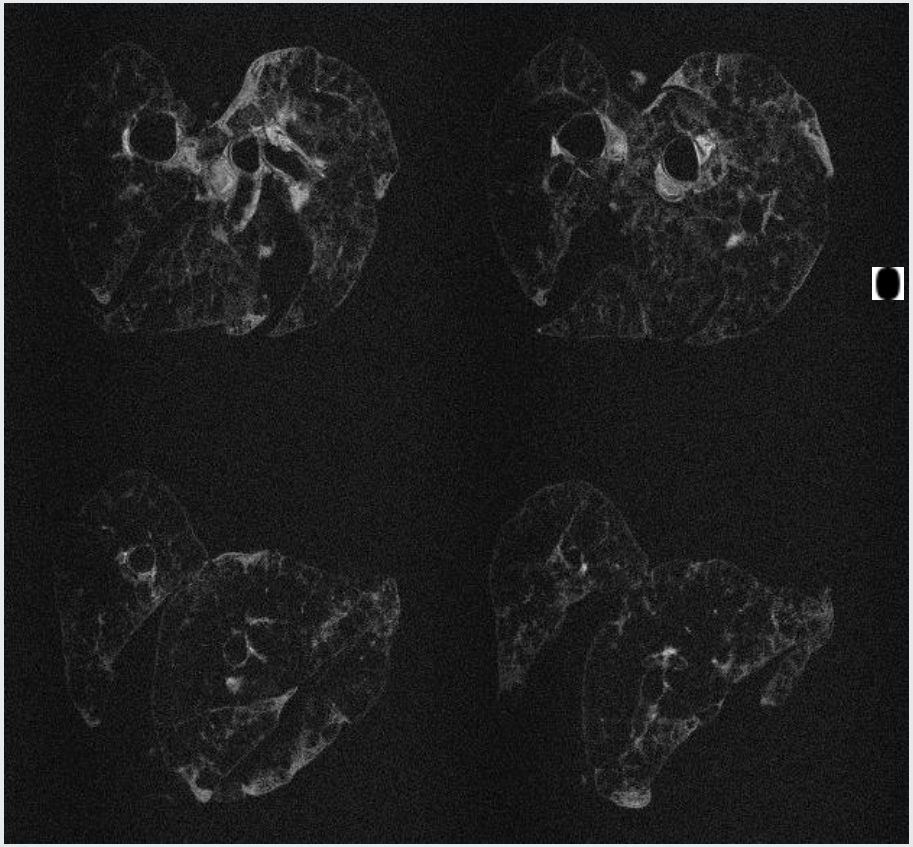
BLM formalin



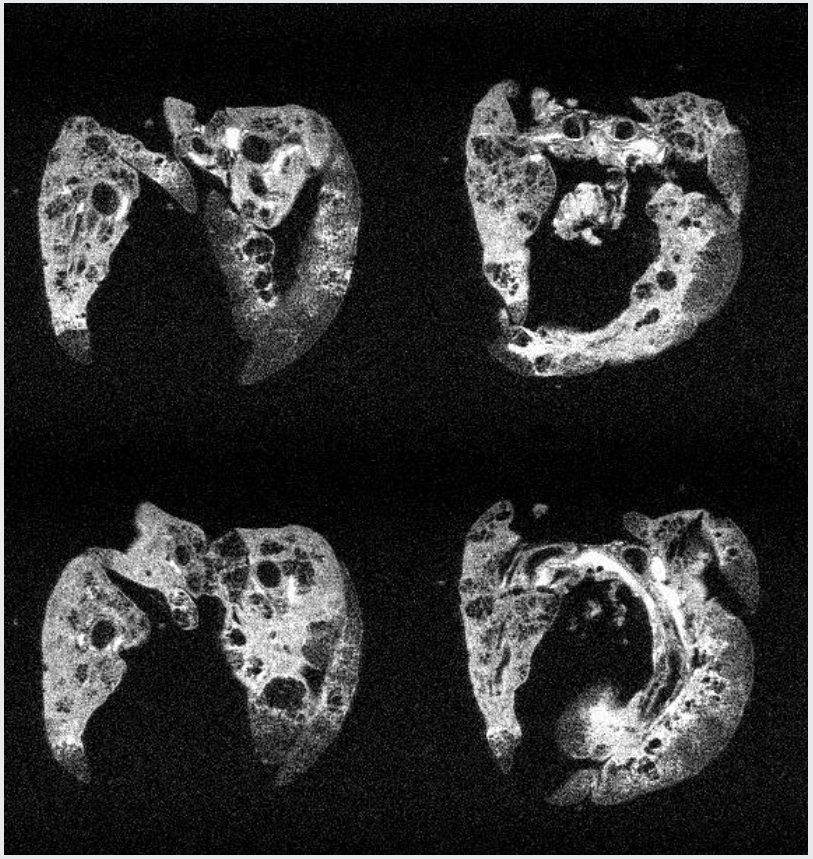
Control vs. Fibrosis – *Ex Vivo* MRI

Day 11 Post Instillation

Control



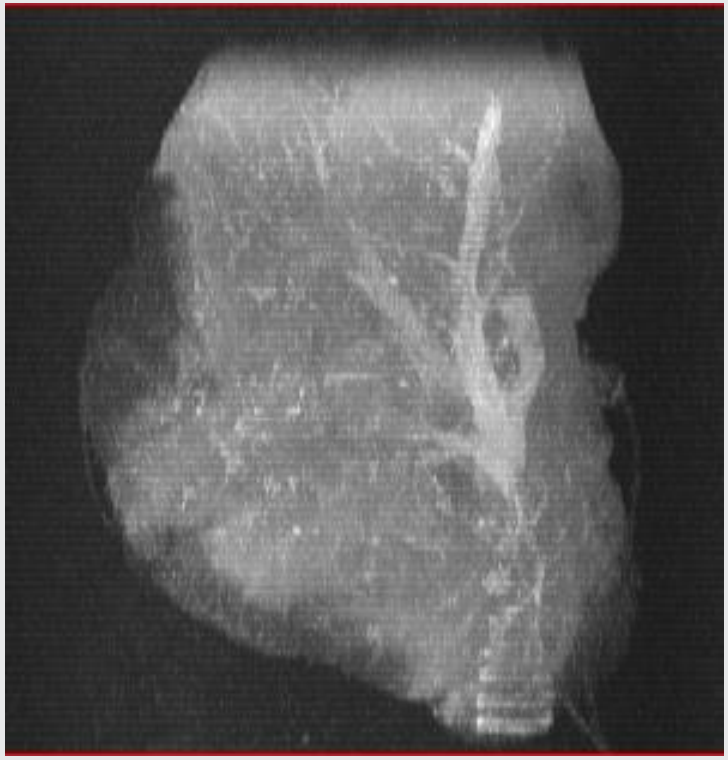
Fibrosis



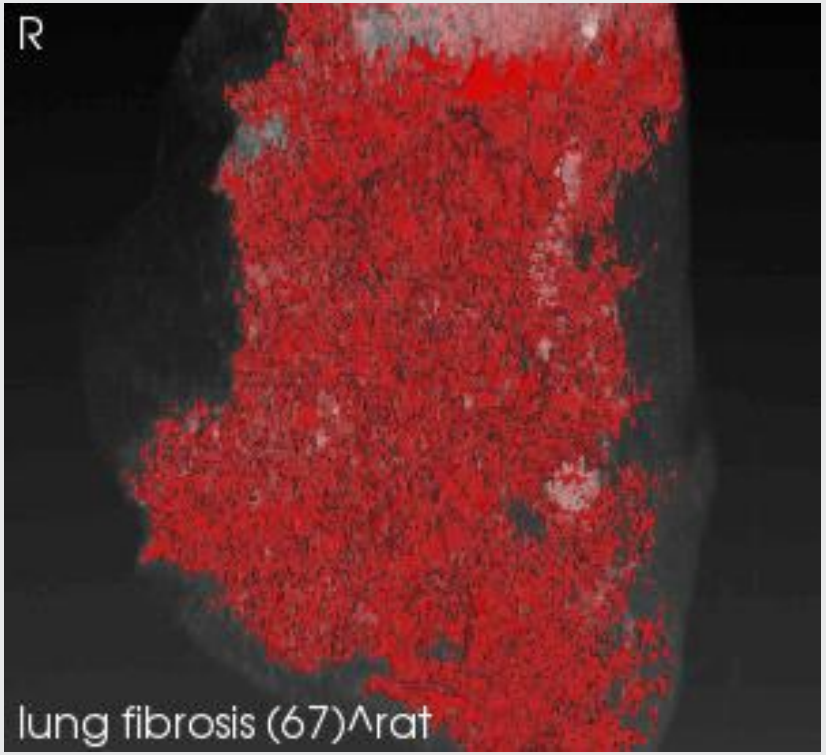
Fibrotic Rat Lung – Volume Quantification Based on *Ex Vivo* MRI

Day 11 Post Instillation

3D rendering

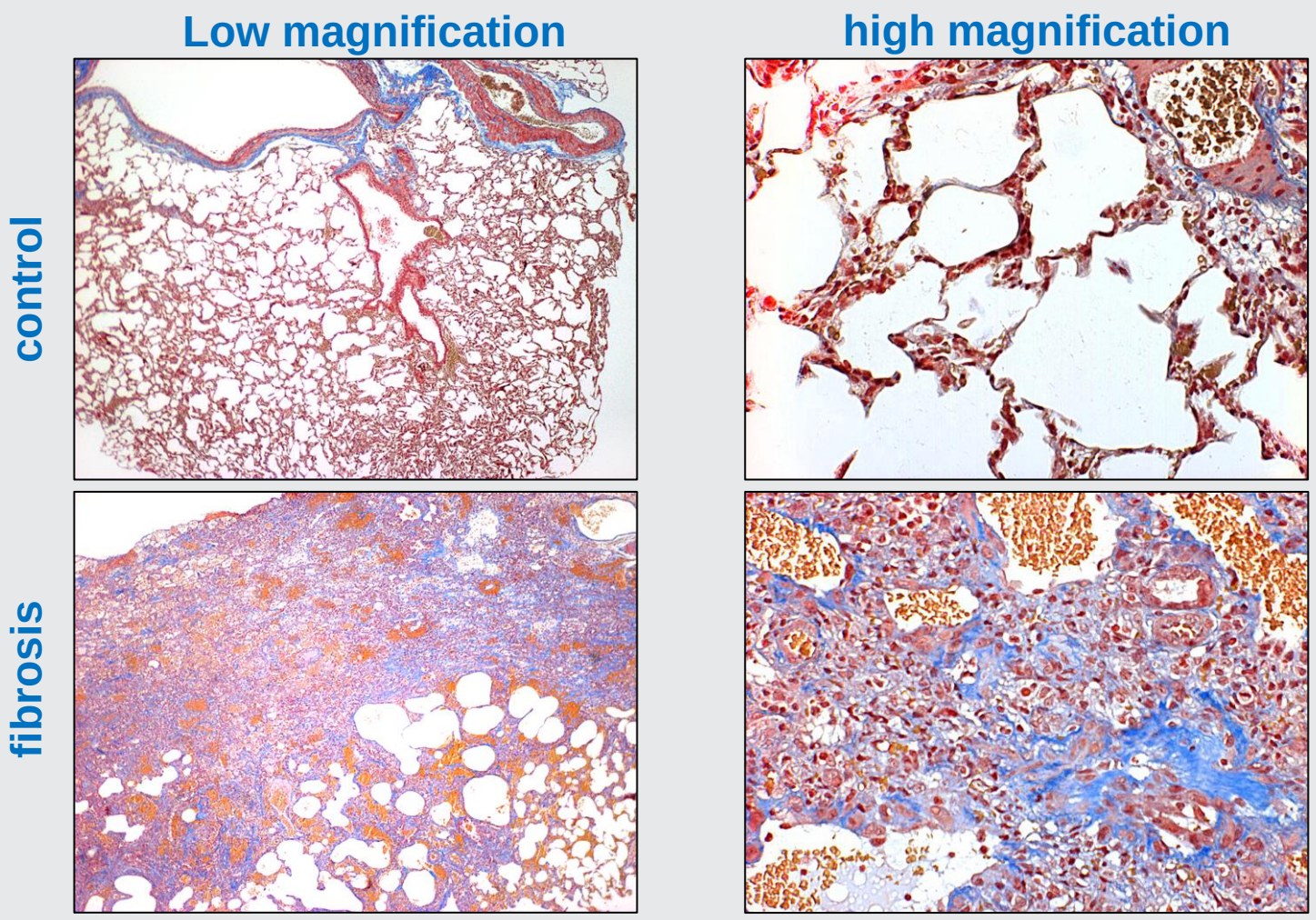


3D rendering + segmentation



Connective Tissue: **1267 mm^3**
Normal Tissue: 1396 mm^3

Histology – Masson's Trichrome



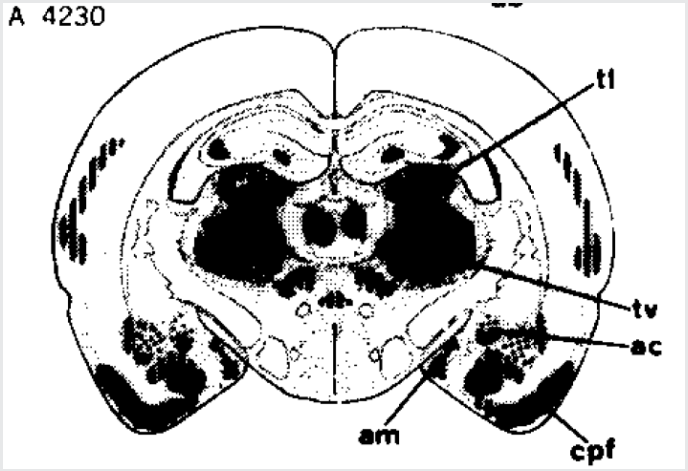
Summary & Comment

- *In vivo* MRI provides a longitudinal evaluation of pulmonary disease progression and regression
- *Ex vivo* MRI in combination with histology provides a quantitative assessment of the components of the interstitial thickening
- Based on the ability to quantify the extent of disease, different therapeutic modalities can be compared for their effectiveness

Pilocarpine- Induced Status Epilepticus

- **Model:** SD male rats treated with LiCl followed by Pilocarpine, a muscarinic cholinergic agonist and accepted model to induce status epilepticus and morphologic damage in rat brain.

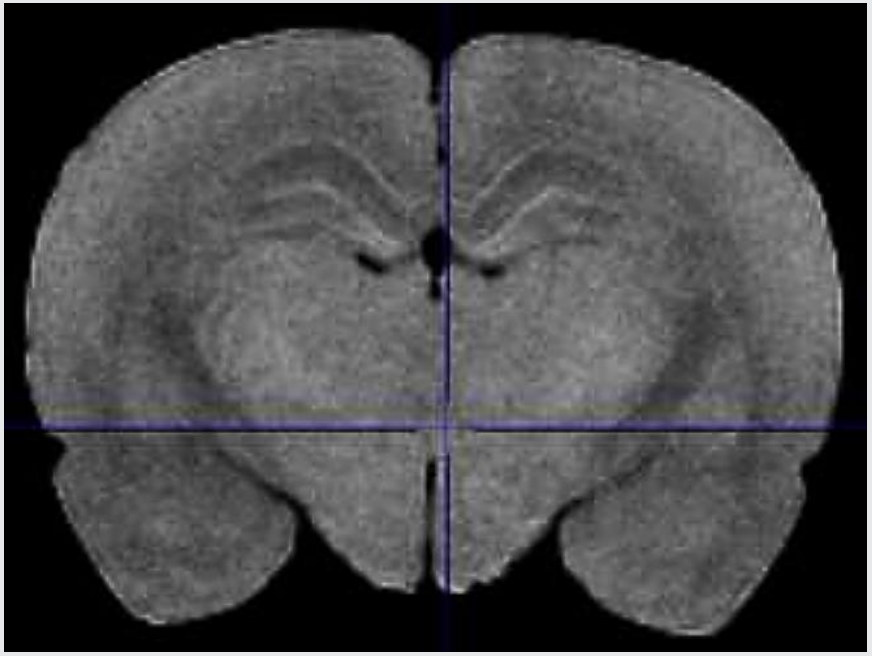
- **Expected outcome**: Neuronal cell degeneration /necrosis



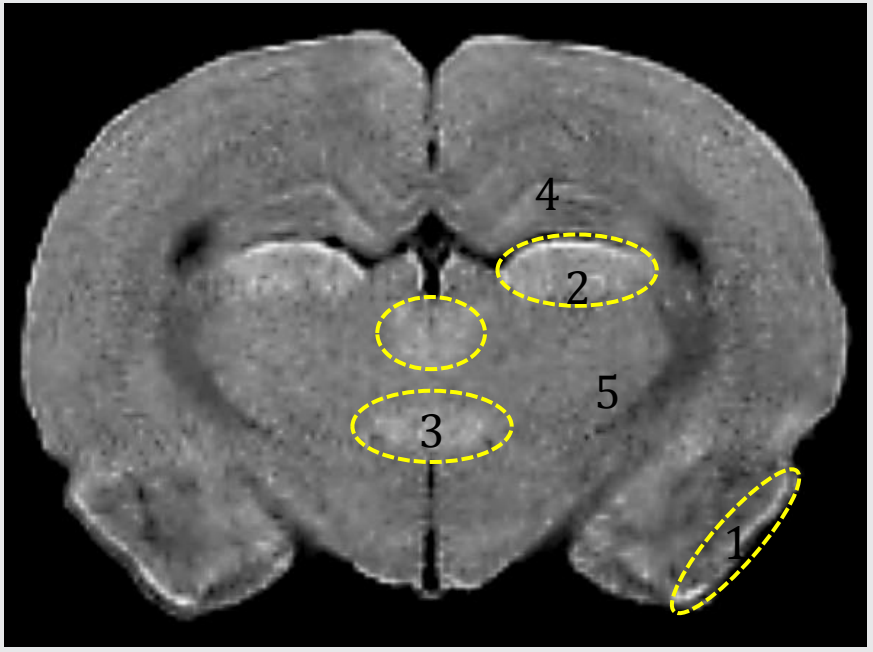
Dark areas : Severe
Hatched areas : Moderate
Dotted areas : Slight

Control vs Pilocarpine
Ex vivo MRI (T1)

control



Pilocarpine

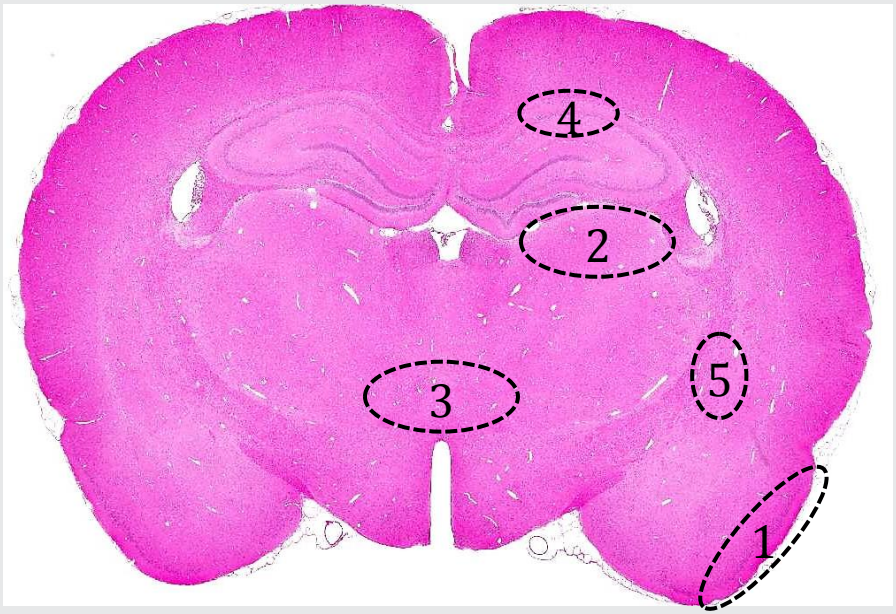


1: Piriform cortex
2: Lateral thalamic nucleus
3: Posterior hypothalamic nucleus
4: Hippocampus
5: Caudate putamen

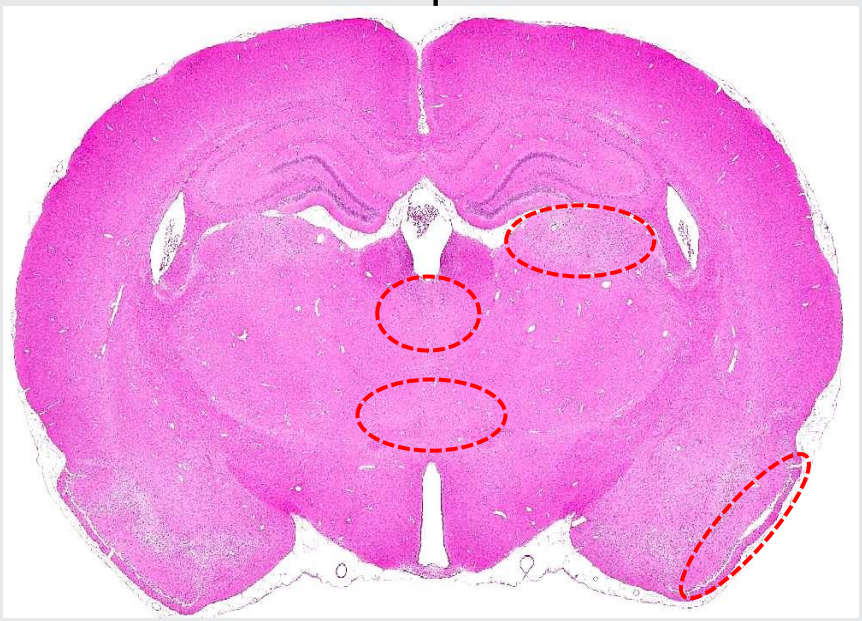
aspectimaging

Control vs Pilocarpine – H&E

control



Pilocarpine

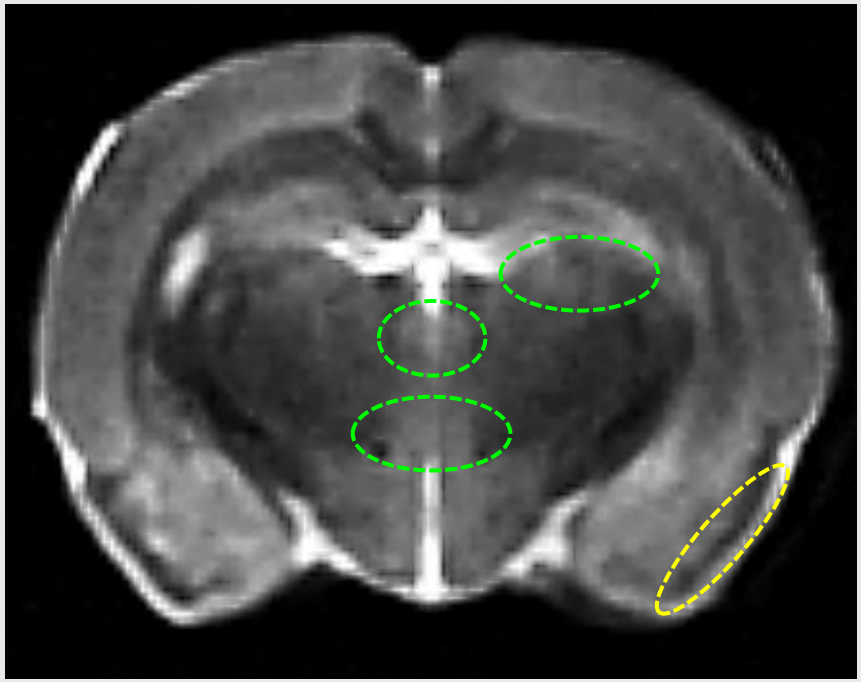


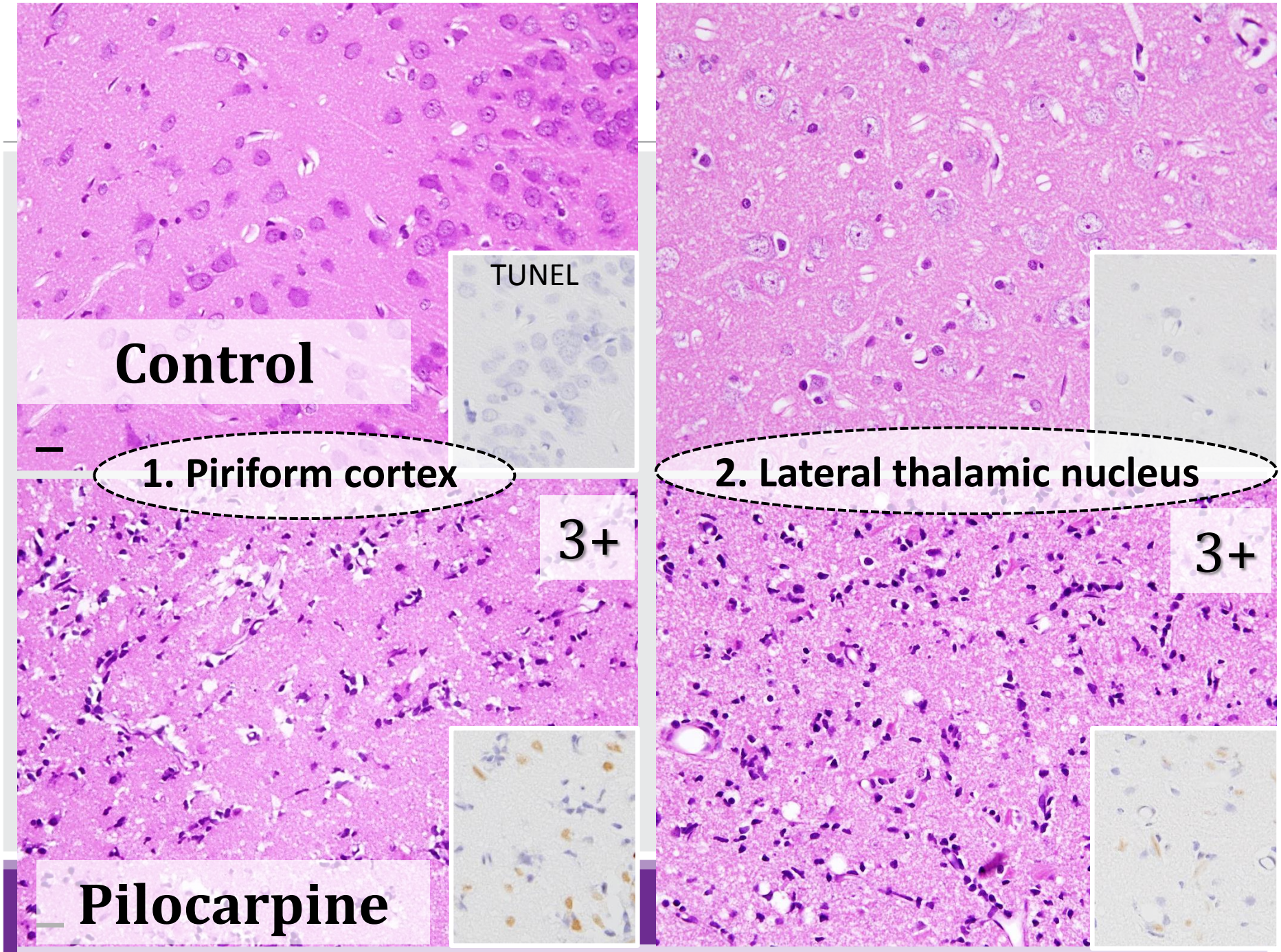
1: Piriform cortex
2: Lateral thalamic nucleus
3: Posterior hypothalamic nucleus
4: Hippocampus
5: Caudate putamen

Control vs Pilocarpine
Ex vivo MRI (T2)

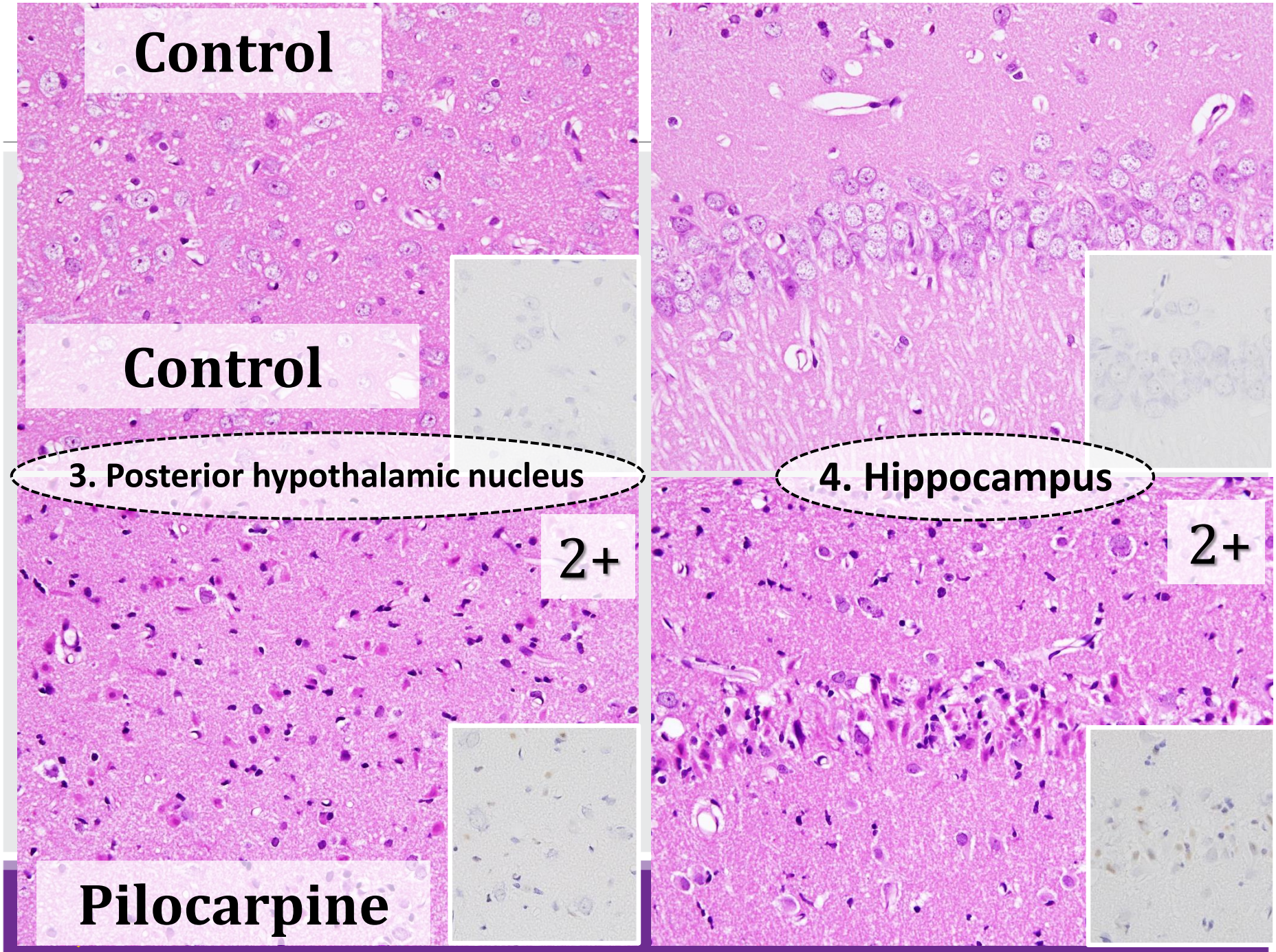
control

Pilocarpine





Bars = 20 μm Pathologic Grade ; 1+ : Slight, 2+ : Moderate, 3+ : Marked

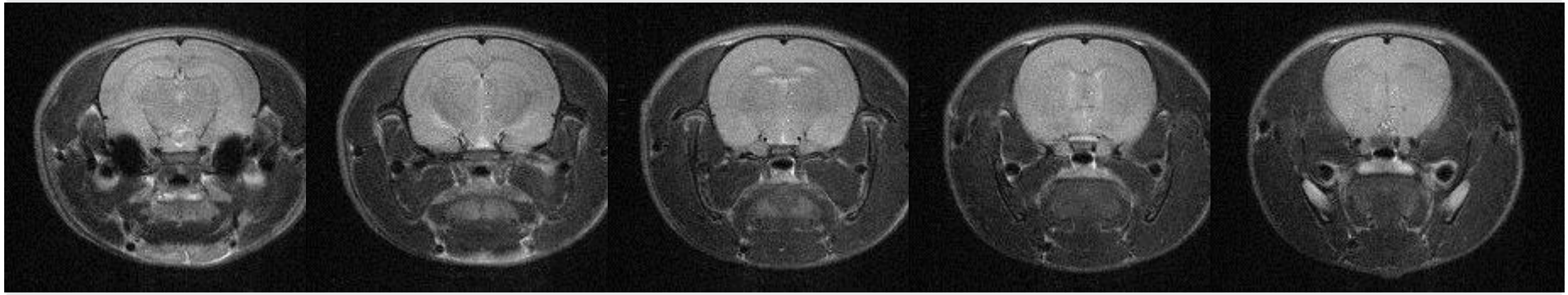

Control
Control
3. Posterior hypothalamic nucleus
2+
Pilocarpine
4. Hippocampus
2+

Summary

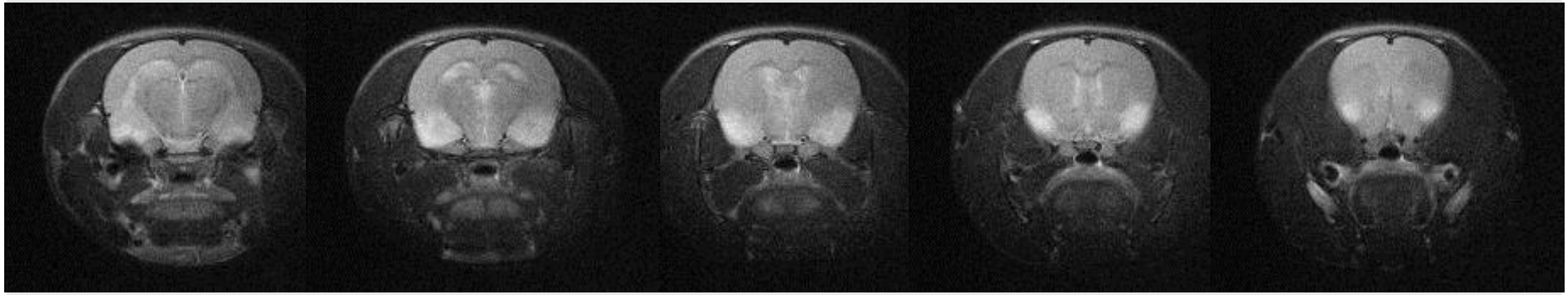
- MRI imaging demonstrated areas of high T1 and low T2 signals compared to controls in the piriform cortex, lateral thalamic nucleus, posterior paraventricular thalamic nucleus, and posterior hypothalamic nucleus of the cerebrum.
- Histopathology showed , neuronal cell degeneration and necrosis accompanied by gliosis in these areas.
- MRI analysis of fixed organs before routine slide preparation could provide useful information for histopathologic evaluation in preclinical toxicity studies

Neurotoxicity induced with Kainic acid

Base line



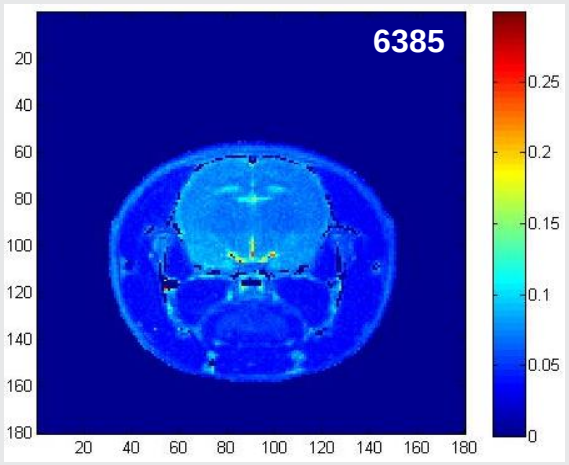
3rd day

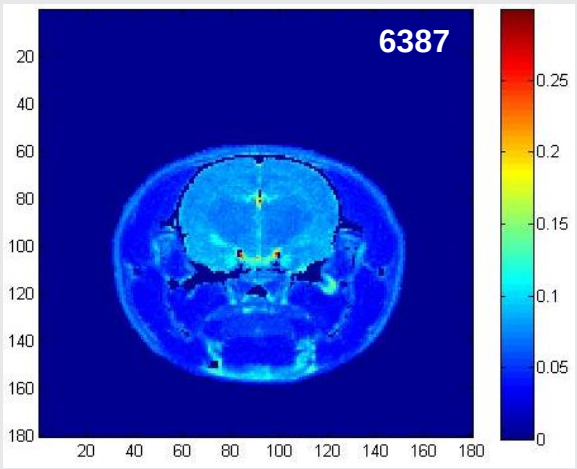


aspectimaging

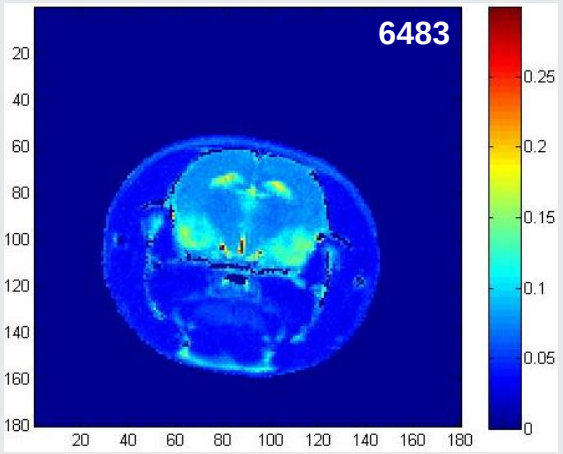
T2 maps following treatment with Kainic acid

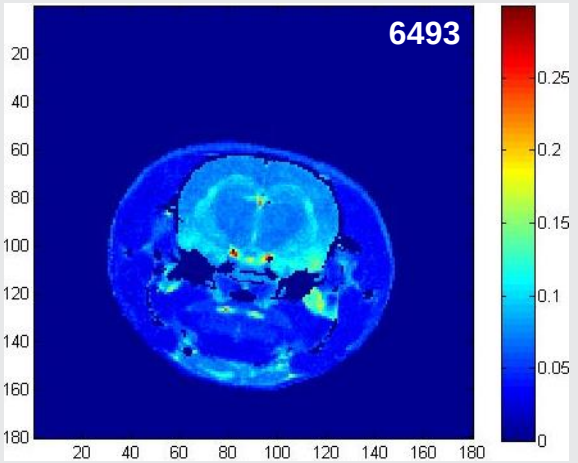
Baseline:



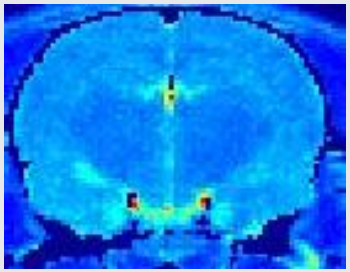
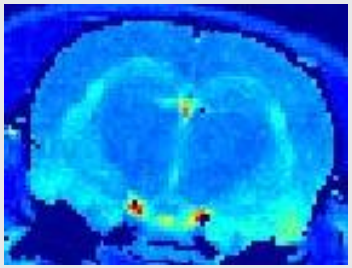
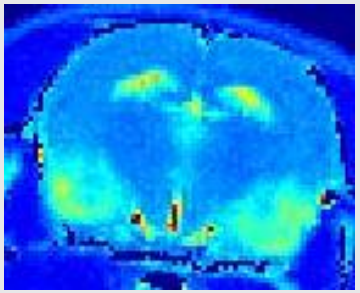
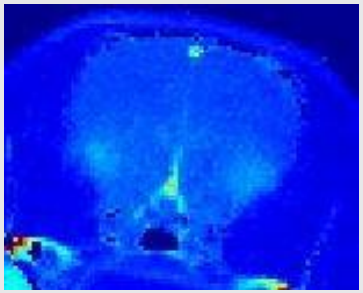
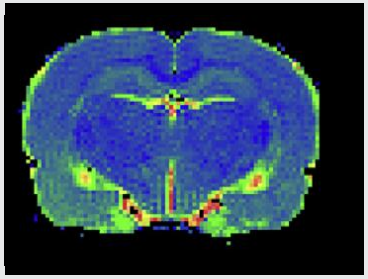
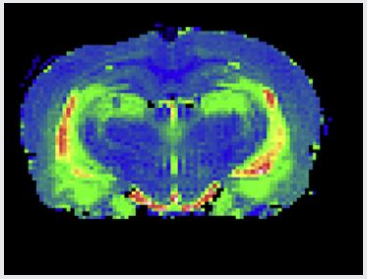
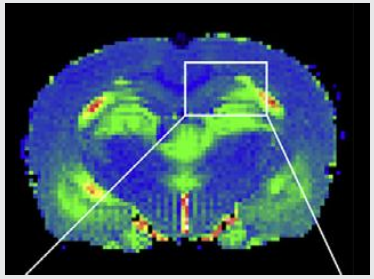
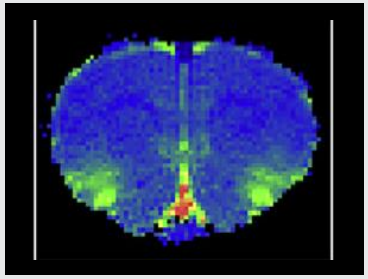


3rd day



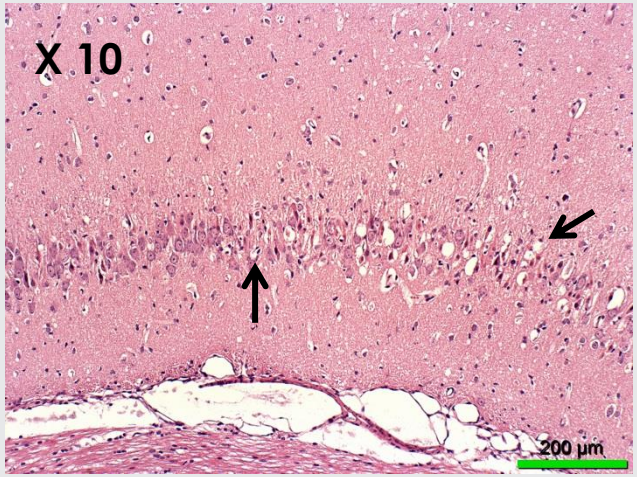


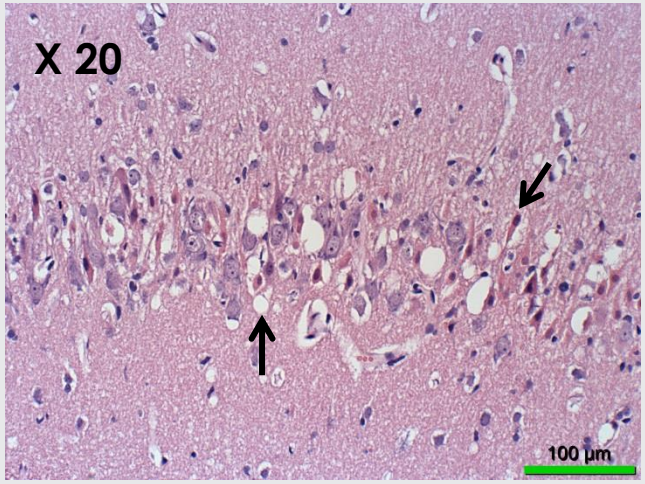
T2 maps following Kainic acid : 1T comparable with 7T

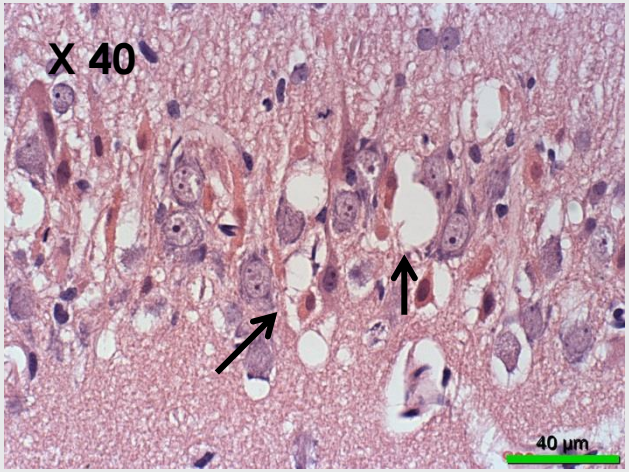
	control	KA	KA	KA
1 Tesla				
	control	edema	neurodegeneration In hippocampus	edema in insular cortex
7 Tesla				

Note the disarrayed cellular layer in the CA3 region of the hippocampus, suggestive of neurodegeneration even in the absence of silver deposition.

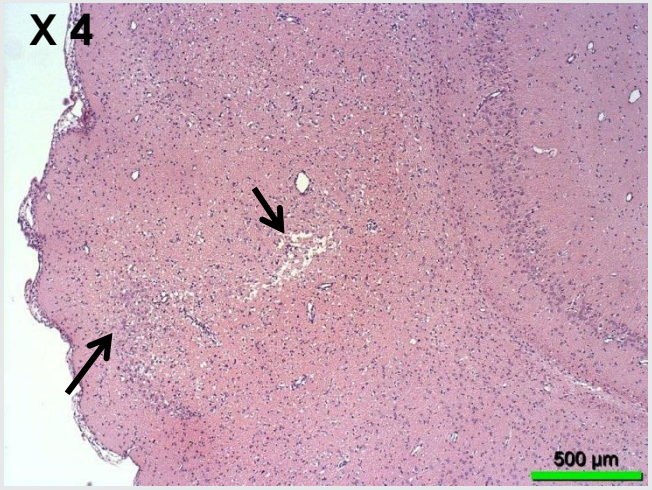
Neuronal degeneration, necrosis and vacuolation in hippocampus

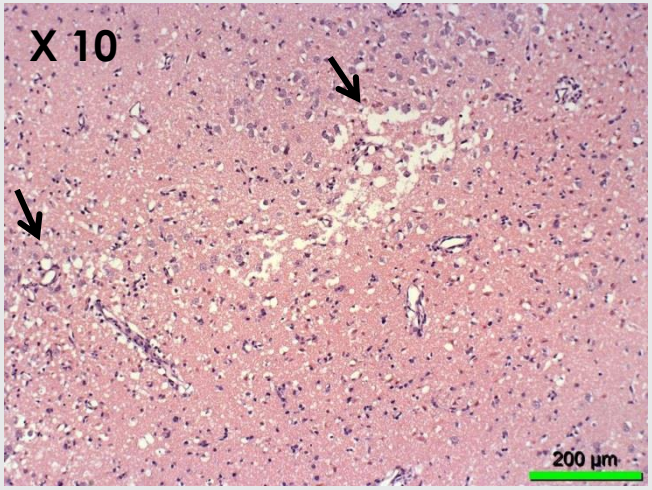


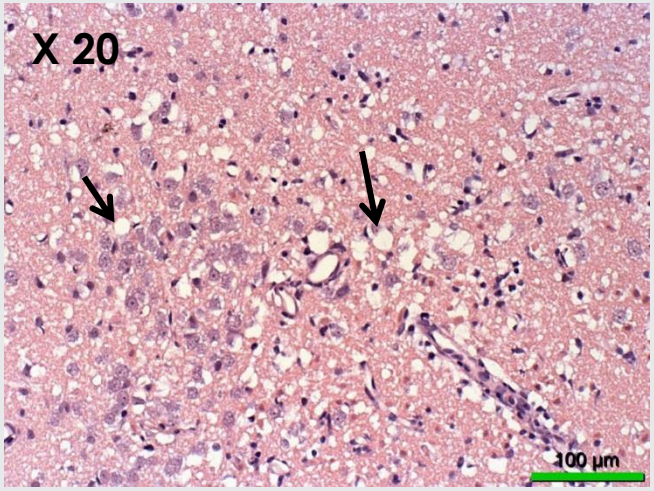


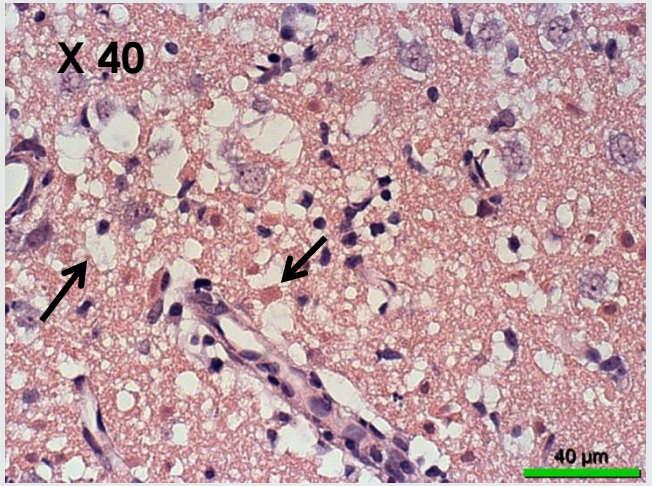


Intramyelinic edema, neuronal necrosis and gliosis in amygdaloid nuclei region









Validation study performed by the FDA regarding the use of MRI in neurotoxicity studies

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The use of MRI to assist the section selections for classical pathology assessment of neurotoxicity

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ARTICLE INFO ABSTRACT

Conclusions: "collect Smart sections…". "..The application of full brain MRI imaging that informs neuropathology offers the potential to dramatically improve detection of neurotoxicity produced by new drugs and facilitate new drug development, review and approval processes, and to qualify an imaging biomarker of neuropathology."

imaging biomarker of neuropathology.

MRI-based Histology- **Smart Sections** Added Value for Lesion Evaluation

- Localize the lesions
- Count the lesions
- Measure lesions volume
- Longitudinal in-vivo follow-up in the same animal
- Information about homogeneity of the lesions

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