WEBINAR: Expanding Preclinical and Histopathology Capabilities with MRI Technology: A Useful Tool for Preclinical Safety and Efficacy Evaluation

Questions and answers from the April 16, 2019 webinar titled "Expanding Preclinical and Histopathology Capabilities with MRI Technology: A Useful Tool for Preclinical Safety and Efficacy Evaluation"

This document includes questions we received and answered during the webinar, as well as those that we did not have time to address. Questions have been grouped into relevant categories.

Magnetic Resonance Histology (MRH) Centric Questions

1. **You mentioned in your seminar that the tissues are transferred to Fluorinert for the MR scanning. Do you envision any damage to the quality of the histology sections used at a later stage for histological validation?**

   From Dr. Nyska's experience the use of Fluorinert to do the scanning on formalin fixed samples has not affected the use of the samples for later conventional histological analysis. Once the MRH scan is complete the samples are returned to 10% formalin for further processing.

2. **How do you immobilize *ex vivo* lungs in Fluorinet to prevent motion during MR imaging?**

   The lungs, along with other fixed tissue samples are typically scanned using an imaging cassette designed specifically for use with the automated sample handling system of the M-Series histology add-on. The imaging cassette has soft flaps within it to help stabilize the fixed tissue sample during imaging.

3. **Are MRI contrast agents used for conventional MR histology, and if not, could contrast agents be used if desirable?**

   The MRH images presented by Dr. Nyska during the webinar did not use any MRI contrast agents, the appearance of the tissue was entirely due to the way in which the image was acquired.

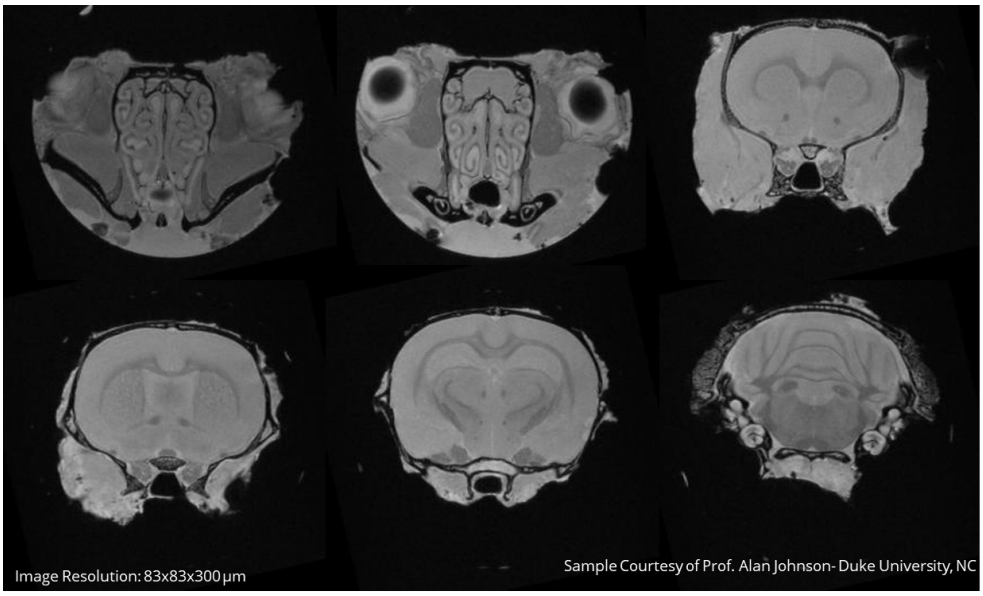
We expanded on this a little bit during the discussion regarding the difference between T1 and T2 weighted images. This is simply a way to acquire images using a specific set of parameters for a pulse sequence. On the M-Series systems there are pre-defined imagine sequences available for those who are less familiar with the physics of MRI, allowing them to acquire very good images without the need to optimize the sequence parameters.
In general, on a T1 weighted image the fat will appear bright, while on a T2 weighted image the water will appear bright. All other tissues will appear as shades of grey depending on their composition. The example of the kidney is a very good one in which the medulla appears quite bright on a T2 weighted image as it contains more water than the cortex for example. Similarly, tumors, inflammation, and many other pathologies appear bright on a T2 weighted image.
Although contrast agents were not used, and are not required to do MRH, you are more than able to use contrast agents if desired to help highlight a specific structure or cell marker in the samples. Commonly used contrast agents include gadolinium and iron oxide.
When using the M-Series systems, as mentioned below, they operate at 1 Tesla. The benefit of working at this field strength is using Gadolinium (Gd) based contrast agents as they show higher sensitivity at lower field strengths than higher ones. This was elegantly shown by Dr. Silvio Aime in a recent publication.

4. **Was there any quantitative validation of MR histology done? What is the sensitivity and specificity of MR histology compared to histopathology?**

   Dr. Nyska refers to the publications relevant to each model he discussed. The links to those publications can be found in the answer to Question #9 below. He routinely does correlation between the MR Histology image and conventional histology.

5. **What is the highest achieved resolution of your MR Histology scans? How long do the scans take to perform?**

   Although not shown during the webinar, the highest resolution images we have seen to date have been on rat whole grain samples scanned *ex vivo*. The in plane resolution was 83x83µm resolution with a slice thickness of 300µm; these images took approximately 2.5 hours to acquire.



Images from Dr. Nyska's webinar ranged in resolution from 270µm in plane resolution with a slice thickness of 1mm, which took 3.5 minutes to acquire; to 117µm in plane resolution with a slice thickness of 0.5mm, which took 56 minutes to acquire.

As discussed during the question and answer session the acquisition time on ex vivo imaging is less important with the automated sample handling as the tissue is fixed and there is no need for intervention or attention from a technician. For in vivo imaging applications acquisition times become more important when taking into account the wellbeing of the animals. The systems do provide inhaled anesthesia, heating support, and physiological monitoring to help support the well-being of the animals and to provide feedback to the operator on how the animal is doing while inside the magnet.

6. **Would it be useful if we could get MRI of paraffin embedded tissue, and is this possible?**

   At this moment Dr. Nyska and the Scintica team do not have any experience in imaging paraffin embedded tissues. The main source of MR signal is from protons within the sample to be imaged, many of them in the form of water. When a tissue sample is dehydrated during the routine steps taken to prepare for embedding the tissue in paraffin, much of the signal is removed.

However, it would appear as though there are some publications in which paraffin embedded tissues have been imaged, so it may be possible, but would require additional testing.

7. **Can you comment on the additional cost of performing MR Histology versus the additional information obtained by using this technique?**

   The cost associated with performing MR Histology using the M-Series system is limited to the cost of purchasing the equipment. No additional infrastructure or shielding is required when installing the system within an existing laboratory space. There is little ongoing operating costs as electricity is only used to operate the electronics during imaging sessions. The system is a permanent magnet, as discussed below, so does not require electricity to create the magnetic field, thus requiring no cooling of the magnet with cryogens or water to prevent quenching. There is really no ongoing maintenance of the system, and associated costs, due to the simplicity of the system.

   The added value of the information provided by MR Histology may be invaluable, especially when considering safety toxicology and efficacy where a specific research group of pharmaceutical company is looking to investigate the effects of a compound on a specific organ such as the lungs for fibrosis, kidney for necrosis, or liver for pre-neoplastic focal lesions. MRH provides an image of the entire organ of interest to detect and quantify lesions. If the number and size of lesions is low these may have been missed using conventional protocols in which a low number of samples are taken from a target organ. The MRH whole organ image can be used to help guide the sectioning of the organ for confirmation of pathology by conventional histology.

   In performing MRH prior to conventional histology one may be able to more confidently evaluate the safety of a target compound prior to its progression along to a clinical trial for efficacy. If a target compound makes it to clinical trials for efficacy and fails due to safety the costs associated with this are immense and far exceed the cost of purchasing a system to perform MRH.

8. **Do you envision MR Histology replacing conventional histopathology in the future?**

   As discussed during the question and answers at the webinar we do not envision this to be the case at all. As Dr. Nyska described, MRH is simply a complimentary technique to be used in conjunction with conventional histopathological examination of tissue. MRH is an efficient tool used to obtain essential information about the entire organ, such as counting lesions, measuring their

size, etc. which is not currently obtained using conventional histological examinations. This information may be used in the safety assessment of a compound, and it may be used to guide the location within the organ or tissue of interest on which further analysis may be performed to confirm the pathological changes observed within the tissue.

Application or Model Specific Questions

9. **Are there any publications or references that relate to the models discussed in today's webinar? Do any of these relate to regulatory aspects of MR Histology?**

   Dr. Nyska and his team have published numerous publications in recent years, please see the listing below:

   - Practical Applications of In Vivo and Ex Vivo MRI in Toxicologic Pathology Using a Novel High-Performance Compact MRI System
   - Compact Magnetic Resonance Imaging Systems – Novel Cost-Effective Tools for Preclinical Drug Safety and Efficacy Evaluation
   - In Vivo Imaging with Confirmation by Histopathology for Increased Rigor and Reproducibility in Translational Research: A Review of Examples, Options, and Resources
   - Histopathology of Biodegradeable Polymers: Challenges in Interpretation and the Use of a Novel Compact MRI for Biocompatibility Evaluation
   - Magnetic Resonance Imaging as a Noninvasive Method for Longitudinal Monitoring of Infusion Site Reactions Following Administration of a Novel Apomorphine Formulation
   - Compact MRI for the Detection of Teratoma Development Following Intrathecal Human Embryonic Stem Cell Injection in NOD_SCID Mice

   Specifically there is one relating to the regulatory aspects of MR Histology:

   - Regulatory Forum Opinion Piece: Imaging Applications in Toxicologic Pathology – Recommendations for Use in Regulated Nonclinical Toxicity Studies

10. **During the discussion of the AKI model, you mentioned there's an enlargement of the kidney after glycerol injection. But it seems the image was not in the same scale among different dates, that the middle two images seems enlarged than the first and the fourth images, is there volume information available for this model?**

    Unfortunately Dr. Nyska does not currently have the scale bars for these images, however a full outline of this study and the findings can be found in a recent publication by Dr. Nyska and his colleagues.

11. A single Purkinje cell is estimated as 4 times the size of a voxel, presuming a voxel is 20x20x20 μm^3, using the formula of $4/3\pi r^3$. Synchronous Purkinje cell deaths ought to enhance MRI signals, leading to a lesion many voxels in size. Can the M-Series system be used to image this size of lesion?

Although Dr. Nyska has not looked at Purkinje cells himself he has suggested the following references regarding this specific question and studying neurotoxicity itself; although the work was not completed using the M-Series systems, the general possibilities of what could be done with MR imaging are discussed. To know for certain the sensitivity of the M-Series system to Purkinje cell death some testing and validation would need to be completed.

Please refer to the references below that Dr. Nyska recommends as a starting point for this discussion:

- Translational Biomarkers of Neurotoxicity: A Health and Environmental Sciences Institute Perspective on the Way Forward
- Quantification and Reproducibility Assessment of the Regional Brain T2 Relaxation in naıve Rats at 7T
- Quantitative Assessment of MRI T2 Response to Kainic Acid Neurotoxicity in Rats in vivo
- The use of MRI to assist the section selections for classical pathology assessment of neurotoxicity

12. Have you tried to correlate your data with PET data using a PET/MR system?

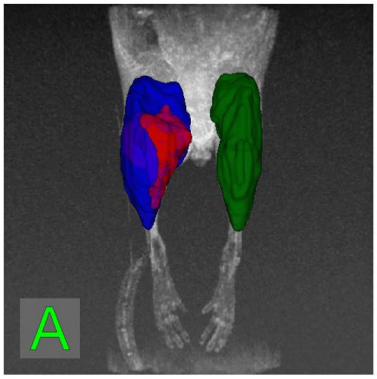
At this time Dr. Nyska has not correlated any of the results presented in the webinar, or others that he has performed over the years with PET data.

There are several PET/MR options available, including the SimPET insert for the M7 system. MR images, either in vivo or ex vivo could be acquired simultaneously with PET images. The coregistered results could provide additional valuable information for specific studies.

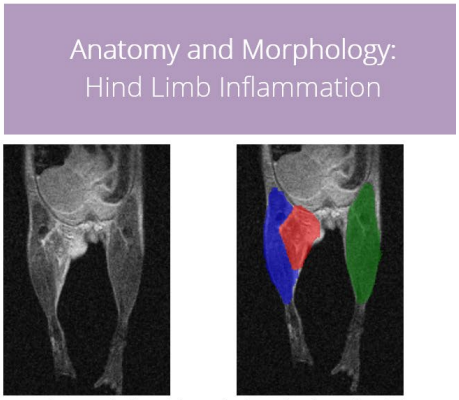
13. Can this technology be used to assess skeletal muscle regeneration and repair?

As Dr. Nyska described he has not yet done this type of imaging for MR Histology, but does believe that it would be possible to see the difference between the muscle and fibrosis.

As Tonya described, she had done an acute inflammatory model on the mouse hind limb and was able to observe the muscles in the hind limb, as well as the inflammatory response. Volumes could be calculated on these different regions, as seen below:



T2 weighted: SE(TE/TR=50/1500, FOV=60mm, Matrix=256x256, Res. 235um, Acq. Time 6:24m:s)



14. Is it possible to use MRI to detect foreign body metallic wear particles from implants (typically submicron to 10µm in size)?

The possibility of detecting the metallic particles would depend on a few things, mainly the type of materials and of course their size.

However, it should be possible to detect their presence in a similar manner as iron oxide nanoparticles are detected. When present on an MR image these particles actually cause a loss of MR signal as they interfere with the homogeneity of the magnetic field.

More details would be needed on the material, and also some trial imaging to detect the sensitivity to the particles and therefore the size or number of particles that would be required to be observed.

M-Series Compact MRI System Questions

15. Which company offers the M-Series Compact MRI system with the histology capabilities? What is the price of these systems?

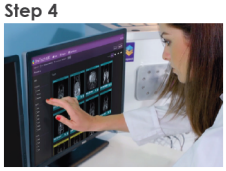
The M-series systems are manufactured by Aspect Imaging. Scintica Instrumentation is a distributor of these systems across North America and Europe. Please contact info@scintica.com for additional information on these systems and the available options. We will be happy to discuss your specific needs and provide you with pricing based on the appropriate configuration. For additional detailed information about the M-Series systems please take some time to watch the on-demand version of a webinar we presented in early 2019.

16. Can you provide us with some more details on the technical details of the system; what is the field strength, does it require liquid helium, etc.?

The M-Series systems operate at a field strength of 1 Tesla. The system is a permanent magnet which is also self-shielded. This allows the system to be quickly installed into existing laboratory spaces and animal facilities, as no additional shielding or infrastructure is required. As it is a permanent magnet there is no heat generated to create the magnetic field so no cryogens (such as liquid helium) are needed, nor is any type of water cooling. There are fans on either side of the magnet to dissipate any heat created during the imaging session which will occur as the gradients (electronic component required to acquire the image) are used.

17. An automated sample handling system was mentioned several times, can you expand on how this works?

The chart below describes the steps taken when using the automated sample handling system of the histology add-on. In this way numerous samples can be run overnight, or throughout the day, without any intervention from a technician to load subsequent samples.

Step 1	Step 2	Step 3	Step 4
			
Samples are placed in cartridges Fixed samples (whole organs or tissue samples) are placed in the cartridges. As MR imaging is non-destructive, the tissue remains unchanged, and routine immunohistochemistry is easily performed following the 3D MR-based histology scans.	**Custom barcodes are printed and samples are scanned** The system produces unique barcode stickers which are applied to each cartridge. The cartridge is scanned and the study software brought up, which allows the operator to enter study-specific and sample-specific data. The appropriate imaging protocol is selected and associated with the sample barcode.	**Up to 9 cartridges are pre-loaded and scanned** The "auto-loader" allows up to 9 cartridges to be pre-loaded. A sample is advanced into the system, its bar code is read and the appropriate protocol run. The next sample is automatically advanced, starting the process again. The samples can be scanned overnight and left unattended.	**High resolution 3D digital images obtained** The system can automatically generate high-resolution 3D data sets of the samples.

18. What is the maximum tissue size that can fit into the MRI system *ex vivo*?

There are two different sized magnets available for the M-Series system, traditionally the M3 will fit samples up to the size of a mouse, while the M7 will fit samples up to the size of a large rat.

The actual size of the tissue which can be imaged depends on the imaging coil being used. For the MR Histology add-on there is a specific coil to fit as tightly to

the imaging cassette as possible – this provides the highest signal to noise, and therefore best image quality.
However, if your sample was larger than what would fit within the imaging cassette you could simple place it in a larger vessel such as a falcon tube, and then select the most appropriate sized coil for imaging. Coils range in inner diameter from 23mm to 71mm.

19. For *in vivo* brain imaging, how large of an animal can fit within the M-Series system?

The M7 system, which is the largest conventional system of the M-Series preclinical systems can fit a large rat, and there is a rat head coil with an inner diameter of 35mm.
If however larger animals are required there are additional magnet options which can be explored, and should be discussed.