# WEBINAR: THE FOUR W'S OF PRE-CLINICAL SMALL ANIMAL IMAGING: WHAT, WHEN, WHERE, WHY

**Questions and answers from the September 24th, 2020 webinar titled "The four W's of pre-clinical small animal imaging: what, when, where, why"**

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.

*Optical Imaging*

1. **Is it possible to integrate optical imaging with CT or X-Ray similar to PET imaging?**

   Yes, there are systems on the market that allow you to integrate both X-Ray or CT with optical imaging, similar to what can be done for PET imaging. This would allow you to have more of an anatomical reference and get a better idea of where your signal is coming from within the body compared to the traditional brightfield image that is often used.

   An alternative to having CT or X-Ray integrated into the same instrument, is to have an imaging cassette which would allow you to transfer the animal from one system to another, with images co-registered in third party software.

   However, additional requirements need to be considered for an optical or stand along system with X-Ray and/or CT capabilities including both infrastructure requirements as well as additional training needed for users since both X-Ray and CT use ionizing radiation.

2. **Is *ex vivo* imaging possible with bioluminescence imaging similar to the images you showed for the fluorescence study?**

   Yes, *ex vivo* imaging is possible with both bioluminescence and fluorescence imaging. For bioluminescence, you need to first understand the enzymatic reaction that happens with the specific luciferase/substrate pair you are using in your model. Firefly luciferase and d-luciferin are the most commonly used pair and require some additional co-factors in order to produce light such as oxygen and ATP. This means that the timeline of *ex vivo* imaging needs to be carefully considered. When you're ready to image, you have two options:

- Inject your animal with the substrate (ie. d-luciferin), wait a couple of minutes to allow the substrate to circulate around the body and then sacrifice the animal. From here, you can choose how quickly you open the animal up based on where your signal is but try to keep it under 30 minutes. For example, if you know your signal is only in the brain, you may want to dissect the brain out as quickly as possible and then image the brain only while there is still adequate oxygen and ATP available. Alternatively, if you have a signal in the abdominal region and you're not sure exactly what organ the signal is originating from, you can dissect and image at each stage, ie. Opening up the skin laterally then taking an image, removing the intestines then taking another image etc. until you localize the signal.

- The other option is dissecting the tissue out and then adding the substrate directly onto the tissue once it is in a petri dish and ready to be imaged. This strategy works nicely for tumors where substrate availability may be limited at endpoint due to restricted tumor vasculature. If unsure, you can inject substrate prior to sacrificing and take a quick *in vivo* image prior to sacrificing to see if there is any signal in the area of interest. Then following dissection, you can always add additional substrate directly to the tissue and image again.

3. **What fluorophore was used in the rats? Were the rats shaved?**

   For the fluorescence study I showed in the webinar, the authors used both Cy3 and FITC. Cy3 fluoresces greenish- yellow with an emission peak of 570nm. FITC is also greenish with an emission peak at 516nm. The hair was removed from the stomach and chest regions but because these fluorophores were used, there is still quite a bit of autofluorescence seen. Below is the reference for the paper for anyone interested in further details of this particular study.

   C. Feng et al., Eur. J.Pharm. Biopharm. (2013).

   For optical imaging, there are many options when choosing a fluorophore. The most common would be GFP, tDTomato and mCherry but any red fluorescent proteins available will have improved sensitivity compared to the GFPs just due to less absorption from tissue. Below is a reference to a paper that nicely compares the difference in signal from GFP, tDTomato and mCherry.

   N.C. Deliolanis et al., J Biomed Opt. (2008).

4. **Can you say more about *ex vivo* imaging in BLI and how that improves issues of limited depth penetration?**

   One of the main disadvantages of optical imaging is the limited depth of penetration, approximately 1mm for fluorescence and 1-2mm for bioluminescence imaging. This is due to the scattering and absorption of light by biological tissues within the body. By removing the organ or tissue of interest from the body, as is the case with *ex vivo* imaging, you're increasing the

amount of light that can be emitted and collected by the CCD camera and ultimately improving the sensitivity.

5. **Optical techniques seem to give detailed cellular information. Have you used this technique to study stomach cells after chemotherapy? This is a problem in cancer treatment.**

   Bioluminescence imaging would be preferred for a stomach study over fluorescence imaging since many things in the gut can auto fluoresce including some types of mouse feed. If you wanted to visualize only live stomach cells following treatment, you could use firefly luciferase as your reporter gene since the requirement of co-factors means that any signal acquired will be representative of live cells only. Alternatively, you could use a different luciferase (i.e. renilla luciferase) which would allow you to visualize all of your engineered cells regardless of whether they were dead or alive. You could choose to either engineer a population of stomach cells (endocrine cells, mucous cells etc.) or deliver a therapy that targets these cell populations and monitor the therapeutic.

*FIVE2 Endomicroscopy*

6. **What is the penetration depth for the FIVE2? How far can it go into the sample?**

   This depends on the sample. For most samples, 200-300µm will be an optimal depth. Whereas for optically clear samples, a depth of 400µm may be possible. For thick tissue like bone, a depth of 100µm or less would be preferred.

7. **How does this type of imaging work with tissues that are moving like the heart and lungs?**

   It is difficult to image the beating heart with this system as the heart pulses faster than the frames per second image rate of this technology. However, certain areas of the lungs can be imaged.

8. **Does the neural imaging require a cranial window?**

   Yes, a cranial window is needed in this case to allow the probe to come within 200µm in proximity to the structure of interest.

9. **Does the animal need to be sacrificed after endomicroscopy imaging or can the animal be monitored over time using this approach?**

   Longitudinal imaging is possible with the FIVE2 system and one can use sterile sheaths to ensure that there is no infections to the animal over time.

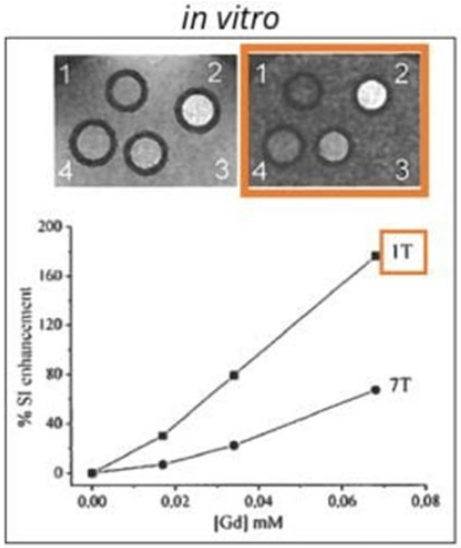
*MRI Imaging*

**10. Are Ugelstad micropellets and Dynabeads or anything similar used in any of your systems?**

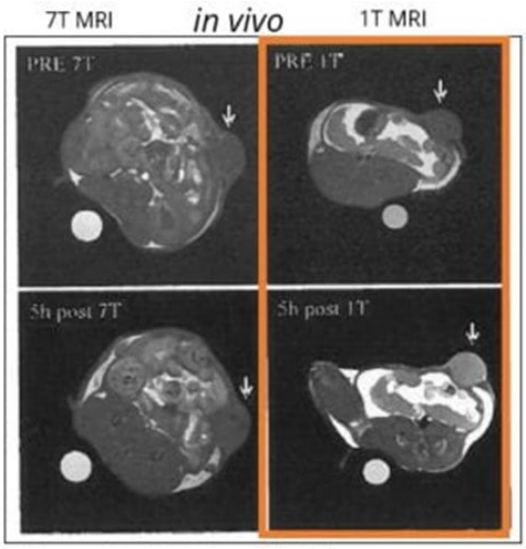
Iron oxide-based contrast agents can be used with our M-series compact MRI systems including paramagnetic, superparamagnetic and ultra small superparamagnetic particles (see reference below). Contrast agents are often used to provide additional information on tumor perfusion and/or specific molecular targets within each animal model.

Daldrup-Link HE, Golovko D, Ruffel B, et al. MR Imaging of Tumor Associated Macrophages with Clinically-Applicable Iron Oxide Nanoparticles. Clin Cancer Res. 2011.

Additionally, the M-Series systems operate at a field strength of 1 Tesla (T), this makes them ideally suited for contrast imaging using Gadolinium (Gd) based agents. Numerous groups, including Dr. Silvio Aime's group at the Molecular Imaging Center at the University of Torino in Italy, have shown the improved sensitivity to Gd contrast agents at 1T when compared to higher field systems.




In vitro and in vivo data from Geninatti-Crich S.et al. *Contrast Media Mol. Imaging*. 2011.




5 hours after injection of the Gd contrast agent, there is a greater enhancement at 1T than 7T:

- 1T: 80±9%
- 7T: 30±13%

*General Questions*

11. **Does the type of mouse used for a study need to be considered when choosing a modality? Ie. Will fur affect the signal?**

    The animal model as well as the type of mouse used for a study should always be considered when choosing a modality. However, specific modalities will be more affected than others.

    For MRI and PET imaging, you may want to consider things like the immunogenicity of your tracer or contrast agent, or whether you're implanting a human vs. murine derived cell line. But these factors will more so affect your model, not the imaging signal itself. Whereas for optical and US imaging, fur on the mouse skin will interfere with signal. For this reason, nude mice or models without hair are always preferred if possible. If it's not possible, shaving or using depilatory cream on the area of interest are very easy and work well to improve the imaging signal.

12. **What is the difference between multiplex and multimodal imaging?**

    Multiplex and multimodal imaging are often used interchangeably. However, there are differences between the two terms.

    Multiplex imaging refers to the simultaneous (or near simultaneous) acquisition of multiple signals transmitted through a shared medium (one or multiple channels) and subsequently de-mutliplexed. For example, two different fluorescent proteins being used to track two different cell lines within the same animal. These signals would be acquired simultaneously on an optical imaging system and then based on their emission spectra can be separated into individual signals.

    Multimodal imaging refers to the combination of two or more imaging modalities in one imaging session for example PET-CT or PET-MRI via distinct lines of transmission. However, many also use multimodal imaging as a general term to describe any study with more than one imaging modality used and not necessarily performed in the same imaging session. For example, using both optical and MRI to monitor tumor growth but the imaging sessions are on alternating days.

    The following review paper provides a nice overview of some of these terms and how they may be used in the imaging field:

    Heinzmann, K., Carter, L., Lewis, J., & Aboagye, E. (2017). Multiplexed imaging for diagnosis and therapy. *Nature Biomedical Engineering, 1*, 697-713.

13. **Do you fuse imaging data from the same experimental animal to get more information than from the image from a single modality?**

There are a couple of ways you can acquire a more complete imaging dataset. Firstly, there are systems that have the capability to acquire two different images on the same animal simultaneously (i.e. PET-CT or PET-MR). This strategy allows you to acquire both anatomical information (CT or MRI) and functional information (PET) from the same animal and often these images are intrinsically co-registered.

Alternatively, you can acquire images on more than one modality independently of each other. In this case, you could use fiducials to co-register the datasets following acquisition or you could just extract the information from each image dataset independently. For example, by using optical and MRI to monitor tumor burden, you would still get both anatomical and functional information however, it doesn't make sense to fuse them since optical is a 2D representation of signal and MRI is a 3D image.