Multimodal Imaging of Mice

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The main objectives of this lecture are to provide instructions/ updates on

(i) existing pre-clinical imaging modalities and their physical principles;
(ii) advantages and disadvantages of each modality;
(iii) multimodality platforms for obtaining maximum imaging-based information;
(iv) contrast agents for multimodality imaging;
(v) appropriate mouse models and practical aspects of animal handling.

1. Existing Pre-Clinical Imaging Modalities: In vivo biomedical imaging involves administering a known amount of energy to the body and measuring, with spatial localization, the energy that is transmitted through, emitted from, or reflected back from various organs and tissues (*Brindle, 2008*). The energy most commonly used is some form of electromagnetic energy, such as X-rays or lights, but occasionally other forms are used such as mechanical energy for ultrasound scans. Imaging the human body began as part of routine clinical care with the development of X-ray imaging by Roentgen (*Serkova et al., 2009a*). Computerized tomography (based on 3D X-ray scan representation) has added immeasurably to the ability to find, measure, and monitor pathologies. The algorithms originally developed by Hounsfield bto produce tomographic images with X-rays have also been extended to nuclear medicine for use with positron emission tomography (PET) and single photon emission computed tomography (SPECT). The development of magnetic resonance imaging (MRI) has provided high levels of contrast with superb resolution in many areas of the body. These modalities have been complemented by ultrasound (US) imaging and more recently by the introduction of new optical imaging (OI).

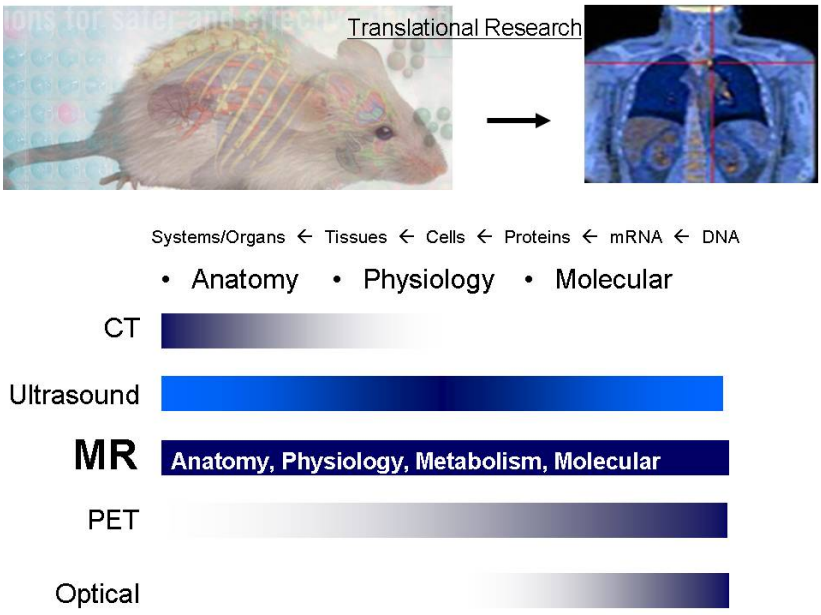

Figure 1: Imaging modalities in bio-medicine and limits of their applications in translational medicine (adapted from *Serkova et al., 2009a*).

Applying imaging modalities in small animals allows for acceleration in the development of new imaging markers and drugs as well as increase in our understanding of pathophysiological processes. Imaging in mice is important because of

the widespread use of genetically engineered mice in biomedical research and the need to measure the in vivo anatomic, functional and molecular phenotypes. Animal imaging is highly attractive because in vivo environment can be successfully captured (in vivo ≠ ex vivo); it is non-destructive (each animal serves as its own control); it can efficiently survey the whole animal; and, finally, it provides translational bridge from animal to human studies (*Weissleder and Mahmood, 2001; Gambhir, 2002*). Advanced technologies developed for imaging in small animals are identical to human imaging modalities (Figure 1) and, therefore, can generally be translated directly for application in clinical scanners. Each of the imaging technologies has its own advantages and disadvantages (see below) in spatial resolution, functional assessment and in imaging of molecular targets. In order to take full advantage of theses imaging modalities for establishing comprehensive anatomical, physiological, metabolic and molecular end-points, one needs to understand basic underlying principles of physics for each modality.

As mentioned above, all imaging modalities are based on physical phenomena which involve interaction of external energy in form of radiofrequency waves (MRI), X-rays (CT), radiation decay (PET) or sound waves (US) with human/ animal body in order to spatially and time-dependently reconstruct anatomical, physiological or molecular images. Here a brief summary is provided on how major imaging modalities function (see also mouse images by different modalities on Figure 2).

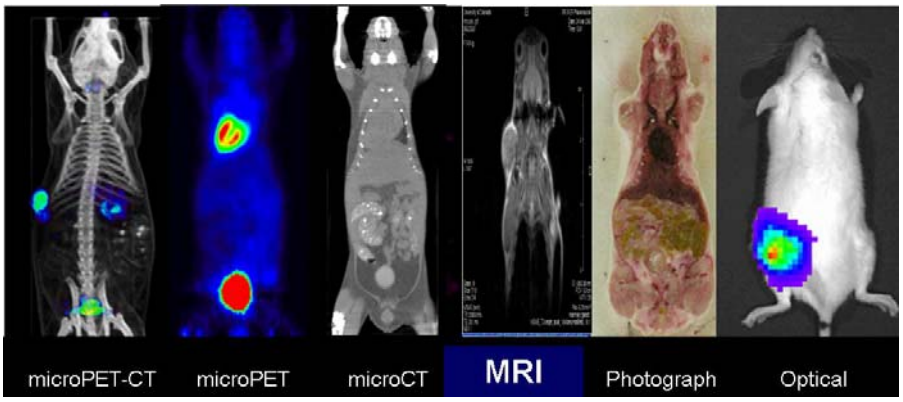

Figure2: Mouse images by different modalities

Magnetic Resonance Imaging and Spectroscopy (MRI and MRS): MRI generates images by applying an external varying magnetic field to the body. The magnetic field aligns hydrogen atoms parallel and anti-parallel to the magnetic field. When a signal in the form of a radio wave pulse is applied to the body using surface coils, atomic distribution between parallel and anti-parallel alignment is changed, and after the pulse is gone, the system relaxes to its original status. Hydrogen atoms in different tissues have different relaxation properties which can be detected by radio-frequency MR receivers. MRI of tissue relaxation characteristics following a radiofrequency pulse of energy can be then translated into information about the concentration, mobility, and chemical bonding of hydrogen and, less frequently, other tissue elements. Although many other MRI techniques exist, the two basic types of images are T1- and T2-weighted MRI. T1-weighted images show fat as a white bright signal, whereas water and cerebrospinal fluid (CSF) are dark. On a T2-weighted image, fat is gray, and blood, edema, and CSF appear white. Unfortunately, calcification is difficult to see on MR images. In addition to anatomical imaging, a physiological assessment of organ perfusion and permeability can be made by injection of gadolinium-based agents and calculation of their uptake into the organ of interest on T1-weighted images. The use of iron-oxide-based nanoparticles (SPIOs and USPIOs), on the other hand, allows for molecular imaging based on T2-weighted scans (see below). Using relaxation properties

of other hydrogen-containing endogenous molecules or other atoms (such as ^{31}P and ^{13}C), metabolic information can be obtain non-invasively in a magnetic resonance spectroscopy (MRS) scan. MRS can be based on observation of protons in various metabolites (such as ^{1}H-MRS of citrate in prostate cancer; ^{1}H-MRS of choline in breast, prostate and brain cancer; ^{1}H-MRS on N-acetyl aspartate and myo-inositol in brain cancer) or phosphorus metabolites by ^{31}P-MRS (membrane phospholipids in tumors, or ATP and phosphocreatine in the muscle). In general, MRI is the method of choice for imaging the central nervous system, musculoskeletal system and stationary soft tissues. There is limited applicability of MR for imaging of lungs. However, one of the most recent discoveries related to MR application in the lung, namely the use of hyperpolarized ^{129}Xe gas for imaging of pulmonary gas transfer, allows a clinician or image recognition program to asses gas exchange and/ or alveolar-capillary barrier status in the lung. In addition to the superior spatial resolution, an advantage of MRI and MRS is that it does not require the use of ionizing radiation; however, MR is one of the most expensive and technically most challenging imaging modalities.

X-ray and Computed Tomography (CT): In biomedical imaging, X-ray techniques, including CT, can reveal intrinsic properties of an object such as its physical and electron density. The X-rays are absorbed in different amounts by the various tissues or materials in the body. Most of the beam is absorbed or scattered, however a small percentage of the beam exits the patient and strikes a detector. CT images are acquired with an X-ray tube passing a rotating fan beam of X-rays through the animal and measuring the transmission at thousands of points with detectors. CT scans are presented as a series of slices of tissue, and the computers can then display the data as a three-dimensional rotating image. Compared with plain X-rays, CT uses 10 to 100 times more radiation. The appearance of tissue on CT scan can be divided into the four basic densities: air is black, fat is dark gray, soft tissue is light gray, and bone or calcium and contrast agents are white. CT is currently one of the most commonly used imaging modalities given faster scanner, widespread availability and high spatial resolution. However, CT has relatively poor contrast resolution in comparison to MRI. This limitation can be partially overcome by the use of contrast agents (see below).

Positron Emission Tomography (PET): Nuclear medicine images are produced by giving the patient short-lived radioactive isotopes and detecting their decay by a gamma camera or positron emission scanner. Nuclear medicine procedures, including PET and SPECT, reveal spatial and temporal distribution of target-specific radiotracers and pharmaceuticals. In PET, a positron-emitting radioisotope is administered in very small doses (usually less than 0.25 mCi for a mouse) intravenously and the distribution of the tracer is imaged. Depending on the application, these data can be interpreted to yield information about properties such as glucose metabolism, blood volume and flow, tissue uptake, receptor binding, and oxygen utilization. Although an extensive array of different radiopharmaceuticals, or molecular probes (including carbon 11, nitrogen 13, oxygen 15 and fluorine 18 based tracers), to image different aspects of pathophysiology and biology are available for PET imaging, the most widely used, and the only clinically approved PET tracer is the fluorinated analogue of glucose, ^{18}fluorine-2-deoxy-D-glucose (^{18}FDG) . The increased uptake of glucose in malignant cells has been known for many years (first described by Otto Warburg in 1930), and the high-uptake of FDG can be used to detect tumor lesions and metastases. There is physiological FDG uptake by brain, heart, kidney and bladder and, in mice, by brown fat on FDG-PET. The major advantage of PET is its ability to assess molecular function; however, PET is limited by poor spatial resolution making it difficult to accurately localize FDG uptake to an anatomical structure. This limitation has been significantly reduced by combining PET with CT, a technique in which both PET and CT are performed sequentially during a

single visit on a hybrid PET/CT scanner (see below). The PET and CT images obtained are coregistered using fusion software, thereby enabling accurate designation of physiologic and molecular data obtained on PET to anatomic structures visualized on CT (an example of a preclinical mouse PET/CT fusion image is presented in Figure 2D). PET/MRI has also been recently explored in animal imaging. In general, PET is a method of choice for physiologic and biochemical information; however, PET studies require radiation exposure. Additionally, a cyclotron facility is required to produce the ultrashort half-life of isotopes making this imaging modality relatively expensive.

Ultrasound (US): Ultrasound uses high-frequency sound waves to make images. Ultrasound image is created with the capture of ultrasound energy reflected from interfaces in the body ("echoes") that separate tissue with different acoustic impedances, where the acoustic impedance is the product of physical density and velocity of sound in the tissue. Typically, a cyst appears sonolucent because it has a few if any echoes (because it is mostly water), while liver and spleen have solid homogenous echo texture due to medium level echoes from the fibrous interstitial tissues. High-intensity echoes (increased echogenicity) are caused by calcification, fat and air. The technology of ultrasound is attractive because it does not use ionizing radiation, can produce real-time image and is less expensive than any other modality.

Optical (OI): In fluorescence imaging, excitation light in the visible region (400-600 nm) is used to excite fluorophores in the tissue, which emit fluorescence at longer wavelengths. Bioluminescence imaging relies on the genetic engineering of tissues to express luciferases. These are photoproteins, isolated from organisms such as the flirefly, which modify their substrates and in so doing produce light, which can be detected using sensitive device cameras. The advantage over fluorescence imaging is that bioluminescence is very sensitive, as there is no background, with detection sensitivity of 10^{-7} – 10^{-5} M. Both techniques are limited by the low depth of penetration (1-2 mm) due scattering and absorption of the emitted photons. They are not really quantitative either. As of today, bioluminescence is exclusively used in mice for molecular imaging

2. Advantages and Limitations: When assessing strengths and weaknesses of each modality, the following important image characteristics should be taken into account:

- Spatial resolution: what is the smallest object I can visualize?
- Signal-to-noise: what is the precision of the measurement?
- Quantitation: what is the accuracy of the measurement?
- Contrast-to-noise: what differential in image intensity must I have to be able to visualize an object of interest?
- Sensitivity: what concentration of a tracer, probe or contrast agent must I have to be able to detect an object?

Therefore, the following advantages and disadvantages can be assigned to each modality which also predict their possible application range in human and animal research (Table 1).

- *CT:* advantages: high spatial resolution; whole body 3D-coverage; moderate costs; short scans; disadvantages: high radiation dose; mostly anatomic information; poor soft tissue contrast.
- *MRI:* advantages: high spatial resolution; excellent soft tissue contrast; outstanding anatomic imaging; provides metabolic, functional and molecular imaging end-points; disadvantages: long scan times for large volume/ high resolution; expensive; complex physics.

- *PET:* advantages: high sensitivity (nM-pM); labeling of small molecules with little or no change in biological action; whole body 3D-volumetric imaging; quantitative; straightforward translation from mouse to man; disadvantages: involves ionizing radiation; access to radiolabeled molecules, especially for short hal-life radionuclides (need for cyclotrons); lower spatial resolution.
- *US:* advantages: real-time; no ionizing radiation; low cost; good spatial resolution; disadvantages: requires direct contact with animals; limited field of view.
- *OI:* advantages: accessible inexpensive technology; low cost; highly sensitive for targets; activatable contrast agents; no ionizing radiation; disadvantages: difficult to translate to man; 3D challenging; not quantitative; contrast agents for protein targeting can be limited by size of fluoroprores.

Table 1: Advantages and disadvantages of single imaging modalities in clinical and pre-clinical cancer research.

Modality	Resolution [mm]		Specificity/ Sensitivity	Anatomic Potential	Functional Potential	Molecular Potential
	Clinical	*Research*				
MRI	5	0.1	high	excellent	excellent	high
CT	5	0.05	high-moderate	excellent	moderate	low
SPECT	10-15	1.5-2	moderate-high	moderate	moderate	high-moderate
PET	10-15	1.5-2	high-excellent	moderate	excellent	high
Ultrasound	5-10	0.2-0.5	moderate	High-moderate	high	moderate-low
Optical	N/A	(0.5)	excellent	moderate-low	moderate	excellent

3. Multimodality Imaging: The combination of molecular-functional-anatomic multiple imaging modalities provides the highest advantage of non-invasive method because, as we could see above, each modality has its own strengths and weaknesses. The simplest way to obtain "multimodality" images is to acquire them at each modality separately and then, by applying an anatomic marker for co-registration, fuse the images using advanced imaging analysis software (*Liao and Li, 2009*). The "multimodal" platform can also be achieved by physically placing two modalities (a) adjunct to each other (for example, docked PET/CT system with a moving animal bed from the PET into CT scanner); or (b) for a simultaneous acquisition, a hybrid scanner could be designed (for example, by placing PET rings inside of an MRI scanner) (*de Kemp et al., 2010*). The first multimodality platform, which has been introduced into clinical setting in 2001, was "docked" PET/CT systems, followed in 2004 by SPECT/CT. In animal research, microPET/CT and microSPECT/CT (mostly based on the docked design), as well as a new generation of triple micro-PET/SPECT/CT scanners are using the high-resolution CT scan (with spatial resolution of the order of 100 microns) to "anatomically" adjust the functional images (PET and SPECT). The total acquisition time for a PET/CT scan in a mouse is approximately 15 minutes (6 min for a PET followed by a 9 min CT scan). However, the use of ionizing radiation from a high-resolution CT scanner is undesirable, particularly in small animal imaging; moreover, CT scans provides anatomic localization only (*Wagenaar et al., 2006*). Most recently, the first attempts have been made (both clinically and pre-clinically) to combine two most sophisticated imaging modalities, namely PET and MRI. Several research groups used different approaches to integrate PET detectors into high-filed MRI. Initially, systems were based on optical fibres guiding the scintillation light to the PET camera, which resides outside the fringe magnetic filed.

Recent advances in gamma ray detector technology paved the way towards the development of fully magnetic-field-insensitive high-performance PET detectors (*Chaudhari et al., 2009; Wagenaar et al., 2006*). Combined PET/MRI allows for multi-parametric imaging and will be, without any doubt, the modality of choice to obtain multiple functional and metabolic imaging end-points along with high-resolution morphology (*Wehrl et al., 2009*). In animal PET/MRI scanners, the performance of all major MR applications, ranging from T1- or T2-weighted images up to echo-planar imaging (EPI) for fMRI as well as MRS, could be maintained when the PET insert was built into the MRI and acquiring PET data simultaneously.

4. Contrast Agents (CAs): Injectable tracers are available for each modality: for CT, MRI and US, they are used to increase tissue contrast and/or obtained dynamic functional information (such as DCE-MRI). For nuclear medicine and optical, injectable tracers are prerequisites for imaging detection (*Hasebroock and Serkova, 2009 and Dobrucki et al., 2010*).

- CT CAs: usually iodine based; however, we can not routinely use human CA in mice because of fast clearance in mice (600 beat/min); Fenestra mouse liver contrast (LC) and Fenestra mouse vascular contrast (VC) are available. For molecular imaging, there are attempts to use gold nanoparticles.
- MRI CAs: Two major classes, (i) Gadolinium (Gd)-based chelates (paramagnetic) reduce T1 resulting in increasing brightness of T1-weighted images; (ii) Iron-oxide nanoparticle based (SPIO, USPIO) superparamagnetic agents, which reduce T2 relaxation times with decreasing signal intensity on T2-weighted images. Gd-chelates: magnevist, omniscan, multihance. SPIO: feridex, resovist.
- PET CAs: they are radiolabeled short-life molecules, peptides, antibodies, cells. Most of the time they need to be produce on-site (half-life times: 18F 110 min; 11C 20 min; 13N 9.9 min; 15O 120 sec).
- US CAs: gas or air-filled microbubbles or lipid microspheres are used which are strong scatteres of US: optison, definity, levovist, echogen, biosphere are intra-vascular; SonoRx is used orally.
- OI CAs: they are mostly fluorescent proteins = enzymes, which catalyze bioluminescent reaction; or fluorescent molecules/ dyes used to label biomolecules and cells.

New targeted agents (including PET radiotracers, MRI nanoparticles and luciferase constructs) are design to visualize genes (Weissleder and Mahmood, 2001), proteins (*Serkova et al., 2010*), tumor pH and microenvironment (*Zhang et al.,, 2010*).

5. Animal Models and Preparation: Mice are the most used animal model in biomedical research (*Gitler et al., 2004* and *de Jong and Maina, 2010*)::

- they are small and prolific breeders;
- they have accelerated lifespan (1 mouse year = 30 human years);
- well characterized anatomically, genetically and physiologically (human/ mouse genes have 85% similarity)
- can be genetically manipulated at molecular levels (transgenic and knock-outs).

Various aspects of animal handling and preparation will be discussed including positioning, anesthesia, the route of contrast agent administration, as well as pulmonary and cardiac gating (*Serkova et al., 2009b*; *Fueger et al., 2006*; *Woo et al., 2008*; *Bartling et al., 2007*)

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