

## **Imaging Technologies II: Comparison of Techniques, Strengths/Weaknesses, Fusion**

Umar Mahmood, MD, PhD, Center for Molecular Imaging Research, Massachusetts General Hospital, Boston, MA

### Introduction

Molecular imaging (MI) encompasses a wide array of biological applications that can be evaluated non-invasively in vivo. To date, much of that evaluation has been in pre-clinical models, mostly mice, with increasing clinical translation expected. Many of the technologies including MRI, PET, SPECT, optical fluorescence (injected probes), CT, and ultrasound are translatable between both realms. Others, such as bioluminescence imaging and fluorescence protein imaging are primarily preclinical tools. The most important point of this lecture is that each biological question one wishes to evaluate non-invasively can be approached using more than one imaging modality. Molecular imaging is multimodal, and each technique has its strengths and weaknesses. The biological question determines the optimal imaging strategy, and often times diverse imaging technologies complement each other to produce information that is greater than the sum of the parts.

### MRI

MRI has very high spatial resolution compared to many of the other modalities; mouse imaging easily achieves  $0.1 \times 0.1 \times 0.5$  mm voxels with reasonable SNR in a matter of minutes and MR microscopy is possible with longer times. Additionally, there is no significant attenuation of signal with depth over the scale of rodents or people at commonly used field strengths. The major weakness of MRI is insensitivity, both with intrinsic contrast imaging, and using administered molecularly-targeted or sensing agents. Several strategies have been employed to increase the sensitivity of detection compared to gadolinium small molecules which are typically detectable in the  $10^{-4}$  M range, including the use of iron oxide nanoparticles (1-3), which have detection thresholds in the  $10^{-8}$  M range, or the use of gadolinium-encapsulated liposomes (4) or gadolinium-conjugated dendrimers (5).

MRI has been used quite extensively for receptor imaging, including receptors on normal tissue (such as asialoglycoprotein receptors present on normal hepatocytes), tumors (e-selectin, folate, her2), abnormal vasculature (alphaVbeta3-integrin), and for “receptors” expressed on cell surfaces when the cells are dying (apoptosis imaging via annexin binding). Given the insensitivity of MRI, these receptors often have to be expressed at moderate to high levels to be visualized, and conjugation to moieties that are detectable in lower concentrations (such as iron oxide nanoparticles) is often required. MRI is a useful modality for cell tracking. The extremely high spatial resolution that can be achieved deep inside tissue compared to other modalities and the associated anatomic information regarding adjacent structures is a benefit; in some cases single cells can be imaged (6, 7). Additionally, the labeling methods for MRI in general do not change viability, function, or differentiation of cells at the labeling levels typically reported (8). However, cell loading of imagable agents (often iron oxide nanoparticles), decreases with cell division so that typically it is an approach best used for short term cell tracking (on the order of one week or less). Additionally, cell viability cannot be determined without the use of a second modality, since the MR reporter in this case is the nanoparticle, which is present in both live and dead cells and moreover can be locally phagocytosed by macrophages clearing dead cells,

occasionally leading to the false impression of persistence of labeled cells. MRI can also be used for reporting of marker genes (9). The advantage of high spatial resolution may be partially offset by the trade off in sensitivity compared to other marker gene approaches. Another important area where MRI has been extensively used is in the evaluation of angiogenesis and vascular imaging in general. Advantages such as high spatial resolution play into MRI's strength, especially for evaluation of vascular volume fraction using blood pool agents or imaging changes in capillary leak using dynamic GdDTPA imaging. Imaging of specific vascular targets has been performed by MRI, but can also be approached by a number of other modalities, especially isotope imaging methods. Finally, imaging enzyme function, such as myeloperoxidase activity has been performed in vivo using probes that change their relaxation properties (10). These smart probes complement optical smart probes which have been optimized for imaging protease activity.

### PET

PET imaging has grown exponentially over the last few years, both clinically (the vast majority of which has been FDG functional glucose imaging), and pre-clinically. PET has relatively low spatial resolution and poor anatomic delineation but extremely high sensitivity. To partially overcome the spatial issues and to improve anatomic correlation of signal, PET-CT combination systems have replaced stand alone PET cameras in many institutions. Given the very high sensitivity, which is among the highest for any molecular imaging modality, especially for deeper tissue, PET imaging is ideally suited for receptor imaging. Additionally, it is well suited for quantitatively evaluating the biodistribution of pharmacological agents, especially in cases where the radiolabeled drug can be synthesized without significantly changing the steric properties of the compound. PET has also been used both preclinically and clinically quite effectively for imaging gene expression using a marker gene paradigm. In this case, the PET imaging agent is trapped inside cells expressing the marker gene. This can be used to track exogenously administered genes for therapy, or can be used to ex vivo label cell populations which can subsequently be imaged in vivo (11). Advantages include the ability to follow cells longer than is typically possible with MRI and iron oxide based approaches. Since the imaging agent is not given until just before imaging, radiotoxicity tends to be less of an issue. Downsides to PET imaging include the increased complexity of synthesis of agents to new targets, and the requirement for agents to reach their molecular target in relatively short time periods (because of radioactive decay).

### SPECT

As a broad generalization, SPECT molecular imaging in many ways is similar to PET imaging. Overall, SPECT devices tend to be less sensitive than PET devices but the synthesis of imaging probes may be easier. SPECT has been used extensively clinically for receptor imaging, as well as imaging processes in which the target is less well defined or depends upon physiologic factors. With respect to ex vivo cell labeling, for example with indium oxine, advantages over MRI include the ability to detect fewer cells. Disadvantages include possible radiotoxicity since the radiolabel is present since cell introduction, limited imaging times (typically somewhat shorter than is possible with MRI), and the possibility of redistribution of tracer to other cell populations with death of labeled cells.

### Near infrared (NIR) optical fluorescence

Fluorescence imaging of exogenously administered fluorochromes has markedly increased over the last few years. Fluorescence is extremely sensitive, and because fluorochromes can be interrogated multiple times, unlike radioisotopes in which one molecule can report with only one decay, in some cases fluorescence imaging may be more sensitive than isotope imaging. Another advantage is the lack of radioactivity. Fluorescence has been used extensively in receptor imaging and many fluorescent analogues of isotope-labeled receptor targeting compounds exist. For cell tracking, a number of commercially available dyes have been used successfully. Like other ex vivo labeling techniques (used in MRI and SPECT) the label is typically visible for about a week or less, as the dye is split into daughter cells or slowly leaks out of the cells over time.

One of the most powerful recent advances in optical imaging has been the introduction of smart fluorescent probes (12). In this case, the administered probes are optically silent (quenched) when injected, but increase their fluorescence up to several hundred fold after target interaction. Proteases are vital to many normal and pathological processes, and the ability to selectively image various proteases, such as cathepsin B, cathepsin D (13), and MMP2, has allowed in situ molecular characterization at the protein and enzyme function level. Moreover, direct imaging of protease inhibition at the protein level (14) opens the possibility for individualized drug dosing of these inhibitors. Additionally, proteases are key common downstream players in diseases such as rheumatoid arthritis, so that their indirect modulation by drug therapy likewise allows determination of appropriate drug dosing on an individualized level (15). In vivo work has been performed using smart MRI probes, such as in the myeloperoxidase imaging example above or in the case of beta-galactosidase MR imaging using an agent which changes its T1 relaxation time after beta-galactosidase cleaves a blocking group that shields a central gadolinium (16). However, optical imaging has had a larger presence to date in the activatable imaging area, and the percentage change in signal intensity after activation is typically far greater by optical than by MRI agents. Ultimately, both will have a role, depending upon lesion location and the required spatial resolution.

Currently, the diverse imaging modalities used to extract molecular information in vivo typically report on one “molecular activity” at a time. For MRI, it is difficult to monitor two agents which both change either T1 or T2, as delivery of imaging agents confounds deconvolution of relaxation changes. Having one agent report a molecular activity as T1 changes and another report a second molecular activity as T2 changes also is difficult, as readout is always somewhat T2 weighted, a problem in that T2 relaxation dominates when the second molecular activity is present at a high level. For PET imaging, all signal is detected as 511 keV photons, making evaluation of even different isotopes difficult. In this case, one depends upon the limited differentiation based on different decay rates, superimposed upon the different kinetics of the compounds reporting on the different molecular activities. A similar problem exists for standard CT scanning in that x-ray attenuation cannot differentiate two different reporters because of the superimposed physiological kinetics of agent distribution. A limited evaluation of different physiological or molecular parameters may be imaged using single photon techniques, by having the two activities report at different energies. However, in cases where two isotopes are present, down scatter of the higher energies may interfere in recording of molecular activity reported at the lower energy. Optical imaging, in contradistinction, can image multiple targets simultaneously (17), opening up the possibility of in vivo mini-arrays which characterize disease

better than single molecular abnormality imaging. An alternative approach is to combine molecular reporting using different imaging modalities, each of which reports a single molecular event type.

Techniques for near infrared optical imaging clinically include tomographic approaches (18), which are ideally suited for human breast imaging, and endoscopic and catheter based imaging (19), which is applicable to imaging human colon, peritoneum, bronchi, lungs, and vessels. Moreover, commercial confocal endoscopic systems are available which allow cellular resolution in vivo and which can be combined with molecularly sensitive fluorescent probes. Thus NIR optical imaging can overcome issues of depth using different approaches, and the spatial resolution in some cases is the highest of any molecular imaging modality.

### CT

CT by itself offers little in the way of molecular imaging capabilities. Exogenously administered agents (often iodinated compounds), are required in high doses to be seen (much higher than the doses of GdDTPA used for nonspecific MRI contrast enhancement). Since many molecular events are relatively uncommon (compared to bulk material such as water), there has been relatively little in the way of CT based targeted agent development. Some early work has been performed using perfusion and leak imaging of small iodinated contrast agents, analogous to the double product vascular imaging that has been performed using dynamic Gd-DTPA MRI. The major use of CT scanning with respect to molecular imaging is the high resolution anatomic detail it provides for multimodality imaging. In particular PET CT has shown its utility in the clinical setting where exact anatomic localization of PET signal often changes patient management. A second smaller and more limited use of CT for molecular imaging falls into preclinical evaluation. In this case, the underlying molecular abnormality is known (e.g. in a transgenic mouse) and efficacy of specific molecular therapy is reported as anatomic changes recorded by multiple CT's over time.

### Ultrasound

Ultrasound to date has had a more limited role in molecular imaging compared with many other imaging modalities, although it lends itself to focal, directed therapeutic intervention combined with imaging. Directed therapy is locally delivered by the use of high powered ultrasound at the site of interest resulting in release of the therapeutic agent by the rupture of the encapsulating microbubbles. A main imaging role in which ultrasound has been evaluated is to look at changes in the number of blood vessels using blood pool agents. This can similarly be performed with other modalities, in particular with MRI using long blood half-life agents such as USPIO's or optical imaging of fluorescent blood pool agents. Targeted ultrasound bubbles typically focus on pathological vascular targets (because these agents have greater difficulty reaching extravascular targets intact).

### Bioluminescence imaging

Bioluminescence imaging is based on the imaging of luciferase, which is either constitutively expressed in cells of interest for cell tracking/detection, expressed under the regulation of promoters of interest to understand which conditions result in gene expression, or are delivered to cells in the setting of gene therapy. Given the expression of a foreign protein (luciferase) in cells, this is likely to remain a preclinical modality. Its forte is imaging at the gene expression

level. A major advantage of the system is very low background. Additionally, it allows very low cell numbers to be tracked when the cells are near a surface (e.g. to follow tumor growth or to see local superficial metastases). A major disadvantage is the high tissue attenuation of the visible wavelength light that is emitted, making the system much less quantitative in deeper settings and markedly increasing the detection threshold for deeper imaging.

#### Fluorescence protein imaging

This is another preclinical imaging modality. In this case, cell tracking, evaluation of protein-protein interactions, and gene expression imaging are main areas where fluorescence protein imaging has a role. Compared with bioluminescence, there is typically more background due to autofluorescence. Like bioluminescence, it suffers from a high degree of attenuation with tissue depth, making the detection of deeper cells more difficult and making quantitation problematic. Fluorescence protein imaging has the advantage of ease of use, and the ability to image at (sub)cellular resolution.

In summary, each molecular imaging modality has strengths and weaknesses. Pick the modality or combination of modalities that will best address the specific biological question at hand.

#### References

1. Josephson L, Lewis J, Jacobs P, Hahn PF, Stark DD. The effects of iron oxides on proton relaxivity. *Magn Reson Imaging* 1988; 6:647-653.
2. Harisinghani MG, Barentsz J, Hahn PF, et al. Noninvasive detection of clinically occult lymph-node metastases in prostate cancer. *N Engl J Med* 2003; 348:2491-2499.
3. Weissleder R, Hahn PF, Stark DD, et al. MR imaging of splenic metastases: ferrite-enhanced detection in rats. *AJR Am J Roentgenol* 1987; 149:723-726.
4. Kabalka G, Buonocore E, Hubner K, Moss T, Norley N, Huang L. Gadolinium-labeled liposomes: targeted MR contrast agents for the liver and spleen. *Radiology* 1987; 163:255-258.
5. Bryant LH, Jr., Brechbiel MW, Wu C, Bulte JW, Herynek V, Frank JA. Synthesis and relaxometry of high-generation (G = 5, 7, 9, and 10) PAMAM dendrimer-DOTA-gadolinium chelates. *J Magn Reson Imaging* 1999; 9:348-352.
6. Heyn C, Ronald JA, Mackenzie LT, et al. In vivo magnetic resonance imaging of single cells in mouse brain with optical validation. *Magn Reson Med* 2006; 55:23-29.
7. Shapiro EM, Sharer K, Skrtic S, Koretsky AP. In vivo detection of single cells by MRI. *Magn Reson Med* 2006; 55:242-249.
8. Dodd CH, Hsu HC, Chu WJ, et al. Normal T-cell response and in vivo magnetic resonance imaging of T cells loaded with HIV transactivator-peptide-derived superparamagnetic nanoparticles. *J Immunol Methods* 2001; 256:89-105.
9. Weissleder R, Moore A, Mahmood U, et al. In vivo magnetic resonance imaging of transgene expression. *Nat Med* 2000; 6:351-355.
10. Chen JW, Pham W, Weissleder R, Bogdanov A, Jr. Human myeloperoxidase: a potential target for molecular MR imaging in atherosclerosis. *Magn Reson Med* 2004; 52:1021-1028.

11. Hung SC, Deng WP, Yang WK, et al. Mesenchymal stem cell targeting of microscopic tumors and tumor stroma development monitored by noninvasive in vivo positron emission tomography imaging. *Clin Cancer Res* 2005; 11:7749-7756.
12. Weissleder R, Tung CH, Mahmood U, Bogdanov A, Jr. In vivo imaging of tumors with protease-activated near-infrared fluorescent probes. *Nat Biotechnol* 1999; 17:375-378.
13. Tung CH, Mahmood U, Bredow S, Weissleder R. In vivo imaging of proteolytic enzyme activity using a novel molecular reporter. *Cancer Res* 2000; 60:4953-4958.
14. Bremer C, Tung CH, Weissleder R. In vivo molecular target assessment of matrix metalloproteinase inhibition. *Nat Med* 2001; 7:743-748.
15. Wunder A, Tung CH, Muller-Ladner U, Weissleder R, Mahmood U. In vivo imaging of protease activity in arthritis: a novel approach for monitoring treatment response. *Arthritis Rheum* 2004; 50:2459-2465.
16. Louie AY, Huber MM, Ahrens ET, et al. In vivo visualization of gene expression using magnetic resonance imaging. *Nat Biotechnol* 2000; 18:321-325.
17. Mahmood U, Tung CH, Tang Y, Weissleder R. Feasibility of in vivo multichannel optical imaging of gene expression: experimental study in mice. *Radiology* 2002; 224:446-451.
18. Ntziachristos V, Yodh AG, Schnall M, Chance B. Concurrent MRI and diffuse optical tomography of breast after indocyanine green enhancement. *Proc Natl Acad Sci* 2000; 97:2767-2772.
19. Funovics MA, Alencar H, Su HS, Khazaie K, Weissleder R, Mahmood U. Miniaturized multichannel near infrared endoscope for mouse imaging. *Mol Imaging* 2003; 2:350-357.