

**The Physical Basis for Molecular Imaging**  
**G. Allan Johnson, Ph.D.**  
**Center for In Vivo Microscopy**  
**Duke Medical Center**

**Introduction**

The term “molecular imaging” is reasonably new. It refers in the broadest sense to the concept of spatially mapping (imaging) the presence of specific molecules, which are presumably of interest in some critical physiologic, biochemical, or disease process [1] [2]. There are a vast number of methods currently under development that easily fit this definition, each with its own advantages and disadvantages. The limited time permitted for this presentation precludes an exhaustive study of each. This presentation will focus on four active modalities: optical imaging, nuclear imaging (PET and SPECT), x ray (conventional radiography and CT), and magnetic resonance imaging (MRI). We will describe the fundamental process that provides image signal for each modality and discuss the impact of this signal on sensitivity, contrast, and spatial resolution. We will draw comparisons between the modalities with specific focus on preclinical methods (small animals). We will give specific examples in which the use of new agents has allowed significant increase in the sensitivity, spatial resolution, or both.

Figure 1 shows the electromagnetic spectrum spanning wavelengths covering 14 orders of magnitude from  $10^6$  microns (radio waves) to  $10^{-8}$  microns (gamma rays). The imaging modalities under discussion span this entire range. Magnetic resonance operates at wavelengths in the radiofrequency part of the spectrum. Optical imaging exploits the near infrared and visible part of the spectrum. X ray imaging is performed at wavelengths  $<10^{-2}$  microns. And nuclear imaging is done at wavelengths of  $\sim 10^{-6}$  microns.

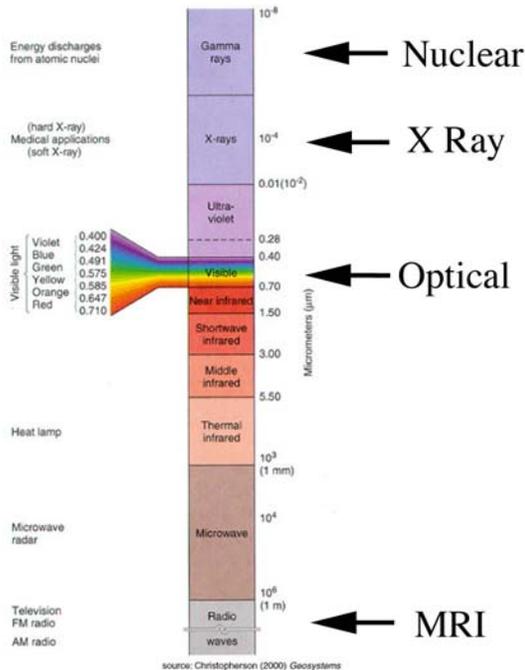


Figure 1. All of the molecular imaging methods employ some sort of electromagnetic radiation. The four methods under discussion span the entire spectrum

**SPECT Imaging**

One could readily argue that nuclear imaging is the most highly developed of the molecular imaging methods, particularly when one takes a narrow view of “molecular imaging”. Nuclear techniques are direct imaging methods where the signal source is the molecule of

interest. The origin of the signal is the decay of an unstable nucleus. The two most common methods of tomographic nuclear imaging are single photon emission computed tomography (SPECT) and positron emission tomography (PET). As the name implies, SPECT exploits single gamma rays from decaying nuclei. There are a number of potential nuclei for SPECT imaging.

Table 1 lists a few of the more common ones:

Isotope	Half Life	Energy(keV)
<sup>67</sup> Ga	78 hrs	93,184,296,388
<sup>99m</sup> Tc	6 hr	140 kev
<sup>111m</sup> In	2.8 days	23,26,173 247
<sup>123</sup> I	13 hr	159
<sup>201</sup> Tl	3 days	80,135,167

Table 1 Common SPECT Isotopes

The majority of the nuclides yield gamma rays at energies > 100 keV, so the radiation exits the patient with only limited attenuation. Since the nuclear detectors are able to count individual gamma rays, nuclear imaging methods are extremely sensitive. Concentrations in the nano or even pico mole can be detected. Thus these are truly trace probes, which have little effect on the local biochemistry.

The spatial resolution is determined by a combination of factors. The collimator is one of the most important. More closely spaced septa or collimators with higher aspect ratios define the solid angle of the radiation better thereby improving the spatial resolution. The energy of the gamma rays also influences the spatial resolution since the higher energy gamma rays can penetrate the septa. As with most imaging systems, there is a trade off between the sensitivity and spatial resolution. As the collimators define narrower acceptance angles, the amount of radiation reaching the scintillator is reduced. The spatial resolution can be very high for pinhole collimators in small animal systems (< 1x 1 x 1 mm) [3].

### PET Imaging

Positron emission tomography is a direct imaging method like SPECT that also detects gamma rays. But the source of the gamma rays is the pair production that accompanies positron decay. An unstable nucleus decays by conversion of a proton to a neutron with the simultaneous expulsion of a positron and a neutrino. The positron travels some distance until it encounters an electron. Since the positron and electron are antimatter/matter, they annihilate each other. As they do, they emit **two** gamma rays, each at 511 keV. The gamma rays are emitted in exactly opposite directions. Table 2 lists some of the most common PET emitters.

Isotope	Half Life	$\beta^+$ Energy(MeV)	$\beta^+$ Range
<sup>11</sup> C	20 min	1	medium
<sup>15</sup> N	10 min	1.2	medium
<sup>15</sup> O	124 sec	1.7	long
<sup>18</sup> F	1.8 hrs	0.6	short
<sup>64</sup> Cu	12.7 hrs	0.7	short

Table 2 Common PET radionuclides

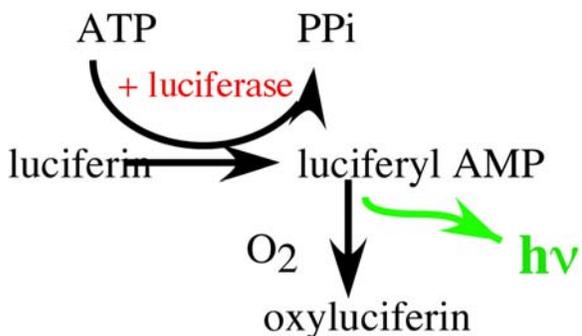
Like SPECT, PET imaging is remarkably sensitive detecting very low probe concentrations (nanomole). Note however the much shorter half life of most PET emitters. This imposes challenges in synthesizing and delivery of specialized probes. While spatial resolution is again dependent on many physical parameters of the scanner, the fundamental resolution limit is imposed by the uncertainty about how far the positron has traveled before it encounters the electron. Not surprisingly then, emitters with more energetic positrons result in lower resolution images. The fundamental resolution limit for most small animal PET systems is ~ 1.5 mm.

### X Ray Imaging

Some might argue that there are really very limited opportunities for radiographic “molecular” imaging. But if one chooses a broad definition of the term “molecular imaging” one can readily conclude that this is one of the more exciting methods particularly with the relatively recent development of microCT [4, 5]. MicroCT is an indirect method in which the molecular probe is not the source of the electromagnetic signal. The probe exerts its effect through a secondary modulation of the incident (xray) electromagnetic radiation. The use of iodinated contrast agents has been common in clinical practice for many years. The more recent introduction of specialized iodinated probes as blood pool agents presents some very interesting possibilities. The spatial resolution for (small animal) radiographic methods is 2-3 orders of magnitude better than the best nuclear imaging methods. Badea et al have recently reported in vivo microCT with isotropic spatial resolution of 100 microns using a new class of molecular probes with extremely high concentrations of iodine [6]. But the high spatial resolution comes at a price. The sensitivity of the microCT is abysmal. The sensitivity is determined by a number of competing issues factors- energy of the radiation, size of the specimen, and most importantly the total radiation flux [7]. In Badea’s work, the 100 micron resolution was achieved with  $\sim 10^{-2}$  molar concentrations of iodine at a total dose of  $\sim 0.15$  Gy.

### Optical Imaging

Optical imaging is another direct imaging method, i.e. a method where the molecule of interest is the source of radiation. There are a host of clever optical methods that have been developed by microscopists and molecular biologists over the years. One of the most popular methods is bioluminescence [8]. Bioluminescent imaging relies on the presence of an enzyme such as luciferase- the molecule responsible for light from the firefly. If the substrate for luciferase (luciferin) is present, the luciferase catalyzes the formation of oxyluciferin and light.



The luciferase gene is used as a reporter gene for the gene of interest. Those cells expressing the gene of interest will also express the marker gene. When the substrate is supplied, those cells will give off the characteristic bioluminescence.

Like PET and SPECT, bioluminescent imaging is very sensitive with detection limits in the nanomole. Bioluminescent imaging has intrinsically very high spatial resolution. Using conventional optical microscopes one can achieve micron levels of resolution. Optical methods do not suffer from the dose limitations of nuclear or radiographic methods since the energy of the optical photons is not sufficient to produce the biological damage done by ionizing radiation. Unfortunately this same attribute (low energy photons) poses one of the major drawbacks of optical methods. Most tissue is relatively opaque to optical radiation so the depth to which one can effectively image is very limited. In addition, optical radiation is scattered heavily by most tissues. Thus most of the molecular imaging done to date has been limited to small animal models. Extension to the clinical domain will require particularly clever approaches that are not entirely apparent at the moment.

### Magnetic Resonance Imaging

The majority of the molecular imaging studies in MRI have employed indirect methods where the molecular probe of interest exerts its influence on the signal from the water protons in some secondary fashion. One of the most successful techniques has been the use of paramagnetic contrast agents. These agents contain a source of unpaired electrons which couple strongly to the water protons. The presence of these paramagnetic agents results in enhanced proton signal. But the sensitivity is again, nowhere near that of optical or nuclear methods. Contrast agents at concentrations of  $10^{-4}$  molar are frequently necessary for major signal enhancement. The use of a wide range of paramagnetic agents is now routine in the clinical arena. But some would argue that this is more physiologic imaging than true “molecular” imaging.

Recent work has resulted in direct imaging methods for MRI where the molecule of interest is the source of the signal. Hyperpolarized  $^{129}\text{Xe}$  imaging was first proposed in 1994 [9]. The method was extended to  $^3\text{He}$  in small animals in 1995 [10] and shortly thereafter to humans. Through the use of optical pumping methods, the signal in the He and Xe atoms can be enhanced some 100-1000 X over that of the natural (unpolarized) substance. The enhanced sensitivity can be used for higher spatial resolution with resolution now on the order of 100 microns for small animal models [11].

### Summary

Comparison of imaging methods that are based on wavelengths that span 14 orders of magnitude, exploiting such a wide range of physical interactions, employing radically different methods of detection will inevitably end up comparing some apples to oranges. The table below is an attempt to make some comparisons of the relative sensitivity and spatial resolution for the four methods under comparison for the limited application of small animal imaging.

Method	Sensitivity	Spatial Resolution	Expense
PET	++++	+	++
SPECT	++++	++	+++
MicroCT	+	++++	+++
Optical	+++	++	++++
MRI	++	+++	+

PET and SPECT are the most sensitive methods. MicroCT is one of the least sensitive methods but the use of new (highly attenuating) agents shows promise. Optical imaging methods are a close second in sensitivity to the nuclear techniques but scatter and limited transmission of light have limited optical methods to 2D images to date. MRI is more sensitive than CT but less than optical and nuclear methods.

PET imaging has the lowest spatial resolution of the methods at  $\sim 1.5 \times 1.5 \times 1.5$  mm ( $3.4$  mm<sup>3</sup>). The resolution for SPECT is somewhat better at  $1 \times 1 \times 1$  mm ( $1$  mm<sup>3</sup>). MicroCT has the very best resolution of any of the methods at  $0.1 \times 0.1 \times 0.1$  mm ( $1 \times 10^{-3}$  mm<sup>3</sup>). Optical methods have excellent spatial resolution in plane. But scatter and penetration have made volumetric imaging difficult. MRI can rival microCT in resolution for fixed specimens, but limited sensitivity requires longer acquisition times than CT limiting in vivo resolution to  $\sim 0.1 \times 0.1 \times 1$  mm ( $1 \times 10^{-2}$  mm<sup>3</sup>).

While the PET cameras are not as expensive as MRI systems, the need for cyclotrons and specialized radiopharmacy equipment on sight imposes a significant additional expense. SPECT cameras are relatively inexpensive and the longer half lives of many of the probes means that they can be purchased from commercial sources that can take advantage of economies of scale. MicroCT is comparable in expense to SPECT. Optical systems are some of the least expensive. MRI systems are generally some of the most expensive systems.

### Conclusion

Molecular imaging is in its infancy. It is unlikely that any single method will become the dominant method. There is little doubt that new methods will continue to arise and existing methods will continue to evolve. Effective use of all of these techniques for preclinical and clinical application will demand an understanding of the fundamental source of image signal to allow informed decision about the relative strengths and weaknesses.

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