An introduction to molecular imaging in radiation oncology: A report by the AAPM Working Group on Molecular Imaging in Radiation Oncology (WGMIR)

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Molecular imaging is the direct or indirect noninvasive monitoring and recording of the spatial and temporal distribution of in vivo molecular, genetic, and/or cellular processes for biochemical, biological, diagnostic, or therapeutic applications. Molecular images that indicate the presence of malignancy can be acquired using optical, ultrasonic, radiologic, radionuclide, and magnetic resonance techniques. For the radiation oncology physicist in particular, these methods and their roles in molecular imaging of oncologic processes are reviewed with respect to their physical bases and imaging characteristics, including signal intensity, spatial scale, and spatial resolution. Relevant molecular terminology is defined as an educational assist. Current and future clinical applications in oncologic diagnosis and treatment are discussed. National initiatives for the development of basic science and clinical molecular imaging techniques and expertise are reviewed, illustrating research opportunities in as well as the importance of this growing field. © 2013 American Association of Physicists in Medicine. [http://dx.doi.org/10.1118/1.4819818]

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1. INTRODUCTION

To address educational and clinical issues for molecular imaging in radiation oncology, the American Association of Physicists in Medicine (AAPM) formed the Working Group on Molecular Imaging in Radiation Oncology (WGMIR). This report from the WGMIR is for educational and reference purposes, and reviews the terminology, basic scientific approaches, clinical applications, and national initiatives in molecular imaging as relevant to cancer diagnosis and treatment.

2. TERMINOLOGY, NOMENCLATURE, AND DEFINITIONS

Molecular imaging is the science whereby cells, or biological species such as proteins and molecules, are imaged or used to noninvasively image in vivo biological structure and/or function at the molecular or cellular level. Definitions of molecular imaging show varied scientific perspectives with common basic principles. In April 2005, the Society of Nuclear Medicine [SNM, now named the Society of Nuclear Medicine and Molecular Imaging (SNMMI)] and the
Radiological Society of North America (RSNA) held a summit meeting on molecular imaging that included representatives of the AAPM and other scientific societies. From this meeting a consensus statement was crafted to define “Molecular Imaging” as

“Molecular Imaging techniques directly or indirectly monitor and record the spatiotemporal distribution of molecular or cellular processes for biochemical, biologic, diagnostic, or therapeutic applications.”

A more descriptive definition was presented by the (former) SNM alone.2

“Molecular imaging is the visualization, characterization, and measurement of biological processes at the molecular and cellular levels in humans and other living systems. To elaborate, molecular imaging typically includes 2- or 3-dimensional imaging as well as quantification over time. The techniques used include radiotracer imaging/nuclear medicine, MR imaging, MR spectroscopy, optical imaging, ultrasound, and others.”

Depending on the molecular imaging technique, the detected signal may arise from an endogenous material or an administered material such as a contrast agent. Additionally, chemical or biological amplification of the number of signal-generating molecular species present may be manipulated to increase signal intensity, and thus detectability. Clinical imaging modalities are considered molecular imaging when physiological, chemical, or functional imaging is performed that images a patient’s biology instead of anatomy alone. For instance, the physiological distribution of a radiopharmaceutical in nuclear medicine imaging has a molecular basis, and the signal intensity in magnetic resonance imaging, though of nuclear origin, is regulated by the local molecular environment and thus also has a molecular basis. Both of these examples conform to the quoted definitions of molecular imaging.

For radiation oncology purposes, molecular imaging that improves detection of tumor cells, including their spatial extent, phenotype, and particular biological character, may be used to more accurately define targets and dose regimens for individual patients. One molecular approach to radiation oncology has been described as a “three-block process” comprising molecular imaging, molecular signature determination (e.g., molecular characterization of a tumor), and molecular therapeutics.3 Molecular therapeutics in radiation oncology refers to the use of ionizing radiation to initiate specific molecular events that are directly therapeutic themselves or to manipulate pathways that lead to an intended therapeutic effect.3 Molecular imaging methods are also being investigated as a direct monitor of the radiation delivery accuracy.4-7

Molecular imaging to assess radiation treatment response may provide the basis for prediction of tumor and normal-tissue responses, initially for populations of patients and later for individual patients as specific molecular signatures become available for clinical use. Tumor and normal-tissue response data may help in the formulation of biophysical response models that could be used to optimize computer-based treatment planning.8

Clinical imaging devices and tools are commercially available to acquire molecular images. For example, positron emission tomography (PET) and magnetic resonance imaging (MRI) systems can provide image data sets that can be correlated with computed tomography (CT) for radiation treatment planning and verification. The role of molecular imaging in cancer treatment will thus continue to increase.

Clinical imaging of a cancer target with conventional MRI and/or CT will show normal anatomy and the target region with variable enhancement due to natural tissue contrast or the administration of a contrast-enhancing agent. The incorporation of molecular, physiological, or other biological characteristics of a tumor into a treatment plan remains the responsibility of the clinician through clinical judgment, a complex process that assimilates laboratory and pathological reports, knowledge of spread of disease pathways, and expected clinical course. However, biological tumor characteristics, such as the presence of hypoxia and proliferation rate, are important determinants in radiation treatment response.9-11 Hypoxia indicates possible regions refractory to radiation treatment, and cell proliferation may indicate regions of active tumor growth. Thus, in vivo molecular imaging of cell hypoxia, proliferation, and other important tumor characteristics and properties is of great interest to the radiation oncology community.12-15 and will enable diagnosis, radiation treatment, and evaluation in the molecular manner described by Coleman and others.3,16,17 Of particular interest to the radiation oncology community is the concept of molecular target credentialing where a specific molecular target is imaged, its molecular signature defined, a treatment given, and the effect of the intervention and the signature is then evaluated. This approach can be used to validate the proposed target as a legitimate one for cancer therapy and to provide the opportunity for individualized therapy on the basis of both the initial characteristics of the tumor and the tumor’s response to an intervention.3

3. MOLECULAR IMAGING MODALITIES AND TECHNIQUES

The molecular imaging field includes multidisciplinary scientific expertise and multimodality imaging to produce anatomical, physiological, or functional images of biological processes over a range of spatial scales from molecular to entire organ levels. The number or density of molecular signals depends on the imaging technique and the targeted biological process or system. Definitions for the multidisciplinary terminology relevant for this field can be found in Cox and Nelson18 and Wagenaar et al.19 and specifically for oncology in Pomper and Gelovani.20

Five modalities are typically used for molecular imaging: (a) PET, (b) SPECT, (c) MRI, (d) optical, and (e) ultrasound. Table I lists these modalities and their corresponding contrast agents, and summarizes their salient physical properties and their respective advantages and disadvantages. These five imaging modalities and their techniques are now reviewed.
TABLE I. Various modalities and contrast agents used for molecular imaging (see Refs. 238–244).

<table>
<thead>
<tr>
<th>Technique</th>
<th>Tracers and probes</th>
<th>Spatial resolution</th>
<th>Signal detection</th>
<th>Sensitivity</th>
<th>Preclinical studies</th>
<th>Clinical studies</th>
<th>Temporal resolution</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>PET</td>
<td>Radiolabeled tracers</td>
<td>High-energy γ-rays (511 keV photons)</td>
<td>$10^{-15}$</td>
<td>1–2 mm</td>
<td>1.8–2.3 mm</td>
<td>Minutes</td>
<td>Very high sensitivity for functional, metabolic and molecular processes</td>
<td>Poor spatial resolution, expensive equipment, requires cyclotron, can monitor only one isotope, exposure to radioactivity</td>
<td></td>
</tr>
<tr>
<td>SPECT</td>
<td>Radiolabeled tracers</td>
<td>Low-energy γ-rays</td>
<td>$10^{-14}$</td>
<td>1–2 mm</td>
<td>3–5 mm</td>
<td>Minutes</td>
<td>High sensitivity, can detect multiple radiotracers and image multiple processes</td>
<td>Poor spatial resolution, expensive equipment, exposure to radioactivity</td>
<td></td>
</tr>
<tr>
<td>MRI</td>
<td>Gadolinium agents (±radiotracers)</td>
<td>Radiofrequency waves</td>
<td>$10^{-9} - 10^{-6}$</td>
<td>50–100 μm</td>
<td>0.25–0.5 mm</td>
<td>Seconds–hours</td>
<td>High soft-tissue resolution and contrast to noise ratio, dynamic studies</td>
<td>Expensive equipment</td>
<td></td>
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<tr>
<td>Optical</td>
<td>Bioluminescent and fluorescent probes</td>
<td>Light (near infrared)</td>
<td>$10^{-12}$</td>
<td>1–10 mm</td>
<td>...</td>
<td>Seconds–minutes</td>
<td>Highly sensitive for molecular functions, no radiation exposure, inexpensive</td>
<td>Depth-dependent attenuation of emitted light</td>
<td></td>
</tr>
<tr>
<td>Ultrasound</td>
<td>Microbubbles (±antigen labels)</td>
<td>Ultrasonic waves</td>
<td>$10^{-8}$</td>
<td>50 μm</td>
<td>0.5–1.5 mm</td>
<td>Subsecond–minutes</td>
<td>No exposure to radiation, quick, inexpensive</td>
<td>Poor imaging in presence of air or bone</td>
<td></td>
</tr>
</tbody>
</table>

*Sensitivity refers to moles of label detected.

3.A. Positron emission tomography

The basic physical event in the formation of PET images is a simultaneous detection of two photons produced by the annihilation of a positron within a targeted tissue in the patient. The positron-emitting radionuclides used are proton-rich isotopes, mostly cyclotron-produced, with half-lives between about 1 min and several days. These radionuclides are attached to a biological tracer molecule designed to localize in vivo in tissues with specific properties such as increased metabolism (e.g., glycolysis, cell proliferation, and protein or membrane synthesis), tumor hypoxia, or disease-specific targeting, as discussed in more detail below.

Positrons are emitted with a continuous energy spectrum with a characteristic maximum energy from 0.63 MeV (for $^{18}$F) to $\sim$ 3.7 MeV (for $^{76}$Br) among clinically used positron-emitting radionuclides. An emitted positron subsequently undergoes mutual annihilation with an atomic electron, generally forming a short-lived (~1 nsec) species known as positronium at or near the end of the positron range — the positron-electron pair then annihilates with the emission of two 511-keV photons that travel in opposite directions (180° in the rest frame). This process is much more common than the emission of three or more photons. The finite positron range (a few mm) and the noncollinearity of the annihilation photons (due to nonzero kinetic energy at the time of annihilation) impose two fundamental limitations on the spatial resolution. The former introduces a position distribution with long tails and full width at 20% of maximum amplitude between 0.5–10 mm, which strongly depends on the positron’s energy spectrum (the radioisotope used) and the type of tissue in which the annihilation occurs. The latter, annihilation photon noncollinearity, blurs the back-to-back emission with an angular dispersion of about 0.47°. The two 511-keV annihilation photons must interact in the detectors (i.e., crystals) of the PET scanner to produce a measured coincident event. In most current scanners, each scintillation crystal face is a rectangular shape with sides typically between 4 and 8 mm, which together with the detector ring radius ($r \approx 35$ cm) have a strong effect on PET spatial resolution. The multiple detectors are aligned in rings orthogonal to the longitudinal table axis in an efficient dual coincident photon detection geometry that for a single table position provides a typical scan length of ~15–18 cm in the longitudinal direction. Current clinical PET scanners have system spatial resolutions typically from 4 to 6 mm full.
width at half maximum (FWHM) and because of partial-volume averaging have difficulty accurately quantifying activity in tumors with diameters smaller than about 25 mm. Tumors with diameters comparable to or even smaller than the system spatial resolution may nonetheless be visualized if the tumor-to-normal tissue contrast is sufficiently large. For details on the types of current scintillation detectors, associated readout electronics, and PET scanner geometry and performance, the reader is referred to several literature reports.28–30

Detected annihilation photons undergo energy discrimination with an energy window centered around 511 keV (for example, from 350 keV to 650 keV). A coincident event is produced if two energy-discriminated photons are detected within a timing coincidence window, usually between 6 ns and 12 ns in duration. If the two photons originate from one positron annihilation then this event is a true coincidence. Accidental detection within the timing window of photons from different annihilation events produces “random (false) events.” If one or both of the photons from an annihilation pair undergoes Compton scatter, but retains sufficient energy to lie within the detection energy window, an erroneously positioned “scatter event” is produced. The rate of random coincidences increases with the total activity in the patient within the longitudinal field-of-view (FOV) of the scanner and with the duration of the coincident timing window. The rate of scatter coincidences depends on the location within the body and the patient diameter and can exceed 60% of the total detected events for large diameter patients.

Some older PET scanners operate in two acquisition modes, two-dimensional (2D) and three-dimensional (3D). In the 2D mode collimating septa extend between adjacent detector rings towards the patient and limit the detection of coincidences only to detectors from the same ring or within a small number of neighboring rings (typically 1 to 5). In 3D mode, the septa are removed and coincidences are allowed between detectors from a large number of rings, which leads to higher detection efficiency. Due to recent improvements of the event processing electronics and of the reconstruction and scatter-correction algorithms, all new PET scanners operate in 3D mode. Standardized procedures for determining the optimal activity concentration, the sensitivity, the resolution, the scatter fraction, and other parameters of the PET scanners have been developed.31,32

The quality of PET images is affected by fundamental physical and technical limitations, as described in part above and elsewhere,29,33 the related loss of contrast for small objects (partial volume effect),34–36 degradations due to attenuation,37–39 random events,40,41 scatter,42–46 sampling,47 are effects (nonequidistant radial sampling which leads to nonuniformities),48 the depth of interaction,49 variable-background activity,50 and organ and patient motion.51,52 Correction techniques have been developed and progress has been made in decreasing the effects of these factors on PET image quality and accuracy.35–37,53–59 Some of these limitations and their impact on the accuracy of PET segmentation of tumors are discussed in greater detail in an open access book chapter.60 Rapidly advancing technology and computing power continue to improve PET scanner performance. Examples of technology improvements include the introduction of smaller and faster responding detectors, more specialized and efficient signal processing electronics, and inclusion of resolution and time-of-flight information in the reconstruction process.49,59,61–63

Molecular imaging with the use of targeted radiolabeled tracers typically involves the following three main steps: (i) design of molecules that can target specific soluble or immobile cell receptors, called ligands; (ii) attachment of radioisotopes that emit photons without changing the biological binding properties of the target molecules; and (iii) injection and in vivo imaging, by using high resolution and high sensitivity devices. PET allows quantitatively accurate detection of molecularly targeted radioisotopes to determine tissue biological properties or processes, such as commonly performed imaging of glycolysis and cell proliferation. However, imaging of hypoxia, gene expression for both intracellular and cell surface receptors and proteins, tumor angiogenesis, and apoptosis are rapidly advancing.9,10,64–67 With direct radioisotope imaging, the radiolabeled material may be attached to a specific molecule that selectively determines the binding location of the labeled material. Examples include imaging of cell-surface epitopes using radiolabeled antibodies and of specific receptors using molecularly targeted peptides (Fig. 1).55,68

In this example, the image intensity distribution directly

![Fig. 1. microPET images of three BALB/c mice implanted with EMT-6 cells and imaged with 18F-FLT (a), and 76Br-labeled antibodies with a specific binding activity to Sigma-II in nonblocked (b) and blocked (c) animals. Animals were imaged in supine position. Reprinted with permission from D. J. Rowland, Z. Tu, J. Xu, D. Ponde, R. H. Mach, and M. J. Welch, “Synthesis and in vivo evaluation of 2 high-affinity 76Br-labeled α2-receptor ligands,” J. Nucl. Med. 47, 1041–1048 (2006). Copyright © 2006 by Society of Nuclear Medicine.](image-url)
correlates with the degree of malignancy, such use of SUV is hardly unambiguous—observed SUVs can be affected by image noise, coarse image spatial resolution, other concurrent nonspecific processes (e.g., perfusion), and user biased ROI selection. An AAPM Task Group is currently working to define the use of SUV for PET-based segmentation. In summary, SUV is a clinically useful quantity for the parameterization of PET images, but care has to be taken with the interpretation of the results.

Other applications of PET for molecular imaging include cerebral blood flow (perfusion) using $^{15}$O H$_2$O, tumor hypoxia (Fig. 2) with $^{18}$F fluoromisonidazole, and $^{64}$Cu-ATSM (diacetyl-bis(N4-methylthiosemicarbazone)), and cell proliferation with $^{13}$C thymidine, $^{18}$F fluorothymidine (FLT), and other radiolabeled thymidine analogs. Although all amino acids have been radiolabeled, methionine and tyrosine have been focused upon with PET imaging. Furthermore, synthetic amino acids such as $^{18}$F-fluoro-aminocyclobutyric acid (FACBC) have also been used for tumor imaging. Other advanced PET applications in oncology include imaging cell permeability, deoxyribonucleic acid (DNA) synthesis, tumor receptors, and monitoring of the distribution of and response to therapeutic agents.

The hybrid PET-CT scanner rapidly replaced PET-only scanners and is contributing to the radiation oncology community because it provides both a CT image with the needed electron density information for accurate radiation dose calculations and an accurately coregistered biomolecular PET image of the patient in the treatment position (Fig. 3). Software tools available in most commercial radiation treatment planning systems allow display and manipulation of the fused anatomic (CT) and biomolecular (PET) images.

### 3.B. Single photon emission tomography

Single photon emission computed tomography (SPECT) is a nuclear medicine tomographic imaging technique that employs the emission of x-rays and noncoincident gamma-rays by radiolabeled agents. Those radiolabeled tracers are injected intravenously in the human body and concentrate in an organ or structure of interest. Radiolabeled tracers are produced by the combination of gamma-emitting radioisotopes and a ligand that is designed to bind to certain molecules thereby targeting a particular anatomic area or metabolic or other biologic process. The emitted photons may pass through a collimator and the detector produces a 2D image of the tissue localization of the radiotracer. Compared to PET, using a collimator has the significant disadvantage of reduction in sensitivity. SPECT is a tomographic technique and produces 2D cross-sectional images at multiple angles around the subject that can be further manipulated for multiplanar or 3D volumetric reformats as needed. In contrast to PET, which detects time-coincident photon emission of positron-electron annihilation occurring in very close proximity to positron generation, SPECT provides direct detection and measurement of overall tissue photon emission that inherently possesses less radiation event localization information and therefore results
FIG. 2. Two separate examples of hypoxia PET imaging (all images are transverse sections). (a)–(c) Brain tumor imaging using (a) T₁-weighted and (b) T₂-weighted MR imaging followed by (c) ¹⁸F-fluoromisonidazole PET hypoxia imaging. (Arrow indicates a hyper-intense region surrounding the hypo-intense center of the tumor) (Images courtesy of JD Bourland, Wake Forest School of Medicine). (d) and (e) Imaging of head/neck cancer using (d) CT and (e) ⁶⁰Cu-ATSM PET hypoxia imaging. From K. S. Chao et al., “A novel approach to overcome hypoxic tumor resistance: Cu-ATSM-guided intensity-modulated radiation therapy,” Int. J. Radiat. Oncol., Biol., Phys. ⁴⁹, 1171–1182 (2001). Copyright © 2001 by Elsevier Inc.

FIG. 3. Fused fluorodeoxyglucose (FDG) PET/CT images in the (a) transaxial, (b) sagittal, and (c) coronal planes of a patient with lung cancer. CT was acquired at 45 mAs, 120 kV, pitch ~1. The PET scan was acquired 60 min after the injection of FDG (3D acquisition at 3 min per bed position). A large tumor in the left lung is apparent (arrow). Also present is malignant lymphadenopathy in the mediastinum (arrow head) and metastatic lesions in the liver (dashed arrow). Image provided by Dr. Bradley Kemp, Department of Medical Physics, Mayo Clinic, Rochester, MN.

in poorer image spatial resolution, typically several millimeters to 1 cm.

The main detector component of a clinical SPECT system is the Anger gamma camera. For each incident photon, a gamma camera collects signals from a number of photomultipliers (usually 50–70) that cover the entire field of view. Those signals are multiplexed by using a resistive network and only four position signals are derived, which are used to calculate the X and Y coordinates of each photon. More details on calculating the coordinates of the incident photons are available in the IAEA Human Health Series Report No. 6.

When the gamma camera is rotated around the object to be imaged, projection data are obtained and tomographic SPECT images are mathematically reconstructed. Typically, projections are acquired every 3°–6° while the camera rotates around the object of interest. A full 360° rotation is necessary to produce a reconstructed image of optimal quality. Depending on the imaging application 60 to 120 projections may be acquired, for example, in cardiac imaging, where the camera rotates 180° from approximately the right anterior oblique to the left posterior oblique to minimize the pronounced attenuation and scattering effects associated with radiation emitted latero-posteriorly from the heart. Correction techniques and reconstruction algorithms can compensate for those effects, but their clinical value and acceptance are still being addressed.

Acquisition of each projection typically lasts 15–20 s, which yields a total scanning time of around
20 min. Scanning times may take as long as 40 min for low administered activities and/or high-energy photon emitters such as gallium-67 and iodine-131. Of interest, accelerated acquisitions can be accomplished by using multihheaded gamma cameras.

In the Anger camera a lead or tungsten collimator, usually with hexagonal parallel holes, determines the photons that will be accepted and measured. The collimator is the main limiting factor of the resolution and sensitivity of clinical SPECT systems and its optimization remains an open issue, whereas in dedicated small animal imaging systems the multipinhole focused collimators appear to be the design of choice.85 A scintillator converts each photon, after passing through the collimator with initial energies that vary between 71 keV (Thallium-201) and 364 keV (Iodine-131), to thousands of optical photons with energies of several eV.86,87 This conversion-amplification task is performed by a combination of a scintillator with a photomultiplier tube (PMT). The scintillator is coupled to the PMT, usually through an optical light-guide such as silicon fluid, grease, or Lucite light pipes. Optical photons strike the PMT’s photocathode, which emits photoelectrons. By applying a high, usually negative voltage (∼1000 V) to a multidynode stage, the photoelectrons are multiplied, forming an electron cloud that hits the PMT’s anode. This charge is detected and further processed by acquisition electronics. Although all clinical SPECT cameras use scintillators made of sodium iodide doped with thallium (NaI-Tl), several new materials including cesium iodide doped with thallium (CsI-Tl), cesium iodide doped with sodium (CsI-Na), and lanthanum bromide doped with cerium (LaBr3:Ce) may offer improved light output, energy resolution or PMT coupling.83 Solid state detectors, which allow direct photon detection and have greatly improved energy resolution, are a relatively recent technological alternative to the conventional scintillator-PMT module.88 Energy discrimination by solid state detection can be crucial in novel applications where multiple radiotracers are administered and detection of gamma ray emission of different energies is necessary in order to differentiate tissue colocalization of different radionuclides.89 Even more importantly, however, may be the improved scatter rejection, and therefore improved contrast and even spatial resolution, resulting from superior energy resolution.

There are two major advantages of SPECT compared to PET. The first is the existence of radiotracers that are based on a variety of radioisotopes with different energies and their relatively long half-life that makes them easily accessible to routine clinical practice. The second is radioisotope availability for academic research, even at centers that are far away from cyclotron facilities. For an extensive description of the various radiotracers available for SPECT imaging and their distinctive properties the reader is referred to the review paper by Loudos et al.90 Currently, the radionuclides most frequently used for SPECT radiolabeling include 99mTc, 111In, 67Ga, 131I, and 201Tl.91–93 Tc-99m and In-111 are gamma emitters that are widely used for research purposes due to their availability, suitable half-lives, and relative ease of attachment to small molecules of interest.94,95 Endogenous ligands such as peptides, antibodies, hormones, and selectins can be relatively easily labeled for SPECT imaging.96,97 Also, because of their size these molecules diffuse slowly into tissue and have slow clearance from blood, which can be of the order of hours or even days. Thus, the long half-lives of commonly used SPECT isotopes makes imaging possible for slow biological processes (e.g., cell division, infection, and inflammation) and for validating the biodistribution of slowly localizing therapeutic radiopharmaceuticals.

Myocardial, brain, thyroid, and whole-body bone SPECT have become routine in the clinic. Myocardial SPECT includes cardiac-gated acquisition to provide quantitative parameters of myocardial function and perfusion such as heart muscle thickness, myocardial contractility, left ventricular ejection fraction, and cardiac output. Other specific applications are being developed.98 Dynamic renal scintigraphy shows that SPECT has the potential to provide valuable dynamic information. These studies are limited to the preclinical level and their clinical merit is to be determined.99 SPECT imaging of inflammatory properties of vulnerable atherosclerotic plaque is an active area of research, in order to preemptively identify high-risk coronary cases and individualize patient treatment.100 In the near future, innovative applications of SPECT imaging may include radioactive labeling and noninvasive in vivo tracking of stem cell trafficking and tissue homing in the interest of targeted cardiovascular stem cell therapies for myocardial infarction and therapeutic angiogenesis.101,102 SPECT has an increasing role in oncology due to a number of novel radiopharmaceuticals that are designed for specific tumors. For example, 99mTc-HYNIC Octreotide (99mTc-Oct) images somatostatin receptor expression and distribution, while 99mTc-Glucarate (99mTc-GLU) has increased tumoral affinity.103–105 SPECT tracers are being used for functional imaging and anatomical characterization of brain tumors,106 and in a preliminary evaluation have been used for tumor diagnosis, staging, monitoring of therapy outcomes, and detection of recurrence in lung cancer and malignant lymphoma.107 In early work, SPECT was used to evaluate pre- and post-treatment lung function and thus plays a role in the optimization of lung radiation treatment plans.108 In a similar manner, reproducible molecular imaging of tumor metabolic activity will allow better localization of tumor regions and early assay for the radiation response of tumors and normal tissues thereby increasing overall treatment safety and efficacy (Figs. 4 and 5).109

In basic oncology research, numerous preclinical studies have developed protocols of molecular imaging of tumor cell receptors, cell apoptosis, hypoxia, and angiogenesis.110–113 SPECT has been used to image integrin αvβ3 expression, VEGF, extracellular matrix, activated endothelial cells, and matrix metalloproteinase, all of the latter being implicated in tumor neovascularization.114 SPECT also has the unique ability to probe two or more molecular pathways simultaneously by dual-isotope/dual-energy imaging;115 thus, different organs or functions can be monitored simultaneously.116 Results from simultaneous imaging of Tc-99m and I-123, and of Tc-99m and Ga-67 are available.117 Multimodality SPECT imaging, usually combined with CT (hybrid SPECT-CT),
FIG. 4. $^{131}$I SPECT study in a female patient with follicular thyroid cancer, presenting with relapse of the disease 2 years after thyroidectomy and radioiodine ablation of the surgical remnant. Images were acquired 6 days after a new therapeutic administration of 4.5 GBq (150 mCi) of $^{131}$I. Acquisition settings were: energy photopeak 364 keV; energy window 20%; angular range 3600; 120 projections; and 20 s per projection. Tomographic reconstruction was accomplished by an iterative algorithm (OSEM, 2 iterations, 10 subsets). Consecutive coronal slices are presented, which show local cancer recurrence (solid arrows), cervical lymph node metastases (dashed arrows), and multiple lung metastases (arrowheads). The high contrast of the images is due to the high administered activity and the high tumor-to-background $^{131}$I uptake ratio, particularly in the neck.

has already evolved to standard practice in several fields of oncology, cardiology, and neuropsychiatry in order to combine functional and anatomical image acquisitions. Hybrid SPECT-CT imaging can achieve accurate coregistration of image data and improve diagnostic evaluation of indeterminate lesions detected on routine cross-sectional imaging of oncologic cases. SPECT is also expected to become a key element of future molecular imaging approaches, along with genomics, proteomics, metabolomics, and radiogenomics, contributing to the convergence of tissue-specific diagnostics and tissue targeted therapeutics, i.e., theragnostics.

3.C. Magnetic resonance imaging

The origin of the signal in magnetic resonance is the magnetic dipole moment, $\mu$, that results from those nuclei in which the net angular momentum is nonzero. In the presence of an external magnetic field ($B_0$) $\mu$ will undergo precession about $B_0$ with an angular frequency, $\omega_0$, defined by the Larmor equation, $\omega_0 = \gamma B_0$, where $\gamma$ is the nuclide-specific constant gyromagnetic ratio. At 1.5 T, this frequency is approximately 64 MHz for protons, placing it within the radiofrequency (RF) portion of the electromagnetic spectrum.

FIG. 5. $^{111}$In-Octreotide (OcteoScanTM) SPECT study in a patient with suspected neuroendocrine tumor of the pancreas. Images were acquired 24 h after radiotracer administration of an activity of 200 MBq. Acquisition settings were: energy photopeaks 171 and 245 KeV; window 20% around each photopeak; angular range 3600; 120 projections; and 30 s per projection. An iterative algorithm (OSEM, 2 iterations, 10 subsets) was employed in tomographic reconstruction. Selected transverse, coronal, and sagittal slices are presented, which show abnormal focal tracer uptake at the site of the pancreatic tumor (red cross). Normal tracer accumulation in the spleen (solid arrow), the lower part of the liver (dashed arrows) and the urinary bladder (arrowhead) is also demonstrated.
Signal formation, signal-to-noise ratio (SNR) and contrast are a result of the complex interaction of $\mu$ with its local environment. MR image formation is achieved by the application of RF pulses followed by linearly varying magnetic fields (gradients) that spatially encode the MR signal. Image contrast is determined by the relative distribution of tissues of differing $T_1$ and $T_2$ relaxation times and the choice of MR imaging parameters. The $T_1$ time constant describes the rate of energy loss to the lattice structure of the material (i.e., spin-lattice) while the $T_2$ constant describes the loss of phase coherence due to spin exchange between nuclei (i.e., spin-spin). A third time constant, known as $T_2^*$, includes the loss of signal due to macroscopic magnetic field inhomogeneities and is related to the $T_2$ constant by the relationship $1/T_2^* = 1/T_2 + \gamma \Delta B_0$ where $\Delta B_0$ describes the inhomogeneity. Parameters that emphasize $T_1$ or $T_2$ differences generate the so-called $T_1$- and $T_2$-weighted images, respectively. A third type of contrast in which neither $T_1$ or $T_2$ relaxivity is emphasized but rather the relative difference in tissue proton density can also be acquired. These images are known as proton density-weighted images.

The SNR in an MR image is in large part determined by the field strength of the main magnetic field, $B_0$, which in commercially available devices is 1.5 T (most common) or 3.0 T (gaining wider acceptance, particularly for neurologic imaging applications). It is noted that within the United States, the US Food and Drug Administration (FDA) has granted 510(k) clearance for commercial distribution of whole body MR scanners at field strengths of 3.0 T or less. Human MR imaging with field strengths above 3.0 T is restricted to research institutions as defined by approved Institutional Review Board (IRB) protocols, with maximum field strengths of 8.0 T for adults, children, and neonates older than one month and 4.0 T for neonates less than one month. Research imaging with humans at field strengths beyond these limits require specific permission by the FDA in the form of an investigative device exception (IDE).

MR spectroscopic imaging (MRSI), a variation of MR spectroscopy (MRS), is an in situ method of determining the relative concentrations (i.e., MRS) and spatial distribution of metabolites containing the nuclide of interest. $^1$H is the most common nuclide imaged using MRSI methods due to its relative abundance compared to other nuclei and has been demonstrated to detect, quantify, and differentiate neoplastic disease processes in the brain, breast, and prostate. Brain tumors are characterized by a loss of N-acetylaspartate (NAA), elevated levels of choline-containing compounds and lactate (in certain tumors), and reduced levels of creatine. Each of these chemicals provides an important signature of tumor growth and behavior: decreased NAA levels are associated with decreased neuronal density in the tumor, elevated choline is indicative of altered-membrane phospholipid metabolism, increased lactate levels result from increased anaerobic metabolism, and decreased creatine levels are indicative of altered bioenergetic pathways. MRSI of the prostate, the key biochemical changes include a three-fold reduction in citrate concentration and a two-fold increase in choline concentration compared to healthy peripheral zone regions. The peak area ratios of choline/citrate and (choline + creatine)/citrate appear to be reliable indicators of cancer in the prostate. Strong linear correlations between Gleason grade and both a decrease in citrate and increase in choline concentrations have been shown, suggesting that MRSI can be used as a metric for the assessment of radiation therapy efficacy. In breast cancer, increases in the levels of choline and its metabolites associated with cell membrane synthesis and turnover resulting from malignant cell growth can be monitored using MRSI to determine the presence/absence of cancer. $^1$H MRSI has also been investigated for diagnosis of neoplasms in a variety of other organs including liver, bone, and salivary glands and for assessing treatment response in head and neck cancers, thyroid, and melanoma. MRSI of nuclei other than hydrogen, such as $^{13}$C, $^{19}$F, $^{23}$Na, $^{31}$P, $^{35}$Cl, and $^{39}$K, is also possible. $^{31}$P is particularly significant because its presence can be used to elucidate intracellular pH and ATP homeostasis. However, the relative abundance of these nuclei is of the order of $10^{-4}$ to $10^{-7}$-fold less than that of $^1$H.

Acquisition of MRSI data can be performed using chemical shift imaging techniques. Figure 6 shows two spectra from voxels within the prostate overlaid onto a $T_2$-weighted image of the gland. The first spectrum, which is located on the left side of the gland within the peripheral zone, identifies a high citrate peak with relatively low choline and creatine peaks typical of healthy prostatic tissue. The second spectrum, taken from a different slice including both the central and peripheral zones, shows an absence of citrate with an elevated choline peak, suggestive of malignancy. Notice also that in this slice the spectroscopic voxel is located over a region of decreased signal on the $T_2$-weighted images and shows the characteristic signal decrease in those regions where cancer is present.

MRSI is technically challenging in comparison to conventional MR imaging methods and as such its integration into routine clinical diagnostic and radiation therapy planning has been slow. MRSI data do not directly lend themselves to familiar grayscale images, which are typically represented in pseudocolor-scale, and no commercial radiation treatment planning systems exist at this time that can readily utilize MRSI data. MRSI exhibits rather coarse spatial resolution, with typical MRSI voxel sizes on the order of 0.1–1 cc. Smaller sampling sizes are possible, but require longer imaging times and/or higher magnetic field strengths.

Perfusion is the mechanism by which nutrients and oxygen are delivered to and waste products are removed from cells. This process can be visualized by MR using a perfusion contrast agent that can be either endogenous or exogenous. Blood is an effective endogenous contrast agent and perfusion imaging with blood as the contrast agent is performed by acquiring two data sets: the first in which the magnetization of the blood on the arterial side of the imaging volume is nulled or saturated (measured signal is set as the
FIG. 6. MR $^1$H spectroscopic profiles characteristic of (a) normal and (b) cancerous prostate tissue. T$_2$-weighted axial images are used to identify the region of interest from which the spectra is sampled (square region) with the associated metabolic profile shown adjacent. Normal peripheral zone tissue is characterized by a high citrate and low choline concentrations as seen in (a). In contrast, the large hypo-intense mass (arrows) seen on the T$_2$-weighted image in (b) shows a marked elevation of choline and no identifiable citrate peak. Metabolite locations are identified by their relative frequency shift from the water resonant frequency in units of parts per million. Metabolite peaks are measured in relative units. Images courtesy of Dr. Akira Kawashima, Department of Radiology, Mayo Clinic.

In diffusion MRI, the relative mobility of water within the imaging volume is the basic property from which signal contrast is derived. Any unbound molecule (water or other) will undergo random translational, or Brownian, motion. Under the influence of a magnetic field gradient, the MR signal, S, from a molecule undergoing diffusion will decrease according to the monoexponential relationship, $S = S_0e^{-bD}$ where $S_0$ is the signal in the absence of diffusion, “b” is a factor proportional to the integral of the applied diffusion gradient.

FIG. 7. Relative cerebral blood volume (rCBV) measurements obtained from an MRI perfusion imaging sequence. Asymmetric perfusion distribution between opposing hemispheres indicates an unequal distribution of blood. The relative increased blood flow around the tumor periphery (arrow) indicates breakdown of the blood-brain barrier while the lack of signal within the central zone of the tumor indicates necrosis/calcification. rCBV values color coded in terms of the number of standard deviations from the mean cerebral blood volume within a normal (i.e., reference) region of the brain. Image provided courtesy of Dr. Kirk Welker MD, Department of Radiology, Mayo Clinic.
sensitizing gradient waveform(s) over their duration (s/mm²) and D is the diffusion coefficient (mm²/s). Acquiring an MRI image with and without the application of this diffusion sensitizing gradient provides a measure of S and S₀, allowing an estimate of D.

The extent of diffusion of water in vivo is highly dependent upon a variety of factors that include fluid viscosity, cellular permeability, active transport mechanisms, and the microstructure of the local environment. As a result of the sensitivity of diffusion MR imaging to these tissue properties, diffusion-weighted MR imaging has been used for a variety of oncologic applications. MRI-derived cellularity differences among tissues have been correlated with diffusion-weighted MRI and have been associated with the onset of neoplastic changes, the ability to differentiate tumor from benign or necrotic tissue, and tumor grade. The serially monitored apparent diffusion coefficient (the measured diffusion value) has also been shown to correlate with response to chemotherapy as well as to be a potentially useful tool for monitoring therapeutic response to radiation therapy of brain cancer. Diffusion tensor MR imaging, which quantifies diffusion anisotropy as a second-rank tensor within each voxel, can be used to track the maximum diffusion value along nerve fibers, providing so-called tractography images, and has been used extensively for mapping white matter tracts in the brain (Fig. 8). Fiber tracking using diffusion tensor information is also providing important treatment planning information for both surgical and radiation therapy approaches, allowing visualization of the spatial relationship between these tracts and tumor and their distortion by the tumor. Because of the technical challenges and dependence upon user input, tractography images are not currently considered sufficiently reliable to be useful for routine radiation therapy planning applications.

Functional MR imaging (fMRI) provides information on the hemodynamic and metabolic changes in the brain following neuronal excitation. It has been shown that during excitation cerebral blood flow can increase between approximately 30%–50%. The increased perfusion provides a concomitant increase in oxyhemoglobin, increasing the relative concentration of oxygen in activated regions. However, oxygen consumption in these regions increases by approximately 5% as the baseline state of aerobic metabolism switches to the active state of anaerobic glycolysis. The disassociation of oxygen supply and consumption forms the basis of the contrast mechanism used in fMRI and is known as blood oxygen level-dependent (BOLD) contrast. The magnitude of the BOLD response is determined by the relative concentrations of deoxyhemoglobin (paramagnetic) and oxyhemoglobin (diamagnetic) within the blood. Paramagnetic substances produce local magnetic field inhomogeneities which result in susceptibility induced dephasing and signal loss. T₂*-weighted gradient echo imaging sequences are used to quantify the signal difference by acquiring two data sets, the first in the resting state in which the brain is saturated with deoxyhemoglobin and the second following neurological activation when the inflow of oxyhemoglobin is greatest. Because it is noninvasive in nature and avoids the use of ionizing radiation, fMRI has been used for the assessment of neuronal function pre- and post-treatment and to identify...
brain regions to be avoided (Fig. 9) with either surgical or radiosurgical treatment.163, 164

For exogenous contrast, and because of its paramagnetic properties, solutions of chelated organic Gadolinium (Gd) complexes are used as intravenously administered MRI contrast agents.165, 166 To avoid Gd$^{3+}$ toxicity, Gd contrast agents are encased in a chelate molecule. Gd-based contrast agents reduce tissue T$_1$ and T$_2$ relaxation times and are used to increase tissue contrast resolution. A new class of Gd contrast agents is currently under investigation as specific agents for molecular imaging. These agents differ from Gd-DTPA complexes by the inclusion of functional elements into the molecular complex that can recognize and bind to specific molecules or in relation to specific molecular processes occurring on or within the cellular membrane. Like targeted drug therapy, these molecular constructs are characterized by a functional unit that provides the specificity necessary to identify the molecular process, a molecular “vehicle,” and the Gd ion(s). Ideally, the concentration of Gd at each cell should be sufficient to identify them individually. However, such a scenario requires voxel dimensions to be of the same resolution as the cell’s diameter, which is not achievable on clinical, whole body MR imaging systems. The functional unit can serve to attach the molecular structure to a specific cell receptor or provide a mechanism by which Gd can be resolved directly across the cellular membrane to accumulate within the cytoplasm of the cell. Aime et al.167 and Sosnovik and Weissleder168 have presented reviews of these processes. Such approaches fall under the current scope of nanotechnology MR imaging.169-171

### 3.D. Optical imaging

Optical molecular imaging is based on detecting visible and infrared photons after they are transmitted through biological tissues. The resulting images are a result of photon emission, reflectance, absorption, scattering, and phase-shift. The advantages of optical imaging are its noninvasiveness, real time observation and acquisition, ease of use, low cost, and minimal toxicity to biological systems. Since energy levels of atoms emitting infrared and optical photons are low, from $\sim$1 to $\sim$10 eV, path-lengths (i.e., penetration depths) in tissue are relatively short (several cm at most). Thus, optical imaging tends to be used for relatively thin geometries, and has been more widely used for in vitro measurements and surface or near-surface in vivo imaging of small animals. Major optical imaging techniques are bioluminescence, fluorescence, and diffuse/coherent tomography,67 ordered from highest to lowest sensitivity.172

Bioluminescence imaging (BLI) uses biologically produced photon emissions. The predominant technique uses firefly luciferase, the enzyme that is responsible for production of the yellow-green light emitted by fireflies173, 174 (Fig. 10). The luciferase gene is cloned and spliced into target DNA of specific cells ex vivo and the cells then infused into the study subject. Other luciferases, such as renilla luciferase, are also used.175 With a biochemical energy source and oxygen, luciferase catalyzes reactions that result in yellow to green photon emission (approximately 500 nm). Self-contained BLI commercial systems are available for small sample sizes. Uses of BLI include imaging of in vivo gene expression, monitoring of protein–protein
interactions, tracking cancer cells, and whole body imaging of small animals. Also, \textit{in vivo} growth and response of tumors and metastases before and after chemotherapy have been studied for prostate and other cancer models. BLI remains largely a planar (or 2D) imaging modality and reconstruction of the 3D \textit{in vivo} distribution of bioluminescence is challenging because of the numerous and complex optical photon interactions in tissue. Bioluminescence tomography (BLT) is being pursued, however, offering the possibility of providing reliable depth information and greater quantitative accuracy.

Fluorescence techniques can be divided into optical and near-infrared (NIR) fluorescence imaging; green fluorescent protein (GFP) is the most widely used of the optical fluorophores. These techniques can be implemented using general-purpose charge-coupled detector (CCD)-based cameras and data acquisition and processing techniques that allow deconvolution of the abundant tissue autofluorescence from that of the signal fluorescence of the specific fluorophore being imaged. GFP imaging has been developed based on the discovery and isolation of that protein from jellyfish and other iridescent creatures. Organic fluorophores have been used for NIR imaging. The wavelengths of light for GFP imaging range between 380 nm and 780 nm while those for NIR imaging range between 700 nm and 1000 nm. Uses of GFP include imaging of \textit{in vivo} gene expression, monitoring of protein–protein interactions, and tracking of protein populations.

The GFP gene is inserted into a genetic construct which in turn is incorporated into the DNA of the cells of interest. \textit{In situ} fluorescence is stimulated by an external excitation visible-light source of the appropriate wavelength. Absorption at 489 nm results in fluorescence at 508 nm without any exogenous agent being administered—the product of the cloned GFP gene leads to fluorescence. The resulting emission intensity pattern can be digitally captured and displayed with a desired grayscale or color-scale, indicating the distribution or intensity of protein activity. GFP has been modified to produce blue, red, yellow, and cyan versions (BFP, RFP, YFP, and CFP, respectively) through wavelength shift mechanisms due to amino acid substitutions in mutant variants. The use of such multiple discrete wavelengths allows discrimination of multiple targets or processes.

Diffuse optical tomography (DOT) is based on NIR light that is transmitted through tissue and detected using an array of sources and detectors distributed over a large region. The NIR light is used to probe the interior of the body for physiological changes and can provide functional data those are derived due to the light absorption spectra of specific molecules. DOT can provide either scalar- or vector-based measurements. Scalar methods measure the optical flux exiting the tissue and the photon path length. The vector methods measure both the optical flux magnitude and the average propagation delay using either a time-domain or frequency-domain system. Time-domain systems use picosecond-wide optical pulses and time-gated photon counting detectors; frequency-domain systems use a RF modulated light source, photomultiplier tubes or fast photodiodes, and RF phase detectors. The maximum imaging depth of DOT is about 1.5 to 2.0 cm. The advantage of using frequency-domain systems is a greater SNR compared to that of time-domain systems. DOT has been used to image the molecular function of breast cancer.

Optical coherence tomography (OCT) uses backscattered light photons and a Michelson interferometry detector system to produce 2D images of the structure of superficial layers of tissue. The principle of OCT is analogous to that of ultrasound B-mode imaging except OCT uses light instead of acoustic waves to differentiate tissue composition by measuring the light reflectance. The technique has been described as “an optical biopsy,” since OCT can produce near-histologic images without excision, with a spatial resolution of 1 to 15 \(\mu\)m. Due to photon absorption and scattering, its sampling depth is limited to within 2–3 mm of the surface in tissues. The 2D images can be assembled to construct a 3D image set. Originally developed for ophthalmology, OCT is being applied to cancer diagnosis and tissue characterization, for instance, for optical imaging of larynx cancer, and may be able to perform imaging at the cellular level. OCT is an example of an imaging technique that acquires signals that have a molecular origin. Thus, it can provide novel anatomical information at a scale finer than that given by CT. Potential future applications of OCT in radiotherapy include refined target and critical organ definitions for treatment planning for areas reachable by endoscopy, after registration to the planning CT.

Recent advances in the development of nanoparticle imaging probes have introduced semiconductor-based quantum dots (QDs) for \textit{in vivo} molecular and cellular optical imaging.
imaging. Quantum dots are luminescent nanocrystals composed of atoms from groups II–VI or III–V elements in the periodic table. These 2- to 6-nm size QDs provide a promising alternative as optical contrast agents to organic dyes and fluorophores. The advantages of using QDs are tunable emission from visible to infrared wavelengths, large absorption coefficients across a wide spectral range, very high levels of brightness, photostability resistant to metabolic degradation, and small size. Bioconjugated QDs offer new possibilities and challenges for highly sensitive optical imaging of molecular targets in genes, proteins, living cells, and animal models and for early cancer detection, tumor cell tracking in real time, and monitoring of cancer treatment in humans.

3.E. Ultrasound

Developments of ultrasound for molecular imaging include the characterization of tissues through spectral analysis (i.e., the spectrum of flow velocities are mapped vs time) and the use of specialized contrast agents that enhance imaging for a specific biological structure or process. Reflected ultrasound carries spectral information that varies with structures and with molecular components that comprise tissues. Tissue identification and distinction of normal and abnormal tissues has been and continues to be investigated. Conventional ultrasound imaging provides structural information. In addition, ultrasound contrast imaging offers functional information such as perfusion status, tumor viability, and molecular expression in intravascular targets. Ultrasound contrast imaging methods include the use of small gas-filled bubbles (microbubbles) distributed to tissues via the vascular system. Microbubbles provide contrast due to the echogenicity of the microbubble gas or its containing shell. Molecular specificity is ensured by the attachment of antibodies, peptides, and other ligands to the microbubble surface. Microbubbles with a stabilizing shell are now FDA-approved. Microbubble diameters range from 1 to 10 μm and shell thicknesses from 10 to 200 nm. The lifetime of microbubbles depends on the characteristics of the gas inside, the bubble shell composition, and the acoustic waveform (intensity and frequency) striking the microbubbles. Ultrasound contrast can also be provided by the use of nonbubble emulsion contrast agents.

Major applications of ultrasound-based contrast imaging are blood vessel detection and assessment of tissue perfusion and vascular delivery of drugs or genes. Ultrasound contrast imaging has succeeded in the detection of inflammation and angiogenesis of tumors, both of which play important roles in tumor growth and metastasis. The general paradigm of ultrasound perfusion imaging is to acquire (i) a baseline (no contrast) image, (ii) subsequent dynamic contrast-enhanced images following a bolus of microbubbles, and (iii) a high power ultrasound burst to rupture the microbubbles and thus eliminate the contrast enhancement. The microbubbles are administrated intravenously and 1–2 ml of contrast agent is sufficient to produce good-quality imaging. Microbubbles manufactured to 2–4 μm diameters can pass through the capillary bed but do not extravasate into the interstitium. Microbubbles may rupture spontaneously when they travel under the ultrasound beam, depending on the level of emitted mechanical energy. Their elimination is achieved via the lungs following their trans-pulmonary passage and diffusion into the alveolar air. Microbubbles are completely eliminated from the body approximately 20 min after intravenous injection.

Advantages that ultrasound offers are real-time imaging, noninvasiveness, nonionizing radiation, portability, low cost, and high spatial and temporal resolution. The resolution of ultrasound molecular imaging ranges from 50 μm to 0.5 mm. This high spatial resolution is attainable by using frequencies from 20 to 100 MHz. The primary competing characteristics for ultrasound molecular imaging are depth of penetration and spatial resolution, which vary inversely and directly, respectively, with increasing frequency.

Recently, targeted ultrasound imaging has been enhanced by developing new contrast agents such as liposomes and nanoparticles, which target particular intravascular receptors or other structures. Molecularly targeted microbubbles offer the potential for personalized diagnostic imaging and therapy through delivery of targeted contrast as well as interventional agents. Use of receptor-specific ultrasound contrast microbubbles to demonstrate highly vascularized tumor periphery is illustrated in Fig. 11.

4. MOLECULAR IMAGING CHALLENGES IN CLINICAL RADIATION ONCOLOGY

4.A. Spatial scale in molecular imaging

The spatial scale of molecular imaging techniques covers approximately 4 orders of magnitude, from the use of optical and nanosensor techniques at the subcellular level (1 μm resolution) up to SPECT and MRSI (10 mm resolution). This wide variation in spatial scale presents challenges with respect to integrating such data into a clinical radiation treatment planning system, such as molecular image registration with the corresponding planning CT. Figure 12 shows the spatial capabilities and relative importance for CT and molecular techniques (see Ref. 212). A more detailed table of the limiting spatial resolution for different imaging modalities is presented in Ref. 213. Although the spatial and temporal resolution of various imaging modalities is improving due to technological advances, fundamental physical considerations in some cases may impose limits on the resolution achievable.

4.B. Image quality

Overall image quality depends on a number of complex interacting factors, including the physical processes affecting the signal and its origination (depth and surrounding tissues), acquisition sampling rates (spatial and temporal), background signal, target-to-background contrast, statistical uncertainty (or “noise”), scanner performance parameters, and the underlying biology which determines the distribution of the
Ultrasound imaging of tumor vasculature by the use of ultrasound contrast microbubbles (MB) conjugated to the tripeptide sequence arginine-arginine-leucine (RRL). Nude mice bearing PC3 human prostate cancer cell lines were imaged 120 s after injection of either MBRRL or a MB control. Transverse ultrasound image depicting persistent contrast enhancement in background subtracted images after injection of MBRRL [(a); white arrow], which is not seen in the control [(b); white arrow]. Noncolor coded portions are not background subtracted. The source of these images (From G. E. Weller et al., “Ultrasonic imaging of tumor angiogenesis using contrast microbubbles targeted via the tumor-binding peptide arginine-arginine-leucine,” Cancer Res. 65, 533–539 (2005). Copyright © 2005 by American Association for Cancer Research.) states that the opacification is matching the anatomic pattern of the tumor vasculature, with a highly vascularized tumor periphery [white arrow in (a)] and a necrotic tumor core [white dashed arrow in (a)].

imaging signal in vivo. For instance, an ionizing photon emission technique will suffer from signal degradation from internal attenuation and scatter, and an optical tomography technique will be dependent on photon scatter and sampling rate. Image quality of molecular images can be assessed using image analysis techniques developed for conventional imaging. Each molecular imaging modality is unique and will require specialized quality assurance and quality control. Some imaging studies may require individual calibration or quality assurance testing for each patient. Standardized phantoms, and QA tests and benchmark data for each imaging modality for a variety of lesion locations would be extremely valuable in helping users detect and/or avoid errors in image acquisition and processing and application to therapy.


4.C. Biologic structure definition and response

The use of molecular images in radiation treatment presents various challenges, including (1) image transmission, (2) registration of multimodality images, (3) knowledge of the information fidelity as well as image interpretation (tumor vs normal tissue vs inflammation) for the molecular image modality in use, and (4) composition of the target and critical volumes from a set of multimodality, correlated image sets. Issues of patient immobilization and motion, image file formats, registration algorithms, and verification of software tools for processing and display also have to be addressed.

Problems associated with multimodality image registration and fusion have been largely resolved for most clinical imaging modalities, including those with noisy (i.e., sparse) data. The introduction of multimodality, hybrid devices (e.g., PET-CT) has further simplified this process. However, molecular image sets may require more robust registration algorithms and image display and analysis capabilities in order to accommodate the wide range of spatial resolution, signal intensity, image matrices and depth, and voxel dimensions encountered across such modalities. Current commercial external-beam radiation treatment planning systems likely cannot accommodate the variety of molecular imaging data sets. The digital imaging and communications in medicine (DICOM) and DICOM - radiation therapy (DICOM-RT) image format standards will be important for “routine” use of molecular images.

Accurate image interpretation is required in order to use molecular images for radiation oncology applications. Correct image interpretation can yield information on the diagnosis, classification (segmentation) of normal and target tissues, evaluation of a treatment plan, and treatment effectiveness. The seemingly simple task of setting the window-level
to manually draw a structure on a molecular image may mislead the user to draw a smaller or larger target volume than would be specified by using a quantitative (albeit a relative) index such as the SUV. Even target volume definition with FDG PET for lung cancer remains an elusive process with quite variable results depending on the approach used for determining the target boundary. Clearly, users of molecular images will need to have experts who can interpret the biological image and understand its clinical characteristics and limitations. Robust software tools are also needed to aid or direct the interpretation and delineation processes.

A fundamental understanding of the physical and technical factors that affect the qualitative appearance and quantitative accuracy and precision of molecular images is needed to safely and meaningfully apply these images to therapy. In other words, the biological significance must be known for the intensity level of a particular voxel for the molecular imaging technique. In vivo molecular imaging has been and will continue to be used to assist in identifying cancerous tissues in the body. The biological attributes of tumor and normal tissues cannot be assessed accurately if the information in the molecular images is biased. Common image artifacts affecting the accuracy of diagnostic PET images are routinely “read through” by the nuclear medicine physician or radiologist. However, if image artifacts were mistaken for areas of active tumor, they might be erroneously targeted for increased radiation dose, with potentially serious negative consequences. The accuracy and precision with which molecular imaging techniques permit localization of regions for increased dose targeting and response assessment are affected by the acquisition, image processing, and display methods.

Even with appropriate image manipulation and registration tools and an understanding of the technical and biological characteristics of molecular images, the clinical use of such images is still challenged by the needs to define a target volume. Current image-based radiation treatments use relatively simple binary (or segmented) targets that define regions to be treated or avoided. With intensity modulated treatment, dose constraints are used to optimize target volume coverage while specifying the dose limits for critical and normal tissue volumes. Although likely to be useful, biological imaging complicates the optimization process by (1) increasing the amount of potentially relevant information to enable “biological” constraints, and (2) providing novel images about which the user may be uncertain regarding image quality or interpretation. These considerations have been discussed in general and for radiosurgery in particular. The reality is that the biological target volumes for multimodality image sets will not be congruent in size or shape. Temporal effects must also be addressed when defining the target, an example of which is given by Sovik et al. In their study, the tissue oxygenation of canine sarcomas was measured by DCE-MRI throughout the course of fractionated radiotherapy to adaptively tailor the IMRT plan to tumor hypoxia. Although hypoxia imaging has been one of the first uses of biologic imaging for clinical radiation oncology purposes, there has not been significant clinical success. Thus, the simple binary anatomical target becomes one with a variable boundary which is biologically matched to a radiation dose distribution of one or more dose levels for an individual patient. For instance, recent work shows that MR nerve tract diffusion tractography may predict for pathways of spread for brain tumors, such that tracts originating in or near the gross tumor volume may require inclusion in the radiation treatment field. A hypothetical clinical target volume is shown, including the obvious diffusion tracks identified via MR diffusion imaging (Fig. 13). Developing a culture in which diagnostic radiologists, radiation oncologists, physicists, pathologists, basic scientists, vendors, and others routinely collaborate together to interpret, use, and perform quality assurance of molecular images is necessary to determine the appropriate clinical applications for these images.

4.D. Biological modeling and application of molecular imaging to radiation oncology both for treatment planning and response assessment

Predictive models based on biological data from molecular images will provide information to the healthcare team when making therapeutic decisions and prognoses. The recently published report of Task Group 166 includes brief descriptions and many references to current models for normal tissue and tumor radiation response. Molecular images should provide accurate spatial distributions of quantitative biological data for tumor and normal tissues, enabling the resulting biophysical models to be applicable to individual patients. Image acquisition and processing techniques should be standardized to insure image quality is acceptable for quantitative use in biological modeling of radiation dose response.

The principles of modern radiation oncology treatments underscore the significance of image-based identification of the actual extent of viable tumors, to maximize tumor dose coverage and uniformity and at the same time protecting surrounding structures. Of particular interest, molecular imaging may be used in radiation oncology to augment both treatment planning and assessment of response to therapy. Modern high-resolution anatomical imaging combined with high-sensitivity molecular imaging can achieve volumetric tumor characterization and quantitative modeling of tissue irradiation. In this way, more accurate planning of targeted and actual gross tumor volume may be achieved, while sparing neighboring sensitive organ structures from unnecessary irradiation. Noninvasive molecular imaging modalities, such as PET and SPECT, are increasingly proposed for the collection of unique information about tumor radiobiology, for example, hypoxia and glycolytic metabolism, and the use of such information allows for optimized “dose-painting” or “dose-sculpting” when implementing modern conformal or intensity-modulated radiation therapies.

5. INITIATIVES IN MOLECULAR IMAGING

Molecular imaging for cancer characterization, diagnosis, and treatment is a national priority. The National Cancer Institute (NCI), through its Cancer Imaging Program (CIP), is providing numerous opportunities for research funding and
FIG. 13. Diffusion track imaging of potential pathways of spread for a primary brain tumor. (a) T1-weighted postcontrast image showing the primary anaplastic astrocytoma and treatment margin (pink line, 90% isodose) obtained from the treatment plan by computed tomography (b). (c) Diffusion tensor imaging reference image \( (b = 0) \) taken at the same time point showing all major diffusion paths passing through the tumor. Two major bundles pierce the treatment margin. (d) T1-weighted postcontrast image 3 months after the time point of (a) showing recurring tumor (green box) just outside of the treatment margin and in the same location as the major posterior bundle (c). (e) The treatment plan that was used for stereotactic radiotherapy (pink line) and the proposed anisotropic treatment plan (green line) with increased dose along the two prominent bundles emanating from the primary tumor and reduced margin along other directions. (f) Depiction of the original (pink) and proposed (green) treatment plans of the follow-up T1-weighted postcontrast image. Their contention was that the proposed plan might have stalled or may have even prevented the onset of the secondary tumor. Reprinted from A. P. Krishnan, I. M. Asher, D. Davis, P. Okunieff, and W. G. O’Dell, “Evidence that MR diffusion tensor imaging (tractography) predicts the natural history of regional progression in patients irradiated conformally for primary brain tumors,” Int. J. Radiat. Oncol., Biol., Phys. 71, 1553–1562 (2008). Copyright © 2008 by Elsevier Inc..

Policy setting in the area of novel cancer imaging with the mission to promote and support “cancer-related research in imaging sciences and technology for the understanding of cancer biology and . . . the management of cancer and cancer risk.” The NCI-driven quantitative imaging initiative requires substantial medical physics input to be successful. Opportunities exist at all levels from the basic to clinical sciences and for academic and commercial entities within the following programs: (1) American College of Radiology Imaging Network, (2) Network for Translational Research: Optical Imaging, (3) Development of Preclinical Drugs and Enhancers, (4) In Vivo Cellular and Molecular Imaging Centers, (5) Interagency Council on Biomedical Imaging in Oncology, and (6) Small Animal Imaging Resource Program. The National Institute for Biomedical Imaging and Bioengineering (NIBIB) has several programs for research funding in the areas of development of novel drug and gene delivery, image-guided interventions, improvements in imaging methods, systems and methods for cellular and molecular imaging, systems and methods for small animal imaging, nuclear medicine, optical imaging and spectroscopy, ultrasound, and x-ray, electron, and ion beam imaging. Other opportunities for education and collaboration have been afforded by the Biomedical Imaging Research Opportunities Workshops (BIROW) and by symposia on molecular and oncologic imaging being hosted by scientific societies such as the AAPM, the RSNA, the American Society of Radiation Oncology (ASTRO), and the American College of Radiology (ACR).

6. CONCLUSIONS

Molecular imaging is the imaging of specific, molecular sources using radioisotope, magnetic resonance, optical, or ultrasound methods. Oncologic molecular imaging has been established as a national priority by government and scientific organizations, and holds great promise for patients through imaging of tumor and normal-tissue environments to provide in vivo characterization of hypoxia, perfusion, apoptosis, and nutritional state. For the clinical application of these techniques, spatial scale issues related to accurate registration and clinical interpretation of data over 4 orders of magnitude need to be resolved. Such an approach is a paradigm shift for the field of radiation oncology. Molecular and biological targets
as well as volumes from digital images may be used to biologically optimize radiation dose distributions. Image information in spatial, temporal, physical, and biological intensity formats may be used quantitatively. Radiation treatment could be accomplished using “conventional” doses delivered with lower doses to yield radiation-initiated molecular events. In particular, PET imaging can readily be found in radiation oncology clinics.

Molecular imaging and advanced imaging in radiation oncology present great challenges and opportunities for collaborations through the convergence of molecular biology, diagnostic radiology, radiation oncology, physics, imaging science, chemistry, and other fields. Integration of diagnostic radiology and radiation oncology, in particular, is underway, with imaging providing the key link among diagnosis, treatment, and response assessment. A growing community of multidisciplinary scientists and clinicians is required to address the acquisition, manipulation, interpretation, and application of molecular and biological images in the clinical settings.

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