Molecular Imaging Applications

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Highlights
Overview of molecular imaging
MR molecular imaging within the context of other imaging modalities
MR molecular imaging strategies

Target Audience
M.D. and Ph.D. researchers with an interest in molecular imaging concepts and applications.

Introduction
Molecular imaging has revolutionized our perceptions of imaging. This high impact field is finding transformative applications in the understanding, detection, and treatment of nearly all diseases [1].

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Figure 1: Overview of molecular imaging and its applications
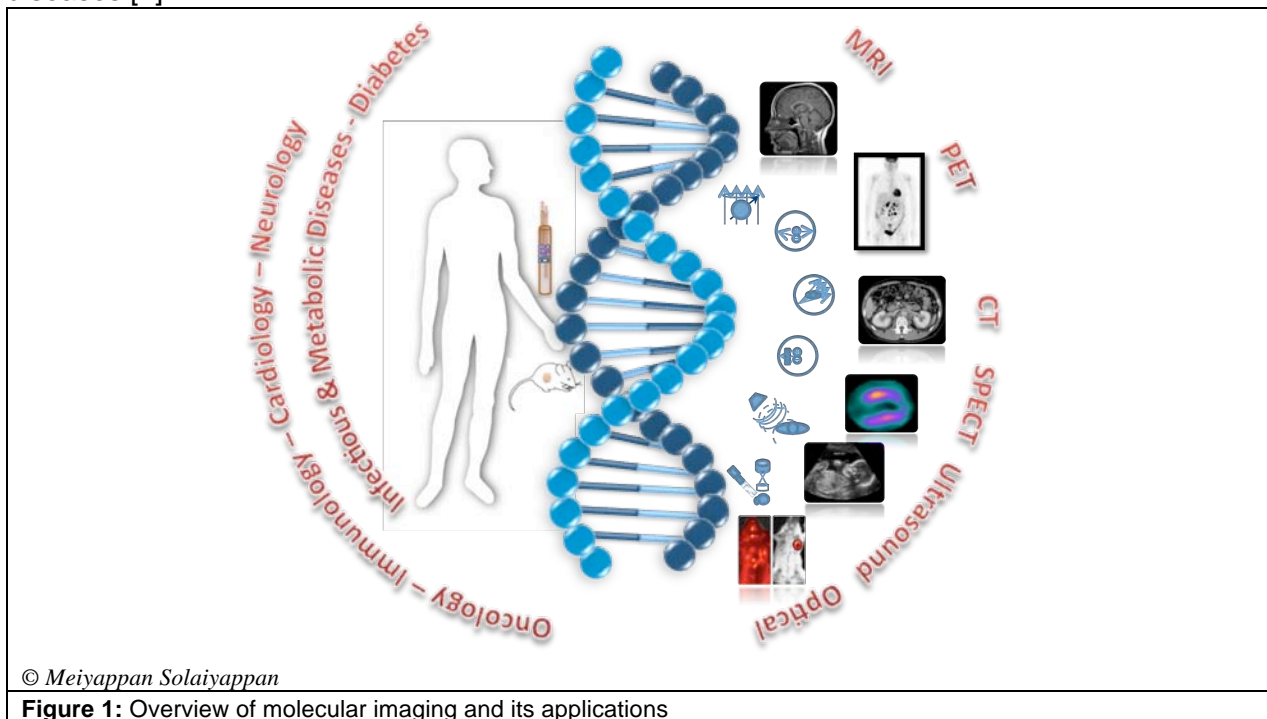
The field of molecular imaging is a an exciting fusion and integration of many different disciplines including molecular biology, chemistry and probe design, imaging technologies, visualization, and image analysis, that are focused on understanding, detecting, and treating oncological, neurological, cardiovascular, inflammatory, metabolic, and infectious diseases (**Figure 1**). Based on their strengths, different imaging modalities provide different but equally valuable information that can be integrated in advancing our understanding of these diseases.

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As the era of 'omics' progresses towards personalized medicine, the field of molecular imaging is finding multiple uses in noninvasive characterization of the molecular features of diseases and their impact on function. In complex diseases such as cancer, with its tremendous genetic diversity, it is becoming increasingly important to develop molecularly-targeted treatment strategies that combine detection with treatment.

MR molecular imaging within the context of other modalities
A summary of different imaging modalities and their sensitivity, spatial resolution, strengths and weaknesses are presented in **Table 1** [2] and a summary of the different applications of MR techniques are summarized in **Table 2**. These tables highlight the advantages of MR and its strengths in imaging metabolism and physiology. However, a drawback of MR methods is limited sensitivity of detection and several innovative strategies are being pursued to increase these limits.

Table 1: Sensitivity, spatial resolution, and clinical translation of molecular-functional imaging (MFI) modalities [2].

Imaging Modality	Sensitivity of detection in MFI	Spatial Resolution *in vivo*	Advantages	Disadvantages
CT	500 micromolar (Gd-DTPA) – low millimolar (Iodine) range	>10 μm	High spatial resolution	Patients are given high radiation doses
MRI	T_2-contrast, iron oxide nano-particles: nanomolar-micromolar range	4 μm (experimental MRI), 250 μm in plane (clinical MRI)	High spatial resolution	Particle size is often large, which restricts *in vivo* delivery
	T_1 contrast, multilabeled targeted Gd-DTPA macromolecules: >10 μM	4 μm (experimental MRI), 250 μm in plane (clinical MRI)	High spatial resolution	Particle size of contrast agent or reporters is relatively large
MRS	Millimolar range (^{1}H at 4.7 – 11 Tesla)	≥0.5 cm (3 Tesla), 0.7 cm (1.5 Tesla)	Detection of endogenous metabolites	Low sensitivity results in low spatial resolution
Optical	Nanomolar range: ≥50 cells (fluorescence); ≥1000 cells (bioluminescence)	>25 μm, intravital microscopy: 1 – 15 μm	High sensitivity, high spatial resolution	Restricted depth detection
PET	Picomolar range	>1 mm^3 (microPET), ~4-5 mm (clinical PET)	High sensitivity, short-lived isotopes	Low spatial resolution, cyclotron required for generating some isotopes
SPECT	Picomolar range	>1 mm (microSPECT), ≥3 mm (clinical SPECT)	High sensitivity	Low spatial resolution, long-lived isotopes
Ultra-sound	>10^6 microbubbles per ml blood	>40 mm	High spatial resolution, cost effective	Few probes available

Table 2: MR biomarkers currently available for applications in diseases

T_1, T_2, T_2* contrast	• Vascular volume • Permeability surface area product • Extraction fraction • Vessel size • BOLD imaging • Extracellular matrix remodeling • Receptor expression on epithelial and endothelial cells • Enzyme activity dependent contrast • pO_2 • pH

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Spectroscopic contrast	• Total choline, phosphomonoesters, phosphodiesters • Lactate • Lipid • pH • Energy (ATP, phosphocreatine, inorganic phosphate) • Drug pharmacokinetics • Labeled substrate utilization
Contrast from apparent diffusion coefficient (ADC) of water	• Cell death • Edema • Fiber mapping
Chemical Exchange Saturation Transfer (CEST), Amide proton transfer (APT) contrast	• pH • Receptor expression • Gene expression

Brief outline of molecular imaging strategies

Some of the strategies in molecular imaging are broadly outlined in Figure 2 and include (a) enzyme-based reporters, (b) receptor based reporters, and (c) transporter based reporters [3].
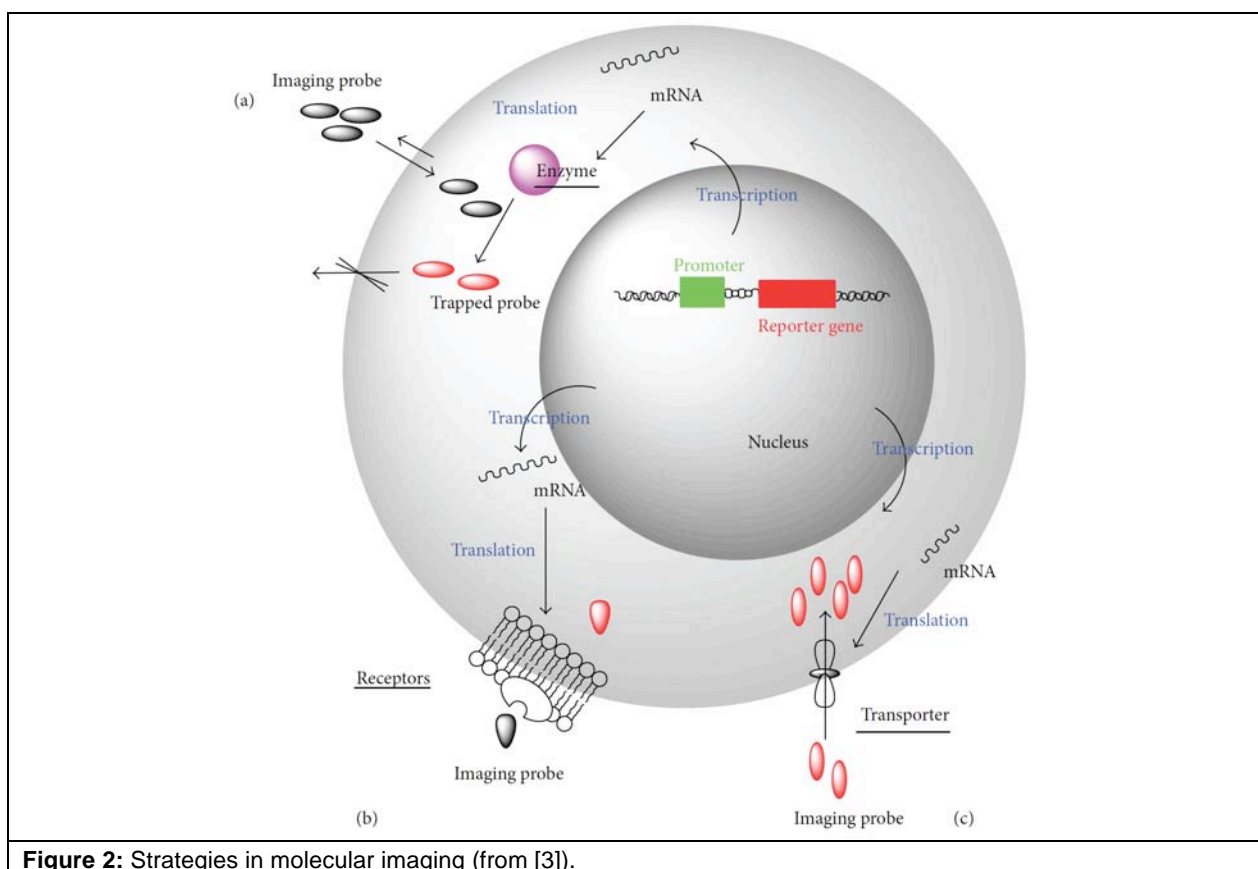

Figure 2: Strategies in molecular imaging (from [3]).

Receptor Expression
Receptors, antigens or proteins expressed on cell surfaces provide available targets for detection and targeting. Labeled antibodies have been used to image antigens overexpressed by cancer cells, such as Her2/neu in breast cancer. MR contrast generated by injecting a biotinylated antibody that binds to a specific receptor, followed by administration of avidin linked to gadolinium-diethylenetriaminepentaacetate (GdDTPA) that binds to the biotin on the antibody [4] can be visualized on T_1-weighted images.

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Integrins and Angiogenesis
Angiogenesis plays an important role in cancer, stroke and cardiac ischemia. Multiple MR probes have been developed to characterize tumor vasculature. Applications of dynamic contrast enhanced (DCE) MRI based on T_1 or T_2 perfusion probes have dominated the use of MRI in the assessment of vascular parameters, such as perfusion, vascular volume, and permeability surface area product [5]. In recent advances, agents have been developed that bind specifically to receptors expressed on tumor vasculature (reviewed in [6] and [5]).

Cell tracking
Another application of MR probes has been in tracking the migration of stromal and immune cells, by pre-labeling them with gadolinium or iron-based contrast agents [7, 8]. Examples of such studies include detecting the migration and homing of cells involved in tumor progression, such as fibroblasts, natural killer (NK) cells, or stem cells [7-9].

Hypoxia
Several noninvasive MR probes detect hypoxia. *In vivo* imaging of tumor oxygen consumption has been performed using ^{19}F MRSI of ^{19}F oxygen reporters such as 2-nitro-α-[(2,2,2-trifluoroethoxy)methyl]-imidazole-1-ethanol (TF-MISO). TF-MISO is a nitroimidazole-based probe that accumulates in hypoxic cells through a biochemical reduction. The reduced probes bind to endogenous cellular molecules and accumulate in hypoxic cells [10, 11] an can be used to report on hypoxia *in vivo* with ^{19}F MRS [12]. Hypoxic regions are identified by imaging the uptake, distribution and retention of the probes by acquiring ^{19}F MRSI [12] and reviewed in [6].

pH
MRS measurements of tumor pH have identified acidic extracellular and neutral to alkaline intracellular environments in cancer cells and tumors. The chemical shift of the primary intracellular inorganic phosphate (Pi) signal, detected by ^{31}P MRS is pH-dependent and provides a measure of intracellular pH [13]. Exogenous pH markers such as 3-aminopropylphosphonate (3-APP) [14] for ^{31}P MRS or (imidazol-1-yl)3-ethyoxycarbonylpropionic acid (IEPA) [14] or 2-(imidazol-1-yl)succinic acid (ISUCA) for ^{1}H MRSI [15] are used to measure extracellular pH. Relaxation enhanced pH measurements can also be performed *in vivo* using gadolinium-based contrast reagents with a pH-dependent relaxivity pH, such as Gd-DOTA-4AmP^{5-} [16].

Proteolytic activity
Several proteases are involved in cancer invasion and metastases. Several new approaches have been explored to image matrix metalloproteinase (MMP) activity *in vivo* with MRI [17, 18] and reviewed in [6].

CEST
Chemical-exchange-dependent saturation transfer (CEST) can be used for several applications such as measuring pH *in vivo*, detecting exogenous CEST-contrast agents, or detecting amide protons of endogenous mobile cellular proteins and peptides, [19, 20]. CEST reporter proteins can be used for molecular imaging studies in gene promoter and expression studies [21] and in detecting enzyme activity [22].

^{13}C MRS and hyperpolarization
The hyperpolarization of spins significantly increases the sensitivity of detection of the MR signal of ^{13}C-labeled substrates by >10,000. Hyperpolarized ^{13}C-labeled substrates injected systemically, allow real-time metabolic mapping [23-25]. Hyperpolarized ^{13}C has been used to

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measure *in vivo* pHe, and investigate metabolic pathways and enzymatic activity by labeling different substrates such as pyruvate, glutamate, or fumarate [26-28].

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