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Nanomedicine strategies for molecular targets with MRI and optical imaging

Dipanjan Pan, Shelton D Caruthers, Junjie Chen, Patrick M Winter, Angana SenPan, Anne H Schmieder, Samuel A Wickline, and Gregory M Lanza

Division of Cardiology, Washington University Medical School, 4320 Forest Park Avenue, Cortex Building, Suite 101, Saint Louis, MO 63108, USA, Tel.: +1 314 454 8813, Fax: +1 314 454 5265

Gregory M Lanza: greg.lanza@mac.com

Abstract

The science of ‘theranostics’ plays a crucial role in personalized medicine, which represents the future of patient management. Over the last decade an increasing research effort has focused on the development of nanoparticle-based molecular-imaging and drug-delivery approaches, emerging as a multidisciplinary field that shows promise in understanding the components, processes, dynamics and therapies of a disease at a molecular level. The potential of nanometer-sized agents for early detection, diagnosis and personalized treatment of diseases is extraordinary. They have found applications in almost all clinically relevant biomedical imaging modality. In this review, a number of these approaches will be presented with a particular emphasis on MRI and optical imaging-based techniques. We have discussed both established molecular-imaging approaches and recently developed innovative strategies, highlighting the seminal studies and a number of successful examples of theranostic nanomedicine, especially in the areas of cardiovascular and cancer therapy.

Nanotechnology is starting to invade different areas of science and ‘theranostic’ biomedical science is no exception [1–4]. The science of **theranostics** plays a critical role in **personalized medicine**, which represents the future of patient management. Nanoparticle-based medicinal approaches have emerged as an interdisciplinary area, that shows promise in understanding the components, processes, dynamics and therapies of disease at a molecular level. The unprecedented potential of nanoplateforms for early detection, diagnosis and personalized treatment of diseases have found application in every biomedical imaging modality.

These include noninvasive cellular and **molecular-imaging** techniques, including ultrasound (US) [5], optical [6], PET [7], computed tomography [8–9] and MRI [10–14].

MRI is a noninvasive diagnostic technique based on the interaction of nuclei with each other and the surrounding molecules in a tissue. The sensitivity of magnetic resonance is low in comparison to nuclear and optical modalities; however, the absence of radiation (transmitted or injected) and high spatial resolution (e.g., sub-millimeter) makes it advantageous over

Correspondence to: Gregory M Lanza, greg.lanza@mac.com.

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techniques involving radioisotopes. The introduction of higher magnetic fields (4.7–14 T) increases the signal-to-noise ratio, permitting higher resolution or faster scanning. The emerging field of hyperpolarized magnetic resonance [12–14] may improve the low sensitivity of the desired nuclei (e.g., ^{13}C) and offer the use of stable isotope precursors for quantitative *in vivo* imaging and real-time metabolic profiling.

Probes for **optical imaging** that are excitable in the near-infrared (NIR) range are preferable for both *in vitro* and *in vivo* imaging. The ‘optical transmission window’ of biological tissues falls within the NIR range ($\lambda = 650\text{--}900\text{ nm}$). Investigation within this range allows for deeper light penetration and reduced light scattering, thus producing increased image contrast with excellent sensitivity of detection. In this review we will particularly emphasize advanced imaging methods and targeted nano-sized contrast agents for MRI and optical imaging modalities.

Molecular MRI at the nanoscale

Basic principle of MRI & prerequisites

An understanding of magnetic resonance contrast agents is founded upon a rudimentary appreciation of MRI and the NMR phenomenon. The basic principles of NMR state that the intrinsic angular momentum or spins of protons (i.e., hydrogen nuclei) and electrons [10–14] when placed in a strong external magnetic field (B_0) orientate themselves either parallel (i.e., spin-up) or antiparallel (i.e., spin-down) to B_0 . The overall impact, which is a function of B_0 , is minute, about 0.01–0.1 eV or approximately $10^{-6}\text{--}10^{-7}$ more spin-up than spin-down states per voxel. Because tissues are predominantly water, this trivial distribution imbalance is perceptible! The ensemble of many spins exhibits a net magnetization that can be ‘tilted’ by magnetic gradients away from the direction of the main magnetic field after absorption of radiofrequency excitation energy. The transition from this excited state (tilted) back to the ground state is known as relaxation. magnetic resonance contrast is defined by the two-principle NMR processes of spin relaxation: T_1 (spin-lattice or longitudinal relaxation time constant) and T_2 (spin-spin or transverse relaxation time constant); relation rates are the inverse of the relaxation times (i.e., $R_1 = 1/T_1$, $R_2 = 1/T_2$) [10–12].

Magnetic resonance contrast agents accelerate the rate of T_1 and T_2 relaxation. Paramagnetic agents principally accelerate longitudinal T_1 relaxation, producing ‘bright’ contrast in T_1 -weighted images (e.g., gadolinium based). Superparamagnetic agents primarily increase the rate of dephasing or transverse T_2 relaxation and create ‘dark’ or negative contrast effects (e.g., iron oxide-based agents). T_1 contrast agents directly influence protons proximate to themselves and are highly dependent on local water flux; whereas T_2 contrast agents disturb the magnetic field beyond and independent of their immediate environment. The contrast impact of T_2 agents extends well beyond their immediate surroundings, while T_1 contrast agents have only very local influence. Thus, paramagnetic metals of T_1 agents must be ideally exposed to water with fast exchange rates within an imaging voxel. In contrast, superparamagnetic metals can be sequestered anywhere within a supporting matrix and still elicit T_2 contrast.

The term relaxivity (r_1 or r_2) refers to the change in relaxation rate (R_1 or R_2) as a function of contrast metal ion concentration and is expressed in $\text{mM}^{-1}\text{s}^{-1}$ (at varying temperatures); this is also referred to as ‘ionic’ relaxivity. However, for nanoconstructs with many metals associated with each particle, the moiety is considered as one unit, in which case the terms ‘molecular’ or ‘particulate’ relaxivity are often applied [15–16].

T_1 -weighted MRI with gadolinium-based nanoparticles

Early attempts to create targeted paramagnetic contrast agents failed, due to meager or poorly effective metal payloads (i.e., exposed to fast water exchange) per homing unit (e.g., antibody).

In the late 1990s, the development of targeted paramagnetic contrast agents was considered infeasible by the magnetic resonance community. However, in 1998, Sipkins demonstrated *in vivo* imaging of **angiogenesis** with paramagnetic polymerized liposomes in the Vx2 tumor model [17] and, the same year, Lanza *et al.* reported fibrin imaging with paramagnetic per-fluorocarbon nanoparticles [18]; the prevailing dogma was proved wrong. Subsequently, alternative nonparticulate approaches to magnetic resonance molecular imaging have found success against abundantly expressed epitopes such as HER-2/*neu* receptors, using an avidin conjugated to gadolinium-DTPA (12.5 Gd/avidin) and a thrombus-avid, fibrin-binding peptide derivatized with gadolinium 19. A few years later, integrin-targeted liposome constructs, similar to polymerized liposome particles of Sipkins were reported by Mulder *et al.* for angiogenesis imaging in rodent cancer models [20–22]. Alternative approaches with paramagnetic lipoprotein mimics were similarly reported for macrophage imaging of atherosclerotic plaque [23–24].

Ligand-targeted paramagnetic liquid per-fluorocarbon (PFC) nanoparticles have been extensively demonstrated *in vivo* for molecular imaging and targeted drug delivery directed against fibrin in ruptured atherosclerotic plaques [25], as well as integrins of angiogenic endothelium in atherosclerosis [4,26–28] and nascent cancers [29–32]. For PFC paramagnetic nanoparticles, the ‘ionic relaxivity’ r_1 is 35 (mM [Gd³⁺] s)^{−1} and molecular r_1 relaxivity is > 2000,000 (mM[paramagnetic particles]s)^{−1} [15–16]. This extraordinarily high molecular relaxivity of targeted nanoparticles permits a voxel containing fewer than 100pM of the agent to be detected conspicuously, with a contrast-to-noise ratio of 5 at 1.5 and 3.0 T [32].

Dendrimer-based approaches—For the PFC nanoparticles, the high surface-to-volume ratio of the nanoparticle accommodated 100,000 chelated gadolinium atoms per particle, resulting in outstanding T₁ signal amplification (bright) per particle bound to a receptor. However, an alternative approach to achieve these T₁ signal amplification with dendrimers utilizes effective cellular internalization and concentration of the agent to build signal. This approach involves clever targeting of a cell membrane receptor specific system to mediate intracellular concentration of the imaging drug delivery agent. Recycling of the receptor between the membrane and the cytoplasm serves to continually amplify the target cell-specific concentration of contrast as described below.

Polyamidoamine dendrimers are an ideal platform for this type of targeted cell uptake and delivery due to their small size, monodispersed population and numerous surface amines to conjugate targeting and imaging molecules. As required, in order to escape the vasculature through vascular pores, dendrimers are less than 50 nm in diameter; this is well below the typical ceiling (150 nm) for endosomal particle internalization in nonphagocytic cells. Two versions of this approach have been used successfully in dendrimers to mark specific cells *in vivo*.

Bulte *et al.* explored the use of magnetic nanoparticle contrast agents as a means of following stem cell dispersal after grafting [33]. Bulte’s research showed that it was possible to label and MRI-track stem and progenitor cells for up to 60 days after grafting them into a rat model. Bulte *et al.* utilized amine-terminated dendrimers that encased iron-oxide particles to promote highly enhanced nonspecific endocytosis by cells. However, these cationic particles can elicit deleterious biological effects, such as the activation of complement and clotting pathways. Despite the uniqueness of this work, the intent of the research is *ex vivo* cell labeling and neither were expected to work *in vivo*.

A second dendrimer-based approach, derived from the seminal work of Wiener *et al.* [34], was extended by Baker and his colleagues. For these works, the dendrimer surface was modified with different chemistries to achieve high cell avidity for monomeric targeting ligands by

attaching multiples of these molecules [35]. The highly specific delivery of drugs to tumor cells overexpressing folate receptors was illustrated *in vivo* with negligible nonspecific binding or toxicity. The Baker group employed this platform to deliver Fe₃O₄ [36,37] and gold [38], complexed within the interior of the dendrimer, as well as Gd⁺³ chelated to the surface to tumor cells with impressive specificity and uptake. MRI imaging demonstrated the specific marker (in this case the folate receptor) on the surface of tumor cells [39] and also provided confirmation of methotrexate drug delivery [35].

Beyond folate targeting, dendrimeric particles offer many opportunities to combine imaging and drug delivery for various cell and tumor markers. Other examples include the use of an anti-HER2 F_(ab) coupled to dendrimers that identified HER2-expressing tumor cells, and a similar approach with anti-EGF binder to delineate EGF-overexpressing tumor cells [40]. At this juncture, almost any cell with a specific membrane marker can be imaged and similarly treated by targeted dendrimeric contrast agents.

Angiogenesis paramagnetic molecular imaging

Neovessel formation (i.e., angiogenesis) is an important biosignature of cancer. One molecular signature, $\alpha_v\beta_3$ -integrin, has received prominent attention for angiogenic-targeting applications, because it is expressed on the luminal surface of activated endothelial cells but not on mature quiescent cells. The $\alpha_v\beta_3$ -integrin, a heterodimeric transmembrane glycoprotein, is expressed by numerous cell types, including endothelial cells [41–42], macrophages [43], platelets [44], lymphocytes [45], smooth muscle cells [45] and tumor cells [46,47]. Fortunately, the steric constraint of perfluorocarbon nanoparticles to the vasculature precludes significant interaction with nonendothelial integrin-expressing cells, which greatly enhances neovascular target specificity [48,49].

$\alpha_v\beta_3$ -integrin-targeted paramagnetic nanoparticles sensitively detected histologically corroborated angiogenic endothelium at 1.5 T in New Zealand White rabbits bearing Vx2 tumors (<1.0 cm) implanted into the hind limb 12 days previously [29]. *In vivo* competition studies in that report demonstrated that the ligand-directed homing was specific for the $\alpha_v\beta_3$ -targeted nanoparticles. Moreover, the T₁-weighted images obtained with $\alpha_v\beta_3$ -targeted nanoparticles differentiated growing tumors from the inflammatory remnants of host-rejected cancer [29]. These tumor remnants were not differentiated from growing cancers using standard T₂-weighted MRI, nor would ¹⁸F deoxyglucose PET imaging be expected to discern the high oxygen utilization of macrophage metabolism from proliferating Vx2 tumor cell demand. This simple example illustrated how magnetic resonance molecular imaging with paramagnetic nanoparticles provides fundamental data that could support medical decisions to biopsy, treat or observe anomalous pathology or to interrogate early lesion response to chemotherapy or radiation treatment.

A follow-up study in athymic mice demonstrated that angiogenesis induced by very minute tumors, less than 40 mm³, could be detected by magnetic resonance within 0.5 h following IV administration of $\alpha_v\beta_3$ -integrin-targeted PFC particles; this contrast signal continued to strengthen over the next 2 h (Figure 1) [30]. Again, *in vivo* competition studies demonstrated the high specificity of ligand-directed targeting, which was further corroborated by $\alpha_v\beta_3$ -targeted bimodal particles (fluorescent and MR) and immune fluorescent microscopy [30].

Angiogenesis image-guided drug delivery

Anti-angiogenesis therapy in conjunction with chemotherapy or radiation therapy has become a well-established treatment for lung, colon and breast cancer [50–52]. However, only a limited subset of patients achieve optimal effectiveness from anti-angiogenic pretreatment; moreover, the clinical timing of the treatment and its duration are not individualized to acute therapeutic

response. PFC paramagnetic nanoagents offer a sensitive and personalized approach for selecting patients for treatment and even delivering the anti-angiogenic therapy itself. In addition to ultrahigh payloads of paramagnetic chelates, enabling diagnostic MRI, $\alpha_v\beta_3$ -targeted nanoparticles can incorporate therapeutic agents for effective targeted drug delivery [1,4,27,28,31,53–56]. Such dual-function agents are referred to as ‘theranostics’. Chemotherapeutics, such as paclitaxel, doxorubicin, rapamycin and fumagillin, as well as toxic peptides, such as mellitin and thrombolytic enzymes, have been effectively incorporated into the surfactant of PFC nanoparticles [2,4,28,31,54–55]. Theranostic agents can noninvasively quantify the delivery of potent chemotherapeutic agents, such as fumagillin, as well as provide quantitative measures of response for improved longitudinal medical management. Moreover, such agents vastly reduce total drug exposure with an anticipated benefit of improved safety.

Fumagillin is a mycotoxin produced by *Aspergillus fumigatus*, which suppresses angiogenesis by inhibition of methionine amino-peptidase 2 (MetAP2) [57–58]. TNP-470, a water-soluble functional analogue of fumagillin, selectively inhibits proliferating endothelial cells (i.e., angiogenesis). The anti-tumor efficacy of TNP-470 was widely demonstrated in rodents [59–62] and later studied in human clinical trials [63–67]. Unfortunately, at dosages required for therapeutic effects, TNP-470 elicited sudden moderately severe symptoms of neurotoxicity, including weakness, nystagmus, diplopia and ataxia [63,64,67].

Inclusion of fumagillin into the surfactant of the $\alpha_v\beta_3$ -targeted nanoparticles enables the delivery of the drug into proliferating endothelial cells via ‘contact-facilitated transport’, which is promoted by the ligand-based tethering of the nanoparticle to the target cell surface and the spontaneous exchange of the lipid surfactant components with similar membrane lipids through hemifusion complexes (Figure 2) [1,53,56].

The anti-angiogenic effectiveness of $\alpha_v\beta_3$ -targeted fumagillin nanoparticles was studied in the syngeneic Vx2 adenocarcinoma rabbit model using a minimal fraction of the TNP-470 dosages previously used in preclinical animal models [4]. The Vx2 tumor volume was reduced by one-half to two-thirds among rabbits receiving $\alpha_v\beta_3$ -targeted fumagillin nanoparticles, when compared with animals given nontargeted fuma-gillin nanoparticles, $\alpha_v\beta_3$ -targeted nanoparticles without drug or saline. Moreover, 3D reconstruction and mapping of the contrast enhanced voxels over a chain model of the tumor surface demonstrated a coherent asymmetric peripheral distribution characterized by dense neovessel regions interspersed with a finer, reticular pattern (Figure 3).

Fluorine (^{19}F)-imaging agents based on PFC nanoparticles

^{19}F presents an excellent probe for quantitative MRI, which is highly enriched in perfluorocarbon nanoparticles. ^{19}F has 100% natural abundance, a spin of 1/2 and a gyromagnetic ratio of 40.08 MHz/T, close to the that of ^1H (42.58 MHz/T), resulting in 83% of the sensitivity of ^1H [68]. In addition, the chemical shift of ^{19}F , due to its seven outer-shell electrons, is sensitive to the molecular environment of the nucleus, including oxygen tension. The ^{19}F spectroscopic signature manifests a range of >200 ppm [69–70], which permits unambiguous identification of distinctive ^{19}F -containing compounds, even at low field strengths. Moreover, no background exists for the ^{19}F signal *in vivo*, providing a unique spectroscopic signature for quantitative MRI.

Interrogation of hypoxia using ^{19}F MRI paramagnetic O_2 mapping

Tissue oxygenation is a major therapeutic concern in many pathologic lesions, particularly in the treatment of cancers [71]. The rapid growth of malignant tumors, combined with formation of dysfunctional neovasculature, results in regional hypoxia, which reduces susceptibility to chemotherapy and radiation therapy [72]. Quantitative ^{19}F MRI of PFCs were explored as an

MRI method for *in vivo* mapping tumor hypoxia [73–75]. Although blood oxygen level dependent (BOLD) has been used in MRI of oxygen utilization and hypoxia in neurology, psychology and oncology [76–81], its qualitative information may be less preferable compared with the quantitative data achievable with PFCs [75]. At a given temperature, the partial pressure of dissolved paramagnetic O₂ (pO₂) in PFCs directly correlated to the relaxation rate of ¹⁹F or R1, which can be estimated as a measure of local *in vivo* tissue oxygenation. Since some PFCs have lower sensitivity to physiological temperature variations (30–42°C), such as hexafluorobenzene (HFB), local ¹⁹F R1 can estimate tissue oxygenation using *a priori* calibrated standard R1–pO₂ standard curves [82]. The precision of the ¹⁹F MRI method can reach 1–3 mmHg in hypoxic region [83], comparable to fine electrode measurements [84]. Moreover, fast ¹⁹F MRI techniques, such as fluorocarbon relaxometry using echo planar imaging for dynamic oxygen mapping (FREDOM), have been developed for dynamic mapping of tumor pO₂ with high precision [85].

Molecular ¹⁹F MRI with site-targeted PFC nanoparticles

The unique capability of ¹⁹F MRI to directly determine the absolute quantity of ¹⁹F atoms has been largely unexploited until the recent advent of PFC nanoparticle-based molecular imaging, beginning in 1996 [86]. Although these targeted nanoparticles were initially used to deliver high payloads of surface gadolinium to enhance T₁-weighted ¹H MRI, as discussed earlier, they inherently resulted in regional deposition of ¹⁹F atoms adequate for quantitative MRI. Using fibrin-targeted PFC nanoparticles, the particles bound on the surface of fibrin clots provided enough ¹⁹F atoms for MRI at 4.7T field strength [87,88]; later, the concept was extended to a clinical scanner at 1.5T [72]. The linear correlation between the quantity of bound ¹⁹F atoms and the measured ¹⁹F magnetic resonance signal from functionalized PFC nanoparticles has been quantitatively mapped in clot and tissue phantoms [88]. PFOB and perfluoro-15-crown-5-ether (PFCE) nanoparticles targeted to the same biological specimen were simultaneously or selectively assessed with ¹⁹F MRI at 1.5T, illustrating the unique spectral opportunity for phenotypic characterization achievable by delineation of multiple pathological biosignatures simultaneously [89]. Subsequently, Neubauer *et al.* further demonstrated that paramagnetic Gd³⁺, closely bound to the surfactant surface of PFC nanoparticles, increased the ¹⁹F R1 fourfold, improving ¹⁹F signal intensity 125% at 1.5 T [90].

Examples of ¹⁹F imaging of sparse epitopes, as presented by biological tissues, has included detection of atherosclerotic valve angiogenesis [91]. Angiogenic valve leaflets in hyperlipidemic rabbits following α_vβ₃-integrin targeted PFC nanoparticles exhibited approximately three-times higher ¹⁹F signal *ex vivo* than similar valve leaflets in atherosclerotic animals treated with untargeted nanoparticles [91]. Similarly, molecular imaging with VCAM-1 targeted PFC nanoparticles in the kidneys of the ApoE mouse showed the potential assessing vascular inflammation [92]. Potentially, *in vivo* quantitative ¹⁹F MRI, using site-targeted PFC nanoparticles, can be complicated by the background signal from unbound particles circulating in the blood pool depending on the dose, timing of imaging and field strength (particularly above 4.7 T); however, the use of diffusion-weighted ¹⁹F MRI techniques to selectively suppress the ¹⁹F signal from circulating nanoparticles versus the targeted bound particles eliminates this background problem. [93]

¹⁹F MRI of monocytes, dendritic & stem cells

Regenerative therapy using stem cells offers great promise for the treatment of many diseases. To date, ¹H MRI using iron oxides has served as a primary method for monitoring stem cell trafficking [94]. Typically, *in vitro* cultured stem cells are incubated with ¹H MRI contrast agents such as superparamagnetic iron oxide (discussed later) or paramagnetic Gd-DTPA [95,96], which results in uptake by endocytosis and modification of the cells as imaging agents.

Labeled stem cells can be detected by ^1H MRI based on the negative (i.e. dark) or 'positive' (i.e. bright) contrast effects, dependent on the labeling approach applied. Moreover, such ^1H MRI techniques appear sensitive to even a single cell under certain circumstances [97].

^{19}F MRI can be used as an alternative method for quantitative trafficking of stem cells *in vivo* [98]. Ahrens *et al.* demonstrated that PFCE nanoparticles could be effectively internalized by dendritic stem cells with the help of a cationic transfection agent and specifically detected *in vivo* by ^{19}F MRI at 11.7 T [99]. Subsequently, phospholipid-encapsulated PFC nanoparticles were shown to internalize into stem/progenitor cells without the need of a transfection agent and be detected by ^{19}F MRI at both clinical (1.5 T) and research (11.7 T) field strengths [100]. Ruiz-Cabello *et al.* recently showed that PFCE nanoparticles with cationic surface charge were also effectively internalized by neural stem cells [101]. In another example, *in vivo* monitoring of the migration of PFCE nanoparticle labeled T-cells 48 h into the pancreas of diabetic mice after intraperitoneal injection [102]. Currently, a minimum of 2000 labeled cells is required for ^{19}F cell-trafficking detectability, versus the single cell possibility with iron oxides [100].

MRI with superparamagnetic nanoparticles

One of the earliest applications of nanotechnology in MRI involved the use of paramagnetic iron-oxide particles. Iron-oxide crystals have long been used as superparamagnetic T_2^* contrast agents for MRI [103–106]. Superparamagnetic iron oxide (SPIO, particle diameter > 50 nm) and ultrasmall super-paramagnetic oxide (USPIO, particle diameter < 50 nm) particles have nonstoichiometric microcrystalline magnetite core(s) and are typically coated with dextran (e.g., ferumoxide) or siloxane (e.g., ferumoxsil) [107]. While at very low doses, circulating iron oxides can decrease the T_1 time of blood for positive contrast enhancement with magnetic resonance angiography, at the usual doses used for molecular imaging, the T_2^* effects predominate resulting in short T_2 relaxation with marked signal loss [105,108–109]. Unfortunately, for molecular-imaging applications, persistent T_2^* effects from circulating iron oxides nanoparticles delays MRI by 24–72 h postinjection [110–112], complicating clinical implementation of these techniques. Moreover, iron oxides concentrated at a target site generate magnetic susceptibility artifacts (dark or negative contrast) with a marked blooming effect, which smear into adjacent nontargeted voxels.

Spontaneous phagocytic uptake of SPIO and USPIOs by macrophages in atherosclerotic plaques was recognized and demonstrated in 2000 and 2001 by Schmitz *et al.* and Ruehm *et al.* in hereditary or diet-induced hyper-lipidemic rabbits [113–116]. In 2003, this finding was extended to include human plaque [117]. Systematic evaluation of USPIO-enhanced MRI contrast in carotid atheroma confirmed that the optimal signal intensity was achieved 24–36 h after administration. Subsequently, the USPIO compound ferumoxytol was compared with ferumoxtran-10 as a marker of macrophage activity in atherosclerotic plaques. While both were reported to be effective, ferumoxytol had optimal luminal signal intensity 3 days post-treatment and ferumoxytol-treated rabbits had peak measurements 5 days after injection [11,8]. Recently, new magnetic resonance pulse sequences and image postprocessing techniques have been developed to reverse the dark contrast appearance into a bright positive contrast effect [119–126]. For example, inversion-recovery with ON-resonant water suppression (IRON) allowed the dark magnetic distortion artifacts associated with T_2^* USPIO imaging to be viewed as positive contrast that correlated with macrophage counts in hyperlipidemic rabbits best approximately 72 h post-treatment [123].

Ligand-directed targeting of T_2^* iron oxide nanoparticles

The development of monocrystalline iron oxide nanoparticles (MION) helped to extend magnetic resonance iron oxide imaging beyond the limitations of passive targeting through

tissue accumulation and particle phagocytosis to ligand-directed or active targeting. MIONs have an average core diameter of 3 nm and can be directly coupled to homing ligands that specifically target epitopes in the tissue of interest. Dextran-coated MION, coupled to human holo-transferrin (Tf-MION), was used to visualize transgene expression in a gliosarcoma mouse model *in vivo* [127]. In those experiments, the cellular uptake of MION increased approximately 500% relative to control cells following overexpression of engineered transferrin receptor. In other experiments, MION was used to estimate blood volume distribution and indirectly assess angiogenesis in brain tumors [128]. The targeting efficiency of iron-oxide particles improved further with the development of dextran cross-linked iron oxide (CLIO) particles [129]. CLIO has been used with a variety of ligands, including E-selectin [130], a peptide sequence from the transactivator protein (Tat) of HIV-1 [131–134], annexin V [110] and VCAM-1 [135]. Although these particles may be demonstrated with histology to target tissue specifically soon after injection, noninvasive MRI remains delayed and the combination of particle clearance into macrophages and nonspecific particle diffusion within tissue convolves the imaging result.

One recent approach to address the marked delay between targeted iron-oxide administration and imaging has been a return to using large microparticulate iron oxides. In high-field (9.4 T) rodent experiments, iron microparticles were targeted to the gpIIb/IIIa receptor of platelets [136], and a leukocyte-mimicking, dual-epitope-targeting approach directed against vascular inflammatory biomarkers was imaged following intracardiac injection [137]. These results suggest a potential role for large particle approaches, although previous issues of intra-vascular agglomeration and rapid pulmonary clearance when administered IV may still need to be addressed.

T₁w imaging with colloidal iron-oxide nanoparticles

Colloidal iron oxide nanoparticles (CIONs) are a vascular constrained T₁w molecular-imaging agent that avoids typical magnetic bloom artifacts, permits rapid *in vivo* molecular imaging without blood pool magnetization interference and supports targeted drug delivery [138,139]. A CION is comprised of iron oxide suspended within a hydrophobic matrix and encapsulated within a partially crosslinked phospholipid capsule, which decreases T₂ effects more than T₁, an entirely unexpected result. A CION has rapid clearance of circulating interference on T₁ contrast (<60 min), while blood T₂ shortening persists well over 2 h, as expected for superparamagnetic agents. Moreover, CION is designed for therapeutic drug delivery (e.g., fumagillin) via a unique mechanism termed ‘contact-facilitated drug delivery’.

Manganese-doped iron oxide nanoparticles

Manganese-doped superparamagnetic iron oxide (Mn-SPIO) nanoparticles were studied to create ultrasensitive MRI contrast agents for liver imaging. Hydrophobic Mn-SPIO nanoparticles encapsulated in block copolymer mPEG-b-PCL micelles form self-assembled small clusters (mean diameter: ~80 nm). Mn-SPIO nanoparticles within the micelles decrease are superparamagnetic at room temperature. At the magnetic field of 1.5 T, Mn-SPIO nanoparticle-clustering micelles have a T₂ relaxivity of 270 (Mn + Fe) mM⁻¹s⁻¹, which is much higher than a single Mn-SPIO nanoparticle in a lipid-PEG micelle. This clustered nanocomposite approach has yielded significant liver contrast with signal intensity decreases of approximately 80% in 5 min after intravenous administration. The time window for enhanced MRI is highly prolonged and the contrast in liver images is marked, offering potential to identify the smallest liver lesions and differential diagnosis of other liver diseases [140]. Similar particles have been reported in combination with chemotherapeutic agents for targeted magnetic resonance detection and treatment of breast cancer [1,14].

Manganese as a paramagnetic contrast agent

Manganese was one of the first reported examples of paramagnetic contrast material studied in cardiac and hepatic MRI, because of its efficient R_1 enhancement. Similar to Ca^{2+} and unlike the lanthanides, manganese is a natural cellular constituent and often a cofactor for enzymes and receptors. Manganese blood-pool agents, such as mangafodipir trisodium, have been approved as a hepatocyte-specific contrast agent but transient side effects due to dechelation of manganese from the linear chelate has reduced their use [142,143].

Aime *et al.* reported Mn(II) complexes bearing benzyloxymethyl functionalities that displayed relaxivity values only slightly smaller than those shown by the most clinically used contrast agents [144]. In these Mn(II) chelates, the exchange rate of the coordinated water was much higher than those reported for Gd(III) complexes with octadentate ligands. This group exploited these rapid coordinated water exchange rates to develop macromolecular adducts with human serum albumin. The albumin-Mn(II) binding attained relaxivity values analogous Gd(III) systems. These results strongly supported the view that Mn(II) complexes, in spite of the lower effective magnetic moment, could be considered viable alternatives to the currently used Gd(III) complexes as contrast agents for MRI applications.

Similarly, Caravan *et al.* designed, synthesized and demonstrated a novel Mn(II) complex based on EDTA, which was functionalized with a bis-phenyl moiety to noncovalently complex with serum albumin, analogous to the gadolinium-based contrast agent MS-325. Albumin-binding measurements revealed that the complex adhered to plasma proteins (93–96%) primarily via serum albumin (rabbit: 89–98%). Relaxivities of the albumin-Mn-EDTA complex were: $r_1 = 5.8 \text{ mM}^{-1}\text{s}^{-1}$ (buffer); $51 \text{ mM}^{-1}\text{s}^{-1}$ (rabbit plasma); and $46 \text{ mM}^{-1}\text{s}^{-1}$ (human plasma). Variable-temperature NMR diffusion profiles indicated that the high relaxivity was due to slow tumbling of the albumin-bound complex and fast exchange of the inner sphere water. Imaging in an injured rabbit carotid artery model clearly showed that the agent could delineate both arteries from veins and differentiate healthy from damaged vessel wall. Again, suggesting that Mn-based agents could be used for T_1 w imaging in lieu of gadolinium [145].

Manganese nanoparticles for T_1 weighted imaging

MRI based on MnO nanoparticles has been used to elicit bright signal enhancement and fine anatomic detail in the T_1 -weighted (T_1 w) magnetic resonance image of a mouse brain. The MnO nanoparticles were targeted to breast cancer and selectively detected breast cancer cells in a metastatic tumor in brain [146]. Subsequently, this Korean group greatly improved the relaxivity of MnO nanoparticles by developing MRI jack-o'-lanterns. These hollow manganese oxide nanoparticles, while retaining efficient cellular uptake, illustrate the potential of this bifunctional theranostic approach [147].

Another unique Mn-based agent was reported as manganese (III)-labeled nanobialys, which were shown to have potential as targeted magnetic resonance theranostic nanoparticles [148]. The nanobialys produced by molecular selfassembly of amphiphilic-branched polyethyl-enimine assumed a toroidal, biconcave shape, which was tunable with regard to particle size and exhibited low polydispersity. The bialys presented Mn(III) in a kinetically stable, porphyrin-coupled complex directly exposed to the surrounding water. The vascular constrained nanobialys (180–200 nm) have ionic r_1 and r_2 relaxivities of $3.7 \pm 1.1 (\text{s} \cdot \text{mmol} [\text{Mn}])^{-1}$ and $5.2 \pm 1.1 (\text{s} \cdot \text{mmol} [\text{Mn}])^{-1}$, respectively and particulate relaxivities of $612,307 \pm 7,213 (\text{s} \cdot \text{mmol} [\text{nanobialy}])^{-1}$ and $866,989 \pm 10704 (\text{s} \cdot \text{mmol} [\text{nanobialy}])^{-1}$, respectively. The concept of Mn(III) nanobialy efficacy was illustrated *in vitro* using antifibrin monoclonal targeting to fibrin-rich clots.

Subsequently, we advanced the concept of 'soft-type' nanocolloids with the development of manganese oxide and manganese oleate nanocolloids, which incorporate divalent manganese [149]. These nanocolloids, encapsulated by phospholipids, are designed for vascular targeting (>120 nm) similar to CION and PFC nanoparticle formulations. However, unlike the paramagnetic gadolinium PFC nanoparticles, the manganese was uniquely entrapped within the core matrix. Manganese nanocolloids presented a multiplicity of specific surface-homing ligands for high avidity and sensitivity for molecular imaging with MRI (excellent 'stick and stay' quality), such as antifibrin antibodies and more recently $\alpha_v\beta_3$ -integrin antagonists.

Magnetic resonance experiments demonstrated the high-resolution T₁w molecular imaging in suspension and against fibrin with ManOC and ManOL. The ionic r₁ relaxivities of ManOC and ManOL were 4.1 ± 0.9 (s·mmol [Mn])⁻¹ and 20.4 ± 1.1 (s·mmol [Mn])⁻¹, respectively, while the particulate relaxivities were 85,099 (s·mmol [ManOC])⁻¹ and 631,208 (s·mmol [ManOL])⁻¹, respectively. The ionic r₂ relaxivities of ManOC and ManOL were 18.9 ± 1.1 (s·mmol [Mn])⁻¹ and 65.6 ± 0.9 (s·mmol [Mn])⁻¹, respectively and the r₂relaxivities were 395,410 (s·mmol [ManOC])⁻¹ and 2,028,925 (s·mmol [ManOL])⁻¹, respectively. The specific relaxivities, obtained by measuring the relaxation rate as a function of the concentration of the contrast agent were also found to be markedly and unexpectedly increased for the ManOL, compared with the ManOC. Fibrin-rich clots were targeted *in vitro* with fibrin-specific monoclonal antibodies, revealing markedly increased T₁w images of the targeted specimens, yielding signal intensities (75 ± 20 and 95 ± 19 au, respectively) versus the control nanocolloid (32 ± 07 au) and background air (7 ± 4 a.u.).

Optical imaging at the nanoscale

Basic principle of optical imaging & prerequisites

Optical imaging is a noninvasive, relatively low-cost technology that uses light to probe cellular and molecular function in the living body. The technique has gained tremendous attention due to its high sensitivity and high suitability for small-animal studies. In fluorescence imaging, the subject of interest is typically illuminated by excitation light and at a shifted wavelength emitted light is collected. The contrast is derived either from the use of exogenous agents or from endogenous molecules with inherent optical properties. Generally, a spatial scale of up to centimeters can be resolved by the optical-based techniques. However, in most cases, tissue auto-fluorescence generates significant background signal. Therefore, the information that is typically derived is not quantitative in nature and image information is mostly surface-weighted due to excessive tissue absorption. With the advent of recently developed advanced techniques such as fluorescence molecular tomography [150] and photoacoustic tomography [151–152], it is expected that optical-based techniques will gain more attention.

NIR optical imaging

The NIR spectral window (700–1000 nm) offers unique opportunities for deep tissue fluorescence imaging [153]. Light in this range can penetrate deep into living tissue, which is not possible below 700 nm. In the visible range (<700 nm) the scattering is high and the presence of strong exogenous absorbers (e.g., water, hemoglobin, deoxyhemoglobin and lipid) does not permit the light to penetrate deep into the tissue. Detection of contrast from the externally administered exogenous contrast is also difficult owing to the high characteristic auto-fluorescence from tissue and cellular components. The penetration of light at this imaging window is at maximum with minimum loss due to hemoglobin and water absorption. Therefore, the feasibility of deep imaging of the cells can be envisioned within this window [154]. The immense potential offered by NIR imaging may enable us to detect physiological, metabolic and molecular function *in vivo*. In the past few years, a considerable amount of progress has

been made in the areas of optical instrumentation and reconstruction algorithm, towards developing smart probes for NIR imaging for various diseases.

Numerous approaches have been proposed for optical imaging in terms of using fluorescent dyes. Some of the prerequisites for these dyes are:

- Strong excitation and emission in the NIR ‘window’;
- Stable fluorescent spectrum over a range of pH;
- Ease of chemical modifications for attaching ligands;
- No photo bleaching;
- No adverse toxicity.

The nature of the materials allows optical contrast agents to be classified into three main categories: organic, inorganic or hybrid in nature (Figure 4). The class of inorganic contrast agents is dominated by quantum dot (QD) particles [155]. Other examples of this class are carbon dots [156] and gold nanoparticles [157]. Gold nanoparticles have been prepared in different shapes and sizes (e.g., spheres, rods or hexagonal) [157]. These exogenous agents can be categorized in two major types: nonspecific and specific to cellular targets [157]. BODIPY [158] and lanthanide chelates [159] are examples of hybrid optical agents. Cyanine dyes are an important class of optical agents, which have gained tremendous attention due to their favorable NIR optical properties (Figure 5) [160]. Tetrapyrroles such as porphyrins, chlorins and phthalocyanines are another example of organic small-molecule dyes [161]. Depending on the nature and mode of their activation, these dyes can be nonspecific, targeted or activatable in nature.

QD-based agents

Quantum dots are among the most widely studied optical contrast agents. They are inorganic fluorophores based on cadmium sulphide- and cadmium selenide-type semiconductor crystals. QDs offer many advantages over traditional organic dyes, such as high photo stability, high quantum yield, continuous emission spectra spanning UV to NIR window, narrow emission spectral band width (20–30 nm) and long fluorescence lifetimes (10 ns or higher). Simulation results suggested two spectral windows for quantum dots (i.e. 700–900 and 1200–1600 nm). The excited state lifetimes (10–50 ns) of QDs are almost one order of magnitude higher than that of small-molecule organic dyes (2–5 ns), which makes them an interesting choice for fluorescence lifetime imaging of cells, tissue specimens and living animals. Since their conception, QDs have found wide applications in cells and in *in vivo* optical imaging, including cell trafficking, sentinel lymph-node imaging, neuro imaging, for example. QDs have been used as a stable fluorescent tracer for nonspecific uptake studies and lymph-node mapping in living animals [162,163]. Earlier in 2002, Akerman *et al.* demonstrated the feasibility of targeted *ex vivo* imaging with QDs [164]. In this seminal work, ZnS-capped CdSe QDs (10 nm) were coated with a lung-targeting peptide that accumulated in the lungs of mice after intravenous injection, whereas two other peptides specifically directed QDs to blood vessels or lymphatic vessels in tumors. They also demonstrated that a polyethyleneglycol coating of the QDs prevented their nonselective accumulation in reticuloendothelial tissues. In another work, QDs have been encapsulated in phospholipid micelles and injected directly into frog oocyte cells for real-time tracking of embryonic development [165].

$\alpha_v\beta_3$ -integrin remains an attractive biochemical epitope that is highly expressed on activated neovascular endothelial cells and which is essentially absent on mature quiescent cells. RGD tripeptide-functionalized QDs ($\lambda_{em} = 705$ nm) exhibited high-affinity integrin-specific binding in cell culture and *ex vivo*. NIR fluorescence imaging was also demonstrated in nude mice

bearing subcutaneous implanted integrin-positive U87MG human glioblastoma tumors [166]. Real-time imaging and tracking of single-receptor molecules has been made possible with antibody-conjugated QDs on the surface of living cells [167,168].

Phospholipid encapsulated QDs encountered some fluorescence loss and stability issues [164,169]. In another important report, Shuming and coworkers designed a polymer-encapsulated QD specific for prostate cancer cells in mice. ABC triblock copolymer uniformly coated the QDs and prevented particle aggregation and fluorescence loss by forming a stable hydrophobic protective layer around single QDs. Prostate specific membrane antigen antibody-coated nanoparticles were highly stable *in vivo* and demonstrated spectral imaging in live animals harboring C4-2 tumor xenografts. The results were supported by histological and immunohistochemical evidence [170].

Very recently, synthesis of a QD linked to α -fetoprotein (AFP) antibody has been reported for studying specific binding AFP, an important marker for hepatocellular carcinoma cell lines. QD-anti-AFPs were found to target the tumors *in vivo* and the authors investigated the inhomogeneous distribution of the probes in the tumor by using a site-by-site measurement method. The technique successfully demonstrated their ability to detect the distribution of the probes within cancer cells [171].

Another interesting example is NIR type II quantum dots. Frangioni and co-workers synthesized 10-nm-sized NIR QDs encapsulated by an oligomeric phosphine coating [172]. This poly-dentate phosphine coating rendered the parent particles soluble, dispersed and stable in serum. When these particles were injected (400 pmol) in mice, sentinel lymph nodes were detected 1-cm deep in real time, using excitation fluence rates of only 5 mW/cm². This approach was considered important for real-time, operating room sentinel lymph node resection, a common procedure in breast cancer surgery.

Typically, semiconductor QDs are prepared in organic solvents under high-temperature condition. Prior to their *in vivo* application, they are rendered water soluble by biocompatible, hydrophilic entrapment. This encapsulation, often challenging, can generate aggregated particles with poor shelf life and *in vivo* stability. Due to their agglomerated size and short circulation half lives, the *in vivo* targeted imaging would be very challenging with these types of particles and very few successful examples have been reported so far.

Quantum dots include heavy metal crystals that could elicit toxicity in their soluble form [173]. Given the known toxicity potential of Cd, cadmium-free examples of QDs, including InAs and CuInSe, have been proposed [174]. Bawendi and co-workers report the development of luminescent QD materials to ternary I-III-VI semiconductor systems by following a modular hot injection synthetic method [175]. Strongly luminescent CuInS₂/ZnS core/shell nanocrystals were synthesized by the same group from copper iodide, indium acetate, zinc stearate and dihydrolipoic acid as a surface ligand. CIS/ZnS NCs are the first example of lower toxicity QDs used for *in vivo* imaging.

Carbon dots

The development of quantum-sized carbon analogues has been reported very recently by Ya-Ping Sun and co-workers [156]. The carbon dots were produced via laser ablation of a carbon target in the presence of water vapor with argon as carrier gas and the surface of the particles was passivated by a diamine-terminated oligomeric PEG H₂NCH₂(CH₂CH₂O)_nCH₂CH₂CH₂NH₂ (average n = 35, PEG_{1500N}). Interestingly, the passivated carbon dots with organic moieties attached to the surface were strongly photoluminescent in suspension and the emissions cover both the visible and NIR wavelengths. Subsequently, the same group of authors demonstrated that carbon dots injected into mice

provided strong fluorescence *in vivo*, which, combined with their biocompatibility and non-toxic characteristics, suggested great potential for optical molecular imaging [176]. Another interesting example of fluorescent carbon nanoparticles (CNPs: 2–6 nm in size) with a quantum yield of approximately 3% was synthesized via nitric acid oxidation of carbon soot [177].

Xanthane dyes

Fluorescein and dyes with related structures (i.e., xanthane dyes) [178,179], have high quantum yields (fluorescein = ~0.8) and have been widely used in optical imaging studies. Numerous fluorescein intermediates and derivatives are commercially available. These probes typically absorb and emit in the visible wavelength and are generally only suitable for superficial imaging. As a result, primary applications have been in laboratory instruments operating at visible wavelengths with limited pharmaceutical development activity to date.

Cyanine & cypate dyes

Cyanine dyes have found numerous applications as nonlinear optical materials [180] and in biomolecular labeling [181–183], DNA sequencing and *in vivo* imaging [184]. They have been employed as labels in fluorescence imaging studies of biological mechanistic pathways. NIR cyanine dyes have an advantage, in that light at their emission and absorption maxima are perfectly tuned in the 650–900-nm range where absorbance by biomolecules is relatively poor and autofluorescence is less. They have found wide interest among synthetic chemists due to their straightforward synthesis [185–186], broad wavelength tunability and large molar absorptivities. Cyanine dyes can be classified into two broad categories based on structural connectivity: basic and adaptable. In basic cyanine dyes, two aromatic rings are connected by a polymethine chain with conjugated C-C double bonds. Adaptable cyanine dyes consist of reactive -Cl or -NH₂ groups in the central *meso* position for postsynthetic modifications via S_{RN}1 reactions with nucleophiles (e.g., amines [187] or thiols [188]).

Targeted cyanine dyes are meant to achieve higher target-to-background signal ratios and may provide more specific information from the diseased tissue of interest than nontargeted dyes. Typically, cyanine dyes are developed carrying functional groups for conjugation to macromolecules (e.g., antibodies, peptides and nanoparticles and polymers). The dye-to-macromolecule ratio needs to be properly optimized to positively impact the fluorescence properties of the conjugates [189].

Novel VCAM-1-targeted MRI and optical-based multimodal imaging agents were synthesized recently. A phage display-derived peptide sequence, containing the VHSPNKK motif, was shown to bind VCAM-1. The peptide sequence and a cyanine 5.5 dye were conjugated to an iron oxide-based (CLIO) nanoparticle. These particles were injected into a cholesterol-fed *apoE*^{-/-} mice to determine whether VCAM-1 expression could be detected *in vivo* in atherosclerotic lesions. Typically, extensive neovascularization and VCAM-1 expression are seen in this model. Extensive decrease in signal intensity associated with iron oxide NP accumulation in atherosclerotic lesions was observed in magnetic resonance and the results were further corroborated by macroscopic epifluorescence imaging of Cy 5.5 [190]. Cy5.5-labeled fluorescent probes for targeting thrombin and activated-platelets were developed based on the glycoprotein IIb/IIIa (GP-IIb/IIIa)-binding sequence, Pro-Ser-Pro-Gly-Asp-Trp. Both linear and branched sequences were synthesized, and provided at least five-times greater signal enhancement than the control (irrelevant peptide) when targeted to *in vitro* plasma clots. [191]

Weissleder and Ching-Tung synthesized a new class of stable, monocarboxylate functionalized cyanine dyes. The synthetic strategy was based on the nucleophilic attack of alkyl-thiols on cyanine dyes bearing chlorosubstituted polymethinic linkers [192]. Similar results were

obtained with monocarboxylate-derivatized fluorochromes (CyTE dyes). NIR fluorescence microscopy was able to monitor the endothelial cell internalization of the CyTE dye labeled VCAM-1 targeting peptide [192].

Licha and co-workers synthesized a group of glucamine and gluosamine-substituted cyanine dyes structurally related to indocyanine green [193]. *In vivo* results in rats indicated that, in order to overcome the limitations in spatial resolution and sensitivity, the strategy might facilitate optical-imaging applications where intrinsic contrast between tumor and healthy tissue needed to be amplified. The direct labeling of phage displaying a VCAM-1-targeting peptide was demonstrated using these fluorophores and the endothelial cell internalization of the VCAM-1-targeted phage was monitored via NIR fluorescence microscopy.

Renard and co-workers reported the synthesis of two novel water-soluble NIR cyanine dyes. When these dyes were connected within a peptide, mutual fluorescence quenching between the dyes was observed at both 705 and 798 nm. Based on this property, an internally quenched caspase-3-sensitive NIR fluorescent probe was prepared [194]. A recent review on targetable optical-imaging probes described the development of these dyes in detail [195].

Activatable 'smart' probes have been designed to morph their physical properties after a molecular interaction. They have been specifically synthesized for reporting activity of proteases such as matrix metalloproteinases, thrombin and cathepsins. The probes are optically nonresponsive in their quenched state and become fluorescent after enzyme-mediated release of the dye. This results in at least 100-fold signal enhancement. The design of the probes allowed us to combine a carrier particle or macromolecule with a NIR dye, via enzyme specific peptide substrates. For an excellent review on these activatable probes, please refer to Mahmood *et al.* [196].

Photoacoustic imaging

One of the most valuable attributes of optical imaging is its favorable safety profile. However, a major drawback is poor deep-tissue penetration. Photoacoustic imaging is a new nonionizing imaging technique that brings together the advantages of US and optical imaging [197–199].

Conventional exogenous imaging agents, including organic small-molecule fluorophores and lanthanide chelates, are generally susceptible to photobleaching, poor quantum yields and a broad emission window [200,201]. Lanthanide chelates have a tendency to distribute nonselectively into extravascular space [202].

Gold nanoparticles, on the other hand, offer unique optical properties and much better compatibility with the cellular environment, in comparison to other nanoparticles. Electromagnetic radiation irradiates the electrons in the outer shell of a gold nanoparticle to oscillate. This phenomenon is known as surface plasmon resonance (SPR). When excited, the SPR of gold particles scatter and absorb light in the visible or the NIR spectrum. The SPR can be controlled by tuning the size, shape and coating of gold nanoparticles. The optical properties of gold nanoparticle-treated cells have been controlled by size [203–205], morphology [206–208] and surface coating [209,210].

Gold nanoparticles (15 nm) have been utilized in a photoacoustic experiment to augment cell contrast upon irradiation by a short pulse laser. The acoustic emissions created by these particles were collected by ultrasonic array to image the target cells. Agarwal and co-workers have utilized these particles for the targeted photo acoustic detection of LnCAP prostate cancer cells [211].

In 2009, in an interesting novel finding, Pan and colleagues reported the development of gold nanobeacons (GNBs) [212]. GNBs represent a novel class of compliant ‘soft’ metal nanocolloids, which incorporate multiple tiny gold nanoparticles (2–3 nm) within a bigger vascularly constrained nanoparticle. The final formulation was encapsulated by phospholipids to produce 120–130-nm-sized GNBs that incorporate 10^3 gold atoms/particle. GNBs provided significant signal enhancement within the NIR window (740–820 nm) relative to blood (Figure 6c). These agents were developed for targeted imaging applications; however, at three-times the expected molecular imaging dose, a strong blood pool signal was seen in rats. Using *in vitro* clot phantoms, fibrin-targeted GNBs provided several-fold signal increase in comparison with the control (no gold) and nontargeted GNB particles (Figure 6A–B).

Kim *et al.* reported the development of a golden carbon nanotube for the photoacoustic detection of sentinel lymph nodes in rats [213]. In this very recent report, gold-coated carbon nanotubes were used for sensitive detection of lymphatic endothelial cells and cancer metastasis within sentinel lymph nodes. Golden nanotubes may present a more efficient *in vivo* biosensor compared with other nanocrystals (e.g., QDs) or fluorescent labels.

Future perspective

Molecular-imaging approaches, particularly those based on nanotechnologies, are expanding as more scientists with new expertises enter the field. In MRI, T_2^* imaging with iron oxide dominated the field 20 years ago. For the iron oxide-based nanoparticle platforms, the superparamagnetic effects induce magnetic susceptibility artifacts, creating dark signal dropouts or negative contrast. Today, there are numerous clinically practical T_1 w imaging approaches based on iron, manganese and gadolinium. Ligand-directed phospholipid-encapsulated PFC nanoparticles are vascularly constrained unique platform technology, which may be applied to clinically relevant modalities including fluorine and proton-based MRI techniques. A clever targeting mechanism of a cell membrane receptor specific system to mediate intracellular concentration of the imaging drug delivery agent is realized with dendrimer-based agents. This alternative approach to achieving these molecular-imaging goals with dendrimers utilizes effective cellular internalization and concentration of the agent to build signal. However, the recent discovery of nephrogenic systemic fibrosis (NSF) associated with gadolinium-based MRI agents have prompted the scientists to explore other paramagnetic metals. With the introduction of colloidal iron oxide-based nanoparticles, T_1 w MRI with iron oxide based agents can now be envisioned.

In optical imaging, where originally small-molecule dyes coupled to homing ligands were common, now nanoparticle concepts incorporating numerous fluorescent and/or NIR dyes into one particle are common. Solid-gold particles originally targeted individually are packaged within nanoparticles for markedly greater signal amplification. Moreover, beyond the imaging opportunities, many nanoplatform technologies are compatible with targeted drug delivery, offering further unique strategies to personalize the diagnosis and management of medical problems. With **nanomedicine** now at or closely approaching the clinic, an accelerated era of learning how to better design and use these new tools has begun.

Executive summary

- ‘Theranostic nanomedicine’ integrates molecular imaging and targeted drug delivery in an effort to optimize and individualize medical management, which represents a growing trend in healthcare. Nanoparticle-based molecular imaging has emerged as an interdisciplinary area showing immense potential for understanding the components and dynamics involved in disease processes at a molecular level. The technologies are among a class of image-guided drug-

delivery opportunities emerging within the MRI and optical imaging space for the detection and treatment of cardiovascular, cancer and inflammatory diseases.

- A variety of MRI nanoparticle agents based on iron, gadolinium and manganese have evolved with theranostic capability. In addition to more familiar site-specific iron oxide (T2 imaging) and gadolinium nanoparticles (T1 imaging), the development of colloidal iron-oxide nanoparticles for rapid T1 imaging, transferrin targeted dendrimeric gadolinium nanoparticles that concentrate intracellularly to enhance signal and manganese-based nanoparticles that provide remarkably high T1 signals are discussed to illustrate the divergence of chemistries observed in the newest generation of nanomedicine technologies.
- Similarly, beyond well known optical properties of quantum dots, the exploration for noncadmium-based agents is yielding new finds, such as the development of carbon dots.
- Moreover, whereas the clinical utility of optical imaging has been considered very limited due to poor deep tissue penetration, photoacoustic imaging, which combines ultrasound and optical imaging, has emerged as an important tool for preclinical research and, soon, clinical applications. In this imaging context, gold nanoparticles in various forms, which have remarkable near infrared properties have found extensive application for optical and photo acoustic based imaging techniques.

Glossary

Theranostics	Technique that integrates diagnostics and therapy to selectively adapt treatment of a patient according to their genotype and details of the disease that have earlier not been localized or differentiated
Personalized medicine	The area of science that helps to identify a person's predisposition to a particular disease using genomic and molecular data
Molecular imaging	The visualization of the cellular function and the follow-up of the molecular process in living organisms in a noninvasive way using diagnostic modalities
Optical imaging	A noninvasive diagnostic technique that uses the near infrared region of the electromagnetic spectrum (650 900 nm)
Angiogenesis	A physiological process that involves the growth of new blood vessels from pre-existing vessels. Angiogenesis is an important bio-signature of cancer
Nanomedicine	In a broad sense, the biomedical application of nanotechnology is referred as nanomedicine

Bibliography

1. Soman N, Lanza G, Heuser J, Schlesinger P, Wickline S. Synthesis and characterization of stable fluorocarbon nanostructures as drug delivery vehicles for cytolytic peptides. *Nano Lett* 2008;8:1131–1136. [PubMed: 18302330]
2. Lanza GM, Yu X, Winter PM, et al. Targeted antiproliferative drug delivery to vascular smooth muscle cells with a magnetic resonance imaging nanoparticle contrast agent: implications for rational therapy of restenosis. *Circulation* 2002;106:2842–2847. [PubMed: 12451012]
3. Crowder KC, Hughes MS, Marsh JN, et al. Sonic activation of molecularly-targeted nanoparticles accelerates transmembrane lipid delivery to cancer cells through contact-mediated mechanisms:

- implications for enhanced local drug delivery. *Ultrasound Med Biol* 2005;31:1693–1700. [PubMed: 16344131]
4. Winter PM, Schmieder AH, Caruthers SD, et al. Minute dosages of $\alpha_v\beta_3$ -targeted fumagillin nanoparticles impair Vx-2 tumor angiogenesis and development in rabbits. *FASEB J* 2008;22:2758–2767. [PubMed: 18362202]
 5. Liang HD, Blomley MJK. The role of ultrasound in molecular imaging. *Brit J Radiol* 2003;76:S140–S150. [PubMed: 15572336]
 6. Kumar S, Kortum RR. Optical molecular imaging agents for cancer diagnostics and therapeutics. *Nanomedicine* 2006;1(1):23–30. [PubMed: 17716206]
 7. Ametamey SM, Honer M, Schubiger PA. Molecular imaging with PET. *Chem Rev* 2008;108(5):1501–1516. [PubMed: 18426240]
 8. Rabin O, Manuel PJ, Grimm J, Wojtkiewicz G, Weissleder R. *Nat Mater* 2006;5(2):118–122. [PubMed: 16444262]
 9. Pan D, Williams TA, Senpan A, et al. Detecting vascular biosignatures with a colloidal, radio-opaque polymeric nanoparticle. *J Am Chem Soc* 2009;131(42):15522–15527. [PubMed: 19795893]
 10. Nelson KL, Runge VM. Basic principles of MR contrast. *Magn Reson Imaging* 1995;7:124–136.
 11. Sosnovik DE, Weissleder R. Emerging concepts in molecular MRI. *Curr Opin Biotechnol* 2007;18(1):4–10. [PubMed: 17126545]
 12. Golman K, Olsson LE, Axelsson O, et al. Molecular imaging using hyperpolarized ^{13}C . *Brit J Radiol* 2003;76:S118–S127. [PubMed: 15572334]
 13. Ross BD, Bhattacharya P, Wagner S, Tran T, Sailasuta N. Hyperpolarized MR imaging: neurologic applications of hyperpolarized metabolism. *Am J Neuroradiol* 2009;1:24–33. [PubMed: 19875468]
 14. Fain SB, Korosec FR, Holmes JH, O'Halloran R, Sorkness RL, Grist TM. Functional lung imaging using hyperpolarized gas MRI. *J Magn Reson Imaging* 2007;25(5):910–923. [PubMed: 17410561]
 15. Winter P, Caruthers S, Yu X, et al. Improved molecular imaging contrast agent for detection of human thrombus. *Mag Reson Med* 2003;50:411–416.
 16. Winter P, Athey P, Kiefer G, et al. Improved paramagnetic chelate for molecular imaging with MRI. *J Magn Magn Mater* 2005;293:540–545.
 17. Sipkins DA, Cheresch DA, Kazemi MR, et al. Detection of tumor angiogenesis *in vivo* by $\alpha_v\beta_3$ -targeted magnetic resonance imaging. *Nat Med* 1998;4:623–626. [PubMed: 9585240]
 18. Lanza G, Lorenz C, Fischer S, et al. Enhanced detection of thrombi with a novel fibrin-targeted magnetic resonance imaging agent. *Acad Radiol* 1998;5(Suppl 1):S173–S176. [PubMed: 9561074]
 19. Botnar RM, Buecker A, Wiethoff AJ, et al. *In vivo* magnetic resonance imaging of coronary thrombosis using a fibrin-binding molecular magnetic resonance contrast agent. *Circulation* 2004;110:1463–1466. [PubMed: 15238457]
 20. Mulder WJ, Strijkers GJ, Habets JW, et al. MR molecular imaging and fluorescence microscopy for identification of activated tumor endothelium using a bimodal lipidic nanoparticle. *FASEB J* 2005;19:2008–2010. [PubMed: 16204353]
 21. Mulder WJ, Strijkers GJ, van Tilborg GA, Griffioen AW, Nicolay K. Lipid-based nanoparticles for contrast-enhanced MRI and molecular imaging. *NMR Biomed* 2006;19:142–164. [PubMed: 16450332]
 22. Mulder WJ, van der Schaft DW, Hautvast PA, et al. Early *in vivo* assessment of angiostatic therapy efficacy by molecular MRI. *FASEB J* 2007;21:378–383. [PubMed: 17202248]
 23. Frias JC, Williams KJ, Fisher EA, Fayad ZA. Recombinant HDL-like nanoparticles: a specific contrast agent for MRI of atherosclerotic plaques. *J Am Chem Soc* 2004;126:16316–16317. [PubMed: 15600321]
 24. Lipinski MJ, Amirbekian V, Frias JC, et al. MRI to detect atherosclerosis with gadolinium-containing immunomicelles targeting the macrophage scavenger receptor. *Magn Reson Med* 2006;56:601–610. [PubMed: 16902977]
 25. Flacke S, Fischer S, Scott M, et al. A novel MRI contrast agent for molecular imaging of fibrin: implications for detecting vulnerable plaques. *Circulation* 2001;104:1280–1285. [PubMed: 11551880]

26. Winter PM, Morawski AM, Caruthers SD, et al. Molecular imaging of angiogenesis in early-stage atherosclerosis with $\alpha_v\beta_3$ -integrin-targeted nanoparticles. *Circulation* 2003;108:2270–2274. [PubMed: 14557370]
27. Winter P, Neubauer A, Caruthers S, et al. Endothelial $\alpha_v\beta_3$ -integrin targeted fumagillin nanoparticles inhibit angiogenesis in atherosclerosis. *Arterioscler Thromb Vasc Biol* 2006;26:2103–2109. [PubMed: 16825592]
28. Winter P, Caruthers S, Zhang H, Williams T, Wickline S, Lanza G. Antiangiogenic synergism of integrin-targeted fumagillin nanoparticles and atorvastatin in atherosclerosis. *J Am Coll Cardiol Img* 2008;1:624–634.
29. Winter PM, Caruthers SD, Kassner A, et al. Molecular imaging of angiogenesis in nascent Vx-2 rabbit tumors using a novel $\alpha_v\beta_3$ -targeted nanoparticle and 1.5 tesla magnetic resonance imaging. *Cancer Res* 2003;63:5838–5843. [PubMed: 14522907]
30. Schmieder AH, Winter PM, Caruthers SD, et al. Molecular MR imaging of melanoma angiogenesis with $\alpha_v\beta_3$ -targeted paramagnetic nanoparticles. *Magn Reson Med* 2005;53:621–627. [PubMed: 15723405]
31. Schmieder AH, Caruthers SD, Zhang H, et al. Three-dimensional MR mapping of angiogenesis with $\{\alpha\}_5\{\beta\}_1(\{\alpha\}\{\beta\}_3)$ -targeted theranostic nanoparticles in the MDA-MB-435 xenograft mouse model. *FASEB J* 2008;22:4179–4189. [PubMed: 18697838]
32. Morawski AM, Winter PM, Crowder KC, et al. Targeted nanoparticles for quantitative imaging of sparse molecular epitopes with MRI. *Magn Reson Med* 2004;51(3):480–486. [PubMed: 15004788]
33. Bulte JW, Douglas T, Witwer B, et al. Magnetodendrimers allow endosomal magnetic labeling and *in vivo* tracking of stem cells. *Nat Biotechnol* 2001;19:1141–1147. [PubMed: 11731783]
34. Wiener E, Konda S, Shadron A, Brechbiel M, Gansow O. Targeting dendrimer-chelates to tumors and tumor cells expressing the high-affinity folate receptor. *Invest Radiol* 1997;32:748–754. [PubMed: 9406015]
35. Kukowska-Latallo JF, Candido KA, Cao Z, et al. Nanoparticle targeting of anticancer drug improves therapeutic response in animal model of human epithelial cancer. *Cancer Res* 2005;65:5317–5324. [PubMed: 15958579]
36. Shi X, Thomas TP, Myc LA, Kotlyar A, Baker JR Jr. Synthesis, characterization, and intracellular uptake of carboxyl-terminated poly(amidoamine) dendrimer-stabilized iron oxide nanoparticles. *Phys Chem Chem Phys* 2007;9:5712–5720. [PubMed: 17960261]
37. Landmark KJ, Dimaggio S, Ward J, et al. Synthesis, characterization, and *in vitro* testing of superparamagnetic iron oxide nanoparticles targeted using folic acid-conjugated dendrimers. *ACS Nano* 2008;2:773–783. [PubMed: 19206610]
38. Shi X, Wang S, Meshinchi S, et al. Dendrimer-entrapped gold nanoparticles as a platform for cancer-cell targeting and imaging. *Small* 2007;3:1245–1252. [PubMed: 17523182]
39. Swanson SD, Kukowska-Latallo JF, Patri AK, et al. Targeted gadolinium-loaded dendrimer nanoparticles for tumor-specific magnetic resonance contrast enhancement. *Int J Nanomedicine* 2008;3:201–210. [PubMed: 18686779]
40. Thomas TP, Shukla R, Kotlyar A, et al. Dendrimer-epidermal growth factor conjugate displays superagonist activity. *Biomacromolecules* 2008;9:603–609. [PubMed: 18193839]
41. Cheresh DA. Integrins in thrombosis, wound healing and cancer. *Biochem Soc Trans* 1991;19:835–838. [PubMed: 1794568]
42. Friedlander M, Theesfeld CL, Sugita M, Fruttiger M, Thomas MA, Chang S, Cheresh DA. Involvement of integrins $\alpha_v\beta_3$ and $\alpha_v\beta_5$ in ocular neovascular diseases. *Proc Natl Acad Sci USA* 1996;93:9764–9769. [PubMed: 8790405]
43. De Nichilo M, Burns G. Granulocyte-macrophage and macrophage colony-stimulating factors differentially regulate α_v integrin expression on cultured human macrophages. *Proc Natl Acad Sci USA* 1993;90:2517–2521. [PubMed: 7681600]
44. Helluin O, Chan C, Vilaire G, Mousa S, DeGrado WF, Bennett JS. The activation state of $\alpha_v\beta_3$ regulates platelet and lymphocyte adhesion to intact and thrombin-cleaved osteopontin. *J Biol Chem* 2000;275:18337–18343. [PubMed: 10751402]
45. Itoh H, Nelson P, Mureebe L, Horowitz A, Kent K. The role of integrins in saphenous vein vascular smooth muscle cell migration. *J Vasc Surg* 1997;25:1061–1069. [PubMed: 9201167]

46. Carreiras F, Denoux Y, Staedel C, Lehmann M, Sichel F, Gauduchon P. Expression and localization of α_v integrins and their ligand vitronectin in normal ovarian epithelium and in ovarian carcinoma. *Gynecol Oncol* 1996;62:260–267. [PubMed: 8751559]
47. Kageshita T, Hamby CV, Hirai S, Kimura T, Ono T, Ferrone S. Differential clinical significance of $\alpha(v)B(3)$ expression in primary lesions of acral lentiginous melanoma and of other melanoma histotypes. *Int J Cancer* 2000;89:153–159. [PubMed: 10754493]
48. Hu G, Lijowski M, Zhang H, et al. Imaging of Vx-2 rabbit tumors with $\alpha_v\beta_3$ -integrin-targeted ^{111}In nanoparticles. *Int J Cancer* 2007;120:1951–1957. [PubMed: 17278104]
49. Weissleder R, Bogdanov A Jr, Tung CH, Weinmann HJ. Size optimization of synthetic graft copolymers for *in vivo* angiogenesis imaging. *Bioconjug Chem* 2001;12:213–219. [PubMed: 11312682]
50. Zetter BR. Angiogenesis and tumor metastasis. *Annu Rev Med* 1998;49:407–424. [PubMed: 9509272]
51. Folkman J. Tumor angiogenesis. *Adv Cancer Res* 1985;43:175–203. [PubMed: 2581424]
52. Carmeliet P. Mechanisms of angiogenesis and arteriogenesis. *Nat Med* 2000;6:389–395. [PubMed: 10742145]
53. Lanza GM, Yu X, Winter PM, et al. Targeted antiproliferative drug delivery to vascular smooth muscle cells with a magnetic resonance imaging nanoparticle contrast agent: implications for rational therapy of restenosis. *Circulation* 2002;106:2842–2847. [PubMed: 12451012]
54. Marsh J, Senpan A, Hu G, Scott M, Gaffney P, Wickline S, Lanza G. Fibrin-targeted perfluorocarbon nanoparticles for targeted thrombolysis. *Nanomedicine* 2007;2:533–543. [PubMed: 17716136]
55. Cyrus T, Zhang H, Allen JS, et al. Intramural delivery of rapamycin with $\alpha_v\beta_3$ -targeted paramagnetic nanoparticles inhibits stenosis after balloon injury. *Arterioscler Thromb Vasc Biol* 2008;28:820–826. [PubMed: 18292395]
56. Partlow K, Lanza G, Wickline S. Exploiting lipid raft transport with membrane targeted nanoparticles: a strategy for cytosolic drug delivery. *Biomaterials* 2008;29:3367–3375. [PubMed: 18485474]
57. Liu S, Widom J, Kemp CW, Crews CM, Clardy J. Structure of human methionine aminopeptidase-2 complexed with fumagillin. *Science* 1998;282:1324–1327. [PubMed: 9812898]
58. Sin N, Meng L, Wang MQ, Wen JJ, Bornmann WG, Crews CM. The anti-angiogenic agent fumagillin covalently binds and inhibits the methionine aminopeptidase, MetAP-2. *Proc Natl Acad Sci USA* 1997;94:6099–6103. [PubMed: 9177176]
59. Bergers G, Javaherian K, Lo KM, Folkman J, Hanahan D. Effects of angiogenesis inhibitors on multistage carcinogenesis in mice. *Science* 1999;284:808–812. [PubMed: 10221914]
60. Castronovo V, Belotti D. TNP-470 (AGM-1470), mechanisms of action and early clinical development. *Eur J Cancer* 1996;32A:2520–2527. [PubMed: 9059342]
61. Konno H, Tanaka T, Kanai T, Maruyama K, Nakamura S, Baba S. Efficacy of an angiogenesis inhibitor, TNP-470, in xenotransplanted human colorectal cancer with high metastatic potential. *Cancer* 1996;77:1736–1740. [PubMed: 8608571]
62. Shusterman S, Grupp SA, Barr R, Carpentieri D, Zhao H, Maris JM. The angiogenesis inhibitor TNP-470 effectively inhibits human neuroblastoma xenograft growth, especially in the setting of subclinical disease. *Clin Cancer Res* 2001;7:977–984. [PubMed: 11309349]
63. Bhargava P, Marshall JL, Rizvi N, et al. A Phase I and pharmacokinetic study of TNP-470 administered weekly to patients with advanced cancer. *Clin Cancer Res* 1999;5:1989–1995. [PubMed: 10473076]
64. Kudelka AP, Verschraegen CF, Loyer E. Complete remission of metastatic cervical cancer with the angiogenesis inhibitor TNP-470. *N Engl J Med* 1998;338:991–992. [PubMed: 9527612]
65. Kudelka AP, Levy T, Verschraegen CF, et al. A phase I study of TNP-470 administered to patients with advanced squamous cell cancer of the cervix. *Clin Cancer Res* 1997;3:1501–1505. [PubMed: 9815836]
66. Logothetis CJ, Wu KK, Finn LD, et al. Phase I trial of the angiogenesis inhibitor TNP-470 for progressive androgen-independent prostate cancer. *Clin Cancer Res* 2001;7:1198–1203. [PubMed: 11350884]
67. Offodile R, Walton T, Lee M, Stiles A, Nguyen M. Regression of metastatic breast cancer in a patient treated with the anti-angiogenic drug TNP-470. *Tumori* 1999;85:51–53. [PubMed: 10228498]

68. Bachert P. Pharmacokinetics using fluorine NMR *in vivo*. Prog Nucl Magn Reson Spectrosc 1998;33:1–56.
69. Wolf W, Presant CA, Waluch V. 19F-MRS studies of fluorinated drugs in humans. Adv Drug Deliv Rev 2000;41:55–74. [PubMed: 10699305]
70. Kaneda MM, Caruthers S, Lanza GM, Wickline SA. Perfluorocarbon nanoemulsions for quantitative molecular imaging and targeted therapeutics. Ann Biomed Eng 2009;37:1922–1933. [PubMed: 19184435]
71. Mason RP, Hunjan S, Le D, et al. Regional tumor oxygen tension: fluorine echo planar imaging of hexafluorobenzene reveals heterogeneity of dynamics. Int J Radiat Oncol Biol Phys 1998;42:747–750. [PubMed: 9845089]
72. Davda S, Bezabeh T. Advances in methods for assessing tumor hypoxia *in vivo*: implications for treatment planning. Cancer Metastasis Rev 2006;25:469–480. [PubMed: 17029029]
73. Mattrey RF, Schumacher DJ, Tran HT, Guo O, Buxton RB. Use of imagent BP (PFOB) in diagnostic imaging and F-19 magnetic resonance for PO₂ measurements. Biomater Artif Cells Immobilization Biotechnol 1991;19:435.
74. Mattrey RF, Schumacher DJ, Tran HT, Guo Q, Buxton RB. The use of imagent(R) BP in diagnostic imaging research and 19F magnetic resonance for PO₂ measurements. Biomater Artif Cells Immobilization Biotechnol 1992;20:917–920. [PubMed: 1391533]
75. Spiess BD. Perfluorocarbon emulsions as a promising technology: a review of tissue and vascular gas dynamics. J Appl Physiol 2009;106:1444–1452. [PubMed: 19179651]
76. Barrett T, Brechbiel M, Bernardo M, Choyke PL. MRI of tumor angiogenesis. J Magn Reson Imaging 2007;26:235–249. [PubMed: 17623889]
77. Iannetti GD, Wise RG. BOLD functional MRI in disease and pharmacological studies: room for improvement? Magn Reson Imaging 2007;25:978–988. [PubMed: 17499469]
78. Matthews PM, Jezzard P. Functional magnetic resonance imaging. J Neurol Neurosurg Psychiatry 2004;75:6–12. [PubMed: 14707297]
79. Merboldt KD, Fransson P, Bruhn H, Frahm J. Functional MRI of the human amygdala? NeuroImage 2001;14:253–257. [PubMed: 11467900]
80. Padhani AR, Krohn KA, Lewis JS, Alber M. Imaging oxygenation of human tumours. Eur Radiol 2007;17:861–872. [PubMed: 17043737]
81. Vaupel P, Mayer A. Hypoxia in cancer: Significance and impact on clinical outcome. Cancer Metastasis Rev 2007;26:225–239. [PubMed: 17440684]
82. Kodibagkar VD, Wang X, Mason RP. Physical principles of quantitative nuclear magnetic resonance oximetry. Front Biosci 2008;13:1371–1384. [PubMed: 17981636]
83. Zhao D, Constantinescu A, Hahn EW, Mason RP. Tumor oxygen dynamics with respect to growth and respiratory challenge: investigation of the Dunning prostate R3327-HI tumor. Radiat Res 2001;156:510–520. [PubMed: 11604064]
84. Mason RP, Constantinescu A, Hunjan S, et al. Regional tumor oxygenation and measurement of dynamic changes. Radiat Res 1999;152:239–249. [PubMed: 10453084]
85. Hunjan S, Zhao D, Constantinescu A, Hahan E, Antich P, Mason R. Tumor oximetry: demonstration of an enhanced dynamic mapping procedure using fluorine-19 echo planar magnetic resonance imaging the Dunning prostate R3327-At1 rat tumor. Int J Radiat Oncol Biol Phys 2001;49:1097–1108. [PubMed: 11240252]
86. Lanza GM, Wallace KD, Scott MJ, et al. A novel site-targeted ultrasonic contrast agent with broad biomedical application. Circulation 1996;95:3334–3340. [PubMed: 8989148]
87. Yu X, Song SK, Scott MJ, et al. Molecular characterization of thrombus using bimodal ¹H/¹⁹F MR imaging with a novel fibrin-targeted nanoparticulate contrast agent. Proc Intl Soc Mag Reson Med 2000;8:465.
88. Morawski AM, Winter PM, Yu X, et al. Quantitative “magnetic resonance immunohistochemistry” with ligand-targeted (19)F nanoparticles. Magn Reson Med 2004;52:1255–1262. [PubMed: 15562481]
89. Caruthers SD, Neubauer AM, Hockett FD, et al. *In vitro* demonstration using ¹⁹F magnetic resonance to augment molecular imaging with paramagnetic perfluorocarbon nanoparticles at 1.5 Tesla. Invest Radiol 2006;41:305–312. [PubMed: 16481914]

90. Neubauer AM, Caruthers SD, Hockett FD, et al. Fluorine cardiovascular magnetic resonance angiography *in vivo* at 1.5 T with perfluorocarbon nanoparticle contrast agents. *J Cardiovasc Magn Reson* 2007;9:565–573. [PubMed: 17365236]
91. Waters EA, Chen J, Allen JS, et al. Detection and quantification of angiogenesis in experimental valve disease with integrin-targeted nanoparticles and 19-fluorine MRI/MRS. *J Cardiovasc Magn Reson* 2008;10:43. [PubMed: 18817557]
92. Southworth R, Kaneda M, Chen J, et al. Renal vascular inflammation induced by Western diet in ApoE-null mice quantified by ¹⁹F NMR of VCAM-1 targeted nanobeacons. *Nanomedicine* 2009;5:359–367. [PubMed: 19523428]
93. Waters EA, Chen J, Yang X, et al. Detection of targeted perfluorocarbon nanoparticle binding using 19F diffusion weighted MR spectroscopy. *Magn Reson Med* 2008;60:1232–1236. [PubMed: 18956417]
94. Arbab AS, Yocum GT, Kalish H, et al. Efficient magnetic cell labeling with protamine sulfate complexed to ferumoxides for cellular MRI. *Blood* 2004;104:1217–1223. [PubMed: 15100158]
95. Arbab AS, Jordan EK, Wilson LB, Yocum GT, Lewis BK, Frank JA. *In vivo* trafficking and targeted delivery of magnetically labeled stem cells. *Hum Gene Ther* 2004;15:351–360. [PubMed: 15053860]
96. Modo M, Mellodew K, Cash D, et al. Mapping transplanted stem cell migration after a stroke: a serial, *in vivo* magnetic resonance imaging study. *Neuroimage* 2004;21:311–317. [PubMed: 14741669]
97. Shapiro E, Sharer K, Skrtic S, Koretsky A. *In vivo* detection of single cells by MRI. *Magn Reson Med* 2006;55:242–249. [PubMed: 16416426]
98. Bulte JW. Hot spot MRI emerges from the background. *Nat Biotechnol* 2005;23:945–946. [PubMed: 16082363]
99. Ahrens ET, Flores R, Xu H, Morel PA. *In vivo* imaging platform for tracking immunotherapeutic cells. *Nat Biotechnol* 2005;23:983–987. [PubMed: 16041364]
100. Partlow KC, Chen J, Brant JA, et al. 19F magnetic resonance imaging for stem/progenitor cell tracking with multiple unique perfluorocarbon nanobeacons. *FASEB J* 2007;21:1647–1654. [PubMed: 17284484]
101. Ruiz-Cabello J, Walczak P, Kedziorek D, et al. *In vivo* “hot spot” MR imaging of neural stem cells using fluorinated nanoparticles. *Magn Reson Med* 2008;60:1506–1511. [PubMed: 19025893]
102. Srinivas M, Morel PA, Ernst LA, Laidlaw DH, Ahrens ET. Fluorine-19 MRI for visualization and quantification of cell migration in a diabetes model. *Magn Reson Med* 2007;58:725–734. [PubMed: 17899609]
103. Stark DD, Weissleder R, Elizondo G, et al. Superparamagnetic iron oxide: clinical application as a contrast agent for MR imaging of the liver. *Radiology* 1988;168:297–301. [PubMed: 3393649]
104. Weissleder R, Hahn PF, Stark DD, et al. Superparamagnetic iron oxide: enhanced detection of focal splenic tumors with MR imaging. *Radiology* 1988;169:399–403. [PubMed: 3174987]
105. Frank H, Weissleder R, Brady TJ. Enhancement of MR angiography with iron oxide: preliminary studies in whole-blood phantom and in animals. *AJR Am J Roentgenol* 1994;162:209–213. [PubMed: 8273667]
106. Kresse M, Wagner S, Pfefferer D, Lawaczeck R, Elste V, Semmler W. Targeting of ultrasmall superparamagnetic iron oxide (USPIO) particles tumor cells *in vivo* by using transferrin receptor pathways. *Magn Reson Med* 1998;40:236–242. [PubMed: 9702705]
107. Jung C, Jacobs P. Physical and chemical properties of superparamagnetic iron oxide MR contrast agents: ferumoxides, Ferumoxtran, ferumoxsil. *Magn Reson Imaging* 1995;13:661–674. [PubMed: 8569441]
108. Anzai Y, Prince MR, Chenevert TL, et al. MR angiography with an ultrasmall superparamagnetic iron oxide blood pool agent. *J Magn Reson Imaging* 1997;7:209–214. [PubMed: 9039617]
109. Loubeyre P, Zhao S, Canet E, Abidi H, Benderbous S, Revel D. Ultrasmall superparamagnetic iron oxide particles (AMI 227) as a blood pool contrast agent for MR angiography: experimental study in rabbits. *J Magn Reson Imaging* 1997;7:958–962. [PubMed: 9400837]
110. Schellenberger EA, Bogdanov A Jr, Hogemann D, Tait J, Weissleder R, Josephson L. Annexin V-CLIO: a nanoparticle for detecting apoptosis by MRI. *Mol Imaging* 2002;1:102–107. [PubMed: 12920851]

111. Kircher MF, Allport JR, Graves EE, et al. *In vivo* high resolution three-dimensional imaging of antigen-specific cytotoxic T-lymphocyte trafficking to tumors. *Cancer Res* 2003;63:6838–6846. [PubMed: 14583481]
112. Kelly KA, Allport JR, Tsourkas A, Shinde-Patil VR, Josephson L, Weissleder R. Detection of vascular adhesion molecule-1 expression using a novel multimodal nanoparticle. *Circ Res* 2005;96:327–336. [PubMed: 15653572]
113. Schmitz SA, Coupland SE, Gust R, et al. Superparamagnetic iron oxide-enhanced MRI of atherosclerotic plaques in Watanabe heritable hyperlipidemic rabbits. *Invest Radiol* 2000;35:460–471. [PubMed: 10946973]
114. Schmitz SA, Taupitz M, Wagner S, et al. Magnetic resonance imaging of atherosclerotic plaques using superparamagnetic iron oxide particles. *J Magn Reson Imaging* 2001;14:355–361. [PubMed: 11599058]
115. Schmitz S, Taupitz M, Wagner S, et al. Iron-oxide-enhanced magnetic resonance imaging of atherosclerotic plaques: postmortem analysis of accuracy, inter-observer agreement, and pitfalls. *Invest Radiol* 2002;37:405–411. [PubMed: 12068163]
116. Ruehm SG, Corot C, Vogt P, Kolb S, Debatin JF. Magnetic resonance imaging of atherosclerotic plaque with ultrasmall superparamagnetic particles of iron oxide in hyperlipidemic rabbits. *Circulation* 2001;103:415–422. [PubMed: 11157694]
117. Kooi ME, Cappendijk VC, Cleutjens KB, et al. Accumulation of ultrasmall superparamagnetic particles of iron oxide in human atherosclerotic plaques can be detected by *in vivo* magnetic resonance imaging. *Circulation* 2003;107:2453–2458. [PubMed: 12719280]
118. Herborn CU, Vogt FM, Lauenstein TC, et al. Magnetic resonance imaging of experimental atherosclerotic plaque: comparison of two ultrasmall superparamagnetic particles of iron oxide. *J Magn Reson Imaging* 2006;24:388–393. [PubMed: 16791857]
119. Cunningham C, Arai T, Yang P, et al. Positive contrast magnetic resonance imaging of cells labeled with magnetic nanoparticles. *Magn Reson Med* 2005;53:999–1005. [PubMed: 15844142]
120. Dharmakumar R, Koktzoglou I, Li D. Generating positive contrast from off-resonant spins with steady-state free precession magnetic resonance imaging: Theory and proof-of-principle experiments. *Phys Med Biol* 2006;51:4201–4215. [PubMed: 16912377]
121. Mani V, Briley-Saebo KC, Itskovich VV, Samber DD, Fayad ZA. Gradient echo acquisition for superparamagnetic particles with positive contrast (GRASP), sequence characterization in membrane and glass superparamagnetic iron oxide phantoms at 1.5T and 3T. *Magn Reson Med* 2006;55:126–135. [PubMed: 16342148]
122. Zurkiya O, Hu X. Off-resonance saturation as a means of generating contrast with superparamagnetic nanoparticles. *Magn Reson Med* 2006;56:726–732. [PubMed: 16941618]
123. Stuber M, Gilson WD, Schar M, et al. Positive contrast visualization of iron oxide-labeled stem cells using inversion-recovery with ON-resonant water suppression (IRON). *Magn Reson Med* 2007;58:1072–1077. [PubMed: 17969120]
124. Korosoglou G, Tang L, Kedziorek D, et al. Positive contrast MR-lymphography using inversion recovery with ON-resonant water suppression (IRON). *J Magn Reson Imaging* 2008;27:1175–1180. [PubMed: 18425827]
125. Korosoglou G, Weiss RG, Kedziorek DA, et al. Noninvasive detection of macrophage-rich atherosclerotic plaque in hyperlipidemic rabbits using “positive contrast” magnetic resonance imaging. *J Am Coll Cardiol* 2008;52:483–491. [PubMed: 18672170]
126. Korosoglou G, Shah S, Vonken EJ, et al. Off-resonance angiography: a new method to depict vessels – phantom and rabbit studies. *Radiology* 2008;249:501–509. [PubMed: 18780823]
127. Weissleder R, Moore A, Mahmood U, et al. *In vivo* magnetic resonance imaging of transgene expression. *Nat Med* 2000;6:351–355. [PubMed: 10700241]
128. Dunn JF, Roche MA, Springett R, et al. Monitoring angiogenesis in brain using steady-state quantification of δR_2 with MION infusion. *Magn Reson Med* 2004;51:55–61. [PubMed: 14705045]
129. Hogemann D, Josephson L, Weissleder R, Basilion JP. Improvement of MRI probes to allow efficient detection of gene expression. *Bioconjug Chem* 2000;11:941–946. [PubMed: 11087345]

130. Kang HW, Josephson L, Petrovsky A, Weissleder R, Bogdanov A Jr. Magnetic resonance imaging of inducible E-selectin expression in human endothelial cell culture. *Bioconjug Chem* 2002;13:122–127. [PubMed: 11792187]
131. Koch AM, Reynolds F, Kircher MF, Merkle HP, Weissleder R, Josephson L. Uptake and metabolism of a dual fluorochrome Tat-nanoparticle in HeLa cells. *Bioconjug Chem* 2003;14:1115–1121. [PubMed: 14624624]
132. Josephson L, Tung CH, Moore A, Weissleder R. High-efficiency intracellular magnetic labeling with novel superparamagnetic-Tat peptide conjugates. *Bioconjug Chem* 1999;10:186–191. [PubMed: 10077466]
133. Dodd CH, Hsu HC, Chu WJ, et al. Normal T-cell response and *in vivo* magnetic resonance imaging of T cells loaded with HIV transactivator-peptide-derived superparamagnetic nanoparticles. *J Immunol Methods* 2001;256:89–105. [PubMed: 11516758]
134. Lewin M, Carlesso N, Tung CH, et al. Tat peptide-derivatized magnetic nanoparticles allow *in vivo* tracking and recovery of progenitor cells. *Nat Biotech* 2000;18:410–414.
135. Nahrendorf M, Jaffer FA, Kelly KA, et al. Noninvasive vascular cell adhesion molecule-1 imaging identifies inflammatory activation of cells in atherosclerosis. *Circulation* 2006;114:1504–1511. [PubMed: 17000904]
136. von zur Muhlen C, von Elverfeldt D, Moeller JA, et al. Magnetic resonance imaging contrast agent targeted toward activated platelets allows *in vivo* detection of thrombosis and monitoring of thrombolysis. *Circulation* 2008;118:258–267. [PubMed: 18574047]
137. McAteer MA, Schneider JE, Ali ZA, et al. Magnetic resonance imaging of endothelial adhesion molecules in mouse atherosclerosis using dual-targeted microparticles of iron oxide. *Arterioscler Thromb Vasc Biol* 2008;28:77–83. [PubMed: 17962629]
138. Caruthers S, Senpan A, Pan D, et al. A novel targeted iron oxide nanocolloid agent for rapid detection of fibrin clots via T₁ and T₂ weighted MRI. *J Cardiovasc Magn Reson* 2008;10(Suppl 1):A384.
139. Senpan A, Caruthers S, Rhee I, et al. Conquering the dark side: colloidal iron oxide nanoparticles. *ACS Nano* 2009;3(12):3917–3926. [PubMed: 19908850]
140. Lu J, Ma S, Sun J, et al. Manganese ferrite nanoparticle micellar nanocomposites as MRI contrast agent for liver imaging. *Biomaterials* 2009;30:2919–2928. [PubMed: 19230966]
141. Yang J, Lee CH, Ko HJ, et al. Multifunctional magneto-polymeric nanohybrids for targeted detection and synergistic therapeutic effects on breast cancer. *Angew Chem Int Ed Engl* 2007;46:8836–8839. [PubMed: 17943947]
142. Federle M, Chezmar J, Rubin DL, et al. Efficacy and safety of mangafodipir trisodium (MnDPDP) injection for hepatic MRI in adults: results of the U.S. multicenter phase III clinical trials. Efficacy of early imaging. *J Magn Reson Imaging* 2000;12:689–701. [PubMed: 11050638]
143. Federle MP, Chezmar JL, Rubin DL, et al. Safety and efficacy of mangafodipir trisodium (MnDPDP) injection for hepatic MRI in adults: Results of the US multicenter phase III clinical trials (safety). *J Magn Reson Imaging* 2000;12:186–197. [PubMed: 10931579]
144. Aime S, Anelli PL, Botta M, et al. Relaxometric evaluation of novel manganese(II) complexes for application as contrast agents in magnetic resonance imaging. *J Biol Inorg Chem* 2002;7:58–67. [PubMed: 11862541]
145. Troughton JS, Greenfield MT, Greenwood JM, et al. Synthesis and evaluation of a high relaxivity manganese(II)-based MRI contrast agent. *Inorg Chem* 2004;43:6313–6323. [PubMed: 15446878]
146. Na HB, Lee JH, An K, et al. Development of a T₁ contrast agent for magnetic resonance imaging using MnO nanoparticles. *Angew Chem Int Ed Engl* 2007;46:5397–5401. [PubMed: 17357103]
147. Shin J, Anisur RM, Ko MK, et al. Hollow manganese oxide nanoparticles as multifunctional agents for magnetic resonance imaging and drug delivery. *Angew Chem Int Ed Engl* 2009;48:321–324. [PubMed: 19040234]
148. Pan D, Caruthers SD, Hu G, et al. Ligand-directed nanobialys as theranostic agent for drug delivery and manganese-based magnetic resonance imaging of vascular targets. *J Am Chem Soc* 2008;130:9186–9187. [PubMed: 18572935]
149. Pan D, Senpan A, Caruthers SD, et al. Sensitive and efficient detection of thrombus with fibrin-specific manganese nanocolloids. *Chem Commun (Camb)* 2009;22:3234–3236. [PubMed: 19587924]

150. Ntziachristos V. Fluorescence molecular imaging. *Ann Rev Biomed Eng* 2009;8:1–33. [PubMed: 16834550]
151. Zhang HF, Maslov K, Stoica G, Wang LV. Functional photoacoustic microscopy for high-resolution and noninvasive *in vivo* imaging. *Nat Biotechnol* 2006;24:848–851. [PubMed: 16823374]
152. Zhang HF, Maslov K, Wang LV. *In vivo* imaging of subcutaneous structures using functional photoacoustic microscopy. *Nature Protocols* 2007;2:797–804.
153. Frangioni JV. *In vivo* near-infrared fluorescence imaging. *Curr Opin Chem Biol* 2003;5:626–634. [PubMed: 14580568]
154. Mahmood U, Weissleder R. Near-infrared optical imaging of proteases in cancer. *Mol Cancer Ther* 2003;2(5):489–496. [PubMed: 12748311]
155. Michalet X, Pinaud FF, Bentolila LA, et al. Quantum dots for live cells, *in vivo* imaging, and diagnostics. *Science* 2005;307:538–544. [PubMed: 15681376]
156. Ya-Ping S, Bing Z, Yi L, et al. *Am. Chem Soc* 2006;128 (24):7756–7757.
157. Kannan, R.; Katti, KV. Targeted gold nanoparticles for imaging and therapy biomedical applications of nanotechnology. Wiley; UK: 2007.
158. Hama Y, Urano Y, Koyama Y, Choyke PL, Kobayashi H. Activatable fluorescent molecular imaging of peritoneal metastases following pretargeting with a biotinylated monoclonal antibody. *Cancer Res* 2007;67(8):3809–3817. [PubMed: 17440095]
159. Griffin JMM, Skwierawska AM, Manning HC, Marx JN, Bornhop DJ. Simple, high yielding synthesis of trifunctional fluorescent lanthanide chelates. *Tetr Lett* 2001;42(23):3823–3825.
160. Licha K, Hessenius C, Becker A, et al. Synthesis, characterization, and biological properties of cyanine-labeled somatostatin analogues as receptor-targeted fluorescent probes. *Bioconjug Chem* 2001;12(1):44–50. [PubMed: 11170364]
161. Chin WW, Thong PS, Bhuvaneswari R, Soo KC, Heng PW, Olivo M. *In vivo* optical detection of cancer using chlorin e6 – polyvinylpyrrolidone induced fluorescence imaging and spectroscopy. *BMC Med Imaging* 2009;9:1. [PubMed: 19133127]
162. Ballou B, Lagerholm BC, Ernst LA, Bruchez MP, Waggoner AS. Noninvasive imaging of quantum dots in mice. *Bioconjug Chem* 2004;15:79–86. [PubMed: 14733586]
163. Kim S, et al. Near-infrared fluorescent type II quantum dots for sentinel lymph node mapping. *Nat Biotechnol* 2004;22:93–95. [PubMed: 14661026]
164. Akerman ME, Chan WCW, Laakkonen P, Bhatia SN, Ruoslahti E. Nanocrystal targeting *in vivo*. *Proc Natl Acad Sci USA* 2002;99:12617–12621. [PubMed: 12235356]
165. Ballou B, Lagerholm BC, Ernst LA, Bruchez MP, Waggoner AS. Noninvasive imaging of quantum dots in mice. *Bioconjug Chem* 2004;15:79–86. [PubMed: 14733586]
166. Cai W, Shin DW, Chen K, et al. Persistent tissue kinetics and redistribution of nanoparticles, quantum dot 705, in mice: ICP-MS quantitative assessment. *Nano Lett* 2006;6:669–676. [PubMed: 16608262]
167. Dahan M, et al. Diffusion dynamics of glycine receptors revealed by single-quantum dot tracking. *Science* 2003;302:442–445. [PubMed: 14564008]
168. Lidke DS. Quantum dot ligands provide new insights into erbB/HER receptor-mediated signal transduction. *Nat Biotechnol* 2004;22:198–203. [PubMed: 14704683]
169. Ness JM, Akhtar RS, Latham CB, Roth KA. Combined tyramide signal amplification and quantum dots for sensitive and photostable immunofluorescence detection. *J Histochem Cytochem* 2003;51:981–987. [PubMed: 12871979]
170. Xiaohu G, Yuanyuan C, Richard ML, Leland W, Shuming N. *In vivo* cancer targeting and imaging with semiconductor quantum dots. *Nat Biotechnol* 2004;22:969–976. [PubMed: 15258594]
171. Yu X, Chen L, Li K, et al. Immunofluorescence detection with quantum dot bioconjugates for hepatoma *in vivo*. *J Biomed Opt* 2007;12:014008. [PubMed: 17343483]
172. Sungjee K, Yong TL, Edward GS, et al. Near-infrared fluorescent type II quantum dots for sentinel lymph node mapping. *Nat Biotechnol* 2004;22(1):93–97. [PubMed: 14661026]
173. Lovric J. Unmodified cadmium telluride quantum dots induce reactive oxygen. *Chem Biol* 2005;12:1227–1234. [PubMed: 16298302]

174. Choi HS, Ipe BI, Misra P, et al. Tissue- and organ-selective biodistribution of NIR fluorescent quantum dots. *Nano Lett* 2009;9(6):2354–2359. [PubMed: 19422261]
175. Allen PM, Bawendi MG. Ternary I-III-VI quantum dots luminescent in the red to near-infrared. *J Am Chem Soc* 2008;130(29):9240–9241. [PubMed: 18582061]
176. Sheng-Tao Y, Li C, Pengju G, et al. Carbon dots for multiphoton bioimaging. *Am Chem Soc* 2009;131(32):11308–11309.
177. Ray SC, Saha A, Jana NR, Sarkar R. Fluorescent carbon nanoparticles: synthesis, characterization, and bioimaging application. *J Phys Chem C* 2009;113:18546–18551.
178. Achilefu S, Jimenez HN, Dorshow RB, et al. Synthesis, *in vitro* receptor binding, and *in vivo* evaluation of fluorescein and carbocyanine peptide-based optical contrast agents. *J Med Chem* 2002;45:2003–2015. [PubMed: 11985468]
179. Folli S, Wagnieres G, Pelegrin A, et al. Immunophotodiagnosis of colon carcinomas in patients injected with fluoresceinated chimeric antibodies against carcinoembryonic antigen. *Proc Natl Acad Sci USA* 1992;89:7973–7977. [PubMed: 1518823]
180. Williams DJ. Organic polymeric and non polymeric materials with large optical non linearities. *Angew Chem Int Ed Engl* 1984;23:690–703.
181. Gomez-Hens A, Aguilar-Caballos MP. Long-wavelength fluorophores new trends in their analytical use. *Trends Anal Chem* 2004;23:127.
182. Patonay G, Salon J, Sowell J, Strekowski L. noncovalent labeling of biomolecules with red and near-infrared dyes. *Molecules* 2004;9:40–49. [PubMed: 18007410]
183. Tung C-H. Fluorescent peptide probes for *in vivo* diagnostic imaging. *Biopolymers* 2004;76:391–403. [PubMed: 15389488]
184. Weissleder R, Ntziachristos V. Shedding light onto live molecular targets. *Nat Med* 2003;9:123–128. [PubMed: 12514725]
185. Mason SJ, Balasubramanian S. Solid-phase catch, activate, and release synthesis of cyanine dyes. *Org Lett* 2002;4:4261–4264. [PubMed: 12443073]
186. Mason SJ, Hake JL, Nairne J, Cummins WJ, Balasubramanian SJ. Solid-phase methods for the synthesis of cyanine dyes. *J Org Chem* 2005;70:2939–2949. [PubMed: 15822952]
187. Peng X, Song F, Lu E, et al. Heptamethine cyanine dyes with a large stokes shift and strong fluorescence: a paradigm for excited-state intramolecular charge transfer. *J Am Chem Soc* 2005;127:4170. [PubMed: 15783189]
188. Song F, Peng X, Lu E, Wang Y, Zhou W, Fan J. Tuning the photoinduced electron transfer in near-infrared heptamethine cyanine dyes. *Tetrahedron Lett* 2005;46:4817.
189. Gruber HJ, Hahn CD, Kada G, et al. Anomalous fluorescence enhancement of cy3 and cy3.5 versus anomalous fluorescence loss of cy5 and cy7 upon covalent linking to proteins and noncovalent binding to avidin. *Bioconj Chem* 2000;11:696–704.
190. Kelly KA, Allport JR, Tsourkas A, et al. Detection of vascular adhesion molecule-1 expression using a novel multimodal nanoparticle. *Circ Res* 2005;96:327–336. [PubMed: 15653572]
191. Tung CH, Quinti L, Jaffer FA, et al. A branched fluorescent peptide probe for imaging of activated platelets. *Mol Pharm* 2005;2(1):92–95. [PubMed: 15804182]
192. Hilderbrand SA, Kelly KA, Weissleder R, et al. Monofunctional near-infrared fluorochromes for imaging applications. *Bioconj Chem* 2005;16:1275–1281.
193. Kai L, Bjorn R, Vasilis N, et al. Hydrophilic cyanine dyes as contrast agents for near-infrared tumor imaging: synthesis, photophysical properties and spectroscopic *in vivo* characterization. *Photochem Photobiol* 2000;72(3):392–398. [PubMed: 10989611]
194. Bouteiller C, Clave G, Bernardin A, et al. Near-infrared fluorescent pH-sensitive probes via unexpected barbituric acid mediated synthesis. *Bioconj Chem* 2007;18:1303–1317.
195. Hilderbrand SA, Weissleder R. Near-infrared fluorescence: application to *in vivo* molecular imaging. *Curr Opin Chem Biol* 2009;14(1):71–79. [PubMed: 19879798]
196. Mahmood U, Weissleder R. Near-infrared optical imaging of proteases in cancer molecular cancer therapeutics. *Mol Cancer Ther* 2003;2:489–496. [PubMed: 12748311]
197. Wang LV. Prospects of photoacoustic tomography. *Med Phys* 2008;35 (12):5758–5767. [PubMed: 19175133]

198. Maslov K, Zhang HF, Hu S, Wang LV. Optical-resolution photoacoustic microscopy for *in vivo* imaging of single capillaries. *Opt Lett* 2008;33:929–931. [PubMed: 18451942]
199. Fang H, Maslov K, Wang LV. Photoacoustic Doppler effect from flowing small light-absorbing particles. *Phys Rev Lett* 2007;99:184501. [PubMed: 17995411]
200. Bruchez M Jr, Moronne M, Gin P, et al. Semiconductor nanocrystals as fluorescent biological labels. *Science* 1998;281:2013–2016. [PubMed: 9748157]
201. Chan WC, Maxwell DJ, Gao X, et al. Luminescent quantum dots for multiplexed biological detection and imaging. *Curr Opin Biotechnol* 2002;13:40–46. [PubMed: 11849956]
202. Sharma P, Brown S, Walter G, et al. Nanoparticles for bioimaging. *Adv Colloid Interface Sci* 2006;123–126:471–485.
203. Turkevich J, Stevenson PC, Hillier J. A study of the nucleation and growth process in the synthesis of colloidal gold. *Discuss Faraday Soc* 1951;11:55–75.
204. Kreibig U, Genzel L. Optical absorption of small metallic particles. *Surf Sci* 1985;156:678–700.
205. Khlebtsov NG, Trachuk LA, Mel'nikov AG. The effect of the size, shape, and structure of metal nanoparticles on the dependence of their optical properties on the refractive index of a disperse medium. *Opt Spectrosc* 2005;98:83–90.
206. Sarkar D, Halas NJ. General vector basis function solution of Maxwell's equations. *Phys Rev E* 1997;56:1102.
207. Jin RC, Cao YW, Mirkin CA, et al. Photoinduced conversion of silver nanospheres to nanoprisms. *Science* 2001;294:1901–1903. [PubMed: 11729310]
208. Murphy CJ, Jana NR. Controlling the aspect ratio of inorganic nanorods and nanowires. *Adv Mater* 2002;14:80–82.
209. Marinakos SM, Novak JP, Brousseau LC, et al. Gold particles as templates for the synthesis of hollow polymer capsules. Control of capsule dimensions and guest encapsulation. *J Am Chem Soc* 1999;121:8518–8522.
210. Caruso RA, Antonietti M. Sol-gel nanocoating: an approach to the preparation of structured materials. *Chem Mater* 2001;13:3272–3282.
211. Agarwal A, Huang SW, O'Donnell M, et al. Targeted gold nanorod contrast agent for prostate cancer detection by photoacoustic imaging. *J Appl Phys* 2007;102:064701.
212. Pan D, Pramanik M, Senpan A, et al. Molecular photoacoustic tomography with colloidal nanobeacons. *Angew Chem Int Ed Engl* 2009;48(23):4170–4173. [PubMed: 19418503]
213. Kim JW, Galanzha EI, Shashkov EV, Moon HM, Zharov VP. Golden carbon nanotubes as multimodal photoacoustic and photothermal high-contrast molecular agents. *Nat Nanotechnol* 2009;10:688–694. [PubMed: 19809462]

Figure 1. Molecular imaging of C-32 human melanoma tumor (2–3 mm) in athymic mouse (A) before, (B) 30min and (C) 120 min after injection of $\alpha_v\beta_3$ -targeted gadolinium perfluorocarbon nanoparticles using 1.5 T Philips NT Gyroscan

Note the bright contrast to localize and characterize nascent melanoma only 30 min after contrast injection.

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Figure 2.

(A) Contact-facilitated drug delivery illustrated with rhodamine PFOB nanoparticle bound to C32 melanoma cell (transfected with Rab5 and Rab 7 GFP endocytic markers). (B) Perfluorocarbon nanoparticle forming a hemifusional complex with surface of endothelial cell. Lipids transfer passively to the target cell outer leaflet and require ATP to flip to the inner membrane leaflets. Reproduced with permission from [1–3].

Figure 3. Tumor response in the Vx2 syngeneic rabbit adenocarcinoma model to fumagillin therapy delivered by $\alpha_v\beta_3$ -targeted perfluorocarbon nanoparticles at minute doses versus dose used in clinical trials with water soluble analogue TNP-470

Note that the tumor is smaller ($p < 0.05$) and the 3D magnetic resonance angiogenic map much more sparse versus tumor surface area than the control animal ($p < 0.05$). Reproduced with permission from [4].

Figure 4. General classification of optical contrast agents

BODIPY: Boron-dipyrromethene; NP: Nanoparticle; PEG: Polyethylene glycol; ZnS: Zinc sulphide.

Figure 5.
Classification of cyanine dyes.

Figure 6. Cross-sectional PA images of an low density polyethylene tube filled with plasma clot Bars between images refer to both images. (A) Control and (B) Targeted with GNBs using a curved-array PA system (≈ 800 nm). (C) PA spectrum of GNBs and rat blood over a 740–820-nm near infrared wavelength range.