SPECIAL FEATURE: PERSPECTIVE

Advancing biomedical imaging

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Imaging reveals complex structures and dynamic interactive processes, located deep inside the body, that are otherwise difficult to decipher. Numerous imaging modalities harness every last inch of the energy spectrum. Clinical modalities include magnetic resonance imaging (MRI), X-ray computed tomography (CT), ultrasound, and light-based methods [endoscopy and optical coherence tomography (OCT)]. Research modalities include various light microscopy techniques (confocal, multiphoton, total internal reflection, superresolution fluorescence microscopy), electron microscopy, mass spectrometry imaging, fluorescence tomography, bioluminescence, variations of OCT, and optoacoustic imaging, among a few others. Although clinical imaging and research microscopy are often isolated from one another, we argue that their combination and integration is not only informative but also essential to discovering new biology and interpreting clinical datasets in which signals invariably originate from hundreds to thousands of cells per voxel.

imaging | intravital microscopy | inflammation

Engineering sciences have played a major role in advancing biomedical imaging by improving and miniaturizing detectors, enhancing system design, increasing speed, sensitivity and resolution, accelerating computational analysis, and developing methods to minimize the side effects of applied energy. Additionally, chemical engineering has produced advanced imaging probes (nanomaterials, labeled small and large molecules, and fluorescent proteins) to improve tissue, cell, and molecular specificity. Currently, imaging is evolving rapidly in three distinct biomedical areas: (*i*) imaging molecular biomarkers or contributing to biomarker analysis, (*ii*) single cell imaging, and (*iii*) imaging therapeutics. Each area has highly significant potential for accelerating progress, as we will discuss below after an overview of available tools.

Engineering Advances Have Yielded Impressive Tools

Clinical Imaging Systems. Modern imaging systems have made great progress since the first devices were developed more than 100 y ago. X-rays, for example, introduced by Wilhelm Röntgen's images of his wife's hand, are now used in sophisticated three-dimensional computed tomography (CT) scans that can detect millimeter-sized pulmonary nodules in high-risk populations (1), among many other applications. The explosion of imaging technologies has also produced complementary information because the energy–matter interaction generates different contrast mechanisms (e.g., magnetic relaxivity, susceptibility, diffusion, temperature, elasticity, electrical impedance, radiation absorption, scattering, and fluorescence) (2). Imaging systems can be grouped according to energy type (e.g., X-rays, positrons, photons, or sound waves), spatial resolution (e.g., macroscopic or microscopic), or obtained information type (anatomical, physiological, cellular, or molecular) (Fig. 1) (2). Macroscopic imaging systems that provide anatomical and physiological information are now in widespread clinical and preclinical use. By contrast, systems that provide microscopic resolution are widely used in basic science (Fig. 1). Imaging modality selection is largely determined by the scientific or medical question at hand. Although current imaging technologies' technical capabilities are often amazing, their future potential is equally exciting. Examples include microscopy performed in live subjects (3, 4) and imaging at extreme resolutions in live cells (5). At these resolutions, real-time observations will provide spectacular insight into the mammalian cells' inner workings.

Research Imaging. Microscopes that allow imaging in live animals have been indispensable in discovering cancer biology (6–9), immunology (10–14), and brain function (15–17). Research microscopy systems are often based on confocal or multiphoton scopes with long working distance objectives, special lasers, and unique motion compensation techniques. In addition to advances in optics and detector technology, imaging's research contributions have been enabled by fluorescent proteins and exponentially expanded computational power. The discovery of fluorescent proteins, for which the Nobel prize was awarded in 2008 (Shimomura, Chalfie, and Tsien), allowed researchers to visualize a broad range of specific proteins or cells for the first time. One stunning example is the combinatorial color-labeling method based on the stochastic expression of several fluorescent proteins (Brainbow) (16). Lichtman and coworkers (16) were able to mark individual neurons with over 100 distinct colors and subsequently trace and reconstruct entire connectome brain maps. Similarly, fluorescent proteins facilitated the development of ultraresolution microscopy techniques for which the Nobel prize was awarded in 2014 (Betzig, Hell, and Moerner). Reporter genes have also been described for other imaging technologies such as MRI (18, 19), nuclear imaging (20, 21), and ultrasound (22). Although computational advances have greatly contributed to new imaging techniques, much work remains to be done, particularly with regard to automated image analysis (23), data mining, integrating complex datasets into multiscale models, and developing new visualization tools.

Chemical Tools for Biomolecular Imaging. Chemical tools are increasingly important in both clinical and research imaging because they can add molecular and cellular specificity and/or enhance physiological data extraction. Additionally, chemical imaging agents have two major advantages over fluorescent proteins although the two are often used complementarily: chemical tools enable imaging in humans and obviate the need for genetically engineered mouse models. A considerable number of imaging agents have been developed over the last decade

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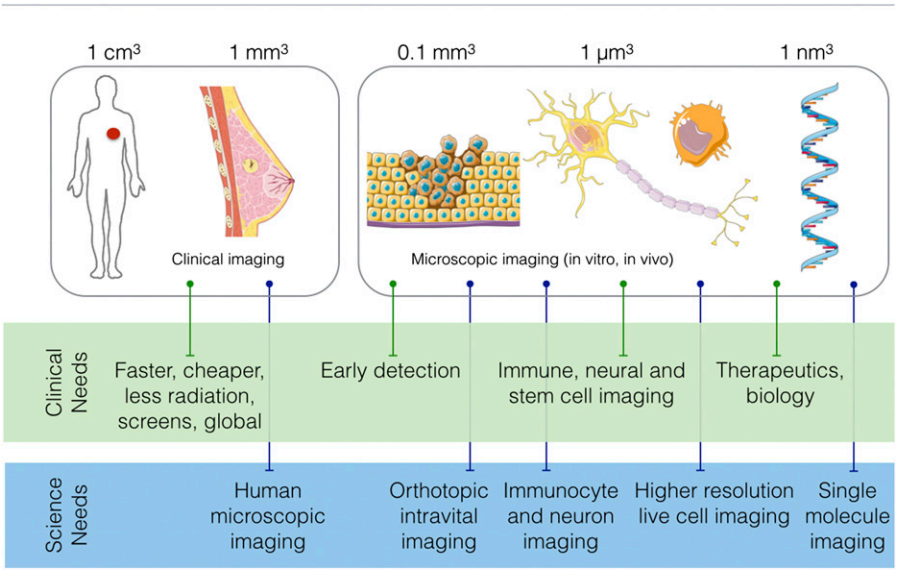

Fig. 1. Overview of clinical and basic science imaging needs.

[Molecular Imaging and Contrast Agent Database (MICAD); www.ncbi.nlm.nih.gov/books/NBK5330/], and some agents are commercially available or even FDA-approved (24). Nanoparticles are particularly promising because they tend to accumulate in innate immunocytes, which are often "first responders" in pathologic processes (25). Further, nanoparticles have unique pharmacokinetics: i.e., they circulate longer and are not immediately cleared renally and can be targeted to specific organs, cells, or proteins. Magnetic nanoparticles, which are detected by magnetic resonance imaging (MRI), are perhaps the best-studied nanoparticle type. Ferumoxytol, for example, is an FDA-approved nanomaterial for iron replacement in treating anemia but has been used to enhance MRI (26); when tagged with fluorochromes, ferumoxytol also doubles as an MR and/or optical imaging agent (12). Quantum dots have been essential in certain microscopic imaging experiments (27, 28), especially in conjunction with environmentally sensitive particles (29), targeted particles, and short wave infrared particles that can be detected much deeper in tissue (30). Labeled antibodies and antibody fragments have long been used for targeted imaging, and the introduction of long-lived imaging isotopes (^{89}Zr, ^{68}Ga, ^{64}Cu,^{124}I) has resulted in some spectacular clinical results (31–33). Newer alpaca-derived antibody fragments currently being developed offer several advantages over traditional antibodies (34, 35). Specifically, single chain camelid antibody fragments lack an Fc portion and are much smaller (~15 kDa) than immunoglobulins (~150 kDa), "diabody" antibody derivatives (~60 kDa), Fab fragments (~50 kDa), or single-chain variable fragments (ScFvs) (~25 kDa). Other important chemical imaging tools now in routine use include a large number of isotope, fluorochrome, or metal-labeled small molecules (24). Finally, there are a number of hyperpolarized C13 metabolites being developed for metabolic MR imaging (36).

Imaging Molecular and Cellular Biomarkers

In vivo imaging of molecular and cellular biomarkers is most useful for studying organs not readily biopsied (such as at the brain), finding early cancers, and mapping disease severity and location. Molecular biomarker development has largely been guided by "omics" techniques and immunopathological studies. Emerging multiplexed imaging (37) and cytometry (38–41) approaches will likely play an important role in defining new imaging targets. Finally, clinical imaging can enhance biomarker information by providing complementary information (42, 43).

Imaging Receptors. Applying imaging technologies to receptors has expanded our knowledge of human biology and improved treatments for numerous conditions. For example, receptor imaging has been used to study the dopamine reward pathway in people with attention deficit hyperactivity disorder (ADHD). One study found that adults with ADHD had fewer D2 and D3 receptors (measured via ^{11}C-raclopride and ^{11}C-cocaine) in their reward circuits and that receptor levels were proportional to inattention symptoms (44). Additionally, ADHD patients' reward circuits were less sensitive. Receptor imaging is also influencing cancer diagnostics. Tumor receptors play an important role in carcinogenesis and tumor growth: relevant receptors include steroid receptors (estrogen receptor in breast cancer and androgen receptor in prostate cancer), somatostatin receptors (SSTR2), and growth factor receptors (EGFR, HER2) among others (e.g., transferrin, folate, and asialoglycoprotein receptors). Tumor receptor imaging has been used to spot cancers (45, 46), understand cancer biology (47), and quantitate the effects of receptor inhibition on tumor growth (48).

Finding Smaller Cancers. Cancer remains the second most common cause of death in the United States. In 2014, there were 1,665,540 new cancer cases diagnosed and 585,720 cancer deaths in the United States. However, the vast majority of cancers are curable when detected early (>90% in stage 1). Most clinical imaging technologies can easily visualize cancers when they approach 1 cm^3, which is equivalent to ~3 billion cells. Through recent advances in image resolution and chemical agents, the detection size boundary is being pushed toward 1mm^3, which corresponds to ~3 million cancer cells. Hopefully even smaller sized cancer lesions will likely be detectable in the future. To achieve this goal, we will need tools to determine which properties of precancerous lesions predict the likelihood of progression to malignant metastatic disease. There are extraordinary opportunities in pushing these boundaries: (*i*) exploring new imaging technologies, sensors, and agents through engineering advances, (*ii*) combining blood biomarkers with imaging, and (*iii*) developing microscopic imaging tools that can be used intraoperatively or during minimally invasive procedures (i.e., microendoscopy) (49). Combining imaging and blood biomarker analysis may be particularly helpful in increasing the accuracy of screening procedures. For example, in addition to identifying easily confirmed lung cancers, low-dose CT scans invariably discover many harmless lesions that require further work-up at high costs. Blood tests for circulating tumor DNA, microvesicles, circulating cancer cells, and/or other makers may increase the accuracy of CT screening (42, 50, 51). Intraoperative imaging with fluorescent affinity ligands or antibodies (52, 53) is now a clinical reality, and reports from the first clinical trials are very promising (49, 54). These approaches will ultimately change cancer surgery standards by giving surgeons real-time feedback about tumor margins and whether any cancer remains. Imaging-facilitated

surgery will likely be more curative and also decrease the rate of repeat surgeries.

Imaging Physiology. Imaging physiology has long been the mainstay of clinical diagnostics. Using a sensitive contrast agent that informs on vascular parameters (density, permeability, etc.) often reveals disease processes. Because many physiological processes simply do not happen ex vivo, the only way to learn about them is to watch them in vivo. A stunning example is certain leukocytes' capacity to crawl along the endothelial surface of small vessels, sometimes even against the flow of blood (55). This patrolling behavior was discovered only because newly developed imaging tools had the sensitivity and resolution to follow cell group interactions distinguished by specific reporter genes. Noninvasive imaging, even clinical imaging, will likely adopt the advantages of spectrally resolving several targets. Key aspects of complex physiology and disease process, including those that seem to conflict one another, occur simultaneously. Integrating comprehensive imaging data can provide unprecedented insight into pathology. For instance, near infrared fluorescence imaging of macrophage presence, angiogenesis, and protease activity in ischemic mouse hearts linked these healing biomarkers to cellular function in the setting of heart failure (56). Translatable PET/MR imaging may enable multispectral imaging, as recently shown in mice with heart failure (57) and Alzheimer's disease (58).

Single Cell Imaging

Intravital microscopy can reveal cells' 3D morphology and interactions with neighboring cells in their native microenvironment. Some emerging discoveries have direct implications for understanding clinical findings.

Immune Cell Imaging. For the most part, immunology is still studied via flow cytometry and genomics (59). Nevertheless, single cell immunocyte imaging has tremendous potential for deciphering cells' in vivo spatial distribution, dynamics, lineage, and behavior in disease. High resolution imaging has recently lead to surprising discoveries. For example, new mouse models with bright fluorescence reporter genes (e.g., $Cx3cr1^{GFP}$ and others) show that macrophages are much more widely distributed than previously thought, that macrophages have projecting dendrites that facilitate sensing (60), and that these cells display remarkable dynamics and effector functions. The heart, for instance, contains a dense network of macrophages whose delicate far-reaching dendrites can be appreciated only by using sensitive 3D microscopy. Similar networks exist in many other healthy and diseased organs, and imaging facilitates exploration of these networks' functions in normal and diseased tissues especially in cancer, myocardial infarction, type 1 diabetes, and autoimmune diseases. Taking cancer as an example, the following are some outstanding questions: (*i*) What is the respective role of tissue-resident (i.e., yolk sac-derived) tissue macrophages versus those recruited from hematopoietic sources during tumor initiation and metastases? (*ii*) Can tumor-associated macrophages be used therapeutically to enhance tumor killing? (*iii*) Why do some patients respond much better than others to emerging immune checkpoint blockades? and (*iv*) Why do some patients experience extraordinary toxicities with immunotherapies whereas others do not ?

Beyond imaging at single cell resolution, reporting on the immune cell populations is becoming feasible in patients. Recent MRI and PET studies in patients with atherosclerosis and acute myocardial infarction relied on macrophage-avid iron oxide nanoparticles or the glucose analog ^{18}F-FDG to study inflammatory responses (61–63). These imaging trials described a systemic activation of the immune system, with accumulation of leukocytes in the ischemic tissue and in remote atherosclerotic plaques. In addition, increased activity was observed in the spleen and the bone marrow. Taken together, the imaging data imply that acute organ ischemia triggers increased bone marrow and splenic production of myeloid cells, which migrate to the ischemic organ but also to remote atherosclerotic plaques, thus promoting disease progression. Translating these insights from mouse to man would likely be impossible without imaging, which, unlike biopsies, can sample the entire human body noninvasively. Newer approaches of cell labeling, reporter gene strategies (64), immune cell imaging (35), and checkpoint blockade imaging [e.g., programmed death-1 (PD1) and cytotoxic T-lymphocyte antigen (CTLA-4)] are also being explored clinically.

Stem Cell Imaging. Important questions that microscopic imaging can help to answer are those related to survival, proliferation, and differentiation of stem and progenitor cells. In vivo microscopy can follow individual fluorescently tagged hematopoietic stem cells over several days and report on their propensity to divide or migrate as a function of their localization in the hematopoietic niche and as a function of disease: for instance, in mice with increased sympathetic tone after ischemic stroke (65). Intravital imaging has become a workhorse for deciphering the role of bone marrow stem cells (66–69). At the whole body level, bioluminescence and PET imaging of reporter gene expression in the tracked cells are leading the field because they can be quite sensitive and tracking labels do not dilute with cell division. In addition, the imaging signal ceases when the cells die. These techniques have been used for tracking cells transplanted into failing mouse hearts, where imaging provided the sobering but important feedback of limited stem cell survival. Clinically, cells have been tagged with magnetic materials (70) and isotopes to monitor their in vivo distribution (64). Excellent reviews exist on these topics (71–73).

Brain Mapping. Arguably, the brain is the organ in which structure–function relationships are the least understood. Neuroscience has driven, and continues to profit from, fast-paced imaging development. In vivo functional MRI (fMRI) and diffusion tensor imaging provide insight on specific brain area functions and interconnections. However, understanding how the brain works necessitates cellular and subcellular resolution and thus relies on microscopic techniques. A particular challenge is the need for techniques that combine high spatial resolution, high cellular and/or molecular specificity, and large-volume imaging capacity. For instance, cellular connectomes track axons with micrometer resolution over long distances through large volumes of the brain and spinal cord. Meeting this challenge now seems within reach: for instance, by combining CLARITY (74) and Brainbow (16). CLARITY ex vivo processing replaces optically dense lipids in cellular membranes with a 3D hydrogel that is cross-linked to proteins and preserves the tissue structure. The procedure increases light penetration depth by at least an order of magnitude and enables imaging of large portions of the mouse brain at cellular resolution. The Brainbow method provides, not unlike color TV, cell-specific coding of ~100 hues through a combination of three to four fluorescent proteins per neuron. When combined, CLARITY and Brainbow may be able to simultaneously visualize a multitude of neuronal circuits in their entirety. Together with functional imaging of firing neurons with calcium and voltage reporters, these approaches demonstrated that brain imaging is at the forefront of imaging technology development and contributes to deciphering how the central nervous system works.

Imaging in Drug Therapy

Imaging has the potential to play a leading role in the routine use of therapeutics, particularly in oncology where drug resistance develops over time and targeted therapies can be extremely expensive. Similarly, therapeutic intervention in Alzheimer's disease may benefit from clinical imaging. The US Food and Drug Administration (FDA) recently approved three PET imaging agents (florbetaben, florbetabir, and flutemetamol) that target amyloid. Unfortunately, because the Centers for Medicare and Medicaid Services (CMS) often does not reimburse use of these and other PET ligands, their use is ironically limited in favor of more costly alternatives. Consequently, most PET imaging is currently performed during clinical trials. Here, imaging is used to enroll patients into specific trials: test drug distribution in phase 1 trials via PET imaging (often ^{11}C-labeled rather than the ^{18}F companion imaging drugs); guide biopsy of specific tissues for pathological analysis, and deliver drugs by image guidance or as a readout of efficacy in therapeutic trials (e.g., tumor shrinkage or change in metabolic activity in target tissue). Imaging can also be used as a companion diagnostic to provide information essential to safe and effective use of a corresponding therapeutic product. In reality, however, the FDA-approved list of companion diagnostics relies much more heavily on in vitro diagnostic devices to interrogate tissue samples (www.fda.gov/MedicalDevices/ProductsandMedicalProcedures/InVitroDiagnostics/ucm301431.htm).

In the research setting, intravital microscopy has been used to study new drugs' pharmacokinetics and pharmacodynamics (3). A growing list of fluorescent companion imaging drugs enables these advances (9, 75–82) and immobilization techniques allow orthotopic imaging (83–85) and methods to study drug/target binding (86). These advances allow detailed insight into when and why drugs fail. Until now, most research on the therapeutics' mechanisms of action and failures has been performed in cell culture, rather than at the cellular level in vivo. Reliance on cell culture leaves unanswered a number of questions regarding delivery to target cells and whether or not the assumed mechanism of drug action occurs in vivo. For example, what are the drug concentrations inside cellular compartments (nucleus vs. cytoplasm)? Is the drug mechanism the same for every cell within the tumor, or is there heterogeneity? Do response mechanisms differ within tumor classes (i.e., different models of ovarian cancer)? How, when, and where does resistance develop? These questions are exemplified by a recent study of eribulin (9), which was developed and FDA-approved as a potent microtubule-targeting cytotoxic agent to treat taxane-resistant cancers. However, recent clinical trials showed that this drug eventually fails in many patient subpopulations for unclear reasons. To investigate eribulin's resistance mechanisms, researchers developed a fluorescent analog, with sufficiently similar pharmacokinetic (PK) properties and cytotoxic activity across a human cell line panel, to study the parent drug's cellular PK and tissue distribution. Results showed that resistance to eribulin and its fluorescent analog depended directly on the multidrug resistance protein 1 (MDR1). In vivo, MDR1-mediated drug efflux and 3D tumor vascular architecture critically determined drug accumulation in tumor cells. Also, standard i.v.-administered third-generation MDR1 inhibitor failed to rescue drug accumulation; however, encapsulating the same MDR1 inhibitor within a nanoparticle delivery system reversed the multidrug-resistant phenotype and potentiated the eribulin effect in vitro and in vivo in mice. This study is just one example of how in vivo imaging of an anticancer drug's cellular PK is a powerful strategy for elucidating drug resistance mechanisms in heterogeneous tumors and evaluating strategies to overcome this resistance. This type of essential information is hard to obtain without imaging.

The Future

If the last decade's rapid advances in imaging and engineering are a good harbinger, then the future looks bright indeed. There are extraordinary opportunities in further advancing imaging capabilities to support basic science and translational and clinical missions (Fig. 1). We argue that these new tools will ultimately allow new types of measurements. The most useful techniques will quantitatively and comprehensively access the cellular and/or subcellular/molecular levels in vivo. In the following, we list some of the current technological challenges (Fig. 1): (*i*) How do we improve clinical detection of earlier forms of cancers to <1 mm^3 (e.g., 0.1 mm^3 consisting of ~10^3 cells)? (*ii*) Can we develop single cell imaging techniques to image beyond the current depth capabilities (i.e., deeper than ~200–500 μm)? (*iii*) Can we develop methods to characterize individual cells' functional states within tissues and tumors? (*iv*) How can we vastly increase data acquisition speeds to accelerate imaging or enable broader coverage (field of view) and how do we increase the spatial resolution by 10- to 100-fold without increasing acquisition times? (*v*) How can we push multiplexing: i.e., simultaneously imaging 10–100 targets? (*vi*) Can we develop advanced chemical tools: e.g., brighter, small footprint fluorochromes that are biocompatible and/or can be used as sensors? (*vii*) Can we develop a complete set of mouse models with genetically encoded fluorescent proteins in all relevant classes of immune cells in addition to lineage tracers for each of these cells? (*viii*) How do we accelerate the development of human microscopic imaging through endoscopes and probes? (*ix*) Can we harness technological advances to develop miniaturized sensors and implantable microscopes for long-term imaging on the scale of days to months?

In summary, imaging has critically contributed to all aspects of basic science, translational studies, and clinical medicine. A world without imaging is clearly not imaginable. We anticipate that future developments will allow us to push the boundaries of what can be measured and detected.

1 Church TR, et al.; National Lung Screening Trial Research Team (2013) Results of initial low-dose computed tomographic screening for lung cancer. *N Engl J Med* 368(21):1980–1991.

2 Weissleder R, Pittet MJ (2008) Imaging in the era of molecular oncology. *Nature* 452(7187):580–589.

3 Pittet MJ, Weissleder R (2011) Intravital imaging. *Cell* 147(5):983–991.

4 Chen BC, et al. (2014) Lattice light-sheet microscopy: Imaging molecules to embryos at high spatiotemporal resolution. *Science* 346(6208):1257998.

5 Gao L, Shao L, Chen BC, Betzig E (2014) 3D live fluorescence imaging of cellular dynamics using Bessel beam plane illumination microscopy. *Nat Protoc* 9(5):1083–1101.

6 Lohela M, et al. (2014) Intravital imaging reveals distinct responses of depleting dynamic tumor-associated macrophage and dendritic cell subpopulations. *Proc Natl Acad Sci USA* 111(47):E5086–E5095.

7 Carmeliet P, Jain RK (2011) Molecular mechanisms and clinical applications of angiogenesis. *Nature* 473(7347):298–307.

8 Pignatelli J, et al. (2014) Invasive breast carcinoma cells from patients exhibit MenaINV- and macrophage-dependent transendothelial migration. *Sci Signal* 7(353):ra112.

9 Laughney AM, et al. (2014) Single-cell pharmacokinetic imaging reveals a therapeutic strategy to overcome drug resistance to the microtubule inhibitor eribulin. *Sci Transl Med* 6:261ra152.

10 Clatworthy MR, et al. (2014) Immune complexes stimulate CCR7-dependent dendritic cell migration to lymph nodes. *Nat Med* 20(12):1458–1463.

11 Lämmermann T, et al. (2013) Neutrophil swarms require LTB4 and integrins at sites of cell death in vivo. *Nature* 498(7454):371–375.

12 Fu W, Wojtkiewicz G, Weissleder R, Benoist C, Mathis D (2012) Early window of diabetes determinism in NOD mice, dependent on the complement receptor CRIg, identified by noninvasive imaging. *Nat Immunol* 13(4):361–368.

13 Abtin A, et al. (2014) Perivascular macrophages mediate neutrophil recruitment during bacterial skin infection. *Nat Immunol* 15(1):45–53.

14 Murooka TT, et al. (2012) HIV-infected T cells are migratory vehicles for viral dissemination. *Nature* 490(7419):283–287.

15 Kuchibhotla KV, Lattarulo CR, Hyman BT, Bacskai BJ (2009) Synchronous hyperactivity and intercellular calcium waves in astrocytes in Alzheimer mice. *Science* 323(5918):1211–1215.

16 Livet J, et al. (2007) Transgenic strategies for combinatorial expression of fluorescent proteins in the nervous system. *Nature* 450(7166):56–62.
17 Hamel EJ, Grewe BF, Parker JG, Schnitzer MJ (2015) Cellular level brain imaging in behaving mammals: An engineering approach. *Neuron* 86(1):140–159.
18 Weissleder R, et al. (2000) In vivo magnetic resonance imaging of transgene expression. *Nat Med* 6(3):351–355.
19 Genove G, DeMarco U, Xu H, Goins WF, Ahrens ET (2005) A new transgene reporter for in vivo magnetic resonance imaging. *Nat Med* 11(4):450–454.
20 Gambhir SS, et al. (2000) A mutant herpes simplex virus type 1 thymidine kinase reporter gene shows improved sensitivity for imaging reporter gene expression with positron emission tomography. *Proc Natl Acad Sci USA* 97(6):2785–2790.
21 Chung JK (2002) Sodium iodide symporter: Its role in nuclear medicine. *J Nucl Med* 43(9):1188–1200.
22 Shapiro MG, et al. (2014) Biogenic gas nanostructures as ultrasonic molecular reporters. *Nat Nanotechnol* 9(4):311–316.
23 Chittajallu DR, et al. (2015) In vivo cell-cycle profiling in xenograft tumors by quantitative intravital microscopy. *Nat Methods* 12(6): 577–585.
24 Chopra A, et al. (2012) Molecular Imaging and Contrast Agent Database (MICAD): Evolution and progress. *Mol Imaging Biol* 14(1): 4–13.
25 Weissleder R, Nahrendorf M, Pittet MJ (2014) Imaging macrophages with nanoparticles. *Nat Mater* 13(2):125–138.
26 Gaglia JL, et al. (2015) Noninvasive mapping of pancreatic inflammation in recent-onset type-1 diabetes patients. *Proc Natl Acad Sci USA* 112(7):2139–2144.
27 Chen O, et al. (2013) Compact high-quality CdSe-CdS core-shell nanocrystals with narrow emission linewidths and suppressed blinking. *Nat Mater* 12(5):445–451.
28 Kim S, et al. (2004) Near-infrared fluorescent type II quantum dots for sentinel lymph node mapping. *Nat Biotechnol* 22(1):93–97.
29 Lemon CM, et al. (2014) Metabolic tumor profiling with pH, oxygen, and glucose chemosensors on a quantum dot scaffold. *Inorg Chem* 53(4):1900–1915.
30 Hong G, et al. (2012) Multifunctional in vivo vascular imaging using near-infrared II fluorescence. *Nat Med* 18(12):1841–1846.
31 Holland JP, et al. (2012) Annotating MYC status with 89Zr-transferrin imaging. *Nat Med* 18(10):1586–1591.
32 Ho AL, et al. (2013) Selumetinib-enhanced radioiodine uptake in advanced thyroid cancer. *N Engl J Med* 368(7):623–632.
33 Pandit-Taskar N, et al. (2014) ^{89}Zr-huJ591 immuno-PET imaging in patients with advanced metastatic prostate cancer. *Eur J Nucl Med Mol Imaging* 41(11):2093–2105.
34 Ashour J, et al. (2015) Intracellular expression of camelid single-domain antibodies specific for influenza virus nucleoprotein uncovers distinct features of its nuclear localization. *J Virol* 89(5):2792–2800.
35 Rashidian M, et al. (2015) Noninvasive imaging of immune responses. *Proc Natl Acad Sci USA* 112(19):6146–6151.
36 Nelson SJ, et al. (2013) Metabolic imaging of patients with prostate cancer using hyperpolarized [1-^{13}C]pyruvate. *Sci Transl Med* 5:198ra108.
37 Angelo M, et al. (2014) Multiplexed ion beam imaging of human breast tumors. *Nat Med* 20(4):436–442.
38 Bendall SC, Nolan GP (2012) From single cells to deep phenotypes in cancer. *Nat Biotechnol* 30(7):639–647.
39 Bodenmiller B, et al. (2012) Multiplexed mass cytometry profiling of cellular states perturbed by small-molecule regulators. *Nat Biotechnol* 30(9):858–867.
40 Fienberg HG, Nolan GP (2014) Mass cytometry to decipher the mechanism of nongenetic drug resistance in cancer. *Curr Top Microbiol Immunol* 377:85–94.
41 Krishnaswamy S, et al. (2014) Systems biology: Conditional density-based analysis of T cell signaling in single-cell data. *Science* 346(6213):1250689.
42 Carlsson A, et al. (2014) Circulating tumor microemboli diagnostics for patients with non-small-cell lung cancer. *J Thorac Oncol* 9(8):1111–1119.
43 Hasan N, Kumar R, Kavuru MS (2014) Lung cancer screening beyond low-dose computed tomography: The role of novel biomarkers. *Lung* 192(5):639–648.
44 Volkow ND, et al. (2011) Motivation deficit in ADHD is associated with dysfunction of the dopamine reward pathway. *Mol Psychiatry* 16(11):1147–1154.
45 Mankoff DA, Link JM, Linden HM, Sundararajan L, Krohn KA (2008) Tumor receptor imaging. *J Nucl Med* 49(Suppl 2):149S–163S.
46 Maurer AH, et al. (2014) Imaging the folate receptor on cancer cells with 99mTc-etarfolatide: Properties, clinical use, and future potential of folate receptor imaging. *J Nucl Med* 55(5):701–704.
47 Wang Y, Fruhwirth G, Cai E, Ng T, Selvin PR (2013) 3D super-resolution imaging with blinking quantum dots. *Nano Lett* 13(11): 5233–5241.
48 van Dijk LK, et al. (2013) Imaging of epidermal growth factor receptor expression in head and neck cancer with SPECT/CT and 111In-labeled cetuximab-F(ab')2. *J Nucl Med* 54(12):2118–2124.
49 Pan Y, et al. (2014) Endoscopic molecular imaging of human bladder cancer using a CD47 antibody. *Sci Transl Med* 6:260ra148.
50 Ilie M, et al. (2014) "Sentinel" circulating tumor cells allow early diagnosis of lung cancer in patients with chronic obstructive pulmonary disease. *PLoS ONE* 9(10):e111597.
51 Ghazani AA, et al. (2014) Molecular characterization of scant lung tumor cells using iron-oxide nanoparticles and micro-nuclear magnetic resonance. *Nanomedicine (Lond Print)* 10(3):661–668.
52 Thurber GM, Figueiredo JL, Weissleder R (2010) Detection limits of intraoperative near infrared imaging for tumor resection. *J Surg Oncol* 102(7):758–764.
53 Kirsch DG, et al. (2007) A spatially and temporally restricted mouse model of soft tissue sarcoma. *Nat Med* 13(8):992–997.
54 van Dam GM, et al. (2011) Intraoperative tumor-specific fluorescence imaging in ovarian cancer by folate receptor-α targeting: First in-human results. *Nat Med* 17(10):1315–1319.
55 Auffray C, et al. (2007) Monitoring of blood vessels and tissues by a population of monocytes with patrolling behavior. *Science* 317(5838):666–670.
56 Leuschner F, et al. (2012) Rapid monocyte kinetics in acute myocardial infarction are sustained by extramedullary monocytopoiesis. *J Exp Med* 209(1):123–137.
57 Majmudar MD, et al. (2013) Monocyte-directed RNAi targeting CCR2 improves infarct healing in atherosclerosis-prone mice. *Circulation* 127(20):2038–2046.
58 Maier FC, et al. (2014) Longitudinal PET-MRI reveals β-amyloid deposition and rCBF dynamics and connects vascular amyloidosis to quantitative loss of perfusion. *Nat Med* 20(12):1485–1492.
59 Benoist C, Lanier L, Merad M, Mathis D; Immunological Genome Project (2012) Consortium biology in immunology: The perspective from the Immunological Genome Project. *Nat Rev Immunol* 12(10): 734–740.
60 Da Silva N, et al. (2011) A dense network of dendritic cells populates the murine epididymis. *Reproduction* 141(5):653–663.
61 Alam SR, et al. (2012) Ultrasmall superparamagnetic particles of iron oxide in patients with acute myocardial infarction: Early clinical experience. *Circ Cardiovasc Imaging* 5(5):559–565.
62 Wollenweber T, et al. (2014) Characterizing the inflammatory tissue response to acute myocardial infarction by clinical multimodality noninvasive imaging. *Circ Cardiovasc Imaging* 7(5): 811–818.
63 Yilmaz A, et al. (2013) Imaging of myocardial infarction using ultrasmall superparamagnetic iron oxide nanoparticles: A human study using a multi-parametric cardiovascular magnetic resonance imaging approach. *Eur Heart J* 34(6):462–475.
64 Kurtz DM, Gambhir SS (2014) Tracking cellular and immune therapies in cancer. *Adv Cancer Res* 124:257–296.
65 Courties G, et al. (2015) Ischemic stroke activates hematopoietic bone marrow stem cells. *Circ Res* 116(3):407–417.
66 Spencer JA, et al. (2014) Direct measurement of local oxygen concentration in the bone marrow of live animals. *Nature* 508(7495): 269–273.
67 Ferraro F, et al. (2011) Diabetes impairs hematopoietic stem cell mobilization by altering niche function. *Sci Transl Med* 3:104ra101.
68 Fujisaki J, et al. (2011) In vivo imaging of Treg cells providing immune privilege to the haematopoietic stem-cell niche. *Nature* 474(7350):216–219.
69 Krause DS, et al. (2013) Differential regulation of myeloid leukemias by the bone marrow microenvironment. *Nat Med* 19(11): 1513–1517.
70 Bulte JW (2013) Science to practice: Can stem cells be labeled inside the body instead of outside? *Radiology* 269(1):1–3.
71 Nguyen PK, Riegler J, Wu JC (2014) Stem cell imaging: From bench to bedside. *Cell Stem Cell* 14(4):431–444.
72 Cromer Berman SM, Walczak P, Bulte JW (2011) Tracking stem cells using magnetic nanoparticles. *Wiley Interdiscip Rev Nanomed Nanobiotechnol* 3(4):343–355.
73 Srivastava AK, Bulte JW (2014) Seeing stem cells at work in vivo. *Stem Cell Rev* 10(1):127–144.
74 Chung K, et al. (2013) Structural and molecular interrogation of intact biological systems. *Nature* 497(7449):332–337.
75 Kim E, Yang KS, Weissleder R (2013) Bioorthogonal small molecule imaging agents allow single-cell imaging of MET. *PLoS ONE* 8(11):e81275.
76 Kim E, Yang KS, Giedt RJ, Weissleder R (2014) Red Si-rhodamine drug conjugates enable imaging in GFP cells. *Chem Commun (Camb)* 50(34):4504–4507.
77 Budin G, Yang KS, Reiner T, Weissleder R (2011) Bioorthogonal probes for polo-like kinase 1 imaging and quantification. *Angew Chem Int Ed Engl* 50(40):9378–9381.
78 Reiner T, et al. (2011) Accurate measurement of pancreatic islet beta-cell mass using a second-generation fluorescent exendin-4 analog. *Proc Natl Acad Sci USA* 108(31):12815–12820.
79 Reiner T, et al. (2012) Imaging therapeutic PARP inhibition in vivo through bioorthogonally developed companion imaging agents. *Neoplasia* 14(3):169–177.
80 Thurber GM, et al. (2013) Single-cell and subcellular pharmacokinetic imaging allows insight into drug action in vivo. *Nat Commun* 4:1504.
81 Yang KS, Budin G, Reiner T, Vinegoni C, Weissleder R (2012) Bioorthogonal imaging of aurora kinase A in live cells. *Angew Chem Int Ed Engl* 51(27):6598–6603.
82 Miller MA, Askevold B, Yang KS, Kohler RH, Weissleder R (2014) Platinum compounds for high-resolution in vivo cancer imaging. *ChemMedChem* 9(6):1131–1135.
83 Lee S, et al. (2012) Real-time in vivo imaging of the beating mouse heart at microscopic resolution. *Nat Commun* 3:1054.
84 Giedt RJ, Koch PD, Weissleder R (2013) Single cell analysis of drug distribution by intravital imaging. *PLoS ONE* 8(4):e60988.
85 Aguirre AD, Vinegoni C, Sebas M, Weissleder R (2014) Intravital imaging of cardiac function at the single-cell level. *Proc Natl Acad Sci USA* 111(31):11257–11262.
86 Dubach JM, et al. (2014) In vivo imaging of specific drug-target binding at subcellular resolution. *Nat Commun* 5:3946.