

Multimodality Molecular Imaging with Combined Optical and SPECT/PET Modalities

Today's medical imaging technologies are expected to furnish anatomic, physiologic, molecular, and genomic information for accurate disease diagnosis, prediction of treatment response, and development of highly specific and sensitive drugs and imaging agents. However, none of the current imaging methods used in humans provides comprehensive medical imaging. To harness the strengths of different imaging methods, multimodality imaging has become an attractive strategy for *in vivo* studies. Beyond small-animal imaging, a less frequently used multimodality imaging strategy is the fusion of radionuclear and optical methods. This less frequent use is probably attributable to some misconceptions, technical difficulties, or a lack of appreciation for the benefits of the 2 methods in patient care. This minireview addresses some of these concerns, with emphasis on the potential applications of multimodality optical and SPECT/PET systems.

Key Words: fluorescence; bioluminescence; diffuse optical tomography; contrast agent; molecular probe

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The roles of imaging in disease diagnosis and treatment monitoring continue to increase because of advances in imaging technologies and concomitant improvements in detection sensitivity, spatial resolution, and quantitative information. In a perfect world, a single imaging method could furnish anatomic, physiologic, and molecular information with high sensitivity and specificity. However, none of the current imaging methods used in humans provides comprehensive medical imaging. Whereas CT and MRI provide high anatomic resolution, the exceptionally high detection sensitivity of optical and radionuclear methods, such as PET and SPECT, enables these techniques to excel at molecular imaging.

To harness the strengths of different imaging methods, multimodality imaging has become attractive for both small-animal and human studies. Naturally, modalities are chosen to furnish synergistic, complementary, or clinically useful information beyond that provided by any individual method. For example, coregistration with CT images provides the anatomic landscape for localizing the functional or molecular data generated by PET (1).

Beyond small-animal studies, the fusion of radionuclear and optical methods is a less frequently used multimodality imaging strategy. This less frequent use is attributable to a variety

of reasons, ranging from some misconceptions and technical difficulties to a lack of appreciation for the benefits of combining the 2 methods. Additionally, the incorporation of optical into conventional imaging methods has been hampered by the small number of optical imaging studies that have been done in humans, the absence of a standard optical imaging system, and the lack of U.S. Food and Drug Administration–approved target-specific optical molecular probes. Some of these concerns may become obsolete because of ongoing efforts toward the clinical translation of optical molecular probes and devices. The recent trend by *The Journal of Nuclear Medicine* to publish studies related to optical imaging will further demonstrate the complementary nature of the 2 modalities and will disseminate the progress made in biomedical optics beyond the optical imaging community. Optical and radionuclear methods have a symbiotic relationship that can be explored in a multimodality imaging framework, which is the focus of this minireview.

OPTICAL IMAGING

Optical imaging and spectroscopy, which use nonionizing radiation in the visible and near-infrared wavelengths (~400–1,500 nm), have preceded all other imaging methods, having been in existence for many centuries. At the macroscopic level, the observation of changes in skin, eye, or tongue coloration remains a primary screening method for a variety of diseases. In this method, the sun or room light serves as the excitation source for endogenous chromophores (light-absorbing molecules) that are upregulated in symptomatic patients and the human eyes serve as the detector. However, such physical manifestations occur at advanced stages of diseases and may be too subtle for detection by human eyes at early stages. For these and smaller lesions, more sensitive optical methods have been developed for imaging biochemical processes at the cellular and molecular levels. Optical imaging has been shown to have sensitivity, specificity, and resolution unparalleled by other biomedical imaging methods. The challenge of modern biomedical optics, however, is to translate these findings from single cells to the complex multicellular milieu in living organisms.

IN VIVO OPTICAL IMAGING METHODS

A variety of contrast mechanisms are available for optical imaging; these include light absorption, scattering, fluorescence, and bioluminescence (for a review, see Ntziachristos et al. (2)). Elegant strategies for designing fluorescent molecular probes have been reviewed (3–5). In bioluminescence imaging, cells transfected to express an enzyme such as luciferase convert an injected substrate into a light-emitting source that is captured by a highly sensitive cooled charge-coupled device camera (6). The absence of an external excitation source leads to

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a signal-to-background ratio higher than that achieved with simple planar fluorescence imaging (2).

Fluorescence imaging is most commonly performed with reflectance planar geometry for simplicity and speed. In fluorescence reflectance imaging (FRI), the light source and detector are on the same side of the sample, separated by appropriate emission filters that block the excitation light from the detector and admit fluorescence light. FRI is surface weighted because the pathlengths of the excitation and emission photons are shortest for fluorophores in superficial tissues.

A computational approach to optical imaging, called diffuse optical tomography (DOT), has emerged as a technique with improved imaging performance relative to that of FRI. In DOT, transmission measurements are made between multiple source–detector pairs and reconstructed into 3-dimensional images by use of algorithms similar to those used in CT. The 3-dimensional DOT images address many limitations of FRI by providing considerably improved deep-tissue sensitivity, volumetric localization, improved resolution, and increased quantitative accuracy (2). For example, DOT approaches have been used to image breast tumors and activity in the brain at tissue depths of several centimeters.

COMBINED DOT/PET INSTRUMENTATION AND DATA FUSION

DOT is most effective when coupled with established imaging modalities. At a basic level, optical images can be simply coregistered with images from an anatomic imaging modality. For example, DOT images of optical contrast agent uptake in human breast tumors have been coregistered with images from anatomic MRI and CT scans (7). However, multimodality data can also be fused more tightly by use of anatomic knowledge from either MRI or radiographic techniques to explicitly aid in light modeling (8–10). Recently, some research groups developed integrated systems for concurrent MRI/bioluminescence tomography (11) and MRI/absorption tomography for small-animal imaging. An unresolved issue with fusing optical images with an alternate modality is determining the optimal mechanism for transferring spatial information between the modalities. One approach is to assign optical properties ac-

ording to anatomic segmentation. Another approach is to assume a correlation between the contrast of the alternate modality and an optical property (e.g., absorption (10) or scattering (12,13)) and to provide a bias to the mean value of each voxel. Both of these approaches are adversely affected when the correlation between the contrast of the optical modality and the contrast of the alternate modality is unknown or unpredictable.

The development of a combined optical/PET system is exciting because of the opportunity to obtain complementary data from both contrast mechanisms within the same device. For the prospect of fusing optical imaging and PET data, there is a high potential for a tight correlation between the contrast data. If both signals are incorporated into a single molecular probe (see later discussion), then the mechanism for integrating and fusing the datasets could be explicitly modeled.

Coregistered and concurrent DOT/PET datasets would be important for data fusion for molecular probes during dynamic distribution and reporting. For small-animal imaging, in which whole-body optical imaging has been demonstrated, a pair of recent simulation studies examined the feasibility of an optical/PET system. In one study, a bioluminescence/optical/PET system was evaluated (14), and another study reported an integrated Monte Carlo approach for modeling both the nuclear and the optical radiation problems (15). These efforts lay the groundwork for future experimental realizations of combined optical and nuclear tomography systems. Although whole-body human scanning is unlikely, the feasibility of DOT for imaging breast cancer, superficial sentinel lymph nodes, the brain surface, arms, and fingers has been shown. Combined DOT/PET could be performed in these regions. Furthermore, minimally invasive procedures carried out by traditional or advanced (e.g., optical coherence tomography) endoscopy techniques permit optical imaging throughout endoscopy-accessible human tissues.

MULTIMODALITY OPTICAL AND SPECT/PET APPLICATIONS

To appreciate the potential benefits of combining optical and SPECT/PET modalities, we have tabulated the similarities and differences for the 2 imaging methods in Table 1. These properties furnish a platform for discussing recent advances

TABLE 1
Overview of Optical Imaging Methods in Relation to PET and MRI

Parameter	Feature	PET	MRI	Optical methods		
				Confocal, multiphoton microscopy	Reflectance and raster scan	DOT
Contrast	Sensitivity to probes	High fM–pM	Low μ M–mM	High fM–pM	High pM–nM	High pM–nM
	Activating probes	No	No	Yes	Yes	Yes
	Intrinsic	No	Yes	Yes		
Imaging	Nonionizing	No	Yes	Yes		
	Resolution	1–5 mm	40 μ m–1 mm	~0.3–10 μ m	0.1–3 mm	0.1–10 mm
	Depth	Not limited	Not limited	0.01–0.5	0.1–2 mm	0.1–50 mm
	Tomography/sectioning	Yes	Yes	Yes	No	Yes

Optical methods have many flexible and powerful modes of molecular and functional contrast. Effect of depth on imaging performance is dramatic.

and potential opportunities for combined optical and SPECT/PET studies.

Cross-Validation of Optical Method for In Vivo Molecular Imaging

The high detection sensitivity is shared by nuclear and optical imaging, prompting the use of both methods for molecular imaging studies and facilitating a direct comparison of the nascent optical imaging performance with the established nuclear imaging performance. Typically, the radionuclides in radiopharmaceuticals are replaced with fluorescent dyes to produce the analogous optical molecular probes. Although initial strategies for tumor-specific imaging used fluorescent dye-labeled antibodies to minimize the effects of the dye on biologic activity and the distribution of the biomolecules, more recent methods have used smaller bioactive peptides and compounds (4,5). In general, studies have shown that replacing a radionuclide with a fluorescent dye may alter the in vivo distribution or rate of uptake in the target tissue without a loss of molecular recognition of the carrier molecule by the receptor, in some cases.

Recently, elegant molecular designs have been developed to take full advantage of the unique high detection sensitivity of both optical and nuclear imaging methods. Instead of preparing 2 different imaging agents (1 for each modality) that are distributed differently in the body, thereby increasing the potential for cumulative toxicity and complicating data fusion, a new trend is to fuse the signaling moieties for the 2 imaging systems into one molecule (monomolecular multimodality imaging agents [MOMIAs]) (16–20). This unique structural feature ensures that both signals emanate from the same source, allowing for the fusion of contrast data with high spatial precision. In addition to single molecules, quantum dots and a cross-linked near-infrared fluorescent polymer core were recently labeled with ^{64}Cu and ^{111}In for dual optical/PET and optical/SPECT studies, respectively (21,22). The polyvalent nature of these nanomaterials is attractive for multimodality imaging because the normalization of differences in the detection sensitivities for different contrast mechanisms can be achieved by incorporating the appropriate number of signaling molecules per particle.

Conversely, optical methods have been used to cross-validate radionuclear images. For example, a trimodal fusion reporter system with the ability to furnish fluorescent, bioluminescent, and PET {with 9-[4- ^{18}F -fluoro-3-(hydroxymethyl)butyl]guanine} contrast data has been developed (23). In metastatic cells transfected with a mutant herpes simplex virus type 1 thymidine kinase enzyme, the phosphorylation of 9-[4- ^{18}F -fluoro-3-(hydroxymethyl)butyl]guanine traps the radiopharmaceutical in the cells, thereby allowing the visualization of primary and metastatic tumors by PET. The highly sensitive and specific bioluminescence imaging and the high resolution provided by fluorescence microscopy of ex vivo tissue are subsequently used to validate the PET data. Variations of transduced multimodality molecular probes for combined optical and SPECT/PET studies are now available and have revealed the unique strengths of each reporter system (23–26). These genetically engineered reporter systems show great promise in preclinical drug development and biomedical research but have limited potential for human applications outside gene therapy.

Quantitation and Whole-Body Biodistribution

Tissue absorption and scattering of the low-energy radiation used in optical imaging limit the penetration depth. The net result is that optical imaging methods can provide micron-level spatial resolution and exquisite detection sensitivity for superficial tissues but rapidly lose these advantages in deep tissues. Conversely, quantitative imaging by SPECT/PET is not limited by tissue depth in either small animals or humans. In a combined system using a simple planar optical imaging approach, the optical contrast data can provide high-throughput imaging of molecular and functional events, and nuclear imaging can provide quantitative data when needed. In combined systems that use DOT, quantitative optical data is possible at depths of up to several centimeters but not at depths sufficient for whole-body human imaging as is possible by SPECT/PET. The MOMIA construct or the dual-reporter gene approach is ideally suited for this complementary imaging strategy.

Longitudinal Studies

A fundamental difference between optical imaging and nuclear imaging is that the emission of radioisotopes is a single event that is governed by the half-life of the radionuclide. This half-life can range from a few seconds to several years. Radionuclides with short half-lives, such as ^{18}F and ^{11}C , are useful for rapid signal acquisition and minimize patient exposure to prolonged radioactivity. Considering that signal regeneration is the hallmark of optical contrast mechanisms, dual imaging with ^{18}F or ^{11}C could provide early pharmacokinetic data by PET followed by longitudinal imaging by DOT. Unlike the high photon fluency often used in fluorescence microscopy, the lower light power used for in vivo optical imaging minimizes photobleaching. In fact, optical signals can be detected in tissues for several weeks after a single administration of the imaging agent. For bioluminescence imaging, a controlled release of enzyme substrates can be used to prolong the light emission during longitudinal studies. Consequently, PET can provide early quantitative data in a target tissue, and subsequent changes can be monitored longitudinally by an optical method.

Intraoperative Procedures

A combination of optical and nuclear methods is routinely used in disease diagnosis and management. For example, PET/CT is used to localize diseased tissue, which is then biopsied for histologic validation by an optical method. Another example is the conventional use of methylene blue or isosulfan blue and $^{99\text{m}}\text{Tc}$ -labeled sulfur colloid to detect sentinel lymph nodes for staging of the axilla in breast cancer patients (27). For the detection of melanoma, $^{123/131}\text{I}$ -radiolabeled methylene blue was delivered to cancerous tissues for subsequent scintigraphy or radiotherapy (28). Although the optical imaging component of the dye was not used in that study, the molecular construct was similar to the MOMIA construct and was clearly amenable to the dual-imaging concept. In general, optical and nuclear MOMIAs can be used in 2 ways. First, the nuclear component can provide a whole-body image and localize diseased tissue, and the fluorescence component can guide tissue biopsy. Second, after nuclear imaging, the optical component can serve as a visual guide during surgery. Along with in vivo

microscopy, high-resolution optical images of tumor boundaries can also be obtained.

Fusion of PET and Optical Data for Improved Optical Image Quality and Unified Molecular Imaging

PET and optical data could be fused to provide a unified report of an imaging probe. Incorporation of PET data into DOT reconstructions could serve as a priori spatial information in the form of tissue segmentations, linear correlations of optical contrast and PET contrast data, or dynamic models of the biochemical relationships between optical contrast and PET contrast data (29,30). Fused images potentially provide the resolution of PET with a joint optical/PET molecular readout. For instance, PET data might indicate the localization of a probe, whereas optical data would report the probe activity. A comprehensive algorithmic would jointly reconstruct both PET and DOT datasets in a manner similar to that used for fused PET/CT reconstructions. If such an approach incorporated a model of optical/PET probe activity, then a single self-consistent readout of all of the molecular information could be obtained.

FUTURE DIRECTIONS

Although reports of multimodality optical and SPECT/PET studies have been predominantly associated with cells and small animals, precedence exists for the use of this strategy in humans. The low cost and flexible contrast mechanisms of optical imaging facilitate its integration into existing PET or SPECT systems, thereby accelerating the use of the combined strengths of both modalities. Adding optical instruments to nuclear scanners would provide physiologic parameters, such as the oxygen extraction factor, oxygen saturation, blood volume, water and lipid contents, and blood flow, without the use of contrast agents. The development of MOMIAs will facilitate human applications of optical molecular probes and provide molecular information through the activation of MOMIAs. Studies on the use of the MOMIA concept in breast cancer imaging and interventional radiology are already in progress at some institutions. Although combined optical and SPECT/DOT is in its infancy, this technique will provide complementary and new information for enhanced patient care.

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