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Radiologic and Near-Infrared/Optical Spectroscopic Imaging: Where Is the Synergy?

Brian W. Pogue^{1,2}, Frederic Leblond¹, Venkataramanan Krishnaswamy¹, and Keith D. Paulsen^{1,3}

¹Thayer School of Engineering, Dartmouth College, 8000 Cummings Hall, Hanover, New Hampshire 03755.

²Department of Surgery, Dartmouth Medical School, Lebanon, New Hampshire.

³Department of Diagnostic Radiology, Dartmouth Medical School, Lebanon, New Hampshire.

Abstract

OBJECTIVE—Optical and radiologic imaging are commonly used in preclinical research, and research into combined instruments for human applications is showing promise. The purpose of this article is to outline the fundamental limitations and advantages and to review the available systems. The emerging developments and future potential will be summarized.

CONCLUSION—Integration of hybrid systems is now routine at the preclinical level and appears in the form of specialized packages in which performance varies considerably. The synergy is commonly focused on using spatial localization from radiographs to provide structural data for spectroscopy; however, applications also exist in which the spectroscopy informs the use of radiologic imaging. Examples of clinical systems under research and development are shown.

Keywords

CT; bioluminescence; fluorescence; optical spectroscopy; radiography; x-ray

The combination of radiologic imaging with near-infrared (NIR) or visible light (VIS) imaging has the potential to merge structure and molecular function into a single approach, and this paradigm has become commercially successful at the preclinical level to facilitate mechanistic and applied in vivo research. Although radiologic imaging is widely used for hard tissues and niche areas of soft-tissue and organ imaging, exploration of temporal and dual-energy methods is growing. In comparison, NIR/VIS spectroscopy of soft tissues for quantifying hemoglobin, oxygen saturation, water, and fat has been applied in many niche applications but has not really been shown conclusively to offer much value in imaging per se. The value of including NIR/VIS spectroscopy and spectroscopic imaging is the molecular-specific information that can be incorporated into the structures imaged by radiography. Although endogenous molecules can be imaged, the growth potential exists in molecular contrast agent development and the realization of methods that allow accurate mechanistic imaging and quantification. These advances are clearly being generated in the preclinical area first, but initial clinical systems and studies have appeared over the past half decade [1]. This article summarizes these developments and explores the potential future synergy.

Preclinical imaging of all types has experienced significant growth, and commercial demand has driven the packaging of a diversity of systems and research into new approaches. Customized systems for radiography, PET, SPECT, ultrasound, and MRI are available and are now commonplace in research laboratories [2]. Rapid development of optical imaging tools has also occurred during the past decade, which has culminated in several systems but also a wide variety of capabilities and prices [3]. Nearly half a dozen NIR/fluorescence systems are commercially available that either include radiologic imaging or the imaging stage can be translated between the radiography system and the NIR/VIS instrumentation, allowing sequential scanning in a seamless manner [3]. Although the integration of these methods has advanced considerably, their true synergy in terms of how they might be combined is yet to be realized. A comprehensive review of current and emerging systems is presented in this article.

Perhaps the most important feature that differentiates radiographic from optical imaging is the dramatic tradeoff between resolution and sensitivity to contrast agents. Clearly, the resolution of radiographs is better in deep tissues relative to NIR/VIS, although contrast degrades as the tissue thickness increases because of increased scatter in the imaging field. In comparison, the resolution of optical imaging is much more sensitive to the thickness of tissue through which the light travels. In the case of visible light, for example, tissue is highly absorbing, leading to light penetration of typically less than 1 mm. In the case of NIR light, absorption by the main tissue chromophores decreases drastically by at least two orders of magnitude when compared with VIS. As a consequence, NIR light is highly scattered in tissue, with an average transport path length near 1 mm, which allows tissue-specific penetration up to several centimeters [4]. The disparity between radiographic and VIS/NIR resolution is so great that they are rarely compared in any meaningful way. Rather, much of the focus of combined imaging centers on optical contrast agents that provide molecular or functional information about the tissue. A significant potential strength of NIR/VIS imaging, which is perhaps still unrealized, is the ability to image multiple fluorescent molecules (hereafter referred to as “fluorophores” for simplicity) at the same time, thereby providing multiple tissue targets. Generally, the sensitivity to concentrations of fluorophore is in the micromolar to nanomolar range with NIR/VIS, whereas contrast agents used with radiography tend to be several orders of magnitude higher in terms of concentration levels, namely in the millimolar or higher ranges. Thus, radiographic contrast imaging is restricted to vascular or gastroscopic use in which the high concentrations can be maintained by the vessels or organ walls being evaluated.

A conceptual illustration of the tradeoff between contrast agent concentration sensitivity and the number of agents that could be imaged in a single scan is shown in Figure 1. The simple idea represented here is that radiographic imaging is rarely used with more than one agent because dynamic spectroscopy or energy resolution has not been routinely integrated into radiography systems, although up to two energy ranges are now available during a single imaging session in many scanners. In comparison, optical spectroscopic imaging can provide resolution of up to 10 different fluorophores without moving the subject to be imaged. Thus, the variety of molecules that can be interrogated is much higher and each provides molecular or functional specificity.

The systems and approaches to combining radiographic and optical methods are reviewed from preclinical imaging through early clinical and emerging systems that are entering clinical trials. The full capacity of molecular sensitivity and the range of molecular species imaged have not yet reached full potential, and systems entering different clinical specialties will likely differentiate in the future to exploit this potential. In the following sections, the fundamental limitations and advantages are outlined, and the systems are reviewed. The article ends with a summary of emerging developments and future potential.

Attenuation and Spectroscopic Imaging

Radiographic projection imaging is based on attenuation of the signal propagating through tissue; in which case the loss of signal is dominated by the bulk tissue photoelectric effects where the atomic mass, to a large extent, dictates the attenuation level and contrast. A set of representative attenuation spectra for different types of human tissues is shown in Figure 2A for the useful diagnostic radiography range. The x-ray attenuation of soft tissue has a relatively featureless spectrum; hence, resolving different types of soft tissues is challenging; yet, these differences are the origin of all organ and tumor contrast in the absence of injected agents. The value of CT results from resolving soft-tissue variation achieved through the use of multiple overlapping projections, which significantly improve the lower limit of contrast recovery (by at least an order of magnitude compared with single projection radiographic imaging). Projection imaging is largely dominated by applications involving bones and air cavities because of the inherent higher contrast between them and the surrounding soft tissue as shown in Figure 2A in the case of bone.

In comparison with radiography, the attenuation of NIR/VIS light is dominated by elastic scatter in tissue [5,6] and has an absorption contribution that is typically 100 times lower than the scattering effect within the NIR/red wavelength range (bounded by $\lambda = 620$ nm to $\lambda = 1,200$ nm). Thus, diffuse transport is possible through this highly scattering regimen, and diffuse imaging has been realized in many preclinical and clinical applications. Diffuse tomography is possible through 10 cm of tissue or more [4,7–10], and transmission-based small animal fluorescence imaging is routinely performed in this wavelength range [11]. Moreover, the strong molecular absorption features in the spectra allow quantitative molecular spectroscopy of tissue through multiwavelength analysis of the data.

Interestingly, multienergy radiographic spectroscopy has been investigated for a long time as well, but only recently have commercial dual-energy scanners become available [12–14]. Because they provide automated segmentation of hard and soft tissues, these scanners have been used for bone densitometry and connective tissue imaging. Multispectral imaging with radiography is still in its infancy, largely due to limitations in efficient radiographic sources and proven practical utility in clinical imaging [15].

Exogenous Contrast Agents

Enhancing contrast in radiography is achieved through injection of ionic and nonionic iodinated compounds for vascular imaging or with barium sulfate-type agents for gastrointestinal imaging [16]. Although research into new agents is not currently growing, several commercial suppliers exist and clinical use is widespread and routine. The attenuation spectra for iodine and barium are shown in Figure 3A in which the monotonic decrease in photoelectric absorption dominates with a large increase from the K-edge effect. When imaging with CT using photon energies (kVp) near 100 keV, Compton scatter is the tissue-dominant interaction, whereas with the addition of a contrast agent, the photoelectric absorption is much higher when localized in vessels or organs. In general, clinical radiography contrast agents are safe and approved ways to enhance contrast. They do not present molecular contrast or tissue-specific contrast but simply highlight the functional and structural features of vessels or the intestinal tract because of their confinement immediately after administration. Research into packaged delivery of agents in liposomes [17–20] or specialized structures such as dendrimers [21] is under way; however, because of the high concentrations, molecular delivery is not considered as a logistically plausible mechanism for imaging with radiography. Animal and human imaging with pegylated and lipid-based CT contrast agents is also being considered [22,23]. Spectral recovery of contrast

concentration is likely possible by resolving the energy below and above the K-edge of the agent, and research is ongoing but no commercial uses have appeared.

In comparison with the monotonic attenuation spectra of radiography contrast agents (except for the K-edge), the range of fluorophores available for biologic contrast enhancement is extremely wide, with just a few of the more relevant ones (in the red and near-infrared part of the spectrum) shown in Figure 3B. Targeted delivery of agents can provide a linkage between the fluorophore and some targeted vehicle [24]. The specificity of agent binding is always a concern and nonspecific binding or uptake is the problem that confounds most receptor-based imaging approaches. Although the approach has been studied extensively with different receptors in animal studies, few of these targeted agents have made their way into human clinical trials. Background fluorescence from tissue is also a major concern when imaging with visible wavelengths [25], and most research systems focus on far red or near-infrared dyes, which have significantly lower autofluorescence from the tissue.

The relatively narrow emissions from most fluorophores allow multiple molecules to be used at the same time as long as spectral filtering or spectrally resolved detection is performed. Nonetheless, most research is completed with one agent at a time to simplify the analysis. As molecular imaging continues to develop, the ability to compare the uptake of molecules or conduct molecular competition studies will become increasingly important. Thus, the ability to follow multiple wavelength tags to molecular tracers will be an important area of emerging research. The opportunities have already been recognized in the preclinical commercial sector, and several options for multiwavelength imaging of rodents are currently available.

Preclinical Systems

Whole-body small-animal imaging based on diffused visible or NIR light defines an ensemble of in vivo imaging techniques that have expanded dramatically in their preclinical use over the past decade [3]. The main application of this type of imaging results from the detection of fluorescent or bioluminescent molecules associated with specific molecular processes that can be used as biomarkers of different types of abnormalities. In biologic applications, fluorescence signals can be associated with different types of processes including, for example, autofluorescence of biologic tissues, exogenous dyes tagged with molecular probes specifically targeting cell receptors, and fluorescent proteins expressed in transgenic rodent models of disease. In a manner that is similar to PET and SPECT, in vivo NIR/VIS imaging can provide biologic scientists with information that is specific to biologic processes of interest with high levels of sensitivity if used in conjunction with targeted probes. High-resolution MRI is also used for functional molecular imaging, albeit with reduced sensitivity when compared with nuclear imaging.

The development of NIR/VIS imaging has not reached the same level of maturity as nuclear imaging; hence, a proper comparison of their specificity and sensitivity is not yet complete. Nuclear imaging is recognized to be more sensitive than NIR/VIS, which in turn is more sensitive than MRI (Fig. 1). The advantages of NIR/VIS relative to nuclear imaging include lower cost of the hardware as well as reduced burden in terms of imaging constraints leading to potentially higher throughput and fewer security concerns and personal training associated with the use of radioactive material. However, NIR/VIS and nuclear imaging are in some ways very similar. For example, because they provide images that are specific to molecular or functional processes, both techniques are more limited in terms of providing anatomic information related to the physical structures of an animal (e.g., exterior contour, delineation of organs). As a result, medical imaging companies, such as Philips Healthcare, GE Healthcare and Siemens Healthcare, have developed systems that combine preclinical

nuclear imaging with techniques such as preclinical CT (Fig. 4A) and high-resolution MRI, which primarily provide structural information.

Structural images are usually superimposed with the nuclear scans, providing users with a more precise reference for the location of the radioactive sources with respect to the major organ systems, thereby compensating for the low resolution ($\sim 1\text{--}2\text{ mm}$) of PET/SPECT. The structural information also is often used to implement tissue attenuation correction in the context of PET or SPECT reconstruction algorithms, which improves the quantitative accuracy of the recovered images. Research as well as commercial avenues that are currently very active in NIR/VIS imaging consist of mirroring preclinical developments in the nuclear world by combining NIR/VIS with structural techniques such as planar radiographic imaging or CT (Figs. 4B and 4D). In terms of research developments, a number of preclinical systems now have NIR/VIS setups that are compatible with radiography/CT platforms, allowing seamless coregistration of the images derived from both systems (Fig. 4C). To maintain coregistration, research laboratories and manufacturers have developed universal animal tables compatible with different imaging techniques. This type of approach typically involves sequentially imaged sessions with both instruments in which the table is physically moved between platforms [26]. Other laboratories have developed multifunctional instruments that integrate the optical imaging hardware within the radiographic imaging system [27, 28]. Because the animal does not need to be moved between imaging sessions, the multifunctionality alleviates some of the burden associated with animal handling by facilitating anesthesia and functional monitoring [29].

However, the space constraints associated with imaging hardware are challenging, and, if not implemented carefully, multifunctional systems can be limited in their tissue sampling in terms of spatial resolution and contrast recovery. For example, the signal-to-noise ratio (SNR) in a micro-CT image pixel is, at constant x-ray exposure, proportional to $(\Delta\chi)^{-2}$, where $\Delta\chi$ is the spatial resolution. Typical resolutions for clinical CT are on the order of 1 mm^3 per voxel. However, because typical organ sizes scale proportionally to body-mass index, the length scales that are targeted by micro-CT are between 50 and $100\text{ }\mu\text{m}^3$. To reach the SNR of clinical devices, the preclinical radiography doses required would be very large, thereby significantly limiting the potential of micro-CT to resolve soft tissues [30]. In fact, many of the applications for preclinical micro-CT are structural and anatomic studies related to skeletal diseases such as osteoporosis and arthritis. Of course, the uses of micro-CT can be extended with radiopaque agents that exhibit preferential uptake in the organs of interest. Concurrently with the development of novel imaging agents and incremental instrumentation improvements, several principles are continuously being explored to improve the sensitivity and contrast range of the technique, including K-edge subtraction, radiography phase delay, x-ray scatter, x-ray diffraction, and x-ray fluorescence [31].

As shown schematically in Figure 1, the molecular sensitivity of nuclear imaging reaches concentrations in the nanomolar range compared with micromolar levels for clinical and preclinical CT. To appreciate how NIR/VIS compares with PET/SPECT, several factors must be considered when evaluating the detection of small variations in fluorescence. Here, sensitivity is defined as the minimum concentration of a dye that can be resolved in vivo against all other sources of fluorescence that would not be specific to the pathologic processes of interest. Preclinical whole-body imaging can be broadly divided into two categories: imaging based on signals acquired in an epi-illumination geometry and signals acquired in transmission across the animal [3]. Moreover, no system is unique within each of these imaging paradigms [3] at both the commercial and research levels because of the wide range of hardware components that can be incorporated into the design. For example, light detection can be performed with several different types of photodetectors, including scientific-grade charge-coupled devices (CCDs) and internal-gain devices such as electron-

multiplied CCDs (EMCCDs), intensified-CCDs (ICCDs), photomultiplier tubes (PMTs), and avalanche photodiodes (APDs). Bioluminescence systems are different because they do not require the use of a light source but, as explained later, are more closely related to transillumination than epi-illumination in terms of sensitivity.

Epi-illumination NIR/VIS systems are generally constructed with a broad-beam light source to excite the fluorophores (e.g., defocused laser diode or arc lamp) and a cooled CCD camera with well-depths allowing digitization, ideally at 16-bits resolution. SNR associated with fluorescence or bioluminescence detection systems can be written as

$$S/N = \frac{I_{\text{target}} t}{\sqrt{(I_{\text{target}} + B_{\text{auto}} + B_{\text{ns}} + B_{\text{bt}}) t + DN^* t + RN}}$$

where I_{target} is the signal of interest, and B_{auto} , B_{ns} , and B_{bt} are the photon signals associated with tissue autofluorescence, fluorophore concentrations in places other than the location of interest (e.g., a tumor), and bleed-through of excitation light across the fluorescence filters. These three sources of noise, along with the photon noise of the signal of interest itself result in what is often called shot noise. Other sources of noise affecting sensitivity are the dark noise (DN) associated with detected photonic events from thermal electrons generated in the absence of any input light signal and the readout noise (RN) associated with the transfer of electrons accumulated in a CCD chip into a digital image. Because it is the only source of noise that does not scale with exposure time, t , of the photodetector, readout-noise can usually be made negligible by increasing the integration time.

Because of tissue absorption and scattering, epi-illumination imaging is highly surface weighted, implying that fluorescence events occurring closer to the surface comprise exponentially more weight within the bit depth of the chip. Furthermore, cellular and extracellular molecules with intrinsic fluorescence in certain spectral bands in the visible and NIR domain exist. In fact, their contribution, labeled B_{auto} , is often the term in the formula that limits the intrinsic SNR, which can be attained with epi-illumination. Bleed-through of excitation light across the filters is another factor that can significantly limit the sensitivity of a system, even in cases in which the autofluorescence is low. That is why contributions associated with dark noise and readout noise are usually considered negligible for epi-illumination, which typically does not require the use of low-noise internal-gain photodetectors such as PMTs, APDs, ICCDs, or EMCCDs. The readout noise of most scientific-grade CCDs is also sufficiently low not to cause sensitivity limitations for epi-illumination. Another factor that can significantly limit the sensitivity of a system is the stochastic noise associated with the photon signal coming from dyes accumulated in locations that are not associated with the disease process under study. The contribution from this type of noise, however, depends on the specific biologic application being considered and is independent of the imaging paradigm that is used—that is, epi-illumination or transillumination. Some control can be gained over the contribution from autofluorescence by using excitation and fluorescence wavelengths that minimize its impact. For example, the contributions from most autofluorescence molecules, such as reduced nicotinamide adenine dinucleotide, collagen, and elastin, become smaller with increasing wavelength and are almost negligible in the NIR.

In contrast to epi-illumination systems, the sensitivity of instruments designed for transillumination fluorescence is not limited as much by tissue autofluorescence because the images are not as heavily surface weighted. As a result, fluorophores at different depths contribute to the signal with the same order of magnitude. Analytic expressions for diffusion from a point source of fluorescence inserted at depth, d , into a tissue with thickness, T ,

indicate the epi-illumination fluorescence signal is proportional to $\exp(-2d\mu)$, whereas the transmission signal scales are proportional to $\exp(-T\mu)$, where μ is the effective attenuation coefficient, which is independent of the depth, up to boundary condition effects and differences in absorption and scattering between excitation and emission. Clearly, however, this approximation shows that fluorescence signals associated with transmission, although less affected by skin autofluorescence, for example, can be orders of magnitude smaller than typical epi-illumination signals. The small signals and the fact that autofluorescence contributes to a lesser extent, explains why transillumination imaging is usually performed with very sensitive photodetectors, such as cooled internal gain CCDs or PMTs. Bioluminescence is similar in this respect because the signals are even more specific to the disease process because the detected molecular events are associated with genetic modifications of specific cells. In fact, bioluminescence signals are typically smaller than transillumination signals, but the sensitivity of these systems is usually superior because of the absence of nonspecific bioluminescence resulting in the absence of the terms B_{auto} and B_{ns} in the SNR equation. Moreover, no excitation bleed-through occurs in bioluminescence.

Transillumination imaging, on the other hand, can still suffer from reduced sensitivity associated with nonspecific signals and bleed-through. However, the contribution from excitation bleed-through can be significantly attenuated through focused detection based on PMTs or APDs, which is more difficult to achieve for wide-field CCD detection. Therefore, for biologic applications in which the nonspecific signal and the autofluorescence are small, the sensitivity of a system to low photon fluxes is limited by either the dark noise or the readout noise. An advantage of PMT detection over CCD detection is that PMTs do not suffer as much from readout noise. Further increases in sensitivity are reached by using time-correlated single-photon counting techniques in conjunction with PMTs. This methodology eliminates most of the dark noise as well as the readout noise, thereby achieving sensitivity that is close to the theoretic lower limit for fluorescence as well as for bioluminescence.

An advantage of detection methods based on CCDs is their ability to provide users with snapshots of an animal with high frame rates compared with PMTs or APDs that are acquiring light signals pixel-by-pixel. The optimal approach for a given biologic application is the one that best trades off between the required level of spatial resolution and sensitivity to low photon fluxes. In summary, CCD-based approaches are sufficient for epi-illumination fluorescence imaging with existing technology, whereas more sensitive approaches can be useful for transillumination fluorescence or bioluminescence. Although epi-illumination imaging is the most widespread NIR/VIS approach, significant progress has been made in the development of transillumination fluorescence tomography systems with sensitivity levels in the nanomolar range. Although significant research has been reported in this area, the level of spatial resolution that is sufficient to fully exploit the potential of fluorescence tomography has not been effectively determined [32–34]. CCD-based tomography has been argued to be the most desirable approach because of the high density of data it provides, which increases the spatial resolution and quantitative characteristics of reconstructed images. However, systems based on pixel-by-pixel focused detection have also been shown to retrieve molecularly targeted contrast with image quality comparable to CCD-based detection. The increased tissue sampling associated with wide-field detection decreases the sensitivity of the tomography inversion problem to stochastic noise in the data, thereby allowing images to be recovered that have superior resolution and contrast. A point is expected to exist past which increasing the sampling does not provide further benefits to imaging, but studies investigating this issue in detail have not been conducted. The conclusion that ever increasing tissue sampling automatically leads to increasing resolution and quantitative information is a misunderstanding of the basic limitations of diffuse optical imaging attributed to tissue scattering.

Early diffuse optical tomography systems were designed to be used as standalone preclinical instruments and presented substantial limitations in terms of their ability to reconstruct small and low-contrast targets. However, in recent years substantial effort has been invested in the development of approaches that acquire optical images in conjunction with other preclinical imaging techniques such as micro-PET, micro-SPECT, high-resolution MRI, or high-resolution ultrasound. Most of the preclinical applications combining radiography/CT with NIR/VIS imaging have been passive—that is, simple coregistration algorithms present researchers with superimposed images of the structural and molecular or functional information [35–42]. For example, Carestream Health (formerly Kodak, Inc.) has developed a multifunctional instrument integrating epi-illumination fluorescence imaging with planar radiographic imaging [3]. Caliper Life Sciences also has developed multifunctional instruments that combine NIR/VIS and radiographic hardware providing a user with the ability to perform planar fluorescence or fluorescence tomography with planar radiographic imaging or micro-CT. Several other preclinical optical imaging companies have developed universal tables, allowing optical images to be registered or fused with planar radiographic or CT systems [3]. However, a growing area of research is moving away from simple multitechnique image registration by actively using the structural information provided by radiography/CT to improve the quantitative information provided by optical NIR [43–45]. In this approach, prior structural information can be used in optical tomography to decrease how mathematically ill-posed the problem is and improve the quantitative nature of the luminescence information that is retrieved. Using prior information in the context of the optical tomography problem is limited in the case of CT by the typically poor soft-tissue contrast associated with micro-CT, which limits the ability to delineate abnormalities such as tumors. For some applications, however, specific tissue contrast based on x-ray absorption can be generated using radiologic agents. In one instance, the prior structural information associated with CT images has been used to implement absorption or scattering correction in an effort to improve forward modeling of light transport and reconstruction [46]. In recent years, several tomography systems have been developed that actively combine NIR or far red imaging with CT, either through the development of universal animal tables [26] or fully integrated multifunctional instruments [27,28,47]. A clear advantage of using prior structural information for imaging-guided fluorescence tomography is a more well-posed problem mathematically, which significantly reduces the need for high-density NIR/VIS measurements. This new avenue opens the door to noncontact focused optical detection methods that are based on highly sensitive photodetectors, such as PMTs [26].

Clinical Systems and Developments

For clinical breast imaging applications (Fig. 5), the synergy between radiographic and optical spectroscopic imaging exists at three levels: optical imaging as an adjunct to improve diagnostic accuracy of mammography, hybrid radiography/optical imaging systems that combine information, and optical spectroscopy in subjects when radiographic imaging is not desirable, such as in assessing cancer risk. Over the past decade, several diffuse optical imaging systems have been developed as an adjunct to standard mammography [48–55]. Advanced Research Technologies developed one of the first commercial optical breast scanning systems for clinical use. The ART Soft-Scan system consists of a multiwavelength, time-correlated, single-photon counting–based time domain diffuse optical tomography system operating in a transmission geometry [56,57]. During an examination, the breast is positioned in a tank and is mildly compressed using plates made of plexiglass. The tank is filled with an index-matching fluid and a raster-scanning technique is used to mechanically scan the source and detection modules to obtain the tomographic projection measurements. The system supports four wavelength channels (760, 780, 830, and 850 nm), with 1,520 projection measurements acquired at each wavelength. Acquisitions at multiple wavelengths

allow reconstruction of the major tissue chromophore concentrations, the blood oxygenation level, and the reduced scattering coefficient using spectral unmixing techniques. The overall scan time is approximately 40 minutes. Figure 4A shows a photograph of this system along with a representative set of images. The images are interpreted with reference to mammography to improve the specificity of a breast examination. The system is approved for sale in Canada and Europe, with clinical trials ongoing to study response to neoadjuvant chemotherapy [58]. A randomized blinded clinical trial of imaging with NIR spectral tomography for women with mammographically detected abnormalities was carried out with age-matched control subjects, looking at the value of NIR imaging and assessing the added value in terms of an NIR examination after mammography [59]. This study showed that there was potential to improve the negative predictive value through the combined examinations, although the value occurred for tumors greater than 6 mm in diameter.

The CT Laser Mammography (CTLM) system developed by Imaging Diagnostic Systems, is another commercial breast imaging system designed as an adjunct to mammography [60,61]. This system operates in a CT-like geometry in which the pendant breast is scanned in a noncontact manner using a continuous wave single wavelength (808 nm) with an examination time near 15 minutes. The one wavelength lies approximately at the isobestic point of oxy- and deoxyhemoglobin, measuring them together with equal weighting, but no ability exists to assess scatter or other absorbers. The system recovers a total hemoglobin map of the breast volume.

The development of hybrid radiographic/optical imaging systems that offer coregistration through coupled hardware integration of these two techniques has recently been realized. The first hybrid radiographic/optical imaging system for clinical breast cancer imaging was developed at the Massachusetts General Hospital (MGH) [62]. The Tomosynthesis/ Optical Breast Imaging (TOBI) system integrated a custom two-wavelength frequency domain diffuse optical tomography system with a commercial digital breast tomosynthesis scanner to allow coregistered acquisition of optical and radiographic images. The system consisted of removable fiber-coupled source and detector cassettes that could be attached to the top and bottom compression plates of the tomosynthesis scanner. This mechanism allowed seamless insertion and removal of the optical cassettes without altering breast position. The source module consisted of two radiofrequency-modulated diode lasers (785 and 830 nm) coupled into a two-stage optical multiplexer that allowed switching between the two lasers and 40 different fiber positions on the source cassette. The detection cassette consisted of nine 3-mm fused silica fiber bundles individually coupled to APD modules. Standard frequency domain tomography techniques were used for radiofrequency modulation and demodulation of the source and detector signals. The system allowed a coregistered radiographic/optical imaging sequence in which the optical acquisition occurred first and with the compression plates held stationary the optical source/detector cassettes were removed and the tomosynthesis scan performed. Because mammography-level compression is typically used during tomosynthesis, the overall acquisition time was limited to 90 seconds to minimize patient discomfort. Frequency domain optical acquisition at two different wavelengths allowed reconstruction of hemoglobin concentration, oxygenation, and reduced scatter coefficient volume images. The initial clinical study using this system focused on obtaining coregistered optical and radiographic images and the optical contrast corresponding to various tissue types was derived by sampling optical data from regions corresponding to different tissue types identified by segmenting the radiographic images. A follow-up study focused on a more synergistic approach to fusing radiographic and optical data by using the prior information on the spatial distribution of tissue types extracted from the tomosynthesis images to directly guide the optical image reconstructions.

Although additional wavelengths and higher scan density can improve optical contrast recovery, the extra data are often not practical to obtain because of acquisition time constraints and other integration issues imposed by the hybrid imaging hardware. Continuous wave acquisition systems, on the other hand, have simple architectures that are cost-effective and easier to implement. They allow simpler wavelength multiplexing schemes, which could be exploited to achieve higher scan densities within a reasonable time. However, pure continuous wave systems are not capable of measuring optical scatter, which is required for accurate recovery of tissue chromophore concentrations and oxygenation. Hence, in many cases, a combination of continuous wave and frequency domain detection is often required to obtain an optimal imaging data set.

Some of these issues were addressed in a second-generation TOBI system that is currently operational at the MGH [63]. This system uses a combination of continuous wave and frequency domain optical acquisitions for data collection. Similar to the previous instrument, the source and detector channels are integrated into removable fiber-coupled cassettes that attach to the bottom and top compression plates. The frequency domain acquisition is architecturally similar to that used in the first generation TOBI system but uses 685 and 830 nm for better quantification of hemoglobin concentration and oxygenation levels. The continuous wave subsystem consists of a combination of galvanometric mirrors for scanning and spatially fixed sources for illumination at multiple wavelengths (685, 810, and 830 nm) and an array of 32 fiber-coupled APDs for detection. The detected electrical signal is then demodulated to identify the wavelengths and the position of the sources. Despite the complex acquisition scheme and the significantly increased number of measurement channels, the overall acquisition time is approximately 1 minute.

One original application using optical spectroscopy of breast tissue was shown and continues to be studied [64–67]. These studies have shown that optical spectral features are correlated with radiographic breast density, which is an important factor in a woman's potential risk of breast cancer. Because the data support the fact that optical measurements are related to this risk factor, the authors have hypothesized that optical density measurements could be a unique predictor of cancer risk. Thus, they are carrying out prospective clinical trials in high-risk populations of women to determine if this is indeed the case. If true, optical spectroscopy could be used as a low-cost, low-risk screening tool for women who are not of age to participate in mammography screening programs. Additionally, women at high risk could potentially be optically screened more frequently than is possible with mammography. In this setting, optical spectroscopy would truly be synergistic with radiographic mammography in that they would be used on different populations of women or at different examination intervals.

Apart from breast imaging, hybrid radiographic/optical imaging has also been developed for diagnosing and monitoring cartilage abnormalities and alteration in the composition of synovial fluid caused by osteoarthritis [68]. This system combines the high-resolution imaging of bone joints using radiographic tomosynthesis with functional optical imaging of the cartilage region to potentially improve diagnostic outcome.

Resolution and Contrast

Spatial resolution often significantly limits the utility of an imaging system; yet, in tomographic systems contrast-resolution performance can be of equal importance because soft-tissue contrast is critical [69]. Contrast-resolution characterizes the ability to detect subtle or low contrast levels, such as the relative contrast between tumor and surrounding normal tissues, which might have very subtle changes in Hounsfield units (or CT number) [70]. Radiographic CT is almost exclusively used for low-contrast resolution of soft tissues,

in which contrast changes as low as 1% are routinely differentiated. In comparison, projection radiographic imaging is rarely used for soft-tissue contrast imaging because it is much less sensitive to these subtle changes although it has higher spatial resolution. Improvements in soft-tissue contrast come in mammography, for example, through reduction of tissue thickness and hence signal scatter via breast compression. In NIR imaging, the contrast resolution relative to the background varies strongly with depth into the tissue and size of the object. Extremely high sensitivity and resolution can exist near the surface of tissue (near 10 μm), but with orders of magnitude worse resolution (10 mm) as a function of depth into thick tissue. The variation in contrast-resolution can range from 0.1% on the surface (with 16-bit detection) to 10% in deep tissue tomography. These values have been quantified in earlier studies of contrast-detail analysis comparing radiographic system performance to optical system performance for thick tissue imaging. A practical rule of thumb in diffuse imaging is that the resolution scales as 20% of the diameter of tissue through which the signal is measured [71], or $\Delta x = 2D$.

In the thickest tissue cases, D might be 10 cm, in which case $\Delta x = 2$ cm. Alternatively, if D is associated with surface imaging, then it is just a few hundred microns (for light going in and out) and Δx is then on the order of tens of microns. Thus, although very approximate, the equation is a reasonable estimate of the resolution for a given tissue thickness. When quantifying contrast in optical imaging, the contrast recovery is rarely ideal except for surface imaging. As in most imaging systems, an inverse relationship exists between the contrast, C , that can be detected and the size of the region to be recovered [4,72,73], such that $C \propto \Delta x^{-1}$. This relationship is true in radiographic imaging [74,75] as well as in optical imaging, but the proportionality coefficients differ in most cases and the relationship can be defined through experimental studies of the contrast-detail curve.

Although fundamental limits to contrast resolution do exist, they are strongly dependent on the size of the region in radiographic imaging. Webb [70] has shown that as the size of the region decreases, the dose needed to achieve the same signal-to-noise level increases with the fourth power of the size: $\text{Dose} \propto \Delta\mu^{-2} \Delta x^{-4}$, where $\Delta\mu$ is the change in attenuation coefficient between the tissue to be detected and the tissue surrounding it, and Δx is the size of this tissue. As Δx decreases by a factor of 10, the dose needed to resolve the region increases by a factor of 10,000, which is an enormous increase that is both technologically and physiologically impossible to achieve even in mouse models. Thus, a fundamental limit exists in terms of resolving small contrast in small regions in radiographic imaging. This effect limits the value of micro-CT for imaging soft-tissue tumors in that they cannot be resolved within reasonable imaging times because the dose needed and signal-to-noise required are insufficient. Indeed, even injected contrast agent imaging does not provide sufficient discrimination for tumor relative to normal tissue except in the case of liver tumors using specialized agents targeted to hepatocyte uptake [76], which yield negative contrast.

Contrast resolution limits place serious constraints on small animal radiographic imaging because tumors can rarely be segmented with confidence due to the lack of apparent contrast, which restricts the synergy that can be achieved between optical and radiographic imaging with small animal technology. Basically, shapes of normal organs can be segmented, especially organs bounded by air or bone, but beyond these conditions the extent of tumors cannot easily be defined. Lipid emulsion contrast agents can help define liver lesions and preclinical agents are available (Fenestra, ART Inc.); however, it is one of the few successful agents for small animal tumor contrast [77]. Thus, optical imaging can only take advantage of the spatial definition of exterior volumes of tissue, rather than segmenting suspected tumor regions within soft-tissue organs, without going to more advanced

subtraction methods [78]. Acquisition times for accurate imaging can be long as well because the dose required for good signal-to-noise can be quite high.

In comparison, human imaging of larger regions is more successful for segmentation because the size scale is larger and the dose required to resolve the regions of interest is consistent with what is technologically possible and logistically feasible. Thus, much more potential synergy in terms of prior information exists between radiographic imaging in humans combined with optical imaging. The combination is still under development and remains to be proven, although physically the arguments seem to be logical.

Areas of Synergy and Conclusions

The real synergy between radiographic and optical systems occurs when the data are used in a way that recovers information in a manner that cannot be obtained without the presence of both radiographic and NIR/VIS images [79]. Perhaps the most important issue is the ability to segment tissues from the radiographic images automatically. The newer clinical dual-energy radiographic CT systems will make this realizable because they provide high-fidelity segmentation without user guidance [80–82] and potentially for automated contrast agent detection as well [83,84]. Automatic segmentation is an important feature because almost all research systems require fairly heavy user guidance in the segmentation process. The ideal outline of synergistic image recovery is illustrated in Table 1, in which the radiographic data are preprocessed by spatial and energy filtering to segment soft and hard tissues. Much of this work is being pioneered in adaptive radiotherapy treatment planning with so called tomotherapy or arc therapy [81,85,86]. Spatial synergy is achieved when structural information from radiographic imaging improves the accuracy of the optical parameter recovery or the optical spectroscopy can be focused onto the region identified in the radiographic imaging. This synergy in spatial localization is commonly being investigated today in preclinical systems as well as in combined systems involving mammography or tomosynthesis. If automated segmentation of the radiographic image is possible, the NIR/VIS data could be spectrally collected and the estimation of fluorophore concentrations could be performed by region-guided updates with constraints given by the spatial and spectral fits.

Additional synergy can be obtained when the information from optical imaging provides added confidence in the need for radiographic imaging examining the potential for optical measurements to predict cancer risk [64–67]. Further work in this area will require validation and testing of cancer incidence versus risk or the merits of optical spectroscopy as a surrogate for radiographic imaging. This already occurs in preclinical imaging in which optical measurements commonly replace radiographic imaging to quantify tumor progression or incidence. Preclinical optical spectroscopy or imaging is significantly less expensive than radiographic imaging; hence, synergy exists in performing optical measurements in place of radiologic imaging. Future areas of synergy will likely be in cases in which optical imaging provides additional replacement value and when optical imaging can be used to determine when radiologic imaging is warranted.

Finally, both radiographic tomography and optical imaging system designs are advancing. As time goes on, the potential to synergistically integrate the two types of information will advance as well. As the value of the different types of information obtained from each system continues to increase, the combination may continue to be synergistic or may become redundant. As systems advance for human use, the synergistic areas will be in data streams that complement each other and provide the most physiologically and pathologically relevant information for the diseases being imaged.

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Fig. 1.

Conceptual schematic shows how different imaging techniques compare in terms of their sensitivity to low molecular concentrations. In particular, radiography can image down to millimolar levels and is mostly used to resolve one agent at time, whereas near-infrared (NIR)/optical fluorescence imaging is sensitive to micromolar to nanomolar concentrations and has potential to image up to 10 agents simultaneously.

Fig. 2. Attenuation spectra for radiography and optical/near-infrared (NIR)

A and **B**, Graphs show spectra for radiology (**A**) and optical/near-infrared (NIR) (**B**) in terms of constituents in most tissues. X-ray attenuation is dominated by photoelectric effect absorption, which increases exponentially at lower energies. NIR/visible wavelengths are dominated by elastic scatter, and window of very low absorption occurs from 620 to 1,050 nm, sometimes referred to as diffuse transmission window of tissue. Molecular constituents of tissue in NIR/visible imaging provide significantly stronger absorption peaks that allow molecular spectroscopy of tissue. Hb = hemoglobin.

Fig. 3. Spectra of contrast available

A and **B**, Graphs show spectra of contrast available in radiography (**A**) and near-infrared (NIR) fluorescence (**B**). Contrast from iodinated agents or barium in attenuating x-rays is dominated by enhanced photoelectric effect in 10–200 keV range due to both K-edge effects and general increase in overall absorption of x-rays. Range of organic or biocompatible fluorophores is illustrated with some representative agents in **B** including protoporphyrin IX, cyanine dyes Cy5.5 and C7, and indocyanine green. Z is atomic number of the element.

Fig. 4. Examples of various systems

A, Example images from Carestream system with radiographic projection and fluorescence within gastrointestinal tract and overlay. This instrument was first system developed for hybrid radiographic/optical fluorescence imaging of mice.

B, Photograph shows newly released Quantum radiographic tomography and optical tomography system from Caliper Biosciences.

C, Example images from optical tomography combined with radiographic CT are shown for glioma tumor imaged with contrast from endogenously induced fluorescence from protoporphyrin IX within tumor.

D, VisEn Medical Systems instrument features animal cassette that allows optical and radiographic tomography in same holder.

Fig. 5. Breast imaging systems

A, Photograph shows SoftScan (ART, Inc.) breast imaging system.

B and **C**, Representative images of total hemoglobin concentration (**B**) and reduced scatter coefficient (**C**) images acquired by SoftScan system are shown along with corresponding radiologic images of breast.

D, Photograph shows CT Laser Mammography (IMDS Inc.) breast imaging hardware.

E and **F**, Representative images of total hemoglobin concentration are shown along with corresponding radiologic images of breast.

G, Photograph of Tomosynthesis/Optical Breast Imaging (TOBI) system from Massachusetts General Hospital. (Copyright IEEE Publishing)

H and **I**, Representative total hemoglobin concentration images are shown along with coregistered tomosynthesis images in plane of interest. (Copyright IEEE Publishing)

TABLE 1

Synergistic Image Recovery

Technique	Preprocessing	Data Reduction	Constrained Estimation
Radiography	Spatial filter, energy filter	Segment in projections	
Near-infrared imaging	Spectral optimization and fitting	Region-guided fitting	Spectral constraint, spatial regularization