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Review

Demystifying optical diagnostics

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Abstract. This paper describes a few optical imaging methods in their translational stage to the clinical phase. The methods are compared to well-established X-ray imaging methods such as computed tomography and mammography. The drawback of the diffuse paths of laser photons compared to the ballistic paths of the X-ray is compensated by coherence methods for tissue characterization adjacent to the surface, and acoustic wave enhanced imaging and fluorescent markers that follow functional changes when deeper tissue is analyzed. This paper presents some of the latest developments in this field as presented in a Bench to Bedside workshop on optical imaging held at the National Institutes of Health.

Keywords: Optical imaging, optical coherence tomography, X-ray imaging, computed tomography, functional imaging

1. Introduction

1.1. Bench to bedside

Optical imaging techniques have been widely investigated over the last decade for biomedical diagnostic applications. The aim of this paper is to introduce optical imaging techniques that are moving from the bench to the bedside and are competing with and complementing X-ray imaging techniques. For an optical imaging technique to be clinically useful, multi-disciplinary and multi-step approaches are required. One should devise quantitative theories, and develop methodologies applicable to *in vivo* quantitative tissue spectroscopy and tomographic imaging. At the bench, one designs and conducts experiments on tissue-like phantoms and runs computer simulations to validate the theoretical findings. The most difficult task is to bring the optical system from the bench to the bedside. The use of animal models, as an intermediate step, for pre-clinical studies is usually necessary. Strong collaborations

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among engineers, physicists, and physicians are needed to identify physiological sites where optical techniques may be clinically practical and offer new diagnostic knowledge and/or less morbidity over existing methods. The optical imaging methods discussed in this paper were presented at the fourth Inter-Institute Workshop on Diagnostic Optical Imaging and Spectroscopy from Bench to Bedside from September 20–22, 2004 at the National Institutes of Health.

Organized by Amir Gandjbakhche from the National Institutes of Health and Israel Gannot from the National Institutes of Health and Tel Aviv University (Israel), more than 300 researchers from academia, government, and industry attended. During the presentations and poster sessions, the latest theoretical and technological advances of optical imaging were discussed. Opportunities and critical technological developments with the greatest potential for clinical and research utilities were also presented.

Each of the workshop's ten sessions was dedicated to a different aspect of the optical imaging field and the translation of the research from the bench to the bedside. The fields highlighted at the conference and in this paper include optical coherence tomography, including a presentation by G.J. Tearney, Massachusetts General Hospital entitled, "Evaluating plaque microstructure in patients by intracoronary optical coherence tomography" and an exciting presentation by Joseph Schmitt from LightLab Imaging Inc. who presented, "Optical coherence tomography: poised to enter the Japanese cardiology market." Ultrasound-aided optical imaging included presentations by Lihong Wang, Texas A&M on ultrasound-aided high-resolution biophotonic imaging and A. Claude Boccara from Ecole de Physique et de Chimie Industrielles de Paris on sensitivity and resolution in acousto-optic imaging. Performance and limits of each modality were highlighted to show how the combination of these modalities allowed for better diagnostics. Optical imaging has benefits over X-ray techniques in cancer and cardiology diagnosis, detection, and treatment. This paper introduces optical methods and compares them to the competing X-ray methods.

1.2. X-ray and optical imaging methods

Diagnostic trends in medicine are being directed toward cellular and molecular processes, where treatment regimens are more amenable for cure. X-ray imaging techniques are currently being used by doctors in hospitals today. Optical imaging is capable of performing cellular and molecular imaging using the short wavelengths and spectroscopic properties of light.

1.2.1. X-ray imaging methods

Two common X-ray imaging methods are radiography and computed tomography (CT). Radiography is the process of creating an image from X-rays that have passed through structures. X-rays are intercepted by a highly absorbing detector and are converted into a corresponding 2D image. X-ray absorption and scattering contribute to contrast in the X-ray image. The interactions pertinent to medical X-ray applications include photoelectric absorption, Rayleigh scattering, and Compton scattering. X-rays exhibit properties of electromagnetic radiation and quantum mechanics [40]. Although quick and inexpensive, radiographs cannot discern tumor tissue, show low-contrast structures with good resolution, and do not provide depth information [5]. X-ray photon energies are high, averaging between 17 and 150 keV, and experience little attenuation. The choice for a particular X-ray imaging application is a trade-off between acceptable radiation doses and achievable image contrast.

CT is widely used for various aspects of radiation oncology. CT reconstructs images from X-rays transmitted transversely onto the patient by an X-ray source and its radiation is then detected by an array of detectors on the opposite side after being transferred through the patient. It produces cross-sectional images that can show anatomical structures with better accuracy and resolution than

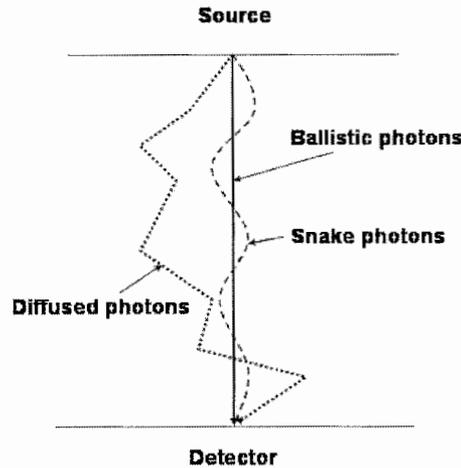


Fig. 1. Schematic diagram of various photon trajectories through tissue medium.

X-ray radiography [5]. However, CT is expensive and like radiography, involves exposure to ionizing radiation. This enables sub-millimeter spatial resolutions to be achieved, with good discrimination between different types of tissues (better than 1% attenuation change) [48]. One limitation of CT is that anatomical information by itself cannot definitively differentiate between tumors and benign tissues. Another limitation is that it cannot completely reveal histopathological and physiological characteristics or be used to assess early response to therapy. Therefore, although CT is particularly good at depicting bone detail, tissue contrast is suboptimal, and intravenously administered contrast agents are frequently required to enhance its tissue-discriminating ability [5].

Comparing radiography to CT, radiography has three times better spatial resolution, ten times better clinical resolution, and has better tumor discrimination as well. The sensitivity and specificity of both techniques are very similar, but the temporal resolution of CT is twice that of radiography [5]. Photon paths in optical imaging are more complicated than X-ray paths. They can be roughly divided into three major path types: ballistic (as in X-ray), snake, and diffused paths (the major amount of energy that passes through tissue) as described in Fig. 1. Because of these phenomena, analyzing optical data is more mathematically complex. Optical imaging methods comparable to the X-ray imaging methods are described below.

1.2.2. Optical imaging methods

Optical imaging modalities are based on physical principles of wavelength-dependent light-tissue interaction, including photon scattering, total internal reflection, absorption, reflectance, polarization, and fluorescence (both endogenous and exogenous). The absorption and scattering of photons within tissue limit the depth of penetration and spatiotemporal resolution of images that can be obtained. However, recent advances in optical contrast agents, optical probes of physiological parameters, light transport theory, light sources, and optical detectors have created conditions for major breakthroughs in optical imaging. Novel imaging modalities with expanding areas of application have emerged, including optical diffusion tomography, optical coherence tomography, and various confocal fluorescence imaging approaches.

Optical imaging shows promise in cancer and cardiology diagnosis, detection, and treatment. Besides its capacity for earlier detection of intraepithelial lesions and small tumors near the surface, optical

imaging can also assess surgical margins in real time in the operating room. There are many reasons why optical imaging has become popular in the biomedical field. Optical techniques are much safer than X-ray techniques because optical imaging uses non-ionizing radiation, the photon energy is approximately -2 eV. Optical techniques have high intrinsic contrast of absorption, scattering, and polarization properties. Optical scattering can show the size of cell nuclei and optical polarization can show the collagen network. Optical imaging is functional imaging of physiological parameters including hemoglobin oxygen saturation, total hemoglobin volume, cell nuclei size, collagen orientation, denaturation of collagen, blood flow, and temperature and pH changes.

There are new noninvasive ways of using NIR light as probes for tissue investigation. The advantage of NIR is that light in this range (650–900 nm) can traverse tissue very efficiently, as absorption in this part of the spectrum is relatively low. This allows penetration to greater depths (in the range of 0.5 to 2 cm) than is achievable using visible light. Since depth penetration is one major limiting factor of *in vivo* optical imaging, the wavelength chosen should ideally be in this diagnostic window. Moreover, imaging in the NIR region also has the added advantage of minimizing tissue autofluorescence, which can improve the target's signal to background noise ratio [23]. Due to the different absorption or scattering properties of tissues at NIR wavelengths, NIR-based optical methods offer the potential to differentiate between soft tissues that are currently indistinguishable using any other modality.

All of the existing imaging modalities require that final diagnosis be determined through tissue biopsy. Optical methods offer the potential to obtain an immediate, definitive, and noninvasive diagnosis; so avoiding the need for a painful biopsy and the associated time delay while the tissue is analyzed by a pathologist. Most conventional imaging systems are unable to provide functional information. By contrast, specific absorption by natural chromophores allows functional information to be obtained using optical imaging methods. Chance has shown that one could determine hemoglobin saturation noninvasively [8]. This method can be useful in order to detect hematomas, strokes, and vascular deficiencies, as well as to investigate muscle and brain physiology.

Optical imaging methods cost significantly less than a CT system and are much simpler to use. Optical methods are simple to set up and do not require dedicated facilities. In addition, optical methods do not expose the patient to any ionizing radiation, nor to any comparable hazard. Therefore, reasonable doses can be repeatedly employed without harm to the patient.

Unfortunately, the ionizing radiation techniques above cannot yet be replaced by the diffuse optical imaging modalities described below. This is because, even at NIR wavelengths, for which the optical attenuation in background tissue is rather small, differences in absorption coefficients between normal and diseased tissue are also quite small. Furthermore, differences in scattering properties, which are likely to be the largest source of contrast, are also fairly nonspecific. This can result in poor specificity and low spatial resolution optical images [14].

The application of optical techniques in biology and medicine has recently become an important field. One such technique is optical coherence tomography (OCT). OCT takes cross-sectional images of tissue by measuring the pathlength traveled by the interrogating beam incident on the tissue sample by using an optical technique known as interferometry. The light source is divided into two identical beams with a Michelson interferometer and one beam is directed to the sample and the other to a reference mirror with a known location. When light returns from the sample and the reference mirror it is recombined at a detector, and the interference between the two beams is registered. The use of temporally incoherent light allows the distance traveled by the sample beam to be determined, since interference can occur only when light from both beams arrives at the detector simultaneously [7]. One limitation of OCT is that it suffers from multiple scattering, which blurs images coming from tissue at depths of greater than 1 mm. Optical methods are adaptable for use to enhance existing diagnostic tools.

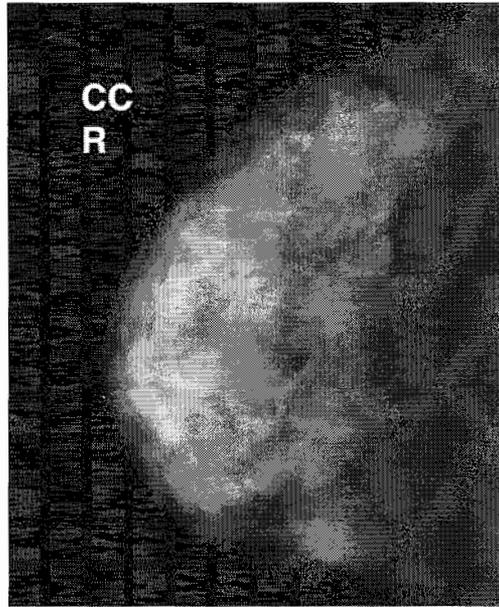


Fig. 2. Dense fibrocystic changes as seen in X-ray mammogram (courtesy of Ville Marie Breast Center).

2. Breast cancer detection

The present standard diagnostic tool for breast cancer diagnosis is X-ray mammography. However, optical techniques such as trans-illumination and diffuse optical tomography imaging are challenging mammography. A mammogram is an X-ray formed from a quasi-point source that irradiates the breast and the transmitted X-rays are recorded by an image receptor. A region of reduced transmission can be a tumor, calcification, or normal fibroglandular tissue. A breast imaging system must have a sufficient spatial resolution to delineate the edges of fine structures in the breast and resolve structural details as small as $50\ \mu\text{m}$. Because the breast is sensitive to ionization radiation, which at least at high doses is known to cause breast cancer, it is desirable to use the lowest radiation dose compatible with excellent image quality. Mammography suffers from both a reliance on ionizing radiation and a large number of false positives, leading to unnecessary biopsies and surgical interventions. Furthermore, X-ray mammography is of limited diagnostic value for young women and dense breasts.

Mammography is used to detect a number of abnormalities, the two main ones being masses and calcifications. Calcifications are tiny mineral deposits within the breast tissue that appear as small white spots, as seen in Fig. 2. Calcifications are divided into two categories, macrocalcifications and microcalcifications. Macrocalcifications are coarse, larger calcium deposits that are often associated with benign fibrocystic change or with degenerative changes in the breasts, such as aging of the breast arteries, old injuries, or inflammation. Macrocalcification deposits are found in about 50% of women over the age of 50 and can also be associated with benign conditions that do not require a biopsy. Microcalcifications are tiny (less than 1/50 of an inch) specks of calcium in the breast. When many microcalcifications are clustered together they may indicate a small cancer. About 50% of the cancers detected by mammography and 90% of ductal carcinoma *in situ* cases are associated with a cluster of microcalcifications. The shape and arrangement of microcalcifications help determine the likelihood of cancer. However, only 17% of calcifications requiring biopsy are cancerous. Another important

change seen on a mammogram is the presence of a mass, which may occur with or without associated calcifications. Since a mass is any group of cells clustered together more densely than the surrounding tissue, a cyst or collection of fluid may appear as a mass on mammography. The difference between a solid mass and a cyst can often be shown with ultrasound. Diagnostic mammography may show that an abnormality has a high likelihood of being benign. Just like with calcifications, the size, shape, and edges of the mass help the radiologist in evaluating the likelihood of cancer. About 65% of all breast lesions that are evaluated with biopsy are found to be benign when microscopically evaluated.

The weakness of mammography is its decrease in accuracy of detecting breast cancer in radiographically dense breasts usually found in younger women. Tumor detection by mammography has a greater than 20% false negative rate in women younger than 50 years old (9000 cases per year) making mammography not clinically useful for women under 50. Greater than 65% density has shown to be a 4–6 fold increase in developing breast cancer, and a mammographically dense breast at 30 is probably also dense at 50+ years old [44]. Mammography alone cannot prove that an abnormal area is cancerous although some abnormalities are very characteristic of malignancy. If mammography raises a significant suspicion of cancer, tissue must be biopsied. Also, 10% of breast cancers go undiscovered with mammography (20,000 cases overall) and more than 50% of surgical biopsies are benign.

One of the most challenging areas to apply diffuse optical imaging of deep tissues is the human female breast [13,19,32,46]. Therefore, there is a need for quantitative noninvasive methods for chemotherapy monitoring. Furthermore, optical methods are free of risk and could therefore be used for continuous check-up during therapies, and for regular examinations of risk groups. Two optical imaging networks for evaluating breast cancer are described in brief below, including in-depth descriptions of a few promising projects.

2.1. European Network on Optical Mammography [37]

The European Network on Optical Mammography (OPTIMAMM) funded from December 2000 to May 2004 aimed to improve detection and clinical evaluation of breast cancer by assessing the diagnostic benefits gained from time-domain optical mammography as a novel noninvasive imaging modality. OPTIMAMM consisted of nine European partners from six European countries. OPTIMAMM's achievements have been performance of clinical studies by scanning laser pulse mammography to develop criteria for malignancy detection, investigation of a small number of patients by tomographic optical mammography and photoacoustic mammography and compare to scanning laser pulse mammography, and investigation of a small number of patients by contrast enhanced optical mammography to further characterize ambiguous breast lesions by vascular contrast agent (indocyanine green) kinetics within the lesion to compare directly to contrast agent kinetics as derived from MR study [34]. Additional accomplishments have been comparison of optical/photoacoustic mammography with conventional imaging modalities, determination of sensitivity and specificity of laser pulse mammography, exploration of methodological and technical improvements and their potential for detection and differentiation of lesions, development of refined, robust data processing algorithms (forward/inverse) and software for generation of low-noise, high-contrast optical mammograms, measurement of scattering and absorption properties of normal breast tissue, benign lesions and tumors (*in vivo* and *ex vivo*) at various wavelengths and creation of a database, and correlation of scattering and absorption properties with physiological and pathological tissue parameters [17].

The Physikalisch-Technische Bundesanstalt (PTB) and Charité Universitätsmedizin have each designed a scanning optical mammography, University College London has designed an optical tomography

instrument [21], and Universiteit Twente has built a photoacoustic mammography system [27]. Retrospective clinical studies have been performed at all locations. The Politecnico di Milano has developed a multi-wavelength time-resolved optical mammography system [35]. It includes seven wavelengths including some in the infrared (IR) waveband. The mammograph detection rate of cancer has been shown as 80%, cysts 82%, fibroadenomas 37%, and other 34% [41,42].

An initial mammograph designed by PTB was three wavelengths, two projections, scanned 1000–2000 positions, and took 3–5 minutes to scan. A newer generation prototype designed with off-sets for optical mammographs to be recorded with four wavelengths (total hemoglobin, oxygen hemoglobin saturation + water + lipid, scatter power). The absorption coefficients are derived from photon counts and the visibility of the tumor is analyzed using a visibility score of 0–5, where 0 signifies nothing was detected, 5 signifies contrast that dominates image and anything above 2 is considered detected. In comparison to X-ray mammography, malignant lesions visible in 2 projections was 72%, visible in one projection was 18%, and not visible was 10%. Tumor physiological parameters and the diffraction of photon density waves were also modeled. The PTB optical scanning mammography project with OPTIMAMM [10] is described below.

Laser pulse mammography aims at improving detection and differentiation of breast tumors by yielding additional information to X-ray mammography. The methods and instrumentation have been developed to image breast tissue *in vivo* by using pulsed laser radiation in combination with time-resolved transmission measurements and to estimate optical properties of different types of breast tissue and tumors.

The breast being slightly compressed between two parallel glass plates is trans-illuminated by short NIR laser pulses (670 and 785 nm) at a large number of scan positions. Multiple scattering of photons inside the breast tissue causes broadening of the laser pulses. At each scan position the number and times of flight of photons leaving the tissue and arriving at the detector are recorded. By analyzing the shape of the transmitted pulses and by applying physical models, optical mammograms are generated and absorption and scattering properties of breast tissue are estimated.

A single-channel apparatus has been developed for detecting optical mammograms at two optical wavelengths. Optical mammograms have been recorded during examinations on patients within a pre-clinical study performed at the Robert Roessle Hospital, Charité, Humboldt University of Berlin. Tumors and cysts have been imaged based on the analysis of photons with selected times of flight through the tissue. Absorption and reduced scattering coefficients of tumor tissue have been measured *in vivo* using diffusion theory of photon transport in highly scattering media. Blood oxygen saturation of normal tissue and tumors from optical mammograms were recorded at two optical wavelengths [18]. Benign and malignant alterations of tissue have been differentiated based on blood content and oxygen saturation. Fig. 3 shows an optical mammography image with mathematical analysis of the optical properties and localization (Table 1) of the tumor centroid. Laser pulse mammography is a noninvasive method to regularly examine risk groups.

2.2. NTROI Network on multi-dimensional diffuse optical imaging [44]

A United States collaboration of scientists, funded by the National Cancer Institute, uses multi-dimensional diffuse optical imaging (MD-DOI) to study breast cancer detection, characterization, and management. DOI is a noninvasive optical technique that employs NIR light to quantitatively characterize the optical properties of thick tissues. Quantitative DOI methods employing time- or frequency-domain photon migration technologies have only recently been used for breast imaging. MD-DOI employs broadband technology in spectral (650–1000 nm) and temporal (1 GHz) domains in order to separate

Table 1
Scattering and absorption coefficients (mm^{-1}) of the tissue background and tumor, estimated for one patient with invasive ductal carcinoma, using random walk methodology [10]. Lateral dimensions of the tumor D were estimated for assumed tumor depths Z

		View	670 nm		785 nm	
			μ'_s	μ_a	μ'_s	μ_a
Background		cc	1.61	0.0042	1.25	0.0033
		ml	1.57	0.0045	1.21	0.0034
Tumor	$Z = 8 \text{ mm}/D = 15 \text{ mm}$	cc	1.47	0.0097	1.08	0.0075
	$Z = 8 \text{ mm}/D = 15 \text{ mm}$	ml	1.81	0.0102	1.71	0.0064

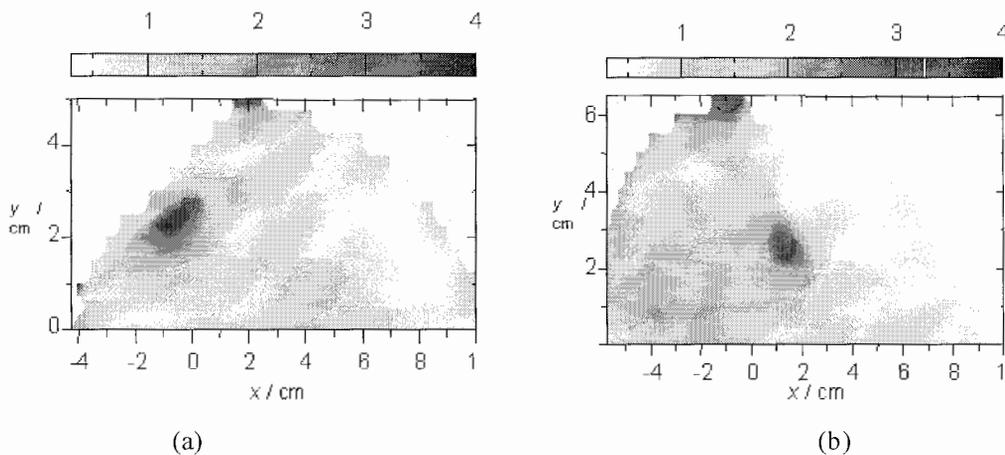


Fig. 3. (a) Craniocaudal view and (b) mediolateral view of 2D optical images (reciprocal normalized photon counts) of the breast with invasive ductal carcinoma [17] (printed with permission from Dr. Grosenick, Physikalisch-Technische Bundesanstalt, Berlin, Germany).

absorption from scattering and quantify multiple molecular probes based on absorption or fluorescence contrast. Additional dimensionality is provided by integrating and co-registering MD-DOI functional information with MRI and X-ray mammography.

A group at Dartmouth has begun a project that involves spatial (tomography) optimization integrated with MRI [36]. A second project with planar tomography with mammography and MRI is a collaborative effort between the University of Pennsylvania and Massachusetts General Hospital [12,25]. This is an integration of frequency domain and broadband approaches; allowing for many sources and detectors and functional sectioning. Another collaborative project between the University of California, Irvine and the University of California, San Francisco involves spectral (functional) optimization. The project is described below and involves a handheld optical scanner as a quantitative spectroscopy device. The patient reclines while the scanner is moved along the breast. The complete absorption and scattering spectrum are recovered in every position of the scan. It involves spectroscopy and tomography integrated with MRI [11,20,28]. The target applications of these devices include monitoring neoadjuvant chemotherapy and screening mammographically dense or high risk women.

Tromberg et al. have developed an optical scanner which takes line scans of tissue [45]. In the absorption spectra, there is a lipid peak at 920 nm and a water peak at 980 nm. There are many properties of tissues that change with disease. These include blood oxygen saturation, total blood volume, lipid content, as well as cells, matrix, and vessels. The water to lipid ratio changes between

normal tissue and lesion (i.e., invasive ductal carcinoma). The lipid content is lower in the tumor, causing a decrease in oxygen saturation, an increase in hemoglobin, and an increase in water content. The tissue optical indices measured in this study were tissue hemoglobin oxygen saturation and total hemoglobin content; however, other parameters will be studied in the future including metabolism, angiogenesis, and extracellular matrix. The goal of the study was to develop tissue optical indices to improve contrast (e.g. normal vs. tumor; malignant vs. benign), diagnostic power (e.g. sensitivity, specificity) and insight into fundamental physiologic and metabolic processes.

In the study comparing malignant vs. normal tissue, linescans were performed on patients with invasive ductal carcinoma. Significant differences were found between the tumor and normal tissue with regards to lipid content, water content, deoxygenated saturation, oxygen saturation, and total hemoglobin concentration. The lipid and water contents were very good indicators, but the strongest indicator by power was the deoxygenated tissue saturation. Significant differences were not observed with the oxygen saturation and scatter power.

Disease prediction is difficult because of overlap in tumor and normal values. There was no change seen when more prediction values were added to deoxygenated tissue saturation because tissue optical properties change with tissue metabolism which is age dependent. Also, contrast may depend upon other factors. Another experiment ran additional indices showed that there was a decrease in normal tissue optical indices with age and that tumor tissue optical indices were greater than normal. The tumor tissue optical indices increased until age 50 then decreased showing that tissue optical indices have low sensitivity. The best parameters for younger people include lipid content, deoxygenated blood saturation, and oxygen blood saturation. For older people, deoxygenated blood saturation, and oxygen blood saturation are the best indicators. This linescan device can be used to monitor neoadjuvant chemotherapy and screen mammographically dense or high risk women.

3. Evaluating coronary arteries and plaque microstructure

Selective coronary angiography has remained the clinical “gold standard” for evaluating the coronary anatomy and defining epicardial coronary artery disease. While conventional invasive coronary angiography provides exceptional spatial resolution and a general map of the coronary system, it is expensive and has a small but definite risk of complications. In addition, it requires either a brief hospitalization or observation period after the procedure. The replacement of even a fraction of these procedures with noninvasive imaging modalities would constitute an important advance in the care of patients suspected of having coronary artery disease. Currently, a number of imaging modalities are used for diagnosing epicardial coronary artery disease. Most identify the luminal diameter or stenosis, wall thickness, and plaque volume [29].

Two X-ray techniques used to evaluate the coronary anatomy are electron beam computed tomography (EBCT) and multi-detector spiral CT (MDCT). EBCT is a highly sensitive noninvasive imaging modality used to detect and quantify coronary artery calcium, which occurs exclusively in atherosclerotic arteries and is absent in the normal vessel. Calcium is quantified with EBCT by screening selected asymptomatic individuals at high risk for developing clinically significant coronary artery disease as well as for the diagnosis of obstructive coronary artery disease in symptomatic individuals. The presence and extent of coronary calcium seems to correlate with overall atherosclerotic plaque burden and might indirectly suggest an increased frequency of the presence of vulnerable plaque. However, the role of calcification in the biology of the vulnerable plaque is unclear, and there is no established consensus regarding the exact impact of calcification on the process or risk of plaque rupture [29].

MDCT scanners have recently been used for coronary artery scanning. The increased scan speed results in improved spatial and temporal resolution. The resolution of MDCT is greater than 500 μm . However, the image quality has been insufficient in a substantial number of cases. Calcifications often hinder evaluation of severely diseased coronary segments, and the coronary arteries are frequently affected by motion artifacts. In addition, it has been observed that the patient's heart rate during the scan critically influences the image quality. Therefore, heart rate control is one of the most important factors in better image acquisition and MDCT requires pre-medication with beta-blockers [29].

One study evaluated the diagnostic accuracy of MDCT angiography in determining significant coronary artery stenoses ($> 50\%$ lumen diameter narrowing in angiography) and occlusions compared with conventional invasive angiography (CAG). Invasive CAG showed that 35 out of 58 patients had significant coronary artery stenoses. MDCT angiography correctly classified 30 out of 35 patients having at least one coronary stenosis (sensitivity 85.7%, specificity 91.3%, positive predictive value 93.8%, and negative predictive value 80.8%). By analyzing each coronary artery, CAG found 62 stenotic coronary arteries in the 229 coronary arteries evaluated. MDCT correctly detected 50 out of 62 stenotic coronary arteries and an absence of stenosis was correctly identified in 156 out of 167 normal coronary arteries (sensitivity 80.6%, specificity 93.4%, positive predictive value 81.9%, and negative predictive value 92.8%) [29]. In another study, MDCT was shown to detect plaque types in nonstenotic major coronary arteries from 22 individuals, compared with intravenous ultrasound (IVUS)-observed plaque types, in a blind study. Although this modality has been shown to characterize calcified plaque well, with a sensitivity and specificity of more than 90%, detection of exclusively non-calcified plaque was still unreliable, yielding only 53% sensitivity, particularly in the distal part of the vessel [31]. The noninvasive technique of MDCT for examining the coronary artery appears to be a useful method for detecting coronary artery stenoses with a high accuracy particularly with the proximal portion and large arteries.

Magnetic resonance imaging (MRI) and IVUS are two noninvasive, non-ionizing methods currently used for detecting and imaging plaques. MRI has a resolution of 100 μm but cannot discriminate between plaque components [7]. IVUS is an invasive approach that provides tomographic visualization of vessel wall structures *in vivo* and allows more precise assessments of lumen narrowing and vessel remodeling. IVUS can determine the reference vessel diameter and location of calcium and confirm stent deployment. However, its low resolution also of 100 μm does not depict the fine structures of plaques. Several retrospective IVUS studies have shown the compensatory enlargement of a vessel as one of the characteristic features of culprit lesions in acute coronary syndrome [31]. A bright IVUS signal from calcifications makes assessment of neighboring tissue difficult due to saturation artifact and attenuation of US by calcifications causes shadowing, which impairs visualization of deeper vessel wall structures and prevents accurate measurement of the depth of calcification [22].

There are two optical imaging techniques popular for evaluating coronary anatomy: spectroscopy and intravascular OCT. Spectroscopy allows accurate analysis of the histological composition of the vessel wall by analyzing the emission and absorption of different wavelengths of light for various chemical components. Currently, two approaches using this technique, Raman spectroscopy and NIR spectroscopy, have been under investigation as alternative modalities for detecting vulnerable plaque in *ex vivo* conditions. Compared with IVUS, Raman spectroscopy provides better detection of cholesterol, as observed by histochemistry in the plaque. Also, in a current *ex vivo* study examining human atherosclerotic plaques, NIR spectroscopy identified the lipid core with 90% sensitivity and 93% specificity. Furthermore, it achieved sensitivities and specificities ranging from 77% to 93% for detection of the fibrous cap and inflammation within the plaque [31]. Another minimally invasive technique is OCT.

OCT is an imaging technique that uses optical echoes of an infrared light source directed at the vessel wall to create high-resolution, tomographic images of a vessel. OCT is an optical analog of time-of-flight

B-mode US because it measures the amplitude of backscattered light returning from a sample as a function of delay [22]. The resolution of OCT ranges from 2 to 10 μm and allows superior depiction of the thin fibrous caps responsible for plaque vulnerability, as well as improved visualization of plaque components, such as regions of lipid-rich plaque. Current limitations of OCT include poor tissue penetration and blood interference. Therefore, this modality requires saline injection with or without proximal vessel balloon occlusion to displace blood to avoid reduction of image quality. However, although the current penetration depth is limited to approximately 2 mm, this depth is sufficient to assess plaque vulnerability because it is predominantly characterized by the morphology of the luminal surface [31]. OCT has the potential for use at the bedside to visualize microscopic plaque structures, and two projects are described below.

3.1. Evaluating plaque microstructure with intracoronary OCT

The ability of OCT to visualize microscopic plaque structures *in situ* can help clarify the intrinsic morphologic features that determine plaque vulnerability and monitor structural changes that occur with plaque regression after genetic and/or pharmacologic intervention. OCT is also able to identify and study vulnerable plaques for which the relevant morphologic features including a thin fibrous cap (less than 65 μm), a large lipid pool, and activated macrophages near the fibrous cap are primarily within 500 μm of the luminal surface. When monitoring stent deployment, the depth of penetration within a vessel wall does not present a limitation [7]. The benefits of OCT relative to histology include no excessive tissue handling, a more realistic geometry and no structural artifacts. Also, OCT imaging can be performed *in vivo*, making it possible to study disease progression and the effect of therapeutic treatments in living patients [9].

Screening long vessel segments is difficult with OCT because clear visualization of the entire vessel lumen is possible only by displacing blood with saline. In order to remove the blood from the field of view, OCT images have been recorded during intermittent 8–10 cc saline flushes through the guide catheter. This flush has added 5–10 min to the imaging procedure [22].

A study by Bouma and Tearney showed the sensitivity and specificity of OCT to be respectively: 89%, 98% for fibrous plaques; 97%, 94% for fibrocalcific plaques; and 84%, 94% for lipid rich fibroatheromas. The precise locations of the struts with respect to the vessel wall were more readily apparent with OCT than IVUS [7].

The results of a study by Jang et al. [22] showed that the layered structure of the normal coronary wall was absent in all OCT images of atherosclerotic plaques; calcifications within plaques were identified by the presence of well-delineated, low backscattered heterogeneous regions; and lipid pools were less well-delineated than calcifications and demonstrated decreased signal density and more heterogeneous backscattering than fibrous plaques. The strong contrast between lipid-rich cores and fibrous regions allowed fibrous caps to be easily identified. The maximum penetration depth of OCT measured 1.25 mm and IVUS measured 5 mm. The OCT axial resolution was $13 \pm 3 \mu\text{m}$ compared to the IVUS axial resolution of $98 \pm 19 \mu\text{m}$. OCT images allowed improved evaluation of the extent of calcifications within plaques and visualization of plaque microstructure adjacent to calcifications. The experiments showed potential for OCT to measure cap thickness at a greater degree of precision than IVUS. OCT also identified two additional plaques with similar characteristics that were not definitely identified by IVUS. All fibrous plaques, calcifications, and echolucent regions identified by IVUS were seen in OCT images. OCT images provided additional morphologic information, which could be used to improve plaque characterization. The high resolution of OCT facilitated the identification of intimal hyperplasia,

the internal and external elastic laminae, and echolucent regions, architecture difficult to discern with IVUS. However, the contrast between calcifications and the surrounding vessel wall was often higher in IVUS images. Lack of saturation and shadowing artifacts in OCT images of calcium deposits allowed calcium within the vessel wall to be located and adjacent tissues to be seen. High resolution permitted measurement of thin fibrous caps (less than $65\ \mu\text{m}$) thought to be present in a majority of vulnerable coronary plaques [22].

OCT is able to image macrophages at the border of lipid pool and fibrous cap junction due to a different OCT signal because of different refractive indices. A clinical study was performed of 85 patients undergoing intracoronary stent procedures with culprit/remote lesions [43]. IVUS did not resolve as many features as OCT. OCT showed that cap macrophage density was higher in acute clinical syndromes in both remote and culprit sites and no significant difference was seen between remote and culprit densities for each clinical subgroup. If there was a high concentration of macrophages in the culprit site, there was a high concentration of macrophages in the remote site. The cap macrophage density was higher at rupture sites than the remainder of the cap and all acute syndrome culprit phages. The surface cap macrophage density was more predictive of clinical syndrome than subsurface macrophage density at culprit, but not remote sites. Fibrous lesions had a higher density of macrophages in acute syndromes. These were the first measurements of key plaque features in living patients. The next generation of OCT technology will include improvements in sensitivity and speed, improved catheters, and will enable comprehensive coronary screening with minimal saline. Taking optical frequency domain images will increase the speed from 4 to 72 frames per second. OCT can also be applied to volumetric microscopic screening for cardiology, ophthalmology, and gastroenterology [43].

3.2. Intravascular OCT: Poised to enter the Japanese cardiology market [38]

LightLab Imaging, Inc. developed an intravascular OCT system (Fig. 4) and was then acquired by Goodman, a Japanese company, which has been marketing the machine in Japan. Japan has a heavy reliance on catheter-based diagnostic technologies. Intravenous ultrasound (IVUS) is used in a large percentage of cases (35–75% in Japan vs. 3–5% in US), and the importance of diagnostic tools is reflected in reimbursements. In Japan, long, complicated minimally invasive procedures are preferred over procedures such as open-heart surgery. The LightLab Imaging system is high resolution ($16\ \mu\text{m}$ (axial) \times $25\ \mu\text{m}$ (lateral)) with a rotary (circumferential) format allowing for 15 frames/sec or 256 lines/frame (approximately 4000 lines/sec) and a 1–5 mm/s pullback rate of the catheter. The compact, digital system contains a digital signal processor, digital video and CD storage, and a photo printer. Key components of the system include a fiber-optic interferometer, 50–60 mm bandwidths, and two channels for polarization desensitizing. The imagewire is a rotating optic fiber that has to bend through radiuses as large as 8 mm. It is 2–4 mm in diameter, has a fused fiber tip that is $125\ \mu\text{m}$ and a 2 mm depth of field. The accuracy of measured diameters exceeds $\pm 2.5\%$. Plaques visualized with OCT can be characterized as normal, highly lipid, or fibro-fatty.

The first generation OCT coronary delivery system had an occlusion balloon, which was very low pressure and over-sized. It allowed a $250\ \mu\text{m}$ view of OCT by itself allowing for a short snapshot. There is a need to use this as a surveying and diagnostic tool to replace some of the functions of ultrasound. A recently designed occlusion balloon provides a bolus flush that allows 35 mm longitudinal scans, tip and back-directed side-hole flushing and has a low profile tip (0.9 mm OD) for crossing tight lesions. It can operate over a large range of low pressures and perform in a large range of diameter sizes. The balloon causes only a mild loss of endothelium, no damage to media and an occasional minor spasm



Fig. 4. LightLab Imaging, Inc. bedside intravascular OCT machine poised to enter the Japanese cardiology market (courtesy of LightLab Imaging).

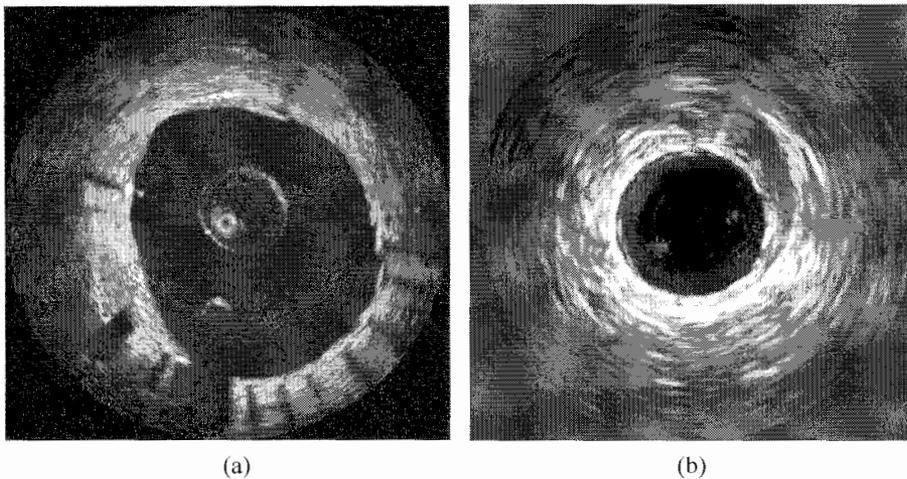


Fig. 5. Tacrolimus six months in-stent comparing (a) optical coherence tomography image to (b) intravascular ultrasound image (courtesy of LightLab Imaging).

under occlusion times of 30–60 seconds. There are 50 OCT systems and 700 disposable ImageWires produced to date. Pilot clinical studies have been performed of 40 percutaneous coronary interventions (PCI) cases, 27 OCT/IVUS comparisons, and 10 OCT/angiography comparisons. Figure 5 shows typical examples of an OCT image compared to an IVUS image. The results have shown that stent placement precision is better with OCT than IVUS. There is great potential for using OCT to study the quality of stent placement.

4. Molecular and functional optical imaging

In vivo imaging of molecular events in small animals has great potential to impact basic science and drug development. It creates also the basis for further development into clinical uses. Fluorescence imaging is emerging as an important alternative because of its operational simplicity, safety, and cost-effectiveness. Fluorescence imaging has recently become particularly interesting because of advances in fluorescent probe technology [1,3,4,16], including targeted fluorochromes [2] as well as fluorescent “switches” sensitive to specific biochemical events. Techniques include fluorescence lifetime imaging (FLI) [15], fluorescence reflectance imaging (FRI), and fluorescence molecular tomography (FMT) [33, 39].

FMT is an evolving technique for quantitative three-dimensional imaging of fluorescence *in vivo* that offers the promise of noninvasively quantifying and visualizing specific molecular activity in living subjects in three dimensions. Fluorescence lifetime imaging (FLI) is a valuable tool for localizing diseased tissues and investigating their functional status because of its decreased dependence on transient changes in the probe concentration and high sensitivity to its local environment, depending on the molecular probe used. All the above mentioned methods have been demonstrated by groups led by Achilefu, Ntziachristos, and Gannot. These groups work on optical imaging of tumors targeted by fluorescence markers. This enhanced method of minimally invasive optical imaging has the potential to become a good detection, screening, and monitoring method avoiding the side effects of X-ray radiation.

5. Ultrasound-enhanced optical imaging techniques

5.1. Acousto-optic imaging [6]

Acousto-optic (AO) imaging is a technique that is able to reveal optical properties in the millimeter range inside scattering media by tagging the photon paths with an ultrasonic beam. The aim of this research is to use the ultrasound to get high resolution and optical properties to get high contrast. The intersection of US and optical field create virtual source of tagged photons. To increase both the contrast and the resolution of the AO images, A. Boccara has used the nonlinear response of the speckle modulation. Variation of the second-harmonic signal as the square of the ultrasonic amplitude has been found, and strong reduction of the tagged zone size has been demonstrated.

Obtaining an optical image with a sufficient resolution of a structure deeply buried in biological tissues is a delicate operation. Solving the inverse problem remains extremely difficult due to the heterogeneity of the optical properties of biological tissues. In the case of breast imaging, it is not possible at this time to spatially resolve tumors of diameter less than roughly 1 cm. This approach aims at overcoming this limitation by “tagging” the photons along their paths by a focused US beam. Therefore, the tissue under study is illuminated by a single frequency laser beam of very long coherence length, and also by a focused US beam in order to periodically modify the phase of the optical waves in the focal zone of the US beam. This approach tends to reveal optical contrasts with a spatial resolution linked to the acoustic resolution. *Ex vivo* experiments have been successful but *in vivo* experiments have to account for blood flow and body movements which decorrelate the speckle field. Another limitation is time because one measurement has to be done in less than 1 ms or contrast will be lost. Experimental schemes able to reach the shot noise limit detection level have been developed and using a large number of speckle grains can overcome the difficulty of low frequency noises and high frequency decorrelation by blood flow. To increase optical and acoustical power, or increase the number of usable speckle grains, one can use many

CCD or CMOS arrays or use photorefractive crystals. This can improve the number of speckle grains from one million to one billion.

The resolution achieved is of the order of 1 mm^3 in a few cm thick samples.

5.2. *Ultrasound-aided high-resolution biophotonic imaging [47]*

With US-modulated optical tomography, a focused US wave encodes diffuse laser light in scattering biological tissue. In photo-acoustic (thermo-acoustic) tomography, a low-energy laser or radiofrequency (RF) pulse induces US waves in biological tissue due to thermoelastic expansion. Spectroscopic oblique-incidence reflectometry can detect skin cancers with 95% accuracy based on functional hemoglobin parameters and cell nuclear size. Unfortunately, electromagnetic (EM) waves in the non-ionizing spectral region do not penetrate biological tissue in straight paths. Consequently, high-resolution diffuse optical tomography (DOT) based on non-ionizing EM waves alone is limited to superficial tissue imaging. Ultrasound has good resolution but poor contrast in early-stage tumors and strong speckle artifacts as well, but DOT has excellent contrast. Wang has developed US-mediated imaging modalities by combining EM and US waves synergistically.

Mueller matrices provide a complete characterization of the optical polarization properties of biological tissue. The separation of benign and malignant lesions by optical contrast in the Mueller matrix leads to $10 \mu\text{m}$ resolution, $\sim 1 \text{ mm}$ imaging depth, the detection of birefringence (density of collagen), orientation (direction of collagen), diattenuation (property of collagen), and orientation of muscle fibers versus depth in rat heart septum. Ultrasound modulated optical tomography (UOT) has its intensity of light modulated by US shifts, parallel lock-in detection of speckles, and instantaneous frequency along the US axis. The experimental configuration has two CCD cameras and an ultrasonic transducer. Laser-induced photoacoustic tomography (PAT) uses a laser pulse ($< \text{ANSI limit}$) that generates local heating (mK), US waves are emitted and detected. The inverse-source problem is solved to detect absorption changes and this technique can image (up to 8 mm deep) if contrast (e.g., indocyanine green) is used. The technique is high resolution $60 \mu\text{m}$, high sensitivity (fmol), resolution is scalable, and speckle free. RF-induced thermo acoustic tomography (TAT) allows for deep penetration, 0.5 mm resolution, no speckle artifacts, and monitoring of treatment. Combining US and EM waves provides improved spatial resolution compared to optical or RF imaging and new contrast mechanisms compared to US imaging. This is because spatial resolution is determined and scalable with US parameters. Contrast is provided by EM properties so deep (cm) that tissue imaging can be achieved and speckle artifacts do not exist. Functional imaging can be accomplished with endogenous agents, non-ionizing radiation, and costs are comparable to those of US systems.

6. Multimodality techniques

Even with innovations in imaging, no single imaging modality can provide the entire picture of tissue profiling, thus making the fusion of anatomical and functional imaging vital. Structural imaging systems have been fused with molecular imaging technologies, and such mergers include positron emission tomography (PET)/CT, single photon emission computed tomography (SPECT)/CT, optical fluorescence imaging, MRI, functional MRI, and magnetic resonance spectroscopy using molecular imaging probes. Nuclear imaging benefits greatly from molecular probes originating from biochemistry and pharmaceuticals with relatively poor spatial resolution. Other imaging approaches like CT suffer from a lack of specificity, optical techniques suffer from low signal penetration through tissue, and

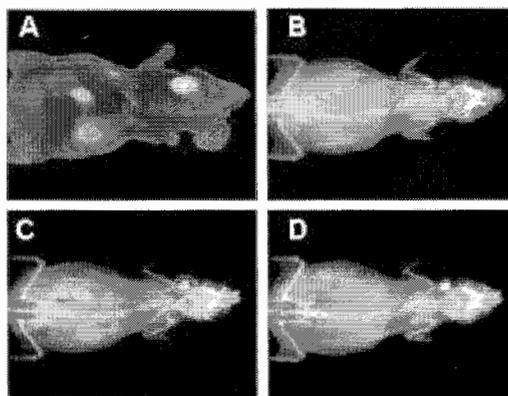


Fig. 6. Laboratory mouse sequentially imaged in (A) fluorescence mode (B) radiography mode. Images were then (C) merged and (D) contrasted and merged (courtesy of Kodak medical imaging unit and Dr. Achilefu from Washington University, St. Louis, OH).

MRI techniques suffer from low signal strength from specific molecular constituents [24]. Therefore, combining noninvasive imaging techniques can provide the best analysis.

A new commercial system allows for multiple labels and imaging modes, including optical and X-ray methods. The KODAK Image Station digital imaging system allows for NIR fluorochromes, visible fluorochromes, chemi- and bio-luminescence, visible stains, and isotopes and X-rays to be used together. Figure 6 shows a laboratory mouse in fluorescence mode (A) and radiography mode (B) using the X-Ray imaging module accessory. Images were then merged (C) and contrasted and merged (D), allowing precise co-registration of the optical molecular imaging signal with anatomical radiograph. The cooled CCD technology, range of illumination and emission wavelengths, and proprietary chamber design provide more versatility than other imaging systems. Long wavelength, full field illumination (green to NIR) enables light penetration into tissue to excite target fluorochromes both *in vivo* or in extracted organs and tissues. UV illumination and laser based scanning systems are extremely limited in this mode of imaging. The choice of illumination and emission wavelengths increases signals for most fluorochromes and significantly decreases background fluorescence from most gel, blot, plate, and tissue materials when compared to broad UV or laser excitation. The system's construction utilizes highly qualified, low fluorescent materials to provide limited auto-fluorescence for very clean imaging throughout the visible and NIR spectrum. The closed optical path image chamber keeps the optical path clear of contaminating fluorochromes for continuous low background imaging. The full field CCD imaging can produce images in seconds while laser scanning of similar fields take from 5 to 30 minutes. The system offers wide angle (20 cm) viewing of larger samples, continuously variable zoom to 2 cm viewing providing detailed 20 $\mu\text{m}/\text{pixel}$, high resolution imaging. The system has been used for many applications including *in vivo* targeting of underglycosylated MUC-1 tumor antigen using a multimodal imaging probe [30], and the feasibility of *in vivo* multichannel optical imaging of gene expression in mice [26].

7. Summary

The fourth Inter-Institute Workshop on Diagnostic Optical Imaging and Spectroscopy from Bench to Bedside allowed diagnostic optical imaging to be discussed in-depth. It enabled us to understand the potential as well as limitations of these proposed methods. At this time, some optical imaging

techniques can compete with X-ray and other noninvasive techniques. Other optical imaging techniques are being combined with other noninvasive techniques to provide better resolution, including acousto-optic imaging and ultrasound-aided biophotonic imaging. X-ray and optical techniques can sometimes be combined to exploit the advantages of both methods for the best contrast, resolution, and functionality expressed in the same images and sometimes used as complimentary methods registered together after taking the images separately with different machines. Further progress in research and development may increase the potential of optical methods being introduced routinely into the medical area for the sake of better diagnostics and treatment in healthcare.

References

- [1] S. Achilefu and R.B. Dorshow, Dynamic and continuous monitoring of renal and hepatic functions with exogenous markers, *Contrast Agents* **222** (2002), 31–72.
- [2] S. Achilefu et al., Novel receptor-targeted fluorescent contrast agents for *in vivo* tumor imaging, *Investigative Radiology* **35**(8) (2000), 479–485.
- [3] S. Achilefu et al., Synthesis, *in vitro* receptor binding, and *in vivo* evaluation of fluorescein and carbocyanine peptide-based optical contrast agents, *Journal of Medicinal Chemistry* **45**(10) (2002), 2003–2015.
- [4] S. Achilefu et al., Development of contrast effectors for optical and multimodal imaging of tumors, *Abstracts of Papers of the American Chemical Society* **228** (2004), U824–U824.
- [5] S. Apisarnthanarax and K.S.C. Chao, REVIEW: Current Imaging Paradigms in Radiation Oncology, *Radiation Research* **163** (2005), 1–25.
- [6] A. Boccara, Sensitivity and resolution in acousto-optic imaging: how far can we go? in: *Optical Diagnostic Imaging from Bench to Bedside*, National Institutes of Health, Bethesda, MD, 2004.
- [7] B.E. Bouma and G.J. Tearny, Special Review: Clinical Imaging with Optical Coherence Tomography, *Acad. Radiol.* **9** (2002), 942–953.
- [8] B. Chance, *Photon Migration in Tissues*, New York: Plenum Press, 1989.
- [9] A.H. Chau et al., Mechanical Analysis of Atherosclerotic Plaques Based on Optical Coherence Tomography, *Annals of Biomedical Engineering* **32**(11) (2004), 1494–1503.
- [10] V. Chernomordik et al., Quantification of optical properties of a breast tumor using random walk theory, *J. Biomed. Optics* **7**(1) (2002), 80–87.
- [11] D.J. Cuccia et al., *In vivo* quantification of optical contrast agent dynamics in rat tumors by use of diffuse optical spectroscopy with magnetic resonance imaging coregistration, *Appl. Opt.* **42**(16) (2003), 2940–2950.
- [12] J.P. Culver et al., Three-dimensional diffuse optical tomography in the parallel plane transmission geometry: evaluation of a hybrid frequency domain/continuous wave clinical system for breast imaging, *Med. Phys.* **30**(2) (2003), 235–247.
- [13] M.A. Franceschini et al., *Frequency-domain techniques enhance optical mammography: Initial clinical results*, in National Academy of Sciences of the United States of America, 1997.
- [14] A.H. Gandjbakhche, R. Nossal and R.F. Bonner, Resolution limits for optical transillumination of abnormalities deeply embedded in tissues, *Med. Phys.* **21** (1994), 185–191.
- [15] I. Gannot et al., Functional optical detection based on pH dependent fluorescence lifetime, *Lasers in Surgery and Medicine* **35**(5) (2004), 342–348.
- [16] E.E. Graves, R. Weissleder and V. Ntziachristos, Fluorescence molecular imaging of small animal tumor models, *Current Molecular Medicine* **4**(4) (2004), 419–430.
- [17] D. Grosenick et al., Time-domain optical mammography: initial clinical results on detection and characterization of breast tumors, *Appl. Opt.* **42**(3) (2003), 170–186.
- [18] D. Grosenick et al., Concentration and oxygen saturation of haemoglobin of 50 breast tumours determined by time-domain optical mammography, *Phys. Med. Biol.* **49**(7) (2004), 1165–1181.
- [19] D. Grosenick et al., Development of a time-domain optical mammograph and first *in vivo* applications, *J. Biomed. Optics* **1** (1999), 342–355.
- [20] G. Gulsen et al., Congruent MRI and near-infrared spectroscopy for functional and structural imaging of tumors, *Technol. Cancer Res. Treat.* **1**(6) (2002), 497–505.
- [21] J.C. Hebden et al., Monitoring recovery after laser surgery of the breast with optical tomography: a case study, *Applied Optics* **44** (2005), 1898–1904.
- [22] I.-K. Jang et al., Visualization of Coronary Atherosclerotic Plaques in Patients Using Optical Coherence Tomography: Comparison With Intravascular Ultrasound, *J. Amer. Coll. Cardiology* **39**(4) (2002), 604–609.
- [23] F.F. Jobsis et al., Reflectance Spectrophotometry of Cytochrome aa 3 *in vivo*, *J. Appl. Physiol.* **43** (1977), 858–872.

- [24] E.E. Kim, Targeted Molecular Imaging, *Korean J. Radiol* **4**(4) (2003), 201–210.
- [25] A. Li et al., Reconstructing chromosphere concentration images directly by continuous-wave diffuse optical tomography, *Optics Letters* **29**(3) (2004), 256–258.
- [26] U. Mahmood et al., Feasibility of *in vivo* multichannel optical imaging of gene expression: experimental study in mice, *Radiology* **224**(2) (2002), 446–451.
- [27] S. Manohar et al., Photoacoustic mammography laboratory prototype: imaging of breast tissue phantoms, *J. Biomed. Optics* **9**(6) (2004), 1172–1181.
- [28] S. Merritt et al., Coregistration of diffuse optical spectroscopy and magnetic resonance imaging in a rat tumor model, *Appl. Opt.* **42**(16) (2003), 2951–2959.
- [29] J.-Y. Moon et al., The Utility of Multi-detector Row Spiral CT for Detection of Coronary Artery Stenoses, *Yonsei Medical Journal* **46**(1) (2005), 86–94.
- [30] A. Moore et al., *In Vivo* Targeting of Underglycosylated MUC-1 tumor antigen using a multimodal imaging probe, *Cancer Res.* **64** (2004), 1821–1827.
- [31] M. Nakamura, D.P. Lee and A.C. Yeung, Identification and Treatment of Vulnerable Plaque, *Rev. Cardiovasc. Med.* **5**(2) (2004), S22–S33.
- [32] S. Nioka et al., Optical imaging of human breast cancer, *Adv. Exp. Med. Biol.* **361** (1994), 171–179.
- [33] V. Ntziachristos et al., Visualization of antitumor treatment by means of fluorescence molecular tomography with an annexin V-Cy5.5 conjugate, *Proceedings of the National Academy of Sciences of the United States of America* **101**(33) (2004), 12294–12299.
- [34] V. Ntziachristos et al., Concurrent MRI and diffuse optical tomography of breast cancer after indocyanine green enhancement, *Proc. Natl Acad. Sci.* **97** (2000), 2767–2772.
- [35] A. Pifferi et al., Four-wavelength time-resolved optical mammography in the 680-980-nm range, *Opt. Lett.* **28** (13) (2003), 1138–1140.
- [36] B.W. Pogue et al., Hemoglobin imaging with hybrid magnetic resonance and near-infrared diffuse tomography, *Adv. Exp. Med. Biol.* **530** (2003), 215–224.
- [37] H.H. Rinneberg, The European Network on Optical Mammography (OPTIMAMM): experiences, major results and outlook, in: *Optical Diagnostic Imaging from Bench to Bedside*, National Institutes of Health, Bethesda, MD, 2004.
- [38] J.M. Schmitt, Intravascular OCT: poised to enter the Japanese cardiology market, in: *Optical Diagnostic Imaging from Bench to Bedside*, National Institutes of Health, Bethesda, MD, 2004.
- [39] R.B. Schulz, J. Ripoll and V. Ntziachristos, Experimental fluorescence tomography of tissues with noncontact measurements, *Ieee Transactions on Medical Imaging* **23**(4) (2004), 492–500.
- [40] J. Seibert and J.M. Boone, X-Ray Imaging Physics for Nuclear Medicine Technologists. Part 2: X-Ray Interactions and Image Formation, *J. Nucl. Med. Technol.* **33** (2005), 3–18.
- [41] L. Spinelli et al., Bulk optical properties and tissue components in the female breast from multiwavelength time-resolved optical mammography, *J. Biomed. Opt.* **9**(6) (2004), 1137–1142.
- [42] P. Taroni et al., Clinical trial of time-resolved scanning optical mammography at 4 wavelengths between 683 and 975 nm, *J. Biomed. Opt.* **9**(3) (2004), 464–473.
- [43] G.J. Tearney, Evaluating plaque microstructure in patients by intracoronary optical coherence tomography, in: *Optical Diagnostic Imaging from Bench to Bedside*, National Institutes of Health, Bethesda, MD, 2004.
- [44] B.J. Tromberg, Multi-dimensional diffuse optical imaging in breast cancer, in: *Optical Diagnostic Imaging from Bench to Bedside*, National Institutes of Health, Bethesda, MD, 2004.
- [45] B.J. Tromberg, Optical indices for detecting breast cancer, in: *Optical Diagnostic Imaging from Bench to Bedside*, National Institutes of Health, Bethesda, MD, 2004.
- [46] T.L. Troy, D.L. Page and E. Sevick-Muraca, Optical properties of normal and diseased breast tissues: prognosis for optical mammography, *J. Biomed. Optics* **1** (1996), 342–355.
- [47] L.V. Wang, Ultrasound-aided high-resolution biophotonic imaging, in: *Optical Diagnostic Imaging from Bench to Bedside*, National Institutes of Health, Bethesda, MD, 2004.
- [48] S. Webb, *The physics of medical imaging*, 3 ed., Bristol, Philadelphia, and New York: Institute of Physics Publishing, 1998, 633.