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INTRODUCTION TO OPTICAL IMAGING IN CLINICAL MEDICINE

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1.1 BRIEF HISTORY OF OPTICAL IMAGING

Throughout history, as soon as a piece of optical apparatus was invented, human beings have used it to gaze both outward and inward. The refractive power of simple lenses made from quartz dates back to antiquity. The modern refractive telescope was invented in the Netherlands by Lippershey in 1608 and refined and used widely by Galileo in Italy during the Renaissance to discover the satellites of Jupiter, among other extraterrestrial objects. The modern microscope was also invented in the Netherlands several years earlier, in 1595, by the same lens and eyeglass makers (Lippershey, Sacharias Jansen, and his son, Zacharias). Soon thereafter it was used to probe the microarchitecture of the human cell. Both

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telescopes and microscopes have evolved considerably over the years, guided by the elegantly simple fundamental physical laws that govern optical image formation by refraction proffered mathematically by Willebrord Snellius in 1621. Indeed, the principles of optics can simultaneously seem exceedingly simple and irreducibly complex. The early work in the Renaissance on the theory of diffraction and dispersion was led by Descartes, Huygens, and Newton, and was followed by the famous double-slit experiment of Young, which was subsequently supported by theory and calculations by Fresnel. The end of the nineteenth century saw the application of interferometry (Michelson) followed by the rise of quantum optics in the twentieth century. These scientific giants and their work provide the framework upon which all the fields and applications discussed in this book are built.

1.2 INTRODUCTION TO MEDICAL IMAGING

The dawn of the modern era of medical diagnosis can be traced to 1896, when Wilhelm Roentgen captured the first x-ray image, that of his wife's hand [1]. The development of radiology grew rapidly after that. Many noninvasive radiologic methodologies have been invented and applied successfully in clinical medicine and other areas of biomedical research. For the first 50 years of radiology, the primary examination involved creating an image by focusing x-rays through the body part of interest and directing them onto a single piece of film inside a special cassette. Later, modern x-ray techniques have been developed to significantly improve both the spatial resolution and the contrast detail. This improved image quality allows the diagnosis of smaller areas of pathology than could be detected with older technology. The next development involved the use of fluorescent screens and special glasses so that the physician could see x-ray images in real time. This caused the physician to stare directly into the x-ray beam, creating unwanted exposure to radiation. A major development along the way was the application of pharmaceutical contrast agents (dyes) to help visualize, for the first time, blood vessels, the digestive and gastrointestinal systems, bile ducts, and the gallbladder. The discovery of the image intensifier in 1955 has also contributed to the further blossoming of x-ray-based technology. Digital imaging techniques were implemented in the 1970s with the first clinical use and acceptance of the computed tomography (CT) scanner, invented by Godfrey Hounsfield [2].

Nuclear medicine (also called *radionuclide scanning*) also came into play in the 1950s. Nuclear medicine studies require the introduction into the body of very low-level radioactive chemicals. These radionuclides are taken up by the organs in the body and then emit faint radiation signals which are detected using special instrumentation. Imaging techniques that derive contrast from nuclear atoms, such as positron emission tomography (PET) and single-photon emission computed tomography (SPECT), reveal information about the spatiotemporal distribution of a target-specific radiopharmaceutical, which in turn yields information

about various physiological processes, such as glucose metabolism and blood volume and flow [3–5]. Magnetic resonance imaging (MRI) provides structural and functional information from concentration, mobility, and chemical bonding of hydrogen [6,7]. All this information is essential for the early detection, diagnosis, and treatment of disease.

In the 1960s the principles of sonar (developed extensively during World War II) were applied to diagnostic imaging. The process involves placing a small device called a *transducer* against the skin of a patient near the region of interest, such as the kidneys. The transducer produces a stream of inaudible high-frequency sound waves that penetrate the body and bounce off the organs inside. The transducer detects sound waves as they bounce off or echo back from the internal structures and contours of the organs. These waves are received by an ultrasound machine and turned into live pictures through the use of computers and reconstruction software. For example, in ultrasonography, images reveal tissue boundaries [6].

In addition to these traditional imaging modalities, optical imaging began to play a significant role in clinical medicine in early 1960. Optical imaging at both the macroscopic and microscopic levels is used intensively these days by clinicians for diagnosis and treatment. New advances in optics, data acquisition, and image processing made possible the development of novel optical imaging technologies, including diffuse tomography, confocal microscopy, fluorescence microscopy, optical coherence tomography, and multiphoton microscopy, which can be used to image tissue or biological entities with enhanced contrast and resolution [8]. Optical imaging technologies are more affordable than conventional radiological technologies and provide both structural and functional information with enhanced resolution. However, the optical techniques still lack sensitivity and specificity for cancer detection. Within the past few years, there has been increased interest in improving the clinical effectiveness of optical imaging by combining two or more optical imaging approaches (or integrating optical imaging technologies into traditional imaging modalities) [9–11].

Emerging optical technologies are now combined with novel exogenous contrast agents, including several types of nanovectors (e.g., nanoparticles, ligands, quantum dots), which can be functionalized with various agents (such as antibodies or peptides) that are highly expressed by cancer receptors [12–15]. These techniques provide improved sensitivity and specificity and make possible in situ labeling of cellular proteins to obtain a clearer understanding of the dynamics of intracellular networks, signal transduction, and cell–cell interactions [16–20]. Ultimately, the combination of these new molecular and nanotechnology approaches with new high-resolution microscopic and spectroscopic techniques (e.g., optical coherence tomography, optical fluorescence microscopy, scanning probe microscopy, electron microscopy, and mass spectrometry imaging) can offer molecular resolution, high sensitivity, and a better understanding of the cell's complex "machinery" in basic research. The resulting accelerated progress in diagnostic medicine could pave the way to more inventive and powerful gene- and pharmacologically based therapies.

1.3 OUTLINE OF THE BOOK

The desire for powerful optical diagnostic modalities has motivated the development of powerful imaging technologies, image reconstruction procedures, three-dimensional rendering, and data segmentation algorithms. In particular, the overwhelming problem of light scattering that occurs when optical radiation propagates through tissue and severely limits the ability to image internal structure is discussed. The broad range of methods that have been proposed during the past decade or so to improve imaging performance are presented. The relative merits and limitations of the various experimental methods are discussed. We consider whether the new advanced approaches will contribute further to the likelihood of successful transition from benchtop to bedside. A brief presentation of each chapter follows.

Chapter 2 outlines traditional clinical imaging modalities and their use in therapy planning and guidance, including ultrasound (US), x-ray imaging, computed tomography (CT), magnetic resonance imaging (MRI), positron emission tomography (PET), and single-photon emission computed tomography (SPECT). Although these imaging technologies provide good structural and functional information, they have inherent safety issues (especially x-ray/CT and those used in nuclear medicine—PET/SPECT), and their resolution is limited. Future advances in medical imaging may be possible by combining these traditional imaging modalities with novel optical, molecular, and nano-imaging approaches. The advantages of these multimodal approaches enable higher-resolution structural and functional imaging and disease detection in its early stage when the therapy success rate is relatively high.

Chapter 3 outlines the current imaging approaches in clinical medicine and is authored by leading clinicians from several prestigious teaching hospitals in the United States. This chapter is organized in four independent sections dealing with ophthalmic imaging, imaging of the gastrointestinal mucosa, cardioimaging, and neuroimaging. Current technological approaches, their limitations, and further needs are presented in detail.

Because the eye is the only transparent organ in the body accessible to in vivo *and* noninvasive examination, it is often the first and best venue for the application of new optical imaging techniques. Optical coherence tomography (OCT) is a prime example. Before this technique spread rapidly to other fields of inquiry, it was perfected in ophthalmology. The optics of the eye can be limiting in terms of theoretical achievable resolution (i.e., ocular aberrations, numerical aperture, etc.) compared to conventional or confocal microscopy. These limitations have, however, provided opportunities for the development of tools to overcome them (e.g., adaptive optics). Chapter 4 is a monograph of the optical-based imaging modalities for ophthalmic use, including conventional fundus imaging, OCT, and scanning laser ophthalmoscopy, and also new technologies such as adaptive optics and polarization imaging.

Confocal microscopy, invented by Minsky in 1957, is an elegant and simple method for achieving high image contrast by reduction of light scatter from

adjacent (lateral and axial) voxels. Chapter 5 focuses on reflectance confocal microscopy (RCM) for skin cancer detection. RCM enables real-time in vivo visualization of nuclear and cellular morphology. The ability to observe nuclear and cellular details clearly sets this imaging modality apart from other noninvasive imaging technologies, such as MRI, OCT, and high-frequency ultrasound. The lateral resolution of RCM, typically 0.2 to 1.0 μm, enables tissue imaging with a resolution comparable to that of high-magnification histology. As a result, RCM is being developed as a bedside tool for the diagnosis of melanoma and nonmelanoma skin cancers. The development and testing of advanced RCM instrumentation by the Memorial Sloan–Kettering cancer microscopy group is discussed in detail in this chapter.

Chapter 6 is devoted to the clinical applications of OCT in gastroenterology, including the esophagus, stomach, colon, duodenum, and the pancreatic and biliary ducts. OCT is an attractive tool for the interrogation of gastrointestinal tissue because it rapidly acquires optical sections at resolutions comparable to those of architectural histology, is a noncontact imaging modality, does not require a transducing medium, relies on endogenous contrast, and unlike the collection of forceps biopsies, is nonexcisional. Traditional assessment of gastrointestinal tissues is typically performed by endoscopy with accompanying forceps biopsy in organs with a larger-diameter lumen (esophagus and colon) or with brush cytology in the pancreatic or biliary ducts. OCT has shown promise for targeting premalignant mucosal lesions, grading and staging cancer progression, and reducing the risks and sampling error associated with biopsy acquisition. Although biopsy has traditionally been considered the gold standard for the diagnosis of gastrointestinal pathology, in many cases this assessment suffers greatly from sampling errors, with only a small percentage of the involved tissue being imaged. This is especially evident in cases where the disease may be focally distributed. Recent use of high-speed Fourier-domain OCT in the gastrointestinal tract has made significant strides toward comprehensive imaging, which may help to reduce the sampling error associated with the current assessment.

The most recent advances in confocal endomicroscopy for gastrointestinal (GI) cancer diagnosis are presented in Chapter 7. Confocal endomicroscopy is a rapidly emerging optical imaging modality that is currently being translated to routine clinical use. Advances in miniaturization have led to the development of numerous fiber optic–based confocal microscopes which may be deployed through the instrument channel of a conventional endoscope, or permanently packaged within the tip of a custom endoscope. Confocal endomicroscopes can enable real-time, high-resolution, three-dimensional visualization of epithelial tissues for improved early detection of disease and for image-guided interventions such as physical biopsies and endoscopic mucosal resection. Current research effort has focused on overcoming the limitations of confocal microscopy. For example, its limited field of view is now overcome by real-time generation of a mosaic of stitched images. The application of confocal microscopy for the interrogation of epithelial surfaces in the GI tract presents unique design challenges.

Therefore, this chapter focuses on clinical motivation, challenges, design parameters, and other fundamental aspects of GI endomicroscopy. Also discussed are the latest efforts to develop miniature confocal microscopes that are compatible with those of GI endoscopy.

Chapter 8 presents recent progress in minimally invasive approaches in interventional cardiology (IC). The diagnosis of vulnerable plaques occupies an important part of this chapter. The structure of these plaques (i.e., hard core, fluid-filled lesions accessible only with depth-resolved techniques) makes them the most difficult to diagnose, yet they seem to be responsible for most deleterious coronary events. Identification and visualization of high-risk plaques are key to designing customized therapeutic approaches. The ultimate goal is to accurately categorize high-risk patients, target therapy to appropriate areas of vulnerable plaque, and thus prevent or reduce the probability of adverse events. A number of minimally invasive imaging modalities currently in use are presented, including intravascular ultrasound (IVUS) and angioscopy. Also presented are promising new investigational methodologies, including OCT, intracoronary thermography, near-infrared spectroscopy, and intracoronary MRI. OCT in particular uniquely enables excellent resolution of coronary architecture and precise characterization of plaque morphology.

An overview of time-resolved (lifetime) laser-induced fluorescence spectroscopy (TR-LIFS) is presented in Chapter 9. TR-LIFS instrumentation, methodologies for in vivo characterization, and diagnosis of biological systems are presented in detail. Emphasis is placed on the translational research potential of TR-LIFS and on determining whether intrinsic fluorescence signals can be used to provide useful contrast for the diagnosis of high-risk atherosclerotic plaque and brain tumors intraoperatively.

Chapter 10 outlines the use of near-infrared spectroscopy (NIRS) for noninvasive monitoring of brain hemodynamics and oxidative metabolism (i.e., oxygenation status). This technology is capable of monitoring cerebral activity in response to various stimuli (motor, visual, and cognitive) and therefore is currently being used to study functional processes in the brain, to diagnose mental diseases, and more precisely, to localize brain injuries. Clinical demonstration and widespread use of this technology is expected to increase in the coming years because of its promise for functional imaging. In particular, major breakthroughs are expected in cutting-edge techniques such as time-domain functional near-infrared spectroscopy. Current research systems employ low-power pulsed diode lasers and complex, efficient detection instrumentation. Future prototype clinical instruments will achieve higher signal/noise ratios through the use of ultrafast (picoseconds), high-power (>1 W) broadband fiber laser and miniaturized, sensitive, and fast photonic crystal devices combined with high-throughput photodetection electronics.

Chapter 11 describes the application of NIRS to mammography. In optical mammography, diagnosis is based on the detection of local differential concentrations of endogenous absorbers and/or scatterers between normal and diseased breast tissue. Various implementation approaches in conjugation with MR

imaging are discussed in detail. The prevalence and implication of age, hormonal status, weight, and demographic factors on complex structural changes in the breast are discussed as well. Such intra- and interpatient variations affect the effectiveness of these imaging modalities adversely and to date have restricted wide clinical use to a specific demographic or time window. As clinical optical mammography matures, its potential impact on cancer management will become more clearly defined. The main applications of this optical technology, as proposed by the Network for Translational Research in Optical Imaging, are monitoring of neoadjuvant chemotherapy response, screening for subpopulations of women in which mammography does not work well, and optical imaging as an adjunct to x-ray mammography.

A promising new optical technology called *photoacoustic tomography* (PAT) is introduced in Chapter 12 for breast imaging. Research interest in laser-induced PAT is growing rapidly, largely because of its unique capability of combining high-contrast optical imaging with high-resolution ultrasound in the same instrument. Recent in vivo studies have shown that the optical absorption contrast ratio between tumor and normal tissues in the breast can be as high as $3:1$ in the near-infrared region, due to significantly increased tumor vascularity. However, optical imaging has low spatial resolution, due to strong light scattering. Ultrasound imaging can provide better resolution than optical imaging due to less scattering of acoustic waves. However, the contrast for ultrasound imaging is low, and it is often incapable of revealing diseases in early stages. In a single hybrid imaging modality, PAT combines the advantages of optical and ultrasound imaging while avoiding the limitations of each. PAT imaging can penetrate to a depth of about 1 cm with an axial resolution of less than 100 μm at a wavelength of 580 nm. PAT has shown the potential to detect breast cancer, to probe brain functioning in small animals, and to assess vascular and skin diseases.

Chapter 13 focuses on the application of optical imaging to tissue angiogenesis monitoring. Angiogenesis is a process fundamental to several normal tissue physiological functions and responses as well as to many pathological conditions. The ability to monitor and quantify angiogenesis clinically could aid in the management of certain diseases, healing responses, and therapies. While several methods for measurement of angiogenesis are under development and are being implemented using conventional medical imaging modalities, optical techniques have also shown potential. To properly design and evaluate optical techniques to measure angiogenesis, an appreciation of the complex physiology of the process is necessary. Methodology to quantify angiogenesis and independent test optical measurements are also needed. The fundamental characteristics of angiogenesis, measurement methods, and the current state of optical techniques are reviewed briefly in this chapter.

Chapter 14 introduces a relatively new technology, called phase-contrast optical coherence tomography. This technique can detect subwavelength changes in optical pathlength (OPL) by measuring the phase of an interference signal. Although phase information is readily available in any interferometric setup, environmental noise corrupts the phase information, rendering it difficult to use.

Robust phase measurement requires interferometer designs that cancel common-mode noise. This can be achieved with common path and differential phase interferometric implementations. Measurement of depth-resolved subwavelength changes in OPL is now possible for novel optical imaging applications. Phase-sensitive low-coherence interferometry in both time- and spectral-domain implementation, as well as their potential for biomedical applications (including surface profilometry, quantitative phase-contrast microscopy, and optical detection of neural action potentials), are described in this chapter.

Polarization is a fundamental property of light that can be harnessed to provide additional contrast without staining or labeling. Imaging technologies that detect and visualize the interaction of tissue with polarized light, revealing structural or chemical characteristics not visible with standard intensity imaging, are described in Chapter 15. The anisotropic real and imaginary parts of the refractive index of tissue are fundamental properties that can be quantified through the detection of transmitted or reflected polarized light. The linear and circular birefringence and dichroism also calculated with polarimetric techniques can help to differentiate normal and diseased tissue. Currently, linear birefringence is most often measured because it is related to the highly organized structure of collagen. Circular birefringence and linear and circular dichroism, which are related to other structural and chemical anisotropies, are generally not explored. Whereas traditional light-scattering techniques normally probe only size distribution and concentration of particles, polarized light scattering is sensitive to shape, orientation, and internal structure of the particles, as well as to structural characteristics of the global system. Applications of this technology to the fields of biomedicine, materials science, and industrial and military sensors are presented in this chapter.

Chapter 16 covers the use of various nanotechnologies for contrast enhancement in optical imaging. Optical imaging technologies are more affordable than traditional radiological technologies and provide both structural and functional information with enhanced resolution. However, they still lack sensitivity and specificity for cancer detection. Therefore, in the past few years there has been increasing interest in improving clinical effectiveness of optical imaging by combining emerging optical technologies with novel exogenous contrast agents, including several types of nanovectors (e.g., nanoparticles, ligands, quantum dots), which can be functionalized with various agents (such as antibodies or peptides) that are expressed highly by cancer receptors. In this way, it becomes possible to label proteins in live cells and obtain a clearer understanding of the dynamics of intracellular networks, signal transduction, and cell–cell interactions in addition to improving sensitivity and specificity. Use of the enhanced sensitivity and specificity of molecular imaging approaches in medicine has the potential to affect positively the prevention, diagnosis, and treatment of various diseases, including cancer. These new molecular and nanotechnology approaches with new developments in microscopic and spectroscopic techniques toward high spatial resolution (i.e., optical coherence tomography, optical fluorescence microscopy, etc.) are presented to some extent in this chapter. Implications of the various

nanotechnologies for more efficient drug delivery and tissue regeneration are discussed as well.

Chapter 17 focuses on the use of molecular probes for optical contrast enhancement of GI cancers. The surface of the GI tract is accessible to direct examination via endoscopy. The most common GI cancers originate in the superficial mucosa, where unscattered ballistic photons can penetrate to depths that are relevant for interrogating tissue properties and detecting cellular markers. Improvements in instrumentation now permit the detection of these photons to depths that were not possible previously. In addition, molecular probes whose binding characteristics are verified by microscopy facilitate rapid wide-field detection of precancerous lesions over large areas, followed by closer inspection with endomicroscopy. Probes that detect GI cancer have been identified and the clinical application of both contrast-enhancing agents and specific molecular diagnostic reagents capable of revealing early disease markers is on the horizon. The effort ongoing toward the development of GI cancer probes and detection reagents is presented in detail in this chapter.

A broad cross section of optical imaging research and clinical technology development for early disease detection and therapy guidance is described in the book. We recognize that the broadness of the biomedical optical imaging field makes it likely that we have omitted many important investigations in our sampling. Moreover, the rapid pace of discovery, although exciting for all the authors, also provided the challenge of trying to hit a moving target. We hope that the book will provide a foundation of knowledge upon which future technological developments in optical imaging will materialize.

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