MULTIMODAL ENDOSCOPY AND ITS APPLICATION

By

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To my daughter, Sophia, Dai
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<td>One-dimensional</td>
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<td>2D</td>
<td>Two-dimensional</td>
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<tr>
<td>3D</td>
<td>Three-dimensional</td>
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<tr>
<td>ARPAM</td>
<td>Acoustic-resolution photoacoustic microscopy</td>
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<td>ANSI</td>
<td>American National Standards Institute</td>
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<tr>
<td>ATF</td>
<td>Amino-terminal fragments</td>
</tr>
<tr>
<td>CNR</td>
<td>Contrast-to-noise ratio</td>
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<tr>
<td>CT</td>
<td>Computed tomography</td>
</tr>
<tr>
<td>CW</td>
<td>Continuous wave</td>
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<td>DAQ</td>
<td>Data acquisition</td>
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<td>DCF</td>
<td>Double-clad fiber</td>
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<tr>
<td>DM</td>
<td>Dichroic mirror</td>
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<tr>
<td>DPSS</td>
<td>Diode-pumped solid-state</td>
</tr>
<tr>
<td>ESD</td>
<td>Electrostatic discharge</td>
</tr>
<tr>
<td>FDA</td>
<td>Food and Drug Administration</td>
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<td>FWHM</td>
<td>Full width at half-maximum</td>
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<td>G1</td>
<td>First generation</td>
</tr>
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<td>G2</td>
<td>Second generation</td>
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<td>GCF</td>
<td>Gold-coated film</td>
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<td>GI</td>
<td>Gastrointestinal</td>
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<td>GRIN</td>
<td>Gradient index</td>
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<tr>
<td>IACUC</td>
<td>Institutional Animal care and Use Committee</td>
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<td>ID</td>
<td>Inner diameter</td>
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<td>IONP</td>
<td>Iron oxide nanoparticle</td>
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<td>Description</td>
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<tr>
<td>IVPA</td>
<td>Intravascular photoacoustic imaging</td>
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<td>IVUS</td>
<td>Intravascular ultrasound</td>
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<tr>
<td>LD</td>
<td>Laser diode</td>
</tr>
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<td>LED</td>
<td>Light emitting diode</td>
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<td>MAP</td>
<td>Maximum amplitude projection</td>
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<td>MMF</td>
<td>Multimode fiber</td>
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<tr>
<td>MOSFET</td>
<td>Metal-oxide-semiconductor field-effect transistor</td>
</tr>
<tr>
<td>MRI</td>
<td>Magnetic resonance imaging</td>
</tr>
<tr>
<td>NA</td>
<td>Numerical aperture</td>
</tr>
<tr>
<td>ND</td>
<td>Neutral density</td>
</tr>
<tr>
<td>NI</td>
<td>National instrument</td>
</tr>
<tr>
<td>NIH</td>
<td>National Institutes of Health</td>
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<tr>
<td>NIR</td>
<td>Near-infrared</td>
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<tr>
<td>NIRS</td>
<td>Near-infrared spectroscopy</td>
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<td>OCT</td>
<td>Optical coherence tomography</td>
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<td>OD</td>
<td>Outer diameter</td>
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<tr>
<td>OPO</td>
<td>Optical parametric oscillator</td>
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<td>OR-PAM</td>
<td>Optical-resolution photoacoustic microscopy</td>
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<td>Acronym</td>
<td>Description</td>
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<tr>
<td>PAI</td>
<td>Photoacoustic imaging</td>
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<td>PAM</td>
<td>Photoacoustic microscopy</td>
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<td>PAT</td>
<td>Photoacoustic tomography</td>
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<tr>
<td>PC</td>
<td>Personal computer</td>
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<tr>
<td>PD</td>
<td>Photodiode</td>
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<tr>
<td>PEG</td>
<td>Polyethylene glycol</td>
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<tr>
<td>PET</td>
<td>Positron emission tomography</td>
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<tr>
<td>RGB</td>
<td>Red, green, blue</td>
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<tr>
<td>ROI</td>
<td>Region of interest</td>
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<tr>
<td>SBR</td>
<td>Signal to background ratio</td>
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<tr>
<td>SH</td>
<td>Sample holder</td>
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<tr>
<td>SMF</td>
<td>Single-mode fiber</td>
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<tr>
<td>SNR</td>
<td>Signal-to-noise ratio</td>
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<tr>
<td>SPECT</td>
<td>Single-photon emission computed tomography</td>
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<tr>
<td>SST</td>
<td>Stainless steel tubing</td>
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<tr>
<td>US</td>
<td>Ultrasound imaging</td>
</tr>
<tr>
<td>UST</td>
<td>Ultrasound transducer</td>
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<tr>
<td>VCSEL</td>
<td>Vertical-cavity surface-emitting laser</td>
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Endoscopic imaging plays an exclusive role in early diagnosis, management, precision medicine of diseases in the internal organs due to its specific capability of directly reaching the surface of internal organs. Particularly, for some internal organs with small size, like artery, advanced endoscopic imaging has already become a sophisticated tool helping doctors make decision. Due to the complexity of many diseases, like atherosclerosis, cancer, a single endoscopic imaging modality could only answer some parts of the questions of interest. Fortunately, many imaging techniques are complementary in nature, thus combining their strengths in a multimodal endoscopy would have the potential to more completely characterize biological tissue.

This dissertation intends to develop several novel endoscopes integrating emerging non-ionizing, non-invasive biomedical imaging techniques, photoacoustic imaging (PAI), optical coherence tomography (OCT) and ultrasound imaging (US), that aim to combine the strengths of each modality and explore the unique ability of multimodal endoscopy for diseases diagnostics and therapeutics. The first part of this dissertation discusses the concept of physically integrating optical-resolution photoacoustic microscopy (OR-PAM), OCT and US into a miniature probe, and then
demonstrates the ability of the integrated tri-modal endoscope with phantom and in vivo animal experiments. This prototype of tri-modal endoscope shows the feasibility of combining strengths of OR-PAM, OCT and US into a single imaging platform.

The second part of this dissertation describes the second generation of tri-modal endoscope, whose probe size is highly reduced into 1 millimeter in diameter. And we explore the applications of the tri-modal endoscope for the characterization of atherosclerotic plaques, minimally invasive molecular imaging of pancreatic cancer, as well as molecular intravascular imaging aided by multifunctional nanoparticles.

The last part of this dissertation demonstrates the feasibility of fiber-free endoscope which fully integrates all the components in a miniature probe based on low-cost compact laser sources. In conventional PAI system, laser’s bulky size, high cost and strict maintenance requirements significantly limit PAI’s practical biomedical applications. Here, we first demonstrate the feasibility of PAI system with low-cost compact light sources. Then, we shows the implementation of fiber-free endoscope and its applications.
CHAPTER 1
INTRODUCTION

Background and Motivations

Endoscopy has been an indispensable tool for diagnosis, management, interventional treatment of internal organ diseases with its specific capability of directly reaching the surface of internal organs. However, due to the complexity of many internal organ diseases, like cancers, a single imaging modality could only provide partial information, thus, it is such difficult for clinicians to make decision through one imaging modality. Naturally, many imaging techniques are complementary.

Thus, multimodal imaging is always attractive and desired since it can combine the strengths of several different imaging modalities, and is able to characterize biological tissue more completely, thus offering the possibility of precision diagnosis of diseases. While multimodality images can be obtained by performing each individual modality separately without integrating them into a single platform, it is, however, time-consuming to acquire multimodality images through such a process, hard to avoid errors from the required complex image registration, and more importantly, impossible to catch dynamic biological processes simultaneously. To overcome these limitations, physically integrating multiple modalities into a single platform is highly desired.

Photoacoustic imaging (PAI) is an emerging non-invasive three-dimensional (3D) technique capable of imaging tissue absorption and providing anatomical, functional, and molecular properties of biological tissue in high resolution. In PAI, an image is formed through the detection of either pulsed or intensity modulated light-induced ultrasound waves. Compared to other modalities, PAI is a multi-scale imaging technique from microscopic to macroscopic...
scales. Through detection of focused pulsed laser-induced ultrasound waves with an ultrasound transducer, optical-resolution photoacoustic microcopy (OR-PAM) can reach as high as sub-micrometer resolution. Acoustic-resolution photoacoustic microscopy (ARPAM) can penetrate into biological tissue as deep as several millimeter with tens of micrometer resolution by focused ultrasonic detection. While, without any focusing mechanism, photoacoustic tomography (PAT) has deepest tissue penetration depth but lowest spatial resolution.

Optical coherence tomography (OCT) is an emerged technique capable of imaging tissue scattering property with a high resolution based on a low coherence interferometer\(^{25, 26}\). While OCT is deal for imaging retina \(^{25, 26, 27, 28, 29}\), it has also been used to visualize other tissues \(^{30, 31, 32, 33, 34}\).

Pulse-echo ultrasound imaging (US) is another emerged imaging technology with the ability of measuring tissue structural characteristics within a relatively deep penetration depth. In US imaging, ultrasonic waves generated by an ultrasound transducer travel through the tissue and then are back scattered by tissue. The strength of the back scatter carries the structural information of tissue. By detection of the back scattered ultrasonic waves, the tissue structure can be mapped.

Using combined PAI and OCT platform, both optical absorption and scattering information of tissue can be obtained \(^{11, 35}\). In this regard, we and other groups have made efforts to develop an integrated platform combining PAI and OCT \(^{35, 36, 37, 38, 39, 40, 41}\). These studies have demonstrated the advantages of such a dual-modality system. For example, for in vivo microcirculation studies, PAI provided absorbing components such as blood vessels, while OCT obtained the fine structures of the surrounding
tissues. By integrating PAI and US with a common ultrasound transducer, it is able to offer structural and functional information of tissue with a relatively deep penetration depth.\textsuperscript{42, 43, 44, 46, 47, 48, 49, 50}

It is conceivable that a multimodal approach combining PAI, OCT and US would provide a more powerful tool for tissue imaging,\textsuperscript{51} which motivates us to investigate such multimodal endoscopic technique and its applications.

**Dissertation Outline**

In this study, I developed several multimodal endoscopic imaging probes and systems for various applications. Chapter 2 and 3 will focus on describing the challenges and development of two generation multimodal endoscope: G1 multimodal endoscope and G2 multimodal endoscope. In Chapter 2, I will introduce the development of our first generation (G1) multimodal endoscope integrating OR-PAM, OCT, and US, towards proof of concept and demonstration of in vivo imaging capability. Our G1 multimodal endoscope designed and implemented has demonstrated, for the first time, that OR-PAM, OCT, US images can be obtained from the same tissue volume coaxially. The capability of providing relatively complete information of biological tissue has shown that our G1 multimodal endoscope is suitable for in vivo imaging of internal organ. However, the size of the probe is 2 mm in diameter which is still too large to be used for clinical intravascular and transurethral imaging or for preclinical studies of internal organs in small animals. For example, internal organs such as colon of nude mouse is so small in size that a 2mm-diameter probe would simply break the colon.\textsuperscript{52}

Although the urethra of an adult is about 5-7 mm in diameter for men and larger for women, the ureteral wall injury happens very often while ureteroscopy is performed.\textsuperscript{53}

In particular, a critical size of 1 mm or smaller is required for clinical intravascular
imaging. Therefore, in Chapter 3, after tackling the challenges of engineering a tiny translatable endoscopic probe, I designed and implemented our G2 multimodal endoscopic probe integrating PAI, OCT, and US with a diameter of only 1.0 mm based on a double-clad fiber (DCF) and a high frequency ultrasound transducer. The challenges and implementation of the probe will be described in detail. And validation experiments including phantom experiments, in vivo experiments will also be shown to demonstrate the capability of the G2 multimodal endoscope.

Chapter 4 and 5 will focus on presenting various applications of the G2 multimodal endoscope. In Chapter 4, I will describe in detail about our G2 multimodal endoscope applied in cancer research, particularly, for pancreatic cancer studies. We enhanced the molecular imaging capability of PAI using targeted multifunctional nanoparticles, and improved the ability of directly accessing pancreas through applying our developed G2 miniature endoscope. A novel fan-shaped scanning mechanism was developed to minimize the invasiveness. Chapter 5 presents the applications of our G2 multimodal endoscope in intravascular imaging, including label-free atherosclerotic plaque characterization and molecular intravascular imaging with targeted multifunctional nanoparticles.

In Chapter 6, a new concept of fully integrated fiber-free endoscope will be introduced. Benefit from the development of light sources including miniature high-power light emitting diode (LED) and high-power high-speed vertical-cavity surface-emitting laser (VCSEL), it is possible to build a compact photoacoustic imaging system with these light sources instead of bulky pulsed lasers in conventional photoacoustic imaging setup. We first validated the possibility of obtaining high-quality photoacoustic
images with LED and VCSEL, respectively. We have demonstrated, for the first time, in vivo feasibility of photoacoustic imaging with a miniature LED excitation, and photoacoustic microscopy with a compact VCSEL excitation. Based on these exploration, we then proposed a total new concept of fiber-free endoscope. Validation experiments will be shown to demonstrate the capability of the proposed endoscope. In our latest generation endoscope, only cable is needed for power supply and data transmission, which will highly increase the practical ability of endoscope.

Finally, in Chapter 7, the summary of my dissertation and future directions will be presented.
CHAPTER 2
G1 MULTIMODAL ENDSOCOPE: CONCEPT AND VALIDATION

Motivations

Optical-resolution photoacoustic microscopy (OR-PAM) is the microscopic scale of PAI modalities that has highest spatial resolution. Since hemoglobin molecules are strong optical absorbers within a unique range of optical spectrum, OR-PAM is ideal for mapping microvasculature in biological tissue through using light with a specific wavelength (such as 532 nm). Through combing OR-PAM and OCT, both optical absorption and back-scattering information can be obtained in high resolution. We and other groups have made efforts to develop an integrated platform combining OR-PAM and OCT. These studies have demonstrated the advantages of a dual-modality system. For example, for in vivo microcirculation studies, OR-PAM provided absorbing components such as blood vessels, while OCT obtained the fine structures of the surrounding tissues. In addition, it is easily to integrate OR-PAM and ultrasound (US) using a common ultrasound transducer. Bai et al. reported a probe with an overall diameter of 1.1 mm, representing the smallest in size in OR-PAM and US combinations.

It is highly desired that a tri-modal approach combining OR-PAM, OCT and US can be built for tissue imaging. Yang et al. took an initial step towards the integration of PAI, OCT and US in a single probe for ovarian tissue imaging. While this study is certainly inspiring, in their probe, PAI, OCT and US were not physically integrated. Moreover, their probe cannot visualize microvasculature due to its relatively poor resolution of PAI.

In this regard, we present a tri-modal approach that integrates optical-resolution PAM (OR-PAM), OCT, and US in a single 2mm-diameter probe. In this novel probe,
OR-PAM and OCT use the same optical path based on a single-mode fiber (SMF), a gradient index (GRIN) lens and a thin gold-coated film, while OR-PAM and US use share a 40 MHz unfocused ultrasound transducer, enabling these three modalities to coaxially obtain images from the same tissue volume. Both phantom and in vivo experiments were performed to demonstrate the capabilities of the integrated tri-modality imaging probe.

**Integrated Probe and System**

The schematic of the integrated miniature probe is shown in Figure 2-1A. In this probe, a single-mode fiber (SMF-28e+, Thorlabs) with a numerical aperture (NA) of 0.14 encapsulated by a ceramic ferrule (CFLC126-10, Thorlabs) with an outer diameter of 1.25 mm that was used to deliver light for both OR-PAM and OCT. A custom-designed GRIN lens with a diameter of 0.7 mm and a working distance of 5 mm was used to focus the light beam from the tip of SMF with 8° angle facial cutting for minimizing back-reflection. The light beam was then reflected into the sample by a home-made thin gold-coated polyester film (48-1F-OC, CS Hyde Company, IL) with a thickness of 25 μm, attached to the tip of the stainless steel tubing (SST1) using epoxy glue. A custom-made unfocused ultrasound transducer with a center frequency of 40 MHz and a dimension of 0.6 mm x 0.5 mm x 0.2 mm (Blatek, Inc., State College, PA) was mounted to the tip-wall of SST1. The ultrasound transducer should be placed coaxially with the optical path with respect to the thin film while fixing. The probe was protected by a second stainless steel tubing (SST2) with an aperture opened at its tip to allow both light and ultrasound to transmit with minimal loss, which ultimately determined the overall size of the whole probe, i.e., 2.0 mm in diameter.
Compared to the conventional way of using 45° prism to reflect light and transmit ultrasound, here, gold-coated film (GCF) was used to reflect light. Since the ultrasound attenuation caused by GCF was significantly smaller, it resulted in improved detection of both photoacoustic and ultrasound signals (in practice, the housing space between the transducer and GCF was filled with deionized water or the whole probe was immersed into a water tank). Further, the attenuation of GCF was quantitatively assessed by using the same ultrasound transducer as the probe and an ultrasound pulser/receiver (5073PR, Olympus) (as shown in Figure 2-2). The ultrasound transducer (UST) and a steel plate (SP) were both placed in a water tank with a certain distance between each other. The echoes reflected by the steel plate were recorded with (Figures 2-2B, 2-2D) and without (Figures 2-2A, 2-2C) GCF between transducer and steel plate. By computing the ratio of peak-to-peak value of echoes with (Figure 2-2C, 0.45 V) and without (Figure 2-2D, 0.43 V) GCF, the two-way ultrasound attenuation caused by GCF was around 5%.

The integrated tri-modality imaging system is schematically shown in Figure 2-3A. The probe was mounted on a linear stage and immersed inside a water tank. A hole was drilled at the bottom of the water tank and sealed with transparent plastic film with a thickness of 50 μm to allow both light and ultrasound to transmit with minimized energy loss.

For OR-PAM and time-domain OCT, a nanosecond pulsed Nd:YAG laser having a repetition rate of 20 Hz and a center wavelength of 532nm and a broadband light source with a full width at half-maximum (FWHM) of 75 nm and a center wavelength of 1310 nm were used for OR-PAM and time-domain OCT, respectively. A data acquisition
(DAQ) board (PCI-5124, National Instrument) with 12-bit resolution, a sampling rate of 200 MS/s, and a signal-to-noise ratio (SNR) of 56 dB was used to resolve signals as small as 1 mV, enhancing the sensitivity of the data acquisition system. For US imaging, an ultrasound pulser/receiver (5073PR, Olympus) with an integrated amplifier and a bandwidth of 75 MHz, synchronized with OR-PAM/OCT, was used to generate ultrasound and receive echoes. By scanning in x-y plane with a two-dimensional (2D) linear stage, a volumetric image of sample could be obtained. Figure 2-3B shows the timing diagrams of the whole scanning, which is synchronized by the pulsed Nd:YAG laser with a repetition rate of 20 Hz (i.e. T=50 ms). The signal acquisition of OR-PAM is triggered by the synchronizing output of pulsed Nd:YAG laser. With a 10 μs (t₁) delay, the time-domain OCT subsystem starts to collect signal. After 20 μs (t₂), US subsystem is triggered to acquire echoes from the sample. Currently, it takes around 25 minutes to acquire data for tri-modal volumetric imaging given a scanning area of 1 × 1 mm² with a 6-μm step size, which is limited by the repetition rate of the pulsed laser (20 Hz) for OR-PAM.

Phantom Validation

The feasibility of the integrated tri-modality imaging system was firstly validated by experiments using a turbid tissue mimicking phantom (as shown in Figure 2-4A). The background of the phantom was composed of 2% Agar, TiO₂, and India ink with absorption coefficient of 0.007 mm⁻¹ and reduced scattering coefficient of 1.0 mm⁻¹. Four simulated targets were used: (1) two pieces of human hairs with a diameter of around 100 μm were embedded at a depth of 1.0 mm under the surface; (2) two pieces of bare optical fibers with a diameter of 0.4 mm were positioned with a depth of 2.0 mm. Figures 2-4B and 2-4C) show the cross-sectional images of the phantom from OR-PAM.
and OCT, respectively. Both the OR-PAM and OCT images show clear boundary
delineation of the hairs at the depth of 1.0 mm, indicating the penetration depth of OR-
PAM and OCT is more than 1.0 mm. Figure 2-4D gives the cross-sectional US image of
the phantom, in which both the hairs and bare fibers were detected, indicating the
imaging depth of US is more than 2.0 mm. Figure 2-4E shows the overlap of tri-modal
image through pseudo RGB color coding with equal ratio of OR-PAM (R, red), OCT (G,
green), US (B, blue), indicating the matched correlation of three modalities.
Furthermore, through examining the images of the hairs, it suggests that the spatial
resolution of US is lower than OR-PAM and OCT. However, US imaging is able to
image deeper targets.

**Spatial Resolution Measurements**

The spatial resolution of OR-PAM was evaluated by imaging a carbon fiber with a
diameter of 6 μm. Figure 2-5A shows the cross-sectional image of the carbon fiber.
Figures. 2-5B and 2-5C, respectively, show the lateral and axial profiles corresponding
to the dotted lines in Figure 2-5A. In Figures. 2-5B and 2-5C, the dotted profile (blue)
represent the experimental data, which is Gaussian fitted (red) indicating a lateral
resolution of 13.6 μm and an axial resolution of 42.1 μm with a signal-to-noise ratio
(SNR) of 28 dB. The axial resolution primarily depends on the bandwidth and central
frequency of the ultrasound transducer, which is close to the axial resolution of 43.0 μm
for US with a SNR of 32 dB estimated by using a tungsten wire with a diameter of 12
μm. We also used the same 6μm-diameter carbon fiber to estimate the spatial
resolution of OCT (see Figure 2-6), which respectively gave a lateral resolution of 13.4
μm and an axial resolution of 14.3 μm with a SNR of 20 dB. The results suggest that
both OR-PAM and OCT have similar lateral resolution determined by the point spread
function of the light focus. Since the axial resolution of OCT is primarily determined by the FWHM of the light source that 75 nm in our case resulting in an axial resolution of 10 μm.

**In Vivo Animal Experiments**

To further demonstrate the potential clinical capabilities of this integrated tri-modal probe, in vivo imaging of a rat ear (rat weight=70g) was performed. Before the experiments, the hairs on the ear were gently removed using a human-hair-removing cream. The rat was placed on a home-made animal holder and anesthetized by a solution of ketamine (85 mg/kg) and xylazine. The rat was sacrificed according to the University of Florida Institutional Animal Care and Use Committee (IACUC)-approved techniques after the experiments. Strict animal-care procedures approved by the University of Florida IACUC and based on guidelines from the National Institutes of Health (NIH) for the Care and Use of Laboratory Animals were followed. The laser exposure was about 15 mJ/cm² at the optical focus inside the ear tissue which is lower than the American National Standards Institute (ANSI) laser safety limit (20 mJ/cm²). The scanning step in the X-Y plane was 6 μm, which is below the spatial resolution of our probe. The tri-modal images of a 1.2 × 1.2 mm² area of the rat ear were obtained without average of the signals, taking around 36 minutes, which is limited by the repetition rate (20 Hz) of our pulse laser for OR-PAM.

The top row of Figure 2-7 shows the maximum amplitude projection (MAP) images of OR-PAM (Figure 2-7A), OCT (Figure 2-7B), US (Figure 2-7C), and fused tri-modal pseudo-color-coded RGB images (Figure 2-7D) obtained with an equal ratio. The 2nd, 3rd, and bottom rows of Figure 2-7 show the cross-sectional images of OR-PAM,
OCT, US and fused tri-modality, corresponding to the three dashed lines in the respective MAP image.

We see that OR-PAM, OCT and US, respectively, image the microvasculature, fine structures surrounding the blood vessels and both superficial and deep tissue structures. To closely interpret these images, blood vessels in the rat ear are mapped by OR-PAM with the highest contrast (Figure 2-7A), epidermis, dermis, and cartilage are identified by OCT (Figure 2-7B), and deep tissues like subcutaneous tissue are visualized by US (Figure 2-7C). By overlapping the OR-PAM, OCT, US images (Figure 2-7D), the structures/tissue morphology are better displayed.

**Conclusion**

In summary, we have developed a novel miniature probe, which coaxially integrates OR-PAM, OCT, and US in a single platform, which is very suitable for in vivo imaging internal organs, such as endoscopic imaging, intravascular imaging. The phantom and in vivo experiments have shown that the tri-modal probe is able to offer high resolution images of tissue absorption and optical back-scattering properties as well as deep tissue structures. For future studies, in order to enable broader potential applications of the integrated tri-modal probe with the imaging system, firstly, we need a rotary device to replace current bulky linear stage. A possible way is to implement an internal scanning mechanism based on micro-motor. Secondly, currently, two lasers are used for OR-PAM and OCT respectively and thus the total cost is relatively high and the whole system is bulky. Moreover, the current imaging speed is mainly limited by the slow repetition rate (20 Hz) of the pulsed laser for OR-PAM. A high-repetition-rate pulsed laser with compact volume and low cost (such as diode-pumped solid-state
(DPSS) laser with a repetition rate of 2-5 kHz) is required to both speed up imaging and reduce the whole volume and cost.
Figure 2-1. Integrated probe. A) schematic, B) photograph (photo courtesy of author). GCF, gold-coated film; UST, ultrasound transducer; GL, gradient index lens; SST1 ~ SST2, stainless steel tubing; CF, ceramic ferrule; SMF, single-mode fiber; USTC, ultrasound transducer cable.
Figure 2-2. Attenuation of gold-coated film. A) without and B) with gold-coated film between ultrasound transducer and steel plate. C) echo of A), D) echo of B). UST, ultrasound transducer; T: transmit wave; R: reflected wave; SP: steel plate; GCF: gold-coated film.
Figure 2-3. Integrated tri-modality imaging system. A) schematic, B) timing diagrams for synchronizing subsystems. T: 50 ms; $t_1$: 10 μs; $t_2$: 20 μs.
Figure 2-4. Cross-sectional OR-PAM, OCT and US images from tissue mimicking phantom. A) phantom geometry with 4 simulated targets, B) OR-PAM image, C) OCT image, D) US image, E) tri-modal color-coded RGB image.
Figure 2-5. The spatial resolution of OR-PAM. A) OR-PAM cross-sectional image of a carbon fiber, B) the lateral resolution, C) the axial resolution, D) high resolution microscopy image of the carbon fiber. PSF, point spread function.
Figure 2-6. The spatial resolution of OCT. A) OCT cross-sectional image of a carbon fiber, B) the lateral resolution, C) the axial resolution. PSF, point spread function.
Figure 2-7. In vivo imaging of rat ear by the integrated tri-modality probe. Maximum amplitude projection (MAP) images (top row) and cross-sectional images (2nd, 3rd and bottom rows) corresponding to the dotted lines shown in the MAP image of OR-PAM A), OCT B), US C) and fused tri-modality D). BV, blood vessel; ED, epidermis; D, dermis; CT, cartilage; ST, subcutaneous tissue.
CHAPTER 3
G2 MULTIMODAL ENDOSCOPE

Motivations

In Chapter 2, we \(^{12}\) demonstrated a miniature tri-modal probe with a diameter of 2 mm, physically integrating OCT, US, and OR-PAM. The use of a gold-coated thin film in this probe allowed us, for the first time, to coaxially acquire OR-PAM, OCT and US images of the same area of biological tissue in vivo. While encouraging, this 2mm-diameter tri-modal probe cannot be used for clinical intravascular and transurethral imaging or for preclinical studies of internal organs in small animals. For example, internal organs such as colon of nude mouse is so small in size that a 2mm-diameter probe would simply break the colon. \(^{52}\) Although the urethra of an adult is about 5-7 mm in diameter for men and larger for women, the ureteral wall injury happens very often while ureteroscopy is performed. \(^{53}\) In particular, a critical size of 1 mm or smaller is required for clinical intravascular imaging. It is, however, rather challenging to engineer a tiny multimodal probe having a diameter of 1 mm or smaller. In the following sections, we describe how we tackled the challenges to develop a novel probe integrating PAI, OCT, and US with a diameter of only 1.0 mm based on a double-clad fiber (DCF).

Material and Methods

Integrated Multimodal Probe

The structure of the probe is schematically shown in Figure 3-1. In this probe, a DCF with a dual cladding structure (DCF13, Thorlabs), in which, single-mode light travels in the Ø9 µm core with a numerical aperture (NA) of 0.12 and a spectrum range of 1250 – 1600 nm, while multi-mode light propagates in the Ø105 µm inner first cladding (NA = 0.2, 400 – 2200 nm), is used to deliver light for both PAI (multi-mode)
and OCT (single-mode). A custom-designed gradient-index (GRIN) lens with a diameter of 0.25 mm and a working distance of 5 mm (GRINTECH GmbH) is utilized to focus the light beam including single-mode (indicated by red dotted line) and multi-mode (indicated by green area) from the tip of DCF with 8° angle facial cutting for minimizing back-reflection. Travelling through a piece of 45°-angle-tilting reflection slide (which is optically transparent but ultrasonically untransparent), the light beam is then reflected into the sample by a 0.5-mm enhanced-aluminum-coated right-angle prism (MPCH-0.5, Tower Optical Corp.). For OCT, the back-scattering light from sample shares the same optical path with light transmission. For PAI, the light-induced photoacoustic signals reflected by prism and reflection slide, are detected by a custom-made unfocused ultrasound transducer with a center frequency of 40 MHz and a dimension of 0.6 mm × 0.5 mm × 0.2 mm (Blatek, Inc., State College, PA) mounted coaxially with respect to the optical path. The generated photoacoustic signal was lost around by ~15% after the two independent reflections, but it was still high enough to obtain good PA images with high contrast. The transducer is also used for US imaging, in which, an image is formed by detecting the echo of the pulsed ultrasound generated by the same transducer. All the components are fixed by three stainless steel tubes (260 µm, 500 µm, and 1000 µm in diameter).

**Assembly of the Integrated Multimodal Probe**

The procedure of assembling the probe is shown in Figure 3-2A. We assembled the probe under a microscope. Firstly, DCF was mounted into stainless steel tubing (SST) 1 (MicroGroup Corp.) with an inner diameter (ID) of 130 µm and an outer diameter (OD) of 260 µm. SST 1 was then fixed with GRIN lens by SST 2 (ID, 270 µm; OD, 500 µm). Two uncoated right-angle prisms (MPU-0.5, Tower Optical Corp.) were
glued using optical adhesive into a prism pair as a reflection slide (Figure 3-1). Since the high acoustic impedance of BK7 glass, of which the prism is made, it was ideal to reflect ultrasound through the hypotenuse of the prism while the bottom of the prism pair was amounted in parallel with the axis of SST 2. Finally, all components were mounted into SST 3 (ID, 920 µm; OD, 1000 µm) using epoxy glue. The axes of these three tubes were in parallel each other. The key to the assembling procedure is that the ultrasound transducer should be placed coaxially with the optical path with respect to the prism pair and coated prism. After the components were fixed in position, we did simple testing for both optics and ultrasound to make sure the assembly was optimized. The photograph of the integrated probe is shown in Figure 3-2B following this procedure.

**Integrated Multimodal Imaging System**

The integrated multimodal imaging system is schematically shown in Figure 3-3. For PAI, a nanosecond pulsed Nd:YAG pumped Optical Parametric Oscillator (OPO) laser having a repetition frequency of 20 Hz was used as light source. The light beam attenuated by a neutral density (ND) filter, and then shaped by a small iris, was focused by a convex lens (L1); the light beam then passed through a 100-µm pinhole for spatial filtering and was coupled into a multimode fiber (MMF) (FG105LCA, Thorlabs). Since a time-domain OCT system was available in our laboratory, hereafter, it was used to demonstrate the feasibility of the integrated probe (this OCT system is compatible to any state-of-art OCT system such as swept source OCT). A broadband light source with a full width at half-maximum (FWHM) of 75 nm and a center wavelength of 1310 nm was utilized for the time-domain OCT system, the optical interface of which was a single mode fiber (SMF) (SMF-28e+, Thorlabs). Through a home-made DCF coupler (Figure 3-4), consisting of four convex lenses and a short-pass dichroic mirror (DM)
(DMSP1180, Thorlabs), the integrated probe was coupled with both PAI and OCT systems based on DCF. A data acquisition (DAQ) card (PCI-5124, National Instrument) embedded in a computer (PC) with 12-bit resolution and a sampling rate of 200 MS/s was utilized as the data acquisition system. For US imaging, an ultrasound pulser/receiver (5073PR, Olympus) with an integrated amplifier and a bandwidth of 75 MHz was utilized to generate ultrasound and to receive echoes. By scanning the probe in x-y plane with a two-dimensional (2D) linear stage, a volumetric image of the sample could be obtained.

Results and Discussion

Phantom Validation

The feasibility of the integrated multimodal probe/imaging system was firstly validated by experiments using a turbid tissue-mimicking phantom (Figure 3-5). The background of the phantom was composed of 2% Agar, TiO2, and India ink with absorption coefficient of 0.007 mm\(^{-1}\) and reduced scattering coefficient of 1.0 mm\(^{-1}\). Two simulated targets embedded in the background phantom with a depth of 0.5 mm and a spacing of 1.0 mm were used: (1) human hair; (2) a piece of pencil lead with a diameter of 0.5 mm. Both hair and pencil lead could be imaged by the three imaging modalities, of which, OCT shows the highest spatial resolution, while US has a relatively lower spatial resolution. For further examination of these images, through pseudo RGB color coding with an equal ratio of PAI (R, red), OCT (G, green), US (B, blue), a fused cross-sectional image is obtained (Figure 3-5E), indicating the matched correlation and complement of the three modalities.
In Vivo Animal Experiments

To demonstrate the capabilities of this integrated multimodal probe, in vivo imaging of a mouse ear (mouse weight=35g) was firstly performed. The laser exposure was about 10 mJ/cm$^2$ at the optical focus inside the ear tissue which is lower than the American National Standards Institute (ANSI) laser safety limit (20 mJ/cm$^2$). An area of 2 mm x 2 mm of the mouse ear was scanned with a scanning step size of 10 μm, which took around 36 minutes.

Before experiment, the hairs on the ear of mouse were gently removed using a human-hair-removing cream. The mouse was placed on a home-made animal holder and anesthetized by a solution of ketamine (85 mg/kg) and xylazine. The mouse was sacrificed according to the University of Florida Institutional Animal Care and Use Committee (IACUC)-approved techniques after the experiment. Strict animal-care procedures approved by the University of Florida IACUC and based on guidelines from the National Institutes of Health (NIH) for the Care and Use of Laboratory Animals were followed.

The top row of Figure 3-6 shows the maximum amplitude projection (MAP) images of PAI (Figure 3-6A), US (Figure 3-6B), OCT (Figure 3-6C), and fused multimodal pseudo-color-coded RGB images (Figure 3-6D) obtained from PAI (R, red), OCT (G, green), US (B, blue). The middle and bottom rows of Figure 3-6 show the cross-sectional images of PAI, US, OCT, and fused multi-modality, corresponding to the two dotted lines in the respective MAP images. We see that PAI, OCT and US, respectively, were able to image the microvasculature, fine structures surrounding the blood vessels and elastic structures. To closely interpret these images, blood vessels in the mouse ear are mapped by PAI with the highest contrast (Figure 3-6A), elastic
structures like cartilage, which have high ultrasound reflection coefficients are clearly visualized by US (Figure 3-6B), while epidermis, dermis, and cartilage are identified in OCT image (Figure 3-6C). Through overlapping the PAI, OCT, and US images (Figure 3-6D), the structures/tissue morphology are better displayed, which shows in vivo imaging feasibilities of the integrated multimodal probe.

**In Vivo Imaging of Human Hand**

We then conducted in vivo imaging of the back of a human hand to explore the potential clinical capabilities of the integrated multimodal probe. A step size of 10 μm was used for scanning the back of hand, and it took around 15 seconds to obtain a b-scan (cross-sectional) image. Figures 3-7A, 3-7B and 3-7C, respectively, show the cross-sectional images of the hand from PAI, US and OCT. Using the same RGB pseudo-coding method as the one used for mouse ear imaging, the fused multimodal image is given in Figure 3-7D. Figure 3-7E is the photograph of the scanning location on the surface of hand indicated by red dotted line.

From Figure 3-7, it is noted that blood vessels (as indicated by a white arrow in Figure 3-7A), superficial fine structures and elastic structures (such as bone, as indicated by a white arrow in Figure 3-7B) are identified by PAI, OCT and US, respectively. Since typically the thickness of mouse ear is less than 2 mm, the deep penetration ability of US was not demonstrated from images shown in Figure 3-6B. Here, in Figure 3-7B, it is evident that US can image the structure of tissue up to 5 mm in depth. Carefully examining Figures 3-7B and 3-7C, we note that superficial surface of tissue having less ultrasonic reflection can be complementarily visualized by OCT with a high resolution. From the fused image (Figure 3-7D), we can see that the morphology of tissue with a depth of up to 5 mm was mapped by our integrated probe.
with a high resolution and that different tissues were imaged by the three different imaging modalities.

**Conclusion**

We have developed a novel miniature device of only 1.0 mm in diameter that integrated PAI, OCT and US in a single probe based on a double-clad fiber. The results from the phantom and in vivo experiments have shown that the integrated multimodal probe has the capability of simultaneously obtaining optical absorption and scattering properties of biological tissue as well as tissue elastic characters in high resolution. We noted that in our experimental setup, the imaging speed was limited by a relatively low repetition frequency of nanosecond pulsed Nd:YAG pumped OPO laser (20 Hz), and a relatively slow approach of time-domain OCT. The low imaging speed would affect the images by sample movements during the acquisition process. For example, in the experiments of in vivo imaging of a mouse ear, for scanning an area of 2 mm x 2 mm of the mouse ear (Figure 3-6) with a scanning step size of 10 μm, it took around 10.8 seconds for obtaining a cross-sectional image and 36 minutes for a volumetric image. Thus, any movements of the mouse ear within 10.8 second would have blurring effect in a cross-sectional image, and within the 36 minutes time period would have blurring effect in the volumetric image. In the experiments of in vivo imaging of human hand, it took around 15 seconds to obtain a cross-sectional image. Thus, any movements within 15 seconds would generate blurring effect in the cross-sectional image. Nevertheless, the advanced probe presented here can be adapted easily with any high-speed PAI system and state-of-art OCT imaging system (such as high-speed swept source OCT). We expect that the speed can reach as high as hundreds of kHz per scanning point using a high repetition rate nanosecond pulsed laser for PAI and a swept source for
OCT. The highly integrating and miniature size of the probe has the potential to generate significant impact in preclinical and clinical applications such as multimodal intravascular coronary imaging, in vivo imaging of internal organs in small animals, ureteroscopy/transurethral imaging, and minimally invasive interventional therapy.
Figure 3-1. The integrated multimodal probe. A) Schematic of the probe, B) Three-dimensional rendering of the probe.

Figure 3-2. Assembling of the integrated multimodal probe. A) Procedure of assembling, and B) Photograph.
Figure 3-3. Integrated multimodal imaging system. ND, neutral density filter; L1, L2, L3, lenses; PH, pinhole; BP, beam splitter; PD, photodiode, AMP, amplifier; RSOD, rapid scanning optical delay; PC, personal computer; MMF, multimode fiber; SMF, single mode fiber; DCF, double-clad fiber.
Figure 3-4. Double-clad fiber coupler. L1, L2, L3, L4, lenses; MMF, multimode fiber; SMF, single mode fiber; DCF, double-clad fiber; DM, dichroic mirror.

Figure 3-5. Cross-sectional images of tissue-mimicking phantom. A) Phantom geometry with 2 simulated targets, B) PAI image, C) US image, D) OCT image, E) Color-coded RGB image. The scale bars indicate 0.3 mm in length.
Figure 3-6. In vivo images of mouse ear by the integrated multimodal probe. Maximum amplitude projection (MAP) (top row) and cross-sectional images (middle and bottom rows) corresponding to the dotted lines shown in the MAP image of A) PAI, B) US, C) OCT, and D) Fused tri-modality. BV, blood vessel; ED, epidermis; D, dermis; CT, cartilage. The scale bars indicate 0.3 mm in length.
Figure 3-7. Cross-sectional images of the skin of human hand. A) PAI image, B) US image, C) OCT image, D) Color-coded RGB image, and E) Photograph of the scanning location (photo courtesy of author). The scale bars indicate 0.3 mm in length.
CHAPTER 4
MULTIMODAL ENDOSCOPE IN CANCER RESEARCH

Motivations

Pancreatic cancer is the fourth leading cause of cancer-related death in the United States (US). It is estimated that 53,670 new cases will be diagnosed and 43,090 pancreatic cancer patients will die in the US in 2017. The Pancreatic Cancer Action Network predicts that pancreatic malignancies will become the second leading cause of cancer-related death by 2020. In pancreatic cancer, the 5-year survival rate is approximately 7%, which is attributed to primary factors including inefficient early diagnostic tools and ineffective treatment methods. Nevertheless, the overall 5-year survival rate is significantly improved to 26% for patients diagnosed in the early stages without metastatic lesions. Therefore, to develop reliable methods that can improve early diagnosis is highly desired.

Conventional diagnostic imaging modalities including X-ray radiography, computed tomography (CT), fluoroscopy, ultrasonography, and magnetic resonance imaging (MRI) have been used for diagnosis and treatment planning for pancreatic cancer medically. However, these conventional imaging modalities provide only structural or anatomical changes which often happen several years after detrimental molecular changes, especially for pancreas-related diseases. The specificity of these conventional imaging techniques for early diagnosis of pancreatic cancer is quite low.

It is believed that molecular imaging is able to offer highly sensitive and specific detection of tumors through sensing the molecular changes. For pancreatic cancer, current existing molecular imaging techniques include positron emission tomography (PET), single-photon emission computed tomography (SPECT), MRI with
contrast agent enhancement (such as magnetic nanoparticles) \(^{64, 65, 66, 67, 68}\), optical/fluorescence imaging \(^{67, 69, 70}\), and photoacoustic imaging (PAI) \(^{71, 72, 73}\). PET and SPECT involve ionization radiation with the long half-life of radiotracers which limits the temporal resolution. In addition, they both have relatively low spatial resolution in localizing the tumors. The relatively slow data acquisition time of MRI often generates motion artifact issue and reduced signal-to-noise \(^{74}\). In optical imaging, fluorescent dyes, quantum dots or nanoparticles conjugated to targeted antibodies or peptides are commonly used as contrast agents. Optical molecular imaging has a relatively high spatiotemporal resolution without ionization radiation. However, the limited tissue penetration capability of light prevents the use of optical techniques for noninvasive imaging of deeply located organs like pancreas.

PAI is an emerging biomedical imaging technique that combines the optical contrast with an increased ratio of imaging depth to spatial resolution capable of providing anatomical, functional, molecular properties of biological tissue with a high resolution \(^{1, 2, 3, 4, 5, 6, 7, 8, 9, 12, 75, 76}\). In PAI, images are formed through detecting pulsed laser-induced wideband acoustic waves. The image contrast in PAI originates from light absorption in tissue. Similar to optical imaging, PAI also suffers from the penetration limitation, and cannot be used to noninvasively image deeply located organs such as pancreas.

The goal of this study is to demonstrate a multimodal endoscopic approach combined with targeted multifunctional IONPs for molecular imaging of pancreatic tumor where the pancreas is directly accessed with minimally invasiveness using a 1mm-
diameter miniature endoscope in combination with a novel fan-shaped two-dimensional (2D) scanning mechanism.

**Materials and Methods**

**Animal model and Multifunctional Nanoparticles**

In this study, mouse pancreatic cancer cell line panc02 derived tumor model was used. Two groups of six mice (three in one group) were respectively received the injection of three types of multifunctional nanoparticles: 1) near-infrared (NIR) 830-maleimide fluorescence dye conjugated to the IONPs without targeting ligands, NIR830-IONP; 2) NIR830-IONP plus conjugation of ATF targeting, NIR830-ATF-IONP; 3) NIR830-ATF-IONP plus polyethylene glycol (PEG) coating, NIR830-ATF-PEG-IONP. PEG was used to stabilize the nanoparticles and modify surface properties to reduce non-specific uptake of nanoparticles by macrophages in the reticuloendothelial system to improve targeted delivery of the nanoparticles.77 Strict animal care procedures approved by the Emory University IACUC and based on guidelines from the NIH, guide for the Care and Use of Laboratory Animals were followed. Mice were sacrificed using Emory University Institutional Animal Care and Use Committee (IACUC)-approved techniques. Then the mice were frozen under negative 80 °C and delivered to University of Florida for imaging experiments.

**Miniaturized Endoscopic Imaging System and Scanning Mechanism**

The multimodal endoscopic probe with a size of 1 millimeter in diameter described in detail before16 was used to directly reach the surface of the pancreas through a hole in the abdomen of mouse. In brief, a double-clad fiber was integrated into the probe for delivering light beam, which was then focused by a gradient-index (GRIN) lens with a diameter of 0.25 mm and a working distance of 5 mm. A custom-
made unfocused ultrasound transducer with a center frequency of 40 MHz and a
dimension of 0.6 mm × 0.5 mm× 0.2 mm was used to detect the photoacoustic signals.
Here, the photoacoustic imaging was performed. The imaging system is schematically
shown in Figure 4-2. The probe was mounted to the stage consists of one-dimensional
(1D) linear stage and a rotator. A nanosecond pulsed Nd:YAG pumped Optical
Parametric Oscillator (OPO) laser having a repetition frequency of 20 Hz was used as
light source. The light beam attenuated by a neutral density (ND) filter, was then split
into two parts. One part reached the photodiode (PD) module to monitor light intensity in
real time for calibration. The other part then shaped by a small iris, was focused by a
convex lens (L1); then the light beam passed through a 100-µm pinhole for spatial
filtering and was coupled into the double-clad fiber in the multimodal endoscopic probe.
A data acquisition (DAQ) card (PCI-5124, National Instrument) embedded in a computer
(PC) with 12-bit resolution and a sampling rate of 200 MS/s was utilized as the data
acquisition system. And, an ultrasound receiver (5073PR, Olympus) with an integrated
amplifier and a bandwidth of 75 MHz was utilized to receive the photoacoustic signal.
The laser exposure was about 8 mJ/cm² at the surface of the tissue which is lower than
the American National Standards Institute (ANSI) laser safety limit (20 mJ/cm²).

To minimize the invasiveness of plugging the endoscopic probe into the
abdomen of a mouse, a novel fan-shaped scanning method was developed. As shown
in Figure 4-3, a hole with a size of slightly larger than 1mm in diameter was drilled
through the abdomen of mouse. A volumetric image of the tissue was obtained by
scanning the probe through the tissue in a two-dimensional (2D) fan-shaped plane with
the hole location as the center. The 2D fan-shaped scanning (20 mm (radius) x 60
degrees) was realized through the combination of a linear translation along radial direction (a step size of 30 μm) and a circular rotation (a step size of 0.3 degree).

**Near-Infrared Planar Fluorescence Imaging System**

A conventional near-infrared planar fluorescence imaging system was built to obtain 2D fluorescence images after each endoscopic imaging experiment for further cross validation. As schematically shown in Figure 3, a CW 785 nm laser (M5-785-0080, Thorlabs) was used as the light source. The light beam was split into two parts that were respectively coupled into two fiber bundles, and then traveled through light diffusers (DG10-1500, Thorlabs) in order to generate homogeneous illumination on the sample mounted on the sample holder. The induced fluorescence signal was collected by a fast charge-coupled device camera (CoolSNAP EZ, Photometrics) with a high performance fluorescent band-pass filter (NT86-381, Edmund Optics) mounted in the front for filtering out non-fluorescence signal. The laser power used for illumination was the same in all experiments.

**Image Processing**

For endoscopic photoacoustic imaging, the collected photoacoustic signals were first processed through Hilbert transform, and then applied with the time-reversal reconstruction algorithm implemented in Matlab 8.6 to obtain 2D images. These reconstructed 2D images were imported into an image processing platform (Amira 5.4.2) to obtain a 3D volumetric image. The photoacoustic signals from each mouse were calibrated with the laser power used and normalized to the same scale. And all endoscopic photoacoustic images were displayed in the form of maximum amplitude projection (MAP).
For fluorescence imaging, images were collected by a program implemented in National Instruments (NI) Labview, followed by a post-processing tool box in Matlab 8.6. Like photoacoustic imaging, fluorescence signals from each mouse were normalized to the same scale for comparison.

**Results and Discussion**

The previous study\(^{78}\) showed that the light absorbance of IONPs increases with decreasing the wavelength, however, considering the fact that tissue penetration depth is greater in the NIR region, light with a wavelength of 730 nm was chosen as the wavelength of the light source for inducing photoacoustic signals in our experiments.

Figure 4-5A-4-5C, respectively, show photoacoustic MAP images from the mice of Group 1 administered with non-targeted nanoparticles NIR830-IONP, targeted NIR830-ATF-IONP, and PEG-coated targeted NIR830-ATF-PEG-IONP. To closely investigate these images, in Figure 4-5A, we can see that, we can see that the contrast of tumor to normal tissue is too low to identify the tumor with non-targeted NIR830-IONP. However, for the tumor having targeted NIR830-ATF-IONP or PEG-coated targeted NIR830-ATF-PEG-IONP, much enhanced contrast of tumor to normal tissue is seen from Figs. 4-5B and 4-5C where the tumor area with a clear tumor boundary is pinpointed. Take a comparison between Figure 4-5B and 4-5C, PEG coating deteriorates the contrast of tumor to the background, but increases the specificity of tumor detection with a more clearly defined tumor boundary. We then selected region of interest (ROI) within the tumor area, and computed the signal to background ratio (SBR). We plotted the SBR for the mice received the injection with three different nanoparticles as shown in Figure 4-5D for quantitatively analysis. From these plots, we can see that mouse with targeted NIR830-ATF-IONP has the highest SBR (\(~ 145,\)
43dB), while the value of mouse with non-targeted NIR830-IONP is the lowest (~ 24, 28dB). PEG coating reduces the uptake of nanoparticles by cells like macrophages within the tumor, which is indicated by the contrast of mouse with targeted NIR830-ATF-PEG-IONP (~ 102, 40dB).

After the endoscopic photoacoustic imaging, we then conducted experiments using the NIR planar fluorescence imaging system. Figure 4-6A ~ 4-6C shows the fluorescence images from mice in Group 1 injected with NIR830-IONP, NIR830-ATF-IONP and NIR830-ATF-PEG-IONP, respectively. The highest contrast in the image for the mouse with non-targeted NIR830-IONP (Figure 4-6A) come from both the tumor (indicated by red arrow) and normal tissue (indicated by blue arrow). Thus, the specificity is too low to identify the tumor. On the contrary, for the mouse having targeted agent of NIR830-ATF-IONP (Figure 4-6B) or NIR830-ATF-PEG-IONP (Figure 4-6C) shows much enhanced image contrast to allow the tumor and the tumor boundary to be identified clearly. Taking a close look at the difference between images for NIR830-ATF-IONP and NIR830-ATF-PEG-IONP, we note that PEG coating reduces tissue uptake of nanoparticles, especially in organs without tumor as indicated by white arrow in Figure 4-6B and 4-6C. We also plotted the SBR of the ROI from these three mice as shown in Figure 4-6D, where we see similar SBR values to that for photoacoustic images (Figure 4-5D). The mouse with NIR830-IONP has a SBR of ~35 (31 dB), while the mice with NIR830-ATF-IONP or NIR830-ATF-PEG-IONP, respectively, have the SBR of ~144 (43 dB), ~101(40 dB).

The endoscopic photoacoustic images and quantitative plots for the second group of mice are shown in Figure 4-7, while the corresponding fluorescence images
and plots are shown in Figure 4-8. These images show image quality that are comparable to that for the first group of mice.

**Conclusion**

This work represents the first report on multimodal photoacoustic-fluorescence molecular endoscopic imaging of pancreatic cancer using a miniature probe and targeted multifunctional magnetic iron oxide nanoparticles in a mouse pancreatic cancer model. With the novel fan-shaped scanning mechanism, the endoscopic probe can directly reach the surface of pancreas through abdomen with minimal invasiveness. The photoacoustic and fluorescence signals of the tumor region were significantly improved with targeted multifunctional nanoparticles. This study indicates the potential of targeted multimodal photoacoustic-fluorescence endoscopic molecular imaging for early detection of pancreatic cancer.
Table 4-1. List of multifunctional nanoparticles with/without targeting

<table>
<thead>
<tr>
<th>Nanoparticle Type</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>NIR830-IONP</td>
<td>Non-targeted, only has the near-infrared (NIR) 830-maleimide dye conjugated to the magnetic iron oxide nanoparticle (IONP)</td>
</tr>
<tr>
<td>NIR830-ATF-IONP</td>
<td>NIR830-IONP + conjugation of amino-terminal fragment (ATF) targeting</td>
</tr>
<tr>
<td>NIR830-ATF-PEG-IONP</td>
<td>NIR830-ATF-IONP plus polyethylene glycol (PEG) coating</td>
</tr>
</tbody>
</table>
Figure 4-1. The spectral characterization (excitation wavelength: 800 nm and emission wavelength: 825 nm) of NIR-830 dye.

Figure 4-2. Endoscopic Imaging System. ND, neutral density filter; L1, L2, L3, lenses; PH, pinhole; BP, beam splitter; PD, photodiode; DAQ, data acquisition; US, ultrasound.
Figure 4-3. Scanning Mechanism.

Figure 4-4. Near-Infrared Planar Fluorescence Imaging System. BP, beam splitter; L1, L2, lens; DF, diffuser.
Figure 4-5. Endoscopic photoacoustic images of pancreatic tumor from mice in Group 1. Maximum amplitude projection (MAP) images of A) mouse injected with NIR830-ATF-PEG-IONP; B) mouse injected with NIR830-ATF-IONP; and C) mouse injected with NIR830-IONP; D) quantitative plot and comparison of average contrast in region of interest (ROI) (pancreatic tumor). The scale bars indicate 3 mm in length.
Figure 4. Fluorescence images of pancreatic tumor from mice in Group 1.
Photographs were merged with fluorescence images from A) mouse injected with NIR830-ATF-PEG-IONP; B) mouse injected with NIR830-ATF-IONP; and C) mouse injected with NIR830-IONP; D) quantitative plot and comparison of average contrast in region of interest (ROI) (pancreatic tumor).
Figure 4-7. Endoscopic photoacoustic images of pancreatic tumor from mice in Group 2. Maximum amplitude projection (MAP) images of A) mouse injected with NIR830-ATF-PEG-IONP; B) mouse injected with NIR830-ATF-IONP; and C) mouse injected with NIR830-IONP; D) quantitative plot and comparison of average contrast in region of interest (ROI) (pancreatic tumor). The scale bars indicate 3 mm in length.
Figure 4-8. Fluorescence images of pancreatic tumor from mice in Group 2. Photographs were merged with fluorescence images from A) mouse injected with NIR830-ATF-PEG-IONP; B) mouse injected with NIR830-ATF-IONP; and C) mouse injected with NIR830-IONP; D) quantitative plot and comparison of average contrast in region of interest (ROI) (pancreatic tumor).
CHAPTER 5
MULTIMODAL ENDOSCOPE FOR INTRAVASCULAR IMAGING

Motivations

Cardiovascular disease is No.1 killer of Americans \(^{79}\). Every year, more than 1 million people in the United States experience cardiovascular events and the major cause of them is the rupture of an unstable atherosclerotic plaque \(^{80,81}\), thus, assessment of vulnerability of these plaques is crucial \(^{82,83,84,85}\). The majority of unstable plaques can be featured as a large lipid core and a thin fibrous cap with a thickness of less than 65 µm infiltrated by macrophages \(^{86,87,88}\). Several noninvasive and invasive methods have been developed to evaluate the atherosclerotic plaques during the past decades. Currently, invasive catheter-based intravascular imaging modalities, such as catheter-based x-ray angiography \(^{89}\), intravascular ultrasound (IVUS) \(^{90}\), near-infrared spectroscopy (NIRS) \(^{91}\), optical coherence tomography \(^{92}\), are still the standard for detection of atherosclerotic plaques with the capabilities of offering 3D structural, functional, compositional, biochemical, molecular information of plaque lesions compared to noninvasive imaging modalities like CT \(^{93}\), MRI \(^{94,95}\), ultrasound \(^{96}\), PET \(^{83}\), SPECT \(^{97}\). However, these techniques still suffer from various limitations for characterization of vulnerable coronary plaques. For example, for all kinds of noninvasive imaging modalities, their specificity and spatial resolution are relatively low and avoiding the cardiac motion artefacts is realistically difficult \(^{98}\). In x-ray angiography, 3D lumen shape can only be approximately estimated by 2D projected silhouette with no compositional distribution \(^{99}\). IVUS can offer lumen geometry and the structural information of vessels with a spatial resolution of ~100 µm, but due to the low contrast of ultrasound between soft tissue types, the sensitivity and specificity for plaque
composition is very limited. Intravascular NIRS can offer biochemical properties of plaques based on the different light absorption and scattering coefficients of different molecules but little structural information and very limited tissue penetration. OCT has a high spatial resolution (~10 µm) and provides better information of lumen structure, fibrous cap thickness, macrophages but with limited penetration depth of 1-2 mm and limited compositional information.

Intravascular photoacoustic imaging (IVPA) is an emerging catheter-based 3D imaging modality with the potential of providing both structural and compositional properties of tissue components in the vessel wall with deep penetration depth and high specificity for lipid type based on the fact that the different optical absorption coefficients of different molecules. However, due to the strong optical scattering in human tissue, spatial resolution and penetration depth are two contrary sides for different photoacoustic imaging modalities. OR-PAM has the highest spatial resolution but smallest penetration depth, while, ARPAM has moderate spatial resolution and penetration depth, and photoacoustic tomography (PAT) has lowest resolution but deepest penetration depth. Presently, for IVPA, most of them use PAT modality to archive deepest penetration and provide biochemical components in the vessel wall for identification of lipid core. But, it is hard to characterize some small components of plaques, such as thin fibrous cap with a thickness of less than 65 µm. In this regard, some studies took an initial step towards intravascular imaging using PAM modality. But, the resolution is not as high as the resolution of OCT for superficial structures. Moreover, the structural information offered by IVPA is not as good as IVUS.
Multimodal intravascular imaging combining PAM, OCT, US capable of providing 3D information of structural, compositional, biochemical and molecular features of coronary lesions will offer clinicians with a critically important tool for the detection and assessment of coronary atherosclerotic plaques. It combines the advantages of functional, compositional and molecular contrast of PAM, high spatial resolution of OCT, broad imaging depth and well structural mapping ability of US.

**Label-free plaque characterization**

**Materials and Methods**

**Human Artery Sample Preparation**

Human artery samples with atherosclerotic plaques were collected from the Department of Surgery of Shands Hospital at the University of Florida. Before the experiments, the samples were immersed into formalin solution. The samples were firstly utilized for multimodal imaging with the integrated probe, and then sent to the Molecular Pathology Core at the University of Florida for histology examination.

**Photoacoustic Microscopic Imaging System**

A conventional acoustic-resolution photoacoustic microscopy setup was built to demonstrate the capability of photoacoustic imaging for characterizing the vessel wall, in particular, artery with atherosclerotic plaques. The system is schematically shown in Figure 5-1. A wavelength tunable nanosecond pulsed OPO laser was used to induce photoacoustic signal. The light beam was split into two beams and delivered by fiber bundles to illuminate test samples. At each imaging probe position, the induced photoacoustic signal was detected by a focused transducer (50 MHz central frequency, 3 mm aperture and 6 mm focal length). The photoacoustic signal was then amplified and digitalized by a 12-bit data acquisition board (PCI-5124, National Instrument) at a
sample rate of 200 MS/s. One or two-dimensional raster scanning of the imaging probe along the horizontal plane coupled with the depth-resolved ultrasonic detection formed a 2D or 3D photoacoustic image.

**Multimodal Endoscopic Imaging System**

The multimodal endoscopic probe with a size of 1 millimeter in diameter described in detail before\(^1\) was used for mapping vessel wall. The integrated multimodal imaging system is schematically shown in Figure 5-2. The probe was mounted to the stage consists of one-dimensional (1D) linear stage and a rotator. The rotator was used to obtain a 2D cross-sectional image. Together with a pull-back scanning driven by 1D linear stage, a 3D volumetric image can be obtained. For photoacoustic imaging, a nanosecond pulsed Nd:YAG pumped Optical Parametric Oscillator (OPO) laser having a repetition frequency of 20 Hz was used as light source. The light beam attenuated by a neutral density (ND) filter, and then shaped by a small iris, was focused by a convex lens (L1); the light beam then passed through a 100-µm pinhole for spatial filtering and was coupled into a multimode fiber (MMF) (FG105LCA, Thorlabs). For OCT, a broadband light source with a full width at half-maximum (FWHM) of 75 nm and a center wavelength of 1310 nm was utilized for the time-domain OCT system, the optical interface of which was a single mode fiber (SMF) (SMF-28e+, Thorlabs). Through a home-made DCF coupler consisting of four convex lenses and a short-pass dichroic mirror (DM) (DMSP1180, Thorlabs) described in detail in Chapter 3, the integrated probe was coupled with both PAI and OCT systems based on DCF. A data acquisition (DAQ) card (PCI-5124, National Instrument) embedded in a computer (PC) with 12-bit resolution and a sampling rate of 200 MS/s was utilized as the data
acquisition system. For US imaging, an ultrasound pulser/receiver (5073PR, Olympus) with an integrated amplifier and a bandwidth of 75 MHz was utilized to generate ultrasound and to receive echoes. The laser exposure was about 10 mJ/cm² at the surface of the tissue which is lower than the American National Standards Institute (ANSI) laser safety limit (20 mJ/cm²).

**Histological Analysis**

After performing multimodal imaging, the human artery samples were immersed into 10% formalin solution. Then the samples were sent to Molecular Pathology Core at the University of Florida for histology examination. Histological sections were applied with H&E stain and analyzed using standard procedures to confirm the presence of atherosclerotic plaques.

**Results and Discussion**

**Plaque characterization with photoacoustic microscopy**

Referring to the optical absorption spectra of various tissue types (Figure 5-3) ¹¹⁰, we used dual wavelength (750 nm and 1210 nm) for photoacoustic imaging lipid-rich plaques (Figure 5-4). Lipid-rich core in the vessel wall have a strong optical absorption around 1210 nm, which can be clearly seen in the photoacoustic microscopic image through detection of the photoacoustic signal by pulsed laser with a wavelength of 1210 nm (Figure 5-4B). For the wavelength of 750 nm (Figure 5-4A), other components such as intima in the vessel wall have strong optical absorptions. Combining the photoacoustic images with the dual wavelength, the compositional properties of plaque can be acquired (Figure 5-4C).

The results have demonstrated that the feasibility of obtain the compositional information of atherosclerotic plaques within the vessel wall through spectroscopic
photoacoustic imaging benefited from the fact that different molecules have different light absorption coefficients.

**Plaque characterization with multimodal endoscopy**

We then conducted ex vivo experiments of human arteries with atherosclerotic plaques to demonstrate the capability of the integrated multimodal endoscope for intravascular imaging. The multimodal images of an artery sample with lipid-rich plaques were acquired by rotatory scanning with 1200 samples/circle and pulling back with a step size of 60 μm. The artery sample was fixed using a cylindrical holder made of 2% agar solution and coupled with the probe by water. For PAI, light with a wavelength of 1210 nm, at which, lipid has an absorption peak, was utilized to excite the photoacoustic signal.

Figures 5-5A, 5-5B and 5-5C, respectively, show the cross-sectional images of one of the samples from PAI, US and OCT. The fused multimodal image using RGB pseudo-coding is given in Figure 5-5D, while the histological image of the sample by H&E stain is shown in Figure 5-5E. Figure 5-6 shows the cross-sectional and three-dimensional PAI, US and OCT images of the sample.

Closely inspecting these images in comparison with the histology (Figure 5-5E), regions of plaque with rich lipid are mapped by PAI with high contrast, as indicated by yellow arrows in Figure 5-5A (the area in the image with high pixel intensity) and Figure 5-5E. The superficial structure of the vessel wall like thin fibrous cap, as indicated by red arrow in Figure 5-5C (a thin bright layer with a dark area) and Figure 5-5E, is clearly imaged by OCT with high resolution, while US is able to obtain the structure of the whole vessel wall (Figure 5-5B), attributing to the strength of deep tissue penetration of
ultrasound. We note that the fused image of PAI, OCT, and US (Figure 5-5D) certainly provides more complete structural and compositional information of the vessel wall/plaque.

To show the capability of the integrated multimodal endoscope, we further conducted spectroscopic photoacoustic imaging on the human artery samples with atherosclerotic plaques using six wavelengths of 710 nm, 800 nm, 900 nm, 1100 nm, 1150 nm and 1210 nm (Figure 5-7). These multi-spectral PAI images illustrate that multiple chemical components of plaque can be inferred/obtained for more accurate correlation with plaque vulnerability. For example, elastin and collagen which have relatively high light absorption coefficients under 1000 nm, would appear as high intensity pixels in the PAI images (Figure 5-7A-C). While lipid, which has an absorption peak around 1210 nm, showed the brightest areas in Figures 5-7E and 5-7F. Combining these chemical compositional information with the structural images obtained from US (Figure 5-5B) and OCT (Figure 5-5C), the properties of artery wall as well as plaques can be visualized more completely.

Figure 5-8 and 5-9 respectively show the cross-sectional and three-dimensional multimodal images from another human artery sample with atherosclerotic plaques, while Figure 5-10 shows the spectroscopic photoacoustic images of the sample. Carefully investigating these images, the results are consist with the results from sample #1.

**Molecular intravascular imaging with targeted nanoprobes**

**Material and Methods**

**Animal model and Multifunctional Nanoparticles**
In this study, two groups of six mice (three in one group) were respectively received the injection of three types of multifunctional nanoparticles: 1) near-infrared (NIR) 830-maleimide fluorescence dye conjugated to the IONPs without targeting ligands, NIR830-IONP; 2) NIR830-IONP plus conjugation of ATF targeting, NIR830-ATF-IONP; 3) NIR830-ATF-IONP plus polyethylene glycol (PEG) coating, NIR830-ATF-PEG-IONP. PEG was used to stabilize the nanoparticles and modify surface properties to reduce non-specific uptake of nanoparticles by macrophages in the reticuloendothelial system to improve targeted delivery of the nanoparticles. Strict animal care procedures approved by the Emory University IACUC and based on guidelines from the NIH, guide for the Care and Use of Laboratory Animals were followed. Mice were sacrificed using Emory University Institutional Animal Care and Use Committee (IACUC)-approved techniques. Then the mice were frozen under negative 80 °C and delivered to University of Florida for imaging experiments. In each experiment, multimodal endoscopic imaging was firstly performed. Then, aorta of the sample was collected and dissected for 2D fluorescence imaging.

**Multimodal Endoscopic Imaging System**

The multimodal endoscopic probe with a size of 1 millimeter in diameter described in detail before was used for mapping vessel wall. The integrated multimodal imaging system was described in detail in the above section (Figure 5-2). In this study, for photoacoustic imaging, the nanosecond pulsed Nd:YAG pumped Optical Parametric Oscillator (OPO) laser was tuned to generate a light beam with a wavelength of 730 nm based on our previous study. And the laser exposure was
about 7 mJ/cm² at the surface of the tissue which is lower than the American National Standards Institute (ANSI) laser safety limit (20 mJ/cm²).

**Near-Infrared Planar Fluorescence Imaging System**

For cross validation, a conventional near-infrared planar fluorescence imaging system was built to obtain 2D fluorescence images after each endoscopic imaging experiment. The system was described in detail in Chapter 4 (Figure 4-4). In brief, a CW 785 nm laser (M5-785-0080, Thorlabs) was used as light source to excite the fluorescence emission with a wavelength of 830 nm that can be detected by a fast charge-coupled device camera (CoolSNAP EZ, Photometrics) with a high performance fluorescent band-pass filter (NT86-381, Edmund Optics) mounted in the front for filtering out non-fluorescence signal. The excitation laser power used for illumination was the same in all experiments.

**Image Processing**

In multimodal endoscopic imaging, each frame contains information of PAI, OCT and US. For PAI, the collected photoacoustic signals were firstly processed through Hilbert transform, then applied with the reconstruction algorithm implemented in Matlab 8.6 to obtain 2D images. These reconstructed 2D images were then imported into an image processing platform Amira 5.4.2 to obtain a 3D volumetric image. The photoacoustic signals from each mouse were calibrated with the laser power and normalized to the same scale. For OCT, the envelope of the detected signals back-scattered from the depth inside the sample that matches the optical path length of the reference arm within the coherence length of the light source was decoded to form the image representing the reflective profile as a function of the depth. For US, the collected
echo of ultrasound was firstly filtered by a band-pass filter (pass band: 10 MHz ~ 70 MHz). B-mode ultrasound images were formed through combining echo of ultrasound. The overlap of tri-modal images were obtained through pseudo RGB color coding with equal ratio.

For fluorescence imaging, images were collected by a program implemented in NI Labview, followed by a post-processing in Matlab 8.6. Like photoacoustic imaging, fluorescence signals from each mouse were normalized to the same scale for comparison.

**Histological Analysis**

After performing multimodal imaging, the aorta samples were sent to Molecular Pathology Core at the University of Florida for histology examination. Histological sections were applied with H&E stain and analyzed using standard procedures to confirm the presence of atherosclerotic plaques.

**Results and Discussion**

Figure 5–11A~5-11C respectively show cross-sectional PAI, OCT, US images from mice in Group 1 with the contrast agent of non-targeted nanoparticles NIR830-IONP (up row), targeted NIR830-ATF-IONP (middle row), and targeted with PEG-coating NIR830-ATF-PEG-IONP (bottom row). From these images, we can see that, PAI provides optical absorption information of the vessel wall, especially, that areas with strong absorption within the wavelength of 730 nm have the highest contrast. While, either OCT or US can offer structural properties of the whole vessel wall since the vessel wall is thin enough (hundreds of micrometer) to be mapped by OCT and US. The difference between OCT and US images lies in two parts: (1) OCT has higher spatial
resolution can provide more fine structure (Figure 5-11B); (2) US can provide mechanical properties of tissue (indicated by blue arrows in Figure 5-11C). Fused images can obviously provide relatively complete structure of vessel wall (Figure 5-11D). Closely investigating these images, we can see that, the contrast of plaque to vessel wall is too low to identify the margin of the plaque with the agent of non-targeted NIR830-IONP (top row of Figure 5-11). Either with or without PEG coating targeted nanoparticles highly enhance the contrast of plaque to vessel wall, which is good enough to pinpoint the plaque area with a clear boundary (the areas indicated by red arrows in Figure 5-11A, or the areas with red color in Figure 5-11D). Particularly, from the middle and bottom row fused images in Figure 5-11D, we can not only get the relatively complete information of vessel wall but also be able to pinpoint the areas with plaque as these areas have the highest contrast enhanced by targeted nanoparticles (NIR830-ATF-IONP and NIR830-ATF-PEG-IONP). Take a comparison between with (bottom row of Figure 5-11) and without (middle row of Figure 5-11) PEG coating, we can see that PEG coating deteriorates the contrast of plaque to the background, but it enhances the specificity. Figure 5-12A~5-12C respectively show the three-dimensional volumetric PAI, OCT, and US images.

After performing endoscopic imaging, the aorta samples were collected and dissected for fluorescence imaging using the near-infrared planar fluorescence imaging system. Figure 5-11E shows the fluorescence images from mice in Group 1 injected with NIR830-IONP (top row), NIR830-ATF-IONP (middle row) and NIR830-ATF-PEG-IONP (bottom row) respectively. Areas with highest contrast in the image from mouse with non-targeted NIR830-IONP (top row in Figure 5-11E) locate both in the plaque
(indicated by white arrow) and normal tissue (indicated by green arrow). On the contrary, targeted agents either NIR830-ATF-IONP (middle row in Figure 5-11E) or NIR830-ATF-PEG-IONP (bottom row in Figure 5-11E) highly enhanced the image contrast in the plaque. The contrast is sufficient enough to pinpoint the plaque and identify the plaque boundary. Take a closely look at the difference between images from NIR830-ATF-IONP and NIR830-ATF-PEG-IONP, PEG coating reduces vessel wall’s uptake of nanoparticles. The blue dashed lines in Figure 5-11E indicate the location of their corresponding endoscopic cross-sectional images. By comparing these images with endoscopic images, it is confident that these results consist with that from endoscopic imaging.

The results of endoscopic imaging and its corresponding fluorescence imaging with mice in Group 2, were respectively shown in Figure 5-13 and 5-14. We can see similar trend as the results from mice in Group 1, which further demonstrated the capability and feasibility of our multimodal endoscope for molecular intravascular imaging with nanoparticles.

**Conclusion**

The application studies in this chapter have demonstrated the capability of our multimodal endoscope for intravascular imaging either label-free or with agent enhancement. We have shown that it is feasible to obtain compositional information of atherosclerotic plaques with spectroscopic photoacoustic imaging. Through performing experiments using human artery samples with atherosclerotic plaques, it has shown that multimodal endoscope integrating PAI, OCT, and US is able to characterize plaques without any exogenous agents labeled to plaques. The multimodal endoscope can not only provide information about plaques which have strong light absorption under certain
illumination but also offer the back-scattering and mechanical structural properties of surrounding vessel wall with high spatial resolution. With the miniature size of 1 mm in diameter, the multimodal endoscope is clinically translatable for intravascular imaging for assessing the atherosclerotic plaques such as vulnerability. With the agent enhancement with multifunctional nanoparticles, the multimodal endoscope was demonstrated to be able to map mouse aorta with tiny plaques with high resolution and specificity. It further shows the feasibility of our developed multimodal endoscope for molecular intravascular imaging on small animals, which will benefit preclinical studies in laboratory.
Figure 5-1. Schematic of acoustic-resolution photoacoustic microscopy. BS, beam splitter; L1, L2, lens; DAQ, data acquisition; PC, personal computer.

Figure 5-2. Multimodal endoscopic imaging system. ND, neutral density filter; L1-L7, lenses; PH, pinhole; BP, beam splitter; PD, photodiode, AMP, amplifier; RSOD, rapid scanning optical delay; PC, personal computer; MMF, multimode fiber; SMF, single mode fiber; DCF, double-clad fiber; DM, dichroic mirror.
Figure 5-3. Optical absorption spectra of various tissue types.

Figure 5-4. Photoacoustic microscopic images of human atherosclerotic artery sample. Photoacoustic images obtained at A) 750 nm and B) at 1210 nm, C) fused image from the two wavelengths.
Figure 5-5. Cross-sectional images of human artery #1 with atherosclerotic plaque. A) PAI image, B) US image, C) OCT image, D) Color-coded RGB image, and E) Histology using H&E stain. The scale bars indicate 2 mm in length.

Figure 5-6. Cross-sectional (top row) and three-dimensional (bottom row) images of human artery #1 with atherosclerotic plaques. A) PAI image, B) US image, C) OCT image. The scale bars indicate 2 mm in length.
Figure 5-7. Spectroscopic photoacoustic images of human artery #1 with atherosclerotic plaques. PA images at 710 nm A), 800 nm B), 900 nm C), 1100 nm D), 1150 nm E), and 1210 nm F). The top and bottom rows, respectively, show the cross-sectional and three-dimensional images. The scale bars indicate 2 mm in length.

Figure 5-8. Cross-sectional images of human artery #2 with atherosclerotic plaque. A) PAI image, B) US image, C) OCT image, D) Color-coded RGB image, and E) Histology using H&E stain. The scale bars indicate 2 mm in length.
Figure 5-9. Cross-sectional (top row) and three-dimensional (bottom row) images of human artery #2 with atherosclerotic plaques. A) PAI image, B) US image, C) OCT image. The scale bars indicate 2 mm in length.
Figure 5-10. Spectroscopic photoacoustic images of human artery #2 with atherosclerotic plaques. PA images at 710 nm A), 800 nm B), 900 nm C), 1100 nm D), and 1210 nm E). The top and bottom rows, respectively, show the cross-sectional and three-dimensional images. The scale bars indicate 2 mm in length.

Figure 5-11. Multimodal endoscopic images and fluorescence images of mice aorta in Group 1. A) PAI images, B) OCT images, C) US images, D) Color-coded RGB image fusing PAI, OCT and US, E) fluorescence images. The scale bars indicate 1 mm in length.
Figure 5-12. Three-dimensional images of mice aorta in Group 1. A) PAI images, B) US images, C) OCT images.
Figure 5-13. Multimodal endoscopic images and fluorescence images of mice aorta in Group 2. A) PAI images, B) OCT images, C) US images, D) Color-coded RGB image fusing PAI, OCT and US, E) fluorescence images. The scale bars indicate 1 mm in length.
Figure 5-14. Three-dimensional images of mice aorta in Group 2. A) PAI images, B) US images, C) OCT images.
CHAPTER 6
FULLY INTEGRATED FIBER-FREE ENDOSCOPE

Motivations

Since the invention of fiber-optic endoscopy\textsuperscript{112}, it has been widely used for visualization of internal organs in human such as stomach\textsuperscript{113, 114, 115}, bowel\textsuperscript{116, 117}, esophagus\textsuperscript{118, 119, 120}, and so on. Fiber-optic endoscopy has become a gold standard tool in clinic for diagnosis and therapy of many internal organs like optical colonoscopy for aiding diagnosis and therapeutics of colorectal diseases\textsuperscript{121, 122, 123, 124}. However, fiber-optic endoscopy is constrained by problems of discomfort and adverse-effect, and limitations of organ reachability. For example, there’re many adverse events reported in the past while using upper gastrointestinal (GI) endoscopy including infection, perforation, bleeding, and so forth\textsuperscript{125}. There’s a clinical need for examining the entire small bowel, however, it is hardly to be done with conventional fiber-based optical endoscopy. To tackle these challenges, swallowable capsules were developed to measure GI physiological parameters including temperature, pressure, and pH value\textsuperscript{126, 127, 128}. As the invention of wireless video-telemetry capsule endoscopy\textsuperscript{129}, a new generation of fully integrated fiber-free endoscope, namely wireless capsule endoscope, has widely used for imaging small bowel with its invasiveness and capability of reaching the entire small bowel. Since 2001 when the Food and Drug Administration (FDA) approved wireless capsule endoscope for clinical use, it has been demonstrated that wireless capsule endoscopy is an extremely efficient tool for offering high quality images of the entire small bowel which were inaccessible to conventional fiber-based optical endoscopy\textsuperscript{130, 131}. 
While inspiring, wireless capsule endoscopy up to date is a typical camera to some extent, it can only provide surface profile information of internal organs. It is highly desired to develop a fully integrated wireless capsule endoscopy which is able to provide functional, compositional, structural, and molecular properties of tissue to advance the aid of diagnosis and therapeutics of internal organ diseases. Before we can get to that step, there’s an important stage we need to reach, that is fully integrated fiber-free endoscopy. In this Chapter, we will describe our efforts in developing fully integrated fiber-free endoscopy, specially, fiber-free photoacoustic endoscopy.

The most critical components in photoacoustic endoscopy is the nanosecond pulsed laser with high energy. At present, bulky size and heavy weight lasers including Q-switched Nd: YAG laser, Ti: Sapphire laser, optical parametric oscillator (OPO), and dye laser are most commonly used as excitation sources in photoacoustic imaging systems. In conventional photoacoustic endoscopy, light from these lasers were delivered by optical fiber or fiber bundles. It is obvious that these bulky lasers cannot be integrated into a tiny endoscopic probe to implement fiber-free endoscopy. Therefore, the first challenge is to prove the feasibility of exciting photoacoustic signals with compact miniature light source with relatively low output energy like light emitting diode (LED) and Vertical-Cavity Surface-Emitting Laser (VCSEL). In the first and second section, we describe our approval of photoacoustic imaging with LED and VCSEL. Then in the last section, we will introduce VCSEL-based fully integrated fiber-free endoscope.

**Photoacoustic Imaging with a Miniature Light Emitting Diode Excitation**

**Motivations**

Nanosecond pulsed lasers with relatively high energies (mJ) including Q-switched Nd: YAG laser, Ti: Sapphire laser, optical parametric oscillator (OPO), and dye
laser, have played an essential role in the development of photoacoustic imaging technique. However, their bulky size, high cost and strict maintenance requirements, significantly limit their practical biomedical applications.

Laser diodes (LDs) are proposed to address some of these drawbacks as LDs are relatively simple, compact, inexpensive, and highly power efficient. Although the output peak power of a single element LD is relatively low (several hundred watts) compared to a widely used photoacoustic excitation lasers like Q-switched Nd: YAG laser, through strategies including composing a single element LD array, focusing the light, coding light excitation, or stacking diode bars, it is possible to image biological tissue with adequate signal-to-noise ratio (SNR) and high spatial resolution using LDs. Furthermore, it is relatively easy for LDs to operate at a high repetition frequency (up to megahertz), which is a significant advantage for real-time imaging applications. However, LDs still have drawbacks. Firstly, most high-power LDs are not miniature enough to be packaged into an endoscope, for example. Secondly, the driving and cooling systems of high-power LDs are still not efficient enough to be a part of a portable handheld or remote device. Thirdly, commercially available high-power LDs have choices of only several wavelengths, limiting their applications in multispectroscopic PAI.

High-power light emitting diode (LED) becomes another attractive alternative, attributing to its low-cost (tens of dollars), miniature size, relatively efficient electronic driving and cooling system, easy-to-integrate, and wide optical spectrum range from UV to near-infrared. In this regard, Hansen and Allen et al. took initial steps towards LED-based photoacoustic imaging. While these studies are certainly inspiring and have
shown the possibility of using high-power LEDs as excitation source for photoacoustic imaging, they did not show the feasibility of in vivo tissue imaging with LEDs. For example, in Hansen’s study, the photoacoustic signals were acquired from a phantom that consisted of a thin stripe of green colored gelatin overlaid by a layer of un-colored gelatin with an excitation of 60ns pulsed light with 6 watts peak power generated by LED. However, LED’s low repetition rate of 200 Hz and the large averaging time of 50,000 greatly limited the in vivo imaging capability of their system. While Allen et al., using vascular phantoms, studied the feasibility of inducing photoacoustic signals with widefield illumination in the visible spectrum range, the low SNR of photoacoustic signals caused by the low light intensity (10μJ/cm²) reduced the possibility of in vivo imaging.

**System Description**

We describe a PAI system based on a low-cost high-power miniature LED that can be used to in vivo map vasculature networks in biological tissue. The LED was overdriven by 200-ns pulses and operated at a repetition rate of 40 kHz, and provided 1.2-W power at 405-nm with a radiation area of 1000μm x 1000μm and a size of 3.5mm x 3.5mm (as shown in Figure 6-1A). We validated the LED-based PAI system using phantoms including black-tape stripes, pencil lead and human hair, and demonstrated its in vivo imaging ability by mapping the vasculature of mouse ear.

Short pulsed or modulated light with certain energy is required to generate detectable photoacoustic signals. Most commercially available LEDs operate at a few watts only on continuous-wave mode, which cannot satisfy the requirement. However, this limitation can be overcome by high-current driving at a low duty cycle. In this study, a super-fast switching high-current LED driving circuit operating on nanosecond-pulse
mode was used. Figure 6-1B shows the schematic of the high-speed high-current driving circuit based on a power metal-oxide-semiconductor field-effect transistor (MOSFET) Q1 (IRF7469, Infineon Technologies). With a rise-time of 2.2 ns and a fall-time of 3.5 ns, the MOSFET was driven by a high-speed driver U1 (ISL55110, Intersil Corp.) powered by the voltage VCC_LOGIC with a minimum pulse width of 6 ns. The peak current of LED (D2) could be adjusted by changing the value of resistor R1 and power supply VCC_LED. A fast-recovery Schottky diode D1 (SB3003CH, ON Semiconductor) was used for electrostatic discharge (ESD) protection of LED. To reduce the optical rise time of LED, a speed-up capacitance C1 was added in parallel to the current-limiting resistor R1, while a sweep-out loop consisting of inductor L1 and resistor R2 was used to improve the optical fall time. In our experimental setup, the following component values were used: R1 = 0.5 Ω, C1 = 1.0 nF, R2 = 2.7 Ω, L1 = 5.6 nH, VCC_LOGIC = 12 V DC, VCC_LED = 15 V DC. Figure 6-1C shows the waveform from the LED D2 measured by an oscilloscope while a 200-ns pulse signal from a function generator was used as the input (PULSE_INPUT) of the circuit. From the waveform, we can see a 200-ns pulse with relatively flat peak and sharp rise-up and fall-down edges was obtained. With this high-speed high-current driver, a peak power of 6.4 watts was achieved for high-power LED (EDC405-1100, Marubeni) operation at a repetition rate of 40 kHz and a pulse width of 200 ns.

The LED-PAI system is schematically shown in Figure 6-2. The light from LED was collimated by an aspheric lens L1, and then focused on the testing sample by an aspheric lens L2. The area of the focal zone was around 1 mm², thus the exposure was about 0.12 mJ/cm² at the optical focus which is lower than the American National
Standards Institute (ANSI) laser safety limit (20 mJ/cm$^2$). The induced photoacoustic signal was detected by an ultrasound transducer with a center frequency of 2.25 MHz and a focal length of 25.4 mm (V304, NDT, Olympus), and then amplified by a pre-amplifier, which was cascaded by two ultrasound receiver (5073PR, Olympus), with a total gain of 70 dB. A data acquisition (DAQ) card (PCI-5124, National Instrument) with 12-bit resolution and a sampling rate of 200 MS/s was used to resolve signals as small as 1mV. Testing sample mounted on the sample holder (SH) was fixed in position, while the LED, lenses, and transducer were mounted on a 3-dimensional (3D) linear stage. Before each experiment, the space between sample holder and transducer was filled with water to achieve the maximum ultrasonic coupling between the sample and transducer. The whole system was synchronized by a computer, which controlled the function generator to provide a 200-ns pulse with a repetition frequency of 40 kHz. Meanwhile, through the DAQ card, the photoacoustic signals were acquired and stored in the computer. By scanning the transducer and the light beam in 2-dimensional (2D) X-Y plane with the linear stage, the volumetric PAI image of a sample could be obtained through combining A-line images that are formed by converting the photoacoustic signal into one-dimensional depth-resolved image based on the sound velocity in soft tissue (1.54 mm/µs). In our experimental setup, a third dimension (Z direction) of linear stage was added to adjust the location of the focal plane in the testing sample.

Results and Discussion

The feasibility of the LED-PAI system was firstly validated by experiments using tissue-mimicking phantoms. The background of each phantom, composed of 2% Agar, TiO2, and India ink with light absorption coefficient of 0.007 mm$^{-1}$ and reduced scattering coefficient of 0.7 mm$^{-1}$, was intended to mimic soft tissue. Three types of
targets (to be embedded in the background phantom) that have different absorption coefficients were used: (1) three pieces of black-tape stripes (two with a width of 0.5 mm each, and one with a width of 1.0 mm); (2) two pieces of pencil lead with a diameter of 0.7 mm each; (3) two pieces of human hair. For each experiment, signal averaging of 2000 times at each scanning point was needed. With the LED repetition frequency of 40 kHz, a scanning speed of 20 A-lines per second was achieved. With a scanning step of 30 µm, it took around 8.5 minutes for scanning an area of 3 mm x 3 mm.

Figure 6-3A shows the geometry of the black-tape-stripe phantom. The top two stripes, separated by a distance of ~1.5 mm, were located near the surface (focal plane), while the third stripe was located at a depth of ~1 mm. Figures 6-3B and 6-3C give the acquired 3D photoacoustic image and one cross-sectional image, respectively.

The geometry of the pencil-lead phantom is shown in Figure 6-3D. Two pieces of lead separated with a distance of ~1 mm were located near the surface (focal plane) and at a depth of ~1 mm. Figures 6-3E and 6-3F present the corresponding 3D photoacoustic image and cross-sectional image, respectively.

Figures 6-3G-3I, respectively, show the geometry, 3D image and cross-sectional image of two pieces of human hair having a separation distance of ~0.5 mm.

From these phantom images, we can see that light absorbers located in the focal plane had higher intensity of photoacoustic signals, due to the higher energy density and higher sensitivity of ultrasound detection in the focal zone. These results demonstrate that the LED-PAI system has the capability of imaging light absorbers in tissue-mimicking phantoms. Further analysis of the photoacoustic signals from these phantom experiments indicated that a SNR of 20 dB could be achieved, which is
adequate to obtain images with a contrast-to-noise ratio (CNR) of as high as 13 dB. In addition, our LED-PAI system could map targets with different optical properties at a depth of at least 1 mm. The spatial resolution of our LED-PAI system was estimated to be as high as 150 μm using a tungsten wire with a diameter of 30 μm based on Gaussian fitting method discussed in 12.

To further demonstrate the potential of the LED-PAI system, in vivo imaging of a mouse ear (mouse weight=35g) was performed. Before the experiment, the hairs on the ear were gently removed using a human-hair-removing cream. The mouse was placed on a home-made animal holder and anesthetized by a solution of ketamine (85 mg/kg) and xylazine. The mouse was sacrificed according to the University of Florida Institutional Animal Care and Use Committee (IACUC)-approved techniques after the experiment. Strict animal-care procedures approved by the University of Florida IACUC and based on guidelines from the National Institutes of Health (NIH) for the Care and Use of Laboratory Animals were followed.

Figure 6-4 shows the photoacoustic signal (as indicated by red arrow, averaged 4000 times) from a point in the vasculature of mouse ear. A SNR as high as 14 dB could be achieved, which is adequate for obtaining volumetric photoacoustic image of biological tissue.

Figure 6-5 gives in vivo images of mouse ear with an area of 4 mm x 4 mm. The scanning step in the X-Y plane was 20 μm, and signal averaging of 4000 times was used at each scanning point. With the LED repetition frequency of 40 kHz, a scanning speed of 10 A-lines per second was achieved for the in vivo imaging. Figures 6-5A~5C, respectively, show the maximum amplitude projection (MAP) image, the cross-sectional
image corresponding to the dotted line in Figure 6-5A, and the 3D volumetric image. Figure 6-5D is the photograph of mouse ear, in which, the red square with dotted line indicates the scanning area.

To closely inspect these images, we note that the blood vessels in the mouse ear were mapped by LED-PAI with high contrast, due to the fact that hemoglobin molecules are strong optical absorbers around 405 nm, compared to other components such as water in the tissue.

**Conclusion**

In summary, we have developed a novel photoacoustic imaging system based on a low-cost high-power miniature LED. The in vivo images obtained indicate the great potential of LED-PAI for label-free biomedical imaging applications. For example, as demonstrated in this work, mapping vasculature networks in tissue could be an important application of LED-PAI. In addition, this study suggests that portable, handheld PAI devices would become possible with further improved LEDs. Currently, the power of LEDs are relatively low compared to LDs or pulsed OPO lasers; however, attributing to LEDs’ miniature size, one attractive way is to make a LED array to achieve higher pulsed light energy.

**Photoacoustic Imaging with a Vertical-Cavity Surface-Emitting Laser Excitation Motivations**

Instead of using nanosecond pulsed lasers with relatively high energies (mJ) such as Q-switched Nd: YAG laser, Ti: Sapphire laser, optical parametric oscillator (OPO), and dye laser, conventional edge-emitting laser diodes(LDs) and high-power LEDs have been proposed for photoacoustic imaging system to avoid the practical limitations due to laser's bulky size, high cost and strict maintenance requirements. LDs
are relatively simple, compact, inexpensive, and have highly power efficient. Through strategies including composing a single element LD array \(^{132}\), focusing the light\(^{133,134}\), coding light excitation\(^{135}\), or stacking diode bars \(^{47,136}\), it is possible to image biological tissue with adequate signal-to-noise ratio (SNR) and high spatial resolution using LDs even though the output peak power of a single element LD is relatively low (several hundred watts). High-power LEDs are low-cost (tens of dollars), easy-to-integrate, miniature in size, and have relatively efficient electronic driving and cooling system and wide optical spectrum range from UV to near-infrared. We and other groups took initial steps towards LED-based photoacoustic imaging. In Hansen’s study \(^{137}\), the photoacoustic signals were acquired from a phantom that consisted of a thin stripe of green colored gelatin overlaid by a layer of un-colored gelatin with a LED excitation. Allen et al. \(^{138}\), using vascular phantoms, studied the feasibility of inducing photoacoustic signals with widefield LED illumination in the visible spectrum range. We, for the first time, demonstrated the feasibility of in vivo tissue photoacoustic imaging with LEDs \(^{75}\). In our system, overdriving with 200 ns pulses and operating at a repetition rate of 40 kHz, a 1.2 W 405 nm LED with a radiation area of 1000 \(\mu m \times 1000 \mu m\) and a size of 3.5 mm\(\times\)3.5 mm was used to excite photoacoustic signals in tissue. In vivo imaging of the vasculature of a mouse ear showed that LED-based PAI could have great potential for label-free biomedical imaging applications where the use of bulky and expensive pulsed lasers is impractical. While inspiring, photoacoustic imaging system using either LDs or LEDs has drawbacks and limitation. For example, most high-power LDs are not miniature enough to be packaged into an endoscope. In addition, the driving and cooling systems of high-power LDs are still not efficient enough to be made
into a tiny device. What’s more, most commercial available LDs cannot work in pulse mode with a pulse width as short as tens of nanosecond, which is critical to obtain high resolution photoacoustic images, especially to photoacoustic microscopy.

Vertical-Cavity Surface-Emitting Lasers (VCSELs), first reported in late 1970s\textsuperscript{141}, are another type of semiconductor laser diodes that the emitted light leaves the device in a direction perpendicular to the chip surface. Over the past decade, high-power VCSELs or VCSEL arrays have experienced rapid development and become increasingly attractive for high-power laser applications due to VCSELs' unique properties including circularly symmetric output beam, narrow spectral linewidth, and low cost\textsuperscript{142}. VCSELs appear to combine the advantages of LEDs and conventional edge-emitting LDs perfectly, even though so far the conversion efficiency of VCSELs are not as high as edge-emitting LDs. Particularly, working in pulse mode with short pulse width less than 1 µs, VCSELs outperform edge-emitting LDs benefiting from their robustness, good beam shape and simple mounting. Table 6-1 shows the comparison of major parameters for VCSELs, LEDs, and edge-emitting LDs\textsuperscript{143}. In this study, we, for the first time, demonstrated the feasibility of photoacoustic imaging with a VCSEL excitation through both phantom and in vivo experiments.

**System Description**

The VCSEL-based PAI system is schematically shown in Figure 6-6. As shown in Figure 6-7A, a two-dimensional (2D) VCSEL array packaged in a TO-46 can that is capable of delivering pulsed Gaussian shaped light beam with a peak power up to 10 W and a pulse width as short as 10 ns was used as the excitation laser source. The VCSEL can emit a narrow light beam with a center wavelength of 860 nm. The VCSEL array was driven by a high-speed high-current laser driver that can provide pulsed
electrical current as high as 9 A with a switching speed up to 200 MHz cascaded to a compact pulse generator capable of outputting pulsed signals with a tunable pulse width ranging from 1 to 64 ns in steps of 0.25 ns and a variable frequency of 1 kHz to 2 MHz (as shown in Figure 6-7B). Here, the pulse width was set to 20 ns, and the repetition frequency was 55 KHz, with which, we can see, a pulsed light beam with a relatively flat peak and sharp rise-up and fall-down edges was obtained (Figure 6-7C). The light from VCSEL was collimated and focused on the testing sample by four pieces of achromatic lenses L1 ~ L4. The area of the focal zone was around 0.003 mm², thus the exposure was about 6.7 mJ/cm² at the optical focus which is lower than the American National Standards Institute (ANSI) laser safety limit (20 mJ/cm²). The induced photoacoustic signal was firstly reflected by a glass slide 45 degree tilted with the direction of light beam between objective lens L2 and sample holder, and then detected by an ultrasound transducer with a center frequency of 10 MHz and a focal length of 15 mm (V327, NDT, Olympus). The detected ultrasound signals were then amplified by a pre-amplifier, which was cascaded by two ultrasound receiver (5073PR, Olympus), with a total gain of 80 dB. A data acquisition (DAQ) card (PCI-5124, National Instrument) with 12-bit resolution and a sampling rate of 200 MS/s was used to resolve signals as small as 1mV. Testing sample mounted on the sample holder (SH) was fixed in position, while the VCSEL, lenses, and transducer were mounted on a 3-dimensional (3D) linear stage. Before each experiment, the space between sample holder and transducer was filled with water to achieve the maximum ultrasonic coupling between the sample and transducer. The whole system was synchronized by the trigger output of pulse generator which is a signal with a repetition frequency of 55 kHz and controlled by a
computer. At the same time, the photoacoustic signals were acquired and stored in the computer through the DAQ card. By scanning the transducer and the light beam in a 2-dimensional (2D) X-Y plane with the linear stage, the volumetric PAI image of a sample could be obtained through combining A-line images that are formed by converting the photoacoustic signal into one-dimensional depth-resolved image based on the sound velocity in soft tissue (1.54 mm/µs). In our experimental setup, a third dimension (Z direction) of linear stage was added to adjust the location of the focal plane in the testing sample. We validated the VCSEL-based PAI system using phantoms including black-tape stripes, pencil lead and human hair, and demonstrated its in vivo imaging ability by mapping the vasculature within the human hand.

**Results and Discussion**

The feasibility of the VCSEL-PAI system was firstly validated by experiments using tissue-mimicking phantoms. The background of each phantom, composed of 2% Agar, TiO2, and India ink with light absorption coefficient of 0.01 mm$^{-1}$ and reduced scattering coefficient of 1.0 mm$^{-1}$, was intended to mimic soft tissue $^{140}$. Three types of targets (to be embedded in the background phantom) that have different absorption coefficients were used: (1) a piece of black-tape stripe and a piece of human hair; (2) three pieces of pencil lead with a diameter of 0.5 mm each; (3) five pieces of human hair. For each experiment, signal averaging of 1000 times at each scanning point was needed. With the VCSEL repetition frequency of 55 kHz, a scanning speed of 55 A-lines per second was achieved. With a scanning step of 30 µm, it took around 3 minutes for scanning an area of 3 mm x 3 mm.

Figure 6-8A shows the geometry of the phantom with the targets of black-tape stripe and human hair. The stripes is about 1 mm in width and a piece of human hair,
separated by a distance of \(~0.5\) mm, were located at a depth of \(~0.5\) mm from the surface. Figures 6-3B and 6-3C give the acquired 3D photoacoustic image and one cross-sectional image, respectively.

The geometry of the pencil-lead phantom is shown in Figure 6-8D. Two pieces of lead separated with a distance of \(~1\) mm were located at a depth of \(~1\) mm from near the surface, while a third piece of lead was crossly fixed on top of these two pieces. Figures 6-8E and 6-8F present the corresponding 3D photoacoustic image and cross-sectional image, respectively.

Figures 6-8G~I, respectively, show the geometry, 3D image and cross-sectional image of five pieces of human hair casually embedded into the background with a depth of less than 1 mm and a separation distance of less than 0.5 mm. All the human hair were mapped by VCSEL-PAI with high contrast and resolution.

These results demonstrate that the VCSEL-PAI system has the capability of imaging light absorbers in tissue-mimicking phantoms. Further analysis of the photoacoustic signals from these phantom experiments indicated that a SNR of 30 dB could be achieved, which is adequate to obtain images with a contrast-to-noise ratio (CNR) of as high as 21 dB. In addition, our VCSEL-PAI system could map targets with different optical properties at a depth of at least 1 mm. The spatial resolution of our VCSEL-PAI system was estimated to be as high as 50 \(\mu\)m using a tungsten wire with a diameter of 20 \(\mu\)m based on Gaussian fitting method discussed before \(^{12}\).

We then conducted in vivo imaging of the back of a human hand to explore the potential capabilities of the VCSEL-PAI system. A step size of 10 \(\mu\)m was used for scanning the back of hand and at each scanning point signal averaging of 1000 times
was taken. Thus, with the VCSEL repetition frequency of 55 kHz, it took around 12 seconds to obtain a b-scan (cross-sectional) image for a length of 6.5 mm. Figure 6-9A shows the cross-sectional photoacoustic image of the hand, while Figure 6-9B is the photograph of the scanning location on the surface of hand indicated by red dotted line. To closely inspect the image, we note that the blood vessels (indicated by red arrows in Figure 6-9A) within the hand were mapped by VCSEL-PAI with high contrast, due to the fact that hemoglobin molecules are strong optical absorbers, compared to other components such as water in the tissue.

**Conclusion**

In summary, we have developed a novel photoacoustic imaging system based on a low-cost high-power Vertical-Cavity Surface-Emitting Laser. This study suggests that portable, handheld PAI devices would become possible with VCSELS. Currently, the power of compact VCSELS are relatively low compared to pulsed nanosecond lasers; however, attributing to VCSELS’ miniature size, good beam quality, it is still able to obtain images as good as conventional photoacoustic imaging system.

**Fiber-free endoscope based on miniature light source**

**Endoscopic Probe**

We then developed a VCSEL-based fully integrated fiber-free endoscope with a frontal view. The structure of the endoscopic probe is schematically shown in Figure 6-10A. In this probe, a two-dimensional (2D) VCSEL array packaged in a TO-46 can that is capable of delivering pulsed Gaussian shaped light beam with a peak power up to 10 W and a pulse width as short as 10 ns was integrated as the excitation laser source, which can emit a narrow light beam with a center wavelength of 860 nm. The emitting light beam is firstly collimated and focused by two achromatic doublets (AC050-008-B,
AC050-015-B, respectively, Thorlabs), then travel through a transparent slide and finally illuminate the sample to excite photoacoustic signals. The induced photoacoustic signals are reflected by a reflection slide made of glass, and detected by a custom-made ultrasound transducer with a center frequency of 40 MHz and a dimension of 0.6 mm × 0.5 mm × 0.2 mm (Blatek, Inc., State College, PA). Further investigating the schematic of the probe, we realized that the reflection structure for photoacoustic signals would worsen the signal-to-noise ratio thus affect the quality of images. Through experiment validation, we found that the photoacoustic signal was lost around by ~8% after the two independent reflections, but it was still high enough to obtain good PA images with high contrast. Figure 6-10B shows the 3D rendering of the structure of the probe. All the components are fixed by a custom-made stainless steel tubing with a cylindrical shape that has a size of 6 mm in diameter and 20 mm in height. The photograph of the endoscope is shown in Figure 6-10C.

Results and Discussion

The capability of the VCSEL-based fully integrated fiber-free endoscope was demonstrated by experiments using tissue-mimicking phantoms. The background of each phantom, composed of 2% Agar, TiO2, and India ink with light absorption coefficient of 0.01 mm⁻¹ and reduced scattering coefficient of 1.0 mm⁻¹, was intended to mimic soft tissue. Two types of targets (to be embedded in the background phantom) that have different absorption coefficients were used: (1) pencil lead with a diameter of 0.5 mm each; (2) human hair. For each experiment, signal averaging of 3000 times at each scanning point was needed. With the repetition frequency of 60 kHz, a scanning speed of 20 A-lines per second was achieved. With a scanning step of 30 µm, it took around 8.4 minutes for scanning an area of 3 mm × 3 mm.
The geometry of the pencil-lead phantom consists of two pieces of pencil lead with a diameter of 0.5 mm is shown in Figure 6-11A. The two pieces of lead separated with a distance of ~1 mm were located at a depth of ~1 mm from near the surface. Figures 6-11B and 6-11C present the corresponding 3D photoacoustic image and cross-sectional image, respectively.

Figures 6-11D~11F, respectively, show the geometry, 3D image and cross-sectional image of six pieces of human hair randomly put in the background.

From these phantom images, we can see that the VCSEL-based fiber-free endoscope has the capability of imaging light absorbers in tissue-mimicking phantoms. Further analysis of the photoacoustic signals from these phantom experiments indicated that a SNR of 25 dB could be achieved, which is adequate to obtain images with a contrast-to-noise ratio (CNR) of as high as 16 dB. In addition, the VCSEL-based fiber-free endoscope could map targets with different optical properties at a depth of at least 1 mm. The spatial resolution of the VCSEL-based fiber-free endoscope was estimated to be as high as 40 μm using a tungsten wire with a diameter of 15 μm based on Gaussian fitting method discussed in 12.

We then conducted in vivo imaging of a human hand to explore the potential capabilities of the VCSEL-based fiber-free endoscope. A step size of 30 μm was used for scanning the hand and at each scanning point signal averaging of 5000 times was taken. Thus, with the VCSEL repetition frequency of 60 kHz, it took around 16.7 seconds to obtain a b-scan (cross-sectional) image for a length of 6 mm, and around 28 minutes to acquire a volumetric image with a scanning area of 6 mm x 3 mm. Figure 6-12A shows the maximum amplitude projection (MAP) photoacoustic image of the hand,
while the cross-sectional image corresponding to the dashed line in Figure 6-12A is shown in Figure 6-12B. Figure 6-12C is the photograph of the scanning location and area on the surface of hand indicated by red dotted line. To closely inspect the image, we note that the blood vessels (indicated by blue arrows in Figure 6-12B) within the hand were mapped by VCSEL-based fiber-free endoscope with high contrast.

**Conclusion**

In summary, we have for the first time developed and demonstrated a novel fully integrated fiber-free photoacoustic endoscope based on a low-cost high-power miniature VCSEL. Complex phantom experiments have been performed to show the feasibility of the implemented VCSEL-based fully integrated fiber-free photoacoustic endoscope, while the capability of the endoscope has been demonstrated by in vivo experiments.
Table 6-1. Comparison of major parameters of VCSELs, LEDs, and edge-emitting LDs

<table>
<thead>
<tr>
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<th>VCSELs</th>
<th>LEDs</th>
<th>Edge-emitting LDs</th>
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<tbody>
<tr>
<td>Efficiency</td>
<td>+</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Modulation speed</td>
<td>+++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Beam shape</td>
<td>+++</td>
<td>+</td>
<td>++</td>
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<tr>
<td>Cost</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>Spectral width</td>
<td>+++</td>
<td>+</td>
<td>++</td>
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<tr>
<td>Spectral shift</td>
<td>+++</td>
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</tr>
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</table>
Figure 6-1. LED and its driver. A) photograph of LED (photo courtesy of author), B) schematic of high-speed high-current driving circuit, C) driving waveform.
Figure 6-2. LED-PAI system. L1, L2, lens; SH, sample holder; UST, ultrasound transducer.
Figure 6-3. Photoacoustic images of tissue-mimicking phantoms. Geometry A), 3D image B), and cross-sectional image C) of black-tape-stripe phantom; geometry D), 3D image E), and cross-sectional image F) of pencil-lead phantom; geometry G), 3D image H) and cross-sectional image I) of human-hair phantom. All the scale bars indicate 0.5 mm in length.
Figure 6-4. Photoacoustic signal of a point in the vasculature of mouse ear.
Figure 6-5. In vivo images of mouse ear by LED-PAI system. (a) Maximum amplitude projection (MAP) image, (b) cross-sectional image, (c) 3D volumetric image, (d) photograph (photo courtesy of author). All the scale bars indicate 0.5 mm in length.
Figure 6-6. VCSEL-PAI system. L1, L2, L3, L4, lens; UST, ultrasound transducer; SH, sample holder; GS, glass slide.
Figure 6-7. VCSEL and its driver. A) photograph of VCSEL (photo courtesy of author), B) high-speed high-current driving circuit, C) driving waveform.
Figure 6-8. Photoacoustic images of tissue-mimicking phantoms. Geometry A), 3D image B), and cross-sectional image C) of black-tape-stripe-and-hair phantom; geometry D), 3D image E), and cross-sectional image F) of pencil-lead phantom; geometry G), 3D image H) and cross-sectional image I) of human-hair phantom. All the scale bars indicate 0.5 mm in length.
Figure 6-9. Cross-sectional images of the skin of human hand. A) PAI image, B) Photograph of the scanning location (photo courtesy of author). The scale bars indicate 1.0 mm in length.
Figure 6-10. The structure of VCSEL-based fully integrated photoacoustic endoscope. A) Schematic, B) 3D rendering, C) Photograph (photo courtesy of author).
Figure 6-11. Endoscopic photoacoustic images of tissue-mimicking phantoms. Geometry A), 3D image B), and cross-sectional image C) of pencil-lead phantom; geometry D), 3D image E), and cross-sectional image F) of human-hair phantom. All the scale bars indicate 0.5 mm in length.
Figure 6-12. Endoscopic photoacoustic images of human hand. A) maximum amplitude projection (MAP) image, B) cross-sectional image, C) location of the scanning area (photo courtesy of author). All the scale bars indicate 1.0 mm in length.
CHAPTER 7
CONCLUSION AND FUTURE WORK

In this dissertation, I have developed two generation of multimodal endoscopy integrating photoacoustic imaging, optical coherence tomography, and ultrasound imaging. The first generation G1 multimodal endoscope was used to proof of the concept and show the advantages of multimodal endoscopy for characterization of biological tissue through both phantom validations and in vivo animal experiments. While, the second generation G2 multimodal endoscope with a state-of-art double-reflection coaxial structure that guarantees it can image the same area of sample simultaneously and a highly miniaturized size of 1 mm in diameter, represents the most advanced multimodal endoscope up to date. After phantom and in vivo validations, we exploited the applications of multimodal endoscope in cancer research and intravascular imaging. Through agents of multifunctional magnetic iron oxide nanoparticles, we applied the G2 multimodal endoscope on a mouse orthotopic human pancreatic cancer xenograft model. With the novel fan-shaped scanning mechanism, the endoscopic probe can directly reach the surface of pancreas through abdomen with a minimized invasiveness. It highly benefited the development of methodologies for aiding diagnosis and therapeutics of pancreatic cancer. In intravascular imaging, our multimodal endoscope was demonstrated to be suitable for either label-free atherosclerotic plaque characterization or molecular imaging with the help of agents of multifunctional magnetic iron oxide nanoparticles. Finally, we proposed a novel concept of developing next generation of fully integrated wireless multimodal endoscopy. However, at present, even reaching an important middle stage of implementation of a
fully integrated fiber-free multimodal endoscopy, especially fiber-free photoacoustic endoscope is rather challenge. Therefore, we took efforts to develop a fully integrated fiber-free photoacoustic endoscope based on the validation of the feasibility of inducing photoacoustic signals in biological tissue with miniature light sources like high-power LEDs and VCSELs.

For future studies, I believe we can continue to exploit the applications of our G2 multimodal endoscopy. For example, in intravascular imaging, we can make efforts on identification of intra-plaque hemorrhage and vasa vasorum density which are two significant predictors for assessment of vulnerability of plaques\textsuperscript{144, 145}. In cancer research, it is possible to apply the G2 multimodal endoscopy for colon cancer studies even on small animals like mouse, which is significant in preclinical cancer studies.

More importantly, for development of multimodal endoscope technology itself, we will take our efforts on developing a next generation of fully integrated wireless multimodal endoscopy which is highly desired in either preclinical studies or clinical practices.
LIST OF REFERENCES


BIOGRAPHICAL SKETCH

Xianjin Dai received his B.S. in electrical engineering and automation, communication engineering, and M.S. in precision instruments and mechanics from University of Electronic Science and Technology of China in June 2006 and June 2009. After working four years as a Research Associate at Chinese Academy of Sciences, he entered the University of Florida in August 2013 for his PhD study in biomedical engineering. His research focuses on multimodal endoscopy and its applications. He received his PhD degree in the fall of 2017.