Optical Coherence Tomography Technology

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Optical coherence tomography (OCT) is an emerging diagnostic technology, developed by our research group and collaborators in 1991, that is being investigated for applications in a number of medical fields.[1] OCT enables visualization of tissue microstructure in situ and in real time with resolutions in the 1-10 µm range and previous studies have demonstrated that changes in tissue architectural morphology associated with neoplasia can be identified.[2,3,4,5] Clinical OCT systems often use superluminescent diodes (SLDs) which enable imaging with 10-15 µm axial resolution. These resolutions are typically insufficient for identifying neoplastic changes for cancer detection or tissue morphological and structural features for visualization of other pathologies. Recent advances in solid-state laser and nonlinear fiber technology have enabled the development of ultrahigh resolution and spectroscopic OCT techniques which promise to improve tissue differentiation and image contrast.

The longitudinal resolution in OCT images is inversely proportional to the optical bandwidth and proportional to the square of the center wavelength of the light source. Enhancing the resolution of OCT images continues to be a very active field of research. Ultrahigh resolution OCT requires extremely broad bandwidths because of this $\lambda^2/\Delta\lambda$ dependence of the longitudinal resolution. This is particularly the case for the spectral region between 1.0 µm and 1.5 µm. This spectral region is of high interest for OCT because of the high penetration depth in biological tissue and the possibility to perform spectroscopic OCT imaging of functional parameters such as water content and tissue oxygenation.[6] We demonstrated previously OCT imaging with resolutions of 1 µm at 800 nm and 5.1 µm at 1300 nm in biological tissue using a solid-state modelocked lasers as well as nonlinear fiber sources.[7,8,9] We have recently developed compact portable ultrahigh resolution systems that enable ultrahigh resolution and spectroscopic imaging to be performed in clinical settings.

References


Compact Ultrahigh Resolution OCT System for Real-time In Vivo Imaging

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One of the key problems in OCT has been the lack of compact, high performance, low coherence light sources with sufficient bandwidth and power to enable high resolution, real time imaging. Compact, diode pumped solid state lasers can generate femtosecond pulses, but do not have sufficient bandwidths for high resolution imaging. High nonlinearity, air-silica microstructure fibers [1] or tapered fibers [2] can generate an extremely broadband continuum using low energy femtosecond pulses. These high nonlinearities are achieved by the anomalous dispersion characteristics of the fibers, which shift the zero dispersion to shorter wavelengths, and the small core diameters, which provide tight mode confinement. We recently demonstrated ultrahigh resolution OCT with an axial resolution of 2.5 µm at 1300 nm center wavelength using continuum generation with femtosecond pulses from a Ti:Sapphire laser coupled into an air-silica microstructure fiber [3]. While this approach achieves high resolutions in the important 1300 nm wavelength range, the Ti:Sapphire laser requires a high power pump laser which makes the light source expensive and bulky.

We report the development of a compact ultrahigh resolution OCT system based on new compact broadband light sources that can emit in either the 1 µm and 1.3 µm wavelength range. The 1300 nm wavelength allows good image penetration depth in tissue, while the 1064 nm wavelength offers a good compromise high axial resolution, but lower penetration depths. The laser and OCT system are compact, robust, transportable and well suited for clinical studies.

Figure 1 shows a schematic of the experimental setup. A compact diode pumped femtosecond Nd:Glass laser generates pulses with 110-150 fs duration, 150 mW average power at 75 MHz repetition rate and 1064 nm wavelength. Either an ultrahigh numerical aperture fiber or a tapered fiber can be used to generate a spectrum centered at 1 µm or 1.3 µm wavelength, respectively. The OCT system consists of broadband 3 dB fiber couplers (FC), and custom designed achromatic lenses (L). In order to accommodate the broad bandwidths and enable real time imaging, delay scanning is performed using a rapid scanning delay line with reflective optics. A hand-held probe was realized for in vivo imaging. An x-galvo is used for transverse scanning at 4 frames per second. Dispersion is matched in the two arms of the interferometer in order to maintain resolution. Dual balanced detection with two photodiodes (D1, D2) is used to cancel the intensity noise of the light source. Polarization controllers (PC1, PC2) are used in the sample arm and the reference arms.

To demonstrate the performance of the system in an in vivo imaging application, an African frog tadpole (Xenopus laevis) was imaged (Fig. 2). The continuum light sources yield an axial resolution of 5-6 µm in air or ~4 µm in biological tissue, which is three times higher than with the conventional semiconductor sources (superluminescent diodes or amplified spontaneous emission light sources). This improved resolution enables the differentiation of small features, which cannot be resolved using conventional light sources.
Figure 1. Schematic of the OCT system using a femtosecond diode pumped Nd:Glass laser (visible in the bottom left corner) with continuum generation either (a) in an ultrahigh numerical aperture single mode fiber or (b) in a tapered single mode fiber. See text for explanation of the acronyms.

Figure 2. In vivo OCT images of an African frog tadpole (Xenopus laevis) (a) at 1064 nm using spectral broadening in a high NA fiber (b) at 1300 nm using continuum generation in a tapered fiber. The axial resolution is 5-6 µm in both cases.

In vivo ultrahigh resolution OCT imaging of the hamster cheek pouch was also demonstrated at 1 µm (Fig. 3a). We chose this tissue it has a morphology consisting of thin layers than can only be well resolved by using an ultrahigh resolution system. Fig. 3b shows an image of human skin, also acquired in vivo and at high speed. The stratum corneum is easily visible. Features as sweat ducts and the dermal-epidermal junction can also be differentiated. The images are 2 mm x 1.6 mm (transverse x axial) in size and contain 500 x 360 pixels.

Figure 3. In vivo OCT images acquired at 4 frames per second with the 1 µm light source. The axial and transverse resolution are 5.5 µm and 11 µm, respectively. (a) Image of a hamster cheek pouch. The junction of 2 blood vessels is visible. (b) Image of human skin. Sweat ducts and the junction between the epidermis and the dermis can be resolved.
These results demonstrate the utility of our system to perform in vivo ultrahigh resolution OCT imaging with a portable light source based on the generation of supercontinuum by nonlinear effects in optical fibers. Such system, with its user-friendly laser, opens the door to the ultrahigh resolution OCT imaging in the clinic, and will be a powerful tool for future studies.

References

Spectroscopic Optical Coherence Tomography

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Standard optical coherence tomography generates a two-dimensional map of backscattered intensity from tissue. Broadband laser sources are used to precisely localize light from small volumes via coherence-gated detection, thereby enabling high-resolution imaging of tissue microstructure. Beyond providing high spatial resolution, the use of broadband sources also provides access to wavelength dependent absorption and scattering in tissue. Spectral information obtained from the OCT interference signal can be used to quantitatively probe the functional state of tissues through correlation of spectral changes with the known absorption or scattering characteristics of tissue constituents. Furthermore, the combination of spectroscopic information with structural images provides an alternate mode of contrast for OCT images. Because intrinsic image intensity contrast in tissue is low, such contrast enhancement is an important area of research to improve the diagnostic capability of OCT.

Relatively few studies on spectroscopic OCT have been performed because suitable broadband light sources have not been available. Spectroscopic OCT was demonstrated initially using a bandwidth of ~ 50 nm at a center wavelength of 1.3 µm [1]. Studies in the spectral region of the water absorption band at 1.45 µm were performed using two different light sources in dual wavelength OCT systems. One wavelength was used for absorption measurements and the second light source for referencing [2, 3]. Our group has had a sustained interest in developing spectroscopic OCT for clinical applications. We initially realized spectroscopic OCT imaging in the wavelength range from 650 nm to 1000 nm using a Kerr-lens modelocked Ti:Sapphire laser with dispersion compensation by double chirped mirrors [4]. Figure 1 shows an image acquired of pathologic gastrointestinal tissue with this system. Green and red hue color scale indicates enhanced optical scattering of short and long wavelengths, respectively.

Figure 1. Spectroscopic imaging of pathology in the human esophagus. Addition of spectral information to standard intensity images provides enhanced contrast to conventional OCT.
We also explored spectroscopic OCT imaging near the water absorption band around 1.45 µm using light obtained by nonlinear continuum generation in an air-silica microstructure fiber [5]. We imaged with a bandwidth selected from the continuum of 200 nm centered at 1400 nm selected from the continuum. Figure 2 shows a schematic of the setup. The phantom, shown in figure 2a, consisted of two microscope cover glass slides of 170 µm thickness confining a water sample. The cover glasses were in contact at one side and had a spacing of ~ 0.8 mm at a lateral distance of 10 mm. The focal position of the beam was ~ 0.4 mm above the bottom of the coverglass. An OCT image of the sample is shown in figure 2b. We computed spectra at several lateral locations in the sample by Fourier transforming the OCT A-scans. Figure 2c shows the reconstructed spectra at the edges of the phantom with one intermediate location. One clearly observes that absorption of the spectral intensity around 1.45 µm increases with increasing absorber thickness and can be quantified from the spectral changes.

Our most recent work involves construction of high-speed, real-time spectroscopic OCT imaging systems for clinical imaging applications. We hope to leverage improved contrast offered by spectroscopic information to enhance the diagnostic capability of OCT for optical biopsy. We have developed compact, portable broadband sources at wavelengths of 0.8 µm, 1 µm, and 1.3 µm. These sources are described elsewhere in this report. The sources provide sufficient bandwidth to offer access to spectral differences in scattering and absorption together with enough power for high-speed imaging. We are combining these sources with a high speed data acquisition system that will acquire the interferometric A-scan signals in real-time. The key component of the data acquisition system is a 14-bit, 64 MHz analog to digital converter. Using fast Fourier transform and Hilbert transform algorithms implemented on state-of-the-art PCs, we plan to spectroscopic OCT to complement our high speed clinical OCT systems.

References

Biopsy Needle for Optical Coherence Tomography

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OCT applications in the past have been limited to the surfaces or lumens of organ systems because the penetration depth of OCT is limited to 2-3 mm in most tissues. There are, however, many clinical scenarios where high resolution imaging of solid tissues is desirable. One promising application of OCT is in imaging pathology and guiding biopsy in solid tissues. This procedure could reduce the sampling error of excisional biopsy in diagnosing cancers in solid organs such as the prostate or breast. Other applications include optical imaging in scenarios where excisional biopsy is hazardous, and in surgical guidance such as in cryosurgery or interstitial photodynamic therapy.

We have developed a prototype of an interstitial imaging needle for OCT that has a diameter as small as 27 Gauge (~410 µm).[1] The OCT imaging needle is inserted directly into soft solid tissues or organs to deliver, scan, and collect a single mode optical beam. Imaging can be performed up to 2-3 mm away from the needle, enabling imaging of a cylindrical volume 4-6 mm diameter by several mm in length. The small size of the OCT imaging needle permits its use in virtually any solid tissue or organ with low resistance and minimal trauma during imaging in direct contact with tissue. The small size also allows the needle to be integrated with standard biopsy devices to provide a “first look” to guide biopsies.

Figure 1A shows a schematic of a representative needle design. A GRIN lens and a micro prism are used to focus and deflect the optical beam which is emitted at an angle of 90 degrees from the axis of the needle. The optical fiber is housed concentrically within a thin wall stainless steel hypodermic tube with a 27 gauge outer diameter. This hypodermic tube protects the optical fiber and facilitates the insertion of the needle into the tissue. The imaging needle has a confocal parameter of ~380 µm, corresponding to a spot size (or transverse resolution) of ~17 µm, with a focal distance ~80 µm outside the optical window. The single mode fiber, the GRIN lens and the micro prism are attached to form a single unit with UV curing optical cement and housed concentrically in a 27 Gauge hypodermic tube. An optical window is ground on the tube and the distal end of the tube is sharpened for easy insertion. The optical beam can be scanned radially by rotating the entire needle along with the distal optics. This technique is similar to that used with acupuncture needles. The OCT imaging plane is perpendicular to the needle axis and the position of the imaging plane can be controlled by varying the depth of needle insertion.

(A)

Hypodermic Tube (27 gauge) Beam Window Microprism
SM Fiber GRIN Lens

Figure 1. A. Schematic of needle design. B. In vivo OCT image of hamster leg muscle acquired with the needle. The needle has focusing optics at the distal end. Muscle fascicles are clearly discerned. Layered appearance resulting from muscle birefringence is also observed (indicated with arrows).
The OCT imaging needles can be inserted into tissue independently or coupled to standard biopsy techniques, such as fine needle aspiration (FNA) or core biopsy. Figure 2 shows one concept of OCT guided biopsy. The OCT needle is introduced first, prior to the biopsy device. If an area of tissue pathology is detected, the biopsy device would then be inserted in parallel, riding the OCT needle in a monorail-like fashion. This approach has the advantage of allowing selective insertion of the larger 18 gauge biopsy devices only when deemed necessary by the imaging. Since the small 27 gauge OCT imaging needle would produce only minimal trauma, more needle insertions could be performed than currently possible, allowing larger volumes of tissue to be screened.

![Figure 2. Monorail biopsy and OCT imaging needle. A monorail design takes advantage of the small size (27 gauge) of the OCT imaging needle, allowing imaging to be performed prior to the introduction of an 18 gauge biopsy needle. This approach reduces trauma, allowing multiple sites to be imaged before a decision to biopsy is made.](image)

Needles of even smaller diameter (200 µm or less) can ultimately be built. The movement of the needle can be performed with minimal resistance from the tissue and minimal trauma. OCT imaging can assess larger volumes of tissue than are possible to excise and imaging can be performed in real time prior to tissue biopsy with minimal tissue trauma. The OCT imaging needle and OCT needle biopsy device would permit the imaging of pathology inside virtually any solid organ or tumor and permit the guidance of biopsy based on microstructural features. This would be an enabling technology for a wide range of research and clinical applications.

**Reference**

Micro-motor Catheter for High Resolution Endoscopic Imaging

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One current constraint in OCT is the trade-off between high transverse resolution and depth of field. Optimum image quality, signal and contrast are only obtained for depth ranges on the order of the optical beam confocal parameter. This necessitates a weakly focused beam in order to image several millimeters into a biological specimen. The ability to adjust beam focus would allow tighter focusing and enable higher transverse image resolution.

Small optical probes such as catheters and endoscopes have been demonstrated for internal body imaging [2-4]. Other imaging probes have been developed that utilize piezo-electric actuated optical fibers or micro-electromechanical (MEMS) chips [5,6]. The simplest catheter design uses a rotating or translating speedometer cable, optical fiber and lens assembly housed in a transparent plastic sheath. This design however does not allow the focal position to be adjusted in real time during OCT image acquisition. Thus, practical transverse image resolutions are severely limited. In addition, rotary probes may have non-uniform rotation distortion (NURD) and can exhibit polarization artifacts due to fiber birefringence.

In this study a new micro-motor endoscope with adjustable focus was developed. By using motor actuation with miniature optics located at the distal end of the probe, the need for a rotating speedometer cable and rotary optical fiber coupling is eliminated. With the ability to adjust the beam focal position, tissue structure can be visualized with high transverse resolution. In addition, focus can be maintained even if the probe is not centered in the luminal structure that is being imaged. An adjustable focus also enables C-mode OCT imaging (acquiring images with several focal planes and fusing them together) to overcome depth of field limitations [7].

Figure 1 shows a schematic of the experimental setup. A compact, broadband Cr$^{4+}$:Forsterite laser combined with a dispersion shifted fiber was used to generate spectral bandwidths of 210 nm at a center wavelength of 1250 nm with 40 mW output power. Light was coupled into a broadband optical circulator and fiber optic beam splitter. Dual balanced detection was used to reduce background intensity noise from the laser source and increase signal to noise. To enable real time imaging a rapidly scanning delay line was used in the reference arm. Polarization controllers (PC) were used in both the sample and reference arms. Dispersion compensating prisms (DCP) and an air gap coupling (AGC) were used to match material dispersion and achieve broadband, high resolution operation.

The endoscope probe consisted of a fiber optic collimator and focusing lens with a rod mirror mounted onto the micro-motor. The endoscope probe and housing fit within a 5 mm diameter sheath. The control wires for the micro-motor were fed through to the proximal end of the endoscope and attached to control electronics. The fiber collimator and focusing lens assembly were mounted at the end of a speedometer cable and the focus could be adjusted distally by translating the assembly with respect to the rotating mirror. Real time imaging was performed at 4 frames per second with 1600 axial scans per second. The OCT signal was filtered and demodulated at a heterodyne frequency of 9.8 MHz. Image data was acquired with a 5 MHz, 12 bit A/D converter and displayed on the computer screen.
The OCT system achieved a transverse resolution of 8\(\mu\)m and an axial resolution of 8\(\mu\)m. Figure 2 shows an image of two finger pads grasping the endoscope sheath housing. Cross correlation processing and bilinear interpolation were employed to create high resolution polar images. Both the stratum corneum and dermal-epidermal junction can be resolved at high resolution with good image contrast. By varying the micro-motor rotation speed, the transverse pixel and frame rate could also be adjusted. For large diameter structures such as the esophagus, high pixel densities are necessary to achieve high transverse resolutions. Novel display methods to visualize these structures are necessary as the image sizes at high resolution will be extremely large.

Figure 1. (a) OCT system using a broadband Cr\(^{4+}\):Forsterite light source. Accurate dispersion matching through the use of an air gap coupling and dispersion compensating prisms enables broadband, high resolution operation. (b) The adjustable focus probe with fiber collimator (FC), focusing lens (FL), rod mirror (RM) and micro-motor (MM). The micro-motor located at the distal end of the endoscope allows accurate scanning of the optical beam with no non-uniform rotation distortion.

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Figure 2. Human finger pad OCT image as the original data set (a) and transformed (b) into an accurate polar mapping. Skin structure is discernable with good demarcation of the stratum corneum, dermal and epidermal layers. Polar conversion of the Cartesian data is performed with a bilinear interpolation algorithm enabling the ability to visualize features at high resolution even with a large diameter radial scan.
To demonstrate focus tracking in scattering media, chicken skin and muscle were imaged *ex vivo*. Figure 3 shows two radial scans acquired with the beam focus at the tissue surface (a) and 1 mm within the sample (b) respectively. The focal position was varied by translating the collimator assembly with an electronically controlled stage. Several features deeper within the tissue can be resolved more clearly at the further beam focus position.

![Figure 3](image-url)

**Figure 3.** Image of chicken muscle and skin tissue *ex vivo* at two different focal positions. Several structural features can be resolved with greater detail using an adjustable focus located deeper within the sample (b). The focused spot size is 8 µm corresponding to a confocal parameter of 75 µm.

We have developed an adjustable focus, micro-motor endoscope for ultrahigh resolution OCT imaging. Tight focusing of the optical beam enabled a transverse resolution of 8 µm with a confocal parameters of 75 µm. Axial resolution of 8 µm was achieved using a broadband Cr:Forsterite laser. The ability to adjust beam focus in real time enabled greater resolution and optimization of image quality. Focus adjustment can allow higher transverse image resolution and visualization of tissue microstructure than were previously possible. Ultrahigh resolution imaging in both transverse and axial dimensions will allow for improved detection of dysplastic and neoplastic changes in endoscopic applications.

References

MEMS-based High Resolution OCT and Optical Coherence Microscopy

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The ability to perform cellular level imaging of structure and pathology using endoscopic and catheter based minimally invasive devices would have a powerful impact on the ability to diagnose disease. This program is a collaboration between investigators at UCLA specializing in MEMS optical technology and investigators at MIT specializing in biomedical optical imaging. We propose to develop new microscanning devices based on MEMS technology and couple these devices with optical coherence tomography, confocal microscopy, and optical coherence microscopy.

Figure 1. Optical micrograph of fabricated MEMS scanner devices. Rectangular and elliptical scanners have been developed.

Current medical imaging technologies can be divided into two general types based on the resolution scales that they measure. Large-scale structural imaging techniques include Xray, CT, MRI, and ultrasound. These modalities permit internal body imaging, but have resolutions limited to fractions of millimeters. Microscopic imaging plays powerful role in medicine, but its application is almost exclusively limited to laboratory use on tissue specimens. Excisional biopsy and pathology is the gold standard for the diagnosis of many diseases including cancers. While biopsy provides unparalleled diagnostic capability, it has the disadvantage of requiring excision and processing of a specimen. In addition, biopsy often suffers from sampling errors if the excised specimen misses the diseased area.

Figure 2. Resonant frequency measurements for MEMS scanner. Inner and outer frame are both near 1kHz operation.
The development of a technology for real time, in vivo, microscopic imaging of structures within the body would have a powerful impact on clinical medicine. Such a technology could guide conventional biopsy improving sampling error, or may ultimately be used directly for tissue pathology screening or diagnosis in real time. Imaging technologies such as scanning confocal microscopy, and multiphoton confocal microscopy, optical coherence tomography, and optical coherence microscopy allow imaging to be performed in living tissues with cellular level resolution. All of these imaging technologies require the delivery and scanning of a single mode optical beam to the site being imaged. Optical fibers can deliver and collect single mode optical beams. However, the development of these technologies for internal body imaging through catheters and endoscopes has not been possible because of the lack of a microscopic optical scanning technology. The integration of MEMS technology, fiber optics, and biomedical imaging can be used to construct third generation medical imaging technologies which can perform microscopic resolution, internal body imaging. This would represent a major advance, enabling a wide range of basic as well as clinical studies. Ultimately, the ability to fabricate these devices in a cost effective manner suitable for medical devices, combined with further clinical research, could significantly improve diagnosis and treatment in many clinical situations. The core MEMS technology and fiber optic integration also has dual use in photonics and display technology applications.

In particular, the development of ultrahigh resolution endocopic MEMS biomedical imaging technology would have important applications for early neoplastic diagnosis and screening. Cancer is the second leading cause of mortality in the industrialized world. Almost all cancers develop over a period of several years and are characterized by progressive mutations and changes of tissue and cellular structure which occur prior to metastasis. The diagnosis of early neoplastic changes is critical to outcome since once metastatic, cure is difficult. Large tumors can be visualized by conventional clinical imaging modalities such as Xray, MRI, or ultrasound. However, in order to improve the detection of early neoplasias, a technology that permits microscopic imaging of tissue internal to the body is necessary. For these reasons, the development of microscopic, in vivo, internal body imaging techniques was recently identified as an important focus area for future research at several NIH workshops. Finally, many of these devices would be used as disposables and thus be attractive for commercialization. Since the medical disposable device market is extremely large, the economic impact of this imaging technology could also be very significant.

We propose to develop several innovative X-Y MEMS scanning devices for endoscope-based imaging applications, and demonstrate ultrahigh resolution OCT, confocal microscope, and coherence-gated confocal microscopes using the MEMS devices. The MEMS scanner developed in this program will have high resolution, low actuation voltage, and high resonant frequency. In addition to scanning micromirrors. Imaging studies will be performed using in vitro specimens as well as in vivo developmental biology models.
Optical Coherence Microscopy Development

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Optical Coherence Tomography (OCT) has shown exciting potential for high resolution imaging of tissue microstructure [1]. OCT combines coherence gating afforded by broadband light sources with heterodyne detection to provide superior signal to noise and image contrast at a range of wavelengths. OCT typically operates with depth priority scanning. A cross-sectional image is generated by slowly scanning the focus transversely at fixed depth and rapidly varying the reference arm delay with respect to this focal position. This scanning technique creates a tradeoff between transverse resolution and depth of field, since adequate depth of field must be preserved over the length of the axial scan. Figure 1 illustrates the compromise.

High resolution OCT can achieve 1-2 μm axial resolution but is limited to a transverse resolution of 5-6 μm. The relatively low lateral resolution achievable with depth priority scanning OCT is generally insufficient for imaging of cellular features and therefore limits the utility of OCT in applications requiring cellular level diagnostics. To extend the imaging power of optical coherence tomography to very high transverse resolution, alternate scanning modalities can be considered. Figure 2 compares scanning techniques available for coherence-gated imaging. Depth priority scanning typically used for OCT imaging is illustrated in figure 2a. One option for overcoming the tradeoff between depth of field and transverse resolution is the use of focus scanning, where the focus position is translated in the sample rather than varying the reference arm path length. Mechanical limitations to rapidly translating the focus
make focus scanning difficult to achieve with depth priority scanning. The use of transverse priority scanning as illustrated in figure 2b, however, enables focus scanning with much lower speed requirements for translating the focus. In transverse priority OCT, the focal position is rapidly scanned transversely and slowly scanned in depth to map out a two-dimensional cross-sectional image. A second technique for improving resolution involves changing the scan plane from cross-sectional to en face. En face imaging as depicted in 2c maps a two-dimensional image by raster scanning the focus over a plane at a fixed depth. The coherence gate can therefore be fixed to the focal depth, eliminating the tradeoff between transverse resolution and depth of field.

Implementation of these alternate scanning techniques for real time, high-resolution OCT imaging requires a high speed, broadband phase modulator for reference arm scanning. Previous studies have used either stretched fiber phase modulators, which have limited scanning speeds, or waveguide phase modulators, which have limited bandwidths. Our group has developed and demonstrated grating phase delay scanners for high speed OCT imaging [2]. These systems are similar to grating-based pulse amplitude and phase shaping techniques in femtosecond optics [3]. The devices use a grating and lens to produce a spectral dispersion combined with a galvanometer-controlled mirror to produce a phase shift in the Fourier plane, resulting in a group and phase delay. The grating phase delay scanner enables group and phase delays to be independently controlled. Recently, grating phase delay scanners have been demonstrated to achieve phase modulation [4]. We have extended the concept of the grating phase modulator to broadband imaging applications using a novel reflective modulator design. The reflective modulator is similar to reflective pulse stretchers and compressors previously developed for femtosecond pulse amplification [5]. The reflective phase delay line is incorporated into a broadband OCT system with a two-axis scanning confocal microscope in the sample arm. Figure 3 illustrates the system schematically. Two 50/50 couplers or a circulator and one 50/50 coupler (not shown) split light from a broadband source into a reference and sample arm. Backreflected light from the sample is recombined with phase shifted light from the reference arm to produce interference at two dual-balanced detectors. Polarization control in both arms aligns the electric field to produce maximal interference. This system design can be implemented at many wavelengths and can support large optical bandwidth from femtosecond laser sources to enable high speed, high-resolution imaging.

The alternate scan techniques were demonstrated using the system implemented for operation at 1300 nm. Imaging was performed on human skin in vivo using light from a superluminescent diode laser centered at 1310 nm with 65 nm bandwidth. A coherence gate of 12 µm was used in combination with a 15 µm focus spot size. Figure 4a presents a standard depth-priority OCT image for comparison. Figure 4b shows a transverse priority OCT image. Fast transverse scanning is performed by driving the X mirror with a triangle waveform to provide 1000 scans per second. The sample arm is translated with a computer-controlled stage to scan the focus position. En face imaging is performed in figure 2c by
scanning the XY galvo mirrors in a raster pattern and fixing the focus depth. The transverse scan OCT image shows higher signal over the imaging depth because the focus is translated to track the imaging plane. The en face image has a uniform intensity because imaging is performed at a constant depth. Ridges at the junction between the dermis and epidermis are visible in all of these images.

Because en face and transverse scanning techniques are not limited by depth of field, they can be extended to very high transverse resolution to enable imaging of cellular level detail. Our group has focused particular effort on the development of the en face imaging technique for very high transverse resolution. This method is typically known as optical coherence microscopy (OCM). OCM essentially combines coherence gated, heterodyne detection from optical coherence tomography with high transverse resolution confocal microscopy. By combining confocal spatial rejection and coherence gating to remove unwanted scattered light from images, OCM can yield improved contrast and greater imaging depths than standard confocal microscopy [6, 7]. Real time, in vivo OCM has been demonstrated for cellular imaging [8].

**Figure 3.** Broadband imaging system using a reflective grating phase modulator and a confocal microscope. The system can be used to achieve alternate scanning modalities to conventional depth priority OCT. Phase modulation in the reference arm enables heterodyne detection to be performed while keeping the imaging position fixed at the focus of the sample arm during scanning.
Confocal laser scanning microscopy (CLSM) has been demonstrated as a powerful tool for \textit{in vivo} cellular imaging [9]. High contrast confocal imaging in scattering tissue, however, requires a confocal axial section thickness of less than approximately 5 \( \mu m \) [10]. This necessitates very high numerical aperture optics, which are typically expensive, bulky, and difficult to design. We investigated the use of enhanced axial sectioning from combined confocal and coherence gating in OCM to relax the microscope design constraints, thereby enabling cellular imaging with miniaturized, low cost probe designs that are unsuitable for confocal imaging alone. We constructed an OCM system for operation at 800 nm using a modelocked Ti:Al\(_2\)O\(_3\) laser with over 100 nm of bandwidth providing a coherence gate of better than 3 \( \mu m \). The confocal axial resolution was set to 30 \( \mu m \), a value which is far too weak for high contrast imaging in standard confocal microscopy. Figure 5 presents the results of imaging human skin \textit{in vivo}. Images were acquired at 4 frames per second with 5 mW of optical power on the skin.

**Figure 4.** Demonstration of alternative scanning techniques for high resolution OCT. A) Conventional depth priority scanning OCT. B) Transverse priority scanning OCT with dynamic focusing. C) \textit{En face} scanning which scans the focus in a raster pattern at a fixed imaging depth.

**Figure 5.** Cellular images of human skin taken with optical coherence microscopy. Cellular structure is visible throughout the epidermis. Coherence gate = 3 \( \mu m \), Confocal gate = 30 \( \mu m \), Field of view = 210 x 144 \( \mu m \).
The transverse spot size at the focus was better than 2 μm. Cellular structure is clearly visible throughout the epidermis. These results demonstrate that a short coherence gate can be used to overcome weak confocal sectioning and provide cellular imaging in situations when confocal microscopy alone would be insufficient. OCM in this operating regime has promise for cellular imaging in a variety of clinical applications currently limited by a lack of suitable probes for confocal imaging.

As a first step toward the development of OCM probes suitable for clinical application, we have developed a compact, handheld microscope. The microscope design is shown schematically in figure 6. The probe uses a pair of closely coupled galvanometer scanners, which impart orthogonal angles to a collimated input beam. The scanners are then images by a relay lens pair to a 30X, 0.9 NA objective lens. The relay pair also serves to magnify the input beam presented to the objective. The probe measures about 15 cm in length and produces a spot size of less than 3 μm. Preliminary OCM imaging results were obtained looking at Xenopus laevis tadpole, a well known animal model useful in developmental biology applications. Figure 7 presents images of cellular detail in the tadpole. The top row of images shows visualization of subcellular detail in tadpole mesenchymal cells. The bottom row presents still frame images excerpted from a movie of blood cells flowing in a vessel.

Figure 6. Handheld microscope for in vivo OCM imaging applications. The probe measures about 15 cm in length and produces a spot size of less than 3 μm.

Figure 7. In vivo imaging of Xenopus laevis using a handheld probe at 1300 nm. Images were acquired at 4 frames per second with 4 mW of power on the sample. Field of view ~140 um x 140 um.
We seek to extend our work on OCM by developing compact systems suitable for clinical studies and miniaturized probe technology for endoscopic applications. We believe that OCM can improve upon and fill important gaps in currently available cellular imaging technology. In addition, we will continue to develop the concept of transverse scanning OCT through the construction of a real-time focus scanning system. This technique offers promise of cellular resolution cross-sectional imaging.

References

Optical Biopsy Using OCT

Optical coherence tomography (OCT) permits the visualization of tissue architecture \textit{in vivo} and \textit{in situ} with resolutions of 1–15 µm. Like conventional biopsy and histology, OCT provides three-dimensional images that may allow differentiation of normal from diseased tissue. However, unlike biopsy, OCT can be performed in real time and without excision, increasing its possible range of utility into situations where excision is impossible or undesirable and perhaps allowing the use of OCT for surgical guidance.

We are investigating the use of OCT in imaging a variety of clinically relevant tissue types and pathologies, both for application in clinical diagnosis and treatment and for basic biomedical research. Integration of high resolution OCT into portable systems with the specialized delivery devices described previously offers promise to expand the use of OCT \textit{in vivo} and \textit{ex vivo} imaging in the clinic and pathology lab.

OCT has already found wide application in ophthalmology, where it has been used in clinical assessment of retinal pathology. Other applications under investigation include imaging of cartilage degeneration for studies of osteoarthritis, imaging of the prostate and breast to explore the use of OCT for differentiation of normal from cancerous or pre-cancerous tissue, and visualization of arterial morphology to aid in diagnosis and treatment of coronary disease.

Ultrahigh Resolution OCT Imaging for Ophthalmology

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OCT has perhaps been most widely investigated in ophthalmology, where it is beginning to make a clinical impact in the assessment of retinal diseases such as macular holes, age-related macular degeneration, glaucoma, and diabetic retinopathy [1-4]. Current clinical practice calls for the development of techniques to diagnose ophthalmic disease in its early stages, when treatment is most effective and significant irreversible damage can either be prevented or delayed. Since the introduction of commercial OCT instrumentation for retinal imaging in 1996 by Humphrey Instruments, now Carl Zeiss Meditec, OCT technology has undergone multiple generations of improvement. The introduction of the commercial instrument OCT3 in 2002 achieved a four-fold increase in imaging speed compared to earlier instruments. The commercial ophthalmic OCT3 system, with ~10 µm axial image resolution, provides more detailed cross-sectional information on retinal pathology than any other previous ophthalmic diagnostic technique (Figure 1). This system is beginning to achieve widespread acceptance as a standard ophthalmic diagnostic tool and is rapidly becoming available in many ophthalmology clinics. However, the detection of many of the early changes associated with diseases can require more accurate quantification of retinal structure than is possible with standard resolution OCT.
Using the broad bandwidth of our ultrahigh resolution (UHR) OCT system, we can image with axial resolutions better than 3 µm in the retina [5], corresponding to a factor of 5 improvement over OCT technology using superluminescent diode sources. The signal to noise ratio for the system is ~95 dB. This system enables a significant improvement in the visualization of intraretinal structures for earlier diagnosis and more precise staging of pathology (Figure 1). To our knowledge, the UHR OCT image shown in Figure 1 represents the highest resolution \textit{in vivo} image ever acquired of the human retina. Standard resolution OCT3 has the ability to visualize major intraretinal morphology such as retinal nerve fiber layer, retinal pigment epithelium, the inner and outer plexiform layers, and the inner and outer nuclear layers. Ultrahigh resolution OCT offers an unprecedented axial resolution to visualize intraretinal morphology such as the external limiting membrane and the photoreceptor inner and outer segments. These intraretinal structures are relevant in a variety of retinal diseases, including age-related macular degeneration, diabetic retinopathy, and glaucoma (the three leading causes of blindness worldwide).

**Figure 1.** \textit{In vivo} standard resolution OCT3 (top) and ultrahigh resolution (bottom) OCT images of a normal human fovea at approximately the same site. Resolutions were ~10-15µm (axial) x 15µm (transverse) and ~3µm (axial) x 15µm (transverse) respectively.

**Figure 2.** Ultrahigh resolution allows for an unprecedented visualization of intraretinal structures that may be quantified to provide an objective measure of retinal disease.
Image processing techniques can be applied to acquired tomograms to quantify retinal and intraretinal structures relevant to disease. Figure 2 illustrates the application of preliminary image processing segmentation algorithms to ultrahigh resolution OCT images to quantify retinal and intraretinal structures. Ultrahigh resolution OCT enables the quantification of layers relevant to retinal disease, which were previously not visualized or quantified using standard resolution OCT. Precise quantification of the retinal thickness is important for the diagnosis and staging of macular edema and diabetic retinopathy. Quantification of the photoreceptor and Henle’s layer may be important in a variety of retinal diseases. Quantification of the ganglion cell layer and the nerve fiber layer is important in retinal diseases such as glaucoma. Figures 3 and 4 demonstrate the ability to use the ultrahigh resolution OCT to quantify and map the different retinal layers in a normal volunteer.

**Figure 3.** *In vivo* two-dimensional retinal thickness map of a normal human macula. This image is constructed from 4200 measurement locations (7 tomograms consisting of 600 A-Scans each). The transverse sampling resolution was 5 µm and the horizontal sampling resolution was 500 µm per pixel.

**Figure 4.** *In vivo* two-dimensional intra-retinal layer thickness map of a normal human macula. This image is constructed from 4200 measurement locations (7 tomograms consisting of 600 A-Scans each). The transverse sampling resolution was 5 µm and the horizontal sampling resolution was 500 µm per pixel.
The visualization and quantification of retinal and intraretinal layers should serve as a valuable clinical tool for the early assessment of ophthalmic disease. This concept has already been demonstrated in a mouse retinal disease model, which allows us to follow and track different retinal diseases in this animal. Using the ultrahigh resolution OCT system, we have imaged and identified the many intraretinal layers of the mouse retina. Figure 5 illustrates the ability for ultrahigh resolution OCT to visualize the intraretinal layers of a normal mouse retina in vivo. When compared with histology taken from the same animal, the ultrahigh resolution OCT image corresponds well with the layers identified in the histology.

![Figure 5. In vivo ultrahigh resolution OCT image of a normal mouse retina and corresponding histology. The layers identified in the OCT images correspond well with the layers in the histology.](image)

In the rhodopsin knockout mouse, the outer plexiform and outer nuclear layers undergo degeneration three months postpartum. Figure 6 illustrates the differences between the in vivo OCT images of a normal mouse retina and a rhodopsin knockout mouse retina. At 5 months of age, the outer plexiform layer and the outer nuclear layer of the rhodopsin knockout mouse retina would have undergone degeneration. When comparing the knockout mouse retina with the normal wild type mouse retina, the OCT image clearly demonstrates this degeneration in the knockout mouse. The in vivo ultrahigh resolution OCT images clearly depict the degeneration of the outer plexiform and the outer nuclear layer in the rhodopsin knockout mouse retina.

![Figure 6. In vivo ultrahigh resolution OCT image of a normal mouse retina (Rd +/- wild type) and a rhodopsin knockout mouse retina (Rd -/-). OCT has the ability to quantify the thickness of the different intraretinal layers as well as track disease progression in a non-invasive manner.](image)
In addition to *in vivo* retinal imaging of normal subject and animal models of retinal disease, we have also started to image ophthalmology patients at the clinic. As described in another section of the progress report, we have developed a clinical compact low-threshold Ti:Sapphire laser that is robust and portable and can be used in the ophthalmology clinic. Imaging with the ultrahigh resolution OCT prototype has been performed in the New England Eye Center of the Tufts-New England Medical Center. The diagnosis of retinal pathology was performed using standard methods including fundus examination, fundus photography and/or fluorescein angiography. A total of >150 patients have been imaged so far at the New England Eye Center. Figure 7 illustrates OCT scans of a patient with macular hole Stage I. Ultrahigh resolution OCT provides unprecedented axial resolution to visualize the intraretinal morphology of retinal diseases. It promises to provide additional information on the understanding of retinal disease morphology, pathogenesis, and management.

**References**


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**Figure 7.** *In vivo* standard resolution OCT3 (top) and ultrahigh resolution (bottom) OCT images at approximately the same site of a patient with stage I macular hole. Resolutions were ~10-15µm (axial) x 15µm (transverse) and ~3µm (axial) x 15µm (transverse) respectively.
In Vivo Imaging of Osteoarthritis

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Osteoarthritis is a degenerative joint disease resulting in several thousand orthopedic procedures every year. Articular cartilage, which is essential for joint function, often exhibits morphological changes during the preliminary stages of many arthritic diseases and could be used as an early indicator of disease.

Current diagnostic techniques such as X-ray radiography, minimally invasive needle arthroscopy, magnetic resonance imaging (MRI) and high frequency ultrasound (HF-US) have been applied to osteoarthritis, however clinical systems have limited resolution (100-250 µm) and can require long acquisition times.

In this work ultrahigh resolution OCT is used to visualize the progression of osteoarthritis in articular cartilage. Osteoarthritis was induced by intra-articular injections of sodium iodoacetate and is a well-established model for chemically induced cartilage damage. Cartilage thinning, osteochondral remodeling and fibrillation of articular surfaces are typically observed within 10-15 days of iodoacetate introduction and similar disease progression was seen in this study.

For this study a newly developed, compact Cr4+:Forsterite modelocked laser was used as the broadband light source. An operating bandwidth of 180-220nm at a center wavelength of 1250nm enabled axial resolutions of 3-5µm. A transverse resolution of 18µm was obtained using a 18mm focal length lens to focus the light onto the sample. Using dual balanced detection to reduce background laser intensity noise, a sensitivity of 95 dB was measured. Polarization controllers (PC) in the sample and reference arms were used to optimize the image contrast. Two dimensional cross sectional images were obtained by scanning the optical beam in the sample arm with a mechanical galvanometer. A 4 Hz repetition rate allowed real-time imaging to be performed with a compact handheld probe.

Experimental osteoarthritis was induced in 12 male Wistar rats (180-200 grams) by injecting 3mg of sodium iodoacetate dissolved in 50µl of sterile saline into the left knee under the patellar ligament. Both knee joints were opened under sterile conditions via a medial parapatellar incision. Once the capsule was opened and cartilage exposed, OCT imaging was performed with a handheld probe. After imaging, each joint was sutured closed under sterile conditions. All procedures were performed with the rats under anesthesia.

OCT imaging was performed on the left and right knees at each time point (Day 0, 5, 10, 20, 30 and 60) by reopening the suture site. At each time point one animal from each imaging group (A and B) was sacrificed. Rat knees were imaged longitudinally along the right and left medial condyles as well as within the sulcus region. During imaging the handheld probe was clamped in place above the knee and accurately positioned with a micrometer stage.

Imaging of normal knees was performed to provide a baseline reference of articular structure in knees without osteoarthritic degeneration. Figure 1a shows an OCT image of the rat knee condyle and sub-patellar region. The cartilage-bone interface can be clearly delineated with the top cartilage layer exhibiting a highly backscattering and laminar appearance. It was also possible to accurately measure a cartilage thickness of 150µm+/-10µm. Below the cartilage was a layer with low signal intensity corresponding to the cartilage-bone interface followed by subchondral bone. Osteochondral features were also resolved at high resolution below the tissue surface (Figure 1b). Cartilage remodeling and fibrillations were apparent.
In addition to normal cartilage structure, several architectural changes were visible by OCT after injection of iodoacetate. Figure 2 shows a time progression of the knee degeneration imaged at Days 0, 20 and 60. Marked cartilage thinning was observed in the load bearing condyle regions indicative of osteoarthritis progression with minimal degeneration seen on the knee sulcus region between the condyles (data not shown).

OCT was capable of clearly visualizing cartilage degeneration in this animal model. Thinning was much more pronounced on knee condyles (both left and right) than on the knee sulcus region indicating deterioration due to wear rather than dissolution from sodium iodoacetate. The ability to assess disease progression in an animal model will have important applications for drug discovery and pharmaceutical trials. Although this study was performed with a hand held probe, OCT imaging is also possible using
arthroscopes or minimally invasive devices such as imaging needles. OCT promises to be a useful imaging modality in both osteoarthritis research and clinical diagnosis.

References

Visualization of Normal and Neoplastic Breast Tissue

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In this work, ultrahigh resolution OCT is evaluated as a diagnostic imaging modality for the detection and differentiation of normal, benign and neoplastic tissue in the breast. Imaging is performed using two broadband, modelocked laser light sources with axial resolutions of 2-5\(\mu\)m.

OCT imaging and histopathology were performed on a total of 12 patients (one with bilateral mastectomy) with 2 biopsies taken from each breast (26 mastectomy specimens). Specimens were obtained at Beth Israel Deaconess Medical Center (BIDMC) and imaged at MIT. Biopsy samples (each 0.5cm x 0.25cm) were taken from areas with grossly palpable lesions as well as from unremarkable breast tissue. Specimens were placed in 10% buffered formalin or phosphate buffered saline (PBS) at BIDMC and transported to MIT for imaging within a 2 hour time frame. Fixation in buffered formalin or PBS did not affect OCT image quality or histological staining. OCT imaging was performed using a benchtop, ultrahigh-resolution imaging system operating at wavelength of 800nm. Axial image resolutions of 2-3 (corresponding to 1-2\(\mu\)m in tissue) and transverse resolutions of 5\(\mu\)m were achieved. Image data was stored on a CD-ROM and reviewed using image-processing software. Following OCT imaging, oriented tissue samples were processed in the histology laboratory and 5mm serial sections (8-10 levels) were obtained in the same plane that the probe had scanned. Standard hematoxylin and eosin (H&E) staining was used for contrast enhancement and tissue differentiation. Digital OCT images and histology sections were then compared and correlated for accuracy. Depth of tissue visualization, tissue architecture, cellular morphology, and the ability to distinguish normal, benign and malignant tissue were evaluated by comparison of histology cross sections and corresponding OCT images.

Mastectomies were performed at BIDMC and tissue samples were transported within a two hour timeframe to MIT for OCT imaging. One of the primary goals of this study was to determine the capability of OCT to visualize normal breast parenchyma as well as palpable benign and neoplastic breast lesions. Figures 1 shows an OCT image and corresponding histology for a normal breast tissue specimen. It was seen that adipose tissue (A) provided the best reflectivity signal (light regions) while stromal areas (S) were more highly scattering with higher optical absorption (dark regions). While adipose membranes were well resolved and readily correlated to histological cross sections, it was in generally difficult to see glandular structures and tissue ducts. Vessels within the tissue could be resolved however were usually located deep within the tissue and beyond the range of the OCT image.

For normal breast tissue it was also possible to distinguish dilated lymphatic (DL) and glandular lobule (GL) structures with OCT. Dilated lymphatics exhibited similar reflectivity characteristics of adipose tissue however lacked the distinct membrane surfaces seen for adipose. Collapsed glandular lobules in the normal specimen were visualized as having a mottled texture. These structures were not as well defined however, possibly due to being collapsed during the imaging process. A dilated duct (D) was observed in the histology but was not seen in the corresponding OCT image. This may be a result of a mismatch between the histology cross section and OCT scan plane or may have been missed if the duct was located deeper than the OCT penetration depth. Tissue calcifications were not seen in the OCT images.
Figure 1. (a) OCT image of normal breast tissue at 800nm with (b) corresponding histology. Adipose cells showed high reflectivity and cellular membranes were well defined. Light scattering and absorption was greater for tissue stroma (S).

For palpable breast lesions, it was possible to resolve several significant architectural features with OCT. Figure 2 shows the imaging results for a benign specimen with fat necrosis. From the histology cross section, necrotic cells are seen infiltrating the stroma and surrounding localized adipose tissue. While the OCT image was not able to resolve nuclear density, it was seen that the adipose cellular membranes exhibited a higher degree of asymmetry and were larger than observed in normal adipose tissue.

Figure 2. Neoplastic breast tissue with fat necrosis at 800nm. Cell infiltration is readily observable in histopathology (b) however necrotic cellular features could not resolved by OCT (a). Adipose membranes however were seen to have larger wall thickness and asymmetry.

The highly scattering regions surrounding the adipose cells seen in Figure 2a may correlate to necrosis however due to the similarity of these OCT images to stromal tissue (Figure 1a) a definitive assessment of necrosis infiltration cannot be inferred.

Imaging of neoplastic specimens was conducted with results for a lobular carcinoma in-situ (LCIS) specimen shown in Figure 3. Ducts expanded by cellular epithelial proliferation of dishesive cells can be
seen in the histopathology. OCT images showed perilobular stroma (PS) made up of loose collagen bundles as well as possible glandular structures (G). Multiple ductal structures both benign (BD) and neoplastic (ND) can be seen with OCT. The neoplastic ducts filled with LCIS correlated well to the terminal ductal lobular units (TDLU) seen in histopathology. To some degree even cellular level detail of dishesive cells within ducts could be resolved with OCT (Figure 3a inset).

![Figure 3](image)

**Figure 3.** Lobular carcinoma in situ imaged at 800nm. Diluted channels and ductal structures can be seen in the OCT image. Cellular nuclei of dishesive cells within the epithelium could be resolved.

The greater awareness of breast cancer and corresponding increase in annual mammography screening has resulted in the detection and diagnosis of earlier stage breast cancer, often as it remains in situ. The capability to visualize and diagnose breast lesions at an early stage in cancer progression remains an important criteria in identifying patient risk and appropriate treatment. Core needle biopsy combined with histopathology still remains the gold standard for detection of atypical or malignant lesions. More frequently, imaging modalities are now being used in conjunction with core needle procedures to enable image-guided breast biopsy. The combination of these techniques has allowed greater diagnostic capability for doctors and reduced false negative biopsy outcomes.

The capability of OCT to image normal breast tissue as well as palpable benign and neoplastic lesions was demonstrated. Several architectural features were visualized with OCT and correlated well to histopathology indicating the potential for OCT to be used for image guidance in excisional breast biopsy procedures.

Because OCT is fiber optically based the use of a minimally invasive imaging probes is possible for a so-called ‘optical biopsy’. Core needle biopsies typically employ 14 Gauge (2.1mm outer diameter) or 11 Gauge (3.0mm outer diameter) vacuum-assisted instruments19,20 which can cause significant patient anxiety and discomfort in screening exams21. The possibility to pre-screen biopsy regions with a minimally invasive imaging probe would allow for multiple regions to be examined easily without major damage to the breast tissue or discomfort for the patient. OCT imaging of tissue microstructure using a 27 Gauge (0.40mm outer diameter) needle in vivo has been previously demonstrated by our group22. In the current study OCT imaging was performed ex vivo however it is anticipated that future studies will incorporate in vivo imaging of breast tissue with minimally invasive probes.

It is clear that greater resolution and specificity are required for diagnostic capability however the potential application of OCT for guided breast biopsy is exciting. The use of minimally invasive OCT for image-guidance during core needle biopsy may enable the detection and treatment of suspect lesions with reduced patient discomfort and decreased false negative outcomes.
References

Ultrahigh Resolution OCT Imaging of Prostate Pathology

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Prostate carcinoma is the second leading cause of death among men in the United States.[1-2] The lifetime risk in Western society of developing histologic evidence of prostate cancer has been estimated to be 30%, with the risk of developing clinical disease 10% and the probability of mortality due to the disease roughly 3%.[3] While core biopsy is the standard method for diagnosing prostate cancer, biopsy techniques often suffer from sampling errors which lead to false negatives. There has therefore been significant interest in novel techniques to improve the diagnostic accuracy in prostate cancer. Ex vivo, ultrahigh resolution OCT imaging studies suggest that differentiation of glandular architectural morphology associated with prostate cancer is feasible.[4] Devices such as OCT needles have been demonstrated which enable imaging in solid tissues or organs.[5] A technology capable of imaging the prostate in vivo at high resolution could be integrated with core biopsy to reduce sampling error.

We have recently developed a portable ultrahigh resolution system that enables ultrahigh resolution imaging to be performed in the clinic. Imaging with increased axial as well as increased transverse resolution reduces speckle noise features, improving image quality. Our OCT system uses an all solid-state, Kerr-lens mode-locked Cr:forsterite laser source operating at 1260 nm center wavelength as the light source.[6] A sterilizeable hand-held surgical probe was developed which enables imaging in the surgical suite and in the pathology laboratory. A low power green aiming beam allows localization of the imaging area and facilitates marking for correlation with histology. The probe has a stainless steel outer tube with a transparent window which can be sterilized using standard sterilization procedures and slipped over the distal end of the probe prior to imaging. The probe produces a focused spot with a 12 µm transverse resolution. The system achieves an axial resolution of 5.5 µm in air or 4.2 µm in tissue. Images can be acquired in real time at 4-8 frames per second.

Figure 1 shows example ultrahigh resolution OCT images of the prostate taken with the Cr:forsterite laser source. Images of the prostate were performed in the pathology laboratory approximately 20 minutes after the prostate was excised. Fine striations in the fibromuscular stroma (A), large glandular structures in seminal vesicle (B), stromal tissue, small glands and the muscular wall of the vas deferens (C) are visible in the OCT images.

Imaging in the pathology laboratory allows precise registration of OCT images with histology and allows access to tissues that are important for diagnostic utility. Figure 2 shows two representative OCT images taken of benign prostatic tissue (A) and an area of prostate adenocarcinoma (B). Higher backscattering intensity similar to the intensity level the epithelial cells lining the glands was observed throughout areas of carcinoma. This may be the result of loss of glandular organization and differentiation, increased nuclear size, and hyperchromasia, although its origin must be further investigated.
Fig. 1. Representative ultrahigh resolution OCT images of structures in the human prostate. A. Striations of the fibromuscular stroma and adipose droplets are visible. B. Large glands in the seminal vesicle. C. Muscular layers of the vas deferens.

Fig. 2. A. Benign glandular tissue. Large glands and glandular distribution can be clearly identified in the image and corresponding histology. B. OCT image of an area of prostate adenocarcinoma.

These preliminary results demonstrate the feasibility of using ultrahigh resolution OCT to image human prostate pathology using a portable ultrahigh resolution system capable of imaging in vivo. Additional studies are in progress to evaluate the ability of OCT to differentiate normal and pathologic conditions in the prostate and other tissues. Spectroscopically-resolved OCT using broadband light sources also promises to improve tissue differentiation and image contrast. These technologies can be extended into the intraoperative setting and should enable comprehensive imaging studies. The ability to visualize tissue pathology in situ and in real time promises to improve both diagnosis and therapy of prostate carcinoma. These studies were conducted in collaboration with Dr. Mike Weinstein and Dr. Anthony D’Amico at Brigham and Women’s Hospital.
References


High Resolution Imaging of Coronary Vessels

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Coronary disease is one of the leading causes of death in the industrialized world. Identification of coronary structure, in particular, coronary plaques and the associated fibrous regions can lead to early detection and reduce the risk of coronary thrombosis. Methods to accurately identify arterial wall components, such as calcium, fibrous tissue and lipids have many potential research and clinical applications in biomedical as well as health-industry related fields. OCT has been shown the capability to resolve arterial morphology with good distinction between various structural features (i). The penetration depth of OCT is typically 1-2mm which is ideal for a coronary vessel model. In addition polarization sensitivity of light can be employed to distinguish collagen within the intima and media from polarization insensitive regions such as calcium deposits. Identification of clinically relevant coronary structures with high resolution, contrast and depth penetration will be investigated with the goal of distinguishing between normal, fibrous and vulnerable plaque vessel morphology. High resolution OCT imaging has the potential to impact coronary diagnosis as well as the guidance of interventional surgeries.

OCT has been shown to be an effective intravascular imaging technique for the discrimination of coronary plaques and assessment of interventional procedures such as arterial stent placement [1,2]. Guided placement of coronary stents have greatly improved the outcome of interventional intravascular procedures and constitutes the majority of percutaneous interventions [3,4]. Current intracoronary stent procedures require a secondary ultrasound (IVUS) catheter to be introduced after removal of a balloon catheter in order to assess stent deployment efficacy. In addition to increased operational time which incurs higher risk to the patient, IVUS resolution is limited to only ~80 µm at an operational frequency of 40 MHz.

OCT has several advantages for intravascular imaging. High resolution OCT imaging with discrimination of sub-cellular features has been demonstrated indicating a resolution capability down to 10-20 µm. Further because OCT is a fiber-optically based imaging technique, extremely small and compact probes have been demonstrated with outer diameters of <500 µm [5]. The simple design and components of OCT catheters make them relatively inexpensive as well as highly compact when compared with IVUS catheters and ultrasound machines. Finally OCT can be performed at 4-8 frames per second allowing near real-time imaging during coronary procedures.

A transverse scanning catheter-endoscope for minimally invasive intraluminal imaging has also been developed (Figure 1). The catheter-endoscope consists of a single-mode optical fiber placed within a cable assembly. The beam from the distal end of the fiber is focused by a gradient index (GRIN) lens and is directed perpendicular to the catheter axis by a microp prism located at the distal end. The catheter is encased in a transparent sheath to enable introduction into body lumens. The beam is scanned circumferentially at 1-4 revolutions per second by rotation of the cable, fiber, and optical assembly inside the plastic housing. The catheter-endoscope had a diameter of 1 mm, which is small enough to allow imaging through the accessory port of a standard endoscope and can be used to image coronary arteries. In vivo, real-time imaging of New Zealand White rabbits was performed.
For the arterial study, ex-vivo imaging of human coronary arteries were been performed. Examples of OCT images of normal, fibrous and plaque-rich arteries are shown in figure 2. These results suggest the feasibility of OCT imaging for improving patient risk stratification in the premalignant conditions and inflammatory diseases. Preliminary results suggest both an ability to distinguish epithelial changes and to identify mucosal abnormalities consistent with early neoplastic changes. Specialized image processing software is also in continuing development to accurately represent high resolution OCT images taken in a polar coordinate basis.

The high resolution of OCT allows imaging to be performed near the resolution of histopathology, offering better discrimination and delineation of arterial structure and morphology. OCT has demonstrated the potential to have a significant impact on both the identification of high risk coronary plaques and in guidance of interventional arterial procedures.

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Ultrahigh Resolution OCT in the Clinic

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We report the development of a portable ultrahigh resolution system that enables ultrahigh resolution imaging to be performed in the clinic. Imaging in the clinic and in the pathology laboratory enables accurate registration of OCT images with histology as well as a comparison of in vivo versus ex vivo imaging. In order to preserve diagnostic integrity, specimens often cannot be removed from the hospital. The capability of performing ultrahigh resolution imaging in the pathology lab setting enables access to tissues that were previously inaccessible and is especially important as a research tool. Imaging with increased axial as well as increased transverse resolution also reduces speckle noise features, improving image quality. Techniques that can visualize microscopic disease in situ and in real time promise to improve diagnostic accuracy. The ability to visualize tissue structures can also be combined with intraoperative studies to facilitate the guidance of microsurgical procedures.[1]

Our OCT system uses an all solid-state, Kerr-lens mode-locked Cr:forsterite laser source operating at 1260 nm center wavelength as the light source.[2] In order to make the system compact and portable, the two arms of the laser cavity are folded to fit the pump head, laser optics, and fiber coupling apparatus within a 60x30 cm footprint box (Figure 1). Broadband light necessary for ultrahigh resolution imaging is generated by spectrally broadening the femtosecond laser pulses using self-phase modulation in a dispersion-shifted single-mode fiber. The sample arm of the system can be intergrated with a wide variety of imaging devices such as OCT catheters, surgical imaging probes, and imaging needles. A low power green aiming beam allows localization of the imaging area and facilitates marking for correlation with histology. The system achieves an axial resolution of 6 µm in air or 4.5 µm in tissue. Images can be acquired in real time at 4-8 frames per second.

Example ultrahigh resolution OCT images taken in the pathology laboratory with the Cr:forsterite laser source are shown in Figure 2. Images were performed in the pathology laboratory from specimens obtained postmortem. Glandular structure and mucosal features are visible in both the trachea and...
bronchus. Sharp boundaries between follicles as well as highly scattering centers which correspond to calcified inclusions are visible in the thyroid.

Figure 2. Example ultrahigh resolution OCT images of trachea, bronchus, and thyroid. Images were taken with the Cr:forsterite laser source in the pathology laboratory.

These preliminary results demonstrate the feasibility of ultrahigh resolution OCT imaging using a portable system in a pathology laboratory environment. Additional studies are in progress to evaluate the ability of OCT to differentiate normal and pathologic conditions in the prostate and other tissues. Spectroscopically-resolved OCT using broadband light sources also promises to improve tissue differentiation and image contrast. These technologies can be extended into the intraoperative setting and should enable comprehensive imaging studies. The ability to visualize tissue pathology in situ and in real time on tissues that were previously inaccessible promises to be an important research tool to improve both diagnosis and therapy. These studies are being conducted in collaboration with Dr. Liron Pantanowitz and Dr. James Connolly at Beth Israel Deaconess Medical Center and Harvard Medical School.

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