Optical Coherence Tomography

Tomography and Optical Imaging

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Introduction

Optical coherence tomography (OCT) is an emerging imaging technique for a wide range of biological, medical, and material investigations. OCT was initially developed for imaging biological tissue because it permits the imaging of tissue microstructure in situ, yielding micron-scale image resolution without the need for excision of a specimen and tissue processing. OCT is analogous to ultrasound B-mode imaging except that it uses low-coherence light rather than sound and performs cross-sectional imaging by measuring the backscattered intensity of light from structures in tissue. The OCT image is a gray-scale or false-color two-dimensional representation of backscattered light intensity in a cross-sectional plane. The OCT image represents the differential backscattering contrast between different tissue types on a micron scale. Because OCT performs imaging with light, it has a one to two order-of-magnitude higher spatial resolution than ultrasound and does not require contact with the specimen or sample.

OCT was originally developed and demonstrated in ophthalmology for high-resolution tomographic imaging of the retina and anterior eye. Because the eye is transparent and is optically accessible, it is well suited for diagnostic OCT imaging. OCT is promising for the diagnosis of retinal disease because it can provide images of retinal pathology with 10 micron resolution, almost one order-of-magnitude higher than previously possible using ultrasound. Recently, OCT has been applied for imaging in a wide range of nontransparent tissues. In tissues other than the eye, the imaging depth is limited by optical attenuation due to scattering and absorption. Ophthalmic imaging is typically performed at 800 nm wavelengths. However, because optical scattering decreases with increasing wavelength, OCT imaging in nontransparent tissues is possible using longer near infrared wavelengths. In most tissues, imaging depths of 2–3 mm can be achieved using a system detection sensitivity of 100 to 110 dB. Imaging studies have been performed in a wide range of biological, medical, and surgical specialties including developmental biology, cardiology, gastroenterology, urology, and neurosurgery. High-resolution OCT using short coherence length, short pulsed light sources has also been demonstrated and axial resolutions less than 2 μm have been achieved. High-speed real time OCT at image acquisition rates of 4 to 8 frames per second have also been demonstrated. OCT has been extended to perform Doppler imaging of blood flow and birefringence imaging to investigate tissue injury. Different imaging delivery systems including transverse imaging catheters and forward imaging devices have been developed to enable internal body OCT imaging. Most recently, an OCT catheter has been combined with endoscope based delivery to perform in vivo imaging in animal models and human patients.

This chapter will provide an overview of the OCT technology, beginning with the basic principles of operation. Technological advancements over the last decade have enabled OCT to transition from laboratory- to clinical-based imaging. These enabling advancements will be discussed, followed by representative applications in biology, medicine, surgery, and materials.

Principles of Operation

OCT is based on optical ranging, the high-resolution, high dynamic range detection of backscattered light. In contrast to ultrasound, the velocity of light is extremely high. Therefore, the echo time delay of reflected light cannot be measured directly and interferometric detection techniques must be used. One method for measuring echo time delay is to use low coherence interferometry or optical coherence
domain reflectometry. Low coherence interferometry was first developed for measuring reflections in fiber optics and optoelectronic devices.

The echo time delay of reflected light is measured by using a Michelson-type interferometer (Figure 1).

The light reflected from the specimen or sample is interfered with light that is reflected from a reference path of known path length. Interference of the light reflected from the sample arm and reference arm of the interferometer can occur only when the optical path lengths of the two arms match to within the coherence length of the optical source. As the reference arm optical path length is scanned, different echo delays of backscattered light from within the sample are measured. The interference signal is detected at the output port of the interferometer, electronically band-pass filtered, demodulated, digitized, and stored on a computer. The position of the incident beam on the specimen is typically scanned in the transverse direction and multiple axial measurements are performed. This generates a two-dimensional data array that represents the optical backscattering through a cross-sectional plane in the specimen (Figure 2). The logarithm of the backscatter intensity is then mapped to false-color or gray-scale and displayed as an OCT image. The interferometer in an OCT instrument can be implemented using a fiber optic coupler and beam-scanning can be performed with small mechanical galvanometers in order to yield a compact and robust system (Figure 3).

In contrast to conventional microscopy, the axial resolution in OCT images is determined by the coherence length of the light source. The axial point spread function of the OCT measurement as defined by the signal detected at the output of the

![Figure 1](image1.png)

**Figure 1** Schematic illustrating the concept of low-coherence interferometry. Using a short coherence length light source and a Michelson-type interferometer, interference fringes are observed only when the path lengths of the two interferometer arms are matched to within the coherence length of the optical source. Abbreviations: BS, beam splitter; \( \Delta L \), coherence length.

![Figure 2](image2.png)

**Figure 2** OCT image generation. The OCT image is typically acquired by performing axial measurements of optical backscatter at different transverse positions on the specimen and displaying the resulting two-dimensional data set as a gray-scale (or false-color) image.
interferometer is the electric-field autocorrelation of the source. The coherence length of the light is the spatial width of the field autocorrelation and the envelope of the field autocorrelation is equivalent to the Fourier transform of its power spectrum. Thus, the width of the autocorrelation function, or the axial resolution, is inversely proportional to the width of the power spectrum. For a source with a Gaussian spectral distribution, the axial resolution $\Delta z$ is given:

$$\Delta z = \frac{2 \ln 2 \cdot \lambda^2}{\pi \cdot \Delta \lambda}$$

where $\Delta z$ and $\Delta \lambda$ are the full-widths-at-half-maximum of the autocorrelation function and power spectrum respectively and $\lambda$ is the source central wavelength. Figure 4 illustrates the dependence of the coherence length (axial resolution) on the bandwidth of the optical source. To achieve high axial resolution requires broad bandwidth optical sources. Resolution is also improved by using shorter wavelengths, however, shorter wavelengths are scattered and absorbed more in biological tissue.

The transverse resolution in an OCT imaging system is determined by the focused spot size in analogy with conventional microscopy and is given by:

$$\Delta x = \frac{4 \lambda}{\pi} \cdot \frac{f}{d}$$

Where $d$ is the spot size on the objective lens and $f$ is its focal length. High transverse resolution can be obtained by using a large numerical aperture and focusing the beam to a small spot size. The transverse resolution is also related to the depth of focus or the confocal parameter $2\varepsilon_R$ (two times the Raleigh range).

$$2\varepsilon_R = \frac{\pi \Delta x^2}{2 \lambda}$$

Thus, increasing the transverse resolution results in a reduced depth of field. Typically, the confocal parameter or depth of focus is chosen to match the desired depth of imaging. Increased resolution may
also be obtained by using a higher numerical aperture lens or objective and spatially tracking the focus through the specimen.

Finally, the detection signal-to-noise is given by the optical power backscattered from the sample divided by the noise equivalent bandwidth:

\[ \text{SNR} = 10 \log \left( \frac{\eta P_{\text{SAM}}}{\frac{\text{FWHM}}{\text{NFB}}} \right) \]

Depending upon the desired signal to noise performance, incident powers of 5–10 mW are typically required for OCT imaging of 250 500 square pixel images at several frames per second. If lower data acquisition speeds or signal-to-noise can be tolerated, power requirements can be reduced accordingly.

**Technological Developments**

Since the inception of OCT in the early 1990s, there has been rapid technological developments aimed at improving the imaging resolution, acquisition rate, and methods for beam delivery to the tissue or sample. Investigators have also explored other imaging methods using the principles of OCT to extract information from the tissue or sample. Some of these methods have included acquiring optical Doppler signals from moving scatterers or structures, obtaining images based on the polarization state of the returned light, and extracting spectroscopic information based on the local absorption or scattering properties of the tissue.

**Optical Sources / High-Resolution Imaging**

The majority of OCT imaging systems to date have used superluminescent diodes (SLDs) as low coherence light sources. SLDs are manufactured with a similar structure to that of laser diodes, but common end facets are angle-cleaved to suppress lasing. These device structures do not support oscillation modes and generate output based on amplified spontaneous emission, resulting in emission spectra that are broader than laser diodes. SLDs are commercially available at a range of wavelengths including 800 nm, 1.3 μm, and 1.5 μm and are attractive because they are compact, have high efficiency, and low noise. However, output powers are typically limited to only a few milliwatts which limits fast real-time acquisition rates, and the available bandwidths are relatively narrow, permitting imaging with 10–15 micron resolution. Recent advances in short-pulse solid-state laser technology make these sources attractive for OCT imaging in research applications. Femtosecond solid-state lasers can generate tunable, low-coherence light at powers sufficient to permit high-speed OCT imaging. Short pulse generation has been achieved across the full wavelength range in titanium:sapphire (Ti:Al₂O₃) from 0.7 μm to 1.1 μm and over more limited tuning ranges near 1.3 μm and 1.5 μm in chromium: forsttrite (Cr³⁺:Mg₂SiO₄) and chromium:yttrium-aluminum-garnet (Cr³⁺:YAG) lasers, respectively. OCT imaging with resolutions of 1 μm and 5 μm has been demonstrated at 800 nm and 1.3 μm respectively using Ti:Al₂O₃ and Cr³⁺:Mg₂SiO₄ sources. More compact and convenient sources such as superluminescent fiber sources, are currently under investigation. The titanium:sapphire laser technology is routinely used in multi-photon microscopy applications for its high peak intensities to enable multi-photon absorption and subsequent emission of fluorescence from exogenous fluorescent contrast agents. Combined OCT and multi-photon microscopy has been used to provide complementary image data using a single optical source.

**Fast Scanning**

The short-pulse solid-state laser technology not only provides broad spectral bandwidths for high-resolution OCT imaging, but also higher output powers to enable fast real-time OCT imaging. Higher incident powers are required to maintain equivalent signal-to-noise ratios when scanning at a faster rate. Linearly translating a reference arm mirror is problematic at high rates and provides axial scan frequencies of approximately 100 Hz, depending on the mirror size and the translating galvanometer. Several investigators have utilized rotating glass cubes, piezoelectric modulators, and multi-pass optical cavities to increase axial scan rates while maintaining scan ranges of 1–2 mm. An optical delay based on the principles used in femtosecond pulse shaping has been demonstrated for OCT. This delay line spectrally disperses the reference arm beam with a grating. The dispersed beam is then focused by a lens on to a rotating mirror mounted on a galvanometer. The mirror, located in the Fourier-transform plane of the lens, imparts a wavelength-dependent phase shift on the light. Subsequently, when re-coupled back into the interferometer, this phase-shift is equivalent to a time-delay in the time domain. The use of high-speed resonant galvanometers has permitted axial scan rates as high as 8 kHz over scan ranges of several millimeters. Depending on the image pixel size (number of axial scans within each image), this scan rate can provide video-rate OCT imaging (30 frames per second). An example of high-speed functional OCT imaging is shown in Figure 5. Images of a
beating *Xenopus laevis* (African frog) tadpole were compared at slow (30 s per image) and at fast (2.50 ms per image) acquisition rates. Artifacts due to cardiac motion were present at slow acquisition rates, but were minimized with acquisition rates of 4 frames per second. Functional cardiac parameters could be measured and high-speed processes such as chamber and valve function could be visualized in real-time.

Depth-priority scanning, as described above, is performed by rapidly varying the optical delay in the reference arm and collecting a single axial scan before translating the beam laterally and repeating this depth-scanning. An alternative method for generating OCT images is with transverse-priority scanning. This is equivalent to optical sectioning in confocal and multi-photon microscopy. Three-dimensional OCT imaging using transverse-priority scanning can be obtained by stepping the position of the reference arm mirror after each *en face* image is acquired. The OCT images produced from transverse-priority scanning can be correlated with confocal or multi-photon microscopy images, which are acquired with the same scanning method. This method can also utilize higher numerical aperture objective lenses to provide high transverse resolutions since a large depth of focus is not needed as in depth-priority OCT scanning. The combination of OCT with high-numerical aperture objectives has been termed optical coherence microscopy.

**Doppler Optical Coherence Tomography**

Conventional OCT detects the amplitude of the optical backscatter versus position within the sample. Doppler OCT is a technique which has been applied to measuring fluid flow within small capillary tubes and in *vivo* vessels in the skin, retina, and heart of small animal models and humans. This technique is based on digitally sampling the interference fringes that are produced from a moving scatterer within the sample or specimen and determining the Doppler frequency shift in the signal due to the moving scatterer. Figure 6 illustrates two- and three-dimensional optical Doppler data for fluid flow through a cylindrical tube. A two-dimensional cross-sectional profile of fluid flow through a silicon tube with a 600 µm inner-diameter is plotted in Figure 6. The acquired data (points) closely match the predicted profile (line) for these tube dimensions. Below the plot, one method of obtaining a three-dimensional fluid flow profile is illustrated. While imaging the tube in cross-section, the fluid flow velocity was altered with a perfusion pump. The detection filter bandwidth of the OCT electronics served a windowing function, detecting only flow velocities with frequencies within the detection bandwidth. For an unknown flow profile, the center frequency of the detection filter can be shifted to map the flow velocities present within the sample.

**Beam Delivery**

The OCT imaging technology is modular in design and a variety of optical instruments can be used to deliver the OCT beam to the tissue or sample. Because OCT is fiber-optic based, single optical fibers can be used to deliver the OCT beam and collect the reflected light. The OCT technology can readily be integrated into existing optical instruments such as research and surgical microscopes, ophthalmic slit-lamp biomicroscopes, and hand-held imaging probes.

Imaging penetration is determined by the optical absorption and scattering properties of the tissue or specimen. The imaging penetration for OCT ranges from tens of millimeters for transparent tissues such as the eye to less than 3 mm in highly-scattering tissues such as skin. To image highly-scattering tissues deep within the body, novel beam-delivery
instruments have been developed to relay the OCT beam to the site of the tissue to be imaged. An OCT catheter has been developed for insertion into biological lumens such as the gastrointestinal tract. Used in conjunction with endoscopy, the 1 mm diameter catheter can be inserted through the working channel of the endoscope for simultaneous OCT and video imaging. Minimally-invasive surgical procedures utilize laparoscopes, which are long, thin, rigid optical instruments that permit video-based imaging within the abdominal cavity. Laparoscopic OCT imaging has been demonstrated by passing the OCT beam through the optical elements of a laparoscope. Deep solid-tissue imaging is possible with the use of fiber-needle probes. Small (400 μm diameter) needles housing a single optical fiber and micro-optic elements can be inserted into solid tissues and rotated to acquire OCT images.

Recently, microfabricated micro-electro-optical-mechanical systems (MEOMS) technology has been used to miniaturize the OCT beam scan mechanism.

**Applications**

**Developmental Biology**

OCT has been demonstrated in the field of developmental biology as a method to perform high-resolution, high-speed imaging of developing morphology and function. Cellular-level imaging is possible, providing a non-invasive technique for visualizing cellular processes such as mitosis and migration. Imaging studies have been performed on several standard biological animal models commonly employed in developmental biology investigations including *Rana pipiens* (Leopard frog), *Xenopus laevis*.
(African frog), and *Brachydanio rerio* (zebrafish) embryos and eggs, and the murine (mouse) model.

A series of cross-sectional images acquired *in vitro* from the dorsal and ventral sides of a Stage 49 (12 day) *Rana pipiens* (Leopard frog) tadpole is shown in Figure 7. Features of internal architectural morphology are clearly visible in the images. The image of the eye differentiates structures corresponding to the cornea, lens, and iris. Internal morphology not accessible in one orientation due to the specimen size or shadowing effects can be imaged by reorienting the specimen and scanning in the same cross sectional image plane. With the OCT beam incident on the ventral side, images of the respiratory tract, ventricle of the heart, internal gills, and gastrointestinal tract were acquired.

These images can be compared with corresponding histology (Figure 7). Histological images are acquired by euthanizing the specimen, immersing the specimen in a chemical fixative, and physically sectioning thin (2–5 micron-thick) slices using a microtome. The slices are placed on a microscope slide, selectively stained to highlight particular features, and viewed with light microscopy. The correlations between OCT and histology images are strong, suggesting that OCT images can accurately represent the *in vivo* specimen morphology. The potential exists to repeatedly image specimens to quantify organo- and morphogenesis throughout development. Technologies such as OCT are likely to become increasingly important in functional genomics, relating genetic features to the morphology and function in living specimens.

OCT images represent the optical backscatter intensity from regions within the tissue or sample. Because OCT relies on the inherent optical scattering changes to produce imaging contrast, no exogenous contrast agents or fluorophores are necessary. This permits long-term sequential imaging of development *in vivo* without loss of specimen viability. Repeated images of a developing zebrafish embryo within its egg beginning immediately after fertilization and up until hatching have been demonstrated without loss of specimen viability or without developmental abnormalities. In this example, the zebrafish egg and embryo were semi-transparent and the use of OCT significantly complemented observations made using light microscopy. By imaging subtle differences in backscattering intensity, interfacial structural layers millimeters deep within specimens can be clearly delineated.

Previous OCT images have characterized morphological features within biological specimens. These structures are static even though they may have been acquired from *in vivo* specimens. *In vivo* imaging in living specimens, particularly in larger organisms and
for medical diagnostic applications, must be performed at high speeds to eliminate motion artifacts within the images. Functional OCT imaging is the quantification of in vivo images which yield information characterizing the functional properties of the organ system or organism. High-speed OCT permits both the positioning and manipulation of specimens as well as imaging in real time and is a powerful technology for functional imaging in developmental biology animal models.

Studies investigating normal and abnormal cardiac development have been frequently limited by an inability to access cardiovascular function within the intact organism. OCT has been demonstrated for the high-resolution assessment of structure and function in the developing Xenopus laevis (African frog) cardiovascular system (Figure 8). The morphology of the in vivo cardiac chambers is clearly delineated. Image acquisition rates are fast enough to capture the cardiac chambers in mid-cycle. With this capability, images can be acquired at various times during the cardiac cycle. These frames can be displayed in real-time to produce a movie illustrating the dynamic, functional behavior of the developing heart. OCT, unlike technologies such as computed tomography and magnetic resonance imaging, provides high-speed in vivo imaging, allowing quantitative dynamic activity, such as ventricular ejection fraction, to be assessed.

**Cellular Imaging**

Although previous studies have demonstrated in vivo OCT imaging of tissue morphology, most have imaged tissue at ~10-15 μm resolutions, which does not allow differentiation of cellular structure. The ability of OCT to identify the mitotic activity, the nuclear-to-cytoplasmic ratio, and the migration of cells has the potential to not only impact the fields of cell and developmental biology, but also impact medical and surgical disciplines for the early diagnostics of disease such as cancer.

The Xenopus laevis (African frog) tadpole has been used to demonstrate the feasibility of OCT for high-resolution in vivo cellular and subcellular imaging. Many of the cells in this common developmental biology animal model are rapidly dividing and migrating during the early growth stages of the tadpole, providing an opportunity to image dynamic cellular processes. Three-dimensional volumes of high-resolution OCT data have been acquired from these specimens throughout development. From this 3-D data set, cells undergoing division were identified and tracked in three dimensions. In a similar manner, 3-D data sets were acquired to track single melanocytes (neural crest cells) as they migrated through the living specimens. The ability of OCT to characterize cellular processes such as mitosis and migration not only are of interest in cell and developmental biology, but also have relevance for cancer diagnostics and tumor metastasis.

An example of cellular-level OCT imaging in these specimens is shown in Figure 8. This composite image (0.83 μm x 1 mm, 1800 x 1000 pixels) was acquired using a titanium:sapphire laser with a broad bandwidth (~260 nm). The axial and transverse resolutions in this image is 1 μm and 5 μm, respectively (see Figure 4). Because the high transverse resolution reduced the depth-of-focus to 49 μm, separate OCT images were first acquired with the focus at different depths within the specimen. These images were then assembled to produce the composite image shown in Figure 8. This type of image construction is similar to C-mode ultrasound. Cellular features including cell membranes, nuclei, and nuclear morphology are clearly observed.

**Medicine – Imaging Barrett's Esophagus**

OCT performs in situ imaging and has the potential to be used as a screening technique of early pathological changes in patients. OCT imaging has the potential to be a general diagnostic in many organ systems,

![Figure 8](image-url)  
*Figure 8* Cellular-level OCT imaging using a broad bandwidth titanium:sapphire laser. Axial and transverse image resolutions are 1 μm and 5 μm, respectively, enabling the visualization of cell membranes, nuclear morphology, and sub-cellular organelles. Cellular processes such as mitosis and migration can be visualized in real-time in living specimens. (Image reprinted with permission from Drozdek W, Morgner U, Kortner FX, et al. (1999) In vivo ultrahigh resolution optical coherence tomography. Optics Letters 24: 1221–1223.)
particularly where current methods of screening by excisional biopsy are limited. Barrett’s esophagus is a condition where the cells of the distal esophagus undergo a metaplastic change, resembling cells of the lower gastrointestinal tract. This condition is believed to be caused by chronic gastroesophageal reflux. Several studies have demonstrated that Barrett’s esophagus is associated with a 30–125 times increased risk of developing adenocarcinoma. For this reason, endoscopic surveillance of Barrett’s epithelium every 12–18 months is recommended. Endoscopic screening currently involves random four-quadrant biopsies every 1–2 cm along the length of suspect mucosa. However, excisional biopsy is prone to sampling errors and small foci of carcinoma or dysplasia may be missed. Because of the imprecision and high cost associated with screening, new methods are being developed to assess patients at increased risk. Endoscopic ultrasonic catheters have been used for imaging the gastrointestinal tract with 50–100 μm resolution. However, these resolutions are insufficient to resolve early epithelial changes that occur in Barrett’s esophagus and the pre-malignant changes that lead to adenocarcinoma of the esophagus.

The ability of OCT to differentiate normal and pathologic tissue is a central question being addressed by many research groups. Comparisons with histology show good correlations between OCT images and histological findings. The image resolution of conventional OCT (10–15 μm) is sufficient to differentiate architectural but not cellular morphology. Still, endoscopic OCT resolution can differentiate normal from Barrett’s epithelium in real-time based on differences in epithelial architecture. Crypt- and gland-like structures that disrupt the relatively uniform layers of squamous epithelium can be readily identified, enabling differentiation between normal and Barrett’s epithelium (Figure 9). The ability to differentiate normal from Barrett’s epithelium suggests that the OCT technology could be used for screening applications.

**Oncology – Identifying Tumors and Tumor Margins**

OCT has been used to differentiate between the morphological structure of normal and cancerous tissue for a wide range of tumors. The use of OCT to identify tumors and tumor margins in situ will represent a significant advancement for medical or image-guided surgical applications. OCT has been demonstrated for the detection of brain tumors and their margins with normal brain parenchyma, suggesting a role for guiding surgical resection. A hand-held surgical imaging probe was constructed for this application. The compact and portable probe permits OCT imaging within the surgical field while

![Figure 9](image-url)  **Figure 9**  Endoscopic OCT imaging in human esophagus shows normal and Barrett’s esophagus. A OCT catheter was inserted into the working channel of an endoscope, guided by video imaging. Regions of normal and Barrett’s esophagus were imaged. Corresponding biopsy histology is shown. OCT can identify Barrett’s esophagus by the presence of gland- and crypt-like structures (arrows). (Images reprinted with permission from Li XD, Boppard SA, Van Dam J, et al. (2000) Optical coherence tomography: advanced technology for the endoscopic imaging of Barrett’s esophagus. *Endoscopy* 32: 031–036.)
the OCT instrument can be remotely located in the surgical suite.

Figure 10 shows a specimen of outer human cerebral cortex with metastatic melanoma. The OCT images in Figure 10a,b were acquired through the tumor. These original images were threshold segmented to identify regions of high backscatter within the tumor. The original images were then overlaid with the segmented data and are shown in Figure 10c,d. The OCT images show increased optical backscattering in the region of the larger tumor (arrows). Smaller tumor lesions also appear within the image (arrows). A shadowing effect is observed below each tumor site due to the increased optical backscatter and the subsequent loss of optical power penetrating beneath the tumor. In Figure 10a,c, the boundary of the tumor can be identified. In Figure 10b,d, the tumor is identified below the surface of normal cortex. The histology in Figure 10e,f confirms the presence and relative size of the tumor.

The image resolutions used to acquire the images in Figure 10 were as high as 16 μm, higher than any current ultrasound, CT, or MRI intraoperative imaging technique. This allowed the tumor-cortex interface and the extent of tumor below the surface to be defined with high resolution. At higher imaging resolutions, it may be possible to image individual tumor cells which have migrated away from the central tumor. OCT represents a new high-resolution optical imaging technology that has the potential for identifying tumors and tumor margins on the micron scale and in real time. OCT offers imaging performance not achievable with current imaging modalities and may contribute significantly toward the surgical resection of neoplasms.

Image-Guided Surgery

The repair of vessels and nerves is necessary to restore function following traumatic injury. Although the repair of these sensitive structures is performed with the aid of surgical microscopes and loupes to magnify the surgical field, surgeons are limited to the en face view that they provide. A technique capable of subsurface, three-dimensional, micron-scale imaging in real-time would permit the intraoperative monitoring of microsurgical procedures. The capabilities of OCT for the intraoperative assessment of microsurgical procedures have been demonstrated. High speed OCT imaging was integrated with a surgical microscope to perform micron scale three-dimensional imaging on microsurgical specimens.

OCT has been used to image in vitro peripheral nerves and identify individual fascicles. Longitudinal tracking of the spatial orientation of rabbit peripheral nerve fascicles is demonstrated in Figure 11. Representative cross-sectional images of the peripheral nerve are shown in Figure 11a–d. For each slice, one fascicle was manually segmented, colored white, and tracked through the acquired volume of data. Forty images at 100 μm spacing were assembled for the 3-D projections shown in Figure 11e,f. The horizontally- and vertically-rotated projections of the peripheral nerve dramatically reveal the twisted path of the segmented fascicle along the longitudinal axis of the nerve. In addition, a branch in an unsegmented fascicle is observed in Figure 11f. The use of OCT to acquire multiple cross-sectional images and threedimensionally reconstruct the peripheral nerve offers the opportunity to determine the relative diameters of individual fascicles and to longitudinally track their spatial orientation. OCT may also be useful at identifying and grading the degree of injury in nerves during surgical repair. These results have shown how 2-D OCT images and 3-D OCT projections can
provide diagnostic feedback to assess microsurgical anastomoses. This previously unavailable diagnostic ability offers the potential to directly impact and improve patient outcome by incorporating high-speed, high-resolution intraoperative image guidance during microsurgical procedures.

Surgical intervention requires visualization to identify tissue morphology, precision to avoid sensitive tissue structures, and continuous feedback to monitor the extent of the intervention. OCT may provide the technological advancements to improve the operative procedure. The feasibility of OCT to perform image-guided surgical intervention has been investigated. OCT has been used to monitor laser ablation therapy in real-time and may enable more precise control of laser delivery. An argon-laser ablation sequence of fresh ex vivo rat rectus abdominis muscle is shown in Figure 12. A pair of blood-filled vessels were located 1.5 mm below the tissue surface and centered within the OCT image. Because blood has a higher absorption coefficient than muscle at the 514 nm argon laser wavelength, coagulation and thermal heating of the blood occurred prior to thermal damage to the overlying tissue. The accompanying histology reveals both the thermally-damaged overlying tissue and coagulated blood present within the vessel pair.

These examples demonstrate the use of OCT for guiding and monitoring surgical intervention. The laser is only a representative interventional surgical technique for a wide range of instruments and techniques including scalpels, electrosurgery, radiofrequency, microwaves, and ultrasound ablation. OCT imaging was performed at 8 frames per second, fast enough to capture dynamic changes in the optical properties of the tissue during thermal ablation. These image sequences provided interesting insight into ablation mechanisms for a variety of tissue types. OCT can monitor the extent of thermal injury below the surface of the tissue by imaging the changes in optical backscatter. OCT imaging can therefore provide empiric information for dosimetry to minimize the extent of collateral injury. The use of OCT for guiding surgical interventions has the potential to improve intraoperative monitoring and more effectively control interventional procedures.

**Materials**

While the majority of OCT applications have been in the fields of biology and medicine, OCT has also been demonstrated in the non-biological areas of materials investigation, optical data storage, and microfluidic devices. The highly-scattering or reflecting optical properties of many materials prohibits deep imaging penetration using OCT. Many material defects, however, originate near the surface or at interfacial boundaries, making the use of OCT a possibility for inspection and quality control. OCT has been used to identify subsurface defects in ceramics and polymer composites. The optical ranging capabilities of OCT through scattering materials has been utilized for increasing the data storage capacity by assembling multiple layers of optically-accessible data.

The advancement of microfabrication techniques has led to increasingly complex microfluidic and bioMEM (biological micro electro mechanical) systems. Microstructures within microfluidic systems range from 10–1000 μm, within both the imaging depth and resolution of OCT. In addition, microfluidic systems are typically fabricated from transparent or semi-transparent substrates, facilitating imaging penetration to deeper three-dimensional features.
OCT is not only capable of imaging three-dimensional microstructures at micron-scale resolutions, but is also capable of obtaining dynamic functional data from microfluidic systems. Microfluidic devices made from polymeric materials are attractive since they provide flexibility in the design of the channels, can be manufactured in large volumes, and are mostly bio-compatible. A three-dimensional imaging technique, such as OCT, will be instrumental in the analysis of these devices and the monitoring of device performance.

Conclusions

The capabilities of OCT offer a unique and informative means of imaging biological specimens and non-biological samples. The non-contact nature of OCT and the use of low-power near-infrared radiation for imaging causes few harmful effects on living cells or damage to materials. OCT imaging does not require the addition of fluorophores, dyes, or stains in order to improve contrast in images. Instead, OCT relies on the inherent optical contrast generated from variations in optical scattering and index of refraction. These factors permit the use of OCT for extended imaging over the course of hours, days, or weeks.

OCT permits the cross-sectional imaging of tissue and samples and enables in vivo structure to be visualized in opaque specimens or in specimens too large for high-resolution confocal or light microscopy.

Imaging at cellular and subcellular resolutions with OCT is an important area of ongoing research. The Xenopus (African frog) developmental animal model has been commonly used because its care and handling are relatively simple while allowing cells with a high mitotic index to be assessed. Many of the cells observed were as large as 100 µm in diameter, but ranged in size down to a few microns, below the resolution of most OCT systems. Imaging human cells in vivo remains a challenge since differentiated human cells are 10 to 20 microns in size, too small for most OCT systems. With further advances in OCT technology, improved discrimination and
imaging of more detailed structures should be possible. New laser sources at other wavelengths in the near-infrared can enhance tissue contrast as well as potentially provide functional information since tissue scattering and absorbance properties in specimens are wavelength dependent. Short coherence length short pulse laser sources have been used to achieve higher axial resolutions on the order of 1 to 3 microns. Unfortunately, unlike superluminescent diode source, these high-speed and high-resolution systems utilize femtosecond lasers that are relatively complex and costly. Developing compact and portable optical sources at near-infrared wavelengths, broad spectral bandwidths, and high output powers is an area of active research.

Optical coherence tomography provides high-resolution morphological, functional, and cellular information of biological, medical, and material specimens and samples. OCT represents a multifunctional investigative tool that not only complements many of the existing imaging technologies available today, but also is poised to become a major optical imaging modality.

**Imaging:** Imaging Through Scattering Media; Interferometric Imaging. **Terahertz Technology:** Coherent Terahertz Sources. **Tomography:** Tomography and Optical Imaging. **Microscopy:** Confocal Microscopy; Imaging multiple Photon Fluorescence Microscopy, Overview.

**Further Reading**


Totowa, NJ: Humana Press, Inc.


**List of Units and Nomenclature**

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<thead>
<tr>
<th>Symbol</th>
<th>Abbreviation</th>
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<tr>
<td>Cr4+Mg2SiO4</td>
<td>chromium:forsterite</td>
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<tr>
<td>Cr4+YAG</td>
<td>chromium:yttrium-aluminum-garnet</td>
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<td>CT</td>
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<td>PSAM</td>
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<td>SNR</td>
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**See also**