Broad-bandwidth optical sources provide increased resolution in optical coherence tomography systems for biomedical imaging.

looking below the surface

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The goal of biomedical imaging is to provide diagnostic information and improve clinical decision-making. As trends in medical and surgical diagnostics and treatments evolve from the morphological (anatomical) level to the cellular and molecular levels, there is an increasing need for diagnostic technologies to follow this same trend. Optical coherence tomography (OCT) has the potential to provide diagnostic information at all of these levels.

OCT is analogous to ultrasound imaging except that the instruments detect reflections of near-IR (NIR) light rather than sound.[1-3] The approach images deep within highly scattering tissues in real time at micron-scale resolution. OCT systems use fiber-based or free-space interferometric techniques to detect magnitude and phase information on the reflections from within tissue. The resultant multidimensional data yields images and volume data sets of the tissue microstructure. Using techniques such as optical Doppler tomography, it is possible to track the velocity of moving scatterers within the tissue, such as blood flow within the microcirculatory system. By measuring the spatial distribution of polarization changes using polarization-

sensitive OCT, researchers and physicians can detect early structural changes in birefringent tissues such as muscle, the retinal nerve fiber layer, and cartilage.

Increasing the resolution and developing practical clinical optical sources are two of the current challenges in OCT. The goal for meeting these challenges is to improve the diagnostic capabilities and the clinical utility of the technology. The potential for OCT imaging has been illustrated in medical and biological imaging applications, from morphology to molecules.

improving resolution

The OCT technology has advanced since its inception in the early 1990s. Advancements in optical sources, interferometer designs, beam delivery systems, and rapid-scanning optical-delay lines have enabled the technology to be applied to a diverse array of biological, medical, surgical, and nonbiological applications. In medicine and biology, it has long been recognized that virtually all pathological processes begin at the molecular and cellular levels, and early detection of these changes has been shown to improve treatment outcomes. Central to the use of the OCT technology is the capability to resolve finer details within tissue. Improving the resolution of OCT has therefore been a challenge addressed by many groups.

A standard OCT configuration acquires cross-sectional images of tissue in depth. The system performs low-coherence interferometry with a Michelson-type interferometer as the optical path length in a reference arm is varied, which corresponds to a scan in depth along a single beam path in the tissue. Subsequently, the beam position on the tissue is translated laterally, and an adjacent axial depth scan is acquired. These columns of data are assembled to produce the cross-sectional image of the tissue.

The axial and transverse resolutions of OCT are independent. The axial resolution (Δz) is defined by the coherence length of the optical source given by

$$\Delta z = \frac{2\ ln\ 2}{\pi} \cdot \frac{\lambda_0^2}{\Delta\lambda} \qquad (1)$$

where λ_0 is the center wavelength of the source and $\Delta\lambda$ is the spectral bandwidth. In other words, the axial resolution can be improved by decreasing the wavelength or by increasing the spectral bandwidth.

The transverse resolution in OCT is determined by the objective or focusing lens. Using standard principles of Gaussian optics, we can define the transverse resolution by

$$\Delta x = \frac{4\lambda_0}{\pi} \cdot \frac{f}{d} \qquad (2)$$

where f is the focal length of the lens and d is the diameter of the beam incident on that lens. Increasing the numerical aperture of the objective increases the transverse resolution but decreases the confocal parameter. The use of high numerical-aperture (NA) objectives requires either transverse-priority OCT (imaging a planar section as in confocal microscopy) or techniques to dynamically scan the focus of the beam in a cross-sectional plane while performing depth-priority scanning.

Improving the transverse resolution is straightforward—simply increase the NA of the lens. Improving the axial resolution in OCT is more challenging. Aside from using shorter wavelengths, it is possible to make significant improvements by increasing the spectral bandwidth of the optical source. Ultrafast lasers such as solid-state diode-pumped titanium-doped sapphire (Ti:sapphire), chromium-doped forsterite (Cr^{4+}:Mg_2SiO_4), and chromium-doped yttrium aluminum garnet (Cr^{4+}:YAG) are commonly used as OCT sources. These sources not only offer broad spectral bandwidths for high-resolution imaging but also high average output

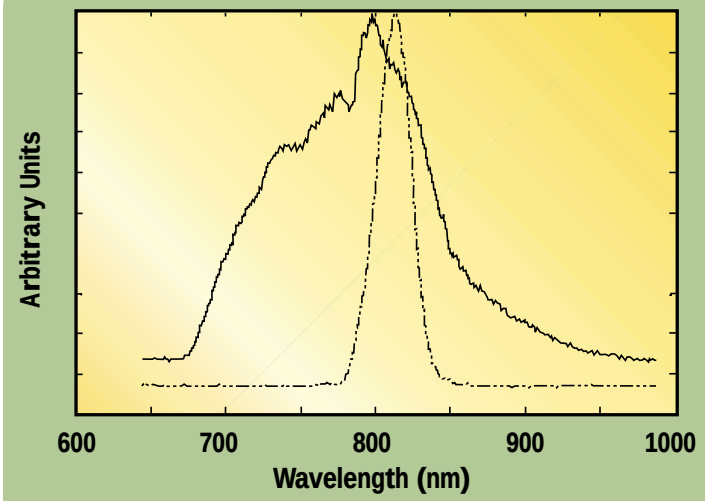

Figure 1 Spectral broadening obtained by pumping high-NA optical fiber with a Ti:sapphire laser can increase OCT imaging resolution.

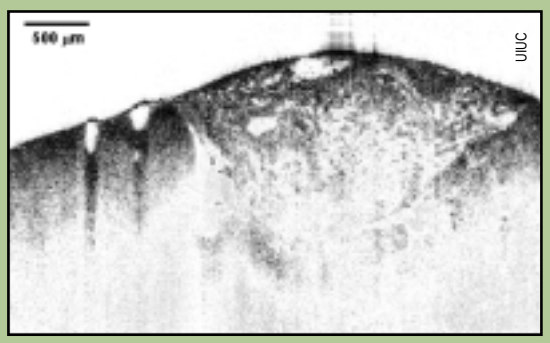

Figure 2 OCT imaging of a sarcoma (muscle tumor) in a dog shows gross structural abnormalities compared to the more homogeneously appearing tissue for normal muscle. Resolution is about 15 µm.

powers for high-speed OCT imaging.

Because of the "biological window" in tissue—a range of wavelengths that are minimally absorbed by biological chromophores and water—the optimal tissue imaging wavelengths are in the NIR spectral region (from 800 to 1300 nm). The Ti:sapphire laser at 800 nm and the Cr^{4+}:Mg_2SiO_4 laser at 1300 nm provide sources at each end of this window. The longer wavelengths in this biological window undergo less scattering, enabling imaging depths as great as 3 mm in highly scattering tissue such as skin or muscle. Although the 800-nm output from the Ti:sapphire laser offers less imaging penetration in highly scattering tissue, the shorter wavelength and broad spectral output can offer high imaging resolution. Spectral bandwidths hundreds of nanometers wide have been generated using Ti:sapphire lasers, enabling axial imaging resolutions as fine as 1 μm in tissue. This laser is also commonly used in multiphoton microscopy, enabling multimodality instruments.

The ultrashort, high-peak intensity pulses from a Ti:sapphire laser can be used to generate nonlinear effects in microstructured and tapered optical fibers, producing even wider spectral bandwidths in a single-mode output suitable for OCT imaging. Spectral broadening can also be induced by pumping high-NA fibers with narrow core sizes that spatially concentrate light and enhance nonlinear processes (see figure 1 on page 29). While the spectrum is not as broad as those from microstructured or tapered fibers, high-NA fiber is inexpensive and readily available.

Preserving the bandwidth generated from the optical source is equally important for optimizing the imaging resolution in OCT. The dispersion properties of instrument optics and biological specimens degrade the imaging resolution. Typically, researchers compensate for this effect by introducing dispersive material into the reference arm of the OCT instrument. This, however, compensates for only a small region within the tissue. Both experimental and computational approaches have been used to compensate for the spatially varying dispersive properties of biological specimens. Using digital algorithms that are amenable to real-time processing, the effects of dispersion can be managed digitally, narrowing the interferogram data from scatterers within the sample and improving the resolution considerably.

imaging

Imaging diagnostic markers of cancer remains a leading goal in biomedical imaging because early diagnosis and treatment of the disease improves the likelihood for a cure. The pathogenesis of cancer, like most diseases, begins at the molecular and cellular levels, progressing to later-stage morphological changes. The data and images presented here were acquired with a diode-pumped Ti:sapphire laser.

Morphology

OCT has been used to image virtually every tissue of the human body at the morphological level. Initial studies were of the eye because of the relative transparency compared to more highly scattering tissues. The 10- to 20-μm resolution currently provided by most OCT optical sources is high enough to resolve the architectural morphology of different tissue types, revealing interesting features that can be correlated to diseases and pathological processes (see figure 2 on page 29). Morphological changes such as those readily apparent in figure 2 can be used to identify suspect areas during image-guided surgery or when taking biopsy specimens for histological processing.

Cellular

Cells in biological tissue vary in size from 5 to 50 μm in diameter. Cells present in developing biological specimens or cells that are undifferentiated (often in tumors) tend to be larger in size with well-defined characteristics that pathologists use to classify cells as normal or abnormal. Normal cells in human tissue tend to be smaller, roughly 5 to 15 μm, and challenge the resolving capability of current OCT systems. Using the broad spectra from ultrafast lasers, researchers have tracked cell morphology and cellular

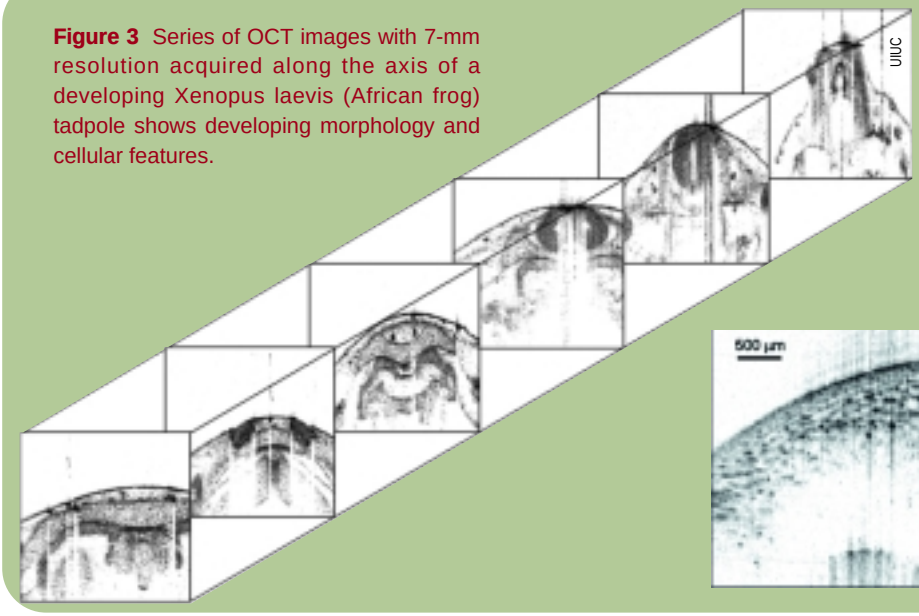

Figure 3 Series of OCT images with 7-mm resolution acquired along the axis of a developing Xenopus laevis (African frog) tadpole shows developing morphology and cellular features.

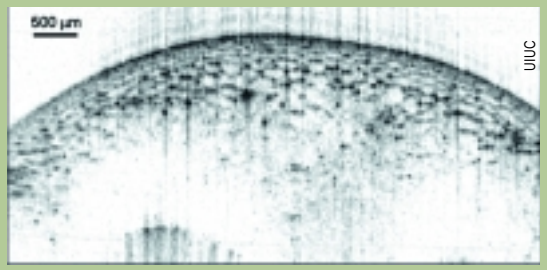

Figure 4 OCT image shows the brick-like arrangement of cells in a botanical specimen. Focal areas within the OCT image that appear highly scattering are individual nuclei within cells.

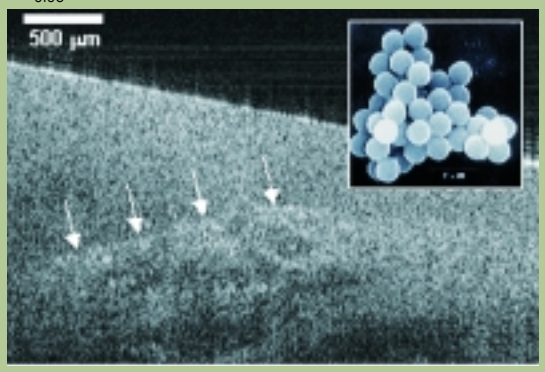

Figure 5 Protein-coated microspheres can be targeted to label specific molecules in tissue. The OCT image shows a layer of these microspheres in a tissue model constructed to mimic the optical properties of human skin.

processes such as cell mitosis (division) and cell migration over time in developing biological specimens (see figures 3 and 4).[4,5]

Molecular

The resolution of OCT is not sufficient to resolve individual molecules. However, it is possible to indirectly detect the presence of specific molecules and map their spatial distribution using the technology. Spectroscopic OCT methods can detect changes in the optical properties of tissue, which allows researchers to identify tissue regions that absorb or scatter wavelengths within the spectral bandwidth of the optical source. A commonly pursued application of this would be the detection and differentiation of oxy-hemoglobin from deoxy-hemoglobin within areas of tissue such as the brain. Spectroscopic techniques may potentially provide additional contrast mechanisms for pathologic regions of tissue. Investigations are underway to determine how these spectral changes correlate with biological and molecular features.

A second indirect means of generating images or maps based on the molecular composition of tissue is to use markers or probes to label targets. Optical contrast agents developed for OCT consist of protein-coated microspheres that can encapsulate a wide variety of materials to enhance detection (see figure 5). The protein coat can be chemically modified to target specific molecules or cell types in tissue.

The OCT technology has emerged as a new high-resolution optical-imaging modality for medicine and biology. Ongoing investigations are beginning to transition the technology from the laboratory to the clinic, and an increasing number of imaging studies are underway on human patients. The technological challenges include not only refinements in imaging resolution and optical sources but also making the technology more clinically useful. As image quality and instrument portability improve, emphasis will be placed on methods to extract clinically diagnostic information from images of tissue morphology, cells, and molecular composition. **oe**

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