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EARLY DEVELOPMENT OF OPTICAL LOW-COHERENCE REFLECTOMETRY AND SOME RECENT BIOMEDICAL APPLICATIONS

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ABSTRACT

This paper explains the term low-coherence interferometry, reviews the early development of optical low-coherence reflectometry, and shows some of the paths that led to the field of biomedical optics. This paper demonstrates that early technical developments in the telecommunications industry resulted in a myriad of technical implementations and applications in biology, medicine, and the explosion of the field in noninvasive biomedical optical techniques. Recent examples of innovative applications of this proliferating technology into the fields of ophthalmology, developmental biology, and endoscopy are described. *© 1999 Society of Photo-Optical Instrumentation Engineers.* [S1083-3668(99)00602-4]

Keywords temporal coherence; optical low-coherence reflectometry (OLCR); optical coherence tomography (OCT); eye; optical biopsy; diagnostics; developmental biology.

1 INTRODUCTION

1.1 LOW-COHERENCE INTERFEROMETRY FOR BIOMEDICAL IMAGING

Low-coherence interferometry is a type of interferometry based on low-coherent light sources.[1] This technique permits the observer to precisely measure the amplitude and the relative phase of reflected or backscattered light. A Michelson optical interferometer consists of a light source, a beamsplitter which splits the light into a reference arm and a measurement arm, and a light detector. The measurement beam is reflected from the specimen with different delay times which depends on the various refractive indexes in the different layers of the specimen. The light reflected from the reference mirror, which is positioned at variable distances, produces a variable time delay. The light from the reference mirror which has a known delay, and the light from the specimen which has multiple delays is correlated and detected. After signal processing the optical distance of the reflecting regions in the specimen is measured. The geometrical distance of these reflecting regions is determined by dividing the optical distance by the group velocity index for each region.

The technology has developed several nomenclature terms. OTDR is optical time domain reflectometry. This technique involves optical ranging with femtosecond laser pulses and nonlinear cross correlation techniques. OCDR is optical coherence domain reflectometry. It is a one-dimensional optical ranging technique where the amplitude and longitudinal delay of backscattering from a sample is resolved using low-coherence Michelson interferometer. Another term with similar meaning is OLCR which is another term for optical low-coherence reflectometry.

OCT is optical coherence tomography. This technique constructs a two-dimensional, transverse image of the sample from a series of one-dimensional scans. OCT is similar to B-scan ultrasound imaging techniques; however, the contrast is derived from differences in optical rather than acoustic backscattering. OCT instruments are constructed by coupling OCDR systems with transverse scanning of the probe beam.

Partially coherent light is defined as light with a short coherence length, but having high spatial coherence. The precision of these methods is from 1.0 to 10 μm depending on the intraocular structure that is measured. In the field of ophthalmology, its advantages for the measurements of intraocular structures include: (1) partial coherence interferometry has high precision, high longitudinal resolution, and low sensitivity to longitudinal eye motion which occur during the measurement time, and (2) for *in vivo* measurements mydriatics are not necessary for inducing pupil dilation.

In recent years it has been possible to produce low-coherence interferometers based on optical

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fibers, optical fiber couplers, and superluminescent diode light sources. Single-mode optical fibers and fiber optical couplers are useful for the construction of modular OLCR systems since the fiber optics simplify transverse mode matching. These compact, stable, and low-cost devices have evolved into devices which are useful for noninvasive evaluation of living biological tissues. This paper discusses how this technology transfer occurred. Indeed, the number of biomedical applications based on OLCR may surpass the number of applications in the telecommunications industry which generated the technology.

The concept of coherence is fundamental to understanding the phenomenon of interference. Although the phenomenon of interference was observed by Newton, its mathematical description and physical optical interpretation is of more recent origin. When the source of light is coherent and has a long coherence length, then interference can be observed for a wide range of relative path lengths of the reference and measurement arms of a Michelson interferometer.

When an interferometer is used for optical ranging it is necessary to measure the absolute positions of structures in the specimen. In this case, the light source should have a short coherence length; e.g., the source should be a low-coherence source. A light source of high coherence would consist of a single optical frequency. A low-coherence light source can be considered to be composed of a superposition of different wavelengths rather than a single wavelength. The coherence length is a measure of the light beam's temporal coherence and is inversely proportional to its frequency content or bandwidth.

What is the coherence time and coherence length of a light wave? Partial coherence of light is due to random fluctuations of light emission. These random fluctuations are normally characterized by a time scale that is much shorter than the time constant of the light detector; therefore, these fluctuations are averaged out in the detection process. In order to measure the degree of temporal coherence it is necessary to measure the autocorrelation function, which is also called the temporal coherence function. The normalized temporal coherence function is a function of the path difference or time delay between the two arms of the interferometer. The coherence time is defined as the time delay which reduces the autocorrelation function, or the normalized temporal correlation function, to one-half or to a value of $1/e$. The definition of the coherence length (L_c) is the propagation distance of the wave packet during the coherence time τ_c with a group velocity v_g.

The reference and the measurement arm of a Michelson interferometer act as delay lines which allow light waves that were generated at different times to interfere. The coherence measured by the Michelson interferometer is called temporal coherence because the two interfering light waves differ in their time origin. Temporal coherence is related to the properties of the wave along the direction of propagation. Another way to understand the concept of coherence time is to define it as the temporal interval over which it is possible to reasonably predict the phase of the light wave at a given point in space.

For a Michelson interferometer with a light source of bandwidth $\Delta\nu$, the time delay, $\Delta\tau_c$, for which interferometric fringes can be observed is limited. This can be expressed as

$$\Delta\tau_c\Delta\nu \leqslant 1. \tag{1}$$

The time delay, $\Delta\tau_c$, is the coherence time of the light:

$$\Delta\tau_c \approx 1/\Delta\nu. \tag{2}$$

A coherence length, or more precisely, the longitudinal coherence length of the light, L_c, can also be defined as

$$L_c = c\Delta\tau_c \approx c/\Delta\nu. \tag{3}$$

The longitudinal coherence length is thus related to the spectral distribution of the light source. Now that we have a general understanding of the meaning of coherence we can proceed to understand why low-coherence light sources are used for optical ranging and OLCR and OCT.

If, instead of the typical laser, e.g., a HeNe laser which is a high coherent light source, a light source of low coherence is used, then a new set of interferometric instruments adapted for biomedical imaging are feasible. Interferometric techniques based on reflectometry with low coherence light sources, such as superluminescent diodes (SLD) and light emitting diodes (LED), permit measurements of optical distance and imaging on living biological specimens including imaging of the human body *in vivo*. An interferometer based on a partial coherence light source has high precision, high longitudinal resolution, and is not very sensitive to longitudinal specimen motion during the measurement. The latter feature is of considerable importance for measurements on the human eye *in vivo*.[2]

With a low-coherence light source as part of an interferometer the detector only responds to interferometric intensity fluctuations when the sample and the reference reflection have traveled through approximately the same lengths of optical group delay. A low-coherence light source has a broad bandwidth; it emits light over a wide range of wavelengths. The measurement principle is as follows: the reference arm delay is scanned at a known speed and the output from the detector is measured; this permits the amplitudes and the longitudinal positions of the reflections from the sample to be measured with high precision. There is no interference effect if the paths lengths of the reference and measurement arms of the interferom-

eter differ by more than the coherence length. In summary, since the interference effect is only observed when the measurement path length and the reference path length are matched to within the coherence length of the light, low-coherence interferometry yields a technique for precisely measuring both the magnitude and the delay of the reflected optical signals.

What determines the spatial resolution of OLCR? Resolution in the axial direction is determined by the coherence length of the light source, which is inversely proportional to the source bandwidth. The resolution also is a function of the dispersion of the object; since dispersion elongates the coherence length. The resolution in the transverse direction is determined by the focusing properties of the optical beam.

1.2 PREVIOUS REVIEWS AND INTRODUCTORY MATERIAL

In 1996 Fercher published a comprehensive review paper which explained the fundamentals of optical coherence tomography (OCT), illustrated the various interferometric modalities, and presented applications in different fields.[3] The paper contains drawings of the optical components in the various types of interferometers which clearly illustrate the design principles. Therefore, these drawings are not reproduced in the current paper. Fercher also discussed recent and possible future developments of OCT. Two other review and didactic articles on low-coherence interferometry and tomography were also published by the Fercher group.[4,5] A recent book on optical coherence tomography of ocular diseases describes the technology and physical principles of OCT instrumentation.[6] This review of the physical principles of OCT may appeal to readers without rigorous training in physical optics. A recent review of novel coherence-domain imaging techniques which covers noninvasive imaging of subsurface structures and blood flows in living biological tissues is recommended.[7] Readers can obtain excellent overviews of current research on low-coherence imaging and diagnostics in *Trends in Optics and Photonics* which is published every two years by the Optical Society of America (OSA).

Theoretical modeling of the interactions of light and heterogeneous tissue provides useful guidelines and insights to experiments. A comprehensive model of OCT is described that includes the interference effects that produce speckle in images of dense heterogeneous tissue.[8] This model is based on the extended Huygens–Fresnel formulation of beam propagation in a turbulent atmosphere, adapted to the analysis of OCT. Pan et al. presented a theoretical model of OCT that related the modulation amplitude to path-length-resolved reflectance.[9] They conclude from the analysis of their model that OCT measures the local relative variations of path-length-resolved reflectance. They conclude that highly scattering tissue should be treated as an optically heterogeneous composition of microstructural segments.

Tuchin has written an excellent tutorial paper on various coherent optical techniques for the analysis of tissue structure and dynamics.[10] These methods include speckle correlation, speckle interferometric, and polarimetric methods and instruments.

Finally, there are several recommended sources which cover interferometry and physical optics.[11–15] Several books of reprinted milestone papers and patents can serve as useful background material for a broader understanding of the relation of optical low-coherence reflectometry to tissue optics, confocal microscopy, and optical tomography.[16–19]

1.3 SCOPE OF THE REVIEW PAPER

This paper reviews the early development of optical low coherence reflectometry (OLCR) and shows some of the paths that led from its early development to the field of biomedical optics. Recent examples of innovative applications of this technology in the fields of ophthalmology, dermatology, and developmental biology are described. These examples, which are grouped in chronological order, were selected in order to augment, but not replicate, the 110 papers cited in the 1996 review by Fechner.[3] Space limitations necessarily restrict the number of citations.

2 DEVELOPMENTS OF OPTICAL LOW-COHERENCE REFLECTOMETRY IN THE TELECOMMUNICATIONS INDUSTRY

Optical low coherence reflectometry (OLCR) is an interferometric technique that allows one to measure the amplitude and relative phase of a reflected or backscattered light wave.[3] It is based on a Michelson type interferometer with a broadband light source. The technique allows one to measure the propagation time with high resolution and to determine the amplitude with high sensitivity. Optical interfaces in complex structures can be precisely located and measured. The technique is based on coherent cross-correlation detection of light reflected from the sample.

In 1976 Barnoski and Jensen developed a novel technique to investigate attenuation characteristics of optical fiber waveguides.[20] This paper is historically significant because it is one of the earliest publications which reported measurements of backscatter in optical fibers using the time-domain method. In the early 1980s the emergence of high brightness semiconductor broadband light sources and single mode optical fibers stimulated the development of these techniques. The important technological development was the development and availability of the superluminescent light emitting diode (SLD) which made low-coherence reflectometry practical

in a fiber-optic system.[21,22] A light source with a large spectral bandwidth, and therefore a short coherence length, could be used to achieve a distance resolution of about 10 μm. Another innovation was the development of fiber optic devices such as couplers which are important in the development of fiber optic based designs. These technological developments are the prerequisites of current devices used for noninvasive optical imaging of living tissues *in vivo*.

The OLCR technique was originally developed by Danielson and Whittenberg, Youngquist et al., Takada et al., among others, for reflection measurements in telecommunications devices with micron resolution.[23–29] These authors described new reflectometer techniques that are based on broadband light sources with short coherence length, and coherent cross-correlation detection methods. These techniques differed from the methods of optical time-domain reflectometry by the use of continuous wave light sources.

Danielson and Whittenberg developed a new type of all-fiber reflectometer with micrometer resolution.[23] Their technique used a scanning Michelson interferometer with a broadband light source and cross-correlation detection. The authors state that the problems which are inherent in conventional time-domain reflectometry, such as bandwidth requirements of the detection electronics, and limited dynamic range, motivated them to different methods of high resolution reflectometers. They used a LED which was operated continuously with a FWHGM spectral width of about 130 nm centered at 1300 nm. An InGaAs detector was operated in the photovoltaic mode.

Youngquist et al., developed optical coherence-domain reflectometery as a new optical evaluation technique to determine the positions and magnitudes of reflection sites with miniature optical assemblies.[25] Their instrument consisted of a Michelson interferometer with a short-coherence-length laser diode operating at 830 nm. The heterodyne frequency of the interferometer signal is generated by the reference mirror which oscillates as it is driven by a piezoelectric transducer. This optical coherence-domain reflectometer has a resolution of 10 μm with an optical dynamic range of more than 100 dB.

Kubota et al. developed an interferometer which measured displacements with 0.02 μm accuracy and distance with 100 μm accuracy.[26] Their system is based on a laser diode light source and a $\lambda/8$ waveplate. The authors suggested the use of polarization-maintaining fiber that would permit remote measurements of relative displacement and absolute distance.

It was the rapid pace of developments in the telecommunications industry which advanced low-coherence reflectometry techniques. The dynamic range of the early reflectometers was about 50 dB and was later improved to 161 dB as reviewed in *Optical Time-Domain Reflectometry*.[28] In 1987 researchers in the telecommunications industry used a superluminescent diode as a low coherent light source in a fiber optic interferometer together with a Michelson interferometer which implemented the dual-beam principle. Two wave packets from the Fabry–Perot interferometer are directed towards the object. This new device was designed to locate faults in optical waveguides and optical fibers.[23,24] In that same year a new, low-coherence domain interferometer was described that is based on a short-coherence length diode laser.[25] The optical device to be evaluated replaces one of the mirrors in the interferometer. The reference mirror is translated to match the optical distance of the point(s) of reflection within the device to be tested. Heterodyne frequency of the interferometer signal is generated by oscillating the reference mirror with a piezoelectric transducer.

The all fiber implementation of OLCR Michelson interferometer has been described in detail by Novàk et al.[30] It was constructed with single-mode polarization-maintaining fibers. Similar implementations of this all fiber OLCR were subsequently constructed and used by the groups of Salathé in Switzerland and Fujimoto in the United States.

A continuing series of advances in OLCR emerged from the telecommunications industry in the next decade.[29–38] These advances resulted in improved spatial resolution, improved sensitivity, and increased scanning speed of the instruments.[29,30,37]

An interesting development of a new type of optical delay line deserves further exposition. Kwong et al., have developed a simple, high speed, nearly vibration free, mechanically scanned, optical delay line suitable for femtosecond time-resolved signal-averaging measurements.[37] They demonstrate a 2 ps time window autocorrelator with a display updated at 400 Hz. The delay line used a dithering planar mirror as a time-varying linear phase ramp in the spectral plane of a modified grating-lens femtosecond pulse shaper. The time delay is linearly proportional to the angular deviation of the mirror. The device is based on a well-known property of Fourier transforms. The use of a linear phase ramp in the frequency domain results in a delayed or advanced, but otherwise undistorted pulse in the time domain. The rapid-scanning phase ramp is achieved by simply tilting a planar mirror that is placed at the lens focal plane of a grating and a lens pulse shaper. The tilted mirror provides a continuous phase ramp and is scanned by dithering the mirror through a small angle. This last example was further developed as a rapid scanning optical delay line for biomedical applications of OLCR and OCT.[7,39]

3 EARLY DEVELOPMENT OF BIOLOGICAL APPLICATIONS BASED ON OPTICAL COHERENCE DOMAIN REFLECTOMETRY

The early developments of OLCR and OCT are adequately covered in a review by Fercher and are not repeated in detail in this review.[3] This review stresses the major themes.

Graduate theses can provide detailed insight into theoretical and experimental developments in an emerging field of technology. Four examples were selected: Hee,[40] Huang,[41] Clivaz,[42] Drexler,[43] and Hee.[44] They are noteworthy in the field of OLCR, and provide insight into the previous sources of technological development and how they influenced the course of research. In addition to these theses there are a number of published reviews, proceeding papers and books which provide introductory materials on the early biological development of interferometry applied to diagnostic biomedical applications.[3–7]

Fujimoto et al. in 1986 used optical time of flight measurements for optical ranging.[45,46] The time delay of femtosecond pulses of light reflected from intraocular structures was determined by correlating the reflected pulses of light with pulses from an optical delay line used as a reference. The correlation was done with second harmonic generation from a mode-locked laser. Pulse durations of 30 fs resulted in distance resolution of about 10 μm.

Another early development from the Fujimoto group was the development of polarization sensitive low-coherence reflectometry for birefringence characterization and ranging.[47] In this paper the authors demonstrate how the birefringence of tissues can be measured by controlling the polarization state of the incident light and determining the polarization state of the reflected light.[47] The device is insensitive to the rotation of the sample in the plane perpendicular to ranging. The instrument can distinguish local variations in birefringence as small as 0.05° with a distance resolution of 10.8 μm and a dynamic range of 90 dB. The nerve fiber layer is the main source of retinal birefringence; therefore, the authors proposed that polarization sensitive low coherence reflectometry could result in enhanced imaging of the nerve fiber layer in the retina.

An early biological application (ophthalmology) of low coherence reflectometry was made by Fercher and Roth in 1986.[48] Their dual beam version of partial coherence interferometry is completely insensitive to longitudinal eye motion. The great advantage of this technique is that it can be used to measure both short and long intraocular distances with high precision. In addition, this technique can obtain topographic and tomographic images of the human retina.

The larger number of publications from the Fercher group are cited in Refs. 3–5. Low-coherence Fabry–Perot interferometers were used to measure axial eye lengths and corneal thickness.[49–52] They used a Fabry–Perot interferometer with a dual-beam technique and a multimode laser diode.[4] In 1991, Hitzenberger described a dual-beam Michelson interferometer that used a Doppler heterodyne technique.[50] This instrument used a multimode laser diode at 780 nm and a coherence length of 100 μm. He later used a superluminescent diode at 830 μm with a coherence length of 20 μm as a light source. Fercher also developed real time retinal imaging. Other types of low coherence Fabry–Perot interferometers were developed by the Fercher group in order to perform noninvasive metrology on the human eye *in vivo*.[51,52] The Fabry–Perot interferometer used the reflection from the air–cornea interface to serve as a reference for the weaker reflections from the retina or the cornea–aqueous interface.

An early application of optical coherence domain reflectometry occurred in 1990 when Clivaz et al. used a LED emitting at 1300 nm and a polarization preserving single mode fiber coupler in a reflectometer to study biological tissue.[53] These studies demonstrated the usefulness of the novel method in this domain. To improve the spatial resolution they replaced the LED source by the fluorescence light emitted by a titanium–sapphire crystal pumped by an argon-ion laser.[53] Clivaz et al. in 1992 performed measurements of attenuation coefficients of optical signals in turbid tissues.[54,55] OLCR was applied to investigate diffusive biological tissue with a single-mode fiber.[56] The authors studied samples of fresh arteries. This noninvasive method allows one to determine optical parameters, such as the index of refraction, the transmission properties, and the tissue thickness.

Schmitt et al. demonstrated that the OLCR can have a high potential for applications in medical diagnostics based on the correlation of optical properties with the functional state of the tissue, e.g., in dermatology.[57]

4 SELECTED RECENT TECHNICAL ADVANCES AND APPLICATIONS IN THE BIOMEDICAL OPTICAL FIELD

4.1 OPHTHALMOLOGY

Applications of OLCR and OCT to the eye have resulted in the development of instrumentation and many clinical studies. Two proceedings papers were selected because of their outstanding pedagogical value.[4,5] Fercher et al. have written a short tutorial on various types of interferometric techniques that can be used in ophthalmology. These techniques include: fringe interferometry for surface topography; interferometric length measurement; and synthetic aperture imaging. The text is illustrated with a copious collection of diagrams which illustrate the concepts and compliment the equations and written description of the various instrumental techniques. Clinical applications of par-

tial coherence interferometry include measurements of corneal thickness, anterior chamber depth, axial eye length, and retinal thickness. In a second tutorial paper Fercher et al. demonstrate how partial coherence tomography or optical coherence tomography synthesizes tomographic images.[5] The information content of partial coherence tomography is described in terms of the distribution of the refractive index. The authors show that only abrupt changes in the scattering potential can be observed by backscattering tomography.

The thickness of the human fundus *in vivo* has been measured by partial coherence tomography. Drexler et al. used the dual-beam low-coherence technique to measure the thickness of the retinal nerve fiber layer.[58] A superluminescent diode was the light source (emission centered at 832 nm, with a coherence length of 26 μm). The standard deviation of repeated measurements at the same position on the retina was 5 μm.

High speed scanning is important for biological and medical diagnostics to reduce image-blurring motion artifacts. For example, optical low-coherence reflectometry with a fast optical delay line, based on a rotating cube, has been developed which provides thickness measurements with a measurement repetition rate of 384 Hz and a longitudinal scanning speed of 21 m/s in air over a range of 3 mm.[59]

A compact clinical pachymeter for the measurement of corneal thickness has been developed based on a fiber Michelson interferometer with a rotating glass cube to generate rapid depth scans. This rapid scanning OLCR device has been incorporated into a small clinical instrument that is attached to a Haag-Streit BQ 900 slit lamp for the rapid and precise measurement of the thickness of the human cornea *in vivo*.[60] The light source is a pigtailed superluminescent diode with an emission centered at 850 nm. The optical principle of the rotating delay line is described in Ref. 59. The detection unit consists of a silicon photodiode, a preamplifier, a filter, a rectifier, and a low-pass filter with a bandpass of 60 kHz. The submicron precision and intraindividual reproducibility of measurements of human corneal thickness with the compact clinical pachymeter have been reported.[61]

An interesting application of partial coherence tomography is the study of anterior chamber depth, the thickness of the crystalline lens, and their changes during the process of accommodation.[62] These studies are important to investigate the mechanism of accommodation in the human eye.

A scanning version of the dual-beam version of partial coherence interferometry was used to measure central and peripheral corneal thickness, the anterior chamber depth, and the lens thickness of the human eye.[63] The authors report a mean geometric precision (standard deviation) of the measurements of central corneal thickness as 0.29 μm. Drexler et al. investigated the dispersion effects in ocular media with the technique of multiple wavelength partial coherence interferometry.[64] They measured the group refractive indices and group dispersion in water, in the cornea, the aqueous humor, the lens, and in intraocular lenses. When the object being measured is dispersive the resolution is limited because of a broadening of the detected signals. Drexler et al. demonstrated that partial coherence interferometry is capable of characterizing eye length changes during accommodation in humans.[65]

Podoleanu et al. have developed an OCT instrument that acquired both longitudinal and transversal images of the *in vivo* human eye.[66] Images of up to 3×3 mm are acquired from the retina in less than 1 s. The authors demonstrated that the OCT transversal images acquired for the retina show details that are unobtainable with the scanning laser ophthalmoscope.

Baumgartner et al. describe two technical improvements to the dual beam version of partial coherence interferometry that has been developed for measuring intraocular distance *in vivo*.[67] First, a special diffractive optical element for matching of the wavefronts of the divergent beam reflected at the cornea and the parallel beam reflected at the retina and collimated by the optical system of the eye. This increases the signal-to-noise ratio of *in vivo* measurements by 20–25 dB. Second, the instrument uses a synthesized light source consisting of two spectrally displaced superluminescent diodes with effective bandwidth of 50 nm, and compensates for the dispersive effects of the ocular media. These improvements result in an instrument capable of acquiring optical coherence tomograms of the retina of the human eye *in vivo* with an axial resolution of 6–7 μm.

Drexler et al. have used a dual beam version of OCT in a clinical setting to obtain cross-sectional of images retinal layers.[68] Images were acquired from normal subjects and subjects with various pathologic cases, including glaucoma, diabetic retinopathy, and different types of age-related macular degeneration. Hitzenberger et al. have improved the resolution of partial coherence interferometry for the imaging of the human eye *in vivo*.[69] They achieved a resolution of 5 μm in the retina. This represents an improvement by a factor of 2–3 as compared to currently used instruments. They achieved this improvement with the use of a dispersion compensating element and a broadband superluminescent diode.

4.2 EMBRYOLOGY AND DEVELOPMENTAL BIOLOGY

Some of the most exciting applications of optical low-coherence tomography have occurred in the basic sciences of embryology and developmental biology. Boppart et al. reported advances in imaging developing neural morphology using optical

coherence tomography.[70] They used a Cr^{4+}:forsterite mode-locked laser as a broad bandwidth light source to image individual cells in a developing specimen of *Xenopus laevis*. The authors propose the use of optical coherence tomography as an alternative to repeated histological preparations over time for the study of neural development. Both sagittal and cross-sectional neural morphology in developing Xenopus embryos were imaged with high resolution and identified.

In another study Boppart et al. have used optical coherence tomography to investigate developing embryonic morphology in *Rana pipiens, Xenopus laevis*, and *Brachydanio rerio*.[71] The authors demonstrate a good correspondence between optical coherence tomographic images and those obtained by histology of sections stained with hematoxylin and eosin (H&E).

The rapid acquisition of *in vivo* biological images by use of optical coherence tomography was demonstrated by Tearney et al.[72] High speed image acquisition is critical for the imaging of living subjects; it permits imaging that is devoid of motion artifacts. The authors achieved image acquisition rates of four images/s. A piezoelectric fiber stretcher was employed to vary the reference arm delay. Faraday rotators were used to compensate for the birefringence that was induced during stretching of the optical fiber. The high speed instrument used a Kerr-lens mode-locked chromium-doped forsterite laser as the low-coherence light source. This light source provided the 2 mW power incident on the specimen to maintain a signal-to-noise ratio of 110 dB during the high speed data acquisition. In order to demonstrate the capability of the system the authors imaged the beating heart of *Xenopus laevis* (African frog).

Boppart et al. demonstrated that optical coherence tomography is suitable for assessing cardiovascular development.[73] They showed that *in vivo* and *in vitro* imaging are performed at similar resolution to that of histopathology without the requirement for animal killing. Studies on the developing *Xenopus laevis* cardiovascular system demonstrated that ventricular size and wall position could be determined at 16 μm resolution. Studies made with optical coherence tomography correlated well with those made with histology.

4.3 OPTICAL BIOPSY IN DERMATOLOGY, ENDOSCOPY, CARDIOLOGY, VASCULAR MORPHOLOGY, GASTROENTEROLOGY, AND DENTISTRY

An interesting application of optical coherence microscopy involving subsurface imaging of living skin was reported.[74] Pan and Farkas[75] demonstrated the potential of OCT for noninvasive imaging of living skin simultaneously at two wavelengths in the near infrared range (839 and 1285 nm). Their instrument is composed of two monomode fiber-optic based Michelson interferometers in parallel.

Häusler and Lindner developed a method called optical coherence profilometry (OCP) which is used to measure the skin surface topology.[76] For clinical applications a fiber optic implementation is used. Image acquisition time is 4 s with a spatial resolution of less than 2 μm. Spectral radar is another technique based on OCT in which the scattering amplitude along one vertical axis from the surface of the skin into the bulk can be measured with one exposure.

A rapid scanning implementation of OCT that is used with a catheter endoscope has been developed by Tearney et al.[77,78] The scanning single-mode fiber optic catheter endoscope has a traverse scanning design. The distal portion of the catheter endoscope uses a gradient-index (GRIN) lens and a microprism to emit and collect a single spatial-mode optical beam. The microprism is mounted at the distal surface of the GRIN lens. It directs the beam perpendicular to the axis of the catheter. The beam is scanned in a circumferential pattern and can image transverse cross sections from the structure into which it is inserted. A unique feature of the catheter endoscope is the 1.1 mm diameter. The authors have demonstrated the device by imaging of an *in vitro* human venous morphology.[79] In another study the same authors demonstrated *in vivo* endoscopic optical biopsy with optical coherence tomography.[80] They used the endoscopic device to obtain cross-sectional images of the rabbit gastrointestinal and respiratory tracts at 10 μm resolution. The instrument consists of a light source with appropriate power and wavelength characteristics, a high speed optical delay line which is based on femtosecond pulse shaping, and a second generation optical coherence tomography catheter endoscope that is 1 mm in diameter. The light source was a short-pulse, Kerr-lens mode-locked Cr^{4+}:forsterite laser. The output power was 30 mW with a Gaussian full width at half maximum (FWHM) spectral bandwidth of 75 nm centered at 1280 nm.

Two papers demonstrate applications of optical coherence tomography to vascular pathology. Brezinski et al. present an *in vitro* study that demonstrates the feasibility of using optical coherence tomography for intracoronary imaging of plaque morphology at a micron scale.[79] The wavelength of the source was 1300 nm with a bandwidth of 50 nm. The resulting axial resolution was 20 μm. The dynamic range was 109 dB, with a power of 160 μm at the sample. The image acquisition time was 40 s. In another *in vitro* study of aorta and relevant nonvascular tissue was imaged to a depth of 1.5 mm with optical coherence tomography.[80] The light source was a 1300 nm wavelength, superluminescent diode with a 50 nm bandwidth. The resulting

axial resolution was 20 μm. The authors showed that optical coherence tomography can achieve high contrast and differentiate between tissue with high lipid content and tissue with high water content. The instrument achieved high contrast images of aortic atherosclerotic plaques from *in vitro* human abdominal aorta specimens.

Optical coherence tomography is a useful technique for imaging in tissue that is important for microsurgical intervention.[81] The authors imaged 50 sites from post-mortem material from ten patients. With a 1300 nm superluminescent diode light source with a 50 nm bandwidth the depth resolution was 16 μm and the traverse resolution was 30 μm. Human nervous, reproductive, and vascular tissues were imaged. After this procedure the tissue samples were fixed, and stained with hematoxylin/eosin (H/E) and prepared for histological study by light microscopy.

Several research groups have applied the technique of optical coherence tomography to gastrointestinal tissue; these studies suggest the feasibility of diagnostic imaging technology which can be integrated with conventional endoscopy. Tearney et al. have imaged normal and diseased postmortem specimens of the esophagus and the colon.[82] Classical histological techniques were used to confirm tissue identity. The authors achieved 16 μm resolution and were able to differentiate the various tissue layers such as mucosa, submucosa, and muscularis. These studies suggest the feasibility of endoscopic applications of optical coherence tomography for diagnostic imaging.

Izatt et al. have demonstrated the utility of optical coherence tomography and microscopy for the imaging of gastrointestinal tissues.[83] The theoretical and technical bases for both techniques are described. Optical coherence tomography achieves high spatial resolution (less than 30 μm in three dimensions) and high dynamic range (greater than 100 dB). Optical coherence microscopy combines optical coherence tomography with the micron resolution of confocal microscopy. The authors demonstrate images which reveal cellular-level microstructure up to several hundred microns deep in gastrointestinal tissues.

Izatt et al. have expanded the discussion of optical coherence tomography for biodiagnostics.[7] One interesting application is bidirectional color Doppler optical coherence tomography.[84] This technique employs coherent signal-acquisition electronics and joint time-frequency analysis algorithms to achieve flow imaging simultaneous with conventional OCT imaging. This technique achieves quantitative blood flow imaging in scattering tissue with micron-scale spatial resolution. Cross-sectional maps of blood flow velocity with less than 50 μm spatial resolution and less than 0.6 mm/s velocity precision were obtained through the intact skin in living hamster subdermal tissue. Chen et al. combined Doppler velocimetry with optical coherence tomography to measure blood flow velocity at discrete spatial locations in the skin.[85]

Sergeev et al. have used endoscopic applications of OCT for *in vivo* studies of human mucosa in respiratory, gastrointestinal, urinary, and genital tract.[86] They have developed an instrument based on the integration of a sampling arm of an all-optical-fiber interferometer into standard endoscopic devices. The light source is a superluminescent diode with a central wavelength at 830 nm, a bandwidth of 30 nm, and a power of 1.5 mW. The in-depth scanning is achieved with an integrated piezo-optical modulator that introduces the difference of length in the interferometer arms. An image of 200×200 pixels is acquired in 1 s. This rate eliminates motion artifacts. With this OCT endoscope the authors have studied mucous membranes of esophagus, larynx, stomach, urinary bladder, uterine cervix, and tumors.

Rollins et al. describe an OCT instrument which can acquire images at speeds up to video rates.[87] The instrument utilizes a high power broadband light source and real time image acquisition hardware. The high speed scanning delay line in the reference is based on Fourier-transform pulse shaping technology. This paper describes the theory of low coherence interferometry with a dispersive delay line and presents the details of the theory and operation. The design equations of the system are presented. Movies of a beating *Xenopus* embryo heart based on images acquired at 8, 16, and 32 images per second are presented.

Bouma et al. have demonstrated two short-coherence length, rare-earth-doped fiber optical sources for performing optical coherence tomography in human tissue.[88] The first light source is a stretched-pulse, mode-locked Er-doped fiber laser with a center wavelength of 1.55 μm, a bandwidth of 80 nm, and a power of 100 mW. The second light source is a Tm-doped silica fiber fluorescent source centered at 1.81 μm with a bandwidth of 80 nm and a power of 7 mW.

Colston et al. developed a compact dental optical coherence tomography system that incorporates the interferometer sample arm and the transverse scanning optics into a hand unit that can be used intraorally to image human dental tissues.[89] The optical unit contains collection optics based on a 0.64 NA GRIN lens/angle prism combination mounted on the end of the sample arm single mode fiber optic. The average imaging depth of the system varies from 3 mm in hard tissue to 1.5 mm in soft tissue. The authors illustrated the potential of the instrument for diagnosis of periodontal disease, detection of dental caries, and the evaluation of dental restoration.

Feldchtein et al. have demonstrated the utility of OCT as a diagnostic imaging modality in clinical and research dentistry.[90] They have imaged healthy oral mucosa as well and normal and abnormal

tooth structure. Single transverse scans were accomplished in 2–5 s. In some cases, scan times of 25 s were used to improve the contrast of the images.

4.4 DEVELOPMENT OF RAPID-SCANNING OPTICAL DELAY LINE

Many of the clinical diagnostic instruments are based on rapid scanning devices for the optical delays. Recent improvements in scanning speeds are based on new technical developments. A few examples are described in some detail. Tearney et al. describe a rapid-scanning optical delay line based on a grating and a scanning mirror.[39] The grating-based phase control delay line has a scanning rate of 6 m/s and a repetition rate of 2 kHz. The device is well suited for applications such as OCT that require high-speed, repetitive, linear delay line scanning with a high duty cycle. The device is similar to that reported by Kwong et al. in 1993.[37] The unique feature of the Tearney et al. device is that there is independent control of the phase- and group-delay scan rates. In applications such as OLCR and OCT it is necessary to scan a reference beam path in an interferometer. In that case the output of the interferometer is modulated by the phase-delay scan velocity, but the ranging distance is controlled by the group-delay scan velocity.

The Fourier transform of a linear phase ramp in the frequency domain results in a delay in the time domain. Therefore, one can achieve a linear phase ramp by folding the pulse shaping apparatus and placing a tilting mirror at the Fourier plane of the lens as was done by Kwong et al.[37] The grating-based phase control delay line can function at high speed because it allows group delay to be produced by scanning the angle of the beam instead of by mechanical translation.

An alternative type of optical delay line has been constructed which is based on spinning glass cubes. These devices based on spinning glass cubes can have high velocities and repetition rates (>20 m/s at 400 Hz). However, these spinning glass cubes have limited duty cycle and nonuniform delay scan rates and can introduce delay-dependent dispersion.[59]

5 DISCUSSION

Optical low coherence reflectometry (OLCR) is an interferometric method that allows one to measure the amplitude and relative phase of a reflected or backscattered electromagnetic field. It is based on a Michelson type interferometer with a broadband light source. The method allows one to measure the field propagation time with high resolution and to determine the field amplitude with high sensitivity. Optical interfaces can be precisely located and measured. The method is noninvasive. The noninvasive techniques of OLCR and OCT offer a method for optical biopsy. These techniques complement other techniques such as multiphoton excitation microscopy and spectroscopy, and *in vivo* three-dimensional confocal microscopy for optical biopsy of thick, highly scattering tissues such as human skin.[91–93]

The optical path length is the quantity that is actually measured with the OCLR. The desired quantity is the geometrical distance. In highly scattering, dispersive, biological specimens, the phase velocity is frequency dependent. Therefore, in order to obtain geometrical distances from OLCR measurements it is necessary to divide the optical path length by the group velocity index of the medium. This presents a difficulty in multilayered biological structures such as the human cornea and retina, and human skin. Typically, an average group velocity index is assumed. However, it is known, in the human cornea, for example, that the hydration and protein content differs as a function of depth within the cornea. This implies that the group velocity index is not a constant for this highly scattering, biological tissue, but is a function of position within the thickness of the tissue; i.e., each layer of a layered structure such as human cornea and retina and human skin has a unique value of the index. The accuracy of the determination of group velocity index affects the accuracy of the resulting geometrical distances that are measured with OLCR.

Since the values of the group velocity index are not always known for complex, layered biological tissues it is suggested that figures showing the one- and two-dimensional images based on OLCR indicate both the measured optical path length and the derived geometrical distance based on a value(s) of the group velocity index. The value of the group velocity index should always be stated as well as the source of the value.

6 CONCLUSIONS

Optical low-coherence reflectometry and its three-dimensional manifestation as optical coherence tomography is rapidly evolving as an important noninvasive imaging technique. Image acquisition up to video rates and axial and transverse resolution in the micrometer range define the temporal and spatial resolution. Applications in the areas of biodiagnostics and optical biopsy are rapidly evolving. An emerging application is the development of a new generation of endoscopes. Technology that was originally developed in the telecommunications industry has been transferred to noninvasive biomedical diagnostics.

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