MICRON~RESOLUTION BIOMEDICAL IMAGING WITH OPTICAL COHERENCE TOMOGRAPHY

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In recent years, a number of powerful techniques have been developed for noninvasive biomedical imaging, including ultrasound, x-ray computed tomography (CT), magnetic resonance imaging (MRI), and positron emission tomography (PET). Each of these methods has different advantages and limitations for particular clinical applications. Optical imaging of tissue offers the potential of a noninvasive, high resolution diagnostic using non-ionizing radiation, and the possibility of using spectroscopic properties to distinguish tissue type and probe metabolic function. Recent advances in optics and photonics technology, including semiconductor lasers, fiber optics, and high quantum efficiency solid state detectors, suggest the possibility of a clinically viable optical biomedical imaging technology.

Information concerning the internal structure of biological tissue may be obtained from the time of flight of light backscattered from or transmitted through the tissue under study. Time gating of light backscattered from within tissue samples localizes the depth or longitudinal position of internal tissue reflections. Micron resolution optical ranging measurements have been demonstrated in the eye and skin using femtosecond laser pulses and nonlinear cross-correlation time gating techniques.[1] Time gating can also be used to enhance transillumination imaging of turbid tissue by preferentially rejecting multiply-scattered diffuse light, which has a longer travel time through the tissue than the unscattered or forward scattered components. However, in traversing thick amounts of tissue, the image-bearing transmitted component is severely attenuated relative to the diffuse component, so high sensitivity and high contrast detection are critical for system performance. Several coherent[2-4] and incoherent[5-8] detection schemes have been developed for time-gated optical transillumination imaging (see also other special issue articles).

An alternative approach to time gating for optical ranging and scattered light rejection is to use the coherence properties of light. White light interferometry is commonly used for dispersion measurements in gases and optical materials because small changes in optical path length through a sample can be measured with great accuracy. An interferometer constructed using a semiconductor[9] or fluorescent[10] source of low-coherence light can provide distance measurements with micron-scale resolution, equivalent to femtosecond resolution in the time domain. Low coherence interferometry has recently been used to obtain high resolution measurements of fiber optic and integrated optic structures,[11,12] and for optical ranging and optical property measurements in the eye and other tissues.[13-17] Low coherence interferometry is also the basis of time-resolved holography.[3,4]

OPTICAL COHERENCE TOMOGRAPHY

In this article, we describe a new optical biomedical imaging technique for micron-scale resolution imaging of biological microstructure that we have developed. Optical Coherence Tomography (OCT)[16] is an extension of low coherence interferometry. Tomographic imaging is performed by transverse scanning the incident optical ranging beam to generate two- or three-dimensional data sets corresponding to slices or blocks of tissue, which may be plotted as false color or gray scale images. Using compact and inexpensive commercially available optoelectronic components, spatial resolutions of <20 μm are achieved with detection sensitivities of greater than 100 dB. OCT thus achieves high spatial resolution and femtowatt sensitivity using a purely linear optical system, without the need for high peak power lasers and nonlinear optical detection systems.

We have implemented OCT in both retroreflection and transillumination imaging geometries. In retroreflection OCT, cross-sectional images of microstructure in tissues are obtained by measuring the depth of reflections versus transverse position. In this configuration, OCT performs noncontact and noninvasive micron scale imaging, and is especially powerful for imaging tissues with low scattering, such as in ophthalmic diagnosis (Figs. 1 and 2). OCT is also capable of performing optical transillumination imaging in highly scattering tissues to obtain projection images of embedded structures. In this configuration, a showgram similar to an x-ray radiograph is constructed by scanning in the transverse plane. We have performed studies in simple model systems and biological specimens that explore the potential of OCT for retroreflection and transillumination tomography.

INTERFEROMETRIC RANGING AND TOMOGRAPHY

Both transillumination and retroreflection optical coherence tomography rely on high resolution optical ranging using low coherence interferometry.[9-12] In low coherence interferometry, light from a broad bandwidth source is used to perform highly accurate distance measurements. When light

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from a narrow band light source such as a laser is incident on a simple Michelson interferometer (Fig. 3), changes in the relative path lengths of the interferometer arms result in sinusoidal interference fringes at the output. If the incident light is broadband, however, the relative phases of the different frequency components add coherently only when the interferometer arm path lengths are matched to within the source coherence length. Thus, the distance to a discrete reflection in the sample may be accurately measured by noting the position of the reference arm at which interference fringes appear at the output. Alternatively, for a sample with distributed reflectivity such as biological tissue, a map of reflectivity versus distance into the sample is obtained by sweeping the reference arm and synchronously recording the interferometric signal.

Our OCT systems are implemented using compact fiber optic interferometers[15,16] (Fig. 4). In the retroreflection geometry, low coherence 830 nm light from a broad bandwidth superluminescent diode (SLD) is coupled into a single mode fiber optic Michelson interferometer. Light exiting the sample arm fiber is collimated and directed into the specimen being measured. Light backscattered from tissue structures is combined in a fiber optic beamsplitter with light from a scanning reference mirror. Since the superluminescent diode source has a short coherence length on the order of 10 μm, high resolution ranging measurements are possible. The sample arm fiber also serves as a modular probe that can be interfaced to a variety of instruments such as medical diagnostic instruments or microscopes. Tomographic images are constructed by combining longitudinal scans with lateral scanning of the probe beam across the sample under study. The digitized scan sequences characterizing the optical reflectivity or backscattering in the tissue cross-section are mapped using image processing software to gray-scale or false-color images.

The transillumination OCT system[18,19] is qualitatively similar to the retroreflection system except that the interferometer is extended to a Mach-Zehnder geometry, and a higher power source is used. Because high detection sensitivites are required for transillumination imaging through thick turbid media, a Kerr-lens-modelocked femtosecond Ti:Al_2O_3 laser is used as a high power short coherence length light source. In this system, light retroreflected from the reference mirror recombines at a second beamsplitter with light transmitted through the sample. Interference occurs only for the component of the transmitted pulse that is coherent with and temporally overlaps the reference pulse. The modelocked Ti:Al_2O_3 laser was chosen because of its high output power and broad wavelength tunability. Although a short pulse source is used, the temporal resolution of the system depends only on the coherence time of the light source so that a short coherence length continuous-wave light source would function analogously.

OCT System Performance

The optical detection scheme used in OCT is equivalent to optical heterodyne detection and yields exceptional measurement dynamic range. Since heterodyne detection is sensitive to the product of field amplitudes, the weak signal beam emerging from the sample is multiplied by the relatively strong reference light to enhance the detection of very weak optical signals in the presence of thermal and other post-detection noise sources. In the retroreflection geometry, Doppler modulation techniques are used to obtain high speed, high dynamic range measurement of the interferometric signal. The reference arm length is repetitively scanned at a constant velocity to produce a Doppler shift of the reflected signal from the reference mirror, which in turn modulates the interference signal. The modulated signal is then demodulated using bandpass filtering and envelope detection, and digitized and recorded on a computer. Using ~500 μW of power at 830 nm coupled into the fiber interferometer with ~200 μW incident on the specimen, a detection sensitivity of 94 dB is achieved in retroreflection.

The transillumination OCT system is used primarily for

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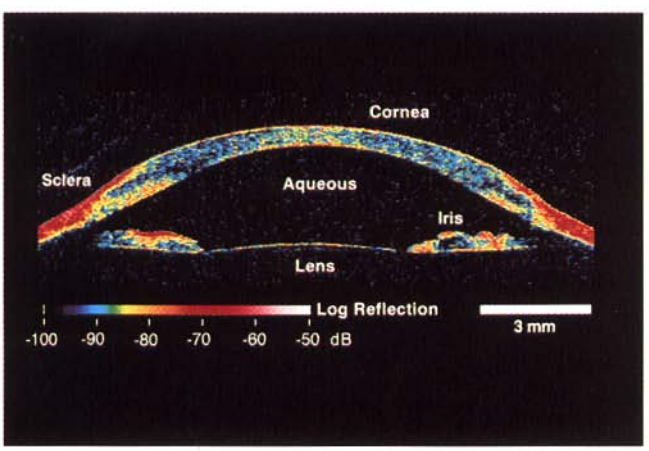

Figure 1. Retro-reflection OCT image of the anterior segment of a human eye obtained *in vitro*, showing large-scale morphology of ocular structures. Clearly identifiable features include the cornea, sclera, iris, anterior angle, and lens anterior capsule. False color scale dynamic range: 50 dB.

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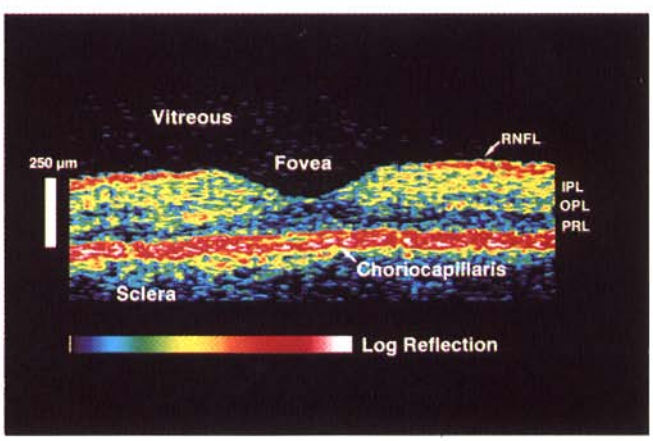

Figure 2. *In vivo* retro-reflection OCT image of the foveal region in the left eye of a human subject. The foveal pit is centrally located; lateral to the fovea, identifiable retinal layers include: RNFL = retinal nerve fiber layer; IPL = inner plexiform layer; OPL = outer plexiform layer; PRL = photoreceptor layer. The vertical scale is expanded by a factor of 2 for clarity. False color scale dynamic range: 35 dB.

en face imaging in the transverse plane, and thus reference arm scanning is impractical. In this case, modulation of the interference signal is accomplished with a piezoelectric fiber stretcher placed in the reference arm. The modelocked laser source provides an average coupled power of ~150 mW of 830 nm light, with ~50 mW incident on the specimen. Using dual balanced detection to cancel excess laser intensity noise, we have achieved quantum shot noise limited detection sensitivity to transmitted signals of up to 130 dB, or 1 part in 10^{13} of the incident optical power.

RETROREFLECTION OCT IN OPHTHALMIC DIAGNOSIS

We have begun preliminary investigations to explore retroreflection OCT in a variety of clinical imaging applications, including skin cancer detection in dermatology, detection and characterization of coronary artery disease in cardiology, and in endoscopic procedures for a new type of "optical biopsy." Several features of retroreflection OCT make it particularly attractive, however, for clinical applications in ophthalmic diagnostics.[20] While retroreflection OCT is an optical analog of ultrasound B-scan, unlike ultrasound it does not require direct contact with the eye or saline immersion of the eye. In addition, the axial ranging resolution (13 μm in our current set-up) is a factor of 5-10 higher than standard ultrasound. The optical sectioning capability and resolution of OCT is similar to that of confocal microscopy, with the additional benefit that the axial resolution is determined by the coherence properties of the source and, thus, does not depend upon the available numerical aperture or the quality of the beam focus. In contrast, for scanning confocal retinal imaging systems, such as scanning laser ophthalmoscopy[21] or scanning laser tomography,[22] the pupil aperture and ocular aberrations limit the axial resolution in the back of the eye to several hundred microns. Finally, the penetration depth of the OCT probe light is essentially unlimited in the transparent medium of the eye. Therefore, deep structures may be visualized without signal or contrast loss due to absorption or multiple scattering of light.

For imaging of the eye and retina for ophthalmic diagnosis, we have coupled the sample arm probe light into a standard slitlamp biomicroscope. Two orthogonal galvonometric scanning mirrors located colinear with the microscope viewing optics scan the probe beam in preset two-dimensional patterns on the retina under computer control. Using our current optomechanics, typical 100 (lateral) × 500 (axial) pixel images require approximately 2.5 seconds for a retinal image. Image acquisition times are currently limited by the linearity and duty cycle of the longitudinal scan system, as well as the available incident power. Acquisition times may also be decreased by reducing axial resolution, signal-to-noise ratio, or the number of lateral pixels sampled.

Figure 1 shows an OCT tomograph of the anterior segment of a human eye obtained *in vitro*. Several structures within the eye are clearly resolved, including the cornea (the clear front part of the eyeball), sclera (the white of the eye), iris (pupil), and lens anterior capsule (the membrane which encloses the lens). Backscattering from within both the nominally transparent cornea and through the full depth of the iris is visible because of the high sensitivity of OCT. Several clinically relevant measurements can be performed using images such as Figure 1. The profile and internal structure (as revealed by backscattering density) of the cornea are important for diagnosing corneal disease. The thickness and curvature of the cornea also provide a highly accurate determination of the corneal refractive power, which is an impor-

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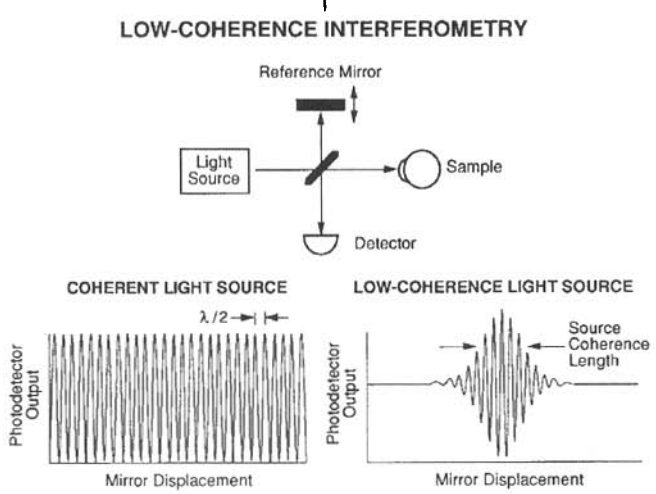

Figure 3. Low coherence interferometry. Interference fringes are observed at the detector output only when the sample and reference arm path lengths are matched to within the coherence length of the light source.

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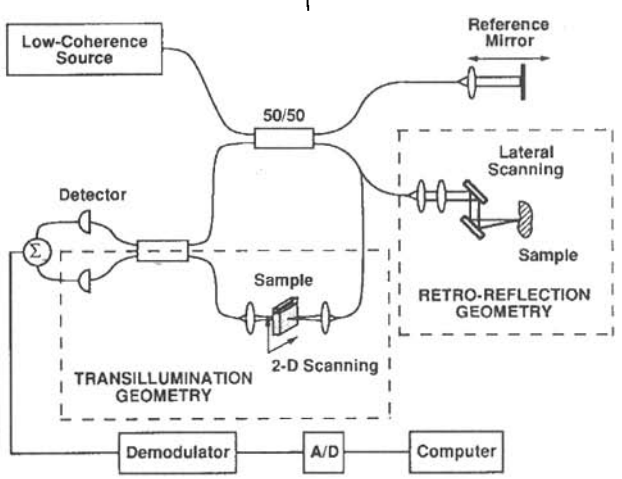

Figure 4. Schematic diagram of the OCT system. In retro-reflection, axial profiles of sample reflectivity versus depth are generated by scanning the reference mirror and synchronously recording the magnitude of the interferometric signal. Tomographic images are constructed from a sequence of multiple laterally displaced axial reflectivity profiles. In transillumination, the reference mirror position selects a "coherence gate" of allowed path length through the sample. 2-D scanning generates shadowgraphs of structures embedded within the turbid media.

tant parameter for contact lens fitting and intraocular lens implant power calculations prior to cataract surgery. Cross-sectional images of the eye may also be useful for identification of masses, tumors, or foreign bodies.

One of the most challenging and successful initial applications of OCT is in cross-sectional imaging of the retina. The retina has important structural features on the micron scale and has very weak backscattering. In addition, since the retina is located at the back of the eye, OCT is one of the only diagnostic techniques capable of performing high resolution cross-sectional imaging in this tissue.

The noninvasive measurement of retinal structure has diagnostic applications for a wide range of retinal diseases. Figure 2 shows one of the first retinal OCT tomographs obtained *in vivo*. This image is a saggittal section taken through the macular region of a human volunteer. The fovea (the region of highest visual acuity) is visible as a characteristic thinning of the retina due to the lateral displacement of the overlying structures in the area of central vision. Lateral to the fovea, layers of alternating high and low scattering reveal the stratified cellular structure of the retina. To compensate for involuntary patient movement during data acquisition, motion artifacts were removed from Figure 2 using cross-correlation image processing techniques. To the best of our knowledge, this and other retinal OCT images are the highest resolution tomographic images of the living retina that have ever been obtained.

Transillumination Imaging in Turbid Media

OCT can be also used to perform transillumination imaging in highly scattering tissues. Recently, there has been interest in transillumination as an alternative to mammography for the early diagnosis of breast cancer. Optical radiation is relatively safe and may be capable of distinguishing malignant tissue based on the differential optical properties of the surrounding neovascularization. Several time- or spatially-resolved optical imaging techniques have been proposed that reduce image degradation in transillumination by discriminating against temporal or directional characteristics of multiply scattered light.[2-8,23,24] OCT performs both temporal and directional gating simultaneously. In OCT, the position of the reference arm sets a "coherence gate" that detects transmitted optical signals at a given time delay, equivalent to performing femtosecond time-resolution gating. The use of single-mode fibers and a confocal sample imaging geometry produces a directional selection of the transmitted optical signal similar to purely spatially resolved imaging techniques such as confocal microscopy, optical heterodyne receivers,[23] and spatial incoherence imaging.[24]

Two-dimensional shadowgrams of objects embedded in scattering media are obtained in transillumination OCT by fixing the reference arm delay to select the earliest arriving transmitted light, and plotting the magnitude of the interference signal while scanning the sample over each spatial resolution element. Figure 5 illustrates a

transillumination OCT image of an Air Force resolution test chart immersed in a suspension of 1 μm diameter latex microspheres 27 scattering mean-free-paths thick. Microspheres were used because their scattering parameters may be accurately computed from Mie theory. The upper-left image in Figure 5 shows a gray scale mapping of the test chart imaged through water alone. The lower right image was obtained through the scattering microspheres with an optimal coherence gate set to select early arriving (unscattered) light. In traversing the microsphere suspension, the unscattered light was attenuated by a factor of −117 dB. The resolution chart is almost perfectly reconstructed with 125 μm spatial resolution, demonstrating that early light retains image information. When the reference delay was increased by only 300 fsec, allowing the detection of some scattered photons, the chart image was significantly degraded but still visible (lower left). When the reference delay was increased to 1.3 psec, the image was overwhelmed by diffuse light and no bars were resolved (upper right). Each 130 × 100 pixel image in Figure 5 required 11 minutes acquisition time.

A surprising result of transillumination OCT illustrated by Figure 5 is that diffuse light was detected with a coherent detection system. This is because the early-arriving diffuse light is the result of primarily small angle scattering events, which cause only minor phase discontinuities. Further experimental results have demonstrated that coherent time-resolved photon migration is qualitatively similar to results of earlier studies using incoherent detection, in that the transmitted light arrives in distinct ballistic and diffuse peaks. Investigations of the attenuation of both components of the transmitted light have been used to established a fundamental quantum noise limit on tissue thickness for ballistic light imaging, based on tissue scattering properties and damage thresholds.[18]

These results suggest that ballistic light imaging with transillumination OCT functions optimally for applications that require very high spatial resolution and relatively shallow tissue penetration (up to a few mm tissue thickness for typical scattering parameters.). Thus, OCT may be useful as an adjunct to conventional light microscopy or as a new tool for histopathology. We have also used transillumination OCT to demonstrate imaging with early arriving, or forward scattered, diffuse light through thick biological media at the expense of reduced spatial resolution.[19] Although OCT is potentially simpler to implement than time-resolved imaging approaches, the use of coherent detection may be a disadvantage because it rejects useful signals contained in other spatial field modes that would be detected using direct detection techniques. The problem of deep tissue imaging is extremely challenging in general and additional investigations need to be perfomed to assess the potential of OCT and other techniques for applications such as in mammography.

OPTICAL RADIATION IS RELATIVELY SAFE AND MAY BE CAPABLE OF DISTINGUISHING MALIGNANT TISSUE BASED ON THE DIFFERENTIAL OPTICAL PROPERTIES OF THE SURROUNDING NEOVASCULARIZATION.

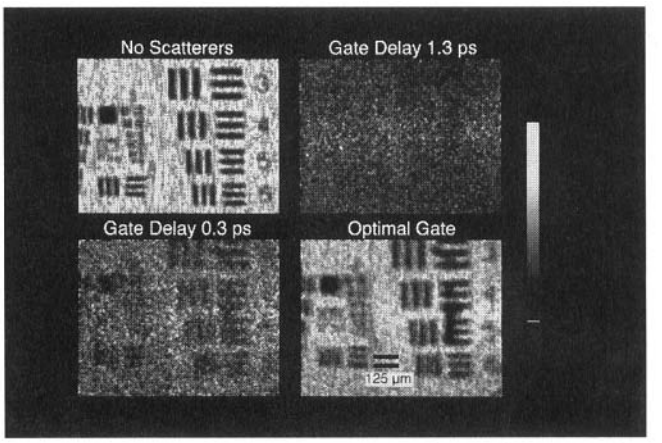

Figure 5. Transillumination OCT images of a resolution test chart embedded in a suspension of 1 μm diameter polymer microspheres, 27 scattering mean-free-paths thick. Images are shown for various coherence-gate delays.

CONCLUSIONS

Biomedical imaging is a fertile area for the application of ultrafast photonics technology. Optical imaging techniques with high spatial resolution such as OCT could have the potential for significant impact in clinical situations where microscopic architectural or anatomical features and noninvasive image acquisition are important to disease diagnosis. In addition, polarization and/or spectral resolution may provide unique sensitivity to tissue properties such as laminar structure, directional orientation, chromophore content and concentration, oxygenation of hemoglobin, and tissue hydration. Fiber optic implementations can extend this capablility through the use of endoscopes and laparoscopes to remote locations within the body.

OCT combines femtowatt detection of backscattered or transmitted light with micron-scale axial and lateral resolution. The optoelectronics may be packaged into a compact, inexpensive diode laser/fiber optic implementation that is readily compatible with existing medical instrumentation. Simple extensions of OCT can be designed to operate at multiple wavelengths for differential wavelength spectroscopy, or to measure tissue birefringence properties. Although the problem of optical imaging through thick tissues remains challenging, we believe that OCT is a promising technique for basic research and clinical applications in a variety of biomedical and medical applications.

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ELECTRONIC HOLOGRAPHY FOR IMAGING THROUGH TISSUE

BY E. LEITH, E. ARONS, H. CHEN, Y. CHEN, D. DILWORTH, J. LOPEZ, M. SHIH, P.-C. SUN, AND G. VOSSLER

Image formation through inhomogeneous media, especially biological tissue, is an important and challenging problem, and is being addressed by many different approaches. Holography is one way that appears quite promising. In fact, there are a variety of holographic methods that may be used individually or in combination.

The earliest application of holography to the problem of imaging through such highly scattering material as biological tissue was the holographic realization of the 1971 Duguay and Mattick principle of the first arriving light.[1]

In this procedure, a narrow pulse of light is passed through a highly scattering medium. The light emerging from the opposite side of the medium (Fig. 1) dribbles out; the light that is scattered least or is scattered mostly in the forward direction emerges first, whereas light that is more severely scattered emerges later. Thus, the emerging light pulse is considerably lengthened, and may easily be hundreds or thousands of times longer than the incident pulse. The light that emerges first, because it has been less severely scattered, is capable of forming the best image. If some small portion of the light has emerged without scatter—light that may be called the specular component, and sometimes the

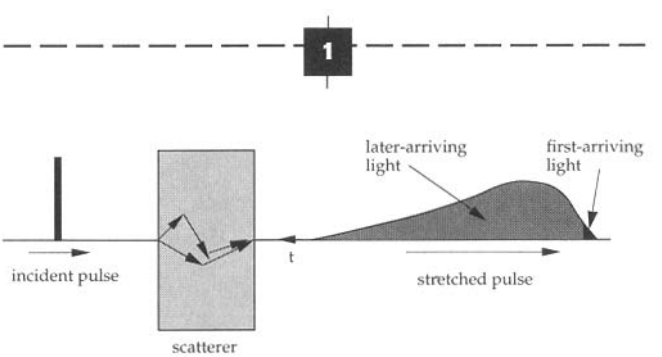

Figure 1. The stretching of a pulse in a scattering medium.