TIME-GATED FOURIER-DOMAIN OPTICAL COHERENCE TOMOGRAPHY

by

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Abstract

Optical coherence tomography (OCT) has been shown to be a versatile three-dimensional imaging tool in diagnostic medicine, combining micrometre-scale resolutions with fast acquisition times. This imaging modality uses the interference between light backscattered from a sample and light that has traversed a known reference path delay to determine the scattering profile over penetration depths of up to several millimetres in tissue.

A novel OCT system is presented that uses nonlinear optics to process the backscattered light in the optical domain prior to standard Fourier-domain OCT acquisition and processing. The nonlinear optical effects experienced between short light pulses are strongly intensity-dependent, occurring only significantly when the pulses are temporally and spatially overlapped. These conditions allow for the creation of a user-controlled time gate that restricts the light backscattered from the sample to a narrow (~ 100 micrometres) depth field of view prior to detection.

When strong and weak scattering interfaces exist across the sample depth range, the signal-to-noise ratio of the weaker scattering sites can be limited by the finite detector dynamic range in Fourier-domain OCT systems. By aligning the time gate temporal delay to the backscatter from the weak interfaces of interest, a user can completely remove the strong backscattered light and enhance imaging contrast. The

nonlinear effect used in the current time-gated OCT design is sum-frequency generation, which provides an additional advantage of imaging at near infrared (1280 nm) wavelengths, used for long penetration depths in tissue, while detection is performed in the visible (504 nm) with silicon-based camera technology. With the reduced depth field of view, the number of sampling points required per depth scan is also proportionately reduced, permitting faster acquisition rates for the time-gated region of interest.

A complete description of the time-gated OCT system design is presented, along with proof-of-concept images demonstrating contrast enhancement and operation in a highly scattering biological medium. Based on its successful initial performance, future development of this system is expected for its eventual use in many OCT imaging applications.

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Chapter 1

Introduction

From its first conceptualization as a three-dimensional biological imaging system in 1991 [1], the development of optical coherence tomography (OCT (Optical Coherence Tomography)) has undergone remarkable progress for a wide range of applications in medicine, biology, data storage and material science [2]. Its real-time operation at high resolution (1 to 15 μm) permits non-invasive imaging at depths of up to several millimetres in tissue.

In contrast with alternative imaging methods, OCT does not provide the highest resolutions, nor the greatest penetration depths available to researchers today. For example, histology, confocal and Coherent Anti-Stokes Raman Spectroscopy (CARS (Coherent Anti-Stokes Raman Spectroscopy)) microscopy are all capable of sub-micrometre transverse resolutions [3, 4, 5], though these modalities can require careful sample preparation, including excision, and are comparatively time-consuming, making them impractical for *in vivo* three-dimensional imaging. On the other hand, ultrasound is capable of clinical imaging at penetration depths of tens

of centimetres. However, its maximum resolutions, in the range of tens of micrometres, can only be achieved by focusing high frequency (~ 100 MHz) pressure waves, which reduce the corresponding penetration depths to millimetres [6, 7]. It is the combination of OCT's capabilities that has provided its growth in niche areas that favour real-time *in vivo* imaging and sub-10 μm resolutions in exchange for limited penetration depths.

Presently, the most mature clinical application of OCT imaging is the diagnosis and monitoring of ocular diseases in the posterior segment of the eye [8]. High OCT resolutions permit quantitative *in vivo* diagnosis and monitoring of age-related degeneration of a patient's retinal thickness, which can be due to retinal disorders such as: macular holes, central serous chorioretinopathy, epiretinal membrane, or macular edema. By imaging the optic nerve head, ophthalmologists are capable of detecting early signs of glaucoma and of monitoring the optic nerve fibre layer in patients suffering from papilledema. This one application of its clinical use draws on the combined high resolution, non-invasive and fast acquisition capabilities that OCT alone can offer. Additional clinical applications include imaging internal organs for tumors and diseases with endoscopic OCT probes, near-surface imaging in dermatology and dentistry and assisting in the guidance of minimally invasive surgical biopsies [9].

All OCT-based systems perform imaging by analyzing the interference between broadband light backscattered from a sample and light reflected in a reference arm with a known path delay. The most common implementation uses a Michelson interferometer, as shown in Fig. 1.1, and determines the backscattered intensity with respect to sample depth at one transverse point on the sample at a time. A three-dimensional image is built up by raster scanning the beam across the sample.

Figure 1.1: The Michelson Interferometer

Over the past sixteen years, a wide variety of OCT systems have been developed to exploit different imaging capabilities, or to enhance contrast for a particular application. For example:

- A Doppler OCT system takes incremental transverse scanning steps and quickly remeasures the depth profile of the sample, converting small scattering site displacements into net flow velocities as small as $10 \mu\text{m}/\text{sec}$. Its applications include endoscopic microvascular flow [10] and blood flow monitoring in the retina [11] and skin [12].
- A polarization-sensitive OCT system separates the polarization states of the interference signal prior to detection to enhance contrast in birefringent samples. The decrease in the degree of birefringence in collagen has been found to be an indicator of thermal damage in tissue caused by burns [13], and thickness measurements of the birefringent retinal nerve fibre layer can be used to detect glaucoma [14].

- A second-harmonic OCT system interferes the second-harmonic generated light that has occurred at nonlinear scattering sites within a sample with frequency-doubled light in the reference arm of the interferometer. As with polarization-sensitive OCT, second-harmonic OCT is used to enhance the contrast of small changes in collagen although in this case, the imaging is sensitive to collagen's nonlinear properties [15].
- An *en face* OCT system raster scans across the sample, acquiring a cross-sectional view of the backscattered intensity at a single depth location. The acquisition process is then repeated at other depths and combined to build a three-dimensional image [16]. Since *en face* systems acquire cross-sectional slices at high speeds, they are most suited to applications that are sensitive to movement, such as ophthalmology [14].

In the present study, a novel OCT system has been designed and developed to enhance imaging contrast by restricting backscattered light from the sample to a narrow time or, equivalently, depth window using nonlinear optics [17]. The region of backscattered light that is rejected from the detector can be easily controlled by the user so that strong sample backreflections can be eliminated from a depth scan, allowing a better view, as quantified by the signal-to-noise ratio, of the weaker scattering sites of interest.

The temporal discrimination, or time gating, provided by nonlinear optics is a well-known phenomenon, finding particular use in current ultrafast pulse characterization methods, such as second-harmonic autocorrelators and frequency-resolved optical gating techniques [18]. In these cases, the nonlinear interaction between the incident electric fields is intensity dependent, occurring only during the temporal and spatial

overlap of short high intensity pulses in the nonlinear medium. Second-harmonic autocorrelators operate by sending an unknown pulse into an interferometer to control the temporal overlap of the recombined pulses. The pulses are sent into a nonlinear crystal and the second-harmonic generated light is detected over a range of relative pulse delays. Due to the intensity dependence of nonlinear optics, an autocorrelation trace of the unknown pulse is produced, from which the pulse duration can be inferred. Frequency-resolved optical gating (FROG) performs the additional step of measuring the spectral content of the nonlinearly generated light at varied relative incident pulse delays. In this case, a separate well-characterized reference pulse can be used to produce a cross-correlation trace, and several FROG (Frequency-Resolved Optical Gating) implementations using nonlinear interactions other than second-harmonic generation have been reported. Iterative post-processing techniques are applied to the detected *time-frequency* trace, resulting in a near-complete phase and amplitude characterization of the unknown pulse [19].

Time gating has also been used for nonlinear time-resolved imaging [20]. In this case, the ballistic light that has transmitted through, or been reflected from, a sample is temporally filtered by its duration of overlap with a separate gating pulse in a nonlinear medium. Time-resolved imaging has been implemented with incoherent time gates formed using second-harmonic generation [21], parametric amplification [22], stimulated Raman scattering [23] and the optical Kerr effect [24]. These techniques have thus far aimed to achieve the shortest time gate possible to maximize the depth resolution and do not perform interferometric-based imaging. A depth resolution of $\sim 10\mu\text{m}$ has been reported using 70 fs pulses in a time gate provided by sum-frequency generation [25].

The novel OCT system presented here, called, “Time-Gated Fourier-Domain Optical Coherence Tomography (TG-FDOCT)”, temporally filters the combined interferometric signal from the Michelson interferometer with incoherent time gating. As opposed to previous time-gated imaging work, the time gate is used here to reduce the depth field of view to approximately $100\text{ }\mu\text{m}$ using pulses with several hundred femtosecond durations. Subsequent standard OCT detection and processing is then performed to achieve $<10\text{ }\mu\text{m}$ depth resolution within the gate window.

Apart from improving contrast of weaker scattering sites, the restriction of the depth field of view provides several further advantages. In this implementation, time gating is achieved using sum-frequency generation, which upconverts the imaging spectrum, centred around 1280 nm , to visible wavelengths, centred around 504 nm , for detection. Since the scattering and absorption coefficients in tissue have been found to decrease with increasing wavelength in the $600\text{--}1300\text{ nm}$ range [26], OCT systems have focused on using longer wavelength sources to increase penetration depths. The upconversion of light to visible wavelengths thus takes advantage of the scattering properties of tissue by imaging in the infrared, while also permitting detection using better developed silicon-based, as opposed to indium gallium arsenide-based, technology.

A major focus of OCT system development has been directed toward maximizing acquisition speeds in order to reduce motion artifacts and blurring that occur in clinical environments. The scanning speed bottle-neck in Fourier-domain OCT systems is the electronic analog to digital conversion process in current line-scan cameras [9, 27]. The number of sampling points, and camera pixels, required for a depth scan at each transverse position scale linearly with the depth field of view over which light is

backscattered from the sample. By restricting the depth field of view, the user has the ability to trade imaging depth for acquisition speed. Applications are envisioned in which standard and time gating OCT scans are performed simultaneously, allowing a user to align the time gate and acquire three-dimensional data at an order of magnitude higher speed for a specific depth region of interest.

A description of the time-gated OCT system design and performance is presented in the following sections. Proof-of-concept images using a microscope slide and mirror, as well as images taken of a highly scattering biological system (onion skin), are presented to illustrate its advantages over current OCT systems and its potential for future development. Currently the high cost of the required ultrafast sources and detector technology are the system's largest inhibitors to its commercialization for clinical use. However, these costs have been decreasing, and applications that already use ultrafast lasers, such as nonlinear imaging and ultrafast machining, could immediately benefit from this work.

Chapter 2

Background and Theory

2.1 OCT System Designs

The earliest OCT systems, commonly referred to as time-domain OCT (TDOCT (Time-Domain Optical Coherence Tomography)), use a reference arm with a path delay that is mechanically stepped across the full sample depth range, as depicted in Fig. 2.1. At each reference arm position, the interferometric intensity is recorded by a photodetector, yielding the scattering depth profile for the reference arm range of motion. The fact that the reference path length must be mechanically swept to obtain an axial scan limits the acquisition speed: using a rapid scanning delay line, a 8 kHz Doppler OCT implementation has been demonstrated [10], and a 3.125 kHz system has been commercialized and adapted for high resolution ophthalmology [28, 29]. The popularity of TDOCT has diminished in recent years due to the development of spectral-domain OCT systems with higher acquisition speeds and sensitivities. However, TDOCT is still useful for ophthalmic applications that generate *en face* cross-sectional scans with combined high axial and transverse resolutions, and in

systems that employ adaptive focusing optics [30].

Figure 2.1: Schematic of a Free-Space Time-Domain OCT (TDOCT) System

More recent Fourier-domain OCT (FDOCT (Fourier-Domain Optical Coherence Tomography)) systems differ from TDOCT in that the reference arm remains fixed, and the spectral interferogram is measured with a spectrometer (Fig. 2.2). An inverse Fourier transform is applied in post-processing to reconstruct the scattering depth profile, achieving the same axial resolution as obtained in TDOCT systems. To prevent the aliasing of scattering interfaces over the processed depth profile, the source spectrum must be measured with a sufficiently narrow spectral resolution, requiring the use of line-scan cameras with typically 512, 1024 or 2048 pixels, depending on the source bandwidth and maximum penetration depth of the sample. The electronic line-scan camera provides a faster scan rate than the mechanical mirror scan rates achieved in TDOCT. In addition, by spreading the imaging spectrum across many pixels, the noise is reduced, permitting a higher sensitivity [31, 32]. Recently, FDOCT systems with axial scan rates of 29.3 kHz [33] and 75 kHz [34] were reported using

a 10 bit 2048 pixel and a 12 bit 512 pixel line-scan camera, respectively. The use of longer OCT source wavelengths to improve tissue penetration depths has negatively affected FDOCT due to the higher read noise of the infrared line-scan detectors currently available.

Figure 2.2: Schematic of a Free-Space Fourier-Domain OCT (FDOCT) System

As opposed to measuring the interferograms with a spectrometer and line-scan camera, researchers have instead developed laser sources that rapidly sweep through their spectral bandwidth with narrow instantaneous line widths. These OCT systems, called swept-source OCT (SSOCT (Swept-Source Optical Coherence Tomography)), use single photodetectors that are temporally synchronized to the spectral content of the interferogram [35]. By using currently available high speed 8 bit digitizer PC cards with two 1 GHz sampling channels [36], the electronic scan speed limitation can be overcome and replaced by the speed at which the source can perform its spectral sweep. As in the case of FDOCT, SSOCT systems use a fixed reference arm and perform an inverse Fourier transform to recover the depth scattering potential.

Currently, the fastest OCT axial scan rates reported have been with SSOCT implementations; a buffered Fourier domain mode locked laser has achieved an axial scan rate of 370 kHz [37]. As opposed to FDOCT, SSOCT systems require dynamic calibration to maintain their time-frequency synchronization. This calibration has been accomplished by monitoring the fringes produced in a separate Mach-Zehnder interferometer at fixed path delay during each spectral sweep / axial scan [38].

2.2 OCT Theory

2.2.1 The Interference Signal

Each of the previously discussed core OCT designs use the same fundamental physics to interferometrically determine the scattering depth distribution of a sample. In the simplest case, consider monochromatic plane-waves sent into the reference and sample arms of a Michelson interferometer. In this case, both the sample and reference arm beams are perfectly reflected using mirrors. The electric fields from each arm, E_{ref} and E_{sam} , are combined at the output of the interferometer, each with respective real amplitudes A_{ref} and A_{sam} and optical path delays z_{ref} and z_{sam} . The optical frequency of the light source is ω and the frequency-dependent propagation constant, $\beta(\omega)$, is the same for each arm. The combined field, using the analytic signal approximation, is

$$E_{ref} + E_{sam} = A_{ref} \exp\{i[\omega t - \beta(\omega)z_{ref}]\} + A_{sam} \exp\{i[\omega t - \beta(\omega)z_{sam}]\}. \quad (2.1)$$

When the interference signal is measured by a single photodetector, the resulting

time-averaged intensity, I is proportional to [39]

$$I \propto \frac{1}{T} \int_t^{t+T} |E_{ref} + E_{sam}|^2 dt = A_{ref}^2 + A_{sam}^2 + 2A_{ref}A_{sam} \cos[\beta(\omega)(z_{ref} - z_{sam})]. \quad (2.2)$$

It is observed that the cosine term contains the interferometric depth information, expressed as the relative optical path difference between the two arms. When extending the above theory to consider the intensity from n backreflections from the sample, equation (2.2) is altered and a fourth, and undesired, term arises due to the interferometric mixing between different sample reflections, given by

$$\begin{aligned} I \propto & A_{ref}^2 + \sum_{k=1}^n A_{sam,k}^2 + 2A_{ref} \sum_{k=1}^n A_{sam,k} \cos[\beta(\omega)(z_{ref} - z_k)] \\ & + 2 \sum_{k=1}^n \sum_{j>k}^n A_{sam,k} A_{sam,j} \cos[\beta(\omega)(z_j - z_k)]. \end{aligned} \quad (2.3)$$

Note that this term, along with the reference and sample DC terms, can mask the true depth structure.

The above theory can be further adapted for the case in which a source with a power spectrum $S(\omega)$ is sent into the interferometer. If the combined sample and reference arm light is measured in a spectrometer with a fixed reference arm position, as in FDOCT systems, the light from all scattering sites across the depth z reaches the detector at the same time, but separated in frequency. The corresponding intensity at a frequency ω is then written in terms of the source frequency and depth dependent field amplitudes as

$$\begin{aligned} I(\omega) \propto & S(\omega) \{ A_{ref}(z_{ref})^2 + \sum_{k=1}^n A_{sam,k}(z_k)^2 \\ & + 2A_{ref}(z_{ref}) \sum_{k=1}^n A_{sam,k}(z_k) \cos[\beta(\omega)(z_{ref} - z_k)] \\ & + 2 \sum_{k=1}^n \sum_{j>k}^n A_{sam,k}(z_k) A_{sam,j}(z_j) \cos[\beta(\omega)(z_j - z_k)] \}. \end{aligned} \quad (2.4)$$

As further developed in [40], the inverse discrete Fourier transform of the collected interferometric spectral intensity, referred hereafter as the interferogram, will yield the axial depth profile. Since the desired cosine interference term in equation (2.4) is multiplied by the source power spectrum, the depth profile will consist of a series of delta function reflections at specific path delays, convolved with the inverse Fourier transform of the spectral envelope of the source. Thus, the point-spread function in an FDOCT system is linked to the inverse Fourier transform of the source spectrum. A higher OCT depth resolution, or narrower point-spread function, is obtained with a greater spectral bandwidth and, unlike other optical imaging modalities, is typically decoupled from its transverse resolution, which is achieved by focusing the beam onto the sample.

2.2.2 Axial and Transverse OCT Resolution

The ideal axial resolution of an OCT system is usually characterized with a Gaussian shaped power spectrum, and is expressed in terms of its centre wavelength λ_0 and full-width half-maximum (FWHM (Full-Width at Half Maximum)) spectral bandwidth $\Delta\lambda$. Using the Gaussian time-bandwidth product of $\frac{2\ln 2}{\pi}$ and assuming a delta function sample reflection, the axial resolution Δz , defined as the Gaussian FWHM of the point-spread function, is given by

$$\Delta z = \frac{2 \ln 2}{\pi} \frac{\lambda_0^2}{\Delta \lambda}. \quad (2.5)$$

The Gaussian power spectrum is typically chosen due to its smooth exponential decay even though, by using a spectrum that more closely resembles a top-hat, it is possible to achieve a higher FWHM axial resolution at the expense of larger side-lobes. However, in virtually all OCT applications, the backscatter intensity varies

by several orders of magnitude along the depth of the sample and the presence of side-lobes near a strong interface can partially or completely mask weaker nearby structure. Researchers have reshaped their source spectrum for OCT imaging, both optically [41, 42] and digitally in post-processing [43, 44], to optimize the point-spread function for a given application.

As opposed to axial resolution, the transverse resolution of an OCT system is determined by how tightly the incident beam can be focused onto the sample. The diffraction-limited spot size, given in terms of the $1/e^2$ beam radius, w_0 , that can be achieved for a Gaussian beam profile depends on the focal length of the lens being used, f , the input beam spot size, d , and the beam wavelength λ . This relationship, developed in [45], is

$$w_0 = \frac{\lambda f}{\pi d}. \quad (2.6)$$

As a beam is more tightly focused, spherical aberration, caused by the shape of the lens, will limit the achievable spot size. Assuming a collimated input beam, the spherical aberration-limited spot size in a plano-convex lens, w_{sph} is found in [46] for a lens numerical aperture NA and focal length f :

$$w_{sph} = 0.067 f (2NA)^3. \quad (2.7)$$

As the transverse resolution is increased, the Rayleigh range, z_R , defined as the distance from the focal point at which the spot size is increased by a factor of $\sqrt{2}$, will be reduced according to

$$z_R = \frac{\pi w_0^2}{\lambda}. \quad (2.8)$$

In typical FDOCT and SSOCCT applications, scanning is performed over a depth field of view of roughly four times the Rayleigh range [47], which is left intentionally

long so that the scattering potential across several millimetres can be measured simultaneously. This also simplifies alignment for many *in vivo* applications, particularly for endoscopy, in which it may be difficult to finely position the focal point at the region of interest. As equation (2.8) shows, a 2 mm depth field of view in a 1.3 μm OCT system results in a diffraction limited spot size of 14.4 μm in air, yielding a 16.9 μm FWHM transverse resolution.

By contrast, confocal microscopy uses high numerical aperture lenses and focuses the backscattered light from the sample through a small pinhole that acts as a spatial filter. In this way, only light with specific spatial wave-vectors (*i.e.*, primarily only single-scattered light returning directly from the sample focal volume) is allowed to reach the detector, while achieving transverse resolutions in the micrometre range. Since traditional confocal microscopy does not use interferometry, the axial resolution is dependent on both the spatial filter aperture and the Rayleigh range, and is greater than the focused spot size transverse resolution. The ideal depth attenuation provided by a confocal circular pinhole aperture is a relatively weak $\left[\frac{\sin(u)}{u}\right]^2$ relationship, with u being directly proportional to the axial distance from the focal point [48].

Researchers have improved the attenuation of signals from outside of the focal volume by performing confocal microscopy with ultrahigh resolution OCT. These hybrid confocal/OCT systems, called optical coherence microscopy (OCM), benefit from both high transverse and axial resolutions, with exponential-decaying point-spread functions and an increased sensitivity in highly scattering samples. Although initial OCM systems employed TDOCT system designs, the difficulty in aligning the reference arm path delay with the confocal spatial filter has led to the more recent development of FDOCT and SSOCT based OCM (Optical Coherence Microscopy)

systems. Transverse and axial resolutions of $0.9 \times 2.2 \mu\text{m}$ have been reported for a 29 kHz FDOCT implementation [49], as well as a $1.6 \times 8 \mu\text{m}$ 42 kHz SSOCT implementation with a $20 \mu\text{m}$ confocal gate width [50].

Nearly all OCT systems designed for turn-key clinical applications use fibre optics whenever possible as a cost-effective way to keep the system aligned and robust to external vibrations. Therefore, the sample arm of the interferometer is usually composed of a length of fibre-optic cable that is free-space coupled to a rapid transverse-scanning galvanometer and focused onto the sample. The returning backscattered light is focused back into the fibre-optic cable that, like a pinhole, acts as a spatial filter. The theoretical confocal gate provided by a single-mode fibre optic cable, developed in [51], is written according to the detected intensity $I(u)$, with respect to optical units u , which are defined by the numerical aperture of the collimating lens, NA , the centre wavelength of the source, λ , and the axial distance from the focal point z . The fibre-optic cable confocal aperture is characterized by its “A-parameter”, found from the pupil radius of the objective, a_0 , the fibre core radius, r_0 , and the fibre-collimating lens distance d . The fibre-optic spatial filtering relationships are given by:

$$\begin{aligned} I(u) &= \left| \frac{A\{1 - \exp[-(A - iu)]\}}{[1 - \exp(-A)](A - iu)} \right|^2, \\ A &= \left(\frac{2\pi a_0 r_0}{\lambda d} \right)^2, \\ u &= \left(\frac{8\pi}{\lambda} \right) z \sin^2 \left(\frac{NA}{2} \right). \end{aligned} \tag{2.9}$$

In the present time-gated FDOCT system, a fibre-optic cable is used as a spatial filter at the output of the Michelson interferometer. Equation (2.9) is used to demonstrate the difference between the predicted attenuation provided by the spatial filter confocal gate and the measured attenuation that was achieved by nonlinear time

gating.

2.2.3 Practical Considerations

When light is sent through a sample medium, a group velocity dispersion imbalance can arise between the arms of the interferometer. As a result, the higher order terms in the Taylor expansion of the propagation constant must be considered, resulting in a chirped interferogram and a spread in the axial point-spread function [39]. Early methods of countering this effect included introducing varying amounts of dispersive material, such as glass, into the reference arm until the depth resolution was maximized [52]. Due to the difficulty in accurately matching the dispersion of a biological sample with artificial materials, methods have been developed to analyze and fit the dispersion to high orders by observing the nonlinear phase in an interferogram of a strong scattering site reflection at a single sample depth. The fringe spacing is fit to the detection wavelength for subsequent dispersion compensation in post-processing [33, 53].

A final consideration is that OCT imaging is performed in highly scattering media, which adds random noise to the interferograms in the form of speckle, and multiple scattering events [54]. As light probes deeper into the sample, the useable signal returned from single backscattering events is attenuated exponentially. Although the scattering properties of biological tissues have not been fully characterized, a study of OCT imaging in freshly excised rat arteries [26] found the attenuation coefficient of the arterial wall to decrease from 14–22 mm^{-1} at 830 nm to 11–20 mm^{-1} at 1300 nm. The lower signal attenuation at higher wavelengths, confirmed in other studies of swine small intestine and of the retina [57, 58], permit greater penetration

depths, which has prompted the development of OCT systems in the 1300 nm and 1550 nm range where fibre-optic telecommunication components may also be used.

A further discussion of scattering theory and methods to reduce speckle are found in [55, 56]. In these works, models to extend the basic FDOCT theory, which assumes delta function sample reflections, are discussed to account for the scattering effects on the beam's spatial and temporal coherence. Monte Carlo models assuming Mie scattering [59], or adapting the extended Huygens-Fresnel theory applied to atmospheric scattering [60], have been proposed but have yet to be thoroughly verified in biological scattering media.

2.3 Fourier-Domain OCT (FDOCT)

The time-gated FDOCT system presented in this thesis requires the use of ultrafast pulses to achieve high instantaneous intensities in a nonlinear crystal. A swept-source, with narrow instantaneous linewidth, and a TDOCT system are both not suitable for its implementation. With a view toward supplementing later system design and performance considerations, some additional FDOCT-specific theory is presented below.

As was explained previously, the scattering potential of the sample depth is obtained at each transverse position in an FDOCT system by performing an inverse Fourier transform of the measured spectrum. As can be seen from the depth dependent FDOCT terms in equation (2.4) on page 12, the frequency of the interferometric fringes detected across the source bandwidth increases as the relative path delay between a reference and a sample arm reflection is extended. According to the sampling theorem of the discrete Fourier transform, at least two samples per fringe period

are required to prevent aliasing. Therefore, with an expected maximum penetration depth Z_{max} in a medium with index n and a source spectrum centred at a wavelength λ , the required wavelength resolution of the spectrometer, $\delta\lambda$, to prevent aliasing is given in [40] as

$$Z_{max} = \frac{1}{4n} \frac{\lambda^2}{\delta\lambda}. \quad (2.10)$$

Note that the maximum depth is inversely proportional to the spectrometer resolution. Thus, when the depth field of view is restricted by time gating, the required spectral resolution and number of detector pixels are reduced for the same source bandwidth. The detected spectral range in the spectrometer must extend beyond the FWHM of a Gaussian source spectrum in order to achieve the depth resolution predicted by equation (2.5) on page 13. Wojtkowski *et. al.* [61] determined the minimum necessary spectrometer spectral range, $\Delta\Lambda$, for imaging with an axial resolution given by a Gaussian FWHM bandwidth, $\Delta\lambda$ to be

$$\Delta\Lambda = \frac{\pi}{2 \ln 2} \Delta\lambda. \quad (2.11)$$

The resulting number of pixels for a given depth range, N , is then found by dividing the spectral range by the spectral resolution required for unaliased imaging:

$$N = \frac{\Delta\Lambda}{\delta\lambda} = \frac{\pi}{2 \ln 2} \frac{\Delta\lambda}{\delta\lambda}. \quad (2.12)$$

The ability to see weak backscattered light in an OCT system is characterized by its sensitivity, which is defined by the maximum amount of attenuation that can be introduced into a sample arm consisting of a single mirror reflection before the signal-to-noise ratio is reduced to 1 [55]. The achievable sensitivity, expressed in decibels, is dependent on the noise sources that are present in the system such as: detector

dark noise, read noise, shot noise and relative intensity noise. Several studies have investigated the theoretical signal-to-noise capabilities of FDOCT systems [31, 32], and sensitivities in excess of 110 dB have been reported [62].

When detecting higher fringe frequencies, which correspond to longer path delays between the reference and sample arm reflections, the pixel size of the line-scan camera becomes significant during post-processing. As described in [31, 63], the measurement of high frequency fringes results in a convolution between the rectangular detector pixel and the interferogram in the frequency domain, causing a reduction in the attainable sensitivity for FDOCT systems. By analyzing the processed peak height of a sample mirror reflection translated over a 0.2 to 1.2 mm depth range, a sensitivity loss of 7.2 dB has been reported [33]. Yun, *et. al.* [63] found that their experimentally measured sensitivity roll-off with depth, $R(z)$, was dependent on the spectral resolution provided by the spectrometer grating, $\delta\lambda$, and the camera pixel wavelength spacing, $\Delta\lambda$. Their sensitivity model for FDOCT systems, with Z_{max} referring to the maximum depth range, is characterized by

$$\begin{aligned} R(z) &= \left[\frac{\sin(\zeta)}{\zeta} \right]^2 \exp \left[-\frac{w^2}{2 \ln 2} \zeta^2 \right], \\ \zeta &= \frac{\pi}{2} \frac{z}{Z_{max}}, \\ w &= \frac{\delta\lambda}{\Delta\lambda}. \end{aligned} \tag{2.13}$$

In highly scattering biological samples, the scattering signal strength returning from different interfaces can vary by several orders of magnitude. Thus, another important OCT specification is the dynamic range of the system, defined as the logarithmic ratio between the strongest and weakest sample intensity signals that can be measured simultaneously. As derived in Wojtkowski *et. al.* [64], the dynamic range for a shot-noise limited FDOCT system is dependent on a line scan camera's

full-well capacity, FWC and number of pixels, N , and is given by

$$DR_{shot}^{FDOCT} = 10 \log \left(\frac{FWC}{8} N \right). \quad (2.14)$$

There exist some disadvantages of FDOCT systems that arise from its previous description and analysis. As shown from the FDOCT interferogram analysis in equation (2.4) on page 12, the scattering potential can be determined by applying an inverse Fourier transform to the detected interferogram. However, the depth profile is based only on the absolute value of the relative path delays. For example, the interferometric fringes will look identical regardless of whether the sample back-reflection occurs with a 1 mm shorter, or a 1 mm longer optical path delay than the reference arm. In order to prevent imaging artifacts caused by this depth ambiguity, early FDOCT systems placed the reference arm zero-point outside the sample so that all back-scattering depth structure is known *a priori* to occur at longer path delays.

A method to resolve the path delay ambiguity has been recently developed, called complex, or full-range FDOCT, which takes additional interferometric data to effectively halve the maximum path delay required in current FDOCT systems. Full-range FDOCT implementations initially relied on recording multiple interferograms for every axial scan, each separated by a relative phase shift introduced either in the reference arm [65, 66], or in fibre-based interferometers [67]. More recently, systems have been developed that apply a phase shift continuously during a transverse scan and distinguish between positive and negative path delays by analyzing the motion of the interferometric fringes with a Hilbert or Fourier transform [68, 69].

Although the full-range FDOCT theory described by Göttinger *et. al.* [66], and implementation described by Leitgeb *et. al.* [65] limits the axial scan speed due to

the use of a piezo-mounted reference mirror, it was adopted in the present proof-of-concept demonstration of the time-gating FDOCT system for its simplicity and effective reduction of noise caused by spectral variations of the optical source. In this case, the depth ambiguity is resolved by acquiring two interferograms with a relative phase shift of approximately 90° between them for each axial scan. The 0° and 90° interferograms are combined as the real and imaginary components of one signal in post-processing. The difference between the inverse Fourier transform of this complex signal and of its conjugate yields the correct positive and negative path delays, and serves to reduce common mode signals such as the autocorrelation and cross-correlation terms present in equation (2.4) on page 12.

An advantage of this algorithm is that it is relatively insensitive to small wavelength-dependent phase errors between the acquired interferograms. If using a mirror to achieve a 90° phase shift at 1280 nm, the far edges of the imaging spectrum, at 1240 nm, will experience a 92.9° shift. As discussed by Leitgeb *et. al.* [65], a polychromatic phase error of this magnitude will only very marginally decrease the signal-to-noise ratio of the processed peak intensity, and can typically be neglected.

Unfortunately, this full-range method suffers from the fact that symmetric depth structure is attenuated. Although Götzinger *et. al.* [66] recognized and developed further image processing techniques to deal with this issue, it was avoided in this implementation by centring the time-gate to one side of the reference path delay. Due to the strong attenuation of backscattered light that is not temporally overlapped with the time-gate, the occurrence of symmetric depth structure is effectively eliminated. For standard FDOCT imaging, the sample was *a priori* positioned to avoid symmetric depth structure as much as possible.

In order to properly process FDOCT interferograms, an accurate pixel to frequency conversion calibration must be performed for a given spectrometer alignment. Typical spectrometer calibration methods involve identifying the camera pixel numbers of well-known spectral lines emitted from a gas source and mapping them to their respective frequencies. An alternative approach was developed here that uses the 0° and 90° interferograms generated from a sample mirror with a known path delay. Considering a single sample reflection in the FDOCT interferogram given by equation (2.4) on page 12, the interferogram intensities, labelled I_0 and I_{90} , at a frequency ω with a ϕ phase shift between them are written as:

$$\begin{aligned} I_0(\omega) &\propto S(\omega) \left\{ A_{ref}^2 + A_{sam}^2 + 2A_{ref}A_{sam} \cos \left[\beta(\omega)(z_{ref} - z_{sam}) - \frac{\phi}{2} \right] \right\}, \\ I_{90}(\omega) &\propto S(\omega) \left\{ A_{ref}^2 + A_{sam}^2 + 2A_{ref}A_{sam} \cos \left[\beta(\omega)(z_{ref} - z_{sam}) + \frac{\phi}{2} \right] \right\}. \end{aligned} \quad (2.15)$$

When the arctangent is taken of $\frac{I_0(\omega)}{I_{90}(\omega)}$, the resulting signal will oscillate in frequency space. These oscillations will cross $\frac{\pi}{4}$ whenever the arctangent's argument is 1. By setting the recorded interferograms equal, all terms except for the cosines cancel, resulting in the crossing point condition

$$\beta(\omega)(z_{ref} - z_{sam}) = n\pi, \quad n = 0, 1, 2, \dots \quad (2.16)$$

Equation (2.16) illustrates that, even when the ϕ phase offset is not 90° , the frequency at the arctangent $\frac{\pi}{4}$ crossing points is related to the known reference and sample arm path delay. By locating the spectrometer camera pixels at which the processed interferograms cross $\frac{\pi}{4}$, the pixels can be mapped to the appropriate fringe frequency spacing for a known calibration path separation, assuming a free-space non-dispersive propagation constant, $\beta = \frac{\omega}{c}$. The mapped pixel to frequency points

are fitted with a second-order polynomial to smooth the calibration data and interpolate across the entire detected bandwidth. Note that when this method is applied using a fixed reference mirror vibration to achieve the 0° and 90° phase shifts, the polychromatic phase error introduced across the spectrum is removed.

2.4 Nonlinear Time Gating

The sum-frequency mixing process used for the time-gated FDOCT system relies on the nonlinear polarization induced by high intensity electric fields in a birefringent crystal. As developed in [70], the inhomogeneous wave equation for the field generated by this induced polarization can be solved easily when a number of simplifying assumptions are made that are listed and explained below:

- The nonlinear effect is weak. This approximation, known as negligible incident beam depletion, means that it is assumed the input fields are not affected by the creation of the sum-frequency electric field and each have the same average power before and after the nonlinear interaction. Since a new electric field is being created during the sum-frequency process, this assumption is not strictly true as it would violate the conservation of energy.
- The input beam pulse durations are longer than several electric field cycles, which allows the Slowly Varying Envelope Approximation. When solving the wave equation, the use of a slowly varying carrier envelope for the electric field permits the removal of higher order derivative terms, which simplifies the analysis.
- The sum of the wave vectors of the incident fields is equal to the sum-frequency

field wave vector in the crystal. This condition, referred to as perfect phase-matching, is strongly dependent on the input beam angles, frequencies and polarizations, as well as the crystal type and cut and tilt angles. When phase-matching is not satisfied, the created electric field is partially or completely cancelled out along the crystal length by the out-of-phase induced polarization. The determination of the proper conditions for phase-matching, as well as the bandwidth over which phase-matching occurs is later simulated, experimentally measured and discussed for the current time gated FDOCT implementation.

- The beams are spatially uniform, there is no diffraction, and the group velocity of the beams is the same for all frequencies. These final assumptions are required to simplify the analysis by neglecting spatial walk-off of the beams within the nonlinear crystal. Some degree of walk-off will be present in practice, which will reduce the length over which the sum-frequency interaction occurs.
- The nonlinear system is non-absorbing so that the induced polarization is instantaneous with the incident electric field.

Under the above assumptions, the solution of the wave-equation yields a generated electric field that is directly proportional to the induced nonlinear polarization, P , which can be represented as a Taylor series of n^{th} order susceptibility terms, $\chi^{(n)}$, about the incident electric field E with an electric permittivity of free space ϵ_0 :

$$P = \epsilon_0[\chi^{(1)}E + \chi^{(2)}E^2 + \chi^{(3)}E^3 + \dots]. \quad (2.17)$$

When the electric field incident on a nonlinear crystal is composed of a sum of two input fields at frequencies ω_1 and ω_2 , the generated output field will include higher order harmonic terms at beating frequencies between the two. By setting the

crystal tilt angle and beam polarization appropriately, the phase-matching condition can be set for a desired nonlinear interaction. To illustrate, consider two incident plane-wave electric fields at frequencies ω_1 and ω_2 , using the same formalism initially used in equation (2.1) on page 11. If phase-matching is arranged for only the sum-frequency ($\omega_1 + \omega_2$) interaction so that all other harmonic terms are suppressed and the susceptibility orders higher than 2 are ignored, the upconverted field E_{SFG} generated in the crystal will be proportional to the square of the incident field:

$$\begin{aligned} E_{incident}(t, z) &= A_1(t, z) \exp\{i[\omega_1 t - \beta(\omega_1)z_1]\} + A_2(t, z) \exp\{i[(\omega_2 t - \beta(\omega_2)z_2)]\}, \\ E_{SFG}(t, z) &\propto A_1(t, z)A_2(t, z) \exp\{i[(\omega_1 + \omega_2)t - \beta(\omega_1)z_1 - \beta(\omega_2)z_2]\}. \end{aligned} \quad (2.18)$$

The above theory can be easily extended to the case in which an incident field at frequency ω_{OCT} is composed of a reference arm and single sample arm reflection from an OCT Michelson interferometer, as considered earlier in equation (2.1) on page 11. When this field is combined with a separate gate field at frequency ω_{gate} for sum-frequency generation in a nonlinear crystal, an output field E_{SFG} at $\omega_{SFG} = \omega_{OCT} + \omega_{gate}$ will be produced. Calculation of the SFG intensity reveals the relative path delay ($z_{ref} - z_{sam}$) phase dependence, permitting subsequent standard FDOCT detection and processing. The SFG electric field and intensity in this case is given by:

$$\begin{aligned} E_{SFG}(t) &\propto A_{gate} \exp\{i[\omega_{gate}t - \beta(\omega_{gate})z_{gate}]\} \\ &\quad \times (A_{ref} \exp\{i[\omega_{OCT}t - \beta(\omega_{OCT})z_{ref}]\} + A_{sam} \exp\{i[\omega_{OCT}t - \beta(\omega_{OCT})z_{sam}]\}), \\ I_{SFG} &\propto \frac{1}{T} \int_t^{t+T} |E_{SFG}(t)|^2 dt \propto (A_{gate}A_{ref})^2 + (A_{gate}A_{sam})^2 \\ &\quad + 2(A_{gate})^2 A_{ref}A_{sam} \cos[\beta(\omega_{OCT})(z_{ref} - z_{sam})]. \end{aligned} \quad (2.19)$$

When adding frequency bandwidth to the OCT source only, and assuming perfect phase-matching across its spectrum, the light upconverted in equation (2.19) is extended across the same frequency bandwidth as the OCT source, permitting the same final FDOCT depth resolution. In the ideal case, the use of a continuous-wave gate results in no change to the interferogram apart from the overall $(\omega_{OCT} + \omega_{gate})$ frequency shift.

When the plane-wave incident gate and imaging fields are replaced by pulses with time-amplitude distributions, their product, which is proportional to the upconverted field, will only be significant when the fields are temporally overlapped within the nonlinear crystal. As such, when considering OCT pulses with short durations relative to the gate pulse, the phase-dependent terms in the upconverted interferogram will be limited to include only the pulses with path delays that fall within the temporal window provided by the gate.

In the frequency domain, the use of pulses in sum-frequency generation results in a convolution between the incident power spectra at each upconverted frequency. To illustrate, consider that sum-frequency generation at $(\omega_{OCT} + \omega_{gate})$ can also occur at $(\omega_{OCT} + \delta\omega) + (\omega_{gate} - \delta\omega)$. Thus, when the light at a given upconverted frequency is detected, the interferometric phase will be composed of a sum of phase terms for each allowable sum-frequency interaction. The convolution between the narrow gate and broad OCT spectra will serve to wash-out the high frequency interferometric fringe information from the detected intensity. This low-pass filtering effect corresponds in the temporal domain to the rejection of backscattered light from outside the time gate.

More detailed mathematical analysis of the sum-frequency interaction between the pulses in the nonlinear crystal, accounting for group velocity mismatch, spatial walk-off and pulse chirp is beyond the scope of the present work. However, a simplified and qualitative understanding of the negative effects of gate pulse chirp on OCT imaging is considered from a frequency domain perspective: When OCT pulses are upconverted at different temporal locations across a linearly chirped gate, their spectra will be upconverted to different centre frequencies. Since interference is only observable at common frequencies across the upconverted bandwidth, the total interferogram bandwidth is reduced and the OCT point spread function is broadened. Based on this basic reasoning, it is desirable to keep the incident gate and imaging pulses as transform-limited as possible.

Chapter 3

System Design

3.1 Overview

The proof-of-concept implementation of time-gated FDOCT, shown in a simplified schematic in Fig. 3.1, uses a modified optical parametric oscillator (OPO (Optical Parametric Oscillator)) source (Coherent Inc. Mira OPO) for imaging with 65–80 nm bandwidth, centred at 1280 nm. The gating pulse is generated with a 76 MHz repetition rate Ti:Sapphire laser (Coherent Inc. Mira 900-F) centred at 830 nm which, when mixed with a 1280 nm imaging signal, upconverts the spectrum to a centre wavelength of 504 nm. The gating pulse is set to convert light from both the reference arm and backscattered light from the sample. The gate width is adjusted by clipping a portion of its 9 nm spectral bandwidth in a 4-f grating pair configuration and the gate delay can be easily set across the sample depth range.

The sum-frequency generated signal is sent free-space to a home-built spectrometer and detected with an area scan 14 bit 35 MHz electron-multiplying CCD (EM-CCD (Electron-Multiplying Charge-Coupled Device)) camera (Andor Technologies

DU-885). To monitor and compare standard infrared FDOCT imaging with time-gated FDOCT, the unconverted infrared signal is picked off after the nonlinear crystal interaction and sent by fiber to a separate home-built spectrometer with a 14 bit InGaAs line scan camera (Goodrich Sensors Unlimited SUI LDV-512LX) (not shown in Fig. 3.1 for simplicity).

Figure 3.1: Schematic Setup of the Time-Gated FDOCT System. NL-XTAL: Non-linear Crystal, EMCCD: Electron-Multiplying CCD, NDF: Neutral Density Filter, HWP: Half-Wave Plate, 4-f GP: 4-f Grating Pair.

The 830 nm Ti:Sapphire laser that provides the time-gating pulse also pumps the OPO source, which produces pulses over a wide cavity-tuned wavelength range in the infrared (1100–1600 nm) that are temporally synchronized to the gating pulses. To achieve a broad imaging bandwidth, the OPO dispersion is compensated by inserting 5.572 mm of SF66 glass into the cavity. The resulting output spectrum is centred about 1280 nm with 60–80 nm bandwidth (~ 8.5 – $12 \mu\text{m}$ axial resolution in air), and

is stable for time scales < 0.4 ms over which axial scans are taken. The shape and bandwidth of the spectrum gradually varies over ~ 1 min time scales and is corrected by applying feedback to the piezo-controlled cavity length. The chirp from optical elements such as the fibre-optic cable is observed in the spectral variation of the sum-frequency generated output while varying the gate delay. A prism pair pulse compressor is used to compensate this dispersion so that the bandwidth (and axial resolution) is maintained across the gate.

Prior to entering the prism pair, the pulses emitted from the OPO are expanded by a factor of ~ 1.375 and collimated using a pair of 8 mm and 11 mm effective focal length aspheric lenses (Thorlabs Inc. C240TM, C220TM). The lenses, not shown in Fig. 3.1 for simplicity, initially focused the beam through a pinhole to ensure the OPO light used for imaging was spatially coherent. It was found that the use of the pinhole did not significantly improve imaging performance and was removed, but the lenses were left in place to provide a fine adjustment of the beam collimation.

Imaging is performed using a Michelson interferometer. The reference arm offset is fixed, as in standard FDOCT, though its mirror is mounted to a piezo transducer. The mirror's small oscillations provide the interferograms with the 0° and 90° phase offsets necessary for full-range imaging. As opposed to using a galvanometer for raster scanning, the sample is mounted to a DC motorized translation stage for proof-of-concept simplicity, and for the ease with which the axis of sample motion can be changed and electronically controlled (axial scanning is performed to obtain the attenuation profile of the time gate, and transverse scanning for creating two-dimensional images).

Time-gating is achieved with a noncollinear type II sum-frequency interaction within a β -barium borate (BBO (Beta-Barium Borate)) nonlinear crystal (length

2 mm). At these mixing wavelengths, phase matching occurs over a bandwidth greater than 150 nm FWHM, verified by tuning the OPO while maintaining the same crystal angle. The conversion efficiency between the infrared beam and the sum-frequency generated output varies from -25 to -34 dB, dependent on the adjustable 84–176 μm -20 dB gate width, provided by the 4-f grating pair.

Axial scanning speed is determined by the rate of the reference mirror’s vibrations, as well as the pixel read off rate of the camera being used. In the first implementation, the EMCCD camera was run in fast-kinetics mode, whereby accumulated charge is quickly shifted to unused pixels in its two-dimensional array prior to read-off. This method permitted axial scan rates that were limited to the 1.3 kHz piezo frequency of the mirror. However, it was discovered that the final electronic charge from incident photons was severely sacrificed in this high-speed operation, permitting an OCT sensitivity of only 69 dB with 18 dB electron-multiplying gain. By running the camera in full-image mode, all of the pixels of the two-dimensional array are read-off twice per axial scan (for both 0° and 90° interferograms), limiting the axial scan rate to 8 Hz. In this case, the mirror is driven by an 8 Hz square-wave and a sensitivity of 88 dB was achieved with 18 dB applied electron-multiplying gain. The speed in this case is below current FDOCT standards due to this particular camera’s inflexibility in the number of pixels that must be read out per frame.

The following sub-sections discuss the design choices and limitations for each of the above components of the time-gated FDOCT system in further detail.

3.2 The OCT Source: A Modified Optical Parametric Oscillator (OPO)

An optical parametric oscillator (OPO) performs parametric frequency downconversion of an incident “pump” beam into two lower frequency products: an “idler” and “signal” beam, with the idler being the lower frequency component of the two. The Mira OPO is designed to be pumped with ultrafast pulses with at least 500 mW average power produced from the Mira Ti:Sapphire laser. Downconversion is achieved with a periodically poled nonlinear crystal that is phase matched across a broad wavelength range ($\sim 1100\text{--}1600$ nm for the signal beam) at room temperature. By constructing a ring cavity to provide temporal overlap between the generated signal pulse and the next incoming pump pulse, it is possible to achieve parametric amplification and a build-up of signal power within the oscillator. An output coupler allows the lower-frequency beam, built-up over many pump pulses, to escape and be subsequently used for other experiments. A simplified schematic of the Mira OPO is shown in Fig. 3.2 to illustrate its operation.

Figure 3.2: Schematic Setup of the Mira OPO for Infrared Pulse Generation. M1, M2: Mirrors 1 and 2.

The Mira OPO typically generates pulses with average power >100 mW in the

infrared region with narrow spectral bandwidths (15–30 nm) when using the supplied 12 mm dispersion block, recommended for >1200 nm operation. Although the nonlinear crystal permits operation across a much wider wavelength range, only narrow bandwidths are emitted at a time due to the dispersion provided by the nonlinear crystal and dispersion block. By tuning the cavity length, pulses with different centre wavelengths are temporally overlapped with the pump pulses and experience intra-cavity gain.

In order to avoid having the time gate drift over the sample region of interest, the pulses used for sum-frequency generation from both the imaging interferometer and time gate must be temporally synchronized. For example, a gate shift of $100\text{ }\mu\text{m}$ corresponds to a timing jitter of roughly 300 fs, and drift would be difficult to avoid if using two separate laser sources. Since the OPO is pumped by the 830 nm pulses eventually used for time gating, this synchronization is assured.

As described by the axial resolution relationship given by equation (2.5) on page 13 a broad imaging source bandwidth is required to achieve high OCT axial resolutions. In regular operation, the narrow bandwidths emitted by the OPO are insufficient. For instance, a 30 nm FWHM Gaussian spectral width centred at 1280 nm will result in only $24\text{ }\mu\text{m}$ axial resolution. However, the OPO spectral output can be extended by compensating its intra-cavity dispersion to achieve temporal overlap over a broad range of wavelengths with the pump pulse.

The group delay dispersion of the OPO was found by first recording the translation stage position of the output coupler (shown in Fig. 3.2) at emitted centre wavelengths from 1100–1400 nm using the 12 mm dispersion block in the cavity. The spectra were

measured by sending a portion of the output signal into a WaveScan laser spectrometer (APE GmbH) that had been supplied with the Mira OPO. In this case, both the 12 mm block of glass and nonlinear crystal contribute to the cavity dispersion. To isolate the dispersion terms, the same cavity position measurement was later performed using the second supplied 5.572 mm dispersion block, normally recommended for 1100–1200 nm operation. The 12 mm OPO cavity position with respect to centre emission wavelength is shown in Fig. 3.3, fitted with a 4th-order polynomial so that a smooth group delay dispersion curve could be derived for the cavity and later used to predict pulse dispersion in the imaging setup.

Figure 3.3: Experimental OPO Operation with 12 mm Dispersion Block Inserted into Cavity

The group delay of half of the cavity, T_g , is found for 12 mm operation by considering the physical length and group index of the nonlinear crystal, L_{XTAL} and $n_{g,XTAL}$, the physical length and group index of the dispersion block, L_{block} and $n_{g,block}$, and of

the remaining air-sections of the OPO cavity, L . The speed of light is denoted by c and the group indices are written as functions of wavelength, λ , resulting in a group delay given by

$$T_g = \frac{L_{XTAL}}{c} n_{g,XTAL}(\lambda) + \frac{L_{block}}{c} n_{g,block}(\lambda) + \frac{L}{c}. \quad (3.1)$$

When multiplying equation (3.1) through by c , the variation of $T_g c$ should match that observed by the cavity length change shown in Fig. 3.3. After inserting the 5.572 mm block in place of the 12 mm block, the new cavity length change $T_{g,5.572}c$ for OPO output wavelengths can be predicted from the previous $T_{g,12}c$ according to

$$T_{g,5.572}c = T_{g,12}c + (L_{block,12} - L_{block,5.572})[1 - n_{g,block}(\lambda)]. \quad (3.2)$$

Since the cavity position and block lengths are readily available, equation (3.2) allows the determination of the type of dispersion glass being used. The group index was calculated using the Sellmeier coefficients for several popular glass types until a match was found for SF66, as shown in Fig. 3.4. The maximum possible error was calculated by estimating the systematic uncertainties of 0.002 mm for the cavity position, 0.005 mm in the block thickness and 0.5 nm in the displayed OPO controller centre emission wavelength.

The group delay dispersion (GDD) is found by taking the derivative of the group delay with respect to the angular frequency. The GDD (Group Delay Dispersion) can be calculated from equation (3.2) in terms of the emission wavelength λ by applying the relationship between angular frequency, ω and wavelength λ ,

$$d\omega = -\frac{2\pi c}{\lambda^2} d\lambda. \quad (3.3)$$

By inserting another $-L_{block}(1 - n_g)$ term into equation (3.2), the OPO GDD can be predicted for the insertion of other types of glass in the cavity. A plot of the

Figure 3.4: Experimental and Predicted OPO Operation with a 5.572 ± 0.005 mm SF66 Dispersion Block Inserted into Cavity

predicted GDD, in units of fs^2 , is shown for a 1100–1400 nm range in Fig. 3.5 using 12 mm and 5.572 mm of SF66 glass, as well as for no block at all, corresponding to the GDD of the nonlinear crystal.

As can be seen in Fig. 3.5, when using the 5.572 mm block above the recommended 1100–1200 nm wavelength range, the GDD is less than 100 fs^2 in the region 1240–1310 nm. This indicates that pulses roughly within this wavelength range can simultaneously achieve temporal overlap with the 830 nm pump pulses from the Ti:Sapphire and experience gain in the OPO. A sample spectrum measured with the APE WaveScan spectrometer when the OPO was aligned to emit a broad spectral

Figure 3.5: OPO Group Delay Dispersion Curves Predicted for Various Operating Conditions

bandwidth is shown in Fig. 3.6. The spectrum is fitted using a second-order super-Gaussian curve, given by

$$y = y_0 + A \exp \left[\frac{-(x - x_c)^4}{2w^4} \right]. \quad (3.4)$$

The broadened spectrum emitted by the OPO is very sensitive, requiring careful initial alignment and also cavity length feedback for further stabilization, which is supplied by a piezo transducer mounted to the end translation stage. The cavity length feedback is provided by the APE OPO controller hardware, which attempts to retune the centre wavelength when it has moved off a user set point. Since the shape of the spectral envelope varies as the cavity length is changed, the piezo feedback is only applied for relatively large centre wavelength shifts (± 2 nm) that occur on minute time-scales to avoid constant readjustments.

During operation, the spectrum can fluctuate on second time-scales from the

Figure 3.6: Sample OPO Broadened Spectrum Obtained by Intra-Cavity Dispersion Matching. Solid Line: Super-Gaussian fit provided by equation (3.4)

super-Gaussian shown in Fig. 3.6 to an asymmetric Gaussian with a narrower FWHM and a slightly higher or lower centre wavelength. In this case, the temporal overlap with the pump pulses in the OPO has slightly changed, restricting the intra-cavity gain. Small-scale spectral variations persist in the proof-of-concept time-gated OCT implementation, resulting in an axial resolution that varies unpredictably from $\sim 8.5\text{--}12\text{ }\mu\text{m}$ in air during imaging. Periods of larger spectral variation appear to be correlated with the times that the air conditioner is activated to stabilize the room temperature in the lab. These temperature variations might slightly change the Ti:Sapphire pulse repetition rate and therefore change the parametric gain-spectral profile inside the OPO cavity.

By processing the 0° and 90° spectral interferograms for full-range imaging, the spectral envelope is ideally removed provided its shape is constant during the interferogram acquisition. Slight power spectrum variations at millisecond time scales will result in DC and low frequency noise differences between the interferograms, which show up around zero-path delay in the processed depth scans. Most OCT systems, including the 0-90 full-range implementation [66], already suffer from increased noise around zero-path delay using standard continuous wave super-luminescent diode OCT sources. The solution commonly taken is to avoid imaging in these areas.

Since the spectral envelope shown in Fig. 3.6 is not purely Gaussian, the axial resolution predicted by equation (2.5) on page 13 will be inaccurate. The point-spread function profile is instead expected to follow the inverse Fourier transform of the second-order super-Gaussian curve given in equation (3.4) on page 38, after frequency rescaling. The predicted OCT logarithmic and linear point-spread functions are calculated and shown in the left and right-hand plots of Fig. 3.7 respectively. Note that the more square-like super-Gaussian curve produces a 20 dB sidelobe in the point-spread function at $\sim 15 \mu\text{m}$ from the main peak, but is capable of a higher FWHM resolution ($8.1 \mu\text{m}$) than that predicted for a regular Gaussian spectral envelope.

Figure 3.7: Predicted OCT Point-Spread Function from Super-Gaussian Fitted Spectrum Emitted by OPO

3.3 Pulse Dispersion Compensation

The pulses emitted from the spectrally broadened OPO will not be transform-limited, and additional chirp will be added from the various lenses, beamsplitter cube and, in particular, the fibre-optic cable at the output of the interferometer, all shown in the system design of Fig. 3.1. Although in typical OCT systems, only the relative GVD between the two arms of the interferometer affects axial resolution, the temporal discrimination provided by time gating could cut bandwidth from the spectrum of a chirped pulse near the gate edge. With a reduced bandwidth in the sum-frequency generated interferogram, the resulting imaging resolution will be degraded.

To compensate for common-path dispersion in the OCT system, a pair of SF11 equilateral glass prisms (Thorlabs Inc. PS853) are inserted into the beam path. A

mirror reflects the beam after the second prism, sending it back through the prism pair at a slight vertical deviation where it is then picked off and sent to the interferometer. By adjusting the path separation between the prisms, negative dispersion can be introduced according to the following equations, adapted for group-delay dispersion (GDD) [72, 73],

$$GDD = -\frac{\lambda^3}{2\pi c^2} \frac{d^2 P}{d\lambda^2}, \quad (3.5)$$

$$\frac{d^2 P}{d\lambda^2} = 4l \left\{ \left[\frac{d^2 n}{d\lambda^2} + \left(2n - \frac{1}{n^3} \right) \left(\frac{dn}{d\lambda} \right)^2 \right] \sin(\beta) - 2 \left(\frac{dn}{d\lambda} \right)^2 \cos(\beta) \right\},$$

where P is the optical path length, found to be dependent on the prism index n , prism apex-to-apex distance l , and angular deviation β . The angular deviation is approximated using an angular dispersion of 67 nm/deg and an offset of 1° at 1200 nm.

The group delay dispersion of the 59±1 cm Corning SMF-28 fibre-optic link, formed by cleaving and splicing together a 2 m patch cable (Thorlabs P3-SMF28-FC-2) is given in units of fs^2 using the dispersion formula specified for 1200–1625 nm operation [74],

$$GDD = -\frac{\lambda^2}{2\pi c} \frac{0.086}{4} \left(\lambda - \frac{\lambda_0^4}{\lambda^3} \right), \quad (3.6)$$

where the zero-dispersion wavelength (1313 nm) is denoted by λ_0 .

The combined group-delay dispersion from the fibre and OPO, along with the final dispersion after compensation using a prism pair apex separation of 55 cm is plotted in Fig. 3.8. Note that the use of the prism pair shifts the GDD zero-crossing point to approximately 1280 nm, corresponding to a local minimum in the group delay spectral profile. The fact that the GDD goes roughly linearly from positive to negative on either side of the zero-point indicates an approximately symmetric nonlinear pulse chirp that could slightly degrade resolution near the gate edges. In order to correct

the remaining nonlinear pulse chirp, a higher order dispersion correction would be required, perhaps through the use of custom-made dielectric chirped mirrors, which was beyond the scope of the present work.

Figure 3.8: Simulated Dispersion Map of OPO and Fibre-Optic Link, with Compensation Provided by a Prism Pair with 55 cm Apex Separation

Final adjustments to the prism apex separation were performed according to experimental measurements of the spectral variation across the gate profile, as discussed further in the Results section. A transmission of 83% was achieved using the prism pair configuration at 1280 nm.

3.4 The Michelson Interferometer

After dispersion compensation, the imaging beam is sent free-space to the Michelson interferometer. The beam was split using a non-polarizing 50:50 BK7 beamsplitter

cube (Thorlabs Inc. BS012), coated for 1100–1600 nm operation. The splitting ratio was found experimentally at 1280 nm to be 59 % transmitted / 41 % reflected, with 40 ± 2 mW being sent to the sample arm. The beam is focused onto the sample using a 15.3 mm effective focal length, 0.16 NA aspheric lens (Thorlabs Inc. C260TM-C), achieving a 12.1 ± 0.2 μm FWHM spotsize. The spotsize was measured by recording the average power as a razor blade is stepped through the beam at its focus. Assuming a Gaussian intensity distribution, the recorded intensity was differentiated and fit in Fig. 3.9, achieving a $1/e^2$ radius of 10.3 ± 0.2 μm .

Figure 3.9: Transverse Beam Intensity Profile, Obtained by Translating a Razor Blade through the Beam Focus and Differentiating the Beam Intensity with respect to Blade Position

The achieved spot size can be compared to the diffraction limit provided by the

aspheric lens according to equation (2.6) on page 14. Using a measured $1/e^2$ radius of 1.86 mm of the collimated input OPO beam, and a specified aspheric focal length of 15.3 mm, the diffraction-limited $1/e^2$ radius was calculated to be $3.4 \mu\text{m}$. The large discrepancy between the theoretical and experimentally achieved spot sizes is primarily attributed to the use of an aspheric lens with a design wavelength of 780 nm. In this case, with the input beam wavelength centred about 1280 nm, the aspheric lens shape will no longer correctly compensate for spherical aberration. For comparison, the spherical aberration limited spot size in a plano convex lens, calculated from equation (2.7) on page 14 using the aspheric lens specifications, is $33.6 \mu\text{m}$. Additional broadening of the achieved spot size could result from transverse coma, caused by a non-normal beam incidence on the lens, or from aberrations in the beam profile caused by the beam expander / collimator provided by the 8 mm and 11 mm aspheric lenses (Thorlabs Inc. C240TM, C220TM) at the output of the OPO.

With a $1/e^2$ spot size radius of $10.3 \pm 0.2 \mu\text{m}$, the Rayleigh range is calculated from the relationship given in equation (2.8) on page 14 to be $260 \pm 5 \mu\text{m}$ in air. According to [47], OCT systems typically scan over a range of four times the Rayleigh range, which is in this case $1.04 \pm 0.02 \text{ mm}$. Given that the infrared spectrometer, used for standard FDOCT, described later in this chapter permits a useful axial path delay range of $\pm 0.79 \text{ mm}$, a depth of focus scanning range of approximately one millimetre is appropriate for viewing multiple interfaces across the sample depth.

The sample is mounted to a translation stage that is controlled with DC servo motorized actuators (Thorlabs Inc. Z625B) using a T-cube USB DC Servo motor controller (Thorlabs Inc. TDC001) interfaced with a computer. When scanning at 8 Hz, the translation stage was set to stop at each measurement position during the

acquisition to avoid fringe washout due to sample motion. In this case, the maximum stage velocity was set to 0.1 mm/sec, with an additional 100 ms delay introduced between the end of a step and the start of an axial scan acquisition.

The beam sent to the reference arm is passed through a variable attenuator wheel to allow the user to optimize the reference arm power for maximum interferometric fringe amplitudes. When imaging a sample with backscatter that is much weaker than the reference arm, the variable attenuator serves primarily to reduce the spectral power until just before the detector saturates. When there exists a sizeable backscattered, or backreflected signal (greater than one quarter of the detector well), the reference arm is attenuated until both arms have roughly equal power spectra, verified by blocking the light in one arm at a time. Both arms are then attenuated equally, achieved by inserting a variable attenuator into the beam path after the interferometer, until the intensity from each arm equals one quarter of the detector well, thus maximizing the fringe amplitudes and dynamic range.

The reference mirror is mounted to a piezo kinematic mount (Thorlabs Inc. KC1-PZ), that introduces a 90° phase shift at 1280 nm for every other acquired interferogram by vibrating at a 160 nm amplitude, equal to $\frac{\lambda}{8}$. The oscillations are controlled using a piezo-electric controller (Thorlabs Inc. MDT693A) that accepts an external waveform input from a 20 MHz function generator (Agilent Technologies Inc. 33220A). To achieve the proper signal amplitude and frequency for a 90° phase shift, the phase delay between the acquired interferograms was measured when using a mirror in the sample arm. In this manner, it was found that a 160 nm vibration amplitude was difficult to reach without risk of mechanical damage at sinusoidal oscillation frequencies greater than 1.3 kHz. For 8 Hz high sensitivity operation, the

amplitude was found in the same manner, though the frequency was low enough that a square-wave control signal could be used, relaxing the camera timing requirements and reducing the possibility of fringe washout due to reference arm motion during acquisition.

The combined reference-sample arm output of the interferometer is free-space coupled into a 59 ± 1 cm single-mode fibre-optic cable, formed by splicing the ends of a 2 m FC/APC patch cable (Thorlabs P3-SMF28-FC-2). Coupling is performed using an aspheric lens fibre coupler (OFR FiberPort PAF-X-11-IR) mounted to a translation stage. The power is optimized by small alignment changes, achieving a reference arm coupling ratio of 45–51 %. When coupling light into the fibre that returns from a focused mirror reflection, significantly higher coupling ratios, between 68 % and 72 %, are attainable. These higher coupling ratios are believed to be either due to the fact that the beam entering the interferometer is not exactly collimated, or the fibre is not exactly located at the focus of the OFR fibre coupler.

The single-mode fibre has a specified cutoff wavelength of 1260 nm and, given the below cutoff wavelengths present in the imaging spectrum of Fig. 3.6, some wavelength-dependent attenuation is therefore expected. However, this effect was found to be minimal due to the good agreement between the shape of the power spectra measured before and after the fibre using the APE Wavescan and infrared OCT spectrometers, respectively.

The fibre-optic link serves to improve the spatial coherence of the backscattered light with respect to the reference arm, and has been demonstrated to improve cellular visibility of OCT images of onion skin [75]. The fibre also decouples the alignments for the OCT interferometer and that required for sum-frequency mixing in the nonlinear

crystal, and provides a spatial filtering effect as discussed previously and predicted according to equation (2.9) on page 16. Light exiting the spatial filter enters a fibre collimator (Thorlabs Inc. F240APC-C) to collimate the free-space beam to a specified $1/e^2$ diameter of 1.40 mm, aligned for 1310 nm.

3.5 Shaping The Gating Pulses

The pulses used for time gating in the current system are partially taken from the Ti:Sapphire output beam prior to pumping the OPO. These pulses can then be shaped by amplitude filtering in the frequency domain, which is accessed using a 4-f grating pair configuration, as described in [76] and shown schematically in Fig. 3.10. The primary motivation for shaping the gating pulse in the current implementation is to control its temporal duration while remaining transform-limited. If the gating pulse duration was instead extended by introducing chirp, the centre wavelength of the imaging spectrum would be shifted depending on whether sum-frequency mixing occurred at the beginning or end of the time gate. At the detector, interference would only be visible over regions of spectral overlap, resulting in a reduced imaging bandwidth and poorer depth resolution.

Figure 3.10: 4-f Grating Pair Schematic. G1, G2: Dispersive Gratings, L1, L2: Focusing Lenses

The 4-f grating pair uses a dispersive grating to spatially separate the power spectrum of the input beam. The dispersed light is collimated and focused by the first lens, L1. The next downstream lens, L2, refocuses the beam onto the second grating that recombines the spatially separated spectrum back into a short pulse. The temporal pulse output can be shaped from the input by modifying the spectral content of the signal at the focus between L1 and L2.

In this implementation, it was desired to increase the pulse duration by avoiding the introduction of any additional chirp. Thus, the gratings and lenses were each placed one focal length apart (i.e. a true 4-f configuration) for initial alignment. The dispersive gratings (Thorlabs GR25-1208) each have 1200 grooves/mm, with approximately 78 % efficiency for s-polarized light at 830 nm in Littrow configuration. The plano-convex lenses (Thorlabs LA1708-B) each had a focal length of 20 cm. The grating angles were set for a Littrow configuration by tuning the Mira Ti:Sapphire to the blaze wavelength (750 nm) and aligning both gratings for a direct back reflection at the input and output of the grating pair.

The pulse autocorrelation trace was measured before and after the 4-f grating pair using a 2-photon autocorrelator (APE GmbH MINI Autocorrelator). The raw input pulse autocorrelation trace FWHM, calculated automatically with the OPO controller using a Gaussian-fit, was matched at the output by adjusting the second focusing lens position to a distance of 20.5 ± 0.1 cm from the second grating. To reduce the bandwidth and extend the pulse duration, an adjustable iris was inserted 11.4 ± 0.1 cm after the first lens. By keeping the iris out of the focal area of the spectrum, the bandwidth clipping is not as sharp, reducing the sidelobes in the temporal gating pulse profile. The iris was aligned by ensuring a symmetric clipping of the output

pulse spectrum about its centre wavelength (830 nm) with the APE WaveScan spectrometer. Several representative gate power spectra are shown with their associated pulse autocorrelation traces in Fig. 3.11 for spectral clipping at various iris openings.

Figure 3.11: 4-f Grating Pair Normalized Power Spectra and Autocorrelation Traces at Several Iris Openings. Autocorrelation Trace Gaussian-fit FWHM values for each iris opening are listed.

Despite a specified grating efficiency of 78 % at 830 nm, the power efficiency measured in the final 4-f grating configuration suggests a 61 % efficiency for each grating, not accounting for losses due to the lenses, mirrors and a $\frac{\lambda}{2}$ plate used to control the gate polarization being sent into the nonlinear crystal. As the iris gradually closes, the spectral bandwidth is narrowed and average power is lowered while the Gaussian-fit FWHM of the temporal duration of the pulses is broadened. Table 3.1

Output Spectral Bandwidth FWHM (± 0.1 nm)	Autocorrelation Trace, Gaussian Fit FWHM (± 4 fs)	Average Output Power (± 0.1 mW)
9.0 nm	132 fs	132.1 mW
8.4 nm	220 fs	94.9 mW
7.5 nm	249 fs	84.2 mW
5.8 nm	291 fs	63.4 mW
3.9 nm	428 fs	39.8 mW

Table 3.1: 4-f Grating Pair Temporal Broadening of the Gate Pulse

summarizes the pulse broadening achieved at several iris openings, with the first entry corresponding to the iris being completely open and no spectral shaping.

The autocorrelation of the pulse is equal to the inverse Fourier transform of its power spectrum. The hard-clipping of the spectrum caused by the iris will thus result in temporal side-lobes on either side of the main pulse, even though they were not visible in the linearly-scaled autocorrelation pulse trace. It is desirable to broaden the time duration of the gate pulses while minimizing the effect of these sidelobes. Ideally, a top-hat temporal pulse shape is desired to strongly reject all backscattered light from outside the time gate, though it can only be achieved by applying a more sophisticated phase and amplitude transfer function to the gate spectrum than can be provided using the iris aperture.

3.6 The Nonlinear Crystal

Time-gated imaging is achieved using type II sum-frequency generation in a β -barium borate (BBO) nonlinear crystal (length 2 mm). The optical properties of this crystal are well-characterized [77, 78], and predictions of its nonlinear performance for various mixing wavelengths are simulated using SNLO software, provided by Sandia National

Laboratories [79].

The birefringence of nonlinear crystals permits sum-frequency generation at specific internal crystal angles for different input pulse polarizations. For a negative uniaxial crystal, such as BBO, a type I interaction consists of input fields with the same polarization, perpendicular to the principal plane (ordinary polarized waves). By contrast, type II sum-frequency generation occurs when one input field has ordinary polarization and the other has extraordinary polarization. In both cases, the polarization of the upconverted field is extraordinary.

Sum-frequency mixing can only noticeably occur when the sum of the input beam wave vectors is equal to the wave vector of the upconverted beam. This condition, known as phase-matching, strongly depends on the crystal index of refraction at various wavelengths and restricts the nonlinear interaction to a phase-matching bandwidth for a given crystal type, length, orientation, and input beam polarizations and frequencies.

Sum-frequency generation between the imaging OPO pulses (centred at 1280 nm) and the gate Ti:Sapphire pulses (centred at 830 nm) was simulated in a BBO crystal using SNLO. Although the nonlinear interaction efficiency was found to be greater for a type I interaction, the predicted phase-matching bandwidth was too small to upconvert the entire imaging spectrum, which would limit the time-gated depth resolution. For a type II interaction with 830 nm extraordinary and 1280 nm ordinary polarizations, a phase-matching bandwidth of 62 nm for a 1 cm long crystal was predicted about 1280 nm, which is sufficient given the OPO imaging spectrum shown in Fig. 3.6 and a ~ 2 mm crystal interaction length.

To determine the final crystal tilt angle to achieve type II sum-frequency mixing,

the cut-angle of the nonlinear crystal with respect to the optic axis, and its orientation with the input beam polarizations must be considered. Typically, the cut-angle is specified by the user for a given application so that the input beams are at normal incidence to the crystal, and losses are minimized. Our BBO crystal, which had not been custom-made for the current application, has a cut-angle of 19.8° and BBAR coating for input wavelengths of 800–1600 nm. Type II sum-frequency generation is predicted by SNLO to occur at an internal crystal angle of 38.6° with respect to the optic axis.

The crystal cut angle of 19.8° was found by observing the second-harmonic generation (SHG) of a 1300 nm beam, polarized in the plane of the table and focused onto the crystal with a 150 mm plano-convex lens (Thorlabs Inc. LA1433-C). At this wavelength, the internal crystal angle is predicted with SNLO to be 20.5° , indicating that SHG should occur at nearly normal incidence to the crystal cut surface. By rotating the crystal mount around the input beam axis while at nearly normal incidence, the SHG power was maximized when the polarization was normal to the principal plane. Using the SNLO predicted internal angles and ordinary and extraordinary indices of refraction at 1280 nm and 830 nm respectively, type II phase-matching was predicted to occur for a 45° crystal rotation about the input beam axis, and a crystal tilt of 50.4° .

Type II sum-frequency generation was first observed without the use of the fibre-optic spatial filter, and the polarizations of the OPO and gate pulses were made perpendicular to one another using $\frac{\lambda}{2}$ plates. Both input beams were aligned for parallel, spatially separated, propagation and were focused with the same 150 mm plano-convex lens onto the crystal for noncollinear mixing. To achieve spatial overlap,

the crystal was tilted to observe the second-harmonic generated light for each beam individually. The focused origin of the second-harmonic generated light was made visible on the crystal surface by directing the focused incident beam onto a strongly scattering crystal defect with a mirror located between the focusing lens and crystal. By switching between the SHG light produced from the gate and OPO spectra, both beams could be aligned to the same scattering point, ensuring spatial overlap. After sum-frequency generation was achieved, found by matching the temporal delay between the incident pulses at the proper crystal tilt angle, the combined beams were moved off the surface defect to maximize the upconverted intensity.

The approximate temporal delay of the gate and imaging pulses were matched by tracing out their respective beam paths with a length of string (total matched optical path length, including the fibre-optic link: 5.9 ± 0.1 m). The gate optical path used a variable translation stage delay for fine adjustment of the time gate position. By rotating the crystal to the approximate tilt angle for type II phase-matching, the gate delay was varied until green light, corresponding to sum-frequency generation between the OPO and gate pulses, was observed. At this point, the gate and OPO beam alignment, polarization, crystal tilt angle and focus were all be finely adjusted to maximize the sum-frequency generated light.

The addition of the fibre-optic link resulted in a slight rotation of the polarization, and introduced a small amount of ellipticity. A linear polarizer (Thorlabs Inc. LPVIS050) was inserted prior to the fibre-optic cable, with a maximum transmission of 83.5 %. After the fibre-optic cable, the maximum transmission was 69.4 %, after a polarization rotation of $45 \pm 2^\circ$ that was partially compensated afterwards using the

OPO $\frac{\lambda}{2}$ plate. To regain temporal overlap, the optical length of the 59 ± 1 cm fibre-optic cable was calculated using a specified group index of 1.47 [74], corresponding to a physical path length increase of 87 ± 1 cm. This path length increase was introduced to the gate optical path by folding the beam back across the table after the 4-f grating pair and prior to the variable delay.

After maximizing the average sum-frequency signal power, a final crystal tilt angle of 48° and rotation of 50° about the beam axis was used for the remaining proof-of-concept experiments. To ensure that the phase-matching bandwidth was indeed large enough to accept the entire OPO imaging spectrum, the OPO infrared and sum-frequency generated average powers were measured after the crystal over a range of wavelengths, with the sample arm of the interferometer blocked. The OPO was operated using the 12 mm dispersion block to maintain relatively narrow (~ 20 nm) bandwidths across the range from 1170–1400 nm, and the gate pulse autocorrelation trace width was kept constant at a Gaussian-fit FWHM of 253 fs. Using the assumptions that the upconverted intensity is directly proportional to the product of the input intensities, and that the OPO pulse durations were approximately constant across this spectral range, the nonlinear efficiency, defined as the ratio of the sum-frequency generated to incident OPO average power, was calculated and normalized, as shown in Fig. 3.12. In the first OPO cavity alignment, the signal power severely dropped off with some spectral broadening at higher wavelengths (depicted by triangular data points). These points were discarded from the phase-matching fit after the OPO alignment was corrected (second alignment, square data points), with the same operating parameters and nonlinear efficiency at several lower wavelengths (1200 nm, 1300 nm) recalculated for verification.

Figure 3.12: Experimental Phase Matching Bandwidth of Type II Sum-Frequency Mixing in BBO with a Constant Gate Intensity

With an external tilt angle of 48° , the refracted beams pass through approximately 2.3 mm of the nonlinear crystal, resulting in a SLNO-predicted collinear phase-matching bandwidth of 270 nm. The discrepancy between this value and the ~ 155 nm bandwidth experimentally found in Fig. 3.12 can be attributed to a greater wave vector mismatch in the crystal caused by the noncollinear arrangement of the incident beams. Nevertheless, Fig. 3.12 indicates that the entire spectral bandwidth emitted by the OPO spectrum can be upconverted for a fixed crystal tilt angle. A more even nonlinear conversion efficiency across the imaging bandwidth depicted in Fig. 3.6 is expected since the crystal tilt angle was readjusted prior to imaging to maximize the sum-frequency average power for the broadened OPO bandwidth.

3.7 Detection and Processing

Due to the noncollinear alignment for sum-frequency mixing, the 1280 nm, 830 nm and 504 nm pulses will each propagate in different directions after exiting the crystal. The unconverted OPO imaging beam will still have very nearly the same average power as it did prior to entering the crystal, as required for the negligible pump depletion approximation. This beam was picked off with a mirror and collimated using a 250 mm focal length plano-convex lens (Thorlabs Inc. LA1461-C) mounted to a translation stage. The beam is then free-space coupled with an aspheric lens coupler (Thorlabs Inc. F240APC-C) into a fibre-optic cable, using fine adjustments to the lens position and collimation to achieve a transmission efficiency of approximately 38 %. With an unattenuated OPO beam sent to only the reference arm of the interferometer (input power of 29 ± 1 mW), after passing through the fibre-optic spatial filter, the nonlinear crystal and several mirrors and lenses, the power output from the final fibre-optic cable was 1.5 mW, corresponding to an approximate 12.6 dB loss in the system. The fibre-optic cable was spliced to a pigtailed polarization controller (General Photonics Corp. PolarITE), allowing the polarization of the beam sent to the infrared spectrometer to be rotated to maximize the diffraction efficiency of the grating.

The beam is collimated at the fibre output using an air-spaced doublet collimator (Thorlabs Inc. F810APC-1310), providing a $1/e^2$ diameter of 6.60 mm at an aligned wavelength of 1310 nm, and sent into the infrared spectrometer. The spectrometer uses a 600 grooves/mm diffractive grating (Thorlabs Inc. GR25-0610), with a blaze wavelength of 1000 nm, providing a specified efficiency of 88 % in Littrow configuration for perpendicular polarization at 1280 nm. The maximum spectral resolution,

$\delta\lambda$, provided by the grating at a centre wavelength, λ , can be calculated according to its resolving power, which, based on the number of grooves illuminated, N , and the diffraction order, n , being observed, is given by

$$\frac{\lambda}{\delta\lambda} = nN. \quad (3.7)$$

Substitution into equation (3.7 using a spot size of 6.60 mm and a 600 grooves/mm grating yields a spectral resolution of 0.32 nm at 1280 nm for first-order diffraction.

The spectrally dispersed infrared beam is focused onto a 14 bit InGaAs line scan camera (Goodrich Sensors Unlimited SUI LDV-512LX) using an air-spaced compound lens system (Soligor GmbH Auto-Zoom 1:35 f=55–135 mm) with adjustable focal length and zoom. The camera was run on its high dynamic range setting, OPR 7, with a 240 μ s exposure time and maximum line-scan rate of 3.725 kHz. Each camera line scan was externally triggered using an 80 MHz function generator (Agilent Technologies Inc. 33250A), which was itself externally triggered from the piezo-driving signal sent to the piezo-electric controller. The amplitude and frequency of the vibrating reference mirror was set to provide a 90° phase shift between consecutive line scans by monitoring the phase shift present in the detected interferograms. The phase lag on the InGaAs camera triggering signal was adjusted to start a line acquisition when the oscillating mirror was at a maximum or minimum position.

The spectrometer focusing optic was set to resolve the high frequency interferometric fringes originating from reference and sample arm mirrors in the Michelson interferometer at long (~ 1 mm) path delays. After ensuring that the oscillating mirror and acquisition timing provided the correct 90° phase shift between consecutive lines, a small path delay was set between the interferometer arms and the calibration method detailed earlier was applied to the acquired 0° and 90° interferograms.

Once calibrated, the path delay was increased and the focus, input beam alignment and beam polarization were finely readjusted to maximize the fringe amplitude and obtain a smooth spectral envelope. After each readjustment, the calibration method was redone to account for any changes in the frequency-to-camera pixel conversion.

Using the final frequency-to-pixel calibration, the frequency resolution of a single camera pixel was calculated to be 0.52 nm. As was previously discussed, the depth range of a FDOCT system is dependent on the maximum interferometric fringe frequency that can be spectrally resolved and detected. In this case, the imaging depth is limited by the pixel-spectral resolution as opposed to the resolution provided by the dispersive grating. The predicted depth range for FDOCT imaging with this grating, lens combination and detector is found to be 0.79 mm using equation (2.10), found on page 19.

The sum-frequency generated beam is collimated by a 250 mm plano-convex lens (Thorlabs Inc. LA1461-A) after the nonlinear crystal and sent free-space to a separate spectrometer, while the 830 nm gating beam is sent to a beam stop after the crystal and is no longer used. Assuming good spatial overlap within the crystal, the sum-frequency beam will be expanded roughly by a factor of 1.67 from the incident OPO beam diameter that was focused onto the crystal with a 150 mm plano-convex lens.

Incoming light to the visible spectrometer is bandpass filtered (Thorlabs Inc. FGB-37) and sent through an achromatic $\frac{\lambda}{2}$ wave plate (Thorlabs Inc. AHWP05M-630) before being focused through a pair of entrance slits, used to accept only a specific narrow input beam angle. The beam is expanded by a factor of 3.33 by using a 45.0 mm focal length achromat doublet lens (Thorlabs Inc. AC254-045-A1) to focus the beam through the slits, and a 150 mm focal length plano-convex lens (Thorlabs

Inc. LA1433-A) to later recollimate the beam. This beam expansion, taken together with the expansion from the crystal and a $1/e^2$ collimated beam diameter of 1.40 mm, yields a final approximate $1/e^2$ diameter of 7.77 mm incident on the grating.

The beam is spectrally dispersed using a 1800 grooves/mm grating (Thorlabs Inc. GR25-1850), with a blaze wavelength of 500 nm, and focused with a 200 mm achromat doublet lens (Thorlabs Inc. AC254-200-A1) onto the 1004x1002 (horizontal x vertical) area scan electron-multiplying CCD (EMCCD) camera (Andor Technologies DU-885). The EMCCD camera was triggered using the same 80 MHz function generator as used for the InGaAs camera, thus preventing the possibility of synchronously imaging in both standard and time gated FDOCT in the current implementation. Although two-dimensional image features were found to be very reproducible using the DC servo motor translation stage actuators, the OPO spectral envelope varies unpredictably during imaging, resulting in slight changes in the point-spread functions between time gated and standard OCT scans.

The EMCCD camera has shown a much higher final electronic charge from incident photons when operated in full-image mode as opposed to fast-kinetics. In this case, accumulated charge isn't quickly shifted to unused pixels on the camera during acquisition, but is instead read off as a frame that includes all the pixels of the two-dimensional array. Although a subset of pixels may be defined by the user as a region of interest, the camera must still shift through all vertical rows during a read cycle, which severely limits its continuous acquisition speed. For full-image operation, the continuous read off rate for one frame is 16 Hz, and the spectrum is focused onto a height of ~ 3 pixels, with the remaining 999 vertical pixels remaining unused. The reason for the large difference in detector response between full-image

and fast-kinetics mode with all other parameters equal remains under investigation by the manufacturer [80].

When operating in full-image mode, the EMCCD camera is set to record two 1004x1002 pixel frames prior to read-out. These frames are recorded at a rate of 16 Hz, allowing the start of an acquisition cycle to be triggered by a single 8 Hz burst that is itself triggered from the piezo-driving square-wave signal. After ensuring the timing is correct for acquiring the 0° and 90° phase-shifted interferograms, the spectrometer alignment was performed using the same procedure described above for the infrared spectrometer at an axial scan rate of 8 Hz. The higher groove density in the visible spectrometer grating provides a predicted spectral resolution of 0.036 nm about 504 nm, allowing imaging over a much wider depth range (1.76 mm) than that needed for a time-gating implementation with typical depth ranges restricted to ~ 0.2 mm. In this case, the 1800 grooves/mm grating was chosen for its high efficiency (80 % for perpendicular polarization) as opposed to its resolving power. As in the infrared case, the pixel resolution using a 12.4 nm bandwidth centred about 504 nm (equivalent in frequency bandwidth as 80 nm about 1280 nm) was calculated to be 0.022 nm. Unlike the infrared spectrometer, the maximum depth range of the time-gated system will be limited according to the resolution provided by the dispersive grating.

Despite slight variations in the National Instruments LabView software commands to interface with each of the cameras for infrared or visible light detection, the processing code is very similar for both imaging systems and can be described according to the block diagram shown in Fig. 3.13.

The EMCCD camera initialization consists of cooling the sensor until stabilized

Figure 3.13: Block Diagram of Computer Post-Processing Algorithm

at the desired temperature, setting the acquisition mode parameters (exposure time, number of consecutive acquisitions per scan, electron-multiplying gain and pixel shift rates) and external trigger. In order to prevent temperature restabilization delays, the sensor temperature is forced to be maintained only below -80°C during image acquisition. These parameters are all accessible using the Andor Software Development Kit LabView VIs that communicate directly with the camera. By contrast, the Sensors Unlimited InGaAs camera options, such as its operational setting, trigger and pixel uniformity correction are all set externally through National Instruments' Measurement and Automation Explorer prior to running the acquisition and processing LabView program.

The dark spectra listed in the block diagram refer to any previously saved 0° and 90° spectra, which are subtracted from their respective interferograms prior to

performing the inverse Fourier transform. When imaging a strongly scattering sample, the reference arm reflection will be much stronger and is typically used as the dark spectrum while the sample arm is blocked. The subtraction of the reference arm power spectrum from the interferogram will reduce the DC noise level in the processed depth scan. A dark spectrum taken with both interferometer arms blocked will subtract off the common-mode background noise detected by the camera.

After the previously saved dark spectrum and calibration data is loaded, and the camera and DC servo motor initialized, the main loop of the program begins. For sample alignment purposes, two-dimensional scanning is not performed, and single depth scans (consisting of a 0° and 90° interferogram) are processed at a time. When desired, a new dark spectrum or spectral recalibration may be performed, taking the place of the previously saved data. After dark subtraction, the intensities at a user-defined frequency stepsize are calculated across the interferogram using cubic spline interpolation. Zero-padding is performed to increase the number of frequency sampling points to the next lowest power of two prior to calculating the discrete fast Fourier transform. The two frequency-scaled interferograms are combined as a complex signal and the inverse Fourier transform is applied to both this signal and its complex conjugate. Their difference is multiplied by the Heaviside step function to resolve positive and negative path delays, as described in the full-range algorithm by Göttinger *et. al.* [66].

Following the determination of the depth profile, the results are plotted and displayed to the user and the loop is restarted. For operation with the EMCCD camera, the temperature stability is rechecked and EM gain reset to the user-specified value during each loop. The user may start a two-dimensional scan after specifying the

number of transverse scan steps and the DC servo motor controlled translation stage stepsize. In this case, the DC servo motor takes a step, the 0° and 90° interferograms are acquired and the motor position recorded before another step is taken. Operation through the rest of the program is essentially the same, compared to the single axial scan case, though processing is now performed on the array of acquired interferograms. The two-dimensional set of depth scans are displayed using a three-dimensional contour plot in the LabView environment.

When exiting the program, the camera and DC servo motor links with the computer are closed and the current dark spectrum and calibration data are saved to text files. The user can choose to save the data acquired, but this option must be set prior to starting the program. Though inconvenient, this implementation prevents large amounts of data from being built up during regular operation and alignment. The raw interferometric data, as well as the processed depth scans and servo motor positions, are all saved to text files for the duration of the program when the save data option is selected.

The structure of the program currently does not support high acquisition speeds, primarily due to the presence of ActiveX controls on the LabView front panel. The T-cube DC servo motor controller and three-dimensional contour plot are both ActiveX components; their removal increases the maximum single depth scan repetition rate from 37 Hz to 246 Hz. The remaining delays, apart from the on-board camera read-out speeds, are caused by the FireWire data transfer rate from the camera to computer, and the signal processing algorithms required to determine the depth profile.

Chapter 4

System Performance & Analysis

4.1 Overview

An overview of the key OCT system specifications for both the standard infrared FDOCT (IR FDOCT) and the time-gated (TG FDOCT) implementations are summarized in table 4.1. The performance of each of these parameters is discussed in further detail in the following sections, with time-gated imaging results of contrast improvement and performance in a highly scattering medium (onion skin) later presented.

The sensitivity given for both systems was measured by inserting neutral density filters into the sample arm to attenuate the backreflected signal from a mirror. The reference arm power was set to just avoid detector saturation and the attenuation increased until the signal was indistinguishable from the processed noise floor. The reference arm power spectrum was subtracted from the interferograms during processing, which reduced the noise by approximately 5 dB. Due to the uncertainty of exactly matching the processed signal to the root-mean-square (RMS) noise level, an

Specification	IR FDOCT	TG FDOCT
Dynamic Range (± 2 dB)	56 dB	48 dB
Sensitivity (Scan Rate 1.3 kHz) (± 2 dB)	94 dB	69 dB
Sensitivity (Scan Rate 8 Hz) (± 2 dB)	86 dB	88 dB
-20 dB Gate Width	—	84 – 176 μm
Axial Resolution (FWHM)	8.5 – 12 μm	
Transverse Resolution (FWHM) (± 0.2 μm)	12.1 μm	
Camera Exposure Time	240 μs	
Sample Arm Power (± 2 mW)	40 mW	

Table 4.1: Final OCT System Specification Summary

estimated error of ± 2 dB is included. As done in other OCT systems, a more accurate measurement of the system sensitivity could be performed using a calibrated attenuator, and measuring the peak height with respect to the RMS (Root Mean Square) noise floor.

The sensitivity of the time-gated system was calculated in the same manner as the standard FDOCT system, with the time gate centred on the sample arm reflection with a relative path delay of 50 μm . In the case of 1.3 kHz operation, the EMCCD camera was run in fast-kinetics mode with an applied electron-multiplying gain of 18 dB. As compared to previous work [17], this represents a 15 dB sensitivity gain, which is consistent with the increased level of electron-multiplying gain applied prior to pixel read-off. When operating the EMCCD camera in full-image mode, its increased electronic charge for incident photons permitted an 88 dB sensitivity with 18 dB electron-multiplying gain. As previously discussed, the full-image mode camera operation requires the read-off of the entire two-dimensional pixel array (1004x1002 pixels) for each interferogram, which limits the axial scan rate to 8 Hz. By contrast,

in fast-kinetics mode the axial scan rate is limited by the 1.3 kHz reference mirror vibration, which is used in this implementation for full-range FDOCT imaging.

Although the use of electron-multiplying gain reduces the effective read-noise of the camera, the dynamic range is eventually decreased as the dark noise is amplified [81]. At 18 dB gain, the dynamic range was found to be limited to 25–30 dB. To avoid this limitation during further system characterization and proof-of-concept images, the camera was run with no applied electron-multiplying gain, permitting a 48 dB dynamic range. Although this trade-off reduces the sensitivity to ~ 52 dB in the case of 1.3 kHz operation, it was found to reduce the sensitivity in the 8 Hz full-image operation to only ~ 77 dB, indicating that the electron-multiplying gain did not improve the signal-to-noise ratio as effectively at very low light levels, where the relative background and random intensity noise levels are more significant.

The dynamic range achieved experimentally for both the standard and time-gated FDOCT systems is lower than that predicted for shot-noise limited performance, given by equation (2.14) on page 21, when inserting the specified 40,000 e^- and 100×10^6 e^- full-well capacities of the 1004 pixel EMCCD and 512 pixel InGaAs cameras, respectively. The predicted dynamic ranges, 67 dB and 98 dB for the time-gated and standard FDOCT systems, are based on a shot-noise limited model, which clearly does not apply for the higher dark and read noise present in InGaAs technology. The InGaAs camera specifications also include a minimum dynamic range of 3000:1, which corresponds to a more realistic ideal interferometric dynamic range of 70 dB. In practice, the experimental dynamic range rarely reaches the theoretical limit due to additional parasitic noise constraints from autocorrelation terms or flicker noise [64], as well as from operation in the non-shot-noise limited regime.

Specification	Contrast Images		8 Hz Onion Skin Images
	Scan Rate 1.3 kHz	Scan Rate 8 Hz	
Gate Autocorrelation Trace Gaussian Fit FWHM	208 fs	279 fs	249 fs
Full-Range Piezo-Driven Reference Mirror Signal	Sine-wave 520 mV amplitude	Square-wave 270 mV amplitude	
EMCCD Electron Multiplying Gain	5.9 dB	0 dB	
EMCCD Sensor Temperature	-85°C		
Post-Processing Frequency Step-size	0.06 THz		

Table 4.2: OCT Operating Specifications for Proof-of-Concept Imaging

The sample arm aspheric lens, used to focus the beam onto the sample, was in place only during the contrast and onion skin imaging and not for the characterization of the point-spread function, sensitivity or dynamic range. It was recognized that the passage of sample arm light through the aspheric lens and either a microscope slide for contrast imaging, or onion skin, will introduce a dispersion imbalance between the two interferometer arms. Although a dispersion mismatch reduces the achievable depth resolution, it was deemed to be a small effect in this case due to the spectrum's proximity to the zero dispersion point of glass. Apart from the variable ND filter used in the reference arm, no additional dispersion matching was performed for the proof-of-concept experiments.

The operational system settings for the contrast enhancement and proof-of-concept images are summarized in table 4.2.

4.2 System Performance

4.2.1 Standard Infrared FDOCT

The infrared FDOCT detection was implemented with the previously discussed infrared spectrometer and InGaAs line-scan camera. The point-spread function and dynamic range were calculated using equal reference and sample arm backreflections from a mirror. A common-path variable attenuator wheel was inserted prior to the fibre-optic spectrometer link to reduce the maximum average power incident on a pixel to just below the saturation point of the detector. The relative path delay between the interferometer arms was approximately 0.2 mm, and a sample point-spread function is shown in Fig. 4.1 in both logarithmic and linear scale. A Gaussian fit was applied to the processed data points shown in the linear plot, indicating an axial resolution (FWHM) of $8.4 \mu\text{m}$.

The achieved point-spread function shows several similar features with that predicted from the sample OPO broadened output spectrum, shown in Fig. 3.7. In both point-spread functions, there are -20--25 dB side-lobes occurring at 15–20 μm from the main peak, and the Gaussian FWHM axial resolutions are comparable (8.5 vs. 8.1 μm) on the linear scale. Note that the PSF shapes are not expected to match exactly since the power spectrum emitted from the OPO was different between the two measurements.

The sensitivity of the infrared FDOCT system was observed to decrease at greater path delays. This effect, already reported in other FDOCT implementations [31, 63], has been attributed to high frequency fringe washout caused by the finite spectrometer resolution and pixel size of the line-scan camera. To characterize the sensitivity loss,

Figure 4.1: Infrared Point-Spread Function on a Linear and Logarithmic Scale

the system dynamic range was calculated for a sample arm mirror that was scanned across the depth range. The source spectrum and sample mirror backreflection are assumed to have equal average powers across the scan, so that a decrease in dynamic range with depth corresponds to a decrease in sensitivity. The final measured infrared sensitivity variation with path delay is shown (points) in Fig. 4.2, overlaid with the predicted sensitivity roll-off given from equation (2.13) on page 20, using the previously estimated infrared spectrometer and pixel spectral resolutions of 0.32 nm and 0.52 nm, respectively.

During the above analysis, it was observed that the processed noise floor also decreased with increased relative path delays. In order to compensate for this roll-off, the RMS noise was calculated at each depth position across 122 axial scans. The logarithmic RMS noise with respect to path delay, Δz , was characterized using a

Figure 4.2: Infrared Sensitivity Loss across the Axial Depth Range Due to Finite Camera Pixel Size and Spectrometer Resolution

parabolic fit: $Noise_{RMS}[dB] = -8.0162(\Delta z)^2$ and was thereafter subtracted to all logarithmically-scaled infrared depth scans to achieve a flat noise floor.

The theoretical FDOCT sensitivity decrease fit the data quite well for path delays less than 0.75 mm when the noise roll-off was not included (i.e. processed peak heights were considered only). However, as shown in Fig. 4.2, the sensitivity roll-off based on the dynamic range at each depth point suggests a higher spectrometer resolution using the model provided in [63]. Beyond 0.75 mm path delays, the sensitivity sharply drops off and the point-spread function broadens before eventually aliasing back in at the opposite end of the depth range. This sudden drop in system performance is consistent with the predicted 0.79 mm depth range caused by the limited 0.52 nm pixel resolution and interfaces of interest were kept to within 0.7 mm path delays

during imaging. The interference from remaining interface backreflections at farther out of focus path delays were attenuated using the spatial filter provided by the fibre-optic cable.

4.2.2 Time-Gated FDOCT

The time-gated system was characterized similarly to standard FDOCT, with additional considerations resulting from the temporal discrimination provided by sum-frequency generation. The temporal profile of the time gate can be partially controlled by the user by adjusting the iris opening in the previously discussed 4-f grating pair. By removing bandwidth from the 830 nm gating pulses, the -20 dB time gate width could be adjusted from 84–176 μm . However, the removal of the gating pulse bandwidth decreased both its average and peak pulse powers, resulting in a trade-off between nonlinear conversion efficiency and gate width. The nonlinear conversion efficiency, defined here as the ratio of upconverted average power to infrared (1280 nm) average power, is given in table 4.3 for various gate pulse widths and average powers found from table 3.1. In this case, the time gate is centred on the pulses reflected from the reference arm, which had an average power of 6.6 ± 0.2 mW incident on the crystal.

Note that in table 4.3 the conversion efficiency roughly decreases proportionately to the gate average power divided by its autocorrelation width. This trend is based on the very simplistic model that the time gate is wider than the OPO pulses, so that conversion efficiency is directly proportional to the gate pulse peak intensity, assuming Gaussian temporal gate shapes. The trend does not hold well for the narrowest gate width (132 fs), at which point it will be similar to the 1280 nm pulse duration and

Gate Avg. Power (± 0.1 mW)	Gaussian FWHM of the Gate Autocorrelation Trace (± 4 fs)	Gate Avg. Power / FWHM (mW/fs)	SFG Conversion Efficiency ($\times 0.001$, ± 0.1)
132.1 mW	132 fs	1.00	3.3
94.9 mW	220 fs	0.43	2.3
84.2 mW	249 fs	0.34	1.8
61.8 mW	320 fs	0.19	1.1
39.8 mW	428 fs	0.09	0.5

Table 4.3: Sum-Frequency Generation Conversion Efficiency for Varied Incident Gate Pulse Durations and Average Powers

the convolution between the pulses must be considered.

In addition to the effect of pulse peak intensity on nonlinear conversion efficiency, the axial resolution of the upconverted signal can suffer if either the gate or the imaging pulses are significantly chirped. The pulse dispersion can be investigated directly by observing the upconverted power spectrum as the gate is shifted across the pulses backreflected from the reference arm only. When the prism pair separation does not provide the proper dispersion compensation, the spectrum will shift depending on the temporal overlap between the pulses. Although this method of observing chirp is qualitative and coarse, the prisms were adjusted to a 55 cm apex separation using this method, without an *a priori* measurement of the separation distance predicted from theory.

A quantitative measurement of the resolution loss at the gate edges due to dispersion can be performed by centring the gate on the reference arm pulses and scanning a sample mirror across the depth field of view. At far path delays, the sample arm

pulses will not experience temporal overlap with the gating pulse, and only the up-converted light from the reference arm will reach the detector. As the sample arm is brought within the temporal gate boundaries, interference will be observed and processed as in standard FDOCT. Assuming a constant source and sample mirror backreflection, the relative intensity of the processed interferograms across the depth field of view provides a cross-correlation attenuation profile of the time gate, as shown in Fig. 4.3, for a gate autocorrelation trace Gaussian FWHM of 407 fs and a 1.3 kHz acquisition rate. The plot is overlaid with the axial resolution measured at several path delay positions. Note that the very centre of the gate attenuation profile is jagged due to detector saturation that was not noted until after the scan.

Figure 4.3: Axial Resolution (points) Measured Across the Time Gate Attenuation Profile (solid line)

The maximum achievable axial resolution was dictated by the spectrally broadened pulses emitted by the OPO. In this case, the broadening was not as extreme as shown in Fig. 3.6, but nevertheless permitted axial resolutions in the range of 11–11.5 μm (Gaussian FWHM bandwidth >60 nm about 1280 nm). Within the gate boundaries, the depth resolution is observed to fluctuate due to the instability of the OPO spectrum. However, at the gate edges, the resolution clearly deteriorates, consistent with the presence of pulse chirp as predicted by the group delay dispersion map in Fig. 3.8.

The hard-clipping of the gate spectrum in the 4-f grating pair is expected to add sidelobes to the temporal profile of the gating pulse, which will be extended to the cross-correlation attenuation profile. For most time-gated imaging applications, it is desirable to position the gate to restrict the depth field of view to a specific window within the sample. With this application in mind, an optimal gate shape would be one that upconverts all backscattered light within the pass-band equally and possesses a very sharp roll-off in the stop-band. The corresponding attenuation profile would look like a top-hat, requiring more complex phase and amplitude spectral control over the gate pulse in the 4-f grating pair. Given that the Mira Ti:Sapphire laser emits pulses with bandwidths up to ~ 10 nm about 830 nm, the temporal shaping capabilities will be limited according to the Fourier transform of the final gate spectrum.

The nonlinear time gate attenuation profile can be compared to the predicted confocal gate provided by spatial filtering in the fibre-optic link. Using equation (2.9), given on page 16, and the specifications for the OFR collimating optic [84] and SMF-28e fibre [74], the *A-parameter* of the spatial filter was found to be 14.0, using an input $1/e^2$ beam radius (a_0) of 1.86 mm, a fibre mode-field radius (r_0) of 4.5 μm ,

centre wavelength (λ) of 1280 nm and fibre-to-collimation lens distance (d) of 11 mm. The specified numerical aperture of the fibre is 0.14, and the overlaid attenuation profiles are shown in Fig. 4.4. It is immediately clear that the spatial filter confocal gate does not provide the sharp attenuation roll-off that is achieved with nonlinear time gating for the beam size, optical fibre and lenses being used.

Figure 4.4: Confocal Spatial Filter and Time Gate Attenuation Profiles

As in the standard infrared FDOCT case, the processed axial point-spread function was measured using a mirror in the sample arm. A gate with an autocorrelation trace Gaussian-fit FWHM of 279 fs was centred on the sample backreflection, and a variable attenuation wheel was used for each arm to optimize the detected interferometric fringe amplitude for a path delay of $\sim 50 \mu\text{m}$. For the below PSF, the OPO spectrum emitted was such that the side-lobes were reduced as compared to Fig. 4.1, but the axial resolution FWHM was broadened to $9.5 \mu\text{m}$.

Figure 4.5: Time-Gated Point-Spread Function on a Linear and Logarithmic Scale

To measure the time-gated sensitivity depth dependence, the reference arm reflection was attenuated so that the upconverted spectrum filled only $\sim 10\%$ of the detector well. The mirror reflection from the sample arm was then adjusted to different path delays and its attenuation set so that the detected interferogram was just below saturation. By readjusting the sample arm power to the same level at each path delay, the attenuation introduced by the time gate is compensated and the remaining loss in fringe amplitude can be attributed to a loss in sensitivity, assuming a flat noise floor. The resulting attenuation profile for a $111\ \mu\text{m}$ -20 dB gate width is overlaid with the relative processed peak intensities after compensating for the gate loss, shown in Fig. 4.6.

The dips in the sensitivity profile correspond with the side-lobe local minima points in the time gate. In these cases, it is expected that a more detailed nonlinear

Figure 4.6: Time-Gated Sensitivity Depth Dependence Across a $111 \mu\text{m}$ Gate. Points: Measured Sensitivity, Line: Cross-correlation Time Gate Attenuation Profile

theory that includes the convolution, group velocity dispersion and walk-off between the pulses would be required to explain the loss of fringe amplitude at the gate nodes, which is beyond the scope of the present work.

4.3 Time-Gated Contrast Enhancement

One of the primary advantages of time gating is its ability to provide contrast improvement when in the presence of strong and weak scattering sites. The situation is illustrated with the schematics shown in Fig. 4.7, using the dynamic range and sensitivity values observed for the 8 Hz slide and mirror axial scans shown in Fig. 4.9.

Figure 4.7: Achievable Contrast for Standard and Time-Gated FDOCT. Left-hand Schematic: Sensitivity Limited Performance. Right-hand Schematic: Dynamic-range Limited Performance.

The left-hand schematic shows the experimental dynamic range window and sensitivity levels of both the time-gated and standard infrared FDOCT systems. As can be seen, the infrared system slightly outperforms the time-gated system specifications. However, the situation changes when a sample is imaged that consists of a strong and a weak scattering interface at two different depths. In standard FDOCT, the light backscattered from both interfaces is incident on the detector. To optimize the dynamic range, the user can match the average reference and sample arm beam powers and then set a common path attenuation so that the interferogram fills the detector well. The noise floor in this case is shifted up from the sensitivity limit to the limit provided by the camera's dynamic range. However, with time gating, the user can isolate the weaker signal of interest and completely remove the signal from

the strong interface. In this case, the time-gated noise floor remains at the sensitivity limit, providing a maximum of 29 dB signal-to-noise, or contrast improvement, to the weaker signal.

To illustrate the contrast improvement made possible with the current time-gated FDOCT implementation, a microscope slide and mirror were placed in the sample arm of the interferometer to provide both strong and weak backreflections over a single axial scan. The mirror, mounted to the DC servo translation stage, was brought very close to the rear surface of the slide, with the beam focus set to maximize the mirror backreflection. Additional multiple reflections with a periodic path delay will occur between the slide and mirror, showing up as peaks in the depth profile. The extra out of focus ~ 1.5 mm optical depth separation with the front microscope slide surface was sufficiently attenuated by the fibre-optic spatial filter to not be observed as an aliased signal above the noise floor.

Although the time gate could be centred on any one of the interfaces present in the axial scan, the weaker multiple reflections were chosen in order to demonstrate the system's contrast enhancement capabilities. The initial demonstration, described in [17], was taken with an electron-multiplying gain of 5.9 dB at a 1.3 kHz acquisition rate and is shown in Fig. 4.8 with each interface labelled across the axial scan. To provide a fair comparison between standard and time-gated FDOCT, the backscattered intensity was offset to match the processed RMS noise floors to 0 dB and the same exposure times were used for both EMCCD and InGaAs (Indium Gallium Arsenide) interferogram acquisition.

At the time of the experiment, the achievable dynamic range of the infrared

Figure 4.8: 13 dB Contrast Enhancement Demonstrated in an Axial Scan of a Microscope Slide and Mirror

FDOCT system was 45 dB [17], and the time-gated sensitivity noise floor was approximately 58 dB, after accounting for the ~ 6 dB applied electron-multiplying gain. Referring back to Fig. 4.7, the maximum contrast improvement for this system is equal to the difference between the infrared dynamic range and time-gated sensitivity noise floors, predicted to be 13 dB, and corresponding to that achieved experimentally. A slight loss from the reported 13.6 dB contrast improvement in [17] occurred due to the reprocessing of data with a 0.06 THz stepsize and recalculation of the RMS noise floor. This discrepancy is expected to fall within the error of the RMS noise floor calculation and depth roll-off correction.

After achieving a much higher OCT sensitivity in full-image mode operation at 8 Hz, the slide and mirror imaging experiment was performed again. In this case, a

much more dramatic 29 dB contrast improvement is possible, as predicted by Fig. 4.7 and demonstrated in Fig. 4.9 for three overlaid axial scans in which the 2nd and 3rd multiple reflections were each time gated separately. In the case of the 3rd multiple reflection, the use of time gating brings the signal completely out from below the dynamic range limited noise floor in standard FDOCT.

Figure 4.9: 29 dB Contrast Enhancement Demonstrated in an Axial Scan of a Microscope Slide and Mirror

4.4 Proof-of-Concept in a Highly Scattering Medium

Beyond the demonstration of contrast improvement, proof-of-concept performance of the time-gated FDOCT system is required in a highly scattering medium to establish its potential for typical biomedical OCT applications. Previous researchers have

demonstrated their OCT imaging capabilities with model eyes for ophthalmic applications [65], and also with onion skin [75, 82, 83] when it is inconvenient to bring biological samples into the laboratory.

An onion skin sample contains sub-surface cellular structure that can be detected with standard FDOCT systems. A two-dimensional scan, performed with the current infrared FDOCT setup, is shown in Fig. 4.10. As was previously discussed, a two-dimensional image is built up using the axial scans acquired while translating the sample through the focused beam. The front surface of the onion is shown at path delays of ~ -0.62 — -0.55 mm across the transverse axis, with greater penetration depths shown at shorter relative path delays. The detail of the cellular structure observed conforms well with images previously reported.

Figure 4.10: Standard FDOCT Two-dimensional Scan of Onion Skin

A primary motivation of time-gated imaging in a highly scattering medium is to establish that the restriction of the depth field of view can be accomplished without adverse effects on imaging performance. To assess the capabilities of the time-gated system at high penetration depths, the onion was aligned so that many scattering peaks were visible across a single infrared axial scan. While keeping the reference arm fixed, the DC servo translation stage was scanned in the axial direction (as opposed to

transverse for two-dimensional imaging) for infrared, and then time-gated, acquisition so that each scattering interface passed through the focal volume. The scattering peaks at a single processed depth position were then plotted over an axial translation of 1.5 mm.

The same process was performed using the time-gated system, with the gate centered at the focal point. Since the infrared noise floor was significantly higher near zero path delay, its scattering peaks were plotted at a fixed path delay of $234\text{ }\mu\text{m}$, as compared to a path delay of $98\text{ }\mu\text{m}$ for the time-gated scan. The comparison plot is shown in Fig. 4.11 after aligning the two axial scans. Penetration into the onion proceeds from left to right, with the first peak corresponding to the onion front surface.

Figure 4.11: Standard and Time-Gated FDOCT Scattering Locations at Various Penetration Depths in Onion

It is observed that the scattering peak locations are roughly aligned between the two scans. However, some mismatch in the scattering intensities is observed and expected since the plots were generated over different processed depth locations and the translation stage motion may not have been purely in the axial direction. Most importantly, matching cellular structure is observed in both scans at penetration depths >1 mm.

In typical FDOCT imaging, the sample is tilted to avoid the strong specular reflection from the front surface. As previously demonstrated in the contrast enhancement analysis, the presence of a strong front reflection in FDOCT will limit the achievable SNR (Signal-to-Noise Ratio) of the weaker subsurface structure of interest. In time-gated imaging, this is not an issue since the gate can be easily adjusted to attenuate, or completely remove the stronger scattering sites.

A two-dimensional scan of onion skin was taken with both standard and time-gated FDOCT when the tilt and focus was set to maximize the front surface reflection. As compared to Fig. 4.10, the infrared scan shows less cellular structure beneath the surface since the noise floor is raised from the sensitivity limit to the dynamic range limit of the InGaAs detector. The time gate was positioned approximately $400\text{ }\mu\text{m}$ beneath the surface, and the two-dimensional scan was performed over the same transverse sample range. For comparison purposes, the images shown in Fig. 4.12 have the same scattering intensity grayscale, with 0 dB set to the RMS noise floor. To focus on the time-gated structure in the image, the grayscale maximum level of both images was set to 40 dB. In this case, the front surface interfaces with >40 dB backscatter intensity in the infrared scan will appear broader than they actually are since their point-spread functions have been clipped to 40 dB.

Figure 4.12: Two-dimensional Standard and Time-Gated FDOCT Scans of Onion Skin

The cellular structure is observed to match in both scans, indicating that time-gated OCT imaging works in a highly scattering sample medium. Note that the cellular walls, shown for path delays $\leq -150 \mu\text{m}$ are weaker in the time-gated images since they are partially attenuated near the edge of the gate, and that some axial lines in the infrared image were corrupted by detector saturation.

Not only does the cellular structure match in both images, but there appears to be better detail present after time-gating. This detail can best be seen when the onion scans of Fig. 4.12 were further processed by resetting the grayscale intensity levels to nonlinearly brighten the weaker backscatter intensities that were at least 6 dB above the noise floor. The axes of the resulting contour plot were also rescaled to zoom in on only the time-gated region, with the dotted boxes in Fig. 4.13 used to highlight

two cells visible in the time-gated image.

Figure 4.13: Rescaled and Zoomed Two-dimensional Standard and Time-Gated FDOCT Scans of Onion Skin. Highlighted: Time gated cells visible at $\sim 250 \mu\text{m}$ beneath the surface.

Chapter 5

Conclusions and Future Work

In the present study, a novel OCT system was designed and developed to enhance imaging contrast by restricting backscattered light from the sample to a narrow time or, equivalently, depth window using sum-frequency generation. The region of backscattered light that is rejected from the detector can be easily controlled by the user so that strong sample backreflections can be eliminated from a depth scan, allowing a better view, as quantified by the signal-to-noise ratio, of the weaker scattering sites of interest.

A proof-of-concept implementation demonstrated a 13 dB and 29 dB time-gated contrast improvement of the multiple reflections between a microscope slide and mirror for axial scan rates of 1.3 kHz and 8 Hz respectively. Imaging in a highly scattering medium was demonstrated using onion skin, whereby matching cellular structure was shown across a two-dimensional scan at penetration depths of several hundred micrometres, as well as at penetration depths >1 mm in a one-dimensional scan.

In the present implementation, the depth field of view was restricted due to the temporal overlap requirements in sum-frequency mixing. The attenuation profile of

the time gate was found to be much stronger than that caused by spatial filtering provided by a fibre-optic cable link. The additional flexibility of having the transverse resolution decoupled from the time gate width, and potential for direct user control over the gate pulse delay and temporal profile, indicates that time-gated FDOCT provides significant advantages over an optical coherence microscopy system adapted for a $\sim 100\ \mu\text{m}$ depth field of view.

The rejection of light from outside the time gate filters out the high interferometric fringe frequencies seen at the spectrometer detector. Since the number of pixels required for unaliased imaging across a fixed frequency bandwidth decreases with the maximum depth range, shown in equation (2.10) on page 19, it is clear that in the present implementation the interferograms are being oversampled by the 1004 pixels in the EMCCD camera. According to the spectral resolution predicted by equation (2.10) and resulting number of detector elements predicted by equation (2.12) on page 19, only 45 sampling points are required for $9\ \mu\text{m}$ resolution imaging across a Gaussian spectrum with 80 nm bandwidth, centred at 1280 nm for a maximum depth field of view of $100\ \mu\text{m}$ in air. When considering a CCD camera that acquires interferometric data from only 45 pixels as opposed to the more typical 900 pixels for a 2 mm depth field of view experienced in standard FDOCT, an axial scan speed increase of a factor of ten over current FDOCT systems should be attainable.

With time-gated detection over only ~ 45 pixels per axial scan, the need to use a pixel-array camera, as in standard FDOCT, is diminished. In confocal microscopy, an array of 32 photomultiplier tubes has already been implemented commercially for hyperspectral imaging (Carl Zeiss LSM 510 META), with reported scan rate capabilities of 500 kHz [85], which is well beyond the current capabilities of even the

fastest swept source OCT systems.

Further time-gated system development can proceed in several directions. As shown in the gate attenuation profiles, sidelobes remain near the gate edges, which are expected to be due to the *sinc*-function temporal profile of the gating pulse. By filtering the phase and amplitude across the entire gate spectrum as opposed to hard-clipping the spectral amplitude with an iris, an attenuation profile with a flatter plateau in the pass-band and reduced sidelobes in the stop-band should be attainable.

Although the current system sensitivity roughly matches that of the standard infrared FDOCT system, it has been shown a further sensitivity increase will allow a greater potential time-gated contrast improvement. By increasing the nonlinear upconversion efficiency, more time-gated light will reach the EMCCD detector. It is expected that with a custom-designed nonlinear crystal, a higher conversion efficiency and corresponding sensitivity improvement should be attainable.

A much greater EMCCD camera electronic response for incident photons has been achieved when acquiring full-images from the pixel array as opposed to using the fast-kinetics mode to shift accumulated charge to unused regions of the sensor. However, the full 1004×1002 pixel read-off limits frame rates to 16 Hz and, with two interferograms per axial scan required for full-range imaging, the axial scan rate is reduced to 8 Hz. According to the camera specifications [81], it should also be possible to define a cropped sub-array region of pixels on the sensor for isolated read-off, providing the higher scan rates associated with a reduced depth field of view. However, due to continued software errors present in the current version of the Andor software development kit, the camera is not able to acquire in isolated crop mode. Either the proper fast kinetics or isolated crop operation will be necessary to increase

axial scan speeds to be competitive with those recently reported in standard FDOCT.

Beyond methods that directly affect time gating, the overall OCT system can be enhanced by implementing design features present in many of the standard models already used for *in vivo* clinical applications. For example, to reduce beam alignment issues, the free-space OCT system can be converted from an optical bench to fibre-optic implementation. In addition, as opposed to translating the sample across a fixed beam path for two- or three-dimensional scanning, typical OCT systems use a galvanometer to quickly raster scan the beam over the sample field of view. Since high intensity ultrafast pulses are being used in the current OCT system, careful attention to avoid detrimental nonlinear effects in optical fibre, and proper dispersion management, must be considered to implement these changes.

The full-range FDOCT method used presently requires the measurement of two interferograms every axial scan. Methods that are sensitive to the relative interferogram shift across a transverse line scan have been recently reported, requiring only a single interferogram measurement per axial position and only slightly more complex post-processing methods [68, 69]. By switching to a full-range design that requires half the recorded interferograms per transverse scan, the overall image acquisition time will also be halved. Alternatively, the use of a full-range algorithm can be removed entirely if a top-hat shaped time gate is positioned to one side of the reference path delay. In this case, all interfaces from symmetric depth locations are effectively removed, resolving the path delay ambiguity.

Based on the presented proof-of-concept results, the time-gated FDOCT system shows potential for future commercial use in current OCT applications. The current design permits synchronous standard and time-gated imaging, allowing a user to

image the complete sample and then dynamically control the gate width and position to optimize the acquisition speed and imaging contrast for their application. The high cost of ultrafast laser systems remains an inhibitor to immediate commercialization, though it has been, and is expected to continue, to decrease as ultrafast lasers are used in an increased number of applications and industries.

List of Acronyms

BBO	Beta-Barium Borate	A nonlinear crystal
CARS	Coherent Anti-Stokes Raman Spectroscopy	CARS is a third-order nonlinear spectroscopy technique
EMCCD	Electron-Multiplying Charge-Coupled Device	A camera that accumulates charge from incident photons with additional “electron-multiplying” gain applied prior to electronic read-off.
FDOCT	Fourier-Domain Optical Coherence Tomography	FDOCT detects the full sample depth field of view in a single shot with a spectrometer
FROG	Frequency-Resolved Optical Gating	A pulse characterization method that provides near-complete phase and amplitude recovery of the measured pulse
FWHM	Full-Width at Half Maximum	
GDD	Group Delay Dispersion	The second derivative of the spectral phase with respect to angular frequency, expressed in femtoseconds-squared, for a given length in a medium

INGaAs.....	Indium Gallium Arsenide	InGaAs is the compound typically used for detection of photons in the infrared (1000–1600 nm)
OCM.....	Optical Coherence Microscopy	Imaging technique that combines confocal microscopy with high resolution optical coherence tomography for higher sensitivity in scattering media
OCT.....	Optical Coherence Tomography	OCT is an interferometric-based imaging technique
OPO.....	Optical Parametric Oscillator	A light source based on parametric difference frequency amplification, requiring an external pump beam. Oscillation within a cavity permits high output powers (>100 mW) for the 76 MHz Mira model across a broad cavity-tuned centre frequency range
RMS.....	Root Mean Square	
SFG.....	Sum Frequency Generation	
SNR.....	Signal-to-Noise Ratio	
SSOCT.....	Swept-Source Optical Coherence Tomography	SSOCT detects the full sample depth field of view by rapidly sweeping through their source spectral bandwidth with narrow instantaneous line widths, requiring a single detector as opposed to a spectrometer in FDOCT
TDOCT.....	Time-Domain Optical Coherence Tomography	TDOCT uses a scanning reference delay line to image at different sample depths

Glossary

confocal microscopy Microscopy technique that uses a spatial aperture to reject out of focus light from reaching the detector

femtosecond 1 femtosecond [fs] is equal to 10^{-15} seconds

full-range FDOCT A Fourier-domain optical coherence tomography system in which positive path delays between the reference and sample arms of the interferometer can be distinguished from negative path delays

full-well capacity The number of photo-electrons that can be held by a camera pixel prior to saturation

histology Traditional optical microscopy of a mounted thin slice of tissue

Littrow configuration Condition in which light incident on a dispersion grating is diffracted back along the same path

sum-frequency generation A second order nonlinear process in which an electric field is created with a carrier frequency equal to the sum of the incident field frequencies interacting in a nonlinear medium

transform-limited A pulse with the minimum temporal profile allowed according to the Fourier transform of its frequency spectrum

ultrasound Clinical imaging technique based on the pulse-echo delay of focused pressure waves

Bibliography

- [1] D. Huang, E. A. Swanson, C. P. Lin, J. S. Shuman, W. G. Stinson, W. Change, M. R. Hee, T. Flotte, K. Gregory, C. A. Puliafito, J. G. Fujimoto, “Optical Coherence Tomography” *Science* **254**, 1178 (1991).
- [2] J. G. Fujimoto, “Optical Coherence Tomography: Introduction,” in *Handbook of Optical Coherence Tomography*, B. E. Bouma and G. J. Tearney, eds. (Marcel Dekker, 2002), pp. 1-40.
- [3] Nikon Instruments Inc., “Eclipse E200 Specifications,” <http://www.nikoninstruments.com/products.php?n=e200&p=Specifications> (accessed December 2, 2007).
- [4] A. L. Carlson, L. G. Coghlan, A. M. Gillenwater and R. R. Richards-Kortum, “Dual-mode reflectance and fluorescence near-video-rate confocal microscope for architectural, morphological and molecular imaging of tissue,” *J. Microscopy* **228**, 11 (2007).
- [5] J.-X. Cheng, Y. K. Jia, G. Zheng and X. S. Xie, “Laser-Scanning Coherent Anti-Stokes Raman Scattering Microscopy and Applications to Cell Biology,” *Biophys. J.* **83**, 502 (2002).

- [6] P. N. T. Wells, "Ultrasound imaging," *Phys. Med. Biol.* **51**, R83 (2006).
- [7] F. S. Foster, C. J. Pavlin, K. A. Harasiewicz, D. A. Christopher and D. H. Turnbull, "Advances in ultrasound biomicroscopy," *Ultrasound in Med. & Bio.* **26**, 1 (2000).
- [8] M. J. Rivellesse and C. A. Puliafito, "Optical Coherence Tomography in the Diagnosis and Management of Posterior Segment Disorders," in *Handbook of Optical Coherence Tomography*, B. E. Bouma and G. J. Tearney, eds. (Marcel Dekker, 2002), pp. 471-485.
- [9] P. H. Tomlins and R. K. Wang, "Theory, developments and applications of optical coherence tomography," *J. Phys. D.: Appl. Phys.* **38**, 2519 (2005).
- [10] V. X. D. Yang, M. L. Gordon, S-J. Tang, N. E. Marcon, G. Gardiner, B. Qi, S. Bisland, E. Seng-Yue, S. Lo, J. Pekar, B. C. Wilson and I. A. Vitkin, "High speed, wide velocity dynamic range Doppler optical coherence tomography (Part III): *in vivo* endoscopic imaging of blood flow in the rat and human gastrointestinal tracts," *Opt. Express* **11**, 2416 (2003).
- [11] S. Yazdanfar, A. M. Rollins and J. A. Izatt, "Imaging and velocimetry of the human retinal circulation with Doppler optical coherence tomography," *Opt. Lett.* **25**, 1448 (2000).
- [12] Y. Zhao, Z. Chen, C. Saxer, S. Xiang, J. F. de Boer and J. S. Nelson, "Phase-resolved optical coherence tomography and optical Doppler tomography for imaging blood flow in human skin with fast scanning speed and high velocity sensitivity," *Opt. Lett.* **25**, 114 (2000).

- [13] J. F. de Boer, S. M. Srinivas and J. S. Nelson, "Polarization-Sensitive Optical Coherence Tomography," in *Handbook of Optical Coherence Tomography*, B. E. Bouma and G. J. Tearney, eds. (Marcel Dekker, 2002), pp. 237-274.
- [14] M. E. J. van Velthoven, D. J. Faber, F. D. Verbraak, T. G. van Leeuwen, M. D. de Smet, "Recent developments in optical coherence tomography for imaging the retina," *Prog. Ret. & Eye Res.* **26**, 57 (2007).
- [15] J. Su, I. V. Tomov, Y. Jiang and Z. Chen, "High-resolution frequency-domain second-harmonic optical coherence tomography," *Appl. Opt.* **46**, 1770 (2007).
- [16] A. G. Podoleanu, J. A. Rogers and D. A. Jackson, "OCT *En-face* Images from the Retina with Adjustable Depth Resolution in Real Time," *IEEE J. Select. Topics Quantum Electron.* **5**, 1176 (1999).
- [17] M. S. Muller, P. J. L. Webster and J. M. Fraser, "Time-gated Fourier-domain optical coherence tomography," *Opt. Lett.* **32**, 3336 (2007).
- [18] X. Gu, S. Akturk, A. Shreenath, Q. Cao and R. Trebino, "The Measurement of Ultrashort Light Pulses—Simple Devices, Complex Pulses," *Opt. Rev.* **11**, 141 (2004).
- [19] R. Trebino, "FROG," in *Frequency-Resolved Optical Gating: The Measurement of Ultrashort Laser Pulses*, R. Trebino, ed. (Kluwer Academic, 2000), pp. 101-115.
- [20] C. Dunsby and P. M. W. French, "Techniques for depth-resolved imaging through turbid media including coherence-gated imaging," *J. Phys. D: Appl. Phys.* **36**, R207 (2003).

- [21] J. G. Fujimoto, S. De Silvestri, E. P. Ippen, C. A. Puliafito, R. Margolis and A. Oseroff, "Femtosecond optical ranging in biological systems," *Opt. Lett.* **11**, 150 (1986).
- [22] C. Doulé, T. Lépine, P. Georges and A. Brun, "Video rate depth-resolved two-dimensional imaging through turbid media by femtosecond parametric amplification," *Opt. Lett.* **25**, 353 (2000).
- [23] M. D. Duncan, R. Mahon, L. L. Tankersley and J. Reintjes, "Time-gated imaging through scattering media using stimulated Raman amplification," *Opt. Lett.* **16**, 1868 (1991).
- [24] T. Yasui, K. Minoshima, E. Abraham and H. Matsumoto, "Microscopic time-resolved two-dimensional imaging with a femtosecond amplifying optical Kerr gate," *Appl. Opt.* **41**, 5191 (2002).
- [25] E. Baigar, C. Hauger and W. Zinth, "Imaging within highly scattering media using time-resolved backscattering of femtosecond pulses," *Appl. Phys. B* **67**, 257 (1998).
- [26] J. M. Schmitt, A. Knüttel, M. Yadlowsky and M. A. Eckhaus, "Optical-coherence tomography of a dense tissue: statistics of attenuation and backscattering," *Phys. Med. Biol.* **39**, 1705 (1994).
- [27] M. Wojtkowski, T. Bajraszewski, P. Targowski and A. Kowalczyk, "Real-time *in vivo* imaging by high-speed spectral optical coherence tomography," *Opt. Lett.* **28**, 1745 (2003).

- [28] P. R. Herz, Y. Chen, A. D. Aguirre, J. G. Fujimoto, H. Mashimo, J. Schmitt, A. Koski, J. Goodnow and C. Petersen, "Ultrahigh resolution optical biopsy with endoscopic optical coherence tomography," *Opt. Express* **12**, 3532 (2004).
- [29] Y. Chen, P. M. Andrews, A. D. Aguirre, J. M. Schmitt and J. G. Fujimoto, "High-resolution three-dimensional optical coherence tomography imaging of kidney microanatomy *ex vivo*," *J. Biomed. Opt.* **12**, 034008 (2007).
- [30] C. C. Rosa, J. Rogers, J. Pedro, R. Rosen and A. Podoleanu, "Multiscan time-domain optical coherence tomography for retinal imaging," *Appl. Opt.* **46**, 1795 (2007).
- [31] R. Leitgeb, C. K. Hitzenberger and A. F. Fercher, "Performance of fourier domain vs. time domain optical coherence tomography," *Opt. Express* **11**, 889 (2003).
- [32] J. F. de Boer, B. Cense, B. H. Park, M. C. Pierce, G. J. Tearney and B. E. Bouma, "Improved signal-to-noise ratio in spectral-domain compared with time-domain optical coherence tomography," *Opt. Lett.* **28**, 2067 (2003).
- [33] B. Cense, N. A. Nassif, T. C. Chen, M. C. Pierce, S-H. Yun, B. H. Park, B. E. Bouma, G. J. Tearney and J. F. de Boer, "Ultrahigh-resolution high-speed retinal imaging using spectral-domain optical coherence tomography," *Opt. Express* **12**, 2435 (2004).
- [34] Y. Zhang, B. Cense, J. Rha, R. S. Jonnal, W. Gao, R. J. Zawadzki, J. S. Werner, S. Jones, S. Olivier and D. T. Miller, "High-speed volumetric imaging of cone photoreceptors with adaptive optics spectral-domain optical coherence tomography," *Opt. Express* **14**, 4380 (2006).

- [35] H. Lim, M. Mujat, C. Kerbage, E. C. W. Lee, Y. Chen, Teresa C. Chen and J.F. de Boer, “High-speed imaging of human retina *in vivo* with swept-source optical coherence tomography,” *Opt. Express* **14**, 12902 (2006).
- [36] Gage Applied Technologies Inc., “CompuScope 82G Specifications,” http://www.gage-applied.com/products/PDF/compuScope_82G.pdf (accessed December 2, 2007).
- [37] R. Huber, D. C. Adler and J. G. Fujimoto, “Buffered Fourier domain mode locking: unidirectional swept laser sources for optical coherence tomography imaging at 370,000 lines/s,” *Opt. Lett.* **31**, 2975 (2006).
- [38] R. Huber, M. Wojtkowski, J. G. Fujimoto, J. Y. Jiang and A. E. Cable, “Three-dimensional and C-mode OCT imaging with a compact, frequency swept laser source at 1300 nm,” *Opt. Express* **13**, 10523 (2005).
- [39] M. R. Hee, “Optical Coherence Tomography: Theory,” in *Handbook of Optical Coherence Tomography*, B. E. Bouma and G. J. Tearney, eds. (Marcel Dekker, 2002), pp. 41-48.
- [40] M. W. Lindner, P. Andretzky, F. Kieseewetter and G. Häusler, “Spectral Radar: Optical Coherence Tomography in the Fourier Domain,” in *Handbook of Optical Coherence Tomography*, B. E. Bouma and G. J. Tearney, eds. (Marcel Dekker, 2002), pp. 335-357.
- [41] A. C. Akcay, J. P. Rolland and J. M. Eichenholz, “Spectral shaping to improve the point spread function in optical coherence tomography,” *Opt. Lett.* **28**, 1921 (2003).

- [42] J. Na, J. Kim, E. Choi, S. Y. Ryu, J. H. Baek and B. H. Lee, "Spectral filtering in OCT system implemented by mechanically induced long-period fiber grating," *Opt. Quantum Electron.* **37**, 1251 (2005).
- [43] R. Tripathi, N. Nassif, J. S. Nelson, B. H. Park and J. F. de Boer, "Spectral shaping for non-Gaussian source spectra in optical coherence tomography," *Opt. Lett.* **27**, 406 (2002).
- [44] J. Gong, B. Liu, Y. L. Kim, Y. Liu, X. Li and V. Backman, "Optimal spectral reshaping for resolution improvement in optical coherence tomography," *Opt. Express* **14**, 5909 (2006).
- [45] P. W. Milonni and J. H. Eberly, *Lasers* (John Wiley & Sons, 1988).
- [46] Melles Griot Inc., *Melles Griot, The Practical Application of Light*, (1999).
- [47] F. I. Feldchtein, V. M. Gelikonov and G. V. Gelikonov, "Design of OCT Scanners," in *Handbook of Optical Coherence Tomography*, B. E. Bouma and G. J. Tearney, eds. (Marcel Dekker, 2002), pp. 125-126.
- [48] S. J. Hewlett and T. Wilson, "Resolution Enhancement in Three-Dimensional Confocal Microscopy," *Mach. Vis. Appl.* **4**, 233 (1991).
- [49] C. Xu, C. Vinegoni, T. S. Ralston, W. Luo, W. Tan and S. A. Boppart, "Spectroscopic spectral-domain optical coherence microscopy," *Opt. Lett.* **31**, 1079 (2006).
- [50] S-W. Huang, A. D. Aguirre, R. A. Huber, D. C. Adler and J. G. Fujimoto, "Swept source optical coherence microscopy using a Fourier domain mode-locked laser," *Opt. Express* **15**, 6210 (2007).

- [51] M. Gu, J. R. Sheppard and X. Gan, “Image formation in a fiber-optical confocal scanning microscope,” *J. Opt. Soc. Am. A* **8**, 1755 (1991).
- [52] I. Hartl, X. D. Li, C. Chudoba, R. K. Ghanta, T. H. Ko and J. G. Fujimoto, “Ultrahigh-resolution optical coherence tomography using continuum generation in an air-silica microstructure optical fiber,” *Opt. Lett.* **26**, 608 (2001).
- [53] R. A. Leitgeb, W. Drexler, A. Unterhuber, B. Hermann, T. Bajraszewski, T. Le, A. Stingl and A. F. Fercher, “Ultrahigh resolution Fourier domain optical coherence tomography,” *Opt. Express* **12**, 2156 (2004).
- [54] J. M. Schmitt, S. H. Xiang and K. M. Yung, “Speckle Reduction Techniques,” in *Handbook of Optical Coherence Tomography*, B. E. Bouma and G. J. Tearney, eds. (Marcel Dekker, 2002), pp. 175-201.
- [55] A. F. Fercher, W. Drexler, C. K. Hitzenberger and T. Lasser, “Optical coherence tomography—principles and applications,” *Rep. Prog. Phys.* **66**, 239 (2003).
- [56] J. M. Schmitt, “Optical Coherence Tomography (OCT): A Review,” *IEEE J. Select. Top. Quantum Electron.* **5**, 1205 (1999).
- [57] W. Y. Oh, B. E. Bouma, N. Iftimia, S. H. Yun, R. Yelin and G. J. Tearney, “Ultrahigh-resolution full field optical coherence microscopy using InGaAs camera,” *Opt. Express* **14**, 726 (2006).
- [58] B. Považay, B. Hermann, A. Unterhuber, B. Hofer, H. Sattmann and F. Zeiler, J. E. Morgan, C. Falkner-Radler, C. Glittenberg, S. Blinder and W. Drexler, “Three-dimensional optical coherence tomography at 1050 nm versus 800 nm in

- retinal pathologies: enhanced performance and choroidal penetration in cataract patients,” J. Biomed. Opt. **14**, 041211 (2007).
- [59] Q. Lu, X. Gan, M. Gu and Q. Luo, “Monte Carlo modeling of optical coherence tomography imaging through turbid media,” Appl. Opt. **43**, 1628 (2004).
- [60] H-W. Wang, J. A. Izatt and M. D. Kulkarni, “Optical Coherence Microscopy,” in *Handbook of Optical Coherence Tomography*, B. E. Bouma and G. J. Tearney, eds. (Marcel Dekker, 2002), pp. 275-298.
- [61] M. Wojtkowski, V. J. Srinivasan, T. H. Ko, J. G. Fujimoto, A. Kowalczyk and J. S. Duker, “Ultrahigh-resolution, high-speed, Fourier domain optical coherence tomography and methods for dispersion compensation,” Opt. Express **12**, 2404 (2004).
- [62] A. H. Bachmann, R. A. Leitgeb and T. Lasser, “Heterodyne Fourier domain optical coherence tomography for full range probing with high axial resolution,” Opt. Express **14**, 1487 (2006).
- [63] S. H. Yun, G. J. Tearney, B. E. Bouma, B. H. Park and J. F. de Boer, “High-speed spectral-domain optical coherence tomography at 1.3 μm wavelength,” Opt. Express **11**, 3598 (2003).
- [64] M. Wojtkowski, R. Leitgeb, A. Kowalczyk, T. Bajraszewski and A. F. Fercher, “*In vivo* human retinal imaging by Fourier domain optical coherence tomography,” J. Biomed. Opt. **7**, 457 (2002).

- [65] R. A. Leitgeb, C. K. Hitzenberger, A. F. Fercher and T. Bajraszewski, "Phase-shifting algorithm to achieve high-speed long-depth-range probing by frequency-domain optical coherence tomography," *Opt. Lett.* **28**, 2201 (2003).
- [66] E. Götzinger, M. Pircher, R. A. Leitgeb and C. K. Hitzenberger, "High speed full range complex spectral domain optical coherence tomography," *Opt. Express* **13**, 583 (2005).
- [67] M. V. Sarunic, M. A. Choma, C. Yang and J. A. Izatt, "Instantaneous complex conjugate resolved spectral domain and swept-source OCT using 3x3 fiber couplers," *Opt. Express* **13**, 957 (2005).
- [68] R. K. Wang, "*In vivo* full range complex Fourier domain optical coherence tomography," *Appl. Phys. Lett.* **90**, 054103 (2007).
- [69] B. Baumann, M. Pircher, E. Götzinger and C. K. Hitzenberger, "Full range complex spectral domain optical coherence tomography without additional phase shifters," *Opt. Express* **15**, 13375 (2007).
- [70] R. Trebino and J. Buck, "Nonlinear Optics," in *Frequency-Resolved Optical Gating: The Measurement of Ultrashort Laser Pulses*, R. Trebino, ed. (Kluwer Academic, 2000), pp. 37-60.
- [71] R. W. Boyd, *Nonlinear Optics*, 2nd ed. (Academic, 2003).
- [72] R. L. Fork, O. E. Martinez and J. P. Gordon, "Negative dispersion using pairs of prisms," *Opt. Lett.* **9**, 150 (1984).
- [73] J. P. Gordon and R. L. Fork, "Optical resonator with negative dispersion," *Opt. Lett.* **9**, 153 (1984).

- [74] Corning Inc., “Corning SMF-28e Optical Fiber Product Information,” www.vinkarola.com/pdf/Corning%20SMF-28e%20Fiber.pdf (accessed November 16, 2007).
- [75] M. Ohmi and M. Haruna, “Ultra-High Resolution Optical Coherence Tomography (OCT) Using a Halogen Lamp as the Light Source,” *Opt. Rev.* **10**, 478 (2003).
- [76] I. Walmsley, L. Waxer and C. Dorrer, “The role of dispersion in ultrafast optics,” *Rev. Scien. Instr.* **72**, 1 (2001).
- [77] V. G. Dmitriev, G. G. Gurzadyan and D. N. Nikogosyan, *Handbook of Nonlinear Optical Crystals*, (Springer-Verlag 1991).
- [78] D. N. Nikogosyan, *Nonlinear Optical Crystals: A Complete Survey*, (Springer Science+Business Media, 2005).
- [79] Sandia National Laboratories, “SNLO Software,” <http://www.sandia.gov/imrl/XWEB1128/snloftp.htm> (accessed December 4, 2007).
- [80] S. Kress and A. Pham, Andor Technology Inc., 425 Sullivan Avenue, Suite #3, South Windsor, CT, USA, 06074 (personal communication, 2007).
- [81] Andor Technology Inc., *Scientific Digital Camera Solutions 2006 Catalog*, (2006).
- [82] B. Bouma, G. J. Tearney, S. A. Boppart and M. R. Hee, “High-resolution optical coherence tomographic imaging using a mode-locked Ti: Al₂O₃ laser source,” *Opt. Lett.* **20**, 1486 (1995).

- [83] Y. Watanabe, Y. Hayasaka, M. Sato and N. Tanno, “Full-field optical coherence tomography by achromatic phase shifting with a rotating polarizer,” *Appl. Opt.* **44**, 1387 (2005).
- [84] OFR Inc., Division of Thorlabs Inc., “OFR FiberPorts,” http://www.ofr.com/fo_paf_models.htm (accessed November 27, 2007).
- [85] P. Constantinou, T. Nicklee, D. W. Hedley, S. Damaskinos and B. C. Wilson, “A High-Resolution MACROscope with Differential Phase Contrast, Transmitted Light, Confocal Fluorescence, and Hyperspectral Capabilities for Large-Area Tissue Imaging,” *IEEE J. Select. Top. Quantum Electron.* **11**, 766 (2005).