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Master Thesis

Visible-Light Swept-Source Optical Coherence Tomography

by

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Abstract: This thesis shows an ultra-high resolution (UHR) swept-source optical coherence tomography (SS-OCT) system that uses the visible part of the electromagnetic spectrum. Light from a Fianium SC400-4 supercontinuum light source was used in the filter with a calculated instantaneous line width of 0.085nm. The filter was built with a galvanometer scanner running at 200Hz and grating of 1800 lines/mm, providing a full width half maximum (FWHM) optical bandwidth of 213nm centred at approximately 600nm. The axial resolution of the system was 1.3mm in air indicating a submicron resolution in tissue. The sensitivity of the system was 85dB with a sensitivity roll-off of 3.20dB/0.1mm and 0.9mm imaging depth. Measurements were obtained from various samples to demonstrate the system’s ability to measure with high axial resolution, and to perform spectroscopic UHR-SS-OCT measurements.

Populaire wetenschappelijke samenvatting (Nederlands):

Optical coherence tomography (OCT) is een medisch toegepaste imaging techniek waarmee doorsnede plaatjes kunnen worden gecreëerd van verschillende biologische weefsels. Zo word deze methode onder andere toegepast bij het in beeld brengen van huid, bloedvaten en ogen. OCT word al gebruikt in een clinische setting om te assisteren bij de diagnose van verschillende oog ziektes waaronder glaucoma, maculaire gaatjes en leeftijdsgewijzigde maculadegeneratie. Door het oppervlak te scannen kan zo een doorsnede worden geherconstueerd. De axiale resolutie van OCT wordt bepaald door de coherentie lengte van het licht. Deze coherentie lengte is inverse proportioneel aan de bandwijdte van het licht, en proportioneel aan de kwadraat van het centrale golflengte. In andere woorden, een lichtbron met een lagere golflengte en een hoge bandbreedte zorgt voor een hoge resolutie. Een veel voorkomende keuze voor lichtbron bij OCT ligt tegen het infrarood aan, rond 800 nm en 1150 nm, omdat er daar een dip zit in het absorptie spectrum van water. Aangezien er veel water aanwezig is in biologische weefsels, en zo ook het oog, is dit belangrijk anders word al het licht geabsorbeerd voordat het aankomt bij het netvlies. Echter is de bandbreedte rond deze golflengtes, door water absorptie, beperkt tot een maximum van ongeveer 100 nm, wat ervoor zorgt dat een maximale axiale resolutie van ongeveer 3 micron kan worden bereikt. Door te kiezen voor zichtbaar licht, wat een lage centrale golflengte (600 nm) heeft en een grote bandwijdde (van 450 tot 750 nm) kan een sub-micron axiale resolutie worden gerealiseerd. Het nadeel van zichtbaar ligt gebruiken om het netvlies te meten, is dat het oog zeer gevoelig is voor zichtbaar licht. Daarom kan er alleen een hele lage hoeveelheid licht worden gebruikt. Om ervoor te zorgen dat het signaal nog boven de ruis uit komt, is in dit onderzoek gekozen voor een zogenaamde swept source OCT oplossing, wat gebruikt maakt van een sweeping filter dat door het gehele spectrum scant in de tijd. Deze oplossing heeft een hogere sensitiviteit dan het alternatief, Fourier domain OCT, waar het spectrum in de ruimte word verspreid. Het tweede voordeel van zichtbaar licht, naast de hoge resolutie, is de mogelijkheid om door middel van spectrum analyse kleur informatie te verkrijgen van het netvlies. Dit kan interessante mogelijkheden bieden voor bijvoorbeeld kleuren visie onderzoek. In deze thesis wordt een VIS-SS-OCT oplossing besproken wat gemaakt is met als doel het systeem geschikt te maken voor het in beeld brengen van het menselijk netvlies. Het systeem is in staat een resolutie van 1.3 micron te bereiken in lucht, wat een sub-micron resolutie betekend in weefsels. Daarnaast worden verschillende kleur-analyse resultaten gepresenteerd en is het systeem gebruikt om biologische samples in beeld te brengen.
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1. Introduction

Optical coherence tomography (OCT) is an imaging technique used in medical science that makes use of light waves to create cross-sectional images of morphological structures. It is non-invasive, hence with OCT one can image biological samples in vivo. Common subjects such blood vessels [1], (skin) cancer [2], [3] and eyes [4], [5] are imaged using OCT. The technique is applied in clinical settings for the evaluation of the retina. It can be used to diagnose for several diseases such as macular holes, glaucoma and age related macular degeneration [6]. In addition, OCT can be used to image the central retina, retinal thickness in case of sub- and intra-macular edema and to monitor the retina-vitreous interface [7].

OCT is based on interferometry, which makes use of a collimated beam that is split by a beam splitter into a sample and reference arm. The reference arm contains a mirror and the sample arm the subject of research. In the case of retinal imaging, this would of course be an eye. As both mirror and sample reflect light back towards the beam splitter, the two beams are joined together and the electric fields will interfere. In the case of Fourier domain OCT (FD-OCT) a signal can be retrieved by Fourier transforming the interference pattern, producing one or more coherence peaks each of which correspond to a reflective layer in the sample. By scanning the surface of the sample a cross sectional image can be created. The axial resolution of OCT is determined by the coherence length of the light source, which in turn is proportional to the square of the bandwidth divided by the centre wavelength. Thus, low coherence light, and hence a high axial resolution, can be achieved by using a light source with either a large bandwidth or a low centre wavelength, or both.

For retinal imaging, the selection of this bandwidth is limited. The chamber of the eye is filled with a water-like substance called vitreous humour, which the light has to pass through in order to reach the retina. To image the human eye using OCT, it is thus essential to take the absorption spectrum of water into consideration. The use of near infra-red (NIR) is common, with centre wavelengths around 800nm or 1150nm, because there is a dip in the absorption spectrum of water. These, so called, optical windows are limited in size to about a maximum of a 100 nm. OCT systems that employ such a bandwidth generally have a resolution of about 3 μm. Although it is common in interferometry, visible light is not often used in OCT. But due to a low centre wavelength (around 600 nm) and a large optical window in the absorption spectrum of water, the visible spectrum allows for light with a very low coherence length. This in turn can create OCT images with a sub-micron axial resolution in tissue [8]. It can thus help image even the smallest of changes within the retina, for example the thinning of the retinal nerve fibre layer which can be an early sign of the onset of glaucoma, even before morphological changes to the optic nerve head [9]. An earlier detection of this thinning can allow for faster diagnosis, which in turn can help prevent damage and vision loss. Additionally, VIS-OCT allows for spectral analysis of the visible spectrum, which can create 3D colour images [8] and can provide depth-resolved colour information. This can offer new insights into colour vision by colour analysis of individual cones or could even be used to pinpoint the location of chromophores such as haemoglobin [10]. ANSI standards [11] limit the imaging power to only a few μW, for example around 20 μW for a maximum illumination time of 20 seconds, but even these levels can be uncomfortable during imaging. By using a swept source solution for a visible light system, the amount of power and total bandwidth can easily be controlled to reduce the risk of damage, and increase the comfort of subjects. In this thesis an OCT system is presented which combines swept source with visible light for the first time, with the goal of creating a system suitable for retinal imaging. First, a brief overview is given of OCT and it's variations. From this the choice for visible light and swept source OCT will be made clear. Second, will be the details on the development and explanation of the setup and intermediary results. Third, the spectral analysis will be explained and finally there will be a discussion and future work will be covered.
2. Optical Coherence Tomography

Optical coherence tomography works on a basis of interferometry. A source emits a beam that is split in two separate arms by a beam splitter, into a reference arm and a sample arm. The beams in both arms are reflected back by a mirror and sample (such as the retina) and the two beams come together again at the beam splitter. When the length of the reference arm ($L_r$) and the length of the sample arm ($L_s$) are the same, or when the arms are at zero optical path length difference (zero OPD) the light coming from both arms will interfere. By moving the reference arm, each reflective surface of the sample can be located, as interference will occur each time when $L_r = L_s$. This is called Time domain OCT (TD-OCT).

For Fourier domain OCT (FD-OCT), interference will show as fringes upon the spectrum of the light source. By doing a Fourier transform of these fringes, different coherence peaks will appear for each reflective layer. This way an entire depth-scan can be done at once. In the following sections each of these methods will be explained in more detail. Finally there will be an explanation of swept source OCT (SS-OCT).

2.1. Time domain OCT

OCT was invented by Fujimoto et al in 1991 [12], whose original schematic for an OCT scanner can be seen in Figure 1. They described a technique that was analogous to ultrasonic echolocation to produce cross sectional images of biological tissue in a non-invasive manner. The technique makes use of low-coherence interferometry, where a collimated beam coming from a light source, such as a super luminous diode (SLD), is split into two arms. The reference arm consists of a lens and a mirror which reflects the light directly back. The sample arm has the same setup but instead the light is focussed onto a sample. For retinal imaging, this would be an eye, and the beam would be focussed onto the retina by the eye lens itself, and sometimes in combination with a second lens to correct for defocus. The light returns from both arms and is combined, hereafter the light is detected by a photodiode. When the reference arm and sample arm are of the same length, when $L_s = L_r$, interference will occur. The accuracy of this measurement depends on the coherence light of the source ($\lambda_c$), and at the same time this is the resolution of the eventual image. The light sent into the sample will scatter and reflect and some of it will return from the sample. When two layers of different composition have a difference in refractive index $n$, there will be more reflection coming back from that position. By moving the reference arm, the different reflective surfaces of the retina can be detected. By measuring the distance over which the reference arm moved compared to the starting position, and relating it to each reflection, a cross sectional image can be recreated. One in-depth scan is called an A-scan. By making several A-scans, a slice of the sample is imaged called a B-scan. One depth within a volume of B-scans is called a C-scan.

Time domain OCT uses the relative distance of the reference arm to reconstruct the cross sectional image of the sample. The process however requires the arm to be moved for each depth-scan, and the method
is now mostly outdated. In the next section, Fourier domain OCT will be discussed, which acquires data over the entire A-scan at once and provided a faster alternative to TD-OCT.

2.2. Fourier domain OCT

It had been theorized before [13], but in 2002 Wojtkowski et al [14] experimentally showed a new way of doing OCT called Fourier domain OCT (FD-OCT) or spectral domain OCT (SD-OCT). With FD-OCT, the reference arm is kept stationary, and the light that is coming out of the interferometer is sent towards a spectrometer. The spectrometer separates the light in space using a grating, and with a lens, this spectrum is focused onto a CCD line scan camera. The camera will detect the spectrum sent out by the source. When interference occurs, an interference pattern will appear on top of the spectrum. By doing a Fourier transform of this pattern, different peaks will appear which represent the reflective surfaces in the sample. By determining the conversion of physical distance to pixel, the image can be reconstructed. Usually this is done by placing a mirror in the sample arm and moving that mirror a set distance between each measurement. The resulting A-scans then represent the different positions of the mirror, which can be related to the physical distance that the mirror moved. The full width half maximum (FWHM) of the amplitude will represent the coherence length of the source, and thus also the resolution of the system in the axial direction.

When the signal is detected by the CCD camera, in reality it is adding smaller individual measurements together and so creating an output that is as approximation of the actual interference pattern. According to the Nyquist theorem, this approximation is sufficient only if the rate at which the signal changes from one distance to another does not exceed the rate at which a new measurement is taken. More exactly in the case of the CCD camera, the frequency of the fringes on the signal cannot exceed the frequency of the pixels on the camera. Even more simply put, if more than one fringe start to fall onto the same pixel, the signal cannot be correctly approximated and the signal will start to disappear. When imaging takes place deeper into the sample, i.e. as the distance of the coherence peak to the zero delay line increases, the frequency of the signal increases. Therefore, the signal cannot be correctly approximated using the CCD camera. In the end its ability to do so is limited by the size of each individual pixel $\delta x$, and the optical resolution of the spectrometer. The optical resolution is an indication of the spectrometers ability to resolve the individual wavelengths of the total bandwidth. Of course, the total spectrum still has to fit on the total size of the camera. Hence, the optical resolution is a combination of choice of grating, focusing lens and CCD camera. If the optical resolution is low, for example by a bad choice in grating, more wavelengths will fall onto a single pixel. Consequently, this will also make the approximation of

Figure 2 a) A recorded signal from a SD OCT system. b) The Fourier transform of the spectrum. From [31].

![Figure 2](image-url)
the signal worse. As the approximation becomes harder, the peak amplitude decreases. This phenomenon is called sensitivity roll off with depth and can be expressed in the following equation:

$$R(z) = \frac{\sin^2(\pi z)}{2Z_{\text{max}}} \exp\left(-\frac{\pi^2 \omega^2}{8 \ln 2} \left(\frac{z}{Z_{\text{max}}}\right)^2\right),$$

where $Z_{\text{max}}$ is the maximum probing depth [15] and $\omega$ the amplitude spectrum of the light source. The first term of this equation is what describes the roll-off as a result from the pixel size. The second term describes the effect from the optical resolution, of which $\omega$ is a direct measure.

In SD-OCT, the image is constructed by Fourier transformation of the interference pattern. The method is widely applied since its experimental demonstration and provided a faster way of obtaining OCT images. The imaging depth and fringe washout are limited by the pixel size of the CCD camera. As these cameras improve and the optics, so will the roll-off, but currently these pixels are limited to about 10 microns in size. The last method that will be discussed, swept source OCT, does not make use of a CDD camera but instead separates the wavelengths in time, and also records the signal in time. The equivalent of the optical resolution in SS-OCT is the instantaneous linewidth, which is the packet of wavelengths ($\delta \lambda$) that arrive at the detector at one instance in time. This instantaneous linewidth, and also the rise time of a photodetector (which could be considered the equivalent of the CCD camera pixel size) are smaller and higher, resulting in less roll-off with depth for otherwise equal systems. Thus, SS-OCT has a sensitivity advantage over SD-OCT. This will be discussed in more detail in the next section.

2.3. Swept Source OCT

Where the signal obtained in SD-OCT is in space Fourier domain, in swept source OCT (SS-OCT) the signal is obtained in time Fourier domain. In this case, the light is separated in time before it goes into the interferometer by use of a filter which sweeps through the entire bandwidth $\Delta \lambda$ with a smaller instantaneous bandwidth $\delta \lambda$. Different filters are available for SS-OCT, such as Fabry-Perot filters [16] although most of these are limited to the 1300 nm range. The introduction of polygon-mirror based swept source filters, opened up the possibility for other wavelength ranges to be used [8, 10]. These filters can be used with a telescope design or without [18] but the principle is the same. The design of the wavelength sweeping filter used in this research is based on a telescopeless polygon-scanning filter.
However, instead of a polygon mirror a galvanometer is used. In the next section mathematical framework will be given for SS-OCT using such a wavelength sweeping filter.

Figure 3 shows such a polygon scanner based filter presented by Lim et al [19]. The light comes into the filter and is split by a beam splitter, after which it hits a grating. The grating disperses the light under angles which can be calculated using the grating equation:

$$\lambda = p(\sin \alpha + \sin \beta)$$  \hspace{1cm} (2)

Here, $\alpha$ is the incident angle on the grating and $\beta$ is the reflective angle and $p$ is the grating pitch. The range of $\beta$ can be calculated with

$$\Delta \beta = \sin^{-1}\left(\frac{\Delta \lambda}{p} - \sin \alpha\right),$$  \hspace{1cm} (3)

which needs to match the acceptance angle of the first lens. For ultra-broadband sources however, chromatic aberrations and dispersion affect system performance. Thus, for this system a telescopeless design was used instead. In that case, the light first hits the polygon scanner, after which it is dispersed by the grating. The Littrow angle is the angle under which both the incident and returning angle are the same, or in other words when $\alpha = \beta$ meaning that equation (2) becomes:

$$\lambda = p(2 \sin \alpha).$$  \hspace{1cm} (4)

By placing the polygon mirror and grating in the Littrow configuration, it is ensured that a part of the spectrum ($\delta \lambda$) is sent back through the same path at all times. By rotating the mirror, the entire bandwidth is swept through. As the mirror turns and reaches a new facet, the sweep begins again.

The signal of a swept source OCT system can be written as

$$i_{\text{swept}} \propto \frac{\eta e}{E_v} S(1 + \alpha + 2\sqrt{\alpha} \cos([k_0 + K(t - t_0)]\Delta L)),$$  \hspace{1cm} (5)

where $t_0 = (L_r + L_s)/c$, $\eta$ is the detector quantum efficiency, $e$ the electron charge, $E_v$ the photon energy of the centre wavelength, $k_0 = 2\pi/\lambda_0$, $S(k)$ is the amplitude of the light source, $\alpha$ is the incident angle and $\Delta L = 2(L_s + L_r)$ [20]. This equation is very similar to that for SD-OCT, but this time containing the linear sweep through the bandwidth as $k(t) = k_0 + Kt$. The maximum imaging depth can be calculated with:

$$Z_{\text{max}} = \frac{\lambda_0^2}{4\delta \lambda}.$$  \hspace{1cm} (6)

This is the same for SD-OCT, where in that case $\delta \lambda$ is the wavelength spacing between pixels on the CDD camera. In the case of SS-OCT, there is no CCD camera and thus no wavelength spacing over the pixels. Instead, a photon detector is used and the spacing is in time. The rise time of a photodetector is the time it takes for a signal to change from a certain low value to a certain high value, and can also be seen as the detectors ability to measure fast change in the intensity of the interference pattern. When comparing to SD-OCT, where the separation was done in space, this can be seen as the individual pixel size of the camera. The optical resolution is a measure of the spectrometers ability to correctly resolve the total bandwidth. Simply stated, this means the better the optical resolution, the better the separation of individual wavelengths. In SS-OCT, this can be related to the instantaneous bandwidth of the swept
source filter. In general, due to high rise times of photodetectors and small instantaneous bandwidth sizes coming from a swept source filter, the roll-off with depth for SS-OCT is less than for SD-OCT.

Noise in an OCT system is built up of three different elements. First is background noise which comes from the detector or other sources and is a constant value, second Bose-Einstein noise and last is shot noise. Shot noise, also known as photon noise, is a direct result of the individual measurements of photons. When the amount of photons increase, so will the shot noise in a linear fashion. An OCT system is said to be shot noise limited when only the shot noise still has an influence on the total measured noise, and the other noise contributions are small enough in comparison to be ignored. To test this the total noise can be measured as a function of power. If the noise increases linearly with the power, the system is shot noise limited. For a shot noise limited SS-OCT system, the signal to noise ration can be described as:

\[
\frac{S}{N} = \frac{\eta P_s \tau}{E_v},
\]

where \(\eta\) is the quantum efficiency of the detector, \(P_s\) the sample arm power, \(\tau\) the time it takes to sweep through the entire spectrum once, and \(E_v\) the photon energy. As mentioned, in this research a galvanometer mirror is used for sweeping, instead of a polygon mirror. The advantage of this is the ability to slow down the imaging speed and thus increasing \(\tau\). For a system using visible light, power must be kept low, and thus increasing \(\tau\) will help to increase an otherwise low SNR. More on this will be covered in section 3.

The axial and lateral resolution in OCT is decoupled, which means that both are independent. The lateral resolution is determined by the optics of the system. When a beam is focussed in a plain by a lens, a circular interference pattern will appear. The best possible spot a perfect lens can create is described by the Airy disk, which is the bright maximum in the middle of this pattern. The lateral resolution can be described as the system’s ability to resolve two individual airy disks. This can be described, for the limited diffraction case, with the following equation:

\[
\Delta x = 1.22 \lambda \frac{f}{D},
\]

where \(\Delta x\) is the separation of the two airy disks, or the lateral resolution, \(f\) is the focal length of the lens, \(\lambda\) is the wavelength and \(D\) the beam size of the incident beam. Generally, by increasing the beam size the lateral resolution can be improved. However, the pupil has a size of about 2 mm in width in undilated situations, and can increase to 8 mm in dilated conditions. A dilated pupil introduces a significant amount of wave front aberrations which lowers lateral resolution. Thus, most OCT systems make use of an incident beam with a width of about 1 – 2 mm. There is a way to compensate for aberrations such that a larger beam size can be used by applying adaptive optics [14,15], which can increase the lateral resolution.

The axial resolution, which is in depth, is determined by the coherence length of the light source.

\[
l_c = \frac{2ln2}{\pi} \frac{\lambda_0^2}{n \Delta \lambda},
\]

in which \(\lambda_0\) is the centre wavelength, \(\Delta \lambda\) is the bandwidth at FWMH and \(n\) the refractive index of the
medium. From equation (9) it is clear that a lower centre wavelength and a higher bandwidth produce a smaller coherence length and thus a higher resolution.

In this section a general background has been given on the different types of OCT, with special attention to a SS-OCT system that uses a polygon mirror based bandwidth filter. It was shown that SS-OCT has an advantage compared with SD-OCT when it comes to sensitivity roll off with depth and penetration depth. In this project, only low power is available to image the eye (a maximum of 7 μW was used) and so this advantage is a necessity. Furthermore, because the polygon mirror was replaced by a galvanometer, there is more flexibility in bandwidth selection and in imaging speed. This selection in bandwidth is mostly important to ensure the safety of subjects during the testing phases of the scanner. By limiting the bandwidth to only the red part of the spectrum, photochemical damage could be prevented when testing the system on a human eye, as this only occurs in lower wavelengths (< 600nm).

Although lower imaging speeds (τ) can be a disadvantage when it comes to motion, it can also be an advantage in the case of low power as it will improve the SNR.

3. Light sources

The body, and also the eye, consists of ~75% water. With biological imaging, and naturally also with retinal imaging, the choice of light source is therefore limited by the absorption spectrum of water and haemoglobin in blood. Around 800 nm, there is a so called ‘optical window’ in which there is very little absorption by those substances. In Figure 4 this optical window is displayed. A smaller region of low absorption can be found around 1150 nm, which can still be seen on the right side of Figure 4.

In order to comply to the ANSI standards for retinal imaging, either the imaging power has to be kept very low or the illumination time very short [11]. For a bandwidth of 300 nm, ranging from 450 – 750 nm, the retina may only be illuminated for 20 seconds when a power of 20 μW is used. By keeping the power far below that (around 7 μW) the integration time can be increased, thereby improving the SNR.

![Figure 4: The optical window of biological tissue, showing the absorption spectra of water (H2O) and haemoglobin. In this 'optical window' there is very low absorption and biological tissue is virtually transparent [32].](image-url)
4. System description

![Diagram of the first setup using a 50:50 beam splitter to send the light towards a 600 l/mm transmissive grating. The galvo and silver coated mirror are in the Littrow configuration, allowing one part of the bandwidth, the size of the instantaneous bandwidth to be sent back towards the beam splitter. As the galvo rotates (at 200 Hz) the total bandwidth is swept through. The two silver coated mirrors are used for alignment and send the beam into the interferometer which consists of a reference and sample arm using an f=100 mm and an f=75 mm air spaced achromatic lens for each arm respectively.]

A schematic of the first setup is shown in Figure 5. The source is the Fianium WhiteLase SC400-2 with an average output of 2mW/nm. The filter consisted of a 600 lines/mm grating at a blaze wavelength of 580 nm. A 50:50 (R:T) beam splitter send a beam with 50% of the original power towards the grating where the light is dispersed. A silver coated galvanometer mirror was placed behind it. This reflected the dispersed light towards a regular silver mirror, which in turn sends the beam back towards the galvanometer and thus returning the beam towards the beam splitter. Again, with 50% of its previous power, the beam is split off and with use of two silver coated mirrors the beam is sent towards a collimator. Due to a Littrow configuration, as the galvanometer rotates, different parts of the dispersed spectrum are sent directly back along the path of the incident beam. This instantaneous bandwidth is sweeping through the entire spectrum at 200 Hz, creating a swept source filter. With use of endlessly single mode fibres (LMA-5, NKT Photonics) the light is sent into an interferometer. The interferometer is purposely kept free space in order to prevent chromatic aberrations, likely to occur with such a broad spectrum (300 nm). With use of the equation [18]:

\[
\partial \lambda = \frac{\lambda_0 \cos(\alpha)}{\pi W} p,
\]

we can calculate the instantaneous bandwidth. Here we have the centre wavelength \(\lambda_0 = 600\ nm\), the incident angle on the grating, which is also the Littrow angle of that wavelength, \(\alpha = 10.37^\circ\), the grating pitch \(p = 600\) lines/mm, the diffraction order number \(m = 1\) and \(W\), the \(1/e^2\) width of the Gaussian beam, is 2.5 mm. This results in an instantaneous bandwidth of 0.32 nm. Using this result the imaging depth can be calculated as:

\[
Z_{max} = \frac{\lambda^2}{4\delta \lambda}\]

(11)
which was determined to be $0.3 \, mm$. The FWHM of the system at this stage was determined to be $100 \, nm$, which would give a theoretical coherence lengths of $l_c = 2.65 \, nm$.

Preliminary results of this first system are shown in Figure 6 which displays an A-scan of a piece of paper. The speckle pattern is clearly visible, but seems to be dispersed. It is clear that further measures needed to be taken in order to correctly map the data to the proper wavelengths, and to compensate for the dispersion. Moreover, the system displayed large amounts of chromatic aberrations at the collimators, even with the taken precautions.

![Figure 6: OCT image of a piece of cardboard. The image is obtained by averaging over 20 B-scans taken at the same position. There was no other post-processing done on the image, and is thus in arbitrary units. The stretching in the image indicates that there was still dispersion present in the system despite efforts to prevent this. Most of this was later compensated in post-processing.](image)

The second stage of the system is shown in Figure 7, containing a few major changes to the design. First, the transmission grating was replaced by a reflective grating of $1800 \, l/mm$, in order to improve the instantaneous bandwidth and in turn the imaging depth. Second, a 90:10 (R:T) beam splitter was used for balanced detection. In the sample arm, two galvanometer scanners- were implemented to allow for surface scanning, still using an f=75mm focussing lens.

![Figure 7: The second setup had replaced the transmissive grating with an 1800 l/mm reflective grating. All collimators were replaced with custom built ones using f=10mm achromatic doublets. A 90:10 (R:T) beam splitter was used for balanced detection. In the sample arm, two galvanometer scanners- were implemented to allow for surface scanning, still using an f=75mm focussing lens.](image)
sample arm allowing the capturing of volume-scans. The sample arm has an x- and y-galvanometer scanner and an \( f = 75\, mm \) air-spaced achromatic focusing lens (Thorlabs).

Results generated with this second setup were mapped to and corrected for dispersion in post-processing, which will be discussed in a later chapter. Despite the extra measures, the sample arm still gave a lot of chromatic aberrations, resulting in a shape change of the spectrum when returning to the detection arm. In Figure 9d shows a comparison of the spectrum that went into the sample arm and that which came back out when using a sample mirror. Due to the loss that occurred in the 600-750 nm region of the spectrum, the balanced detection was insufficient and had to be taken out.

The final stage of the setup is shown in Figure 8. The filter had one major change to increase the overall power coming out of the filter namely, the use of a galvanometer scanner (as opposed to a polygon scanner), which allowed for the freedom to change the angle not only in the x-y-plane, but also in the z-plane. By doing so, and also placing the grating in the same angle, we could create an offset beam that

Figure 8: The final system design. The laser sends the beam towards a galvanometer scanner operating at 100 Hz, which send the light towards an 1800 l/mm grating. The dispersed light is send back to the galvanometer mirror. Both grating and galvanometer are placed under a slight angle in the z-plane, thus creating a return beam that is offset from the incident beam. A small mirror placed underneath the incoming beam reflects the return beam to two silver coated mirrors that were used for alignment. All collimators are custom made to accommodate the broadband light source using \( f=10 \) mm achromatic doublets. Furthermore, endlessly single mode fibres were used of a short length (5 mm) to prevent dispersion. The sample arm was changed to a reflective sample arm to decrease the beam size to about 1 mm, suitable for retinal imaging. The glass slide that was used for mapping (see section 5) had another purpose of being used for balanced detection. This not only increased the SNR but had the added benefit of improving the glass slide signal for better calibration.

returned from the grating. A small mirror was placed underneath the incoming beam to reflect the beam towards the two silver mirrors used for alignment. This allowed for a 70% increase of power at the first collimator. The sample arm was replaced by a reflective sample arm. Doing so prevented the achromatic aberrations that were apparent with the previous configuration. Also this meant we could again implement the balanced detection. This time, instead of a beam splitter a glass slide was used to reflect the light towards another mirror, which reflected it into the balanced detector. The glass slide served
another function which was for the post-processing mapping. How this was done and why will be discussed in section 5. The reference arm consists of a collimator, a focusing lens of $f = 50\text{mm}$ and a mirror. The reflected light returns towards the beam splitter for interference and is detected by a New Focus 2107-FC-M balanced detector.

The sensitivity roll-off of the system was determined by measuring the height of the point spread function of a mirror in the sample arm. From this the roll-off was determined to be $3.2\text{ dB} \text{ per 0.1 mm}$, which corresponds to the theoretical value of $3.22\text{ dB} \text{ per 0.1 mm}$. The result can be seen in Figure 9b). The sensitivity was measured by attenuating the signal by $40\text{ dB}$ and again measuring the peak height compared to the noise floor. The noise floor is defined as the standard deviation of the Fourier transform when blocking the signal from the sample arm. From this a sensitivity of $85\text{ dB}$ was measured. The theoretical value was about $93\text{ dB}$, which indicates a loss of about $8\text{ dB}$. The loss is attributed to imperfect polarization state matching, which due to the shortness of the fibres was not corrected for, source noise and optical inefficiencies in the system. The total imaging depth can also be seen from Figure 9b) as being $0.9\text{ mm}$, which corresponds to the theoretical value of $1\text{ mm}$. Matching to the measured imaging depth is an instantaneous bandwidth of $0.1\text{ nm}$. This is slightly larger than the

Figure 9: a) The SNR of the system using 40dB attenuation. b) The sensitivity roll-off with depth measurements and a simple second degree polynomial fit to show the average roll-off. The roll off is $3.2\text{ dB} \text{ per 0.1 mm}$ with a maximum imaging depth of $0.9 \text{ mm}$. c) Measurement of reference arm noise of power increase showing a linear trend, indicating a shot noise limited system. d) The spectrum returning from the sample arm (black) together with the spectrum that went into the sample arm (grey) showing a loss of FWHM in the region of 600-750 nm. This might be a cause of the lower axial resolution that was found.
theoretical value of 0.085 nm, which we attribute to beam clipping in the filter. Another cause is the low power output, causing the signal to drop into the noise floor at distances close to the theoretical $Z_{\max}$. Because the sensitivity was on the lower side, it was made sure that the system was shot noise limited, by measuring the noise level of the reference arm with increase of power. From Figure 9c) it can be seen that this becomes linear so we were dealing with a shot noise limited system.

The coherence length, or the width of the point spread function coming from a mirror measurement, is 1.3 μm. This is smaller than the theoretical value of 0.9 μm. The cause of this is the loss of FWHM in the spectrum returning from the sample arm (Figure 9d), which may be due to chromatic aberrations.

5. Data acquisition and processing

The processing of the data was done in two steps: Mapping and dispersion compensation. In this section, the manner of acquisition will be explained first, following by the process of mapping the data to the correct wavelengths and correcting for dispersion in the system. For both these processes an algorithm was written in MatLab.

![Figure 10: a) Spectrum of the glass slide before (blue) and after mapping (red) showing the shift in the phase that occurs when correcting. b) Fourier transform of the spectrum before (blue) and after mapping (red). The sharpness of the peak and its symmetry indicates that the spectrum is correctly mapped and evenly spaced in $k$-space.](image)

The signal is detected by a New Focus 2107-FC-M balanced detector and acquired at a sampling rate of 2 MS/s and each acquired spectrum consists of 4992 samples. Because a galvanometer scanner was used in the filter, two spectra will arrive at the detector, one sweeping from blue to red, and visa versa. It was attempted to combine these two spectra in post processing, by inverting one of the spectra so it would match the first in sweep sequence. However, due to small fluctuations in the spectrum they could not be matched exactly and it only further complicated the process. Thus, only one of the sweeps (from blue to red) was recorded and used for capture.

The data was mapped to the correct wavelengths using an auto calibration method given by Mujat et al [23]. The authors make use of a known modulation on the spectrum to create a mapping $k$-vector. By placing a glass cover slide directly in the beam, the light passing through the slide will interfere with the light reflected twice before exiting. This results in an interference pattern appearing on the spectrum with a stable frequency. The Fourier transform of this spectrum has a peak at the same position throughout capture. The pattern should be a perfect sinusoid as a function of $k$. In general the signal
detected is not perfectly evenly spaced in $k$, and therefore results in a broadening of the peak. The method alters the wavelength assignment in such a way that the resulting spectrum is evenly spaced in k-space. As the slide will create the same peak in each a-scan, the method can be used for all individual data acquisitions, and can be easily subtracted as background noise for the final result. Equally easy is isolating the peak from each measurement, so only the modulation remains by averaging over multiple a-scans. Only the background signal remains, and the modulation peak can be isolated from other background signals such as the DC peak. Figure 10 left shows the modulation created by the glass slide on our data created by averaging over 8000 a-scans. The spectrum is then manually assigned to an estimated k-array, which was calculated using the grating equation, by interpolation. For a perfect sinusoid the phase should be linear with $k$. This is used to calibrate the spectrum. By calculating the phase, the assigned $k$-array can be altered using:

$$k_{\text{new}} = k + \frac{\varphi(k)}{z_{\text{peak}}}$$

$$z_{\text{peak}} = 2\pi \frac{i_{\text{peak}}}{(k_{\text{max}} - k_{\text{min}})}$$

In which $i_{\text{peak}}$ is the index of the peak created by the glass slide in index space, the space in which the raw data is represented in a vector of 4992 samples, and $\varphi(k)$ is the phase. The phase is simply retrieved unwrapping the angle of the data using built in MatLab functions. The values of $k_{\text{max}}$ and $k_{\text{min}}$ are retrieved from the new k-array. The new array is again used to interpolate the spectrum, which results in a (more) linear phase. Estimations, noise and low signal at the wings of the spectrum can influence the end result, which is why it usually takes a few iterations to get a correctly mapped spectrum. When the spectrum is calibrated, the phase will be linear, and the resulting coherence peak will show the coherence length of the source. The final k-array can then be used to interpolate the captured data which is cleaned by removal of the calibration and DC peak. Using equations (12) and (13), the values of $k_{\text{max}}$ and $k_{\text{min}}$ will also fluctuate and not stay fixed on the values put into the system based on the grating equation. Through testing it became clear that the initial input did change the output of the mapping process slightly. Unfortunately, further study of the method did not reveal much insight into where this came from. However, these small fluctuations are corrected for during dispersion compensation and so had little to no effect on the end result images. It was thus that, mostly due to time constraints, it was not pursued further.

The glass slide was not only used for mapping, but the reflection of from the surface was also used for balanced detection. This had two advantages. One being that extra loss was prevented because it eliminated the use of another beam splitter to create a balanced detection arm. Second, by using this beam for balanced detection, the glass slide signal was increased which provided a better basis for the calibration method.
Second part of the data processing is of the removal of dispersion artefacts in the spectrum. Dispersion is caused by a difference of refractive index $n$ between different wavelengths, thus resulting in each wavelength taking a slightly different path through the optics. This difference is seen in the data as a wavelength-dependent phase shift and therefore a broadening of the coherence peak. For data of this system, an algorithm was used which tries to find this phase by iteration and beforehand estimated values. When the spectrum is then multiplied with this created phase, and the phase is correct, they will cancel out and the broadening disappears. It is also possible to calculate the actual phase, but for that a sharp coherence peak is needed, i.e. from a mirror. But since there can be slight difference in dispersion between different data sets, due to pressure, heat or moisture, and because the used bandwidth is so large, this did not result in the most optimal compensation. The algorithm creates a polynomial phase function of the form $a_0 + a_1 x + a_2 x^2 + a_3 x^3$, where the values of $a_0$ and $a_1$ are set to 0 (they represent a linear shift and can be ignored). $a_2$ and $a_3$ are estimated beforehand, and the program searches over a small range for the most optimal values for a particular a-scan in a dataset.

Although the methods for mapping and dispersion compensation both work with the phase, they are not the same and cannot be combined into a single step. The mapping method is based on the a priori knowledge of what the glass slide modulation will look like. This is not known for the dispersion and must be retrieved from the data itself. If only method two were to be used for both adjustments, then there would be no basis for the mapping. Combining both steps into a single would result in correction that is only valid for a single dataset, corresponding to a single depth-scan. Applying this correction to the other sets would result in broadening of the coherence peaks.

The end result of the two steps is an accurate k-array and dispersion compensation phase, which can separately be applied to the rest of the a-scans in a rapid fashion. The LabView program shows a live feed of the capture at a frame rate of 0.4 Hz with 500 a-scans per B-scan. It utilizes the last k-array and

![Graph showing CIE colour space functions for a standard observer. The functions are multiplied with the obtained data to separate those parts representing blue, green and red. Mapping and dispersion compensation is done beforehand, only converting to OCT image is done separately. The three obtained (greyscale) images per B-scan are combined into three channels of an RGB image.](image)

Figure 11: CIE colour space functions for a standard observer.
phase that were determined by the algorithm, to optimize the feed without slowing down the program dramatically, as would happen with live compensation calculations. Resulting data sets were transformed into three dimensional reconstructions and en-face images using Amira.

The final operation that was done to some of the data in post processing, was spectral analysis. Since we are using white light, spectral analysis of the data allows for RGB imaging [8]. In order to obtain RGB images, the data needed to be separated into the three different colours, red, green and blue. This can be achieved by taking the corrected data, and multiplying it by a function representing each of the colours. For true colour representation, the CIE (International Commission on Illumination) wavelength to RGB colour standard functions [24] were used, displayed in Figure 11, whose numerical formulas are also known as the CIE standard observer. Each B-scan was thus represented in the red, green and blue. Each of these sets was then transformed into a B-scan image and set as the respective colour channel in the RGB image. The results are colour images of the different samples as discussed in section 6.2.

As each RGB image consists of three colour channels, and each channel consists of different parts of the complete spectrum, the bandwidth of each channel of the RGB image is approximately 3 times smaller than the total spectrum. This results in RGB images with a lower resolution (~3 micron) than the black and white images, which use the entire spectrum for analysis.

### 6. Results

The following section contains the images that were obtained with the system. Visible light has a very low penetration depth. Thus, most light gets absorbed and reflected on or near the surface of most objects. The goal of this project was to create setup for retinal imaging. Most layers of the retina are transparent, and due to the low absorption of water in the visible range, visible light will reach each layer. Unfortunately, finding a good phantom sample that correctly models the eye is not easy [25]. For that reason, the samples that are chosen are either of paper with different patterns (such as money or holographic seals), and other (semi-)transparent objects such as tape and plastic novelty glasses. There are roughly three categories of results presented below. The first set is of all non-biological samples that show the systems capabilities of creating cross-sectional images at high resolution. The second section is focussed on the colour analysis of different samples. These are also non-biological and are created from different combinations of tape, paper and marker. The last section will discuss some of the biological samples that were obtained.

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Figure 12: 3D image of a 5000 yen bill. An image of a character appears together with all the small groves on the paper. The ink of the character occasionally gives a strong reflection which appears to be higher than the rest of the paper, as would be expected of ink.
6.1. Cross sectional and three dimensional reconstructions of non-biological samples.

Figure 13: A pair of HoloSpex™ glasses which produce far field holograms when put on. The image was acquired with 400 by 400 A-scans and imaged a surface of 1.9 by 3.4 mm in size. The distance between the two layers was determined to be 80 microns, which would be the thickness of the first layer.

First images were created by using paper as a sample, mostly to use this as a reference for post-processing calibration. Still, there were some interesting samples such as a 5000 yen bill (Figure 12), from which a symbol was imaged as well as a holographic seal.

In Figure 13 a 3D-reconstruction of a pair of HoloSpex™ far-field holographic glasses is shown. The samples consisted of multiple (semi-) transparent layers that we imaged using our VIS-SS-OCT system. Two different sets of glasses were sampled, each with different holographic elements and results.

The first set of glasses created an image of a snowman around point light sources. The sample was imaged with 400 by 400 A-scans of a surface that was 1.9 by 3.4 mm in size. The system was able to image two separate layers of the glasses, where one seems to be a plastic cover and the other layer contains the holographic elements. With a resolution of 1.3 micron, small fluctuations in the surface of both layers are visible, such as scratches, dents and thinning of the layers in the circular elements.

The second pair of holographic glasses was of an image of a Sakura, a Japanese cherry blossom flower. These glasses were of a slightly different structure. Again there were multiple layers, except this time the holographic element seemed to be a zig-zagging structure of blue coloured print underneath a protective layer. As the second layer has some thickness, the protective layer also appears to contain the impression of the holographic element.

Figure b shows a reference photograph of the holographic image. Here you can clearly see the structure in blue. Figure b) shows the en face reconstruction of the OCT image, which shows a clear match with the reference image below.
Figure 14: Second pair of Holospex™ glasses obtained with 400 by 400 A-scans of a surface of 1.9 by 3.4 mm. a) Shows the 3D reconstruction of two layers. The top layer appears to be a continuous plastic cover. The imprint of the lower layer is also visible. In the lower layer the holographic structure is visible and corresponds to the structure as it was photographed with a microscope seen in b). The layers are offset in Amira for better visualization.

Figure a) shows the 3D reconstruction of the OCT images, where the layers are artificially offset for better visualisation. It is interesting to see that the second layer has clear holes in it, and thus the difference in thickness is represented in the first layer, where the imprint is seen. However, in the first layer it is clear that it is continuous surface, and so it can be concluded that they are two separate layers.

Figure 15: Results on the computer circuit board. Image a) shows the 3D reconstruction transposed with the B-scan shown in c). Image b) displays the en face image of the board. Below it image d) shows a reference photograph of the imaged surface. The data was obtained with 500 by 400 A-scans, imaging a surface of about 2.0 by 3.5 mm in size. The data revealed that there were actually two layers on the board, which became visible around the metallic elements as seen in image c). The thickness of the plastic layer was determined to be 23.

Another interesting sample is of a computer circuit board. From this we were able to determine that the system was able to correctly image complex structures and it could be used to differentiate between different materials. The board was imaged with 500 A-scans by 400 B-scans and covered an area of approximately 2.0 by 3.5 mm. As can be seen in Figure 15, a clear distinction can be made between the
different materials of the letters and numbers, and the board itself which is much more reflective. Interestingly enough, we were able to image the plastic cover on top of the metallic elements of the circuit board, as is clear from image Figure 15c. This layer was not visible by eye, and was determined to be 23 microns thick. The cross sectional image seen in c is taken at the location indicated by the black line on Figure 15b.

6.2. Spectral analysis results for RGB images

The next section will show the results of the spectral analysis. For this purpose, custom phantom samples were created to test the capabilities of imaging in colour. These samples were created mostly with paper, clear tape and different coloured dyes. The images were created by taking the average of a 100 B-scans, taken over a very small region of approximately 1 mm, to avoid speckle.

![Sample made of clear tape with different colours of marker on it. From left to right: Purple, blue, red, green and nothing (white). The different penetration depths of different parts of the visible spectrum are quite visible. The image was obtained by averaging over a 100 B-scans taken over a region smaller than 1 mm, to get rid of the speckle pattern.](image)

The first sample is of a piece of clear tape with coloured dye on top from different markers. The results immediately showed a large difference between the blue part of the spectrum, and the red. More light was able to penetrate the sample below the red dye. Of course, this is due to the poor penetration depth of blue light. This effect is also clear from the purple part of the sample to the far right. Here the blue part of the spectrum does not penetrate the sample at all, and blocks most other light as well. However, the red is still visible underneath. Unfortunately, the individual colours do not appear at the surface part of the image, which can be attributed to specular reflections occurring at the surface. Thus most light there is reflected throughout the spectrum, causing the image to appear white – yellowish. That the specular reflection appears more yellow than white is due to the shape of the spectrum, which has a higher intensity in the orange and green parts of the spectrum, than it does in the blue and red. Correction methods for this were tested, but not perfected and thus are not represented in the end results.
Figure 17: Two OCT images of the same sample, which is a piece of paper with different colours of pen (blue, green and red respectively). The image not only shows the different penetration depths of the colours, it also shows the grooves created by the pen stroke. Additionally, the top image was taken at a different position than the lower image. The position of the top image had less green ink in the middle compared to the lower image. This is also apparent from the OCT image.

Figure 17 shows two sets of cross sectional images of the sample displayed in the upper left corner, consisting of a white piece of paper with three coloured lines on top. The image again shows a clear distinction between the different coloured regions because of penetration depth. Because there are no specular reflections in this image, the colours are more apparent at the surface and in depth of the image. Additionally, the grooves created by the pen strokes and the build-up of ink near the sides of a single stroke are visible in the image. Especially in the upper image, which was taken at the region indicated on the sample picture, the green appears much less green and more a combination of the different colours. It corresponds with the fact that in the middle of that stroke there appears to be a groove containing almost no ink, and thus the paper underneath is seen. The lower image (b), has a much more distinct green colour, corresponding to the amount of green ink in the region indicated by b on the sample photograph. Finally, between the two images it is clear that the white regions reflect light at random, and not all parts of the spectrum reflect are reflected from the same location.

Figure 18: Sample of clear tape with red and blue dye on the bottom of the tape, placed on white paper. The image shows the top layer with a clear reflection. The bottom layer (paper) hardly shows up but for a thin line in the blue region. The red region is clearly visible and colours the paper below it.
The next sample, seen in Figure 18, consisted of a piece of clear tape which was coloured on the bottom instead of the top, and then placed on white paper. As expected, the top layer shows some specular reflections and appears white. On the bottom of the image, it is seen that the red light is able to reach the coloured part of the sample and also appear on the image. The blue light is able reach that far, as indicated by the thin blue line appearing on the bottom of the tape layer, however it does not penetrate the rest of the sample and so the paper does not appear. Even so, although the red part of the spectrum does reach that far, it isn’t able to image the blue part of the sample. This indicates that the colour of the sample has a great impact on how much it can be imaged with visible light, and that the spectral analysis works. It is also interesting to note that due to the red coloured tape, the paper underneath appears red even though it is not coloured itself.

Figure 19: Paper that is coloured with different shades of marker, blue, red, green and orange respectively, covered by clear tape. The blue is almost not visible, but the green appears in the middle. Also the red and orange have a slightly different shade, indicating that the spectral analysis works for colours in between red, green and blue.
To determine whether or not the system was able to distinguish between different shades, the following sample was created. It is again a piece of paper coloured with different markers. A light blue, red, green and orange. A piece of clear tape was placed on top to see how much of an effect the specular reflection would have on each shade. It appears that the red and orange sections are brighter as compared to the green and blue. There is a difference between the red and orange colour. Furthermore, although the blue part is almost not visible, it can be said that also between them there is a distinction, showing that the

Figure 20: White paper that is coloured red, with clear tape on top that is coloured green. The part indicated by the red square was imaged. On the left the tape has a high reflection, but the paper underneath is not visible. The part that is coloured green also appears that way in the OCT image. The red coloured dye appears even under the green layer and makes the paper visible.

Figure 21: White paper with coloured dots on it (red, green and blue) covered with Scotch tape which is more scattering than clear tape. The image was taken as an average over a 100 A-scans taken at the same position. Thus, in this image there is more speckle than in the previous images. The colours coming from the dots are still visible underneath the more scattering scotch tape. The blue lines running through the image at the bottom are a left over artefact from the glass slide.
spectral analysis is able to create colours that are not solely blue, green and red, but also shades in between.

Figure 20 shows a sample consisting of a piece of paper that was dyed with red marker, with clear tape on top that was partly coloured green. The part of the sample that was imaged is indicated by the red box on the sample photograph. In the left corner, the tape is not coloured and appears as a bright reflection with nothing appearing underneath. The part of the tape that is coloured green appears that way on the OCT image as well, with underneath the red paper appearing. As became clear from Figure 18, the paper underneath the red tape started to appear red even though it was not coloured itself. This sample shows that it is still possible for the system to obtain colour information with depth, even though the layer above is coloured. This indicates that the system is only limited in this by the penetration depth of the different parts of the visible spectrum.

The next samples are again of paper with coloured dots, this time however imaged without a second galvanometer and thus the sample was scanned at the same position. The images thus have more speckle and less colour separation. The sample seen in Figure 21 made use of Scotch tape to cover the paper, which was more scattering than the clear tape used in the previous samples, as well as having less specular reflections. The image shows the top layer of the Scotch tape and the coloured dots underneath. To the far left the red colour appears, with part green and blue in the middle, and

![Figure 22: The same sample as Figure 21 but taken at lower power. When the image is processed in the normal way there are no distinct other reflections visible besides the top layer. The grey line is again a left over artefact from the glass side. However when the image is processed with colour analysis small reflections appear below the surface.](image)

again red to the right. The blue line appearing across the image is a background noise artifact left over from the glass slide and can be ignored.

For Figure 22 the same sample was imaged but at lower power. When the image was processed in the normal way, without spectral analysis, the second layer did not show up. The grey line appearing underneath is again a background noise artefact that was not successfully removed during post-processing. When the image was processed using spectral analysis however, small speckle reflections
in red became distinct from the background noise. When comparing to the upper image, the same spots can be identified. However, without the aid of the colour analysis image, these spots would not be identified as structures. This shows that the method of spectral analysis could be used as a contrast enhancing tool, and perhaps aid in differentiation between structures. This also becomes apparent when we consider the sample of Figure 18, where if we switch to black and white the red structure disappears, as is shown in Figure 23.

![Figure 23: Black and white image of Figure 14 where the red is not visible anymore.](image)

In this section the results were presented which were obtained using spectral analysis of the visible spectrum to obtain RBG images. The samples presented were different combinations of paper and tape, showing that the colour information can be gathered in depth, in different shades and from different materials. Furthermore, the benefit of using colour analysis to enhance contrast and obtain a better distinction between different structures was shown.
6.3. Biological samples

The imaging of biological samples was also attempted during the research. For one, images were taken of a small transparent fish called *Oryzias latipes* also known as the Japanese rice fish or Medaka (メダカ). However due to the low power and low penetration depth the imaging was restricted to the surface of the fish. Furthermore, the fish was alive during imaging only paralyzed. Because of the slow imaging speed of the system, the heartbeat of the fish caused a large disturbance throughout the OCT image. When the fish was no longer living, a surface image was captured of the head of the fish as seen in Figure 24. It appeared to be surface only; however a small internal structure was detected at the top layer, most likely of the layers of scales.

Another sample was taken of the shell of a small water snail seen in Figure 26. The top and the bottom of the snail’s outer shell were successfully imaged. However no further details or internal structures were identifiable from the image. As a final test, a human thumb was imaged by pressing the thumb against the hole of a mirror mount where other (non-biological samples) were placed. The images are again obtained with low penetration depth due to the range of wavelengths used in the system, and thus mostly show the surface of the thumb. Figure 25 shows one of many B-scans taken at approximately the same position on the thumb. As the imaging speed is slow, there was movement during the capture which reflects in the images. Still the system was capable to capture individual grooves in the thumb.

All biological samples (with exception of the thumb), were imaged during stage II of the system setup. During this time, SNR and power were still low (around 2 µW of power onto the sample). The sensitivity of the system was thus too low for biological imaging, which explains why only surface areas could be seen on the OCT images. In the final stage of the project it was difficult to image both the fish and the snail due to practical reasons. The beam could no longer be pointed down, and the fish and the snail could not be imaged vertically.
In conclusion, due to slow imaging speeds, low imaging depth and low power, the system was not fully capable of imaging of biological samples. However, as the image of the thumb shows, higher power improved this capability significantly and thus when the system is able to image at a faster speed (and adjustments to the imaging beam direction), the imaging other biological samples in vivo, such as the Medaka, should be no issue.

7. Discussion
This research was conducted with the goal of creating a system suitable for retinal imaging using visible light. The use of visible light with OCT has been demonstrated before [19, 20] as well as with spectral analysis for RGB images (which is called METRiCS OCT) by Robles et al [8]. All these systems demonstrated their work on in vivo mouse skin with the goal of mapping the amount of haemoglobin. In order to apply the same principal for retinal imaging, the power output of the laser has to be far below the power output used in these studies. For a power output of 20 µW maximum illumination time of 20 seconds (without scanning) is allowed before damage occurs to the retina [11]. For safety, the power was kept far below this value, not exceeding a power output of 7 µW. It was also for this reason that a swept source approach was used. Namely, a SS-OCT system has the advantage of having a higher SNR than SD-OCT, which would be required when using lower power. Moreover, by using a galvano-mirror based swept source filter, in contrast to a polygon mirror based filter, the speed of the system could be slowed down, increasing the SNR and making it safer for retinal imaging. Also, it allowed for a flexible bandwidth selection to have the ability of restricting the light to the red part of the spectrum if necessary.

Although the lower speed of the system can be an advantage as it increases the SNR, it can also be a disadvantage when it comes to motion artefacts. Due to the low speed of the system (200 Hz sweeping rate), in vivo imaging of a Medaka fish was unsuccessful. To speed up the imaging, a polygon mirror can be used. Considering the broad bandwidth, the range of the Littrow angle \( \Delta \alpha_{lit} = 40.27^\circ \) according to equation 2 and using a grating of 1800 lines per millimetre. The angle of between two adjacent facets on the polygon needs to be \( \theta = \Delta \alpha / 2 = 20.14^\circ \). Thus, to accommodate the entire spectrum the polygon would need to have 18 facets. With a rotation speed of 4000 rpm this would result in a sweep speed of 1200Hz, which is already 6 times faster than the galvanometer-scanner. Off course this way the
flexibility of the bandwidth selection will be lost, plus the ability to offset the incoming and returning beam from the filter as in Figure 8.

Difficulties with imaging depth are hard to solve due to the inherent low penetration depth of visible light. Also, the low SNR and high amounts of dispersion cause the signal to be lost in the noise, even if that signal would be visible from the background noise after dispersion compensation. However, finding that signal (especially in retinal imaging) is not easy without already having some dispersion compensation present during capture. A combination of both visible and NIR or IR light could be a good way to get ‘the best of both worlds’. The high penetration depth of NIR, combined with the higher power allowed into the eye and lower dispersion, could help with locating the reflective surfaces. The Fianium WhiteLase SC400-4 has a full range of 450 – 2500 nm. The filter that was used to limit the bandwidth to 450 – 750 nm, also has an output for > 750 nm. Both beams could be send towards separate filters, and combined at a later stage to be both be sent into the eye, as is being done now for certain adaptive optics setups [29].

The system recorded a SNR of 85dB, a value which makes biological imaging very difficult, as is also evident from the biological samples presented here. For this system we estimate that a SNR of a 95-100dB should be minimum in order to capture the retina. By looking at equation 7, the SNR can be improved by either increasing the power or the integration time \( \tau \). Motion artefacts are already an issue, so slowing down the system is not recommended. Furthermore, increasing the power brings it in close range of the limit of 20 \( \mu \)W for retinal imaging. Thus, noise needs to be reduced. The higher amount of noise in the system is attributed to three things. One is imperfect balanced detection caused by loss of spectrum in the sample arm (Figure 9d). If the spectra that fall on both sensors on the detector do not match, the DC will not be cancelled out and can cause small artefact in the FFT close to zero optical path length difference. Second is shot noise. When looking at Figure 9d, the trend appears linear. However for lower powers, there is an outlier which might indicate the system is not perfectly shot noise limited. The last reason for why the SNR is low is due to fringe washout. Fringe washout was detected when using a double-axis galvo which was vibrating slightly in time. This caused the spectrum to fluctuate in time and the fringes decreased. If fringe washout is still occurring in the system, it could account for the 8 dB of loss missing. The method by Mujat et al allowed for a cheap and efficient way of mapping the data to the correct wavelengths. Although the method worked well and the theory was sound, some questions remained about the implementation and the method would benefit from a more practical guide. One main issue that remains was the shifting values of \( k_{\text{max}} \) and \( k_{\text{min}} \). When looking at the equations it is evident that these values will change and mathematically this should not matter. But as they represent physical values, which can be determined beforehand, they should remain constant. Changing the initial k-values did not have a large impact on the end result besides wrong wavelengths shown in the end plot, as small fluctuations were corrected for in step two (dispersion compensation). But the end values of the k-array are important when applying the colour analysis, as wrongly mapped wavelengths will result in wrong colour representations. Further study of the method and experimentation with the program is needed to accurately determine how the start and end values of the k-array can remain fixed, without compromising the mapping process.

The colour analysis, or METRiCS method [8], provided an simple and clean way of obtaining RGB images of the OCT data. The trade-off is a lower resolution as the total bandwidth was cut in three. By employing dual window method, or short-time Fourier transform (STFT) this might be circumvented [30]. Furthermore, the overall shape of the spectrum also had an influence on the final images. For example, the light intensity was much higher in the yellow and orange regions than in the blue, resulting in an overall more yellow hue. Compensation was attempted by dividing the end result by the incoming
spectrum. However, as the incoming spectra did not match the data exactly, this resulted in over compensation in the other areas of the spectrum. Perhaps an average spectrum can be extrapolated from the data itself, or a function can be used that approximates it. This would require some further programming and testing.

8. Conclusion

In conclusion, the system presented was able to create images with a 1.3 $\mu m$ axial resolution. The systems capabilities were shown on non-biological samples. Additionally, because of the use of visible light, spectral analysis allowed for RGB images and colour information in depth. These results were also presented on non-biological samples. Biological samples as the Medaka fish, a snails shell and a human thumb were also presented. However, low penetration depth and slow imaging speed proved \textit{in vivo} imaging challenging.

The goal was to create a system ready for retinal imaging. As far as the requirements set at the beginning, which were a small imaging beam (approx. 1mm), high flexibility of bandwidth selection, and low power onto the sample, the system meets the criteria. However, low SNR prevented any retinal images despite efforts. A higher SNR is thus critical if the system is to succeed in this regard. As far as the spectral analysis goes, the system and the algorithm behaved as expected.

Considering the scope of the research, and the time available (10 months), it was not enough time to obtain retinal images. However, results have shown great promise in applying VIS-SS-OCT for retinal imaging. In doing so, the submicron resolution can be used to detect the earliest symptoms of diseases such as glaucoma. The in depth colour information can provide new insights into colour vision by colour analysis of individual cones and can be used to locate chromophores. Based on the results obtained thus far, retinal imaging is only the next step.
9. Bibliography


