## **Development of a Coherent Optical Imaging System for Clinical Dermatology**

**by**

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**B.S.,** Korea Advanced Institute of Science and Technology (2010)

Submitted to the Department of Mechanical Engineering in partial fulfillment of the requirements for the degree of

Master of Science in Mechanical Engineering

at the

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#### **Abstract**

The abnormal structure of cutaneous capillaries is associated with many skin diseases including skin cancer and port wine stain. Consequently, the demand for an imaging modality that can provide non-invasive visualization of capillary level blood flow is high. The major challenge in such imaging is to achieve high resolution and great flow sensitivity over a sufficient depth. Numerous imaging techniques derived from optical coherence tomography have provided a technical breakthrough and produced promising images of capillary networks of retina and brain tumors. However, these modalities have never been applied in clinical dermatological studies.

This thesis illustrates the process of design and complete construction of an optical frequency domain imaging (OFDI) system for dermal vasculature imaging that can be used in a clinical environment. The system consists of optical hardware, electronics, and a microscope; every part is contained in a portable cart that can be readily carried to a clinic. The optical subsystem includes a wavelength-swept laser source, a fiber optic interferometer with a delay stage and a polarization-sensitive balanced receiver. **All** power supplies, control drivers and monitoring circuits are integrated and enclosed in a case with a control interface. The microscope is attached to an articulating arm to be positioned as desired while the patient sits at ease.

The system performance is summarized as  $10 \mu$ m resolution with frame rate of **100** frames per second. Further studies, in collaboration with dermatologists, will involve imaging the vascular structure of port wine stain lesions and investigating their correlation to laser treatment.

Thesis Supervisor: Benjamin **J.** Vakoc Title: Assistant Professor of Dermatology and Health Sciences and Technology, **HMS,** MGH

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## **Chapter 1**

## **Introduction**

Non-invasive assessment of skin microvasculature is essential for diagnosis, treatment monitoring and pathological investigation in dermatology. Cutaneous microvascular structures are **highly** affected **by** skin cancers, as cancer cells induce angiogenesis to supply oxygen and nutrients. Capillary malformations result in pink or red lesions on the skin, and if formed in certain locations, can lead to glaucoma or Sturte-Weber syndrome. Dermal irritation, burn, and wound healing processes also involve injury and regeneration of blood vessels. As a result, the demand for an imaging modality that can provide non-invasive visualization of capillary level blood flow is very high.

The major challenge in microvasculature imaging is to achieve high resolution and great flow sensitivity over a sufficient depth. Specifically, microvessels are located in the dermis up to  $\sim$ 2 mm in depth, and their diameter measures  $\sim$ 10  $\mu$ m. The flow velocity in capillary is as slow as  $\sim 0.5$  mm/s at rest [1].

## **1.1 Related Works**

During the past two decades, various derivatives of optical coherence tomography **(OCT)** have found applications in imaging blood flow. Early studies combined the concept of laser Doppler velocimetry with time domain **OCT,** which maps the Doppler frequency shift of the **OCT** signal that is caused **by** moving scatters in the blood **[5, 8].** Here, the minimum detectable flow velocity was inversely coupled to the spatial resolution through the short time Fourier transform window. This undesirable coupling was eliminated **by** phase-resolved imaging techniques [22]. Phase-resolved time domain **OCT(TD-OCT)** determines the phase information **by** Hilbert transforming the **TD-OCT** signal and calculates the Doppler frequency shift from the phase information [21].

Frequency domain approaches brought significant advances in **OCT** imaging technology **[10].** These approaches can be categorized in two different types: **(1)** the spectral domain approach where a broadband light source is used and the output signal is detected **by** a spectrometer and (2) the frequency domain approach where a wavelength-swept laser is used as a source and a single element photodiode as a detector [4]. These two approaches are also referred to as spectral domain **OCT (SD-OCT)** and optical frequency domain imaging (OFDI), respectively. Both types of imaging systems have been applied to imaging blood flow **by** mapping the phase shift of the interferometric output signal, and have shown promising images of human retina **[23]** and mouse brain tumor microvasculature (Figure 1-1-B) **[16].** However, these phase resolved **OCT** techniques are **highly** sensitive to bulk tissue motion.



Figure **1-1:** Recent images of microvasculature obtained **by (A) OMAG** : images of human palm capillary networks in different depth  $(A-A)$  400-450  $\mu$ m,  $(A-B)$  450-**650** pm, **(A-C) 650-780** tm, and **(A-D) 780-1100** pm **[1];** and (B) OFDI **:** images depth-projected vasculature within the first 2 mm of mouse brain tumor **[16]**

Optical micro angiography **(OMAG)** contrasts blood flow with static tissue **by**

processing a modulated spectral **OCT** signal. **A** piezoelectric scanner is added to the reference mirror of a conventional spectral domain **OCT** system. The modulated spectral interferogram undergoes a series of Hilbert and Fourier transforms after which the signals from the moving blood cells and the static tissue are separated into the positive and negative frequency space of fast Fourier transform (FFT) **[17, 1].**

Variance of speckle signals is also proved to provide blood flow contrast. This approach does not require any phase information. The speckle variance between the intensity image frames is larger in a static fluid than in a static tissue because of the Brownian motion of moving caterers in the fluid. This enables the mapping of the flow regardless of the Doppler angle, and can detect the capillaries that are parallel to the incident beam [12].

Interestingly, these **OCT** derived imaging modalities have not been applied to studies in clinical dermatology. Although the **OMAG** technique demonstrated promising preliminary images as shown Figure **1-1-A),** the inherent complexity of the system has delayed its application in clinical studies.

### **1.2 Motivation**

The main motivation of this thesis is to develop and construct an OFDI system for clinical studies of dermal microvasculature. Preliminary studies on a bench top system have been successful in imaging microvasculature of a mouse brain tumor. This study aims to build a clinical system that is specifically tailored for skin imaging based on the current laboratory system. **A** potential application of this study is to investigate vascular biology of port wine stain (PWS) in response to pulsed-dye laser (PDL) treatment.

#### **1.2.1 Port Wine Stain (PWS)**

PWS is **a** vascular birthmark that consists of dense and dilated capillaries in the skin. The name is derived from its reddish or purplish discoloration. The occurrence is approximately **0.3%,** but the pathology is poorly understood. While benign, they commonly present on the face and neck, and often cause measurable stress to children and young adults. As the discoloration does not fade over time, treatment is often recommended.

The most common method of PWS treatment employs a pulsed-dye laser to destroy excessive blood vessels. Laser pluses of wavelengths that match the absorption band of hemoglobin cause local heating within the vessel, and the heat causes thermal damage to the vessel. Current protocols use wavelengths from **577-600** nm and pulses with a duration ranging form 0.45-40 ms [14, **15].** While PDL therapy has proven beneficial to most patients, very few patients experience complete fading of the lesion. Additionally, as shown in Figure 1-2, some patients with initial improvement experience redarkening after a few years, whereas some patients do not respond to the therapy at all **[7, 9].** Interesting to note is that it is not possible to predict the efficacy of the therapy before the treatment, and mechanisms underlying these poorly responding cases are unknown.



Figure 1-2: Redarkening of PWS after PDL treatment. Each panel shows a patient before treatment (left), after six treatments of PDL (middle), and at follow up examination after **9** years (Panel **A)** of **10** years (Panel B,C, and **D). [7]**

To improve the current therapy, we must first advance our understanding of underlying vascular biology of PWS treatment. Our OFDI system is expected to play a vital role in such clinical studies. Until now, there have been limited techniques to assess the vasculature before and after the treatment. Histological examinations do not provide a non-invasive visualization of the vessels. Confocal microscopy provides *in vivo* imaging with fluorescent dyes but offers limited fields and penetration. **A** clinically deployable imaging system with capillary level high-resolution and sufficient penetration through the dermis would enable a better understanding of PWS physiology and improve the treatment.

### **1.3 Thesis Organization**

Chapter **1** introduces the clinical motivation of this thesis regarding its application to study of port wine stain response to pulsed-dye laser therapy. The background study of optical coherence tomography **(OCT)** and optical frequency domain imaging (OFDI), the second-generation technology of the **OCT,** is covered in Chapter 2. The basic principles and theoretical performance limits of both techniques are explained. The detailed design and construction of the system is presented in Chapters **3** in the following order: the optical hardware; the electronics and control device; and the microscope interface. Chapter 4 evaluates system performance, presents a promising image of human dermal vasculature from the system, and concludes with the discussion of future work.

## **Chapter 2**

## **Background**

## **2.1 Optical Coherence Tomography**

Optical coherence tomography **(OCT)** is an imaging modality derived from low coherence interferometry that can provide non-invasive high-resolution imaging of the subsurface structure of scattering materials. Figure 2-1 displays a schematic of a standard **OCT** system. **A** beamsplitter divides the incident light and directs it to the reference mirror and the sample. The reflected light from the reference mirror and the backscattered light from the sample recombine and interfere at the beamsplitter output.



Figure 2-1: Schematic of **OCT** system. BS **:** Beamsplitter

Consider a simple case with a single mirror in the sample arm. If the source is monochromatic (coherent), the intensity of the recombined field output is proportional to the cosine of the optical path difference (OPD)  $\Delta l$  between the reference and the sample arms, as given **by** Equation 2.1.

$$
I_o \sim |A_r|^2 + |A_s|^2 + 2A_r A_s \cos[k\Delta l(t)],\tag{2.1}
$$

where  $k = 2\pi/\lambda$  is the wavenumber;  $A_r$  and  $A_s$  are field amplitudes incident on the reference mirror and the sample mirror, respectively. However, if the source contains a spectrum of wavelengths or frequencies, the interference is observed only for the OPD values within the coherence length. The coherence length is inversely proportional to the source spectrum. **OCT** uses a broadband source (low coherence), because a short coherence length leads to the 'depth selection' ability. The peak value of the output envelope is proportional to the reflectivity of the sample at the certain depth of path-match. Hence, the reflectivity profile is obtained **by** scanning the reference mirror.

#### **2.1.1 Performance Limits of OCT**

As described in [2], the depth resolution of **OCT** imaging is directly related to the coherence length of the source and is inversely proportional to the source bandwidth. For a source with a Gaussian spectrum, the axial resolution  $\delta z$  is given by

$$
\delta z = \frac{2 \ln 2}{\pi} \left( \frac{\lambda_c^2}{n \Delta \lambda} \right),\tag{2.2}
$$

where *n* is the refractive index of the sample,  $\Delta\lambda$  is the full width at half maximum (FWHM) of the source power spectrum, and  $\lambda_c$  is the center wavelength of the spectrum. The transverse resolution of **OCT** imaging is defined as the minimum spot size of the sample beam, which is determined **by** the focusing property of the beam. The transverse resolution  $\delta x$  is given by

$$
\delta x = \frac{4\lambda}{\pi} \left( \frac{f}{d} \right) \tag{2.3}
$$

where  $d$  is the beam diameter on the lens, and  $f$  is the focal length. A high numerical aperture **(NA)** results in a smaller spot size for the same beam, which leads to high transverse resolution. However, this high transverse resolution is achieved at the expense of reduced depth of focus **,** which is given **by**

$$
b = \pi \frac{\delta x^2}{2\lambda}.\tag{2.4}
$$

The depth of focus is interpreted as the depth in the sample that can be imaged, and is also referred to as the depth range. In **OCT,** the coherence gate decouples the axial resolution from the beam profile. Therefore, **OCT** imaging typically employs a low **NA** objective compared to confocal imaging, in order to achieve a large ranging depth.

#### **2.1.2 Scanning Procedures and Image Display**

To obtain three-dimensional information with **OCT,** it is necessary to implement the scanning mechanism in three directions. Depth scan, which is also called A-scan, in z direction is accomplished **by** scanning the reference mirror. Transverse scan, or B-scan, can be implemented either **by** a scanning mirror in the sample arm or translating the sample.

Different planes can be imaged with different scanning priorities of directions. The most common scanning method of **OCT** is the depth priority scan, which yields a tomographic imaging plane **by** measuring depth profiles at different lateral positions along a line. *En* face imaging is also possible **by** performing a two-dimensional transverse scan while holding the reference mirror fixed.

## **2.2 Optical Frequency Domain Imaging**

Optical Frequency Domain Imaging (OFDI) is one of the two frequency domain alternatives for conventional (time domain) **OCT.** OFDI, like the other frequency domain alternatives, e.g. spectral domain **OCT,** enables much faster imaging with greater sensitivity **by** eliminating the need for scanning the reference mirror.

As discussed in [20], the principle of OFDI is based on optical frequency domain reflectometry (OFDR). Figure 2-2 shows the basic configuration of a fiber-based OFDR system. The source output splits into the reference and the sample arms. The interference is detected while the wavelength of a narrowband laser is swept while the reference mirror is fixed.



Figure 2-2: Basic configuration of OFDR system

The detector current is given **by**

$$
i_{det}(t) = \frac{\eta q}{h\nu} \Big( P_r + P_s \int r^2(z) dz + 2\sqrt{P_r P_s} \int r(z) \Gamma(z) \cos(2k(t)z + \phi(z)) dz \Big), \quad (2.5)
$$

where  $\eta$  is the detector sensitivity,  $q$  the quantum of electric charge  $(1.6 \times 10^{-19})$ coulomb),  $h\nu$  the single photon energy,  $P_r$  the optical power reflected from the reference arm,  $P<sub>o</sub>$  the optical power illuminating the sample. The third term represents the interferometric signal, whereas the first and second terms contribute to the background. The coordinate z is defined along the depth into the sample where  $z = 0$ is the path-match between the reference and the sample arm.  $r(z)$  and  $\phi(z)$  are the amplitude and the phase of the sample reflectivity at a depth  $z$ .  $\Gamma(z)$  is the coherence function of the instantaneous laser output, and  $k(t) = k_i + \alpha t$  is the wavenumber that

is varied monotonically over time t with a slope  $\alpha$ .

If we consider, for simplicity, the case with a single reflector in the sample arm at  $z = z_0$  where the input is considered coherent within the range that  $\Gamma(z) = 1$ , the signal current can be simplified:

$$
i_{sig}(t) = \frac{\eta q}{h\nu} 2\sqrt{P_r P_s} r(z_0) \cos\left(2(k_i + \alpha t) z_0\right).
$$
 (2.6)

It is clear that the signal is proportional to the reflectivity  $r(z_0)$ , with the signal frequency in time  $(\Omega = 2\alpha z_0)$  encoding the depth  $z_0$ . Thus the depth reflectivity profile can be obtained **by** Fourier transforming the fringe signal, without having to scan the reference mirror.

### **2.2.1 Performance Limits of OFDI**

The performance of OFDI is analyzed in the same way that conventional time domain **OCT** is analyzed. For a tuning source with a Gaussian-shaped spectrum, the axial resolution is given **by [11]**

$$
\delta z = \frac{2\ln 2}{\pi} \left( \frac{\lambda_c^2}{n \Delta \lambda} \right),\tag{2.7}
$$

which is the same as Equation 2.2. Likewise, the transverse resolution is governed **by** the beam profile, and given **by** the equation **2.3.**

The depth range  $\Delta z$  is related to the sampling frequency of the digital acquisition **(DAQ)** board. According to the sampling theorem, the sampling frequency must be twice the maximum frequency of the signal from the maximum depth that can be imaged  $(\Delta z)$  in this case. The depth range is given by [6, 20]

$$
\Delta z = \frac{\lambda_0^2}{4n\delta\lambda},\tag{2.8}
$$

where  $\delta \lambda = \Delta \lambda / N_s$  is the sampling wavelength interval, and  $N_s$  is the number of samples within the FWHM of the spectrum  $\Delta \lambda$ . This sampling interval  $\delta \lambda$  should be smaller than the instantaneous linewidth of the source in order to take advantage of the coherence of the narrowband. It is also shown in [20] that the signal-to-noise

ratio (SNR) of the OFDI system is improved to

$$
(SNR)_{OFDI} = \frac{N_s}{2}(SNR)_{TD-OCT}
$$
\n(2.9)

compared to the SNR of time domain **OCT (TD-OCT).**

#### **2.2.2 Wavelength Swept Laser**

As mentioned above, OFDI is operated **by** a tunable laser source with narrow linewidth. The laser characteristics that are most significant for OFDI can be summarized as the wavelength sweep range, the sweep repetition rate, and the instantaneous linewidth. The wavelength sweep range  $(\Delta \lambda)$  is related to the axial resolution  $(\Delta z)$ , as can be seen in Equation **2.7.** For imaging of a biological sample with a center wavelength at  $1.3 \mu$ m, the scanning range has to be wider than 100nm in order to achieve the order of 10  $\mu$ m resolution. The sweep repetition rate determines the imaging speed, since one sweep delivers one A-line profile. The instantaneous linewidth is relevant to the ranging depth in a way that the narrower the linewidth, the longer the coherence length that the signal from deeper point can be detected. An instantaneous linewidth of 0.1-0.2 nm is sufficient for many biomedical applications. **[3]**



Figure **2-3:** Schematic of polygon mirror based wavelength-scanning laser. **[18]**

One of the common implementations of this tunable laser source for OFDI systems is a semiconductor laser with a polygon mirror scanner and a diffraction grating based wavelength-scanning filter **[18].** Figure **2-3** presents the schematic of the filter. The reflection-type filter consists of three parts: a grating that splits the incident broadband light into narrowband wavelength components; an afocal telescope with two lenses that redirects the diverging beam from the grating to the polygonal mirror while controlling the beam diameter; and a polygonal scanner that reflects the narrowband component that is incident perpendicular to its facet.



Figure 2-4: Schematic of the wavelength-scanning laser **[18].**

As illustrated in Figure 2-4, only the reflected wavelength component returns into a fiber-ring laser cavity, where it is amplified **by** a semiconductor optical amplifier **(SOA).** Rotation of the polygonal mirror sweeps the wavelength of the amplified output.

The equations of the parameters of this wavelength swept laser are summarized in Table 2.1. Note that a **100%** duty cycle is achieved when all beams fall within a facet without clipping. The sweep range is determined **by** the free spectral range (FSR) of the laser. It has been demonstrated that the FSR can be twice as large when the wavelength-scanning filter is configured with an end reflector **[13],** as shown in Figure **2-5.** This effect can be translated to a faster A-line rate **by** increasing the number of facets to **2N** and keeping the same FSR.

Diffraction wavelength	$\lambda = p(\sin \alpha + \sin \beta)$	$p:$ grating pitch $\alpha$ : incident angle $\beta$ : diffracted angle
Center wavelength	$\lambda_0 = p(\sin \alpha + \sin \beta_0)$	$\beta_0$ : angle between the optical axis and the grating normal
Free spectral range	$\Delta\lambda = p \cos\left(\frac{F_1}{F_2}\theta\right)$	$F_1, F_2$ : Focal lengths of the lenses
Instantaneous linewidth	$\delta\lambda = \sqrt{4 \ln 2} \lambda_0 p \cos \alpha / (W\pi)$	$W: 1/e^2$ beam width
Facet-to-facet polar angle	$\theta = 2\pi/N \approx L/R$	$N:$ number of facets $R:$ polygon diameter
Polygon facet width	$L = 2R \tan(\theta/2)$	
Beam width at polygon facet	$W' = WF_2 \cos \beta / F_1 \cos \alpha$	

Table 2.1: Important parameters of the wavelength swept laser



Figure **2-5:** The configuration of the wavelength-scanning filter with an end reflector [13].

#### **2.2.3 Optical Frequency Shifter**

In **OFDI,** the ranging depth is limited **by** the coherence length of the instantaneous output of the wavelength swept laser. The fringe visibility decreases due to the finite extent of the coherence length, as seen by the term  $\Gamma(r)$  in Equation 2.5. Moreover, the ranging depth is restrained **by** the inability to distinguish the positive and the negative frequency in an argument of a cosine function in Equations **2.5** and **2.6.**

One approach to achieve the maximum raging depth within the coherence length is to use an optical frequency shifter in the interferometer to shift the frequency of the detector signal **[19].** Figure **2-6** illustrates a schematic of an OFDI system with an optical frequency shifter.



Figure **2-6: A** basic configuration of an OFDI system with a frequency shifter **FS :** frequency shifter **[191.**

The signal current can be expressed as

$$
i_{sig}(t) = \frac{\eta q}{h\nu} 2\sqrt{P_r P_s} \int r(z) \Gamma(|z|) \cos(2k(t)z + \phi(z) + 2\pi \Delta ft) dz \Big), \tag{2.10}
$$

where  $\Delta f$  is the round-trip frequency shift. Note it is the third term in Equation 2.5 with the frequency shift term  $\Delta f$ . With a linear sweep in wavenumber,  $k(t) = k_i + \alpha t$ ; then the detector signal frequency is given **by**

$$
f_{sig} = \left| \frac{\alpha}{\pi} z + \Delta f \right|.
$$
 (2.11)

Therefore, as shown in Figure **2-7,** now the negative frequency can be mapped in the positive frequency domain, shifted by  $\Delta f$  and both sides of the coherence range can be used.



Figure **2-7:** Illustration of the ranging depth (a) without and **(b)** with a frequency shifter **[19].**

### **2.2.4 Balanced Detection**

As seen in Equation 2.1, the **OCT** signal comprises the background **DC** terms and the **AC** term that provides the sample information. Balanced receiver detects the oscillating term of the interferometric output **by** subtracting the quadrature signals of the beamsplitter. The quadratures can be detected because the transmitted field and the reflected field from the beamsplitter have a  $\pi/2$  phase difference. Figure 2-8 illustrates a conceptual schematic of a balanced receiver.



Figure **2-8:** Schematic of a balanced receiver concept.

The intensities at Detectors **A** and B are given **by** the following:

$$
I_A = |E_A|^2 = |iE_1 + iE_2|^2 = E_1^2 + E_2^2 + 2\cos(2k\Delta l),\tag{2.12}
$$

$$
I_B = |E_B|^2 = |E_1 + i^2 E_2|^2 = E_1^2 + E_2^2 - 2\cos(2k\Delta l), \qquad (2.13)
$$

where  $E_A$  and  $E_B$  are the fields at each detector;  $E_1$  and  $E_2$  are the fields incident to mirrors 1 and 2 divided from the beamsplitter, represented **by** the orange and green colors, respectively;  $k$  is the wave number; and  $\Delta l$  is the OPD. The balanced detection is implemented **by** subtracting Equation **2.13** from Equation 2.12.

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## **Chapter 3**

## **System Design and Construction**

This chapter describes the design and the construction of an OFDI system for dermal vasculature imaging that can be used in a clinical environment. The system consists of optical hardware, electronics, and a microscope. The whole systems is encased in a portable cart **(61** cmx101.4 cmx92.9 cm, WxDxH) with a monitor and the microscope attached to it.

### **3.1 Optical Subsystem**

There were three constraints in designing the optical hardware. First, to obtain images of capillary flow, it is necessary to achieve the performance of  $5 \mu m$  resolution over a ranging depth of **3** mm. The source center wavelength was chosen to be **1300** nm, because it has low absorption in biological samples while penetrating to sufficient depth. To satisfy the specification with the chosen center wavelength, the source must be configured to achieve an FSR greater than **100** nm and a coherence length greater than 2 mm on both sides of the signal spectrum.The second constraint is the short imaging time imposed **by** the clinical environment. In this thesis, the imaging speed was aimed at **100** frames per second, which is equivalent to an A-line rate of **80** kHz. Last, all opto-mechanical components had to be confined to an optical breadboard of size 42 cmx **66** cm. The breadboard was mounted on a sliding shelf in the cart. Optical fibers were laid out on a panel, which was fixed under the sliding shelf.

#### **3.1.1 Optical Source: Short Cavity Wavelength Swept Laser**

Conventional OFDI systems employ a fiber-ring cavity wavelength swept laser, which is illustrated in Section 2.2.2, Figure 2-4, where a narrow linewidth spectral filter is inside a fiber-ring cavity with a broadband gain medium. In order for one narrowband pulse to build up sufficient gain while maintaining the instantaneous linewidth and the sweep rate, the cavity length is must be as short as possible. For the desired sweep rate of **80** kHz, the total cavity length must be shorter than 2 m. However, due to the fiber length required **by** polarization controllers in the cavity, it was difficult to reduce the fiber length below the required level. The alternative approach was to replace the fiber coupler with a free-space beamsplitter. **A** schematic of this new set up is illustrated in Figure **3-1.**



Figure **3-1:** Schematic of the short cavity wavelength-scanning laser. **SOA :** Semiconductor Optical Amplifier; PBS **:** Polarization beamsplitter; FR: Faraday rotator; HWP **:** Half wave plate; Col **:** Collimator

To ensure stability in the lasing direction, we used a polarization beamsplitter (PBS) with a Faraday rotator(FR) and a half-wave plate (HWP). Collimator **A** is configured so that the incident field is split into a **50/50** ratio at the PBS, while the first order diffraction is maximum at the grating so that power loss through grating is

minimum. The FR shifts the polarization **by 450** in both directions of travel, whereas the HWP shifts 90° in one direction and reverses the shift to -90° in the opposition direction. Therefore, the polarization of the filter input field is perpendicular to the filter output; the output is directed to Collimator B. Collimator B is configured to have the maximum power from the transmission of the PBS, which is equivalent to the maximum coupling of the reflected output to the cavity.

As a result, the total cavity length including the double path within the filter is reduced from 3.4 m (fiber-ring) to 1.2 m. The alignment process is summarized in Appendix **A**

#### **3.1.2 Interferometer**

The system is based on a fiber-optic interferometer, which is more robust in terms of physical alignment. The schematic of the interferometer is provided in Figure **3-2.**

To achieve a full ranging depth, an acoustooptical frequency shifter is inserted in the reference arm. The amount of optical frequency shift  $\Delta f$  was calculated from

$$
\Delta f = \frac{f_{samp}}{4},\tag{3.1}
$$

where  $f_{\text{samp}}$  is the sampling frequency of the data acquisition  $(DAQ)$  instrument.

Note we implemented a mirror to calibrate the signal in the post-processing process. This calibration compensates for the nonlinearity of the sampling as well as dispersion. **A** computer-controlled shutter is implemented to block the light from the calibration mirror during the imaging process. The calibration mirror and the reference mirror are fixed on a motorized delay stage. The alignment process is explained in Appendix B.

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Figure **3-2:** Schematic of the optical subsystem.

FBG: Fiber Bragg grating; LM **:** Laser monitor; **FS :** Frequency shifter; pol **: 450** polarizer

#### **3.1.3 Polarization Sensitive Balanced Receiver**

The system employs a polarization sensitive balanced detection, as illustrated in Figure **3-2.** The reference arm polarization is controlled to be 45 degrees at the beamsplitter. To compensate for the dispersion caused **by** the crystal in the optical frequency shifter in the reference arm, the same type of crystal is inserted in the sample arm. **A** computer-controlled shutter is installed in the sample arm to block the backscattered sample field while only the reference field is recorded to obtain a background signal that is used in the post processing process.

#### **3.1.4 Breadboard Assembly**

The breadboard construction of the whole optical subsystem is photographed in Figure **3-3** and Figure **3-5.**



Figure **3-3:** Photograph of the optical breadboard on a sliding shelf.

To construct the system in a compact space, aluminum mounts were designed for the beamsplitters, the polygonal mirror, and the **SOA.** The designed mounts are presented in Figure 3-4. The design considerations include compatibility with

commercial opto-mechanical products; placement of the optical post and the clamping forks of surrounding elements; and flexibility in position adjustment. The drawings for each component are provided in Appendix **C.**



Figure 3-4: The designed mounts for **(A)** the beamsplitters in receiver, (B) the **polyg**onal scanner and **(C)** the **SOA.**



Figure **3-5:** Photograph of the optical breadboard in the cart (top view) **37**

### **3.2 Control Box**

The metal case that contains all electronics will be referred to as the control box from this point. The control box is mounted on a sliding guiderail and placed in the portable cart.

#### **3.2.1 Case Design**

The case was designed with Protocase Designer 4 software. Figure **3-6** presents the design of the case. The width and the depth were constrained to fit in a standard server rack in the cart, and the height was 4 **U** (rack server units).



Figure **3-6:** Schematic of the empty Control Box.

As seen in Figure **3-7,** the front panel of the case includes ventilation holes, power switches, and **BNC** outputs for monitoring the laser and the galvanometer scanner. The back panel serves as a connection interface where all wires are mounted with a mate-and-lock cable connectors. The construction involved re-assembling of all cables with appropriate connectors. For cables with high currents, we installed mechanical



screw fixers to fix the cables with cable ties on the screws.

Figure 3-7: The Control Box interface viewed from (A) the front and (B) the back.

### **3.2.2 Power Connection**

The control box encloses all the power supplies of the system components. Each component was matched with an appropriate power supply. The scheme of the power control is illustrated in Figure 3-8. The main switch connects 115 V AC input to the main LED and other first level electronics such as ventilation fans; a temperature monitor; the delay stage with the reference and the calibration mirrors; the balanced detectors in the polarization sensitive receiver part; and the motorized linear stages in the microscope. The power adapters that connect components to AC power are not depicted in the diagram. Secondary switches connect the 115V DV input to the optical frequency shifter, the power supplies of the galvanometer scanner

driver, the polygon driver, and the laser driver. Enable switches are installed for the galvanometer scanner, the polygon scanner, and the **SOA.**



Figure **3-8:** Control Box power connection. **PS** : power supply

#### **3.2.3 Component Layout**

One of the challenges in the construction was placing all components in the compact space. To make the best use of the space, some components were stacked on a plate supported **by** aluminium posts. The bottom of the control box was designed so that the components can be fixed with standard screws or cable ties. Figure **3-9** presents a photograph of the completed control box taken from the top without the top panel.



Figure **3-9:** Photograph of the control box in the cart (top view)

### **3.3 Microscope**

The microscope provides the interface between the sample and the optical subsystem. The microscope subsystem comprises an objective lens, a two dimensional linear stage, and a galvanometer mirror scanner. The microscope is attached to the cart via an articulating arm so that it can be placed as desired while imaging patients.

#### **3.3.1 Lens selection**

IAs mentioned in Section 2.1.1, a good transverse resolution is achieved via a high **NA** at the expense of large depth of focus. In the ideal case, it is desired to maintain high transverse resolution over the full depth of focus (Figure **3-10).** One of the



Figure **3-10:** The transverse resolution and the depth of focus **-** coupled with the **NA**

ideas to address this problem is to image three different depth sections with three separate light sources that are centered at different wavelengths. **By** taking advantage of chromatic aberration, different sources can be focused at different depth planes, which is illustrated in Figure **3-11.**

To select the objective lens that provides sufficient chromatic aberration to focus different source at enough separation distance, beam diameters over the depth have been calculated with ZEMAX software. **LSM02** from Thorlabs was chosen, since the total focal shift was **1** mm in free space, which translates to sufficient depth of focus when imaging biological samples. Figure **3-12** plots the root mean square (RMS) beam



Figure **3-11:** Improvement in transverse resolution with tri-band source

spot radius along the depth. The focal shift  $\Delta f$  was calculated from the equation:  $\Delta f = z_0 - z$ , where  $z_0$  is the back focal length from the lens surface at the wavelength **1300** nm.



Figure **3-12:** RMS Spot radius.

### **3.3.2** Transverse Scanning

We have implemented an one-dimensional galvanometer scanner to achieve a tomographic cross-sectional image, and a bi-directional motorized linear stage to move the lens with respect to the sample to obtain volume information as well as to adjust the lens position. The scan priority is diagrammed in Figure **3-13**



Figure **3-13:** Schematic of the scanning mechanism. red **:** z-direction depth scan provided **by** the laser sweep; blue **:** x-direction transverse scan provided **by** the galvanometer; green **:** y-direction transverse scan provided **by** the linear stage.

#### **3.3.3 Objective Interface Design**

The objective interface is designed to image the dermal microvasculature of human patients. To facilitate the process, a commercial adhesive window for dermal confocal imaging is employed. The window is designed to interface a magnetic ring and the imaging site. The magnetic ring attaches to an adapter piece, which can be adjusted to find the best focus. The magnet mechanism enables simple attachment and detachment with an outer ring. The basic concept is diagramed in Figure 3-14, and a photograph of the microscope is shown in Figure **3-15.**



Figure 3-14: Schematic of the skin-objective interface **(A)** without the outer ring and (B) with the outer ring.



Lens **(inside)**

Figure **3-15: A** photograph or the microscope.

## **3.4 Construction**

The whole system is encased in a portable cart  $(61 \text{ cm} \times 101.4 \text{ cm} \times 92.9 \text{ cm}, W \times D \times H)$ with a monitor and the microscope attached to it. **A** computer (Windows **7,** a **2.67** MHz quad core **CPU,** a 12 GB RAM) of a height of **1 U** (rack server unit) is inserted at the top. The optical breadboard where all optical elements are fixed with clamping forks is mounted on a sliding server rack self. **A** thin aluminum panel was attached under the sliding shelf. On the panel, fiber based elements such as couplers and circulators were laid out. The control box is mounted on a set of guide rail and installed under the optical breadboard. At the bottom of the cart, an electrical power isolator is installed.

The microscope is mounted on an articulating arm (42 inches maximum length). The double arm is gas spring assisted so it can be effortlessly pulled and positioned as desired. It provides up to 42" of vertical and horizontal adjustment with **180** degrees rotation. The head part is ball-jointed to allow the fine-tuning of the position. The arm is fixed on the left panel of the cart. Cables that connects controllers in the controller box to the galvanometer and the linear stage are fixed tight with tie straps so the connections are robust. The Optical fiber in the sample arm is protected in a **3** mm diameter outer jacket and also tied securely to the microscope with tie straps.

Figure **3-16** shows a schematic of the complete system on the cart. **A** complete list of parts is included in Appendix **C** and a list of connectors and electronic cable components is included in Appendix **D.**

#### **3.4.1 Challenges**

The main challenge of this work was building the system from scratch. Component purchases involved comparing similar products from different manufacturers and selecting the ones that meet desired specifications. Simulation and design required softwares (Zemax and Solidworks) that **I** have never been exposed to before. Basic resources such as screws and bolts also had to be purchased as well as electrical cable connectors and wire crimps.



Figure **3-16:** System. **(A) A** Schematic and (B) a photograph.

Like other experimental studies, this project required solid understanding of the theory as well as experience and insight in laboratory. As I did not have a background in Optics, understanding the physics of **OCT** and OFDI was challenging in the beginning. In the laboratory, aligning all free-space optical components to minimize power loss have been quite tedious.

Planning had gone through a great amount of trial-and-error, specifically for the planning the work in between the purchase and the delivery of a component. Devices occasionally broke down, identifying the cause and repairing them also took some unexpected time.

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## **Chapter 4**

## **Performance and Future Work**

This chapter presents the performance of the system. The image obtained from the system is what we believe to be the first image of human dermal vasculature imaged without any exogenous agents. The system will be further improved and applied for clinical studies in dermatology in the future.

### **4.1 Laser Performance**

The performance of the system is largely determined **by** the source characteristics, since the balanced receiver and **DAQ** are commercial products and the imageprocessing algorithm is already optimized for vasculature OFDI.

#### **4.1.1 Coherence Length**

The coherence length was measured **by** finding the calibration mirror delay that causes the fringe amplitude to decrease to the half of the path-match fringe amplitude. The path-match was found with an RF spectrum analyzer when the peak occurred at a 40 MHz signal frequency. The coherence length was measured to be **3.15** mm to the positive side of the spectrum and **3.30** mm to the negative side of the spectrum, which satisfies the goal described in Chapter **3.1.** Figure 4-1 shows the oscilloscope signals at the pathmatch and at the maximum ranging depths.



Figure 4-1: The oscilloscope signals.

### **4.1.2 Source Spectrum**

As described in Section 2.2.1, the source spectrum determines the axial resolution of OFDI. The source had a bandwidth of 140 nm, as measured **by** an optical spectrum analyzer **(OSA).**

### **4.2 Image and Discussion**

The vasculature of human cheek skin is imaged for testing purposes. The adhesive window with index-matching gel was placed on the cheek of a subject while the person was sitting on a chair. The field of view was set at  $7.2 \text{ mm} \times 5.6 \text{ mm}$ . Data acquisition time was 54 seconds. Figure 4-2 displays an *en face* image of human cheek vasculature. The vessels are color-mapped along the depth into the skin: yellow (superficial) to red (deep).

We believe optimization of the processing algorithm and scan pattern will provide more contrast. In addition, employing thicker objective window with anti-reflective



Figure 4-2: *In-vivo* image of the human cheek vasculature.

coating will also remove the undesired vertical striation.

## **4.3 Future Work**

Plans for further research can be summarized as **(1)** application of the system for the study of port wine stain (PWS) treatment and (2) optimization of optical system performance.

After the system is validated, we will investigate the kinetics of microvascular response of PWS to the pulsed-dye laser (PDL) therapy. We will be working with dermatologists in Massachusetts General Hospital and Brigham and Women Hospital. We will recruit and obtain consent from a group of adult volunteers seeking treatment for PWS. The patients will be treated up to **8** times, and image of capillary structure in lesion will be taken prior and immediately after the treatments, and also at follow-up appointments. This study aims to provide guidelines for the most effective parameters for the therapy through understanding of the biology at microvascular level.

While the current system presents valuable and promising imaging of capillary structure, its performance can still be improved. To better discriminate capillaries in skin, a uniform transverse resolution throughout the depth is advantageous. In the current OFDI system, high transverse resolution is limited to 25-30m for a 1mm depth of field. Higher resolution is usually achieved at the expense of penetration depth. I have planned to develop a laser source that can penetrate deeper (2mm) in the skin with better resolution  $(\sim 10m)$ . The concept has been validated through simulation results. As discussed in Section **3.3.1,** the lens was chosen so that it provides sufficient focal shifts between three different bands of wavelengths **(1000** nm, **1300** nm, and **1700** nm).

The shorter and longer regime sources will be incorporated into the current system. Since different wavelengths focus at different depths with longer wavelength at a deeper position, three separate band of signal is obtained. These data can be merged in to one image at the processing step.

## **Appendix A**

## **Short Cavity WSL Alignment**

- **1.** Collimation adjustment (with a collimator to mirror distance of **25** cm)
	- (a) Adjust collimator(3) first.
	- **(b)** Collmator(1) and (2) are connected to the PM fiber which has a different **NA** to **SMF,** so they can not be collimated **by** monitoring the coupled power. Adjust collimation **by** observing the beam size so that it is maintained constant over the distance.
- 2. Height Matching
	- (a) **All** heights should match the opening of the polygon mirror.
	- **(b)** Adjust collimators first, and also adjust the PBS mount so that the beam does not bend up or down after the PBS. Also adjust the vertical angle of the grating.
- **3.** Layout and polarization adjustment
	- (a) Mount the **SOA** so that its arrow points toward the direction of collima $tor(1)$ .
	- **(b)** Adjust the mounting angle of collimator(1) to have **50/50** split after the PBS.
- (c) Adjust the mounting angle of collimator(2) to have maximum light going into the telescope.
- 4. Output coupling
	- (a) Maximize the coupling between the collimator(1) and **(3) by** adjusting the knobs on the output mirror and collimator(3).
- **5.** Source alignment
	- (a) Place a temporary mirror in place of the grating, and align to gain lasing power of 50mW at least in the free space before the collimator(3). **Ad**just the knobs on the temporary mirror first and then the ones on the collimator(2).
- **6.** Telescope alignment
	- (a) The half wave plate can be either at **22.5** or **67.5.** Choose the angle that makes the 0th order diffraction power of the grating the minimum.

## **Appendix B**

## **Frequency Shifter Alignment**

- **1.** Match heights of the collimators and the **AO** holes.
- 2. Obtain a good 0th order coupling with the **AO** off. With the **AO** off, diffraction does not occur so the beam must be in a regular circular shape. If it is chipped, this indicates that it does not go through the crystal properly.
- **3.** Turn the **AO** on. Twig the rotation mount slightly to make one of the first order beam is the brightest. Place the IR card long enough away from the beam so there is clear separation. Taking out the receiving collimator may help. Check whether it is the first order **by** switching the **AO** on and off. With the **AO** off, only the 0th order exists.
- 4. Put the receiving collimator back in, and turn the **AO** off. Align again for a good Oth order coupling. Do not touch the first collimator or the **AO.** Only adjust the second collimator. (This coupling need not be as good as the coupling at Step 2.)
- **5.** Turn the **AO** on, adjust lateral angle of the second collimator to find the 1st order spot. **Try** to remember where the bright 1st order was and turn to that direction. This turn may be quite far. Note that 1st order power can be very small.

**6.** Slowly optimize this 1st order coupling, **by** adjusting the collimators, then adjusting the **AO.** Care must be taken not to lose the 1st order coupling.

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# **Appendix C**

# **Components List**







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# **Appendix D**

# **Control Box Components**





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