Development of Hyperspectral Imagers for Snapshot Optical Coherence Tomography

by

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ABSTRACT

Development of Snapshot Optical Coherence Tomography via Hyperspectral Imaging for Biomedical Studies

by

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Optical Coherence Tomography (OCT) is an established interferometry-based technique for volumetric tissue imaging with micrometer resolution, best known in many medical applications such as ophthalmologic imaging and endoscopy. Several clinically recognized examples include retinal imaging to detect glaucoma and age-related macular degeneration (AMD) or cardiovascular imaging when employed with a catheter. Scanning mechanism presenting in all current OCT technology requires moving parts, often limiting the system’s compactness, compromising light throughput and risking unwanted movement. Snapshot imaging thus allows fast and high-throughput acquisition while minimizing motion artifacts caused by instrumental vibration or samples’ transient nature. This thesis presents novel work contributing to the development of a snapshot 3-Dimensional OCT (3D-OCT) system. With theoretical and experimental evaluations, different hyperspectral imaging designs were surveyed to provide enhancements such as high throughput, dense spectral sampling, high sensitivity toward the appropriate spectrum and spatial-spectral tunability. A proof-of-concept snapshot 3D-OCT system is introduced to simultaneously collect signals of a volumetric datacube, enabling cellular visualization of scattering biological samples. This system affords diffraction-limited performance with reduced motion and requires minimal computational time.
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Chapter 1

Introduction

1.1 Objectives and specific aims

Optical Coherence Tomography (OCT) is an established optical technique for imaging inside tissue volumes by recording interferometric information from the sample and a flat reference surface. OCT's unique capability to obtain 3D images of tissue microstructure within a scattering medium is useful when high-resolution, sub-surface information is required for disease diagnosis and treatment. However, OCT still heavily relies on bulky scanning mechanism(s) which introduces motion artifacts, resulting in blurred or non-continuous images and possible inaccurate clinical interpretation [1].

To improve the performance of current OCT technologies, new snapshot OCT modalities were examined to combine OCT’s interferometry with snapshot hyperspectral imaging concept and thus, to deliver high-resolution and light-sensitive volumetric datacube in one time frame. The overall goal of the thesis is to create enabling technologies toward a snapshot 3D-OCT system. To achieve this goal, my work is divided into three aims as follows:
1.1.1 Specific aim 1

Determine the design requirements for building a snapshot OCT system based on hyperspectral imaging.

This involves the comparison and evaluation of available hyperspectral imaging technologies to determine the suitable methods for OCT. Number of spectral sampling, datacube size and spectral range were defined. A complete analysis on the relationship between 2D cameras, its spectral sensitivity, pixel count and the system datacube’s size and penetration depth was carried out.

1.1.2 Specific aim 2

Build a hyperspectral imaging snapshot technology that can provide fast acquisition and light sensitivity with near infrared (NIR) detection dedicated to OCT.

The purpose of this work is to verify and develop the hyperspectral imaging modalities to provide a datacube with low noise, sufficient spectral sampling, and rapid acquisition in OCT’s imaging spectral range. This aim incorporates a hyperspectral imager capable of imaging at 100 frames/second rate in the 750-850 nm range as well as development of techniques (for example fiber-based), to provide high spectral sampling which directly translates to higher imaging depth of snapshot OCT systems.

1.1.3 Specific aim 3

Develop a proof-of-concept snapshot 3D-OCT.
A proof-of-concept snapshot 3D-OCT system was designed and characterized. Depth assessment, 3D visualization and initial biological imaging were performed to demonstrate the snapshot OCT capability. An analysis of the theoretical constraints was conducted to guide developments of future systems with increased imaging depth and improved axial and lateral resolutions.

1.2 Overview

The dissertation, with the focus on developing a novel snapshot OCT system, describes designs of hyperspectral and interferometry systems, their characterizations and experiments to improve snapshot OCT qualities. Below is the detailed dissertation’s outline accompanied by a graphical illustration (Figure 1-).

Chapter 1 provides a summary of the work in its entirety, including how individual designs contribute to making a high-performance snapshot 3D OCT.

Chapter 2 introduces both the medical and technical backgrounds of OCT to create a baseline for system’s further improvements. This chapter also compares and contrasts various hyperspectral imaging modalities and determined the suitable techniques for OCT application.

Chapter 3 explains the required design space to develop a snapshot OCT system using a snapshot image spectrometer. As the design guideline for the work in the following chapters, Chapter 3 covers requirements in spectral ranges and pixel counts among other design decisions.
Chapter 4 focuses on the design of an IMS-based hyperspectral system as the preliminary step for 3D-OCT. This chapter describes an experimental comparison between this design and the previous hyperspectral imagers as well as a study of transient biological experiments.

Chapter 5 describes the design and characterization of the proof-of-concept 3D-OCT system based on image mapping. It also examines the system’s performance for further improvements.

Chapter 6 explores the evolutions of material choices and fabrication processes to develop a fiber-based component. This work focuses on adjusting the datacube size to enhance OCT’s imaging depth.

Chapter 7 concludes the thesis with the summary results of the above research, and discusses future developments of the snapshot 3D-OCT and plans for system’s experimental verification in my post-doctoral work.
Figure 1: Work flow presented in this thesis organized according to topics and chapters
Chapter 2

Background

2.1 OCT in biomedical and clinical applications

While OCT’s applications cover widely across many different sub-categories in biomedical imaging, ophthalmology is one of its most significant applications. The technology has been established as a standard-of-care for glaucoma and AMD, two leading causes of adult blindness [2] as well as other ophthalmologic diseases including central serous chorioretinopathy (CSCR), macular holes, photoreceptor atrophies and white dot syndrome. Many new imaging techniques have been developed to improve ophthalmological care such as ultrasonography, confocal microscopy and scanning laser ophthalmoscopy [3]. However, they either require physical contact and/or provide low axial resolution and depth range, making them inappropriate for in vivo cross-sectional retinal imaging with high resolution [3, 4, 5, 6]. OCT is preferred over other techniques thanks to its non-invasive, quantitative and accurate qualities in disease diagnosis and treatment. Since OCT requires back-reflected signal from the sample, the high transmittance of the ocular media makes OCT an ideal method for imaging. Different from confocal microscopy, OCT balances spatial resolution for the unique long working distance and deep penetration. Typical, depth range of OCT is from 1 to 2 mm, while spatial
resolution varies between 10 and 20 μm [7]. Axial resolution depends on the coherence length of the source, and is from 5 to 10 μm [7]. Such ophthalmological 3D structures are crucial for many early disease detection [8]. Additionally, the depth resolution of OCT does not depend on the beam aperture of the sample, making it ideal to image the highly sensitive layer structure at the eye’s fundus [9]. As a result, OCT has become the routine test to examine the posterior eye [8].

AMD is well-known in both developed and developing countries as the main cause of irreversible blindness in patients 60 years old and above. Figure 2-a shows one of the most recent studies where OCT was used to diagnose and monitor patients of wet and dry AMD by measuring the choroidal thickness. The images on the left show the color fundus photographs, while those on the right display the choroidal layers and their variability in thickness for statistical studies.

**Figure 2:** OCT imaging for AMD and glaucoma assessment.

a: Choroidal thickness correlated to AMD severity [10]. b: Sub-retinal delinearization and different degrees of retinal pigment epithelium’s detachment showing in a serious case of AMD [8]. c: Optic nerve head in a significant glaucoma case imaged by Spectral domain optical coherence tomography. BV: blood vessel, G/IPL: ganglion cell/inner plexiform layer. INL: inner nuclear layer. ONL: outer nuclear layer. OPL: outer plexiform layer. RNFL: retinal nerve fiber layer [10].
The diagnosis of the second-leading cause of blindness in the world, glaucoma, is also an important OCT application. Accounting for more than 2.5 million cases of blindness in America and many more worldwide, glaucoma will potentially have 3.36 million more patients in 2020 due to the aging population [11, 12, 13]. The incidences of glaucoma could be significantly greater given the symptomless onset and many unregistered blindness cases around the world due to lack of clinical availability [14, 15].

Without the use of OCT, it has been shown that the current "gold-standards" in this irreversible disease diagnosis are highly subjective and dependent on individual physicians, which could leave many patients untreated and create unnecessary stressful false positives [16, 17]. Meanwhile, the need for early detection is crucial to slow down, or in some cases, to stop the progress of the disease before permanent vision loss occurs [14]. The most distinguishable marker in glaucoma is the thinning of the optic nerve fiber layer, which can be imaged distinctively with the use of OCT as shown in Figure 2-c.

Figure 2-c: Cross-sections and reconstructed longitudinal coronary images
Apart from its application in ophthalmology, OCT is also an emerging optical modality for many different medical applications, especially when it is integrated with other imaging techniques. Among the established and commercialized applications is cardiovascular OCT, an invasive catheter-based technique which offers a much higher resolution of coronary arteries compared with conventional ultrasound as seen in Figure 2- [18]. This technique brings detailed information for coronary artery disease diagnosis. A wavelength around 1300 nm is usually chosen due to the scattering and absorption characteristics of tissue [18].

![Figure 2-: Example of OCT-integrated endoscopy](image)

a: Comparison between tubular adenoma and normal colon tissue [8].
b: Images of gastric tissue biopsy [19]

In gastrointestinal (GI) imaging, OCT is usually adapted to endoscopy to deliver cross-sectional images with high resolution to assist conventional analyses (Figure 2-) [19]. It has been proved in GI pathologies that early detection and the pre- and post-treatment assessments all require characterization of 3D architectural morphology [20]. OCT is a
relevant tool to detect early stages of dysplasia in Barrett’s esophagus and acute inflammatory bowel diseases [21, 22, 23]. When analyzing other types of cancer such as skin cancer, OCT is also applied to characterize the abnormal accumulation of melanin by recording the interference from the highly scattering skin layers [24].

2.2 OCT Principle

Analogous to ultrasound, OCT measures the back-scattered signals to obtain depth-resolved information. The concept behind OCT is based on encoding sample volume reflectivity in the Fourier-domain coefficients of the interferogram spectrum. While ultrasound uses high frequency sound waves echoed from tissue to characterize different structures, OCT offers a much more refined resolution of tissue-level structures by using scattering light from the sample. The time delays in ultrasound are slow enough for direct electronic detection, since its velocity is 1500 m/s in water in contrast with the light’s velocity of $3 \times 10^8$ [25]. OCT signals are detected by correlating lights from a flat, reflective reference surface and the sample. Another attribute that sets OCT apart from ultrasound is that OCT does not require direct contact with the sample or liquid immersion [25]. Depth resolution below 5 μm is usually reported for OCT [26, 27].

OCT delivers high axial resolution and fast response with an increased depth range compared with confocal microscopy and other optical techniques. As shown in Figure 2-a, the simplest OCT system consists of light coming from a low coherent source into a beam-splitter, where it is divided between the sample and reference arms. In the common arm where the two beams are recombined, interference is formed between the electric fields
of the sample and reference arms $E_{sam}$ and $E_{ref}$, respectively, and is recorded at the detector’s plane. This interference fringe can only be obtained if the offset in path lengths of two arms are within the coherence length of the source.

![Diagram of OCT Principle](image)

**Figure 2**: OCT Principle

a: Setup of a simple OCT system. b: Effect of objective’s NA in OCT’s depth of focus [25]

The irradiance $I$ measured by the detector, on the other hand, is directly related to $E^2$. Ignoring the constant scaling, the total irradiance is given by:

$$I = (E_{sam} + E_{ref})^2 = I_{sam} + I_{ref} + \sqrt{I_{sam}I_{ref}\cos(kz + \phi)}$$

The third term in the equation above is the interference of interest caused by the path length mismatch between the two arms. This path length difference can be reconstructed by taking the Fourier transform of the interference spectrum in wave-number domain, i.e. in k-space. The interference signal detected by the camera can be rewritten as a function of wave-number ($k$), the light source’s signal ($S$) and the path lengths in the system ($r$ as path length of reference arm and $z$ as path length mismatch).
Additional terms in the above equation are the refractive index $n$ and the light’s amplitudes from the two arms ($a_R$ and $a(z)$).

Spatial resolution in OCT is directly related to the numerical aperture (NA) of the beam, similar to the conventional confocal microscopy. OCT systems are usually designed to have low NA beams so that the depth of field is longer than the coherence length, as its spot size is given by $\Delta x = \frac{4\lambda f}{\pi d}$ (Figure 2-b) [25]. As expected, the depth of field in OCT is higher with a lower NA: $2z_R = \pi(\Delta x^2)/2$. The axial resolution, however, depends on the coherent length of the source, thus is independent of OCT’s depth of field: $\lambda z = \frac{\ln 2}{\pi} \frac{\lambda^2}{\Delta \lambda}$. The axial depth range depends on the spectral sampling $\delta \lambda$ at the center wavelength: $z = \frac{2(\lambda_0^2)}{4n\delta \lambda}$.

Considerable research has been carried out to develop and enhance OCT for better imaging quality and easier accessibility. Current OCT technologies surpass the simple system (Figure 2-) to deliver greater speed, less encumbrance and more precision. OCT is generally divided into two types depending on system configuration and image acquisition.

**Time-Domain OCT (TD-OCT)**

The first and simplest OCT type is TD-OCT which is based on the simple system presented in Figure 2-a. Within every scan, the detector measures the interference from the back-reflected and back-scattered light at a specific axial and transverse location.
Therefore, TD-OCT requires lateral and axial scanning for both reference and sample arms to obtain a complete 3D datacube. Due to the requirement of a cumbersome scanning mechanism in both arms, TD-OCT is prone to instability and vibration. Its lack of high speed is also another drawback, making this setup less desirable for many biological and medical imaging applications [28].

**Fourier-Domain OCT (FD-OCT)**

The next category of OCT technology is termed FD-OCT, which has advanced over the slower and lower SNR conventional TD-OCT [29]. Its two variations include SD-OCT and Swept-source OCT (SS-OCT).

In SS-OCT, a tunable monochromatic source is used to illuminate the reference and sample arms, while the interference signal is detected by a point photodiode [30]. SD-OCT usually utilizes a broadband superluminescent diode laser (SLD) to carry interferometric beam in the spectral domain instead of sweeping a narrow-band laser across its spectrum like SS-OCT. Developments in SD-OCT recently established the significant increase in detection sensitivity for FD-OCT over TD-OCT, which can be used to increase imaging speed and signal-to-noise ratio (SNR). FD-OCT also has less dependence on vibration and more mechanical instability [1, 31]. Its speed is fast enough for video-rate and real time performance [32, 33, 34].

**SD-OCT description**

A conventional SD-OCT system for retinal imaging consists of a NIR SLD source. The Gaussian shape in the source’s spectrum is usually preferred due to its ability to generate
non-rippled Gaussian shape coherence peaks in the Fourier domain. Linnik geometry is more stable and creates less aberration from the beam-splitter compared with Michelson and Mirau geometries [25, 35]. The two identical objectives for OCT tend to have low NA (e.g. 0.1) to increase the depth of field. The beam is dispersed by a diffraction grating and imaged onto a line-scanner.

Figure 2: FD-OCT layout and results
a: System Layout. b: Interferometric fringes obtained with a line scanner. c: Depth profile of a flat surface after Fourier transform. d: Position of mirror surface relative to zero-OPD position. e: Different fringe frequencies indicating different sample’s depths. f: widening effect related to position of a moving mirror on the sample arm.

Data Acquisition and Calibration

As the line-scanner records the signal in spectral domain, calibration steps are required to generate depth profile. Background subtraction and flat-field correction are applied to the signal by imaging a dark spectrum and a white source, respectively. The dispersion by the grating is approximately linear in wavelength domain, while the Fourier relationship between the spectrum and depth profile requires the spectrum linear in wave numbers. Fourier interpolation (i.e. zero-padding) is applied to increase the signal’s digital sampling. Although this step does not provide any additional information about the signal,
it makes the next step, linear interpolation for wavelength-wavenumber conversion, more effective. Such interpolations are required since Fourier transform does not allow unequal sampling.

There are other alternatives for Fourier transform, though they usually suffer from slow computations, making them unsuitable for real-time imaging. One example is the Lomb-Scargle method which is based on the calculations of least squares and sinusoids of data and thus, does not require multiple interpolation steps for linear sampling [36]. Lomb-Scargle was tested in real time together with the conventional Fourier transform for result comparison which verifies its slow computation. As a result, Fourier transform are used for depth reconstruction in all of the OCT experiments mentioned here. Note that Fast Fourier transform for unevenly sampled data is also available and can be further explored [37].

Additional post-processing procedures are also carried out to reduce certain OCT’s inherent artifacts, including point-spread-function (PSF) removal, dispersion compensation and depth decay reduction. Dispersion compensation is required due to the variation in the sample’s medium. Such refractive index mismatch can be digitally corrected fairly easily by multiplying the signal with an exponential term representing the effective path length difference. Another calibration obstacle in SD-OCT is the loss of sensitivity as the sample moves away from the zero optical-path difference (OPD) position. The decay along the depth (z axis) can be observed in Figure 2-f and quantified in the equation below:
\[ R(z) = \frac{\sin^2 \left( \frac{\pi z}{2d} \right)}{\left( \frac{\pi z}{2d} \right)^2} e^{\left( -\frac{\pi^2 \omega^2 z^2}{8 \ln^2 \left( \frac{z}{d} \right)} \right)} [33] \]

where \( \omega \) is the ratio of spectral resolution and the sampling interval, while \( d \) is the maximum scan depth. Not only does the sensitivity of the signal suffer at a further depth, but the resolution is also reduced because the sample moves away from the focal plane, especially in complex tissue structure. This artifact can be easily observed when the sample arm is mounted on a high-precision translation stage for a uniform movement. Figure 2-f displays the movement of a mirror moving away and back toward the zero OPD position. As expected, as the mirror goes away from the zero-optical path length (OPD), the signal becomes slightly dimmer and broadened.

2.2.1 Motivation for high resolution, fast and stable OCT system

Although Fourier-Domain OCT (FD-OCT) is now firmly recognized and widely used, both spectral-domain and swept-source/optical frequency domain imaging embodiments still require scanning elements. More research has been done to minimize scanning, which results in the line illumination and CCD-based spectrometer to obtain a 2D subsurface image in snapshot [38]. With the implementation of a one-axis moving part (galvano scanner, moving stage), the technique images a line of the sample and reference mirror, thus requires only one scanning axis to obtain a 3D structure [39, 40, 41, 42]. Moving parts can limit the system’s compactness, which is an important factor in systems miniaturized for endoscopic applications, and can introduce motion artifacts.
Moving parts can limit the system’s compactness, which is an important factor in systems miniaturized for endoscopic applications, and can introduce motion artifacts. The artifacts caused by movements and vibrations of the sample or of the scanning mechanism itself can result in blurred or non-continuous images, and potentially inaccurate clinical interpretation [43]. This effect is worsened when the samples are dynamic objects (Figure 2-) [1, 33]. Snapshot imaging modalities capture light in parallel instead of raster scanning a focused beam, potentially allowing imaging with reduced illumination power or increased frame rate [44].

Figure 2: Heartbeat-Induced Axial Motion Artifacts in Optical Coherence Tomography Measurements of the Retina [43]

Efforts to reduce the number of scanning elements has led to line-illumination [21, 40] and full-field [45] approaches in OCT. The former technique images a line on the sample and reference mirror, hence requires only one scanning axis to obtain a 3D structure [39, 41, 42]. Full-Field OCT as exemplified by AC Boccara et al. can provide real time in vivo imaging without lateral scanning, albeit with acquisition of multiple phase-shifted images, rather than single-shot [46]. Subhash et al demonstrated a version of FF-
OCT where the requisite phase-stepped images are all captured in a single camera snapshot by distributing each image to a separate region of the image sensor [35, 47]. This method could, therefore, deliver snapshot en face (XY) FF-OCT imaging at a single axial (Z) location; however, generation of a 3D volume required recording of multiple camera acquisitions. Therefore studies were carried out to investigate methods of obtaining (x,y,λ) in one camera frame.

2.3 Snapshot hyperspectral imaging

Hyperspectral imaging has been a crucial tool in a variety of biological and biomedical research. Not only is the method indispensable in multi-fluorescence detection and tracking, the datacube (x,y,λ) generated from hyperspectral imaging can also be converted to the Fourier domain for depth assessment in OCT. Furthermore, the similarities between their developments enable interchangeable snapshot 3D research. Hyperspectral imaging methods capture spectral information at each spatial (XY) location in a 2D scene, but have traditionally required spectral or spatial scanning to acquire the full spectral datacube.

2.3.1 Imaging spectrometers in biomedical imaging

One of hyperspectral imaging’s main applications in biomedical optics is fluorescence imaging, which can act as an important complimentary method with OCT. Its common applications include the tracking of sub-cellular dynamics [48] and studying of gene expressions [49] by staining cells with one or more fluorophores to create high-contrast and colorful images. The expansions of fluorescence imaging such as creating
more fluorescent proteins [50] and nanoparticles [51, 52], especially those with similar spectral signatures, together with the development of multiplexed staining methods [53] have been the driving force for more rigorous imaging technologies to capture all the spatial and spectral details. Currently, there are different imaging technologies with various strengths when applied to fluorescence microscopy. For example, scanning confocal spectral imaging can yield fine spatial resolution with high-throughput result, but lacks in speed and uniformity due to its point-by-point spatial scanning approach. The technique’s typical performance is 5 frames/s for the size of 512-512 pixels [54]. The implementation of acousto-optic tunable filter (AOTF) or liquid crystal tunable filter (LCTF) for fast wavelength switching [55, 56] suffers from poor throughput due to filters’ light loss. Other scanning modalities including the frequency-scanning Fabry-Perot arrays [57], the phase-scanning Fourier-transform imaging spectrometers [58] and scanning hyperspectral techniques can have fast performance but generate scanning-related artifacts [59]. Therefore, it is desirable to employ snapshot imaging concept for hyperspectral imaging to quickly and uniformly obtain high-throughput datacube of (x,y,λ).

2.3.2 Snapshot hyperspectral imagers

Snapshot hyperspectral imaging can capture the full datacube within one camera’s integration span. This emerging and fast-growing field can be roughly divided into two subcategories of indirect and direct imaging. The indirect imaging approach implies complex post-processing computations from an encoded image, while direct approach involves a direct division of amplitude, field or spectrum [59].
Figure 2: Examples of computationally intensive techniques.
Top: CASSI’s optical train in the single-disperser configuration, and example of the raw data when the image has three wavelengths. Bottom: CTIS’ system layout [59]

Computed Tomography Imaging Spectrometer (CTIS) [60, 61], Coded Aperture Snapshot Spectral Imager (CASSI) [62, 63] and Snapshot Hyperspectral Imaging Fourier Transform Spectrometer (SHIFT) [64, 65] are examples that demand extensive computations which slow down the acquisition and data reconstruction, and generate computational artifacts. SHIFT utilizes tilted birefringent crystal prisms and a lenslet array. Each sub-field formed by the array is subject to a different OPD, creating a 3D interferogram cube. Thus Fourier transform is required to reconstruct spectral data. The system was reported to be very compact and easy to fabricate. In CTIS, tomographic
reconstruction is required to separate the mixed spatial and spectral data from multiple projections on the image (Figure 2-). The technology offers a compact system, although common to other similar computation-based imagers, CTIS requires complex post-processing computations [59]. Its missing cone effect caused by the lack of information at the vertex of the multi-directional projections also renders it challenging to reconstruct low-frequency signals.

Similarly, CASSI allows a compact setup to capture 3D hyperspectral cube. The technique applies compressive sensing algorithm to convert a vectorized 2D image into a 3D datacube. It relies on the number of constraints in the algorithm to limit the unknowns for better datacube accuracy. It has been shown that a series of image snapshots are often required to provide a successful reconstruction [66].

IRIS is an example of the spectral division technique in which a full-field image is divided into a finite number of monochromatic images recorded simultaneously. In addition to its polarization-dependent chromatic aberrations, IRIS requires a large-format Wollaston polarizers with sufficient birefringence and can only provide up to 8-32 spectral bands so far [67]. Although the spectral sampling is too low to be considered for OCT application, IRIS is the first system that can provide multi-spectral bands optically without any data reconstruction.

Direct imaging is preferred for system simplicity and significant reduction of computational artifacts. System iterations and developments have been carried out over recent years for the field-division hyperspectral imaging technique. Several snapshot
Hyperspectral imagers based on the direct division of the image field have been developed and commercialized. Only recently have these techniques become more feasible with the development of large-format camera. Among the prominent systems are the microlens array-based HyperPixel Array™ Imager (Bodkin Design & Engineering, LLC) [68], the fiber-based HyperVideo (Opto-Knowledge Systems, Inc.) [69] and the faceted mirror-based IMS.

The techniques mentioned above produce direct spatial and spectral imaging by separating an image into spatially different zones without complex computation. In the HyperPixel Array design, the 2D image is resampled by an array of pinholes. A prism is placed at the direction that provides the longest dispersion for rays from the pinholes without overlapping data (Figure 2-). The setup of this system is not only compact, but it also affords simple calibration thanks to its uniform pupil geometry. The spatial and
spectral samplings are dependent on the number of lenslet elements in the micro-lens array and the lenslet pitch respectively.

Figure 2: 2D fiber bundle redistributed into one -directional fiber line [70]

The second technique, HyperVideo, can provide a higher spectral resolution which is more desirable for OCT applications. Due to technological difficulties, the current limitation of the technique is the simple fiber geometry which leads to low sampling. The newest system can create a datacube \((x,y,\lambda)\) of \((44\times40\times300)\) by incorporating 4 cameras [69]. Similar approaches were investigated by different groups to align a rectangular fiber bundles into a straight line for one-directional dispersion [71, 70, 72]. Despite the limited spatial sampling, this system can give a higher spectral resolution without system limitation.

IMS is a recently developed hyperspectral imaging modality which can map 3D information \((x,y,\lambda)\) onto a 2D detector in a single camera integration (Figure 2-) [73]. The concept is based on a modified slicing technique originally developed for astronomy applications, where the image is “sliced”, \(i.e.\) divided into segments with the use of a
custom faceted mirror. These segments are then dispersed to provide spectral image in snapshot mode. Image slicing uses separate detection system for each segment and thus consists of as many spectrometers as segments of an image. IMS is a special implementation of slicing were image strips are grouped to allow acquisition of a spectral image using single image sensor and allow to limit the number of used spectrometers. The groups are defined as spaced lines of an image deflected into same direction. Several strips/lines are imaged by same re-imaging optics which allows to make system compact.

Figure 2-: IMS layout
a: Detailed IMS system adapted to a Zeiss inverted microscope (Key components highlighted in red boxes) b: Simplified IMS layout with key components [67]

The key component of the IMS is a mapping mirror called also mapper, which consists of multiple facets of different 2D tilt angles. By slicing the large image into discrete strips and regrouping these strips with void space in between them, the mapper creates a pupil array equally spaced for dispersion later in the optical train. The system consists of as many sub-re-imaging systems as the number of segment groups. The number of
spectral samples achievable by IMS is directly proportional to the number of these groups. While IMS is compact and light efficient it also requires tedious and more complicated calibration process comparing to other direct-imaging techniques (HyperVideo and HyperPixel™ Array) due to the non-uniformity among individual facets in the fabrication process as well as different alignment of re-imaging sub system.

2.3.3 Selection of snapshot hyperspectral imager for OCT imaging

A quick comparison of the techniques mentioned above can be found in Table 2- where their advantages and disadvantages are listed side-by-side. The decision about the potential techniques suitable for OCT imaging is based on four criteria. First and foremost, the OCT system must have a comparable depth range to the current scanning modalities. This translates to finding an imager capable of providing high spectral sampling. Second, the system should have the potential to provide a relatively large field of view (e.g. 100x100 samples). Third, light coherence has to be maintained throughout the system to ensure the depth information carried in the signal’s phase is not mixed. Fourth, the system should be easy to calibrate for clinical setting and not introduce additional computational errors.

Since OCT post-processing procedure requires data reconstruction from the spectral to depth domains, additional computation would extend the necessary processing time as well as make processing more prone to artifacts. Both CTIS and CASSI are already subject to reconstruction artifacts which can potentially compromise the overall imaging quality. These artifacts can be somewhat mitigated with increased
iterations which however prolongs the reconstruction cycle. Therefore, the risk of compromising the signal quality deems the indirect imaging techniques less suitable for OCT imaging.

According to Table 2-, both of the micro-array concept behind the HyperPixel Array™ Imager and the fiber bundle concept from the HyperVideo system can be compact and allow simple calibration. The spatial sampling of the former technique depends on the number of lenses in the lenslet array. Spectral sampling on the other hand is a function of lenslet pitch and the size of the image sensor. Lenslets with higher power to provide smaller point spread function are critical to high spectral sampling systems. Prior implementations demonstrated relatively small datacubes mainly due to application of off-the-shelf lenslet arrays [68]. They reported spectral sampling of 90 with spatial sampling of 55x44. System implementations often require pinhole array (to limit crosstalk) which can limit the overall light throughput. Meanwhile, the spatial sampling of the latter is directly related to the fiber counts. Prior literature reports image geometry capable to provide low spatial sampling (10×10 [71], 16×16 [70], 14×14 [72] and 44×40 [69]) but sufficient spectral resolution for OCT. Although the current reported datacubes from either of the techniques do not have high sampling, both can give promising results with system redesign and be applicable for OCT. One important consideration for spectral imaging for OCT system is the necessity to maintain interference signature throughout the system. Therefore fiber based spectrometer must utilize single mode fiber to transfer interference between bundle’s input and output.
The IMS system provides high light throughput and spatial resolution. For example, previously reported IMS systems were capable of obtaining spatial sampling of 285x285 and 350x350 while light throughput was 50-80% [74, 75]. Spectral sampling on the other hand was 45-60 depending on the implementation. To achieve higher spectral sampling, system needs to be redesigned to allow larger number of sub-systems (re-imaging) and larger number of tilts implemented in the mapping mirror. Another alternative is to vary spatial sampling in two imaging directions and allow more void space between the lines for more spectral sampling (This approach was applied in the proof-of-principle implementation - see Chapter 5).

Based on above analysis we decided to pursue two development directions: (1) development of a proof of concept OCT system based on image mapping and (2) development of fiber bundle technology for snapshot spectrometers. First implementation allows to quickly reach spatial resolution of 100-200 spectral bins and demonstrate the snapshot OCT principle. This approach can also provide high light throughput critical in bio-imaging applications. I will present a snapshot proof-of-concept OCT system based on this technique, and deliver thorough analysis and design to evaluate this system. Detailed designs and fabrication techniques in both cases are described in Chapter 5. The results from this implementation will provide an important input to future advancement of IMS for high spectral sampling. On the other hand, development of custom fiber bundle technology has a great potential as it can result in simple optical layout system, simple calibration and high spectral/spatial resolution. The fiber-based technology was selected as there is no fundamental system limitation for spectral
sampling besides the sensor’s size. In addition, this system has the potential to be tunable and to balance spectral/spatial parameters in real time.
### Examples of Indirect Imaging

**System's name**

- CTIS
- CASSI
- SHIFT
- IRIS
- HyperPixel Array™ Imager
- HyperVideo
- IMS

**Technique’s concept**

- Using a 2D disperser to create raw image
- Separating overlapping data with computed tomography
- Using a binary-coded mask to encode the image
- De-multiplexing raw image in post-processing
- Using tilted birefringent crystal prisms and a lenslet array to create various optical path differences
- Using Wollaston beamsplitting polarizer to apply wavelength retardation
- Using micro-lens array to pixelize and break down images
- Using tilted birefringent crystal prisms and a lenslet array to create various optical path differences
- Using a fiber bundle to separate image in one direction for dispersion
- Using a faceted mirror to redistribute images

**Advantages**

- Compact
- Efficient in using pixel counts
- Simple setup and calibration
- Compact
- Simple calibration
- High throughput

**Disadvantages**

- Difficult to manufacture the dispersive element and to calibrate
- Difficult to implement hardware
- Time-consuming algorithms
- Prone to artifacts
- Substandard result, especially for large datacube [66]
- Suffering from parallax effect [65]
- Require Fourier transform to convert to \((x,y,\lambda)\)
- Polarization-dependent chromatic aberration
- Limited spectral band (up to 8 in current system)
- Datacube size dependent on micro-lens array
- Spectral and spatial sampling limited by array's pitch
- Current system requiring multiple cameras
- Dependent on the number of lenslet array elements for spectral sampling
- System performance

**Reported \((x,y,\lambda)\) cube size**

- 203x203x55 [61]
- 604x480x53 [76]
- 250x250x16 [65]
- 55x44x90 [77, 78]
- 44x40x300 [69]
- 44x40x300 [72]
- 355x350x41 [74]

Table 2:: Comparison of multiple hyperspectral imagers
Chapter 3

Design space for snapshot 3D-OCT

*Parts of this chapter’s contents have been published in the following journal article: T.-U. Nguyen, M. C. Pierce, L. Higgins, and T. S. Tkaczyk, Snapshot 3d Optical Coherence Tomography system using image mapping spectrometry. Opt Express 21, 13758–13772 (2013) and the preparation of the following journal articles: T.-U. Nguyen and T. S. Tkaczyk, Development and characterization of high-throughput and fast-performance Image Mapping Spectrometer (IMS) (2014)

3.1 Design choice for OCT’s interferometer

3.1.1 Determining spectral range of operation

Typical spectral ranges in OCT include the regions around 800 nm, 1000 nm and 1300 nm depending on different cell types (e.g. retinal imaging vs. skin imaging), as discussed in Section 2.1. In the development of snapshot 3D-OCT, 800 nm was selected to be the main imaging range. The NIR region is the spectrum of interest in ophthalmic imaging, arguably one of the most common applications for OCT. The region offers reduced scattering and increased penetration depth compared to the visible wavelength range, while avoiding increased attenuation within the vitreous due to higher absorption of water in higher spectral region. Additionally for the purpose of snapshot OCT imaging, a 2D camera is required instead of the typical point sensor or line scanner. Considering the spectral sensitivity of a typical camera later shown in Figure 3-, 2D large-format image acquisition at 1000 nm or 1300 becomes unlikely with the current silicon-based sensor
technology. Note that this decision was not a result of a conceptual limitation but rather instrumentation availability.

3.1.2 Choosing SD-OCT as the snapshot OCT’s interferometric component

As Time-Domain OCT requires multi-dimensional scanning, FD-OCT became an obvious choice for snapshot OCT (see Section 2.2). SS-OCT requires an expensive light source and a point photodiode, making it challenging to acquire all spatial and spectral data at the same time. Thus SD-OCT was chosen to build a snapshot OCT system. A complete SD-OCT system was built and described in the description of OCT’s background (Section 2.2).

3.1.3 Light source requirements

The interferometric technique and the operational spectral range are the deciding factors in light source selection. SD-OCT demands a low coherent broadband superluminescent diode laser (SLD) on which the interferometric fringes are superimposed. As the optimal spectral range of roughly 800 nm lies beyond the visible spectrum, the first snapshot OCT system (Chapter 5) was built with a red LED for ease of alignment and system’s evaluation. The spatially incoherent LED source (\(\lambda=633\) nm, FWHM=13.5 nm) covers a relatively low spectral range of 50 nm, limiting the axial resolution \(\lambda z\) (see Section 2.2). Additionally, the low central wavelength of 633 nm is proportional to the depth range to the 2\(^{nd}\) order, as shown in the following equation: 

\[
z = \frac{2(\lambda_0^2)}{4n\delta\lambda},
\]

thus significantly reduces the depth range. This parameter is heavily
dependent on the central wavelength ($\lambda_o$) and spectral sampling ($\delta\lambda$). The light source selection is also based on the criteria of NIR range and optimally its large bandwidth.

![Graphs showing spectral intensity and coherence function](image)

**Figure 3:** Relationship between light source and axial resolution

Figure 3- shows two promising light sources and their corresponding axial resolution. Although the first light source generates more ripples due to its non-Gaussian spectral shape, the two diodes incorporated in this light source enable a higher axial resolution (5.4 vs. 6.5 µm). Thus, the SLD 35 HP (Superlum Diode, Ltd. $\lambda_0$=825nm, $\lambda_{FWHM}$=62nm) was selected for the design of further implementations of snapshot OCT system. This light source covers the spectral band of 100 nm, which significantly increases the axial resolution (see Section 5.8.1 for more design explanation).
3.2 OCT’s spectrometer considerations

Developing a snapshot OCT system relies mainly on converting a single line spectrometer in SD-OCT into multiple miniature line- or point- spectrometers by separating the image into different zones. Coupled with the interferometer, this spectrometer has to preserve the light coherence, maintain high throughput in the NIR range and provide spectral sampling dense enough to provide a depth penetration of 2 mm, while still delivering high spatial sampling.

3.2.1 Effect of sampling-pixel relationship on datacube size

To illustrate the potential for future development and scaling of the snapshot 3D-OCT, the fundamental relationships between spatial and spectral-depth imaging parameters, as well as resolution were investigated. Using a single image sensor (CCD or CMOS) to capture hyperspectral data \((x,y,\lambda)\) requires a trade-off between pixels used for spatial and spectral resolution. When this datacube is converted from \((x,y,\lambda)\) to \((x,y,k)\) to \((x,y,z)\) to obtain a 3D volume in this snapshot 3D-OCT, the original trade-off becomes one involving spatial points, axial range, and axial resolutions. In conventional spectral-domain OCT, axial range is determined by the system's spectral resolution, while axial resolution is inversely related to the spectral bandwidth collected. Given a finite number of pixels in a SD-OCT line-scan camera, one can only increase axial range at the expense of axial resolution, and vice-versa [79]. In snapshot 3D-OCT, the product of spatial and spectral pixels \((N_x \times N_y \times N_\lambda)\) cannot exceed the total number of camera pixels.
Figure 3 presents the relationship between the number of resolvable spatial points in each of the X and Y directions, imaging depth, and camera pixel count, in IMS-OCT. This analysis assumes that the system accommodates sufficient spectral bandwidth to achieve 10 µm axial resolution, and that there are two axial pixels per axial resolution element (Nyquist's criterion is exactly met).

![Figure 3: Effect of camera pixel count on 3D datacube size for a system operating at 10 µm axial resolution](image)

3.2.2 Camera selection

Based on the sampling-pixel relationship, as well as the requirements for low readout noise and high spectral response in the NIR range, there were three cameras utilized in this dissertation: the Apogee camera with large chip and low readout noise, Imperx with the largest chip for a maximized datacube, and the pco.edge which can
provide the best sensitivity in NIR and fast readout. The advantages and disadvantages of the cameras were tested in different optical systems throughout this dissertation.

The first camera was an Apogee Alta U16M, 16 MPxl, 9 µm pixel size. It was connected to a laptop via a USB cable and controlled with the LabVIEW 2009 environment. Designed for astronomy applications, the camera could perform well under low light condition. One of previous IMS systems developed in Tkaczyk lab was also built around this camera, making it convenient to develop a proof-of-concept IMS-based snapshot OCT system. For alignment and other fast acquisition purposes, the camera can bin images prior to acquisition and/or capture 12-bit images. Otherwise, operating in snap-shot mode, the camera can capture a full-frame 16-bit image containing 4096×4096 pixels. Despite its advantages, the camera’s connection to the computer results in slow data readout. Typically, a period of roughly 10 s was required after every snapshot to completely transfer the full image to the computer, making it more challenging for system alignment and high speed imaging.

The proof-of-concept system described in Chapter 5 used the Apogee 16 MPxl camera, but collected light from only every fourth facet of the mapper (Section 5.3.2). This arrangement uses only 3.5 MPxl, with (85×356) spatial points and 117 spectral pixels. Binning consecutive groups of 4 pixels in the direction along the facet length allowed the presentation of the image data in Figure 3- with an equal points in X and Y (85×85). Figure 3- illustrates how the use of all 16 MPxl’s would have enabled either deeper imaging, or
additional spatial points. While theoretically feasible, use of all camera pixels would require a redesign of the IMS optical train.

<table>
<thead>
<tr>
<th></th>
<th>Imperx Bobcat camera</th>
<th>Pco.edge sCMOS camera</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Spectral sensitivity at 800 nm (%)</strong></td>
<td>10</td>
<td>30</td>
</tr>
<tr>
<td><strong>Acquisition speed (fps)</strong></td>
<td>4.65</td>
<td>100</td>
</tr>
<tr>
<td><strong>Dynamic range (dB)</strong></td>
<td>66</td>
<td>88.6</td>
</tr>
<tr>
<td><strong>Potential data cube size (x,y,λ)</strong></td>
<td>256x256x400</td>
<td>96x96x600</td>
</tr>
<tr>
<td><strong>Depth range (mm)</strong></td>
<td>1.22</td>
<td>1.83</td>
</tr>
</tbody>
</table>

Table 3: Performance comparison between Imperx Bobcat camera and Pco.edge sCMOS camera

The Bobcat Imperx 29 MPxl (B6640) with Camera Link® Medium, 5.5 pixel size is an improvement over the Apogee 16 Mpxl thanks to its live preview, 4-tap capability and high pixel counts. As illustrated in Figure 3-, this pixel count can potentially allow imaging to depths of over 1 mm in tissue, with 256×256 lateral pixels. The camera was incorporated in the optical train to test the fabrication process of the Fiber Bundle Imaging Spectrometer system in Chapter 6. While the large format makes the camera attractive for snapshot imaging modalities, its quantum efficiency of less than 10% at 800 nm (Figure 3-), high noise and maximum full-frame speed of 4.65 frame-per-second (fps) make the camera not ideal for live imaging [80]. Note that this is by far the largest widely available chip, thus can afford the largest datacube size.
The third camera, pco.edge sCMOS camera (2560x2160, 6.0 µm pixel size), was manufactured based on a scientific-grade CMOS (sCMOS) sensor. It was the largest widely available sCMOS chip, and was incorporated in a hyperspectral imager design described in Chapter 4 to confirm its performance. The camera was connected to a full 10-tap Camera Link PCI-Express card via two Camera Link cables. The computer was specially built with 24 MB of RAM and a motherboard capable of fully keeping up with the camera frame rate. With 16-bit Analog to Digital Converter (A/D) and the dynamic range of 27,000:1 (88.6dB vs. 66dB of the Bobcat), the camera could acquire data at up to 100 fps. Thus this camera can perform much faster data acquisition without compromising on light efficiency, as its
quantum efficiency at 850 nm is 30% at the same NIR range [81]. Although lower pixel counts compared to the Bobcat can imply less pixels dedicated for spectral sampling, as reflected in Figure 3-, the fabrication of the fiber-based device built in Chapter 6 promises greater separation between two adjacent spatial fields. Thus the benefits of the sCMOS camera outweigh its pixel counts.

### 3.2.3 Requirements for high spectral sampling

Imaging depth range is the key consideration in snapshot OCT design due to the 2D camera limitation previously explored. As the snapshot OCT system takes advantage of the SLD-35-HP Superlum Diode parameters, the imaging depth can be represented as a function of spectral sampling in Figure 3-. This calculation assumes the central wavelength of 780 nm, and the total illuminated spectral band of 100 nm.

![Graph showing imaging depth as a function of spectral sampling.](image)

**Figure 3-**: Imaging depth as a function of spectral sampling

Figure 3- supports the sampling-pixel relationship analyzed earlier in which over 1mm of depth range can be fulfilled with the 29 Mpxl Bobcat camera (specifically 400 spectral bins are required and image size could be 256x256). To further increase the depth
to approach 2 mm, it is required to have at least 650 pixels dedicated for spectral
dispersion. On the other hand, to improve sensitivity and imaging rate, the system can be
designed with lower spatial sampling, e.g. 96x96x600 when incorporated with the fast
pc.o.edge sCMOS camera. Note that high frame rate OCT implementation can be
combined with effective video mosaicking to increase imaged area and at the same time
minimize motion artifacts.
Chapter 4

Development and characterization of high-throughput and fast-performance IMS*

*The contents of this chapter are being prepared for submission as the following manuscript: T.-U. Nguyen and T. S. Tkaczyk, Development and characterization of high-throughput and fast-performance Image Mapping Spectrometer (IMS) (2014).

This chapter introduces a new implementation of an Image Mapping spectrometer. The system is capable of delivering the datacube \((x, y, \lambda)\) of \((210, 210, 60)\) in snapshot mode. All of the prior IMS generations were limited by the readout rate (see Section 4.4.2) and were designed for operation in a visible spectral range. The system described here provides ability to image in the NIR region (quantum efficiency of the chip spans over 1000 nm while comfortable bandwidth is in 800-900 nm range) and can perform data acquisition at up to 100 fps. To the best of my knowledge, it is the fastest snapshot hyperspectral imager reported. The system’s performance was dictated by chip optical parameters and physical dimensions and drove an entirely new optical design of optical and opto-mechanical systems. To maintain the system’s telecentricity and maximize sampling, a compact prim-lenslet module was integrated in close proximity to the sensor. Experiments were conducted to compare the light throughput of this new IMS model versus the previous IMS systems in literature.
4.1 System description

The system was built as a stand-alone hyperspectral imager to isolate its performance from the interferometry component in the OCT system. It can be applied in a wide-field setup or and in microscopy applications. The input of the system, also the conjugate plane of the camera, is placed at the image plane from the fore-optics such as a microscope.

IMS provides a direct approach for hyperspectral imaging, enabled by advances in large format detectors and the development of a component termed image mapper. The mapper was custom-fabricated by micromachining. It contains long, thin tilted mirror facets to redistribute the image zone into separate sub-imaging systems. The mapper breaks down an image spatially into a 5x6 pupil array and regroups facets 30 elements apart into the same sub-pupil. This facet geometry allows void space among facets of the same sub-pupil for prism dispersion. The system records this hyperspectral image onto a large format camera via an array of lenslets.

This image is relayed and magnified to match the mapper size by a pair of Zeiss 2.5x objective and tube lens enclosed in a long lens tube. The spot size created at the mapper is designed to be 75µm, same as the width of the mapper’s facet for diffraction limited performance, which then sampled at 2 pixels to maintain Nyquist sampling. To capture the large and high-NA beam reflected by the mapper facets, the Olympus MVPLAPO 1X objective (NA=0.25, WD= 65mm, FOV=34.5mm) was used to prevent vignetting or clipping.
Figure 4: System layout

a: System schematic overlaid on SolidWorks design. (1) Field stop (2) 2.5X Zeiss EC Plan Neofluar® Objective, (3) spectral filters, (4) Zeiss tube lens, (5) Image mapper, (6) MVXPLAPO 0.63X Olympus objective, (7) Custom-made prisms array and 30 achromatic doublet lenses with specially designed precision array holder, (8) pco.edge sCMOS camera. b: Actual system mounted on the optical table.

A pupil array of 5x6 sub-pupils can be observed after the objective, at which a prism-lenslet array were inserted to individually image them onto a large sCMOS camera. This PCO pco.edge camera (pixel=6.5µm, 2560×2160) was selected thanks to its high
speed, high dynamic range and low readout noise. The prism-lenslet array contains a custom-designed array of 5 long prisms (H-ZF62, 20°) and 30 achromatic doublets (d=2mm, f=12mm).

4.2 Design and fabrication

The system is mounted on a breadboard using commercial and custom-made optical and mechanical components. What set this IMS system apart from other IMS designs in literature is its high speed, sensitivity and capability of imaging in both visible and NIR bands. The design criteria were dictated by sCMOS chip parameters: its physical dimensions, pixel size and total pixel count.

4.2.1 Fabrication of mapping mirror

In the image mapper, group of facets deflected selected image lines into specific direction so it passes through unique area of stop of the large collimating lens. Therefore each tilt direction uses different area of the pupil. The sub-pupils are further coupled with dedicated small re-imaging lens. This lens creates an image of image lines (of selected group) a unique zone of sCMOS detector. The IMS has 30 re-imaging systems with prisms placed in the pupil plane. The pupil image can be observed in Figure 4-e. An objective was placed after the mapper to collect these reflective rays into a 5x6 array.

Mapper design

Figure 4-a shows that the mapper consists of 7 identical V-shaped blocks of 30 facets. One block of 30 facets (Figure 4-b) constitutes to 6 sets of different y-tilts and
redirects light into 6 columns in Figure 4-e. The 5 sub-pupil rows in Figure 4-e are the result of the different x-tilts within one group, each has 5 facets (Figure 4-c).

Therefore, the 210 facets are distributed so that every 30 consecutive facets belong to different sub-fields in the 5x6 matrix, i.e. two adjacent facets’ images in the same sub-field are 30 facets apart on the mapper. For example, the second facet and facet #32 on the mapper counting from one side reflect light to the sub-pupil in the first row and second column. Figure 4-d shows the actual mapper’s group under a white light interferometer (Zygo New View) to confirm its optical mirror quality and uniformity.

Figure 4:- Mapper visualization

a: Mapper viewing from one side. b: One block of 30 facets (as shown within the red rectangle from part a) with exaggerated x-tilts. c: One group of mapper (as shown within the blue rectangle from part b) with exaggerated y-tilts. d: microscopic image of 5 consecutive facets of the same y-tilt and different x-tilts. e: 30 zones separated by the mapper.
Mapper fabrication

The mapper was created from a long rod of pure Aluminum (Al) due to the metal’s properties including malleability and low carbon impurities to produce low roughness. A thin metal slice (roughly an inch thick) was hand-sawed from the rod before mounting into a CNC machine (Hass Automation®, Inc.) to create the mapper substrate, as seen in Figure 4-b. (see more details about mapper substrate fabrication in Appendix A)

![Mapper fabrication images](image)

Figure 4: Mapper fabrication

The substrate had a protruding 1×1” rectangle surrounded by four through holes in the corners for mounting on the diamond turning machine. To achieve the precise angle tilts and optical surface quality, the substrate was fixed on a four-axis Nanotech® Ultra Precision milling machine. Two ruling tools were mounted orthogonally onto a stationary machine spindle. In the ruling procedure, one tool at a time was ruled orthogonally to the
mapper surface in the direction of the designed facets. The two tools were precisely secured and positioned to make sure their tips touched the mapper substrate at an exact height (within ~1 µm of precision) when each tool was rotated orthogonally to the mapper’s surface. The precision of this crucial alignment is aided with microscope mounted next to the diamond turning for live adjustment. Test cuts were also made on a substrate with both tools. The substrate was then examined under the white light interferometer for iterative tool adjustments until the two cuts were less than 1 µm apart in height. The carbide tool created the rough cuts, accounting for the different depths across the facet length to create the y-tilts.

After the rough cut step, the thickness of the deepest cut to the very top surface was 803 µm. Next, the machine spindle tilted the 75-µm wide diamond tool to different angles ranging from -1.83° to 1.83°. This angled tool carved into the substrate one facet at a time before the spindle changed its tilt to create 5 rows in the pupil image. To maintain the surface quality of the substrate and the tools’ longevity, the depth of cut of every pass was maintained to be from 20 µm to 2 µm until the desired depth was achieved. The final mapper (Figure 4-d) had the overall surface waviness of ~65 nm when measured under the white light interferometer. This effect was caused by the tool vibration during fabrication, but has not created any discernable artifact in imaging experiments. The mapper’s local roughness was under 10 nanometers for mapper reflectivity. More description of the ruling technique applied for this mapper can be found in literature [82].
4.2.2 Prism-lenslet module design

The prism-lenslet module was designed so that the final dispersed image could match the camera size and the dispersion could cover beyond visible range without compromising imaging quality. Depending on different filter sets, the system can cover several sets of spectral range including 410-532 nm, 514-850 nm (Figure 4-d) or the whole visible range of 480-660 nm. Note that non-linear dispersion is the reason for the difference in spectral spread.

To accommodate the sensor chip size while maintaining the system’s telecentricity and resolution, the system entails a set of 30 miniature lenslets with short focal length (12mm). The size of these lenslets was considerably smaller than those in previous IMS designs (2mm in diameter vs. 6.5mm). This setup required a more difficult packaging design involved high-precision opto-mechanics in close proximity to the camera. The doublets’ focal length and the camera’s cover glass effectively reduce the working distance between the camera and the prism-lenslet down to roughly 5 mm. The high dispersion required for long spectral spread also dictated the material and angle of the prisms and their short distance to the lenslets (Figure 4-c).

Since the system can pan from 514 nm to 850 nm in wavelength, the effect of point-spread-function (PSF) broadening and nonlinear dispersion can be observed. Figure 4-a illustrates the spectral nonlinearity specific to the prism nature in the design, where the lower wavelengths cover more pixel counts on the camera chip. At the average wavelength of 620 nm, the system can perform at diffraction limit, depending on the sub-
pupil location (Figure 4-b). The PSF is approximately 18 µm to satisfy Nyquist criterion at the camera sensor.

Figure 4:- Prism-lenslet optical design
a: Dispersion curve along the spectrum. b: Point-spread function at 620 nm. Black curves show Airy disk size. c: design of 2 prisms and lenslets in the array. d: spectral spread from 514 nm to 850 nm in one sub-image

For a broad spectral spread, a highly dispersive element (H-ZF62) was chosen with the refractive index of 1.92 at 600 nm. The 2mm×16 mm prisms have the wedge angle of 20° and can disperse light from 6 lenslets on the same row. The distance from every two adjacent rows is 2.67 mm, thus a thin shim made of black plastic was inserted between two prisms to maintain the correct spacing and block stray light. The angle between the lenses and prisms are illustrated in Figure 4-b. A rectangular Aluminum alloy block was machined with a corresponding angled opening, where the prisms and shims were glued together by a UV-curing optical adhesive (Figure 4-b and Figure 4-).
Figure 4-: Prism-lenslet array

a: Previous potential designs for lenslet holder. b: Metal prism holder design c: Final design of prism and lenslet holders looking from the opposite side of the camera. d: Actual prism-lenslet assembly looking from the camera side held together by two dowel pins and screws on the two sides (not visible in picture). f: Assembly of prism-lenslet array onto the camera.

Manufacturing the lenslet holder, however, required more attempts due to the lenslets’ tight tolerance, small size and their requirement for repeatability (for 30 lenslets). Traditional machining and 3D printing were unsuitable, since the former option used hard metal and risked chipping the lenslets; and the latter contains oily, waxy residue which could damage the lenslets’ surfaces. The inconsistency in 3D printing shrinking factor along different directions also makes it less ideal for fabricating the lenslet holder.
Hence, I considered an alternative strategy - a laser-cutting of opaque polymer material whose thickness was roughly the same as the lenslets’. Figure 4-a shows two of different lenslet designs based on laser cutting on a 2-mm black sheet of polyoxymethylene, a thermoplastic called Delrin®. On the left image of Figure 4-a, the lenslets can lean against two walls created by adjusting two different laser powers for cutting, while the opening sides can secure the lenslets with a rubber band. The image on the right displays another option where small cantilevers were cut out from the material to exert a small force against the lenslets to keep them in place. Although these options might be useful in similar designs, their complications relating to choosing miniature rubber bands, and to maintaining the cantilever’s precision along the material thickness without burning the material deem them unreliable. Laser cutting was still crucial in creating small fixtures. As the laser power is the strongest at the waist of the laser’s depth of focus, the laser produces the sharpest cut at this plane. Careful alignment can place the
laser focal plane on the top surface of the material for cutting so that the cut-out shape resembles half of the beam waist. The resulting opening’s cross-section after laser-cut is shown in red in Figure 4-b-d. This opening can sufficiently hold the lenslet on one side; while the other side is covered and secured with a thin black Delrin® cutout (~50 µm in thickness). The thin lens cover was aligned with the lenslet holder by a pair of dowel pins. Two miniature screws attached the lenslet holder and cover to the machined metal prism holder’s threaded holes. This composite was inserted to a metal circular plate that fitted outside the camera outer ring, hence creating a secured unit of prism-lenslet-camera.

4.3 Image Processing

4.3.1 Data Acquisition

The raw image includes 30 sub-images of the object imprinted on the mapper facets, as the camera is placed at the conjugate plane of the object and mapper surface (Figure 4-a).

4.3.2 Calibration

The Fast IMS system was calibrated based on the procedure described in Bedard et al [74]. Enhancement and further work were made to improve the calibration speed and quality as well as to adapt to the system’s specific requirements, such as employing an automatic filter wheel for fast filter transition, using a different set of filters and applying a de-striping method to further enhance the uniformity of the final image. As a consequence of the broad spectral range, a different set of narrow-band filters were
employed including 532, 589.6, 632.8, 656.2 and 785 nm. Note that the 785 nm filter has strong transmission at the range lower than 600 nm, so another high pass filter was employed to prevent spectral leaking.

The input image is mapped onto the mapper surface, and then imaged at the camera sensor. Thus the exact relationship between one point at the mapper and the corresponding pixel location is vital in image reconstruction. The calibration process can be divided into three steps. First, an experiment was carried out which involved recording images of a 5µm-wide slit moving across the FOV in both X and Y directions. This procedure not only repositioned the facets but also located the exact locations of 5 wavelengths from each facet by rotating the automatic filter wheel containing 5 narrow band filters through the system. In the X-scan, the slit was mounted on a translation stage and positioned parallel to the mapper’s facets. A series of different facets’ locations created from the X-scan were added up to create a location-dependent weighted sum. A partial of this weighted image can be observed in the left image of Figure 4-, where the brightness of the facets trails off along the vertical direction of the image. In Y-scan, the slit is positioned orthogonally to the mapper facets with the presence of a narrow-band filter, effectively creating a sampling point for both spectral and spatial directions as can be seen in Figure 4-. Second, all the calibration images were processed for centroid fitting, facet repositioning and spectral polynomial fitting [74].
Figure 4-a-b shows an all-white image after the second step when all of the facets were rearranged to the correct positions. The look-up table was generated for further imaging. Different from the first two calibration steps which are only required once, the third step, flat-field correction, needs to be repeated before every experiment session, and when there is a change in lighting condition. It consists of taking a white-field image for flat field correction to correct the irregularities on the mapper surface, the optical train, the CMOS sensor and the stray light from the environment. For example, as a US Air Force (USAF) resolution target was placed in the microscope connected to the system, the camera displayed the raw image as in Figure 4-d. The \((x,y,\lambda)\) datacube was then created from the previously calibrated look-up table. This image was divided by the full-field image, i.e. white image in Figure 4-a and b. The result was a full-field corrected datacube, one of whose spatial surface is shown in Figure 4-c.
Figure 4-: Image reconstruction example

a: Raw image of white image. b: Reconstructed image of white image without flat-field correction. c: Image of an USAF resolution target after flat-field correction. D: Raw image of USAF resolution target.

Minor post-processing can also be further introduced such as applying a de-striping technique, and to add a virtual mask when necessary to digitally crop out the image. Striping is unnoticeable in most situations but in a few fluorescence imaging experiments. Since the signal is created from the sample itself, it is more challenging to create a flat-field correction image through the same optical train. The de-striping process includes three steps: (1) creating a 2D low pass filter with a very wide and not very tall window, (2) creating a 2D high pass filter with very wide and very short window and (3) adding these two filtered images to create a stripe-free image [83].
4.4 Experiments

4.4.1 High throughput fluorescence experiment on BPAE cells:

The new design and fabrication processes allow this IMS system to acquire images much faster than previous generations. In this experiment, a prepared BPAE slide of MitoTracker® Red CMXRos, Alexa Fluor® 488 Phalloidin, and DAPI (FluoCells®) was used to initially validate the system’s sensitivity. The sample was imaged with a microscope objective (5X Zeiss EC Plan Neofluar® Objective) and relayed onto the front end of the IMS system, thus the total system magnification is 2.3x throughout the entire optical train between the sample and sensor.

Figure 4- shows that MitoTracker® Red CMXRos and DAPI can be easily observed, and the system can provide sufficient visuals for fluorescence imaging at a frame rate of 50 fps, much faster than the previous IMS systems whose highest frame rates in brighter conditions were recorded to be 5.2 fps [84] and 7.2 fps [74, 85].
Figure 4: Examples of fluorescence imaging over different integration times. 

a, b, c, d represents images taken with 500 ms, 200 ms, 100 ms and 50 ms integration time, respectively. Left: spatial cross-section at 536 nm. Middle: spatial cross-section at 626 mm. Right: RGB images. Note: all images were background subtracted and normalized.

An identical imaging experiment of the same prepared sample was taken with one of the first IMS systems when one image was obtained at the rate of every 10 seconds.
[67]. In this new system, the obtained datacube consists of 60 single wavelength images. Selected wavelength images are shown in Figure 4-.

4.4.2 Side-by-side throughput comparison between the ‘Fast’ IMS system and previous generations

More detailed comparison between the Fast IMS and the most recent IMS generation was carried out to ensure the experimental conditions were identical. The same prepared slide of BPAE cells were used as the standard imaging target for both systems.

Figure 4-: Throughput comparison between the previous and current IMS systems

Note: all images were background subtracted and normalized.

Figure 4- demonstrates a series of experiments with the two systems at different frame rates. At 100 ms of integration time, the images on the bottom of Figure 4- displays higher contrast images taken with the Fast IMS than acquired with prior IMS implementation (top). As the frame rate increases, the performances become more
noticeable, as graininess is clearer in previous system regardless of the post-processing improvements including intensity normalization and background subtraction. Spectral separation at 50, 20 and 10 ms observed on the top of Figure 4- further proves the previous generation’s inability to deliver an image well above noise level compared with the current system.

4.4.3 Fast experiment with Flp-In-CV-1 cell line

Another hyperspectral experiment was carried out in collaboration with Diehl Lab, Rice University. In this experiment, the sample was Flp-In-CV-1 cell line (Life Technologies™) that had EGFP-SKL fusion stably transfected using the PiggyBac cumate switch (Systems Biosciences, LLC). Flp-In-CV-1 cells, taken from African Green Monkey kidney, are rapid generation of stable cell lines which contains a single integrated FRT site from pFRT/lacZeo. The cells was induced with 30ug/ml cumate for 24 hours by David Tsao from Diehl Lab. A 60x oil-immersion Zeiss objective was used to match the sample size, creating a total magnification of ~28x. Figure 4- illustrates the abundant amount of light captured by this IMS system with frame rates far higher than ever reported. The datacube contains 60 spectral images, 4 of which are displayed along the columns of Figure 4-. They display consecutive spectral sampling from 509 nm to 518 nm within the emission filter window. Different rows of the figure show the exposure times of 100, 50, 20 and 10 ms, which effectively indicate the frame rates of 10, 20, 50 and 100 fps, respectively and in chronological order.
Figure 4: Fluorescence imaging result of Flp-In™-CV-1 Cell Line at different integration times

As can be seen from the images of 20 ms and 10 ms of exposure times, there is a visible decrease in dynamic range when imaging at high frame rate, as the noise floor and system’s artifacts can be noticeably observed. However, the signal is still highly discernable and useful for visual assessment. It means that not only video rate imaging would be possible, but also minimal amount of vibration would be averaged within each integration time. Additionally, when a video with high frame rate is taken, the rapid movement of the cells can also be observed for the study of cell dynamics. Small cross-sections from exposures of 50 ms and 20 ms were separated and analyzed in Figure 4. The graph further indicates the still discernable signal over the increasing noise floor as
the exposure changed from 50 ms to 20 ms. It also showed the movement of the signal from peaks 1a to 1b, and 2a to 2b during the experiment.

![Comparison of cross-sectional intensity](image)

Figure 4-: Comparison of cross-sectional intensity of the sample F1p-CV-1 Cell Line

In conclusion the study of the Fast IMS system theoretically and experimentally provided designs and fabrication methods to simultaneously obtain a hyperspectral datacube with high frame rate. Common OCT’s issues relating to motion-artifacts and lack of signals in low-light conditions are mitigated as the system has high acquisition rate. This enhances the system’s sensitivity to OCT’s faint scattering biological signals while still operating within the safe input power for biomedical applications.
Chapter 5

Proof-of-concept snapshot 3d optical coherence tomography system using image mapping spectrometry*

*Parts of this chapter’s contents have been published in the following journal article: T.-U. Nguyen, M. C. Pierce, L. Higgins, and T. S. Tkaczyk, Snapshot 3D Optical Coherence Tomography system using Image Mapping Spectrometry. Opt Express 21, 13758–13772 (2014).

In addition to hyperspectral imager designs, a proof-of-concept snapshot OCT system was built. The lateral (XY) dimension is acquired by use of wide-field Koehler illumination, while depth (Z) information is encoded in the interference fringe pattern captured by the IMS system's spectral (λ) dimension. This system can give depth information (Z) at different spatial positions (XY) within one camera integration time to potentially reduce motion artifact and enhance throughput. The (x,y,λ) datacube of (85×356×117) provides a 3D visualization of sample with 400 mm depth and 13.4 mm in transverse resolution. Axial resolution of 16.0 mm can also be achieved in this proof-of-concept system. To the best of my knowledge, this is the first demonstration of a snapshot 3D-OCT imaging using a hyperspectral imaging technique to provide volumetric data. This system also has the capability of increasing spectral sampling through system redesign elaborated in Section 5.8.
5.1 Adapting SD-OCT to a previously built IMS system

The original snapshot 3D-OCT system is based on the combination of Spectral Domain OCT (SD-OCT) and Image Mapping Spectrometry (IMS). In SD-OCT, interference patterns recorded on the line scanner carry information of optical path length mismatch between the sample and reference arms, indicating the depth distribution of scattering layers within the sample. The IMS technique is used as the spectral measurement component in the snapshot 3D-OCT system. A large-format 2D camera captures images of the array to obtain both surface and spectral information of the sample. The requirements for a successful 3D-OCT include no involvement of moving part/scanning stages, enough depth range and resolution for biological imaging.

5.1.1 Preliminary attempt to build a snapshot OCT system

Initially, the snapshot 3D-OCT design process was started by utilizing the IMS system described in Gao 2010. In the previous IMS configuration, a single prism (ZF6,10°) was employed to disperse light spanning over a wide spectrum (450-650 nm) into 60 spectral bins [67], resulting in a datacube of 285×285×60. However, OCT requires long imaging depth which depends on spectral sampling, and 60 spectral bins at 633 nm, 50 nm band width can only give 167 μm depth. Due to the requirement of high spectral resolution within a narrower optical bandwidth for FD-OCT, I used a ruled diffraction grating (600 lines/mm) for higher dispersion. This high dispersion could potentially lead to spectral overlapping among sub-images within one sub-field, thus the number of sub-images was reduced to give more space for spectral information from dispersion. The set
of lenslet array holders in Gao et al were redesigned to accommodate the new pupil geometry. For initial assessment, a mask for the mapper was 3D-printed to cover all but only one group of mapper facets so that there would be only one sub-image within one sub-field, leaving more space for dispersion. For simplification, the design and layout description of this intermediate system are not discussed here since the more completed layout will be shown in the later system iteration.

Figure 5: Preliminary fringes obtained when previous IMS and SD-OCT are combined

a: Raw data of 25 sub-images
b: Reconstructed depths corresponds to fringes in 25 sub-images
c: Cross-sectional depth (A-line) from the center sub-image

The dark and bright horizontal bands in one sub-image are spatial features from the USAF resolution target embedded with interferometric fringes. This fringe indicates the resolution target’s position relative to the reference mirror’s position. The bottom
section of Figure 5- illustrates the uniformity of the 25 spectra's shapes across the large field CCD.

This preliminary setup proved that the IMS system has the ability to obtain at least 25 sub-images encoded with both spatial and spectral information, thus providing a full 3D datacube for OCT requirements. However, the system can only image a very small section of the mapper to prevent spectral overlapping, resulting in very limited spatial region (only 25 out of 285 mapper facets). Further designs were made to more appropriately match the specifications of OCT and the IMS system.

5.1.2 Preliminary IMS-OCT system specification

The previous setup was able to show fringe visualization. A proof-of-concept 3D-OCT system was built afterward to reconstruct 3D features with higher depth and resolution. This system can provide a datacube of 85×356×117 pixels. The designed specifications are shown below:
<table>
<thead>
<tr>
<th>Description</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Center wavelength ($\lambda_0$)</td>
<td>633 nm</td>
</tr>
<tr>
<td>Spectral bandwidth ($\lambda_{FWHM}$)</td>
<td>13 nm</td>
</tr>
<tr>
<td>Total capture bandwidth ($\Delta \lambda$)</td>
<td>50 nm</td>
</tr>
<tr>
<td>Number of spectral pixels (N)</td>
<td>110</td>
</tr>
<tr>
<td>Wavelength captured at each pixel ($\delta \lambda$)</td>
<td>0.1 nm</td>
</tr>
<tr>
<td>Axial range (z)</td>
<td>0.40 mm in air</td>
</tr>
<tr>
<td>Lateral resolution ($\Delta x$)</td>
<td>13.4 µm</td>
</tr>
<tr>
<td>Axial resolution ($\Delta z_{FWHM}$)</td>
<td>16.0 µm</td>
</tr>
</tbody>
</table>

Table 5: Proof-of-concept system’s design specifications

5.2 Proof-of-concept 3D-OCT system principles

In the standard scanning FD-OCT, a beam is focused to a single point at the sample. However in this snapshot 3D OCT system, a full-field OCT configuration was set up with Koehler illumination in a similar fashion to the full-field OCT technique [45]. Since spatial and spectral information of a full-field image cannot be successfully extracted with a 1-dimensional linear array-based spectrometer, the system relayed an image of the overlapping sample and reference beams to the IMS.

Adapting IMS to the unique requirements of OCT requires redesign of previous IMS modalities. This new concept for snapshot OCT requires the IMS system to perform high spectral sampling within a narrow bandwidth (over 100 spectral bins within a bandwidth of 50-150 nm in the read/NIR region), in contrast to the earlier IMS systems which achieved lower spectral sampling (60 spectral bins across the entire visible range) [67].
5.2.1 Interferometry Arm

A spatially incoherent LED source (\(\lambda=633\) nm, FWHM=13.5 nm) was attached to an engineered diffuser for source pattern removal. The diverging beam was collimated by a condenser lens (\(f=40\) mm). An iris was placed next to the collimator for FOV control. Koehler illumination was established with the combination of lens L2 (\(f=75\) mm) and a microscope objective for full-field imaging. The Michelson-type interferometry objective (Zygo 2.5x, NA=0.074, WD=10.3 mm) had a built-in reference arm to minimize alignment variations between the two arms. A 300 mm focal length lens (L3) after the (50/50 non-polarizing) beam splitter BS1 collected the overlapping sample and reference arm beams and images them onto the mapper. The magnification (3.75x) created by the objective and lens L3 ensured that the FOV covered the entire mapper surface (roughly 1×1" square). 50% of the light exiting the interferometer was reflected at the second beam splitter (BS2) towards a reference camera (RC), which was used to capture the full-field surface image of the sample.

5.2.2 IMS Arm

Since general IMS modalities had been previously reported in literature [67, 73, 74, 86, 87] only key redesigns to meet OCT imaging' criteria are highlighted. Different from other IMS configurations [73], this OCT-adapted IMS system had the mapper positioned perpendicular to the incoming beam to achieve a uniform focal plane across the mapper surface. This setup reduced sensitivity to artifacts like sub-field image vignetting and pupil plane distortions, therefore simplified the mapper facet angle calculations [75]. In
addition, this configuration minimized adjacent facet blockage, as individual facets had different heights, which could potentially block parts of the light from other facets if the mapper is placed at an angle to the incoming beam.

Figure 5:- System Layout. a: System Schematic


Each facet deflected light to different angles toward the collecting lens L4 (f=80mm). Lens L4 organized the high NA incoming beams into different pupils, with the specific destination pupil depending on the mapper's facet tilts. A beam expander
consisting of two lenses, a 2" diameter, 50 mm focal length (L5) and a 3" diameter, 200 mm focal length (L6) lens, was used to match the pupil array size to the image sensor dimensions without clipping of the large array.

In previous IMS systems, a single prism (ZF6, 10°) was employed to disperse light spanning a wide spectrum (450-650 nm) into 60 spectral bins [67]. However, due to the requirement of much higher spectral resolution and narrower spectral bandwidth for FD-OCT, a ruled diffraction grating (300 lines/mm) was implemented for greater dispersion. The dispersed array of beams carrying spatial and spectral information in two directions were simultaneously mapped onto a large-format CCD camera (Apogee Alta U16M, 16 MPx, 9 μm square pixel size) by a lenslet array with adjustable focal length. This array set had two plates, each containing 25 lenslets, 6.25 mm in diameter to create telephoto lens combinations. The lenslet array's geometry was designed to match the dispersion angle from the diffraction grating.

5.3 3D-OCT mapper design and pupil distribution

5.3.1 Mapper fabrication method

Fabricated in-house, the image mapper was made of high purity aluminum (5N 99.999%) for high malleability and reflectivity. The earlier mappers used in IMS were fabricated using a raster-fly cutting technique on a four-axis Nanotech Ultra Precision milling machine [67]. Here I used a ruling technique which has been shown recently to exhibit several advantages over raster-fly cutting [88].
The previously used raster-fly cutting process is significantly slower than ruling, and it creates a large inconsistency in facet widths. In the ruling technique, the tool moves into the substrate from one side with a predefined cutting depth, gradually scooping the raw material out while translating across the substrate from left to right, as shown in Figure 5-a. This process creates a clean, highly uniform, reflecting surface as one thin film of aluminum is removed on each tool pass. The final surface roughness in ruling is under 10 nm and comparable with raster-fly cutting [87]. The included angle of the diamond tool can potentially damage adjacent facets during the ruling process. This challenge can be overcome by utilizing a tool with a narrower included angle to maintain the uniformity of the facet width. By using a tool with 5° included angle, the facet’s width variability was measured to be within 6.7%, in good agreement with previous mappers’ quality [74].

For fabrication, the aluminum substrate was mounted on a stage which can be translated along the machine's y axis as shown in Figure 5-a. To obtain sub-micron accuracy in tilt angles and surface flatness across each facet, two tools were mounted on the machine's spindle. For the initial rough cuts, a carbide tool created seventeen 1.5 mm wide passes across the 1” square substrate by maintaining the carbide tip stationary and orthogonal to the mapper substrate. During that time, the mapper substrate moved along the y axis with depth (x) values varying along the pathway. For the fine cuts, the machine spindle created pre-programmed tilts (x-tilts) before the 75-µm diamond tool cut into the substrate to create 20 uniform 75-µm wide facets, within each 1.5 mm wide carbide-tool pass. In a similar fashion to the rough cut, the mapper substrate traveled across the stationary and tilted diamond tip with very fine cutting depths, ranging from 20 µm down
to 2 µm in multiple iterations.

Figure 5: Image mapper fabrication

a: Mapper in fabrication. The substrate is mounted on the Nanotech milling machine. Two tools are placed on spindle prior to cutting facets. b: Reflection of ruler’s straight edge on the finished mapper. c: Mapper looking from the top. Different facet tilts are shown as variations in depth of cuts. d: Examination of mapper’s facets with white-light interferometer. e: Mapper looking from the front. f: Enlarged section of mapper looking from the front showing finer cuts for individual facets.

While the IMS-OCT mapper was designed to have 300 facets, each 75 µm in width, the actual fabricated component included 40 extra facets as a safety factor in fabrication,
and also to enable testing of the system in alternative configurations. As a result, some sub-fields contain images from 3 facets while others have 4. Divided into identical "blocks" of 100 facets, the entire mapper with 17 rough-cut passes thus comprise 3.4 blocks. Each facet in a single block has a unique two-dimensional angle to deflect light towards the collecting lens. Figure 5-b shows a ruler's straight edge being reflected as a zigzag pattern on the 17 rough-cut passes; a few of the individual facets can be seen in the white-light interferometry image in Figure 5-d.

5.3.2 Mapper design and pupil distribution

Each block of 100 facets is tilted so that light from the interferometer is reflected into 35 sub-pupils (Figure 5-). In this first-generation design, light from only every 4th facet, i.e. facets 1, 5, 9 ... are collected (Figure 5-b-c); light from the remaining facets is discarded outside the lenslet array in order to maintain enough void space in between pupils for subsequent dispersion. This results in 85 out of the 340 facets being used to direct light from the OCT interferometer to the camera. Starting from one end of the mapper, the first 20 facets share the same y-tilt and therefore redirect light onto the same horizontal row at the lenslet array. Facets spaced 20 steps apart (e.g. facets 1 and 21 in Figure 5-c) have the same x-tilt and thus, redirect light to a common column.
Figure 5: Mapper facet and pupil distribution

a: Facet tilt directions relative to mapper. b: Pupil distribution from one block of mapper (100 facets). Facets whose numbers are not shown are discarded in the leftmost and rightmost columns. c: Grouping and order of facets. Facet of the same y-tilt correspond to light grouped in the same row; and those of the same x-tilt correspond to the same columns. Thus two facets which are 100 facets apart have the exact same x and y tilts.

This geometry is repeated across the entire surface of the mapper such that the corresponding facets within each block (facets 1, 101, 201 ...) have identical x and y tilts, and therefore direct light to the same sub-pupil. Since facets 1, 101 and 201 are 100 facets \( \times 75 \, \mu\text{m} = 7.5 \, \text{mm} \) apart at the mapper, this distance between the images of facets 1 and 101 at the image plane creates the necessary empty space to be filled in with later dispersion from the diffraction grating.
5.4 Additional 3D-OCT design considerations

5.4.1 Cross-talks

There were two categories of cross-talk occurring in the system. The first arose from diffraction due to the 75-μm wide mapper’s facets, leading to light leaking from one sub-pupil to neighboring sub-pupils. This effect was termed spatial cross-talk [87]. With the use of a pupil mask in the pupil array plane, the spatial cross-talk level was reported to be 6% [86]. This level strongly depended on the surface quality of the mapper’s facets and the sub-pupil separation. When previously using the raster-fly cutting technique, the mapper facets were not perfectly flat, but had an optical power which broadened the beam and increased light leaking [87]. The new ruling technique used in this paper achieved facet flatness in the sub-micron range, ensuring that cross-talk caused by facets’ non-uniformity is minimized. The second type of cross-talk spectral, arises from the dispersion of individual sub-images within the tightly-packed array, with the red end of one spectrum potentially overlapped with the blue end of the next. To minimize spectral cross-talk, a band-pass filter (OD6) was inserted into the system, leading to spectral leakage in the 0.001% range.

5.4.2 NA matching

To maximize the ability of the system to produce high resolution, the sample resolution should match the facet width of the mapper and be sampled by at least by two pixels to satisfy Nyquist requirement. The resolution of the system is determined by various factors. Here, the basic equation $PSF=1.22\times\lambda fd$ is used where $\lambda$ is the center
wavelength of the light source, $f$ is the focal length and $d$ is the beam’s diameter. After mapping the interference signal from the sample and reference arms to the mapper, there is one more mapping step onto the camera’s pixels. The geometric relationship between the resolution at the mapper $x_{\text{mapper}}$ and the sensor $x_{\text{camera}}$ is given by:

$$x_{\text{camera}} = x_{\text{mapper}} \times \frac{f_{\text{array}}}{f_{\text{collect}}} \times \frac{75\mu m \times 61mm}{80mm \times 4} = 14.3\mu m$$

where $f_{\text{array}}$ and $f_{\text{collect}}$ are the focal lengths of the lenslet array and the collecting lens, respectively, and $M$ is the magnification of the beam expander. The focal lengths of all elements in the lenslet array are identical and can be obtained by adjusting the spacing between two array plates.

5.4.3 Grating Geometry and Lenslet Array Holder Design

The implementation of the diffraction grating instead of a prism changed its geometry in the dispersion direction, since the incoming and dispersing beams are of different angles. The incoming beam comes to the grating at $34^\circ$ relative to the grating’s orthogonal axis, while the dispersed beam ($21.5^\circ$) are of different angles with the grating’s normal axis. Therefore, one dimension of the square pupil array is stretched. The scaling factor for the horizontal axis is $\cos 15.5^\circ \div \cos 40^\circ = 1.26$. Depending on this ratio, a rectangular lenslet holder was fabricated using the 3D printing technology. This holder was designed to fit into the window of the camera for the shortest working distance possible. It is limited by the shutter of the camera, which is 26 mm away from its sensor.
5.5 Calibration for proof-of-concept 3D-OCT system

5.5.1 Data acquisition

The image is stored and opened in MATLAB® for further data processing. All images shown in this chapter were acquired with an exposure time of 125 ms.

5.5.2 IMS- OCT Calibration

Unlike other hyperspectral modalities, IMS does not demand extensive computation requirements for every image acquisition to generate spectrally-resolved images [67]. Post-processing for IMS-OCT includes one-time data extraction and alignments, followed by the same standard SD-OCT calibrations as described previously in section 0. The flow chart of the calibration steps can be seen in Figure 5-.

The raw image obtained from the proof-of-concept includes 25 vertically oriented sub-fields. Initial data processing starts with subtracting background to eliminate stray light by blocking signals from both reference and sample arms. Individual sub-images are extracted; and a 3D matrix of (x,y,n) is created. x is the transverse axis obtained by stacking multiple sub-images, while y is the axis along the facet length, and n is the dispersed spectral information in pixels. This extraction is determined by a relative threshold values for both horizontal and vertical directions. The blank space which separates the sub-images after dispersion is discarded. The order of captured facet images is designed prior to fabrication, thus can be easily determined. Verification of this order can be done by translating a mask at an image plane after the mapper and
observing the illuminated sections on the camera (Figure 5-a).

After the spatial calibration process rearranges the sub-images to their correct positions, an initial wavelength calibration is carried out. Before, calibrations were made by recording the signal when a narrow-band (NB) filter at 632.8 nm were placed after the light source. When the filter is tilted with respect to the optical axis, the transmitted spectrum is blue-shifted. This phenomenon gives the advantage of pinpointing two spectral values over a narrow bandwidth by mounting the filter at two different angles. The pixel locations of the two wavelengths were recorded by both of the IMS-OCT system and Ocean Optics spectrometer as the reference. Since dispersion from the IMS grating is linear in wavelength, the wavelength-pixel relationship can be easily calculated with two known locations. However, NB filters are designed for orthogonal illumination; and tilted beam compared with the filter surface can result in spectral widening and shifting [89], rendering this method unsuitable for the pupil-array concept in IMS. A different approach is made in which the source’s spectrum from both the reference spectrometer and the 3D-OCT system is fitted with a Gaussian shape as the spectrum’s height and FWHM are calculated (Figure 5-b). This calibration step generates the \((x,y,\lambda)\) datacube.
Given the estimated spectral values from the linear calibration mentioned above, the measured fringe data are then zero-padded and interpolated so that they are evenly...
spaced in wavenumber (k). A Fourier transform of the re-sampled spectra generate the
OCT axial scattering profile (A-line) for each individual spectral line. The spectral phase
obtained from an image of a simple reflector is used to iteratively adjust spectral values
based on the process described by Mujat et al. [90]. The calculated nonlinearity in phase
φ(k) is removed to compensate for errors in spectrometer calibration or dispersion
mismatch between sample and reference arms [91, 92]. Since the bandwidth of the LED
used here is relatively narrow (50 nm), dispersion mismatch effects are relatively minor.
A flat mirror was mounted on an axial translation stage to record different sample
positions for a one-time depth calibration. The corrected pixel-wavelength assignments
and depth scale are then applied on all subsequent data sets.

This one-time calibration series to convert the raw 2D image into a (x,y,λ)
datacube for subsequent image acquisitions Depth calibration is carried out by mounting
a flat and reflective surface on an axial translation stage; and different depth position is
recorded. DC components are also removed so only the interferometric components will
be considered for the next step (Figure 5-d).

Imaging a 1 mm grid provides sharp lines in the raw image which can be used to
vertically align each sub-image as can be seen in Figure 5-e. Further processing is also
required to minimize effects from distortion and magnification variation among the sub-
images. Gradients along the facet length are calculated so all the sudden changes in
gradient, i.e. the edges of the sample’s sharp lines, can be aligned and interpolated to fix
misalignment and distortion. Assuming that distortion along facet length is linear, the
new aligned and distortion-corrected data set is generated by linear interpolation.

Intensity correction is also carried out to compensate for brightness variation across different sub-images. The image of only the OCT reference arm is recorded. Ideally, this image should give a flat transverse image and a near-Gaussian shape in the spectral axis. Thus, all data points from the same wavelength, for example, from the images of a 1 nm narrow-band filter, are selected. All variations in this 2D image are compensated so this en-face image becomes a flat field (Figure 5.5.2e). The 2D scaling factor is stored and applied to the en-face surfaces of all wavelength, since it is assumed that the intensity variations are the same for all wavelength to simplify the calibration process.

After all of the calibration steps mentioned above, any raw image taken by the system can be readily processed for quick data reconstruction. A predefined mask extracts the sub-images; and wave-number interpolation is carried out given the known wavelength array. Converting into Fourier space, the depth profiles of all spatial points can be quantitatively reconstructed and visualized.
Figure 5 - OCT calibration steps

a: A segment of a raw sub-image with horizontal features from sample and vertical interferometric fringes. b: One spectral cross-section taken from (a). c: Calibrated spectra corresponding to the raw image in (a). Spectra along the facets form a gradient from black (610 nm) to white (640 nm). d: The initial wavelength-pixel relationship is fitted to a third-order polynomial. e: The calibrated wavelength after zero-padding to 512 data points to prepare for depth reconstruction. f: A spectrum of interferometric fringes with DC components removed. g: Depth profile reconstructed from the fringes shown in f. h: Relationship between wavelengths and the array indices. For a narrow spectral band such as that used here, this relationship is almost linear.

5.6 3D-OCT Imaging Parameters Characterization and Experiments

5.6.1 Preliminary experiments

Depth assessment

Figure 5 - shows data from a flat, reflective surface taken from the large 3D datacube at multiple depth positions, as the sample is mounted on a translation stage for this calibration experiment.
Figure 5: Snapshot 3D-OCT system’s depth assessment

a: Different depth positions of a flat, reflecting mirror mounted on a translation stage. b: Measured axial resolution from one representative transverse location. c: Relationship between peak pixel position and mirror physical position. Note that at the position around 400 µm, peak positions become undetectable, indicating the end of the imaging depth. d: Linear regression of the relationship between peak pixel position and mirror position.

Adjacent positions are 25.4 µm apart (Figure 5-a). After zero-padding and phase linearization processes, the average axial resolution was measured to be 20.9 µm over the depth range (Figure 5-b). Axial resolution of 16.0 µm can be obtained near the zero optical path difference (OPD) position. In addition, the axial position of each coherence peak is plotted against translation stage position in Figure 5-c. This confirms the expected depth
range of approximately 400 µm. The physical depth and pixel value relationship is established and fitted to a linear equation (Figure 5-c). The measured SNR for the coherence peak at a depth of 50 µm was 43dB.

**Multi-layer detections**

Another short experiment shows the 3D structure of impurity on the sample, glued by a clear tape (Figure 5-). Three sub-images are extracted and examined. Standard simple OCT calibration generates several different layers of surfaces, whose cross sections can also be observed. Inverted gray scale is applied in this case for clearer visualization.

![Figure 5: Preliminary multi-fringes in snapshot 3D-OCT system](image)

Fringes and depths obtained when a clear tape with impurity is applied on a mirror.
In this first-generation system, the performance is evaluated by imaging a USAF resolution target with clear tape on the front surface to produce 3D structures. The raw image of 4096×4096 pixels can be seen in Figure 5-a, while both the target's bars and interferometric fringes due to reflections at the clear tape can be observed in Figure 5-b.

![Figure 5-a: Simultaneous spatial and spectral visualization](image)

- a: Spatial features from resolution target.
- b: Interferometric fringes caused by resolution target.
- c: Interferometric fringes caused by clear tape.

### 5.6.2 3D visualization

The current system provides a datacube (85x356x127) from 85 facets of 356 pixels in length, being dispersed in 127 pixels. The final image shown in Figure 5- demonstrates a simple experiment in which 3D structure can be visualized after calibration algorithms are applied. The result was shown in the open-source MicroView 3D Image Viewer (Parallax Innovations).
Multiple surfaces can be observed along the depth in the 3D display as well as in the XZ and YZ cross-sections. Note that the dark bands on the 1st and 3rd surfaces from the right shown in Figure 5-c come from the resolution target’s spatial features. The second surface from the right was created by the interference between the tape’s two reflective surfaces, thus indicates the tape’s actual thickness. The bright DC component is left intact in Figure 5- for illustration.

Figure 5-: 3D structure recorded in snapshot mode from the 3D-OCT system. 

a: Reconstructed structure of clear tape on USAF target. b,c: Its XZ and YZ cross-sections. 

d: Transverse image from the reference camera.
5.6.3 Experiment with scattering samples

After interference fringes and 3D datacube were recorded from reflective objects, I tested the system with a simple but more scattering sample. A US dime was placed at the image plane (photographed with a conventional camera in Figure 5-a) and the ear's 3D shape on the dime was recorded and calibrated to obtain a 3D datacube of the same size as the previous experiment. However, due to the mapper's design (see Section 5.3.2), the resolution along the x-axis is four times higher than that along the y-axis. To maintain uniform sampling across the FOV, X-axis binning was carried out, as seen in the composite transverse view in Figure 5-b and transverse cross-sections at different depths of the ear in Figure 5-c. Four out of 85 curvatures on the dime's surface from 85 mapper facets are displayed in Figure 5-d.

Figure 5: System evaluation with simple 3D structural sample
a: A 2D image of an US dime taken with reference camera. b: Corresponding transverse surface acquired with snapshot 3D-OCT system. c: Transverse surfaces at different depths. d: Cross-sections along the depth range.
To investigate the potential for the IMS-OCT system to image biological samples, a 3D volume of a piece of onion was acquired. The power at the sample was measured to be $3.1 \text{mW/cm}^2$. Figure 5-a shows the regular en face 2D image taken from the reference camera, while Figure 5-b displays the reconstructed en face image obtained from the OCT system. Five transverse slices in the XY plane at various locations along the axial (Z) axis are shown in Figure 5-c, indicating different structures within the onion depth.

Figure 5:- 3D snapshot of a layer of onion placed on top of a scattering metal surface

a: Image of a layer of onion (bottom) on a metal surface (top) acquired with the reference camera. b: Transverse surface acquired with snapshot 3D-OCT system. c: Representative transverse sections at different (z) depths.

5.7 Proof-of-concept system’s evaluation

The proof-of-concept system has several compromises in the design. First, since only one out of every 4 facets are used for imaging, the sampling across the facet width is effectively four times lower than that along one facet. Second, the implementations of
two beam-splitters result in unwanted reflection from the walls of the cubes. The first beam-splitter creates an inverting shadow in all sub-fields on the sensor. This issue is temporarily circumvented by slightly offsetting the image’s FOV and implementing a mask to partially cover each facet image on an image plane. The second beam-splitter, on the other hand, creates a strong reflection to the middle sub-field, which is on the optical axis. Third, spatial resolution in this current system is limited by the lenslet array, due to the working distance limitation caused by the bulky shutter of the camera. Last, the tightly packed sub-images within one sub-field does not allow broader dispersion. This consequently affects the spectral sampling, and as a result, the imaging depth.

The depth range, axial resolution, and FOV for the system reported here were chosen to enable a first proof-of-concept demonstration of the IMS-OCT concept for 3D volumetric imaging. This setup is able to provide 85 sub-images in 25 sub-fields; each sub-image carries spatial features along one mapper facet's length as well as the interferometric fringes created from the reference mirror and sample. The lateral resolution (13.4 µm) and depth range (400 µm) meet the expected performance, while the averaged axial resolution of 20.9 µm is slightly larger than the expected value mostly due to the broadening effect along the depth of range in OCT. However, the measured axial resolution near zero OPD position (16.0 µm) where the broadening effect is insignificant meets the theoretical calculation of 15.9 µm.

The system described here uses a 16 MPx camera, but collects light from only every fourth facet of the mapper (Section 5.3). This arrangement uses only 3.5 MPx, with
(85×356) spatial points and 117 spectral pixels. Binning consecutive groups of 4 pixels in the direction along the facet length allowed us to present the image data in Figure 3- with an equal points in X and Y (85×85). Figure 3- illustrates how the use of all 16 MPxI's would have enabled either deeper imaging, or additional spatial points. While theoretically feasible, use of all camera pixels would require a redesign of the IMS optical train. The next generation system aims to take advantage of 29 MPxI image sensors which are currently on the market. As illustrated in Figure 3-, this pixel count will allow imaging to depths of over 1 mm in tissue, with 256×256 lateral pixels.

5.8 Improved design for high-performance snapshot 3D-OCT System

The experimental setup was not fully optimized for light efficiency in the first 3D-OCT system. An incoherent LED was used as the light source, which is replaced by an SLD source in the next generation system to increase the illumination power across the full FOV. The two 50/50 beamsplitters (BS1 and BS2 in Figure 5-) simplified alignment of the system, but reduced overall throughput by 75%. These components can easily be replaced by polarizing beamsplitters and waveplates to circulate light from the source to the detector (via the sample) more efficiently. At the IMS arm, the next system was designed with a different geometry, aiming to reach light efficiency at the level of 50-60% achieved in previous IMS systems [74]. Reducing the overall number of surfaces in the light path also reduces losses due to Fresnel reflections.

With improved light illumination and overall system throughput, future IMS-OCT systems can be expected to image with shorter exposure times (125 ms), with
corresponding increases in frame rates. The previously reported IMS systems demonstrated imaging in live biological tissue [74], therefore, it is believed there are no system-related limitations for future live biological tissue imaging with IMS-OCT. Currently for the 16 Megapixel camera in this proof-of-concept system, it typically takes 10s to transfer all acquired data to the computer via a USB port, and 9 s to generate a 3D (x,y,z) volume using MATLAB, on an Intel® Core™ 2 Duo chip.

A significant improvement involves higher density and more uniform sampling at the specimen. The proof-of-concept mapper only collects light from every 4th facet, discarding spatial information from the rest of the mapper to save space for dispersion. The setup leads to higher sampling along the facet length than across the facets, and requires necessary vertical binning for uniform sampling, as shown in the previous experiment. This uneven sampling issue is overcome in the high-performance system by redesigning the mapper facet geometry to utilize all the facets on the mapper. Not only is this an enhancement for spectral sampling and imaging depth, the beam expander removal also minimizes the blurring and distortion on the corner sub-fields.

The performance of this snapshot 3D-OCT system is tailored toward the requirements of OCT’s clinical applications. With spatial and axial resolutions comparable to regular OCT specification (5μm and 3.4μm, respectively), this system can provide valuable information for time-sensitive experiments and/or dynamic objects. The system is designed to be adaptable with a higher NA objective in front of the sample, thus pushing the system toward Optical Coherence Microscopy for a datacube of high spectral and
spatial resolutions. Combined with image mosaicking and effective data processing [93], this modality can provide a large structural field of data with minimal vibrational residues. The system also has a potential to be combined with fluorescence imaging to access both structural and chemical information within the epithelial layers.

5.8.1 IMS large datacube system design

In this the next IMS-OCT design, high sampling density enables biological imaging with greater depths and higher resolutions. Designed specifications include a 224×275×400 datacube with spatial resolution of 10μm. The spectrum of interest is the NIR region (λo=825 nm) to enable deeper imaging than the proof of concept system, while still using a silicon based camera. A 29-Megapixel camera will be used to accommodate this larger datacube.

The improved IMS-OCT system is designed to meet performance standard for OCT in biomedical applications, while uniquely provides the snapshot capability. As can be seen from Table 5-, spatial resolution of 10 μm and axial resolution of 4.84 μm are expected, and also typical for retinal imaging or other OCT modalities [7, 94, 95].

Based on the previous proof-of-concept design, this ultimate system will also consist of a full-field interferometer and a hyperspectral imager (Figure 5-). The NIR SLD source (SLD-35-HP Superlum Diode, Ltd. λo=825nm, λ_{FWHM}=62nm) was utilized for this new system to ensure the appropriate wavelength for biological applications, especially in ophthalmological imaging. Containing two diodes, the new light source creates a much
broader spectral range for high axial resolution. Koehler illumination is still maintained in the interferometric section.

<table>
<thead>
<tr>
<th>Description</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Center wavelength ($\lambda_0$)</td>
<td>825 nm</td>
</tr>
<tr>
<td>Spectral bandwidth ($\lambda_{FWHM}$)</td>
<td>62 nm</td>
</tr>
<tr>
<td>Total collected bandwidth ($\Delta\lambda$)</td>
<td>100 nm</td>
</tr>
<tr>
<td>Number of spectral pixels (N)</td>
<td>400 pixels</td>
</tr>
<tr>
<td>Wavelength spacing between pixels $\delta\lambda$</td>
<td>0.22</td>
</tr>
<tr>
<td>Axial range ($z_{RD}$)</td>
<td>777 $\mu$m</td>
</tr>
<tr>
<td>NA</td>
<td>0.053</td>
</tr>
<tr>
<td>Lateral resolution ($\Delta x$)</td>
<td>10 $\mu$m</td>
</tr>
<tr>
<td>Axial resolution ($\Delta z_{FWHM}$)</td>
<td>4.84 $\mu$m</td>
</tr>
<tr>
<td>Confocal parameter $b$</td>
<td>598 $\mu$m</td>
</tr>
</tbody>
</table>

Table 5-: Proposed system’s parameters

The interferometric objective is still employed for easier alignment and stability. All the components in the system were redesigned to match the new spectral range, mapper and camera. Figure 5- shows the elimination of the second beam-splitter to prevent unwanted reflection. Instead, the mapper facet angles will be designed to redirect light to a large mirror without obstructing the incoming beam.

The new system is expected to provide a uniform and large datacube of higher axial resolution, continuous spatial sampling and higher imaging depth. The optimal solution for higher spectral dispersion is to design a new mapper. As mentioned in the previous subsection, the proof-of-concept system has 100 facets of different angles
repeating themselves about three times so that each sub-field has 3 or 4 sub-images. Since it was proved to be a limiting factor in capturing more spectral pixels, the new mapper has $14 \times 16 = 224$ facets of all different angles.

Figure 5: Proposed schematic of the high performance snapshot 3D-OCT system

This change in design eliminates the non-uniformity in sampling across the facets while improving depth range with higher numbers of spectral pixels. In this setup, the beam-expander is discarded to eliminate additional aberrations and potential clipping, and to reduce the optical path length, effectively making the system more compact. Instead, the mapper is designed to have steeper tilt angles to create a larger pupil array.
The new camera (Imperx-Bobcat ICL-B6620 29 Megapixel) can provide easy continuous shooting for alignment purpose; and without a shutter, it also does not provide any physical limitation to the lenslet array position like in the current setup. Thus the designed lenslet array’s focal length is guaranteed not to limit the diffraction-limited spatial resolution determined by the objective. The new pupil array geometry of even numbers in both directions (14×16) will separate the sub-pupil with the second beam-splitter’s reflection along the optical axis. The previous method of implementing a mask at an image plane is still acceptable since the sacrifice in spatial sampling is relatively insignificant.

5.8.2 New fabrication processes

For the new mapper design, different materials are researched to enhance the mapper’s surface quality. Among the potential materials includes a new aluminum alloy (RSA-6061 from RSP Technology), whose datasheet shows an RMS surface roughness of about 2 nm after being diamond machined. To obtain the performance listed in Table 5-. This design asks for the first mapper of which the facet width is lower than 70 μm, and thus, a new diamond tool of finer width (50 μm). Ruling technique is still preferred over raster-fly cut to fabricate the new mapper.

The 14×16 pupil array requires at least 224 imaging lenslets. This number can be doubled if telephoto sets are required like in the current setup. Individually inserting each lenslet into the holder is inconvenient and expensive. Instead, a new lenslet array is designed to be fabricated in-house with the Ultra-Precision Lathe machine. This newly designed lenslet array contains 14×16 lenslets, 1.26 mm in diameter and 1.6 mm apart
with 13 mm in focal length. Since the NA of each lenslet is low (0.05), the array is not limited by design and fabrication constraints. The Apogee camera (previously implemented for the proof-of-concept system) is no longer used so the working distance limitation caused by the moving shutter is eliminated. However, working distance of at least a few centimeters is ideal to prevent contact between the array and the camera sensor.

![Figure 5: Lenslet array fabrication using diamond turning machine.](image)

*Left: Lenslet array in relationship the image plane. Middle: Plastic miniature objective containing stacks of lenslets with self-alignment features. Right: Actual example of array [96, 97].*

Potentially, the lenslet array will be made from optical grade plastic such as Zeonex and polystyrene. For prototyping, this plastic array can be fabricated with single step diamond turning process [98]. To avoid the tedious process of putting individual lenslets into the holder, a whole lenslet plate will be manufactured with a 3D-printed and black dyed mask attached to prevent cross-talk and scattering. In the case when a combination of two lenslets are required to meet the design requirement, lenslet plates’ design requires cut with self-alignment features as described in McCall 2011 for stability [98].
Thus, the next generation of IMS-OCT is an improvement on the previously described proof-of-concept system as it minimizes the current setback of motion artifacts created by moving parts. However, the design iteration presented above leads to a rigid and cumbersome system with many light folded path. In addition to its lack of compactness, the system is inflexible in design. Its datacube size has to be predetermined prior to the design process, with the spectral sampling capped by the design and pixel counts. Parallel to the IMS-OCT approach, a new light guiding component was studied to circumvent the inherent rigidity in image mapping concept and is presented in the following chapter.
Chapter 6

Fabrication of a distributed fiber bundle for snapshot spectral imaging

6.1 Creating a tunable component for image separation

6.1.1 Introduction

This Chapter discusses a method to provide high spectral sampling for OCT application. A proof-of-concept tunable Spectrometer using custom distributed fiber bundle was designed to divide a full-field image into different spatial zones, and to potentially make space for dispersion. This chapter focuses on the development of the key component in the system: a fiber-based unit with large and tunable spacing among the fibers to improve spectral sampling and match the output image with the camera aspect ratio. The system’s tunability also enables the use of smaller but more sensitive camera sensor to detect low-light and high dynamic cellular activities without sacrificing imaging depth.

The preliminary work of fiber assembly was done with multi-mode fiber for affordability, but the ultimate fiber OCT system will be built with single-mode fiber to maintain the light coherence. Since the bundle device was designed to have short length, the throughput attenuation is negligible.
Literature research shows that the concept of resampling the image plane into individual fibers for later dispersion was explored by several groups. The largest reported spatial samplings was $44 \times 40$ [69]. Their low spatial samplings, however, make them undesirable for biological imaging, let alone for OCT’s requirement to create a valid depth range. The objective of this chapter is to investigate methods of fabrication that leads to a compact and tunable hyperspectral device that can promise higher spectral density.

6.1.2 Design of a distributed fiber bundle

The key element of this tunable spectrometer is the distributed coherent fiber bundle. The fibers are tightly packed together and expanded toward the output 5 inches away. The end facing the sample is referred to as the input, and the end facing the sensor is the output. In the final fiber bundle design, the input will contain a $76 \times 144$ multi-mode fiber bundle closely stacked together, while the output consists of 19 stacks of fibers, each has 4 layers, pressed against flexible thin rubber inserts. The setup allows adjustability between space dedicated for spatial and spectral data by compression/decompression of the fiber bundle. As mentioned above, Figure 6- illustrates the design in which four layers of fibers are stacked against each other to create enough dispersion space while maintaining an acceptable output size which can match with commercial coupling optics. Consequently, a simple, compact fiber-based component is created that can break the image down to many smaller segments without any special fabrication or complicated data reconstruction.
6.2 Testing system setup

An optical system was setup to examine the fiber quality. The fiber bundle was mounted on the orange clamp seen in Figure 6- on the image plane. The system was illuminated with a white LED. As the clamp compressed and decompressed the output end of the bundle, the distance between two adjacent stacks of fiber could be adjusted depending on the desired pixel numbers dedicated for later dispersion. Note that for simplicity sake, the intended prism for dispersion was not included in this testing system.

The NA at the output of the fiber bundles is 0.275, and its dimension is roughly 2” for one side. To accommodate such a large beam, a large-format, high NA lens was required to capture all the light without introducing clipping. A Hasselblad 150 mm prime lens was employed to focus the light at infinity. The image was then reimaged onto the camera by a 75 mm 2-inch doublet. Initially, a USB power color Lumenera Infinity camera was used to detect and prism dispersion due to its ease of use. Afterward, the prism was removed to simply the throughput verification process, and the Imperx 29 Megapixel CCD
camera was employed. This 4-tap Imperx camera could provide live imaging as it transferred images to the computer via two mini-Imperx cable and the standard mini Imperx frame grabber.

Figure 6-: Optical system to verify fiber bundle performance

6.3 Fiber assembly methods

The concept of fiber bundle fabrication requires three requirements. First, the fibers have to be packed as tightly as possible to avoid spatial loss, and to limit the bundle size for smaller imaging optics. Second, the fibers have to be roughly of the same length so light from all fibers are not obscured. Third, the geometry of the output has to accommodate gaps in between rows of fibers to create void space for dispersion.

6.3.1 Fiber types in used for fiber bundle fabrication

In the first approach, a plastic fiber of 500 µm in diameter were used for initial assessment. This fiber was not only large and easy to maneuver but also capable of
delivering high throughput thanks to its large core (486 µm), which made it perfect for prototyping. The material of the fiber also allows experiment with laser cutting, since this method can produce straight cut for one or more layers of fibers. The second category of fiber was chosen for its significantly smaller cores: 50 µm (Corning ClearCurve OM2 50/125um Bare Fiber) and 62.5 µm (62.5/125µm InfiniCor® 300 Multimode Optical Fiber-OM1). Since they were specially manufactured for telecommunication, their spectrum for peak performance is in the infrared range. However, our design limits the length of the fiber to be under 5”, thus the light loss caused by the non-optimal spectral range is negligible. The final fiber type studied in this project was a 12-fiber ribbon for prototyping. Using fiber ribbon considerably reduced the assembly time while maintaining acceptable gaps between fibers, and improving the uniformity of fiber alignment. In the rest of this chapter, the experiments of fiber assembly methods were carried out with a mix of all the fibers mentioned above.

Figure 6: Corning OM2 multimode fiber and its nominal dimensions
6.3.2 Fiber assembly using weaving

The first challenge was to bind the fibers into a controlled 2D block. The fibers were weaved with standard threads and yarns on a flip folding rigid heddle loom to create a 1D sheet. Since thread’s thickness was roughly 100 µm, the fibers were expected to be compactly weaved. The white threads were distributed alternatingly through the heddle and attached to the warp beam, while a stick shuttle carried the fibers back and forth line by line to create a sheet of fiber ‘fabric’. A thick black yarn was inserted after every 10 fibers to create folding gaps (Figure 6-a-b).

Figure 6-: Processes of weaving fibers
a: fibers weaved with white thread and black yarn on a mechanical loom. b: the edge of weaved fibers. c: weaving fibers with (thin) threads on one end and (thick) yarn on the other hand for tunability. d: weaving fiber ribbons with threads and yarns.
After examining that the fibers were uniformly aligned by the threads, the next step was to verify whether weaving would be a viable option to pack the fibers tightly on one end (input), while creating bigger gaps on the other end (output). This was carried out by replacing half of the vertical threads attached to the warp beam (Figure 6-a) with thick black yarn, as can be seen on Figure 6-c. Since the rod structure of the fiber was much smaller than the black yarn thickness, the black yarn could not pack the fibers uniformly. This attempt was experimented and confirmed with fiber ribbons of 12 counts (Figure 6-).

The weaving approach, though promising for further development in the future, affected the compactness of the system. As the threads were straightened to firmly keep the fibers in place, the discrepancy between the sizes of the threads and the fibers created a gap between the two materials and prevent fibers to be packed against each other. For future work, the weaving technique should be modified so that fiber tightening would not stretch out the threads, for example by investigating in compressible threads. Another disadvantage of this method is the more random elasticity of the commercial yarn. Due to the time limitation and the main objective of this research, other methods to control the spacing between the fiber stacks were explored.

6.3.3 Fiber assembly by using adhesives

To address the issue of fiber bundle compactness, I chose to assemble fibers with adhesives for better control of fiber locations. The adhesives experimented were commercial double sided tape and various kinds of gel epoxies from DoubleBubble®. The
very high-peel strength epoxy was selected for further study due to its opaque color and ease to use.

![Image of opaque epoxy](image)

Figure 6-: Opaque epoxy for fiber adhesion and light blocking

a: fiber ribbons attached by opaque epoxy. b, c: unmixed epoxy

The epoxy contained two ingredients which required thorough mixing to be converted from their gel form into solid polymerization. No unexpected stray light leaked through the space among the fibers as the epoxy seeped through the cracks between the individual elements (Figure 6-). Effect of epoxy in laser cutting and light throughput will be further discussed in the following sections.

### 6.4 Methods for cutting and polishing fiber bundle

The fiber bundle is created by stacking fiber layers on top of each other with different materials in between. This can lead to noticeably uneven output surface (up to 1 or 2 mm) across all fibers, making traditional fiber polishing challenging. Thus, other fiber processing methods were examined.
6.4.1 Laser cutting

Laser cutting requires a high and localized power laser beam to heat up and evaporate materials. It can create the sharpest cut at the waist of the laser beam, as described in the lenslet holder’s fabrication in Section 4.2.2. Furthermore, the laser’s effectiveness depends on the coupling objective that decides the laser’s NA, its depth of cut and consequently the sharpness at the beam waist. Figure 6-a-c illustrates the first attempt of cutting at different laser power and speed.

Figure 6-: Initial laser cutting experiment on plastic fiber  
a, b, c: visual assessment of laser-cut cross-sections at different laser power and speed.  
d: fiber shape with the input on the left and output on the right. e: overall laser cut surface. f, g: images of fibers dispersed by a prism recorded by a color camera.

The cut appeared not to be even, due to the differences in laser power and speed, and the height of the sample with respect of the laser. Note that the fibers were aligned with different geometry between the output and input so that there were two layers at the input corresponding with one output layer in an attempt to manipulate the aspect
ratio change from the input to the output end of the device (Figure 6-d). The results were observed on a color Lumenera Infinity camera. Figure 6-g shows the dispersed light from the fiber layers was slightly pushed toward each other compared with Figure 6-f, showing the first sign of tunability.

Figure 6-: Laser cutting performance.

a: loose fibers adhered to each other before laser cutting. b: bundles after one single laser cut looking from the top surface. c: from the side. d: 4 fiber layers laser-cut simultaneously. Note the cores shown dark dots in the middle of each fiber. e: light throughput examination under a microscope. f: illustration of burned fibers during laser cutting.

Further laser cutting experiments were carried out with smaller core fibers. Multiple layers were cut at the same time to determine the optimal depth the laser cutter could perform. Highlighted observations of these experiments are shown in Figure 6- with part a showing the pre-cut fiber bundle piece. This experiment gave significant insights in how laser cutting potentially affected fiber design and polishing. For example, due to different melting points, the plastic contributing to the fiber’s coating burned faster, exposing the fiber cores as observed from the top of the bundle in Figure 6-b.
Figure 6-: Light throughput initial challenges.

a: Uneven illumination. b: Light coupled to the coatings but the cores. c: Combination of challenges from a and b. d: Occasional light leaking

Comparisons between Figure 6- c and f show the impacts from laser power and speed, as low laser power could not completely burn off the plastic, creating a black ash residue on the surface. The ash residue, if covering the fiber coatings, could alleviate stray light going through the coating, but risk blocking the fibers’ cores completely. Therefore, really high laser power at low speed was preferred. The fiber bundle was then observed under the microscope for light throughput and surface assessment in Figure 6- d and e. Note that the small, black dots in the middle of every fibers on the left of Figure 6- d indicates the visibility of the fibers’ cores. These dots are completely missing in the third layers, suggesting that the cores were burned and fused into the cladding material, resulting in poor light transmittance. This was reconfirmed in Figure 6-a as the fiber bundle was placed into the testing optical system.
Another issue found in the fiber cutting experiments were the occasional light transmittance within the coating, creating a doughnut shapes instead of a point source for every fiber. There are two reasons behind this phenomenon: First, the fibers were designed for long range telecommunication purposes, so all the light coupled into the cladding and coating would be significantly dampened by the time it reaches the destination. This effect therefore does not affect applications that involve short fiber lengths. Second, the inconsistency in this phenomenon suggests that in the case of fibers attached in epoxy versus clear tape, the epoxy created another layer of material on top of the fibers’ coatings to create a lower NA ratio, minimizing light leaking. The last issue observed was the light leaking in between the fibers, which could be alleviated with opaque epoxy explained previously.

![Figure 6: Fiber ribbon layers](image)

While epoxy cannot be applied pre-laser cutting due to its high flammability, applying epoxy post-cutting can compromise the cross-sectional cuts. To overcome this issue, I decided to use fiber ribbons with pre-manufactured color plastic protector to limit coating’s stray light, and used clear tape to bond the fibers. The cutting quality, as seen in
Figure 6-, still produces weak and uneven light transmission when observed under the microscope.

6.4.2 Diamond turning fiber bundle

Another more precise and controlled method of polishing the fiber bundle was needed. Diamond-turning machine came up as an option to resurface all layers simultaneously. This idea required a special mounting fixture to attach the fiber bundle onto the vacuum chuck. As the fiber bundle revolves along the machine axis, the diamond tool gradually moves forward to take off layers 20 microns at a time. To ensure no vibration from the edge fiber layers, both ends of the bundle were dipped into oblique and machinable epoxy to create a solid block (Figure 6-b). The fibers were left for 24 hours for the epoxy to be completely dry.

A fiber holder was fabricated to mount fibers onto the diamond turning machine’s vacuum chuck. The holder was designed to have a 1mm indentation at the bottom in the middle, surrounded by \( \frac{1}{2} \)" thick flush surface to seal the vacuum. Figure 6-a shows the top of the holder with fiber ribbon bundles attached on both side for symmetry and spinning balance. The holder was then mounted and balanced on the vacuum chuck of a diamond turning machine. As the holder and fiber bundles rotated along the machine axis, a fixed synthetic diamond tool was translated along the x axis to complete a pass. The tool was then moved 20 µm into the fiber bundle to slowly resurface the fiber bundle. The total of 150 passes were made to completely remove any uneven roughness on the bundle surface.
Figure 6-: Fiber bundle mount on the diamond turning machine.

a: fiber bundle mounted on the fiber holder. b: fiber bundle with ends sealed in epoxy.

c: holder mounted and balanced on the diamond turning machine.

d: fiber bundle being resurface

The result of the diamond turned fiber bundle is shown in Figure 6-. Part a displays the output side where every stack of fiber was sandwiched by two layers of gray natural gum. Processing with synthetic PCV tools provided sufficient quality to verify uniformity of the fabricated bundle (Figure 6-a,b). To further prove this throughput improvement, the bundle was inserted again into the testing optical system, where the output and input can be observe in part d and e, respectively. The result observed on the sensor can be seen in part f, where the aperture stop was closed to the smallest position to prevent saturation on the sensor, which did not occur in the case of laser cut fibers.
As it promised a much higher throughput through the fiber bundle, this fabrication process was favored over laser cutting. While surface roughness using synthetic tools is not optimal, it is expected to be greatly improved with natural diamond tools. Additional improvements can contribute to a more uniform result including applying a reduced feed rate in the final cuts for flatter cross-section quality. To further avoid debris that can cover the fiber cores, the fiber bundle will be dipped into isopropyl alcohol and blown dry.

While further work needs to be done to complete building the device, preliminary efforts on fiber bundle fabrication confirms the method to provide OCT’s high spectral sampling by manipulating the input-output geometry and by adjusting the compression
among the fiber stacks with substantial throughput. Also, system’s tunability provides live switching between a large region-of-interest with low depth range and a small field-of-view (FOV) with high depth.
Chapter 7
Conclusion

This dissertation has described in detail the design conditions and options, available approaches and hardware to implement hyperspectral imaging spectrometers to snapshot OCT imaging. In addition a novel proof-of-concept snapshot 3D-OCT technique for biomedical imaging was built and characterized.

The design space of hyperspectral imaging spectrometers was studied to pave the way for snapshot 3D-OCT system. Two separate hyperspectral imager models were conceptualized to explore their potentials in OCT application. The first imager with IMS system implementation, captures snapshot 3D datacube while allowing data acquisition up to 100 frames/s. This is the fastest snapshot hyperspectral imager ever been reported. Designed to operate in visible and NIR spectral regions typical of OCT retinal imaging, the system can deliver high throughput in low light conditions and produce less motion artifact due to its light sensitivity.

The second developed device, fiber bundle based system, can provide dense spectral sampling to enable deep tissue imaging. The fiber bundle based spectrometers are built around a custom made and adjustable coherent fiber bundle assembly. This component provides a unique functionality of spectral range and field-of-view tuning. It is the first step to ensure spectral sampling dense enough for 2 mm depth in snapshot mode, comparable to current cutting-edge scanning OCT technologies.
A new generation of compact snapshot OCT will be enabled by utilizing the fiber bundle based spectrometer described in Chapter 6 for dense spectral sampling and application of sCMOS camera (Chapter 4) providing NIR sensitivity and high acquisition rate.

Thus the work described in this dissertation has provided the design and fabrication methods that allow construction of a novel snapshot OCT systems with minimized motion-related artifacts and low level illumination. At the same time they match current clinical and research OCT machines in resolutions and depth range.
Bibliography


[54] C. Zeiss, Germany, LSM 510 META Product Brochure.


[70] N. Gat, G. Scriven, J. Garman, M. D. Li, and J. Zhang, Development of four-dimensional imaging spectrometers (4D-is), Imaging Spectrometry XI (2006).


[80] Imperx, 6421 Congress Avenue, Boca Raton, FL 33487, USA, Imperx ICL-B6640 User Manual.


Appendix A

Fabrication parameters to create mapper substrate

This Appendix concerns the fabrication of mapper substrate using the CNC machine (Hass Automation®, Inc.). The process of mapper substrate fabrication contains four major steps. (1) A design is sketched with the appropriate dimensions for the selections of milling and drilling tools, as well as the mounting requirements to create mapper facets, which will be described in the next Appendix. The drilling and milling parameters were calculated based on the material and tools shown in Table A-. (2) Tool and part alignments were carried out by eye-balling followed by the machine automatic procedures. (3) The pure Aluminum piece was resurfaced on both side with an end mill tool. This step is crucial for accurate results in later steps of mapper fabrication, since it plays an important role in creating the precise angle tilts. This same end mill tool was also used to create the two sides of the mapper substrate as can be seen in the image below. (4) Four holes were created which match the ruling fixture exactly (as seen in Figure 4-).

Below are the codes specific to the dimensions of the mapper substrate and the parameters determined for the fabrication.

CNC codes
- **Flatten surface:**

  T8 M06;
  G90 G54 G00 S1085 M03;
  X-1. Y-0.45;
  G43 H08 Z0.3 M08;
  G01 Z-0.09 F4.341;
  X1.;
  G00 Z1. M09;
  M30;

- **Drilling**

  T2 M06
  G90 G54 G00 X0.637 Y0.331 S3730 M03
  G43 H09 Z0.1 M08;
  G83 Z-0.7 Q0.05 R0.1 F7.46;
  X-0.637 Y0.331;
  X-0.637 Y-0.331;
  X0.637 Y-0.331;
  G80 G00 Z1.0 M09
  M30

- **Creating two sides**

  T8 M06
  G90 G54 G00 S1085 M03;
  X0.6549 Y0.75;
  G43 H08 Z0.3 M08;
  G01 Z-0.25 F4.341;
  Y-0.75;
  G00 Z1. M09;
  M30;

---

**Drilling parameters**

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<tr>
<td>Spindle speed</td>
<td>3730 rpm</td>
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<tr>
<td>Feed rate</td>
<td>0.002 ipr</td>
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<tr>
<td>Cutting feed</td>
<td>7.460 ipm</td>
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**Milling parameters**

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<td>Feed per tool</td>
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<td>0.004 ipr</td>
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</table>

Table A: CNC drilling and milling parameters
Appendix B
Code for micro-milling

The mappers mentioned in this thesis were fabricated on a micromilling machine. The procedure’s description can be found in 5.3.1. The CNC code to create the mapper’s fine cuts is provided in this Appendix. Note that the rough cut with the carbide tool would be the same as the fine cut, except for the tool’s parameters and the omission of parameter #526 value for every facet.

( FAST SYSTEM FINE CUT)
(L= 23MM, LEAD IN = 1MM, W=22MM BLK=11, Width = 0.375, FACET WIDTH 0.075)
(TOOL RADIUS: FLAT. tool angle = 2.5 each side )

G71 G01 G18 G40 G90 G94 G61

( =========== SECTION - COMMANDS =========== )
#500 = 11  (# OF SURF. BLOCK PASSES )
#501 = 0.0  ( BLOCKS COUNTER )
#503 = 30  (# OF CUTS PER BLOCK )
#506 = 0.0  ( Y TILT COUNTER )
#507 = 0.0  ( START VARIABLE )
#508 = 0.0  ( END VARIABLE )
#509 = 0.0  ( Y LOCATION )
#510 = 0.0745  ( TOOL WIDTH )
#513 = 0.02  ( DOC FOR 1 ROUND)
#516 = 40  ( # OF DOC PASSES)
#517 = 0.0  ( DOC COUNTER )
#518 = 35  (# OF DOC PASSES TO START 0.002 DOC)
#521 = 1  ( TOOL PATH START )
#522 = 26  ( TOOL PATH LENGTH )
#525 = 0.0  ( TOOL COMP COUNTER )
#523 = #521 - #522  ( TOOL PATH END )
#514 = -#513  ( INITIAL X SHIFT )

Y3.000000 F500  ( PARKING POSITION - Y )
Z5.000000  ( PARKING POSITION - Z )
X5.000000  ( PARKING POSITION - X )
C0.000000  ( PARKING POSITION - C )

Y1.0 Z1.0 X3.0 F500
X2.0000000 F100
M27  ( TURN MIST #2 ON )
N30  ( LABEL THIS LINE TO BE 30 FOR LOOPS )

IF[#506EQ0]THEN #507 = #522*0.0365/2  ( Facet 1 X = 0.0000, Y = 0.0365 )
IF[#506EQ0]THEN #508 = -#522*0.0365/2
IF[#506EQ0]THEN #526 = 0.0000  ( X-tilt angle in degrees )

IF[#506EQ1]THEN #507 = #522*0.0365/2  ( Facet 2 X = -0.0296, Y = 0.0365 )
IF[#506EQ1]THEN #508 = -#522*0.0365/2
IF[#506EQ1]THEN #526 = -1.6971  ( X-tilt angle in degrees )

IF[#506EQ2]THEN #507 = #522*0.0365/2  ( Facet 3 X = 0.0296, Y = 0.0365 )
IF[#506EQ2]THEN #508 = -#522*0.0365/2
IF[#506EQ2]THEN #526 = 1.6971  ( X-tilt angle in degrees )

IF[#506EQ3]THEN #507 = #522*0.0365/2  ( Facet 4 X = -0.0148, Y = 0.0365 )
IF[#506EQ3]THEN #508 = -#522*0.0365/2
IF[#506EQ3]THEN #526 = -0.8485  ( X-tilt angle in degrees )

IF[#506EQ4]THEN #507 = #522*0.0365/2  ( Facet 5 X = 0.0148, Y = 0.0365 )
IF[#506EQ4]THEN #508 = -#522*0.0365/2
IF[#506EQ4]THEN #526 = 0.8485  ( X-tilt angle in degrees )

(REPEAT FOR A TOTAL OF 30 FACETS AFTER APPLYING ANGLE CORRECTION FORMULA)

#507 = #507 + #522*0.0365/2  ( SHIFT START TOWARD TOP OF SUBSTRATE )
#508 = #508 + #522*0.0365/2  ( SHIFT END TOWARD TOP OF SUBSTRATE )
G52 X[#514]  ( X SHIFT FOR DOC )
Y[#509]  F300  ( FEEDRATE = 300 MM/MIN )
C[#526]  ( TILT TOOL FOR X TILT )
X[#507] Z[#521]  ( START )
M01
X[#508] Z[#523]  ( END )
M01
X3.000000  ( CLEAR )
Z[#521] F1000  ( REWIND )

#509 = #509 + #510  ( Y TOOL SHIFT TO CUT THE NEXT FACET DOWN )
#506 = #506 + 1  ( INCREMENT Y COUNTER )
IF[#506LT#503]GOTO30

#506 = 0  ( RESET Y TILT COUNTER )
#501 = #501 + 1  ( INCREMENT BLOCK COUNTER )
IF[#501LT#500]GOTO30
#509 = 0  \quad \text{(RESET Y POSITION INITIAL)}
#501 = 0  \quad \text{(RESET BLOCK COUNTER)}
#514 = #514 - #513  \quad \text{(INCREMENT X SHIFT FOR DOC)}
#517 = #517 + 1  \quad \text{(INCREMENT DOC COUNTER)}

\text{IF[#517GT#518] THEN #513 = 0.002 (**********IMPORTANT - CHANGE DOC**********)}
\text{IF[#517LT#516]GOTO30}

#514 = 0  \quad \text{(RESET X-SHIFT)}
#517 = 0  \quad \text{(RESET DOC COUNTER)}
(G52 X0.0 Z0.0 Y0.0  \quad \text{(SHIFT LOCAL COORD. TO O.O)})
G64  \quad \text{(TURN OFF EXACT STOP)}
M28  \quad \text{(TURN MIST #1 OFF)}
M29  \quad \text{(TURN MIST #2 OFF)}

Y-5.000000 F500  \quad \text{(PARKING POSITION - Y)}
Z10.000000  \quad \text{(PARKING POSITION - Z)}
X5.000000  \quad \text{(PARKING POSITION - X)}
C0.000000  \quad \text{(PARKING POSITION - C)}
M30