SOFTWARE IMPLEMENTATION OF A COMBINED FLUORESCENCE AND SWEPT SOURCE OPTICAL COHERENCE TOMOGRAPHY IMAGING SYSTEM

By

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As members of the Master’s Committee, we certify that we have read the thesis prepared by ***Matthew Sassu***, titled ***Software Implementation of a Swept Source Optical Coherence Tomography System*** and recommend that it be accepted as fulfilling the thesis requirement for the Master’s Degree.

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Final approval and acceptance of this thesis is contingent upon the candidate’s submission of the final copies of the thesis to the Graduate College.

I hereby certify that I have read this thesis prepared under my direction and recommend that it be accepted as fulfilling the Master’s requirement.

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##### Abstract

This paper serves as a detailed overview of the software developed for the Falloposcope endoscope being developed by the Jennifer Barton Optics Tissue Laboratory. The Falloposcope is designed for a screening procedure for early detection of ovarian cancer in the fallopian tubes. The software controls a charge-coupled device (CCD) camera to perform reflectance and fluorescence imaging, which serves to navigate the endoscope to the fallopian tubes and surveil suspicious tissue regions. It also controls a swept source optical coherence tomography (SS-OCT) imaging system to provide high resolution, cross-sectional tomographic images of these tissue regions. CCD imaging is performed using a Princeton Instruments Pixis 1024B Digital CCD camera system. OCT imaging is performed using a Santec HSL-2100 swept source infrared laser and BPD-200-ST photodetector, as well as an AlazarTech ATS-9462 data acquisition board. Data from these instruments is displayed on a graphic user interface written in C++/CLI. This paper details how each of these imaging systems functions, from hardware control through data processing and visualization, as well as how more challenging programming tasks were completed. The core functionality of each system is complete, but there remain some parameters of the OCT data display which remain to be finalized. The available options, as well as the standard practices within the medical industry around OCT imaging, are discussed.

# Falloposcope System

The purpose of the Falloposcope is to scan for early stage ovarian cancer in the fallopian tubes. The Falloposcope is an experimental endoscope, which is being developed by the Jennifer Barton Optics Tissue Laboratory. Fluorescence and reflectance imaging provide navigation and surveillance for suspicious regions of tissue. OCT imaging scans suspicious regions with high resolution for cancer detection (Yuan, 2011).

The purpose of this thesis is to review the data acquisition hardware selected, and the software developed to control this system. The Falloposcope itself is a miniature (0.8 mm diameter), flexible endoscope containing a multimode fiber for tissue illumination, a lensed fiber optic bundle for returning reflectance and fluorescence light, and a single mode fiber with fused focusing elements for emitting and returning the infrared laser light used for OCT imaging. A diagram of the Falloposcope is shown in figure 1, and its imaging modalities are described in figure 2.

A close up of a device

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Figure 1: Falloposcope Diagram, courtesy of Gabriella Romano at the Optics Tissue Laboratory

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Figure 2: Falloposcope Imaging Modes

The fiber elements of the Falloposcope plug into a console that contains multiple illumination laser diodes for the reflectance/fluorescence system, the OCT swept source laser, OCT fiber optic elements, the CCD camera, and the OCT photodetector. The console also holds the computer system and display monitor.

This thesis focuses on the computer interface including the control of data acquisition hardware that collects signals from the CCD and photodetector, data processing on the CPU and GPU, and software required to acquire, process, and display data in a manner usable to a physician.

Fluorescence and Reflectance Imaging

Fluorescence is the emission of light from certain substances, which have absorbed light of an appropriate wavelength and emitted in another (usually longer) wavelength. In testing performed by the Optics Tissue Laboratory and others, it was found that benign, cancerous, and normal tissue have unique fluorescence and reflectance signatures, i.e. the relative irradiance of remitted light as a function of wavelength is statistically significantly different in each category (Tate, 2015) (Utzinger, 2003). The Falloposcope contains a multimode fiber to carry four different wavelengths of light through the endoscope and to illuminate the tissue. The remitted reflectance and fluorescence will be returned by the lensed fiber optic bundle, passed through a filter wheel and imaged by the Pixis 1024B CCD camera. This process is shown in Figure 3. This portion of the Falloposcope is an endoscopic realization of the multispectral fluorescence imaging system developed by Dr. Urs Utzinger. (Utzinger, 2003) (Tate, 2015).

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Figure 3: Fluorescence and Reflectance Imaging Diagram

SS-OCT Overview

OCT imaging utilizes backscattered near-infrared light to create a two-dimensional image of microstructures in tissue. OCT imaging will be implemented for depth scanning of regions of interest in the fallopian tube epithelial layer. This is shown in Figure 4.

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Figure 4: OCT Imaging Diagram. There are 3 discrete tissue layers backscattering light into the single mode fiber.

The method is non-invasive, and depending on the specifications of the setup, has a resolution both axially and laterally of a few microns (Huang, 1991). Three-dimensional images can also be rendered using an appropriate sweeping pattern and image processing.

OCT imaging uses low coherence interferometry, which is implemented using a Michelson interferometer. This setup, as well as the wave equations describing the interference and the final irradiance distribution on the detector, are shown in Figure 5 (Drexler, 2015). Figure 4 shows 3 discrete tissue layers; in practice, there are multiple layers within a sample which will backscatter. The effects of imaging in tissue are discussed in the mathematics walkthrough in Section 4.1.

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Figure 5: Michelson Interferometer, the single mode fiber will serve as the sample arm in the Falloposcope system. 3 discrete tissue layers are sampled, as shown in Figure 4.

The initial wave equation of the light source is represented by . The beam is divided into reference and sample beams by the 50:50 beam splitter. The reference beam (is reflected off the reference mirror at a distance . The sample beam ( is backscattered off the discrete tissue layers present in the sample, each of which are at a distance . To demonstrate the mathematics, the three discrete tissue layers will be represented by In practice, there may be more tissue layers, or fewer. The portion of the sample beam reflected off the beam splitter interferes with the portion of the reference beam transmitted through the beam splitter and produces an interference pattern on the detector. Plugging the sample and reference wave equations and into the power density profile equation returns the power density profile incident on the detector. The detector sensitivity is represented as the scalar ρ. This power density profile, often called the spectral interferogram, contains the signal used to create an OCT image (Drexler, 2015). Figure 6 shows this equation.

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Figure 6: Spectral Interferogram: power density profile, a real result for the detection current as a function of wave number

The power density profile is a function of wave number (k) and consists of three terms: 1) the DC signal, which is path length independent and is simply the reflectance of the source from reference and sample, 2) the cross-correlation terms, which contain the interference patterns between the reference beam and the reflectance from tissue layers of the sample, and 3) the auto-correlation terms, which represent interference within the sample beam between the discrete tissue layers.

The spectral interferogram is shown in Figure 7. This is the result for a perfect reference reflector rR = 1, and three discrete tissue layers rS1 = 0.1, rS2 = 0.15, and rS3 = 0.2. The reflectors are located at zS1 = 1 mm, zS2 = 1.25 mm, and zS3 = 1.5 mm. This is simulated data, shown to demonstrate the mathematics.

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Figure 7: Spectral Interferogram (power density profile). This is the profile for three discrete reflectors with reflectance rS1 = 0.1, rS2 = 0.15, and rS3 = 0.2. The locations of the reflectors are zS1 = 1 mm, zS2 = 1.25 mm, and zS3 = 1.5 mm. The reference mirror is a perfect reflector (rR = 1) at OPD = 0 mm. Plot Generated in Matlab.

Calculating the Fourier Transform of the power density profile returns the auto correlation function (Wiener-Khinchin Theorem) (Chatfield, 1989), which is the depth profile of the tissue sample, also called an A-Scan. The irradiance of the A-Scan is a relative value, based upon the reflectivity of the reference mirror and the sum of reflected light from all tissue layers in the sample. Figure 8 shows the A-Scan resulting from the inverse Discrete Fourier Transform (DFT) of the spectral interferogram from Figure 7, providing the spatial positions of each layer.

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Figure 8: A-Scan, the result of calculating the inverse DFT of the Spectral Interferogram in Figure 7. A peak appears for each discrete tissue layer, and the height of each peak corresponds to the reflectivity of the tissue layer. Autocorrelation terms are weaker and appear closer to OPD = 0. Note that autocorrelation terms for zS1-zS2 and zS2-zS3 are both at +/- 0.25 mm because the OPD difference is the same. Plot Generated in Matlab.

The brightest reflector, zS3 at OPD 1.5 mm, has the tallest peak in the A-Scan, as expected. The FFT operation generates positive and negative spatial values; the depth profile we are interested in is only the positive range, while the negative values are mirror image artifacts which can be discarded.

Arranging in software a series of A-Scans, obtained while scanning the sample beam over the tissue, creates a cross-sectional image of the tissue called a B-Scan (Drexler, 2015). Each column of a B-Scan is an A-Scan, with new A-Scans added as columns to the right side of the B-Scan image and all other data shifted to the left. This process is shown in detail in Section 4.5.

The original version of OCT imaging, referred to as Time Domain OCT, required movement of the reference arm to construct the power density profile (Huang, 1991). This movement took time and limited the possible implementations of OCT imaging. The development of Fourier Domain OCT negated the need for reference arm scanning, at the expense of requiring a swept laser source or a broadband source with a spectrometer detector, as well as the need to perform a Fourier transform, allowing for real time imaging (Choma, 2003).

In Swept Source OCT imaging, the low coherence white light source used in time domain OCT is replaced with a sweeping laser which emits near infrared light, often centered at 1300 nm, with a narrow instantaneous bandwidth (Podoleanu, 2012). The resulting power spectral density profile may be similar to a broadband source, but data is gathered as the laser sweeps through the spectral bandwidth and the profile (A-scan) is reconstructed in software (Choma, 2003). The remaining steps of arranging A-Scans into a B-Scan, remains the same.

One common issue with Swept Source OCT systems is that the sweeping laser is not linear in wavenumber. The signal from the photodetector is obtained as a function of time. Our laser sweeps very linear in wavelength as a function of time, so that the signal can be easily converted to a function of wavelength. The power density profile needs to be linear in wavenumber, so a conversion must be performed. There are a variety of ways to do this, one common method for lasers that are non-linear in either wavelength or wavenumber is the use of a Mach-Zehnder Interferometer to generate a clock signal for the DAQ board. However, many conversion methods exist (Jung, 2011) (Azimi, 2010) (Vergnole, 2012) (Morosawa, 2007) (Chong, 2008). The conversion method for the Falloposcope system has not yet been selected but given the stability of our laser and the fixed relationships between time, wavelength, and wavenumber, will likely be a simple look up table obtained through calibration.

## Software Functional Requirements

The Falloposcope imaging system displays fluorescence/reflectance and OCT images to the operator. The software must provide control for the data acquisition, as well as processing, display, and saving for the Falloposcope imaging system. The overall system requirements for the software are:

* **Simple, Intuitive Operation:** For this iteration of the Falloposcope, the system will be controlled by a trained engineer/researcher. However, the software should be designed with an eye to the future, including control and display comprehensible by a physician/surgeon or a technician.
* **Translational Ability:** This system does not need to meet full FDA requirements; however, decisions should be made that facilitate eventual FDA approval.
* **Compact Computer System:** The software and data acquisition must run on a standard tower computer.
* **Real-Time Operation:** The software must enable real-time control of hardware and display of data.
* **Hardware Control:** The software must send commands to a sensitive CCD camera (PIXIS 1024B) appropriate to assure quality reflectance and fluorescence images (e.g. integration time). The software must also control a data acquisition (DAQ) board reading data from the photodetector and external trigger of the swept source laser.
* **Image Acquisition:** The software must enable beginning and ending acquisition of images from the fluorescence imaging system and acquisition of a-scans from the OCT imaging system (separate control, one or both systems operating).
* **Image Display:** Two windows should be available, one for real-time display of CCD images with refresh speeds limited by the camera not software, the other for “waterfall” display of processed OCT a-scans with processing and refresh speeds that enable smooth-appearing progression of the waterfall and avoid loss of data.
* **Image Saving:** Individual CCD images should be saved with a click of a button; images should be saved in full resolution, non-compressed format. OCT A-Scans should be saved with a click of a start and stop button, with some practical limit on A-Scan number. OCT A-Scans should be saved as processed floating point, non-compressed arrays.
* **Procedure Saving:** Save a video of the screen, plus audio commentary. Should be enable at the click of a button, to document an entire procedure.
* **Utilize Existing Laboratory Hardware and Equipment (where appropriate):** As an established medical imaging laboratory, the optics tissue laboratory has relevant hardware from prior projects and experiments. Some of this equipment and software is appropriate for this project.

## Background

The component pieces to perform CCD and OCT imaging exist, but there is no software which meets the needs of the combined Falloposcope system in a simple and efficient format. Software to simultaneously perform CCD and OCT imaging utilizing existing laboratory hardware required custom development.

To perform CCD imaging, the system utilizes a Pixis 1024B CCD camera from Princeton Instruments. The Pixis 1024B has C++ support, as well as sample code which performs continuous measurements. This sample code was modified and incorporated into the Falloposcope software, with functionality added to display images in the GUI and save data.

To perform OCT imaging, the system utilizes a Santec HSL-2100 Swept Source laser and accompanying BPD-200-ST photodetector. Not originally available was a data acquisition board capable of real-time processing of the A-Scans in a manner acceptable for the Falloposcope system. After considerable discussion with vendors and analysis of capabilities and needs, a decision was made to purchase the AlazarTech ATS-9462 DAQ board. The AlazarTech software development kit (ATS-SDK) is written in C++. AlazarTech also offers a library for performing the data processing steps required for real time OCT imaging. The sample code provided by AlazarTech to interface with the ATS-9462 DAQ board and perform the OCT data processing steps was incorporated directly into the Falloposcope software. Parameters were changed to allow the DAQ board to sample the HSL-2100 output and perform measurements continuously. Additions were made to display A and B-Scans in the GUI and save sections of data specified by the operator.

# Software Implementation Overview

The software itself is written in C++/CLI, which meets the requirements of being written in C++, and allows for the use of .NET objects to create a graphic user interface (GUI) which displays data from the CCD and OCT systems. This software was developed in the Community Edition of Visual Studio, 2017.

The core of the software is the GUI, from which all other functionality is controlled. The three primary functions are screen recording, which should run continuously throughout any procedure, as well as the ability to continuously capture CCD and OCT data. All three of these processes run on separate threads, allowing them to run simultaneously.

The Screen capture function is the simplest, being initiated and stopped with the push of a button. The CCD and OCT threads connect to the Pixis 1024B and ATS-9462 respectively and take measurements continuously. For each device, there is an option to save data on the GUI. The operator has the choice of starting or stopping acquisition at any time.

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Figure 9: Diagram of software threads initiated on the GUI

The use of these three distinct threads was decided upon by considering the needs of the operator running the procedure. The operator will need to initialize the screen recording at the start of the procedure, which can be done, and will then run in the background with no additional input required. The CCD and OCT imaging functions need to run simultaneously, requiring that each be run on a separate thread. This allows data to be acquired from each instrument and uploaded to the GUI, without interrupting the other. The operator will need to run each instrument for an unknown length of time while exploring the tissue sites in the fallopian tubes; this exploration is captured by the screen recording function. High-resolution data from the Pixis 1024B and ATS-9462 is gathered and discarded continuously until specified by the operator.

This functionality is controlled from the main GUI, which consists of the following controls to operate the 3 independent threads of the Falloposcope software.

Screen Recording:

* Start/stop recording procedure button

CCD Imaging:

* CCD camera user-defined parameters (integration time, etc.)
* Contrast maximization option check box
* Start/stop CCD imaging button
* CCD image display box
* Box for CCD image file name
* Save CCD image button

OCT Imaging:

* Start/stop OCT imaging button
* OCT waterfall display able to show 3 seconds of data
* Box for OCT B-Scans file name
* Start/stop save OCT A-Scans button

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Figure 10: Falloposcope Software GUI

The following sections discuss how these threads are run, parameters are set, and data is acquired and saved, in greater detail.

# Implementation: CCD Imaging

The CCD imaging system will image light reflected from or emitted by fluorophores inside the fallopian tubes, returned via the fiber optic bundle of the Falloposcope. The CCD camera used for this project is the Pixis 1024B CCD imaging system from Princeton Instruments.

* 1 mega-pixel camera; 1024x1024 resolution
* Data connection via USB 2 port
* Produces 16-bit greyscale images

### Software Implementation

Included with the camera is C++ sample code which performs continuous measurements. The following measurement settings are used:

* Number of readouts = 1
* Number of frames per readout = 1
* Regions of interest = 1 (consisting of the full 1024x1024 CCD pixel area)

With these settings, for each image captured by the camera, a buffer is returned containing 1 image consisting of 1024x1024 pixels of greyscale data. Each pixel is 16 bits, meaning each buffer is 2,097,152 bytes. Additionally, a callback function is set which is executed each time a buffer is available from the CCD. This callback function processes and displays the image on the GUI (Princeton Instruments, 2018).

The primary addition to the provided sample code is processing the data into a bitmap image. The .NET framework’s Graphics Device Interface (GDI) has a bitmap object, where each pixel is given a red, green, blue, and alpha (transparency) value (Microsoft Corporation, 2018). Each of these values is a Byte, while the data returned from the camera is 16-bit greyscale (no separate red, greed, or blue data is provided). Speed is less of an issue for the fluorescence measurements; the CCD exposure time and readout time is the bottle-neck so the computer has time to render each image between measurements. Despite this, the fastest possible rendering method is still utilized.

To display an image using GDI objects, the short data from each pixel needs to be converted to a byte, and set to be the value of the blue, green, and red components of each pixel. Each alpha value must be set to 255 for maximum opacity. To convert from short to byte, each value must be multiplied by 255 / 65535 = 0.00389105, with the decimal truncated or rounded.

The fastest way to write the byte values to each pixel is to write each byte value directly to the pixel’s RGB locations in memory (Microsoft Corporation, 2018). This is the process:

1. Lock the buffer containing the bitmap’s pixel data.
2. Write the byte value of each greyscale pixel to the Blue, Green, and red pixels in the bitmap object.
3. Unlock the buffer.

Once these steps are complete, the bitmap can be sent to the main thread for rendering on the GUI. The diagram in Figure 11 visualizes this byte writing process.

A picture containing object

Description automatically generated

Figure 11: Diagram showing the assignment of greyscale pixel values to the blue, green, and red values in each pixel. Alpha = 255, this process is repeated for all greyscale values and their corresponding pixels in the bitmap

NOTE: In scaling and converting each pixel to a byte from a short, resolution is lost. Most screens are only capable of displaying 8-bit data, so there is no visible difference. To preserve the full resolution of data provided from the CCD, a separate array is created and saved in a format which can be viewed by a specialty viewer like ImageJ. The advantage of having both files is that the 8-bit image can be opened in all common image display programs for ease of viewing, while the higher 16-bit data are still saved if additional analysis needs to be performed. This process is also implemented to display B-Scan images, which are discussed in detail in Section 4.5.

### Contrast Maximization

There is an option on the GUI to perform contrast maximization on the image before it is displayed. This option can be enabled or disabled while performing continuous measurements. If selected, the greyscale values are interpolated; 1% of outliers in either direction are discounted, which prevents outlying overexposed or dead pixels from adversely affecting the new image. The new maximum greyscale value is set to 255, the minimum is set to 0, and all pixels in between are scaled to fit this new range. Any outliers above the chosen maximum are set to 255, and any outliers below the minimum are set to 0 (Princeton Instruments, 2018).

Figure 12 is an image of a test fiber for the Falloposcope imaging a standard test pattern. There is no contrast maximization present, and the range of greyscale values is between 3 and 95.

A picture containing invertebrate, animal

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Figure 12: Pixis Image, without Contrast Maximization. the image is cropped down from its original 1024x1024 size to 339x306 for inclusion in this document.

Compare this to Figure 13, the Pixis image with contrast maximization; the new range of greyscale values runs from 0 to 255.

A picture containing animal, invertebrate, photo

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Figure 13: Pixis image, with contrast maximization enabled. The image is cropped from its original size of 1024x1024 to 339x298 for inclusion in this document.

NOTE: The images contain a slight blur intentionally, which is implemented by physically moving the target out of focus. This blur minimizes the visible distraction of the honeycomb pattern created by the fiber bundle.

### Saving Data and Images

Pixis images are saved with a push of a button in the GUI. Images are saved in the 8 bits per color, 4 colors per pixel format. The images in Section 3.2 were captured using this function. The image displayed in the GUI is saved, meaning if contrast maximization is enabled, the contrast maximized image will be saved. However, the saved 16-bit data are not manipulated. This is to preserve the raw, higher resolution data captured by the camera.

# Implementation: OCT Imaging

This section applies the SS-OCT theory presented in Section 1.2, walking through the steps to scan a sample, read the resulting power spectral density function from the photodetector, and construct a B-Scan image.

To read the power spectral density function from the BPD-200-ST, the laboratory purchased an AlazarTech ATS-9462 board, as well as the ATS-SKD, ATS-GMA, and ATS-GMA-OCT software packages for capturing data and performing OCT data processing. The ATS-9462 DAQ board, along with the Santec HSL-2100 laser source and BPD-200-ST photodetector, constitute the major hardware components for this application:

Santec HSL-2100

* + Center wavelength; 1320 nm
  + Bandwidth; 170 nm
  + Pulse Width; 50 s

Santec BPD-200-ST photodetector

* + Sampling Speed: 80 MHz

#### Mathematics Walkthrough

This section walks through the mathematical theory which led to the hardware selections and parameter settings used for the Falloposcope system. These equations are outlined in detail by Nakamura et al. in the paper “Optical frequency domain ranging by a frequency-shifted feedback laser” (K Nakamura, 2000). The required theoretical imaging depth is 3mm, and the required resolution is 5 m. Both requirements are met in this application. In the end, we will arrive with these parameters, which will be used for hardware selection and software settings:

* + Minimum DAQ sampling rate: 32.942 Mega Samples per second (MS/sec)
  + Minimum number of data points pre FFT: 1646 points

**Step 1, laser characteristics:** The rate at which the frequency of the laser changes is the total chirp frequency, and the average chirp rate is the change in frequency during one pulse width.

* : 1320 nm
* Bandwidth: 170 nm
* : 1235 nm
* : 1405 nm
* Pulse Width: 50 s

**Step 2, laser characteristics in tissue:** Given the characteristics of the swept source laser, we can calculate the sampling interval (SI) of the laser in meters and Hz.

* Imaging Depth: 3mm
* Tissue Refractive Index (n): 1.4

Once we have the sampling intervals, we can calculate the resolution we can achieve with this source in tissue.

And finally calculate the number of data points required to fully display the required range of the system.

**Step 3, data acquisition:** To sample this data, and avoid imaging anomalies such as aliasing, we need to specify the maximum beat note of the system. This is the frequency we expect to see at the maximum desired imaging depth, based on the characteristics of the swept source laser. This is the highest frequency we need to be able to sample.

The number of data points we need is double the maximum beat note, which will provide 2 data points for each period in the frequency. This is the minimum required sample rate of the DAQ board and should be used as the minimum spec when sourcing and purchasing a DAQ board.

Finally, we can calculate the theoretical resolution of this system based on the speed of light in tissue, and the chirp rate of the swept source laser. This is very close to our result from equation 4.6, the maximum resolution possible with the swept source laser.

**Step 4, data points per laser sweep:** From the minimum sample rate, we calculate the number of data points per sweep.

As a quick sanity check, the number of expected data points can be calculated from the characteristics of the laser and the sampling interval. The mathematics performed to arrive at this value are based on the laser characteristics in tissue and should return the same required number of data points.

#### Hardware Specifications

A DAQ board with an acquisition speed of 50 MS/s is required; we specify this because it is above the theoretical minimum of 39.2 MS/s and is available in off the shelf hardware. The ATS-9462 DAQ board has a maximum acquisition rate of 180 mega samples per second (MS/s), which is approximately 6 times the required 32.9 MS/s. In software, the board can be set to acquire at 50 MS/s, which is better aligned with our needs. This sampling speed provides a maximum of 2500 data points per laser sweep.

In practice, this number is reduced due to the requirement that the board have 32 clock sample cycles to rearm itself for the next acquisition. This reduces the number of sample points to 2468.

The number of data points required can be reduced further by viewing the data captured by the photodetector. The BPD-200-ST has the capability of subtracting the DC terms from the power density profile discussed in Section 1.2 (Santec Corporation, 2016), leaving only the cross-correlation and auto-correlation terms. Figure 14 shows an example readout from the BPD-200-ST with a single reflector (mirror) in the sample arm, as displayed on an oscilloscope, along with the external trigger output from the HSL-2100. A single laser sweep is displayed.

A close up of a screen

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Figure 14: Oscilloscope display of the power density profile minus the DC terms, as well as external trigger output from the HSL-2100. One 50 s pulse width is displayed, with the external trigger of the HSL-2100 and the output from the BPD-200-ST.

With each sweep, the HSL-2100 emits an external trigger from a coaxial port on the back of the device (Santec Corporation, 2016). The oscilloscope shows how the power density profile overlaps with the trigger signal; and indicates how the ATS-9462 data acquisition parameters need to be set in software. The falling edge is used as the zero point. The power density profile starts at approximately -5 s and continues to +35 s. Given the DAQ board sample rate of 2500 samples per sweep, we need to use the following settings:

* Pre-trigger samples = 250
* Post-trigger samples = 1750

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Figure 15: Illustration of the signal in Figure 14, with pre and post trigger samples shown.

It is necessary to perform Fast Fourier Transforms (FFTs) on the data as it’s acquired. The number of data points must be a power of 2, meaning we need to pad the data with 48 zeroes to bring the total number of pre FFT data points to 2048 (2^11) (AlazarTech, 2018) (Steven W Smith, 1997). This is above the required minimum of 1646 pre FFT data points calculated in Section 4.1. With the increase in the number of data points gathered our imaging depth is approximately 4 mm instead of the required 3 mm, but beyond 3 mm the signal is extremely low.

The computer’s hardware must be capable of performing these calculations. Based on recommendations from AlazarTech, the following computer was procured:

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Figure 16: Computer Specifications

This computer system has the necessary hardware, and bandwidth between the GPU, DAQ board, and onboard memory, to perform high resolution OCT imaging in real time (Intel, 2018) (AMD, 2018). Not listed is the ATS-9462 board itself, as well as the accompanying AMD WX7100 GPU, which were purchased separately. The GPU is inserted into the PCIe x 16 slot on the motherboard, and the ATS-9462 is inserted in the PCIe x 4 slot.

Finally, the coaxial cables from the BPD-200-ST and the external trigger from the back of the HSL-2100 need to be connected to the ATS-9462 in the Channel A and External Trigger coaxial ports.

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Figure 17: Data and external trigger cables (from the BPD-200-ST and HSL-2100 respectively) connected to the ATS-9462 DAQ board

#### Software Implementation

AlazarTech provides the ATS-GMA-OCT library for performing OCT data processing in real time. 4 buffers are set, together forming what is effectively a circular buffer, which follows these steps:

* Data is acquired by the ATS-9462 board and is stored in an onboard buffer.
  + Acquisition time per buffer = 0.04 seconds
  + A-Scans acquired per buffer = 800
* After acquiring, the full buffer is transferred to the GPU directly, and pre-processing is performed.
  + Wavenumber linearization calibration (not included): transforms the data set from its default linearity in wavelength to linearity in wavenumber. An accurate FFT requires data with a constant frequency, so a calibration must be included (Jung, 2011). The calibration method for the Falloposcope has not been selected.
  + Windowing Function (Hanning): performing a Discrete Fourier Transform on a finite, non-repeating data set causes errors in the transformation, which show up as high frequency noise. Windowing reduces the amplitude of the pre-FFT data to zero at the ends, artificially making the data set repeating, and reducing the high frequency noise (National Instruments, 2018).

The higher end CPU and motherboard is required for this step due to the increased number of channels connecting different PCIe ports on the motherboard. In lower end motherboards, the physical slots are present (i.e. 4x and 16x slots), but there may only be 1 lane connected to either port. Effectively, the transfer speed is cut by 1/4 or 1/16 (Silent PC, 2018).

* The GPU performs the pre-processing steps and the FFT and allows the buffer to be accessed by local memory on the computer. NOTE: Data must be red out of the buffer at this time. It is critical that the data read from the current buffer be completed before the next buffer is full, otherwise the buffers will fill with data faster than they are cleared and there will be an overflow. The size of the post FFT data set is 1024, half the size of the input array because only the positive depth profile is used, which is half the length of the input array (Steven W Smith, 1997).
* The buffer is posted back to the ATS-9462 board, allowing old data to be overwritten by new data. This serves as a circular buffer, allowing data to be acquired in a new buffer while the previous buffer is read out and processed.
  + Buffers acquired per second = 25

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Figure 18: Data gathering and processing steps for a single buffer, with respective hardware.

800 A-Scans are available with each new buffer posted. Section 4.4 discusses how to display them, and Section 4.5 discusses organization into a B-Scan.

#### A-Scan

The first A-Scan from each buffer is displayed in the A-Scan graph, corresponding to 25 frames per second. The A-Scan is only displayed as an additional tool for the operator. This is only 1 out of 800 A-Scans, so a significant amount of data is not displayed.

Figures 19 and 20 show A-Scans with the reference mirror in different positions. The x axis is in pixels and the y axis is in arbitrary units.

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Figure 19: A-Scan; OPD = 0 mm

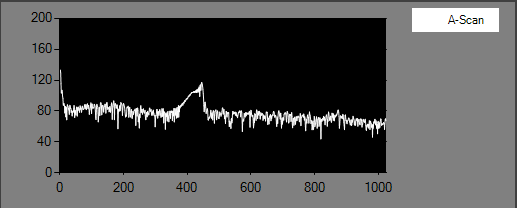


Figure 20: A-Scan; OPD 1.96 mm

NOTE: As discussed in Section 1.2, there is not currently a conversion from wavelength to wavenumber. The results can be seen in the A-Scan, where the signal has a much wider FWHM. When a calibration method is implemented, this will become a narrow peak.

#### B-Scan

A B-Scan is created by arranging a series of A-Scans into the columns of an image. This is done in the Falloposcope software by converting the A-Scan values into pixel values and displaying them in a Bitmap. Writing A-Scan values to pixels follows the same process described in Section 3.1 to display CCD images. As the Falloposcope scans, new A-Scan data is added to the right side of the B-Scan image, and existing data is shifted to the left.

On the GUI, the B-Scan image is refreshed at 25 Hz, the same refresh rate as the A-Scan graph in Figures 19 and 20. Each A-Scan consists of 1024 data points, and each B-Scan consists of 1024 A-Scan arranged into columns. With a refresh rate of 25 Hz, this image displays 41 seconds of data.

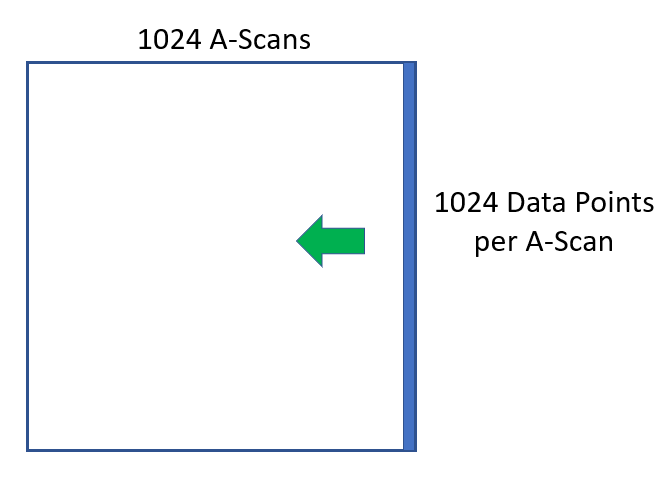


Figure 21: B-Scan image displayed on the GUI

NOTE: As described in Section 4.3, 800 A-Scans are gathered per buffer. However, only 1 A-Scan per buffer is displayed, meaning 799 A-Scans are discarded. With a new buffer available every 0.04 seconds, and a refresh rate of 25 Hz, 41 seconds of data is displayed in a B-Scan image. The desired parameters for the Falloposcope display have not been finalized, but a standard B-Scan typically displays 3 seconds of data at one time, with a resolution of 5,000 A-Scans per second.

To realize this desired display, the following B-Scan arrangement remains to be programmed. The Santec Laser sweeps at a rate of 20,000 scans per second, resulting in 20,000 A-Scans. Averaging every 4 A-Scans results in a desirable noise reduction and a total of 5,000 A-Scans per second of data. Given the fixed monitor refresh speed of 25 Hz, we need to display 200 A-Scans per refresh. To display 3 seconds worth of data, the image needs to be 15,000 columns long.

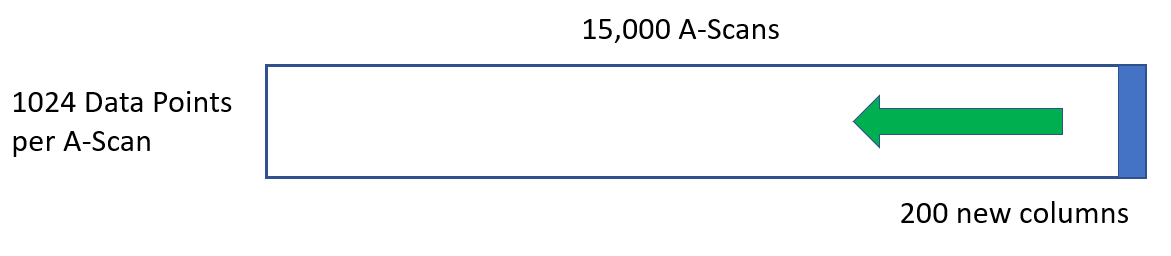


Figure 22: B-Scan in line with standard medical imaging applications

This setup is not programmed at present but will likely be the final version of the B-Scan which is used for the Falloposcope software.

#### Saving B-Scans

At present, a simple function to save the displayed 1024x1024 bitmap is implemented. Below is one of these bitmaps. Shown on the image is the reference mirror moving through the full possible imaging region, ranging from OPD -4.5 mm, through ODP 0 mm, and ending at OPD + 4.5 mm. This is above the required 3mm due to the additional data points collected. This movement is done manually, so the motion of the reference mirror is not constant. In OCT implementations, the reference mirror is positioned so the OPD 0 position is slightly above the sample, and all reflections from the sample are in the positive OPD direction.

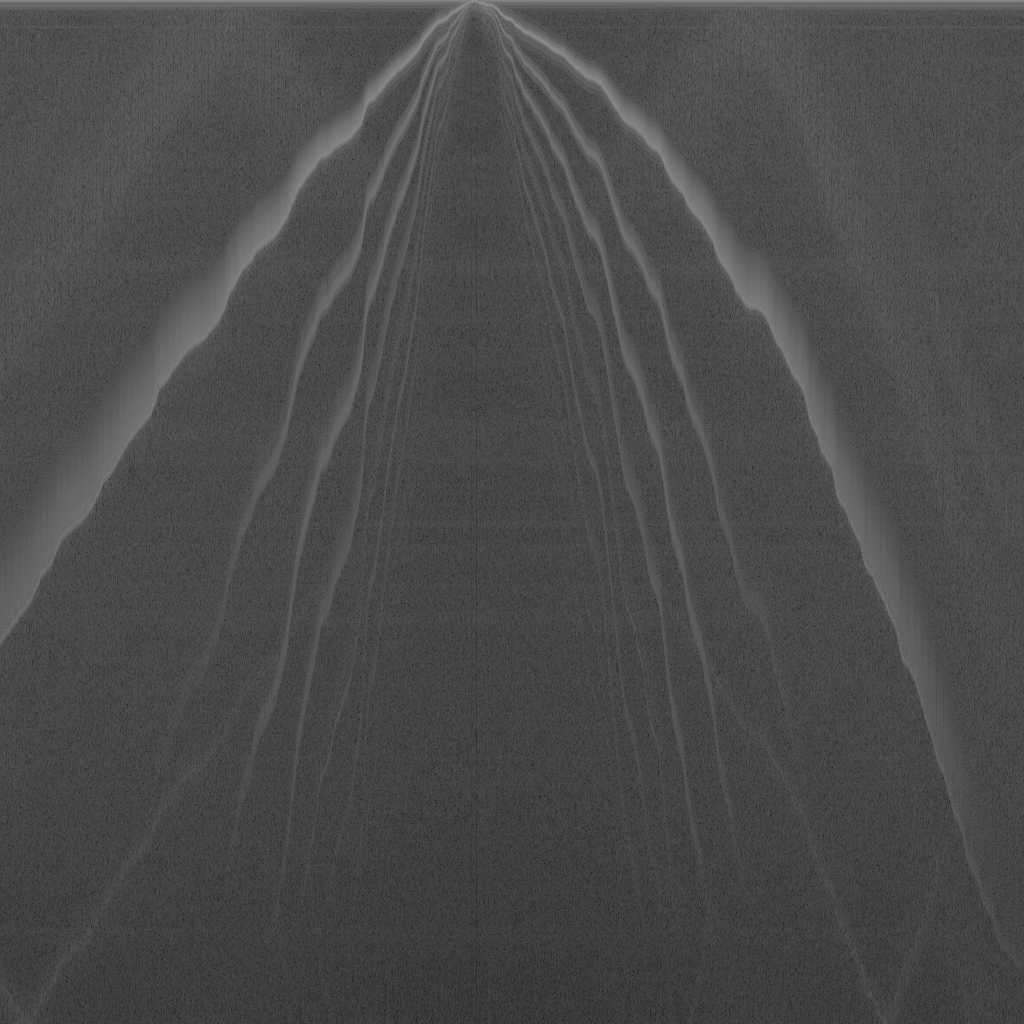


Figure 23: B-Scan image, reference mirror moving through full imaging region over a period of 41 seconds. Movement of the reference arm is done manually.

# Conclusion and Next Steps

We have reviewed the theory behind fluorescence imaging and SS-OCT and given a brief description of the Falloposcope system. We have implemented the Falloposcope’s CCD and SS-OCT imaging system into a single computer system and software package, which can be installed in a laboratory or medical facility to operate the Falloposcope endoscopic system.

Some features still need to be implemented. The B-Scan display needs to be finalized and programmed, as discussed in Section 4.5. Also, the calibration method of the SS-OCT system needs to be determined and programed, as discussed in Section 4.4. Finally, additional hardware control will need to be included to control the light emitted during the fluorescence and reflectance imaging. Controls for this can be added in the GUI in the lower left corner, while the code can be added anywhere, as long as C++ programming rules are followed.

##### Appendix A: Computer Setup and Software Installation

This is a complete walkthrough of the computer setup and software installation process performed on October 12, 2018 on the dedicated Falloposcope computer. For completion, this description assumes that you are setting up a completely new system and walks through all steps required. Please follow these instructions exactly. **NOTE:** It is possible, with ongoing updates to drivers and Windows 10 itself that things will not work in the future exactly as they did before. This walkthrough includes as much information as possible to explain not only what was done, but why. Hopefully this will help with your troubleshooting!

1. **Computer Specifications:** If you’re starting from scratch, these are the settings of the computer the laboratory ordered from NorTech:

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The critical components are the X299 chipset, as well as the AMD Pro WX7100 GPU. The high-end chipset provides enough lanes for data transfer between the AlazarTech board and the GPU. Without them data processing cannot happen as quickly. If you are considering a different configuration, this functionality is what’s required.

1. **Install Updated GPU Drivers, Enable Direct GMA:** You will need to change the driver settings of the AMD card to allow for Direct Graphics Memory Access (GMA). This will allow data from the AlazarTech board to be written directly to the AMD card. The drivers used by the optics tissue laboratory are saved (win10-64bit-radeon-pro-software-enterprise-18.q3.1-oct1.exe), as well as the installation instructions (AMD Driver Installation Guide), but it’s recommended you download the latest driver software from the AMD website.

Install all possible packages and restart the computer. When this is complete, you should be able to right-click to access the FirePro settings.

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Select “Advanced”, then “SDI/DirectGMA”. Check the box and move the slider as high as it will go.

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Restart the computer for this change to take effect.

1. **Install Visual Studio 2017 Community Edition:** All software was developed in Visual Studio 2017 for C++/CLI. At the time of this writing, the Community version of Visual Studio is free, hopefully this is still the case or Microsoft will have difficulty attracting 3rd party developers to its platform. Anyway, several sub-packages must also be installed. Download and run the Visual Studio 2017 Installer, then select the following packages to include in the installation of the Community Edition. The total file size should be around 8 GB.
   1. .NET Desktop Development
   2. Desktop Development with C++
   3. Along the right hand side, there is a section titled “Installation Details”. Under “Desktop Development with C++”, be sure to check “C++/CLI Support” or you will be unable to debug the software.
   4. With these three options selected, click “Install While Downloading” in the lower right-hand corner.
   5. Restart the computer when prompted.

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1. **Install AlazarTech Packages:** There are 3 CDs of material to install. For each, run the Setup on each CD to the default folder, all 3 should install to the main folder “C:\AlazarTech”. Do NOT modify this unless you know what you’re doing! The Falloposcope software has file dependencies in the various directories.
   1. ATS-SDK
   2. ATS-GMA v4.0.0
   3. ATS-GMA-OCT v4.0.0

Once this is complete, you need to move the .dll files to the appropriate locations.

1. **Install PICam:** Run the installation for “PICam.exe”. There may be updated versions of this, but you should be fine with the saved version.
   1. Install to the default directory “C:\Program Files\Princeton Instruments\PICam”.
   2. Select “Custom” installation and check the box next to “SDK” to install the PICam software development tool kit.
   3. As it installs, it will ask about various sub-packages. Click “Yes” as they come up.
   4. Restart the computer.
2. **Install Microsoft Expression Encoder 4:** Run the executable “Encoder\_en\_installer.exe”. This will install the video codec required to play back the video files of the screen recordings. This may not be available elsewhere, so it’s recommended you use the installer. **NOTE:** There is a separate program which performs the screen recording. Source code is in a separate folder named “ScreenRecorder” and it’s written in a 32 bit C++ console application. This doesn’t affect you unless you’re replacing the screen recording functionality.

##### Appendix B: Software Foundations

**Changes to AlazarTech Sample Code “inversion\_application”:** This section details changes made to the AlazarTech sample code “inversion\_application”, specifically changes to the board parameters required to successfully run the sample code. Following this, the code will be copied to the Falloposcope Software V1.

1. Update code to be compatible with VS2017 Community Edition. This is done automatically by running the .sln file.
2. Edit function “AlazarSetCaptureClock()”, change sample rate to “SAMPLE\_RATE\_180MSPS” to match the sample rate of the ATS-9462 board.
3. On line 249, change the line to read “autoDMAFlags = ADMA\_EXTERNAL\_STARTCAPTURE | ADMA\_NPT”; by default the last section is “ADMA\_CONTINUOUS\_MODE”
4. Comment out lines 333-338, which sets the pack mode. Comment out lines 291-292, which calls the function to change the pack mode. Comment out line 270, which sets this variable in the first place (it’s not necessary anywhere four our setup, the board doesn’t support it, and when you try to call the function it throws an error.
5. Set the trigger source ID to TRIG\_EXTERNAL. This is done in line 131, it is the 4th parameter in the function AlazarSetTriggerOperation().
6. //Change UserDefinedContext to True, the default is false. This is in line 273.

**Rendering Bitmaps:** Both the CCD and OCT imaging functions require data to be rendered as a Bitmap. The following are a few critical notes on building the Bitmaps from the raw data returned from each device.

1. **Use Lockbits:** The fastest way to read and write data from the bitmap is to lock the bits in the bitmap object to read and or write data directly to them. The bitmap object is a managed object in the .NET framework, and it is necessary to perform the specific Lockbits operation to remove this automatic management, allowing data to be written directly. C++ allows for direct memory writing using pointers. Please see the code for how this is implemented.
2. **Do NOT use ImageLockMode::ReadWrite:** There are several modes to use to read and write data, and this one is incredibly slow. In the case of the B-Scan imaging, it is necessary to read old data, and write new data, to the same image. The fastest way to do this is to first use ImageLockMode::ReadOnly, read the existing data to a new buffer, and unlock the pixel buffer. Then, lock the pixel buffer again with ImageLockMode::WriteOnly, and perform the writing operation.
3. **To display the Bitmap in the PictureBox, use AutoSize::Zoom:** There are several ways to resize bitmaps to fit in the available space on the GUI. Zoom mode is one of the fastest as no interpolation is used to render the image as long as the aspect ratio is the same for both the bitmap and the PictureBox it is displayed in. Be careful not to use an image resizing mode like stretch, which will perform an interpolation on the bitmap and slow down rendering speeds considerably.

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