

TOOLS USED IN THE DEVELOPMENT AND SUPPORT OF AN
ANTI-OXIDANT BIO-PHOTONIC SCANNER

by

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ABSTRACT

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I report on the development and production of the Pharmanex S2 Biophotonic Scanner. A brief description of measuring carotenoid concentrations using Resonance Raman Spectroscopy (RRS) and a history of the S2 are given. The S2 uses light emitting diodes (LEDs) and bandpass filters to excite a Raman response at two wavelengths. Four photomultiplier tubes with corresponding bandpass filters detect four points of the emission curves. Using a divided shifted technique the two emission curves provide a measure of the carotenoid signal strength. I designed a set of computer controlled tests using the LabVIEW™ programming language used in the production of the S2. These tests validate the quality of the scanners produced through visual and pass/fail criterion. A description of the hardware and software of the “Scanner Setup Tool” and “FG5 Scanner/Filter Monochromator Tool” are given in detail.

ACKNOWLEDGEMENTS

I would like to acknowledge Scott Bergeson and Justin Peatross from BYU who both acted as my advisors during this project. I would also like to thank John Fralick, a consultant on this project, for his mentorship and friendship. From Pharmanex: Scott Ferguson, David Rockhill, Jaime Salas, and, Clay Keisel. Thanks for putting up with me. From National Instruments: Garth Black, for all the technical support and programming advice that I crucially needed for this project.

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Chapter 1 INTRODUCTION

Automated test and measurement tools are widely used in today's manufacturing environments [1]. Industries use these tools for a wide range of task. Examples of these tools are smart camera systems that can visually check that a part is assembled correctly, or environment regulators that maintain temperature and humidity. Even products that are assembled by hand require computer-controlled quality assurance tests to ensure manufacturing standards are met. Computer automated tools can improve production efficiency, reduce error, and boost product quality [1].

For the past four years I worked as a research assistant for the locally based company, Pharmanex, a division of Nuskin Enterprises. As part of a five-member team, I helped develop a second-generation instrument to measure anti-oxidant levels in a person's skin using resonant Raman spectroscopy. In this thesis, I will describe a set of computer controlled tests I designed to help in the development and production of Pharmanex's Biophotonic Scanner.

1.1 RESONANCE RAMAN SPECTROSCOPY

The Biophotonic scanner uses the Raman Effect to detect specific nutrients in human tissue. The Raman Effect results from the inelastic collision of photons and certain molecules [2]. These molecules have vibrational energy stored in spring like oscillations of their constituent atoms. As an example, consider a photon of given wavelength (color) that interacts with a molecule. The molecule absorbs a photon and then releases another of less energy (longer wavelength) by the amount of energy in the vibrational mode. The intensity of the Raman scattering depends, among other things, on the wavelength of light used to excite the molecules. Raman scattering increases by several orders of magnitude when the excitation wavelength coincides with the molecule's electronic absorption band [2].

Carotenoids are one type of molecule that experiences this increase. Two examples of carotenoids are β -carotene and lycopene which give color to many vegetables and act as anti-oxidants. As discussed in Reference 3, the human body generates highly reactive oxygen species (ROS) and free radicals. These ROS, with their high-energy internal state, can react with and destroy important organic molecules like proteins, lipids, and cellular DNA. Carotenoids act as quenchers of singlet oxygen by readily absorbing and dissipating the energy of singlet oxygen. This quenching effect protects cell membranes from oxidative damage. The presences of carotenoids can be thought of as the first line of defense against harmful oxidants by providing the easiest target. By attacking the carotenoids first, the oxidant concentration is decreased, leaving the cells better protected. Carotenoids therefore contribute to nutritional protection and enhance the body's antioxidant defense system.

1.2 S1 BIO-PHOTONIC SCANNER

Scientists at the University of Utah developed technology to measure carotenoid concentration in living human tissues using Raman spectroscopy. They collaborated with Pharmanex to develop a table-top instrument to detect lycopene and β -carotene in human skin. These first relatively expensive and large footprint instruments were called “gold-standards” because of their high precision. Using these devices as a model, Pharmanex developed a portable Biophotonic scanner. This first-generation scanner used a low-power blue laser (473 nm) to illuminate a small spot on the hand. It used a spectrometer to measure fluorescence and the faint Raman signature of anti-oxidant molecules in the green wavelength band. The Raman signal occurs at a specific wavelength (509.8 nm) shifted 1525 cm^{-1} from the excitation wavelength. The difficulty in accurately measuring the Raman signature is that the skin has a strong fluorescence background approximately 100 times stronger than the Raman peak (Fig.1). While this first-generation device was successful in measuring the Raman signature, Pharmanex wanted a lower-cost device with high reliability in the field. [4]

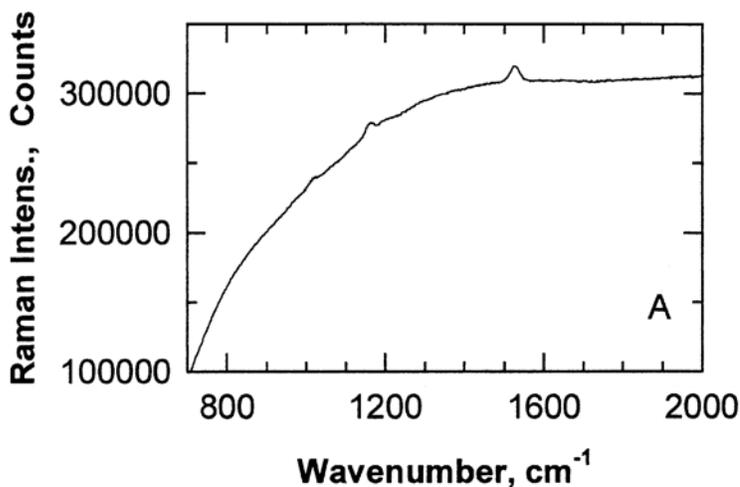


Figure 1: Raman “bumps” with large Fluorescence Background [5]

1.3 SECOND GENERATION S2 SCANNER

The S1 scanner was a complex instrument. It was expensive to build, required a narrow range of operating temperatures, and tended to bleach skin samples. Professors Justin Peatross and Scott Bergeson of the Department of Physics and Astronomy at BYU closely examined the scanner and proposed a new scanner that would eliminate many of the short comings in the S1 design.

Their second-generation scanner, called the S2, replaces the expensive laser used in the S1 with an inexpensive Light Emitting Diode (LED) source. However, because an LED is less coherent than a laser, the illuminated spot size on the hand tends to be considerably larger. The LED light must be spectrally filtered to achieve a sufficiently narrow wavelength range for Raman spectroscopy. The resulting large spot size and low light level present a significant challenge for detector design. The detector receives insufficient light to use a standard grating spectrometer. Instead we use four high-gain photomultiplier tubes (PMTs) with associated bandpass filters to observe wavelengths on and near the emitted Raman peak (Fig. 2).

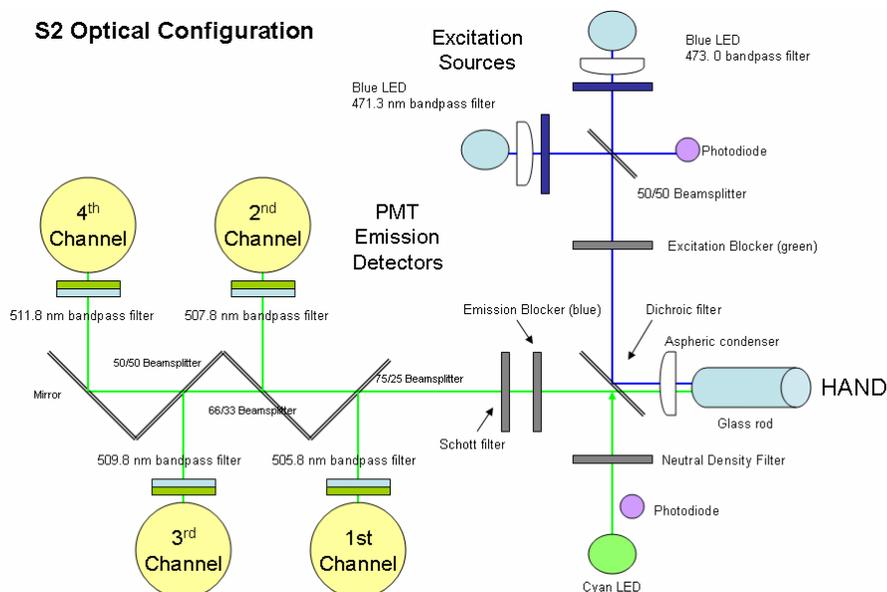


Figure 2: S2 Optical Layout

The use of PMTs presents its own challenges. An accurate detection of the anti-oxidant signal requires a precision of near a few parts in 10,000. The relative efficiencies of the four PMTs can vary by a few percent while taking a measurement. If not properly handled, this can cause errors that can overwhelm the Raman signal. This problem is avoided by measuring the ratio of light intensities for two different excitation wavelengths. As will be shown in the following section, the variation in the PMT gain divides away in the analysis.

1.3.1 SCANNING METHODOLOGY

The S2 scanner excites the Raman line alternately at two wavelengths and measures the fluorescence in four wavelength channels. The smooth lines in Fig. 3 show simulated Raman signals. The horizontal bars represent the spectral bandpass of the four fluorescence measurement channels. The first excitation wavelength puts the Raman line in the 2nd detection channel shown in the top panel of Fig. 3. The other three channels are

background fluorescence only. The second excitation wavelength puts the Raman line in the 3rd detection channel shown in the bottom panel of Fig. 3. The scanner measures these two shifted spectra at a 5 Hz repetition rate, and averages the measurements for 90 seconds. The two sets of averaged measurements are then divided into each other, dividing away detector efficiency variations.

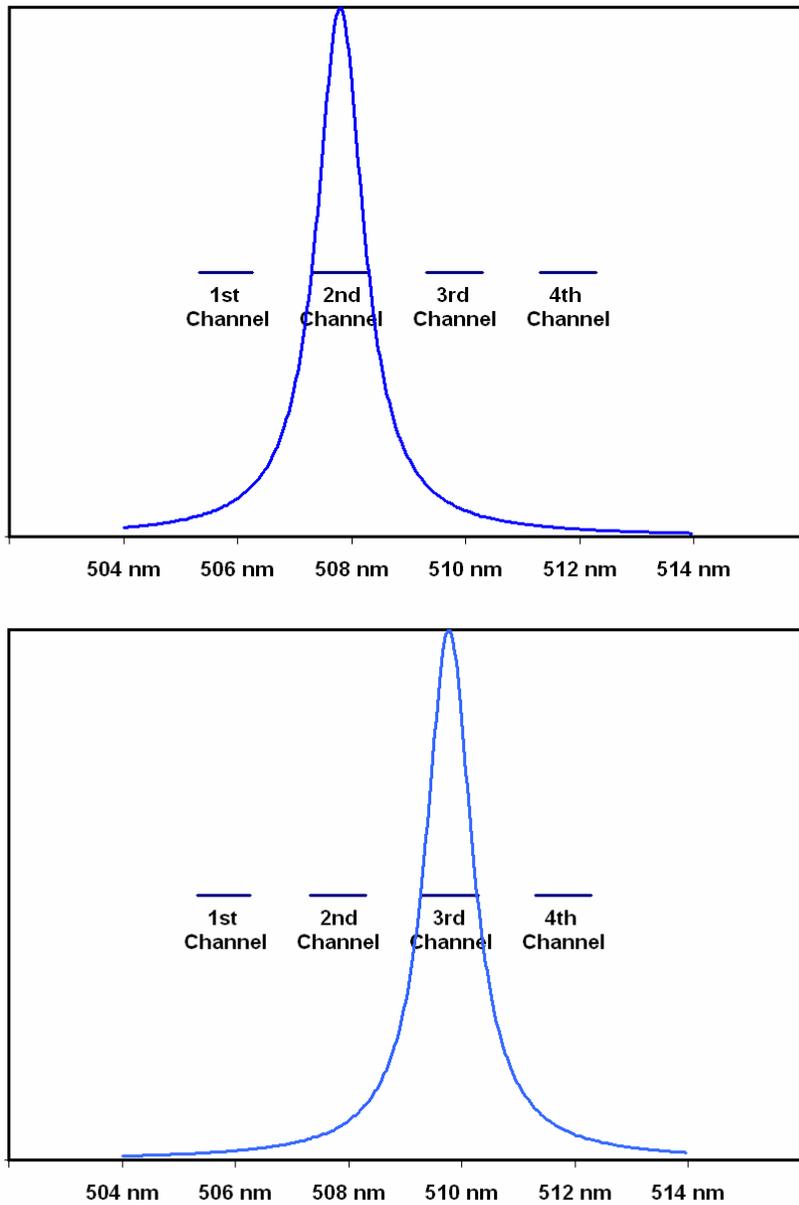


Figure 3: Detection Channels at Two Excitation Wavelengths

The strong background fluorescence causes the ratios of the four channels to all fall close to one with only slight variation introduced by the presence of the Raman signal. Depending on the excitation wavelength, either channel 2 or 3 has a tiny extra amount of signal due to the Raman response of the carotenoids. This added signal creates the shape of the spectral curve shown in Fig. 4, which is sampled by the four PMTs at the points indicated. The ratios represented by the four points are:

Equation 1: Analyzer points

$$\frac{\text{Flour 1 at Excit 1}}{\text{Flour 1 at Excit 2}}, \frac{\text{Flour 2 at Excit 1}}{\text{Flour 2 at Excit 2 + Shifted Raman}}, \frac{\text{Flour 3 at Excit 1 + Shifted Raman}}{\text{Flour 3 at Excit 2}}, \frac{\text{Flour 4 at Excit 1}}{\text{Flour 4 at Excit 2}}$$

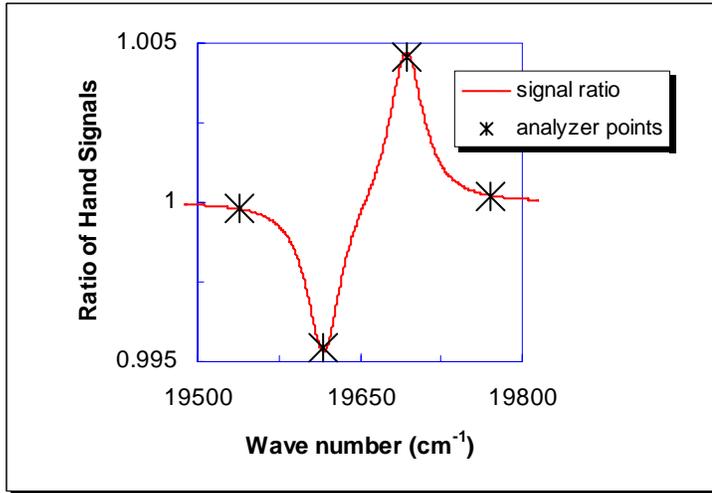


Figure 4: Ratio of two shifted Raman spectra

1.3.2 DATA ANALYSIS

The four points are used to determine a background level and a Raman signal strength. Mathematical uniqueness requires that the curve representing the background be a polynomial of order two or less – a parabola or a line. We find that a parabolic

background provides the best measurement reliability. The parabola is constrained to pass through the two endpoints, which always represent background fluorescence. We also require that points 2 and 3 are an equal distance above and below the parabola.

The distance from point 2 to the parabola is labeled D, and signifies the relative strength of the anti-oxidant signal compared to the background fluorescence (Fig. 5). Ideally, for the PMTs used in the S2 scanner, a sample containing no anti-oxidant signal should yield a value of D less than 2×10^{-4} , while a typical Raman peak would be greater than 100×10^{-4} . The value of D is put onto an absolute scale by multiplying by the average of the PMT signals used in the denominator of the ratios. This new value is what we call the Carotenoid-count (C-count). Overall machine calibration is achieved through a multiplicative factor obtained by measuring the C-count from a substance of known carotenoid concentration [6].

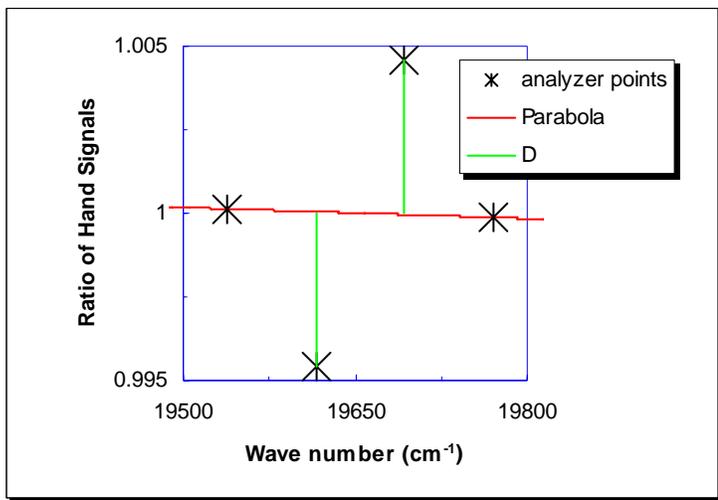


Figure 5: Parabola and D

1.3.3 BANDPASS FILTER SELECTION

A significant calibration problem arises from errors in the spectral spacing of the excitation and emission filters. Because β -carotene is an organic compound, it decays too quickly to be used as a standard reference source. Instead, a silicone plastic is mixed with a chemical known to give a similar Raman peak to β -carotene. Since the signal from the standard does not have precisely the same Raman Shift as in human tissue, the spacing of the bandpass filters needs to be precisely matched to obtain the same response to both the calibration standard and human skin across different machines.

The proper spacing of bandpass is achieved by a two step process. First, the filter spectra are measured using a monochromator to determine the bandpass wavelength of each filter. Second, six filters having the correct spacing are installed into a machine. The machine is then partially assembled and tested using a monochromator to verify that the bandpass spacing is correct. As discussed later in this thesis, I wrote the program to measure the filter wavelengths and to test whether an assembled machine meets the acceptance criterion.

1.3.4 ADVANTAGES OF THE S2 SCANNER.

In comparison and validation tests, the S1 and S2 scanners performed similarly in terms of accuracy and repeatability. The major advantage of the S2 is that it scans in one-third the time of the S1 and requires fewer recalibrations. A second advantage is the wider range of environmental conditions in which it can operate normally. The scan operators were formerly required to operate the S1 scanner in a low-humidity environment within a temperature range of 75° to 85° F. Now the restriction is 50° to 95°

F in a non-condensing environment [7]. Pictures of the S1 and S2 Biophotonic Scanners appear in Figs. 6 and 7.



Figure 6: S1 vs. S2 Size Comparison



Figure 7: S2 Side Profile

1.4 TOOLS USED IN THE DEVELOPMENT AND PRODUCTION OF THE S2

This Thesis discusses a few of the many LabVIEW™ computer programs I wrote and that are used in the developing and manufacturing the S2 scanner. Over the course of the project, I wrote dozens of programs for a variety of specialized tests and applications. Besides the general scanner operating software, I programmed the LED and PMT burn-in and test tool, the LED acceptance tester, the self-calibrating scanner set-up, and several others, all of which had several generations. The first few programs were modeled after ones written by my advisor, but as time went on I became the local expert and programmed independently.

My most widely-used programs are the “scanner setup” tool and the “monochromator” tool. These are used in the day-to-day assembly and testing of the scanners in production. These programs operate scanner hardware and external test equipment, are used to select LEDs, PMTs, and filters. They are used to test the overall performance of each scanner and are the subject of this thesis.

1.5 PUBLICATIONS

I am a co-author of an article that reports on the S2 Biophotonic Scanner which was accepted for publication in the Journal of Biomedical Optics on Feb 5, 2008, and will appear in print around June of this year. The article, entitled “Resonance Raman measurements of carotenoids using light emitting diodes”, outlines in detail the design of the S2.

The S2 technology is also described in a patent application entitled “Raman Instrument for Measuring Weak Signals in the Presence of Strong Background Fluorescence”. Abstracts for the article and application appear in Appendix D.

The S2 scanner has been presented at two conferences. First, BIOS2008, the Society of Photographic Instrumentation Engineers (SPIE) conference on biomedical optics, held on January 19, 2008. That presentation will be published as part of the conference proceedings: “Divided shifted Raman spectroscopy for carotenoid detection,” *Biomedical Vibrational Spectroscopy V: Advances in Research and Industry*, BIOS2008, San Jose, CA.

The other conference was the 16th Annual International Laser Physics Workshop held on Leon Mexico in Aug, 2007. The presentation was entitled “Reliable Detection of Carotenoid Molecules in Human Skin Using Light Emitting Diodes,” and was presented by Justin Peatross.

1.6 THESIS CONNECTION TO MY HONORS EXPERIENCE

The S2 Biophotonic Scanner is a successful marketing tool for selling dietary supplements. The C-count maps onto a scale that ranges from 10,000 to 90,000, with higher numbers indicating a better antioxidant score. Pharmanex guarantees that anyone using their dietary supplements will see their score increase. This promise has motivated thousands to buy Pharmanex supplements and make other life changes to improve their health.

It is interesting to think about the role of numbers in a person's view of their health. Some people obsess over numbers such as weight, body mass index, cholesterol level, blood pressure, and so forth. Over time we have learned that these numbers can only be partially controlled by lifestyle choices, and target values tend to be redefined as our knowledge increases.

Our culture is driven in part by the concept of 'if you are healthy you are happy.' Increased measurement capabilities give us more numbers and if we are not careful these numbers can be used as the criterion for happiness. Numbers provide one measure of health, which can influence happiness, but there are other important aspects of happiness that are not easily quantified, and that should not be neglected.

As stated by Viktor Frankl, "It [does] not really matter what we [expect] from life, but rather what life [expects] from us. We [need] to stop asking about the meaning of life, and instead to think of ourselves as those who were being questioned by life - daily and hourly. Our answer must consist, not in talk and meditation, but in right action and in right conduct. Life ultimately means taking the responsibility to find the right answer to its problems and to fulfill the tasks which it constantly sets for each individual." [8]

Stated differently, numbers alone cannot bring more meaning to life. However, they can be used in formulating choices that promote good health.

Chapter 2 LABVIEW™ SOFTWARE AND HARDWARE INTERFACES

LabVIEW™ is a fully graphical programming language developed by National Instruments (NI) and is used for all tools described in this thesis. Code is organized into programs called virtual instruments or VIs. It is assumed the reader is familiar with the basics of LabVIEW™. Appendix B provides an overview of LabVIEW™ for the novice user.

One powerful aspect of LabVIEW™ is its ability to communicate with almost any scientific instrument. Any device that has an interface connection such as, GPIB, VXI, PXI, Serial, Ethernet, or USB can communicate with LabVIEW™ through built in I/O protocols. The S2 is controlled using a data acquisition board that interfaces with the computer through USB.

This chapter describes the method LabVIEW™ uses to communicate with instruments and gives overviews of two specific devices used in the manufacturing and production of the S2.

2.1 LABVIEW™ VISA

LabVIEW™ can communicate with instruments through the Virtual Instrument Software Architecture (VISA). VISA is a standard for configuring instrumentation systems including GPIB, Serial, Ethernet, and USB interfaces. VISA functionality appears as nodes on the block diagram. In order to communicate with a device using RS232 protocol, the device port and a string command is required. The string command is unique to a particular device and causes it to perform tasks. These commands are contained in the device's operating manual. With this input, the 'VISA Write' node converts the string to machine code and sends the command to the device port. The 'VISA Read' node then requests data from the port and converts it into a string value. Depending on the operation, this string can be used in subsequent operations or calculations [9].

2.2 NATIONAL INSTRUMENTS USB-6009

The USB-6009 is the data acquisition device used in the S2. Manufactured by NI, the USB-6009 provides connections to eight analog input (AI) channels, two analog output (AO) channels, 12 digital input/output (DIO) channels, and a 32-bit counter through a USB interface. The AI resolution is 14 bits differential and 13 bits single-ended with a maximum sampling rate of 48 kS/s while the DIO can be configured as an open-drain or push-pull. [10]

For integration as a component in the S2 a custom OEM version of the USB-6009 was engineered. It differs from the standard USB-6009 in several respects. First the external cover of the hardware package is removed and the original connectors are replaced with a socket for a ribbon cable making installation into another device easier (see Fig. 8). Also there are custom modifications to the electronics and firmware that change four of the DIO channels into 11 bit AI channels and allow access to the onboard EEPROM for storing operating parameters.



Figure 8: NI USB-6009

The USB-6009 Interactive Control Panel, shown in Fig. 9, provides basic interaction between computer and instrument. In the center of the front panel is a diagram of the board with pin locations of the ribbon cable socket indicated. Each control or indicator associated with the I/O function has a label indicating the pin or pins it links to. The interface is very useful for initial setup and testing of any application using the USB-6009.

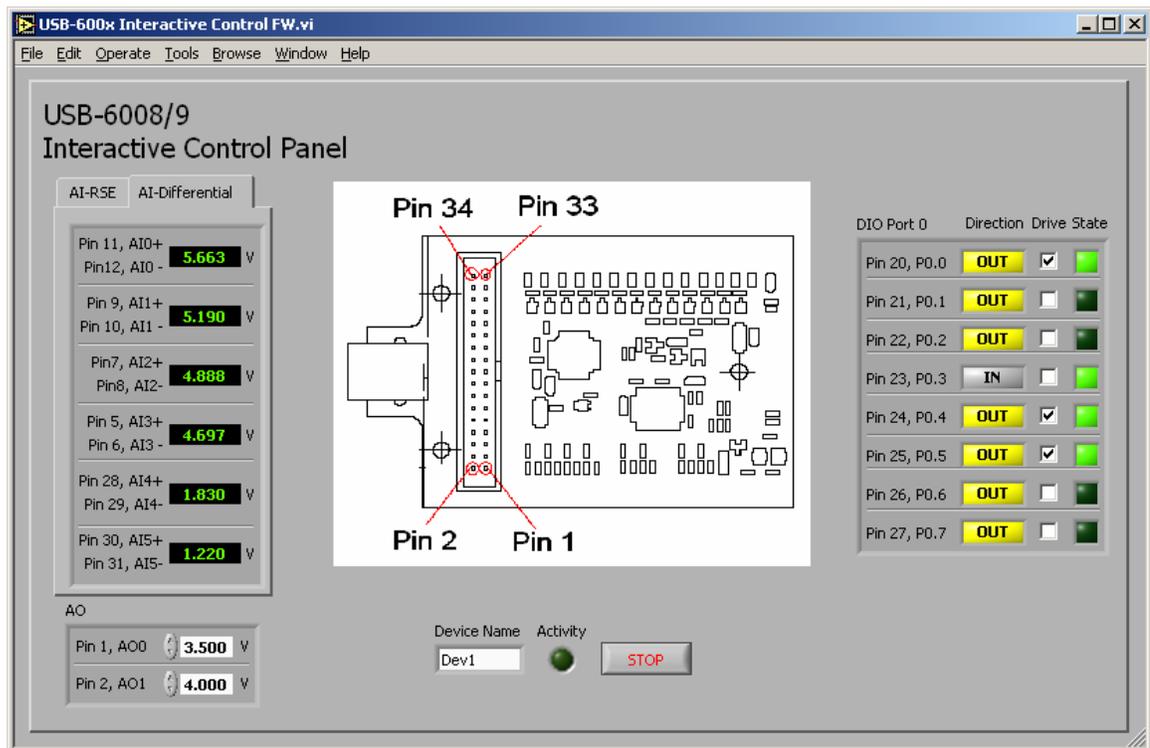


Figure 9: USB-6009 Interactive Control Panel

The USB-6009 also uses VISA to communicate with LabVIEW™, although the procedure to communicate with USB devices is much more involved than RS232. To simplify the code interface, NI developed a driver called NI-DAQ™-mx Base which adds device specific VIs and functions to LabVIEW™. As an example, Fig. 10 demonstrates the methodology of communicating with the USB-6009 to acquire data through the AI. The first subVI (see Appendix B.4) creates a task that is registered into the computers

memory. The next two subVIs configure the task based on their inputs. In this instance the task is configured to acquire two AI channels in differential mode with a range of -20 to 20 Volts at a rate of 1000 samples per second for one sample. The task is then started or written to the USB-6009. Next the task is executed and the data is transferred to the block diagram. Finally the task is stopped on the USB-6009 and cleared from the computers memory.

The figure also shows three examples of polymorphic subVI. These VIs have drop down menus where various configuration options are available. The subVI that reads the data is currently set to read a 1-Dimesonal double precision array, it can also be set to a 2-Dimensional double precision array for AI function or several different options for DIO and Counter functions.

Variations of the basic setup below are used in all communication between the S2 Scanner and the computer.

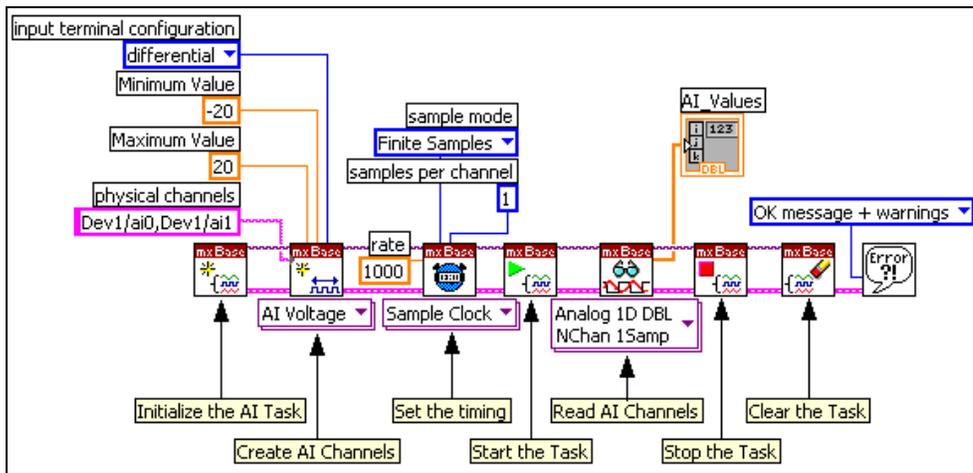


Figure 10: DAQ mx BASE AI Example

2.3 ORIEL MONOCHROMATOR

The bandpass filter selecting process mentioned in section 1.3.3 uses a Cornerstone™ 260 ¼ Monochromator manufactured by Oriel Instruments (see Fig 11). The monochromator has a 1200 lines/mm grating with 10 µm fixed slits at the input and output. This setup gives a spectral resolution of about a tenth of a nanometer. [11]

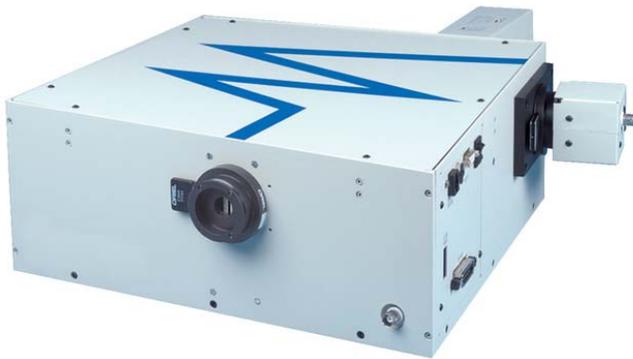


Figure 11: Cornerstone™ 260 ¼ Monochromator

The Cornerstone™ 260 ¼ model communicates with a computer either through Serial RS232 or GPIB. Sample LabVIEW™ interfaces for both communication types are included as part of the device. This project uses the RS232 interface to use the monochromator as both a spectrometer and a monochromatic light source.

The RS232 interface VI provides control of the basic functions of the monochromator. Indicators show the present wavelength, the type of grating and filter installed, and a status indicator for the shutter. The controls perform various actions, like repositioning the grating to a specific angle, scanning over a specific range in specified increments, and opening or closing the shutter. All of this is done using VISA

functionality. The Cornerstone™ 260 ¼ does not acquire any data from the light passing through it so all of the commands sent and data retrieved are short strings. This allows for acceptable speeds of operation with the bandpass filter matching.

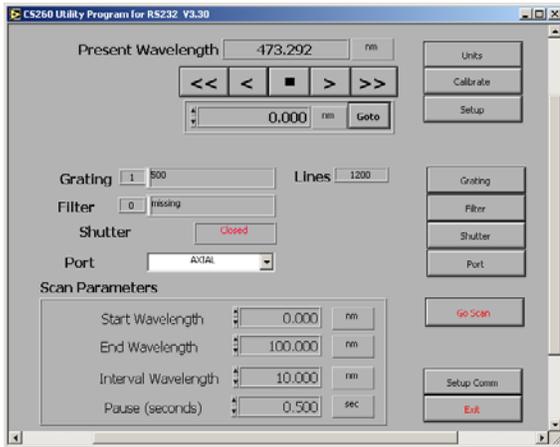


Figure 12: Oriel Monochromator LabVIEW™ Interface

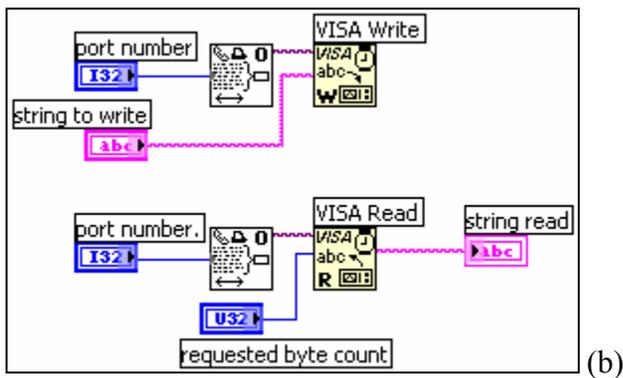


Figure 13: Oriel Monochromator LabVIEW™ VISA code

Chapter 3 SETUP AND QUALITY TESTING IN SCANNER MANUFACTURING

The S2 Biophotonic Scanner is manufactured at a plant located in Shanghai, China. Most of the employees don't speak English, and communicating with the few who do is challenging. It can be difficult to implement changes in factory procedures. These limitations make it important that the tools I develop can be easily operated while maintaining all required functionality.

I developed six LabVIEW™ interfaces currently in operation at the China plant: the filter matching tool, sled tester, LED tester, 24 hour burn-in tool, scanner setup tool, and the monochromator tool. Each interface varies in its complexity for user interaction. The two tester tools and burn-in tool require almost no user input to the interface and give simple readouts so that virtually any employee can use them without confusion.

The monochromator and scanner setup tools ideally require a well qualified technician who understands the S2 technology and design to operate the user interfaces. However, none of the technicians fit this profile. To compound this problem, the original

interface version contained many of my idiosyncrasies. Though the format and flow seems logical to me I need to consider the point of view of the end user. This constrains the kinds of functions and data I can allow the technicians to access. For example, when I give too much information, the technicians try to recalculate the pass/fail criteria by hand which leads to confusion and error. Other times they try to somehow force their measurements to meet the pass/fail criteria, also resulting in non-optimized scanners.

Through repeated errors resulting from misuse of the program I have made many changes to provide a simpler user interface that is less inclined to confuse the technicians. The next two sections will give overviews of the scanner and monochromator setup tools. Each interface presented is of the current software revision implementing the lesson and considerations mentioned above. I will describe these tools and step the reader through each tool's functions with comments about how each function is used in the assembly process.

3.1 SCANNER SET-UP TOOL

The factory uses the scanner setup tool, or “Scanner Tool S2.vi,” for setting the gain on the PMT and photodiode signals. The user activates the VI and performs a series of steps involving changing settings on the front panel or making adjustments to the scanner electronics. Figures 14 and 15 show the front panel. To better understand the tools functionality, this section will describe the three major areas of the front panel and briefly describe the VIs role in the scanner setup process.

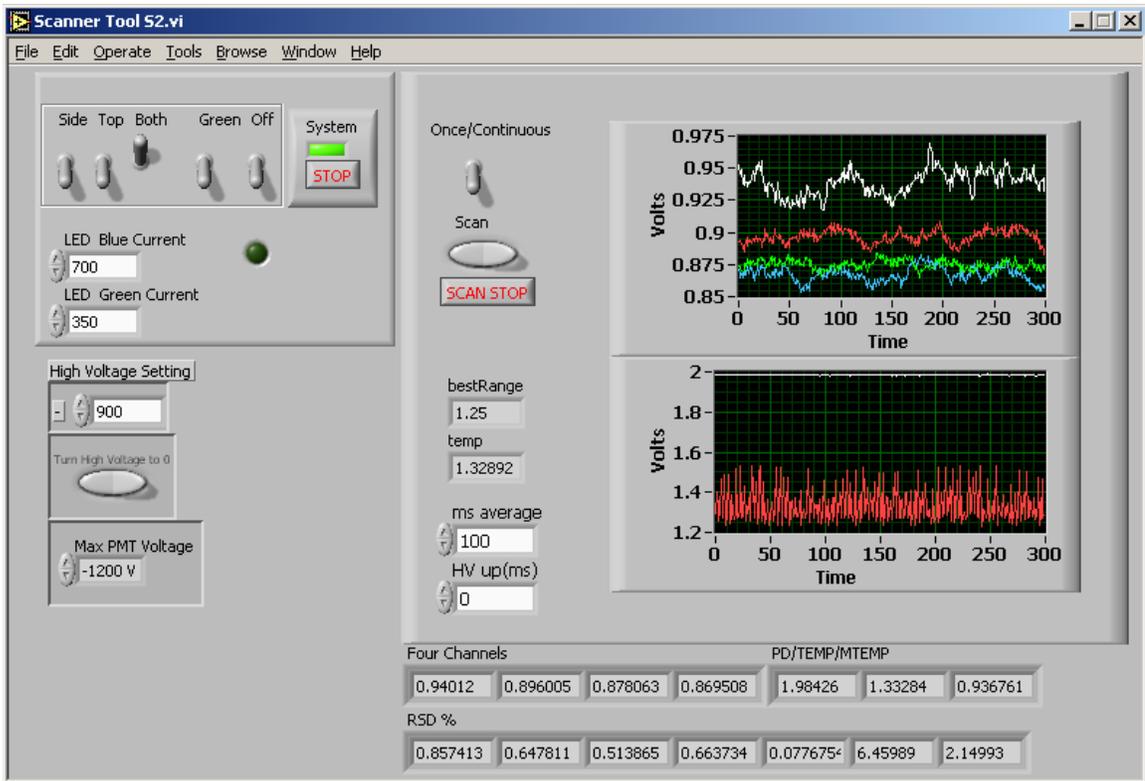


Figure 14: Scanner Set-Up Tool Front Panel

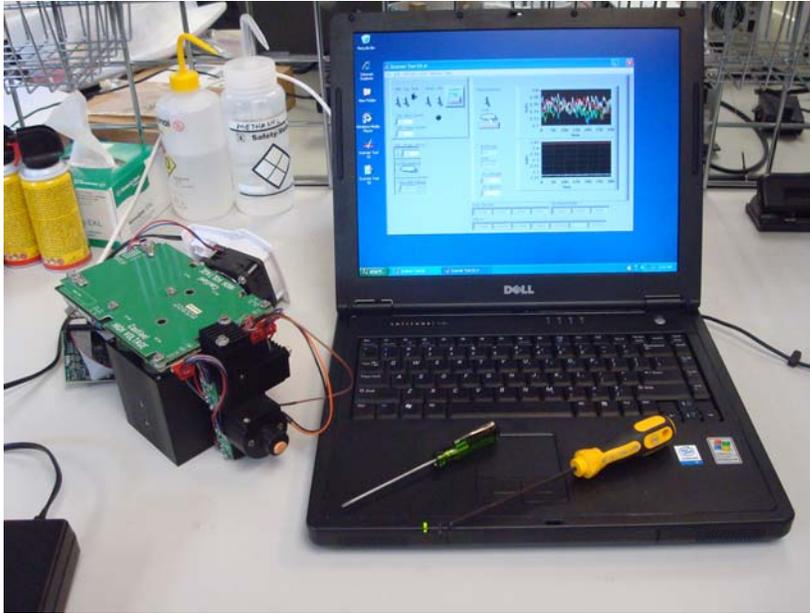


Figure 15: Scanner Set-Up Picture

The user controls the scanner LEDs through the controls in the upper left hand corner of the front panel (Fig. 16). Using the indicators that look like switches the LEDs are turned on and off while the two numerical controls set the current levels. To protect the LEDs, the switches are programmed so that only one can be on at a time and the Blue/Green Current boxes have maximum current restrictions. With these controls the user can check that all the LEDs function correctly and change the current in conjunction with steps in the setup procedure.

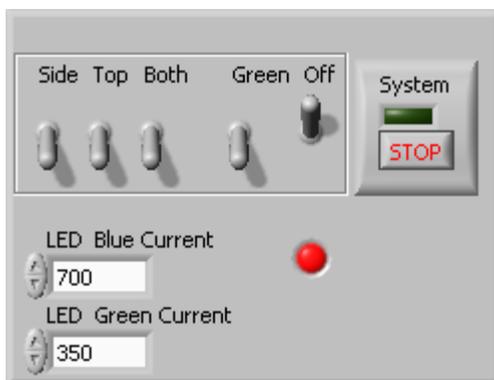


Figure 16: LED Controls

The user visually checks the AI signal of the PMTs and photodiodes using the controls and indicators on the right side of the front panel (Fig. 17). After pressing the ‘Scan’ control the program acquires data for the amount of time specified in “ms average”. The upper graph displays the four PMT voltages, while the lower one graphs the photodiode signal (white) and the temperature (red). The ‘High Voltage’ controls (Fig. 18) change the voltage for the PMTs and can be seen real-time in the upper graph while changes in the LED current will be seen in the white line of the lower graph. The PMTs are protected from overdriving by the ‘Max PMT Voltage’ control and be set to zero using the associated rocker switch.

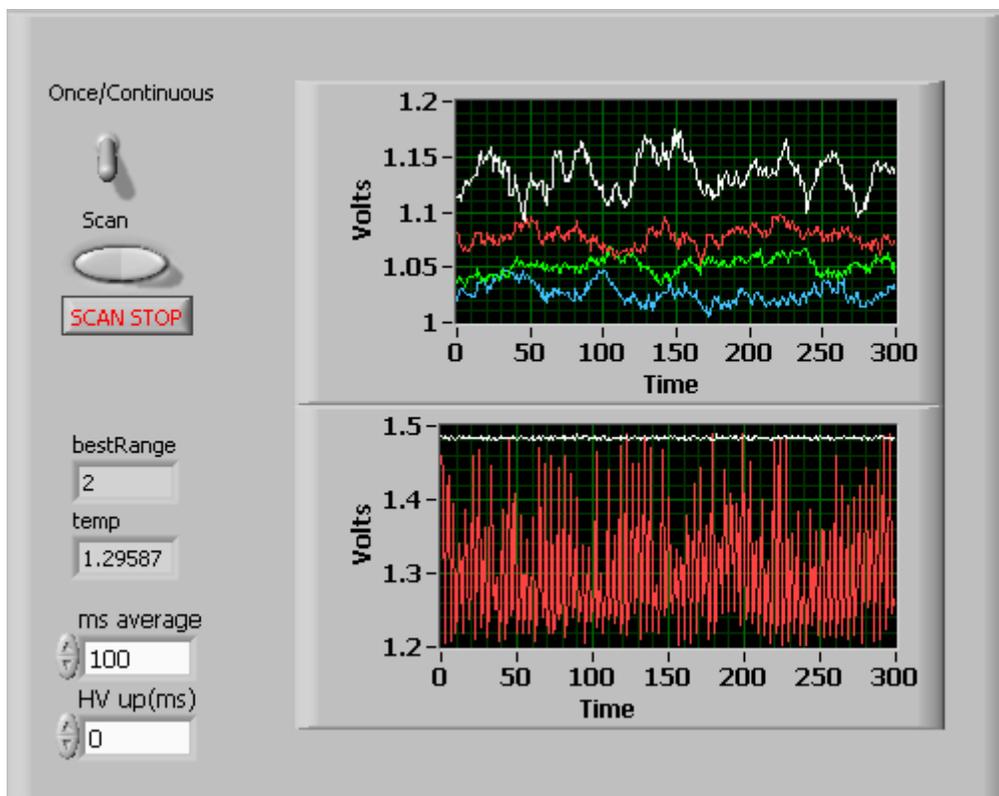


Figure 17: AI Data Acquisition

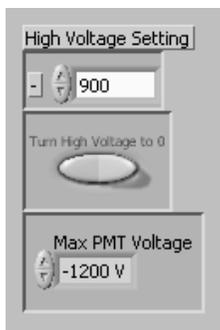


Figure 18: High Voltage Control

Using a blank calibration standard as a fluorescence reference, these controls and graphs are used to adjust the positions of LEDs to achieve maximum throughput (Figs. 19 and 20). Next, the blue LED with the lowest average signal amplitude is used to illuminate the standard in order to set the gain of each PMT. Initially, each PMT has different amplitudes, using variable resistors the gain of each PMT is adjusted until they all read the same. A similar procedure is used to set the gain of the two photo diodes.

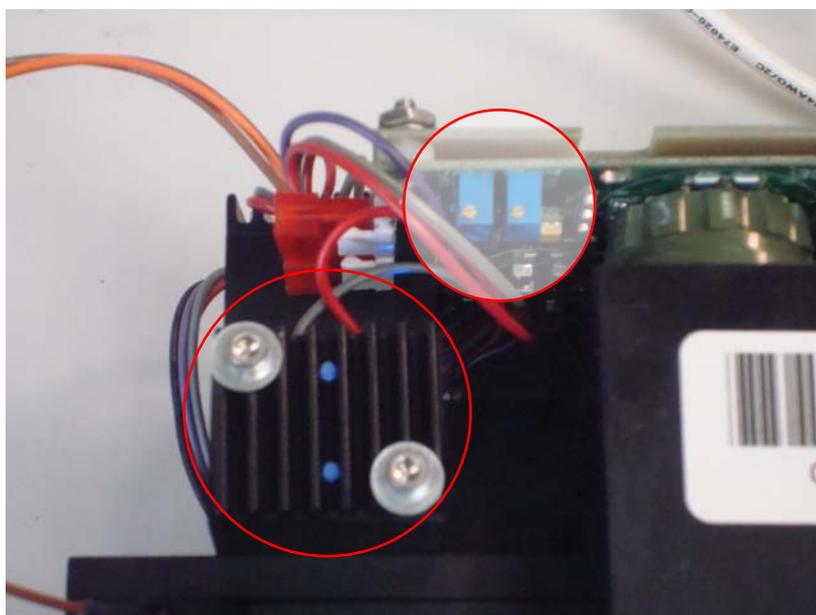


Figure 19: LED Alignment and Gain Adjustment

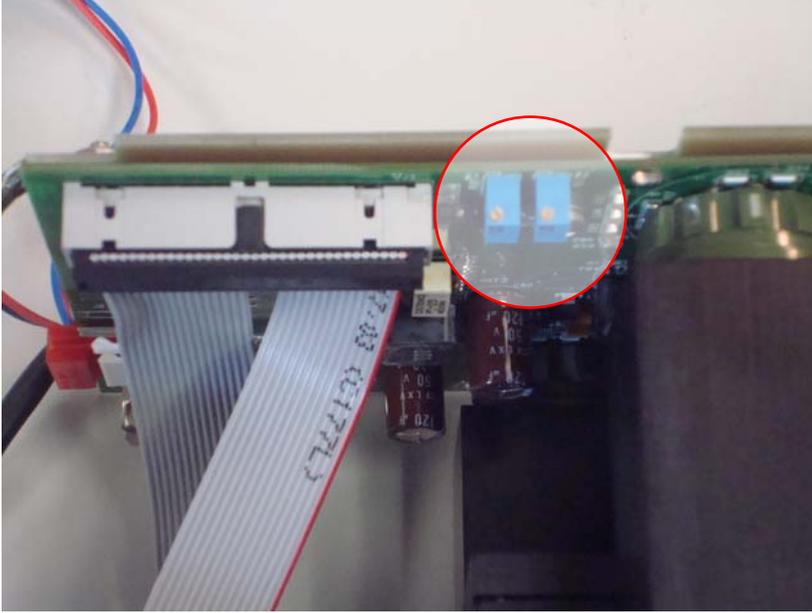


Figure 20: PMT Gain Adjustment

A description of the “Scanner Tool S2.vi” block diagram is found in appendix C.1.

3.2 FG5 SCANNER/FILTER MONOCHROMATOR TOOL

The filter matching setup consists of a Cornerstone™ 260 ¼ Monochromator, a modified S2 scanner (MS2), and either an S2 scanner or filter to be tested. Depending on the configuration, two or three devices are controlled by the FG5 Scanner/Filter Monochromator Tool (or FG5). The FG5 interface communicates with the one or two scanners and the monochromator's serial port simultaneously to take spectral measurements.

The FG5 is designed to control three separate configurations: auto-calibration, filter transmission measurements, and filter set testing. While all configurations can be controlled from the basic interface; in practice FG5 is packaged into two executables, one for a scanner and another for filters. The calibration configuration can be used from either executable.

3.2.1 THREE FG5 CONFIGURATIONS

Configuration #1: monochromator calibration

The first configuration calibrates the monochromator (Fig. 21). After repeated use, the monochromator alignment changes slightly, causing errors in the filter matching process. To avoid this, the monochromator is calibrated daily. A cadmium lamp that produces narrow spectral lines in the 470 to 520 nm range is placed in front of the focusing optics. The FG5 performs a 50 nm scan, measuring the cadmium spectra. Using this data and the known values for the Cadmium lines, calibration coefficients are generated for the monochromator.

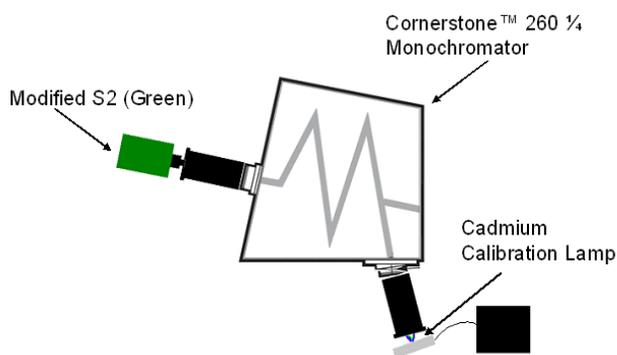


Figure 21: FG5 Monochromator Calibration: Configuration #1

Configuration #2: Filter transmission measurement

The calibrated monochromator is then used to characterize the S2 transmission filters. The FG5 Filter transmission measurement setup uses one of two filter holders with blue or green LED light sources. The set up is shown in Fig. 22. With these holders the user can obtain spectral profiles for both excitation and emission filters. In operation, a filter holder is attached and the LED is connected to an external power supply. The FG5 turns on the PMTs of the MS2 and scans the wavelength range of the filter. To protect the PMTs from direct exposure to the LED light, 15 nm bandpass blocking filters are placed in the filter sockets of the MS2. Two different blocking filters allow two PMTs to measure blue light and the other two green.

The monochromator transmits light with 0.1 nm bandwidth. The wavelength of this passband is swept across the transmission profile of the filters in the MS2 and the PMT voltage is recorded. These measured transmission curves are used to create a matched set, as described previously.

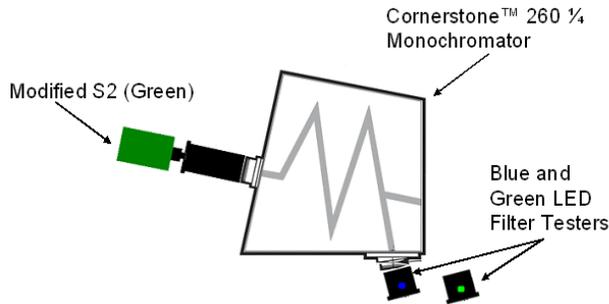


Figure 22: FG5 Filter Transmission Measurement: Configuration #2

Configuration #3: Filter set testing

Finally, a matched filter set is installed into a scanner. Before final assembly, this matched set is measured again. When a matched set is installed into a scanner and tested again, the bandpass wavelengths of the different filters do not always match up. This is probably due to variations in the blocking filters and also to slight misalignments in the filters. When a filter is tilted, its bandpass wavelength shifts.

To test an installed filter set, we need to measure the blue excitation filters using emission measurements, and the green emission filters using transmission measurements. We do this using the MS2, the monochromator, and the installed set.

A diagram of the two-scanner setup is shown in Fig. 23. In order to obtain a spectral profile of the six bandpass filters, three light sources must be used. FG5 first turns on one of the S2s Blue LEDs sending light through its excitation filter. The light continues through the monochromator and into the MS2. Next, the MS2's PMTs power up and the monochromator scans incrementally through the wavelength range of the excitation filter. At each increment, FG5 collects data from the MS2 and reads the wavelength from the monochromator. This produces a transmission curve for the

excitation filter. This process is repeated with the second blue S2 LED to obtain the second filter's transmission curve.

The third light source is a green LED in the MS2 located where one of the blue LEDs normally resides. This time, green light from the MS2 passes through to the installed filter set in the S2. The monochromator scans through the wavelength range of all four emission filters and the PMTs record transmission curves for the four filters.

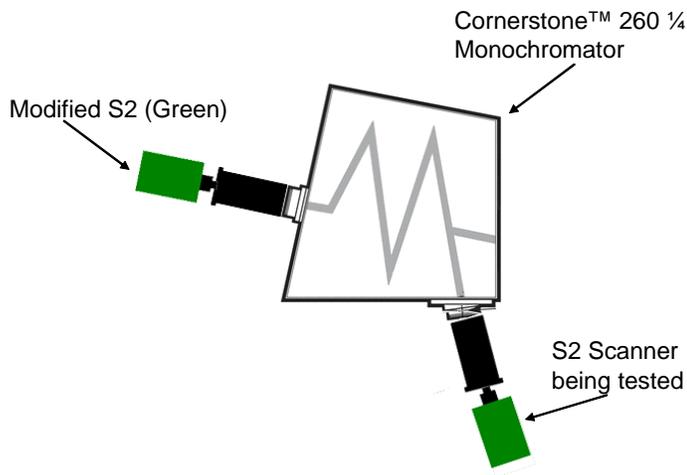


Figure 23: FG5 Filter Set Testing: Configuration #3

3.2.2 FILTER LETTER CODE

The FG5 Scanner and Filter setups assign letter codes to filters. When filters are received from the manufacturer they are labeled with their target wavelength. For use in the factory a single digit number is assigned. The four emission filters are numbered 0-3 and the excitation filters are numbered 5 and 6. With each filter there is a certain tolerance in the transmissions peak wavelength. Each filter is sorted into bins that are defined by a maximum and minimum difference from the target wavelength. Letters A-Z identify each bin with N being the bin closest to target. The letter code is formed by the

filter number and the bin letter. A matched set consist of six numbered filters all with the same letter. For example 5F, 6F, 0F, 1F, 2F, 3F form a matched set.

In certain cases a filter has two letter codes. The bins farthest away from N for one filter can overlap with the farthest bins of another. These filters receive a double letter code. The first code starts with filter types number and its letter code. The second is number and letter of the overlapping bin. For example: 0Z – A1.

3.2.3 FG5 USER INTERFACE

The FG5 Front Panel is designed for both simple and advanced functionality (Fig. 24). For normal operation the user has access to a few controls and a limited amount of data. Advanced options are accessed through the menu toolbar and are used for troubleshooting and maintenance of the FG5.

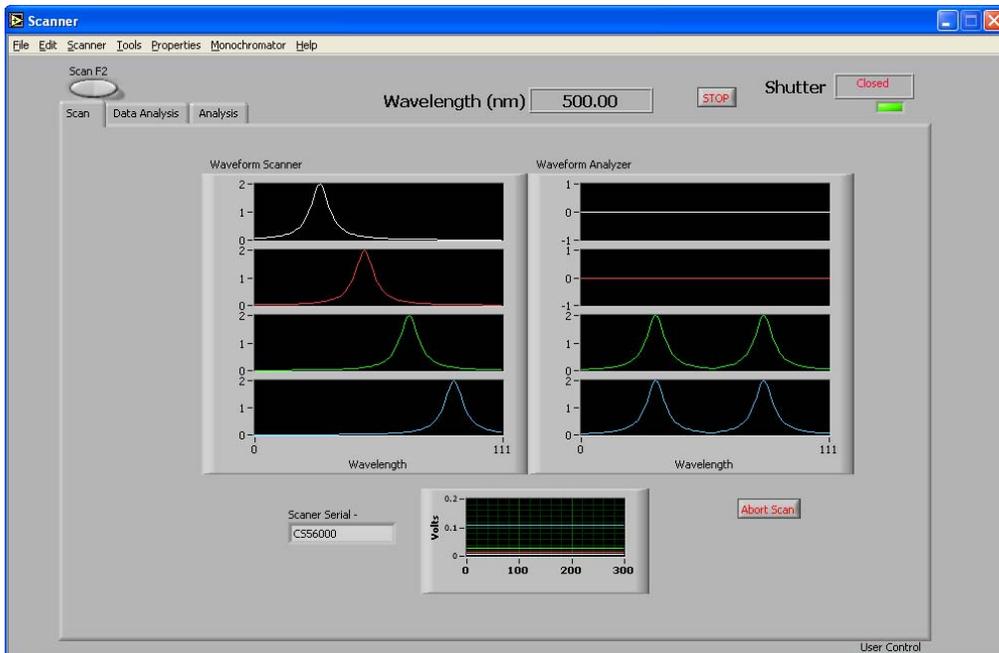


Figure 24: FG5 Front Panel

Across the top of the front panel are the most basic controls and indicators. The ‘Scan F2’ control initiates either a scanner or filter scan while the ‘Stop’ control stops the VI. Two of the indicators show information about the monochromator’s current wavelength and shutter status. The LED indicator on the far right tells whether or not the most recent calibration is being used.

The FG5 Front panel displays most of the scan data in one of three tabs shown below the ‘Scan F2’ rocker switch. The three tabs are called ‘Scan,’ ‘Data Analysis,’ and ‘Analysis’ (also seen in Fig. 24). The two graphs named ‘Waveform Scanner’ and ‘Waveform Analyzer’ display the PMT voltages of the S2 and MS2 PMTs respectively. While the bottom graph shows the most recent PMT data read from either scanner. The ‘Abort Scan’ button terminates the scan without stopping the VI. This is useful when something is amiss in an FG5 configuration. The last indicator, ‘Scanner Serial -’, shows the serial number of the S2 under test.

The ‘Data Analysis’ tab displays results of a filter’s transmission curve being fitted to a Lorentzian (Fig. 25). The user can select which filter to plot using the ‘Graph’ control in the upper left-hand corner of this tab. The ‘XY Graph’ displays the raw data, the best fit, residuals calculated from the difference of the raw data and best fit. Below the graph, the fit parameters are displayed: the center wavelength, bandpass width, and estimated errors. Also shown are the current filter label, the scanner serial number, and the fit type. The ‘Analysis’ tab changes what it displays depending on whether the FG5 is in the Filter transmission or Filter set configuration.

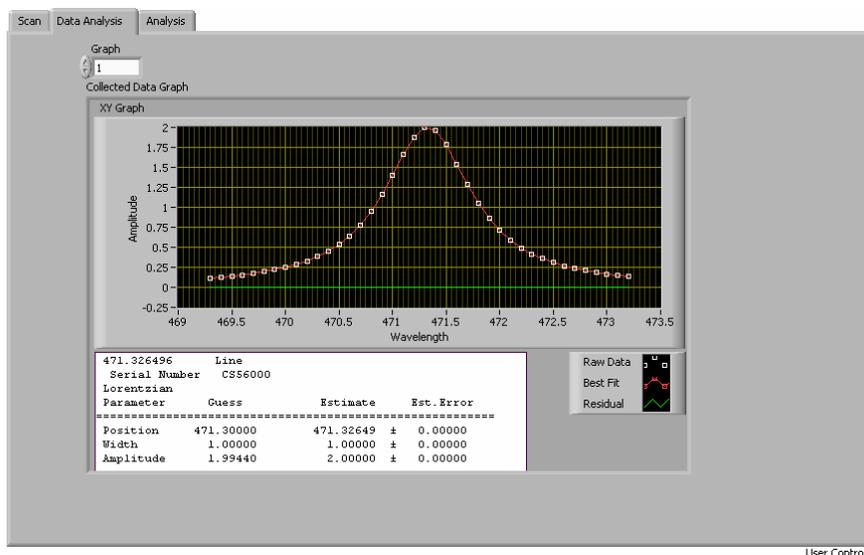


Figure 25: Data Analysis Tab

The FG5 monochromator auto-calibration has been integrated into both the scanner and filter executables for easier operation (Fig. 26). At the commencement of an auto calibration, the FG5 turns off the existing calibration settings and scans cadmium's 467.81nm and 480.00 nm lines followed by the 508.58 nm line. The center wavelength and voltage amplitude of each line is calculated. These scan a repeated adjusting for the scan range and PMT sensitivity. The FG5 then scans each line three times and then averages the data for each line (Fig 27). A Lorentzian fit is generated to find the center wavelength of each line. These values are compared to the established line values of cadmium resulting in a polynomial fit. The polynomial coefficients are then used to measure the 480 nm line again to verify its measured center wavelength is close to the established value. If the calibration is successful, the new calibration information is displayed. If it fails an error is reported and a suggestion is given for resolving the issue.

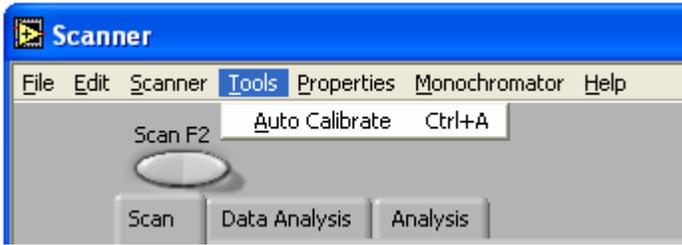


Figure 26: Auto-Calibrate Menu Bar

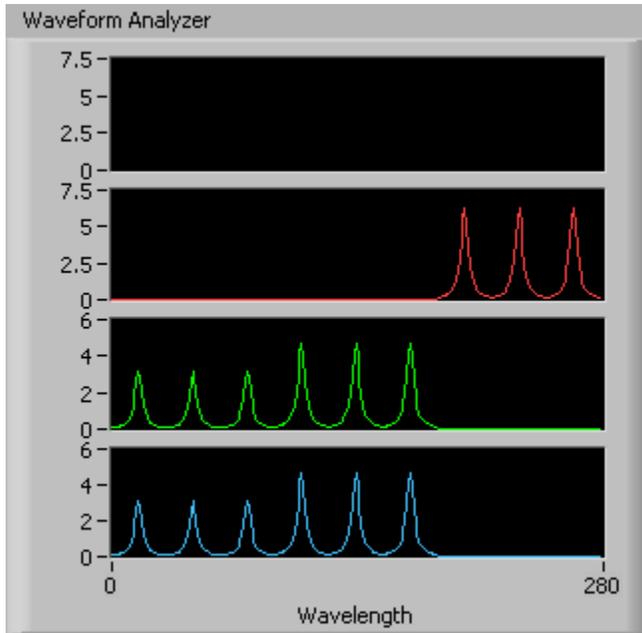


Figure 27: 467.81, 480.00 and 508.58 nm averages

Depending on which is executable is selected the menu toolbar, 'Analysis' tab, and window label, are configured for either transmission measurements or set testing (Fig. 28). When the 'Scan F2' rocker is pushed in the filter transmission configuration the 'Scan' tab is brought to the front and a popup window appears (Fig. 29). The user then selects a filter from the list on the left-hand side. When a custom scan is selected, additional options appear on the screen. These options, shown in Fig. 30, allow the user to choose the initial scan wavelength, the width of the scan, and the expected filter center wavelength. The user presses the 'OK' button and the scan begins.

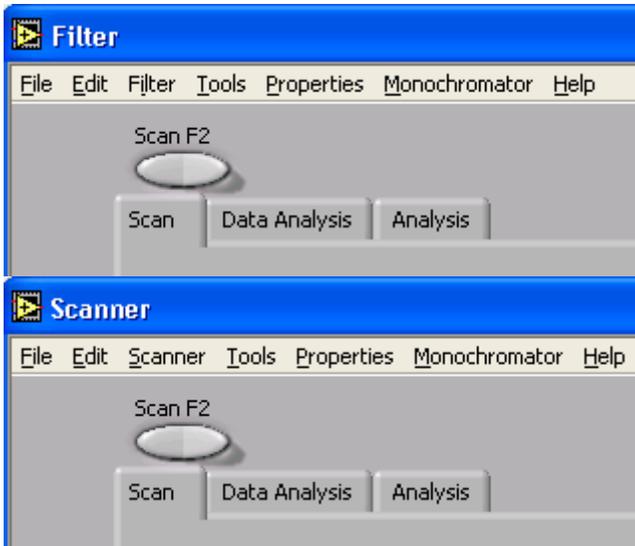


Figure 28: Window Label and Menu Bar

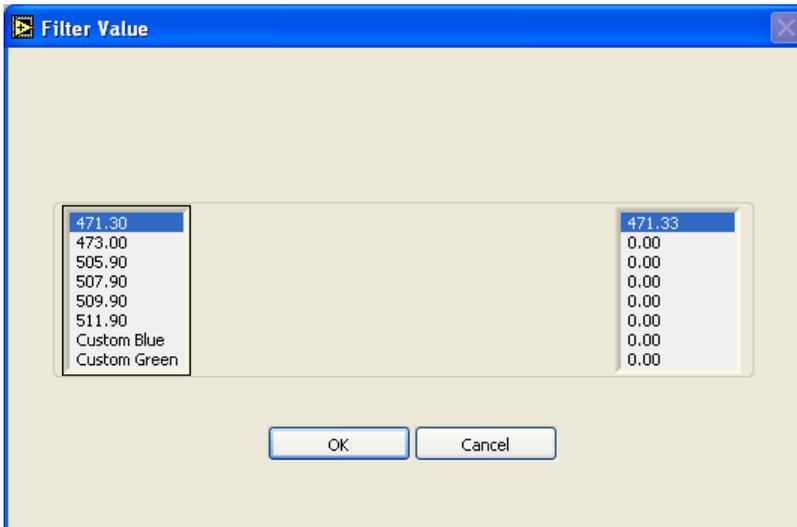


Figure 29: Filter Value Popup Window

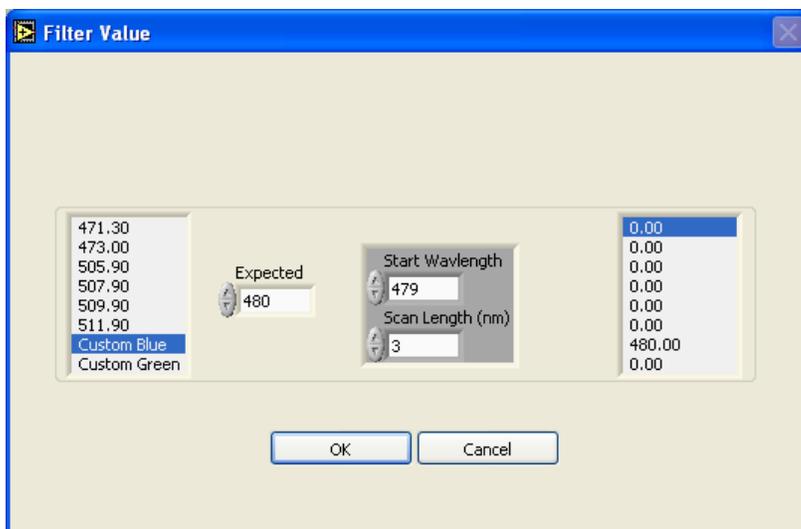


Figure 30: Custom Filter Value Popup Window

The transmission data for the emission (green) or excitation (blue) filter is plotted in the ‘Waveform Analyzer’ chart. The top two windows display data from green filters while the bottom two display data from blue filters (Fig. 31). The data from the two significant windows is averaged together for use in the spectral analysis.

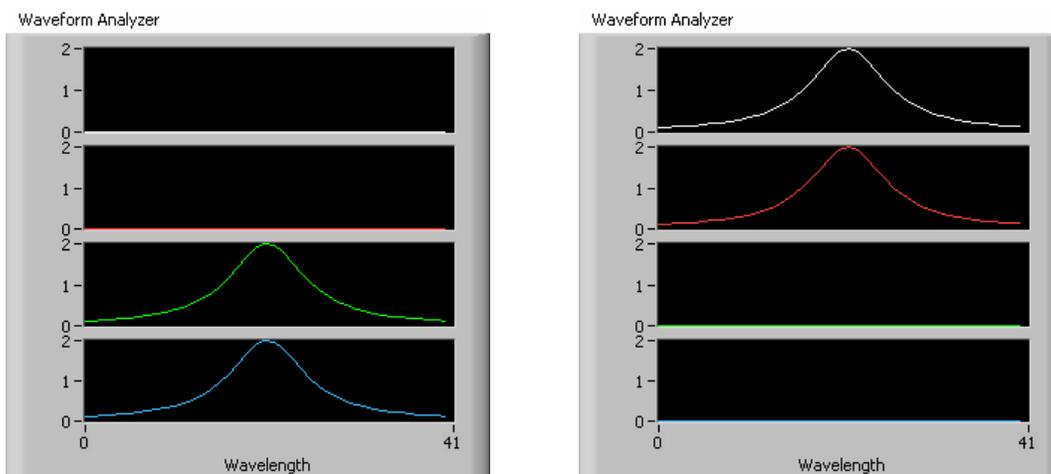


Figure 31: Filter Waveform Analyzer Graphs

After fitting the data to a Lorentzian the results appear on the ‘Analysis’ tab (Fig. 32). For filter transmission measurements, the ‘Analysis’ tab duplicates the graph and table shown on the ‘Data Analysis’ tab. To the right of this is a box labeled ‘Letter Code

Information' containing three indicators: 'Measured', 'Difference From Ideal', and 'Filter Code'. After a successful scan, the 'Filter Code' indicator displays the letter code of the filter.

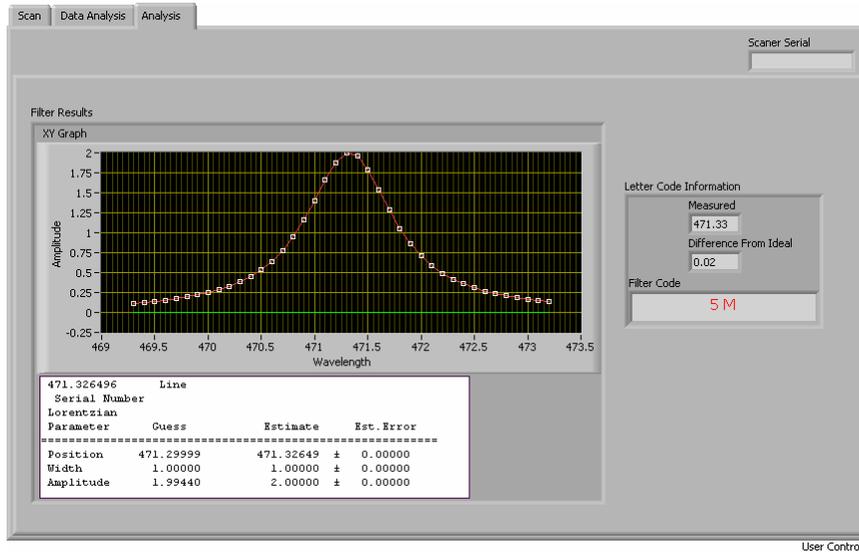


Figure 32: Filter Analysis Tab

When using the filter set testing configuration, pushing the 'Scan F2' rocker opens a pop-up window asking the user to manually enter the scanner serial number. During the scan, the PMTs are read and their voltages are plotted in the 'Waveform Scanner' and 'Waveform Analyzer' graphs. Figure 33 depicts the spectral curves of the six filters in a set after a scan is complete.

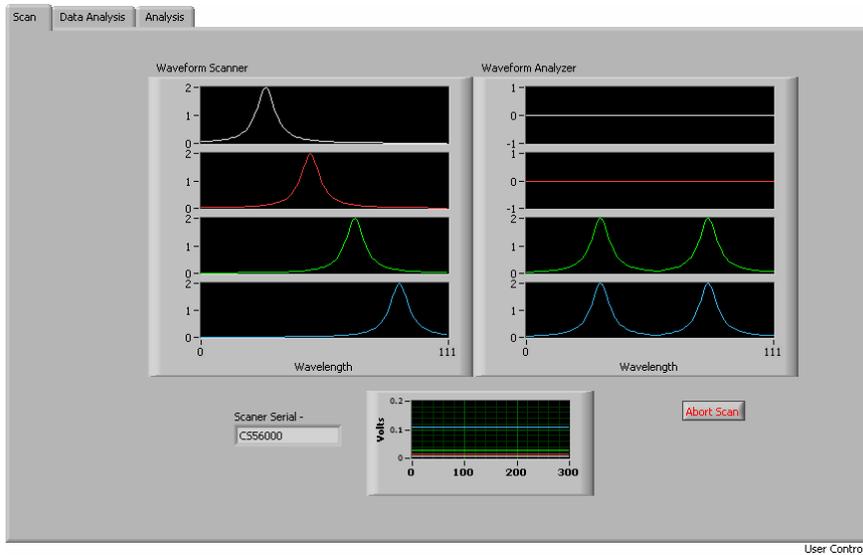


Figure 33: Filter Set Scan Tab

Each filter is fitted to a Lorentzian and then compared to the spacing of an ideal filter set calculated from one of the filters center wavelength. Figure 34 shows the ‘Analysis’ tab that is configured for set testing. The tab displays the pass/fail status of the filter set along with a summary of the filter wavelengths, spectral widths, and scanner letter code. A star symbol denotes the reference filter from which the matched set is calculated. The data summary for each filter that falls into this matched set displays a “thumbs-up”. If a filter falls outside of the matched set, the summary shows a “thumbs-down” symbol. If all six filters pass, then the summary displays a “Pass.” Otherwise, if any of the filters fail, the summary displays “Fail.” Tables 1 and 2 list the possible responses given at the end of a scan.

To understand the LabVIEW™ coding methodology for the FG5, an overview is provided in appendix C.2.

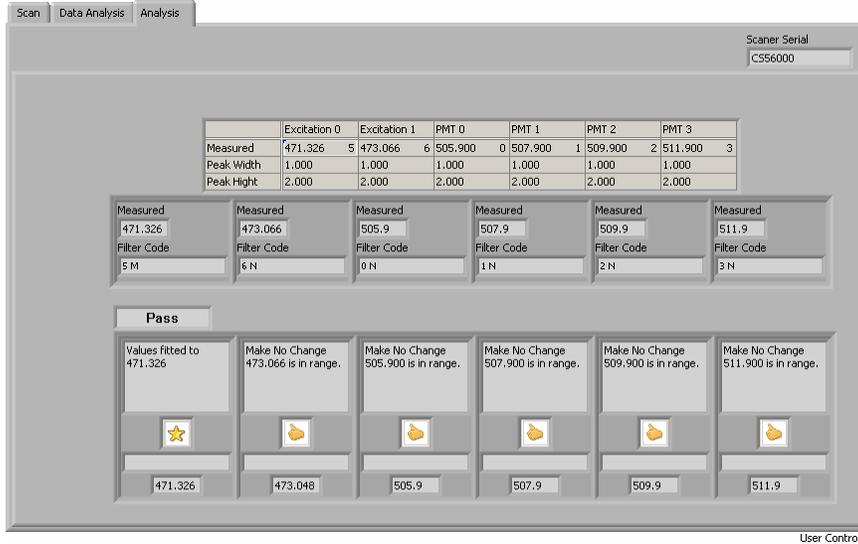


Figure 34: Analysis Tab

Scanner Passes	Fail
Scanner Fails	Pass
One or more filters data maximum is above given voltage range	Voltage High
One or more filters data maximum is below given voltage range	Voltage Low
Scan was aborted	Abort

Table 1: Scanner Pass/Fail

Filter Condition	Message	Icon	Filter Suggestion	Best Fit Value
Filter is in Range	Make No Change 000.000 is in range.		✓	✓
Filter is not in Range	The filter value 000.000 is out of range.		✓	✓
Filter is used to create Best Fit values	Values fitted to 000.000		✓	✓
Filter data maximum is below given voltage range	Voltage is below 1.5 Volts Change HV			
Filter data maximum is above given voltage range	Voltage exceeds 8 Volts Change HV			
Scan was aborted	Aborted			
Another Filter is not in voltage range				

Table 2: Filter Pass/Fail

Chapter 4 CONCLUSION

This thesis describes two (of many) tools I created using LabVIEW™ for use in scanner production and testing. Specifically, I outline the scanner and monochromator setup tools in detail, providing the important aspects of the user interface and LabVIEW™ coding as appendices. As part of ongoing revisions to these tools, I have made considerations to adjust for the abilities and qualifications of the end user.

The S2 Scanner is manufactured in Shanghai, China. Most of the assembly is done by hand, so the tools I have developed have been integrated into the production process to act as vital test for ensuring that manufacturing standards are met. As a result, every S2 Scanner produced is tested using these tools.

The S2 Scanners measure carotenoids in human tissue using an innovative Raman Spectroscopy technique using LED and PMTs. This new technique provides reliable data that can be obtained in varying environmental conditions. The technology of the S2 scanner has been published in a peer-reviewed journal and presented at a national biomedical optics conference and a patent covering the scanning technique is pending.

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- [4] W. Gellermann, I. V. Ermakov, R. W. McClane, and P. S. Bernstein, "Raman imaging of human macular pigments", *Opt. Lett.* 27, 833-835 (2002).
- [5] T. R. Hata, T. A. Scholz, I. V. Ermakov, R. W. McClane, F. Khachik, W. Gellermann, and L. K. Pershing "Non-Invasive Raman Spectroscopic Detection of Carotenoids in Human Skin", *JID* 115, 441-448 (2000)
- [6] S. D. Bergeson, S. Ferguson, J. F. Fralick, and J. Peatross, U. S. Patent Application PCT/US2006/038282.
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- [12] National Instruments, *LabVIEW™ 7 Express User Manual*, (National Instruments, Austin, 2003).
- [13] National Instruments, *LabVIEW™ 7 Express User Manual*, (National Instruments, Austin, 2003), pp 2-1,2-2.

ANNOTATED BIBLIOGRAPHY

Books:

J. R. Ferraro, K. Nakamoto, and C. W. Brown, *Introductory Raman Spectroscopy*, 2nd Ed. (Academic Press, San Diego, 2003).

This is a general text on the subject of Raman Spectroscopy covering the history and scope of the science. This book gives insight to general practices of this field and discusses various applications in different fields of study.

J. J. Laserna, *Modern Techniques in Raman Spectroscopy*, (Wiley, West Sussex, 1996).

This book covers ten fields of research in Raman Spectroscopy. Various ideas for experimental implementation were found in this book. The most helpful section was on time-resolved resonance Raman Spectroscopy, because it is the general idea behind my own research.

D. A. Long, *The Raman Effect: A Unified Treatment of the Theory of Raman Scattering by Molecules*, (Wiley, West Sussex, 2002).

This text is an in depth treatment of the subject, including an extensive mathematical treatment of Raman scattering. For example it includes a full mathematical treatise of Rotational and Vibrational-Rotation Resonance Raman Scattering

R. L. McCreery, *Raman Spectroscopy for Chemical Analysis*, (Wiley, West Sussex, 2000).

This text covers specific techniques in chemical analysis. Its discussion on Raman signals in human tissue and methods for improving measurement is directly helpful to understanding my own research.

E. Smith and G. Dent, *Modern Raman Spectroscopy: A Practical Approach*, (Wiley, West Sussex, 2005).

This text discusses advances in Raman Spectroscopy in recent years, as well as gives a general treatment of the subject. The techniques in this book provide for an idea of how to improve my own work.

Articles:

I. V. Ermakov, M. R. Ermakova, R. W. McClane, and W. Gellermann, “Resonance Raman detection of carotenoid antioxidants in living human tissues”, *Opt. Lett.* 26, 1179-1181 (2001).

This article discusses the use of Raman technology to measure carotenoids in human tissue using non invasive techniques. This article directly talks about the technology that is implemented in my current project.

I. V. Ermakov, R. W. McClane, W. Gellermann, and P. S. Bernstein, “Resonant Raman detection of macular pigment levels in the living human retina”, *Opt. Lett.* 26, 202-204 (2001).

This article discusses the early development of an instrument that the concentration of macular carotenoid pigments in the living human retina. This is an early treatment of this technology before it was developed into a commercial device.

W. Gellermann, I. V. Ermakov, R. W. McClane, and P. S. Bernstein, “Raman imaging of human macular pigments”, *Opt. Lett.* 27, 833-835 (2002).

This article discusses the use of Raman imaging to measure relative concentrations of carotenoids in the human eye. This information can be used to diagnose certain types of macular degeneration. This is the predecessor to the device used to measure human hand tissue.

W. Gellermann, I. V. Ermakov, M. R. Ermakova, P. S. Bernstein and R. W. McClane, “In vivo resonant Raman measurement of macular carotenoid pigments in the young and the aging human retina”, *J. Opt. Soc. Am. A* 19, 1172-1186 (2002).

This is another article that discusses the use of Raman imaging to measure relative concentrations of carotenoids in the human eye. This information can be used to diagnose certain types of macular degeneration. This is the predecessor to the device used to measure human hand tissue.

T. R. Hata, T. A. Scholz, I. V. Ermakov, R. W. McClane, F. Khachik, W. Gellermann, and L. K. Pershing "Non-Invasive Raman Spectroscopic Detection of Carotenoids in Human Skin", JID 115, 441-448 (2000)

This article discussed the use of Non-Invasive Raman Spectroscopy in the field of dermatology. It is believed that high concentration levels of anti-oxidants in human tissue protects against oxidative stress. The article covers the important of these anti-oxidants and relates how this new technology can improve the study of the skin. This is a similar implementation of technology used in my research.

C. R. Smidt, "Clinical Screening Study: Use of the Pharmanex® BioPhotonic Scanner to Assess Skin Carotenoids as a Marker of Antioxidant Status", Pharmanex Study Report, (2003)

An overview of the first generation Bio-Photonic Scanner and the results of a clinical study validating the technology. My research was to make a device with comparable results to this study, while improving on cost and temperature stability.

C. R. Smidt and D. S. Burke, "Nutritional Significance and Measurement of Carotenoids", Current Topics in Nutraceutical Research Vol. 2 No. 2 pp. 79-91, 2004

This article talks about the specific benefits of carotenoids in the human body. It also gives an overview of the best sources for these nutrients. The article helps validate the importance of this research in the medical field.

BPX 473 Configuration

Updated: 9/29/2005

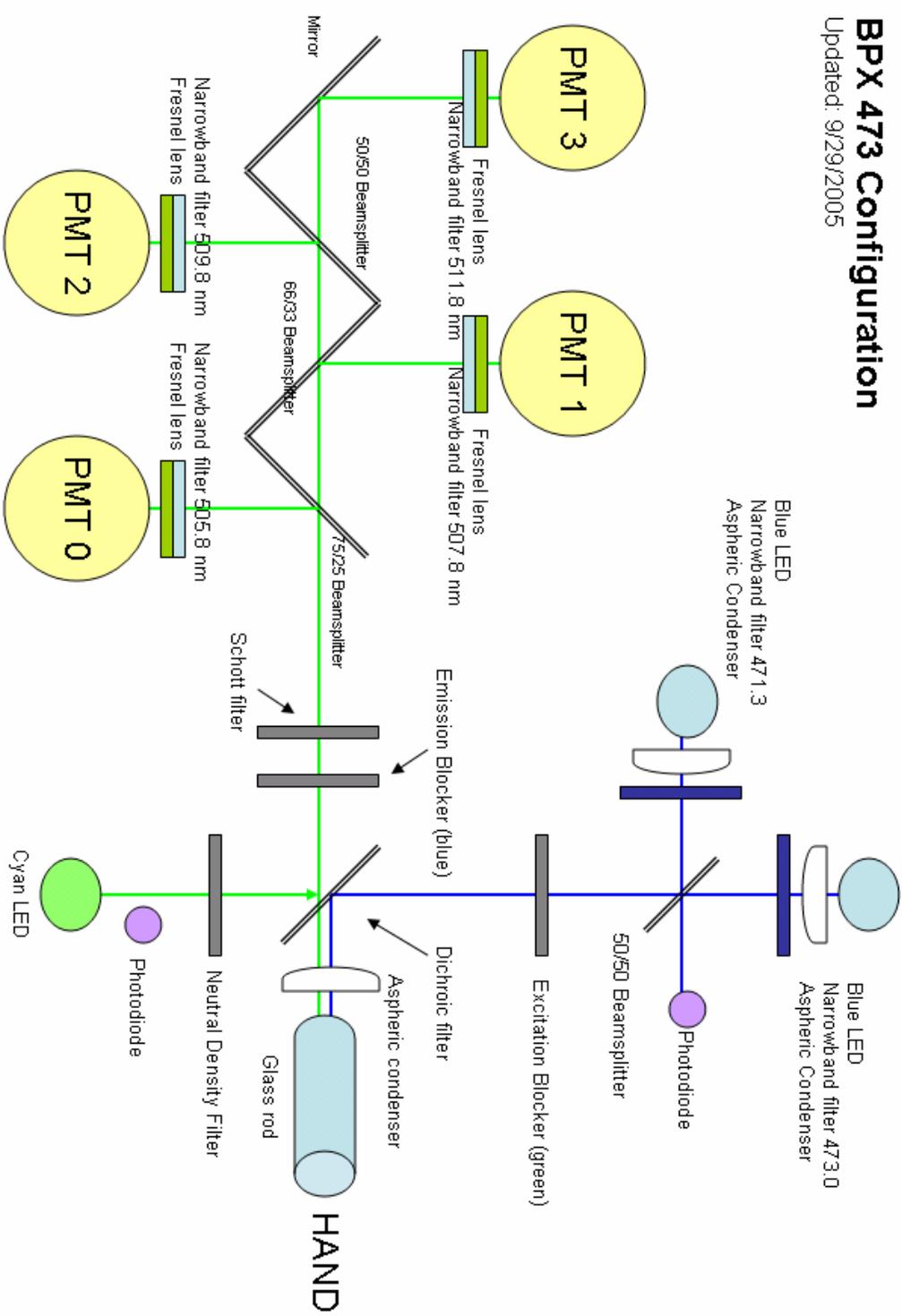


Figure 35: S2 Optical Layout

APPENDIX B: INTRODUCTION TO LAB VIEW

B.1 BASIC METHODS OF LABVIEW™ PROGRAMMING

LabVIEW™ is a fully graphical programming language that can be used without writing any traditional line code. The programming interface is primarily two windows: the Front Panel and Block Diagram. User I/O, graphs, mathematical analysis, and countless other programming objects are placed in one of these two areas. Together the front panel and block diagram form a virtual instrument or VI. A short discussion of LabVIEW™ is given below. A more detailed discussion can be found in the reference [12].

B.2 THE FRONT PANEL

The front panel in LabVIEW™ is designed to function similarly to an interface panel on a scientific instrument. The front panel uses controls and indicators for input and output terminals of the VI. Examples of controls are knobs, push buttons, and dials, while indicators are graphs, LEDs, and other objects that output data. Controls are used as the means to supply data to the block diagram. Indicators then display the data the block diagram acquires or generates. [13]

An example front panel is shown in Fig. 36. This VI produces a sine wave with the amplitude defined by the value of the control named ‘Amplitude’. The two indicators in this example are a graph of the sine wave and an LED Boolean which is activated when the amplitude of the sine wave is zero.

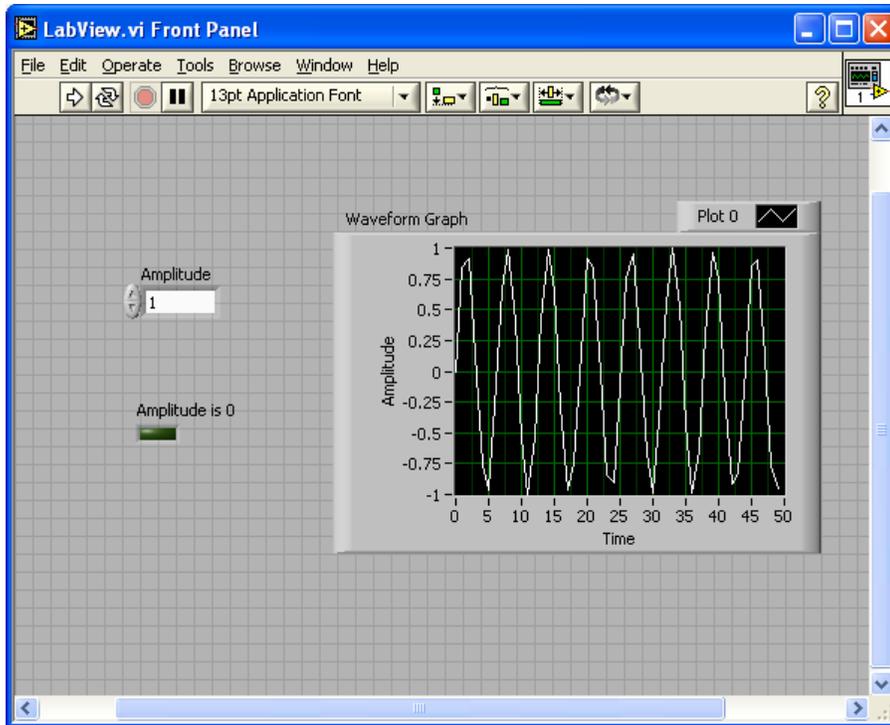


Figure 36: Example of the Front Panel

B.3 THE BLOCK DIAGRAM

After building the front panel, code is added by using graphical representations of functions to control the front panel objects. The controls and indicators on the front panel all appear as objects called terminals on the block diagram. Other objects, called nodes, perform actions when a VI is executed. Terminals and nodes are connected via graphical wires that change appearance based on the type of data being passed. The actions of the nodes are analogous to those in text based languages. There are nodes for mathematical operations, subroutines, instrument communication, etc. [13]

Figure 3 shows examples of terminals, nodes, and wires. The three terminals correspond to the objects on the front panel in Fig. 37. The terminal 'Amplitude' passes its value to a 'for loop' node using a wire that represents a double precision number. The 'for loop' will execute the number of times indicated by the constant attached to the input

in the upper left hand corner. Inside the 'for loop,' three nodes produce the points of sine wave. The blue node outputs the current iteration of the 'for loop' as an integer value and passes it to the 'Sine' node. The 'Sine' node takes the sine of the input and outputs another double precision number. The 'Amplitude' and 'Sine' values then enter the 'Multiply' node where the product of the two values is outputted to an array node on the edge of the 'for loop.'

After the final iteration of the 'for loop' a wire passes a double precision numerical array to the terminal 'Waveform Graph' which appears as the sine wave on the front panel. Another branch of wire connects to the 'Mean' node which passes the mean of the array as double precision number to the 'Equal to 0?' which compares the input to zero and outputs a Boolean value to the wire connected to the 'Amplitude is 0' terminal.

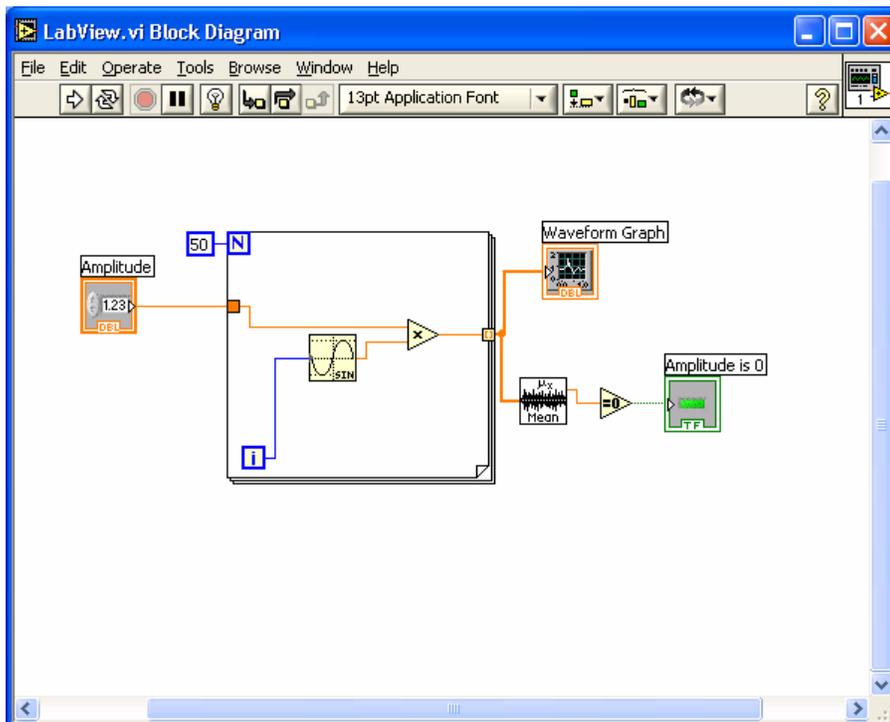


Figure 37: Example of the Block Diagram

B.4 SUBVIS

The VI described previously can execute as an independent program or as a subroutine in another VI. The subroutine VI appears as a subVI node when called by another VI. As an example, Fig. 38 shows a node called “LabView.vi” which represents the code shown in Figs. 36 and 37. Terminals of the same name and data type connect to the input and outputs of the “LabView.vi” subVI. The result of execution is the same, only the way you see the code has changed.

It is advantageous to use subVIs to control communication with external instruments. The interface between a computer and instrument is complex involving many calls and requests as well as the conversion of data into formats that can be transferred through the interface connection.

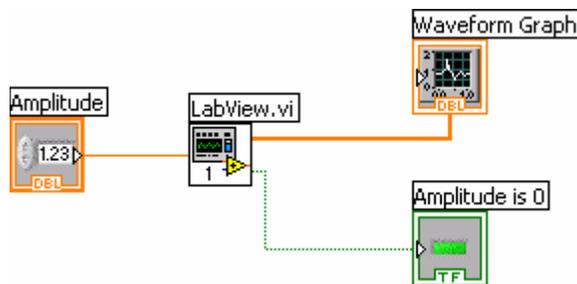


Figure 38: Example of the SubVI

APPENDIX C: TOOL LABVIEW™ CODE

C.1 SCANNER TOOL S2 BLOCK DIAGRAM

The block diagram of the “Scanner Tool S2.vi” is divided between three while loops that correlate to the three main front panel sections. Aside from these while loops, starting and stopping procedures are controlled by a few subVIs.

The first while loop contains the code for controlling the LEDs (Fig. 39). The ‘One Choice LED’ terminal outputs the value of the switch controls defining the state of the LEDs. This value is then past to two ‘index array’ nodes that reduce the ‘Which Current’ and ‘Which LEDs’ arrays to a single set of parameters. The selected ‘Which Current’ is a Boolean value that determines which LED current setting is used. The ‘Which LEDs’ array contains three numbers that define which LED gets turned on or off and is passed to a DIO subVI that switches the state of LEDs

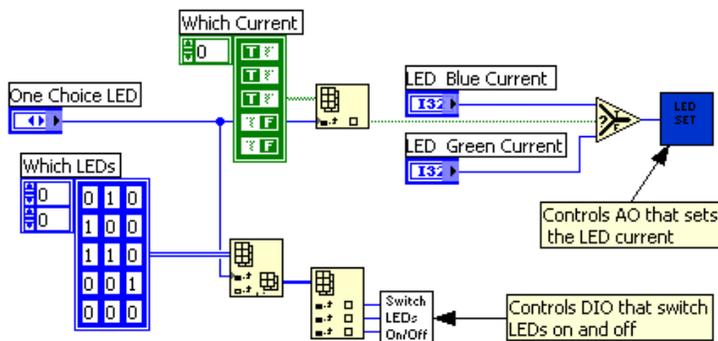


Figure 39: LED code

The second while loop is divided into three sub tasks which execute with each loop iteration (Fig. 40). First, the voltage range is determined by ‘Max PMT Voltage.’ The ‘Turn High Voltage to 0’ value is then used to decide whether to keep the existing ‘High Voltage’ value or set it to zero. The third task compares the ‘High Voltage’ and ‘Max PMT Voltage’ values to reference values. If there is a change in either value a

“true” Boolean value is passed to the case structure. The high voltage changes and reference values are updated.

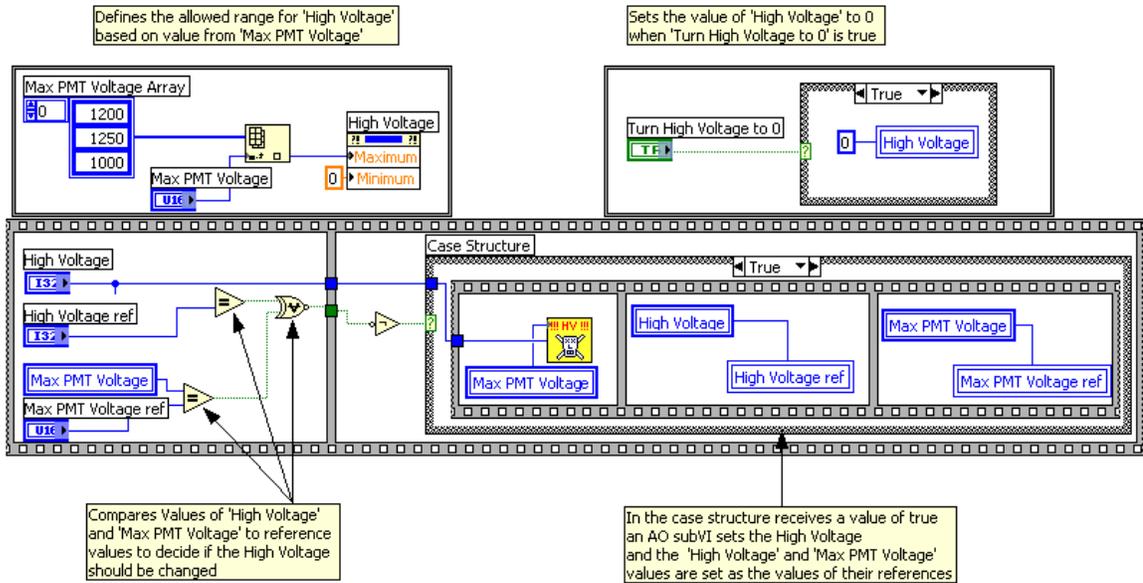


Figure 40: High Voltage Code

The final while loop contains the code controlled by the ‘Scan,’ ‘Once/Continuous,’ and ‘ms average’ terminals. Figure 41 displays two case structures that execute at each iteration. The top case structure use the value of ‘Once/Continuous’ to set the ‘stop Scanning’ control either to stop the scan after the first iteration or to scan continuously. The second case structure receives the value of the ‘Scan’ terminal and performs a scan if it is “true.” Within the case structure an AI task is performed to determine the appropriate data range for the scan. The calculated ‘best Range’ value is then used to reconfigure the AI task. The AI tasks in this code are divided into three separate subVIs. This allows the AI task to be placed in the ‘Scan While Loop’ without having to use CPU time to create and clear tasks. The ‘Scan While Loop’ while continue to execute until either the ‘stop Scanning’ or ‘stop’ Boolean terminals are true.

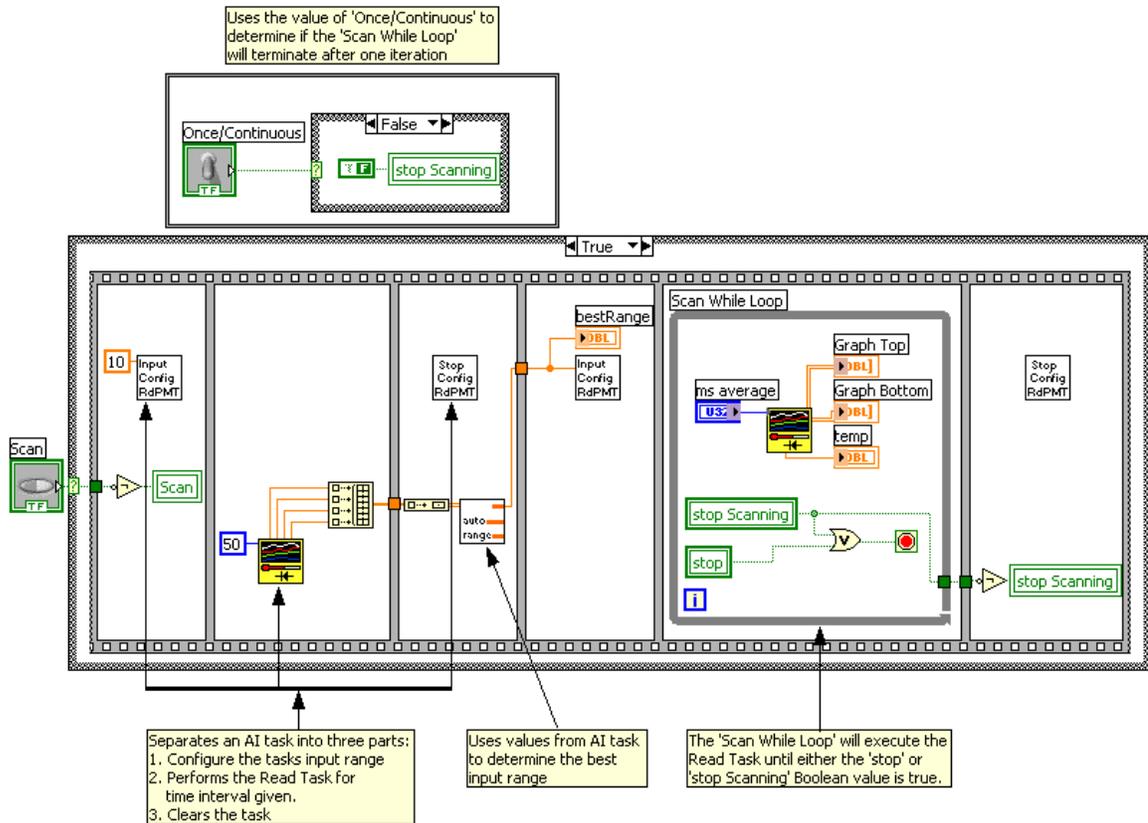


Figure 41: Scan Code

C.2 FG5 LABVIEW™ METHODOLOGY

The FG5 Block Diagram contains more than 30 interacting subVIs, making it too complex to fully document here. Instead I will explain the contents of the “Entire Scan.vi” VI that manages the monochromator scanning. Within “Entire Scan.vi” is another set of VIs that breakdown the Scanner/Filter/Calibration scans into manageable sub routines. Figure 42 shows the layout and gives brief descriptions of these VIs.

The methodology of this VI starts with the data in the ‘Start Up Cluster’ terminal. The current FG5 configuration, device port location, and other parameters are carried to a VI that uses this information to initialize the USB-6009s and monochromator. The VI that follows controls the popup window the user sees and uses the inputted data to create

an array of grouped scan parameters. The number of groups in the array is defined by the type of scan. In Scanner mode three groups are formed, one for each of the excitation filters and one for all four emission filters. Only one group is needed in Filter mode and numerous are used during calibration. After the groups are defined the data is passed to a VI that initializes various Front Panel objects and passes the array to the input node of a 'for loop'.

The 'for loop' executes once for each group of scan parameters. Inside the loop are three VIs and a 'while loop.' The first VI sets the LED currents and PMT voltage while moving the monochromator to its starting position. The next VI is within the 'while loop' and is responsible for the AI from the USB-6009(s). During each iteration of the 'while loop' this VI takes a reading and then moves the monochromator to a new position. This continues until the VI recognizes it is at the end of the scan and sends a command to stop the while loop. After the 'while loop' the data is grouped and if necessary prepares for another 'for loop' iteration. The next VI takes all the data from the 'for loop' and arranges it into an array of clusters. These clusters contain certain parameters from the scan and the raw data. This data is then passed to the Data Process VI which numerically fits the data, assigns a filter code, computes Pass/Fail criteria, and then displays the data on the Front Panel. The final VI generates a printed report when in Scanner mode. This report is used as part of the documentation that outlines a scanners assembly history.

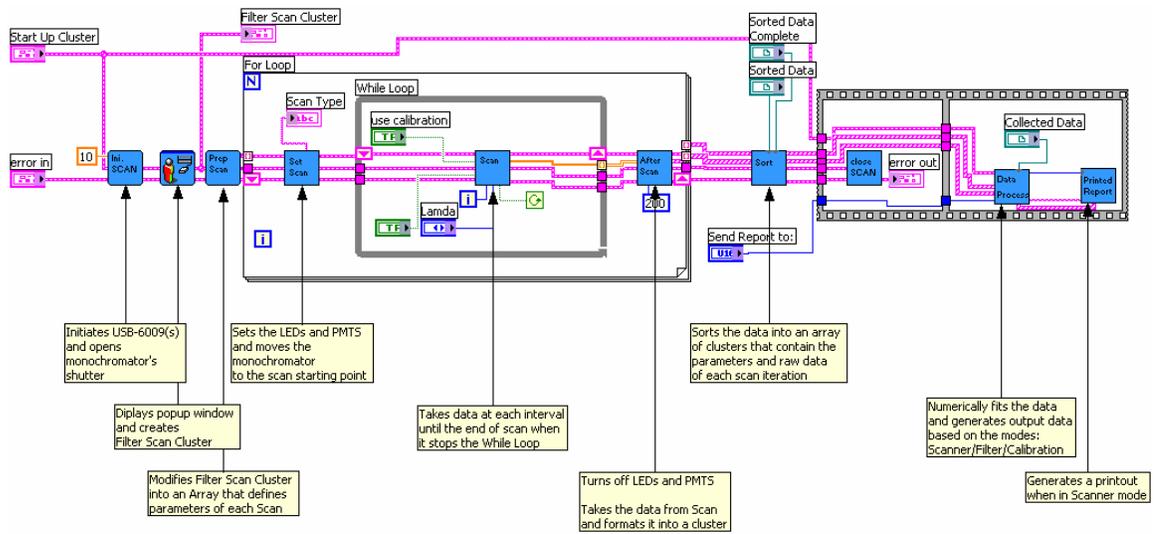


Figure 42: FG5 Methodology

APPENDIX D: PUBLICATIONS AND PATENTS

In the Journal of Biomedical Optics:

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Resonance Raman measurements of carotenoids using light emitting diodes

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We report on the development of a compact commercial instrument for measuring carotenoids in skin tissue. The instrument uses two light emitting diodes (LEDs) for dual-wavelength excitation and four photomultiplier tubes for multichannel detection. Bandpass filters are used to select the excitation and detection wavelengths. The $f/1.3$ optical system has high optical throughput and single photon sensitivity, both of which are crucial in LED-based Raman measurements. We employ a signal processing technique that compensates for detector drift and error. The sensitivity and reproducibility of the LED Raman instrument compares favorably to laser-based Raman spectrometers. This compact, portable instrument is used for non-invasive measurement of carotenoid molecules in human skin with a repeatability better than 10%.

Keywords: resonance Raman spectroscopy, carotenoids, β -carotene, light emitting diodes

I. INTRODUCTION

Carotenoids are important in maintaining proper human health. They protect tissues from oxidative stress that might otherwise lead to premature macular degeneration [1], cataract formation, sun burns [2], premature skin aging, and basal cell and squamous cell carcinomas [3, 4]. Carotenoids protect cellular DNA [5] and play a role in the recovery of burn patients [6]. Reliable monitoring of carotenoid levels in tissue may aid studies to better understand how they perform these functions.

Resonance Raman spectroscopy has been demonstrated as a rapid, non-invasive approach to monitoring carotenoid concentrations in human tissues [7, 8]. The method's success is primarily due to the low fluorescence quantum yield of these molecules. While the Raman scattering cross-section increases dramatically on resonance, fluorescence from carotenoids remains low.

Several compact laser-based Raman spectrometers have been reported [4, 7, 8, 9, 10, 11, 12]. Typically these use optical fibers to deliver laser light to the sample and to collect scattered light. Grating spectrometers and CCD detectors are used to disperse and detect the scattered light spectrum. This approach offers wide spectral coverage, aiding in the identification of several biomolecules in the sample. Fiber coupling of the laser and fluorescence collection is convenient because the mode diameter and numerical aperture of the fiber is a good match for both the laser and the spectrometer. These systems often require environmentally stable operating conditions. The performance of the lasers and CCD detectors are sensitive to temperature variations. In an unstable environment, frequent recalibrations are required.

The large resonance Raman scattering cross section

makes it possible to use incoherent excitation sources, such as light-emitting diodes (LEDs). These sources can offer higher stability in a wider range of environmental conditions at a lower cost and in a small form factor. However, these sources have broad spectral bandwidths, do not couple well into optical fibers, and are poorly matched to the slit area and numerical aperture of grating spectrometers. Using a bandwidth-narrowed LED source with a conventional Raman spectrometer results in recorded spectra that are one to two orders of magnitude weaker than those measured using a laser source.

This lower signal level can be overcome in part with sensitive detectors. For example, an instrument for measuring carotenoids in the macula was reported [13]. A photomultiplier tube (PMT) was used to detect the Raman signal transmitted through a narrowband interference filter. By tilting the filter it was possible to change the passband wavelength enough to determine the baseline fluorescence level at a nearby wavelength and to subtract this from the Raman signal. Because the signal-to-background levels were relatively high (0.20), changes in the background fluorescence only had a small influence on these carotenoid measurements.

In this paper we describe an instrument that uses spectrally-filtered LEDs for resonance Raman spectroscopy of carotenoids in skin tissue. For a narrowband excitation source, the signal to background ratio is 20 times smaller in the skin than in the macula, 1% being a typical value. For a spectrally filtered LED, this ratio is even smaller, owing to the 1 nm spectral width of the bandwidth narrowing filter. Such an excitation source emits only 0.5 mW of optical power, resulting in an order of magnitude less light exposure to the skin tissue and therefore much low signal levels compared with a typical laser-based instrument.

We also describe techniques we developed to overcome the difficulties in using the low-intensity excitation sources. We use PMTs to measure the skin fluorescence for increased sensitivity. To compensate for both short-

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