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Prototype Automated Flow-Through Sensor for Measuring Waterborne Microbial Concentrations Using Bulk Fluorescence

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PROTOTYPE AUTOMATED FLOW-THROUGH SENSOR FOR MEASURING

WATERBORNE MICROBIAL CONCENTRATIONS USING BULK

FLUORESCENCE

A Thesis Presented

by

SUSAN M. SAVILL

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Applied Physics Program

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WATERBORNE MICROBIAL CONCENTRATIONS USING BULK

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ABSTRACT

PROTOTYPE AUTOMATED FLOW-THROUGH SENSOR FOR MEASURING

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FLUORESCENCE

December 2011

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Directed by Associate Professor Stephen Arnason

Timely and inexpensive monitoring of microbial ecology in the world's water

supplies is crucial to the study of environmental and human impact on water quality and

the prevention of disease outbreaks. Current technology is lacking in its ability to

accurately measure and predict the presence of possible disease pathogens in a timely and

cost effective manner. This paper describes the construction and initial testing of an

automated prototype water sensor intended to detect fluctuations in microbial density in

real-time by using bulk fluorescence of SYBR Gold stained bacteria. The sensor is

comprised of off-the-shelf hardware and an in-house designed and built flow-through

fluorometer. A flow-through design allows water to be channeled through filters,

injected with a fluorescent dye, and then held in the fluorometer while its bulk

fluorescence is measured. Preliminary testing has confirmed the prototypes' ability to

reproduce a series of dilutions of fluorescein consistent to within 0.8% of a similar

manual series; consistently measure the bulk fluorescence of SYBR Gold for specific

Lambda DNA concentrations; differentiate between Lambda DNA dilutions as close as 0.05 μg DNA mL^{-1} Milli-Q water; and repeatedly create and measure a dilution of SYBR Gold in Instant Ocean which varied 3.4% from its average. Additional testing is needed to study filter performance and longevity, the prototypes' performance using SYBR Gold with sea water, and the correlation between bulk fluorescence and current water quality testing methods. Items not currently considered include SYBR Gold containment and waste, the use of DNase to improve fluorescence, and gross filtration for larger particles and debris.

ACKNOWLEDGEMENTS

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A special thanks to: Paul for spending so much of his free time with me, giving me advice and keeping me on track; Bridget for being the driving force behind most of the testing, providing great insight and keeping me on my toes; Bala and Steve for the generosity they showed me while I was sick for which I am eternally grateful; and Steve especially for all he has taught me over the years and his patience for allowing me so much time to complete my degree.

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CHAPTER 1

INTRODUCTION

1.1   *Waterborne Pathogens*

Disease outbreaks of gastroenteritis, respiratory illness, dermatitis and other skin conditions with moderate to sometimes severe symptoms (1) have been linked to recreational swimming in marine, chlorinated and fresh water. In many instances, researchers have found the culprits to be waterborne pathogens; bacteria, viruses and protozoa, often present when water is fecally contaminated. To illustrate the severity of the water quality problem one study has estimated that in the year 2000, between 627,800 and 1,479,200 people suffered from gastrointestinal illness (GI) as a result of swimming at Los Angeles and Orange County beaches alone (2).   Our nation's water resources are periodically monitored for evidence of certain indicator bacteria as a means to estimate possible fecal contamination and thus disease outbreaks. The Natural Resource Defense Council (NRDC) which compiles water quality and public notifications data at US beaches, found 24,091 days worth of beach closures or advisories in 2010, 70% of which were due to high bacteria count and another 23% based on the possibility of high bacteria due to severe rain or sewerage overflows (3). This represented the second highest level of closings and advisories in 21 years even though less frequent beach monitoring occurred.

1.2 *Current EPA Microbial Water Quality Requirements*

The Beach Act of 2000 requires states to adopt into their standards EPA-approved criteria for monitoring waterborne pathogens. Attempts to directly measure the disease causing microbes have proven difficult due to a lack of technology and expense. The criteria developed by the EPA therefore require the enumeration of certain indicator organisms. An indicator organism is a bacterium the does not necessarily cause disease but whose presence indicates fecal contamination and consequently the possible presence of other harmful pathogens. For decades the standard indicator organisms have been E.coli and enterococci for marine water and E.coli for fresh water (4).

1.3 *Drawbacks of the Current Monitoring System*

Current microbial sampling techniques and test scheduling are limiting in their scope.

- The majority of recreational waters are checked weekly, monthly or sometimes not at all. EPA-approved microbial enumeration techniques require 18 to 24 hours (5) to get results. Such delay in data retrieval may lead to possible pathogen exposure.

- Indicator organisms must be easily collected, not grow in water or on beaches and have a relatively short life span such that the amount of bacteria present will indicate recent fecal contamination and corresponding pathogens. However, both E.coli (6, 7) and enterococci (7, 8) have been shown to persist and regrow under certain environmental conditions. Recent research has also shown that E.coli may be able to survive in sand for at least a year (6) and in soil for up to 9 years (9), thus allowing it to re-infect water without additional fecal matter being present. Based on such

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studies, enumeration of E.coli and enterococci may not be well-correlated to recent water contamination by fecal matter.

- Current accepted indicator bacteria do not always correspond to incidence of disease (10). In some cases, the presence of indicator organisms did not correlate with disease outbreak, while in other cases a lack of indicator organisms failed to predict incidents of viral infection. In general, it has been recognized that E.coli and enterococci are not good indicators of viral and protozoan pathogens. Although the presence of the current indicator organisms in recreational waters may require beach closure, it may not be indicative of a health hazard.

- The EPA gathers data from known disease outbreaks. Though many waterborne diseases can be traced back to their source, others can not. This lack of knowledge makes it difficult to monitor water effectively.

- Current indicator organisms are correlated to GI illness only. The probability of experiencing other health effects such as respiratory infection and rash are unknown.

1.4   *Current Investigations*

The EPA is required by the Clean Water Act, as amended by the Beach Act, to conduct studies on pathogens and pathogen indicators in coastal recreational waters and publish water quality criteria recommendations based on those studies. As part of that requirement, the EPA is focused on the following goals (11):

- *Assess human health risk by performing epidemiologic studies and quantitative microbial risk assessments*. These studies will expand the amount of observed

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illnesses by including upper respiratory illness (URI), rash, eye ailment, and ear ache along with the standard GI.

- *Identify more appropriate indicators that better correlate indicator concentrations to health effects*. Options currently being evaluated include bacteroides (rod shaped bacteria found in human and animal digestive systems), viruses, phages (virus to a bacterium), pharmaceuticals, caffeine and even laundry detergent.

- *Evaluate changes in microbial concentrations in time and location*. Recent research indicates that the concentration of indicator organism can vary on a time scale of minutes to decades as well as vary with location.

- *Develop protocols for more timely and accurate evaluation of indicators*. New methods are yielding results in hours however their correlation with health effects needs more study.

- *Develop predictive models and tools to help better predict and monitor water quality*. Site-validated statistical models that relate water quality to wind speed, rainfall, tide level and/or E.coli levels have been able to reliably predict water quality in a timely manner. How models behave with changing water type; fresh or marine, or climate needs to be studied.

1.5   *One New Method of Microbial Enumeration*

The rapid detection of microbial concentrations in marine, estuarine and fresh water using bulk fluorescence has been demonstrated in a recent paper by Wegley, et al (12). Microbial cells in a 0.45 μm filtered water sample were reliably enumerated after staining

with SYBR Gold DNA stain. SYBR Gold adheres to all DNA in a sample and fluoresces

when excited by light of a proper frequency. A simple fluorometer can then be used to

determine fluorescence which in turn is proportional to microbe concentration.

Although not currently an EPA approved method for indicating fecal contamination, it is

believed that this method could prove useful in monitoring microbial water quality in

real-time.

1.6   *Prototype Automated Microbial Water Sensor*

The study described in this thesis investigates the possibility of creating a self-

contained, portable, automated water sensor based on the Wegley (12) bulk fluorescence

protocol. The automated sensor would monitor fluctuations in microbial concentrations

in real-time, possibly while positioned on a buoy. The current fecal indicator organisms

of E.coli and enterococci are filtered out of the sample, however bacteroids, all phages

and viruses pass through. This type of sensor has the potential to

- Allow for measurements of water samples that include some disease causing

  pathogens and some potential new indicator organisms.

- Improve predictive models by relating real-time microbial concentrations with

  parameters such as temperature, PH, dissolved Oxygen, turbidity (commonly

  measured items), rainfall, tide level, etc.

- Be customized to allow for slight variation in target microbes or measurement of

  fluorescent indicators such as detergents.

The goal of this paper is to present the initial design and testing of a prototype automated water sensor that uses fluorescence to determine relative fluorescein and SYBR Gold stained DNA concentrations. It is hoped that the information presented here-in will provide a stepping stone for further research and development of a real-time water quality sensor that will reliably measure fluctuations in microbial concentrations.

CHAPTER 2

METHODOLOGY

2.1 *Bulk Fluorescence Protocol*

An automated prototype water quality sensor was developed to test the feasibility of

automating the real-time measurement of microbial density fluctuations using the bulk

fluorescence of SYBR Gold stain as recommended by the Wegley, et al paper (12).   The

recommended protocol suggests filtering 1 mL of water through a 0.45 μm filter for a

sample or a 0.02 μm filter for a blank, adding the water to 1μL of 10000X SYBR Gold

and 13 units ml^{-1} of DNase I, incubating the sample for 1 minute, then measuring the

emission spectrum of 450-650 nm with a 495 nm excitation.

2.2 *Overall Prototype Design*

In order to automate the bulk fluorescence protocol described in Section 2.1 the

prototype must be able to draw water into the system, filter water through a 0.45 μm filter

or a combination of 0.45 μm and 0.02 μm filters, inject SYBR Gold and DNase into the

sample, incubate the sample for one minute, pass the sample into a fluorometer where the

bulk fluorescence can be measured, present a result that is proportional to the amount of

microbes in the sample, and test samples one after another without contamination from a

prior sample. An earlier version of the prototype was assembled and tested as described

in Appendix I. The redesign of the existing prototype and its testing involved the following steps:

1. Hardware was chosen then tested to confirm that water could be drawn into the system, filtered, injected, and measured.

2. Software was designed to control the flow of fluid through the system and to record the appropriate data.

3. The pumping and injection systems were tested using fluorescein to assure that specific and consistent dilutions could be achieved.

4. The sensitivity and repeatability of the fluorometer was tested using manual dilutions of Lambda DNA and SYBR Gold.

5. The entire automated water sensor system was tested by repeatedly creating a dilution of SYBR Gold in Instant Ocean (For aquarium use, a powder added to fresh water to create sea water).

6. The ability to clean the flow-through spectrophotometer cell between samples was tested by running Instant Ocean through the cell for various lengths of time.

    The addition of DNase and the incubation time were not included in these initial tests. Further discussion on this topic can be found in section 4.10. The details of the listed steps are presented below.

2.3   *Hardware Design*

    To minimize cost, all hardware is off-the-shelf with the exception of the fluorometer which was designed and fabricated in-house. The water sensor is controlled by a

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National Instrument CompactRIO, a reconfigurable embedded control and acquisition system. The CompactRIO is composed of a real-time controller (NI cRIO-9014), a 4 slot reconfigurable field-programmable gate array (FPGA) chassis (NI cRIO-9103) and two input/output (I/O) modules; a 4-channel 24-bit universal analog input module (NI 9219), and an 8 channel solid-state relay sourcing or sinking digital output module (NI 9485). The photodiode output is connected to the NI 9219 module. All other hardware is connected to the NI 9485 module.

A schematic of the automated water sensor prototype is shown in Figure 2.1. The structure of the fluorometer is shown in Figure 2.2. The hardware and how it is used in the design is presented below.
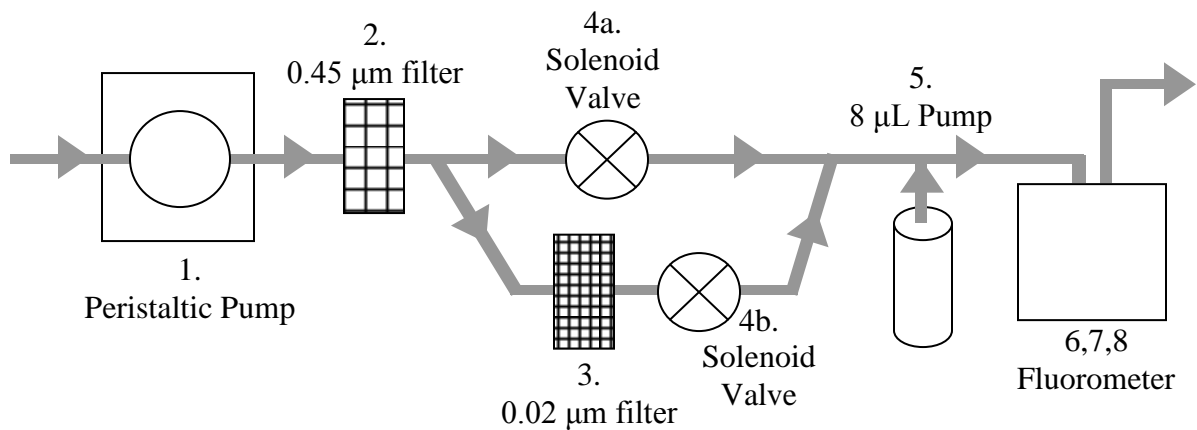
Figure 2.1: Flow through design of the prototype.

1. *Water is driven through the system using a single-channel Cole Parmer peristaltic pump (EW-77122-02). By using Tygon 3350 silicon 3/32 X 5/32 tubing a flow rate of 1.5-0.5 mL min^{-1} can be maintained (as determined by testing).*

2. *Water is filtered through* a *0.45 μm (Sterlitech CA04525100) low protein binding cellulose acetate 25 mm membrane filter.* Its purpose is to eliminate larger microbes such as E.coli and enterococci in order to improve fluorescence.   The filter is housed in a stainless steel syringe filter holder (Advantec MFS In KS25 c/N 301200) with a 3.8 cm^2 filtration area.

3. *When a background sample is needed, the water is also filtered through a 0.02 μm, low protein binding, Anopore inorganic membrane filter (Anodisc 25, Whatman 6809-6002).* The small pore size should stop all bacteria and most viruses from passing through, thus providing a good background sample. The filter is housed in an identical stainless steel syringe filter holder (Advantec MFS In KS25 c/N 301200).

4. *Two solenoid valves (Peter Paul 42X00090GM) are opened or left closed to establish the path the water takes, through either the 0.45 μm filter only (a) for a sample or through the combination of 0.45 μm and 0.02 μm filter (b) for a blank.*

5. *A fluorescent dye (SYBR Gold or fluorescein) is injected into the moving stream of water using a Cole Parmer 8 μL diaphragm pump (EW 73120-02).* Varying flow rates can be achieved by changing the stroke timing.

6. *Water then enters a semi-micro Starna flow through quartz spectrophotometer cell (73.65F-Q-10) housed in an in-house designed and built fluorometer (Fig 2.2).* Here the water flow is stopped so that the fluorescence can be measured. The flow through cell has a 10 mm path length, 15 mm Z-height and a 0.715 mL capacity. The cells volume was chosen to coincide with the 1mL suggested sample volume in the Wegley paper (11).

7. *An optically filtered blue green Nichia NSPE510S LED centered at 514 nm, housed in the fluorometer, excites the sample.* The optical filter used is a 492/10 nm Newport bandpass filter (CFS-001809) needed to make sure the LED doesn't interfere with emissions. The LED flashes at 217 Hz for the entire time the CompactRio is on.

8. *Two SI S8745-01 photodiodes situated opposite each other and at right angles to the LED (Fig 2.2) measure the emission fluorescence.* One photodiode is filtered with a Newport XMS-540A / 25 nm optical filter needed to filter out light from the LED. The photodiodes are attached to a custom built circuit board which isolates and amplifies the signal and sends the resulting voltage to the CompactRio. The unfiltered photodiode was saturated therefore its data was not used.
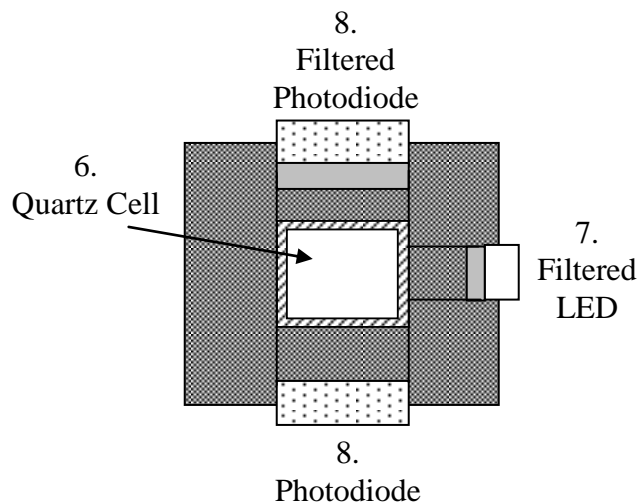
Figure 2.2: Fluorometer housing. Contains the quartz spectrophotometer cell, the two photodiodes and the LED.

2.4   *Software Design*

The CompactRIO is programmed using the graphical programming platform

LabVIEW. For the purpose of testing, two main programs were created; one which ran

the pumping system, runpumps.vi, the other which read the photodiodes, readdiodes.vi

(The actual LabVIEW programming can be found in Appendix 2). For ease of testing,

the two programs were run separately throughout all experiments.

Runpumps.vi: The pumping program allows the user to choose the filter or filters for the

water to pass through, the amount of time to run the peristaltic pump prior to and after the

running of the 8 µL pump, the timing of the 8 µL pump stroke, and the number of 8 µL

pump strokes. Once those values are set, the program runs the peristaltic pump drawing

water into the system, opens a solenoid valve to allow the water to pass through either

one or both filters, runs the 8 µL pump, then continues to pump until the flow through

cell is filled. This same program is used to flush the cell by running the peristaltic pump

for a set amount of time while not running the 8 µL pump.

Readdiodes.vi: The photodiodes send a constant stream of data to the CompactRio. The

program reads these voltage measurements in 14 ms intervals and records 1000 data

points. All data points from each photodiode are sent to both file and screen. In addition

to the data points, the number and time each data point was read is sent to the screen,

along with the 1000 point average for each photodiode. The values recorded throughout

the report are the 1000 point averages of the filtered photodiode readings as manually

recorded from the screen.

2.5 *Selection and Testing of the Peristaltic Pump*

The peristaltic pump was the only addition to the original hardware. It was chosen so that we could see the pumping process, keep the water sample from making any unnecessary contact with container walls, and provide more pressure to force water through the filters. The pump's size was determined based on the low achievable flow rates needed to provide better control over mixing and filling the spectrophotometer cell. Replacing an earlier diaphragm pump that broke during testing, the peristaltic pump, like its predecessor, was unable to pull Milli-Q water through the filters, but did quite well pushing the water through.

The flow rate of water through the system is determined by the speed of the peristaltic pump and the inside diameter of the tubing used. In order to determine maximum flow rate, a number of timed tests were performed where water was pushed by the peristaltic pump through the 0.45 μm filter and caught by a 5.0 mL cylinder with 0.1 mL increments. Measurements were read manually. Since the dial regulating pump speed has no markings, it was calibrated in the same manner for flow rates of 2/3 and 1/3 maximum speed. Results are presented in Section 3.2.

2.6 *Automated Dilutions of Fluorescein*

The water sensor must be able to create the proper dilution of DNase and SYBR Gold necessary for testing. Fluorescein was used to test the ability of the pumping system to produce correct and consistent dilutions. Fluorescein was chosen due to its lower cost, ease of handling and similar absorption and emission spectra to that of SYBR Gold

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(fluorescein has an absorption max at 494 nm, and emission max around 520 nm compared to that of SYBR Gold which has an absorption at 495 nm and emission max at 537 nm).

A manually made series of dilutions consisting of 50.9 μM concentrated fluorescein (New England BioLabs, MA) and Milli-Q water were produced and the fluorescence measured in the fluorometer. The dilutions were as follows: 1 part fluorescein: 1000 parts Milli-Q water (1:1000), 1:2000, 1:4000, 1:8000 1:16000, and 0 (the blank).

A 1:2000 automated dilution was created by drawing Milli-Q into the system using the peristaltic pump, injecting the 1:1000 manually created dilution into the stream using the 8 μL diaphragm pump, and collecting the sample in a plastic cuvette. The cuvette was placed in the fluorometer and the fluorescence measured. The remaining automated dilutions, 1:4000, 1:6000, 1:8000, 1:10000 and 1:16000, were created in a similar manner using the 1:2000 manually made dilution in the 8 μL pump. The switch from 1:1000 to 1:2000 dilution was due to a lack of prepared 1:1000 dilution. Specific automated dilutions were created by varying the speed of the peristaltic pump and the stroke timing of the 8 μL pump as tabulated in Appendix 3.

2.7   *Fluorometer Sensitivity and Repeatability using SYBR Gold and Lambda DNA*

The fluorometer needs to demonstrate that it can provide measurable readings of low microbial concentrations, consistent readings when measuring identical as well as changing microbial concentrations, and minimal resolution when measuring fluctuations

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in microbial density. The fluorescence measurements of SYBR Gold stained dilutions of Lambda DNA were used to evaluate the fluorometer.

Lambda DNA was chosen as a means to control the amount of DNA in the sample. The following Manual dilutions of Lambda DNA in Milli-Q water were produced; 0 (the control), 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, and 1.0 μg DNA ml^{-1} Milli-Q water.   SYBR Gold was added to each dilution of Lambda DNA at a concentration of 200 μL of 100X SYBR Gold into 2 mL of Lambda DNA dilution (creating a 10X solution). Each dilution was placed in a separate plastic cuvette and placed into the fluorometer.

In order to show that the fluorometer is consistent in its measurements, the fluorescence of each dilution was measured once (Test 1) and then the entire series in the same exact cuvettes was measured again (Test 2). While using SYBR Gold we discovered that the plastic of the cuvette held on to some of the fluorescence. Cleaning with ethanol and then Milli-Q seemed to solve the problem. In order to test the cleaning method, all dilutions (from Test 1 and 2) were placed one at a time into a single plastic cuvette and measured (Test 3). The cuvette was cleaned with ethanol and then Milli-Q in between samples.

The fluorometer is expected to provide voltage readings that should indicate the concentration of DNA in the sample. The resulting fluorometer readings were therefore corrected by subtracting the voltage of the control (Lambda DNA concentration of 0) from the voltage of each reading.

The resolving power of the fluorometer (the ability of the fluorometer to measure small changes in DNA concentration) along with consistency in measurement were

analyzed by calculating the mean, sample standard deviation (SD), standard deviation of the mean (SDM=SD/SQRT(3)), and 95% confidence interval (1.96*SDM) at each concentration. The 95% confidence intervals indicate the range of values where there is 95% certainty that the fluorometers actual mean will occur within those values.

Since the control itself had error associated with it, subtracting it from the other data would compound that error. In order to improve the error associated with the statistical analysis, the data in its un-corrected form (the control concentration was not subtracted) was also analyzed.

2.8   *Automated Water Sensor System Repeatability*

The entire automated water sensor system must be able to consistently produce and accurately measure specific concentrations of SYBR Gold (1 µL 10000X SYBR Gold mL^{-1} water sample). Finding that SYBR Gold fluoresced without any DNA present, it was decided to use SYBR Gold and Instant Ocean only to test the system. Since the 8µL pump would not be able to reduce the commercially prepared 10000X SYBR Gold down to the required 10X needed for the sample, it was decided to start instead with a 100X SYBR Gold stock (10 µL 10000X SYBR Gold: 990 µL Milli-Q water).

The test was performed by drawing the Instant Ocean through the system using the peristaltic pump at maximum speed, injecting 100X SYBR Gold into the stream using the 8 µL pump at 1200 ms per stroke for 75 strokes to achieve a sample with a 10X SYBR Gold dilution (1 µL 100X SYBR Gold: 9 µL Instant Ocean) and passing the sample into the flow-through cell in the fluorometer where the flow was stopped and it's fluorescence

measured. The procedure was repeated 4 times using a 90 s flush in between samples to

clean out the cell. Two of the samples were measured twice by the fluorometer. A

manual dilution of 10X SYBR Gold in Instant Ocean contained in a plastic cuvette was

also placed into the fluorometer and measured.

In order to verify that the pumping system created consistent concentrations of SYBR

Gold the data was analyzed by computing the average, standard deviation, SDM and 95%

confidence interval.

2.9   *Cleaning the Flow-Through Spectrophotometer Cell*

The flow-through spectrophotometer cell needs to be cleaned between samples to

assure that all traces of the prior sample have been removed. While performing other

tests, it was noted that some fluorescence would remain in the quartz cell when flushed

with only Milli-Q.   The effect was less pronounced when flushed with sea water. The

test was performed by manually pipetting left over Lambda DNA dilutions (0.4 and 0.5

μg DNA ml^{-1} Milli-Q) directly into the flow-through cell then measuring the fluorescence

with the fluorometer. The cell was then hooked up to the pumping system and flushed

with filtered sea water for various lengths of time, pausing at those times for the

fluorescence to be measured. The test was repeated three times.

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2.10 *Percent Error and Percent Difference*

Sections 3.3, 3.4 and 3.5 use percents to describe error. The following equations are used to determine the percent difference in the listed section.

- 3.3 slope comparison: $Percent\ Difference = \dfrac{slope_{manual} - slope_{pumped}}{slope_{average}} x\ 100$

- 3.4 slope comparison: $Percent\ Difference = \dfrac{slope_{max} - slope_{min}}{slope_{average}} x\ 100$

The following equations are used to determine the percent error in the listed sections

- 3.3: $Percent\ Error = (1 - slope_{figure3.4})\ x\ 100$

- 3.5 $Percent\ Error = \dfrac{voltage_{max} - voltage_{average}}{voltage_{average}} x\ 100$

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CHAPTER 3

RESULTS

3.1 *Prototype Automated Water Quality Sensor*

The prototype was able to draw water into the system for the specified amount of time, filter through the 0.45 μm filter, or through the combination of a 0.45 μm and 0.02 μm filter as specified, inject the sample with a specified amount of SYBR Gold or fluorescein, measure the samples fluorescence and send the results to the computer. The entire process, neglecting any incubation period, is accomplished in under five minutes. The tabulated test data can be found in Appendix 4.

3.2 *Peristaltic Pump Flow Rate and Calibration*

The flow rate of water through the system is determined by the speed of the peristaltic pump and the inside diameter of the tubing used. In order to determine flow rate, a timed test was performed where water was pushed by the peristaltic pump through the 0.45μm filter and caught in a 5.0 mL cylinder with 0.1 mL increments. The results are shown in Figure 3.1. Numerous tests performed at maximum speed yielded a flow rate of 0.025 mL s^{-1}.   Since the dial regulating pump speed has no markings, it was calibrated using flow rates of 2/3 and 1/3 maximum speed (Figure 3.2

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Peristaltic Pump Flowrate Test
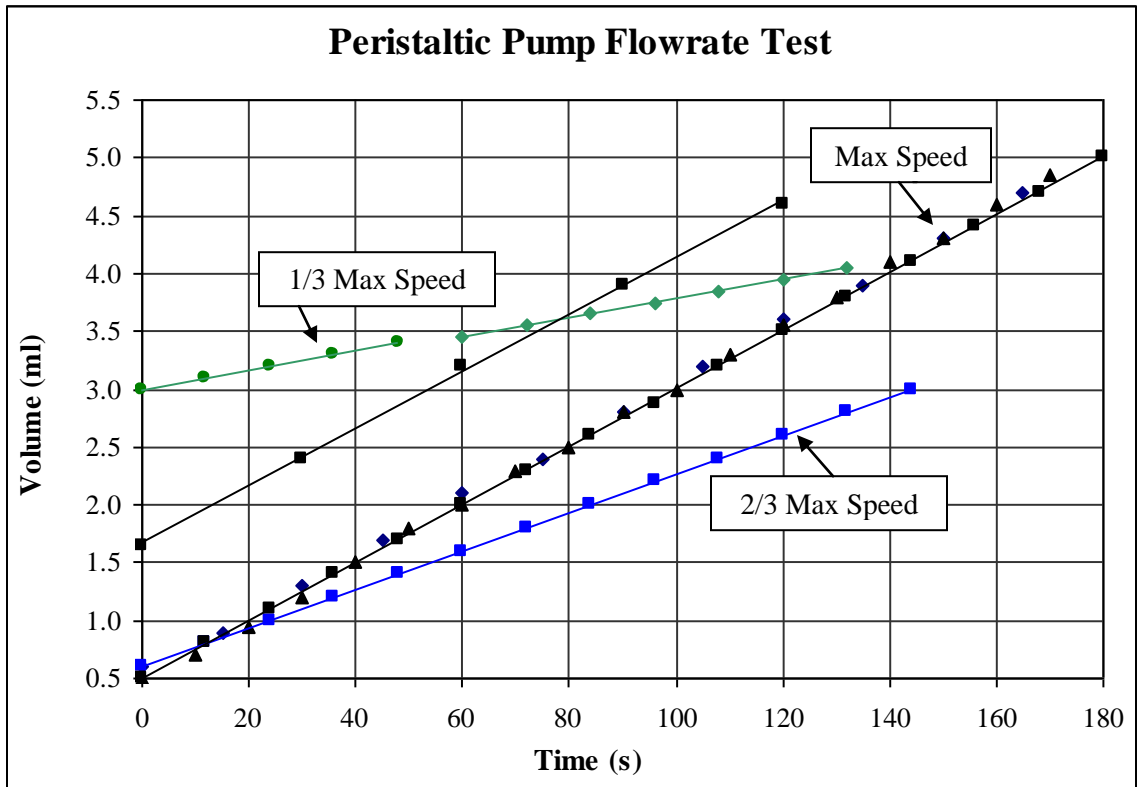
Figure 3.1: Flow rate testing of the peristaltic pump. Water was pushed through a 0.45 μm filter.   Multiple tests were performed at maximum speed. The tests at lower speeds were done to calibrate the pumps dial.
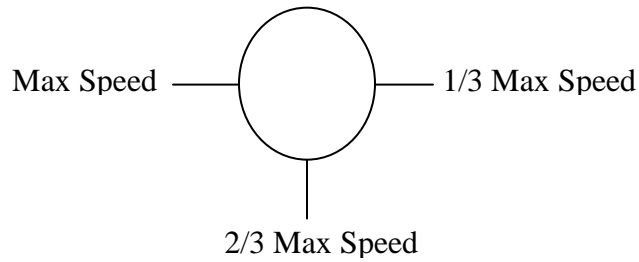
Figure 3.2: Peristaltic pump dial. The dial has a pointer but no markings. Lines indicate the direction to point the dial in order to achieve the desired pump speed.

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3.3 *Automated Dilutions of Fluorescein*

Fluorescein dilutions ranging from 1:2000 to 1:16000 were created by pumping Milli-Q water through the system and injecting it with a 1:1000 then 1:2000 fluorescein dilution. The dilutions were caught in a plastic cuvette and manually placed into the fluorometer.   The automated pumping system was able to produce a specified series of dilutions as evidenced by a well correlated linear trend line with an R^2 of 0.9978 and a 0.8% variation in trend line slope when compared to a similar manual series (Figure 3.3). The accuracy of the pumping system when producing a specific dilution is illustrated by the graph in Figure 3.4. Comparison of individual data points by plotting pumped vs manual dilutions yields a 6.2% variation from the ideal slope of 1 (Figure 3.4).

Fluorometer Results vs Fluorescein Dilution

manual: $y = 5.17x - 1.39$
$R^2 = 0.9878$

pumped: $y = 5.13x - 1.17$
$R^2 = 0.9978$

(Y-axis: Photodiode Voltage (mV); X-axis: mL Fluorescein ml^{-1} milli-Q x10000)
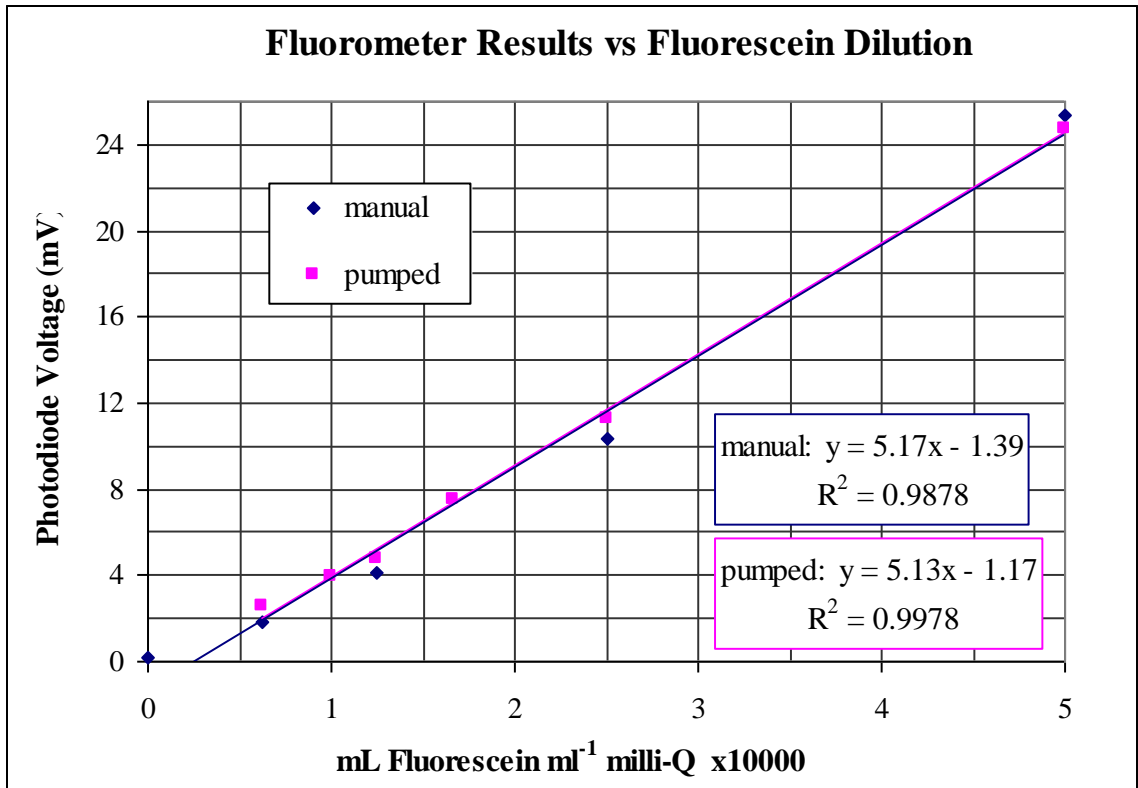
Figure 3.3: Comparison of manual vs automated fluorescein dilutions. The linear trend lines have slopes that differ by less than 0.8%. Manual: Manually produced series of dilutions ranging from 1:2000 – 1:16000. Pumped: Automated series of dilutions created by pumping water through the system and injecting fluorescein with the 8 μL pump.

Pumped vs Manual Photodiode Voltage

$y = 0.938x + 1.100$
$R^2 = 0.9989$

(Y-axis: Pumped (mV); X-axis: Manual (mV))

Figure 3.4:   Comparison of manual to pumped photodiode voltage. The 0.938 slope is within 6.2% of 1. (note that not all dilutions could be plotted)

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3.4   *Fluorometer Sensitivity and Repeatability using SYBR Gold and Lambda DNA*

Manual dilutions of Lambda DNA in Milli-Q water stained with SYBR Gold were placed in a plastic cuvette then placed into the fluorometer.   The resulting photodiode readings were corrected by subtracting the reading for a control dilution composed of Milli-Q water and SYBR Gold only.   Three trials were conducted.

Measurement of the manually produced SYBR Gold stained dilutions of Lambda DNA in Milli-Q water show a linear increase in voltage reading with a corresponding increase in DNA, indicating the fluorometers ability to identify increasing concentrations of DNA in the sample (Figure 3.5). Nearly parallel trend lines among the three trials illustrate the fluorometers ability to consistently measure changing amounts of DNA (Figure 3.5). The trend lines of the three trials have a difference in slope of at most 3%. Individual data points for each trial at each concentration were compared by examining the average at each concentration and the corresponding 95 % confidence intervals shown as error bars in Figure 3.6. 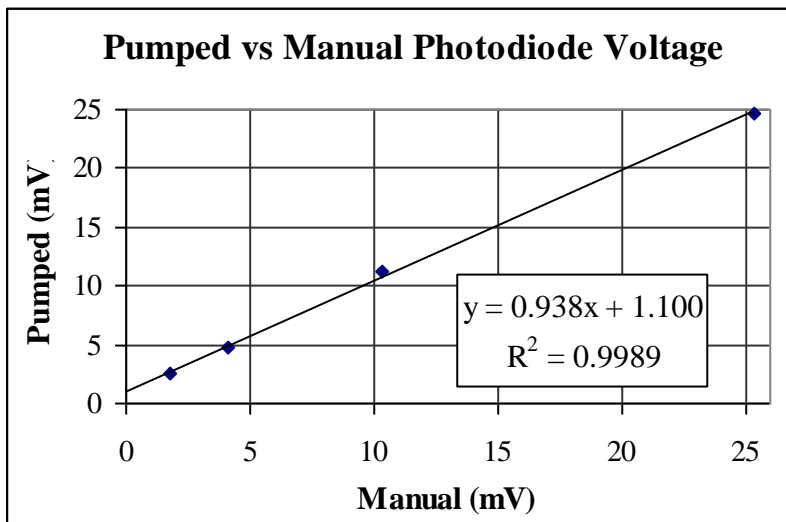 The variation in individual data points at each concentration ranged from a minimum 95% confidence interval of 0.04 mV at .3 µg DNA ml^{-1} Milli-Q to a maximum of 0.17 mV at 0.4 µg DNA ml^{-1} Milli-Q. This corresponded to a standard deviation of the mean that ranged from 0.02 mV to 0.09 mV.

In order to eliminate the error associated with the measurement of the blank, the average and 95 % confidence intervals were recalculated for the uncorrected data (Figure 3.7).   The individual variation in uncorrected data points at each concentration showed improvement over the corrected version, ranging from a minimum 95% confidence interval of 0.01 mV at .3 µg DNA ml^{-1} Milli-Q to a maximum of 0.14 mV at 0.2 µg DNA

ml^{-1} Milli-Q which corresponds to a standard deviation of the mean ranging from 0.01mV to 0.07mV.

Since no error bars overlap there is a statistical difference between each of the measured data points in Figures 3.6 and 3.7. The fluorometer therefor can differentiate between dilutions as close as 0.05 μg DNA mL^{-1}.

Fluorometer Results vs DNA Concentration

$y = 5.183x + 0.1169$
$R^2 = 0.9793$

$y = 5.083x + 0.0302$
$R^2 = 0.9682$

$y = 5.244x + 0.0581$
$R^2 = 0.9948$
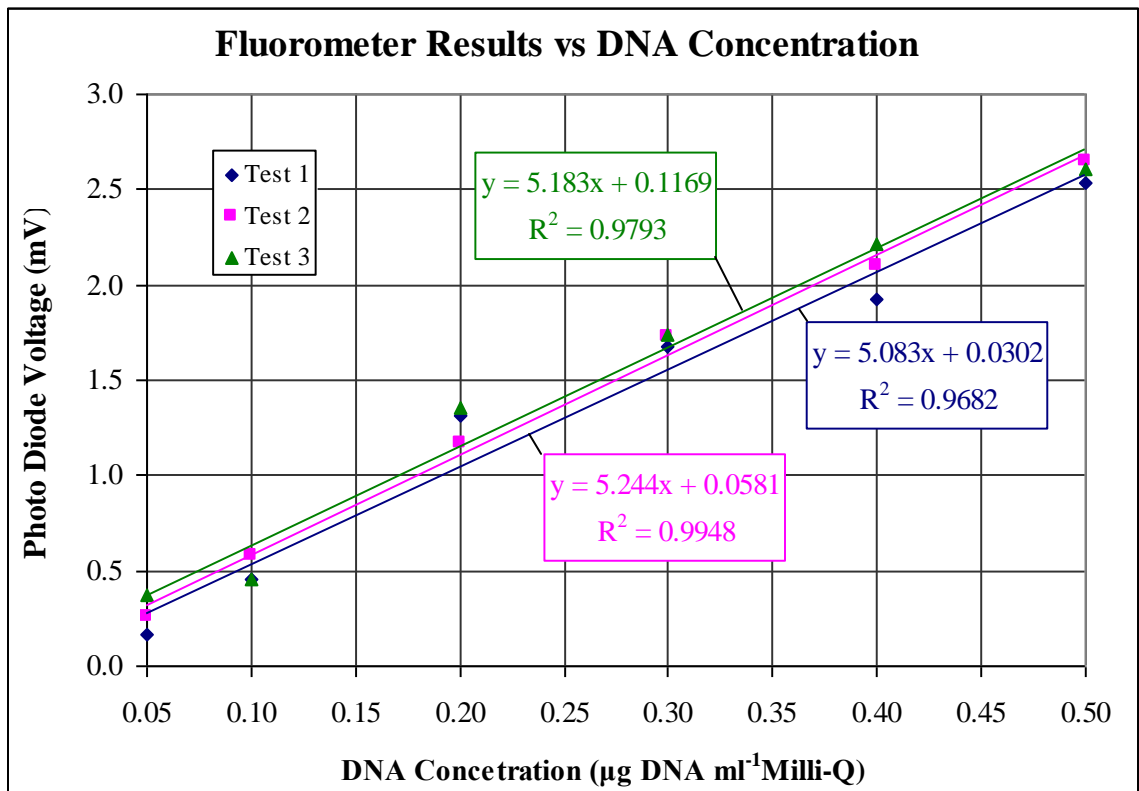
Figure 3.5: Variability of the photodiode as tested in the fluorometer
Test 1 = manual dilutions using different cuvettes
Test 2 = Re-measuring the exact test 1 cuvettes
Test 3 = Placing same dilutions into same cuvette, cleaning with ethanol and Milli-O between each measurement.
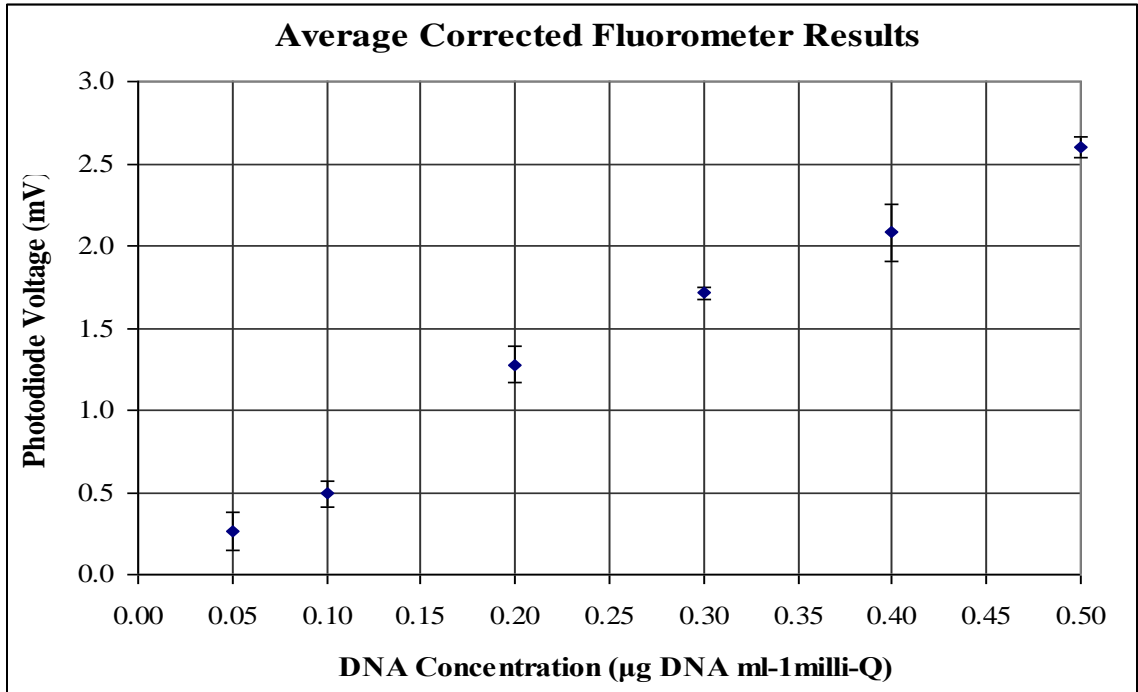
Figure 3.6: The average of the photodiode measurements presented in Figure 3.5. Error bars depict the 95% confidence intervals.
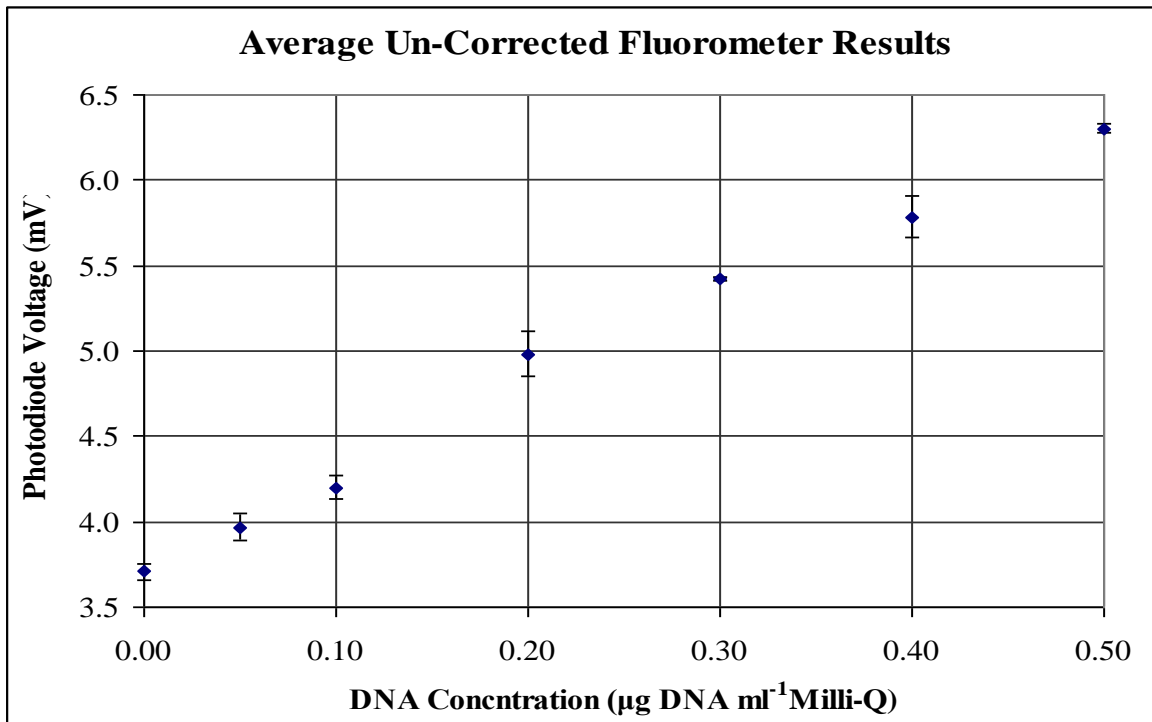
Figure 3.7: The average of the un-corrected photodiode measurements presented in Figure 3.5. Error bars depict the 95% confidence intervals.

3.5 *Automated Water Sensor System Repeatability*

The variability of the automated sensor system as a whole was tested by pumping

Instant Ocean through the system, injecting it with 100X SYBR Gold, and measuring the

fluorescence of the resulting 10X dilution in the flow-through cell and fluorometer.   The

process was repeated four times (Table 3.1). The fluorescence of a manually prepared

dilution of SYBR Gold and Instant Ocean was measured in a plastic cuvette in the

fluorometer and found to be 3.89mV. Since testing was to determine the repeatability of

the entire system, the photodiode values have not been corrected. All automated values

fall within 3.4% of the mean. The corresponding 95% confidence interval is +/- 0.13mV.

Comparison of the 4 trial average with the manual dilution of SYBR Gold yields a 6.7%

error.

Trial	Measured Photodiode (mV)	Average Photodiode (mV)
1	4.29	4.29
2	4.23	4.23
3	3.97	4.01
	4.06	
4	4.00	4.08
	4.16	
avg		4.15
stdev		0.13
sdm		0.06
95%CI		0.13

Table 3.1: The automated pumping system is used to inject
SYBR Gold into the Instant Ocean being pumped
through the system. All values are within 3.4%
of the average with a 95% confidence level of +/-
0.13mV

3.6   *Cleaning the Flow-Through Spectrophotometer Cell*

The flow-through spectrophotometer cell needs to be cleaned between samples to assure that all traces of the prior sample have been removed. Using the automated system, the cell was flushed with Instant Ocean to see how long it would take to get a minimal photodiode reading. Flushing with Instant Ocean for two minutes between samples was shown to clean the cell to a baseline level of approximately 1 mV. Flushing the cell without pausing resulted in the baseline being reached in about 1 minute. (Figure 3.8).
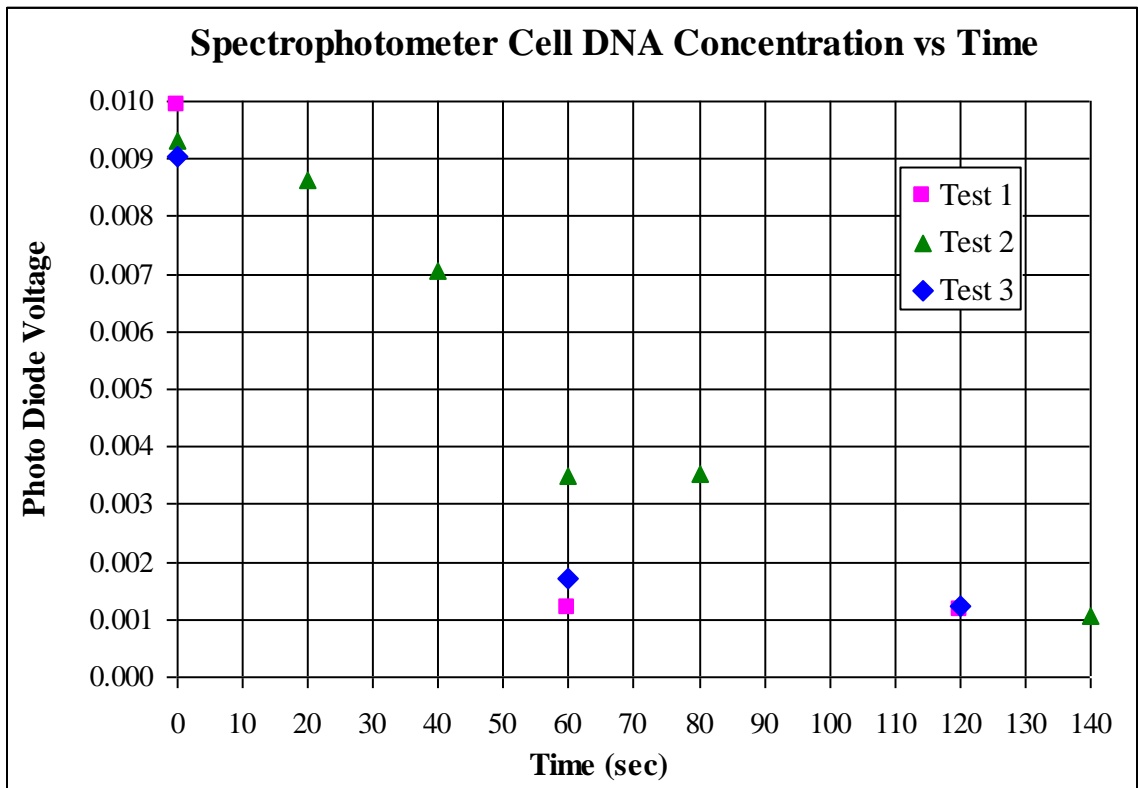
Figure 3.8: Time needed to clear the cell of any remaining residue from the previous sample. Stopping and starting the test a number of times as shown by Test 2 is not as effective as running the system non-stop for a greater length of time.
Test 1= 0.4 µg DNA ml^{-1} Milli-Q, Test 2 = 0.4 µg DNA ml^{-1} Milli-Q,
Test 3 = 0.5 µg DNA ml^{-1} Milli-Q

CHAPTER 4

DISCUSSION

4.1   *Peristaltic Pump Flow Rate and Calibration*

The flow rate of water through the system is controlled by an unmarked dial on the

peristaltic pump.   The dial must be physically turned to change the flow rate. Testing

was performed by manually measuring water levels in a 5ml cylinder with 1 mL

graduations. Exact measurements were difficult and inadvertent rounding of values may

have occurred leading to the near perfect data (Figure 3.1). However, these flow rate

values were later used to successfully calculate fluorescein dilutions (Figure 3.3). The

2/3 and 1/3 max pump speeds were also used during testing to achieve some of the larger

concentrations.

4.2   *Errors in testing*

Two sources of error should be considered while examining the results:

- All manual dilutions have an inherent random error associated in their production.

  Manual dilutions were created with pipettes of various sizes and therefore various

  volumetric errors. The manual dilutions of fluorescein as plotted in Figure 3.3 as well

  as those of Lambda DNA and SYBR Gold plotted in Figure 3.5 appear to exhibit a

  random error due their variation from the linear trend line. The difference between

the automated and manual dilution of SYBR Gold and Instant Ocean may also be due

to this random error.

▪ There may be error associated with the fluorescence of the sample itself. It is

understood that larger concentrations of fluorescent may decrease the fluorescence

intensity due to the fluid absorbing some of that fluorescence. The emission and re-

absorption of the SYBR Gold fluorescence was not tested.

4.3   *The 8 μL diaphragm pump*

The low concentrations of fluorescein and SYBR Gold when mixed with Instant

Ocean were difficult to achieve. While running the SYBR Gold and Instant Ocean test, a

significant amount of time was spent altering the number of 8 μL strokes and stroke

timing in order to create a sample that matched the fluorometers reading of the manually

produced sample.   The data presented in Table 3.1 represents the final test with the

peristaltic pump at max speed and the 8 μL pump at 1200 ms per stroke. One possible

cause for this inability to accurately produce samples with low fluorescent dye

concentration is the required slow pump stroke. The long pause between half strokes

may have caused the fluorescein and the SYBR Gold to be shot into the main stream in

spurts resulting in uneven concentration throughout the stream. A pump that delivers a

smoother flow would be preferred. One possible, though expensive alternative is a

syringe pump which could provide a more uniform flow of SYBR Gold into the sample

stream while providing an easy means for storage and temperature control. A second

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alternative would be a more affordable peristaltic pump which would however require a separate compartment to store and cool the SYBR Gold.

4.4 *Filters*

Further testing must be done to better understand the filters performance during and after operation. Initial prototype testing was focused on passing water through the filters, which was accomplished. No attempt was made to determine how long the filters would last, or if the filters were functioning properly.

The membrane filters are held in a stainless steel housing integral to the prototype making replacement difficult. It is suggested that a disposable filter be considered as a means to allow filters to be easily changed in the field.

4.5 *Spectrophotometer cell*

There was difficulty in cleaning the quartz spectrophotometer cell between samples. A small but consistent difference in the fluorometer measurement of a blank taken before and after testing is evident. It is believed that the SYBR Gold itself was adhering to the sides of the quartz container. Manually flushing the cell with alcohol appeared to eliminate the problem; however there is no plan for alcohol to be used in the system. Automated flushing of the cell for two minutes with Instant Ocean between samples resulted in a minimum measurement of 1mV every time (Figure 3.8). This value is larger than the initial blank reading of 0.9 mV taken prior to testing. Continued flushing produced no change. During testing, the blanks 1mV reading after the first sample

remained fairly constant for all subsequent readings and should therefore not cause a problem. However, further testing should include consideration of the blank reading before and after testing and its effect on the fluorometer's output.

According to the data presented in Figure 3.8, a minimum of 1 minute of flushing is required to achieve the 1mV minimum reading. It is believed that the long flushing time may be due to the current spectrophotometer cell design which has its input and output at its top. During flushing, it was observed that the sample seemed to get stuck in the cell. A more efficient design may consider using a cell with the input at the top and the output at the bottom, allowing the cell to be more easily flushed.

4.6 *Pumping System*

Fluorescein was used to test the ability of the pumping system to produce a specified set of dilutions. The pumping system clearly demonstrated this ability by producing a nearly perfect linear trend (Figure 3.3) with determination coefficient of 0.9978 and a slope within 0.8% of its manual counterpart. Since all but the first of the automated dilutions were made from a single manual dilution, any observed measurement error should be due only to the pumping system or the fluorometer. It is presumed that comparison to the slope of the trend line of the set of manual dilutions alleviates error associated with comparison to the individual manual measurements as discussed in Section 4.2.

Although a comparison of pumped vs manual fluorometer measurement was shown in Figure 3.4, the cause of the 6% error is uncertain. It is believed that the largest source

of error is the variation in manual dilutions as discussed in Section 4.2. It is also possible

that error exists in the pumping system or the fluorometer; however, given the high

determination coefficient, this error appears to be small. Further testing involving more

than four data points should be performed to confirm the correlation between manual and

automated individual dilutions.

It should be noted that manual dilutions of fluorescein presented in Figure 3.3 do fit a

polynomial trend line with a determination coefficient of 0.9995. The possibility that the

proper curve fit for the fluorescein dilutions is a polynomial due to some unknown

fluorescein characteristic has not been ruled out. Though if this were the case, then the

linearity of the automated series of dilutions is odd.

4.7   *Fluorometer Testing*

Testing using SYBR Gold and DNA was performed to demonstrate that the output of

the fluorometer provides voltage measurements in proportion to the fluorescence of the

sample.   Three sets of measurements of manually produced SYBR Gold stained dilutions

of Lambda DNA in Milli-Q water successfully exhibited similar linear increases in

voltage reading with corresponding increases in DNA (Figure 3.5).

As an afterthought comparison of the three measurements at each DNA dilution is

plotted in Figures 3.6 and 3.7 with error bars indicating the corresponding 95%

confidence intervals. Error bars that do not overlap indicate a statistical difference in

measurement at each concentration.   Based on Figures 3.6 and 3.7, the smallest

statistically significant interval between dilutions is 0.05 µg DNA ml^{-1} Milli-Q. There

are however two caveats to keep in mind. First, this testing was not specifically done to show the minimum difference in concentration and it is possible that more testing will result in a smaller difference being discovered. Second, three data points are not enough to say with confidence that the fluorometer has this type of precision.   More testing would need to be done to further test the consistency and minimum concentration difference that the photodiodes could measure.

Two versions of average fluorometer results were given, corrected (Figure 3.6) and un-corrected (Figure 3.7). It was initially thought that the blank value should be subtracted as a means to determine the actual fluorometer values. Both Figure 3.5 and 3.6 present the corrected data. In order to examine error, it was decided not to use the corrected value since the error associated with the blank would increase the error in all the corrected measurements. It is also believed that the water sensor should provide relative concentrations as opposed to exact concentrations. For these reasons, the un-corrected version of Figure 3.7 was presented.

4.8   *Water Flow Path*

While testing the system using SYBR Gold and Instant Ocean it was noticed that SYBR Gold was spreading from the junction of the 8µL pump and the main stream back towards the solenoid.   Believing that this back flow may have been causing the unexpected fluorescence readings discussed in 4.3, we tried rearranging the system by putting a solenoid valve at that junction. The relocation of the solenoid seemed to make

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things worse, however it was later realized the photodiode was not seated properly and light may have been leaking in. The issue has not been resolved.

4.9 *SYBR Gold Storage*

SYBR Gold stock comes in a 10000X concentration. The prototype's pumping system is unable to reduce the 10000X stock solution to the recommended 10X concentration needed to stain the samples. For this reason testing with SYBR Gold and Lambda DNA used a manually prepared 100X stock solution in the 8 µL pump. Some sources suggest that 100X SYBR Gold stock can be stored at -20°C for 1 to 2 weeks (13). Smaller concentrations are too unstable for storage. Further testing should be done to confirm this observation.

4.10 *DNase and 1 minute Incubation*

Initial testing did not include the use of DNase or the 1 minute incubation as suggested by the Wegley protocol described in section 2.1. In the Wegley paper, DNase is used to eliminate extra DNA from the sample in order to achieve a clearer fluorescent signal. Since initial testing did not use sea water, the DNase was not needed. In the future, a second 8 µL diaphragm pump is on hand and can easily be incorporated into the design. Initial tests performed without an incubation time seemed to work properly and so it was avoided throughout the remainder of the experiments. The LabVIEW software can be easily modified to include both the additional pump and the 1 minute incubation.

4.11 *Circuit Board*

The circuit board diagram used by the fluorometer to collect and amplify the photodiode output is presented in Appendix 5. The circuit board was part of the initial design. It was constructed without its needed capacitors. Capacitors were added prior to our testing. However, earlier versions of the circuit diagram were fuzzy and the capacitor values could not be easily read. The following capacitors were thus added to the circuit: $C21=C23=100$ μF, $C22=0.1$ μF, $C24 = 1$ μF. It is unknown if the given circuit board is the ideal configuration for this fluorometer. Every photodiode value presented is a 1000 point average and thus has associated with it a 1000 point data file. Examination of the 1000 point data files seemed to indicate a cyclical variation in the values. It is unknown if this variation is a flaw in the design or is a natural output. Further consideration should be given to the overall circuit design to maximize the photodiode output.

CHAPTER 5

RECOMMENDATIONS

5.1 *Further Testing*

Further prototype testing should include the following.

- The filtering system should be assessed for filter type including membrane vs depth, and performance to verify proper function during testing. Timed testing should also be done to determine the filters longevity.

- Comparison of microbial concentration in sea water samples using manual count and the automated prototype should be performed to verify that results are consistent.

- The need for DNase should be examined.

5.2 *Hardware*

- Replacement of the 8 uL pump should be considered. A peristaltic pump would enable SYBR Gold to be delivered at a steady rate. In addition to a steady flow, a syringe pump would also provide a more manageable means of storing and cooling the SYBR Gold. A comparison of benefit vs cost would be needed.

- The use of disposable filters would ease filter replacement in the field.

- A gross filter or a pair of filters at the inlet of the pumping system would be necessary to keep out larger debris that could clog the system.

- The use of depth filters as pre-filters or as the filters themselves may improve filter longevity.

- Replacement of the current quartz cell to one with input at the top and output at the bottom may improve upon the necessary flushing time between samples and thus a smaller waste containment vessel.

- Slight redesign of the flow path of the water may be needed to prevent backflow of the SYBR Gold.

- Consideration should be given to storage of SYBR Gold and DNase. Both need refrigeration.

- Consideration should be given to disposal of SYBR Gold and DNase. Both are toxic. The cell must be emptied into some type of storage container that would be replaced when filled.

- Improvements to the fluorometer circuit board may be necessary in order to improve the photodiode output.

CHAPTER 6

CONCLUSION

A prototype water quality sensor has been developed to test the feasibility of

measuring microbial density fluctuations in real time using the bulk fluorescence of

SYBR Gold stain.   Initial design and testing confirmed the following:

- The prototype was able to draw water through the 0.45 μm and 0.02 μm filters, inject

  it with fluorescent dye, and contain the sample in the fluorometer where its bulk

  fluorescence could be measured.

- The LabVIEW software was able to control the motion of fluid through the system

  and to record the appropriate measurement data either as a file or on screen according

  to the experimenters input of filter or filters to pass through, amount of time to run the

  peristaltic pump, timing of the 8 μL pump stroke, number of 8 μL pump strokes and

  data file name.

- The pumping and injection system were able to reproduce a series of fluorescein

  dilutions that when plotted yielded a constant slope that was within 0.8% of the slope

  of a similar manual dilution series.

- The fluorometer was able to repeatedly measure specific dilutions of Lambda DNA

  and SYBR Gold to within a 95% confidence interval of at most 0.17 mV with

corresponding standard deviation of the mean of 0.09 mV and was able to differentiate between dilutions as close as 0.05 μg DNA mL^{-1}.

- The entire prototype was able to repeatedly create and measure a dilution of SYBR Gold in Instant Ocean with a standard deviation of the mean of 0.06 mV and a 95% confidence interval of 0.13 mV.

- The measurement of the "blank" was returned to a minimum value of 1 mV between samples after flushing the flow-through spectrophotometer cell with Instant Ocean for at least 1 minute.

Based on the small amount of data obtained, further testing should be done to confirm these results. Additional testing should examine filter performance and prototype performance using SYBR Gold with sea water both with and without DNase. Factors to consider prior to field testing should include SYBR Gold and DNase containment and disposal, and filtration for large particulates and debris.

APPENDIX 1

PRIOR WORK

The initial form of the prototype was designed, constructed and tested by Renee Parry, a former graduate student at UMass Boston. The flow-through fluorometer with circuit board was designed and built by Steve Rudnick of the UMass EEOS Department. Through Renee's efforts, the initial prototype was able to pull water through a filterless system using a 250 μL diaphragm pump, inject fluorescein or SYBR Gold with the 8 μL diaphragm pump, and measure the bulk fluorescence of dilutions of fluorescein or SYBR Gold with DNA when samples were collected and manually placed into the fluorometer. LabVIEW programming controlled the pumps, and was used to record the fluorescence, recording three sets of 1000 data points and sending it to a file. It was later realized that the circuit board used to gather the photodiode signal was without its capacitors. The data gathered during this initial phase is therefore questionable.

APPENDIX 2

LabVIEW PROGRAMMING

Runpumps.vi
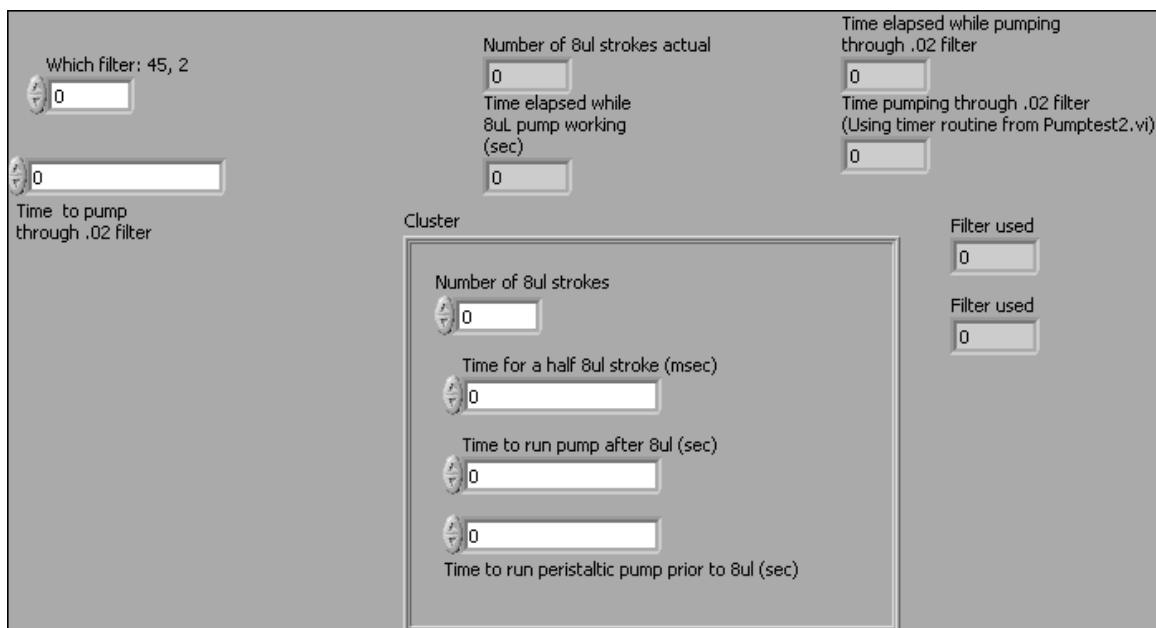
Figure A2.1: The main screen for Runpumps.vi. The user inputs the following: If passing
through the 0.45 μm filter only, enter 45 where it asks "which filter", then enter the amount of
time to run the peristaltic pump prior to and after the running of the 8μL pump, the timing of the
8 μL pump stroke, and the number of 8 μL pump strokes. If passing through both the 0.45 μm
and 0.02μm filter, enter 2 where it asks "which filter" then enter a time into the "time to pump
through .02 filter" The remainder of the screen contains values returned to the user during and
after the program is run.

Figure A2.2: The LabVIEW programming that runs when "Which filter" from Runpumps.vi is not equal to 45. The program calls pumptest2.vi and spits out time elapsed calculated in two ways, and displays "Filter used" as 2.
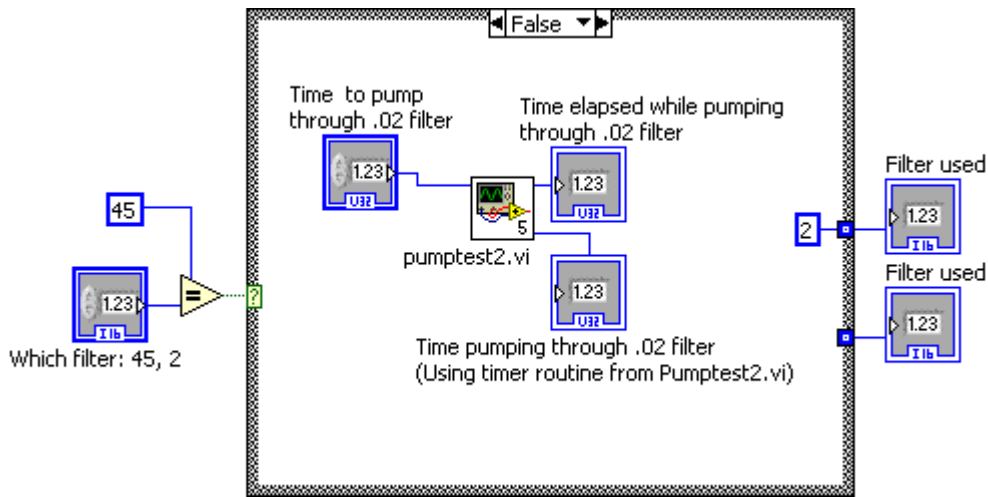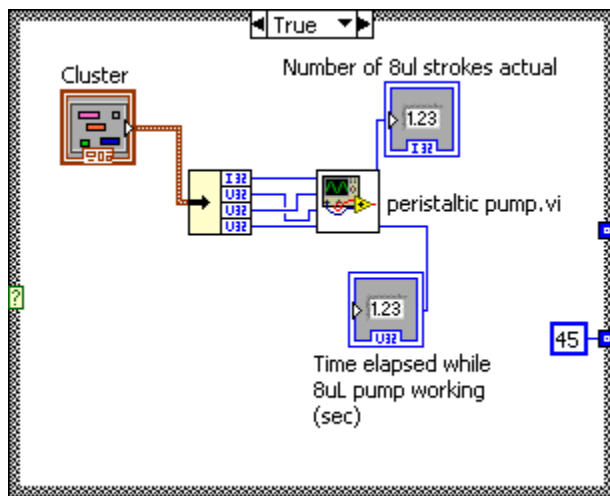

Figure A2.3: The LabVIEW programming that runs when "Which filter" from Runpumps.vi is equal to 45. The program calls peristaltic pump.vi and spits out the actual number of 8 µL strokes, the time elapsed while the 8 µL pump is running, and the filter used as 45.

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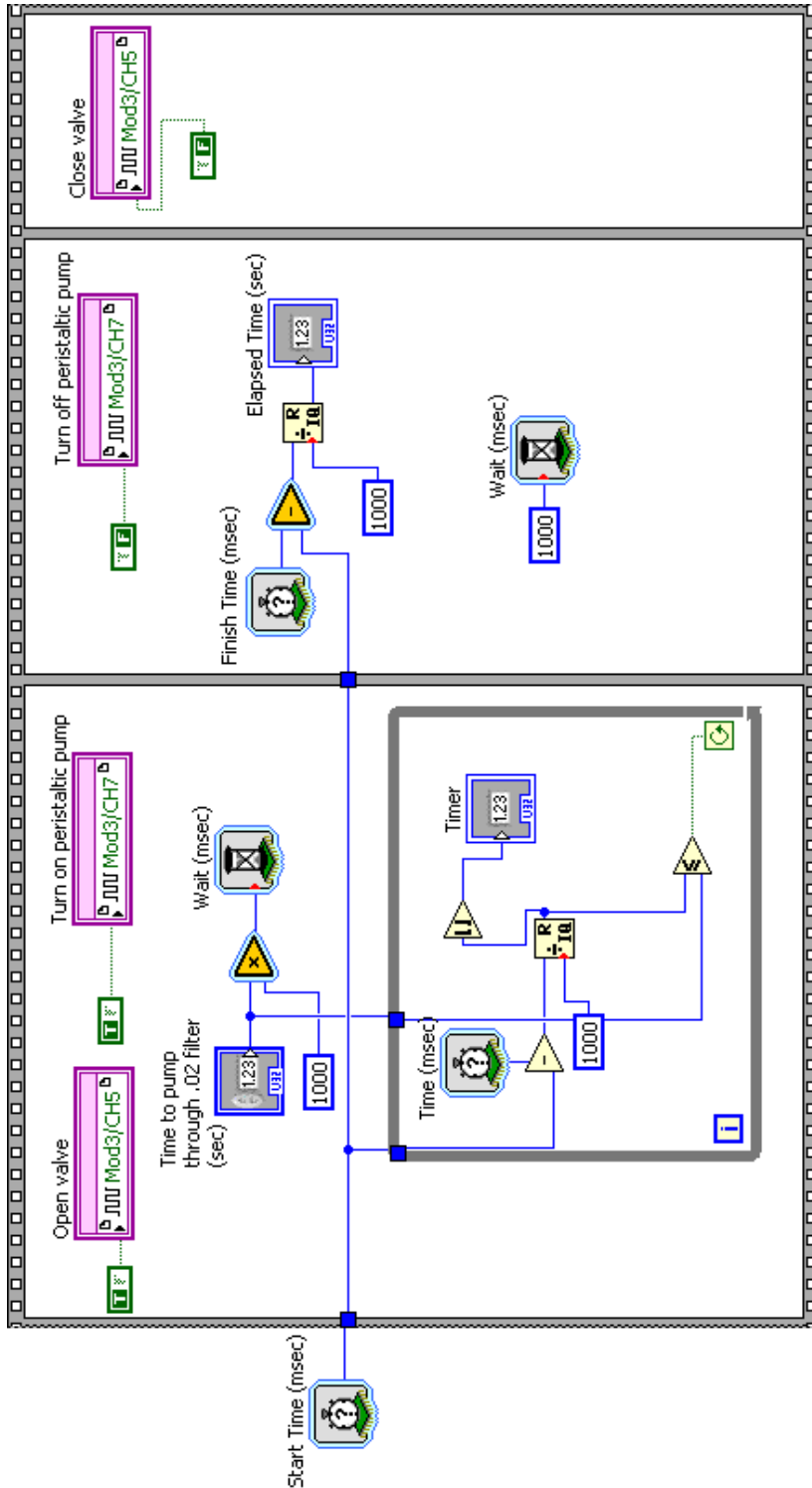
Figure A2.4: Pumptest2.vi. This program allows water to pass through the 0.02 μm filter by opening the solenoid valve nearest that filter (4b in Figure 2.1) and running the peristaltic pump for the length of time input in "time to pump through .02 filter" on the Runpumps.vi main screen.

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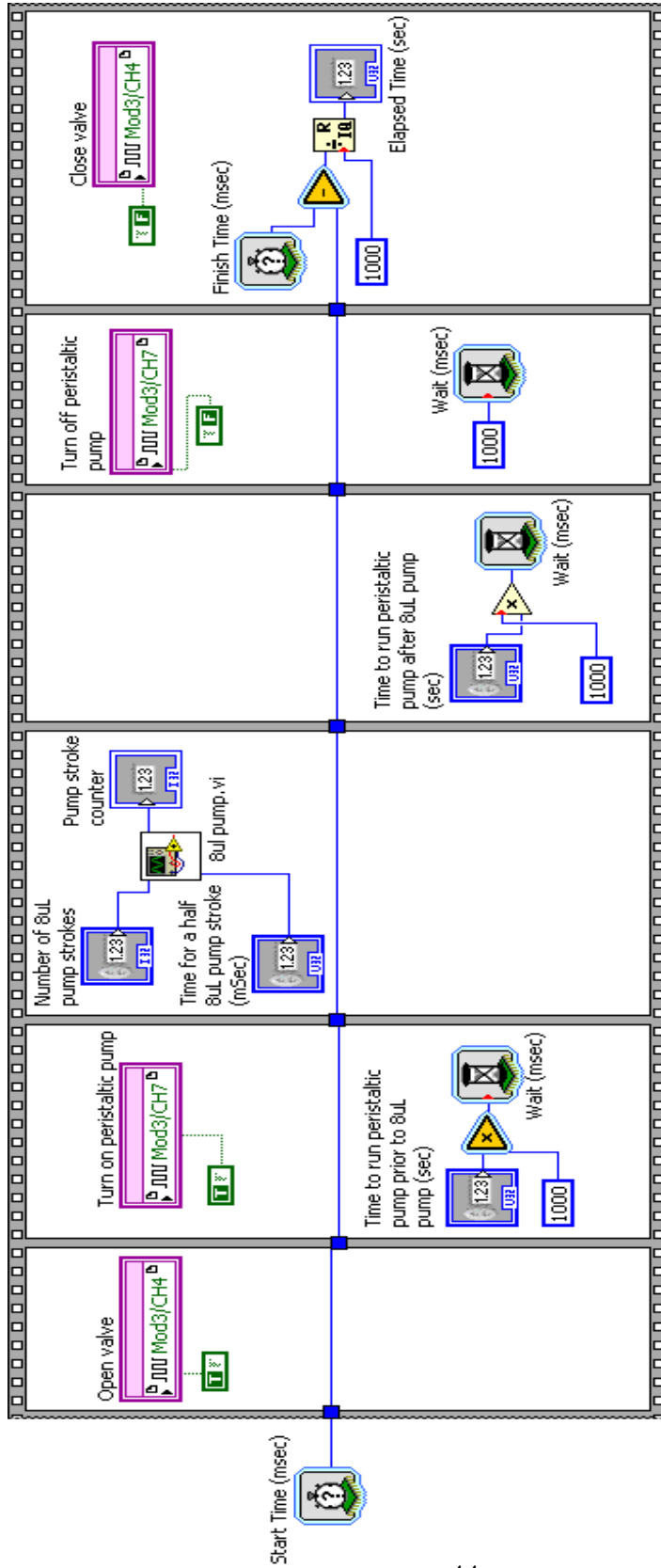
Figure A2.5: <u>Peristaltic pump.vi</u>. This program does the following:
- Opens the solenoid valve nearest the 0.45 μm filter (4a in Figure 2.1)
- Runs the peristaltic pump for the amount of time entered in <u>Runpumps.vi</u> "time to run the peristaltic pump prior to 8 μL"
- Opens <u>8ul pump.vi</u> which will use the data entered into <u>Runpumps.vi</u> to run the 8 μL pump
- Runs the peristaltic pump after the running of the 8 μL for the length of time indicated in <u>Runpumps.vi</u>
- Turns off the peristaltic pump, pauses then closes the solenoid valve.
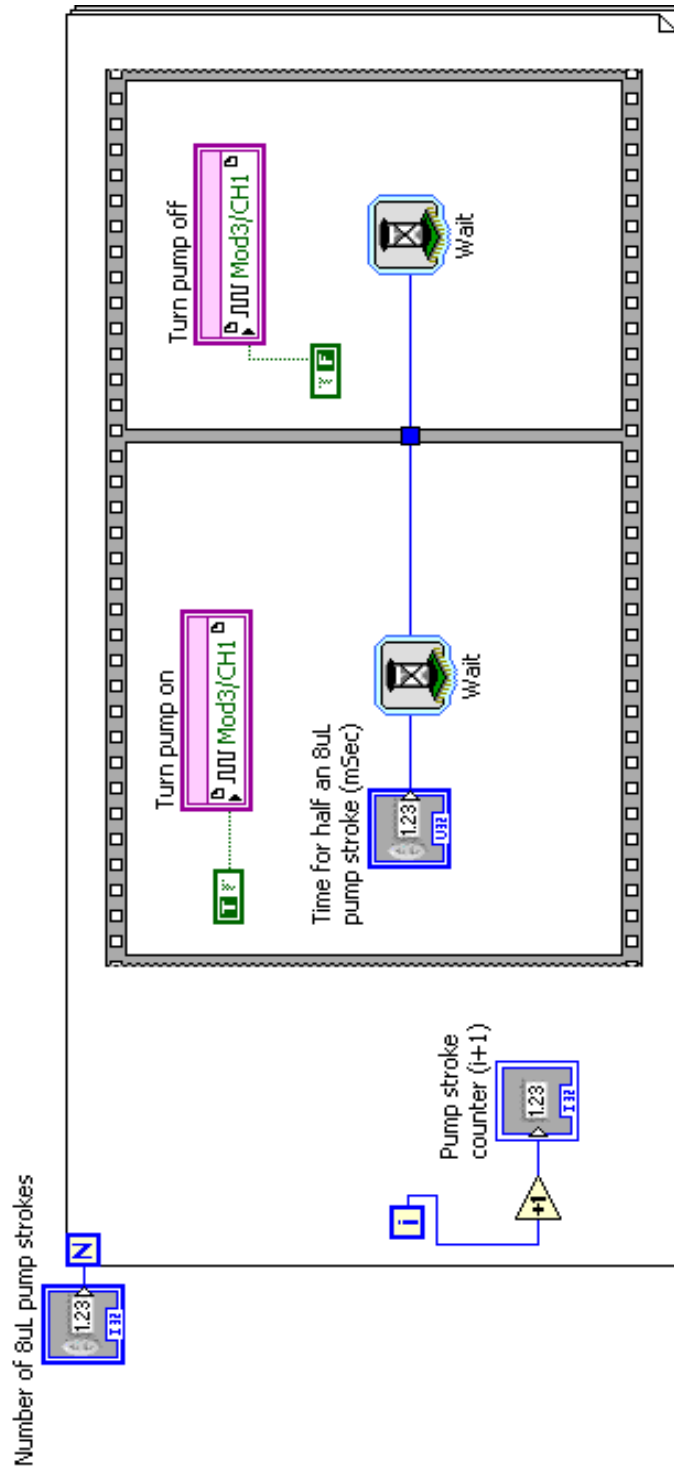
Figure A2.6: <u>8ul pump.vi</u>. The only way to operate the diaphragm pump is to continually turn the pump on and off. The number of strokes entered into the <u>Runpumps.vi</u> is thus controlled by using a loop. The timing of the half stroke as entered in <u>Runpumps.vi</u> is used to "wait" in both parts of the on-off cycle.

Readdiodes.vi
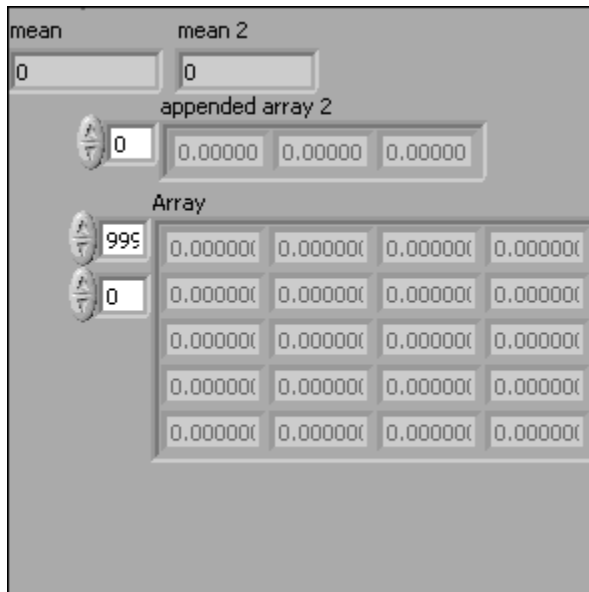
Figure A2.7: The main screen for Readdiodes.vi. There is no input here. The user simply runs the program. "Mean" and "mean 2" are the 1000 point averages of each of the photodiodes. The array lists the individual photodiode readings, the number of the data point, and the time the data was recorded.
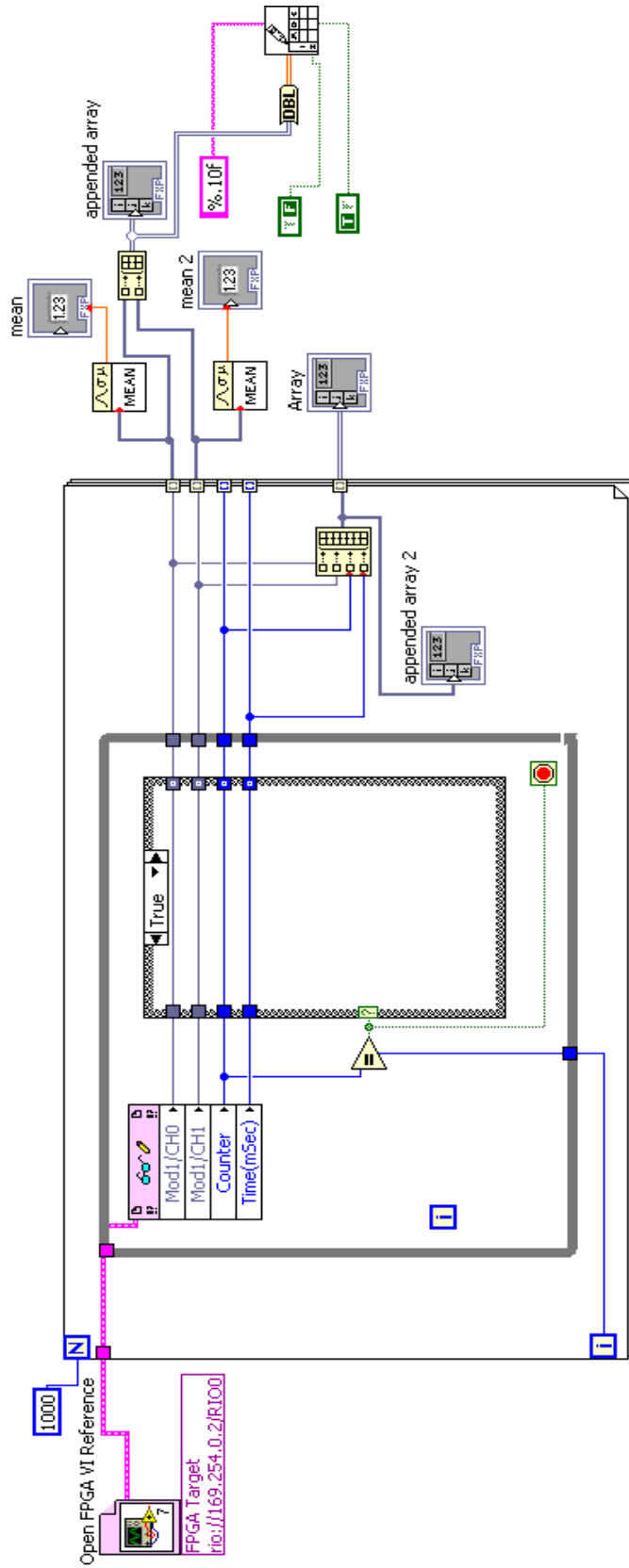
Figure A2.8: <u>Readdiodes.vi</u>. The program simply reads the photodiodes. It must be looped the way it is to make sure the program does not record the same data point twice.   If the value of the counter equals the loops iteration (i), the data point will be recorded. Otherwise, the point is not recorded. The program sends the data to an array and sends the array to both the screen and to a file that you name. (Earlier forms of this program that did not contain the true/false portion always recorded the same data point multiple times.)

FLUORESCEIN DILUTIONS

Input dilution	Output dilution	Peristaltic pump flow rate (ml/s)	8 μL pump flow rate (ml/s)	Total flow rate in main tube (ml/s)	Time to fill 3.5 cm of cuvett (s)	8 μl pump half stroke time (ms)	8 μl pump number of strokes to fill
1000	2000	0.0083	0.00834	0.01667	210.0	479.71	218.9
2000	4000	0.0083	0.00830	0.01660	210.8	481.69	218.8
2000	6000	0.0167	0.00835	0.02505	139.7	478.80	145.9
2000	8000	0.0250	0.00834	0.03334	105.0	479.76	109.4
2000	10000	0.0250	0.00625	0.03125	112.0	639.68	87.5
2000	16000	0.0250	0.00357	0.02857	122.5	1119.44	54.7

Table A3.1: Calculating pump parameters for automated fluorescein dilutions

1. Input dilution = manually made dilution in the 8uL pump
2. Output dilution = automated dilution filling the plastic cuvette
3. Peristaltic pump flow rate: max = .025 ml s^{-1}, 2/3 max = .0167 ml s^{-1}, 1/3 max = .0083 ml s^{-1}
4. 8 μL pump flow rate = (Input +1) x peristaltic pump flow rate (3) / (Output – Input) which comes from a ratio of 8 μL pump to peristaltic.
5. Total flow rate = peristaltic pump flow rate (3) + 8 μL pump flow rate (4)
6. Time to fill cuvette = 3.5 mL / Total flow rate (5)
7. Half Stroke = .008 x 500/flow rate (4) where 0.008 mL = volume of 8 μL pump stroke, and the 500 is converting seconds to ms.
8. Number of Strokes to fill = Time to fill (6) x 500 / half stroke (7)

Input dilution	Output dilution	No of 8μL Strokes	Time for a half 8μL stroke (ms)	Time to run pump after 8μL (s)	Time to run peristaltic pump prior to 8μL (s)
1000	2000	239	480	15	5
2000	4000	239	480	15	5
2000	6000	166	479	5	10
2000	8000	124	479	6	10
2000	10000	98	640	5	10
2000	16000	60	1119	10	10

Table A3.2: Data input to Readiodes.vi for automated fluorescein dilutions

1. The number of 8 μL strokes does not match those calculated in Table A3.1. The number of strokes calculated in Table A3.1 are the exact amount needed to fill the cuvette. Extra strokes were added to allow for wiggle room when collecting the sample.
2. The time to run the peristaltic pump was based on the length of tubing and the time needed for the old sample to be cleared and the new sample to get to the cuvette. The length of tubing has since changed.

APPENDIX 4

DATA TABLES

dilution	10000/dilution	avg pd ~ manual (mV)	avg pd ~ pumped (mV)	pumped - manual (mV)	% error
2000	5.000	25.33	24.70	-0.63	2.5
4000	2.500	10.30	11.27	0.97	9.4
6000	1.667		7.50		
8000	1.250	4.10	4.80	0.70	17.1
10000	1.000		3.90		
16000	0.625	1.80	2.60	0.80	44.4
	0	0.20			

Table A4.1: Results of fluorescein testing used in Figures 3.3 and 3.4.
pd = photodiode

$$\% \text{ error} = \frac{|pumped - manual|}{manual} \times 100$$

DNA Concentration (μg DNA ml⁻¹ Milli-Q)	Test 1 (mV)	Test 1 – Control (mV)	Test 2 (mV)	Test 2 – Control (mV)	Test 3 (mV)	Test 3 – Control (mV)
Control (0)	3.758	0	3.681	0	3.681	0
0.05	3.920	0.162	3.936	0.255	4.053	0.372
0.1	4.211	0.453	4.256	0.575	4.135	0.454
0.2	5.070	1.312	4.846	1.165	5.033	1.352
0.3	5.434	1.676	5.413	1.732	5.414	1.733
0.4	5.677	1.919	5.785	2.104	5.896	2.215
0.5	6.296	2.538	6.327	2.646	6.290	2.609
1.0	10.567	6.809	10.640	6.959	10.628	6.947

Table A4.2: Fluorometer results vs DNA concentration. The manually made DNA concentration of 1.0 was not used in Figure 3.3 due to its lack of linearity with the remaining data.

DNA Concentration (μg DNA ml⁻¹ Milli-Q)	Avg (mV)	SD (mV)	SDM (mV)	95% CI (mV)
0.05	0.263	0.105	0.061	0.119
0.1	0.494	0.070	0.041	0.079
0.2	1.276	0.098	0.057	0.111
0.3	1.714	0.033	0.019	0.037
0.4	2.079	0.150	0.086	0.169
0.5	2.598	0.055	0.032	0.062
1.0	6.905	0.083	0.048	0.094

Table A4.3: Average corrected fluorometer results.

DNA Concentration (μg DNA ml^{-1} Milli-Q)	Avg (mV)	SD (mV)	SDM (mV)	95% CI (mV)
Control (0)	3.707	0.044	0.026	0.050
0.05	3.970	0.073	0.042	0.082
0.1	4.201	0.061	0.035	0.069
0.2	4.983	0.120	0.069	0.136
0.3	5.420	0.012	0.007	0.013
0.4	5.786	0.110	0.063	0.124
0.5	6.304	0.020	0.011	0.022
1.0	10.612	0.039	0.023	0.044

Table A4.4: Average uncorrected fluorometer results.

Test 1: 0.4ug DNA 1ml^{-1} Milli-Q

Time (s)	Voltage (v)
0	0.00719
5	0.01248
15	0.01379
35	0.01211
55	0.00844
75	0.00500
95	0.00399
135	0.00149
175	0.00129
235	0.00114
Milli-Q only	0.00081

Test 2: 0.4ug DNA 1ml^{-1} Milli-Q

Time (s)	Voltage (v)
Milli-Q only	0.00084
0	0.00995
60	0.00119
120	0.00115

Test 3: 0.5ug DNA 1ml^{-1} Milli-Q

time	Voltage (v)
0	0.00932
20	0.00863
40	0.00706
60	0.00351
80	0.00352
140	0.00105
200	0.00104

Table A4.5: Spectrophotometer cell concentration vs time.
Milli-Q only test occurs after cleaning with ethanol and Milli-Q.
After about 140s, value change very little so these values weren't plotted.

APPENDIX 5

CIRCUIT DIAGRAM
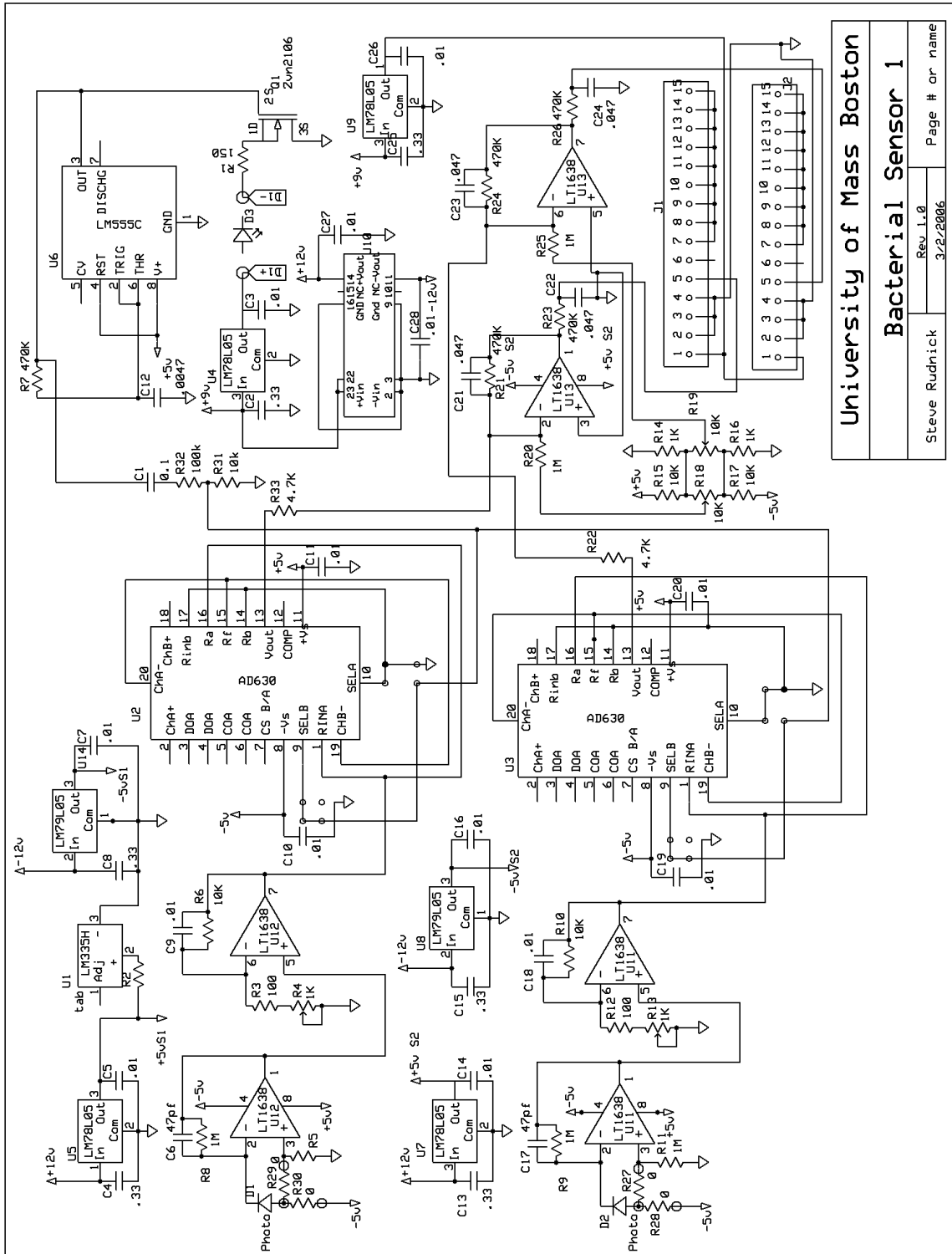
University of Mass Boston

Bacterial Sensor 1

Steve Rudnick

Rev 1.0

3/2/2006

Page # or name

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