Development of a Low Cost Handheld Microfluidic Phosphate Colorimeter for Water Quality Analysis

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ABSTRACT

Development of a Low Cost Handheld Microfluidic Phosphate Colorimeter for Water Quality Analysis

This thesis describes the design, fabrication, and testing process for a microfluidic phosphate colorimeter utilized for water quality analysis. The device can be powered by, and interfaced for data collection with, a common cell phone or laptop to dramatically reduce costs. Unlike commercially available colorimeters, this device does not require the user to measure or mix sample and reagent. A disposable poly(dimethylsiloxane) (PDMS) microfluid chip, powered by an absorption pumping mechanism, was used to draw water samples, mix the sample at a specific ratio with a molybdovanadate reagent, and load both fluids into an onboard cuvette for colorimetric analysis. A series of capillary retention valves, channels, and diffusion pumping surfaces passively controls the microfluidic chip so that no user input is required. The microfluidic chip was fabricated using a modified SU-8 soft lithography process to produce a 1.67mm light absorbance pathlength for optimal Beer Lambert Law color absorbance. Preliminary calibration curves for the device produced from standard phosphate solutions indicate a range of detection between 5 to 30mg/L for reactive orthophosphate with a linearity of $R^2=91.3\%$ and precision of 2.6ppm. The performance of the PDMS absorption driven pumping process was investigated using flow image analysis and indicates an effective pumping rate up to approximately 7 μ L/min to load a 36 μ L sample.

Keywords: Microfluidics, colorimeter, orthophosphate, polydimethylsiloxane, lab on a chip

Acknowledgements

For my wife Melissa.

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List of Equations

$$K_n = \frac{\lambda_{mfp}}{D_{characteristic}} \quad (2.1)$$

$$\tau = -\mu \frac{du}{dz} \quad (2.2)$$

$$\frac{1}{r} \cdot \frac{\partial}{\partial r} \cdot (r \cdot u_r) + \frac{\partial u_z}{\partial z} = 0 \quad (2.3) \quad (2.3)$$

$$\rho \left(u_r \cdot \frac{\partial u_r}{\partial r} + u_r \cdot \frac{\partial u_r}{\partial z} \right) = \rho \cdot g_r - \frac{\partial p}{\partial r} + \mu \left[\frac{\partial}{\partial r} \left(\frac{1}{r} \cdot \frac{\partial}{\partial r} \cdot (r \cdot u_r) \right) + \frac{\partial^2 u_r}{\partial z^2} \right] \quad (2.4)$$

$$\rho \left(u_r \cdot \frac{\partial u_r}{\partial r} + u_r \cdot \frac{\partial u_r}{\partial z} \right) = \rho \cdot g_r - \frac{\partial p}{\partial z} + \mu \left[\frac{1}{r} \frac{\partial}{\partial r} \left(r \cdot \frac{\partial}{\partial r} \cdot u_z \right) + \frac{\partial^2 u_z}{\partial z^2} \right] \quad (2.5)$$

$$0 = -\frac{\partial p}{\partial z} + \mu \cdot \frac{1}{r} \cdot \frac{\partial}{\partial r} \left(r \cdot \frac{\partial}{\partial r} \cdot u_z \right) \quad (2.6)$$

$$u_z = -\frac{1}{4\mu} \cdot \frac{dP}{dz} (R^2) \quad (2.7)$$

$$u_x(y, z) = -\frac{c^2}{2\mu} \cdot \frac{\partial p}{\partial x} \left[1 - \left(\frac{z}{c} \right)^2 + 4 \sum_{k=1}^{\infty} \frac{(-1)^k}{\alpha_k^3} \cdot \frac{\cosh(\frac{\alpha_k y}{c})}{\cosh(\frac{\alpha_k b}{c})} \cdot \cos(\frac{\alpha_k z}{c}) \right] \quad (2.8)$$

$$\bar{u} = \frac{(D_h)^2}{S\mu} \cdot \frac{\Delta P}{L} \quad (2.9)$$

$$D_h = \frac{4 \cdot Area}{Perimeter} = \frac{2hw}{(h+w)} \quad (2.10)$$

$$R_e = \frac{\rho u D_h}{\mu} \quad (2.11)$$

$$v = i \cdot r \rightarrow \Delta P = Q \cdot R_{fluid} \quad (2.12)$$

$$\bar{u} = \frac{(D_h)^2}{S\mu} \cdot \frac{\Delta P}{L} \rightarrow Q = \frac{(D_h)^2}{S\mu} \cdot \frac{\Delta P}{L} \cdot A_{cross} \quad (2.13)$$

$$v = \Delta P; \quad i = Q; \quad r = R_{fluid} = \frac{S\mu L}{(D_h)^2 \cdot A_{cross}} \quad (2.14)$$

$$\sum i_i = 0 \rightarrow \sum Q_i = 0 \quad (2.15)$$

$$R_{total} = \left[\frac{1}{\sum_1^3 \left(\frac{1}{R} \right)} \right]^{-1} + R_4 + R_5 \rightarrow R_{fluid} = \left[\frac{1}{\sum_1^3 \left(\frac{(D_h)^2 \cdot A_{cross}}{S\mu L} \right)} \right]^{-1} + 2 \cdot \left(\frac{S\mu L}{(D_h)^2 \cdot A_{cross}} \right) \quad (2.16)$$

$$\Delta P = \gamma \left(\frac{1}{R_1} + \frac{1}{R_2} \right) = 2 \cdot \frac{\gamma}{D_h} = \gamma \left(\frac{1}{h} + \frac{1}{w} \right) \quad (2.17)$$

$$\Delta P = \frac{2\gamma \cos \theta}{R_1} = 2\gamma \cos \theta \left(\frac{1}{h} + \frac{1}{w} \right) \quad (2.18)$$

$$Ca = \frac{\mu \cdot U_x}{\gamma} \quad (2.19)$$

$$Bo = \frac{\rho \cdot a \cdot (D_h)^2}{\gamma} \quad (2.20)$$

$$Pe = \frac{u_x \cdot l}{D} \quad (2.21)$$

$$x^2 \propto D \cdot t \quad (2.22)$$

$$L \cong \frac{w \cdot Q}{hD} \quad (2.23)$$

$$T = \frac{l}{l_o} = 10^{-\varepsilon(\lambda) \cdot l \cdot c} \quad (3.1)$$

$$-\log_{10} T = A(\lambda) = \varepsilon \cdot l \cdot c \quad (3.2)$$

$$A(\lambda) = (\varepsilon(\lambda) \cdot l) \cdot c + error \quad (3.3)$$

$$R_\lambda = \frac{l_p}{p} \quad (3.4)$$

$$F = D \frac{\partial c}{\partial x} \Big|_{x=l} \approx \frac{2D(C_{atm}-C_{degass})}{l} \cdot \exp\left[\frac{-\Pi^2 D}{4(l)^2} \cdot t\right] \quad (5.1)$$

$$Q \approx F \cdot A_{flux} \quad (5.2)$$

$$L_{Diff} = \left[\frac{2 \cdot (D_h)^2 \Delta P \cdot t}{S \cdot \mu} \right]^{1/2} \quad (5.3)$$

$$A = -\log_{10} \left(\frac{V_{Sample}-V_{Dark}}{V_{Blank}-V_{Dark}} \right) \quad (5.4)$$

$$P_{interface} = \frac{2l_p \cdot \gamma_{water}}{A_{cross}} \quad (7.1)$$

$$Q_{calc.} = \frac{(P_{measure}-P_{interface})}{R_{fluid}} \quad (7.2)$$

$$C_l = \left[(C_s - C_o) \left(1 - \operatorname{erf} \left(\frac{l}{2\sqrt{Dt}} \right) \right) \right] + C_o \quad (7.3)$$

$$P_{vacuum} = (P_{atm} - P_{diff}) \quad (7.4)$$

$$P_{diff} = \frac{P_{atm} \cdot (V_{atm} - V_{diff})}{V_{atm}} \quad (7.5)$$

$$V_{diff} = V_{PDMS} \cdot C(l, t) \quad (7.6)$$

$$P_{vacuum} = P_{atm} - \left(\frac{P_{atm} \cdot (V_{atm} - V(t)_{PDMS} \cdot C(t))}{V_{atm}} \right) \quad (7.7)$$

$$y = A \cdot (1 - e^{(-B \cdot x)}) \quad (7.8)$$

$$V_{PDMS}(t) = V_{max}(1 - e^{-C \cdot t}) \quad (7.9)$$

$$u_x(y) = -\frac{c^2}{2\mu} \cdot \frac{\partial p}{\partial x} \left[1 - 4 \sum_{k=1}^{\infty} \frac{(-1)^k}{\alpha_k^3} \cdot \frac{\cosh(\frac{\alpha_k y}{c})}{\cosh(\frac{\alpha_k b}{c})} \right] \quad (7.10)$$

Chapter 1 Broader Impacts of Water Analytics and Project Overview

1.1 Compelling need for a low cost portable water analysis system

Water quality issues are currently a pressing research and development topic. A cornerstone for future sustainable development is a clean and secure water supply. The objective of this project is to investigate the development of a low cost, handheld, rapidly deployable water analytics device for emergency response situations, long term environmental monitoring, on site field testing, low cost routine testing, and many others. According to the World Health Organization's Report on Global Water and Sanitation Assessment (Global Water and Sanitation Assessment Report, 2000) a lack of water quality data from both water sources and points-of-use has impeded the development of effective water quality management programs. In order to equip both government and commercial entities with the data they need to monitor a variety of water parameters, new monitoring systems should be developed. (Flynn et al., 2007) (Ailamaki et al., 2003) With these instruments water quality databases can be enhanced by including point-of-use data which is normally cost prohibitive using traditional water monitoring techniques (Gundry, Wright, & Conroy, 2004). This project is directed towards the development of enhancing point-of-use water testing networks for both developed and emerging nations. Lab-on-a-chip microfluidic technologies are employed to lower the cost and usability barriers associated with traditional environmental water analytical methods. By integrating semiconductor manufacturing based lab-on-a-chip technologies with traditional water testing, the economies of scale which have long been recognized by the

microelectronics industry can be applied to addressing the problem of generating widespread water quality databases.

1.2 Eutrophication from excess phosphate concentrations as a source of water pollution

The microfluidic colorimeter developed for this project was designed to detect dissolved reactive phosphate, also known as orthophosphate which is a stable form of phosphorus. Phosphorus was chosen since it is a major water pollutant in China and the U.S. (Wei & Rongliang, 2007) (Fareed & Ansani, 2005). Algae growth in bodies of water is often limited by the availability of nutrients such as nitrogen and phosphorus (Sawyer, McCarty, & Parkin, 2003). If these nutrients are available in abundance algae growth rates are accelerated. Water bodies are termed eutrophic if they contain excess algae concentrations leading to high turbidity and large variations in dissolved oxygen. beyond the natural algae decomposition or removal rate. Point and non-point nutrient sources resulting from human activities greatly accelerate this process (Fareed & Ansani, 2005) (Anderson, Glibert, & Burkholder, 2002). Agricultural land has become a primary non-point source of phosphorus run off into surface waters (Wei & Rongliang, 2007) (Fareed & Ansani, 2005) (Hasler, 1969). Wastewater treatment plants also are significant point sources (Wei & Rongliang, 2007) (Anderson, Glibert, & Burkholder, 2002) of phosphorus originating from detergents and human waste among others. Eutrophication has long been a problem in the U.S. (Hasler, 1969) and an accelerating one in China (Wei & Rongliang, 2007). Algae blooms are responsible for massive fish kills, toxic water quality, repugnant smelling waters, and unsightly views of once pristine lakes. Economic ramifications of eutrophication include; water unsuitable for industrial cooling, loss of

aquaculture, loss of tourism and higher costs for drinking water treatment. Examples of social and environmental impacts are job losses from reduced tourism, reduced biodiversity, and elevated toxicity from certain algae blooms (Smith, Tilman, & Nekola, 1999). Removal of phosphorus is costly. Therefore, it is imperative that phosphorus concentrations of point and non-point sources are measured to aid in the development of sound environmental management decisions.

1.3 Project initiation and inspiration

This project was initiated while a group of Cal Poly students were in rural China during the summer of 2008. During this trip students traveled to Chongming Island, China on the outskirts of Shanghai to investigate opportunities for sustainable engineering design problems that matched Cal Poly's technical capabilities and the needs of the local residents. Water quality was a major issue for all of the communities visited, but no widespread universal water testing infrastructure existed. Limited water testing was being performed by researchers on the island, but required significant knowledge of water chemistry, laboratory methods, and specialized equipment. In order for water testing to be more easily performed outside the localized research realm, instruments which could be mass produced, easily used, and were affordable would need to be developed. Researchers had proposed (Bruzewicz, Reches, & Whitesides, 2008) (Whitesides, 2006) that lab-on-a-chip microfluidics could address many of these needs by using the immense economies of scale associated with the semiconductor industry to drive down costs of traditional water analytics. The same methods used for water analytics could be scaled down, automated, and made more affordable by lab-on-chip processes. Various microfluidic technologies that were already being pursued at Cal Poly

were considered a good fit for developing a low technology water testing device. The capstone goal of this project was to begin the development of practical technologies through applied engineering and science which address the immediate concerns of society.

Chapter 2 Fluid Transport on the Micron Scale

2.1 Fluid scaling laws and key micron scale fluid transport definitions

The purpose of this section is to familiarize the reader with the fundamental concepts of fluid dynamics at the microscopic scale. Microfluidics is the study of fluid dynamics on the micron and scale. In the process of scaling fluid dynamics down from the familiar macro scale to the microscopic scale the dominant parameters affecting fluid flow changes. As pipes, reservoirs, pumps and such are shrunk in dimension, the bulk properties are diminished and surface properties are inherently more important. The microscopic relationship between a dynamic fluid and a static solid surface has a profound effect on both the characteristic properties of the fluid and the calculations required to predict or model the fluid transport phenomena.

An important scaling law is the surface area to volume ratio. Decreasing the characteristic length scales of a fluid reservoir has a profound effect of increasing the surface area to volume ratio of the fluid. For example, Table 1 shows the surface area to volume ratios of three fluid pipes representing a range of dimensional scales. It is evident that the properties associated with the interface between the reservoir wall and fluid will be magnified.

Table 1: Decreasing the radial pipe dimension magnifies the surface to volume ratio.

Part	Diameter	Surface Area : Volume
Water main pipe	15 cm	.27 : 1
Fish aquarium air line	15 mm	2.7 : 1
Microfluidic channel	15 μm	2700 : 1

If the dimensions of a fluid handling reservoir are further reduced from the micron scale to the nanoscale, the reservoir dimensions approach the molecular mean free path of the fluid. The ratio of molecule to interface and molecule to molecule interactions increases to the point where discontinuities within the fluid exist. The Knudsen number captures this effect and is used to determine if a classical fluid dynamics approach can be taken or whether alternative analysis techniques are warranted. In general when Knudsen numbers approach unity continuum fluid dynamics breaks down.

$$K_n = \frac{\lambda_{mfp}}{D_{characteristic}} \quad (2.1)$$

Typically microfluidics utilizes channels with micron scale dimensions which contain fluids not gases. Therefore, Knudsen numbers will generally be much less than one. For example a 100 μm tall channel filled with water with a mean free path approximating an average molecular width of 1.9 $\text{\AA}$ will have $K_n = 1.9 \times 10^{-5}$ which is clearly in the continuum regime.

To understand the transport phenomena of fluids at the microscopic scale it is useful to examine and carefully define what a fluid is. Generally defined a fluid is a substance which constantly deforms under the presence of an applied shear force. In most

cases a fluid has the same resistance to the applied stress over a given time period and can be mathematically defined by Newton's Law of Viscosity:

$$\tau = -\mu \frac{du}{dz} \quad (2.2)$$

Where τ is the applied stress normal to the fluid (Pa), μ is the fluid viscosity (Pa · s) u is the axial fluid velocity (m/s), and z is the channel height (m). Fluids exhibiting this behavior are classified as Newtonian fluids. Examples of non-Newtonian fluids may be honey or wax whose viscosities change with temperature. For this project only Newtonian fluids are considered. A Newtonian fluid will assume the shape of its containment reservoir, but the process in which this occurs is the fundamental question of fluid dynamics. In order to solve fluid dynamics problems in a cohesive manner it is first necessary to establish a coordinate system to base all subsequent problems on and define key parameters of the input and output variables for the governing equations. Figure 1 depicts the Cartesian coordinate system which is utilized for describing dimensions and directions for various hydrodynamic phenomena.

Figure 1: On the left is the hydrodynamic coordinate system using Cartesian coordinates and on the right is the cylindrical hydrodynamic coordinate system.

Microfluidic channels built by a soft photolithography process generally have rectangular cross section geometries. The two axis symmetry of the channel has been exploited so that each cross section is divided into four parts where b equals the channel half width and c equals the channel half height. The Cartesian coordinate system is convenient for describing these rectangular cross sections, but tends to complicate the hydrodynamic solutions for fluid flow. Therefore, a hypothetical cylindrical coordinate system is used to initially derive the equations for fluid flow.

The final objective of the hydrodynamic calculations for this thesis is to understand the relationship between the forces acting on a Newtonian fluid contained within the channel walls and the resultant fluid response to those forces for a given set of fluid properties. Gravity (g) and applied pressure gradient along a rectangular channel length (dp/dx) are the two forces acting on the fluid. The fluid response of interest is the fluid velocity ($\bar{u}$), and the resultant distribution across the cross section ($u(y,z)$), parallel to the channel length. The fluid properties influencing the hydrodynamic calculations are density (ρ) and viscosity (μ). The collection of governing equations describing fluid motion for a Newtonian incompressible fluid are referred to as the Navier-Stokes equations. This system of equations has two components; continuity and conservation of momentum.

The derivation of, and solution to, the Navier-Stokes equations requires a number of simplifying assumptions to be made. Full explanations of why these assumptions are valid can be found elsewhere (Bird, Stewart, & Lightfoot, 1960) (Alexandrou, 2001) (Papanastasiou, Georgiou, & Alexandrou, 2000). For the Navier-Stokes equations to be valid the fluid in question must be Newtonian, within an isothermal system,

incompressible, and undergoing laminar flow so that all convective terms are non-existent. The delineation between laminar and convective motion will be discussed in section 2.3. To simplify the partial differential Navier-Stokes equations into an analytical result requires further key assumptions which are discussed within the derivation of the analytical solution for laminar pipe flow.

The Hagen-Poiseuille solution from the Navier-Stokes Equations:

For a control volume of infinitesimally small volume contained within cylindrical channel (Figure 2) the Navier Stokes governing equations are given as follows.

Figure 2: Control volume and velocity distribution for Hagen-Poiseuille pipe flow.

Continuity

$$\frac{1}{r} \cdot \frac{\partial}{\partial r} \cdot (r \cdot u_r) + \frac{\partial u_z}{\partial z} = 0 \quad (2.3)$$

r-momentum

$$\rho \left(u_r \cdot \frac{\partial u_r}{\partial r} + u_r \cdot \frac{\partial u_r}{\partial z} \right) = \rho \cdot g_r - \frac{\partial p}{\partial r} + \mu \left[\frac{\partial}{\partial r} \left(\frac{1}{r} \cdot \frac{\partial}{\partial r} \cdot (r \cdot u_r) \right) + \frac{\partial^2 u_r}{\partial z^2} \right] \quad (2.4)$$

z-momentum

$$\rho \left(u_r \cdot \frac{\partial u_r}{\partial r} + u_r \cdot \frac{\partial u_r}{\partial z} \right) = \rho \cdot g_r - \frac{\partial p}{\partial z} + \mu \left[\frac{1}{r} \frac{\partial}{\partial r} \left(r \cdot \frac{\partial}{\partial r} \cdot u_z \right) + \frac{\partial^2 u_z}{\partial z^2} \right] \quad (2.5)$$

The continuity equation stipulates that the fluid mass contained within the channels of a closed system is conserved. If fluid flow in the channel is assumed to be steady and fully developed, then the continuity equation can be further reduced. Fully developed flow implies that the velocity distribution across the channel cross section is constant, but not necessarily equal. Therefore, any change in fluid velocity along the length of the channel is zero. In addition, the continuity equation requires that flow in any direction not parallel to the length of the channel is zero. A key assumption to simplify both the continuity equation and the momentum equations is that the fluid velocity at the channel wall is zero. This so called ‘no slip condition’ is characteristic only of highly laminar flows. Applying these assumptions to the continuity equation reduces the equation to the velocity across the channel and at the channel walls is zero. The momentum equation yields the most important result of the laminar pipe flow equations. Using the assumptions discussed above plus neglecting the force of gravity the r-momentum equation drops out leaving the reduced z-momentum equation as:

$$0 = -\frac{\partial p}{\partial z} + \mu \cdot \frac{1}{r} \cdot \frac{\partial}{\partial r} \left(r \cdot \frac{\partial}{\partial r} \cdot u_z \right) \quad (2.6)$$

Applying a further assumption that the pressure gradient within the pipe is only parallel to the channel length reduces the momentum equation to a separable differential equation. Following separation of the equation and double integration results in the important Hagen-Poiseuille velocity profile:

$$u_z = -\frac{1}{4\mu} \cdot \frac{dP}{dz} (R^2) \quad (2.7)$$

It is apparent from equation 2.7 that the cross sectional flow profile will be parabolic with the maximum fluid velocity at the channel centerline.

2.2 Circular versus square cross sections of fluid channels

The Hagen-Poiseuille analytical solution for pipe flow problems is essential to easily solving fluid transport problems. However, for most microfluidic systems built using a photolithography process the channel cross sections are rectangular (Figure 1) and thus, requires the Hagen Poiseuille solution to be in Cartesian coordinates (Papanastasiou, Georgiou, & Alexandrou, 2000).

$$u_x(y, z) = -\frac{c^2}{2\mu} \cdot \frac{\partial p}{\partial x} \left[1 - \left(\frac{z}{c}\right)^2 + 4 \sum_{k=1}^{\infty} \frac{(-1)^k}{\alpha_k^3} \cdot \frac{\cosh(\frac{\alpha_k y}{c})}{\cosh(\frac{\alpha_k b}{c})} \cdot \cos(\frac{\alpha_k z}{c}) \right] \quad (2.8)$$

Where: $u_x(y, z)$ is the axial fluid velocity, $\alpha_k = (2k - 1) \cdot \frac{\pi}{2}$, for $k = 1, 2, 3 \dots$ and μ is the fluid viscosity, c is the channel half height, b is the channel half width, $\frac{\partial p}{\partial x}$ = pressure drop axial to fluid flow direction

The Cartesian coordinate solution is rather complex and cumbersome to use, therefore a third variation of the pipe flow equation was developed utilizing friction factors to account for different channel cross sectional geometries including rectangular shapes of varying aspect ratio (Blevins, 1984).

$$\bar{u} = \frac{(D_h)^2}{S\mu} \cdot \frac{\Delta P}{L} \quad (2.9)$$

Where: S is the shape factor determined by the friction coefficient and the relative width and height aspect ratio of the channel and can be found in (Blevins, 1984). The non differential form of the constant pressure drop (ΔP) parallel to the pipe length measured across L is the length of the pipe, and D_h is the hydraulic diameter of the channel cross section. For a rectangular cross sectional channel the hydraulic diameter is:

$$D_h = \frac{4 \cdot Area}{Perimeter} = \frac{2hw}{(h + w)} \quad (2.10)$$

The Hagen-Poiseuille analytical solution using the cross sectional shape factor is particularly useful and is used extensively throughout this project. The obvious limitation to this analytical solution is that only the average fluid velocity is calculated. It is important to note that all three of the pipe flow velocity equations apply exclusively to steady, pressure driven, incompressible Newtonian fluids and assumes a purely laminar flow. While these assumptions are useful to derive analytical relationships from the Navier Stokes equations, they often do not reflect the situation at hand inside the microchannel. Therefore, it is important to identify the relative degree of laminar flow using the Reynolds number which is a non-dimensional hydrodynamic parameter.

2.3 Implications of low Reynolds flow regimes

The Reynolds number categorizes fluid transport into two major regimes; viscous force dominated flow and momentum force dominated flow. These two regimes can be conceptualized by looking at smoke emanating from a cigarette. Where the smoke is highly columnar and ordered the flow rate and direction is dominated by the fluid viscosity. As the smoke rises and travels further from its origin it begins to mix with the

air around it and disperse in an unpredictable manner. This is the turbulent regime where the fluid velocity and mass dictate the fluid transport behavior. The Reynolds number originates out of the non-dimensionalization of the time and pressure terms in the Navier Stokes momentum equations and is defined as:

$$R_e = \frac{\rho u D_h}{\mu} \quad (2.11)$$

Where ρ is the fluid density and u is the fluid velocity in the direction of interest.

Traditionally the boundary between laminar and turbulent flow is where the Reynolds number is greater than 2,300 however, a clear distinction between regimes is not always evident (Purcell, 1977) (Tabeling & Cheng, 2005). Typically microfluidic systems which employ only fluids in the liquid state operate with $R_e = 10$ or less (Stone, Stroock, & Ajdari, 2004). There are profound implications associated with flow at low Reynolds numbers. E.M.Purcell (Purcell, 1977) has included a number of practical examples to conceptualize what low Reynolds numbers imply. One of these examples provides a sense of scale for the Reynolds number by comparing the Reynolds number between three common life forms swimming in water. For a scuba diver $R_e \cong 10^4$, a gold fish $R_e \cong 10^2$, and a microorganism $R_e \cong 10^{-4}$ assuming all the life forms are swimming at a constant velocity and producing a propulsion reasonable for their capabilities. For both the goldfish and the microorganism, which reside well into the laminar flow regime, gravity and the time domain have a negligible effect.

Extremely low Reynolds number ($R_e \ll 1$) flow regimes, such as those in which some microfluidic systems operate, fluid transport is called Stokes flow. Stokes flow is where the inertial term in the Navier Stokes equation can be completely neglected so that

all terms with a time variable are eliminated (Tabeling & Cheng, 2005). The practical implications for Stokes flow is that the hydrodynamic equations which model flow in one direction can be reversed and applied in the other direction. Since little turbulence is present within the system, the flow is ordered and columnar in both directions.

2.4 Modeling fluid systems by the equivalent circuit method

A particularly useful application of the Hagen-Poiseuille pipe flow equations is calculating the flow rates for laminar fluids inside pipe networks. The same assumptions for laminar, steady state, incompressible, and Newtonian fluids applies. Laminar flow hydrodynamics can be analogous to equations for electrical circuitry, principally Ohms and Kirchoff Laws, and solved in the same manner as a system of interconnected resistors.

To utilize Ohm's law in solving for an equivalent fluid channel system the voltage, current and resistance variables are equated to pressure drop (across the pipe length), flow rate, and fluid resistance by:

$$v = i \cdot r \rightarrow \Delta P = Q \cdot R_{fluid} \quad (2.12)$$

The relationship between pressure drop, flow rate and fluid resistance are conveniently described by the Hagen Poiseuille pipe flow analytical solution (Aumeerally & Sitte, 2006). The average fluid velocity in the channel is converted to flow rate by multiplying the velocity by the channel cross section so that:

$$\bar{u} = \frac{(D_h)^2}{8\mu} \cdot \frac{\Delta P}{L} \rightarrow Q = \frac{(D_h)^2}{8\mu} \cdot \frac{\Delta P}{L} \cdot A_{cross} \quad (2.13)$$

In this manner the following analogous relationships (Ajdari, 2004) for a rectangular channel of dimensions h and w which is the channel height and width (equation 2.10), respectfully:

$$v = \Delta P; \quad i = Q; \quad r = R_{fluid} = \frac{S\mu L}{(D_h)^2 \cdot A_{cross}} \quad (2.14)$$

Similarly, Kirchoff's Law can be used in conjunction with the above analogous variables to determine the flow rates entering and leaving fluid junctions so that:

$$\sum i_i = 0 \rightarrow \sum Q_i = 0 \quad (2.15)$$

Two common examples where equivalent circuit modeling can be used is illustrated in Figure 3.

Figure 3: Equivalent circuit analogy for analyzing fluid dynamics of a typical microfluidic mixing junction.

Here a three way channel junction is modeled as three resistors arranged in parallel and connected to two more resistors connected in series. The equivalent circuit solution for this junction is:

$$R_{total} = \left[\frac{1}{\sum_1^3 \left(\frac{1}{R} \right)} \right]^{-1} + R_4 + R_5 \rightarrow R_{fluid} =$$

$$\left[\frac{1}{\sum_1^3 \left(\frac{(D_h)^2 \cdot A_{cross}}{S \mu L} \right)} \right]^{-1} + 2 \cdot \left(\frac{S \mu L}{(D_h)^2 \cdot A_{cross}} \right) \quad (2.16)$$

Where R_{total} = total electrical resistance for the circuit and R_{fluid} = total fluidic resistance for the three way fluid junction. In this manner complex pipe flow problems can be broken down into straightforward analytical equations to solve for pressure drop and flow rate. Equivalent circuit solutions are utilized in section 5.3 in the design of the microfluidic device.

2.5 The influence of surface effects on micron scale fluids

The large surface area to volume ratio for microfluidic channels magnifies the effects caused by three phase interfaces. A common example of a three phase interface would be the curved interface on the leading end of an advancing fluid column. The surface tension associated with the fluid along the three phase interface can be defined as a change in work required to increase the surface area of the interface. An important implication of the surface tension associated with a curved interface inside a channel is the Young – LaPlace Equation:

$$\Delta P = \gamma \left(\frac{1}{R_1} + \frac{1}{R_2} \right) = 2 \cdot \frac{\gamma}{D_h} = \gamma \left(\frac{1}{h} + \frac{1}{w} \right) \quad (2.17)$$

Where γ is the surface tension and R_1 and R_2 are the radii of curvature inside the channel. The Young-LaPlace equation results in significant pressure drops across a three phase

interface and must be taken into consideration while calculating the pressure drop and corresponding flow rate of a microfluidic device. A practical design equation to account for this phenomenon is the capillary pressure equation (Butt & Graf, 2006):

$$\Delta P = \frac{2\gamma \cos \theta}{R_1} = 2\gamma \cos \theta \left(\frac{1}{h} + \frac{1}{w} \right) \quad (2.18)$$

Where θ is the contact angle between the channel wall and the liquid to gas interface. The direction of the pressure gradient is affected by the contact angle. Hydrophobic channel wall surfaces result in a pressure gradient opposed to the axial flow direction.

Hydrophilic surfaces result in a pressure gradient which assists the fluid flow.

Two dimensionless terms which categorize the effect of surface tension on fluid flow are the Capillary number (Ca) and the Bond number (Bo) (Tabeling & Cheng, 2005).

$$Ca = \frac{\mu \cdot U_x}{\gamma} \quad (2.19) \quad Bo = \frac{\rho \cdot a \cdot (D_h)^2}{\gamma} \quad (2.20)$$

Where ρ = fluid density, a = acceleration associated with the body force, U_x = fluid velocity in the axial direction. For microfluidic devices which have a low Capillary or Bond number, flow is dominated by interfacial surface tension forces. In these devices the presence of an interface will require using the capillary pressure equation to correct the pressure drop predicted by the conventional hydrodynamic pipe flow equations.

2.6 Calculating diffusion controlled mixing processes

Mixing fluids on a micron scale is almost always a diffusion controlled process. As was shown previously by the Reynolds number, fluid flow is highly laminar inside microfluidic channels. For this reason mixing due to advection is limited while diffusion, which is an atomic level phenomenon, is virtually unchanged by downscaling. The ability for a fluid to diffuse into another fluid is characterized by the diffusion coefficient which is equivalent to the number of molecules moving through a cross sectional area per time (Atkins & de Paula, 2006). A range of diffusion coefficients for various fluids in water is shown in Table 2. The diffusion coefficient is related to molecular weight and with the exception of several long molecular chained fluids, diffusion coefficients are of similar size (Tabeling & Cheng, 2005). This is important from a microfluidic design standpoint since the diffusion coefficient of many specific fluids is not known, but can be inferred by other similar fluids.

Table 2: Range of diffusion coefficient values for fluids common in microfluidics.

<u>Fluid Type in Water at 25°C</u>	<u>Diffusion Coefficient (10⁻¹⁰ m²/s)</u>
Air	2
Sulfuric Acid	1.73
Water	2.2
Hemoglobin	.069

A dimensionless number used to characterize the ratio of advection to diffusion controlled mixing phenomenon is the Peclet number:

$$Pe = \frac{u_x \cdot l}{D} \quad (2.21)$$

Where l is the characteristic hydraulic dimension (m) and D is the diffusion coefficient (m^2/s). A large Peclet number indicates a strong advection mixing effect. Unlike the Reynolds number there is no characteristic Peclet number to clearly delineate between advection and diffusion regimes (Tabeling & Cheng, 2005).

Determining the residence time required for diffusion mixing to occur inside a microfluidic channel is a common design problem. There are various approaches (Tabeling & Cheng, 2005) (Zhang, Zhao, & Xiao, 2008) (Nam-Trung & Wu, 2005) to modeling diffusion processes in micro channels. One simple analytical approach is to statistically model a fluid molecule by one dimensional random walk theory. This model results in the random walk distance being proportional to the diffusion coefficient by (Atkins & de Paula, 2006):

$$x^2 \propto D \cdot t \quad (2.22)$$

Where x is the random walk distance and t is the residence time. From a design standpoint this relationship can be used to quickly estimate the dimensions of a diffusion mixer. The random walk distance is the largest cross sectional dimension of the channel (see Figure 1) and time would be the residence time required for the fluid to transport from inlet to outlet. For example, if the largest cross sectional dimension is the channel

width and both the flow rate and diffusion coefficient for the fluids to be transport is known, then all the variables can be correlated by (Tabeling & Cheng, 2005):

$$L \cong \frac{w \cdot Q}{hD} \quad (2.23)$$

Where Q is the average flow rate ($\mu\text{L}/\text{min}$) and L is the diffusion mixer length (mm).

This equation is used to design the approximate diffusion mixer length of the microfluidic device described in section 5.4. Since the exact diffusion coefficient for one fluid into another is unknown more detailed equations than this analytical solution are often unnecessary, however more complex models exist using computational fluid dynamics (CFD) (Soleymani, Kolehmainen, & Turunen, 2008) if required.

Chapter 3 Light Absorption Spectroscopy

3.1 Beer-Lambert Law

Both the Beer and Lambert Laws are empirical results which describe the relationship between the absorption of light by a chemical species and concentration. Beer Law states that light absorption is proportional to the concentration of a species while the Lambert Law describes the relationship between distances light must travel through a species and the resulting degree of absorption. The combined Beer-Lambert Law can be written in terms of the proportion of light transmission (Atkins & de Paula, 2006) lost by:

$$T = \frac{I}{I_o} = 10^{-\varepsilon(\lambda) \cdot l \cdot c} \quad (3.1)$$

Where $T(\lambda)$ is the light transmitted through the chemical species, I is the intensity of light emerging from the chemical species (W/m^2), I_o is the intensity of incident light, $\varepsilon(\lambda)$ is the absorptivity coefficient ($L/mg \cdot cm$), l is the optical path length through the chemical species (cm), and c is the concentration of absorbing species (mg/L). Often light absorbance is used in lieu of transmittance so that:

$$-\log_{10} T = A(\lambda) = \varepsilon \cdot l \cdot c \quad (3.2)$$

Where $A(\lambda)$ is the light absorbance as a function of wavelength resulting from the chemical species concentration and the optical pathlength. The molar absorptivity coefficient is related to the molecular light transmittance cross section and is unique to a given chemical species. Chemicals with high molar absorptivity coefficients have large cross sections capable of absorbing a greater percentage of light and are desired for spectroscopy techniques based on Beer-Lambert Law since lower concentrations of the species can be detected. Long optical pathlengths are advantageous to increase light absorption for a given chemical species and corresponding concentration (Atkins & de Paula, 2006).

The Beer Lambert Law can be used in spectroscopy to measure the concentration of an analyte based on light absorption measurements. To produce a light absorption signal which is directly proportional to the concentration of an analyte, a chemical reagent must be reacted with the analyte to form a product.

The analyte is the species measured and the matrix is any chemical species such as water which does not participate in the chemical reaction. The reaction forms a chemical

product of a concentration directly proportional to the analyte concentration and has a molar absorptivity coefficient distinct from both the reagent and analyte. An ideal reaction uses a reagent which has no absorption signal for the wavelengths of light being analyzed. However, most reagents produce an absorption signal which must be zeroed from the measurement process using a no-analyte blank. In this manner, the absorption signal used in the measurement process derives only from the product and not from the reagent.

To use the Beer Lambert Law for spectroscopy, light absorption measurements are related to the analyte concentration using a standard calibration curve (Figure 4). The light absorption caused by the reagent is first zeroed from the instrument. A series of light absorption measurements are taken of the chemical product which is produced from a series of known analyte concentrations. From this data, a calibration curve is generated which is used to determine unknown concentrations of analyte in a sample from subsequent light measurements.

Figure 4: A standard calibration line generated from known concentrations of analyte (x-axis). Once generated, the standard calibration line is used to find the unknown concentrations of analyte. Light absorption is measured (1.) then input into the calibration line (2.) to determine analyte concentration (3.).

The Beer Lambert Law is used to generate a standard calibration curve by:

$$A(\lambda) = (\epsilon(\lambda) \cdot l) \cdot c + error \quad (3.3)$$

$$y = m \cdot x + b$$

The grouped terms $(\epsilon(\lambda) \cdot l)$ are dependent on the optical pathlength of the instrument and molar absorptivity coefficient of the chemical product being measured and are fixed values for measurement process. Error in the calibration curve is the aggregate error of the instrument and chemical reaction that results in a non-zero light absorption measurement for a zero analyte concentration. Reactions to form a light absorbing product have limited concentration ranges where the Beer Lambert Law can be applied (Boltz, Lueck, & Jakubiec, 1978). The limits to the range of linearity should be

determined for each chemistry and instrument combination. The extreme ends of this linear relationship contribute towards the minimum and maximum detection limits.

3.2 Components of a colorimeter instrument

A colorimeter is an instrument used for light absorption spectroscopy. The function of this instrument is to measure the amount of light absorbed by a chemical solution. A spectrophotometer measures light absorbance at a specific wavelength through a chemical sample; however it is different from a colorimeter in several ways (Sawyer, McCarty, & Parkin, 2003). There are three basic components for any colorimeter or spectrophotometer; a light source, cuvette, and light detector (Figure 5).

Figure 5: Basic components of a colorimeter. A tungsten lamp can be used in conjunction with a light filter or a LED without filter. The photodiode measures light power leaving the cuvette as an output current which is then electronically processed to determine light absorbance.

Colorimeters use a monochromatic light source which is obtained either by using a full spectrum light source and color filter, or a narrow spectrum light emitting diode (LED). Conversely, spectrophotometers use a full spectrum light source and diffraction

grating or prism system to deliver monochromatic light over a range of wavelengths.

Both instruments operate in the visible spectrum; however spectrophotometers are capable of near ultra violet operation as well. For light detection equipment colorimeters generally use photodiodes while spectrophotometers use spectrometers. The light source and detector system is matched so that the spectral intensity of the source matches the responsivity of the detector. Responsivity is defined as (Cvijetic, 2004):

$$R_{\lambda} = \frac{I_p}{P} \quad (3.4)$$

Where I_p is the detector photocurrent output (amp) and P is the incident light power (watts). Spectrophotometers deliver greater versatility since the operator can perform a variety of light absorption tests at various wavelengths without having to change filters or detectors. The light absorbance data from a spectrophotometer is more informative than a colorimeter equipped with a photodiode since the absorbance data is broken down per wavelength instead of one integrated absorbance output for all wavelengths of light. Sample cuvette sizes and shapes vary between instrument type and manufacturer (Sawyer, McCarty, & Parkin, 2003). A typical spectrophotometer cuvette is 1cm by 1cm at the base by 3cm tall. The optical pathlength, which is the distance the light must transmit through the liquid sample before reaching the light detector, for this size cuvette is 1 cm. For a colorimeter, the cuvette is generally larger with a typical size of one inch in diameter, to yield a one inch optical pathlength, and a volume between 10 to 25mL. The optical pathlength for colorimeters is longer to improve the Beer-Lambert light absorbance since the light source and detection systems are less sensitive than in spectrophotometers (Cvijetic, 2004). Benchtop sized spectrophotometers offer better

performance and versatility and are particularly suitable for laboratory research work. Colorimeters have fewer complex components which reduce the cost and performance, but increase the suitability for rough onsite field testing and downscaling the instrument for handheld operation. The specific optical pathlength used for the microfluidic colorimeter and its effect on instrument sensitivity is described in Sections 5.6 and 5.7.

Chapter 4 Literature Review of Microfluidic Colorimeter Instruments

4.1 Literature review of microfluidic colorimeter instruments

Numerous microfluidic colorimeter instruments have been designed, fabricated, and tested for use in water quality monitoring. Many of these have been developed specifically to test for phosphate in water (McGraw et al, 2006) (Doku & Haswell, 1999) (Mas-Torres et al, 1997) (Cleary et al, 2008) (Bowden & Diamond, 2003) (Daykin & Haswell, 1995). These microfluidic instruments generally share the same basic light source and detection components as found in conventional colorimeters and utilize the Beer-Lambert Law to determine chemical species concentrations. A major departure of microfluidic systems from conventional ones is the size and configuration of the cuvette and the method in which water samples and reagent are measured, mixed, and loaded into the cuvette. All of the colorimeter instruments reviewed are micro- fluid injection analysis (μ FIA) systems, meaning that sample and reagent are pumped into and out of the fluid handling system along a continuous piping network. The μ FIA colorimeters in this review all have the same general components; a light source and detector, fluid pumps,

and a micron scale fluid handling device which measures, mixes, and positions the sample for analysis.

Two general configurations for the light source and detection components for a μ FIA system are shown in Figure 6 and Figure 7. The planar design directs light in the plane of the microfluidic chip using either light waveguides (Song, 2007) (Demming, 2009), fiber optics (Doku & Haswell, 1999) (Daykin & Haswell, 1995), or “Z” shaped liquid capillary flow cells (Zhang J.-Z. , 2000) Figure 6. A distinct advantage of the planar design is an increased optical pathlength which improves the signal to noise ratio for low absorbance species at low concentrations. A significant drawback is the increased cost and complexity to integrate optical components which require high tolerances within a microfluidic chip.

Figure 6: Planar light source and detector configuration. This design results in a longer optical pathlength for greater light absorption sensitivity; however design and fabrication of the microfluidic device is more difficult and costly. This depiction incorporates fiber optics to guide light into and out of the cuvette which is common for this configuration.

An instrument which orients the light source and detector perpendicular to the microfluidic chip is also shown in Figure 7. This arrangement was used in variety of μ FIA systems with success (Cleary, et al, 2008) (Rainelli & et.al, 2003) (Mas-Torres, et al. 1997) (Pamula & et.al, 2005) (Adams, et al, 2003). A perpendicular light and detector arrangement is easier to design and fabricate, however the optical pathlength is restricted to the height of the microfluidic channel which is typically in the range of 150 μ m (Rainelli, et al, 2003) to 400 μ m (Bowden & Diamond, 2003). The shorter Beer-Lambert Law optical pathlength can reduce minimum detection limits and decrease the signal to noise ratio (O' Toole & Diamond, 2008). Variations between the planar and perpendicularly orientated light source and detectors arrangements have been proposed (O' Toole & Diamond, 2008). These systems employ reflective mirror surfaces to direct light down a longer pathlength without placing the light source and detector within the plane of the microfluidic chip. Presently, it appears the predominant optics configuration is the perpendicularly orientated setup (O' Toole & Diamond, 2008) until the design fabrication of planar optical systems becomes more practical.

Figure 7: Perpendicular light source and detector configuration. This design results in an optical pathlength equal to the height of the microfluidic channel which is substantially shorter than the planar design. The perpendicular configuration is used for μ FIA systems since it is easy to fabricate and requires lower alignment tolerances. In this depiction a photodiode and LED is used in lieu of a spectrometer and tungsten light source; however both light sources and detectors can be used interchangeably.

Three predominant fabrication techniques and materials are used to create micron scale fluid channels; deep etching silicon substrates, micromilling plastic substrates, and elastomer molds built using a softlithography process (Section 6.1). The deep etching and softlithography processes were adapted from the semiconductor industry (Whitesides, 2006) (Reyes, 2002). Each fabrication process has specific advantages and disadvantages for building microfluidic colorimeters depending on the type of application and desired performance. The advantages and disadvantages of each fabrication process is described below.

Deep etched silicon substrates using either wet etching or dry etching processes were used to create some of the first microfluidic devices (Reyes, 2002) (Terry, Jerman, & Angell, 1979) (Raymond, Manz, & Widmer, 1995). One of the first silicon based microfluidic systems, a gas chromatographic air analyzer built at Stanford, (Terry,

Jerman, & Angell, 1979) used wet etched silicon as a channel substrate and a mechanically bonded glass top. The advantages of using etched silicon are that smaller features with finer resolutions can be built and well established lithography processes can be utilized (Wolf, 2004). Three distinct disadvantages in using deep etched silicon are the high costs and complex fabrication process associated with working in silicon and the non-optically clear properties of silicon substrates. For these reasons few silicon substrate based colorimetric instruments have been successfully made (Adams, et al, 2003) (Reyes, 2002).

Fabricating micron scale channels from plastic using either micro-milling, hot embossing or laser ablation is a popular fabrication process for creating μ FIA colorimetric instruments (Cleary, et.al, 2008) (Rainelli & et.al, 2003) (Becker & Locascio, 56, 2002) (Locascio, et al, 2006). Polymers exhibit several properties such as optical transparency, a wide range of surface properties depending on formulation, low cost, machineability, and moldability, which make them attractive for use in colorimetric measurements (Becker & Locascio, 2002). Common forms of plastic for μ FIA colorimetric instruments include poly(methyl methacrylate) (PMMA) (Cleary, et al, 2008) (Rainelli, et al, 2003), polyethylene (PE), and silicone (Becker & Locascio, 2002). Micron scale fluid channels can be machined using high frequency cutting spindles to form trapezoidal channels several hundred microns deep and equally wide (Rainelli, et al, 2003) (Bowden & Diamond, 2003). UV laser ablation forms micron scaled channels by localized decomposition of a UV sensitive plastic (Becker & Locascio, 56, 2002). Hot embossing is a fabrication technique which shows promise to commercially mass produce microfluidic devices. In this process a hot mold is pressed into a hard plastic,

typically either polyvinylchloride (PVC) or polyethylenetetrathalate (PETG), to imprint the channel features (Becker & Locascio, 2002) (Becker & Heim, 2000). Disadvantages to plastics as substrates for μ FIA colorimetric instruments include; rough micron scaled features, less control over fabrication process including feature sizes and repeatability, and a porous substrate which can absorb chemicals, thereby affecting test results (Bowden & Diamond, 2003).

Soft lithography is an effective fabrication technique for rapidly producing microfluidic prototypes. After the soft lithography process was discovered by the George Whitesides research group an explosion in microfluidic development followed (Cooper & Whitesides, 2002) (Xia & Whiteside, 1998) (Zhao, Xia, & Whitesides, 1997). In this process a female mold is built on top of a silicon wafer with SU-8, a UV light curable polymer, using photolithography. Once the mold is made an elastomer, typically poly(dimethylsiloxane) (PDMS), is poured over the female mold. After the elastomer dries it is peeled away from the mold, thereby creating a flexible microfluidic device. The primary advantages for using soft lithography are that prototypes with small features at fine resolutions can be quickly and affordably fabricated. PDMS as substrate for colorimetric measurements also has the advantage of being optically clear and resilient to many acid based reagents (Xia & Whiteside, 1998). A permanently installed μ FIA colorimetric instruments built from PDMS, or similar elastomer, would be a poor choice of material since the porosity of the substrate would result in chemicals slowly diffusing into the substrate, thus degrading the performance of the instrument with time.

The pumping mechanism employed in the μ FIA colorimetric instruments, previously reviewed, can be categorized in three groups; micron scale mechanical pumps

integrated into the microfluidic chip, large external mechanical pumps, and integrated non-mechanical pumps. Several types of micro-mechanical pumps include electrostatic, electromagnetic, and electrochemical actuated pumps (Trung & Wereley, 2002) (Ho-Kim & et.al, 2008). Common to all these systems is a mechanical diaphragm which pistons in such a way to create a positive displacement. Micro-mechanical pumps are complex and prone to failure (Rainelli, et al, 2003). However, newer pumps such as the electrochemical pump, can achieve high flow rates up to 50 μ L/min while using a robust elastomer membrane (Ho-Kim, et.al, 2008). External peristaltic and syringe pumps are common to μ FIA systems due to their low cost, high reliability, and high flow rates (Cleary J. e., 2007). The disadvantages of external pumps are the increased power consumption, size, and minimal flow rate control (Rainelli, et.al, 2003) (Cleary J., 2007). Pumping rates of 100 μ L/min are easily maintained however, become oscillatory at low flow rates due to the motion of the stepper motor advancing the pumping piston at an unsteady rate (Rainelli, et al, 2003). Integrated non-mechanical pumping is usually accomplished by applying a large voltage potential across the fluid channel to establish electroosmotic flow as charged chemical species move through the conductive liquid. The advantages for such a pumping system are controlled low flow rates and no moving parts to cause mechanical failure (Doku & Haswell, 1999) (Tang, Li, & Tao, 2009). To generate an effective flow rate using charge potential, the pH level for the pumping fluid must generally be acidic depending on the channel material type (Rainelli & et.al, 2003) (Doku & Haswell, 1999) (Butler, Mills, & Hauser, 1997). Other disadvantages include; high voltage potentials required which are impractical for battery operation, low flow rates around 1 μ L/min (Doku & Haswell, 1999), and conflicting pH requirements in the

fluid stream between ideal pumping conditions and ideal light absorbance conditions (Rainelli & et.al, 2003). Pumping fluids through μ FIA systems has proved to be a non-trivial problem and continues to present challenges for microfluidic development.

The performance of several types of μ FIA colorimetric systems indicate that microfluidic based instruments could be utilized for field based work. To date, microfluidic colorimetric instruments which detect phosphate (McGraw & et.al, 2006) (Doku & Haswell, 1999) (Mas-Torres, et.al, 1997) (Cleary, et.al, 2008) (Bowden & Diamond, 2003) (Daykin & Haswell, 1995), ammonia (Sequeira & et.al, 2002), nitrite and nitrate (Pamula & et.al, 2005) (Zhang J.-Z. , 2000), cobalt, and cadmium (Tan & et.al, 1994) have been developed. Two examples of μ FIA instruments for phosphate detection built by Diamond, et. al (McGraw & et.al, 2006) and Haswell, et. al (Doku & Haswell, 1999) provide relative performance standards for subsequent microfluidic phosphate colorimeters. Diamond's system utilizes the yellow vanadomolybdophosphoric acid method to detect reactive (ortho) phosphate down to .3mg/L and to a maximum of 20mg/L. The microfluidic chip for this system is made from PMMA and uses a perpendicularly orientated 370nm LED and photodiode sensor arrangement with a 200 μ m optical pathlength. The flow rate of the reagent and sample mixture is 2 μ L/min which is provided by an external peristaltic pump. Total measurement time is 15min per sample with the instrument capable of intermitted testing for seven days. The system built by Haswell uses the more sensitive (Boltz, Lueck, & Jakubiec, 1978) blue ascorbic acid and ammonium molybdate method to detect orthophosphate. The minimum detection limit for this instrument is .1mg/L with a maximum range to 10mg/L. The deep etched glass microfluidic chip uses planar orientated fiber optics for a long optical pathlength of

6mm. The LED peak output is 700nm and uses a small spectrometer for light detection. Fluid flow is accomplished inside the microfluidic channels by electroosmotic action. The time for each measurement is one minute using .6 μ L of sample and reagent mixture.

The literature review demonstrates that microfluidic technologies have been successfully employed to perform colorimetric analysis to determine phosphate concentrations in water. All of the field deployable systems reviewed use a flow injection analysis approach to load reagent and sample into a chip, perform the light absorbance measurement, then eject the fluid waste. No handheld, single use, and disposable chip system for colorimetric measurements was found. Either a planar or perpendicular light source and detector arrangement was used by a most researchers. The perpendicular arrangement was used more frequently due to ease of fabrication and lower cost; however at the expense of a shorter optical pathlength. No arrangement for increasing optical pathlength for a perpendicularly orientated system was found. The effectiveness of LED light sources as a low power and low cost alternative to traditional tungsten light sources was validated (McGraw & et.al, 2006), as well as the use of single photodiodes in lieu of spectrometers (O' Toole & Diamond, 2008). Both the LED and photodiode represent significant cost savings potentials for a future colorimetric instrument. Pumping of sample and reagent was predominantly done with peristaltic pumps externally mounted from the microfluidic chip. This pump type is expensive and thus represents a significant cost of the total system. No low cost, automated pumping system to load a sample and reagent into a colorimetric instrument was found.

Chapter 5 Theory and Design of the Microfluidic Colorimeter

5.1 Overview of functional requirements for the microfluidic colorimeter

A microfluidic colorimeter instrument was designed and fabricated to test reactive phosphate in water using the yellow molybdovanadate method. The instrument consists of three main components; a disposable, single use microfluidic chip, and a chip reader interfaced to a USB equipped data acquisition electronics package (Figure 8). The functional requirements of the microfluidic chip is to measure the ratio of molybdovanadate (reagent) and water (sample), completely mix the two fluids to form a fluid product, then fill an optical cuvette with the fluid for analysis. Once the microfluidic chip has finished loading the cuvette, the chip is inserted into the chip holder and aligned by a magnetic positioning mechanism. The function of the chip holder is to house the LED and photodiode, in a perpendicular configuration with respect to the microfluidic chip, so that the light output is aligned to transmit through the cuvette and onto the active surface of the photodiode (Figure 9). The intensity of light striking the photodiode is a function of the concentration of orthophosphate in the sample which is in turn converted to a voltage signal by the data acquisition electronics. From this voltage light absorbance measurements can be calculated, compared to a master calibration curve, and the concentration of orthophosphate output to the instrument user.

Figure 8: Schematic of the microfluidic colorimeter instrument. The microfluidic chip is designed for one time use and is responsible for measuring and mixing a water sample and reagent, then filling the optical cuvette. The chip holder is designed to house the LED and photodiode which are aligned perpendicular to the microfluidic chip.

Figure 9: Schematic of the microfluidic chip held inside the chip holder and the microfluidic chip components. For the chip holder the LED light output is aligned to transmit through the cuvette and onto the photodiode. The three functional requirements of the microfluidic chip is to measure the reagent and sample, mix the two fluids, and fill an optical cuvette for analysis.

5.2 Absorption driven pumping mechanism

An innovative power free pumping mechanism for PDMS based microfluidic devices was first presented by Hosokawa (Hosokawa & et.al, Power free PDMS microfluidic devices for gold nano-particle based DNA analysis, 2004). This mechanism utilizes the high solubility of air in a vacuum depleted PDMS substrate to create a diffusion gradient within the elastomer, which in turn creates a pressure differential inside the fluid channels. The pressure differential is used to draw fluids into channels. Air dissolved in PDMS follows Henry's Law so that the concentration of gas dissolved in PDMS is proportional to the partial pressure of gas around the elastomer (Merkel & et.al, 2000). The advantages of this pumping mechanism are that no moving mechanical parts, power supply, or other external equipment are required since the substrate material acts as the pump.

The three step absorption drive pumping mechanism is illustrated in Figure 10, Figure 11, and Figure 12. In step one a microfluidic channel and reservoir made from PDMS with one inlet is first degassed inside a vacuum chamber (Figure 10). Inside the vacuum chamber oxygen and nitrogen are drawn out of the PDMS substrate. The concentration profile of air inside the elastomer as a function of diffusion distance (l) and time from the exposed substrate surfaces are depicted in each step. As per Henry's Law the concentration for a volume of air inside a given volume of PDMS is found by multiplying the solubility coefficient for oxygen and nitrogen in PDMS by the surrounding air pressure (Merkel, et al, 2000). This concentration profile, according to Hosokawa, is exponential and follows Fick's First Law of Diffusion (Hosokawa, et al., 2004) (Hosokawa, et al, 2006). It is shown that the exposed substrate surfaces are depleted first and to a lower air concentration than the inner substrate portions. This

process in effect “primes” the device for the subsequent fluid pumping process. In step two the PDMS device is removed from the vacuum chamber causing oxygen and nitrogen to rapidly diffuse into the exposed substrate surfaces (Figure 11). The air flux into the exposed inside channel and reservoir walls and into the outer exposed substrate is equal. In step three, the air flux through the open fluid channels into the exposed inside channel walls has been cut off by droplets of liquid introduced at the channel inlet (Figure 12). With the main air flux path now blocked by liquid, air trapped inside the fluid channels and reservoirs will preferentially diffuse into the PDMS substrate. The net effect is a decrease in moles of oxygen and nitrogen inside the fluid channels and, as per the ideal gas law, creating a pressure differential which can draw liquid further into the reservoirs. In steps two and three the initial concentration of air into the PDMS substrate exponentially decreases with diffusion distance. With time the concentration gradient diminishes in turn, reduces the pumping power. Therefore, there is a limited time and capacity for which the absorption pumping mechanism can work.

Figure 10: Step one of absorption pumping process. The microfluidic device is degassed to remove air from the PDMS substrate. At Time: 1 the degassing process has just begun so the concentration of air dissolved in the substrate is uniform throughout the elastomer thickness. At interim Time: 2 air diffuses out of the PDMS as the concentration profile is approximated by Fick's First Law. Once the entire substrate is depleted to a concentration equal to the vacuum degass pressure the device is "primed" and ready for pumping.

Figure 11: In step two the device is exposed to atmospheric pressure. The low concentration of air inside the PDMS substrate at Time: 1 begins to diffuse out of the substrate. As with step 1, diffusion occurs most rapidly where the distance (l) is minimal. Both the fluid channel surfaces and the outer exposed substrate surfaces offer cross-sectional diffusion pathways. The diffusion rates inside and outside the device are equal at Time: 2.

Figure 12: In step three liquid has been introduced at the channel inlet. With the air flux into the reservoir cut off by the liquid and the air inside the reservoir continuing to diffuse into the PDMS substrate, vacuum pressure is generated inside the reservoir. As time progresses the PDMS substrate is saturated by air which eliminates the concentration gradient and thus ends the pumping process (Time: 3).

There are several desirable performance objectives to be met for using the absorption pumping mechanism in a microfluidic colorimeter. High flow rates and pressure differentials are required to rapidly fill the cuvette for fast analysis times while overcoming the fluid friction pipe flow losses. Repeatable and adjustable flow rates are needed for instrument precision and to fine tune the residence times of fluids inside diffusion mixers. These attributes can be achieved using absorption driven pumping provided the mechanism is characterized in such a way that provides a set of design guidelines to achieve certain fluid pumping criteria. Hosokawa, et. al has partially characterized this pumping mechanism by modeling the diffusion of air in PDMS by Fick's First Law of Diffusion (Hosokawa & et.al, Power free PDMS microfluidic devices for gold nano-particle based DNA analysis, 2004). Using the degas and atmospheric pressures as boundary conditions yielded:

$$F = D \frac{\partial c}{\partial x} \Big|_{x=l} \approx \frac{2D(C_{atm} - C_{degass})}{l} \cdot \exp \left[\frac{-\pi^2 D}{4(l)^2} \cdot t \right] \quad (5.1)$$

Where F is the volume of air diffusing into or out of a given PDMS substrate area per time ($\text{cm}^3(\text{STP})/\text{s cm}^2$), D is the diffusion coefficient of air into PDMS (cm^2/s), C_{atm} is the concentration of air in PDMS at atmospheric pressure ($\text{cm}^3(\text{STP})/\text{cm}^3$), and l is the diffusion distance. Hosokawa relates F to the flow rate of fluid into the device by:

$$Q \approx F \cdot A_{flux} \quad (5.2)$$

Where A_{flux} is the cross-sectional area of the substrate where air can diffuse into the PDMS substrate. These equations result in three design parameters which need to be optimized in order to meet the desired pumping attributes. For high flow rates and pressure differentials the flux area should be maximized while the total pumping volume minimized. To reach the maximum pumping rate quickly the diffusion length should be minimized. However, total pumping capacity is related to the volume of PDMS available for diffusion, and therefore the diffusion length should not be too short so as to diminish total fluid pumping capacity.

Four absorption pumping configurations for the microfluidic colorimeter were designed and tested to optimize the absorption pumping process. The overall objective in each design was to couple the maximum pumping surface (A_{flux}) to the smallest volume of fluid to be pumped (the cuvette volume). Few microfluidic devices (Han, Tang, & Zheng, 2007) (Hosokawa, et al., 2004) (Hosokawa, et al., 2006) have been built which utilize absorption drive pumping so the first design attempts were to get a rough idea of the pumping capabilities afforded by this mechanism. In all three designs capillary

retention valves (CRV) were used to couple the cuvette to the absorption pumping surfaces (see Figure 9) with a unique “starburst” pattern. CRV’s are essentially narrowed sections of fluid channels which serve to selectively restrict liquid flow through the channel based on the pressure difference driving the fluid, and the contact angle between the fluid and channel wall (Cho et al., 2007). Section 5.5 has an in depth discussion of CRV’s. By connecting the absorption pumping surfaces to the cuvette using CRV’s, the entire cuvette could be filled without filling the absorption pumping surfaces. Anytime a pumping surface is in contact with water it is incapacitated as a flux surface for air to diffuse directly into the PDMS substrate. Therefore, it is desirable to couple the pumping surface and volume to be filled with a valve mechanism that will selectively pass air, but not fluid.

Designs one, two, and three were configured to vary the pumping surface area, the width of the CRV’s, and the volume of the cuvette to get a general sense of the pumping capacity for each design (Figure 13). Preliminary tests were conducted on three sets of each “starburst” pattern with 1/8”, 1/16”, and 1/32” deep and equal diameter cuvettes. See section 6.1 for fabrication details. Each set of devices were made out of 2.9mm thick PDMS castings and were degassed for greater than 1hr 20min at -60kPa (gauge) to “prime” each device. The devices were then removed from the vacuum chamber, dye placed on the fluid inlets, and the time to fill each cuvette measured (Figure 14). As was expected the smaller cuvettes coupled to the larger pumping surfaces filled faster while the large 1/8” deep cuvette could not be completely filled by any of the configurations. “Starburst” surfaces (b.) and (c.) each exhibited elements for an optimal design. Both designs were capable of filling a 1/16” deep by 3/8” diameter cuvette in approximately

6.5 to 8 minutes. The larger pumping surface with closely spaced “fingers” in design (b.) resulted in a higher diffusion air flux and a shorter diffusion distance however; it lacked CRV’s so the pumping surface would prematurely fill with fluid and thus slow the pumping process. Design (c.) lacked a large pumping surface however; the CRV’s demonstrated the capability of selectively filling the cuvette first. With these initial designs and tests the general design configuration was proved to be a valid concept and the successful elements of each design were incorporated into the final design.

Figure 13: First three designs for the absorption pumping “starburst” surfaces built to initially gauge the pumping capabilities for each design coupled to a different cuvette size. Designs (b.) and (c.) proved to be the most effective; however (b.) lacked CRV’s that were incorporated in (c.) while (c.) lacked the larger effective pumping surface of (b.). Design (a.) proved to have too small of a pumping surface for an effective design.

Figure 14: Image of initial “starburst” patterns being tested. Note that two of the four designs have identical surface areas, but different CRV widths. Glass slides have been placed on top the devices to enhance the diffusion flux inside the pumping surfaces. All of the “starburst” patterns shown are with 1/32” deep by 3/16” diameter cuvettes.

In the final absorption pumping configuration the pumping surface area was dramatically increased by adding larger “fingers” in a tightly spaced “starburst” pattern. This change reflects the positive attributes found in designs (b.) and (c.). Additionally, smaller CRV’s, from 50 μ m to 10 μ m, connect the pumping surface and cuvette to insure the cuvette fills first. A 1/16” deep by 3/16” diameter cuvette dimension was used in the final device. Table 3 gives an overview of the pumping surface and resulting surface area to volume ratio for a 1/16” x 3/16” cuvette. The final device has approximately 41 times more pumping area for a given cuvette volume than if only the walls and faces of the cuvette acted as a diffusion surface. This design is a radical improvement over previous designs by Hosokawa (Hosokawa, et al. , 2004) (Hosokawa, et al., 2006) in terms of available pumping surface while consuming less of a device footprint than a design by Han (Han, Tang, & Zheng, 2007). A detailed account covering the test procedures and results of pumping performance by the final design is given in Sections 7.1 and 7.2.

Figure 15: Final design of the “starburst” absorption pumping configuration. The total pumping surface area consists of $(53) \times 17.8\text{mm}^2$ individual “fingers” connected to the 5mm diameter cuvette by $15\mu\text{m}$ wide CRV’s (a.). Image in (b.) is the cuvette completely filled with only one “finger” filled. In this manner the 945mm^2 of pumping surface remains liquid free until the cuvette fills and the CRV’s burst as shown in (c.).

Table 3: Overview of absorption driven pumping surface areas and surface area to cuvette volume ratios for each design.

<u>Design</u>	<u>Pumping Surface Area (mm^2)</u>	<u>Surface Area / Volume (mm^{-1})</u>
Star (a.)	136	6.4
Star (b.)	289	14
Star (c.)	271	13
Final Design	945	44
Cuvette Only	65.2	3.1

5.3 Ratio mixer

To add the correct amount of reagent to sample a ratio mixer was designed which uses variations in pipe flow resistance to alter the flow rate ratios of the two fluids. In this manner a desired fixed volumetric ratio of reagent to sample could be realized. Using fluid resistance to vary flow rate ratios is a effective method to passively control fluid flow (Lam & et.al, 2006) (Stiles & et.al, 2005); however using this principle for a “T” junction mixer is unique. Generally, microfluidic designers use external variable flow peristaltic pumps (Stone, Stroock, & Ajdari, 2004) to drive fluids into the device at the desired flow rate ratio. This device is unique in that a vacuum pressure is developed downstream from the ratio mixer, therefore requiring the flow rate ratio to be established between the fluid inlet and the fluid junction.

There are two performance requirements for the ratio mixer; mix the molybdovanadate reagent (Cat. 20760-32, Hach Corporation, Loveland CO) and water sample to a one to twenty volumetric ratio, respectively, and direct the stream of reagent into the sample such that rapid diffusive mixing can occur. The target mixing ratio was determined based on the chemical manufacturer recommendation. The first ratio mixer design incorporated a basic two channel “T” junction connected to the diffusion mixer in Figure 16. The difference in dimensions between the reagent and sample channels alters the fluidic resistance as per Hagen-Poiseuille pipe flow (Equation 2.9). When the junction channels are collectively modeled as a resistive circuit network (Equations 2.14-2.16), the resistance in the reagent channel is twenty times that of the sample. An important assumption made in the pipe flow equations is that the reagent and sample viscosities are the same. This assumption was made since the reagent contains 60% water

and 30% sulfuric acid. No published viscosity data was available for the reagent so it was assumed to have to viscosity of water. The fluid resistance ratio for the reagent and sample channel(s) is the first ratio mixer design is calculated below:

$$\frac{R_{reagent}}{R_{sample}} = \frac{30 \cdot (.001Pas) \cdot (9.5mm)}{\left(\frac{2 \cdot 75\mu m \cdot 75\mu m}{75\mu m + 75\mu m}\right)^2 \cdot 75\mu m \cdot 75\mu m} \cdot \frac{\left(\frac{2 \cdot 50\mu m \cdot 1.25mm}{50\mu m + 1.25mm}\right)^2 \cdot 50\mu m \cdot 1.25mm}{30 \cdot (.001Pas) \cdot (20.5mm)} = 20$$

Figure 16: Dimensions of the first “T” ratio mixer. Fluids were drawn into the ratio mixer by an externally connected pumping reservoir. Sample freely entered into the diffusion mixer, but reagent could not due to the high capillary pressure which resisted the forward motion of the reagent fluid column.

During initial tests using an absorption pumping reservoir attached to the diffusion mixer, the reagent did not enter the ratio mixer on account of the large difference in capillary pressure (Butt & Graf, 2006) caused by the reagent interface contained within a small hydraulic diameter. The difference between capillary pressures which resisted the forward motion of the reagent and sample fluid streams would have to be minimized by balancing the dimensions of the channels.

In the second ratio mixer design the simple “T” junction was replaced by six sample inlets and one reagent inlet to form a “pinwheel” arrangement. All channels feeding into a central circular channel junction connected to the diffusion mixer (Figure 17). By dividing the sample inlet into five separate channels the difference between the sample and reagent capillary pressures in the channels is 10%, and assumes both the reagent and sample have similar contact angles and surface tensions. The six sample inlets are modeled in the pipe flow equation as a parallel network which results in a fluid resistance ratio of:

and is close to the target flow ratio.

This ratio mixer design was incorporated into the first full microfluidic chip. To test the performance of the ratio mixer the device was degassed to charge the pumping reservoir, quickly removed from the vacuum chamber, placed under a microscope, and droplets of water based dye placed over the inlets. The progression of the fluids entering the ratio mixer and subsequently into the diffusion mixer was monitored. The “pinwheel” design was capable of drawing both the sample and reagent streams into the diffusion mixer.

Figure 17: On the left are the dimensions for the “pinwheel” design which shows six 100µm wide sample channels and one 75µm wide reagent channel connected to a 500µm diameter intersection chamber. On the right is an image of the ratio mixer injecting a yellow dye which is simulating reagent into a green dye which is simulating the sample.

However, the reagent stream was delayed in entering the ratio mixer which resulted in several micro liters of sample only liquid entering the diffusion mixer. Having both reagent and sample streams quick reaching a flow rate equilibrium is important so that the initial aliquot of sample and reagent are mixed to the correct ratio. The “pinwheel” design had two additional shortcomings; it occupies a large area on the chip, and does not direct the reagent stream down the middle of the diffusion mixer channel width. Having the reagent stream inject into the channel middle reduces the diffusion distance of the reagent and sample.

A third ratio mixer was designed to encompass the balanced capillary pressure attribute of the “pinwheel” design and the compactness of the “T” mixer design. An “interdigitated” ratio mixer was designed so that the reagent stream would be focused into

Figure 18: On the left are the dimensions of the “interdigitated” ratio mixer. An exploded view of the sample and reagent channel intersection is shown. Pictured at right the ratio mixer being used to inject a stream of black dye to simulate the reagent which is approximately 10µm wide and surrounded by clear streams of water on either side.

the center of the diffusion mixer channel. Designs similar to the “interdigitated” arrangement have been used (Zhang, Zhao, & Xiao, 2008) (Knight & et.al, 1998)

however; these mixers were designed principally for rapid mixing by constraining the width of a fluid stream using liquid sheathing. The “interdigitated” ratio mixer consists of twenty parallel sample streams, ten to each side, and one reagent stream directed at the middle of a converging junction, Figure 18. In this manner the fluidic resistance provided by each of the twenty sample channels is equivalent to the reagent channel. When the sample channels are modeled as a parallel resistive circuit the reagent channel has twenty times more fluid resistance. Preliminary tests of this ratio mixer design were conducted in the same manner as the “pinwheel” design. The initial results showed the sample streams could converge nearly simultaneously, but that the reagent stream was delayed in entering the mixer junction by approximately one minute. This delay is attributed to slight differences in the length of the channels, $9687\mu\text{m}$ for the reagent versus $9620\mu\text{m}$ for the sample channel and small air bubbles being trapped in the reagent. Fortunately the microfluidic chip uses absorption pumping so that the depleted PDMS elastomer quickly removes any residual air bubbles, effectively removing the interfacial capillary pressure, which allow flow to be established in the channel. Figure 19 shows an image sequence of the “interdigitated” ratio mixer reaching equilibrium flow rate between reagent and sample in approximately one minute. To account for the initial one minute aliquot of sample a set of diversion chambers were added downstream from the ratio mixer (see section 5.5). The “interdigitated” ratio mixer design did result in the reagent stream being directed down the center of the diffusion mixer (see Figure 19) while occupying a smaller footprint than the “pinwheel” mixer. For these reasons the “interdigitated” mixer design was selected to be incorporated into the final microfluidic chip prototype. Verification of the flow rate ratios between the sample and reagent is discussed in Section 7.4 of this paper.

Figure 19: Image sequence of the “interdigitated” ratio mixer being used to draw blue dye (sample) and yellow dye (reagent) into the diffusion mixer. In (a.) the sample has just entered into the sample channels. Three seconds later in (b.) the individual sample channels are approaching the mixer junction at nearly equal velocity. In (c.) the sample channels have combined at the junction six seconds after entering the sample channels. Note the yellow dye has not reached the junction yet. One minute later (d.) the blue and yellow dyes have reached equilibrium.

5.4 Diffusion mixer

The diffusion mixer is located downstream from the ratio mixer to mix the sample and reagent prior to the fluid mixture entering the cuvette. As previously discussed earlier, flow through most microfluidic devices is highly laminar so mixing is principally by diffusion. To accomplish complete mixing by diffusion, the fluids to be mixed need long residence times together and a short diffusion distance. Three diffusion mixer design parameters can be adjusted to achieve this result; fluid velocity through the mixer, mixer length, and width. The fluid velocity and mixer length effect the fluid residence time while the mixer width influences the diffusion distance. For the microfluidic colorimeter two diffusion mixer designs were used. The first design was built and tested to make preliminary measurements of the fluid flow through the diffusion mixer, and to determine where along the diffusion mixer width the reagent stream would be injected by the ratio mixer.

To determine the length and width of the first diffusion mixer design a series of analytical equations were used. First, the pressure drop across the length of the mixer was estimated by determining the maximum capillary pressure required to advance a fluid interface through the channel (Equations 2.17 and 2.18). The channel width and height were arbitrarily chosen to be 250 μ m by 50 μ m, respectively. For these dimensions the required pressure for driving a curved interface of water through the mixer is 1183 Pa, assuming a PDMS to fluid contact angle of 110°. The residence time required for the sample and reagent to mix was estimated by Equations 2.2 and 2.3. Assuming the diffusion distance equals the mixer width and using the more conservative diffusion coefficient for sulfuric acid (reagent contains 10% sulfuric acid by volume) the required residence time is 36 seconds. The final diffusion mixer length is calculated by integrating the fluid velocity (Equation 2.13) with respect to time and solving for the mixer length so that:

$$L_{Diff} = \left[\frac{2 \cdot (D_h)^2 \Delta P \cdot t}{S \cdot \mu} \right]^{1/2} \quad (5.3)$$

Where t is the residence time of fluids in the mixer, and L_{Diff} is the total length of the diffusion mixer. Based on this calculation, the minimum total diffusion mixer length needed to be 140mm however, the first mixer was made 157mm long as a conservative estimate.

Preliminary tests to determine the pressure differential and fluid velocity through the diffusion mixer incorporated into the various ratio mixer designs (see Section 5.3) were conducted to determine if the correct diffusion mixer length was used. The pressure drop and fluid velocity were independently determined by two methods. First the pressure drop between the fluid inside the device and atmosphere was measured using a pressure sensor (Freescale Semiconductor MPX5050VC6T1 -7.25 to 0 psi) connected to the device via flexible tubing (Figure 20). Based on the measured pressure differential, L_{Diff} could be recalculated. A second method for determining fluid velocity involved taking images of the advancing fluid column using a specially built camera stand then

Figure 20: On the left is the measured pressure drop inside the microfluidic device while pumping fluid through the diffusion mixer. On the right is a schematic of the experimental setup. The pressure sensor measured the pressure inside the unfilled portion of the channels while they were filling up.

measuring the change in fluid column length with time on the digital images using Image J software (rsbweb.nih.gov/ij/). For both methods the microfluidic device was degassed to -96kPa gauge for greater than 1.5 hours to “prime” the PDMS. The device was then

removed from the vacuum chamber for 2 minutes prior to starting each experiment which was initiated by placing droplets of dye into the sample and reagent inlets.

Based on the initial tests of fluid dye running through the “interdigitated” ratio and diffusion mixers it was evident that the reagent stream injected through the middle of the diffusion mixer (Section 5.3). This reduced the effective diffusion distance from 250 μ m to 125 μ m which should decrease the minimum diffusion mixer length. However, the results from the pressure sensor tests indicated that a higher than anticipated pressure differential inside the diffusion mixer resulted (Figure 20). The average measured pressure was 7.85kPa gauge in the first five minutes compared to the estimated 1.183kPa pressure initially used to design the mixer. Using the higher pressure differential and shorter diffusion distance in equation 5.3 the new minimum diffusion mixer length should be approximately 210mm.

The velocity of the advancing fluid column was measured to verify the new minimum diffusion mixer length based off pressure sensor readings. Images of the fluid and air interface were taken at one second intervals with a Canon 20D digital camera controlled by Canon TC-80N digital timer. Both the camera and 18-55mm Canon lens were mounted to a fix base. Camera settings were: speed =250, aperture = 5.0 and ISO =100.

Figure 21: Pictured on the left is the fluid column advancing through the diffusion mixer. There is a 12 second time gap between each image from (a.) to (d.). The diagram on the right shows the digital camera mounted over the microfluidic device. To run the experiment droplets of dye were placed on the device inlets 2 minutes after pulling it from the vacuum chamber. The device was positioned under the camera system, the lens focused, and then the image sequence started.

The initial velocity tests suggested the fluid velocity inside the diffusion mixer was slower than predicted by the pipe flow equations which used the pressure drop measured in the previous experiment. The measured fluid column velocity was between 4.5 to 9mm/sec for the 30 second duration it took the fluid to air interface to transit through the diffusion mixer. This velocity range is almost half the 11.5mm/sec that the pipe flow equations predict based on the 7.85kPa measured pressure drop. The reason for such a disparity is the presence of the small radii of curvature fluid interface during the velocity tests by image analysis which creates a large capillary pressure opposing the advancing fluid column. In addition, the pressure differential inside the device is not steady, therefore the modeling the velocity of the fluid inside the channels using pressure alone is not trivial. Based on the measured fluid velocities and the new diffusion distance of 125 μ m, the minimum diffusion mixer length should be 81mm to 163mm using equation 5.3.

A range of Peclet and Reynolds numbers can be calculated using the fluid velocity data found in the previous experiments. The Peclet number range is from 650 to 1300 which is not indicative of diffusion controlled mixing, however, the Reynolds number is from .037 to .074 which suggests flow is highly laminar and that advection mixing will be minimal. The high Peclet number is likely from using the axial fluid velocity which is high, instead of the much lower perpendicular fluid velocity which is in the direction of the diffusion gradient. Based off the previous experiments a second diffusion mixer design was made that is 250 μ m wide x 60 μ m deep x 258mm long. This design represents the most conservative fluid velocity estimates to insure complete mixing occurs prior to the fluids reaching the cuvette. A complete performance analysis of the final design is given in section 7.3.

5.5 Capillary Retention Valves

A capillary retention valve (CRV) is a microfluidic channel section that is narrower than the surrounding channel dimensions so that a combination of Laplace pressure and the three phase liquid interface forces act to stop the flow of liquid. The primary benefits for using this type of valve are that it is passively controlled, and simple to construct. Some common applications of CRV's are temporary valves which stop fluid from flowing until a minimum pressure difference driving the fluid inside the column is reached and selective valves which permit gases to pass through, but stop liquids from entering a channel. One drawback for this valve is that it only works when a liquid, air, and solid interfacial region exists, once the valve "bursts" it no longer can stop the flow of liquid.

A variety of theories have been proposed and tested to fully understand the mechanisms which determine how CRV's work (Cho & et.al, 2007) (Huang, Huang, & Lee, 2008). Two mechanisms dominate how much pressure is required for the liquid column to pass through the CRV. The first is the increase Laplace pressure (section 2.6) acting against the advancing fluid column caused by sudden decrease in hydraulic diameter as the channel cross section is reduced. The second mechanism is less understood however; Cho (Cho & et.al, 2007) proposes that the transition of the liquid to PDMS channel wall contact angle from the inside CRV channel wall to the outer channel wall requires an even greater pressure. Both mechanisms are shown and described in Figure 22.

Figure 22: CRV burst sequence. In (1.) the liquid column encounters the smaller hydraulic diameter and the higher Laplace pressure slows or stops the advancing column. From (2.) to (3.) the column has moved through the smaller hydraulic diameter portion of the valve and the liquid interface becomes “pinned” at the channel corners. To advance the column from (3.) to (4.) the liquid contact angle must transition 90° to wrap around the channel wall. This process is stopped short by the channel walls as seen in (5.) which results in a new three phase interface further from the CRV. Soon afterward the liquid column can freely flow through the CRV (6.).

For the microfluidic colorimeter chip CRV's were used to connect the cuvette and absorption pumping surfaces and to divert the initial aliquot of liquid mixture from the diffusion mixer into two dump chambers. In the final microfluidic prototype, sixty 10µm x 60µm CRV's connected the cuvette to the absorption pumping surfaces (Figure 15).

These valves were successful in passing only air through to the pumping surfaces before blocking the liquid filling the cuvette. Once the cuvette filled the CRV's burst nearly simultaneously. The dump chamber arrangement was built in response to problems associated with the reagent and sample streams entering the ratio mixer at the same time (Section 5.3). During the initial filling of the microfluidic chip sample fluid would fill the diffusion mixer before reagent ever entered the mixer and consequently, the initial aliquot of fluid into the cuvette was sample rich. Dump chambers with a CRV located downstream were fabricated to divert the first approximately 3 μ L of sample rich liquid so that it would not enter the cuvette and affect the light absorption measurement. Figure 23 depicts the process of the dump chamber and CRV working to divert the initial sample from the cuvette.

Figure 23: Dump chamber filling sequence. (1.) When the microfluidic chip begins pumping only sample (blue) fills the diffusion mixer. (2.) The CRV stops the initial aliquot of sample and diverts it into the dump chambers. (3.) As the vacuum pressure in the device increases the CRV bursts which allows liquid to flow into the cuvette. The reagent (yellow) has now reached the diffusion mixer so that both liquids now fill the diffusion mixer at the correct ratio (green) and flow directly into the cuvette.

In operation, the dump chamber and CRV arrangement did not perform as expected. In all the prototypes the CRV burst prematurely so that the dump chambers never filled and the initial aliquot of sample rich liquid went directly into the cuvette. Closer examination of the CRV bursting revealed why it burst prematurely. An image sequence (Figure 24) of dye moving through the retention valve was taken at thirty frames per second through 10X magnification. Using vacuum pressure measurements as a function of time (Section 5.4), the pressure differential across the fluid interface during this image sequence was estimated at -4kPag. For the CRV to function correctly, it must be able to resist this pressure for a long enough duration to allow the dump chambers to fill. Since the fluid moves through the CRV in slightly more than half a second it clearly cannot withstand the -4kPa vacuum.

Figure 24: 30 FPS image sequence of dye passing through a 40µm dump chamber CRV. As the fluid column first penetrates the narrow channel capillary pressure acts to slow the fluid interface velocity. As the interface transitions back into the wider channel section the curved interface grows until contacting the channel wall.

To calculate the theoretical back pressure a CRV can withstand before bursting both the Laplace pressure and the contact angle transition must be accounted for. Using

the 40 μ m wide by 60 μ m tall dump chamber CRV as an example, the theoretical pressure the valve can withstand due to Laplace pressure alone is:

$$P_{vac} = -2 \cdot .072 \text{ N/m}^2 \cos 110^\circ \left(\frac{1}{60\mu\text{m}} + \frac{1}{40\mu\text{m}} \right) = 2052 \text{ Pa}$$

Where .072N/m² is the surface tension of water, P_{vac} is the gauge vacuum pressure generated across the liquid interface, and 110° is the contact angle for water on PDMS. Huang (Huang, Huang, & Lee, 2008) and others realized the Laplace pressure inside the narrow section of the CRV alone does not reflect the ability of the CRV to withstand bursting. Since the CRV is connected to a 250 μ m wide channel (which is also 60 μ m tall) the curved interface will prematurely contact the walls of the outer channel before bursting as shown in Figure 23 and Figure 24. This effect reduces the back pressure to:

$$P_{vac} = -2 \cdot .072 \text{ N/m}^2 \cos 110^\circ \left[\left(\frac{1}{60\mu\text{m}} + \frac{1}{40\mu\text{m}} \right) - \left(\frac{1}{60\mu\text{m}} + \frac{1}{250\mu\text{m}} \right) \right] = 1034 \text{ Pa}$$

Clearly, both these pressures are lower than the 4kPa vacuum pressure that drew the liquid through the CRV in Figure 24 and accounts for the rapid bursting of the valve. To account for the contact angle transition from the inner CRV wall to the outside channel, Cho (Cho & et.al, 2007) proposes that the contact angle effectively increased by the angle which these two walls intersect. In this prototype the inner CRV wall and the outside channel are perpendicular to each other so the effective contact angle is 110°+90°=200° however; contact angles over 180° are not possible so the effective contact angle is 200°-180°=20°. Using this result the new pressure is:

$$P_{vac} = -2 \cdot .072 \text{ N/m}^2 \cos 20^\circ \left[\left(\frac{1}{60\mu\text{m}} + \frac{1}{40\mu\text{m}} \right) - \left(\frac{1}{60\mu\text{m}} + \frac{1}{250\mu\text{m}} \right) \right] = 2841 \text{ Pa}$$

This higher pressure seems to agree with observations of liquid dye moving through CRV since the valve did momentarily slow the advancing fluid column and begin filling the dump chamber inlets. Unfortunately, this higher pressure still is unable to withstand the 4kPa vacuum pressure generated by the microfluidic chip in operation. For this reason, none of the dump chamber and CRV combinations proved effective. In future designs, the CRV could be made effective by increasing the dimensions of the channel on the downstream portion of the CRV and/or reducing the CRV width. For example if the CRV outlet channel were made 2.5mm wide and 100 μ m tall, it could withstand a vacuum pressure of 4258Pa which is enough to partially fill the dump chambers before the CRV bursts. During the characterization study (Chapter 8) the microfluidic colorimeter generated satisfactory phosphate analysis tests without the dump chambers working properly so this design element was not corrected. However, for a more comprehensive characterization study which employs lower concentration phosphate analysis the dump chamber and CRV design will likely need to be used. Therefore this design element should be corrected in future prototypes.

5.6 Selection of chemical reagents, cuvette pathlength, and light source for reactive phosphate detection

A number of colorimetric testing methods have been developed for determining phosphorus concentrations in aqueous environments (Eaton, Clesceri, & Greenberg, 1995) (Boltz, Lueck, & Jakubiec, 1978). These methods are divided into three categories and each one is used to measure different forms of phosphorus. The three prominent

forms of phosphorus are; condensed phosphates, organic phosphates, and orthophosphates (Section 1.2). The concentration of any of these forms of phosphorus can be used to calculate the total phosphorus in water. Orthophosphate can be measured by direct colorimetric analysis using the molybdovanadophosphoric acid, stannous chloride, or ascorbic acid methods. To measure condensed phosphates, also called the total acid hydrolyzable phosphorus, an acid hydrolysis step is first performed to convert the condensed phosphates to orthophosphates. Following acid hydrolysis, the sample is measured by either direct colorimetric method. Similarly, organic phosphates are measured using a preliminary acid digestion step to oxidize organic matter to release phosphorus as orthophosphate. The resulting orthophosphate can be measured using either one of the direct colorimetric methods.

To eliminate the complexities of sample preparation by acid hydrolysis or acid digestion, the direct colorimetric reaction method was chosen. Of the three direct colorimetric methods, commercially prepared reagents for the molybdovanadophosphoric acid and ascorbic acid methods were readily available. Both of the reagent types contained all the chemicals required to perform the colorimetric analysis and buffering agents to keep the sample within the pH limits for each test. In the molybdovanadophosphoric acid method ammonium molybdate reacts with orthophosphate in an acid medium to form a mixed phosphate and molybdate complex. In the presence of vanadium a yellow molybdovanadophosphoric acid is formed which exhibits peak light absorption below 470nm (Eaton, Clesceri, & Greenberg, 1995). In the ascorbic acid method orthophosphate and molybdate ions combine in an acid solution to form a

phosphomolybdate complex which is subsequently reduced to form a heteropoly blue complex (Eaton, Clesceri, & Greenberg, 1995) (Boltz, Lueck, & Jakubiec, 1978).

To determine which two methods to use for the microfluidic colorimeter, a series of light absorption tests were conducted to determine the sensitivity of each method and the wavelengths where maximum absorbance occurs. An Ocean Optics DH-2000-BAL light source with 500 μ m fiber optic cable was connected to a 1cm square cuvette holder with collimating lens which in turn was connected to an Avantes Avaspec 2048 spectrometer through another 500 μ m fiber optic cable. Plastic cuvettes with a 1cm pathlength which were rated to transmit between 220-900nm were used for both methods. The deuterium and halogen combination light source has a wide range of light output that covers both the UV, visible, and near IR wavebands while the spectrometer has an operating range between 200 to 1000nm. Using this equipment, wide absorbance spectrums could be produced to determine which wavelengths would be optimal for use in the final microfluidic colorimeter. Phosphate solutions of 0.25, 0.5, 0.75, 1, 5, 10, 25 mg/L concentrations were prepared by diluting 100mg/L standard solution (HACH Corporation catalog no. 14242-32) and de-ionized water (Barnstead B-pure). Each phosphate solution was mixed with the appropriate amount of reagent and a light absorption measurement taken using a 2ms integration time and 20 scan average spectrometer setting. Figure 25 shows two representative absorbance spectrums from both methods using a representative 5mg/L orthophosphate solution.

Figure 25: Absorbance spectrums from the molybdovanadophosphoric and ascorbic acid colorimetric analysis for orthophosphate methods. Note: the ascorbic acid spectrum is based on a 1mg/L spectrum which has been normalized to the molybdovanadophosphoric spectrum for comparison purposes. Both methods have peak absorbance responses at different ends of the UV-VIS-IR light spectrum.

According to the reagent manufacturer the molybdovanadophosphoric acid method is capable of detecting 0.3 to 45mg/L orthophosphate, therefore 1, 5, 10, and 25mg/L standard phosphate solutions were for light absorbance measurements. The ascorbic acid method is more sensitive and is rated from 0 to 2.5mg/L orthophosphate, therefore 0.25, 0.5, 0.75, and 1mg/L standard phosphate solutions were used. Both methods produced spectrums whose absorbance magnitude was proportional to the orthophosphate concentration as per the Beer Lambert Law (equation 3.2).

To decide which method to use based on the absorbance spectrums the maximum absorbance signal was compared. It was important to select the method which could generate the highest absorbance signal because the microfluidic colorimeter uses a short pathlength which linearly diminishes the signal (see section 3.1). The ascorbic acid

method gave higher absorbance peaks than the molybdovanadophosphoric acid method when adjusted for orthophosphate concentration. However, due to the dramatically lower upper permissible orthophosphate range for the ascorbic acid method the maximum absorbance would be lower than for a high orthophosphate concentration molybdovanadophosphoric acid test. For example, using a 1cm pathlength cuvette in an ascorbic acid test would generate an absorbance signal of approximately 0.3 for a 2.5mg/L orthophosphate solution measured at 890nm. Contrasting to that, using a 1cm pathlength cuvette for a molybdovanadophosphoric acid test to measure a 25mg/L orthophosphate solution would generate a 0.9 absorbance signal while measured at 400nm (see Figure 26). From a design perspective the molybdovanadophosphoric test offered a conservative approach to ensuring a strong enough absorbance signal that could be obtained by simply using higher phosphate solutions to verify the microfluidic colorimeter concept. Ideally the ascorbic acid method would be used when designing an instrument capable of lower detection limits. In addition the molybdovanadophosphoric reagent comes in liquid form that can be injected into a microfluidic device without further modification. The ascorbic acid reagent come in powdered form so there were concerns as to whether or not the powdered reagent could be suspended in liquid form while maintaining its original properties. For these reasons the molybdovanadophosphoric acid method was chosen for the microfluidic colorimeter.

Figure 26: Comparison of molybdovanadophosphoric method (lines in blue) and ascorbic method (lines in red). The molybdovanadophosphoric method can be used for higher phosphate concentrations; therefore can generate higher absorbance signals despite its lower sensitivity when compared to the ascorbic acid method. To illustrate this absorbance signal at point (a.) is approximately three times higher than point (b.); however the phosphate concentration is 25 times higher at (a.). Generating high absorbance signals for the microfluidic colorimeter prototype was important to improve signal to noise ratios for this proof of concept device.

The minimum dimensions of the cuvette and the optimal corresponding light source were calculated from absorbance measurements using the molybdovanadophosphoric acid method. From a design point of view using the shortest cuvette pathlength decreases the cuvette volume which shortens the cuvette fill time for a given absorption pumping surface. Faster fill times result in shorter instrument measurement time. However, decreasing the pathlength also decreases the absorbance signal to noise ratio and thus the instrument's sensitivity. Ideally the cuvette would be long and narrow, such as in the planar μ FIA colorimeters described in Section 4.1, to reduce volume while increasing pathlength. Such a system was impractical for this microfluidic colorimeter so the cuvette dimensions were limited by the capabilities of the absorption pumping device (Section 5.2), the manufacturing process (Section 6.1),

minimum cross section to cover the light detector (Section 5.7), and the minimum absorbance signal to noise ratio.

To determine the minimum cuvette pathlength and corresponding light source wavelength, absorbance measurements using the molybdovanadophosphoric acid method were performed using a Shimadzu UV-1700 Pharmaspec UV-VIS spectrophotometer. By measuring the absorbance spectrum for various concentrations of orthophosphate the absorptivity coefficient for the molybdovanadophosphoric reaction could be measured. The light absorbance for various cuvette pathlengths could be then calculated using Beer Lambert Law. Standard solutions of 5, 10, 20, 30, 40, and 50mg/L orthophosphate were mixed with reagent and poured into 1cm cuvettes. Absorbance spectrums were measured from 400 to 500nm and are shown in Figure 27. This wavelength range was chosen based on recommended values found in literature (Eaton, Clesceri, & Greenberg, 1995) for the molybdovanadophosphoric acid method. The average absorptivity coefficient for select wavelength was calculated based on the absorbance spectrums as is listed in Table 4. Using the absorptivity coefficient, the theoretical absorbance for three possible cuvette pathlengths was calculated as shown in Table 5. The three cuvette pathlengths were selected based on fabrication limitations described in Section 6.1. Based on the capabilities of both the spectrophotometer and the deuterium / halogen light source setup, it was estimated that the minimum reliably detectable absorbance signal is .01. This value was assumed to be the minimum for the microfluidic colorimeter. Using the theoretical absorbance calculations for the molybdovanadophosphoric method for various pathlengths it was determined that the 1.59mm cuvette coupled with a 400nm light source would generate a sufficiently strong absorbance signal for the microfluidic colorimeter.

This combination is a compromise between pathlength and light source wavelength. A longer pathlength would result in too large of a cuvette volume for the absorption pumping mechanism. A light source below 400nm could have been used however; measurements taken close to the peak absorption spectrum (Figure 25) result in large signal deviations between subsequent measurements (Boltz, Lueck, & Jakubiec, 1978). In addition, light sources which approach the UV spectrum are more expensive than similar types of light sources which emit higher wavelengths.

Figure 27: Absorbance spectra generated by a UV-VIS spectrophotometer using the molybdovanadophosphoric acid method and a 1cm cuvette. The orthophosphate concentrations tested span the range of this method's capabilities. A higher absorbance magnitude for a given phosphate concentration is desired so using a lower wavelength light source for this reaction chemistry is beneficial.

Table 4: Absorbance coefficients for all phosphate concentrations at select wavelengths

Absorbance Coefficient (L/mg cm)							
Wavelength	5 (mg/L PO ₄)	10	20	30	40	50	Average ϵ
500nm	-4.4E-03	-1.2E-03	5.0E-05	2.7E-04	6.8E-04	9.0E-04	1.4E-04
440nm	2.0E-03	7.1E-03	6.7E-03	6.6E-03	6.9E-03	7.4E-03	6.9E-03
400nm	-1.5E-02	8.7E-03	1.3E-02	1.4E-02	1.6E-02	1.7E-02	1.4E-02

Table 5: Calculated absorbance values for three different cuvette pathlengths

Theoretical Absorbance for .794mm Pathlength						
Wavelength	5 (mg/L PO ₄)	10	20	30	40	50
500nm	0.000	0.000	0.000	0.000	0.000	0.001
440nm	0.003	0.005	0.011	0.016	0.022	0.027
400nm	0.005	0.011	0.022	0.033	0.044	0.054
1.59mm Pathlength						
500nm	0.000	0.000	0.000	0.001	0.001	0.001
440nm	0.005	0.011	0.022	0.033	0.044	0.055
400nm	0.011	0.022	0.044	0.065	0.087	0.109
3.18mm Pathlength						
500nm	0.000	0.000	0.001	0.001	0.002	0.002
440nm	0.011	0.022	0.044	0.066	0.088	0.110
400nm	0.022	0.044	0.087	0.131	0.174	0.218

5.7 Light source, light detector and signal processing package

A light source and detector pair was selected for the microfluidic colorimeter based on cost, sensitivity, and ease of implementation into a prototype system. The light wavelength range required for the instrument needed to be centered around 400nm and of sufficient intensity at that wavelength range to transmit through the PDMS cuvette walls, a glass slide, and approximately 1.59mm of reagent and sample mixture (Figure 9). A detector capable of converting the transmitted light intensity into an amperage signal with a high signal to noise ratio was selected based on the light source type. To convert the detector's amperage into voltage, then amplify the voltage and finally analyze the voltage a custom signal processing package was developed.

The available light source options for the prototype included deuterium, tungsten, halogen, and LED sources. The deuterium source has good intensity below approximately 500 nm while the tungsten and halogen sources have higher intensities above approximately 500nm. As opposed to the other light sources, LEDs have “tuned” peak light intensities to generate high spectral intensity distributions centered on specific wavelength ranges, including near the 400nm range. An InGaN LED (LED405E Thor Labs, Newton NJ) with a peak power of 6mW at 405 nm with a 15nm FWHM inside a standard T-1 ¾ package was selected as the light source. Compared to the other light sources, LEDs are low cost, compact, and have low power consumption. Another benefit of using an LED source is that bandpass filters, which allow only specific wavelength ranges of light to transmit through them, are not needed since LEDs can be made to emit only narrow peaked light spectrums. Colorimeters require narrow wavelength ranges of light output so that only specific areas of the absorbance spectrum are tested at one time.

A third benefit of using this specific 405nm output LED is that the radial light intensity distribution is concentrated directly in front of the LED, thereby creating a narrow view half angle of only 5°. Therefore, lens systems to focus the light output are not needed for this LED which further reduces cost and complexity for the instrument.

The initial light detector selected for the instrument was a silicon based photodiode (SMO5PD2A Thor Labs) with a .8mm² active area and peak spectral responsivity (see section 3.2) between 600-800nm. During preliminary tests of the microfluidic colorimeter using standard phosphate solutions this detector exhibited poor signal to noise ratio at all phosphate concentrations. The high noise from this diode was likely due to the low responsivity of the detector around the 400nm range. A second photodiode made from gallium phosphorus with a 4.8mm² active area (SMO5PD7A Thor Labs) and a high responsivity to the 405nm LED light intensity was substituted for the silicon photodiode. In this manner, the light source and detector are paired so the photodiode can detect subtle changes in the 405nm wavelength light which is transmitted through the microfluidic cuvette (Figure 28). The performance of the microfluidic colorimeter using the 405nm LED light source and the GaP photodiode is detailed in Section 8.1.

Figure 28: 405nm LED output intensity spectrum collected with a USB4000 spectrometer overlaid onto the GaP photodiode responsivity distribution provided by the photodiode manufacturer. This type of photodiode was selected to improve signal to noise ratio over the silicon photodiode due to the improved response at lower wavelengths.

A custom signal processing package was built by Alex Korzh from the Electrical Engineering Department at California Polytechnic State University for the microfluidic colorimeter. This package consists of a trans-impedance amplifier to boost the signal from the GaP photodiode, a data acquisition system (Dataq Instruments, Akron, OH), a USB connection, custom software written in Visual Basic 6.0, and various miscellaneous electrical hardware components. The data acquisition functions as the analog to digital converter so that the incoming voltage from the amplifier is converted to a digital signal which can be processed by a computer.

Figure 29: Wiring schematic for the signal processing package. All electronics components, including the LED, are powered by a 5V USB connection. In this manner the microfluidic colorimeter can be run using any USB equipped laptop, or similar device, making the system completely field portable.

The key features to this signal processing package are that the 10 bit data acquisition system is powered by, and controlled through, a USB connection so that any USB equipped laptop, or similar device capable of running Visual Basic can be used. Another feature is that the device is controlled via software which permits greater flexibility in changing the signal processing features of the device.

The main functions of the software are to first convert a voltage signal from the electronics package into a light absorbance value and then calculate the concentration of orthophosphate in the sample. A copy of the Visual Basic software program is included in appendix A. The voltage signal read by the electronics package is inversely proportional to the intensity of light transmitted through the cuvette. Low concentrations of phosphate result in high transmittance of light intensity, which corresponds to more light reaching the photodiode, and thus a higher voltage read by the data acquisition system. Transmission is inversely, and logarithmically, related to absorbance which is calculated by:

$$A = -\log_{10} \left(\frac{V_{Sample} - V_{Dark}}{V_{Blank} - V_{Dark}} \right) \quad (5.4)$$

Where V_{Sample} is the voltage measured by the instrument when a phosphate containing microfluidic chip is inside the colorimeter, V_{Dark} is the voltage while the LED is turned off, V_{Blank} is the voltage when a microfluidic chip containing de-ionized water and reagent is in the instrument. Using this equation absorbance values are measured in a three step process. First, a dark measurement is taken by turning a switch to the LED in the off position and allowing the software to read the voltage. The dark measurement records any response from the photodiode as a result of ambient light striking the active surface. The ambient light must be subtracted from each measurement so that only the light absorbance associated with the product is analyzed. In the second step a reference microfluidic chip containing only de-ionized water and reagent is inserted into the instrument and the transmission voltage read. The last step is to measure a microfluidic chip containing an unknown concentration of orthophosphate and reagent. From these three voltages the absorbance for the sample is calculated and input into a master calibration line (see section 3.1) whereupon the concentration of phosphate is calculated and displayed by the software. For enhanced flexibility the software also allows the user to generate a separate calibration line using two sample points generated from two additional microfluidic chips. This option would be used if the instrument had been altered in a way that may lead the user to suspect that the master calibration curve is not valid at the time. An example may be if the laptop powering the instrument has a low battery which would cause the LED intensity to be adjusted, and thereby affect the light transmissions tests.

Chapter 6 Fabrication of the Microfluidic Colorimeter

6.1 Modified soft lithography process to fabricate the fluid chip

The microfluidic colorimeter chips were made of cast PDMS from SU-8 epoxy molds built using a soft lithography process pioneered by George Whitesides (Xia & Whiteside, 1998) (Zhao, Xia, & Whitesides, 1997). It was Whitesides and his research group who initially demonstrated that an epoxy based negative photoresist could be patterned and developed on top of a silicon wafer using semiconductor manufacturing equipment to form a male mold. PDMS, a common electronics packaging elastomer, could then be poured over the mold, cured, and removed from the SU-8 mold thereby creating micron scale features inside a cheap elastomer casting. Unlike glass etching and micromilling techniques, soft lithography is cheap, rapid, and a relatively easy process for building microfluidic channels. The discovery of this process sparked the rapid growth of microfluidics technologies which continues to this day (Whitesides, 2006).

For this project the soft lithography process was chosen for a number of reasons. The pumping process relies on a PDMS substrate for air to diffuse into the material to generate a vacuum. Since the PDMS absorption pumping process has not been thoroughly researched, numerous prototypes had to be made in order to change the feature sizes and configurations for multiple devices. Soft lithography is a cheap and rapid process for making microfluidic devices so that numerous prototypes can be tested to refine the design quickly and economically. Coupled with high resolution transparency masks, a device mold can be made using soft-lithography in several days for less than \$100.00. Compared to etched glass or micro-milled plastic the turnaround time and costs for soft-lithography make it a very attractive option. Lastly, the microfluidic chip

contains small feature sizes (13 μ m minimum) with complicated arrangements which makes lithography based manufacturing processes ideally suited for this device.

There is a six step process for manufacturing the microfluidic chips using soft lithography. The same general process for all the various microfluidic devices was similar, only subtle fabrication changes were needed to either improve the mold quality or achieve a different mold thickness. A total of ten molds were made for all the prototypes. The fabrication process outlined below is for the final microfluidic chip prototype.

Step 1:

A 500 μ m thick <100> orientated silicon wafer serves as a working platform to build the SU-8 polymer mold on top of. The smooth polished surface of the silicon wafer provides a mold base which produces uniformly flat bonding surfaces between the top and bottom PDMS castings. The silicon wafer is cleaned first with piranha acid etchant (mixture of hydrogen peroxide and sulfuric acid) for approximately 10 minutes at 70°C then stripped of any residual oxide by immersion in buffered oxide etch (mixture of hydrofluoric acid and buffering agents) for 5 minutes at room temperature. Removing the native oxide helps both SU-8 adhesion to the silicon wafer and facilitates removal of the PDMS casting by decreasing the PDMS to silicon bond strength. The last silicon wafer preparation stage is to dehydrate bake the silicon wafer at 150°C for approximately 5 minutes to improve the adhesions of SU-8 to the silicon wafer.

Step 2:

SU-8 epoxy based negative resist (MicroChem Corporation, Newton, MA) is spun onto the silicon wafer to produce a uniformly thick resist layer. The 2050 SU-8 formulation was used since it has a viscosity optimized for making 50 μ m resist coatings which is close to the target resist thickness for this project. For a target resist thickness of 60 μ m the spin coat machine turned the silicon wafer at 400 RPM for 20 seconds then accelerated at a rate of 602 RPM per second to 2,500 RPM for 35 seconds. A combination of spin speed RPM versus target thickness graphs from both MicroChem and Cal Poly Microfabrication Lab were used to decide the final planarizing speed of 2,500 RPM. Immediately after the spin coat program was finished a chemical wipe soaked in high purity acetone was used to manually remove the SU 8 edge bead. The SU 8 coated silicon wafer was then placed on a slow turning platform for approximately 10 minutes at room temperature for stress relief within the resist and to further planarize the resist coating along the edge of the silicon wafer.

Figure 30: On the left is a cleaned blank silicon wafer (step 1). On the right is the same wafer with a 60 μ m thick SU 8 negative photoresist layer.

Step 3:

The 60 μ m thick SU 8 resist layer is partially thermally cured in a soft bake step. The wafer is heated at 55°C on a laboratory hot plate for 12 minutes then immediately moved to a separate hot plate set at 85°C for another 25 minutes. Maintaining constant hot plate temperatures is important to produce consistent film qualities, therefore each hot plate is monitored using a thermocouple and the hot plate is permitted to reach a stabilized temperature long before it is used.

Step 4:

Negative photo resist cross links and cures when exposed to UV light. Transparency masks are clear where the resist will remain and darkened by emulsion where no UV light will reach the resist so that it can be later developed away. To keep UV light from penetrating the darkened portions of the mask through sub-micron gaps between the emulsion ink droplets a high resolution printer system is required. For this project transparency masks (see appendix B) were created on AutoCAD and printed by CAD Art Services (www.outputcity.com; Bandon, OR) at a resolution of 20,000 dpi. For the photolithography process the SU 8 coated wafer was placed on top of a 4" x 4" glass surface, the transparency (emulsion side down) which was taped to a another glass square which was placed in conformal contact on top of the SU 8. The glass squares, transparency, and wafer were all taped together and placed inside an aligner equipped with a high pressure mercury arc lamp. MicroChem recommends using only 365nm wavelength or lower light sources to expose SU 8. To ensure this a 360nm optical filter (Omega Optical, Brattleboro, VT) was placed on top the transparency and wafer layers so

that only sub-360nm light would pass through to the negative photoresist. The wafer assembly was exposed for 150sec as per Micro Chem's recommended exposure dose and based on previous experience using this light source for soft lithography.

Figure 31: On the left is a depiction of the photolithography process (step 4). A 20,000 dpi transparency mask selectively exposes SU 8 to form the device pattern. Following a post exposure bake the outline of the device can be seen in the photoresist as seen on the right (step 5).

Step 5:

Following the exposure step the SU 8 coated wafer was thermally cured in a post exposure bake process. The wafer was heated to 55°C for 12 minutes then 85°C for 25min which is the same as in the soft bake step. The post exposure bake process is to further cure the SU 8, particularly in the regions where cross linking was initiated by UV exposure. During this process a faint outline of the device can be seen which indicates an adequate UV dosage was given.

Step 6:

The wafer was submerged in a organic solvent based developer (MicroChem) for approximately 10min to develop away all the negative photoresist not exposed to UV light. After development the wafer was rinsed with isopropyl alcohol to ensure full development had occurred. If there was an residual unexposed SU 8 the alcohol would create a thin white film on the SU 8 surface indicating the wafer needed more development time.

Figure 32: On the left is the final SU 8 mold following development (step 6). On the right is an image of the mold which is 60 μ m tall throughout the SU-8 portions of the device. Magnets were placed on top the SU-8 to achieve feature sizes between 1/16" to 1/8" in height.

Step 7:

Post development it was discovered that many of the molds exhibited numerous cracks throughout the SU 8 layer. To eliminate these cracks the wafer was hard baked at 150°C for 15min. Hard baking also assisted in giving the SU 8 a final cure so that the resulting mold would be hard and robust to stand up to multiple PDMS castings being

lifted off the mold. Figure 33 and Figure 34 are microscope images of the final prototype device mold before and after the hard baking step.

Step 8:

After the hard baking step the mold was examined under the microscope to take measurements of critical dimensions on the mold. For the final prototype the mold dimensions were between 3-16% larger than designed however; PDMS shrinks after curing so the dimensional increase was generally negligible. The largest increase was in the “starburst” CRV’s where the final width was 11.6 μ m instead of 10 μ m and the effect of which is described in Section 7.2. It was also interesting to note that the CRV’s were severely bent prior to hard baking, but straightened out afterwards. This is likely due to the high residual stresses imposed in the SU-8 layer during curing which contracts the epoxy based photoresist, thus “pulling” the CRV’s straight and simultaneously closing in the numerous cracks. MicroChem and others (Lorenz & et.al, 1997) suggest that cracking is associated with underexposed SU 8. The UV exposure dose was not increased despite the initial cracks because it would lead to a further increase in the mold dimensions.

The SU 8 thickness was verified using an Ambios XP 1 profilometer (Ambios Technology, Santa Cruz, CA) and found to be approximately 59-65 μ m with good uniformity for the final prototype. Measuring the channel width and thicknesses verified that the hydrodynamic equations used to predict fluid flow were based on accurate dimensions.

Figure 33: To the left are images of the SU 8 mold prior to hard baking. On the right is the same feature post hard bake. Images (a.) and (b.) show the intricate sample channels which are 60μm wide separated by 25μm wide channel walls. The smallest device features are shown in (c.) and (d.) where approximately 10μm wide CRV's connect the absorption pumping surface and the cuvette.

Figure 34: Images (a.) and (b.) show any early prototype CRV before and after hard baking. High residual stresses within the photoresist cause the narrow CRV to pull straight once heated to 150°C. Image (c.) shows a CRV on the final prototype after hard bake. The crisp features and accurate dimensions indicate the correct exposure dosage was used.

Step 9:

In order to fabricate meso-scale features within the microfluidic device an alternative approach to soft lithography was required. For a sufficient light absorbance signal the required optical pathlength would have to be approximately 1.5mm or more (see section 5.6). Since the orientation of the light source and detector is perpendicular to the microfluidic channels and cuvette this would require that a mold greater than 1mm in depth be made. Researchers have demonstrated that thick SU 8 features with high aspect ratios can be fabricated using soft lithography (Conradie & Moore, 2002). This process would necessitate a complex fabrication process involving multiple SU 8 layers spun coat on top each other with two or three masks to produce the various SU 8 thicknesses.

The meso-scale cuvette height was produced by placing a NdFeB magnet (K & J Magnetics, Inc., Jamison, PA) directly on top the SU 8. These magnets are manufactured to .002" tolerance and come in 1/32" thickness graduations. For the final prototype a disc shaped 1/16" tall by 3/16" diameter NdFeB magnet was placed on top a 5mm diameter SU 8 feature which was continuously connected to the absorption pumping surfaces and the diffusion mixer outlet. In this manner a cuvette mold with an effective optical pathlength of 1.65mm (1.58mm magnet plus 60 μ m SU 8) was built without any complex softlithography processes. However; the magnet required careful manual positioning with tweezers so that the 4.76mm diameter magnet would align over the 5mm diameter SU 8 cuvette feature. Each magnet position was verified under an optical microscope. Additional disc magnets were placed over reagent and sample inlets (1/16"x1/8"), the dump chambers (1/32"x1/32"), and the magnetic positioning "pins"(1/8"x1/8"). To assist in aligning the magnets and to secure the magnets in place a layer of magnets were placed

underneath the wafer so that the upper and lower magnets held each other in place by magnetic attraction as shown in Figure 35.

Figure 35: In step 9 small magnets were placed on top the SU 8 mold and below the silicon wafer. The combined magnetic attraction held them in place for later casting in PDMS. The magnets on top the SU 8 define the meso scaled dimensions of the cuvette and fluid inlet reservoirs.

Step 10:

Multiple PDMS castings were made from a single modified SU 8 mold by pouring the elastomer into a “boat” to contain the liquid with the wafer and mold at the bottom as shown in Figure 36. Dow Corning Sylgard 184 elastomer (Essex Group, Ontario, CA) was mixed at a ten parts elastomer to one part catalyst ratio, degassed for approximately 30 minutes to remove air bubbles, and then poured directly on top the mold. The aluminum foil or petri dish “boat” determined the volume of PDMS required to produce the casting. The absorption pumping mechanism is influenced by the depth of the PDMS casting, therefore it was important to make each casting to as near uniform thickness as possible. For the final prototype the target thickness was 3mm. To facilitate

this the same “boat” was used for each casting so that a known volume of PDMS could be poured onto the device each time. For a planarized thickness the entire boat and aligning magnets assembly was placed on a slow turning platform. This rotating platform was in turn placed inside a furnace and the PDMS was thermally cured for approximately two to three hours at 70°C and left to sit overnight at room temperature.

Figure 36: Schematic of the “boat” used to contain the PDMS for casting. The depth of the PDMS casting was important since it influenced the absorption driven pumping mechanism. Therefore care was taken to ensure that the same volume of PDMS and identical “boats” were used to fabricate each device.

Step 11:

Once cured the PDMS casting was removed from the SU 8 and magnet mold. The final device was assembled by first cutting the PDMS casting down to approximately 2”x3”. Magnets (1/8”x1/8”) were placed into impressions left by the mold into four corners to act as magnetic positioning “pins” which are further described in Section 6.2. The casting was then placed on top a 2”x 3” glass slide with the channel features facing up. A separate 3mm thick cured slab of PDMS with no features was cut down to 2”x 3”. Three holes for the reagent and sample fluid inlets were then cored out of the slab using a sharpened section of 1/8” diameter stainless steel tubing. The slab was then aligned over

the PDMS casting to match up the channel inlets before conformally bonding the pieces together (see Figure 37).

Figure 37: A schematic and image of the final microfluidic chip. The 2"x3" glass slide, PDMS casting, and slab are all conformally bonded for later disassembly to clean the device between experiments. The final cuvette depth is 1.65mm and while the fluid channels average 62 μ m deep.

Approximately forty PDMS devices were fabricated for the various device prototypes and test structures. A total of eight final prototype PDMS devices were fabricated for final water phosphate concentration testing. The average PDMS thickness for each final prototype device ranged from 6.4 to 6.7mm with good uniformity across the device. Since the devices were assembled by conformal contact they could be disassembled after each test for cleaning and reassembly.

6.2 Construction of the light source and detector assembly

An aluminum prototype to hold the LED light source, photodiode, and microfluidic chip was fabricated on a milling machine. The functional requirements for this assembly were to align the LED, cuvette, and photodiode along an axis perpendicular to the face of the microfluidic chip. All optical components were purchased from Thor Labs and were designed to easily screw together to rapidly change the prototype

configuration as necessary (see Figure 38 and Figure 39). The three aluminum plates were individually milled out. The hole configurations for the magnetic “pins” and the lens tubes were accurate to within .001” as per the CNC mill software. For final assembly the aluminum plates were bolted together using 1/8” diameter bolts. Magnets were inserted into 1/8” diameter holes on the base plate to serve as the stationary magnetic “pins”.

The self aligning magnetic “pin” arrangement was devised to solve the problem of reliably aligning the cuvette between the photodiode and LED. This mechanism works by the attractive magnetic force of the magnets embedded into the chip and the magnets mounted in the base plate pulling the chip into the LED and photodiode assembly until the cuvette is aligned. Due to fabrication limitations it was not possible to cut the PDMS casting accurately along the device perimeter. For this reason the dimensions of the microfluidic chip change slightly between castings and therefore cannot be used to align the device. The magnetic aligning mechanism instead relies on the photolithography process to produce highly accurate dimensions between relative features. In other words the dimensions of the magnetic “pins” on the chip relative to the cuvette are accurate. Since the stationary magnetic “pins” on the aluminum plate could be fabricated within .001” relative to each other, theoretically the microfluidic chip could align itself over the photodiode with .001” accuracy. In use it was found that the magnetic “pin” arrangement was very effective in aligning the cuvette over the LED and photodiode axis. There was initial concern that the cuvette would only partially cover the photodiode’s active surface however; the large diameter cuvette coupled with the self aligning magnetic “pins” reliably placed it over the photodiode each time the chip was inserted.

Figure 38: Solidworks schematic of the light source and detector assembly. Stationary magnetic “pins” on the assembly and magnets embedded in the microfluidic chip provided a reliable alignment mechanism to position the cuvette over the photodiode. All optical components were modular and threaded for easy assembly and reconfiguration as needed.

Figure 39: Image of the final colorimeter prototype assembly.

Chapter 7 Testing Procedures, Results, and Discussion for the Microfluidic Chip

7.1 Device flow rate calculated by image analysis

The flow rate of the reagent and sample filling the microfluidic chip was quantified in order to characterize the pumping capabilities of the integrated absorption driven pump. Characterizing the flow rate within the device is important so that the residence time inside the diffusion mixer can be estimated and the total time require to perform each water test is known. Furthermore, the flow rate results achieved by this microfluidic chip could be used to design future absorption driven PDMS devices.

The flow rate was calculated by image analysis using the experimental setup described in Section 5.4. The vacuum pressure generated inside the device was simultaneously measured using the pressure sensor setup also described in section 5.4. Knowing the vacuum pressure in the device and the flow rate assisted in back calculating the hydrodynamic equations for calculated flow rate versus measured flow rate. For this set of tests the pressure sensor output was recorded every second by a NI-USB 6225 data acquisition unit (National Instruments, Austin, TX) for later analysis. A custom PDMS device was built for these tests using the final prototype casting and partially plasma treated PDMS slab. Plasma treated PDMS results in a permanent bond which can withstand the pressure caused by the pressure sensor tubing. Approximately a 5mm diameter circle of PDMS surrounding the pressure sensor inlet was plasma treated so that the pressure sensor was securing held in place by the substrate. This ensured an air tight seal for accurate vacuum pressure measurements. The remainder of the PDMS device

was conformally bonded so that it could be partially disassembled for cleaning between experiments.

The flow rate analysis experimental procedures were common for all replications. Six experiments were conducted; two replicated tests for each of three different degas pressures. In this manner the flow rates achieved by variations in degas pressure could be analyzed. Prior to each test the PDMS microfluidic chip was degassed for greater than 1.5 hours inside a vacuum chamber at either -47, -71, or -94kPag to “prime” the device. Each test was initiated by starting the data acquisition unit to begin recording pressure as a function of time, but without the pressure sensor connected to the device. After one minute the vacuum chamber was opened which exposed the device to atmosphere and thus started the process of air diffusing into the PDMS. Once out of the vacuum chamber the device was placed under the digital camera, the tygon pressure sensor tube plugged into the device, and the camera lens adjusted to bring the device into focus. This process took approximately one minute, but to ensure that the time between exposing the device to atmospheric pressure and initiating the flow rate experiment was kept constant, the this time period was kept a constant two minutes for all experiments. After two minutes had passed the pumping sequence was initiated by placing droplets of dye into the sample and reagent inlet reservoirs. Blue dye was used to simulate water sample and yellow dye to simulate reagent. After one more minute the camera began taking images once every two, three, or five seconds depending on the degas pressure. It took between 6.5 to 13.3 min to fill the microfluidic cuvette which resulted in between 160 to 200 high resolution digital images that would have to be analyzed. The elapsed experiment time starts when the last fluid channel was blocked by a droplet of dye. Due to the complexity of the test

arrangement, and the work required to analyze the images only two runs per each degas pressure was conducted.

To calculate the flow rate two methods involving image analysis were used. In the first approximately 50 seconds of the microfluidic chip filling up, the fluid column advanced through the diffusion mixer. The flow rate is calculated in the same manner as described in section 5.4. Once the fluid enters into the cuvette the flow rate is calculated by measuring the change in area the fluid occupies between each image. Sixty images were loaded at a time into Image J and converted to eight bit grayscale images. Each image sequence was cropped and scaled using the scale bars built into the device. On average the images scaled to 96 pixels per millimeter. The sequence was adjusted for correct threshold so that area with fluid could be distinguished from areas without fluid and then converted to binary images (see Figure 40). To perform the flow rate calculation, the fluid area for each image was calculated by performing a pixel count of the dark pixels then converting pixels to millimeters squared according to the scaling factor. This calculation yielded change in area of fluid inside the cuvette as a function of time. Flow rate in cubic millimeters (or micro liters) was obtained by multiplying the cuvette depth by the change in area. To reduce scatter between flow rate measurements using this technique only every third image was measured, or one image every six to nine seconds, resulting in a larger change in area per measurement.

Figure 40: Conversion of digital camera full color images (top) to binary images (bottom) to calculate change in fluid area inside the cuvette as a function of time. From the change in area flow rate into the cuvette can be calculated. In (a.) the fluid column has advanced into the cuvette and forms a growing curved interface (b.) until a critical radius of curvature is obtained and wetting along the cuvette walls occurs (c.).

The results of the flow rate and internal vacuum pressure as functions of elapsed experiment time for each degas pressure are shown in Figure 41, Figure 42, and Figure 43. For these figures flow rate and internal vacuum pressure have been overlaid on the same graph by inverting negative vacuum pressures and plotting them as positive values.

Analysis of the experimental data revealed several trends and phenomena of the flow rate into the device as a function of vacuum pressure and elapsed experiment time. For all six experimental runs there were distinct fluctuations in the flow rate at specific intervals of time. These fluctuations are broken down into four trends that represent time intervals where the three phase fluid, PDMS, and air interface is located within specific features of the device (see Figure 44). The trends are; filling the diffusion mixer, fluid column entering the cuvette, initial cuvette filling, and final filling of the device. It is

hypothesized that this interface, in combination with the fluid resistance inside the various channels, resulted in radical changes in the flow rate during the course of the experiment. Analysis of these trends is important to provide a set of design tools for predicting fluid flow rate for future devices.

Prior to analyzing the changes in flow rate the experimental procedure was partially verified by calculating the theoretical average flow rate and comparing it to the averaged measured flow rate. The theoretical average flow rate is equal to the total microfluidic device volume (cuvette and diffusion mixer) which is equal to 33.21 μ L divided by the total time required to fill the cuvette. The measured average is the sum of the weighted measured averages divided by the total experimental time. The results are found in Table 6. This result indicates that measuring flow rate by image analysis is a suitable technique for yielding approximate results. The broad trends identified by this analysis are logical in that flow rate increases with degas pressure. Detailed analysis of degas pressure and vacuum pressure achieved inside the device is given in section 7.2.

Table 6: Summary of flow rate data from all tests.

<u>Degas Pressure (kPa)</u>	<u>47</u>	<u>71</u>	<u>94</u>
Time to Fill Cuvette (sec)	745	396	308
Calculated Flow Rate (μ L/min)	2.7	5.0	5.1
Measured Flow Rate (μ L/min)	2.8	4.3	5.3
Percent Error	4	15	4

Figure 41: Flow rate and vacuum pressure inside the device as a function of time for a microfluidic chip degassed at -96kPag for greater than 1.5hr. It takes approximately 40sec to develop a measurable vacuum pressure from when fluid is placed at the inlet reservoirs (t=0sec). Vacuum pressure steadily increases until it begins to approach a maximum towards the experiment end. The large changes in flow rate are due to surface interfacial phenomena as the fluid column moves through the various microfluidic features. These changes are detailed in later figures.

Figure 42: Vacuum pressure and flow rate for the same microfluidic chip as in Figure 41 except the device was degassed at -71kPag. The same trend in flow rate fluctuations can be seen. Total time to fill the cuvette has increased from the -94kPag test as was anticipated.

Figure 43: Vacuum pressure and flow rate for the same microfluidic chip degassed at -47kPag. Again the same trends in pressure and flow rate that were measured for the previous tests. The premature reduction in vacuum pressure approximately half way through the experiment is likely due to a leak in the pressure sensor tubing to PDMS connection.

The four trends in flow rate as a function of pressure and experiment time were analyzed using a -71kPag experimental run. This run was selected since it represents roughly the midpoint degas pressure used in all six experiments.

As the diffusion mixer is filled by the advancing fluid column two opposing forces are influencing the fluid interface velocity, which in turn affects the flow rate. Vacuum pressure is increasing as air rapidly diffuses into the PDMS substrate which increases average flow rate as per equations 5.1 and 5.2. Simultaneously, flow rate is decreased by the increasing fluid resistance due to increased fluid column length as determined by equation 2.12. Both pressure and column length has a first order effect on flow rate therefore; whichever changes faster will dictate whether flow rate increases or decreases. In the first -71kPag run from $t=69\text{sec}$ to $t=129\text{sec}$ the flow rate decreases from

3.3 to 2.6uL/min in an exponential fashion. The vacuum pressure inside the device for this same time period increases from 1.21kPag to 4.14kPag while the fluid column

Figure 44: From (1.) to (2.) the diffusion mixer is filled which results in a corresponding decrease in flow rate. At point (3.) the fluid interface enters the relatively larger cuvette which rapidly reduces capillary pressure thus resulting in a spike in flow rate. As the interface expands from (3.) to (4.) the flow rate again decreases until at (5.) a critical fluid diameter is reached and fluid wets the cuvette wall. The cuvette then fills at an increasing rate proportional to the vacuum pressure from (6.) to (7.).

increases from 20 to 124mm. This implies that flow rate should increase as graphed in

Figure 45 which conflicts with the measured flow rate data. Flow rate in this, and all

other cases, is calculated using the equivalent circuit method and equations 2.12-2.16.

Capillary pressure associated with the presence of a curved interface is also account for

using equation 2.18. Assuming that the pressure data changes with experiment time are

perhaps incorrect a second calculation can be performed to explain the measured decrease in flow rate. If only the average vacuum pressure is used to calculate flow rate as a function of increasing column length then the calculated and measured flow rates are in close agreement (see Figure 45). The implications for understanding pressure and fluid velocity inside a diffusion mixer driven by absorption drive flow are important. By balancing the competitive forces of vacuum pressure caused by the diffusion of air in PDMS and resistance caused by the dimensions of the diffusion mixer, a fluid interface can be drawn through the diffusion mixer at a constant rate. This would allow a designer to achieve a constant residence time for the interface in the diffusion mixer.

Figure 45: At left the difference in measured and calculated flow rate using instantaneous pressure measurements. At right is the difference in flow rates assuming a constant vacuum pressure. The advantage of increasing vacuum pressure with time is that flow rate is held relatively constant as the competitive effects of pressure and fluid resistance negates each other.

Another general trend measured in all the experiments was a sudden spike in the flow rate as the fluid interface first entered the cuvette (see Figure 44 and Figure 45). This increase in flow rate is attributed to the sudden reduction in capillary pressure associated with the expanding hydraulic diameter from $97\mu\text{m}$ inside the diffusion mixer

to approximately 1.65mm inside the cuvette. Using Equation 2.18 the capillary pressure associated with the three phase interface decreases from -1018 to -30Pa. Using the equivalent circuit modeling approach the theoretical flow rate change can be calculated using the pressure change associated with the reduction in capillary pressure and the increase in vacuum pressure with time. Taking the measured flow rates at $t = 129$ sec, which represents the time of last flow rate measurement inside the diffusion mixer, and $t = 156$ sec, which is the first measurement inside the cuvette, the flow rate increases 3.6 μ L/min. During this same time interval the measured vacuum pressure increases from 4141 to 4980Pa. Assuming the fluid resistance is constant and equal to the equivalent resistances of the ratio and diffusion mixers, the pressure increase is directly proportional to the flow rate increase.

$$\Delta Q_{t=129 \rightarrow 156 \text{ sec}} = \frac{\Delta P}{R_{\text{fluid}}} = \frac{1827 \text{ Pa}}{5.87 \times 10^{13} \text{ Pa} \cdot \text{s} \cdot \text{m}^{-3}} = 1.9 \mu\text{L}/\text{min}$$

Figure 46: Regions of the flow rate curve where the magnitude of the flow rate change may be questionable. In (a.) the flow rate measurement technique changes which may influence accuracy. In (b.) the change in flow rate is so low that performing accurate measurements by image analysis is less practical.

This calculation only accounts for approximately half the measured flow rate increase which suggests the measurement technique is inaccurate in measuring this flow rate transition. As was mentioned earlier the average measured flow rate was 15% greater for the -71kPag experiments than calculated, this could account for part of this inaccuracy. A second source of error is that the flow rate measurement technique changes at this critical juncture (see Figure 46). Not data points are collected for 27 seconds since the image analysis requires large changes in area to accrue before a valid measurement can be taken. Attempts to decrease the time between analyzed images resulted in high scatter among the data points. Unfortunately this results in lost measurements during critical events in the filling process. For this reason the magnitude of the flow rate change as the fluid first enters the cuvette may be questionable. The same can be said for later in the cuvette filling process as the fluid droplet reaches a critical dimension (see Figure 40). Regardless, the general trends in flow rate can be seen and it is important to note that radical changes in interfacial dimensions will influence the flow rate and should be considered during the design process.

A third flow rate trend common to all experiments was a dramatic decrease in flow rate as the curved droplet fluid interface initially expanded inside the cuvette (see Figure 40). Initially this was thought to be an error in the image analysis technique however; the same trend was recorded during six experiments and the average weighted flow rate (calculated earlier) confirmed that a major reduction in flow rate was occurring. Literature (Adzima & Velankar, 2006) for modeling droplets of two immiscible fluids inside microfluidic channels suggest that the pressure required to form and collapse droplets is proportional to the channel cross section area and flow rate. Relating the force

to form, enlarge, then release droplets from vertical capillary tubes has been used extensively (Butt & Graf, 2006) to calculate surface tension of liquids. In both instances the pressure, or force, required to expand the droplet is related to the length of the three phase interfacial region and the surface line tension. For this microfluidic device the air, water, and PDMS interfacial lines extend the length of the curved liquid perimeter and are located along the top and bottom faces of the cuvette (see Figure 47).

Figure 47: Diagram of the cuvette being initially filled by the advancing fluid interface.

The differential vacuum pressure required to lengthen these interfacial lines and thus draw fluid into the cuvette is the sum of the pipe flow and interfacial pressures. In this case the interfacial pressure is opposite to the pipe flow pressure so that it impedes fluid flow. For this situation the interfacial pressure drop is found by:

$$P_{interface} = \frac{2l_p \cdot \gamma_{water}}{A_{cross}} \quad (7.1)$$

Where l_p = line perimeter, γ_{water} = surface tension for water, and A_{cross} = effective channel cross section which the capillary force acts over. Since the hydraulic dimension

of the cuvette is large compared to the micron scaled channels the fluid resistance is considered negligible. If the flow rate is calculated by the measured pressure drop and the fluid resistance using the equivalent circuit, the theoretical flow rate using equation 2.12 can be found by:

$$Q_{calc.} = \frac{(P_{measure} - P_{interface})}{R_{fluid}} \quad (7.2)$$

The results comparing the measured versus the calculated flow rate is shown in Table 7.

Table 7: Comparison of initial flow rates into the cuvette

<u>Elapsed Experiment Time</u> (sec)	<u>Calculated Flow Rate</u> (uL/min)	<u>Measured Flow Rate</u> (uL/min)
150	3.8	6.0
162	3.2	5.1
174	3.1	4.9
186	3.0	4.3
198	3.1	4.1
210	3.1	3.6
222	3.0	3.5
234	3.2	Not Available
246	3.4	0.2
Average	3.2	4.0

These calculations confirm with good approximation the reduction in flow rate trend and the average flow rate into the cuvette which could be expected given the measured

vacuum pressure. The dramatic measured flow rate drop from 6 μ L/min to almost zero was not predicted in the calculation which again calls into question the measured flow rates at both extremes of this trend. Most importantly, it is evident that a severe reduction in flow rate can be expected as the initial three phase interface expands into the cuvette. Ultimately, this interface reaches a critical dimension whereupon the contact angle of the interface with respect to the cuvette walls reaches the dynamic critical contact angle and wetting occurs (Cho & et.al, 2007). This phenomena was described in section 5.5 in which the contact angle transition which governs the action of the CRV also controls the critical dimension inside the cuvette.

The final trend measured in the flow rate experiments was that of a consistently increasing flow rate proportional to the measured vacuum pressure once the fluid inside the cuvette began wetting along the cuvette side walls (see Figure 44). This occurred once the cuvette was approximately half full and continued right up to the point where the CRV valves to the absorption pumping surfaces filled. This trend was predicted by flow rate calculations which used the equivalent circuit method to correlate measured vacuum pressure and fluid resistance. To simplify the calculations the fluid resistance was assumed constant and equal to a point where the cuvette was approximately three quarters full, or in the middle of this last trend.

All of the flow rate trend calculations were combined and compared to the measured flow rates and are plotted in Figure 48. The flow rate calculations for all the trends except the initial filling of the diffusion mixer are similar. When the weighted averages for the calculated flow rates are compared to the average flow rate calculated in Table 6 for the -71kPag experiments, there is only a 7% error versus a 15% error for the

measured flow rates. This result partially verifies the methods used to calculate flow rates inside the device for a variety of scenarios including; filling a serpentine channel, fluid transitioning from small to large hydraulic diameter, and filling a reservoir experiencing constant fluid resistance. Collectively, these experiments highlight the pumping capabilities for this microfluidic device which is able to obtain high flow rates over a sustained time period. The result from these pumping curves suggest that future microfluidic devices can benefit from using this pumping mechanism to obtain moderate flow rates over relatively long time periods using an extremely simple pump configuration.

Figure 48: The average calculated flow rate was within 7% of the measured flow rate. The measured trend in (a.) was opposite to that calculated. Trends (b.) and (c.) are in close agreement between calculated and measured values. The less dramatic flow rate fluctuations calculated in (b.) and (c.) highlight the limitations for using image analysis to measure flow rate on a micron scale.

7.2 Vacuum pressure achieved by the absorption driven pump

A unique absorption powered pumping mechanism first utilized by Hosokawa (Hosokawa & et.al, Power free PDMS microfluidic devices for gold nano-particle based DNA analysis, 2004) (Hosokawa & et.al, Power free sequential injection for microchip immunoassay toward point-of-care testing, 2006) and described in section 5.2 was used to fill the microfluidic chip with reagent and sample fluids. To effectively design microfluidic devices using this unique pumping mechanism the variables affecting the process should be characterized. As an initial step the final microfluidic chip prototypes were tested using various degas pressures. This was done so that the optimal degas pressure to achieve a specific vacuum pressure, and thus flow rate inside the device, could be obtained for final testing. The ultimate goal is to achieve an appropriate flow rate inside the diffusion mixer so that the correct residence time of the reagent and sample could be achieved.

Initial tests were conducted to measure the vacuum pressure as a function of time for a device without any fluid inside it. Degas pressure is an important design variable since it can be quickly and incrementally adjusted whereas variables such as PDMS substrate thickness and diffusion area (Section 5.2) require completely new prototypes for each test. All tests to measure the vacuum pressure generated inside the microfluidic prototypes used the same experimental setup described in sections 5.4 and 7.1. Four degas pressures or -96, -71, -47, and -31kPag were selected based on the vacuum chamber pump limitations and the pressures which would yield the most useful experimental data. The first PDMS device tested was approximately 5.5mm thick and conformally bonded to a 2" x 3" glass slide. As was expected the vacuum pressure at first

increased rapidly with time before leveling off as the maximum vacuum pressure was achieved (Figure 49). This result is predicted by the Hosokawa solution for air flux as a function of time which represents the solution to a Fick's Second Law of Diffusion problem that was described in section 5.2 (Figure 50).

Figure 49: Vacuum pressure generated inside an initial PDMS microfluidic device as a function of time and degas pressure. The direct relationship between maximum vacuum pressure and degas pressure can clearly be seen.

From these initial experiments it was made clear that the degas pressure was directly, and linearly related to the vacuum pressure inside the device. The practical implications of this were that fluid flow rates inside the microfluidic channels, which are dependent on the pressure differential driving the fluid column, could be controlled by varying the degas pressure. The direct linear relationship between degas pressure and vacuum pressure in the device improves with elapsed experiment time as can be seen by the improving linear fit for the pressure readings taken at 3,000 seconds versus those taken at 100 seconds as shown in Figure 51.

Figure 50: Theoretical volume of air diffusing into the PDMS substrate based on the air flux model proposed by Hosokawa. This plot curve follows an exponential shape which is a result of the solution to Fick's Second Law of Diffusion. The similar shape between the theoretical air flux curve and the measured vacuum pressure curve is notable.

Figure 51: Relationship between vacuum pressure generated inside the device as a function of time and the degas pressure of the vacuum chamber. A distinct linear relationship exists between the degas pressure and the maximum vacuum pressure ($t=3000$ sec) generated inside the device. Based on this plot one can predict the vacuum pressure the device will generate for any degas pressure between approximately -30 to -100kPag.

To better model the vacuum pressure curves generated by each microfluidic device, an analytical equation which relates the internal vacuum pressure, degas pressure, absorption pumping surface area, PDMS substrate volume, and time is proposed. By developing an

analytical equation that relates these design and performance parameters microfluidic devices could be developed that would generate targeted internal vacuum pressures and thus the desired fluid flow rate. The derivation for this proposed analytical equation begins by examining the cause of the vacuum pressure. Fick's Second Law states that gas molecules will absorb into the PDMS elastomer in a non steady state fashion so that a concentration gradient develops along this thickness of the PDMS substrate (Merkel & et.al, 2000) (Atkins & de Paula, 2006). This gradient changes as a function of time and the relative starting concentrations of air in PDMS at the extremes of the gradient distance. The driving force for air to diffuse into the elastomer is a function of the concentration gradient along the diffusion length. Mathematically this is expressed as (Callister, 2003):

$$C_l = \left[(C_s - C_o) \left(1 - \operatorname{erf} \left(\frac{l}{2\sqrt{Dt}} \right) \right) \right] + C_o \quad (7.3)$$

Where l is the diffusion distance (PDMS substrate thickness), D is the diffusion coefficient for oxygen and nitrogen in PDMS which is $3.4 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$ (Merkel & et.al, 2000), C_x is the concentration of air in PDMS at the prescribed diffusion depth ($\text{cm}^3(\text{STP})/\text{cm}^3 \text{ PDMS}$), C_s is the concentration on the inside PDMS substrate face, C_o is the concentration on the outside PDMS substrate face. Both boundary condition concentrations are found by multiplying the solubility coefficient for oxygen and nitrogen gas into PDMS by the surrounding pressure. Before the pumping mechanism starts the concentration of air inside the device is at atmospheric pressure. As the number of moles of oxygen and nitrogen leave the internal volume of the microfluidic channels and cuvette and absorb into the surrounding PDMS substrate the concentration, and thus

pressure, of the internal volume falls below atmospheric pressure. Mathematically, the gauge vacuum pressure inside the microfluidic device can be related by:

$$P_{vacuum} = (P_{atm} - P_{diff}) \quad (7.4)$$

Where P_{atm} = the starting atmospheric pressure inside the device, and P_{diff} = the pressure resulting from the gas molecules diffusing into the PDMS substrate. Using the ideal gas law and assuming the temperature and the internal microfluidic channel volume is constant:

$$P_{diff} = \frac{P_{atm} \cdot (V_{atm} - V_{diff})}{V_{atm}} \quad (7.5)$$

Where V_{atm} = the volume of air inside the internal microfluidic channels at the start of the diffusion pumping process and V_{diff} = volume of air that has diffused into the PDMS substrate. Only the V_{diff} term changes with time and thus influences the vacuum pressure at any given time. The volume of air which has diffused into the PDMS substrate is related to the volume of PDMS available for air to diffuse into, and the extent of the diffusion gradient which is determined by equation 7.3. Mathematically this is expressed as:

$$V_{diff} = V_{PDMS} \cdot C(l, t) \quad (7.6)$$

Unfortunately, finding the volume of air which has diffused into the PDMS as a function of time is not a trivial problem since the concentration gradient varies as a function of distance. For this discussion the PDMS volume available for diffusion is assumed to be

equal to the fixed cross-sectional area multiplied by the maximum diffusion depth which changes with time. Combining terms the calculated vacuum pressure inside the device is:

$$P_{vacuum} = P_{atm} - \left(\frac{P_{atm} \cdot (V_{atm} - V(t)_{PDMS} \cdot C(t))}{V_{atm}} \right) \quad (7.7)$$

Using this analytical result to match measured vacuum pressure as a function of time proved difficult. The $V(t)_{PDMS}$ term was the most problematic since it was difficult to determine for the complex absorption surface geometry used in each prototype. Curve fitting software (XLFit, IDBS Corporation, www.idbs.com) was used to determine the closest fitting analytical model from the measured data. Using data from the final microfluidic device prototype that was degassed to -96kPag and measured using the experimental setup described previously, the following analytical model was a close fit:

$$y = A \cdot (1 - e^{(-B \cdot x)}) \quad (7.8)$$

Where $A = 39.69\text{kPa}$ and $B = .0027 \text{ sec}^{-1}$ for two averaged runs. The sum of squares for this fit was $r^2 = .99959$. The coefficient “A” determines the maximum vacuum pressure to be achieved while “B” determines the rate at which that maximum is reached. This analytical equation was used to solve for P_{vacuum} by inputting fixed V_{atm} and V_{PDMS} terms that were measured on the prototype based on the transparency mask dimensions. The curving fitting software generated a fit where $A = 112\text{kPa}$ and $B = .0023 \text{ sec}^{-1}$ while $r^2 = .99834$. This result suggests the model accurately depicts the rate at which the vacuum pressure changes with time however; the maximum theoretical vacuum pressure is approximately three times higher than the measured value (see Figure 52). One reason for the wide discrepancy between the analytical model and the measured value is the

V_{PDMS} term is held constant. This implies that the maximum volume of PDMS available for air to diffuse into is immediately occupied at the initial concentration of air in PDMS and extends through the entire diffusion depth. To form a more accurate model the V_{PDMS} term was calculated according to:

$$V_{PDMS}(t) = V_{max}(1 - e^{-C \cdot t}) \quad (7.9)$$

Where V_{max} = the maximum volume of PDMS which is available for air to diffuse into which is the cross sectional diffusion area multiplied by the diffusion depth, C = rate constant proportional to the change in maximum concentration diffusion depth. This equation was used since it resembled the measured data curve fit. Using this model with a rate constant $C = .0026 \text{sec}^{-1}$ and $V_{max} = 1.95 \text{cm}^3$ the maximum vacuum pressure was even higher so that $A = 151.7 \text{kPa}$ and $B = .0017 \text{sec}^{-1}$ however; the initial P_{vacuum} more closely matched the measured data. To match the measured and modeled data required the maximum PDMS diffusion volume to be reduced to $V_{max} = .55 \text{cm}^3$ and the rate constant increased to $C = .004 \text{sec}^{-1}$ which yielded $A = 40 \text{kPa}$ and $B = .0027 \text{sec}^{-1}$ with an $r^2 = .99973$ (see Figure 52). A clear shortcoming of this microfluidic prototype is the complex features which form the device make characterization of the absorption driven pumping process difficult. Further analysis using simplified microfluidic devices is required so that analytical models capable of accurately predict the pumping behavior can be developed. By better understanding the variables which affect the absorption pumping process optimized microfluidic devices can be built without having to built multiple prototypes a relying on empirical results.

Figure 52: Plots of the analytical models to predict vacuum pressure as a function of time for the final microfluidic prototype. Model #3 used a smaller volume of PDMS for air to diffuse into than models 2 and 3 in order to match the measured vacuum pressure curve.

7.3 Diffusion phase diagram

A process was developed to determine the correct diffusion mixer length driven by the absorption pumping mechanism using a specific degas pressure. As was discussed in section 2.7 the purpose of the diffusion mixer is to mix two or more fluids together by diffusion instead of convection. To achieve this a minimal residence time of the different fluids in close contact with each other is required. The residence time can be varied by changing the velocity of the fluid through the mixer and/or change the length of the diffusion mixer. To change the fluid velocity the pipe flow equations (see section 2.2) stipulate that the channel length, hydraulic diameter and pressure differential driving the fluid can be altered. When using the absorption pumping mechanism a number of variables influence the final pressure differential achieved inside the channel (see sections 7.1 and 7.2). Collectively, these variables made the determination of the residence time for fluids inside the diffusion mixer for the microfluidic colorimeter

difficult. As a result, an empirical method for designing and using the diffusion mixer within the microfluidic colorimeter was developed.

The first microfluidic prototypes employed a diffusion mixer which was designed based off analytical results (section 5.4). After these prototypes were built an empirical approach was discovered which allowed the relative residence times of two fluids in the diffusion mixer to be observed. This technique permits a designer to vary both the diffusion mixer length and degas pressure to “tune” the optimal fluid residence time. Changing the diffusion mixer in large graduated steps would affect the fluid residence time dramatically however; a new device needs to be made and thus is not efficient for small changes in residence time. The direct relationship between degas pressure and fluid flow rate had been previously established (section 7.1, 7.2) so that flow rate, and thus residence time, inside the device could be finely controlled. A combination of these two variables would permit a design to find the optimal diffusion mixer length and degas pressure couple.

Three absorption driven microfluidic devices which employed the same “starburst” patterns used in the final microfluidic device (section 5.2) were fabricated using soft lithography (section 6.1). Each “starburst” pattern of the same dimensions was coupled to either a 63, 250, or 500mm length diffusion mixer and “T” junction (section 5.3). Each device had the same diffusion mixer width and depth ($250\mu\text{m} \times 60\mu\text{m}$). A copy of the transparency mask can be found in appendix B. A single PDMS casting approximately 3mm thick was made from the SU 8 mold. Two inlet holes for each device were punched through the casting which was then conformally bonded to another 3mm thick PDMS substrate. The entire casting comprised of three devices was degassed at -31,

-47, -71, and -96kPag inside a vacuum chamber. After approximately 1.5 to 2 hours the casting was removed from the vacuum chamber and droplets of blue and yellow dye placed at the “T” junction fluid inlets. Blue dye was placed into the right hand side inlet, yellow into the left hand side. The dyes were drawn into the device by the absorption pumping surfaces which in turn filled the 1/32” deep cuvette then the pumping surfaces themselves. Digital images of each “starburst” were taken immediately after they filled up using a mounted Canon 20D camera with remote shutter switch. The devices were lit from underneath using a digital projector to minimize shadowing caused by the fluid. The images were compiled for each degas pressure and are presented as a phase diagram in Figure 53.

Figure 53: Diffusion phase diagram showing the relative degree of mixing achieved by each diffusion mixer for different combinations of mixer length and degas pressure. For all images yellow dye was placed in the left side of the “T” mixer and blue dye in the right.

The results shown in the phase diagram confirm that increasing the diffusion mixer length and decreasing the degas pressure increases the residence time which in turn increases mixing between the two dyes. In the upper left region of the diffusion mixer the high degas pressure coupled with the short diffusion mixer results in a high fluid velocity over a short distance thus the yellow and blue dyes are unmixed by the time they reach the cuvette. Once inside the cuvette, the relatively large hydraulic dimensions (.85mm x 5mm) reduce mixing by diffusion so that the different colored dyes moved along their

respective sides and flowed into the closest absorption pumping surface. This results in a distinct color contrast between the left and right sides of the “starburst” pattern.

Conversely, in the lower right portion of the diffusion phase diagram the long mixer and low degas pressure result in complete diffusion mixing due to the low pressure differential driving the fluid over a long distance. No distinct color gradient is seen between the left and right sides of the “starburst”. The optimal diffusion mixer length and degas pressure is where near complete mixing has occurred such as the -47kPag and 250mm mixer length couple. At this point the diffusion mixer is not so long so as to require a large device footprint, but not so short that an unreasonably low degas pressure is required. This point represents where the minimum residence time is permissible for near complete mixing to occur so that any instrument employing this mixer and pumping surface combination time will have the shortest analysis time.

The relationship between diffusion mixer length and degas pressure is non-trivial and requires an empirical approach. The absorption pumping mechanism is influenced by the cross sectional area of the PDMS available for air to diffuse into (section 5.1) Adding long diffusion mixers changes the absorption driven pumping parameters by increasing this cross sectional area, but only before fluid fills the mixer. The 62mm diffusion mixer comprises of 3.9% of the total PDMS surface area, 14% for the 250mm mixer, and 25% for the 500mm mixer. Therefore, the 500mm mixer employs more absorption pumping surface than the other mixers and thus begins to draw fluid into the mixer sooner and at a higher velocity. This advantage decreases as the mixer fills, eventually the longer mixer draw fluids at a lower velocity than the other mixers because of the higher fluid resistance for a 500mm channel. To further complicate matters the diffusion process is

non-steady state therefore; the fluid velocity changes with time even though the fluid resistance is held relatively constant after the diffusion mixer fills. The diffusion phase diagram represents to collective variables, known and unknown, which influence to total residence time of the two fluids inside the mixer. Due to its simple and empirical nature, similar phase diagrams for other devices could be constructed based off several prototypes which eliminate the need for complex CFD or similar analysis. For these reasons this process represents a significant design tool for absorption pumping devices.

7.4 Confirming ratio mixer accuracy by flow reversibility and interface boundary image analysis

Two experiments were conducted to confirm that the ratio mixer was delivering the correct 20:1 sample to reagent volumetric flow rate ratio into the cuvette. Both these experiments were to test only the steady state condition of the ratio mixer which occurs once the reagent and sample streams have reached the diffusion mixer. The correct flow rate ratio between the sample and reagent is important for accurate and repeatable phosphate measurements. Too much reagent will result in excess color in the cuvette which will give an artificially high phosphate measurement. Insufficient reagent will cause not all the sample to react and form a low amount of product which will give an artificially low phosphate measurement.

The first experiment utilizes the Stokes flow regime (see section 2.4) to reversibly flow liquid through the microfluidic device and measure the quantity of liquid collected in the sample and reagent inlets. In operation the microfluidic chip has a maximum

Reynolds number of approximately 10 which implies that flow through the device will be the same in both directions. The ratio mixer was designed based on adjusting the relative fluid resistances between the sample and reagent (section 5.4 and 2.5). If fluid is pumped into the diffusion mixer outlet, through the ratio mixer, and out the channel inlets the difference in fluid resistance caused by the sample and reagent channels should result in twenty times more volume of fluid to flow through the sample channels than the reagent channels.

To conduct this experiment the final microfluidic chip prototype was connected to a syringe pump (NE-300 New Era Pump Systems, Wantagh, NY) using .02"ID Tygon tubing (see Figure 54)). Three vials to collect liquid from each microfluidic inlet were cleaned, baked, and weighed to within one milligram. Equal lengths of .02"ID Tygon tubing were used to connect the sample and reagent inlets to the vials. Small pieces of tape were placed over the vials to minimize weight loss due to evaporation. The syringe pump was run at a constant flow rate for one hour or more. Immediately afterward each vial was weighed three times. The difference in fluid weights of the combined sample vials to the reagent vial determines the flow rate ratio. The results from this experiment are summarized in Table 8.

Figure 54: Experimental setup to confirm the ratio mixer performance using reverse flow.

Table 8: Summary of flow rate ratio test using reverse flow.

<u>Experiment</u>	<u>Flow Rate ($\mu\text{L}/\text{min}$)</u>	<u>Pumping Time (hrs)</u>	<u>Flow Rate Ratio</u>
1	30	1	20:1
2	10	3	27:1
3	50	1.5	16:1
4	50	1.5	18:1
5	50	1.5	18:1
6	50	1.5	14:1

The results from this experiment proved the ratio mixer was able to produce approximately a 19:1 flow rate ratio however; there were wide discrepancies between tests. These discrepancies were due to the difficulty in consistently and accurately measuring the small changes of the vial weights. Furthermore, the flow rates and direction in this experiment did not mimic the actual performance of the microfluidic device which increased the uncertainty of the results.

A second experiment was conducted to confirm flow rate ratio by comparing the relative widths of the sample and reagent streams at the entrance of the diffusion mixer. For this experimental setup the final microfluidic prototype chip was degassed at -71kPag for more than 1.5 hrs in a vacuum chamber. The prototype was then removed from the vacuum chamber and placed under a microscope. Droplets of de-ionized water were placed over the sample inlets then a droplet of ink dye placed over the reagent inlet. As the two liquids entered the ratio mixer, images were taken at 500X of the area where the diffusion and ratio mixer connect (see Figure 55). From these images the widths of the left and right water streams and the width of the ink dye stream were measured. Images were taken approximately every 30 seconds for the 5 min it took to fill each cuvette. This was done to insure the relative stream widths were not significantly fluctuating. A total of twelve runs were made using the same microfluidic chip and the average stream widths for each run were used to calculate the relative flow rates.

Figure 55: Image of ink dye and water streams flowing into diffusion mixer. The relative streams widths of both liquids were used to calculate the relative flow rate ratios after correcting for the parabolic velocity distribution across the channel as is plotted on the right.

To convert the relative stream widths into flow rates, the velocity profile across the channel cross section had to be accounted for. As was discussed in section 2.3 the fluid

velocity along the channel walls obeys a “no slip” condition for laminar flow regimes. As a result the velocity profile taken across the channel is parabolic with the maximum velocity in the center. Using the coordinate system found in Figure 1 only a quarter section of the channel was analyzed then symmetry applied to reduce the number of computations. Additionally, since imaging across the z-plane was not possible the velocity profile along this plane was assumed constant. The velocity distribution along the channel half width was found by solving equation 2.8 for a constant z so that:

$$u_x(y) = -\frac{c^2}{2\mu} \cdot \frac{\partial p}{\partial x} \left[1 - 4 \sum_{k=1}^{\infty} \frac{(-1)^k}{\alpha_k^3} \cdot \frac{\cosh(\frac{\alpha_k y}{c})}{\cosh(\frac{\alpha_k b}{c})} \right] \quad (7.10)$$

The full explanation of this equation can be found in section 2.3. Once the velocity distribution across the full channel width is known, the average relative velocity of the reagent or sample stream across a section of the channel equal to the measured width can be calculated by integration (Figure 56). The relative velocity of the sample and reagent can be converted to relative flow rate by multiplying the cross sectional area of the stream by the average velocity within that stream.

Figure 56: The relative average reagent and sample velocities are found by correcting for the “no slip” condition along the channel walls. The velocity distribution along the z-plane was assumed constant.

The effect of adjusting the relative flow rates using the parabolic distribution can be seen in the experiment results listed in Table 9. The uncorrected flow rate ratio of sample to reagent is high, which would suggest the reagent (ink dye) stream is too narrow relative to the sample (water) stream. By correcting for the parabolic velocity distribution it is shown that the reagent, which flows down the middle of the channel, is traveling at a higher velocity, therefore occupies a narrower stream width while achieving the flow rate ratio closer to the targeted value.

Table 9: Results of sample to reagent flow rate ratios.

<u>Experiment</u>	<u>Uncorrected Flow Rate Ratio</u>	<u>Corrected Flow Rate Ratio</u>
1	28:1	21:1
2	31:1	24:1
3	28:1	21:1
4	34:1	25:1
5	34:1	25:1
6	34:1	25:1
7	34:1	25:1
8	39:1	30:1
9	34:1	26:1
10	35:1	26:1
11	31:1	24:1
12	31:1	24:1

The results from this experiment find the sample ratio to be higher than the target value of 20:1 by approximately 13%. This is likely due to the difference in viscosity caused by using shellac based ink dye rather than de-ionized water. A lower viscosity ink would flow faster and thus occupy a narrower cross section of the channel. Both flow rate ratio experiments suggest that the ratio mixer is achieving roughly a 20:1 volumetric flow rate ratio of sample to reagent. Further analysis using particle image velocimetry (PIV) and a confocal microscope could more accurately calculate the relative flow rate ratios if needed.

Chapter 8 Gauge Characterization Study of the Microfluidic

Colorimeter

8.1 Gauge characterization design of experiment and experimental setup

To test the performance of the microfluidic colorimeter a gauge characterization study was conducted. A HACH DR 700, which is a commercially available colorimeter system, was tested in the reproducibility study to serve as a second gauge (see Figure 57). Two light absorption measurement instruments were used for the microfluidic colorimeter. The first was the GaP photodiode used in the colorimeter prototype and second was a USB 4000 OceanOptics spectrometer. The accurate and sensitive spectrometer served as a secondary light absorption measurement technique to verify and compare the performance of the low cost and less sensitive GaP photodiode setup. The microfluidic colorimeter was powered and controlled by a battery powered Asus 10" EeePC Netbook (Asus International, www.eeepc.asus.com) to simulate a field deployable system.

Figure 57: Image of the HACH DR 700 Colorimeter.

A set of test procedures were established to reduce the number of uncontrolled variables and maximize the data obtained from each run. For the microfluidic colorimeter six reusable and identical devices were fabricated and were the only devices used throughout the reproducibility study. The HACH DR700 used six matching glass cuvettes which were labeled and used throughout the duration of the study. A total of five runs were conducted in which each run used six devices or cuvettes. For each run one device or glass cuvette was used to first zero the instrument using a blank solution which left five others for light absorption tests. Standard solutions of phosphate were made from dilutions of a 500ppm solution of standard phosphate solution (HACH Corporation) and 13m Ω -cm de-ionized water (Barnstead B-pure). Each run for the microfluidic colorimeter consisted of taking each device one at a time out of a vacuum chamber, waiting two minutes then placing droplets of phosphate standard solution and molybdovanadate acid reagent into the inlet channel wells. The time to fill the cuvette of each device was recorded to calculate the fluid flow rate. After each cuvette was filled, the microfluidic device was inserted into the LED and light detector holder, and light

absorption measurements were taken every six seconds for a total of thirty seconds. For the HACH DR700 colorimeter each glass cuvette was filled with 25mL of standard phosphate solution and mixed with 1mL of molydovanadate acid reagent for thirty seconds. The mixture was set aside for three minutes and then analyzed three times by the colorimeter taking care to zero the instrument each time with the blank vial.

A design of experiment was organized to collect the requisite data for a gauge repeatability study. See Table 10 for an excerpt of the experiment design. One operator performed all the tests so no attempt to isolate the gauge reproducibility was made. The testing order for each device and standard phosphate concentration was randomized to assist in normalizing the output data. Runs for the microfluidic colorimeter were conducted over multiple days while all runs for the HACH DR700 were performed in one day. Tests within each run were conducted at approximately five to ten minute intervals. Five absorption measurements were taken at six second intervals to measure signal stability for both the GaP and spectrometer systems.

Table 10: Excerpt of the design of experiment for the microfluidic colorimeter.

Operator	Run & Test #		Device #	PO4 (mg/L)	Time (l/min.)	Time (min.)	Detector	AU Measurements					
								1	2	3	4	5	Avg.
Sean	1	1	3	0	1.7	5.3	P						
Sean	1	2	5	5	2	5.3	P	0.017	0.019	0.017	0.017	0.019	0.018
Sean	1	3	6	30	1	5.7	P	0.101	0.101	0.101	0.101	0.101	0.101
Sean	1	4	1	10	2.2	4.6	P	0.052	0.050	0.052	0.054	0.052	0.052
Sean	1	5	2	2.5	2	4.9	P	0.011	0.013	0.011	0.011	0.013	0.012
Sean	1	6	4	20	1.5	5.1	P	0.092	0.092	0.094	0.092	0.092	0.092
Sean	2	1	6	0	1.4	5	P						
Sean	2	2	1	20	1.5	5.6	P	0.063	0.063	0.065	0.065	0.065	0.064
Sean	2	3	2	30	1.5	5.6	P	0.076	0.076	0.078	0.076	0.076	0.076
Sean	2	4	3	10	1	5.5	P	0.027	0.026	0.027	0.027	0.027	0.027
Sean	2	5	4	5	1.4	5.5	P	0.019	0.019	0.021	0.018	0.019	0.019
Sean	2	6	5	2.5	1	5.6	P	-0.006	-0.006	-0.006	-0.007	-0.006	-0.006

There are four basic outcomes for this gauge study. See Table 11 for a summary of the gauge study configuration. First is to measure light absorption signal stability for both gauges over time to determine short term repeatability. Next is to measure the response of the microfluidic gauge based on the two different light detection configurations. Third is to determine the data output linearity for each gauge. Last is to quantify the different levels of precision for both gauges.

Table 11: Summary of the gauge repeatability parameters.

Gauge 1: Microfluidic colorimeter
Gauge 2: HACH DR700 colorimeter
Artifacts: 2.5, 5, 10, 20, 30 ppm phosphate standards
Operator: Sean Kaylor
Microfluidic colorimeter configuration 1: GaP photodiode
Microfluidic colorimeter configuration 2: USB 4000 spectrometer

8.2 Light absorption signal stability

To determine the stability of the absorption signal for the microfluidic colorimeter one measurement was recorded every six seconds for thirty seconds. Instability in the absorption signal would likely stem from problems with the data acquisition electronics due to excessive noise or due to incomplete mixing of the sample and reagent so that the molybdovanadate and phosphate reaction is actively occurring while light absorption is being measured. Figure 58 shows that the signal was stable over the thirty second period. On average the signal fluctuated 2.2% which indicated that the average light absorption signal over a thirty second period could be used for all subsequent measurements.

Figure 58: Light absorption signal stability for the initial test using the microfluidic colorimeter equipped with a GaP photodiode.

8.3 Variability between spectrometer and photodiode detectors

A spectrometer or a GaP photodiode can be used in the microfluidic colorimeter to measure light absorption. The performance of the GaP photodiode and supporting electronic package was verified with the spectrometer. Ten measurements were made on a 20ppm phosphorus standard solution using both the spectrometer and GaP photodiode. A paired t-test was used to statistically compare the mean phosphate concentration that was measured by each light detection system. Before using the paired t-test, the ten phosphate measurements were tested for normality using the Anderson Darling test using a 95% confidence interval (see Figure 59). Due to the design of the gauge study both spectrometer and photodiode measurement results are dependent on each other.

Figure 59: Histogram of ten 20ppm phosphate measurements to compare the spectrometer and GaP photodiode setups.

The Anderson Darling test failed to reject the hypothesis that the data is not normal.

Having confirmed that the assumptions for the paired t-test are valid the t-test was run using the following hypotheses:

$$H_0: \mu_{Difference} = 0$$

$$H_A: \mu_{Difference} \neq 0$$

Here the mean absorption signal from the spectrometer and photodiode are being evaluated. The paired t-test results in a p-value greater than 0.050 therefore; fail to reject the null hypothesis which indicates the population means do not have a significant difference for a 95% confidence interval. From an experimental perspective this results means that either the spectrometer or photodiode detector system can be used for the microfluidic colorimeter without affecting the absorption measurement. Based on the results from the previous paired t-test, the light absorption measurements using the

photodiode system could be used without having to compare the results to the measurements based on the spectrometer.

8.4 Test gauges for linearity

One indicator of gauge precision for a colorimeter is how linear the output light absorption data is compared to a set of standard solutions with varying concentrations of contaminants. In the gauge study, runs twenty five measurements were taken to determine how well each colorimeter could generate a linear line for phosphate solutions ranging from 2.5 to 30ppm. Preliminary review of the data for the microfluidic colorimeter showed three suspect points where the absorption signal was off do to negligent operator error. Two of the discarded measurements recorded a negative absorbance signal. This was likely due to an incorrect dark measurement which led to the instrument recording an increase in light transmission between the blank and 2.5ppm phosphate readings. A third measurement taken at 30ppm phosphate was discarded since the absorbance measurement was lower than thirteen other 20ppm phosphate measurements. The lower absorbance was likely due to an error in the assembly of the microfluidic device. In this case there was less transmission losses because the cuvette was not properly aligned with the photodiode so that light absorbance was measured only through the PDMS substrate. With these points removed the sample size for the microfluidic colorimeter was $n = 22$ and $n = 25$ for the HACH DR700 colorimeter.

To test for linearity, a general linear model (GLM) was first generated for each gauge to determine which variables in the gauge study were affecting the output data.

This model uses a three factor designed experiment with one response. Factor one is phosphate concentration which is fixed and consists of five levels; 2.5, 5.0, 10, 20, and 30ppm concentrations. Factor two is the run order which is five levels and randomized. The microfluidic chip (sample vial for HACH DR700 colorimeter) is the last factor and has six levels randomized between runs. The three assumptions for the GLM were validated by; comparing residuals for each sample set and confirming the data is normal, testing for equal variance using the Levene's and Barlett's Tests, and verifying that the gauge study used random ordering and that each test variable was kept independent. Figure 60 contains the results for the residuals plot which shows the data for both gauges are normal.

Figure 60: Plots of residuals from 25 phosphate measurements using both the microfluidic colorimeter and the HACH DR700 Colorimeter.

For the equal variance tests the hypotheses are:

$$H_o: \sigma_{2.5ppm PO4} = \sigma_{5ppm PO4} = \sigma_{10ppm PO4} \dots$$

$$H_A: \sigma_{2.5ppm PO4} \neq \sigma_{5ppm PO4} \neq \sigma_{10ppm PO4} \dots$$

Both the Levene's and Barlett's Tests have p-values greater than 0.050, therefore the test fails to reject the null hypothesis. This result implies that the variance in light absorption caused by different phosphate concentrations is statistically equal throughout the range of standard phosphate solutions.

The assumption that each test variable is independent and random ordering was used is confirmed by the design of experiment. Each device was paired randomly to multiple phosphate concentrations and the test order was randomized. Having confirmed the assumptions required to perform the GLM, a multiple level GLM was computed for both gauges using Tukey comparisons. All confidence intervals were 95%. The results from the GLM for the microfluidic colorimeter are summarized in Figure 61.

Factor	Type	Levels	Values			
PO4	fixed	5	2.5, 5.0, 10.0, 20.0, 30.0			
Run	random	5	1, 2, 3, 4, 5			
Chip No.	random	6	1, 2, 3, 4, 5, 6			
Analysis of Variance for Light Absorbance, using Adjusted SS for Tests						
Source	DF	Seq SS	Adj SS	Adj MS	F	P
PO4	4	0.0164863	0.0069984	0.0017496	87.21	0.000
Run	4	0.0006035	0.0005093	0.0001273	6.35	0.013
Chip No.	5	0.0005907	0.0005907	0.0001181	5.89	0.014
Error	8	0.0001605	0.0001605	0.0000201		
Total	21	0.0178410				
S = 0.00447898 R-Sq = 99.10% R-Sq(adj) = 97.64%						

Figure 61: Results of the GLM for the microfluidic colorimeter showing the distinct relationship between phosphate concentration and light absorbance signal.

The GLM indicates that the light absorbance response is dependent principally on the phosphate concentration, and to a lesser extent, on the specific microfluidic chip used and

the order in which each test was performed. Since the p-value for the run order and microfluidic chip number is less than 0.050 these control variables present a problem when using a linear regression analysis to derive the master calibration curve. Run order likely influenced the phosphate measurement because with each subsequent test the porous PDMS microfluidic chips absorbed residual phosphate from the previous test.

The results from the HACH DR700 colorimeter GLM are similar to the microfluidic colorimeter (see Figure 62). Again, a strong linear relationship between phosphate concentration and light absorbance was established. Unlike the microfluidic colorimeter, the p-values for both the vial type and run order were greater than 0.050 which implies that these random factors can be disregarded when building the master calibration line.

Factor	Type	Levels	Values			
[PO4]	fixed	5	2.5, 5.0, 10.0, 20.0, 30.0			
Vial No	random	6	1, 2, 3, 4, 5, 6			
Run	random	5	1, 2, 3, 4, 5			
Analysis of Variance for AU light absorbance, using Adjusted SS for Tests						
Source	DF	Seq SS	Adj SS	Adj MS	F	P
[PO4]	4	0.427572	0.221342	0.055335	504.33	0.000
Vial No	5	0.001443	0.001228	0.000246	2.24	0.123
Run	4	0.000203	0.000203	0.000051	0.46	0.762
Error	11	0.001207	0.001207	0.000110		
Total	24	0.430425				
S = 0.0104748 R-Sq = 99.72% R-Sq(adj) = 99.39%						

Figure 62: Results from the GLM of phosphate measurements taken using the HACH DR 700 colorimeter.

To form the master calibration line a linear regression for both the microfluidic colorimeter and the HACH DR700 colorimeter was performed. For the linear curve fit

the light absorbance signal was the response and the phosphate concentration was the predictor. In the microfluidic colorimeter GLM the microfluidic chip number and run order did have a statistical affect on the light absorbance response. However, these variables were disregarded to simplify the linear regression fit since it was considered impractical to take into consideration the chip number and run order for a master calibration line. The results of the linear regression line fit for the microfluidic colorimeter are depicted in Figure 63 and Figure 64. The R-squared value of 91.3% is considered low for research or laboratory work; however it is within acceptable limits for an instrument prototype designed for fieldwork.

The regression equation is					
Light absorbance = - 0.000796 + 0.003621 PO4					
S = 0.00881949 R-Sq = 91.3% R-Sq(adj) = 90.8%					
Analysis of Variance					
Source	DF	SS	MS	F	P
Regression	1	0.0162853	0.0162853	209.37	0.000
Error	20	0.0015557	0.0000778		
Total	21	0.0178410			

Figure 63: Results from the linear regression curve fit for the microfluidic colorimeter which shows the master calibration equation and the goodness of fit to a linear line.

Figure 64 shows the regression line which corresponds to the master calibration line and the 95% prediction and confidence intervals for the linear regression line. Both the prediction and confidence intervals indicate the gauge precision over the range of phosphate concentrations. The prediction interval is a more conservative estimate of precision than the confidence interval since it calculates the future outcome of an individual value whereas the confidence interval estimates only for the sample mean.

Neither of these intervals is linear therefore; the precision of the gauge varies with phosphate concentration.

Figure 64: Plot of the master calibration equations with 95% prediction and confidence intervals for the microfluidic colorimeter.

The same linear regression analysis for the HACH DR700 colorimeter yields the results found in Figure 65. The HACH DR700 colorimeter has a higher R-squared value which indicates the relationship between the light absorbance signal and the phosphate concentration corresponds well to a linear fit.

The regression equation is					
Light absorbance = 0.03970 + 0.01528 [PO4]					
S = 0.0175803 R-Sq = 98.3% R-Sq(adj) = 98.3%					
Analysis of Variance					
Source	DF	SS	MS	F	P
Regression	1	0.423317	0.423317	1369.67	0.000
Error	23	0.007109	0.000309		
Total	24	0.430425			

Figure 65: Results of the linear regression plot for the HACH DR700 colorimeter which shows the master calibration equation and the goodness of fit to a linear line.

In Figure 66 the master calibration line and the corresponding confidence and prediction intervals are displayed. It is evident that both intervals were grouped closer to the master calibration line which indicates the precision for this gauge is higher than that of the microfluidic colorimeter.

Figure 66: Plot of the HACH DR700 colorimeter master calibration equation. Both the confidence and prediction intervals suggest this system is more precise than the microfluidic colorimeter for this range of phosphate concentrations.

8.5 Microfluidic and HACH DR700 colorimeter precision

As a broad indicator of instrument precision, the standard deviation of the light absorbance signal that was calculated in the linear regression analysis can be used to back calculate the corresponding phosphate concentration.

$$AU = .003621 \cdot [PO_4^{3-}] - .00076 \rightarrow [PO_4^{3-}] = \frac{(AU + .00076)}{.003621} \rightarrow$$
$$[PO_4^{3-}] = \frac{(.0088195 + .00076)}{.003621} = \mp 2.6ppm [PO_4^{3-}] \quad (8.1)$$

The standard deviation of the light absorbance signal and master calibration equation calculated in the regression plot for the HACH DR700 colorimeter can also be used to estimate the precision of the instrument.

$$AU = .01528 \cdot [PO_4^{3-}] + .03970 \rightarrow [PO_4^{3-}] = \frac{(AU - .03970)}{.01528} \rightarrow$$
$$[PO_4^{3-}] = \frac{(.0175803 - .03970)}{.01528} = \mp 1.4ppm [PO_4^{3-}]$$

To determine if there was any significant variance between the HACH DR700 and microfluidic colorimeters thirteen 20ppm phosphate solution measurements were evaluated. A two tailed paired t-test was used to calculate the variance between the gauges as they repeatedly measured the 20ppm phosphate standard. The master calibration equation for each instrument was used to convert light absorbance into phosphate concentration. The results from the paired t-test are depicted in Figure 67. The

hypothesis statement used for this test was:

$$H_o: \mu([PO_4^{3-}]_{(microfluidic)} - [PO_4^{3-}]_{(HACH)}) = 0$$

$$H_A: \mu([PO_4^{3-}]_{(microfluidic)} - [PO_4^{3-}]_{(HACH)}) \neq 0$$

The variation in means between the two gauges is not statistically significant; therefore the paired t-test cannot reject the null hypothesis.

Figure 67: Results from the paired t-test showing that the variation between the two colorimeters is not statistically significant to a 95% confidence interval.

In this gauge characterization study the variance between using the spectrometer and photodiode light detection systems for the microfluidic colorimeter was found statistically negligible using a paired t-test. A GLM test was then performed on each gauge. Prior to using the GLM three assumptions concerning the data were verified; the sample sets were normal, of equal variance, and were random and independent. The GLM revealed that the microfluidic colorimeter output was being influenced by the order in which the tests were performed and the microfluidic chip being used for each test. The

HACH DR700 colorimeter output was affected only by the phosphate concentration which was the desired result. Although the microfluidic colorimeter output was affected by factors other than phosphate concentration, these factors were disregarded to simplify the linear regression analysis. A linear regression plot was made for each gauge to derive the master calibration equation. This equation was used to calculate phosphate concentration based on a light absorbance signal. Based on the linear regression plot the precision of the microfluidic gauge was estimated to have a standard deviation of 2.6ppm phosphate and 1.4ppm for the HACH DR700 for a 95% confidence interval. A second paired t-test was used to determine the variance between gauges while repeatedly measuring a 20ppm phosphate standard solution. Although the microfluidic colorimeter had a higher standard deviation between tests points, the sample means for both gauges were statistically equal.

Chapter 9 Future Work

9.1 Empirical equations describing the absorption pumping process

A series of equations were offered in section 7.2 to predict the absorption pumping performance for a given PDMS device. The objective of these equations was to calculate the vacuum pressure inside the device at a specific time given a certain absorption pumping surface area, degas pressure, and PDMS substrate thickness. While equation 7.7 was capable of predicting the rate at which the vacuum pressure would increase, it failed to accurately predict both the starting and final vacuum pressures. The final prototype device yielded desirable pumping characteristics but proved too difficult

to model analytically given the complex absorption pumping arrangement. In order to generate a system of equations to predict the absorption pumping mechanism more empirical results needs to be collected.

A series of absorption pumping prototypes which have varying surface areas and PDMS substrate thickness should be used to first empirically model the absorption pumping mechanism. By building a series of PDMS prototypes using soft lithography (section 6.1) and testing the vacuum pressure generated from each device (section 5.4) the pumping surface area, PDMS substrate thickness, and degas pressure terms could be isolated. The resulting empirical data would then be analyzed using curve fitting software to determine whether equation 7.8 correctly models the data. For example, Hosokawa's model of air flux diffusing into PDMS (Figure 50 and equation 5.1) and the model proposed in equation 7.7 and 7.8 predict that increasing the absorption pumping surface will decrease the time required for the device to reach the maximum vacuum pressure while increasing the total maximum vacuum pressure. Since all the microfluidic chip prototypes have the same absorption pumping surface it is impossible to empirically verify whether the analytical models are correctly predicting the effect of changing the pumping surface.

While measuring the vacuum pressure inside the microfluidic chips both with fluid filling the device and while the device was dry an interesting affect on the vacuum pressure curve was noticed. When the vacuum pressure generated inside the device was tested without fluid the pressure as a function of time was higher than when tested while fluid was filling the channels. If the dry vacuum pressure was subtracted from the vacuum pressure measured while filling, the difference was linear with time (see Figure

68). Essentially, as time progressed the effectiveness of the vacuum pressure generated inside the device while the device decreased at a constant rate. This trend was measured for the -47, -71, and -94kPag degas pressures with a high degree of linearity. Furthermore, it was discovered that the difference between the vacuum pressures was also related to the degas pressure. The measured vacuum pressures differences increased with degas pressure, also in a linear fashion. In this way the effective vacuum pressure inside the device while it was filling with fluid could be inferred based on the degas pressure alone.

Figure 68: Plot of the vacuum pressure inside the microfluidic chip while filling with fluid and while dry and the linear difference between the two plots.

The reason why the measured vacuum pressure inside the device decreased while filling with fluid was never resolved however; it may have important design implications if a microfluidic designer where to use this pumping mechanism. Measuring the vacuum pressure inside the device while it is filling is time consuming and difficult since the prototype must be specially prepared to connect with the pressure sensor line. If this linear reduction in vacuum pressure can be expected for other devices then the vacuum

pressure can be measured while the device is dry and the effective vacuum pressure for when the device is in use calculated based on the degas pressure. More absorption pumping device need to be built for testing so that more empirical data can be gathered to support the observations made with the microfluidic colorimeter chip.

The end result of developing a system of analytical equations which relate the pumping area, PDMS thickness and degas pressure is to equip microfluidic engineers with a set of tools to design absorption pumping devices without first building prototypes. This pumping mechanism has attractive merits and is a natural fit for disposable microfluidic devices. However, without further design tools to predict the pumping behavior an engineer might expect to achieve from their devices it remains impractical for everyday use.

9.2 Field testing the microfluidic colorimeter system

The gauge characterization study performed in Chapter 8 utilized only laboratory prepared orthophosphate standard solutions. To fully characterize the performance of the microfluidic colorimeter it is imperative that the device be tested under field conditions at both point and non-point pollution sources. Typical phosphate concentration encountered in field applications will have lower phosphate concentrations than used to characterize the microfluidic colorimeter. Performing light absorption tests with field samples presents several challenges which are not encountered in laboratory samples such as uncontrolled temperatures and risk of contamination. Analytical challenges such as color, suspended solids, and other interfering constituents are common to both microfluidic and

conventional test methods. Field samples often contain high turbidity associated with both dissolved and un-dissolved suspended solids. In addition, contaminants other than what is being measured can interfere with colorimetric measurements.

The concentration range of standard phosphate samples utilized in the gauge characterization study (Chapter 8) is higher than what can be expected in field measurements. The minimum phosphate solution utilized had a concentration of 2.5ppm which is over twice the concentration that would likely be measured in the field. Therefore, it is imperative that the statistical minimum detection level of the instrument be determined first in a laboratory setting then in the field. Once the minimum detection level has been determined then it can be improved upon with subsequent prototype microfluidic instruments until they are comparable to conventional colorimeter instruments.

Turbidity or color in the samples can lead to artificially high phosphate measurements due to the absorption associated with the suspended solids (Sawyer, McCarty, & Parkin, 2003). Since calibration curves are generated with clear phosphate solutions, the only light absorption associated with the measurement is a direct result of the colorimetric reaction. To improve field test results as much turbidity and color should be removed as is practical. This can be done most conveniently by a combination of filtration and dilution. For conventional colorimeters which use 10mL or more of sample, filtration is usually done through glass fiber filters with a 2-60 μ m nominal pore size connected to a vacuum pump to draw the sample through the filter in a timely manner. Conversely, the microfluidic colorimeter uses approximately 37 μ L of sample and thus requires only a small glass fiber syringe filter to prepare several droplets of sample. The

microfluidic colorimeter does have the distinct disadvantage of small inlet channels which are susceptible to clogging by suspended solids. For reliable usage samples should be filtered with a minimum of 2 μ m pore size to prevent clogging in the 60 μ m x 60 μ m inlet channels. However, standard methods recommend 0.45 μ m filtration to distinguish between dissolved and particulate phosphorus. If filtration alone cannot reduce the residual color in the sample, the sample can be diluted with de-ionized water so long as the instrument's minimum detection level is not approached.

Interfering contaminants can adulterated the water sample and result in either positive or negative interferences. An advantage of the molybdovanadate reaction is that there are fewer interfering compounds than with the ascorbic acid method (section 5.6) however; silica, sulfide, and ferrous iron can interfere with the sample (Boltz, Lueck, & Jakubiec, 1978). To verify the accuracy of both the colorimeter and the colorimetric chemistry a matrix spike should be performed on the field sample. In this manner any interfering compounds, or excess turbidity, can be isolated from the measurement process and the accuracy of the method confirmed. To perform a matrix spike known quantities of orthophosphate standard solution are added to samples. The total matrix spike volume should be kept to a minimum by using highly concentrated standard solution to avoid adulterating the sample. Performing a field test measurement and matrix spike comparison between the conventional and microfluidic colorimeters would allow the two instruments to be compared in more meaningful way and bring to light any distinct advantages or disadvantages between the two.

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Appendix A

Copy of the Visual Basic software program used to control the microfluidic colorimeter.

Welcome screen:

Option Explicit

' Exits program

Private Sub CancelButton_Click()

End

End Sub

' Launches the chinese version of program (to be developed)

Private Sub Command1_Click()

Call MsgBox("Chinese Language To Be Developed", vbExclamation, "In Development")

End Sub

' Launches the english version of program

Private Sub OKButton_Click()

English.Visible = True

Dialog.Visible = False

End Sub

Main:

Dim DataCap(0 To 4) As Single ' Stores voltages captured directly

Public AvgData As Single ' Holds average of voltages captured

Public AU As Single ' Calculated concentration

Public Dark As Single ' Dark voltage

Public Blank As Single ' Blank voltage

Public Sample As Single ' Sample voltage

Public StdMeas1 As Single ' Known value 1 voltage

Public StdMeas2 As Single ' Known value 2 voltage

Public StdMeas1Con As Single ' Known concentration 1 value

Public StdMeas2Con As Single ' Known concentration 2 value

Public CustSlope As Single ' Custom slope calculated value

Public CustB As Single ' Custome B variable calculated value

Public PO4 As Single ' PO4 calculated concentration

```

Dim i As Integer          ' index variable

Dim TimeLeft As Integer   ' counter variable for timing

Private Sub Command2_Click()

WinDaq1.Stop ' Stops capture from windaq

End ' Closes the program

End Sub

Private Sub Command1_Click()

WinDaq1.Start ' Starts capture from windaq

Call DarkMeas ' Calls the dark measurement function

Label1(4).Caption = Dark

Call BlankMeas

Label1(5).Caption = Blank

Dim MastCal As Integer

MastCal = MsgBox("Would you like to use the master calibration?", _
vbQuestion + vbYesNo, "Please Choose")

' If statement to choose between using the master or custom calibration
If MastCal = vbYes Then

Call MastCalib

Else

Call CustCalib

End If

End Sub

' Function to get the dark measurement and store dark value
Private Sub DarkMeas()

Call MsgBox("Please turn off LED for Dark Reference Measurement."
+ " Press OK when ready.", vbOKOnly)

Call CaptureLoop ' CaptureLoop function reads in da

Dark = AvgData ' sets the master dark variable

End Sub

' Function to get the blank measurement and store blank value
Private Sub BlankMeas()

Call MsgBox("Please insert Blank Reference and turn on LED for Blank Reference Measurement." _

```

```

+ " Press OK when ready.", vbOKOnly)

Call CaptureLoop ' CaptureLoop function reads in data

Blank = AvgData ' Sets the master blank variable

End Sub

' Delay code by nSeconds (timer module)

Sub Pause(ByVal nSecond As Single)

Dim t0 As Single

t0 = Timer

Do While Timer - t0 < nSecond

Dim dummy As Integer

dummy = DoEvents()

' if we cross midnight, back up one day

If Timer < t0 Then

t0 = t0 - CLng(24) * CLng(60) * CLng(60)

End If

Loop

End Sub

' Function to capture data from data aquisition unit and calculate average

Private Sub DataCapture()

DataCap(i) = Format$(WinDag1.GetScaledData(0), "0.00") ' Gets reading from DataAq

' Averages the 5 readings stored in DataCap array

If i = 4 Then

AvgData = (DataCap(0) + DataCap(1) + DataCap(2) + DataCap(3) + DataCap(4)) / 5

End If

End Sub

' Function to uniformly capture data based on TimeLeft seconds and place in

' proper DataCap index for use in calculations

Public Sub CaptureLoop()

TimeLeft = 26 ' Total time in seconds for reading

i = 0

' Initialize array index to 0' Loop to repeat until TimeLeft seconds have passed once per Pause function pass

Do While Not TimeLeft = 0

```

```

Label1(1) = TimeLeft ' Displays current time left in seconds

If TimeLeft Mod 6 = 2 Then ' Calls DataCapture function every 6 seconds

Call DataCapture

Label1(i + 8).Caption = DataCap(i)

i = i + 1

End If

Call Pause(1) ' Pause for condition number of seconds

TimeLeft = TimeLeft - 1

Loop

End Sub

' Uses master calibration to get and interperet data

Private Sub MastCalib()

Call MsgBox("Please insert Sample and make sure LED is turned on." _
+ " Press OK when ready.", vbOKOnly)

Call CaptureLoop ' CaptureLoop function reads in data

Sample = AvgData

AU = -Log((Sample - Dark) / (Blank - Dark)) ' Calculates absorbance

PO4 = (AU + 0.002223) / 0.003673 ' Calculates PO4 concentration using absorbance

Label1(7) = PO4 ' Displays PO4 level

End Sub

Private Sub CustCalib()

' Initializes custom calibration by opening up form custom1

English.Visible = False

Custom1.Visible = True

End Sub

Public Sub CustCalc()

'Calculates the custom slope and B value based on known measurements obtained

CustSlope = ((StdMeas1 - StdMeas2) / (StdMeas1Con - StdMeas2Con))

CustB = (StdMeas1 - (CustSlope * StdMeasCon1))

End Sub

' Clears all the labels

Private Sub Command3_Click()

```



```

Dim i As Integer

i =

Do While i < 10
Label1(i).Caption = ""
i = i + 1
Loop
End Sub

Custom Calibration 1:

Option Explicit

Private Sub OKButton_Click()

English.StdMeas1Con = Text1 ' Saves known concentration

Custom1.Visible = False

English.Visible = True

Call English.CaptureLoop ' CaptureLoop function reads in data

English.Sample = English.AvgData

English.AU = -Log((English.Sample - English.Dark) _
/ (English.Blank - English.Dark)) ' Calculates absorbance

English.StdMeas1 = English.AU

' Closes english window, displays custom2

English.Visible = False

Custom2.Visible = True

English.Label1(13) = English.StdMeas1

English.Label1(14) = English.StdMeas1Con

End Sub

Custom Calibration 2:

Option Explicit

Private Sub OKButton_Click()

English.StdMeas2Con = Text1 ' Saves stdmeas2con value

Custom2.Visible = False

English.Visible = True

Call English.CaptureLoop ' CaptureLoop function reads in data

English.Sample = English.AvgData

```

```

English.AU = -Log((English.Sample - English.Dark) _
/ (English.Blank - English.Dark)) ' Calculates absorbance

English.StdMeas2 = English.AU

English.Label1(13) = English.StdMeas2

English.Label1(14) = English.StdMeas2Con

Call English.CustCalc ' Calculates the custom equation based on known samples

Call MsgBox("Please insert Sample and make sure LED is turned on." _
+ " Press OK when ready.", vbOKOnly)

Call English.CaptureLoop ' CaptureLoop function reads in data

English.Sample = English.AvgData

' Calculation of absorption and PO4 concentration values

English.AU = -Log((English.Sample - English.Dark) / (English.Blank - English.Dark))

If English.AU > 0 Then

English.PO4 = (English.AU + English.CustB) / English.CustSlope

Else

Call MsgBox("Negative absorbance detected, please run again.", vbOKOnly)

End If

English.Label1(7) = English.PO4

End Sub

```

Appendix B

Copies of the transparency masks used during the soft lithography process.

Figure 69: Mask 1 with the initial starburst patterns on the left and ratio mixer with diffusion mixer on the right.

Figure 70: Mask 2 with three initial prototypes of the microfluidic chip.

Figure 71: Mask 3 with the final two prototype microfluidic chips on top. The three different diffusion mixers connected to the starburst on the bottom was used to construct the diffusion phase diagram in section 7.4