# OPTO-ELECTRONICS BASED FLOW ANALYZER FOR MICROFLUIDIC SYSTEMS

A THESIS

submitted by

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## Thesis Certificate

This is to certify that the thesis titled of **OPTO-ELECTRONICS BASED FLOW ANALYZER FOR MICROFLUIDIC SYSTEMS**, submitted by **NILESH MARSHKOLE**, to the Indian Institute of Technology Madras, Chennai for the award of the degree of Master of Technology, is a bona fide record of the research work done by him under our supervision. The contents of this thesis, in full or in parts, have not been submitted to any other Institute or University for the award of any degree or diploma.

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I would like to dedicate this thesis to my amazingly loving and supportive parents who have always been with me, no matter where I am.

## Abstract

Keywords : Microfluidic, Poly Dimethyl Siloxane (PDMS), Soft-lithography.

Flow analyzer is used to quantify the flow rate of microfluidic particles for the devices which analyze the characteristics of microfluidic particles precisely. As accurate flow rate needed to devices which quantify the microfluidic particles with minimum sample volume. The microchannel is fabricated on PDMS material because of bio-compatible, good optical properties etc. using soft-lithography technique. Soft lithography is a non-conventional procedure for replicating a pattern and, it is very fast and easy process to fabricate pattern on PDMS. Generating air droplets (bubbles) inside the microchannel to measure the flow rate of fluid because droplets will move with the flow rate of fluid. The interrogation point at the pattern, where flow channel and both fiber channels meets, as droplets will pass through this point because of change in refractive index between two fibers, amount of coupling light will decrease and output of the detector will goes down. Microcontroller analyzing detector output continuously to measure the traverse time of droplets through interrogation point. Flow rate of fluid would calculate with the traverse time and if we have size of the droplets.

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## Abbreviations

**CAD** - Computer Aided Device

**PDMS** - Poly Dimethyl Siloxane

**UV** - Ultra Voilet

**KOH** - Potassium hydroxide

**LASER** - Light Absorption Stimulated Emission Radiation

**TEM** - Transverse Electromagnetic

**LNA** - Low Noise Amplifier

**DC** - Direct Current

**SNR** - Signal to Noise Ratio

**BW** - Bandwidth

**GBW** - Product of Gain and Bandwidth

**TIA** - Transimpedence Amplifier

**TQFP** - Thin Quad Flat Package

**PLL** - Phase Locked Loop

**ADC** - Analog to Digital Convertor

**PWM** - Pulse Width Modulation

**UART** - Universal Asynchronous Receiver Transmission

**SAR** - Successive Approximation Register

## Notations

$\mu$l - micro liter

nl - nano liter

ml - mili liter

min - minute

$\mu$m - micro meter

$\eta$ - viscosity of the stream

$\nu$ - velocity of the stream

$\gamma$ - interfacial tension between two immiscible fluid

$r$ - distance from the center

$w_o$ - beam waist

$\lambda$ - wavelength

$Z_r$ - Rayleigh range

# Chapter 1

## Introduction:Flow Analyzer

### 1.1 Overview

The ability to measure flow rate of fluid within micro liter volumes, is very important. It helps us verify the capability of the microfluidic analysis system in applications like liquid chromatography or in metering mixing, flow cytometer[1]. There are many principles have been used to measured flow rate of fluid, e.g. thermal flow sensor, pressure sensor, electrical admittance of a microfluidic channel, or by monitoring the optical image of a moving object.[2]

Our work is based on opto-electronics where the measurement is done by collecting light signal after passing through the fluid. Fluid flow is within a microchannel fabricated in a polymer, PDMS. A flow analyzer will measure the flow rate of the microfluidic droplets/particles in the fluid which are typically used in a microfluidic device, with the aim to analyze, detect and sort microfluidic particles.

A flow cytometer will analyze blood cells by injecting blood sample at particular flow rate. But it is not necessary that cell population would be same in all blood samples. The aim of our work on the opto-electronics flow analyzer is to quantify the number of cells within the fluid sample. In the analysis of microfluidic particles, it is important that we investigate each particles. Consequently we use hydro-dynamic focusing to create a laminar flow of the sample fluid and ensure that the particles flow in a single file down the device.

The need for analysis of microfluidic particles is such type of structure which has dimension in micrometer and takes minimum amount of sample. The fabrication of the device for microfluid analysis needs that device should provide good optical properties and surface chemistry. The materials which being use in the fabrication should be inexpensive and bio-compatible[3].

## 1.2 Motivation of work

System based on a lab-on-chip design have grown increasingly popular. The main aim of the system is to make whole bio/chemical laboratories on a chip, fabricated on silicon or polymer. It has scaled down the laboratories size by the factor of hundreds of decimeter to hundreds of micrometer. And by reduction of size it required less amount of sample in $\mu$l or $n$l volume[4]. The reduction in size of the device increases resolution, but the small fluid channels make particle detection more demanding[3][4].

Microsystems are easy to print on a polymer surface using soft-lithography. As a material, use of polymers will reduce time, cost and complexity of the prototyping and manufacturing. There are so many system which still working on the microfluidic like miniaturized analytical system, biomedical devices, tools for chemistry and biochemistry, and systems for fundamental research[5]. There are such application which rely on the accurate flow rate for high throughput like flow cytometry and particles sorting/counting[6].

## 1.3 Thesis Outline

We begin by describing the work done by us on polymer device fabrication, fluid flow in microchannel and light coupling between two bare fiber inside the polymer structure. The third chapter describes the working of electronics and semiconductor components, and micro-controller being used for flow analyzer. In the fourth chapter, we explain the working of flow analyzer, combining all the components which being used in the previous sections. In the fifth chapter we give the results of the flow analyzer and discuss them. In the final chapter, we summarize our work and outline the scope for future work.

# Chapter 2

## Micro-Channel Pattern For Flow Analyzer

### 2.1 Overview

In bio-medical analysis, we need to minimize the quantity of sample to better analyze. A microfluidic device has a typical dimension of 10-1000 $\mu$m which provides for several uses in biomedical devices and microchemistry, and also for fundamental research. In order to make these systems successful, there must have some required physical property for that particular application, e.g. optical properties, surface chemistry and materials used in fabrication should be inexpensive and easily available. In the early stages of microfluidic devices, silicon and glass were the materials used for fabrication. Techniques were expensive, time consuming and required specialized facilities. Fabrication in polymer is quite easy and as a material it gives several advantage in prototyping and manufacturing like low cost, less time and complexity. Polydimethylsiloxane (PDMS) has been one of the most actively developed polymer for microfluidics[5].

## 2.2 Fabrication of the Pattern

Several methods are there to fabricate the micro-structure. Our approach is to fabricate the microfluidic devices based on the techniques of soft lithography. It gives rapid prototyping and replica molding.

### 2.2.1 Soft Lithography

Soft lithography is a non-conventional procedure for replicating a pattern. We start the prototyping by creating a design for a device in a computer-aided design (CAD) program. For transparency in the print design need high resolution image setter. This transparency act as the photo-mask of device in contact photo-lithography to create a positive relief of photo-resist on silicon wafer. This positive relief of photo-resist will be a *master* which used for the casting of PDMS devices[3][7]. The detail procedure of the soft lithography is explained in the Figure 2.1.

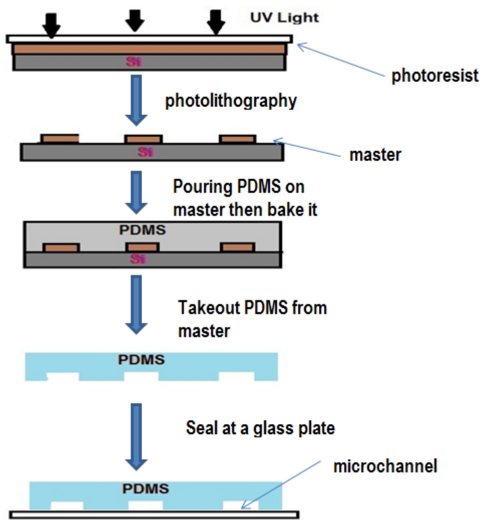



Figure 2.1: Scheme for casting master and molding PDMS device

The photo-mask is kept on the silicon wafer and we expose the wafer to ultra-violet (UV) light. After the UV exposure we get a positive relief of the required pattern[3][7]. SU8-2050 photoresist is commonly used, though the type of photo-resist can depend on the application and the size of the pattern going to fabricate. Photo-resist is a photo-curable epoxy, laid on the silicon wafer. It is durable and provides us with a master that can be used repeatedly, although a failure can occur if the silicon wafer breaks or the photoresist is released from the silicon wafer as we peel the PDMS from the master.

### 2.2.2 Fabrication of Master For Device

The protocols which being to use during prototyping of master of the required microstructure are:

1. Take Si wafer and immerse in isopropanol and KOH for 30 seconds, and handle wafer with tweezers.
2. Placed wafer on spindle and put a big glob of photoresist (SU8-2050) on wafer then run the spin coater.
3. Take out wafer from spin coater and placed on hot plate.
4. Kept mask on the wafer and expose UV light.
5. Again placed on hot plate for post exposure baking.
6. Place master in developer and when it's developed, dip in isopropanol.
7. Blow dry to the master.

The Figure2.2 shows the master which will look after fabrication.

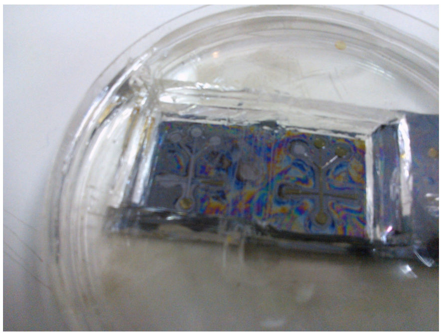


Figure 2.2: Master of the device

### 2.2.3 Print Micro-Structure Pattern In PDMS

The procedure which follow to replicate pattern into PDMS are:-

1. Make PDMS by mixing base and curing agent in the 10:1 ratio.
2. If bubbles are present in PDMS then remove it using vacuum pump.
3. Take master in petri dish, and pour PDMS on master.
4. Bake overnight.
5. Remove PDMS chip.
6. Punch holes for inlets and outlets on PDMS device.
7. Plasma cleaning then stick on the glass plate.

The PDMS device will get after these steps that shown in Figure 2.3.

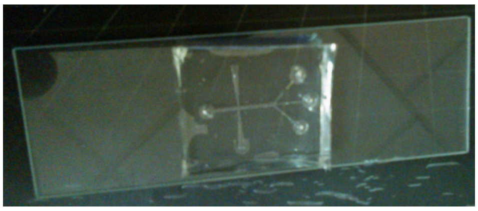


Figure 2.3: Molded PDMS device

## 2.3 Fluid Flow In Micro-Channel For Investigation

Micro-channel devices are widely used for analyzing microfluidic particles. Micro-fluid particles are analyzed as they flow through the micro-channel. As the physical length decreases, gravity has less importance, and surface tension begins to dominate[8]. In this section, we explain the proper technique which used to analyze the microfluidic particles and then our approach that use to analyze microfluidic droplets.

### 2.3.1 Hydro-Dynamic Focusing

PDMS device has a dimension in micrometer and fluid flow in the micro channel is little bit challenging task. The width of the flow channel of the device is 500 $\mu$m and width of the inlet channel is 150 $\mu$m. PDMS device is shown in Figure2.4 . To analyzing microfluidic particles accurately the most important requirement we must fulfill is that microfluidic particles should pass in a single file from the interrogation point. In the PDMS device the flow channel has a dimension of 500 $\mu$m and particles size generally has 10-20 $\mu$m, so if particles flow through 500 $\mu$m size of channel then possibility is there that several particles can pass in the bunch. If space will reduce in the flow channel for the micro-fluidic particles then we can make like particles would pass only one at a time.

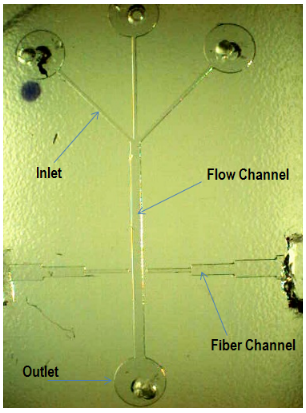



Figure 2.4: PDMS Device

Hydro-dynamic focusing is a means to make the sample fluid much narrower, and allow passage to only a single file of micro particles[9]. To get the hydro-dynamic focusing in the micro-channel device, the device should have three inlets. When two fluids are injected in the microchannel with different flow rates then the fluid of higher flow rate constricts the fluid with the lower flow rate. To study hydrodynamic focusing in our micro-channel device, we inject water in both side inlets and ink in the middle inlet. By applying a higher flow rate for water than ink, we observed that width of the ink stream becomes narrow as flow rate of water is increasing. Syringe pumps are used to inject fluid in the micro-channel device, and provide injection that supports flow rates in the range from $m$l/min to $\mu$l/min.

We perform an experiment by keeping ink flow rate at 15.61 $\mu$l/min, as we vary the flow rate of water. We observe that width of the sample (ink) varies with a change in the flow rate of water. We get a narrow width of sample, of around 10 $\mu$m, by hydrodynamic focusing. Some sample pictures of hydrodynamic focusing are shown in Figure 2.5. The variation in the width of the sample fluid with different flow rate of water is tabulate in Table2.1.

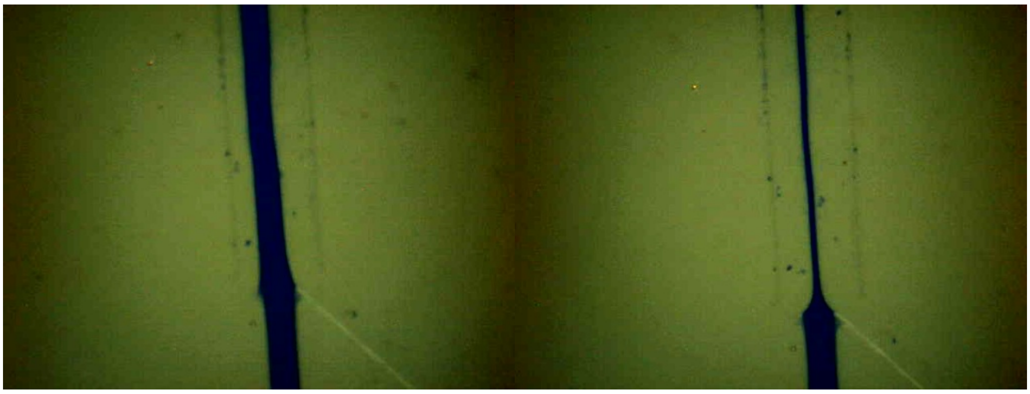


Figure 2.5: Hydrodynamic Focusing

| **Flow Rate of Water** ($\mu$l/min) | **Width of the Sample** ($\mu$m) | **Width of the Sample** ($\mu$m) |
|---|---|---|
| 55 | 220 | 250 |
| 100 | 200 | 210 |
| 120 | 170 | 180 |
| 145 | 150 | 160 |
| 165 | 130 | 140 |
| 190 | 100 | 115 |
| 210 | 85 | 100 |
| 230 | 70 | 75 |
| 255 | 60 | 65 |
| 275 | 50 | 55 |
| 320 | 30 | 45 |
| 365 | 20 | 40 |
| 410 | 10 | 30 |

Table 2.1: Tabulate variation in width of ink stream with variation of water's flow rate

We performed the experiment with two different flow rate of sample (ink) and observed that width of the sample (ink) is broader with higher flow rate as compared to the low flow rate of sample (ink), shown in Figure2.6.

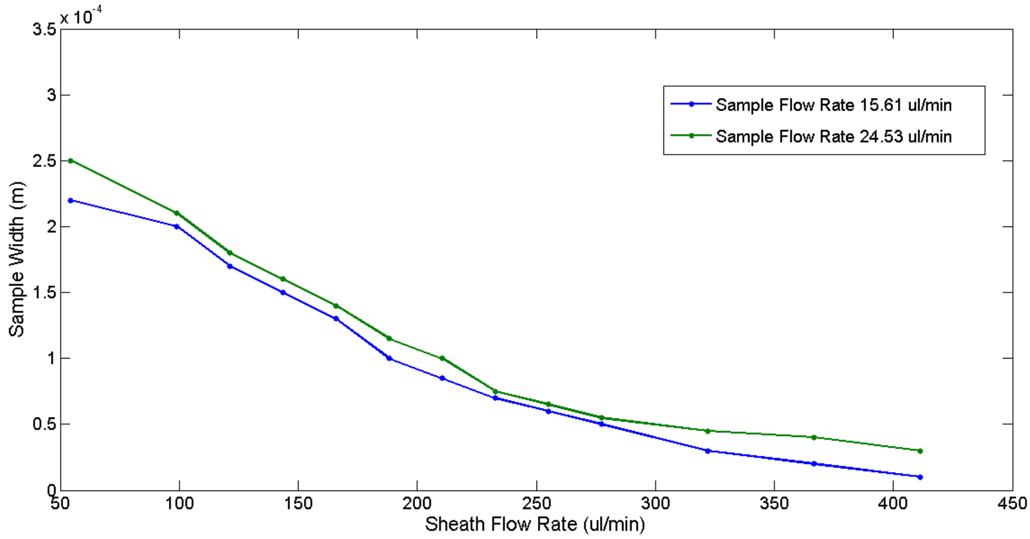



Figure 2.6: Sample Width vs Flow Rate of Sheath

### 2.3.2 Generation of Droplet

As mentioned earlier, micro-fluids have uses in bio-medical, micro-chemistry etc. There are also some application where droplet based micro-fluidics are used, e.g. in diagnostic chips and biomolecule synthesis. Micro-fluidic droplets are generated by mixing two different stream of fluid which are immiscible with each other . The shear force generated on the discontinuous phase, causes one of the fluids to break into discrete droplets.

The property of the droplets depends on the capillary number defined by[8]-

$$Ca = \frac{\eta\nu}{\gamma} \tag{2.1}$$

where $\eta$ and $\nu$ are viscosity and velocity of the the stream respectively and $\gamma$ is the interfacial tension between two immiscible fluid. Droplets formation starts when the capillary number is above the critical point, which depends on the geometry of the device. To analyze the flow rate of the microfluid, we generate the droplet in the microfluid, by controlling the flow velocity of the micro-fluid. In the PDMS, generating a droplet of oil is tough because oil sticks on the wall of the device and minimizes the chances of drop formation. We can avoid the sticking oil injecting KOH solution. Instead of attempting oil droplets in water, it was easier to generate air droplets (bubbles) in water and then measured the flow rate of these bubbles. The droplets are formed as the stream of water was sheared into the air, as shown in Figure2.7.

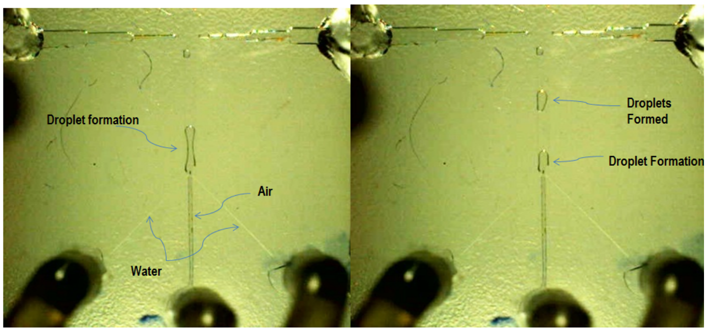



Figure 2.7: Droplet formation

## 2.4 Gaussian Beam

In optics, we always play with the light and it's property. The light source which used commonly is LASER, which emits a beam with Gaussian profile. Lasers typically operate in the transverse mode or TEM mode[10]. Gaussian optics describes the interaction of the beam with other optical components. The profile of laser light is shown in Figure2.8.

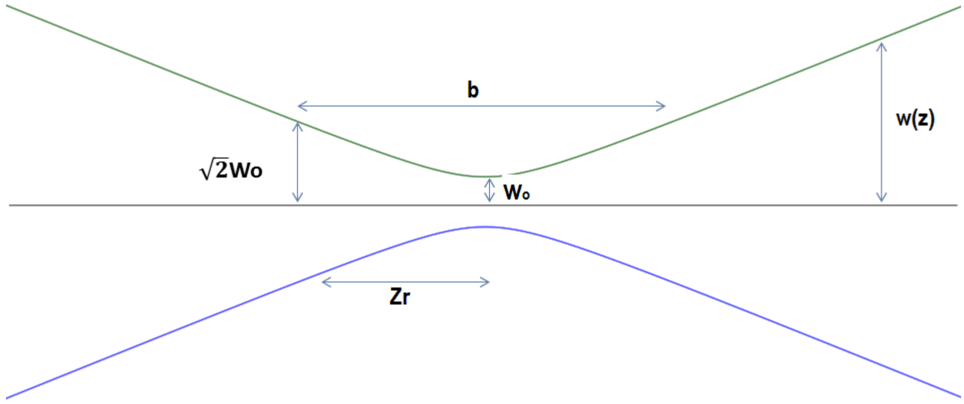



Figure 2.8: Gaussian beam

The Gaussian beam is radially symmetrical distribution and it's change in electric field is defined by the equation[10] -

$$E = E_o \exp\left(-\frac{r^2}{{w_0}^2}\right) \tag{2.2}$$

where $r$ is distance from the center of the beam and $w_0$is the radius at which the amplitude drop by $1/e^2$ of it's value on the axis. When the Gaussian beam propagates in free space, then at $z = 0$ the spot size of the beam has its minimum value along the beam axis, and is known as the "Beam Waist". The spot size of the beam at any point along axis is calculated by

$$w(z) = w_0 \sqrt{1 + \left(\frac{z}{z_r}\right)^2} \tag{2.3}$$

The Rayleigh range is a distance from beam waist where spot size of the beam become $\sqrt{2}w_0$ and is

$$z_r = \frac{\pi w_0^2}{\lambda} \tag{2.4}$$

The radius of curvature $R(z)$ is defined by the wavefront of the beam and $R(z)$ is -ve for a converging beam, +ve for a diverging beam, and infinity at beam waist.

$$R(z) = z\left[1 + \left(\frac{z_r}{z}\right)^2\right] \tag{2.5}$$

## 2.5 ABCD Matrix For Gaussian Beam

Light diverges when it comes out from an optical fiber. To quantify the microfluidic particles accurately it is better to have light focused on the particles. A focusing lensed fiber will provide a focused beam that improves the interrogation of fluid particles. If spot size of the light beam relatively same as microfluidic particles so, only one particles will pass through the light beam and focused light provides more coupling between two bare fibers. As per datasheet of the focusing lensed fiber, specification of the fiber is given for the free space but in the PDMS device include several layers between two fiber channel.

To characterize Gaussian beam propagation through the several layers of the device which have different physical properties (i.e. refractive index, width), we are using ABCD Matrix for Gaussian beam. ABCD matrix is used for ray tracing in the optical systems particularly LASER. It relate the output of the optical components with the input. ABCD matrix of some optical components which present in the PDMS device is shown in Table2.2 -

| Elements | Matrix | Remarks |
|---|---|---|
| Propagation in free space or in any medium | $\begin{pmatrix} 1 & d \\ 0 & 1 \end{pmatrix}$ | $d$ = distance |
| Refraction at a flat interface | $\begin{pmatrix} 1 & 0 \\ 0 & \frac{n_1}{n_2} \end{pmatrix}$ | $n_1$= initial refractive index |

Table 2.2: Transfer matrix of optical components

In a Gaussian beam of wavelength of $\lambda$, radius of curvature $R$, beam spot size $w$ and refractive index $n$ then complex parameter $q$ can be defined as -

$$\frac{1}{q} = \frac{1}{R} - \frac{i\lambda}{\pi n w^2} \tag{2.6}$$

The output of the optical system is determine by using ABCD matrix as -

$$\begin{pmatrix} q_2 \\ 1 \end{pmatrix} = \begin{pmatrix} A & B \\ C & D \end{pmatrix} \begin{pmatrix} q_1 \\ 1 \end{pmatrix} \tag{2.7}$$

$$q_2 = \frac{Aq_1 + B}{Cq_1 + D} \tag{2.8}$$

The structure of the device is shown in Figure2.4 while width and refractive index of each layers are shown in Table2.3.

| Layer | Refractive Index | Width of the medium |
|---|---|---|
| IMF | 1.45 | 10 $\mu$m |
| PDMS | 1.41 | 50 $\mu$m |
| Sheath | 1.40 | 140 $\mu$m |
| Sample | 1.33 | 10 $\mu$m |

Table 2.3: Dimension and refractive index of different layers in PDMS device

As per given parameter's values in the data-sheet of the focusing lensed fiber are -

Operating Wavelength $\lambda$= 1550 nm
Spot Size = 4 $\mu$m
Working Distance = 150$\mu$m
Distance from fiber end to beam waist = $\frac{150\mu\text{m}}{2}$= 125 $\mu$m

So, using (2.6), the complex $q$ parameter of the Gaussian beam at beam waist is -

$$\frac{1}{q_1} = \frac{1}{R} - \frac{i\lambda}{\pi n w^2}$$

$$\frac{1}{q_1} = \frac{1}{0} - \frac{i1550\ \text{nm}}{3.14 * (2\,\mu\text{m})^2}$$

.

$$q_1 = i8.10\,\mu\text{m}$$

We know the complex $q$ parameter 125 $\mu$m from the end of the fiber. Now, to determine $q$ parameter at the end of the fiber we use the ABCD matrix

$$\begin{pmatrix} A & B \\ C & D \end{pmatrix} = \begin{pmatrix} 1 & -125\,\mu\text{m} \\ 0 & 1 \end{pmatrix}$$

Rearranging the respective element values in (2.7) -

$$q_2 = \frac{Aq_1 + B}{Cq_1 + D}$$

$$q_2 = -125\,\mu\text{m} + i8.10\,\mu\text{m}$$

This is the complex $q$ parameter at the end of the fiber and from (2.6), we convert into the form of $R(z)$ and $w(z)$, to obtain

$$R(z = -125\,\mu\text{m}) = -125.52\mu\text{m}$$

$$w(z = -125\,\mu\text{m}) = 30.91\mu\text{m}$$

Here, we have the complex $q$ parameters at the end of the fiber which defines the radius of curvature $R(z)$ and beam waist $w(z)$ at $z = -62.5\,\mu$m from the minimum beam waist. By considering the $q$ parameters as initial complex $q$ parameters of the device then with respect to that we can determine the complex $q$ parameters of whole device. The final $q$ parameters defines the other parameters of the propagating Gaussian beam in the PDMS device.

But, this method is time consuming to calculate the $q$ parameters at each points of the device to observe the propagating beam profile through out the device. We tried to make this simple by write python code for Gaussian beam where it gives the plot of waist size $w(z)$ profile of the propagating beam inside device but it can't give the radius of curvature because at one point radius of curvature become infinity where beam waist $w(z)$ is minimum and python not support the infinity figure. In the other case, if we needs to change any parameters of the device like width of the any layer, refractive index of medium etc. then we need to calculate again complex parameter at each points while python code provides free to change any parameters.

In the Figure2.9 and2.10, we observe the variation in waist size of Gaussian beam in the air while in the Figure beam profile of the propagating light beam inside the PDMS device.

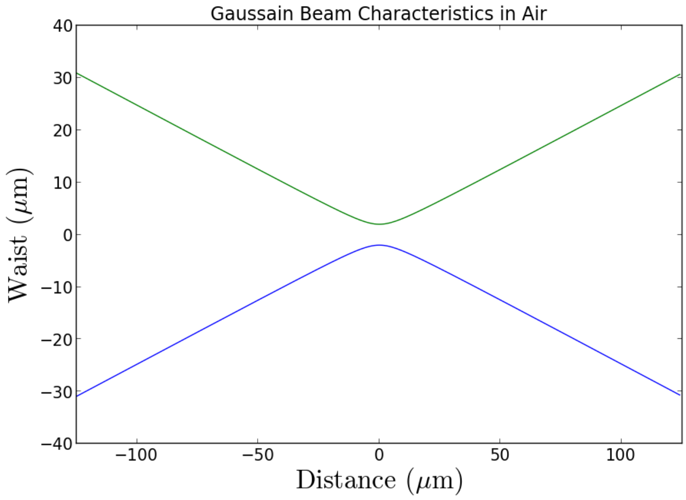



Figure 2.9: Beam Profile in the air

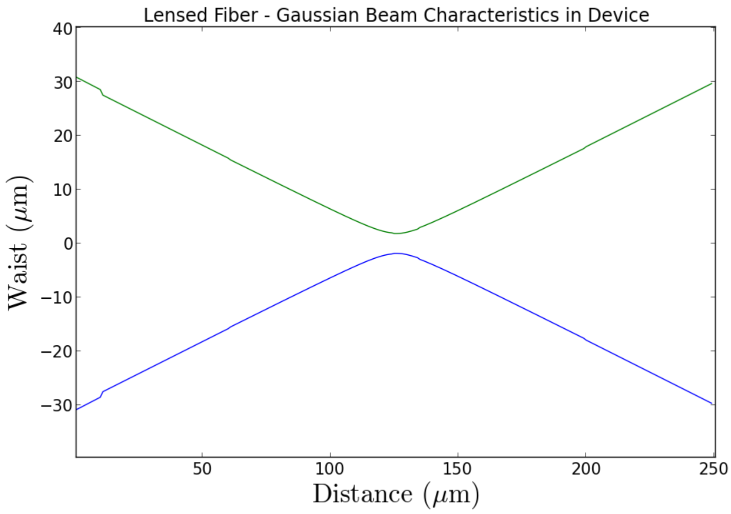



Figure 2.10: Beam profile of the propagating light beam inside the device

DVI, $\sigma$

# Chapter 3

## Opto-Electronics and Micro-Controller & It's Programming

### 3.1 Overview

As explained earlier, the measurement of flow rate of microfluid is done by generating droplets in the microfluid which pass through light path at one point. At the interrogation point, the laser beam passes through the microfluid and falls on the detector. In this section, we will see the working of a laser driver, used to drive the laser diode as a light source and a transimpedance low noise amplifier (LNA) that is generally used to convert an optical signal to an analog signal. In the end, we describe the micro-controller which we used to generate a pulse signal for the laser driver and also for monitoring the analog output from the LNA.

## 3.2 Design Of Transmitter and Receiver Board

### 3.2.1 Laser Driver

In the present day, so many type of light sources are available but we used semiconductor laser diode with fiber coupled. Due to our requirement, source should be fiber coupled and should be coherent. A laser diode is very sensitive to the fluctuations in current and those fluctuations can damage or reduce the lifetime of laser diode. To use the laser diode in proper manner, and for it to last long, the applied current should be constant and free of spikes. The circuit that delivers the constant current, linear, accurate and noiseless current is referred to as the laser driver. Laser drivers always drive a constant current, sometimes as a pulse train with a low duty cycle, and we set the current required for the laser diode to operate. The maximum current rating of the laser diode depends on the device and it's varies with different laser diodes. Before we use any type of laser diode, we first refer to the data-sheet and follow the specifications and characteristics.

Fiber-coupled Laser Diode is using as a light source for analyzing the droplets to measure the flow rate of micro-fluid droplets. The specification of the diode is given below -

Wavelength $\lambda = 1307.74$ nm

$I_{\max} = 1000$ mA

$P_{\max} = 167$ mW

Pulse Width = 10 $\mu$s

Duty Cycle = 1 %

The schematic of Laser driver is shown in Figure3.1. In our laser driver, we used a IC-HK laser switch that delivers the constant current to diode and the output current of the IC-HK is depend on the voltage of pin 8 and resistor R4 & R6. The pin diagram of IC-HK is shown in Figure. IC-HK gives the spike free switching of laser diode with current pulse at range of DC to 155 MHz. The integrated thermal shutdown feature prevents damage from excessive temperature.

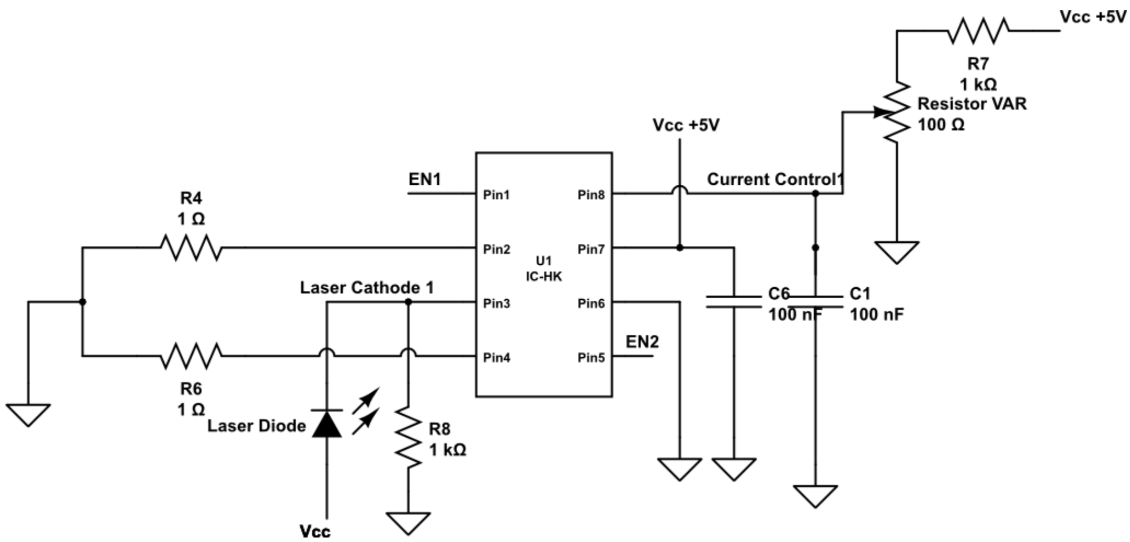



Figure 3.1: Circuit diagram of Laser Driver

### 3.2.2 Low-Noise Amplifier (LNA)

The light after passing through the micro-fluid will fall on the detector and the detector will convert the energy into an electrical signal which will then be converted from analog to digital signal by micro-controller. The optical to electrical conversion is achieved by a InGaAs p-i-n photodiode followed by the trans-impedence amplifier. The circuit diagram of trans-impedence amplifier is shown in Figure3.2.

The transimpedence amplifier is mainly use to convert the current into voltage because of the following reasons:

- Simple configuration, very few components required
- Large bandwidth for a desired gain, since bandwidth scales as $\frac{1}{\sqrt{\text{Gain}}}$
- Relatively high SNR since noise scales as $\sqrt{R_f}$

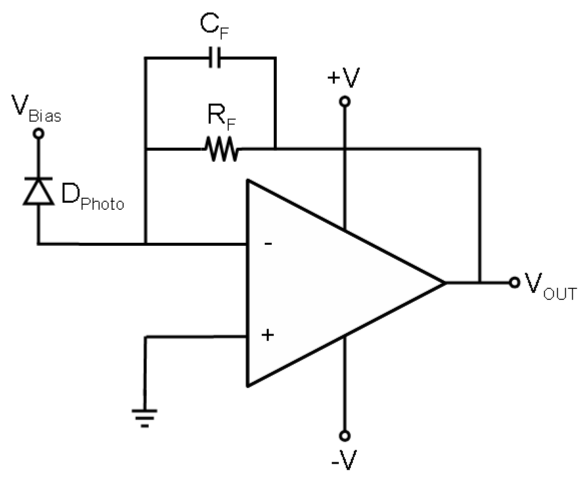



Figure 3.2: Circuit diagram of Trans-impedence Amplifier

There are so many design of transimpedence amplifier for different applications. In the design of our circuit, we have used a transimpedence amplifier with OPA656 IC is called Low noise Amplifier (LNA). OPA656 IC provides very wide-band, unity-gain stability, voltage feedback op-amp and extremely low DC error give good precision in optical application [11]. Wide-band photo-diode amplification is one of the application of OPA656. The schematic of Low noise amplifier is shown in Figure3.3.

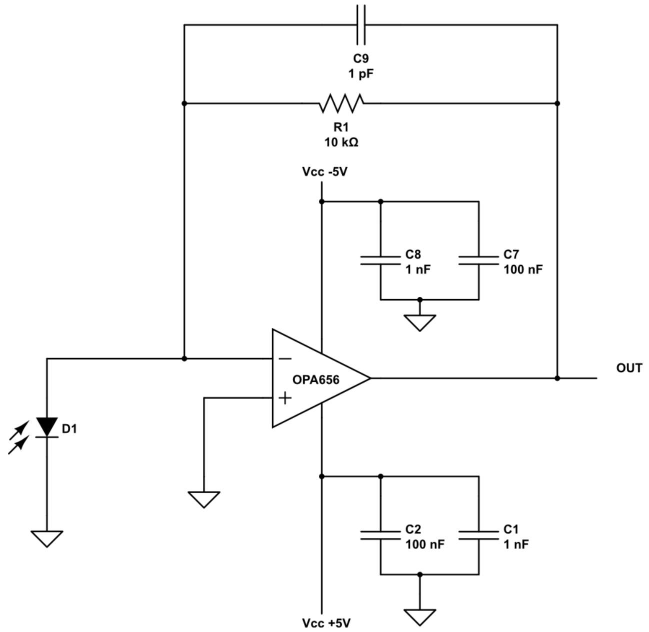



Figure 3.3: Circuit Diagram of Low noise amplifier (LNA)

The bandwidth of the LNA is -

$$\text{BW} = \sqrt{\frac{\text{GBW}}{2 \times \pi \times R_f \times (C_f + C_p)}} \tag{3.1}$$

where, Gain Bandwidth Product (GBW) is 230 MHz

Gain of TIA $R_f$ is 10 kΩ

Feedback Capacitance $C_f$ is 1 pF

Photo-diode capacitance $C_p$ is 0.7 pF

BW = 46.415 MHz

## 3.3 Micro-controller and It's Programming

In the previous section, we discussed about the light source and detector part. The receiver will convert the optical signal to electrical signal and the output signal from the detector will go to micro-controller for examining the analog signal, where micro-controller will convert the incoming analog signal to digital signal. Here, we are using micro-controller for two purpose -

- Generates pulse signal for laser driver
- Monitor incoming analog signal from the detector

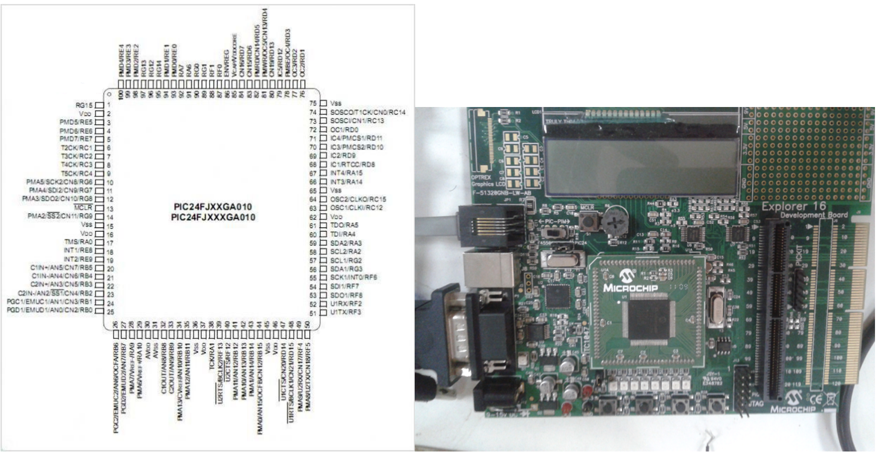
Next in this section, we mention some of the important specification of micro-controller then followed by procedure require for micro-controller to generates pulse signal and in the last data acquisition for the flow analyzer.

### 3.3.1 Specification of Micro-Controller

Flow analyzing is doing with help of **PIC24FJ128GA010** micro-controller of 100-pins with TQFP package. To use this micro-controller, **Explorer-16 Development Board** using to interface with micro-controller while **MPLAB ICD3 Debugger** and **MPLAB** software used to communicate with micro-controller. In the MPLAB software, simply C language is use to write the program. As mentioned earlier, we are analyzing flow rate of micro-fluid droplet using laser beam where laser driver is pulsing laser beam to get high peak power. The pulse signal for driver is got from the micro-controller and the microcontroller is also used for monitoring the analog signal of LNA board. The details of these functions is described in the next sections.

There are some specification of the PIC micro-controller which we are going to use in our task.

- It is 16-bits micro-controller.
- It has 8 MHz internal oscillator with 4 ×PLL (Phase Locked Loop) and multiple divide option.
- It has 10-bits ADC with upto 16 channel and conversion rate of 500 ksps.
- It has five 16-bits timer with programmable prescaler.
- It has five 16-bits compare/PWM outputs.




(a) Pin diagram of PIC24 micro-controller (b) Explorer-16 Development Board for 100-pins

Figure 3.4: PIC micro-controller and it's development board

### 3.3.2 Programming of Generation Of Pulse Signal To Drive Laser Driver

Pulse signal is required to pulsing the laser diode with 1 % duty cycle. Micro-controller is generates the pulse signal with Output Compare module. The output compare module just compare the value of the time base with register value. It can generates the single pulse or train of pulse which depends on configuration. When compare events happens then it generates the interrupts also if enable. The microcontroller has five output compare module and time base for these module, it has five timers.

The following configuration of module needed to generate the continuous pulse signal with pulse width modulation (PWM) are -

1. Set the PWM period in the selected Time Period Register (PRy).
2. PWM duty select by value of OCxRS register.
3. Initial duty cycle select by OCxR register.
4. Interrupt can enable if required for timer and output compare modules.
5. Set the PWM mode by Output Compare mode bit OCM<2:0> of register OCxCON<2:0>.
6. Set timer prescaler value by TMRy register and enable the timer by setting bit TON = 1 of TxCON<15>.

The period of the pulse signal is determine by PRy register and PWM period is calculated by -

$$\text{PWM period} = [(PRy) + 1] \times T_{cy} \times \text{Timer Prescaler Value} \quad (3.2)$$

where $T_{cy}=\frac{F_{osc}}{2}$

By following all the above steps we prepare code for pulse signal of period 1 ms with 1 % duty cycle and details of the code is in Appendix A.

### 3.3.3 Programming of Data Acquisition For Flow Analyzer

The main part of the program for analyzing flow rate is the monitoring analog output signal of the detector without any failures. The logic behind this program is that light beam of laser would be fall continuously on the detector by pass through fluid and detector output is going to monitor continuously by Analog-to-Digital Converter module. When microfluidic droplet will pass through the light beam then detector output will change and respective change in the signal will be come in the monitored data. For monitoring the detector output signal, there are two module going to use one is Analog-to-Digital Converter (ADC) module and other is Universal Asynchronous Receiver Transmission (UART) module.

#### Analog-to-Digital Converter:

In PIC24FJ128GA010, ADC module that is 10-bits high speed converter. The key feature of the ADC module is -

- Successive Approximation (SAR) conversion
- It has up-to 16 input pins.
- External voltage reference voltage.
- It has conversion speed up-to 500 ksps.
- 16-word conversion result buffer.

The steps which should follow to configure the ADC module are -

1. Select port as analog input (AD1PCFG<15:0>) and declare same port as input port separately.

2. Select voltage reference source which depends on the expected range of input analog signal (AD1CON2<15:13>)

3. Select conversion clock of analog signal (AD1CON3<7:0>).

4. Select sample/conversion sequence (AD1CON3<7:0> and AD1CON3<12:8

5. Select form of data which store in the buffer (AD1CON1<9:8>)

6. Select the interrupt matching condition (AD1CON2<5:2>).

7. Turn ON module (AD1CON1<15>).

In this case, output analog signal of detector has period 1 ms with 1 % duty cycle means 10 $\mu$m of positive width. So, during conversion of analog signal to digital signal require at-least 4 sample point on positive width of 10 $\mu$m for differentiate between low and high level. The conversion rate of 2.5 $\mu$m being use for the ADC module.

The resolution of the ADC is 3.22 mV which is calculated by

-

$$\text{Resolution} = \frac{\text{Reference Voltage}}{2^{\text{No. of bits}}} \tag{3.3}$$

where Reference voltage is 3.3 V and ADC is 10-bits ADC.

$$\text{Resolution} = \frac{3.3}{2^{10}} = 3.22\,\text{mV}$$

**Universal Asynchronous Receiver Transmission (UART):**

To analyze the ADC data we need to send data to the computer by interfacing the micro-controller with a computer. The micro-controller uses a UART module to communicate with the computer.

After completion of each conversion the micro-controller will send a corresponding digital value to the computer over UART. But due to limitation of UART transmission rate that is maximum 1 Mbps. If we send data continuously then there is a chance of loose some data point. So, to avoid loosing data it is better to first store data into the memory of micro-controller, wait for memory to fillup, and then start the transmission of data.

The steps to configure UART are -

1. Initialize UxBRG register by setting proper baud rate.
2. Set number of data bit, stop bit and parity selection by setting PDSEL<1:0> (UxMODE<2:1>) and STSEL (UxMODE<0>) bits.
3. Set the UxTXIE if interrupt required.
4. Enable UART by setting UARTEN (UxMODE<15>) bit.
5. Enabling of transmission by setting UTXEN (UxSTA<10>) bit.
6. Load data to the UxTXREG register. Data will store in the word and bytes with 9-bits and 8-bits transmission respectively.

**UART Baud Rate Generator:**

The transmission speed of the UART is decide by the baud rate. Baud rate defines the number of symbol changes occur in one second. The use of bit rate and baud rate is different but in this case both are same because UART module follow the standard Non-Return-to-Zero (NRZ) format and in the NRZ format has only two symbol which is 0 and 1 for each bit.

The value of baud rate register is calculate by -

$$\text{Baud Rate} = \frac{F_{cy}}{16 \times (\text{UxBRG} + 1)} \tag{3.4}$$

In our case, we choose 115200 baud rate and $F_{cy}$ is 32 MHz so, the UxBRG value is 8.

# Chapter 4

## Micro-Fluidic Flow Analyzer

### 4.1 Overview

Before this section, we described all the components in part by part which going to use in flow analyzer like fabrication of PDMS device, fluid flow in a micro-channel, coupling light between the bare fibers, electronics circuits used and the micro-controller. For examining the flow rate of droplets in the micro-fluid we are linking all these components and measuring flow rate of droplets. In this section, we mention the initial approach for measuring flow rate (velocity) of different size of object, then go through the experimental setup for analyzer and in the last data analysis of collection of data.

### 4.2 Working Principle

Before we measure the flow rate of droplets in the micro-fluid, we describe a visitor counter. In the visitor counter, we measured the number of people entering and exiting our lab. We kept a light source at one end and detector at another end, and laser light falls continuously on the detector. Meanwhile if any person passes through the laser beam path, the detector output changes from high level to low level. The low level of detector output tells the someone is traverse through it and micro-controller increments the counter by one. After the visitor counter, we decided to measure the velocity of a small fan by using the relation of speed, distance and time. When we placed fan between source and detector, the vanes of the fan cuts the laser beam. The vanes also take some time to traverse the laser beam. The time taken by vanes to pass through the laser beam is decrease as fan speed increase by applying more voltage to fan. The relativity between time taken and speed of fan is shown in Figure4.1. Having built some confidence on our setup and algorithms, we moved to the next experiment where we measure the flow rate of bubbles in glass tube by producing bubbles from the aerator. The setup of experiment is shown in Figure4.2a. We place a water tube between the laser source and detector and whenever bubbles cross detector output goes low. In all the above cases, the output of detector goes to the input of comparator module in micro-controller. As the detector output goes below a reference level, the comparator gives a high level, as shown in Figure4.2b.

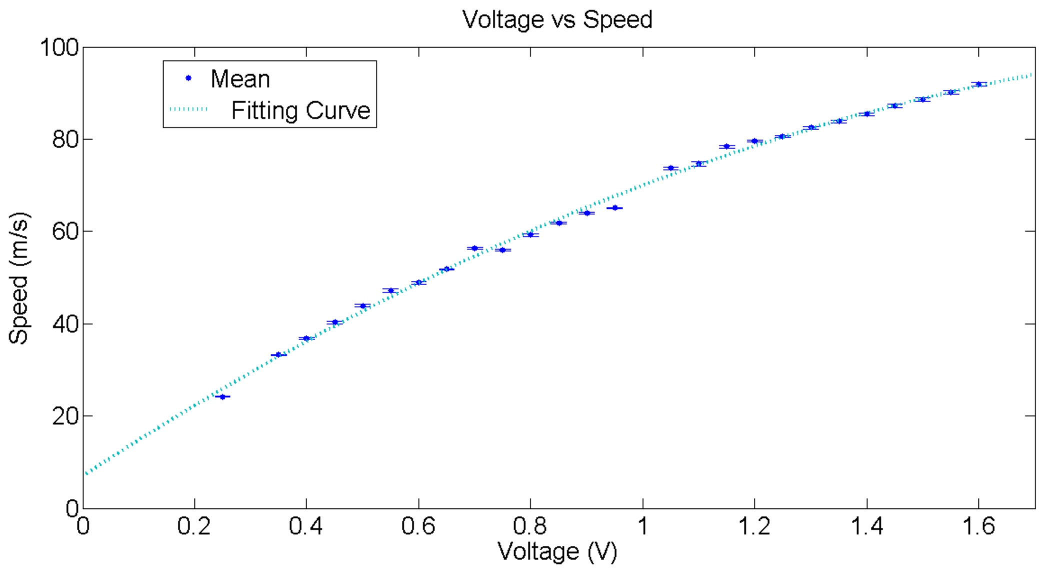
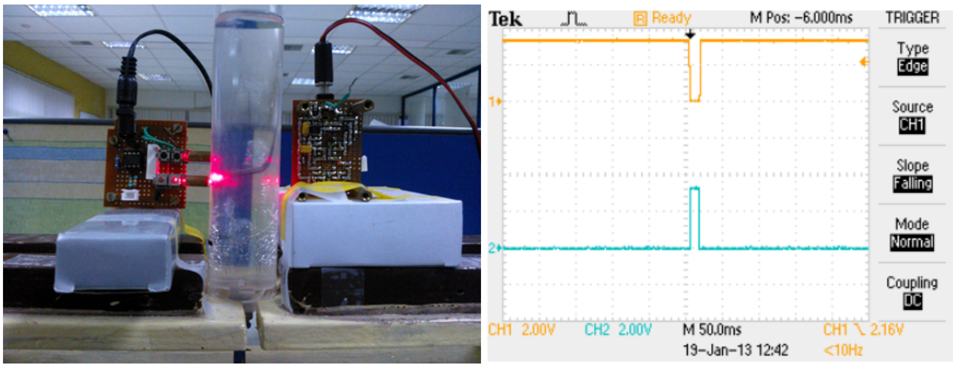



Figure 4.1: Applied voltage versus speed of fan

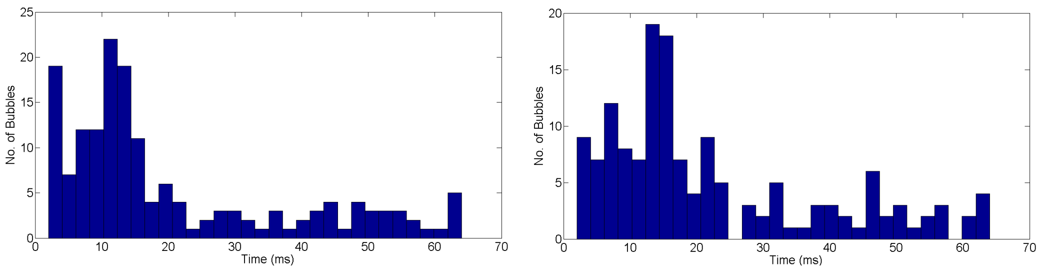



(a) Setup for measurement of bubbles velocity in the glass tube

(b) Comparator Response with input signal

Figure 4.2: Experimental setup and comparator response

The time taken by the respective object is determined through a measurement of the width of the these pulses. In the case of glass tube, we collect data of the traversal time of the bubbles in the laser beam path, with different flow rates and plot the histogram shown in Figure4.3.




(a)

(b)

Figure 4.3: Traverse time of bubbles at different flow rate

We followed the same approach with the micro-fluid but also introduced some modification. For the PDMS device, we used fiber coupled components while before we used only free space components. We also used a pulsed laser source. The previous time calculation was performed by measuring the pulse width, but now source itself is pulsed. The main reason to use pulse source instead of continuous is to get high peak power which is more desirable.

So, here micro-controller monitors the detector output continuously and when it's go below the predefined reference value then micro-controller starts counter and as detector output reaches above the reference level then micro-controller stops counter. The detail working of flow analyzer for droplets in the micro-fluid is explain in the next section.

## 4.3 Experimental Setup For Flow Analyzer

The experimental setup for flow analyzer is made by putting together all those components which we discussed earlier and arrangement of the components is shown in Figure4.4.

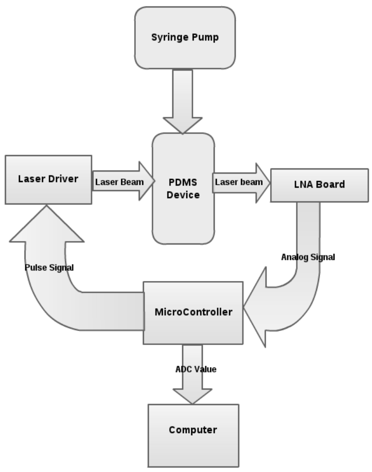



Figure 4.4: Block Diagram of Flow Analyzer

Th micro-controller will generate a pulse signal which goes to the driver circuit, which pulsed the laser source. The light reaches the PDMS device on fibers. Meanwhile the syringe pump will start to inject fluid in the flow channel. The flow channel has width of 450 $\mu$m and height of 150 $\mu$m. The relation between the flow rate and velocity is -

$$\nu = \text{Flow rate } \times 1 \times 10^{-3} \times \frac{1}{\text{width } \times \text{height}} \tag{4.1}$$

At the interrogation point, where both fiber channels and flow channel meet, light will couple from one fiber to other fiber across the fluid channel. The amount of coupled light between two fiber will be vary as a droplet passes through it because air droplets (bubbles) create refractive index mismatch. Refractive index of air is lower than water so, light will couple more in the case of water as compared to air. Based on the coupled light in the fiber, detector gives the analog voltage. The 1% duty cycle allows us to use high power laser diodes, with a peak output power of 7.94 dBm. For the detector, we use InGaAs p-i-n photodiode, followed by a low-noise amplifier (LNA) with gain of 10 kΩ. Without any bubbles, we observed a peak input power of -14.26 dBm at the detector. So, whenever droplets will go through the interrogation point then power will drop by 22.2 dBm.

## 4.4 Data Analysis and Interpretation

The analysis for flow rate of microfluidic droplet tested by micro-controller program whose flow chart is shown in Figure4.5.

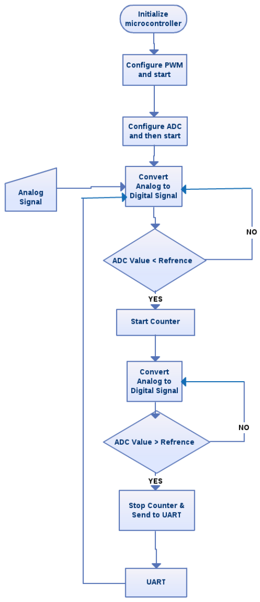
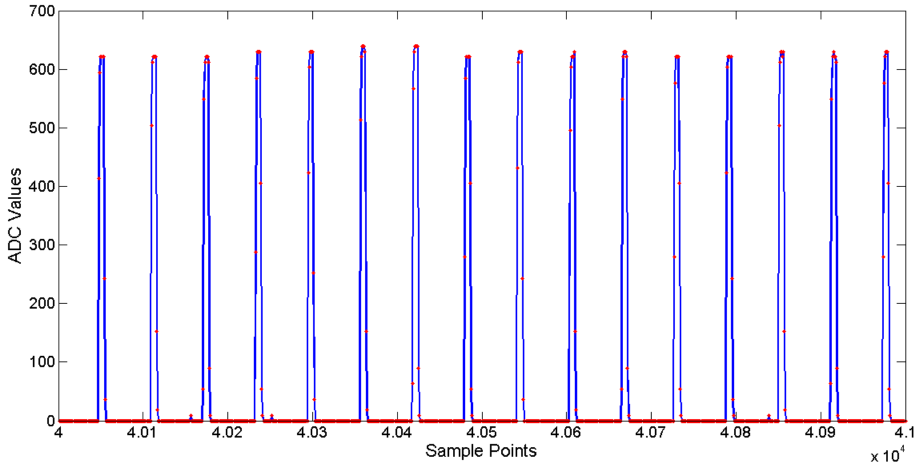
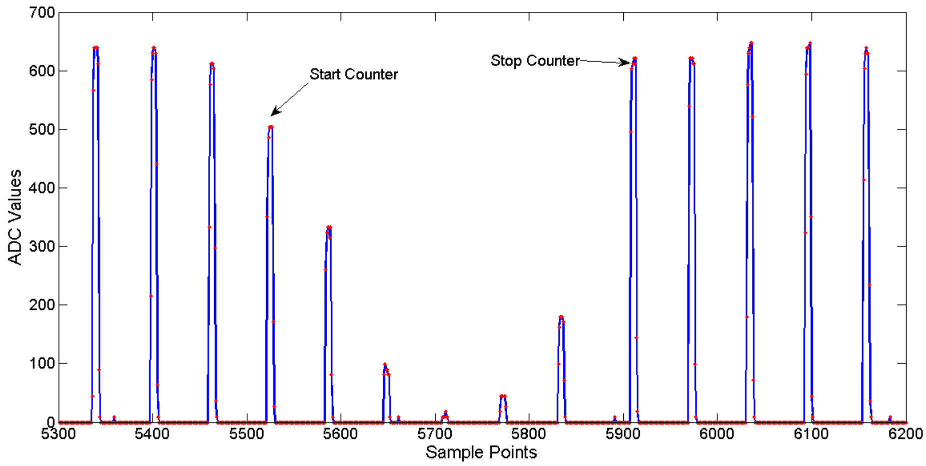



Figure 4.5: Flow Chart of Flow Analyzer

We perform the experiment by generating air droplet (bubbles) in the water and collect the data from the micro-controller which signify two things that in the absence of droplet detector comes like Figure4.6a. Whenever any droplets cross the laser beam then the detector output looks like Figure4.6b. The time duration between first point till maximum voltage represent time taken by droplet to pass beam in the Figure4.6b.




(a) ADC data output with absence of droplets




(b) ADC data output with presence of droplet

Figure 4.6: ADC data output with two different cases. (a) ADC data waveform shows that no droplets are traversing through light path. (b) ADC data waveform shows that refractive index change take place there, so intensity of the light goes low.

In the flow chart of Figure4.5, the micro-controller is also monitoring the ADC value continuously. When the ADC value goes smaller than a predefined reference value, then the micro-controller starts a counter which counts while subsequent ADC values do not become greater than the reference value. Finally, the counter value is sent over UART to the computer.

# Chapter 5

## Results and Discussion

Now, we measured the traverse time of droplets with different flow rate set in the syringe pump and after collect data we observe that droplet's traverse time is less for high flow rate and vice-verse. Here we are working in the micrometer scale of size and droplets size is not same always because of that traverse time is fluctuating. We run the experiment for 15 minutes, and plot a histogram of collected data. We can observe the traversal time for particular flow rate and comparing traversal times for all the droplets. The histograms of the collected data, with flow rate of 250 $\mu$l/min and 350 $\mu$l/min, are shown in Figure5.1 &5.2 respectively.

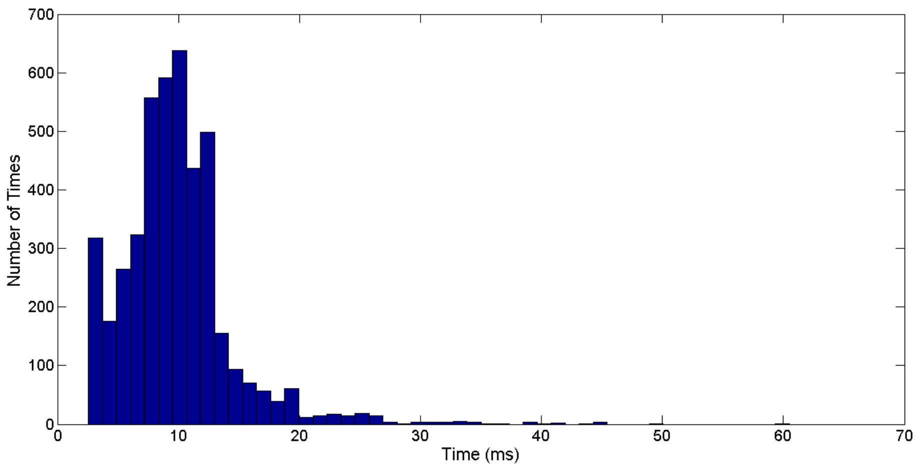



Figure 5.1: Histogram of droplet's traverse time with 250 $\mu$l/min flow rate

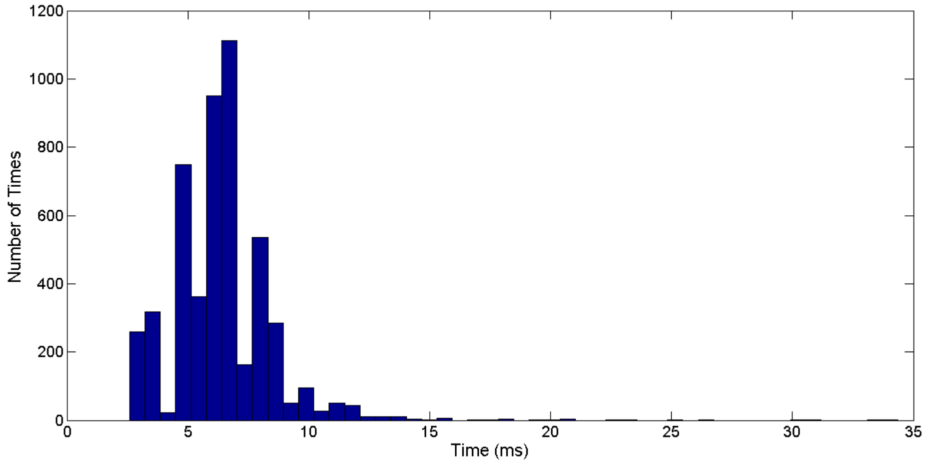



Figure 5.2: Histogram of droplet's traverse time with 350 $\mu$l/min flow rate

In this case, measured the size of the droplets using traverse time and velocity of the fluid. The velocity of the fluid can be calculate by equation (13), velocity of the fluid is 0.0617 $\frac{\mathrm{m}}{\mathrm{s}}$ and 0.0864 $\frac{\mathrm{m}}{\mathrm{s}}$ for flow rate of 250 $\mu$l/min and 350 $\mu$l/min respectively. So, the size of the droplets is -

- At 250 $\mu$l/minthe size of the droplets is approximately 617 $\mu$m.
- At 350$\mu$l/min the size of the droplets is approximately 518.51 $\mu$m.

By knowing the flow rate of the fluid we determined the size of the droplet same if we know the size of the flow rate then we can calculate the flow rate of the fluid.

We repeated this exercise with several flow rates and also check the uncertainly in the measurement, which we plot using error bars. The error plot is shown in Figure5.3, where we observed that traverse time varies more with low flow rate because size of the generated droplets varies with low flow rate of fluid by large amount as compared to high flow rate of fluid.

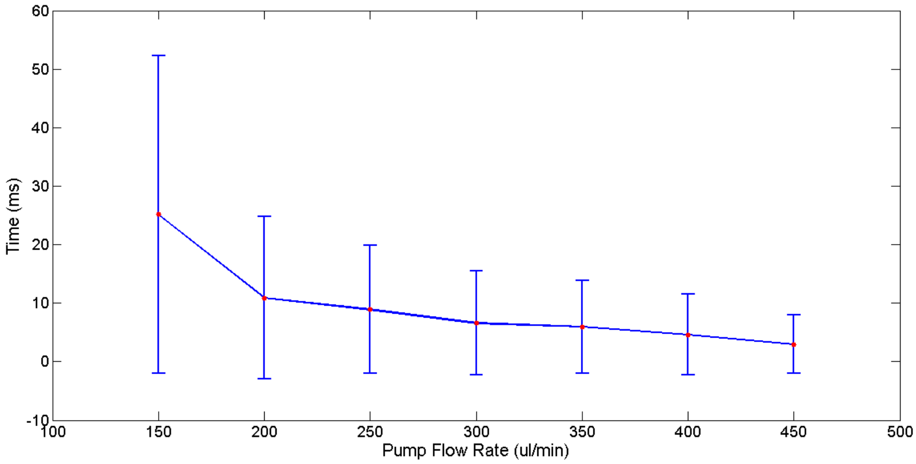



Figure 5.3: Error plot of Pump flow rate versus traverse time

This principle of the fluidic analyzer has ability to measure the any flow rate of fluid. To get the accurate results, microfluidc particles should block large number of positive pulses when it pass through the laser light. In our experiments, laser light is pulsed by 1 ms with 1 % duty cycle and 99 % of the pulse signal which contains no information. So, we measured the traverse time by monitor the ADC signal but in this method 2 ms of maximum error is possible. Because of the limitation of ADC in microcontroller we are using 1 ms of pulse signal. For the lower flow rate of fluid this pulse rate will provide enough accuracy in the result. If measurement of higher flow rate required then just decrease the pulse repetition rate, but make sure that particles should block enough number of pulses to get the more accurate result.

# Chapter 6

## Summary & Future Scope

The aim of the thesis was to develop the device which can quantify the flow rate of microfluid in the microchannel. We fabricated the microchannel using PDMS as a material by soft-lithography because PDMS is bio-compatible, non-expensive and soft-lithography is fast replicating and easy process. A laser driver is used to pulse the laser diode, with a period of 1 ms period with a 1 % duty cycle. On the detector side, we used an InGaAs p-i-n photodiode followed by a LNA board. In our experiment, we can determine the size of the droplets because we know the flow rate of fluid and traverse time. If we know the exact size of the droplets then we can calculate the flow rate of droplets exactly.

The flow analyzer can be used in any microfluidic device which used to measure the physical and chemical properties of the microfluidic particles. We are going to use flow analyzer in the flow cytometer to analyze the flow of blood cells to quantify the physical and chemical properties of blood cell accurately.

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[11] OPA656, Wideband, Unity-gain stable, fet-input operational amplifier, Datasheet.

# APPENDICES

# Appendix A

## Pulse Generator

The following codes are written in MPLab software -

```
#include <p24FJ128GA010.h>
#define FCY 16000000UL
#include <libpic30.h>
_CONFIG1(WDTPS_PS1 & FWPSA_PR32 & WINDIS_OFF & FWDTEN_
OFF & ICS_PGx2 & COE_OFF & BKBUG_OFF & GWRP_OFF & GCP_OFF
& JTAGEN_OFF )
//Configuration bits for more details look at the header file.
_CONFIG2( POSCMOD_HS & OSCIOFNC_OFF & FCKSM_CSDCMD &
FNOSC_PRIPLL & IESO_OFF )
void main (void)
{
PWM_Init1(); // Initialize PWM module
__delay_ms(2); // Delay of 2ms
T2CONbits.TON=1; // Turn ON TIMER
__delay_us(100); // Delay of 100 us
while(1) // Infinite loop
{ }
}
void PWM_Init1()
{
OC2CON = 0x0000; // Turn off Output Compare 1 Module
OC2R = 160; // Set intial duty cycle
OC2RS = 160; // Set final duty cycle
OC2CON = 0x0006; // Load new compare mode to OC1CON with
```

```
    TIMER2
PR2 = 16000; // Period setting (16000*62.5*10e-9)
}
```

# Appendix B

# Measurement of traverse time

The following codes are written in MPLab software -

```
/*******************************************************
It is mandatory to set configuration bits on GUI as
1.Primary OScillator is select :HS
2.Primary oscillator output function : OSC2/CLKO/RC15 functions
as CLKO (FOSC/2)
3.Clock switching and fail-safe clock monitor are : disabled
4.Primary oscillator : HSPLL
5.IESO mode :disabled
6.Watch dog timer : disabled
7.Emulator/debugger uses : EMUC2/EMUD2
8.Writes to program memory : allowed
9.Code protection : disabled
10.JTAG:disabled
*******************************************************/
//Headder Files
#include <p24FJ128GA010.h>
#include "uart2.h"
#include <stdlib.h> // adds data conversion library
#include <string.h> // adds string tools
#include <stdio.h>
#define FCY 16000000UL
#include <libpic30.h>
/********************************************************************/
```

```
_CONFIG1(WDTPS_PS1 & FWPSA_PR32 & WINDIS_OFF &
FWDTEN_OFF &ICS_PGx2 & COE_OFF & BKBUG_OFF&
GWRP_OFF & GCP_OFF & JTAGEN_OFF )
//Configuration bits for more details look at the header file.
_CONFIG2( POSCMOD_XT & OSCIOFNC_OFF & FCKSM_CSDCMD &
FNOSC_PRIPLL & IESO_OFF) //prototype Declaration of functions
/*********************************************************************/

unsigned int ADCValue=0;
int *ADC16Ptr;
char mem[6060]={0}; //maximum usable memory is 6060 Bytes
char array[30];
int location=1;
unsigned int ADCValue1;
unsigned int i=0;
unsigned int j=0;
float rxData;
char str[10];
char str1[10];
unsigned int maximum;
unsigned int counter=0; //initializes a software counter
//Main Function
int main(void)
{
/***********************************/
//Set up I/O Port
/***********************************/
int ADCValue=0;
int *ADC16Ptr;
AD1PCFG = 0xFFFF; //set to all digital I/O
TRISFbits.TRISF5 = 0; //configure PortF 5th pin as output and 4th as input
TRISFbits.TRISF4 = 1;
TRISDbits.TRISD0 = 0;
PORTB = 0x0000;
TRISB = 0x0428;
/**************************************/
//Main Program Functions
/**************************************/
```

```
PWM_Init(); //Initialize PWM module
__delay_ms(2);
T2CONbits.TON=1; //Start PWM
ADCInit(); //Initialize the A/D converter
ADCStart();
UART2Init(8); //Initiate UART1 to 115200 at 32MHz OSCI
__delay_ms(1000); //Delay to stablize UART module
while(1)
{
do {
ADCValue1 = readADC();
ADCValue=0,i=0,j=0;
if(15<ADCValue1<130)
{
ADCValue1 = readADC();
if(15<ADCValue1<130)
{
do
{
ADCValue++;
ADCValue1 = readADC();
if(ADCValue==1023)
{
ADCValue=0;
i++;
}
} while(ADCValue1<130);
}
}
} while(i<1);
rxData=(((1023*i)+ADCValue)*(2.5/1000));
sprintf( str, "%f", rxData);
UART_puts(str);
UART_putc('\n');
UART_putc('\r');
} //calling the function to read data from memory and send
    through UART
return(0);
}
/**********************************************************/
```

```
/************************************/
//Initailization of PWM
/************************************/
void PWM_Init()
{
OC1CON = 0x0000; // Turn off Output Compare 1 Module
OC1R = 0x0010;
OC1RS = 0x0010; // Initialize Secondary Compare Register1
with 0x0001 (DUTY CYCLE)
OC1CON = 0x0006; // Load new compare mode to OC1CON with TIMER2
PR2 = 800-1; //period setting
}
/************************************/
//Intialization of ADC
/************************************/
void ADCInit(void)
{
AD1CON1 = 0x00E0; // integer format, auto conversion,sample after
conversion ends
AD1CON2 = 0x0000;
AD1CON3 = 0x0801;// AD Clock as derivative of system clock ,13TAD,
//16 samples are collected in 1mSec,Fcy = 32 Mhz, 1Tcy=62.5 ns
AD1CHS = 0x0005;// Positive sample input channel for MUX A to use AN5,
//Negative input channel for MUX A to use VR-
AD1PCFG = 0xFFDF; //AN5 analog pin
AD1CSSL = 0x0000; // Channel Scanning Disabled
T3CONbits.TON=1; //Start the timer
}
void ADCStart(void)
{
AD1CON1bits.ADON = 1; // Turn on the A/D converter
}
void readADC(void)
{
ADC16Ptr=&ADC1BUF0; //define pointer
IFS0bits.AD1IF=0; //Reset Interrupt
```

```
AD1CON1bits.ASAM=1; //auto start sampling
while(!IFS0bits.AD1IF); //check for interrupt(wait until interrupt occurs)
AD1CON1bits.ASAM=0; //If interrupt occured, conversion happened,
then stop the sampling/conversion
ADCValue = *ADC16Ptr; // Read value from buffer to variable return ADCValue; }
/*************************************/
//Transmission of data through UART
/*************************************/
void UART_putc(char data)
{
U2TXREG = data;
while(IFS1bits.U2TXIF == 0);
IFS1bits.U2TXIF = 0;
}
void UART_puts(char *s)
{
do
{
UART_putc(*s);
s++;
}
while(*s != 0);
}
/*********************************************************************
```