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FOREWORD

First and foremost, we would like to extend our sincere appreciation and utmost gratitude to Associate Professor Dr. Ngah Ramzi Hamzah, Rector of UiTM (Pulau Pinang), Dr. Mohd Mahadzir Mohammud@Mahmood, Deputy Rector of Academic Affairs and Dr. Mohd Subri Tahir, Deputy Rector of Research, Industry, Community & Alumni Network for their generous support towards the successful publication of this issue. Not to be forgotten also are the constructive and invaluable comments given by the eminent panels of external reviewers and language editors who have worked assiduously towards ensuring that all the articles published in this issue are of the highest quality. In addition, we would like to thank the authors who have submitted articles to EAJ, trusting Editor and Editorial Board and thus endorsing a new initiative and an innovative academic organ and, in doing so, encouraging many more authors to submit their manuscripts as well, knowing that they and their work will be in good hands and that their findings will be published on a short-term basis. Last but not least, a special acknowledgement is dedicated to those members of the Editorial Board who have contributed to the making of this issue and whose work has increased the quality of articles even more. Although there will always be cases in which manuscripts will be rejected, our work so far has shown that the board members' motivation has been, and will be, to make publications possible rather than to block them. By means of intensive communication with authors, academic quality is and will be guaranteed and promising research findings are and will be conveyed to the academia in a functional manner.

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CHARACTERIZATION OF HYDRODYNAMIC FLOW FOCUSING SINGLE PARTICLE DETECTION

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ABSTRACT

The focus of this paper is the development of a device to detect single particle in a diluted liquid suspensions by using hydrodynamic flow optical detection system. To encounter the robustness and cost effectiveness, the device developed in this work uses Polymethylmethacrylate (PMMA) as a base material that was fabricated using standard machining processes. This project explores the feasibility to construct a much larger micro-flow dimensions (approximately 0.5mm to 1.0mm channel width) using standard polymer fabrication techniques which does not require high-end silicon / MicroElectroMechanical System (MEMS) fabrication facilities. The focused stream width has been obtained within the range of 5μm to 50μm which is appropriate to allow confinement of particle or cell to flow continuously in single file. The analytical model has been established to predict the focused stream width formation and its relation to the flow rates ratio. The characterization of hydrodynamic flow has been constructed by using PMMA as the base and optical fiber as a detector for the desired size of 70mm x 40mm, the thickness of 5mm while the depth of each channel was 0.5mm.

Keywords: micro-flow cytometer; fabrication; optical fiber; microelectromechanical system (MEMS).

1. INTRODUCTION

1.1 Flow Cytometer

Flow cytometer is a technique to analyze microscopic particles or cells individually and has been widely used in biomedical engineering for counting and sorting blood cells (Sobek Young, Gray, & Senturia, 1993; Wlodkowic, Skommer, Akagi, Fujimura, & Takeda, 2013; Lee, Chang, Huang, & Yang, 2006), analyzing cell's morphology (Rieseberg, Kasper, Reardon, & Scheper, 2001) and for high throughput single cell analysis (Larsen, Blankenstein, & Branebjerg, 1997). The flow cytometer principle lies behind the ability to isolate and interrogate microscopic particles individually (Cubaud & Mason, 2008). In a

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conventional flow cytometer, the flow stream of sample solution is hydrodynamically focused to establish a continuous flow of cell or particle in single file (Kanda et al., 2001). Individual particle of cells will flow pass a detection region. Work on micro-cytometer had previously been demonstrated where the analysis provided valuable evidence of cancer progressions, cancer activity status etc (Thangawng et al., 2010; Nasir, Justin, Shriver-Lake, Golden, & Ligler, 2010). Nasir et al. (2010) had reported of a a label-free DC impedance-based micro-cytometer for Circulating Tumor Cells (CTCs) by exploiting the difference in size between CTCs and blood cells. The detection system is from the changes in DC impedance between Polyelectrolytic Gel Electrodes (PGEs) under low DC voltages.

Miniaturizing such a large-scaled analytical instrument is an ever challenging task, but with the rapid progress in MicoElectroMechanical System (MEMS) technology supported with high sensitivity sensing mechanism, the move towards miniaturizing a medical laboratory within a small chip device is now possible to be achieved (Thangawng et al., 2010). Early development of microdevices had used silicon wafer as a base material and were processed using standard semiconductor process step. However, the fabrication facilities would require high-end and expensive equipment as well as dedicated clean-room facilities. Due to unreasonable initial investment cost that was required, most of the small scale research laboratories could not initiate the research in this area. Therefore, this project focuses on the development of a micro-flow cytometer using a polymer based material which is lower in cost compared to standard mechanical workshop facility. Due to the nature of the machining processes, the micro-channel size is also much bigger than the typical micro-flow cytometer as reported elsewhere.

2. MATERIALS AND METHOD

2.1 Microdevice Design

The microdevice before the fabrication process is a PMMA-based design, as can be seen in Figure 1. The design and fabrication of micro-channels for generation of hydrodynamic flow focusing consists of the sample inlet, Qi, the sheath inlet, Qs, the Y-channel, the exit channel, the outlet, Qo and a pair of fiber optic channel.

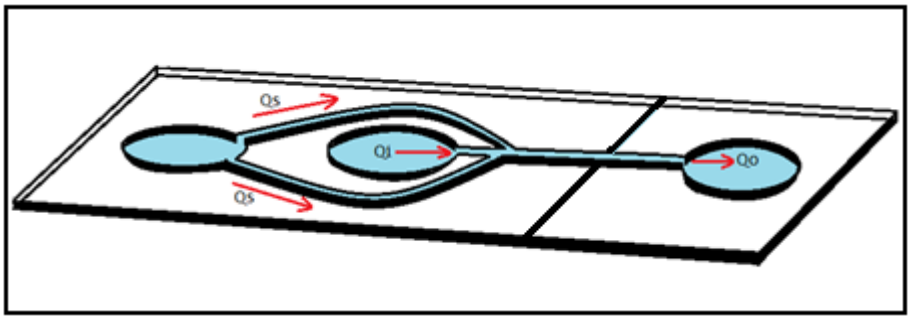

Figure 1: PMMA Plain Structure Design.

2.2 Fabrication

The micro-channels were fabricated from a plain structure (planar structure) using polymethylmethacrylate (PMMA). The desired size of PMMA fabrication was 70mm x

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40mm, the thickness was 5mm and the depth of each channel was 0.5mm. Figure 2 depicts the desired dimension of the PMMA design that included the etched fiber cable channels embedded within PMMA glass in 1mm diameter.

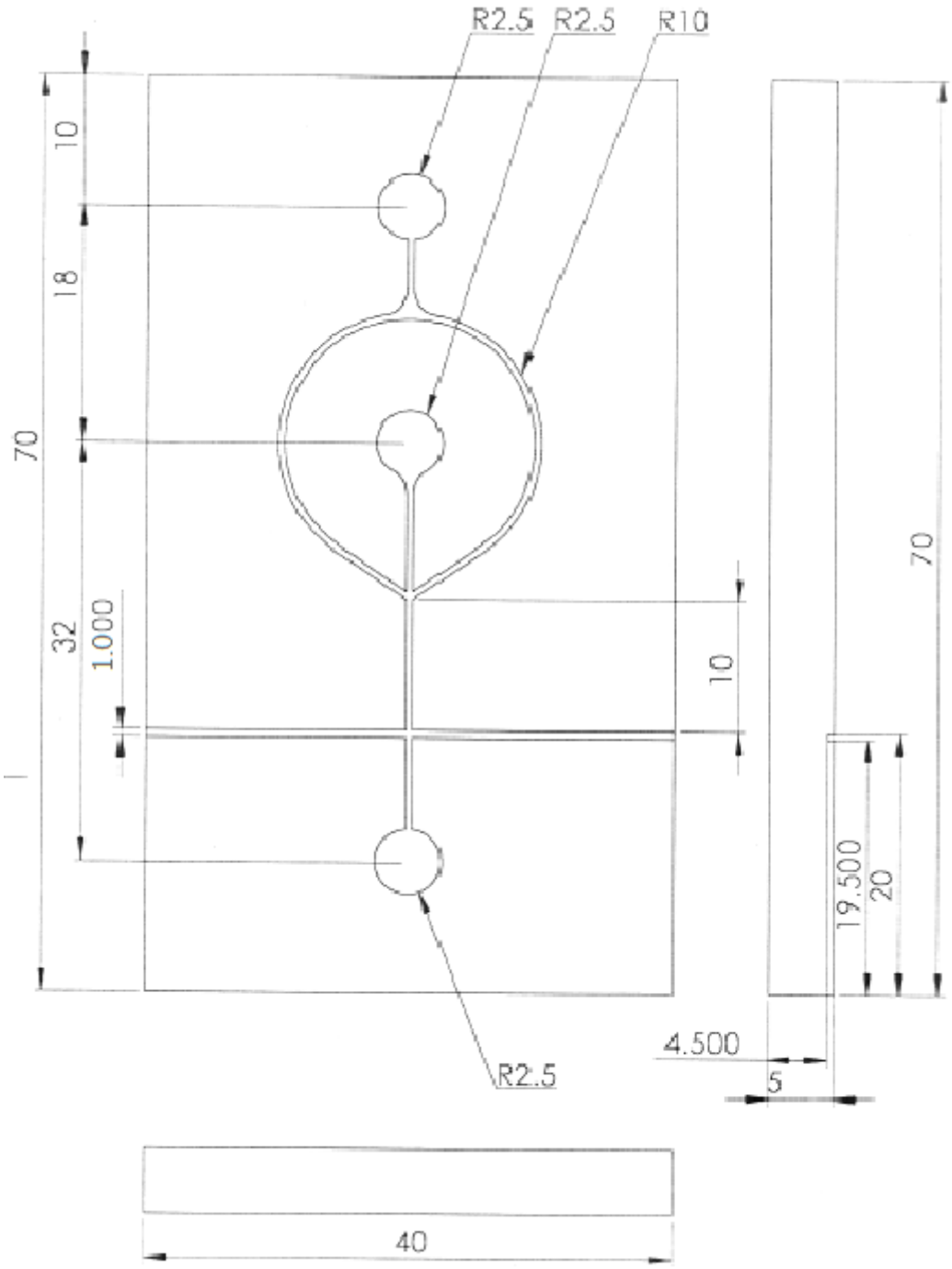

Figure 2: Dimension of PMMA Design with the Etched Fiber Cable Channels.

This fabrication of PMMA-based process was done by using the CNC machining process. Machining of the PMMA was divided into two parts; bottom and top. The bottom part

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consists of the micro-channel fabrication. The top part consists of three liquid access holes: - two holes for inlets and one hole for outlet. The type of material used was Makrolon due to its ease of fabrication and also cost effectiveness. The size of driller used was 4mm in diameter to suit the diameter of tube fittings in order to ensure its compatibility to the holes so that the flowing liquid will not spill out.

The optical alignment test was conducted on the top and bottom parts of the PMMA. This was important to ensure that the transmitting signal between these two parts occurred before they were glued together. Once the fabrication of the PMMA plain structure was completed, the etch channels was fitted with the diameter of cable.

2.3 Optical Sensing

The fiber optic splicing machine was used to join the two or more fiber cables. The types of cables that can be joined are single mode and multimode fiber optic cables. A hot stripper machine was then used to cut and cast aside the unwanted coating from the fiber cables.

Ethyl Alcohol was used to clean the dusty fiber glass when exposed in the air after the cutting process to cast aside the coating. Next, the fiber glass was cut by using the Optical Fiber Cleaver in order to ensure the angle is 90 °. This step is important to ensure that the signal was transmitted well from the emitter to the receiver.

The cutting process required three steps. Firstly, it was required to cover the fiber cable holder so that the cable will remain static and stable with the thumb pressing on that cover. Then, the cleaver had to be pushed to the front drastically. Finally, the holder had to be pressed to open the cover.

Figure 3 illustrates the assembly of micro-fluidic PMMA plain channels with optical fiber cables at both sides. The size of the etch channels must be of the same diameter as the fiber cables. Therefore, to make it tighter, the channels and cables were glued together.

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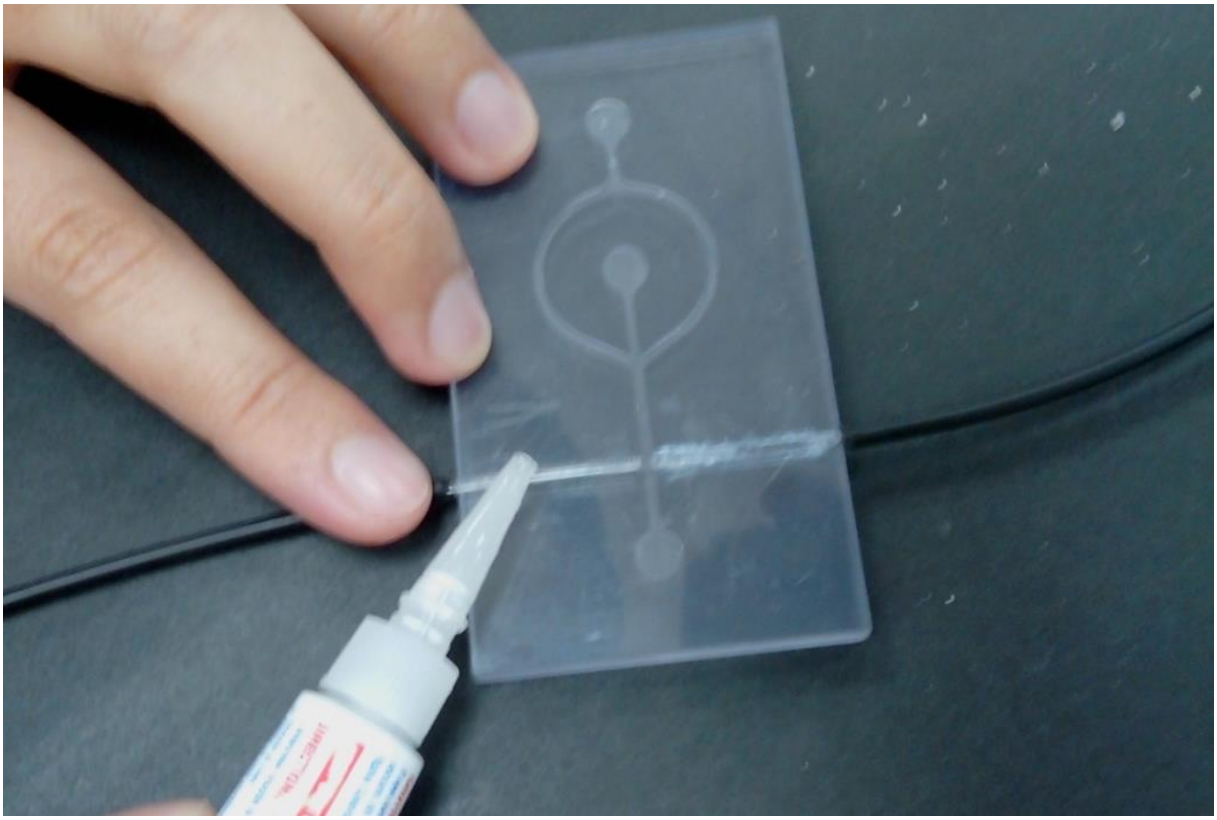

Figure 3: Assembled Micro-Fluidic Channel with Optical Fiber Cables.

2.4 Liquid Driving System

The plain PMMA was covered with 1mm thick Makrolon which needed three holes to be drilledfor the liquid inlets and outlet by using the CNC Drilling Machine. The diameter of the holes were 4 mm in size as to ensure it fits with the tube. The piece of Makrolon was glued to the plain PMMA on top using Elephant Glue, as well as the three tubes and holes which were then glued together to make them tighter. Figure 4 shows the completed glued tube fittings to the Makrolon with optical glass fiber.

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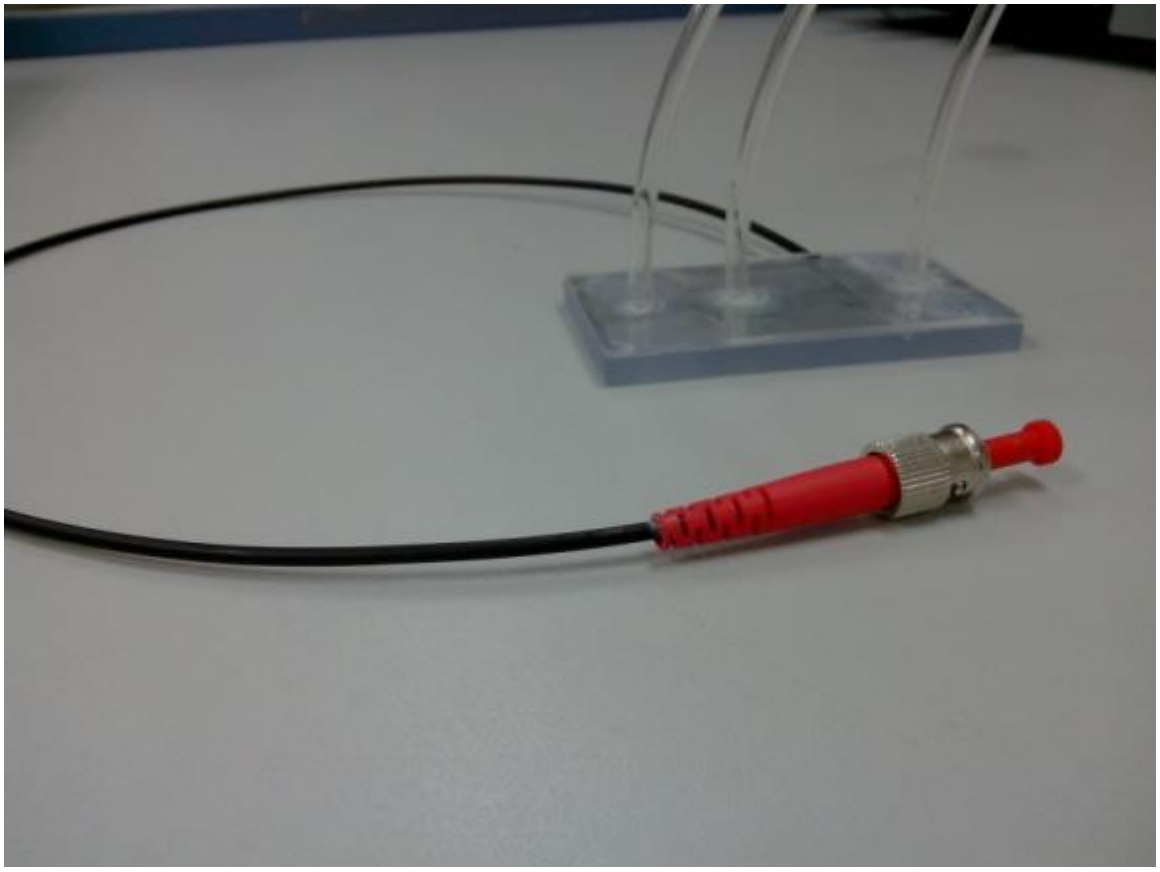

Figure 4: Completed Glued Tube Fitting.

3. RESULTS AND DISCUSSION

3.1 Sensor Readout

A complete microchannel sensor as in Figure 4 was integrated with an existing optical equipment as shown in Figure 5. Red light beams of 850nm from Light Emitting Diode (LED) was chosen in order to see the transmitted signal through the fiber glass. The transmission signal would be unable to occur if the optical fiber was not in the right place. During the optical fiber alignment, the separation of fiber optics needed to be tested for its transmitting signal via an oscilloscope to show the incident. The displayed square wave signal on the output will prove if the transmitting signal had occurred.

During the transmission of the red light beam to the Receiver Module, the output on this red light beam at the other end of the Receiver Module was measured in watt units or dBm. The reading for both input and output via oscilloscope were in frequency; Hz unit. The reading was taken from the difference in frequency; $\Delta f = f_i - f_o$, due to the changes of signal that could only be seen through the input (f_i) and output (f_o) value of frequency. The change in frequency proved that the detection of single particle had occurred.

Figure 6 illustrates the image of 1mm width of the downstream that was captured using CCD Camera before applying the sheath and sample fluid. The red light through the fiber glass proved that the transmission signal was occurring and it was in good condition for

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transmitting light based on the same frequency;1kHz via the oscilloscope. The optical fiber cable that was tested for its transmission signal before and after the cutting process showed that the frequency from the input to the output was the same but only differed in its amplitude value. Table 1 tabulates these readings from the transmitting signal before the cutting process, while Table 2 tabulates these readings from the transmitting signal after the cutting process.

Figure 5: Completed microchannel sensor integrated with optical equipment.

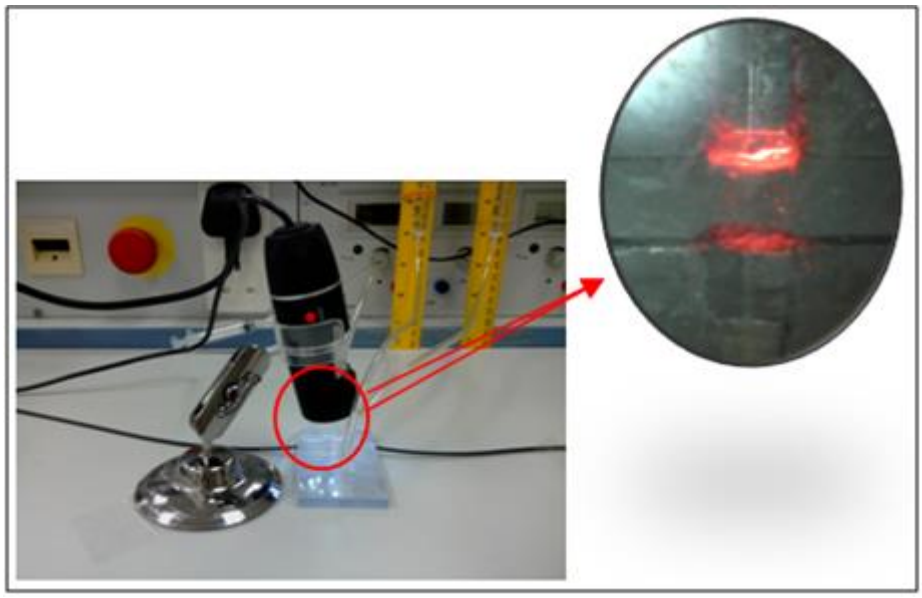

Figure 6: Image of 1mm width of the downstream is captured by using CCD Camera. Red light proved the occurence on the signal transmission.

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Table 1: Readings of Transmitting Signal before Cutting Process.

TYPES OF SIGNALS	INPUT (YELLOW COLOUR)	OUTPUT (BLUE COLOUR)
Frequency	1kHz	1kHz
Amplitude	67.0mV	56.0mV

Table 2: Readings of Transmitting Signal after Cutting Process.

TYPES OF SIGNALS	INPUT (YELLOW COLOUR)	OUTPUT (BLUE COLOUR)
Frequency	1kHz	1kHz
Amplitude	67.0mV	49.0mV

3.2 Analysis Of Hydrodynamic Flow Focus Stream

In this experiment, ink as the sample liquid and water as sheath liquid were used. The densities of the two liquids were assumed as 1000 kg/ms^3 respectively Figure 7 shows a schematic diagram of a single particle detection experimental setup for this experiment. As shown earlier in Figure 1, the stream in interest, Qi was focused and sheathed downstream. By manipulating the flow rates of the focused flow, location of the focused sheet can be found to achieve the best focused stream width (W_f). The flow cytometer technique was applied for counted and examined the single particles since it provided a precise control on the focused stream width.

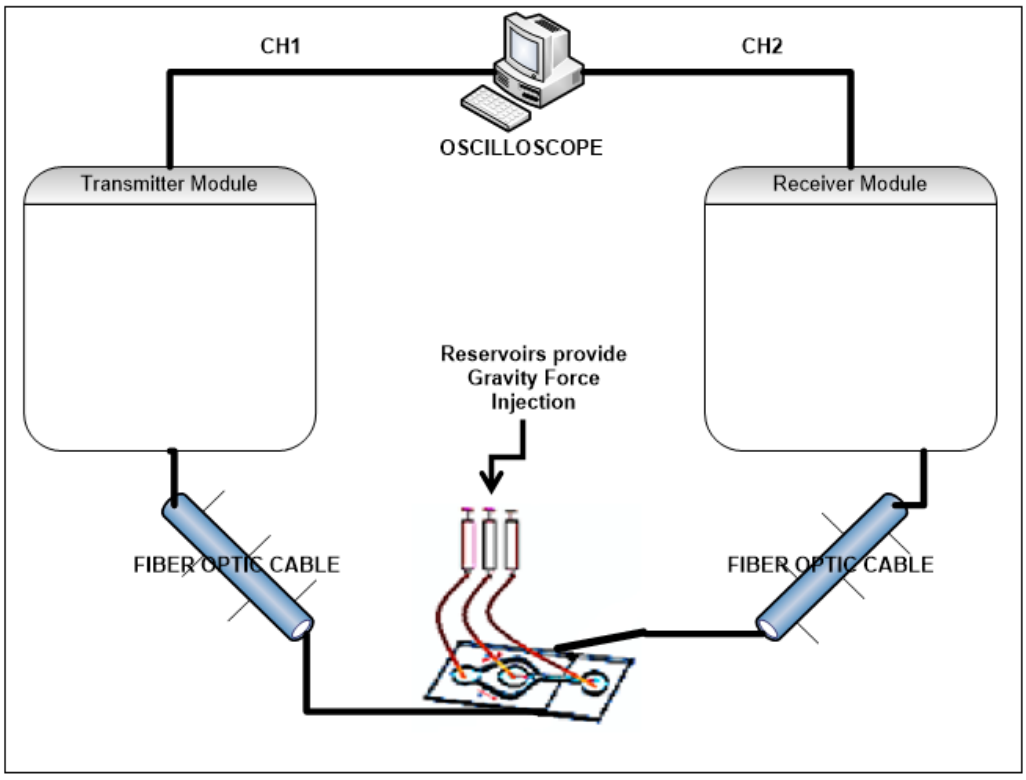

Figure 7: Schematic Diagram of the Single Particle Detection Experimental Setup.

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For the Y-junction micro-channels, the relationship between the focused stream width (W_f) and the volumetric flow rates of the inlet channel (Qi) and the sheath inlet (both Qs) (Rieseberg et al., 2001), can be expressed as (1);

$$\frac{W_f}{W_o} = \frac{Q_i}{Q_i + 2Q_s} \tag{1}$$

where,

W_f = focused stream width (m)

W_O = outlet channel size

Qi = inlet channel flow rate

Q_S = sheath inlet flow rate

So,

$$\frac{W_o}{W_f} = \frac{Q_i + 2Q_s}{Q_i}$$

$$\frac{W_o}{W_f} = 1 + 2\frac{Q_s}{Q_i}$$

Assume that the flow rates ratio as n, thus,

$$\frac{Q_s}{Q_i} = n$$

Therefore,

$$1 + 2n = \frac{W_o}{W_f}$$

$$\frac{W_o}{1 + 2n} = W_f \tag{2}$$

Using this simplified equation as in (2), the data for n and W_f with fixed outlet channel was tabulated in Table 3. Subsequently, the graph in Figure 8 showed that W_f was exponentially proportionate to n. Therefore, results obtained from Table 3 and Figure 8 show that the micro-flow cytometer device that has been developed in this project had fullfiled the theoretical value as in (2).

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Table 3: Result obtained from the experimentation.

n	W_f (mm)=W_o/(1+2n)	W_o (mm)
15	0.016129032	5.00E-01
14	0.017241379	5.00E-01
13	0.018518519	5.00E-01
12	0.020000000	5.00E-01
11	0.02173913	5.00E-01
10	0.023809524	5.00E-01
9	0.026315789	5.00E-01
8	0.029411765	5.00E-01
7	0.033333333	5.00E-01
6	0.038461538	5.00E-01
5	0.045454545	5.00E-01
4	0.055555556	5.00E-01
3	0.071428571	5.00E-01
2	0.100000000	5.00E-01
1	0.166666667	5.00E-01

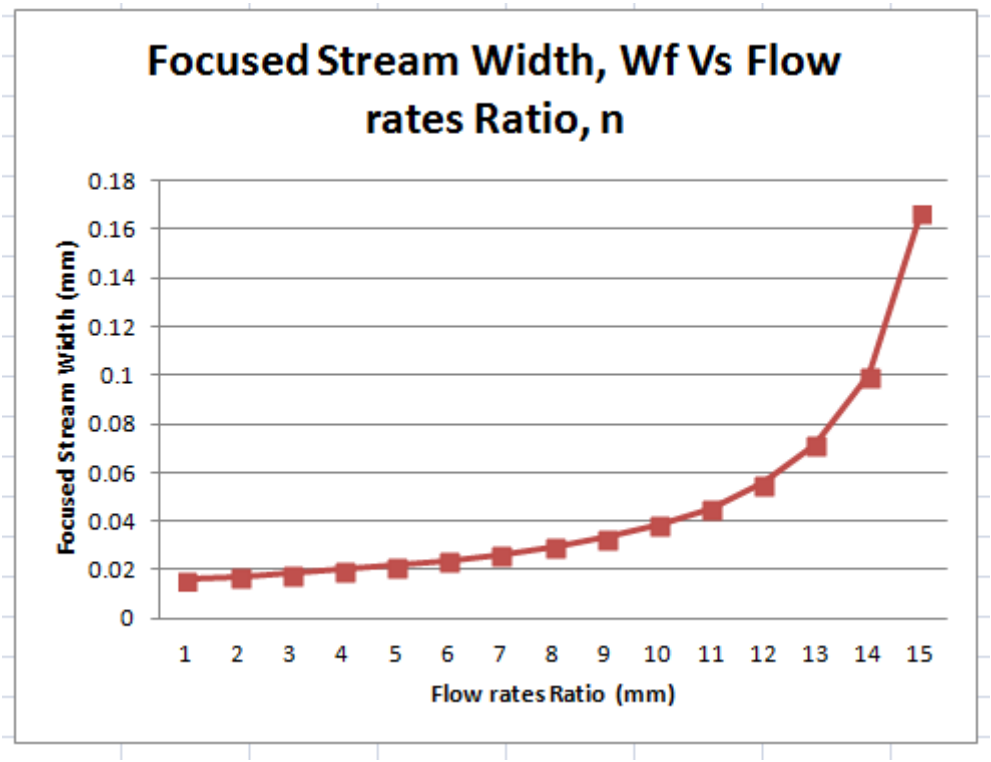

Figure 8: Data obtained from the experimental setup.

4. CONCLUSION

The fabrication of a microdevice for the desired size of 70mm x 40mm with the thickness of 5mm and depth of each channel at 0.5mm was completely done with the development of the micro-flow cytometer device. The fabricated micro-channel can be used as a reusable device to detect a single particle. The characterization of hydrodynamic flow had been constructed

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by using the main device which was the optical fiber for a single particle detector. The experimental setup to evaluate the flow rates ratio towards focused stream width was found to have fulfilled the theoretical equation in which the focused stream width had increased exponentially proportionate to the increase in the value of the flow rates ratio.

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