Research Title:

Development of Digital Holographic Microscope for Three-Dimensional (3D) Microfluidics Application

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1.0 Introduction

Mixing process in microfluidic devices has been widely employed in bio-, nano-, and environmental technologies for biomedical and health related issues. It also extensively applied in food and chemical industries. Many micromixers have been developed before for the aforementioned applications. The word 'mixing' defines a physical process where both stirring and diffusion occur at the same time.

In biomedical application, Tay et al. (2016) used micromixer for early detection of malaria and diagnosis to enhance the sensitivity of the test. This microfluidic device is very practical as a processing step to remove interfering blood cells to focus on malarial parasites. Next, Yao et al. (2013) used micromixer to monitor the level of blood sugar in a body. Owing to its short analysis time, low reagent consumption and multi-process integration, this microfluidic mixer is replacing the conventional diagnostic system. On the other hand, in chemical application, Karthikeyan & Sujatha (2017) improved mixing performance by fabricating a herringbone type of micromixer to detect heavy metal ions in groundwater. In particular, Arsenic and mercury ions in water can cause liver damage, discoloration of hands and feet, kidney damage, lung cancer and neurotic disorder. Therefore, detection and quantification of these ions are essential for health care and serious diseases prevention.

According to Lee et al. (2011), the purpose of microfluidic mixing process is to improve the efficiency of mixing by reducing the characteristics size of microscale devices within shorter mixing channels. Besides that, it aims to attain a thorough and rapid mixing of multiple samples in microfluidic devices. Sample mixing is obtained by improving the diffusion effect between different fluids flow. They also emphasized on the advancement of efficient mixing process to realize the overall microanalysis system approach and lab-on-a-chip systems. According to Capretto et al. (2011), microfluidic mixing can be classified as either "active" or "passive". In an active mixing scheme, to accelerate the mixing process, an external energy force is exerted to perturb the fluids sample. Meanwhile, passive mixer used specially designed microchannel to reduce the diffusion length, and maximize the samples' contact area and contact time.

2.0 Problem Statement

Othman et al. (2015) studied micro mixing of water and organic phase in a co-flow glass capillary device to produce synthetic polymeric biodegradable nanoparticles. They carried out the measurements using transmission electron microscopy. Characterization of any sample using the microscope is technically very challenging, as it needs extremely thin sections of specimens, usually about 100 nm. Chen et al. (2016) conducted intensive numerical and experimental study to assess the species mixing performance of micromixer with serpentine microchannels. The concentration profiles of the species were measured using a stereoscopic microscope. However, this type of microscope has a low magnification and makes it difficult to distinguish individual cells. In a different study, Maeki et al. (2017) analysed the effect of lipid concentration and mixing performance on the lipid nanoparticles' sizes within microfluidic devices using a confocal microscope. Although they managed to understand lipid nanoparticles formation mechanism and its fluid dynamics, this type of microscope is not a truly three-dimensional measurement technique.

Following this, a number of studies have been conducted using digital holographic microscopy to overcome the aforementioned limitations. For instance, Ooms et al. (2009) experimented a three-dimensional flow in a T-shaped micromixer by means of digital holographic microscopy (DHM). It is to overcome smaller-depth-of-field associated with conventional optical microscopy. Besides that, Bettenworth et al. (2014) also used the microscopy technique in monitoring wound healing in vitro and providing multimodal quantitative information of cellular changes upon cytokine stimulation. This is because the non-imaging electrical analysis approach lack the ability for simultaneous assessment of cellular morphology and mass alterations. To overcome the limitation of conventional digital microscope, Zetsche et al. (2016) used DHM to study behaviour and physiology of mucus released by the cold-water coral *Lophelia pertusa*. Detailed microscale observation and visualization of transparent mucoid substances in real time without staining can be easily achieved using DHM. Campos et al. (2018) also successfully demonstrated the application of DHM as a label-free and non-pertubing means to quantify lipids droplets in differentiating adipocytes in a robust medium to high throughput manner.

In short, digital holographic microscopy is a promising three-dimensional fluid flow measurement technique. However, commercially available digital holographic microscopes are

prohibitively expensive and beyond the means of most research institutions with tight budget. The commercial microscope is also strictly limited to fluid moving in horizontal direction. Therefore, it is imperative to develop a custom-made digital holographic microscope with the same capabilities of the commercial ones. In addition, the proposed holographic microscope would have an extra capability to observe and visualize fluid under the influence of gravitational force i.e., in vertical direction. For this reason, the research objectives have been set as follow.

3.0 Objectives

The main objectives of this project are:

(a) To design, manufacture, assemble and calibrate digital holographic microscope.

(b) To design and fabricate a microfluidic Y-junction micromixer by laser micromachining.

(c) To experimentally investigate 3D flow dynamics of miscible fluids using the developed digital holographic microscope.

4.0 Literature Review

4.1 Microfluidic Mixer

Ooms et al. (2009) experimented a three-dimensional flow in a T-shaped micromixer using digital holographic microscopy to overcome smaller depth-of-field associated with conventional optical microscopy. In this experiment, a Nd: YLF laser with a wavelength of 527 nm and maximum pulse energy of 10 mJ was used. To reduce the deformation of the light beam passing through the T-mixer, high quality glass plates microchannel were used. The system consists of a pressure vessel made of stainless steel and provides constant mass flows to both inlets using two controllers. It can be concluded that digital holographic microscopy is a suitable method to carry out three-dimensional velocity measurements of time independent microscopic flows with sufficient accuracy and spatial resolution. Using high speed multiple frame measurements to record the particles in multiple consecutive holograms proved that microscopy could successfully follow the particles in a three-dimensional measurement domain.

4.2 Three-Dimensional Measurement Technique

Bishara et al. (2010) implemented lens-free on-chip holographic microscopy over a wide field-of-view using a sub-pixel shifting based super-resolution algorithm. This is to solve the problem at the detector array, which involved the finite pixel size, and to retrieve digitally recorded holograms of the objects with much higher resolution. The technique applied was Pixel Super-Resolution. To get a better approximation of image sampling on a higher resolution grid, they used diversified lower-resolution images. It deviated to one another by division of low-resolution grid constant. A light source with wavelength of 500-600 nm, 50 µm core size of optical fiber and CMOS sensor with 2.2 µm × 2.2 µm pixel size were used. In order to evaluate the improvement of spatial resolution due to Pixel Super-Resolution, they fabricated a calibration object consisting of 1-µm wide lines etched into a glass cover slide with 1 µm separation between the lines. The result showed that the fine features of the object could be seen clearly by applying the Super-Resolution hologram. The experiment was furthered by applying this resolution scheme to illustrate a sample of whole blood smear. A source with wavelength of 500 nm was used and 36 sub-pixel shifted holograms have been captured. The technique used permitted resolving cell clusters and it would be difficult to resolve from

processing a single lower-resolution hologram. In conclusion, this Pixel Super-Resolution approach is a big success since it can effectively retrieve much higher resolution digital holograms of the objects, allowing sub-micron spatial resolution to be gained across the entire sensor chip active area.

Phillips et al. (2011) combined the scanning probe microscopy (SPM) and optical tweezers to diversify the orientation and location of probe held by two optical traps. The problems with SPM is that it can only work in a direction that is perpendicular to the surface while optical tweezer is sensitive to low forces restricted by Brownian motion and the size of the particle. In this study, they used an inverted bright field microscope with numerical aperture of 1.4, lens with magnification of 100×, motorized XY stage and a closed loop piezoelectric objective lens positioning system. The trapping beam is supplied by a 3.2 W Nd:YAG laser with 1064 nm emitted wavelength. A type of microorganism known as diatom Nitzschia acicularis of cylindrical in shape was used in the study to characterize their proposed technique.

4.3 Digital Holography Method

Anand et al. (2010) investigated the dynamics of phase objects using real-time digital holographic microscopy. This is to overcome the limitation of the conventional microscopy to image the micro-objects as most of them are transparent to the visible radiations and it only provides the object information at certain object plane due to the definite depth of focus. A He-Ne laser with wavelength of 611 nm and maximum output power of 2 mW, a 10× microscopic objective with numerical aperture of 0.25 and CCD camera with 8-bit dynamic range were used in the study. They used several phase object i.e., onion skin and a section of polymethyl methacrylate (PMMA) slab with an embossed letter on it for realization of phase contrast imaging. The embossed PMMA slab was exposed to iron cylinder with diameter of 6 mm and temperature up to 393 K (heat source) and acted as dynamic micro-object. It was proved that digital holographic microscopy could be an effective tool to analyse the dynamic phenomena occurred in phase object when it was subjected to heat.

Shin et al. (2010) presented an optofluidic three-dimensional sensing using a microfluidic device and digital holographic microscopy. A recognition system using statistical algorithms was employed for real-time identification of microorganisms. In this study, they used a beam splitter, He-Ne laser with 633 nm wavelength, a microscope objective with magnification of 40× and numerical aperture of 0.65, single hollow channel with 200 μm wide

and coverslip glass with 170 µm thick. For the microorganisms, they used 20-40 µm of Euglena acus and Chilomanas. As a result, the sensing and recognition of three-dimensional microorganisms for the samples was successful. Thus, it was proved that optofluidic three-dimensional sensing and identification system of microorganisms with digital holographic microscopy is a feasible technique.

Choi et al. (2011) investigated the direction of particles migrate in a square microchannel by using digital holographic microscopy technique. The problem arose when the flows in the channel were largely disturbed by the presence of the particles, causing bad interactions between particles and the wall. In this experiment, polystyrene microspheres with mean diameters of 7 µm to 15 µm were used at a dilution of 0.05% water solution. Other than that, 100 mm of two transparent borosilicate square capillaries with diameters of 84 µm and 94.3 µm were used as the microchannels. Several governing factors affect the particle separation. These include size of the particle, channel length and width, and channel Reynolds number (Rc). This study concluded that the particles migrate in lateral direction before cross-laterally toward the four equilibrium positions.

4.4 Three-Dimensional Microfluidics Application

Langehanenberg et al. (2009) studied the sedimentation of red cells and fibrosarcoma cells in a collagen tissue model by using digital holographic microscopy (DHM). The purpose of this study is to ensure that DHM able to track three-dimensional cell dynamically and automatically without mechanical focus adjustment. The Germany Zeiss-type microscope lens and tube lens were used to magnify the object wave. Meanwhile, a 1280x960 pixels digital image sensor recorded the super-position of object wave. The sedimentation process of red cell in human was done in physiological solution onto a zwitterionic-polymer-coated cover slip and result in a phase unwrapping efficiency of 100%. On the other hand, human fibrosarcoma tumor cells were observed in a Petri dish. High phase noise level in collagen tissue model caused its performance decreased to an average of 75%. At the end of this study, digital holographic microscopy succeeded in tracking and analysing the shape of cells on the coated surfaces. This proves that DHM can provide quantitative phase contrast with multifocus imaging from a single recorded hologram.

Tamrin et al. (2015) studied and analysed the aggregation in blood related products by means of digital holographic microscopy. This developed technique aimed to maximize the

amount of detected particle images in real time and spatially remove the noise contributed by the undiffracted beam and virtual images. A 523 nm wavelength Elforlight diode pumped solid state Nd:YLF laser was used in this study. They also used a 50× long working distance microscope objective of 0.55 numerical aperture. A closed path was constructed in PVC tubing and a Watson-Marlow peristaltic pump at 110 rpm drove its flow. The background noise can be removed by coherent subtraction using a reference hologram. Based on the result, all the holograms showed an increased probability of particles clustering with a peak in the radial distribution function at a radial distance of about 5 µm. It also showed that approximately 10 particles pairs from the 1500 particles cohort were aggregating and these numbers were not a time-controlled as they did not change as the time increase. In conclusion, digital holographic microscopy is able to record the instantaneous three-dimensional position of particles in flow volume.

Zetsche et al. (2016) used digital holographic microscopy to study the behaviour and physiology of mucus released by the cold-water coral Lophelia pertusa. Previously, conventional light microscopy was confronted with an important limitation where the technique can only track the opaque particles i.e., graphite, disallowing the visualization of transparent mucus in aqueous solutions, making it difficult to distinguish different types of mucus. For the experiment, they used L. pertusa fragments consisting 9-15 polyps, acrylic cylinders flow chambers with inner diameter of 9.9 cm, and peristaltic pump to ensure the chambers' water circulation. An off-axis self-interference approach based on the principle of differential digital holographic microscopy with a 630 nm red LED light and Leica 4× objective were used. Based on the measurement of optical path length difference with a state-of-the-art holographic microscope, in response to different stimuli, ranging from mucus strings, sheaths and 'string balls', the result demonstrated that L. pertusa produced various type of mucus. In conclusion, digital holographic microscopic technology allows microscale observations and permits the real time visualization of transparent mucoid substances without staining through optical path length difference measurements in L. pertusa.

Campos et al. (2018) presented digital holographic microscopy as a label-free and nonperturbing means to quantify lipid droplets in differentiating adipocytes in a robust medium to high throughput manner. Most of the previous method depended on the steps of liquid handling, included the procedures of imaging and image analysis, which needed lengthy process optimizations and thus making it challenging to reproduce quantitatively. For the materials, they used the bone marrow-derived MSC line OP9 cells, acoustic dispenser, and

differentiation cocktail containing dexamethasone, insulin and isobutyl-methylxanthine. A low intensity laser with wavelength of 684 nm, black wall imaging plates precoated with poly-ornithine, healthcare-imaging system, and microscope objectives with 10× magnification, 0.3 numerical aperture and 20× magnification, 0.4 numerical aperture were used. A MATLAB software was used to reconstruct the best-focus images automatically, and the phase signal or difference in optical path was automatically measured using a fixed threshold value. Digital holographic microscopy proved to be more sensitive than fluorescence microscopy, allowing detection of differences in adipocyte differentiation up to three days earlier. In conclusion, besides lowering the costs in consumables, this lens-free holography microscope is a powerful tool that can give various information related to cell morphology in a nonpertubing manner, therefore reflecting true physiologically relevant measurements.

4.5 Summary

In summary, there are various microscopy techniques to visualize, detect and locate three-dimensional particles in microfluidics devices. All the techniques described have their own pros and cons. With advanced technology, it is important to develop a low-cost imaging technique with high resolution and high magnification in investigating and probing three-dimensional flows in microfluidics devices. Therefore, a custom made digital holographic microscope will be developed with extra capability as it can measure and characterize the fluid under the influence of gravitational force.

5.0 Proposed Method

5.1 Development of Digital Holographic Microscope

In order to observe and visualize three-dimensional flows in microfluidics device with high accuracy, the design parameters for such a development including setup of the holographic system are crucial. Figure 5.1 shows the development method used for developing digital holographic microscope, and Figure 5.2 shows the CAD design of optical setup.

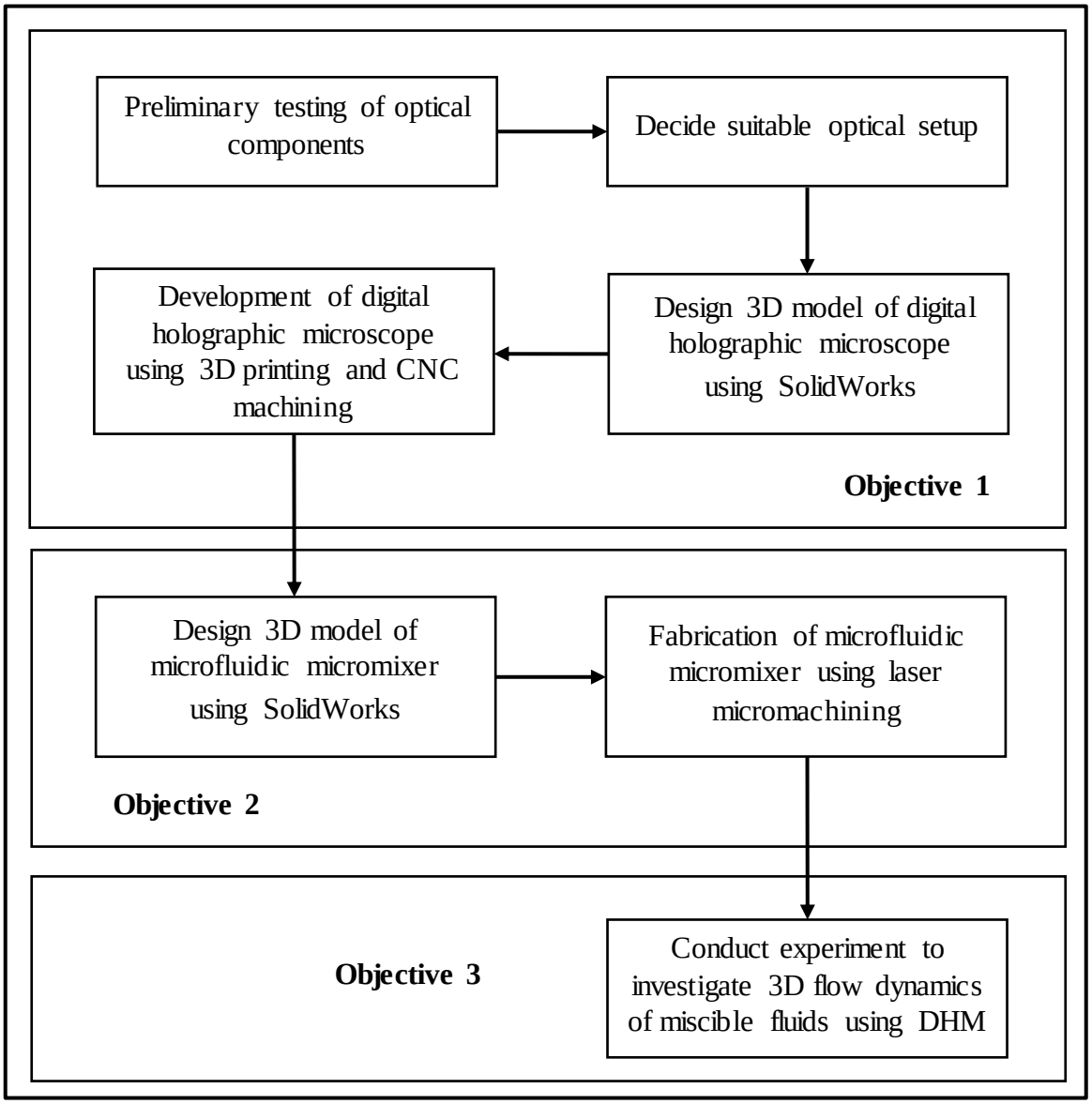

***Figure 5.1**: Methodology for developing digital holographic microscope*

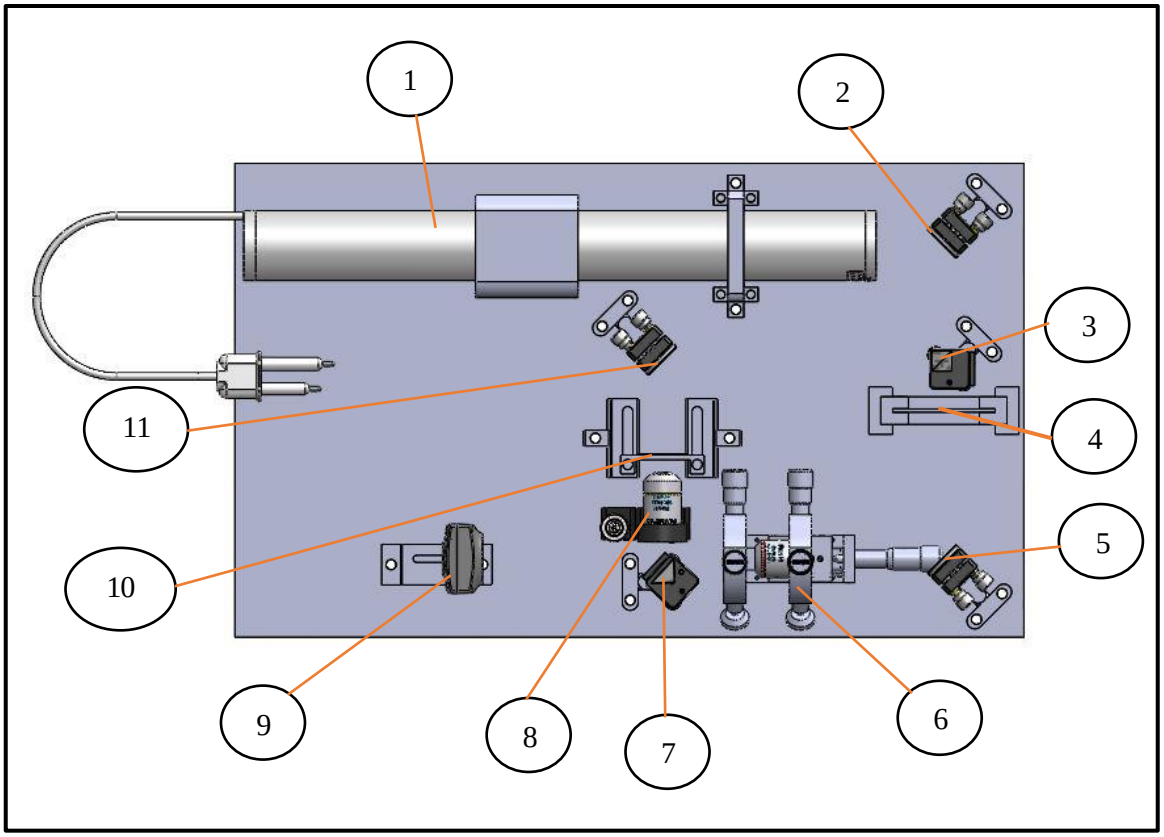

***Figure 5.2**: CAD design of optical setup*

***Table 5.1**: List of optical components*

No.	Optical component
1	He-Ne laser
2, 5, 11	Round mirrors
3	Beam splitter
4	Linear step ND filter
6	Spatial filter
7	Right angle prism mirror
8	10× microscope objective
9	CMOS camera
10	Sample (miscible fluids)

5.1.1 Component Fabrication by CNC Machining

A multi-axis milling machine (X-Carve) will be used to fabricate the entire base plate (300 mm × 500 mm × 8 mm) to mount all optical components. As shown in Figure 5.3, it is powered by a DeWalt 611 spindle.

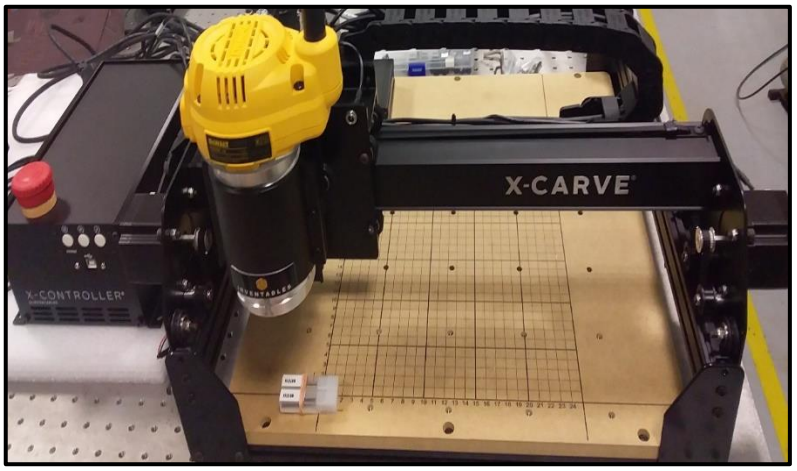

***Figure 5.3**: 500 mm × 500 mm multi-axis milling machine*

5.2 Fabrication of Microfluidic Channel

The microfluidic Y-junction micromixer will be fabricated using the CO_2 FABOOL Laser Mini (Figure 5.4) by FABOOL software. Two different types of liquids will be respectively channeled into reservoir 1 and reservoir 2, as shown in Figure 5.5, by using a dual syringe pump (Figure 5.6). The liquids flow to the inlet 1 and inlet 2 and then mixed at the Y-junction.

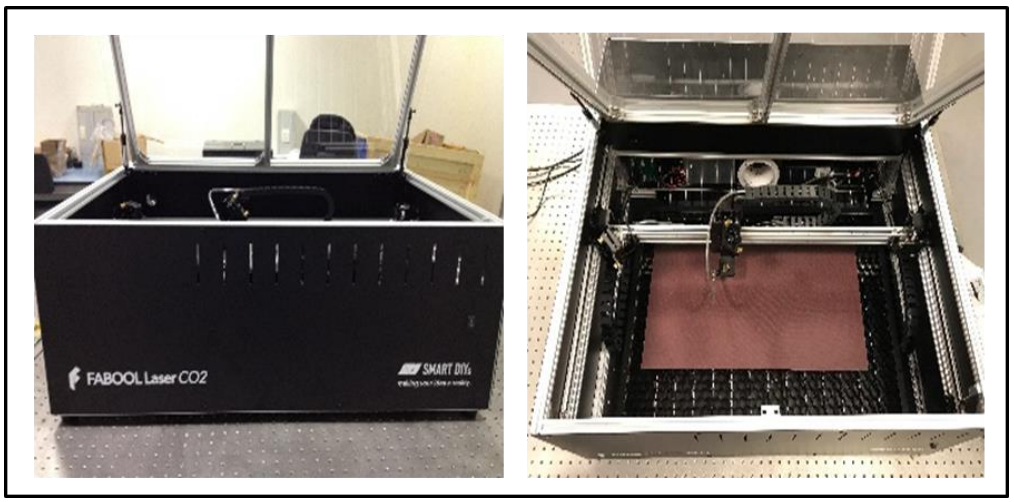

***Figure 5.4**: CO_2 FABOOL Laser Mini*

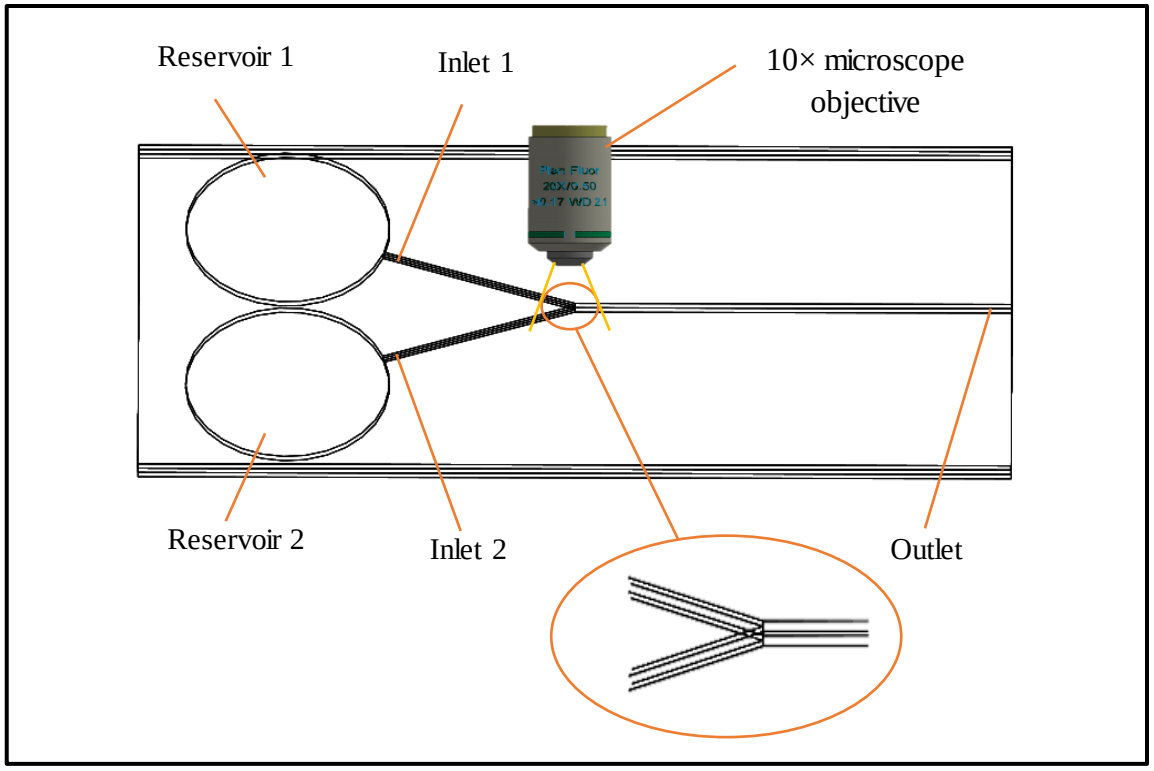

***Figure 5.5**: 3D model of 30 mm × 15 mm microfluidic Y-junction micromixer*

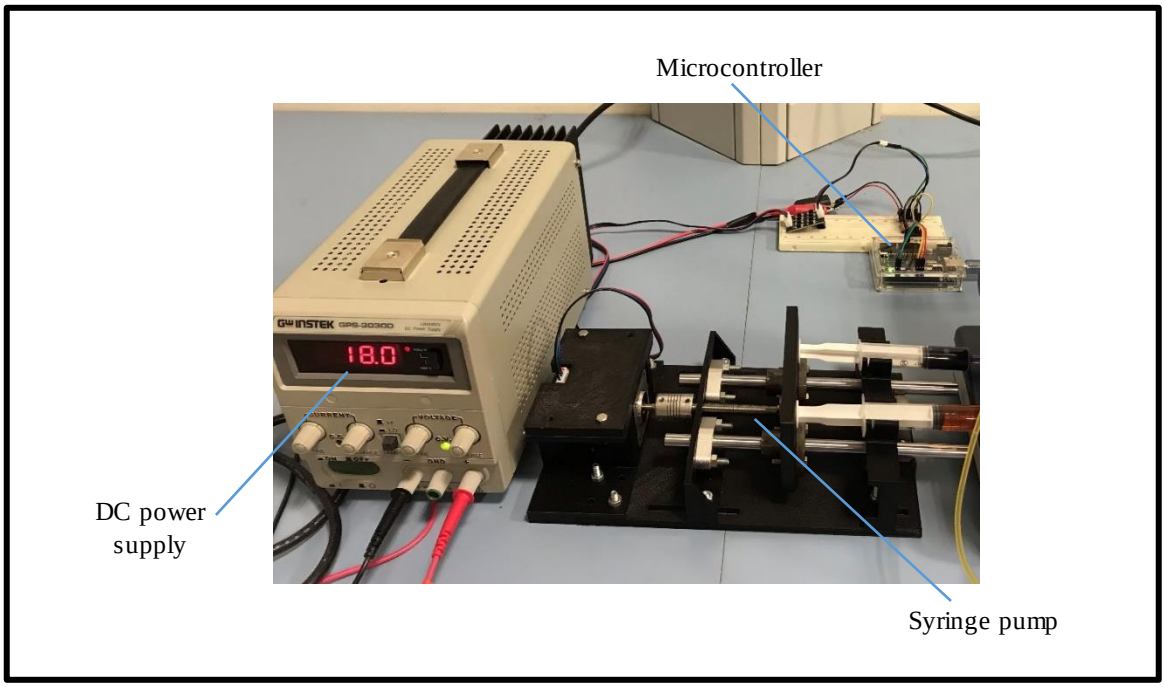

***Figure 5.6**: Syringe pump setup*

6.0 Proposed Conclusion

Upon the completion of this project, this research expects outcomes as below:

1. A low-cost and functioning digital holographic microscope will be manufactured and calibrated. This process will involve programming work to interface synchronize CMOS camera and illumination source.
2. The developed microscope will be able to distinguish individual particles and measure micrometer thin sections of specimens.
3. Custom-made digital holographic microscope can be used to observe and visualize miscible fluids that move either in horizontal or vertical direction, under the influence of gravitational force.

7.0 Proposed Timeline

***Table 7**: Project timeline*

Gantt Chart																
	Year	**2018**				**2019**										
	Month	**Sept**	**Oct**	**Nov**	**Dec**	**Jan**	**Feb**	**Mar**	**Apr**	**May**	**June**	**July**	**Aug**	**Sept**	**Oct**	**Nov**
1	**Literature study**															
2	**Optical alignment, calibration and code programming of DHM**															
3	**Experimental investigation of optimum laser micromachining parameters for fabricating microfluidic Y-junction micromixer**															
4	**Preliminary experiment**															
5	**3D trajectories of moving particles in miscible fluid assessment in Y-junction micromixer by DHM**															
6	**Documentation**															

8.0 References

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