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DEVELOPMENT OF AN OFF-AXIS DIGITAL HOLOGRAPHIC MICROSCOPE FOR THREE-DIMENSIONAL (3D) MICROFLUIDICS APPLICATION

Hawa Anak Ringkai

Bachelor of Engineering with Honours

(Mechanical and Manufacturing Engineering)

2019

UNIVERSITI MALAYSIA SARAWAK

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Final Year Project Report ☑

Masters ☐

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DEVELOPMENT OF AN OFF-AXIS DIGITAL HOLOGRAPHIC MICROSCOPE FOR THREE-DIMENSIONAL (3D) MICROFLUIDICS APPLICATION

HAWA ANAK RINGKAI

A dissertation submitted in partial fulfilment of the requirement for the degree of

Bachelor of Engineering with Honours

(Mechanical and Manufacturing Engineering)

Faculty of Engineering

Universiti Malaysia Sarawak

2019

Dedicated to my beloved family members and friends.

ACKNOWLEDGEMENT

I would like to express my deepest appreciation to Dr. Khairul Fikri bin Tamrin, my supervisor for the assistance and helpful guidance throughout this final year project. I am also grateful for the professionalism, compassion and patience shown by him during this period that helped me a lot toward the completion of this project.

To the lecturers and friends, especially from the Department of Mechanical and Manufacturing Engineering, Faculty of Engineering UNIMAS, I would like to express my sincere gratitude for the continuous support I received throughout this project period. Finally yet importantly, I would like to pay my highest respect to my family, who keeps giving me positive motivation towards life and challenges, to strengthen me, both physically and mentally.

Thank you.

ABSTRACT

Mixing process in microfluidic devices has been widely employed in bio-, nano-, and environmental technologies for biomedical and health related issue i.e., early detection of malaria and diagnosis, and monitor the level of blood sugar in a body. It also extensively applied in chemical industries to detect heavy metal ions in groundwater. Digital holographic microscopy (DHM) is a promising three-dimensional fluid flow measurement technique as it can easily measure detailed microscale observation and visualization of flow field in real time. DHM uses digitally recorded hologram and computer algorithm to calculate the image of the object wave front. However, commercially available digital holographic microscope is not affordable to most research institutions and it is strictly limited to fluid moving in horizontal direction only. Therefore, it is imperative to develop a custom-made digital holographic microscope with an extra capability to observe and visualize fluid under the influence of gravitational force. The optical system was initially designed using a CAD software. Optical component and holders were fabricated using a 3D printer while the housing for the entire optical system was machined using a multi-axis milling machine. Linear step neutral density (ND) filter was used for calibration purpose. Once calibration of the optical system using Fourier Transform method was done, a custom-made microchannel was laser-cut to ensure precise dimensional accuracy. Thereafter, three-dimensional experimental flow mixing of 10-µm polystyrene microsphere suspended in water with Magnaflux Carrier II petroleum distillate carrier oil was conducted inside the fabricated microchannel. To get better and clearer images, the working distance of 10× microscope objective was increased from 16.5 mm to 18 mm. With resolution of 1280 pixels × 1024 pixels ThorCam camera, 10 µm microspheres in water can be seen clearly inside the 634 µm interior wall of the fabricated microchannel. The calculated total field of view of the camera is 853 µm × 683 µm. The experiment was successfully demonstrated using the developed digital holographic microscope. In order to improve the overall performance of the system, higher power objective lens and higher power laser are suggested to be used in the future.

ABSTRAK

Proses pencampuran dalam peranti mikrofluidik telah digunakan secara meluas dalam bio-, nano-, dan teknologi persekitaran untuk isu berkaitan biomedikal dan kesihatan, iaitu pengesanan awal malaria dan diagnosis, dan memantau tahap gula darah dalam tubuh. Ia juga digunakan secara meluas dalam industri kimia untuk mengesan ion logam berat di dalam air bawah tanah. Mikroskop hologram digital adalah teknik pengukuran aliran cecair tiga dimensi yang menjanjikan kerana ia dapat mengukur dan memerhatikan skala mikro secara terperinci dan menggambarkan medan aliran dalam masa nyata. Mikroskop ini menggunakan hologram digital dan algoritma komputer untuk mengira imej objek gelombang depan. Walau bagaimanapun, mikroskop hologram digital yang tersedia secara komersial sangat mahal dan di luar kemampuan kebanyakan institusi penyelidikan dan ia hanya terhad kepada cecair yang bergerak dalam arah mendatar sahaja. Oleh itu, adalah penting untuk mencipta mikroskop hologram digital dengan keupayaan tambahan untuk memerhatikan dan menggambarkan cecair di bawah pengaruh daya graviti. Sistem optik pada mulanya direka menggunakan perisian CAD. Komponen dan pemegang optik telah direka menggunakan pencetak 3D manakala perumah bagi keseluruhan sistem optik telah dimesin menggunakan mesin penggilingan pelbagai paksi. Penapis ketumpatan neutral (ND) langkah linear digunakan untuk tujuan penentukuran. Sebaik sahaja penentukuran sistem optik menggunakan kaedah Fourier Transform dilakukan, sebuah saluran mikro dibuat khas untuk mengurangkan ketepatan dimensi yang tepat. Selepas itu, percampuran aliran percubaan tiga dimensi mikrosfera polistirena 10-μm dalam air dengan minyak pembawa minyak penyulingan Magnaflux Carrier II telah dijalankan di dalam saluran mikro yang direka. Untuk mendapatkan imej yang lebih baik dan jelas, jarak kerja 10 × mikroskop objektif telah meningkat daripada 16.5 mm hingga 18 mm. Dengan resolusi 1280 piksel × 1024 piksel kamera ThorCam, 10 μm mikrosfera dalam air dapat dilihat dengan jelas di dalam 634 μm dinding dalaman saluran mkro yang direka. Medan pandangan yang dikira adalah 853 μm × 683 μm. Eksperimen ini berjaya ditunjukkan menggunakan mikroskop hologram digital yang dibangunkan. Untuk meningkatkan prestasi keseluruhan sistem, lensa objektif kuasa yang lebih tinggi dan laser kuasa yang lebih tinggi dicadangkan untuk penggunaan pada masa akan datang.

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LIST OF ABBREVIATIONS

3D3C	-	Three-Dimensional Three-Component
ArF	-	Argon Fluoride
ATCC	-	American Type Culture Collection
CAD	-	Computer-Aided Design
CCD	-	Charge-Coupled Device
CFD	-	Computational Fluid Dynamics
CHT	-	Circular Hough Transform
CMOS	-	Complementary Metal-Oxide Semiconductor
CNC	-	Computer Numerical Control
DDPIV	-	Defocusing Digital Particle Imaging Velocimetry
DHM	-	Digital Holographic Microscopy
DOC	-	Depth of Correlation
DPSS	-	Diode-Pumped Solid-State
EWOD	-	Electrowetting-on-Dielectric
He-Ne	-	Helium-Neon
ITO	-	Indium-Tin Oxide
LED	-	Light-emitting Diode
MATLAB	-	Matrix Laboratory
Nd: YAG	-	Neodymium-doped Yttrium Aluminium Garnet
Nd: YLF	-	Neodymium-doped Yttrium Lithium Fluoride
p-ATP	-	p-Aminothiophenol
PIV	-	Particle Image Velocimetry
PMMA	-	Poly-methyl Methacrylate
PTV	-	Particle Tracking Velocimetry
PVC	-	Polyvinyl Chloride
SERS	-	Surface-enhanced Raman Spectroscopy
SPM	-	Scanning Probe Microscopy
SPSS	-	Statistical Package for the Social Science
TIRFM	-	Total Internal Reflection Fluorescence Microscopy

LIST OF SYMBOLS

λ - Light wavelength

C - Width of aperture

De - Dean number

D_h - Microchannel hydraulic diameter

k - Coherent light constant

n_1 - Refractive index of air

n_2 - Refractive index of cover glass before detector array

NA - Numerical aperture of imaging system

R - Radius of convex surface’s curvature

Re - Reynolds number

Rc - Channel Reynolds number

R_T - Theoretical resolution

$S_{aperture}$ - Aperture shift

$S_{hologram}$ - Hologram shift

t - Integration time of recording device

v - Turbid medium velocity

z_1 - Distance between object plane and fiber end

z_2 - Distance between object and detector plane

CHAPTER 1

INTRODUCTION

1.1 Background

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 C.-Y. 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.

1.2 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. X. 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.

1.3 Objectives

The main objectives of this project are:

(a) To design, manufacture, assemble and calibrate an off-axis digital holographic microscope.

(b) To fabricate a microfluidic mixer by laser micromachining.

(c) To experimentally demonstrate flow mixing of dissimilar fluids using the developed digital holographic microscope.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Chapter 2 provides a summary of literature study related to the three-dimensional measurement technique that have been conducted by number of researchers before. The researcher objective, problem statement, apparatus and material, and the result for each literature study have been stated briefly in every section. Besides that, the study of three-dimensional microfluidics in biological field by means of digital holographic microscope also included in this chapter.

2.2 Microfluidic Mixer

Kim & Breuer (2007) introduced the use of bacterial carpets to enhance microfluidic mixing. This is because it is difficult to find a fluidic actuator to improve the mixing between parallel streams of fluids. Attachment of flagellated bacteria onto the solid surface can activate a solid-fluid interface, so that the cell and working fluid will be unmixed. In this study, they used 1 µm diameter and 2 µm long of Serratia marcescens, a wild American Type Culture Collection (ATCC 274). For microfabrication, soft lithography technique was used to fabricate the fluid devices. The fabricated Y-junction microchannel consisted of two arms, and each arm carried fluid into a main mixing channel having dimension of 28 mm long, 15 µm high and 200 µm wide. At the end of this study, it showed that the bacterial carpets could improve the mixing between two streams in the microchannel. However, due to falling pH, which is affected by high carpet metabolism, the device performance decreased. Thus, bacterial carpet is still unsuitable to replace other conventional technique though it is robust and easy to manufacture.

Kuntaegowdanahalli et al. (2009) studied the principle of Dean-coupled inertial migration in spiral microchannels for separating different size of particles. This is because the use of porous membrane filter is not efficient at separating various size particles due to complex fabrication of 3D structures. Standard soft lithography method was used to fabricate five-loop spiral microchannel with two inlets and eight equally spaced outlets. They used polystyrene particles of varying diameters i.e., 10 µm, 15 µm and 20 µm. All these particles come in one mixture. The flow description in the channel is based on Dean number (De) which can be defined as:

$$De = Re\sqrt{\frac{D_h}{2R}} \qquad \text{(Eq. 2.1)}$$

where Re : Reynolds number

D_h : Microchannel hydraulic diameter

R : Radius of convex surface's curvature

The microchannel successfully separated all the particles with efficiency of 90%. They further demonstrated the application of their technique for separating neural cells, achieving 80% efficiency.

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.

Xu et al. (2011) presented a flexible integration of high efficiency silver surface enhanced Raman scattering (SERS) monitor in an extended microfluidic channel.

Previously, the laser-induced fluorescence was used as a high sensitivity optodetection technique but it has some limitations pertaining to detect non-fluorescent product and providing sufficient structural information of the product. In this study, they used a mixture solution of 0.06 M trisodium citrate and 0.08 M silver nitrate aqueous solution. The combination of photolithography and wet-etching technique were used to fabricate the microfluidic channel. An oil immersion objective lens with a numerical aperture of 1.40 and magnification of 100×, 800 nm central wavelength of femtosecond laser pulse, two-galvano-mirror set, and Shimadzu spectrometer were used in the experiment. They managed to fabricate microchannel bed of 75 µm wide and 20 µm deep. In the test of p-aminothiophenol (p-ATP), the detection for SERS signals were chose at three locations, and peak intensity error was not more than 3%, specifying that SERS substrate be in an excellent uniformity. In conclusion, integrated SERS substrates allows the detection of products in real time during chemical reactions, therefore giving credibility for *in situ* detection at various positions.

2.3 Three-Dimensional Measurement Technique

Zhang & Menq (2008) implemented a microscope off-focus image to measure and analyse three-dimensional particle dynamics in a wide-ranging frequency. One limitation of optical tweezer technique is that the used of quadrant photodiodes in the detection system only provides three dimensional position one particle at a time with subnanometer precision. Another limitation is limited measurement range confined by the size of the laser focus of an approximately 1 µm in each axis. To overcome these limitations, they used a modified inverted microscope with total optical magnification of 54× and a complementary metal-oxide semiconductor (CMOS) camera. It is noted that sampling rate is indirectly proportional to the image size. Other than that, thresholding approach was adopted to minimize the errors caused by non-uniform background noise, and to increase the resolution of the measurement. The experimental results proved that all three axes of subnanometer resolution at 40 Hz sampling rate could be obtained using the CMOS camera.

Tien et al. (2008) modified the original single charge-coupled device (CCD) implementation by placing variation of colour filters over each pinhole and used three CCD colour cameras for image acquisition. This method attempts to solve the identification issue of overlapping particle exposures and image saturation caused by numerous exposures of individual particle. A single method with two important modifications have been proposed. First was applying different colour filter onto each of the pinhole apertures. Next modification was by modifying the test section for seeding and illumination methods. These modifications are useful for particle identification as they provided a good contrast between background and the particles' image. The frame grabber was used to capture the images into a computer, and the particle locations have been identified using four stage image-processing procedures. The measured particle location had uncertainty of 0.15% in the in-plane direction and 1.5% of the full depth range in the out-of-plane direction. They concluded that the color-coded backlighted single camera system is suitable for visualization and quantification of micro-scale fluid flows.

Lu et al. (2008) used micro particle image velocimetry to investigate the flow inside droplets actuated by electrowetting-on-dielectric (EWOD) in air. It is to overcome the thin and flat droplet which lead to irrotational and inefficient for mixing. In this study, they used square shaped electrodes with area of 600 µm × 600 µm, an indium-tin oxide (ITO) coated glass wafers, silicon oxide with 500 nm blanket layer, deionized water as working fluid and 2 µm red coloured fluorescent polystyrene microbeads. Besides that, they also used two frequency-doubled Nd: YAG lasers with wavelength of 523 nm, Nikon inverted microscope, CCD camera and 10× objective lens. The result showed that flow of droplet in three dimensions indeed deviated significantly from the parabolic flow profile used in current flow simulations. The two-dimensional data showed two symmetric circulations were within the moving droplet. A simple forward-and-back motion is inefficient for mixing due to the reversibility of the flow.

Speidel et al. (2009) used three-dimensional back focal plane interferometry technique to scan line optical tweezers in one or two dimensions. This improved method uses oscillating lasers to trap and track the particles in all three dimensions. The purpose of this technique is to overcome the inflexibility using single particle video tracking in holographic technique due to physical intrinsic problems concerning axial position tracking. Usually, it requires recording calibration curves for each particle type before measurements and subsequent two-dimensional curve fitting. The setup had two divisions, which were scanning line optical tweezers found in manipulation unit and inline interferometric tracking unit together with two quadrant photodiodes. They used one Watt Nd: YAG laser with wavelength of 1064 nm, Germany acousto-optic modulator, two galvanometric scanning mirrors, 1 mm diameter reference diode, water immersion microscope objective lens, 2× and 4× two beam expanders and 63× water immersion dipping lens in this study. For the sample cell, open chamber was used which consists of 150 µm thick fully transparent cover slip and 200 µl of ultra pure H_20 with 2 µl bead solution. The nominal bead has diameter of 970 nm, density of 1.96 g/cm^3 and 1.37 refractive index. By the end of this study, they concluded that optical traps enable dynamic long-range interaction between particles with enhanced contact probability. The technique used increases the possibility to track several particles' motions in three-dimensional with nanometres precision.

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. The aperture shift and the hologram shift at the detector plane can be determined using simple geometrical optics approximation.

$$\frac{S_{hologram}}{S_{aperture}} = \frac{z_2}{z_1} \times \frac{n_1}{n_2} \qquad \text{(Eq. 2.2)}$$

where $S_{hologram}$: Hologram shift, in µm

$S_{aperture}$: Aperture shift, in µm

z_1 : Distance between object plane and fiber end, in mm

z_2 : Distance between object and detector plane, in mm

n_1 : Refractive index of air (n_1 = 1)

n_2 : Refractive index of cover glass before detector array (n_2 = 1.5)

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