Light Field Imaging Applied to Reacting and Microscopic Flows

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Jonathon R. Pendlebury

A thesis submitted to the faculty of
Brigham Young University
in partial fulfillment of the requirements for the degree of

Master of Science

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December 2014

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ABSTRACT

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Master of Science

Light field imaging, specifically synthetic aperture (SA) refocusing is a method used to combine images from an array of cameras to generate a single image with a narrow depth of field that can be positioned arbitrarily throughout the volume under investigation. Creating a stack of narrow depth of field images at varying locations generates a focal stack that can be used to find the location of objects in three dimensions. SA refocusing is particularly useful when reconstructing particle fields that are then used to determine the movement of the fluid they are entrained in, and it can also be used for shape reconstruction. This study applies SA refocusing to reacting flows and microscopic flows by performing shape reconstruction and 3D PIV on a flame, and 3D PIV on flow through a micro channel. The reacting flows in particular posed problems with the method. Reconstruction of the flame envelope was successful except for significant elongation in the optical axis caused by cameras viewing the flame from primarily one direction. 3D PIV on reacting flows suffered heavily from the index of refraction generated by the flame. The refocusing algorithm used assumed the particles were viewed through a constant refractive index (RI) and does not compensate for variations in the RI. This variation caused apparent motion in the particles that obscured their true locations making the 3D PIV prone to error. Microscopic PIV (µPIV) was performed on a channel containing a backward facing step. A microlens array was placed in the imaging section of the setup to capture a light field from the scene, which was then refocusing using SA refocusing. PIV on these volumes was compared to a CFD simulation on the same channel. Comparisons showed that error was most significant near the boundaries and the step of the channel. The axial velocity in particular had significant error near the step were the axial velocity was highest. Flow-wise velocity, though, appeared accurate with average flow-wise error approximately 20% throughout the channel volume.

Keywords: synthetic aperture PIV, light field imaging, particle image velocimetry, microscopic light field particle image velocimetry
ACKNOWLEDGMENTS

I would like to acknowledge the help and mentoring of my advisor Dr. Truscott and for all the time and effort he put into these projects. His patience and enthusiasm for the field was always motivating and enlightening.

I would like to thank my committee for their support.

Bryce McEwen performed the most difficult task of starting this experiment from the ground up as well as the numerical simulations used in this study. Without his contributions this thesis would not have been possible.

Jesse Belden has been a significant help throughout my graduate career, his knowledge and insight into this field was extremely valuable.

I would like most of all to thank my wife, Robyn, for her patience, kindness and encouragement throughout my studies.

This material is based upon work supported by the National Science Foundation under Grant No. CMMI-1126862. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the author(s) and do not necessarily reflect the views of the National Science Foundation.
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CHAPTER 1. INTRODUCTION AND BACKGROUND

Light field photography is a rapidly growing field with diverse applications including image-based rendering, microscopy, and three dimensional Particle Image Velocimetry (3DPIV). Light field photography differs from traditional photography in that it records the direction in addition to the position of a light ray. One advantage of recording a light field is the ability to redefine certain image parameters, such as depth of field, post-capture. A light field can be recorded by a single camera moved to many different positions or an array of individual cameras. The images from the cameras can be combined to create a single image with a large simulated aperture, called a synthetic aperture. This provides a photographic technique for focusing on a single thin plane in the field of view where only objects on that specific plane are sharply in focus. In addition, with a large enough aperture, sufficient light rays pass around foreground objects rendering them effectively transparent. This ability is the primary focus of the techniques used in this study.

The light field is defined as the radiance at a point in a given direction [1]. If the space is free of obstacles, called occluders, the radiance of a light ray is constant along its path, and the light field can be reduced to a 4D function. Levoy and Hanrahan [1] propose a method of parameterizing a light field by defining two planes, \((u, v)\) and \((s, t)\), and finding the intersection of a light ray on both planes. If the planes are fixed in space, the intersection points define the position and direction of the light ray. Knowing the direction of the light rays allows the pixels associated with a specific focal plane to be gathered and combined into a new image. Figure 1.1 shows a diagram representation of recording a light field with two cameras. To record a light field using the two-plane method, Levoy and Hanrahan [1] built a gantry with a single camera whose position was controlled via computer. Assuming the cameras can be modeled as pinholes, the position of the camera becomes the first intersection point because all light rays must pass through the pin hole, while the pixels on the image sensor become the second intersection point. Using this gantry
Levoy and Hanrahan were able to produce accurate image-based renderings of the objects imaged. Levoy et al. [2] built a larger, portable gantry to record light fields of the statues of Michelangelo.

Using a single camera on a gantry limits the recording of light fields to static objects. To remove this limitation, Isaksen et al. [3] used an array of cameras to capture a light field, and demonstrated two possibilities when combining the images: 1) the aperture, or depth of field, of the final image can be changed, and 2) the focal plane can be arbitrarily positioned. By combining the camera images, a single image with a narrow depth of field, or a large aperture, is produced, which results in a single, thin plane in the scene that is in focus. It was also shown that the focal plane could be changed by shifting the images with respect to each other, moving the focal plane arbitrarily throughout the volume. As previously stated, any object not on the plane of focus that is smaller than the simulated aperture does not obstruct the view, but merely decreases the contrast slightly. Vaish et al. [4] showed that if the images were warped as well as shifted, the focal plane could be repositioned such that it was not parallel to the plane defined by camera position. If focal planes are created throughout the volume with different positions, a focal stack is created.

Wilburn et al. [5] used 100 inexpensive web cameras to build a large array of imaging devices. The cameras could be synchronized to acquire images simultaneously, or they could acquire images sequentially, making it possible to record a light field video [6]. Belden et al. [7]
used a camera array to perform 3D Particle Image velocimetry (PIV), termed Synthetic Aperture PIV (SAPIV), on a particle-laden flow. By generating a focal stack of large aperture images, the particles in the foreground that would normally obstruct the particles in the background become large and blurred creating a “see-through” effect, allowing all the particles in the volume to be seen and tracked. By filtering the focal stack images, the noise can be reduced and a volume with particle locations can be generated. Using cross-correlation a full three dimensional-three component (3D-3C) of velocity field can be measured. Because focal stacks can be generated at each time instance, a fully time resolved velocity field can be measured [7]. Belden et al. [8] also showed that the technique could be used to locate bubbles in a bubble field. These methods all rely on using multiple cameras to record a light field, but it can also be done with a single camera without a gantry.

Ng et al. [9] placed an array of microlenses, each approximately 125µm in diameter, into a camera at the intermediate imaging plane and moved the camera sensor back a distance equal to the focal length of the microlens array. Each microlens became a separate viewing angle and took the place of a pinhole camera. This allowed a single camera to capture a light field with each image. Figure 1.2 shows a schematic of the light field camera [9]. Levoy et al. [10] adapted this technique by adding a microlens array to a microscope imaging system, and recorded a light field from a microscope. This provided the ability to refocus scenes from a microscope using synthetic aperture refocusing, as well as the ability to change the perspective of the scene, which is not normally possible for standard microscopes.

This thesis contains two different procedures based on synthetic aperture refocusing. The first section contains a description and results of the shape reconstruction and 3D PIV of a flame. The second section contains a paper that will be submitted to Lab on a Chip in 2014. Bryce McEwen was a coauthor on this paper and some of the sections are taken from his masters thesis. My thesis uses the same principle and methods, but its main contributions lay in the results. Bryce’s thesis examined the flow measurement capability with a channel of varying width and constant height, thus little velocities in the third dimension are present. This study seeks to determine the ability of light field µPIV to determine the movement of the flow in 3 dimensions by examining a channel of constant width and variable height. I have updated the introduction to include some newly publish material. I also adapted the setup to make the calibration process more user-friendly.
Figure 1.2: Image showing the configuration of the plenoptic camera designed by Ng et al. [9]. (Reproduced from [9])

The experiments were all performed by me, but the computational simulations were run by Bryce. The results section was wholly written by me.
CHAPTER 2. 3D RECONSTRUCTION AND PIV ON FLAMES USING LIGHT FIELD PHOTOGRAPHY

2.1 Introduction

Flames are a part of everyday life. Every human on the planet has seen, interacted with, or felt the effects of a flame. They are a source of heat, light and energy for many people, and yet accurate measurements of this everyday occurrence are still difficult. Fuel only burns in a thin section of the flame called the flame front. In turbulent flames the turbulent fluid structures create a shearing strain (Equation 2.1) that stretches and manipulates the flame front, causing an increase in surface area allowing more fuel to burn in the same amount of time. This is beneficial in most circumstances; however, the shearing strain can become large enough to break the flame front, allowing fuel to pass through the front without burning. This can lead to inefficiencies in the combustion processes. The interaction between the flame and turbulent flow is complicated and many experts in the field are still trying to understand this interaction [11]. An improved understanding of this interaction can help increase efficiency in combustion.

In experiments, the strain rate is typically determined by means of an equation relating rate of strain to a velocity field. This equation has nine terms and is formulated as

$$\varepsilon = \left[ \begin{array}{ccc} \frac{\partial u}{\partial x} & \frac{1}{2} \left( \frac{\partial u}{\partial y} + \frac{\partial v}{\partial x} \right) & \frac{1}{2} \left( \frac{\partial u}{\partial z} + \frac{\partial w}{\partial x} \right) \\ \frac{1}{2} \left( \frac{\partial v}{\partial x} + \frac{\partial u}{\partial y} \right) & \frac{\partial v}{\partial y} & \frac{1}{2} \left( \frac{\partial v}{\partial z} + \frac{\partial w}{\partial y} \right) \\ \frac{1}{2} \left( \frac{\partial w}{\partial x} + \frac{\partial u}{\partial z} \right) & \frac{1}{2} \left( \frac{\partial w}{\partial y} + \frac{\partial v}{\partial z} \right) & \frac{\partial w}{\partial z} \end{array} \right]$$  \hspace{1cm} (2.1)

where the values $u$, $v$, and $w$ refer to the velocity components, and $x$, $y$, and $z$ are the coordinate directions.

A more accurate understanding of the turbulence/flame interaction can increase our knowledge of when and where the strain rate might be high enough to cause these localized extinctions.
However, measurements of flames are difficult and often inaccurate. An instrument inserted into the flame can cause disturbances that will change the inherent flame characteristics.

Even modeling the combustion process is extremely difficult, as Jacqueline Chen of Sandia National Labs noted [12]. Chen showed that only in the last few years has computing power applied to turbulent combustion become sufficient that simulations can be completed without any averaging or closure models for the turbulence, allowing a direct numerical simulation (DNS) for the flow field. These simulations have allowed insights into the turbulent combustion interactions of certain types of flames. However, the mesh generally contains billions of nodes and the simulation requires millions of CPU hours to complete, requiring peta-scale super computers. Even with immense computing capacity, current computing power only allows the modeling of small volumes of specific types of flames.

Optical methods are an attractive option because they remove the intrusive nature of measurement devices and rely only on cameras. Flow velocity measurements are typically done using Particle Image Velocimetry (PIV). Micro particles are entrained in the flow, and two images are taken of the laser-illuminated flow. Particle displacement is found using digital image correlation between two consecutive images resulting in a vector field. Usually this is done with special optics to create a laser light sheet. By illuminating only one plane of the volume of interest, issues of parallax are removed. This method results in two-dimensional, two component (2D-2C) velocity fields. Planar Laser Induced Fluorescence (PLIF) is typically used to find general flame structures and relies on specific wavelengths of light that cause specific molecules to fluoresce. Certain molecules exist in different concentrations throughout the flame. For example, OH molecules exist in higher concentrations in the unburned fuel regions of the flame, and CH molecules exist in higher concentrations near the flame front. Knowing the position of these concentrations can help identify the location of the flame front.

As explained above, PIV with a light sheet can only resolve 2D-2C velocity fields, and 4 velocity gradients. Tanahashi et al. [13] at the Tokyo Institute of Technology performed an experiment simultaneously measuring stereoscopic PIV, CH PLIF, and OH PLIF to determine if standard 2D PIV is sufficient to estimate the rate of shearing strain. Stereoscopic PIV can measure the third component of velocity by using parallax to determine the motion in and out of the light sheet (z-direction). Their results showed significant velocity components in the z-direction, indicating a
2D-2C estimate of a 3D flow field is an unreliable estimate of the rate of shearing strain within the flame. Shimura et al [14], at the Tokyo Institute of Technology, later performed a more extensive experiment using dual-plane stereoscopic PIV. Two independent stereoscopic PIV systems were used, imaging from laser sheets separated by a distance of 340 µm. The particles could be tracked moving from one plane to the other, giving 3D-3C velocity measurements. Using this setup, they were able to fully resolve the strain tensor in that specific area of the flame. They found that the single plane stereoscopic PIV underestimated the shearing strain by nearly 10% compared to the dual-plane case. Because the effort is to understand the interaction between turbulent flow and the flame, it is desirable to have the most accurate measurement of the strain rate possible. It appears that a full 3D-3C velocity field of a flame must be known to accurately calculate the rate of shearing strain.

Belden et al. [7] described a method for measuring volumetric 3D-3C velocity fields using synthetic aperture (SA) refocusing. This study seeks to apply this method to reacting flows by fully resolving a flame velocity field in time. Reconstructing the flame was also attempted using SA refocusing in a processes similar to the visual hull method described by Adhikari and Longmire [15].

During this study we discovered that PIV in particular, presents a difficult problem because reacting flows generate a large amount of heat and various gases that can significantly change the index of refraction from that of the surrounding air. The generated refraction field is inhomogeneous and continuously varying, making a standard model for error prediction difficult. This can cause significant errors in determining true particle positions, making accurate PIV measurement impossible. Interestingly, this effect is often ignored by those performing PIV measurements on flames [14] [16] [17] [13]. A more general study of this error source will be covered in this paper.

2.2 Experimental Methods

This section contains a detailed description of the experimental setup used for the flame experiment. An explanation of the setup and processing procedure for the general Synthetic Aperture Imaging (SAI) is given first, followed by a description of the additions made to the general SA setup for the 3D reconstruction experiment. Finally, the changes and additions made to the general setup in order to perform the 3DPIV experiment is given.
2.2.1 Synthetic Aperture

As Wilburn et al. [6] described, by using an array of video cameras synchronized together to capture images simultaneously, a time resolved light field can be recorded. Each individual frame recorded from the video camera array defines a light field. Using SA refocusing, each recorded light field can be reparameterized into a stack of narrow depth of field images where only objects on that plane will be sharply in focus while off-plane objects are blurred. This method can also be used to capture light fields at high frame rates.

A high speed SA array consists of several high speed cameras placed in different positions, viewing the desired scene from different angles. The position of the cameras is arbitrary as long as they can view the entire scene independently, although some configurations yield better results. As outlined by Belden et al. [7], while using the SA setup for 3D PIV experiments, the number of cameras in the array is dependent on the required experimental fidelity. The particle field reconstruction quality was shown to improve with increasing camera numbers until the range of 10-15 cameras was reached, at which point the reconstruction quality leveled off. During the experiments herein we were limited to the eight Photron SA3 high speed cameras available which is an acceptable amount of cameras according to Belden et al. [7] but is not the optimum amount. Belden et al. also showed that particle field reconstruction quality decreased as spacing between the cameras increased because significant warping was required for images with steep angles, as described shortly. Typically in these experiments cameras were mounted on an aluminum frame in two rows as seen in Figure 2.1, and the spacing had a practical lower limit equal to the size of the camera bodies.

Figure 2.1: The high speed SA camera array taking images to be reconstructed.
As with all multi-camera configurations, the SA setup must be calibrated to create a relationship between 2D image coordinates and 3D world coordinates. If the world coordinates of the imaged points are known, the internal and external parameters of the cameras can be found easily; however, the precision required to image a set of precisely known world coordinates makes this process difficult and often impossible. For the described system we use an open-source bundle adjustment method written in MATLAB by Svoboda et al. [18], which estimates the camera parameters and world coordinates iteratively using only point correspondences. Originally the software was written to find its own correspondence points by tracking an LED moved through an experimental volume. This point-finding approach was modified to calibrate using a checkerboard pattern on a flat plate. The pattern was sized to fit the region of interest, and the corners of the pattern were used as correspondence points in the images. The corner coordinates were located in each image using a corner pattern sampled from the images themselves and cross correlation, and correspondingly fed into the calibration software. Figure 2.2 shows a calibration grid used during the experiment from the perspective of the eight different cameras.

![Figure 2.2: An example view of the calibration grid from each camera in a typical eight-camera setup. The top row shows the view from the top cameras, while the bottom rows the view from the bottom cameras. All images must be projected onto a known reference geometry before being shifted and combined. The cameras on the outer edge need to be warped significantly, which can cause error in the final volume reconstruction.](image-url)
Post processing includes projecting all images onto a known reference geometry then shifting the images to define the focal position. Projection involves transforming the images from the 2D camera coordinate system to the 3D world coordinate system using a homography and some known world geometry. Although the geometry is not required to be planar, this experiment used the grid defined during the calibration step. As shown by Belden et al. [7] the transformed images can be shifted to their corresponding focal plane location using equation 2.2.

\[
\begin{pmatrix}
    x'' \\
    y'' \\
    1
\end{pmatrix} =
\begin{pmatrix}
    1 & 0 & \mu_k \Delta X_{Ci} \\
    0 & 1 & \mu_k \Delta Y_{Ci} \\
    0 & 0 & 1
\end{pmatrix}
\begin{pmatrix}
    x' \\
    y' \\
    1
\end{pmatrix}
\] (2.2)

where \( \mu_k \) is the constant to identify the k-th focal plane, and \( \Delta X_{Ci} \) and \( \Delta Y_{Ci} \) are the relative camera locations for the i-th camera found during calibration. The projected and shifted images are combined using either an additive or multiplicative combination to produce a final image. In additive combination (2.3) the individual images are averaged together, causing the points where particles overlap to increase in intensity relative to other points [7]. Whereas, in multiplicative combination (2.4) the images are multiplied together, causing only the pixels that contain non-zero intensities in all images to contribute to the final image.

\[
I_{SAk} = \frac{1}{N} \sum_i I_{FKi}
\] (2.3)

\[
I_{SAk} = \prod_i I_{FKi}
\] (2.4)

The multiplicative combination was used in the flame reconstruction so only parts of the images that contained flames would contribute to the volume. During the PIV experiments the additive combination was used.

2.2.2 3D Reconstruction of a Flame

The 3D reconstruction experiment used the setup shown in Figure 2.1 with the eight cameras set with three on top and five on bottom. The five cameras on the lower row were spaced to image the flame at higher angles in the attempt to reduce the elongation experienced by Adhikari
and Longmire [15]. Two Bunsen burners were chosen, having circular outlets of approximately 13mm and 30mm in diameter. Both burners produced a partially premixed flame using natural gas at a pressure of 0.5 psig. The burners were positioned and cameras adjusted to capture a significant portion of the flame shape in the field of view. A frame rate of 500 fps was chosen because it was fast enough to capture a smooth transition between flame shapes, but slow enough to provide a variety of shapes to reconstruct.

Both flames were filmed with the room completely dark to produce a higher contrast between the flame and its surroundings. The images were projected and shifted according to the calibration and combined using the multiplicative algorithm (Eq. 2.4) to create a focal stack. In theory, each image of the focal stack will contain only the portion of the flame associated with that focal location, thus tracing the outline of the flame surface. Isosurfaces were created from each focal plane and stacked together to generate a volume which approximately characterized the flame envelope. This method is similar to volumetric reconstruction or visual hull, as described by Adhikari and Longmire [15], who used a tomographic setup to generate visual hulls of objects falling through a particle-laden flow. The method outlined by Adhikari and Longmire used binarized silhouettes of the objects from each camera, backprojected through a volume to determine the shape of the object. They reported difficulty with accurate reconstruction because of the lack of ability to resolve concave surfaces, as well as elongation in one dimension resulting from a small number of cameras viewing the objects from primarily one direction. The latter limitation was also experienced during this study.

2.2.3 3D PIV Seeding Methods

The 3D PIV setup was similar to the one used during the 3D reconstruction experiment, with the main difference in the configuration of the camera positions. Figure 2.3(a) shows the positioning of the cameras for this experiment with four cameras on the top row and four cameras on the bottom row. The spacing between the cameras was smaller than the previous setup to increase the particle reconstruction accuracy. A frame rate of 1000 frames per second was chosen, because it is the highest frame rate possible at full resolution. To illuminate the seeding particles a 100 W, Nd:YLF double pulse laser with a wavelength of 527nm was used (see Figure 2.3(b)) with a beam expander to increase the diameter of the beam to a column roughly 2 inches in diameter, allow-
ing a volume of particles to be illuminated. With laser illumination, the camera frame rate is less important because the laser pulse governs the time step. A double pulse laser contains two lasers capable of pulsing independently, each with a pulse width of approximately 120ns. The two laser pulses were separated by 100µs, and set to straddle the camera shutter. A timing diagram is shown in Figure 2.4. From preliminary experiments it was determined that this allowed the particles to move between 10-20 pixels per time step, providing adequate particle motion to determine the flow velocity within the flame.

(a) Image of the 3D PIV camera array.  
(b) Diagram showing laser position

Figure 2.3: Setup

Figure 2.4: Illustration of the laser trigger timing in relation to the camera shutter. With the setting of 1000 frames per second, and an exposure set for the entire frame, the shutter of the camera was open for 1000µs. A single laser pulse lasting 120ns happens 50µs before the end of the first image and illuminates the particles at that point in time. The second laser pulse happens 50µs after the start of the second image, creating two particle images with a separation of 100µs.
The flame used in the 3D PIV experiment was produced by a Bunsen burner with a circular outlet of approximately 17mm in diameter. Propane was fed to the burner at a pressure of 5-10 psig, which was necessary to operate the particle seeder. An adjustable needle valve within the burner was used to control the fuel flow rate.

A particle seeding chamber was placed inline with the fuel flow to seed the flame for PIV measurements (see Figure 2.5). The seeding chamber was designed to induce a swirl into the flow upon entering, which entrains particles and through swirling motion causes the larger particles to propagate to the edge and remain in the cylinder.

![Image of custom build seeding chamber](a) ![Image of seeding chamber open](b)

**Figure 2.5:** Images of the custom build seeding chamber. Figure (b) shows the container open with the inlet nozzle on the upper half of the cylinder.

Normally with PIV, the density of the particle material is chosen to match the fluid density as closely as possible, allowing the particles to be neutrally buoyant so the only flow is with the motion of the fluid. In flames, however, neutrally buoyant particles cannot survive the extreme temperatures of the flame front and therefore can only be tracked before being destroyed. To track velocities throughout the flame, a temperature resistant material must be chosen, such as ceramic particles. Aluminum oxide, titanium dioxide, and silicon dioxide, as well as others, have a melting point beyond the expected flame temperature of a propane flame, but have a density that is roughly three orders of magnitude greater than propane gas. To counteract this extreme difference in density, researchers have used smaller particles. For example, Filatyev et al. [17] used
particles 0.5 $\mu m$ in diameter, and Tanahashi et al. [13] used particles 0.18 $\mu m$ in diameter. For this experiment aluminum oxide particles with a diameter of approximately 1-5$\mu m$ in diameter were chosen because they have a sufficiently high melting temperature, and they were readily available for our preliminary experiments.

Generally in PIV, better accuracy is achieved when the particles are 2-5 pixels in diameter in the image; however, because of the relatively small particle size in this experiment, the diameter in the images was in the range of 1-3 pixels. A 105mm lens was used to increase the recorded particle diameter into the usable range.

2.3 Results

2.3.1 3D Reconstruction

Reconstruction of the flame in time shows the general flame envelope shape progression. Figure 2.6 shows the reconstruction of a partially-premixed flame from a Bunsen burner with a 30mm outlet diameter. The top row shows the actual image of the flame from a single camera in the array through six sequential time steps. The bottom row shows a frontal view of the flame visual hull using volumetric reconstruction with SA refocusing. During this discussion the z dimension will be parallel to the viewing axis of the cameras, the x dimension will be across the width of the flame, and the y dimension will be along the axis of the flame from bottom to top.

A rough comparison was performed to quantify reconstruction accuracy. With the grid used for calibration, the pixel-to-mm ratio can be calculated by determining the average number of pixels per square of the pattern. Using this ratio the rough size of the flame in the original images and the size of the reconstruction could be compared and were found to be within 10% from the frontal view. As the window is rotated, however, the difference becomes much more significant. Figure 2.7 shows the reconstructed flame rotated about the y-axis by different angles. It is obvious from these images that the reconstruction is much longer in the z-direction than it is in the x-direction. No cameras were positioned at 45° and 90° rotations, and therefore no information is available to compare the z-dimension directly to the actual flame; however, the outlet of the Bunsen burner is circular, and it can be expected that the flame would be roughly axi-symmetric about the
Figure 2.6: From left to right, a 3D reconstruction of a partially-premixed methane flame from a Bunsen burner. The bottom row shows the reconstruction of the time step shown in the top row.

Figure 2.7: These images show the rotation of the reconstructed flame. Image (a) is the front view, image (b) shows the flame rotated by 45° about the axis of the Bunsen burner body, and image (c) shows the flame rotated by 90° about the burner axis.

y-axis. The reconstruction in these images shows an elongation of 2.5 to 3 times in the z-direction which is common in SA refocusing as shown by Belden et al. [7].
The flame from the 13mm bunsen burner was also reconstructed using the same method and setup. Figure 2.8 shows a frontal view of the reconstruction of the smaller flame. The elongation of this flame is comparable to the larger flame.

Figure 2.8: Similar to the previous flame reconstruction. The top row shows the time step of the flame, with the corresponding reconstruction being shown in the bottom row.

To further test this method, two 5mm diameter spheres were reconstructed using a different configuration. A setup similar to the 3D PIV was used with four cameras on top and four on bottom as well as being closer together. We would expect this configuration to increase the elongation, as there is less peripheral information for the object. Figure 2.9 shows the reconstruction results, which indicate elongation in the z-dimension much like the flame. As expected, the elongation is greater for the spheres, on the order of 3 to 4 times the width of the frontal section. This follows from the concept of the visual hull, which combines line-of-sight silhouettes from each view point [15]. With no views of the object from the side, there is no information for the shape perpendicular to the main camera viewing angle.

One possibility to better determine the shape of the object is to move from a planar view of the scene to a surround view. This poses problems with the current processing as the images must be projected to a reference plane and the steeper the camera viewing angle the higher the error associated with the warp.
Figure 2.9: These images show the reconstruction of two spheres. These illustrate the problem of elongation in the depth dimension.

2.3.2 3D PIV

PIV was performed on the particle-seeded flow to determine the velocity field within the flame. Initially the particles were hardly distinguishable in the images and post processing was required. Raw images from the Photron cameras were saved in 12-bit format and bit-shifting the images increased the contrast considerably. The nozzle of the burner was masked out to remove the reflected glare from the laser. Figure 2.10 shows example images of the original and post processed images.

The focal stack images were thresholded to remove the noise from out of focus particles; however, several of the thresholded focal planes produced no in-focus particles. The volume reconstruction resulting from these focal stacks, therefore, is extremely sparse, making it difficult to obtain results from SAPIV. As described previously, the index of refraction generated by the flame obscure the true position of the particle, this effect will be covered in the following section.

MatPIV was used to perform 3D cross correlation of sequential time steps to measure the velocity field. A multipass algorithm was used with decreasing interrogation volume size starting 128x128x64 and ending at 64x64x32 voxels. Global and median filtering were used to smooth the results but nan-interpolation was not used as PIV calculations generally produced a significant number of NaN’s in the velocity field. For more information about this software the user is referred to [19]. Most time steps did not return good results because of the limitations recently discussed. Figure 2.11 shows the results of 25 PIV calculations averaged together. The general trend in the
velocity field shown is encouraging. The vectors are mostly in the flow direction and the velocities measured near the burner exit are slower than those measured further away as expected with flow acceleration after the flame front. The magnitude of the velocity is also on the order expected from this setup. However, gaps can be seen near the base of the vector field (y ≈ 700), and another spot near the top of the field (y ≈ 200), which indicate the lack of usable data in those regions.

Two representative slices of this vector field in the x-y plane are shown in Figure 2.12. These show more detail concerning the gaps in the PIV processing.

One possible source of error could be the use to thresholding as described by Belden et al. [7] for noise removal. Figure 2.13 shows histograms of a sample focal plane from a refocused particle field. A refocused particle field in air can be modeled using a gaussian function with the particles on that plane at a higher intensity. Thresholding the images using this histogram removes most of the noise, leaving only the particles. The histogram from a particle field in a flame however shows a histogram skewed to the lower intensity values. These images can still be thresholded, but the risk of removing particles that do not coalesce is higher, because they may not be high enough in intensity to remain after the thresholding.
Figure 2.11: Figure of the velocity vectors of the Bunsen burner flame. These vectors are averaged over 25 time steps. Generally vector fields are averaged over 100 or more time steps, but variations in the particle locations caused many of these vector plots to have holes. It can be seen in this figure near the base of the flame there is a region of no data, this is caused by the lack of usable correlation data in those areas of the images. In this plot, y=0 corresponds to the top of the flame.

Figure 2.12: This image shows two representative planes from the 3D flow field shown in Figure 2.11
Other noise removal techniques may provide better results. Deconvolution is one such method, and is used during the microscopic PIV experiments and is discussed in more detail there. Deconvolution uses a method for removing noise from an image produced by the optical system and may result in better particle locations.

2.3.3 Refractive Index

As mentioned previously, the refractive index generated by heat and gases produced in a flame is a source of error in velocity measurements.

If a refocused particle-laden flow is viewed from the side, the particles appear as the lines from each camera [?]. The spots where the lines converge indicates the location of the particle. If a flow in air is viewed from the side (see Figure 2.14(a)), the cameras’ line-of-sight can be seen converging into concise particle locations. If a refocused particle-laden flow in a flame is also viewed from the side (see Figure 2.14(b)), the areas of convergence appear as large blobs, as shown in the red circle, while other locations narrowly miss or don’t converge, as shown in the yellow circle. This is an indication that the particle locations appear shifted. This will, at the least, introduce error into the particle locations, and will at worst cause particles not to reconstruct at all.

In order to quantify the amount of possible error in particle locations due to refractive index, a grid of dots was placed behind the Bunsen burner. The dots appeared roughly 5 pixels in
Figure 2.14: Y-Z slices of reconstructed particle-laden volumes. (a) shows the slice of a particle-laden volume in air; (b) is the volume in flame. Reconstruction in flame can introduce aberrations caused by the refractive index, which is visible as the particles in (b) converge to a larger blob or not at all, as opposed to a discreet point as seen in (a).

diameter in the image which corresponds to the range of particle sizes used in this experiment. An image with no refractive gradient was taken as a calibration image to represent the true location of the dots. High speed video was collected with the flame-induced refractive index between the camera and pattern. By implementing a cross correlation algorithm with the calibration image as baseline, the apparent motion of the dots was calculated. Figure 2.15(a) shows the results of 1361 dot movement images averaged together, and Figure 2.15(b) shows one standard deviation from the mean. Figure 2.15(c) shows a single movement calculation indicating that fluctuations in the index of refraction can cause much higher errors than those seen in the average.

To determine if the separation distance between the flame and the dot pattern had an effect on the amount of apparent motion, the grid was placed at four different distances from the flame. The processes of determining the amount of apparent motion was repeated for each distance, which were nine, ten, eleven, and twelve inches as measured from the center of the flame. Figure 2.16 shows the results of the nine inch case and the twelve inch case averaged over 1361 measurements.

The average range of movements in each case is approximately equal. The case not shown, eleven inches distance, is similar in magnitude with a maximum average movement of 2.8 pixels.
Figure 2.15: Images showing the apparent dot movement of a grid, placed 10 inches behind the flame. Figure (a) shows the apparent movement averaged over 1361 steps and Figure (b) shows one standard deviation from the mean. Figure (c) shows a single movement image.

Figure 2.16: Two figures showing the difference in apparent shift based on the distance the dots are placed behind the flame average over 1361 steps. In Figure (a) the dots are placed 9 inches from the center of the Bunsen burner outlet, whereas Figure (b) the dots are placed 12 inches behind.

The distance from the flame, therefore, is not a significant factor in the error introduced by the refractive index. This indicates that the apparent movement of the dot pattern is representative of the maximum possible apparent movement of particles within the flame. With typical PIV algorithms, an average displacement of 10 pixels between images is ideal, and thus a refraction movement of 3-4 pixels could introduce significant error.
2.4 Conclusions and Future Work

This study focuses on reconstruction and 3D PIV of flame using light field imaging, specifically synthetic aperture refocusing. Reconstruction of the flame was done using SA refocusing to create a visual hull of the flame envelope. The frontal view of the reconstructed flame is accurate in shape and size, but because of lack of view angles imaging the sides, the reconstruction suffers from elongation in the z-direction, which is also apparent in the reconstruction of two 5mm spheres. More cameras at higher angles would decrease this error, but pose problems with warping images to a reference plane. Velocity fields of particle-laden flows can be generated using SA refocusing. Flames pose a difficult problem with refocusing because the refractive index generated by the heat and gases produced by a flame create error, obscuring the true position of the particle. This shift at best introduces error into the particle position and at worst can impair particle reconstruction completely. Current knowledge does not provide a solution for the error associated with the refractive index. If the index of refraction field is known, the true position of the particles may be calculated. A promising study has been done by Atcheson et al. [20] in which sixteen cameras were set in a semi-circle around a camp stove viewing a noise background with the camp stove between the cameras and background. The refractive field created apparent movement in the background that was determined using an optical flow algorithm. By combining the apparent movement of each background, they were able to determine a discretized 3D refractive index field produced by the flame. Because the refractive index field is known it might be possible to correct the apparent movement of the particles; however, the experimental setup for using this method in combination with SAPIV would be difficult. Both setups would need to run simultaneously, with eighteen cameras for the noise background, and eight for the SAPIV setup. Lighting for the two setups would be opposite, as the background images need to be visible to the cameras, but the PIV needs no lighting so the particles are illuminated only by the laser. The complexity of this setup would make it prone to error, and difficult to implement. Another possible why to improve the results of the PIV would be to use a deconvolution for noise removal as opposed to the standard thresholding method.
CHAPTER 3. MICROSCOPIC LIGHT FIELD PARTICLE IMAGE VELOCIMETRY

As explained earlier, this is a paper to be submitted to Lab-on-a-Chip in 2014, co-authored by Bryce McEwen. Some of the sections are taken from his thesis. My contributions to this field are outlined in the Introduction and Conclusions sections.

3.1 Introduction

Advances in microfabrication processes have lead to increased development of microfluidic devices in several areas, especially in the biomedical field. The ability to obtain experimental measurements of flow properties in microfluidic devices is essential for validating numerical models, designing new devices, and improving existing designs. This is especially important for fluids with non-uniform chemistries, non-uniform geometries, unsteady shear responses (non-Newtonian), and particulate flows (e.g., blood flow). Time-resolved, three-dimensional velocity components can provide important, detailed information about flow field properties. One minimally intrusive method for measuring the velocity components of a flow field is particle image velocimetry (PIV). Originally, PIV was used as a means of effectively measuring two-dimensional flow fields. Improvements in digital imaging and computational capabilities have lead to the development of methods capable of measuring all three components (3C) of velocities of particles in three-dimensional space (3D). Often these methods are relatively complex, time intensive and scarcely seeded. Micro-scale 3D-PIV (3D \( \mu \)PIV) methods have further complications and limitations due to size constraints and lighting difficulties. Some current 3D \( \mu \)PIV methods include confocal [21–23], stereoscopic [24, 25], defocusing [26, 27], volumetric correlation [28] and holographic \( \mu \)PIV [29]. Depending on the requirements of the measurement, the attributes of one technique may be favored over another as there is no “one size fits all” system. Here, we develop a light field (LF) \( \mu \)PIV method with the goals of: (1) providing a low-cost, easy-to-use 3D \( \mu \)PIV
technique and (2) enabling large enough seeding density to instantaneously resolve unsteady flow fields.

Development of novel and existing 3D µPIV systems continues to be an active area of research. [30] Confocal laser scanning microscopy (CLSM) uses hardware to produce high-contrast images at different focal planes. [22, 23] Applying conventional PIV to the images yields two-component velocity vectors in planes perpendicular to the optical axis of the microscope (i.e., 3D-2C velocity fields). The method does not instantaneously measure 3D velocity vectors. Lindken et al. [24] present a method for obtaining three component velocity vectors from two-dimensional planes (2D-3C) using stereoscopic µPIV. Two cameras are used to simultaneously capture images at different viewing angles and measurements are made along several planes in the z dimension in order to get velocity vectors throughout the entire flow field. They report that one of the limitations of stereoscopic µPIV is a decreased resolution along the optical axis (z-direction) due to a relatively large depth of focus of the stereoscopic objective lenses.

A method for obtaining three-dimensional, three component (3D-3C) flow velocities in a micro volume based on the macro scale defocusing digital PIV method [31] was presented by Yoon and Kim [26]. By placing a mask of three pinholes, arranged in an equilateral triangle configuration, between the objective lens and image plane the depth of the particles could be correlated by the size of the resulting triangle pattern observed. In this method particle seeding must be low because the triangle pattern becomes less distinguishable with increasing particle density. Light intensity is also a challenge because light transmission is limited by the pinhole mask. Tien et al. [27] modified the setup to reduce this limitation by using a color camera and color filters over each of the pinholes effectively making each color channel a separate image sensor. This method was refined to instead use three different colored light sources at different angles to produce the triangle patterns. [32] The 3D locations of particles are computed and velocities found using particle tracking velocimetry (PTV); the authors demonstrated the ability to instantaneously resolve unsteady velocity fields. Fouras et al. [28] demonstrated that volume correlation could be used to determine vector velocity fields.

Other methods have been developed to determine the velocity field in micro flows with complex flow fields. Digital holographic PIV (DHPIV) records light interference patterns as a hologram [29] and through post processing the 3D field can be reconstructed. By finding the
movement of particles from two time steps the velocity field is generated. Wavefront sensing and
astigmatism PTV [33, 34] are similar techniques that use a cylindrical lens to generate an anamor-
phic image set where out-of-focus particles deform by different amounts in different directions.
The difference in deformation can be correlated to the axial location of the particles.

Increasingly, concepts from light field imaging and computational photography are being
exploited to make fluids measurements. As described by Levoy [10], light field imaging attempts
to measure the radiance of light along many rays that intersect a scene. This enables post-capture
computational photography operations, such as the ability to refocus a scene at different depths
thereby generating a focal stack of images that span a 3D volume.

On the macroscopic scale, one of the more prevalent 3D PIV methods is Tomographic
PIV (Tomo-PIV). [35] Multiple synchronized cameras view an entire measurement volume from
different angles and 3D particle fields are reconstructed using optical tomography to obtain instan-
taneous, 3D-3C velocity measurements. Belden et al. [7] present a similar method that uses the
concepts of LF imaging and synthetic aperture refocusing known as Synthetic Aperture Particle
Image Velocimetry (SAPIV). Images from an array of cameras are combined using an SA refo-
cusing algorithm to generate a focal stack of narrowly focused synthetic images. The 3D particle
field can be reconstructed by either thresholding the focal stack [7] or performing 3D deconvo-
lution [36] and the seeding density can be large. Both SAPIV and Tomo-PIV require cameras to
view the entire volume in focus (i.e., large depths of field), which inhibits their translation to the
microscale. Microscope objective lenses have very small depths of field and working distances on
the order of millimeters. Small focal lengths in comparison with the objective diameters make it
spatially impossible to arrange a set of more than two higher-magnification microscope objectives
to focus on a single point.

An alternative means of sampling a light field is to insert a microlens array between the
main lens and image sensor of an imaging system, so as to measure ray radiance and angle. [9].
This approach introduces a trade-off of lateral resolution for angular resolution. A macro 3D PIV
method using this so-called plenoptic camera was proposed by Lynch et al. [37,38]. SA refocusing
can be performed on plenoptic light field images as well, yielding a focal stack of synthetic images
covering a 3D measurement volume.
Levoy et al. [10] developed a method for converting a conventional microscope into a light field microscope. A microlens array is placed at the intermediate image plane between the objective and a camera. In this paper, microscopic LF imaging is employed to perform 3D µPIV measurements. The use of a microlens array provides a practical and relatively simple means of sampling ray radiance and direction on the microscale. Using SA refocusing and 3D deconvolution, we robustly reconstruct 3D particle fields. The seeding density is relatively large (e.g., 8 particles within a $24 \times 24 \times 8$ voxel interrogation volume) thus allowing velocity field computation via PIV. Measurements of velocity in a microchannel with a backward facing step are compared with CFD and show small deviation over time indicating the method is viable for instantaneous 3D µPIV measurement. Limitations of the method arising from the trade-off between lateral and angular resolution are also discussed.

### 3.2 Light Field Micro-imaging

A four-dimensional light field written as $L(u,v,s,t)$ describes the radiance along all rays that intersect a scene as parameterized by the intersection of the rays with two specific planes: the $uv$ and $st$ planes [1]. Placing an array of microlenses (lenslets) at the intermediate image plane of an imaging system (as shown schematically in Figure 3.1) enables capture of a sampled version of a four-dimensional light field in a single photograph [10] [9]. The microlenses define the $st$ plane and the camera sensor defines the $uv$ plane. A light field can be reparameterized to recover information in the depth ($Z$) dimension, which requires trading lateral spatial resolution for angular resolution [9]. For a lenslet-based light field microscope, the spatial resolution is controlled by the size of the lenslets, while the depthwise (or axial) resolution is governed by the number of resolvable spots behind each lenslet [10].

#### 3.2.1 Synthetic Refocusing

Figure 3.1 shows a schematic of a light field microscope in which a microlens array has been placed at the intermediate image plane of a microscope with an infinity corrected objective. The sensor plane ($uv$) is placed behind the microlens plane at a distance equal to the lenslet focal length, $f_l$. Rays from a point on an in-focus object in the imaging volume (solid lines in Fig-
Figure 3.1: (a) Ray diagram of a light field microscope that makes use of an infinity corrected objective. An in-focus object projects to a point on the microlens plane (red rays), while rays from an out-of-focus point span multiple microlenses (blue rays); depth is thus encoded by the microlens array. (b) A synthetic image is formed on the \( s't' \) plane by reprojecting pixels through their parent lenslets.

Rays from a point in the imaging volume not at the focal depth (dashed lines in Figure 3.1(a)) do not meet a point at the lenslet plane and thus span multiple microlenses. Therefore, the depth information about this out-of-focus point is encoded in certain pixels behind more than one lenslet. A particular depth in the imaging volume can be brought into focus by reparameterizing the light field post-capture. In other words, the light can be reprojected to a new imaging plane (the \( s't' \) plane) behind the tube lens, and a refocused image can be synthesized to effectively move the plane of focus of the microscope.

Refocused images are formed using geometry that assumes each lenslet acts as a pinhole. Therefore, each pixel is reprojected along the direction given by the vector connecting the pixel and the center of its parent lenslet. Thus, the first step in refocusing the light field involves pairing each pixel on the sensor \((uv)\) plane with its parent microlens by determining which pixels are behind each microlens on the \( st \) plane. Each pixel is then reprojected to the desired synthetic focal plane \( s't' \) located at a distance \( d \) from the lenslet plane (note: \( d \) is positive if \( s't' \) is to the right of \( st \) and negative if to the left of \( st \)). As depicted in Figure 3.1(b), the \( s't' \) coordinates of a reprojected pixel can be found from similar triangles and are given by

\[
\begin{bmatrix}
s_p' \\
t_p'
\end{bmatrix} = \frac{d}{f_i} \begin{bmatrix}
u_p \\
v_p
\end{bmatrix} + \left(1 - \frac{d}{f_i}\right) \begin{bmatrix}
s_l \\
t_l
\end{bmatrix}
\]  
(3.1)

\[
\begin{bmatrix}
s_p' \\
t_p'
\end{bmatrix} = \frac{d}{f_i} \begin{bmatrix}
u_p \\
v_p
\end{bmatrix} + \left(1 - \frac{d}{f_i}\right) \begin{bmatrix}
s_l \\
t_l
\end{bmatrix}
\]  
(3.1)
where \((u_p, v_p)\) are the coordinates of the pixel at the sensor plane and \((s_l, t_l)\) are the coordinates of the parent lenslet center at the lenslet plane. The \(uv\) and \(st\) plane are assumed to have origins on the \(Z\) axis.

Refocusing on a synthetic image plane effectively moves the object plane of the microscope by a distance \(\delta\). For a general optical system, one would expect the magnification to change with a change in the image and object plane locations. However, for an infinity corrected objective (as used herein), the total magnification is a constant and is given by \(M = -f_t/f_o\), where \(f_t\) and \(f_o\) are the focal lengths of the tube lens and objective lens, respectively. It can then be shown that the new synthetic image plane and object plane displacements are related by

\[
\delta = \frac{d}{M^2} \quad (3.2)
\]

A refocused image is formed at the \(s't'\) plane by integrating all reprojected pixels over a discretized synthetic image plane. Because the magnification is constant, the spatial resolution of the discretized synthetic image plane should be the same for every new focal depth. Levoy et al. [10] showed that the lateral spatial resolution of the light field equals the size of the lenslets, \(p_l\). Therefore, we sample the discretized synthetic image plane with resolution \(p_s = p_l\) and an overall size of \(N_s \times N_t\), where \(N_s\) and \(N_t\) are the number of lenslets in the \(s\) and \(t\) dimensions, respectively. A bilinear interpolation scheme is used to sample the synthetic images. It should be noted that by tracing the marginal rays from a given pixel (shown by the dashed lines in Figure 3.1(b)), the area of intersection with the synthetic image plane will be smaller than the lenslet area because the rays converge to a point on the tube lens. However, in order to reduce computational complexity when sampling a new synthetic image, it is assumed that the light from a given pixel intersects the \(s't'\) plane over an area equal to the lenslet area. Refocusing algorithms were implemented in Matlab and the code can be found online [39].

The sacrifice in lateral resolution is made to gain angular resolution of the light rays, which can be exploited for resolution in the axial (i.e., depth, \(Z\)) dimension. The axial resolution of a LF microscope was derived by Levoy et al. [10] and relevant aspects are presented here for context. The angular resolution is described by the number of resolvable spots behind each lenslet computed
as

\[ N_u = \frac{p_l^2}{R_{obj}} \]  

(3.3)

where lenslets are assumed to have equal length \( p_l \) in the \( s \) and \( t \) dimensions. \( R_{obj} \) is the smallest resolvable distance between two points on a specimen imaged by a microscope and is given as

\[ R_{obj} = \frac{0.47\lambda}{2NA^2}M \]  

(3.4)

where \( \lambda \) is the wavelength of light, \( NA \) is the numerical aperture of the objective and \( M \) is the magnification. The pixel pitch, \( p \), of the image sensor must be less than \( p_l/N_u \) or the pixel size will limit the angular resolution. Levoy et al. [10] define axial resolution in several ways, but most relevant for the present work is the depth of field of synthetically refocused images, which is computed as

\[ D_{tot} \approx \frac{(2 + N_u)\lambda n}{2NA^2} \]  

(3.5)

where \( n \) is the index of refraction of the medium in which the objective is immersed (herein the medium is air, \( n=1 \)). Considering that \( N_u \) is the number of non-overlapping depths within a SA focal stack [10], the expected total resolvable volume depth can be estimated as

\[ D_v \approx N_uD_{tot} \]  

(3.6)

To avoid under sampling, it is common to generate more than \( N_u \) synthetic focal planes; therefore, the focal stacks used in this work are generated with depth spacing of

\[ \delta_{FP} \approx \frac{D_{tot}}{3} \]  

(3.7)

In 3D \( \mu \text{PIV} \) applications, light rays experience a refractive index change as they pass from the working fluid in a microchannel into the medium surrounding the objective (air). The relationship between synthetic image plane and synthetic object plane displacement in equation 3.2 assumes that the object is in the same medium as the objective and must be corrected for this
refractive index change. The true object plane displacement is given by

$$\delta' = \frac{n_f}{n} \delta$$  \hspace{1cm} (3.8)$$

where $\delta$ is the apparent object depth and $n_f$ is the refractive index of the working fluid. The apparent object depth is the object plane location that would occur with a uniform refractive index. Similarly, the depth of field, total volume depth and focal plane spacing derived in equations 3.5-3.7 are corrected by substituting $D_{tot2}$, $D_v$ and $\delta_{FP}$ for $\delta$ in equation 3.10, respectively.

To demonstrate light field refocusing, a glass slide containing fluorescent particles was angled with respect to the optical axis and imaged without a microlens array at three different depths. A light field image of the slide was also captured and synthetically refocused at the three different depths. Figure 3.2(a) shows a raw light field microscope image of the glass slide. The top of the slide is farther from the microscope objective than the bottom. A laser (532 nm) illuminates the particles, which fluoresce at 560 nm. The focal plane is slightly above the center of the image. In Figure 3.2(b)-3.2(d), the lenslet array has been removed from the microscope and the microscope lamp provides the illumination for the glass plate and particles. The focal plane locations are set by translating the objective to $Z = 68.5 \mu m$, $0 \mu m$ and $-73.5 \mu m$ for Figure 3.2(b)-3.2(d), respectively. Figure 3.2(e)-3.2(g) show the result of refocusing the light field image from Figure 3.2(a) at depths nearly equal to those shown in Figure 3.2(b)-3.2(d). The refocused light field images match well with the conventional micrographs with some reduction in resolution.

3.2.2 3D Deconvolution

The blurring effect evident in the refocused images in the bottom row of Figure 3.2 would introduce significant noise into the PIV measurements if not removed. Fortunately, Levoy et al. [10] showed that this blurring can be mitigating through a 3D deconvolution approach. We consider the synthetic focal stack to be a volume with spatial intensity $i(x,y,z)$. Under the assumptions of linearity and shift-invariance in formation of the 3D refocused volume, the formation process can be modeled as a 3D convolution [10, 40] written as

$$i(x,y,z) = h(x,y,z) \otimes o(x,y,z)$$  \hspace{1cm} (3.9)$$

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Figure 3.2: Refocusing of a light field image. (a) The original, raw light field image. (b)-(g) Comparisons between refocused light field images and micrographs without a microlens.
where \( o(x,y,z) \) is the true object and \( h(x,y,z) \) the 3D point spread function (PSF) of the optical system. 3D deconvolution attempts to invert equation 3.9 to estimate \( o \) given \( i \) and an estimate of \( h \) [40]. Figure 3.3 shows a graphical representation of Equation 3.9 where the slice from the deconvolved volume is an estimation of the true object. Regardless of the algorithm used to perform deconvolution, an accurate estimate of the PSF is essential. For light field microscopy, Levoy et al. [10] recommend empirically estimating the PSF by imaging a sub-resolution fluorescent bead in the center of a lenslet and refocusing the light field thus generating a focal stack of the PSF. Because it is critical to maintain the same optical characteristics for the PSF images as for the PIV experiment, it may not be practically feasible to image a sub-resolution bead in the center of a lenslet. Therefore, we form an estimate of the PSF by refocusing sparsely seeded light field PIV images, and averaging sub-volumes corresponding to several different particles. Although the particles are likely larger than is desirable, we have achieved good 3D deconvolution results.

Levoy et al. [10] found that an iterative deconvolution algorithm is appropriate for volumes reconstructed with limited angular resolution, as is the case for light field imaging. In this work, the iterative Richardson-Lucy algorithm, commonly applied in traditional optical microscopy [40], is used with 50 iterations. Figure 3.3 shows slices from the refocused volume, the average PSF and the estimated object obtained; images are from actual PIV microchannel data. The blurring that is very apparent in the refocused volume is largely removed in the deconvolved volume. Levoy et
al. [10] performed optical performance tests and observed that refocused images at locations far from the original focal plane, but still within the range over which the light field microscope could be refocused, were not as sharply focused and the cause was attributed to uncorrected spherical aberrations in the objective lens. In our system, this contributes to velocity measurement errors at the extreme depths of the volume. Finally, although the blurring artifacts are reduced through 3D deconvolution, the reconstructed particles are elongated in the axial dimension by a factor of $\approx 4$. This is a result of the reduced axial resolution and has an effect on the PIV results as discussed in Section 3.4.

3.3 Experimental Methods

3.3.1 Setup

A diagram of the experimental setup is shown in Figure 3.4(a). The light field microscope system consists of a Zeiss Axiovert 200 inverted microscope with an EC Plan-Neofluar 20x/(NA = 0.5) objective lens, a 21 Megapixel Canon EOS Mark II 5D DSLR camera (pixel pitch = 6.4 µm) with a 105 mm Nikon macro lens. A microlens array manufactured by Adaptive Optics Associates consists of plano-convex lenses with a pitch of 125 µm and a focal lengths of 2.5 mm. Using equations 3.3-3.6, we find this light field arrangement gives $N_u = 12.5$ resolvable spots, $D_{tot_2} = 20.6$ µm and $D_v = 257$ µm, where each length has been corrected for the refractive index change using equation 3.10 with $n_f = 1.333$ for water.

A collimated laser beam with a 532 nm wavelength reflects off of a dichroic mirror and passes through the objective lens, illuminating the tracer particles in the flow volume. The tracer particles are 1.7 - 2.2 µm Nile Red fluorescent spheres (Spherotech), which fluoresce above 550 nm. A filter allows only the higher wavelengths emitted from the fluorescent particles to pass, removing unwanted reflections of the laser light off of the channel walls, significantly increasing the signal-to-noise ratio. A syringe pump (Brain Tree Scientific, BS-300) drives a 1:5 solution of fluorescent particles to distilled water in a 25 µL syringe (Hamilton) through PVC micro-tubing (508 µm I.D.), where it connects though a micropipette tip to the PDMS microchannel containing a backward facing step. Images are captured at the camera’s maximum frame rate of 3.9 frames per second, and stored on a computer for processing. The low frame rate limits the maximum
Figure 3.4: (a) Experimental setup of the light field microscope and microchannel flow. (b) Image of the microlens array, which is placed at the intermediate image plane of the microscope. The back focal plane of the microlens array is imaged with a 1:1 DSLR camera.

Results for each flow rate are averaged over 143 and 200 image pairs, respectively. Roughly 900 µm of the channel length in the flow direction are visible in the field of the view of the microscope. The backward facing step is positioned roughly in the middle of the field of view, with the flow-wise direction moving from the thin section into the thick section.

3.3.2 Microchannels

The geometry of the microchannels used in these experiments is shown in Figure 3.4(a). The width of the microchannel is approximately 415 µm and the depth in the thin and thick sections is 70 µm and 145 µm, respectively. The channels are constructed from Polydimethylsiloxane (PDMS, Sylgard 184). Photolithography is used to create a positive mold from SU-8 photoresist on a silicon wafer. PDMS is cast around the positive mold and cured in an oven at 90°C for approximately 20 minutes. The channels are then cleaned with ethyl alcohol and bonded to a glass
coverslip. The height of the channel at the mid section and at the high section is measured using the focusing dial on the microscope, which has a resolution of the 1 μm. The microscope was focused on particles stuck to the base of the channel, then focused on particles stuck on other parts of the channel and the difference in position of the dial corresponded directly to the height of the channel.

3.3.3 Calibration method

Proper calibration of the light field microscope requires that the camera sensor lies on the focal plane of the lenslet array and that the lenslet array lies on the intermediate image plane of the microscope. Setting the correct distance between the camera sensor and the lenslet focal plane, \( f_i \), is the most important part of calibration and is necessary for producing valid light field images. The following steps are performed to calibrate the system:

1. A slide with fluorescent particles is brought into focus in the eyepiece of the microscope.

2. The lenslet array is removed from the optics train so the camera images the viewport directly. The camera is set in live mode and with the macro lens on the camera set to a magnification of 1:1, the camera is positioned such that the particles in the viewport are in focus in the camera. The particles should now be in focus in the microscope and the camera.

3. The lenslet array is returned to the optics train between the camera and the viewport (Figure 3.4(b)) and positioned such that a grid pattern appears overlayed on the particle image. When the grid pattern appears, as seen in Figure 3.5(a), the microlens array plane is conjugate with the camera focal plane [41]

4. The lenslet array is adjusted until the micro lenses are vertically and horizontally aligned with the camera sensor.

5. The camera is moved back until it is conjugate with the focal plane of the lenslet array. When the camera is properly focused on the back focal plane of the lenslet array, each lenslet subimage will be nearly uniformly filled for objects that are in focus, as seen in Figure 3.5(b).
6. When all adjustments are complete, an image is captured of an in-focus white background illuminated by a mercury vapor lamp (Zeiss HBO 100) and used as a calibration image to define the \((s,t)\) locations of the lenslet centers.

7. An image is taken of a single particle directly behind a single lenslet. This particle is used to generate the PSF used for deconvolution.

### 3.3.4 Rendering Algorithms

To produce a stack of refocused images from a single light field image, the focal plane spacing must be specified, along with the upper and lower bounds of the refocusing range. Refocusing algorithms were implemented in MATLAB. Code and example files are posted online and can be found at [39]. The spacing and bounds must correct for any refractive index changes. The apparent depth of an object transitioning from a medium of higher refractive to a medium with a lower refractive index is given

\[
q = \frac{n_2}{n_1} p
\]
where \( q \) is the apparent depth, \( n_2 \) is the lower refractive index, \( n_1 \) is the higher refractive index, and \( p \) is the actual depth. When imaging with a dry objective lens, the actual depth is the apparent depth multiplied by the refractive index of the medium containing the fluorescent particles. Thorough descriptions of how the refocusing algorithms function can be found in literature [1, 9], including sample MATLAB code [37].

Focal stack images are post processed in order to reduce blurring and background noise, and produce higher-quality particle images. Post processing techniques implemented in this work include 3D deconvolution. The concepts of 3D deconvolution and its implementation were discussed in Section 3.2.2.

3.3.5 PIV

An open-source code, called matpiv [7], was adapted to perform three-dimensional PIV. The algorithm includes a multi-pass 3D cross correlation between two intensity fields. The user provides interrogation regions sizes for each pass and an interrogation overlap percentage. For more details on matpiv, the reader is referred to [19] and [7].

3.4 Results and Discussion

3.4.1 Channel Flows

Three dimensional velocity fields were generated using the method described above. The averaged vector field for the 0.4\( \mu L/min \) case is shown in Figure 3.6, with several vectors removed for clarity. For this experiment the \( y \) direction (v velocity) corresponds to the flow wise direction, the \( z \) direction (w velocity) is the axial direction and the \( x \) direction (u velocity) is the transverse direction. The vectors are color coded based on their velocity magnitude. Qualitatively, the figures look promising. A profile is clearly visible from top to bottom and side to side, and velocities in the thin section are higher than velocities in the thick section. To get a better idea of the three dimensionality of the flow, a cross section of a \( y-z \) plane was taken halfway between the side walls and is shown in Figure 3.7(a). Some axial movement of the flow can be seen in the section near the step at \( y = 475 \mu m \).
Figure 3.6: Three dimensional vector field of the micro channel flow. A box is drawn around the field to indicate the walls of the channel. In this coordinate system, y is the flow wise direction, z is the axial direction, and x is the transverse direction.

The error of the PIV measurements was obtained by comparison with numerical simulations of the channel. The model used in the simulation was a half channel with a plane of symmetry to reduce computational time. The width was 210µm, with a thin section height of 70µm and a thick section height of 145µm, which is the same as the experimental section. The mesh was composed of hexahedral cells with a near wall prism layer. The boundary conditions consisted of a mass flow inlet, and a split outflow at the outlet, a no-slip boundary condition was imposed at the wall. As the entry length for these Reynolds numbers would be on the order of 1µm and the length of the experimental channel before the test section is several orders of magnitude larger than this, the inlet flow of the CFD simulation was imposed as fully developed. The mass flow at the inlet was set to correspond to the volume flow rate of the syringe pump and the density of water, or 6.7µg/s and 10µg/s for the two flow rates respectively. A y-z plane in the same position as Figure 3.7(a) was taken of the numerical simulation results and is shown in Figure 3.7(b). The vectors are scaled with the same factor and can thus be compared directly. The v velocities appear similar throughout the channel, especially midway along the axial direction. The most significant difference can be seen in the w velocities near the step (y = 475µm), where the PIV appears to underestimate the values produced in the numerical simulation.

To have a better understanding of the differences quantitatively, the error of the measurements was calculated using
Figure 3.7: Comparison of the CFD and PIV data of a y-z slice at $x = 187.5$. (a) shows PIV vector fields, (b) shows the same plane with vectors in the same position of the numerical simulation. The flow in the x direction is neglected in these plots.

$$U_{error} = \frac{U_{cfd} - U_{piv}}{U_{cfd}} \times 100 \quad (3.11)$$

where $U_{cfd}$ are the component velocities predicted by the CFD simulation and $U_{piv}$ are the component velocities measured from PIV.

PIV is not able to resolve the flow near the boundaries, therefore the errors in the flow-wise direction are expectedly high there (Flow wise average error $\approx 2500\%$ with a median value $\approx 33\%$). Neglecting the top, bottom, and side boundaries the average and median errors drop to $\approx 175\%$, and $\approx 18\%$ respectively. The errors in the 0.6 $\mu$L/min are similar, with an average of $\approx 3300\%$, and a median of $\approx 37\%$ for the entire field, and $\approx 40\%$, and $\approx 20\%$ respectively with the disregarded boundaries.

When using the standard error calculation (3.11) the extreme values near the boundaries make it is difficult to visualize the error distribution using a color map. To better visualize the distribution of error through the volume and to help determine some possible sources of error, the color values were clipped at a maximum of 100%.

$$U_\Delta = U_{cfd} - U_{piv} \quad (3.12)$$
Figure 3.8 shows the clipped error images for the v velocities throughout the channel on the 0.4 $\mu$L/min case. The y-z slices are taken from side wall to side wall with the distance from the wall shown in the figure. These images indicate that as you move away from the boundaries the error throughout the channel are relatively low. They also show pockets of high differences at the upper corner of the step ($y \approx 500$).

Some possible explanations of the error in these regions is the effect of the walls on the refocusing. As light field imaging takes rays from multiple directions, some of these rays may pass through multiple water-PDMS boundaries before entering the objective, obscuring or distorting that ray. This could particularly happen at the upper corner of the step. Another possible source of error comes from particles clumping together and adhering to the walls of the channel, creating an obstacle for the flow to move around. Imperfections in the channel geometry due to manufacturing could cause the same issues.

Figure 3.9 shows the v velocity profile preceding the step, and the development of the profile after the step of both the PIV and CFD simulation. The horizontal lines indicate one standard deviation from the mean based on all the time steps averaged of the PIV data. The profiles in Figure 3.9(a) are on the y-z plane at $x = 187.5\mu m$ which corresponds to Figure 3.8(e). The profiles in Figure 3.9(b), which are on the x-y plane at $z = 43.9\mu m$, show good agreement with the numerical simulation in the center of the channel, and also illustrate the error associated with the boundary.

The axial direction shows a significant amount of error near the step. The numerical simulation predicts high w velocities in this region, as shown in Figure 3.10, and the PIV significantly underestimates these velocities. Because of the trade off in axial resolution as described in [10] and in section 3.2.2 there are fewer resolvable planes in the z direction and the particles become elongated. In order to perform PIV with the elongated particles the axial dimension of the interrogation volumes must be increased in size, therefore lowering the resolution of the PIV in the axial direction.

The error of the flow in the transverse direction is shown in Figure 3.11(a). This is another high error source, however, this is most likely not due to interrogation volume size. The numerical simulation contains very little velocity in high PIV errors. The most likely source for flow in this direction seen in the channel is imperfections in the channel, or the bonding. Particles stuck to the
Figure 3.8: Slices in the y-z plane showing the difference in the v velocity between the CFD simulation and the PIV measurements for an inlet flow rate of 0.4\( \mu \)L/min. The x value indicates the distance from the side wall. Areas of high and low error can be seen in these images. Away from the boundaries the differences is consistently low. Near the step (y = 475 \( \mu \)m) the error is higher.

walls of the channel could also cause flow in the transverse direction were the ideal case in the simulation will not contain any of these obstacles.

3.5 Conclusions

A method was presented to find the 3D-3C velocity field of flow through a micro channel. The setup is simple and inexpensive (\( \approx \)$1000 without the camera) and can be applied to an existing system without modifying the microscope. Flow-wise velocities are well resolved throughout the channel with low error, except near the boundaries. Due to the lower resolution in the axial direction the axial velocities were underestimated near the step. High seeding densities can be
Figure 3.9: Velocity profiles showing the development of the flow profile throughout the channel. Figure (a) is a y-z plane located at $x = 187.5\mu m$, figure (b) is a x-y plane located at $z = 43.9\mu m$. The error at the boundaries is clearly visible in these figures.

Figure 3.10: A line plot comparing the w velocity of the PIV, and the numerical simulation. The velocities are located at $x = 187.5\mu m$ and $y = 487.5\mu m$, closely following the step. The numerical simulation predicted high velocities at this point reaching roughly $120\mu m/s$. The PIV, however, significantly underestimated this value by nearly one order of magnitude.
Figure 3.11: A slice of the three dimensional error field positioned 187.5 µm from the side of the channel. Figure (a) shows the velocity error in the x-direction. Figure (b) shows the error in the y-direction, or flow direction. The error throughout the center of the volume is 10-20%. Figure (c) shows the error in the z-direction. This is significant near the step of the channel.

used and instantaneous velocities can be found with every time step. A comparison to other µPIV methods is shown in Table 3.1.
Table 3.1: Comparison of the different $\mu$PIV methods discussed in this section.

<table>
<thead>
<tr>
<th>Method</th>
<th>Dimensional Capacity</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Defocusing</td>
<td>3D-3C</td>
<td>Simple setup can be applied to an existing system.</td>
<td>Very low lighting, very low seeding density.</td>
</tr>
<tr>
<td>LFPIV</td>
<td>3D-3C</td>
<td>Simple, inexpensive setup can be applied to and existing system. Instantaneous vector field for every time step with high seeding densities.</td>
<td>Lateral and axial resolution limited by lenslet and image sensor size.</td>
</tr>
</tbody>
</table>
CHAPTER 4. CONCLUSION

4.1 Summary

Light field imaging applied to fluid flow measurements is a growing field, and has the potential to increase the capability of present 3D PIV techniques. This thesis contains the efforts of applying light field imaging, specifically synthetic aperture refocusing to the reconstruction of the shape and 3D PIV of a flame, and performing μPIV on a micro channel with a backward facing step. Results from the experiment show difficulty using SA refocusing on reacting flows were reconstruction is elongated in the direction of the optical axis of the cameras, and SAPIV is impaired by the error associated with the refractive index. The reconstruction quality might be improved by widening the spacing of the cameras to give more information about the sides of the flame, however, this could cause error from significant warping of the cameras at higher angles. The error associated caused by the refractive index was estimated and shown to be in the range of 0-3 pixels on average. The μPIV results show good agreement with numerical data in the flow wise direction, but significant error in the axial direction. Although this makes the axial velocities unusable, the flow wise velocities are accurate to within 20% on average throughout the volume. The setup for LF μPIV is easy to use and inexpensive, it also allows higher seeding densities that can be resolved for instantaneous velocities and used for unsteady flows.

As mentioned previously, this thesis builds on the work presented by Bryce McEwen in his thesis, and Chapter 3 is a paper that will be submitted to Lab-on-a-Chip in 2014 co-authored by Bryce. Some of the sections of this paper, therefore, are taken from his thesis. My contributions to this work include:

1. Experiments performed using SA refocusing to reconstructed a flame envelope.

2. Determined reconstruction using SA refocusing can cause significant elongation in the z-dimension.
3. SAPIV experiments performed on flames.

4. Determined the refractive index can introduce significant error into the flow velocity calculations.

5. Performed experiments to determine the rough amount of error associated with the refractive index.

6. Determined the apparent movement of the pixels could be in the range of three pixels on average resulting in significant error in particle locations.

7. Literature review of recent contributions of µPIV included in the introduction of the µPIV section.

8. A complete redesign of the optical train movement system in the microscope experimental setup for improved calibration ability and ease of use.

9. Perform µPIV on channels containing backward facing steps creating significant flow parallel to the optical axis, or in the axial direction.

10. Asses the abilities of the method for resolving the axial flow velocities.

11. Determined the flow measurements in the flow-wise direction correspond well to the numerical simulation throughout the channel, and the axial flow measurements significantly underestimate the numerical simulation.
REFERENCES


