Microscopic Light Field Particle Image Velocimetry

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ABSTRACT

Microscopic Light Field Particle Image Velocimetry

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This work presents the development and analysis of a system that combines the concepts of light field microscopy [1] and particle image velocimetry (PIV) to measure three-dimensional velocities within a microvolume. Rectangular microchannels were fabricated with dimensions on the order of 350-950 µm using a photolithographic process and polydimethylsiloxane (PDMS). The flow was seeded with fluorescent particles and pumped through microchannels at Reynolds numbers ranging from 0.016 to 0.028. Flow at Reynolds numbers in the range of 0.02 to 0.03 was seeded with fluorescent particles and pumped through microchannels. A light field microscope with a lateral resolution of 6.25 µm and an axial resolution of 15.5 µm was designed and built based on the concepts described by Levoy et al. [1]. Light field images were captured continuously at a frame rate of 3.9 frames per second using a Canon 5D Mark II DSLR camera. Each image was post processed to render a stack of two-dimensional images. The focal stacks were further post processed using various methods including bandpass filtering, 3D deconvolution, and intensity-based thresholding, to remove effects of diffraction and blurring. Subsequently, a multi-pass, three-dimensional PIV algorithm was used to measure channel velocities. Results from PIV analysis were compared with an analytical solution for fully-developed cases, and with CFD simulations for developing flows. Relative errors for fully-developed flow measurements, within the light field microscope refocusing range, were approximately 5% or less. Overall, the main limitations are the reduction in lateral resolution, and the somewhat low axial resolution. Advantages include the relatively low cost, ease of incorporation into existing µPIV systems, simple self-calibration process, and potential for resolving instantaneous three-dimensional velocities in a microvolume.

Keywords: micro PIV, microPIV, particle image velocimetry, light field, synthetic aperture, SAPIV, microscopic channel flow, bryce mcewen, tadd truscott, jesse belden, lightfield microscope
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<td>$\mu$</td>
<td>Micro Particle Image Velocimetry</td>
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<tr>
<td>$v$</td>
<td>Velocity component in $y$</td>
</tr>
<tr>
<td>$w$</td>
<td>Velocity component in $z$</td>
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CHAPTER 1. INTRODUCTION

This work describes the development and analysis of a microscopic particle image velocimetry system based on light field imaging. This chapter begins by describing the objectives of this work and how they were met. Next, a background is provided on recent work and developments in micro- and macroscopic particle image velocimetry methods. Finally, the theory behind light field imaging and light field microscopy is described. Subsequent chapters include information on the experimental methods, results and discussion, and conclusions.

1.1 Objectives

The objectives of this work are to combine the concepts of light field microscopy and particle image velocimetry to create a system capable of measuring three-dimensional velocities of flow in microchannels, to take measurements using the system, to quantify the system error by comparison to analytical and numerical solutions, and to explore possible applications of the system. To achieve these objectives, a light field microscope was designed, assembled, and calibrated. Computational algorithms were created for rendering focal stacks from light field micrographs, post processing the focal stacks to remove out-of-focus information and achieve quality particle image volumes suitable for performing three-dimensional particle image velocimetry. Image stack post-processing methods included adaptive intensity thresholding, three-dimensional deconvolution, and bandpass filtering. Three-dimensional particle image velocimetry methods were implemented to obtain velocities in microchannels. Microchannels were fabricated and flow through the channels was seeded with particles of appropriate size and driven by a syringe pump. Analytical solutions matching full-developed flow conditions were defined and calculated for measurement comparison. Computational models were created for conditions in which no known closed-form analytical solutions exist. Analysis of the measurements and error were performed and described.
Possible applications of the system were explored by researching areas where volumetric microscopic particle image velocimetry methods are being implemented.

1.2 Background

Advances in microfabrication processes have led 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 numeric models, designing new devices, and improving existing designs. Time-resolved, three-dimensional velocity components can provide important, detailed information about flow field properties. This is especially important for fluids with non-uniform chemistries, non-uniform geometries, non-linear shear responses (non-Newtonian), and particulate flows (e.g. blood flow).

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 led to the development of methods capable of measuring velocities of particles in three-dimensional space (3D) with vectors having three-dimensional components (3C). Often these methods are relatively time intensive, scarcely seeded, and/or expensive. Micro-scale 3DPIV methods generally have further complications and limitations than macro-scale techniques, due to various geometric constraints and lighting difficulties.

The first µPIV system was developed by Santiago et al. [2] in 1998 using an epifluorescent microscope with a mercury lamp and a CCD camera. The system was capable of obtaining two-dimensional velocity vectors spaced 3.45 µm apart over a 120 µm x 120 µm field. Since the introduction of the first µPIV system, several methods have been developed to accommodate more complex flows, especially those involving three-dimensional characteristics. There are several existing µPIV systems with the capability to measure velocity components in three dimensions and developing such systems continues to be an active area of research. Some current 3D-macro-PIV (3D-µPIV) methods include Confocal, Stereoscopic, Defocusing, Volumetric Correlation, and Holographic µPIV.

Park et al. [3] present a µPIV method capable of obtaining 3D-2C measurements through the use of confocal laser scanning microscopy (CLSM). Confocal laser scanning microscopy fo-
cuses a laser at a point to excite a fluorescent signal which passes through a pinhole aperture. The aperture only accepts rays originating from the focal point. A spinning disk is used to scan the laser throughout the focal plane. The scanning/imaging process is repeated at different depths to reconstruct a volume from the optically sliced images. Applying conventional PIV to the images will provide two-component velocity vectors in several planes along the optical axis (3D-2C). The continuity equation can be applied to obtain out-of-plane velocities. Lima et al. [4] have applied confocal µPIV to measure blood flow in microchannels, and report the ability to obtain in-focus, high-contrast particle images with optical thickness of less than 1 µm [5]. This method is not ideal for higher flow velocities because of the time required for scanning, and does not directly measure three-dimensional velocity vectors.

Lindken et al. [6] present a method for obtaining three-dimensional velocity vectors from two-dimensional planes (2D-3C) using stereoscopic µPIV with a self-calibration technique. Two cameras are used to simultaneously capture images from different viewing angles. They report that one of the major limitations of stereoscopic µPIV is due to a relatively large depth of focus of the stereoscopic objective lenses. A large depth of focus decreases the resolution along the optical axis (z-direction) because fewer unique focal slices can be obtained throughout the volume depth. Since stereoscopic µPIV is a 2D-3C method, measurements must be made along several sections in the z-direction in order to obtain velocity vectors throughout the entire flow field (3D-3C). Stereoscopic µPIV has been used by several other researchers for microflow measurements [7–9].

A method for obtaining 3D-3C flow velocities in a micro volume using defocusing is described by Yoon and Kim [10]. They use a mask with three pinholes placed between the objective lens and the image plane. The distances between the three pinholes are all equal, forming an equilateral triangle. The reference plane is located along the optical axis (z-axis), and is the plane located at the focal point of the objective lens. A particle located in the reference plane will appear as a single, in-focus point on the imaging plane. When light from a particle outside of the reference plane passes through the holes in the mask, it will appear as three equidistant points on the imaging plane. The distance between points is correlated to the distance of a particle from the reference plane. The z-direction is determined by the orientation of the triangles. Two major drawbacks of this method are the inability to resolve higher seeding densities and poor light intensity. Particle seeding must be very low because the sets of three points become less distinguishable.
when particles crowd the image plane, thus, the defocusing technique is more of a particle tracking velocimetry (PTV) method than a PIV method. Light intensity is poor because light transmission only occurs through the three pinholes in the mask.

Fouras et al [11] present a technique for resolving 3D-3C velocity fields, similar to the defocusing concept used by Yoon and Kim [10], through the use of volumetric correlation and the continuity equation. If all seed particles have the same diameter, the system can be calibrated to provide a correlation between the diameter and intensity of a detected image particle, and its depth along the optical axis. This method can be applied to conventional macro-PIV systems by illuminating the flow field with a laser volume, rather than a sheet. The focal plane \((z = 0)\) is usually located at one end of the channel depth at a wall boundary. Particles that would otherwise be out of focus, adding background noise, contain information about the locations and displacements in the z-direction. An advantage of this technique is that it can be easily and inexpensively applied to existing PIV systems. Depth correlation is limited by signal-to-noise ratio and bit depth, especially at distances far from the calibration plane.

Kim and Lee [12] report on using digital holographic particle tracking velocimetry (DH-PTV) to measure laminar flow velocities inside of a microtube. Digital holographic methods record interference patterns arising from the intersection of light scattered from an object and a coherent reference light beam. The hologram is recorded on a camera sensor and can be used to determine the distance of particles from the sensor. Kim and Lee [12] use an angular spectrum algorithm to reconstruct particle fields and a particle tracking technique to obtain velocity measurements. They conclude that the method was successful at accurately resolving particle velocities in a 100\(\mu\text{m}\)-diameter channel.

Advantages and disadvantages of the various \(\mu\text{PIV}\) methods are summarized in a Table 1.1. Clearly, no single method has properties that are ideal for all situations. Knowing the advantages and disadvantages of each can help researchers choose an appropriate method to meet the needs of their research objectives. Likely, the future of experimental microfluidic measurements will rely on a variety of methods suited to particular problems, rather than a universal technique.

Blood flow is an important area in the study of microfluidic devices. Increasing understanding of blood flow phenomena could lead to improvements in the design of artificial organs, and the understanding of thromboembolism pathology. Thromboembolism is the process of blood
clots forming (thrombosis) and detaching from their point of origin (embolism). Thrombosis and thromboembolism involve complex interactions of convective transport, chemical reactions, and fluid shear and adhesive forces [13]. Understanding how surface chemistry, geometry, and flow characteristics affect thromboembolism is vital in order to improve numerical models of thromboembolism in biomedical devices [14–17]. Several researchers have used μPIV to measure flow characteristics of blood in a microchannel. As mentioned previously, Lima et al. [4] used confocal μPIV to obtain velocity measurements in a 300μm × 45μm rectangular microchannel. Patrick et al. [14] used confocal μPIV to investigate the intermittency of two-phase cellular-level flow of blood in a rectangular microchannel. Raz et al. [18] used μPIV and CFD to explore the effect of flow patterns and shear stresses on platelet activation of blood flowing through a model of a stenotic vessel. Their PIV measurements were used to validate the CFD model, and there was good correlation, however they indicated the need for more complex, three-dimensional flow models and experimental validation. These studies indicate that there is a high demand for accurate 3D-3C μPIV systems, especially in the biomedical field.

The μPIV method presented in this work makes use of light field imaging. A light field can be defined as radiance of a 4D function of axial and angular position [19]. Light field imaging is the process of capturing a set of rays from a scene, such that they can be reparameterized to render new scenes post capture. Light field imaging and its applications have become more popular over last decade. Several light-field-based PIV techniques exist in the macroscopic realm that are capable of resolving three-dimensional fluid velocities. Here, we present a few state-of-the-art light field-based PIV techniques currently applied to macroscopic fluid flow fields.

Elsinga et al. [20] describe the principles of Tomographic PIV, which uses multiple synchronized cameras at different viewing angles to obtain instantaneous, 3D-3C velocity measurements. Unlike conventional 2D PIV methods where only a plane of the flow field is illuminated, tomographic-PIV illuminates and images a volume of the flow field. The cameras have large depths of field in order to image the whole volume with all particles in focus. The images are reconstructed into three dimensions using optical tomography, a method that reconstructs a volume through stacks of 2D intensity planes. They conclude that the technique is well suited for studying complex 3D flows. However, tomographic-PIV is not well suited for micro-scale flow measurements. Microscope objective lenses with higher magnifications have very small depths
Table 1.1: Properties of volumetric $\mu$PIV methods. Park et al. [3] explored two different objective lens magnifications in their confocal laser scanning microscopy (CLSM) experiments, and they are represented in two separate columns. The lateral and axial resolutions, frame rates, particle density, volume size, interrogation region resolution, disadvantages and advantages are presented.*May produce 3D-3C data by taking measurements at different depths. **May produce 3D-3C data through continuity.

<table>
<thead>
<tr>
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<tbody>
<tr>
<td>Lateral Res. [\mu m]</td>
<td>0.829 9.323 25 fps 5.5 x 5.5</td>
<td>0.331 1.268 25 fps 44 x 44 x 15</td>
<td>0.595 10 $\mu$s 2D-3C* NA NA</td>
<td>NA</td>
<td>0.71 NA</td>
<td>NA 500 $\mu$s 3D-3C Unlimited</td>
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<td>Axial Res. [\mu m]</td>
<td>3D-2C**</td>
<td>3D-2C**</td>
<td>0.002%</td>
<td></td>
<td>768 x 388 x 50 9.4x 5 x 5 x 1</td>
<td>350 x 100 x 100 60x NA</td>
</tr>
<tr>
<td>Max. Velocity</td>
<td>900 x 680 x 200 44 x 44 x 15</td>
<td></td>
<td>Relatively inexpensive, three-component vectors</td>
<td>Simple setup, adaptable to existing systems</td>
<td>Very low lighting, very low seeding density Correlation depth limited by camera bit depth and signal-to-noise ratio</td>
<td>Long data processing times, low particle density</td>
</tr>
<tr>
<td>Dimensions</td>
<td>195 x 99 x 99</td>
<td>760 x 516 x 516</td>
<td></td>
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<tr>
<td>P.Density</td>
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<td></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Volume</td>
<td>10 x 22</td>
<td></td>
<td></td>
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<tr>
<td>Magnification</td>
<td>Particle contrast, depth-wise resolution</td>
<td></td>
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</tr>
<tr>
<td>Magnification Resolution [\mu m]</td>
<td>3D-2C**</td>
<td>3D-2C**</td>
<td></td>
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<tr>
<td>Advantages</td>
<td>Particle contrast, depth-wise resolution</td>
<td></td>
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</tr>
<tr>
<td>Disadvantages</td>
<td>Complex, relatively expensive setup, two-component vectors, depth-wise scanning time</td>
<td>Complex, relatively expensive setup, two-component vectors, depth-wise scanning time</td>
<td>Large depth of focus decreases depth-wise resolution, complex calibration, only two-dimensional slice of instantaneous vector plane</td>
<td>Very low lighting, very low seeding density</td>
<td>Correlation depth limited by camera bit depth and signal-to-noise ratio</td>
<td>Long data processing times, low particle density</td>
</tr>
</tbody>
</table>
of field and working distances—on the order of millimeters. Small focal lengths in comparison with the objective lens diameters make it spatially impossible to arrange a set of more than two higher-magnification microscope objectives to focus on a single point.

Belden et al. [21] present a method that uses the concepts of light field imaging and synthetic aperture refocusing to produce 3D-3C velocity measurements (Synthetic Aperture Particle Image Velocimetry). An array of synchronized cameras with large depths of field capture directional information from light rays and a synthetic refocusing algorithm is used to digitally refocus on different planes throughout the flow field. They report the ability to image flows with high seeding densities and even multi-phase and bubbly flows. The concepts behind SAPIV show potential for application to micro-scale flows.

Both Tomographic and Synthetic Aperture PIV techniques use an array of camera sensors to capture light field information. It is also possible to use an array of lenses with a single camera sensor to sample light fields [1, 22, 23]. Lynch [24] investigated a method of using a plenoptic camera to capture light field images of macroscopic flow fields, and using refocused images to perform 3D-3C PIV measurements. He performed a numerical evaluation and concluded that the method is feasible and, due to its simplicity, can be used as a compliment or inexpensive alternative to 3D-3C multi-camera techniques.

Many macro-scale PIV methods cannot be applied to µPIV systems because wave optics can no longer be neglected as length scales approach the diffraction limit of light. Levoy et al. [1] have developed a method for converting a conventional microscope into a light field microscope capable of producing a three-dimensional volume from a single photograph. An array of microlenses is placed at the intermediate image plane of a microscope, between the objective and a camera. Each lenslet in the array provides a slightly different viewing angle, thus capturing light field information that can be used to create three-dimensional focal stacks and perspective views. In a light field microscope, some lateral resolution is sacrificed to provide better angular resolution. The experiments described in this work use focal stacks produced by a light field microscope [1] to quantify microchannel flow using three-dimensional particle image velocimetry.
1.3 Light Field Micro-imaging

A light field, as discussed in this work, is radiance as a four-dimensional function of angular and spatial location, and it is possible to create an optical system capable of capturing a light field in a single photograph [1, 19, 22]. A four-dimensional light field written as \( L(u, v, s, t) \) describes the radiance along all rays that intercept a scene as parameterized by the intersection of the rays with two specific planes: the \( uv \) and \( st \) planes [19]. Placing an array of microlenses (lenslets) at the intermediate image plane of an imaging system (as shown schematically in Figure 1.1) enables capture of a sampled version of a four-dimensional light field in a single photograph [1, 22]. As depicted in Figure 1.1, the microlenses define the \( st \) plane and the camera sensor defines the \( uv \) plane. A light field can be reparameterized to recover information not just at the capture (image) plane, but in depth (\( Z \)) as well, which requires trading spatial resolution for angular resolution [22]. 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 pixels behind each lenslet [1]. For more details regarding light field microscope resolution, see section 1.5.

1.3.1 Light Field Refocusing

Figure 1.2 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 Figure 1.2) meet at a point at the lenslet plane, and thus spread in a circular pattern (for a circular aperture) behind a single lenslet. Rays from a point in the imaging volume not at the focal depth (dashed lines in Figure 1.2) do not meet a point at the lenslet plane and thus span multiple microlenses. There-
Figure 1.2: Ray diagram of a light field microscope that makes use of an infinity corrected objective.

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. The spatial resolution of this synthetically refocused image will be \( N_s \times N_t \), where \( N_s \) and \( N_t \) are the number of lenslets in the \( s \) and \( t \) dimensions, respectively. This represents the lateral resolution of the light field microscope. In microscopy, axial resolution is typically given as the depth of field of the imaging system. Levoy [1] describes three cases that should be considered when discussing the axial resolution of the light field microscope. These three cases include the depths of field of the microscope alone (\( D_{tot1} \)), the microscope with the microlenses (\( D_{tot2} \)), and a single microlens pixel (\( D_{tot3} \)). The equations for each these depths of field as derived in [1] are:

\[
D_{tot1} \approx \frac{\lambda n}{NA^2} 
\]

(1.1)

\[
D_{tot2} \approx \frac{(2 + N_u) \lambda n}{2NA^2} 
\]

(1.2)

\[
D_{tot3} \approx \frac{(2 + N_u^2) \lambda n}{2NA^2} 
\]

(1.3)

where \( \lambda \) is the light wavelength, \( n \) is the refractive index of the objective lens (\( n = 1 \) for a dry lens), \( NA \) is the numerical aperture of the objective lens, and \( N_u \) is the number of resolvable spots.
behind each lenslet. $D_{tot 2}$ represents the depth of field of a rendered light field image. $D_{tot 3}$ is the theoretical range over which light field images can be refocused.

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 1.3, the $s' - t'$ coordinates of a reprojected pixel can be found from similar triangles and are given by

$$\begin{bmatrix} s'_p \\ t'_p \end{bmatrix} = \frac{d}{f_t} \begin{bmatrix} u_p \\ v_p \end{bmatrix} + \left( 1 - \frac{d}{f_t} \right) \begin{bmatrix} s_l \\ t_l \end{bmatrix}$$

(1.4)

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 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

$$\delta = \frac{d}{M^2}$$

(1.5)

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. In terms of geometric optics, the spatial resolution of the light field equals the size of the lenslets, $p_l$. 

10
Therefore, the discretized synthetic image plane is sampled with resolution \( p_s = p_l \) and overall size of \( N_s \times N_t \). Herein, a bilinear interpolation scheme is used to sample the synthetic images.

Note that by tracing the marginal rays from a given pixel (shown by the dashed lines in Figure 1.3), we find 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.

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 1.4(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 Figures 1.4(b)- 1.4(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 Figures 1.4(b)- 1.4(d), respectively. Figures 1.4(e)- 1.4(g) show the result of refocusing the light field image from Figure 1.4(a) at depths nearly equal to those shown in Figures 1.4(b)- 1.4(d). The refocused light field images match well with the conventional micrographs with some reduction in resolution.
Figure 1.4: 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.
1.4 3D Deconvolution

The purpose of deconvolution is to undo image formation to estimate the original object. Imperfections introduced in the image formation process include effects such as blurring, noise, diffraction, etc. If the nature of the imperfections can be quantified, one can perform a reverse convolution (deconvolution) that would ideally result in the original object. Deconvolution is a very useful tool in the field of microscopy, as it is used to remove out-of-focus information from adjacent planes in a focal stack. The formation process can be modeled as a 3D convolution under the assumptions of linearity and shift-invariance in formation of the 3D volume [1, 25]. Letting $o(X,Y,Z)$ be the object and $h(X,Y,Z)$ the 3D point spread function (PSF) of the optical system, then the volume formation is given by

$$i(X,Y,Z) = h(X,Y,Z) \otimes o(X,Y,Z)$$  \hspace{1cm} (1.6)

The field of 3D deconvolution is concerned with effectively inverting equation 1.6 to estimate $o$ given $i$ and an estimate of $h$ [25]. Regardless of the algorithm used to perform deconvolution, an accurate estimate of the PSF is essential. For light field microscopy, Levoy et al. [1] 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 reasonable 3D deconvolution results.

Levoy et al. [1] 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 [25], is used. Figure 1.5 shows volume renderings of the tilted glass slide, the average PSF, and the estimated object obtained after 100 iterations of the 3D deconvolution algorithm. The volume was refocused from $-120 \mu m$ to $120 \mu m$ in $5 \mu m$ increments and volume renderings were produced. The effect of blurring is very apparent in the raw volume renderings, especially when viewing...
the volume at an angle of 90°. An average PSF was constructed using the method described above. The average PSF is shown in Figures 1.5(e)-1.5(h) and was used in the deconvolution procedure. The deconvolved volume renderings are shown in Figures 1.5(i)-1.5(l). Blurring is significantly removed after 100 iterations of the deconvolution algorithm. It is important to note, however, that particles located at ends far from the original focal plane are very blurry even after deconvolution. Levoy et al. [1] 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 than they would have liked, and the cause was attributed to uncorrected spherical aberrations in the objective lens. For the system used in this work, \( D_{\text{tot}} \) is around 160 \( \mu \text{m} \), which gives a range of 80 \( \mu \text{m} \) on either side of the original focal plane (see Section 2.1 for details on system specifications). The particles that are in focus in Figures 1.4(b) and 1.4(d) are located at the far ends of the total refocusable depth of field, and are thus not as sharply focused in Figure 1.5(i).

1.5 Limitations

The reader is referred to Figures 1.1 and 1.3 for visual aid of the following discussion. As mentioned in section 1.3, there is a trade-off between lateral and axial resolution in a light field microscope. The number of pixels in a refocused image is limited by the number of lenslets in the microlens array. Increasing the number of lenslets will decrease the number of pixels on the sensor behind each lenslet. The number of pixels behind each lenslet is related to the refocusing range and the number of distinct focal planes in a rendered volume. This presents a trade-off that must be made between lateral and axial resolution. Large camera sensors are necessary because the number of pixels of a refocused light field image will be equal to the number of lenslets in the microlens array. Small pixel sizes are required in order to adequately sample images from the microscope. The smallest separation distance that can be resolved between two point sources imaged by a microscope is known as the Sparrow Limit and is given by Inoue [26] as

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

(1.7)
Figure 1.5: Volume renderings of refocused light field images and the microlens array PSF from $-100 \mu m$ to $100 \mu m$ in $5 \mu m$ increments. (a)-(d) Volume rendering of fluorescent particles on tilted microscope slide. (e)-(h) Volume rendering of average single lenslet images. (i)-(l) Volume rendering after 100 iteration deconvolution using single lenslet PSF.
The pixel pitch on the camera sensor must be smaller than this value or there will be an additional reduction in resolution. One major limitation of large sensor sizes with small pixel pitches is the camera frame rate, which limits the maximum velocities that can be measured in microchannels. Camera sensors with small pixels normally have small sensor sizes and therefore smaller fields of view, which translates into fewer microlens images that can be projected onto the sensor. This reduces maximum field of view that can be measured. Illumination for a light field microscope must also be spatial and angularly uniform [1], otherwise illumination intensity will fall off towards the periphery of the images or of the microlens subimages [1]. Fortunately, fluorescent particles are used in $\mu$PIV, which emit light at an angular distribution that is independent of the excitation light source. This means that spatial uniformity will result in angular uniformity.

1.6 Conclusions

Several methods exist and are being developed for performing three-dimensional $\mu$PIV, each with its own set of limitations and advantages. Microfluidic devices have several applications in the biomedical field, such as quantifying blood flow properties and phenomena. Light Field Microscopy has the ability to capture a light field image and render images at arbitrary depths along the optical axis to produce a three-dimensional focal stack. Thus, a three-dimensional volume can be captured in a single photograph. Three-dimensional deconvolution can be used to remove out-of-focus information from adjacent planes in a focal stack, thereby reconstructing the original volume. The main limitations of Light Field Microscopy are the loss of lateral resolution and the trade-off between lateral and axial resolutions. Light Field Microscopy shows good potential for $\mu$PIV applications notwithstanding some of the main resolution limitations. The proceeding chapters contain information on the experimental methods, results and discussion, and conclusions and suggested future work. Chapter 2 describes the equipment and properties of the light field microscope and PIV system, the process whereby the microchannels were created, including the microchannel geometries, the calibration requirements and procedures, the algorithms used for light field refocusing and PIV, and the approaches used to validate measured velocity data. Chapter 3 presents the results and discussion of several experiments, as well as CFD simulations used for comparisons. Chapter 4 provides conclusions and a brief, and outlines suggestions for future work.
CHAPTER 2. EXPERIMENTAL METHODS

This chapter contains a description of the experimental setup, microchannels, system calibration, light field processing algorithms, PIV algorithms, and validation methods. The equipment and properties of the experimental setup, including the light field microscope, and PIV system components and settings are discussed in detail. The microchannel fabrication methods, materials, and geometries are described. Light field microscope calibration procedures are outlined, the implementation of light field rendering algorithms is explained, PIV algorithms are listed, and the vector field validation methods used herein are introduced.

2.1 Experimental Setup

A typical µPIV system consists of a microscope, an illumination source, seeding particles (usually fluorescent), a camera, a pump, and a flow channel. Typical algorithms used to process acquired images include image processing algorithms to increase particle image quality, correlation algorithms to obtain particle image displacements, vector post-processing algorithms to correct spurious vectors, and algorithms to analyze measured data. This section provides descriptions of the equipment and algorithms used in this work. A diagram of the setup is shown in Figure 2.1(a). The light field microscope system consists of a Zeiss Axiovert 200 inverted microscope with an EC Plan-Neofluar 20x/0.5 objective lens, a 21 Megapixel Canon EOS Mark II 5D DSLR camera with a 105 mm Nikon lens, and a microlens array manufactured by Adaptive Optics Associates. The individual lenslets in the microlens array are plano-convex with pitches of 125 µm and a focal lengths of 2.5 mm. The depth of field of rendered light field images ($D_{\text{tot}_2}$) and refocusing range ($D_{\text{tot}_3}$) for this microscope objective and lens are approximately 15.5 µm and 160.1 µm, respectively. Details of how the microlens array parameters were selected can be found in Levoy et al. [1]. A columnated 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 $\mu$m 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 mixture of distilled water and fluorescent particles in a 1 cc syringe (B-D) through PVC micro-tubing (0.02” I.D.), where it connects to a PDMS microchannel though a micropipette tip (see Figure 2.1(b)). Images are captured at a the camera’s maximum frame rate of 3.9 frames per second, and stored on a computer for processing. The low frame rate significantly limits the maximum flow rates that can be measured in the channel.

2.2 Microchannels

The microchannels used in these experiments were constructed from Polydimethylsiloxane (PDMS, Sylgard 184). PDMS is a polymer that is widely used for microfluidic devices. It has good optical transparency, excellent casting properties, and is biocompatible in that it does not react strongly with biofluids, such as blood. [27]. This section contains detailed information on
Table 2.1: Settings used for coating the silicon substrate with SU-8 2075 photoresist.

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<tr>
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<th>Speed [rpm]</th>
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</table>

the fabrication of microchannels and the final channel geometries used for the experiments in this work.

2.2.1 Fabrication Methods

The microchannels were created by casting PDMS over a positive mold made from of SU-8 2075 photoresist. After casting, the PDMS was cured and a thin PDMS bottom layer was attached though a stamp and stick method. The following sections provide detailed process descriptions for photolithography, casting, and bonding.

Photolithography

1. A silicon substrate is prepared by submerging in a Hydrofluoric acid solution for approximately 30 seconds.

2. The substrate is rinsed thoroughly with deionized water for at least 2 minutes and dried with a nitrogen gun.

3. The wafer is centered on a Laurell Spinner and the vacuum is turned on to hold the wafer firmly against the chuck. A quarter-sized amount of SU-8 2075 is poured onto the wafer surface.

4. A three-step process is used to spin the SU-8 to a uniform thickness using the settings shown in Table 2.1.

5. The wafer is placed on a hot plate for a soft bake at 60°C for 2 minutes followed by 95°C for 7 minutes.
6. The mask, used for exposure, is created by printing the channel pattern onto a transparency using a high-resolution printer. The transparency is attached to a glass plate for use in the aligner.

7. A Karl Suss mask aligner is used for the exposure process. The exposure time is set to 18s, the alignment gap is set to 80 µm, hard contact delay is set to 10 s, and resist type is set to negative. The mask is inserted to the aligner and the exposure process takes place. The wafer and the mask are removed from the aligner.

8. The wafer is placed on a hot plate for a post exposure bake at 60°C for 2 minutes followed by 95°C for 7 minutes.

9. The wafer is placed into a developer solution and agitated for approximately 15 minutes. After development, the surface is washed with isopropyl alcohol and dried with a nitrogen gun.

10. The wafer undergoes a final hard bake on a hot plate at 150°C for 2 minutes.

**Casting**

The following processes were used to cast the PDMS microchannels used in this work.

1. Sylgard 184 is a two-part elastomer kit produced by Dow Corning. One part curing agent is mixed with ten parts of the base to begin the cross-linking process. For one batch of microchannels, 4.5 grams of developer is mixed with 45 grams of base.

2. The two parts are mixed thoroughly and placed in a vacuum chamber until all bubbles are removed. This process normally takes about 15-20 minutes.

3. The silicon wafer containing the SU-8 mold is clamped into the acrylic casting device shown in Figure 2.2. Micropipette tips (GeneMate Ultramicro 0.1 - 10 µL) are used to create the holes that connect to the channels. Micropipette tips are inserted into holes in the top plate. Before inserting the micropipette tips, the tip holes are plugged using putty that can withstand the temperatures reached during curing. The reason for plugging the holes is to
prevent capillary rise of the PDMS into the pipette tip. If only the top portions of the tips are sealed, the air inside of the pipette tips will expand during heating and exit out of the bottom, producing bubbles in the PDMS. The bubbles will usually rise high enough above the channel that the channel will remain unaffected, however it is recommended that only the bottom portion of the pipette tip be plugged. Machine screws (8-23 2") are inserted through the holes in the plate corners and the first set of nuts is used to clamp the wafer between the retaining wall and the base plate. Another set of nuts is added to the screws and will be used to hold the top plate at the correct distance above the wafer. The top plate is placed on the second set of nuts and the final set of nuts holds the top plate firmly in place. Final adjustments of the micropipette tips are made so that they align with the channel entrances and exits, and the PDMS is poured into the casting device.

4. The casting device is placed in an oven to cure at 90°C for approximately 30 minutes.

5. A small layer of PDMS (~ 0.7 mm) is poured onto a smooth silicon wafer and cured at 90°C for approximately 20 minutes.

**Bonding Method 1**

1. After curing in the oven, the casting device is removed and disassembled. The top plate, holding the micropipette tips, is carefully pulled from the PDMS and the PDMS is peeled from the SU-8 mold master. The thin layer of PDMS is carefully peeled from the smooth silicon wafer.

2. A smooth wafer is cleaned with acetone and isopropyl alcohol and then placed onto the Laurell Spinner. The vacuum is turned on to hold the wafer firmly against the chuck. A quarter-sized puddle of PDMS curing agent is poured onto the wafer and spun to a very thin layer by using the settings shown in Table 2.2.

3. The wafer is removed from the spinner and the cured channel section of PDMS is carefully placed, with the channel face down, onto the wafer with the curing agent. The channel section is carefully removed from the wafer and brought into contact with the thin PDMS layer, creating the channel floor.
Figure 2.2: Acrylic device used for pouring PDMS over SU-8 mold. The retaining wall holds the wafer in place and allows the PDMS to be poured to the appropriate height. Before pouring, micropipette tips are inserted through holes in the tip holder and the height of the holding plate is adjusted such that the micropipette tips are brought into contact with the microchannel reservoirs. The PDMS flows around the pipette tips, forming a hole into which a micropipette tip is later inserted for fluid connections, similar to a method used by Lima et al. [4].

Table 2.2: Settings used for spinning a thin layer of Sylgard 184 curing agent onto a smooth silicon wafer for stamp and stick bonding.

<table>
<thead>
<tr>
<th>Step</th>
<th>Speed [rpm]</th>
<th>Acceleration [rpm/s]</th>
<th>Time [s]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Step 1</td>
<td>500</td>
<td>86</td>
<td>6</td>
</tr>
<tr>
<td>Step 2</td>
<td>2000</td>
<td>344</td>
<td>60</td>
</tr>
<tr>
<td>Step 3</td>
<td>6000</td>
<td>6020</td>
<td>2</td>
</tr>
</tbody>
</table>
4. The PDMS is placed into an oven at 90°C to cure for 10 minutes. If done properly, this method creates an excellent permanent bond between the two PDMS sections.

5. Channels used for experiments are sliced from the main piece of PDMS. The channel bottom and a glass slide are cleaned with ethyl alcohol, and the channel forms a reversible bond when brought into contact with the glass slide.

6. To create fluid connections to the channels, micropipette tips are inserted to the inlet and outlet holes, and PVC microtubing is inserted into the micropipette tips as shown in Figure 2.1(b).

**Bonding Method 2**

As an alternate bonding method, the PDMS channel is bonded directly to a glass coverslip rather than adding a PDMS floor. The PDMS floor is more suitable for applications where biocompatibility is essential, such as blood flow. The main drawback of using PDMS for the channel floor, however, is the reduction in optical performance. Objective lenses are highly optimized optics designed for very specific conditions. The objective lens used in this work is a dry lens, designed to view specimens in air. The lens has a built-in correction for a 0.17 mm coverslip. Imaging through the bottom layer of PDMS causes spherical aberrations due to the index of refraction change and distortions due to anisotropy in the PDMS, thus a coverslip is used to provide optical axis to the channel. The coverslip is bonded to the PDMS through the following Oxygen plasma treatment using a Planar Etch II (PE2) plasma etcher.

1. The casting device is disassembled and the channels are carefully peeled cut and peeled from the silicon wafer.

2. One or more channels is/are cut from the main channel block. The channels must be cut so that they can fit onto a 24 mm x 60 mm coverslip.

3. The coverslip and microchannel surfaces are cleaned with Acetone, followed by Isopropyl alcohol and finally Methanol.
4. The vacuum and power to the PE2 machine are turned on and the Oxygen supply valve is opened.

5. With the vacuum valve closed, the vent switch is turned on to vent the chamber with Nitrogen and bring it to atmospheric pressure.

6. The chamber is opened and the channel(s) and coverslip(s) are placed in the chamber so that the surfaces to be bonded are near, but not in contact with, the floor.

7. The vacuum valve is opened slowly in order to avoid displacing the samples until the chamber pressure reaches approximately 100 mTorr.

8. Once the chamber pressure decreases below 100 mTorr, the valve may be opened more. The chamber is vacuumed to a pressure of about 40 mTorr.

9. The Oxygen switch is turned on and the Oxygen valve is adjusted until the pressure reaches 600 mTorr.

10. The power is set to 20 Watts. To ensure that the appropriate power is used, it is recommended that the machine be run and adjusted until the desired power is reached before the samples are placed in the chamber.

11. The Generator Power switch is turned on and the samples are exposed to the plasma treatment for 30 seconds.

12. The Power Generator switch is turned off, the Oxygen switch is turned off and once the Oxygen flow returns to 0, the Vent switch is briefly switched on and then off again to vent potentially toxic gases from the chamber. When the chamber pressure returns to 40 mTorr, the vent switch is briefly switched on one more time.

13. The vacuum valve is closed and the chamber is vented back to atmospheric pressure.

14. The chamber is opened and the channel(s) and coverslip(s) are brought into contact as quickly as possible.

15. The bonded channel(s) are placed on a hotplate at 75°C for 5 minutes.
2.2.2 Channel Geometries

The channels used in these experiments were rectangular with sudden expansions and contractions. They were originally designed for performing blood flow experiments similar to those described in Goodman et al. [17]. Figure 2.3 shows the parameters of the channel dimensions. For the photomask, the lengths, $L_1$ and $L_2$, were both specified at 1 mm and the widths, $W_1$ and $W_2$ were specified at 955 $\mu$m and 430 $\mu$m, respectively. The channel depths were measured by focusing the microscope on particles adhering to the channel floor and then moving the objective until it focused on particles adhering to the channel ceiling. The distance between the floor and the channel was measured with the focusing drive, which has a 1 $\mu$m resolution. To correct for refractive index changes, the measured distance was multiplied by the refractive index of water (1.333) to obtain the actual channel depth. To validate this measurement technique, a channel was cut in half, mounted to a glass coverslip, and imaged with the microscope. Figure 2.4(a) shows an image of a channel cross section. The image shown in Figure 2.4(b) was taken of a calibration grid (Thorlabs R1L3S3P) with 50 $\mu$m spacings. The image was read into MATLAB and a region of grid cells was selected, cropped, and binarized. The centroid locations for each of the grid cell elements were calculated to sub-pixel accuracy using the `regionprops` function in MATLAB, and a plot of the centroids and binarized image is shown in Figure 2.4(c). The means and standard deviations of the distances between neighboring centroid locations in $x$ and $y$ were calculated. The mean distance between grid cells in $x$ is 159.4 px with a standard deviation of 0.475 px, and the mean distance between grid cells in $y$ is 159.4 px with a standard deviation of 0.445 px. To convert distances measured in pixels to real space, the pixel distance is divided by the mean distance between grid cells.
(pixels/grid cell) and multiplied by 50 µm per grid cell. The grayscale images were viewed with the colormap shown in Figure 2.4(d) to more easily distinguish between shades of gray. A region of interest was selected, cropped, and enlarged. The $x$ locations of the walls were specified and the depth of the channel shown in Figure 2.4 was measured to be 434.9 µm ± 0.95 µm. In comparison, the depth measured by focusing on the top and bottom of the channel and multiplying the distance (323 µm) by the refractive index of water gives 427.9 µm ± 2.5 µm, which matches within 1.6% of the method of the cross section imaging method. While the measurement uncertainty for cross section method is lower, the result may be less accurate because the cut may not have been made perfectly perpendicular to the channel. For example, if the actual channel depth is 430 µm and a cut is made with a 10° deviation from the depthwise ($z$) axis, the resulting cross-sectional depth will be 436.6 µm—a 1.5% difference. Therefore, the focusing measurement method will be used for determining all microchannel depths. Measurements for channel widths were made using the raw light field images and converting between pixels and object space through:

$$L_{obj} = \frac{L_{px}P_s}{M}$$  \hspace{1cm} (2.1)  

where $L_{obj}$ is the length in object space, $L_{px}$ is the measured length of the photograph in pixels, $M$ is the magnification, and $P_s$ is the pixel pitch on the camera sensor in µm per pixel. More details on measurement methods, including the uncertainty analysis can be found in Appendix B.

The velocity measurements were performed in two different channels having depths of 427.9 µm ± 2.5 µm and 373.2 µm ± 2.5 µm.

2.3 Calibration

Calibration consists of proper positioning and alignment of optical components, and registering lenslet locations and sizes in order to parameterize the light field. This section discusses the procedures for calibrating the system.
Figure 2.4: (a) Cross section of a PDMS microchannel. (b) Calibration grid with 50 μm squares used to calibrate distance measurements. (c) Plot of centroids locations of grid cells. (d) Channel cross section viewed with a ‘jet’ colormap. (e) Wall locations are specified by user interface.
2.3.1 Positioning and Alignment

Proper calibration of the light field microscope requires that the camera sensor lie on the focal plane of the lenslet array and that the lenslet array lie on the intermediate image plane of the microscope. Setting the camera sensor accurately at the lenslet focal plane, \( f \), 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 covered with fluorescent seeding particles is brought into focus in both the microscope eyepiece and the microscope camera port.

2. The lenslet array is inserted into the optical train and positioned such that there is a grid pattern overlaying the image. When the grid pattern appears, as seen in Figure 2.5(a), the microlens array plane is conjugate with the camera focal plane [28]. To cut off uncorrected aberrations from the objective lens, a C-mount camera adapter with a 20 mm aperture is inserted into the camera port. To simplify the step of positioning the microlens array, a nylon spacer is placed against the barrel adapter to position the array where the a standard C-mount camera sensor would normally be.

3. The camera focal plane 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 2.5(b).

4. The camera is set to live view while the lenslet array is rotated until the lenslet rows and columns are parallel and perpendicular with the sensor pixels. The rendering algorithms used by Levoy and Ng [1, 22] use Fourier slice refocusing and the calibration grid used in LFDisplay [29] can take into account the rotation of microlenses with respect to the camera sensor. Lynch [24] reports that the bilinear interpolation methods used in their refocusing algorithms to rotate images and ensure that the correct number of pixels are behind each lenslet degrade the quality of particle images. The calibration in this work uses the same type of lenslet registration as described in [24] and therefore the lenslet grid must not be rotated relative to the sensor grid.
5. When all adjustments are complete, an image is captured of an in-focus white background illuminated by a green mercury vapor lamp (Zeiss HBO 100) and used as a calibration image to define the \((s, t)\) locations. To ensure the appropriate number of pixels behind each lenslet, an iterative process can be used whereby a calibration image is captured and read into LFDisplay to measure the number of pixels between lenslet centers. This process is repeated until the number of pixels behind each lenslet is correct.

### 2.3.2 Lenslet Registration

Lenslet registration is necessary for reparameterizing the light field so that ray tracing can be performed. The registration method used in this work is based on the process described in [24]. Each lenslet is assigned a spatial \(s, t\) coordinate relative to the optical axis and each pixel on the camera sensor is assigned an angular \(u, v\) coordinate relative to the optical axis. The steps for completing lenslet registration are described below.

1. The microscope is focused on a white background illuminated by a green mercury vapor lamp and a light field image is captured. This image is used as a calibration image to determine lenslet centroid locations with sub-pixel accuracy.
2. The calibration image is read into LFDisplay to determine the center lenslet location and appropriate grid spacing. This step is not necessary as it can be performed in MATLAB, however, LFDisplay provides a convenient and fast user interface for determining lenslet spacings. Figure 2.6 shows the grid aligned at the center lenslet. The red lines mark the center lenslet location. Figure 2.7 shows the grid after adjusting the grid size to match the lenslet sizes. The lenslet size information is saved to a text file.

3. The calibration image is read into MATLAB and the user selects lenslets at the periphery of the image so that the image can be cropped to removed unused lenslets. A circle is fit to the selected periphery lenslets and the grid information is read in from the lenslet text file or provided interactively by the user if no lenslet file exists. A grid is fit to the bounding box of the circle and the user specifies the region of lenslets to use.

4. A two-dimensional Guassian distribution is fit to the intensity image for each grid cell to find the sub-pixel location of each lenslet centroid.

5. All radiance elements (pixels) in the light field image are assigned to their parent lenslets.

2.4 Light Field Image Processing

Focal plane spacing and the upper and lower bounds of the refocusing range are specified to produce a stack of refocused images from a single light field image using the concepts described in Section 1.3.1. Refocusing algorithms were implemented in MATLAB and the code can be found in Appendix A. 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 by equation 2.2

\[ q = \frac{n_2}{n_1} p \]  

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
Figure 2.6: LFDisplay is used to provide the center lenslet location. This figure shows the grid centered at the microscope aperture.

descriptions of how the refocusing algorithms function can be found in literature [19,22], including sample MATLAB code [24].

Focal stack images are post processed in order to reduce noise and produce better quality particle images. Post processing techniques implemented in this work include 3D deconvolution, bandpass filtering, and intensity thresholding. The concepts of 3D deconvolution and its implementation are discussed in Section 1.4. Bandpass filtering discards frequencies that are outside of a range. A particle image diameter is specified and image data outside of the diameter range are discarded. The intensity-based thresholding technique divides each image into windows of a specified size. An intensity distribution is calculated for each window and used to determine a threshold value. For example, an intensity distribution is calculated for a given window having a mean, $\mu$, and standard deviation, $\sigma$. A threshold value may be set to $t = \mu + k\sigma$, where $k$ is some constant, and all pixel intensities that are less than $t$ are set to 0. This thresholding technique was used by Belden et al. [21] and Lynch [24].
2.5 Particle Image Velocimetry Methods

An open-source code, called matpiv [21], 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 [30] and [21].

2.6 Vector Field Validation Methods

Several approaches are used to validate the PIV results, including experimental measurements, computational analysis, and comparison to known theoretical solutions. The performance of the system described herein was analyzed by displacing particles by a known amount between image pairs, and also by comparing channel velocities to CFD results and known analytical solutions. Exact solutions exist for fully-developed flows in channels with simple geometries, such as cylinders and rectangular ducts. The entry length required to reach fully-developed flow in a uni-
Table 2.3: Predicted entry lengths required for fully-developed flow, calculated using equation 2.3, for various rectangular channel dimensions. The channel dimensions are based off of the measurements of the SU-8 master mold.

<table>
<thead>
<tr>
<th>Width [µm]</th>
<th>Depth [µm]</th>
<th>Flow Rate [µL/min]</th>
<th>Re_Dh</th>
<th>L_e [µm]</th>
</tr>
</thead>
<tbody>
<tr>
<td>449.5 ± 1.34</td>
<td>427.9 ± 2.5</td>
<td>0.65 ± 0.1</td>
<td>0.0246 ± 0.004</td>
<td>219.76 ± 0.73</td>
</tr>
<tr>
<td>986.3 ± 1.38</td>
<td>427.9 ± 2.5</td>
<td>0.65 ± 0.1</td>
<td>0.0152 ± 0.002</td>
<td>298.88 ± 1.23</td>
</tr>
<tr>
<td>449.5 ± 1.34</td>
<td>373.2 ± 2.5</td>
<td>0.65 ± 0.1</td>
<td>0.0262 ± 0.004</td>
<td>204.44 ± 0.80</td>
</tr>
<tr>
<td>986.3 ± 1.38</td>
<td>373.2 ± 2.5</td>
<td>0.65 ± 0.1</td>
<td>0.0159 ± 0.002</td>
<td>271.18 ± 1.32</td>
</tr>
</tbody>
</table>

Form duct is given by equation 2.3, where $D_h$ is the hydraulic diameter, and $Re_{D_h}$ is the Reynolds number using hydraulic diameter as the characteristic length.

$$\frac{L_e}{D_h} = 0.5 + 0.05Re_{D_h}$$

Entry lengths are shown in Table 2.3 for the case of water flowing through a rectangular duct. The equation given for entrance length assumes that the flow enters the duct with a uniform velocity profile, and applies better to cases where flow enters a narrow channel section from a wider channel section, than for cases where flow enters a wider section from a narrower section. Therefore, a CFD model will be used to predict where flow reaches a fully-developed state.

2.7 Conclusions

The experimental setup for a light field µPIV system is relatively inexpensive and the equipment is easily accessible to laboratories whose focus is on microfluidics and µPIV. A microlens array designed for a specific objective lens, or range of objective lenses, and a camera that meets the resolution criteria are all that is needed to convert a standard microscope to a light field microscope. Biocompatibility, casting properties, and optical access make PDMS a good candidate for microchannel fabrication and biomedical experiments and applications. Microchannels used in this work were successfully created through photolithography and PDMS. Calibration of the light field microscope is relatively simple. Required precision in optical alignment and positioning can present challenges in producing quality light field images, and small adjustments may be time consuming. It may be possible to design components that facilitate accurate positioning with good
repeatability, such as permanently attaching a microlens array to a camera sensor, but such equipment can become expensive. Velocity validation using prescribed particle displacements, as well as CFD and known solutions, will be discussed in Chapter 3.
CHAPTER 3. EXPERIMENTAL RESULTS AND DISCUSSION

This chapter contains the results and discussion of CFD simulations and PIV experiments using the LF microscope. The first experiment consists of a device used to prescribe particle offsets in order to compare the measured and known values. Subsequent experiments are performed using the LFPIV system and microchannels described in Chapter 2. The results of CFD simulations are presented and used to determine what sections of the channels are fully developed. The results are compared to known analytical solutions, and are also used in comparison with experimental methods for cases in which a known analytical solution does not exist.

3.1 Validation

Solid Body Rotation

A series of tests were conducted using particles dispersed in a solid PDMS block to calculate error between expected and measured displacement values. A low concentration of fluorescent particles were mixed with uncured PDMS and, after curing, the PDMS block was bonded to an empty plastic Petri dish. The dish was mounted to the device shown in Figure 3.1, which allowed it to rotate about the optical axis. Using a known angular velocity, \( \omega \), the \( u \) and \( v \) velocity components were predicted for each \( x \) and \( y \) location as:

\[
\begin{align*}
    u(x, y, z) &= \omega \sqrt{x^2 + y^2} \cos \left( \tan^{-1} (y, x) + \frac{\pi}{2} \right) \\
    v(x, y, z) &= \omega \sqrt{x^2 + y^2} \sin \left( \tan^{-1} (y, x) + \frac{\pi}{2} \right)
\end{align*}
\]  

The rotation device was mounted to the microscope stage and the microscope objective was focused on a plane 100 \( \mu m \) above the bottom of the PDMS. A laser pointer marked the angle on a polar grid of two degree increments that was centered on the rotational axis. A light field image
Figure 3.1: Device used for PIV validation. A solid PDMS block containing fluorescent particles is mounted to a plastic petri dish and rotated about the optical axis to simulate solid body rotation. A laser pointer marks the angle on a polar grid.

was taken of the PDMS, the block was rotated by $4^\circ \pm 1^\circ$, the objective was translated in $z$ by 10 $\mu m \pm 0.5 \mu m$, and a subsequent light field image was captured.

The light field images were refocused from -200 $\mu m$ to 200 $\mu m$ in 5 $\mu m$ increments. Correcting for the change in refractive index when light rays transition from PDMS to air, this range corresponds to -290 $\mu m$ to 290 $\mu m$ in 7.25 $\mu m$ increments. The theoretical depth over which the light field microscope image can be refocused as given by Levoy et al. [1] and discussed in Section 1.4 is approximately 160 $\mu m$ (see equation 1.3).

Three-dimensional PIV was performed on the two refocused images using the 3D intensity fields. Three different types of image post processing were applied to the raw refocused images before the PIV analysis including adaptive thresholding, bandpass filtering, and three-dimensional deconvolution. Deconvolution was performed using the method described in section 1.4 with 100 iterations. After deconvolution, images from the focal stack were convolved with a 2D Gaussian...
kernel to restore particle shape. A three-dimensional cross-correlation PIV algorithm was used on refocused image sets in the range \( z = -145 \, \mu \text{m} - 145 \, \mu \text{m} \). Various \( z \)-depths were explored to find the PIV best-case interrogation region. The RMS error was calculated for each of the velocity components, \( u, v, \) and \( w \) using:

\[
RMS_V = \sqrt{\frac{\sum^N_{i=1} (V_{m,i} - V_{th,i})^2}{N}} \quad (3.3)
\]

where \( V \) is the velocity component \( u, v, \) or \( w \), \( V_m \) is the measured velocity, \( V_{th} \) is the theoretical velocity, and \( N \) is the total number of valid vectors. The total RMS magnitude was calculated using:

\[
RMS_{tot} = \sqrt{RMS^2_u + RMS^2_v + RMS^2_w} \quad (3.4)
\]

A triple-pass, 3D cross-correlation PIV analysis using an initial interrogation region size of 32 x 32 x \( z_{depth} \) and final size of 16 x 16 x \( z_{depth} \) with 50% region overlap was performed for each of the different types of post-processed images, where \( z_{depth} \) is the depth of the interrogation region in voxels. Figure 3.2 shows the RMS errors for each velocity component as well as the total RMS error for the different processing techniques at various interrogation region depths. Using raw refocused images exhibited the worst performance in all cases with the exception of the error in \( w \) for a \( z_{depth} \) of 8 voxels, where intensity-based thresholding performed worse. Intensity-based thresholding improved with increasing \( z_{depth} \) for all three velocity components because the particle count increased. At a \( z_{depth} \) of 24 pixels it performed slightly better than deconvolution for \( v \) and \( w \) components and only slightly worse overall (\( RMS_{tot} \)). Deconvolution and bandpass filtering yielded the best results across the range of interrogation region sizes and their results will be compared for a \( z_{depth} \) of 24 voxels.

For the focal stack used in this analysis, a depth of 24 voxels covers 174 \( \mu \text{m} \), which is much larger than the 15.5 \( \mu \text{m} \) focal depth (\( D_{tot} \)). The larger interrogation depth probably yielded the best results because the displacement in \( z \) was uniform and the seeding density was low, so that larger interrogation regions were required to increase the number of particles per voxel. PDMS is very viscous and therefore the particles did not disperse well in the PDMS and had a tendency to clump together.
Figure 3.2: RMS error calculations for various interrogation region depths ($z_{size}$) using raw re-focused images and three different post-processing techniques.
Figure 3.3: (a) Velocity field superimposed on a focal slice for a 16 x 16 x 24 interrogation region with 50% overlap. The green cross marks the calculated axis of rotation and the dashed red line marks the mask boundary in both (a) and (b). (b) Theoretical (red) and measured (green) displacement vectors superimposed on a focal slice for a 16 x 16 x 24 interrogation region with 50% overlap. The white dashed line shows a region where particle density is low or nonexistent near the edge of the particle field. Errors in this region are very high.

One plane of vectors was plotted and used to provide an initial guess for the location of the axis of rotation. The initial guess was then used to find the sub-pixel location of the rotational axis through an iterative minimization of overall $u$ and $v$ errors, with the assumption that the rotational axis is colinear with the optical axis of the objective lens. Figure 3.3(a) shows a top view of one plane of masked vectors superimposed on a corresponding image from the center of the interrogation region of the focal stack. Figure 3.3(b) shows a similar plot with red vectors denoting the theoretical displacements. A mask was drawn prior to performing PIV analysis and is marked by the dashed red line in Figure 3.3. All vectors outside of the mask were ignored. The white rectangle highlights an example of a region where particle seeding is practically nonexistent. Vectors in this region have very low magnitudes because there is insufficient image data for good correlation. Vectors along the edges of the field of view generally have high errors due to low particle count, which leads to poor correlation.
Figures 3.4, 3.5, and 3.7 show the percent errors of the measured displacements relative to the theoretical displacements for all three velocity components. The errors for the three components of displacement were calculated using:

$$
\varepsilon_i = \frac{|\delta_{i,th} - \delta_{i,m}|}{\delta_{i,th}} \times 100\% \tag{3.5}
$$

where $\delta_{i,th}$ and $\delta_{i,m}$ are the theoretical and measured displacements of an interrogation region, $i$, in either $x$, $y$, or $z$. The $x$ and $y$ displacements ($u$ and $v$) show accuracy within about 10% in many regions with the exception of high errors on lines that cross the axis of rotation, areas of very low or nonexistent seeding, and measurements near the edges of the mask. Relative errors are high along lines that cross the axis of rotation because theoretical displacements in $x$ and $y$ approach zero. Figure 3.6 shows the relative percent error of the in-plane velocity displacement magnitudes defined as:

$$
\|\delta_i\| = \sqrt{\delta_{u,i}^2 + \delta_{v,i}^2} \tag{3.6}
$$

The relatively high errors move from the $x$ and $y$ axes to the axis of rotation since the theoretical displacements approach zero there. Errors in $u$, $v$ magnitudes are high near the mask boundaries, where seeding density is very low and neighboring vectors are invalid (outside of the masked area). The deconvolved set has regions of high error in locations where $x$ is low and $y$ is around 300 $\mu$m - 500 $\mu$m (upper-left edge of the region of interest) as seen in Figure 3.6(b). The errors for bandpass filtering in this same region are lower than the deconvolved set. The bandpass-filtered results have large errors, on the order of 40% to 60%, near the lower edge of the region of interest. The errors in this region are higher for the deconvolved results (on the order of 40% to 70%) but they are more localized. The bandpass results have a region of high error in the lower-right portion of the particle field while errors for the deconvolved results in the same region are much lower. The reason for the high errors is most likely due to too few particles. Figure 3.8 shows a plot of particle count, and it is clear that regions of high error correspond to low particle count when compared to Figures 3.4-3.7.

The $w$ velocity components show much higher error than the $u$ and $v$ components. This is most likely due to low, non-uniform particle density, a small displacement in $z$ (10 $\mu$m) relative to the axial resolution (15.5 $\mu$m), and lower axial resolution than lateral. Bandpass filtering and
deconvolution performed similarly and large \( z \)-depths for the interrogation region provided the best results. It is expected that large interrogation regions would not perform as well if relatively high gradients in \( z \) were present. For example, if the top of the PDMS block were sheared during rotation, such that the top portion rotated at a significantly higher rate than the bottom, large gradients \( \left( \frac{\partial v}{\partial z}, \frac{\partial u}{\partial z} \right) \) would exist. If a large interrogation region in \( z \) were used, the \( x \) and \( y \) displacements would be much higher at the top portion of the interrogation region than the bottom portion, and the final displacement calculated from correlation would not be accurate. Certainly, higher seeding densities would allow for smaller interrogation region depths. Therefore, the size of interrogation region that provided the best results in the experiment cannot be expected to give the best performance for all cases, but it does demonstrate the need for adequate seeding density for good correlation results and shows that the accuracy for \( z \) components of velocity will generally be lower than the \( x \) and \( y \) components.

### 3.2 Channel Section Measurements and Simulations

This section presents the PIV and CFD results for the channel sections shown in Figure 3.9. The PIV results are compared to a CFD simulation created in FLUENT 12.0 and, where applicable, with exact, analytical solutions for steady, laminar, fully-developed flow in a rectangular duct. Table 3.1 shows the channel dimensions, volumetric flow rates, and PIV settings for each channel.
section. The pump flow rate for all cases was set at 0.65 μL/min, and was chosen such that the flow through the small section of the channels would be slow enough to perform PIV and avoid particle streaking. The Reynolds numbers for the various channel dimensions are listed in Table 3.1. The CFD simulation and the results will be presented and discussed, followed by PIV results.
3.2.1 CFD Simulation

A CFD simulation of the channel flow was created for comparison to PIV results in channel sections where known analytical solutions do not exist. FLUENT was used to create the simulation of flow through microchannels with depths of 373.2 $\mu$m and 427.9 $\mu$m, and volumetric flow rate of 0.65 $\mu$L/min. The meshes were created in Gambit 2.3.16, and a steady, laminar, pressure-based solver was used in FLUENT. The pressure-velocity coupling scheme was set as SIMPLE and the
Figure 3.9: Scale drawing of regions of the channel where PIV was performed. The circles represent the field of view of the microscope objective for each of the experimental observations.

Table 3.1: Channel, PIV, and flow properties for the sections outlined in Figure 3.9. R is the final interrogation region size used in PIV analysis, $\delta_z$ is the distance between focal stack slices, and O is the percent overlap between interrogation regions. The channel widths are based off of measurements made using raw light field images.

<table>
<thead>
<tr>
<th>Sec.</th>
<th>Width [$\mu$m]</th>
<th>Depth [$\mu$m]</th>
<th>$Q_{[\mu L/min]}$</th>
<th>Re</th>
<th>$\delta_z$ [$\mu$m]</th>
<th>R [vx]</th>
<th>O</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>950 ±14</td>
<td>427.9 ±2.5</td>
<td>0.65 ±0.1</td>
<td>0.016 ±0.002</td>
<td>9.31</td>
<td>8x16x8</td>
<td>50%</td>
</tr>
<tr>
<td>S2</td>
<td>915 ±14→386±14</td>
<td>373.2 ±2.5</td>
<td>0.65 ±0.1</td>
<td>NA</td>
<td>9.31</td>
<td>8x16x8</td>
<td>50%</td>
</tr>
<tr>
<td>S3</td>
<td>386 ±14</td>
<td>373.2 ±2.5</td>
<td>0.65 ±0.1</td>
<td>0.028 ±0.004</td>
<td>6.65</td>
<td>8x16x8</td>
<td>50%</td>
</tr>
<tr>
<td>S4</td>
<td>386 ±14→915 ±14</td>
<td>372.2 ±2.5</td>
<td>0.65 ±0.1</td>
<td>NA</td>
<td>6.65</td>
<td>8x16x8</td>
<td>50%</td>
</tr>
<tr>
<td>S5</td>
<td>915 ±14</td>
<td>373.2 ±2.5</td>
<td>0.65 ±0.1</td>
<td>0.017 ±0.003</td>
<td>6.65</td>
<td>8x16x8</td>
<td>50%</td>
</tr>
</tbody>
</table>

spatial discretization gradient, pressure, and momentum were set to Least Squares Cell Based, Second Order, and Second Order Upwind, respectively. A mass flow rate was provided as a mass inlet condition and the exit was defined as an outflow boundary. The fluid was defined as water at 298 K. To conduct a grid independence study, a simulation was run to convergence, the grid sizes were refined and, with each refinement, velocity profiles across $x$ and $z$ were extracted. The RMS error between the CFD solution and fully-developed solution was used as a metric for determining grid independence. When the difference of RMS error between subsequent profiles became very small, in this case on the order of $5 \times 10^{-9}$, the solution was considered to be grid independent. Figure 3.10 shows that the solution converged around 2500 iterations using a single-precision solver.
A known solution exists [31] for fully-developed, laminar, incompressible flow in a rectangular channel and is shown in equations 3.7 ans 3.8, where \( \alpha_n = \left( n - \frac{1}{2} \right) \pi \), \( u_y \) is velocity in the y-direction, \( a \) is the channel width, and \( b \) is the channel height.

\[
\begin{align*}
  u_y(x,z) &= \frac{\chi + \rho g y b^2}{2\mu} \left[ 1 - \frac{z^2}{b^2} + 4 \sum_{n=1}^{\infty} \frac{(-1)^n \cosh(\alpha_n \frac{x}{b}) \cos(\alpha_n \frac{z}{b})}{\alpha_n^3 \cosh(\alpha_n \frac{a}{b})} \right] \\
  Q &= \frac{\chi + \rho g y}{3\mu} 4ab^3 \left[ 1 - \frac{b}{a} \sum_{n=1}^{\infty} \frac{1}{\alpha_n^5} \tanh(\alpha_n \frac{a}{b}) \right]
\end{align*}
\] (3.7) (3.8)

The CFD simulations were validated by comparing the fully-developed portions of the channel to Equations 3.7 and 3.8. The theoretical entry length for the narrow channel section is given in Table 2.3 and is 219.8 \( \mu m \) for the 427.9 \( \mu m \) deep channel, which is less than a quarter of the length of the narrow section. Therefore, it is assumed that the flow half-way along the narrow section of the channel (\( y = 3500 \mu m \)) is fully developed. Figure 3.11 shows the CFD results plotted against the fully-developed solution for the narrow portion of the channel with a depth of 427.9 \( \mu m \). Figure 3.11(a) shows the \( x \) profile plotted at \( z = 0 \mu m \) and \( y = 3500 \mu m \) for the CFD result and the theoretical result. The theoretical profile is plotted as the red line and the CFD data points are the blue squares. The error of the CFD simulation relative to the theoretical is shown in Figure 3.11(b). All errors are less than 0.24%. Figures 3.11(c) and 3.11(d) show plots of velocity profiles in \( y \). The maximum percent error of the CFD analysis relative to the theoretical profile in \( y \) is less
Figure 3.11: Comparison of theoretical and computational velocity profiles in the narrow channel section, $S_3$. The channel dimensions for the CFD simulation are shown in row 1 of Table 2.3. (a) Profile comparison of $v$ across $x$ at $z = 0 \, \mu m$ and $y = 3500 \, \mu m$. The red line represents the theoretical profile and the squares represent the CFD results. (b) Percent error relative to the theoretical result for plot in (a). (c) Profile comparison of $v$ across $z$ at $x = 0 \, \mu m$ and $y = 3500 \, \mu m$. The red line represents the theoretical solution and the squares represent the CFD results. (d) Percent error relative to the theoretical result for plot in (c).

than 0.16%. These results indicate that the CFD simulations are valid and have a level of accuracy sufficient for comparison with measured values.
Figure 3.12 shows the comparison of profiles of $v$ across $x$ from the CFD results with the known solution for fully-developed flow for the wider portion of the channel with the dimensions shown in row 2 of Table 2.3. The profiles are located in the center of the wider section, $S_5$, shown in Figure 3.9. The profile of $v$ across $x$ at $y = 4500 \mu m$ and $z = 0 \mu m$ is shown in Figure 3.12(a). The percent error relative to the fully-developed solution is shown in Figure 3.12(b). Plots of profiles of $v$ across $z$ at $x = 0 \mu m$ and $y = 4500 \mu m$ are shown in Figures 3.12(c) and 3.12(d). These plots show that the flow does not reach a fully-developed state in section $S_5$ of the channel. The CFD results were used to determine which channel regions can be compared to the known analytical solution for fully-developed flow. Figure 3.13 shows a plot of the CFD results for $v$ along the $y$-axis, where $x = 0$ and $z = 0$. A drawing of the channel is superimposed with dashed lines that mark the predicted entry lengths from equation 2.3. The equation accurately predicts the location where the flow reaches a fully-developed state in the narrow channel section; however, it does not apply to the wider section of the channel because equation 2.3 assumes that flow enters the entry region with a uniform profile. The flow entering the narrower section from the wider section is close enough to being uniform that it accurately predicts the entry length for the narrower section. Flow will only be fully developed in the narrow sections and the longer entry section, which correspond to sections $S_3$ and $S_1$ in Figure 3.9. Therefore, Equations 3.7 and 3.8 will be used in comparing PIV results for sections $S_1$ and $S_3$, and the CFD results will be used for comparing PIV results for the expansion and contraction regions ($S_4$ and $S_2$).

3.2.2 PIV Results for Fully-developed Sections

This section presents the measurements made in channel sections where flow was fully developed. Fully-developed flow occurs in sections $S_1$ and $S_3$. The data are compared with the analytical solution for fully-developed, laminar, steady flow in a rectangular channel.

Measurements for $S_1$

Channel section $S_1$ is located in the long portion of the channel entry as shown in Figure 3.9. The CFD simulation predicts fully-developed flow in this region. After measuring the entry widths of several different channels with an optical microscope, it was determined that the PDMS channel
Figure 3.12: Comparison of theoretical and computational velocity profiles in the wider channel section, $S_5$. The channel dimensions for the CFD simulation are shown in row 2 of Table 2.3. (a) Profile comparison of $v$ across $x$ at $z = 0 \mu m$ and $y = 4500 \mu m$. The red line represents the theoretical profile and the squares represent the CFD results. (b) Percent error relative to the theoretical result for plot in (a). (c) Profile comparison of $v$ across $z$ at $x = 0 \mu m$ and $y = 4500 \mu m$. The red line represents the theoretical solution and the squares represent the CFD results. (d) Percent error relative to the theoretical result for plot in (c).
Figure 3.13: Plot of CFD results for $v$ along the $y$-axis ($x = 0, z = 0$) for the 427.9 $\mu$m-deep channel. A scaled drawing of the channel is superimposed on the graph, with the two dashed red lines marking the theoretical entry lengths given in Table 2.3. The flow reaches a fully-developed state at the predicted entry length for the narrow section. Equation 2.3 does not apply to the wider section because the flow is entering from a smaller cross-sectional area. This plot shows that the flow only reaches a fully-developed state in the narrow channel sections and the long section in the range of approximately 2000 $\mu$m to 1000 $\mu$m, which correspond to sections $S_1$ and $S_3$ in Figure 3.9. The channel dimensions are based off of the measurements of the SU-8 master mold.
dimensions vary from the widths of the SU-8 mold, therefore a more reliable measurement for the channel width is to measure the width of the channel in pixels using the raw light field images, and convert the width to microns using the pixel pitch size of the camera sensor. Corresponding CFD models were created for the new dimensions. The width of section $S_1$ was measured at $950 \, \mu m \pm 14 \, \mu m$.

Light field images were refocused from -100 $\mu m$ to 100 $\mu m$ in 7 $\mu m$ increments to produce three-dimensional focal stacks for use in PIV. The focal stacks were then post processed to remove blurring and noise. Figure 3.14 shows the results of refocusing and thresholding. The first column of images (a, d, g) are the raw refocused images at $z = -77 \, \mu m$, -3 $\mu m$, and 63 $\mu m$. The second column (b, e, h) were thresholded using a bandpass filter. The third column (c, f, i) used the adaptive thresholding method based on sub-image intensities, as discussed in Section 2.4. An interrogation window of 10 x 10 pixels was used and the thresholding level was set at 3.4 standard deviations above the mean. All three methods were used for PIV analysis.

Light, emitted by the particles, transitions from water ($n = 1.333$) to air ($n = 1$); therefore, a 7 $\mu m$ increment used in refocusing corresponds to 9.33 $\mu m$ in real space. Focal plane indices were mapped to the focal depth vector (a vector, ranging from -100 to 100 microns in 7 micron increments, multiplied by the refractive index of water) in order to convert the $z$ indices to physical coordinates. To convert the $x$ indices from pixels to world coordinates, a draggable rectangle was created in MATLAB using the `imrect` command, and the width of the rectangle, in pixels, was set by dividing the channel width, in microns, by the number of microns per pixel in a rendered light field image. Each lenslet images a 6.25 $\mu m$ width in object space ($s_{xy} = \frac{p_l}{M} = \frac{125 \, \mu m}{20}$). Therefore, a width of 950 $\mu m$ in object space is equivalent to 152 px in a rendered light field image. The pixel indices of the image in $x$ range from 1 to $N$, where $X_i = [1,2,\ldots,N]$ and $N$ is the image width in pixels. The draggable rectangle has a pixel width of $W_{rect}$. Equation 3.9 is used to convert the index vector, $X_i$, to a world-coordinate vector, $X_w$, where $x_0$ is the coordinate of the left edge of the rectangle in pixels and $s_x$ is the width of the width of a lenslet sub-image in object space.

$$X_w = \left( (X_i - 1) - \left( x_0 + \frac{W_{rect}}{2} \right) \right) s_x \tag{3.9}$$
Figure 3.14: Refocused particle images of channel section $S_1$. (a,d,g) Raw refocused images at $z = -77, -3, \text{ and } 63 \, \mu\text{m}$. (b,e,h) Images after bandpass filtering. (c,f,i) Image after applying intensity thresholding.
This equation puts the origin of the $x$-axis at the horizontal center of the draggable rectangle. The top of the rectangle is moved to a specified $y$ value. For example, for section $S_1$ the images were taken with the field of view centered at $y = 1500 \ \mu m$, as depicted in Figure 3.9. The rectangle would be moved so that the top edge is in the vertical center of the image and equation 3.10 provides the conversion between pixel and world coordinates, where $y_{ch}$ is the target $y$-coordinate of the channel. For example, $y_{ch} = 1500 \ \mu m$ for $S_1$.

$$Y_w = ((Y_i - 1) - y_0)s_y + y_{ch} \quad (3.10)$$

Three-dimensional PIV was performed on each of the image stacks shown in Figure 3.14 using a triple-pass cross-correlation with an initial interrogation region size of $32 \times 32 \times 8$ voxels, and a final volume size of $8 \times 16 \times 8$ voxels with 50% overlap. The interrogation region size was set smaller in $x$ than $y$ because of higher velocity gradients in $x$, especially near the walls. There is no velocity gradient with respect to $y$ for fully-developed flow. Figure 3.15 shows the $y$, $x$, and $z$ velocities in pixels per frame, through time, at the vector nearest to the channel center. The figure shows the unsteadiness of the pump, as the $y$-velocity components vary between 1 and 3 pixels per frame, where each pixel represents 6.25 $\mu m$ in physical space (lenslet pitch divided by magnification). Variations in $u$ and $w$ are two orders of magnitude smaller than variations in $v$. Histograms of the velocities are also plotted in Figure 3.15. The curved red lines in the histograms are normal distributions fitted to the data, and the red, vertical lines represent expected values. The expected flow rate is 0.65 $\mu L/min$, or 2.11 pixels per second. The $x$ and $z$ velocity components resemble normal distributions, but the $y$ component of velocity does not fit the normal distribution as well, as there are high peaks on both sides of the mean in Figure 3.15(d). It is likely that if more image pairs were to be averaged, the histogram would approach a normal distribution centered about the expected value. The larger magnitude of the variations and the non-normal distribution of velocities in $v$, indicate the fluctuations in $v$ are most likely due to pump unsteadiness and not random error from PIV algorithms.

Particle count per interrogation region is an important property that has a large effect on PIV accuracy. Particle density was measured by binarizing focal stacks and counting the number of connected components for each interrogation volume using the `bwconncomp` function in
Figure 3.15: Instantaneous and mean velocities measured at the interrogation region nearest the channel center at section $S_1$. The red lines in the velocity plots represent a running mean velocity. Corresponding histograms with fitted normal distributions are shown below the velocity plots. The red vertical lines in the histogram plots represent the ideal velocities. (a) Velocities in the $y$-direction. (b) Velocities in the $x$-direction. (c) Velocities in the $z$-direction. (d) Histogram of $v$. The $v$ components do not correspond well to a normal distribution, indicating that the variation in velocity is most likely due to an unsteady flow rate rather than random error. (e)-(f) Histograms of the $x$- and $z$-velocities, respectively. The variations in $u$ and $w$ more closely resemble normal distributions, indicating that variation is most likely due to random error.

MATLAB. The particle counts per interrogation volume are plotted in Figure 3.16. Figure 3.16(b) shows the mean particle count per interrogation volume for 50 image pairs. The maximum density is slightly higher than 6 and regions nearest the original focal plane have particle densities that are mostly in the range of 3 to 5. The number of particles decreases significantly near the refocusing limits. The slices shown in Figure 3.16 are centered at the following locations in $z$: 81.3, 44.0, 6.7, -30.7, -68.0, and -105.3 (all units given in $\mu$m). The interrogation depth was specified at 8 pixels, which is equivalent to a depth of 74.6 $\mu$m in object space. Therefore, each interrogation re-
Region contains focal stack information that extends 37.3 \( \mu \text{m} \) in the positive and negative \( z \)-direction. Only the interrogation regions centered at \( z = 44.0, 6.7, \) and \(-30.7 \mu \text{m} \) cover focal stack information that is contained within the refocusing limit \( (D_{\text{tot}}) \) of \( z = -80 \) to \( z = 80 \mu \text{m} \). For example, the regions centered at \( z = -68.0 \mu \text{m} \) will extend from \( z = -30.7 \mu \text{m} \) to \( z = -105.3 \mu \text{m} \). The number of particles and quality of particle images decrease as the refocusing limits are approached. The three regions nearest \( z = 0 \) have the highest particle density. The outer regions, centered at \( z = 81.3 \mu \text{m} \) and \( z = -105.3 \mu \text{m} \) have 59\% and 41\% fewer particles, respectively, than the region centered at \( z = 6.7 \mu \text{m} \). Streaks of higher particle concentrations parallel to the \( y \)-axis can be seen Figure 3.16(b). This effect could be caused by inadequate particle mixing prior to pumping and/or particle sedimentation occurring within the syringe during pumping. Particle concentrations also decrease near the edges of the region of interest, which is likely caused by poor illumination and lenslet image quality.

Figure 3.17 shows the errors for the velocity components in the \( y \)-direction throughout the volume. The percent error was calculated using:

\[
e = \left| \frac{\bar{v}_m (x,y,z) - v_{th} (x,y,z)}{v_{th} (x,y,z)} \right| \times 100\% \quad (3.11)
\]

where \( v_{th} (x,y,z) \) is the theoretical \( y \)-component of velocity at location \( x,y,z \) based on the analytical solution, and \( \bar{v}_m (x,y,z) \) is the measured velocity at that point averaged over 47 image pairs.

To calculate a theoretical velocities at given \( x, y, \) and \( z \) locations using equation 3.7, the flow rate must be known. The pump flow rate was set at 0.65 \( \mu \text{L/min} \); however the flow rate was found to be unsteady and, therefore, the flow rate was calculated based on mean velocity vectors near the channel center. The center vector and its two neighbors were extracted from each dimension of the mean velocity vector matrix. The flow rate was calculated for each of the extracted vectors using equations 3.7 and 3.8, where \( u_y (x,z) \) is the vector measurement at location \( (x,z) \). This calculation resulted in a 3 x 3 x 3 matrix of flow rates, which were all averaged to obtain a single flow rate measurement. This method of measuring flow rate yielded 0.667, 0.685, and 0.684 \( \mu \text{L/min} \) for the raw refocused, bandpass filtered, and thresholded data sets, respectively. These flow rates correspond to respective percent differences between the measured and the target flow rates of 2.6\%, 5.3\%, and 5.2\%.
Figure 3.16: Particle counts per interrogation volume for $S_1$. (a) Particle count for a single focal stack (b) Average particle count over 47 focal stacks.

There are high errors at the edges of the vector planes shown in Figure 3.17. The main causes of higher errors are most likely due to low particle count, particles entering and exiting the field of view, and inaccuracies in world coordinates. The low particle counts around the edges of the slices can be seen in Figure 3.16. As discussed previously, low particle concentration at the edges is most likely due to reduced illumination and lenslet image quality. Particles entering and exiting the field of view lead to poor image correlation because part of the interrogation image no longer exists in the field of view. The velocity gradient, $\frac{\partial v}{\partial x}$, increases near the wall, which increases the sensitivity of $v$ with respect to $x$. Therefore, error in wall location measurement has a larger effect on $v$ in regions nearer to the wall. Error is amplified even more in near-wall locations.
Figure 3.17: Relative percent error of $v$ for raw-refocused, bandpass-filtered, and intensity-thresholded images sets at $S_1$. All results are averaged over 47 image pairs. The upper colorbar limit was clipped at 30% in order to show finer levels of error. (a) Error for PIV using raw refocused images. (b) Error for PIV using bandpass filtered images. (c) Error using thresholded images.

because the theoretical velocity approaches zero near the wall and, therefore, the relative percent error will be higher because of low values in the denominator of equation 3.11.

Interrogation regions beyond the refocusing limits show high errors, which are mainly caused by low particle concentrations and poor image quality. As discussed previously, only three of the slices shown have interrogation regions that are within the refocusing limits. Particle concentration in these three regions is much higher, and particle image quality is significantly better than regions that are beyond the refocusing range. The errors in the three slices near the original focal plane are much lower than the other three three slices located at $z = 81.3$, -68.0, and -105.3 $\mu$m. Interrogation regions within the refocusing limits have streaks of low error, which correspond to regions of high particle density.

Relative percent error is in the range of 10-15% near the walls of the channel. The errors in this region can be seen in more detail in Figure 3.18, which shows profiles of $v$ across $x$ at various $z$ values and for $y = 1500 \mu$m. Measured velocities are lower than the theoretical velocities near the walls and higher than theoretical values near the center of the channel. The profiles actually
resemble a developing profile. Figure 3.12(a) shows a developing profile for the CFD results with similar characteristics to the profiles shown in Figure 3.18. It is likely that the microscope was not positioned precisely in $y$ because all channel reference features in $y$, such as the expansions and contractions, were outside of the field of view and the microscope stage did not have precise markings for $x$ and $y$ translation. The microscope may have been centered closer to $y = 2000 \, \mu m$, and Figure 3.13 shows that the flow is beginning to accelerate just beyond $y = 2000 \, \mu m$.

The measured velocities at $z = -68.0 \, \mu m$ are much higher than the corresponding theoretical profile. Recall that the interrogation region centered at $z = -68.0 \, \mu m$ contains image information from focal depths of -105.3 $\mu m$ to -30.7 $\mu m$, which extends beyond the theoretical refocusing range, and the seeding density is lower in this region. Interrogation regions contain information from eight focal stack images. Each focal stack image is at a different depth, and therefore particles within each depth location have different theoretical velocities. If the velocity gradient in $z$ is high, and the particle distribution is not uniform throughout $z$, the final interrogation region displacement will tend toward the region where particle densities are highest. This is a possible explanation for why the measured velocities over-predict the theoretical velocities at focal depths where seeding density is low.

Velocities from the bandpass-filtered set begin to converge back to the theoretical profile as the channel walls are approached, but velocities from the raw-refocused set tend to diverge and over-predict the theoretical velocities. This over-prediction can be explained by the combined effects of insufficient particle seeding, larger velocity gradients, and out-of-focus particles. Referring again to Figure 3.16, the number of particles per interrogation region decreases near the channel walls. Out-of-focus particles have larger diameters than in-focus particles and will appear in a larger number of adjacent interrogation regions than for focal stacks where out-of-focus particles have been thresholded out. The out-of-focus particles farther away from the channel walls will have higher velocities and out-of-focus particles can have a large affect on regions where particle density is low and velocity gradients in $x$ are high. This explains why the velocities near the channel walls, plotted in Figure 3.18(b), are higher than the theoretical values.

Figure 3.19 shows a plot of instantaneous velocity profiles across $z$ at $x = 4.2 \, \mu m$ and $y = 1520 \, \mu m$, and their corresponding errors. The red profile, $v_{\text{ideal}}$, is the ideal velocity profile based on a flow rate of 0.65 $\mu L/min$. The mean, minimum, and maximum velocity measurements
Figure 3.18: Plot of measured and theoretical $v$ across $x$ at various $z$ locations for $S_1$. The $y$ value is fixed at approximately $y = 1500 \mu$m.
Table 3.2: The total error in $v$ for each plane of vectors calculated using equation 3.12 for raw-refocused, bandpass-filtered, and intensity-thresholded image focal stacks for $S_1$. Interrogation regions that are completely within the refocusing range, $D_{tot}$, are bolded.

<table>
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<tr>
<th>$z$ [µm]</th>
<th>Raw $\varepsilon_k$ [µm]</th>
<th>Bandpass $\varepsilon_k$ [µm]</th>
<th>Thresh $\varepsilon_k$ [µm]</th>
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<td>-105.3</td>
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<td>5.73e-3</td>
<td>5.47e-3</td>
</tr>
<tr>
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</tr>
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<td>3.83e-3</td>
<td>3.46e-3</td>
<td>3.33e-3</td>
</tr>
</tbody>
</table>

and profiles are plotted, and show the variation in pump flow rates. Relative errors for the three planes within the refocusing range have errors of less than 5%. As distance from $z = 0$ is increased, the measured velocities tend to be higher than the theoretical velocities. The reason for the over-prediction was discussed previously for the case of the profile shown in Figure 3.18, where $z = -60.8$ µm. Velocity measurements for planes with negative $z$ locations tend to diverge from the theoretical curve more than velocity measurements for planes with positive $z$ locations. Movement of the focal plane in the negative $z$-direction corresponds to movement away from the objective lens and, therefore, increases the number of particles and the amount of water between the objective lens and the focal plane. Increasing the number of particles between the focal plane and the objective lens decreases the signal-to-noise ratio, and increasing the amount of water increases spherical aberrations for the dry objective lens used in these experiments. Decreasing signal-to-noise ratio and increasing spherical aberrations will result in poor image correlation and poor image quality, and likely causes more divergence from the theoretical values in the negative $z$-direction.

The total error in $v$ for each plane of vectors in $z$ was calculated using equation 3.12, where $i, j, k$ are the indices of the three-dimensional velocity matrix, $\varepsilon_k$ is the total sum of error elements in plane $k$, and $v_m(i, j, k)$ and $v_{th}(i, j, k)$ are the measured and theoretical values of $v$ at $(i, j, k)$.

$$\varepsilon_k = \sum_{i=1}^{M} \sum_{j=1}^{N} \sqrt{(v_m(i, j, k) - v_{th}(i, j, k))^2}$$  \hspace{1cm} (3.12)

Table 3.2 shows the total error values for each plane of vectors for the raw-refocused, bandpass-
Figure 3.19: (a, c) Instantaneous $v$ profiles at $x = 4.2 \, \mu m$ and $y = 1520 \, \mu m$ for $S_1$. The ideal curve, $v_{\text{ideal}}$ is the theoretical flow velocity for the pump flow rate of 0.65 $\mu$L/min. Other profile flow rates were based on instantaneous velocity measurements as described Section 3.2.2. (b, d) Errors between the theoretical and measured profiles.

filtered, and intensity-thresholded image sets. For the vector slices within the refocusing limits, bandpass filtering had the lowest total error, followed by intensity thresholding and raw refocused images. Total error within the refocusing limits for the intensity-thresholded and raw-refocused image sets were 4.4% and 18.4% higher than the bandpass-filtered set, respectively.

Percent errors in $u$ and $w$ velocities were calculated relative to the theoretical $y$-velocity component, since the theoretical values are equal to zero. Equation 3.13 is used to calculated the
error, where $V_m$ is the velocity component in the $x$ or $z$ direction.

$$\varepsilon = \frac{|V_m(x,y,z)|}{v_{th}(x,y,z)} \times 100\% \quad (3.13)$$

Figure 3.20 shows the relative percent errors in $u$ for raw-refocused, bandpass-filtered, and intensity-thresholded image sets. Errors for the raw-refocused and bandpass-filtered image sets are within 4%, and mean errors for each plane are within 1%. Errors in $u$ for the intensity-thresholded images are higher, with means errors for each plane ranging from 10% to 17%. Figure 3.14 shows that several particle sizes for the intensity-thresholded images are only one to two pixels, whereas the bandpass-filtered images contain particles that are generally around three pixels in diameter. Algorithms used in PIV have sub-pixel evaluation processes which rely on particles to have diameters larger than a single pixel, otherwise integer biasing can occur when particles sizes are less than 2 pixels [24]. Therefore, the cause of higher error for the intensity-thresholded images is most likely due to small particle sizes. The errors in $w$ for the three different image sets are shown in Figure 3.20. The results are similar to the errors in $u$. The magnitudes of the errors for intensity-thresholded set are higher in $w$ than $u$.

**Measurements for $S_3$**

Channel section $S_3$ is located in the narrow channel portion, centered at $y = 3500 \, \mu m$, where flow should become fully developed within about 204 $\mu m$ of the inlet and outlet (see Figures 3.13). Figure 3.22 shows examples of the refocused images. The width and depth of the narrow section are 386 $\mu m \pm 14 \, \mu m$ and 373.2 $\mu m \pm 2.5 \, \mu m$, respectively. The pump flow rate was set to 0.65 $\mu L/min$. The light field images were refocused from -302 $\mu m$ to 231 $\mu m$ in 6.65 $\mu m$ increments. Only the refocused images from -133 $\mu m$ to 107 $\mu m$ were used for PIV. The refocusing limits were non-symmetric because the measurements were taken at 26.6 $\mu m$ above the channel center. The same image post processing and PIV parameters presented in Section 3.2.2 were used for $S_3$.

The approximate number of particles per interrogation region for the $S_3$ focal stacks is shown in Figure 3.23 for a single focal stack as well as the average number per interrogation region for 47 focal stacks. Four of the nine interrogation region planes shown in Figure 3.23
Figure 3.20: Percent error of $u$ relative to $v$ for raw-refocused, bandpass-filtered, and intensity-thresholded image sets at $S_1$. The upper colorbar limit was clipped at 10% in order to show more detail. (a) Error for PIV using raw refocused images. (b) Error for PIV using bandpass filtered images. (c) Error using thresholded images.

Figure 3.21: Relative percent error of $w$ for raw-refocused, bandpass-filtered, and intensity-thresholded image sets at $S_1$. The upper colorbar limit was clipped at 10% in order to show more detail. (a) Error for PIV using raw refocused images. (b) Error for PIV using bandpass filtered images. (c) Error using thresholded images.
Figure 3.22: Refocused particle images of channel section $S_3$. (a,d,g) Raw-refocused images at $z = -73, 0,$ and $67 \ \mu m$. (b,e,h) Images after bandpass filtering. (c,f,i) Image after applying intensity thresholding.
Figure 3.23: Particle count per interrogation volume for channel section $S_3$. (a) Particle count for a single focal stack. (b) Particle count over 47 focal stacks.

contain focal stacks that are completely within the refocusing range, $D_{tot3}$, and are centered at $z = -6.7, 20.0, 46.7, 73.3 \, \mu m$. These planes have the highest particle counts, with the exception of the plane located at $z = 73.3 \, \mu m$, which has slightly lower particle density than the plane located at $z = -33.3 \, \mu m$. The particle density images also show streaks of high and low particle counts, which are likely due to insufficient particle mixing and/or particle sedimentation in the syringe during data collection. The edges of particle density images, parallel to the flow, have very low particle counts. The particle count per interrogation region is lower for $S_3$ than for $S_1$ because the distance between focal planes for $S_3$ focal stacks was 6.7 $\mu m$, rather than the 9.3 $\mu m$ used for $S_1$.

The relative percent errors in $v$, averaged over 47 image pairs, are plotted in Figure 3.24. The edges of all of the regions of interest, specifically the edges parallel to the channel walls and
Figure 3.24: Relative percent error of \( v \) for raw-refocused, bandpass-filtered, and intensity-thresholded image sets at \( S_3 \). The upper colorbar limit was clipped at 30% in order to show more detail. (a) Error for PIV using raw refocused images. (b) Error for PIV using bandpass filtered images. (c) Error using thresholded images.

The inlet edge (\( y = 3000 \, \mu m \)), have very large errors. Errors in regions along the edge where \( x \sim -192 \, \mu m \) and \( x \sim 182 \, \mu m \) are high due to the lack of particles in those regions. Particle density is particularly low along those edges because some portions of the interrogation regions are outside of the channel walls. The high errors along the inlet edge are most likely due to new particles entering the field of view. Regions where particle density is in the range of five to six particles per interrogation region have errors within 5%. Interrogation regions that contain images outside of the refocusing range have very high errors in the negative \( z \)-direction and range from about 15% to as high as 80%.

Profiles of \( v \) across \( x \) at \( y \sim 3500 \, \mu m \) and various \( z \) locations are plotted in Figure 3.25 for raw-refocused and bandpass-filtered image sets. Measured velocities match the theoretical velocities within 5% for planes that are within \( D_{tot3} \). The interrogation region centered at \( z = -33.3 \, \mu m \) contains refocused images that are outside of \( D_{tot3} \) and the measured velocities are higher than the theoretical, for reasons discussed in Section 3.2.2. The data points begin to diverge from the theoretical profile near the walls, where the velocity gradients are higher. The cause of
Table 3.3: The total error in $v$ for each plane of vectors in $S_3$, calculated using equation 3.12 for raw-refocused, bandpass-filtered, and intensity-thresholded image focal stacks for $S_3$. Interrogation regions that are completely within the refocusing range, $D_{tot}$, are bolded.

<table>
<thead>
<tr>
<th>$z$ [$\mu$m]</th>
<th>Raw $\varepsilon_k$ [$\mu$m]</th>
<th>Bandpass $\varepsilon_k$ [$\mu$m]</th>
<th>Thresh $\varepsilon_k$ [$\mu$m]</th>
</tr>
</thead>
<tbody>
<tr>
<td>-113.305</td>
<td>6.01e-3</td>
<td>1.16e-2</td>
<td>8.92e-3</td>
</tr>
<tr>
<td>-86.645</td>
<td>5.20e-3</td>
<td>8.50e-3</td>
<td>6.27e-3</td>
</tr>
<tr>
<td>-59.985</td>
<td>4.51e-3</td>
<td>6.04e-3</td>
<td>4.64e-3</td>
</tr>
<tr>
<td>-33.325</td>
<td>3.92e-3</td>
<td>4.17e-3</td>
<td>3.66e-3</td>
</tr>
<tr>
<td>-6.665</td>
<td>3.44e-3</td>
<td>3.02e-3</td>
<td>3.08e-3</td>
</tr>
<tr>
<td>19.995</td>
<td>3.06e-3</td>
<td>2.60e-3</td>
<td>2.74e-3</td>
</tr>
<tr>
<td>46.655</td>
<td>3.11e-3</td>
<td>2.68e-3</td>
<td>2.69e-3</td>
</tr>
<tr>
<td>73.315</td>
<td>3.70e-3</td>
<td>3.09e-3</td>
<td>2.97e-3</td>
</tr>
<tr>
<td>99.975</td>
<td>3.90e-03</td>
<td>4.16e-03</td>
<td>4.34e-03</td>
</tr>
</tbody>
</table>

this divergence is also discussed in Section 3.2.2. Figure 3.26 shows the relative percent errors for profiles of $v$ across $x$ at $y \sim 3500$ $\mu$m and all $z$ locations. Planes within the refocusing range have errors within approximately 5%, while planes outside the refocusing range have errors that increase with distance from $z = 0$.

The errors in $v$ for each of the planes were calculated using equation 3.12. The bandpass-filtered set had the lowest overall error for the planes within the refocusing range, followed by the intensity-thresholded and then raw-refocused image sets. The total error for each plane is shown in Table 3.3.

Errors in $u$ and $w$, relative to $v$, are shown in Figures 3.27 and 3.28. The results are similar to those of $S_1$, where the raw-refocused and bandpass-filtered image sets have low error and the intensity-thresholded image set has large errors. Reasons for the large errors in the intensity-thresholded image set are discussed in Section 3.2.2. The corners of the data sets around $y = 4000$ $\mu$m is higher because flow is developing in those regions.

Figure 3.29 shows the instantaneous velocity profiles and corresponding errors. For the raw-refocused image set, the mean velocity matches well in the positive $z$ direction but diverges from the curve in the negative $z$-direction. This same divergence was observed for section $S_3$. Divergence is mainly due to low seeding density, low signal-to-noise ratio due to higher numbers of particles between the objective lens and the focal plane, and images outside of $D_{tot}$. 66
Figure 3.25: Plot of measured and theoretical $v$ across $x$ at various $z$ locations and $y \sim 3500 \mu m$ for $S_3$. 

67
Figure 3.26: Error between measured and theoretical values of \( v \) across \( x \) at various \( z \) locations for \( S_3 \).
Figure 3.27: Relative percent error of $u$ for raw-refocused, bandpass-filtered, and intensity-thresholded image sets at $S_3$. The upper colorbar limit was clipped at 10% in order to show more detail. (a) Error for PIV using raw refocused images. (b) Error for PIV using bandpass filtered images. (c) Error using thresholded images.

Figure 3.28: Relative percent error of $w$ for raw-refocused, bandpass-filtered, and intensity-thresholded image sets at $S_3$. The upper colorbar limit was clipped at 10% in order to show more detail. (a) Error for PIV using raw refocused images. (b) Error for PIV using bandpass filtered images. (c) Error using thresholded images.
3.2.3 PIV Results for Developing Flow Sections, S₄ and S₂

This section presents a brief qualitative analysis on the measurements made in sudden expansion and contraction portions of the channel. The expansion and contraction correspond to section S₄ and S₂ in Figures 3.9 and 3.13. Global coordinates were specified using the same process described in Section 3.2.2. The CFD simulations were used to extract data to compare to PIV results.

Section S₄ is located in the portion of the channel where the narrow section expands into the wide section. Profiles of velocity magnitude across x at various y and z locations are plotted in Figure 3.30. The PIV data used in these plots come from a bandpass-filtered image set. The light field images were refocused from -302 μm to 231 μm in 6.65 μm increments. Only the refocused images from -133 μm to 107 μm were used for PIV. The refocusing limits were non-symmetric because the measurements were taken at 26.6 μm above the channel center. The final interrogation region size was 8 x 16 x 8 voxels. The three z locations plotted are within the refocusing limits. The channel expands from a width of 386 μm ±14 μm to 915 μm ±14 μm. The flow rate, as measured from the S₃ results was 0.57 μL/min.

The PIV data generally follow the CFD data. Close to the expansion, at y = 4000 μm, The PIV data are slightly lower than the CFD values near the centerline, and slightly higher in positions.
more than approximately 100 µm from \( x = 0 \). This may be due to the actual channel width being slightly larger than the measured channel width. The PIV data at \( y = 4153 \) µm appear to be slightly shifted to the right of the corresponding CFD profile, and the PIV data at \( y = 3853 \) µm appear to be slightly shifted to the left of the corresponding CFD profile. The differences between the PIV data points and the CFD-generated profiles are consistent for the three \( z \)-locations. Differences between the PIV and CFD data are most likely due to inaccuracies in the measured channel dimensions and flow rates, and the channel being slightly angled relative to the camera sensor.

Section \( S_2 \) is located in the portion of the channel where the wide section contracts into the narrow section. Profiles of velocity magnitude across \( x \) at various \( y \) and \( z \) locations are plotted in Figure 3.31. The PIV data used in these plots come from a bandpass-filtered image set. The light field images were refocused from -133 µm to 128 µm in 9.31 µm increments. The final interrogation region size was 8 x 16 x 8 voxels. The three \( z \) locations plotted are within the refocusing limits. The channel contracts from a width of 915 µm ±14 µm to 386 µm ±14 µm. The flow rate, as measured from the \( S_3 \) results was 0.57 µL/min. The comparisons between the PIV and CFD data for the contraction are similar to the expansion results of \( S_4 \).

### 3.3 Conclusions

The results presented in this chapter have shown that it is feasible to perform \( \mu \)PIV experiments using a light field microscope. The solid body rotation experiments were successful at resolving velocities in the \( x - y \) plane within about 10%, despite the poor seeding dispersion and low seeding density in the PDMS block. The displacement measurements in the \( z \)-direction for the solid body rotation experiment had higher errors than in-plane measurements, due to low seeding density, lower axial resolution than lateral, and a displacement value lower than the distance between distinct focal planes.

PIV measurements taken in fully-developed channel sections had relative errors within 5% for planes within \( D_{tot3} \). Seeding density must be increased in order to use smaller interrogation region depths. Bandpass-filtered image sets performed better than raw-refocused and intensity-thresholded sets for the channel flow measurements within the refocusing range. Small particle sizes for intensity-thresholded sets led to higher errors in \( u \) and \( w \). PIV measurements taken in developing channel matched closely with the profiles generated from CFD models.
Figure 3.30: Profiles of velocity magnitude across $x$ at various $y$ locations for the channel expansion ($S_4$). The CFD results (dashed lines) are compared with PIV results (data points). The three $z$ locations are within the refocusing limits.
Figure 3.31: Profiles of velocity magnitude across $x$ at various $y$ locations for the channel expansion ($S_2$). The CFD results (dashed lines) are compared with PIV results (data points). The three $z$ locations are within the refocusing limits.
CHAPTER 4. CONCLUSIONS

4.1 Summary

Particle Image Velocimetry is a useful tool for obtaining experimental velocity measurements. Several methods of PIV have been developed to measure various types of flow fields. In this work the concepts of microscopic light field imaging were used to experimentally obtain flow velocities in a microchannel with the motivation of applying the technique to measure blood flow. The results were compared with known analytical solutions to determine measurement accuracy. The results show good promise, despite some limitations, and future work should be pursued to determine if improvements to image processing and system components can yield more accurate results.

4.2 Future Work

This work has shown that light field \( \mu \text{PIV} \) using a plenoptic camera is a feasible method, but there are several aspects of the system and analysis that require future work and improvements to better determine the full potential of plenoptic light field \( \mu \text{PIV} \). Areas where further research and analysis are required include numerical simulations, system components and equipment, channel fabrication, and blood flow experiments.

4.2.1 Numerical Simulations

Simulations provide valuable information about best-case scenarios, and there are several aspects of the system used in this work that require simulation to determine optimal parameters to improve performance of refocusing, post processing, and PIV techniques. Lynch [24] provided an excellent numerical parametric study of macro-scale plenoptic light field PIV, and a similar analysis should be performed on the micro scale.
Seeding density is one of the most important PIV parameters. The seeding density must be high enough to provide good cross correlation, however there are limits to the number of particles that can be introduced into the flow, especially for 3D-3C methods. If too many particles are used, the signal-to-noise ratio will be decreased and particles located outside of the microscope focal plane will be blocked out. A simulation could determine how well occluded particles that lie along the optical axis at different depths can be resolved. For example, if there is a particle located at \( P_1(x,y,z) = (0,0,0) \) and others located at \( P_2 = (0,0,dz) \) and \( P_2 = (0,0,-dz) \), a simulation would determine how accurately the particle locations could be resolved in \( z \). Experiments should be performed to determine an optimal seeding density.

It is mentioned in [24, 32] that bilinear interpolation methods, used to adjust light field images such that there are an equal number of pixels behind each lenslet, degrade particle image quality and for this reason Fourier slice refocusing was not used in this work or the work of Lynch [24]. Fourier slice refocusing is computationally much faster [24] than the methods used herein and a simulation could be used to show if the negative effects of bilinear interpolation are significant.

The ability to specify exact particle locations and sizes will be useful for determining which post-processing techniques are the most accurate and effective at thresholding out particles that do not lie within the focal plane of interest. The techniques explored in this work include bandpass filtering, 3D deconvolution, and intensity thresholding using a sliding window. Each of these methods, and additional methods, could be explored further to determine if there is a technique that works best for reconstructing microscopic particle fields with high enough quality to perform more accurate PIV analysis.

### 4.2.2 System Components and Equipment

There are several system components and pieces of equipment that could be upgraded to provide better data acquisition and to better control experimental parameters. The syringe pump used in this work was inexpensive and, unfortunately, not well suited for providing a steady flow rate at small scales and low flows. A pump designed for microscale flows would provide a much more consistent flow rate and therefore better mean results. Lindken et al. [33] suggest using either a hydrostatic pump, or a syringe pump driven by a servo rather than a stepper motor.
As previously mentioned, light field microscopes require that the illumination be both spatially and angularly uniform [19]. A laser was used to illuminate fluorescent particles and the direction of light emitted from the microspheres should be independent of the direction of illumination, however, the laser light had significant spatial non-uniformities due to laser speckle. Non-uniformities in the illumination negatively affect image quality for PIV as well as light field imaging. A particle that is brightly illuminated in one frame may be significantly dimmer in the next frame, reducing the correlation between image pairs. This is especially a problem when resolution is low. Lindken et al. [33] suggest using a holographic diffuser plate to help eliminate speckle. Illumination used for calibration could also be improved in future work.

In this work, the camera was adjusted with a micrometer stage until it was at the correct distance from the microlens array. Trial and error was used until the lenslet images filled the correct number of pixels on the camera sensor. Using a condenser with a pinhole aperture as mentioned in [28] may be more robust and provide a better calibration image than the Mercury vapor lamp used in this work.

It is also suggested that a more precise and robust optical positioning system be used for the camera and lens array, perhaps even using a camera with a lens array mounted on the sensor. With the array mounted to the sensor, only the camera position requires adjustment and the camera could be placed close to the camera port on the microscope, reducing the effect of positioning errors. The lenslet grid would also be permanently aligned with the pixel grid on the camera sensor.

Using a different camera could also be explored. The microscope used in this work had a 20x objective lens which magnified a 1 mm field of view to 20 mm—slightly less than the 24 mm height of a full-frame DSLR camera. If the region of interest does not cover the entire field of view, a smaller camera sensor may be used as long as the sensor pixels are small enough to adequately sample the light field images. Higher frame rates could be used to measure higher flow rates, especially if the camera is capable of operating in double shutter mode.

To improve the image quality an oil or water immersion objective lens could be used. As mentioned previously, refractive index changes introduce spherical aberrations when the lens does not have a built-in correction. Objects in water imaged through air appear closer than their actual locations due to this effect. If an immersion lens were used, the refractive index could be matched all the way to the objective lens, reducing spherical aberrations.
Finally, particle sizes that are too small will lead to integer biasing and higher error. Larger particles should be used with larger particle diameters on the image sensor. The particles used herein had 2 \( \mu m \) diameters, which is smaller than the imaging width of each lenslet in the microlens array (6.25 \( \mu m \)). A rendered light field image will have a pixel for each microlens in the array. The particles were below the resolution of the microlenses, which will result in particle diameters of about a pixel. Larger particles will also improve deconvolution results. These suggested improvements have the potential to save time used to position and set up the system and improve image quality, which could lead to significantly better results.

4.2.3 Channel Fabrication

If the channels described in this work are to be used in blood flow experiments, the channel floors would need to be constructed out of PDMS, which is much more biocompatible than glass [4]. A technique for constructing the channel floors is discussed in Section 2.2.1. If these channel floors were to be used, they would need to be made as thin and uniform as possible to reduce degradation in image quality. In order to get the floor layer to be thinner and more uniform, it should be spun onto a smooth substrate, such as a silicon wafer, using a spinner and carefully removed from the substrate before it is bonded to the other half of the PDMS channel. The channels would also need to be more mass producible when several experimental runs are required. A more robust casting device, perhaps fabricated out of aluminum, could be used to decrease the time required to align the channels and pipette tips. New channels should also be constructed with depths that are within the refocusing range of the light field microscope. The depths of the channels used in this work were more than twice as large as the refocusing range. Using a shallower channel will decrease noise caused by particles outside of the measurement volume.

4.2.4 Blood Flow Experiments

The motivation behind this work was to explore a method to resolve blood flow velocities and thrombus growth in three dimensions in time. The results indicate that it may be possible to apply the method to blood flow, but further investigation is required. One of the major limitations is frame rate. The blood flow experiments performed by Goodman et al. [17] use much higher flow
rates than the experiments performed in this work. A smaller camera with high light sensitivity and high quantum efficiency would be necessary to capture images at higher flow rates. The blood would also need to be marked with a fluorescent dye or mixed with fluorescent particles, both of which would affect the way that blood reacts to the channel surfaces and shear stresses.

4.2.5 Conclusion

The results presented in this work warrant further research and development into the use of light field microscopy for performing \( \mu \text{PIV} \). The main limitation of the method presented herein is the resolution of the light field microscope. In instances where high resolution measurements are required, light field \( \mu \text{PIV} \) may not be adequate. However, there are several advantages to light field \( \mu \text{PIV} \) that may appeal to microfluidic researchers, such as the relatively low cost, ease of calibration, and potential to measure instantaneous, 3D-3C vectors.
REFERENCES


APPENDIX A. MATLAB LIGHT FIELD REFOCUSING CODES

%% Light Field Rendering Codes for LF Microscope

direc = 'E:\'; % Directory containing light field image data
calibimg = 'calibration1'; % Name of the calibration image. Should be % located in direc
lenslet_name = 'lenslet.txt'; % Text file generated by LFDiplay containing % the x and y coordinates of the center lenslet, and the x and y grid % spacings
setname = 'S1'; % Name the calibration set so that additional % images from the same set can use the same calibration information
imname = 'IMG.'; % The root light field image name
infmt = '%03d'; % The format of the image numbers
imgext = 'JPG'; % Extension of the LF images
img_nums = 173:212; % The numbers of the images to refocus
% Create a cell array containing the file names of the LF images, excluding % the extension
imgname = {};
for i = 1:length(img_nums)
    imgname{i} = [imname num2str(img_nums(i), infmt)];
end
filename = [direc imgname{1} '.' imgext];

% Define Experimental Parameters
% * ALL DIMENSIONS mm *
% Optical parameters
optics.si = 164.5;
optics.fl = 2.5; % Focal length of lenslet array
optics.pl = 0.125; % Pitch length of lenslets
optics.M = -20; % Magnification of the objective lens
optics.ft = 164.5; % Focal length of the tube lens
optics.fo = optics.ft/abs(optics.M);
optics.pp = 6.4/1000; % Camera pixel pitch
% Specify the focal plane locations IN OBJECT SPACE
% so = 0 will correspond to the plane in focus at the time of capture.
% Positive values are away from the main lens, negative values are towards % the main lens.
zthickness = 5;
% Enter the z values for refocusing
so = [-200:zthickness:200]/1000;
% Specify the type of optical system you have (e.g. ICO = infinity % corrected objective)
optical_sys = 'ICO';
% thresh_percent is used for image binarization of lenslet subimages for % determining the bounds of the image used for the 2D Gaussian fit
thresh_percent = 0.5;
% dist_thresh_percent is the threshold for deviations from the calculated % spacing
% between lenslets
dist_thresh_percent = 0.5;
% Read in the lenslet data produced by LFDiplay
lenStr = importdata([direc lenslet_name]);
lenStr = regexp(lenStr{1}(2:end-1),',','split');
xc = str2double(lenStr{1});
yc = str2double(lenStr{2});
szx = str2double(lenStr{3});
szy = str2double(lenStr{6});
save([direc lenslet_name '.mat'], 'xc', 'yc', 'szx', 'szy'); % Save the data

%% LF calibration
I = imread([direc calibimg '.JPG']);
Get the lenslet centroid locations, cx and cy
fprintf('Creating calibration grid and calculating lenslet locations.\n');
tic;
[cx, cy, xc, yc, nLs, nLt, nL_sc, nL_tc] = ...
    lf_calibration_JB(I, direc, thresh_percent, dist_thresh_percent, init_lenslets, lenslet_name);
toc;
t_calib = toc;
% Convert lenslet centroids to physical coordinates
cs = optics.p_p*(cx - xc);
cy = -optics.p_p*(cy - yc);
optics.nL_s = nL_s;
optics.nL_t = nL_t;
save([direc 'lenslets_centroids' setname '.mat'], 'cs', 'ct', 'cx', 'cy', 'xc', 'yc', 'nL_s', 'nL_t', 'nL_sc', 'nL_tc', 't_calib')

%% Manually adjust the lenslet offsets for PIV images
% OPTIONAL, BUT USUALLY NECESSARY
load([direc 'lenslets_centroids' setname '.mat']);
optics.nL_s = nL_s;
optics.nL_t = nL_t;
[lenslet] = lenslet_shift(filename, cx, cy, xc, yc);
% Convert lenslet centroids to physical coordinates
cs = optics.p_p*(cx - xc);
ct = -optics.p_p*(cy - yc);
save([direc 'lenslets_centroids_PIV' setname '.mat'], 'cs', 'ct', 'cx', 'cy', 'xc', 'yc', 'nL_s', 'nL_t', 'nL_sc', 'nL_tc')

%% Define the pixel-lenslet associations
load([direc 'lenslets_centroids_PIV' setname '.mat']);
optics.nL_s = nL_s;
optics.nL_t = nL_t;
tic;
[lenslet] = lf_radiance_gen_JB(filename, cs, ct, xc, yc, optics.p_p);
toc;
t_registration = toc;
save([direc 'LF_registration' setname '.mat'], 'lenslet', 't_registration')

%% Refocus the LF at different depths
load([direc 'lenslets_centroids' setname '.mat']);
optics.nL_s = nL_s;
optics.nL_t = nL_t;
load([direc 'LF_registration' setname '.mat'])
vol = [];
for j = 1:length(imgname)
    filename = [direc imgname{1} '.' imgext];
    save_dir = [direc imgname{1} '/refocused/'];
    mkdir([direc imgname{1}]);
    mkdir(save_dir);
tic;
    for i = 1:length(so)
        fprintf(['Image ' num2str(i) ' of ' num2str(length(so)) '; set ' ...
            num2str(j) ' of ' num2str(length(imgname)) '\n']);
    end
end
SA_image = lf_render_JB(filename, lenslet, so(i), optics, ...
    optical_sys);
vol(:,:,i) = SA_image;
end
toc;
t_refocusing = toc;
vol_raw = vol;
vol = uint8(vol_raw.*255/max(vol_raw(:)));
for k=1:length(so)
    SA_image = vol(:,:,k);
imnum = num2str(k,infmt);
imwrite(SA_image,[save_dir ...
    'fp_',imnum,'.tiff'],'tiff','Compression','none');
end
focal_planes = [1:length(so);so];
save({save_dir 'focal_planes.mat'},'focal_planes','t_refocusing')
save({save_dir 'vol.mat'},'vol','vol_raw')
clear vol vol_raw
end
clear('SA_image','t_refocusing','focal_planes');

function [cx,cy,xc,yc,nL_x,nL_y,nL_xc,nL_yc,sz] = ...
    lf_calibration_JB(I,direc,thresh_percent,dist_thresh_percent,init_lenslets)
% This code defines the centroid of a lenslet array from a calibration
% and estimates the size of the lenslet subimages.
% OUTPUTS:
% [cx,cy,xc,yc,nL_x,nL_y,nL_xc,nL_yc,sz] = ...
% lf_calibration_JB(I,direc,thresh_percent,dist_thresh_percent,init_lenslets)
% cx,cy are matrices of lenslet centroid positions
% xc,yc is the x,y location of the center lenslet
% nL_x,nL_y are the number of lenslets in x and y
% nL_xc,nL_yc are the indeces of the center lenslet
% sz is the size of the lenslet subimages
%
% INPUTS:
% I is the raw light field image
% direc is the directory of the lenslet file
% thresh_percent is the percent threshold used for image binarization to
% get the bounds of the lenslet subimage
% dist_thresh_percent is used for removing lenslet outliers. Any calculated
% lenslet spacings that differ by more than dist_thresh_percent*sz are
% thrown out
% init_lenslets is a boolean value. If it is true, the parameters from
% lenslet.mat are used. If it is false, the user interactively provides ...
% the intial
% lenslet spacings
% Jesse Belden – November 8, 2011
%
[m,n,d] = size(I);
if d == 3
    I = rgb2gray(I);
end
I = im2double(I);
[m,n] = size(I);

%%%% Select circle contour points
figure(1);
imshow(I);
title('Click at least 3 points to define lenslet contour (more points is ...
    better), hit enter when done selecting')
disp('Click at least 3 points to define lenslet contour (more points is ... 
    better), hit enter when done selecting');
fprintf('
')
prev = 0;
i = 1;
loop_flag = 1;
while loop_flag == 1;
    title('Zoom in on a lenslet at the periphery, then hit right arrow ... 
    when done zooming')
    disp('Zoom in on a lenslet at the periphery, then hit right arrow when ... 
    done zooming');
    zoom on;
    pause;
    zoom off;
    [xtemp,ytemp] = ginput(1);
    if ~isempty(xtemp)
        xi(i) = xtemp;
        yi(i) = ytemp;
    end
    if i == 1 || length(xi) > prev;
        loop_flag = 1;
    else
        loop_flag = 0;
    end
    prev = length(xi);
    i = i+1;
    figure(1);clf;
    imshow(I); hold on;
    title('Click at least 3 points to define lenslet contour (more points ... 
    is better), hit enter when done selecting')
    disp('Click at least 3 points to define lenslet contour (more points ... 
    is better), hit enter when done selecting');
    fprintf('
')
    plot(xi,yi,'b.');
end

[x0y0, rad, residual] = fitcircle([xi',yi']);
x0 = x0y0(1);
y0 = x0y0(2);
a = rad;
b = rad;
alpha = 0;
h = ellipse(a,b,alpha,x0,y0,'b');
plot(x0,y0,'ro','linew',2,'markersize',10)

%% Select center lenslet and lenset width
if init_lenslets == 1
    load([direc 'lenslet.mat'])
nL_x = 2*(floor(rad/szx)-1) + 1;
nL_y = nL_x;
nL xc = floor(rad/sz);
nL yc = nL xc;
cindx_vec = (1:nL_x) - nL xc;
cindy_vec = (1:nL_y) - nL yc;
[idx,indy] = meshgrid(cindx_vec,cindy_vec);
indx = index(:)';
indy = indy(:)';
cx_vec = szx*cindx_vec + xc;
cy_vec = szy*cindy_vec + yc;
[xi,yi] = meshgrid(cx_vec, cy_vec);
xi = xi(:)';
yi = yi(:)';
else
    win = 60;
    figure(1);
    axis([-x0-win/2 x0+win/2 y0-win/2 y0+win/2 ])
    title('Select the center of the center lenslet')
    disp('Select the center of the center lenslet')
    fprintf('
')
    [xc,yc] = ginput(1);
    plot(xc,yc,'g.' )
    title('Select the center of the lenslet one to the right of the center ... lenslet')
    disp('Select the center of the lenslet one to the right of the center ... lenslet')
    fprintf('
')
    [xR,yR] = ginput(1);
    plot(xR,yR,'g.' )
    title('Select the center of the lenslet one above the center lenslet')
    disp('Select the center of the lenslet one above the center lenslet')
    fprintf('
')
    [xT,yT] = ginput(1);
    plot(xT,yT,'g.' )
    dR = sqrt( (xR-xc).^2 + (yR-yc).^2 ) ;
    dT = sqrt( (xT-xc).^2 + (yT-yc).^2 ) ;
    sz = mean([dR,dT]);
    szx = abs(xR-xc);
    szy = abs(yT-yc);
    sz = mean([dR,dT]);
    % Find centers using 2D Gaussian fit
    [xc,yc] = gaussian2D_peakfind(I,xc,yc,sz);
    [xR,yR] = gaussian2D_peakfind(I,xR,yR,sz);
    [xT,yT] = gaussian2D_peakfind(I,xT,yT,sz);
    plot(xc,yc,'b.' )
    plot(xR,yR,'b.' )
    plot(xT,yT,'b.' )
    dR = sqrt( (xR-xc).^2 + (yR-yc).^2 ) ;
    dT = sqrt( (xT-xc).^2 + (yT-yc).^2 ) ;
    sz = mean([dR,dT]);
    szx = abs(xR-xc);
    szy = abs(yT-yc);
    % Select more lenslets to estimate initial grid
    figure(1);clf;
    imshow(I);hold on;
    plot(xc,yc,'b.' )
    title('Select more lenslet centers (at least 4) to estimate initial grid')
    disp('Select more lenslet centers (at least 4) to estimate initial grid')
    fprintf('
')
    prev = 0;
    i = 1;
    loop_flag = 1;
    clear xi yi indx indy
    while loop_flag == 1;
        title('Zoom in, then hit right arrow when done zooming')
        disp('Zoom in, then hit right arrow when done zooming');
        zoom on;
        pause;
        zoom off;
        [xtemp,ytemp] = ginput(1);
        if ~isempty(xtemp)
            xi(i) = xtemp;
            yi(i) = ytemp;
        end
        if i == 1 || length(xi) > prev;
[xi(i),yi(i)] = gaussian2D_peakfind(I, xi(i), yi(i), max(szx, szy));
dx = xi(i) - xc;
dy = yi(i) - yc;
indx(i) = round(dx/szx);
indy(i) = round(dy/szy);
loop_flag = 1;
else
    loop_flag = 0;
end

figure(1);clf;
imshow(I);hold on;
plot(xc,yc,'b.');</ref>

% Select more lenslet centers (at least 4) to estimate initial grid')
disp('Select more lenslet centers (at least 4) to estimate initial grid');
fprintf('
');
plot(xi,yi,'r.');</ref>

prev = length(xi);  
i = i+1;
end

% Use the initial homography to estimate the other grid point locations
nL_x = 2*(floor(rad/sz)-1) + 1;
nL_y = nL_x;
nL_xc = floor(rad/sz);
nL yc = nL_xc;

% Homography estimates from the selected points
ref_points = [indx;indy;ones(1,length(indx))];
img_points = [xi;yi;ones(1,length(xi))];
cindx_vec = (1:nL_x) - nLxc;
cindy_vec = (1:nL_y) - nLyc;
[cindx,cindy] = meshgrid(cindx_vec,cindy_vec);
pts = [cindx(:);cindy(:);ones(1,length(cindx(:)))];

[XY,H,Hinv] = apply_homog(img_points, ref_points, pts);
sz = mean([Hinv(1,1),Hinv(2,2)]);

% Construct grid and search for other lenslet centers


cx = reshape(XY(1,:),[nL_y,nL_x]);
cy = reshape(XY(2,:),[nL_y,nL_x]);
plot(cx,cy,'y+');
title('Select the upper left, then lower right bound of the region of lenslets to keep')
disp('Select the upper left, then lower right bound of the region of lenslets to keep');

% Remove lenslets outside circle or off image plane
[xcrop,ycrop] = ginput(2);
if isempty(xcrop)
    ind = find(round(cy-sz)<ycrop(1)); cx(ind) = NaN; cy(ind) = NaN;
    ind = find(round(cy+sz)>ycrop(2)); cx(ind) = NaN; cy(ind) = NaN;
    ind = find(round(cx-sz)<xcrop(1)); cx(ind) = NaN; cy(ind) = NaN;
    ind = find(round(cx+sz)>xcrop(2)); cx(ind) = NaN; cy(ind) = NaN;
    ind = find( sqrt((cx-xc).^2 + (cy-yc).^2) > rad ); cx(ind) = NaN; ... 
    cy(ind) = NaN;
end
cx_old = cx;
cy_old = cy;
for i = 1:nL_y...
for j = 1:nL
    if isnonan(cx(i,j))
        x0 = cx(i,j);
        y0 = cy(i,j);
        Igrid = I(round(y0-sz/2):round(y0+sz/2),... 
            round(x0-sz/2):round(x0+sz/2));
        BW = im2bw(Igrid,thresh_percent*max(Igrid(:)));
        [iy,ix] = find(BW==1);
        x0 = sum(ix)/length(ix) + round(x0-sz/2);
        y0 = sum(iy)/length(iy) + round(y0-sz/2);
        [cx(i,j),cy(i,j)] = gaussian2D_peakfind(I,x0,y0,sz);
    end
end
toc;
elapsed = toc;
xc = cx(nL,yc,nL,xc);
yc = cy(nL,yc,nL,xc);
dist = sqrt((cx-cx_old).^2 + (cy-cy_old).^2);
ind = find(dist > dist_thresh_percent*sz);
cx(ind) = NaN;
cy(ind) = NaN;
end

function [cx,cy,xc,yc] = lenslet_shift(filename,cx,cy,xc,yc)
% This code shifts the results of the lenslet calibration to fit the PIV
% image lenslet locations.
% INPUTS:
% filename is the light field image file name
% cx,cy are the lenslet centroid x,y locations
% xc,yc is the x,y location of the center lenslet
% OUTPUTS:
% new cx,cy,xc,yc values
% Written by Jesse Belden — January 23, 2012
% Read Image
I = imread(filename);
% I = im2double(rgb2gray(I));
I = im2double(I);

% Select circle contour points
figure(1);
imshow(I);hold on;
plot(cx,cy,'+');
title('Shift the lenslet centers to line up with the PIV image centers')
disp('Shift the lenslet centers to line up with the PIV image centers');
fprintf('n');
title('Zoom in to check lenslet center alignment, then hit right arrow ... 
when done zooming')
disp('Zoom in to check lenslet center alignment, then hit right arrow when ... 
done zooming')
zoom on;
pause;
zoom off;
title('Select the location of what you think is a lenslet center on the ... 
PIV image')
disp('Select the location of what you think is a lenslet center on the PIV ... 
image');
[xtemp,ytemp] = ginput(1);
if ~isempty(xtemp)
    xi = xtemp;
    yi = ytemp;
end

d = sqrt((xi-cx).^2 + (yi-cy).^2);
ind = find(d == min(d(:)));
xshift = xi-cx(ind);
yshift = yi-cy(ind);

figure(1);
imshow(I);hold on;
plot(cx+xshift,cy+yshift,'c+');
title('Zoom in/out to see other lenslets, then hit right arrow when done ...
      zooming')
disp('Zoom in/out to see other lenslets, then hit right arrow when done ...
      zooming')
zoom on;
pause;
zoom off;

loop_flag = input('Hit enter if the lenslets look good, enter 1 to adjust ...
      further => ');

%% Adjust further
while loop_flag

figure(1);
imshow(I);hold on;
plot(cx+xshift,cy+yshift,'c+');
title('Shift the lenlset centers to line up with the PIV image centers')
disp('Shift the lenlset centers to line up with the PIV image centers');
fprintf('
')
title('Zoom in/out to see other lenslets, then hit right arrow when ... 
      done zooming')
disp('Zoom in/out to see other lenslets, then hit right arrow when ... 
      done zooming')
zoom on;
pause;
zoom off;

xs = input('Enter the x−shift to try => ');
ys = input('Enter the y−shift to try (remember, y is positive down) => ');
xshift = xshift+xs;
yshift = yshift+ys;

figure(1);
imshow(I);hold on;
plot(cx+xshift,cy+yshift,'c+');
title('Shift the lenlset centers to line up with the PIV image centers')
disp('Shift the lenlset centers to line up with the PIV image centers');
fprintf('
')
title('Zoom in/out to see other lenslets, then hit right arrow when ... 
      done zooming')
disp('Zoom in/out to see other lenslets, then hit right arrow when ... 
      done zooming')
zoom on;
pause;
zoom off;

loop_flag = input('Hit enter if the lenslets look good, enter 1 to adjust ...
      adjust further => ');

end

cx = cx+xshift;
cy = cy+yshift;
xc = xc+xshift;
yc = yc+yshift;
end

function [lenslet] = lf_radiance_gen_JB(filename, cs, ct, xc, yc, p_p, sz, ...
    cs_offset, ct_offset)
% Light field parameterization
% INPUTS:
% filename is the filename of the image to be refocused
% cs, ct are the lenslet centroid locations in physical coordinates
% xc, yc is the x,y location of the center lenslet
% p.p is the pixel pitch on the camera sensor
% sz is the lenslet spacing
% cs_offset, ct_offset are optional parameters for offset in the physical
% lenslet locations
%
% OUTPUTS:
% lenslet is a structured array of the parameterized light field. Each
% pixel in the image is assigned to a parent lenslet.
% Kyle Lynch (lynchkp@auburn.edu) October 4, 2010
% Modified by Bryce McEwen October 2011
% Modified by Jesse Belden — November 14, 2011
% Re-wrote much of the code

if nargin < 3
    fprintf('Not enough input arguments.\n');
    return;
end

% Read Image
img = imread(filename);

% Determine the average lenslet domain size (physical units)
if nargin <= 6
    dcs = abs(diff(cs'));
dcs = dcs(:);
dct = abs(diff(ct));
dct = dct(:);
indx = find((dcs - mnanmean(dcs(:)' ) ) > mnanstd(dcs(:)' ));
indy = find((dct - mnanmean(dct(:)' ) ) > mnanstd(dct(:)' ));
dcs(indx) = [];
dct(indy) = [];
avg_x = mnanmean(dcs(:)' );
avg_y = mnanmean(dct(:)' );
if abs(avg_x-avg_y)/max(avg_x,avg_y) > 0.1
    fprintf('Error: Lenslet Size differs by > 10 percent in x-y');
    return;
else
    sz = mean([avg_x,avg_y]);
r = sz/2;
end
if nargin < 8
    cs_offset = 0;
c_offset = 0;
end

% Convert all pixels into s-t coordinates (physical units)
[nL_t, nL_s] = size(cs);
[npt, nps] = size(img);
[px,py] = meshgrid(1:nps,1:npt);
p = p.p*(px - xc)-cs_offset;
p = -p.p*(py - yc)-ct_offset;

% Find parent lenslet for each pixel
k = 1;
for i = 1:nL_t
    for j = 1:nL_s
        if ~isnan(cs(i,j))
            lenset(k).s = cs(i,j);
            lenset(k).t = ct(i,j);
        end
    end
end

% Find pixels that fall within lenslet domain
% % Radius method
% dist = sqrt( (ps - cs(i,j)).^2 + (pt - ... 
    ct(i,j)).^2 );
% ind = find(dist <= r);
% Square lenslet method (much faster)
ds = abs(ps(1,:) - cs(i,j));
dt = abs(pt(:,1)' - ct(i,j));
is = find(ds < sz/2);
it = find(dt < sz/2);
% Define the s,t coordinates and radiance of pixels behind the ...
lenslet
lenalet(k).ps = ps(it,is);
lenalet(k).pt = pt(it,is);
lenalet(k).s_ind = is;
lenalet(k).t_ind = it;
k = k+1;
end
end
end

function [SA_image] = lf_render_JB(filename, lenslet, so, optics, optical_sys)
% LF_RENDER Light Field Rendering/Refocusing
% [SA_image] = lf_render_JB(filename, lenslet, so, optics, optical_sys)
% integrates signal from each lenslet, rendering an image
% INPUTS:
% filename is the file name of the light field image being rendered
% lenslet is the structured array produced by lf_radiance_gen_JB containing
% light field parameterization information
% so is the z-location where refocusing will take place
% optics is a structured array containing information about the light field
% microscope
% optical_sys the type of optical system you have (e.g. ICO = infinity
% corrected objective)
% OUTPUTS:
% SA_image is a 2D rendered image refocused at so
% Kyle Lynch (lynchkp@auburn.edu) October 4, 2010
% Modified by Bryce McEwen October 2011
% Modified by Jesse Belden - November 14, 2011
% Re-wrote much of the code
% Read Image
img = imread(filename);
[m,n,d] = size(img);
if d == 3
    img = im2double(rgb2gray(img));
end
img = im2double((img));
radiance = img;

nL = length(lenslet);
% Optical parameters
si = optics.si;
f_l = optics.f_l;
p_l = optics.p_l;
nL_s = optics.nL_s;
nL_t = optics.nL_t;
M = optics.M;
fo = optics.fo;
% Define scaling of pixels and location of image plane
if strcmp(optical_sys,'ICO');
    [d, scale] = ICO(fo, ft, p_l, so);
end

%% For each lenslet, project each ray (pixel)
% Define size of refocused image
SA_image = zeros(nL_t, nL_s);
tic;
for k = 1:nL_lenslet
    lenslet(k).radiance = radiance(lenslet(k).t_ind, lenslet(k).s_ind);
    radiancetest = find(lenslet(k).radiance > 0);
    for j = 1:length(radiancetest)
        ray = radiancetest(j);
        ray_radiancetest = lenslet(k).radiance(ray);
        ray_s = lenslet(k).s;
        ray_t = lenslet(k).t;
        ray_u = lenslet(k).ps(ray);
        ray_v = lenslet(k).pt(ray);
        % Project ray to s'−t' plane
        sp = ray_s*(1 + d/f_l) - ray_u*d/f_l;
        tp = ray_t*(1 + d/f_l) - ray_v*d/f_l;
        % Convert to synthetic sensor coordinates
        initial_x = scale*sp + ceil(nL_s/2);
        initial_y = -scale*tp + ceil(nL_t/2);
        if initial_x == round(initial_x)
            L_x = floor(initial_x);
            R_x = ceil(initial_x)+1;
            L_x_coeff = R_x - initial_x;
            R_x_coeff = initial_x - L_x;
        else
            L_x = floor(initial_x);
            R_x = ceil(initial_x);
            L_x_coeff = R_x - initial_x;
            R_x_coeff = initial_x - L_x;
        end
        if initial_y == round(initial_y)
            B_y = floor(initial_y);
            T_y = ceil(initial_y)+1;
            B_y_coeff = T_y - initial_y;
            T_y_coeff = initial_y - B_y;
        else
            B_y = floor(initial_y);
            T_y = ceil(initial_y);
            B_y_coeff = T_y - initial_y;
            T_y_coeff = initial_y - B_y;
        end
        TL = T_y_coeff*L_x_coeff;
        TR = T_y_coeff*R_x_coeff;
        BL = B_y_coeff*L_x_coeff;
        BR = B_y_coeff*R_x_coeff;
        if T_y > 0 && T_y <= nL_t
            if L_x > 0 && L_x <= nL_s
                SA_image(T_y, L_x) = SA_image(T_y, L_x) + ray_radiancetest*TL;
            end
        end
        if R_x > 0 && R_x <= nL_s
            SA_image(T_y, R_x) = SA_image(T_y, R_x) + ray_radiancetest*TR;
        end
    end
end
if $B_y > 0 \&\& B_y \leq nL_t$
    if $L_x > 0 \&\& L_x \leq nL_s$
        $SA\_image(B_y, L_x) = SA\_image(B_y, L_x) + \text{ray\_radiance}\times BL$;
    end
    if $R_x > 0 \&\& R_x \leq nL_s$
        $SA\_image(B_y, R_x) = SA\_image(B_y, R_x) + \text{ray\_radiance}\times BR$;
    end
end
end
toc
APPENDIX B. CHANNEL MEASUREMENTS AND UNCERTAINTY

This appendix provides information on how uncertainties of image-based length measurements, depth measurements, and values for $L_e$ and $Re_{D_h}$ were estimated.

B.1 Image-based Length Measurements

The image-based length, $L$, is measured by interactively drawing a line representing the length to be measured. The length is converted from pixels to $\mu$m using a conversion factor, $K$. The conversion factor is obtained using the grid method described in Section 2.2.2. The equation for the physical length can be written as

$$L = \sqrt{(x_1 - x_2)^2 + (y_1 - y_2)^2} K$$  \hspace{1cm} (B.1)

where $(x_1, y_1)$ and $(x_2, y_2)$ represent the two end points of the line. For the case of measuring the SU-8 mold width (represented by a distance in $y$), the $y$ uncertainty was estimated at about $\pm 3$ pixels. This value was chosen because the edge thickness in the image was approximately 6 pixels wide. The uncertainty in $x$ is calculated by obtaining an uncertainty in $x_1 - x_2$, since this difference would theoretically be zero in the case of a vertical line measurement. This $x$-component uncertainty is calculated by creating several line measurements and defining $x_2$ as

$$x_2 = \frac{y_2 - y_1}{m} + x_1$$  \hspace{1cm} (B.2)

where $m$ is the line slope. Therefore, the uncertainty in $x_2$ is

$$u_{x_2} = \pm \sqrt{\left(\frac{\partial x_2}{\partial y_1} u_{y_1}\right)^2 + \left(\frac{\partial x_2}{\partial y_2} u_{y_2}\right)^2 + \left(\frac{\partial x_2}{\partial m} u_m\right)^2 + \left(\frac{\partial x_2}{\partial x_1} u_{x_1}\right)^2}$$  \hspace{1cm} (B.3)
The value of $u_{x_1}$ is zero because only the relative distance between $x_1$ and $x_2$ is important. The value of $u_m$ is calculated by

$$u_m = \pm t_{\nu,95\%} \frac{\sigma_m}{\sqrt{N}}$$  \hspace{1cm} (B.4)

where $t_{\nu,95\%}$ is the inverse student-t distribution value at 95% confidence and $\nu$ degrees of freedom, $\sigma_m$ is the standard deviation of slope measurements, and $N$ is the number of slope measurements. The uncertainty in $K$ is given by

$$u_K = \pm t_{\nu,95\%} \frac{\sigma_K}{\sqrt{N}}$$  \hspace{1cm} (B.5)

using the centroid measurements from the binarized calibration grid to obtain values of $\sigma_K$, $\nu$, and $N$. The uncertainty in $L$ is then given by

$$u_L = \pm \sqrt{\left( \frac{\partial L}{\partial y_1} u_{y_1} \right)^2 + \left( \frac{\partial L}{\partial y_2} u_{y_2} \right)^2 + \left( \frac{\partial L}{\partial K} u_K \right)^2 + \left( \frac{\partial L}{\partial x_2} u_{x_2} \right)^2}$$  \hspace{1cm} (B.6)

### B.2 Depth Measurements

The channel depth can be written as

$$D = (d_1 - d_2)n_f$$  \hspace{1cm} (B.7)

where $d_1$ and $d_2$ are the depths of the floor and ceiling of the channel, as measured by the objective lens translation knob, and $n_f$ is the refractive index of the fluid. The uncertainties, $u_{d_1}$ and $u_{d_2}$, are specified at half of the depth of field of the objective lens. The uncertainty in $D$ is

$$u_D = \sqrt{\left( \frac{\partial D}{\partial d_1} u_{d_1} \right)^2 + \left( \frac{\partial D}{\partial d_2} u_{d_2} \right)^2 + \left( \frac{\partial D}{\partial n_f} u_{n_f} \right)^2}$$  \hspace{1cm} (B.8)

### B.3 Entry Length and Reynolds Number Uncertainties

The uncertainties of the entry length and Reynolds numbers were obtain by rewriting the equations in terms of flow rate, channel width and depth, and kinematic viscosity. Reynolds num-
ber for flow in a rectangular duct may be rewritten as

\[ Re_{Dh} = \frac{2Q}{(w+d)\nu} \]  

(B.9)

where \( Q \) is the flow rate, \( \nu \) is the kinematic viscosity, \( w \) is the channel width, and \( d \) is the channel depth. Assuming a negligible uncertainty in \( \nu \), the uncertainty is given by

\[ u_{Re} = \sqrt{\left( \frac{\partial Re}{\partial Q} u_Q \right)^2 + \left( \frac{\partial Re}{\partial w} u_w \right)^2 + \left( \frac{\partial Re}{\partial d} u_d \right)^2} \]  

(B.10)

where \( u_w \) and \( u_d \) are obtained from the channel measurements described above. The flow rate uncertainty was estimated at 0.1 \( \mu \text{L/min} \). The equation for entry length can be written as

\[ L_e = \frac{wd}{(w+d)} \left( \frac{0.2Q}{(w+d)\nu} \right) \]  

(B.11)

and the uncertainty is given by

\[ u_{L_e} = \sqrt{\left( \frac{\partial L_e}{\partial Q} u_Q \right)^2 + \left( \frac{\partial L_e}{\partial w} u_w \right)^2 + \left( \frac{\partial L_e}{\partial d} u_d \right)^2} \]  

(B.12)