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Application of non-invasive flow measurement techniques for quantitative analysis of a biomedical device

Amit Chainani

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APPLICATION OF NON-INVASIVE FLOW MEASUREMENT TECHNIQUES FOR QUANTITATIVE ANALYSIS OF A BIOMEDICAL DEVICE

By Amit Chainani

A Thesis Submitted in Partial Fulfillment of the requirement for the

Master of Science
In
Mechanical Engineering

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January 2011
APPLICATION OF NON-INVASIVE FLOW MEASUREMENT TECHNIQUES FOR QUANTITATIVE ANALYSIS OF A BIOMEDICAL DEVICE

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I would like to thank Dr. Kathleen Lamkin-Kennard and Dr. Wayne Walter for their valuable time and patience. I would also like to thank Dave Hathaway, Robert Kraynik and Steven Kosciol for their guidance and expertise. Finally, I would like to thank my parents, specially my father, for putting me through school and always believing that I can achieve anything thing I want.
Abstract

Researchers have extensively studied the techniques and applications of Particle Image Velocimetry (PIV) and Planar Laser Induced Fluorescence (PLIF). Developments in computing and imaging techniques have significantly aided the accuracy and efficiency of these techniques which are now commonly used to study velocity vectors, predicting acceleration and mixing profiles in modern fluid dynamics.

This project focused on analyzing the flow field inside a MicroWell, a component of the assay kits that are processed by the Vitros ECi analyzer. The assay kits and analyzer are manufactured by Ortho Clinical Diagnostics, Rochester, NY. Reagents and samples are mixed in the MicroWell during various operations of the analyzer. This process was extensively examined and quantified using the said techniques.

This study involved building a test rig that emulates the injection and mixing process of the actual system and allows access for extensive PIV and PLIF analysis as well as optimization and synchronization of both systems for accurate real-time results. Further, results obtained from both experiments were studied in conjunction and then quantified for an aid of future assay and Immunodiagnostics development.
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Chapter 1: Specific Aims

This study is intended to experimentally investigate the fluid flow inside a vessel (MicroWell) during the convective mixing processes initiated by the injection of a reagent into the vessel containing a blood serum sample (primary mixing) and the oscillation of the vessel containing a blood serum sample and an injected reagent (secondary mixing). A motor causes angular oscillations of an incubator ring with respect to its center. This movement is transferred as a lateral oscillatory motion to the stationed MicroWells. A thorough analysis of the effects of this system requires the use of non-invasive flow measurement techniques such as Particle Image Velocimetry (PIV) and Planar Laser Image Fluorescence. This study is intended to precisely measure variations in flow characteristics during the course of the process for a range of parameters. Specific aims are:

1. Design and set an experimental rig for a real-time evaluation of the incubator mixing process of the Immunodiagnostic Analyzer using flow visualization.
2. Develop technique and metric to quantify mixing in a vessel as function of space and time.
3. Compare overall mixing process with pure diffusion using developed techniques.
4. Determine the effect of primary mixing. Specifically, quantify mixing as a function of reagent to sample volume(3) and injector speed(3)
5. Determine the effect of secondary mixing on a single reagent. Specifically, quantify mixing as function of time over five jiggles and reagent to sample volume(3)
6. Evaluate the quality of the current prescribed (nominal) mixing process and parameters by comparing final results of the process with an ‘ideal’ mix of the components using developed techniques.
Chapter 2: Background and Significance

The Ortho Clinical Diagnostics (OCD) Vitros ECi immunodiagnostic system is currently used in many laboratories around the world for thyroid, oncology, cardiac, reproductive, endocrinology, anemia, bone metabolism, and infectious disease testing (OCD, 2007). This device is capable of running up to 60 tests simultaneously and reporting up to 90 results per hour with good accuracy.

An important part of the Vitros ECi system is its incubator ring and the tiny MicroWells in which the reagent and blood serum are mixed. The system’s stepper motor is geared to the incubator ring holding the MicroWells. In addition to moving the MicroWells to various locations within the analyzer, the stepper motor occasionally produces angular oscillations (jiggles) of the ring which are intended to stimulate convective mixing within the MicroWells. Even though the process is well controlled and various inputs such as the speed and steps of the stepper motor can be precisely altered, little is known about the actual fluid motion within the MicroWell or the impact of the ‘jiggle’ on the mixing process.

Previous attempts to better understand this mixing process have included Computational Fluid Dynamics (CFD) using commercially available CFD codes and experimental analysis using food dyes and high speed cameras. Yet, these attempts have only been able to go as far as to qualitatively predict the fluid profiles during the process with little or no real time synchronization with the stepper motor that controls the process.

To begin research in this extensive and highly complex stream of fluid dynamics, literature related to sloshing, fluid mixing and experimental techniques of Particle Image Velocimetry

Figure 2-1: The OCD Vitros ECi system
(PIV) and Planar Laser Induced Fluorescence (PLIF) has been analyzed. Previous research dealing with similar problems is described in the following sections (fluid mixing and sloshing).

2.1 Slosh Dynamics

Slosh Dynamics or the problem of liquid boundary movement within moving containers has been researched by a number of scholars in recent history. The sloshing phenomenon has broadly been classified into free and forced sloshing (Ibrahimm et al., 2001). Free sloshing or free liquid oscillations are characterized by the vibration excitation of the liquid at its natural frequencies. Forced sloshing, on the other hand, is generally stimulated by an external force or displacements applied to the container or an influx or outflux of fluid within the container.

The diverse application of sloshing has attracted many researchers from the fields of aerospace, civil, naval and nuclear engineering that have had varied interests in the problem depending on the application at hand. Early analytical studies in this field concentrated on modal analysis of the liquid free surface of the partially filled containers based on the Fourier representation of the free surface. This research was then extended to oscillating containers (Abramson et al., 1961). Studies (Armstrong, 1961, Graham, 1951) showed that the forced sloshing free surface has two mechanical components. One of these components is related to the lateral movement of the container and is proportional to the containers acceleration while the second component can be adequately modeled using an equivalent combination of pendulum or mass-spring-dashpot systems. These methods ensure preservation of mass and moments of inertia, center of gravity, modes of oscillations of the free surface and force components of the system while evaluating the dynamics of the liquid-air interface. Models include linear and more recently non-linear (Figure 2-2) solutions describing flows in rectangular, conical and spherical geometries.
Epperson et al. (1961) experimentally analyzed dynamic loads exerted on a scaled model of a missile fuel tank that caused by the oscillation of the fuel inside the tank as the missile undergoes extreme accelerations and deceleration. Epperson et al. concluded that the “pattern” of flow was independent of the magnitude of the acceleration of the tank and sensitive to the shape of the tank and its angle of inclination.

Abramson et al. (1966) conducted compelling studies that demonstrated the need of further developing non-linear analytical methods to accurately explain modes and force responses of liquid sloshing within cylindrical, spherical and rectangular geometries. This was a pioneering experimental effort that detected various modes of the free surface in each case using a strain sensing instrumentation system that sensed the force response of the system in parallel and normal direction to the excitation. The study reported that even though the force responses and modes predicted by linear analytical studies were in close range with the data collected for low amplitude sloshing within rectangular and cylindrical geometries, in cases of containers with compartment or in cases of high wall interaction, these studies were inaccurate.

Analytical attempts to describe the flow field during free as well as forced sloshing have been made by Green (1959) and Budiansky (1958). These studies have used the Navier-Stokes equations coupled with Newtonian force balances to solve the problem. This has been done by treating the container as rigid and impermeable, and the fluid as being inviscid, incompressible,
and initially irrotational. Generally, the solution is obtained using three boundary conditions (Ibrahim et al., 2001). These conditions are:

1. The zero normal velocity component at the wetted boundary wall
2. Zero pressure at free surface.

Using these boundary conditions, solutions for the flow field inside rectangular and conical geometries (Ehrlich, 1959, Green, 1959, Gavrilyuk et al., 2005) have been obtained. Non-linear fluid free surface boundary conditions when applied to the fluid field equations result in major difficulties for most researchers. This has stimulated experimental and computational fluid dynamicists to step in and model complex geometries that are being used in the fields of nuclear aerospace and mechanical design. Dodge et al. (1965) conducted and experimental and theoretical investigation on the longitudinal excitation of cylindrical containers to conclude that all the characteristics exhibited by the free surface of the fluid in the container depended upon the amplitude and frequency of tank excitation. Dodge et al. supported their conclusion by comparing experimental data collected using liquid displacement transducers with theoretical estimation of force and mode of the free surface.

More recently, smoothed-particle hydrodynamic and level-set techniques have been used by many scholars (Chen et al., 2009, Colagrossi et al., 2004, Delorme et al., 2005) to numerically simulate lateral sloshing inside rectangular and cylindrical geometries. Chen et al. (2009) have shown that generally during lateral vacillations in a partially filled rectangular container, the combination of the fluid inertia and sudden wall displacement produces a cyclical pressure and volume imbalance inside the container. In the case of an accelerating container, there is a high pressure region developed close to the lagging wall of the container caused by the inertia of the fluid. This also causes a change in the free-surface shape as there is a fluid displacement towards the high-pressure region. However, as the container slows down or changes direction, the whole pressure system reverses, and a high pressure is developed on the opposite wall. High oscillation frequencies and changes in amplitude result in changes in variation in the magnitude of pressures developed.

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Research conducted by Pal et al. (2001) on the effects of sloshing compared experimental analysis with results from finite element analysis. Pal et al. created an experimental setup consisting of complex container geometry being excited by the use of a controlled ‘shaking platform’ to produce a fluid sloshing within the container. The change in the level of the fluid within the container was measured by wall sensors on the container walls. The data hence collected was processed to calculate the ‘slosh-response’ of the system. The slosh frequency, slosh displacement and slosh damping for 30, 60, 90, 120 and 150 mm liquid depths were compared to FEM results. In conclusion, the authors compared the three dimensional free-surface profiles of liquid at different time steps from both the experimental and computational analysis and reported that the results were in good agreement.

Experiments conducted by Akyildiz et al. (2005) on a similarly excited sloshing systems within a rectangular container were some of the first attempts to analyze the 3-dimensional pressure distribution during the phenomenon. Using an arrangement of pressure transducers, Akyildiz tested a variety of volume and ‘roll frequencies’ for the given geometry and produced time dependent pressure functions for data obtained at various sites. Akyildiz also reported a linear relationship between fill depth and maximum pressure recorded.

Evidently, experimental fluid visualization of sloshing has been primarily endeavored in relation to nuclear engineering. Self-induced sloshing of coolant in reactor vessels of a fast breeder nuclear reactor (a fast neutron reactor designed to produce more fissile fuel than it consumes) has been visualized using PIV by Okamoto et al. (2004). As described by Okamoto, self-induced sloshing is the oscillation of fluid generally excited by a steady flow pattern that causes gradual instabilities in the fluid-air surface. In their research, Okamoto et al. replicated this effect in their PIV rig using an optically accessible flow loop illustrated in Figure 2-3.
Figure 2-3: Flow loop (Okamoto et al., 2000)

The velocities of a fluid continuously entering and leaving a rectangular section of the tank were captured by the use of a CCD camera focused along the vertical plane of the tank where as the orientation of the liquid-air interface were approximated by fluid level sensors across the tank. Data captured by the PIV and level sensing system were both used to calculate the 'sloshing energy' (\( \Delta E(x, y) \)) at all coordinates of the flow field. This quantity is expressed as:

\[
\Delta E(x, y) = \int_{T_s} \Delta E(x, y, t) \cdot dt
\]

Equation 2-1

where \( x \) and \( y \) are the coordinated of the location; \( dt \) is the window of time between two consecutive measurements; and \( \Delta E \) is the oscillation energy at the location. The authors concluded that the technique enabled highly accurate measurements of the growth mechanism of the self-induced sloshing and that the results were in good agreement with previous analytical and experimental work done in relation to self-induced sloshing and showed

In spite of these remarkable efforts, visualization of forced slosh dynamics remains an almost uncharted territory and the use of imaging techniques in the field has been very limited. Among others, the inaccuracies related to data collection and velocity and phase calculations due to the
movement of the container being imaged are an area of high pose major obstacles in the way of progress in this effort. To the best of the author's knowledge, visualization of slosh dynamics as a technique for initiating mixing has yet not been attempted and it can be anticipated that the results of such a study would accentuate the current scope of knowledge in this field.
Table 2-1: Summary of literature review relating to slosh dynamics

<table>
<thead>
<tr>
<th>Author(Year)</th>
<th>Study Time</th>
<th>Description</th>
<th>Geometries Investigated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Budiansky (1958)</td>
<td>Analytical</td>
<td>Theoretical models describing natural modes and natural frequencies of small-amplitude sloshing of liquids in partially filled containers investigated</td>
<td>Cylindrical and conical</td>
</tr>
<tr>
<td>Green (1959)</td>
<td>Analytical</td>
<td>Approximated the eigenvalues for sloshing of propellant in a rectangular tank using the method of separation of variables</td>
<td>Conical</td>
</tr>
<tr>
<td>Ehrlich (1959)</td>
<td>Analytical</td>
<td>Obtained some exact solutions of the boundary value problem of liquid sloshing within fuel tanks.</td>
<td>Cylindrical</td>
</tr>
<tr>
<td>Abramson et al.(1961)</td>
<td>Experimental</td>
<td>Sloshing response forces in both parallel and normal directions to the excitation acquired and then compared to theoretical results derived by Hutton’s theory</td>
<td>Cylindrical and spherical</td>
</tr>
<tr>
<td>Armstrong (1961)</td>
<td>Analytical</td>
<td>Developed analytical model for sloshing of propellant within a fuel tank</td>
<td>Rectangular</td>
</tr>
<tr>
<td>Author(Year)</td>
<td>Study Time</td>
<td>Description</td>
<td>Geometries Investigated</td>
</tr>
<tr>
<td>-------------------------</td>
<td>-----------------------</td>
<td>-----------------------------------------------------------------------------------------------</td>
<td>------------------------------------------</td>
</tr>
<tr>
<td>Epperson et al. (1961)</td>
<td>Experimental</td>
<td>Force based study carried out to accurately predict the wall forces during slosh impacts within the fuel tank bulk-head of a missile. High speed camera images used to relate mode shapes to the observed forces.</td>
<td>Conical and Spherical</td>
</tr>
<tr>
<td>Dodge et al. (1965)</td>
<td>Theoretical and Experimental</td>
<td>Effects low-frequency harmonic oscillation to the liquid free surface studied analytically by solving Laplace's equations using Bessel functions. Results for free surface variations are compared to experimental analysis.</td>
<td>Cylindrical</td>
</tr>
<tr>
<td>Abramson et al. (1966)</td>
<td>Experimental</td>
<td>Force and Mode experiments carried out for partially filled containers to verify previously proposed linear mechanical models for the liquid free surface during forced sloshing.</td>
<td>Rectangular, Cylindrical and Conical</td>
</tr>
<tr>
<td>Hashimoto and Sudo (1988)</td>
<td>Experimental</td>
<td>Velocity vectors obtained through particle image velocimetry used to calculate fluid forces and sloshing energy distributions during self induced sloshing.</td>
<td>Rectangular</td>
</tr>
<tr>
<td>Author(Year)</td>
<td>Study Time</td>
<td>Description</td>
<td>Geometries Investigated</td>
</tr>
<tr>
<td>----------------------</td>
<td>-----------------------------</td>
<td>------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
<td>--------------------------</td>
</tr>
<tr>
<td>Pal et al. (2001)</td>
<td>Experimental and Numerical</td>
<td>Liquid-level variations and slosh mode configurations measured using liquid sensors on the walls of the container. Data and 3-D free surface mode shapes compared to FEM model</td>
<td>Conical/Spherical</td>
</tr>
<tr>
<td>Ibrahim et al. (2001)</td>
<td>Theoretical, Experimental and Numerical</td>
<td>An extensive review of significant efforts and advances in the research of slosh dynamics</td>
<td>-</td>
</tr>
<tr>
<td>Colagrossi et al. (2004)</td>
<td>Numerical</td>
<td>Results for wave height and free surface shape compared between Smoothed particle hydrodynamics (SPH) solution and high speed camera images.</td>
<td>Rectangular</td>
</tr>
<tr>
<td>Akyildiz and Unal (2005)</td>
<td>Experimental</td>
<td>Pressure distribution during fluid sloshing (close to Natural Frequency) in a partially filled container investigated using strategically placed pressure transducers.</td>
<td>Rectangular</td>
</tr>
<tr>
<td>Author(Year)</td>
<td>Study Time</td>
<td>Description</td>
<td>Geometries Investigated</td>
</tr>
<tr>
<td>-------------------</td>
<td>---------------------</td>
<td>-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
<td>-------------------------</td>
</tr>
<tr>
<td>Gavrilyuk et al. (2005)</td>
<td>Analytical</td>
<td>Moiseyev asymptotics theory used to study linear and non-linear mechanical models describing the free surface during sloshing in a curvilinear coordinate system</td>
<td>Conical</td>
</tr>
<tr>
<td>Delorme et al. (2005)</td>
<td>Numerical</td>
<td>Dynamic loads, velocity vectors and Wall forces during sloshing simulated using smoothed particle hydrodynamics.</td>
<td>Rectangular</td>
</tr>
<tr>
<td>Chen et al.(2009)</td>
<td>Numerical and Experimental</td>
<td>Sloshing numerically investigated using Level-set method coupled with RANS equations. Results compared to a pressure based experimental study.</td>
<td>Rectangular</td>
</tr>
</tbody>
</table>
2.2 Particle Image Velocimetry (PIV)

Particle Image Velocimetry is an optical flow visualization technique that utilizes tracer particles suspended within a fluid to calculate velocities. During a PIV experiment the light of the high power laser sheet (Figure 2-4a) is dispersed by the tracer particles as it passes through the fluid medium. This dispersed light is the signal captured by the camera, stored as images. Two such consecutive images (separated by a small window of time) are then examined by first dividing them into a number of interrogation regions and then determining displacement and velocities (Figure 2-4b) of the regions.

There has been extensive research done in the field of Particle Image Velocimetry. Adrian (1991) made an effort to collectively describe the method and important modes of operation. Adrian’s paper discusses a number of non-invasive techniques to analyze fluid flow. Adrian introduces Pulsed Laser Velocimetry and then moves on to the technique of PIV to explain its fundamentals and experimental setup. At the time Adrian assessed that using the basic two – dimensional PIV, one can accurately predict the instantaneous fields in flows of the order of only millimeters per second.
Huang et al. (1997) commented on the errors of Digital PIV and claimed that for a 300,000 displacements within an interrogation area size of 21 X 21 pixels and an image size of 768 X 480 pixels the RMS error recorded was 0.0044 pixels. The authors noted that the error was largely attributed to CCD camera noise and resolution. It may be noted that with improved resolution cross-correlation accuracy increases greatly.

2.3 Planar Laser-Induced Fluorescence (PLIF)

Planar Laser Induced Fluorescence is a flow visualization technique that uses fluorescence intensity data to compute the local concentration in a flow field. Fluorescing dye molecules are subjected to a high powered laser that excites the dye causing the molecule to fluoresce. An optical filter is used to filter the light collected by camera so that emission wavelength is visible and can be captured by the camera. Multiple frames these images are then co-related to analyze concentration distribution within the flow field. Figure 2-5 below illustrates a schematic of the technique.

![Figure 2-5: PLIF Rig Setup (www.wikipedia.com)](image-url)
The PLIF technique captures images containing planar data intensity data using a CMOS camera. In theory, the intensity of this signal depends upon a number of factors including concentration of dye, laser power, image depth and exposure among others. The use of PLIF as a reliable and accurate tool to measure concentration changes within a flow has been limited until recently. This maybe largely attributed to the lack of computational resources available to read and track concentration changes accurately.

Law and Wang (2000) used Beer-Lambert law to evaluate the concentration within a cell after eliminating effects of laser attenuation and absorption. This technique was also used by Muhlfriedel and Baumann (2000), who furthered the technique to calculate the mass transfer of Rhodamine -B across an interface between two partial immiscible liquids. Muhlfriedel and Baumann’s study showed that this technique can be used to calculate mass transport over fluid-fluid boundary. The study however did not quantify the local concentration data per pixel. Fall et al. (2001) developed statistical methods to study fluorescent concentration change within a steadily fed vessel.

Brown et al.(2004) used PLIF for accurate concentration based mixing analysis of two fluids. Brown proved that images of a fluorescent dye such as Rhodamine can be used to deduce accurate instantaneous concentration of the dye. Grayscale image intensities could be calibrated based on a known concentration.

Golnabi (2006) furthered the method to precisely analyze CCD images for PLIF images. The method analyzed PLIF images of flow system using Matlab. The images captured by the camera in JPEG or TIFF formats can be directly read by the Matlab program to provide intensity and color map matrices. Further, the program enables precise signal calculation in fluorescing cells within the region of interest. An important technique highlighted by Golnabi is cell count accumulation along rows and columns to calculate intensity fluctuation from image to image.

Neal et al.(2008) attempted to develop mixing performance criteria for a vessel with a rising free-surface. Neal’s study focused on implementing PLIF for visualization of mixing dynamics in a vessel being filled by a fluorescent pouring out a ‘fill pipe’. Neal developed a code that could track tracer concentration in a region of interest within the images acquired by the CCD
camera which then was used to analyze the overall performance of the system and develop a criterion for mixing.

In reference to the problem at hand, methods used by Law and Wang, Golnabi, Brown et al, Neal et al. and Korczyk et al. can directly be used to physically track the dissolution of a solute within a solvent in a situation where the each image taken during a dissolution sequence can be directly normalized against an image of the well containing a fully dissolved mixture. However, since the well is continuously moving during this process, a technique to relate images taken during various spatial positions needs to be employed to accurately quantify mixing using these techniques.

2.4 Simultaneous PIV and PLIF

PIV and PLIF are closely related techniques and have generally have similar experimental set ups. This has inspired a number of attempts (Borg et al., 2001, Law and Wang, 2000) to combine the two for synchronized analysis of various flows. The previously discussed paper by Law and Wang (2000) was a pioneering effort in the technique of conducting PIV as well as PLIF on a desired flow field simultaneously. Essentially, the authors have tried to combine the two systems into one by synchronizing the PIV and PLIF camera so that the images taken by the two cameras are over coinciding intervals.

An important aspect of the experiment highlighted by the authors (Law and Wang) is the seeding and dyeing of the fluid being analyzed and the experimental setup. It can be understood that PIV and PLIF are two processes that would effectively reduce the accuracy of each other if they are conducted at the same time, the concentration of the seed particles as well as the dye must be controlled so as to give suitable results for both process.

The system is tested for gathering flow field data of a turbulent jet stream mixing into stagnant fluid. For this purpose the authors seeded both the turbulent and the stagnant media with PIV particles of the same concentration and dyed the jet stream to get an understandable visualization of the process. It is found that the combined PIV and PLIF system can obtain mass transport characteristics for the mixing process satisfactorily and the possibility of error due to interference
of particles between the systems can be limited by optimization of dye and particle concentration.

However the authors do agree on the limitations of the process and attribute them to the fact that both processes limit the accuracy of the results that can be obtained from one and the other.
<table>
<thead>
<tr>
<th>Author (Year)</th>
<th>PIV</th>
<th>PLIF</th>
<th>Simultaneous PIV/PLIF</th>
<th>Application</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adrain (1991)</td>
<td>PIV</td>
<td></td>
<td></td>
<td>A review of effort in the field of PIV and other optical measurement techniques.</td>
</tr>
<tr>
<td>Northrup et al. (1991)</td>
<td>PIV with fluorescent particles</td>
<td>-</td>
<td>-</td>
<td>Measurement of Blood Flow</td>
</tr>
<tr>
<td>Huang et al. (1997)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Muhlfriedel and Baumann (2000)</td>
<td>Digital PIV</td>
<td>PLIF using Rhodamine B and a Nd-Yag Laser</td>
<td>PIV and PLIF systems were run simultaneously and measurements correlated</td>
<td>Mass-transfer across liquid-liquid boundaries between two immiscible fluids</td>
</tr>
<tr>
<td>Law and Wang (2000)</td>
<td>Digital PIV</td>
<td>PLIF using Rhodamine B. Images were calibrated to</td>
<td>Simultaneous PIV and PLIF systems used to capture turbulent mass-transfer characteristics</td>
<td>Turbulent jet experiments</td>
</tr>
<tr>
<td>Borg et al. (2001)</td>
<td>Digital PIV</td>
<td>PLIF using Rhodamine B and a Nd-Yag laser</td>
<td>Simultaneous PIV and PLIF systems used to interpolate concentration and velocity measurements at the same spatial locations. These were used to correlate velocity and concentration fluctuations</td>
<td>Turbulent jet experiments</td>
</tr>
<tr>
<td>Author (Year)</td>
<td>PIV</td>
<td>PLIF</td>
<td>Simultaneous PIV/PLIF</td>
<td>Application</td>
</tr>
<tr>
<td>--------------------</td>
<td>-----</td>
<td>------------------------------------------------</td>
<td>-----------------------</td>
<td>--------------------------------------------------</td>
</tr>
<tr>
<td>Fall et al. (2001)</td>
<td>-</td>
<td>PLIF used to calculate Reduced mean concentration, Mean mixing index and variation in Mean mixing index</td>
<td>-</td>
<td>Characterization of mixing in a stirred tank</td>
</tr>
<tr>
<td>Brown et al. (2004)</td>
<td>-</td>
<td>PLIF</td>
<td>-</td>
<td>Developed statistical methods to study concentration change in a steadily fed vessel</td>
</tr>
<tr>
<td>Korczyk et al. (2006)</td>
<td>Digital PIV used to generate velocity fields and velocity distribution histograms</td>
<td>-</td>
<td>Mixing of Cloud and Clear air</td>
<td></td>
</tr>
<tr>
<td>Golnabi (2006)</td>
<td>-</td>
<td>CCD images analyzed pixel-by-pixel using Matlab to accurate estimate concentration changes</td>
<td>-</td>
<td>Analysis of Dye covered sections in a flow loop</td>
</tr>
<tr>
<td>Author (Year)</td>
<td>PIV</td>
<td>PLIF</td>
<td>Simultaneous PIV/PLIF</td>
<td>Application</td>
</tr>
<tr>
<td>---------------------</td>
<td>-----</td>
<td>------------------------------------------------</td>
<td>-----------------------</td>
<td>---------------------------</td>
</tr>
<tr>
<td>Neal et al. (2008)</td>
<td>-</td>
<td>PLIF images processed using previously</td>
<td></td>
<td>Mixing within vessel</td>
</tr>
<tr>
<td></td>
<td></td>
<td>established method and then used to</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>characterize degree of mixing</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Takahashi et al. (2008)</td>
<td>-</td>
<td>PLIF data used to identify density</td>
<td></td>
<td>Supersonic mixing</td>
</tr>
<tr>
<td></td>
<td></td>
<td>distribution and a number of cross-sections</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>within the flow field</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
2.5 Significance

At current stage of research, scholars have used PIV to analyze the flow field in the case of self induced sloshing. The PLIF technique has also been used by researchers to estimate concentration fields and mass transfer. However, there are still significant gaps in the understanding of convective mixing caused as a result sloshing. Furthermore, no such effort has been made to study or quantify the cause and effects of primary and secondary mixing within the MicroWell.

These circumstances present a unique opportunity to build on research and apply PIV to analyze fluid flow as well as PLIF for concentration studies in relation to a biomedical device. The application of both techniques will increase our knowledge of the convective effects of the jiggle and its impact on the mixing of the reagent and the assay. This study will also produce a method to accurately quantify the mixing within the MicroWell which can then be applied to study the effects of other parameters such as fluid volume and injection height.

It is hoped that this knowledge will help the future design and development of assays and analyzers aiming to improve performance and disease detection.
Chapter 3: Experimental Setup

3.1 Rig Setup and Index Matching

Optical accessibility and Acrylic Well: The construction of the analyzer makes the MicroWell inaccessible to the laser or the camera. To overcome this, a number of components that do not contribute directly to the mixing process but block the incubator ring were removed. These components include the Sample supply, Feeder Tray, Luminometer and Sample Metering among others. Also, a major portion of the housing of the system was removed leaving the incubator ring exposed for experimentation.

However, the MicroWells are suspended within the incubator ring optical exposure of the ring still did not give access to the inside of the MicroWells. For this purpose, an optically accessible acrylic MicroWell with flat exterior surfaces was manufactured to the internal dimensions of the MicroWell produced by Ortho Clinical Devices and was installed on top of the incubator ring as shown in Figure 3-1a & Figure 3-1b below.

The accuracy of the manufactured acrylic MicroWell was checked using an optical comparator. Table 3-1 compares some of the dimensions of the acrylic MicroWell with the suggested dimensions of the MicroWell and show that the machining errors of these characteristic dimensions of the MicroWell are insignificant. Table 3-1 organizes these dimensions according to dimension types and points out that the magnitude of errors in each dimensions are related to the dimension type. Maximum error (2.32%) was identified in the spherical radius at the bottom of the MicroWell.
Table 3-1: Comparison of suggested dimension of the MicroWell to the dimensions of the acrylic MicroWell

<table>
<thead>
<tr>
<th>Dimension Type</th>
<th>Suggested Dimension (in)</th>
<th>Acrylic MicroWell Dimension (in)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diameter</td>
<td>0.291</td>
<td>0.291</td>
</tr>
<tr>
<td>Diameter</td>
<td>0.280</td>
<td>0.280</td>
</tr>
<tr>
<td>Diameter</td>
<td>0.199</td>
<td>0.199</td>
</tr>
<tr>
<td>Diameter</td>
<td>0.164</td>
<td>0.164</td>
</tr>
<tr>
<td>Spherical Radius</td>
<td>0.088</td>
<td>0.0861</td>
</tr>
<tr>
<td>Depth</td>
<td>0.078</td>
<td>0.077</td>
</tr>
<tr>
<td>Depth</td>
<td>0.361</td>
<td>0.365</td>
</tr>
</tbody>
</table>

(a) CAD of the Acrylic MicroWell atop the incubator ring

(b) Acrylic MicroWell atop the incubator ring
Laser and CCD camera alignment and Sheet Generation: Both PIV and PLIF techniques require perpendicular placement of the camera capturing images and the laser ‘sheet’ illuminating the plane of interrogation. As seen in Figure 3-2, the laser beam passes through a biconcave and a spherical lens which together vertically elongate the light passing through and create a ‘light sheet’. With the aid of mirrors and kinematic mounts the laser sheet was directed such that the light sheet coincided with the vertical center plane of the MicroWell in focus. Lastly an Iris was added to the path of the laser sheet to control the height of the section the MicroWell being illuminated.

Figure 3-3 shows an overhead view of the setup and the path of the laser beam from its housing to the Acrylic MicroWell.

Figure 3-2: Laser Sheet Creation
Height mismatch of the laser sheet camera and other optics with the MicroWell was adjusted using 4 mechanical scissor jacks as illustrated by Figure 3-4 to avoid a force imbalance. The final vertically aligned arrangement can be seen in Figure 3-5.
In contrast with the straight light sheet generated by the optics, the movement of the MicroWell during the jiggle is angular with respect to the center of the incubator ring and therefore not along a straight plane. This may pose a problem as the MicroWell would potentially move in and out of the plane of the light sheet during the motion. However, since the maximum displacement of the MicroWell between two consecutive images has been calculated to be under 0.05 mm, the variation caused in the plane of focus is limited to 1% of the MicroWell diameter (approx 7.4 mm) as seen in Figure 3-6.
Fluorescent Dye and optic Filter selection for PLIF: The application of the Laser Induced Fluorescence (LIF) technique requires that a fluorescent dye that absorbs light of the wavelength produced by the laser be used. The dye absorbs the high intensity laser light to fluoresce or emit light of different wavelength. Further, to filter out the wavelength of the laser (532nm) while acquiring the signal from the dye a 532nm optical filter is used that blocks the undesired band of wavelength.

After extensive research, Sulfurhodamine –B (IUPAC: 2-(3-diethylamino-6-diethylazaniumylidene-xanthen-9-yl)-5-sulfo-benzenesulfonate) was chosen for the experiment on the basis of the properties displayed in the spectra chart shown in Figure 3-7 as well as its insensitivity towards pH dependent light absorption.

![Figure 3-7: Plot comparing the emission and absorption spectra of the dye with respect to the laser wavelength and Optic Filter (http://www.fluorophores.tugraz.at)](http://www.fluorophores.tugraz.at)

Figure 3-8a and Figure 3-8b show the application of an optical filter to filter out the absorption wavelength and allow the emission wavelength to be seen by the camera.
Refractive Index Matching: Previous research has shown that if the fluid being analyzed has a similar refractive index to that of its container, uneven refractive bending of the laser sheet used for PIV and PLIF at curved surfaces can be prevented. This calls for the creation of an ‘analog’ that matches the desired physical properties of the actual fluid as well as the refractive index of the container.

Therefore, to ensure a viscosity match (± 0.2cP) with the blood serum and reagents (2.5-3 cP) generally used in the ECi analyzer and refractive index(1.4875 ± 0.0002) match with acrylic MicroWell produced, a custom blood serum and reagent analog was developed.

Using an Ostwald viscometer and a refractometer the viscosity and refractive index of aqueous sodium iodide were measured at various concentrations (Figure 3-9). This data, when compared to the target dynamic viscosity and refractive index shows that an aqueous sodium iodide with a refractive index of 1.4875 has a dynamic viscosity within acceptable range of the target (Figure 3-10). The final results of this experiment are illustrated in Figure 3-11 which shows that by matching either the refractive index or the viscosity of the sodium iodide–water solution the other property can also be matched within good range.
Figure 3-9: Concentration versus refractive index and dynamic viscosity for aqueous sodium iodide.

Figure 3-10: Dynamic viscosity versus refractive index for aqueous sodium iodide solution.
3.2 Linux Code Manipulation

The ECi analyzer unit (Figure 3-12) provided to RIT is a Linux based system and that uses relevant Real-time Transport Protocol (RTP) commands with a limited number of parameters to run various assays. Under usual circumstances these parameters are preloaded to the script and the program calls all the devices relevant to the assay such as the incubator ring, the reagent metering, the blood serum metering and Luminometer.

Figure 3-11: Image showing the results of Index Matching

(a) Acrylic MicroWell partially filled with index matched 
    Analog

(b) Acrylic MicroWell completely filled with index 
    matched Analog

Figure 3-12: The Vitros ECi Analyzer

Chainani. A
Since this study is focused on analyzing only the fluid dynamics within the MicroWell during the convective mixing process, a customized script that is not only focused on running the motions of the incubator ring and the reagent metering but also enables variation of the following parameters was developed in conjunction with OCD.

1. The angular velocity of the incubator ring
2. The slot number (1-60) holding the desired MicroWell
3. The reagent injection volume
4. The number of jiggles

The script was further customized for PIV and PLIF analysis of primary and secondary mixing. Flow charts describing the procedures for both scripts are shown in Figure 3-13 & Figure 3-14. The first script only allows for selection of the number of jiggles and the slot number for a given experimental run and was used for PIV analysis of secondary mixing. The second script allows for selection of reagent volume, MicroWell volume slot number, number of jiggles for a given experimental run. This script was used for PLIF and PIV analysis of primary mixing (number of jiggles set to zero).

For PLIF analysis of secondary mixing the script was further altered to control and minimize the effects of the injection jet into the MicroWell, the injector height was lowered to within 4 mm of the acrylic MicroWell surface and the injector flow rate was reduced to a level where the reagent drops into the MicroWell primarily due to gravity. These steps ensured that little or no damage is done to the air-blood serum interface during the injection process and effects of the convective mixing process are accurately measured.
Input: MicroWell Slot No. of “jiggles”

The incubator ring rotates the optically accessible MicroWell placed on top of it from the home position to the point where the injector introduces the dye to the MicroWell.

The incubator “Jiggles” the Microwell with a specified attributes and then stops back at the home position.

5V signal is outputted at each step of the motor.

Figure 3-13: Script 1 Flow Chart
Figure 3-14: PIV Script Flow Chart

Chainani, A
3.3 Synchronization

Figure 3-15 shows the path of the MicroWell during an experimental run. During an experimental run, the MicroWell starts at the “home” position, then moves in a clockwise direction to the “start-up” position, does a pre-mixing jiggle, moves counter-clockwise to the “mean” where it does five jiggles, and finally returns to the home position.

One jiggle, which is, a sequence of sudden movements characterized by 6 reversals of direction takes place in less than a third of a second under nominal speed of the stepper motor. During a jiggle, the MicroWell moves from the mean position to the right extreme position, back to the mean, then to the left extreme and then back to the mean. This sequence occurs twice for each jiggle.

To ensure repeatability of the experiment and compare between data acquired for various assays it is essential to capture images at the same spatial position of the MicroWell at every crossing during the jiggle. For this reason, the camera and the laser were “synchronized” with the movement of the MicroWell. Initially, a step-count triggering method was developed to trigger the camera and the laser but was later replaced with a voltage-level triggering system for better accuracy. Sections 3.3.1 and 0 give details of the two triggering methods.
Figure 3-15: Path of the MicroWell
3.3.1 Original Method for Synchronization: Step-Count Triggering

Figure 3-16 shows a schematic of the synchronization system initially developed for this application.

![Figure 3-16: Schematic showing the synchronization system](image)

At every step of the stepper motor, the RTP board of the analyzer outputs a 5 volt signal. The signal from the RTP board is in accordance with the trajectory of the MicroWell. Figure 3-17 illustrates the trajectory of the MicroWell during a Jiggle and the show the signals outputted by the LabVIEW program. These signals are then used by the synchronizer box to trigger the camera and laser. Each trigger from the LabVIEW program corresponds to a single image pair. These plots show that, that the system works well as long as there are no delays or discrepancy in the movement of the MicroWell. However, upon further testing temporal variation in the spatial position of the MicroWell was discovered. Figure 3-18 shows the variation in the MicroWell trajectory over eight runs. This graph was obtained by taking high speed images of the MicroWell during the eight runs. The variation causes a mismatch in step numbers and the actual position of the MicroWell further causes errors in data acquisition. To address this issue step-count triggering was replaced with voltage-level triggering.
3.3.2 **Improved Method for Synchronization: Voltage-Level Triggering**

The voltage –level triggering mechanism uses two LabVIEW programs to analyze the trajectory of the MicroWell and to trigger various spatial locations of the MicroWell. The real time position of the MicroWell during a “sample” run is traced using a Hall-effect sensor (HES) (Figure 3-19) which detects the position of a magnet attached to the acrylic MicroWell. The first program is used to capture a voltage trajectory (Figure 3-20) of the MicroWell. The second program then uses information processed using the voltage trajectory to trigger specific locations of interest in trajectory of the MicroWell.

Chainani. A
**HES Voltage Capture and Processing:** Since the “mean” position of the MicroWell shifts each time the system is started, the range, maximum and minimum voltage of the trajectory vary. A HES voltage trajectory is captured for calculation of the center location and the range of the jiggle trajectory each time the analyzer is restarted.
To analyze the effects of change in velocity of the MicroWell on the fluid within, velocity vectors before and after change in velocity must be analyzed and therefore PIV image pairs before and after a velocity change are required. However, pulsing limitations of the Nd-Yag laser limit the time between two image pairs to a minimum of approximately 0.07 s and the signaling limitation of the DAQ card being used limit the number of triggered voltage ranges to just one per run. Therefore multiple runs are needed to capture image pairs at different locations in the MicroWell trajectory. Each run can capture only two locations per jiggle.

**Triggering for Image Capture:** Five voltage bands (Figure 3-21), relative to the range of the MicroWell trajectory, are created using pre-determined bandwidth ratios that section the trajectory. The band ratios ensure correlation between points trigger after each time the machine is restarted. Transition points and transition direction of the MicroWell between the voltage bands represent eight locations of interest in the trajectory of the MicroWell. The MicroWell trajectory crosses each location ten times over the course of five jiggles (twice per jiggle). The eight locations are listed in Table 3-2.

For instance, if the Post-Center-Right location is to be triggered using the system, each time the MicroWell transitions from band 3 to band 2 a five volt pulse will be sent to the camera and the laser. This will ensure that the image is taken within one millisecond of transition between band 3 and 2 as well as that the MicroWell is moving towards the right and not the left when the image is triggered. The run would result in a total of 10 image pairs at the Post-Center-Right location over 5 jiggles.

<table>
<thead>
<tr>
<th>Location No.</th>
<th>Location Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Post-Center-Right</td>
</tr>
<tr>
<td>2.</td>
<td>Pre-Extreme-Right</td>
</tr>
<tr>
<td>3.</td>
<td>Post-Extreme-Right</td>
</tr>
<tr>
<td>4.</td>
<td>Pre-Center-Right</td>
</tr>
<tr>
<td>5.</td>
<td>Post-Center-Left</td>
</tr>
<tr>
<td>6.</td>
<td>Pre-Extreme-Left</td>
</tr>
<tr>
<td>7.</td>
<td>Post-Extreme-Left</td>
</tr>
<tr>
<td>8.</td>
<td>Pre-Center-Left</td>
</tr>
</tbody>
</table>
Figure 3-20: Voltage Trajectory of the MicroWell during five jiggles

Figure 3-21: Locations of interest (Table 3-2) shown as transition lines between voltage bands

Chainani. A
This triggering mechanism ensures that the maximum delay between the time the MicroWell reaches the desired location and the trigger to the synchronizer box is less than one millisecond. With each run, six or eight (depends upon the position) “junk” image pairs are captured that are discarded at post processing at the post-processing stage. A total of eighty image pairs are collected over eight runs to complete the PIV experiment for one assay.
Chapter 4: Image Processing Methods

4.1 PLIF Signal Optimization

Preliminary fluorescence analysis was focused on optimizing the signal captured by the camera and developing a function for dye concentration and signal intensity using various powers of the Nd-Yag laser and the Sulfurhodamine-B dye. Four laser powers and four dye concentrations were chosen for this purpose. Table 4-1 shows the details of the power and concentrations tested. The laser power was measured using a photosensitive laser meter and the intensities read in to MATLAB as TIFF images were measured on a scale of 0 to 255.

Table 4-1: Mean captured intensity for the concentration-laser power experiment

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Laser Power(mW)</th>
<th>117</th>
<th>225</th>
<th>380</th>
<th>597</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0050%</td>
<td>52</td>
<td>78</td>
<td>103</td>
<td>126</td>
<td></td>
</tr>
<tr>
<td>0.0025%</td>
<td>31</td>
<td>46</td>
<td>66</td>
<td>86</td>
<td></td>
</tr>
<tr>
<td>0.0010%</td>
<td>28</td>
<td>43</td>
<td>55</td>
<td>65</td>
<td></td>
</tr>
<tr>
<td>0.0005%</td>
<td>22</td>
<td>32</td>
<td>42</td>
<td>48</td>
<td></td>
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</tbody>
</table>

Results of the experiment showed that at the tested dye concentrations and laser powers the concentration-intensity functions for the dye were close to linear (Figure 4-1). The results (Figure 4-2) obtained were in accordance with Beer-Lambert Law and the results published by Borg et al. (2001).

![Figure 4-1: Dye concentration vs. mean pixel intensity for Laser Power of 380mW](image-url)
The above results are valuable for data processing and prove that pixel intensity levels of each pixel within an image can be linearly correlated to the local dye concentration at that point.

Further, the reagent concentration calculations were carried out so as to ensure that the final (post complete mixing) concentration of all assays was equal (0.000005 gm per ml of index matched NaI). This would consequently ensure signal optimization of all assays. Equation 2 shows the volumetric relationship of the system. Equation 3 is used to find the mass volume percentage of the dye in the reagent analog, \( mv\%_f \).

\[
V_r + V_s = V_f \quad \text{Equation 2}
\]

\[
mv\%_f \times V_f = mv\%_r \times V_r \quad \text{Equation 3}
\]

where, \( mv\%_f \) is the mass volume percentage of the dye in the final mixed assay, \( V_f \) is the total volume of the assay, \( V_r \) is the volume of the reagent analog, and \( V_s \) is the total volume of the blood serum analog. Knowing \( V_r \) and \( V_s \), \( V_f \) is found.

Table 4-2 shows details of dye concentrations used for the various assay combinations for which data was acquired. These concentrations were determined by trial and error to obtain an
acceptable signal. Figure 4-3a shows an image before optimization and Figure 4-3b shows the image after optimization.

Table 4-2: Reagent concentration

<table>
<thead>
<tr>
<th>S.No</th>
<th>Sample Volume $V_s$ (µl)</th>
<th>Reagent Volume $V_r$ (µl)</th>
<th>Total Volume $V_f$ (µl)</th>
<th>Final Conc. $m%_f$ (%)</th>
<th>Total Dye weight (g)</th>
<th>Dye conc. in reagent, $m%_r$ (%)</th>
<th>Weight (g) of dye 20ml of index matched NaI Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>80</td>
<td>20</td>
<td>100</td>
<td>0.005</td>
<td>0.0000050</td>
<td>0.0250</td>
<td>0.0050</td>
</tr>
<tr>
<td>2</td>
<td>80</td>
<td>55</td>
<td>135</td>
<td>0.005</td>
<td>0.0000067</td>
<td>0.0122</td>
<td>0.0024</td>
</tr>
<tr>
<td>3</td>
<td>25</td>
<td>50</td>
<td>75</td>
<td>0.005</td>
<td>0.0000037</td>
<td>0.0075</td>
<td>0.0015</td>
</tr>
</tbody>
</table>

a) Before intensity optimization  
b) After intensity optimization at 0.005% dye $m\%_r$ concentration

Figure 4-3: PLIF signal Optimization

4.2 PLIF Processing Techniques

To efficiently apply techniques that have been discussed, it is essential to quantify the changes in the mixing process as parameters, such as reagent volume and jiggle amplitude, are changed.

Chainani. A
Ideally, one would like to quantify a process using a single metric that is standard in various cases but there are a number of ways this process could be quantified.

In order to do so, TIFF images acquired were cropped so that the MicroWells in each image case had the same pixel alignment. A ‘gage-well’ next to the MicroWell was filled with fluorescent dye, the intensity of which was then used to detect and adjust pixel position of the MicroWell in each image. This was done by locating the outer edge of the gage well filled within each image (Figure 4-4)

![Gage Well](image)

**Figure 4-4: Gage Well**

The intensity data was then extracted from the images and normalized against a ‘baseline’ image containing intensity data of MicroWell containing uniformly mixed Sulfurhodamine B. The following equation can better help understand this, pixel-by-pixel transformation. $I^*(i,j)$ is the normalized intensity of pixel $(i,j)$ in the image being analyzed and is given by:

$$I^*(i,j) = \frac{I(i,j)}{I_b(i,j)}$$

where, $I(i,j)$ is the local intensity of pixel $(i,j)$ in the image being analyzed. $I_b(i,j)$ is the local intensity of pixel $(i,j)$ in the baseline image being used for normalization.
Since concentration of the baseline image is known (0.005%), the local concentration matrices can generated for each image. Results can be displayed as concentration contours. The MicroWell was divided into 4 regions of interest where initial examination showed unique mixing patterns. Figure 4-5 illustrates these four regions of interest. A region of interest covering the entire fluid, region 5 (Figure 4-6) was analyzed in addition to the aforementioned regions of interest.

![Figure 4-5: Regions of interest 1-4](image1)

![Figure 4-6: Region of Interest 5](image2)

Normalized intensity data obtained in various regions of interest was compared to an ‘ideal’ or constant concentration mix to quantify the process at various times. For example, in a situation where dye enters a previous ‘reagentless’ or dark region of the MicroWell, if a pixel from the sequence of images, from the region of interest is 60% as bright as its corresponding pixel in the baseline or ideally mixed image, it may be said that the pixel corresponds to 60% mixed within that region. Utilizing this conclusion, a summation of such ‘percentages of mixedness’, X% of all the pixels in a desired region (mxn) would accurately predict the percentage of mixedness within that region. This is done using the following equation.
4.3 **MicroWell Velocity Calculation for PIV**

In the case of stationary PIV systems, the container containing the fluid does not generally move and the typical PIV processing codes do not accommodate for this. However, in the case of this study, The MicroWell moves at continuously varying velocities along the horizontal axis. Therefore, to accurately isolate velocities within the MicroWell, the instantaneous velocities calculated by the PIV code must be corrected by subtracting the instantaneous velocity of the MicroWell.

The instantaneous velocity of the MicroWell can be accurately calculated by using the same PIV technique used for calculating particle velocities and then changing the interrogation cell size (from 32 pixels to 512 pixels) as illustrated by Figure 4-7a & Figure 4-7b.

\[
X\% = \left[1 - \frac{\sum_{j=1}^{m} \sum_{i=1}^{n} \text{abs}(1-I^*(i,j))}{m \times n}\right] \times 100
\]

This metric was calculated and plotted for primary and secondary mixing and diffusion for all assays.

4.4 **Binary Masking Technique for PIV**

Using the above described interrogation regions; velocities for particles within can be calculated. However, for acrylic regions, outside the MicroWell, that have no tracer particles and hence the correlation produces zero displacement and consequently zero velocity. Subtracting the velocity...
of MicroWell from these null vectors produces negative velocities for regions that should theoretically have no velocities as they are solid parts of the acrylics MicroWell.

A binary mask (Figure 4-8a) of the acrylic MicroWell (Figure 4-8b), expressing the internal parts of the MicroWell as a logic “TRUE” and the solid acrylic as a logic “FAIL”, is created. By placing this mask within a black (“FALSE”) region of pixel size 1280 X 1024 (size of images captured) according to the lateral position of the MicroWell within an image of selected image pairs, a binary mask of the internal parts of the MicroWell for the image pair can be approximated (Figure 4-8c and Figure 4-8d). Using this binary mask, a mask for the fluid within the MicroWell can be created by isolating and deleting the area above the free surface of the fluid in the selected image pair (Figure 4-8e).

Based on this binary mask, the velocity of the MicroWell is subtracted from the particle velocities for the regions that have a pixel logic “TRUE”. For regions that have a pixel logic “FALSE” null vectors replace the velocities calculated by WIDIM (Window-Displacement-Iterative-Multigrain). This masking technique allows the isolation of contours/vectors (Figure 4-8f) calculated within the MicroWell which can then be used to calculate the velocities within, while producing null vectors for the vector field covering the solid acrylic parts of the MicroWell.

a) Image of the MicroWell used to create the Binary mask
b) Binary mask of the MicroWell

Chainani. A
Figure 4-8: Binary masking technique for PIV
Chapter 5: PIV Results

5.1 Experimental Accuracy

5.1.1 Variation in MicroWell Position

Data collected for all three assay volumes for consecutive jiggles at nominal stepper motor speed was analyzed for variation in MicroWell position (pixels) and velocity to compare the accuracy of the voltage level triggering method with the previously used step-count triggering method (Appendix III). Figure 5-1 shows the actual pixel locations of the extreme positions captured using the step-count method superimposed over a typical trajectory of the MicroWell. The acquired positions in this case are not accurate and have a range of up to 200 pixels. This inaccuracy makes it hard to precisely control the positions at which data is controlled as seen in preliminary studies.

![Figure 5-1: Captured Extreme Positions using Step-Count Method](image_url)

The accuracy of the voltage level triggering mechanism depends upon two factors, the acquisition rate which would determine the temporal accuracy of the system and voltage resolution of the sensor which determines the spatial accuracy. Since the acquisition rate (LabVIEW Program) was kept under one millisecond and the HES being used has a spatial resolution of 0.01V, this system is adequate for data collection at the nominal speed of the stepper motor. Figure 5-2 confirms this as it shows minimal variation in the spatial locations of various positions (as measured by the camera) at which data has been collected for Assay 1.
Similar results were obtained for assays 2 and 3 can be found in the “Master” tables for the respective assays in Appendix IV.

![Figure 5-2: Spatial accuracy of positions captured using the voltage](image)

### 5.1.2 Variation in MicroWell Displacement

Experimental accuracy can also be quantified by analyzing the displacement of the MicroWell during the time between the two consecutive PIV images. Considering the design of the ECi system, it can be assumed that the MicroWell travels at the same speeds each time crosses specific location. Hence, image pairs triggered at the same locations should show similar displacements of MicroWell.

This hypothesis is supported by Table 5-1 which shows repeatable trends in displacement of the MicroWell from jiggle one through jiggle five for Assay one. Positive displacements value represent motion in the left direction negative displacements represent motion in the right direction. Table 5-1 also shows that the maximum variation in the displacements between images A and B of the acquired data remains under 2 pixels for all positions.
Table 5-1: MicroWell Displacements between images A and B for various positions of Assay 1

<table>
<thead>
<tr>
<th>Position Type</th>
<th>Jiggle 1</th>
<th>Jiggle 2</th>
<th>Jiggle 3</th>
<th>Jiggle 4</th>
<th>Jiggle 5</th>
<th>Max Variation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Post-Center-Left 1</td>
<td>7</td>
<td>8</td>
<td>7</td>
<td>7</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>Pre-Extreme-Left 1</td>
<td>6</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>Post-Extreme-Left 1</td>
<td>-9</td>
<td>-8</td>
<td>-8</td>
<td>-9</td>
<td>-9</td>
<td>1</td>
</tr>
<tr>
<td>Pre-Center-Left 1</td>
<td>-3</td>
<td>-3</td>
<td>-3</td>
<td>-4</td>
<td>-5</td>
<td>2</td>
</tr>
<tr>
<td>Post-Center-Right 1</td>
<td>-9</td>
<td>-9</td>
<td>-9</td>
<td>-9</td>
<td>-9</td>
<td>0</td>
</tr>
<tr>
<td>Pre-Extreme-Right 1</td>
<td>-5</td>
<td>-5</td>
<td>-5</td>
<td>-5</td>
<td>-5</td>
<td>0</td>
</tr>
<tr>
<td>Post-Extreme-Right 1</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Pre-Center-Right 1</td>
<td>3</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>Post-Center-Left 2</td>
<td>8</td>
<td>7</td>
<td>7</td>
<td>8</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>Pre-Extreme-Left 2</td>
<td>5</td>
<td>4</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>Post-Extreme-Left 2</td>
<td>-8</td>
<td>-7</td>
<td>-8</td>
<td>-8</td>
<td>-8</td>
<td>1</td>
</tr>
<tr>
<td>Pre-Center-Left 2</td>
<td>-3</td>
<td>-3</td>
<td>-3</td>
<td>-4</td>
<td>-3</td>
<td>1</td>
</tr>
<tr>
<td>Post-Center-Right 2</td>
<td>-6</td>
<td>-4</td>
<td>-5</td>
<td>-5</td>
<td>-6</td>
<td>2</td>
</tr>
<tr>
<td>Pre-Extreme-Right 2</td>
<td>-2</td>
<td>-2</td>
<td>-3</td>
<td>-2</td>
<td>-2</td>
<td>1</td>
</tr>
<tr>
<td>Post-Extreme-Right 2</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td>Pre-Center-Right 2</td>
<td>3</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>4</td>
<td>2</td>
</tr>
</tbody>
</table>

5.1.3 Effect of Number of Jiggles on Velocity Vectors

The ECi analyzer implements convective mixing in a way such that in a multiple “jiggle” assay sequence there is a pause after each “jiggle”. After a “jiggle” the MicroWell heads to the “home position” where it pauses and then heads back to the jiggle position to continue with the next jiggle. To understand the effects of this pause on the velocity field within the MicroWell, velocity vectors for all five jiggles at all eight position types were compared. From this it was observed that the general shape of velocity field for a given position type remained unchanged. Also, there is no increasing trend in the momentum within the fluid as the velocity magnitudes and direction relative to various regions of the MicroWell stay consistent in almost all jiggles.

This observation is supported by Figure 5-3, which shows the velocity vectors at the Post-Center-Left positions for jiggles 1-5 for assay 1. These velocity vectors show that there are two general directions of the particle velocity along the Y-axis. Fluid in the top right corner of the MicroWell is moving in a downward direction whereas fluid in the top left corner is rising. This imbalance in the magnitude and direction of velocities in the fluid causes a deformation in the
free surface as the MicroWell continues to move toward the left extreme. It may be noted that the magnitude of the velocities along the Y-axis for jiggle 3 is slightly lower than the magnitude in all the other jiggles. This may have been as a result of the occasional inaccuracy in the speed of the MicroWell which may result in a slight loss of momentum within the fluid.

It is clear that the above phenomenon is repeated for five consecutive jiggles. Since the velocity profile is repeatable with magnitudes in several regions in MicroWell within the same range for each jiggle, it can be inferred that the pause between jiggles ensures that there is little or no momentum carried over from one jiggle to the next and that the momentum generated with each jiggle is responsible for the flow direction during that jiggle only.

Similar comparison can be made for Pre-Extreme-Left and Pre-Extreme-Right positions as shown in Figure 5-4 and Figure 5-5 for the 1st and 5th jiggles respectively. Both velocities in the X and Y direction have been contoured.
Figure 5-3: Velocity Vectors for Post-Center-Left positions for Assay 1 showing the MicroWell accelerating towards the left (contours scaled to velocity along the Y-axis)
Figure 5-4: Velocity Vectors for Pre-Extreme-Left positions for Assay 1 showing the MicroWell decelerating towards the Left
Figure 5-5: Velocity Vectors for Pre-Extreme-Right positions for Assay 1 showing the MicroWell decelerating towards the Right
5.2 Primary Mixing

To understand the convective effects of the reagent injection on the overall mixing within the MicroWell, PIV data was collected and analyzed for primary mixing while altering the parameters listed below:

1. **Reagent to Sample Volume:** The reagent and sample volumes were varied in accordance with the assay ratios generally used for immunodiagnostic tests. The assays tested are listed in Table 5-2

<table>
<thead>
<tr>
<th>Assay No.</th>
<th>Sample (µl)</th>
<th>Reagent A (µl)</th>
<th>Reagent Volume %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>80</td>
<td>20</td>
<td>20.000</td>
</tr>
<tr>
<td>2</td>
<td>80</td>
<td>55</td>
<td>40.741</td>
</tr>
<tr>
<td>3</td>
<td>25</td>
<td>50</td>
<td>66.667</td>
</tr>
</tbody>
</table>

2. **Injection Speed:** The ECi analyzer is equipped with an injector capable of injecting fluid into the MicroWell in the range of approximately 85-210µl/sec (1000-2500 steps/sec). To analyze the effect of this range of injection speeds three injector speeds were analyzed. These speeds are 1500, 2000 and 2500 steps/second.

3. **Injection Height:** The ECi Analyzer system injects the reagent at a default height of 1.2 cm above the brim of the MicroWell. Initial experiments showed that injecting the reagent at this height produced vigorous primary mixing further producing large instantaneous velocities for PIV measurements and demonstrating almost instantaneous mixing for the PLIF analysis. This coupled with the previously discussed pulsing limitations of the Nd-Yag laser restricted the application of both optical techniques for measurements of secondary mixing. However, by lowering it closer to the brim of the MicroWell for all secondary mixing tests, various parameters such as number of jiggles were isolated. Nonetheless the effects of the injector height were isolated and then quantified by analyzing primary mixing within the MicroWell at original height (1.2 cm) of the injector as well as the lowered height (0.1 cm).
Using the above parameters, experiments listed in Table 5-3 were conducted. Since primary mixing requires no movement of the MicroWell, synchronization of the movements of the MicroWell to the laser was not applicable. Instead, just one trigger from the previously discussed synchronization system was used to trigger the laser and camera as the MicroWell reached the mean position.

<table>
<thead>
<tr>
<th>Data Sets collected for nominal injector height</th>
<th>Injector speed(steps/second)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assay No.</td>
<td>1500</td>
</tr>
<tr>
<td>1</td>
<td>X</td>
</tr>
<tr>
<td>2</td>
<td>X</td>
</tr>
<tr>
<td>3</td>
<td>X</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Data Sets collected for lowered injector height</th>
<th>Injector speed(steps/second)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assay No.</td>
<td>1500</td>
</tr>
<tr>
<td>1</td>
<td>X</td>
</tr>
<tr>
<td>2</td>
<td>X</td>
</tr>
<tr>
<td>3</td>
<td>X</td>
</tr>
</tbody>
</table>

5.2.1 Effect of Reagent and Sample Volume

The effect of the reagent and sample volume can be better understood with the help of Figure 5-6 which compares the three assay ratios side by side. Velocity vectors are shown for all three assay ratios that represent the flow field at 0.069 and 0.276 seconds after injection. The vectors show that with increase in the reagent volume there is an increase in the region of initial disturbance (Time = 0.069 seconds) within the fluid. The comparison of vectors of assays 1 and 2 specifically shows this trend as the reagent volume changes from 20 µl to 55 µl between the two assays while the sample volume remains constant at 80 µl. Along the same line, the comparison of vectors for assays 2 and 3 show that a decrease in the sample volume while keeping the reagent volume the same generates velocities of the same magnitudes as Assay 3.

Velocity vectors calculated 0.276 seconds after injection show a decrease in the magnitude of velocities in all regions in all assays. At this point it is clear that many regions of the fluid are not excited even if the reagent to sample ratio is increased to 2:1.
Figure 5-6: Vectors showing the flow field within the MicroWell after the injection (Assays 1, 2 & 3, Nominal Injection height, Injector Speeds: 2000 steps/sec)
5.2.2 Effect of Injection Speed

Figure 5-7 compares the vertical velocities produced within the MicroWell during primary mixing of assay 1 at injector speeds of 1500, 2000, and 2500 steps/sec respectively (lowered injection height). The figure shows velocity vectors have been calculated 0.069 sec and 0.276 seconds after the reagent is injected into the MicroWell. The first set of vector fields (0.069 sec) shows that with increase in injection speed, from 1500 to 2500 steps/sec, there is a significant increase initial velocities within the MicroWell as well as the region of the MicroWell stimulated by primary mixing as well.

Figure 5-7 also shows that the reagent dropped in the MicroWell displaces a large part of the fluid initially. However, this agitation is concentrated in the top-center region of the MicroWell and quickly begins to die down with time. Vector fields calculated for all three injection speeds at 0.276 sec show a slow vortex like formations with vertical velocity magnitudes in the range of -0.005 – 0.01 m/s. Vector fields taken after this point in time show vertical velocities much lower in magnitudes and a clear disorientation of flow direction. It can be assumed that after 0.69 sec mixing within the MicroWell may no longer be an effect of the reagent injection.

Further, with increase in the reagent volume, there is an increase in the velocity and stimulated regions for all three injection speeds as seen in Figure 5-8. Figure 5-8 also shows that at the higher reagent volume of 55 ml, there is an increase in the initial velocities caused within the MicroWell in relation to increase in injection speed.

Some image pairs in Figure 5-7 and Figure 5-8 also show a large region of distortion in the image caused by the light reflected from the “unstable” free surface broken by the reagent as it is injected into the MicroWell. It may be noted that the calculated particle velocities in these regions of high intensities may have some error.

At the nominal injection height, the initial velocities produced in the MicroWell are largely a result of the accelerated fluid deforming the fluid and seem to depend upon the height of the injector and the injection volume instead of the injection speed. Therefore no observable trends in the velocity field were found while varying this parameter at the nominal injection height.
Figure 5-7: Vectors showing the flow field within the MicroWell after the injection (Assay 1, Nominal Injection height, Injector Speeds: 1500 2000 & 2500 steps/sec)
Figure 5-8: Vectors showing the flow field within the MicroWell after the injection (Assay 2, Nominal Injection height, Injector Speeds: 1500 2000 & 2500 steps/sec)
5.2.3 Effect of Injector Height

Figure 5-9, Figure 5-10 and Figure 5-11 show the velocity fields after reagent injection from nominal injector height for assays 1, 2 and respectively. The figures show velocity vectors spaced apart by similar time periods seen in section 5.2.1. The vectors shown in Figure 5-9 once again show high velocities right after the injection which damp out as time goes by. An important difference however between the previously discussed velocity vectors and the ones seen in Figure 5-9, Figure 5-10 and Figure 5-11 is the magnitude of the velocities generated which is almost six times higher in first 0.069 seconds for the nominal injection height as compared to the velocities produced at the lowered injection height.

It appears that injecting the reagent at the nominal height of injection invigorates almost all regions of the fluid within the MicroWell while deforming the free surface of the fluid enough that even particles at the bottom of the MicroWell are significantly displaced.

Vector fields displayed in Figure 5-10 and Figure 5-11 show unique patterns initially which may be attributed to the change in the volume of the reagent injected into the MicroWell in both cases and the uncertainty in the exact region of the free surface most disturbed during the injection process. Nonetheless, as the flow is taken over by diffusive effects as time goes by, a repeatable flow direction seems to emerge in all three assays. Decremented circulation around the central region of the MicroWell is assumed 0.276 seconds after injection in all three cases.

It can now be said that although the convective mixing caused by the injection process is significant at the nominal injection height, it is only instantaneous and therefore may not be able to completely mix the reagent and the sample. Further, larger displacements are only produced in the central region of the MicroWell which makes it difficult for mixing to occur in other parts of the MicroWell.
Figure 5-9: Velocity Vectors showing the flow field within the MicroWell after the injection (Assay 1, Nominal Injection height, Injector Speed: 2000 steps/sec)
Figure 5-10: Velocity Vectors showing the flow field within the MicroWell after the injection (Assay 2, Nominal Injection height, Injector Speed: 2000 steps/sec)
Figure 5-11: Velocity Vectors showing the flow field within the MicroWell after the injection (Assay 3, Nominal Injection height, Injector Speed: 2000 steps/sec)
5.3 Secondary Mixing

PIV data was collected for the following assay volumes \((V_f)\) to analyze the flow field within the MicroWell during secondary mixing.

<table>
<thead>
<tr>
<th>Assay Name</th>
<th>Total Volume, (V_f) (µl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assay 1</td>
<td>100</td>
</tr>
<tr>
<td>Assay 2</td>
<td>135</td>
</tr>
<tr>
<td>Assay 3</td>
<td>75</td>
</tr>
</tbody>
</table>

Figure 5-12 shows velocity vectors for the first four positions listed in Table 5-1 from jiggle 1 during the secondary mixing of Assay 1. The vectors have been contoured to show the vertical (Y) component of Velocity. First vector field (Post-Center-Right-1) is generated as the MicroWell leaves its mean position and starts accelerating towards the right extreme position and therefore shows that the general direction of the flow is towards the left. The magnitude of this vector field also shows a pattern in the Y-component of velocity that can be correlated to the images captured for the position. The images show that the free surface in the left half of the well is moving in an upward direction and the free surface on the right side moving downward. This is in agreement with the Y-velocity vectors for this position which show significant upward and downward velocities for the top left and right regions of the fluid respectively. The vertical component of velocity shows a significant loss in magnitude to 0 m/s as we move down to the bottom region of the MicroWell.

The next image pair analyzed is at the Pre-Extreme-Right location. At this location, the MicroWell is still moving toward the right extreme position but is decelerating causing the fluid furthest away from the right wall of the MicroWell to move downward. As the MicroWell moves past the Post-Extreme-Right location, it is moving leftward causing the inertia driven flow within the MicroWell to move right. As the MicroWell reaches the Pre-Center-Right location, it starts slowing down. This velocity transition is evident in the fact the free surface of fluid is now
leveling and fluid closest to the free surface is moving in a direction so as to balance it back from its orientation at the previous location.

As the MicroWell moves further to the left (Figure 5-13) and on the Post-Center-Left location, the fluid within the MicroWell moves towards the right and deforms the free surface as it climbs up the right wall of the MicroWell. At the Pre-Extreme-Left location, the MicroWell is once again decelerating to change direction and this causes a change in the Y-component of the velocity vectors at this location. Since the MicroWell is no longer accelerating towards the left the fluid in the top right region starts moving downward to once again balance the free surface before there is a rightward change in direction.

At the Post-Extreme-Left location, the vectors show the fluid within the MicroWell moving to the left while the free surface of the fluid is tilted right as MicroWell accelerates to the Mean position. Finally, at the Pre-Center-Left location the fluid particles in the top left and right region move in against each other so as to balance the free surface as the MicroWell slows down and approaches the mean position.

This sequence of moves is repeated twice per jiggle after which the MicroWell briefly pauses at the “home” position. This pause causes a loss in momentum within the fluid which can be confirmed as there is no incremental gain in the magnitudes of velocity within the MicroWell from one jiggle to another.

Figure 5-14 through Figure 5-17 show velocity vectors at the various locations of interest for the secondary mixing of assays 2 and 3 respectively. For assay 2 velocity magnitude especially in the top right and left regions are considerably higher than those for Assay 1. This can be attributed to the higher volume of assay 2. In the case of assay 3, the velocity magnitude decreases with a decrease in the total volume of fluid within the MicroWell.
Figure 5.12: Velocity fields for positions 1-4 (jiggle 1) for Assay 1 being mixed at the nominal speed of the stepper motor.
Figure 5-13: Velocity fields for positions 5-8(jiggle 1) for Assay 1 being mixed at the nominal speed of the stepper motor
Figure 5-14: Velocity fields for positions 1-4 (jiggle 1) for Assay 2 being mixed at the nominal speed of the stepper motor
Figure 5-15: Velocity fields for positions 5-8(jiggle 1) for Assay 2 being mixed at the nominal speed of the stepper motor.
Figure 5-16: Velocity fields for positions 1-4 (jiggle 1) for Assay 3 being mixed at the nominal speed of the stepper motor.
Figure 5-17: Velocity fields for positions 5-8 (jiggle 1) for Assay 3 being mixed at the nominal speed of the stepper motor
Chapter 6: PLIF Results

6.1 Primary Mixing

In conjunction with the PIV, PLIF data was also taken while varying the parameters of reagent to sample ratio, injection speed and injection height. Table 6-1 lists the PLIF tests conducted to analyze primary mixing in the MicroWell. Sections 6.1.1 and 6.1.2 discuss the effects of the variation of the various parameters on the mixing within the MicroWell.

Table 6-1: PLIF Experiments for Primary mixing analysis

<table>
<thead>
<tr>
<th>Assay No.</th>
<th>Injector speed(steps/second)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1500</td>
</tr>
<tr>
<td>1</td>
<td>X</td>
</tr>
<tr>
<td>2</td>
<td>X</td>
</tr>
<tr>
<td>3</td>
<td>X</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Assay No.</th>
<th>Injector speed(steps/second)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1500</td>
</tr>
<tr>
<td>1</td>
<td>X</td>
</tr>
<tr>
<td>2</td>
<td>X</td>
</tr>
<tr>
<td>3</td>
<td>X</td>
</tr>
</tbody>
</table>

6.1.1 Effect of Injection Speed

To analyze the effect of variation in injection speed, reagent analog (index matched NaI mixed with fluorescent dye) was introduced into the MicroWell at three injection speeds for both the nominal and the lowered injection height. Figure 6-1 visually compares the acquired PLIF data for the primary mixing of Assay 1 at the three injection speeds (lowered injection height). The PLIF images shown in Figure 6-1 were captured at 0.07, 0.7 and 1.4 s after injection and visually show the effect of the injection speed on the primary mixing at the lowered injection height.
(a) PLIF image 0.07s after injection (Injection Speed = 1500)

(b) PLIF image 0.07s after injection (Injection Speed = 2000)

(c) PLIF image 0.07s after injection (Injection Speed = 2500)

(d) PLIF image 0.7s after injection (Injection Speed = 1500)

(e) PLIF image 0.7s after injection (Injection Speed = 2000)

(f) PLIF image 0.7s after injection (Injection Speed = 2000)
In all three cases, the dye breaks the free surface of the fluid in the MicroWell and then is decelerated by viscous forces as it gets closer to the lower surface of the MicroWell. This causes the mixing in regions that fall in the path of the dye while other regions that are further away from the path are relatively unaffected. As the injection speed is changed from 1500 steps/sec to 2000 steps/sec, the initial momentum of the reagent analog increases which causes air bubbles as dye breaks the free surface.

Figure 6-2 shows the change in percentage of mixedness within the entire volume of assay 1 at injection speeds of 2500, 2000 and 1500 steps/sec. The figure shows that at the lowered injection height, increasing the injection speed from 1500 to 2500 steps per second improves the overall mixing of the assay only by about 7%. Although the final mixing percentages of assay 2 and 3 (Figure 6-3 & Figure 6-4) are incrementally higher than the final mixing percentage Assay 1 (for all injection speeds), it can be said that increasing the injection speed improves the overall mixing similarly (approx. 11% and 13% respectively).
Figure 6-2: Change in the percentage of mixedness during primary mixing of assay 1 at injection speeds of 1500, 2000 & 2500 steps/sec

Figure 6-3: Change in the percentage of mixedness during primary mixing of assay 2 at injection speeds of 1500, 2000 & 2500 steps/sec
6.1.2 Effect of Reagent and Sample Volume

In accordance with the PIV results, PLIF results show that assay volume ratios significantly affect the quality of mixing within the MicroWell. Comparing the results for the three assays tested shows that mixing within the MicroWell may be proportional to the reagent to sample volume ratio.

Figure 6-5, Figure 6-6 and Figure 6-7 compare the change in mixedness of the three assays ratios at injection speeds of 1500 steps/sec, 2000 steps/sec and 2500 step/sec. The three figures confirm that the overall mixing rate increases as the reagent to sample ratio increases from 20:80, 20:55 to 50:25 in Assays 1, 2 and 3 respectively. It can therefore be derived that initial mixing within the MicroWell is related upon the amount of sample liquid displaced by the reagent and since, a high ratio combination of reagent to sample volume would result in higher displacement.

Chainani. A
of the sample volume, it would result in a higher percentage of mixedness.

Figure 6-5: Change in the percentage of mixedness during primary mixing of assays 1, 2 & 3 at an injection speed of 1500 steps/sec

Figure 6-6: Change in the percentage of mixedness during primary mixing of assays 1, 2 & 3 at an injection speed of 2000 steps/sec
Figure 6-7: Change in the percentage of mixedness during primary mixing of assays 1, 2 & 3 at an injection speed of 2500 steps/sec

6.2 Secondary Mixing

PLIF data was taken for secondary mixing of the combinations following assays combinations (Table 5-2) while running the stepper motor at nominal speed and was compared to data collected for no movement (diffusion).

<table>
<thead>
<tr>
<th>Assay No.</th>
<th>Sample (µl)</th>
<th>Reagent A (µl)</th>
<th>Reagent Volume %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>80</td>
<td>20</td>
<td>20.000</td>
</tr>
<tr>
<td>2</td>
<td>80</td>
<td>55</td>
<td>40.741</td>
</tr>
<tr>
<td>3</td>
<td>25</td>
<td>50</td>
<td>66.667</td>
</tr>
</tbody>
</table>

Figure 6-8a - Figure 6-8h show the raw data collected for assay combination one for the time course of one jiggle. The image names ‘a’ and ‘b’ denote images taken using pulse A and pulse B of the laser, respectively. The images indicate visually that the dye is not well mixed after one jiggle. Similar images were captured for all five jiggles which allowed the dye seeded reagent to further dissipate well within the MicroWell in the case of convective mixing.
Figure 6-9 and Figure 6-10 compare some of the data collected for diffusive and convective mixing of assay number 1. Figure 6-11 and Figure 6-12 compare convective mixing of assays numbers 2 and 3 respectively. From these images it can be inferred that convective mixing does promote the dissipation of the dye within the MicroWell more than diffusion. Also, that the amount of liquid present in the MicroWell has some effect on the mixing.
Figure 6-9: Diffusive Mixing of Assay No. 1

Post injection state  
Approximately 1.25 seconds after injection  
Approximately 2.5 seconds after injection

Figure 6-10: Convective Mixing Assay No. 1

Post injection state (0 jiggles)  
Approximately 1.25 seconds after injection (1.5 jiggles)  
Approximately 2.5 seconds after injection (4 jiggles)
The percentage of mixedness was calculated and plotted for both convective mixing and diffusion processes for assays 1, 2 and 3. Figure 6-13 to Figure 6-17 show plots for each of the 5 regions for assays 1-3.
Figure 6-13: Change in Percentage of Mixedness for Secondary Mixing of Assays 1, 2 & 3 (ROI 1)

Figure 6-14: Change in Percentage of Mixedness for Secondary Mixing of Assays 1, 2 & 3 (ROI 2)
Figure 6-15: Change in Percentage of Mixedness for Secondary Mixing of Assays 1, 2 & 3 (ROI 3)

Figure 6-16: Change in Percentage of Mixedness for Secondary Mixing of Assays 1, 2 & 3 (ROI 4)
It is evident that convective mixing does produce better results than diffusion. Figure 6-17, illustrates mixing for the entire MicroWell and shows that convective mixing has a much higher mixing rate than diffusion. In comparison to convective mixing, diffusion produces little or no change in the overall state of the mix during the given time period.

However, individual analysis of the four regions of interest shows that convective mixing as applied may not be very effective in some regions of the MicroWell. As seen in Figure 6-13 - Figure 6-16 the convective mixing process works better in regions 1 and 2 than in regions 3 and 4. In regions 3 and 4, the ‘mixedness’ slope even during convective mixing is shallow and therefore changes in the percentage of mixedness over time are not very large and tend to lie in close proximity to the values showed by the diffusion process. This is a remarkable observation and is in accordance with PIV results discussed previously, where velocities recorded in the lower half of the MicroWell are much lower and at times negligible as compared to the top those in the top half.

Figure 6-18 shows a plot of rate of mixing as a function of assay type, for data listed in Table 6-3 for three assays, and for each of the 5 regions. The ‘mixing rate’ in this case is defined as the linear slope between the maximum mixedness and minimum mixedness during any given sequence.
Both convective and diffusive mixing are shown for each region. It is clear from Figure 6-18 that convective mixing of the overall assay (region 5) in each of the volume ratios is more effective than diffusive mixing. Also, it is evident that mixing of assay 1 and 2 are comparable and much larger than mixing in assay 3. It may be noted, that for regions 3 and 4 (lower half) of the MicroWell, the mixedness in case of assay 3 is much larger than that of assays 1 and 2. This could be attributed to the difference in effective interface diameter (diameter of the well at the liquid air interface), which is much smaller for assay 3 than for assay 1 and 2, since the total volume of assay 3 is lower.

Table 6-3: Mixing Rates

<table>
<thead>
<tr>
<th>Test Name</th>
<th>Assay No.</th>
<th>Mixing Type</th>
<th>Zone</th>
<th>Mixing Rate (percent per second)</th>
<th>Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>058test</td>
<td>1</td>
<td>Convective</td>
<td>1</td>
<td>13.76</td>
<td>-1.53%</td>
</tr>
<tr>
<td>058test</td>
<td>1</td>
<td>Convective</td>
<td>2</td>
<td>16.54</td>
<td>-2.06%</td>
</tr>
<tr>
<td>058test</td>
<td>1</td>
<td>Convective</td>
<td>3</td>
<td>7.01</td>
<td>-0.33%</td>
</tr>
<tr>
<td>058test</td>
<td>1</td>
<td>Convective</td>
<td>4</td>
<td>6.71</td>
<td>-0.21%</td>
</tr>
<tr>
<td>058test</td>
<td>1</td>
<td>Convective</td>
<td>5</td>
<td>11.23</td>
<td>-0.86%</td>
</tr>
<tr>
<td>064test</td>
<td>1</td>
<td>Diffusive</td>
<td>1</td>
<td>4.53</td>
<td>-0.31%</td>
</tr>
<tr>
<td>064test</td>
<td>1</td>
<td>Diffusive</td>
<td>2</td>
<td>6.42</td>
<td>-1.12%</td>
</tr>
<tr>
<td>064test</td>
<td>1</td>
<td>Diffusive</td>
<td>3</td>
<td>5.28</td>
<td>-0.15%</td>
</tr>
<tr>
<td>064test</td>
<td>1</td>
<td>Diffusive</td>
<td>4</td>
<td>4.29</td>
<td>-0.03%</td>
</tr>
<tr>
<td>064test</td>
<td>1</td>
<td>Diffusive</td>
<td>5</td>
<td>5.58</td>
<td>-0.29%</td>
</tr>
<tr>
<td>065test</td>
<td>2</td>
<td>Convective</td>
<td>1</td>
<td>16.03</td>
<td>-1.33%</td>
</tr>
<tr>
<td>065test</td>
<td>2</td>
<td>Convective</td>
<td>2</td>
<td>16.09</td>
<td>-1.27%</td>
</tr>
<tr>
<td>065test</td>
<td>2</td>
<td>Convective</td>
<td>3</td>
<td>2.43</td>
<td>0.09%</td>
</tr>
<tr>
<td>065test</td>
<td>2</td>
<td>Convective</td>
<td>4</td>
<td>3.71</td>
<td>0.26%</td>
</tr>
<tr>
<td>065test</td>
<td>2</td>
<td>Convective</td>
<td>5</td>
<td>9.59</td>
<td>-0.24%</td>
</tr>
<tr>
<td>071test</td>
<td>2</td>
<td>Diffusive</td>
<td>1</td>
<td>8.06</td>
<td>-1.65%</td>
</tr>
<tr>
<td>071test</td>
<td>2</td>
<td>Diffusive</td>
<td>2</td>
<td>8.2</td>
<td>-1.24%</td>
</tr>
<tr>
<td>071test</td>
<td>2</td>
<td>Diffusive</td>
<td>3</td>
<td>2.4</td>
<td>0.18%</td>
</tr>
<tr>
<td>071test</td>
<td>2</td>
<td>Diffusive</td>
<td>4</td>
<td>2.4</td>
<td>0.23%</td>
</tr>
<tr>
<td>071test</td>
<td>2</td>
<td>Diffusive</td>
<td>5</td>
<td>3.27</td>
<td>-0.10%</td>
</tr>
<tr>
<td>072test</td>
<td>3</td>
<td>Convective</td>
<td>1</td>
<td>9.14</td>
<td>-0.87%</td>
</tr>
<tr>
<td>072test</td>
<td>3</td>
<td>Convective</td>
<td>2</td>
<td>10.28</td>
<td>-1.33%</td>
</tr>
<tr>
<td>072test</td>
<td>3</td>
<td>Convective</td>
<td>3</td>
<td>9.9</td>
<td>0.35%</td>
</tr>
<tr>
<td>Assay Test</td>
<td>Assay #</td>
<td>Assay Type</td>
<td>Region</td>
<td>Mixing Rate (Percent/s)</td>
<td>Percentage Error</td>
</tr>
<tr>
<td>-----------</td>
<td>---------</td>
<td>------------</td>
<td>--------</td>
<td>-------------------------</td>
<td>------------------</td>
</tr>
<tr>
<td>072test</td>
<td>3</td>
<td>Convective</td>
<td>4</td>
<td>9.48</td>
<td>0.62%</td>
</tr>
<tr>
<td>072test</td>
<td>3</td>
<td>Convective</td>
<td>5</td>
<td>6.08</td>
<td>-0.26%</td>
</tr>
<tr>
<td>077test</td>
<td>3</td>
<td>Diffusive</td>
<td>1</td>
<td>8.7</td>
<td>-1.13%</td>
</tr>
<tr>
<td>077test</td>
<td>3</td>
<td>Diffusive</td>
<td>2</td>
<td>7</td>
<td>-0.73%</td>
</tr>
<tr>
<td>077test</td>
<td>3</td>
<td>Diffusive</td>
<td>3</td>
<td>1.06</td>
<td>0.08%</td>
</tr>
<tr>
<td>077test</td>
<td>3</td>
<td>Diffusive</td>
<td>4</td>
<td>1.98</td>
<td>0.20%</td>
</tr>
<tr>
<td>077test</td>
<td>3</td>
<td>Diffusive</td>
<td>5</td>
<td>3.14</td>
<td>-0.13%</td>
</tr>
</tbody>
</table>

Figure 6-18: Plot Comparing Various Assays
Chapter 7: Conclusions and Limitations

In accordance with the specific aims set forth by this document, the following objectives have been achieved:

- **The development of a testing environment** that allowed optical access to the contents within the MicroWell geometry for a high speed camera and a laser sheet. This included refractive index and viscosity matching of a blood serum analog and reagent analog.
- **The application and optimization of PIV and PLIF techniques.** The study included selection of PIV particles, PLIF dye and PLIF filter and optimization of parameters such as dye/particle concentrations in blood serum analog and reagent analog, laser power, signal intensity, capture time and time between image pairs.
- **Development and Implementation of data processing techniques to accurately quantify the process.** In the case of PIV, code was used to detect MicroWell positions, MicroWell Displacement and Velocity, Fluid Velocity and then compute the velocity of the fluid relative to MicroWell walls. For PLIF, Matlab code was used to convert intensity data captured as images to concentration. This data was the used to further quantify the process using the metric of "percentage of mixedness".
- **Utilization of developed techniques and metrics to compare diffusive and convective mixing within various regions of the MicroWell.** The study found that convective mixing produced significantly better results than diffusive mixing in certain (upper) regions of the MicroWell while having limited effects in the lower regions.
- **Employment of developed techniques to analyze primary mixing as a function of time.** Primary mixing analyses showed that there was a clear relationship between the reagent to sample volume, injection speed and injection height, and the change in percentage of mixedness as time passes after initial injection. To further analyze and demonstrate these relationships, the variation in results for three assays was recorded at three injection speeds and two injection heights.
Nonetheless, the following limitations were encountered during the course of this study and have confined its accuracy:

- Data for the study has been collected using an Nd-Yag laser that allows up to microsecond time differences between image pairs. However, the laser also limits the number of images pairs captured to 14.5 images per second. This limitation allows us to only capture about 16 good image pairs in the time it takes for the MicroWell to complete on jiggle. This temporally limits the accuracy of this quantitative analysis. Therefore it is encouraged that future studies use a continuous laser which would enable data collection of up to 139 image pairs per jiggle and would therefore allow the production of a more continuous velocity field from position to position.

- The images captured in the current data sets clearly show changes in the shape of the free surface of the fluid within the MicroWell at all positions. The scope of this project has limited us from studying this part of the phenomenon in detail (literature shows that the free surface shapes can be directly related to slosh-dynamics and fluid velocities). Developing techniques to accurately visualize and analyze the free surface shape would be extremely useful in fully understanding the fluid dynamics within the MicroWell.
References


Appendix I: PIV code

File 1: JJ_Batch.m

%clear all;

if sb>0.5;
    disp('***');
else
    sequence = 'Water 1330rpm'
    Interval = 1
    startnum=1
    endnum=10
end

inprefix=['E:\PIVData\OrganizedTests\ sequence ' '\ run ' '\RawData\ run];
outprefix=['E:\PIVData\OrganizedTests\ sequence ' '\ run ' '\fim\ run];

for i = startnum:endnum;
    if i<10;
        extrazeros='00000';
    else if i<100;
        extrazeros='0000';
    else if i<1000;
        extrazeros='000';
    else if i<10000;
        extrazeros='00';
    else extrazeros='';
    end;
    Chainani. A
end;
end;
end;

infilename=[inprefix extrazeros num2str(i) '.T000.D000.P000.H001.L' 'a' ];
outfilename=[outprefix extrazeros num2str(i) 'A' ];
%
%
% %%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%% %
% %
% % Program: fim <-> tif 
% % Authors: I. Dias & F. Scarano
% % Date: 3.09.97
% % Task: Performs conversion of fim to tiff images 
% % and vice versa
% %%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%% %
%
opt=2;

choice='KO';
while strcmp(choice,'OK')~=1
    switch opt
    case 1,
        disp(' ');
        inname=input('Type the input filename (no extension): ','s');
        outname=input('Type the output filename (no extension): ','s');
        choice='OK';
    case 2,
        disp(' ');
    end
end
%
% inname=input('Type the input filename (no extension): ','s');
inname = infilename;
%
% outname=input('Type the output filename (no extension): ','s');
outname = outfilename;
%

Chainani. A
```matlab
outname=outfilename;

choice='OK';
otherwise disp(' ');
    disp('    hey amico I just asked you 1 or 2! :/ ');
    disp('    Try once again :-( ');
    disp(' ');
    opt=input('    Type option and press enter: ');
end;
end;

%----------------- FIM --> TIF CONVERSION -------------------
if opt==1
    disp(' ');
    disp('----- TIF2FIM CONVERTER -----');

% FIM IMAGE READING
    filename=[inname '.fim'];
    disp(' ');
    disp(['    Reading ' inname '.fim']);
    fid=fopen(filename);
    lixo=fread(fid,[1 12],'uchar');
    ncol=hex2dec(fread(fid,[1 4],'uchar'));
    nlin=hex2dec(fread(fid,[1 4],'uchar'));
    cc=20;
    lixo2=0;
    while lixo2~=[13
        cc=cc+1;
        lixo2=fread(fid,1,'uchar');
    end;
    matrix=fread(fid,[ncol+1 nlin],'uchar');
```

Chainani. A
matrix1=matrix(1:ncol,:);
matrix2=uint8(matrix1');
fclose(fid);
image(matrix2)
colormap(gray(256))

% WRITE TIF FILE
outname=[ outname 'tif'];
disp('');
disp([' Writing ' outname ']);
imwrite(matrix2,outname,'tif');
close;

%------------------ TIF --> FIM CONVERSION -------------------
elseif opt==2
  disp('');
disp('---- TIF2FIM CONVERTER -----');

% IMAGE READING
filename=[inname '.tif'];
disp('');
disp([' Reading ' inname '.tif']);
matrix=imread(filename);

% WRITE FIM FILE
outname=[ outname '.fim'];
numcolrow=size(matrix');
numrow=numcolrow(1);
umcol=numcolrow(2);
strlin=num2str(dec2hex(numrow));
strcol=num2str(dec2hex(numcol));
matrix1=[matrix 13*ones(numrow,1)];

if numcol<16
    zcol='000';
end
if (numcol > 16) & (numcol < 256)
    zcol='00';
end
if (numcol > 256) & (numcol < 4096)
    zcol='0';
end

if numrow<16
    zlin='000';
end
if (numrow > 16) & (numrow < 256)
    zlin='00';
end
if (numrow > 256) & (numrow < 4096)
    zlin='0';
end

disp(' '); disp([' Writing ' outname']);
 fid=fopen(eval('outname'),'w+b');
 fprintf(fid,'%s%s%s%s','LBIC00000000',zcol,strcol,zlin,strlin);
 fprintf(fid,'%107c',' ');
 fprintf(fid,'%c',13);
fwrite(fid,(double(matrix1))','uint8');
clear matrix;
clear matrix1;
fclose(fid);
end;
end;

---

File 2: Well Mask Generator

% Well Mask Generator
% Created by Amit Chainani
% For OCD ECi @ RIT
% Rochester Institute of Technology
% Department of Mechanical Engineering
%
% This Program reference a MicroWell image mask saved in the directory 'E:\PIV PROCESSING\wellmask.TIF' and image 'A' from a given image set. It then creates masks for each of the images referenced.

clc
clear all
% A = imread('E:\PIVData\OrganizedTests\repeatabilitytests\031_test\RawData\031_test000000.T000.D000.P000.H001.LA.TIF');
% BW = roipoly(A);
% s = find(BW(20,:),1,'first');
% f = find(BW(20,:),1,'last');
% wellmask = BW(:,s:f)

BW = imread('E:\PIV PROCESSING\wellmask.TIF');
l = size(BW);

Chainani. A
initial=0;
final= 15;
experiment = 'HES_data';
testname = '056_test';
inprefix=['E:\PIVData\OrganizedTests\' experiment '');testname \RawData\testname ']
outprefix = ['E:\PIVData\OrganizedTests\' experiment '');testname \WellMasks\testname ']
mkdir(['E:\PIVData\OrganizedTests\' experiment '');testname \',\'WellMasks'
for i = initial:final

if i<10;
extrazeros='00000';
else if i<100;
extrazeros='0000';
else i<1000;
extrazeros='000';
end;
end;
imagei = imread(['inprefix extrazeros num2str(i) '.T000.D000.P000.H001.LA.TIF']);
imshow(imagei)
h = impoint;
position = wait(h);
mask = zeros(size(imagei));
mask(:,position(1,1):(position(1,1)+ l(1,2)-1)) = BW;
imwrite(mask,['outprefix 'mask extrazeros ,num2str(i),'.TIF'],\TIFF');
close
end

**File 3: Super_Batch.m**

if ssb>0.5;
disp(['*** Executing Super Super Batch *** for Sequence' sequence ]); 
imageleader=sequence;
else

Chainani. A
sequence = 'test2'
    imageleader='test2'
    maxfilenumber=1 %134
startnum=1 % This should always start with 1.  FXLink starts with 0 and this corrects for that.
    interval=1
    step=1

sb=1;

endnum=maxfilenumber+1; % don't change this

% ----------------- Choose one of the following -----------------------------

%RGBtoMono
%RGBtoMonoRotate
%RGBtoMonoRotateRootDir;
%ChangeNumberVideo2

endnum=endnum-interval;

% ----------------- Choose one of the following -----------------------------
%FormatforPIVBackward
%TSI_FormatforPIV
%FormatforPIVShiftFirstLeft  % shift1
%FormatforPIVShiftSecondRight; % shift2

TSI_JJ_Batch;

sb=0;

File 4: Super_Super_Batch.m
ssb=1;

sequence = 'primarymixing';

run = '093_test';
file = run;
startnum = 0;
maxfilenumber = 79;
imageleader = run;

h=1;
step=1;
interval=1;
TSI_JJ_CreateandCopy;
TSI_JJ_SuperBatch;

ssb=0;

File 5: Case_File_gen.m

%   Case File Generator
%   Created by Amit Chainani
%   For OCD ECi @ RIT
%   Rochester Institute of Technology
%   Department of Mechanical Engineering
%   This program generates two case files (two interrogation cell
%   sizes) containing parameters for widim.
clc
clear all
first= 57;
last= 80;
for i = first:last
    testnumber = ['0' num2str(i)];
    disp(['for ', testnumber])
    experiment = 'Secondarymixing_thesis';
    testname = [testnumber '_test'];
    initial = 0;
    listing=dir(['E:\PIVData\OrganizedTests\' experiment '\' testname 'fim']);
    final = (numel(listing)-2)/2 -1;
    fid = fopen(['E:\PIV PROCESSING\widim\x1-processing\test-x1-\' testnumber,'.cas'], 'wt');
    fprintf(fid, '*****Picture_parameters*****
E:\PIVData\OrganizedTests\%s\%s\fim\%s000\n',experiment,testname,testname);
    fprintf(fid, '%i
',initial);
    fprintf(fid, '%i
',final);
    fprintf(fid, '1
1280
1
1024
1
64
64
1
1
1.00000
1.00000
1.00000
1.00000
1
1
1
16
16
1.00000
1.00000
1.00000
n
Processing_parameters
nno_raw
nno_centroid
ngaussian
no_whittaker
n3
n3
n10
n3
n2
nfft
n4
n
Validation_procedure_parameters
nfft
n
Processing_parameters
n
Output_parameters
ntecplot
auto
E:\PIVData\OrganizedTests\%s\%s\vector\%s000-x1-\n',experiment,testname,testname);
    fprintf(fid, 'no_map\n1\n1
Optional_parameters
NOT_DEFINED
ndisabled\n1\n0.00000
0.00000
n');
    fclose(fid);
    disp('Saved x1')
    clear fid;
    fid = fopen(['E:\PIV PROCESSING\widim\x2-processing\test-x2-\' testnumber,'.cas'], 'wt');
    Chainani. A
File 6: Velocity_correction.m

%   Velocity Correction
%   Created by Amit Chainani
%   For OCD ECi @ RIT
%   Rochester Institute of Technology
%   Department of Mechanical Engineering

%This program referenced vector plots for the MicroWell Velocity and

clear all
clc
for testnumber = 57:80
    initial = 0;
    experiment = 'Secondarymixing_thesis';
    testname = ['0' , num2str(testnumber) , '_test'];
    listing=dir(['E:\PIVData\OrganizedTests\' experiment '\\' testname '\\' fim']);
    final = (numel(listing)-2)/2 -1;
    inprefix1=['E:\PIVData\OrganizedTests\' experiment '\\' testname '000-x1-'];
    inprefix2=['E:\PIVData\OrganizedTests\' experiment '\\' testname '000-x2-'];
    maskprefix = ['E:\PIVData\OrganizedTests\' experiment '\\' testname 'WellMasks\' testname ];
    outprefix=['E:\PIVData\OrganizedTests\' experiment '\\' testname 'CorrectedVectors\' testname];
    outpdir=['E:\PIVData\OrganizedTests\' experiment '\\' testname 'CorrectedVectors'];
    urang=[3,2,12,2];
    mkdir(['E:\PIVData\OrganizedTests\' experiment '\\' testname ],'CorrectedVectors');
    wellwidthp = size((imread('E:\PIV PROCESSING\wellmask.TIF')),2);
    wellwidthm = (0.0254*0.2877);
    correcting
    for n = initial:final;
        if n<10;
            extrazeros='00';
        else if n<100;
            extrazeros='0';
        else n<1000;
            extrazeros='';
        end;
    end;
    pltfile(:,1:8)= dlmread([inprefix1 extrazeros num2str(n) '.plt'] ,',',3,0);
    du = mean(dlmread([inprefix2 extrazeros num2str(n) '.plt'] ,',',urang));
    [p,q]= size(pltfile);
    mask = imread([maskprefix , 'mask' , extrazeros , '000' , num2str(n) , '.TIF']);
    Chainani. A
resizedmask = imresize(mask, 1/16);  
resizedmask = (resizedmask');  
%    mask = mask';  
resizedmaskf = flipr(resizedmask);  
%    maskf = flipr(mask);  
for i = 1:p  
uvelocity((pltfile(i,1)),(pltfile(i,2)/16)) = pltfile(i,3);  
vvelocity((pltfile(i,1)/16),(pltfile(i,2)/16)) = pltfile(i,4);  
pltfile(i,5) = pltfile(i,6);  
end  
vcorr = vvelocity;  
ucorr = zeros(79,63);  
for g = 1:79  
    for h = 1:63  
        if resizedmaskf(g,h) > 0  
            ucorr(g,h) = uvelocity(g,h) - du;  
        end  
        if resizedmaskf(g,h) == 0  
            vcorr(g,h) = 0;  
        end  
    end  
end  
for i = 1:p  
    pltfile(i,6) = ucorr((pltfile(i,1)/16),(pltfile(i,2)/16));  
    pltfile(i,7) = vcorr((pltfile(i,1)/16),(pltfile(i,2)/16));  
    pltfile(i,8) = pltfile(i,6)*(wellwidthm/wellwidthp)*1000;  
    pltfile(i,9) = pltfile(i,7)*(wellwidthm/wellwidthp)*1000;  
end  

fid = fopen([outprefix 'corrected' extrazeros num2str(n) '.plt'], 'wt');
fprint(fid, 'TITLE="%s image%s"
', testname, num2str(n));
fprint(fid, 'VARIABLES="X","Y","U","V","SN","UCorr","VCorr","Umps","Vmps"
');
fprint(fid, 'ZONE T="Velocity field", I=79, J=63\r\n');

for e=1:4977;
    for f = 1:9;
        fprintf(fid, '%8.4f\t', pltfile(e,f));
    end;
    fprintf(fid, '\r\n');
end
fclose(fid);
disp(['Corrected ', num2str(n+1)));
end

declare

File 7: Fluid_mask_gen.m

% MicroWell Fluid Mask Generator for Primary Mixing
% Created by Amit Chainani
% For OCD ECi @ RIT
% Rochester Institute of Technology
% Department of Mechanical Engineering
% This Program reference a MicroWell image mask saved in the directory 'E:\PIV PROCESSING\wellmask.TIF' and image 'A' from a given image set. It then creates masks for each of the images referenced.

clear all
for testnumber = 81:89
    initial=11;
    final= 11;

Chainani. A
experiment = 'primarymixing';
testname = '081_test';
BW = imread('E:\PIV PROCESSING\wellmask.TIF');
l = size(BW);
inprefix=['E:\PIVData\OrganizedTests\' experiment ' testname ' RawData ' testname ];
outprefix = ['E:\PIVData\OrganizedTests\' experiment ' testname ' WellMasks ' testname ];
mkdir(['E:\PIVData\OrganizedTests\' experiment ' testname ' WellMasks']);
wellsize = 810;
for i = initial:final
    if i<10;
        extrazeros='00000';
    else if i<100;
        extrazeros='0000';
    else i<1000;
        extrazeros='000';
    end;
end;
imagei = imread([inprefix extrazeros num2str(i) '.T000.D000.P000.H001.LA.TIF']);
imshow(imagei)
h = impoint;
position = wait(h);
mask = zeros(size(imagei));
mask(:,position(1,1)-wellsize:(position(1,1)-wellsize+ l(1,2)-1)) = BW;
h = imfreehand;
var1 = createMask(h);
for j= 1:1024
    for k = 1:1280
        mask(1:200,k)= 0;
        if var1(j,k)== 1
            mask(j,k)=0;
        end
    end
    Chainani. A
imwrite(mask,[outprefix 'mask','.TIF','TIFF']);
close
imshow(mask);
end
end
Appendix II: PLIF code

File 1: Image_copy_rename.m

%   File Copy and Rename
%   Created by Amit Chainani
%   For OCD ECi @ RIT
%   Rochester Institute of Technology
%   Department of Mechanical Engineering
clc
clear all

% initial= input('First File Number =');
% final= input('Last File Number =');
% experiment = input('Experiment Name =','s');
% testname = input ('Test Name=','s');
for testnumber=84:84
    initial= 0;
    experiment = 'Primary_mixing';
    testname = ['0',num2str(testnumber),'_test'];
    final = 31;
    inprefix=['E:\PLIF Data\Organized Tests\' experiment ' \testname \RawData\' testname];
    outprefix=['E:\PLIF Data\Organized Tests\' experiment ' \testname \RenamedData\' testname];
    outpdir=['E:\PLIF Data\Organized Tests\' experiment ' \testname \'];
    mkdir([outpdir],'RenamedData')
    for i = initial:final;
        if i<10;
extrazeros='00000';
else if i<100;
    extrazeros='0000';
else if i<1000;
    extrazeros='000';
else if i<10000;
    extrazeros='00';
else extrazeros='';
end;
end;
end;

if i<5;
    extrazerosn='00000';
else if i<50;
    extrazerosn='0000';
else if i<500;
    extrazerosn='000';
else if i<5000;
    extrazerosn='00';
else extrazerosn='';
end;
end;
end

infilenamea=[inprefix extrazeros num2str(i) '.T000.D000.P000.H001.L' 'A.TIF' ];
outfilenamea=[outprefix extrazerosn num2str(((i+1)*2)-1) '.TIF' ];
infilenameb=[inprefix extrazeros num2str(i) '.T000.D000.P000.H001.L' 'B.TIF' ];
outfilenameb = [outprefix extrazerosn num2str(((i+1)*2)) '.TIF '];

if i == 4
    outfilenameb=[outprefix '0000' num2str(((i+1)*2)) '.TIF'];
else if i == 49
    outfilenameb=[outprefix '000' num2str(((i+1)*2)) '.TIF'];
else if i == 499
    outfilenameb=[outprefix '00' num2str(((i+1)*2)) '.TIF'];
else
    outfilenameb=[outprefix extrazerosn num2str(((i+1)*2)) '.TIF'];
end
end
end

copyfile([infilenamea],[outfilenamea]);
copyfile(infilenameb,outfilenameb);

disp 'Copied Set Number';
disp(i);

disp(1+i);
end
end

File 2: Image_select_crop.m

%  Image Crop
%  Created by Amit Chainani
%  Rochester Institute of Technology
%  Department of Mechanical Engineering

Chainani. A
The following program uses the lateral pixel position of the outer edge of the gage well and the pixel size of the MicroWell to accurately crop out the MicroWell in each image of a given set of images.

```
initial = 1;
final = 64;
experiment = 'Proposaldata';
testname = '078test';
wellsize = 520;

inprefix = [E:\PLIF Data\Organized Tests\ experiment ' testname ' RenamedData\ testname];
outprefix = [E:\PLIF Data\Organized Tests\ experiment ' testname ' CroppedData\ Cropped' testname];
outpdir = [E:\PLIF Data\Organized Tests\ experiment ' testname \];
mkdir([outpdir], 'CroppedData');

for i = initial:final;
    if i<10;
        extrazeros='00000';
    else if i<100,
        extrazeros='0000';
    else if i<1000;
        extrazeros='000';
    else if i<10000;
        extrazeros='00';
    else extrazeros='';
    end;
end;
end;

imagei = imread([inprefix extrazeros num2str(i) '.TIF']);
```

Chainani. A
figure, imshow(imagei);
position = impoint;
position = wait(position);%
position(1,1)
p = round (position(1,1));

close
welledge= p-800;
croppedimage = zeros(1024,wellsize);

for j= 1:1024
    for k= 1:wellsize
        croppedimage(j,k)= imagei(j,k+welledge);
    end
end

imwrite((croppedimage/256),([outprefix extrazeros num2str(i) '.TIF'])))
disp 'Cropped and Saved Image Number';
disp(i);
end

File 3: image_calliberation.m
%
Image Average
% Created by Amit Chainani
% For OCD ECi @ RIT
% Rochester Institute of Technology
% Department of Mechanical Engineering
% This program averages images A and B from 30 image pairs to create a
% set of average calliberation images for normalized intensities.

Chainani. A
initial= input('First File Number =');
final= input('Last File Number =');
experiment = input('Experiment Name =','s');
testname = input ('Test Name =','s');

inprefix=['E:\PLIF Data\Organized Tests\' experiment ' testname 'CroppedData\Cropped' testname];
outpdir=['E:\PLIF Data\Organized Tests\' experiment ' testname '\];
outprefixodd =['E:\PLIF Data\Organized Tests\' experiment ' testname 'AveragedData\testname 'odd.TIF'];
outprefixeven = ['E:\PLIF Data\Organized Tests\' experiment ' testname 'AveragedData\testname 'even.TIF'];
I = ((final -initial)+1)/2;

totintensity = zeros(I,2);

for i = initial:1:final;
    if i<10;
        extrazeros='00000';
    else if i<100,
        extrazeros='0000';
    else if i<1000;
        extrazeros='000';
    else if i<10000;
        extrazeros='00';
    else if i<10000;
        extrazeros='0';
    else extrazeros='';
    end;
end;
if i <=(initial+1);
    imagei=imread([inprefix extrazeros num2str(i) '.TIF ']);

    Chainani. A
if rem(i,2)== 1
imagei1 = uint16(imagei);
initialodd = imagei1;
end
if rem(i,2)== 0
imagei2 = uint16(imagei);
initialeven = imagei2;
end
end
if i > (initial+1)
    imagei = imread([inprefix extrazeros num2str(i) '.TIF']);
    if rem(i,2)== 1
        imagei1 = initialodd + uint16(imagei);
        initialodd = imagei1;
    end
    if rem(i,2)== 0
        imagei2 = initialeven + uint16(imagei);
        initialeven = imagei2;
    end
end
[m,n] = size(initialodd);
totintensity(i,1) = sum(sum(imagei))/(m*n);
disp 'Added Set Number';
disp(i);
end
mkdir([outpdir],'AveragedData')

avgoddimage = (initialodd/I);
avgevenimage = (initialeven/I);
for i=initial:1:final
    if rem(i,2)== 1
        totintensity(i,2)= sum(sum(avgoddimage))/(m*n);
        totintensitydev(i,1)= totintensity(i,1) - totintensity(i,2);
    end
    if rem(i,2)== 0
        totintensity(i,2)= sum(sum(avgevenimage))/(m*n);
        totintensitydev(i,1)= totintensity(i,1) - totintensity(i,2);
    end
end

imwrite(uint8(avgoddimage),[outprefixodd],'TIF')
imwrite(uint8(avgevenimage),[outprefixeven],'TIF')

figure, plot(totintensity)
figure, plot(totintensitydev)

File 4: Percentage_mixedness.m

% Percentage of Mixedness
% Created by Amit Chainani
% For OCD ECI @ RIT
% Rochester Institute of Technology
% Department of Mechanical Engineering
This program Normalizes Images from a selected run and then calculates the "Percentage of Mixedness" of each pixel within the image. The percentages are saved as the file ['E:\PLIF Data\Organized Tests\experiment ' testname 'Results\' testname extrazeros num2str(i)]

clc
clear all
initial= 29;
final= 150;
experiment = 'Primary_mixing';
testname = '089_test';
testnamecal = '088_test';

inprefix = ['E:\PLIF Data\Organized Tests\' experiment ' testname 'CroppedData\Cropped' testname];
outpdir = ['E:\PLIF Data\Organized Tests\' experiment ' testname 'Results'];
calimagenamea = ['E:\PLIF Data\Organized Tests\' experiment ' testnamecal 'AveragedData\' testnamecal 'odd.TIF'];
calimagenameb = ['E:\PLIF Data\Organized Tests\' experiment ' testnamecal 'AveragedData\' testnamecal 'even.TIF'];
calimagea = 1.+imread([calimagenamea]);
calimageb = 1.+imread([calimagenameb]);
mkdir([outpdir],'Results')
I= final - initial + 1;
[m,n]= size(calimagea);
hunderedmat = int16(ones(m,n))*100;
t = 0;
for i = initial:final;
    tic
    if i<10;
        extrazeros='00000';
    else if i<100,
        extrazeros='0000';
    else if i<1000;
        extrazeros='000';
    else if i<10000;
        extrazeros='00';
    else extrazeros='';
    end;
end;
end;
imagei= 1.+imread(['inprefix extrazeros num2str(i) 'TIF']);
if rem(i,2)== 1
    imageiN = abs((int16(imagei)*100)./(int16(calimagea))-hunderedmat); % Perecentage of mixedness of array
end
if rem(i,2)== 0
    imageiN = abs((int16(imagei)*100)./(int16(calimageb))-hunderedmat); % Perecentage of mixedness of array
end
outprefix = ['E:\PLIF Data\Organized Tests\' experiment township Results\' testname extrazeros num2str(i)];
csvwrite([outprefix],imageiN)
t= t +toc;
disp ('[Analyzed Image ', num2str(i) ']' Time elapsed = ' num2str(t) ' Time left(mins) = ' num2str((t/i)*(final-i)/60))
end
Appendix III: Variations in step-count triggering method

While using the step-count triggering method it was observed that even with the application of high precision synchronization techniques discussed previously the captured positions of the MicroWell in the PIV images were not repeatable.

To further investigate this issue, the trajectory of the MicroWell during a jiggle was traced repeatedly using a high speed camera. Figure 0-1 shows these trajectories and the variation in the motion of the MicroWell from run to run during fifteen runs.

The results show severe temporal variation of the center and extreme positions of the MicroWell from run to run. However, there is little or no variation in the spatial center and extreme positions of the MicroWell in these results. Therefore, using the results from the repeatability tests a median path of the MicroWell was created (Figure 0-2). This median path clearly has all the spatial and temporal characteristics of a ‘typical’ trajectory of the MicroWell during the motion and therefore can be used to calculate the ‘typical’ temporal position of a MicroWell in a given image using its temporal position and velocity.

Figure 0-1: MicroWell Motion Variation

Figure 0-2: Median Path of MicroWell
To temporally correct the positions of the MicroWell in various images, a continuous function representing the median path of the MicroWell can be generated using a Fourier series that accurately described all the spatial and temporal characteristics of the trajectorial curve with an average temporal error of less than one image pulse number.

The temporal position of each acquired PIV image pair was shifted so that they all lie along the media trajectory shown in Figure 0-2. The corrected temporal positions were calculated by iteratively solving a Fourier series within a given temporal interval using the spatial position of each MicroWell in the respective image.

Figure 0-3 & Figure 0-4 show the raw and corrected spatial positions of 40 PIV images taken over 8 runs intended to capture the center positions during a jiggle.
To further enhance the data set available for analysis, data was collected aiming for extreme positions during the motion and the same process was used to temporally correct the acquired data. Figure 0-5 & Figure 0-6 illustrate the raw and corrected MicroWell positions respectively.
**Appendix IV: HES Positions Master table**

Table 7-1: Master table showing the list of positions captured using hall effect sensors.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Position Name</th>
<th>S. No.</th>
<th>Position Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Post-Center-Right-1-Jiggle-1</td>
<td>41</td>
<td>Post-Center-Right-2-Jiggle-3</td>
</tr>
<tr>
<td>2</td>
<td>Pre-Extreme-Right-1-Jiggle-1</td>
<td>42</td>
<td>Pre-Extreme-Right-2-Jiggle-3</td>
</tr>
<tr>
<td>3</td>
<td>Post-Extreme-Right-1-Jiggle-1</td>
<td>43</td>
<td>Post-Extreme-Right-2-Jiggle-3</td>
</tr>
<tr>
<td>4</td>
<td>Pre-Center-Right-1-Jiggle-1</td>
<td>44</td>
<td>Pre-Center-Right-2-Jiggle-3</td>
</tr>
<tr>
<td>5</td>
<td>Post-Center-Left-1-Jiggle-1</td>
<td>45</td>
<td>Post-Center-Left-2-Jiggle-3</td>
</tr>
<tr>
<td>6</td>
<td>Pre-Extreme-Left-1-Jiggle-1</td>
<td>46</td>
<td>Pre-Extreme-Left-2-Jiggle-3</td>
</tr>
<tr>
<td>7</td>
<td>Post-Extreme-Left-1-Jiggle-1</td>
<td>47</td>
<td>Post-Extreme-Left-2-Jiggle-3</td>
</tr>
<tr>
<td>8</td>
<td>Pre-Center-Left-1-Jiggle-1</td>
<td>48</td>
<td>Pre-Center-Left-2-Jiggle-3</td>
</tr>
<tr>
<td>9</td>
<td>Post-Center-Right-2-Jiggle-1</td>
<td>49</td>
<td>Post-Center-Right-1-Jiggle-4</td>
</tr>
<tr>
<td>10</td>
<td>Pre-Extreme-Right-2-Jiggle-1</td>
<td>50</td>
<td>Pre-Extreme-Right-1-Jiggle-4</td>
</tr>
<tr>
<td>11</td>
<td>Post-Extreme-Right-2-Jiggle-1</td>
<td>51</td>
<td>Post-Extreme-Right-1-Jiggle-4</td>
</tr>
<tr>
<td>12</td>
<td>Pre-Center-Right-2-Jiggle-1</td>
<td>52</td>
<td>Pre-Center-Right-1-Jiggle-4</td>
</tr>
<tr>
<td>13</td>
<td>Post-Center-Left-2-Jiggle-1</td>
<td>53</td>
<td>Post-Center-Left-1-Jiggle-4</td>
</tr>
<tr>
<td>14</td>
<td>Pre-Extreme-Left-2-Jiggle-1</td>
<td>54</td>
<td>Pre-Extreme-Left-1-Jiggle-4</td>
</tr>
<tr>
<td>15</td>
<td>Post-Extreme-Left-2-Jiggle-1</td>
<td>55</td>
<td>Post-Extreme-Left-1-Jiggle-4</td>
</tr>
<tr>
<td>16</td>
<td>Pre-Center-Left-2-Jiggle-1</td>
<td>56</td>
<td>Pre-Center-Left-1-Jiggle-4</td>
</tr>
<tr>
<td>17</td>
<td>Post-Center-Right-2-Jiggle-2</td>
<td>57</td>
<td>Post-Center-Right-2-Jiggle-4</td>
</tr>
<tr>
<td>18</td>
<td>Pre-Extreme-Right-1-Jiggle-2</td>
<td>58</td>
<td>Pre-Extreme-Right-2-Jiggle-4</td>
</tr>
<tr>
<td>19</td>
<td>Post-Extreme-Right-1-Jiggle-2</td>
<td>59</td>
<td>Post-Extreme-Right-2-Jiggle-4</td>
</tr>
<tr>
<td>20</td>
<td>Pre-Center-Right-1-Jiggle-2</td>
<td>60</td>
<td>Pre-Center-Right-2-Jiggle-4</td>
</tr>
<tr>
<td>21</td>
<td>Post-Center-Left-1-Jiggle-2</td>
<td>61</td>
<td>Post-Center-Left-2-Jiggle-4</td>
</tr>
<tr>
<td>22</td>
<td>Pre-Extreme-Left-1-Jiggle-2</td>
<td>62</td>
<td>Pre-Extreme-Left-2-Jiggle-4</td>
</tr>
<tr>
<td>23</td>
<td>Post-Extreme-Left-1-Jiggle-2</td>
<td>63</td>
<td>Post-Extreme-Left-2-Jiggle-4</td>
</tr>
<tr>
<td>24</td>
<td>Pre-Center-Left-1-Jiggle-2</td>
<td>64</td>
<td>Pre-Center-Left-2-Jiggle-4</td>
</tr>
<tr>
<td>25</td>
<td>Post-Center-Right-2-Jiggle-2</td>
<td>65</td>
<td>Post-Center-Right-1-Jiggle-5</td>
</tr>
<tr>
<td>26</td>
<td>Pre-Extreme-Right-2-Jiggle-2</td>
<td>66</td>
<td>Pre-Extreme-Right-1-Jiggle-5</td>
</tr>
<tr>
<td>28</td>
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