Development and Applications of Film-based and Digital Holographic Particle Image Velocimetry for Both Large and Small Scale Flow Measurements

by

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Abstract

In this dissertation, new advances on Holographic Particle Image Velocimetry for fluid flow measurements and their applications are presented. At first, a film-based holographic particle image velocimetry system is improved upon the hybrid HPIV (Zhang et al. Exp. in Fluids, 1996) system. By introducing a mirror into the recording setup, two orthogonal views of the same particle field is recorded onto a same recording media. By carefully matching each individual particle from two views, we obtain 3D particle locations with 7µm uncertainty in all three directions. A proof-of-principle system is constructed and used to measure the flow behind a rising bubble. With advances in digital recording media, digital holography becomes a very promising 3D flow measurement technique. The basic principles of digital holographic particle image velocimetry are presented in this dissertation first, and a system incorporating single-beam two-view concept is used to measure the flow around a free swimming copepod. To address the need in understanding small scale near wall flow, a high-resolution Digital Holographic Microscope (DHM) is developed. Digital Holographic Microscopy (DHM) enables measurements of 3D locations and displacements of microscopic objects in space. It has the potential of revolutionizing microscopy, especially while studying small-scale dynamic phenomena. The dissertation introduces this technique, and then demonstrates its implementations in tracking microorganisms, and in performing 3D velocity measurement of turbulent shear flows. The primary focus is placed upon the near-wall region of a turbulent boundary layer over a smooth wall, covering the viscous sublayer, buffer layer and lower portion of logarithmic layer (0<\text{y}^+<150.) The Reynolds number based on \( u_r = \sqrt{\tau_w / \rho} \) is 1,400. The measurements are performed at a resolution
of one wall unit in all directions. The resolution is sufficient for resolving buffer layer structures and for measuring instantaneous wall shear stress distributions from velocity gradients in the sublayer. The data provides detailed statistics on the spatial distribution of wall shear stress along with the characteristic flow structures. Included are streamwise counter-rotating vortex pairs, multiple streamwise vortices and other structures. Conditional sampling based on local shear stress magnitudes identifies characteristic length scales of $\Delta z^+=70$ and $\Delta y^+=\sim10$, and its associated deterministic flow pattern. In the region of high stress, the conditionally averaged flow consists of a sweeping motion induced by a counter rotating pair of streamwise vortices. These vortices seem to be the major contributors to the local high shear stress but not the only ones. Statistics on the local strain and geometric alignment between strain and vorticity shows that the high shear generating vortices are inclined to the free stream direction at $45^\circ$. The dissertation will conclude with the studies using digital holographic microscope to investigate the swimming behaviors of dinoflagellates and how their behavior is modified by interacting with themselves and stimuli. Our studies, for the first time, quantitatively show the trends on behavioral modifications of dinoflagellates in the presence of prey and kinematical quantities that distinguish the differences between species.
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6.5. REFERENCES
1. Introduction
The physical world that we are living in is multi-dimensional. The phenomena occurred in it, physical or biological, are inherently complex and involve the interactions between physical systems at different length and time scales, most critically in four physical dimensions (time and space). In fluid mechanics research, the abilities of capturing these phenomena or interactions in experiments either advance or limit one’s understanding of the fundamental physics. For instance, How to accurately model turbulent flows near a boundary is a long standing challenge facing the fluid mechanics research community. Great advances have been made in Large Eddy Simulation (LES) modeling of near-wall flows. Many models have been proposed and been successfully applied to predict specific types of near-wall flows. Yet, all models require certain ad hoc “tweaking” of simulation parameters that severely limit their predictive power under unknown boundary conditions. The very fact that all near-wall models require ad hoc parameterization suggests the needs for more comprehensive and complete understanding of the physical processes involved. Despite the best efforts made in the past decade, our experimental techniques primarily remain two-dimensional (two velocity components on a 2D plane by various planar velocimetry techniques) or two and half dimensional at the best (three velocity components on a 2D plane by stereoscopic planar velocimetry techniques or scanning planar techniques.) This inability of obtaining volumetric three-component velocity vector field with high spatial resolution and large length scale separation hinders the efforts in understanding the relationships and interactions relating to various tensor and vector fields, i.e. strain, stress, vorticity, and helicity; or in gaining the intuitive
insights, for better model development. The work presented in this dissertation represents our “relentless” drive to attain such ability.

It is our belief that holography offers the solution to provide high resolution volumetric flow field measurements in both spatial and temporal dimensions. Continuing the previous research works on film based holographic particle image velocimetry, we simplify the original film-based optical setup by introducing an inclined mirror into the recording path. Consequently two orthogonal views of the same velocity field are recorded onto a single film. Owing to the simplification, the measurement uncertainty is improved to a few micrometers from a few millimeters. The technique is presented in Chapter 2. Chapter 3 presents a digital holographic particle velocimetry system with the same two-view recording concept. In answering the call of measuring 3-dimensional near wall flows with extremely high spatial resolution and the needs in biological flow studies, a digital holographic microscope is developed and presented in Chapter 4. Chapter 5 and 6 present the studies and results on the near-wall turbulent flows using existing Particle Image Velocimetry technique and newly-developed Digital Holographic Microscope. In Chapter 7, we present a study on swimming behavior of marine microorganisms in population and the behavioral alterations in the presence of stimulus (prey) using digital holographic microscopic cinematography (a 4-dimensional microscope), before we conclude in Chapter 8.
2. One-Beam Two-View Holographic Particle Image Velocimetry

2.1 Background and Rationale
Holographic Particle Image Velocimetry (HPIV) is presently the only method that can measure the instantaneous three-dimensional velocity distribution in a finite volume at high Reynolds numbers. This technique is based on recording double exposure holograms of a flow field seeded with small tracer particles. The holograms are reconstructed (optically or numerically) and a variety of methods are used for measuring the 3-D distribution of the particle displacements.

Several HPIV systems have already been constructed and implemented over the years. Barnhart et al. (1994) were the first to introduce a phase-conjugate off-axis recording system and use it to obtain a 3-D velocity distribution. Meng and Hussain (1995), in an attempt to simplify optical setup, proposed an in-line recording and off-axis viewing method, which combines the simplicity of in-line recording, and the high signal-to-noise ratio (SNR) of off-axis holography. Since in-line recording involves a reference beam passing through the sample volume, the particle seeding density is limited, which in turn limits the spatial resolution of the measurement.

The primary obstacle to implementation of HPIV has been the inherent “depth of focus” problem, i.e., the substantially larger measurement uncertainty, typically in the 200 µm – 1.5 mm range, along the depth direction (the illuminating light direction), compared to about 10 µm in the other two lateral directions. Since the depth of focus decreases with increasing scattering angle (increasing numerical aperture), Pu and Meng (1999) introduced a wide-angle, side-scattering, off-axis HPIV. Their 90º side-scattering,
which is weaker than near-forward scattering, reduces the depth-of-focus problem but does not eliminate it. Zhang et al. (1997) and subsequently Tao et al. (2000 and 2002) circumvented this problem by recording two perpendicular holograms of the same flow field, each consisting of near-forward scattering, high-pass filtering and an off-axis reference beam. This method was applied to measure the flow within a square duct and the data was used for studying the structures of turbulence.

Considering all the experience gained by the previous studies, we believe that near-forward scattering off-axis holography with two orthogonal views is an optimal approach for obtaining improved accuracy and resolution. Our rationale includes the following: First, high pass filtered, near-forward scattering provides the strongest and a theoretically axisymmetric signal for recording a hologram. Consequently, the signal to noise ratio is high and the reconstructed particle shape is less sensitive to speckle noise, as compared to methods involving non near-forward off-axis holography. Second, multiple views of a single particle in space provide sufficient constraints to determine the particle location accurately, avoiding the need to estimate the axial location of the particle from its elongated trace, a procedure that is inherently inaccurate and unavoidably exhaustive. Third, with redundant information recorded, the system can be extended to study various multi-phase flows, e.g. by measuring the morphology of and the velocity around dispersed objects. The biggest shortcoming of this approach is the complex optical system, and that it requires four windows (two for each hologram), limiting the type of facilities where this technology can be implemented.
2.2 Methodology

2.2.1 Principles
This paper introduces an off-axis HPIV system that reduces the complexity of the two perpendicular systems of Zhang et al. (1997), yet provides two simultaneous perpendicular views of the same particle. The principles of the “single-beam two-view” holography system are illustrated in Figure 2.1. In the recording phase (Figure 2.1a), a mirror is inserted in the flow field and as a result each particle is illuminated in two different directions. The first beam (Ray 1) illuminates the particle before it is reflected by the mirror, and the second beam (Ray 2) illuminates the particle after being reflected. In the setup shown these two rays illuminate the same particle in orthogonal directions, but configurations involving other angles are possible as well. Consequently, two spatially separated particle images, one being the “original image” (denoted by solid lines); and the other being the “mirror image” (dotted line) are formed and recorded on the same hologram.

During reconstruction (Figure 2.1b) both views (images 1 and 2) are reconstructed simultaneously but at different locations in space, along with a virtual mirror, which is also indicated by dotted lines. Due to the depth of focus problem, both particle traces are elongated along the direction of the optical axis of the reconstructed wave. However, since the two views are perpendicular to each other, the exact 3-D location of the particle can be determined by combining the data provided by these views. To achieve this goal one has to devise a technique to accurately measure the orientation and location of the reconstructed mirror plane. The resulting “mirroring constraint” is then used for matching the two orthogonal views, each providing information in directions that are perpendicular to its optical axis. The 3-D location of the particle is determined by
combining the data from the two views at the same level of accuracy, avoiding the uncertainties associated with the depth-of-focus problem.

The single-beam two-views method has several advantages. First, since the mirror is placed inside the facility, for example on one of its walls, the test facility requires only one window, instead of four. Thus, the type of facilities where HPIV can be implemented is extended. Second, having to record only one hologram requires considerably fewer optical components compared to the Zhang et al. (1997) setup. However, for the same size of sample volume the required window is larger and the sample volume has a triangular shape. Third, as demonstrated in this paper, the known 3-D coordinates of the particles enable us to quadruple the spatial resolution of the velocity distributions, and significantly increase the accuracy in velocity measurements. A reconstructed flow field containing more than 200 particles/mm$^3$ in the wake of a rising bubble enables us to calculate the 3-D velocity distribution using an interrogation volume of $220 \times 154 \times 250 \, \mu$m, and a vector spacing of half this distance. In the Zhang et al. (1997) measurements the sample volume is $930 \times 930 \times 930 \, \mu$m.

The following section provides information on the optical setup data analysis methods. Subsequently, experiments with sparse and dense particle concentrations are used for: (1) demonstrating the feasibility of the single-beam two-view HPIV method; (2) measuring and quantifying the spatial resolution of the 3-D particle coordinates; (3) testing the data processing techniques; (4) using the 3-D particle coordinates in order to increase the spatial resolution of the velocity distributions; and (5) evaluating the data quality by determining how well we satisfy the continuity equation for incompressible flow (conservation of mass).
2.2.2 Optical Setup – Recording and Reconstruction

Following Zhang et al. (1997) and Tao et al. (2000 & 2002), near-forward off-axis holography is used for recording the holograms. We focus on developing a method to position the particle in space precisely, and as a result do not incorporate (yet) means to overcome the directional ambiguity problem in double exposure images. Thus, the reference beams of the first and second exposures share the same optical path. This problem can be easily resolved by using two separate reference beams inclined at different angles, similar to the approach used by Barnhart et al. (1994) and Pu and Meng (1999). As shown in Figure 2.2, the light source for recording holograms is an injection seeded, frequency doubled (wavelength – 532 nm), 15 Hz, 400 mJ/pulse, dual head Nd:YAG laser (Spectra Physics, PIV-400). A single pair of laser pulses, separated by a short time interval, is gated by a mechanic-optical shutter, in order to record a single double exposure hologram. An adjustable beam attenuator consisting of a λ/2-waveplate and a polarized beam splitter cube (PBS) reduces the output laser energy to a desired energy level. The laser beam is then split by a set of λ/2-waveplates and a PBS to object (illuminating) and reference beams. The reference to illuminating beam intensity ratio is maintained at 1:9 (Zhang et al., 1997). The object beam is expanded, collimated and directed into the flow facility. Illumination of the test section at an angle generates multiple reflections from the glass windows. Inserts (I) containing liquids are placed in the path of the object beam to overcome this problem. A mirror installed on one of the interior walls of the facility walls reflects the object beam, illuminating the particles in the triangular overlapping volume twice. Thus, two separate images, each with a different view are formed. In order to increase the SNR and reduce the speckle noise⁴, the object beam passes through a high-pass filter assembly consisting of a pair of relay lenses (L6 &
L7 in fig. 2) and a small pin located at the focus of the first lens. The collimated, directly transmitted beam (DC component) is focused onto the pin and blocked out, whereas the light scattered by the particles is allowed to propagate to the holographic plate, essentially undisturbed. The relay lenses also bring the image of the sample volume closer to the vicinity of the plate. The reference beam is spatially low-pass filtered using a 250 µm pinhole (made of a 200 µm thick stainless-steel shim), expanded and collimated. In order to prevent the ionization of the air, a combination of lens array (L3 & L4 in fig. 2) is used to gradually focus the 15 mm diameter laser beam into an approximately 250 µm diameter spot. The focused power density is estimated as $5 \times 10^7$ W/cm², slightly below the breakdown power level for air ($7 \times 10^7$ W/cm²). The angle between the reference and object beams is 20°.

A CW Argon-Ion laser is used as a light source for reconstruction. It is integrated into the optical path of the primary reference beam through the beam splitter and illuminates the hologram. The developed hologram is placed in its original location, but is rotated by 180° along with the relay lenses (L6) and the high-path filter. Thus, the reconstructed wave front propagates backwards through the original relay lenses to form a phase-conjugate reconstruction of the real image (dotted lines). This method minimizes the aberration introduced by the relay lenses. The pin (high-pass filter) is left in the path of the reconstruction beam, and is matched with its reconstructed image as a calibration check point, ensuring conjugate reconstruction. Since our reconstruction laser is operating at $\lambda_c=514$ nm rather than the recording wavelength, $\lambda_r=532$ nm, it is necessary to modify the reconstruction angle according
\[
\sin \theta = \left( \frac{\lambda_c}{\lambda} \right) \sin \theta_r
\]  

(2.1)

where \( \theta_c \) and \( \theta_r \) are the angles between the reference and object beams during reconstruction and recording, respectively\(^7\). The low energy (50 mW) CW laser is used during reconstruction for convenience (and since it is available, a CW Nd:YAG laser would be a better choice), and the angle is adjusted using mirror M1. As illustrated in Figure 2.2, two particle fields, the original and its mirror image, are reconstructed at different locations in space. The reconstructed images are scanned using a 640×484 pixels video camera at a spatial resolution of 3.452×3.505 \( \mu \)m/pixel. The camera is mounted on a 3-D motorized traversing stage, and the images are recorded every 2.2 mm in \( x \), 1.69 mm in \( y \) and 250 \( \mu \)m in \( z \), providing a continuous record in the \( x \) and \( y \) directions.

### 2.2.3 Data Analysis Procedure

#### 2.2.3.1 Outline

To obtain the 3-D velocity vectors from the two different holographic images, Zhang et al. (1997) and Tao et al. (2000 & 2002) scan the reconstructed field, record 2-D slices of particle traces and use 2-D PIV techniques to compute the velocity. The 3-D vector field is obtained by combining the two 3-D distributions of two velocity components. Due to the depth of focus effect, each 2-D section through the reconstructed field contains traces of particles that are located within about 1mm from this plane (Malkiel et al., 1999). Consequently, the velocity is effectively integrated (low-pass filtered) in the depth (axial) direction, limiting the minimum volume used for obtaining a single velocity vector. They do not (and can not) match the perpendicular particle traces since that would require
matching of the two separately reconstructed fields to within a few microns, which is a formidable task (attempts were not successful). In the current system, the two orthogonal views are recorded onto and reconstructed from a single hologram, providing data that can be used for matching the two fields. Utilizing this advantage, the analysis procedures are modified in order to reduce the effect of spatial filtering and resolve the flow at smaller scales. Thus, data processing consists of two steps. The first, discussed in section 2.3.2, determines the 3-D location of particle centroids, and the second, discussed in section 2.3.3, calculates the 3-D velocity vectors using the known particle locations. Two optional methods for calculating the velocity have been considered, but only one has achieved the desired accuracy. This method utilizes the measured centroids in order to “trim” the elongated particle traces in the original scanned images. The velocity is calculated from the trimmed traces.

2.2.3.2 Measuring the 3-D Particle Centroid

Implementation of the mirroring constraints requires matching of the reconstructed and original (physical) coordinates since the mirroring constraint is only valid in the physical space. Due to the differences in media (water/air) and laser wavelength (532nm/514nm), the two spaces are different. The higher refractive index of the sample volume (water) introduces compression along the normal direction of the water-air interface, whereas the wavelength difference introduces expansion. For convenience, we select a coordinate system that matches that of the 3-D traversing system used for scanning the hologram. In the following analysis, all the variables with tilde are expressed in terms of the measured (reconstructed) coordinates and the rest refer to the physical space. A transformation from the physical to measured spaces can be expressed as:
\[ \tilde{x}_i - \tilde{x}_0 = c_{ij} x_j \]
\[ x_j = \tilde{c}_{ij} (\tilde{x}_i - \tilde{x}_0) \]  
(2.2)

where \( c_{ij} = (c - 1)n_i^s n_j^s + \delta_{ij} \), i.e.,

\[
c_{ij} = \begin{bmatrix}
(c-1)n_1^s n_1^s + 1 & (c-1)n_1^s n_2^s & (c-1)n_1^s n_3^s \\
(c-1)n_2^s n_1^s & (c-1)n_2^s n_2^s + 1 & (c-1)n_2^s n_3^s \\
(c-1)n_3^s n_1^s & (c-1)n_3^s n_2^s & (c-1)n_3^s n_3^s + 1
\end{bmatrix}
\]  
(2.3)

Here \( \tilde{c}_{ij} \) is the inverse of \( c_{ij} \), \( \tilde{n}^s \) is the normal vector of the water-air interface, and \( \tilde{x}_0 \) is the origin in the measured space, conveniently set to zero. The compression scalar constant, \( c \), is (Collier et al., 1971)

\[
c = \frac{n_{\text{air}} \cdot \hat{\lambda}_{\text{record}}}{n_{\text{water}} \cdot \hat{\lambda}_{\text{recon}}}. \]  
(4)

As noted before, the particle traces from the original and mirror views are obtained by scanning the entire reconstructed volume in planes spaced 250 µm apart. A sample image of an elongated particle trace is presented in Figure 2.3a. Its 3-D boundaries are defined using a simplified segmentation procedure (for explanation on segmentation in general, see Pratt, 1992). To accelerate the process, the segmentation is performed separately for each line using a thresholded image. Adjacent line segments that form a continuous particle are joined to define a 2-D boundary (cross-section) of a particle. This procedure is repeated at different depths, generating a series of 2-D traces. By defining a similar join operator for the planar segments the 2-D segments are united into a 3-D particle trace. Thus, each 3-D particle is composed of several connected 2-D “blobs” with known centers and mean radii. In subsequent calculation, in order to increase the speed of analysis, each 3-D particle trace is replaced by a cylinder with a finite length and
a mean radius, defined by \( \{ \tilde{x}_s, \tilde{x}_e, \tilde{D}_p \} \), where \( \tilde{x}_s \) and \( \tilde{x}_e \) are the starting and ending point of this trace, respectively, and \( \tilde{D}_p \) is the mean particle diameter. The cylinder is aligned with a line (least square) fitted through all the measured centers of the 2-D “blobs” (Figure 2.3b). This linear fitting procedure reduces the influence of noise, and simplifies the following analysis procedures. The original and mirror views generate two perpendicular fitted cylinders for each particle.

Once the particle tracers are identified in both views, the mirroring process is employed to match them, provided that the mirror plane, defined in eqn. 2.5, is known.

\[
n^m_x x + n^m_y y + n^m_z z + d = 0, \tag{2.5}
\]

Here \( \vec{n}^m \) is the normal vector of the mirror plane and \( d \) is the distance of the mirror plane from the origin. The mirror particle trace in the reconstructed space is reflected and positioned in the original space (and the original space is reflected and positioned on the mirror space) using the following procedure:

\[
\{ \tilde{x}_s, \tilde{x}_e, \tilde{D}_p \} \overset{C}{\longrightarrow} \{ \tilde{x}_s, \tilde{x}_e, D_p \},
\]

\[
\tilde{x}_{s,e}^\prime = \tilde{x}_{s,e} - 2(\vec{n}^m \cdot \tilde{x}_{s,e} + d)\vec{n}^m, \tag{2.6}
\]

\[
\{ \tilde{x}_s, \tilde{x}_e, D_p \} \overset{C}{\longrightarrow} \{ \tilde{x}_s, \tilde{x}_e, \tilde{D}_p \},
\]

where \( \tilde{x}_{s,e}^\prime \) are the starting and ending points of the reflected particle trace in the physical space. In this procedure the reconstructed space is converted to the physical space, the particle trace is reflected with respect to the mirror, and then converted back to the reconstructed space. Consequently, the reflected mirror traces (cylinders) are positioned
in the original space. Then, a matching process is used for finding the corresponding traces of the same particle. We compute the shortest distances between the reflected and original traces, i.e. between two perpendicular 3-D straight lines with finite supports. If the shortest distance falls within the mean diameter of the particle traces, and the crossing points are within their supports, these two traces are considered as matched. Equation 2.7 represents these conditions in a mathematical form:

\[
0 \leq \lambda_r \leq \left| \frac{\vec{x}_e - \vec{x}_s}{\vec{x}_e - \vec{x}_r} \right|
\]

\[
0 \leq \lambda_o \leq \left| \frac{\vec{x}_e - \vec{x}_s}{\vec{x}_e - \vec{x}_o} \right|
\]

\[
|D| \leq \frac{\tilde{D}_r + \tilde{D}_o}{2}
\]

(2.7)

where \( \lambda_r \) and \( \lambda_o \) are the distances between the starting points on the reflected (subscript \( r \)) and original (subscript \( O \)) traces and the crossing point, respectively. The subscripts \( s \) and \( e \) represent the starting and ending points, respectively, and \( |D| \) is the shortest distance between two traces. They are computed from

\[
\begin{bmatrix}
\frac{\dot{y}_e - \dot{y}_s}{\ddot{y}_e - \ddot{y}_s} & \frac{\dot{y}_e - \dot{y}_o}{\ddot{y}_e - \ddot{y}_o} & \frac{\dot{y}_e - \dot{y}_o}{\ddot{y}_e - \ddot{y}_o} \\
\frac{\dot{z}_e - \dot{z}_s}{\ddot{z}_e - \ddot{z}_s} & \frac{\dot{z}_e - \dot{z}_o}{\ddot{z}_e - \ddot{z}_o} & \frac{\dot{z}_e - \dot{z}_o}{\ddot{z}_e - \ddot{z}_o} \\
\frac{\dot{x}_e - \dot{x}_s}{\ddot{x}_e - \ddot{x}_s} & \frac{\dot{x}_e - \dot{x}_o}{\ddot{x}_e - \ddot{x}_o} & \frac{\dot{x}_e - \dot{x}_o}{\ddot{x}_e - \ddot{x}_o}
\end{bmatrix}
\begin{bmatrix}
\lambda_o \\
-\lambda_r \\
-D
\end{bmatrix}
= \frac{\vec{x}_e - \vec{x}_s}{\tilde{D}_r + \tilde{D}_o}
\]

(2.8)

For a matched pair that satisfies Equation 2.7, the centroid of the particle is positioned at the center of the line defined by the shortest distance between the two traces.

The elongated particle trace shown in Figure 2.3 appears to have “bumps”, and one may be curious whether this trace is actually a combined image of two (or more) particles. Although such a possibility exists, especially when the flow field is densely seeded, the matching procedure would identify such an event. An elongated trace created
by more than one particle would be matched with the orthogonal view more than once, at
different depth. Presently, the analysis procedure is programmed to discard such a trace.
This event is extremely rare, at least in the present flows, which contain more than 200
particles (20 µm diameter) per mm$^3$. In the case of the image in Figure 2.3, there is only
one matched trace in the orthogonal view, and the bumps are most likely a result of
speckle noise.

2.2.4 3-D Velocity Measurements

2.2.4.1 Discrete 3-D Correlations Using Particle Centroids

Once the particle centroids are identified at an acceptable level of accuracy, one can
construct a 3-D correlation algorithm by using the spatial distributions of particle
centroids. One can represent the 3-D intensity distribution of each particle, $I_p(\vec{x})$, as a
scalar function, $I_s(\vec{x})$, that has a finite support, convoluted with a delta function with a
shift centered at the particle centroid:

$$I_p(\vec{x}) = I_s(\vec{x}) \otimes \delta(\vec{x} - \vec{x}_i)$$  \hspace{1cm} (2.9)

where $\vec{x}_i$ is the centroid of the $i$th particle. The scalar function is modeled as an “ideal”
particle with intensity distribution that is a function of distance from its centroid, i.e.

$$I_s(\vec{x}) = \left\{ \begin{array}{ll} f(|\vec{x}|) & |\vec{x}| < r_p \\ 0 & \text{otherwise} \end{array} \right. \quad \text{if } s=1$$  \hspace{1cm} (2.10)

In this way, we can represent the intensity distribution of all the particles in an
interrogation cube (IC), $I_s(\vec{x})$, as the ideal particle intensity distribution convoluted with
a series of delta functions with different shifts,

$$I_s(\vec{x}) = I_s(\vec{x}) \otimes \sum_{i=1}^{N} \delta(\vec{x} - \vec{x}_i)$$  \hspace{1cm} (2.11)

Therefore, the direct correlation between two exposures within an IC can be written as
\[ R_{12}(\vec{r}) = \int [I_1^1(\vec{x})I_2^2(\vec{r} + \vec{x})] \cdot d\vec{x} \quad (2.12) \]

where \( I_{1,2} \) represents intensity distribution of the 1st and 2nd exposure. By substituting eqn. 10 into eqn. 11, the 3-D correlation function is no more than a self-correlation function of the ideal particle scalar function convoluted with a series of delta functions with different centroids, namely

\[ R_{12}(\vec{r}) = R_{ss}(\vec{r}) \otimes \sum_{i,j}^{N_1 \times N_2} \delta(\vec{r} - \vec{x}_i + \vec{x}_j) \quad (2.13) \]

where \( N_{1,2} \) are numbers of particles in the first and second images, and \( R_{ss} \) is the auto-correlation function of \( I_s \), defined as,

\[ R_{ss}(\vec{r}) = \int I_s(\vec{x}) \cdot I(\vec{x} + \vec{r}) \cdot d\vec{x} \quad (2.14) \]

Since \( I_s \) is a scalar function of finite support, its auto-correlation function is a scalar function of finite support too. Any displacement more than twice the diameter of a particle has a zero correlation value. Thus, a fast implementation of eqn. 2.14 can be realized by pre-sorting the list of particle. The asymptotic complexity of the algorithm is reduced from \( O(m^2N_1N_2) \) to \( O(m^2 \cdot \max(N_1, N_2)) \), where \( m \) is the range of values of \( \vec{r} \) over which the correlation is computed. Furthermore, if \( I_s \) is prescribed as a 3-D Gaussian or a top-hat function, its auto-correlation function is well defined and can be tabulated for fast computations. Simulations show that with 20 particles in an interrogation cube, the algorithm for computing the 3-D velocity can reach a speed of 20 vectors/s. This computational cost seems high, but we have not made a substantial effort to improve the efficiency of this procedure, mainly since we have encountered difficulties in achieving the desired level of accuracy in displacement measurements.
The present 3-D correlation method would offer high measurement accuracy and good resolution as long as the particle centroid measurements are accurate, and the number of mis-identified particles is low. The latter condition is satisfied, as demonstrated later in this paper. It also provides a good foundation for tracking each individual particle. Unfortunately however, the uncertainty associated with the 3-D location of particle centroids propagates into the velocity measurements. In the present experiments, the particle displacements are typically about 60 \( \mu \text{m} \), and the uncertainty in determining the location of the centroids is 7\( \mu \text{m} \) (details follow), less then the particle diameter but sufficient to introduce a displacement measurement uncertainty of about 10\%. This problem prevents us from pursuing this approach any further.

2.2.4.2 2-D Correlations Using Filtered Scanned Images
Similar to Zhang et al. (1997), this 2-D correlation approach is based on using the original scanned images for calculating two components of the velocity, and determining the 3-D vectors by combining the two data sets. However, the analysis is performed only after “trimming” the elongated traces using the measured location of particle centroids. This procedure erases the particle traces form all scanned planes whose distance from the calculated centroid location exceeds a certain prescribed distance. In the present data we scan the reconstructed field every 250 \( \mu \text{m} \), and as a result particles with centroids located more than \(~125 \ \mu \text{m}\) from the scanned plane that is closest to the centroid are erased, leaving only traces within a 250 \( \mu \text{m} \) “sheet” centered on the scanned plane. Figures 2.3 and 2.4 illustrate the trimming process and its effect on the number of particles in an interrogation window. The original elongated \(~1 \text{ mm}\) long trace of a 20 \( \mu \text{m} \) diameter particle is shown in Figures 2.3a and b. Figure 3a is obtained by scanning the image in
many closely spaced planes, and Figure 3b shows the five cross sections of this particle that would be obtained during typical scanning every 250 µm. Also shown is the cylindrical, least-square-fitted line segment that is used for matching with the perpendicular view and determining the location of the centroid. The traces are removed from four of the five planes, and are left only on the plane located closest to the 3-D centroid (Figure 2.4a). Figures 2.4b and c show the same doubly exposed, 192×192 µm² area (that includes the particle of Figure 2.4a) before and after filtering. Particle traces that are not located within the ±125 µm range are removed from the original image. Repeating this procedure for all the particles effectively eliminates the adverse, low-pass filtering effect of the elongated traces.

The filtered images are used to determine the two in-plane velocity components following typical 2-D PIV analysis procedures. Combining the data from the two perpendicular views, one can obtain the 3-D vector for each interrogation volume, 220×154×250 µm³ in the present experiments. Reconstruction of the 3-D vector is based on solving the over-determined Equation 15 backward by singular value decomposition, which essentially provides a least-square-fit solution:

\[
\begin{bmatrix}
U^m_x \\
U^m_y \\
U^o_x \\
U^o_y \\
U^o_z
\end{bmatrix} =
\begin{bmatrix}
1 - 2n^m_x & -2n^m_y & -2n^m_z \\
-2n^m_x n^m_y & 1 - 2n^m_y & -2n^m_y n^m_z \\
0 & 1 & 0 \\
0 & 0 & 1
\end{bmatrix}
\begin{bmatrix}
U_x \\
U_y \\
U_z
\end{bmatrix}
\]

(2.15)

where \(U^m_{x,y}\) are the measured velocity components in the mirror view, \(U^o_{x,y}\) are the components in the original view, \(U_{x,y,z}\) are the 3D velocity components to be reconstructed, and \(n^m_{x,y,z}\) are normalized normal vector for the mirror plane. Details on
the auto-correlation methods used for determining the 2-D in-plane velocity components, including uncertainty analysis, are provided in Zhang et al. (1997), Tao et al. (2000 & 2002) and Roth et al. (2001). The methods for determining the mirror plane are discussed in the following section.

2.2.5 On-line Calibration of the Mirror Location and Orientation
The success of our measurement procedure hinges upon whether the location of mirror plane can be determined down to the required resolution. Unfortunately, the reconstructed mirror plane (Equation 2.5) may change slightly with each reconstruction, as we remove the hologram, develop it, and remount it in the system during reconstruction. Therefore, an on-line calibration method is essential to determine the exact location and orientation of the mirror plane during each reconstruction. As described in this section, we have devised a dual-step, on-line calibration procedure, the first one providing a “coarse” (± 100 µm) resolution, and the second providing a “fine” (± 10 µm) resolution. Subsequent error analysis shows that the error associated with mirror orientation affects the velocity measurement, but the error associated with the location of the mirror is a constant and only affects vector position. Thus, determining the orientation of the mirror is more crucial.

2.2.5.1 Coarse Calibration
The coarse measurement is performed by marking the corner of the mirror with four crosses and pre-measuring the physical distances between any two crosses to within pixel accuracy (3.5 µm). During reconstruction we only measure the $x$ and $y$ (in plane) coordinates of these crosses and compute the depth difference between any two crosses using Equation 2.16
\[
\left( \tilde{x}_i - \tilde{x}_j \right)^T \tilde{A} \cdot \left( \tilde{x}_i - \tilde{x}_j \right) = D_{ij}, \tag{2.16}
\]

Here \( \tilde{x}_{i,j} \) are the location of \( i \)-th and \( j \)-th crosses in the reconstructed space, \( \tilde{A} = \tilde{C}^T \tilde{C} \), \( \tilde{C} \) is defined in equations 2 and 3, and \( D_{ij} = \| \tilde{x}_i - \tilde{x}_j \|^2 \). All the quantities in Equation 11 except for \( \tilde{z}_i - \tilde{z}_j \) can be measured or computed down to a resolution of about 3.5 \( \mu \)m.

Therefore, the depth difference, \( \tilde{z}_i - \tilde{z}_j \), should also have the same order of resolution.

Considering that the light propagation direction may be different from the \( z \) coordinate (e.g. due to slight misalignment of the hologram relative to the scanning system), a correction, \( \Delta \tilde{x} \), needs to be added into Equation 2.5, once \( \Delta \tilde{x}_{ij} = \tilde{x}_i - \tilde{x}_j \) is obtained. Equation 17 gives the final form of this correction.

\[
\Delta \tilde{x} = \Delta \tilde{z} \cdot \tilde{K}
\]

\[
\left( \tilde{K} \tilde{A} \tilde{K}^T \right) (\Delta \tilde{z})^T + \left[ \tilde{K} \tilde{A} \Delta \tilde{x}_{ij}^T + \left( \tilde{K} \tilde{A} \Delta \tilde{x}_{ij}^T \right)^T \right] \cdot (\Delta \tilde{z}) + \left( \Delta \tilde{z} \cdot \tilde{A} \cdot \Delta \tilde{x}_{ij}^T - D_{ij} \right) = 0 \tag{2.17}
\]

where \( \tilde{K} \) is the light propagation vector, which has the form, \([k_x, k_y, 1]\). Our measurements show that the uncertainty in mirror orientation associated with this calibration method is quite low, about 0.1°, which is almost sufficient for precise matching of the two views. The fine calibrations, discussed below, confirm this estimate.

Once the relative distances between crosses on the reconstructed mirror are known, only one measurement of depth is needed to determine the depth of all the other points. In order to perform this measurement during the coarse calibration phase, we make use of an observation that out-of-focus “large” linear objects form an identifiable diffraction pattern (Pratt, 1992). When the distance to the focal plane is large, i.e. it exceeds a few
hundreds of object diameters, the spacing of the pattern increases linearly with the
distance to the focal point. Thus, one can scan the reconstructed image of the cross at
different depths, and identify the intensity peaks of the first order diffraction grating on
both sides of the focus plane, and on both sides of the center of the image. By fitting two
lines that connect the peaks on one side of the focus plane with the opposing peaks on the
other side of the focus plane, the crossing point of these lines provides an estimate for the
focal point of the cross. When this method is compared with the depth of a manually
focused image (whose accuracy is also limited by the depth of focus effect), we have
found that the uncertainty between them is around 100µm, which is far from the required
resolution. Nevertheless, this result provides a starting point for a finer calibration.

2.2.5.1 Fine Calibration
Uncertainties of 0.1° for orientation and 100 µm for location of the mirror, obtained by
the coarse calibration, are still far from the resolution one hopes to achieve.
Consequently, the coarse results are followed with a fine calibration procedure. We first
manually identify a set of particle traces at various depths and locate their mirrored
counterparts. Then, using the reflecting and matching procedure described in Section
2.3.2 (which require use of the mirror parameters), we determine the distance between the
two views of the particle traces. The mirror parameters are then optimized by finding the
parameters that minimize the distances between the original and mirror views. This
procedure refines the measured mirror parameters, and reduces the uncertainty in
orientation to about 0.1 mrad, and that of the location down to ~10 µm. The 0.1 mrad is
estimated based on a calibration grid spacing of 38.1 mm and uncertainty in location of 1
pixel (3.5 µm). The 10 µm is based on the uncertainty in the location of the 3-D particle
centroid, as determined by our calibration experiments discussed in the next section. In
order to distinguish these particles and find their counterparts easily during experiments,
we seed the flow with a very small fraction of larger “mirror calibration particles,” with
size of 50-100 µm, and concentration that would leave 3-5 particles in the sample volume
(along with the high concentration of 20 µm flow tracers). When the image is
reconstructed, both images of these particles can be easily found and matched. Then, the
mirror parameters that minimize the distance between them are identified.

2.3 Calibrations and Velocity Measurements

2.3.1 Experimental Setup and Sample Images
In the present “proof of concept” experiments we use a 100 mm high tank with a 63.5 ×
63.5 mm triangular cross section, as sketched in Figure 2.5. The mirror is mounted
internally as the back wall of the tank. The reason for selecting a triangular testing tank
is not due to a limitation of the present technique, but for convenience and simplicity of
construction. In fact, trials using a rectangular test facility with an external triangular
chamber to minimize the multiple reflections, as illustrated in Figure 2.2, achieve the
same result. The quiescent fluid (water in current case) is seeded with particles and a
train of bubbles is injected periodically from a 6.26 mm orifice located 18.78 mm from
both windows. The orifice is connected to a pressure-regulated air supply line, and the
pressure is maintained at a level that causes the bubbles to be pinched off at the rate of
0.02 Hz, generating 6~8 mm diameter bubbles. The characteristic rise velocity of these
bubbles is 20 cm/s. A system consisting of a He-Ne laser pointing at a photo-diode
located on the other side of the facility is positioned 25.4 mm above the orifice. Once the
bubble is detected by the photo-diode, the next available pulse pair is gated out by
shutter, and a double-pulse off-axis hologram of the wake behind a rising bubble is recorded. The Nd:YAG laser is not triggered directly to generate a pulse pair since the laser must be fired continuously fired at 15Hz in order to match the laser cavity length, at its thermal equilibrium state, with the wavelength of the injection seeder.

Two experiments have been performed. The purpose of the first is to demonstrate the feasibility and accuracy of the present two-view technique. Thus, the flow is seeded only with “large” polystyrene particles, ranging from 20 to 130 µm in diameter, at a low seeding density of 0.3 particles/mm³. The sample volume has mean distance of 25 mm from the mirror plane with maximum and minimum distances of 35 mm and 2 mm, respectively. The results of this experiment are used for evaluating the data processing procedures, including the on-line calibration, particle identification, mirroring, matching and determination of the particle centroids. Due to the low concentration the particles traces can also be identified and matched manually. The results of this test are also used for determining the uncertainty in locating the particle centroid.

The second experiment is performed using 20 µm mono-dispersed polystyrene particles at a high concentration of about 200-300 particles/mm³. The purpose of this test is to demonstrate the system performance under conditions that enable velocity measurements at the desired data density. Based on numerous calibration tests of 2-D PIV data analysis procedures (e.g. Keane and Adrian, 1995; Roth et al, 2001 and 1999; Sridhar and Katz, 1995), each interrogation area used for calculating one velocity vector should contain at least 5 particle pairs. Thus, a characteristic interrogation volume of 0.0016 mm³ requires about 300 particles/mm³. The total sample volume is 38×38×38 mm and the delay between laser pulses is 4.5 ms.
Figure 2.6 shows two reconstructed views of the same rising bubble exposed twice with a high particle concentration around it. The shapes and displacement of the bubble are clearly reconstructed in both views. As is evident from the magnified subsections of these images, the reconstructed images of the 20 μm particles on both sides of the mirror give similar image quality, without any deterioration on either side. The measured image signal to noise ratio on both sides are comparable, demonstrating that introducing the mirror into the path of the object beam, and letting the object beam pass through the particle field twice does not increase the noise level (at least for the present depth).

2.3.2 Locating the Particle Centroid and Its Uncertainty
An 8×8×8 mm cube of the sample containing sparsely seeded large particles recorded in the first experiment is scanned along with its mirror image, but this time the volume is slightly larger, 14×8×8 mm. The larger domain in the second volume accounts for the axial compression due to recording and reconstruction at difference wavelength (including the effect of water), and the elongated traces that may extend beyond the boundaries of the cube. Figure 2.7a shows 174 pairs of identified and matched particle traces, along with a magnified view of one of the pairs. Each particle is shown as an ellipsoid with the measured particle length (depth) as its major axis and the measured mean diameter as its minor axis. Clear crossings between the elongated traces of the two views are evident from the x-z projection (Figure 2.7b). The fact that the images match very well can also be seen from x-y and y-z planes (Figures 8c and d, respectively). The probability density (pdf) of the shortest distances between matched traces (3-D line segments) is plotted in Figure 2.8. The pdf of the absolute distance between two line segments, shown in Figure 2.8a, has a mode of about 4μm and a rather narrow standard
deviation of 6.8 μm. It also indicates that the mirrored traces tend to lie at a mean distance of 3 μm under their counterparts (average value). Therefore, one can confidently estimate that the systematic error in centroid location is 3 μm, and the uncertainty is 6.8 μm, the latter corresponding to about twice the camera resolution during reconstruction (3.5 μm/pixel). Figure 8b shows the same distribution normalized with the mean diameter of the particle traces used in the second experiment (20 μm). Here the systematic measurement error is 15% and the uncertainty is 26% of the particle diameter.

Both the mirror location and mirror orientation contribute to the error in centroid calculations. From the reflection equation (Equation 2.6), one can determine that the error in mirror location introduces a constant shift, which is equal to twice the distance of this point from to the mirror. This error does not affect the displacement vector measurement, since the relative distance between particles is not altered. Conversely, the error in determining the mirror orientation, $\Delta \theta$, introduces an error that is linearly proportional to the distance from the mirror, $\Delta d = 2\Delta \theta d$, where $d$ is the distance from the mirror. Thus, the error in mirror orientation affects the distance between particles, i.e. the velocity measurements. The present measurements indicate that the total error in centroid location, including the contributions of mirror location orientation and errors, is well bounded within 10 μm over a domain extending to 15 mm away from the mirror. With the help of fine calibration, this error can be reduced even further, especially when the calibration particles are all located far away from the mirror. As noted before, this uncertainty is sufficient for trimming the elongated particle traces from the original
scanned images, but is not sufficient for using 3-D correlations to determine the velocity directly from the centroids.

2.3.3 Velocity Measurements and Their Uncertainty
In order to demonstrate the potential of the current method, this section presents a sample part of the 3-D velocity distributions in the wake behind the rising bubble shown in Figure 2.6. Figures 2.9a and b show sample particle images obtained from both views, each with a physical dimension of 2.2×1.69 mm. The corresponding velocity maps are calculated using 2-D PIV method after trimming/filtering the images from out-of plane particle traces, and using the software described in Tao et al (2000 & 2002). The analysis is performed using an interrogation window of 64 by 44 pixels, corresponding to physical dimensions of a 220×154 µm, with 50% overlap between windows. Thus, the velocity vector spacing is 110 µm in x and 77 µm in y, a significantly higher resolution than any of our previous results (interrogation window of 0.93×0.93 mm). This increased spatial resolution is made possible to a great part due to the trimming process that removes the “out of plane” particle traces. Consequently, the correlation peaks are not contaminated by the effect of distant particles, enabling us to resolve the high velocity gradients within the bubble wake.

The 2-D PIV vector maps obtained in both views of the sample volume are combined to determine the 3-D velocity vectors, using Equation 2.10. Figure 2.10 shows part of the resulting 3-D, instantaneous, velocity field in the bubble wake, containing 96×110×61 vectors within a volume of 10.5×8.5×20 mm. The z dimension corresponds to 61 planes spaced 250 µm apart in the reconstructed image (333 µm spacing in the physical space). To check the accuracy of our measurement, one can examine whether the results satisfy
the continuity equation, i.e. conservation of mass for an incompressible flow. Following
Zhang et al. (1997), we calculate the normalized divergence,

\[
\sigma = \frac{(\sigma_x^2 + \sigma_y^2 + \sigma_z^2)}{(\overline{\sigma_x^2}) + (\overline{\sigma_y^2}) + (\overline{\sigma_z^2})},
\]

(2.17)

where the “over bar” denotes spatial averaging of the velocity using a 3-D box filter over
a certain length scale, \( \Delta \) (volume \( \Delta^3 \)). The average of \( \bar{\sigma} \) over an entire sample volume
varies from zero, when the continuity equation is satisfied identically at every point, to
1.0 for random data. Figure 2.11 shows the cumulative pdfs of \( \sigma \) of the current data for
spatial averaging scales of \( \Delta = 0.25, 0.5, 1.0 \) and 2.0 mm, and compares them to the data in
Pu and Meng (1999), averaged at \( \Delta = 0.93 \) mm and 7.44 mm. Several trends can be
observed from these results. First, \( F(\bar{\sigma}) \) increases rapidly with increasing \( \Delta \). Second, for
the same length scale, e.g. \( \Delta = 1 \) mm in the present measurements \( \Delta = 0.93 \) mm in Zhang et
al. (1997), the present data converges to \( F(\bar{\sigma}) = 1 \) much faster. In fact, at the 90\(^{th}\)
percentile, the present \( \bar{\sigma} \) is five times smaller. Third, the divergence of the present
velocity distribution averaged over a 1x1x1 mm volume is comparable to the divergence
of Zhang et al. (1997) data over a 7.44x7.44x7.44 mm volume. Clearly, the present
results satisfy the continuity equation significantly better than the original data of Zhang
et al. (1997). We believe that the primary causes for improvement are the trimming
process that removes out-of-focus particles from the sample volume, and the substantially
higher seeding density in the current experiments. Consequently, unlike our previous
measurements (Pu and Meng, 1999, Zhang et al. 1997, and Bao et al. 2000), the velocity
in each interrogation volume is calculated based only on the displacement of particles that are located within this volume.

The uncertainty in $\bar{U}$ is introduced both by the measured $\bar{U}^m$ and $\bar{U}^o$ as well as by the mirror parameters. The contribution of each variable/parameter can be estimated by performing error propagation analysis using Equation 2.10. The displacement uncertainty associated with 2-D PIV measurements of $\bar{U}^m$ and $\bar{U}^o$ is about 0.4 pixel, or 1.4 µm, using a conservative estimate based on twice the standard deviation of the difference between exact and measured displacements (Bao et al., 2000&2002; and Roth et al. 2001), while the uncertainty in mirror orientation, $[-0.7176 \; 0.0057 \; -0.6964]$, is about $10^{-4}$ radians. For the present characteristic particle displacement between exposures, ~20 pixel, the uncertainty in 2-D PIV is about 2%, dominating the ~0.1% uncertainty associated with the mirror orientation. Combining both effects, the total uncertainty in velocity measurement is about 2.5%.

2.4 Conclusions and Discussion
Recording of two perpendicular holograms simultaneously overcomes the depth of focus problem in holographic PIV at the cost of added complexity, but does not overcome the spatial, low-pass filtering caused by the elongated particle traces. This paper introduces a method that records two orthogonal views of the same sample volume on a single hologram, requiring only one window and one recording system. Furthermore, data analysis procedures are developed to overcome the adverse low-pass filtering effect, enabling us to quadruple the spatial resolution of the velocity distribution. The technique is based on placing a mirror in the test section that reflects the object beam at an angle of 45°. Particles located in the volume in which the incident and reflected beams from the
mirror overlap are illuminated twice in perpendicular directions. The optical setup consists of the previously proven off-axis holography with near-forward scattering from particles, high-pass filtering of the object beam and conjugate reconstruction. Both views are reconstructed simultaneously but at different locations in space. Consequently, the two views can be scanned at the same time and maintain their relative orientation. As demonstrated by in this paper, a high signal to noise ratio is maintained in both views.

In addition to the reduced number of optical elements, a primary advantage of the present system in terms of application is that it requires only one optical port for illumination of the sample volume instead of four in our previous setup. Consequently, single beam, two views HPIV can be implemented in a substantially wider range of facilities, where the mirror can be inserted, for example, on the wall opposing the object beam port (Figure 2) or in the middle of the facility, if it does not disrupt the flow. There is a wide range of flows/problems, especially near-wall (single and multi-phase) flows, where the setup of Figure 2.2 provides an effective method for mapping the 3-D velocity and particle distributions.

However, the sample volume covered by the two views has a triangular shape, which may be a problem in certain applications, and the single port must be larger than that used in our original setup. If a cubic volume is essential, and the sample volume has to be located away from the wall, the optical setup can be modifies, as illustrated in Figure 2.12. This system still requires only one large optical window, or multiple smaller windows on the same side of the facility. Using two sets of relay lenses with different focal lengths enables us to bring both views to the vicinity of the holographic plate, optimizing the image quality. Still, both views are recorded on the same (larger)
holographic plate, enabling simultaneous processing, precise matching of the two views, trimming of the particle traces, and velocity measurements at high spatial resolution. Another drawback of the current setup is the directional ambiguity, since we presently record and reconstruct the two exposures simultaneously. This problem can be resolved by using reference beams with different orientations for the each of the exposures. This improvement will be incorporated in future studies.

During data analysis the two views are matched to find the 3-D coordinates of the particle centroids. Associated procedures for precise on-line calibration of the mirror location and orientation are addressed in detail. A method for calculating the 3-D velocity field using 3-D correlations of the measured location of particle centroids is introduced, but the present 7 µm uncertainty in the 3-D location of the particle causes an unacceptable error level with the present particle displacement and seeding density. As an alternative, we use the measured location of particle centroids for trimming the elongated traces from the original scanned reconstructed images. This procedure erases all the particle tracers whose centroids are located at distances exceeding half the distance between scanned planes (125µm in the present study) from each scan of the sample volume. Only these filtered images are used to determine the two in-plane velocity components, following typical 2-D PIV analysis procedures. The data from the two perpendicular views are then combined to determine the 3-D velocity vector for each interrogation volume. This trimming procedure eliminates the low-pass filtering effect caused by the elongated traces of “distant” particles, effectively quadrupling the spatial resolution and substantially increasing accuracy of the data.
Calibration tests using sparse particle distributions demonstrate that it is indeed possible to match perpendicular views. The standard deviation of the distance between matched particle traces is about 7 µm, and the systematic error is 3 µm, both of which are smaller than the particle diameter. Thus, we can conservatively claim that the uncertainty in locating the particle (and position of velocity vector) in all directions is about 10 µm. Sample three dimensional velocity measurements are performed within a densely seeded flow field in the wake behind a rising bubble. The high particle concentration (~200 particles/mm³) and trimming process enable us to resolve several particles within interrogation volumes of 220×154×250 µm (the latter dimension is the spacing between scanned planes), and use them for PIV measurements. With 50% overlap, the vector spacing is 110×77×250 µm³. A sample with 96×110×61 is used for examining whether the results satisfy the continuity equation. The present trimmed and more densely seeded holograms clearly show substantial improvements over our previous data.

Reference


**Figures and Tables**

**Fig. 2.1** Principle of the single beam two-view holographic particle image velocimetry system. (a) Recording phase. (b) Reconstruction phase.

**Fig. 2.2** Optical layout of holographic recording (solid lines) and reconstruction setup (dashed lines) using a near-forward scatting off-axis scheme.

**Fig. 2.3** Reconstructed image of a single particle and its model as a cylindrical line. (a) Iso-surface of an identified particle with 1.5 mm length in the depth direction. (b) The line fitting process. 2D slices are contour plots of cross sections of the particle.

**Fig. 2.4** Particle trimming process used in velocity calculation. (a) a single 2D image is left in the plane, which is closest to the particle centroid is preserved from the image in Figure 4 (b) same sample area of 192 µm$^2$ (64×64 pixel) containing the particle in (a) before filtering (c) the same area after filtering.
Fig. 2.5  Sketch of experimental setup. The He-Ne laser and a photodiode are used to detect the passage of a rising bubble.

Fig. 2.6  Reconstructed double exposure images of the same bubble in the two perpendicular views.

Fig. 2.7  Results of tests with sparse particle concentration containing 174 particles, identified and matched within a cube of 8 mm×8 mm×8 mm. The particles are represented as ellipsoids with major and minor axes determined by experiment. (a) A 3-D plot of matched particles from the 1st and 2nd views overlapped, showing also a magnified subsection of the cube. (b), (c) and (d) are projections of the same data.

Fig. 2.8  Probability density function of distance (mainly Δy) between the matched traces in the two views. (a) Absolute distance (b) Distance normalized by the measured particle diameter.

Fig. 2.9  Two typical perpendicular filtered particle image pairs (a and b) and their corresponding velocity maps analyzed by auto-correlation 2-D PIV algorithm. Interrogation window size is 64×44 pixel (220µm×154µm), and the vector spacing is 110µm in x and 78µm in y.

Fig. 2.10  A 3D volumetric velocity distribution in the wake of the rising bubble. Total number of vectors is 96×110×61 over a 10 × 10 × 20 mm³ test volume.

Fig. 2.11  Cumulative distribution of $\sigma$ defined in eqn. 13. The numbers in legend indicate the length scales over which the data was averaged prior to calculating $\sigma$. Solid symbols indicate the present data and the hollow symbols present the data by Zhang et al. The insert shows the 90th percentile of $F(\sigma)$ as a function of averaging length scale.

Fig. 2.12  Optional optical setup for recording a single-beam two view hologram with a cubic sample volume located away from the wall.
Fig. 2.1 Principle of the single beam two-view holographic particle image velocimetry system. (a) Recording phase. (b) Reconstruction
Fig. 2.2 Optical layout of holographic recording (solid lines) and reconstruction setup (dashed lines) using a near-forward scattering off-axis scheme.
Fig. 2.3 Reconstructed image of a single particle and its model as a cylindrical line. (a) Iso-surface of an identified particle with 1.5 mm length in the depth direction. (b) The line fitting process. 2D slices are contour plots of cross sections of the particle.
Fig. 2.4. Particle trimming process used in velocity calculation. (a) a single 2D image is left in the plane, which is closest to the particle centroid is preserved from the image in Figure 4 (b) same sample area of 192 μm² (64×64 pixel) containing the particle in (a) before filtering (c) the same area after filtering.
Fig. 2.5 Sketch of experimental setup. The He-Ne laser and a photodiode are used to detect the passage of a rising bubble.
Fig. 2.6. Reconstructed double exposure images of the same bubble in the two perpendicular views.
Criterion for matched particle views:
Distance between line segments ≤ particle diameter
Fig. 2.7 Results of tests with sparse particle concentration containing 174 particles, identified and matched within a cube of 8 mm×8 mm×8 mm. The particles are represented as ellipsoids with major and minor axes determined by experiment. (a) A 3-D plot of matched particles from the 1st and 2nd views overlapped, showing also a magnified subsection of the cube. (b), (c) and (d) are projections of the same data.
Fig. 2.8 Probability density function of distance (mainly $\Delta y$) between the matched traces in the two views. (a) Absolute distance (b) Distance normalized by the measured particle diameter.
Fig. 2.9 Two typical perpendicular filtered particle image pairs (a and b) and their corresponding velocity maps analyzed by auto-correlation 2-D PIV algorithm. Interrogation window size is 64×44 pixel (220µm×154µm), and the vector spacing is 110µm in x and 78µm in y.
Fig. 2.10 A 3D volumetric velocity distribution in the wake of the rising bubble. Total number of vectors is $96 \times 110 \times 61$ over a $10 \times 10 \times 20$ mm$^3$ test volume.
Fig. 2.11 Cumulative distribution of $\bar{\sigma}$ defined in eqn. 13. The numbers in legend indicate the length scales over which the data was averaged prior to calculating $\bar{\sigma}$. Solid symbols indicate the present data and the hollow symbols present the data by Zhang et al. The insert shows the 90th percentile of $F(\bar{\sigma})$ as a function of averaging length scale.
Fig. 2.12 Optional optical setup for recording a single-beam two view hologram with a cubic sample volume located away from the wall.
3. Digital Holographic Recording and Reconstruction Using One-Beam Two-View

3.1 Background and Rationale
The film-based holographic system provides unequivocally high spatial resolution, often around the order of micrometers, and at the same time large measurement volume, usually at the order of decimeter. The ratio between the largest resolvable scale and smallest scale thus ventures into four decades, that provides an excellent experimental tool for studying the phenomena with wide spectra of scales like turbulence. Yet, due to the requirement of wet processing of recording film (developing and fixation processes), film based holographic techniques are simply too cumbersome to be suitable for measuring dynamical phenomena with wide temporal spectra, particularly in the area of biological flows which involve 4-dimensional (spatial and temporal) locomotion of organisms and 4-dimensional flows surrounding them. A more specific example is marine copepods. It has been estimated that the average concentration of marine copepods is at about one per liter, making them the most numerous multi-cellular animals on earth (Boxshall 1998.) These micro-crustaceans form major links between the plankton world and nektonic organisms. The rate and specificity of their feeding can control phytoplankton population growth, while copepods themselves are an important source of food for fish, euphausiids and coelenterates (Mann and Lazier, 1996). Along with food availability and predator pressure, the copepod population dynamics are affected by swimming behavior and the types of flow fields they generate to capture food, which is species dependent (Strickler, 1982). Locomotion and the generation of
feeding currents increase the encounter rates with food, and can enhance the copepod’s detection ability by stimulating escapes in motile prey (Yen and Strickler, 1996). Alternately, motionless sinking can make the copepod less conspicuous to predators or prey. Within the species, advertisement is important for reproduction (Doall et al., 1998; Yen and Strickler, 1996). Because copepods sense their environment utilizing antennules studded with arrays of setae that respond to fluid deformation, the flow field that a swimming animal generates is a ‘lens’ through which the copepod views its surroundings. Knowledge about this flow field is important for understanding the impact of swimming behavior on the success of a copepod in locating food, avoiding predators and finding mates.

Current knowledge is largely based on video observations, including a Schlieren system for visualizing wakes (Strickler, 1977), high-speed cinematography for understanding how food particles are captured and feeding currents are generated (Koehl and Strickler, 1981; Strickler, 1984), and threedimensional (3-D) video tracking of free-swimming copepods in particle fields (Paffenhöfer et al., 1995; Strickler, 1985). The latter combines particle tracks at different times to observe two-dimensional (2-D) flow fields in lateral and dorsal views. More recently, Particle Image Velocimetry (PIV) has been used to map instantaneous 2-D flow fields around tethered specimens (van Duren et al., 1998). However, since flow fields around copepods are 3-D, unsteady and vary with swimming speeds and orientations (Bundy and Paffenhöfer, 1996; Strickler, 1982; van Duren et al., 1998), 3-D measurements are essential. Considering the importance of copepods in the aquatic and marine food webs, and 30 years of related hypotheses, it seems worthwhile to investigate whether digital cinematographic holography may break
barriers and allow us to test these hypotheses. Unlike video microscopy, holography maintains the same lateral resolution over a substantial depth (Vikram, 1992). This advantage has led to the development of several submersible holography systems for studying plankton, starting with a sample volume of a few ml (Carder et al., 1982), to samples of one liter and above (Katz et al., 1999; Malkiel et al., 1999; O’Hern et al., 1988; Watson et al., 2001). The latter utilize pulsed lasers and emulsions as recording media. Cinematographic holography was introduced for laboratory research decades ago (Heflinger et al., 1978; Knox and Brooks, 1969), but difficulties in acquiring and processing data resulted in limited applications. Recent development in digital imaging, which simplifies the acquisition, and computing power, which enables numerical reconstruction, has led to renewed interest in cinematic holography (Kebbel et al., 1999; Owen and Zozulya, 2000; Xu et al., 2001). The limited resolution of digital imaging, which is at least an order of magnitude lower than that of holographic emulsions, restricts us to in-line holography. This technique (details follow) maximizes the fringe spacing. Because the reference beam typically passes through the sample volume, it becomes increasingly degraded with increasing particle concentration. Consequently, the reconstructed images are noisier and the maximum particle concentration is lower compared to off-axis holograms (Zhang et al., 1997).

Holographic PIV is the only technique to date that can measure a 3-D instantaneous velocity distribution over a finite volume (Barnhart et al., 1994; Pu and Meng, 2000; Sheng et al., 2003; Tao et al., 2002; Zhang et al., 1997) at a resolution of millions of vectors. The velocity is obtained by recording two exposures of a flow field seeded with microscopic particles, and measuring the displacement of these particles. However, the
depth coordinate of a reconstructed particle is less accurate than the lateral coordinates, severely reducing the ability to estimate the corresponding velocity component. This problem has been solved by recording two inclined holograms, using each for determining a 3-D distribution of two velocity components, and matching the two sets to obtain the 3-D velocity. Utilizing emulsion and off-axis holography, Tao et al. (2002) measured 136×130×128 3-D velocity vectors in a cubic sample with sides of about 45 mm. By inserting an inclined mirror in the path of the illuminating beam inside the test facility, the incident and reflected beams create two views that can be recorded on the same emulsion (Sheng et al., 2003). Using off-axis holography, this method enables measurement of particle locations to within 7 µm, and resolves about 200 particles mm–3. We adopt this approach, but use digital inline holography to record a time series of a free-swimming copepod and the flow field surrounding this animal. To our knowledge, this is the first time that digital holographic PIV has been implemented as a tool for simultaneous observation of the animal’s behavior and measurement of the complex flow around it.

3.2 Digital Holographic Recording and Reconstruction

3.2.1 Generic Mathematical Models for Digital Holographic Recording and Reconstruction

In digital holographic recording, we replace the recording film with a digital recording media like a digital CCD camera, sketched in fig. 3.1. The interference patterns previously formed on the recording film is directly registered as digital gray scale images. As time progresses, a stream of holograms can then be recorded and stored on a computer. Theoretically, this recording arrangement would not alter the outcome of the reconstruction, had the recording media have infinite resolution and size. The following
mathematical treatment hence assumes the holographic recording occurred in the continuous domain regardless of recording schemes (in-line or off-axis.)

3.2.1.1 Mathematics for Recording

We treat the light ($O$) scattered or emitted by the $i$th object located at $(x_i, y_i, z_i)$ as a volume integral of point emitters with the complex optical strength $\tilde{p}_i(x-x_i, y-y_i, z-z_i)$,

$$\tilde{O}_i(x,y;z;x_i,y_i,z_i) = \iiint \tilde{p}_i(x_i,y_i,z_i)\tilde{h}(x,y,z;x_i,y_i,z_i)dx_i dy_i dz_i,$$  \hspace{1cm} (3.1)

where $\tilde{h}$ is the point source emitter propagation function, which has the general form as,

$$\tilde{h}(x,y,z;x_i,y_i,z_i) = \frac{\exp\left(\frac{jk\sqrt{(x-x_i)^2 + (y-y_i)^2 + (z-z_i)^2}}{j\lambda\sqrt{(x-x_i)^2 + (y-y_i)^2 + (z-z_i)^2}}\right)}{\sqrt{(x-x_i)^2 + (y-y_i)^2 + (z-z_i)^2}}.$$ \hspace{1cm} (3.2)

It is clear that Eqn. 3.1 represents a “distribution” function of a phase function located at $(x_i, y_i, z_i)$ with a finite support $V \in \mathbb{R}^3$ convoluting with a scattering function, $\tilde{h}$. For simplicity, the volume integral in 3.1 will be denoted as an operator “$\otimes$” hereinafter.

Hence, the superposition of optical wave fronts from a cluster of scattering objects can then be written as

$$\tilde{O}(x,y,z) = \sum_i \tilde{O}_i(x,y,z;x_i,y_i,z_i)$$

$$= \sum_i \tilde{p}(x,y,z;x_i,y_i,z_i) \otimes \tilde{h}(x,y,z),$$ \hspace{1cm} (3.3)

assuming the high-order scattering is negligible. Using Schwatz distribution functions, eqn. 3.3 can be rewritten in its continuous form (3.4),

$$\tilde{O}(x,y,z) = \tilde{P} \otimes \tilde{h},$$ \hspace{1cm} (3.4)
where $\tilde{P}$ is a distribution function of complex phase in a volume in which all the scatters are located. When the recording plane is located at $z=0$ and a reference illumination with known phase distribution, $\tilde{R}(x, y, z) = A(x, y, z)e^{i\phi(x, y, z)}$, is illuminating the hologram plane, the resultant optical field is written as the superposition of two optical fields, $O$ and $R$,

$$\tilde{H}(x, y) = \tilde{O} + \tilde{R}, \quad (3.5)$$

and the irradiance of two superposed optical fields is,

$$I_H(x, y) = \tilde{H} \cdot \tilde{H}^* = A^2 + Ae^{i\phi}(\tilde{P}^* \otimes \tilde{h}^*) + Ae^{-i\phi}(\tilde{P} \otimes \tilde{h}) + |\tilde{P} \otimes \tilde{h}|^2, \quad (3.6)$$

where “$*$” denotes the complex conjugate, and “$|$” the modulus. Equation 3.6 represents the interference patterns recorded on the digital medium, since only the irradiance can be converted directly to electron here. Physically, the first term in 3.6 represents the background set by the reference beam. It provides a much-needed offset for recording full peak-to-peak interference waveforms. A value of the immediate level within the dynamic range of the recording medium is preferred in practice. For example, for an 8-bit gray scale camera, reference beam intensity alone should register a grayscale value around 128 to expose a good hologram. The second term represents the amplitude modulation of the scattered beam, $\tilde{P} \otimes \tilde{h}$, and reference beam, $\tilde{R}$. The term assumes the form of an amplitude modulation, i.e. a carrier function multiplying a signal function. In this case, the carrier function is the reference beam and the signal is the scattered beam. In the reciprocal space, the amplitude modulation represents a convolution of scattered light spectra and reference light spectra. In special cases, the reference beams have
limited wave numbers in their reciprocal spaces, i.e. band limited. The reference beam would shift the spectra of also band-limited scattered beams in the reciprocal space. A properly designed filter in the reciprocal space can thus isolate the scattered light from the reference light, the basic principle used in off-axis recording scheme. The third term is the phase-conjugate of the second term. It generates a similar amplitude modulation as the second term would. For instance, a diverging point source generates the same interference pattern as a converging point source does. Therefore, the third term represents a source of “colored” noise, i.e. noise has the same spectrum distribution as the signal. The last term represents the secondary scattering. It can be modeled as probability density function of secondary scattering at current location convoluted with a point source function, i.e. has the same form as the 2nd and 3rd terms. The probability density function increases as the density of scatters, void fraction of the scatters, and strength of individual scatter. The fourth term is also a noise term that tends to be much smoother and Gaussian-like at large scales but produces non-Gaussian-like statistics at small scales. In high-density particle holography, this is the very culprit for the degradation of signal to noise ratio.

3.2.1.2 Mathematics for Reconstruction

In optical reconstruction of a transmission hologram, the hologram is illuminated with a conjugate reference beam, i.e. a “backward propagating reference beam”, \( \bar{R}^* = Ae^{-i\phi} \). Mathematically, the process can be simply described as a transmission density aperture directly proportional to the recorded hologram intensity is illuminated by a known optical field and then is allowed to propagate forward using Hygen’s principle,

\[
\phi_r(x_r, y_r, z_r) = \int (1-tI_h)\bar{R}^*(x, y, 0)\tilde{h}(x_r, y_r, z_r; x, y, 0)dxdydz,
\]

(3.7)
where \( t \) is the transfer coefficient with no direct consequence to the final result. Equation 3.7 can be decomposed into two integrals,

\[
\varphi_r = \tilde{R}^* \bigg|_{z=0} \otimes \tilde{h}(x_r, y_r, z_r) + (I + \bar{R}) \bigg|_{z=0} \otimes \tilde{h}(x_r, y_r, z_r).
\] (3.8)

The first integral is wave front propagation of \( \tilde{R}^* \), and the second one is the reconstructed wave front containing scattering light. Substituting 3.6 into 3.8, \( \varphi_r \) results in

\[
\varphi_r = \tilde{R}^* \bigg|_{z=0} \otimes h(x_r, y_r, z_r) + \left\{ t \left[ A^2 + A e^{i\theta} \left( \tilde{P}^* \otimes \tilde{h}^* \right) + A e^{-i\theta} \left( \tilde{P} \otimes \tilde{h} \right) \right] \right\} \otimes \tilde{h}(x_r, y_r, z_r). \] (3.9)

Equation 3.9 can be rearranged and simplified as 3.10 by neglecting secondary scattering

\[
\varphi_r = \tilde{R}' \otimes \tilde{h}' + \tilde{A}' \tilde{P}^* \otimes \tilde{h}^* \otimes \tilde{h}' + \tilde{A} e^{-i\theta} \tilde{P} \otimes \tilde{h} \otimes \tilde{h}', \] (3.10)

where \( \tilde{R}' = \tilde{R}^* + tA^2(x, y, 0) \), and \( \tilde{A}' = tA^2 \). Now, it is clear that the first term is the propagation of the modified reference beam that sets the background, the second term is the reconstructed image (will be proven later), and the last one is the virtual image generated at the twice distance away from the hologram plane (will shown later.)

**Real Image:** Since the operator, “\( \otimes \)”, is commutable, thus the proof of the reconstruction lies in the properties of the point source propagation function, \( \tilde{h} \). If the original scatter is located at \( (x_{ob}, y_{ob}, z_{ob}) \), the hologram is at \( (x_h, y_h, z_h = 0) \), and the reconstruction location is at \( (x_r, y_r, z_r) \), a conjoined point response function, \( \tilde{h}' = \tilde{h}^* \otimes \tilde{h} \), can be expressed as,

\[
\tilde{h}'(x_r, y_r, z_r; x_{ob}, y_{ob}, z_{ob}; x_h, y_h, z_h) = \tilde{h}^* (x_h - x_{ob}, y_h - y_{ob}, z_h - z_{ob}) \otimes \tilde{h} (x_r - x_h, y_r - y_h, z_r - z_h) .
\]

The further derivatives are carried out in the Fourier space without any assumptions for the form of the function, \( \tilde{h} \), as long as the continuous Fourier transformation exists.
Taking Fourier transformation on both sides and applying the fact that $\tilde{h}$ is an even function, we obtain

$$
\tilde{H}'(\omega_x, \omega_y, \omega_z) = \tilde{H}^*(\omega_x, \omega_y, \omega_z) e^{j(\sigma_x x_b + \sigma_y y_b + \sigma_z z_b)} \cdot \tilde{H}(\omega_x, \omega_y, \omega_z) e^{-j(\sigma_x x_b + \sigma_y y_b + \sigma_z z_b)}
$$

$$
= |\tilde{H}|^2 \cdot e^{-j[\omega_b(x_b - x_{ob}) + \omega_b(y_b - y_{ob}) + \omega_b(z_b - z_{ob})]}
$$

(3.11)

where $\tilde{H}$ is the Fourier transform of $\tilde{h}(x, y, z)$, and $|\tilde{H}|$ is the magnitude. Thus, the conjoined transfer function, $\tilde{h}'$, is a delta function with a constant scaling factor in physical space,

$$
\tilde{h}' = a \delta(x_r - x_{ob}) \delta(y_r - y_{ob}) \delta(z_r - z_{ob}),
$$

(3.12)

Substituting (3.12) into the second term in (3.10), we can obtain

$$
A'(\tilde{P}^* \otimes \tilde{h}')_{x,y,z} = A' a \tilde{P}^* \otimes \delta(x_r - x_{ob}, y_r - y_{ob}, z_r - z_{ob})
$$

$$
= (A' a) \tilde{P}^* (x_{ob}, y_{ob}, z_{ob})
$$

(3.13)

Consequently, Equation 3.13 states that the optical field at $(x_r, y_r, z_r)$ during the reconstruction is the exact conjugate field of the field at the same 3-D location during the recording. Thus, the numerical reconstruction process indeed recovers the 3D locations of scatters by simply backward-propagating the recorded hologram using (3.10).

**Virtual Image:** The term, $\tilde{h} \otimes \tilde{h}_r$, represents physically that a optical field is propagated to the hologram plane and then re-propagate further to a location at $(x_r, y_r, z_r)$. To satisfy the basic Heygen’s principle, the function must have the following property,

$$
\tilde{h}(x_h, y_h, z_h; x_{ob}, y_{ob}, z_{ob}) \otimes \tilde{h}(x, y, z, x_h, y_h, z_h) = \tilde{h}(x_r, y_r, z_r; x_{ob}, y_{ob}, z_{ob})
$$

$$
= \tilde{h}(x_r - x_{ob}, y_r - y_{ob}, z_r - z_{ob})
$$

(3.14)

It needs to point out that the aforementioned property of $\tilde{h}$ is the intrinsic requirement for Hygen’s principle to hold. The proof of such property can be demonstrated using
Fresnel’s formula of \( h \) that assumes the validity of the binomial expansion of point source. Applying (3.14) into (3.10), we can observe that the reconstructed optical field form the third term is a “diffraction” field propagated from the original field \( \tilde{P} \) with a phase shift, \( 2\phi \).

3.2.2 Several Special Cases

3.2.2.1 In-line Holography of Real Particles

Fig. 3.1 show a typical in-line holographic recording scheme, the particle field is illuminated with a single collimated coherent light. The scattered portion by the particles is object beam, and the undisturbed part of the illumination beam is reference beam. When the particle is opaque (or large refractive index difference between particle and surrounding medium), the scattered light is essentially dominated by the diffraction. The optical field, \( \tilde{P} \), can be expressed as,

\[
\tilde{P} = \sum_{i} A a_i \left( x - x_i, y - y_i, z_i \right) \otimes \tilde{h},
\]

(3.15)

where \( a_i \) is a two-dimensional aperture function of i-th particle defined as a heaviside step function with 1 inside the cross-section and 0 otherwise. The reference beam here is simply uniform plane wave with the amplitude, \( A \),

\[
\tilde{R} = A.
\]

(3.16)

Combining (3.10), (3.15) and (3.6), the recorded hologram is expressed as

\[
I_h = A^2 - \sum 2 A a_i \otimes \text{Re} \left( \tilde{h} \right) + O \left( A \sum a_i \right)^2.
\]

(3.17)

The useful interference fringes are in fact registered as the cosine transformation of the aperture function. The second term in Eq. 3.17 has a negative sign here since the object
is opaque. During the reconstruction (Eq. 3.10), the reconstructed optical field at \((x_r, y_r, z_r)\) becomes,

\[
\varphi_r = I_h \otimes \tilde{h}(x_r, y_r, z_r) = A^2 \otimes \tilde{h}(x_r, y_r, z_r) - \sum Aa_i \otimes \delta - \sum Aa_i \otimes \tilde{h}_{z=z_i} + O(\cdot)
\]

and the reconstructed irradiance field, \(I_r = \|\varphi_r\|^2\), will form in-focus images of the real particles simplified as 2-D cross sections.

### 3.2.2.2 In-line Holography of Phase Particles

For in-line recording of phase particles, the aperture function used in Eq. 3.15 is replaced by a phase function on a unit circle, \(\tilde{a}(x, y, 0) = e^{i\phi(x,y)}\), representing the phase alteration to the illumination light. The hologram recorded becomes,

\[
I_h = A^2 + A \sum \text{Re}(\tilde{a}_i) \otimes \text{Re}(\tilde{h}) - A \sum \text{Im}(\tilde{a}_i) \otimes \text{Im}(\tilde{h}) + O,
\]

where the second is a cosine transformation of the aperture function and the third term a sine transformation. To extract the phase object during the reconstruction, one needs to follow the step in Eq. 3.18. The reconstructed intensity is a phase-gram of \(\varphi_r\) rather than the intensity \(I_r\). This recording scenario is exceedingly useful for study the deformation of a transparent boundary such as a flexible boundary.

### 3.2.2.3 In-line Recording with a Divergence Illumination

This recording scheme is proven to be particularly useful when a high-resolution imaging is required. However, in the next chapter, we will introduce a much simpler system that provides higher spatial resolution and less complexity. Assuming the object is real and is located at \(p_0 = (x_0, y_0, z_0)\), a point source centered at \(p_1 = (x_1, y_1, z_1)\) is used to illuminate and record the hologram. The light scattered by the object is,
\[ P = \left[ a(x - x_0, y - y_0; z_0) \frac{e^{j|p - \tilde{p}|}}{j\lambda|\tilde{p} - \tilde{p}|} \right] \otimes \tilde{h}. \] (4.20)

And the reference beam at hologram plane \((x_h, y_h; z_h = 0)\) is,

\[ R = \frac{e^{j|p - \tilde{p}|}}{j\lambda|\tilde{p}_h - \tilde{p}|}, \] (4.21)

where \(\tilde{p}_h\) is the position vector of a point on the hologram. The hologram is then,

\[ I_h = -\left(\lambda|\tilde{p}_h - \tilde{p}_l|\right)^2 + \frac{e^{-j|p - \tilde{p}|}}{j\lambda|\tilde{p}_h - \tilde{p}|} \left[ a(x - x_0, y - y_0; z_0) \frac{e^{j|p - \tilde{p}|}}{j\lambda|\tilde{p} - \tilde{p}_l|} \right] \otimes \tilde{h} + \]

\[ \frac{e^{-j|p - \tilde{p}|}}{j\lambda|\tilde{p}_h - \tilde{p}|} \left[ a(x - x_0, y - y_0; z_0) \frac{e^{-j|p - \tilde{p}|}}{j\lambda|\tilde{p} - \tilde{p}_l|} \right] \otimes \tilde{h}^* + O(\cdot) \] (4.22)

During the reconstruction, we will “illuminate” the hologram, \(I_h\), with the conjugate reference beam, i.e. a convergence beam focusing at the point, \(\tilde{p}_l\), and then propagate forward. The process is best described mathematically as

\[ \varphi_r = (I_h R^*) \otimes \tilde{h} \]

\[ = -\left(\lambda|\tilde{p}_h - \tilde{p}_l|\right)^2 + \frac{e^{-j|p - \tilde{p}|}}{j\lambda|\tilde{p}_r - \tilde{p}_l|} \left[ a(x - x_0, y - y_0; z_0) \frac{e^{-j|p - \tilde{p}|}}{j\lambda|\tilde{p} - \tilde{p}_l|} \right] \otimes \tilde{h}^* \otimes \tilde{h} + \]

\[ \frac{e^{-j|p - \tilde{p}|}}{j\lambda|\tilde{p}_h - \tilde{p}|} \left[ a(x - x_0, y - y_0; z_0) \frac{e^{-j|p - \tilde{p}|}}{j\lambda|\tilde{p} - \tilde{p}_l|} \right] \otimes \tilde{h} \otimes \tilde{h} + O(\cdot) \]

\[ = -\left(\lambda|\tilde{p}_h - \tilde{p}_l|\right)^2 + \left[ a(x - x_0, y - y_0; z_0) \frac{e^{-j|p - \tilde{p}|}}{j\lambda|\tilde{p} - \tilde{p}_l|} \right] \otimes \delta + \]

\[ \frac{e^{-j|p - \tilde{p}|}}{j\lambda|\tilde{p}_h - \tilde{p}|} \left[ a(x - x_0, y - y_0; z_0) \frac{e^{-j|p - \tilde{p}|}}{j\lambda|\tilde{p} - \tilde{p}_l|} \right] \otimes \tilde{h}(\tilde{p} - 2\tilde{p}_l) + O(\cdot) \]

It is clear that a carrier function is modulated onto the aperture function, which needs to be demodulated by simply multiplying the phase conjugate of the carrier function or by
simply taking the amplitude of $\varphi_r$. The importance of this recording and reconstruction model lies in the applications when a focused backlit microscope with coherent light illumination when intense illumination and larger magnification are required.

### 3.2.2.4 Off-axis Recording with a Uniform Collimated Reference Beam

The particle scattering field is the same as Eq. (3.15). The reference wave can be written as Eq. (3.23):

$$R = Ae^{j(k_\delta x_0 + k_\delta y_0)}$$  \hspace{1cm} (3.23)

where $\vec{k}$ is the propagation vector, $\vec{n}_{hx}$ and $\vec{n}_{hy}$ are the unit vectors of the hologram plane in x and y directions. When reference beam is collimated and the hologram is a plane, the coefficients, $\vec{k} \cdot \vec{n}_{hx}$ and $\vec{k} \cdot \vec{n}_{hy}$, are constant. The recorded hologram is expressed as

$$I_h = A^2 + A \sum a_\delta \Re\left(e^{-j(k_\delta x_0 + k_\delta y_0)\vec{n}_h}\right) + O\left(\left(A \sum a_\delta\right)^2\right)$$  \hspace{1cm} (3.24)

where the interference pattern (2nd term) is a cosine transformation of the aperture function with a phase shift. The role of such a phase shift is to shift the propagation in the angular spectrum, i.e., the light propagates in a shifted direction. The simple demodulation procedure can be performed before the hologram is reconstructed. However, the demodulation can also be attained through the normal reconstruction process by multiplying the hologram with a conjugate reference beam,

$$R^* = Ae^{-j(k_\delta x_0 + k_\delta y_0)}.$$  \hspace{1cm}

In this case, simple derivation can show that the reconstructed field is the same as those in the in-line case, except that the virtual image is modulated in propagation direction rather than in the propagation depth.

$$\varphi_r = I_h \otimes \tilde{h}(x_r, y_r, z_r)$$

$$= A^2 \otimes \tilde{h}(x_r, y_r, z_r) - \sum A a_i \otimes \delta - \sum A a_i \otimes \Re\left(e^{-2j(k_\delta x_0 + k_\delta y_0)\vec{n}_h}_{z, z_i}\right) + O(\cdot)$$
3.2.3 Simplification and Discrete Implementation

The first simplification comes from the point source propagation function, $\tilde{h}$. When the condition $z^2 >> x^2 + y^2$ is satisfied, the point source function can be expanded in the binomial series,

$$
\tilde{h}_z = \frac{1}{j\lambda z} e^{jkz} \exp \left[ j \frac{\pi}{\lambda z} (x^2 + y^2) \right],
$$

(3.26)

which is also known as Huygens-Fresnel approximation. The first part of Eq. 3.26 is a constant phase shift for a given $z$. It is of little consequences if the irradiance is our only concern, and is often neglected in most application. Yet it has decisive effects when a precise phase representation of the reconstruction is needed such as in phase object holography. Table 3.1 provides a list of properties of $\tilde{h}_z$ used in the analysis and implementation.

To numerically reconstruct a digitally recorded hologram, a digital implementation of the continuous system, Eq. (3.10), is required. It is noticed that the point source kernel, $\tilde{h}_z$, is neither band-limited nor space-limited. The direct consequence is the discrete implementation of continuous function does not exist. However, owing to discretization and limited size, the digitally recorded hologram itself is fortunately both band-limited and space-limited. The recorded hologram is thus considered as a real hologram, $I_h$, passing through an ideal low-pass filter, $g_{LP}$, with cut-off frequencies $U_c$ and $V_c$, i.e.,

$$
I_{hLP} = I_h \otimes g_{LP}.
$$

(3.27)

Thus, the reconstructed field, $\varphi_z$, is

$$
\varphi_z = I_h \otimes g_{LP} \otimes \tilde{h}_z = I_h \otimes \tilde{h}_{LP} .
$$

(3.28)
What shown above is, in fact, the direct consequences of linearity: the assumption that the input is band-limited is equivalent to the assumption that the reconstruction kernel is band-limited. Therefore, the discrete form of \( \tilde{h} \) can be obtained from the continuous function by sampling at the rate of twice of the bandwidth. One observation is that when the band-limit condition on the recorded hologram is violated, i.e. in the cases that particle scattering has a very wide range of angles due to metallic coating of a particle or optical aberration, the discrete implementation does not exist. In this situation, no linear discrete system can produce faithful reconstruction without introducing severe aberrations. Another observation is made based on the fact that the band-limited function, \( \tilde{h}_{L.P} \), drops quickly to zero beyond some spatial limits \( X_c \) and \( Y_c \). In other words, it is “almost” space-limited. So, the approximate cut-off points in space are,

\[
X_c = \pm \frac{\lambda}{2\pi} U_c, \quad Y_c = \pm \frac{\lambda}{2\pi} V_c, \tag{3.29}
\]

Thus, the approximate spatial extent of the kernel can be deduced as,

\[
N_h = 2 \left\lfloor \frac{X_c}{\Delta X} \right\rfloor + 1, \quad M_h = 2 \left\lfloor \frac{Y_c}{\Delta Y} \right\rfloor + 1 \tag{3.30}
\]

where \( \lfloor \cdot \rfloor \) denotes the lower bound integer of the operand. From Eq. 3.29 and 3.30, one can design the recording system consistent with the band-limit and space-limit requirements.

In order to increase computational efficiency, circular convolution can be used instead of linear convolution. It needs to point out that the implicit periodicity assumption of using circular convolution affects the results in the form of overlapping and wrapping. If the size of the convolution is not large enough to cover the size of the resultant field, then overlapping occurs. This phenomenon is more detrimental to the
reconstruction since the size of the reconstructed field is significantly larger than that of recording. For fast implementation, continuous analytical Fourier transform of \( \tilde{h}_z \), is sampled to yield the discrete form for the band-limit version,

\[
\tilde{H}_z = \exp\left\{-j2\pi \left( \frac{n^2}{(N\Delta x)^2} + \frac{m^2}{(M\Delta y)^2} \right) \right\}, \tag{3.31}
\]

where \( N \) and \( M \) are total number of samples in \( x \) and \( y \) directions. Formula 3.31 allows the digitization of the hologram using different spacing for each axis separately.

### 3.3 Digital Holography System Using One Beam Two View Concept

The 80 ml sample volume is illuminated with a collimated, 3 mW He-Ne laser beam (Fig. 3.3). Part of this beam is diffracted by objects in the sample volume – seed particles and a copepod – and interferes with the undisturbed part of this beam. The interference pattern is recorded by a lens-less 2048 × 2048 pixel camera with a pixel pitch of 7.4 µm, and a 15×15 mm field of view. Two perpendicular views are obtained by inserting mirrors on the walls of the test chamber. Objects located in regions where the incident and reflected beams overlap are illuminated twice in perpendicular directions. The two views are recorded on the same charge-coupled device (CCD) chip, but laterally separated. (Fig. 3.4). Use of two mirrors (Fig. 3.3) doubles the triangular overlapping volumes.

Because the objects are moving, the exposure time must be short enough to prevent the smallest resolvable fringe spacing from smearing. Here, the velocity does not exceed 4 mm s\(^{-1}\) (540 pixels s\(^{-1}\)), and the electronic shuttering of the camera (0.1 ms exposure time) is sufficient for reducing the movement during exposure to much less than one pixel. Faster flows (~1 m/s) require high-power pulsed lasers. Images are recorded at 15 frames per second, while buffering 10 s segments (150 frames). Acquisition starts when a
copepod appears in the field of view. Successful sequences, which capture copepods in a region with two views, and sufficiently far from the wall (>4 mm), are stored.

Our animal, a female *Diaptomus minutus*, was freshly caught in Lake Michigan and transported to Baltimore in a Dewar’s jar. The vessel contained artesian well water from the Pryor Street well in Milwaukee. It was seeded with monodisperse, 20 µm diameter polystyrene spheres (specific gravity 1.05) at a concentration of 4 mm⁻³. Although denser seeding would improve the spatial resolution, it would also reduce the signal to noise ratio of reconstructed images. Fig. 2 is a sample digital in-line hologram containing two interference patterns (“shadows”) of the same copepod, along with the invisible signatures of the seed particles.

The digital holograms are reconstructed numerically using a Kirchhoff integral

\[
U(\mathbf{r}_0) = \iint \frac{I(\mathbf{r})}{\lambda r_0} \exp\left(j k r_0\right) \cdot \cos(\mathbf{n} \cdot \mathbf{r}_0) \cdot d\mathbf{s}
\]

which relates the intensity of light at a point in the reconstruction plane, \(I(\mathbf{r}_0) = UU^*\) (where \(U\) is the complex light amplitude and \(U^*\) is the complex conjugate), to the intensity in the hologram plane, \(I(\mathbf{r}_1)\). Here \(r_0\) and \(r_1\) share the same origin, \(\mathbf{r}_{01} = \mathbf{r}_0 - \mathbf{r}_1\), \(\mathbf{n}\) is the normal to the hologram, and \(k\) is the wavenumber of the reconstruction wavelength, \(\lambda\). Imposing the paraxial approximation,

\[
U(x, y, z) = \iint \frac{I(x, y)}{\lambda z} h_0(\xi - x, \eta - y) \cdot d\xi d\eta = I \otimes h_c
\]

where \(z\) is the distance between the hologram and the reconstruction plane. This convolution integration (Fresnel transformation) is performed over the entire hologram.
for each desired depth. Analysis in the spectral domain reduces the computation time. A similar approach is presented in Milgram and Li, 2002.

Subsequent analysis consists of applying a mirroring transformation to overlap the two reconstructed volumes. Using proximity criteria to match perpendicular images of the same particle, we can determine the 3-D location of this particle to within microns (29). Cross-correlation of reconstructed areas in successive frames, which contain traces of the same particle, is used for determining the displacement of each particle. In-house developed software is used for this purpose (Roth and Katz, 2001; Roth et al., 1999; Shridhar and Katz, 1995). The 3-D velocity is determined by matching the data from the two views.

### 3.4 Results

Fig. 3.5 shows reconstructed perpendicular views of the copepod and seed particles, computed from the hologram shown in Fig. 3.4. Note that the two views are reconstructed at different depths. Clearly, digital reconstruction of in-line holograms can resolve fine details of the copepod structure. Sample instantaneous distributions of 3-D velocity vectors in the vicinity of this copepod in the laboratory and copepod reference frames are presented in Figs. 3.6a and b, respectively. The graphic representation of the copepod has the correct scales. Vectors that are located within the central 2/3 span of the antennules are black; vectors outside of this span are gray. A recirculating flow pattern is evident in the copepod frame of reference, which sinks at an average speed of 0.29 mm s$^{-1}$. Similar sets of 3-D velocity distributions are obtained from each pair of reconstructed holograms.
The reconstructed images from 15 sequences consistently show that the copepod sinks for several seconds. It then executes a short hop upwards and resumes sinking (see supporting information). As it sinks, the copepod generates a feeding current by moving its feeding appendages. The present 15 Hz recording rate may not be sufficient for following the (high frequency) motion of the appendages, but different phases of their motion are discernable (Fig. 3.5). The “high” speed flow generated by the appendages (Fig. 3.6a) is most evident in the ventral region of the copepod (Z<79.5 mm), but extends to the anterior and posterior regions. The feeding current generates a reaction force that propels the copepod. This vertically directed force acts against the copepod’s excess weight (weight minus buoyancy) and drag, reducing its sinking rate compared to the terminal speed (speed with no feeding current).

The recirculating flow pattern generated by the copepod is clearly demonstrated in Fig. 3.7 by combining reconstructed images, shifted so that the image of the copepod is fixed. Blurring of the copepod occurs due to slight variations in sinking speed, and motion of the appendages. Streaks generated by the seed particles are clearly evident in both views. Above the copepod, several particles are drawn towards the center of the copepod. Below and to the sides of the copepod, particles are ejected downwards, subsequently looping around and migrating upward (relative to the copepod), some of them touching its antennules. Quantitative data on the trajectory and velocity along the path of selected particles are presented in Figs. 3.9 and 3.10. The velocity peaks in a narrow domain near the tips of the appendages, reaching 3.6 mm/s, i.e. 12.5 times the sinking velocity. We use these data to estimate the propulsive force generated by the copepod.
3.5 Discussion

Excess Weight and Propulsive Force: The particles slightly beyond the reach of the antennules circumvent the copepod, creating a closed streamline pattern, without a separated wake. This pattern is characteristic of low Reynolds numbers flows ($Re_L = UL/\nu$, $U$ and $L$ being characteristic velocity and length scales, respectively, and $\nu$ is kinematic viscosity), such as Stokes flows with $Re<<1$, or Oseen flows with $Re~1$ (Pozrikidis, 1992). The present $Re$, based on the sinking velocity and copepod prosome length, or diameter of the recirculation zone are 0.29 or 1.2, respectively. Thus, the flow lies in the transition region between Stokes and Oseen flows. Here we model it using the simpler Stokes flow solutions to estimate the magnitude of the forces produced by the copepod.

The horizontal ($u$) and vertical ($v$) velocity components induced by a vertical point force (Stokeslet) in Stokes flow (34, 35) are

$$v = \frac{f}{8\pi \mu L} \left( \frac{1}{r/L} + \frac{(y/L)^2}{(r/L)^3} \right)$$

$$u = \frac{f}{8\pi \mu L} \left( \frac{x y / L^2}{(r/L)^3} \right),$$

(3.34)

where $f$ is the force, $\mu$ is the liquid viscosity, $x$ and $y$ are coordinates, and $r$ is distance from the origin. The Stokeslet realistically describes the far-field flow but neglects the fact that the object has a finite size. The far-field net effect of the copepod on the surrounding flow, its excess weight ($w_{excess}$), can be modeled as a Stokeslet.

The flow pattern for a Stokeslet in an absolute frame of reference is illustrated in Fig. 3.8a, with $U_{ref} = w_{excess}/8\pi \mu L$. A recirculating flow pattern appears in a frame of reference moving downward at a fraction of $U_{ref}$ (Fig. 3.8b). For the recirculation to form, there must be a residual downward velocity component (jet) below the object in its own reference frame. This flow can only be generated by a propulsive force. Thus, in reducing
its sinking speed and generating a propulsive feeding current, the copepod creates a recirculating pattern that extends slightly beyond its antennae. This combination of sinking and feeding is not discussed in detailed conceptual and numerical analyses of the forces acting on a swimming copepod under various conditions (Jiang et al., 2002a; Jiang et al., 2002b). They include cases of stationary bodies producing feeding currents (hovering or tethered) and freely sinking bodies, both of which do not generate recirculating patterns.

The data can be used for estimating $w_{\text{excess}}$. In a reference frame sinking at $v_{\text{sink}}$, the vertical velocity component vanishes at $y_o = \pm w_{\text{excess}}/4 \pi \mu v_{\text{sink}}$. Estimating $y_o$ as half the distance between the points with zero velocity in Fig. 3.7 ($y_o=2$ mm), and since $v_{\text{sink}} = 0.29$ mm/s, one obtains $w_{\text{excess}} = 7.2 \times 10^{-9}$ N. With a volume of $1.1 \times 10^{-10}$ m$^3$, determined following Chojnacki (1983), the estimated excess density of the copepod is 6.7 kg/m$^3$ (1006.7 kg/m$^3$). This value falls in the range measured by Svetlichny (1980) and Knutsen et al. (2001). The same analysis can be performed in any frame, including one without recirculation, by measuring the velocity and distance between points above and below the copepod with the same velocity.

One can also estimate the propulsive force, $P$, generated by the feeding appendages. Averaging $v$ of a Stokeslet at $y=0$ over a certain radius, $R$, one obtains $\bar{v} = \pi / (4 \pi \mu R)$. Based on Figs. 3.8-3.9, there are two regions with radius of 200 $\mu$m and characteristic peak velocity of $\bar{v} = 3.6$ mm/s in the vicinity of the feeding appendages. Combining the two regions, $P = 2(4 \pi \mu R \bar{v})$, i.e. $P=1.8 \times 10^{-8}$ N, 2.5 times higher than $w_{\text{excess}}$. At a constant sinking velocity, $P$ must balance the sum of $w_{\text{excess}}$ and the drag force (Jiang et al, 2002a). Since the relative velocity around the copepod is downward (Fig. 3.6-3.9), so is the drag,
requiring $P$ to be larger than $w_{\text{excess}}$. Thus, the estimated drag is about 1.5 times the excess weight. The propulsive force also generates a moment (negative $x$ direction), since it is applied at about 30 $\mu$m in front of the centerline of the prosome, based on the location of maximum $\vec{v}$. The magnitude of this moment is about $5.4 \times 10^{-13}$ Nm. To overcome this moment and avoid tumbling, the copepod turns its tail, creating a velocity bias in the positive $z$ direction (see insert in Fig. 3.9). Turning the flow creates a reaction force and a moment in the positive $x$ direction. Based on the average velocity bias (~0.1 mm/s, Fig. 3.9), over a radius of 250 $\mu$m (half the tail length), the force is $0.6 \times 10^{-9}$ N. Multiplying it by the distance to the center of mass of the copepod (1 mm), the estimated moment is $6 \times 10^{-13}$ Nm. Thus, the moment generated by the tail’s redirection of fluid is of sufficient magnitude to compensate against the moment of the propulsive force, allowing the copepod to maintain a relatively constant orientation.

**Copepod Behavior:** While generating the feeding current (Strickler, 1982; Strickler, 1984) the mouthparts, studded with chemoreceptors (Friedman and Strickler, 1975), scan the water flowing by them. When the presence of a food particle is perceived, additional movements of the mouthparts capture the particle and bring it to the mouth (Koehl and Strickler, 1981; Strickler, 1984; Strickler, 1985). Sensory setae on the stretched out antennules (Fig. 3; Huys and Boxshall, 1991) are mechanoreceptors (Strickler and Bal, 1973), as well as chemoreceptors (Bundy and Paffenhöfer, 1993). These receptors enlarge the volume of water scanned for food (Strickler, 1985; Jiang et al., 2002c). Particles that do not smell “good enough” are either actively rejected after capture by the mouthparts, or passively rejected – allowed to pass without being captured. For a
stationary hovering copepod, these rejected particles remain in the water below the copepod, and are not entrained into the feeding current again (Strickler, 1982).

The recirculating pattern in Figs 3.6-3.9 is, to our knowledge, the first report of a multi-encounter feeding pattern in calanoid copepods. The combination of feeding and sinking results in particles being drawn toward the copepod with its feeding current, passively rejected and then recirculated. The recirculation is interrupted aperiodically, after 8.7 s in the present example, when the copepod hops. During a hop, the copepod jumps about 0.5 mm upward to a position where its mouthparts are just below the stagnation point of the previous recirculation zone. If it were not for the hop, the copepod would never encounter new particles, due to the closed recirculation pattern. The recirculation allows the copepod to taste some of the particles that have passed near the mouthparts once more, this time with different, perhaps even more sensitive chemoreceptors on its antennules. Considering that the 20 µm polystyrene, spherical particles are mechanically desirable but chemically undesirable, this additional sensing by the sensors on the antennules ensures that the animal does not forfeit potentially good food. The available sequences of holograms suggest that the timing between hops is sufficient for a rejected particle to reach the antennule (about half the recirculation cycle). We speculate that once the copepod senses, using its antennules, a particle that has already been tasted and rejected (and is still not acceptable); it hops to another volume to look for different food. These assertions require testing by altering the properties of the particles, e.g. replacing them with desirable food, and observing the behavior of the copepod.
This chapter shows that digital holographic PIV is a relatively simple, but powerful tool for analysis of 3-D copepod (or other organism) dynamics and its interaction with its local environment, be it with other organisms or the local 3-D flow field.

References


Knutsen, T., Melle, W. and Calise, L. (2001). Determining the mass density of marine copepods and their eggs with a critical focus on some of the previously used methods. J. Plankton Res. 23 859-873.


**Figures and Tables**

\[
1 \otimes \tilde{h}_z(x, y) = 1 \quad \text{A.1}
\]

\[
\tilde{h}_z(x, y) \otimes \tilde{h}_z^*(x, y) = \delta(x, y) \quad \text{A.2}
\]

\[
\tilde{h}_{z_1}(x, y) \otimes \tilde{h}_{z_2}(x, y) = \tilde{h}_{z_1+z_2}(x, y) \quad \text{A.3}
\]

\[
\Im \{\tilde{h}_z\} = \exp\left[-j\frac{\lambda z}{4\pi} \left(\frac{u^2 + v^2}{x} \right)\right], \text{ where } u, v = \frac{2\pi}{x}, \frac{2\pi}{y} \quad \text{A.4}
\]

\[
\Im \{\tilde{h}_z\} = -j\lambda z \tilde{h}_z^*(x, y) \quad \text{A.5}
\]

\[
\Im \{\Re(\tilde{h}_z)\} = \Re(\Im \{\tilde{h}_z\}) = \cos \frac{\lambda z}{4\pi} (u^2 + v^2) \quad \text{A.6}
\]

\[
\Im \{\Im(\tilde{h}_z)\} = \Im(\Im \{\tilde{h}_z\}) = -\sin \frac{\lambda z}{4\pi} (u^2 + v^2) \quad \text{A.7}
\]

Table 3.1 Properties of Fresnel point source kernel, \( \tilde{h}_z \)

**Fig. 3.1** Sketch of inline digital holographic recording apparatus

**Fig. 3.2** Formation of a hologram
Fig. 3.3 Optical setup for digital in-line holography and test section.

Fig. 3.4 A sample digital hologram containing two views of the same swimming copepod in a seeded test section. Scale is 1 mm.

Fig. 3.5 In-focus, numerically reconstructed, dorsal (left) and lateral (right) views of the same swimming copepod, from the hologram of Fig. 2. (S) setae on antennule; (F) feeding appendages; (P) tracer particle (there are many). Inserts show the feeding appendages in up-stroke and down-stroke positions. Scale is 1 mm.

Fig. 3.6 Measured instantaneous velocity near the copepod (A) in the ambient frame of reference, and (B) in the copepod frame of reference.

Fig. 3.7 Particle streaks in the copepod reference frame obtained by combining 130 appropriately shifted reconstructed images. In all cases the dorsal and lateral views are in focus.

Fig. 3.8 Flow field generated by a Stokeslet (Eqn. 3) at: A. Absolute reference frame; B. Reference frame sinking at $0.33U_{ref}$.

Fig. 3.9 Selected particle tracks in 3 dimensions.

Fig. 3.10 Speeds of selected particles approaching feeding appendages. Inset: Horizontal velocity near tail along line A-A. Z axis origin at center of mass.
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4.1 Background

There is a growing interest in understanding micro-scale bio-physical processes, such as the kinematics and dynamics of swimming micro-organisms, e.g., bacteria, dinoflagellates or nauplii, and their interactions with the surrounding fluids (DeAngelis and Gross, 1992; Fennel and Neumann, 2004; Fennel and Osborn, 2004). Direct observations on such processes require suitable tools that are capable of resolving both temporal and spatial scales at the appropriate levels. The readily available candidate is the optical microscope. However, as shown in Figure 4.1, as the power of the microscope increases and the lateral resolution \(1.22\lambda/\text{NA}\), where \(\lambda\) is the wavelength and \(\text{NA}\) is numerical aperture of the entire optical system) improves, the field of view and depth of field decrease nonlinearly to a very thin layer. For example, increasing the power from 10X to 40X reduces the theoretical depth of field from 12\(\mu\text{m}\) to 2\(\mu\text{m}\), greatly limiting the size of resolvable volume.

Holography, on the other hand, is capable of recording a 3D volumetric field on a single plane (hologram plane) and later reconstructing it. With the recent development of in-line digital holography for particulate flows (Malkiel et al, 2003, Pan and Meng, 2003), it is now possible to record the hologram on a digital medium, and then reconstruct the sample volume numerically (Schnars and Juptner, 2002; Milgram and Li, 2002). However, previous implementations of digital holography have been hindered by the spatial resolution of the digital media, i.e., the pixel resolution of a camera. To overcome
this limitation, Xu, *et. al.* (2001) used a point laser source for illuminating the sample volume, and recorded the hologram on a lens-less camera. They successfully resolved (~60 µm) *D. brightwellii* in great detail, and subsequently used this method for 4D (space and time) tracking of microstructures and organisms (Xu et al., 2001&2003). Point source and lens-less recording do seem to improve the resolution in all directions, but this approach still has a limited depth-of-field, and is inherently limited by the pixels resolution of the recording medium.

Several recent studies(Carl *et al.*, 2004; Colomb *et al.*, 2005; Coppola *et al.*, 2004; Dubois *et al.*, 2004; Ferraro *et al.*, 2003; Marquet *et al.*, 2005; Repetto *et al.*, 2004; Dubois *et al.*, 2002) utilized a Mach-Zender interferometer configuration with coherent or partially coherent light source to record intensity and phase-contrast digital microscopic holograms of a 3D object on a glass slide. They successfully demonstrated the ability of digital holographic microscopy in resolving micro-structures of the phase object at high spatial resolution, often down to hundreds of nanometers. These studies focused on a few isolated stationary objects located within microns from the imaging plane. To the best of our knowledge, digital holographic microscopy has never been applied to examine the spatial distribution and velocity of a dense cloud of particles with an extended depth.

In this paper, we combine in-line digital holography and a conventional microscope objective in order to circumvent the obstacles associated with the limited resolution of a digital recording medium. Using the same setup as an optical microscope, we replace the light source with a collimated, coherent laser beam, and record a stream of magnified holograms on a CCD camera. The 3D fields can be reconstructed from these magnified
holograms at almost the same resolution as the optical microscopes. Reconstructed holograms of sample volumes with depth of 1 to 10 mm, containing particles ranging in size between 0.75 to 3 µm, demonstrate the efficacy of the Digital Holographic Microscope (DHM) as viable means of extending the depth of field of a microscope by almost three orders of magnitude. Detailed data on the spatial resolution in all directions confirms that a DHM maintains the lateral resolution of the microscope, and substantially reduces the depth of focus, enabling reconstruction of the 3-D coordinates of thousands of particles in a dense cloud. Finally, we also demonstrate the feasibility of using this technique to study particle dynamics by recoding a time series of particle traces and the trajectory of a nauplius.

4.2 Methodology

4.2.1 Optical Setup
As illustrated in Figure 4.2, the optical setup is very similar to a conventional transmission light microscope, but instead of using white light, we replace it with a coherent laser beam. In the current setup, we spatially filter a 3mw, He-Ne laser beam using a 25µm pinhole, expand and collimate the beam to 30mm diameter, and then illuminate the sample volume. Since the resulting intensity, 0.33mW/cm², is still too high, we use a variable Neutral Density (ND) filter to further attenuate the beam. In most of the tests, a filter of ND=1 is used, reducing the illumination intensity on the specimen to ~30µW/cm². A bright field microscope objective with proper tube length is used to imaging the optical field (hologram) onto the digital recording medium (CCD sensor). Note that the object plane is located outside of the sample volume. A 15 frames per second digital CCD camera (Kodak ES4.0), with a 2048x2048 pixels sensor and 7.4µm
pixels pitch, is used as recording medium. The theoretical background provided in the next section proves that the microscope objective enables us to overcome the limited pixel resolution. In fact, it is the magnification of objective that determines the resolution of the recording and reconstruction process.

4.2.2 Analysis of Microscopic Holography

A hologram is a record of interference between light scattered from objects, e.g. micron or sub-micron particles, and a reference beam with known phase distribution (Goodman, 1968). One can represent the optical field at the hologram plane \((x, y, 0)\) as

\[
U_H(x, y) = A \cdot e^{j k \cdot n_H} + \sum_i \left[ a_i(x', y'; z_i) h_z(x - x', y - y'; z_i) dx' dy' \right],
\]

where \(k_r\) is the propagation vector of the reference beam and \(n_H\) is the norm vector of the hologram plane. The first term represents the optical field of the reference beam, where the phase accounts for its angle with the scattering light, assuming that the hologram is perpendicular to the scattering axis. In the following analysis we assume that this angle is zero. The second term is the superposition of light scattered from discrete particles located at a distance \(z_i\) from the hologram plane, and produce (by being illuminated) fields with local distributions of \(a_i(x', y')\). Thus, each particle is considered as a superposition of point sources, whose individual fields are \(h_z(x, y; z_i)\). Using a paraxial approximation for particles much smaller than \(z_i\),

\[
h_z(x - x', y - y'; z_i) = \frac{1}{j \lambda z_i} \exp \left\{ j \frac{k}{2 z_i} \left[ (x - x')^2 + (y - y')^2 \right] \right\}
\]

If the scattering is diffraction dominated, as in in-line holography, each particle can be considered as a 2D aperture with a shape equal to its cross-section normal to the incident light. Thus, scattering from an individual particle is a convolution of a 2D aperture with
the impulse response function (Eq. 4.2). The resulting interference intensity on the hologram plane, \( I_z(x,y) \), is

\[
I_z(x,y) = U_H U_H^* = A^2 - A \sum a^*(x,y) \otimes h_z(x,y) - A \sum a(x,y) \otimes h_z(x,y) + \sum \sum |a(x,y) \otimes h_z(x,y)|^2
\]  

(4.3)

where \( \otimes \) indicates a convolution integral. To determine the effect of the microscope objective, we model its compound lens system as a perfect thin lens. The optical field at the distance \( d_i \) behind the lens, resulting from an optical disturbance, \( U_0(x_o,y_o,d_0) \), where \( d_0 \) the object distance before the lens, is

\[
U_i(x_i,y_i,d_i) = \int \int h_l(x_i,y_i;x_o,y_o) U_0(x_o,y_o) dx_o dy_o ,
\]

where

\[
h_l(x_i,y_i;x_o,y_o) = \frac{1}{M} \delta\left(\frac{x_i}{M} + x_o, \frac{y_i}{M} + y_o\right) \exp\left[ j \frac{k}{2Md_i^2} \left( x_i^2 + y_i^2 \right) \right] \exp\left[ j \frac{k}{2d_o} \left( x_o^2 + y_o^2 \right) \right],
\]

and \( M = d_i/d_0 \) is the magnification. Replacing \( U_0(x_o,y_o) \) with \( U_H(x_o,y_o) \) and performing the integration, the optical field generated by the hologram at the image plane is

\[
U_i(x_i,y_i,d_i) = \frac{1}{M} U_H \left( -\frac{x_i}{M}, -\frac{y_i}{M} \right) \exp\left[ j \frac{k}{2M^2d_i^2} \left( x_i^2 + y_i^2 \right) \right] \exp\left[ j \frac{k}{2d_o} \left( x_o^2 + y_o^2 \right) \right]
\]  

(4.4)

Thus, the image plane contains a magnified hologram plane with a phase correction that becomes unity when the magnification is sufficiently large. The intensity distribution in the image plane simply becomes a magnified hologram

\[
I_i(x_i,y_i) = \frac{1}{M^2} U_H \left( -\frac{x_i}{M}, -\frac{y_i}{M} \right) J_{iH} \left( -\frac{x_i}{M}, -\frac{y_i}{M} \right).
\]  

(4.5)

which contains the four contributors presented in Eq. 4.3. This true magnified hologram enables us to drastically relax the spatial resolution requirement of recording medium. Furthermore, we can use the magnification as a means of matching the desired resolution with that of the recording medium. As shown in this paper, the magnified holograms have little difficulty in resolving sub-micron particles and follow their trajectory in space.
Several simple methods can be used for improving the reconstructed image quality. For example, one can reduce the contamination generated by fixed specks on windows or by non-uniform illumination by subtracting a time average of a series of holograms from each instantaneous hologram. A similar effect can be achieved by recoding and subtracting a background hologram that does not contain particles. Both methods greatly reduce the background non-uniformities, preserving only the records of interference patterns generated by moving particles.

4.3 Result and Discussion

4.3.1 Sample Images and 3-D Resolution
To demonstrate the capability of in-line digital holographic microscopy, this paper focuses on its advantages over a conventional microscope and over a lens-less in-line holography. Using 10X, 18X and 40X objectives, we record holograms of sample volumes containing mono-dispersed, polystyrene spherical particles with nominal sizes of 3.189µm and 0.75µm. The particles are dispersed in distilled water at a high concentration of about 2000 particles/mm³. The dimensions of the rectangular container are 10×30×10 mm (the latter one is depth), but the actual depth of liquid with particles is varied between 1 - 10mm in order to examine various aspects of the technique. Table 4.1 presents all the present test conditions. For clarity, unless specified otherwise, the data and sample images presented hereafter refer to holograms recorded with a sample depth of 1mm. Figure 4.3 showcases a typical hologram and several reconstructed images of a sample containing 3.189µm particles recorded using a 10X objective. A 512 × 512 pixel (400 × 400 µm²) section of the original in-line hologram (2048×2048 pixels or 1.5×1.5
mm$^3$), showing interference patterns generated by particles located at various depths, is presented in Figure 4.3a. Sample images reconstructed at three different depths, obtained using equations 4.2, 4.6, and 4.7, are shown in Figures 4.3b–d. In focus images of particles, appearing as dark circular spot in the bright background, along with dark concentric rings associated with close-by, out-of-focus particles, can be clearly observed. Close inspections indicate that the in-focus particle traces possess optical properties that are similar to those observed under a conventional microscope, i.e. a sharp dark edge with a bright spot in the middle. A combined/compressed image showing all the particles reconstructed from the sample hologram segment is presented in Figure 4.3e. To bring all the particles in a volume into focus in one plane, we assign each pixel with the lowest intensity obtained over the entire depth, i.e.

$$I_{\text{combined}}(x,y) = \min_z I(x,y,z)$$

(4.8)

The three dimensional distribution of all the particles within the reconstructed volume is presented in Figure 4.3f. To determine the 3-D coordinates of each particle we use a segmentation method discussed in Sheng et al. (2003). The reconstructed planes are thresholded based on the local SNR at first, i.e. based on \[ \frac{[I(x,y,z) - \bar{I}_{x\epsilon V}]}{\sigma_{x\epsilon V}} \], where \( \bar{I}_{x\epsilon V} \) is the mean intensity of a small volume around the particle of interest, and \( \sigma_{x\epsilon V} \) is the standard deviation of intensity over this volume. Scanning through the images provides a list of line segment, which are combined into 2-D planar blobs using a join operator. Repeating this procedure at different depths, and using a join operator for the planar segments, we unite 2-D segments into 3-D particle traces. The location of a particle center is then estimated using the centroid of the 3-D blob. In the example
presented in this paper, the sample volume contains 5679 identified particles, i.e. more than 2500 particles/mm$^3$.

Clearly, the example demonstrates the efficacy of a DHM in extending the depth of field of a conventional microscope. However, the DHM also inherits the shortcomings of in-line holography, most prominently the elongated depth of focus. In conventional (non magnified) in-line holography, reconstruction of a spherical particle creates an elongated ellipsoidal image, whose length in the depth direction is typically two orders of magnitude higher than the lateral dimensions (Meng and Hussain, 1995; Zhang et al., 1997). This inherent problem greatly reduces one’s ability to determine the $z$ coordinate of a particle, and requires additional means, e.g. multiple views or multiple holograms (Sheng et al., 2003; Zhang et al., 1997) to determine the 3-D coordinates of a particle at the same accuracy. Thus, it is important to quantify the depth-of-focus of the DHM, and determine whether magnifying the hologram helps in reducing the depth of focus. The measurements are performed under all the conditions and parameters presented in Table 4.1. A sample measured 3-D shape of reconstructed 3 µm particle using a 10x objective is shown in the insert included in Figure 4.4. The total length of the elongated trace is about 40 µm, i.e. about ten particle diameters, much less that the typical 0.5-1 mm (forty particle diameters) depth of focus of a non-magnified hologram.

Figure 4.4 shows the ensemble-averaged distributions of

$$\frac{\Delta I_{center}(z)}{\Delta I_{center}(z_{min})} = \left[ \frac{\int_{x \in V}(z) \cdot I_{center}(z)}{\int_{x \in V}(z_{min}) - I_{center}(z_{min})} \right]$$

along the depth direction over all the particles for each experimental condition. Here $z_{min}$ is the plane with minimum intensity, and the subscript “center” refers to the center of the particle. Each case contains at least 1000 particles, i.e. the database is sufficiently large
for obtaining converged statistics. All the profiles peak when the particle is in focus and decrease away from the center. For the 3µm particles, the widths of the peaks, defined based on 75% of their peak values, decrease from 25µm to 4µm with increasing magnification, as also specified in Table 4.1. The width of the peak also decreases with decreasing particle diameter, from ~4µm for the 3µm particle to 2.5µm for the 0.75µm particle (at 40X). Thus, the effect of size is less pronounced compared to that associated with magnification.

The intensity profiles are asymmetric, and oscillate at increasing level (i.e. display improved contrast) with increasing magnification and decreasing particle size. These oscillations arise from interference between light scattered from the particle with the reference light. With increasing magnification, the higher numerical aperture enables the reconstruction process to resolve increasing number of consecutive constructive and destructive interference patterns. The profiles in the forward direction \((z - z_{\text{min}} < 0)\) are longer than those in the backward direction, consistent with the intensity distribution associated with Mie scattering by a spherical particle. As the particle size approaches the wavelength of the illumination light, the asymmetry becomes less pronounced, as is evident by comparing the profiles of 0.75µm and 3µm particles at 40X magnification.

Figure 4.5 summarizes the effect of magnification and particle size on the depth of focus, \(L_z\), and detected size of the reconstructed particle, \(D_d\), both based on the points with 75% of peak intensity. The four rows represent test conditions A-D, whose parameters are provided in Table 4.1. The columns show (from left to right) the distributions of \(D_d\), \(L_z/D_d\) and \(L_z\). The mean values are indicated by \(<>\). The first three rows indicate that for a constant particle diameter, \(D_d\) decreases with increasing magnification, but the change
is particularly evident at a magnification of 40X. At the latter magnification, the detected diameter of the 0.75µm particles remains equal to the actual particle size. A plausible explanation for this trend is provided in the following section.

The mean depth of focus decreases slightly, by 26%, as the magnification increases from 10X to 18X, and then substantially, by 75%, as the magnification increases from 18X to 40X. Thus, for conditions A and B, $\langle L_z \rangle / \langle D_d \rangle$ is still in the 6.3-7.6 range, while for condition C, $\langle L_z \rangle / \langle D_d \rangle$ decreases to 3.4. With the 40x objective, the mean depth of focus reduces to slightly above the actual diameter of the 3.2µm particle and to 3.3 times the diameter of the sub-micron particles. These values are substantially lower than the typical depth of focus of ~40 particle diameters for a lens-less in-line hologram. We believe that the improvement is a direct result of magnifying the hologram, which provides a much better recording of the high frequency part of the interference pattern generated as a point source interferes with the reference beam (see next section). The depth information is exclusively encoded in the fringe spacing, and should improve with increasing number of fringes resolved by the recording medium. Clearly, a DHM is not only capable of recording and reconstructing micron and sub-micron particles, which is normally impossible for lens-less in-line holography (especially in liquid over a large depth), it also reduces depth-of-focus by at least one order of magnitude. With increasing magnification, the depth of focus is reduced down to a few particle diameters.

Conclusions based on the depth extent of present tests are summarized in Figure 4.6. It shows the largest depth we have successfully recorded and reconstructed for each test case, $Z_d$, normalized by the theoretical depth-of-field ($\Phi$) of the corresponding microscope objective (provided in Figure 4.1). These numbers are lower than the
maximum depth that can be reconstructed, but not substantially. Clearly, a DHM extends the depth-of-field by at least two orders of magnitude, increasing with decreasing magnification, and reaching almost 1000 times at the present lowest magnification. The values of $Z_d/\Phi$ decrease with particle size. At the same time, DHM substantially reduces the depth of focus problem of lens-less in-line holography. The mean depth-of-focus normalized by the actual particle diameters ($D_p$), ranges between 1-6, decreasing as the microscope power increases. On the other hand, the detected particle size based on the 75% intensity also decreases with increasing power (Figure 4.5). Thus, one needs to consider the bandwidth limits of this method while interpreting the shape of particles, as discussed in the following section.

Before concluding, we would like to demonstrate that digital holographic microscopy can be used for tracking of particles by recording a sequence of holograms. Figure 4.7a shows combined/compressed five exposure tracks of all the 3µm particles located within a 1mm deep sample. They are recorded using a 10X objective and the delay between exposures is 66ms. Variations in the velocity of the particles at different depths can be clearly observed from the differences in displacements. Figure 4.7b shows tracks of 0.75µm particles over seven exposures, which are recorded using a 40X objective and combined over a depth of 100µm. In both examples the particle traces are clear, and the latter displays the variations in trajectory due to Brownian motion. Clearly, cinematographic digital holographic microscopy is suitable for studying the micro-scale dynamics of particles. To further demonstrate the capability of DHM in bio-fluid applications, we used cinematographic DHM with 10X objective to record the behavior of a free-swimming copepod nauplius (size ~100µm) located in a 25mm deep container.
The 3-D trajectory, presented in Figure 4.8, shows the nauplius engaged in a 3D downward helical motion extending over 1mm in all three dimensions. Images of five swimming postures at different phases of the cycle clearly show the complex motion of its swimming appendages. These images demonstrate that the 3D morphology of this marine organism, including the setae at the tip of its swimming appendages, which have typical width of about 1µm, is clearly resolved. Note that due to the size of the nauplius, not all of its parts are in focus in a certain plane. Different parts come into better focus as the reconstruction plane is varied.

4.3.2 Considerations Related to Bandwidth Limitations
As noted before and demonstrated in Figure 4.5, we can resolve increasingly smaller objects and the depth of focus decreases with increasing magnification. However, the size of the reconstructed image also decreases, requiring us to proceed with care. Especially noticeable is the reduction in the reconstructed size of the 3µm particle to about one third of its actual value at a magnification of 40X. Both the recording and reconstruction processes can contribute to the loss of shape information. In this section we discuss the effect of the field of view on the image. During recording, the particle depth is encoded as a high frequency “carrier” function, and its shape information is stored as a low frequency amplitude modulation, i.e. as the “envelope,” as illustrated in Fig. 4.9. The solid lines show a line cut through a hologram of a 15 mm wide, one-dimensional slit. The envelope, marked by dashed lines, is the diffraction pattern of an aperture. The high frequency real fringes are generated by interference between diffracted and undisturbed planar reference waves, i.e. due to the angle between the inclined diffracted light and the
collimated reference beam. The fringe spacing is a function of the origin of the diffracted light (Vikram, 1992).

The wavelength of the envelope is inversely proportional to the original object size, but the carrier function is a 2D chirp function, in which the frequency increases linearly with distance from the center of the object (Eq. 4.1 and 4.2). Consequently, the choices of recording parameters affect the bandwidth of the results. A reduced field of view due to increasing magnification limits the extent of the envelope being recorded, which would result in a smoothed boundary (gradually diminishing in the background) during reconstruction. If a constant threshold level were applied during the segmentation process that defines the object boundaries, the object would appear smaller.

To demonstrate the effect of field of view, we can compare it to the size of the airy pattern, i.e., the diameter of the first lobe of the envelope modulation, \(D_{\text{airy}} = \frac{1.22 \lambda \cdot z}{d}\), where \(d\) is the object diameter. For a 3.2\(\mu\)m particle located 1 mm from the hologram plane and \(\lambda=633\)nm, one obtains \(D_{\text{airy}}=0.24\)mm, which is only slightly lower than the entire field of view of the camera at a magnification of 40X (0.375mm, Figure 4.1). Thus, the camera only records the complete airy patterns of particles located very close to the center of the field of view of the objective. The rest will only register a portion of the pattern, degrading the recorded particle shape. Since the Airy pattern increases with depth, the detected diameter should also decrease with depth. Indeed, as illustrated in Figure 4.10, conditionally sampling the present particle sizes base on depth shows a decrease in the characteristic detected diameter with depth. However, in spite of the limitations in determining particle size/shape, its depth is correctly reconstructed since the depth information is encoded in the high-frequency fringe pattern (carrier frequency).
The higher the recording resolution is, the better resolved these fringes are, and consequently, the axial resolution is improved. Our assertion is partially supported by a recent study by Yang et. al. (2005). They proposed to equalize the intensity of fringe pattern, i.e. demodulate the envelope function from carrier function, so that a high accuracy of the particle location can be achieved. In our case, the limited numerical aperture of the microscope recording, in fact, limits fringe recording within a small range of intensity variation, i.e., our N.A. help us demodulate the envelope function in high magnification recording.

4.4 Summary
This paper introduces the application of in-line digital holographic microscopy to measure the spatial distributions of micron (3.2µm) and sub-micron (0.75µm) particles in dense liquid suspensions with depths of 1-10mm. It consists of illuminating a sample with a collimated laser beam, and using a microscope objective to digitally record a magnified image of the optical field created in a plane located outside of the sample volume. Thus, the image is a magnified digital in-line hologram, as demonstrated by a brief analysis. A series of tests with different objectives and particle diameters of 0.75 and 3 µm are used for characterizing the performance of this method. We demonstrate that DHM is capable of recording and reconstructing the traces of several thousands of particles located within a sample whose depth extends to almost 1000 times the depth of field of a conventional microscope. Yet, it maintains the lateral spatial resolution of the conventional microscope over the entire volume. Clearly, DHM is capable of recording and reconstructing micron and sub-micron particles, which is normally impossible for lens-less in-line holography, especially in liquid. The typical elongation on the depth
direction, the so called depth of focus problem that characterizes in-line holography, still exists, but it is at least one order of magnitude smaller that that of a lens-less in-line holograms. The depth of focus decreases with increasing magnification, ranging between ten to two particle diameters.

An automated segmentation method maps the 3D coordinates of the particles with submicron resolution in the lateral direction, and two-ten diameters in the depth direction. With a 10X objective, we successfully reconstruct the coordinates of particles in a dense suspension containing 2000 particles per mm³. With a 40X objective we successfully reconstruct the traces of 0.75µm sub-micron particle over a depth of 1mm. Cinematographic DHM records the particle motion, which can be used for determining their velocity. Clearly, this method is capable of tracking the three dimensional motion of particles, such as swimming behavior of microscopic organisms, and study their interactions with other particles as well as the surrounding flow fields.

**Figures and Tables**

<table>
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<th>Resolution of imaging system (µm/pixel)</th>
<th>Depth of sample (mm)</th>
<th>Mean reconstructed diameter at 75% of peak intensity (µm)</th>
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</table>

Table 4.1. Parameters of the present tests, and resulting mean diameters and depth of focus of the reconstructed particle traces.

**Fig. 4.1** The theoretical depth-of-field (DOF) as a function of resolution or field of view (S. Inoue and K.R. Spring, 1997). M refers to the power of a microscope objective.
**Fig. 4.2** Optical setup of the Digital Holographic Microscope (DHM).

**Fig. 4.3** (a) Part of a hologram recorder using a 10X objective, containing 3.189 µm diameter particles in a 1mm deep solution. (b - d) Reconstruction of planes located 120µm, 580µm and 800 µm from the hologram plane. In focus particles appear as dark spots on the bright background. (e) A combined/compressed image containing all the particles covered by the hologram section shown in a. (f) The location of all the particles detected within the entire 1.5x1.5x1 mm³ volume, totaling 5769 particles.

**Fig. 4.4** Ensemble averaged intensity distribution along the depth direction. For definitions of terms, see Eq 9. The insert is an iso-intensity surface plot of a typical reconstructed particle at 75% of its peak intensity. The depths of the sample are 1mm for the 3 µm particles and 0.1 mm for the 0.75 µm particle.

**Fig. 4.5** Statistics on the properties of reconstructed particle traces. Rows are arranged as experimental conditions A-D (Table 1), with the following magnifications, particle diameters and sample depth. A: 10X, 3.189 µm; 1mm; B: 18X, 3.189 µm, 1mm; C: 40X, 3.189 µm, 1mm; and D: 40X, 0.75 µm, 0.1mm. The columns are (from left to right): Dₜ, Lz/Dₜ; Lz; where Dₜ is the detected particle diameter, and Lz is the depth of focus, both based on 75% of the peak intensity. The numbers in <> above the distributions indicate mean values.

**Fig. 4.6** Summary of the present test conditions, including largest successfully tested sample depth (Zₜ) and depth of focus normalized by the nominal particle diameter and theoretical depth of field (Φ). Letters indicate test conditions (Table I and Figure 5).

**Fig. 4.7** A demonstration of a cinematographic DHM. (a) Combined/compressed tracks, consisting of 5 exposures, of 3.189µm particle located within a 1mm deep sample; (b) Sample tracks, consisting of 7 consecutive exposures, of 0.75µm particles, combined over a depth of 100µm.

**Fig. 4.8** A 3D, four seconds trajectory and behavior of a free swimming copepod nauplius, stage VI, obtained using cinematographic DHM. The images in (A)-(E) show reconstructions of planes dissecting the center of the organism at different times, as indicated in each frame.
**Fig. 4.9** Intensity distribution of a hologram generated by a single 15µm wide, one-dimensional slit, located 1mm away from the hologram plane. The solid line represents intensity distribution of fringes in the hologram, while the dashed line outlines their envelope.

**Fig. 4.10** The detected particle size distribution of Case C (3µm particles, 40X objective) conditionally sampled based on distance from the hologram plane.

**Reference**


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Fig. 4.10 The detected particle size distribution of Case C (3μm particles, 40X objective) conditionally sampled based on distance from the hologram plane.
5. Low Shear Near Wall Turbulence Boundary Layer

5.1. Introduction

Understanding the interaction of turbulence with a solid wall is of fundamental importance to turbulence modeling in many applications. Numerous well-studied interactions involve near wall turbulence production and dissipation due to mean shear. Other, less investigated mechanisms are associated with shear-free turbulence interacting with a wall. In this case the origin of the turbulence is located mostly away from the wall, allowing careful investigations of how the solid boundary influences the turbulence without the local production that dominates the shear flows. Understanding these mechanisms is also particularly helpful in modeling of turbulence in flow with weak local shear, such as near stagnation points, within separated regions, etc.

Several studies have already investigated the influence of a wall on turbulence in the absence of mean shear. So far, these studies have mainly focused on the initial response of decaying turbulence as a solid wall is suddenly introduced into a previously isotropic field. Uzkan & Reynolds (1967) conducted the first experiment in a water-tunnel with grid-generated turbulence at low Reynolds numbers passing near a belt moving at the same velocity as the mean flow. They show that a viscous length scale can be used to normalize the distributions of streamwise turbulence intensity at different downstream locations. Thomas & Hancock (1977) conducted a similar experiment at higher Reynolds numbers. They find that the streamwise turbulence intensity is augmented near the wall, in contrast to Uzkan & Reynolds’ results. Nevertheless, both identify a conceptual mechanism whereby the main effect of the wall is “wall blocking” or “wall reflection”,

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which generates a splating effect, and as a result transfers energy from wall-normal component to tangential components. Hunt & Graham (1978) attempt to reconcile the differences between the above-mentioned studies by theorizing that they are caused by competing mechanisms, one viscous and one kinematic, the latter being the wall blocking effect. They argue that due to the low Reynolds number in the Uzkan & Reynolds’ experiment, the viscous mechanism dominates; whereas kinematics dominates the higher Reynolds number experiments in the Thomas & Hancock’s experiments.

On the computational front, Biringen & Reynolds (1981) use large-eddy simulation to simulate turbulence above a solid wall. The results show qualitative agreement with the low Reynolds number experimental results. Perot and Moin (1995a, b) use DNS to extensively study the shear-free turbulent boundary layers. Various boundaries, including shear-free surfaces, solid and permeable walls are simulated. They find no persisting peaking of streamwise turbulence intensity near the wall, which indirectly support the results of Uzkan & Reynolds (1967). Perot & Moin show that energy transfer between Reynolds stress components (inter-component energy transfer) occurs through the work of the pressure-strain term. However, all the other terms in the Reynolds stress energy budget are also significant. The inter-component transfer is caused by imbalance between splats (impinging local “stagnation point” flow) and antisplats (“ejections”). Since there are splats and antisplats but no inter-component transfer in the case of a free surface, they argue that the imbalance is caused by viscous near wall effects. Recently, Aronson et al. (1997) conducted wind tunnel experiments with a moving boundary. They find that the initial response of the near-wall turbulence to the moving boundary is consistent with the model of Hunt and Graham (1978), causing transfer from wall-normal
to wall-tangential components. Further downstream this effect diminishes, the tangential components decay and eventually vanish. They also observe that unlike other regions, in a thin layer near wall, the pressure strain transfers energy from the wall normal to the wall-parallel components tending to make the flow more anisotropic.

Limited by physical constraints or computational tools, all the previous experimental and numerical studies do not reach “steady state” conditions, which are free of initial conditions. In the experiments involving decaying turbulence in a wind/water tunnel with a moving wall, the test section has not been long enough to be free of the effect of transition between stationary and moving walls. In the numerical studies of Perot & Moin (1995), the length scale of the wall-parallel components is $\sqrt{\nu}$, i.e. the paper focuses on transient phenomena. In the present paper we examine shear-free turbulence near a solid wall using an experimental setup that enables measurements under steady state boundary conditions, i.e. the turbulence does not decay in time. Our main objective is to determine how does the presence of a stationary solid wall with no mean shear influences the structure of otherwise isotropic turbulence generated by another source. As will be demonstrated, our results confirm the conclusions of Perot & Moin (1995) about the dominant effect of pressure-velocity correlations on the energy balance, as well as the effect of pressure strain on the inter-component energy transfer. However, under steady conditions the dissipation rate and the terms involving the pressure peak away from the wall. Furthermore, we provide clear evidence that the wall causes breakup of large eddies into smaller structures, thus altering (reducing) the local length and time scales. The peak in dissipation rate occurs in the eddy breakup region and is directly caused by the formation of energetic small structures.
The experimental apparatus, error analysis, and characterization of the mean flow parameters are presented in section 5.2. The results, consisting of Reynolds stresses, energy, vorticity and dissipation spectra, as well as all the terms in the evolution equations of turbulent kinetic energy and Reynolds stresses are presented in Section 5.3. Section 5.4 contains discussions on the dominant flow phenomena generating the dissipation peak away from the wall, as well as comparisons of the estimated pressure strain to different models for this term. The conclusions are presented in Section 5.5.

5.2 Setup, Procedures and Flow Characterization

5.2.1 Setup and Procedures

The experiments have been performed in a test facility designed to generate isotropic turbulence at high Reynolds numbers, but with almost zero mean velocity (Friedman and Katz, 2002). As illustrated in figure 5.1, the desired flow is generated by four rotating grids located symmetrically, separated by 76.2 cm in length and 21.6 cm in width, in the corners of a 1.22×0.46 m water tank. Each spinning grid consists of four 20.3×5.1 cm meshes with blockage ratio of 40% attached to a central shaft. The meshes are bounded on both ends by 11.4 cm diameter perforated disks in order to minimize the pumping action caused by spinning baffles. An independently controlled, induction-motor drive each grid, maintaining its speed within ± 0.4RPM (~1%) of the prescribed values. Every two adjacent grids rotate in opposite directions, creating symmetry and as a result low mean velocity at the center of the tank. In the present study the grids are rotating at 400RPM, at about 50% of its maximum capability. Baffles installed over the top of the grids prevent the air entrainment and surface wave generation on the free surface. A flat,
A smooth solid plate is also installed at the center of the tank, between the four grids, 20.3 cm above the bottom and parallel to it. The elevation of the plate can be precisely varied.

Two-dimensional Particle Image Velocimetry (PIV) is used to measure the velocity distribution within the tank. A dual head ND:YAG laser beam is expanded to a thin sheet with a waist thickness about 0.2 mm using a combination of a spherical and cylindrical lenses. The locations of the 50×50 mm² sample areas are indicated in figure 5.1. The image pairs are acquired at 3 Hz using a 2K×2K, Kodak ES4.0 “cross-correlation” digital camera. The time interval between exposures is 3 ms. The velocity is determined using in-house developed software (Roth et al., 1999, Roth and Katz, 2001). The interrogation window size is 16×16 pixels (390 µm), and the vector spacing is ~195 µm (50% overlap), providing a resolution that is comparable to the Kolmogorov length scale (~145 µm, details follow). Consequently, the data enables us to estimate the dissipation rate directly from the statistics of velocity gradients. We record 1200 image pairs (velocity fields) in each of the measurement domains in order to obtain converged statistics of the turbulence parameters.

The absolute uncertainty associated with displacement measurement is about 0.2 pixel (~5 µm), which for a typical displacement of 14 pixels results in a relative uncertainty in instantaneous velocity of 1.5% or 1.6 mm/s. In calculating ensemble-averaged quantities (denoted by \( \overline{U}(x_1,x_2) = \langle U(x_1,x_2,t) \rangle \)), the uncertainty, estimated using \( \Delta \overline{U}_i = \Delta U_i / \sqrt{N} \), where \( N \) is the number of measurements (typically 1200), decreases to very small values, i.e. 0.05 mm/s for velocity components. The uncertainty for second order moments, determined using \( 2 \Delta U_i / \sqrt{N} \) (0.09 mm/s), is about 0.1% of the typical rms velocity fluctuations, 80 mm/s. The resulting errors in velocity derivatives, estimated at
$\Delta(\mathcal{U}_j/\partial x_j) = 8 \text{ 1/s}$, is about $\sim 8\%$ of the typical magnitude of instantaneous velocity derivatives ($\sim 100 \text{ 1/s}$). In accordance, the uncertainty associated with the statistics obtained from the velocity derivatives squared (e.g. dissipation rate), and other terms in the turbulent kinetic energy (TKE) transport equations, have uncertainty of about $2\Delta(\partial U_i/\partial x_j)/\sqrt{N} = 0.46 (1/\text{s}^2)$, and a relative value of about 0.4%.

### 5.2.2 Mean Flow, Fluctuating Components and Scales

Figures 5.2(a) and (b) show the mean velocity distributions covering an area extending from the wall ($x_2=0$) down to 150mm away from the wall. The mean wall normal component, $U_2$, vanishes at the wall and gradually increases to 0.035m/s at $x_2=130$mm. The mean wall-parallel component, $U_1$, remains at about 0.01m/s almost homogeneously throughout the three measurement regions (indices 1 and 2 are assigned to the $x$ and $y$ directions, respectively). The r.m.s value in the wall normal direction (figure 5.2c), $\langle u'u'_2 \rangle^{0.5}$ where $u'_i = U_i - \overline{U}_i$, vanishes in the vicinity of the wall and gradually increases to 0.07m/s at $y=150$mm. The r.m.s value of the wall parallel component starts from 0.04m/s close to the wall, and increases to 0.08 m/s at $y=150$mm. Both the mean velocity and its variance show a strong influence of the wall on the wall normal component, and less noticeable effects in the wall-parallel direction. The influence of wall seems is most prevalent at $x_2 \leq 30$ mm. A statistically homogeneous, isotropic field (at least as far as mean and variance are concerned), with spatial variations of less than 0.005m/s, in both mean and r.m.s values, is established at $x_2>30$ mm. Around $x_2=50$ mm both components of the mean velocity drop to very low level, with magnitudes at or below 0.01 m/s, while the rms values are about 0.06 m/s, establishing essentially a turbulent domain with essentially no mean flow.
Close to the wall, the characteristic magnitude of the mean shear strain rate is about 1 \(1/\text{s}\), only about 1% of the instantaneous turbulence-generated instantaneous levels (~100 \(1/\text{s}\)), and only 6-8% of the rms values of velocity gradients (detailed distributions follow). Thus, our experimental setup creates an almost shear-free “boundary layer,” similar to the conditions established using DNS by Perot and Moin (1995). However, the present measurements are performed under stationary conditions, free of transient effects created shortly after the wall is introduced into the flow. Due to the symmetry of our system, it is rather safe to assume that the flow and turbulence are homogenous along wall-parallel directions (as confirmed experimentally for this facility in Friedman and Katz, 2002), and that the ensemble-averaged parameters close to the center of the plate have axisymmetric distributions with respect to the wall normal direction.

Sample one-dimensional energy spectra, \(E_{11}(k_1)\) and \(3/4E_{22} (k_1)\), using all the 256 data points along the homogeneous direction (\(x\)) are presented in Figure 5.3. We use FFT, with no windowing function and no zero padding, but with removal of mean and linear de-trending, following procedures discussed in detail in Doron et al. (2001). These samples reveal that isotropic turbulence over the entire spectrum is established as close as 40 mm away from the wall. The spectra change significantly (a detailed discussion is presented in Section 5.3.1) and turbulence becomes anisotropic, both at large and small scales, in the vicinity of the wall, consistent with the previously observed transient cases (Thomas and Hancock, 1977; Perot & Moin, 1995; Aronson et al., 1997). The spectra at \(x_2=40\) mm have a clear inertial range with a \(-5/3\) slope, enabling us to estimate the dissipation rate away from the wall using (Tennekes and Lumley, 1972),

\[
E(k_1) = \frac{18}{55} \cdot 1.6 \cdot \varepsilon^{2/3} k_1^{-5/3}
\]  

(5.1)
The estimated dissipation rate at $x_2=40$ mm is $\varepsilon \approx 2.6 \times 10^{-3}$ m$^2$/s$^3$. In the following section we’ll calculate the dissipation rate directly from the data and obtain $\varepsilon_\infty \approx 2.8 \times 10^{-3}$ m$^2$/s$^3$.

The resulting Kolmogorov length scale, $\eta = (\nu^3 / \varepsilon)^{1/4}$, is 145 µm, the Taylor Micro-Scale, $\lambda \approx (15 \nu k / \varepsilon)^{1/2}$ ($k=3.7 \times 10^{-3}$ m$^2$/s$^2$ is the turbulent kinetic energy, details follow), is 4.6 mm, and the integral scale $L_\infty = k^{3/2} / \varepsilon_\infty$ is 8.04 cm. Thus, $Re_\lambda = \sqrt{k \lambda / \nu}$ is 280 and the turbulent Reynolds number, $Re_T$, estimated using $Re_T = \frac{3}{20} Re^2_\lambda$, is $1.17 \times 10^4$. These results are similar to prior data obtained in the same facility (Friedman and Katz, 2002). The slight difference occurs, presumably, since the previous measurements were performed at the center of the facility and without the plate. The present measurements are also being performed at a higher (four times) spatial resolution.

5.3 Results

5.3.1 Turbulence and vorticity Spectra

As discussed before, and illustrated in more detail in Figure 4, at $x_2 \approx 300 \eta$ ($0.6 L_\infty$) the turbulence appears to be nearly isotropic, although $3/4E_{22}(k_i)$ is slightly higher than $E_{11}(k_i)$ at intermediate and low wave-numbers. With decreasing distance from the wall, anisotropy becomes more and more pronounced. Figure 5.4(a) compares $E_{11}(k_i)$ and $3/4E_{22}(k_i)$ for three distances from the wall, Figures 5.4(b-c) focus on the low wave-numbers at higher magnification and more examples; and Figures 4d and e focus on the high wave-number range. Focusing on $E_{11}(k_i)$, one can make several observations: (1) At large scales ($k_i \eta < 0.026$) $E_{11}$ decreases with decreasing $y$ in the in the $x_2 > 87 \eta$ range, remains unchanged in the $61 \eta < x_2 < 87 \eta$ range, and decreases slightly closer to the wall. (2) At high wave-numbers, i.e. $\eta k_i > 0.026$, $E_{11}$ of the free stream turbulence has a significantly higher slope than that of all the other near wall spectra ($x_2 \leq 87 \eta$), i.e. small-
scale structures near the wall are significantly more energetic than structures located far from the wall. (3) At small scales \((k_1\eta>0.026)\) and \(x_2 \leq 87\eta\) there is a distinguishable build-up of energy, i.e. a bump forms in the distributions of \(E_{11}\). This energy build-up peaks at \(x_2=61\eta\) and \(x_2=36\eta\) and decreases slightly with further decreasing distance from the wall \((x_2=10\eta)\).

The spectra of wall-normal fluctuations (Figures 5.4c, e) demonstrate that at large scales \((k_1\eta<0.026)\) \(E_{22}\) is suppressed significantly with decreasing distance from the wall, becoming much weaker than the wall-parallel component. In the high wave number range, \(E_{22}\) of the far field has a larger slope and is significantly lower than the corresponding levels in the near wall spectra. The small-scale energy peaks in the \(x_2=36\eta-87\eta\) range, and then diminishes with further decrease in distance from the wall. Very close to the wall, at \(x_2=10\eta\), the high wave-number spectral bump diminishes significantly. The presence of a solid wall suppresses the wall-normal, large-scale fluctuations, and to a much lesser extent also in the wall parallel fluctuation. However, the wall promotes the small-scale fluctuations causing both \(E_{22}\) and \(E_{11}\) to develop spectral bumps, especially in the \(x_2=36\eta-61\eta\) range. Thomas and Hancock (1977) also observe high frequency bulging of \(E_{11}(k_1)\) as the wall is approached. Their \(E_{22}\) decreases rapidly with distance from the wall at low wave numbers, in agreement with the Hunt and Graham’s (1978) theory, and remains unchanged at high wave numbers. They conclude that the small eddies are less influenced by the wall than the larger ones. In the present results, the wall increases both the wall-normal and wall-parallel small scale fluctuations.

By calculating the vorticity distributions, and from them the vorticity spectra, one can examine the effect of the wall on the characteristic energy of turbulent eddies. The
spectra of $\omega_z$, shown in Figure 5, demonstrate the following trends: (1) Far (266$\eta$) from the wall, the vorticity spectra have a range of scales with the expected $k^{-8/3}$ slope, consistent with trends of isotropic turbulence. (2) The energy/strength of large scale eddies decreases with distance from the wall, especially at $x_2 \leq 36\eta$. As expected, close to the wall, at $x_2 = 10\eta$, the low wave-number eddies are significantly weaker. (3) At high wave-numbers, the vorticity spectra in the $36\eta \leq x_2 \leq 87\eta$ range have higher energy than those of the corresponding far-field isotropic turbulence, peaking at $36\eta - 61\eta$. With further decrease in distance, the high wave-number vorticity energy decreases again. Note that the wavelength at the spectral bump, $\sim 10\eta$, is substantially smaller (by 6-8 times) than their distance from the wall ($61\eta \leq x_2 \leq 87\eta$). Thus, the results suggest that the presence of the wall causes breakup of the large eddies into smaller structures. Since the most energetic small scale eddies are located clearly away from the wall, and the energy near the wall is significantly lower, the breakup cannot occur as a result of direct collision with the wall. Instead, as shown in Sections 5.3.3 and 5.4.1, the breakup occurs as a result of interaction of the wall-induced pressure field with the turbulence.

5.3.2 Dissipation Rate and Reynolds Stresses

The measured normal stresses contributing to the turbulent kinetic energy, along with the Reynolds shear stress, i.e. $\langle u_i u_j \rangle$, $i, j = 1, 2$, are plotted in Figure 5.6. Each term is normalized by its value at $x_2/L_\infty = 0.6$ ($x_2/\eta = 330$). With decreasing distance from the wall the wall-parallel component decreases very slightly and then increases again, whereas the wall-normal component decreases to a very small value. The Reynolds shear stress has a very low value everywhere (note the differences in scales), one order of magnitude lower than normal stresses away from the wall, diminishing to almost zero near the wall. Thus,
the shear stress plays a very limited role in the dynamics of shear-free turbulence near a wall.

Since the present measurement resolves scales down to the Komogorov scale, we can calculate the distributions of dissipation rate directly from

$$\varepsilon = 2\nu \left\langle s_{ij} s_{ij} \right\rangle; \quad s_{ij} = 0.5 \left( \frac{\partial u_i}{\partial x_j} + \frac{\partial u_j}{\partial x_i} \right)$$  (5.2)

where \( s_{ij} \) is the strain rate tensor of the fluctuating components. However, the 2-D PIV data enables us to calculate only four of the six strain rate terms, \( s_{11}, s_{12}, s_{22} \) directly, and \( s_{33} \) using the continuity equation. The other two terms, \( s_{13} \) and \( s_{23} \), need to be approximated. Thus, we can only calculate five of the nine terms in Equation 5.2, \( s_{11}s_{11}, s_{22}s_{22}, s_{12}s_{12} = s_{21}s_{21}, s_{33}s_{33} \). Considering the symmetry of our facility, and consistent with the results in Friedman and Katz (2002), we believe that locally axis-symmetry and wall-parallel homogeneity may be assumed for the statistical properties of the current flow, with the axis of symmetry aligned with the wall-normal direction. In the Appendix, consistent with the results of George and Hussain (1991), we show that for axisymmetric turbulence the dissipation rate \( \varepsilon \) can be estimated from

$$\varepsilon = 4 \left[ \frac{\left( \frac{\partial u_2}{\partial x_1} \right)}{\left( \frac{\partial u_2}{\partial x_2} \right)^3} + \frac{\left( \frac{\partial u_1}{\partial x_1} \right)}{\left( \frac{\partial u_1}{\partial x_2} \right)^3} + \frac{\left( \frac{\partial u_1}{\partial x_2} \right)}{\left( \frac{\partial u_2}{\partial x_1} \right)^3} \right]$$  (5.3)

Figure 5.7(a) shows the distributions of the four available velocity derivatives \( \left\langle (\partial u_2/\partial x_1)^2 \right\rangle, \left\langle (\partial u_1/\partial x_2)^2 \right\rangle, \left\langle (\partial u_2/\partial x_1)^2 \right\rangle \) and \( \left\langle (\partial u_2/\partial x_2)^2 \right\rangle \), averaged over the presumed homogeneous (wall parallel) direction. Far from the wall \( \left\langle (\partial u_1/\partial x_1)^2 \right\rangle \) and \( \left\langle (\partial u_2/\partial x_2)^2 \right\rangle \) are close to each other, and so are \( \left\langle (\partial u_1/\partial x_2)^2 \right\rangle \) and \( \left\langle (\partial u_2/\partial x_1)^2 \right\rangle \). The ratio between the longitudinal and cross derivatives (squared) increases with distance from the wall, approaching about 1.5 at \( x_2 \geq 300\eta \ (0.6L) \) i.e. not a ratio of two, the expected value for
isotropic turbulence. Due to related initial concerns on the effect of high frequency noise on the validity of the derivatives, we spatially filter the data using a box-filter of 4×4 vectors, and repeat the derivations. This scale corresponds to $k_1\eta=0.18$, i.e. slightly lower than the wave-number at which the energy spectra (Figure 5.4) start flattening. The results, shown in Figure 5.7(b), are quantitatively slightly different, as expected, but the ratio between longitudinal and cross derivatives at $x_2=0.6L_\infty$ remains unchanged. Furthermore, the variations with distance from the wall appear to be very similar. Thus, for the rest of the analysis we proceed using the original, unfiltered data. With decreasing distance to the wall all the velocity derivatives decrease slightly, and then increase again to maxima at $x_2/\eta\sim50$. As the maximum is approached the differences between the four terms diminish, with $\langle(\partial\bar{u}_2/\partial x_2)^2]\rangle$ only slightly lower than the other three. With further decrease in distance from the wall, all the four terms decrease again. Thus, all the available terms that contribute to the dissipation rate peak away from the wall.

Figure 5.8 presents the dissipation rate estimated using Equation 5.3 and averaged along the wall parallel direction. “Far” from the wall ($\geq300\eta$), $\varepsilon_\infty \approx 2.8 \times 10^{-3} \text{ m}^2/\text{s}^3$ is of the same order of magnitude as the estimate based on curve fitting to the energy spectrum ($2.6 \times 10^{-3} \text{ m}^2/\text{s}^3$, see Section 5.2.2). With decreasing distance from the wall, $\varepsilon$ first decreases slightly, reaching a minimum at $x_2/\eta\sim200$, and then increases again, peaking at $x_2/\eta=50$ with a level ($\varepsilon=4.05\times10^{-3}$) that is substantially higher than the far field values. Closer to the wall $\varepsilon$ decreases again, i.e. the dissipation peak is clearly located away from the wall. In summary, the overall dissipation rate and all the components contributing to it have maxima away from the wall. We will attempt to explain the mechanism generating the local dissipation maximum away from the wall as the results unfold.
Figure 5.8 also shows the distribution of turbulent kinetic energy, $k$, estimated assuming axisymmetry (see Appendix), i.e.

$$ k = \left\langle u_1^2 \right\rangle + \frac{1}{2} \left\langle u_2^2 \right\rangle $$

(5.4)

The trends of $k$ are mostly caused by the wall-normal component. Thus, with decreasing distance from the wall $k$ decreases monotonically, but the rate of decay seems to increase at $x_2 < 100 \eta$, in the region where the dissipation maximum starts forming. A complete analysis of the evolution equations of turbulent kinetic energy is presented in Section 5.3.3.

To obtain a fuller picture on the distributions of turbulent structures and their role in the dissipation rate, Figure 5.9 presents one-dimensional, so called “dissipation spectra”, $k_1^2 E_{11}$ and $k_1^2 E_{22}$, each showing a magnified high wave-number range. Away from the wall both dissipation spectra peak at $k_1 \eta = 0.011 - 0.014$ (rad/m). With decreasing distance from the wall these low wave-number peaks become smaller. The levels in $k_1^2 E_{22}$ keep on decreasing up to the wall, whereas the peaks in $k_1^2 E_{11}$ remain essentially unchanged at $x_2 \leq 36 \eta$. However, another dissipation peak appears at higher wave-numbers, in the $k_1 \eta = 0.048 - 0.071$ rad/m range. These high wave number peaks reach maximum levels at $36 \eta \leq x_2 \leq 61 \eta$, i.e. in the region with local dissipation maximum. Closer to the wall, at $x_2 = 10 \eta$, the high-wave number peaks become smaller, especially in the distributions of $k_1^2 E_{22}$, but they are still evident.

Clearly, the presence of the wall suppresses the turbulent energy at large scales ($k_1 \eta < 0.036$), especially of the wall-normal component, but to a lesser extent, also of the wall-parallel component. However, energy is added to both components at high wave-numbers, generating small scale energetic eddies that dominate the dissipation rate. There
There is a correspondence between the local maximum in $\varepsilon$ at $x_2=50\eta$, and the high wave-number peak in the dissipation spectra at the same location. These trends indicate that the local dissipation maximum is caused by breakup of the large eddies to small-scale energetic structures. Consistent with Perot and Moin (1995), the next section shows that the wall affects the turbulent kinetic energy balance through the pressure-velocity correlations. The wall causes breakup of large structures into smaller ones that are more dissipative. The increase in dissipation rates at $36\eta \leq x_2 \leq 61\eta$ is directly related to build up of energy at small scales.

As the wall is approached, i.e. at $x_2<36\eta$, the dissipation rate decreases for two reasons. First, as we will show in the next section, the local production of turbulence is weak, and second, the energy generated elsewhere and diffusing towards the wall is already “consumed” due to high dissipation away from the wall. With $k$ and $\varepsilon$ available, one can calculate the “shear parameter”, $s^* = \frac{S}{\nu \varepsilon}^{1/2}$, introduced by Corrsin (1958), Komogorov (1962) and Saddoughi & Veeravalli (1994) to determine whether local isotropy can be assumed. In our case, $s^*$ away from the wall varies between 0.001 to 0.003, i.e. it is very small.

### 5.3.3 Budgets of Turbulent Kinetic Energy and Reynolds Stresses

To compare and quantify the phenomena affecting the structure of shear-free turbulence near the wall, including the role of pressure strain, we examine the evolution of turbulent kinetic energy (Pope, 2000)

$$\frac{\partial k}{\partial t} + U_j \frac{\partial k}{\partial x_j} + \frac{\partial P}{\partial x_i} = P - \varepsilon \quad (5.5)$$

$$P = -\langle u_i u_j \rangle \tilde{S}_{ij} = -\frac{1}{2} \langle u_i u_j \rangle \left( \frac{\partial U_i}{\partial x_j} + \frac{\partial U_j}{\partial x_i} \right)$$
\[ T_i = \frac{1}{2} \langle u_i u_j \rangle + \frac{1}{\rho} \langle pu_i \rangle - 2v \langle u_j s_{ij} \rangle \]

where \( P \) is the mean-shear turbulence production, and \( T_i \) is the turbulence advection term.

As outlined in the appendix, assuming axisymmetry and wall-parallel homogeneity,

\[ P = -4 \langle u_i^2 \rangle \frac{\partial \bar{U}_i}{\partial x_1} - 2\langle u_1 u_2 \rangle \left( \frac{\partial \bar{U}_1}{\partial x_2} + \frac{\partial \bar{U}_2}{\partial x_1} \right) \left( \langle u_1^2 \rangle + \langle u_2^2 \rangle \right) \]  \hspace{1cm} (5.6)

\[ \frac{\partial \langle u_j s_{ij} \rangle}{\partial x_i} = 4 \frac{\partial \langle u_1 s_{11} \rangle}{\partial x_1} + \frac{\partial \langle u_1 s_{21} \rangle}{\partial x_1} + \frac{\partial \langle u_2 s_{21} \rangle}{\partial x_1} + \frac{\partial \langle u_2 s_{22} \rangle}{\partial x_2} + \frac{\partial \langle u_1 s_{22} \rangle}{\partial x_2} + \frac{\partial \langle u_2 s_{22} \rangle}{\partial x_2} \]  \hspace{1cm} (5.7)

\[ \frac{\partial \langle u_j k \rangle}{\partial x_i} = \frac{\partial \langle u_j u_i u_j \rangle}{\partial x_i} = 2 \frac{\partial \langle u_j k \rangle}{\partial x_1} + \frac{\partial \langle u_j k \rangle}{\partial x_2} \]  \hspace{1cm} (5.8)

\[ \frac{\partial k}{\partial t} + \bar{U}_j \frac{\partial k}{\partial x_j} = 2\bar{U}_1 \frac{\partial k}{\partial x_1} + \bar{U}_2 \frac{\partial k}{\partial x_2} \]  \hspace{1cm} (5.9)

where in Equation 5.9 we also assume a steady flow. Subject to the same assumptions, the missing term, which involves the pressure-velocity correlations, can be estimated from a balance of all the other terms, i.e.

\[ \frac{1}{\rho} \frac{\partial}{\partial x_i} \langle pu_i \rangle = P - \varepsilon - \frac{\partial \langle u_j k \rangle}{\partial x_i} - 2v \frac{\partial \langle u_j s_{ij} \rangle}{\partial x_i} - \bar{U}_j \frac{\partial k}{\partial x_j} \]  \hspace{1cm} (5.10)

Note that an assumption of wall-parallel homogeneity implies that \( \frac{\partial \langle u_j p \rangle}{\partial x_1} = \frac{\partial \langle u_2 p \rangle}{\partial x_2} \) and that \( \frac{\partial \langle u_j k \rangle}{\partial x_1} = \frac{\partial \langle u_2 k \rangle}{\partial x_2} \) in Equation 5.8. However, in computing \( \frac{\partial \langle u_j k \rangle}{\partial x_i} \) we examine all the terms in order to confirm the validity of our assumptions.

Figure 5.10 shows the turbulence production estimated using Equation 5.6, along with the contributing terms, all scaled with the dissipation far from the wall (\( \varepsilon_\infty \)). The total production seems to decrease almost monotonically as the wall is approached. Far from the wall \( P = \varepsilon \), i.e. there is production-dissipation balance. Near the wall, including the vicinity of the dissipation peak the local production is only about 20% of the local dissipation rate. A comparison between the terms contributing to \( P \) shows that the
contribution of the shear stress is negligible, that $-4\langle u_i^2 \rangle \partial U_i / \partial x_i$ contributes a low constant value, and that $-\left( \langle u_i^2 \rangle + \langle u_2^2 \rangle \right) \partial U_2 / \partial x_2$ is the dominant term, which causes the decreasing trends with distance from the wall.

Figure 5.11(a) shows the wall-normal turbulent transport terms of $k$, i.e. $\langle u_2 u_1^2 \rangle$, $\langle u_2 u_2^2 \rangle$ and $\langle u_2 k \rangle$. As is evident, near the wall the curves are smooth, but with increasing distance from the wall the results oscillate. We believe that the cause of these oscillations is insufficient convergence of the triple correlation terms away from the wall, even with 1200 instantaneous measurements (the oscillations increase with when the data base is reduced). This insufficient convergence causes patchiness in the (2D) distributions of the triple correlation terms away from the wall, which disappears in the region with smaller length scales near the wall. Since our primary interest is the turbulence near the wall, the present data is sufficient for showing the significant trends. Interestingly, $\langle u_2 u_2^2 \rangle$ is always negative, implying energy flux towards the wall and $\langle u_2 u_1^2 \rangle$ is always positive, implying energy flux away from the wall. Combined, $\langle u_2 k \rangle$ is negative but quite small at $x_2<140 \eta$, i.e. there is turbulent kinetic energy flux towards the wall, and positive farther away from the wall. Since the evolution equation of turbulent kinetic energy contains the spatial gradient of this term, we smooth it by fitting a 6th order polynomial to the data and use it in subsequent analysis. In Figure 5.11(b) we show that $\partial \langle u_2 k \rangle / \partial x_2$ is substantially larger than $\partial \langle u_1 k \rangle / \partial x_1$, in agreement with our assumptions of wall-parallel homogeneity. Thus, only wall-normal gradients in turbulent kinetic energy flux contribute to the overall balance.
All the terms in the evolution equation of $k$ with appropriate signs to indicate gain or loss, including $-\frac{1}{\rho} \partial \langle \partial x_j \rangle \langle pu_i \rangle$, calculated using Equation 5.10, are presented in Figure 5.12.

The advection by mean flow and the viscous diffusion terms are very small, leaving $\varepsilon$, $P$ and $\partial \langle u_z \rangle / \partial x_z$ as the only terms contributing to the estimate of the pressure-velocity correlations. Consequently, $-\frac{1}{\rho} \partial \langle pu_i \rangle / \partial x_i$ (or $\frac{1}{\rho} \partial \langle pu_2 \rangle / \partial x_2$ assuming wall-parallel homogeneity) peaks at $x_2 = 0.1 L_{\infty} (52 \eta)$, very close to the location of maximum dissipation rate. Near the dissipation peak the pressure velocity-correlation term is 70% of the dissipation rate, indicating that work of the pressure produces most of the energy being dissipated. Away from the wall, where production-dissipation balance exists, the pressure-velocity correlation term diminishes. Clearly, the wall impacts the turbulence budget through the pressure-velocity correlations. The existence of a peak in the pressure-velocity correlation term away from the wall is consistent with the observations by Perot and Moin (1995a).

Subject to the assumptions of steady flow, axisymmetry and wall-parallel homogeneity, the data can also be used for examining the terms in the transport equations for the normal Reynolds stresses

$$\frac{\partial \langle u_1^2 \rangle}{\partial t} = -\overline{u}_k \frac{\partial \langle u_1 \rangle}{\partial x_k} - 2 \overline{u}_k \langle u_1 u_k \rangle - 2 \overline{\partial \langle u_1 \rangle / \partial x_1} \frac{\partial \langle u_1 \rangle}{\partial x_k} + \nu \frac{\partial^2 \langle u_1^2 \rangle}{\partial x_k \partial x_k} - \varepsilon_{11} + 2 \left\langle \frac{\partial u_1}{\partial x_1} \right\rangle$$

$$\frac{\partial \langle u_2^2 \rangle}{\partial t} = -\overline{u}_k \frac{\partial \langle u_2 \rangle}{\partial x_k} - 2 \overline{u}_k \langle u_2 u_k \rangle - 2 \overline{\partial \langle u_2 \rangle / \partial x_2} \frac{\partial \langle u_2 \rangle}{\partial x_k} + \nu \frac{\partial^2 \langle u_2^2 \rangle}{\partial x_k \partial x_k} - \varepsilon_{22} + 2 \left\langle \frac{\partial u_2}{\partial x_2} \right\rangle$$

where $\varepsilon_{ij} = 2 \nu \langle \partial u_i / \partial x_k \cdot \partial u_j / \partial x_k \rangle$ is the so-called dissipation tensor (pope, 2000). As shown in the Appendix, assumptions of axisymmetry and wall-parallel homogeneity lead to

$$\varepsilon_{11} = 2 \nu \left\langle \left( \frac{\partial u_1}{\partial x_1} \right)^2 + \left( \frac{\partial u_1}{\partial x_2} \right)^2 + \frac{1}{2} \left( \frac{\partial u_1}{\partial x_1} \right) \left( \frac{\partial u_2}{\partial x_2} \right) \right\rangle$$

$$\varepsilon_{22} = 2 \nu \left\langle \left( \frac{\partial u_2}{\partial x_1} \right)^2 + \left( \frac{\partial u_2}{\partial x_2} \right)^2 \right\rangle$$

(5.12)
\[
\frac{\partial \langle u_k u_i^2 \rangle}{\partial x_k} = 4 \frac{\partial \langle u_i u_i^2 \rangle}{\partial x_1} + \frac{\partial \langle u_i u_i^2 \rangle}{\partial x_2} \tag{5.13}
\]
\[
\frac{\partial \langle u_i u_i^2 \rangle}{\partial x_k} = 2 \frac{\partial \langle u_i u_i^2 \rangle}{\partial x_1} + \frac{\partial \langle u_i u_i^2 \rangle}{\partial x_2} \tag{5.14}
\]
\[
\frac{\partial \langle u_i u_i^2 \rangle}{\partial x_k} = 2 \frac{\partial \langle u_i u_i^2 \rangle}{\partial x_1} + \frac{\partial \langle u_i u_i^2 \rangle}{\partial x_2} \tag{5.15}
\]
\[
\frac{\partial \langle u_i u_i^2 \rangle}{\partial x_k} = \frac{\partial \langle u_i u_i \rangle}{\partial x_1} + \frac{\partial \langle u_i u_i \rangle}{\partial x_2} \tag{5.16}
\]

Then, and imposing steady state conditions (\(\frac{\partial}{\partial t} = 0\)), and relying on wall-parallel homogeneity to assume that \(\partial (pu_i) / \partial x_i = 0\), the transport equations for the normal Reynolds stresses are reduced to

\[
0 = -\frac{\overline{U_k}}{\partial x_k} \frac{\overline{u_i u_i}}{\partial x_k} - 2 \frac{\overline{\partial u_i}}{\partial x_k} \langle u_i u_i \rangle - 2 \nu \frac{\partial^2 \langle u_i^2 \rangle}{\partial x_k^2} - \left( \frac{\partial^2 \langle u_i^2 \rangle}{\partial x_k^2} - \epsilon_{11} \right) + 2 \left( \frac{1}{\partial x_k} \right) \tag{5.17}
\]

where explicit estimates for the different terms are presented in Equations 5.12-5.16.

These equations still maintain some of the measurable terms that can be neglected under the wall-parallel homogeneity assumption, e.g. \(2 \nu \frac{\partial^2 \langle u_i^2 \rangle}{\partial x_i^2}\), in order to demonstrate that they are negligible. It is essential to impose \(\partial (pu_i) / \partial x_i = 0\) since the evolution equations for \(k\) can only estimate the magnitude of \(\partial \langle u_i p \rangle / \partial x_i\), and the values of \(\partial \langle pu_i \rangle / \partial x_i\), which are
assumed to be the dominant part of the pressure-diffusion term, are needed in the equations for \( \langle u_2^2 \rangle \). Subject to this assumption, all the terms in Equation 5.15 except for the pressure strain are known. Thus, the pressure strain can be estimated from the balance of the other terms, as illustrated in Figure 5.13.

In the balance of \( \langle u_1^2 \rangle \) (Figure 5.13a), advection by mean flow, production, \( \nu \partial \langle u_i^2 \rangle / \partial x_k \partial x_k \) and \( \partial \langle u_i^3 \rangle / \partial x_i \) are negligible, the latter one supports the wall-parallel homogeneity assumption. Consequently, the pressure strain term balances the triple correlation and dissipation terms. Away from the wall the pressure triple correlation, which is dominated by \( \partial \langle u_2 u_i^2 \rangle / \partial x_2 \), balances \( \varepsilon_{11} \), namely the turbulent flux of wall-parallel normal stress is balanced by the dissipation. However, with decreasing distance from the wall, the pressure strain term becomes the primary source for \( \langle u_i^2 \rangle \). The contribution of the pressure strain to the wall-parallel component peaks away from the wall, consistent with Perot and Moin (1995a). Conversely, in the present data \( \varepsilon_{11} \) peaks clearly away from the wall and the viscous diffusion term is very small, whereas in Perot and Moin \( \varepsilon_{11} \) peaks with very large values at the wall, and it is balanced to a great part by gain generated by the viscous diffusion term. It is quite likely that the discrepancy is associated with Perot and Moin studying transient phenomena whereas the present data represents steady state conditions. An increase in \( \varepsilon_{11} \) at the wall (but to a lesser extent) is also reported by Aronson et al. (1997) in wind tunnel measurements of decaying grid-generated turbulence near a wall moving at the same speed as the mean flow. In their case the advection by mean flow balances the dissipation away from the wall, and the
effect of pressure becomes noticeable only at $x_2/L_\infty < 0.1$, significantly less than the present results.

In the balance of $\langle u_2^2 \rangle$ (Figure 13b), the primary contributors to gain are the pressure-velocity correlation and production terms, with a small contribution near the wall from the triple correlation. The losses are mostly due to $\varepsilon_{22}$ and the pressure strain. Like all the other present dissipation terms $\varepsilon_{22}$ peaks at $x_2/L_\infty = 0.1$ whereas in Perot and Moin’s (1995) results it decreases monotonically with decreasing distance from the wall. Away from the wall the effect of the pressure strain diminishes, but near the wall it is very large. Consistent with the results of Perot and Moin, near the wall the pressure diffusion term increases the $\langle u_2^2 \rangle$, and the pressure strain term decreases it. In their simulations these effects also peak away from the wall, at $x_2/L_\infty \sim 0.04$, whereas in the present measurements the peak is at $x_2/L_\infty = 0.1$.

5.4. Discussion

5.4.1 Dominating Dissipation Mechanisms

As the present data shows, steady shear-free turbulence near a wall generates a dissipation peak near but away from the wall. In this section we attempt to explain the underlying mechanism for this phenomenon. We have shown in previous section that the wall interacts with the turbulence through the pressure. The balance of turbulent kinetic energy shows that the primary source near the wall is pressure diffusion with some (much smaller) contribution from production. “Far” from the wall, at $x_2/L_\infty = 0.6$, there is a production-dissipation balance. Near the wall the dominant source of the wall-normal component of the Reynolds stress is the pressure-velocity correlations, whereas $\varepsilon_{22}$ and the pressure strain depletes this energy. The contribution of the triple correlation term
(turbulent diffusion) to the wall-normal component is small. On the other hand, the pressure strain is the primary source of energy for the wall-parallel component, which is depleted mostly by $\varepsilon_{11}$. Near the wall the contribution of the triple correlation term to the wall-parallel component is much smaller than the pressure strain (or dissipation), but far from the wall $\varepsilon_{11}$ is balanced by turbulent diffusion. Thus, the pressure strain is clearly the primary mechanism of inter-component energy transfer. All the dissipation terms and the parameters involving pressure peak at $x_2/L_\infty = 0.08 - 0.1$. At $x_2/L_\infty = 0.6$, the pressure strain and pressure-diffusion terms diminish.

As the present energy and dissipation spectra show, the enhanced dissipation at $x_2/L_\infty = 0.1$ is caused by energetic small-scale eddies, which form at the expense (due to breakup) of the large structures that dominate the turbulence far from the wall. Depletion of the energy of the large structure is more pronounced in the spectra of the wall-normal component than in the wall-parallel component. However, at the scales of the dissipating small energetic eddies, there is an increase in energy of both the wall-normal and wall-parallel components. This phenomenon is illustrated by a sample instantaneous velocity distribution shown in Figure 5.14. Here, the original velocity field is spatially filtered using a $21 \times 21$ vectors box filter (4.2$\times$4.2 mm), which correspond to $\kappa_1=1500$ rad/m, i.e. a wave-number slightly smaller than the high wave-number peaks in the dissipation spectra. In the low-pass-filtered velocity distribution (Figure 5.14a) most of the energetic structures, as demonstrated by the vorticity magnitude, are located away from the wall. By subtracting the filtered velocity from the original data we also obtain the “high-pass” filtered flow structure (Figure 5.14b). Clearly, most of the energetic small structures are concentrated in the high dissipation region, in the vicinity of $x_2/L_\infty = 0.1$. The same
qualitative phenomenon repeats itself, but with considerable spatial variations, over the entire data set. Note that consistent with the spectra, the energetic small structures are significantly smaller than the distance between them and the wall. Using the peak in dissipation spectrum ($\kappa_1=4500$ rad/m), the characteristic size of a dissipating eddy is 1.3 mm, more than five times smaller than $0.1L_\infty$ (8 mm).

In support to the claim that breakup of large eddies accelerates close at the wall, Figure 5.15 presents probability density functions of $u_z$ at various distances from the wall, as well as the skewness of this component, i.e. $\xi_2 = \langle u_z u_z^3 \rangle / \langle u_z^2 \rangle^{3/2}$. To illustrate the level of asymmetry in the pdf’s, each plot also shows a flipped pdf in dashed line.

Several trends become clearly evident. First, the pdf’s become wider and the skewness decreases (in magnitude) with increasing distance from the wall. Second, near the wall, including the high dissipation region, the negative “tails” of all the pdf’s are distinctly larger than the positive tails. On the other hand, the number of small positive values is larger than the negative ones. The larger negative tail represents large, energetic, but less frequent sweeping events moving towards the wall, whereas the small but more frequent positive events represents broken-up, less energetic eddies. Clearly, structures traveling away from the wall are smaller than those traveling towards the wall. Third, similar to the trends of turbulent kinetic energy, there is a noticeable increase in the slope of the skewness at $0.06 < x_2/L_\infty < 0.2$, i.e. in the region of the dissipation peak. This trend is consistent with the accelerated breakup process, which alters the local length and time scales. Finally, at $x_2/L_\infty < 0.06$ the slope of the skewness decreases again and the negative tail in the pdf is not as obvious. Thus, the breakup process slows down in this region.
Further insight, in an attempt to understand the differences between trends of \( u_2^2 \) and \( u_1^2 \), can be obtained by examining the relationship between pressure diffusion and distribution of turbulent kinetic energy. A monotonic increase in \( -\langle u_2 p \rangle \) with \( x_2 \) \((\partial \langle u_2 p \rangle / \partial x_2 < 0)\) implies that \( \langle u_2 p \rangle \) is negative, i.e. on average the pressure is higher when \( u_2 < 0 \). This trend may be related to the skewness, i.e. to the fact that there are less, but more powerful negative \( u_2 \) events near the wall (further discussion follows). Since, presumably, the effect of the wall on the \( u_2 - p \) correlation decreases with distance from the wall, a positive \( \partial \langle u_2 p \rangle / \partial x_2 \) implies that \( u_2 \) must be increasing at a faster rate to make up for the increasing \( x_2 \). Thus, the rapid increase in \( u_2^2 \) with \( x_2 \) (Figure 5.6), especially the higher gradients in the \( x_2/L_\infty < 0.2 \) range (aided by increased skewness in this domain), is essential for maintaining a positive \( \partial \langle u_2 p \rangle / \partial x_2 \). In other words, for the pressure diffusion to be a source of turbulent kinetic energy \( u_2^2 \) must decrease rapidly as the wall is approached, consistent with the present (and all previous) results. Very close to the wall, i.e. at \( x_2/L_\infty < 0.05 \), \( u_2^2 \) decreases to less than 20% of its far field value, and it can no longer sustain the gradient that exists in the region of maximum dissipation (and pressure diffusion). Indeed, the slope of \( u_2^2 \) vs. \( x_2 \) decreases slightly near the wall, and as a result \( \partial \langle u_2 p \rangle / \partial x_2 \) decreases. This trend is also consistent with the decrease in the slope of the skewness, and the reduction in the asymmetry in the pdf very near the wall (Figure 5.15). Conversely, \( u_1^2 \), which is being fed by the pressure strain, actually increases slightly with decreasing distance from the wall, at least for \( x_2/\eta > 10 \) (the present range of measurements).
This type of logic, along with dimensional arguments, would lead to models for the
temperature-velocity correlation of the type \(-\frac{1}{\rho} \langle u_z p \rangle \propto \langle u_2 u_2 u_2 \rangle\), which is conceptually
consistent with the model introduced by Lumley (1978) for nearly homogeneous
turbulence, namely \(\frac{1}{\rho} \langle u_z p \rangle = -\frac{1}{5} \langle u_2 u_k u_k \rangle\). As Figure 5.12 shows, trends of the triple
correlation term, which is dominated by \(\langle u_2 u_k u_k \rangle\), differ significantly from those of the
pressure diffusion term. If we fit a smooth curve to \(\langle u_2 u_2 u_2 \rangle\) and differentiate it (Figure
5.11b), the fitted \(\partial \langle u_2 u_2 u_2 \rangle / \partial x_2\) has a maximum away from the wall, but unfortunately it is
located at \(x_2/L_\infty = 0.05\), noticeably closer to the wall than the peak of the pressure
diffusion term \(x_2/L_\infty = 0.1\). The same discrepancy occurs if we try 
\(-\frac{1}{\rho} \langle u_z p \rangle \propto \xi_2 k^{3/2}\) instead (not shown). Thus, the actual process is more complicated than the present arguments.

Perot and Moin (1995a) conclude that the inter-component transfer is caused by
imbalance between splats and antisplats, which is caused by viscous near-wall effects.
Indeed, in their computations, the near wall viscous diffusion effects are substantial. The
present instantaneous velocity distributions also show many events that would qualify as
splats and antisplats. However, our results indicate that the inter-component transfer
peaks away from the wall, and that it involves breakdown of large structures into small
energetic eddies, which dominate the dissipation process. In fact, as discussed above,
very near the wall there is very little wall-normal component left that can be transferred
to the wall-parallel direction. The reasons for the differences are speculative. However,
they are most likely related the steady boundary conditions, and very high Reynolds
number in the present measurements (\(~Re_T=12,000\), v.s. less than 400 in Perot and Moin).
5.4.2 Comparisons to Pressure Strain Models

It is also tempting to compare the present pressure strain terms to some of the many models developed to-date (see Pope, 2000, for a summary). However, due to discrepancies in the underlying assumptions, the results are disappointing, in agreement with the conclusions of Aronson et al. (1997). The Rotta (1951) “return to isotropy” model is

\[
\frac{1}{\rho} \left\langle \rho \partial_t (\epsilon \partial x_j) \right\rangle^R = -2c_1 \epsilon \delta_{ij} - 2c_1 \epsilon \left( \frac{u_i u_j}{u_k u_k} - \frac{1}{3} \delta_{ij} \right)
\]

(5.18)

Inherently, since \( b_{22} \) is negative near the wall \( \left\langle \rho \partial_t (\epsilon \partial x_j) \right\rangle^R \) is positive, in contrast to the present data. Near the wall the pressure strain enhances the anisotropy, and as a result it cannot be modeled as a mechanism for reducing the anisotropy. The model introduced by Lumley (1978) and by Shih and Lumley (1986), as simplified by Perrot and Moin (1995b), is

\[
\frac{1}{\rho} \left\langle \rho \partial_t (\epsilon \partial x_j) \right\rangle^L = \epsilon_{ij} - \frac{2}{3} \epsilon \delta_{ij} - 2\epsilon \left( \frac{u_i u_j}{u_k u_k} - \frac{1}{3} \delta_{ij} \right) = \epsilon_{ij} - 2\epsilon \left( \frac{u_i u_j}{u_k u_k} \right)
\]

(5.19)

Thus, \( \left\langle \rho \partial_t (\epsilon \partial x_j) \right\rangle^L = \epsilon_{22} - 2\epsilon \langle u_2 u_2 \rangle/(2 \langle u_1 u_1 \rangle + u_2 u_2 \rangle) \). Near the dissipation peak \( \langle u_2 u_2 \rangle \) is small, making the second term on the right hand side substantially smaller than the first. Thus, the pressure strain term still has the wrong sign. The model introduced in Perot and Moin (1995b), which matches their data very well, is

\[
\frac{1}{\rho} \left\langle \rho \partial_t (\epsilon \partial x_j) \right\rangle^{P&M} = C \epsilon_{ij} - 2\beta \epsilon \delta_{ij}
\]

\[
\epsilon_{ij} = \epsilon_{ij} - \nu \frac{\partial^2 \langle u_i u_j \rangle}{\partial \xi_k \partial \xi_k} - \frac{2}{3} \epsilon \delta_{ij}
\]

\[
\epsilon = \frac{1}{2} \left( \epsilon_{ij} - \nu \frac{\partial^2 \langle u_i u_j \rangle}{\partial \xi_k \partial \xi_k} \right)
\]

(5.20)
We do not know which coefficients to choose, so we use the values, $C_\pi=2.3$ and $\beta=1$ (based on the present Reynolds number). Since the viscous diffusion terms in the present study are very small (unlike the much lower $Re_\tau$ data of Perot and Moin) this model is reduced to

$$\frac{1}{\rho} \left\langle p \hat{\partial}_i u_j / \hat{\partial} x_j \right\rangle^{P&M} \approx C_\pi \left( \varepsilon_{ij} - \frac{1}{3} \varepsilon_{ll} \delta_{ij} \right) - \beta \varepsilon_{ij} b_{ij}. $$

Using the assumptions of axisymmetry and wall-parallel homogeneity, one obtains

$$\frac{1}{\rho} \left\langle p \hat{\partial}_i u_i / \hat{\partial} x_1 \right\rangle^{P&M} \approx C_\pi \left( \frac{1}{2} \varepsilon_{11} - \frac{1}{3} \varepsilon_{22} \right) - \beta \left( 2 \varepsilon_{11} + \varepsilon_{22} \right) \left[ \frac{1}{2} \left( u_1 u_1 \right) + u_2 u_2 \right] - \frac{1}{3} $$

$$\frac{1}{\rho} \left\langle p \hat{\partial}_i u_i / \hat{\partial} x_2 \right\rangle^{P&M} \approx C_\pi \left( \frac{1}{2} \varepsilon_{22} - \frac{1}{3} \varepsilon_{11} \right) - \beta \left( 2 \varepsilon_{11} + \varepsilon_{22} \right) \left[ \frac{1}{2} \left( u_2 u_2 \right) + u_1 u_1 \right] - \frac{1}{3} $$

(5.21)

As Figures 5.13(a) and (b) show, the first term on the right hand side is small, especially near the wall, where $\varepsilon_{11} \approx \varepsilon_{22}$. Thus, the sign of the pressure strain is determined by the anisotropy tensor, leading to conflicting signs with the present (and expected) trends, much like the Rotta and Lumley models. Models in which the pressure strain promotes return to isotropy, are not consistent with the present trends, in which the pressure strain is a primary contributor to the anisotropy. In spite of differences in the experimental results, this statement agrees with the conclusions of Perot and Moin (1995a, b) and with Aronson et al. (1997). In the present data, Figures 5.13(a) and (b) show that the terms affecting the pressure strain significantly are

$$\frac{1}{\rho} \left\langle p \hat{\partial}_i u_i / \hat{\partial} x_1 \right\rangle \approx \frac{1}{2} \left( \varepsilon_{11} + \partial \left( u_1^2 u_k \right) / \partial x_k \right) $$

$$\frac{1}{\rho} \left\langle p \hat{\partial}_i u_i / \hat{\partial} x_2 \right\rangle \approx -\frac{1}{2} \varepsilon_{22} + \frac{1}{\rho} \partial \left( u_2 p \right) / \partial x_2 - \frac{1}{2} P_{22} + \partial \left( u_2^2 u_2 \right) / \partial x_2 $$

(5.22)

The significance of the different terms varies with distance from the wall. In the vicinity of the dissipation peak $\frac{1}{\rho} \left\langle p \hat{\partial}_i u_i / \hat{\partial} x_1 \right\rangle \approx \frac{1}{2} \varepsilon_{11}$ and $\frac{1}{\rho} \left\langle p \hat{\partial}_i u_i / \hat{\partial} x_2 \right\rangle \approx -\frac{1}{2} \varepsilon_{22} + \frac{1}{\rho} \partial \left( u_2 p \right) / \partial x_2$.

### 5.5 Summary and Conclusions

Isotropic turbulence with very low mean velocity, at moderately high Reynolds numbers ($Re_\tau=280$), is generated using symmetrically positioned four active grids. A
solid wall placed in this turbulent domain enables us to examine the interaction of nearly shear free turbulence with this wall under steady state conditions. The data base consists of 1200, ensemble averaged, 2-D velocity distributions, obtained using PIV, with spatial resolution that matches the Kolmogorov scale. Assumptions of axisymmetry and wall-parallel homogeneity enable us to directly determine all the terms in the evolution equations for the turbulent kinetic energy and Reynolds stresses, except for the terms involving the pressure. The latter are evaluated by balancing the equations.

The results show an increasing anisotropy with decreasing distance from the wall, as the wall-normal component of the Reynolds stress decreases and the wall-parallel component remains unchanged, and even increase slightly. This trend agrees with the results of Thomas and Hancock (1977), Perot and Moin (1995a) and Aronson et al (1997). The dissipation rate peaks at $x_2/L_\infty = 0.08 - 0.1$, i.e. near but clearly away from the wall. The primary source term of turbulent kinetic energy near the wall is pressure diffusion. Pressure diffusion is also the dominant source for the wall-normal component of the Reynolds stress near the wall, whereas $\varepsilon_{22}$ and the pressure strain deplete this component. Conversely, pressure strain is the primary source for the wall-parallel component and it is depleted by $\varepsilon_{11}$. Thus, in agreement with Perot and Moin (1995a), inter-component energy transfer caused by the pressure strain enhances the anisotropy. Consequently, pressure strain models that are based on the anisotropy tensor, i.e. assume that the pressure strain reduces the anisotropy lead to the wrong results. The contributions of turbulent diffusion and production to the balance of energy and Reynolds stresses near the wall are smaller than the terms involving dissipation and pressure. However, far from
the wall the production and triple correlation terms become significant and balance the dissipation terms.

Samples of instantaneous flow structure, pdf’s of velocity magnitude (along with skewness), as well as energy, vorticity and dissipation spectra are used for elucidating the process generating enhanced dissipation at $x_2/L_\infty = 0.1$. They clearly show that the high dissipation rate is caused by breakup of the large eddies, which dominate the turbulence far from the wall, into energetic small-scale structures. Consequently, even as the overall wall-normal Reynolds stress diminishes near the wall, the energy at small scales of both components increases. The resulting much shorter length and time scales enhance the dissipation rates. These small structures are significantly smaller (by 6-8 times) than their distance from the wall. Since turbulent diffusion near the wall is small, the breakup process must occur in the vicinity of the dissipation peak (i.e. locally), due to interactions between the turbulence and the wall-induced pressure field (or image of the flow). Very near the wall, at $x_2/L_\infty < 0.06$, the wall-normal component and the pressure diffusion that feeds it diminish. As a result, the inter component transfer and dissipation rate decrease. However, since the wall-parallel component is still substantial, significant levels of dissipation must persists all the way to the wall.

Throughout the paper we compare the present steady data and trends to the DNS results of Perot and Moin (1995a), and to the experimental data of Aronson et al. (1997) and Thomas & Hancock (1977). We agree, at least qualitatively, with the important role of pressure strain on the inter-component energy transfer. The numerical results also show that the effect of pressure diffusion on the wall-normal component peak away from the wall. However, there are also some disagreements. Unlike Perot and Moin’s data, in
the present system, the effect of viscous diffusion is minimal and the effect of turbulent
diffusion is significant only far from the wall. Accordingly, Perot and Moin conclude that
viscous-induced imbalance between splats and antisplats is responsible for the inter-
component energy transfer, whereas in the present data the transfer is associated with
pressure-induced breakup of the turbulence to small energetic structures. Note, however,
that since the present \( u_1^2 \) is still very high at \( x_2 = 6 \eta \), viscous effects most likely become
significant closer to the wall. The reasons for the differences may be the steady boundary
conditions, and high Reynolds number of the present measurements.

**Appendix**

In Section 5.3 we make an assumption of axisymmetry with respect to the \( y (x_2) \) axis.

Applying the rotational invariance constraint around the wall-normal direction on the 81-
term 4\(^{\text{th}}\) rank tensor, \( Q_{ijkl} = < s_{ij} s_{kl} > \), inherently

\[
\begin{align*}
Q_{1111} &= Q_{3333} \\
Q_{1212} &= Q_{3232} 
\end{align*}
\]  

(A1)

In general, tensor rotation can be defined as

\[
Q_{ijkl}' = J_{i1} J_{j2} J_{k3} J_{l4} Q_{ijkl}
\]  

(A2)

where the second order tensor \( J_{ii} \) is the Jacobian of rotation. An assumption of
axisymmetry implies that rotation around the \( x_2 \) axis would not alter the terms of the 4\(^{\text{th}}\)
order tensor \( Q_{ijkl} = < s_{ij} s_{kl} > \). Rotation around the \( x_2 \) axis implies that the Jacobian terms are

\[
J_{11} = J_{33} = \cos \theta, \quad J_{31} = -J_{13} = \sin \theta \quad \text{and the rest of the terms are zero.}
\]

Performing the rotation, one obtains that

\[
Q_{TTFF} = Q_{1111} \cos^4 \theta + Q_{3333} \sin^4 \theta + A_{ijkl} \cos^2 \theta \sin^2 \theta + B_{ijkl} \cos^3 \theta \sin \theta + C_{ijkl} \cos \theta \sin^3 \theta \]  

(A3)
Since axisymmetry implies that $Q_{1111} = Q_{3333}$, then $B_{ijkl} = C_{ijkl} = 0$ and $A_{ijkl} = 2Q_{1111}$.

Thus,

$$A_{ijkl} = 2Q_{1111} = Q_{1133} + Q_{1313} + Q_{3113} + Q_{3131} + Q_{3311}$$  \hspace{1cm} (A4)

In cases where indices $i$ and $j$, as well as $k$ and $l$ are symmetric,

$$Q_{1133} + Q_{1313} + Q_{3113} + Q_{3131} + Q_{3311} = 2Q_{1133} + 4Q_{1313}$$  \hspace{1cm} (A5)

Thus, axisymmetry implies that $2Q_{1111} = 2Q_{1133} + 4Q_{1313}$, i.e.

$$Q_{1313} = \frac{1}{2} [Q_{1111} - Q_{1133}]$$  \hspace{1cm} (A6)

Another way to obtain Equation A6 is to rely on relationships developed based on two-point velocity correlations (Batchelor, 1946, Chandrasekhar, 1950, George & Hussein, 1991). For the present coordinates, assuming wall-parallel homogeneity and axisymmetry, George & Hussein (1991) show that:

$$\begin{align*}
\left\langle \frac{\partial u_i}{\partial \chi_m} \frac{\partial u_j}{\partial \chi_n} \right\rangle &= (-2\alpha_{02} + 2\alpha_{22} - 2\beta_{02}) \delta_{im} \delta_{jn} + \delta_{in} \delta_{jm} + \delta_{mn} \delta_{ij} + \\
&+ \left\{ (8\alpha_{02} - 4\alpha_{22} + 6\beta_{02}) \delta_{mn} - (4\alpha_{02} - 2\beta_{22} - 16\alpha_{22}) \delta_{2m} \delta_{2n} \right\} \delta_{ij} + \\
&+ \left[ (4\alpha_{22} - 6\beta_{02}) \delta_{mn} - 2\beta_{22} \delta_{2m} \delta_{2n} \right] \delta_{ij} - \\
&- \left(4\alpha_{22} - 2\beta_{02} \right) \left[ \delta_{2m} \delta_{2n} + \delta_{jm} \delta_{in} + \delta_{im} \delta_{jn} \right] \\
\end{align*}$$  \hspace{1cm} (A7)

where

$$\begin{align*}
\alpha_{02} &= \frac{1}{3} \left( \frac{\partial \bar{u}_2}{\partial \bar{u}_1} \right)^2 \hspace{1cm} \beta_{02} = \frac{1}{6} \left( \frac{\partial \bar{u}_1}{\partial \bar{u}_3} \right)^2 + \left( \frac{\partial \bar{u}_2}{\partial \bar{u}_3} \right)^2 - \frac{3}{2} \left( \frac{\partial \bar{u}_2}{\partial \bar{u}_1} \right)^2 \hspace{1cm} \alpha_{22} = \frac{1}{4} \left( \frac{\partial \bar{u}_2}{\partial \bar{u}_2} \right)^2 - \frac{1}{2} \left( \frac{\partial \bar{u}_2}{\partial \bar{u}_3} \right)^2 \hspace{1cm} \beta_{22} = \frac{1}{2} \left( \frac{\partial \bar{u}_1}{\partial \bar{u}_2} \right)^2 + \left( \frac{\partial \bar{u}_1}{\partial \bar{u}_2} \right)^2 - \frac{10}{3} \left( \frac{\partial \bar{u}_1}{\partial \bar{u}_3} \right)^2 - \frac{1}{3} \left( \frac{\partial \bar{u}_1}{\partial \bar{u}_1} \right)^2 - \frac{1}{3} \left( \frac{\partial \bar{u}_1}{\partial \bar{u}_3} \right)^2 \right. \\
\end{align*}$$  \hspace{1cm} (A8)

are invariants of the $4^{th}$ rank velocity derivative tensor. Substituting for $Q_{1113} = \langle S_{13} \rangle$ and $0.5[Q_{1111} - Q_{1133}] = 0.5(\langle S_{11} \rangle - \langle S_{13} \rangle)$ one can readily show that both are equal to $3\alpha_{02} - \alpha_{22} + 2\beta_{02}$, verifying Equation A6.
Consequently, for axisymmetric, and wall-parallel homogeneous turbulence, only four terms are needed to fully determine the dissipation rate, $\varepsilon = 2\nu Q_{ijkl} \delta_{ik}\delta_{jl}$. Explicitly, using Equations A7 and A8,

$$
\varepsilon = \nu \left[ 2\left( \frac{\partial u_2}{\partial x_1} \right)^2 - \left( \frac{\partial u_2}{\partial x_2} \right)^2 \right] + 8\left( \frac{\partial u_1}{\partial x_1} \right)^2 + 2\left( \frac{\partial u_1}{\partial x_2} \right)^2 \right] (A9)
$$

In the evolution Equation for the turbulent kinetic energy we also need to calculate the mean shear production term

$$
P = -\langle u_i u_j \rangle S_{ij} = -\frac{1}{2} \langle u_i u_j \rangle \left( \frac{\partial U_i}{\partial x_j} + \frac{\partial U_j}{\partial x_i} \right). (A10)
$$

We use the same assumptions of wall-parallel homogeneity and axisymmetry, which lead to relations between terms of the 4th rank tensor $\langle u_i u_j \rangle S_{ijkl}$ (note that $P = \langle u_i u_j \rangle S_{ijkl} \delta_{ik}\delta_{jl}$) that are to equivalent to those indicated in Equations A1 and A6, i.e.

$$
\begin{align*}
\langle u_1 u_1 \rangle &\frac{\partial U_1}{\partial x_1} = \langle u_3 u_3 \rangle \frac{\partial U_3}{\partial x_3} \\
\langle u_1 u_2 \rangle \left( \frac{\partial U_1}{\partial x_2} + \frac{\partial U_2}{\partial x_1} \right) &= \langle u_3 u_2 \rangle \left( \frac{\partial U_3}{\partial x_2} + \frac{\partial U_2}{\partial x_3} \right) \\
\langle u_3 u_3 \rangle &\frac{\partial U_3}{\partial x_3} = -\langle u_1 u_1 \rangle \left( \frac{\partial U_1}{\partial x_1} + \frac{\partial U_1}{\partial x_2} \right) \\
\langle u_1 u_3 \rangle &\left( \frac{\partial U_1}{\partial x_3} + \frac{\partial U_3}{\partial x_1} \right) = \frac{1}{2} \langle u_1 u_1 \rangle \frac{\partial U_1}{\partial x_1} - \langle u_3 u_1 \rangle \frac{\partial U_3}{\partial x_3} \right) (A11)
\end{align*}
$$

Consequently, the mean shear production can be estimated from

$$
P = -4\langle u_1^2 \rangle \frac{\partial U_1}{\partial x_1} - 2\langle u_1 u_2 \rangle \left( \frac{\partial U_1}{\partial x_2} + \frac{\partial U_2}{\partial x_1} \right) \left( \langle u_1^2 \rangle + \langle u_2^2 \rangle \right) \frac{\partial U_2}{\partial x_2} \right] (A12)
$$

Similarly, to calculate the turbulent viscous diffusion term $-2\nu \langle u_j s_j \rangle / \partial x_j$, we use
\[ \partial u_{s_1} / \partial x_1 = \partial (u_{s_3}) / \partial x_3 \]
\[ \partial u_{s_2} / \partial x_1 = \partial (u_{s_2}) / \partial x_3 \]
\[ \partial u_{s_3} / \partial x_1 = \partial (u_{s_1}) / \partial x_3 \]
\[ \partial u_{s_1} / \partial x_2 = \partial (u_{s_3}) / \partial x_2 \]
\[ \partial u_{s_3} / \partial x_1 = \frac{1}{2} \left( \partial (u_{s_1}) / \partial x_1 - \partial (u_{s_3}) / \partial x_1 \right) \]
\[ \partial u_{s_3} / \partial x_1 = -\partial (u_{s_1} + s_{22}) / \partial x_1 \]

Consequently:
\[ \frac{\partial (u s_{ij})}{\partial x_i} = 4 \frac{\partial (u_1)}{\partial x_1} + \frac{\partial (u_2)}{\partial x_2} + \frac{\partial (u_2)}{\partial x_2} + \frac{\partial (u_1)}{\partial x_1} + \frac{\partial (u_1)}{\partial x_1} + \frac{\partial (u_2)}{\partial x_2} \] (A14)

For estimating the turbulent advection term of \( k = 0.5 u_j u_j \), axisymmetry implies that
\[ \partial (u_k) / \partial x_i = 2 \partial (\partial x_k / \partial x_i) + \partial (\partial x_2 / \partial x_2) \] (A15)

Wall-parallel homogeneity would imply that first term on the right hand side vanishes.

To estimate the terms in the Reynolds stress transport equations
\[ \frac{\partial (u^2_1)}{\partial t} = -2 \frac{\partial (p u_1)}{\partial x_1} + v \frac{\partial^2 (u^2_1)}{\partial x_1^2} - \frac{\partial (u_1 u_2)}{\partial x_1} - \frac{\partial (u_1 u_2)}{\partial x_1} + 2 \left\{ \frac{\partial (u_1)}{\partial x_1} \right\}, \]
\[ \frac{\partial (u^2_2)}{\partial t} = -2 \frac{\partial (p u_2)}{\partial x_2} + v \frac{\partial^2 (u^2_2)}{\partial x_2^2} - \frac{\partial (u_1 u_2)}{\partial x_2} - \frac{\partial (u_1 u_2)}{\partial x_2} + 2 \left\{ \frac{\partial (u_2)}{\partial x_2} \right\} \] (A16)

it is necessary to estimate the dissipation tensor, \( \varepsilon_{ij} \)
\[ \varepsilon_{ij} = 2 v \left( \partial u_i / \partial x_j \cdot \partial u_j / \partial x_k \right) \]
\[ \varepsilon_{11} = 2 v \left( \left( \partial u_1 / \partial x_1 \right)^2 + \left( \partial u_1 / \partial x_2 \right)^2 + \left( \partial u_1 / \partial x_3 \right)^2 \right) \] (A17)
\[ \varepsilon_{22} = 2 v \left( \left( \partial u_2 / \partial x_1 \right)^2 + \left( \partial u_2 / \partial x_2 \right)^2 + \left( \partial u_2 / \partial x_3 \right)^2 \right) \]

Applying the axisymmetry conditions (Equation A6) and continuity,
\[ \left\{ \left( \partial u_1 / \partial x_1 \right)^2 \right\} = \left\{ \left( \partial u_1 / \partial x_1 \right)^2 \right\} + \frac{1}{3} \left\{ \left( \partial u_1 / \partial x_1 \right) \left( \partial u_2 / \partial x_2 \right) \right\} \] (A18)

Thus,
\[ \varepsilon_1 = 2 \nu \left( 2 \left( \frac{\partial u_1}{\partial x_1} \right)^2 + \left( \frac{\partial u_1}{\partial x_2} \right)^2 + \frac{1}{2} \left( \frac{\partial u_1}{\partial x_1} \right) \left( \frac{\partial u_2}{\partial x_2} \right) \right) \]

\[ \varepsilon_2 = 2 \nu \left( 2 \left( \frac{\partial u_2}{\partial x_1} \right)^2 + \left( \frac{\partial u_2}{\partial x_2} \right)^2 \right) \]  

(A19)

Due to the wall-parallel homogeneity in the present system, consistent with the experimental results, only the terms involving \( \frac{\partial u_i}{\partial x_2} \) contribute significantly to the evolution of the Reynolds stresses. We also have to evaluate

\[ \frac{\partial^2 u_i u_j}{\partial x_k \partial x_l} = \frac{\partial^2 u_i u_j}{\partial x_1^2} + \frac{\partial^2 u_i u_j}{\partial x_2^2} \]  

(A20)

The third term on the right hand side can be written as \( \frac{\partial^2 u_1 u_2}{\partial x_3^2} \) and using Equation A6 \( \frac{\partial^2 u_1 u_2}{\partial x_3^2} = \frac{1}{3} \left( \frac{\partial^2 u_1 u_2}{\partial x_1^2} - \frac{\partial^2 u_1 u_2}{\partial x_2^2} \right) \), namely \( \frac{\partial^2 u_1 u_2}{\partial x_3^2} = \frac{1}{3} \frac{\partial^2 u_1 u_2}{\partial x_1^2} \). Thus,

\[ \frac{\partial^2 u_1 u_2}{\partial x_3^2} = \frac{4 \partial^2 u_1 u_2}{3 \partial x_1^2} + \frac{\partial^2 u_1 u_2}{\partial x_2^2} \]  

(A21)

However, as the data shows (Figure 12), due to the horizontal homogeneity the first term on the right hand side is very small, i.e. \( \frac{\partial^2 u_1 u_2}{\partial x_3^2} \approx \frac{\partial^2 u_1 u_2}{\partial x_2^2} \). Axisymmetry also implies that \( \frac{\partial^2 u_1 u_2}{\partial x_3^2} = \frac{\partial^2 u_1 u_2}{\partial x_1^2} \), i.e.

\[ \frac{\partial^2 u_1 u_2}{\partial x_3^2} = \frac{2 \partial^2 u_1 u_2}{\partial x_1^2} + \frac{\partial^2 u_2 u_2}{\partial x_2^2} \]  

(A22)

and wall-parallel homogeneity implies that \( \frac{\partial^2 u_2 u_2}{\partial x_2^2} \) is dominant. Similarly,

\[ \frac{\partial^2 u_1}{\partial x_3^2} = \frac{\partial^2 u_1}{\partial x_1^2} \] and \( \frac{\partial^2 u_2}{\partial x_3^2} = \frac{\partial^2 u_2}{\partial x_2^2} \), namely

\[ \nu \frac{\partial^2 u_1}{\partial x_3 \partial x_k} = 2 \nu \frac{\partial^2 u_1}{\partial x_1^2} + \nu \frac{\partial^2 u_1}{\partial x_2^2} \]

\[ \nu \frac{\partial^2 u_2}{\partial x_3 \partial x_k} = 2 \nu \frac{\partial^2 u_2}{\partial x_1^2} + \nu \frac{\partial^2 u_2}{\partial x_2^2} \]  

(A23)

Axisymmetry also implies that the terms involving transport by mean flow are
\[ U_k \frac{\partial u_i^2}{\partial x_k} = 2U_1 \frac{\partial u_i^1}{\partial x_1} + U_2 \frac{\partial u_i^2}{\partial x_2} \]
\[ U_k \frac{\partial u_i^3}{\partial x_k} = 2U_1 \frac{\partial u_i^1}{\partial x_1} + U_2 \frac{\partial u_i^3}{\partial x_2} \]

(A24)

To determine the production tensor terms, we need to evaluate \( \langle u_i u_i \rangle \alpha \partial x_i / \alpha x_3 \). Using Equation A6 and imposing that \( \langle u_i u_i \rangle = \langle u_3 u_3 \rangle \),

\[ \frac{\alpha U_1}{\alpha x_3} \langle u_i u_i \rangle = \frac{1}{2} \left[ \frac{\alpha U_1}{\alpha x_1} \langle u_1 u_1 \rangle - \frac{\alpha U_1}{\alpha x_1} \langle u_3 u_3 \rangle \right] = 0 \]

(A25)

Thus, the production tensor terms are,

\[ \frac{\alpha U_1}{\alpha x_k} \langle u_i u_k \rangle = \frac{\alpha U_1}{\alpha x_1} \langle u_1 u_1 \rangle + \frac{\alpha U_1}{\alpha x_2} \langle u_1 u_2 \rangle \]
\[ \frac{\alpha U_2}{\alpha x_k} \langle u_2 u_k \rangle = 2 \frac{\alpha U_2}{\alpha x_1} \langle u_2 u_1 \rangle + \frac{\alpha U_2}{\alpha x_2} \langle u_2 u_2 \rangle \]

(A26)

Assuming steady state conditions (\( \partial / \partial t = 0 \)), and relaying on wall-parallel homogeneity to assume that \( \alpha(p u_i) / \alpha x_1 = 0 \), the transport equations for the normal Reynolds stresses are reduced to

\[ 0 = -U_k \frac{\partial u_i^1}{\partial x_k} - 2 \frac{\alpha U_1}{\alpha x_k} \langle u_1 u_k \rangle - 2 \nu \frac{\partial^2 \langle u_i^1 \rangle}{\partial x_k \partial x_k} - \frac{\alpha u_i^1 u_i^3}{\alpha x_3} - \varepsilon_{11} + 2 \left( p \frac{\partial u_i^1}{\partial x_1} \right) \]
\[ 0 = -U_k \frac{\partial u_i^2}{\partial x_k} - 2 \frac{\alpha U_2}{\alpha x_k} \langle u_2 u_k \rangle - 2 \nu \frac{\partial^2 \langle u_i^2 \rangle}{\partial x_k \partial x_k} - \frac{\alpha u_i^2 u_i^2}{\alpha x_2} - \varepsilon_{12} + 2 \left( p \frac{\partial u_i^2}{\partial x_2} \right) \]

(A27)

These equations still maintain some of the measurable terms that can be neglected under the wall-parallel homogeneity assumption in order to demonstrate that they are negligible. However, is essential to impose \( \alpha(p u_i) / \alpha x_i = 0 \) since the evolution equations for \( k \) can only estimate the magnitude of \( \alpha(u_i p) / \alpha x_i \), and the values of \( \alpha(p u_2) / \alpha x_2 \) are needed in the equations for \( \langle u_2^2 \rangle \).
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**Figures**

**Fig. 5.1** Experimental setup for studying steady, shear-free turbulence near a solid wall. Top: side view Bottom: top view.

**Fig. 5.2** Distributions of mean (ensemble averaged) velocity components and their r.m.s. values, as measured in three 5x5 cm sample areas. The side panels show the profiles averaged over the wall-parallel direction. All units are m/s. (a) Mean wall-normal velocity component, $\overline{U}_2$. (b) Mean wall-parallel velocity component, $\overline{U}_1$. (c) R.M.S. of wall-normal component, $\langle u_2 u_2 \rangle^{0.5}$. (d) RMS of wall-parallel component, $\langle u_1 u_1 \rangle^{0.5}$.

**Fig. 5.3** Samples of 1D energy spectra, $E_{11}(k_1)$ and $3/4 E_{22}(k_1)$, near ($x_2$=10$\eta$) and “far” ($x_2$=266$\eta$) from the wall. Solid lines with filled symbols - $E_{11}(k_1)$; Dashed lines with hollow symbols - $3/4 E_{22}(k_1)$.
Fig. 5.4 One-dimensional energy spectra scaled with $(e_\infty \nu^\gamma)^{3/4}$ ($e_\infty$ is shown in Figure 8). (a) $E_{11}(\kappa_1)$ and $3/4E_{22}(\kappa_1)$ at $x_2=266\eta$ (top), $x_2=61\eta$ (middle) and $x_2=10\eta$ (bottom). (b-e) Magnified sections of the spectra: (b) $E_{11}$ at low wave numbers ($1/\kappa=20\eta\sim1000\eta$). (c) $3/4E_{22}$ at low wave numbers. (d) $E_{11}$ at high wave numbers ($1/\kappa=4\eta\sim20\eta$) (e) $3/4E_{22}$ at high wave numbers.

Fig. 5.5 One dimensional spectra of the out-of-plane vorticity ($\omega_3$) scaled with $(e_\infty \nu^3)^{3/4}$ ($e_\infty$ is shown in Figure 8). The insert shows magnified, high wave number portions of the spectra.

Fig. 5.6 Reynolds stresses, $\langle u_i u_j \rangle$, $i,j=1,2$, averaged along the wall-parallel direction, and scaled with their far field ($x_2=310\eta$) values.

Fig. 5.7 (a) Distributions of $\langle \hat{\epsilon} u_i / \hat{\epsilon} x_j \rangle$ $i,j=1,2$, averaged along the wall-parallel direction. (b) Distributions of spatially filtered velocity fluctuations derivatives squared. A 4 X 4 vectors box filter ($\Delta=0.8\text{mm}$) is used.

Fig. 5.8 The dissipation rate estimated using Equation 3, and turbulent kinetic energy estimated using Equation 4. Both are averaged along the wall-parallel direction, and scaled with their value at $x_2=310\eta$, denoted as $e_\infty$ ($=2.8\times10^{-3} \text{ m}^2\text{s}^{-3}$) and $k_\infty$ ($=3.7\times10^{-3} \text{ m}^2\text{s}^{-2}$).

Fig. 5.9 One dimensional dissipation spectra scaled with $(e_\infty \nu^3)^{3/4}$. (a) $k_1^2E_{11}$; and (b) $k_1^2E_{22}$.

Fig. 5.10 Mean shear production turbulent of kinetic energy estimated using Equation 6, along with the terms contributing to it. The data are averaged along the wall-parallel direction.

Fig. 5.11 (a) Scaled wall-normal turbulent transport, $\langle u_2k \rangle$, along with the terms contributing to it. (b) Scaled terms contributing to the turbulent transport flux, $\hat{\partial}\langle u_2k \rangle / \hat{\partial} x_1$ (according to Equation 8), along with $\hat{\partial}\langle u_2^3 \rangle / \hat{\partial} x_2$. The data are averaged along the wall-parallel direction.
Fig. 5.12  Scaled terms contributing to the turbulent kinetic energy budget. Proper signs are assigned to each term to indicate gains and losses. The data are averaged along the wall-parallel direction.

Fig. 5.13  Scaled terms contributing to the Reynolds stress budgets. Proper signs are assigned to each term to indicate gains and losses. (a) Wall-parallel component; (b) wall-normal component. The data are averaged along the wall-parallel direction.

Fig. 5.14  Sample instantaneous velocity and vorticity distributions. (a). Spatially, low-pass filtered vorticity using a $\Delta=21\eta$ ($\kappa=1500$ rad/m) box filter. (b) A magnified portion of the velocity and vorticity distributions, as marked in (a), showing characteristic large-scale structures in the far field. (c) High-pass filtered ($\Delta<21\eta$) vorticity distribution of the same flow field. (d) A magnified portion, as marked in (c), of the high-pass filtered velocity map showing typical small-scale structures in the vicinity of the wall.

Fig. 5.15  The skewness and several probability density functions of the wall-normal velocity fluctuations (solid lines). The dashed lines show flipped pdf’s in order to highlight the asymmetry. The data are averaged along the wall-parallel direction.
Fig. 5.1 Experimental setup for studying steady, shear-free turbulence near a solid wall. Top: side view Bottom: top view.
Fig. 5.2 Distributions of mean (ensemble averaged) velocity components and their r.m.s. values, as measured in three 5x5 cm sample areas. The side panels show the profiles averaged over the wall-parallel direction. All units are m/s. (a) Mean wall-normal velocity component, $U_z$. (b) Mean wall-parallel velocity component, $U_1$. (c) R.M.S. of wall-normal component, $\langle u_z^2 \rangle^{0.5}$. (d) RMS of wall-parallel component, $\langle u_1^2 \rangle^{0.5}$. 
Fig. 5.3 Samples of 1D energy spectra, $E_{11}(\kappa)$ and $3/4E_{22}(\kappa)$, near ($x_2=10\eta$) and “far” ($x_2=266\eta$) from the wall. Solid lines with filled symbols - $E_{11}(\kappa)$; Dashed lines with hollow symbols - $3/4E_{22}(\kappa)$. 

Fig. 5.4 One-dimensional energy spectra scaled with $\left(\frac{\varepsilon_0 v_s}{\varepsilon_\infty}\right)^{1/4}$ ($\varepsilon_\infty$ is shown in Figure 8). (a) $E_{11}(k_\parallel)$ and $3/4E_{22}(k_\parallel)$ at $x_2=266\eta$ (top), $x_2=61\eta$ (middle) and $x_2=10\eta$ (bottom). (b-e) Magnified sections of the spectra: (b) $E_{11}$ at low wave numbers ($1/k_\parallel=20\eta$–$1000\eta$). (c) $3/4E_{22}$ at low wave numbers. (d) $E_{11}$ at high wave numbers ($1/k_\parallel=4\eta$–$20\eta$) (e) $3/4E_{22}$ at high wave numbers.
Fig. 5.5 One dimensional spectra of the out-of-plane vorticity ($\omega_3$) scaled with $\left(\varepsilon^3 / \nu^3\right)^{1/4}$ ($\varepsilon^3$ is shown in Figure 8). The insert shows magnified, high wave number portions of the spectra.
Fig. 5.6 Reynolds stresses, $\langle u_i u_j \rangle$, $i,j = 1,2$, averaged along the wall-parallel direction, and scaled with their far field ($x_2 = 310\eta$) values.
\[
\begin{align*}
\left\langle \left( \frac{\partial u_i}{\partial x_j} \right)^2 \right\rangle
\end{align*}
\]
Fig. 5.7 (a) Distributions of $\left( \frac{\partial u_i}{\partial x_j} \right)^2$ for $i, j = 1, 2$, averaged along the wall-parallel direction. (b) Distributions of spatially filtered velocity fluctuations derivatives squared. A 4 X 4 vectors box filter ($\Delta=0.8$mm) is used.
Fig. 5.8  The dissipation rate estimated using Equation 3, and turbulent kinetic energy estimated using Equation 4. Both are averaged along the wall-parallel direction, and scaled with their value at $x_2=310\eta$, denoted as $\varepsilon_\infty (=2.8\times10^{-3} \text{ m}^2\text{s}^{-3})$ and $k_\infty (=3.7\times10^{-3} \text{ m}^2\text{s}^{-2})$. 
Fig. 5.9  One dimensional dissipation spectra scaled with $(\varepsilon^3/\nu)^{1/4}$. (a) $k^2 E_{11}$; and (b) $k^2 E_{22}$.
Fig. 5.10  Mean shear production turbulent of kinetic energy estimated using Equation 6, along with the terms contributing to it. The data are averaged along the wall-parallel direction.
Fig. 5.11  (a) Scaled wall-normal turbulent transport, $\langle u_z k \rangle$, along with the terms contributing to it. (b) Scaled terms contributing to the turbulent transport flux, $\partial \langle u_z k \rangle / \partial x_1$ (according to Equation 8), along with $\partial \langle u_2^3 \rangle / \partial x_2$. The data are averaged along the wall-parallel direction.
Fig. 5.12  Scaled terms contributing to the turbulent kinetic energy budget. Proper signs are assigned to each term to indicate gains and losses. The data are averaged along the wall-parallel direction.
\[
\frac{\partial u_1}{\partial x_1} - \frac{\partial u_k}{\partial x_k} - \frac{\varepsilon_{11}}{-4/3} \frac{\partial u_3}{\partial x_1} - P_{\nu} \frac{\partial u_1}{\partial x_k}
\]

(a)
Scaled terms contributing to the Reynolds stress budgets. Proper signs are assigned to each term to indicate gains and losses. (a) Wall-parallel component; (b) wall-normal component. The data are averaged along the wall-parallel direction.
Fig. 5.14 Sample instantaneous velocity and vorticity distributions. (a) Spatially, low-pass filtered vorticity using a $\Delta=21\eta$ ($\kappa=1500$ rad/m) box filter. (b) A magnified portion of the velocity and vorticity distributions, as marked in (a), showing characteristic large-scale structures in the far field. (c) High-pass filtered ($\Delta<21\eta$) vorticity distribution of the same flow field. (d) A magnified portion, as marked in (c), of the high-pass filtered velocity map showing typical small-scale structures in the vicinity of the wall.
Fig. 5.15 The skewness and several probability density functions of the wall-normal velocity fluctuations (solid lines). The dashed lines show flipped pdf’s in order to highlight the asymmetry. The data are averaged along the wall-parallel direction.
6. Simultaneous Measurements of Wall Shear Stress and 3-dimensional Near Wall Flow Structures

6.1. Background
The relationship between the near wall structures and wall shear stress in a wall-bounded shear flow has been the subject of both computational and experimental studies for many decades. It is a common consensus that streamwise vortices dominate in the inner layer of the turbulent boundary layer and are involved in “bursting” processing (Brooke&Hanratty, 1993, Hamilton, et al., 1995, Jimenez, et al., 2005, Kawahara&Kida, 2001, Kim, et al., 1987, Robinson, 1991, Schoppa&Hussain, 2002, just name a few.) Recently, equipped with more quantitative measurement technique, Particle Image Velocimetry (PIV), Adrian and his coworkers (Adrian et al., 2000) studied flow structures in the logarithmic layer and concluded that hairpin structures form and travel as packets. Most insight on buffer layer dynamics and its interaction with the wall have been obtained largely through Direct Numerical Simulations (DNS) at low Reynolds numbers. Kravcheko et al. (1993), using a DNS database by Kim et al. (1987), found that a streamwise vortex situated very close to the wall is strongly correlated with the local high skin friction. These findings have motivated numerous attempts to develop schemes for drag reduction, e.g. Kim et al. (2003).

Experimental assessment of relationships between turbulent structures and wall stress require simultaneous measurement of both with sufficient spatial resolution. Various techniques have been developed and implemented for measuring the wall shear stress, using oil film interferometry, chemical probes, or hot-wire MEMS sensors as described in
reviews by Ho&Tai (1998), Lofdahl&Gad-el-Hak (1999), Naughton&Sheplak (2002). However, to the best of our knowledge, we do not have 3D experimental data on buffer layer flow structures and wall stress simultaneously.

In this paper, we apply a recently developed technique, in-line Digital Holographic Microscopy (Sheng et al., 2006), to simultaneously measure both components of the instantaneous wall shear stress and velocity distribution in the $0<y^+<100$. The measurement resolution is equivalent to current DNS, 3 wall units in the stream- and span-wise directions, one wall unit in wall-normal direction. Our main objectives are to examine the relationships between wall shear stress and near-wall coherent structures. Brief descriptions on the experimental setup, measurement technique and accuracy are provided in 6.2. Data on characteristic length scales of mean flow are provided in 6.3.1, followed by sample flow structures and wall stress distributions 6.3.2, and statistics of wall stresses in 6.3.3. Results of conditional sampling of flow and stresses based on local stress maxima and minima are presented in 6.3.4. Characteristic length scales of buffer layer structures are determined in 6.3.4 and 6.3.5, before concluding in 6.4.

6.2. Facility, Analysis and Uncertainties

Measurements are performed in a vertical 55 x 55mm square duct facility (Tao et al., 2002, Zhang et al., 1997), and the sample volume is situated at 3.3m (~60 diameter) downstream to a honeycombed entrance. As illustrated in Fig. 6.1, the flow is seeded locally with $2\mu m$ polystere particle through a set of five $50\mu m$ injectors located 40mm (~800 diameter) upstream to the sample volume. The seeding fluid with 1% solid concentration (~10,000 particles/mm$^3$) is injected at 0.05ml/s by a motorized syringe. The exit velocity is 2mm/s, i.e. 0.1% of the centerline velocity, $U_c=2 m/s$. At this
velocity, the fluid remains close to the wall (Gopalan, et al., 2004), as confirmed by observation. Even 75mm downstream of the injector the penetration depth is about 2mm. This low injection speed and proper distance from the sample volume insure that the effect of injection on the near-wall flow is negligible. The measurement volume has dimensions of 1.5mm × 2.5mm × 1.5mm in streamwise, wall-normal, and spanwise directions, denoted as x, y, z respectively, which is equivalent to 88×145×88 wall units. The corresponding velocity components are u, v, and w. To prevent adverse effects of surface discontinuities, the inner surface is intact, i.e. the bore containing the microscope objective does not penetrate.

To perform 3D velocity measurement of a dense cloud of particle in a flow with an extended depth, we have recently introduced the in-line digital holographic microscope (DHM). This method (fig. 6.1) records an enlarged in-line hologram. The microscope objective focuses the hologram plane, located outside of the sample volume (dash-dot line in fig. 6.1), onto a CCD array, i.e. the image is an interference pattern between light scattered from objects located in the sample volume and the illuminating collimated reference beam. It does not contain in-focus images of these objects. Theoretical analysis shows that the image plane contains a magnified hologram with a phase correction that becomes unity when magnification is sufficiently large. The magnified instantaneous 3D particle field is reconstructed numerically using Fresnel diffraction formula, i.e. by convoluting the digital hologram with the impulse response function (Malkiel et al., 2003, Milgram&Li 2002). In Sheng et al. (2006), we provide detailed data on resolutions, image quality and depth of focus. As shown, the digital holographic microscope substantially reduces the “depth-of-focus” problem, namely that
reconstruction of a spherical particle creates an elongated ellipsoidal image, whose length in the depth direction is typically two orders of magnitude larger than the lateral dimensions. With a 10X objective, DHM reduces the depth-of-focus of a particle down to 6~10 times its diameter.

A 2K×2K CCD camera (Kodak ES 4.0) is used to record a pair of in-line particle holograms with in-pair time interval of 80µs. Numerical reconstruction of 2D wall-parallel planes is performed every 2µm, totaling 1250 planes for each hologram. Three-dimensional segmentation then identifies the particle locations and intensity distribution (Sheng et al. 2003, 2006). Particle tracking is used to measure the velocity, i.e. each particle pair provides one vector. To improve the efficiency and accuracy of this tracking process, the 2D slices of images are first collapsed into sets of 2D images, each combining slices within a 30µm thick layer. Conventional PIV is applied to each image to obtain velocity distributions, which help in defining search ranges for the particle-tracking algorithm. Other criteria are also used for improving track matching accuracy, such as size and scattering intensity, nearby velocities, and 3D image correlation of volumes centered at the assumed pair. At the present concentrations, we have encountered very few cases with ambiguity, and discarded them. The velocity is then determined from the displacement of the particle centroid, as obtained from the 3D intensity distribution. The streamwise and spanwise resolutions are 0.7182 µm/pixel, corresponding to ~1mm/s. The resolution in wall normal direction is lower due to the depth-of-focus problem. Although a great improvement over lens-less systems, it is still not adequate for accurately determining the wall-normal velocity. However, keeping in mind that: (a) we have data on the well-resolved intensity distribution with the elongated
spheroids, and (b) the shapes of traces of the same particle are very similar since they are subjected to the same recording conditions (e.g. intensity of laser and optical distortions), one may still determine the wall normal velocity based on the displacement of the centroid of elongated trace (as done in film based holography by Pu&Meng (2005). To determine how accurate this measurement is, we calculate the probability distributions of the normalized velocity divergence, \( \sigma = \left( \frac{\partial^2 u + \partial^2 v + \partial^2 w}{\partial^2 u + \partial^2 v + \partial^2 w} \right) \), over 100 realizations. The average value of \( \sigma \) varies between 0, if the velocity distribution is divergence free, and 1 for random data (Zhang et al. 1997). Figure 6.2a compares the present PDF and cumulative distribution of \( \sigma \) to those of our previous 3D velocity distributions, obtained using two perpendicular views and complex off-axis optical setup (Sheng et al., 2003; Tao et al., 2002; Zhang et al., 1997). Clearly, the present results are substantially more divergence free than our previous data. To quantify the error in wall-normal direction, we added random errors with standard deviation denoted as \( \varepsilon \), to our measured wall-normal component and reevaluate the normalized divergence. As is evident, the divergence free condition deteriorates very quickly from \( \varepsilon=1.25 \text{ mm/s} \) to that at 2.5mm/s. Figure 6.2a suggests that our measurements have uncertainties of about 1mm/s in all directions.

The number of resolved vectors in individual measurement for the present data varies from 2,000 to 10,000 with a mean nearest neighbor distance of 50 – 100 \( \mu \text{m} \), i.e. 3~8 wall units (provided later in 6.3.1). However, the particle are most concentrated near the wall (<1mm), where they provide an averaged mean nearest neighbor distance in wall normal direction of less than 15\( \mu \text{m} \) (< 1 wall unit) and about 3 wall units in streamwise and spanwise directions.
Instantaneous wall shear stresses, \( \tau_{xy} = \mu \frac{\partial u}{\partial y} \bigg|_{y=0} \) and \( \tau_{zy} = \mu \frac{\partial w}{\partial y} \bigg|_{y=0} \), are estimated from the slope of local velocity profiles in the viscous sublayer, \( y < 75\mu m \) or \( y^+ = \sim 4.5 \). To determine this slope, we divide the viscous sublayer into sub-volumes of \( 150 \times 75 \times 150 \mu m \), and apply linear regression over all velocity vectors measured within each volume (e.g., fig 6.2b). Thus, the spatial resolution of skin friction presented here is \( 150 \mu m \), \( \sim 9 \) wall units. The standard deviation of scatter around regression lines is \( \sim 8\% \). This uncertainty is well below measured spatial variations in stress, \( 60\% - 300\% \), hence has minor consequence in interpretation of results.

**6.3 Results**

**6.3.1 Sample Data and Mean Scales**

The measured velocity map is initially unstructured, and is then interpolated onto a grid with spacing of \( 20 \times 15 \times 20 \mu m \) using first order Taylor expansion based on the surrounding 30 points and least square solutions. The present analysis is based on the first 100 3D velocity vector maps. Fig. 6.3 shows a sample instantaneous velocity distribution, in this case at low seeding concentration, containing 1,228 vectors. A “large-scale” streamwise vortex is, nonetheless, observable in the projected y-z view. Superimposing \( u \) of all realizations, we can estimate the mean wall unit by linear regression to the velocity profile within the viscous sublayer, as illustrated by the inserts in fig. 6.4. The mean wall shear stress, \( \langle \tau_{xy} \rangle \bigg|_{y=0} \) (the \( y=0 \) will be omitted for brevity), is 3.19 N/m, i.e. the friction velocity, \( u_r = \sqrt{\tau_{xy} / \rho} \) is 56.5mm/s, the wall unit, \( \delta_{w} \), is 17\( \mu m \), the Reynolds number, \( Re_r \), based on friction velocity and half channel height (\( \delta^+ = 1500 \)) is 1400, and the Reynolds number, \( Re_\delta \), based on outer variables, is 50,000.
As shown in fig 6.4, the mean streamwise velocity profile scaled with the inner variables produces the characteristic law of wall of mean streamwise velocity in the viscous sublayer, buffer layer, and lower logarithmic layer. Since the present results are based on the 100 realizations, the profile in the log layer is not fully converged yet. Distributions of Reynolds stresses and turbulent kinetic energy (not shown) have the expected near-wall distributions, as obtained in DNS of channel flow (Kim et al., 1987), but our profiles are still jittery. For example, in our data $u'^2$ peaks at $y^+$=12.2 where $u'^2 / k = 1.61$. In Kim et al. (1987), the corresponding values are 11.8 and 1.7.

6.3.2 Characteristic Near-Wall Flow Structures and Associated Wall Stress
Simultaneous observations on near wall flow structures and wall stress provide insight on relationships between them. Spatial topologies of near-wall coherent structures can be visualized using $\lambda_2$ (Jeong&Hussain, 1995) or swirling strength (Adrian et al., 2000). We have found that iso-surfaces of both quantities capture similar “large-scale” flow structures, so only the $\lambda_2$ distributions are presented. Classifying structures is a subjective process. Nonetheless, we have identified the following:

- **Counter Rotating Pair of Streamwise Vortices with Similar Strength**, shown in fig. 6.5a-b, appear in 30 of the 100 realizations. These pairs of vortices are typically inclined at various angles, but frequently close to 45°, to the downstream direction. Pure streamwise alignment is rare. These vortices often seem to be originated from the wall, as in fig. 6.5a, and then quickly lift off. Once extending away from the wall into the lower logarithmic layer, they tend to swerve partially towards the spanwise direction. The centers of these pairs are located in the $3<y^+<40$ range, but mostly around $y^+= 20$, and the spacing between them is $z^+= 50 – 70$. Their normalized streamwise vorticity,
\( \omega_s \delta_u / u_r \), often exceed 1,000, i.e. 2/3 of the spanwise shear. Depending on the direction of rotation, the flow induced by a pair is either a downward, stagnation-like flow (“sweeping”) or “de-straining” motion away from the wall (“ejection”). During ejection, there is a stress minimum between the vortex pair (fig. 6.5a). Conversely, during a sweep a stress maximum develops on the wall. The spatial variations of wall stress magnitudes associated with these structures are often large, ranging between 0.4 to 3\(<\tau_{xy}\>.

**Multiple Quasi-Streamwise Vortices:** We have observed 30 cases that have multiple quasi-streamwise vortices coexisting within the buffer layer. These structures are also primarily aligned in the streamwise direction, with tilting angle of less than 20°, but have much larger range of orientation in the spanwise direction compared to the counter-rotating pairs. They have a wide variety of spatial arrangements with spacing between vortices ranging from 5 to 90 \( \delta_y \). Their core sizes as determined based on the magnitude of \( \lambda_2 \), vary from 5-10 \( \delta_y \) in the \( y^+ = 5\sim12 \) range, but extend to 20-40\( \delta_y \) in the upper parts of the buffer layer. There seems to be no preferred direction of rotation, and no correspondence in magnitude of neighboring vortices. In fig. 6.5c-d, four streamwise vortices coexist in the sample volume, with one of them (bottom-right in fig. 6.5d) situated very close to the wall, initially at \( y^+ = 5 \). Complex vortex-vortex interactions, i.e. vortices interlacing around each other, and vortex-wall interactions, i.e. lateral movement near the wall with significant distance from other structures, are clearly evident, even in the viscous and buffer layers. The resulting induced flow field is not as distinct as in the previous cases. The \( \omega_s \delta_u / u_r \) ranges from \(-600\) to \(+600\), i.e. \( \pm45\% \) of mean spanwise shear. Spatial distributions of wall stresses beneath such complex flows do not display clear causal relationships, as it does for counter rotating vortex pairs. Their “footprints”
on the wall stresses, nonetheless, bear some signatures when they are close to the wall (fig. 6.5c). Yet, the streamwise coherence of $\tau_{xy}$ is far less prominent, and the stress magnitudes vary only between 0.8~1.2$<\tau_{xy}>$. However, these vortices contribute to ~35% of mean wall shear stress.

- In 38 of the 100 cases, we do not see “small” buffer layer structures. Among them there is evidences of induced motion by structures that are larger than the sample volume, e.g. a “large” scale sweeping flow or circular motion associated with large vortices. The associated stress magnitudes vary substantially.

- **Spanwise Structures**: Spanwise structures residing entirely in the buffer layer are rare, two in the present set, but do exist. A clear snapshot of a newly generated hairpin is present in fig. 6.5e-f. Due to the limited data, we cannot attach statistical significance for this phenomenon. The “hairpin” has a shape of an “Ω” whose legs are deeply embedded in the buffer layer, at $y^+=12$, whereas the head extends to $y^+\approx45$. The legs are initially aligned with the spanwise direction, and then turn upward at an angle of 45° to the streamwise direction. The spacing between legs is ~45 $\delta_y$.

It is worth noticing that there is a spot with elevated wall shear stress in the vicinity of the kinked leg. This spot does not appear to be associated with entrainment, but may be related to the torque necessary to bend the vortex tube towards the streamwise direction from its initial spanwise orientation. At the wall, $\rho \partial \omega_x / \partial x = \partial \tau_{zy} / \partial x$, $\rho \partial \omega_y / \partial y = \partial \tau_{xy} / \partial z - \partial \tau_{zy} / \partial x$, i.e. spatial gradients of the wall shear stress must be involved with bending of the legs.
6.3.3 Statistics of Wall Shear Stresses

Figure 6.6(a-d) shows two randomly selected but representative instantaneous wall stress distributions, the contour plot displaying $\tau_{xy}(x,z)$, and the vector plots showing both components. Both have streak-like structures aligned in the streamwise direction. The vector plots reveal a divergent pattern on both sides of a stress maximum, and a convergent pattern on both sides of a minimum. As will be shown, the divergent pattern is the result of local stagnation flow by a strong sweeping event and the convergent pattern is caused by a local ejection. However, a significant fraction of the stress maps, ~50%, shows no streak-like distribution.

Compiled over 10,000 local wall stress measurements from the 100 realizations, joint probability density (PDF) distribution of streamwise and spanwise wall stresses (fig. 6.7) shows a symmetric distribution of the spanwise component, and a long tail of high streamwise component. Skewness and kurtosis for $\tau_{xy}$ are 0.9 and 5.2, respectively, in very good agreement with wind tunnel data at comparable Reynolds numbers (Kimura et al., 1999; Miyagi et al., 2000; Ruedi et al., 2004). The PDFs of both components show strong non-Gaussian distribution at large values, as revealed in log-linear plot (not shown), indicating strong intermittency. The width of spanwise shear stress distribution increases with increasing streamwise shear, i.e. a large $\tau_{xy}$ is often accompanied by a large $\tau_{zy}$. This relationship suggests that both are caused by the same phenomenon, e.g. near wall vortical structures.

6.3.4 Conditionally Averaged Wall Stresses and Flow Structures

To identify characteristic length scales and spatial structure in wall stress, we use conditional averaging (Kim et al., 1987) of stress distribution, centered on local minima.
and maxima of streamwise stress. The conditions are \( \tau_{xy}(x,z) > \alpha \langle \tau_{xy} \rangle \) for maxima and \( \tau_{xy}(x,z) < \beta \langle \tau_{xy} \rangle \) for minima, where \( \alpha = 1.2, 1.4, 1.6, 18 \) and \( \beta = 0.6, \) and 0.8 respectively.

The conditional distribution of \( \tau_{xy} \) based on local maxima (fig. 8a) shows a slight reduction in characteristic length scales with decreasing stress magnitude. For maxima at \( z^+ = 0 \), the minima are located at \( z^+ = \pm 35 \), followed by additional maxima at \( z^+ = \pm 70 \). Conversely, the spanwise stress distribution (fig. 6.8b), has the highest slope at \( z^+ = 0 \), and broad peaks with opposite signs starting at \( z^+ = \pm 25 \). As shown below, these minima and maxima are associated with local sweeping and ejection events, which are generated by near wall structures. The trends are reversed in the conditionally averaged distributions based on streamwise stress minima (fig. 6.8c-d), but the length scales remain similar. A length scale of \( z^+ = 70 \), i.e. the distance between minima in fig. 6.8a, is smaller but of the same order as the well-known length-scale of 100 wall units associated with low-speed streaks (Robinson, 1991).

To identify the near wall structures responsible for mitigating and augmenting local stresses, we perform conditional averaging of velocity and vorticity, based on local streamwise stress extremes. Averaging is performed over time and streamwise direction i.e. assuming that the process is stationary and homogenous in horizontal direction. Figure 6.9 shows the conditionally averaged results in the y-z plane. Since in both cases all quantities are expected to have good reflectional symmetry with respect to \( z^+ = 0 \) and due to space limitation, only halves of the plots are shown. The left column contains the averaged flow pattern, \( \omega_x \) and \( \omega_y \) for \( \tau_{xy} < 0.6 < \tau_{xy} \rangle \), and the right column is for the results for \( \tau_{xy} > 1.8 \langle \tau_{xy} \rangle \). Only 18 and 20 instantaneous cases of the 100 satisfy the
minimum and maximum stress conditions, hence the database is small and “jitter” is evident.

For local high stress (fig. 6.9d-f), a distinct pair of counter-rotating vortices (only the right one is shown) is located at $z^+ = \pm 27$ and $y^+ = 8$, and between them, there is strong downward flow, creating a 2D stagnation flow pattern. The divergent wall stress pattern (fig. 6.6) is the direct consequence of this sweeping motion. The streamwise vorticity also shows a counter rotating vortex pair with a peak vorticity located slightly above the center of the near-wall structure. The peak of $\left. \frac{\delta \tau}{\delta u} \right|_{\tau^{<} > 1.8 < \tau^{<}}$ is 170, i.e. 4 times stronger and closer to the wall than results reported by (Kravchenko et al., 1993). The wall normal vorticity (fig. 6.10c) also contains a pair of vortices. The magnitudes of conditionally averaged $\omega_x$ and $\omega_y$ are similar, suggesting structures aligned at a $\sim 45^\circ$ angle to the freestream.

For local stress minima (fig. 6.9a-c), a pair of counter rotating vortices (only the left one is shown) are located at $z^+ = \pm 25$ and $y^+ = 18$, and between them, there is an upward 2D de-straining flow pattern (ejection). The convergent wall stress pattern (fig. 6.6c) is generated by this ejection. Both vorticity components have peak values of 170, again suggesting structures aligned at a $\sim 45^\circ$ angle to the freestream. More than 50% of the data used for both conditional samplings are the same instantaneous realization shifted based on stress magnitude. However, the upward ejection motion is weaker and the conditionally averaged structures are located further away from the wall compared to the sweeping motion. These trends suggest that local entrainment by a streamwise vortex pair is dominant in the sweeping-high-stress cases, and important, but not the only
mechanism, in the ejection-low-stress case. Some other phenomena, e.g. pressure
gradients generated by outer layer structures, may also have significant effects.

Conditionally sampled spanwise vorticity for the high-stress case shows a series of
local peaks separated by $z^+=60-70$. This pattern is caused by elevated $\partial u / \partial y$ in the
region where the sweeping motion brings high momentum fluid closer to the wall, i.e. by
thinning the shear layer. The local minima are caused by reduced $\partial u / \partial y$ in the region
where the ejection motion catapults low speed near-wall fluid away from the wall. The
characteristics length scales are clearly evident and in good agreement with fig. 6.8.

6.3.5 Alignment of Vorticity and Local Strain
Several studies have shown that in turbulent flows away from boundaries, the vorticity is
preferentially aligned with the intermediate strain-rate (Ashurst et al., 1987, Tao et al.,
2002, Tsinober et al., 1992). Following Tao et al. (Tao et al., 2002), we first examine the
strain parameters in the near-wall region. We also investigate the relative alignment
between vorticity and eigenvectors of the strain rate tensor, denoting $\alpha$, $\beta$, and $\gamma$ as the
most extensive, intermediate and most compressive eigenvalues, respectively. Figure
6.10 shows a joint PDF of alignment angles of the conditionally sampled fluctuating
vorticity with the overall strain-rate (mean+fluctuating). The condition is $|\lambda_2 \delta / u_*| >$
160, i.e. intermediate to powerful vortices. This joint PDF shows the cosine of angle
between the eigenvector of $\gamma$ and $\vec{\omega}$, $\cos(\phi(\omega-\gamma))$, and the angle between the eigenvector
corresponding to $\beta$ and $\vec{\omega}$, $\theta(\omega-\beta)$. If the analysis includes all the data, the peak would
be concentrated at $\cos \phi(\omega-\gamma)=0$ and $\theta(\omega-\beta)=0$, i.e. the vorticity is aligned with the
intermediate strain (spanwise) direction. In the conditioned data, there are two peaks, one
close $\theta=0^\circ$ and the other near $\theta=45^\circ$. As the vorticity threshold level increases (not
shown), the peak at $45^\circ$ becomes more prominent and the one close to $0^\circ$ diminishes. Since the mean strain dominates the eigenvector directions and the mean extensive strain is aligned at $45^\circ$ to the streamwise direction, this trend implies that powerful vortices lies in a plane inclined at $45^\circ$ to the streamwise direction (upward), and form an angle of $45^\circ$ with the spanwise direction ($\beta$). Clearly the alignment trends of near-wall vortices differ from those away from walls.

Before concluding, a short note on the alignment of the strain-rate tensor is provided. Previous studies have shown that in unbounded turbulent flows the most prevalent structures are axial extension, i.e. the strain parameter, defined as $s^* = -\sqrt{6\alpha\beta\gamma/\left(\alpha + \beta + \gamma\right)^{3/2}}$, tends to 1 ($s^*=1$ for axial extension and $s^*=-1$ for axial compression). Measurements performed at the center of the present facility show the same trend (Tao et al., 2002). However, the strain parameter for the inner part of turbulent boundary layer (inserted in figure 6.10) shows a preferred value of $s^*=0$, which corresponds to planar shear. Thus, the near wall turbulence is distinctively different from that away from the wall.

**6.4. Conclusion**

Digital holographic microscopy is used to simultaneously measure the spatial distribution of instantaneous wall shear stresses and the 3-dimensional near wall flow in a fully developed turbulent square duct flow at $Re_\tau = 1,400$. The spatial resolution is $3\times1\times3$ wall units and the volume is $90 \times 120 \times 90 \delta_v$.

A wide variety of buffer layer flow structures are observed and some are deeply embedded in the viscous sublayer. Their sizes in the buffer layer are in the $5\sim10 \delta_v$ range, but they grow while migrating away from the wall. In 62 of the 100
measurements, we clearly observe distinct structures, amongst them 30 are counter-rotating pairs, 30 contain multiple quasi-streamwise vortices, and two are “baby” hairpins. Counter rotating vortex pairs seem to have the strongest impact on wall stresses. The interaction of multiple quasi-streamwise vortices with the wall is more complex, resulting in smaller spatial variation of wall stress. Thus, consistent with previous studies, the mechanism for augmenting and reducing wall stress is by near-wall structures that involve momentum exchange between outer and inner layer flows. In 38 of the 100 realizations, there are no buffer layer structures. In these cases, the wall stress distributions are characterized by large-scale variations in stress magnitude with little spatial modulation over the sample area. Other mechanisms related to kinking vortices also involve significant spatial gradients of wall stresses.

Conditionally averaged velocity, vorticity, and stress magnitude reveal that sweeping motion by stagnation-like flows generated by counter rotating vortex pairs located at \( y^+=\pm8 \) and \( z^+=\pm25 \) is responsible for stress maxima. Ejection motion by de-straining like flow away from the wall, also created by counter rotating pairs located at \( y^+=\pm18 \) and \( z^+=\pm25 \) is responsible for stress minima. The peak streamwise vorticity is \( \omega_x\delta/u_r=170 \), and the structures are aligned at \( 45^\circ \) to the freestream direction. Both the shear stress and spanwise vorticity distributions in extreme events have characteristic spacing of \( z^+=70 \). We also observe that the alignment between vorticity fluctuations of large magnitude and local strain rate eigenvectors differs from that measured in isotropic turbulence. Powerful buffer layer structures tend to be located in the \( \alpha-\beta \) plane and preferentially aligned at \( 45^\circ \) to either eigenvectors.
**Figures**

**Fig. 6.1** Experimental setup including the digital holographic microscope.

**Fig. 6.2** (a) Cumulative probability distribution of the present normalized divergence, \(\sigma\), in comparison with previous measurements and artificially introduced events with standard deviation of \(\varepsilon\). The insert is the probability density function. (b). Illustration of local wall shear stress measurement using linear regression of velocity in the viscous sublayer.

**Fig. 6.3** (a) A sample 3D instantaneous velocity distribution with 1228 vectors, and (b) end view (y-z projection) of the same flow. The location of a particle in the first exposure is marked by a dot, and in the second by an \(\times\).

**Fig. 6.4** Measured mean streamwise velocity profile compared to the Law of the wall. Insert: data points from 100 realizations and linear regression used for calculating \(u_\tau\).

**Fig. 6.5** Left: Instantaneous distributions of wall shear stress and iso-surfaces of \(\lambda_2\), denoting near wall structures. (a) counter rotating pair; (c) multiple quasi-streamwise vortices, and (d) a “baby” hairpin structure. Right: Interpolated velocity distributions in a y-z plane corresponding to structures on the left. (b). \(x^+=40\), (d) \(x^+=25\), and (f) \(x^+=65\).

**Fig. 6.6** Left: Sample instantaneous streamwise wall stress distributions. Right: corresponding vectors showing both components of the wall stress.

**Fig. 6.7** Top left: Joint PDF of streamwise and spanwise shear stress components. Right: PDF of spanwise component. Bottom left: PDF of streamwise components.

**Fig. 6.8** Distributions of conditionally averaged (a) \(\tau_{xy}/<\tau_{xy}>\) and (b) \(\tau_{zy}/<\tau_{xy}>\) in regions where \(\tau_{xy}>\alpha<\tau_{xy}>\), with the maximum centered at \(z^+=0\); (c) \(\tau_{xy}/<\tau_{xy}>\) and (d) \(\tau_{zy}/<\tau_{xy}>\) in regions where \(\tau_{xy}<\beta<\tau_{xy}>\) with the minimum centered at \(z^+=0\).

**Fig. 6.9** Conditionally averaged velocity and vorticity distributions around regions with high wall stress, \(\tau_{xy}>1.8<\tau_{xy}>\) on the right side, and low wall stress,
\( \tau_{xy} < 0.6 < \tau_{xy} \) on the left. The stress maxima and minima are located at \( z^+ = 0 \). For the low stress: Left halves of (a) velocity, (b) \( \omega_x \) and (c) \( \omega_y \) for low wall stress. For the high stress: Right halves of (d) velocity, (e) \( \omega_x \) and (f) \( \omega_y \) for high wall stress. (g) full \( \omega_z \).

Fig. 6.10 Alignment of vorticity fluctuations relative to eigenvectors of the local strain rate tensor. Insert: PDF of strain parameter of local strain (\( s^* \)) and strain fluctuation (\( s' \)).

Reference


Fig. 6.1 Experimental setup including the digital holographic microscope.
Fig. 6.2  (a) Cumulative probability distribution of the present normalized divergence, $\sigma$, in comparison with previous measurements and artificially introduced events with standard deviation of $\varepsilon$. The insert is the probability density function. (b). Illustration of local wall shear stress measurement using linear regression of velocity in the viscous sublayer.
Fig. 6.3  (a) A sample 3D instantaneous velocity distribution with 1228 vectors, and (b) end view (y-z projection) of the same flow. The location of a particle in the first exposure is marked by a dot, and in the second by an ×.
Fig. 6.4 Measured mean streamwise velocity profile compared to the Law of the wall. Insert: data points from 100 realizations and linear regression used for calculating $u_\tau$. 

\[ \exp u^+ = y^+ \]
Fig. 6.5 Left: Instantaneous distributions of wall shear stress and iso-surfaces of $\lambda_2$, denoting near wall structures. (a) counter rotating pair; (c) multiple quasi-streamwise vortices, and (d) a “baby” hairpin structure. Right: Interpolated velocity distributions in a $y$-$z$ plane corresponding to structures on the left. (b) $x^+=40$, (d) $x^+=25$, and (f) $x^+=65$. 
Fig. 6.6   Left: Sample instantaneous streamwise wall stress distributions. Right: corresponding vectors showing both components of the wall stress.
Fig. 6.7 Top left: Joint PDF of streamwise and spanwise shear stress components. Right: PDF of spanwise component. Bottom left: PDF of streamwise components.
Fig. 6.8 Distributions of conditionally averaged (a) $\tau_{xy} / < \tau_{xy} >$ and (b) $\tau_{zy} / < \tau_{xy} >$ in regions where $\tau_{xy} > \alpha < \tau_{xy} >$, with the maximum centered at $z^i=0$; (c) $\tau_{xy} / < \tau_{xy} >$ and (d) $\tau_{zy} / < \tau_{xy} >$ in regions where $\tau_{xy} < \beta < \tau_{xy}$ with the minimum centered at $z^i=0$. 
Fig. 6.9  Conditionally averaged velocity and vorticity distributions around regions with high wall stress, \( \tau_{xy} > 1.8 < \tau_{xy} > \) on the right side, and low wall stress, \( \tau_{xy} < 0.6 < \tau_{xy} > \) on the left. The stress maxima and minima are located at \( z^+ = 0 \).

For the low stress: Left halves of (a) velocity, (b) \( \omega_x \) and (c) \( \omega_y \) for low wall stress.

For the high stress: Right halves of (d) velocity, (e) \( \omega_x \) and (f) \( \omega_y \) for high wall stress. (g) full \( \omega_z \).
Fig. 6.10 Alignment of vorticity fluctuations relative to eigenvectors of the local strain rate tensor. Insert: PDF of strain parameter of local strain ($s^*$) and strain fluctuation ($s'*$).
7 Measurements on Unicellular Phytoplankton Swimming Behavior Using Digital Holographic Microscopic Cinematography

7.1 Background
Motions of swimming micro-organisms have long fascinated cell biologists. Dinoflagellates are among the most conspicuous marine phytoflagellates because of their abundance in accessible coastal waters, their unusually large cell size range and distinctive morphology. Moreover, these phytoplankton exhibit unique population dynamics – algae bloom. Severe harmful algae blooms have been documented to cause massive fish kills, destructions to delicately balanced coastal ecosystems, and endangerment to public health. Many biological and environmental factors for harmful algae bloom have been identified or speculated, however, many fundamental questions regarding the dynamics of algae bloom remain unanswered. Apart from the complexity of the problem, it is largely due to the lack of experimental observations on the swimming behaviors (measurable physical responses to any stimuli) of cells in their natural environment, i.e. free-swimming in large concentration free from the boundary effects.

To obtain an accurate and faithful description on swimming behaviors of cells in large population, we need to resolve motions both spatially and temporally. It is our intention to simultaneously quantify both swimming mechanisms of each individual cell and collective behavioral modifications due to the alterations of physical and/or chemical environments, i.e. cell – cell interactions, predator – prey interactions and cell – environment interactions (chemotaxis, gravitaxis, gyrotaxis, and phototaxis, etc.) These
interactions often occur at different length and time scales with large scale separation. For instance, the beating of the longitudinal flagella (200nm in width, 5-10µm in length) is typically at 50–60Hz with an amplitude of 2–5 µm, meanwhile the cell body travels at the speed up to 1mm/s and rotates along its anterior-posterior direction at the speed up to 20 rad/s (the values presented here are measured directly from our experiments, since literatures on the subject are very limited due to the 3-dimensional nature of the motion.) On the other hand, interactions between cells occur on much grander length scales (~mm) and longer time scales (from seconds to hours). Furthermore, to examine the “normal” cell behaviors, cells should be allowed to swim freely in normal concentration (~100,000cells/ml) free from the boundary effects that often plague the observations made on microscopy slides. Digital Holographic Microscopic Cinematography is the ideal choice for such a demanding task. Digital Holographic Microscopy itself will provide detailed 3D information on cells in motion with high spatial resolution as well as good length scale separation. Combining Holographic recording with high speed cinematography provides a unique way to examine cell motions in great temporal resolution. We believe that this is the first “functioning” system capable of performing high resolution 4D tracking on a large collection of swimming cells simultaneous over a substantial volume, and most importantly capable of providing robust statistics on population dynamics from thousands trajectories.

In this chapter, we present quantitative measurements and descriptions on the “normal” swimming behaviors of *Karlodinium veneficum* 2064 (a mixotroph) and *Pfiesteria piscicida* (a heterotroph) at first. The behaviors are fully quantified in the form of the distributions of helix parameters (radius, pitch, speed, and angular velocity)
obtained by the instantaneous helix decomposition of a 3D trajectory. And later the predator-prey interaction between *Pfiesteria piscicida* (predator) and *Rhodomonas* (prey) is studied quantitatively by comparing the distributions of helix parameters with and without the presence of preys. Clear evidences of collective modifications on swimming behavior and cell spatial distribution in the presence of food will also be provided.

### 7.2 Materials and Methods

#### 7.2.1 Experimental Setups

Optical setup is shown in fig. 7.1. A cuvette (Spectrum Cell, inc.) with an inner dimension of 3mm × 3mm and 40mm in vertical direction is used as the measurement container. Large horizontal dimensions ensure that cells suspended in the cuvette are less likely influenced by the boundaries. Vertical Length of the cuvette is intentionally chosen to be long for considerations on the possible vertical migration of cells due to phototaxis or gravitaxis. All outside surfaces of the cuvette are coated with anti-reflection coating to prevent the interference from multiple reflections. A Q-Switched diode-pumped ND:YLF (λ=660 nm, multiple longitudinal modes, Crystal Lasers, inc.) is used to illuminate the cuvette. The illumination is reshaped, filtered and collimated into a Gaussian beam of 3mm in diameter through a 10 μm pinhole assembly. A high-speed digital holographic microscope (Sheng et. al. 2006) is used to image the swimming cells in the cuvette. The high-speed digital holographic microscope consists of a commercial 20X objective lens (Edmund Scientific) and a high-speed 1K × 1K CMOS camera (maximum speed at 2000 frame per second up to 3 seconds recording, Photron). The objective lens is located at 160mm away from the sensor forming a typical configuration as a conventional light microscope. The sensor has pixel size of 19 μm × 19 μm, thus a
physical dimension of 19.5mm × 19.5mm. The current setup has a spatial resolution of 0.975µm on planes parallel to the hologram plane (Sheng et. al. 2006.) The object plane is located at 100 µm away from the inner wall of the cuvette. The entire measurement volume is 0.8mm×0.8mm×3mm. A right hand coordinate system is used as a fixed reference frame with the positive y direction pointing downwards and the positive z direction into the paper. In current experiments, holograms are recorded at the rate of 60 fps instead of 2,000 fps and total duration of 13s for each recording.

7.2.2 Materials
Three dinoflagellates: *Oxyrrhis marina*, *Karlodinium veneficum* 2064, and *Pfiesteria piscicida*, are examined in this study. Figure 7.2 shows SEM and light microscope images of these micro-organisms, and their physical dimensions are listed in Table 7.1:

<table>
<thead>
<tr>
<th>Species</th>
<th>Length (µm)</th>
<th>Width (µm)</th>
<th>Relationship</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Oxyrrhis marina</em></td>
<td>12-35</td>
<td>8-25</td>
<td>Predator</td>
</tr>
<tr>
<td><em>Karlodinium veneficum</em></td>
<td>12-15</td>
<td>8-10</td>
<td>Prey of <em>Oxyrrhis</em></td>
</tr>
<tr>
<td><em>Pfiesteria piscicida</em></td>
<td>8-10</td>
<td>6-8</td>
<td>Predator</td>
</tr>
</tbody>
</table>

Table 7.1 Physical parameters of marine dinoflagellates.

*Oxyrrhis marina* and *Karlodinium veneficum* are cultured and prepared by Jason Adolf and Allen Place from UMIBI Center of Marine Biotechnology. And *Pfiesteria piscicida* and its prey, *Rhodomonas*, are cultured and prepared by Robert Belas of UMIBI.

The experiments were conducted at 10:30am, beginning of presumed diurnal vertical migration cycle. A single cuvette was used for recording the motions of all species.
Premixed cultures were introduced to the cuvette by a transfer syringe. Both cuvette and syringe were washed with cleaning solution (50:50 mixture of 5% HCl and 100% Methanol) in sonic bath for 5 minutes and rinsed with media before each transfer to prevent cross contamination. The cultures were allowed to equilibrate in the cuvette for 5 minutes before each recording, in the attempt to maintain quiescent flow condition. Each experiment was conducted within 15 minutes after the transfer in the hope of preventing the culture from heating up by the illuminating laser light. The recording was conducted under the normal ambient lighting; no specific arrangement was made either to suppress or to induce the phototaxis. Table 7.2 lists the experimental conditions.

<table>
<thead>
<tr>
<th>Cultures</th>
<th>Recording Conditions</th>
<th>Temperature</th>
<th>Sampling Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Oxyrrhis marina</em> (alone)</td>
<td>15,000 cells/ml</td>
<td>t = 11:35am</td>
<td>23.5 °C</td>
</tr>
<tr>
<td><em>Karlodinium veneficum</em> (alone)</td>
<td>50,000 cells/ml</td>
<td>t = 11:50am</td>
<td>60 fps</td>
</tr>
<tr>
<td><em>Pfiesteria piscicida</em> (alone)</td>
<td>100,000 cells/ml</td>
<td>t = 12:07pm</td>
<td></td>
</tr>
<tr>
<td><em>Pfiesteria piscicida</em> (predator) + <em>Rhodomonas</em> (prey) 50% mixtures (100,000 cells/ml)</td>
<td>t = 12:35pm</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 7.2 recording conditions.

**7.2.3 Recording and Analysis**

**Recording:** The holographic movies were recorded at the rate of 60 fps (Frame per second) and camera shutter speed at 1/100,000 sec (laser pulse width was ~10ns). CMOS camera was synchronized with laser, and each exposure was triggered by Q-SW firing signal. The digital holograms were temporally stored on the camera memory (8 GBytes) and later transferred to computer storage via a firewire (IEEE 1394/400) connection.
Each recording lasted ~13sec, resulting in a sequence of 780 holograms. Two recordings were taken for each experimental condition. Over six Gigabytes of data were recorded which contained several Terabytes of 4D information.

**Hologram Preprocessing:** To remove the time-invariant defects from the recorded moving cells, we have subtracted the time averaged hologram of the time sequence from each hologram (a technique often employed to reduce the background noise, described in Sheng et. al. 2006.) A rescaling procedure was also applied to the original holograms before subtraction in the attempt to equalizing the time-varying laser intensity such that the background intensity of each hologram in each sequence remains constant. Thereinafter, each hologram was over sampled to 2K×2K image from the original 1K×1K one in the hope of improving reconstruction resolution.

The practice seems to be counter-intuitive, since over-sampling seldom provides extra information, but it appears to be very effective according to my experiences. The notion, which can be proved rigorously using communication theory, will not be provided here. On the contrary, I will attempt to provide a simple justification. First, the holographic recording is a reciprocal space recording technique (the smaller an object is, the larger its interference pattern is). Less recording resolution can in fact register interference patterns generated by much finer objects (higher resolution). For instance, a 1μm object generates much larger interference pattern than the real object does. Thus, the information one needs for reconstructing high resolution object is already recorded in the hologram. Due to the Fourier transform implementation of our reconstruction procedure (Sheng et. al, 2006, Malkiel et. al, 2003), the reconstructed space (images) unfortunately shares the same sampling resolution as its reciprocal space (recorded hologram).
Consequently, information on the object with higher spatial resolution, which is however properly recorded, is lost forever during the reconstruction. For example, a 1µm object physically requires 0.5µm sampling resolution to distinguish itself from the background noise. A simple way to rectify such resolution imbalance is artificially increasing the reconstruction resolution by over-sampling the hologram. The technique does not improve the recording resolution, but surely improve the reconstruction resolution. One, however, needs to tack precaution while applying such a technique indiscriminately. If the interference of the object is not recorded, artificially increasing sampling rate simply “does not help”. But it proves to be useful in the current experiments. Hereinafter, we will use the over-sampled spatial resolution as our measurement resolution, i.e. 0.48 µm in two directions parallel to the hologram plane.

**Hologram Reconstruction:** Each hologram is reconstructed at an interval of 10µm in z direction (perpendicular to the hologram) over a range of 3mm. Three hundred 2K × 2K gray scale images are generated for each hologram, totaling ~1 Tera-bytes of images for each time sequence. The reconstruction calculation is distributed to a heterogeneous loose-coupled cluster made of seven Intel Pentium® 4 computers via Message Passing Interface (MPI) protocols under a collective workers’ paradigm. Entire reconstruction for each time sequence takes seven days at the current computer speed.

**Post-processing:** Once a stack of images are reconstructed, information on cell locations is extracted using a hybrid two-step auto-focusing routine. 3D Cell images are first processed by a 3D segmentation algorithm (Sheng *et. al.* 2006.) to determine the 3-dimensional cell centroids. It is found that due to the unique scattering of cells, scattering intensity forms two peaks which often saddle the in-focus point. The aforementioned 3D
segmentation, primarily rely on the intensity, hence produces an in-focus centroid biased towards either of intensity peaks, about ±20 µm away. To improve the measurement accuracy in the z direction, second procedure, relying on the sharpness of cell image (an often used criteria for determining in-focus images, Malkiel et al., 2006), is applied to the images within ±100µm around the cell centroid determined by the first procedure. The measurement of sharpness, i.e. surface integral of the Laplacian derivatives of the entire image, along the z direction is thus fitted with a Gaussian curve to achieve sub-pixel resolution. And centroid measurements in x and y directions are further computed by the intensity weighted averaging a 2D segment of the in-focus cell image. Further information on each cell, such as cross section dimension, total reconstructed volume, and in-focus snapshots, is extracted as well for later use in Lagrangian cell tracking.

**3D Lagrangian Tracking of Cell Motion:** A multi-layer decision tree tracking algorithm combined with a Supported Vector Machine classifier (Sheng et. al. 2006b) is used for tracking cell motion in 3D and in large concentration. Detailed algorithms and necessary background for SVM classifier and decision tree are far beyond the scope of this dissertation, thus only general concept is provided. A multiple layer decision tree, currently 16-layer, is sliding along the time axis. Only the top layer connects to the developed trajectories or a new trajectory. Each top node forms a depth-first decision tree with a maximum of 6 child-nodes, and each child-node forms another nested depth-first tree under it with a maximum of 6 grandchild-nodes. It is a recursive definition of a decision making forest. Each layer of a tree represents a specific time along the time line, and each node in the layer represents a possible candidate for a specific trajectory (identified by the top node) at this time. Thus a branch (a route, depth first) connecting
the top node to a bottom node represents a possible trajectory segment preceded by a known trajectory. An evaluation value is assigned to each bottom node (16 layer, i.e. 16 time steps below the top node.) by a SVM classifier, which utilizes the multiple variables such as the smoothness of the segment, similarity of cell size and shape within the segment, instantaneous velocity and acceleration, to determine the likelihood of such segment. Once all possible branches, i.e. all possible candidate segments, are evaluated, the best “branch” with maximum likelihood will be selected. And the second-top layer node associated with the best “branch” is chosen as top layer node for the next time step decision making. All siblings next to the chosen node are “trimmed”. The development process iterates itself until the positions at all time steps are determined. The guiding principle is try-and-error based on the time history that is mimicking human’s decision process under the same circumstances. It is believed that such process improved our tracking accuracy substantially with no defects of similar commercial tracking software packages.

\subsection*{7.2.3 Quantifying Swimming Behavior using Instantaneous Helix Fit and Phase Space Decomposition}

Prior observations on micro-organism locomotion (Crenshaw \textit{et al.} 2000) showed that micro-organism tends to move helicoidally, i.e. a trajectory as a 3-D curve in general. Our latest measurements support earlier observations (Crenshaw \textit{et al.}, 2000). However, pure linear trajectories do exist in our measurement due to various biological reasons. In this work, we adopt the methods from differential geometry to directly quantifying the measured trajectories, and later modeling each trajectory as a helix with several time-dependant variables. Figure 7.3 shows a 3-D measured trajectory of \textit{Karlodinium} and its associated coordinates used in the decomposition analysis. The trajectory of a moving
organism is obtained in a fixed laboratory coordinate system \((\tilde{x}, \tilde{y}, \tilde{z})\). A local orthogonal coordinate system \((\tilde{T}, \tilde{N}, \tilde{B})\) also known as Frenet trihedron; where \(\tilde{T}\) is the tangential vector, \(\tilde{N}\) is the normal vector, and \(\tilde{B}\) is the binormal vector; is attached to the center of cell body. It moves along the trajectory whereas rotates about an arbitrary axis, \(\tilde{K}(t)\), with the angular velocity, \(\omega\). It can be shown that the rotation axis, \(\tilde{K}(t)\), is in fact the center-line direction of the instantaneous helix. And the rotation of Frenet trihedron can be completely defined by trajectory’s curvature, \(k\), and torsion, \(\tau\).

Coordinate system, \((\tilde{T}, \tilde{B}, \tilde{N})\), can be defined by the position vector and its derivatives as the following,

\[
\tilde{T} = \frac{\hat{r}}{|\hat{r}|}, \quad \tilde{N} = \frac{\tilde{r}}{|\tilde{r}|}, \quad \tilde{B} = \tilde{T} \times \tilde{N} = \frac{|\tilde{r}| \left(\tilde{\dot{r}} - \tilde{\ddot{r}} + |\tilde{\dot{r}}|^2 \tilde{\ddot{r}} / |\tilde{\dot{r}}|\right)}{|\tilde{\dot{r}}, \tilde{\ddot{r}}, \tilde{\dddot{r}}|}, \quad (7.1)
\]

where \(|\cdot|\) denotes the norm of a vector, \([\cdot]\) represents the determinant of a second order tensor, ‘\(\cdot\)' time derivative of order one, and \(\hat{r}(t)\) is the position vector of trihedron’s origin in the fixed reference frame. The motion of TNB is completely defined by its translation and rotation. The translation is simply given by the translational velocity, \(V\),

\[
V = |\hat{r}|, \quad (7.2)
\]

which lies along the axis of \(\tilde{T}\) at any given moment. The rotation of TNB is given by the curvature \(k\) and the torsion \(\tau\) of the trajectory. \(k\) defines the rate of rotation about the axis \(\tilde{B}\) and is given by:
\[ k = \frac{\mathbf{r} \times \ddot{\mathbf{r}}}{|\mathbf{r}|}. \]  
(7.3)

\( \tau \) defines the rate of rotation about the axis \( \vec{T} \) and is given by (shown in fig. 7.3):

\[ \tau = \frac{(\dot{\mathbf{r}} \times \ddot{\mathbf{r}}) \cdot \mathbf{r}}{|\dot{\mathbf{r}} \times \ddot{\mathbf{r}}|^2}. \]  
(7.4)

Angular velocities associated with \( k \) and \( \tau \) are thus defined as \( \ddot{\omega}_k = V\mathbf{k}\vec{B} \) and \( \ddot{\omega}_\tau = V\tau\vec{\mathbf{r}} \).

The total angular velocity is hence the linear combination of both, i.e. \( \mathbf{\omega} = V\sqrt{k^2 + \tau^2} \) for the magnitude and \( \vec{K} = \frac{(k\dot{\mathbf{r}} + \tau\ddot{\mathbf{r}})}{\sqrt{k^2 + \tau^2}} \) for the rotation axis in the fixed reference frame.

With time history of translational velocity \( (V) \), curvature \( (k) \), and torsion \( (\tau) \), one can reconstruct the 3D trajectory by simple path integration (eqn. 7.5) by advancing the homogenous transformation (eqn. 7.6) along the time line given the initial position and direction of \( \mathbf{TNB} \) in the reference frame of \( \mathbf{XYZ} \):

\[ \vec{r}(t) = \int_0^t V(t)\vec{r}(t)dt. \]  
(7.5)

The transformation of \( \mathbf{TNB} \) at any given time, \( t + dt \), is given in a recursive form:

\[ [\vec{\mathbf{T}}, \vec{\mathbf{N}}, \vec{\mathbf{B}}]_{t+dt} = [\vec{\mathbf{T}}, \vec{\mathbf{N}}, \vec{\mathbf{B}}] \times \begin{bmatrix} K_1^2 + (1-K_1^2)\cos \theta & K_1K_2(1-\cos \theta) + K_3\sin \theta & K_1K_3(1-\cos \theta) - K_2\sin \theta \\ K_1K_2(1-\cos \theta) - K_3\sin \theta & K_2^2 + (1-K_2^2)\cos \theta & K_2K_3(1-\cos \theta) + K_1\sin \theta \\ K_1K_3(1-\cos \theta) + K_2\sin \theta & K_2K_3(1-\cos \theta) - K_1\sin \theta & K_3^2 + (1-K_3^2)\cos \theta \end{bmatrix}_{t,dt} \]  
(7.6)

where \( \theta_{t,dt} = \omega(t)dt \) and \( K_{1,2,3} \) are three components of the instantaneous rotation axis at any given time \( t \), \( \vec{K}(t) = k(t)\mathbf{B}(t) + \tau(t)\mathbf{r}(t) \). It is clearly advantageous to transform a 3D trajectory in a fixed Cartesian frame to a moving curvilinear coordinate system since the kinematics information is incorporated into the coordinate system. The kinematics
parameters, \( V \), \( k \), and \( \tau \), in fact forms a 3-dimensional phase space (it is a Hilbert space, proof not provided here.) to faithfully describe kinematical features of a trajectory. Nice as it is, there are a few singularities in the phase space. Tables 7.3 shows several extreme cases and the ways how they are treated in our analysis.

1. **When trajectory is a single point, i.e. the organism is immobile.** The tangential velocity \( V \) is zero, and both curvatures (\( k \) and \( \tau \)) are undefined. To ensure continuous transform from trajectory to \( Vk\tau \) phase space, we assign the origin \((0,0,0)\) to this case.

2. **When the trajectory is a straight line, i.e. \( \vec{r} \) is a null vector.** \( k \) is zero whereas \( \tau \) is undefined. We assign the point \((V,0,\text{infinite})\) to such a case.

3. **When the motion is 2-dimensional, i.e. velocity and acceleration vectors are collinear,** curvature \( k \) is zero, and torsion \( \tau \) is undefined. We assign both curvatures to be zeros.

4. **When a trajectory is a 2-dimensional circle,** curvature \( k \) is a constant, and \( \tau \) is zero.

5. **When a trajectory is a perfect helix,** both curvatures are non-zero constants.

---

We can further represent the trajectory in phase space \( Vk\tau \), in terms of helix parameters for easy interpretation since most micro-organism moves helicoidally. We call this procedure – instantaneous helix fit. Equations 7.7 shows the transformation from \( Vk\tau \) space to the helix parameters (also illustrated in fig. 7.3): radius \((R)\), pitch \((P)\), translational velocity \((V)\) and angular velocity of a helix \((\omega)\).
\[
R = \frac{\vec{T} \times \vec{K}}{\sqrt{k^2 + \tau^2}}, P = 2\pi \frac{\vec{T} \cdot \vec{K}}{\sqrt{k^2 + \tau^2}}, V_p = V\left(\vec{T} \cdot \vec{K}\right) \tag{7.7}
\]

The accuracy of helix representation hinges heavily upon the accuracy in measuring the instantaneous helix axis, \(\vec{K}(t)\). In theory \(\vec{K}(t)\) can be obtained simply by eqn. (7.1)-(7.4) so long as the position vector \(\vec{r}\) and its high order derivatives are sufficiently resolved and smooth. Although the trajectories are usually resolved adequately and relatively smooth, high order derivatives are not, especially in the case of torsion and binormal unit vector calculation (third order derivative). The uncertainty in high order derivations then hinders the accurate estimation of helix rotation axis, \(\vec{K}\), subsequent affects all helix parameters estimated.

In this work, we use only the normal unit vectors to estimate the helix axis to improve the accuracy and smoothness. It is easy to prove that at any given time the helix axis should be perpendicular to all normal unit vectors within an infinitesimal time period (Crenshaw 1989.) Therefore, helix axis is given by, \(\vec{K}(t) = \vec{N}(t) \times \frac{d\vec{N}(t)}{dt}\), or is estimated by \(\vec{K}(t) = \vec{N}(t) \times \vec{N}(t + dt)\). It needs to point out that when a helix is left-handed, resultant helix radius \(R\) is negative; and the sign reverses if a helix is right-handed. Similarly in \(Vk\) space, a left-handed helix has negative torsion value and a right-handed helix has position one.

### 7.2.4 Measurement Resolution and Uncertainty

Lagrangian measurement of 3D locations of a moving cell is crucial for later behavioral analysis. Current measurements are conducted with single-view holographic microscopy which provides higher lateral resolutions (two directions parallel to the sensor) and lower
resolution in the depth direction (normal to the sensor). The lateral resolutions are equivalent to the digitizing resolution, i.e. 0.485 μm. The resolution in depth direction is however directly determined by particle size and magnification (Sheng et. al. 2006), typically 1~2 times of particle diameters at 20X, i.e. 10~20μm. Figure 7.4 shows a series of reconstructed images of a single moving *Karlodinium* cell (~15 μm). Each image is reconstructed at the interval of 10 μm (approximately 1 body length). The image in the center of the series (8th image) is the in-focus image determined by the auto-focusing algorithm. The in focus plane is clearly located by the algorithm. A sub-pixel algorithm is used to find the presumable center of the cell. As shown in 7.4b, edge sharpness values measured along the depth direction has a distinguishable peak around the in focus location and sharply drops to zero. Subsequently, one can use Gaussian curve fit to obtain the center of the cell with sub-pixel resolution. Simple as it is, quantifying the sub-pixel resolution is rather difficult and arbitrary. Here we quantify the sub-pixel resolution by measuring the standard deviations of the difference between measured trajectory and its smoothed counterpart by a 11-point top-hat smoothing kernel (1/6 second). The histogram of such sub-pixel resolution is provided in fig. 7.5a. The distribution peaks around 3 μm, and has a mean of 5.5 μm, subsequently gives a sub-pixel resolution of 0.2-0.4 pixel in the depth direction (reconstruction step in z) or 0.2-0.4 body length. It is a great improvement and sufficiently accurate to determine the 3D trajectory, but is still 5~10 times worse than two lateral directions. A typical trajectory is shown in fig. 7.5b.
7.3 Results

7.3.1 Snapshots of Individual Swimming Cell
The power of digital holographic microscopic cinematography is fully demonstrated in figures 7.6 and 7.7. A short time sequence of a free swimming *Oxyrrhis marina* cell is displayed in figure 7.5. The inner one is a superimposed image from all instances within 0.3 second time period. The relative displace is shown in the real scale. The outer ones are in-focus images artificially repositioned to show the details of the cell body and fully resolved longitudinal flagellum (dinoflagellates have two flagella: one wraps around the cell body, often called the transverse flagellum; the other one trails the cell body, usually termed as the longitudinal flagellum.) It is very surprising to us that the longitudinal flagellum of the moving cell; of which the normal width is 200nm and the length is 5~10 µm; is nicely resolved, since the spatial resolution (even artificially over sampled) is only 0.485 µm. It is well known that a linear line object leaves much more coherent signature during holographic recording and reconstruction (see the reconstructed setae on copepod’s antenna, Malkiel et al. 2003.) Yet, it is the first time that we show that digital holographic microscope can resolve finer structures than its recording resolution even with low-grade commercial optics. The reconstructed longitudinal flagellum as expected has very short depth of focus, ±3 µm. In order to properly visualize the flagellum, the reconstruction has to be conducted with much finer pitch in depth, i.e. 1 µm between two adjacent slices. Since the longitudinal flagellum of dinoflagellates is offset from its anterior-posterior axis, in focus image of the flagellum is several micrometers from the in focus plane of the cell body depending on the posture of the cell. The distance between two in-focus images is measured at ~7µm (maximum), consistent with the mean width of
an *Oxyrrhis marina* cell. The images shown are the in-focus ones of the longitudinal flagellum, hence the slightly enlarged cell body.

The sequence starts at the middle-right image (marked with a smaller time stamp) and progresses counter-clockwise to form half of a helix. The trajectory is in fact 3-dimensional, i.e. the helix axis is pointing out of the paper (not shown in figure). As observed in previous studies, *Oxyrrhis* translates through the water while the cell body rotates about its anterior-posterior axis in a clockwise direction if viewed from the posterior. One could draw the conclusion by noticing the evolution of the positions of the “finger-like” protrusion. It rotates slowly (very slowly) from the left in the beginning to the center towards the end of the sequence, and from the back to the front (not demonstrated here). The longitudinal flagellum seems to “host” less-than one wavelength (often ¾ wavelength) of the traveling wave. There is no apparent evidence to support the claim that waveform of the flagellates has diminishing amplitude towards the tip (as described in Cosson et al., 1988), on the contrary our measurement seems to suggest the otherwise (see the amplified waveform in images). Judging by the actuation mechanisms of the longitudinal flagellum, i.e. two linearly-arranged microtubule arrays sliding against each other, it is not clear why diminishing amplitude of traveling wave should be the consequence. The longitudinal flagellum is oddly aligned at an angle to the anterior-posterior axis, and is always pointing inwards to the center of the helix axis (see in fig. 7.6), which contradicts the conventional wisdoms about the flagellum alignment claiming that the longitudinal flagellum is always pointing outwards. The images of other free swimming *Oxyrrhis* cells in the same experiment confirm such a controversial observation. It appears that the cell uses its longitudinal flagellum to generate a torque.
for turning the cell body by either angling the trust or creating a drag coefficient difference by changing the symmetry of body. The first scenario is similar to a zodiac, and the second scenario is likening the longitudinal flagellum to a rudder. Both explanations seem to be plausible but cannot be verified at this stage. Nevertheless, our quantitative observation indicates that the longitudinal flagellum is responsible for the cell body rotation in the dorsal direction, thus, the curving of the trajectory in space. Further studies by mapping the external flow around the moving cell and its longitudinal flagellum waveform simultaneously with digital holographic microscope will provide more definitive understandings to the functionalities of the longitudinal flagellum.

A trajectory of a free swimming *Karlodinium* cell, superimposed with in-focus images sampled at every 20 frames (1/3 of a second), is shown in figure 7.7. The trajectory is color-coded with its translational velocity (velocity magnitude.) A legend of color coding is displayed in the 3-d rendering of the trajectory (inserts at the bottom). Only half sequence of images are shown in the open stretch of the trajectory for clarity purposes. The enlarged image (on the top) shows the details of a reconstructed cross-section. A “V” notch structure at the top of the image indicates the groove hosting the transverse flagellum. The longitudinal flagellum is not shown here for it is in focus at different depth. The trajectory is chosen to provide the anecdotal evidence that cell-cell interaction affects cell’s kinematics. It is shown that the cell speeds up to ~200 µm/s (red) from ~100 µm/s (green) when a fellow *Karlodinium* cell is in close proximity, and quickly returns to its original speed after they cross each other. The interacting cell is only shown in part at the first and in full just before it crosses the responding cell. A little latter, the primary cell slows down to ~50 µm/s (blue) from 100 um/s (green), when a
second cell appears on its “radar” screen. The primary cell seems to be “engaged” itself in a search-and-survey mission during the time of interactions (cruising at large translational velocity – mean velocity of *Karlodinium*, 50 µm/s – while it covers a large path length; and then lingering within a small volume with multiple helical cycles.) It is plausible that the first response of a cell to another cell/particle in its close proximity is to change the translational velocity.

### 7.3.2 Gallery of Motions – Individual Description on Swimming Behaviors of Different Species

**Karlodinium**: 3D trajectories of *Karlodinium veneficum* 2064 are shown in fig. 7.7a. Two close-ups from different z locations are shown in fig. 7.7(b) and (c). All trajectories are color coded by their velocity magnitudes. It is noted that the trajectories are three dimensional, and helices with various radii, pitches, translation velocities and mean orientations are observed. Helices with larger radii are often associated with larger translational velocity (the relationship will be further elaborated at latter sections.)

To describe the swimming behavior and its “aerobatic” ability of a species, a few characteristic trajectories are selected to demonstrate both commonality and diversity of swimming behavior, shown in figure 7.8. Each trajectory is presented in pairs, a 2D projection and an isometric 3D rendering. The scales displayed in the figure are 50 µm. Figure 7.8(a) and (b) show that *Karlodinium* can move in very complicated paths while confined in a very small volume, ~100µm (<10 body length). The complexity of the trajectory arises mainly from the drastic directional changes of the helix axis; meanwhile the radius and pitch remain relatively constant. It appears that the cell “lingers” to “survey” the region more thoroughly. In this example, the cell moves at a relatively slow speed, 50µm/s, which may contributes to the relatively compact investigation region.
This lingering and surveying behavior can also occur at large speed (100µm/s) shown in figs. 7.8(c)-(d) and at extreme speed (>200µm/s) shown in fig. 7.8(e)-(f). In Fig. 7.8(c) and (d), a *Karlodinium* cell migrates over a large distance at its seemingly “cruising” speed (~100µm/s) and at the end “lingers” again around a confined volume. To “survey” a larger volume, *Karlodinium* employs the same tactics, i.e. changing the helix axis rapidly and keeping radius and pitch of the helix constant, with much larger translational velocity. It is also noticeable that the radius of helix increases when the translational velocity increases (from the smallest in fig. 7.8a-b to the largest in fig. 7.8e-f.) Another noticeable time-varying behavior is altering the topological characteristics of trajectories without changing the translational speed shown in fig. 7.8g-h. A *Karlodinium* cell moves in the helix with large radius and pitch initially, and then increases the pitch by reducing the radius, and finally increases the radius by shortening the pitch. Quick succession in characteristics changing is conducted with no apparent modification to the translational velocity. High-speed maneuver (fig. 7.8i-j) to change the main migration direction with grace, or low-speed alteration of direction (fig. 7.8k-l) by forming a cusp (stopping distance is short due to the viscosity and the low Re, typically ~0.001), are part of the game as well. And at last there is always a “perfect” cell moving in a “perfect” helix (fig. 7.8m-n) at its “perfect” speed (will shown later that ~65µm/s is the mean velocity of *Karlodinium veneficum* 2064.)

**Pfiesteria piscicida:** 3D trajectories of *Pfiesteria piscicida* in isolation are shown in figure 7.9a and two close-ups in figures 7.9(b) and (c). The total number of the trajectories is almost 3 times higher than that of *Karlodinium*. The maximum velocity magnitude is ~2.5 times higher than that of Karlodinium (comparing the legends on color.
coding in fig. 7.9 and 7.8). As shown in fig. 7.9, 3D helices are clearly the dominant swimming mode of *Pfiesteria piscicida*. However, there are “rain-forest-like” trajectories (shown in fig. 7.9c) that are predominantly aligned in the vertical direction. This type of trajectories, constituting 20% of entire measured trajectories, is not linear but quadratic with very small amplitude undulation (<2 µm). The cells engaged in such motions appear to move at the low translational velocities, often less than 50 µm/s. Later we will show that these cells are the ones drifting with the background convection flow established by the introduction of the culture. These cells, judging from their slight small dimensions and semi-linear trajectories, are believed to be the remaining of feeding dinoflagellates (food) to *Pfiesteria piscicida*. Subsequently, these cells are excluded from the behavioral analysis of *Pfiesteria*, and their vertical motions are used to construct the background convection flow of which velocity components are removed from the motion of *Pfiesteria*.

The global picture of trajectories right after feeding dinoflagellates (*Rhodomonas*) are introduced to the starved *Pfiesteria* is shown in fig. 7.10a and its close-ups in figs. 7.10(b) and (c). Comparing with figure 7.9a, it is obvious that the number of the trajectories with large translational velocities (red and green colors) increases significantly, and often they cover large distances (i.e. large integral volume per unit time.) Shown in close-ups (fig. 7.10b and 7.10c), trajectories are much more closely-spaced and more “chaotic” in appearances, yet the total number of trajectories (701 tracks vs. 689) has not changed substantially. The aforementioned “rain-forest-like” trajectories constitute the half of the data this time, which confirms that these trajectories represent the motions of *Rhodomonas* since the mixing ratio is 50-50. It is believed that
Rhodomonas is incapacitated due to the initial salinity shock, thus drifts with the “current”.

Pfiesteria (a heterotroph – need to consume prey) clearly moves very differently from Karlodinium (a mixotroph – survive on photosynthesis or consuming prey). The individual Pfiesteria (shown in fig. 11) exhibits more colorful and complicated swimming patterns than Karlodinium does. It is our intention to demonstrate such varieties of swimming patterns with a series of representative trajectories. Figures 7.11(a) and (b) show a Pfiesteria cell is engaged in a “search and survey” mission, during which the cell keeps its translational velocity unaltered. In the “search phase”, the cell reduces the helix radius and increases the helix pitch; while the “survey” region is reached, it reduces the pitch and increases the radius. Figures 7.11(c) and (d) show another example of such behavior, in which the survey motion is in greater details. Unlike Karlodinium, Pfiesteria appears to use narrower ranges of translational velocities, 100~150 µm/s, for such actions. Like Karlodinium, Pfiesteria is capable of altering the pitch and the radius of a helix without changing the mean speed, shown in figs. 7.11e-f (in median speed) and 7.11m-n (in high speed). It needs to point out that 2D project can be potentially misleading as it is shown in fig. 7.11(m) and (n). A 3D trajectory primarily moving in the direction of z (fig. 7.11n) is warped in the 2D projection (fig. 7.11m). Caution is required in drawing conclusions from 2D projections alone. Unlike Karlodinium that primarily moves in a right-hand helix only temporarily switches to a left-hand helix and quickly returns to the original right-hand helix state, Pfiesteria seems to be capable of maintaining a left-handed helicoidal motion (fig. 7.11g and h) and performing a “zigzag” maneuvering at the same time. Figures 7.11(i) to (l) demonstrate
the extreme ability of a *Pfiesteria* cell to change all three helix parameters: pitch, radius and translational velocity at the same time, in the attempt of drastically altering the traversing directions. Especially in the case shown in figs. 7.11(k) and (l), the cell traveling at an extremely high velocity (~350 µm/s) in an almost linear trajectory (very small radius and very large pitch) comes to a dead stop, reverses its direction, and then migrates at a very slow pace (50µm/s) in a trajectory with a small pitch and radius. Figures 7.11(o) and (p) show a *Pfiesteria* cell moving high speed doubles back to its original location. Unlike *Karlodinium* whose helix parameters are confined in rather narrow ranges, *Pfiesteria*, on the other hand, presents a much wider spectrum of helical motions with constant parameters: A helix with large pitch, radius and speed is shown in figs. 7.11q-r, a tight helix in figs 7.11s-t, a very tight helix in fig. 7.11u-v, and a extremely compact helix in fig. 7.11w-x. Scales drawn in figures are 100µm/s.

**Rhodomonas and Background Flow:** As it is discussed in the previous paragraph, *Rhodomonas* can be rendered immobile due to the salinity shock, since *Rhodomonas* and *Pfiesteria* are cultured in the solutions with different salinities. Therefore, *Rhodomonas* tends to drift with the background flow initially. Using the criteria of motion pattern and cell size (*Rhodomonas* is slight smaller than *Pfiesteria*), we separate *Pfiesteria* population from *Rhodomonas*’ for later behavioral analysis. And the motions of *Rhodomonas* are used to deduce the background flow. Figure 7.12 shows the mean vertical velocities of each individual trajectory plotted against their mean z locations. Circle represents the trajectory from *Rhodomonas* population and cross from *Pfiesteria*. *Pfiesteria* population is homogeneously scattered around the plot (no wall), but *Rhodomonas* population is clustered around zero to form a typical shear layer with zero velocity at both walls.
Notice that the positive velocity points downwards and the wall with the smaller z value is the laser exit surface. It is conceivable that this shear layer represents a large convection cell in our cuvette with the maximum vertical velocity of 50µm/s. The velocity components of the background flow are thus removed from the motions of for *Pfiesteria* population.

### 7.3.3 Alteration on Nearest Neighbor Distance Distribution – An Evidence on Predator – Prey Interaction

Nearest Neighbor Distance (NND) measurements on three experimental conditions: *Karlodinium* alone (50,000 cells/ml), *Pfiesteria piscicida* (100,000 cells/ml), and *Pfiesteria* with prey *Rhodomonas* (mixture concentration at 100,000 cells/ml); are conducted at each time step. The probability density distributions of NND are computed and then time-averaged over 13 seconds for each condition. The mean distributions for the first two conditions are shown in fig. 7.13a, and the mean distribution on the mixture of the third is shown separately in fig. 7.13b. Solid lines show the NND of 2000 computer generated random distributions at the same cell concentrations equivalent to those in the sample volume, i.e. 92 cells for *Karlodinium*, 250 cells for *Pfiesteria* alone, and 263 cells for *Pfiesteria* with *Rhodomonas*. In the cases of *Karlodinium* and *Pfiesteria* in isolation, the two distributions collapse nicely with the random distributions, i.e. the cells are randomly distributed in space. Two-tails hypothesis tests, 
\[
\left( \langle NND_{exp} \rangle - \langle NND_{random} \rangle \right) (s/N),
\]
on the measured and corresponding random distributions, show a statistical significance less than 1, i.e. further confirm that *Karlodinium* and *Pfiesteria* alone indeed form two randomly distributed particle fields. It is conclusive that at the current cell concentration, neither *Karlodinium* (a mixotroph) or
*Pfiesteria* (a heterotroph) shows any signs of collective interactions between them. Both *Karlodinium* and *Pfiesteria* are neither attracted to nor repelled by the presence of their fellow cells when they are in isolation. The dinoflagellates, most likely, “choose” to ignore the mechano- or chemo-signals generated by the fellow cells. Each individual cell may however react to the presence of another cell due to the extreme proximity (shown in fig. 7.7) or to avoid a direct collision (also in fig. 7.7). But this individual behavior alteration does not rise up to the level of collective behavioral modification, at least not at current cell concentrations.

The story however takes a dramatic turn when the preys are introduced. As *Rhodomonas* is mixed with *Pfiesteria*, the spatial distribution of the cell ceases to be random (fig. 7.13b). Cells are clearly clustered, for instance a bulge around 10µm to 30µm shown in fig. 7.13b and a deficit at larger length scales. Two-tail hypothesis test between the measured NND distribution and its corresponding random one (solid line) is computed at -2.6, which suggests two statistically different distributions, i.e. the cells (*Pfiesteria* and *Rhodomonas*) are not randomly distributed. When we separate the population of *Pfiesteria* from *Rhodomonas* (classifying our cells), each population alone is randomly distributed (shown in fig. 7.14a). As one measure the nearest distance of *Pfiesteria* relative to *Rhodomonas*, the distribution becomes non-random again (shown in 7.14b). Two-tail hypothesis is measured this time at an elevated value of -3.2. It is rather instrumental to conclude that the predators are clustered around the preys with a clustering distance ranging from 2 to 10 body length away (20~100 µm, the bulge in the NND distribution).
7.3.4 Swimming Behavioral Modification due to Predator-Prey Interaction – How Does Pfiesteria Alter Its Behavior to Hunt Down the Prey

To quantitatively describe the collective behavior modifications, we examine several measurable quantities: instantaneous helix parameters including pitch, radius, and tangential velocity; angular velocity distribution and characteristic time scales from velocity and acceleration autocorrelations. In the hope of comparing these quantities to examine the species dependency and behavioral modification as the stimulus is introduced, we present the quantities in all three conditions: Karlodinium alone, Pfiesteria alone, Pfiesteria in the presence of Rhodomonas. The measured quantities in the presence of prey exclude the population of prey, Rhodomonas.

Translational Velocity Distribution: Figure 7.15 shows the probability distribution of translational velocity for all three conditions. Insert shows the same distributions in log-log scales to highlight the differences. First of all, the distribution of translational velocity is highly species dependent, as shown in the cases of Karlodinium and Pfiesteria alone. Pfiesteria moves much faster than Karlodinium, and has a wide range of speeds from 0 to 500µm/s comparing to a range from 0 to 200µm/s for Karlodinium. The maximum speed of Pfiesteria can in fact reach 1mm/s, whereas Karlodinium can only reach 500µm/s. The difference in motility is likely due to the fact that Pfiesteria is a heterotroph relying on external energy source to survive. When Rhodomonas (the prey) is introduced, a clear deficit in speed ranging from 70µm/s to 220µm/s, mid-range speeds for Pfiesteria, occurs and an increment in the range from 250µm/s to 450µm/s also occurs, i.e. a great number of cells speed up from the mid-range speeds to large-range speeds (they almost double their original speeds.)
Helix Parameters – Radius, Pitch and Tangential Velocity: Figures 7.16a-c paint a much clearer topological picture about trajectories for each experimental conditions based on the instantaneous helix decomposition. *Karlodinium* moves in a characteristic helix with 3–4 µm as the radius, ~12π µm as the pitch and ~50µm/s as the tangential velocity. The peak in fig. 7.16a for Karlodinium (left panel) is very compact, i.e. the variations on the characteristic helix for *Karlodinium* are very limited. On the other hand, *Pfiesteria* has many types of characteristic helices, as first described in the Gallery of Motion. Shown in fig. 7.16a (mid-panel), the contour is elongated in the radius direction suggesting a wide range of characteristic radii ranging from 6 to 13 µm. The pitch of the helices for *Pfiesteria* is concentrated in the region ranging from ~18π to 30π and centered at 24π µm. Comparing to *Karlodinium*, *Pfiesteria* moves in helices with larger radii, 2-4 times larger and a longer pitch, also 2 times longer. Unlike Karlodinium whose characteristic velocities are confined in a small region, hence the contours (fig. 7.16b-c left panels) are aligned primarily in the radius and the pitch directions; the characteristic velocities of *Pfiesteria* are clearly aligned in the velocity direction, i.e. a wide range velocity scales verse a narrow range of length scales. When *Pfiesteria* is alone, the probability density function peaks from 70µm/s to 200µm/s and centers around 140µm/s, ~2.5 times faster than *Karlodinium*.

As the preys are introduced, Joint probability density functions on all helix parameters are modified substantially (shown in right panels of fig. 7.16.) First the characteristic radius increases from 6~13µm to 12~18µm, its corresponding pitch reduces from ~24π to ~16π µm. As for the characteristic velocity, *Pfiesteria* with prey moves at the speeds ranging from 280 to 320um/s, a 50~100% increase compared to *Pfiesteria* in
isolation. It is intriguing that the peak of pdf on velocity and radius in the case of 
Pfiesteria in isolation disappears completely in the case of Pfiesteria with preys. A bi-modal distribution is formed on both sides of the original distribution (fig. 7.16c.)

**Helix Angular Velocity:** Distributions of Angular velocities about the helix axis are measured in three experimental conditions, and results are shown in fig. 7.17. The distribution on Karlodinium alone peaks sharply at ~4 radian/s, which translates to a time scale of 1.6 second. The distribution of Pfiesteria alone peaks around 7 radian/s with a slightly expanded width, equivalent to a time scale of 0.89 second. Comparing to Karlodinium, Pfiesteria spins around the helix axis twice faster than Karlodinium does. When the preys are introduced, the distribution of Pfiesteria forms a plateau centered at ~12 radian/s with a full width of 8 radian/s. The resultant time scale (center of the plateau) is 0.52 seconds. It is conclusive that Pfiesteria in the presence of food tends to spin 50% faster and take 50% less time than those swimming in isolation.

**Characteristic Time Scales:** We further the discussion on the characteristic time scales using ensemble autocorrelation functions of velocity and acceleration components. The integrals of autocorrelation functions provide integral length scales associated with motion and dynamics forcing. Figure 7.18a presents the autocorrelation coefficient functions for all three velocity components in three experimental conditions. Consistent with time scales obtained from angular velocity, Karlodinium has the largest integral time scales, and Pfiesteria in the presence of prey has the smallest. It is clear that autocorrelation coefficient functions of two horizontal components in all three conditions show no significant difference, but those of vertical components show substantial deviations, i.e. the motions of cells are axial symmetric along the vertical direction. One
may speculate that due to the gravitaxis cells would perform random walk in both horizontal directions, but not in the vertical one. It is noticeable that vertical autocorrelation coefficients of Karlodinium and Pfiesteria alone overlap surprisingly well with each other, at least in the first minimum and zero crossing points. It is a possible indication of positive gravitaxis performed by both cells in natural environment (Both of them tend to migrate towards the bottom, a confirmative observation by biologists.) As the collective speed increases in the case of Pfiesteria with Rhodomonas, the differences between the vertical component and two horizontal ones are mitigated, i.e. tends to return to isotropy. It is my speculation that in the attempt to actively hunting for preys, the effect of gravitaxis or phototaxis is thus overwhelmed by seemingly isotropic “random” walk since the preys are shown to be randomly distributed (fig. 7.14a) in space.

The effects of gravitaxis and the speculation of returning to isotropy can be further demonstrated in the autocorrelation function of accelerations. The overlap between two vertical components of Karlodinium and Pfiesteria (two solid lines with diamond symbols) are much clear this time. It is not surprising since gravitaxis is often modeled as a forcing term in the dynamical system, hence the better match in the acceleration correlations. Again the difference between acceleration autocorrelations in three components remains unchanged comparing to the velocity autocorrelations, i.e. the shapes and the distances between each other. It strongly suggests that motivation from the food nearby is a much stronger forcing than the gravity in the presence of preys. Therefore, a possible forcing with an isotropic property should be used for modeling the predator-prey interactions in cell migrations.
In conclusion, when the preys are present, *Pfiesteria* are shown to double its velocity, increases its helix radius by 50%, reduces the pitch by 50%, and increase its angular velocity by another 50%, collectively, in the hope of actively “hunting” down the prey. The evidences on collective behavioral modifications are apparent and conclusive in our measurements; however, the causes for such alterations are not clear to us.

**7.3.6 Observed Scaling and Dynamical Constraints on Swimming Dinoflagellates**

As derived in the previous section, one can obtain the dynamical significant and biological relevant quantities: anterior-posterior angular velocity, $\omega_t$ and dorsal-radial angular velocity $\omega_k$. Figure 7.19a shows the measured dorsal-radial angular velocity distributions for three experimental conditions. It is observed that *Karloridinium* alone peaks at the smallest angular velocity, and *Pfiesteria* when mixed with *Rhodomonas* peaks at the largest value, suggesting the species dependency and behavioral modification. It is intriguing that three distributions can be scaled based on their means and standard deviations (shown in the insert of fig. 7.19a), suggesting a universality of propulsion mechanism associated with dorsal-radial angular velocity. Similarly, the anterior-posterior angular velocity also shows the same scaling property in fig. 7.19b. One would notice that the distributions of *Karlogidinium* and *Pfiesteria* alone are very similar already, with a slight difference in the mean, i.e. only one variable is needed to scale them. This reduction in the number of scaling parameters suggests the same driving mechanisms for cell body rotation along the anterior-posterior axis between the species. As the preys are introduced, the peak broadens, yet the distributions can still be scaled by its mean value and standard deviation.
We also observed another pair of quantities which are not independent with each other. Figures 7.20a-c show the scatter plots of translational velocity verses radius of curvature in log-log scale. All three experimental conditions are plotted separately. A remarkable clustering of data points in all three cases is clearly observable regardless of species and behavior modifications. A relationship of power law can be easily established as a matter of fact, suggesting a relationship that a smaller tangential velocity results in a smaller turning radius, and a larger one mandates a larger turning radius. Since the true nature of turning radius and tangential velocity is related directly to the viscous stresses and moments imposed on the cell body by the surrounding flow, we can only satisfy ourselves by speculating that viscous stresses and moments on the cell body are inter-related and constrained at this moment.

7.4 Summary
Digital Holographic Microscopic Cinematography has been successfully applied to study the population dynamics of marine micro-organisms: harmful algae such as _Karlodinium veneficum_ and _Pfiesteria piscicida_. By recording a time-sequence of magnified digital holograms of free swimming dinoflagellates using digital holographic microscope (DHM, Sheng et. al. 2006), one can track the movement of each individual cell in a cloud. Hence the trajectories of free swimming dinoflagellates in large population can be faithfully obtained. An algorithm modeling the 3-dimensional trajectory as time evolution of an instantaneous helix decomposes each trajectory into several time-dependent helix parameters: Pitch ($P$), Radius ($R$), tangential velocity ($V$) and helix angular velocity ($\omega$). The first three variables, $P$, $R$, and $V$, constitute three axes of a Hilbert phase space, with each axis describing one topological and kinematical feature of
a trajectory. Hence, to a certain degree, a “trajectory” in such a phase space describes how a cell moves topologically and kinematically. An orbit, a cluster, or a non-random distribution of these “trajectories”, would represent a quantitatively measurable behavior. Histograms of such orbits, clusters, or distributions in that matter provide collective measurements of entire population. A change in the phase space distributions reflects a modification in the swimming behavior. And a change in the histograms or probability distributions thus describes a behavior alteration of the entire population. Shown in our results, such phase decomposition is highly suitable for quantitatively describing the swimming behavior of micro-organisms, provided one can measure 3D trajectory accurately. Digital Holographic Microscopic Cinematography proves to be highly effective in measuring the trajectories of densely populated micro-organisms over a substantial volume.

The swimming behaviors of three dinoflagellates: *Oxyrrhis marina*, *Karlodinium veneficum* 2064 and *Pfiesteria piscicida*, were studied in our experiments. And one pair of predator and prey relationship was investigated, *Pfiesteria piscicida* and *Rhodomonas*. The results on *Oxyrrhis marina* demonstrated that digital holographic microscope is capable of resolving the trajectories of hundreds of cells in a volume of 0.8×0.8×3mm³ and at the same time the fine details of each cell like longitudinal flagellum (200nm in width.) The exceptional imaging resolution allows us to investigate the swimming mechanisms of each individual at will. It is the first time to my knowledge that one is capable of imaging both cell body and the waveform of the flagellum from a free-swimming cell far away from boundaries. Most exceptionally, the details on *every cell* in the measurement volume can be resolved. From the samples of individual trajectories by
Karlodinium and Pfiesteria, one can conclude that both cells are capable of swimming in complicated manners, such as altering any three helix parameters in flight, sudden directional changes, fast migration and local survey missions, etc. In comparison, Karlodinium being a mixotroph has a much more compact phase space than the natural hunter Pfiesteria (a heterotroph), i.e. Karlodinium moves slower and has fewer varieties of helical trajectories. The differences are later quantified in the marginal joint PDF distributions of radius, pitch and velocity.

The biggest finding of current experiment comes from the predator and prey experiment. First, it is found that both Karlodinium and Pfiesteria establish probability density distributions of nearest neighbor distance (NND) as a group of randomly distributed particles when they are in isolation. Once the preys (Rhodomonas) are introduced to Pfiesteria, the NND distribution indicates Pfiesteria cells are clustered around their preys (Rhodomonas), i.e. the distribution ceases to be random any more. Second, the joint PDFs of radius vs. pitch, radius vs. velocity and pitch vs. velocity paint a very clear picture of how the Pfiesteria population reacts collectively to “accommodate” the preys. Pfiesteria doubles its tangential velocity, expands the helix radius by 50%, correspondingly reduces the pitch by 50% and at the same time increases the angular velocity about the helix axis by 50%. They are moving in an “excited” state. The most extraordinary thing is the PDFs clearly show the entire population changes its collective swimming behavior. It is our belief that such an alteration in swimming pattern helps Pfiesteria in capturing its prey. But we cannot pinpoint exactly how it helps. Several hypotheses are touted at the moment: (1) Large and fast helical swimming trajectory increases the sampling volume per unit time, hence increases the encounter rate; (2)
Large helical radius helps better triangulation of the prey via chemo- or mechano-sensors; (3) Simply speeds up to catch the preys. No matter what the advantages are, the change of collective behavior is very evident in our measurement.

Reference

Figures
Fig. 7.1 Optical setup for digital holographic cinematography
Fig. 7.2 (a) SEM image of an Oxyrrhis marina. (b) Light microscope image of a Karlodinium veneficum. (c) DIF image of a Pfiesteria piscicida. (courtesy of Haruyoshi Takayama)
Fig. 7.3 decomposing a 3D trajectory with instantaneous helix fit. XYZ is a fixed reference frame. TBN is the local moving frame of reference.
Instantaneous helix parameters are radius \( R \), pitch \( P \) and tangential velocity \( V \).

**Fig. 7.4**
Top: Reconstructed images of a *Karlodinium* cell at consecutive depth locations. \( z_0 \) represents the plane the cell is in focus. The last image shows the edge (white line) of the in focus cell identified by the segmentation algorithm. (b) Integral of edge sharpness value vs. the distance to the hologram. The integral peaks when the cell is in focus. Gaussian curve fitting around the peak provides sub-pixel resolution in determining in-focus location.

**Fig. 7.5**
(a) Histogram of sub-pixel resolution in depth direction, defined as the standard deviation between the measured and the spatially filtered \( z \) positions of each trajectory. (b) A typical 3D trajectory measured in the fixed reference frame. Insert: 3D rendering of the trajectory.

**Fig. 7.6**
Time sequence of a swimming *Oxyrrhis* with its longitudinal flagella fully reconstructed. Time interval is 1/60 sec. The center graph is the superimposed in-focus images with their original lateral displacements \((\Delta x, \Delta y)\). The surrounding images are in-focus images artificially positioned to show the waveforms of the longitudinal flagella at each time instance. Scale: 80 \( \mu \)m.

**Fig. 7.7**
A trajectory of a free-swimming Karlodinium cell (2D projection in x-y plane) superimposed with the reconstructed in-focus images sampled at every 1/3 second. The trajectory is color-coded with the velocity magnitude \( V \). The closeup on the top shows the groove where the traverse flagellum is situated. Bottom: 3D render

**Fig. 7.7**
(a) 3-D trajectories of *Karlodinium veneficum* in the measurement volume. (b) and (c) close-ups

**Fig. 7.8**
(a)-(p) individual trajectories and their corresponding 3D renderings. Trajectories are color coded with velocity magnitude, ranging from 0 to 200 \( \mu \)m/s

**Fig. 7.9**
(a) 3-D trajectories of *Pfiesteria piscicida* (alone) in the measurement volume. (b) and (c) close-ups
Fig. 7.10  (a) 3-D trajectories of *Pfiesteria piscicida* and *Rhodomonas* together in the measurement volume. (b) and (c) close-ups

Fig. 7.11  (a)-(x) individual trajectories and their corresponding 3D renderings. Trajectories are color coded with velocity magnitude, ranging from 0 to 450 µm/s

Fig. 7.12  Mean vertical velocity of individual trajectory vs. its mean z-location. Circle: *Rhodomonas*. Cross: *Pfiesteria*. Background convection flow is indicated by the mean vertical velocity distribution of Rhodomonas. The gravity points toward the positive velocity direction.

Fig. 7.13  Probability density distributions of Nearest Neighbor Distance (a) *Karlodinium* and *Pfiesteria* alone, and (b) *Pfiesteria* with *Rhodomonas*. Solid line with symbols: experimental measurement; solid line: Random distribution of particle field with the same cell concentration.

Fig. 7.14  Probability density distributions of Nearest Neighbor Distance when *Pfiesteria* is mixed with *Rhodomonas*. (a) solid line with squares: NND between *Pfiesteria* themselves; solid line with circles: NND between Rhodomonas themselves, and (b) NND from *Pfiesteria* to *Rhodomonas*. Solid line with symbols: experimental measurement; solid line: Random distribution of particle field with the same cell concentration.

Fig. 7.15  Probability density distribution of tangential velocity (velocity magnitude). Insert: PDFs in log-log scale. Line with diamonds: *Karlodinium* in isolation; line with circles: *Pfiesteria* in isolation; line with crosses: *Pfiesteria* cells (separated from Rhodomonas) when they are mixed with *Rhodomonas*

Fig. 7.16  Joint PDFs of helix parameters. Left panel: *Karlodinium* in isolation, middle panel: *Pfiesteria* in isolation; Right panel: *Pfiesteria* when mixed with *Rhodomonas*. (a) Joint PDFs of radius and pitch (b) tangential velocity vs. radius and (c) tangential velocity vs. pitch.

Fig. 7.17  Probability density distributions of angular velocities about the helix axis.

Fig. 7.18  Autocorrelation coefficients of (a) velocity (b) acceleration
Fig. 7.19  Probability density distributions of (a) angular velocities about dorsal radial direction (b) angular velocities about anterior-posterior direction. Inserts: PDFs scaled with the mean values and standard deviations.

Fig. 7.19  Dynamic constraint on the tangential velocity and radius of curvature: Larger velocity has larger radius. (a) Karlodinium in isolation (b) Pfiesteria in isolation (c) Pfiesteria when mixed with Rhodomonas (statistics on Rhodomonas are not included)
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Fig. 7.6 A trajectory of a free-swimming Karlodinium cell (2D projection in x-y plane) superimposed with the reconstructed in-focus images sampled at every 1/3 second. The trajectory is color-coded with the velocity magnitude (V). The closeup on the top shows the groove where the traverse flagellum is situated. Bottom: 3D render
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Fig. 7.10 (a) 3-D trajectories of *Pfiesteria piscicida* and *Rhodomonas* together in the measurement volume. (b) and (c) close-ups
Fig. 7.11 (a)-(x) individual trajectories and their corresponding 3D renderings. Trajectories are color coded with velocity magnitude, ranging from 0 to 450 µm/s
Fig. 7.12 Mean vertical velocity of individual trajectory vs. its mean $z$-location. Circle: *Rhodomonas*. Cross: Pfiesteria. Background convection flow is indicated by the mean vertical velocity distribution of Rhodomonas. The gravity points toward the positive velocity direction.
\[
\rho(NND)
\]

(a) Karlodinium Meas.
Random Distrib.
Pfiesteria Meas.
Random Distrib.
Fig. 7.13 Probability density distributions of Nearest Neighbor Distance (a) *Karlodinium* and *Pfiesteria* alone, and (b) *Pfiesteria* with *Rhodomonas*. Solid line with symbols: experimental measurement; solid line: Random distribution of particle field with the same cell concentration.
Fig. 7.14 Probability density distributions of Nearest Neighbor Distance when Pfiesteria is mixed with Rhodomonas. (a) solid line with squares: NND between Pfiesteria themselves; solid line with circles: NND between Rhodomonas themselves, and (b) NND from Pfiesteria to Rhodomonas. Solid line with symbols: experimental measurement; solid line: Random distribution of particle field with Random Distribution.
Fig. 7.15 Probability density distribution of tangential velocity (velocity magnitude). Insert: PDFs in log-log scale. Line with diamonds: Karlodinium in isolation; line with circles: Pfiesteria in isolation; line with crosses: Pfiesteria cells (separated from Rhodomonas) when they are mixed with Rhodomonas.
Karlodinium 2064 alone

Pfiesteria alone

Pfiesteria & Rhodomonas
Fig. 7.16 Joint PDFs of helix parameters. Left panel: *Karlodinium* in isolation, middle panel: *Pfiesteria* in isolation; Right panel: *Pfiesteria* when mixed with *Rhodomonas*. (a) Joint PDFs of radius and pitch (b) tangential velocity vs. radius and (c) tangential velocity vs. pitch.
Fig. 7.17 Probability density distributions of angular velocities about the helix axis.
Velocity Autocorrelation $R_{ui, i=1,2,3}$
Fig. 7.18 Autocorrelation coefficients of (a) velocity (b) acceleration
$\omega_k$ (rad/s)

pdf($\omega_k$)

- Pfiesteria+Rhodomonas
- Pfiesteria alone
- Karlodinium alone

$(\omega_k - \omega_k^*)/\omega_k$
Fig. 7.19 Probability density distributions of (a) angular velocities about dorsal radial direction (b) angular velocities about anterior-posterior direction. Inserts: PDFs scaled with the mean values and standard deviations.
Karlodinium alone

Translational Velocity (µm/s)

1/k (µm)
Pfiesteria alone

Translational Velocity (µm/s)

10^3

10^2

10^1

10^0

10^{-1}

10^0

10^1

10^2

10^3

10^4

1/K (µm)
Fig. 7.20 Dynamic constraint on the tangential velocity and radius of curvature: Larger velocity has larger radius. (a) Karlodinium in isolation (b) Pfiesteria in isolation (c) Pfiesteria when mixed with Rhodomonas (statistics on Rhodomonas are not included)
8. Conclusions

The work presented in the dissertation mostly consists of development of 3-dimentional flow measurement techniques and their applications in fundamental studies of wall-bounded turbulent and biological flows. Substantial efforts have been invested in the methods using multiple-exposure particle field holography. The research has dealt with all aspects of Holographic Particle Image Velocimetry (HPIV) field: from simple in-line holography to an advanced two-view off-axis system, from the cumbersome yet highly accurate film-base holography to easy-to-use digital holographic PIV, from a large-scale system to a digital holographic microscope, from low sample rate recording to high-speed holographic cinematography. The applications of these techniques have spanned from high Reynolds number turbulence to micro-scale biological flows of self-propelled micro-organisms.

To perform high-resolution volumetric 3D turbulence measurements and reduce the complexity of previous off-axis holographic PIV systems, an innovative single-beam two-view off-axis HPIV system has been introduced. This method consists of inserting a mirror in the path of an inclined illumination beam. Consequently, the volume near the mirror is illuminated twice by the incident and reflected beams in two different directions. Two orthogonal views of the same particle field are reconstructed simultaneously but are separated in space. Matching two views provides the same measurement accuracy in all directions. For a volume of $50 \times 50 \times 50$ mm$^3$, this method has reduced the vector spacing down to 200µm.
A digital HPIV system has been introduced, taking full advantage of recent availability of large format CCD cameras and simple implementation of in-line holography. Digital HPIV combined with the single-beam, two-view concept was successfully applied to visualize and measure the 3D flow pattern generated by a free-swimming copepod for the first time. It was found that the velocity field and trajectories of particles entrained by the copepod have a re-circulating pattern in the copepod’s frame of reference. This pattern is caused by the copepod sinking at a rate that is lower than its terminal sinking speed, due to the propulsive force generated by its feeding current. Use of Stokeslets to model the velocity distributions enables us to estimate the excess weight, density and the propulsive force generated by a copepod.

Digital Holographic has been further extended to high-speed holographic cinematography. We have used it to study the motion of a free-swimming nauplius (a “baby copepod”, ~60um) and the consequential hydrodynamic signals produced by its motion. The results are vital for understanding and modeling the sensory perception in predator-prey relationship for small marine organisms. The 2µm-resolution holographic measurements reveal that the well-known helical “hops” made by a nauplius (a prey) are actually composed of sequences of high frequency oscillatory motions. This motion, in fact, helps in reducing the overall strength of the detectable hydrodynamic signals “broadcasted” to the surroundings. There is a clear discrepancy, up to two orders of magnitude, between the experimentally measured disturbance field and the one predicted by a widely used self-propelled sphere model. My observation helps explaining that discrepancy.
To observe 3-dimentional near-wall flow structures and their imprints on wall shear stresses, a digital holographic microscope (DHM) has been developed. The DHM provides very high resolution of ~200 nm with the extended measurement depth of more than 500 times depth of field of a conventional microscope. It also substantially reduces the depth of focus of traditional in-line holography, making it possible to obtain full 3D data from a single view hologram. These features make it a highly suitable tool for studying 3-D small-scale dynamical phenomena. The DHM is presently being applied to study the imprints of near-wall structures on the wall shear stress distribution at high Reynolds numbers using data with vector spacing of 15 µm (compared to the wall unit of 17µm.) In particular, we have focused on the signatures left by the vortical structures in the viscous sub-layer, “buffer” and lower log layer, of which all are confined to a depth of less than 1mm from the wall. It is the first time that the 3-dimentional near-wall coherent structures at high Reynolds numbers are being measured experimentally at better than DNS (Direct Numerical Simulation) resolution. Simultaneously, the spatial distributions of the instantaneous wall shear stress, a crucial quantity for boundary layer modeling, are also measured directly from local velocity gradients at the wall. These data enable us to statistically analyze structure-stress, strain-stress, and vorticity-strain relationships in the near wall region. The results reveal a rich composition of buffer-layer structures, varying from counter-rotating vortex pairs to “infant” hairpins. A strong correlation exists between the spatial variability of wall stresses and the presence of near-wall structures. Furthermore, the vorticity of powerful vortical structures shows a preferred alignment to the local velocity strain that differs from that of the background shear flow.
To understand the population dynamics of harmful algae bloom, digital holographic microscopy is extended to digital holographic microscopic cinematography. The motion of dinoflagellates: *Oxyrrhis*, *Karlodinium* and *Pfiesteria*, in high concentration is recorded over a substantial volume of $0.8 \text{ mm} \times 0.8 \text{ mm} \times 3 \text{ mm}$ at the rate of 60Hz. The spatial resolution is sufficient enough to visualize the free swimming cell in great details, i.e. longitudinal flagella of the cell (200nm in width) is clearly captured and reconstructed in our measurements. The measurements also provide large sample volume that long-term trajectories of moving cells in high concentration are captured, from which the quantitative measurements on swimming behavior of each species are obtained via instantaneous Helix decomposition. It is found that *Karlodinium* being a mixotroph moves 2.5 times slower and has fewer varieties of helical trajectories than *Pfiesteria* (a heterotroph). The angular velocity about the helix axis of *Karlodinium* is also 50% smaller than *Pfiesteria*, and the integral time scale of *Karlodinium*’s swimming motion is 2 times larger that of *Pfiesteria’s*. Species dependent swimming behaviors are observed in individual trajectories and quantified in probability density distributions of instantaneous helix parameters. The experiment of examining the predator – prey interaction effects on swimming behavior has been conducted with *Pfiesteria* and its prey *Rhodomonas*. When preys are mixed with *Pfiesteria*, the spatial distribution of the cells (Near Neighbor Distance Distribution) is changed into one showing clear sign of clustering different from a pure random distribution. Further inspection reveals that *Pfiesteria* are clustered around preys. Moreover, the swimming behavior of *Pfiesteria* is also changed dramatically in the presence of food. The cells swim twice faster, their
helix radii are 50% larger, and the angular velocities about the helix axis are 50% faster than the usual (without food presence).