HPIV using Polarization Multiplexing Holography in Bacteriorhodopsin (bR)

by

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ABSTRACT

This paper demonstrates the use of bacteriorhodopsin (bR) as the holographic recording medium for a holographic particle image velocimetry (HPIV) system. Using an off-axis reference beam and forward-scattered holographic geometry, dual-exposure particle images in a fluid flow have been recorded successfully onto the bR film. Photo-induced anisotropy in bR was used to multiplex two polarization holograms at the same location of the holographic film. The transmission holographic recording geometry has been simplified, because polarization multiplexing allows two common beam paths to be used for the two pairs of object and reference waves, respectively. Furthermore, we have also demonstrated that, with a sequential holographic read-out method, the two recorded holograms can be retrieved independently during the holographic reconstruction. This system allows directional ambiguity to be removed in the HPIV flow data (see figure A).

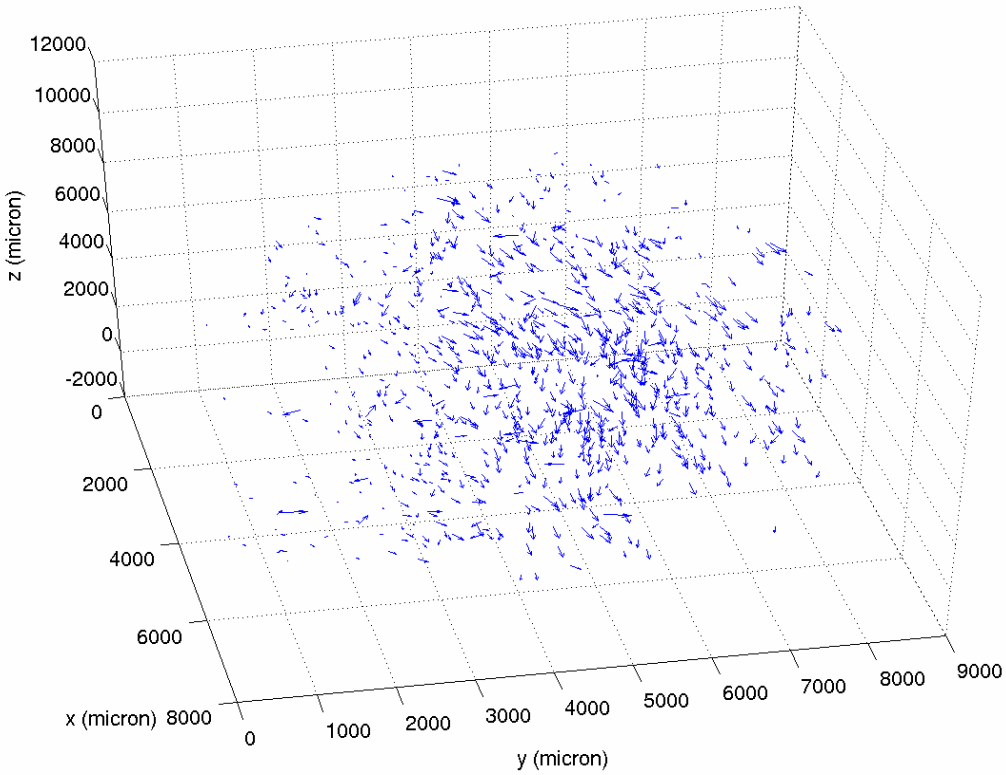

Figure A: Extracted HPIV vectors, the jet is flowing from top to bottom and towards the observer.

1. INTRODUCTION

Recently, in HPIV, there is a shift of emphasis from traditional holographic recording using silver-halide based films (Hinsch 2002, Barnhart *et al* 2002a, Herrmann and Hinsch 2004) to direct digital recording of holograms on CCD (Meng *et al* 2004, Coupland 2004, Ooms *et al* 2004). Due to the limited size of a CCD pixel (typical size of 7µm), direct registration of a hologram on a CCD array requires a recording geometry of a low numerical aperture (NA) in-line transmission configuration. This imposes a limit on the Mie-scattering angle of no more than a few degrees (Meng *et al* 2004, Ooms *et al* 2004). Moreover, the limited serial data transfer rate (typically = 20 MHz) of a CCD chip hinders the development of ultra high resolution sensor large format detectors (giga-pixels camera) which could offer high spatial resolution digital holographic recording. A CMOS sensor that offers parallel transfer rate may eventually overcome this problem. However, at the meantime, bR still offers one of the best solutions before this current bottleneck can be overcome. Barnhart *et al* (2002b) realized the potential of applying bR for holographic particle image velocimetry (HPIV), and subsequently pointed out that a centimeter-square area of bR film has the same information capacity for several hundred state-of-the-art CCD cameras. Barnhart *et al* (2004) discussed the advantages and limitations of using bR as a recording medium for HPIV

Bacteriorhodopsin (bR) is a photochromic protein which has been optimized by nature for millions of years. bR is found in two dimensional crystalline patches in the cell membrane of the Halobacterium Salinarum. Utilizing photosynthesis, the bacterium uses bR to convert light directly into chemical energy (Oesterhelt and Stoeckenius 1971). Specially fabricated and genetically modified bR films offer spatially resolvable optical resolution of the order of several thousand-line pairs per millimeter (Hampp 2000). An optical data storage material that requires no processing, no unwanted optical shrinkage with the recorded data, and with read and write cycles over millions of times without degradation of the film quality, quickly attracts interest from scientists and engineers.

Shown in figure 1 is a simplified two state photo-cycle of bR. Since there is a shift of more than 150 nm between the absorption spectra of the B and the M state, as well as a change in the index of refraction, information can be stored in a bR film by converting a modulated recording intensity into a modulation of the B and M populations. After the information has been recorded (in the form of a hologram), molecules in the M state will eventually fall back to the B state due to the thermal transitions. This obviously results in a loss of modulation, and therefore a loss in the reconstructed signal strength, and is referred to as thermal erasure. When reconstructing the hologram, absorption will cause some bR molecules to transit from the B to the M state. This loss of modulation is referred to as photo-induced erasure, not to be confused with the intentional optical erasure which is done to prepare the film for a new recording (410 nm) - see figure 1. Because of the wide absorption band of the B state, that is ranging approximately from 500 nm to 640 nm (see figure 2), commercially available high power pulsed frequency-doubled Nd:YAG lasers (532 nm) can be used for recording holograms in bR.

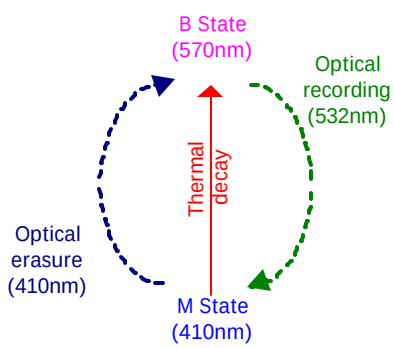

Figure 1: *Simplified two-state photo-cycle of bR (After Hampp 2000).*

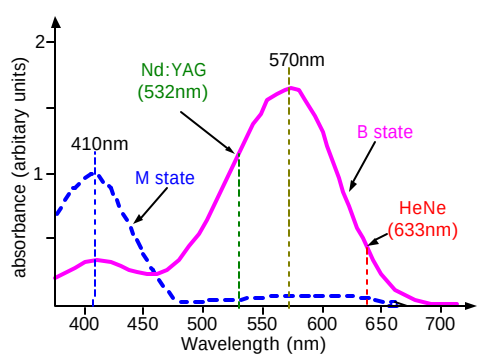

Figure 2: *Absorption spectra of the B and M states of bR (After Hampp 2000).*

As has been discussed in Chan *et al* (2004), various multiplexing methods can be used to record multiple holograms onto a same bR film. Polarization multiplexing by photo-induced anisotropy in bR offers the possibility in removing directional ambiguity in the HPIV data (Koek *et al* 2004).

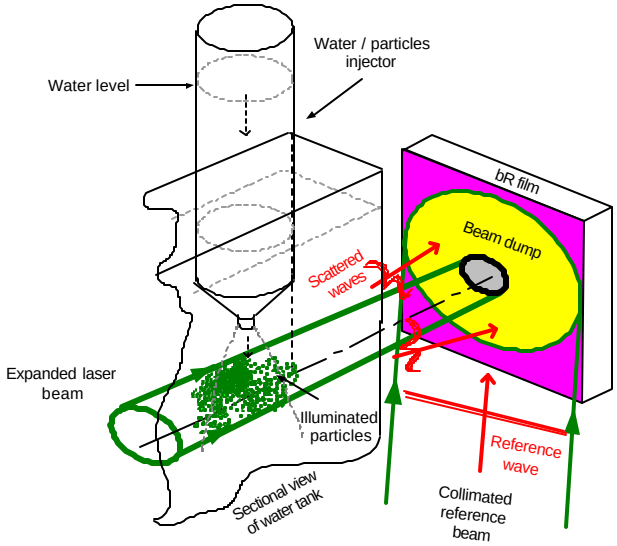

Figure 3: *Schematic of recording a hologram on bR using forward scattering on off axis geometry with a small beam dump.*

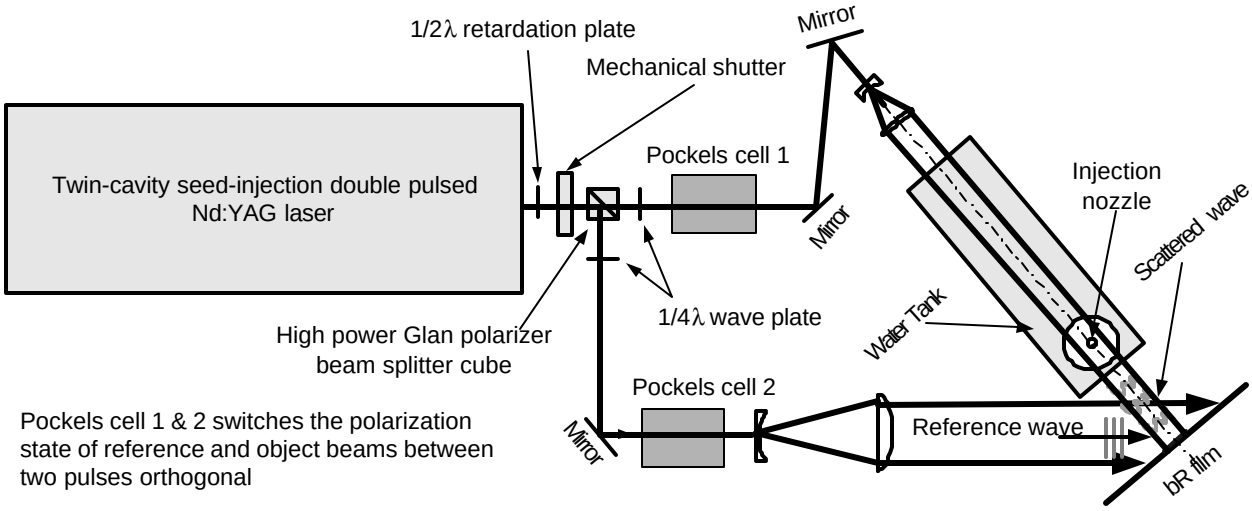

Figure 4: *Schematic of the recording set up for the HPIV experiment.*

2. EXPERIMENTAL SET UP FOR HOLOGRAPHIC RECORDING

As a proof of principle, an experiment was performed on a water jet seeded with 100 µm solid glass sphere. This jet was injected into a clear tank of water through a syringe nozzle. The nozzle has an exit diameter (D) of 2 mm. Since the width of the tank was equal to 50 mm, the wall boundaries of the tank should have little influence on the near field of the flow. The central position of the measurement control volume was located at 12 diameters downstream from the nozzle exit (i.e. X/D=12), which led to a measurement window that was ranging from X/D=8 to X/D=16 along the streamwise direction.

It must be stressed that the glass spheres used in this study were heavier than water. The glass spheres are not "ideal' tracer particles and that their presence may influence the fluid flow, however, they acted as micro-ball lenses that focused the scattered light towards the holographic plate, resulting in a high contrast hologram to be recorded. In order to maximize the signal to noise ratio of the hologram still further, we have adopted a forward scattering geometry with a high numerical aperture (NA) configuration. This led to the recording of diffraction-limited particle images (Chan *et al* 2004). To protect the film from permanent optical bleaching (or damage) by the high energy object laser beam, a beam stop was incorporated on the surface of the film. The specification of the bR film used in this study is listed in table 1.

Table 1. Specification of the used bR film made by MIB GmbH (http://www.mib-biotech.de/)

Film Type	Film Size	Film Thickness	Initial Optical Density	Refractive Index	Thermal Relaxation Time
D96N	100 x 100 mm^2	30 μm	1.5 (570 nm)	1.45-1.55	40-80 s

In the current set up as shown in figures 3 and 4, a small expanded laser beam from a dual-cavity seed-injection pulsed Nd:YAG (532 nm) with output energy of 300 mJ per pulse with pulse duration of 7 ns, was used to illuminate the flow volume twice with a pulse separation time of 230 μs. The object and reference beam fluences were adjusted to be equal to 80 mJ/cm^2 (expanded beam diameter = 9 mm) and 5 mJ/cm^2 (expanded beam diameter onto the bR film = 80 mm) respectively, where it is noted that only a small fraction of the object light scatters onto the bR film. The ratio of the object and reference beam power can be adjusted using a 1/2λ retardation wave plate and a Glan laser polarizer (see figure 4). The two individually positioned quarter wave plates rotated the object wave (horizontal polarization) and the reference wave (vertical polarization) into left and right circular polarizations, respectively. The seeded flow field was illuminated by the object beam in forward scattering direction. The ongoing object beam eventually terminated at a beam dump that mounted on the surface of the holographic plate. The reference beam illuminated the hologram at a subtended angle of 40° against the optical axis of the object beam. The resulting measurement volume was approximately equal to the size of the spreading jet. No signal was recorded outside the jet because the ambient water was not seeded with particles.

When considering polarization holography in bR for HPIV, the recording of a hologram involves a two-step procedure (Chan *et al* 2004). During the first exposure both Pockels cells were switched off, such that the object and the reference wavefront would have left hand circular polarization and right hand circular polarization, respectively, at the plane of the hologram. For the second exposures the Pockels cells were supplied with the half wave voltage, resulting in an interchange of the object and reference polarization. With this set up, we have multiplexed two time separated holograms inside a single film. The recording and reconstruction procedures are summarized in table 1.

Table 2. Polarization configurations for recording and reconstructing the two bR holograms.

Reference Wave Polarization 1	Object Wave Polarization 1	Reference Wave Polarization 2	Object Wave Polarization 2	Reconstruction Wave Polarization 1	Reconstruction Wave Polarization 2
Right Circular R	Left Circular L	Left Circular L	Right Circular R	Left Circular L	Right Circular R

3. DATA EXTRACTION RESULTS AND DISCUSSION

The collimated reconstruction beam for the read-out of a hologram has an intensity of 130 μW cm^{-2}. During the phase conjugated reconstruction, a 1/2λ wave plate was mounted on a mechanical switch that was present in the reconstruction path of the CW laser (see figure 5). With the switch activated the right hand circularly polarized reconstruction beam has resulted in the reconstruction of the left hand circularly polarized first particulate image onto a CCD camera. Similarly, when deactivating the switch the second particulate image was reconstructed having right hand circular polarization.

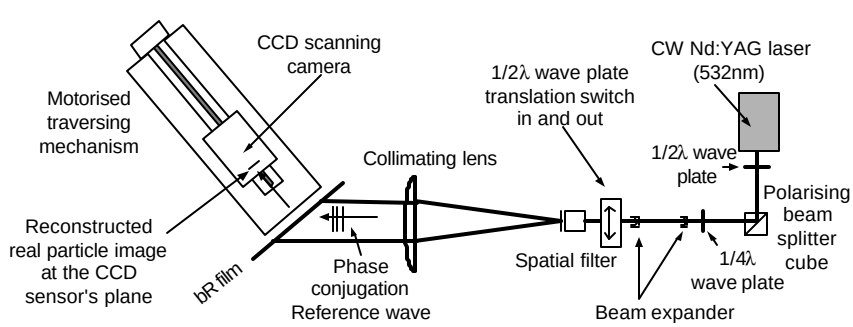

Figure 5: *Reconstruction of two sequentially multiplexed polarization holograms.*

Since the two holograms were stored in a volatile material such as bR, the read-out process is time limited (Chan *et al* 2004). In order to maximize the reconstruction efficiency, the holograms were subsequently reconstructed, scanned and digitized at a constant velocity by the CCD camera (1280 x 1024 pixels, 6.7 µm x 6.7 µm pixel size) that was mounted on a motorized traversing gear. Hence, the switching time of the 1/2 λ wave plate for reconstructing the multiplexed holograms, was determined by the speed of the traversing mechanism. The exposure time of the CCD camera was set to a period that no saturation of the particle images could occur. This was optimized for the local maxima signal intensity analysis.

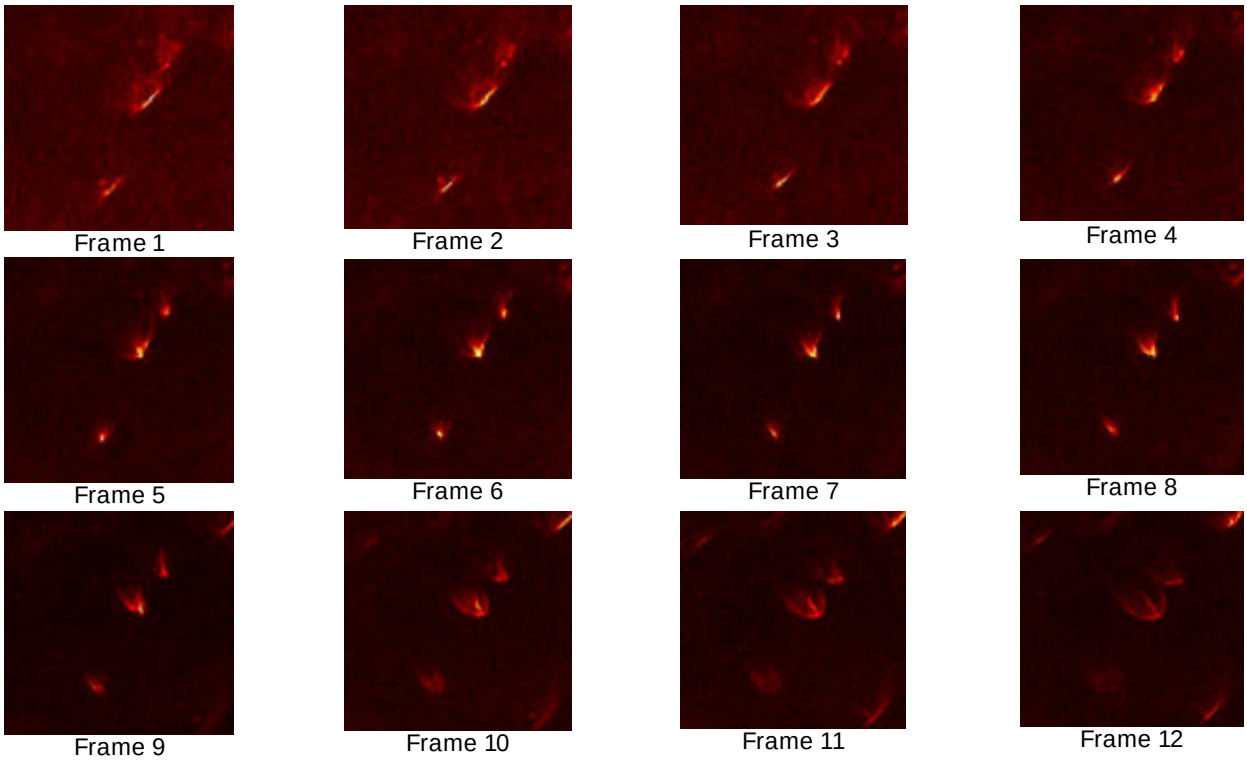

Figure 6: *A selected region of the reconstructed particles images, focusing through 50mm step between each frame*.

Figure 6 shows the reconstructed particle field of one of the two multiplexed holograms through different focal depth (picture resolution = 100x100 pixels). As can be seen in figure 7, the two data sets had been retrieved independently with no cross-talk between the two holographic exposures. In this figure, we have reconstructed around 750 particles within a measurement volume of 7x8x9 mm^3. The magnified view in figure 7 shows each string as a reconstructed particle. Three-dimensional image intensity analysis was performed to determine all velocity components from the recorded flow holograms, e.g. this is done in the following way: 280 continuously scanned image slices were digitized onto the computer of which 140 of them were belong to one of the two holograms. The odd numbers of the image slices belongs to the first hologram and the even numbers of images were from the second multiplexed hologram. Each digitized slice has a resolution of 50µm that was currently limited by the number of images that can be stored on the computer memory buffer. Image processing was performed on each slice to enhance detectability of the reconstructed particles. This was achieved by a special phase preserving denoising image processing routine outlined by Kovesi (1999). In this routine the threshold of the images were determined automatically by wavelet responses in the images. Thresholding of wavelet responses in the complex domain allows one to ensure that perceptually important phase information in the image is not corrupted (Kovesi 1999). All the data extraction and image filtering processes were carried out in MATLAB.

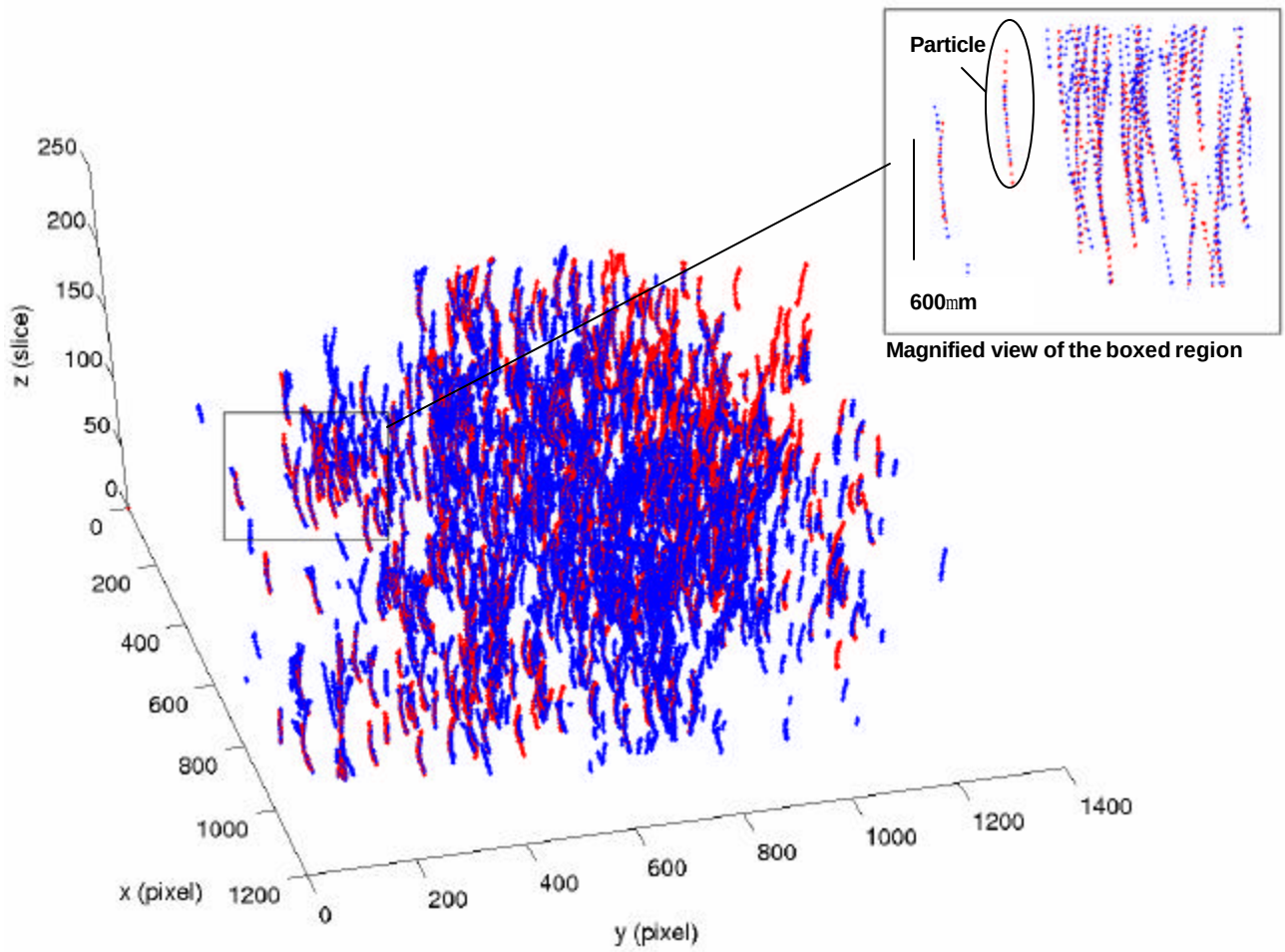

Figure 7: *Two independently reconstructed holographic particle fields superimposed on top of each other. Directional ambiguity is resolved by polarization multiplexing two holograms independently in bR.*

Particle detection was performed by cross-correlating each slice with a particle "mask", a 5x5 Gaussian model particle. Subsequently, the local maxima in the resulting correlation plane were detected (which correspond to particle centers). All the corresponding images from each slice were re-stacked to form particles intensity streaks along the focal depth of the hologram as shown in figure 7. Each streak contained a corresponding intensity value along its trail. This position of the intensity value was obtained by repeating the aforementioned particle detection procedure but without the denoising image processing technique, because this technique stretches the contrast of image to an artificially high value.

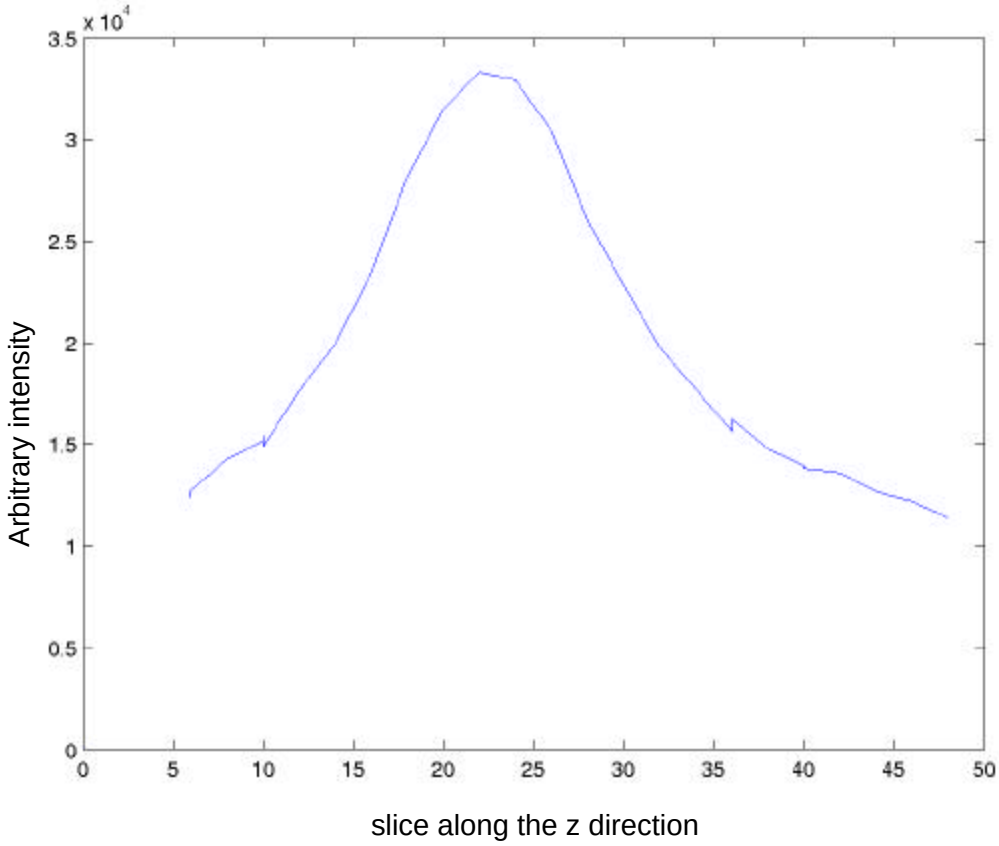

Figure 8: *One of the reconstructed particle strings to show the intensity profile along the slice.*

To extract the velocity vectors, a nearest neighborhood search routine was performed on detected particle centers between the two stacks of images. The maximum intensity value along the image trail was used to determine the particle location (see figure 8).

The directionally resolved data HPIV is presented in figure 9. The vector field exhibits a certain preferential orientation that indicates the jet flowing direction. The obtained maximum flow velocity in the streamwise direction is approximately = 0.65 ms^{-1}. Noting that, the reconstruction was performed in air therefore, the velocity value was corrected for the refractive index in water. The fluid shows a meandering flow profile which could be attributed to the fact that the heavier-than-water particle was used to seed the jet. This is further supported with figure 10, which indicates the jet center line velocity profile was skewed. It is also interesting to note that the velocity values near the edge of the jet did not decay to smaller amplitudes (particle slip velocity ~ 0.2 ms^{-1}), this effect was again due to the condition of the seeding particles.

The result indicated that we have successfully demonstrated that polarization multiplexing in bR offers the capability in removing directional ambiguity in the HPIV data. Currently, the turnaround time for each experiment which includes the recording, digitizing and refreshing of a hologram is approximately 10 minutes. The experimental procedures can be performed routinely by a single operator.

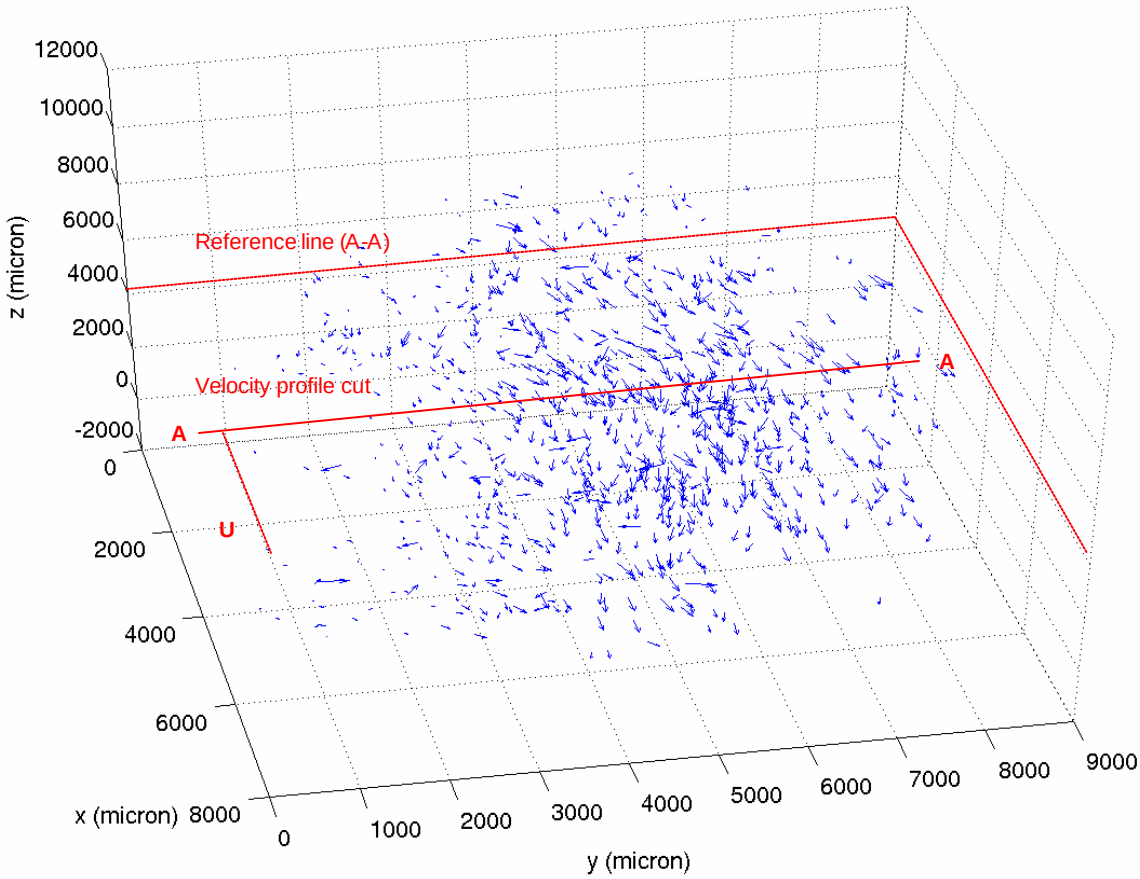

Figure 9: *Extracted HPIV vectors from figure 7, the jet is flowing from top to bottom.*

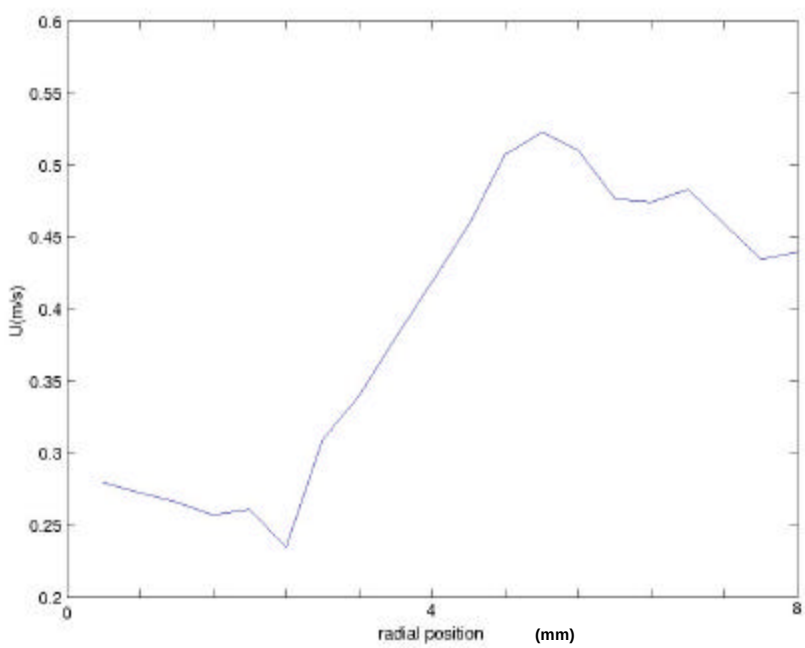

Figure 10: *Interpolated streamwise velocity profile (U) along the line A-A (figure 9) at the center of the jet (X/D=12).*

4. CONCLUSION

As a proof of principle, an experiment was conducted on a particle laden water jet. A dual off-axis transmission hologram was recorded which maximizes the signal to noise ratio of particle images. Volumetric three-dimensional velocity information was extracted from the holograms by tracking the particle positions using the local maxima image intensity analysis. The current result was based on a single holographic measurement, and this could be extended to multiple batches of fluid experiments in "an hour to hour basis" in the near future.

For the first time, we have demonstrated the feasibility in using bR for the recording of dual-exposure polarization multiplex particulate holograms to remove directional ambiguity in HPIV. The system can be operated routinely by a single operator.

ACKNOWLEDGEMENT

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