Three-dimensional microflow measurement by using hybrid multiplexed holography

by

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ABSTRACT

A hybrid technique of multiplexing holography as shown in figure below was developed to characterise microfluidic behaviors for the needs from microfluidics. Following the spirit of holographic PIV (HPIV), a high sensitive photopolymer (CROP), a ferroelectric liquid crystal (FLC) shutter and a rotational stage were introduced to constitute an novel system for 3D3C microflow measurements. CROP photopolymer in the system serves as an intermediate recording media, while FLC shuttle associates with rotational stage serves as a hybrid mechanism of holographic multiplexing. A high-sensitivity CCD on a traverse stage was used to retrieve the holographic images for post processing to derive a 3D instant flow field based on a concise cross-correlation algorithm (CCC). To evaluate the practicability, a microchannel was measured of its inner free flow with a preset exposure time of 6 ms for each image. The flow in a 550 µm × 26 µm microchannel was calculated of its average velocity to be 9.18 µm/s and central velocity to be 12.2 µm/s. The profile accorded with the simulated laminar flow situation. The results thus proved the practicability of the innovative design. Some encountered difficulties in the experiments will also be expatiated for future improvement. In addition, a visualisation technique based on the effect that light of different wavelength results in different refractive index was employed to rapidly view through the whole 3D flow field.

Depiction of the hybrid multiplexed manipulations for recording stereo-images: Two image pairs are shown above. The first multiplexing is operated by using a FLC shutter to divert the path of reference beam. The rapid non-mechanical switching is proper to control the time interval between image 1:1 and image 1:2 (∆t). The second multiplexing is a mechanically angular rotating which require a longer time (T), and is thus used to prepare memory for the next image pair (image 2:1 and image 2:2). Repeat the two multiplexing operations, serial image pairs can be stored in the photopolymer simultaneously.
1. INTRODUCTION

Some measurement techniques have been developed to keep pace with the emerging development of microfluidics. μPIV (Santiago et al., 1998), for instance, is one of the most accepted technique. The technique can measure flows in microchannels with high resolution. Though favorable for planar measurement, the restriction from finite focal depth implies demands to develop new ways to measure simultaneous full-field 3-D micro flow.

For 3D flows, Holographic PIV is often employed (eg. Royer, 1997; Pu et al., 2000) that two instantaneous images are exposed on the same storage material. Holographic plates made of silver halide or dichromated gelatin are commonly used in HPIV so that wet processing is necessary to develop the images. Besides, a CCD is also required to retrieve the stereoscopic image slice by slice in a scanning way while placing the plates back to its original positions. However, inherent low dynamic range and inconvenient wet processing confine its applicability. Furthermore, the analysed results are found deviated due to off-position. Lately, digital holography (DH) (Pan et al., 2001; Xu et al., 2001; Dubois et al., 1999) has been proposed with convenient procedure and real-time process. Nevertheless, low CCD resolution (< 100 lp/mm) and apparent speckle noise still restrict its applications. Recording material is also an essential issue to improve holography. Photopolymer (Waldman et al., 1998; Dhar et al., 1999) and bacteriorhodopsin (BR) (Hampp et al., 2000) are two materials that stress on huge storage capacity, high sensitivity and fast response time down to microsecond. BR also possesses multi-rewriting capability while photopolymer is a write-once-read-many (WROM) media.

In this paper, an experimental facility was setup by incorporating the adoption of microscopy, photopolymer and multiplexed exposure operation. First, images of the microchannel flow were magnified and transmitted via the microscopy. Serial volume holograms could be stored in a high sensitive photopolymer of 300-µm thick (CROP, Aprilis, centered at wavelength of 532 nm) by a new hybrid multiplexed optical operation based on beam polarisation and angular rotation. Scanning the retrieved holograms, the 3D micro flows could then be reconstructed by using a revised concise cross-correlation algorithm. To verify the practicability of the setup, a free flow seeded with 1-µm particles in a straight microchannel was tested. The velocity was calculated to be 9.18 µm/s averagely and 12.2 near the central region. Nevertheless, attributing to the focal elongation effect, the z-axis resolution was apparently larger than other two axes. Overall, the new design has shown its practicability to microflow applications.

2. EXPERIMENTAL METHOD and PROCEDURES

2.1 Experimental Set-up

Figure 1 shows the schematic diagram of the designed system. A flow chamber with channel section of 550 µm × 26 µm, shown in Figure 2, was measured of its inner flow for test. Polystyrene particles of 1-µm were seeded in the straight region to follow the existed fluid motion. Both signal and reference beams in figure 1 were generated by a 200 mW DPSS green laser (532 nm) combining with an electronic shutter, thus resulting in a series of pulse trains. Meanwhile, an inverted microscopy equipped with a 40X objective lens was also incorporated in the device to magnify the

Fig. 1. Schematic diagram of the hybrid multiplexed holography for 3D microfluidic measurement

2
microfluidic image. To compensate the optical path difference, a dove prism was inserted in the path of reference beam. The special arrangement in the system is the use of a FLC shutter for multiplexing. As sketched, FLC shutter serves as a switch that alternates the polarisation state (0° and 90°) with a rate of up to several tens kHz. Light at state $p$ passes the PBS and light at state $s$ reflects at the PBS so that an angular multiplexing can occur. Thus, an image pair can be recorded without mechanical movement while the beams interfered with the signal beam in the recording medium in sequence. Then, rotating or shifting the recording medium by a stage, the next image pair can be recorded.

After recording pairs of images in the medium, a high-sensitivity CCD (PCO PixelFly) on a linear traverser was employed to capture the retrieved holograms. The traverser enables scanning the stereoscopic image slice by slice in sequence. All the relative pages would be finally pieced together to reconstruct the spatial map of particle location in the computer. Figure 3 depicts an actual disposal and operations of the hardware.

2.2 Operational Principles

The operation of the new setup comprises two stages: storage and reconstruction. To record and store successive image pairs in the photopolymer rapidly, the FLC shutter, electric shutter, and rotation stage connected in parallel and activated synchronically. Ideally, the current approach can reach several hundred holograms in the recording medium. In the practice, at least four holograms were well distinguished from the numerous fluidic motions that were frozen in the photopolymer to make sure the feasibility technically. The CCD, as mentioned, scans out the stored images by focusing on different image plane inside the hologram and spatial locations can hence be determined.

To store multiple volume holograms in a medium, holographic multiplex technique and the use of thick media are two essential solutions. Numerous multiplexing techniques such as angular (Mok, 1993), wavelength (Rakuljic et al., 1992), phase-coded (Denz et al., 1998), peristrophic (Curtis et al., 1994), spatial shift (Barbastathis et al., 1996) have been proposed and adopted. Nevertheless, mechanical manipulations limit the operational speed. In our previous study (Yang et al. 2003), phase-code technique has shown its practicability to 3D microflow measurements, but cross-talk between holograms existed. In this study, a hybrid multiplexing technique was proposed. The first-stage multiplexing operation is based on fast alteration of light polarization. As shown in figure 4, incoming horizontally-polarized beam is turned vertical if the FLC shutter is at $s$ state, then is reflected in PBS cube (noted as Ref 1). Contrariwise, the polarization of the beam keeps horizontal at $p$ state, and then passes the PBS (noted as Ref 2). The half-wave plate turns the polarization of Ref 2 to vertical. Both Ref 1 and Ref 2 then interfere with the signal beam in sequence.

Fig. 2. Photo of the microchannel installed on microscopy with a 40X objective lens  
Fig. 3. Photo of the system arrangement  
Fig. 4. Depiction of the polarizing switch mechanism.
The second multiplexing is an angular operation that the recording medium is rotated about 0.4° each time by using a stage. As depicted in figure 5, the first multiplexing can operate rapidly and is suitable to capture an image pair; while the second multiplexing is a mechanical operation and is suitable to refresh memory for the next image pair. Repeat the two multiplexing operations, serial image pairs can be stored in the photopolymer simultaneously.

Fig. 5. Depiction of the hybrid multiplexed manipulations. Time between each image in a pair (Δt) is much shorter than the time between two pairs (T). Δt is determined by the first multiplexed operation, and T by the second.

Selection of suitable storage medium is an influential investigation. CROP photopolymer is primarily composed of vinyl monomer, photosensitizer, and binder. Photosensitizer optimized at 532 nm, can be activated by green light to form initial phase gratings and thus induces ordered variations of concentration. To compensate the imbalance, the un-reacted monomers diffuse to their vicinity of gratings so that a permanent refractive index change forms. Because different exposing procedures are independent to each other, the diffraction efficiency of each exposing can remain to its maximum. Compared to other holographic materials such as Fe:LiNbO₃ or BaTiO₃, commercial CROP photopolymer hold the edge of high sensitivity (1.53 cm/mJ), large dynamic range (M# > 3.24), low shrinkage (<0.1%) and write-once-read-many capability (WORM), thus was employed in the test. Controlled properly, the recording time could be less than 5 ms. To keep the medium from unexpected exposing, the entire measurement is essential to process in a darkroom condition by using Roscolux #27 or Abrisa #201.

Depth conversion ratio should be pre-calibrated. Referring to a standard pattern of 26 µm and focusing the CCD on both the sides in turn, the conversion ratio reached 1.3×10⁻³ µm/step. In addition, the phenomena of focal elongation during the CCD moving due to the extreme magnification of macro lens set will delimit the resolution in depth. In the test, the delimitation was around 1.3 µm. Preliminary tests also showed that the focal elongation might cause a deviation in particle locating up to 10%, which is apparently larger than the other axes.

2.3 3D Velocity Extraction

To figure the velocity distribution out of the reconstructed images, a concise cross correlation (CCC) algorithm (Pu, 2002) was introduced. As shown in figure 6, the second image of an image pair is shifted in a defined range and direction to fit the first image. The similarity between the two images can be estimated by a statistical process. Comparing the similarities obtained at different shifts, the mean displacement of the imaged particles between the two images can be determined by the maximum. The correlation of each axis can be calculated individually so that the efficiency to determine particle displacements in 3D space increases dramatically when comparing with conventional algorithms as expressed in Eq.(1).

\[
time | (l × l) · (X × Y × Z) | >> \time | (l × (X + Y + Z)) |
\]

The adjustment is based on the assumption that particles in the two images can be successfully paired, whereas actual probability of pairing are reduced because of bad discriminability and confined interrogation cell. To describe the detail of the algorithm, a general intensity-domain correlation can be mathematically formulated as

\[
C(x, y, z) = \sum_{j=1}^{m} \sum_{p=1}^{n} \Gamma_y(x, y, z; r)
\]
where \( m, n \) (\( m = n \)) are the number of particles in the first and the second holograms; \( r \) denotes the radius of sphere. \( G \) is a formula of cross-correlation which is defined as

\[
\Gamma_{ij}(x, y, z, r) = \exp \left\{ - \frac{\left[ x - (x_i - x_j) \right]^2 + \left[ y - (y_i - y_j) \right]^2 + \left[ z - (z_i - z_j) \right]^2}{2r^2} \right\}
\]  

(3)

Based on Eq.(2), search in three directions is essential to derive all three components of the 3D displacement. To reduce the complexity of computation, the original 3D model can be decomposed into three one-dimensional correlation that can be revised as

\[
C(x, y, z) = \sum_{i,j=1}^{N} \Gamma_{ij}(x)\Gamma_{ij}(y)\Gamma_{ij}(z)
\]  

(4)

where \( \Gamma(x) = \Gamma_{ij}(x; r) \cdot \Gamma(y) = \Gamma_{ij}(y; r) \cdot \Gamma(z) = \Gamma_{ij}(z; r) \)

If the statistical peak occurs when image 2 moved a displacement \((x_0, y_0, z_0)\) to match image 1, the correlation along an individual axis can be assumed to obtain peak at \(x_0, y_0, z_0\), respectively. Thus Eq.(4) can be simplified as

\[
C(x, y_0, z_0) = \sum_{i,j=1}^{N} \Gamma_{ij}(x)
\]  

(5)

where \( ? = G_{ij}(y_0)G_{ij}(z_0) \) is a constant. Similar expression hold for \( C(x_0, y, z_0) \) and \( C(x_0, y_0, z) \) to derive correlations that correspond to the same particles.

3. RESULTS AND DISCUSSIONS

3.1 Reconstruction of Flows in Microchannel

Before the measurement of flows in a straight microchannel, a free flow sandwiched in between two glass plates was preliminary tested. The result in figure 7 agrees with preset flow condition that the fluid was driven from left to right, indicating the feasibility of the design.

Applying to the microchannel, the time interval between the two images in an image pair (\( \Delta t \)) was 100 ms that the average particle displacement was about 6 pixels. The incident light intensity of each beam was about 25 mW, and the exposure time for each hologram could thus be <6 ms. At least two hologram pairs were scanned in the experiment. In the image retrieving process, the total scanning depth was about 19.5 \( \mu \)m with an increment of 0.65 \( \mu \)m; while the field of view was 232 \( \mu \)m \( \times \) 186 \( \mu \)m. About 1/4 of the cross section of the channel was investigated. Two obtained image pairs that corresponding to figure 5 are exhibited in figure 8 in which the scanned raw images were focused on the middle depth.
After extracting the particle information from image pairs, spatial maps of particle distribution could be established as shown in figure 9. Where two neighboured instantaneous distributions was put together: red dots denote particles from the first image and yellow dots for the second image. To enhance discriminability, particle concentration should be controlled to avoid apparently reciprocated interference. The probability of successful pairing was estimated < 60%, indicating that considerable particles were unavailable or out of pairing in the post-processing. Increasing the probability of successful pairing is an essential work in the improvement.
PTV and CCC algorithm were both applied in this study to analyse 3D velocity field. The result analysed by PTV is shown in figure 10; (a) and (b) demonstrate different perspective views of the 3D flow filed, respectively. The average velocity was estimated to be 9.18 µm/s. The velocity vectors are slightly fluctuated that might be attributed to Brownian motion in such a low Re condition.

![Fig. 10. Velocity vector distributions derived by PTV method. (a) and (b) are observed from different perspectives.](image)

In CCC calculation, interrogation cell was slightly extended (256 pixel × 256 pixel × 2.6 µm, 50% overlap) to ensure sufficient particles. The results are shown in figure 11 and 12. The streamwise velocity profiles consist with Low-Re laminar flow and all the three velocity components agree with simulations. The velocity around the central region of the channel is 12.2 µm/s and the estimated average velocity agreed well with PTV result. Figure 12 shows the 2D streamwise velocity contour, mapped from the data in figure 11, clearly implying that flows of extreme low Re also feature similar flow profiles as that in macro scale. Our previous effort (Chuang et al., 2002) has shown the same experimental evidence. Those evidences proved that the 3D information techniques for image capture, analysis and display can provide a complete solution for all kinds of unknown flow fields.

![Fig. 11. Velocity profiles with respect to X and Z axes. ? and ? in both figures denote streamwise velocities (V) at three locations from the wall. The other symbols near zero velocity are for lateral velocities U and W.](image)

### 3.2 Auxiliary 3D Display

To enhance the visualized display of analyzed velocity field, the system also equipped with a brilliant design by using “Chroma 3D” (Richard, 1991) technique to impress the observers with immediately comprehension of the entire flow field. The design comprises 3D image rotation and chromatic visualization. The chromatic visualization technique adopts the effect that different wavelengths result in different refractive angles. By encoding the velocity vectors obtained by CCD algorithm with a chromatic scale depending on their locations in space, a planar picture could
represent the scene of 3D configuration. Operationally, colors of long wavelength are sensed near the eyes when wearing chroma eyeglasses, so that the flow vectors near the observers were drawn red; contrariwise, distant velocity vectors were drawn colors of short wavelength based on predefined chromatic scale.

For the case in this experiment, the measured flow pattern in a straight microchannel was vectorized and colored in figure 13. Wearing a pair of diffraction eyeglasses, a 3D stereoscopic flow field can be viewed by the observers. Furthermore, by rotating the diagram in different perspectives, observers can obtain the entire and clear outlook of the flow situation. This additional function is also helpful to investigators to create rapid and complete flow configuration in microfluidics design.

![Figure 13. Color-encoded velocity vectors of the flow in the microchannel. The near-wall velocity in this figure is colored red.](image)

### 3.3 Error Sources

Though the system was evaluated of its feasibility by many measurements that showed potential practicability of its design, system improvements are required to further enhance measurement accuracy. The sources that influence the measurement accuracy and the uncertainty include:

1. The focal elongation induced from magnification of macro lens set obscured the exactly discrimination of particle location along the z-axis. According to the past estimation, the uncertainty could be as large as 10%.

2. Slight deviations existed between the reconstructed images when being diffracted by Ref.1 and Ref.2. Therefore, the
distance mismatch should be removed before calculations.

(3) The holograms were so sensitive to environment that the slight fluctuation of the rotation stage had contributed to a slight deviation in reconstructed images. It concluded that strictly control of measurement environment should be enhanced.

(4) The discernibility of particles were decreased because the forward scattering light resulted in background noise. Contrast improvement is thus essential. Eliminating the background noise by using a high-pass filter was under study to optimize contrast.

4. CONCLUSIONS

In this paper, a design based on hybrid multiplexing of holography technique was proposed for 3D3C microfluidic measurement. Derived from HPIV, the approach consisted of holographic multiplexing, a new recording medium, microscopy and a CCC algorithm. In the hologram recording process, the required extra-short time interval between two successive images was determined by a non-mechanical optical diverter; while the time interval between two image pairs was determined by mechanically rotating the recording medium. By adopting CROP photopolymer, the system could capture and store several tens of image pairs simultaneous, and exposure time down to 5 ms was available in recording hologram. However, measurement environment and lens system should be improved to enhance the discriminability of particles.

A straight microchannel was investigated of its inner flow that DI water seeded with 1-µm particles was driven by pressure difference. The reconstructed images were analyzed by both PTV and CCC algorithm respectively to obtain 3D velocity profiles. PTV analysis resulted in average velocity of 9.18 µm/s and the probability of particle pairing was < 60%, which was attributed to the low contrast and significant background noise. CCC algorithm resulted in the same average velocity as PTV analysis, and a mean velocity of 12.2 µm/s at the central region of rectangular microchannel. In addition, this paper showed a concise approach to visualize the colored 3D flow vectors.

Briefly, transient 3D velocity diagram could be derived time-independently by this holography-based approach, and the results could be viewed by using a chromatic approach. Though some influential factors on experimental operation should be further considered to improve the discrimination and thus the accuracy and uncertainty, the proposed design has showed its applicability to microfluidics in the near future.

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