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PARTICLE IMAGE IDENTIFICATION, CLASSIFICATION AND CORRECTION
TECHNIQUES IN DIGITAL HOLOGRAPHIC MICROSCOPY

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ABSTRACT

This thesis aimed to investigate digital methods for identification, classification, and correction of particle images using digital holographic microscopy. The work begins with a micro-scale flow experiment employing a digital off-axis holographic microscope. To overcome noise from out-of-focus particle images due to the increase in the particle concentration, a new particle image identification algorithm with three-dimensional reconstruction was introduced. It shows that individual particle images could be identified at about 62% of the expected number of particles. However, a cylindrical micro-channel used in the experiment was found as the main source of astigmatism. Following this, an automatic image classification using neural network was proposed to classify reliable and astigmatic particle images. A feed-forward backpropagation neural network with two class classifier was trained, achieving overall accuracy of 99.8%. Next, an original method of aberration correction using *a priori* information and digital wavefront aberration processing was applied. Astigmatism introduced by the micro-channel was modelled according to quadratic phase function and optimized using peak detection algorithm. The results show that astigmatism in the detected particle images was effectively compensated. A variant digital off-axis holographic microscope was later developed for large-scale flow measurement, allowing for the first time both amplitude and phase of the holographically reconstructed images to be registered simultaneously. The aberration introduced by a tilt in the optical hologram during the reconstruction was effectively corrected using adaptive optics. In overall, the work discussed in the thesis has proven effective to overcome noise and aberration. The main implication is that the holographic techniques can be successfully employed in complex three-dimensional fluid flow measurements.





TEKNIK MENGENALPASTI, MENGLASIFIKASI DAN MEMPERBAIKI IMEJ ZARAH DALAM MIKROSKOP HOLOGRAFI DIGITAL

ABSTRAK

Kajian ini bertujuan untuk menyiasat pelbagai teknik digital menggunakan mikroskop holografi untuk proses mengenalpasti, mengklasifikasi, dan membaiki imej zarah-zarah bagi mengatasi masalah hingar dan aberasi. Kajian ini bermula dengan menjalankan eksperimen berskala kecil menggunakan mikroskop holografi digital. Untuk mengatasi masalah hingar yang berpunca oleh imej zarah-zarah yang tidak fokus, satu teknik pengenalpastian imej telah disyorkan. Hasilnya, sebanyak 62% imej zarah-zarah berjaya dikenal pasti. Eksperimen ini mendapati penggunaan kolong saluran mikro merupakan punca utama astigmatisma. Klasifikasi imej secara automatik menggunakan rangkaian neural disyorkan untuk mengklasifikasi imej zarah-zarah yang bagus dan yang dipengaruhi oleh astigmatisma. Klasifikasi imej dua kelas berdasarkan rangkaian neural feed-forward backpropagation telah dilatih dan hasilnya mampu untuk mengenalpasti imej pada kadar ketepatan 99.8%. Satu kaedah telah dihasilkan untuk mengatasi masalah astigmatisma dengan menggunakan pemprosesan aberasi digital dan informasi priori. Astigmatisma yang wujud akibat penggunaan kolong saluran mikro telah dimodelkan berdasarkan fungsi fasa kuadratik dan dioptimumkan menerusi algoritma pengesanan puncak. Astigmatisma yang terkandung dalam imej zarah-zarah berjaya diperbetulkan. Sebuah mikroskop holografi digital yang tidak berpaksi telah dibina untuk eksperimen berskala besar. Untuk pertama kalinya, kedua-dua amplitud dan fasa yang terhasil daripada proses rekonstruksi imej secara holografi berjaya direkodkan serentak. Imej zarah-zarah yang direkodkan menggunakan filem hologram didapati dipengaruhi oleh aberasi yang berpunca akibat kedudukan hologram yang serong. Aberasi tersebut berjaya diperbetulkan menggunakan optikal adaptasi. Secara keseluruhannya, teknik-teknik digital yang telah disyorkan di dalam tesis ini telah terbukti efektif untuk mengatasi masalah hingar dan aberasi. Implikasinya, teknik-teknik holografi ini mampu menjayakan pengukuran bendalir kompleks yang bersifat tiga dimensi.





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NOMENCLATURE

A	amplitude
a_{0-5}	scalar constants of a polynomial fit
D	optical density
DoF	depth-of-focus
DHPIV	digital holographic particle image velocimetry
DHM	digital holographic microscopy
d	diameter of a diffraction-limited particle
d_f	distance between two consecutive interference fringes
E	energy of a reconstructed particle image
f	focal point
$g(r_i)$	separation distance of particle pairs
HPIV	holographic particle image velocimetry
$I(x,y)$	intensity of interference pattern at the recording plane (x,y)
I_{ccd}	interference recorded on the CCD plane
I_o	intensity of a focussed particle image
k	spatial frequency (k_x, k_y, k_z)
L	depth volume
MSE	mean squared error
N	total number of particles
N_i	number of particle pairs separated by a distance ($r_i + dr$)
N_p	total number of correlation pairs
NA	numerical aperture
NIST	National Institute of Standards and Technology



NN	neural network
n	refractive index
n_s	particle density
O	complex amplitude of the scattered field
P	scattering power of a diffraction-limited particle
R	complex amplitude of the reference field
\mathbf{r}	3D position vector (r_x, r_y, r_z)
r_i	inter-particle distance
SNR	signal-to-noise-ratio
s_d	shadow density
T	exposure time
t	time
\mathbf{t}	amplitude transmittance of a hologram
t_0	constant background transmittance
$U_o(\mathbf{r})$	3D reconstruction of a complex amplitude
V	total volume of a system
(x,y)	recording plane
(x_i, y_i, z_i)	coordinate of a virtual image
(x_o, y_o, z_o)	coordinate of a real image
(x_R, y_R, z_R)	coordinate of a reference field
α	$\sin \alpha$ is the numerical aperture of a focussed image
β	slope of t versus E of a photographic material
γ	film gamma
λ	wavelength
Ω	angular aperture
σ	standard deviation of an intensity due to background noise
η	$\sin \eta$ is the numerical aperture of a converging beam



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θ	angle of incidence
θ_{wedge}	wedge angle
θ_{ccd}	intersecting angle between the object and the reference fields on the CCD plane
Δx	pixel size
ΔV_i	volume of a discrete shell located at r_i



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CHAPTER 1

INTRODUCTION



Swirls, tornados and cyclones are interesting examples of turbulent flows that exist in nature. In industry, turbulence plays some important roles to increase rate of mixing and heat transfer (Pope, 2000) that in turn depend on the formation of vortices (eddies) which appear on many different length scales. On the other hand, blood flow inside the human body is by nature pulsatile and laminar, ensuring consistent delivery of vital nutrients, oxygen and antibodies throughout the system (Lima, Ishikawa, Imai, and Yamaguchi, 2012). However, the blood flow becomes turbulent when blood vessels are abnormally constricted. In general, turbulent flows are highly complex and inherently three-dimensional. In order to understand and characterise the physics which underpin various turbulent phenomena, it is important to resolve both large and



small scale vortices (Choi and Lee, 2009; Glezer and Coles, 1990; Liu, Dey, Boss, Marquet, and Javidi, 2011; Von Ellenrieder, Kostas, and Soria, 2001; Westerweel, Elsinga, and Adrian, 2013; Zhang, Tao, and Katz, 1997).

There are several flow measurement techniques commonly used and commercially available. These include hot wire anemometry (HWA), laser Doppler anemometry (LDA), particle image velocimetry (PIV), and holographic particle image velocimetry (HPIV). Both hot wire anemometry and laser Doppler anemometry are established point measurement technique where the latter is more favorable since it is a non-intrusive optical measuring technique. Meanwhile, particle image velocimetry in its original form is limited to in-plane two-dimensional two-component (2D-2C) displacement and velocity measurements. Although it is possible to extract the out-of-plane component through stereoscopic viewing (2D-3C), the useful measurement volume remains limited to a light sheet of several millimeters thick. On the other hand, integration of holography in the particle image velocimetry technique has finally realised whole-field, three-dimensional three-component (3D-3C) displacement and velocity measurements (Hinsch, 2002). In brief, holography was invented by Gabor to correct for the spherical aberration of the electron lenses by means of two-step imaging process: (a) recording and (b) reconstruction steps (Gabor, 1948; Gabor, 1949).

Holographic particle image velocimetry is a promising technique to probe and characterize complex flow dynamics (Hinsch, 2002; Katz and Sheng, 2010). It simply records the coherent light scattered by small seeding particles (several micrometres in diameter) that are assumed to faithfully follow the flow and uses it to reconstruct the event afterward. A hologram records objects in the form of complex interference fringes and contains useful amplitude and phase information that provide the means to make three-dimensional three-component measurements. Displacement (or velocity) of particle images can be extracted by focusing the images using a travelling video microscope. Due to cumbersome chemical processing associated with holographic films as recording medium, attention of the fluid community has shifted to using CCD sensors. This offers enormous advantages since digital hologram of particle images can be analysed numerically. As a result, 3D flow measurement technique using holography eventually has emerged in the form of digital HPIV (DHPIV) and micro-digital HPIV (μ DHPIV) techniques.

1.2 Problem Statement

Characterization of both large and small scale vortices is directly related to spatial resolution which in turn depends on concentration of the seeding particles. Although it is desirable to seed the flow under investigation with a relatively high particle concentration for more precise characterization, this consequently results in the



increase of noise contributed by out-of-focus particle images. This type of noise increases with the increase of particle concentration (Katz and Sheng, 2010; Royer, 1974; Sheng, Malkiel, and Katz, 2006), causing problems especially in (a) differentiating between noise and particle image, and (b) unintentionally masking neighbouring particles that lie above and below, in which the mask is applied to avoid detecting the same particle twice. It is noted that this particular noise remains exist even though the employed optical imaging system is ideally free from other physical noise.



Another dominant problem that exists in these three aforementioned measurement techniques (HPIV, DHPIV and μ DHPIV) is aberrations. Aberration is detrimental as it causes reconstructed particle images to appear in the shape of complex 3D morphology, making identification of particle images difficult and inaccurate determination of particle centroid. Aberration also severely restricts the maximum number of particle images that can be extracted. Sources of aberrations include: (a) experimental apparatus (Verrier, Coëtmellec, Brunel, and Lebrun, 2008; Verrier, Remacha, Brunel, Lebrun, and Coëtmellec, 2010), (b) optical misalignment (Cho, Kim, Yu, Shin, and Jung, 2009; Colomb, Montfort, Kühn, Aspert, Cuche et al., 2006; Zhang et al., 1997), and (c) non-uniform shrinkage of a holographic film (Barnhart, Adrian, and Papen, 1994). For example, often cylindrical channels are utilised in the laboratory setup to simulate actual flow down to the minutest details, however, this causes aberration (for example, astigmatism) in the particle images. In large-scale flow, use of corrective optics was found useful to correct for the distortion





introduced by a thick cylindrical window (Alcock, Garner, Halliwell, and Coupland, 2004). Nonetheless, this approach is not foreseen as a practical step in a micro-scale flow.

In comparison to digital techniques (DHPIV and μ DHPIV), conventional HPIV recording using holographic films is often subject to aberrations introduced in the reconstruction step. Correction of the aberrations is usually done optically and laboriously time consuming (Hinsch, 2002; Katz and Sheng, 2010), whereby the resulting intensity image is continuously recorded and checked using the travelling video microscope. In this process, the phase information of the reconstructed particle image is completely loss and this is considered as the main drawback. To the best of author's knowledge, none of the reported holographic reconstruction methods in the literature has managed to record both amplitude and phase simultaneously, limiting the possibility to perform digital aberration correction.

In order to extract as greater number of particle images as possible thereby offering increased spatial resolution, care must be taken to consider the effects of noise and aberration. This thesis deals with the techniques to maximize detection of particle images, automate pattern recognition for image classification and correction of the aberrations in the particle images recorded either using CCD sensors or/and holographic films.

