Digital holographic systems for particulate processes

Mohammad Nurur Rahman

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DIGITAL HOLOGRAPHIC SYSTEMS FOR PARTICULATE PROCESSES

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List of Symbols

A	Amplitude
d	Reconstruction distance
D	Particle diameter
E	Complex amplitude
$I_H(x,y)$	Optical hologram
$I_H(k,l)$	Digital hologram
$U(\xi,\eta)$	Reconstructed wave
w	Angular frequency
Φ	Spatial phase
Φ_e	Detection efficiency
λ	Wavelength
Θ	Orientation along the optical axis
L_P	Projected length of a fiber
L_R	Real length of a fiber

Abbreviations and Acronyms

2-D	Two dimensional
3-D	Three dimensional
ALD	Axis length distribution
API	Active pharmaceutical ingredient
CCD	Charge-coupled device
CLD	Chord length distribution
CMOS	Complementary metal oxide semiconductor
DH	Digital holography
DHPV	Digital holographic particle velocimetry
DIH	Digital inline holography
DLS	Dynamic light scattering
DOF	Depth of field
ESZ	Electrical sensing zone
FBRM	Focused beam reflectance measurement
FFT2	Two dimensional fast Fourier Transform

IFFT2	Inverse two dimensional fast Fourier Transform
FPS	Frames per second
GPU	Graphics processing unit
ISPV	In situ particle viewer
IA	Image analysis
LD	Laser diffraction
MSZW	Metastable zone width
MO	Microscope objective
PAT	Process analytical technology
PIA	Process image analyzer
PIV	Particle image velocimetry
PSD	Particle size distribution
PVM	Particle vision and measurement
UAS	Ultrasonic attenuation spectroscopy

Publications Related to the Thesis

Journal Articles

[J1] T. Khanam, **M. Nurur Rahman**, A. Rajendran, V. Kariwala, and A. K. Asundi, "Accurate size measurement of needle-shaped particles using digital holography," Chemical Engineering Science **66**, 2699-2706 (2011).

[J2] **M. N. Rahman**, A. Rajendran, V. Kariwala, and A. K. Asundi, "Effect of particle concentration and turbidity on particle characterization using digital holography," Chemical Engineering Research and Design **92**, 249-255 (2014).

[J3] **M. N. Rahman**, K. Lee, and A. K. Asundi, "Mixed morphological particulate system characterization using digital holography," Applied Optics, Submitted (2013).

Conference Presentations

1. T. Khanam, **M. N. Rahman**, A. Rajendran, V. Kariwala*, and A. K. Asundi. Direct Measurement of Length and Orientation of Microfibers in Solution. Presented at AIChE Annual Meeting, Salt lake City, 2010.
2. T. Khanam*, **M. N. Rahman**, A. Rajendran, V. Kariwala, and A. K. Asundi. Direct measurement of length and orientation of microfibers in solution. Presented at Crystal Growth of Organic Material 9, Singapore, August 2010. (poster)

Abstract

Digital holography (DH) is an established three dimensional (3-D) imaging tool to measure particle size and shape. In this thesis, the effect of particle concentration and turbidity is studied on the efficiency of digital inline holography (DIH) used for particle characterization. This study is important to assess the performance of the technique under practical situations where a wide range of particle concentrations may be employed, and very often the solution in which the particles are present is turbid. A theoretical relationship is also deduced here between the particle size and the digital image sensor resolution, both for collimated and diverging illumination, considering DIH system. This relationship provides information about how small particle size can be measured with a given optical and digital system before using any magnifying optics. From the algorithm development side, two computer programs are also developed for more complicated particulate systems unlike spherical, such as needles and mixed morphologies. Single crystal study and its results are also described in this thesis.

To check the effect of particle concentration on DH for particle characterization, two sample depths are analyzed. It is demonstrated that measurement performance can be significantly improved by reducing the sample depth. The results are analyzed based on the two metrics, i.e., detection efficiency and mean size of the particle population. Considering 50% detection efficiency as a threshold, it is concluded that DH works well up to 0.1 v/v% and 0.2 v/v% particle concentrations for 10 mm and 5 mm sample depths, respectively. From the turbidity tests, it is found that DH works well up to 150 nephelometric turbidity units (NTU) turbidity level for 10 mm sample depth. From the theoretical derivation, the required digital image sensor resolution (pixel dimension) can be recommended for resolving particular particle size. Finally, the two developed algorithms have been applied in the real particulate systems and the results quite match well with the known values measured with another independent technology. Single crystal study's results will direct this tool in the pharmaceutical industries.

Chapter 1

Introduction

1.1 Background and Motivation

A narrow range of particle size is required for many industrial applications, since the regularity in particle size and shape possesses good storage and transportation properties and a free-flowing nature [1-2]. Not only the physical properties of the crystal such as solubility, dissolution, and bioavailability but also downstream processing steps such as filtration, drying, transport, and storage are greatly affected by the crystal size distribution (CSD) [3]. A good automatic control scheme is necessary with the crystallization processes to meet the desired qualities in crystal size, shape, composition, and internal structure via online monitoring of the CSD [4]. Off-line CSD analysis, as for example, sieving, microscopy, and sedimentation are not suitable for online controlling of the particulate processes, since real-time measurement is not possible from these techniques [2]. Recently, some in-process characterization techniques have been come into focus to facilitate process monitoring for different applications [5]. Among them, laser diffraction (LD), ultrasonic attenuation spectroscopy (UAS), dynamic light scattering (DLS), and electrical sensing zone (ESZ) only provide one dimensional information of a particle (i.e., volume based equivalent diameter) [6]. Now a days, mostly used characterization technique, as a process analytical technology (PAT) tool, is based on the back scattering of light, which is called focused beam reflectance measurement (FBRM) [7-8]. FBRM measurements are not easy to interpret, since chord length distribution (CLD) is obtained instead of particle size distribution (PSD) from the measurement. Usually, moments of CLD are empirically correlated with the moments of PSD [9]. Only for the spheres and octahedra, successful application for converting CLD to PSD is reported [10]. Yet, it provides statistically stronger result than any other characterization technique, since laser rotational velocity is high [11]. Using FBRM, a variety of applications had been carried out and reported in the literature, including crystallization monitoring [9], polymorphic transformation [12], characterization of the metastable zone width (MSZW), and determination of the solubility curve [7].

Comparing to the traditional particle characterization techniques, photographic imaging provides great advantage from application point of view extracting both the size and shape distribution of the particulate process. For imaging technique, optical microscopy is the conventional approach to obtain particle size and shape as well. Real sizes of the particles are obtained from the microscope. Microscopy is not suitable for monitoring the process, since it is an off-line approach. As a result, some imaging instrumentations have already been developed to characterize the particulate processes such as the particle vision and measurement (PVM) of Lasentec and Mettler Toledo Ltd., the process image analyzer (PIA) of MessTechnik Schwartz GmbH, in situ particle viewer (ISPV) of Perdix, and the on-line microscopy systems of GlaxoSmithKline [13-17]. With these tools, researchers have been trying to make image analysis (IA) algorithm for in-process particle characterization. It is very tedious work to do, since particle images in the suspension are quite noisy [18]. Image analysis algorithms are found out based on multi-scale segmentation approach [19], model based object recognition [15, 20], and multivariate statistical technique [10]. Though there are some image analysis algorithms for the particulate system characterization at the present time, their efficacy dramatically drops down at high particulate concentration. Moreover, it is valid to think that the ability of photographic imaging to handle high solid concentration is low as each particle image should be revealed separately on the single image plane. Normal photographic imaging technique always provides the two-dimensional (2-D) projection of a real 3-D object, suffers limited depth of field (DOF) due to the required magnification, and hence requires refocusing along the object specimen by mechanical means [14]. Moreover, one must be care about inferring 3-D information from the 2-D photographic image [21].

On contrary to the 2-D photographic imaging technique, holography provides access to exploit the full capability of 3-D imaging and the real size of an object can be obtained through it [22-25]. But digital holography (DH) prefers to optical holography, since complex and cumbersome wet chemical processing after recording is completely eliminated [22-24]. Moreover, phase contrast imaging is only possible through DH [26]. Due to the extended DOF, DH is now gaining as an emerging and promising characterization tool in many scientific and engineering fields. DH has been successfully applied in the numerous fields such as micro-structure testing [27],

investigation of red blood cell [28], in spray analysis [29], microscopy [30-32], bubble characterization [33], particle characterization [34-37], particle image velocimetry (PIV) [38], and biological applications [39-40]. In spite of these advantages, DH has inferior qualities in terms of pixel resolution and image dimension [25]. Therefore, angular aperture of DH is much smaller than that of film-based holography. And hence, information capacity or particle concentration of DH is much lower. Particle detection procedure is sever adversely affected with high concentration and DH due to the presence of speckle noises in the hologram recording and the reconstruction steps [43]. Today's CCD has sufficient frame rate (frames per second, FPS) which can capture scenes at high temporal resolution. The noises of electronic sensors can be suppressed by digital image processing algorithms.

Since particle size and shape distribution measurement methods using DH are established in literatures [37, 41-42], this thesis has been addressed to find out the practical limitations and applications of the digital inline holographic (DIH) system on particle characterization concerning particle concentration and turbidity effects [43], and to develop some computer algorithms to estimate particle size and shape distribution in population unlike spherical, especially for more complicated particulate systems such as needles and mixed morphologies [44-45]. Single crystal study is also performed. Further, a theoretical relationship is deduced here between the particle size and the digital image sensor resolution (pixel), both for collimated and diverging illumination, considering DIH system.

1.2 Objectives and Scopes of the Study

In DH, depth resolution is poor due to the larger pixel size of the digital image sensor compared with the microscopic interference fringes [28]. This problem is also exacerbated by the highly loaded particle field. A few studies in the literature have studied the effect of particle concentration on holographic measurements. Malek et al. [46] showed how the percentage of extracted particles is affected by the shadow density and the particle sample depth. Kim and Lee [47] demonstrated the effect of particle number density in inline digital holographic particle velocimetry (DHPV) using 40 μm particles. They found that 13–17 particles per mm^3 as optimal particle number density for their DHPV system. Most studies have considered the effect of particle concentration on the ability to localize particles in a three dimensional volume.

However the effect of particle concentration on the size measurement has not been evaluated. This study is important to assess the performance of the technique under practical situations where a wide range of particle concentrations may be employed, and very often the solution in which the particles are present is turbid. In this study, we analyze the effect of particle concentration and turbidity on the number of identified particles as well as particles sizes. We also consider the effect of depth of field on the performance of DH by analyzing two sample depths for different particle concentrations.

Particle size measurement is limited by the spatial resolution of the digital image sensor, and hence microscopic objective (MO) lens should be used in the digital holographic setup to characterize near micron-range particulate system [35, 48]. For this, it is necessary to know how small particle size can be measured with a given optical and digital system before using any magnifying optics. A theoretical relationship is deduced here between the particle size and the digital image sensor resolution, both for collimated and diverging illumination, considering DIH system. From this derivation, the required digital sensor resolution (pixel dimension) can be recommended for resolving particular particle size.

In the past years, several researchers have tested various online particle characterization tools to characterize non-spherical particles [10, 14, 49-52]. One of the important and most available tools is the FBRM. This tool measures the CLD instead of the PSD of particulate process. Converting the CLD to PSD requires a non-trivial inversion algorithm (i.e., particle shape dependent) [5, 51, 53-54]. Another in situ tool, based on direct imaging, is the particle vision measurement (PVM). PVM is more effective tool for measurement of the size and shape of non-spherical particles [4, 55-56]. Although it is a powerful tool for size and shape extraction, 2-D imaging systems suffer from the out-of-focus issues due to the limited depth of field (DOF) imposed by the required magnification [4, 14]. Further, 2-D imaging systems provide projections of the particles on the 2D image plane. Extracting the real size of needle-shaped particles from the measured projected lengths requires information about the angle of orientation along the optical axis. This information is difficult to obtain for particles oriented randomly in 3D volume [56]. A novel and efficient algorithm, based on digital inline holography (DIH) to measure size, position and orientation of a population of microfibers or needles in a 3-D volume, has been developed [44]. The

image analysis method is simpler and computationally less intensive compared to our past method [42]. The efficacy of this method is compared with the previous ones [37, 42] using a single fiber of known length and tilt. Further, the new method has been demonstrated to a population of microfibers in an aqueous suspension and the measurements are validated with the real PSD of the same microfiber population measured by the microscopy.

In literature, most of the algorithms are designed for the spherical particle field characterization using digital holography [25, 31-32, 37, 41, 48, 57-60], and a few algorithms demonstrate capabilities to measure the sizes and shapes of needle shaped particles [42, 44, 61-62]. However, there is no such algorithm for characterization of both spherical and needle shaped particles simultaneously, or mixed morphological particulate system. The algorithm which is suitable for mixed morphological particulate system characterization is especially important in the manufacturing, monitoring, and control of active pharmaceutical ingredients (APIs) in the pharmaceutical industries [12, 63-66]. In this case, polymorphism is a common phenomenon. Polymorphs are the substances, which are similar chemically but different in physical forms. (L)-glutamic acid is one of the examples, which shows two polymorphs: the prismatic alpha and the needle shaped beta form [63]. Another chemical is sodium chlorate (NaClO_3) which has two polymorphs i.e., cubic and tetrahedral shapes [21]. As a result, they show distinct characteristics with respect to each other in terms of physical properties such as solubility, dissolution, bioavailability and so on [4]. Product quality assurance is always of utmost importance in the industries. Therefore, there is a strong requirement to develop an algorithm for characterization, which will be suitable for particles having different shapes in the special cases, for better design, monitoring, and control of the particulate processes. In this work, an image analysis algorithm, based on DIH, is developed to characterize sphere and needle populations simultaneously (i.e., diameter distribution for sphere population and real length distribution for needle population). The algorithm combining two distinct computer programs is employed to quantify two populations of the mixed morphological particulate system [37, 44]. In addition, particle classification and separation are carried out determining a geometric feature (i.e., eccentricity) for each particle image within the reconstruction volume. The method has also been demonstrated considering three different ratios of the spherical particle and needle

populations. In all cases, the eccentricity distributions and axis length distributions (ALDs) show clear separation (i.e., two regions completely perceived) between the two populations. In addition to the particles separation, particle size distributions (PSDs) (diameter distribution for spherical particle population and real length distribution for needle population) match very well with the expected values [37, 44]. This algorithm can successfully be used to track and quantify the different polymorphs for APIs in the crystallization processes, especially for two extreme shapes of the particulate system (e.g., spheres or cuboids and needles). Single crystal study is also performed which will direct this tool in the pharmaceuticals. It is noted that traditional approaches for the classification of different polymorphs are the spectroscopic techniques such as Raman spectroscopy but their results provide only the types of polymorphs and concentrations [4]. These tools do not provide size and shape information of particles which are important characteristics of any particulate system.

1.3 Contributions Obtained from the Thesis

The effect of particle concentration and turbidity on the number of identified particles and the particle mean size measured using inline transmission based digital holography (DH) is investigated [43]. It is demonstrated that measurement performance can be significantly improved by reducing the sample depth though lower concentration loading is preferred in DH. The results presented in this thesis provide information regarding the practical applications and limitations of the DH system for particle characterization.

A theoretical relationship is deduced here between the particle size and the digital image sensor resolution, both for collimated and diverging illumination, considering DIH system. From this derivation, the required digital image sensor resolution (pixel dimension) can be recommended for resolving particular particle size. This relationship indicates how small particle size can be measured with a given optical and digital system before using any magnifying optics.

A novel and efficient algorithm, the ‘Superimposition Method’, for the measurement of length, position, and off-axis tilt of needle-like particles, has been developed without any prior assumption [44]. The method is demonstrated using fiber of known length and orientation. The method has also been applied to an aqueous suspension of fibers in population to measure the real length distribution and their angles of orientation along the optical axis. The obtained real length distribution shows good match with that obtained using the microscopy.

An algorithm, based on digital inline holography, is developed to characterize mixed morphological (here for spheres and needles) particulate system. This particular algorithm combines other two published algorithms of which one is suitable for spherical particulate system and another for needles. In addition, particle classification and separation is carried out determining a geometric feature (i.e., eccentricity) for each particle image within the reconstruction volume. Then, the algorithm has been demonstrated in real mixed morphological particulate system with varying proportions of the spherical particle population to needle population. The algorithm successfully works for the different proportions of needles to spheres. The result shows this algorithm can be successfully used to track and quantify the different polymorphs for APIs in the crystallization processes, especially for two extreme shapes of the

particulate system (e.g., spheres or cuboids and needles). Obvious limitation is that overall particle concentration should be low in order to get better accuracy. Single crystal study is also performed.

1.4 Organization of the Thesis

The thesis is structured as follows: the digital holographic principle and its implementation methods to micro-objects analysis are summarized in Chapter 2 followed by the works conducted throughout this research study in the subsequent chapters. In Chapter 2, a theoretical relationship is also deduced between the particle size and the digital image sensor resolution, both for collimated and diverging illumination, considering DIH system. This relationship indicates how small particle size can be measured with a given optical and digital system before using any magnifying optics.

In Chapter 3, the effect of particle concentration and turbidity on the number of identified particles and the particle mean size measured using inline transmission based digital holography (DH) is described. The method used for this objective and achieved results, discussions, and conclusions are presented in a simple and nice manner. The results presented in this chapter provide information regarding the practical applications and limitations of the DH system for particle characterization.

In Chapter 4, a novel and efficient algorithm, ‘The Superimposition Method’, for the measurement of length, position, and off-axis tilt of needle-like particles, has been described without any prior assumption. The method is demonstrated using fiber of known length and orientation. The method has also been applied to an aqueous suspension of fibers to measure the real length distribution and their angles of orientation along the optical axis. The obtained real length distribution shows good match with that obtained using the microscopy.

In Chapter 5, an algorithm, based on DIH, is described to characterize mixed morphological (here for spheres and needles) particulate system. This particular algorithm combines other two published algorithms of which one is suitable for spherical particulate system and another for needles. In addition, particle classification and separation is carried out determining a geometric feature (i.e., eccentricity) for each particle image within the reconstruction volume. Then, the algorithm has been

demonstrated in real mixed morphological particulate system with varying proportions of the spherical particle population to needle population. The algorithm successfully works for the different proportions of needles to spheres. The result shows this algorithm can be successfully used to track and quantify the different polymorphs for APIs in the crystallization processes, especially for two extreme shapes of the particulate system (e.g., spheres or cuboids and needles). Single crystal study and its results are also analyzed.

At last, in Chapter 6, the conclusions of these studies mentioned above and some new directions for future research are discussed.

Chapter 2

Digital Holography and Its Applications to Micro-objects

2.1 Introduction

In this chapter, the difference between photographic (2-D) and holographic (3-D) imaging is described first. Then, digital holographic principle, its implementation methods and applications are described precisely and briefly, for micro-objects analysis. As a thesis work, a theoretical relationship is deduced here between the particle size and the digital image sensor resolution, both for collimated and diverging illumination, considering DIH system. From this derivation, the required digital sensor resolution (pixel dimension) can be recommended for resolving particular particle size.

2.2 Difference between Photographic and Holographic Imaging

Light is an electromagnetic wave, which varies spatially and temporarily, is a small part of total electromagnetic radiation field ranges [67-70]. Only the electric field strength is considered in many scientific and technological applications. The simplest mathematical form of a light wave is,

$$E(z,t) = Ae^{-i(\omega t - \phi)} \dots\dots\dots(2.1)$$

Where, $E, A, \omega (= 2\pi f), \Phi$ denote the complex amplitude, amplitude, angular frequency, and spatial phase respectively.

Conventional photographic imaging techniques record only the time-averaged intensity of the incident light providing 2-D size and shape of an object. On the other hand, holography is an interesting development in modern optics, which includes two nonlinear optical phenomena i.e., interference recorded between reference and object beams followed by diffraction of the reconstruction beam with that hologram [71-73]. It refers a technique for three dimensional recording and reconstruction of images without any lens. For recording holograms, both reference and object beams must be coherent and laser is usually used for this purpose. The main advantage of holography is to record and reconstruct complex amplitude of the optical field rather than only amplitude in case of photography. Hence, it is possible to image a 3-D volume instead

of a 2-D plane. Table 2.1 provides some basic differences between photography and holography.

Table 2.1 Difference between photography and holography.

Features	Photography	Holography
Recorded Entity	Only intensity	Interference pattern
Wave Recording Accessibility	Object wave can never be completely restored	Object wave can be completely restored
Result	2-D image	3-D image

Traditional 2-D based photographic imaging technique suffers problem regarding limited DOF imposed by the required magnification [37]. Figure 2.1(a) shows an extreme case highlighting the limited DOF in case of normal photographic imaging technique, whereas one fiber is completely un-focused among the fibers in the image plane. It does not give information about the orientation of a particle along the optical axis. Therefore, real size measurement is not possible from the photographic imaging when particles are randomly oriented in 3-D volume. Moreover, particles segregated in the 3-D space might appear on the same image plane. Figure 2.1(b) shows aggregated particles on the single image plane. In this case, strong image analysis algorithm is required to understand real problem properly. Holographic tool can be used to face these challenges, since it has the access of extended DOF imaging capabilities.

Fig. 2.1 Limitations of photographic imaging technique. (a) out of focused particle on a photographic image plane; (b) aggregated particles on the image plane.

2.3 Digital Holographic Principle

Optical holography depends on the photographic film material (e.g., silver halide), whose spatial resolution is one order of magnitude higher than today's digital image sensors such as charge-coupled device (CCD) or complementary metal oxide semiconductor (CMOS) arrays [22]. Due to the high spatial resolution and large angular aperture of the optical hologram, measurement accuracy is high, though high level of technical intricacy is involved. After recording the hologram optically, the processing of the hologram by wet chemical procedures is quite complex and laborious. A breakthrough in this field occurred, when photographic film was replaced by digital image sensors, e.g. CCD. As a result, cumbersome wet chemical processing is completely eliminated and hologram can be captured in the digital format [24].

2.3.1 Digital Hologram Recording

Digital holography refers to the combination of two steps i.e., direct recording of holograms by a CCD or CMOS array and numerical reconstructions of these holograms [22, 24]. The digital hologram $I_H(k,l)$ of size $N \times N$ results from the two dimensional sampling of the optical hologram, $I_H(x,y)$, by the CCD camera [74] is given by,

$$I_H(k,l) = I_H(x,y) \text{rect}\left(\frac{x}{L}, \frac{y}{L}\right) \times \sum_{k=-\frac{N}{2}}^{\frac{N}{2}} \sum_{l=-\frac{N}{2}}^{\frac{N}{2}} \delta(x - k\Delta x, y - l\Delta y) \dots \dots \dots (2.2)$$

Where, k, l are integers, $L \times L$ is the area of CCD chip, and $\Delta x, \Delta y$ are the pixel size of the CCD.

2.3.2 Numerical Reconstruction by the Fresnel Approximation

If the same reference wave illuminates the hologram with an amplitude transmittance $I_H(x,y)$, the approximated Fresnel-Kirchhoff integral provides a complex amplitude, $U(\xi,\eta)$, in the real image plane [22-24],

$$U(\xi,\eta) = \frac{\exp(i2\pi d)}{i\lambda d} \exp\left[\frac{i\pi}{d\lambda}(\xi^2 + \eta^2)\right] \times \iint I_H(x,y) \exp\left[\frac{i\pi}{\lambda d}(x^2 + y^2)\right] \exp\left[\frac{-i2\pi}{\lambda d}(\xi x + \eta y)\right] dx dy \dots \dots \dots (2.3)$$

where, λ is the wavelength and d is the reconstruction distance. Intensity image of the reconstruction plane is expressed as,

$$I(\xi, \eta) = U(\xi, \eta) \times U^*(\xi, \eta) \dots \dots \dots (2.4)$$

Geometry of DIH is shown in Fig. 2.2.

Fig. 2.2 Geometry of DIH.

For numerical computation, the Fresnel-Kirchhoff integral can be represented in the spatial frequencies of ξ and η by Fourier Transformation of the function $I_H(x, y) \exp[\frac{i\pi(x^2 + y^2)}{\lambda d}]$. For rapid calculation, two-dimensional fast Fourier-Transformation can be introduced directly. MATLAB programming tools are used to compute the reconstruction process.

2.3.3 Numerical Reconstruction by the Convolution Approach

Here, the diffraction integral is a superposition integral in the convolution approach. Therefore, complex amplitude of the reconstruction can be calculated-

$$U = IFFT2\{FFT2\{I_H \cdot E_R\} \cdot FFT2\{g\}\} \dots \dots \dots (2.5)$$

where, I_H , E_R and g are the hologram, reference wavefront and the impulse response for free space propagation, respectively.

There is a basic difference between Fresnel approximation and the convolution approach. In the Fresnel approximation, reconstruction plane is calculated in the frequency domain where the pixel size of the reconstructed image varies with distance

and wavelength. In the convolution approach, reconstruction plane is calculated in the frequency domain followed by the spatial domain. As a consequence, the pixel size of the reconstructed image now is the same as hologram plane. For this, the convolution approach is useful for reconstructing inline holograms of the particle field since reconstructions at various depths are to be compared.

2.4 Implementation of Digital Holography for Micro-particles or Objects

In the digital particle holography, first step is to record the instantaneous volume of the particle field using a digital image sensor such as CCD or CMOS array. Though twin image effect is also retained in the reconstruction process, digital inline holography (DIH) is preferred for particle field measurement [75]. In the DIH setup, digital camera resolution requirement is less stringent for recording and full aperture of the camera plane can be used in the reconstruction process [76-77]. Secondly, numerical reconstruction is carried out to obtain the image of the particle field. There are two common approaches for numerical reconstruction of the digital hologram. One approach is the Fresnel approximation, which uses single Fourier Transform to calculate the diffraction integral in the reconstruction plane. The other approach considers diffraction formula as the convolution integral, which takes double or triple Fourier Transform. The main variation between two approaches is in the pixel dimension of the reconstructed image. The spatial resolution in the reconstructed image is dependent on the physical parameters used in the set-up (i.e., wavelength, and reconstruction distance) in case of Fresnel approximation. In case of convolution based reconstruction algorithm, the pixel size in the reconstruction plane does not depend on the reconstruction depth and no approximation is required [57, 78-79]. As a result, the convolution approach is advantageous for reconstructing inline holograms of the particle field, where reconstructions at different depths are to be compared, and is used in this work.

In digital particle holography, one of the crucial tasks is to identify the position of a particle within the reconstruction volume [41, 80]. Depth resolution is worse than the lateral resolution of the optical system and it adversely affects while detecting the depth position of a particle. There are various approaches in the literature to identify particle focal plane in the axial direction of the reconstruction volume. As for example, peak or dip value of the mean intensity of the particle image determines the axial focal

position of that particle [41, 47, 58]. Particle localization in the axial direction is also identified by the correlation coefficient method [81]. In this case, particle axial position is detected, whereas maximum correlation coefficient is obtained. A better method than the commonly known intensity method has also been developed using complex amplitude of the reconstructed wave for finding depth position (i.e., best focal plane) of a particle [82]. Obtaining the depth position of a particle, other objectives such as 3-D coordinate of the particle for velocimetry measurement or particle size and shape can be estimated by using image processing methods. Figure 2.3 presents the schematic which presents the step-wise implementation of DH for micro-objects analysis such as particles, cells, micro-organisms, bubbles etc. Due to the extended DOF, DH is now gaining as an emerging and promising characterization tool in many scientific and engineering fields. DH has been successfully applied in the numerous fields which have been mentioned in the introduction section for 3-D object detection and measurement.

Fig. 2.3 Schematic representation showing step-wise implementation of DH for micro-objects analysis.

2.5 The Relationship between Particle Size and Pixel Size of the Digital Image Sensor

Particle size measurement is limited by the spatial resolution of the digital image sensor and hence microscopic objective (MO) lens should be used in the digital holographic setup to characterize near micron-range particulate system [35, 48]. For this, it is necessary to know how small particle size can be measured with a given

optical and digital system before using any magnifying optics. A theoretical relationship is deduced here between the particle size and the digital image sensor resolution, both for collimated and diverging illumination, considering DIH system. From this derivation, the required digital image sensor resolution (pixel dimension) can be recommended for resolving particular particle size.

2.5.1 For Collimated Illumination

Hologram must be recorded with central maxima and at least three side bands of its diffraction pattern to obtain a good quality image of a particle in reconstruction. For this, the waves travel at a maximum angle [70],

$$\theta_{\max 1} = \frac{4\lambda}{D} \dots\dots\dots(2.6)$$

where D is the particle diameter, λ is the wavelength of light, and which can be visualized in the Fig. 2.4. Maximum spatial frequency resolved by a CCD camera between the two beams (i.e., reference beam and object beam) is given by the following eq. [22] where Δx is the pixel size of the CCD.

$$\theta_{\max 2} = \frac{\lambda}{2\Delta x} \dots\dots\dots(2.7)$$

Fig. 2.4 Diffraction pattern of a particle onto the CCD array.

The figure 2.5 shows the physical interpretation regarding spatial frequency requirement criterion by a digital image sensor.

Fig. 2.5 Maximum spatial frequency resolved by a CCD array.

If reference beam is plane, the two angles i.e., Θ_{max1} and Θ_{max2} are equal in-mathematics. So, we can easily conclude that how much least diameter of particle can be resolved by a particular resolution (pixel size) camera or which camera is required to resolve particular diameter of particle properly. Θ_{max1} and Θ_{max2} will be maximum when D and Δx are minimum respectively. Equating the past two equations, we have

$$D_{min} = 8\Delta x_{min} \dots\dots\dots(2.8)$$

Therefore, it can be concluded that to resolve a particular dimension of particle, pixel size of the CCD array should be 8 times smaller than the particle's size.

2.5.2 For Diverging Illumination

A particle illuminated with diverging wave is shown in Fig. 2.6. From this optical geometry, following relationships can be formulated.

$$\tan \theta_e = \frac{2\lambda}{r}$$

If θ_e is small, then $\tan \theta_e \approx \theta_e$

Hence, maximum spatial frequency resolved by the sensor between diverging wave and object wave is

$$\theta_f = \theta_p - \theta_e$$

$$\theta_p = \theta_f + \theta_e$$

Fig. 2.6 Particle with diverging illumination.

$$\theta_p = \theta_d = \frac{4\lambda}{D} \quad (\text{For maximum spatial frequency } D \text{ would be min.})$$

$$\theta_f = \frac{\lambda}{2(\Delta x)} \quad (\text{For maximum fringe frequency } (\Delta x) \text{ would be min.})$$

$$\frac{4\lambda}{D_{\min}} = \frac{\lambda}{2(\Delta x)_{\min}} + \frac{2\lambda}{r}$$

$$\frac{1}{D_{\min}} = \frac{r + 4(\Delta x)_{\min}}{8(\Delta x)_{\min} r}$$

$$D_{\min} = \frac{8(\Delta x)_{\min} r}{r + 4(\Delta x)_{\min}}$$

$$D_{\min} = 8(\Delta x)_{\min} \times \left(\frac{1}{1 + \frac{4(\Delta x)_{\min}}{r}} \right) \dots\dots\dots (2.9)$$

Here, we see denominator of the bracket term (coefficient) is larger than the numerator, so this coefficient is smaller than 1. This means for diverging waves, resolution requirement for digital image sensor is less stringent. If $(\Delta x)_{\min}/r$ is 0.05, then $D_{\min}$ would be

$$D_{\min} = 6.67(\Delta x)_{\min}$$

If r tends to infinite, the coefficient refers to 1. In that case, diverging waves would be converted as collimated waves. Resolution requirement would be exactly same as that for the plane waves.

2.6 Chapter Summary

This chapter explicitly describes the principle of holography and DH from the wave optics point of view. It also presents a methodology for micro-objects characterization (i.e., 3-D position and size-shape measurement) surveying state-of-the-art of knowledge of this technique from the literature. The methodology, presented here, can be used for micro-objects analysis and part of this is used throughout this research study. Therefore, this chapter provides the fundamental understanding of this field which is the basis for further development of the technique and its applications.

As a thesis work, a theoretical relationship is deduced here between the particle size and the digital image sensor resolution, both for collimated and diverging illumination, considering DIH system. From this derivation, the required digital sensor resolution (pixel dimension) can be recommended for resolving particular particle size. This relationship indicates how small particle size can be measured with a given optical and digital system before using any magnifying optics. This equation is particularly useful to decide digital sensor requirement for better reconstructed particle image in reconstruction if particle size range is known approximately.

Chapter 3

Effect of Particle Concentration and Turbidity on the Efficiency of Particle Detection and Reconstructed Particle Size in DIH*

3.1 Introduction

DH has been successfully used in microscopy, particle imaging, and particle image velocimetry (PIV) [31, 83-84]. Digital holographic methods have been used to measure the 2-D sizes and shapes of the population of spherical and needle-shaped particles [37, 59-60]. The potential of the technique has been extended to obtain 3-D size and shape information [42, 44].

In DH, depth resolution is poor due to the larger pixel size of the digital image sensor compared with the microscopic interference fringes. This problem is also exacerbated by the highly loaded particle field. A few studies in the literature have studied the effect of particle concentration on holographic measurements. Malek et al. [46] showed how the percentage of extracted particles is affected by the shadow density and the particle sample depth. Kim and Lee [47] demonstrated the effect of particle number density in inline digital holographic particle velocimetry (DHPV) using 40 μm particles. They found that 13–17 particles per mm^3 as optimal particle number density for their DHPV system. Most studies have considered the effect of particle concentration on the ability to localize particles in a three dimensional volume. However the effect of particle concentration on the size measurement has not been evaluated. This study is important to assess the performance of the technique under practical situations where a wide range of particle concentrations may be employed, and very often the solution in which the particles are present is turbid. In the present study, we analyze the effect of particle concentration and turbidity on the number of identified particles as well as particles sizes. We also consider the effect of depth of field on the performance of DH by analyzing two sample depths for different particle concentrations.

*M. N. Rahman, A. Rajendran, V. Kariwala, and A. K. Asundi, "Effect of particle concentration and turbidity on particle characterization using digital holography," *Chemical Engineering Research and Design* **92**, 249-255 (2014).

3.2 Experimental Setup, Procedure, and Image Analysis

3.2.1 Materials

National Institute of Standards and Technology (NIST) certified polymer microspheres, with diameter ($39.82 \pm 0.24 \mu\text{m}$), were purchased from Duke Scientific Corporation, USA. The particle density of the sphere is 1.05 gm/cm^3 . The particles are supplied as a suspension and the original sample concentration is approximately 0.7 v/v%. The study of turbidity was performed using consumable milk (Fraser and Neave Ltd., Singapore) as a proxy for a turbid solution. A turbidity meter (WQC-24, DKK-TOA Corporation) was used to measure the turbidity of the solutions. Figure 3.1 shows the materials used in these experiments.

Fig. 3.1 Materials used for particle concentration and turbidity effects. (a) for concentration effect; (b) for turbidity effect.

3.2.2 Optical Setup

The optical setup, shown in Fig. 3.2, is lensless, inline, and transmission based with 1 μm pinhole diverging system. In this experiment, a laser source (Lambda Photometrics DPGL3020 (30 mW), Harpenden, UK) of 532 nm wavelength and a CCD (The Imaging Source DMK41BF02, Bremen, Germany) of $N_x \times N_y = 1280 \times 960$, with 4.65 μm pixel size, were used. A spatial filter system with 60x MO and two types of cuvettes (12.5 mm(L) $\times$ 12.5 mm(W) $\times$ 48 mm(H) and 7.5 mm(L) $\times$ 12.5 mm(W) $\times$ 45 mm(H)) were used in this setup. In this particular case, the pinhole to CCD distance was 55 mm, whereas CCD to object depth was about 22–32 mm in case of 10 mm sample depth and 22–27 mm for 5 mm depth. The exposure time of the CCD was 0.25 ms.

Fig. 3.2 Optical setup of the digital inline holographic microscopy.

3.2.3 Experimental Procedure

Particulate suspensions of different particle concentrations, ranging from 0.01 v/v% to 0.3 v/v%, were prepared by mixing appropriate amount of the original particle sample and de-ionized (DI) water. For each sample, 30 holograms were recorded and processed by the image analysis algorithm. The results were analyzed considering two sample depths—namely 10 mm and 5 mm.

Various turbid solutions, ranging from 0 to 400 NTU, were prepared by mixing consumable milk with DI water. For each turbid sample, 100 μL of particulate suspension and 2000 μL of turbid solution were mixed. 30 holograms for each sample were recorded and processed. The results were analyzed considering two particle concentrations, namely, 0.007 and 0.014 v/v%. It is worth noting that owing to the rather small concentration, the particle suspension had a minor effect on the solution turbidity. Two concentrations were used to check the amount of deviation of the results by varying particle concentrations. In this case, experimental assumption is that overall turbidity effect will come from milk particle suspension as long as particle concentration is low enough.

The two main objectives of this work are to quantify the effect of particle concentration and turbidity on detection efficiency (Φ_e) and size measurements. The detection efficiency is defined as

$$\text{Detection efficiency } (\Phi_e) = \frac{\text{Number of detected particles}}{\text{Expected number of particles}} \dots\dots\dots(3.1)$$

where the numerator refers to the number of particles detected in a hologram and the denominator refers to the expected number of particles present in the volume imaged in the hologram. The trapezoidal volume that is imaged was calculated shown in Fig. 3.3, from the dimensions of the setup, to be 65 mm³ and 26 mm³ for the 10 mm and 5 mm cuvettes, respectively. The expected number of particles was calculated from the suspension concentration and the imaged volume under the assumption that the system is well mixed. Particularly at low particle concentrations, it is possible that more particles than what was theoretically calculated could be present in the measured volume, leading to detection efficiencies larger than 1. The mean particle diameter of each sample was calculated from the particles identified by the image processing algorithm.

Fig. 3.3 Schematic for the calculation of sample volume imaged onto the camera.

3.2.4 Hologram Recording, Reconstruction, and Image analysis

The extraction of particle characteristics involves three steps: hologram recording; reconstruction, and image analysis. In the recording step, the interference pattern, namely hologram, is created by the superposition of two beams, i.e., the object beam and the reference beam. This hologram is recorded by a CCD/complementary metal oxide semiconductor (CMOS) camera and stored in a computer for reconstruction and digital image processing. The hologram contains the information about the entire

sample volume. In order to obtain the information at a particular depth, numerical reconstruction (focusing) is performed.

Numerical reconstruction is usually carried out by the Fresnel-Kirchhoff diffraction integral using Fresnel approximation or convolution approach [22-23]. In case of convolution based reconstruction algorithm, the pixel size in the reconstruction plane does not depend on the reconstruction depth and no approximation is needed [57, 79]. As a result, the convolution approach is advantageous for reconstructing inline holograms of the particle field, where reconstructions at different depths are to be compared, and is used in this work. It should be mentioned that the setup used in this work does not use microscopic objective (MO); it uses a diverging beam to introduce magnification into the system. Magnification is a function of the reconstruction distance and has to be properly included into different reconstructions [37].

In digital particle holography, one of the crucial tasks is to identify the position of a particle within the reconstruction volume [41, 80]. For this purpose, we suitably adopt the digital hologram processing algorithm proposed earlier [37, 59-60]. In the first step, the recorded digital hologram is reconstructed at several depths with 25 μm intervals. Following reconstruction, image segmentation is carried out on each reconstruction plane to identify the particles using a single threshold value of 25 (out of 0 to 255). Pixels having gray level value below 25 are considered as particle and the other pixels are taken as background. Further, image processing operations such as filling, erosion, dilation, noise removal and border clearing are applied on the particle images of each reconstruction plane. Further details are provided elsewhere [37, 59-60]. Detected particles outside the range of 30 to 80 μm are neglected. Particle size is measured automatically based on the equivalent diameter defined in the MATLAB Image Processing Toolbox (IPT). While the lower limit is employed to reduce the noise that can be counted as particles, the upper limit is enforced to avoid the counting of aggregates as particles. A particle can be identified at several depths due to the large depth of focus of the DIH system. The best focused distance of each particle is identified by comparing the mean intensities of the particle at different reconstruction distances. The reconstruction distance at the minimum mean intensity is then specified as the best focused distance and the particle size is measured at that reconstruction distance. It is worth pointing out that Canny edge detection technique [85-86], used in

the previous papers [37, 59-60], is not applied here as it is well-suited only for dilute systems, where the particles reveal strong edges [37].

3.3 Results and Discussions

In this section, results obtained, after conducting the experiments and using processing algorithm, are discussed thoroughly. Results are analyzed considering two metrics, such as detection efficiency and mean size of the particle population. Every possible reason for the detrimental effect of particle concentration and turbidity on this DH based tool for particle characterization is explained. Finally, conclusion is drawn based on 50% detection efficiency as the threshold. However, the performance can be improved from the optical development and/or image processing side.

3.3.1 Effect of Particle Concentration

Effect of particle concentration on the detection efficiency and the reconstructed particle size is described here. Two sample depths are considered and it is demonstrated that measurement performance can be improved significantly by reducing the sample depth. It is also observed that high particle concentration provides adverse effect on particle detection procedure significantly.

3.3.1.1 Detection Efficiency

Figure 3.4 shows the relationship between Φ_e and the particle concentration. At low particle concentration, Φ_e is more than 90%, while at increased concentration, Φ_e decreases. It can also be seen that, as expected, Φ_e for the 5 mm cuvette is consistently higher than the 10 mm. At lower particle concentration, holograms are relatively cleaner and particle images with high resolution can be reconstructed, which leads to higher Φ_e . Figure 3.5(a) shows the reconstructed image of a hologram recorded for 0.01 v/v% particle concentration at 10 mm depth. Well separated particle images with good quality background enable easy detection by the image processing algorithm. The Φ_e decreases at higher particle concentration due to the increase in noise level and overlapping interference pattern. Figures 3.5(b) and (c) show the reconstructed images of a hologram recorded for 0.05 v/v% particle concentration at 10 mm depth. At higher particle concentration, particles are present closely in space, resulting in strong interaction of interference patterns from multiple particles on the focused reconstruction plane. Under these circumstances as shown in Figs. 3.5(b) and (c), the

correct particle outline is not identified making these particles fall outside the 30 to 80 μm range. Further, the high concentrations of the particles reduce the intensity of the light that passes through the sample thus providing less contrast between the particle and the background. These effects result in lowering the detection efficiency.

Fig. 3.4 Effect of particle concentration on detection efficiency.

The effect of sample depth on the measurements is illustrated in Fig. 3.6. From the reconstructed images it is clear that the 5 mm sample volume results in a better image quality compared to the 10 mm cell, i.e., Fig. 3.6(b) contains more overlapping interference patterns than Fig. 3.6(a). Further, owing to the shorter path length that the light travels in the 5 mm cell, the background of reconstructed image is found to be clearer in Fig. 3.6(c) than in Fig. 3.6(d). Hence, Φ_e for 5 mm depth is larger than that for the 10 mm depth. Considering $\Phi_e = 50\%$ as the threshold, it can be concluded that the digital holographic system works well up to 0.1 v/v% for 10 mm sample depth and 0.2 v/v% for 5 mm depth. This observation highlights that particle concentration can be doubled by halving the sample depth while providing the same level of measurement performance. Several researchers have reported the significance of the effect of sample depth on digital particle holography [25, 46]. These experiments confirm the earlier findings by showing that Φ_e can be significantly improved by reducing the sample depth. This is likely due to the lesser number of the overlapping interference patterns, when the sample depth is reduced. It is worth noting that 5 mm sample depth still leads to a large DOF compared to microscopic imaging analysis

which has a DOF of the order of few micrometers [35]. On contrary to the photographic imaging technique, holographic method can handle more solid concentration as particle images are located on different reconstruction planes. In addition, renowned non-holographic method such as FBRM can be used to handle high solid concentration but it does not provide particle's size and shape information [8].

3.3.1.2 Particle Size Distributions (PSDs)

The PSDs and particle sizes obtained at different particle concentrations are shown in Fig. 3.7 for both sample depths, i.e., 10 mm and 5 mm. PSDs for 10 mm depth are shown in Fig. 3.7(a). At lower concentration, a narrower size distribution is achieved since well separated particle images with clear background are identified and measured. At higher concentration, the distribution is broader on both sides due to the addition of speckle noise and overlapping interference pattern. Speckle noise is inherently related with the 'information capacity' of hologram which depends on both particle concentration and depth of the sample volume [25]. The shift to larger sizes can be caused by various reasons; including interference between focused and partially focused particle images and focusing of multiple particles at the same reconstruction plane, as shown in Fig. 3.5(b). The shift of the PSD to lower sizes can be attributed to the reduced resolution due to increase in noise, as shown in Fig. 3.5(c). PSDs measured at 5 mm depth at various particle concentrations are shown in Fig. 3.7(c). PSDs for 5 mm depth are narrower than the PSDs measured at 10 mm depth. This is due to the lower number of overlapping interferences observed in case of 5 mm depth, which leads to fewer particle-particle interactions.

Image processing methods, especially morphological process can significantly affect the original object shape and size, and this can lead to errors in the size measurements. The sequence of image analysis methods applied in the algorithm are fit enough to the requirements. Moreover, the algorithm used default MATLAB parameters which are the smallest and suitable for small particles. Morphological processes in step-by-step with each processed image are described in Fig. 3.8.

Fig. 3.5 Reconstructed images for 10 mm depth. (a) 0.01 v/v% particle concentration; (b) 0.05 v/v% particle concentration showing particle-particle interaction; (c) 0.05 v/v% particle concentration showing size reduction due to the noise (particle outlines at both lower and higher concentrations are shown in the inset for comparison). The green region is the area identified as the particle by the image processing algorithm.

Fig. 3.6 Holograms and reconstructed images at 0.01 v/v% particle concentration. (a) hologram for 5 mm depth; (b) hologram for 10 mm depth; (c) reconstructed image at 25.55 mm for the hologram shown at (a); (d) reconstructed image at 29.95 mm for the hologram shown at (b).

The Fig. 3.8(a) shows a reconstructed intensity image at 26.575 mm for 10 mm depth and 0.01 v/v% concentration. Fig. 3.8(b) is the image after thresholding operation. Afterwards, some basic image processing methods (i.e., bridging, filling, dilation and erosion) have been applied from which the resulting image expresses the Fig. 3.8(c). Finally, noises and particles (attached with the border) removal operations are carried out in order to calculate particle characteristics. It is noted that all particle accurate diameters are not measured from this reconstructed plane. The diameter is measured finally after employing the focusing algorithm.

Fig. 3.7 PSDs and particle sizes at different concentrations. (a) PSDs for 10 mm depth; (b) Errorbar plot of the particle sizes for 10 mm depth; (c) PSDs for 5 mm depth; (d) Errorbar plot of the particle sizes for 5 mm depth. Errorbar plots are drawn based on the means and standard deviations of the distributions.

Fig. 3.8 Illustrations explaining the basic image processing methods. (a) reconstructed intensity image at 26.575 mm for 10 mm depth and 0.01 v/v% concentration; (b) thresholded binary image of (a); (c) image after bridging, filling, dilation and erosion operation on (b); (d) binary image after noises and particles (attached with the border) removal operation on (c).

3.3.2 Effect of Turbidity

Effect of turbidity on the detection efficiency and the reconstructed particle size is described here explicitly. Two particle concentrations are selected for mixing with turbid solutions in order to see whether the particle concentration affects the measurement or not. It is found that from the results; see Fig. 3.9; the particle suspension had a minor effect on the solution turbidity. Φ_e is almost constant up to 50 NTU and starts to decrease as turbidity increases.

3.3.2.1 Detection Efficiency

Figure 3.9 illustrates the relationship between Φ_e and the turbidity levels of the different samples for two particle concentrations. The Φ_e for both particle concentrations (0.007 and 0.014 v/v%) are very close to each other, which implies that the turbidity effect from the particle suspension can be ignored. The Φ_e is higher for sample with low turbidity and it decreases with the increase in turbidity level. It is found that Φ_e is almost constant up to 50 NTU.

Considering $\Phi_e = 50\%$ as the threshold, it can be concluded that the digital holographic system works well up to 150 NTU turbidity level for 10 mm sample depth. At lower turbidity level, light scattering from the milk particles is low; holograms are relatively cleaner and hence particles outlines are easily identified in the reconstructed planes. As shown in Fig. 3.10(a) the hologram recorded at 0 NTU turbidity for 0.014 v/v% particle concentration shows less grainy spots (speckle). The reconstruction, shown in Fig. 3.10(c), reveals that the particle shape and size can be detected quite accurately. At high turbidity, more light is scattered from the milk particles resulting in more speckle noise in the hologram as shown in Fig. 3.10(b). Reconstructing this hologram and applying the image processing algorithm results in poor estimate of the particle contour, as shown in Fig. 3.10(d).

3.3.2.2 Particle Size Distributions (PSDs)

The PSDs and particle sizes obtained at different turbidity levels are shown in Fig. 3.11. Owing to reduced light scattering at low turbidity values, the detected particle sizes are more accurate than the ones at high turbidity. Reconstructed image for hologram recorded at 0 NTU turbidity for 0.014 v/v% particle concentration is shown in Fig. 3.10(c), where the particle shape is correctly detected. At higher turbidity level, scattering from the milk particles is more pronounced and more speckle noise is present in the hologram.

Fig. 3.9 Effect of turbidity of the sample on detection efficiency.

Resolution of the reconstructed image gets reduced and the particle shape is not correctly identified. These particles can be ignored by the algorithm depending on the threshold used. Unreliable particle shape in the reconstructed image of hologram measured at 400 NTU turbidity for 0.014 v/v% particle concentration is shown in Fig. 3.10(d).

Figs. 3.11(b) and (d) show the mean diameters of the particles at various turbidity levels for two particle concentrations. Mean diameters of the particles are almost the same around $40\text{ }\mu\text{m}$ and their distributions are the same at all turbidity levels in both cases.

Fig. 3.10 Holograms and reconstructed images at two different turbidity levels for 0.014 v/v% particle concentration. (a) hologram at 0 NTU turbidity level; (b) hologram at 400 NTU turbidity level; (c) reconstructed image of hologram (a) at 25.48 mm; (d) reconstructed image of hologram (b) at 28.75 mm.

Fig. 3.11 PSDs and particle sizes at different turbidity levels. (a) PSDs for 0.007 v/v% particle concentration; (b) Errorbar plot of the particle sizes for 0.007 v/v% particle concentration; (c) PSDs for 0.014 v/v% particle concentration; (d) Errorbar plot of the particle sizes for 0.014 v/v% particle concentration. Errorbar plots are drawn based on the means and standard deviations of the distributions.

3.4 Chapter Summary

In this chapter, the effect of particle concentration and turbidity on the number of identified particles and the particle mean size measured using inline transmission based digital holography (DH) is described. It is demonstrated that measurement performance can be significantly improved by reducing the sample depth though lower concentration loading is preferred in DH. Taking 50% detection efficiency as the threshold, it can be concluded that the DH setup works well up to 0.1 v/v% and 0.2 v/v% particle concentrations for 10 mm and 5 mm sample depths, respectively, and 150 NTU turbidity level for 10 mm sample depth.

Chapter 4

Real Length Measurement of Needle Shaped Particle Population using DIH*

4.1 Introduction

In the past years, several researchers have tested various online particle characterization tools to characterize non-spherical particles [10, 14, 49, 51-52, 87]. One of the vital and most available tools is the FBRM. This tool measures the CLD instead of the PSD of particulate process. Converting the CLD to PSD requires a non-trivial inversion algorithm (i.e., particle shape dependent) [5, 51, 53-54]. Another in situ tool, based on direct imaging, is the particle vision measurement (PVM). PVM is more effective tool for measurement of the size and shape of non-spherical particles [4, 55-56]. Although it is a stronger tool for size and shape extraction, 2D imaging systems suffer from the out-of-focus issues due to the limited depth of field (DOF) imposed by the required magnification [4, 14]. Further, 2-D imaging systems provide projections of the particles on the 2D image plane. Extracting the real size of needle-shaped particles from the measured projected lengths requires information about the angle of orientation along the optical axis. This information is difficult to obtain for particles oriented randomly in 3-D volume [56]. This limitation requires developing 3-D imaging method capable of providing 3-D object information.

A novel and efficient image analysis algorithm, based on DIH, has been developed to characterize needle-shaped particles (i.e., length, position, and orientation) without any prior information about the orientation of particles. This method is, namely, called as ‘Superimposition Algorithm’. The performance of this algorithm is demonstrated using a single fiber with known size and orientation. The result is comparatively better than other two hologram processing algorithms, which have been published in the earlier times [37, 44]. Finally, this algorithm has been applied to an aqueous suspension of fiber, which shows good results comparing with the result obtained from optical microscopy measurement.

*T. Khanam, M. Nurur Rahman, A. Rajendran, V. Kariwala, and A. K. Asundi, "Accurate size measurement of needle-shaped particles using digital holography," *Chemical Engineering Science* **66**, 2699-2706 (2011).

Related to the contributions of this author in this work, activities carried out from the author are provided as follows-

- 1) Considering the proper digital holographic optical setup for conducting the experiments;
- 2) Conducting the experiments with single fibers (both long and short) on a glass slide at different orientations (from 0^0 to 65^0 at 5^0 interval);
- 3) Conducting the experiments with aqueous suspensions of fibers in the cuvette (total number of holograms captured 514);
- 4) Processing all the recorded digital holograms with the developed method (for both single fibers and aqueous suspensions of fibers experiments);
- 5) Representing, analyzing, and discussing the results part (i.e., the graphs for comparing between the 'Superimposition' and the '3D Point Cloud' algorithms, and the explanations regarding the errors of the results and limitations of the Superimposition algorithm at larger orientations and shorter fibers).

4.2 The Superimposition Algorithm

4.2.1 Recording and Numerical Reconstruction

Recording setup used for this experiment is shown in Fig. 4.1 which is the same to the one described in previous publications [37, 42, 59-60].

Fig. 4.1 Digital holographic experimental setup.

A recorded digital hologram of a tilted fiber is shown in Fig. 4.2(a). After recording digital hologram, numerical reconstructions are carried out with the convolution method in order to focus the image of a particle at several depths. The number of reconstruction steps is a critical parameter. Small depth interval between two successive reconstructions result in increased computational load and larger increments between two consecutive reconstructions might lead to errors in measuring the lengths. The distance between two consecutive reconstructions is dependent on the minimum size of the particles to be analyzed and the depth resolution of the system. It should be mentioned that the optical setup used in this work does not use any microscopic objective (MO); it uses a diverging beam to introduce magnification into the system. Magnification is a function of the reconstruction distance and has to be properly considered into different reconstructions [37].

4.2.2 Image Segmentation

The complex valued reconstructions are transferred to gray scale images (a scale from 0 (black) to 255 (white)) after the reconstructions have been executed. Then intensity images are converted into binary images by choosing an appropriate threshold. Selection of the threshold value for image segmentation is not trivial in DH since

intensity variations due to laser imperfection, multiple object light scattering and related non-uniform illumination have to be considered. The proposed algorithm firstly reconstructs the hologram at half depth of the 3-D volume and the average intensity of the resulting image is computed. A fraction of this average intensity is selected as the threshold value for all other holograms measured for the particular experiment. The fraction is chosen, manually by visual testing, in such a way that only the darkest pixels, which correspond to pixels in focus, are considered as being part of fiber and are set to '1' in the binary image. In this work, 10% and 20% of the mean intensity are used as threshold values for the analysis of holograms of dry particles and particles in suspension, respectively. It should be mentioned that for dry particles with clean background nearly identical results were obtained for threshold values ranging from 5% to 15% of the mean intensity. For particles in aqueous suspension, where background is noisier, the threshold values ranging from 18% to 25% of the mean intensity provided nearly the same results. Fig. 4.2(b) illustrates an example of the segmented images of reconstructions of a titled fiber obtained at various depths.

4.2.3 Superimposition

After image segmentation, the thresholded reconstructed images are superimposed onto the preceding image and this process is resumed until the reconstruction of the whole sample volume is covered. In this way, all the focused parts of a single fiber at different reconstructions are merged together comprising a full projection of the fiber on a single 2D image plane; see Fig. 4.2(c). The resulting superimposed image is then converted to a binary image. After this process, MATLAB function 'bridge' for morphological operation is performed onto the superimposed binary image to connect the zero valued pixel gap between two non-zero neighbours. After bridging, dilation followed by erosion is applied to merge the fragmented portions belonging to a particular fiber. In addition, a suitable co-linearity method that compares the slope and proximity of two line segments is used to join broken fibers. In this method, if the difference in slopes of the two line segments is less than three degrees and the closest distance between any two extremes of the two line segments are smaller than 15 pixels, the two segments are taken to be parts of a single fiber. Hence, from the superimposed image the projected length of the individual fiber is obtained. It is worth pointing out, unlike 3-D point cloud method, all image processing operations are performed on a 2D image plane and hence saving the memory and computational load.

Finally, all the detected fibers are labelled and the major axis length of the fitted ellipse around the fiber on the 2D image plane is measured. This major axis is then multiplied by a factor of $\frac{\sqrt{3}}{2}$ to get the projected lengths [37].

4.2.4 Localization of a Tilted Fiber

After the superimposition step, the projected length of the fiber is known. In order to obtain the real length of fiber, its orientation along the optical axis needs to be measured. The localization step detects the position of the fiber in the 3-D volume and estimates its out-of-plane tilt. For this purpose, the algorithm selects two end regions by sectioning the projected length of the individual fiber into four equal segments; see Fig. 4.3(a). In general, the number of segments can be different from four and should be chosen such that each segment possesses at least 15–20 pixels to allow reliable localization of the segment based on intensity variation. It is noting that the smaller the number of pixels in each segment, the higher the chances of obtaining noisy ‘intensity depth’ profile. This can lead to an erroneous measurement of the angle of tilt. The depth profiling is performed by calculating the mean intensity of the two end regions for all reconstruction depths within the reconstructed volume. In this approach, the two selected end regions are expected to have the minimum mean intensity at their best focused planes. The best focused depths of the two end sections provide the axial locations of their centroids.

Fig. 4.2 Key steps of the superimposition algorithm. (a) hologram of a fiber of length $1320\text{ }\mu\text{m}$ and off-axis tilt of 50° ; (b) numerically reconstructed images after applying threshold at different depths; (c) final superimposed image constituting the projected image of the fiber.

The depth profiling for finding focused positions of the two end regions of a single tilted fiber is shown in Fig. 4.3(b). Once the projected length and axial positions of two terminal segments of a tilted fiber are known, the real length is calculated as,

$$\theta = \tan^{-1} \frac{\Delta d}{L_c} \dots\dots\dots(4.1)$$

$$L_R = \frac{L_P}{\cos \theta} \dots\dots\dots(4.2)$$

where Θ is the angle of orientation along the optical axis, Δd is the distance between two axial positions (along the optical axis, Z) of the terminal segments of a tilted fiber. L_c , L_P , L_R are the centroid to centroid distance between two terminal sections of the projected fiber (see Fig. 4.3(a)), projected length and real length, respectively.

Fig. 4.3 Localization of the 50° tilted fiber shown in Fig. 4.2. (a) sectioning of the superimposed fiber for calculating average intensity of the terminal sections. The circles indicate four equal sections. (b) depth profiling of the two terminal sections determining the tilted position of the fiber.

As can be seen from Eqns. (4.1) and (4.2), the measured Θ depends on L_c and Δd , which affects the measurement of the real length. It is worth pointing out that if the projected length of a fiber is close to the depth of focus of the system, it affects the measurement of Θ significantly, as the two end positions of the tilted fiber are not clearly distinguishable. This case can be occurred when lengths of the needles are small, or bigger needles at larger orientations. This is particularly observed at the large out-of-plane tilts, as the projected length becomes shorter.

4.3 Experiments and Results

The experimental setup used is illustrated in Fig. 4.1. In this setup, a laser source (Lambda Photometrics DPGL3020, Harpenden, UK, 30 mW) with a wavelength of 532 nm is focused onto a 1 μm pinhole which is located at a distance of 64 mm from a CCD camera (The Imaging Source DMK41BF02, Bremen, Germany) with 1280 $\times$ 960 pixels with $4.65 \times 4.65 \mu\text{m}$ each. Two systems - namely a single microfiber placed on a rotating mount and a suspension of microfibers in solution, were studied. For microfiber suspension, the experiment was conducted using a cuvette (12.5 mm(L) $\times$ 12.5 mm(W) $\times$ 48 mm(H)) containing the suspension of a population of carbon fibers (Asbury PAN type carbon fiber, USA) in water.

4.3.1 Single Fiber on a Glass Slide

In this section, the performance of the superimposition method is compared with the '2D projection algorithm' and the '3D point cloud algorithm'. In order to validate the algorithm, it is applied to holograms of a single fiber of known length and off-axis tilt. For this purpose, the experimental setup shown in Fig. 4.1 was used. In this setup, a single fiber of known length was placed on a glass slide. The glass slide along with the fiber was held by a rotating mount capable of providing a known off-axis tilt. Two fibers with lengths 1320 μm (called long fiber) and 176 μm (called short fiber), were rotated from 0° to 65° tilt at 5° intervals. A hologram was recorded for every tilt and processed with 100 reconstructions using the method described in Section 4.2. From the recorded hologram the projected length, angle of orientation and real length were measured and compared with the known values. Figure 4.4 shows comparison of the projected lengths measured by the two algorithms, 2-D projection and superimposition method, along with theoretically expected values. For the long fiber, the 2-D projection algorithm successfully measures the projected length only up to $\Theta = 15^\circ$. For $\Theta > 15^\circ$, the 2-D projection algorithm fails to detect the fiber; see [37] for details. In comparison, the superimposition algorithm successfully measures the projected length even at high degree tilts. For short fiber, both methods provide good measurements of projected lengths up to 55° tilt. At tilts larger than 55° , the 2-D projection fails to detect the fiber, while the superimposition algorithm detects the fiber for all tilts; see Fig. 4.4(b). It is noting that the projected length of fiber at large tilts becomes comparable to the depth of focus of the optical system, hence, the

chances of detecting projected lengths of short fiber at large tilts by 2-D projection method increases. In Figs. 4.4(a) and (b), it is also observed that at tilts above 45° , projected length is overestimated by both 2-D projection and superimposition methods. This overestimation might occur due to the refraction of the laser light by the tilted glass slide.

Fig. 4.4 Projected length measurements of a single fiber. (a) long fiber (1320 μm); (b) short fiber (176 μm). The error bars are theoretically calculated based on the resolution of the measurement systems.

Figure 4.5 shows the relative error in the measured real length for different out-of-plane tilts using superimposition and 3-D point cloud algorithm. As can be seen from Fig. 4.5(a), the superimposition method accurately measures the length of the long fiber over a large range of tilts with relative error less than 4%. Here, the measurement accuracy for both 3-D point cloud algorithm and superimposition method is comparable. For the short fiber, the relative error in the measured length is comparable for both superimposition and 3-D point cloud method only up to 45° tilt; see Fig. 4.5(b). The maximum error obtained from superimposition method is 13%, while the 3-D point cloud method significantly overestimates the length for $\Theta > 45^\circ$. In summary, this experiment confirms the accuracy of the superimposition method over 3-D point cloud method. Furthermore, the superimposition algorithm requires significantly lower computational load in comparison to the 3-D point cloud method. For the holograms of single fiber, the superimposition method usually took only 2 minutes, while the 3-D point cloud method took approximately 3 hours to process the same hologram, although the numbers of reconstructions were the same.

Fig. 4.5 Measurement of the real length of a single fiber. (a) long fiber (1320 μm); (b) short fiber (176 μm). Symbols represent the experimental measurements while the lines show the trend.

4.3.2 3-D Measurements of Microfibers in Suspension

Next, the method was applied to the fiber population in an aqueous suspension, a situation observed in practice. For validation purposes, carbon fibers lengths were measured using an optical microscope. Since all fibers were on flat glass surface, hence the measured lengths are their real lengths. More than 600 microscopy images were captured and the lengths of fibers were measured by image analysis algorithm. Only the superimposition algorithm is considered for the holographic measurements as this method yields accurate results than previously developed algorithms. Each hologram is reconstructed at 400 image planes within 8 mm reconstruction depth. The algorithm consumes approximately 7 minutes to process a single hologram with 400 reconstructed images. A total of 514 holograms of fiber suspension were captured and analyzed by the developed algorithm. A sample digital hologram of fiber suspension, the corresponding superimposed image on the last reconstruction plane, and 3-D distribution of the detected fibers are shown in Figs. 4.6(a), (b) and (c), respectively. A micrograph of the same fiber population is also shown in Fig. 4.6(d). Figure 4.7 illustrates the length distribution of fiber population in suspension. Fibers with tilts larger than 60° and lengths smaller than 176 μm are neglected to avoid any erroneous measurements at large tilts due to the limitations of DOF. 176 μm is chosen as the minimum length, because this value is close to the length of the shortest fiber that measured on a glass slide accurately. For this, many particles are missed since a large number of particles are small contained in the sample. Another limitation of that

algorithm is to neglect the overlapped needles contained on the last reconstructed plane. This case is few as the number of bigger particles are low. Quantitative measurement of those missed particles is difficult due to the algorithm limitation and larger length range of needles. Only development of the advanced algorithm can enhance the measurement performance. A total of 2338 fibers are identified and measured using holography. The resulting real length distribution of the fiber population obtained by analyzing the holograms is represented in Fig. 4.7(a). The distribution of real lengths from analysis of the microscopy images of the same fiber population is also shown in Fig. 4.7(a). For a fair comparison, fibers with length larger than $176\text{ }\mu\text{m}$ are considered from the microscopy data. As can be seen from Fig. 4.7(a), projected length distribution obtained using holography is moved to the left from the real length distribution obtained using microscopy measurements, while the real length distribution obtained by incorporating the angle of orientation in holography measurement matches very well with the microscopy measurements. The corresponding distribution of angle of orientation of the fiber population is presented in Fig. 4.7(b). Since no tools are available for measuring the true tilt distribution of the population of particles, the accuracy of the measured tilt distribution cannot be verified. However, a few observations can be built. Firstly, the fibers show a range of orientation. Secondly, most of the fibers in this experiment have tilts less than 20° and there seems to be a preferred orientation for this system. It refers to the fact that the orientations of fibers depend on the particular system that is being investigated. Hence, the assumption of equal probabilities for all orientations, which is a frequently used assumption for the 2-D inversion techniques [55, 88], is not necessarily valid.

4.4 Chapter Summary

In this chapter, a novel and efficient algorithm, the superimposition method, for the measurement of length, position and off-axis tilt of needle-like particles without any prior information about the orientation of particles, has been described. The method was demonstrated using fibers of known length and orientation. The method was also applied to a suspension of fibers to measure the real length distribution and their angles of orientation in the 3-D volume. The obtained real length distribution showed good matches with that obtained using optical microscopy.

Fig. 4.6 (a) A sample hologram of fiber suspension in a cuvette; (b) the corresponding superimposed image of the reconstructions; (c) three-dimensional distribution of identified fibers showing their orientations and lengths; (d) sample microscopy image of same fiber population on a glass slide.

Fig. 4.7 Characterization of fibers in suspension. (a) length distribution of fiber population in suspension (for fibers longer than 176 μm). Dotted line represents the projected length distribution directly obtained by holography without incorporating the angle of orientation in suspension. (b) corresponding angle distribution of the fiber population in suspension.

Chapter 5

Mixed Morphological Particulate System and Crystal Characterization using DIH*

5.1 Introduction

One of the most popular methods for morphological monitoring is digital inline holography (DIH), where with single shot of optical image acquisition one can reconstruct optical images of a sample at arbitrary focal point, thereby obtaining complete 3-dimensional positional information of whole sample [22-24, 83, 89-91]. Monitoring sizes and shapes of micro-particulate system is very important in chemical engineering, especially in the context of crystallization studies [4, 14]. In literature, most of the algorithms are designed for the spherical particle field characterization using digital holography [25, 31-32, 37, 41, 48, 57-59], and a few algorithms demonstrate capabilities to measure the sizes and shapes of needle shaped particles [42, 44, 61-62]. However, there is no such algorithm for characterization of both spherical and needle shaped particles simultaneously, or mixed morphological particulate system.

The algorithm which is suitable for mixed morphological particulate system characterization is especially important in the manufacturing, monitoring and control of active pharmaceutical ingredients (APIs) in the pharmaceutical industries [12, 63, 65-66]. In this case, polymorphism is a common phenomenon. Polymorphs are the substances, which are similar chemically but different in physical forms. (L)-glutamic acid is one of the examples, which shows two polymorphs: the prismatic alpha and the needle shaped beta form [63]. As a result, they show distinct characteristics with respect to each other in terms of physical properties such as solubility, dissolution, bioavailability and so on [4]. Product quality assurance is always of utmost importance in the industries.

*M. N. Rahman, K. Lee, and A. K. Asundi, "Mixed morphological particulate system characterization using digital inline holography," Submitted to Applied Optics (2013).

Therefore, there is a strong requirement to develop an algorithm for characterization, which will be suitable for particles having different shapes in the special cases, for better design, monitoring and control of the particulate processes. In this work, an image analysis algorithm, based on DIH, is developed to characterize sphere and needle populations simultaneously (i.e., diameter distribution for sphere population and real length distribution for needle population). The algorithm combining two distinct computer programs is employed to quantify two populations of the mixed morphological particulate system [37, 44]. In addition, particle classification and separation are carried out determining a geometric feature (i.e., eccentricity) for each particle image within the reconstruction volume. The method has also been demonstrated considering three different ratios of the spherical particle and needle populations. In all cases, the eccentricity distributions and axis length distributions (ALDs) show clear separation (i.e., two regions completely perceived) between the two populations. In addition, particle size distributions (PSDs) (diameter distribution for spherical particle population and real length distribution for needle population) match very well with the expected values [37, 44]. This algorithm can be successfully used to track and quantify the different polymorphs for APIs in the crystallization processes, especially for two extreme shapes of the particulate system (e.g., spheres or cuboids and needles). Traditional approaches for the classification of different polymorphs are the spectroscopic techniques such as Raman spectroscopy but their results provide only the types of polymorphs and concentrations. Comparison between the spectroscopic techniques and the DH is illustrated in Table 5.1.

Table 5.1 Difference between spectroscopy and digital holography.

Features	Spectroscopy	Digital Holography
Type of Technique	Non-imaging	3-D imaging
Input Parameter	Measurement carried out from the absorption spectra	Measurement carried out from the optical wavefront
Output Result	The type of chemical and concentration can be measured	3-D size and shape can be measured

As an application in crystallization study using DIH tool, an experiment with single Potash Alum crystal on microscope slide was conducted. Results of the crystal study are also described.

The organization of this chapter is as follows. The principle of digital inline holographic microscopy and the method used in this chapter are described in Section 5.2. Then, materials used in the experiments and the optical setup are described in Section 5.3. Section 5.4 presents experimental results and discussions. Finally, conclusions are drawn in Section 5.5.

5.2 Digital Holographic Method used for Mixed Morphological Particulate System Characterization

5.2.1 Recording and Numerical Reconstruction of a Digital Hologram

The experimental setup used for recording the digital hologram of a mixed particles suspension is shown in Fig. 5.1. Recording is the first step in digital holography (DH). Hologram is created by the interference of two beams, i.e., the object beam and the reference beam. Charge-coupled device (CCD)/complementary metal oxide semiconductor (CMOS) camera is used for recording the hologram and the digital hologram is stored for reconstruction and digital image processing in a computer. The hologram holds about the entire 3-D volume information [22-24, 83, 89-91].

Fig. 5.1 Digital inline holographic setup.

Numerical reconstruction (digital focusing) of that digital hologram is carried out in order to obtain the information at various depths. Fresnel-Kirchhoff diffraction integral using Fresnel approximation or convolution approach is used for the numerical

reconstruction. Convolution based reconstruction algorithm does not change the pixel size in the reconstruction planes along the axial position and no approximation is required [78, 80]. And hence, the convolution approach is useful for reconstructing inline holograms of the particle field since reconstructions at various depths are to be compared. Convolution based reconstruction algorithm is used in this paper. It is noted that the diverging beam introduces magnification into the optical system. Magnification depends on the reconstruction distance and has to be accounted properly into different reconstructions [37].

5.2.2 Processing of the Reconstructed Planes

Firstly, hologram is recorded using a CCD camera. The digital hologram with mixed morphological particulate system (spheres and needles) in an aqueous suspension is shown in Fig. 5.2(a). Following recording, numerical reconstructions of that hologram are carried out at several depths in order to focus the particle images within the object region. A representative reconstructed intensity image of the hologram of Fig. 5.2(a) at 23.475 mm depth is presented in Fig. 5.2(b). From each reconstruction plane, particle images are segmented using a single threshold value (i.e., 25 out of 0 to 255). Pixels having intensity value below 25 are considered as particle and others are taken as background. The binary image of Fig. 5.2(b) is shown in Fig. 5.2(c) which reveals focused particles including un-focused particles and noises. Afterwards, some image processing methods (i.e., bridging, filling, dilation, erosion, noise removal and border clearing) are applied to smoothen the boundaries of the focused particle images and remove noises. Some details of the MATLAB code is given as follows-

```
Id_cent = uint8(Id*255/max(Id(:))); % conversion from double to uint8 where Id is
intensity image
```

```
threshold = 25;
```

```
Id_thr = Id_cent < threshold;
```

```
Id_thr = bwmorph(Id_thr, 'bridge'); % bridging operation
```

```
Id_thr = imfill(Id_thr, 'holes'); % filling holes
```

```
Id_thr = bwmorph(Id_thr, 'dilate'); % dilation operation
```

```
Id_thr = bwmorph(Id_thr, 'erode'); % erosion operation
```

```
Id_thr = bwareaopen(Id_thr, round(pi*(15/2)^2)); % noises and un-focused particles removal
```

```
Id_thr = imclearborder(Id_thr); % removal of particle images attached with the border
```

```
Particle_labels = bwlabel(Id_thr) % labeling
```

Reconstructed image of a sphere is quite different from the image of a needle. The reconstructed images resemble the actual shapes of the particles. Therefore, it is not difficult to identify and separate the particles based on their geometries. An ideal sphere has an eccentricity value of 0 and the eccentricity value of a perfect needle is 1 [92]. In this work, eccentricity, a geometric feature, is used to distinguish between a sphere and a needle. Eccentricity value below 0.5, a particle blob is considered as a sphere and above 0.5 considered as a needle. Eccentricity value of 0.5 is chosen for particle classification since two extreme types of the particulate system, i.e., spheres and needles are used here. Fig. 5.2(d) shows the structure of a sphere and a needle after reconstruction, image segmentation and basic image processing steps mentioned earlier.

Due to the large depth of focus of the digital holographic setup, a particle is identified at several reconstructed planes. For the spherical particles, particle localization is employed to identify the best focused plane of each particle by comparing the mean intensities of the particle at different reconstruction planes. The reconstruction distance which corresponds to the minimum mean intensity is then specified as the best focused distance and the particle characteristics such as size is measured at that reconstruction distance. Further details are provided elsewhere [37]. The first reconstruction plane containing only the needle shaped particles (separation carried out based on the eccentricity parameter) is superimposed on the second reconstruction plane (also containing only the needle shaped particles). Superimposition process is carried out up to the last reconstruction plane. The superimposed image on the 200th reconstruction plane contains only the needle shaped particles which is shown in Fig. 5.2(e). The superimposed image on the last reconstruction plane which is shown in Fig. 5.2(f) containing one more needle image than Fig. 5.2(e), which is detected from the last 200 reconstructions.

Fig. 5.2 Illustrations obtained from the hologram processing algorithm. (a) digital hologram of spheres and needles from 15 v/v% needle population; (b) reconstructed intensity image of that hologram at 23.475 mm depth; (c) binary image of (b) based on a threshold value of 25; (d) geometry of a sphere and a needle after basic image processing applied on the image (c); (e) superimposed needle images on 200th reconstruction plane; (f) the last reconstruction plane containing superimposed needle images.

From this superimposed image, we can obtain only the 2-D projected length. To determine the real length of a needle, a proposed algorithm is applied to the superimposed needle shaped particle images which is described elsewhere [44]. The brief flow chart of this algorithm is illustrated in Appendix section.

For crystal study, the algorithm used for the spherical particles has been employed for finding the best focal plane.

5.3 Experimental

5.3.1 Materials

Three samples having different proportions of spherical particles (NIST certified diameter $39.82 \pm 0.24 \mu\text{m}$ from Duke Scientific Corporation, USA) and needles (TOHO Tenax Type 383 with $\approx 9 \mu\text{m}$ diameter) in de-ionized (DI) water were prepared. The samples proportions, 10, 15 and 25 v/v% needles approximately, were calculated from analysis of the holograms by the algorithm. This was carried out to check the performance of the developed algorithm while varying proportion of the spherical particles to needles. For each sample, 100 holograms were recorded and processed with the developed algorithm.

Potash Alum, $\text{KAl}(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$, was used for the crystal experiment. Firstly, saturated solution was prepared at 15°C . Some more crystals were added to the solution. These crystals were dissolved by providing small heat. Then it was cooled down to the room temperature. At the room temperature, some bigger crystals were observed at the bottom of the beaker. That crystal was used in the experimentation.

5.3.2 Optical Setup

The optical setup is shown in Fig. 5.1 which was lensless, inline, and transmission based with $1 \mu\text{m}$ pinhole diverging system. In this experiment, a laser source (Lambda Photometrics DPGL3020 (30 mW), Harpenden, UK) of 532 nm wavelength and a CCD (The Imaging Source DMK41BF02, Bremen, Germany) of $N_x \times N_y = 1280 \times 960$, with $4.65 \mu\text{m}$ pixel size, were used. The spatial filter system with 60x MO and a cuvette of $12.5 \text{ mm (L)} \times 12.5 \text{ mm (W)} \times 48 \text{ mm (H)}$ were also used in this setup. In this particular case, pinhole to CCD distance was 55 mm, whereas CCD to object depth was about 20-30 mm. The exposure time of the CCD was 0.85 ms.

In the single crystal study experiment, microscope slide was used instead of the cuvette.

5.4 Results and Discussions

5.4.1 Eccentricity Distributions and ALDs of the Mixed Populations

Eccentricity distributions and ALDs obtained from the image analysis algorithm, for the three samples, are shown in Fig. 5.3. In case of spherical particles in all the Figs. (i.e., 5.3 (a), (c) and (e)), eccentricity values are shifted from 0 while eccentricity values for the needle shaped particles are near to 1. Spread of the eccentricity distributions for spherical particles are also larger than that of the needle shaped particles. It means that image of a needle particle is reconstructed in better shape than the image of a spherical particle in the reconstruction process. The errorbars of the eccentricity for the spherical and needle-shaped particle populations measured by the algorithm are shown in Fig. 5.4. However, two regions for the two populations of each sample are well separated, as shown in the figures. The ALDs Figs. (i.e., 5.3 (b), (d) and (f)) have also corroborated these findings. It is noted that eccentricity and axis length of a spherical particle is calculated from its best focused image and those of a needle are calculated from the last reconstructed superimposed image. The eccentricity of a needle is measured automatically by MATLAB Image Processing Toolbox function (`regionprops('Eccentricity')`) which provides the value from the ellipse that has the same second-moments as the image region.

5.4.2 PSDs of the Sphere and Needle Populations

The PSDs from all the samples for spheres and needles (in terms of real length distribution) obtained from the image analysis algorithm are shown in Fig. 5.5. The PSD for spheres matches well with the expected one. The mean diameter of the spherical particle population is about $39.62 \pm 2.93 \mu\text{m}$. The mean length of the needle population is about $323.78 \pm 216.37 \mu\text{m}$. The real length distribution of needles also complies well with the result which has been obtained in other work. It is noted that the number of spherical particles is much larger than that of needles.

Fig. 5.3 Eccentricity distributions and ALDs for different samples. (a) and (b) for 10 v/v% needles; (c) and (d) for 15 v/v% needles; (e) and (f) for 25 v/v% needles.

Fig. 5.4 Errorbars of eccentricity for the spherical and needle-shaped particle populations.

Fig. 5.5 PSDs for mixed morphological particulate system obtained from three samples (diamter for sphere and real length for needle).

5.4.3 Single Crystal Study Results

Microscopic image and the hologram of a single crystal are shown in Figs. 5.6(a) and (b), respectively. This hologram is focused at 21.60 mm from the CCD plane in reconstruction. The reconstructed focused image of that crystal is shown in Fig. 5.6(c). The 2-D projected outline obtained from 3-D view within $\pm 50 \mu\text{m}$ from the best focal plane of that crystal is shown in Fig. 5.6(d). The zigzag bend in one part of the crystal is also clearly observed in the 2-D projected image which also exists in the photographic image shown in Fig. 5.6(a).

Fig. 5.6 Results on single crystal experiment using Potash Alum. (a) microscope image; (b) digital hologram; (c) reconstructed intensity image of (b) at 21.6 mm; (d) The 2-D projected outline obtained from 3-D view within $\pm 50 \mu\text{m}$ from the best focal plane of that crystal.

5.5 Chapter Summary

An algorithm, based on digital inline holography, is developed to characterize mixed morphological (here for spheres and needles) particulate system. This particular algorithm combines other two published algorithms of which one is suitable for spherical particulate system and another for needles. In addition, particle classification and separation is carried out determining a geometric feature (i.e., eccentricity) for each particle image within the reconstruction volume. Then, the algorithm has been demonstrated in real mixed morphological particulate system with varying proportions of the spherical particle population to needle population. The algorithm successfully works for the different proportions of needles to spheres. The result shows this algorithm can be successfully used to track and quantify the different polymorphs for APIs in the crystallization processes, especially for two extreme shapes of the particulate system (e.g., spheres or cuboids and needles). Obvious limitation is that overall particle concentration should be low in order to get better accuracy. Here, it shows that DH has the capability to image 3-D view of a crystal. This experimental procedure can be used further to observe the growth rate of different facets within the crystal.

Chapter 6

Conclusions and Directions for Future Research

6.1 Conclusions

The effect of particle concentration and turbidity on the number of identified particles and the particle mean size measured using inline transmission based digital holography (DH) is investigated [43]. It is demonstrated that measurement performance can be significantly improved by reducing the sample depth though lower concentration loading is preferred in DH. Taking 50% detection efficiency as the threshold, it can be concluded that the DH setup works well up to 0.1 v/v% and 0.2 v/v% particle concentrations for 10 mm and 5 mm sample depths, respectively, and 150 NTU turbidity level for 10 mm sample depth. Nevertheless, the results presented in this thesis provide information regarding the practical applications and limitations of the DH system for particle characterization.

A theoretical relationship is deduced here between the particle size and the digital image sensor resolution, both for collimated and diverging illumination, considering DIH system. From this derivation, the required digital image sensor resolution (pixel dimension) can be recommended for resolving particular particle size. This relationship indicates how small particle size can be measured with a given optical and digital system before using any magnifying optics.

A novel and efficient algorithm, the ‘Superimposition Method’, for the measurement of length, position, and off-axis tilt of needle-like particles, has been developed without any prior assumption [44]. The method is demonstrated using fiber of known length and orientation. The method has also been applied to an aqueous suspension of fibers in population to measure the real length distribution and their angles of orientation along the optical axis. The obtained real length distribution shows good match with that obtained using the microscopy.

An algorithm, based on digital inline holography, is developed to characterize mixed morphological (here for spheres and needles) particulate system. This particular algorithm combines other two published algorithms of which one is suitable for spherical particulate system and another for needles. In addition, particle classification

and separation is carried out determining a geometric feature (i.e., eccentricity) for each particle image within the reconstruction volume. Then, the algorithm has been demonstrated in real mixed morphological particulate system with varying proportions of the spherical particle population to needle population. The algorithm successfully works for the different proportions of needles to spheres. The result shows this algorithm can be successfully used to track and quantify the different polymorphs for APIs in the crystallization processes, especially for two extreme shapes of the particulate system (e.g., spheres or cuboids and needles). Obvious limitation is that overall particle concentration should be low in order to get better accuracy. Single crystal study is also performed.

In summary, this thesis has been addressed to find out the practical limitations and applications of the digital inline holographic (DIH) system on particle characterization concerning particle concentration and turbidity effects [43], and to develop some computer algorithms to estimate particle size and shape distribution in population, especially for more complicated particulate systems such as needles and mixed morphologies [44-45]. Further, a theoretical relationship is deduced here between the particle size and the digital image sensor resolution (pixel), both for collimated and diverging illumination, considering DIH system.

6.2 Directions for Future Research

Usually, convolution based reconstruction algorithm is being used in the digital particle holography, which uses equivalent three 2-D fast Fourier Transformation (FFT2) computational intensive calculations for each reconstruction [22, 78]. Though high performance computing system is available at the present days, a reasonable amount of time has to be paid to reconstruct a sample volume. Hence, there is a scope to find an alternate solution, whether by developing of a method or hardware implementation, such as graphics processing unit (GPU) [93-95].

In the today's digital holography, the resolution of CCD or CMOS arrays is one order of magnitude lower than that of the classical photographic film [24]. Due to the poor spatial resolution of the available solid state imaging sensor, DH prefers lower concentration in transmission mode [25]. As a result, DH will take off as a widely embraced tool for particle characterization at the time of its transition from transmission to the reflection mode. So far, some literature has been found out in

which 90° scattering of the object wave front was captured by the digital sensors [25, 36]. No literature is reported about the reflection based digital particle holography. There is a scope to develop the reflection based digital particle holography that can fill the demand of continuous development of the PAT tools for monitoring particulate processes.

Recently, nanotechnology is emerging subject matter in every discipline. For characterizing nano-materials or particles, optical system can be improved by using lower wavelength laser source, as for example ultraviolet (UV) range light, implementation of improved optics and digital image sensor. System could also be upgraded adopting rigorous image analysis methods.

At last, digital holography is a special tool for phase imaging [82, 96], unlike optical holography or photography. It provides direct access to the phase of the object wave which shows phase perturbation. Phase of the light wave from the phase perturbation of the phase objects, such as crystals, living cells, and microorganisms, could be measured through the direct access of DH.

Appendix

Flow chart of the algorithm for mixed morphological particulate system characterization using digital inline holography

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