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2009

Emmanouil, D., Taslima, K., Rajendran, A., Kariwala, V., Naughton, T. J., & Anand, A. (2009). Microparticle characterization using digital holography. *Chemical Engineering Science*, 65(2), 1-34.

<https://hdl.handle.net/10356/91815>

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Microparticle characterization using digital holography

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Abstract

Digital holography is an effective three dimensional imaging technique, with the potential to be used for particle size measurements. A digital hologram can provide reconstructions of volume samples focused at different depths,

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18 overcoming the focusing problems encountered by other imaging based tech-
19 niques. Several particle analysis methods discussed in the literature consider
20 spherical particles only. With the object sphericity assumption in place, anal-
21 ysis of the holographic data can be significantly simplified. However, there are
22 applications, such as particle analysis and crystallization monitoring, where
23 non spherical particles are often encountered. This paper discusses the pro-
24 cessing of digital holograms for particle size and shape measurement for both
25 spherical and arbitrarily shaped particles. An automated algorithm for iden-
26 tification of particles from recorded hologram and subsequent size and shape
27 measurement is described. Experimental results using holograms of spheri-
28 cal and non-spherical particles demonstrate the performance of the proposed
29 measuring algorithm.

30 *Keywords:* Particle; Particulate processes; Crystallisation; Digital Holography;
31 Particle Size; Image processing.

32 1 Introduction

33 The size and shape in many cases, govern the properties of particulate mat-
34 ter [Winn and Doherty, 2000]. Characterizing these properties accurately is
35 an important step towards tailoring them to suit process and product require-
36 ments. Although the measurement of particle size is a mature technique, shape
37 measurement is rather new. Advancements in imaging based techniques (for
38 example, particle vision measurement) has made it possible to obtain both
39 size and shape of particles through 2D imaging tools followed by the use of
40 an image processing algorithm. Online measurement techniques are also being
41 continuously investigated [Ruf et al., 2000, Abbas et al., 2002, Patience and
42 Rawlings, 2001, Larsen et al., 2007, Wang et al., 2008]. The techniques based
43 on 2D imaging systems suffer from limited depth of focus imposed by the
44 required magnification. As a consequence, captured images contain blurred
45 and out-of-focus objects limiting the successful application of image analy-
46 sis [Larsen et al., 2007, Wang et al., 2008]. Hence there is a strong need to
47 develop online sensors and imaging tools capable of providing accurate size
48 and shape information of a population of particles.

49 Holographic particle analysis has been reported as an effective technique for
50 measurement of the position, size distribution, and speed of particles or other
51 micro objects suspended in fluids [Vikram, 1992]. It provides 3D volume infor-
52 mation from a single hologram acquisition. The actual location of the particle
53 can be determined subsequently by reconstructing the recorded hologram at
54 different depths. Thus holography does not encounter the problem of out-of-
55 focus particles as classical imaging tools. In comparison with classical film
56 based holography, use of digital holography is more attractive due to the ease

of set-up and recording process using a digital camera [Schnars and Juptner, 2005, Frauel et al., 2006, Asundi and Singh, 2006]. The application of digital holography for particle analysis has been discussed in literature [Xu et al., 2002, Schnars and Juptner, 2005, Asundi and Singh, 2006]. Similar techniques have been used for several applications including the study of plankton in sea water [Sun et al., 2007] and holographic particle image velocimetry [Hinsch, 2002].

In this work, we benchmark digital holographic microscopy for measurement of particle size distribution of a population of microparticles. For this purpose, we use non-crystalline opaque microparticles, which are representative of particles encountered in real particle characterization situations. In particular, we investigate two classes of particles, namely spheres and fibers which represent the extremes of particle shapes encountered in real world situations, i.e. spheres and needles and are hence ideal for benchmarking.

An algorithm is required for processing digital holograms to extract size and shape information. Although fringe analysis based methods are available for estimating the size or the focusing point of particles recorded in digital holograms without reconstruction [Onural and Ozgen, 1992, Buraga-Lefebvre et al., 2000, Denis et al., 2006, Soontaranon et al., 2008], these methods assume spherical shape of particles and hence are not applicable for the measurement of other shapes. In this paper, we propose an algorithm that uses hologram reconstructions at several depths to extract size and shape information. In this algorithm, particles are segmented from the background using Canny edge detection [Canny, 1986] and best focusing depth for each particle is identified based on a focusing metric. The size and shape information are extracted using the reconstructed image at the best focusing depth. The proposed algo-

83 rithm is automated, but has a few tuning parameters. The performance of the
84 algorithm, however, is not very sensitive to the choice of tuning parameters.
85 This is verified by applying the proposed algorithm to experimentally recorded
86 holograms of particles with different shapes (spherical and needle shaped), size
87 ranges ($10\text{ }\mu\text{m}$ —few hundred μm) and experimental conditions (static, suspen-
88 sion, and flow-through). In every case, the particle size distributions (PSD)
89 measured by the algorithm closely matches with the expected PSD. In sum-
90 mary, this paper successfully establishes digital holography as a technique for
91 accurate measurement of PSD and opens up avenues for potential applications
92 to particle characterization and analysis.

93 The rest of the paper is organized as follows: In Section 2, the principles
94 of holography, recording setup and fundamentals of digital holography are
95 discussed. In Section 3, the algorithm used for the processing of the recorded
96 digital holograms is described. In Section 4, the experiments used to verify the
97 accuracy and the effectiveness of the algorithm are presented and Section 5
98 concludes the paper.

99 **2 Holography and holograms**

100 *2.1 Principle*

101 Holography is an optical phenomenon based on the fundamental theory of
102 diffraction and interference. To explain this phenomenon, a typical optical
103 set-up for traditional free-space in-line digital holographic microscopy, shown
104 in Fig. 1 (a), is used. In this configuration, a strongly coherent light source
105 (laser) illuminates an object. A part of the light is diffracted by the object cre-

106 ating the ‘object beam’, while another part, called “reference beam” remains
 107 undiffracted. The object and reference beams interfere with one another and
 108 form an interference pattern which is recorded by a light sensitive medium,
 109 *e.g.* photographic film [Schnars and Juptner, 2005]. The recorded interference
 110 pattern is called a hologram. When the hologram recorded by the photo-
 111 graphic film (after subsequent chemical film development) is illuminated by
 112 the original reference wave in the absence of the object, it enables the recon-
 113 struction of the original object beam at the previous object position as shown
 114 in Fig. 1 (b). The reconstruction results in a virtual image of the original
 115 object which exhibits the effects of perspective and depth of focus. Hence,
 116 in comparison with other 2D imaging techniques, holography offers a unique
 117 characteristic of restoring 3D volume information from a single hologram ac-
 118 quisition [Schnars and Juptner, 2005].

119 Classical film based holography provides high resolution hologram recording,
 120 but suffers from an increased processing time required for the cumbersome
 121 recording and subsequent chemical film development for reconstruction pro-
 122 cess. The use of charged couple device (CCD) as the holographic recording
 123 medium makes the technique more attractive by eliminating the aforesaid
 124 complications associated with classical film based holography. This technique,
 125 termed digital holography, provides the possibility to perform reconstructions
 126 numerically, which has several advantages, as illustrated in the next section.

127 *2.2 Digital in-line holographic microscopy*

128 Figure 2 shows the schematic of the digital in-line holography setup used to
 129 record the holograms. The setup consists of a laser source and a microscope

130 objective lens that focuses the laser beam on a pinhole located at a distance
 131 D from the recording camera. The sample whose center is located at a dis-
 132 tance d from the recording camera is illuminated by the resulting spherical
 133 diverging beam. One part of the illuminating beam passes through the sample
 134 undiffracted and acts as the reference beam. The other part of the illumi-
 135 nating beam is diffracted by the particles within the sample generating the
 136 object beam. The interference pattern created by the object and the reference
 137 beam, called as the hologram, is recorded by the recording camera. Figure 3(a)
 138 shows an example of a digital hologram of a population of polymer spheres
 139 suspended in water.

140 The reconstruction of the scene at a desired distance can be obtained by multi-
 141 plying the recorded interference pattern with the reference wave and propagat-
 142 ing it through a Fresnel-Kirchhoff integral that can be evaluated numerically
 143 using the convolution method [Schnars and Juptner, 2005]. Figures 3(b) and
 144 (c) show sample reconstructions, at two different depths, obtained from the
 145 hologram shown in Fig. 3(a). As can be seen, objects at different positions
 146 along the z axis (optical axis) come into focus as d changes. It is worth em-
 147 phasizing that the two reconstructions are obtained from the same hologram
 148 and hence it is possible to overcome the focusing issue that is encountered in
 149 classical imaging techniques. This is an unique advantage of holography. It is
 150 worth noting that the diverging beam used in the current set-up introduces
 151 a magnification. This magnification, which is a function of the reconstruc-
 152 tion distance, has to be properly accounted for in order to perform quantita-
 153 tive measurements from the reconstructed images [Schnars and Juptner, 2005,
 154 Vikram, 1992].

155 3 Digital hologram processing for particle measurements

156 There are several algorithms available for extraction of particle properties,
157 such as size and location, from the hologram without reconstruction [Buraga-
158 Lefebvre et al., 2000, Denis et al., 2006, Soontaranon et al., 2008]. These
159 algorithms, however, assume that the particles are spherical and hence cannot
160 be used for accurate size and shape measurement of non-spherical particles. In
161 this section, we propose a method for obtaining the particle information from
162 reconstructions of recorded hologram at several depths. For easier illustration,
163 the overall algorithm is pictorially represented in Fig. 4. Details of the pro-
164 posed algorithm for processing the hologram and subsequent image analysis
165 for particle characterization are discussed in the following sections.

166 3.1 Numerical reconstruction

167 Naturally, the first step after hologram acquisition, is reconstruction of the
168 hologram at several depths. The distance between successive reconstructions
169 depends on the minimum size of the particles to be studied and the depth
170 resolution of the system. In general, this distance should be similar to the
171 minimum particle size that needs to be identified, if not smaller.

172 3.2 Image segmentation

173 A possible approach for image segmentation involves thresholding the inten-
174 sity of the reconstruction [Malkiel et al., 2004]. In threshold based segmen-
175 tation, pixels with intensity value lower than the threshold are considered to

176 belong to a particle, while pixels with intensity values higher than the thresh-
177 old are considered to be background [Calderon De Anda et al., 2005, Sarkar
178 et al., 2009]. In general, the selection of an appropriate threshold value can
179 be difficult. An inappropriate choice for threshold value results in identifica-
180 tion of noise, *e.g.* impurities in the sample solution, shot noise from the laser,
181 diffraction effects from multiple objects, etc., present in reconstructed holo-
182 grams as particles or unidentified particles. Furthermore, imperfections such
183 as non-uniform illumination of the hologram lowers the performance of thresh-
184 old based segmentation. Some additional details regarding the drawbacks of
185 threshold based segmentation are given elsewhere [Darakis et al., 2008].

186 In this paper, we use the Canny edge detection technique for particle seg-
187 mentation [Canny, 1986]. In this technique, a Gaussian filter with standard
188 deviation, σ , is first applied to the reconstruction to reduce the effect of noise.
189 Subsequently, the edges are detected by examining the gradients of intensity
190 for local maxima. Here, thresholding with hysteresis is used to classify the
191 identified maxima using two threshold values, t_L and t_H ($t_H > t_L$). Max-
192 ima with a value lower than t_L are identified as not being edges. Similarly,
193 maxima with a value higher than t_L are identified as edges. Maxima with
194 value between t_L and t_H are identified as edges only if they are connected to
195 identified edges [Sarkar et al., 2009]. Apart from good localization, the use of
196 edge detection-based particle segmentation on digital holograms also has the
197 advantage that highly unfocused particles are not detected, as particles are
198 usually surrounded by strong edges only close to their best focusing point.

199 For every reconstruction, the edge detection algorithm results in a binary
200 matrix with ones corresponding to pixels where edges are detected and ze-
201 ros elsewhere. To identify particles from this matrix, dark areas completely

enclosed by edges are filled to form blobs, which are considered as possible particles. Open ended lines are removed by erosion followed by dilation in order to eliminate noisy formations that frequently appear in the background. This, in principle, does not change the size of the identified blobs. To avoid identifying noise as particles, blobs with diameter less than a cut-off value are removed. Further, blobs touching the edges of the reconstruction window are also removed, as these particles are partially located outside the reconstruction. Finally, a set of blobs corresponding to particles detected in the reconstruction under consideration is obtained.

The edge detection algorithm requires the selection of 3 parameters: the standard deviation of the filter, σ , and the two thresholds, t_L and t_H . A large σ value results in a Gaussian filter with large width, which may affect the size of small particles causing erroneous size measurements. Thus, σ is selected such that the filtering operation does not alter the size of the particles under investigation. On the other hand, the thresholds t_L and t_H only affect the number of identified particles and not their sizes. High values lead to identification of fewer particles (particles with very sharp edges), while lower values lead to false positives (areas surrounded by sharp edges). The false positives correspond to fringes caused by the twin image or out of focus particles and are much brighter than focused particles. As a result, they can be easily identified and neglected based on their average intensity. The performance of the algorithm is not very sensitive to the selection of these parameters. In this paper, t_L and t_H are chosen as 0.3 and 0.6, respectively, and the same set of parameters have been used for the different experimental results presented in Section 4 and were found to give good results for holograms recorded under different conditions and containing particles of different size ranges.

228 3.3 Localization of a particle

229 As the particle segmentation step is applied to every reconstruction of the
230 hologram, a single particle may be identified in several reconstructions. Fur-
231 ther, the magnification depends on the reconstruction distance. Thus, the best
232 focusing depth needs to be determined for accurate size and shape measure-
233 ment, *i.e.* particle localization has to be performed.

234 To find the best focusing depth, blobs with overlapping spatial positions at dif-
235 ferent reconstruction depths are given identical labels. Subsequently, the mean
236 intensity and the variance of each reconstruction is calculated. The reconstruc-
237 tion depth at which these parameters show a minimum is taken as the best
238 focused depth. Particles with minimum of the focusing metric occurring at
239 the first or last examined depth, are likely to be located outside the examined
240 depth range and hence are ignored to avoid erroneous measurements.

241 The determination of focusing metric has been independently verified [Darakis
242 et al., 2008]. In the verification experiments several ceramic beads (average
243 diameter 80 microns) were positioned on a glass slide which was placed on
244 a movable mount. The slide was then positioned at a known distance along
245 the optical axis and the best focused depth was calculated according to the
246 algorithm mentioned above. It was found that the maximum observed error
247 between the measured and expected slide position was 120 μm over a scanned
248 depth of 14 mm. This translates into a 0.35 % error in the magnification,
249 which is negligible.

250 3.4 Particle size and shape measurements

251 Particle segmentation and focusing provide the area occupied by each particle
252 at its best focusing depth. In general, the particles are not spherical and thus
253 the identified regions are not circular. As a result, it is not always easy to
254 measure their sizes. Although several metrics are available for characterizing
255 non spherical particles, we limit ourselves to the following two as they represent
256 quantities that are easy to measure and are capable of classifying particle
257 shape:

- 258 (1) Equivalent diameter, corresponding to the diameter of a circle with the
259 same area as the identified area. This parameter is used for the systems
260 that are spherical, or close to spherical.
- 261 (2) Major and Minor axes lengths, corresponding to axes lengths of an ellipse
262 with the same normalized second central moment as the identified area.
263 This metric is used for non-spherical particles that have one characteristic
264 length sufficiently longer than the others [Kempkes et al., 2008].

265 Naturally, the distribution of equivalent diameter corresponds to the particle
266 size distribution (PSD) of particles, if the particles are spherical.

267 As mentioned earlier, the system introduces magnification. Thus, the measured
268 values (equivalent diameter and axes lengths) need to be converted to true
269 values using the magnification factor M as

$$r = \frac{r_{pixels} \Delta x}{M(d_o)}, \quad (1)$$

270 where r_{pixels} is the measured size of the particle in pixels, Δx the size of a
271 pixel on the CCD camera, and d_o is the best focusing depth of each particle.

272 4 Experiments and results

273 In this section, several experiments are presented to verify the performance
274 of algorithm described in Section 3. In these experiments, the recording setup
275 shown in Fig. 2 was used. The setup uses a laser source with a wavelength
276 $\lambda = 532$ nm. A $60\times$ microscope objective lens focuses the laser beam on a $1\text{ }\mu\text{m}$
277 pinhole located at a distance $D = 63.3$ mm from the recording camera (The
278 Imaging Source DMK41BF02, Bremen, Germany), with $N_x \times N_y = 1280 \times 960$
279 monochrome pixels measuring $4.65 \times 4.65\text{ }\mu\text{m}$ each. The threshold values and
280 the standard deviation of the Gaussian filter were chosen as $t_L = 0.3$, $t_H = 0.6$,
281 and $\sigma = 1.5$, respectively. These parameters were retained for all experimental
282 systems listed in Table 1.

283 The experiments were designed to achieve two primary goals. Firstly, to demon-
284 strate the applicability of digital holography for size and shape measurement
285 of populations of spherical and non-spherical particles. Secondly to demon-
286 strate this under situations that are encountered in practice, namely dry and
287 wet (particles suspended in solution) conditions and flowing suspensions. The
288 results are presented in order of the complexity of the measurement scenario.
289 Firstly, characterization under dry and wet condition of spherical particles are
290 presented. Then the measurement of needle shaped particles on a glass slide
291 and in suspension is presented.

292 4.1 Ceramic beads on glass slide

293 In order to verify the performance of the particle measurement algorithm, ce-
294 ramic beads placed on a glass slide were used. The slide was positioned normal
295 to the optical axis and a hologram was recorded. This procedure was repeated
296 several times to account for a population of particles. Fig. 5(a) shows one of
297 the recorded holograms and Fig. 5(b) shows a sample reconstruction. A total
298 of 31 holograms were processed using the procedure described in Section 3.
299 Here, a depth of 3 mm with a step size of $50\text{ }\mu\text{m}$ was used for each hologram
300 resulting in identification of 377 different particles.

301 In addition, scanning electron microscopy (SEM) was used to record several
302 images of particles taken from the same population. One such image is shown
303 in Fig. 5(c). To segment the SEM images and extract particles (bright areas),
304 Canny edge detection was used with the same parameters as used for the seg-
305 mentation of reconstructed holograms. The focusing was performed manually
306 during the recording and the size of each particle was measured considering
307 the magnification of the SEM. Overall, 615 different particles were measured
308 from the SEM captured images.

309 Figure 5(d) shows the resulting distributions of equivalent diameters of ceramic
310 beads obtained from the holography and the SEM experiments. The mean
311 particle size identified from the holographic microscopy and the SEM are 80.24
312 $\pm 14.46\text{ }\mu\text{m}$ (mean $\pm$ standard deviation) and $79.23 \pm 13.79\text{ }\mu\text{m}$, respectively,
313 showing good agreement. Hence, this experiment verified the accuracy of the
314 digital holography based measurement algorithm.

315 4.2 Polymer particles in flow-through system

316 For this experiment, the object used in the set-up consisted of a flow cell with
317 flowing particle suspensions. National Institute of Standards and Technology
318 (NIST) certified polymer microspheres manufactured by Duke Scientific Cor-
319 poration, USA with diameter of $40.25 \pm 0.32 \mu\text{m}$ were continuously pumped
320 from a beaker through circulation loop using a peristaltic pump. The particles
321 flowed through a quartz flow cell with dimensions $12.5 \text{ mm(L)} \times 12.5 \text{ mm(W)}$
322 $\times 65 \text{ mm(H)}$ and optical path length of 10 mm at a flow rate 5 mL/min.
323 Holograms were captured at the rate of one hologram per second. Reconstruc-
324 tions were carried out with a distance of $50 \mu\text{m}$ between each other covering
325 a volume with a depth of 8 mm.

326 Figure 6(a) shows one of the recorded holograms for the polymer particles
327 and a sample reconstruction is shown in Fig. 6(b). By analyzing 9 holograms,
328 the algorithm identified 437 different particles. The resulting distribution of
329 equivalent diameter is shown in Fig. 6(c). The obtained mean particle size was
330 $43.89 \pm 3.38 \mu\text{m}$. There is an error of $\approx 4 \mu\text{m}$ between the actual and measured
331 particle size, which is below the resolution limit of the system ($\approx 7 \mu\text{m}$). In
332 Fig. 6(c), the presence of particles with diameter $\approx 60 \mu\text{m}$ can also be noted.
333 This indicates the presence of agglomerated particles.

334 4.3 Characterization of fibers

335 The measurement of fibers deserves special attention. Unlike the case of spheres,
336 where each particle is best focused on a particular plane, the case of fibers is
337 not straight-forward. Fibers that lie orthogonal to the optical axis, will be

338 focused at a particular depth. However, for fibers that are not orthogonal to
 339 the optical axis, only a part of the fiber will be focused at particular depth.
 340 Under these situations, the algorithm would measure only the length of their
 341 projections on the reconstruction plane. In order to study this, the first set of
 342 experiments involved the measurement of a single fiber placed on a glass slide.
 343 In the second set of experiments, measurements of a population of fibers in
 344 a solution are reported. In this experiment, particles with needle shape were
 345 studied using carbon fibers (TOHO Tenax Type 383) suspended in water.

346 *4.3.1 Single fiber on a glass slide*

347 In the first set of experiments, a single fiber was placed on a glass slide con-
 348 nected to a rotatable mount that can be adjusted to obtain a desired out-of-
 349 plane tilt. Two fibers with lengths 150 μm and 1320 μm respectively, were
 350 allowed to rotate from 0° to 55° tilt at 5° interval with respect to the opti-
 351 cal axis. The holograms were processed according to the algorithm discussed
 352 earlier. Since the fibers are clearly non-spherical, the major and minor axis
 353 obtained by fitting an ellipse were used characterize the sizes. It is worth not-
 354 ing that while the fibers are rectangular, fitting an ellipse leads to an over
 355 prediction of the axis lengths. Hence, the measured length of the major and
 356 minor axis are multiplied by a factor of $\sqrt{3}/2$ to convert them to fiber length
 357 and diameter, respectively [Eggers et al., 2008].

358 Based on the length and the angle of tilt the expected projected length, as
 359 shown by the continuous lines in Fig. 7(a) and Fig. 7(b), can be calculated.
 360 The comparison between the measured and calculated projected lengths is
 361 shown in Fig. 7(a). As it can be seen the algorithm successfully estimated the

362 projected length of the $150\text{ }\mu\text{m}$ long fiber within the expected accuracy even
 363 for large out-of-plane tilts. For the $1320\text{ }\mu\text{m}$ long fiber, the algorithm mea-
 364 sured the fiber only for small tilts (cf. Fig. 7(b)). For higher tilts, long fibers
 365 are not entirely focused on a single reconstruction and hence the algorithm
 366 neglects such fibers, as they are not enclosed by strong edges. In this way,
 367 erroneous measurements are avoided but false negatives are introduced. It is
 368 worth pointing out that in the case of fibers the measured lengths that are
 369 eventually identified correspond not to the real lengths but only the projected
 370 lengths. In this case rigorous comparison with other measurements is diffi-
 371 cult, unless complex inversion techniques to convert projected lengths to real
 372 lengths for a population of particles are considered [Eggers et al., 2008]. To
 373 overcome the limitation associated with long tilted fibers as well as complex
 374 inversion techniques to convert projected lengths to real lengths, we recently
 375 developed a technique to measure the real lengths of fibers from hologram
 376 reconstructions directly without a priori knowledge of fiber tilt, position and
 377 length [Kempkes et al., 2009]. However it is computationally intensive and its
 378 applicability to fibers shorter than $\approx 200\text{ }\mu\text{m}$ needs to be improved. Hence,
 379 this technique is not applied in this work.

380 4.3.2 *Carbon fibers suspended in water*

381 Based on the understanding gained from the first set of experiments, mea-
 382 surements were performed with a population of carbon fibers contained in a
 383 quartz cuvette with dimensions $12.5\text{ mm(L)} \times 12.5\text{ mm(W)} \times 48\text{ mm(H)}$ with
 384 an optical path length of 10 mm . The recorded holograms were reconstructed
 385 with a step size of $20\text{ }\mu\text{m}$ between each other to cover a depth of 8 mm . One
 386 of the recorded holograms and a sample reconstruction are shown in Fig. 8(a)

and Fig. 8(b), respectively. Using the procedure described in Section 3, 13 holograms were analyzed to identify 283 fibers. The resulting major and minor axis length distributions are shown in Fig. 8(c) and Fig. 8(d), respectively. The obtained mean major and minor axis lengths of these fibers were 138.74 μm and 13.34 μm , respectively.

The axis length distribution shown in Fig. 8(d) is indicative of the shape of the particles and can be used to classify particle shape. While spherical particles tend to lie close to the diagonal, needle shaped particles with a very high major to minor axis ratios tend to lie near the ordinate axis. This information is of importance for researchers interested in particle characterization and is typically not obtained in conventional particle analysis systems. From Fig. 8(d), the ALD clearly shows that the population contains needle shaped particles with similar widths but varying lengths.

5 Conclusions

In this paper, a particle size measurement methodology based on digital holographic microscopy was presented. The algorithm proposed for hologram processing is largely automated, but requires the selection of a few tuning parameters for particle segmentation. The effectiveness of the algorithm, however, is not very sensitive to these parameters, as shown through a series of experiments conducted for particles with different shapes, size ranges, and conditions. Even for the special case of randomly oriented fiber, the algorithm successfully measured the projected lengths with good accuracy, unless the fiber is long enough. Hence, the size and shape measurements obtained using digital holography microscopy show good agreement with the given sizes and

411 shapes measured using independent techniques. The results obtained in this
412 study extend the potential of digital holography for applications in particle
413 characterization and online monitoring of particulate processes.

414 Digital holography microscopy opens up promising possibilities in particle
415 characterization including 3D measurements. Our future work will be directed
416 at demonstrating the 3D measurements for non-symmetric particle population
417 such as fibers and validation for false positives/negatives etc. Besides these,
418 digital holographic based technique deserves special attention in dealing with
419 high suspension density, transparent and semi-transparent particles, which will
420 also be worth investigated in future.

421 **Acknowledgments**

422 Partial funding from the Irish Research Council for Science, Engineering and
423 Technology under the National Development Plan through grant no. PD/2007/11
424 and partially by the Office of Research, Nanyang Technological University
425 through grant no. RG25/07 is acknowledged. The authors would also like to
426 thank Vijay Raj Singh and Qu Weijuan for the assistance.

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505 **List of Figure Captions**

506 Figure 1:

507 Principle of holography: (a) A typical set-up for recording of a hologram; Solid
508 lines correspond to reference beam and dotted curves correspond to object
509 beam. (b) Reconstruction of the hologram.

510 Figure 2:

511 Schematic of digital holographic recording setup.

512 Figure 3:

513 Digital holographic microscopy of polymer spheres suspended in water (a)
514 Hologram; (b) a sample reconstruction at distance d_1 ; (c) reconstruction at
515 a different distance d_2 . Solid circles indicate particles focused at the chosen
516 reconstruction depth.

517 Figure 4:

518 Steps of hologram processing algorithm. (a) Numerical reconstruction at sev-
519 eral depths; (b) Image segmentation using Canny edge detection; (c) Local-
520 ization of a particle; and (d) Particle size and shape measurement, d_{eq} denotes
521 the equivalent diameter for spherical objects, while m_1 and m_2 represent the
522 major and minor axis for non-spherical objects.

523 Figure 5:

524 Digital holographic microscopy data of ceramic beads on glass slide: (a) One
525 of the recorded digital holograms, (b) Example of a reconstructed image. (c)

526 Example of an SEM image of ceramic beads. Bright areas correspond to par-
527 ticles whereas dark circular areas on the background are irregularities of the
528 sample holder. (d) Comparison of PSD obtained from digital holography and
529 SEM.

530 Figure 6:

531 Size measurements for polymer particles in flow-through system (a) Hologram;
532 (b) sample reconstruction; (c) distribution of equivalent diameter. Measured
533 mean equivalent diameter is $43.89 \pm 3.38 \mu\text{m}$ where as expected mean diameter
534 is $40.25 \pm 0.32 \mu\text{m}$.

535 Figure 7:

536 Measurement accuracy of the algorithm for fibers for out-of-plane tilt. (a) 150
537 μm long fiber; (b) 1320 μm long fiber. The error bars take into account the
538 system resolution and the uncertainty in the measurement of the tilt.

539 Figure 8:

540 Size measurements for carbon fibers suspended in water (a) Hologram; (b)
541 sample reconstruction; (c) Major axis length distribution; and (d) Axis length
542 distribution.

543 **List of Table Captions**

544 Table 1:

545 Characteristics of various particles used in the holography measurements.

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Fig. 4. Steps of hologram processing algorithm. (a) Numerical reconstruction at several depths; (b) Image segmentation using Canny edge detection; (c) Localization of a particle; and (d) Particle size and shape measurement, d_{eq} denotes the equivalent diameter for spherical objects, while m_1 and m_2 represent the major and minor axis for non-spherical objects.

(a)

(b)

(c)

(d)

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(a)

(b)

(c)

(d)

Fig. 8. Size measurements for carbon fibers suspended in water (a) Hologram; (b) sample reconstruction; (c) Major axis length distribution; and (d) Axis length distribution.

Experimental system	Average size	Shape
Ceramic beads on glass slide	Diameter $\approx 80 \mu\text{m}$	Spherical to elliptical
Polymer particles in flow-through system	Diameter $40 \mu\text{m}$	Spherical
Carbon fibers	Diameter $\approx 8 \mu\text{m}$, length $\approx 50\text{--}500 \mu\text{m}$	Needle

Table 1

Characteristics of various particles used in the holography measurements.