Three dimensional digital holographic profiling of micro-fibers

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Abstract: A method to measure the size, orientation, and location of opaque micro-fibers using digital holography is presented. The method involves the recording of a digital hologram followed by reconstruction at different depths. A novel combination of automated image analysis and statistical techniques, applied on the intensity of reconstructed digital holograms is used to accurately determine the characteristics of the micro-fibers. The performance of the proposed method is verified with a single fiber of known length and orientation. The potential of the method for measurement of fiber length is further demonstrated through its application to a suspension of fibers in a liquid medium.

OCIS codes: (090.1995) Digital holography; (100.5010) Pattern recognition; (100.6890) Three-dimensional image processing; (110.6880) Three-dimensional image acquisition; (180.6900) Three-dimensional microscopy; (350.4990) Particles

References and links

1. U. Schnars and W. Jueptner, “Digital Holography: Digital Hologram Recording, Numerical Reconstruction, and Related Techniques,” (Springer, Berlin, 2005).
2. Y. Frauel, T. J. Naughton, O. Matoba, E. Tajahuerce, and B. Javidi, “Three-dimensional imaging and processing using computational holographic imaging,” Proc. IEEE **94**, 636-653 (2006).
3. J. Garcia-Sucerquia, W. Xu, S. K. Jericho, P. Klages, M. H. Jericho, and H. J. Kreuzer, ”Digital in-line holographic microscopy,” Appl. Opt. **45**, 836-850 (2006).
4. O. C. Chee, V. R. Singh, E. Sim, and A. K. Asundi, “Development of a simple user-friendly commercial digital holographic microscope,” Proc. SPIE **6912**, 69120V (2008).
5. E. Darakis, T. Khanam, A. Rajendran, V. Kariwala, A. K. Asundi, and T. J. Naughton, “Processing of digital holograms for size measurements of microparticles,” Proc. SPIE **7155**, 715524 (2008).
6. T. Khanam, E. Darakis, A. Rajendran, V. Kariwala, A. K. Asundi, and T. J. Naughton, “On-line digital holographic measurement of size and shape of microparticles for crystallization processes,” Proc. SPIE **7155**, 71551K (2008).

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7. M. Malek, S. Coëtmellec, D. Allano, and D. Lebrun, "Formulation of in-line holography process by a linear shift invariant system: Application to the measurement of fiber diameter," Opt. Commun. **223**, 263-271 (2003).
8. D. Lebrun, A. Benkouider, S. Coëtmellec, and M. Malek, "Particle field digital holographic reconstruction in arbitrary tilted planes," Opt. Express **11**, 224-229 (2003).
9. L. Denis, T. Fournel, C. Fournier, and D. Jeulin, "Reconstruction of the rose of directions from a digital microhologram of fibres," J. Microsc. **225**, 283-292 (2007).
10. S. Wold, K. Esbensen, and P. Geladi, "Principal component analysis," Chemom. Intell. Lab. Syst. **2**, 37-52 (1987).

1. Introduction

Digital holography is an established 3D imaging technique [1, 2]. In this technique, a hologram of a scene is recorded digitally and numerical processing yields reconstructions focused at different depths of the scene. By using an appropriate recording setup, the scene can be magnified enabling digital holographic microscopy measurements [3, 4].

Earlier work dealing with holographic localization and size measurement of micro-particles dealt predominately with spheres. In the case of spherical particles, several localization algorithms have been developed [1, 5]. A few studies have dealt with the development of localization algorithms for fibers. These studies have thus far been limited to the analysis of the projections of particles in the plane of the camera [6], or require the knowledge about the orientation or the size of the object [7, 8, 9]. However, in several practical situations e.g. for a population of fibers in a solution, such information is seldom known limiting the application of these techniques. In this work we develop a novel method based on a simple optical setup to obtain 3D orientation, location, and size of micro-fibers.

The proposed method is benchmarked with a single fiber of known length and orientation. The potential of the method for accurate measurement of fiber length is further demonstrated through application to a suspension of fibers of unknown lengths and with unknown orientations. Since the algorithm can be applied with no information about the orientation or the size of the fiber, it opens up the possibility of using digital holography for particle characterization where determining the size and shape of non-spherical particles is still a major challenge.

2. Hologram acquisition and reconstruction

2.1. Recording setup

Figure 1 shows a schematic of the setup used to record digital holograms. The setup consists of a laser source (Lambda Photometrics DPGL3020, Harpenden, UK) with a wavelength $\lambda = 532$ nm. The beam is focused on a 1 μm pinhole by a microscope objective. The resulting monochromatic spherical diverging wave passes through the sample to a CCD camera (The Imaging Source DMK41BF02, Bremen, Germany), with $N_x \times N_y = 1280 \times 960$ pixels measuring 4.65×4.65 μm each, positioned at a distance of $D = 63.3$ mm from the pinhole.

Part of the light passing through the sample is diffracted by the fibers, creating an object beam. The remainder of the light passes through the sample undiffracted, thereby providing the spherical diverging reference beam.

The reference and object beams interfere on the camera sensor which records the amplitude of the interference pattern.

To obtain a reconstruction, the hologram is multiplied by the numerically approximated reference wave and the result is propagated to the desired distance d using the Fresnel-Kirchhoff diffraction integral [1].

3. Processing of the reconstructed holograms

Previous studies have addressed the issue of fiber measurements for a predefined off-axis tilt [7, 8]. The tilt was used to identify the pixels of the fiber. Although an important result, these

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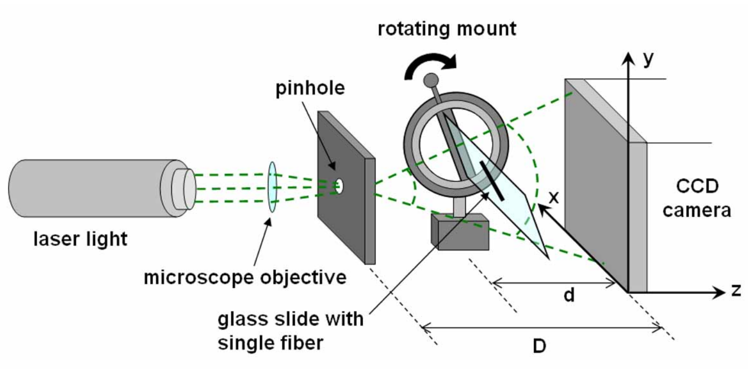
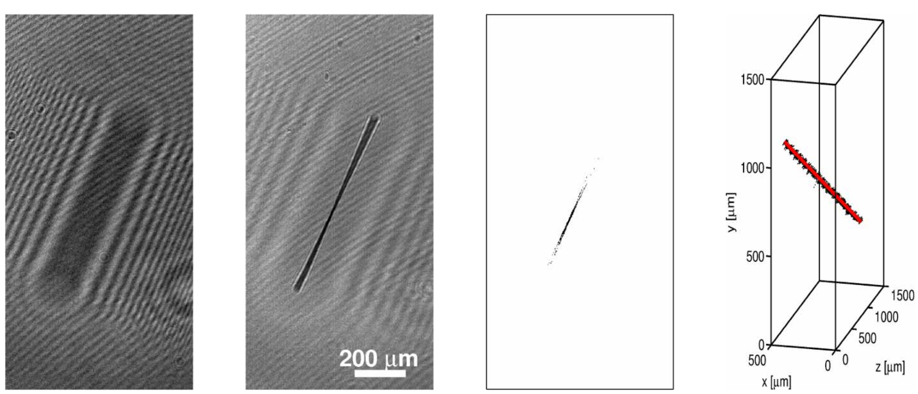


Fig. 1. Digital holographic recording setup.

methods require the knowledge of the tilt, a parameter which is seldom known in real world settings. To overcome this limitation we propose an algorithm that uses image analysis to determine the tilt and the length of the fibers. To describe the algorithm the hologram shown in Fig. 2 is used. The hologram was recorded using an opaque carbon fiber (type T383, Toho Tenax Europe GmbH, Wuppertal, Germany). The diameter and the length of the fiber, measured by a microscope were 7 μm and 1320 μm, respectively.

The fiber shown in Fig. 2 is tilted to an angle of 60° along the optical axis. Figure 2(b) shows a reconstruction of the hologram where it can be seen that only the middle part of the fiber is in focus at this particular reconstruction distance. The reconstructed hologram shows the projection of the fiber on the *xy* plane, from where the length of the fiber would be erroneously measured by an image analysis method as ~ 700 μm, unless the out-of-plane tilt was known. The automated technique described below measures the fiber length within a volume and not on a single projection, yielding correct size measurements without *a priori* knowledge of the fiber's tilt or position.



(a) (b) (c) (d)

Fig. 2. Three dimensional profiling of a tilted fiber: (a) recorded hologram; (b) a sample reconstruction; (c) thresholded reconstruction; and (d) point cloud with fitted line.

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Thresholding The hologram is reconstructed at N_z equally spaced intervals (taken as $N_z = 300$ with 20 μm increment in this paper). Histogram equalization is performed on the intensity of the complex valued reconstructions to account for variations of the intensity's range. The gray scale images are then converted to binary images using a simple threshold. The threshold is chosen in such a way that only the darkest pixels, which correspond to pixels in focus, are accepted as being part of fiber and are set to 1 in the binary image. The rest of the pixels are considered to be background and their value is set to 0 in the binary image. In this paper, a threshold of 5 (on a scale of 0 (black) to 255 (white)) was used for the equalized images and almost identical results were obtained for threshold values up to 15. Figure 2(c) shows a thresholded reconstruction. In-focus parts of the fiber appear in black.

The binary images obtained after thresholding are stored in a 3D matrix A of size $N_x \times N_y \times N_z$. The matrix A contains different clouds of points that correspond to the focused pixels of a fiber at different reconstruction depths. Pixels that are connected in 3D are assigned identical group labels, so that each point cloud corresponds to a different fiber. 3D dilation followed by 3D erosion is applied on A to merge parts of fibers that might appear broken up. To avoid identifying noise as fibers, groups with number of connected pixels smaller than a cut-off value are removed. Groups of connected pixels touching the reconstruction edges are also removed, since they are likely to be part of a fiber partially located outside the reconstruction volume. Following this, connected pixels within A are grouped together. Figure 2(d) shows the point cloud of the fiber in black.

Size and orientation measurement by 3D line fitting Since the micro-fibers are elongated rods, fitting a straight line on each group of pixels yields the orientation and length. Traditional regression methods, such as least squares, are inadequate as they are based on the assumption of error free predictors. However, as it can be seen in Fig. 2(b), the intensity of the reconstruction is contaminated by speckle noise resulting in noisy point clouds that can be seen in Fig. 2(d). Hence, tools such as the principal component analysis (PCA) that can account for errors in the predictors have to be utilized [10]. Figure 2(d) shows in red the line that was fitted in the point cloud of the tilted fiber using PCA. From this line the characteristics of the corresponding fiber (location, size, and orientation) can be measured. Results verifying the accuracy of the proposed method are discussed in the following section.

4. Results

4.1. Tilted fiber

The method outlined in Section 3 was applied for validation purposes first to the fiber of known length and orientation shown in Fig. 2. In this experiment, the fiber was positioned on a glass plate, which was fixed in a revolving mount. The revolving mount allowed for a setting of the out-of-plane-tilt of the sample with an accuracy of 1°, and was positioned between the pinhole and the camera. A total of 17 different angles were used, varying from 0°, where the fiber is positioned perpendicular to the optical axis of the system, to 80°. For angles larger than 80°, the glass plate prevented the fiber from being seen in the reconstruction. In Fig. 2(d), the point cloud for the fiber at an out-of-plane-tilt of 60°, along with the line fitted to it, are shown.

Figure 3 shows the comparison of the resulting orientation and length of the fiber with the true orientation and length. As can be seen from Fig. 3, the method accurately measures the fiber orientation with less than 3 degrees error. The same conclusion also holds for the measured fiber length, which corresponds very well to the true fiber length over a large range of tilts with relative errors below 4%. Overall, the ability of the proposed method to accurately determine fiber location, length, and orientation has been demonstrated.

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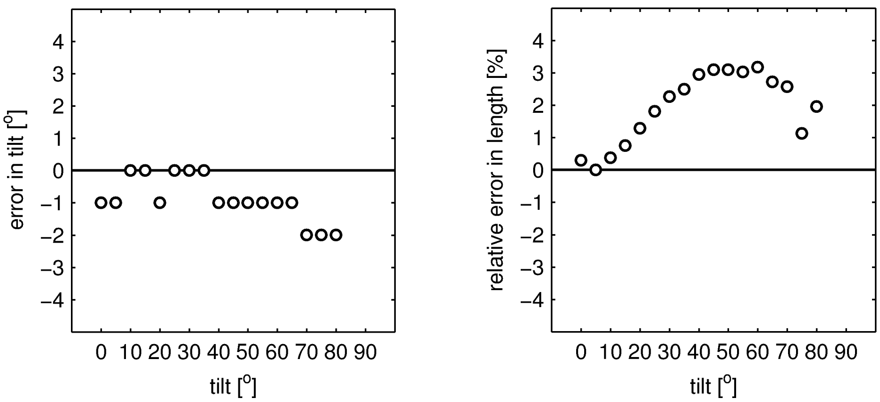
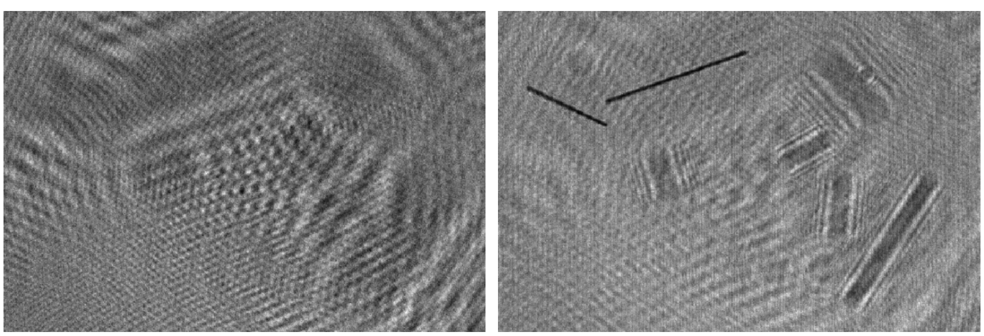


(a) (b)

Fig. 3. Measurements for single fiber. (a) Error in the measurement of the fiber's orientation, and (b) relative error in the measured fiber length for different out-of-plane-tilts of the fiber.

4.2. Fibers in suspension

The proposed method was then applied in a real particle analysis setting, an area where the technique can have useful applications. For this experiment, a suspension of unknown length carbon fibers in water was prepared and contained in a quartz cuvette with dimensions $12.5 \times 12.5 \times 48$ mm. The cuvette was positioned at a distance of 35 mm to 45 mm from the recording camera and the hologram shown in Fig. 4(a) was recorded. Figure 4(b) shows a reconstruction of the hologram, where a few focused and several out of focus fibers at this particular reconstruction distance can be seen. The algorithm was applied on the recorded hologram and the resulting 3D image of the volume is shown in Fig. 5 and the corresponding measurements are listed in Table 1. The algorithm has successfully identified 7 fibers and measured their position, length, and orientation within the studied volume.



(a) (b)

Fig. 4. (a) Hologram of a suspension of fibers in liquid; and (b) a sample reconstruction.

5. Conclusions

A technique has been presented for simultaneous measurement of length, orientation, and location of opaque fibers. The potential of the technique was demonstrated using a single fiber

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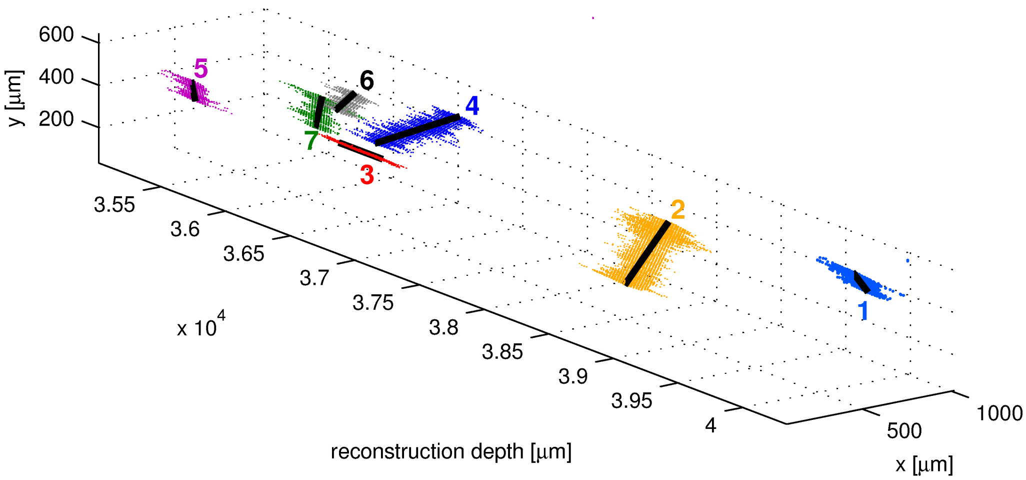

Fig. 5. 3D image of a volume of carbon fibers in suspension, viewed from two different directions and showing both the lines fitted by PCA (black lines), as well as the point clouds (colored dots).

Table 1. Tilt, length and location measurements of identified fibers in Fig. 5.

	centroid location			orientation		
fiber	x [μm]	y [μm]	z [mm]	xy [°]	yz [°]	length [μm]
1	766	498	39.967	-48	14	166
2	918	176	38.094	57	16	352
3	169	512	36.913	-26	-38	226
4	435	538	36.985	19	59	407
5	425	372	35.264	-74	4	111
6	779	419	35.953	30	-2	133
7	876	275	35.615	85	13	159

of known length and orientation and subsequently using a suspension of fibers with random orientations and unknown lengths. Unlike other methods reported in the literature, the method presented in this paper requires no *a priori* information about the size or the orientation of the object, hence it opens up promising possibilities in the field of particle analysis and characterization where the simultaneous measurement of both particle size and orientation is of great interest.

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