Holographic particle-streak velocimetry

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Abstract: We present a way to measure the positions and instantaneous velocities of micrometer-scale colloidal spheres using a single holographic snapshot obtained through in-line holographic video microscopy. This method builds on previous quantitative analyses of colloidal holograms by accounting for blurring that occurs as a sphere moves during the camera’s exposure time. The angular variance of a blurred hologram’s radial intensity profile yields both the magnitude and direction of a sphere’s in-plane velocity. At sufficiently low speeds, the same hologram also can be used to characterize other properties, such as the sphere’s radius and refractive index.

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References and links
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1. Introduction

Particle image velocimetry (PIV) is widely used to map fluid flows at scales ranging from a few micrometers to many meters [1, 2]. Typical implementations measure a tracer particle’s velocity by comparing its position in a sequence of images separated by known time intervals. Here, we describe how to extract this dynamical information from a single holographic snapshot, taking advantage of blurring due to motion during the camera’s exposure time. Under appropriate conditions, the blurred holographic image still can be used to measure each particle’s three-dimensional position and properties with high resolution [3, 4, 5].
2. **Holographic Video Microscopy**

Our in-line holographic video microscope [3, 4, 5, 6], shown schematically in Fig. 1, is based on a commercial inverted microscope (Zeiss Axiovert 100 STV) outfitted with a $100\times$ oil immersion objective with numerical aperture 1.4 (Zeiss S Plan Apo). We replace the microscope’s conventional incandescent illumination with the collimated coherent beam from a solid-state laser (Coherent Verdi 5W) at a vacuum wavelength of $\lambda = 532$ nm. The illuminating beam’s irradiance is on the order of 1 nW/μm², which is comparable to that of conventional microscope illumination. A colloidal sphere in a sample mounted on the microscope’s stage scatters a small proportion of this incident beam. The scattered light interferes with the unscattered portion in the focal plane of the microscope’s objective lens. This interference pattern is magnified and projected onto the detector of a low-noise gray-scale video camera (NEC TI 324 IIA), with a total system magnification of 101 nm/pixel. The video stream is recorded as uncompressed digital video with a digital video recorder (Pioneer H520S). Each snapshot in this holographic video stream then can be analyzed to obtain information about the position, velocity and properties of the scattering object.

The measured intensity at point $\mathbf{r}$ in the focal plane,

$$I(\mathbf{r}, t) = \left| E_0(\mathbf{z} - \mathbf{z}_p(t)) + E_S(\mathbf{r} - \mathbf{r}_p(t)) \right|^2$$

(1)

results from the superposition of the incident plane wave, $E_0(\mathbf{z})$, propagating along $\hat{z}$ and the scattered wave, $E_S(\mathbf{r} - \mathbf{r}_p(t))$, that propagates from the particle’s position $\mathbf{r}_p(t)$ to the point of observation, $\mathbf{r}$. This scattered field is described by Lorenz-Mie theory [7] and depends not only on the particle’s position, but also on its radius, $a_p$, and its refractive index, $n_p$ relative to the refractive index of the surrounding medium, $n_m$. Consequently, recorded images such as the example in Fig. 1 can be fit to Eq. (1) with $\mathbf{r}_p(t)$, $a_p$ and $n_p$ as adjustable parameters [3, 4, 5]. The computed uncertainties in the fit parameters are found to accurately assess each such measurement’s precision [3, 4]. This procedure routinely yields the position of a micrometer-

Fig. 1. In-line holographic video microscope. Objects illuminated by a collimated laser beam scatter some of the light to the microscope’s focal plane, where it interferes with the unscattered portion of the beam. The interference pattern is magnified and its intensity pattern recorded by a video camera. Each particle’s holographic image can fit to Lorenz-Mie theory to obtain the particle’s position, size and refractive index. The images show a typical hologram of a 1.5 μm diameter polystyrene sphere in water together with such a fit.
scale sphere to within a nanometer, and its radius and refractive index to within a part per thousand [3, 4, 8].

3. Motion Blurring

If a particle moves during the exposure time $\tau$ of the camera, the recorded image is the incoherent superposition

$$I_\tau(r,t) = \int_0^\tau I(r,t+t') dt'.$$

(2)

Such blurring has been used to estimate particles’ in-plane velocities in conventional colloidal imaging through a technique known as particle-streak velocimetry [1, 9]. Obtaining accurate results, however, requires the sphere to lie near the focal plane and to move large distances during the exposure. Holographic imaging, by contrast, captures particles’ motions over a much larger axial range [3, 4, 6, 10]. The fine structure, strong gradients and large number of pixels in holographic images also make possible far more sensitive measurements of a particle’s displacement during the exposure time.

Figure 2(a) demonstrates how a sphere’s hologram (Fig. 2(a.i)) becomes blurred as it moves parallel to the imaging plane by one wavelength of light in the shutter interval $\tau$ (Fig. 2(a.ii) and (a.iii)). These holograms were computed [3, 4, 7] for a 1 $\mu$m diameter polystyrene sphere in water. The color table was selected to emphasize the suppression of contrast along the direction of motion. Contrast is more strongly suppressed at larger distances from the center of the pattern where the spacing between fringes becomes comparable to the displacement.

Despite anisotropic contrast suppression, the spacing between fringes is not affected substantially by a modest amount of motion blurring. The blurred hologram thus can be analyzed [3, 4, 5] to obtain the particle’s three-dimensional position, radius and complex refractive index. This is consistent with the previous demonstration of such fits’ robustness against motion blurring at displacements up to 7 wavelengths [4].

Contrast also is suppressed when particles move along the optical axis. In this case, fringes blur isotropically, with the finer fringes at larger radii being most sensitive to small axial displacements. Such isotropic contrast degradation also is caused by imperfections in illumination, and so is more difficult to interpret quantitatively. We focus, therefore, on measuring motion in the plane.

4. Velocimetry with Blurred Holograms

The directionally-dependent suppression of contrast in a motion-blurred hologram is well described by the phenomenological model

$$I_\tau(r,t|v(t)) = I(r,t) \left[ 1 - \left( \frac{v(t)}{v_0(\tau)} \right) \cos^2 \left( \theta + \frac{\phi(t)}{2} \right) \right].$$

(3)

where $I(r,t)$ is the intensity of the unblurred hologram at time $t$ and $v(t)$ is the instantaneous speed of the particle traveling at angle $\phi(t)$ relative to the $\hat{x}$ axis. The degree to which the contrast is diminished depends on the particle’s speed relative to a scale $v_0(\tau)$ that depends both on exposure time and also on details of the sphere’s light scattering properties. For simplicity, we treat $v_0$ as an adjustable parameter.

Angular moments of a measured hologram’s contrast thus yield the particle’s velocity without reference to an explicit model for its undistorted hologram. To extract $v(t)$ and $\phi(t)$ from
the blurred hologram, we define

\[ S(t) = \int I(r, v, t) \sin(2\theta) \, dr \, d\theta \quad \text{and} \]
\[ C(t) = \int I(r, v, t) \cos(2\theta) \, dr \, d\theta, \]

as illustrated in Figs. 2(b) and (c), respectively. Equation (3) then yields

\[ v(t) = v_0 \sqrt{S^2(t) + C^2(t)} \quad \text{and} \]
\[ \phi(t) = \arctan \left( \frac{S(t)}{C(t)} \right). \]

5. Results

To assess the effectiveness of angular moment analysis for snapshot holographic velocimetry, we use Eqs. (4) through (7) to analyze simulated holograms blurred according to Eq. (2) with \( I(r, t) = I(r - vt) \). The data in Fig. 3 show how errors in the estimated displacement, depend on the magnitude and direction of a particle’s actual displacement \( v \tau \), during the exposure time. Relative errors in the estimated speed, Fig. 3(a), are quite large for displacements smaller than one wavelength of light. They rapidly fall to a small fraction of a percent for larger displacements. Estimates for the direction of travel are reliable for even smaller displacements, as can be seen in Fig. 3(b). In both cases, the magnitude of the error depends strongly on direction relative to the pixel array.
We obtain comparably good results for particles at heights \( z_p \) above the focal plane ranging from 20 \( \mu \)m to 100 \( \mu \)m. Particles closer to the focal plane produce holographic images whose fringes are too fine to resolve with our camera. Conversely, particles at too large an axial range yield images whose contrast is too poor to analyze.

Errors also increase for in-plane displacements larger than roughly ten wavelengths as diffraction rings from different orders begin to overlap. The resulting constructive superposition of light and dark fringes causes a modulation in the radial contrast that is not accounted for by Eqs. (6) and (7). This vernier-like effect could be used to extend holographic velocimetry to larger displacements. Blurring can be kept within the domain of validity of the present method by adjusting the camera’s exposure time. Limiting the blurring in this way has the additional benefit that information on the particle’s characteristics and three-dimensional position can be obtained simultaneously.

Figure 4 shows typical experimental results for a 1 \( \mu \)m diameter polystyrene sphere (Polybead Microspheres #07310) conveyed by flowing water. The sample is contained in a 100 \( \mu \)m deep microfluidic channel formed by bonding the edges of glass microscope cover slip to the surface of a glass slide. Flows ranging in speed from 50 \( \mu \)m/s to 100 \( \mu \)m/s were induced by wicking water from one side of the channel with absorbent paper. Setting the camera’s exposure...
Fig. 5. Distribution of estimated instantaneous speeds as a function of trajectory-averaged speed obtained from 6,500 snapshots of 1,500 polystyrene spheres flowing down a microfluidic channel. The dashed line of slope 1 is a guide to the eye.

to $\tau = 1/60$ s yields displacements ranging from 2 to 8 wavelengths of the laser light used for imaging. This is well within the anticipated domain of validity of our technique, particularly because the flow is aligned with the camera. The drift is slow enough that each sphere may be captured in several holograms as it moves across the $64 \mu m$ field of view. The superimposed images in Fig. 4 show such a sequence of snapshots at $1/6$ s intervals. Superimposed arrows indicate where the sphere should appear in the subsequent snapshot based on its estimated instantaneous velocity. Discrepancies between predicted and observed positions are consistent with the sphere’s Brownian motion.

Tracking the sphere’s centroid through a sequence of snapshots allows us to measure its velocity by standard methods of particle-image velocimetry [1, 2, 4, 5]. These trajectory-averaged results can be compared with the estimated instantaneous velocities. Figure 5 shows the measured distribution of instantaneous and trajectory-averaged speeds for 6,500 holograms of 1,500 spheres, using $v_0(\tau) = 60 \mu m/s$ for the scale factor. As anticipated, the velocity estimated from blurring scales linearly with the PIV estimate. The transverse width of this distribution is consistent with the error estimate for the instantaneous speed in Fig. 3(a). Consistent values for single-particle properties were obtained simultaneously from Lorenz-Mie fits over the entire range of speeds.

6. Conclusion

Using both simulation and experiment, we have demonstrated that motion blurring of holographic images can be used to estimate the instantaneous in-plane velocity of colloidal spheres. Accurate results can be obtained over a range of velocities determined by the camera’s exposure time. The amount of blurring required for holographic particle streak velocimetry is small enough that the blurred holograms also may be analyzed to measure the moving particles’ sizes and optical properties using methods developed for stationary particles [3, 4].

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