Robustness of holographic video microscopy against defects in illumination

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Abstract: We numerically investigate how defects in the illumination system of a holographic microscope, in particular divergence and tilt of the illuminating laser beam, affect the measured properties of colloidal spherical particles. We show that under typical experimental conditions divergence and tilt of the laser beam do not affect the measured parameters significantly, confirming the robustness of the technique against such defects.

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References and links

1. Introduction

In-line holographic video microscopy (HVM) images [1, 2] can be interpreted with predictions of the Lorenz-Mie theory of light scattering [3] to simultaneously track and characterize individual colloidal particles [4]. Previous studies have demonstrated tracking resolution finer than 1 nanometer in the focal plane and 10 nanometers in the axial direction over ranges extending to hundreds of micrometers [4, 5, 6]. The same analysis also yields the particle’s radius and refractive index with a claimed part-per-thousand resolution [4, 5, 7]. The extraordinary quantity and quality of information provided by quantitative HVM has enabled such applications as holographic microrheology [8], nanometer-resolution particle-image velocimetry [5, 6, 9], particle-resolved porosimetry [10], and label-free molecular binding assays [5]. The analysis underlying all previous studies, however, has treated the incident illumination as a plane wave propagating along the optical axis. Here, we address how deviations from this idealized model degrade the performance of quantitative HVM. In particular, we assess through simulations how uncompensated curvature and tilt of the illuminating beam’s wavefronts affect extracted values for particle position, size and refractive index obtained in real-world implementations.

An in-line hologram is created when light scattered by an object interferes with the remainder of the illuminating beam, as shown in Fig. 1. In our implementation, the interference pattern is magnified by a conventional microscope before being recorded with a video camera. Each snapshot in the resulting video stream records the intensity

\[ I(r) = |E_0(r) + E_s(r - r_p)|^2 \]  

(1)

at position \( r \) in the microscope’s focal plane due to the superposition of the time-averaged

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Fig. 1. Schematic representation of image formation by in-line holography. (a) Ideal configuration, with scatterer at height \( z_p \) above the imaging plane. (b) Diverging illumination. (c) Inclined illumination. Grayscale images are computed holograms illustrating the influence of illumination defects.
incident electric field, $E_0(r)$, and the field $E_s(r - r_p)$ scattered by an object centered at position $r_p$. Assuming the object to be small compared with the typical length scale for variations in the illumination, the scattered field may be approximated as

$$E_s(r) = E_0(r_p)f(k(r - r_p)),$$

where the scattering function $f(kr)$ describes the outgoing wave that is created by illuminating the particle with the incident wave. The scattering function may be expressed in generalized Lorenz-Mie theory as a series expansion [11, 12]

$$f(kr) = \sum_{m=3}^{\infty} \sum_{n=1}^{\infty} \left\{ r_{mn} \left[ M_{mn}^{(3)}(kr) - im_{mn}^{(3)}(kr) \right] - is_{mn} \left[ N_{mn}^{(3)}(kr) - in_{mn}^{(3)}(kr) \right] \right\},$$

in the vector spherical harmonics, $M_{mn}^{(3)}(kr), M_{mn}^{(3)}(kr), N_{mn}^{(3)}(kr)$ and $N_{mn}^{(3)}(kr)$. The expansion coefficients $r_{mn}$ and $s_{mn}$ depend on the structure of the illuminating beam and the properties of the scatterer. For the special case of scattering by a sphere, they reduce to [12]

$$r_{mn} = i^{n+3m-|m|} \frac{2n+1}{n(n+1)} \frac{(n-m)!}{(n+|m|)!} g_{n,TE}^{m} \beta_{n}(ka_p,n_p/n_m)$$

and

$$s_{mn} = i^{n+3m-|m|} \frac{2n+1}{n(n+1)} \frac{(n-m)!}{(n+|m|)!} g_{n,TM}^{m} \alpha_{n}(ka_p,n_p/n_m),$$

where $\alpha_{n}(ka_p,n_p/n_m)$ and $\beta_{n}(ka_p,n_p/n_m)$ are the familiar Lorenz-Mie coefficients [3] for scattering of a plane wave by a sphere. These, in turn, depend on the sphere’s radius $a_p$, and its complex refractive index, $n_p$, relative to that of the medium $n_m$. The transverse electric (TE) and transverse magnetic (TM) beam shape coefficients, $g_{n,TE}^{m}$ and $g_{n,TM}^{m}$, account for the structure of the incident illumination. Equations (4) and (5) are the complex conjugate of the expressions in Ref. [12] because here we assume a time dependence $\exp(-i\omega t)$. For consistency with earlier HVM studies.

Previous implementations of Lorenz-Mie microscopy [4, 5, 6] have treated the incident field as a linearly polarized plane wave, $E_0(r) = E_0 \exp(ikz)\hat{x}$, propagating precisely along the optical axis, for which only the beam shape coefficients with $m = \pm 1$ differ from zero and are given by $-ig_{n,TE}^{-1} = g_{n,TE}^{+1} = g_{n,TM}^{+1} = 1/2$. This plane-wave approximation also justifies normalizing the measured hologram by the background intensity $I_0(r) = |E_0(r)|^2$ to suppress artifacts due to extraneous scattering centers in the optical train [4]. The normalized hologram then may be fit to the simplified expression

$$B(r) = \frac{I(r)}{I_0(r)} \approx \left| \hat{x} + \alpha e^{-ikr} f(k(r - r_p)) \right|^2,$$

to obtain estimates for the particle’s position $r_p$, its radius $a_p$, and its refractive index, $n_p$. The phenomenological factor $\alpha$ in Eq. (6) is intended to account for diffuse scattering due to surface roughness and for variations in illumination. It typically has values in the range $0.5 < \alpha \lesssim 1$.

In the present study, we assess discrepancies in the estimates for $r_p, a_p$ and $n_p$ that arise when the plane-wave approximation, Eq. (6), is used to interpret holograms that are formed with more realistic models for the incident illumination. Specifically, we identify errors arising from divergence and tilt in the illuminating beam. These simulations establish limits on systematic errors for these parameters that can be expected in practical implementations. Under typical experimental conditions, with divergence angles smaller than 1 mrad and tilt angles less than 10 mrad, the resulting errors are found to be an order of magnitude smaller than numerical uncertainties in the fit parameters [4, 5]. From this we conclude that other factors have a more substantial influence on measurement errors in Lorenz-Mie microscopy and that the analysis technique is robust against this class of illumination defects.
2. Influence of beam divergence

To study the effects of diverging illumination, we model the incident light as a Gaussian beam of vacuum wavelength $\lambda$ and wave number $k = 2\pi n_m / \lambda$ propagating along $+\hat{z}$ through a medium of refractive index $n_m$. Rather than being collimated as in Fig. 1(a), the beam diverges from a focus of half-width $w_0$ at a height $z_0$ above the focal plane of the microscope, as shown in Fig. 1(b). Its electric field is then given in the paraxial approximation by

$$E_0(r) = D(z) \exp \left( -D(z) \frac{x^2 + y^2}{w_0^2} \right) \exp (ik [z + z_0]) \hat{x},$$

(7)

where

$$D(z) = \left( 1 + 2i \frac{z + z_0}{kw_0^2} \right)^{-1}. $$

(8)

Equations (7) and (8) accurately describe a weakly divergent beam with $kw_0 \gg \pi$. Assuming that the particle is centered on the optical axis at height $z_p$ above the focal plane, the beam shape coefficients are [11]

$$-ig_{n,TE}^{-1} = ig_{n,TE}^{-1} = g_{n,TM}^{-1} = \frac{1}{2} \exp \left( -D(-z_p) \frac{1}{[kw_0^2] \left[ n + \frac{1}{2} \right]^2} \right).$$

(9)

We use Eqs. (7) through (9) as inputs to Eqs. (1) through (3) to compute simulated holograms of spherical particles with specified radii and refractive indexes at specified heights above the center of the focal plane. We then use Eq. (6) to extract estimates $\bar{a}_p$, $\bar{n}_p$ and $\bar{z}_p$ for each particle’s characteristics and position under the plane-wave approximation. The differences $\Delta a_p = \bar{a}_p - a_p$, $\Delta n_p = \bar{n}_p - n_p$, and $\Delta z_p = \bar{z}_p - z_p$ represent the errors incurred by fitting the data with a simplified model. Typical results for the relative errors are plotted in Fig. 2 as a function of the beam divergence angle

$$\Omega = \tan^{-1} \left( \frac{2}{kw_0} \right).$$

(10)

The parameters chosen for these simulations are intended to mimic the conditions in recently published experiments [13, 14] on micrometer-diameter colloidal silica spheres in water with $a_p = 0.75 \, \mu m$, $n_p = 1.5$, and $z_p = 13.5 \, \mu m$ in a medium with $n_m = 1.33$ at $\lambda = 640 \, nm$. Taking the position of the illuminating laser’s output coupler to be the center of curvature, these experiments have $z_0 = 10 \, cm$ and $\Omega = 1 \, mrad$, using the manufacturer’s specification for
Fig. 3. Relative errors in (a) radius $a_p$, (b) refractive index $n_p$ and (c) axial position $z_p$ as a function of angle of inclination of the illumination.

beam divergence. The corresponding simulations yield relative discrepancies $\Delta a_p/a_p \leq 10^{-5}$, $\Delta n_p/n_p \leq 10^{-4}$ and $\Delta z_p/z_p \leq 10^{-4}$. This is at least one order of magnitude better than the 1 nm resolution reported for $a_p$, the 10 nm resolution reported for $z_p$ and the part-per-thousand resolution claimed for $n_p$ [13, 14].

The data in Fig. 2 suggest that divergence-induced errors scale as $1/z_0^2$ when the source is positioned beyond the Rayleigh range

$$z_R = \frac{1}{2} \frac{k w_0^2}{},$$

and are maximized at $z_0 = z_R$. Even in this worst-case configuration, the estimated error is smaller than the claimed resolution for $\Omega \leq 2$ mrad, which is a reasonable value for a well-collimated beam.

### 3. Influence of beam inclination

We now study the influence of tilted illumination. In this case, the incident light is a plane wave with wave vector $k$ that makes an angle $\theta$ with respect to the $\hat{z}$ axis as shown in Fig. 1(c). The axis of inclination is chosen to maximize rotation of the polarization vector $\hat{e}$, thereby maximizing the influence of the beam’s inclination. Treating the incident beam as a plane wave is justified by the results of the previous section. The incident field then has the form

$$E_\theta(r) = \exp(ik \cdot r) \hat{e}.$$  

(12)

The scattered field then is given by the plane-wave scattering function Eq. (3) rotated by an angle $\theta$ about the particle position, around the direction $\hat{n} = \hat{z} \times k / |\hat{z} \times k|$. We therefore rewrite the scattered field as $E_s(r) = e^{ik \cdot r} f_\theta(k (r - r_p))$, in terms of the scattered field

$$f_\theta(kr) = R_{\hat{n}}(\theta) f (R_{\hat{n}}^{-1}(\theta) kr)$$

(13)

that is rotated by about the center of the particle by the rotation matrix $R_{\hat{n}}(\theta)$.

As in the previous section, we use Eqs. (12) and (13) as inputs to Eq. (1) as a basis for computing simulated holograms of spherical particles. These holograms are then fit to Eq. (6) to extract the estimates $\bar{a}_p$, $\bar{n}_p$ and $\bar{z}_p$ for the particle’s radius, index of refraction and axial position, respectively.

Figure 3 shows the relative discrepancies for the radius, index of refraction and axial position for a particle with $a_p = 0.75 \mu m$ and $n_p = 1.5$ in a medium with $n_m = 1.33$ as a function of the tilt angle of the laser for different values of the axial position $z_p$. Whereas the errors due to
divergence plotted in Fig. 2 vary smoothly with wavefront curvature, those due to tilt feature
discrete jumps. This is because inclination distorts a hologram’s circular interference fringes
into ellipses, thereby breaking the radial symmetry implicit in the fitting procedure. Jumps in
Fig. 3 reflect changes in the best circularly symmetric fit with increasing distortion.

Even after accounting for ellipticity in the inclined images, the relative discrepancy in all fit
parameters is less than a part per thousand for tilt angles smaller than 1° (17 mrad) over the
experimentally accessible range of the axial position. This falls within the previously estimated
range of errors, and confirms that inclination of the illumination is not the dominant source
of error in Lorenz-Mie microscopy. Local inclination due to divergent illumination similarly
causes negligible offsets for particles located off the optical axis, at least for experimentally
relevant values of the beam divergence.

Although divergence and tilt do not appreciably influence the resolution of Lorenz-Mie track-
ing and characterization, they do introduce systematic correlations between the estimates for
the in-plane and axial coordinates so that $\bar{x}_p = \bar{x}_p(z_p)$ and $\bar{y}_p = \bar{y}_p(z_p)$. Such correlations may
be ignored in conventional microscopy because of the comparatively small depth of focus. They
may become apparent, however, over the large axial range covered by holographic microscopy.
In measurements of positional fluctuations, for example, the apparent in-plane fluctuations will
be augmented by a proportion of axial fluctuations. Neither divergence nor tilt causes a measur-
able dependence of the apparent axial position, $\bar{z}_p$, on the in-plane coordinates, $x_p$ and $y_p$, in the
experimentally relevant range of parameters. Consequently, the correlation may be removed by
subtracting off linear trends [13, 14].

The observed robustness of Lorenz-Mie microscopy against errors in optical alignment leaves open the question of the technique’s ultimate resolution. The principal underlying ap-
proximation, invoked in Eq. (6), involves normalizing the recorded hologram $I(r)$ with an esti-
mate $I_0(r)$ for the background illumination. Correlated artifacts introduced by departures from
ideal normalization can influence fits to Eq. (6). Equation (6), moreover, does not account for
phase disorder in the illumination, which may introduce additional correlated artifacts. Finally,
issues such as the signal-to-noise ratio of the recording, the linewidth of the source and the
coherence length of the illumination have yet to be considered. The present study thus adds
support to the claimed resolution of Lorenz-Mie microscopy while hinting that substantial ad-
ditional gains may yet be achieved.

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