Lifting degeneracy in holographic characterization of colloidal particles using multi-color imaging

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Abstract: Micrometer sized particles can be accurately characterized using holographic video microscopy and Lorenz-Mie fitting. In this work, we explore some of the limitations in holographic microscopy and introduce methods for increasing the accuracy of this technique with the use of multiple wavelengths of laser illumination. Large high index particle holograms have near degenerate solutions that can confuse standard fitting algorithms. Using a model based on diffraction from a phase disk, we explain the source of these degeneracies. We introduce multiple color holography as an effective approach to distinguish between degenerate solutions and provide improved accuracy for the holographic analysis of sub-visible colloidal particles.

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1. Introduction

Holographic microscopy is a powerful tool for characterizing sub-visible particles in suspensions. When illuminated by a laser, a particle scatters light that interferes with the remainder of the incident beam to create an in-line hologram. Each hologram can be analyzed with Lorenz-Mie theory to determine the size and refractive index of an individual particle with high precision and accuracy [1]. The particle’s scattering cross section determines its diameter, its scattering center locates the particle’s 3D position, and the particle’s composition determines its refractive index with respect to its surrounding medium. Holographic microscopy accurately characterizes the size, refractive index and 3D position of spherical particles [2,3]. This analysis is also robust to imperfections in the illumination [4] and departures from sphericity, such as in protein aggregates and nanoparticle agglomerates [5,6]. It can accurately determine the polydispersity of a population of colloidal particles and is consistent with confocal microscopy measurements [7]. Particle-resolved measurement of refractive index with holographic microscopy distinguishes multiple species of similarly-sized particles in a single sample, as in the case of multiple contaminants in a wastewater sample [8]. In the pharmaceutical industry, holographic characterization can be used to differentiate protein aggregates from other contaminants, such as silicone oil emulsion droplets. Protein aggregates reduce formulation efficacy and can cause detrimental immune responses while silicone oil emulsion droplets are largely harmless. Biologic pharmaceutical development and manufacturing benefit from the ability to independently and quantitatively monitor both species simultaneously.

Holographic microscopy has been shown to produce robust and accurate results for small low-index particles (radius \( \leq 5 \mu m \), refractive index \( \leq 1.7 \)). Extending the technology to larger, higher index particles poses a challenge. The analysis relies on a multi-parameter search algorithm on a 5 dimensional \( \chi^2 \) space that contains multiple local minima. The measurement can return inaccurate results when the fitting process gets stuck in a local minimum. The \( \chi^2 \) space becomes more complex for larger and higher index particles where there are more closely spaced local minima. Although Lorenz-Mie theory is an exact theory when applied to homogeneous spherical particles of any size in plane wave illumination, two spheres with different properties may
produce almost identical holograms. In fact, we show that such near-degenerate solutions exist, and that they are more prevalent for large, high-index particles. The presence of noise in actual experimental data increases the challenge in distinguishing between these similar solutions. In this work, we explore the cause of these near-degenerate holograms and introduce strategies for increasing the accuracy of this technique by removing the degeneracy with the use of multiple wavelengths of laser illumination.

The degenerate solutions observed experimentally occur as a series of peaks with similar size and regularly spaced indexes of refraction. The repeating nature of these solutions as a function of refractive index suggests that phase delays could explain the effect. Both Rayleigh scattering and the Rayleigh-Gans-Debye approximation [9, 10] are single-scattering theories and do not include a phase lag. The phase lag, such as caused by light traveling through a medium, is caused by multiple scattering of point scatterers in the medium [11]. The discrete dipole approximation, which allows for interaction between the dipole scatterers [12, 13], should be able to reproduce this effect, but does not provide a conceptual model of the effect.

![Fig. 1. Schematic for models of holographic microscopy. (a) Thin disk model for hologram formation where incident light, represented by green arrows, impinges on the particle represented by the orange disk. The transmitted light is delayed by the disk, and then diffracts and interferes with the incident light. This interference creates the hologram represented by the ringed pattern below the plane of the disk. The hologram shown is data collected from a sample of 1.5 µm polystyrene particles suspended in DI water and illuminated by a 532nm laser. (b) The particle as a Fabry-Perot etalon where the orange cylinder represents a particle and the green arrows represent light that reflects from or transmits through the particle.](attachment:image.png)

The interference structure in extinction coefficients as a function of refractive index presents a similar oscillatory behavior. Extinction coefficients for particles much larger than the wavelength of light oscillate around 2 [9]. The asymptotic value can be explained by modeling the particle as an opaque disk: the diffraction from the edge of the particle doubles the amount of light taken from the incident beam from absorption alone. But the opaque disk approximation does not capture the interference structure. Several theories go beyond the opaque disk approximation. For low index, large spheres the oscillation is described by anomalous diffraction theory [14, 15]. Quadratic phase and quasi-spherical phase approximations of the pupil function of a particle have been explored by Coëtmellec et al., who have represented these phase functions using circle polynomials [16]. For more complex particles, such as burning fuel particles, Wu et al. have developed models based on Debye series and on generalized Lorenz-Mie theory and diffraction theory [17]. Although these models have utility for accurately representing various particles, the interference structure of extinction coefficients can be explained by a geometric optics argument [9]. Light passing through the particle experiences a phase lag relative to the rest...
of the illumination and creates interference. We argue that the nearly degenerate holograms are caused by this effect.

We model the particle as a transparent disk and find this model illustrates the genesis of the secondary minima with minimal complexity. We approximate the vectorial scattering of a sphere in a plane wave with a scalar model where the sphere is represented by a phase delaying disk. Scalar diffraction theory is used to reconstruct a hologram generated by the interaction of the incident light with the particle as shown schematically in Fig. 1(a). The circular shape of the particle’s cross-section leads to a diffraction pattern similar to that of an Airy disk. The interference with the incident beam creates the characteristic rings of the hologram. We also include a Fabry-Perot etalon effect shown schematically in Fig. 1(b).

This model explains the existence of degenerate solutions as a resonance phenomenon. The particle hologram is dominantly caused by the phase delay that the particle imposes on the transmitted light. Higher index particles cause a larger phase delay, but when the phase delay gains $2\pi$ in phase then the hologram returns to its initial form.

This approximate model provides a qualitative explanation for the degenerate solutions, but it is surprisingly accurate and captures much of the phenomena that we see in the exact Lorenz-Mie theory and in experiments. For particles smaller than the wavelength of light this model is close to exact. In addition, the model naturally explains aspects of the hologram, such as the beating behavior seen in larger particle’s hologram profiles. Finally, this model motivates the use of multiple colors to identify the global minimum among a host of spurious local minima. We show here the promise of the use of multiple wavelengths to expand the range of sizes and refractive indexes for holographic microscopy.

2. Experimental evidence of degenerate solutions

We explore the existence of multiple Lorenz-Mie solutions by analyzing selected experimentally measured holograms. Here we consider 1.5 µm diameter polystyrene (PS) beads (Bangs Labs NT16N, Lot# 12035), which have a refractive index of 1.6, suspended in deionized (DI) water. We measured these particles with Spheryx xSight, a holographic microscopy system, modified to be used at three different wavelengths: blue (450 nm Thorlabs CPS450 collimated laser diode), green (532 nm Thorlabs CPS532 collimated laser diode), and red (635 nm Thorlabs CPS635 collimated laser diode). These measurements (one per illumination wavelength) return the diameter, $d_p$, refractive index, $n_p$, and height above the focal plane, $z_p$, for each particle, extracted from parametric fits of the corresponding particle holograms. The particles are tracked over several frames to provide more accurate measurements for each particle [18, 19]. For each illumination wavelength, the hologram of one typical particle is shown in Fig. 2(b)-2(d). Below the experimental holograms, the best fit theoretical hologram is shown in Fig. 2(e)-2(g), which is generated by using parametric optimization of Lorenz-Mie theory using a Levenberg-Marquardt fitter [20, 21]. The initial parameter estimates given to the fitter were the nominal size and refractive index of the particles (1.5 µm in size, and 1.6 in refractive index) and a typical height above the focal plane for this xSight based on our previous measurements (60 µm). Note that the xSight software is able to fit particle holograms without any input guesses, but here we explicitly provide these guesses to explore the nearly degenerate solutions in Lorenz-Mie theory. From three sets of initial parameter guesses for each experimental hologram (labeled “N=0, 1, 2”) the fitting algorithm delivered three different solutions, as shown in Fig. 2(a) and Fig. 2(h)-2(j).

With the nominal values as guesses, the theory gives excellent fits for these three particles. The theoretical holograms in Fig. 2(e)-2(g) closely match the experimental data in Fig. 2(b)-2(d). To more closely compare the theoretical and experimental results, we examine the radial profile, which is the average intensity around the center of the hologram as a function of the radial distance from the center of the hologram (i.e. the azimuthal average). The experimental and theoretical radial profiles agree well as shown by the curves labeled “N=0” in Fig. 2(h)-2(j).
Fig. 2. Experimental evidence of degenerate solutions. Images (b-d), marked “Data”, are experimentally measured holograms of 1.5 µm diameter polystyrene spheres. Each hologram was measured with a different illumination wavelength, 450 nm, 532 nm and 635 nm respectively. Below, images (e-g), marked “Sphere”, represent the best fit using Lorenz-Mie theory to the corresponding experimental hologram, with an initial guess in diameter, $d_p$, and refractive index, $n_p$, at the expected values of 1.5 µm in diameter and 1.6 in refractive index. In (a), additional local minima are found at slightly different size and significantly different refractive index values. The color of the points corresponds to the illumination wavelength, blue: 450 nm, green: 532 nm, and red: 635 nm. For each point in (a), we plot the radial profile, or azimuthal average, of the theoretical hologram corresponding to a sphere with such size and refractive index values in (h-j). The theoretical radial profiles are grouped and colored according to their wavelength. Each theoretical radial profile is compared to the corresponding experimental radial profile (black curve) for that wavelength.

Finally, the size and refractive index parameters returned from the fits match closely to the nominal values, as can be seen by the points labeled “N=0” in Fig. 2(a).

Different initial guesses with larger refractive index, deliver additional local minima for all illumination wavelengths. The size and refractive index of these degenerate solutions are shown in Fig. 2(a), as the points labeled “N=1” and “N=2”, and their radial profiles are compared to the experimental radial profiles in Fig. 2(h)-2(j). The degenerate solutions do not fit the experimental
data as well as the best fit solution, but there is a striking similarity between these solutions. To assure accurate results, the challenge is distinguishing the correct solution from the spurious local minima. The optimization process may encounter many local minima and must compare the solutions at these various local minima to determine the global minimum. The existence of similar holograms for particles with very different refractive indexes increases the probability that the algorithm will mistake one of these near-degenerate local minima for the global minimum. The genesis of these degenerate solutions will inform the resolution of this near-degeneracy problem. Using our model, we explore the nature of these near-degenerate solutions.

3. Transparent disk model

Consider a sphere of radius \( d_p \) and refractive index \( n_p \) illuminated by a plane wave with an electric field,

\[
E_0(\mathbf{r}) = E_0 \exp(ikz)\hat{x}
\]  

(1)

where \( k = 2\pi n_m / \lambda \) and the surrounding medium has refractive index \( n_m \). The vacuum wavelength of the plane wave illumination is \( \lambda \), and it is traveling downward along the \( \hat{z} \) axis with a polarization \( \hat{x} \) in the \( x-y \) plane. The sphere interacts with the incident illumination and produces a scattered field \[1\] \[22\],

\[
E_s(\mathbf{r}) = E_0 \exp(ikz_p)\hat{x}(k(\mathbf{r} - \mathbf{r}_p))
\]  

(2)

where \( z_p \) is the height of the particle above the focal plane, and \( \hat{x}(k(\mathbf{r} - \mathbf{r}_p)) \) is the vector scattering function that is accurately described by Lorenz-Mie theory \[9\]. This scattered field interferes with the incident field to create a hologram whose normalized intensity is given by \[23\],

\[
b(\mathbf{r}) = \frac{|E_s(\mathbf{r})|^2}{E_0^2} = |\hat{x} + \exp(ikz_p)\hat{x}(k(\mathbf{r} - \mathbf{r}_p))|^2. \]  

(3)

The hologram, as shown in Fig. 2(b), contains detailed information about the particle. Our model explains key features of the observed hologram with a minimum of theoretical complexity. We approximate the sphere as a homogeneous cylindrical dielectric slab of diameter \( d_c \), height \( d_c \) and refractive index \( n_p \). To set the cylinder volume equal to that of the modeled sphere, we set \( d_c = \left(\frac{2}{3}\right)^{1/3} d_p \).

To a first approximation, the particle acts as a phase delay for light that passes through it. This single observation explains the fact that there are spurious local minima, and also explains why smaller low index particles are easier to fit. Light passing through the disk experiences a phase delay relative to the light that is not altered by the disk. This phase delay is given by,

\[
\Delta \phi = \frac{2\pi}{\lambda}(n_p - n_m)d_c. \]  

(4)

Using scalar diffraction theory, we can propagate the light after it interacts with the disk to find the light field in a plane below the particle

\[
E(\mathbf{r}) = E_0(\mathbf{r}) + E_d(\mathbf{r})(\exp(i\Delta \phi) - 1)
\]  

(5)

where the scalar plane wave field is given by,

\[
E_0(\mathbf{r}) = E_0 \exp(ikz)
\]  

(6)

and the diffracted field from a circular aperture is given by \[24\].
\[
E_d(r) = -ik \frac{\exp(ikr)}{r} \frac{d^2}{4} \frac{J_1(k \frac{d}{2} \sin \theta)}{k \frac{dc}{2} \sin \theta}
\]

where \( \tan \theta = \frac{d}{z} \) and \( r = \sqrt{z^2 + \rho^2} \) with \( \rho \) the radial distance from the z axis. The interference between the incident light and the light that diffracts due to the particle creates a hologram as shown in Fig. 1(b). Only the phase delay matters, so a hologram created from a disk with a phase delay of \( \Delta \phi_0 \) will create the same hologram as a disk with phase delay \( \Delta \phi_0 + 2\pi N \) where \( N \) is an integer, creating degeneracies at intervals of \( 2\pi \). In addition, particles with different refractive indexes could create identical holograms, for example when a first order resonance coincides with a higher order resonance of a particle with a different index. We can use this fact and Eq. 4 to find the refractive index of such a particle,

\[
n_N = \frac{N \lambda}{d_p} + n_p.
\]

This result is consistent with the interference condition for extinction coefficients described by [9]. To our knowledge, this interference condition described by Eq.8 has not been applied to in-line particle holograms. Using this equation, the calculated resonances are consistent with the spacing that we see experimentally in Fig. 2. It is important to note that \( n_p \) depends on wavelength due to dispersion. Degeneracies may also exist in other parameters such as particle size or height above the focal plane, but they are not the focus of the present work.

One missing piece from the model is that the experimental near degenerate holograms are not perfectly equal. As a way to see where these differences can come from, we incorporate the interaction between the particle and the incident illumination. In our model using a cylindrical particle, the light can reflect back and forth inside the particle, simulating a Fabry-Perot interferometer [25], specifically an etalon. Each time the light encounters an interface between the particle and the medium its reflectivity is given by,

\[
R = \left( \frac{E_r}{E_i} \right)^2 = \left( \frac{n_p - n_m}{n_p + n_m} \right)^2
\]

where \( E_i \) is the field incident on the surface and \( E_r \) is the reflected field. By using these reflection equations to relate the fields on either side of the interface, we calculate the field after the particle based on the incident field at the particle surface as,

\[
t_c = \frac{E_t}{E_i} = \frac{(1 - R) \exp(i \Delta \phi)}{1 - R \exp(i2 \Delta \phi)}.
\]

Using this knowledge of the field after the particle, Eq. 5 becomes:

\[
E(r) = E_0(r) + E_d(r)(t_c - 1).
\]

The degeneracy condition Eq. 8 still holds, but now the disk hologram of index \( n_N \) will not be exactly the same as the corresponding disk hologram with index \( n_p \) due to the reflectivity factor \( R \).

4. Application of transparent disk approximation

We apply the transparent disk model from Section (3) above to the analysis of the experimental holograms presented in Section (2). We find that this simple disk model is surprisingly effective at modeling the experimental data, and furthermore replicates the degenerate solutions found with Lorenz-Mie theory. Again we examine the experimental holograms of 1.5 \( \mu \)m PS particles shown in Fig. 2(b)-2(d) and replotted in Fig. 3(b)-3(d). Each hologram corresponds to a different
Fig. 3. Comparison of fits using Fabry-Perot disk model to experimental holograms. Images (b-d), marked “Data”, are experimentally measured holograms of 1.5 µm diameter polystyrene spheres repeated from Fig. 2. Below, images (e-g), marked “Disk”, represent the best fit using the transparent disk model to the corresponding experimental hologram, with an initial guess in size and refractive index near the expected values of 1.5 µm in size and 1.6 in refractive index. Similar to Lorenz-Mie theory, additional local minima are found at slightly different size and significantly different refractive index values. These points are shown in (a) as stars with the corresponding points from Lorenz-Mie theory replotted from Fig. 2(a). The color of the points corresponds to the illumination wavelength, blue: 450 nm, green: 532 nm, and red: 635 nm. For each point in (a), we plot the radial profile, or azimuthal average, of the theoretical hologram corresponding to a disk with such size and refractive index in (b-j). The theoretical radial profiles are grouped and colored according to their wavelength. Each theoretical radial profile is compared to the experimental radial profile, colored black, for that wavelength.

wavelength, 450 nm, 532 nm, and 635 nm respectively. Below the experimental holograms, we show the theoretical hologram, in Fig. 3(e)-3(g), this time generated by using parametric optimization of the disk model using a Levenberg-Marquardt fitter. Specifically, theoretical holograms for the disk model are calculated using Eq. 11 which depends on Eqs. 4, 6, 7, 9 and 10 for each position r in the hologram. Again, we tried three sets of initial parameter guesses
(labeled “N=0, 1, 2”) for each experimental hologram. The first set of initial parameters (N=0) close to the nominal values were found using an iterative trial and error process. The next two sets of parameters (N=1, 2) were found by applying Eq. 8 with the $n_p$ and $d_p$ values equal to the values used for N=0 initial parameters. Similar to the use of the Lorenz-Mie theory, we found that from each set of initial parameters the disk model fitter optimizes to a different local minimum near-degenerate solution.

With the N=0 guesses (corresponding to the nominal values for the Lorenz-Mie fitting), the disk model also provides excellent fits for these three particles at the three different wavelengths. The theoretical holograms, shown in Fig. 3(e)-3(g), closely match the experimental holograms, shown in Fig. 3(b)-3(d). In addition, the accuracy of the fit is confirmed by their radial profiles shown in Fig. 3(h)-3(j) labeled “N=0”. The similarity is surprising, given the approximation of a spherical particle with a cylinder in the theory.

As in the results from Lorenz-Mie theory, different initial guesses with larger refractive indexes, deliver additional local minima for all illumination wavelengths. The size and refractive index results for these degenerate solutions are shown in Fig. 3(a) as the points labeled “N=1” and “N=2”. Points in Fig. 3(a) correspond to solutions due to Lorenz-Mie theory (dots) and the disk model presented here (stars). Although the points corresponding to the disk model and Lorenz-Mie theory have quantitative differences in size and refractive index, they show strikingly similar trends. We also compared radial profiles of the near-degenerate solutions to the experimental radial profiles in Fig. 3(h)-3(j), and find excellent agreement, as in the results using Lorenz-Mie theory shown in Fig. 2(h)-2(j).

The similarity between the Lorenz-Mie theory solutions and disk model solutions suggests that there is a similar underlying mechanism for the observed degeneracies. The wavelength dependence provides additional convincing evidence that the same mechanism causes the degenerate solutions in both models. In the disk model using Eq. 8, the spacing in refractive index between degenerate solutions is proportional to the wavelength, as seen for the disk model points shown as stars in Fig. 3(a). This same spacing occurs for the points obtained from the Lorenz-Mie theory fits also shown in Fig. 3(a). Spheres in Lorenz-Mie theory act analogously to the cylinders in the model presented here.

5. **Distinguishing particles using multi-color illumination**

Measurements made at multiple wavelengths give additional information that can help identify and avoid near-degenerate local minima. To explore the feasibility of distinguishing the correct results from the ones due to local minima we performed further analysis on our data from 1.5 μm PS spheres and took further measurements on 9.7 μm PS spheres (Bangs Labs NT27N, Lot # 12826). In these experiments, we examined the squared differences between experimental holograms and holograms calculated using the two models at various wavelengths. Plotted in Fig. 4 are these squared differences as a function of refractive index for three different wavelengths (450 nm, 532 nm, 635 nm) and two different particle sizes (1.5 μm and 9.7 μm). The normalized squared differences are given by,

\[
\text{Norm Sq. Diff.} = \frac{\sum_i (I(r_i) - I_{\text{model}}(r_i))^2}{\sum_i |I(r_i) - 1|}.
\]

(12)

where $I(r_i)$ is the experimentally measured intensity at pixel position $r_i$ and where $I_{\text{model}}(r_i)$ is the corresponding theoretical intensity. We use this normalized sum of squared differences as a “goodness of fit” metric. We calculate this metric as a function of the refractive index, holding the other parameters to their best fit values given the N=0 initial guess. The minima in the resulting curves are the values of refractive index corresponding to parameters determined by the fitting algorithms.
Both the disk model (dashed lines) and Lorenz-Mie theory (solid lines) have multiple solutions, evidenced by the multiple minima in the curves. For the 1.5 μm PS particle calculations in the left column, the location of the local minima coincide for the two calculations, consistent with the results in Section 4 above. In addition, as our disk model predicts, the spacing of these local minima depends on wavelength. At this smaller particle size, the shift in refractive index from dispersion is small relative to the spacing of the degenerate solutions. In contrast, the 9.7 μm PS particles present much more tightly spaced false local minima in refractive index which is comparable to the shift in index from dispersion. Although the disk model deviates from the experimental data as evidenced by the large peaks in squared differences, it continues to match the periodicity shown by the Lorenz-Mie theory.

Fig. 4. Comparison of errors of Lorenz-Mie theory (solid lines) and the disk model (dotted lines) as a function of refractive index for two PS spheres: 1.5 μm (left column) and 9.7 μm (right column). In each panel, the normalized sum of the squared differences between the theoretical hologram and the experimental hologram are plotted versus the refractive index while all other fit parameters are held constant. Each row corresponds to data at a specific wavelength: (a-b) 450 nm, (c-d) 532 nm, and (e-f) 635 nm.
Although there are multiple local minima at various values of refractive index, the global minimum at each wavelength matches the expected refractive index for PS particles. The etalon part of our disk model is an example of how such differences could form between holograms that are degenerate according to Eq. 8. Differences could also result from higher order resonances, which are known to occur in spherical particles. These resonances can be involved in optical forces [26], for sorting microspheres with optical resonances [27], and even in Doppler cooling a microsphere [28].

Differences between the near degenerate solutions make possible the determination of the correct solution from spurious solutions from single wavelength holograms, but the process is made much more robust by measuring microspheres at multiple wavelengths. The previously reported success of holographic video microscopy [1–3,23] depends on these differences. Further advances in accurate holographic characterization of larger particles will benefit from understanding the existence and location of the various local minima solutions given by the disk model.

6. Finding the global minimum

When experimental results have peaks at multiple refractive indexes at the same size, it can be difficult to know if the sample contains multiple species of the same size, but different refractive indexes, or if false local minima have confounded the fitting algorithm. First, we can check to see if the spacing of the peaks is consistent with Eq. 8. If this condition is satisfied, then the peaks may be degenerate solutions. Next, the sample can be measured using a laser source of different wavelength. If the peaks are degenerate solutions then they should also satisfy Eq. 8 with a different spacing that depends on the wavelength. It is unlikely that the wavelength dependent dispersion of multiple species would conspire to match degeneracy condition [29]. Once the peaks are confirmed to be local minima of the same particle distribution, then the global minimum can be determined from the most populous peak across wavelengths. Experimental noise will cause some holograms to have best fits in some of the nearly degenerate false local minima, but the slight advantage of the true global minimum will show up after many particles have been measured. Finally with the global minimum determined, the particle holograms associated with the local minima can be reanalyzed to determine the true values.

We demonstrate the process of finding the global minima for a set of experimental holograms using information from measurements at multiple wavelengths and our knowledge of the degenerate solutions. We measured hundreds of 9.7 µm PS particles (described in the previous section) using laser sources at 450 nm and 532 nm. Each particle was tracked as it passed through the field of view of the xSight and several holograms were recorded for each particle. Again the holograms were fit to Lorenz-Mie theory using a Levenberg-Marquardt fitter. The resulting refractive index distributions from the fits of all the particle holograms are show in Fig. 5(a)-5(b). There are sharp peaks in the distributions corresponding to the various minima identified in Fig. 4. For the 450 nm measurements, we detect six different peaks in the refractive index distribution shown in Fig. 5(a). We identify the predicted degenerate peaks by the dashed vertical lines in the figure using the value of the peak near refractive index 1.6 as the value of \( n_p \) in Eq. 8. The spacing of the peaks is consistent with the predicted values, but these peaks could have been caused by six distinct species in our sample. The data from 532 nm illumination experiment are used to confirm the degeneracy. The refractive index distribution from our measurements of the same sample in 532 nm illumination also exhibits peaks that have positions consistent with Eq. 8, confirming that these peaks are caused by nearly degenerate holograms, but correspond to the same species of particles.

With the false local minima identified, we can refit the particle holograms to get the correct values for the refractive index. First we need to determine which peak corresponds to the true global minimum. We pick the peak near refractive index 1.6 because it is the most prominent in
Fig. 5. Experimental distributions of particle hologram fits to Lorenz-Mie theory as a function of refractive index. Panels (a) and (b) show the fit distributions of 9.7 µm PS particles measured in 450 nm and 532 nm illumination respectively. The vertical dashed lines represent expected position of degenerate peaks. After the true peak is determined, the particles are refit using the true values as starting parameters, and the resulting narrow distributions are plotted in (c) for 450 nm and (d) for 532 nm. In panels (c) and (d), the global minimum is indicated by the vertical gray line.

the 532 nm data and is one of the largest peaks in the 450 nm data. The choice also agrees with the nominal value for the refractive index of PS at both wavelengths. After refitting with initial guesses centered on the global minimum refractive index for the species, the multiple peaks collapse into a single peak as shown in Fig. 5(c)-5(d). The global minimum well is narrow in parameter space in terms of the fitting metric, therefore, the corrected results have high precision. With this iterative process based on our understanding of the false local minima, we are able to produce precise and accurate results.
7. Conclusion

We have found the existence of near-degenerate particle holograms generated by Lorenz-Mie theory for particles with significantly different refractive indexes. These solutions can complicate analysis in holographic video microscopy when fitting experimental holograms with Lorenz-Mie theory. To better understand the mechanism behind these nearly degenerate solutions we developed a simple model of particle scattering where the particle is modeled as a cylinder or disk instead of a sphere. In this disk model, the particle acts as a phase retarding plate, and the resulting light that transmits through the particle diffracts toward the focal plane and interferes with the incident light to form the particle hologram.

This simple model captures the effect that causes the observed degenerate solutions. The disk model describes how particles induce a phase delay in the scattered light and how particle holograms will be nearly identical if the phase delay is a multiple of $2\pi$. We observe two orders of degenerate solutions to experimental holograms taken with three different illumination wavelengths. The wavelength dependence of these degenerate solutions in Lorenz-Mie theory matches the dependence predicted by the disk model.

Finally, we explore how this new understanding can help address the problem of degenerate solutions. By measuring spheres with multiple colors of laser illumination we were able to identify degenerate peaks and confirmed that they satisfied the interference relationship of Eq. 8. Using this information we show that the data can be reanalyzed such that the measured refractive indexes collapse around the correct value. Although degeneracies may occur, this proof-of-principle experimental demonstration suggests they can be lifted, resulting in precise and accurate measurements.

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