Holographic tomography of fractal aggregates

Rafe Abdulali,1 Lauren E. Altman,2 and David G. Grier2

1Packer Collegiate Institute, Brooklyn, NY 11201, USA
2Department of Physics and Center for Soft Matter Research, New York University, New York, NY 10003, USA

Holographic particle characterization uses quantitative analysis of holographic microscopy data to precisely and rapidly measure the diameter and refractive index of individual colloidal spheres in their native media. When this technique is applied to inhomogeneous or aspherical particles, the measured diameter and refractive index represent properties of an effective sphere enclosing each particle. Effective-sphere analysis has been applied successfully to populations of fractal aggregates, yielding an overall fractal dimension for the population as a whole. Here, we demonstrate that holographic characterization also can measure the fractal dimensions of an individual fractal cluster by probing how its effective diameter and refractive index change as it undergoes rotational diffusion. This procedure probes the structure of a cluster from multiple angles and thus constitutes a form of tomography. Here we demonstrate and validate holographic tomography through experimental studies on aggregates of silica nanoparticles grown under a range of conditions.

I. INTRODUCTION

Many colloidal particles of natural and industrial interest are formed by random aggregation of nanometer-scale monomeric units. Examples include protein aggregates in biopharmaceutical products [1], haze in beer, soot from flames, astronomical dust particles, nanoparticle agglomerates in precision polishing slurries [2] and microplastics in the environment [3]. Standard optical methods for particle characterization, such as static and dynamic light scattering, yield population-averaged views of such particles’ structural properties [4, 5]. Particle-resolved characterization measurements based on holographic microscopy in principle could probe the structure of individual fractal aggregates [6], but so far have been used to study the average properties of populations of aggregates [7–9]. Here, we demonstrate a fast and effective method to assess the structural properties of individual colloidal fractal aggregates from sequences of holograms recorded at multiple angles.

Each hologram of a fractal aggregate encodes information about the particle’s three-dimensional structure [6,8]. Extracting that information with light-scattering theory is a high-dimensional inverse problem [10] whose numerical convergence is both slow and uncertain. The corresponding analysis for a homogeneous sphere, by contrast, converges rapidly and yields precise values for the sphere’s diameter and refractive index [11]. Applying the efficient spherical-particle analysis to a hologram of a fractal cluster yields values for the diameter and refractive index that characterize an effective sphere [7,9] comprised of the cluster itself and the fluid that fills its pores. This approach builds on the recent observation that effective-sphere analysis can be used to measure the orientation of colloidal dimers in shear flows [12]. For an irregularly shaped cluster, these effective-sphere values depend on both the cluster’s orientation and also its morphology [6, 7]. Previous experimental studies recorded a single hologram of each cluster in a population and combined effective-sphere results to infer information about the particles’ morphology [7, 8]. This approach presumes that all of the clusters in the population are grown under similar conditions so that their results can be combined meaningfully.

Here, we record holographic videos of individual fractal clusters as they undergo translational and rotational diffusion. This protocol yields holograms of each cluster in multiple orientations. When analyzed with the effective-sphere analysis, the set of measurements provides insights into the morphology of that specific cluster. Single particle morphological analyses then can be compared with holographic analysis of the population as a whole performed with single snapshots of clusters in a flowing fluid.

FIG. 1. (a) Schematic representation of holographic characterization of a diffusing fractal aggregate in a microfluidic channel. The aggregate, passing through the illuminating laser beam, scatters light that interferes with the rest of the laser beam to create a hologram. The two images are experimental holograms of a single aggregate in different orientations with fits to Lorenz-Mie theory superimposed in the lower-right quadrants. (b) Effective values for $d_p$ and $n_p$ for the aggregate in multiple orientations. Each plot symbol corresponds to a single observation of the freely diffusing cluster and is colored by the density of observations, $P(d_p, n_p)$. Red and green symbols denote results from the holograms in (a).
II. HOLOGRAPHIC PARTICLE CHARACTERIZATION IN THE EFFECTIVE-SPHERE APPROXIMATION

In-line holographic microscopy uses a collimated laser beam to illuminate the sample [13]. Light scattered by colloidal particles in the sample interferes with the rest of the illumination. An optical microscope magnifies the interference pattern and relays it to a video camera, which records its intensity. Each image in the video stream is a hologram of the particles in the observation volume that encodes information about the particles’ positions and compositions. That information can be extracted by fitting a recorded hologram to a generative model for the image formation process.

We model the illumination as a monochromatic plane wave that is linearly polarized along \( \hat{z} \) and propagates along \( \hat{z} \):

\[
E_0(r) = u_0 e^{ikz} \hat{z}.
\]

(1)

A small spherical particle located at \( r_p \) scatters a wave,

\[
E_s(r) = E_0(r_p) f_s(k(r - r_p)),
\]

(2)

that is proportional to the incident field at its position. The structure of the scattered wave is described by the Lorenz-Mie scattering function, \( f_s(kr) \) [14,16], which is parameterized by the sphere’s diameter, \( d_p \), and its refractive index, \( n_p \). The superposition of the incident and scattered waves creates an interference pattern whose intensity is recorded by the camera [11]:

\[
b(r) = |\hat{x} + e^{-ikr_p} f_s(k(r - r_p))|^2,
\]

(3)

where we have normalized the recorded image [11] so that \( u_0^2 = 1 \). Distances are measured relative to the vacuum wavelength of light, \( \lambda \), through the wave number, \( k = 2\pi n_m/\lambda \), in a medium of refractive index \( n_m \). In typical implementations, \( \lambda, n_m \), and the magnification of the microscope are treated as fixed instrumental parameters. This generative model for the image-formation process can be fit to a measured hologram by optimizing \( r_p, d_p \) and \( n_p \). When applied to homogeneous colloidal spheres, this procedure finds the center of particle with a measurement error of \( \sigma_x = \sigma_y = 2 \text{ nm} \) and \( \sigma_z = 5 \text{ nm} \). The same measurement yields the sphere’s diameter with a precision of \( \sigma_d = 2 \text{ nm} \) and its refractive index with a precision of \( \sigma_n = 0.001 \) [17].

Equation (3) can be generalized to accommodate aspherical and inhomogeneous particles by selecting a suitable form for the scattering function [18]. Such generalizations, however, dramatically increase the time and resources needed to seek optimal solutions to the inverse problem [19]. The effective-sphere approximation avoids this computational cost by analyzing holograms of aspherical particles using the Lorenz-Mie scattering function for homogeneous spheres. In this case, optimal values for the diameter and refractive index reflect the properties of an effective sphere encompassing the particle whose index may be interpreted with Maxwell Garnett effective-medium theory [20] to obtain information about the particle’s true underlying properties [17,19]. A porous sphere, for example, consists of a base material of refractive index \( n_0 \) that comprises a fraction, \( \phi \), of its volume. The rest of the volume is filled with the fluid medium at refractive index \( n_m \). The effective refractive index for such a two-component system is [9,21]

\[
n_p = \sqrt{\frac{1 + 2\phi L(n_0/n_m)}{1 - \phi L(n_0/n_m)}},
\]

(4a)

where the Lorentz-Lorenz function is

\[\]

\[
L(m) = \frac{m^2 - 1}{m^2 + 2}.
\]

(4b)

Effective-sphere analysis can be applied to aggregates of nanoparticles, which tend to be aspherical as well as porous, provided that the aggregates’ asperities are small enough for effective-medium theory to apply [6,8]. The number, \( N = (d_p/d_0)^3 \), of monomers of diameter \( d_0 \) in a cluster of diameter \( d_p \) depends on the aggregation mechanism and is characterized by the fractal dimension, \( D \). The monomers in such a cluster occupy a fraction

\[
\phi(d_p) = \frac{N d_0^3}{d_p^3} = \left(\frac{d_p}{d_0}\right)^{-3-D}
\]

(5)

of the aggregate’s volume, the rest being filled with the medium. This suggests that the effective refractive index of a fractal aggregate should scale with its diameter as [7]

\[
\ln\left(\frac{L(m_p)}{L(m_0)}\right) = (3 - D) \ln\left(\frac{d_p}{d_0}\right),
\]

(6)

where \( m_p = n_p/n_m \) and \( m_0 = n_0/n_m \). Equation (6) has been used successfully to assess the fractal dimension of populations of protein aggregates [8], and nanoparticle clusters [7], and has been validated through simulations of light scattering by fractals [8]. These numerical studies also suggest that Eq. (6) should hold for individual fractal clusters.

III. HOLOGRAPHIC TOMOGRAPHY

As illustrated in Fig. 1(a), a fractal aggregate’s hologram changes as the aggregate rotates, leading effective-sphere analysis to settle on different values for the effective diameter and refractive index. The data in Fig. 1(b) were obtained for a single fractal silica aggregate with each point representing one measurement of the freely-diffusing cluster. The two representative holograms in Fig. 1(a) are called out with large plot symbols in Fig. 1(b) and generally span the range of effective properties exhibited by this aggregate in different orientations. Such a distribution of values can be interpreted
FIG. 2. Characterization data for two populations of silica aggregates compared with characterization data for a single aggregate isolated from the populations. Characterization data for \( d_p \) and \( n_p \) have been scaled according to Eq. (6). In both cases, single-cluster results (scatter) are consistent with population averages (ellipses). (a) Aggregation at a monomer concentration of \( c = 1.4 \times 10^{15} \text{mL}^{-1} \) is consistent with the population-averaged measurement of \( D = 1.39 \pm 0.33 \). (b) Aggregation at \( c = 5.6 \times 10^{15} \text{mL}^{-1} \) is consistent with the population-averaged measurement of \( D = 1.27 \pm 0.34 \).

with Eq. (6) to obtain a tomographic estimate for the cluster’s fractal dimension, \( D \).

The tomographic value of \( D \) for an individual aggregate can be compared with the average scaling behavior obtained from single-hologram analysis of multiple clusters. This comparison constitutes a test of the assumption underlying previous holographic characterization studies [7, 8] that all of the fractal aggregates in a population scale in a similar way and that their scaling behavior can be captured by effective-sphere analysis. This test can be made more rigorous by performing measurements over a range of growth conditions designed to yield aggregates with different fractal dimensions.

IV. HOLOGRAPHIC TOMOGRAPHY OF MODEL FRACTAL CLUSTERS

Our model system consists of dispersions of silica nanospheres (Ludox TMA colloidal silica, Aldrich catalog no. 420859) that are induced to aggregate through the addition of salt. The spheres in this system have mean diameter \( d_0 = 20 \text{nm} \) as determined by dynamic light scattering (LS Spectrometer, LS Instruments; Zetasizer Nano ZS, Malvern Instruments). The stock dispersion has 34 wt% solids, which corresponds to a number density of \( c = 5 \times 10^{16} \text{mL}^{-1} \) assuming the density of silica to be \( \rho_{\text{SiO}_2} = 2.2 \text{g cm}^{-3} \). This stock solution is diluted with deionized water (MilliQ Ultrapure Water System, MilliporeSigma) and is destabilized by adding 0.1 M MgCl\(_2\) (Sigma-Aldrich, CAS no. 7791-18-6). Aggregation is allowed to proceed for 1 h before the dispersion is further diluted with deionized water to arrest growth. Clusters from the diluted dispersion are then sampled immediately for analysis.

Holographic characterization of populations of aggregates is performed with a commercial instrument (xSight, Spheryx, Inc.). This instrument automatically records and analyzes holograms of thousands of particles by drawing up to 6 mL of sample through a microfluidic observation volume in a pressure-driven flow. Each detected particle is characterized by a single value for the diameter and refractive index. The microfluidic channel used for these measurements (xCell, Spheryx, Inc.) has a 25 \( \mu \text{m} \) minimum dimension, which means that the concentration of micrometer-scale particles can be measured accurately at concentrations up to \( 10^7 \text{mL}^{-1} \) before neighboring particles’ holograms overlap significantly.

Individual particles from each sample also were analyzed in a custom-built holographic microscope operating at a vacuum wavelength of \( \lambda = 0.447 \mu \text{m} \) (Coherent Cube) and an effective system magnification of 48 nm/pixel. Samples were contained in microfluidic chambers formed by sealing the edges of a #1.5 glass cover slip to the surface of a standard glass microscope slide with UV-cured optical adhesive (NOA81, Norland Products). The 30 \( \mu \text{m} \) nominal thickness of these chambers is large enough for an aggregate to diffuse freely in three dimensions. The holographic microscope therefore observes the aggregate in all orientations over time. The particle’s tendency to diffuse out of the field of view was controlled using a holographic optical trapping system integrated into the instrument [23]. This instrument’s camera (Flea3 monochrome USB3.1, Teledyne FLIR) records holograms at 30 frame/s with an exposure time of 10 \( \mu \text{s} \), which is fast enough to avoid motion blurring [24, 25]. Holograms may suffer some blurring due to rotation given the typical rotational diffusion coefficient,

\[
D_0 \approx \frac{k_B T}{\pi \eta d_p^2} = 10^9 \text{s}^{-1},
\]

for a micrometer-scale aggregate in water with a viscosity
of η = 1 mPas. Single-particle holograms were analyzed with the open-source `pylorenzmie` package.

Aggregation at vanishingly low monomer concentration allows clusters to grow through diffusion-limited aggregation (DLA) [26] and yields clusters with fractal dimensions as large as $D = 2.3$. Destabilizing a more concentrated dispersion allows for diffusion-limited cluster aggregation (DLCA), which leads to more open and spindly structures with fractal dimensions around $D = 1.3$ [27].

The data in Fig. 2(a) and (b) were obtained from silica aggregates grown at starting monomer concentrations of $c = 1.4 \times 10^{15} \text{mL}^{-1}$ and $5.6 \times 10^{15} \text{mL}^{-1}$, respectively. Each data set shows characterization results for a single cluster together with the 2-σ confidence interval for a population of 5000 clusters drawn from the same sample. The data are rescaled according to Eq. (6) so that the slope of the distribution yields a measurement of the fractal dimension, $D$. In both cases, the fractal dimension of the single-particle distribution agrees with that of the corresponding population. Aggregates grown at lower monomer concentration have an inferred fractal dimension of $D = 1.39 \pm 0.33$ while those grown at higher concentration have a slightly lower value, $D = 1.27 \pm 0.34$, which is consistent with expectations for three-dimensional DLCA [27].

![Figure 3. Measured population-average fractal dimension as a function of initial nanoparticle concentration. Trend lines for both individual aggregates (blue circles) and populations of aggregates (red triangles) suggest an inverse relationship between concentration of nanoparticles $c$ and fractal dimension $D$. The dashed line at $D = 1$ represents the lower physical limit.](image)

Figure 3 shows how the population-averaged fractal dimension varies with monomer concentration over two decades from $c = 10^{14} \text{mL}^{-1}$ to $10^{16} \text{mL}^{-1}$. As expected, the clusters’ fractal dimension is anticorrelated with the initial monomer concentration.

Measurements with xSight show an overall concentration of $10^6$ aggregates/mL in the most dilute samples, with a typical aggregate containing an estimated 5000 monomers. This suggests that no more than $5 \times 10^9$ monomers/mL have aggregated into clusters that are large enough to be detected. Most of the nanospheres therefore either remain in monomeric form or have formed aggregates smaller than the measurement technique’s detection limit of $d_p \lesssim 500 \text{nm}$. This means that the initial concentration of monomers is high enough to support cluster-cluster aggregation over the full range of growth conditions considered in this study.

V. DISCUSSION

Our results demonstrate that effective-sphere analysis of holographic microscopy data can provide useful information about the morphology of individual fractal clusters. Rather than relying on population averages, this approach draws insights from the distribution of effective-sphere characterization results obtained as the particle rotates in three dimensions. This morphological information, in turn, offers insights into the mechanisms for those clusters’ formation. Holographic tomography therefore provides a fast and cost-effective way to analyze aggregation in commercial products.

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DISCLOSURES

DGG is a founder of Spheryx, Inc., the company that manufactures the xSight particle-characterization instrument used in this study.
DATA AVAILABILITY

Data underlying the results presented in this paper are not publicly available at this time but may be obtained from the authors upon reasonable request.

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