

Evolution of Polycrystallinity in Homogeneously Nucleated Colloidal Crystals

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We investigate how polycrystallinity evolves in homogeneously nucleated crystals in an experimental hard-sphere-like colloidal system using three-dimensional confocal microscopy. We capture the whole process of nucleation and crystal growth and use a recently developed method to resolve grain structure and misorientation between adjacent grains and nearest-neighbor clusters. Closely following nucleation, crystals are shown to give rise to grains through the emergence of stacking faults and twinning. The birth rate of new grains from a single nucleation event are measured, observed to increase, and then plateau. We visualize the spatial structure of a nucleus which comprises a highly ordered core and a radial increase in misorientation angles. Finally, we use local misorientation to formulate a measure for positional order which reveals a sharp decoupling between the development of orientational order and positional order at times significantly after nucleation.

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I. INTRODUCTION

Elucidating thermodynamic and kinetic pathways in the transformation of a supercooled liquid into a crystal is of fundamental significance to our understanding of condensed matter [1,2]. One of the most well-studied crystallizing systems is that of hard spheres, which can be experimentally realized using colloidal particles. While the crystallization of hard spheres has been studied widely using light scattering [3–6] and optical microscopy [4,7–10], a less understood aspect is the emergence of polycrystallinity, whereby many differently oriented crystalline domains (so-called grains) appear. Both experiments [4,8] and simulations [11,12] have found that hard-sphere crystals feature a randomly hexagonal-close-packed (rhcp) structure, containing both face-centered-cubic (fcc) and hexagonal-close-packed (hcp) grains.

Polycrystallinity can have a significant impact on material properties in crystalline materials, including fracture

mechanics [13], ductility [14], transport [15], and melting [16]. In hard spheres, the emergence of grains [17] has been recently proposed as a contributing factor to the discrepancy between crystal nucleation rates in experiment [5,9,18–21] and simulation [9,10,22], with numerous other solutions proposed over the years [22–28]. Salient features of the model proposed [17], particularly how the generation of grains changes during crystalline growth, are yet to be explored experimentally. Another topic is the coupling between density and bond-orientational order formation [4,29–32] during crystallization. While there have been claims that bond-orientational order develops first and creates a precursor from which crystals nucleate [4,32–37], recent numerical work using “order-agnostic” machine-learning algorithms have shown that bond-orientational order and density develop in sync with each other [38], as also proposed in previous simulations [39]. Given these discrepancies, a new means to characterize local order which takes the grain structure into account may offer a new perspective. However, identifying grains in crowded three-dimensional crystalline structures with standard bond-orientational order analysis [36,40] remains challenging.

In the present work, we undertook a three-dimensional confocal laser scanning microscopy (CLSM) experiment observing the nucleation and growth of crystals in a supercooled hard-sphere colloidal suspension (volume fraction $\phi \approx 0.52$) through single-particle tracking and

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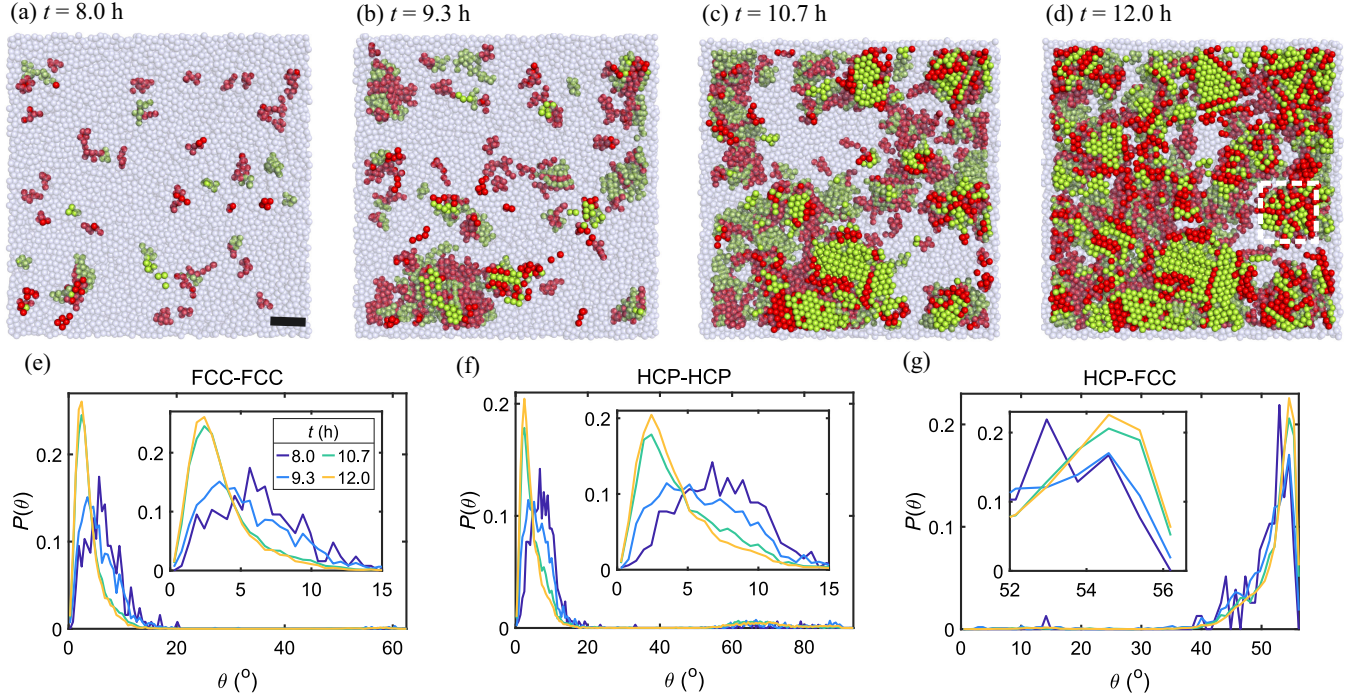


FIG. 1. (a)–(d) 3D rendering of particles at different time points during crystal nucleation and growth. fcc particles are colored yellow, hcp particles red, and amorphous particles translucent blue. In (d), the white box contains a pentatwin. The scale bar in (a) corresponds to 10 μm and applies to (a)–(d). (e)–(g) Probability distributions of misorientation angles between neighboring pairs of crystalline particles of different local structure, (e) fcc-fcc, (f) hcp-hcp, and (g) hcp-fcc. The insets show enlarged comparison of major peaks.

structural analysis. Our experiments are distinguished from previous approaches in several ways. First, we characterized real space crystal structures over time using a recently developed analysis method, BLOSSOM [41], which allows the extraction of the orientation of individual nearest-neighbor clusters (NNCs), which, in turn, yields a measurement of the misorientation between adjacent NNCs. Not only does this give direct access to grain structure, but it becomes possible to distinguish orientational and positional order in nucleated crystals. Second, our experiments span a timescale which captures both crystal nucleation and growth. This enables a detailed assessment of the evolving rhcp structure of crystallites associated with individual nucleation events as well as subsequent development of a polycrystalline structure. Our experiments and analysis give two key findings: First, we observe and measure the incidence of grains and the evolution of inter- and intragrain misorientations during crystal nucleation and growth; Second, we use the same analysis to disentangle the contribution of misalignment between adjacent crystalline nearest-neighbor clusters from their internal positional crystalline order, elucidating a pronounced decoupling between bond-orientational and positional order during crystal growth. We emphasize that both these aspects derive from our focus on the polycrystalline nature of crystal structures, where misorientations between adjacent nearest-neighbor clusters identified using BLOSSOM are

used to both identify and assess grain boundaries, and examine local ordering in a new light.

II. RESULTS AND DISCUSSION

A. Evolution of misorientation angles during crystallization

During a typical experiment (details in Sec. IV A), fewer than ten individual nucleation and growth events are captured within our field of view; snapshots are given in Figs. 1(a)–1(d). Note that nucleation in our experiments followed a long period of time, approximately eight hours. We believe this is largely due to the time it takes for macroscopic flows to settle after the sample is put inside the observation cell. Some of the observed nuclei grow as time progresses, while others dissolve into the amorphous phase due to their precritical sizes. Even at early times, we can see that newly nucleated single-domain crystals have already begun to form multiple grains of fcc and hcp, as shown in Figs. 1(a) and 1(b) (see Sec. IV B for details of analysis). Given the sparsity of nucleation events, these are *not* the product of individual nuclei meeting and forming boundaries but are apparently the result of stacking faults accumulating as a crystal grows.

We confirm this by considering the distribution of misorientation angles within nuclei, as shown in Figs. 1(e)–1(g). First, looking at fcc-fcc and hcp-hcp misorientation angles,

we note a prominent peak around $\theta \approx 0^\circ$ at all times during the crystal growth. This is consistent with the continued formation of hexagonal planes, including stacking faults. However, between fcc and hcp NNCs, we see a peak in the vicinity of $\theta \approx 56.6^\circ$, an angle associated with a geometrically perfect stacking fault as defined by the Blackburn relation (reviewed in Ref. [42]). Therefore, the nuclei have an rhcp structure containing a mixture of fcc and hcp grains related exclusively by stacking faults.

Looking at Fig. 1(e), we see a small population of misorientations between fcc NNCs at $\theta \approx 60^\circ$. This peak is due to twinning, the result of a stacking fault followed by an odd number of hcp layers and another stacking fault. Additionally, we find a small proportion of misorientations between hcp NNCs at $\theta \approx 70^\circ$ [see Fig. 1(f)] corresponding to hcp neighbors meeting at the center of a pentatwin, the result of a succession of five fcc twinning events, each separated by a single layer of hcp. An example of a complete pentatwin is shown in the white box in Fig. 1(d); structures due to fewer than five twinning events may also contribute to the peak. While these have been noted in simulation studies [12], there have been few experimental reports in homogeneously nucleated crystals. Here, not only do we confirm isolated instances directly, but we can find them explicitly in the distributions as a low but significant peak in the misorientation distributions of hcp NNC pairs.

We now consider the evolution of the distributions [Figs. 1(e) and 1(f)] over time. If we look at the dominant peaks at $\theta \approx 0^\circ$ in fcc-fcc and hcp-hcp misorientation angles (see insets), they start broad at early times $t \approx 8$ h with a peak value of $\theta \approx 7^\circ$ before it sharpens and shifts toward lower angles $\theta \approx 2.5^\circ$ at $t \approx 12$ h. This demonstrates that the fcc and hcp crystal structures that first appear during nucleation are significantly more orientationally disordered than their later time counterparts. Note that a perfect crystal would have misorientations of exactly $\theta = 0^\circ$. Looking at the hcp-fcc boundaries, we observe a similar trend. The peak begins broader, at $\theta \approx 53^\circ$ which sharpens toward $\theta \approx 55^\circ$. The direction of the movement is toward larger angles, in this case, toward a geometrically perfect stacking fault as discussed previously. Furthermore, the similarity of the evolutions seen in Figs. 1(e)–1(g) suggests that the settling of particles in hexagonal planes occurs on the same timescale as within purely fcc or hcp domains. Note, however, that the initial peak in the misorientation distribution for hcp is slightly broader and more shifted to higher misorientations than for fcc, with peak values closer to $\theta = 7.5^\circ$ and $\theta = 6.0^\circ$, respectively. This is perhaps a consequence of the lower rotational symmetry of hcp crystals.

B. Development of polycrystallinity over time

Identification of misorientation angles allows us to distinguish *grains* within individual rhcp nuclei during the early stages of growth and consider how their number

evolves over time. Here, we consider seven post critical nuclei, those that grow instead of redissolving, as shown in Fig. 2(a). Note that Fig. 2(a) shows the same field of view as Fig. 1(d) but now showing only the seven postcritical nuclei and ignoring the remaining crystalline particles. We consider small fields of view restricted to the volume of these nuclei (called subvolumes) and trace their nucleation and growth from early times. While the independent emergence of these nuclei is apparent visually, the way in which they were identified is described in Sec. IV C. Figure 2(c) shows the size of the largest nucleus within each subvolume, while the shaded region shows the standard deviation across the seven nuclei. Note the relatively small deviations with respect to the mean; this suggests that growth traces for individual nuclei are well overlapped and share a similar nucleation time, at around $t = 8.5$ h. On nucleation, we see a marked increase in growth rate; the size of these nuclei suggests a critical nucleus size of roughly 20 particles. This is consistent with the upper bound for subcritical nuclei reported previously [8], albeit in a more charged system. Recent studies on similar systems also show critical nuclei sizes on the same order, between 50 and 100 particles [43]. We note that the packing fraction ϕ of the particles calculated from their local Voronoi construction (see Sec. IV D) around the nucleation time is 0.53, which is slightly larger than the initial volume fraction of 0.52 but still within the uncertainty for measuring volume fractions in hard spheres [44]. Recent work on hard-sphere nucleation [45] reports a critical nucleus size of the order of magnitude we have observed at this volume fraction $\phi = 0.53$. Hence, we note that, while departures from an ideal hard-sphere system are undeniable [44], the system is still a good approximation to hard spheres.

Now, we consider grain structure. In Fig. 2(b), we color individual grains within the nuclei; it is clear that each nucleation event leads to the development of multiple grains. Looking at the number of grains per nucleus $N_G(t)$ in Fig. 2(d), each nucleus begins as a single correlated grain, as expected, and is highlighted by the dashed line. At the estimated nucleation time $t = 8.5$ h, the number of grains per nucleus remains around one but rises shortly after. Given that stacking fault grain boundaries are exclusively generated during nucleation (see Sec. II A), the physical origin of polycrystallinity can be understood as random stacking sequences of hexagonal plane *A*, *B*, or *C* positions (which may have different normal directions) which either induce hcp (*ABAB*) or fcc-like (*ABCABC*) ordering. Importantly, the interfacial energy cost of stacking faults is very low [46]. Added to this, the free energy difference between the two competing morphologies in a hard-sphere fluid (fcc and hcp) is very small, and, hence, a random hexagonal-close-packed phase results, as shown extensively in scattering experiments [47–49] and computer simulations [11]. Thus, it becomes unlikely for a

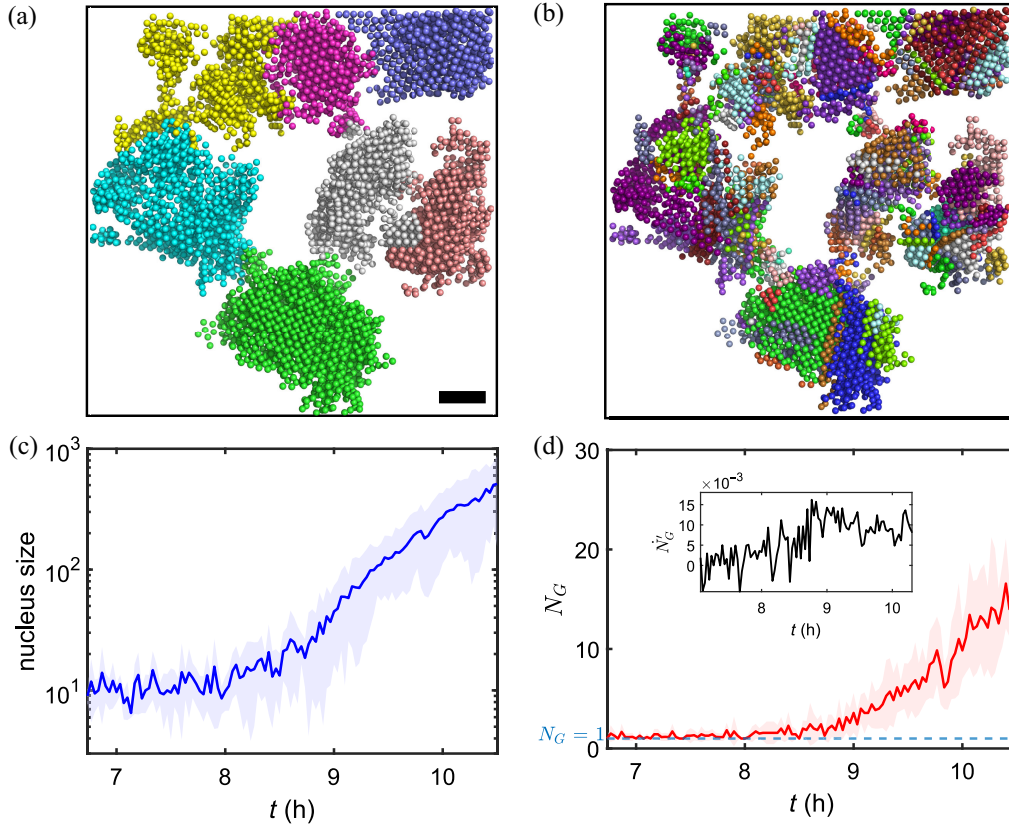


FIG. 2. (a) Seven postcritical nuclei at $t = 12$ h (each nucleus is given a different color). The scale bar is $10 \mu\text{m}$. (b) The nuclei in (a) with individual grains visualized in different colors. (c) Time evolution of the sizes of the nuclei in (a), plotted against the time elapsed since the beginning of the experiment. (d) Time evolution of the number of grains per nucleus N_G . A dashed line corresponding to $N_G = 1$ is shown. Solid lines in (c) and (d) are means at each time point, while the shaded region shows the standard deviation over the seven nuclei. The inset in (d) shows the normalized derivative of the number of grains per nucleus, $\dot{N}'_G$.

nucleus to remain purely fcc. In Fig. 2(d), we see that, within a short time, the structure begins to become polycrystalline, which corresponds to the incidence of stacking faults. We note that the standard deviation in the number of grains is still confined closely to the mean. This suggests that the rate at which stacking faults are introduced into nuclei is consistent between individual nucleation events.

By looking at the derivative of $N_G(t)$ in Fig. 2(d), we estimate the rate at which grains are created within nuclei, as shown in the inset. Note that N_G is smoothed before finding the derivative; the trends are independent of smoothing methods. Grains are produced at the surface; to normalize by the surface area, we take the first derivative $\dot{N}_G(t)$ and scale by the nucleus size to the power of $2/3$, giving a grain incidence rate per surface area, $\dot{N}'_G \sim \dot{N}_G(t)/\text{size}^{2/3}$. We observe an increase in the rate as the nucleus grows; this is most likely correlated with a reduction in the curvature of the nucleus surface, making it easier for stacking faults to form. To the best of our knowledge, this is the first time the grain production rate during crystal nucleation and growth has been measured

experimentally. At first glance, the observed trend is inconsistent with the constant incidence rate of grains adopted in recent theoretical work [17]. However, we note that the distribution of nuclei over space in the same work [17] is much more sparse than in our experiments. In this case, for the majority of the growth time, the rate at which grains are produced would follow the plateau seen in the inset in Fig. 2(d), yielding a constant domain incidence rate. Conversely, the influence of curvature on grain incidence rate would be significant if nuclei are closer together, i.e., if the crystal nucleation rate density is higher. Thus, we may expect the variation in grain production rate over time to be more significant at higher volume fractions within the coexistence window.

C. Spatial distribution of misorientation angles

We consider the structure of individual nuclei in more depth. The same misorientation angles measured previously may be mapped over space. In Figs. 3(a)–3(d), we visualize the misorientations in our field of view by plotting intermediate points between particles and coloring them according to the misorientation angle between their NNCs.

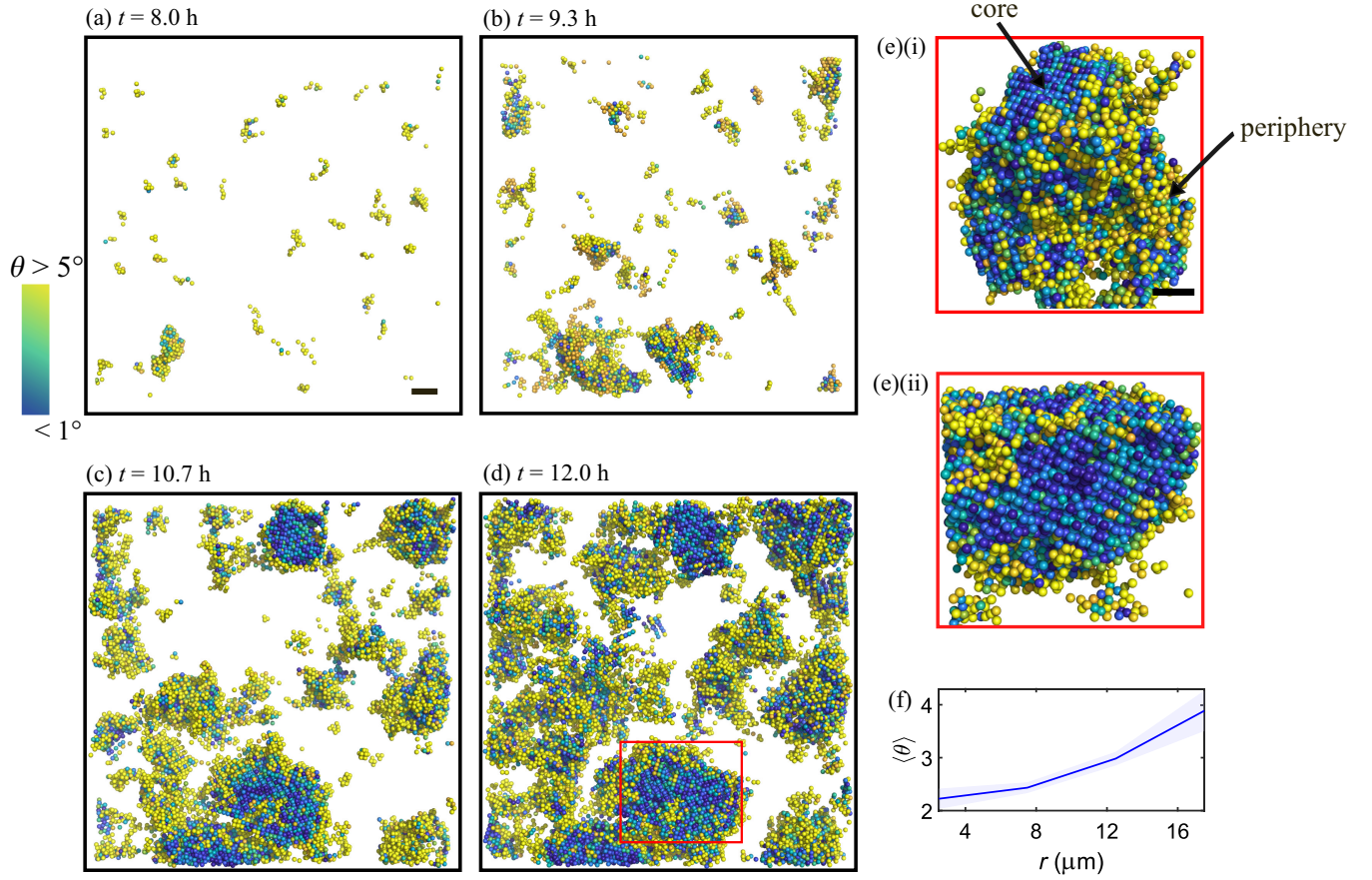


FIG. 3. (a)–(d) Snapshots showing spheres located between NNCs and colored according to the misorientation angle between them θ (color map on the left) at different time points during the nucleation. The scale bar in (a) applies to (a)–(d) and equals 10 μm . A small subvolume is shown exclusively [region inside the red box in (d)] in (e)(i) and (e)(ii) to identify the core and the periphery of the nucleus. The same subvolume is shown in two different viewing angles in (e)(i) and (e)(ii). Scale bar: 5 μm . (f) Radial profile of the nucleus with respect to mean misorientation angle θ . The thick blue line shows the mean, while the shaded region corresponds to the standard deviation over time in the interval 10.5–12.0 h, the latest times sampled.

Misorientations for fcc and hcp NNCs are given as differences from the Blackburn relation [42]. It can be seen that the interiors of the nuclei are characterized by smaller misorientation angles, while angles at the peripheries closer to the disordered fluid phase are larger.

A close-up of a single nucleated crystal [region inside the red box in Fig. 3(d)] is shown in Figs. 3(e)(i) and 3(e)(ii). The radial profile of the misorientation angle is given in Fig. 3(f); this confirms the increase in angle with distance from the center. We note that this agrees broadly with findings for bond-orientational order and density, reported in previous studies using real space imaging of nucleation [8], as well as computer simulations [50,51]. The radial shells over which θ is averaged pass through a range of structures, including stacking faults and particles within the same grain. Given the small standard deviation in these values over each shell, as shown by the shaded region behind the line in Fig. 3(f), it is clear that stacking faults do not seem to influence the radial profile of the misorientation angle. Thus, θ is not sensitive to the presence of stacking faults or grain boundaries but sensitive to the proximity of

the crystal to the fluid phase. While stacking faults separate the grains, they do not seem to influence the kinetic ordering pathway taken for the nucleus seen as a whole. Uneven crystal growth in different directions has also been observed in previous works [8] and seems to be reflected in the off-spherical shape of the crystallite.

D. Measurement and evolution of positional order

Crucially, the assignment of a misorientation angle allows the extraction of a new measure of order, i.e., how the positions of adjacent NNCs are displaced from each other when this rotational misalignment is explicitly removed. Existing measures such as bond coherence [24] may quantify the similarity in the orientation of NNCs in a purely rotational sense but do not provide a means to separate alignment of bond-orientational order and how far the arrangement of particles is from producing a perfect lattice.

Here, we formulate a new *orientation-independent* positional order parameter Δ , where neighboring NNCs

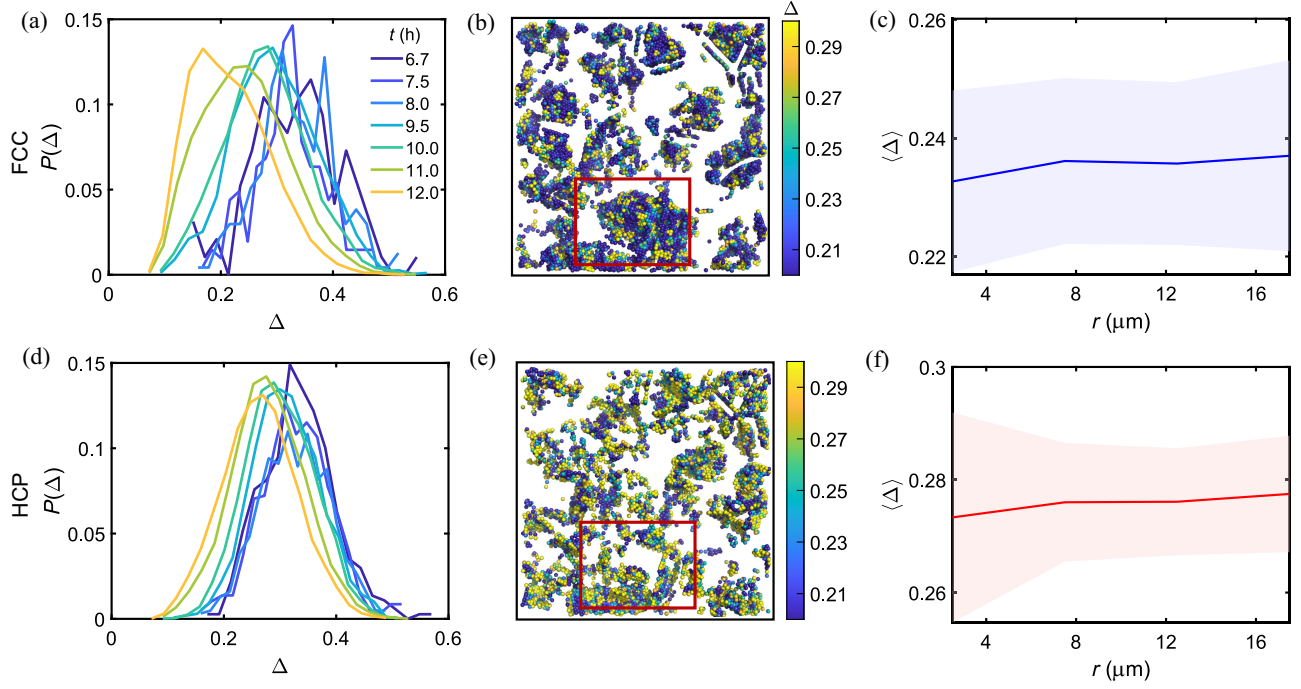


FIG. 4. Probability distributions of Δ for fcc-like NNCs in (a) and hcp-like NNCs in (d) at various time points [legends in (a) apply to (d)]. Spatial map of the Δ parameter at $t = 12$ h for fcc-like NNCs in (b) and hcp-like NNCs in (e), plotted in a similar way for the same field of view ($100 \times 100 \mu\text{m}$ in x - y) as θ in Fig. 3(d). Note that (b) and (e) are 2D projections of the 3D field of view. The region containing one nucleus is drawn in red in (b) and (e). The corresponding radial profile of this highlighted nucleus is shown in (c) and (f) for the fcc-like and hcp-like NNCs, respectively. Thick blue and red lines show the mean, while the shaded region corresponds to the standard deviation over time in the interval 10.5–12.0 h, the latest times sampled.

are first analyzed to find a misorientation angle and then rotated such that the contribution due to this misalignment is removed; the mean relative displacement of particles from each other can then be calculated. Specifically, we take the mean displacement of the positions of particles, $\vec{r}_i$, in neighboring NNCs, i and j , normalized by the mean interparticle spacing in the final crystal, $\bar{d}$:

$$\Delta = \frac{1}{\bar{d}N_{\text{pair}}} \sum_1^{N_{\text{pair}}} \|\vec{r}_i - \vec{r}_j\|_{\text{pair}}, \quad (1)$$

where N_{pair} is the number of NNC pairs. The NNC coordinates are paired by a point set registration step which matches NNC particles with their corresponding reference NNC point and, thus, finds the corresponding points in neighboring NNCs. This point set registration is performed as part of the orientation measurement procedure as described in detail in Ref. [41]. Note that $\vec{r}_i$ and $\vec{r}_j$ are positions relative to their respective central NNC particle such that $\vec{r}_i = \vec{r} - \vec{r}_{\text{central}}$, where $\vec{r}$ is the absolute coordinate.

First, we consider the evolution of $P(\Delta)$, the distribution of Δ over the field of view for fcc- and hcp-like NNCs, as given in Figs. 4(a) and 4(d), respectively. For both morphologies, it is clear that the growth of crystals leads to a reduction in Δ , though the shift is smaller for hcp than

in fcc. Mapping Δ over space [see Figs. 4(b) and 4(e)] reveals that the spatial variation of Δ within individual nuclei occurs differently compared to the spatial map of θ in Fig. 3(d). Analysis of variation of Δ with distance from the center of the same nucleus as in Fig. 3(f), averaged over the same time interval, does not show the same steady increase with distance as seen for misorientation angle θ [compare Figs. 4(c) and 4(f) with Fig. 3(f)]. On closer inspection of Figs. 4(b) and 4(e), regions which have a low Δ value are scattered throughout crystallites in groups of tens of particles, not clustered at the core. One nucleus, whose radial profile is plotted in Figs. 4(c) and 4(f) is marked for clarity in Figs. 4(b) and 4(e) in red. Note that the spatial maps Figs. 4(b) and 4(e) are 2D projections of the 3D field of view of the same region as shown in Fig. 3(d) for θ . For the Δ spatial maps, because the low Δ value regions are scattered around, we identify the nuclei by comparing with the θ spatial maps, as they show the nuclei distinctly with regions of small θ surrounded by larger θ .

We next follow the positional order parameter Δ and misorientation angle θ of individual crystalline NNCs over the field of view and trace it over time, as shown in Fig. 5. The peak of the distribution is also given as a black line. At $t = 11$ h [see Figs. 5(b) and 5(f)], we see results which resemble plots of ϕ and Q_6 (see Fig. 8) shown in Ref. [29], where the lack of correlation evidences a decoupling.

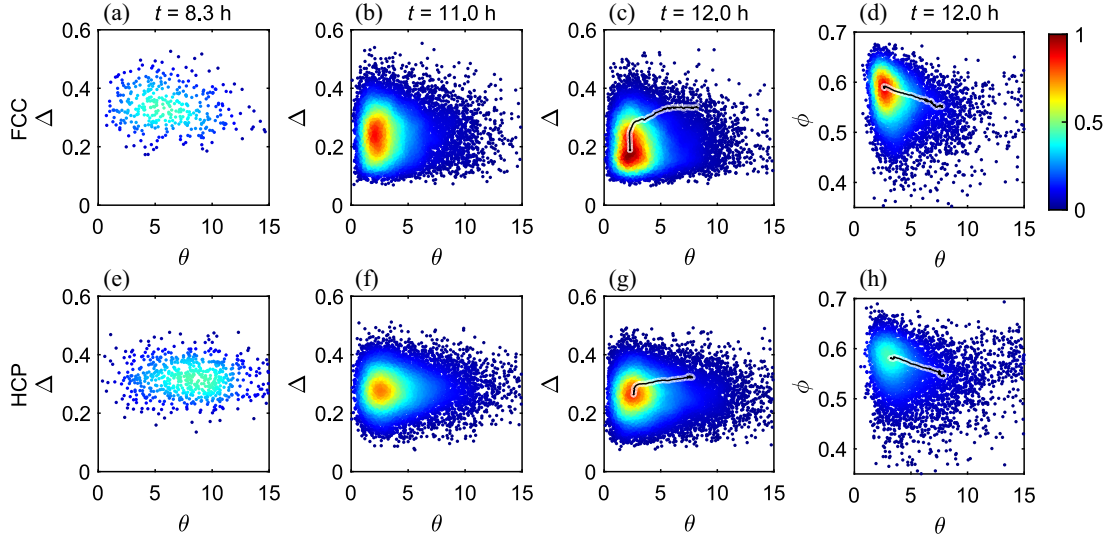


FIG. 5. (a)–(c) and (e)–(g) show how the positional order parameter Δ and misorientation angle θ evolve for fcc-like and hcp-like NNCs over time. A trajectory of the peak of the distribution over time is given in (c) and (g) and in (d) and (h) for the same misorientation angle θ plotted against density ϕ . The density of points is normalized across all the frames. The scale bar for the color map shown on the top right applies to all the plots.

However, as growth continues, there is a marked shift in the distribution downward. Looking at how the peaks change over time [see Figs. 5(c) and 5(g)], we see a trajectory characterized by a reduction in misorientation angle with little change in Δ , followed by a marked kink and rapid drop in Δ . Note that this is not visible when the same misorientation angle average over time is plotted against the local volume fraction ϕ of the same particles [see Figs. 5(d) and 5(h)].

It is clear that a reduction in misorientation angle occurs first, followed by a rapid reduction in positional order. We believe this to be the clearest measurement to date which shows that order based purely on bond-orientational order is the first to develop, followed by local positional ordering, as characterized by relative displacements of the structure of adjacent nearest-neighbor clusters, Δ . We note that the bulk of discussion on decoupling between bond-orientational and translational measures of order focuses on precursor formation and nucleation, i.e., times preceding and including the nucleation time. Here, we show evidence for a decoupling at significantly later times, during crystalline growth, as shown not only by shifts in distributions, but a distinct variation of Δ and θ over space within individual crystallites.

We see a marked difference in evolution of θ with ϕ to that of Δ with ϕ . Replacing θ with Q_6 yields the same effect (see the Appendix). Both θ and Q_6 quantify the orientation of bonds but are not sensitive to bond length and, therefore, do not quantify periodicity in particle positions; this is unlike Δ , which is sensitive to bond lengths. Local volume fraction ϕ , while correlated to positional order Δ via steric packing, is clearly insufficient to detect the specific

development of periodicity. Our results show that, as a crystal becomes denser during nucleation and growth, orientational order appears before positional order. This suggests that crystallization may be promoted by the facilitation of orientational ordering. While ways to selectively enhance bond-orientational order may not be obvious, one might consider reducing misorientation angle (planar alignment) by applying external fields to help the formation of planes. A well-documented example of this is shear-induced crystallization, where parallel hexagonal planes of particles (orientational order) appear before 3D crystalline positions between the layers (positional order) is established [52]. Our study suggests that external fields that promote crystallization do so because they reduce the misorientation angle.

Interestingly, we confirm that the distributions of Δ are significantly different between fcc and hcp grains. Although they begin with similar distributions peaked at $\Delta \approx 0.35$ and reduce around the same time, the extent to which the values drop is higher for fcc compared to hcp [see Figs. 5(b), 5(c), 5(f), and 5(g)]. This suggests that positional order is established more readily in fcc grains than for hcp. One plausible explanation for this is that, given the greater number of hexagonal slip plane directions in fcc (four) compared to hcp (one), particle movement is confined in more directions within the lattice in fcc. There have been numerous reports investigating the prevalence of the fcc phase in crystalline hard-sphere colloidal systems compared to hcp [8,12,43,48,53,54]. Whether the ease with which grains adopt more ordered configurations in fcc seen here is a relevant factor for these findings remains for further investigation.

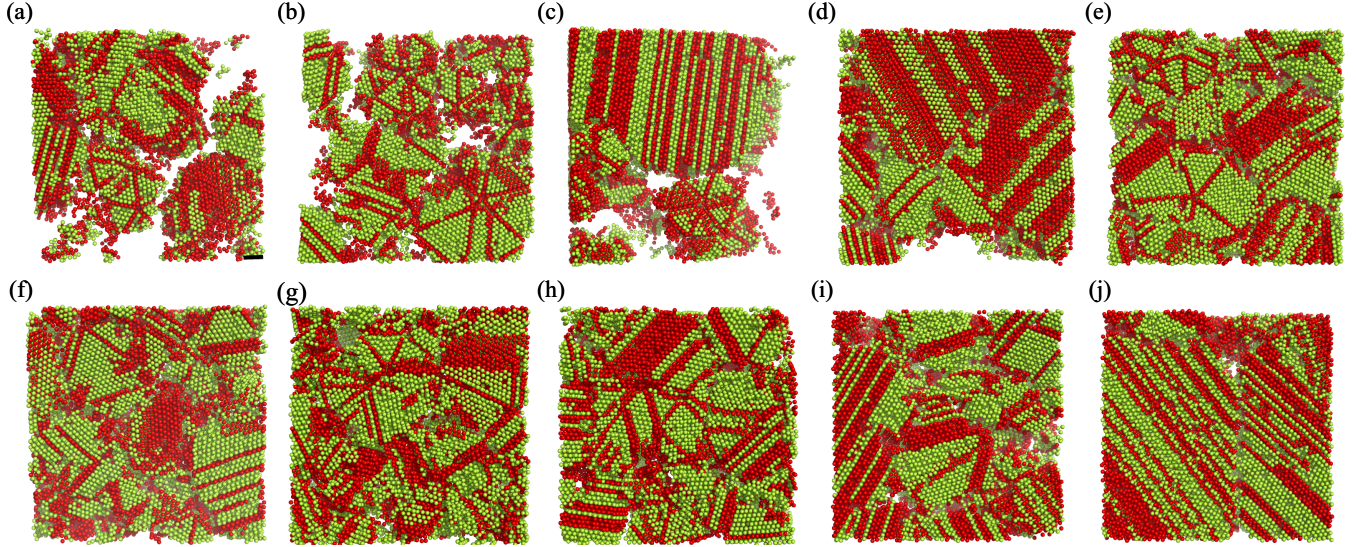


FIG. 6. Crystalline particles detected in the same sample volume in different fields of view. Particles with fcc structure colored yellow and hcp structure colored red. Scale bar [in (a)]: 10 μm and applies to (a)–(j).

III. CONCLUSIONS

We have explored the development of polycrystallinity experimentally in a colloidal model system of PMMA spheres. We observe the birth and growth of homogeneously nucleated colloidal crystals and identify grains as they are formed by measuring the orientation of neighboring NNCs and the misorientations of adjacent clusters. Looking at distributions of misorientation angles, we identify the development of stacking faults and pentatwins. Ordering in NNCs with the same crystal geometry and those at stacking faults seem to occur on the same timescale. We explicitly count the incidence of grains with crystal growth, leading to tens of grains per growing nucleus. We find an initial increase in the production rate of grains with decreased curvature before quickly reaching a plateau. The radial profiles of individual nuclei are also studied; the misorientation angle is found to increase with radial position, consistent with existing bond-orientational order and density characterizations. Finally, by separating misorientation of NNCs from mean displacement from each other, we find a clear decoupling between an initial development of bond-orientational order and a subsequent development of positional order later on during crystal growth, one which is not apparent when looking at local density and bond-orientational order.

We consider the generality of our findings. Many of the findings of this paper such as the dominance of stacking faults and twinning are specific to the fcc or hcp polymorphism present in hard-sphere fluids, a model system still considered a key benchmark in the study of condensed matter [55]. However, other points—such as the radial misorientation profile and the decoupling of misorientation angle and positional order—may not be morphology specific and may prove universal. Comparisons with other crystallizing systems such as charged Yukawa systems,

particularly those with distinct polymorphisms, remain promising avenues of inquiry. The incidence dynamics of grains is also expected to apply to heterogeneous nucleation due to their resemblance to nuclei in our experiments at a late stage of growth, where curvature effects are no longer present. Regardless, the misorientation-based framework that is introduced here has the scope to unravel grain growth dynamics and ordering in a wide range of contexts.

IV. MATERIALS AND METHODS

A. Experimental

The colloidal suspension consists of core-shell PMMA particles (fluorescently labeled core and nonfluorescent shell) [56,57] of diameter 2.0 μm and polydispersity of nearly 2.4% (determined from a dried dispersion that formed a monolayer crystalline array of particles). The particles are dispersed in a density and refractive index matched organic solvent mixture of cyclohexyl bromide and cis-decahydronaphthalene (decalin) saturated with tetrabutylammonium bromide. The synthesis is detailed in Ref. [58]. Crystal nucleation experiments are performed in custom-made sample cells that consist of a cut glass vial top glued onto a microscope coverslip. To prevent heterogeneous nucleation from the base coverslip, a rough layer of polydisperse PMMA particles was spin coated and heated for adhesion. The sample was vortex mixed in a separate vial before being gently injected into sample cell and sealed with a vial top. Observation was carried out with an Andor Dragonfly Multimodal Confocal Spinning Disk unit coupled to an inverted Nikon Eclipse Ti microscope. The scan volume (xyz) was 104.40 $\mu\text{m} \times 104.36 \mu\text{m} \times 40 \mu\text{m}$ in size. Stacks were captured at a rate of one stack every 2 min, typically over 8–12 h. Our observation of both nucleation and growth

was enabled by this low time resolution, with frames too spaced apart to characterize the single-particle diffusion timescales and the nucleation rate. After completion, crystallites can be seen within our field of view as well as outside. An example of the structures seen throughout the same sample volume in different fields of view is shown in Fig. 6. Standard particle detection routines [59] using MATLAB are employed for obtaining the 3D coordinates of all the particles in the field of view. Structural characterization was performed using the BLoSSOM software [41], which uniquely characterizes the misorientations between nearest-neighbor clusters. The 3D rendering of particles was performed using open source PyMOL software [60]. Orientational order analysis was performed using the Steinhardt bond order parameter [36].

B. Analysis of polycrystalline nuclei

Analysis of the colloidal particle coordinates obtained from three-dimensional CLSM images was performed using a recently developed method by Orr *et al.* [41]. The algorithm is able to assign an orientation to local NNCs and, thus, measure misorientation angles between them. Briefly, this method begins by finding the NNCs of each particle using a k - d tree search to locate the 12 nearest particles within a distance cutoff R_{cut} . This cutoff is equal to the first minimum in the radial distribution function of particle coordinates. The relative positions of particles in the NNC with respect to the central particle are determined and then compared with a set of reference NNCs with perfect crystalline structures for each of the hard-sphere crystal polymorphs, hcp and fcc. Note that we also included a search for body-centered-cubic (bcc): No clusters of bcc NNCs were found in our experiments. An orientation calculation is performed to optimally align the measured NNC onto each reference; the angle by which a specific NNC needs to be rotated to match onto its reference provides a measure of its direction in space. Consequently, the difference between the directions of adjacent NNCs corresponds to the misorientation angle. The polymorph reference NNC which minimizes the root mean squared distance to the measured NNC is assigned as the structure of the particle.

Next, crystalline grains are identified as particles with the same crystalline structure which are adjacent in space and have a misorientation angle below a threshold of $\theta_{\text{cut}} = 11^\circ$. Crystalline particles are subsequently identified as those that belong to a grain containing at least six particles. The misorientation angle threshold and grain member threshold were chosen so that the number of crystalline particles agrees with that calculated using the commonly used Steinhardt bond coherence (d_6) order parameter (see Sec. IV D and Fig. 7). Those that are not part of a grain are characterized as amorphous and are plotted in translucent blue in Figs. 1(a)–1(d). In our experiments, we exclusively find fcc and hcp grains, which are plotted as yellow and red, respectively, in Figs. 1(a)–1(d).

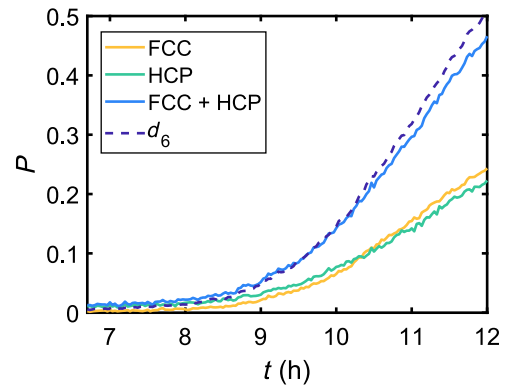


FIG. 7. Proportion of crystalline particles: comparison between BLoSSOM and d_6 .

Misorientation distributions within nuclei are obtained by calculating misorientations between adjacent NNCs. Only particles that are crystalline are included. By including a distance threshold, we ensure that only misorientations within isolated nuclei are counted. As there are fcc and hcp polymorphs, three misorientation distributions are calculated, between fcc neighbors, between hcp neighbors, and between neighboring fcc and hcp neighbors.

C. Identification of individual nuclei

To identify the nuclei in our field of view, we use the same idea of grouping particles into grains based on distance and misorientation angle thresholds. Importantly, we incorporate the step that, if the misorientation is calculated between fcc and hcp, grains can be connected if the misorientation angle is within θ_{cut} of the Blackburn orientation relation. Note that, late into the nucleation, the nuclei identified this way are not well separated and the grains of adjacent nuclei often grow into each other. To study individual nucleation events, we use their spatial profile as visualized by misorientation angles in Fig. 3(d). Specifically, regions of small misorientations at the latest times, corresponding to the nucleus core, are used to manually define subvolumes, seven of which are shown in Fig. 2(a). To study the time evolution of the nucleus growth and the number of grains per nucleus [Figs. 2(c) and 2(d)], we go back to the state of these subvolumes at early times and follow these steps: (i) The size of the largest nucleus in the subvolume is obtained in each time frame. (ii) The number of grains that make up this nucleus is found, and only those grains are counted whose *threshold number* of particles are present in the subvolume at each time frame. We chose a consistent threshold number six, equal to the grain size threshold mentioned previously. The grains comprising the nuclei satisfying the threshold are shown in Fig. 2(b).

D. Calculation of density and bond coherence

The local density of particles is obtained by first calculating the volume of the Voronoi cell of each particle using MATLAB's built-in `convhulln` function. The local

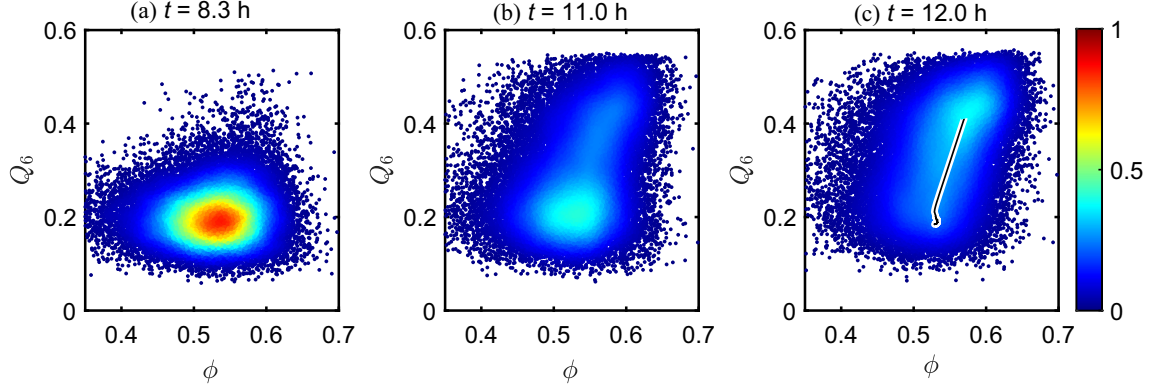


FIG. 8. Evolution of ϕ and Q_6 of individual crystalline NNCs over the field of view. A trajectory of the peak of the distribution over time is given in (c).

density or the Voronoi volume fraction ϕ of every particle is then obtained by dividing the particle volume by its Voronoi volume.

Bond coherence measures the correlation of the bond-orientational order of two neighboring particles [8,24,40,61,62]. The bond-orientational order of particle i with $N_b(i)$ number of neighbors is given by [24,40]

$$q_{6,m}(i) = \frac{1}{N_b(i)} \sum_{j=1}^{N_b(i)} Y_{l,m}(\vec{r}_{ij}), \quad (2)$$

where j runs over the $N_b(i)$ nearest neighbors. $Y_{6,m}$ is a spherical harmonic of the order of 6 (owing to the hexagonal symmetry) and degree m (runs from -6 to 6). Bond coherence between particles i and j is given by

$$d_6 = \sum_{m=-6}^6 \hat{q}_{6,m}(i) \hat{q}_{6,m}^*(j), \quad (3)$$

where

$$\hat{q}_{6,m} = \frac{q_{6,m}}{(\sum_{m=-6}^6 q_{6,m} q_{6,m}^*)^{1/2}}. \quad (4)$$

The value of d_6 ranges from -1 to 1 . A particle is considered locally crystalline if it has six or more neighbors with $d_6 > 0.7$ [24]. The proportion of crystalline particles, P , is given by the number of crystalline particles divided by the total number of particles in the field of view and is plotted in Fig. 7 as a function of time. Note that a coarse-grained form of q_6 , Q_6 , may be defined by a vectorial summation of $q_{6,m}(i)$ over all particles in a nearest-neighbor cluster followed by transformation into a rotationally invariant form [63].

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DATA AVAILABILITY

The data that support the findings of this article are not publicly available upon publication because it is not technically feasible and/or the cost of preparing, depositing, and hosting the data would be prohibitive within the terms of this research project. The data are available from the authors upon reasonable request.

APPENDIX: DECOUPLING BETWEEN ϕ AND Q_6

Decoupling between local volume fraction ϕ and bond-orientational order parameter Q_6 in early, prenucleation supercooled liquids was shown previously [29]. We do the same for our crystallizing system (see Fig. 8), where a similar trend is seen later on during crystal growth. We can see that there is no kink in the profile like when θ and Δ are plotted together.

- [1] G. C. Sosso, J. Chen, S. J. Cox, M. Fitzner, P. Pedevilla, A. Zen, and A. Michaelides, *Crystal nucleation in liquids: Open questions and future challenges in molecular dynamics simulations*, *Chem. Rev.* **116**, 7078 (2016).
- [2] K. Kelton and A. L. Greer, *Nucleation in Condensed Matter: Applications in Materials and Biology* (Elsevier, New York, 2010), Vol. 15.

- [3] H.J. Schöpe, G. Bryant, and W. van Meegen, *Two-step crystallization kinetics in colloidal hard-sphere systems*, *Phys. Rev. Lett.* **96**, 175701 (2006).
- [4] P. Tan, N. Xu, and L. Xu, *Visualizing kinetic pathways of homogeneous nucleation in colloidal crystallization*, *Nat. Phys.* **10**, 73 (2014).
- [5] M. Franke, S. Golde, and H.J. Schöpe, *Solidification of a colloidal hard sphere like model system approaching and crossing the glass transition*, *Soft Matter* **10**, 5380 (2014).
- [6] S. Golde, T. Palberg, and H.J. Schöpe, *Correlation between dynamical and structural heterogeneities in colloidal hard-sphere suspensions*, *Nat. Phys.* **12**, 712 (2016).
- [7] M. Elliot, S. Haddon, and W. Poon, *Direct observation of pre-critical nuclei in a metastable hard-sphere fluid*, *J. Phys. Condens. Matter* **13**, L553 (2001).
- [8] U. Gasser, E.R. Weeks, A. Schofield, P. Pusey, and D. Weitz, *Real-space imaging of nucleation and growth in colloidal crystallization*, *Science* **292**, 258 (2001).
- [9] J. Taffs, S.R. Williams, H. Tanaka, and C.P. Royall, *Structure and kinetics in the freezing of nearly hard spheres*, *Soft Matter* **9**, 297 (2013).
- [10] N. Wood, J. Russo, F. Turci, and C.P. Royall, *Coupling of sedimentation and liquid structure: Influence on hard sphere nucleation*, *J. Chem. Phys.* **149** (2018).
- [11] S. Auer and D. Frenkel, *Prediction of absolute crystal-nucleation rate in hard-sphere colloids*, *Nature (London)* **409**, 1020 (2001).
- [12] B. O'Malley and I. Snook, *Crystal nucleation in the hard sphere system*, *Phys. Rev. Lett.* **90**, 085702 (2003).
- [13] Z. Shan, E. Stach, J. Wiecek, J. Knapp, D. Follstaedt, and S. Mao, *Grain boundary-mediated plasticity in nanocrystalline nickel*, *Science* **305**, 654 (2004).
- [14] T. Mukai, M. Yamanoi, H. Watanabe, and K. Higashi, *Ductility enhancement in AZ31 magnesium alloy by controlling its grain structure*, *Scr. Mater.* **45**, 89 (2001).
- [15] F. Chiabrera, I. Garbayo, L. López-Conesa, G. Martín, A. Ruiz-Cardad, M. Walls, L. Ruiz-González, A. Kordatos, M. Núñez, A. Morata *et al.*, *Engineering transport in manganese by tuning local nonstoichiometry in grain boundaries*, *Adv. Mater.* **31**, 1805360 (2019).
- [16] A.M. Alsayed, M.F. Islam, J. Zhang, P.J. Collings, and A.G. Yodh, *Premelting at defects within bulk colloidal crystals*, *Science* **309**, 1207 (2005).
- [17] W. Wöhler and T. Schilling, *Hard sphere crystal nucleation rates: Reconciliation of simulation and experiment*, *Phys. Rev. Lett.* **128**, 238001 (2022).
- [18] K. Schätzel and B.J. Ackerson, *Density fluctuations during crystallization of colloids*, *Phys. Rev. E* **48**, 3766 (1993).
- [19] Y. He, B.J. Ackerson, W. van Meegen, S.M. Underwood, and K. Schätzel, *Dynamics of crystallization in hard-sphere suspensions*, *Phys. Rev. E* **54**, 5286 (1996).
- [20] J.L. Harland, S.I. Henderson, S.M. Underwood, and W. van Meegen, *Observation of accelerated nucleation in dense colloidal fluids of hard sphere particles*, *Phys. Rev. Lett.* **75**, 3572 (1995).
- [21] S. Iacopini, T. Palberg, and H.J. Schöpe, *Crystallization kinetics of polydisperse hard-sphere-like microgel colloids: Ripening dominated crystal growth above melting*, *J. Chem. Phys.* **130**, 084502 (2009).
- [22] J. Russo, A.C. Maggs, D. Bonn, and H. Tanaka, *The interplay of sedimentation and crystallization in hard-sphere suspensions*, *Soft Matter* **9**, 7369 (2013).
- [23] S. Auer and D. Frenkel, *Suppression of crystal nucleation in polydisperse colloids due to increase of the surface free energy*, *Nature (London)* **413**, 711 (2001).
- [24] P. Pusey, E. Zaccarelli, C. Valeriani, E. Sanz, W.C. Poon, and M.E. Cates, *Hard spheres: Crystallization and glass formation*, *Phil. Trans. R. Soc. A* **367**, 4993 (2009).
- [25] M. Radu and T. Schilling, *Solvent hydrodynamics speed up crystal nucleation in suspensions of hard spheres*, *Europhys. Lett.* **105**, 26001 (2014).
- [26] M. Tateno, T. Yanagishima, J. Russo, and H. Tanaka, *Influence of hydrodynamic interactions on colloidal crystallization*, *Phys. Rev. Lett.* **123**, 258002 (2019).
- [27] G. Fiorucci, G.M. Coli, J.T. Padding, and M. Dijkstra, *The effect of hydrodynamics on the crystal nucleation of nearly hard spheres*, *J. Chem. Phys.* **152**, 064903 (2020).
- [28] S. Ketzetzi, J. Russo, and D. Bonn, *Crystal nucleation in sedimenting colloidal suspensions*, *J. Chem. Phys.* **148** (2018).
- [29] J. Russo and H. Tanaka, *The microscopic pathway to crystallization in supercooled liquids*, *Sci. Rep.* **2**, 505 (2012).
- [30] J. Russo and H. Tanaka, *Role of bond orientational order in the crystallization of hard spheres*, *AIP Conf. Proc.* **1518**, 232 (2013).
- [31] J. Russo and H. Tanaka, *Crystal nucleation as the ordering of multiple order parameters*, *J. Chem. Phys.* **145**, 211801 (2016).
- [32] J. Russo and H. Tanaka, *Nonclassical pathways of crystallization in colloidal systems*, *MRS Bull.* **41**, 369 (2016).
- [33] T. Kawasaki and H. Tanaka, *Formation of a crystal nucleus from liquid*, *Proc. Natl. Acad. Sci. U.S.A.* **107**, 14036 (2010).
- [34] T. Schilling, H.J. Schöpe, M. Oettel, G. Opletal, and I. Snook, *Precursor-mediated crystallization process in suspensions of hard spheres*, *Phys. Rev. Lett.* **105**, 025701 (2010).
- [35] G.I. Tóth, T. Pusztai, G. Tegze, G. Tóth, and L. Gránágy, *Amorphous nucleation precursor in highly nonequilibrium fluids*, *Phys. Rev. Lett.* **107**, 175702 (2011).
- [36] W. Lechner, C. Dellago, and P.G. Bolhuis, *Role of the prestructured surface cloud in crystal nucleation*, *Phys. Rev. Lett.* **106**, 085701 (2011).
- [37] J.F. Lutsko and G. Nicolis, *Theoretical evidence for a dense fluid precursor to crystallization*, *Phys. Rev. Lett.* **96**, 046102 (2006).
- [38] M. de Jager, F. Smalenburg, and L. Filion, *In search of a precursor for crystal nucleation of hard and charged colloids*, *J. Chem. Phys.* **159**, 134902 (2023).
- [39] J.T. Berryman, M. Anwar, S. Dorosz, and T. Schilling, *The early crystal nucleation process in hard spheres shows synchronised ordering and densification*, *J. Chem. Phys.* **145**, 211901 (2016).
- [40] P.J. Steinhardt, D.R. Nelson, and M. Ronchetti, *Bond-orientational order in liquids and glasses*, *Phys. Rev. B* **28**, 784 (1983).
- [41] Nicholas H. P. Orr, T. Yanagishima, E. Maire, and Roel P. A. Dullens, *Grain boundary characterization from particle coordinates*, *Phys. Rev. Mater.* **5**, 123605 (2021).

- [42] R. Krakow, R. J. Bennett, D. N. Johnstone, Z. Vukmanovic, W. Solano-Alvarez, S. J. Lainé, J. F. Einsle, P. A. Midgley, C. M. Rae, and R. Hielscher, *On three-dimensional misorientation spaces*, *Proc. R. Soc. A* **473**, 20170274 (2017).
- [43] W. Gispen, G. M. Coli, R. van Damme, C. P. Royall, and M. Dijkstra, *Crystal polymorph selection mechanism of hard spheres hidden in the fluid*, *ACS Nano* **17**, 8807 (2023).
- [44] C. P. Royall, W. C. Poon, and E. R. Weeks, *In search of colloidal hard spheres*, *Soft Matter* **9**, 17 (2013).
- [45] S. Kale, A. Lederer, and H. J. Schöpe, *The missing billions in hard sphere nucleation*, *Soft Matter* **21**, 8100 (2025).
- [46] J. J. Hoyt and M. Asta, *Atomistic computation of liquid diffusivity, solid-liquid interfacial free energy, and kinetic coefficient in au and ag*, *Phys. Rev. B* **65**, 214106 (2002).
- [47] P. N. Pusey and W. Van Megen, *Phase behaviour of concentrated suspensions of nearly hard colloidal spheres*, *Nature (London)* **320**, 340 (1986).
- [48] P. N. Pusey, W. Van Megen, P. Bartlett, B. J. Ackerson, J. G. Rarity, and S. M. Underwood, *Structure of crystals of hard colloidal spheres*, *Phys. Rev. Lett.* **63**, 2753 (1989).
- [49] J. Zhu, M. Li, R. Rogers, W. Meyer, R. Ottewill, S.-S. S. Crew, W. Russel, and P. Chaikin, *Crystallization of hard-sphere colloids in microgravity*, *Nature (London)* **387**, 883 (1997).
- [50] P. Rein ten Wolde, M. J. Ruiz-Montero, and D. Frenkel, *Numerical calculation of the rate of crystal nucleation in a Lennard-Jones system at moderate undercooling*, *J. Chem. Phys.* **104**, 9932 (1996).
- [51] R. Ohnesorge, H. Löwen, and H. Wagner, *Density functional theory of crystal-fluid interfaces and surface melting*, *Phys. Rev. E* **50**, 4801 (1994).
- [52] N. H. Orr, T. Yanagishima, I. P. Dolbnya, A. V. Petukhov, and R. Dullens, *Single-orientation colloidal crystals from capillary-action-induced shear*, *J. Chem. Phys.* **157**, 224903 (2022).
- [53] S. Heitkam, W. Drenckhan, and J. Fröhlich, *Packing spheres tightly: Influence of mechanical stability on close-packed sphere structures*, *Phys. Rev. Lett.* **108**, 148302 (2012).
- [54] F. Leoni and J. Russo, *Nonclassical nucleation pathways in stacking-disordered crystals*, *Phys. Rev. X* **11**, 031006 (2021).
- [55] C. P. Royall, P. Charbonneau, M. Dijkstra, J. Russo, F. Smallenburg, T. Speck, and C. Valeriani, *Colloidal hard spheres: Triumphs, challenges, and mysteries*, *Rev. Mod. Phys.* **96**, 045003 (2024).
- [56] R. P. A. Dullens, Claesson, D. Derks, A. van Blaaderen, and W. K. Kegel, *Monodisperse core-shell poly (methyl methacrylate) latex colloids*, *Langmuir* **19**, 5963 (2003).
- [57] S. M. Klein, V. N. Manoharan, D. J. Pine, and F. F. Lange, *Preparation of monodisperse pmma microspheres in nonpolar solvents by dispersion polymerization with a macromonomeric stabilizer*, *Colloid Polym. Sci.* **282**, 7 (2003).
- [58] M. Tateno, T. Yanagishima, and H. Tanaka, *Microscopic structural origin behind slowing down of colloidal phase separation approaching gelation*, *J. Chem. Phys.* **156**, 084904 (2022).
- [59] J. C. Crocker and D. G. Grier, *Methods of digital video microscopy for colloidal studies*, *J. Colloid Interface Sci.* **179**, 298 (1996).
- [60] Schrödinger, LLC, *The PyMOL molecular graphics system, version 3.1.8*, software available at <https://pymol.org> (2026).
- [61] J. S. Van Duijneveldt and D. Frenkel, *Computer simulation study of free energy barriers in crystal nucleation*, *J. Chem. Phys.* **96**, 4655 (1992).
- [62] P. Rein ten Wolde, M. J. Ruiz-Montero, and D. Frenkel, *Simulation of homogeneous crystal nucleation close to coexistence*, *Faraday Discuss.* **104**, 93 (1996).
- [63] W. Lechner and C. Dellago, *Accurate determination of crystal structures based on averaged local bond order parameters.*, *J. Chem. Phys.* **129**, 114707 (2008).