Quantitative optical microscopy of colloids: the legacy of Jean Perrin

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Abstract

When the *discontinuous structure of matter* was yet an intriguing hypothesis, Jean Perrin performed a set of elegant and pioneering experiments that marked the birth of what today we consider *quantitative optical microscopy*. Picking up the baton from Perrin, today microscopists face incredible challenges, aiming to extract quantitative information from the increasingly content-rich and complex images made available by modern microscopy techniques. Here, I provide an overview of these challenges and describe the solutions adopted to succeed in this complex task when investigating colloidal systems or systems in which colloidal particles are embedded as microrheological probes.

1. Introduction

Jean Perrin (1870-1942) has been a pioneer of quantitative optical microscopy. Reading his 1909 report in the *Annales de Chimie et de Physique* (English translation [1]) is a stimulating and rewarding experience for any perspective scientist, in particular for experimentalists. In his manuscript, he describes a set of experiments that were stimulated by Einstein's 1905 predictions on Brownian motion [2]. Using a microscope equipped with a *camera lucida*, Perrin and his collaborators observed colloidal suspensions of mastic and gamboge particles at constant temperature. At a fixed height within the sample, the particles' radius R was estimated and their position was tracked in time (every 30 seconds) to extract their diffusion coefficient D. Einstein theory, providing a quantitative link between D and R, was then exploited by Perrin to estimate the Avogadro number. Perrin also reports several other observations, including an independent estimate of the Avogadro number based on the determination of the equilibrium sedimentation profile of the colloids, the measurement of the rotational diffusion coefficient of large spherical inclusions, and the onset of colloidal aggregation experiments performed by adding coagulants. The value of Perrin experiments was immediately recognized by his contemporaries. In a letter to Perrin dated 1909, Einstein himself wrote: "Ich hätte es für unmöglich gehalten, die Brownsche Bewegung so präzis zu untersuchen; es ist ein Glück für diese Materie, dass Sie sich ihrer angenommen haben.", which in English reads: "I would have thought it impossible to investigate Brownian motion so precisely; it is fortunate for this matter that you have embraced it.". While not the first to provide an estimate for the Avogadro number, the meticulous care put by Perrin in all his experiments provided a strong, irrefutable argument in favor of the *molecular reality* of matter and, for this work in particular, he was awarded with the 1926 Nobel prize in Physics.

Retrospectively, we can today consider Perrin the first exponent of *quantitative microscopy*, intended in the present review article as the art of pushing microscopy to its limits of temporal and spatial resolution by combining a set of diverse competences and skills borrowed from different disciplines such as physics, biology and engineering. Given the complexity and the multidisciplinary nature of this art, it is thus not surprising that over the years, the heritage of Perrin has been collected by scientists of different branches, whose work contributed to shape a vast and variegated landscape that in this review article I will only be able to scratch.

The modern approach to quantitative microscopy originates from efforts that have been very discontinuous in time. Indeed, up to about twenty years ago, optical microscopy was used mainly in a qual-

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itative way, with a few notable exceptions that are pointed out very effectively in the introduction of Ref. [3]. In the last twenty years, by contrast, we witnessed to a striking resurgence of quantitative optical microscopy experiments, mostly in connection with the impressive evolution of technology: microscopes have been pushed to unprecedented limits both in terms of optics (e.g. optical resolution enhancement) and of mechanics (e.g. fast automation, high-precision positioning, integration with microfluidics); the computing power of central processing units (CPU) has increased constantly and conformed to Moore's law far beyond any reasonable expectations; the parallel use of graphics processing unit (GPU) made possible the high-speed real-time or post-acquisition analysis of microscopy data, with speed-up factors exceeding 100X; pixelated image sensors can now measure single photons per pixel, can reach 10 KHz acquisition speed at full frame ($\sim$ 1 megapixel) and can acquire more than 10^6 images per second at reduced image resolution, an incredible set of advances since the first demonstration of the charge-coupled device (CCD) in 1969.

In this review article, I will try to cover the potentially very wide area of quantitative optical microscopy of colloidal systems, for which the relatively large length-scale is accompanied by dynamics and kinetics that are slower, and in turn easier to monitor, compared to molecular systems. The article is organized as follows: after a short section sketching the main advantages of optical microscopy, I will briefly describe in Section 3 the most recent efforts to push quantitative optical microscopy beyond the known resolution limits: it will be discussed how super-resolution techniques have been used in colloidal science so far and also what are the current performances of particle tracking algorithms. In Section 4, the recently introduced idea that microscopes can be used as very powerful light scattering machines will be described. In particular, I will show how microscope movies obtained by imaging a sample in real-space with one or more image contrast mechanisms (e.g. absorption, dephasing, scattering, fluorescence) can provide wave-vector resolved scattering information about it. Finally, Section 5 will be devoted to the use of microscopy, possibly in combination with rheology, to link the macroscopic mechanical properties of colloidal crystals and amorphous solids to the structural and dynamical properties.

2. Advantages of optical microscopy

Optical microscopy is one of the several tools used to characterize colloidal systems. Others are for instance (scanning or transmission) electron microscopy and atomic force microscopy; static and dynamic scattering (or diffraction) of light, neutrons or X-rays; sedimentation, centrifugation, field-flow fractionation; optical and ultrasonic spectroscopy; electro- and thermo-phoresis; viscometry and rheology.

Compared to all these methods, optical microscopy has some characterizing advantages. With respect to neutron, electron or x-ray microscopy it allows accessing shorter time scales over typically longer experimental durations and larger length scales. The extracted real-space information is complementary to the one obtained from scattering methods that usually access smaller length-scales (especially with X-rays and neutrons) and shorter time-scales, owing to the use of fast and sensitive detectors such as photomultipliers or avalanche photodiodes. We will show that the existing dichotomy between microscopy and scattering can be partly reconciled by using the ideas described in Section 4.

Microscopes can be very easily coupled with other tools, such as rheometers, optical tweezers, microfluidic chips. Also, typical samples for microscopy can be rather thin (down to a few tens of μm) along the optical axis, which helps in reducing multiple scattering from optically dense colloidal systems and unwanted convective flows.

Light intensity in micrographs can map absorption or dephasing fluctuations within the sample, as well as the distribution and conditional activity of fluorophores or depolarizing structures, providing thereby an extremely rich portfolio of probes. The spatial and directional properties of the illuminating light can be matched to an arbitrary degree with the collection optics, which enables to range from a fully three-dimensional (3D) detection (e.g. bright field microscopy with coherent illuminations) to an almost two-dimensional (2D) one (e.g. confocal or light-sheet microscopy) and permits the use of sophisticated optical schemes such as phase-contrast, dark-field or differential-interference contrast (DIC) that, at least in commercial setups, can be combined with others and alternated in a simple and quick way.

Finally, science-grade microscopes are ubiquitous in research laboratories, either academic or in-

dustrial, with an ample price range that can offer a good match for almost every interested new adopter. Readers interested in knowing in more detail how typical microscopy techniques work and about their applications in soft matter science may refer to Refs. [4, 5].

3. Pushing the limits of quantitative optical microscopy

Some of the limitations of optical microscopy of colloids are technological in nature, such as the signal to noise ratio in microscope images, which is key in discriminating a colloidal particle from the background signal and in determining the particle localization uncertainty. Some others are of fundamental nature. For instance, according to classical linear optics, diffraction poses an ultimate limit to the detection of small colloidal particles. In this Section, I will discuss how both technological and fundamental limitations have been tackled in the last years, with particular reference to colloidal systems.

Super-resolution studies of colloids

When considering recent improvements in optical microscopy, the first thought goes to the so called super-resolution techniques, such as stimulated emission depletion (STED) [6], photo-activated localization microscopy (PALM) [7], and stochastic optical reconstruction microscopy (STORM) [8]. With these revolutionary techniques, some nonlinearities in the emission and/or in the detection of light are exploited to smash the diffraction barrier and achieve resolutions of the order of few tens of nanometers. For introducing such groundbreaking ideas, Eric Betzig, Stefan W. Hell and William E. Moerner shared the 2014 Nobel Prize for Chemistry.

It is quite curious that the first adopters of these methods were life scientists rather than chemists or physicists, despite the fundamental contribution of the latter in developing these tools. In particular, colloid scientists started using super-resolution optical microscopy with a notable delay, which is partly justified by the fact that some of the most common super-resolution approaches require specialized fluorescent probes and/or are intrinsically limited in terms of temporal resolution.

One of the early application of STED to colloidal science was demonstrated in Refs. [9, 10], where (i) the crystalline structure formed by 200 nm large particles, with voids in the crystal as small as 30 nm, was unveiled with unprecedented lateral (43 nm) and axial (125 nm) resolution and (ii) the formation of the colloidal crystals was monitored with a temporal resolution of 5 ms, which allowed visualizing the annealing of potential point defects. More

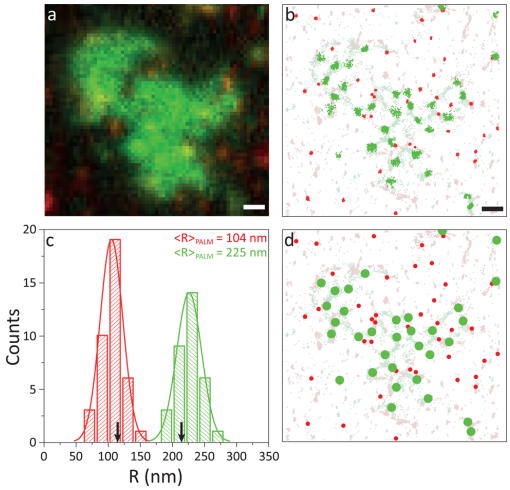

Figure 1: (a) Two-color wide-field image acquired in cyclohexane with 2% v/v isopropanol of a mixed cluster of colloidal particles of $R \sim 110$ nm labeled with a red-emitting fluorophore and $R \sim 220$ nm labeled with a green-emitting fluorophore. (b) Super-resolved image of the field of view shown in (a). Scale bar 1 μm. (c) Radii distribution of the nanoparticles identified and reconstructed in the mixed sample; black arrows indicate the mean radii determined by SEM.(d) Schematic representation of the beads identified in (b). Reprinted with permission from Ref. [11]. Copyright (2016) American Chemical Society.

recently, Aloi et al. [11] demonstrated the possibility to perform high-accuracy super-resolution microscopy with various organic solvents by using PALM to obtain the size distribution of binary mixtures of nanoparticles in close contact. The usefulness of this approach can be appreciated in Fig. 1, where we show the wide-field image of the binary mixture (Fig. 1 a) together with the corresponding super-resolved image of the same sample (Fig. 1 b). Despite the close proximity of most of the particles, the reconstruction of their position and size can be done very precisely to obtain a quantitatively sound particle size distribution (Fig. 1 c) that would be impossible to draw with the wide-field images. The same group also showed how a particular implementation of super-resolution microscopy (iPAINT: interface Point Accumulation for Imaging in Nanoscale Topography) permits nanoscale

imaging of solid/liquid, liquid/liquid and liquid/air interface [12]. They used iPAINT to investigate at the nanoscale the morphology of complex coacervate core micelles in aqueous solutions, revealing a concentration-induced morphological transition of the micelles [13]. Similar to PALM and STORM, iPAINT is based on the localization of single isolated fluorescent molecules. This class of methods may benefit from the recent demonstration that combining them with multivariate statistical analysis opens the way to the 3D reconstruction of nano-structures from 2D super-resolution projections [14].

Another very interesting application is represented by the use of super-resolution imaging to inspect the interior of colloidal particles, in particular of polymer microgel particles [15–17]. Microgel particles of PNIPAM, a polymer that exhibits a reversible volume phase transition at a well prescribed temperature of 32 ^{o}, were studied with STORM with lateral and axial resolution of the order of 30 nm and 60 nm, respectively [15]. This approach allowed reconstructing the internal structure of the microgel particles, and in particular their radial density profile, in different conditions, for instance, of temperature, solvent. The obtained results open the way to the tuning of the internal structure of the microgel particles that may be crucial in their optimization as drug delivery carriers.

Improving the accuracy of particle tracking algorithms

One of the ways to improve the quantitativeness of optical microscopy experiments is based on increasing the precision and accuracy of the image processing. A paradigmatic example in this respect is the need of obtaining very accurate estimates of the particle localization in tracking experiments, which can be used for several purposes. For example, following Perrin we can use the particle mean square displacements to determine diffusion coefficients or, more generally, the mechanical moduli of a soft material in which the colloidal particles have been embedded as probes, which is the realm of microrheology [18, 19].

Camera-based particle tracking determines the position of a particle either by using Gaussian fitting or by calculating the intensity-based centroid, or by locating the local intensity maximum. The best performances of camera-based particle tracking using these approaches are currently of the order of 10 KHz and 3 A° for the temporal and spatial resolution, respectively [21–23] and it is likely that technological advances will improve these performances.

Another route toward improving particle tracking is based on making use of sophisticated image processing algorithms with standard instrumentation. A first example of application of this idea is represented by Bayesian localization microscopy, which allows extremely accurate particle localization to be extracted from wide-field images and, quite importantly, where labeling is obtained with standard fluorescent proteins [24]. A Bayesian analysis of blinking and bleaching (3B analysis) leads to an impressive 50 nm resolution on a 4 s timescale, which is achieved by accurately modeling the blinking and bleaching of the fluorophores so to extract their most likely spatial distribution also when they are overlapping. This property represents an advantage over PALM- and STORM-like approaches, whose image reconstruction algorithm requires several tens of thousands of frames, each one of them obtained with non-overlapping fluorophores. Such advantage comes at the expense of a quite long computational time, about one day of computation time being needed for reconstructing a 50 μm x 50 μm region on a state-of-the-art desktop computer. However, it was shown in Ref. [25] how an improvement by a factor of 60 can be achieved by using cloud computing based on commercially available and accessible solutions.

A second example of analytical approach based on standard instrumentation is represented by super-resolution radial fluctuations (SRRF) microscopy [26], which combines considerations about the radial symmetry property of fluorophore emissions and a temporal fluctuation analysis of the frames to extract super-resolution images (at 1 frame per second and with a spatial resolution down to 60 nm) with conventional fluorophores, low-intensity illumination and about 100 frames. Similar to the 3B analysis, also in SRRF a super-resolution image is obtained without fluorophore detection and localization, which makes both of these techniques very appealing not only for use in the life science community in which they originated, but also for colloid scientists, which would surely benefit from the possibility of using conventional fluorophores in their experiments.

Finally, in Ref. [20], an accurate reconstruction of colloidal particles positions and sizes was achieved in standard confocal microscopy experiments by modeling the image formation process

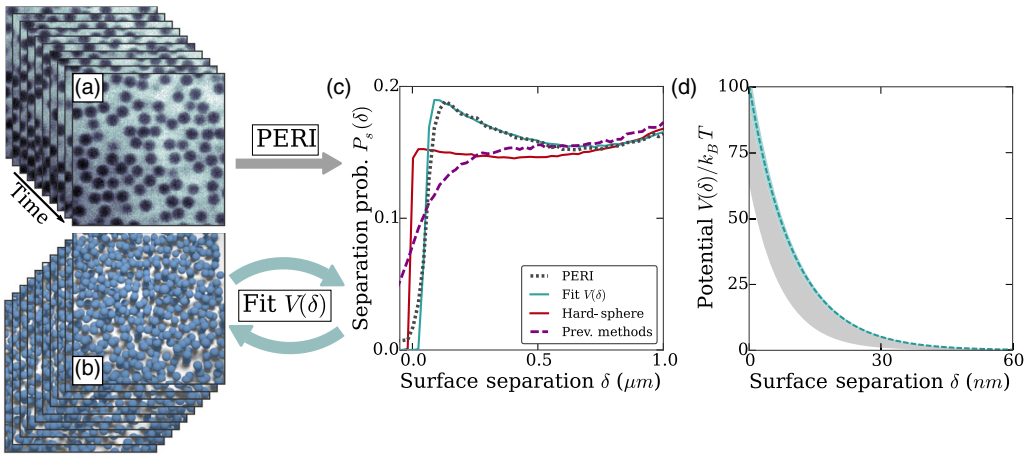

Figure 2: PERI is used to extract the interaction potential of a dilute suspension of $\sim$ 1200 Brownian particles with diameter 1.3 μm. From the microscope images (a) the experimental probability $P_s(\delta)$ of finding a pair of particles with surface-to-surface separation δ is extracted and shown in (c) as a gray dashed line. In panel (c), we also show the best fit (solid cyan line) of the experimental data, which was obtained by running molecular dynamics simulations (b) and iteratively update the interaction potential $V(\delta)$ to match the experimental data. For comparison, the results obtained with a simple hard-sphere potential (red line) and with a standard particle-tracking approach (purple line) are also shown in (c). From the best-fit simulation, the interparticle potential is extracted (d). The shaded bands show the uncertainty in the potential, with the teal band describing uncertainty in the fit and the gray band the uncertainty due to systematic errors. Reprinted under CC BY 4.0 license from Ref. [20]. DOI:10.1103/PhysRevX.7.041007

and fitting the model parameters to the experimental data. This approach, termed parameter extraction from reconstructed images (PERI), was demonstrated in a proof-of-principle experiment in which both the radius and position of 1.3 μm particles were determined to within 3 nm and, by using 1200 particles, it was possible to reconstruct the repulsive interaction potential between the particles (Fig. 2). Once again at the expense of a very long computational time, a reconstructive generative model approach such as PERI outperforms heuristic approaches for tracking [27], such as the ones based on intensity centroids or on local fitting of the image intensity maxima to a Gaussian function, providing a minimally-biased though computationally-intensive tool for particle tracking [28].

4. Digital Fourier Microscopy of colloids: quantitative microscopy flirts with light scattering

A notable dichotomy in modern colloid science is the one occurring between real- and reciprocal-space sample observations. For years, optical microscopes have been used to perform real-space experiments, building on the fact that microscope movies capture the position of the colloidal particles in space and time. At the other extreme, we find light scattering methods that are based on experimental schemes to capture a far-field, reciprocal-space version of the same process. Of course, whether the 'glasses' used to watch the movie are built to probe the sample in real- or in reciprocal-space depends on the needs, skills and tools that are available to the experimenter. After the invention of the laser, scattering methods have dominated for long time the colloidal science laboratories, especially when small-sized, sub-diffraction particles were to be characterized. Recent advances, some of which are discussed in Section 3, slowly subtracted users to light scattering methods, with the main drive that watching in real space how a system looks gives more direct information. However, extracting this information to provide unbiased and robust statistical estimators of physical quantities is not a simple task. In addition, despite the technological and methodological progresses made, the signal-to-noise ratio, the resolution needs and the multiple-scattering limitations still make real-space approaches very challenging for dense suspensions of small colloidal particles. This is true in particular when the optical contrast of the single particles is too small, which affects the signal to noise ratio, or

too large, which easily causes multiple scattering.

While these limit cases can still be safely managed with light scattering techniques [29], it was proposed in Ref. [30] that one can perform light scattering experiments on colloidal samples from the analysis of real-space movies. This approach, termed Differential Dynamic Microscopy (DDM), is based on combining image subtractions and spatial Fourier transforms to obtain a characterization of the static and dynamic scattering signals from the sample in the reciprocal space (q_x, q_y). With DDM, the characterization of the sample dynamics is calibration-free and can be almost automated. By contrast, static scattering information can be extracted with some additional calibration steps. Readers interested in the detail of this procedure, and in general in all the steps of the DDM analysis, can refer to the original papers [30, 31] or to recent reviews [32, 33]. Another recent review article [34] describes DDM in the framework of Digital Fourier Microscopy (DFM) techniques, which have in common that, under proper conditions, they recover scattering information from a digital Fourier transform of real-space images.

A first notable feature of DDM is that it can be implemented with a variety of imaging contrast mechanisms that can operate in various conditions of signal-to-noise ratio, resolution needs and multiple scattering. Since its introduction about ten years ago, DDM was used to study colloids in bright-field [35–41], phase-contrast [42], dark-field [43, 44] and depolarized [45] microscopy. It has also been demonstrated with fluorescent particles in wide-field [36, 46, 47], confocal [48, 49] and light-sheet [50, 51] configurations.

Second, the requirements of DDM in terms of signal-to-noise ratio, resolution and absence of multiple-scattering are way less severe than for real-space tracking experiments, because in DDM: (i) particle localization is not needed and the signal of interest can be even considerably (*e.g.* a factor 400 in [52]) smaller than the noise, (ii) bright-field DDM is intrinsically more insensitive to multiple scattering than far-field scattering because the use of partially coherent light permits using very thin samples (of the order and below 100 μm) and also strongly reduces the effective thickness of the sample and the effects of multiple scattering.

It was recently shown that DDM can be used for performing microrheology experiments [53, 54]. DDM-microrheology (DDM-μr) is an extension of the original Perrin experiment in at least two respects: (i) it determines the particle mean square displacement in a host medium not from the tracks of individual particles but from the study of the intensity fluctuations associated to their motion; (ii) in the original spirit of microrheology [18], it is based on a generalized Stokes-Einstein relation, which converts the mean square displacement of the tracer particles in the frequency-dependent storage (G') and loss (G") moduli. The results obtained in Refs. [53, 54] show independently and very convincingly that DDM can obtain high quality microrheology data bypassing two of the major limitations of particle tracking, which are (i) the selection of the suitable reconstructed particle trajectories and (ii) the need of using colloidal tracers with a sufficiently large optical contrast. In addition, in Ref. [53] an optimization-based method is introduced that grants a calibration-free DDM-μr experiment, which represents an additional advantage over particle tracking microrheology. The performance of DDM-μr can be appreciated by inspecting Fig. 3, where the storage and loss moduli of a 2% PEO polymer solution in water obtained with different methods are shown. The DDM results (black dashed and continuous lines) are found in excellent

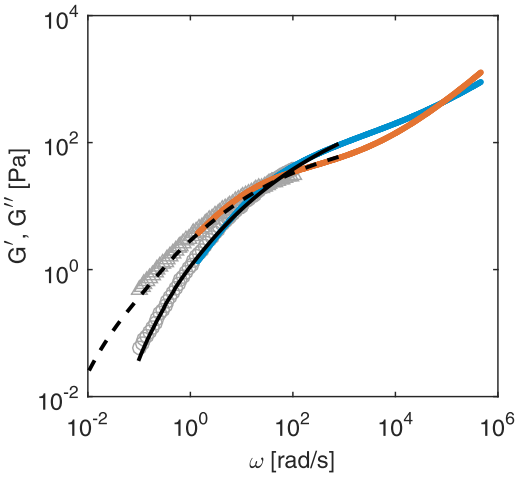

Figure 3: Comparison of the viscoelastic moduli G and G of a 2% PEO polymer solution in water, obtained with different methods. Gray circles (triangles): G (G) obtained with a rheometer; continuous blue (orange) line: G (G) obtained with Diffusing Wave Spectroscopy using 500 nm tracers; black continuous (dashed) line: G (G) obtained with DDM microrheology (weighted average of the results of 100 nm and 500 nm tracers). Reprinted figure with permission from Ref. [53] Copyright (2017) by the American Physical Society.

agreement both at small frequencies with mechanical rheometry (gray circles and triangles) and at large ones with Diffusing Wave Spectroscopy microrheology (orange and cyan continuous lines).

Interestingly, DDM was also used for accurate microrheology experiments probing both the translational and the rotational degrees of freedom of embedded tracer particles [53] and also in optically dense fluids [54], in both cases outperforming particle tracking.

5. Poking while watching: quantitative microscopy meets rheology

The mechanical properties of amorphous materials such as glasses (polymeric, metallic, and colloidal), gels (polymeric and colloidal), foams, or emulsions are not yet fully understood and are the subject of an intense research activity [55]. When subjected to an external action, these materials initially deform in a reversible, elastic fashion. By contrast, a stress that is sufficiently large in amplitude or long in duration may result in plasticity and flow, similar to a viscous liquid. Yielding of the material and the subsequent plastic flow are determined by a series of microscopic events that eventually may lead to fracture and whose nature and properties have not yet been fully elucidated [56, 57].

Theoretically, fracture and plasticity in crystalline solids are well understood, with defects in the crystalline lattice (dislocations) playing a key role [58]. By contrast, understanding the microscopic origin of yield and failure in amorphous solids is certainly far from complete. Most of the difficulty resides in the absence of a symmetric structure, which could serve as reference to detect defects. During the last years, important progresses have been made also for amorphous materials. Theoretical work and simulation have suggested that plastic deformation results from the microscopic occurrence of shear transformations, local rearrangements involving few particles (of the order of 5-10) in well-defined regions, the so-called shear transformation zones (STZ) or 'soft spots' [59]. Such events originate anisotropic stresses (strains) known as Eshelby stresses (strains), anisotropic perturbations with a characteristic quadrupolar spatial correlation, which perturb the otherwise isotropic surrounding elastic medium [60–62]. Simulation also shows that STZ manifest themselves in sheared liquids, seemingly suggesting that they are not peculiar to plastic deformation [63].

How far STZ can account for the failure of the wide variety of amorphous systems exhibiting similar mechanical failure modes is not yet established. In particular, the role of thermal noise has not been addressed in a systematic manner. This, however, is essential to bridge the gap from molecular to granular systems, including the intermediate soft glassy materials (SGM), all of these systems exhibiting similar failure modes.

As far as experiments are concerned, imaging atoms or molecules under mechanical stress is actually beyond reach since the constituents are too small and move too quickly. This is why during the last about fifteen years sheared colloidal gels, glasses and crystals have been widely investigated with quantitative microscopy. Much remains to be done, despite the relevant advances made. For instance, recent experiments with hard-sphere colloids show that STZ surprisingly exist not only in deformed glasses [64] but also in undeformed glasses [65] and defective crystals [66].

The striking observation of Eshelby strains in quiescent colloidal glasses, reported in [65], is well described in Fig. 4, where we show the results of quantitative confocal microscopy experiments in which roughly 50000 monodisperse hard-spheres belonging to a colloidal glass are tracked in $3D$ in the absence of an externally applied strain. While in the presence of an external shear the authors observe STZ that exhibit a bias in the shear strain direction and, in turn, contribute to the macroscopic deformation, the results in Fig. 4 show that STZ are also present in quiescent glasses, as they are activated by the thermal energy. However, they are weaker than the deformed case and they do not contribute to the macroscopic strain because there is no coupling with an external force field. If the glass is deformed above the yield point, some of the STZ do not relax back to the undeformed state contributing to an irreversible plastic deformation. Forcing the material back to an overall zero macroscopic strain causes the emergence of inclusions made of few particles that cancel out the strain contribution of the irreversible STZ.

It should be noted that rheo-microscopy (i.e. confocal microscopy under shear) experiments have been very popular to investigate, among several other phenomena, the structure [67] and the slip [68] of colloidal gels under steady shear. Similarly, when used on colloidal glasses, they have been pro-

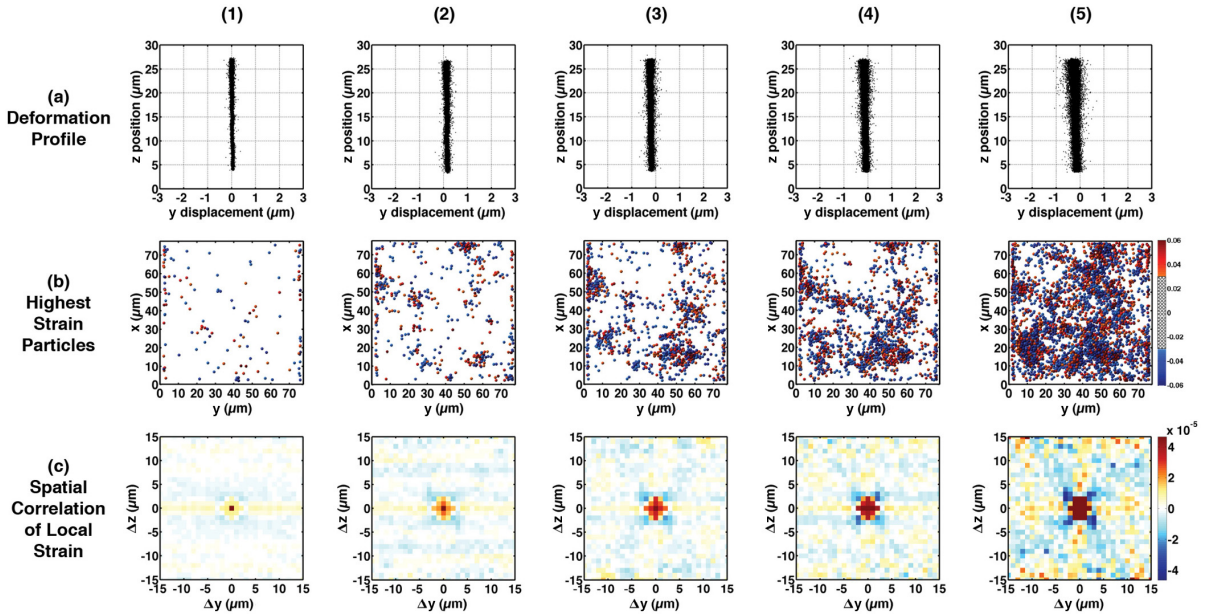

Figure 4: Evolution of strain and strain correlations in a quiescent colloidal glass at different time intervals. The deformation profiles (a) show broadening but no deformation. Row (b) shows top-view reconstructions showing only those particles with individual strain $|\varepsilon_{yz}| > 0.03$, colored according to their strain. At any time, there are equal numbers of high positive (red) and negative (blue) particles. Row (c) shows the spatial $y-z$ plane strain correlations of ε_{yz}, showing the evolution of the fourfold Eshelby signature. Reprinted figure with permission from Ref. [65] Copyright (2016) by the American Physical Society.

viding useful information, for instance, on shear banding [69], shear concentration coupling [70] and creep [71]. In addition, they have been also used to probe the effect of the stress overshoot during start-up shear [72–74] and of a sudden cessation of shear [75]. It would be interesting to use smaller collloidal particles to investigate how an increase of thermal noise would impact on the coupling between rheology, structure and dynamics. A possible way to overcome the limitations of real space approaches in tracking a crowded collection of small particles may be offered by Differential Dynamic Microscopy, which was described in Section 4.

Before ending this Section, I would like to consider polycrystals, an intermediate case between crystals and amorphous solids. Polycrystals are made of crystalline domains (grains) that are separated by grain boundaries, which are defect surfaces for 3D polycrystals or lines for polycrystalline monolayers. The authors of Ref. [76] performed a very thorough quantitative microscopy study in which oscillatory shear deformations were applied with a magnetically oscillating microdisk to polycrystalline monolayers of soft repulsive colloids trapped at a fluid interface. At the same time, an optical microscope was used to image the structure of the polycrystal at various distances from the edge of the disk.

The potential of this kind of experiments can be appreciated by inspecting Fig. 5 in which a Voronoi reconstruction of a $\phi = 13.5$ % monolayer is shown before shear is applied (Fig. 5a). The color in Fig. 5a encodes the number of sides of the polygon. Hexagons (gray) are the majority but pentagons (yellow), heptagons (cyan) and octagons (orange) are also observed. As an oscillatory flow of the microdisk (dark gray with two holes) is applied with amplitude $\theta = 10\ ^o$ and frequency $\omega = 0.1$ Hz, the defects start to move with spatially heterogeneous and temporally intermittent dynamics (Fig. 5b) and to interact (see for instance the annihilation of a pentagon and an hexagon from $t = 20$ s to $t = 70$ s). Panels (c) and (d) show the phase angle of the orientational bond order parameter $\psi_6 = \sum_m e^{6i\theta_m}$ immediately before (c) and 20 min after the application of the shear. Color in panels (c) and (d) corresponds to the orientation of a single grain, which shows how the application of a moderate shear amplitude increases the quality of the crystalline order through defect annihilation. For larger shear am-

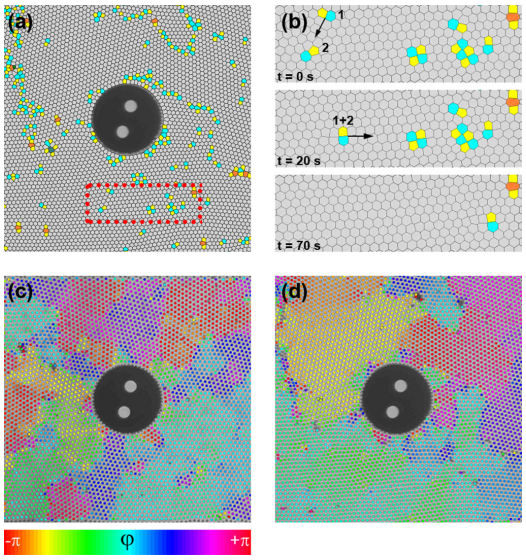

Figure 5: (a) Voronoi diagram of a colloidal monolayer with $\phi = 13.5$ % and (b) time evolution of the region enclosed by the red rectangle. The gray cells are hexagonal, whereas the colored cells denote the local defects of the structure coming from particles with different numbers of neighbors, e.g., 5 (yellow) and 7 (cyan). (cd) Phase angle of the local ψ_6 function for a monolayers sheared at $\theta = 10\ ^o$, $\omega = 0.1$ Hz for 20 min. (c) Initial and (d) final state. Reprinted figure with permission from Ref. [76] Copyright (2017) by the American Physical Society.

plitudes (data not shown), a critical strain exists above which the monolayer becomes fluid-like.

6. Conclusions and Outlook

In this short review, I have tried to give a personal overview of a few hot spots in the use of quantitative optical microscopy for the study of colloidal system. Of course, several at least equally important studies were not mentioned here for space limitations. For instance, there is an increasingly large activity on the sedimentation kinetics and equilibrium of active colloids that would fit perfectly within the scope of the present article but was not discussed here. Similar comments hold for other very active areas such as Digital Holographic Microscopy, for microscopy combined with optical/magnetic tweezers and for many others that I will not even mention here.

The cases presented here are, to some degree at least, ideal continuation of the original work of Perrin, in which the microscope was not used as a mysterious box for qualitative observation but rather a very sophisticated instrument for quantitative and precise estimates to be compared with theoretical predictions and models. Also, all the treated cases exhibit a very high potential of future development, for which we all hold high expectations.

There is a need for more precise particle localization tools as well as for faster super-resolution microscopies that may be used to track small (and thereby fast) colloidal particles. We would like to push even more the rheo-microscopy experiments described in Section 5, to finally understand, for example, how materials fracture and if there are any precursors to this fracture. Finally, it is likely that DDM-μr could be extended to materials that are inhomogeneous at the scale of the probe particles, or to situations in which the probe particles are interacting or polydisperse. All these extensions may one day bring microrheology to the industrial research labs, surpassing the adoption barrier that prevented this to occur with particle tracking microrheology.

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