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Rapid Analysis of the Internal Configurations of Droplets of Liquid Crystal using Flow Cytometry

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Abstract

We report the use of flow cytometry to identify the internal ordering (director configurations) of micrometer-sized droplets of thermotropic liquid crystals (LCs) dispersed in aqueous solutions of adsorbates (surfactants and phospholipids). We reveal that changes in the configurations of the LC droplets induced by the adsorbates generate distinct changes in light scattering plots (side versus forward scattering). Specifically, when compared to bipolar droplets, radial droplets generate a narrower distribution of side scattering intensities (SSC, large angle light scattering) for a given intensity of forward scattering (FSC, small angle light scattering). This difference is shown to arise from the rotational symmetry of a radial LC droplet which is absent for the bipolar configuration of the LC droplet. In addition, the scatter plots for radial droplets possess a characteristic “S-shape”, with two or more SSC intensities observed for each intensity of FSC. The origin of the experimentally observed S-shape is investigated via calculation of form factors and established to be due to size-dependent interference effects that differ for the forward and side scattered light. Finally, by analyzing emulsions comprised of mixtures of bipolar and radial droplets at rates of up to 10,000 droplets per second, we demonstrate that flow cytometry permits precise determination of the percentage of radial droplets within the mixture with a coefficient of determination of 0.98 (as validated by optical microscopy). Overall, the results presented in this paper demonstrate that flow cytometry provides a promising approach for high throughput quantification of the internal configurations of LC emulsion microdroplets. Because large numbers of droplets can be characterized, it enables statistically robust analyses of LC droplets. The methodology also appears promising for quantification of chemical and biological assays based on adsorbate-induced ordering transitions within LC droplets.

Keywords

Flow cytometry; liquid crystal droplets; ordering transition; amphiphile; light scattering; interference effects

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1. Introduction

Recent studies demonstrate that micrometer-sized liquid crystalline droplets dispersed in water (i.e. liquid crystal (LC)-in-water emulsions) can be employed to report the presence of a variety of different analytes based on adsorption of the analytes at the droplet interface and/or the self-assembly of analytes within the droplets.¹⁻⁴ For example, LC emulsions have been shown to form the basis of analytical methods for detection of lipids,⁵⁻⁷ surfactants,^{5, 8-9} proteins,^{5, 10-13} bile acids,¹⁴ charged macromolecules,¹⁵ bacteria,¹⁶ and viruses¹⁶ in aqueous solutions. Upon interaction with LC droplets, these analytes trigger changes in the configurations of the LC within the droplets (a so-called “ordering transition”), which results in changes in the optical properties of the droplets (e.g. the LC droplets appear different when observed using polarized light microscopy before and after interaction with the analyte). One example of this phenomenon is an analyte-triggered bipolar-to-radial ordering transition. Prior to the transition, the LC droplets are dispersed in water in the absence of analyte and assume a bipolar director configuration.^{5-7, 17-19} In this configuration, the LC (director) is aligned tangential to the droplet interface and two diametrically opposite point defects (so-called “boojums”) are present at the poles of each LC droplet (Figure 1A and C).^{5, 20-24} Upon interaction with an analyte such as a surfactant, the LC droplets transform to a radial director configuration,^{5-7, 17-19} with the LC director aligned normal to the droplet surface and a single point defect (hedgehog) is present at the core of each droplet (Figure 1B and D).^{5, 21, 24-25} Between these limiting conditions, LC droplets can adopt a range of director configurations and defect types.^{5-6, 24-27} Here we investigate the potential utility of flow cytometry for characterization of the configurations of LC droplets using model systems that contain mixtures of bipolar and radial droplets.

Although LC-in-water emulsions have been shown to possess attributes that make them promising systems for the detection and quantitation of a range of aqueous analytes, current methods for quantitation of the configurational states of the LC droplets are not optimal. Typically, quantitation has involved analysis of a large number of polarized light micrographs to determine the ordering within hundreds of LC droplets contained in emulsions.^{6-7, 14, 17, 28} This process is not well-suited as the basis of an analytical method for three reasons. First, optical imaging and analysis of images of individual LC droplets is laborious (typically, hundreds of LC droplets must be analyzed to obtain statistically robust results). Second, obtaining easily interpreted micrographs of water-dispersed LC droplets is difficult because the droplets are mobile, often translating at velocities greater than 1 $\mu\text{m/s}$ (they quickly move out of the plane of focus).²⁹ Finally, LC droplets in water undergo sedimentation.^{7, 29} A past study has observed that droplet-surface interactions that result from sedimentation of droplets onto surfaces can influence the configurations of LC droplets.²⁹ These challenges, when combined, motivated the study reported in this paper in which flow cytometry is investigated as the basis of a high throughput method for characterization of the ordering of LCs within the droplets of LC-in-water emulsions.

Flow cytometry is widely used for analysis of mammalian and bacterial cells.³⁰⁻³⁶ Typical analysis rates are thousands of cells per second. In flow cytometry, the objects to be characterized are focused hydrodynamically along the center-line of a capillary by injecting an aqueous dispersion of the objects into the center of a co-flowing stream of sheath fluid

under laminar flow conditions.³⁰⁻³² After flow focusing, the objects pass through a beam of polarized light from a laser, and the intensity of light scattered by each object at both small (0.5-15°, so-called “forward scattering”; FSC) and large scattering angles (15-150°, “side-scattering”; SSC) is measured.³⁰⁻³² The light scattered by the objects (and the internal structure of the objects) gives rise to interference effects that are generally described by a form factor.³⁷⁻³⁸ At small detection angles, the form factor is nearly unity, and the intensity of the scattered light (i.e. FSC) is approximately proportional to the volume of the object. At large detection angles (SSC), the scattering of light is more sensitive to interference effects arising from multiple scattering centers within the object and the form factor is non-unity. Thus, SSC in flow cytometry is generally viewed as being strongly influenced by the internal complexity of an object (e.g. the granularity of a cell).³⁰⁻³²

Unlike biological cells, LC droplets are largely homogeneous in terms of material composition. However, LC droplets are strongly inhomogeneous optically. The director **n** that indicates the average orientation of molecules within the LC is also the local optic axis. Since LCs are birefringent, different internal configurations of the droplets result in different patterns of the optic axis and effective refractive indices. These differences manifest themselves in the scattering of light from micrometer and larger-sized LC droplets. Specifically, previous studies have established that bipolar and radial LC droplets exhibit different light scattering properties.^{21, 39-40} We hypothesized, therefore, that flow cytometry could be used to rapidly analyze director configurations of LC droplets contained in LC-in-water emulsions.

2. Experimental Section

A list of materials and detailed experimental procedures are provided in the SI. We briefly describe our experimental methods here.

Preparation of LC Emulsions

LC-in-water emulsions were prepared according to previously published procedures.^{6-7, 29} Briefly, the emulsions were formed by sequential sonication and vortex mixing of 4 μ L of nematic 5CB in 1 mL of Milli-Q water at 25°C. Five cycles each comprising 10 seconds of vortex mixing (at 2500 rpm) and 10 seconds of sonication yielded milky white emulsions. This procedure results in a polydisperse population of LC droplets with diameters ranging from 1 to 15 μ m (see Figure S-1 of SI).^{7, 41} To ensure that differences in polydispersity did not interfere with our characterization of the response of the LC droplets to analytes, we prepared a “stock” LC-in-water emulsion and divided it into aliquots. The aliquots were then added to solutions containing different analytes/concentrations. This ensured that the distribution of sizes of the droplets were similar in the comparative studies reported in this paper. Isotropic oil-in-water emulsions were prepared in a similar fashion, with the exception that the emulsions were formed in the presence of 5.5 mM SDS to promote emulsion stability (unlike LC droplets, droplets of isotropic oils do not possess an energy barrier associated with internal reorganization of LC during the process of coalescence,⁴² and thus they rapidly coalesce in the absence of surfactant).

Preparation of Phospholipid-Coated LC Emulsions

The interfaces of LC droplets were decorated with phospholipid (DLPC or DPPC) by equilibrating the LC droplets in an aqueous dispersion of vesicles (prepared according to previously published methods;^{5, 43} see SI) for 1 hour. Prior to characterization of the resulting phospholipid-coated LC emulsions using flow cytometry, the droplets were rinsed three times in Milli-Q water to remove excess phospholipid from the bulk aqueous phase. The rinsing steps involved sedimenting the coated-LC droplets using centrifugation (5,000 rpm for 5 minutes), removing the resulting supernatant, and resuspending the droplets in fresh Milli-Q water.

Optical Characterization and Flow Cytometry of Emulsions

Prior to characterization by flow cytometry, LC emulsions were examined using an Olympus IX71 inverted microscope (Center Valley, PA) configured to perform both bright field and polarized light microscopy, and an objective magnification power of 100x (an oil-immersion lens), according to previously published methods (see SI).^{6-7, 41} Following optical characterization, light scattering plots of the intensity of side light scattering (SSC) plotted against the intensity of forward light scattering (FSC) were obtained for both LC-in-water and isotropic oil-in-water emulsions using a BD Accuri C6 flow cytometer (Ann Arbor, MI). FSC was measured at a detection angle of $0^\circ \pm 15^\circ$, and SSC was measured at a fixed detection angle of $90^\circ \pm 15^\circ$.⁴⁴ All flow cytometry measurements were performed at room temperature, and we are confident that any local heating of the LC droplets in the flow cytometer (e.g. by the laser) did not disrupt the nematic phase of the droplets (see SI for more detail). Unless indicated otherwise, in the experiments below, the emulsions were pumped through the flow cytometer at a flow rate of 14 $\mu\text{L}/\text{min}$ and the scatter plots were constructed from the measurement of 50,000 LC droplets.

3. Results and Discussion

We performed flow cytometry using droplets of nematic 4'-pentyl-4-cyanobiphenyl (5CB) dispersed in either water or an aqueous solution of 5 mM sodium dodecyl sulfate (SDS) because these two solution conditions have been demonstrated previously to promote either bipolar or radial configurations of the LC droplets, respectively.^{1, 3-7, 17-18} The concentration of SDS was selected to avoid formation of surfactant micelles (the critical micelle concentration (CMC) of SDS is 8.1 mM;⁴⁵ solutions of SDS only (no LC) were confirmed to not contribute to a significant number of light scattering events detectable by the flow cytometer). Prior to flow cytometry, we used polarized light microscopy to confirm that the 5CB droplets dispersed in water exhibited bipolar configurations (Figure 1A), and that the LC droplets in aqueous 5 mM SDS adopted radial (or near radial, see below) configurations (Figure 1B). Subsequently, the two LC emulsions were pumped through the flow cytometer at a rate of 14 $\mu\text{L}/\text{min}$, and the intensities of both forward and side scattered light (FSC and SSC, respectively) were measured for 50,000 LC droplets in $\sim 2 \mu\text{L}$ of emulsion. In the analysis below, the data is presented according to the standard flow cytometry convention of plotting SSC as a function of FSC.³⁰⁻³² Such a plot is referred to as a "scatter plot" in the remainder of this paper.

From the scatter plots displayed in Figure 2, we make several qualitative observations. First, the scatter plot obtained using the LC droplets in the bipolar configuration (Figure 2A) exhibits a broad distribution of SSCs at each FSC value. In contrast, the scatter plot measured for the LC droplets in the radial configuration (Figure 2B) exhibits a narrower distribution of SSCs at each FSC value. In addition, we note that the scatter plot of radial LC droplets exhibits a characteristic “S-shape”, with two or more values of SSC observed for each value of FSC that lies between ~30,000 and ~60,000 arbitrary units (a.u.). The physical origins of these qualitative features will be discussed in detail below. Overall, we note that the scatter plots obtained for the two configurations of the LC droplets are strikingly different.

We performed three additional experiments to provide support for our conclusion that the differences in the above-mentioned features of the scatter plots shown in Figure 2 arise from differences in the internal configuration of the LC within the droplets (and not other factors, as discussed below). First, we measured the characteristic features of the scatter plots of the bipolar and radial droplets (as described above) to be largely preserved over the operating range of flow rates of the flow cytometer (14 – 66 $\mu\text{L}/\text{min}$; see Figure S-2 of SI). From this result, we conclude that the scatter plots displayed in Figure 2 were not influenced by shear stresses imposed on the droplets during flow focusing in the cytometer. Next, we measured scatter plots for LC-in-water emulsions that contained radial 5CB droplets induced by different molecular amphiphiles (see Figure S-12 of SI) to determine the extent to which the different amphiphiles would influence the scatter plots. The amphiphiles tested were the cationic surfactant cetyltrimethylammonium bromide (CTAB), the phospholipids 1,2-dilauroyl-*sn*-glycero-3-phosphocholine (DLPC) and 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC), and the bacterial lipopolysaccharide endotoxin. All four amphiphiles generated scatter plots with qualitative features similar to the LC emulsions containing SDS-coated radial droplets (Figure 2B), further supporting our conclusion that the major features of the scatter plots are determined by the internal configuration of the LC within the droplets, independent of the choice of amphiphile (for the five amphiphiles investigated).

Past studies have established that micrometer-sized 5CB droplets can possess increasingly complex internal configurations as a function of increasing droplet size.^{7, 46-49} For example, Figure 3A demonstrates that when the diameters of nematic 5CB droplets in 5 mM SDS exceed 5 μm , the extinction bands present in polarized light micrographs of the LC droplets begin to twist relative to the orientation of the crossed polars, indicating the presence of twist deformation of the LC within the droplets. In contrast, LC-in-water emulsions with droplets of smectic octylcyanobiphenyl (8CB) exhibit radial configurations that do not permit the presence of twist (Figure 3B). To determine if size-dependent configurations of LC droplets (e.g. twist within large (> 5 μm) droplets) might be influencing the qualitative features of scatter plots of the LC droplets, in the third experiment we measured a scatter plot for a LC-in-water emulsion that contained radial 8CB droplets coated by SDS (Figure 3D) and compared it to a scatter plot measured for radial 5CB droplets (Figure 3C). Inspection of Figure 3 reveals that the qualitative features of the scatter plot obtained from radial 8CB droplets (narrow distribution of SSCs at a fixed FSC and a S-shape) were similar

to the features of the scatter plots obtained from 5CB droplets. We comment that the higher level of structural detail evident in the scatter plot for 8CB droplets at high FSC values (Figure 3D) is likely due to a bigger population large droplets in the 8CB emulsion (see below for more detail). We conclude, therefore, that scatter plots of emulsions containing radial nematic LC droplets are not significantly influenced by the presence of any twist near the cores of droplets exceeding 5 μm in diameter (likely reflecting that fact that the majority of the volume of LC within the droplets is not twisted, see Figure 3A). We note that ~33% percent of the droplets contained in a typical 5CB-in-water emulsion used in this study have diameters larger than 5 μm (see Figure S-1 of SI).

The results presented above suggest that the differences observed in the scatter plots of emulsions containing either bipolar or radial LC droplets (Figure 2) arise largely from differences in the symmetry of the two droplet director configurations.²¹ Specifically, because LC droplets in a radial configuration are centrosymmetric, the droplets present a rotationally invariant distribution of refractive indices (n) to an incident beam of light.⁵⁰ In contrast, LC droplets in the bipolar configuration present refractive indices to an incident beam of light that depend on the orientation of the symmetry axes of the bipolar droplets relative to the polarization of the incident light. The broad distribution of SSC intensities at a fixed FSC intensity observed for bipolar droplets (Figure 2A) suggests the droplets are not substantially aligned along the direction of flow (also, see comments above and in SI related to the effects of flow rate). To test further the role of the symmetry of the LC droplets in determining the scatter plots, we performed flow cytometry on several *isotropic* oil-in-water emulsions (Figure 4). The isotropic oils tested were heptane, dodecane, immersion oil B, and benzonitrile (refractive indices at 25°C shown in Figure 4). The oil-in-water emulsions were formed using 5.5 mM SDS to stabilize the emulsions.^{37, 45} Two important results emerged from our measurements of the scatter plots of these isotropic oils. First, the scatter plots for each of these four emulsions possessed a narrower distribution of SSCs at each FSC than the scatter plots obtained from LC-in-water emulsions that contained bipolar droplets (see Figure S-14 of SI). Second, as the refractive index of the oil in the emulsions approached the average refractive index of nematic 5CB ($n_{\text{av}} = (2n_o + n_e)/3 = 1.597$), the scatter plot measured by flow cytometry exhibited the S-shape characteristic of the radial LC droplets. Specifically, the scatter plot obtained using benzonitrile (which has a refractive index closest to the average refractive index of nematic 5CB) closely resembles the scatter plot obtained for radial 5CB droplets (compare Figure 4D to Figure 4E). This result demonstrates that the S-shaped scatter plot characteristic of radial LC droplets is not unique to LC droplets, but is also observed with isotropic droplets.

To provide further insight into the physical origin of the S-shaped scatter plots displayed in Figure 4, we considered the intensity of light scattered by a droplet of isotropic oil to be described by:

$$I = \Delta\rho^2 V^2 P(R, q) \quad (1)$$

where ρ is the difference in scattering length density between the droplet and the solvent ($\rho_{\text{droplet}} - \rho_{\text{solvent}}$), V is the volume of the droplet, and $P(R, q)$ is a form factor that depends on both the size and shape of the droplet and accounts for interference effects arising from

multiple scattering centers within the droplet.^{37-38, 51} The form factor is a function of both the radius of the droplet (R) and the magnitude of the scattering vector (q), which is dependent on the angle of detection (θ) and λ , and is expressed as $q = 4\pi \sin \theta / \lambda$. As shown in equation 1, the intensity of light scattered is proportional to $P(R, q)$, which we discuss below.

We treat the droplets of isotropic oil contained in the emulsions as isotropic spheres. The form factor of an isotropic sphere is given as:⁵¹

$$P(R, q) = \left(3 \frac{\sin qR - qR \cos qR}{(qR)^3} \right)^2 \quad (2)$$

Inspection of equation 2 shows that the form factor is unity for $q = 0$, and thus the intensity of forward scattered light is proportional to the square of the volume of a droplet ($I \propto V^2$).³¹⁻³² However, the angle of detection of FSC in a flow cytometer is non-zero because, at a detection angle of 0° , the intensity of the transmitted light would damage the detector.³⁷ The exact forward scattering angle of the BD Accuri C6 flow cytometer used in our experiments is not disclosed by the manufacturer, but is stated to be between 0° and 15° .⁴⁴ For the purposes of the calculations presented below, we assume a forward scattering angle of 5° . The conclusions of our analysis do not depend strongly on the exact forward scattering angle. When a droplet passing through the flow cytometer has a radius that exceeds a critical value, interference effects lead to size-dependent intensities of forward scattered light ($V^2P(R)$ for $\theta = 5^\circ$; Figure 5A). A similar effect is seen for SSC ($75^\circ < \theta < 105^\circ$ in the flow cytometer used in our study), but the period of the interference bands is much shorter ($V^2P(R)$ for $\theta = 90^\circ$; Figure 5B). Past studies of x-ray scattering from homogenous spherical particles have demonstrated that when the period of interference bands becomes sufficiently small, a continuous curve connecting the maxima of each predicted band is measured.⁵¹ We propose that the SSC measured in our flow cytometer is also a curve that connects the maxima of the intensity bands predicted by light scattering theory (red line, Figure 5B).

An important consequence of the above prediction regarding SSC in our experiments is that SSC is expected to be strongly correlated to droplet size. We comment that this interpretation differs from the conventional interpretation of flow cytometry scatter plots measured for mammalian cells, where FSC is used to characterize cell size and SSC is used as a measure of the internal granularity of a cell (see Introduction for further detail).

Figure 5C shows $V^2P(R)$ calculated at $\theta = 90^\circ$ plotted against the same quantity at $\theta = 5^\circ$. Comparison of Figure 5C to experimentally determined scatter plots for both radial LC droplets and droplets composed of isotropic oils (Figures 2 through 4) reveals qualitative similarity. Specifically, Figure 5C predicts the characteristic S-shape of the experimental plots, where two or more intensities of SSCs are observed for a given value of FSC. In addition, we note that the calculated scatter plot can be fit to an experimentally measured scatter plot for radial LC droplets (Figure 5D) by rescaling the axes of the calculated scatter plot by factors of $10^{-10.3}$ for $\theta = 90^\circ$ and $10^{-14.3}$ for $\theta = 5^\circ$. These constants account for the excess scattering length density of the droplets (ρ), as well as several instrument

parameters that influence the detector output (e.g. differences in the calibration of the forward and side light scattering detectors). While quantitative calculation of the scatter plots lies beyond the scope of this paper, the results above provide key insight into the origins of the difference of the qualitative features of the scatter plots for bipolar and radial LC droplets. Specifically, the characteristic S-shaped plot that allows us to identify the radial configuration of the LC droplets arises because of size-dependent interference effects. We emphasize that the S-shaped curve would not be observed for a sample of monodisperse LC droplets, and thus, the use of polydisperse samples can be used to advantage in identifying radial droplets in a mixture of radial and bipolar droplets (see below).

To be a useful tool for analyte quantification using LCs, a flow cytometer must be able to report the percentage of LC droplets contained in an emulsion that exhibit the director configuration induced by the analyte.^{5-7, 14, 17, 28} Because the analytes used in this study induce a bipolar-to-radial ordering transition, we measured scatter plots for 5CB-in-water emulsions containing different percentages of radial LC droplets. The emulsions were prepared by mixing bipolar LC droplets (bare aqueous-LC interfaces) with DPPC-coated radial droplets. DPPC was used to induce the radial configuration because DPPC monomers are essentially insoluble in aqueous solutions,⁵² and thus they were not expected to redistribute between the two populations of LC droplets after mixing. In the analysis below, Φ_{DPPC}^{PM} indicates the percentage of radial LC droplets contained in an emulsion, as determined from analysis of polarized light micrographs.^{6-7, 17, 28}

Figure 6 shows that an increase in Φ_{DPPC}^{PM} resulted in a continuous evolution of the scatter plots from those with features characteristic of bipolar droplets to those with features characteristic of radial droplets. Because the S-shaped region of the scatter plots of the radial droplets occurred between FSC values of 30,000 a.u. and 60,000 a.u. (red dashed lines in Figure 6G), we hypothesized that the total number of light scattering events measured within this range of FSC intensities would increase with an increase in the percentage of radial droplets. To test this hypothesis, we plotted frequency histograms of FSC for each Φ_{DPPC}^{PM} displayed in Figure 6 (Figure 7A). The frequency histograms reveal that a peak located between ~30,000 a.u. and ~60,000 a.u. indeed grows in intensity with an increase in Φ_{DPPC}^{PM} (red dashed lines in Figure 7A). To quantify this growth, we integrated the area under each peak. The area under the peak at $\Phi_{DPPC}^{PM}=0\%$ was subtracted from each peak as a baseline, (see SI for more details) and the resulting area was normalized by the area under the peak for $\Phi_{DPPC}^{PM}=100\%$ (Φ_{DPPC}^{FC}). Figure 7B demonstrates that this metric of the scatter plot correlates closely with the percentage of radial droplets, as determined from polarized light micrographs of the same emulsions (Φ_{DPPC}^{PM}) linear regression of the data in Figure 7B with $\Phi_{DPPC}^{FC}=0\%$ set as the intercept results in a slope of 1.01 and a coefficient of determination (R^2) of 0.98. This result leads us to conclude that flow cytometry can be used to quantify the percentage of radial droplets contained in a LC-in-water emulsion comprised of a mixture of bipolar and radial droplets.

Here we emphasize the capability to quantify the fraction of radial droplets in a mixture of radial and bipolar droplets is a useful one as our past studies have demonstrated that important biological analytes, such as bacterial endotoxin, lead to LC droplets that assume either bipolar and or radial configurations (configurations other than radial and bipolar do not appear to be stable in the presence of endotoxin).⁶⁻⁷ Our results suggest that quantification of the percentage of radial droplets contained in these systems is possible using a flow cytometer. In addition, however, we note that other analytes can lead to LC droplet configurations other than radial and bipolar.^{5, 24-27} In future studies, to enable analysis of such systems, we will explore methods of analysis of scatter plots that lead to identification of these other LC droplet configurations.

We end this study by briefly comparing flow cytometry to optical microscopy as an analytical methodology to quantify the number of droplets contained in a LC emulsion that exhibit a particular director configuration. First, flow cytometry requires a lower sample volume than optical microscopy. For example, the scatter plots used to determine Φ_{DPPC}^{FC} in Figure 7B were obtained using 50,000 droplets and required a sample volume of 2 μ L. In contrast, determination of Φ_{DPPC}^{PM} for each data point displayed in Figure 7B required the analysis of at least 100 LC droplets in 20 μ L of sample. Second, the rate of data acquisition is much faster for flow cytometry (up to 10,000 droplets/s) than for optical microscopy (~ 20 droplets/min in the present study). Third, calculation of Φ_{DPPC}^{FC} from flow cytometry data can be accomplished in several minutes, which is much faster than the hours of image analysis required to calculate Φ_{DPPC}^{PM} from optical micrographs. Fourth, flow cytometry does not suffer from the challenges associated with droplet sedimentation which can influence the number of droplets observed as well as lead to droplet-surface interactions that change LC droplet director configurations while making observations using an optical microscope.²⁹ Finally, the cost of a flow cytometer is comparable to the cost of a polarized light microscope (the cost of the flow cytometer used in the present study was ~\$20,000). Overall, we conclude that flow cytometry has a number of advantages relative to optical microscopy for characterization of the internal configurations of LC emulsions.

4. Conclusion

The results presented in this paper demonstrate that flow cytometry can be used to characterize the internal configuration of LC droplets contained in LC-in-water emulsions. Specifically, LC droplets with rotational symmetry, such as droplets in the radial configuration, generate a characteristic S-shaped scatter plot. The S-shape was shown (by calculation of form factors) to arise from size-dependent interference effects that differ for the two detection angles in a flow cytometer. The different light scattering properties of bipolar versus radial LC droplets leads to systematic differences in scatter plots measured for LC-in-water emulsions containing mixtures of droplets in these two director configurations. This permits the use of flow cytometry as an analytical method to quantify the percentage of droplets in an emulsion that exhibit a radial director configuration with high precision (in a mixture of radial and bipolar droplets). When compared to optical microscopy for such analyses, flow cytometry is faster and more statistically robust (it

involves the analysis of >10,000 droplets compared to ~ 100 droplets for optical microscopy). Overall, these results suggest that flow cytometry can be used as a high throughput alternative to microscopy for read-out of chemical and biological assays based on stimuli-induced ordering transitions within LC droplets.¹⁻⁴ The results also suggest that flow cytometry could be utilized to sort LC droplets based on their different director configurations. In future studies, we will explore this possibility.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

(A and B) Polarized light micrographs of a droplet of nematic 4'-pentyl-4-cyanobiphenyl (5CB) dispersed in either (A) water or (B) aqueous 5 mM SDS. (C and D) Schematic illustrations of the director configurations within the (C) bipolar LC droplet imaged in (A), and the (D) radial LC droplet imaged in (B). The solid black lines within the droplet boundaries in (C) and (D) represent the orientation of the LC director and the black spots represent defects. Double headed arrows indicate the orientation of the crossed polars.

Figure 2.

(A and B) Scatter plots obtained by flowing aqueous dispersions of nematic 5CB droplets in either bipolar (A) or radial (B) configurations through a flow cytometer. The 5CB droplets in (A) were suspended in water; whereas the 5CB droplets in (B) were suspended in an aqueous solution of 5 mM sodium dodecyl sulfate (SDS). Both scatter plots show 50,000 events recorded at a flow rate of 14 $\mu\text{L}/\text{min}$. (C) Molecular structure of SDS.

Figure 3.

(A and B) Polarized light micrographs of (A) the radial or twisted-radial configuration exhibited by nematic 5CB droplets dispersed in an aqueous solution of 5 mM SDS and (B) the radial configuration exhibited by smectic 8CB droplets in an aqueous solution of 5 mM SDS. Double headed arrows indicate the orientation of the crossed polars. Scale bars, 5 μm. (C and D) Scatter plots obtained by flowing either (C) nematic 5CB droplets or (D) smectic 8CB droplets suspended in a 5 mM aqueous solution of SDS through a flow cytometer. The scatter plots comprise 50,000 events recorded at a flow rate of 14 μL/min.

Figure 4. (A-D) Scatter plots obtained by flowing (A) heptane, (B) dodecane, (C) immersion oil B, (D) benzonitrile droplets, or (E) nematic 5CB droplets dispersed in a 5.5 mM aqueous solution of SDS through a flow cytometer. The refractive indices of each isotropic oil at 25°C and the average refractive index of nematic 5CB at 25°C is displayed in the bottom right corner of each respective scatter plot. Note that different SSC axis scales were used for the isotropic oil-in-water emulsions because these emulsions led to lower values of SSC. Each scatter plot represents 50,000 events recorded at a flow rate of 14 $\mu\text{L}/\text{min}$.

Figure 5.

Calculated effect of droplet size on the intensity of light scattered ($V^2P(R)$) at detection angles (θ) of 5° and 90° . (A) Detection angle of 5° . (B) Detection angle of 90° . (C) $V^2P(R)$ at $\theta = 90^\circ$ plotted against $V^2P(R)$ at $\theta = 5^\circ$. (D) Fit of the predicted scatter plot (red line) to the experimentally observed scatter plot for radial 5CB droplets coated by DLPC (as shown in Figure S-12D of SI).

Figure 6.

Scatter plots obtained by flowing nematic 5CB-in-water emulsions containing mixtures of bipolar and radial 5CB droplets through a flow cytometer. The percentage of radial droplets in the mixture, as determined by optical microscopy (Φ_{DPPC}^{PM}), is indicated. Each scatter plot represents 50,000 events recorded at a flow rate of 14 $\mu\text{L}/\text{min}$. The red dashed lines in (G) highlight the area of the scatter plot used to calculate the percentage of radial LC droplets in LCin- water emulsions Φ_{DPPC}^{FC} , see Figure 7 and text for details).

Figure 7.

(A) Frequency histogram for FSC obtained from Figure 6. Bin size, 1,000 a.u. (B) Percentage of radial LC droplets in a mixture of radial and bipolar droplets, as calculated from flow cytometry (Φ_{DPPC}^{FC}), plotted against the percentage of radial LC droplets calculated from polarized light micrographs of the same emulsions (Φ_{DPPC}^{PM}). The red dashed lines in (A) highlight the peak analyzed to calculate Φ_{DPPC}^{FC} .