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Structure and Dynamics of Liquid Crystalline Pattern Formation in Drying Droplets of DNA

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We investigate the formation of ringlike deposits in drying drops of DNA. In analogy with the colloidal “coffee rings,” DNA is transported to the perimeter by the capillary flow. At the droplet edge, however, DNA forms a lyotropic liquid crystal (LC) with concentric chain orientations to minimize the LC elastic energy. During the final stages of drying, the contact line retracts, and the radial stress causes undulations at the rim that propagate inward through the LC and form a periodic zigzag structure. We examine the phenomenon in terms of a simple model based on LC elasticity.

Drying droplets of colloidal suspensions form “coffee ring” stains due to the capillary flow caused by contact line (CL) pinning [1]. This phenomenon has been used for the assembly of nanoparticles [2,3] and diblock copolymers [4]. For gene expression profiling, an array of DNA droplets is often dried on a glass surface for hybridization studies [5]. Since DNA can be stretched by flow [6–9] and “molecular combing” [10], single DNA chains usually align perpendicular to the CL of a drying drop. In this Letter, we show that, when a concentrated DNA droplet is dried, a coffee ring stain is also formed, but, contrary to intuition, the extended DNA chains trace out zigzags along the CL and form a periodic pattern (Fig. 1). During most of the drying time, the contact line is pinned, and DNA chains from the droplet interior are transported to the perimeter. At the droplet edge, a lyotropic liquid crystal (LC) is formed, in which DNA chains align along the droplet edge to minimize LC elastic and surface anchoring energies. During the final stages of drying, the CL retracts, and radial dilative stress causes undulations at the rim that propagate inward through the elastic LC medium, giving rise to the domain pattern. A simple model based on LC elasticity explains the observations.

The experiments are conducted using λ-DNA (contour length \( L_c = 16.3 \, \mu \text{m} \), 48,502 bp, persistence length \( L_p = 50 \, \mu \text{m} \)), obtained from New England BioLabs, Inc. The pattern is observed for a broad range of initial DNA concentrations \( C_i = (0.02–5) \, \text{mg/ml} \), initial droplet volumes \( V_i = (1–50) \, \mu \text{l} \), and for surfaces with different hydrophilicity (clean glass plates, SiO, and indium tin oxide coatings, etc.; contact angle \( 15^\circ \leq \alpha \leq 110^\circ \)). The droplet’s radius \( R = (0.5–12) \, \text{mm} \) is varied by changing \( V_i \) and \( \alpha \). The pattern consists of the herringbonelike domains with a self-similar periodic structure (Fig. 1). The periodicity \( L \) and deposit thickness \( d \) increase with \( V_i \) and \( C_i \); in the deposits, \( L \) is a monotonously increasing function of \( d \) [Fig. 1(a)], measured using confocal microscopy vertical cross sections [Fig. 1(f)]. For example, \( L \) can be varied from \( L < L_c \) to \( L > L_c \) and \( d \) from submicron to tens of microns by changing \( V_i = (1–50) \, \mu \text{l} \) [Fig. 1(a)]. For DNA rings of \( d < 10 \, \mu \text{m} \) obtained with droplets of \( V_i < 30 \, \mu \text{l} \) at 1 mg/ml of DNA, the polarizing microscopy (PM) textures appear gray [Fig. 1(c)]. PM images are polychromatic if \( V_i \simeq 30 \, \mu \text{l} \) [Fig. 1(d)], indicating that, for wavelengths \( \lambda \) in the visible spectral range, \( \Delta n d \sim \lambda \), where \( \Delta n = n_e - n_o \) and \( n_e, n_o \) are extraordinary and ordinary refractive indices. We estimate \( \Delta n \) by correlating texture colors and the measured \( d: \Delta n = -0.011 \pm 0.004 \) for a

FIG. 1 (color). DNA pattern at the perimeter of a dried drop: (a) periodicity \( L \) vs thickness \( d \) in the central thickest part of the ring [obtained by varying \( V_i = (1–50) \, \mu \text{l} \)]; the inset shows a typical birefringent ring; (b) PM texture of the pattern colocalized with the fluorescence signal from a small number of molecules marked by YOYO-1; (c)–(e) PM textures of the pattern inside (c) a thin (<10 \( \mu \text{m} \)) ring and (d),(e) a thick (>20 \( \mu \text{m} \)) ring obtained (c),(d) without and (e) with a 530 nm retardation plate; (f) a vertical confocal microscopy cross section (all DNA labeled) showing the bell-shaped profile of the ring. The crossed polarizer and analyzer are parallel to the vertical and horizontal directions in the plane of the images in (a)–(e), and the retardation plate is at 45° to them in (e). The CL is on the right side of the ring fragments in (b)–(f).

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dried film of ads-DNA ($\Delta n < 0$ because of the conjugated bonds in base pairs) and even smaller in the lyotropic LC observed during drying. Droplets of $V_i < 1 \mu l$, such as those in DNA chips, also produce ringlike deposits, but the pattern is barely observable in PM because $\Delta nd$ is small.

The PM texture of the pattern (Fig. 1) changes upon rotation of the sample; each part can be brought to extinction at a well-defined orientation with respect to the crossed polarizers. This indicates that the director $\hat{n}$ (average local orientation of DNA chains) is not twisted along the normal to the substrate, in agreement with the theoretical predictions [11] that twist is suppressed with increasing the polymer contour length and in phases with positional order. When the sample is rotated in the presence of a 530 nm retardation plate between crossed polarizers, continuous changes of alternating blue and orange bands are observed [Fig. 1(e)]. This allows one to deduce the thickness-averaged in-plane $\hat{n}$. The result agrees well with the fluorescence signal from the dyed DNA molecules within unlabeled DNA [12] colocalized with the PM texture [Fig. 1(b)]. The extended DNA and $\hat{n}$ are uniformly oriented at $\pm (30^\circ - 45^\circ)$ to the CL within the stripes and the in-plane $\hat{n}$ traces out zigzags from stripe to stripe.

In order to elucidate the 3D structure of $\tilde{n}(x, y, z)$, we use fluorescence confocal polarizing microscopy (FCPM) [13] and stain the DNA with acridine orange dye (AO, Aldrich Chemical Co.), which intercalates parallel to the base pairs [14], i.e., approximately perpendicular to the DNA axis (Fig. 2). When studied with polarized FCPM light, the spatial pattern of fluorescence signal visualizes the director structure [15]. To resolve $\hat{n}$ across the film thickness, we study thick, $d = (20-50) \mu m$, deposit rings obtained after drying of droplets of $V_i = 50 \mu l$ at (1–2) mg/ml of DNA. The FCPM resolution ($=1 \mu m$) is sufficient to resolve the structure of $\hat{n}$ in 3D: The pattern is composed of stripes with essentially thickness-independent periodically alternating $\hat{n}$ parallel to the substrate and at $\pm (45^\circ - 60^\circ)$ to the radial direction (Fig. 2). The vertical wall defects run across the whole thickness of the ring [Figs. 2(c)–2(h)] and are due to the discontinuous jumps of $\hat{n}$ between the stripes.

To get insights into the physics of this phenomenon, we experimentally study dynamics of the droplet drying. At the initial stages, it resembles coffee ring formation [1]. As long as the CL is pinned (Fig. 3), the evaporation flux is the strongest next to the edge of the drop. As evidenced by tracking fluorescently labeled tracer DNA, the solute flows from the drop interior toward the periphery to compensate the evaporation losses. This radial outward flow transports random-coiled DNA ($\sim 1 \mu m$ globules) to the drop’s perimeter. DNA concentration at the perimeter and the local effective viscosity gradually increase so that DNA Brownian motion is hindered (as observed by tracking fluorescently dyed molecules). The Stokes drag becomes strong enough to extend DNA [7–9], driving the LC self-organization at the droplet rim [16]. The drop at this stage has a birefringent wedge-shaped LC ring with $\hat{n}$ parallel to the CL, which is still pinned at the initial location. The DNA alignment parallel to the drop’s edge is counterintuitive because DNA is expected to extend along the flow [7–9], which is mainly in the radial direction. To understand these experimental observations, we consider the LC surface anchoring and bulk elastic properties [11,16–21]. Although long DNA chains usually have random coil conformations, it has been demonstrated that “molecular crowding” can straighten the random coils and lead to LC phases [16]. The macroscopic elasticity can be described in a framework similar to other polymer LCs composed of long flexible chains [11,16–21]. The elastic distortions of the LC director $\hat{n}$ are associated with free energy density [20,21]:

$$f = \frac{K_1}{2} (\nabla \cdot \hat{n})^2 + \frac{K_2}{2} (\hat{n} \cdot (\nabla \times \hat{n}))^2 + \frac{K_3}{2} (\hat{n} \times (\nabla \times \hat{n}))^2 + \frac{E \delta \rho_p}{\rho_p^0}.$$  \hspace{1cm} (1)

Here $K_1$, $K_2$, and $K_3$ are Frank elastic constants for splay, twist, and bend deformations of $\hat{n}$, respectively, $\delta \rho_p$ is variation of the local polymer density, $\rho_p^0$ is the mean polymer density, and $E$ is the osmotic compressibility.
phase before the CL depinning. The minimum of LC elastic free energy corresponds to the concentric \( \hat{n} \); hence, the radial dilative stress in the vicinity of the receding CL competes with the LC elasticity, resulting in a periodic distortions of \( \hat{n} \) [Figs. 1, 2, and 3(g)]. Formed wavy undulations are reminiscent of the undulations induced by a dilative mechanical stress in columnar LCs [17,28]. At the onset of pattern formation, the distortions first appear next to the CL [Figs. 3(g) and 3(h)] and then expand to the whole width of the ring with concentric \( \hat{n} \). The ripples of \( \hat{n} \) at the CL propagate in the radial direction \( n_r = \cos qx \times \exp[-q^2r/\sqrt{E + q^2K_1}] \) and decay at the characteristic length \( l_r = q^{-2} \sqrt{(E + q^2K_1)/K_3} \) [Figs. 3(g) and 3(h)], where \( q = 2\pi/L \); this is analogous to the response of a polymer nematic to a grooved surface [21]. For experimental \( L = (3-150) \mu m \) and typical \( \sqrt{K_3/E} = (5-10) \) nm [21], \( E \approx q^2K_1 \), one obtains \( l_r = (10-10000) \mu m \), depending on \( L \). This explains why the ripples of \( \hat{n} \) occurring at the CL penetrate deep into the LC ring [Figs. 3(g) and 3(h)]. The periodic stripes eventually run across the whole width \( \Delta R \) of the birefringent ring and act as a “template” for the deposition of remaining DNA. When the remaining water evaporates, solidification of the DNA deposit is followed by formation of defect walls and cracks, evidenced by additional sharp lines (Fig. 1) that are typical for phases with positional order. Interestingly, the final structure and the pattern of DNA orientation (Figs. 1, 2, and 4) resemble the “sawtooth-type” buckling instability observed at high applied stresses in LCs with partial positional order [17,28]. The sequence of pattern formation and the final director structure are summarized in Fig. 4.

In summary, we describe the periodic pattern formed in the coffee rings of DNA drops and explain it in terms of a
simple model based on the LC nature of the ring at the drying droplet edge. Similar phenomena are observed for other semiflexible biopolymers [23,29]. Questions related to details of the pattern formation remain, such as the precise mechanism of wavelength selection and the relative role of the capillary flow in the LC with viscosity coefficient anisotropy [30], as well as convection [9] and Marangoni flows [26], which modify the pattern formation at the late stages of the drying process. Since essentially all the DNA is concentrated in a narrow ring threaded by a network of defect walls with almost no DNA in the rest of the stain, these effects should be accounted for in the design of microarrays in which DNA droplets are sequen-

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Without optimization of the wetting conditions, it is possible
to damage the DNA chains or “miss” all the DNA in the
ring stain of a dried droplet even if perfect center-of-
mass droplet alignment is achieved.

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[12] We labeled ~1/10000 DNA chains with dye YOYO-1
(Molecular Probes, 488 nm Ar-laser excitation and 510–
550 nm fluorescence detection) so that there is one
YOYO-1 molecule per 15 bp of DNA.
[15] AO dye (488 nm excitation and 510–550 nm detection) is
doped at (10−3–10−4) wt. % into a 1 mg/ml aqueous
DNA solution. In the used epifluorescence mode, the
strongest signal corresponds to the FCPM polarization
perpendicular to \( \hat{n} \) and the weakest signal when it is
parallel to \( \hat{n} \). Obtaining fluorescence textures for different
polarizations, we extract \( \hat{n} \) [13].
Liquid Crystals (Academic, New York, 1982).
[22] Using FCPM and fluorescence imaging of single dyed
DNA within unlabeled DNA molecules, we found that
both the droplet-air interface and the solid substrates
impose tangential boundary conditions on \( n \) at the
interfaces. This implies that the surface anchoring energy
is minimized for tangential \( n \) (consistent with Meyer’s en-
tropy considerations for long chains at the surfaces with
no chemical binding [21]). DNA orientation at the air
interface was additionally confirmed by visualizing
\( n \) structures around air bubbles injected into the DNA LC.
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