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Controlling microdrop shape and position for biotechnology using micropatterned rings

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Abstract

Photolithographic micropatterning is used to achieve topographic rather than chemical control of the static shape and position of microdrops on solid substrates in a gaseous ambient. Micrometer cross-section, millimeter-diameter circular rings with steep sidewalls strongly and robustly pin contact lines of nanoliter to 100 microliter liquid drops, increasing the maximum stable drop volume and eliminating contact line motion due to transient accelerations. Physical and chemical processes involving two-phase transport within these drops are more reproducible, and automated image analysis of the evolving drop contents is greatly simplified. This technique has particular promise for high-throughput protein solution screening in structural genomics and drug discovery.

Keywords

Microfabricated patterns; drop pinning; contact line; protein crystallization; microarrays

1 Introduction

Many biotechnologies including those for high-throughput macromolecular crystallization (Stevens 2000) and gene expression analysis (Skena 2000) could be improved if liquid drops in a gaseous ambient could be produced at precisely defined locations and with exactly the same shape. For example, in macromolecule crystallization and screening — essential steps in modern structural genomics and drug discovery — nanoliter to microliter macromolecule-containing drops are dispensed through air onto injection-molded polymer multiwell plates. The drops then equilibrate via vapor diffusion with reservoir solutions that draw solvent from the drop. The drop shape controls the drop surface-to-volume ratio and solvent evaporation rate, which strongly affect crystal nucleation and growth kinetics and the competing kinetics of precipitation. Shape and position irregularities from drop to drop complicate automated image analysis of crystallization experiment outcomes. Surfactant-containing drops, used for crystallizing membrane proteins, and drops that are inverted to a hanging configuration (to reduce sedimentation and adhesion of crystals, precipitate and other solution components to the substrate) are especially prone to mechanical distortions. Although fully integrated microfluidic alternatives exist (Hansen *et al.* 2002; Zheng *et al.* 2003), a hybrid platform that combines microfluidic concepts with popular high-speed liquid dispensing robots to produce nanoliter to microliter drops with well-defined positions and shapes is likely to prove more flexible and cost effective in many protein crystallization and bioreactor applications. Drop position, shape and uniformity are also important issues in the manufacture and use of microarrays.

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How can drop position and shape be controlled? Several surface treatment techniques have been demonstrated to produce more reproducible or even movable drops (Abbott *et al.*, 1992; Jackman *et al.* 1998; Berejnov *et al.* 2005; Yang *et al.* 2006). Most rely on selectively modifying the surface chemical properties to produce regions of high and low surface energy for the dispensed liquid. However, there is no universal chemical treatment that provides high surface energy contrast for all liquids. Instead, the surface must be customized for each liquid. Liquid contact angles and contact angle hysteresis are typically less than 120° and 90°, respectively (Shafrin *et al.* 1964; de Gennes *et al.* 2004), limiting drop stability and the maximum liquid volumes that can be pinned to treated areas. Surfactant solutions with low surface tensions are particularly difficult to contain (Rosen 1978). Surfactants can also diffuse through the three-phase contact line, and facilitate drop spreading over both hydrophobic and hydrophilic surfaces (Eriksson *et al.* 2001).

An alternative approach to controlling drop position and shape is through surface topography rather than chemistry. On a *flat* substrate, the drop contact line will not advance (recede) until the contact angle reaches the critical advancing (receding) contact angle θ_a (θ_r) determined by the gas, drop and substrate properties at the three-phase line. Suppose that the substrate surface is locally inclined at angle ϕ with respect to the macroscopic average plane. As shown in Fig. 1 (b), the local advancing contact line will not displace until the local contact angle θ_a with respect to the locally inclined surface exceeds the critical advancing angle. The apparent advancing angle measured with respect to the macroscopic surface plane will then be $\theta_a^* = \theta_a + \phi$ ($\theta_a^* \leq 180^\circ$) (Shuttleworth *et al.* 1948; Dyson 1988). Similarly, the apparent receding contact angle, Fig. 1 (c), will be reduced by ϕ : $\theta_r^* = \theta_r - \phi$ ($\theta_r^* \geq 0$). This behavior is summarized by the Gibbs inequality (Oliver *et al.* 1977; Dyson 1988).

$$\theta_r^* = \theta_r - \phi \leq \theta \leq \theta_a + \phi = \theta_a^*$$

Surface corrugations can thus increase the apparent contact angle hysteresis $\theta_r^* - \theta_a^*$ by up to 2ϕ (Johnson *et al.* 1964; Huh *et al.* 1977).

Corrugations in the form of rings with steep sidewalls, shown in Fig. 1, can in principle inhibit drop contact line motion by this mechanism. As the ring is filled with liquid, the contact line eventually moves across the ring's flat top and becomes pinned at its outer edge. When liquid is withdrawn, the contact line remains pinned at the ring's inner edge. Increasing the wall steepness ϕ increases the apparent contact angle hysteresis $\theta_r^* - \theta_a^*$ and the strength of drop pinning. Undercutting the ring's inner edge prevents its dewetting by the receding contact line, and undercutting the outer edge can render it unwettable by the advancing line. The contact line's motion over the entire stable range will be limited to the width of the ring, and can thus be made negligible by making the ring width small compared with its diameter.

Here we demonstrate a simple surface micropatterning technique to produce micrometer-cross-section rings for pinning nanoliter through milliliter drops. These rings provide strong contact line pinning, reproducible drop shapes and positions, and excellent drop stability for a wide range of liquids. This in turn leads to more reproducible chemistry within the drops and simplified automated analysis of experimental outcomes. This technique is likely to prove useful in a broad range of biotechnology applications.

2 Materials and methods

For our demonstration, rings were fabricated using photolithography and standard photoresists on silicon wafers, Pyrex glass substrates and Mylar films. Prior to fabrication the silicon wafers were cleaned with boiling Nanostrip mix and a 10% HF solution, to obtain hydrophilic surfaces. Patterns were designed using CAD software (L-Edit, Tanner Research, Inc.) and masks fabricated using a GCA/MANN 3600F optical pattern generator capable of $\sim 1\ \mu\text{m}$ lateral resolution. Shipley SC1827 photoresist was spun at 4000 rpm for 30 s, baked on a hotplate for 90 s at 115°C , exposed to a broadband dose of $56\ \text{mJ}/\text{cm}^2$ using a contact aligner (EV620) in soft contact mode, developed in AZ 300MIF developer for 80 s and post-baked on a hotplate for 60 s at 115°C . The resulting film and rings had a thickness of $\sim 3\ \mu\text{m}$ as measured with an optical interferometer. SEM images were used to determine ring cross-sections and side-wall angles. Figure 1 (d) shows an SEM image of a photoresist ring on a silicon substrate. The ring has the steep sidewall angles φ needed for strong contact line pinning. These angles were reproducible from ring to ring and wafer to wafer to within $\pm 7^\circ$.

To evaluate ring pinning properties relevant to common biotechnology applications, we used distilled deionized water (NANOpure II, Barnstead, Boston MA), an aqueous solution of the ionic surfactant sodium dodecyl sulfate (SDS) (Sigma, 0.29 kD, 99%), and an aqueous protein solution. The protein solution was obtained by dissolving six times recrystallized hen egg white lysozyme (Seikagaku America, Falmouth MA, Mr $\sim$ 14 kD, lot LF1121) in a 0.1 M sodium acetate buffer with pH = 4.8.

Contact angle hysteresis for pure water on unpatterned silicon substrates does not exceed 60° . On substrates uniformly covered by the photoresist used to fabricate the rings, the advancing and receding contact angles are $\theta_a \sim 80^\circ$, $\theta_r \sim 30^\circ$ for pure water; $\theta_a \sim 35\text{--}40^\circ$, $\theta_r = 0^\circ$ for a 1.0 mg/ml SDS solution; and $\theta_a \sim 70^\circ$, $\theta_r = 0^\circ$ for a 15.0 mg/ml lysozyme solution. On patterned substrates with sidewall angles φ close to 58° (as measured by SEM), the apparent contact angle hysteresis reaches 165° for pure water, (close to the 180° limit), $\sim 100^\circ$ for SDS solutions and $\sim 130^\circ$ for lysozyme solutions. Experiments repeated using the same wafer two months after fabrication yielded the same contact angle hysteresis, indicating the stability of pinning provided by the rings.

3 Results and discussion

As shown in Fig. 2, the effects of micrometer cross-section rings on drops even millimeters in diameter are dramatic. The same ring can hold both large volumes, Fig. 2 (a), corresponding to overhanging drops pinned at the outer ring edge, and small volumes, Fig. 2 (d), down to films a few micrometers thick pinned at the inner ring edge. The ring ensures that a drop's contact line remains pinned as the drop evaporates.

Micrometer rings dramatically increase drop stability against perturbations. These may include the mechanical vibrations that occur during drop dispensing by high speed liquid handling robots and during automated plate handling for storage and inspection. As shown in Fig. 2 (e), after the substrate is rotated from 0° to 90° and back to 0° (at a rate of ~ 20 degrees per second), even drops with very large volumes remain fully pinned to the ring and return to their original shape. For large water drops with a diameter of 6.0 mm, the maximum volume that remains pinned increases by a factor of 4 when compared with drops on an unpatterned silicon substrate, as shown in Fig. 2 (f). This maximum volume is a factor of two larger than is obtained using shallow "wells" having the same diameter, produced by patterning holes in a continuous photoresist film to expose the Si wafer below. For surfactant (SDS) solutions (Fig. 3) and lysozyme solutions, rings increase the maximum volume by up to 200% and 170%, respectively, relative to photoresist-defined wells, where

pinning at the advancing edge is due primarily to surface energy contrast and not to topography. This comparison shows that both the inner and outer edges of the ring are essential to produce the observed pinning enhancement.

The pinning strength of a ring is independent of ring width for widths between at least 2.0 μm and 50.0 μm and drop volumes between at least 100 nL and 100 μL . The pinning strength increases with ring height for heights between 0.2 μm to 2 μm , and then is constant at larger heights (although the reduction in pinning at small height may be due to fabrication issues.) Ring continuity is also important: a single gap as small as 4 μm wide reduces the maximum advancing angle for pure water from 165° to 120°. Wider rings are less likely to have gap defects, and are more readily visible to the eye. A ring width of roughly 8.0 μm provides a good compromise between naked eye visibility, ease of fabrication without defects, and precise drop positioning (within 2% of the diameter) for drop volumes down to ~100 nL.

The strong contact line pinning provided by narrow rings simplifies drop image analysis. The contact line always has the same position and shape, and the shape of the drop's surface is always smooth and simple, producing predictable optical distortion that can be calibrated out. Furthermore, the contact line retains its size and shape as the drop evaporates. Figure 4 shows how images of an evaporating drop can simply be subtracted pixel by pixel to observe, e.g., crystals that have formed within it.

Rings are not restricted to circular shapes. We have demonstrated strong drop pinning to elliptical, "comet" and donut-shaped rings and to rings with sharp corners. Adjacent rings can be joined by channels to allow liquid-liquid diffusion between drops.

Since for nL and larger drops the ring widths are small compared with their diameter, multiple concentric rings can be patterned on the same site, as shown in Fig. 5 (a). This allows a wide range of drop volumes to be pinned at the same position, and thus more flexible use of the patterned area. Closely spaced (50-100 μm) rings surrounding a drop-containing ring can further inhibit drop spreading due to ring defects or large accelerations. If the contact line depins from the ring, it can be caught by a neighboring ring, minimizing the change in the drop shape and position. More generally, the very small ring cross-sections required to obtain strong topographic pinning provide significant advantages for pattern layout and miniaturization.

Rings can be fabricated from a wide variety of chemically stable materials including photoresists and polyimides, and on a wide variety of substrates. We have used polished silicon wafers with native oxides (Figs. 2 and 3), Pyrex glass (Figs. 4 and 5), mylar sheet and polypropylene film. In all cases, pinning is primarily determined by the properties of the rings (for rings at least ~ 2 μm high), not the substrate.

Since the enhanced pinning is primarily a topographic effect, chemical contrast between ring and substrate is not necessary, so that the rings can be fabricated from the same material as the substrate. We have created rings by mechanically embossing 12 μm polypropylene films, and observed enhancements in contact angle hysteresis and maximum stable drop volume comparable to those obtained with photoresist rings on silicon.

As shown in Figs. 5 (b) and (c), this simple drop pinning technology provides the basis for a hybrid high-throughput protein crystallization/screening plate, comprised of a micropatterned optically transparent sheet or film attached to a frame that defines independent cells. The micropatterned surface provides excellent drop position and shape accuracy and excellent drop stability against vibrations during manual or automated handling. The plate can be inverted after dispensing, Fig. 5 (c), to obtain hanging drops, and the optically transparent film can also be X-ray transparent to allow *in-situ* examination of

crystal structure. Multiple film patterns optimized for different applications can be used with the same frame, minimizing the need for expensive new injection molds. Micropatterned rings may also prove useful in microarrays and in other hybrid microfluidic devices where the control of liquid spreading on the microscale is desired.

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Fig. 1.

(a) A drop pinned to a microfabricated ring R on a tilted substrate. (b) and (c) At the advancing (receding) edge, the drop is pinned by the ring's outer (inner) sidewall, producing an apparent advancing (receding) angle θ_a^* (θ_r^*). (d) An SEM image of a 20 μm wide and 2.7 μm high ring fabricated using Shipley SC1827 photoresist

Fig. 2.

(a)-(d) Drops of different volumes pinned to the same ring, illustrating the large range of stable contact angles. (e) and (f) Stability of an individual drop to rotations on (e) a Si substrate patterned with a ring and (f) an unpatterned Si substrate. Images of the drop immediately following dispensing on the horizontal substrate are superimposed on images of the same drop when the substrate is tilted 90° . In (e), a $72\ \mu\text{l}$ water drop stays pinned to the ring and recovers its initial shape when the substrate is rotated back to 0° . In (f), an $18\ \mu\text{l}$ water drop displaces and distends, and does not recover its initial shape or position.

Fig. 3.

Comparison of the maximum volume of a 1% aqueous solution of SDS that can be pinned to an area of a given radius on a vertically inclined substrate, defined by 3.5 μm thick, 4.0 μm wide photoresist rings (▲) and by 3.5 micron deep “wells” obtained by removing photoresist to expose bare wafer disks in an otherwise continuous film (■). The maximum volume pinned by rings saturates above photoresist thicknesses of 2 μm . Pinning by the “wells” does not depend on the photoresist thickness in the range of 0.4 μm to 5.4 μm .

Fig. 4.

Precisely defined contact lines and drop shape simplify recognition of drop contents. A 20 μl drop of a 15 mg/ml lysozyme solution is dispensed and pinned to a 5 mm diameter ring on a Pyrex glass substrate, and allowed to slowly evaporate. (a) Immediately following dispensing the drop contains 2 bubbles. (b) 18 hours later the bubbles are gone, the drop volume has decreased by $\sim 1/5$, and lysozyme crystals have appeared. Direct subtraction of (a) from (b) with contrast enhancement and no additional processing reveals the bubbles and crystals (c).

Fig. 5.

(a) Multiple concentric rings for enhanced drop pinning. Widely separated rings can pin drops of different volumes, while closely spaced rings (inset) prevent drop spreading due to manufacturing defects or large accelerations. (b) An array of water drops dispensed onto a Pyrex glass substrate with microfabricated ring patterns similar to those in (a). Drop volumes are 60 and 20 μl and the rings are 8.0 μm wide. A plastic frame defines and isolates individual cells for independent experiments. Strong pinning allows this array of very large drops to be handled and inverted to the hanging drop configuration (c) with no motion of drop contact lines.