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Automated Droplet Manipulation Using Closed-Loop Axisymmetric Drop Shape Analysis

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Abstract

Droplet manipulation plays an important role in a wide range of scientific and industrial applications, such as synthesis of thin-film materials, control of interfacial reactions, and operation of digital microfluidics. Compared to micron-sized droplets, which are commonly considered as spherical beads, millimeter-sized droplets are generally deformable by gravity, thus introducing nonlinearity into control of droplet properties. Such a nonlinear drop shape effect is especially crucial for droplet manipulation, even for small droplets, at the presence of surfactants. In this paper, we have developed a novel closed-loop axisymmetric drop shape analysis (ADSA), integrated into a constrained drop surfactometer (CDS), for manipulating millimeter-sized droplets. The closed-loop ADSA generalizes applications of the traditional drop shape analysis from a surface tension measurement methodology to a sophisticated tool for manipulating droplets in real time. We have demonstrated the feasibility and advantages of the closed-loop ADSA in three applications, including control of drop volume by automatically compensating natural evaporation, precise control of surface area variations for high-fidelity biophysical simulations of natural pulmonary surfactant, and steady control of surface pressure for *in situ* Langmuir–Blodgett transfer from droplets. All these applications have demonstrated the accuracy, versatility, applicability, and automation of this new ADSA-based droplet manipulation technique. Combining with CDS, the closed-loop ADSA holds great promise for advancing droplet manipulation in a variety of material and surface science applications, such as thin-film fabrication, self-assembly, and biophysical study of pulmonary surfactant.

Graphical Abstract

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Author Contributions

K.Y. and J.Y. contributed equally to the content of this paper.

Notes

The authors declare no competing financial interest.

Supporting Information

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Detailed development of the feedback control system; additional figures that show control of droplet properties and reproducibility of experiments (PDF)

Drop volume control for pure water (AVI)

Surface area control for pure water (AVI)

Surface area control for dynamic cycling of Infasurf under physiologically relevant conditions (AVI)

Surface pressure control for quasi-equilibrium compression of DPPC monolayer at room temperature (AVI)

INTRODUCTION

Effective manipulation of liquid droplets plays an important role in a wide range of scientific and industrial applications, such as synthesis of thin-film materials,¹ control of interfacial reactions,² study of surface rheology,³ and operation of digital microfluidics.⁴ In these applications, droplets serve as a miniaturized platform that provides a number of unique advantages over traditional interfacial models such as the classical Langmuir trough.⁵ First, the small volume of droplets facilitates study of scarce or expensive samples, such as biological or clinical fluids. Second, compared to bulk systems, droplets possess a much larger surface-area-to-volume ratio that makes droplets an ideal soft platform for studying surface reaction and interfacial assembly. Third, due to system miniaturization, it is feasible to ensure a rigorous environmental control, such as temperature and experimental atmosphere, thus allowing for environmental studies across the droplet surfaces.⁶

Compared to the control of a flat surface as in the classical Langmuir trough, effective control and manipulation of a single droplet is not a trivial task. In general, the profile of a freestanding droplet is determined by the balance of gravitational and surface tension forces as predicted by the Bond number, $Bo = \rho g R^2 / \gamma$, where ρ is the density difference across the drop surface, g is the gravitational acceleration, R is the characteristic radius of the droplet, and γ is the surface tension. Droplets with very small Bond numbers, such as micron-sized droplets, are generally considered as spherical beads.⁷ The key parameter for manipulating such droplets is their volume, while the surface area can be calculated by a spherical approximation and the surface tension is routinely assumed to be a constant.⁸ A number of techniques have been developed to manipulate the volume of micron-sized droplets.^{7–9}

However, when the Bond number is sufficiently large, e.g., for millimeter-sized droplets and/or at the presence of surfactants, the drop profile can be significantly deformed by gravity to assume a Laplacian shape.^{5,10} In these cases, the surface area of the droplet cannot be correlated with its volume using a simple spherical approximation. Meanwhile, the surface tension usually varies with the surface area at the presence of surfactants. Hence, all droplet properties, including its volume, surface area, and surface tension, need to be individually controlled for effective droplet manipulation. Nevertheless, in most applications, one can only control the drop volume using a high-precision syringe pump. Neither surface area nor surface tension of the droplet can be directly controlled with current techniques. Cabrerizo-Vilchez and co-workers have developed a fuzzy-logic algorithm to

control the surface pressure or surface area of a pendant drop.¹⁰ In spite of a suitable control method, the fuzzy-logic algorithm requires the definition of input and output fuzzy sets for every control process, which is not a trivial task.

In this study, we developed a novel feedback control method for manipulating millimeter-sized droplet based on axisymmetric drop shape analysis (ADSA). ADSA is a computer-based surface tension measurement methodology first developed by Neumann and co-workers.^{11,12} ADSA determines surface tension, surface area, and volume of a droplet by numerically fitting the experimental drop profile to theoretical profiles integrated with the numerical Laplace equation of capillarity. We have developed a high-speed ADSA algorithm capable of real-time analysis of droplet properties, including its volume, surface area, and surface tension. By coupling real-time ADSA with a proportional-integral-derivative (PID) controlled motorized syringe, we have developed the first closed-loop ADSA that is capable of precisely controlling the volume, surface area, and surface tension of a millimeter-sized droplet.

To demonstrate the feasibility and advantages of this droplet manipulation method, we have engaged it in three applications of importance in various surface science studies. In the first application, we maintained the volume of a water droplet over a prolonged time period by automatically compensating natural evaporation. In the second application, we demonstrated the feasibility of controlling the surface area of an oscillating droplet for *in vitro* biophysical simulations of natural pulmonary surfactant. By precisely controlling surface area variations during dynamic droplet oscillation, our method allows high-fidelity *in vitro* biophysical simulations of respiratory mechanics. In the third application, we demonstrated the effective control of surface pressure during compression of a self-assembled phospholipid monolayer. By controlling surface pressure over time, we implemented the first *in situ* Langmuir–Blodgett (LB) transfer from a monolayer-covered droplet. All these applications have demonstrated that closed-loop ADSA is highly practical in automating droplet manipulation in a variety of material and surface science applications, such as thin-film fabrication, self-assembly, and biophysical study of pulmonary surfactant.

MATERIALS AND METHODS

Development of Closed-Loop ADSA for Droplet Manipulation

Since its development, ADSA has been routinely used as an open-loop system for determining properties of drop/bubble (i.e., surface tension, surface area, and volume) by analyzing its images.^{11,12} Here, we further developed ADSA into a closed-loop system in which droplet properties determined in real time are used as feedback control parameters to actuate the droplet toward desired targets. A diagram of the feedback control loop is illustrated in Figure 1. A digital PID controller was directly integrated into ADSA. In the closed-loop feedback control system, ADSA continuously calculates the error, i.e., the difference between the target values and the current values, and thereafter automatically actuates the droplet toward the target values using a motorized syringe (consisting of a high-precision servomotor and a piston–syringe system) with PID control. The transient state of the droplet can be controlled by administering proper PID gains. The feedback control system was modeled in the time domain, the Laplace domain (in which the time domain

representation is transformed into a frequency domain representation), and the Z-domain (in which the digital discrete time representation is transformed into another frequency domain representation).¹³ Detailed development of the feedback control system can be found in the Supporting Information.

Here, it is important to emphasize on the advantages and novelties of the closed-loop ADSA in comparison with previous droplet manipulation methods. (1) To the best of our knowledge, the closed-loop ADSA is the first method capable of controlling the volume, surface area, or surface tension of millimeter-sized single droplets. Previous methods can only control drop volume. However, knowledge about the surface area and surface tension of the droplet can be only obtained by postexperimental analysis of drop images acquired during the experiments. Hence, control parameters such as area compression ratios and target surface tension values cannot be set *a priori*. In comparison, the closed-loop ADSA analyzes the deformed drop shape in real time and hence eliminates the need of prior image acquisition, which makes possible for live manipulation of a droplet. (2) Since the closed-loop ADSA manipulates the droplet by directly controlling properties of the target droplet, it is independent of the physical methods of fluid actuation, regardless of whether the fluid is driven by a motorized syringe, a piezoelectric transducer, or any other means. (3) Being a free-standing method, although the closed-loop ADSA is demonstrated here with a sessile drop setup, it can be readily applied to manipulate other drop or bubble shapes, such as pendant drop or captive bubble, provided that ADSA is applicable.

Materials

The water used in this study was Milli-Q ultrapure water (Millipore, Billerica, MA). It has a resistivity greater than 18 MΩ-cm at room temperature. The pulmonary surfactant used in this study was Infasurf (Calfactant), which was a gift from ONY Inc. (Amherst, MA). Infasurf was purified from whole-lung bronchopulmonary lavage of newborn calves. Through an extraction process, Infasurf retained all of the hydrophobic components of bovine endogenous surfactant including phospholipids, cholesterol, and most hydrophobic surfactant proteins (SP-B and SP-C).^{14,15} Infasurf has a total phospholipid concentration of 35 mg/mL and is stored frozen in sterilized vials. On the day of experiments, it was thawed and diluted to a phospholipid concentration of 5 mg/mL with a saline buffer of 0.9% NaCl, 1.5 mM CaCl₂, and 2.5 mM HEPES, adjusted to pH 7.0. Dipalmitoylphosphatidylcholine (DPPC) was purchased from Avanti Polar Lipids (Alabaster, AL) and used without further purification. DPPC was dissolved in chloroform to form a 1 mg/mL stock solution.

Experimental Methods

To demonstrate the capacity of the automated control system, we used an experimental setup called the constrained drop surfactometer (CDS), recently developed in our laboratory.^{6,16} Figure 1 shows the schematic of the CDS setup and the closed-loop ADSA-based feedback control system. The CDS is a miniaturized surface tensiometer that utilizes a single liquid droplet confined on a 3–5 mm diameter drop pedestal. The hydrophilic pedestal features a knife-sharp circular edge that maintains droplet integrity and prevents film leakage even at low surface tensions.¹⁷ The droplet is manipulated using a programmable motorized syringe (2.5 mL Gastight) with a maximum displacement speed of 5 mm/s and a resolution of 0.1

μm (equivalent to a volume resolution of approximately 4 nL). The droplet is illuminated with a red-color parallel backlight and is visualized with a machine vision camera with a maximum resolution of 1280×1024 pixels. The volume, surface area, and surface tension of the droplet are determined from real-time image analysis (i.e., no prior image acquisition was needed) with an ultrafast ADSA algorithm capable of analyzing the drop shape at a frequency of 10 Hz. This processing speed is mainly limited by the computational demands of real-time ADSA analysis.

For biophysical simulations of pulmonary surfactant, detailed experimental protocols can be found elsewhere.^{6,16} Briefly, a droplet ($\sim 10 \mu\text{L}$) of 5 mg/mL Infasurf was dispensed onto the CDS drop pedestal, maintained within an environmental chamber that controls the physiologically relevant conditions, i.e., 37°C and 100% relative humidity. After drop formation, the surface tension quickly decreased to an equilibrium value of approximately 22–25 mN/m,¹⁸ indicating formation of an adsorbed surfactant film at the air–water interface. Once the equilibrium was established, the adsorbed surfactant film was compressed and expanded at a rate of 5 s per cycle by withdrawing and injecting water into the droplet using a motorized syringe. The compression ratio was controlled to be less than 20% of the initial surface area to simulate normal tidal breathing. A minimum of five continuous compression–expansion cycles were studied for each droplet. It was observed that the cycles became repeatable after the first cycle.

For the study of self-assembled phospholipid monolayers at room temperature, a tiny amount of 1 mg/mL DPPC stock solution was spread using a microsyringe onto a droplet of pure water of which the surface tension was recorded as γ^0 .¹⁹ The droplet was then slowly expanded to increase surface tension (γ) until the corresponding surface pressure ($\Pi = \gamma^0 - \gamma$) was reduced to ~ 1 mN/m. The droplet was left undisturbed for an additional 1 min to allow evaporation of the solvent. The spread DPPC monolayer was subsequently compressed at a quasi-equilibrium rate of $0.06 \text{ cm}^2/\text{min}$, corresponding to 0.17% initial surface area per second. Langmuir–Blodgett (LB) transfer from the droplet was implemented by lifting a small piece of freshly peeled mica sheet at a speed of 1 mm/min, under controlled surface pressure. Topographical images were obtained using an Innova AFM (Bruker, Santa Barbara, CA). Samples were scanned in air in contact mode with a silicon nitride cantilever of a spring constant of 0.12 N/m and a tip radius of 2 nm. Lateral structures were analyzed using Nanoscope Analysis (version 1.5).

RESULTS AND DISCUSSION

Control of Drop Volume

In this study, we demonstrate direct control of drop volume with the closed-loop ADSA. Figure 2 shows the manipulation of a $60 \mu\text{L}$ water droplet on the CDS platform at room temperature. The volume of the droplet (panel c) was decreased and increased stepwise with a step of every $10 \mu\text{L}$, followed by steps of every 20 and $40 \mu\text{L}$, respectively. Images of this droplet at different volumes are shown as insets in panel a. It is clear that the volume change was controlled to be largely linear with respect to time and without significant overshoot once reaching the target values within a 1% tolerance. Response to the control was fast. Regardless of the magnitude of volume change, the target values were reached within 1 s. It

can be also seen that during the manipulation of drop volume the surface tension (panel a) of pure water fluctuates at 71 ± 2 mN/m, in agreement with the literature value.¹⁹ The deviation mostly takes place upon reducing the drop volume. This is most likely due to traces of impurities (that cause surface tension decrease) and/or intrinsic artifacts of drop shape analysis at small volumes (that cause surface tension increase).^{20,21} Video S1 in the Supporting Information shows the dynamic process of the volume control.

We further demonstrate the usefulness of the ADSA-based volume control by solving a real-world problem, i.e., droplet evaporation. Evaporation is an inevitable problem of any droplet-based apparatus in which the drop volume decreases with time due to evaporation at a rate affected by the surface area of the droplet, volatility of the liquid, and the environmental conditions.²¹ For instance, protein adsorption may take several hours to complete, during which evaporation may cause significant artifact by contracting the air–water interface of the droplet.^{22,23} Another example in which the compensation of droplet evaporation is essential is the study of gas adsorption at the air–water interface.²⁴ Volume loss is especially problematic for small droplets as the highly curved drop surface increases the chemical potential of evaporation.²⁵ Here, we demonstrate that the ADSA-based volume control maintains a constant drop volume over a prolonged time period by automatically compensating evaporation. As shown in Figure 3, without control, the volume of a 60 μ L water droplet shrinks by 30% over 30 min due to natural evaporation. In comparison, with the ADSA-based control, the drop volume was maintained at constant over the same time period with volume variations less than 0.5%.

It should be noted that it is also possible to control the volume of a droplet using a high-precision syringe pump, if only the linear displacement of the syringe pump is properly calibrated and converted to variations in the drop volume. However, our ADSA-based method represents a novel feedback control system that drives the motor to directly control the drop volume with the value determined in real-time by ADSA. Hence, our method is more accurate, faster, independent of the physical means of fluid actuation, and does not require calibration. The entire process is automatic without the need of any human intervention.

Control of Surface Area

Compared to the control of drop volume, the control of surface area and surface tension of the droplet is exceedingly complicated due to the nonlinearity of these two properties. To demonstrate the nonlinearity of surface area and surface tension with respect to the drop volume, we have conducted a comparative experiment of shrinking three droplets of the same initial volume. These are a pure water droplet, a DPPC-covered water droplet, and an Infasurf droplet. Figure 4 shows the surface area (panel a) and surface tension (panel b) of these three droplets as a function of the reducing volume. It is clear that only the surface area of the pure water droplet is nearly linearly correlated with the drop volume. Nonlinearity in surface area exists for both DPPC-covered and Infasurf droplets due to drop deformation upon reducing volume. Surface tensions of all three droplets are nonlinearly correlated with the drop volume, in which the surface tension of pure water remains unchanged while reducing volume, and the surface tensions of DPPC-covered and Infasurf droplets decrease

quickly to near-zero value upon reducing volume (due to compression of lipid monolayer at the air–water interface).

Nonlinearity of the surface area and surface tension renders the control of these two droplet properties using a regular syringe pump impossible. However, being a feedback control system, our closed-loop ADSA can readily control the surface area or surface tension of the droplet without additional difficulties compared to the control of drop volume. In this study, we demonstrate the direct control of droplet surface area with closed-loop ADSA. We first demonstrate the ADSA-based area control with a similar experiment using water droplets as shown in Figure 2. These results can be found in Figure S1 and Video S2.

We further demonstrate the usefulness of the ADSA-based area control for solving a research problem, i.e., *in vitro* biophysical simulations of pulmonary surfactant (PS). PS is a phospholipid–protein mixture that is synthesized by alveolar type II epithelial cells. The primary biophysical function of pulmonary surfactant is to reduce the alveolar surface tension, thus contributing to the elastic recoil of the lungs and increasing the pulmonary compliance.²⁶ A key requirement of *in vitro* biophysical simulations of PS is to mimic the intra-alveolar environment of the lungs. This requires not only rigorous control of experimental conditions to physiological relevance, such as the core body temperature of 37 °C and 100% relative humidity, but also high-fidelity simulation of respiratory cycles during normal tidal breathing, such as the respiratory rate (e.g., 5 s per cycle) and the control of surface area variations to be less than 20% per cycle.²⁷ Numerous studies have demonstrated that variations in alveolar surface area during respiration are small.^{27–29} Surface area of the lungs does not change more than 30% during a deep breath between 40 and 100% total lung capacity (TLC). During normal tidal breathing between 40 and 50% TLC, the area variation is less than 10%.^{27–29} All this physiological evidence suggests that natural PS films must have a very low compressibility contributing to lung recoil.³⁰ However, direct control of surface area during droplet oscillation was not possible in previous *in vitro* simulations. The area compression ratio of surfactant films can be only determined after experiments by postanalysis of drop images stored during the experiments.²⁶

Figure 5 demonstrates the advantages of closed-loop ADSA in biophysical simulations of a natural PS, Infasurf, under physiologically relevant conditions. It can be seen that with surface area control, the adsorbed surfactant film was compressed precisely by 15% area reduction (mimicking exhalation), and subsequently expanded back to its original area (mimicking inhalation). A minimum surface tension of ~2 mN/m was reached with the 15% compression, indicating a low film compressibility of approximately 0.5 (mN/m)^{-1} . The compression and expansion curves coincide closely with each other without showing a significant hysteresis, which represents an ideal biophysical simulation of the respiratory cycle of natural PS.

In contrast, without surface area control, the actual compression ratio of surfactant films cannot be set *prior to* the dynamic cycling experiments. Consequently, the surfactant films are commonly overcompressed or undercompressed, thus failing to mimic the physiological conditions of respiration. As the example shown in Figure 5, the surfactant film without area

control was compressed by 22% area reduction. It is found that as soon as the area was reduced beyond 15%, the surface tension did not decrease anymore. Rather, the compression curve leveled off at the minimum surface tension, indicating extensive film collapse. Collapse of the alveolar surfactant film is uncommon in mammalian lungs under normal conditions.³¹ Hence, this extra area compression is considered to be “overcompression” that is an artifact introduced by the *in vitro* biophysical simulation.³² Because of this overcompression, the compression–expansion cycle shows a significant hysteresis loop. The area enclosed in the hysteresis loop indicates the amount of mechanical energy loss per respiratory cycle, which is again an artifact for *in vitro* biophysical simulation of natural PS.²⁶

Reproducibility of these compression–expansion curves of Infasurf, with and without the feedback control, can be found in Figure S2. Figure S3 further demonstrates the differences upon the cycles made with and without the feedback control. Video S3 shows the dynamic cycling of Infasurf recorded with the ADSA-based area control.

Control of Surface Tension/Pressure

To demonstrate the control of surface tension/pressure, we used the CDS as a miniaturized Langmuir balance to study the compression isotherm of a self-assembled DPPC monolayer at room temperature. Figure 6 shows the surface pressure of the DPPC monolayer as functions of the relative surface area (Π – A curve) and the compression time (Π – t curve). It can be seen that the Π – A curve is consistent with well-characterized DPPC compression isotherms reported in the literature.^{14,33,34} At large surface area and low surface pressure, the DPPC monolayer is in a fluid-like liquid-expanded (LE) phase, while the surface pressure only increases slowly with area reduction. After passing a phase transition plateau at about 8.5 mN/m, the DPPC monolayer is transformed into a solid-like tilted-condensed (TC) phase at which the surface pressure increases rapidly with compression, indicating a very low film compressibility for the TC phase DPPC monolayer.³⁵

As demonstrated by the Π – t curve shown in Figure 6, with the ADSA-based surface tension/pressure control, we were able to compress the DPPC monolayer to a target surface pressure (i.e., 8.5, 20, 30, 40, 50, and 60 mN/m) and maintain it over a 100 s period. It can be seen that when the controlled pressure reaches 30 mN/m, a small overshoot appears due to hysteresis incurred by stiction of the piston–syringe system. However, the controller is able to correct the overshoot and bring the steady-state surface pressure to the target value. The steady-state error of this control is <1.5%. Reproducibility of this control process can be found in Figure S4. Video S4 shows the process of the ADSA-based surface pressure control.

The precise control of surface tension/pressure over time allows extended investigation of self-assembled monolayers and thin-films fabricated at the droplet surface. One such application is *in situ* Langmuir–Blodgett (LB) transfer from the droplet surface. To date, LB transfer of films from the air–water interface to a solid substrate under controlled surface pressure is primarily implemented with a Langmuir trough.³⁵ After LB transfer, the film can be studied in detail by various microscopic and spectroscopic techniques for characterizing its lateral structure and molecular organization.^{14,26,36} Here, taking advantage of the ADSA-

based surface tension/pressure control, we have implemented the first *in situ* LB transfer of DPPC monolayer from the droplet surface. During the LB transfer, ADSA analysis was restricted to partial drop profiles that were not disturbed by the substrate passing the air–water interface. The inset in Figure 6 shows an AFM topographic image of the DPPC monolayer transferred within the phase transition plateau region, i.e., 8.5 mN/m. The AFM image shows clearly coexistence of the LE phase and the signature kidney-shaped TC domains well-documented in the literature.^{14,37}

CONCLUSIONS

We have developed a novel closed-loop ADSA, integrated into the constrained drop surfactometer (CDS), for manipulating millimeter-sized droplets. The closed-loop ADSA extends applications of the traditional drop shape analysis from a surface tension measurement methodology to a sophisticated tool for automatically manipulating droplets in real time. We have demonstrated the feasibility and advantages of closed-loop ADSA in three applications, including control of drop volume by automatically compensating natural evaporation, precise control of surface area variations for high-fidelity biophysical simulations of natural pulmonary surfactant, and steady control of surface pressure for *in situ* Langmuir–Blodgett transfer from droplets. All these applications have demonstrated the accuracy, versatility, applicability, and automation of this new ADSA-based droplet manipulation technique. Combining with CDS, the closed-loop ADSA holds great promise for advancing droplet manipulation in a variety of material and surface science applications, such as thin-film fabrication, self-assembly, and biophysical study of pulmonary surfactant.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Diagram of the closed-loop axisymmetric drop shape analysis (ADSA) feedback control system integrated into the constrained drop surfactometer (CDS). In CDS, a liquid droplet is “constrained” on a 3–5 mm carefully machined pedestal with a knife-sharp edge. The droplet is continuously monitored by ADSA in real-time. Properties of the droplet, including its volume, surface area, and surface tension, are controlled with the closed-loop ADSA. An integrated PID controller continuously calculates the error, i.e., the difference between the target values and the current values, and converts the error into a command signal with proper PID gains. The PID controller is modeled by

$u(t) = k_p e(t) + k_i \int_0^t e(\tau) d\tau + k_d (de(t)/dt)$, where k_p is the proportional gain, k_i is the integral gain, k_d is the derivative gain, $e(t)$ is the value of the error, and $u(t)$ is the value of the command signal. The resultant command signal controls a motorized syringe, consisting of a high-precision servomotor and a piston–syringe system, to actuate the droplet toward the target values.

Figure 2.

Demonstration of the closed-loop ADSA for controlling drop volume. The volume of a 60 μL water droplet (panel c) was decreased and increased stepwise with a step of every 10 μL , followed by steps of every 20 and 40 μL , respectively. Images of this droplet at different volumes are shown as insets in panel a. It is clear that the change of volume was controlled to be largely linear with respect to time without significant overshoot once reaching the target values within a 1% tolerance. During manipulation of drop volume, the surface tension (panel a) of pure water is largely unchanged, and the surface area (panel b) of the water droplet also varies largely linearly with respect to time. The dynamic process of the volume control is shown in Video S1 in the Supporting Information.

Figure 3.

Demonstration of the closed-loop ADSA for maintaining a constant drop volume by automatically compensating evaporation. Without the control, the volume of a 60 μL water droplet shrinks by 30% over 30 min due to natural evaporation. With the control, the drop volume was maintained at constant over the same time period with volume variations less than 0.5%. Results are shown as mean $\pm$ SD averaged from three repetitions. Insets are drop images at different time points indicated by red solid symbols on the curves, showing changes in drop volume.

Figure 4.

Nonlinearity of surface area (a) and surface tension (b) with respect to the drop volume. Three droplets, i.e., a pure water droplet, a DPPC-covered droplet, and an Infasurf droplet, were shrunk linearly in volume. It is clear that only the surface area of the pure water droplet is nearly linearly correlated with the drop volume. Nonlinearity in surface area exists for both DPPC-covered and Infasurf droplets due to drop deformation upon reducing volume. Surface tensions of all three droplets are nonlinearly correlated with the drop volume, in which the surface tension of pure water remains unchanged while reducing volume, and the surface tensions of DPPC-covered and Infasurf droplets decrease quickly to near-zero value upon reducing volume. Insets are drop images at different volumes of the three droplets indicated by red solid symbols on the curves.

Figure 5.

Demonstration of the closed-loop ADSA for controlling area variations in biophysical simulation of a natural pulmonary surfactant, Infasurf, under physiologically relevant conditions. With the area control, the surfactant film was compressed precisely by 15% area reduction and subsequently expanded back to its original area. A minimum surface tension of ~2 mN/m was reached with the 15% compression. The compression and expansion curves coincide closely with each other without showing a significant hysteresis. Without the area control, the actual compression ratio of surfactant films cannot be set *prior to* the dynamic cycling experiments. In the example shown in this figure, the surfactant film was compressed by 22% area reduction. When the area was reduced beyond 15%, the surface tension did not decrease but leveled off at the minimum value, indicating extensive film collapse, which is an artifact due to overcompression of the surfactant film. Images of the droplets along the cycle path are demonstrative of surface tension being a function of drop shape. Reproducibility of these compression–expansion curves are shown in Figure S2. Video S3 shows the dynamic cycling of Infasurf recorded with the ADSA-based area control.

Figure 6.

Demonstration of the closed-loop ADSA for controlling surface pressure for *in situ* Langmuir–Blodgett (LB) transfer of DPPC monolayers from the droplet. The obtained surface pressure–area curve is consistent with well-characterized DPPC compression isotherms reported in the literature. The surface pressure–time curve shows the compression of the DPPC monolayer to a target surface pressure (i.e., 8.5, 20, 30, 40, 50, and 60 mN/m) and maintained over a 100 s period. When the controlled pressure is beyond 30 mN/m, a small overshoot appears due to the hysteresis incurred by stiction of the piston–syringe system. However, the controller is able to correct the overshoot and bring the steady-state surface pressure to the target value. The steady-state error of this control is <1.5%. Reproducibility of this control process can be found in Figure S4. Video S4 shows the process of the ADSA-based surface pressure control. The inset shows an AFM topographic image ($100 \times 100 \mu\text{m}$; z -range 5 nm) of the DPPC monolayer transferred under a controlled

surface pressure within the phase transition plateau region, i.e., 8.5 mN/m. The image shows clearly coexistence of the LE phase and the signature kidney-shaped TC domains well-documented in the literature.

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