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Interfacial microrheology and tensiometry in a miniature, 3-d printed Langmuir trough



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ABSTRACT

We introduce a new approach for manipulating and studying complex fluid interfacial systems, that enables simultaneous tensiometry to measure surface pressure and high-performance interfacial shear microrheology. In contrast to existing techniques, we use a miniature Langmuir trough produced by 3-d printing methods, which fits on the stage of a conventional inverted microscope and which allows compression and stretching of the fluid-fluid interfaces at a constant bulk fluid volume. The tensiometry measurement relies on the quantitative imaging of a curved meniscus separate from the flat interface being imaged under the microscope. We first demonstrate that this approach can measure the interfacial tension of simple fluid-fluid and polymer-laden interfaces with an accuracy comparable to the pendant drop apparatus and Wilhelmy plates. We then study the mechanics of an adsorbed protein layer formed at an air-water (a-w) interfacial microrheology, micrometer-sized probes spread on the interface are tracked to 5 nm precision with very low drift, yielding measurements of interfacial viscosity having a wide dynamic range and high sensitivity $(10^{-9} - 10^{-1} \text{ Nsm}^{-1})$.

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1. Introduction

Complex fluid interfacial systems, formed of liquid-air or liquidliquid interfaces decorated with particles in combination with macromolecules and amphiphiles, are at the heart of many commercial products consisting of foams or emulsions [1–3] as well as biologically important interfaces [4]. Fluid-fluid interfaces of proteins [5,6] and polymers [7,8] lead to the formation of viscoelastic skins [9–11], and display jamming and glassy behavior [12–14]. The effects of colloidal interactions and particle assembly [15] on the stability and the formation of Pickering emulsions [16,17] and their relation to surface coverage, surface pressure, and surface rheology remain poorly understood. While many instruments have been developed to measure the rheology of such complex interfaces, the relation between the structure of the interfaces and their interfacial rheology requires further exploration [18].

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Here we report a new instrument that enables the measurement of the surface tension of complex interfaces and the manipulation of their surface area, while simultaneously performing interfacial two-point microrheology [19,20]. The complex fluid interface is stretched and contracted by pinning it to the edge of disks which are rotated out of the plane by varying amounts. The surface tension is determined by quantifying the deformation of the resulting meniscus under gravity; while microscopy is performed on a different portion of the pinned interface which is flat.

In what follows, we briefly review the currently available instruments, the design of our apparatus and the steps required to image the meniscus, extract the surface tension, and to determine the viscoelastic response of the interface by microrheology. To verify the performance of the instrument, we measure the surface tension of simple a-w and oil-water interfaces as well as the dynamic surface tension of a polymer-laden interface. Finally, we study the mechanics of an adsorbed protein layer on an a-w interface over time. We simultaneously measure the dynamic surface surface tension as protein adsorbs on the interface and measure the viscosity of the layer as surface pressure increases. Our results demonstrate the compatibility of the new apparatus with microscopic imaging, particle tracking, and microrheology. This inexpensive and 3-d printable apparatus promises to improve our understanding of the physico-chemical phenomena on many different kinds of particle-laden interfaces,

2. Instrumental concept

There are a number of existing approaches for forming, manipulating and measuring the interfacial tension of complex fluid interfaces. Tensiometery devices can be classified into several groups [21]: those based upon directly measuring small forces, such as the Wilhelmy plate [22] and the Du Noüy ring [23], pressure-based approaches [24] and gravity based devices, that utilize the equilibrium between gravity and capillary forces. One gravity-based tensiometer, the pendant drop apparatus [25]. images the shape of a droplet under gravity and compares the droplet silhouette to numerical solutions of the Young-Laplace equation [26]. Recently microfluidic platforms have also demonstrated the measurement of dynamic surface tension using the balance of hydrodynamic and capillary forces [27–29]. Interfaces are most famously formed and manipulated in Langmuir-Blodgett (L-B) troughs, where the interface can be compressed by a movable barrier. Alternatively, in the pendant drop apparatus, the interface can be expanded and contracted by changing the drop volume [30].

Our method, like the pendant drop apparatus, deduces surface tension by modeling the shape of a fluid meniscus deformed by gravity, as shown in Fig. 1a. Here, we create a deformed interface by tilting circular disks inserted into flat interfaces in circular wells; the interface is pinned at the edges of the disks and wells by sharp edges. Tilting the disk about a horizontal axis pulls the meniscus against gravity in a symmetric manner, whose shape is determined by an equilibrium between surface tension and gravity, and which notably does not change the bulk volume. The details of the modeling will be discussed below. Moreover, by tilting the disk to different angles, as shown in Fig. 1a, we can also increase or decrease the surface area of the meniscus which results in expansion or compression of the interface.

This simple concept offer several advantages over both the pendant drop apparatus and Langmuir trough: (*I*)Optical access to a flat *interface:* The device contains a channel with a flat pinned interface between the two circular wells, allowing microscopy with highmagnification objectives, as with a Langmuir trough [31–35], but with the advantage that its smaller scale reduces drift and vibration to levels comparable to the small chambers often used in



Fig. 1. The computed meniscus of the deformed interface between two liquids, which depends on the surface tension. (a) Top right: A circular disk, shown in white, is laying flat with its edge pinned on the interface. Center: The disk rotates around a horizontal axis shown with the blue line, with tilt angle θ , and deforms the interface against gravity. The surface is color coded by the height of the meniscus. Left panel: rotating the disk to larger tilt angles deforms the interface more and increases the total interfacial area. A cross section of the surface, shown by the black line, is analyzed to determine the surface tension. (b) The cross section of the numerically simulated interface with y = 0 plane (shown in a) at different Bond numbers (uniformly spaced), for $\theta = 25^\circ$, and zero excess volume V = 0. Inset: Oblique view of the modeled interface. Surface S is confined with boundaries C_1 and C_2 . C_1 is the edge of the disk tilted out of the plane, C_2 is contained completely in the plane z = 0. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

interfacial microrheology experiments [36-41]. (II)Constant volume: Unlike the case for other methods, because of the symmetric design of the trough, there is no volume change or bulk or interfacial flow at the center of the central channel when the interface is compressed or expanded, which facilitates microscopy. (III)Versatile interface formation: Our approach is well suited to either the study of liquid-liquid or liquid-air interfaces, while troughs are typically used for the latter, with notable exceptions [42,43]. The flat interface facilitates the use of spreading techniques to form the interfacial layers, which is possible but challenging for a pendant drop arrangement [44–46]. (IV)Low cost: The new device is made by 3-d printing, with a small wetted portion and a larger portion to adapt it to the microscope stage. The printing/material cost of the wetted portion is at the single US\$-scale, enabling single use devices. The amount of the sample required for each experiment is also small, only 1.6 ml, further reducing the costs of experiments.

3. Design of apparatus

3.1. 3-d printed trough

A rendering of the 3-d printed apparatus is shown in Fig. 2. A photograph of actual setup is available in Supporting Information, Fig. S1. The trough features two cylindrical wells (with tiltable circular disks) connected by a narrow rectangular channel. The top and bottom halves of the trough are separated by a sharp edge, which pins the interface of the subphase and the superphase (if not using air). The pinned contact line enables the modeling of the interface without knowing the value of the intrinsic contact angle between the interface and the 3-d-printed material. Two glass windows in one side of the trough are used to provide optical access to image the curved meniscus. Two identical disks, part 2, with diameter of D = 1 cm are rotated around a horizontal axis to deform the interface. The knife edge on one side of the disk where it pushes the interface down is flipped to provide a maximal tilting range before contact line de-pinning occurs. Part 3 is used to hold and tilt the disk. The rotational axes of the disks sit on the



Fig. 2. (a) 3-d design of the trough on a microscope stage and (b) cross section view A. Part ①: trough to hold the fluids and form the interface. Part ②: disks with knife edges to deform the interface. Part ③ and part ①: designed to synchronously tilt the disks. Part ③: custom designed microscope stage. Part ⑥: mirror to reflect the image of the meniscus to the camera. Some details are deleted from the diagram to provide a clear picture of the trough. (c) Schematic representation of the apparatus from side view. Interface of fluid I and fluid II (red curve) is deformed by two tilted black disks. Left corner: an image of a-w meniscus with red line showing the fitted curved on the interface. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

interface and intersect with the centers of the disks and cylindrical chambers. Two connectors (part 4) are used to synchronize the symmetric motion of the two disks (one is deleted from the scene). Part 5 is a custom designed microscope stage insert to locate the trough on the microscope; this non-wetted portion of the instrument can be reused and easily adapted to different brands of microscope. Depending on the liquids that are tested in the trough, different materials can be used to 3-d print the trough. The design files¹ of the trough for 3-d printing will be available on GitHub, a free online depository for software and design files. The material used for the trough in this study is a polycarbonate-like material (VisiJet® SL Clear). After 3-d printing, the trough was conditioned before use by simply washing it with isopropanol and submerging it in DI water for 24 h to remove possible contaminants and surface-active compounds. Before any experiments, the trough was washed again with isopropanol alcohol and water and dried over a hot plate at low temperature ($\sim 40\ensuremath{\,^\circ C}$). Control experiments described in Section 4.1 show little leaching of surface active compounds over experimental timescales (less then 0.04 mNm⁻¹ reduction of surface tension per hour).

3.2. Modeling the meniscus

We use a standard energy minimization technique to model a meniscus with known interfacial tension. Since the two sides of the instrument are symmetric we only model one cylindrical well and plate. The energy of the surface *S* bounded by curve C_1 and C_2 (shown in Fig. 1b (inset)) is evolved to find the energy minimum. C_2 is the knife edge of the trough (part 1 in Fig. 2) which pins

the outer perimeter of the fluid interface. C_1 is the edge of the circular disk (part 2 in Fig. 2) which sits on the interface of two phases (the upper one may be a liquid or air) with a tilt angle θ from the horizontal plane. *S* is controlled by changing θ . Compression and expansion of the interface is slow enough for the interfaces to be assumed to be quasi-steady. In the simulation, hence, viscous energy damping is neglected, and, surface and gravitational energy are the only energies in the system,

$$E = \int_{S} \Delta \rho g z^{2} \vec{k} \cdot \vec{n} dS + \int_{S} \gamma dS.$$
⁽¹⁾

The first term in Eq. (1) is the gravitational energy, and the second term represents the surface energy. g, $\Delta\rho$, and γ are gravity constant, density difference between subphase and superphase, and interfacial tension respectively. \vec{k} and \vec{n} are unit vectors of gravity and surface element *dS*. By setting the maximum height of the disk, $h = \frac{1}{2}Dsin\theta$, as the length scale, Eq. (1) is nondimensionalized,

$$E' = Bo \int_{S'} z^{\prime 2} \overrightarrow{k} \cdot \overrightarrow{n} dS' + \int_{S'} dS', \qquad (2)$$

where $z' = \frac{z}{h}$, $S' = \frac{S}{h^2}$, $Bo = \frac{g\Delta\rho h^2}{\gamma}$, and $E' = \frac{E}{\gamma h^2}$. The minimum E' is then determined while satisfying two constraints. The first constraint is the boundary of the interface. The second constraint relates to the volume of the subphase fluid. When the disks are not tilted, $\theta = 0$, the edge of the disks are coplanar with the edge of the trough, i.e. curves C_1 and C_2 lie on a same plane. If the volume of the subphase is the same as the volume of the bottom section of the trough, the interface will be flat, while in the excess or lack of the subphase fluid it will not. The second constraint, therefore, is the difference between the volume of the subphase and the volume of bottom section of the trough, V. Note that, in the experiments, we aim to have zero excess volume (V = 0). However, setting the volume constraint remove the influence of potential errors on the simulation and corrects for the effects of evaporation during the experiment. Applying these constraints, the energy minimization is performed using Surface Evolver [47], an open source software program, based on Eq. (2). An example of simulated 3-d interfaces are shown in Fig. 1. The solid black line on the interfaces shows the cross section of the meniscus through the y = 0 plane, corresponding to the imaging plane in experiments. The shape of the curve determines the Bond number and the excess volume. Fig. 1b shows the profile of the simulated curves at $\theta = 25^{\circ}$ and V = 0 for series of different Bond numbers. Surfaces with larger Bond numbers, i.e. smaller surface tension or larger density difference, deform more under gravity than those with smaller Bond numbers.

3.3. Surface tension measurement

To start the experiment, the subphase fluid is added to the trough followed by adding the superphase fluid, if any. To measure the surface tension, the disk is tilted at different angles, and an image of the meniscus is recorded. The imaging setup is similar to any pendant drop apparatus. Briefly, the meniscus is illuminated by a telecentric light source. The image of the meniscus is relayed to a CMOS camera, Fig. S2. The image of the meniscus is analyzed to locate its edge. More detail on imaging optics is provided in section S1.1 of the Supporting Material. We determine the surface tension of the interfaces by comparing numerically generated interfaces to the edge of the experimentally recorded meniscus, Fig. S3. The fitting procedure is also similar to pendant drop tensiometry, since both methods involve iterative fitting of the equations that balance gravitational deformation of the meniscus with the restorative interfacial tension. In pendant drop tensiometry,

¹ https://github.com/mehdimolaei/UPIT.

the edge of a droplet is modeled by Young-Laplace equation [25]. In our method, a table of simulated profiles for different Bond numbers, different excess volumes of the sub-phase fluids, and different tilting angles were pre-computed using Surface Evolver and stored in the fitting software, Fig. 2b. The detailed steps required to measure the surface tension are illustrated in SM Section 2.

The sensitivity of the tensiometer is limited by the smallest detectable change in the Bond number. In pendant drop tensiometry, the sensitivity of the system depends on the size of the needle and the Bond number defined as $Bo = \frac{g\Delta\rho b^2}{v}$, where *b* is the droplet size. In our system, the relative sensitivity of the measurement depends on the tilting angle of the disk and the Bond number. To examine the sensitivity of the surface tension measurement, the following tests were performed. At a fixed tilting angle and a fixed sample volume, the change in the curvature of the meniscus indicates the change in the surface tension and hence the Bond number. The resolution in the Bond number corresponds to the smallest change in surface tension that displaces the interface by the resolution of the imaging system and edge location algorithm, which control measurements show is about 0.5 pixels in a single image. Fig. S4 illustrates how the sensitivity of the system varies with Bond number. At a moderate Bond number, $Bo \sim 1$, relatively small changes in the Bond number leads to large, readily detectable detectable displacements to the edge of the meniscus; however, similar perturbations when the Bond number is smaller, Bo $\sim < 1$, shift the edge of the meniscus by only a few pixels.

Fig. 3a shows the sensitivity of the system versus Bond number at three different tilting angles. As expected, the sensitivity of the system is greater for large Bond numbers. The sensitivity when the tilting angle is 10° is comparable with the sensitivity at tilting angle 30°; thus, the measurement of the interfacial tension is reliable for the tilting angle larger than 10°. Fig. 3a also shows that at Bo > 0.5 the precision of the surface tension measurement is better than 1%. We also determine the surface area expansion ratio at different tilting angle, Fig. 3b. increasing θ from 10° to 35° provides $\approx 40\%$ increase in interfacial area. While not as large as achievable



Fig. 3. (a) A sensitivity analysis for tensiometry at three different tilting angles. Solid lines show the required change in bond number which result in a measurable deformation in the edge of meniscus. Dashed lines indicate the corresponding precision of the surface tension measurement. (b) Area expansion ratios at different tilting angles for three different surface tension values with $g\Delta\rho = 9800 \text{ Nm}^{-3}$. A_0 is the surface area when the tilting angle of the disk is zero, $\theta = 0$.

with a Langmuir trough, this is comparable to that in pendant drop tensiometry, and should suffice to enable perturbation/relaxation experiments to gain insights into interfacial relaxation processes.

3.4. Interfacial microrheology

We utilize the middle part of trough where the interface is flat to image the interface. Particularly, colloidal probes spread on the interface are imaged to perform microrheology and measure the viscoelastic response of the interface. The spreading solution of spherical probe beads with 1 μ m diameter was prepared by mixing 12.5 µL carboxyl-functionalized polystyrene spheres (Invitrogen) in 450 µL DI water and 250 µL dioxane and spread on the interface. Carboxyl groups on the particles release ions when in contact with polar fluids leading to strong particle binding to the interface and giving rise to a long-ranged electrostatic repulsion between particles [48,49]. Normally, spreading solution are prepared using isopropanol [50,51]. We replaced isopropanol with dioxane which has similar density to water and slightly higher boiling point with respect to isopropanol, greatly reducing subphase convection due to residual spreading solvent. The trough was sealed carefully to minimize evaporation and reduce any perturbation of the interface by air currents.

The colloidal probes at the interface are imaged by bright-field microscopy with a 40x long working distance air objective (Leica, NA 0.65). The videos are recorded with a CMOS camera (Point Grey) at 25 fps and a projected pixel scale of 0.15 µm. Probe trajectories are extracted from the video using a custom Matlab implementation of a common particle-tracking algorithm [52]. Inset to Fig. 4a shows segment of a sample micrograph of the passive tracers on the interfaces. The 2-d vector displacements of the tracers were determined from the particle trajectories, $\Delta r_x^i(t,\tau) = r_x^i(t+\tau) - r_x^i(t)$, where *i* and *x* represent different particles and coordinates respectively, and t and τ are the absolute time and the lag time. We carefully eliminate any small drift of the sample from the vector displacements by subtracting the motion of the particles' computed center of mass motion. Fig. 4a shows sample trajectories for time span of 20 s. We also measure the surface tension of the particle-laden interface to test if adding the spreading solution of probe particles reduces the surface tension of the interfaces. The tensiometry measurement indicates a very small surface pressure, that is less than our measurement sensitivity.

The viscosity of interfaces is measured by adapting the two-point microrheology technique pioneered by [20]. Briefly, we calculate the ensemble averaged tensor product of the tracer displacements [53], $D_{xy}(\tau, R) = \left\langle \Delta r_x^i(t, \tau) \Delta r_y^j(t, \tau) \delta \left[R - R^{ij} \right] \right\rangle_{i \neq j, t}$, where R^{ij} is the distance between particle i and j, and x and y indicates different coordinates. In particular, we measure D_{rr} which is the correlated motion parallel to the line connecting the two particles' centers. $D_{rr}(\tau, R)$ is a function of inter-particle distance, and the shape of the function depends on the viscosity of the 2-d interface and viscosity of the bulk fluid [19]. By fitting D_{rr} to the theoretically expected form, we can quantitatively extract the viscoelastic response of the interface with high sensitivity [19,20].

The use of two-point microrheology (instead of single particle methods) facilitates the measurement of the surface viscosity of highly viscous interfaces. In conventional microrheology, the ensemble Mean Squared Displacement (eMSD, $\langle \Delta r^2(\tau) \rangle_{t,i} \rangle$ of single particles is measured, and the viscoelastic response of the medium is extracted via the Generalized Stokes Einstein Relation [54]. However, inevitable static noise in particle tracking typically limits the detection of particle motion, particularly at short lag time [55], and leads to the under-reporting of the shear modulus at high frequencies. Fig. 4b shows the MSD of tracer particles spread at a clean a-w



Fig. 4. (a) Trajectories of 1 µm size charge-stabilized spherical particles on a clean a-w interface over 20 s; scale bar is 20 µm. Top right inset: sample micrograph of the interface; bottom left inset: the drift displacement over 20 s, red line, compared to the size of the microparticle, starting position is the solid circle, ending is the dashed circle. (b) Two-dimensional mean square displacement of the particles on a-w interfaces. Circles: clean interface and square: adsorbed layer of β -lactoglobulin. The dashed line indicates the static noise floor. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

interface and at a viscous protein layer. The static noise level, estimated from clean interface data [55], dashed line in Fig. 4b, reveals a 5 nm uncertainty in particle centroid locations. The MSDs of particles in a protein layer at short lag times overlay on the estimated static noise floor as expected. Because it is a covariance to two particles motion, such static error does not contribute a noise floor to D_{rr} .

4. Results

4.1. Surface tension

Several experiments were performed to test the performance, precision, and repeatability of the new instrument. We first test the device by measuring the surface tension of a clean a-w interface, over an extended period of time. The surface tension of water with air depends on the temperature and concentration of any surface active impurities in the water. The surface tension of HyPure Molecular Biology Grade water (HyClone) was measured over 5 h, at 25 °C, to analyze the measurement error and to assess if any surface active solutes leached from the device. The meniscus of water in the trough when the disk is tilted by 25.1° is presented in Fig. 5a. The green line is the fitted profile on the edge of



Fig. 5. Meniscus of a-w, a, and oil-water, b, interfaces formed in the trough. Inset to (a) Gray bars show the average values of 100 surface tension measurements separated by one hour, with standard error of 0.04 mNm^{-1} . At the end of hour 5 the measurement was repeated at three different tilting angle to confirm the repeatability of the data. The black dashed line shows the value of a-w surface tension as reported in literature [56–58].

meniscus following the method described earlier in Section 3.3. Gray bars in inset to Fig. 5a shows the measured value of the surface tension compared to reported value at literature, $72.14 \pm 0.15 \text{ mNm}^{-1}$, shown with the dashed line [56–58]. Each data set is the average of 100 surface tension measurements from the images of the meniscus recorded over 20 s, which were found to have a standard deviation of 0.40 mNm⁻¹. The solid line in the inset to Fig. 5a is linear fit for the surface tension measurements over 5 h with a slope of 0.04 mNm⁻¹h⁻¹ with rms error of 0.08 mNm⁻¹ from linear regression. The decay in surface tension could be the result of the surface active solutes or a small change in the water temperature. The initial measured value differs from the literature value for pure water by < 0.5%, providing an estimate of the absolute calibration of our instrument. The final measurement (at hour 5) is then repeated at three more different tilting angles to confirm the repeatability of the measurement at different tilting angles. The result is shown with open bars in inset to Fig. 5.

To illustrate the capability of the new trough to investigate fluid-fluid interfaces, we formed a decane-water interface. In Fig. 5b the sub phase is water phase and the middle light area is decane, with the decane-air interface at the very top. The best fit meniscus is shown by the green curve. The computed surface tension value $\gamma = 51.6 \pm 0.72 \text{ mNm}^{-1}$ matches well with values in the literature $\gamma = 52.19 \pm 0.04 \text{ mNm}^{-1}$ [59].

4.2. Dynamic surface tension

To highlight the stability of our instrument and its ability to manipulate the interfacial area, we study the surface tension of a polymer solution at several concentrations, subjected to interfacial compression and expansion. Poly vinyl alcohol (PVA, MW: 22 KDa, Sigma), was chosen as a surface active polymer with conveniently slow interfacial equilibration. The slightly amphiphilic nature of PVA with alternating hydrophobic methylene and hydrophilic hydroxymethine groups is responsible for its weak surface activity in the dilute regime [60]. PVA was dissolved in DI water at different weight percentages. The solutions were then transferred to the trough, and surface tension measurements were performed over the time. The surface tension of solutions of PVA in water at different concentrations were shown in Fig. 6a. Each profile shows slightly time dependency in the beginning and then reaches equilibrium. Since the solutions were premixed, the adsorption was controlled by transferring of the polymers from subsurface to the interface. As expected, higher polymer concentrations in bulk fluid, C_b reduce the surface tension [61,62]. We estimate the equilibrium surface tension from the asymptote values of the surface tension at long time, Fig. 6b. The equilibrium surface tension depends on the bulk concentration as modeled by Langmuir isotherm and Von Szyszkowski surface equation of state



Fig. 6. (a) Surface tension of PVA solutions with different concentrations over the time. (b) Equilibrium surface tension different PVA concentrations, shown with similar symbols as in (a); solid line: the best-fit of Langmuir isotherm model to the data $\gamma_{eq} = \gamma_0 + R_B T \Gamma_{\infty} ln(1 + C_b/C)$. (c) Surface tension of PVA solution (bottom/circles) with the bulk concentration of $C_b = 5$ nM and pure water (top/squares) while the interface undergoes compression and expansion. Bottom row: sample images of the meniscus PVA solution at three different stages of the experiment; S1, start of experiment, S2) after stretching the interface, and S3) after interface compression to the original surface area. Black line shows the smoothed data for three stages. A second order diffusive scheme with fixed boundaries is used to smooth the data. Diffusive coefficient used for smoothing is 0.1, and number of smoothing steps is 200. For comparison, we repeat the compression-expansion experiment with clean water; results are shown at the top (squares). The value of surface tension does not change substantially after changing the surface area, suggesting a low incidence of surface active impurities.

 $\gamma_{eq} = \gamma_0 + R_B T \Gamma_{\infty} ln (1 + C_b/C)$, where R_B is the ideal gas constant and Γ_{∞} and C are fitting parameters. C depends on the tendency of the molecules to adsorb at the interface, and Γ_{∞} indicates the maximum packing on the interface [63].

To observe dynamic surface tension by changing the interfacial area, we prepared the $0.01\%\,(w/w)$ solution of PVA in DI water. The

rate of approach to the equilibrium surface tension for macromolecules like PVA is slow compared to conventional surfactants that makes the study of dynamic surface tension feasible [62]. After setting up the interface, the disks were tilted to 18.1°, and surface tension measurement were started immediately (Fig. 6c bottom left inset with red border). The earliest segments in Fig. 6c, red circles, represents the measured surface tension before the increase in the surface area. After 5 min, the tilting angle was increased to 26.2° to stretch the interface, (Fig. 6c middle inset with blue border). The measured surface tension, blue circles in the Fig. 6c shows an immediate surge after the surface area increased, associated with the decrease in the concentration of the polymer on the interface. With time, polymer from the bulk diffused and adsorbed to the interface and reduced the surface tension to the same equilibrium value. When the equilibrium surface tension was re-established, the disks were tilted back to 18.0° (Fig. 6c bottom right inset with green border). Dropping the tilting angle reduced the surface area and increased the concentration of the polymer on the interface. Hence, slight decrease in the surface tension was observed, green circles in Fig. 6c. Over time the surface tension reached to the same equilibrium by desorption of the excess polymer from the interface. As a control test, we repeated the expansion-compression experiment with pure water to rule out possible effect of leached surface active molecules and impurities from 3-d printing material on the surface tension. Ultra pure water was left in trough for one hour before starting the control experiment. We then measured the surface tension at tilt angles of 18.2°, 25.1°, and 17.8° for time interval of \sim 5 min, plotted by red, blue, and green squares respectively in Fig. 6c. The average values of surface tension for three stages of the experiment, shown by dashed lines in Fig. 6c varied by $< 0.1 \text{ mNm}^{-1}$.

4.3. Adsorbed protein layer on an a-w interface

The viscosity of adsorbed and spread layers of proteins on a-w interfaces have been investigated with different approaches, notably, interfacial stress rheometery using a magnetic needle [64], passive microrheology [51,36,65], active microrheology [51,39] and techniques based on two orthogonal Wilhelmy plates [66,67]. Here, we measure the time-dependent viscosity of an adsorbed protein layer, using two-point microrheology, as the protein coverage (and surface pressure) increases due to progressive adsorption to the a-w interface.

A solution of the milk protein β -lactoglobulin (Sigma-Aldrich, L3908) containing 3 mg/mL protein was prepared in 10 mM phosphate buffer, pH = 5.2. First, 1.66 mL phosphate buffer was added to the trough, and then 3 µL of spreading solution containing probe particles was placed in contact with the interface of air and the buffer solution. We wait ~10 min to stabilize the interface and then measure the surface tension of the neat a-w interface in the absence of the protein molecules. To form the protein layer at the interface, the buffer solution of protein was injected into the trough at three different locations to reach the final bulk protein concentration of 50 µg/mL. As time progresses, protein molecules adsorbs to the interface, and the surface tension gradually decreases, Fig. 7a.

At different ages of the interface (measured from protein addition to the subphase), interfacial two-point microrheology was performed to measure the viscosity of the interfaces, η_s , simultaneously with surface tension measurement. We first measure the correlated motion of the particles embedded at the interface projected along their line of centers, $D_{rr}(R, \tau)$, as described in Section 3.4. Fig. S5 shows D_{rr} at different lag times and different inter-particle distances for the interface at age of 22 and 71 min. For viscous interfaces, D_{rr} increases linearly with τ [19,20].



Fig. 7. Surface pressure, Π and viscosity, η_s of the adsorbed β -lactoglobulin layer at the a-w interface. (a) Surface tension over time since injection of protein solution to the bulk fluid, inset: surface viscosity as measured by two-point microrheology, versus time. (b) Surface viscosity as a function of surface pressure, inset: Surface viscosity as function surface pressure, φ which is computed from surface pressure based on the results of [68]. The black square is a datum from [68] for a spread layer of β -lactoglobulin measured by interface stress rheometer.

The *R* dependence of D_{rr} strongly depends on the viscosity of the interface. At low surface viscosity, $D_{rr} \sim 1/R$ (Fig. S5a), but at higher surface viscosity D_{rr} decays logarithmically (Fig. S5b) revealing the characteristic 2-d response of the interface [19,20].

Since D_{rr} scales linearly with the lag time, the averaged normalized correlation function, $\langle D_{rr}/\tau \rangle_{\tau}$ is only a function of R, where $\langle \cdot \rangle_{\tau}$ indicates averaging over different lag times. Following the method proposed by Prasad *et. al.* [20], we then normalized the timeindependent correlation function with $D_s = k_B T / 4\pi \eta_s$ and fit the dimensionless correlation function, $\bar{D}_{rr} = \langle D_{rr}/\tau \rangle_{\tau} / D_s$ to the theoretical function [19],

$$\bar{D}_{rr} = \frac{\pi}{\beta} H_1(\beta) - \frac{2}{\beta^2} - \frac{\pi}{2} [Y_0(\beta) + Y_2(\beta)],$$
(3)

where $\beta = R\eta/\eta_s$ is the normalized separation function, and *H* and *Y* are the Struve function and the Bessel function of the second kind, respectively. By fitting to this master curve, we estimated η_s for different interfaces as shown in inset to Fig. 7a. Fig. S6 shows \bar{D}_r plotted against normalized separation distances for different age of the interfaces with the solid line indicating the master curve. Since the cross-correlated motions of particles is due to excitations of long-wavelength modes in the system, it is independent of the local environment of the colloids including a possible depletion layer around them or their immersion depth of the interface, providing a strong advantage over conventional one-point microrheology. However, for interfaces with very low viscosity, where η_s/η is smaller than

the size of the probe particles, interfacial two-point microrheology will become insensitive and fail to provide proper estimation of η_s . In this regime, stresses within the bulk fluid will mainly drive the cross correlated motion of particles. A similar limitation, however, exists for one-point microrheology, since the motion of individual particles strongly depends on the bulk fluid in that limit. Therefore, although we have performed microrheology measurement for the interface younger than 20 min, $\eta_s/\eta < 0.1 \,\mu\text{m}$.

Since the surface pressure, $\Pi(t) = \gamma_0 - \gamma(t)$, is also measured over time, we can determine the $\eta_s - \Pi$ relation for the system as plotted in Fig. 7b. At early age of the interface while surface pressure increases up to 2 mNm⁻¹, the surface viscosity remains very small, $< 1 \text{ nNsm}^{-1}$. The long delay in the formation of a viscous film from adsorbed β -lactoglobulin has been observed previously [51]. η_s shows an initial upturn at $\Pi = 3 \text{ mNm}^{-1}$. Although in the current measurement we do not have access to surface coverage, Φ , of the adsorbed molecules, we can estimate it from the $\Pi - \Phi$ isotherm reported by a previous study [68]. For an spread monolayer of β -lactoglobulin on a-w interface Cicuta et al. have measured viscosity at different surface concentrations. In that study, the shear modulus shows its first rise at $\Phi = 0.77$, and loss modulus dominates elastic modulus for $\Phi < 0.87$. Interestingly, for the adsorbed monolayer in this study, the sudden increase in viscosity we observe occurs at $\Phi = 0.74$ (inset to Fig. 7b), and a further increase of only 7% surface converge leads to a 4 orders of magnitude increase in surface viscosity. The result at high surface coverage is in a good agreement with that of measured by interface stress rheometer shown with a black square in the inset of Fig. 7b [68]. At still higher surface pressures, the adsorbed protein layer starts to reveal a viscoelastic response, which will be the focus of a future publication.

Here, we reported the surface viscosity of a 2-d interface with viscosity values that varies more than 4 orders of magnitude. While previously reported results of one-point microrheology for highly concentrated adsorbed β -lactoglobulin on a-w interface deviates from the result obtained from active microrheology [51], the two-point microrheology gives values for the viscosity that are comparable to the active method. The viscosity of an interface with a very dilute concentration of adsorbed molecules, and therefore a small surface pressure, is still inaccessible by two-point microrheology due to the background of correlated motion of particles due to bulk fluid viscosity. Therefore, we did not report estimated surface viscosities for $\Pi < 1 \text{ mNm}^{-1}$.

5. Conclusion

We have demonstrated a powerful and inexpensive new technique for forming, manipulating and imaging complex fluidfluid interfaces using a miniature 3-d printed trough. The new instrument is a versatile tool to explore the relationships between interfacial structure, pressure and rheology. The technique is wellsuited to the application of emerging interfacial microrheology techniques for interfaces requiring surface area manipulation, precision tensiometry or both simultaneously. The accuracy, precision and repeatability of the new tensiometry approach is comparable to the pendant drop apparatus [25] and Wilhelmy plates [22], and demonstrated by measuring the surface tension and dynamic surface tensions of clean and polymer-laden interfaces. Although the current design of the apparatus limits the area compression ratio to 40%, this is more than sufficient to observe surface tension changes for adsorbed macromolecules. Modifying the current design to achieve higher area compression is the focus of future work.

The study of the protein-laden interface demonstrates the suitability of the instrument for high resolution microscopy, particle tracking, and state of the art two-point interfacial microrheology. Utilizing the new approach, we measure the interfacial shear response of the protein layer as the surface pressure increases over the time. Previously, the shear viscosity of similar interfacial layers measured using different techniques including macrorheology [69,64,39] and passive microrheology [70,71,20] have yielded conflicting results [72]. This is presumably due to a lack of understanding of the hydrodynamic condition of the measurements, the flow and displacement induced by the probes, and the coupling of the bulk fluid and the 2-d interfacial layer [72]. In some cases, the values reported for interfacial viscosity obtained from conventional approaches and microrheology differ by more than three orders of magnitude [51,41]. The sensitivity of one-point microrheology to heterogeneity in the interface, noise in particle tracking, and overly simplistic models describing the motion of probes on the interface have been hypothesized as possible reasons for this difference. Previously, microrheology measurements were performed in a small rheology cell or a petri dish [36], with little to no information of surface pressure or coverage which makes comparing result from microrheology and macrorheology even more challenging. The ability to perform low noise, low drift particle tracking in our instrument with simultaneous tensiometry promises to alleviate much of these difficulties. Indeed, the precision of particle tracking in our instrument is comparable with that performed on well controlled rheology cells [51]. The interfacial viscosity of the protein layer we observe at high surface pressure obtained using two-point microrheology is in a excellent agreement with results obtained from interfacial stress rheometer on the Langmuir trough [68]. Future measurements are underway to study the rheology of soft glassy interfaces including adsorbed protein layer on a-w and oil-water interfaces.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.jcis.2019.09.112.

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