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OPTICAL FACTORS IN THE RAPID ANALYSIS OF CAPTIVE BUBBLES

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Abstract

Bubbles and droplets offer multiple advantages over Langmuir troughs for compressing interfacial films. Experiments, however, that manipulate films to maintain constant surface tension (γ) present problems because they require feedback. Measurements of bubbles and droplets calculate γ from the shape of the interface, and calculations in real time based on finding the Laplacian shape that best fits the interface can be difficult. Faster methods obtain γ from only the height and diameter, but the bubbles and droplets rest against a solid support, which obscures one section of the interface and complicates measurements of the height. The experiments here investigated a series of optical variables that affect the visualized location of the different surfaces for captive bubbles. The pitch of the support and camera as well as the collimation of illuminating light affected the accuracy of the measured dimensions. The wavelength of illumination altered the opacity of turbid subphases and hydrated gel used to form the solid support. The width of all visualized edges depended on the spectral width and collimation of the illuminating light. The intensity of illumination had little effect on the images as long as the grayscale remained within the dynamic range of the camera. With optimization of these optical factors, the width of all edges narrowed significantly. The surfaces away from the solid support approached the infinite sharpness of the physical interface. With these changes, the grayscale at the upper interface provided the basis for locating all surfaces, which improved real-time measurements based on the height and diameter.

INTRODUCTION

Bubbles and droplets provide multiple advantages over the more classical Langmuir trough for the manipulation of interfacial films. The method of determining surface tension (γ), however, complicates one particularly useful type of experiment. Isobaric measurements, which hold films at constant surface pressure (π), provide quantitative information concerning molecular fluxes¹ to and from the interface during adsorption² and collapse,¹ as well as detecting the abrupt changes in molecular area of phase transitions during heating.³ Maintaining isobaric conditions requires feedback and measurements of γ in real time. In contrast to experiments with a Langmuir trough, which obtains γ by measuring force directly, experiments with bubbles and droplets instead determine γ indirectly from the interfacial shape.⁴ Because the differential equation that describes the shape has no analytical solution,⁵ determining γ requires numerical integration of the Young-Laplace equation to find the Laplacian shape that best fits the actual interface.⁶ These calculations limit the rate of processes that can be held at constant π .

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The studies here concern an alternative approach for finding γ that uses faster calculations but that introduces an additional problem. Semi-empirical analytical expressions, obtained by numerical fits to Laplacian profiles, calculate γ from only the height and diameter.^{7,8} These algorithms greatly simplify the calculations, but they also require localization of an interfacial section that is partially obscured. Sessile droplets rest upon a solid support, and captive bubbles float against a slightly concave ceiling molded into the surface of a hydrated gel.⁹ The solid support in both cases alters the appearance of the air/water interface, complicating determination of its position and of the bubble's height. Methods that find the Laplacian shape which best fits the interface avoid this problem because the surface away from the support contains sufficient information to determine γ . The experiments here examined the specific case of captive bubbles commonly used to study films of pulmonary surfactant. We investigated the optical factors that affect visualization of the air/water interface, particularly at the upper surface, and how best to determine the two dimensions that rapidly establish γ .

MATERIALS & METHODS

Materials

Dipalmitoyl phosphatidylcholine (DPPC), dioleoyl phosphatidylcholine (DOPC), and 1-palmitoyl-2-oleoyl phosphatidylcholine (POPC) were obtained from Avanti Polar Lipids (Alabaster, AL) and used without further analysis or purification. Polystyrene spheres (0.1 μm diameter, Polysciences, Warrington, PA) were diluted to the desired turbidities. A polished sapphire sphere (3 mm diameter) and spheres with low densities (3 μm diameter, 0.9 g/cm³ density) were obtained from Edmund Optics (Barrington, NJ) and Hoover Precision Products (Cumming, GA), respectively.

Methods

Apparatus—Experiments with the captive bubble used a previously described (2,4,6) apparatus constructed according to diagrams provided by Dr. Jon Goerke (University of California - San Francisco). The instrument used an optical cuvette with a 1-cm pathlength, the bottom of which was filled with 1% (w:w) agarose and molded with a milled rod to produce a conical indentation with a depth of $\sim 100 \mu\text{m}$. After filling the chamber with buffer, sealing with a gasket, and inverting, injection of $\sim 50 \mu\text{l}$ air provided the captive bubble. The chamber was mounted on a horizontal rail that served as an optical bench, and that was split to provide easy access for tubing that communicated with the chamber through the sealing gasket.¹⁰ A charged-coupled device (CCD) camera, mounted on an x-y-z stage supporting a tilt-tip stage to allow proper alignment, recorded images of the bubble along the horizontal axis.

Cameras—With the exception of one set of images, these studies used a camera with a resolution of 640×480 pixels (TM-7EX, Pulnix, Sunnyvale, CA). The images in Figure 5 used a camera with a higher resolution of 1360×1024 pixels (Allied Vision, Stadroda, Germany). Both devices used simple (bw) detectors without filtering to sense color. The cameras used either a 50 mm lens (Tamron, Commack, NY) with an extension tube, or a telecentric lens (Opto Engineering, Mantova, Italy). Both optical arrangements achieved a magnification of 1.0x. The images were recorded directly to computer via frame-grabber for further analysis.

The camera was aligned relative to the gravitationally vertical axis. Roll of the camera (Fig. 1) was set such that the image of a plumb line fell vertically, parallel with a single column of pixels.¹¹ Pitch of the camera (Fig. 1) was then adjusted to minimize the visualized length of an illuminated, leveled plate.¹²

Lightsources—Different lightsources produced red light (panel of light emitting diodes (LEDs), spectrum centered at 660 nm, Phoenix Lighting, Livonia, MI), green light (LEDs, 14 watt, 535 nm, enLux Lighting, Tempe, AZ), or white light (incandescent bulbs). Band-pass filters (Edmund Optics, Barrington, NJ) placed in front of the white lightsources provided red (central wavelength at 660 nm, with a bandwidth of 10 nm), green (535 ± 10 nm), or blue (460 ± 10 nm) light. To eliminate ambient stray light, the apparatus was masked with black tape or the experiments were conducted in a darkened room. A variable-output power supply controlled the intensity of illumination.

Calibration—The factor for converting dimensions within images into actual distances was determined using a reticule (Klarmann Rulings, Litchfield, NH) inscribed with a square grid. The conversion-factor was reproducible on repeated measurements within $\pm 0.2\%$. Although the lines had relatively low contrast, the grid avoided inaccuracies in finding the edges of solid objects such as ball bearings. The grid also provided access to the aspect ratio, which can affect results obtained for the profile of bubbles.^{13,14} For our optics, the aspect ratio was 1:1.

Software—Analysis of the images used two approaches to determine γ from the shape of the bubble. The first used a program constructed with a graphical user-interface (LabVIEW, National Instruments, Austin, TX) to measure the height and diameter of the bubble, and to calculate its γ and area from those variables.^{7,8} The program converted the original image to a black-and-white binary grayscale, with the value for each pixel assigned according to its original grayscale relative to a set threshold value. The maximum horizontal and vertical dimensions of the black bubble then provided the height and diameter used to calculate γ according to a semi-empirical polynomial using published coefficients.⁸

The second approach for obtaining γ used a purchased program to find the Laplacian shape, calculated by numerical integration of the equation of Young and Laplace, that best fit the actual profile. The program ADSA (A.W. Neumann, University of Toronto), first described almost three decades ago,⁶ continues to evolve.^{15–18} The version used for these calculations (2.0) includes the algorithm for detecting edges developed by Canny¹⁹ and was current for analyzing captive bubbles as of November, 2008 (A.W. Neumann, personal communication).

Films—Experiments with monolayers used films formed by depositing $\sim 0.05 \mu\text{l}$ of phospholipid dissolved in chloroform to achieve an initial $\gamma \approx 33 \text{ mN/m}$ for isobars and $\approx 62 \text{ mN/m}$ for isotherms. Exhaustive exchange of the subphase removed the spreading solvent.³ A syringe pump manipulated by a computer-controlled stepper-motor then regulated the volume of the subphase, thereby controlling the volume of the bubble, and compressing or expanding the interfacial film.

RESULTS

The problem—The appearance of the air/water interface away from and adjacent to the agarose dome was substantially different (Fig. 2B). The contrast between grayscales on opposite sides of the upper interface was less than for the other surfaces, and the change extended over a larger distance (Fig. 2). For illumination that produced a variation in grayscale across three pixels for the other surfaces, the change at the upper edge occurred over nineteen pixels. These differences were generally consistent with the expected effects of viewing the upper interface through the lip of the dome, and of the blurring caused by the agarose gel. The images failed, however, to distinguish the air/water interface against the background of the gel. The raw images (Fig. 2A,B), the image convoluted to show the

gradient of intensities rather than the intensities themselves (Fig. S-1), and the histogram of intensities (Fig. 2C,D) provided no discrete features that located the upper interface.

To evaluate methods for finding the upper surface in real time, our studies required a means of establishing its true position. Two objects, the entire shape of which could be known, addressed that problem. In both cases, extension of the lower profile, observed away from the dome, using the known shape determined the location of the upper surface. The first object was a sphere slightly less dense than the aqueous medium that floated against the dome. Its known diameter placed the upper edge at a specific distance above the lower surface. The second shape was a captive bubble, the Laplacian profile of which also determined the location of its upper surface. A sphere and a bubble, manipulated together in the same cuvette, established the upper surface at the identical location. These results demonstrated that for any bubble, the Laplacian shape determined from its lower silhouette could establish the location of its upper surface.

Alignment—Our experiments considered a series of optical factors that seemed likely to affect the appearance of the air/water interface, particularly at the upper surface. Accurate images of the bubble's meridional profile required correct alignment of the agarose dome to the gravitational frame of reference, and of the camera to the vertical axis of the bubble. Methods for analyzing the profile of a bubble all assume that the imaged silhouette accurately portrays its axisymmetric shape, viewed along a horizontal axis. Misalignment of the support, although having no effect on the actual air/water interface away from the gel, would alter the appearance at the upper surface. Incorrect orientation of the camera relative to the bubble would produce skewed images.

Our studies considered alignment in terms of the rotational orientations of yaw, roll and pitch in orthogonal planes relative to the gravitational horizontal and vertical axes (Fig. 1). Because of the bubble's axisymmetric shape, yaw was unimportant. Roll was easily set according to the image of a plumb line.¹¹ Incorrect roll was both readily apparent and susceptible to mathematical correction.^{20,21} Pitch, however, produced subtle changes without deviations from axisymmetry. Incorrect pitch increased the apparent depth of the dome, producing a wider region in which the location of the upper surface was obscured. Intentional misalignment of the dome quantitated the effect of incorrect pitch. After published procedures¹² first set the camera and dome correctly in the gravitational frame of reference, we then pitched the dome by 0.4° from the gravitational horizontal. Because this misalignment had minimal effect on the Laplacian shape that best fit the lower profile, the correct location of the upper surface could be determined for each image. At the upper surface, the region of variable grayscale shifted by 0.05 mm relative to the location determined by fitting the lower interface (Fig. 3), resulting in a change in the calculated γ of ~12%. The errors occurred without changes that were obvious to casual inspection of the images.

Wavelength—The spectral width of the illuminating light produces polychromatic aberration of the optical components,¹⁷ which affects the sharpness of an imaged interface. With colored light, restricted to a narrow band of wavelengths, the width of the imaged air/water interface away from the agarose was less than with white light (Fig. S-2). Initial studies used a green lightsource based on the specified sensitivity of the camera. The known spectral dependence of scattering, however, suggested that the opacity of the gel should decrease at longer wavelengths. Measurements with white light projected through band-pass filters, when adjusted to produce the same transmittance through the subphase, showed a brightening of grayscale for the gel from 115 a.u. for blue light (band-pass centered at 460 nm) to 129 a.u. for green (535 nm) and 139 a.u. for red light (660 nm). While scattering diminished with higher wavelengths, the breadth of the air/water interface remained the

same for the three colors tested. Red light, obtained either from a panel of red LEDs or from white light projected through a band-pass filter, therefore optimized both the width and the contrast across the interface.

The most striking effect of wavelength occurred with a turbid subphase. Vesicles of phospholipids in studies of adsorbed films formed by pulmonary surfactant make the subphase turbid. The profile of a bubble that was completely obscured by turbidity when observed with blue light remained readily evident with red light (Fig. 4). These experiments used polystyrene spheres to generate a subphase with reproducible turbidity. Different kinds of particles, including phospholipid vesicles, would scatter differently at any given wavelength. The well established spectral dependence of scattering, however, suggested that red light should be optimal for all experiments because of the turbid agarose, and essential for measurements when a subphase has turbidity above a minimal level.

Collimation—For an object such as a captive bubble with a reflective surface, orientation of the illuminating light can affect the perceived dimensions.²² Illumination ideal for generating an accurate silhouette would consist of backlighting with all rays parallel to the optical axis. Nonparallel rays, whether generated directly from the lightsource or scattered from the agarose dome, that reflected from the front surface of the object would shrink its apparent size.²² Geometric considerations indicated that both the size of the lightsource and its distance behind the illuminated object should affect reflections from the front surface. To test these possibilities, an iris with a variable aperture placed behind the object controlled the size and distance to the effective lightsource. Measurements with a smooth sapphire sphere of known dimensions confirmed that both smaller size of the aperture and greater separation of the iris from the illuminated object produced better agreement between the imaged and actual dimensions (Fig. 5).

Better collimation affected the breadth of the imaged interface. This effect was most evident with a telecentric lens, which eliminates light that is insufficiently collimated by placing its aperture at its focal point.²³ With a distant lightsource and a telecentric lens, the width of the visualized interface in some images reached the physical limit. Grayscale changed across a lateral edge from the bright value for the subphase in one pixel to the dark value for air in the next (Fig. S-3). At the resolution of individual pixels for our particular camera, the interface achieved the infinitely sharp transition expected for the actual surface of the bubble.

Images with well-collimated light having a narrow spectrum also introduced an additional characteristic at the surfaces. One or more faint, bright halos appeared immediately outside the dark bubble (Fig. 6). A holographic diffuser, the multiple reflections of which eliminate the coherence of the transmitted light, removed the halos. The observed effect was consistent with the lower order interference fringes produced by edge-diffraction (Fig. 6).²⁴

Better collimation of the illuminating light also produced detrimental effects. All interfaces became more apparent. Spurious edges in both the agarose and the subphase (Fig. 4C) that were invisible with a closer lightsource became prominent features with illumination from a greater distance. The dome of the agarose also became darker, consistent with the loss of light refracted from the curved surface that failed to reach the detector (Fig. 4C). The grayscale of the dome lateral to the bubble approached the value for air. Consequently a value of grayscale no longer uniquely identified the air/water interface.

Intensity—Although the intensity of illuminating light seemed likely to alter the appearance of the upper surface, that effect was unimportant. With suitable adjustments in the gain of the camera, made manually rather than with the “auto” setting, comparison of

histograms and line-profiles across the surfaces showed that images obtained with different light-intensities were indistinguishable (Fig. 7). As long as all detected intensities remained within the dynamic range of the camera, the images were qualitatively unaffected by the intensity of illumination. If uncorrected by adjusting gain, the intensity of light would change the value of the grayscale at the interface, but the location of the surface by all other criteria would remain the same.

The change in transmitted intensity represented the greatest effect of a turbid subphase. Scattering blurred the imaged interface, but the reduction in grayscale of the imaged subphase was usually substantially more prominent. Correction for this effect often required only more intense illumination or an increased gain on the camera. This compensation reached its upper limit when light transmitted through the agarose exceeded the camera's dynamic range. With red light, however, adjustments in the illuminating intensity and camera easily allowed visualization of bubbles in dispersions of phospholipids at 4 mM.

Locating the upper interface—For simple objects with a uniform appearance, grayscale represents the simplest and fastest means for locating the surface. A threshold value between the grayscales of the object and the surroundings distinguishes the two substances, and identifies the position of the interface. For bubbles, the difference between the appearance of the interface adjacent to and away from the solid support complicated that approach. With the initial optics (Fig. 2), before optimization, the values of grayscale at the upper and lower surfaces were different, suggesting that a single value of grayscale would be unable to locate both sections accurately.

One approach to finding an interfacial grayscale was simply to choose a threshold value that produced the correct γ . Analysis of an initial image of a bubble in buffer, obtained before compressing a film, provided the Laplacian shape that best fit the lower profile and the value of γ . A threshold grayscale provided a height and diameter that yielded the correct γ (Fig. 2C), and provided the basis for calculating γ in real-time during an experiment. With the original optics (Fig. 2), using polychromatic, poorly collimated light, that approach yielded erroneous results. Experiments designed to produce isobaric compressions successfully maintained γ at reasonably constant values calculated from the height and diameter (Fig. 8A). Images captured during the experiment were then analyzed subsequently by finding the best Laplacian shape. The γ 's obtained by the two methods diverged progressively (Fig. 8A). These observations suggested that the selected interfacial grayscale yielded the correct initial γ by compensating errors rather than by accurately locating the interface.

Collimated, colored light simplified the problem of finding the correct threshold grayscale for determining the height and diameter. For the interfaces away from the solid support, the narrow distance over which grayscale varied (Fig. 6, S-3) meant that the threshold value became unimportant. At the resolution of individual pixels emphasized here, any interfacial grayscale over a broad range returned the same position. The grayscale at the upper surface then accurately located the entire interface.

The darker dome, however, that resulted from better collimation introduced an additional complication. The grayscale at the upper air/water interface also identified pixels extending laterally along the dome, beyond the lateral dimensions of the bubble. These dark pixels prevented the rapid analysis of the bubble's diameter. A second lightsource directed at the upper interface addressed this problem. The greater illumination brightened the dome more than the upper surface of the bubble. The grayscale at the upper surface then identified only the air/water interface, and provided the basis for rapidly calculating γ .

This method produced good agreement between γ 's calculated during an experiment according to height and diameter, and values obtained after the experiment by fitting the lower profile. Isobaric compressions of collapsing films maintained γ that was constant according to calculations based on the lower profile as well as on the height and diameter (Fig. 8B). Compression isotherms for DPPC obtained by the two methods agreed well over the full range of possible γ 's (Fig. 8C). Because calculations based on the height and diameter are especially sensitive to inaccurate dimensions at high γ ,⁸ compression of a clean bubble, without an intentional interfacial film, provided perhaps the most rigorous test. The two methods yielded values of γ that agreed well (Fig. 8C).

DISCUSSION

These studies address a problem which complicates experiments that use feedback in manipulating captive bubbles. The need to determine γ in real-time puts a premium on the efficiency of calculations. The algorithm that calculates γ from the height and diameter of a bubble provides a major advantage because of its simplicity. Manipulation of bubbles using feedback with current computational speeds has generally used γ calculated from the two dimensions. Faster computers in the future may allow feedback based on fits of Laplacian shapes to the interface, but the simpler algorithm that requires only the two dimensions will always be able to track faster processes.

The solid surface against which the bubble floats obscures the location of the upper surface, making the height difficult to determine. The dome is essential. Bubbles must be large enough so that buoyancy influences their shape.⁸ Bubbles of that size are prone to move laterally, especially with a stirred subphase, if unconstrained by a concave surface. Contact with the sides of the chamber can radically alter the behavior of a film, inducing overcompressed metastable films^{25,26} to collapse, consistent with heterogeneous nucleation of collapsed structures. The requirement for the dome and the resulting difference between the appearances of the different edges raises the question of how best to locate the interface. Appropriate optical adjustments simplify the process of finding the upper surface, measuring the height, and rapidly calculating γ .

For many experiments with captive bubbles, the obscured location of the upper surface is unimportant. If an experiment changes a bubble in a predetermined manner, such as by continuous compression to smaller volumes, then feedback is unnecessary. Images captured during the experiment can be analyzed subsequently to determine γ . Without restrictions on the speed of the analysis, the Laplacian shape that best fits the lower profile can provide γ . The shape of the lower profile, away from the dome, includes sufficient information to determine γ . These experiments can avoid the problem of the surface obscured by the solid support.

Experiments that use feedback, however, provide important information. A fixed γ is required to maintain constant thermodynamic conditions, which greatly simplify the analysis of most processes. During collapse of overcompressed films, constant γ ensures that the thermodynamic motivation remains fixed.¹ To the extent that γ is directly related to surface concentration, the change in area at constant γ is proportional to molecular flux.¹ These measurements with feedback provide crucial, quantitative information for understanding interfacial phenomena.

The optical effects investigated here address both the accuracy and the precision of the interfacial measurements. The alignment of pitch and the collimation of the illuminating light both improve the accuracy by removing factors that tend to shift the interfacial location erroneously. Better collimation and a narrower spectrum of illumination improve the

precision by decreasing the width of the visualized interface. The narrower interfacial width, which allows any grayscale over a broad range to locate the lower surface correctly, is essential for using grayscale to identify the position of the surfaces with different profiles. The simplest and most effective step in achieving better collimation is the elimination of stray light. Additional factors include the use of a telecentric lens, and the correct size and positioning of the lightsource. Greater distance of the light behind the viewed object achieves better collimation, but shrinking the size of the lightsource can produce the same result at a smaller distance more convenient for a laboratory with limited space.

Better collimation does significantly darken the surface of the agarose dome. The grayscale at the interface between agarose and subphase approaches the value at the air/water interface. The darker surface of the dome extends beyond the lateral dimensions of the bubble, and complicates measurements of its diameter. Illumination with longer wavelengths, which improves the transparency of the agarose, brightens the dome, but does not eliminate the problem. Imperfect collimation, produced by decreasing the distance to the lightsource or increasing its size, can improve the illumination of the dome with a small decrease in the accuracy of measurements. A second lightsource achieves the same effect without sacrificing accuracy.

Illumination with better collimation and a narrower spectral width also produces edge-diffraction. This effect introduces an additional consideration in locating the interface. In an image of an infinitely sharp surface that has a finite width because of blurring, the maximum gradient of grayscale corresponds to the location of the physical interface.²⁷ With light diffracted at an edge, the bending of light brightens the outer region of the imaged profile, and the actual interface shifts to a point on the profile with a lower grayscale. Diffraction at a surface with a larger radius of curvature than for a blade is more complicated,²⁸ and these studies make no attempt to address those issues. Although the location of the visualized interface should be unaffected for resolution at the level of individual pixels used here, the consequences of the shift produced by diffraction when working at subpixel resolution could be significant.

The problem of finding edges that have different visual characteristics within an image is well known. Multiple methods locate edges based on the characteristics of a region within the image, most commonly using the spatial gradients of intensity.²⁹ We view the optical improvements addressed here as complementing those methods. Analytical segmentation of images to find edges will always benefit from better optics. For the relatively simple objects considered here, our results indicate that optimizing the optics can eliminate the need for segmentation altogether, providing accurate results with the simpler analysis based on a single, global threshold.

A myriad of modifications might further improve the approach described here for localizing the upper surface and determining γ . We have considered the following possibilities:

Change in solid support: A hydrated gel with the refractive index of water would be invisible. Polyacrylamide gels can provide suitable transparency, but the extent of cross-linking necessary to produce useable domes produced significant refraction.

Flatter dome: A dome with less indentation would obscure a smaller portion of the bubble. A flatter dome should lessen the problem at the upper surface, but not eliminate it.

Other protocols for interpreting the interfacial profile: The process of determining γ from the interfacial shape of bubbles and droplets is the subject of an extensive literature. Current procedures determine the γ that produces the Laplacian shape that best fits the actual surface at multiple points. Earlier approaches, developed before the

advent of fast, readily available computers, instead determined γ from the interfacial characteristics at one or a few points.³⁰ Suitable adaptation of these methods might provide the desired speed while avoiding the upper surface.

Fixed position of the upper surface: Rather than measuring the location of the upper interface for each frame, its position could be established based on the lower profile in an initial image, obtained before the start of the experiment, and provided as a set parameter for analysis of subsequent images. Although the camera and cuvette must remain fixed to within the dimension of an individual pixel, that requirement may be achievable.

Perhaps the most interesting characteristic of captive bubbles has been their ability to sustain films at very low γ without the escape from confinement that confounds experiments with Langmuir troughs. The optical issues delineated here are less important for those conditions. Low values of γ are relatively insensitive to errors in the dimensions of the bubble.^{8,31} The dramatic changes in shape that occur at low γ are readily apparent by visual inspection without microscopic assistance, and unaffected by the effects discussed here. The marked slowing of collapse at low γ ^{26,32} is obvious from the stability of the profiles. For other experiments, however, these optical effects are crucial. Isobaric measurements of collapse just below the equilibrium spreading tension of ~ 24 mN/m remain in the region where small changes in the dimensions of the bubble significantly alter the calculated γ . In this range, the kinetics of collapse vary dramatically with γ , increasing rapidly to a maximum that approaches a singularity before decreasing at lower γ .^{26,33} For quantitative experiments that use feedback to measure collapse at these γ , the optical adjustments necessary to obtain accurate γ are essential.

Conclusions—Experiments that maintain the interfacial characteristics of bubbles and droplets at constant values require accurate location of two edges, at the upper and lower surfaces, that differ in appearance. Correct alignment of pitch and the use of well-collimated, red light simplify those measurements.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

Adjustable orientations of the cuvette and camera. Rotations orient the camera and the solid support relative to the gravitationally vertical axis and to each other. The three possible rotations are: roll within the vertical plane perpendicular to the optical axis; pitch in the vertical plane that includes the optical axis; and yaw within the horizontal plane.

Figure 2.

Analysis of a captive bubble visualized with poorly collimated light. A lightsource with a diffuser provided illumination from ~6 cm behind a bubble in water at the ambient temperature ($23 \pm 1^\circ\text{C}$). A. Image of the bubble. B. Quantitative variation of grayscale across the upper and lower surfaces. The grayscale values are averaged laterally over the five pixels indicated by width of the vertical line in panel A. The upper and lower axes are shifted so that the dashed vertical line indicates the location of each interface determined by fitting the profile away from the agarose gel. C. Height, diameter, and γ determined with different values of interfacial grayscale. Separate left axes indicate: the measured height and diameter of the bubble, obtained with the interfacial grayscale set to different values; and the γ calculated from those values of height and diameter. The dashed horizontal line indicates the γ that generates the Laplacian shape which best fit the lower profile, away from the solid support. The right axis gives the number of pixels with each grayscale. There were 60,108 pixels in the red region with a grayscale = 0; that count is off the scale of the y-axis set to illustrate the distribution of pixels at other grayscales. D. Location within the image of pixels that fall into the four major groups identified by the histogram in panel C, with colors in the two panels corresponding to the same pixels.

Figure 3.

Effect of camera-pitch. A film of DPPC was spread on the surface of the captive bubble and compressed to achieve a relatively flattened shape. After adjusting the pitch of the camera to the gravitational frame of reference,¹² the cuvette was adjusted so that the solid support was level within the camera's frame of reference.¹² Images were then obtained with the cuvette pitched 0.4° towards and away from the camera. The lower profile of the bubble, away from the solid support, was unaffected by changes in pitch of that magnitude. The Laplacian fit to that profile yielded a height for the bubble of 125 pixels and a surface tension of 3 mN/m for all three images. A. Image of the complete bubble with the camera leveled. B.-D. Images of the solid/air interface above the bubble, with the solid support pitched to different angles. The horizontal, dashed, white lines indicate the location of the upper interface obtained from the best Laplacian fit to the lower profile. E. Variation of grayscale across the upper interface, with the solid support pitched to the three different angles. The vertical location on the x-axis in each case is expressed relative to the position of the interface located by the Laplacian fit.

Figure 4.

Images of a captive bubble observed through a turbid subphase using light with different wavelengths. The subphase contained polystyrene spheres (100 nm mean diameter). Variation of the voltage applied to the lightsource achieved the same incident intensity, measured without the cuvette in place. White light from an incandescent bulb transmitted through band-pass filters provided illumination with: A, blue light (centered at 460 nm); B, green light (535 nm); and C, red light (660 nm).

Figure 5.

Effect of lighting on the perceived dimensions of a reflective object. A polished sapphire sphere with a diameter of 3 mm was placed in the inverted chamber normally used to contain the captive bubble. An iris with a circular aperture of variable diameter placed between the lightsource and the sphere controlled the effective size of the lightsource and its location. Solid lines give the least-squares fit to the measured data. The horizontal, dashed grey lines give the manufacturer's stated tolerance for the actual diameter of the sphere. With the iris 280 mm behind the object, the deviation of the measured diameter by $\sim 1 \mu\text{m}$ below the manufacturer's specifications was less than the $6 \mu\text{m}$ dimension of an individual pixel.

Figure 6.

Variation of grayscale at edges illuminated with well-collimated light. A green lightsource placed 180 cm behind the visualized object provided illumination. A. The imaged edge of a razor blade. B. Variation of grayscale across the edge, averaged laterally between the two dashed lines. C. Imaged left surface of a captive bubble. D. Variation of grayscale recorded along a horizontal line across the edge, averaged vertically between the dashed lines in panel C.

Figure 7.

Variation of images with light intensity. A. and B. Images with different intensities of illumination, indicated by the voltage applied to the lightsource. The gain of the camera was held constant to illustrate the variation of lighting. C. Histogram of grayscales obtained at different light-intensities, but with the gain of the camera adjusted in each case to superimpose the pixels in the subphase to a grayscale of ~ 220 a.u. D. Variation of grayscale along the vertical line in panel B at different light-intensities with gain of the camera adjusted to produce the same histogram (C.). Traces with different colors represent the same conditions as in panel C.

Figure 8.

Comparison of γ determined by two methods during compression of captive bubbles. Results were obtained by calculating γ from: the height and maximum diameter (red dots) during the compression, with the interface located according to grayscale; the Laplacian shape that best fit the lower profile, away from the agarose gel (black curves) in images captured during the compression and analyzed subsequently. A. Isobaric compression during collapse of DOPC, using poorly collimated light. After an initial pulsed compression of a spread monolayer, the bubble was manipulated to maintain a constant γ . B. Isobaric compression during collapse of POPC, using well collimated light. Results provide the γ (left axis) and height (right axis) determined by the two methods. C. Continuous compression of bubbles with and without a spread film of DPPC. The compressions used a constant infusion of $\sim 6.6 \mu\text{l}/\text{min}$ into the subphase.